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Isabelle Treilleux, Marie-cecile Michallet, Jenny Valladeau-Guilemond, Antoine Marçais, Thierry Walzer, Philippe Krebs, Adelheid Cerwenka, Margaux Hubert, Christophe Caux, Nathalie Bendriss-Vermare

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1 **IL-33 drives polyfunctionality and antitumor activity of a unique ST2+ NK cell population**

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23

24 One sentence summary: The IL-33/IL-33R(ST2)/NK cell axis is a key determinant of cancer immunity and  
25 immunotherapy.

26

27 **Abstract**

28

29 Natural Killer (NK) cell subsets differ to ensure complementary and crucial roles in tumor  
30 immunosurveillance. Their biology is critically regulated by cytokines. Here, we show that IL-33  
31 synergizes with IL-12 to strongly activate a subset of CD56<sup>dim</sup> NK cells acquiring ST2 expression.  
32 Transcriptomic and biological analysis of human ST2<sup>+</sup> CD56<sup>dim</sup> NK cells revealed a distinct intermediate  
33 differentiation state between canonical CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells, combining high proliferative  
34 properties, cytokines/chemokines production, and cytotoxicity. NK cells expressing ST2 protein or  
35 exhibiting a ST2-linked transcriptional signature were identified in human and mouse tumors.  
36 Accordingly, IL-12 unleashes human breast tumor ST2<sup>+</sup> NK cell potential to produce IFN- $\gamma$  in response to  
37 IL-33 and IL-33/IL-12 co-injection resulted in a NK-dependent IFN- $\gamma$  secretion and anti-tumor effects in  
38 murine mammary tumors. An *IL33<sup>hi</sup>-NK<sup>hi</sup>* score in solid tumors correlated with increased progression-  
39 free patient survival. Our findings thus identify polyfunctional ST2<sup>+</sup> NK cells which effector functions can  
40 be harnessed by IL-33 to boost anti-tumor immunity.

41

42 **Keywords:** NK cells, IL-33, ST2/IL1RL1, cancer immunosurveillance, cancer immunotherapy

43

## 44 **Introduction**

45 Natural Killer (NK) cells are major innate effector cells involved in host defense against viral  
46 infections and tumors (1). NK cells have evolved into a spectrum of effector subsets, including the main  
47 CD56<sup>bright</sup> and CD56<sup>dim</sup> subsets in humans, diverging in terms of phenotype, function, location, and  
48 responsiveness to activating stimuli to ensure complementary roles in innate immune responses (2).  
49 Indeed, CD56<sup>bright</sup> NK cells are predominant in lymphoid tissues, secrete high levels of cytokines and  
50 chemokines including Interferon-gamma (IFN)- $\gamma$ , Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), CCL3/4  
51 (Macrophage Inflammatory Protein 1  $\alpha/\beta$ ), and CCL5 (RANTES) and undergo robust proliferation after  
52 cytokine stimulation (3). Conversely, CD56<sup>dim</sup> NK cells are the largest circulating population, show a  
53 greater cytotoxic ability but a lower cytokine production and proliferation compared to CD56<sup>bright</sup> NK  
54 cells (2). CD56<sup>dim</sup> NK cells also express the activating Fc receptor CD16, endowing them with antibody-  
55 dependent cellular cytotoxicity (ADCC) function, a central mechanism in antibody-based antitumor  
56 therapies (4).

57 Developmental relationships between NK cell subsets remain partially solved, with CD56<sup>dim</sup> NK  
58 cells likely originating from differentiated CD56<sup>bright</sup> NK cells (5, 6), and evidences of intermediate subsets  
59 corroborating this developmental trajectory (7). CD56<sup>dim</sup> NK cells can then give rise to a variety of  
60 phenotypically and functionally distinct subsets including terminally differentiated CD56<sup>dim</sup> CD57<sup>+</sup> (8, 9),  
61 memory-like/adaptive (10) or hypo-responsive exhausted-like NK cells (11). These NK cell subsets are  
62 characterized by distinct transcriptional programs (12, 13) regulated by profound epigenetic  
63 modifications and chromatin re-arrangements (14, 13). Therefore, NK cell diversity is broader than  
64 originally depicted and delineates functionally distinct subsets, which may be alternatively exploited for  
65 the development of therapeutic strategies (15).

66 NK cells are highly potent in tumor elimination by directly killing cancer cells and supporting  
67 effective innate and adaptive antitumor immune responses (16). Many tumor cells express high levels  
68 of ligands for activating NK cell receptors, leading to efficient recognition by NK cells (17). Accordingly,  
69 high intratumoral infiltration by NK cells is associated with improved prognosis for cancer patients (18–

70 20). However, chronic exposure to activating signals, presence of inhibitory ligands and  
71 immunosuppressive molecules in tumor microenvironment favor NK cells dysfunction, leading to tumor  
72 escape (21). In this context, NK cells are emerging as promising targets for cancer therapy with a growing  
73 number of therapeutic agents aiming to unleash their full antitumor potential.

74 The activation of NK cells depends on the signaling balance between activating and inhibitory  
75 receptors. However, numerous cytokines such as Interleukin(IL)-2 (22), IL-12 (23), IL-15 (24), IL-18 (25),  
76 and type I IFN (26) also regulate NK cell maturation, activation, and survival by acting alone or in  
77 cooperation. The identification of cytokines (*i.e.* IL-21, IL-23, IL-27, IL-33) regulating NK cells is continually  
78 increasing (27). The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of non-immune  
79 cells at epithelial barriers (28). In response to tissue injury, cellular stress, and inflammation, IL-33 is  
80 released and functions as an alarmin by activating immune cells expressing its heterodimeric receptor,  
81 composed of ST2 (encoded by *IL1RL1* gene) and ILRAcP accessory chain (29). The IL-33/ST2 axis was  
82 originally described to support type 2 immunity (30). Also, studies showed a pivotal role of the IL-33/ST2  
83 pathway on the biology of a subset of regulatory T cells (Tregs) constitutively expressing ST2 and  
84 involved in tissue repair (31) and immunomodulation (32). In contrast, IL-33 is now emerging as a  
85 regulator of Th1 responses during viral infection and chronic immune pathologies in mice, enhancing  
86 IFN- $\gamma$  production in CD4<sup>+</sup> cells, CD8<sup>+</sup> T cells, and NK/NKT cells that acquire ST2 expression under  
87 inflammatory conditions (33–35). However, the role of IL-33/ST2 axis in human NK cell subsets biology  
88 remains scarcely addressed and its impact on tumor development has revealed opposing effects  
89 depending on the tumor type and the microenvironment (36). Indeed, IL-33 can contribute to tumor  
90 progression and metastasis by promoting immune cell-dependent (*i.e.*, recruitment of immune  
91 suppressive cells, activation of type 2-mediated inflammation, and inhibition of effector CD8 T and NK  
92 cells) and immune cell-independent (*i.e.*, tumor angiogenesis, cancer cell stemness, tumor cell  
93 proliferation) mechanisms. In contrast, systemic and/or local production of IL-33 can inhibit tumor  
94 growth and metastasis by enhancing both adaptive (*i.e.*, effector Th1 and CD8 T cells) and innate (*i.e.*,  
95 NK cells, DCs, and eosinophils) immune responses.

96           In this study, we evaluated the role of IL-33 in NK cell biology in physiological and tumor context  
97   in human and mouse models. We found that IL-12 upregulates ST2 expression on a subset of human NK  
98   cells, in a STAT4-dependent pathway, promoting NK cell activation by IL-33. Following IL-33 stimulation,  
99   human ST2<sup>+</sup> NK cells displayed polyfunctional activity featuring increased secretory and cytotoxic  
100   functions as well as a higher proliferative ability. RNA sequencing (RNA-seq) analysis identified human  
101   ST2<sup>+</sup> CD56<sup>dim</sup> NK cells as an unprecedentedly characterized intermediate population between canonical  
102   CD56<sup>bright</sup> and ST2<sup>-</sup> CD56<sup>dim</sup> NK cells. We also identified NK cells expressing ST2 protein in human breast  
103   tumors or exhibiting an ST2-linked transcriptional signature in human and mouse tumor public scRNA-  
104   seq datasets. In accordance, IL-12 unleashes the potential of ST2<sup>+</sup> NK cells from the blood and tumor  
105   tissue of breast cancer patients to respond to IL-33 by producing IFN- $\gamma$ . In a murine mammary tumor  
106   model, IL-33 and IL-12 combination had a potent NK-dependent antitumor effect associated with IFN- $\gamma$   
107   production. Finally, pan-cancer analysis in TCGA database showed that an *IL33<sup>hi</sup>-NK<sup>hi</sup>* score correlates  
108   with good prognosis in several solid cancers including breast cancer. Therefore, our results reveal ST2<sup>+</sup>  
109   NK cells as a hitherto unappreciated polyfunctional subset potentially involved in tumor immune  
110   surveillance and identify IL-33 as a promising immunomodulator for NK cell-based cancer therapy.

111

112

113 **Results**

114 **IL-12/STAT4 signaling drives ST2 expression on human NK cells endowed with polyfunctionality in**  
115 **response to IL-33**

116 As previously reported for mouse NK cells in infectious contexts (35), IL-33 was a particularly potent IFN-  
117  $\gamma$  inducer in human NK cells when combined with IL-12 (from  $\approx$ 500- to  $\approx$ 1800-fold as compared to IL-12  
118 alone) (Fig. 1A), similarly to IL-18, a well-known IFN- $\gamma$  trigger for NK cells, and was 4-fold more potent  
119 than IL-1 $\alpha$ / $\beta$ . IL-33 also synergized with IL-2 (60-fold), IL-15 (7-fold) but not with anti-NKaR agonistic  
120 antibodies or K562 target cells (fig. S1A-C). IL-12 and IL-33 synergy was dose-dependent (fig. S1D) and  
121 IFN- $\gamma$  was detected within a few hours to peak at 72 h (fig. S1H) reaching comparable levels ( $> 1 \mu\text{g/mL}$ )  
122 to those induced by IL-12/IL-18 co-stimulation. At low doses (1 ng/mL), IL-33 even induced higher levels  
123 of IFN- $\gamma$  than IL-18, when combined with IL-12 (fig. S1D). The combination of IL-33 and IL-12 also  
124 enhanced NK cell cytotoxicity towards K562 target cells (Fig. 1B, fig. S1I), and induced their expression  
125 of CD25 and CD69 activation markers (Fig. 1C). These results indicate that IL-33, in the presence of IL-  
126 12, is nearly as effective as IL-18 to trigger polyfunctional activation of NK cells. Blocking experiments  
127 using specific antagonistic molecules against IL-1 family cytokines or receptors demonstrated that ST2  
128 is specifically involved in the synergistic effect of IL-33 (Fig. 1D and fig. S2A) with IL-12 on IFN- $\gamma$   
129 production. The analysis of ST2 expression showed that *ST2* mRNA expression was very low in resting  
130 NK cells but increased up to 15- and 35-fold upon stimulation with IL-12 alone or combined with IL-33,  
131 respectively (Fig. 1E), to reach a plateau after 48h (fig. S2B). Levels of *IL1RAC1* co-receptor transcripts in  
132 NK cells were not similarly regulated (Fig. 1E and fig. S2B). Accordingly, ST2 protein was not detected on  
133 resting NK cells, but  $\sim$ 20% of NK cells, expressing intermediate CD56 levels, acquired ST2 surface  
134 expression upon IL-12 stimulation (Fig. 1F). IL-12 mainly activates STAT4-dependent transcription  
135 programs (37). The treatment of IL-12-activated NK cells with lisofylline, a specific inhibitor of STAT4  
136 phosphorylation (fig. S2C), resulted in a decrease in ST2 expression in NK cells (Fig. 1F). *In silico* analysis  
137 of the *ST2* promoter identified several potential STAT4 binding sites (fig. S2D). The binding of STAT4 to  
138 the *ST2* promoter was confirmed in IL-12-stimulated NK cells by chromatin immunoprecipitation (Fig.

139 1G). Taken together, these data indicate that IL-12 stimulation induces *ST2* transcription and protein  
140 expression in a subset of NK cells, through direct binding of STAT4 to the *ST2* promoter, leading to potent  
141 IFN- $\gamma$  production in response to IL-33.

142

### 143 **IL-33 is a potent activator of a subset of human CD56<sup>dim</sup> NK cells in the presence of IL-12**

144 Next, we aimed to characterize more deeply the NK cells subset acquiring *ST2* expression in response to  
145 IL-12. IL-12 triggered *ST2* expression selectively in ~20% of CD56<sup>dim</sup> NK cells (Fig. 2A) but not in CD56<sup>bright</sup>  
146 NK cells, although STAT4 activation was observed in both subsets in response to IL-12 (fig. S3A). We then  
147 used NF- $\kappa$ B (p65) (38), MAPkinases (p38) (39), and mTOR (S6) (40) signaling pathway activation as  
148 surrogates for *ST2*/IL-33 signaling. In line with *ST2* expression, IL-12 and IL-33 co-stimulation of sorted  
149 NK cell subsets selectively induced p65, p38, and S6 phosphorylation exclusively in ~25% of CD56<sup>dim</sup> NK  
150 cells (Fig. 2B and fig. S3B), while each cytokine alone was ineffective. In agreement, a much greater  
151 proportion of CD56<sup>dim</sup> NK cells produced IFN- $\gamma$  and co-expressed CD25 (Fig. 2C) or CD69 (fig. S3C) in  
152 response to IL-12 and IL-33 combination, as compared to CD56<sup>bright</sup> NK cells. Coherently with CD25  
153 expression, the highest IL-2-triggered proliferation was observed in CD56<sup>dim</sup> NK cells following pre-  
154 activation with IL-12 and IL-33 combination (Fig. 2D). Of note IL-33 was more potent than IL-18 in  
155 inducing IL-2 dependent proliferation. In addition, IL-12 and IL-33 combination promoted a specific  
156 inflammatory cytokine secretion profile in CD56<sup>dim</sup> NK cells (Fig. 2E), involving TNF- $\alpha$ , CCL3, and CCL4. As  
157 a comparison, the other IL-1s cytokines preferentially promoted the activation (Fig. 2, B and C, fig. S3B  
158 and C), the proliferation (Fig. 2D), and the cytokine production (Fig. 2E) in CD56<sup>bright</sup> NK cells (for IL-1 $\alpha$   
159 and IL-1 $\beta$ ) or in both NK subsets (for IL-18). CD56<sup>dim</sup> NK cells represent a heterogeneous population  
160 based on CD57 and KIRs surface expression (9, 8). In IL-12 context, while IL-18 preferentially triggered  
161 IFN- $\gamma$  production in CD56<sup>bright</sup> NK cells, the effect of IL-33 was more prominent in CD56<sup>dim</sup> CD57<sup>-</sup> cells NK  
162 cells (Fig. 2F), correlating with *ST2* expression (Fig. 2G). Altogether these results highlight differences in  
163 the regulation of NK cell activation by IL-1s family cytokines, IL-33 being highly selective for CD56<sup>dim</sup> NK  
164 cells, as summarized in Fig. 2H.



165

166 **ST2<sup>+</sup> NK cells show a unique transcriptional signature with intermediate features between CD56<sup>bright</sup>**  
167 **and CD56<sup>dim</sup> subsets**

168 To further characterize human ST2<sup>+</sup> NK cells, we performed RNA-seq analysis from three different  
169 donors on eight FACS-sorted NK cell subsets, based on surface expression of CD56, CD57, and ST2  
170 following 24-hour culture in medium or IL-12 to induce ST2 expression (Fig. 3A). Principle Component  
171 Analysis (PCA) revealed that the PC1 axis (39% variance) segregated NK cells according to CD56  
172 expression, whereas the PC2 axis (27% variance) discriminated samples according to IL-12 stimulation.  
173 Interestingly, ST2<sup>+</sup> CD56<sup>dim</sup> NK cells clustered together between ST2<sup>-</sup> CD56<sup>bright</sup> and ST2<sup>-</sup> CD56<sup>dim</sup> NK cells  
174 along the PC1 axis (Fig. 3B). Focusing on IL-12 culture conditions, 2,399 genes displayed differential  
175 expression between ST2<sup>-</sup> CD56<sup>bright</sup>, ST2<sup>-</sup> CD56<sup>dim</sup>, and ST2<sup>+</sup> CD56<sup>dim</sup> NK cells (fig. S4A). Analysis of genes  
176 specifically upregulated in each subset compared to the two others allowed us to define transcriptomic  
177 signatures specific for each NK cells subsets (Fig. 3C and Tables 1-3). The ST2<sup>+</sup> CD56<sup>dim</sup> NK cells signature  
178 contained 233 genes, including genes encoding surface proteins (e.g., *IL23R*, *IL1RL1*, *TRPV1*, *SLC16A1*,  
179 *ITGA3*, *MT1X*, *PTGER3*, *ULBP1*, *ICAM1*, *HPSE*, *TREML2*, *LRP8*, *EDA2R*), secreted proteins (*VEGFA*, *GZMA*,  
180 *IFNG*), and transcription factors (TFs) (*DMRTA2*, *E2F2*, *E2F1*, *EZH2*, *ATF3*) (Fig. 3C, fig. S4B, and Table 1).  
181 We then evaluated the enrichment score for signatures of ST2<sup>-</sup> CD56<sup>bright</sup> (418 genes), ST2<sup>-</sup> CD56<sup>dim</sup> (396  
182 genes), and ST2<sup>+</sup> CD56<sup>dim</sup> NK cells (233 genes) in each subset respectively by single sample gene set  
183 enrichment analysis (ssGSEA). Strikingly, ST2<sup>+</sup> CD56<sup>dim</sup> NK cells displayed an intermediate enrichment  
184 score for both ST2<sup>-</sup> CD56<sup>bright</sup> and ST2<sup>-</sup> CD56<sup>dim</sup> NK cell signatures (Fig. 3D). Indeed, besides genes  
185 specifically enriched in ST2<sup>+</sup> CD56<sup>dim</sup> NK cells, we also observed sets of genes encoding TFs, surface  
186 markers or soluble mediators exclusive for ST2<sup>-</sup> CD56<sup>bright</sup> and ST2<sup>-</sup> CD56<sup>dim</sup> NK cells and expressed at  
187 intermediate levels in ST2<sup>+</sup> CD56<sup>dim</sup> NK cells (Fig. 3E and fig. S4A). Interestingly, TFs defining the CD56<sup>bright</sup>  
188 NK cell program were absent in ST2<sup>+</sup> CD56<sup>dim</sup> NK cells (e.g., *BACH2*, *LEF1*, *TCF7*), whereas those  
189 specifically regulating the CD56<sup>dim</sup> NK cell program (e.g., *MAF*, *NFIL3*, *PRDM1*, *TBX21*, *ZBTB16*, *ZEB2*)  
190 were all (except for *NFIL3*) expressed at intermediate levels in ST2<sup>+</sup> CD56<sup>dim</sup> NK cells (Fig. 3E). Among

191 genes shared between ST2<sup>+</sup> CD56<sup>dim</sup> NK cells and ST2<sup>-</sup> CD56<sup>bright</sup> NK cells were secreted molecules (*TNF*,  
192 *XCL1*, *XCL2*, *GZMK*); chemokine receptors (*CCR1*, *CCR5*, *CCR6*) and cytokine receptors (*IL2RA*, *IL2RB*,  
193 *IL12RB2*). Genes shared with ST2<sup>-</sup> CD56<sup>dim</sup> NK cells encoded differentiation markers (*FCGR3A*, *KIR2DL1*,  
194 *KIR2DL3*, *KIR3DL1*, *KIR3DL2*), chemotactic receptors (*CXCR1*, *CXCR2*, *CX3CR1*, *S1PR5*), inflammatory  
195 chemokines (*CCL3*, *CCL4*, and *CCL5*), and cytotoxic molecules (*GZMH*) (Fig. 3E). Among DEG between ST2<sup>-</sup>  
196 CD56<sup>bright</sup> and ST2<sup>-</sup> CD56<sup>dim</sup> NK cells, 75.1 % of genes displayed an intermediate expression in ST2<sup>+</sup>  
197 CD56<sup>dim</sup> NK cells (Fig. 3F). By using a permutation test to randomly shuffle labels of all samples (n = 1000  
198 times) and assess samples distribution, we statistically validated the intermediate transcriptional  
199 pattern of ST2<sup>+</sup> CD56<sup>dim</sup> NK cells (p = 0.012) (Fig. 3F). We then performed functional enrichment analysis  
200 for gene signatures selected from Gene Ontology (GO), Hallmarks, and KEGG databases. Based on the  
201 literature (41) and our own data (Fig. 2E,3E, and fig. S4A), we also defined specific cytokine and homing  
202 signatures for CD56<sup>bright</sup> (CYTOKINES\_NKbright and HOMING\_NKbright) and CD56<sup>dim</sup> NK cells  
203 (CYTOKINES\_NKdim and HOMING\_NKdim). ST2<sup>+</sup> CD56<sup>dim</sup> NK cells were specifically enriched in signatures  
204 involved in cell proliferation (*i.e.* cell cycle) and metabolism (*i.e.* oxidative phosphorylation, glycolysis,  
205 PI3K/AKT/mTOR signaling), indicating their higher activation state as compared with ST2<sup>-</sup> CD56<sup>bright</sup> and  
206 ST2<sup>-</sup> CD56<sup>dim</sup> NK cells. Furthermore, ST2<sup>+</sup> CD56<sup>dim</sup> NK cells showed an enrichment in biological processes  
207 associated with either CD56<sup>bright</sup> (*i.e.* CYTOKINES\_NKbright, HOMING\_NKbright, and regulation of T-  
208 helper 1 immune response) or CD56<sup>dim</sup> (*i.e.* CYTOKINES\_NKdim, HOMING\_NKdim, and cytolysis) NK cell  
209 subsets (Fig. 3G), in line with their polyfunctionality. In agreement, the GO term associated with NK cell  
210 differentiation was progressively enriched in ST2<sup>+</sup> CD56<sup>dim</sup> NK and ST2<sup>-</sup> CD56<sup>dim</sup> NK cells, consistent with  
211 a ST2<sup>-</sup> CD56<sup>bright</sup> → ST2<sup>+</sup> CD56<sup>dim</sup> → ST2<sup>-</sup> CD56<sup>dim</sup> differentiation trajectory. Collins *et al.* recently reported  
212 a continuum in NK cell differentiation, with CD57<sup>+</sup> CD56<sup>dim</sup> NK cells representing a terminally-  
213 differentiated state (13). In concordance with this study, CD57<sup>-</sup> ST2<sup>+</sup> CD56<sup>dim</sup> NK cells expressed higher  
214 levels of genes (*LEF1*, *XCL1*, *TNF*, *GZMK*) and gene signatures/pathways of CD56<sup>bright</sup> NK cells, whereas  
215 CD57<sup>+</sup> ST2<sup>+</sup> CD56<sup>dim</sup> NK cells overexpressed genes (*FCGR3A*, *CXC3CR1*, *KIR2DL1*, *KIR2DL3*, *KIR3DL1*,  
216 *KIR3DL2*, *NFIL3*, *PRDM1*, *ZEB2*, *GZMH*) and gene signatures/pathways shared with CD56<sup>dim</sup> NK cells (Fig.

217 3B-E,G). Altogether, this analysis revealed that ST2<sup>+</sup> CD56<sup>dim</sup> NK cells display a unique, highly activated,  
218 and polyfunctional profile, intermediate between IL-12-activated ST2<sup>-</sup> CD56<sup>bright</sup> and ST2<sup>-</sup> CD56<sup>dim</sup> NK  
219 cells.

220

### 221 **ST2<sup>+</sup> NK cells infiltrate tumors and are functionally competent in response to IL-33 and IL-12 activation**

222 We then addressed the functional relevance of the ST2<sup>+</sup> NK cells in cancer. First, we analyzed previously  
223 published datasets of single cell (sc)-RNAseq from NK cells infiltrating human melanoma metastases  
224 (GSE139249) (42) and mouse lung tumors (GSE123534) (43) to investigate the presence of ST2<sup>+</sup> NK cells  
225 in tumor tissues. Unsupervised clustering of all sequenced intratumoral NK cells revealed nine and eight  
226 distinct clusters based on transcript signatures in human melanoma metastases and in mouse lung  
227 tumors respectively (fig. S5), with human cluster #5 and murine clusters #4 and #7 displaying a higher  
228 ssGSEA score for the 233-gene expression signature associated with ST2 expression in NK cells  
229 (representing 3%, 9.4% and 2.4% of total NK cells respectively) (Fig. 4A). By analyzing human breast  
230 tumor resections, where NK cells were detected in 85% of cases (40/46) and represented up to 7% of  
231 total infiltrating immune cells, regardless of the breast cancer molecular subtype (fig. S6A,B), we  
232 identified ST2<sup>+</sup> NK cells in tumors representing ≈5 to 10% of total tumor-infiltrating NK cells, but not in  
233 patients' blood (Fig. 4B). As a surrogate for IL-33 responsiveness, we monitored p65 phosphorylation in  
234 *ex vivo*-stimulated tumor-infiltrating and circulating NK cells. While IL-33 had no effect alone, its  
235 association with IL-12 induced p65 phosphorylation in a subset of circulating and tumor-infiltrating NK  
236 cells (Fig. 4C), highlighting the potential of NK cells to respond to IL-33 in cancer patients. Consistently,  
237 IL-12 and IL-33 co-stimulation enhanced IFN-γ-production by circulating and tumor-infiltrating NK cells  
238 from breast cancer patients, as compared to IL-12 alone (Fig. 4D). Collectively, these results indicate that  
239 ST2<sup>+</sup> NK cells are present in the microenvironment of different tumor types and that they can respond  
240 to IL-33 resulting in IFN-γ production, suggesting a potential antitumor function of IL-33.

241

### 242 **Combination of IL-33 with IL-12 promotes NK cell antitumor activity *in vivo***

243 As for human NK cells, IL-12 was required to upregulate ST2 expression on the surface of a subset of  
244 mouse spleen NK cells (fig. S7A), which subsequently produced IFN- $\gamma$  in response to IL-33 (fig. S7B). Next,  
245 using the mouse E0771 triple negative breast tumor cell line (44) we addressed the functional relevance  
246 *in vivo* of IL-33 NK-dependent antitumor activity. Mice were orthotopically injected in the mammary  
247 gland with E0771 tumor cells and then treated with IL-33 and IL-12 alone or in combination via  
248 peritumoral injection (Fig. 5A). We observed a marked reduction in primary tumor growth in IL-12/IL-33  
249 co-treated mice compared to the control group and, and to a lesser extent, to IL-12 or IL-33  
250 monotherapy (Fig. 5A). Consistently, co-injection of IL-12 and IL-33 increased long-term survival of  
251 tumor-bearing mice (Fig. 5B). These antitumor effects were linked to higher levels of systemic IFN- $\gamma$  (Fig.  
252 5C). Strikingly, the effects of IL-33/IL-12 co-treatment on tumor growth, survival, and IFN- $\gamma$  production  
253 were abrogated when NK cells were depleted (Fig. 5D-F) while they were maintained in T and B cell-  
254 deficient *Rag2*-KO mice, in an NK cell-dependent manner (fig. S8A-C). Altogether, these results  
255 demonstrate that IL-12 and IL-33 co-stimulation induces anti-tumor NK cell functions *in vivo*.

256

### 257 **An $NK^{hi}$ -*IL33*<sup>hi</sup> score predicts improved cancer patients survival**

258 To assess the clinical relevance of our findings, we analyzed *IL33* and *IL1RL1* (ST2) gene expression in  
259 human cancers using The Cancer Genome Atlas (TCGA) transcriptomic database. Although detected in  
260 virtually all cancer types, *IL33* expression was lower in tumor as compared to adjacent non-tumoral  
261 tissue in 13/30 cancers, including invasive breast carcinoma (IBC), while it was upregulated in five  
262 cancers (Fig. 6A). Similarly, *IL1RL1* was downregulated in 20/30 cancers as compared to adjacent normal  
263 tissues, with some cancers displaying barely detectable *IL1RL1* expression (Fig. 6A). These observations  
264 suggest a protective role of the IL-33/ST2 pathway in human cancers. We confirmed the presence of IL-  
265 33 soluble protein in 95% of breast tumor supernatants, with the highest levels detected in luminal  
266 tumors compared to Her2<sup>+</sup> and triple negative tumors (Fig. 6B). While absent from the nuclei of tumor  
267 cells, IL-33 was expressed in the nuclei of epithelial cells from normal acini, of endothelial cells as  
268 previously reported (45), and of isolated stromal cells morphologically resembling immune cells or

269 cancer-associated fibroblasts (Fig. 6C). Further analysis from public transcriptomic datasets from  
270 microdissected tissues (46, 47) confirmed that *IL33* expression was mainly restricted to the stroma, and  
271 higher in non-tumoral tissue and early non-invasive lesions (DCIS) compared to advanced IBC (Fig. 6D),  
272 consistent with a loss of IL-33 expression in cancer cells during breast tumorigenesis. In addition, we  
273 found that the expression of *IL33* and *IL1RL1* in TCGA data sets for human cancers was frequently  
274 associated with an improved Progression-Free Survival (PFS) (fig. S9A-C). Notably, in breast cancer, *IL33*-  
275 based patient stratification was independently prognostic when adjusted for age, molecular subtype,  
276 and tumor stage (fig. S9D).

277 We then examined whether high *IL33* expression in conjunction with a high *NK* cell infiltration predicted  
278 improved patients survival as compared with these parameters considered separately. TCGA clinical  
279 cases were stratified as *IL33*<sup>high</sup> vs *IL33*<sup>low</sup> expression and as *NK*<sup>high</sup> vs *NK*<sup>low</sup> score, using a previously  
280 defined *NK* cell gene signature (48) (Table 4). *NK*<sup>hi</sup> tumors displayed higher expression of *IL33*, indicating  
281 a positive association between these two parameters (Fig. 7A). An *NK*<sup>high</sup>-*IL33*<sup>high</sup> score was associated  
282 with improved PFS in six cancers including breast cancer (BRCA), whereas *NK*<sup>high</sup>-*IL33*<sup>low</sup> score had no  
283 prognostic value with the exception of Liver Hepatocellular Carcinoma (LIHC) (Fig. 7, B and C, and fig.  
284 S9E). Notably, multivariate analysis in breast cancer patients highlighted that an *NK*<sup>high</sup>-*IL33*<sup>high</sup> score  
285 remained a marker of better PFS regardless of the molecular subtype, the stage of the disease, and the  
286 age of patients (Fig. 7D). Altogether, these results reveal a strong favorable prognostic value of the IL-  
287 33/ST2 axis in particular when associated with a high *NK* score in several cancers including breast cancer.

288

## 289 Discussion

290 Previous studies have established the important role for IL-33 in type 2 immunity, but its contribution  
291 to type 1 immune responses remains poorly understood (36). Indeed, the role of IL-33/ST2 pathway in  
292 antitumor immunity remains elusive, in particular through NK cell modulation. Our results demonstrate  
293 that a specific subset of human CD56<sup>dim</sup> NK cells express ST2 in response to IL-12 and is endowed with  
294 polyfunctional activity in response to IL-33. This unique subset represents an intermediate  
295 differentiation stage between less mature secretory CD56<sup>bright</sup> and more differentiated cytotoxic CD56<sup>dim</sup>  
296 NK cells, with high activation potential and anti-tumor activity. Indeed, tumor-infiltrating NK cells were  
297 found to express ST2 and to produce IFN- $\gamma$  in response to IL-12/IL-33 co-activation. Accordingly, IL-33  
298 and IL-12 combination inhibits mammary tumor development in mice in an NK cell-dependent manner  
299 and an *IL33<sup>hi</sup>/NK<sup>hi</sup>* transcriptomic score predicts better survival in breast cancer and other cancers. These  
300 findings uncover an important role for ST2<sup>+</sup> NK cells in antitumor immunity which is relevant in cancer  
301 immune surveillance and cancer immunotherapy.

302 Our data indicate that IL-33 by itself is not able to activate NK cells. Indeed, ST2 protein  
303 expression in human and mouse NK cells requires IL-12, in agreement with previous works but only  
304 shown at the transcriptional level (33). Mechanistically, we show that IL-12 induces and stabilizes *ST2*  
305 expression in human NK cells through a direct binding of STAT4 in *ST2* promoter, corroborating previous  
306 results in mouse Th1 cells (49). IL-12 appears as the most potent signal to enable NK cells to respond to  
307 IL-33, highlighting a major role of STAT4 as a master regulator of *ST2* expression. Synergistic activity with  
308 IL-12 was also reported for other members of the IL-1 cytokine family, such as IL-18. Indeed, IL-12 and  
309 IL-18 coactivation induces STAT4/c-jun complexes which bind to *IFNG* promoter (50) and activates p38-  
310 MAPK pathway which enhances stability of *IFNG* transcripts in human NK cells (51). Similarly, IL-33 was  
311 shown to activate c-jun (52) and p38-MAPK (our results and (53)), therefore, IL-33 and IL-12 synergy  
312 likely acts through pathways similar to those described for IL-18 to trigger high IFN- $\gamma$  production by NK  
313 cells. However, our study identifies specific regulation of NK cell functions by IL-33 compared to other  
314 IL-1 family cytokines including IL-18. Indeed, IL-33 was unique in its ability to induce the concomitant

315 secretion of IFN- $\gamma$  and a broad spectrum of pro-inflammatory cytokines and chemokines in CD56<sup>dim</sup> NK  
316 cells. In addition, while CD56<sup>dim</sup> NK cells are known for their low proliferative ability (54), only IL-33  
317 strongly potentiates their IL-2-triggered proliferation, consistent with the upregulation of CD25. Our  
318 findings therefore highlight differences in NK cell subsets' ability to respond to IL-1 family members upon  
319 co-stimulation with IL-12, with unique activation features acquired in response to IL-33.

320

321 Our results show that IL-12-induced ST2<sup>+</sup> NK cells stand as an intermediate stage between  
322 conventional CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells, with a mixed transcriptomic profile, consistent with their  
323 polyfunctional activity in response to IL-12 and IL-33. Previous studies reported NK cell differentiation  
324 intermediates, based on their surface expression of CD94/NKG2A, CD62L, CX3CR1, KIRs and CD57 (9, 8,  
325 7, 55) and transcriptional and epigenetic programs governing differentiation from immature CD56<sup>bright</sup>  
326 to terminally differentiated CD56<sup>dim</sup> (56, 12, 13). ST2 expression is restricted to human CD56<sup>dim</sup> NK cells,  
327 although IL-12/STAT4 signaling is active in CD56<sup>bright</sup> NK cells, suggesting differences in transcriptional  
328 regulation by STAT4 among NK cells subsets, as previously evidenced for adaptive memory NK cells (14).  
329 Genes encoding BACH2 and TCF7, master transcription factors of CD56<sup>bright</sup> NK cells, were specifically  
330 downregulated in ST2<sup>+</sup> NK cells and might therefore act as transcriptional repressors preventing ST2  
331 expression in CD56<sup>bright</sup> NK cells, as previously shown to inhibit CD56<sup>dim</sup> transcriptional program (13). The  
332 absence of CD56<sup>bright</sup> specific transcription factors in ST2<sup>+</sup> NK cells argue for a differentiation model where  
333 ST2 is upregulated on NK cells after they first lose the transcription factors governing the CD56<sup>bright</sup>  
334 subset. Furthermore, ST2 expression was preferentially observed in a subset of CD57<sup>-</sup> CD56<sup>dim</sup> NK cells  
335 and lost during terminal differentiation alongside with the acquisition of CD57. Together, our results  
336 point toward a model in which ST2<sup>+</sup> CD56<sup>dim</sup> NK cells originate from CD56<sup>bright</sup> NK cells to emerge as an  
337 intermediate stage between CD56<sup>bright</sup> and terminally differentiated CD56<sup>dim</sup> CD57<sup>+</sup> NK cells.

338

339 We also report NK cells expressing ST2 protein and exhibiting an ST2-linked transcriptional  
340 signature in human and mouse tumors. Of note, we previously reported significant levels of IL-12p40

341 and IL-12p70 in supernatants obtained from advanced breast tumor (57) that may be released in tumors  
342 by activated DCs (58) to locally license NK cells to respond to IL-33 by inducing ST2 expression. IL-12 may  
343 also be produced in tumor-draining lymph nodes by DCs, in close vicinity with NK cells. Interestingly, a  
344 recent study suggested that DC-derived IL-12 is essential for the anti-tumor activity of NK cells (59), while  
345 NK cell-derived IFN- $\gamma$  sustains the production of IL-12 by cDC1 (60), hence delineating a positive feedback  
346 loop. In this context, loss of IL-12 expression in advanced tumors may represent an escape mechanism  
347 preventing NK cells' activation by IL-33. In conclusion, we suggest that NK cells are fine-tuned by the  
348 cytokine microenvironment with IL-12 driving IL-33 activity towards an anti-tumor immune response via  
349 NK cells.

350  
351 Our study reveals that IL-33 protein is present in human breast tumors. Nevertheless, we  
352 document a frequent downregulation of *IL33* expression in tumors and a positive impact of *IL33*  
353 expression on patients' survival in several cancer types, including breast cancer. Consistently, previous  
354 studies reported a positive prognostic impact of IL-33 in hepatocarcinoma, kidney, salivary glands or  
355 prostate cancers (61–63) and IL-33 levels are inversely correlated with tumor grade and tumor size in  
356 lung adenocarcinomas (64) and osteosarcomas (65). These observations support an anti-tumor role for  
357 endogenous IL-33 that could be released in the microenvironment of early-stage tumors following cell  
358 death and stress. Furthermore, our analysis of TCGA database reveal a strong favorable prognostic value  
359 of IL-33 in particular when associated with a high *NK* score in several cancers, including breast cancer,  
360 independently of other clinical parameters, highlighting a selective advantage of combined IL-33 and NK  
361 cells. Our observation complements recent findings showing that a *NK-IL18-IFNG<sup>high</sup>* signature has a  
362 positive prognosis value for cancer patients' survival (43). Although NK cells in advanced solid tumors  
363 are characterized by a dysfunctional state (21), we show here that *ex vivo* stimulation with IL-33 and IL-  
364 12 triggered IFN- $\gamma$  production by human breast tumor-infiltrating NK cells. Consistently, our *in vivo* study  
365 demonstrates a potent antitumor role for IL-33/IL-12 on primary mammary tumor growth in mice via  
366 the activation of tumor-infiltrating NK cell resulting in systemic IFN- $\gamma$  production, in line with previous



367 reports following ectopic expression of IL-33 (66) or systemic administration of IL-33 (67, 68). Systemic  
368 IL-33 was also shown to inhibit the development of metastatic tumor in 4T1 mouse model in an NK cell-  
369 dependent manner (68). Together, these results highlight the potential of IL-33 as a novel therapeutic  
370 strategy to promote NK cells anti-tumor activity.

371

372 In conclusion, our analysis of human and mouse tissues identifies a novel NK cell subset  
373 upregulating ST2 upon IL-12 activation, which display high proliferative abilities and polyfunctionality.  
374 IL-33/ST2 axis plays a protective role in cancer and is of clinical relevance for cancer patients' outcome.  
375 Our results also reveal a promising role of ST2<sup>+</sup> NK cells to be harnessed for cancer treatment.  
376 Deciphering NK cell differentiation paths and identifying stimuli promoting distinct maturation stages  
377 and functions is crucial to achieve tailored harnessing of NK cell features for therapeutic benefit in a  
378 variety of pathological contexts.

379

380

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408

409 **Author contributions**

410 N.B.-V., A.E., E.B., and C.C. designed the study. A.E., E.B., V.P., Y.R., A.V., F.O., C.R., B.E., L.M., E.C., and  
411 T.R. did the experiments. A.E., E.B., V.A., M.A., X.W., A.S., and M.H. collected the data. A.E., E.B., V.A.,  
412 and M.H. interpreted the results and prepared the manuscript. M.H. performed RNAseq data analysis.  
413 V.A., M.A., L.T., X.W., A.S., and M.H. performed TCGA data analysis. L.M., M.-C.M., J.V.-G., and A.C.  
414 provided expertise. E.C., T.W., and A.M. helped in the design of phosphosignaling analysis by flow  
415 cytometry and mouse experiments. T.R. and P.K. provided retrospective cohorts of breast tumors. O.T.  
416 and I.T. provided prospective breast tumor tissues. V.P., Y.R., A.M., T.W., and P.K. read the manuscript  
417 and provided valuable input. A.E., E.B., C.C., and N.B.-V. wrote the manuscript.

418

419 **Competing interests**

420 The authors declare no competing interests.

421

422

423 **Fig. titles and legends**

424 **Fig. 1. IL-12/STAT4 signaling drives ST2 expression and polyfunctionality of human NK cells in response**  
425 **to IL-33**

426 **(A)** Quantification of IFN- $\gamma$  secretion by healthy donors' blood NK cells upon stimulation with IL-33, IL-  
427 18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination with IL-12 (10 ng/mL) for 24 h. Histogram bars  
428 represent the median (n = 4 individual experiments). Friedman test with Dunn's multiple comparisons  
429 test was performed.

430 **(B)** NK cells were activated for 24 h with IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination  
431 with IL-12 (10 ng/mL) and then co-cultured with calcein-loaded K562 target cells for 4 h at a 5:1 effector  
432 to target ratio. Calcein release in the supernatant was quantified by fluorometry to calculate lysis  
433 percentage. Results are expressed as mean + SEM (n = 9 individual experiments). One-way repeated  
434 measures ANOVA with Dunnett's multiple comparisons test against levels in medium was performed.

435 **(C)** NK cells were activated for 24 h with IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination  
436 with IL-12 (10 ng/mL) and then stained for CD25 and CD69 (black line) or corresponding isotypic control  
437 (grey). Representative histogram plots (left) and quantification (%) (right) of CD25<sup>+</sup> or CD69<sup>+</sup> NK cells  
438 after cytokine stimulation. Symbols represent paired individual experiments (n=7). Results are expressed  
439 as mean + SEM. One-way repeated measures ANOVA with Dunnett's multiple comparisons test against  
440 levels in IL-12 was performed.

441 **(D)** IFN- $\gamma$  secretion by healthy donors' blood NK cells upon stimulation for 24 h with IL-33 and IL-12 (10  
442 ng/mL each) in the presence of anti-ST2 (10  $\mu$ g/mL), anti-IL-18 (1  $\mu$ g/mL) blocking antibodies, IL-1RA  
443 antagonist (100 ng/mL) or mIgG1 control antibody (1 or 10  $\mu$ g/mL as control for anti-IL-18 or anti-ST2,  
444 respectively). Results are expressed as mean + SD and are representative of three individual  
445 experiments. One-way repeated measures ANOVA with Tukey's multiple comparisons test was  
446 performed.

447 **(E)** Real-time quantitative PCR (RT-qPCR) analysis of *IL1RL1* (ST2) and *IL1RAcP* mRNA expression in  
448 resting or activated NK cells with IL-12, IL-33, or the combination (10 ng/mL each for 24 h). Results are

449 expressed as mean + SEM of three individual experiments. Each symbol represents one donor. Two-  
450 tailed paired Student *t*-test was performed.

451 **(F)** NK cells were activated with IL-12 (10 ng/mL) in the presence or not of lisofylline (500  $\mu$ M) for 24 h  
452 prior to ST2 surface expression analysis by flow cytometry. Representative dot plots (left) and  
453 quantification (%) (right) of ST2<sup>+</sup> NK cells after cytokine stimulation. One-way repeated measures ANOVA  
454 with Tukey's multiple comparisons test was performed; n = 3.

455 **(G)** p-STAT4 was immunoprecipitated in NK cells following activation or not with IL-12 (10 ng/mL) for 24  
456 h and p-STAT4 enrichment in *ST2* promoter region was measured by RT-qPCR analysis. *PERFORIN-1*  
457 (*PRF1*) and negative control sets 1 and 2 were used respectively as positive and negative controls for p-  
458 STAT4 binding. Results are expressed as mean + SEM of two individual experiments.

459

460 **Fig. 2. IL-33 strongly activates a subset of CD56<sup>dim</sup> NK cells in the presence of IL-12**

461 **(A)** Healthy donors' blood NK cells were activated with IL-12 (10 ng/mL) or not (medium) for 24 h prior  
462 to flow cytometry analysis of ST2, IL-18R1, IL-1R1 expression (grey) or corresponding isotypic control  
463 (black line). CD56 surface expression levels were used to discriminate CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells.  
464 Representative histogram plots (left) and quantification (%) (right) of ST2<sup>+</sup> NK cells after medium or IL-  
465 12 culture. Histogram bars indicate the median. Wilcoxon matched pairs signed-rank test was  
466 performed; n = 12 experiments.

467 **(B)** FACS-sorted CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells were activated with medium or IL-12 for 24 h prior to the  
468 addition of IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$ , supplemented or not with IL-12. Each cytokine was used at  
469 10ng/mL. p65 (NF- $\kappa$ B) phosphorylation was analyzed by flow cytometry 5 min after the addition of IL-1  
470 family cytokines. Representative histogram plots (left) and quantification (%) (right) of p-p65<sup>+</sup> NK cells  
471 after cytokine activation. Histogram bars indicate the median. Two-way ANOVA with Bonferroni's  
472 multiple comparisons test was performed; n = 4 experiments.

473 **(C)** Healthy donors' blood NK cells were activated as indicated for 24 h, each cytokine was used at 10  
474 ng/mL. CD25 surface and IFN- $\gamma$  intracellular expression was analyzed by flow cytometry. CD56 surface

475 expression levels were used to discriminate CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells. Representative dot plots  
476 (left) and quantification (%) (right) of CD25<sup>+</sup> IFN- $\gamma$ <sup>+</sup> NK cells after cytokine activation. Histogram bars  
477 indicate the median. Two-way ANOVA test with Bonferroni's multiple comparisons test was performed;  
478 n = 5 experiments.

479 **(D)** FACS-sorted CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells were pre-activated as indicated, labeled with CTV,  
480 and cultured in low dose IL-2 (100 UI/mL). In vitro NK cell proliferation was analyzed at day 3 for  
481 CD56<sup>bright</sup> and day 6 for CD56<sup>dim</sup> NK cells. Expansion Index (Exp. Index) and Replication Index (Rep.  
482 Index) determine the fold-expansion of the overall culture and of the responding cells only, respectively.  
483 Data shown are representative of three individual experiments.

484 **(E)** Supernatants from FACS-sorted CD56<sup>bright</sup> (empty histogram bars) and CD56<sup>dim</sup> (filled histogram bars)  
485 NK cells were collected to quantify cytokine and chemokine release by Luminex assay. Of note, IL-2, IL-  
486 3, IL-4, IL-5, IL-10, IL-13, IL-17A, IL-22, and G-CSF were not detected (not shown). Results are expressed  
487 as mean + SEM (n = 3 individual donors). One-way repeated measures ANOVA with Tukey's multiple  
488 comparisons test was performed.

489 **(F)** Healthy donors' blood NK cells were activated with combinations of IL-12 and IL-33 or IL-12 and IL-  
490 18 (10 ng/mL of each cytokine) for 24 h prior to flow cytometry analysis of intracellular IFN- $\gamma$  expression  
491 in NK cell subsets based on CD56 and CD57 surface expression. Results are expressed as mean +/- SEM  
492 (n = 7 individual experiments). Two-way ANOVA with Bonferroni's multiple comparisons test was  
493 performed.

494 **(G)** Healthy donors' blood NK cells were activated with IL-12 (10 ng/mL) for 24 h prior to flow cytometry  
495 analysis of ST2 expression on NK cell subsets based on CD56 and CD57 surface expression. Results are  
496 expressed as mean + SEM (n = 12 experiments). One-way repeated measures ANOVA with Tukey's  
497 multiple comparisons test was performed.

498 **(H)** Graphical summary of biological functions differentially regulated in CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells  
499 in response to IL-1 family cytokines (IL-33, IL-18, IL-1 $\alpha$  and IL-1 $\beta$ ) when combined with IL-12.

500

501 **Fig. 3. ST2<sup>+</sup> CD56<sup>dim</sup> NK cells display a unique gene signature compared to CD56<sup>bright</sup> and CD56<sup>dim</sup> NK**  
502 **cells**

503 **(A)** Schematic representation of FACS sorting strategy to isolate eight different subsets sorted from  
504 healthy donors' blood NK cells based on CD56, CD57 and ST2 surface expression. Three and five subsets  
505 were obtained after activation for 24 h with medium or IL-12 respectively.

506 **(B)** Principal component analysis for eight FACS-sorted NK cell subsets based on expression of the top  
507 500 most variant genes obtained from 3 individual healthy donors.

508 **(C)** Heatmap representing upregulated genes in each sorted NK cell subset compared to the two other  
509 NK cell subsets following IL-12 activation for 24 h (n = 3 individual healthy donors). Black squares identify  
510 specific transcriptional signatures for ST2<sup>-</sup> CD56<sup>bright</sup>, ST2<sup>-</sup> CD56<sup>dim</sup> NK cells, and ST2<sup>+</sup> CD56<sup>dim</sup> NK cells.

511 **(D)** ssGSEA analysis of the NK signatures defined by genes specifically upregulated in each sorted NK cell  
512 subset (see Table 1) following IL-12 activation for 24 h (n = 3 individual healthy donors). Kruskal-Wallis  
513 test with Dunn multiple comparisons test was performed.

514 **(E)** Heatmap showing normalized expression for selected genes related to NK cell phenotype and  
515 functions.

516 **(F)** Sampling distribution of genes with an intermediate expression in ST2<sup>+</sup> NK cells (CD56<sup>bright</sup> > ST2<sup>+</sup> >  
517 CD56<sup>dim</sup> or CD56<sup>bright</sup> < ST2<sup>+</sup> < CD56<sup>dim</sup>) with n = 1000 random shuffling of all sample labels. p value was  
518 computed with a permutation test.

519 **(G)** ssGSEA analysis of selected biological pathways in each sorted NK cell subset following IL-12  
520 activation for 24 h (n = 3 individual healthy donors).

521  
522 **Fig. 4. Peripheral and tumor-infiltrating NK cells from breast cancer patients respond to the IL-33 and**  
523 **IL-12 combination**

524 **(A)** Violin plots representing the distribution of ssGSEA score for ST2<sup>+</sup> NK cells signature (233 genes, see  
525 Table 1) for tumor NK cell clusters identified from sc-RNAseq analysis of tumor-infiltrating NK cells  
526 isolated from (left panel) melanoma patients (GSE139249) (42) and (right panel) a mouse model of lung

527 cancer (GSE123534) (43). Kruskal-Wallis test with Dunn multiple comparisons test was performed using  
528 cluster 5 for the melanoma dataset and cluster 4 (turquoise) or cluster 7 (pink) (fig. S5) as references.

529 **(B)** Flow cytometry analysis of ST2 protein expression on the surface of CD56/NKp46<sup>+</sup> NK cells among  
530 PBMCs and tumor cell suspensions from breast cancer patients (n = 6). Representative dot plots (left)  
531 and quantification (%) (right) of ST2<sup>+</sup> NK cells. Results are expressed as mean + SEM. Paired two-tailed  
532 Student *t*-test was performed.

533 **(C)** PBMCs (n = 7) and tumor cell suspensions (n = 4) from breast cancer patients were activated with  
534 medium or IL-12 for 24 h prior to the addition of medium or IL-33. p65 phosphorylation was analyzed 5  
535 min after the addition of IL-33 by flow cytometry in CD56<sup>+</sup> CD7<sup>+</sup> NK cells. Representative histogram plots  
536 (left) and quantification (%) (right) of p-p65<sup>+</sup> NK cells after IL-12 and IL-33 combination. Results are  
537 expressed as mean + SEM. Unpaired two-tailed Student *t*-test was performed with no statistically  
538 significant difference.

539 **(D)** PBMCs and tumor cell suspensions from breast cancer patients (n = 5) were activated as indicated  
540 (10 ng/mL of each cytokine) for 24 h prior to flow cytometry analysis of intracellular IFN- $\gamma$ .  
541 Representative dot plots (left) and quantification (%) (right) of IFN- $\gamma$ <sup>+</sup> NK cells. Symbols represent  
542 individual breast cancer patients and histogram bars the median. Wilcoxon matched-pairs signed rank  
543 test was performed.

544

545 **Fig. 5. The combination of IL-33 and IL-12 promotes antitumor functions in an NK-cell-dependent**  
546 **manner**

547 **(A)** WT mice were injected intra-mammary with 2.5x10<sup>5</sup> E0771 cells on day 0 and then treated in the  
548 tumor area with NaCl solution (n = 6), 10 ng/mouse rIL-12 (n = 9) or 100 ng/mouse rIL-33 (n = 9) or  
549 in combination (n = 10) twice a week from day 2 to day 26. Primary tumor growth was monitored in  
550 mice treated with, rIL-12, rIL-33 or with the two cytokines. Two-way ANOVA with Tukey's multiple  
551 comparisons test was performed.



552 **(B)** Kaplan-Meier survival plots of WT mice treated with NaCl, rmIL-12, rmIL-33 or the two cytokines.  
553 Mice were sacrificed when longest side of primary tumor reached 17 mm. Log-rank test was performed  
554 and the p-value is indicated for NaCl vs rmIL-12 + rmIL-33 comparison.

555 **(C)** Serum was retrieved from blood collected at day 5, 4 h after NaCl or cytokine injection into the  
556 peritumoral area. IFN- $\gamma$  concentration was measured by Elisa in the serum of mice treated with NaCl,  
557 rmIL-12, rmIL-33 or the two cytokines. Histogram bars represent the median. Kruskal Wallis test with  
558 Dunn's multiple comparisons test was performed.

559 **(D)** WT mice were injected intra-peritoneally with NaCl or anti-NK1.1 depleting antibody prior to intra-  
560 mammary injection with  $2.5 \times 10^5$  E0771 cells on day 0 and then treated in the tumor area with NaCl or a  
561 combination of 10 ng/mouse rmIL-12 and 100 ng/mouse rmIL-33 twice a week from day 2 to day 23.  
562 Primary tumor growth was monitored in non-depleted WT mice treated with NaCl (n = 4) or with rmIL-  
563 12 and rmIL-33 combination (n = 5) or in NK cell-depleted WT mice treated with NaCl (n = 5) or with  
564 rmIL-12 and rmIL-33 combination (n = 5). Two-way ANOVA with Tukey's multiple comparisons test was  
565 performed.

566 **(E)** Kaplan-Meier survival plots of non-depleted WT mice treated with NaCl (n = 4) or with a combination  
567 of rmIL-12 and rmIL-33 (n = 5) or in NK cell-depleted WT mice treated with NaCl (n = 5) or with rmIL-12  
568 and rmIL-33 combination (n=5). Mice were sacrificed when longest side of primary tumor reached 17  
569 mm. Log-rank test was performed and the p-value is indicated for non-depleted mice treated with rmIL-  
570 12 + rmIL-33 vs NK cell-depleted mice treated with rmIL-12 + rmIL-33.

571 **(F)** Serum was retrieved from blood collected at day 5, 4 h after NaCl or cytokine injection into the  
572 peritumoral area. IFN- $\gamma$  concentration was measured by ELISA in the serum of non-depleted WT mice  
573 treated with NaCl (n = 4) or with rmIL-12 and rmIL-33 combination (n = 5) or in NK cell-depleted WT mice  
574 treated with NaCl (n = 5) or with rmIL-12 and rmIL-33 combination (n = 5). Histogram bars represent the  
575 median. Kruskal Wallis test with Dunn's multiple comparisons test was performed.

576

577 **Fig. 6. IL-33 is expressed in tumors and downregulated during cancer progression**

578 **(A)** *IL33* and *IL1RL1* (ST2) gene expression in tumoral (T) (orange) and normal (N) (grey) tissues extracted  
579 from TCGA and GTEX databases (ACC: Adrenocortical carcinoma, T=77, N=128; BLCA : Bladder  
580 carcinoma, T=404, N=28; BRCA : Breast invasive carcinoma, T=1095, N=291; CESC: Cervical squamous  
581 cell carcinoma and endocervical adenocarcinoma, T=306, N=13 ; CHOL: Cholangiocarcinoma, T=36, N=9;  
582 COAD : Colorectal adenocarcinoma, T=275, N=349; DLBC: Lymphoid Neoplasm Diffuse Large B-cell  
583 Lymphoma, T=47, N=337 ; ESCA: Esophageal carcinoma, T=182, N=286 ; GBM: Glioblastoma multiforme,  
584 T=163, N=207 ; HNSC : Head and neck squamous cell carcinoma, T=519, N=44 ; KICH: Kidney  
585 Chromophobe, T=66, N=53 ; KIRC : Kidney renal clear cell carcinoma, T=523, N=100 ; KIRP: Kidney renal  
586 papillary cell carcinoma, T=286, N=60 ; LGG : Brain lower grade glioma, T=518, N=207 ; LIHC: Liver  
587 hepatocellular carcinoma, T=369, N=160 ; LUAD : Lung adenocarcinoma, T=483, N=347 ; LUSC: Lung  
588 squamous cell carcinoma, T=486, N=338 ; OV : Ovarian serous cystadenocarcinoma, T=426, N=88 ; PAAD  
589 : Pancreatic adenocarcinoma, T=179, N=171 ; PCPG: Pheochromocytoma and Paraganglioma, T=182,  
590 N=3 ; PRAD: Prostate adenocarcinoma, T=492, N=152 ; READ: Rectum adenocarcinoma, T=92, N=318 ;  
591 SARC: Sarcoma, T=262, N=2 ; SKCM : Skin cutaneous melanoma, T=461, N=558 ; STAD : Stomach  
592 adenocarcinoma, T=408, N=211 ; TGCT: Testicular Germ Cell Tumors, T=137, N=165 ; THCA : Thyroid  
593 cancer, T=512, N=337; THYM: Thymoma, T=118, N=339 ; UCEC: Uterine Corpus Endometrial Carcinoma,  
594 T=174, N=91 ; UCS: Uterine Carcinosarcoma , T=57, N=78).

595 **(B)** IL-33 protein was quantified by Luminex assay in breast tumor-derived supernatants (n = 89). Kruskal-  
596 Wallis test with Dunn multiple comparisons test was performed.

597 **(C)** IL-33 expression was analyzed on breast tumor FFPE slides by Immunohistochemistry (IHC). Non-  
598 invasive Ductal Carcinoma *In Situ* (DCIS) and invasive Breast Cancer (IBC) lesions were identified based  
599 on anatomopathological tissue observations. Red arrows indicate blood vessels, orange arrows indicate  
600 peritumoral normal breast acini and green arrows indicate isolated stromal cells positive for IL-33  
601 staining. Images are shown at 20x magnification. Dotted squares represent enlarges areas.

602 **(D)** Analysis of *IL33* expression in laser-microdissected stromal versus epithelial zones from DCIS and IBC  
603 lesions (GSE41228) (46) and in healthy mammary tissue versus DCIS and IBC (GSE21422) (47). Kruskal-  
604 Wallis test with Steel-Dwas-Fligner multiple comparisons test was performed.

605

606 **Fig. 7. An  $NK^{hi}/IL33^{hi}$  transcriptomic score is associated with improved progression-free survival**

607 **(A)** Boxplots display *IL33* gene expression in  $NK^{hi}$  vs  $NK^{low}$  breast cancer patients from the TCGA database.  
608 An unpaired Student *t*-test was performed.

609 **(B)** Summary of p-values associated with the log rank test performed to evaluate prognostic value of  
610  $NK^{high}/IL33^{high}$ ,  $NK^{high}/IL33^{low}$  and  $NK^{low}/IL33^{high}$  scores as compared to  $NK^{low}/IL33^{low}$  in 32 human cancers  
611 from TCGA database (legend is the same as in panel A, MESO: Mesothelioma ; UVM: Uveal Melanoma  
612 were added). Results are represented as a bubble map showing positive (green) or negative (red) impact  
613 on progression-free survival. Dots size represents p-values obtained by log-rank test. An unpaired  
614 Student *t*-test was performed.

615 **(C)** Kaplan-Maier curves for progression-free survival for  $NK^{high}/IL33^{high}$ ,  $NK^{high}/IL33^{low}$  and  $NK^{low}/IL33^{high}$   
616 scores as compared to  $NK^{low}/IL33^{low}$  in breast cancer dataset from TCGA. p-values were obtained with  
617 log-rank test.

618 **(D)** Multivariate Cox analysis of the impact of  $NK^{high}/IL33^{high}$  score on prognosis in breast cancer patients  
619 from TCGA dataset regarding the age, molecular subtype, and stage of the tumors. p-values were  
620 obtained with log-rank test.

621

622 **Tables with titles and legends**

623 **Table 1.** List of ST2<sup>+</sup> NK cell signature genes used in this study, Related to Fig. 3C,D,F,G, 4A

624 **Table 2.** List of ST2<sup>-</sup> CD56<sup>bright</sup> NK cell signature genes used in this study, Related to Fig. 3C,D,F,G

625 **Table 3.** List of ST2<sup>-</sup> CD56<sup>dim</sup> NK cell signature genes used in this study, Related to Fig. 3C,D,F,G

626 **Table 4.** List of NK cell signature genes used in this study (from (48)), Related to Fig. 7A-D, fig. S9E

627

628 **Materials and Methods**

629

630 **Animals and in vivo tumor models.**

631 C57BL/6J mice were purchased from Charles River Laboratories. *Rag2*-KO mice were bred and  
632 maintained at the P-PAC animal facility of the Cancer Research Center of Lyon, under specific pathogen-  
633 free conditions in accordance with all standards of animal care. Experiments were conducted with  
634 female mice aged between 8-12 weeks. All animal experiments were approved by the ethics committee  
635 (approval number CECCAPP-CLB-2019-017). Mice were orthotopically injected into the 4<sup>th</sup> mammary fat  
636 pad with  $5 \times 10^4$  or  $2.5 \times 10^5$  E0771 tumor cells in 200  $\mu$ L of sterile NaCl at day 0. For NK cell depletion  
637 experiments, mice were injected intra-peritoneally with 200  $\mu$ g/mouse of anti-NK1.1 antibody (BioXcell)  
638 or an equivalent volume of NaCl solution one day prior to tumor cell injection and then once a week. To  
639 evaluate NK cell depletion efficiency, mice were treated with tetracaine analgesic eye drops and  
640 maintained under anesthesia using isoflurane to perform retro-orbital blood collection. Blood NK cell  
641 frequency was measured by flow cytometry. For therapeutic experiments, mice were injected with 10  
642 ng/mouse of rmIL-12 (R&D) or 100 ng/mouse of rmIL-33 (Biolegend) alone or in combination in 50  $\mu$ L  
643 directly in the tumor area twice a week from day 2 to day 26. Control mice were injected with an equal  
644 volume of NaCl solution. Tumor sizes were monitored with a digital caliper (Mitutoyo) twice a week and  
645 expressed as volume of a sphere ( $(\text{smaller length})^2 \times \text{longer length}$ ) from day 9 to the end of experiments.  
646 Mice were sacrificed when longer length of the primary tumor reached  $> 17 \text{ mm}^2$ . All cells were tested  
647 for mycoplasma prior to injection using MycoAlert kit (Lonza). For serum collection, blood was collected  
648 as described above on day 5, 4 h after cytokine injection. Blood was then incubated at room temperature  
649 for 30 min to allow coagulation, centrifuged at 15,000rpm for 15 min and the serum was collected and  
650 aliquoted for preservation at  $-80^\circ\text{C}$  and IFN- $\gamma$  quantification.

651

652 **Tumor cell lines.**

653 The mouse E0771 (derived from a spontaneous triple negative mammary tumor that arose in a C57BL/6  
654 mouse) and the human K562 (originally generated from a female leukemia patient) cell lines were  
655 cultured in RPMI-1640 (Sigma) supplemented with 10% FCS (GIBCO), 2mM-glutamine, and 100 U/ml  
656 penicillin/streptomycin (ThermoFisher).

657

#### 658 **Human breast tumors and blood.**

659 Fresh tumors and blood samples (collected in EDTA anticoagulant-containing tubes) from breast cancer  
660 patients were provided by the tissue bank of CLB (BB-0033-00050, CRB-CLB, Lyon, France, French  
661 agreement number: AC-2019-3426), after approval from the institutional review board and ethics  
662 committee (L-06-36) and patient written informed consent, in accordance with the Declaration of  
663 Helsinki. Healthy donor (HD) blood samples were obtained from the 'Etablissement Français du Sang'  
664 (Lyon). Next-generation tissue microarray (ngTMA<sup>®</sup>) ([www.ngtma.com](http://www.ngtma.com)) (69) generated from human  
665 breast tumors was provided by the Tissue Bank Bern and approved by the Cantonal Ethics Committee  
666 of Bern (200/2014 and 2018-01502).

667

#### 668 **Human peripheral blood NK cell isolation and culture.**

669 Peripheral Blood Mononuclear Cells (PBMCs) were isolated from whole blood of healthy donors by  
670 density gradient centrifugation on Lymphocyte Separation Medium (Eurobio). Total NK cells were  
671 purified from PBMCs by negative immune-selection using the Human NK cell isolation kit (Miltenyi)  
672 following the manufacturer's instructions and purity always exceeded 95%. Cells were cultured in RPMI  
673 GlutaMAX/10% FBS/1% penicillin-streptomycin (complete RPMI, cRPMI). Human PB NK cells were  
674 cultured in cRPMI supplemented with IL-12 (Miltenyi), IL-15 (Peprotech), IL-2 (Chiron), IFN- $\alpha$ 2b  
675 (Schering-Plough), IL-1 $\alpha$  (Peprotech), IL-1 $\beta$  (Peprotech), IL-18 (MBL) or IL-33 (Miltenyi) at concentrations  
676 indicated in Fig. legends. Anti-NKp46 and NKp30 agonist antibodies (R&D) were coated (1  $\mu$ g/mL) in  
677 NUNC 96-well plates (Thermofisher) overnight at 4°C. For blocking experiments, cells were incubated

678 with anti-ST2 (10 µg/mL, R&D), anti-IL-18 (1 µg/mL, MBL), mIgG1 (1 or 10 µg/mL, R&D) antibodies or IL-  
679 1RA (100 ng/mL, Peprotech).

680

#### 681 **Human peripheral blood NK cell sorting**

682 For RNA-seq analysis, MACS-purified total NK cells were stained with antibodies for CD3, CD56, CD16,  
683 CD57 (see table S1) as previously described, FACS sorted using a FACS Aria II (BD), and collected in cRPMI.  
684 Cell viability was determined by DAPI staining (1 µg/mL, Invitrogen).

685

#### 686 **Preparation of single cell suspensions from human breast tumor tissue**

687 Sections of the resected tumor area selected by the pathologists were placed in RPMI supplemented  
688 with 100 IU/mL penicillin (Invitrogen) and 100 µg/mL streptomycin (Invitrogen). Tissues were  
689 mechanically disrupted, supernatant was collected and frozen for subsequent cytokine and chemokine  
690 quantification by Luminex assay following the manufacturer's instructions. Tumor pieces were then  
691 digested for 45 min at 37°C in RPMI supplemented with 100 IU/mL penicillin (Invitrogen) and 100 µg/mL  
692 streptomycin (Invitrogen) using 1 mg/mL of collagenase IV (Sigma Aldrich) and 20 µg/mL of DNase I  
693 (Sigma Aldrich). Digested samples were then filtered on a 70 µm cell strainer and re-suspended in cRPMI  
694 for activation and flow cytometric analysis.

695

#### 696 ***Ex vivo* stimulation of mouse splenic NK cells**

697 Single-cell suspensions were prepared from spleens from C57BL/6J mice (CharlesRiver) after red blood  
698 cell lysis with Pharm Lyse™ lysing buffer (BD Biosciences). Splenocytes were activated in cRPMI  
699 supplemented with IL-12 (Miltenyi), IL-1α (Miltenyi), IL-1β (Miltenyi), IL-18 (MBL) or IL-33 (Miltenyi) for  
700 24 hr. Cells were surface stained for surface markers, fixed, and permeabilized, followed by intracellular  
701 staining of IFN-γ.

702

#### 703 **Flow cytometry**

704 Cells were stained for the expression of surface markers using antibodies listed in table S1 for 30 min at  
705 4°C. Cell viability was determined using Zombie dye (Biolegend) following the manufacturer's  
706 instructions. For intracellular IFN- $\gamma$  staining, GolgiPlug (BD Bioscience) was added to culture media 4 h  
707 prior to the staining. Cells were stained for the expression of surface markers as previously described,  
708 fixed and permeabilized using FoxP3/Transcription Factor Staining Buffer Set (eBioscience) prior to  
709 intracellular staining for 30 min at 4°C. Cells were washed twice with PBS before acquisition on a LSRII  
710 Fortessa flow cytometer (BD Biosciences). Results were analyzed using FlowJo software (Tree Star Inc.).

711

### 712 **Phosphorylation flow cytometry analysis**

713 For p-STAT4 analysis, NK cells were activated or not with IL-12 (0.1 ng/mL; 1 ng/mL or 10 ng/mL) for 1  
714 h. For p-NF- $\kappa$ B, p-p38 and p-S6 analysis, NK cells were activated or not with IL-12 (10 ng/mL) for 24 h.  
715 When indicated, IL-1 family members were added during 5min for p-NF- $\kappa$ B and p-p38 and 1 h for p-S6  
716 analysis. Cells were then fixed with Lyse/Fix Buffer (BD Biosciences) for 10 min at 37°C and permeabilized  
717 with Perm Buffer III (BD Biosciences) for 30 min on ice following the manufacturer's instructions. Cells  
718 were then stained for 45 min at room temperature, washed, and directly acquired on a Fortessa  
719 cytometer (Becton Dickinson) as previously described.

720

### 721 **Killing assay**

722 PB NK cells were activated with IL-1 family members (10 ng/mL) alone or in combination with IL-12 (10  
723 ng/mL) for 24 h and washed 3 times. K562 cells were labeled with calcein (Invitrogen) at 10  $\mu$ L/mL in  
724 cRPMI for 30 min at 37°C, washed twice, and fixed with sulfinpyrazone (4 mM, Sigma-Aldrich). NK cells  
725 were incubated with K562 target cells at indicated effector:target ratios for 4 h à 37°C. Calcein release  
726 was measured using Clariostar (Labtech) and the percentage of specific lysis was calculated as followed:  
727 % of lysis =  $((Fluo_{measured} - Fluo_{spontaneous\ release}) / ((Fluo_{max} - Fluo_{spontaneous\ release})) \times 100$

728

### 729 **Proliferation assay**

730 NK cells were stained with 5  $\mu$ M Cell Trace Violet (CTV) (Invitrogen) for 20 min at room temperature,  
731 washed twice, and activated with IL-1 family members (10 ng/mL) with or without IL-12 (10 ng/mL) for  
732 24 h in RPMI supplemented with 20% human AB serum. After three washes, IL-2 (100 UI/mL, Chiron)  
733 was added and CTV signal was analyzed by flow cytometry on day 3 and 6 for CD56<sup>bright</sup> and CD56<sup>dim</sup>,  
734 respectively.

735

#### 736 **IFN- $\gamma$ ELISAs**

737 Human IFN- $\gamma$  levels were measured in culture supernatants using the human IFN- $\gamma$  ELISA kit (R&D)  
738 following the manufacturer's instructions. Mouse IFN- $\gamma$  levels were measured in serum using the Extra  
739 Sensitive IFN gamma Mouse ELISA Kit (ThermoFischer) following the manufacturer's instructions.

740

#### 741 **Luminex immunoassay**

742 Culture supernatants of activated NK cells were harvested to perform IFN- $\gamma$ , TNF- $\alpha$ , GM-CSF, IL-8, CCL3  
743 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), CCL5 (RANTES), IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17A, IL-22, and G-CSF  
744 quantification using a human Bio-plex PRO assay (Biorad) following the manufacturer's instructions and  
745 acquired with the Luminex200 instrument (Luminex).

746

#### 747 **mRNA isolation and quantitative PCR**

748 Total RNA extraction was performed using NucleoSpin RNA Kit (Macherey-Nagel) following the  
749 manufacturer's instructions. Reverse transcription was performed with the iScript Reverse Transcription  
750 kit (BioRad) using 350 ng total RNA extract. Real Time quantitative PCR (RT-qPCR) analysis was  
751 performed using TaqMan gene expression assay with *ST2* and *IL-1RAcP* specific primers  
752 (Hs00249384\_m1 and Hs00895050\_m1 respectively, Life Technologies). Normalization of *ST2*  
753 expression was performed using *GADD45a* (Hs00169255\_m1, Life Technologies) expression.

754

#### 755 ***ST2* promoter region analysis**



756 The human *ST2* gene sequence was obtained from Gen-Bank and then submitted to Eukaryotic Promoter  
757 Databank (EPD)(70) to identify the promoter region. Putative binding sites for transcription factors in  
758 the *ST2*-regulating region were identified using the PROMO database (71).

759

#### 760 **p-STAT4 Chromatin Immunoprecipitation (Ch-IP)**

761 PB NK cells were stimulated or not with IL-12 as previously described and Ch-IP was performed using  
762 ChIP-IT PBMCs Kit (Active Motif) according to the manufacturer's instructions. Briefly, cells were cross-  
763 linked with 1% formaldehyde then sonicated on ice for 6 min (6 cycles of 1 min; 30 s on / 30 s off) at 4°C  
764 using a water bath-sonicator (Diagenode). Chromatin fragments were reverse cross-linked overnight at  
765 65°C, and 500 ng of each input DNA was run on a 1% agarose gel to confirm that fragment size ranged  
766 between 200 and 1500 bp. p-STAT4 immunoprecipitation was performed overnight at 4°C using anti-p-  
767 STAT4 antibody (Cell signaling). p-STAT4 immunoprecipitation was validated by Western blot using pre-  
768 and post-immunoprecipitated samples stained with anti-p-STAT4 (Cell Signaling). Isolated DNA  
769 fragments were purified and quantitative PCR analysis was performed using 1 µg of DNA as template.  
770 Primers for *ST2* promoter region were extracted from(72) and we used *PRF1* (Cell Signaling) and  
771 Negative Primer Set 1 and 2 (Active Motif) as positive and negative controls for p-STAT4 binding (see  
772 table S1 for sequences). The Ct Value of each sample was normalized to input DNA fraction and fold-  
773 change for p-STAT4 binding was calculated using  $\Delta\Delta\text{Ct}$  method. qPCR analysis was conducted in triplicate  
774 for each sample.

775

#### 776 **RNA-seq**

777 PB NK cells were FACS-sorted as described above. 400,000 cells from each population were resuspended  
778 in 350 µL TCL lysis buffer (Qiagen) supplemented with 1 %  $\beta$ -mercaptoethanol. Total RNA extraction was  
779 performed using the Single Cell RNA Purification Kit (Norgen) following the manufacturer's instructions,  
780 including an additional treatment with rDNase (Qiagen) to avoid DNA contamination. RNA quality was  
781 addressed using 4200 TapeStation (Agilent) automated electrophoresis with RIN always exceeding 8,

782 and total RNA was quantified using Qubit™4 Fluorometer (ThermoFischer). Reverse transcription and  
783 DNA amplification was performed with SmartSeqV4 (Takara) using 10 ng of RNA as template. DNA library  
784 was prepared with Nextera XT DNA library Prep kit (Illumina), hybridized on NovaSeqS1 Flow cell and  
785 sequenced on NovaSeq sequencing platform (Illumina) with a paired-end protocol. Raw sequencing  
786 reads were aligned on the human genome (GRCh38) with STAR (v2.7.3a) and the annotation of known  
787 genes from gencode v33. Gene expression was quantified using Salmon (v1.1.0) and the annotation of  
788 protein coding genes from gencode v33.

789

### 790 **RNA-seq bioinformatics analysis**

791 All analyses were performed in R programming language (v3.6.3). DESeq2 (v1.26.0) was used to  
792 normalize raw counts, to generate principal component analysis (PCA) plots based on the 500 most  
793 variable genes and to identify differentially-expressed genes (DEGs). To identify NK subset signatures,  
794 DEGs between one subset and the two other subsets of NK cells were filtered considering a log<sub>2</sub> fold  
795 change (LFC) > 1 and an adjusted p-value < 0.001, resulting in 234 genes enriched in ST2<sup>+</sup>, 396 genes  
796 enriched in CD56<sup>dim</sup> and 418 genes enriched in CD56<sup>bright</sup> NK cells. These signatures were scored by  
797 ssGSEA using the GSVA package. The Morpheus tool (Broad Institute) was used to generate a heatmap  
798 representing the relative expression of these DEGs, using an unsupervised hierarchical clustering of  
799 genes and samples based on the one minus Pearson correlation metric. To evaluate the intermediate  
800 state of ST2<sup>+</sup> NK cells compared with CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells, DEGs between one subset and the  
801 two other subsets of NK cells were filtered considering a log<sub>2</sub> fold change (LFC) > 1 and an adjusted p-  
802 value < 0.05. The Morpheus tool (Broad Institute) was used to generate a heatmap representing their  
803 relative expression. For this heatmap, genes were classified as follows: upregulated DEGs in CD56<sup>bright</sup>  
804 NK cells (n = 828 genes), downregulated DEGs in CD56<sup>dim</sup> NK cells (n = 1,348 genes), upregulated DEGs  
805 in ST2<sup>+</sup> NK cells (n = 547 genes), downregulated DEGs in CD56<sup>bright</sup> NK cells (n = 1,009 genes) and  
806 upregulated DEGs in CD56<sup>dim</sup> NK cells (n = 1,024 genes). Intermediate transcriptional profile of ST2<sup>+</sup> NK  
807 cells was validated using a permutation resampling strategy. Briefly, among all genes differentially

808 expressed between CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells, the observed number of genes with an intermediate  
809 expression in ST2<sup>+</sup> NK cells (CD56<sup>bright</sup> > ST2<sup>+</sup> > CD56<sup>dim</sup> or CD56<sup>bright</sup> < ST2<sup>+</sup> < CD56<sup>dim</sup>) was evaluated and  
810 compared to a sampling distribution obtained after n = 1000 permutations of sample labels. p value was  
811 computed with a permutation statistical test.

812

### 813 **Analysis of cancer patient data**

814 Upper-quartile normalized expression (UQN) and clinical outcome datasets from The Cancer Genome  
815 Atlas (TCGA) were downloaded from the [Pan-Cancer](https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) Atlas (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>) for 33 tumor types: adrenocortical carcinoma  
817 (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical carcinoma  
818 (CESC), cholangiosarcoma (CHOL), colorectal adenocarcinoma (COAD), diffuse large B-cell lymphoma  
819 (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell  
820 carcinoma (HNSC), kidney chromophobe carcinoma (KICH), kidney clear renal cell carcinoma (KIRC),  
821 kidney papillary cell carcinoma (KIRP), lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC),  
822 lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), ovarian  
823 serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), paraganglioma &  
824 pheochromocytoma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ),  
825 sarcoma (SARC), skin cutaneous metastatic melanoma (SKCM), stomach adenocarcinoma (STAD),  
826 testicular germ cell cancer (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus  
827 endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS) and uveal melanoma (UVM) to compare  
828 gene expression in tumor *versus* peritumoral healthy tissue. For breast cancer analysis, tumors were  
829 further stratified according to their molecular subtype to analyze IL-33 log<sub>2</sub> (RSEM+1) normalized  
830 expression and clinical impact. Overall survival analyses and plots were performed with R software v  
831 3.6.0, using the packages survival and survminer. For each gene, patients were stratified into terciles  
832 according to normalized gene expression levels in log<sub>2</sub> (RSEM+1) to compare top (*i.e.* high) and bottom  
833 (*i.e.* low) terciles. The log-rank test was used to determine statistical significance for overall survival

834 between the two groups of patients. For multivariate analysis in the breast cancer dataset, a cox model  
835 including the main prognostic factors (age, molecular subtype and stage) was used. Gender was  
836 removed from the model due to the low number of males in the study.

837

### 838 **IL-33 immunohistochemistry on FFPE human breast tumor ngTMA**

839 IL-33 staining was performed automatically using a BOND RX autostainer (Leica Biosystems). Sections  
840 were first deparaffinized and antigen was retrieved using Tris buffer (pH 9.0) for 30 min at 95°C. Sections  
841 were then stained with goat anti-human IL-33 (R&D Systems, # AF3625; dilution of 1:400 for 30 min)  
842 primary antibody. A rabbit anti-goat antibody (Agilent, # E0466) was used as secondary antibody, at a  
843 dilution of 1:400 for 15 min. Specific binding of primary antibodies was visualized using a polymer-based  
844 visualization system with horseradish peroxidase as the enzyme and 3,3-diaminobenzidine (DAB) as a  
845 brown chromogen for IL-33. Sections were counterstained with hematoxylin, dehydrated and mounted  
846 with Tissue-Tek Glas Mounting Medium (Sakura). Slides were scanned in high resolution on whole slide  
847 scanners Panoramic 250 Flash (3DHISTECH) or NanoZoomer S360 (Hamamatsu).

848

### 849 **Data analysis and statistics**

850 Statistical analyses were performed using the GraphPad software and tests conducted are indicated in  
851 the Fig. legends. P-values lower than 0.05 were considered to be significant, with stars corresponding to  
852 \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  and \*\*\*\*  $p < 0.0001$ . If no stars are indicated, no statistically  
853 significant difference was found.

854

### 855 **Data Availability**

856 The bulk RNA-seq data generated during this study are deposited in Gene Expression Omnibus  
857 repository (GEO) with accession number GEO: GSE199134. The raw and processed data are publicly  
858 available as of the date of publication. Accession numbers are listed in the table S1. This paper does not  
859 report original code. The RNA-seq data from laser-microdissected stromal versus epithelial zones from

860 DCIS and IBC lesions (46, 47) and from healthy mammary tissue versus DCIS and IBC (46, 47) were  
861 downloaded from GEO with accession number GEO: GSE41228 and GSE21422 respectively. The scRNA-  
862 seq data derived from NK cells infiltrating human melanoma metastases (42) and mouse lung tumors  
863 (43) were downloaded from GEO with accession number GEO: GSE139249 and GSE123534, respectively.  
864 The Cancer Genome Atlas data were downloaded from Firehose (<https://gdac.broadinstitute.org/>).  
865 Genes Signatures used in this study are listed in Tables 1-3.  
866

867 **List of Supplementary Materials**

868 Fig. S1. IL-33 in combination with IL-12 strongly activates NK cell secretory and cytotoxic functions

869 Fig. S2. IL-12 induces ST2 expression on a subset of NK cells in a STAT4-dependent manner

870 Fig. S3. IL-33 preferentially activates a subset of CD56<sup>dim</sup> NK cells

871 Fig. S4. ST2<sup>+</sup> NK cells display a unique gene signature compared to CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells

872 Fig. S5. Unsupervised clustering of sc-RNAseq datasets

873 Fig. S6. Gating strategy to identify NK cells in tumor cell suspensions by flow cytometry

874 Fig. S7 | IL-12 sensitizes mouse splenic NK cells to the production of IFN- $\gamma$  in response to IL-33

875 Fig. S8. Antitumoral effect of IL-33 and IL-12 combination is dependent on NK cells in *Rag2*-KO mice

876 Fig. S9. IL-33 is associated with increased overall patient survival for several cancers

877 Table S1. Reagent and resources table

878

879 **References**

880

- 881 1. E. Vivier, E. Tomasello, M. Baratin, T. Walzer, S. Ugolini, Functions of natural killer cells. *Nat.*  
882 *Immunol.* **9**, 503–510 (2008).
- 883 2. A. G. Freud, B. L. Mundy-Bosse, J. Yu, M. A. Caligiuri, The Broad Spectrum of Human Natural Killer  
884 Cell Diversity. *Immunity* **47**, 820–833 (2017).
- 885 3. A. Poli, T. Michel, M. Thérésine, E. Andrès, F. Hentges, J. Zimmer, CD56bright natural killer (NK) cells:  
886 an important NK cell subset. *Immunology* **126**, 458–465 (2009).
- 887 4. C. Lo Nigro, M. Macagno, D. Sangiolo, L. Bertolaccini, M. Aglietta, M. C. Merlano, NK-mediated  
888 antibody-dependent cell-mediated cytotoxicity in solid tumors: biological evidence and clinical  
889 perspectives. *Ann Transl Med* **7** (2019), doi:10.21037/atm.2019.01.42.
- 890 5. C. Romagnani, K. Juelke, M. Falco, B. Morandi, A. D’Agostino, R. Costa, G. Ratto, G. Forte, P. Carrega,  
891 G. Lui, R. Conte, T. Strowig, A. Moretta, C. Münz, A. Thiel, L. Moretta, G. Ferlazzo, CD56brightCD16-  
892 killer Ig-like receptor- NK cells display longer telomeres and acquire features of CD56dim NK cells upon  
893 activation. *J. Immunol.* **178**, 4947–4955 (2007).
- 894 6. N. D. Huntington, N. Legrand, N. L. Alves, B. Jaron, K. Weijer, A. Plet, E. Corcuff, E. Mortier, Y.  
895 Jacques, H. Spits, J. P. Di Santo, IL-15 trans-presentation promotes human NK cell development and  
896 differentiation in vivo. *J. Exp. Med.* **206**, 25–34 (2009).
- 897 7. J. Yu, H. C. Mao, M. Wei, T. Hughes, J. Zhang, I. Park, S. Liu, S. McClory, G. Marcucci, R. Trotta, M. A.  
898 Caligiuri, CD94 surface density identifies a functional intermediary between the CD56bright and  
899 CD56dim human NK-cell subsets. *Blood* **115**, 274–281 (2010).
- 900 8. N. K. Björkström, P. Riese, F. Heuts, S. Andersson, C. Fauriat, M. A. Ivarsson, A. T. Björklund, M.  
901 Flodström-Tullberg, J. Michaëlsson, M. E. Rottenberg, C. A. Guzmán, H.-G. Ljunggren, K.-J. Malmberg,  
902 Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation  
903 uncoupled from NK-cell education. *Blood* **116**, 3853–3864 (2010).

904 9. S. Lopez-Vergès, J. M. Milush, S. Pandey, V. A. York, J. Arakawa-Hoyt, H. Pircher, P. J. Norris, D. F.  
905 Nixon, L. L. Lanier, CD57 defines a functionally distinct population of mature NK cells in the human  
906 CD56dimCD16+ NK-cell subset. *Blood* **116**, 3865–3874 (2010).

907 10. T. E. O’Sullivan, J. C. Sun, L. L. Lanier, Natural Killer Cell Memory. *Immunity* **43**, 634–645 (2015).

908 11. S. J. Judge, W. J. Murphy, R. J. Canter, Characterizing the Dysfunctional NK Cell: Assessing the  
909 Clinical Relevance of Exhaustion, Anergy, and Senescence. *Front Cell Infect Microbiol* **10**, 49 (2020).

910 12. A. Crinier, P. Milpied, B. Escalière, C. Piperoglou, J. Galluso, A. Balsamo, L. Spinelli, I. Cervera-  
911 Marzal, M. Ebbo, M. Girard-Madoux, S. Jaeger, E. Bollon, S. Hamed, J. Hardwigsen, S. Ugolini, F. Vély, E.  
912 Narni-Mancinelli, E. Vivier, High-Dimensional Single-Cell Analysis Identifies Organ-Specific Signatures  
913 and Conserved NK Cell Subsets in Humans and Mice. *Immunity* **49**, 971-986.e5 (2018).

914 13. P. L. Collins, M. Cella, S. I. Porter, S. Li, G. L. Gurewitz, H. S. Hong, R. P. Johnson, E. M. Oltz, M.  
915 Colonna, Gene Regulatory Programs Conferring Phenotypic Identities to Human NK Cells. *Cell* **176**, 348-  
916 360.e12 (2019).

917 14. C. M. Lau, N. M. Adams, C. D. Geary, O.-E. Weizman, M. Rapp, Y. Pritykin, C. S. Leslie, J. C. Sun,  
918 Epigenetic control of innate and adaptive immune memory. *Nat. Immunol.* **19**, 963–972 (2018).

919 15. J. A. Myers, J. S. Miller, Exploring the NK cell platform for cancer immunotherapy. *Nat Rev Clin*  
920 *Oncol* **18**, 85–100 (2021).

921 16. N. D. Huntington, J. Cursons, J. Rautela, The cancer–natural killer cell immunity cycle. *Nat Rev*  
922 *Cancer* **20**, 437–454 (2020).

923 17. D. H. Raulet, N. Guerra, Oncogenic stress sensed by the immune system: role of natural killer cell  
924 receptors. *Nat Rev Immunol* **9**, 568–580 (2009).

925 18. S. Rusakiewicz, M. Semeraro, M. Sarabi, M. Desbois, C. Locher, R. Mendez, N. Vimond, A. Concha,  
926 F. Garrido, N. Isambert, L. Chaigneau, V. Le Brun-Ly, P. Dubreuil, I. Cremer, A. Caignard, V. Poirier-  
927 Colame, K. Chaba, C. Flament, N. Halama, D. Jäger, A. Eggermont, S. Bonvalot, F. Commo, P. Terrier, P.  
928 Opolon, J.-F. Emile, J.-M. Coindre, G. Kroemer, N. Chaput, A. Le Cesne, J.-Y. Blay, L. Zitvogel, Immune



929 infiltrates are prognostic factors in localized gastrointestinal stromal tumors. *Cancer Res.* **73**, 3499–  
930 3510 (2013).

931 19. K. C. Barry, J. Hsu, M. L. Broz, F. J. Cueto, M. Binnewies, A. J. Combes, A. E. Nelson, K. Loo, R.  
932 Kumar, M. D. Rosenblum, M. D. Alvarado, D. M. Wolf, D. Bogunovic, N. Bhardwaj, A. I. Daud, P. K. Ha,  
933 W. R. Ryan, J. L. Pollack, B. Samad, S. Asthana, V. Chan, M. F. Krummel, A natural killer-dendritic cell  
934 axis defines checkpoint therapy-responsive tumor microenvironments. *Nat. Med.* **24**, 1178–1191  
935 (2018).

936 20. J. Cursons, F. Souza-Fonseca-Guimaraes, M. Foroutan, A. Anderson, F. Hollande, S. Hediye-Zadeh,  
937 A. Behren, N. D. Huntington, M. J. Davis, A Gene Signature Predicting Natural Killer Cell Infiltration and  
938 Improved Survival in Melanoma Patients. *Cancer Immunol Res* **7**, 1162–1174 (2019).

939 21. B. Cózar, M. Greppi, S. Carpentier, E. Narni-Mancinelli, L. Chiossone, E. Vivier, Tumor-Infiltrating  
940 Natural Killer Cells. *Cancer Discov* (2020), doi:10.1158/2159-8290.CD-20-0655.

941 22. G. Trinchieri, M. Matsumoto-Kobayashi, S. C. Clark, J. Sehra, L. London, B. Perussia, Response of  
942 resting human peripheral blood natural killer cells to interleukin 2. *J. Exp. Med.* **160**, 1147–1169 (1984).

943 23. M. Kobayashi, L. Fitz, M. Ryan, R. M. Hewick, S. C. Clark, S. Chan, R. Loudon, F. Sherman, B.  
944 Perussia, G. Trinchieri, Identification and purification of natural killer cell stimulatory factor (NKSF), a  
945 cytokine with multiple biologic effects on human lymphocytes. *J. Exp. Med.* **170**, 827–845 (1989).

946 24. W. E. Carson, J. G. Giri, M. J. Lindemann, M. L. Linett, M. Ahdieh, R. Paxton, D. Anderson, J.  
947 Eisenmann, K. Grabstein, M. A. Caligiuri, Interleukin (IL) 15 is a novel cytokine that activates human  
948 natural killer cells via components of the IL-2 receptor. *J. Exp. Med.* **180**, 1395–1403 (1994).

949 25. H. Okamura, H. Tsutsi, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y.  
950 Nukada, K. Hattori, Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature*  
951 **378**, 88–91 (1995).

952 26. L. Müller, P. Aigner, D. Stoiber, Type I Interferons and Natural Killer Cell Regulation in Cancer. *Front*  
953 *Immunol* **8**, 304 (2017).

954 27. G. M. Konjević, A. M. Vuletić, K. M. Mirjačić Martinović, A. K. Larsen, V. B. Jurišić, The role of  
955 cytokines in the regulation of NK cells in the tumor environment. *Cytokine* **117**, 30–40 (2019).

956 28. C. Garlanda, C. A. Dinarello, A. Mantovani, The interleukin-1 family: back to the future. *Immunity*  
957 **39**, 1003–1018 (2013).

958 29. C. Cayrol, J.-P. Girard, Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family. *Immunological*  
959 *Reviews* **281**, 154–168 (2018).

960 30. F. Y. Liew, J.-P. Girard, H. R. Turnquist, Interleukin-33 in health and disease. *Nat. Rev. Immunol.* **16**,  
961 676–689 (2016).

962 31. W. Kuswanto, D. Burzyn, M. Panduro, K. K. Wang, Y. C. Jang, A. J. Wagers, C. Benoist, D. Mathis,  
963 Poor Repair of Skeletal Muscle in Aging Mice Reflects a Defect in Local, Interleukin-33-Dependent  
964 Accumulation of Regulatory T Cells. *Immunity* **44**, 355–367 (2016).

965 32. L. D. Faustino, J. W. Griffith, R. A. Rahimi, K. Nepal, D. L. Hamilos, J. L. Cho, B. D. Medoff, J. J. Moon,  
966 D. A. A. Vignali, A. D. Luster, Interleukin-33 activates regulatory T cells to suppress innate  $\gamma\delta$  T cell  
967 responses in the lung. *Nat Immunol* **21**, 1371–1383 (2020).

968 33. M. D. Smithgall, M. R. Comeau, B.-R. Park Yoon, D. Kaufman, R. Armitage, D. E. Smith, IL-33  
969 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive  
970 Th2 cells, iNKT and NK Cells. *Int Immunol* **20**, 1019–1030 (2008).

971 34. W. V. Bonilla, A. Fröhlich, K. Senn, S. Kallert, M. Fernandez, S. Johnson, M. Kreutzfeldt, A. N.  
972 Hegazy, C. Schrick, P. G. Fallon, R. Klemenz, S. Nakae, H. Adler, D. Merkler, M. Löhning, D. D.  
973 Pinschewer, The Alarmin Interleukin-33 Drives Protective Antiviral CD8+ T Cell Responses. *Science* **335**,  
974 984–989 (2012).

975 35. J. T. Clark, D. A. Christian, J. A. Gullicksrud, J. A. Perry, J. Park, M. Jacquet, J. C. Tarrant, E. Radaelli, J.  
976 Silver, C. A. Hunter, G. T. Belz, C. V. Rothlin, M. R. Starkey, Eds. IL-33 promotes innate lymphoid cell-  
977 dependent IFN- $\gamma$  production required for innate immunity to *Toxoplasma gondii*. *eLife* **10**, e65614  
978 (2021).

979 36. G. K. Dwyer, L. M. D’Cruz, H. R. Turnquist, Emerging Functions of IL-33 in Homeostasis and  
980 Immunity. *Annual Review of Immunology* **40**, null (2022).

981 37. G. Trinchieri, Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nature*  
982 *Reviews Immunology* **3**, 133–146 (2003).

983 38. J. Schmitz, A. Owyang, E. Oldham, Y. Song, E. Murphy, T. K. McClanahan, G. Zurawski, M. Moshrefi,  
984 J. Qin, X. Li, D. M. Gorman, J. F. Bazan, R. A. Kastelein, IL-33, an interleukin-1-like cytokine that signals  
985 via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*  
986 **23**, 479–490 (2005).

987 39. D. E. Ochayon, A. Ali, P. C. Alarcon, D. Krishnamurthy, L. C. Kottyan, M. T. Borchers, S. N. Waggoner,  
988 IL-33 promotes type 1 cytokine expression via p38 MAPK in human NK cells. *J. Leukoc. Biol.* (2020),  
989 doi:10.1002/JLB.3A0120-379RR.

990 40. R. J. Salmond, A. S. Mirchandani, A.-G. Besnard, C. C. Bain, N. C. Thomson, F. Y. Liew, IL-33 induces  
991 innate lymphoid cell–mediated airway inflammation by activating mammalian target of rapamycin. *J*  
992 *Allergy Clin Immunol* **130**, 1159-1166.e6 (2012).

993 41. N. K. Björkström, H.-G. Ljunggren, J. Michaëlsson, Emerging insights into natural killer cells in  
994 human peripheral tissues. *Nat Rev Immunol* **16**, 310–320 (2016).

995 42. L. F. de Andrade, Y. Lu, A. Luoma, Y. Ito, D. Pan, J. W. Pyrdol, C. H. Yoon, G.-C. Yuan, K. W.  
996 Wucherpfennig, Discovery of specialized NK cell populations infiltrating human melanoma metastases.  
997 *JCI Insight* **4**, 133103 (2019).

998 43. J. Ni, X. Wang, A. Stojanovic, Q. Zhang, M. Wincher, L. Bühler, A. Arnold, M. P. Correia, M. Winkler,  
999 P.-S. Koch, V. Sexl, T. Höfer, A. Cerwenka, Single-Cell RNA Sequencing of Tumor-Infiltrating NK Cells  
1000 Reveals that Inhibition of Transcription Factor HIF-1 $\alpha$  Unleashes NK Cell Activity. *Immunity* **52**, 1075-  
1001 1087.e8 (2020).

1002 44. M. M. Tu, M. M. A. Rahim, C. Sayed, A. B. Mahmoud, A. P. Makrigiannis, Immunosurveillance and  
1003 Immunoediting of Breast Cancer via Class I MHC Receptors. *Cancer Immunol Res* **5**, 1016–1028 (2017).

1004 45. C. Moussion, N. Ortega, J.-P. Girard, The IL-1-like cytokine IL-33 is constitutively expressed in the  
1005 nucleus of endothelial cells and epithelial cells in vivo: a novel “alarmin”? *PLoS ONE* **3**, e3331 (2008).

1006 46. S. Lee, S. Stewart, I. Nagtegaal, J. Luo, Y. Wu, G. Colditz, D. Medina, D. C. Allred, Differentially  
1007 expressed genes regulating the progression of ductal carcinoma in situ to invasive breast cancer.  
1008 *Cancer Res* **72**, 4574–4586 (2012).

1009 47. C. Kretschmer, A. Sterner-Kock, F. Siedentopf, W. Schoenegg, P. M. Schlag, W. Kemmner,  
1010 Identification of early molecular markers for breast cancer. *Molecular Cancer* **10**, 15 (2011).

1011 48. J. P. Böttcher, E. Bonavita, P. Chakravarty, H. Blees, M. Cabeza-Cabrerizo, S. Sammicheli, N. C.  
1012 Rogers, E. Sahai, S. Zelenay, C. Reis e Sousa, NK Cells Stimulate Recruitment of cDC1 into the Tumor  
1013 Microenvironment Promoting Cancer Immune Control. *Cell* **172**, 1022-1037.e14 (2018).

1014 49. C. Baumann, W. V. Bonilla, A. Fröhlich, C. Helmstetter, M. Peine, A. N. Hegazy, D. D. Pinschewer, M.  
1015 Löhning, T-bet- and STAT4-dependent IL-33 receptor expression directly promotes antiviral Th1 cell  
1016 responses. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 4056–4061 (2015).

1017 50. M. Nakahira, H.-J. Ahn, W.-R. Park, P. Gao, M. Tomura, C.-S. Park, T. Hamaoka, T. Ohta, M.  
1018 Kurimoto, H. Fujiwara, Synergy of IL-12 and IL-18 for IFN-gamma gene expression: IL-12-induced STAT4  
1019 contributes to IFN-gamma promoter activation by up-regulating the binding activity of IL-18-induced  
1020 activator protein 1. *Journal of immunology (Baltimore, Md. : 1950)* **168**, 1146–53 (2002).

1021 51. A. Mavropoulos, G. Sully, A. P. Cope, A. R. Clark, Stabilization of IFN-gamma mRNA by MAPK p38 in  
1022 IL-12- and IL-18-stimulated human NK cells. *Blood* **105**, 282–288 (2005).

1023 52. M. Fang, Y. Li, K. Huang, S. Qi, J. Zhang, W. Zgodzinski, M. Majewski, G. Wallner, S. Gozdz, P. Macek,  
1024 A. Kowalik, M. Pasiarski, E. Grywalska, L. Vatan, N. Nagarsheth, W. Li, L. Zhao, I. Kryczek, G. Wang, Z.  
1025 Wang, W. Zou, L. Wang, IL33 Promotes Colon Cancer Cell Stemness via JNK Activation and Macrophage  
1026 Recruitment. *Cancer Res.* **77**, 2735–2745 (2017).

1027 53. D. E. Ochayon, A. Ali, P. C. Alarcon, D. Krishnamurthy, L. C. Kottyan, M. T. Borchers, S. N. Waggoner,  
1028 IL-33 promotes type 1 cytokine expression via p38 MAPK in human NK cells. *J Leukoc Biol* **107**, 663–671  
1029 (2020).

1030 54. C. T. Lutz, A. Karapetyan, A. Al-Attar, B. J. Shelton, K. J. Holt, J. H. Tucker, S. R. Presnell, Human  
1031 natural killer cells proliferate and die in vivo more rapidly than T cells in healthy young and elderly  
1032 adults. *J Immunol* **186**, 4590–4598 (2011).

1033 55. I. Hamann, N. Unterwalder, A. E. Cardona, C. Meisel, F. Zipp, R. M. Ransohoff, C. Infante-Duarte,  
1034 Analyses of phenotypic and functional characteristics of CX3CR1-expressing natural killer cells.  
1035 *Immunology* **133**, 62–73 (2011).

1036 56. J. Hanna, P. Bechtel, Y. Zhai, F. Youssef, K. McLachlan, O. Mandelboim, Novel insights on human NK  
1037 cells' immunological modalities revealed by gene expression profiling. *J. Immunol.* **173**, 6547–6563  
1038 (2004).

1039 57. M. Hubert, E. Gobbin, C. Couillault, T.-P. V. Manh, A.-C. Doffin, J. Berthet, C. Rodriguez, V. Ollion, J.  
1040 Kielbassa, C. Sajous, I. Treilleux, O. Tredan, B. Dubois, M. Dalod, N. Bendriss-Vermare, C. Caux, J.  
1041 Valladeau-Guilemond, IFN-III is selectively produced by cDC1 and predicts good clinical outcome in  
1042 breast cancer. *Sci Immunol* **5** (2020), doi:10.1126/sciimmunol.aav3942.

1043 58. L. Zitvogel, G. Kroemer, CD103+ dendritic cells producing interleukin-12 in anticancer  
1044 immunosurveillance. *Cancer Cell* **26**, 591–593 (2014).

1045 59. D. Mittal, D. Vijayan, E. M. Putz, A. R. Aguilera, K. A. Markey, J. Straube, S. Kazakoff, S. L. Nutt, K.  
1046 Takeda, G. R. Hill, N. Waddell, M. J. Smyth, Interleukin-12 from CD103+ Batf3-Dependent Dendritic  
1047 Cells Required for NK-Cell Suppression of Metastasis. *Cancer Immunol Res* **5**, 1098–1108 (2017).

1048 60. Y. O. Alexandre, S. Ghilas, C. Sanchez, A. Le Bon, K. Crozat, M. Dalod, XCR1+ dendritic cells promote  
1049 memory CD8+ T cell recall upon secondary infections with *Listeria monocytogenes* or certain viruses.  
1050 *Journal of Experimental Medicine* **213**, 75–92 (2015).

1051 61. S. M. Brunner, C. Rubner, R. Kesselring, M. Martin, E. Griesshammer, P. Ruemmele, T. Stempfl, A.  
1052 Teufel, H. J. Schlitt, S. Fichtner-Feigl, Tumor-infiltrating, interleukin-33-producing effector-memory  
1053 CD8(+) T cells in resected hepatocellular carcinoma prolong patient survival. *Hepatology* **61**, 1957–  
1054 1967 (2015).

1055 62. I. Saranchova, J. Han, H. Huang, F. Fenninger, K. B. Choi, L. Munro, C. Pfeifer, I. Welch, A. W. Wyatt,  
1056 L. Fazli, M. E. Gleave, W. A. Jefferies, Discovery of a Metastatic Immune Escape Mechanism Initiated by  
1057 the Loss of Expression of the Tumour Biomarker Interleukin-33. *Sci Rep* **6**, 30555 (2016).

1058 63. M. Rössle, G. Cathomas, L. Bonapace, M. Sachs, S. Dehler, M. Storz, G. Huber, H. Moch, T. Junt, K.  
1059 D. Mertz, Interleukin-33 Expression Indicates a Favorable Prognosis in Malignant Salivary Gland  
1060 Tumors. *Int. J. Surg. Pathol.* **24**, 394–400 (2016).

1061 64. M. Yang, Y. Feng, C. Yue, B. Xu, L. Chen, J. Jiang, B. Lu, Y. Zhu, Lower expression level of IL-33 is  
1062 associated with poor prognosis of pulmonary adenocarcinoma. *PLOS ONE* **13**, e0193428 (2018).

1063 65. R. Koster, O. A. Panagiotou, W. A. Wheeler, E. Karlins, J. M. Gastier-Foster, S. R. Caminada de  
1064 Toledo, A. S. Petrilli, A. M. Flanagan, R. Tirabosco, I. L. Andrulis, J. S. Wunder, N. Gokgoz, A. Patiño-  
1065 Garcia, F. Lecanda, M. Serra, C. Hattinger, P. Picci, K. Scotlandi, D. M. Thomas, M. L. Ballinger, R.  
1066 Gorlick, D. A. Barkauskas, L. G. Spector, M. Tucker, D. H. Belynda, M. Yeager, R. N. Hoover, S.  
1067 Wacholder, S. J. Chanock, S. A. Savage, L. Mirabello, Genome-wide association study identifies the  
1068 GLDC/IL33 locus associated with survival of osteosarcoma patients. *Int. J. Cancer* **142**, 1594–1601  
1069 (2018).

1070 66. X. Gao, X. Wang, Q. Yang, X. Zhao, W. Wen, G. Li, J. Lu, W. Qin, Y. Qi, F. Xie, J. Jiang, C. Wu, X.  
1071 Zhang, X. Chen, H. Turnquist, Y. Zhu, B. Lu, Tumoral expression of IL-33 inhibits tumor growth and  
1072 modifies the tumor microenvironment through CD8+ T and NK cells. *J. Immunol.* **194**, 438–445 (2015).

1073 67. A. Long, D. Dominguez, L. Qin, S. Chen, J. Fan, M. Zhang, D. Fang, Y. Zhang, T. M. Kuzel, B. Zhang,  
1074 Type 2 Innate Lymphoid Cells Impede IL-33–Mediated Tumor Suppression. *The Journal of Immunology*  
1075 (2018), doi:10.4049/jimmunol.1800173.

1076 68. L. Qi, Q. Zhang, Y. Miao, W. Kang, Z. Tian, D. Xu, W. Xiao, F. Fang, Interleukin-33 activates and  
1077 recruits natural killer cells to inhibit pulmonary metastatic cancer development. *Int. J. Cancer* **146**,  
1078 1421–1434 (2020).

1079 69. C. Laedrach, B. Salhia, N. Cihoric, I. Zlobec, C. Tapia, Immunophenotypic profile of tumor buds in  
1080 breast cancer. *Pathol Res Pract* **214**, 25–29 (2018).

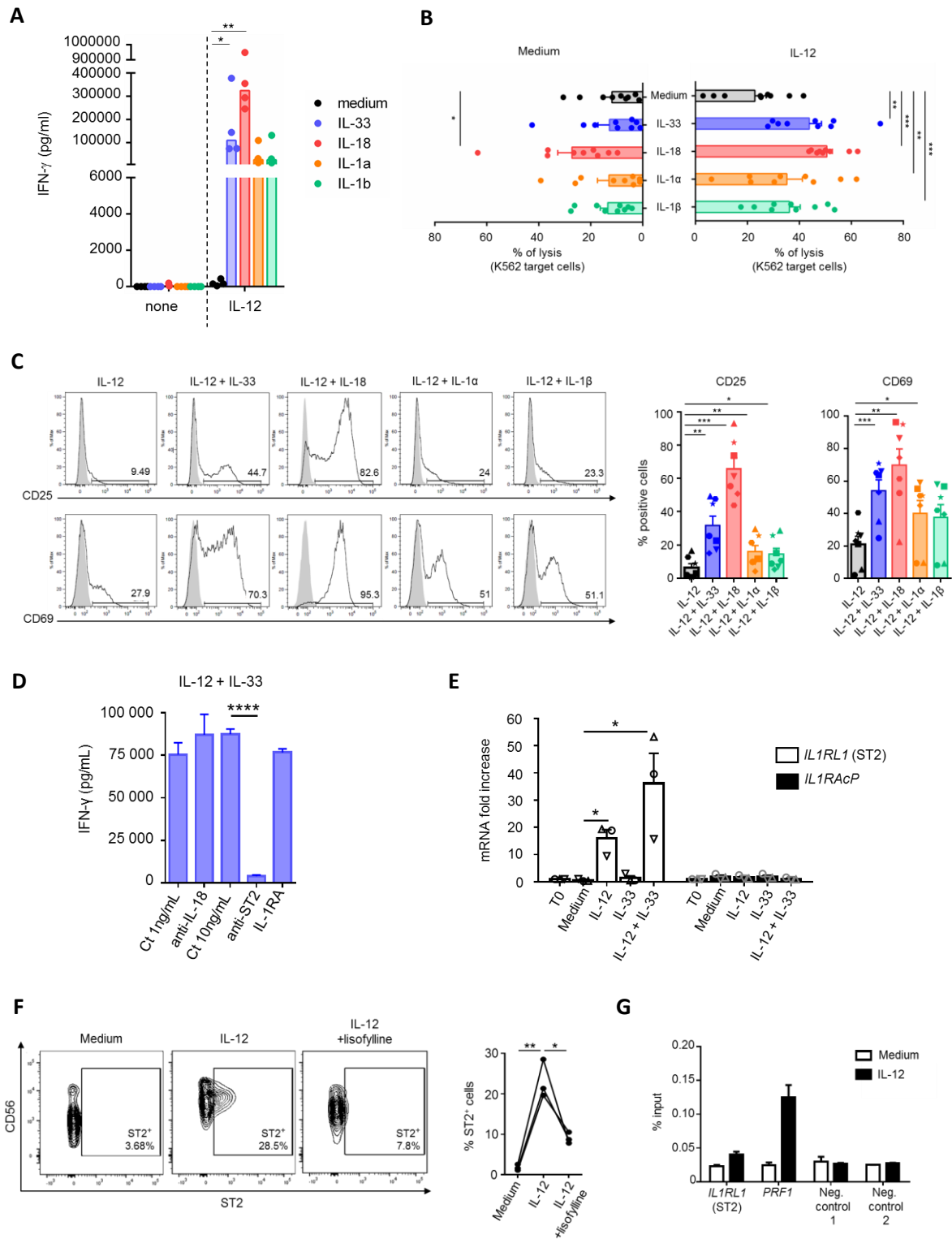
1081 70. R. Dreos, G. Ambrosini, R. C. Périer, P. Bucher, The Eukaryotic Promoter Database: expansion of  
1082 EPDnew and new promoter analysis tools. *Nucleic Acids Res* **43**, D92-96 (2015).

1083 71. D. Farré, R. Roset, M. Huerta, J. E. Adsuara, L. Roselló, M. M. Albà, X. Messeguer, Identification of  
1084 patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. *Nucleic Acids Research*  
1085 **31**, 3651–3653 (2003).

1086 72. Y. Baba, K. Maeda, T. Yashiro, E. Inage, K. Kasakura, R. Suzuki, F. Niyonsaba, M. Hara, A. Tanabe, H.  
1087 Ogawa, K. Okumura, Y. Ohtsuka, T. Shimizu, C. Nishiyama, GATA2 is a critical transactivator for the  
1088 human IL1RL1/ST2 promoter in mast cells/basophils: opposing roles for GATA2 and GATA1 in human  
1089 IL1RL1/ST2 gene expression. *J. Biol. Chem.* **287**, 32689–32696 (2012).

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1093 **Fig. 1. IL-12/STAT-4 signaling drives ST2 expression and polyfunctionality of human NK cells in**  
 1094 **response to IL-33**

1095 **(A)** Quantification of IFN- $\gamma$  secretion by healthy donors' blood NK cells upon stimulation with IL-33, IL-  
 1096 18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination with IL-12 (10 ng/mL) for 24 h. Histogram bars



1097 represent the median (n = 4 individual experiments). Friedman test with Dunn's multiple comparisons  
1098 test was performed.

1099 **(B)** NK cells were activated for 24 h with IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination  
1100 with IL-12 (10 ng/mL) and then co-cultured with calcein-loaded K562 target cells for 4 h at a 5:1 effector  
1101 to target ratio. Calcein release in the supernatant was quantified by fluorometry to calculate lysis  
1102 percentage. Results are expressed as mean + SEM (n = 9 individual experiments). One-way repeated  
1103 measures ANOVA with Dunnett's multiple comparisons test against levels in medium was performed.

1104 **(C)** NK cells were activated for 24 h with IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination  
1105 with IL-12 (10 ng/mL) and then stained for CD25 and CD69 (black line) or corresponding isotypic control  
1106 (grey). Representative histogram plots (left) and quantification (%) (right) of CD25<sup>+</sup> or CD69<sup>+</sup> NK cells  
1107 after cytokine stimulation. Symbols represent paired individual experiments (n=7). Results are expressed  
1108 as mean + SEM. One-way repeated measures ANOVA with Dunnett's multiple comparisons test against  
1109 levels in IL-12 was performed.

1110 **(D)** IFN- $\gamma$  secretion by healthy donors' blood NK cells upon stimulation for 24 h with IL-33 and IL-12 (10  
1111 ng/mL each) in the presence of anti-ST2 (10  $\mu$ g/mL), anti-IL-18 (1  $\mu$ g/mL) blocking antibodies, IL-1RA  
1112 antagonist (100 ng/mL) or mIgG1 control antibody (1 or 10  $\mu$ g/mL as control for anti-IL-18 or anti-ST2,  
1113 respectively). Results are expressed as mean + SD and are representative of three individual  
1114 experiments. One-way repeated measures ANOVA with Tukey's multiple comparisons test was  
1115 performed.

1116 **(E)** Real-time quantitative PCR (RT-qPCR) analysis of *IL1RL1* (ST2) and *IL1RAcP* mRNA expression in  
1117 resting or activated NK cells with IL-12, IL-33, or the combination (10 ng/mL each for 24 h). Results are  
1118 expressed as mean + SEM of three individual experiments. Each symbol represents one donor. Two-  
1119 tailed paired Student *t*-test was performed.

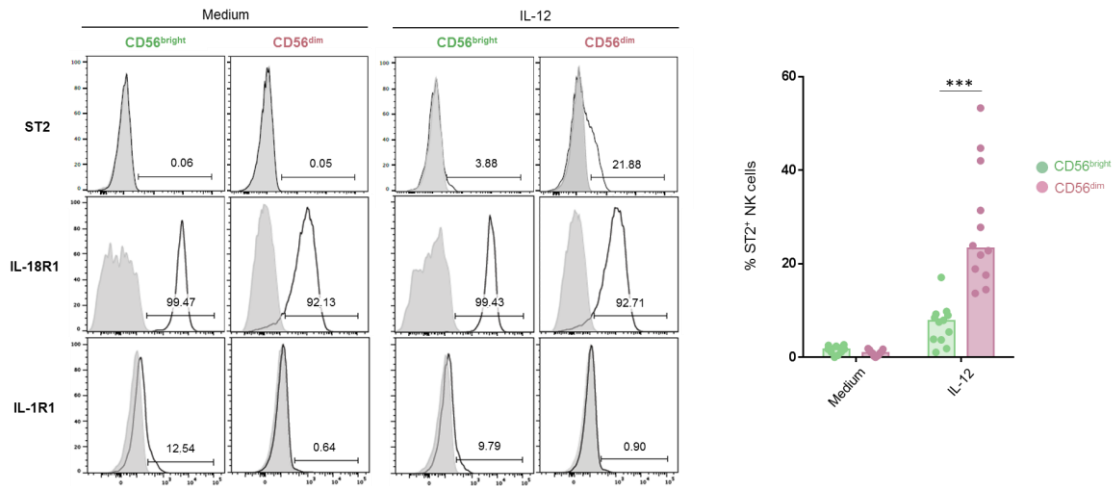
1120 **(F)** NK cells were activated with IL-12 (10 ng/mL) in the presence or not of lisofylline (500  $\mu$ M) for 24 h  
1121 prior to ST2 surface expression analysis by flow cytometry. Representative dot plots (left) and

1122 quantification (%) (right) of ST2<sup>+</sup> NK cells after cytokine stimulation. One-way repeated measures ANOVA  
1123 with Tukey's multiple comparisons test was performed; n = 3.

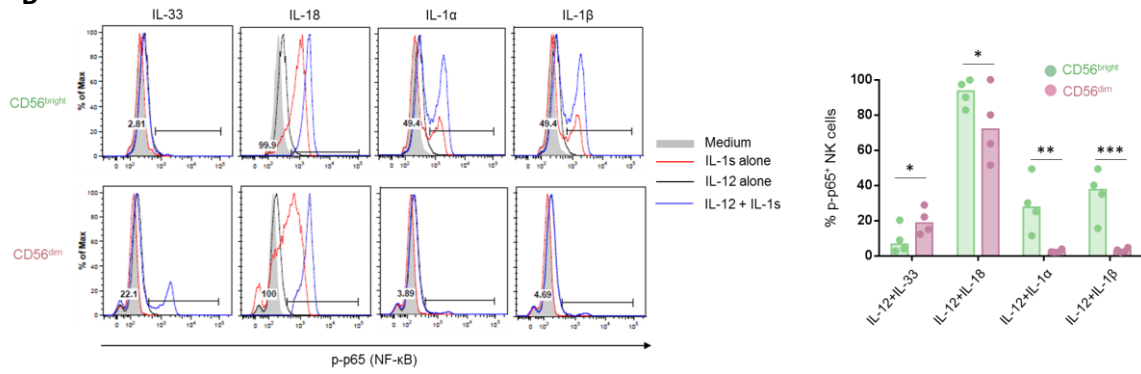
1124 **(G)** p-STAT4 was immunoprecipitated in NK cells following activation or not with IL-12 (10 ng/mL) for 24  
1125 h and p-STAT4 enrichment in *ST2* promoter region was measured by RT-qPCR analysis. *PERFORIN-1*  
1126 (*PRF1*) and negative control sets 1 and 2 were used respectively as positive and negative controls for p-  
1127 STAT4 binding. Results are expressed as mean + SEM of two individual experiments.

1128

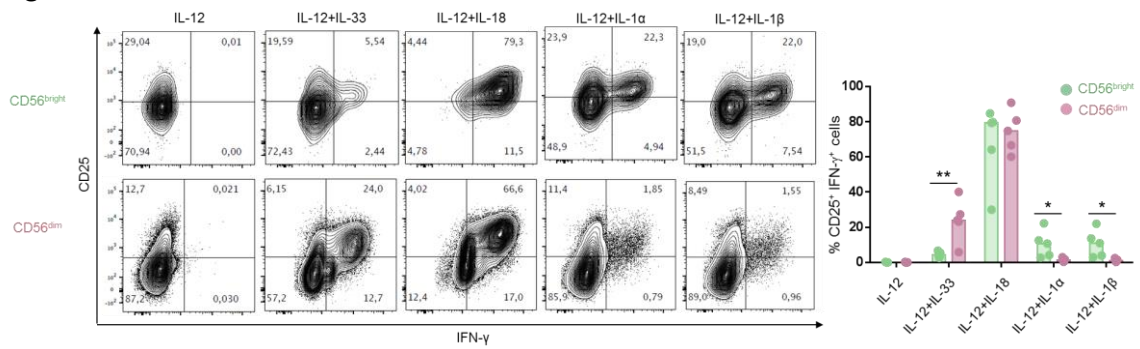
**A**



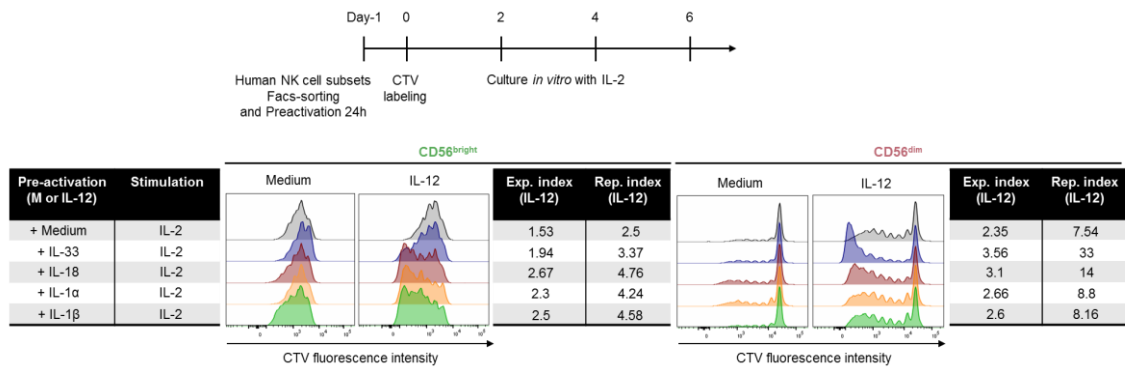
**B**

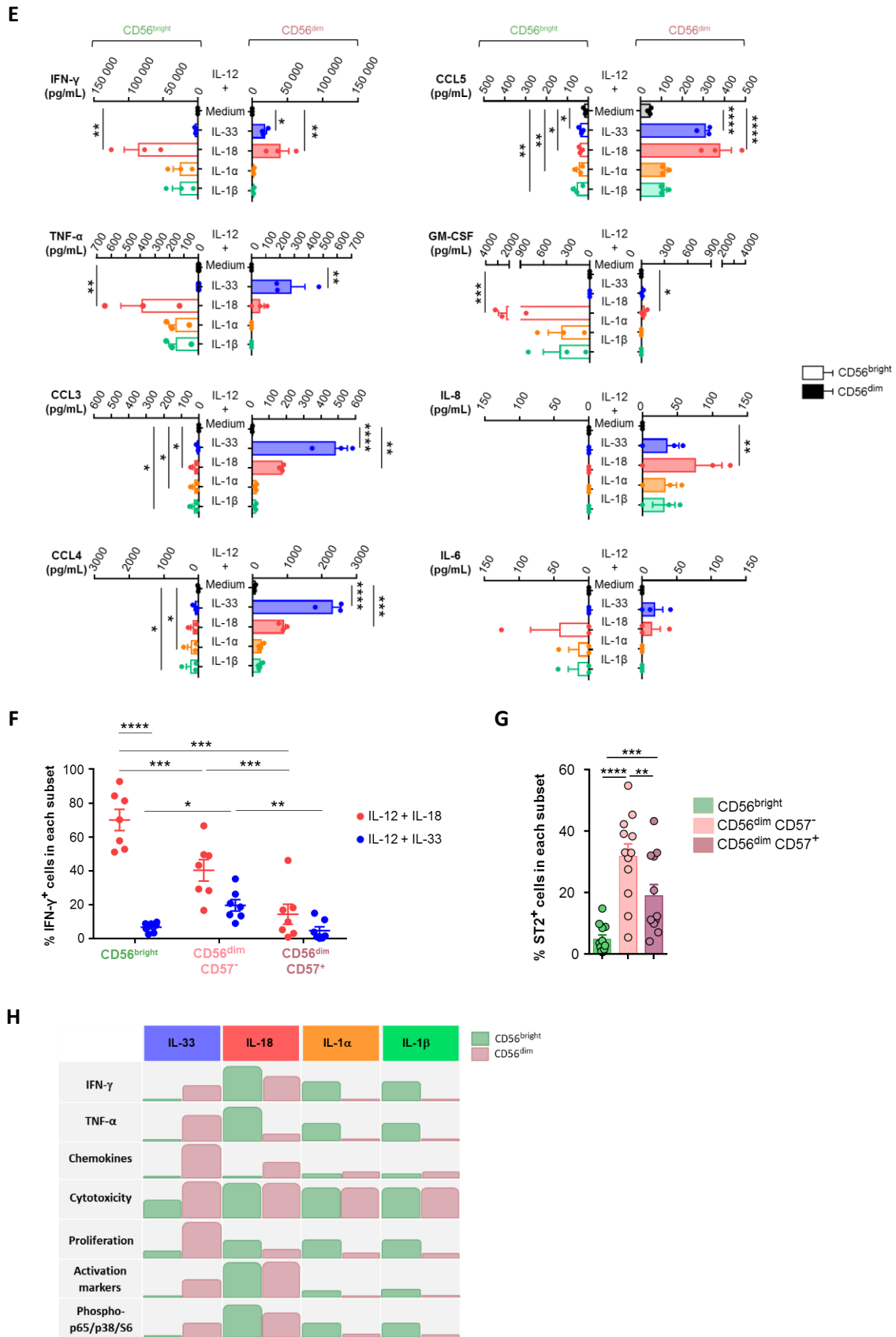


**C**



**D**





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1131 **Fig. 2. IL-33 strongly activates a subset of CD56<sup>dim</sup> NK cells in the presence of IL-12**

1132 **(A)** Healthy donors' blood NK cells were activated with IL-12 (10 ng/mL) or not (medium) for 24 h prior  
1133 to flow cytometry analysis of ST2, IL-18R1, IL-1R1 expression (grey) or corresponding isotypic control  
1134 (black line). CD56 surface expression levels were used to discriminate CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells.  
1135 Representative histogram plots (left) and quantification (%) (right) of ST2<sup>+</sup> NK cells after medium or IL-  
1136 12 culture. Histogram bars indicate the median. Wilcoxon matched pairs signed-rank test was  
1137 performed; n = 12 experiments.

1138 **(B)** FACS-sorted CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells were activated with medium or IL-12 for 24 h prior to the  
1139 addition of IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$ , supplemented or not with IL-12. Each cytokine was used at  
1140 10ng/mL. p65 (NF- $\kappa$ B) phosphorylation was analyzed by flow cytometry 5 min after the addition of IL-1  
1141 family cytokines. Representative histogram plots (left) and quantification (%) (right) of p-p65<sup>+</sup> NK cells  
1142 after cytokine activation. Histogram bars indicate the median. Two-way ANOVA with Bonferroni's  
1143 multiple comparisons test was performed; n = 4 experiments.

1144 **(C)** Healthy donors' blood NK cells were activated as indicated for 24 h, each cytokine was used at 10  
1145 ng/mL. CD25 surface and IFN- $\gamma$  intracellular expression was analyzed by flow cytometry. CD56 surface  
1146 expression levels were used to discriminate CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells. Representative dot plots  
1147 (left) and quantification (%) (right) of CD25<sup>+</sup> IFN- $\gamma$ <sup>+</sup> NK cells after cytokine activation. Histogram bars  
1148 indicate the median. Two-way ANOVA test with Bonferroni's multiple comparisons test was performed;  
1149 n = 5 experiments.

1150 **(D)** FACS-sorted CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells were pre-activated as indicated, labeled with CTV, and  
1151 cultured in low dose IL-2 (100 UI/mL). *In vitro* NK cell proliferation was analyzed at day 3 for CD56<sup>bright</sup>  
1152 and day 6 for CD56<sup>dim</sup> NK cells. Expansion Index (Exp. Index) and Replication Index (Rep. Index) determine  
1153 the fold-expansion of the overall culture and of the responding cells only, respectively. Data shown are  
1154 representative of three individual experiments.

1155 **(E)** Supernatants from FACS-sorted CD56<sup>bright</sup> (empty histogram bars) and CD56<sup>dim</sup> (filled histogram bars)  
1156 NK cells were collected to quantify cytokine and chemokine release by Luminex assay. Of note, IL-2, IL-  
1157 3, IL-4, IL-5, IL-10, IL-13, IL-17A, IL-22, and G-CSF were not detected (not shown). Results are expressed

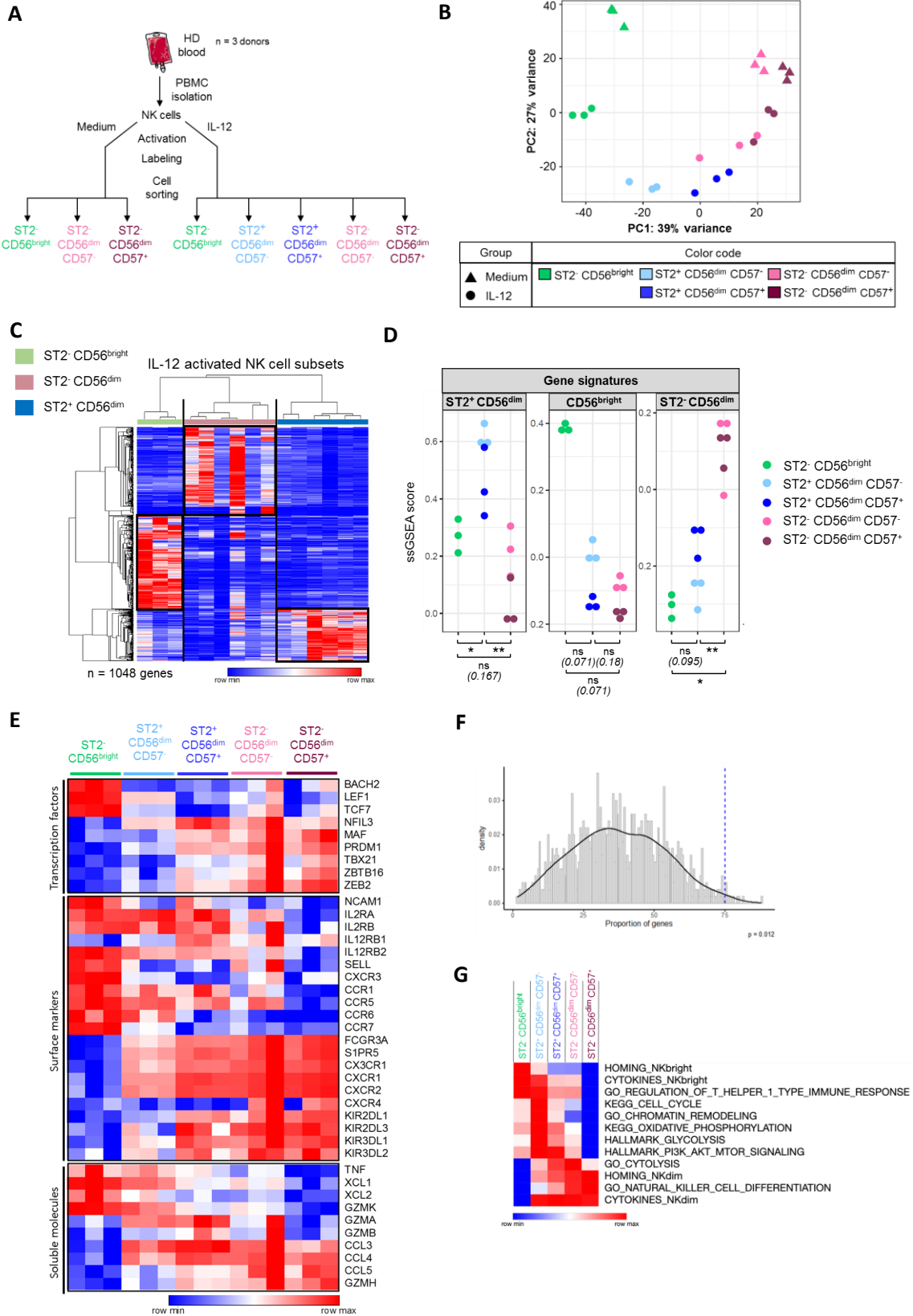
1158 as mean + SEM (n = 3 individual donors). One-way repeated measures ANOVA with Tukey's multiple  
1159 comparisons test was performed.

1160 **(F)** Healthy donors' blood NK cells were activated with combinations of IL-12 and IL-33 or IL-12 and IL-  
1161 18 (10 ng/mL of each cytokine) for 24 h prior to flow cytometry analysis of intracellular IFN- $\gamma$  expression  
1162 in NK cell subsets based on CD56 and CD57 surface expression. Results are expressed as mean +/- SEM  
1163 (n = 7 individual experiments). Two-way ANOVA with Bonferroni's multiple comparisons test was  
1164 performed.

1165 **(G)** Healthy donors' blood NK cells were activated with IL-12 (10 ng/mL) for 24 h prior to flow cytometry  
1166 analysis of ST2 expression on NK cell subsets based on CD56 and CD57 surface expression. Results are  
1167 expressed as mean + SEM (n = 12 experiments). One-way repeated measures ANOVA with Tukey's  
1168 multiple comparisons test was performed.

1169 **(H)** Graphical summary of biological functions differentially regulated in CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells  
1170 in response to IL-1 family cytokines (IL-33, IL-18, IL-1 $\alpha$  and IL-1 $\beta$ ) when combined with IL-12.

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**Fig. 3. ST2<sup>+</sup> CD56<sup>dim</sup> NK cells display a unique gene signature compared to CD56<sup>bright</sup> and CD56<sup>dim</sup> NK**

1175 **cells**

1176 **(A)** Schematic representation of FACS sorting strategy to isolate eight different subsets sorted from  
1177 healthy donors' blood NK cells based on CD56, CD57 and ST2 surface expression. Three and five subsets  
1178 were obtained after activation for 24 h with medium or IL-12 respectively.

1179 **(B)** Principal component analysis for eight FACS-sorted NK cell subsets based on expression of the top  
1180 500 most variant genes obtained from 3 individual healthy donors.

1181 **(C)** Heatmap representing upregulated genes in each sorted NK cell subset compared to the two other  
1182 NK cell subsets following IL-12 activation for 24 h (n = 3 individual healthy donors). Black squares identify  
1183 specific transcriptional signatures for ST2<sup>-</sup> CD56<sup>bright</sup>, ST2<sup>-</sup> CD56<sup>dim</sup> NK cells, and ST2<sup>+</sup> CD56<sup>dim</sup> NK cells.

1184 **(D)** ssGSEA analysis of the NK signatures defined by genes specifically upregulated in each sorted NK cell  
1185 subset (see Table 1) following IL-12 activation for 24 h (n = 3 individual healthy donors). Kruskal-Wallis  
1186 test with Dunn multiple comparisons test was performed.

1187 **(E)** Heatmap showing normalized expression for selected genes related to NK cell phenotype and  
1188 functions.

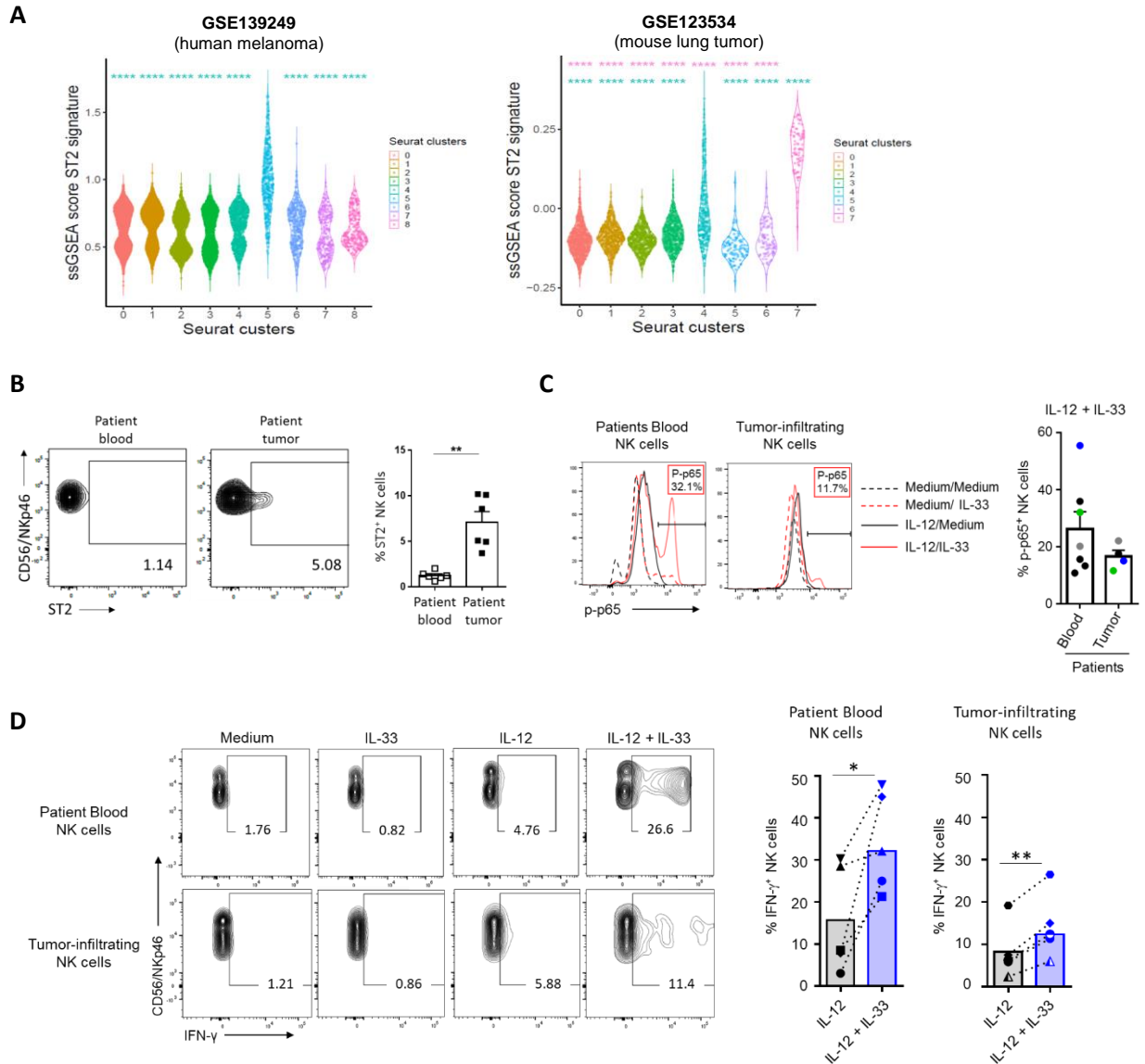
1189 **(F)** Sampling distribution of genes with an intermediate expression in ST2<sup>+</sup> NK cells (CD56<sup>bright</sup> > ST2<sup>+</sup> >  
1190 CD56<sup>dim</sup> or CD56<sup>bright</sup> < ST2<sup>+</sup> < CD56<sup>dim</sup>) with n = 1000 random shuffling of all sample labels. p value was  
1191 computed with a permutation test.

1192 **(G)** ssGSEA analysis of selected biological pathways in each sorted NK cell subset following IL-12  
1193 activation for 24 h (n = 3 individual healthy donors).

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1198 **Fig. 4. Peripheral and tumor-infiltrating NK cells from breast cancer patients respond to the IL-33 and**

1199 **IL-12 combination**

1200 **(A)** Violin plots representing the distribution of ssGSEA score for ST2<sup>+</sup> NK cells signature (233 genes, see

1201 Table 1) for tumor NK cell clusters identified from sc-RNAseq analysis of tumor-infiltrating NK cells

1202 isolated from (left panel) melanoma patients (GSE139249) (42) and (right panel) a mouse model of lung

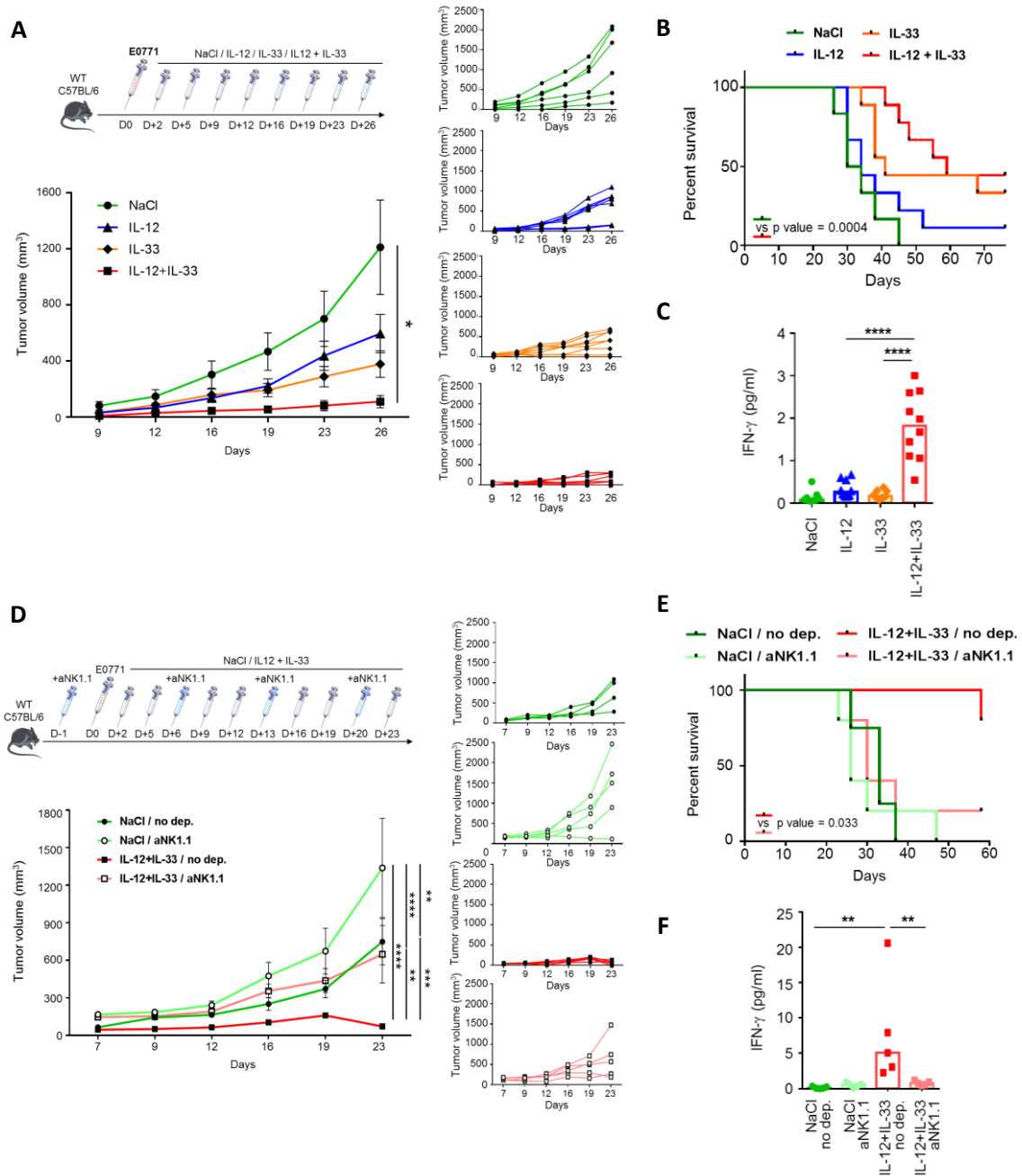
1203 cancer (GSE123534) (43). Kruskal-Wallis test with Dunn multiple comparisons test was performed using

1204 cluster 5 for the melanoma dataset and cluster 4 (turquoise) or cluster 7 (pink) (fig.S5) as references.

1205 **(B)** Flow cytometry analysis of ST2 protein expression on the surface of CD56/NKp46<sup>+</sup> NK cells among  
1206 PBMCs and tumor cell suspensions from breast cancer patients (n = 6). Representative dot plots (left)  
1207 and quantification (%) (right) of ST2<sup>+</sup> NK cells. Results are expressed as mean + SEM. Paired two-tailed  
1208 Student *t*-test was performed.

1209 **(C)** PBMCs (n = 7) and tumor cell suspensions (n = 4) from breast cancer patients were activated with  
1210 medium or IL-12 for 24 h prior to the addition of medium or IL-33. p65 phosphorylation was analyzed 5  
1211 min after the addition of IL-33 by flow cytometry in CD56<sup>+</sup> CD7<sup>+</sup> NK cells. Representative histogram plots  
1212 (left) and quantification (%) (right) of p-p65<sup>+</sup> NK cells after IL-12 and IL-33 combination. Results are  
1213 expressed as mean + SEM. Unpaired two-tailed Student *t*-test was performed with no statistically  
1214 significant difference.

1215 **(D)** PBMCs and tumor cell suspensions from breast cancer patients (n = 5) were activated as indicated  
1216 (10 ng/mL of each cytokine) for 24 h prior to flow cytometry analysis of intracellular IFN- $\gamma$ .  
1217 Representative dot plots (left) and quantification (%) (right) of IFN- $\gamma$ <sup>+</sup> NK cells. Symbols represent  
1218 individual breast cancer patients and histogram bars the median. Wilcoxon matched-pairs signed rank  
1219 test was performed.



1220

1221 **Fig. 5. The combination of IL-33 and IL-12 promotes antitumor functions in an NK-cell-dependent**  
 1222 **manner**

1223 **(A)** WT mice were injected intra-mammary with  $2.5 \times 10^5$  E0771 cells on day 0 and then treated in the  
 1224 tumor area with NaCl solution (n = 6), 10 ng/mouse rIL-12 (n = 9) or 100 ng/mouse rIL-33 (n = 9) or  
 1225 in combination (n = 10) twice a week from day 2 to day 26. Primary tumor growth was monitored in  
 1226 mice treated with, rIL-12, rIL-33 or with the two cytokines. Two-way ANOVA with Tukey's multiple  
 1227 comparisons test was performed.

1228 **(B)** Kaplan-Meier survival plots of WT mice treated with NaCl, rmIL-12, rmIL-33 or the two cytokines.  
1229 Mice were sacrificed when longest side of primary tumor reached 17 mm. Log-rank test was performed  
1230 and the p-value is indicated for NaCl vs rmIL-12 + rmIL-33 comparison.

1231 **(C)** Serum was retrieved from blood collected at day 5, 4 h after NaCl or cytokine injection into the  
1232 peritumoral area. IFN- $\gamma$  concentration was measured by Elisa in the serum of mice treated with NaCl,  
1233 rmIL-12, rmIL-33 or the two cytokines. Histogram bars represent the median. Kruskal Wallis test with  
1234 Dunn's multiple comparisons test was performed.

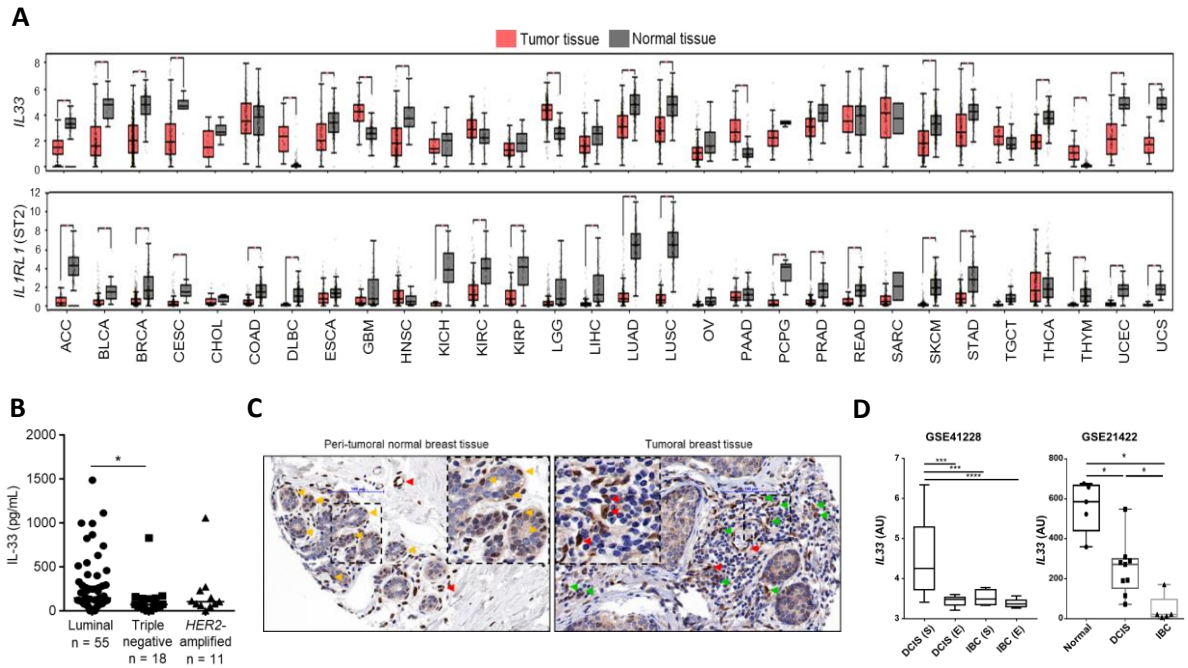
1235 **(D)** WT mice were injected intra-peritoneally with NaCl or anti-NK1.1 depleting antibody prior to intra-  
1236 mammary injection with  $2.5 \times 10^5$  E0771 cells on day 0 and then treated in the tumor area with NaCl or a  
1237 combination of 10 ng/mouse rmIL-12 and 100 ng/mouse rmIL-33 twice a week from day 2 to day 23.  
1238 Primary tumor growth was monitored in non-depleted WT mice treated with NaCl (n = 4) or with rmIL-  
1239 12 and rmIL-33 combination (n = 5) or in NK cell-depleted WT mice treated with NaCl (n = 5) or with  
1240 rmIL-12 and rmIL-33 combination (n = 5). Two-way ANOVA with Tukey's multiple comparisons test was  
1241 performed.

1242 **(E)** Kaplan-Meier survival plots of non-depleted WT mice treated with NaCl (n = 4) or with a combination  
1243 of rmIL-12 and rmIL-33 (n = 5) or in NK cell-depleted WT mice treated with NaCl (n = 5) or with rmIL-12  
1244 and rmIL-33 combination (n=5). Mice were sacrificed when longest side of primary tumor reached 17  
1245 mm. Log-rank test was performed and the p-value is indicated for non-depleted mice treated with rmIL-  
1246 12 + rmIL-33 vs NK cell-depleted mice treated with rmIL-12 + rmIL-33.

1247 **(F)** Serum was retrieved from blood collected at day 5, 4 h after NaCl or cytokine injection into the  
1248 peritumoral area. IFN- $\gamma$  concentration was measured by ELISA in the serum of non-depleted WT mice  
1249 treated with NaCl (n = 4) or with rmIL-12 and rmIL-33 combination (n = 5) or in NK cell-depleted WT mice  
1250 treated with NaCl (n = 5) or with rmIL-12 and rmIL-33 combination (n = 5). Histogram bars represent the  
1251 median. Kruskal Wallis test with Dunn's multiple comparisons test was performed.

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1255 **Fig. 6. IL-33 is expressed in tumors and downregulated during cancer progression**

1256 **(A)** *IL33* and *IL1RL1* (ST2) gene expression in tumoral (T) (orange) and normal (N) (grey) tissues extracted  
 1257 from TCGA and GTEX databases (ACC: Adrenocortical carcinoma, T=77, N=128; BLCA : Bladder  
 1258 carcinoma, T=404, N=28; BRCA : Breast invasive carcinoma, T=1095, N=291; CESC: Cervical squamous  
 1259 cell carcinoma and endocervical adenocarcinoma, T=306, N=13 ; CHOL: Cholangiocarcinoma, T=36, N=9;  
 1260 COAD : Colorectal adenocarcinoma, T=275, N=349; DLBC: Lymphoid Neoplasm Diffuse Large B-cell  
 1261 Lymphoma, T=47, N=337 ; ESCA: Esophageal carcinoma, T=182, N=286 ; GBM: Glioblastoma multiforme,  
 1262 T=163, N=207 ; HNSC : Head and neck squamous cell carcinoma, T=519, N=44 ; KICH: Kidney  
 1263 Chromophobe, T=66, N=53 ; KIRC : Kidney renal clear cell carcinoma, T=523, N=100 ; KIRP: Kidney renal  
 1264 papillary cell carcinoma, T=286, N=60 ; LGG : Brain lower grade glioma, T=518, N=207 ; LIHC: Liver  
 1265 hepatocellular carcinoma, T=369, N=160 ; LUAD : Lung adenocarcinoma, T=483, N=347 ; LUSC: Lung  
 1266 squamous cell carcinoma, T=486, N=338 ; OV : Ovarian serous cystadenocarcinoma, T=426, N=88 ; PAAD  
 1267 : Pancreatic adenocarcinoma, T=179, N=171 ; PCPG: Pheochromocytoma and Paraganglioma, T=182,  
 1268 N=3 ; PRAD: Prostate adenocarcinoma, T=492, N=152 ; READ: Rectum adenocarcinoma, T=92, N=318 ;  
 1269 SARC: Sarcoma, T=262, N=2 ; SKCM : Skin cutaneous melanoma, T=461, N=558 ; STAD : Stomach  
 1270 adenocarcinoma, T=408, N=211 ; TGCT: Testicular Germ Cell Tumors, T=137, N=165 ; THCA : Thyroid

1271 cancer, T=512, N=337; THYM: Thymoma, T=118, N=339 ; UCEC: Uterine Corpus Endometrial Carcinoma,  
1272 T=174, N=91 ; UCS: Uterine Carcinosarcoma , T=57, N=78).

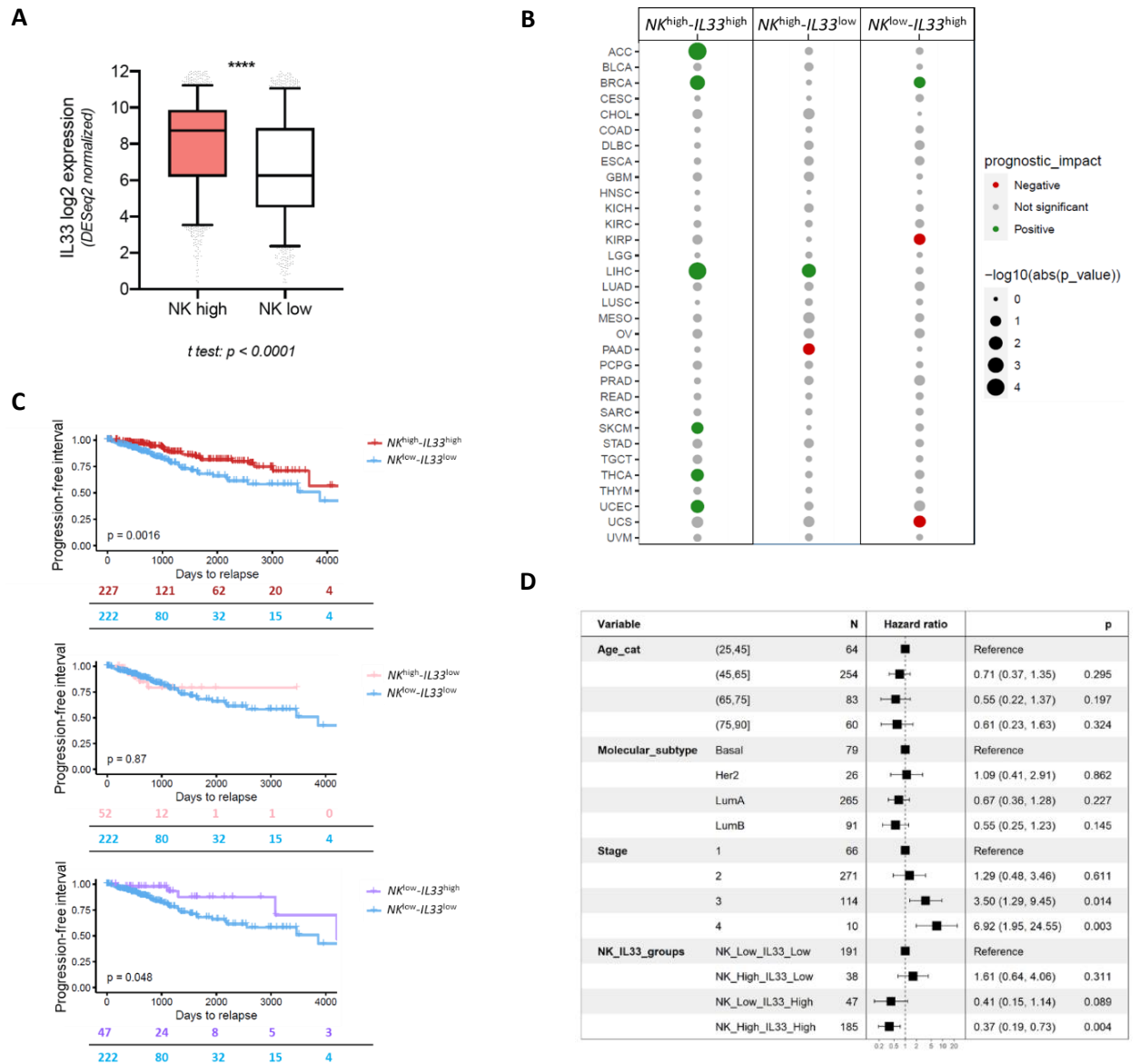
1273 **(B)** IL-33 protein was quantified by Luminex assay in breast tumor-derived supernatants (n = 89). Kruskal-  
1274 Wallis test with Dunn multiple comparisons test was performed.

1275 **(C)** IL-33 expression was analyzed on breast tumor FFPE slides by Immunohistochemistry (IHC). Non-  
1276 invasive Ductal Carcinoma *In Situ* (DCIS) and invasive Breast Cancer (IBC) lesions were identified based  
1277 on anatomopathological tissue observations. Red arrows indicate blood vessels, orange arrows indicate  
1278 peritumoral normal breast acini and green arrows indicate isolated stromal cells positive for IL-33  
1279 staining. Images are shown at 20x magnification. Dotted squares represent enlarges areas.

1280 **(D)** Analysis of *IL33* expression in laser-microdissected stromal versus epithelial zones from DCIS and IBC  
1281 lesions (GSE41228) (46) and in healthy mammary tissue versus DCIS and IBC (GSE21422) (47). Kruskal-  
1282 Wallis test with Steel-Dwas-Fligner multiple comparisons test was performed.

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1287 **Fig. 7. An  $NK^{\text{hi}}/IL33^{\text{hi}}$  transcriptomic score is associated with improved progression-free survival**

1288 **(A)** Boxplots display  $IL33$  gene expression in  $NK^{\text{hi}}$  vs  $NK^{\text{low}}$  breast cancer patients from the TCGA database.

1289 An unpaired Student  $t$ -test was performed.

1290 **(B)** Summary of  $p$ -values associated with the log rank test performed to evaluate prognostic value of

1291  $NK^{\text{high}}/IL33^{\text{high}}$ ,  $NK^{\text{high}}/IL33^{\text{low}}$  and  $NK^{\text{low}}/IL33^{\text{high}}$  scores as compared to  $NK^{\text{low}}/IL33^{\text{low}}$  in 32 human cancers

1292 from TCGA database (legend is the same as in panel A, MESO: Mesothelioma ; UVM: Uveal Melanoma

1293 were added). Results are represented as a bubble map showing positive (green) or negative (red) impact

1294 on progression-free survival. Dots size represents p-values obtained by log-rank test. An unpaired  
1295 Student *t*-test was performed.

1296 **(C)** Kaplan-Maier curves for progression-free survival for  $NK^{high}/IL33^{high}$ ,  $NK^{high}/IL33^{low}$  and  $NK^{low}/IL33^{high}$   
1297 scores as compared to  $NK^{low}/IL33^{low}$  in breast cancer dataset from TCGA. p-values were obtained with  
1298 log-rank test.

1299 **(D)** Multivariate Cox analysis of the impact of  $NK^{high}/IL33^{high}$  score on prognosis in breast cancer patients  
1300 from TCGA dataset regarding the age, molecular subtype, and stage of the tumors. p-values were  
1301 obtained with log-rank test.

1302



1303 **Table 1.** List of ST2<sup>+</sup> NK cell signature genes used in this study, Related to Fig. 3C,D,F,G, 4A

1304

gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
IL23R	840,503787	2,42322895	0,24889705	9,81765284	9,45E-23	8,62E-19
TRPV1	585,713744	1,5368841	0,1666148	9,18767638	4,01E-20	1,46E-16
IL1RL1	1126,7443	2,61252558	0,29381202	9,01318107	2E-19	5,21E-16
NDFIP2	620,975976	1,96393908	0,2429768	8,14409229	3,82E-16	4,35E-13
VEGFA	800,797748	2,08492082	0,27235779	7,70971428	1,26E-14	9,58E-12
DESI1	1249,1091	1,1262011	0,14710845	7,65517735	1,93E-14	1,34E-11
FRMD4B	1643,87039	1,81739229	0,24219981	7,53071051	5,05E-14	2,79E-11
DMRTA2	122,468676	3,05535882	0,41505092	7,31571058	2,56E-13	1,11E-10
FAM89A	545,929495	1,18540785	0,16358858	7,24271616	4,4E-13	1,82E-10
CDC7	1128,13727	1,06925225	0,15213337	7,02091227	2,2E-12	6,82E-10
STRIP2	140,760635	1,29741714	0,1881047	6,89072171	5,55E-12	1,33E-09
NCS1	468,813606	2,07555351	0,31228121	6,84559957	7,62E-12	1,76E-09
SLC16A1	1512,26695	1,9475331	0,28782674	6,72865566	1,71E-11	3,55E-09
CLU	915,149362	1,8992889	0,28769004	6,59283755	4,31E-11	7,36E-09
CHAC2	356,999118	1,25300861	0,19181008	6,53944106	6,17E-11	1,01E-08
ZNF367	984,442668	1,89172016	0,28799764	6,51164619	7,43E-11	1,18E-08
PSAT1	3785,47966	1,67376617	0,26455322	6,44648195	1,14E-10	1,71E-08
GLB1L2	151,981654	1,46163105	0,22563266	6,44192863	1,18E-10	1,75E-08
WDR76	1241,79085	1,43758613	0,2229631	6,43924506	1,2E-10	1,77E-08
CYCS	7640,67913	1,02715495	0,16080527	6,38946825	1,66E-10	2,32E-08
ITGA3	253,34202	1,30398949	0,20420147	6,38357806	1,73E-10	2,39E-08
MT1X	692,256455	1,30721854	0,20490144	6,36783592	1,92E-10	2,55E-08
C4orf46	658,702544	1,03853939	0,16290331	6,3707641	1,88E-10	2,55E-08
CDKN2A	155,551181	1,68677994	0,26579413	6,34882571	2,17E-10	2,85E-08
WEE1	2447,37932	1,44249365	0,22840625	6,28343908	3,31E-10	4,14E-08

FANCB	202,276777	1,19938518	0,19410873	6,18297309	6,29E-10	7,21E-08
ODC1	3337,57699	1,214216	0,19669728	6,16936299	6,86E-10	7,72E-08
PMAIP1	3335,93606	1,18029835	0,19394516	6,13557786	8,49E-10	9,34E-08
MCM3	10000,7616	1,06949838	0,17412227	6,13221471	8,67E-10	9,35E-08
LIMK2	3104,25713	1,15832666	0,18776431	6,12878921	8,86E-10	9,5E-08
MCM5	9509,01304	1,19770655	0,19494071	6,12424439	9,11E-10	9,66E-08
FANCC	557,126559	1,17140782	0,19204919	6,10582487	1,02E-09	1,07E-07
CTNNAL1	508,701126	1,79673214	0,29391591	6,09584392	1,09E-09	1,11E-07
GIN54	525,980957	1,73755156	0,28580647	6,05885887	1,37E-09	1,34E-07
ANKRD9	504,828651	1,19109652	0,19590466	6,05394548	1,41E-09	1,37E-07
GZMA	74121,1277	1,08182451	0,17931976	6,04154076	1,53E-09	1,46E-07
NMB	106,524871	1,14403357	0,19049991	5,99940616	1,98E-09	1,81E-07
TMEM106C	2456,27398	1,26007271	0,21005254	5,98257723	2,2E-09	1,99E-07
MT1E	44,050544	1,95169574	0,32328037	5,95810717	2,55E-09	0,00000022
CENPS-CORT	55,7971453	2,46977763	0,41342915	5,95115155	2,66E-09	2,27E-07
PRXL2A	237,702271	1,05442176	0,17889782	5,94511089	2,76E-09	2,34E-07
MCM7	14752,7609	1,37191183	0,2297699	5,94314722	2,8E-09	2,36E-07
CSTF2	2575,74527	1,09307847	0,18452897	5,9067807	3,49E-09	2,78E-07
PACSIN3	25,0768206	2,26253594	0,38409283	5,83189903	5,48E-09	0,0000004
FAM222A	180,250612	1,40349469	0,23981944	5,80219939	6,55E-09	4,57E-07
AL121985.1	40,7926711	1,16589314	0,20293076	5,75067325	8,89E-09	5,85E-07
MARCHF3	783,211555	1,17359882	0,20479762	5,73444119	9,78E-09	6,32E-07
TIMELESS	4418,15331	1,08590411	0,18925965	5,72959812	1,01E-08	6,49E-07
AL354718.3	26,2533188	1,7417743	0,29946638	5,72789903	1,02E-08	6,53E-07
ACTN1	1511,11114	1,74503563	0,30449433	5,72027976	1,06E-08	6,79E-07
CCDC74A	251,973341	1,31883746	0,2376231	5,71927486	1,07E-08	0,00000068
GIN53	467,902103	1,33967219	0,23328208	5,71328543	1,11E-08	6,94E-07
H4C2	16,5518026	1,95322135	0,34238088	5,70051711	1,19E-08	7,43E-07

AL133215.2	30,1675575	1,42266749	0,25229359	5,63269336	1,77E-08	0,00000101
WDHD1	1158,87928	1,46188406	0,26192838	5,5726197	2,51E-08	0,00000137
PPIF	4031,87853	1,15942507	0,20940214	5,55032627	2,85E-08	0,00000153
TUBB	35257,5275	1,17169715	0,21471365	5,44816951	5,09E-08	0,00000244
PTGER3	1122,67732	1,94573883	0,35633685	5,42295615	5,86E-08	0,00000274
NUDT1	1001,90806	1,00733025	0,18719371	5,38299465	7,33E-08	0,00000331
CCDC167	992,092596	1,07088026	0,20061089	5,34769926	8,91E-08	0,00000382
PGD	6735,92045	1,48019965	0,27474308	5,3438772	9,1E-08	0,00000388
AL645939.6	104,812089	1,27138805	0,23475732	5,33692254	9,45E-08	0,00000396
RPP25	367,831914	1,47390104	0,27692459	5,32409178	1,01E-07	0,00000418
HDAC9	472,156038	1,47723458	0,28428291	5,3202141	1,04E-07	0,00000427
RPS6KL1	45,2426705	1,51043197	0,28647669	5,30331887	1,14E-07	0,00000452
C17orf58	532,027454	1,01441751	0,19141547	5,30104302	1,15E-07	0,00000454
SLC43A3	1071,36813	1,13990933	0,21499718	5,27929886	0,00000013	0,00000504
SNORD3A	146,092244	1,13049166	0,21362145	5,27808359	1,31E-07	0,00000506
MTFP1	1468,3341	1,157943	0,21913998	5,27760423	1,31E-07	0,00000506
ZNRF1	3820,68738	1,16731492	0,2234197	5,2740492	1,33E-07	0,00000511
PPFIA3	324,368873	1,55820112	0,29729033	5,27239408	1,35E-07	0,00000515
UCK2	2079,14805	1,13481736	0,21676644	5,22789662	1,71E-07	0,00000624
AC011447.3	57,3186488	1,11553004	0,21343931	5,22242262	1,77E-07	0,00000638
HASPIN	554,208648	1,68583729	0,32282234	5,21282615	1,86E-07	0,00000661
ULBP1	149,625849	1,51410848	0,29748326	5,21097287	1,88E-07	0,00000665
SLC6A9	187,199925	1,70333199	0,33635265	5,18997273	0,00000021	0,00000735
RPA3	1636,73819	1,00311422	0,19366157	5,1705612	2,33E-07	0,00000798
ARHGAP11B	278,264429	1,20368016	0,23432331	5,16331317	2,43E-07	0,00000825
CHAC1	481,352013	1,23939592	0,24088971	5,14265704	2,71E-07	0,00000902
CDK4	3873,60144	1,11749802	0,21784441	5,12557997	2,97E-07	0,00000971
LRR1	920,996475	1,0036983	0,19575589	5,12386851	2,99E-07	0,00000974

AP1S1	105,27595	1,0450958	0,20455019	5,11990457	3,06E-07	0,00000991
SHMT2	9828,60995	1,1975069	0,23618809	5,10614466	3,29E-07	0,0000106
DNAJC9	3188,18598	1,22199097	0,2387053	5,09616228	3,47E-07	0,000011
PSMC3IP	350,481732	1,4581779	0,28547784	5,0661719	4,06E-07	0,0000126
GLP1R	296,527103	1,49740713	0,30734149	5,03306041	4,83E-07	0,0000144
ICAM1	6843,1637	1,04169586	0,20732701	5,03330967	4,82E-07	0,0000144
TCF19	2640,77586	1,43029337	0,2832562	5,02682044	4,99E-07	0,0000147
CHPF	3180,3357	1,13623626	0,22647359	5,02329478	5,08E-07	0,000015
UACA	101,587297	1,28522978	0,25825954	4,97927636	6,38E-07	0,0000182
HPSE	246,082026	1,25104351	0,24899855	4,96053958	7,03E-07	0,0000195
ZNF90	85,3883948	1,07121192	0,21718338	4,94198616	7,73E-07	0,0000211
CHD5	40,98575	2,40552677	0,45150459	4,93564678	7,99E-07	0,0000216
STEAP1	38,756016	1,82746385	0,36064085	4,92882198	8,27E-07	0,000022
TMEM97	753,470908	1,44688755	0,29464752	4,9218635	8,57E-07	0,0000226
PCNA	5824,63591	1,46770467	0,29734296	4,91964178	8,67E-07	0,0000228
DHCR7	2278,22228	1,01502954	0,20677943	4,91212558	9,01E-07	0,0000235
ATAD2	3755,90616	1,2943769	0,26245318	4,90082092	9,54E-07	0,0000246
CKS2	1407,44132	1,31951811	0,26895317	4,89882553	9,64E-07	0,0000248
DSN1	1613,8097	1,02750526	0,21012032	4,88135299	0,00000105	0,0000265
POLD3	1710,94088	1,00011605	0,20433338	4,88106533	0,00000106	0,0000265
PHLDA3	77,6403238	1,36984895	0,27522185	4,87388501	0,00000109	0,0000272
AC079209.1	41,3225837	1,8582839	0,39181777	4,86322585	0,00000115	0,0000283
CENPP	386,136493	1,29006839	0,26693782	4,84742954	0,00000125	0,0000303
PKM	24972,8962	1,13454211	0,23415197	4,83603007	0,00000132	0,0000319
TESC	1490,65093	1,18929034	0,2514482	4,83364582	0,00000134	0,0000322
MCM6	4653,0983	1,57235012	0,32417432	4,82704181	0,00000139	0,000033
MSH6	3869,11953	1,05660942	0,21928593	4,80328372	0,00000156	0,0000361
RFC4	1553,09963	1,031953	0,21441157	4,78866885	0,00000168	0,0000385

CHAF1A	2149,62629	1,28745474	0,27220876	4,72032172	0,00000235	0,0000514
H2AC13	51,7125691	1,05210436	0,22268468	4,70945125	0,00000248	0,0000537
SPTY2D1OS	42,2396604	1,29347563	0,27513413	4,70686789	0,00000252	0,0000543
GPT2	960,333544	1,47635661	0,30788401	4,69049231	0,00000273	0,0000578
MCM4	6340,26235	1,63367485	0,34634032	4,68454086	0,00000281	0,0000591
NDC80	776,695235	1,29472715	0,27563296	4,67623182	0,00000292	0,000061
GMNN	934,054353	1,43630881	0,30640338	4,67506347	0,00000294	0,0000613
E2F2	802,219169	1,65972969	0,3514081	4,66431846	0,0000031	0,0000641
P2RX5-TAX1BP3	1342,34518	1,52101022	0,3204367	4,66403728	0,0000031	0,0000642
PDLIM1	1187,47176	1,03361573	0,2224437	4,6617062	0,00000314	0,0000647
TMEM154	574,226839	1,00573313	0,2150905	4,64587086	0,00000339	0,0000683
CHEK1	825,482205	1,73191025	0,36953111	4,64538288	0,00000339	0,0000683
TREML2	235,222525	1,45436846	0,32219791	4,64228458	0,00000345	0,000069
EME1	314,144262	1,57289255	0,33841067	4,62692244	0,00000371	0,0000731
MCM2	8689,40083	1,61040092	0,34557034	4,62485422	0,00000375	0,0000737
GINS2	1161,25528	1,8507702	0,39762798	4,61960492	0,00000384	0,0000753
ORC6	684,604254	1,57585647	0,33937762	4,61923814	0,00000385	0,0000753
EPOP	77,9565208	1,32065759	0,28593559	4,61440444	0,00000394	0,0000769
POLD2	3182,659	1,11906962	0,24281803	4,60941177	0,00000404	0,0000783
TMPO-AS1	283,780703	1,71981254	0,36963206	4,59856749	0,00000425	0,0000818
IFNG	6396,66052	1,45636818	0,31848246	4,58996006	0,00000443	0,0000847
RASD1	606,327077	1,55681397	0,33333489	4,58647174	0,00000451	0,0000858
DHCR24	7856,12245	1,11331258	0,24256226	4,58412271	0,00000456	0,0000863
TFDP1	4133,97738	1,30153913	0,28366491	4,57958167	0,00000466	0,0000877
AP1S3	369,438627	1,3518563	0,29480193	4,56559322	0,00000498	0,0000928
BRCA1	930,477735	1,39840757	0,30518946	4,56130619	0,00000508	0,0000945
SLC7A1	2343,405	1,10529514	0,24349859	4,55492539	0,00000524	0,0000965
DHFR	2151,02731	1,55704343	0,33954021	4,5402195	0,00000562	0,00010235

ATAD5	805,154511	1,10530992	0,2427956	4,53887588	0,00000566	0,00010269
RANP1	16,5239844	1,32440045	0,28989191	4,51475446	0,00000634	0,00011231
BZW1P2	22,4181532	1,12484237	0,47018698	4,51482661	0,00000634	0,00011231
S100P	87,3413641	1,82640658	0,3899944	4,51308538	0,00000639	0,00011298
STAP2	242,784642	1,09058482	0,24286098	4,51313801	0,00000639	0,00011298
FEN1	5528,4219	1,38578289	0,30732008	4,49108564	0,00000709	0,00012315
E2F1	2025,17146	1,7319208	0,38283899	4,47953839	0,00000748	0,00012825
AMOTL1	496,757411	1,02596064	0,22667277	4,47446543	0,00000766	0,00013064
TUBAP2	38,2765439	1,53130728	0,34105492	4,47299192	0,00000771	0,00013098
DTYMK	2010,46089	1,02403528	0,22848002	4,47138208	0,00000777	0,00013168
NET1	461,954204	1,2765251	0,28560765	4,46968512	0,00000783	0,00013211
CENPN	782,642068	1,53981103	0,34494963	4,46054026	0,00000818	0,00013737
AC092718.4	63,7504111	1,4920021	0,33635788	4,45531478	0,00000838	0,0001405
LIPG	5,89092867	1,93128208	0,49660756	4,42884074	0,00000947	0,00015449
AC002116.2	25,5918918	1,19588495	0,27276091	4,41044863	0,0000103	0,00016411
UNG	2362,03983	1,32634165	0,29975081	4,40692892	0,0000105	0,00016607
RAD54B	266,75802	1,16939837	0,26554358	4,39421167	0,0000111	0,00017323
ORC1	691,813587	1,6666604	0,37795945	4,3824423	0,0000117	0,0001824
WDR62	766,596341	1,45094258	0,33134967	4,37240158	0,0000123	0,00018922
CLSPN	1091,54245	1,67501566	0,38017063	4,36742483	0,0000126	0,00019244
SCD	1345,74203	1,35532059	0,31142925	4,35959145	0,000013	0,00019813
WWC1	9,77638638	1,85241231	0,49501321	4,3548267	0,0000133	0,00020148
SOCS1	5198,56908	1,17308325	0,27012128	4,34892689	0,0000137	0,00020477
RNF157	4457,47736	1,28354426	0,30207097	4,33638366	0,0000145	0,00021452
TUBBP1	8,52784924	1,50653657	0,34610586	4,33590032	0,0000145	0,00021481
FANCI	4280,14541	1,21764881	0,28034532	4,33554899	0,0000145	0,00021498
AC125611.3	368,112707	1,08632677	0,25174306	4,32071096	0,0000156	0,00022702
CHAF1B	957,421705	1,31490521	0,30328712	4,31485291	0,000016	0,00023054

BAG2	521,183057	1,05120342	0,24327784	4,30709311	0,0000165	0,00023727
CENPH	576,293978	1,26026279	0,2925594	4,30587288	0,0000166	0,0002382
DUT	4484,90877	1,2199032	0,28278906	4,29737298	0,0000173	0,00024617
EIF4EBP1	1072,5969	1,20523444	0,27988365	4,29345192	0,0000176	0,00024939
FOXRED2	439,287578	1,4681439	0,34151791	4,2862685	0,0000182	0,00025619
ASRGL1	198,029471	1,164368	0,2682786	4,28482002	0,0000183	0,00025747
PIR	89,949917	1,65357487	0,38886029	4,27296141	0,0000193	0,00026824
AL353135.2	14,2246629	1,62814621	0,3797066	4,26149956	0,0000203	0,00027982
ADM2	283,026873	1,01121121	0,23931693	4,24890986	0,0000215	0,0002927
PSPH	366,751383	1,07179051	0,25048272	4,23729156	0,0000226	0,00030506
GGH	647,481775	1,55943325	0,36825399	4,23288001	0,0000231	0,00031019
LRP8	2217,7952	1,10561244	0,26234263	4,22833651	0,0000235	0,00031513
TLCD3A	160,982726	1,43553228	0,33736838	4,22412508	0,000024	0,00031955
SMTN	270,762571	1,126887	0,26663656	4,22404222	0,000024	0,00031955
TUBA1B	60578,4037	1,20501359	0,2849711	4,22013433	0,0000244	0,00032373
KNTC1	1787,11296	1,11477093	0,26350939	4,22033697	0,0000244	0,00032373
KIF18A	417,335287	1,31788213	0,31169429	4,21896245	0,0000245	0,0003247
AC004816.1	14,306372	1,65661376	0,39618463	4,202751	0,0000264	0,0003456
MASTL	864,89905	1,02468418	0,24379548	4,18746011	0,0000282	0,00036448
DTL	2028,0242	1,63425478	0,39575132	4,13128745	0,0000361	0,00044953
POLE2	409,461561	1,53115468	0,369125	4,1304297	0,0000362	0,00044998
TUBG1	2203,28342	1,02452182	0,24807305	4,1290456	0,0000364	0,00045177
FAM72A	220,279994	1,15187674	0,28159687	4,12615562	0,0000369	0,00045593
TEDC2	431,086628	1,64867763	0,39625457	4,12497492	0,0000371	0,00045766
P2RX5	22126,469	1,50476936	0,3635052	4,12437677	0,0000372	0,00045823
ARHGAP11A	1730,04945	1,25299442	0,30371393	4,12055956	0,0000378	0,00046369
CDC25A	699,947246	1,81511065	0,44397787	4,10696078	0,0000401	0,00048693
EXO1	713,754785	1,68789674	0,40358897	4,10386851	0,0000406	0,00049185

TUBA1C	3622,14416	1,09707551	0,26829002	4,09586354	0,0000421	0,0005068
B4GALNT1	10,60565	2,15643736	0,49332317	4,08855336	0,0000434	0,00052029
CCDC74B	104,686214	1,11756828	0,31918457	4,08830793	0,0000435	0,00052045
EZH2	1948,0683	1,01462741	0,24857909	4,0719677	0,0000466	0,00054938
AC007952.4	77,532975	1,16366725	0,29066496	4,06902588	0,0000472	0,00055426
DCTPP1	2597,99471	1,11717813	0,27411709	4,0667583	0,0000477	0,00055819
WARS1	28550,4623	1,08044355	0,26992507	4,0640571	0,0000482	0,00056313
BRI3BP	1335,34543	1,04957051	0,25882536	4,04814242	0,0000516	0,00059269
CDC6	1410,79264	1,63800346	0,40051232	4,04157944	0,0000531	0,00060609
HMGA1	1798,81596	1,24562942	0,30887844	4,02957083	0,0000559	0,00062962
RFC3	814,51005	1,30070738	0,32074068	4,02572616	0,0000568	0,00063763
CKS1B	1636,82627	1,22029692	0,30256448	4,02273939	0,0000575	0,00064432
HELLS	911,07159	1,542782	0,38126874	4,01564698	0,0000593	0,00066022
TCAM1P	25,8600269	1,02260311	0,47239221	4,01360092	0,0000598	0,00066435
EDA2R	20,8387114	1,82530656	0,43498774	4,00318835	0,0000625	0,00069177
HMGB2	8675,27654	1,00558925	0,251096	4,00110979	0,000063	0,00069702
CDT1	1368,90856	1,61856181	0,40331384	3,97889661	0,0000692	0,00075425
FZD5	41,668731	1,30012035	0,32147065	3,97882493	0,0000693	0,00075425
CENPU	902,785896	1,51007305	0,38358497	3,96187243	0,0000744	0,00080079
RAD51AP1	490,051196	1,42774933	0,35682273	3,9615959	0,0000745	0,00080124
PAQR4	1091,18271	1,35723293	0,34178392	3,95995024	0,000075	0,00080489
HDC	30,8090943	2,33219202	0,48736777	3,9581798	0,0000755	0,0008096
POLR3G	120,074947	1,3316655	0,33481957	3,95740384	0,0000758	0,00081112
XRCC2	370,305949	1,40619326	0,35690285	3,94694686	0,0000792	0,0008401
UBE2T	901,685734	1,49786465	0,37668238	3,94064866	0,0000813	0,00085717
AC011511.5	26,6442249	1,29618176	0,33396073	3,94086894	0,0000812	0,00085717
DONSON	949,085575	1,09582141	0,27649212	3,94055698	0,0000813	0,00085717
CDKN1A	2383,78661	1,5829976	0,40776128	3,94030883	0,0000814	0,00085756



MTRF2	320,179139	1,39504069	0,35070288	3,93936383	0,0000817	0,00085995
ELFN1-AS1	19,3850066	1,67751136	0,42667302	3,92620031	0,0000863	0,00089647
PHGDH	1154,77976	1,46015007	0,36922628	3,92534315	0,0000866	0,00089916
THSD8	364,425606	1,19026425	0,30716875	3,92487223	0,0000868	0,0009004
STIL	666,799496	1,43452061	0,36383328	3,92234751	0,0000877	0,00090731
ATF3	271,145157	1,08657261	0,28875395	3,91571619	0,0000901	0,00092945
ERCC6L	216,289004	1,44576283	0,36810749	3,91545896	0,0000902	0,00092992
TTK	499,963879	1,57513627	0,39649228	3,91459441	0,0000906	0,00093175
SLC35G1	79,1619926	1,16368713	0,29752546	3,91427716	0,0000907	0,00093185
TIPIN	806,5352	1,15405363	0,29753383	3,89998518	0,0000962	0,00097923
RAB3D	1128,09782	1,01239501	0,26084934	3,89714692	0,0000973	0,00098867
HSF5	107,39634	1,25325507	0,32196466	3,89543348	0,000098	0,00099502

1305

1306

1307 **Table 2.** List of ST2<sup>-</sup> CD56<sup>bright</sup> NK cell signature genes used in this study, Related to Fig. 3C,D,F,G

1308

gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
MARCKSL1	2140,369762	1,740882999	0,116707873	14,91550027	2,61E-50	4,39E-47
SGSM3	8319,280225	1,301010684	0,099219228	13,1115542	2,83E-39	2,71E-36
TMEM123	8464,060835	1,625281824	0,129219167	12,58132481	2,68E-36	2,16E-33
LRRC75B	456,6176087	2,416210354	0,198847881	12,15071388	5,69E-34	3,70E-31
EVA1B	263,1092393	2,229323471	0,188111393	11,85127264	2,12E-32	1,16E-29
IL4R	3450,852494	1,499947728	0,126745269	11,84412664	2,31E-32	1,22E-29
CYSLTR1	649,5300538	1,515430844	0,129159975	11,73268544	8,67E-32	4,26E-29
MAN1C1	565,7106081	3,237435298	0,286233564	11,29381958	1,41E-29	6,17E-27
ANKRD13C	2134,558285	1,002724262	0,089928491	11,15065986	7,11E-29	2,87E-26
P2RX4	2152,637806	1,272120915	0,115924558	10,97495534	5,04E-28	1,92E-25
CD82	977,0473825	2,847624791	0,264383983	10,78862923	3,90E-27	1,38E-24
PPARD	1041,824462	1,179517604	0,111025009	10,62431354	2,30E-26	7,85E-24
CD44	17644,69997	2,466187162	0,235781727	10,46120997	1,30E-25	4,37E-23
CACNB1	672,7485259	1,620194479	0,155622145	10,41380983	2,14E-25	7,09E-23
AKTIP	1505,369451	1,445792576	0,139440905	10,37395255	3,26E-25	1,06E-22
FUCA1	2045,337861	1,842417316	0,17993438	10,23962265	1,32E-24	3,97E-22
LINC00996	319,627706	2,609455123	0,256710702	10,15786989	3,06E-24	8,56E-22
FXYS5	4272,477726	1,196803035	0,121577711	9,843066925	7,34E-23	1,85E-20
AFAP1L2	218,6357125	3,082245531	0,312139865	9,810966072	1,01E-22	2,51E-20
RAB38	243,3177113	3,055826055	0,313133124	9,801295845	1,11E-22	2,70E-20
AHI1	1559,477129	2,477023515	0,255683885	9,685122861	3,49E-22	7,81E-20
SOX4	196,001897	2,237665613	0,23699926	9,444790764	3,56E-21	7,25E-19
KIAA1211L	88,75908207	2,71897675	0,294775003	9,213852772	3,15E-20	5,97E-18
IGFLR1	3854,076961	1,143615923	0,124148381	9,209966716	3,26E-20	6,09E-18
TLE3	1567,104006	2,590685551	0,282744437	9,166413642	4,89E-20	8,80E-18

RARA	600,9572647	1,318907813	0,145484053	9,062144833	1,28E-19	2,22E-17
STBD1	219,2555171	1,822615346	0,209614914	8,717454048	2,85E-18	4,41E-16
FUT8-AS1	67,90486061	2,855822847	0,328357416	8,695225091	3,46E-18	5,29E-16
MMP25	1398,742723	1,40354112	0,162428028	8,639506398	5,65E-18	8,43E-16
FAM102A	2599,12843	1,914468847	0,222371703	8,607765357	7,45E-18	1,09E-15
AL034397.3	264,4978444	2,197245925	0,256021938	8,601655494	7,86E-18	1,14E-15
TMEM200A	142,3148917	2,89847412	0,337566616	8,571643818	1,02E-17	1,47E-15
KIR2DL4	5176,736346	3,205020743	0,372271606	8,56145511	1,11E-17	1,56E-15
SPTBN1	5050,407322	1,454201938	0,169895179	8,562040027	1,11E-17	1,56E-15
GRAMD4	1739,584588	1,256608365	0,148997408	8,426004572	3,58E-17	4,74E-15
AXIN2	122,846397	4,198344719	0,496186938	8,424218323	3,63E-17	4,79E-15
TRAF5	5084,644085	1,264733035	0,151119937	8,371710643	5,68E-17	7,29E-15
AC136475.3	181,4399858	3,322393183	0,393086018	8,331814546	7,96E-17	1,00E-14
AC008691.1	15,3365338	3,974901787	0,471562234	8,271214677	1,33E-16	1,61E-14
PRAM1	324,8694184	3,248314446	0,39599038	8,151417564	3,60E-16	4,12E-14
FOXC1	42,81840529	4,257531481	0,513555488	8,146888353	3,73E-16	4,25E-14
FUT8	857,1317759	2,256320072	0,277545555	8,121125486	4,62E-16	5,20E-14
AREG	49,15236748	2,404981214	0,298520266	8,0837208	6,28E-16	7,04E-14
ANKRD6	380,9412248	1,408207328	0,176085911	8,082307646	6,36E-16	7,08E-14
HVCN1	1192,528257	2,419885321	0,301033606	8,025608906	1,01E-15	1,09E-13
MPZL3	2867,960136	1,96187017	0,244903273	8,005583963	1,19E-15	1,27E-13
LRFN1	903,3538527	1,46235046	0,183524361	7,969598699	1,59E-15	1,66E-13
BACH2	1055,397026	2,043278573	0,256745168	7,956655052	1,77E-15	1,83E-13
IFITM10	79,33410328	2,819037525	0,358126867	7,908149319	2,61E-15	2,67E-13
LMNA	764,4263779	3,588425802	0,460469059	7,795625921	6,41E-15	6,37E-13
MAML2	405,3947041	1,969330901	0,254612167	7,733434442	1,05E-14	1,01E-12
TIAM1	2217,418454	3,446940657	0,443312808	7,712998259	1,23E-14	1,18E-12
LDLRAD2	37,78263563	2,864315899	0,37090984	7,706069704	1,30E-14	1,23E-12

PRCD	141,6670188	2,886854421	0,384456276	7,657090385	1,90E-14	1,76E-12
KLHL6	2736,886324	1,449384166	0,189438168	7,656948369	1,90E-14	1,76E-12
ZFH3	162,5904485	2,099702068	0,274363937	7,648660179	2,03E-14	1,87E-12
FLNB	1219,316738	2,293855562	0,300509328	7,632018925	2,31E-14	2,10E-12
C19orf38	314,3637498	2,865738545	0,381649932	7,60950385	2,75E-14	2,46E-12
TEC	310,8560866	2,442201965	0,320801105	7,608939573	2,76E-14	2,46E-12
DOK7	143,5755317	2,752814433	0,364946495	7,596460325	3,04E-14	2,68E-12
LDOC1	547,4136479	1,203333137	0,159103248	7,587139728	3,27E-14	2,85E-12
IER5L	207,4254029	3,928176017	0,512131998	7,561144111	4,00E-14	3,43E-12
STARD10	965,4029696	1,517324896	0,202110986	7,524072375	5,31E-14	4,52E-12
CAPN12	1574,643552	1,986846274	0,264452466	7,514838013	5,70E-14	4,81E-12
RRM2P3	33,88671537	1,906501022	0,255080085	7,514772312	5,70E-14	4,81E-12
PLK2	98,37650677	2,344139862	0,312635658	7,475686672	7,68E-14	6,37E-12
PRPF40B	405,9369242	1,255426299	0,168137217	7,466021351	8,27E-14	6,83E-12
IL7R	1849,842269	3,752113699	0,497696008	7,41606311	1,21E-13	9,85E-12
FAAH	346,5778786	1,680102357	0,22747979	7,382156404	1,56E-13	1,24E-11
TNFSF9	132,6527701	3,637122844	0,475030088	7,330987379	2,28E-13	1,78E-11
MBOAT7	1550,633095	1,332621699	0,182816461	7,288449341	3,14E-13	2,39E-11
DST	209,6756475	1,816584497	0,250223788	7,282464592	3,28E-13	2,48E-11
ZBTB46	128,1886131	3,641740392	0,496162257	7,228772817	4,87E-13	3,61E-11
MAFF	2257,925472	1,879053686	0,260093808	7,224527248	5,03E-13	3,70E-11
ZNF217	1450,119102	1,287753617	0,178824246	7,201997933	5,93E-13	4,32E-11
UNC93B1	3043,964328	1,750329949	0,243608534	7,184379072	6,75E-13	4,90E-11
TPCN1	1699,033407	1,53151534	0,213456563	7,17768457	7,09E-13	5,11E-11
ZNF296	727,0590777	1,305088768	0,182369891	7,156348565	8,29E-13	5,90E-11
PLEKHN1	206,4025607	2,085181376	0,292403136	7,122297134	1,06E-12	7,46E-11
SMG6	1585,486005	1,057027036	0,148854315	7,10138665	1,24E-12	8,59E-11
XYLT1	1465,872637	1,156545617	0,163384884	7,077618012	1,47E-12	1,01E-10

SLC39A10	1623,403389	1,212661365	0,173634103	6,988704433	2,77E-12	1,87E-10
HID1	39,66821395	2,205264018	0,320151044	6,966474531	3,25E-12	2,16E-10
RRAD	58,48146211	2,953436907	0,428687381	6,90543153	5,01E-12	3,28E-10
MPG	2611,401069	1,003775495	0,145291907	6,904810892	5,03E-12	3,28E-10
FBXL16	35,19675056	3,730713076	0,511691174	6,896414366	5,33E-12	3,47E-10
PLPP1	233,9239877	1,219327306	0,178811692	6,843240224	7,74E-12	4,92E-10
PLXNA4	961,9891073	2,158678049	0,318267672	6,83276983	8,33E-12	5,26E-10
TBXT	24,26215193	5,06557221	0,664143384	6,814910581	9,43E-12	5,87E-10
METTL24	29,66113697	2,986686143	0,454225853	6,799242063	1,05E-11	6,45E-10
GPR68	3491,357851	1,549838753	0,22953245	6,781940691	1,19E-11	7,20E-10
SNX30	333,4991655	1,840690351	0,272887511	6,764333072	1,34E-11	8,09E-10
PDE6G	738,1239368	1,855810087	0,275709691	6,751372835	1,46E-11	8,76E-10
AL158211.5	55,05019248	3,18301925	0,471540867	6,699949582	2,08E-11	1,22E-09
THEM4	1339,204355	1,240865036	0,187320759	6,627140101	3,42E-11	1,94E-09
GATA6	148,983322	3,806964818	0,557597993	6,618724163	3,62E-11	2,05E-09
SPIN3	579,5682663	2,150287743	0,325929848	6,604060126	4,00E-11	2,23E-09
GOLGA6L10	65,06390646	1,759747802	0,268302629	6,598321389	4,16E-11	2,31E-09
GRAMD1B	353,2229995	2,83284927	0,428341998	6,586356155	4,51E-11	2,49E-09
CD300C	662,9534538	2,141666401	0,329522641	6,574886891	4,87E-11	2,67E-09
S100A13	51,69556697	1,57700331	0,241041752	6,54188058	6,08E-11	3,27E-09
EXT1	752,9336282	1,058061661	0,162404986	6,516422519	7,20E-11	3,81E-09
SH3D21	63,91059322	2,212082091	0,339848211	6,508575435	7,59E-11	4,00E-09
ERCC6	1325,076914	1,196918771	0,184243302	6,498806178	8,10E-11	4,24E-09
SUOX	514,9009048	1,922142601	0,295842521	6,492744365	8,43E-11	4,39E-09
IRF2BPL	951,7592032	1,293991659	0,199419253	6,490984681	8,53E-11	4,43E-09
AL157935.2	12,34158825	2,475195319	0,383015829	6,451818604	1,11E-10	5,67E-09
GPR35	331,9485478	1,26233446	0,196491366	6,414399391	1,41E-10	7,11E-09
ERMP1	6432,990792	1,050931817	0,164670882	6,400523906	1,55E-10	7,69E-09

PPP1R9A	493,7632895	1,981004521	0,309699355	6,391466414	1,64E-10	8,14E-09
TAF9B	820,1818757	1,285949427	0,201407611	6,384789994	1,72E-10	8,42E-09
LRP5	94,50272901	3,172582403	0,489045222	6,380459222	1,77E-10	8,61E-09
ITGAX	12415,82202	2,079106969	0,329943746	6,35380944	2,10E-10	1,01E-08
KLRC2	726,1948602	1,217769369	0,191269433	6,347808523	2,18E-10	1,04E-08
ZNF629	237,397175	1,314223877	0,207922122	6,339709292	2,30E-10	1,09E-08
CXCR3	3176,952781	2,727739219	0,432006382	6,289496674	3,18E-10	1,47E-08
DND1P1	139,7377777	1,591147032	0,253169883	6,281714343	3,35E-10	1,54E-08
RNASE6	49,3304587	1,8336818	0,294251275	6,241170135	4,34E-10	1,95E-08
LINC02273	120,6854075	2,104712474	0,334746798	6,237198999	4,45E-10	2,00E-08
NOD2	627,9389415	1,373877227	0,221025473	6,236011813	4,49E-10	2,01E-08
ZEB1	804,8908063	1,574559748	0,252463737	6,234338719	4,54E-10	2,02E-08
LINC00271	60,35176693	2,032485041	0,326992021	6,22232025	4,90E-10	2,18E-08
COL4A4	136,6893954	2,877693674	0,464567299	6,190854914	5,98E-10	2,60E-08
RHOB	142,5630848	1,646035215	0,266532796	6,153458318	7,58E-10	3,23E-08
WNT11	357,6166372	2,750818924	0,447235458	6,140192481	8,24E-10	3,48E-08
ITM2C	313,5989235	3,078454925	0,495274515	6,138832445	8,31E-10	3,51E-08
PATJ	832,4500235	2,026383599	0,331901279	6,116181619	9,58E-10	3,98E-08
CLSTN3	2662,810035	1,451565636	0,237248483	6,116238957	9,58E-10	3,98E-08
SUPT3H	215,3559279	1,029318783	0,169361342	6,078184656	1,22E-09	4,95E-08
TP53I11	303,9506515	1,927634969	0,330736618	6,072075791	1,26E-09	5,13E-08
ITM2A	2462,385495	1,136864047	0,187341643	6,070902966	1,27E-09	5,16E-08
PI16	42,04003912	4,176725001	0,65065832	6,049442295	1,45E-09	5,84E-08
BEX4	1500,538493	1,086656664	0,180329332	6,028447297	1,66E-09	6,56E-08
RGS10	579,2833941	1,532265566	0,254414155	6,020657274	1,74E-09	6,81E-08
AC002383.1	10,8397303	4,32815078	0,680315877	6,015521954	1,79E-09	7,00E-08
CD55	3052,146888	1,09862975	0,18266267	6,015323622	1,80E-09	7,00E-08
BEX2	385,7628247	1,249224469	0,208923302	6,002445603	1,94E-09	7,52E-08

XIRP1	57,22071206	3,683841193	0,573027442	5,982503354	2,20E-09	8,41E-08
CDHR1	819,9741477	2,677331521	0,444707403	5,959096751	2,54E-09	9,51E-08
CRYBG3	125,5555099	2,063911456	0,35247776	5,942522032	2,81E-09	1,05E-07
RAB43	2036,021922	1,001743046	0,168646318	5,941980609	2,82E-09	1,05E-07
TMEM38A	82,82198594	3,210889069	0,538726068	5,937159296	2,90E-09	1,07E-07
ARMC5	1780,369751	1,389518757	0,234393229	5,927321137	3,08E-09	1,14E-07
CLEC12A-AS1	46,22362818	1,931710213	0,330869574	5,923534282	3,15E-09	1,16E-07
RELL1	724,4778869	1,283650735	0,216901888	5,921056996	3,20E-09	1,17E-07
ACVR2A	307,8483385	1,435989021	0,243605706	5,915557483	3,31E-09	1,21E-07
RCBTB2	3063,875493	2,182264742	0,368944091	5,907303203	3,48E-09	1,27E-07
AL022322.2	67,76634459	1,801975645	0,307307468	5,888891436	3,89E-09	1,41E-07
SCARF1	156,4142594	2,842376226	0,482660895	5,876623721	4,19E-09	1,50E-07
BGLAP	96,04635907	3,538994106	0,589813919	5,862238586	4,57E-09	1,63E-07
SV2A	48,2437807	2,445816786	0,44854443	5,852427352	4,84E-09	1,71E-07
SMIM10L2A	64,30658026	3,508776996	0,594553252	5,837677632	5,29E-09	1,87E-07
PLEC	2056,099146	1,861878277	0,320559179	5,836288724	5,34E-09	1,88E-07
FXD7	38,81660948	3,469733075	0,578446437	5,830312225	5,53E-09	1,94E-07
RAB3GAP1	663,7483876	1,089492619	0,187676911	5,809340557	6,27E-09	2,17E-07
EXD2	489,2651708	1,22891323	0,21183615	5,806800475	6,37E-09	2,20E-07
PRDM8	497,0459425	1,594431879	0,274486306	5,790720871	7,01E-09	2,41E-07
AL357033.4	101,5975193	1,590155519	0,27616053	5,765928866	8,12E-09	2,74E-07
LTC4S	42,46148258	2,210105354	0,382728867	5,760233058	8,40E-09	2,82E-07
COL9A2	666,9338283	2,977350103	0,517402243	5,751054742	8,87E-09	2,96E-07
AL031736.1	103,687593	1,950697715	0,33843021	5,742233571	9,34E-09	3,10E-07
GAB1	175,9057184	2,737081522	0,477591498	5,708355806	1,14E-08	3,73E-07
SSBP2	719,3021611	1,973183258	0,345798583	5,705182996	1,16E-08	3,79E-07
DEPDC7	126,9076612	2,000178345	0,352564952	5,698178526	1,21E-08	3,93E-07
AHR	1030,419847	2,406975171	0,421207467	5,693457474	1,24E-08	4,01E-07

PLCH2	1656,044125	1,331782742	0,23544833	5,659271744	1,52E-08	4,83E-07
AL132996.1	6,930815148	3,865822551	0,658816638	5,655974876	1,55E-08	4,91E-07
CRTAM	3258,918725	2,879944593	0,498895482	5,635140157	1,75E-08	5,49E-07
EPAS1	235,0594084	1,780244369	0,315446327	5,624532859	1,86E-08	5,82E-07
RPS6KA2	603,4370845	2,081404584	0,375350496	5,615442365	1,96E-08	6,13E-07
AC021066.1	445,2279295	1,806776785	0,322754978	5,588460676	2,29E-08	7,08E-07
SPECC1	1212,882897	1,008531761	0,180580378	5,582913532	2,37E-08	7,28E-07
MTDHP3	18,53111258	3,651885327	0,630130448	5,57811123	2,43E-08	7,47E-07
AL603783.1	43,40415723	2,919117033	0,516839812	5,577366312	2,44E-08	7,48E-07
NBPF15	523,6238388	1,003156505	0,179966039	5,577463011	2,44E-08	7,48E-07
AC073332.1	50,46757368	2,079539706	0,374584686	5,568107727	2,58E-08	7,88E-07
AC011445.1	149,0232011	1,448193495	0,261854134	5,539578792	3,03E-08	9,14E-07
TCF7	3711,66176	2,801294186	0,501319966	5,532091697	3,16E-08	9,52E-07
AL022341.2	64,00754303	2,406238912	0,436859003	5,525605382	3,28E-08	9,82E-07
NELL2	528,3413071	2,958904755	0,536983549	5,519567819	3,40E-08	1,01E-06
GPR155	584,2908913	1,362453331	0,246781698	5,518453369	3,42E-08	1,02E-06
GORASP1	727,874936	1,159369536	0,212809321	5,517578996	3,44E-08	1,02E-06
MAML3	227,3004088	2,910517687	0,520119301	5,507021751	3,65E-08	1,07E-06
NCKAP5L	557,8377929	1,019341234	0,185736999	5,495749561	3,89E-08	1,14E-06
NBPF9	286,8213266	1,104871219	0,201721668	5,487263097	4,08E-08	1,19E-06
AC068580.4	1237,365465	1,09636765	0,199802887	5,481917224	4,21E-08	1,22E-06
PRSS2	20,42490575	3,435662521	0,640356433	5,468191576	4,55E-08	1,31E-06
SEC22B4P	427,8475782	1,068157178	0,195364259	5,467566083	4,56E-08	1,32E-06
AC136475.9	40,38923353	2,683705638	0,492099609	5,467015518	4,58E-08	1,32E-06
KLHDC9	16,03763148	2,554000388	0,46130946	5,46690161	4,58E-08	1,32E-06
PLA2G6	1633,729643	2,025368206	0,371533287	5,451318684	5,00E-08	1,42E-06
NUDT16P1	132,0159874	2,394800836	0,440749668	5,433913468	5,51E-08	1,56E-06
IFITM3	7923,417658	1,451337304	0,267556571	5,43083565	5,61E-08	1,58E-06



MTM1	1010,089782	1,886289418	0,347656721	5,420132252	5,96E-08	1,68E-06
GPR82	337,6226796	2,635019703	0,481344315	5,419871453	5,96E-08	1,68E-06
ATP6V0E2-						
AS1	108,2815423	1,244807485	0,230542859	5,410393293	6,29E-08	1,76E-06
LINC01871	1830,467521	1,540975424	0,285096179	5,408546324	6,35E-08	1,78E-06
LSR	887,6666794	2,840944598	0,518173198	5,401460927	6,61E-08	1,85E-06
AL034550.2	33,02488769	3,172151524	0,559528571	5,395879874	6,82E-08	1,90E-06
AC092368.3	24,78169397	1,512806742	0,283628302	5,379991707	7,45E-08	2,06E-06
TPD52L1	51,71867641	2,422752867	0,455664353	5,373551856	7,72E-08	2,13E-06
CD2	9315,600078	1,454866474	0,272244595	5,363995817	8,14E-08	2,24E-06
PFKFB3	1945,504031	1,000897819	0,187849186	5,329454109	9,85E-08	2,65E-06
LINC02446	16,30915541	2,489644747	0,459313591	5,324837854	1,01E-07	2,72E-06
ANKDD1B	17,25372673	1,915061785	0,364217268	5,324244425	1,01E-07	2,72E-06
EPHX1	54,71961291	2,458322408	0,471892105	5,292055384	1,21E-07	3,18E-06
EPHA1	89,4450334	1,590839766	0,303668555	5,283878035	1,26E-07	3,32E-06
PALD1	72,54013307	2,564431066	0,484668735	5,283191913	1,27E-07	3,33E-06
GSN	11477,76579	1,080001767	0,204600157	5,282113632	1,28E-07	3,34E-06
SLC17A9	1020,722123	2,025156259	0,383167497	5,274721728	1,33E-07	3,48E-06
ZNF667	63,99101562	3,275472613	0,616486896	5,226061181	1,73E-07	4,42E-06
SPACA9	113,6159267	2,142499804	0,408667207	5,222418166	1,77E-07	4,50E-06
CSF2	167,7107889	3,291839501	0,596805233	5,214685992	1,84E-07	4,67E-06
ZNF471	57,49951783	2,226779851	0,434540854	5,214527878	1,84E-07	4,67E-06
ARMCX2	60,84843318	1,884167269	0,360777333	5,214744992	1,84E-07	4,67E-06
EGR3	43,79934598	2,259073472	0,432640676	5,206379511	1,93E-07	4,85E-06
TTC23	29,86479356	1,512348688	0,295236661	5,206164654	1,93E-07	4,85E-06
BEND5	33,96012583	2,096798976	0,408007954	5,199110676	2,00E-07	5,03E-06
TRPM8	20,34419262	3,04250246	0,678290192	5,195196907	2,05E-07	5,12E-06
WDR86-AS1	5,171232188	3,921026082	0,684325649	5,193465345	2,06E-07	5,16E-06

SLC49A3	485,7237538	1,312431718	0,253372909	5,184921568	2,16E-07	5,39E-06
TUBA8	95,46203126	2,550813663	0,490022933	5,170630572	2,33E-07	5,79E-06
RNF144A	1772,527623	1,289472324	0,250586361	5,162317942	2,44E-07	6,03E-06
SPRY2	473,2348813	2,107772018	0,408888309	5,147224675	2,64E-07	6,51E-06
DDIT4	5551,55676	1,362385866	0,26530604	5,144237245	2,69E-07	6,61E-06
SLC44A1	1742,706472	1,401151872	0,273392571	5,125205954	2,97E-07	7,23E-06
ASPH	249,8983469	1,797842619	0,35231292	5,105993649	3,29E-07	7,92E-06
AC009404.1	43,90260786	1,29603386	0,254206162	5,101933528	3,36E-07	8,08E-06
ENPP3	119,5988475	2,667883964	0,57024697	5,093649621	3,51E-07	8,39E-06
CD27	111,4046967	3,032675704	0,579396161	5,06209634	4,15E-07	9,84E-06
CPNE7	81,84795563	1,838326943	0,363674022	5,0473816	4,48E-07	1,05E-05
LINC02391	9,538284846	2,131988111	0,422294001	5,046154098	4,51E-07	1,06E-05
MFGE8	399,3689337	1,248535075	0,247873273	5,045848157	4,52E-07	1,06E-05
AC004687.3	18,53533533	3,779035129	0,68637623	5,04379158	4,56E-07	1,07E-05
SLC29A4	26,32821855	2,370306808	0,468206092	5,043496764	4,57E-07	1,07E-05
TRO	241,9601661	1,037435872	0,207147565	5,021933504	5,12E-07	1,19E-05
AC011298.1	12,69198312	3,717939806	0,684965986	5,015416738	5,29E-07	1,22E-05
AC136475.5	16,14527145	2,820416285	0,563897138	5,015266466	5,30E-07	1,22E-05
COX6A2	11,17509384	3,840578061	0,686505729	4,995326291	5,87E-07	1,35E-05
AC097658.2	6,571846465	2,389027138	0,470204318	4,983528216	6,24E-07	1,42E-05
PLCB4	8,966236706	3,114548233	0,662253761	4,977668738	6,44E-07	1,46E-05
FXVD2	28,56930801	3,374607679	0,679677749	4,973572964	6,57E-07	1,49E-05
MS4A1	103,7166229	2,651249066	0,544912467	4,960162463	7,04E-07	1,59E-05
SRGAP3	102,6996534	2,09325268	0,424847963	4,953356723	7,29E-07	1,64E-05
IL9RP3	32,18469003	2,965586928	0,614113692	4,945474988	7,60E-07	1,70E-05
EGR2	775,1497977	1,551361391	0,312263	4,943510388	7,67E-07	1,72E-05
CASS4	376,6299138	1,107652538	0,224036719	4,943290919	7,68E-07	1,72E-05
CAV1	12,3243057	3,000225407	0,669579558	4,936992385	7,93E-07	1,77E-05

SEPTIN11	3368,522987	1,230166792	0,249533059	4,93445316	8,04E-07	1,79E-05
DAPP1	453,4751878	1,810976987	0,378679342	4,933798851	8,06E-07	1,79E-05
KCTD9	622,9600217	1,554305672	0,315884338	4,932125087	8,13E-07	1,81E-05
NR4A3	60,41180688	2,390151939	0,482092819	4,931824622	8,15E-07	1,81E-05
AL390719.1	21,22682362	2,173717679	0,438728664	4,924569418	8,45E-07	1,87E-05
AC093890.1	84,29101585	1,850806298	0,37780216	4,919792993	8,66E-07	1,92E-05
LINC01862	165,8059183	3,407342196	0,647630517	4,918208601	8,73E-07	1,93E-05
NPTX2	11,95920836	2,674611464	0,647295525	4,903056914	9,44E-07	2,07E-05
AC246785.2	54,40084889	1,103043325	0,224853183	4,902309554	9,47E-07	2,07E-05
GNG7	32,13907297	2,162926917	0,455478684	4,901646418	9,50E-07	2,08E-05
FHL1	356,3105433	1,851953834	0,378469867	4,898587642	9,65E-07	2,11E-05
TNFSF11	290,4070602	2,662973414	0,535577669	4,898267525	9,67E-07	2,11E-05
E2F3	2431,09033	1,106357893	0,227053379	4,881546688	1,05E-06	2,27E-05
SERPINE1	24,51291118	3,069040041	0,611586793	4,879453656	1,06E-06	2,29E-05
AL512625.1	136,192134	1,160967664	0,239929247	4,87860271	1,07E-06	2,30E-05
LEF1	2008,647706	2,61189181	0,527151981	4,872815174	1,10E-06	2,36E-05
DCAF4	454,5373992	1,152808831	0,236887987	4,871929897	1,11E-06	2,37E-05
PIK3R6	267,3136241	2,728241743	0,551094203	4,869987304	1,12E-06	2,39E-05
TNFSF10	2868,030902	1,088892881	0,223705676	4,865157215	1,14E-06	2,45E-05
A1BG	168,3731151	1,775440392	0,365395825	4,855055251	1,20E-06	2,56E-05
AC016026.1	86,36717872	1,052261414	0,217943588	4,848636915	1,24E-06	2,64E-05
PGBD1	81,40353523	1,717651033	0,362680229	4,847121884	1,25E-06	2,65E-05
MMRN1	259,7084239	3,018811421	0,620565044	4,841825249	1,29E-06	2,72E-05
CCR2	145,1278363	2,860956803	0,574266949	4,83652106	1,32E-06	2,78E-05
RGS9BP	34,79056254	2,361203239	0,496212139	4,830697487	1,36E-06	2,86E-05
LGALS9C	706,5233468	1,509910795	0,322621982	4,828798788	1,37E-06	2,88E-05
SUCLG2-AS1	107,4804422	1,589844771	0,330428536	4,814550363	1,48E-06	3,07E-05
PGGHG	7170,992647	1,625079721	0,338907522	4,784349433	1,72E-06	3,54E-05

EFHC2	306,4969901	1,666384281	0,349250247	4,779806872	1,75E-06	3,61E-05
GNG4	13,38166738	3,909794942	0,683043142	4,773653198	1,81E-06	3,71E-05
UBE2Q2P2	175,2414379	1,404562422	0,295652145	4,76984627	1,84E-06	3,77E-05
TNFRSF10D	45,93736542	2,132218736	0,478037828	4,756439585	1,97E-06	4,02E-05
ZNF667-AS1	191,2023662	2,490988306	0,525674202	4,7365302	2,17E-06	4,38E-05
CUEDC1	35,54985212	2,292075626	0,481057487	4,724454283	2,31E-06	4,63E-05
EGLN3	44,92095041	2,24550515	0,479632739	4,711643955	2,46E-06	4,90E-05
INSC	48,77728206	3,098483111	0,623070967	4,709394843	2,48E-06	4,95E-05
EMILIN1	128,8480127	1,639857566	0,347426603	4,704790036	2,54E-06	5,05E-05
MAL	39,57340648	3,464877836	0,669770372	4,694105682	2,68E-06	5,29E-05
RORC	26,13664694	2,777760689	0,587674155	4,684977087	2,80E-06	5,50E-05
PTK2	351,4638039	2,477553128	0,521587879	4,684769133	2,80E-06	5,50E-05
GYPE	142,7763228	1,891528989	0,401385726	4,684246085	2,81E-06	5,51E-05
AGAP3	949,5526516	1,041453001	0,222716515	4,677427311	2,90E-06	5,67E-05
NLRP6	362,0418812	1,688567398	0,382816417	4,676545909	2,92E-06	5,69E-05
CCR3	57,93643764	2,803902646	0,582266843	4,674047945	2,95E-06	5,75E-05
MYO7A	33,55637115	3,737964548	0,684756816	4,66937877	3,02E-06	5,86E-05
NEIL1	1497,44199	1,453142161	0,310129545	4,667908927	3,04E-06	5,90E-05
ZMAT4	216,068965	2,640467911	0,558154412	4,643769643	3,42E-06	6,54E-05
GATA6-AS1	11,54308557	3,066840867	0,629454234	4,630479567	3,65E-06	6,93E-05
KLRC1	9685,022474	1,004146993	0,21694846	4,629659228	3,66E-06	6,95E-05
TNFSF4	139,7637985	2,011664798	0,427744978	4,626434948	3,72E-06	7,02E-05
JUP	58,15443641	2,526880336	0,546290366	4,616890407	3,90E-06	7,28E-05
BTBD6P1	302,4428418	2,666889986	0,563167772	4,614916427	3,93E-06	7,32E-05
KLHL29	33,08738174	1,946606948	0,416347485	4,61404557	3,95E-06	7,34E-05
LINC01619	61,33308047	2,086410116	0,459723481	4,610883455	4,01E-06	7,44E-05
PER2	289,3529778	1,149889389	0,249251779	4,609300045	4,04E-06	7,49E-05
NAB2	282,4119613	1,650115906	0,354901133	4,605926938	4,11E-06	7,58E-05

PDK4	62,19623696	2,612015818	0,625512614	4,597283482	4,28E-06	7,87E-05
OR2W3	8,537974039	2,71906961	0,575346458	4,588680942	4,46E-06	8,17E-05
RNA5-8SN2	83,66427081	1,424126424	0,309865244	4,576279252	4,73E-06	8,61E-05
TRIM47	230,944992	2,515587478	0,57857664	4,576015626	4,74E-06	8,62E-05
PDE9A	28,46432687	2,11835182	0,463611347	4,5757863	4,74E-06	8,62E-05
RAMP1	1416,771374	2,003743871	0,436861022	4,569071259	4,90E-06	8,87E-05
FES	14096,02622	1,218324888	0,267207028	4,565206498	4,99E-06	9,01E-05
AC012435.2	34,70171456	2,115485105	0,462083466	4,556526026	5,20E-06	9,35E-05
CAMK1	712,271817	1,318506905	0,289591149	4,541444188	5,59E-06	9,97E-05
CKMT2	21,75960717	2,975202182	0,64829	4,540229443	5,62E-06	0,000100095
CYP2E1	41,85003279	1,868522619	0,4155738	4,538337814	5,67E-06	0,000100908
MCF2L	98,84718277	2,428602121	0,531561162	4,537497838	5,69E-06	0,000101221
HDAC11	197,7692003	1,658304137	0,367992609	4,521049269	6,15E-06	0,000108744
AC092171.3	4,066207505	3,35422722	0,680565705	4,517883731	6,25E-06	0,000110189
LINC01232	60,31393813	1,043585838	0,231126021	4,512542166	6,41E-06	0,000112804
UBR5-AS1	44,37993739	1,718926174	0,381103323	4,504273936	6,66E-06	0,000116878
WNT6	5,052215367	3,331058752	0,675564228	4,500825894	6,77E-06	0,000118378
DIO3OS	25,74357413	3,117712453	0,653715159	4,496033198	6,92E-06	0,000120658
FNBP1L	19,56801807	2,777742174	0,598214518	4,494566007	6,97E-06	0,000121283
JHY	255,9646704	1,520655684	0,340394715	4,494675056	6,97E-06	0,000121283
XCL2	7701,308734	1,762187493	0,390203808	4,49211141	7,05E-06	0,000122479
CSTF2T	3840,28611	1,005275341	0,22378182	4,490371606	7,11E-06	0,000123377
PRKAR2B	149,6290659	2,645619867	0,579069628	4,480688106	7,44E-06	0,000128777
PLS3	37,18201753	2,874024246	0,664410669	4,478669708	7,51E-06	0,000129778
CITED4	54,10013354	1,04923707	0,233115122	4,465966524	7,97E-06	0,00013737
MGAT5B	72,42671081	2,704211638	0,596935983	4,462644629	8,10E-06	0,00013928
BMS1P1	120,6512927	1,155984636	0,261025163	4,453962399	8,43E-06	0,000144666
DOCK1	19,87347216	2,858351435	0,618826085	4,453308541	8,46E-06	0,000144984

MPV17L	18,52482589	2,933379987	0,630792433	4,438740939	9,05E-06	0,000153713
KANSL1L-AS1	57,80704568	1,175597107	0,265021626	4,438865951	9,04E-06	0,000153713
FAM117B	398,6916273	1,703561568	0,38370197	4,431446356	9,36E-06	0,00015834
AC009133.3	89,18313721	1,272998877	0,288556644	4,428772591	9,48E-06	0,000159779
AC008014.1	81,58722507	1,565423115	0,352980341	4,420221681	9,86E-06	0,00016568
PAOX	277,1754213	1,163805828	0,264060277	4,410719008	1,03E-05	0,000172546
P4HA2	62,19190001	2,074598291	0,470662803	4,403734272	1,06E-05	0,000177903
CCDC121	55,15150199	1,36373148	0,310334972	4,399393755	1,09E-05	0,000180899
IL3	7,263746933	3,032465126	0,685089311	4,39804176	1,09E-05	0,000181879
HOXA5	29,22251332	2,663573703	0,628071499	4,396650806	1,10E-05	0,000182746
TNFRSF11A	900,3745533	3,043200414	0,652565419	4,393640665	1,11E-05	0,000184991
PCNX2	244,8780474	1,353694323	0,30927685	4,37962019	1,19E-05	0,000196172
KRT81	219,3263906	1,852860789	0,420643076	4,376345734	1,21E-05	0,000198816
AC123912.4	57,7867272	3,215074261	0,670750072	4,370088695	1,24E-05	0,000203932
BMF	108,750818	1,85337081	0,424311083	4,354033382	1,34E-05	0,000217868
HSPG2	17,117014	2,187090022	0,677115263	4,335947056	1,45E-05	0,000235248
SLC5A2	15,49691469	2,370559637	0,57441768	4,324722237	1,53E-05	0,000247153
RASSF2	2129,258914	1,4305824	0,330369984	4,321751259	1,55E-05	0,000250104
CEP68	758,1683459	1,69136332	0,390381931	4,320330329	1,56E-05	0,000251116
NEK6	534,3137535	1,209174135	0,281884172	4,313087311	1,61E-05	0,000259282
AL136456.1	15,04858929	2,661349463	0,605572047	4,297893561	1,72E-05	0,000275504
CYGB	340,0955644	2,588938845	0,590623579	4,292018938	1,77E-05	0,000282226
UBASH3A	71,54453592	2,887247625	0,635371147	4,289809408	1,79E-05	0,000284823
CSPG4P12	36,1560947	1,837829876	0,435732667	4,287569921	1,81E-05	0,000287255
NFIX	60,68310508	2,522417396	0,573915819	4,28194832	1,85E-05	0,000293454
PECAM1	2770,661028	1,234226615	0,29372387	4,27280116	1,93E-05	0,000304801
TLL10	55,17994789	3,165533647	0,677545215	4,266103255	1,99E-05	0,000312867
CARD10	195,7946157	3,162507469	0,681788746	4,254657742	2,09E-05	0,000328161

BASP1	89,17467743	2,459015851	0,534617824	4,252059772	2,12E-05	0,000331342
AC093157.1	224,0533513	1,180037207	0,276519418	4,250369494	2,13E-05	0,000333594
AC079779.1	58,29398965	2,129901217	0,592652134	4,246268326	2,17E-05	0,000338968
WASIR2	8,636171076	2,893649754	0,687707799	4,245385515	2,18E-05	0,000339256
CELSR1	44,13666181	2,481443117	0,603597699	4,245644287	2,18E-05	0,000339256
PKIA	78,85937452	2,204573982	0,521138808	4,238829798	2,25E-05	0,00034824
PDGFA	21,16065015	2,465664476	0,576959244	4,220608974	2,44E-05	0,000376173
BEX5	89,58593359	1,367130389	0,322809632	4,216438277	2,48E-05	0,000382609
CLEC3B	9,930720348	2,837184486	0,656641233	4,211959984	2,53E-05	0,000389978
AK8	8,325888658	2,258884646	0,536740694	4,201875242	2,65E-05	0,000405598
AC005083.1	9,836329602	2,579657893	0,645084134	4,201039134	2,66E-05	0,00040679
INPP5F	829,2714252	1,197923774	0,28491769	4,190833732	2,78E-05	0,000423278
HOXA-AS3	29,76508842	2,251069243	0,535071714	4,188372622	2,81E-05	0,000427249
PDCD4	11931,50272	1,092133935	0,260805255	4,186306354	2,84E-05	0,000430181
FUT6	3,392327544	2,858222968	0,677162137	4,18386062	2,87E-05	0,000433859
AL022238.4	222,1358653	1,115870744	0,267411959	4,183134943	2,88E-05	0,00043492
CSPG4P10	50,37921758	1,015984266	0,242249925	4,177084633	2,95E-05	0,000445644
IGLV2-18	3,924084235	2,611510241	0,663150589	4,173288094	3,00E-05	0,000451786
TLCD2	26,04526924	1,633004101	0,410515131	4,169477262	3,05E-05	0,000458039
AC025164.2	773,1323373	1,464111102	0,353146935	4,16917934	3,06E-05	0,000458298
AC068775.1	884,7281123	1,09932535	0,260790028	4,165733973	3,10E-05	0,000463895
MVB12B	297,7737086	1,406102328	0,347569303	4,156290574	3,23E-05	0,000479572
TTC24	68,77921346	2,267286146	0,562949671	4,149431782	3,33E-05	0,000492719
EPS8	127,5161962	1,415747403	0,355555925	4,13439404	3,56E-05	0,000521907
CARD19	1476,40296	1,100074256	0,26648286	4,126729715	3,68E-05	0,000537257
PLD1	141,5092009	1,383133178	0,339080294	4,125699483	3,70E-05	0,000538894
RETREG1	94,16902923	2,07510904	0,512433511	4,114913381	3,87E-05	0,000560662
MCC	133,7813786	1,435245654	0,362675489	4,109445865	3,97E-05	0,000572868

STXBP1	166,6807384	1,773062233	0,430974083	4,105823205	4,03E-05	0,000580547
ZEB1-AS1	164,9176877	1,566477904	0,388516549	4,102143778	4,09E-05	0,000589145
NECTIN3	65,65558893	2,109821104	0,522511446	4,100453329	4,12E-05	0,000593043
BMP8B	158,3523606	1,797732771	0,428864179	4,094667742	4,23E-05	0,000605456
TMEM14C	657,2655348	1,810962691	0,442101686	4,077193222	4,56E-05	0,000647929
EPHA4	1050,413239	1,557123797	0,38537098	4,065706375	4,79E-05	0,000676663
FAM30A	12,43469716	2,62472664	0,687276262	4,064282968	4,82E-05	0,00068015
LGALS3BP	455,2933785	2,01736213	0,498440034	4,063485753	4,83E-05	0,000681702
CNR2	2219,199414	2,444235085	0,585082679	4,058692112	4,93E-05	0,000692456
ZNF843	15,55036673	2,364271664	0,592664421	4,057948375	4,95E-05	0,000693699
AC036176.1	50,40960634	1,284314447	0,317099019	4,055862318	4,99E-05	0,000698464
CYP4F22	115,5672067	1,023809783	0,254208061	4,05374212	5,04E-05	0,000703365
SIX4	5,032002761	2,972599722	0,687818501	4,049103353	5,14E-05	0,000714977
DTX1	44,33735311	2,597561359	0,615597976	4,038247269	5,39E-05	0,000744769
PCBP3	14,05993545	2,732766327	0,655409566	4,033752225	5,49E-05	0,000757091
COL15A1	46,51217042	2,389977593	0,542911588	4,032154516	5,53E-05	0,000761736
AL138828.1	4,740326734	2,687186184	0,658844736	4,030351437	5,57E-05	0,000767079
AL135818.2	33,98399152	1,492962774	0,370720041	4,027813163	5,63E-05	0,000774348
SERPINB9P1	37,81299154	1,718317668	0,425459062	4,022438429	5,76E-05	0,00079062
H3-2	22,33116103	1,607520491	0,393806175	4,018284767	5,86E-05	0,00080359
LTB	7685,961389	1,885747477	0,465054567	4,015437499	5,93E-05	0,000811148
MEST	60,3144696	1,50647934	0,379673843	4,006372315	6,17E-05	0,00083779
HOXA10	474,6676523	1,586323541	0,395748298	4,00277112	6,26E-05	0,000848934
KL	6,183304183	2,588017678	0,687354751	3,999147664	6,36E-05	0,000861015
SELL	61133,19386	1,046801587	0,263254611	3,99281777	6,53E-05	0,000881824
NR2F6	281,6955243	1,506947823	0,37795197	3,99255195	6,54E-05	0,000882222
RGS6	4,488837285	2,58082069	0,666501808	3,987713893	6,67E-05	0,000897999
YWHAEP7	38,3378469	2,636322159	0,645170748	3,984996126	6,75E-05	0,00090592



CHMP4C	34,73443287	2,848940037	0,671522152	3,970921553	7,16E-05	0,000954791
ABTB2	42,18544019	1,874409248	0,473912327	3,968552991	7,23E-05	0,000961785

1309

1310 **Table 3.** List of ST2<sup>-</sup> CD56<sup>dim</sup> NK cell signature genes used in this study, Related to Fig. 3C,D,F,G

1311

gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
FGFBP2	8144,153518	3,202150845	0,320967185	10,00234058	1,49E-23	9,84E-20
RNF166	8329,004271	1,572015459	0,157231451	10,01458146	1,32E-23	9,84E-20
RASGRP2	4442,914764	1,388044602	0,146010973	9,525583883	1,64E-21	6,51E-18
DPEP2	1526,11809	1,609829657	0,190756897	8,483359095	2,19E-17	4,34E-14
AL353622.1	297,1803946	1,685555006	0,200170725	8,443427493	3,08E-17	5,56E-14
KLF3	2317,609775	1,540112022	0,183942681	8,40503739	4,28E-17	7,07E-14
CEP78	4905,929088	1,044810306	0,126366368	8,259780569	1,46E-16	2,07E-13
LINC00944	44,76489599	3,21482747	0,372462803	8,19595732	2,49E-16	3,29E-13
DYRK1B	672,4335557	1,118315017	0,137280114	8,165574615	3,20E-16	3,86E-13
P2RY8	7887,303735	1,486780838	0,189003159	7,86287299	3,75E-15	3,24E-12
GPA33	161,1699077	2,418507998	0,327658938	7,546200536	4,48E-14	3,07E-11
AC245407.2	406,0213999	1,859932789	0,248929722	7,516379506	5,63E-14	3,72E-11
TMEM71	1574,466452	1,954352228	0,268749796	7,358825676	1,86E-13	1,12E-10
AL138824.1	129,6429159	2,422070604	0,336058541	7,33023171	2,30E-13	1,30E-10
LINC00861	3061,05769	1,53354884	0,210763177	7,323411026	2,42E-13	1,33E-10
KLF2	6970,676213	1,560628347	0,216566128	7,216426459	5,34E-13	2,71E-10
WAKMAR2	491,0159819	1,331281734	0,186211266	7,186350801	6,65E-13	3,30E-10
RASSF1-AS1	238,3238734	1,416845962	0,196731197	7,170698638	7,46E-13	3,52E-10
MCM3AP-AS1	326,1585037	1,138706429	0,161235728	7,053431474	1,75E-12	7,37E-10
AC005332.2	94,85373685	2,095310385	0,293433439	6,976915203	3,02E-12	1,11E-09
BISPR	1681,320381	1,060832931	0,152324166	6,960487766	3,39E-12	1,22E-09
SELPLG	21499,71541	1,283065741	0,185716133	6,934799516	4,07E-12	1,39E-09
OAS1	1195,66649	1,211755432	0,175809612	6,871013811	6,37E-12	2,07E-09
AL662844.4	967,2315141	1,207738148	0,177139872	6,840280625	7,90E-12	2,53E-09
IFIT2	1117,024786	2,133274979	0,315695154	6,834721634	8,22E-12	2,59E-09

RASSF1	11231,77473	1,301637387	0,192592714	6,758344733	1,40E-11	3,90E-09
STMN3	137,9011327	2,372036606	0,352935904	6,73337158	1,66E-11	4,44E-09
AC073130.3	171,1204581	1,663210743	0,247089371	6,720080459	1,82E-11	4,56E-09
HSBP1L1	213,0495397	1,096455411	0,163684666	6,690234449	2,23E-11	5,46E-09
KIFC3	745,2824955	2,12908314	0,321380577	6,679504561	2,40E-11	5,66E-09
AL592295.5	158,8162533	1,063282332	0,160343558	6,661930468	2,70E-11	6,16E-09
HEATR9	73,23745131	2,555095193	0,387866469	6,65222127	2,89E-11	6,36E-09
AC055839.2	298,5800799	1,540998324	0,233523327	6,636494986	3,21E-11	7,00E-09
NDRG1	1077,893796	1,288213421	0,195811703	6,60289876	4,03E-11	8,38E-09
PLEKHG3	8342,958982	1,360164232	0,206675366	6,586993772	4,49E-11	9,18E-09
PARP12	6641,277294	1,200871467	0,18278299	6,561481629	5,33E-11	1,07E-08
FGR	17120,1803	1,4607729	0,222708926	6,556003566	5,53E-11	1,09E-08
ZFH2-AS1	761,7303199	1,065192305	0,162302597	6,536673788	6,29E-11	1,19E-08
THRA	284,6004293	1,592549138	0,2455052	6,533669042	6,42E-11	1,20E-08
ICAM2	3207,579092	1,124983666	0,172111497	6,529980219	6,58E-11	1,22E-08
MHENCN	224,0159648	1,361939827	0,209491017	6,521826752	6,95E-11	1,28E-08
RENBP	199,7535366	1,483512111	0,229877225	6,484212226	8,92E-11	1,62E-08
TTC38	8039,041486	1,003443505	0,159254813	6,304267394	2,90E-10	4,54E-08
NATD1	671,0286088	1,052857164	0,167698493	6,275188997	3,49E-10	5,25E-08
CTSF	1165,042147	1,941891463	0,322774126	6,247305527	4,18E-10	6,09E-08
SFXN3	1817,137939	1,061585468	0,170287364	6,239116516	4,40E-10	6,35E-08
DLGAP1-AS1	330,5404792	1,279252449	0,205533663	6,233047148	4,57E-10	6,41E-08
TTLL3	2088,501966	1,21159376	0,194420136	6,232022848	4,60E-10	6,41E-08
AC244157.2	11,20080967	3,129269147	0,478217362	6,211872602	5,24E-10	6,97E-08
PATL2	4331,057083	2,073692218	0,339076125	6,142247184	8,14E-10	1,01E-07
SNAI3	591,4513799	1,664734223	0,274430177	6,141609469	8,17E-10	1,01E-07
ZMYND10	90,55930117	1,479130226	0,24144219	6,144421879	8,03E-10	1,01E-07
ZNF276	8281,915905	1,111608858	0,18130732	6,140495038	8,23E-10	1,01E-07

TRIM22	16223,92092	1,132720837	0,186423812	6,070675197	1,27E-09	1,47E-07
AL139246.5	210,2164121	1,289055593	0,214307842	6,02576554	1,68E-09	1,81E-07
BTN3A3	7879,327212	1,001665785	0,167620932	5,979652651	2,24E-09	2,27E-07
CCDC88C	5657,368226	1,023107223	0,171581502	5,974768528	2,30E-09	2,33E-07
SLAMF6	4918,979345	1,245100796	0,210034001	5,943449696	2,79E-09	2,74E-07
FCGR2A	84,56463711	2,695351865	0,468057246	5,934925165	2,94E-09	2,83E-07
CDC14B	109,090885	2,543603082	0,417798191	5,929046971	3,05E-09	2,91E-07
DEGS2	171,5630993	2,213052873	0,37606735	5,890150226	3,86E-09	3,54E-07
FAM13A-AS1	86,14944071	1,321665729	0,224337026	5,88294354	4,03E-09	3,60E-07
LINC002481	1047,622692	1,077615531	0,183488496	5,87767994	4,16E-09	3,65E-07
NLRP1	11216,96825	1,109626639	0,189979648	5,855860413	4,75E-09	4,06E-07
EFEMP2	692,2367593	1,341301299	0,231765649	5,815466941	6,05E-09	4,96E-07
AC006369.1	101,647403	1,990066631	0,35352843	5,813465678	6,12E-09	4,96E-07
TRGV10	614,0797893	1,416106431	0,241708205	5,811665992	6,19E-09	4,97E-07
PRR29	306,0053646	1,386872293	0,238445664	5,79664335	6,77E-09	5,35E-07
ZFP36L2	18485,19308	1,390293461	0,239873773	5,788011561	7,12E-09	5,58E-07
METTL7A	1308,623924	1,73695712	0,300854347	5,780965031	7,43E-09	5,80E-07
SEPTIN4	75,97567771	2,698194832	0,452517403	5,75264716	8,79E-09	6,63E-07
AL391987.4	20,05432848	2,197526065	0,378590689	5,734426497	9,78E-09	7,21E-07
BCL9L	506,2869482	1,200452362	0,211099255	5,706615393	1,15E-08	8,25E-07
C3orf18	189,7095457	1,494737274	0,261434877	5,677302098	1,37E-08	9,52E-07
DNAI2	26,98773266	2,158699082	0,377378171	5,675041668	1,39E-08	9,62E-07
AC008555.4	342,8896863	1,305132998	0,230246837	5,671671372	1,41E-08	9,74E-07
AC083862.3	93,04402387	1,605951846	0,285482801	5,666288702	1,46E-08	9,98E-07
AOAH	15162,22478	1,177424409	0,212304547	5,666244743	1,46E-08	9,98E-07
SCIMP	212,0349226	2,783228915	0,450582846	5,664457042	1,47E-08	1,01E-06
A2M-AS1	33,70740077	2,023754653	0,369565554	5,654876374	1,56E-08	1,04E-06
PROCR	189,2710625	1,782388913	0,308461157	5,655020804	1,56E-08	1,04E-06

HLA-DPB1	3374,062135	1,455744026	0,254509239	5,591878485	2,25E-08	1,37E-06
THEMIS2	6429,654639	1,318426828	0,243695999	5,581361969	2,39E-08	1,45E-06
TTYH2	119,2345397	1,912936214	0,330242527	5,570418879	2,54E-08	1,50E-06
FCHO2	354,5028786	1,313542877	0,235595382	5,550212185	2,85E-08	1,64E-06
SYNE1	7987,350074	1,037980237	0,187983316	5,549434563	2,87E-08	1,64E-06
ANO8	97,04963606	1,230117118	0,221829997	5,547661657	2,90E-08	1,65E-06
AL731571.1	505,1583218	1,013699118	0,182893325	5,543829085	2,96E-08	1,66E-06
ARVCF	833,3125729	2,408541464	0,439432257	5,524252065	3,31E-08	1,81E-06
SMAD7	2965,651036	1,11518809	0,202018956	5,511917559	3,55E-08	1,91E-06
UCP3	56,41155725	1,573797693	0,285729882	5,507325675	3,64E-08	1,95E-06
LIME1	942,3741088	1,479021292	0,267520994	5,506762372	3,65E-08	1,95E-06
AC133552.2	182,303471	1,054617444	0,192419877	5,500330174	3,79E-08	1,99E-06
NUAK2	1515,700571	1,857743056	0,334990288	5,499802944	3,80E-08	2,00E-06
AP001372.2	131,5564837	1,148468837	0,210424808	5,469934728	4,50E-08	2,30E-06
AL160269.1	17,96116089	2,732927978	0,50061574	5,464840779	4,63E-08	2,36E-06
MC1R	484,7686664	1,07744908	0,196701472	5,458544783	4,80E-08	2,40E-06
AC015911.3	80,38809773	1,337714978	0,247579608	5,45226435	4,97E-08	2,47E-06
CLIC3	9459,677847	1,481450713	0,279632872	5,441172466	5,29E-08	2,57E-06
PRR5	1701,267869	1,29351842	0,235458066	5,439890922	5,33E-08	2,59E-06
C8G	45,28819125	1,659126755	0,308272489	5,436019139	5,45E-08	2,63E-06
VIPR2	140,3095731	2,66019421	0,459253371	5,43117563	5,60E-08	2,68E-06
AMZ2P1	484,3583591	1,158245381	0,213752863	5,421662242	5,90E-08	2,77E-06
TMEM191A	149,1107394	1,328338074	0,245783935	5,419654336	5,97E-08	2,77E-06
AC093010.2	133,6541972	1,438341241	0,262590937	5,414098905	6,16E-08	2,82E-06
LINC01801	99,14112127	1,834082888	0,337712839	5,409646324	6,31E-08	2,86E-06
CXXC4	7,761928407	3,341815889	0,592915329	5,401131918	6,62E-08	2,96E-06
COL6A2	1751,980505	2,12446352	0,391644071	5,398399147	6,72E-08	3,00E-06
LINC00565	51,79099498	2,056481532	0,380334703	5,383884267	7,29E-08	3,17E-06

AC021188.1	181,0353108	1,059744646	0,197249743	5,382760231	7,34E-08	3,18E-06
ZNF683	1353,273478	2,206563322	0,407730698	5,365112229	8,09E-08	3,39E-06
HCG27	22,85714239	2,036093232	0,387623816	5,365334247	8,08E-08	3,39E-06
LINC00943	31,06372798	3,170285752	0,524871488	5,363474766	8,16E-08	3,40E-06
AC015911.11	643,1416956	1,104234871	0,207477992	5,354340959	8,59E-08	3,53E-06
RASA3	14648,7847	1,227943988	0,230592384	5,350087119	8,79E-08	3,59E-06
MXD4	4367,991898	1,008548164	0,189256948	5,34861958	8,86E-08	3,60E-06
TSPAN32	1680,349979	1,79932189	0,336957099	5,343106309	9,14E-08	3,68E-06
CARMIL3	114,0695523	2,419235059	0,442115745	5,337392111	9,43E-08	3,77E-06
AC008878.3	449,526451	1,286863372	0,24350788	5,302891786	1,14E-07	4,37E-06
DBP	1128,896084	1,281597154	0,24346066	5,294708607	1,19E-07	4,55E-06
BBS2	1317,400007	1,010588346	0,191435061	5,286580295	1,25E-07	4,69E-06
LEXM	191,9846548	1,794520893	0,34204509	5,279096728	1,30E-07	4,84E-06
ZBP1	2571,005471	1,248883048	0,237996161	5,27659424	1,32E-07	4,86E-06
ADGRG1	24045,91616	2,134403793	0,408032775	5,273768679	1,34E-07	4,92E-06
DGKD	7479,620703	1,010624725	0,191786194	5,273526694	1,34E-07	4,92E-06
KLF9	95,97313961	1,239751056	0,234357911	5,271941387	1,35E-07	4,95E-06
AL645933.3	72,22059645	1,355849793	0,256626744	5,26739582	1,38E-07	5,05E-06
LINC00299	1692,671679	1,19453555	0,22752385	5,265035978	1,40E-07	5,10E-06
PHOSPHO1	155,6505971	1,102879801	0,209418228	5,260066897	1,44E-07	5,22E-06
VASH1	473,3051921	1,054677812	0,194473484	5,257675292	1,46E-07	5,28E-06
AL365272.1	94,70735444	1,693224309	0,322038434	5,241850783	1,59E-07	5,63E-06
KDM7A	806,6152019	1,000202276	0,191750705	5,240127635	1,60E-07	5,67E-06
AC026748.1	43,85490864	2,347312926	0,472272602	5,23597803	1,64E-07	5,77E-06
AC004408.2	17,0308697	2,529599962	0,597942865	5,232422875	1,67E-07	5,85E-06
NMUR1	2270,621657	1,684435022	0,324979943	5,230283425	1,69E-07	5,91E-06
LY9	479,5011827	2,118962474	0,391487208	5,228320333	1,71E-07	5,95E-06
TSPOAP1	3229,099018	1,739234161	0,330229789	5,221416885	1,78E-07	6,12E-06

CDC42-AS1	31,27226099	1,79126482	0,358407045	5,218163321	1,81E-07	6,20E-06
AL590560.3	30,87246874	2,067096386	0,398254939	5,208159089	1,91E-07	6,44E-06
C9orf139	1590,305386	1,005446539	0,192864819	5,19139162	2,09E-07	6,92E-06
AC023908.3	109,2582264	1,041530409	0,20068853	5,186997842	2,14E-07	7,04E-06
LINC00987	56,43830273	2,101577291	0,388294364	5,160013637	2,47E-07	7,86E-06
SLC43A2	246,8527537	1,658615653	0,308859536	5,157946714	2,50E-07	7,91E-06
LINC00528	455,6077964	1,04871322	0,2034194	5,149984738	2,61E-07	8,16E-06
AC009133.1	143,5085013	1,162587828	0,223297213	5,144641867	2,68E-07	8,37E-06
ATP8A1	2191,085121	1,016084287	0,197428	5,142980308	2,70E-07	8,42E-06
SORL1	15644,15909	1,237782888	0,241760048	5,13654422	2,80E-07	8,59E-06
AC008115.3	12,80872293	1,749697322	0,341449513	5,121549903	3,03E-07	9,07E-06
TLR1	991,0522492	1,011983935	0,19931512	5,097226114	3,45E-07	1,00E-05
NME4	398,7327413	1,185412126	0,246136621	5,078882468	3,80E-07	1,08E-05
LINC02580	103,136312	1,927552183	0,381713593	5,075481389	3,87E-07	1,09E-05
FCMR	3082,195454	1,525517697	0,30064693	5,06963301	3,99E-07	1,12E-05
ADAMTS10	1817,806232	1,476035116	0,291159586	5,06860452	4,01E-07	1,12E-05
AXIN1	2828,725292	1,107000537	0,21911331	5,054831921	4,31E-07	1,19E-05
PPM1N	111,0466079	1,86650293	0,365824576	5,048706704	4,45E-07	1,22E-05
AC017104.6	281,1891744	1,924773209	0,390438273	5,043971505	4,56E-07	1,24E-05
AC084018.2	126,5141249	1,028639788	0,204398709	5,041739495	4,61E-07	1,26E-05
ADHFE1	1716,108649	1,300878706	0,261954489	5,026136826	5,00E-07	1,34E-05
CALCOCO1	8382,387021	1,036633552	0,20669138	5,018369601	5,21E-07	1,38E-05
YPEL3	5250,87534	1,182445039	0,23676953	5,002213151	5,67E-07	1,48E-05
AC020656.2	18,1122843	1,867029789	0,377135657	4,988305634	6,09E-07	1,55E-05
AC087741.1	361,5262212	1,184731562	0,240995022	4,978428649	6,41E-07	1,62E-05
ASPRV1	58,1557482	1,222666349	0,244679157	4,968705871	6,74E-07	1,69E-05
MAP2K6	110,4938357	1,232926195	0,251886494	4,966838123	6,81E-07	1,70E-05
AC245100.8	344,612282	1,124740314	0,228653101	4,952502178	7,33E-07	1,79E-05

IL13RA1	81,74065765	2,92075194	0,514790173	4,939134248	7,85E-07	1,90E-05
AC134669.1	372,670439	1,042972577	0,211665566	4,925676539	8,41E-07	1,98E-05
IFIT1	167,5701181	1,990677333	0,432179887	4,91394281	8,93E-07	2,08E-05
BTN3A1	11982,80094	1,076439364	0,219833583	4,910972132	9,06E-07	2,10E-05
C13orf46	219,4481016	1,604370654	0,343040709	4,908335023	9,19E-07	2,12E-05
IL24	14,13899697	1,829517236	0,368439861	4,904722928	9,36E-07	2,15E-05
TXNIP	87373,28167	1,198611463	0,24632488	4,892742155	9,94E-07	2,25E-05
PPP2R5C	10495,0938	1,212018087	0,248196189	4,881872109	1,05E-06	2,35E-05
PRR5-ARHGAP8	30,04910832	2,233599954	0,459502379	4,857117848	1,19E-06	2,60E-05
TRIM73	113,5137089	1,326773633	0,270785003	4,832941441	1,35E-06	2,83E-05
MST1L	35,53330089	1,843963043	0,39785379	4,826925457	1,39E-06	2,90E-05
AC009093.10	70,90865127	1,303881935	0,271860718	4,825710292	1,40E-06	2,91E-05
NR1D2	1901,099841	1,061516657	0,220954674	4,805364677	1,54E-06	3,14E-05
SLFN12L	388,6582447	1,016747552	0,212823659	4,804996628	1,55E-06	3,14E-05
DAPK2	315,8775139	2,269275673	0,46000889	4,803430519	1,56E-06	3,16E-05
AL162457.1	60,01120291	1,80702327	0,371410401	4,798244632	1,60E-06	3,22E-05
NHSL2	1708,883997	1,543158551	0,327110587	4,794206828	1,63E-06	3,27E-05
UNC5CL	93,2975135	1,57197324	0,329561404	4,788580335	1,68E-06	3,33E-05
AL162458.1	165,1223187	1,127248748	0,23570295	4,789075463	1,68E-06	3,33E-05
AC010319.1	43,13147385	1,502921542	0,313091723	4,786588652	1,70E-06	3,36E-05
DLGAP1-AS2	58,74548733	1,427003542	0,297323381	4,786150878	1,70E-06	3,36E-05
ZNF208	25,17114763	1,843243073	0,394563582	4,783253633	1,72E-06	3,40E-05
AC243829.1	40,37717938	2,533252857	0,52705675	4,780751422	1,75E-06	3,43E-05
CCDC65	186,1724057	2,427617547	0,513646417	4,779372081	1,76E-06	3,45E-05
SAT1	3820,59353	1,023904591	0,214341376	4,773329599	1,81E-06	3,52E-05
ZNF831	1478,234274	1,148277783	0,240483824	4,772403024	1,82E-06	3,53E-05
AC018809.1	30,58052564	1,127351416	0,235538174	4,758501657	1,95E-06	3,71E-05
FRMPD3	25,27426586	2,242602646	0,492422158	4,745952303	2,08E-06	3,90E-05



C1orf21	3800,709353	1,152410123	0,243213472	4,745811927	2,08E-06	3,90E-05
AC099489.1	97,36576781	1,972012994	0,414899076	4,739572019	2,14E-06	3,99E-05
PLCD1	824,4581922	1,334017381	0,282996846	4,739078882	2,15E-06	3,99E-05
SIRT4	98,34753543	1,075089499	0,227828607	4,733254327	2,21E-06	4,07E-05
AP003068.2	69,49145467	1,045748728	0,221320079	4,731441888	2,23E-06	4,09E-05
MTSS1	2929,102962	1,654146357	0,351096483	4,727358026	2,27E-06	4,15E-05
AL590560.2	120,164635	1,814906046	0,398136941	4,708077351	2,50E-06	4,48E-05
AC124016.2	116,854978	1,002133543	0,214280142	4,704480502	2,55E-06	4,54E-05
AC025279.1	104,2758127	1,381314656	0,294837335	4,703887732	2,55E-06	4,55E-05
AL773545.3	27,83370224	1,160252068	0,246928811	4,693325472	2,69E-06	4,74E-05
PSD2	17,42910173	1,849647571	0,390947834	4,681260096	2,85E-06	4,95E-05
AC023794.4	11,47496082	1,601383024	0,339309844	4,680697826	2,86E-06	4,95E-05
COLGALT2	870,3854753	1,353701533	0,298558899	4,673802619	2,96E-06	5,05E-05
ATG9B	111,1433503	1,447918986	0,316929422	4,673272382	2,96E-06	5,05E-05
FCGBP	212,86402	1,405748668	0,304885636	4,664369744	3,10E-06	5,24E-05
LINC00954	421,6966756	1,163749282	0,248363315	4,660070335	3,16E-06	5,31E-05
SULF2	74,31806132	2,635487063	0,508138654	4,656477327	3,22E-06	5,39E-05
PROX2	60,31267389	1,344959973	0,29151711	4,63502145	3,57E-06	5,86E-05
HSPA7	98,06520416	2,172463402	0,47461447	4,6257089	3,73E-06	6,05E-05
AC092070.2	271,9266929	1,044125945	0,225528349	4,625397712	3,74E-06	6,05E-05
IFT172	739,4401397	1,046835161	0,226649969	4,61768802	3,88E-06	6,22E-05
LRRN1	199,3817219	1,67634591	0,347861825	4,613286527	3,96E-06	6,30E-05
AC107884.1	149,9546605	1,098534222	0,238546131	4,607222473	4,08E-06	6,43E-05
LRFN2	17,33028618	2,70347866	0,589952673	4,602924909	4,17E-06	6,54E-05
LINC00891	99,51639127	1,211701174	0,27010786	4,596032167	4,31E-06	6,73E-05
FCGRT	188,1205837	1,714697178	0,365707243	4,593517411	4,36E-06	6,79E-05
LINC-PINT	939,3163	1,052297189	0,231429274	4,586378535	4,51E-06	6,97E-05
UNC45B	67,03005005	1,69186889	0,370619754	4,578527977	4,68E-06	7,19E-05

FCGR2B	178,910675	2,286289763	0,494827636	4,573149683	4,80E-06	7,34E-05
AC010883.2	20,891705	1,984927898	0,446983453	4,562862989	5,05E-06	7,61E-05
OPRD1	7,304310896	2,099294135	0,457757727	4,558500739	5,15E-06	7,75E-05
GPRASP1	292,3099084	1,65327553	0,351655055	4,558420211	5,15E-06	7,75E-05
PRSS40A	19,76720964	2,409698917	0,534900922	4,549321221	5,38E-06	8,02E-05
AC211476.12	52,38657243	1,113237922	0,242282423	4,548969966	5,39E-06	8,02E-05
RTCA-AS1	86,85366497	1,308702077	0,290320204	4,545842086	5,47E-06	8,10E-05
CCDC114	62,46447889	1,596195989	0,350021629	4,545020059	5,49E-06	8,12E-05
AL353748.3	22,42083074	1,620815243	0,371708927	4,53980668	5,63E-06	8,27E-05
AC015911.10	459,6133838	1,073288441	0,239562632	4,536841491	5,71E-06	8,35E-05
AP000977.1	104,011099	1,901781356	0,427486371	4,535458552	5,75E-06	8,40E-05
P2RY6	102,6792379	2,707602737	0,563857161	4,529894188	5,90E-06	8,57E-05
FRAT1	637,5847984	1,044245964	0,230046085	4,523603261	6,08E-06	8,78E-05
MAN1B1-DT	90,43292212	1,17205302	0,266235582	4,521034025	6,15E-06	8,87E-05
DLG4	58,07181287	1,200925482	0,267174539	4,506728855	6,58E-06	9,29E-05
KRT2	24,47030025	1,962531153	0,45089209	4,503145747	6,70E-06	9,42E-05
CD68	340,2825221	2,88669529	0,563558472	4,496760346	6,90E-06	9,66E-05
AC015911.8	32,80239405	1,306274083	0,294328639	4,493626462	7,00E-06	9,77E-05
TLR8	63,06937408	2,818121661	0,544355561	4,493254349	7,01E-06	9,78E-05
ZNF540	192,2027719	1,014889414	0,226165718	4,492982309	7,02E-06	9,79E-05
AC114490.1	68,7453404	1,42591301	0,315481587	4,492207674	7,05E-06	9,81E-05
ZEB2	5870,147002	1,905679567	0,428102631	4,490713028	7,10E-06	9,85E-05
SGSM1	415,2285547	1,377020187	0,307687888	4,487318345	7,21E-06	9,94E-05
HELZ2	1472,231384	1,081424223	0,242364264	4,476578523	7,58E-06	0,000102887
AC010175.1	27,9370756	1,801279253	0,435967861	4,468299007	7,88E-06	0,000105815
AZIN2	87,19863322	1,544194386	0,352785449	4,467055383	7,93E-06	0,000106216
C11orf21	3310,830889	1,394613926	0,312979942	4,45381841	8,44E-06	0,00011133
KLRC4	422,5199519	1,085847636	0,246319418	4,448547078	8,65E-06	0,000113795

AC008750.7	26,8318274	1,615791861	0,364256153	4,44163176	8,93E-06	0,000116874
SERPINA1	465,6871084	2,858234421	0,560442559	4,440305821	8,98E-06	0,00011723
AC022182.2	150,9571258	1,087910973	0,246185903	4,437422784	9,10E-06	0,000118576
RNF43	227,8255452	1,259155595	0,285216739	4,43645344	9,15E-06	0,000119033
KLRC4-KLRK1	1187,083535	1,055601882	0,244961622	4,435937577	9,17E-06	0,00011924
LINC00612	9,628627634	2,245717697	0,47550919	4,434097608	9,25E-06	0,000120027
ZNF600	6875,594315	1,157301054	0,259148588	4,428094487	9,51E-06	0,000122773
PIK3IP1	3313,24148	1,549054891	0,352505797	4,42026686	9,86E-06	0,000126646
SMTNL1	26,20968107	1,206617562	0,272854977	4,419653143	9,89E-06	0,000126924
AC010247.2	108,6006819	1,751829787	0,408572456	4,416610391	1,00E-05	0,000128474
FPR2	32,26783206	2,812957924	0,586331833	4,414977997	1,01E-05	0,000129113
TBKBP1	190,9601071	1,155598435	0,269633612	4,410544963	1,03E-05	0,000131278
FBXO32	634,638113	1,334966847	0,300758638	4,409183759	1,04E-05	0,000131915
EZH1	2760,488621	1,024724935	0,233506286	4,398972741	1,09E-05	0,000136894
MST1P2	44,49123567	1,487308001	0,338995118	4,391979895	1,12E-05	0,000140659
CERCAM	358,6930088	2,05338782	0,465546825	4,369907416	1,24E-05	0,000152669
ZNNT1	14,06611698	1,666753235	0,384730813	4,365328721	1,27E-05	0,000155134
HHIPL1	12,29385891	2,440397057	0,586643925	4,36195048	1,29E-05	0,000157065
AGBL2	172,1027624	1,091825321	0,251245593	4,342605766	1,41E-05	0,000168555
AJM1	493,903758	1,166318095	0,270096934	4,337255959	1,44E-05	0,000172086
MMP23B	565,0281546	1,426836318	0,330894457	4,336378185	1,45E-05	0,000172493
ACRBP	71,76783533	1,5565221	0,354867671	4,335078283	1,46E-05	0,000173278
LILRA1	34,58448064	2,671567669	0,590158482	4,33373998	1,47E-05	0,000173918
AC015813.2	194,8450986	1,409014415	0,321690641	4,333027371	1,47E-05	0,000174273
TNNT3	49,39452397	1,804851787	0,41390741	4,329582053	1,49E-05	0,000176365
ARHGAP8	41,14702571	1,869067694	0,391968748	4,328443884	1,50E-05	0,000176883
AC087500.1	71,25157823	1,36274409	0,313124684	4,328552407	1,50E-05	0,000176883
SIGLEC14	32,97944356	2,867917613	0,586000779	4,314342775	1,60E-05	0,0001858

GIPR	247,2273223	1,265795748	0,294605051	4,311569226	1,62E-05	0,000187271
ITPKB-IT1	11,65616568	1,94870385	0,442908753	4,304491259	1,67E-05	0,000192127
AL031432.2	48,63230437	1,149306495	0,267555358	4,303230646	1,68E-05	0,000192889
FCN1	240,7170617	2,239097406	0,572694685	4,300606497	1,70E-05	0,000194625
AC241377.4	88,72225019	1,163268268	0,257997161	4,29405576	1,75E-05	0,000199312
SMARCD3	82,5657991	1,061315237	0,245133676	4,290751423	1,78E-05	0,000201632
CHI3L1	105,0187819	2,764764411	0,561411352	4,290005909	1,79E-05	0,000202171
CXCL9	1631,844563	3,019144376	0,567702029	4,286376323	1,82E-05	0,000205034
CCL4L2	2083,779693	1,332132681	0,317476168	4,280361404	1,87E-05	0,000209541
AL109955.1	56,45528254	1,22339593	0,287083275	4,280156044	1,87E-05	0,000209541
AC012020.1	150,8591323	1,13377389	0,262738046	4,279761268	1,87E-05	0,000209791
AC117503.5	55,09409353	1,086407585	0,258588869	4,27956127	1,87E-05	0,00020986
CLEC10A	73,08940149	2,590400054	0,552820393	4,271991036	1,94E-05	0,000215653
DLEC1	189,4312435	1,269736394	0,295617882	4,270105333	1,95E-05	0,000217241
LAX1	1875,573381	1,124027965	0,265052092	4,269530701	1,96E-05	0,000217679
MTMR9LP	120,9932721	1,196541494	0,279272188	4,250639616	2,13E-05	0,000233348
AC008555.1	65,24832511	1,529956752	0,355594055	4,24861174	2,15E-05	0,00023508
AL031432.4	52,55290791	1,406199828	0,328327625	4,248006873	2,16E-05	0,000235456
ERBB2	2779,98432	1,771240615	0,422205266	4,237211366	2,26E-05	0,00024371
IFIT3	988,5316983	1,879000492	0,446826736	4,225013506	2,39E-05	0,000253305
ST3GAL5-AS1	15,98539508	1,361023574	0,322771102	4,225133523	2,39E-05	0,000253305
AC015911.7	168,4716196	1,194825632	0,286871494	4,221939215	2,42E-05	0,000255829
NLRC4	20,05947594	2,21351362	0,491287507	4,221693233	2,42E-05	0,000255972
C1QB	392,594276	2,765195687	0,57671314	4,217893765	2,47E-05	0,000259494
NSG1	213,3118137	2,151324676	0,465034021	4,200775413	2,66E-05	0,000275964
SBK1	2498,439639	1,486623003	0,352244724	4,198557899	2,69E-05	0,000278098
CETP	25,78379367	1,520827665	0,34856314	4,197411794	2,70E-05	0,000279071
MORN3	119,5276288	1,181763759	0,283665883	4,196401452	2,71E-05	0,000280027

SNX29P2	64,91047058	1,145671423	0,271929173	4,192631513	2,76E-05	0,000283689
AC022382.2	13,66233815	1,420709546	0,337932477	4,192213526	2,76E-05	0,000283842
U73169.1	44,52090523	1,071515801	0,256254032	4,191664825	2,77E-05	0,000284017
OR52N4	23,66026102	1,561418814	0,377858277	4,175564778	2,97E-05	0,000300827
NF1	645,8109695	1,044720041	0,252128524	4,164946871	3,11E-05	0,000311834
ZNF528-AS1	80,98846976	1,268779464	0,305086055	4,161081028	3,17E-05	0,000315726
C9orf163	18,5394897	1,187612285	0,286313822	4,159935506	3,18E-05	0,000316677
TET1	95,91593596	1,087796479	0,269314783	4,154274202	3,26E-05	0,000323323
CEBPA	273,3535858	1,693595605	0,383895284	4,152059581	3,29E-05	0,000325817
LGALS2	79,70384392	3,037782072	0,581743211	4,146918286	3,37E-05	0,0003314
SIRPA	69,69050234	2,514324763	0,548528424	4,145239844	3,39E-05	0,000333341
PLXDC1	148,4655896	1,737290221	0,37078693	4,143083583	3,43E-05	0,000335165
MAFB	77,03530969	2,44252124	0,582044745	4,14144348	3,45E-05	0,000337238
MARCKS	50,14277391	2,558047711	0,565758684	4,132939356	3,58E-05	0,000347048
LINC02384	974,945981	1,646170751	0,423241969	4,128697116	3,65E-05	0,000351807
AL160313.2	18,36002482	1,9082612	0,443445282	4,127559352	3,67E-05	0,000353379
TCAF2P1	90,61487765	1,196699269	0,344802197	4,118903513	3,81E-05	0,000364591
PER3	406,3538786	1,176375143	0,286096826	4,117082009	3,84E-05	0,000367152
ARHGEF25	12,10795433	1,523866138	0,369421632	4,11067572	3,95E-05	0,000375858
AC010894.5	15,68235509	1,429634561	0,352352394	4,104378682	4,05E-05	0,000384238
AC138150.2	20,99619197	1,611050011	0,395209006	4,096971637	4,19E-05	0,000394637
AC008033.3	24,92576769	1,644614108	0,439318701	4,090677198	4,30E-05	0,000403584
S1PR1	5902,533667	1,650275277	0,405233926	4,089638087	4,32E-05	0,000404631
AC010332.3	220,6792336	1,18054779	0,287886468	4,087329077	4,36E-05	0,000408292
AL732406.1	25,77180853	2,525015641	0,595819635	4,082382507	4,46E-05	0,000416102
ZEB2-AS1	20,92822888	2,041743092	0,543004141	4,078380522	4,54E-05	0,000421781
A2M	46,10435988	1,855720802	0,465311273	4,077395203	4,55E-05	0,000422936
C1QA	275,1619046	2,666743726	0,577682916	4,072474985	4,65E-05	0,000430965

ALDH2	138,8625881	2,151838446	0,478658617	4,07101691	4,68E-05	0,00043319
LINC00469	44,735068	2,329501796	0,595299585	4,066003525	4,78E-05	0,000440019
ABTB1	3730,092258	1,015826688	0,250840392	4,060212195	4,90E-05	0,000450033
TGFBI	240,632952	2,378503177	0,564127078	4,04868559	5,15E-05	0,000466731
HEXD-IT1	99,19477023	1,606417503	0,393003783	4,047096326	5,19E-05	0,000468839
SECTM1	210,7848666	2,539037872	0,566820489	4,038806098	5,37E-05	0,00048264
FCGR1B	32,76756643	2,529744718	0,598428639	4,038521574	5,38E-05	0,000483007
HCK	238,8229275	2,62929151	0,577984795	4,036712715	5,42E-05	0,000485864
AL627422.2	7,307006393	2,088340758	0,579863734	4,033784066	5,49E-05	0,000491075
TMEM132C	15,95285903	2,113240572	0,570097812	4,028123874	5,62E-05	0,000499218
INGX	56,6903233	1,013427798	0,252357648	4,024988721	5,70E-05	0,000503984
AC011468.5	33,83511075	1,00624306	0,25045443	4,02197368	5,77E-05	0,000508793
CCL5	26076,1303	1,350996157	0,338240824	4,018892107	5,85E-05	0,000513853
DNAH10OS	40,39125171	1,458902435	0,369652043	4,017319822	5,89E-05	0,00051619
LITAF	23187,8389	1,162951381	0,291672676	4,00566205	6,18E-05	0,000536211
AC006252.1	52,37109194	1,009691775	0,250258005	4,004858797	6,21E-05	0,000537282
CTSL	74,21962201	2,454853756	0,540016838	4,001871625	6,28E-05	0,000542219
Z97989.1	67,93343703	1,135059575	0,283732596	3,995354494	6,46E-05	0,00055494
SNPH	180,8298809	1,253589867	0,315616339	3,994005513	6,50E-05	0,000557626
LINC00896	28,77510339	1,411251209	0,353359802	3,991520666	6,57E-05	0,000562285
PLBD1	20,7191469	2,333156757	0,597824193	3,990912588	6,58E-05	0,000563
AC009093.8	58,26159984	2,08156925	0,511004232	3,989005573	6,64E-05	0,000566811
SYNGR1	567,9251608	1,927252588	0,483645475	3,98663967	6,70E-05	0,000571506
AC099521.3	4,465709563	1,915216771	0,507221182	3,985714789	6,73E-05	0,00057349
LINC00243	64,48078132	1,561970772	0,389064624	3,985374808	6,74E-05	0,000574066
AC092636.2	7,528712191	2,192861718	0,592925011	3,98377413	6,78E-05	0,000577452
AC002316.1	1373,011225	1,781229551	0,444075125	3,979749852	6,90E-05	0,000585806
AMY2B	265,8743418	1,086094794	0,269209238	3,977767361	6,96E-05	0,000589217

EFNA1	14,24715828	1,423949984	0,353903706	3,976513335	6,99E-05	0,000591301
AL592295.4	142,3120406	1,031011265	0,262967147	3,973726899	7,08E-05	0,000595923
LSMEM1	18,07762195	1,192849374	0,300746055	3,970909637	7,16E-05	0,000601029
CD4	240,2007002	2,606279363	0,593337825	3,96790605	7,25E-05	0,000606599
C1QC	207,6228252	2,627950954	0,578340915	3,966688121	7,29E-05	0,000608936
AGPAT4	825,0728962	1,142423688	0,292463785	3,951534591	7,77E-05	0,000641791
NPC1	6856,804572	1,1169577	0,283898687	3,951619429	7,76E-05	0,000641791
SSBP3-AS1	83,55414745	1,35851243	0,348467799	3,949429756	7,83E-05	0,000645846
SPTBN5	56,12225854	1,790237666	0,449479988	3,94387235	8,02E-05	0,000658542
LRRC25	99,37734494	2,366414987	0,554275373	3,943665224	8,02E-05	0,000658838
MGAM2	16,39325032	2,038434496	0,600942011	3,936225634	8,28E-05	0,000675674
LIPC	100,0814985	1,466979253	0,372709729	3,932402949	8,41E-05	0,000684541
PRR5L	5668,248956	1,120823026	0,286527703	3,922323325	8,77E-05	0,000708018
LYZ	2603,852795	2,274736707	0,559041505	3,921639446	8,79E-05	0,000709165
AL445228.3	17,73035404	1,353675163	0,341398838	3,910800302	9,20E-05	0,000734566
CCDC183	36,65153314	1,398895017	0,37414863	3,902339346	9,53E-05	0,000753179
LINC00921	253,0803091	1,17283326	0,298308603	3,900763022	9,59E-05	0,000757195
AC106782.1	18,21332579	1,688685299	0,428966603	3,89942032	9,64E-05	0,000759893
PLIN1	28,93255006	1,540497579	0,401704587	3,892711527	9,91E-05	0,000778133
AC108704.2	273,4213432	1,200214784	0,31201904	3,891357084	9,97E-05	0,000781872
LILRB2	146,8986618	2,51266224	0,5753606	3,87920882	0,000104797	0,000813637
FAM110C	211,470031	1,787405297	0,45720726	3,879197894	0,000104801	0,000813637
COL6A4P2	11,74889994	1,809747089	0,570450663	3,86995067	0,000108857	0,000839866
AC092117.1	95,12539326	1,010526045	0,260581465	3,861426274	0,000112727	0,000863341
AC107375.1	27,79797232	1,208929691	0,315845007	3,849790212	0,000118219	0,000896747
H2AC3P	7,187114823	1,946149143	0,495613703	3,846985969	0,00011958	0,000904992
FCGR1A	120,6255376	2,35160559	0,598788438	3,846140739	0,000119993	0,000907772
CARNS1	481,2979576	1,02262037	0,26340231	3,842597591	0,000121739	0,00091818

MAP3K6	269,7151428	1,021393561	0,265297573	3,837253772	0,000124418	0,000934477
AC067945.4	14,57270036	1,306885021	0,34569316	3,83336978	0,0001264	0,000946495
ITGA5	7566,222291	1,017538868	0,267460512	3,82982707	0,000128233	0,000956795
HSD11B1-AS1	10,3404823	1,498716783	0,39565234	3,829069372	0,000128629	0,000958948
AC009951.4	24,76922044	1,812496874	0,465994527	3,826523032	0,000129966	0,000965545
ADAMTS6	6,127241271	2,131827374	0,582871238	3,824732893	0,000130914	0,000971133
SERPING1	254,6799424	2,48752146	0,58399494	3,820698331	0,000133074	0,000984581

1312

1313



1314 **Table 4.** List of NK cell signature genes used in this study (from (48)), Related to Fig. 7A-D, fig. S9E

Name	Gene symbols
NK cell signature	NCR3, KLRB1, PRF1, CD160, NCR1

1315

1 **Supplementary information**

2 **Fig. S1. IL-33 in combination with IL-12 strongly activates NK cell secretory and cytotoxic functions**

3 **(A-C)** Quantification of IFN- $\gamma$  secretion by healthy donors' blood NK cells upon stimulation with IL-33,  
4 IL-18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination with **(A)** IL-15 (10 ng/mL), IL-2 (500 UI/mL),  
5 IFN- $\alpha$  (500 UI/mL), **(B)** anti-NKp30 (1  $\mu$ g/mL), anti-NKp46 (1  $\mu$ g/mL) agonistic antibodies or **(C)** K562  
6 target cells for 24 h. Histogram bars represent the median (n = 4 individual experiments). Friedman  
7 test with Dunn's multiple comparisons test was performed.

8 **(D-G)** Healthy donors' blood NK cells were isolated from the blood of healthy donors and activated for  
9 24 h with IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$  (0.1 to 100 ng/mL of each) alone or in combination with **(d)** IL-12  
10 (10 ng/mL), **(E)** IL-2 (500 UI/mL), **(F)** IL-15 (10 ng/mL) or **(G)** IFN- $\alpha$  (500 UI/mL) prior to supernatant  
11 collection and IFN- $\gamma$  quantification by ELISA. Results are expressed as mean +/- SEM (n = 4 to 8  
12 individual experiments). One-way ANOVA with Tukey's multiple comparisons test was performed for  
13 each cytokine concentration from 1 ng/mL.

14 **(H)** Healthy donors' blood NK cells were activated as described above and supernatants were collected  
15 at several time points prior to IFN- $\gamma$  quantification by ELISA (n = 1).

16 **(I)** Healthy donors' blood NK cells were activated with indicated cytokines (10 ng/mL for each cytokine)  
17 for 24 h and co-cultured with calcein-loaded K562 target cells for 4 h at indicated effector to target  
18 ratios. Calcein release in the supernatant was quantified by fluorometry to calculate lysis percentage.  
19 Results are expressed as mean +/- SEM (n = 3 individual experiments).

20 **(J)** Healthy donors' blood NK cells activated with indicated cytokines (10 ng/mL for each cytokine) for  
21 24 h were exposed to K562 cells in a direct cytotoxic assay. Percentage of CD107+ NK cells are shown.  
22 Results are expressed as mean + SEM (n = 2 individual experiments).

23

24 **Fig. S2. IL-12 induces ST2 expression on a subset of NK cells in a STAT4-dependent manner**

25 Healthy donors' blood NK cells were activated as indicated (10 ng/mL of each cytokine).

26 **(A)** Healthy donors' blood NK cells were activated by different cytokine combinations in the presence  
27 of anti-ST2 (10 µg/mL), anti-IL-18 (1 µg/mL), control mIgG1 antibodies (1 or 10 µg/mL as control for  
28 anti-IL-18 or anti-ST2, respectively) or IL-1RA antagonist (100 ng/mL). Supernatants were collected  
29 after 24 h and IFN-γ secretion was quantified by ELISA. Results are expressed as mean + SD and are  
30 representative of three individual experiments. Two-way ANOVA with Tukey's multiple comparisons  
31 test was performed.

32 **(B)** *IL1RL1/ST2* and *IL1RAcP* relative mRNA expression was analyzed by RT-qPCR at indicated times.  
33 Results are expressed as mean + SD and are representative of two individual experiments. Two-way  
34 ANOVA with Tukey's multiple comparisons test was performed.

35 **(C)** NK cells were treated or not with lisofylline (500 µM) for 24 h prior to activation with IL-12 (0.1 to  
36 10 ng/mL) for 5 min and p-STAT4 intracellular levels were analyzed by flow cytometry (n = 3 individual  
37 experiments).

38 **(D)** *In silico* analysis of putative STAT4 binding sites in *ST2* promoter region. Dissimilarity indicates the  
39 deviation between the identified sequence and the STAT4 consensus binding site. RE and RE query  
40 values indicate the probability to randomly finding the motif respectively considering a model with  
41 equiprobability of the four nucleotides and a model with the same nucleotide frequency as the query  
42 sequence. A value of 0.001 means that the hit is only expected to occur by chance once in 1 Mb of  
43 sequence.

44

45 **Fig. S3. IL-33 preferentially activates a subset of CD56<sup>dim</sup> NK cells**

46 **(A)** Healthy donors' blood NK cells were activated with IL-12 (10 ng/mL) or not (medium) for 5 min and  
47 p-STAT4 intracellular levels were analyzed by flow cytometry on gated CD56<sup>bright</sup> vs CD56<sup>dim</sup> subsets.  
48 Data are representative of three individual experiments.

49 **(B)** FACS-sorted CD56<sup>bright</sup> and CD56<sup>dim</sup> healthy donors' blood NK cells were activated with medium or  
50 IL-12 alone for 24 h prior to the addition of IL-33, IL-18, IL-1α or IL-1β, supplemented or not with IL-12.  
51 Each cytokine was used at 10ng/mL. p38 (MAPK) and S6 (mTOR) phosphorylation as analyzed 5 min

52 or 1 h after the addition of IL-1 family cytokines, respectively. Representative histogram plots (left) and  
53 quantification (%) (right) of p-p38<sup>+</sup> or p-S6<sup>+</sup> NK cells after cytokine activation. Histogram bars indicate  
54 the median. Two-way ANOVA with Bonferroni's multiple comparisons test was performed; n = 4 to 5  
55 experiments.

56 **(C)** Healthy donors' blood NK cells were activated as indicated for 24 h, each cytokine was used at 10  
57 ng/mL. CD69 surface and IFN- $\gamma$  intracellular expression was analyzed by flow cytometry. CD56 surface  
58 expression levels were used to discriminate CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells. Representative dot plots  
59 (left) and quantification (%) (right) of CD69<sup>+</sup> IFN- $\gamma$ <sup>+</sup> NK cells after cytokine activation. Histogram bars  
60 indicate the median. Two-way ANOVA test with Bonferroni's multiple comparisons test was  
61 performed; n = 4 experiments.

62

63 **Fig. S4. ST2<sup>+</sup> NK cells display a unique gene signature compared to CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells**

64 **(A)** Heatmap representing all differentially-expressed genes (upregulated and downregulated)  
65 (n=2399 genes) in each sorted healthy donors' blood NK cell subset compared to the two other subsets  
66 following IL-12 activation (n = 3 individual donors).

67 **(B)** Expression levels of DEG (up and down) presented in heatmap in Fig. 3E.

68

69 **Fig. S5. Unsupervised clustering of sc-RNAseq datasets**

70 UMAP plots of NK cells clusters annotated from human melanoma metastases (GSE139249) (42) and  
71 mouse lung tumors (GSE123534) (43) sc-RNAseq public datasets.

72

73 **Fig. S6. Gating strategy to identify NK cells in tumor cell suspensions by flow cytometry**

74 **(A)** Tumor cell suspensions were stained for CD45, CD3, CD56, and CD7 expression and tumor-  
75 infiltrating NK cells were identified using gating strategy as illustrated. Cell viability was determined  
76 using Zombie dye (Biolegend).

77 **(B)** Cell suspensions were obtained from fresh breast tumors to analyze NK cell frequency among total  
78 infiltrating cells by flow cytometry according to BC molecular types (n = 46 patients). Kruskal Wallis  
79 test with Dunn's multiple comparisons test was performed.

80

81 **Fig. S7 | IL-12 sensitizes mouse splenic NK cells to the production of IFN- $\gamma$  in response to IL-33**

82 **(A)** Splenic cells were activated with IL-12 (20 ng/mL) or not for 24 h prior to flow cytometry analysis  
83 of ST2 surface expression (black line) or corresponding isotypic control (grey). CD45, NK1.1, and CD3  
84 were used to identify NK cells. Representative histogram plots (left) and quantification (%) (right) of  
85 ST2<sup>+</sup> NK cells after medium or IL-12 culture. Horizontal bars represent the mean + SEM (n = 4 individual  
86 mice). Paired two-tailed Student's *t*-test was performed.

87 **(B)** Splenic cells were activated as indicated (20 ng/mL of each cytokine) for 24 h prior to flow  
88 cytometry analysis of IFN- $\gamma$  intracellular expression. CD45, NK1.1, and CD3 were used to identify NK  
89 cells. Plots are representative of four individual experiments with 3 to 4 mice/experiment and results  
90 for each individual mouse are presented in the right panel. Results are expressed as mean + SEM. One-  
91 way repeated measures ANOVA with Tukey's multiple comparisons test was performed.

92

93 **Fig. S8. Antitumoral effect of IL-33 and IL-12 combination is dependent on NK cells in *Rag2*-KO mice**

94 **(A)** *Rag2*-KO mice were injected intra-peritoneally with NaCl or anti-NK1.1 depleting antibody prior to  
95 intra-mammary injection with  $2.5 \times 10^5$  E0771 cells on day 0 and then treated in the tumor area with  
96 NaCl or a combination of 10 ng/mouse rIL-12 and 100 ng/mouse rIL-33 twice a week from day 2 to  
97 day 23. Primary tumor growth was monitored in non-depleted *Rag2*-KO mice treated with NaCl (n = 3)  
98 or with rIL-12 and rIL-33 combination (n = 3) or in NK cell-depleted WT mice treated with NaCl (n  
99 = 3) or with rIL-12 and rIL-33 combination (n=4). Two-way ANOVA with Tukey's multiple  
100 comparisons test was performed.

101 **(B)** Kaplan-Meier survival plots of non-depleted *Rag2*-KO mice treated with NaCl (n = 3) or with rIL-  
102 12 and rIL-33 combination (n = 3) or in NK cell-depleted WT mice treated with NaCl (n = 3) or with

103 rmIL-12 and rmIL-33 combination (n=4). Mice were sacrificed when longest side of primary tumor  
104 reached 17 mm. Log-rank test was performed and the p-value is indicated for non-depleted mice  
105 treated with rmIL-12 + rmIL-33 vs NK cell-depleted mice treated with rmIL-12 + rmIL-33.

106 **(C)** Serum was retrieved from blood collected at day 5, 4hrs after NaCl or cytokine injection into the  
107 peritumoral area. IFN- $\gamma$  concentration was measured by ELISA in the serum of non-depleted *Rag2*-KO  
108 mice treated with NaCl (n = 3) or with rmIL-12 and rmIL-33 combination (n = 3) or in NK cell-depleted  
109 WT mice treated with NaCl (n = 3) or with rmIL-12 and rmIL-33 combination (n = 4). Histogram bars  
110 represent the median. Kruskal Wallis test with Dunn's multiple comparisons test was performed.

111

112 **Fig. S9. IL-33 is associated with increased overall patient survival for several cancers**

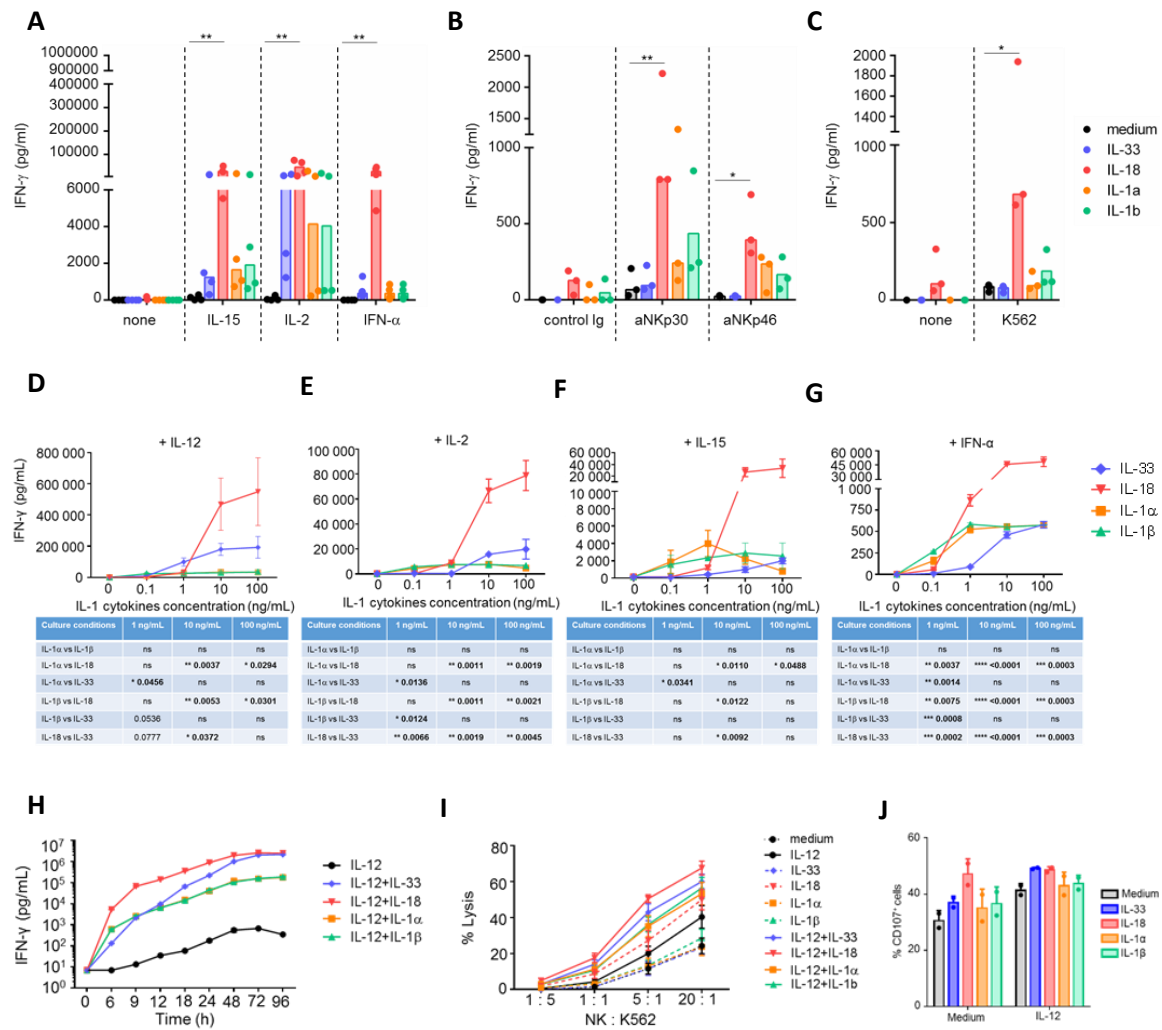
113 **(A)** Summary of p-values associated with the log rank test performed to evaluate prognostic value of  
114 *IL33* and *IL1RL1* (ST2) in 32 human TCGA cancer data sets (the same as in Fig. 6A, MESO: Mesothelioma  
115 ; UVM: Uveal Melanoma were added). Data are represented as a bubble map showing positive (green)  
116 or negative (red) impact on progression-free survival. Dots size represents p-values obtained by log-  
117 rank test.

118 **(B)** Patients from TCGA database were stratified as high or low for *IL33* and progression-free survival  
119 was analyzed in all cancer data sets. Kaplan-Meier survival curves for ACC, BRCA, KIRP, LIHC, SKCM,  
120 THCA, and UCEC patients are represented and p-values were obtained with log-rank test.

121 **(C)** Patients from TCGA database were stratified as high or low for *IL1RL1* (ST2) and progression-free  
122 survival was analyzed in all cancer data sets. Kaplan-Meier survival curves for BRCA, KIRC, KIRP, LIHC,  
123 and UVM patients are represented and p-values were obtained with log-rank test.

124 **(D)** Multivariate Cox analysis of *IL33* impact on prognosis in breast cancers from TCGA dataset  
125 regarding the age, molecular subtype, and stage of the tumors p-values were obtained with log-rank  
126 test.

127 **(E)** Kaplan-Maier curves for progression-free survival for  $NK^{high}/IL33^{high}$ ,  $NK^{high}/IL33^{low}$  and  $NK^{low}/IL33^{high}$   
128 scores as compared to  $NK^{low}/IL33^{low}$  in cancer datasets from TCGA that showed statistical significance  
129 in Fig. 7B. p-values were obtained with log-rank test.  
130  
131



132

133 **Fig. S1. IL-33 in combination with IL-12 strongly activates NK cell secretory and cytotoxic functions**

134 **(A-C)** Quantification of IFN- $\gamma$  secretion by healthy donors' blood NK cells upon stimulation with IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$  alone (10 ng/mL) or in combination with **(A)** IL-15 (10 ng/mL), IL-2 (500 UI/mL), IFN- $\alpha$  (500 UI/mL), **(B)** anti-NKp30 (1  $\mu$ g/mL), anti-NKp46 (1  $\mu$ g/mL) agonistic antibodies or **(C)** K562 target cells for 24 h. Histogram bars represent the median (n = 4 individual experiments). Friedman test with Dunn's multiple comparisons test was performed.

139 **(D-G)** Healthy donors' blood NK cells were isolated from the blood of healthy donors and activated for 24 h with IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$  (0.1 to 100 ng/mL of each) alone or in combination with **(D)** IL-12 (10 ng/mL), **(E)** IL-2 (500 UI/mL), **(F)** IL-15 (10 ng/mL) or **(G)** IFN- $\alpha$  (500 UI/mL) prior to supernatant collection and IFN- $\gamma$  quantification by ELISA. Results are expressed as mean +/- SEM (n = 4 to 8 individual experiments). One-way ANOVA with Tukey's multiple comparisons test was performed for each cytokine concentration from 1 ng/mL.

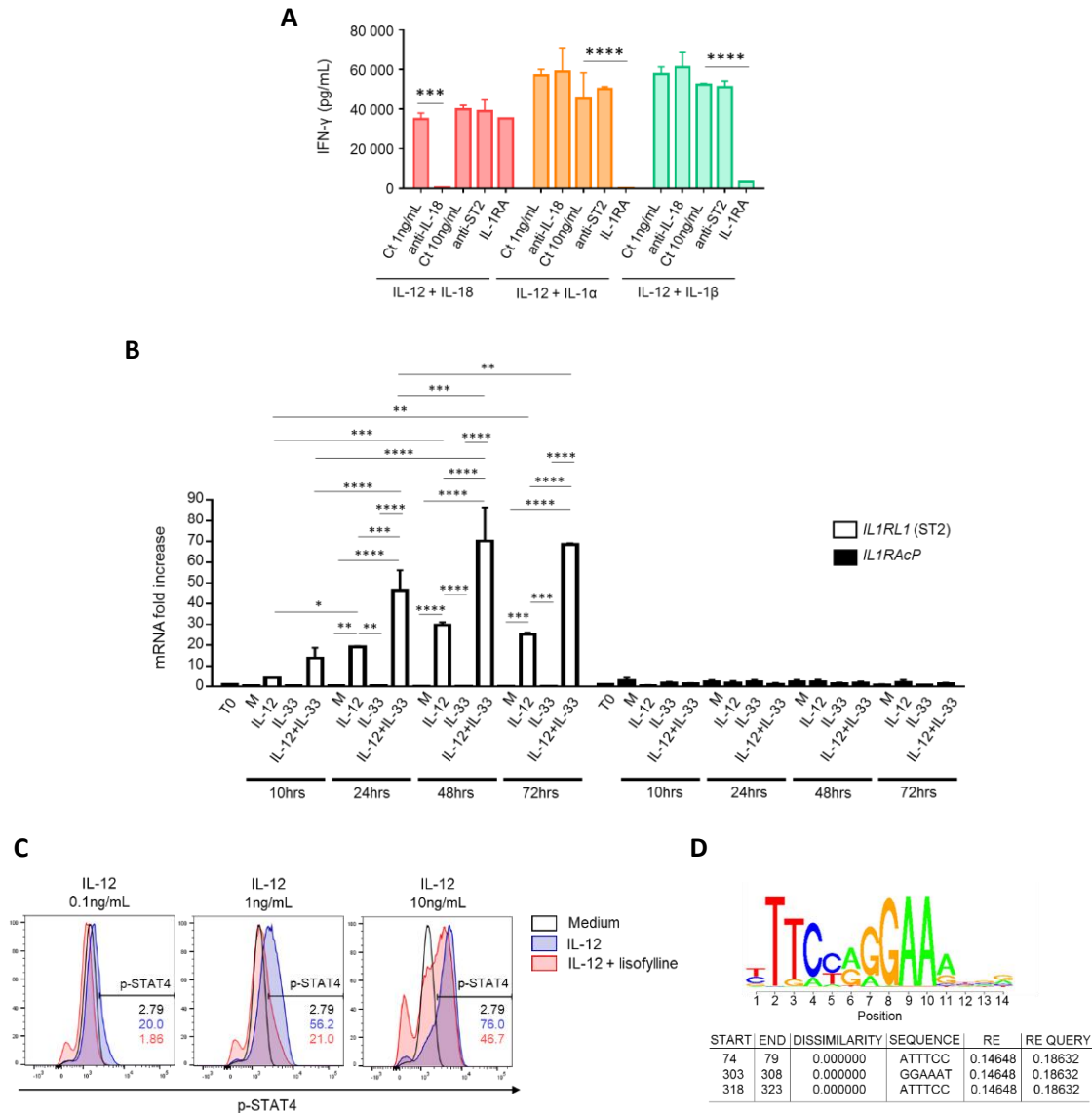


144 **(H)** Healthy donors' blood NK cells were activated as described above and supernatants were collected at several  
145 time points prior to IFN- $\gamma$  quantification by ELISA (n = 1).

146 **(I)** Healthy donors' blood NK cells were activated with indicated cytokines (10 ng/mL for each cytokine) for 24 h  
147 and co-cultured with calcein-loaded K562 target cells for 4 h at indicated effector to target ratios. Calcein release  
148 in the supernatant was quantified by fluorometry to calculate lysis percentage. Results are expressed as mean  
149 +/- SEM (n = 3 individual experiments).

150 **(J)** Healthy donors' blood NK cells activated with indicated cytokines (10 ng/mL for each cytokine) for 24 h were  
151 exposed to K562 cells in a direct cytotoxic assay. Percentage of CD107+ NK cells are shown. Results are expressed  
152 as mean + SEM (n = 2 individual experiments).

153



154

155 **Fig. S2. IL-12 induces ST2 expression on a subset of NK cells in a STAT-4-dependent manner**

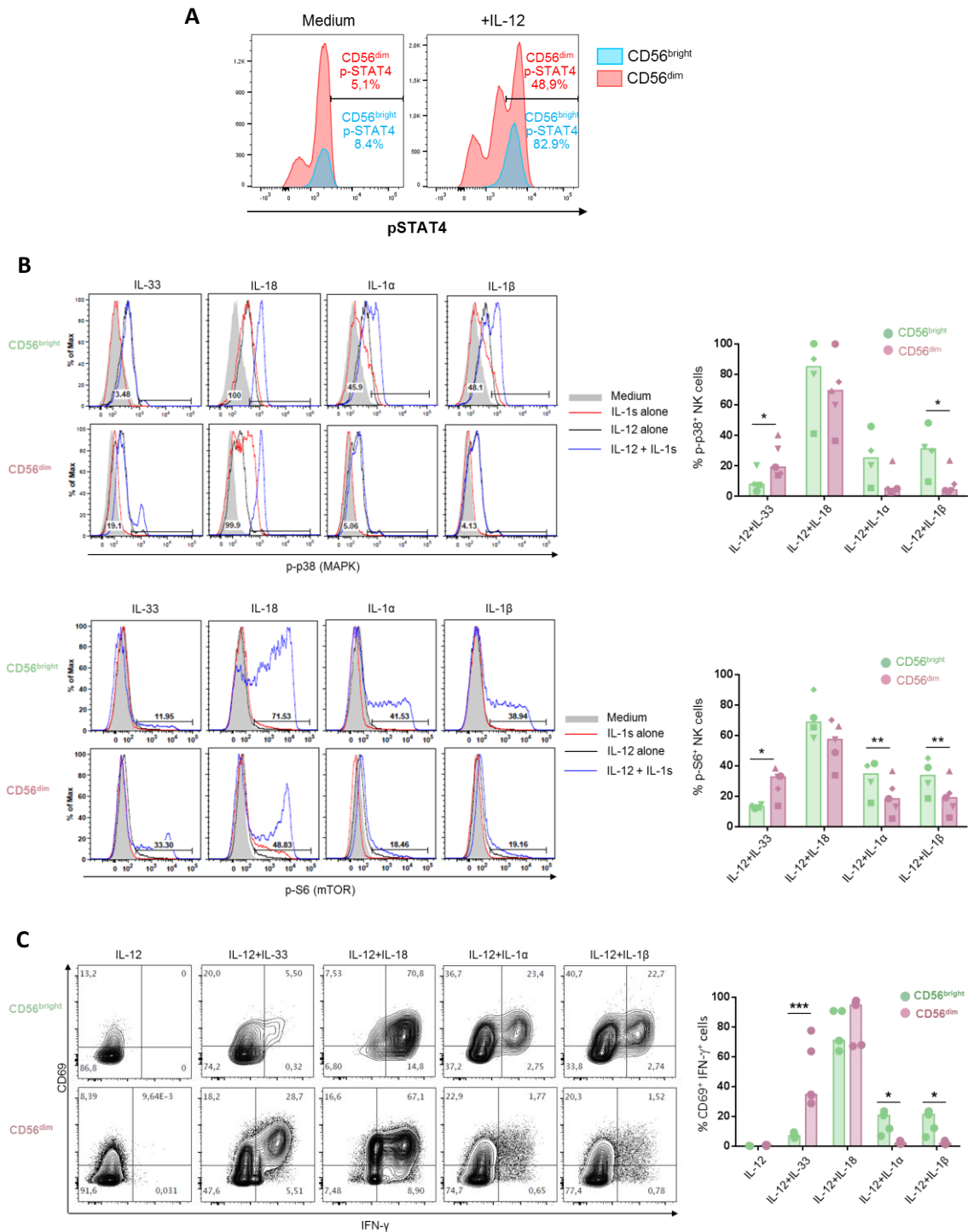
156 Healthy donors' blood NK cells were activated as indicated (10 ng/mL of each cytokine).

157 **(A)** Healthy donors' blood NK cells were activated by different cytokine combinations in the presence of anti-ST2  
 158 (10 µg/mL), anti-IL-18 (1 µg/mL), control mlgG1 antibodies (1 or 10 µg/mL as control for anti-IL-18 or anti-ST2,  
 159 respectively) or IL-1RA antagonist (100 ng/mL). Supernatants were collected after 24 h and IFN-γ secretion was  
 160 quantified by ELISA. Results are expressed as mean + SD and are representative of three individual experiments.  
 161 Two-way ANOVA with Tukey's multiple comparisons test was performed.

162 **(B)** *IL1RL1/ST2* and *IL1RAcP* relative mRNA expression was analyzed by RT-qPCR at indicated times. Results are  
 163 expressed as mean + SD and are representative of two individual experiments. Two-way ANOVA with Tukey's  
 164 multiple comparisons test was performed.

165 **(C)** NK cells were treated or not with lisofylline (500  $\mu$ M) for 24 h prior to activation with IL-12 (0.1 to 10 ng/mL)  
166 for 5 min and p-STAT4 intracellular levels were analyzed by flow cytometry (n = 3 individual experiments).

167 **(D)** *In silico* analysis of putative STAT-4 binding sites in *ST2* promoter region. Dissimilarity indicates the deviation  
168 between the identified sequence and the STAT-4 consensus binding site. RE and RE query values indicate the  
169 probability to randomly finding the motif respectively considering a model with equiprobability of the four  
170 nucleotides and a model with the same nucleotide frequency as the query sequence. A value of 0.001 means  
171 that the hit is only expected to occur by chance once in 1 Mb of sequence.



172

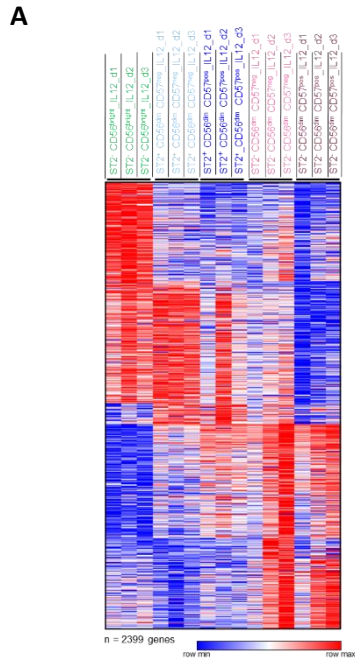
173 **Fig. S3. IL-33 preferentially activates a subset of CD56<sup>dim</sup> NK cells**

174 **(A)** Healthy donors' blood NK cells were activated with IL-12 (10 ng/mL) or not (medium) for 5 min and p-STAT-  
 175 4 intracellular levels were analyzed by flow cytometry on gated CD56<sup>bright</sup> vs CD56<sup>dim</sup> subsets. Data are  
 176 representative of three individual experiments.

177 **(B)** FACS-sorted CD56<sup>bright</sup> and CD56<sup>dim</sup> healthy donors' blood NK cells were activated with medium or IL-12  
178 alone for 24 h prior to the addition of IL-33, IL-18, IL-1 $\alpha$  or IL-1 $\beta$ , supplemented or not with IL-12. Each cytokine  
179 was used at 10ng/mL. p38 (MAPK) and S6 (mTOR) phosphorylation as analyzed 5 min or 1 h after the addition  
180 of IL-1 family cytokines, respectively. Representative histogram plots (left) and quantification (%) (right) of p-  
181 p38<sup>+</sup> or p-S6<sup>+</sup> NK cells after cytokine activation. Histogram bars indicate the median. Two-way ANOVA with  
182 Bonferroni's multiple comparisons test was performed; n = 4 to 5 experiments.

183 **(C)** Healthy donors' blood NK cells were activated as indicated for 24 h, each cytokine was used at 10 ng/mL.  
184 CD69 surface and IFN- $\gamma$  intracellular expression was analyzed by flow cytometry. CD56 surface expression levels  
185 were used to discriminate CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells. Representative dot plots (left) and quantification (%)  
186 (right) of CD69<sup>+</sup> IFN- $\gamma$ <sup>+</sup> NK cells after cytokine activation. Histogram bars indicate the median. Two-way ANOVA  
187 test with Bonferroni's multiple comparisons test was performed; n = 4 experiments.

188



**B**



189

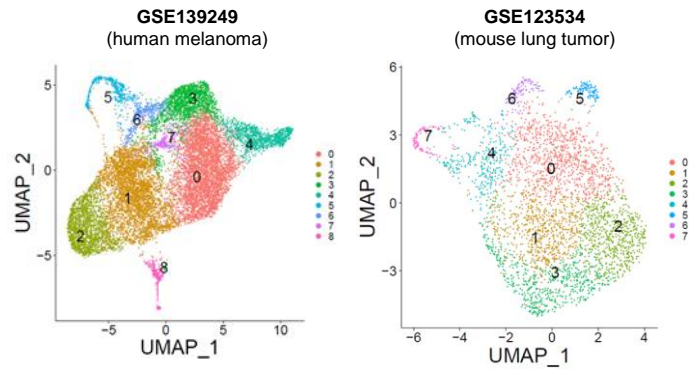
190 **Fig. S4. ST2<sup>+</sup> NK cells display a unique gene signature compared to CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cells**

191 **(A)** Heatmap representing all differentially-expressed genes (upregulated and downregulated) (n=2399 genes) in

192 each sorted healthy donors' blood NK cell subset compared to the two other subsets following IL-12 activation

193 (n = 3 individual donors).

194 **(B)** Expression levels of DEG (up and down) presented in heatmap in Fig. 3E.



195

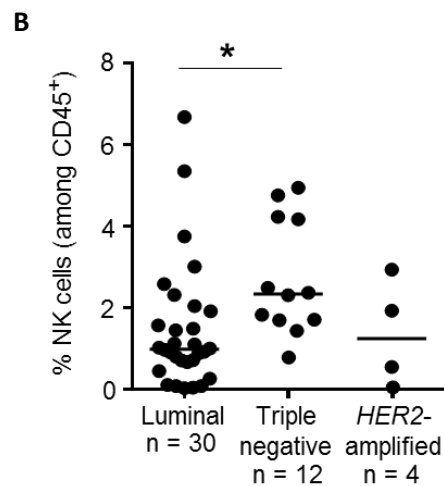
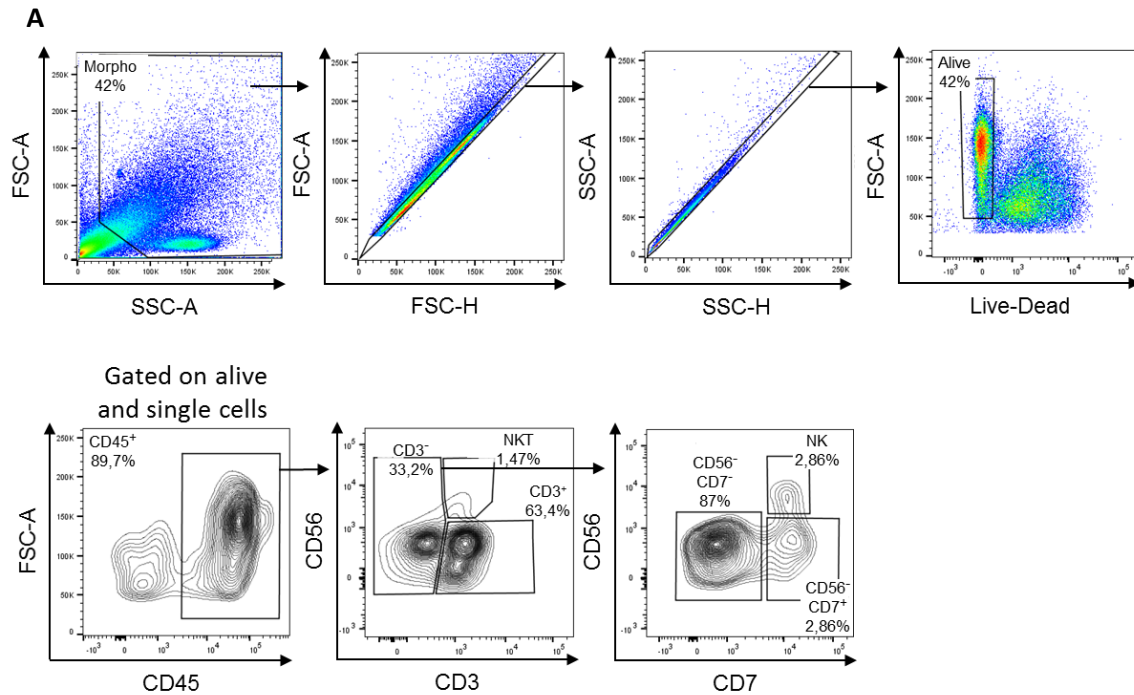
196 **Fig. S5. Unsupervised clustering of sc-RNAseq datasets**

197 UMAP plots of NK cells clusters annotated from human melanoma metastases (GSE139249) (42) and mouse lung

198 tumors (GSE123534) (43) sc-RNAseq public datasets.

199

200



201

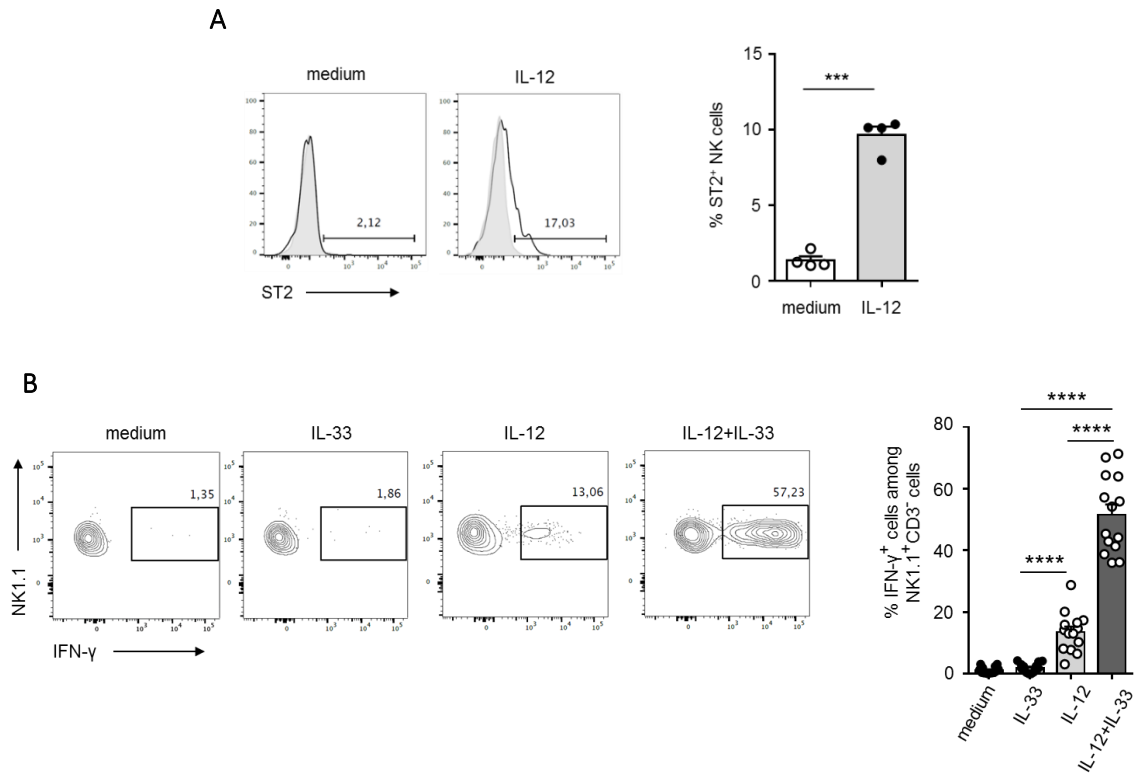
202 **Fig. S6. Gating strategy to identify NK cells in tumor cell suspensions by flow cytometry**

203 **(A)** Tumor cell suspensions were stained for CD45, CD3, CD56, and CD7 expression and tumor-infiltrating NK cells  
 204 were identified using gating strategy as illustrated. Cell viability was determined using Zombie dye (Biolegend).

205 **(B)** Cell suspensions were obtained from fresh breast tumors to analyze NK cell frequency among total infiltrating  
 206 cells by flow cytometry according to BC molecular types (n = 46 patients). Kruskal Wallis test with Dunn's multiple  
 207 comparisons test was performed.

208





209

210 **Fig. S7. IL-12 sensitizes mouse splenic NK cells to the production of IFN- $\gamma$  in response to IL-33**

211 **(A)** Splenic cells were activated with IL-12 (20 ng/mL) or not for 24 h prior to flow cytometry analysis of ST2

212 surface expression (black line) or corresponding isotypic control (grey). CD45, NK1.1, and CD3 were used to

213 identify NK cells. Representative histogram plots (left) and quantification (%) (right) of ST2<sup>+</sup> NK cells after medium

214 or IL-12 culture. Horizontal bars represent the mean + SEM (n = 4 individual mice). Paired two-tailed Student's

215 *t*-test was performed.

216 **(B)** Splenic cells were activated as indicated (20 ng/mL of each cytokine) for 24 h prior to flow cytometry analysis

217 of IFN- $\gamma$  intracellular expression. CD45, NK1.1, and CD3 were used to identify NK cells. Plots are representative

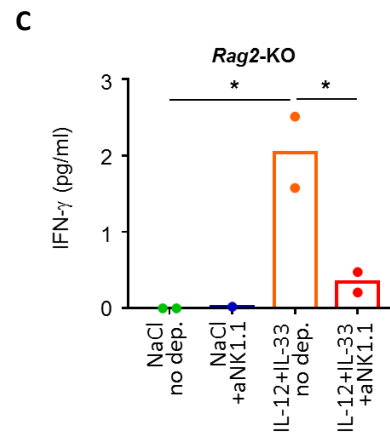
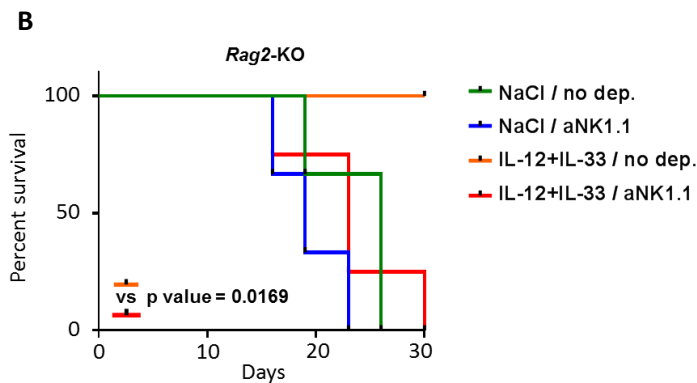
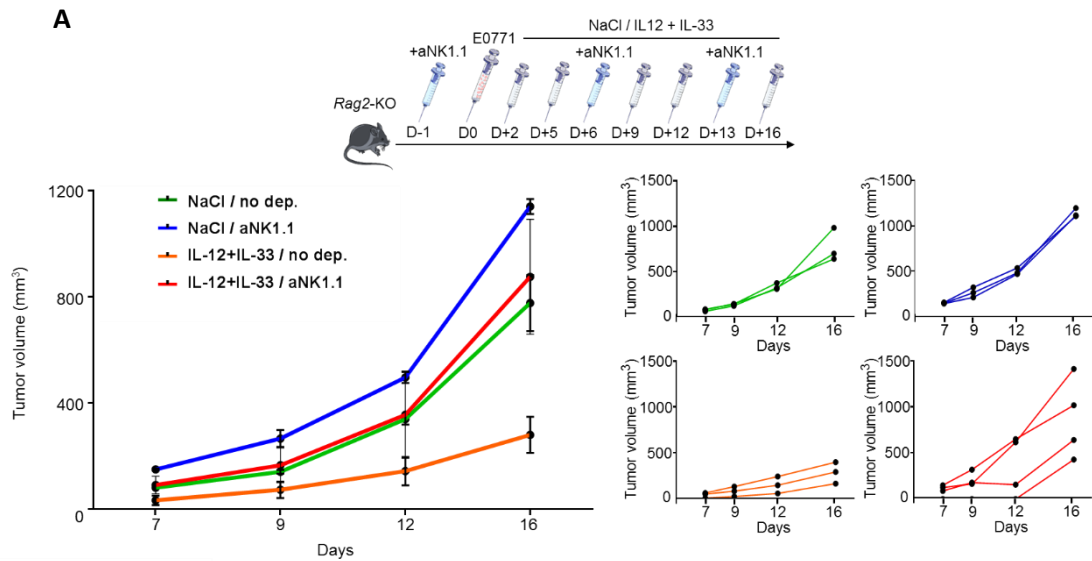
218 of four individual experiments with 3 to 4 mice/experiment and results for each individual mouse are presented

219 in the right panel. Results are expressed as mean + SEM. One-way repeated measures ANOVA with Tukey's

220 multiple comparisons test was performed.

221

222



223

224

225 **Fig. S8. Antitumoral effect of IL-33 and IL-12 combination is dependent on NK cells in *Rag2*-KO mice**

226 **(A)** *Rag2*-KO mice were injected intra-peritoneally with NaCl or anti-NK1.1 depleting antibody prior to intra-  
 227 mammary injection with  $2.5 \times 10^5$  E0771 cells on day 0 and then treated in the tumor area with NaCl or a  
 228 combination of 10 ng/mouse rIL-12 and 100 ng/mouse rIL-33 twice a week from day 2 to day 23. Primary  
 229 tumor growth was monitored in non-depleted *Rag2*-KO mice treated with NaCl (n = 3) or with rIL-12 and rIL-  
 230 33 combination (n = 3) or in NK cell-depleted WT mice treated with NaCl (n = 3) or with rIL-12 and rIL-33  
 231 combination (n=4). Two-way ANOVA with Tukey's multiple comparisons test was performed.

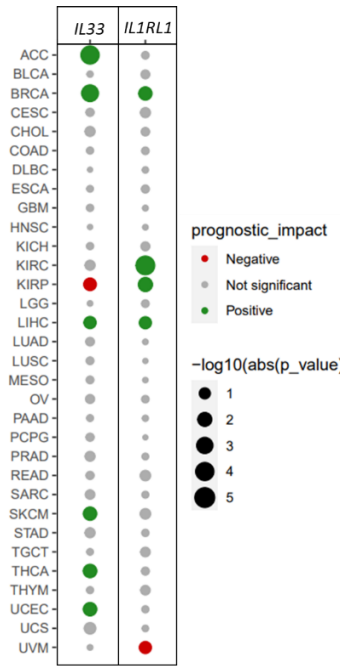
232 **(B)** Kaplan-Meier survival plots of non-depleted *Rag2*-KO mice treated with NaCl (n = 3) or with rIL-12 and rIL-  
 233 33 combination (n = 3) or in NK cell-depleted WT mice treated with NaCl (n = 3) or with rIL-12 and rIL-33  
 234 combination (n=4). Mice were sacrificed when longest side of primary tumor reached 17 mm. Log-rank test was

235 performed and the p-value is indicated for non-depleted mice treated with rmlL-12 + rmlL-33 vs NK cell-depleted  
236 mice treated with rmlL-12 + rmlL-33.

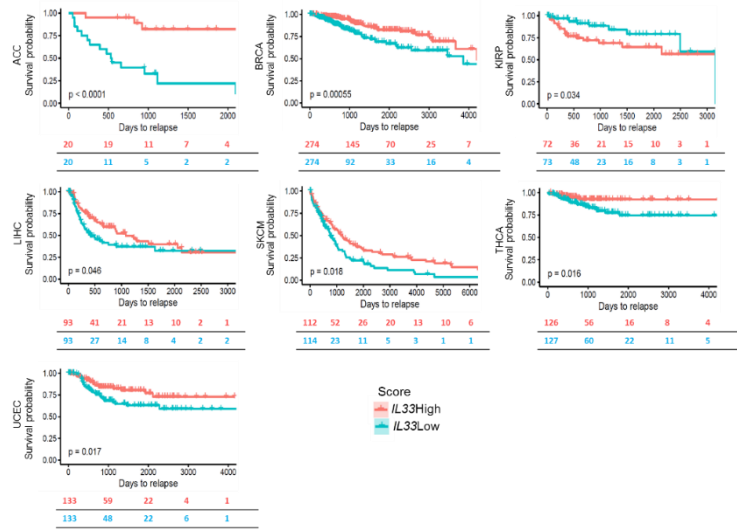
237 **(C)** Serum was retrieved from blood collected at day 5, 4hrs after NaCl or cytokine injection into the peritumoral  
238 area. IFN- $\gamma$  concentration was measured by ELISA in the serum of non-depleted *Rag2*-KO mice treated with NaCl  
239 (n = 3) or with rmlL-12 and rmlL-33 combination (n = 3) or in NK cell-depleted WT mice treated with NaCl (n = 3)  
240 or with rmlL-12 and rmlL-33 combination (n = 4). Histogram bars represent the median. Kruskal Wallis test with  
241 Dunn's multiple comparisons test was performed.

242

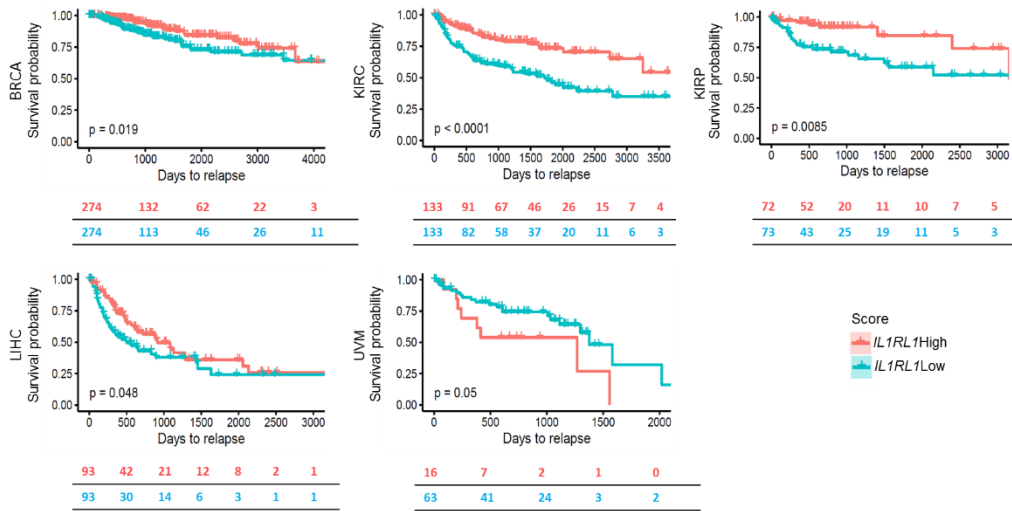
**A**



**B**



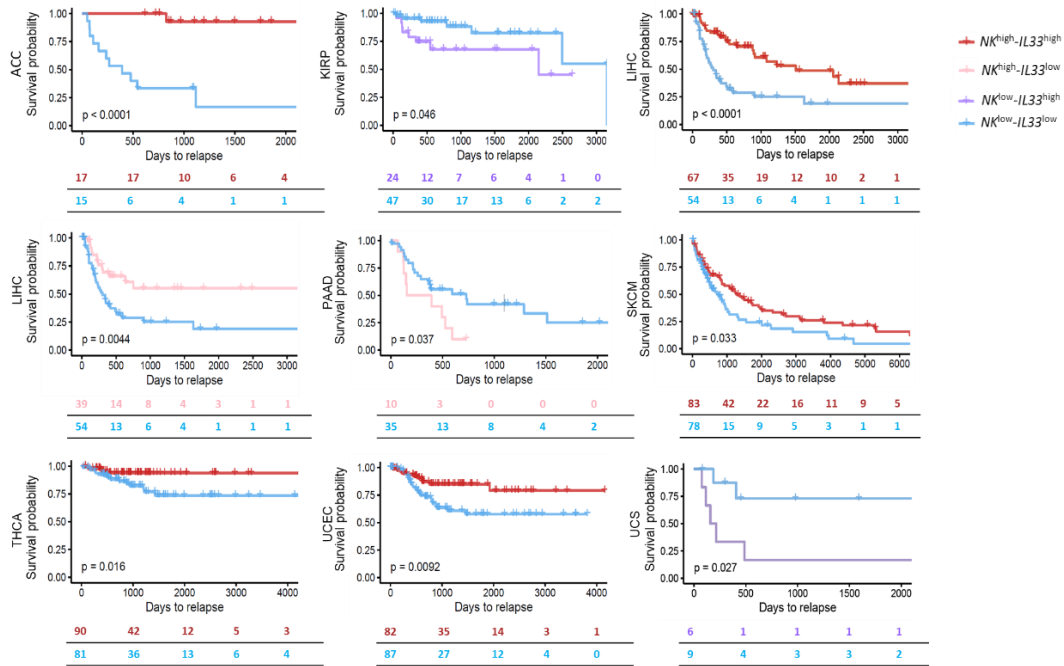
**C**



D

Variable	N	Hazard ratio	p	
Age_cat	(25,45]	150	Reference	
	(45,65]	500	0.71 (0.44, 1.16)	0.171
	(65,75]	166	0.76 (0.40, 1.45)	0.406
	(75,90.1]	106	1.17 (0.58, 2.35)	0.654
Molecular_subtype	Basal	167	Reference	
	Her2	71	0.80 (0.39, 1.66)	0.551
	LumA	495	0.57 (0.35, 0.91)	0.019
	LumB	189	0.43 (0.23, 0.80)	0.008
Stage	1	152	Reference	
	2	536	2.03 (0.95, 4.33)	0.067
	3	218	4.94 (2.28, 10.70)	<0.001***
	4	16	20.39 (7.94, 52.35)	<0.001***
IL33_cat	Low	229	Reference	
	Intermediate1	227	0.46 (0.26, 0.81)	0.007**
	Intermediate2	234	0.65 (0.38, 1.10)	0.107
	High	232	0.45 (0.26, 0.79)	0.006**

E



244

245 **Fig. S9. IL-33 is associated with increased overall patient survival for several cancers**

246 **(A)** Summary of p-values associated with the log rank test performed to evaluate prognostic value of *IL33* and  
 247 *IL1RL1* (ST2) in 32 human TCGA cancer data sets (the same as in Fig. 6A, MESO: Mesothelioma ; UVM: Uveal  
 248 Melanoma were added). Data are represented as a bubble map showing positive (green) or negative (red)  
 249 on progression-free survival. Dots size represents p-values obtained by log-rank test.

250 **(B)** Patients from TCGA database were stratified as high or low for *IL33* and progression-free survival was  
251 analyzed in all cancer data sets. Kaplan-Meier survival curves for ACC, BRCA, KIRP, LIHC, SKCM, THCA, and UCEC  
252 patients are represented and p-values were obtained with log-rank test.

253 **(C)** Patients from TCGA database were stratified as high or low for *IL1RL1* (ST2) and progression-free survival was  
254 analyzed in all cancer data sets. Kaplan-Meier survival curves for BRCA, KIRC, KIRP, LIHC, and UVM patients are  
255 represented and p-values were obtained with log-rank test.

256 **(D)** Multivariate Cox analysis of *IL33* impact on prognosis in breast cancers from TCGA dataset regarding the age,  
257 molecular subtype, and stage of the tumors p-values were obtained with log-rank test.

258 **(E)** Kaplan-Maier curves for progression-free survival for  $NK^{high}/IL33^{high}$ ,  $NK^{high}/IL33^{low}$  and  $NK^{low}/IL33^{high}$  scores as  
259 compared to  $NK^{low}/IL33^{low}$  in cancer datasets from TCGA that showed statistical significance in Fig. 7B. p-values  
260 were obtained with log-rank test.

261

262 **Table S1. Reagent and resources table**

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
<b>Flow cytometry antibodies (Human)</b>		
Anti-CD3-BV711	BD Bioscience	Clone UCHT1 Mouse IgG1 κ
Anti-CD3-PerCP	Miltenyi	Clone BW264/56, Mouse IgG2a κ
Anti-CD3-V450	BD Bioscience	Clone UCHT1 Mouse IgG1 κ
Anti-CD4-BB515	BD Bioscience	Clone RPAT4 Mouse IgG1 κ
Anti-CD4-FITC	BD Bioscience	Clone RPAT4 Mouse IgG1 κ
Anti-CD7-BV650	BD Bioscience	Clone M-T701, Mouse IgG1 κ
Anti-CD7-BV711	BD Bioscience	Clone M-T701, Mouse IgG1 κ
Anti-CD7-FITC	Beckman Coulter	Clone 8h8.1, Mouse IgG2a κ
Anti-CD8-PerCP-Cy5.5	BD Bioscience	Clone RPA T8, Mouse IgG1 κ
Anti-CD14-BV510	BD Bioscience	Clone MφP9, Mouse IgG2b κ
Anti-CD16-V500	BD Bioscience	Clone 3G8, Mouse IgG1 κ
Anti-CD25-PE	BD Bioscience	Clone M-A251, Mouse IgG1 κ
Anti-CD45-AF700	BD Bioscience	Clone HI30, Mouse IgG2b κ
Anti-CD45-BV510	BD Bioscience	Clone HI30, Mouse IgG2b κ
Anti-CD56-PEVio770	Miltenyi	Clone AF12-7H3, Mouse IgG1 κ
Anti-CD56-VioBright-FITC	Miltenyi	Clone AF12-7H3, Mouse IgG1 κ
Anti-CD57-APC	BD Bioscience	Clone HNK-1, Mouse IgM κ
Anti-CD69-APC	BD	Clone FN50, Mouse IgG1 κ
Anti-CD127-APC	eBioscience	Clone eBioRDR5, Mouse IgG1 κ
Anti-CD158(KIR2DL1/S1/S3/S5)-PE-Cy7	Biolegend	CloneHP-A4, Mouse IgG2b κ
Anti-CD158e1(KIR3DL1)-PE-Cy7	Biolegend	Clone DX9, Mouse IgG1 κ
Anti-IFNγ-BV421	Biolegend	Clone 4SB3, Mouse IgG1 κ
Anti-IL-1R1-BV421	BD Bioscience	Clone 89412, Mouse IgG1 κ
Anti-IL-18Ra-PE	BD Bioscience	Clone H44, Mouse IgG1 κ
Anti-p-p38-PE	BD Bioscience	Clone 36/p38, Mouse IgG1 κ

Anti-p-p65-PE	BD Bioscience	Clone K10-895.12.50, Mouse IgG2b κ
Anti-p-S6	Cell Signaling Technology	Clone D57.2.2E, Rabbit IgG
Anti-p-STAT4-PE	BD Bioscience	Clone 38/p-Stat4, Mouse IgG2b κ
Anti-ST2-PE	R&D	Polyclonal goat

#### **Flow cytometry antibodies (Mouse)**

anti-CD11b-APCeFluor 780	eBioscience	Clone M1/70, Rat IgG2b κ
anti-CD27-PerCPeFluor 710	eBioscience	Clone LG.7F9, Haster IgG
anti-IFN-γ-PE	Biolegend	Clone XMG1.2, Rat IgG1 κ
anti-IL-1R-BV421	BD Bioscience	Clone 4E2, Rat IgG2a κ
anti-IL-18Rα-PE	eBioscience	Clone P3TUNYA, Rat IgG2a κ
anti-NK1.1-APC	BD Bioscience	Clone PK136, Mouse IgG2a κ
anti ST2-PE	BD Bioscience	Clone U29-93, Rat IgG2a κ

#### **Other antibodies**

Anti-goat	Agilent	E0466
Anti-IL-18	MBL	Clone 125-2H, Mouse IgG1 κ
Anti-IL-33	R&D	Clone AF3625, Goat
Anti-NK1.1	BioXCell	Clone PK136
Anti-NKp30	R&D	Clone 210845 Mouse IgG2a κ
Anti-NKp46	R&D	Clone 195314, Mouse IgG2b κ
Anti-PanCK	Agilent	clone AE1-AE3,
Anti-p-STAT4	Cell Signaling Technology	Y693, Rabbit IgG
Anti-ST2	R&D	Clone 97203, Mouse IgG1 κ

#### **Reagents**

Calcein	Invitrogen	C1430
CTV	Invitrogen	C34557

#### **Continued**

<b>REAGENT OR RESOURCE</b>	<b>SOURCE</b>	<b>IDENTIFIER</b>
DAPI	Invitrogen	D1306



DNase I	Sigma-Aldrich	D4263
GolgiPlug	BD bioscience	555029
Lisofylline	Cayman chemicals	10010785
Lymphocyte Separation Medium	Eurobio	CMSMSL0101
Pharm Lyse™ Buffer	BD Biosciences	555899
Lyse/fix Buffer Phosflow	BD Biosciences	558049
Perm Buffer III Phosflow	BD Biosciences	558050
RPMI 1640 Medium, GlutaMAX	Gibco	61870036
Sulfinpyrazone	Sigma-Aldrich	S9509
Type IV collagenase	Sigma-Aldrich	C5138
Zombie Dye Aqua	Biolegend	423101

### Cytokines

Recombinant Human IFN- $\alpha$ 2b	Schering-Plough	
Recombinant Human IL-1 $\alpha$	Peptotech	200-01A
Recombinant Human IL-1 $\beta$	Peptotech	200-01B
Recombinant Human IL-1RA	Peptotech	200-01RA
Recombinant Human IL-2	Chiron	
Recombinant Human IL-12	Miltenyi	130-096-704
Recombinant Human IL-15	Peptotech	200-15
Recombinant Human IL-18	MBL	B003-5
Recombinant Human IL-33	Miltenyi	130-109-378
Recombinant Mouse IL-1 $\alpha$	Miltenyi	130-094-050
Recombinant Mouse IL-1 $\beta$	Miltenyi	130-094-053
Recombinant Mouse IL-12	Miltenyi	130-096-707
Recombinant Mouse IL-12 ( <i>in vivo</i> )	R&D	419-ML
Recombinant Mouse IL-18	MBL	B002-5
Recombinant Mouse IL-33	Miltenyi	130-112-958
Recombinant Mouse IL-33 ( <i>in vivo</i> )	Biolegend	580502

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**Kits**

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Bio-Plex Pro™ Human Cytokine 17-plex	BioRad	M5000031YV
ChIP-IT High Sensitivity	Active Motif	53040
Extra Sensitive IFN gamma Mouse ELISA Kit	ThermoFischer	BMS609
FoxP3/Transcription Factor Staining Set	eBioscience	00-5523-00
Human IFN-γ DuoSet ELISA kit	R&D	DY285B
iScript Reverse Transcription kit	BioRad	1708840
MycoAlert™ Mycoplasma Detection Kit	Lonza	LT07-118
NK Cell Isolation Kit, human	Miltenyi	130-092-657
NucleoSpin RNA Kit	Macherey-Nagel	740955

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**Oligonucleotides**

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<i>GADD45a</i> TaqMan	ThermoFischer	Hs00169255_m1
Negative Primer Set 1 and 2	Active Motif	71001 and 71002
<i>IL1Rap</i> TaqMan	ThermoFischer	Hs00895050_m1
<i>IL1RL1</i> TaqMan	ThermoFischer	Hs00249384_m1
<i>IL1RL1</i> (promoter) Forward	Agilent	5'-GTGATCATCGGGTTCAGCTTATC-3'
<i>IL1RL1</i> (promoter) Reverse	Agilent	5'-GCTTTACTAAATACAACAGCCAGCCT-3'
SimpleChIP Human <i>PRF1</i> Primers	Cell Signaling Technology	9014S

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