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Pharmacological targeting Netrin-1 inhibits EMT in cancer

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36

37 Epithelial-to-Mesenchymal transition (EMT) regulates tumour initiation, progression,
38 metastasis and resistance to anti-cancer therapy¹⁻⁷. Whereas great progress had recently
39 been made in understanding the role and mechanisms that regulate EMT in cancer, no
40 therapeutic strategy to pharmacologically target EMT had been identified so far. Here,
41 we found that Netrin-1 is upregulated in a primary mouse model of skin squamous cell
42 carcinoma (SCCs) presenting spontaneous EMT. Pharmacological inhibition of Netrin-1
43 by administrating NP137, an anti-Netrin-1 blocking monoclonal antibody currently used
44 in clinical trials in human cancer⁸, decreased the proportion EMT tumour cells in skin
45 SCCs, as well as decreased the number of metastasis and increased the sensitivity of
46 tumour cells to chemotherapy. Single-cell RNA-seq revealed the presence of different
47 EMT states including epithelial, early and late hybrid EMT as well as fully EMT states in
48 control SCCs. In contrast, administration of NP137 prevents the progression of cancer
49 cells towards a late EMT state and sustains tumour epithelial states. ShRNA knockdown
50 (KD) of Netrin-1 and its receptor Unc5b in EPCAM+ tumour cells inhibited EMT *in vitro*
51 in the absence of stromal cells and regulated a common gene signature promoting tumour
52 epithelial state and restricting EMT. To assess the relevance of these findings to human
53 cancers, we treated mice transplanted with A549 human cancer cell line that undergoes
54 EMT following TGF- β 1 administration^{9,10} with NP137. Netrin-1 inhibition decreased
55 EMT in A549 cells *in vivo*. Altogether, our results identify a new pharmacological strategy
56 to target EMT in cancer opening novel therapeutic interventions for anti-cancer therapy.
57

58 **Introduction**

59 Epithelial-to-Mesenchymal Transition (EMT) in which tumour cells (TCs) lose their
60 epithelial characteristics and acquire mesenchymal features, is a key driver of tumour
61 heterogeneity and has been associated with tumour initiation, progression, metastasis and
62 resistance to chemotherapy or immunotherapy^{2,4-7,11,12}. Whereas great progress in the
63 understanding of the role and the mechanisms by which EMT regulates these different cancer
64 features has been achieved, there is still very little non-genetic pharmacological interventions
65 that allow to inhibit EMT in primary tumours, decrease their metastatic potential or potentiate
66 the response to anti-cancer therapy.

67

68 **Netrin-1 is expressed in EMT skin SCC**

69 To identify novel therapeutic strategies that can inhibit EMT, we searched in RNA-
70 sequencing from epithelial and mesenchymal TCs isolated from a skin squamous cell
71 carcinoma (SCC) model that presented spontaneous EMT
72 (*Lgr5CREER/KRasG12D/p53cKO/Rosa-YFP or LKPR*)¹³⁻¹⁵ for secreted factors preferentially
73 expressed by mesenchymal EPCAM- TCs compared to epithelial EPCAM+ TCs and for which
74 specific therapy targeting this factor was available and currently administrated to cancer
75 patients. We found that *Netrin-1* and its receptor *Unc5b* were overexpressed in EPCAM- EMT
76 TCs as compared to EPCAM+ epithelial TCs (Fig. 1a, b). Netrin-1 has been shown to regulate
77 tumour progression in multiple cancer models by preventing apoptosis of TCs, promoting neo-
78 angiogenesis and controlling the pro-tumorigenic cancer associated fibroblast function¹⁶⁻²⁷. The
79 therapeutic anti-Netrin-1 blocking antibody NP137 is currently tested in clinical trial for the
80 treatment of cancer patients⁸.

81

82

83 **Netrin-1 overexpression increases EMT**

84 To assess Netrin-1 promotes EMT, we first overexpressed human NETRIN-1 in LKPR
85 mouse (*Lgr5CREER/KRasG12D/p53cKO/Rosa-YFP/Rosa-NETRIN-1*) (Extended data 1a-c).
86 Following NETRIN-1 overexpression, there was an increase in the number of tumours per
87 mouse (Fig. 1c) and the proportion of TCs that underwent EMT as compared to control mice
88 (Fig. 1d, Extended data 1d). As previously reported, EMT is not a binary process and occurs in
89 a step wise manner through the presence of intermediate states of EMT^{3,6,7,15,28-30}. We have thus
90 assessed by histological analysis and by immunofluorescence the impact of NETRIN-1
91 overexpression on the different tumour states including epithelial (KRT14+/VIM-), hybrid
92 EMT (KRT14+/VIM+) and late EMT (KRT14-/VIM+) previously described in this model¹⁵.
93 Immunostaining revealed that control skin SCCs were heterogeneous and 20% of them were
94 epithelial (KRT14+/VIM-), 35% presented hybrid EMT (KRT14+/ VIM+) and 45% had full
95 EMT (KRT14-/VIM+) (Fig. 1e, f). By contrast, overexpression of NETRIN-1 led to a
96 significant increase in tumours with full EMT (70%) and a decrease of SCCs with epithelial
97 phenotype (3%) (Fig. 1e, f), in good accordance with the EPCAM expression quantified by
98 FACS (Fig. 1d). These data demonstrate that overexpression of NETRIN-1 promotes tumour
99 initiation and EMT in a primary model of skin SCC.

100

101 **Targeting Netrin-1 inhibits EMT**

102 Although previous studies reported *in vitro* an upregulation of Netrin-1 occurring in
103 several cancers presenting EMT³¹⁻³⁴, the effect of pharmacological inhibition of Netrin-1 on
104 EMT *in vivo* has not been investigated so far. To assess whether the pharmacological inhibition
105 of Netrin-1 inhibits EMT, we administrated an anti-Netrin-1 blocking monoclonal antibody
106 (NP137) to LKPR mice 4 weeks following Tamoxifen administration and determined the
107 impact of Netrin-1 inhibition on tumour formation and EMT (Extended data 1e). NP137

108 administration three times per week led to a decrease of 50% in the number of SCCs per mouse
109 (Fig. 1g). FACS quantification of the percentage of YFP+/EPCAM+ TCs (i.e., epithelial cells)
110 and YFP+/EPCAM- TCs (i.e. cells that underwent EMT) showed that NP137 administration
111 decreased the proportion of EPCAM- TCs that underwent EMT (41% in NP137-treated vs 77%
112 in control mice) in primary skin SCCs presenting spontaneous EMT, showing that in the LKPR
113 mouse model, pharmacological targeting of Netrin-1 inhibits EMT (Fig. 1h). To determine
114 whether Netrin-1 inhibition differentially affects the different EMT states, we assessed by
115 immunofluorescence the proportions of tumours states. NP137 administration increased the
116 proportion of epithelial state (54% in NP137-treated vs 31% in the control mice), whereas the
117 proportion of hybrid EMT was unchanged (16% in NP137-treated vs 12% % in the control
118 mice) and the proportion of late EMT state was decreased (30% in NP137-treated vs 56% in
119 the control mice) (Fig. 1i, j). These data demonstrate that pharmacological targeting of Netrin-
120 1 inhibits EMT in primary mouse model of cancer.

121

122 **Targeting Netrin-1 inhibits metastasis**

123 EMT has been shown to play an important role for metastasis formation in different
124 mouse models and is associated with resistance to anti-cancer therapy^{1,4-6,11,12,35}. We first
125 assessed whether pharmacological inhibition of Netrin-1 in LKPR mice decreased primary
126 metastasis formation. NP137 administration strongly decreased the number of lung metastasis
127 in LKPR mice (Fig. 2a), suggesting that Netrin-1 is important for the formation of spontaneous
128 lung metastasis. As the number of tumours was reduced in LKPR mice treated with NP137, it
129 is possible that the reduction of the number of lung metastases was the consequence of the
130 reduced number of tumours. To clarify this issue, we injected YFP+ TCs intravenously and
131 assessed the number of lung metastases upon pharmacological Netrin-1 inhibition. NP137
132 administration strongly decreased the number of lung metastases following tail vein injection

133 of FACS isolated YFP+ TCs from LKPR mice, demonstrating that Netrin-1 inhibition directly
134 inhibits metastasis formation (Fig. 2b).

135

136 **NP137 sensitizes cancer cells to chemotherapy**

137 EMT has been shown to promote resistance to chemotherapy in skin SCCs^{2,36}. To assess
138 whether pharmacological targeting of Netrin-1 can sensitize TCs to chemotherapy, we
139 administrated Cisplatin/5-Fluoracil (5-FU), a standard chemotherapy for the treatment of
140 human SCCs^{37,38} and assessed the impact of Netrin-1 inhibition on the response to
141 chemotherapy. The combination of NP137 with Cisplatin/5-FU significantly potentiated the
142 effect of Cisplatin/5-FU on the inhibition of tumour growth in primary SCCs from LKPR mice
143 (Fig. 2c). These data demonstrate that pharmacological targeting of Netrin-1 can decrease the
144 cancer features associated with EMT including metastasis and resistance to therapy.

145

146 **Effect of NP137 on stromal cells**

147 To assess in a comprehensive manner the impact of Netrin-1 inhibition on the different
148 EMT tumour states and the composition of the tumour stroma, we performed single-cell RNA-
149 sequencing (10x Genomics Chromium) of TCs and their associated stromal cells in 2 control
150 and 2 NP137-treated primary skin SCCs. Unsupervised clustering revealed the presence of
151 different cell populations in primary skin SCCs (Extended data 2), including TCs identified by
152 *Yfp* and expression and *Epcam* (Extended data 3a, b), immune cells (marked by *CD45/Ptprc*
153 expression), combined each other with *CD86* for myeloid cells, *Cxcr2* for neutrophils or *Cd3d*
154 for lymphocytes T cells), cancer associated fibroblasts (CAFs, expressing *Acta2* and *Pdgfra*
155 and negative for *Yfp*), and endothelial cells (expressing *Pecam1*) (Extended data 3c, d). This
156 clustering reveals that *Ntn1* is expressed in EMT TCs and CAFs in skin SCC from LKPR
157 presenting spontaneous EMT (Extended data 3e).

158 After integration of the tumour of the tumour microenvironment across the conditions, we
159 observed that NP137 administration decreased the proportion of TCs and changed the
160 composition of the tumour stroma, with a relative increase in the proportion of CAFs (Extended
161 data 4a-d). Using scCODA to perform differential abundance analysis, we found no significant
162 difference in the proportion of the different CAF clusters (3 myCAFs, 2 iCAFs, proliferative
163 and glycolysis CAFs) or gene expression within these clusters between the two control and two
164 NP137-treated mice (Extended data 4e-h). Immunostaining analyses confirmed the relative
165 increase of CAFs following NP137 administration (Extended data 4i).

166

167 **Impact of NP137 on tumour cell states**

168 To assess more specifically the impact of Netrin-1 inhibition on the tumour states, we
169 clustered at higher resolution the YFP⁺ TCs of control and NP137-treated mice (Fig. 3a, b). In
170 the control tumour, different clusters corresponding to distinct EMT cell states ranging from
171 epithelial (*Epcam*⁺/*Krt14*⁺/*Vim*⁻), early hybrid EMT (*Epcam*⁻/*Krt14*⁺/*Vim*⁺), late hybrid EMT
172 (*Epcam*⁻/*Krt14*⁻/*Krt8*⁺/*Vim*⁺/*Pdgfra*⁻), and late EMT (*Epcam*⁻/*Krt14*⁻, *Krt8*⁻/*Vim*⁺/*Pdgfra*⁺)
173 were identified (Fig. 3a, Extended data 5a). NP137 administration was associated with an
174 increase in the proportion of epithelial TC state, a similar proportion of early, a decreased
175 proportion of late hybrid EMT and a strong decrease in the late EMT as compared to control
176 (Fig. 3b, c, Extended data 5b, c). *In situ* immunofluorescence for KRT14, VIM, KRT8 and
177 PGFRA confirmed the increase of epithelial states and the decrease of the late EMT tumour
178 state upon NP137 administration (Fig. 3d).

179

180 Spatial transcriptomic analysis using Visium 10x unravelled the spatial localization of
181 the different tumour states identified by single-cell RNA-seq and showed that they were
182 localised in distinct niches as previously reported¹⁵. NP137 administration increased the

183 epithelial state (*Epcam*⁺/*Krt14*⁺) and inhibited the occurrence of late EMT state (*Krt14*⁻/*Krt8*⁻
184 */Vim*⁺) blocking EMT progression at the hybrid EMT state (*Krt8*⁺/*Vim*⁺) (Fig. 3e, f, Extended
185 data 6).

186

187 To further understand the mechanisms and signalling pathways by which NP137
188 administration regulates EMT, we have performed pathway analyses using the MSigDB
189 Hallmark genesets³⁹ on the single-cell RNA-seq data. These analyses revealed that pathways
190 associated with EMT, hypoxia, angiogenesis and inflammatory response were all significantly
191 decreased in TCs following Netrin-1 inhibition (Fig. 3g).

192

193 Lineage trajectory analysis revealed that two distinct lineage trajectories could be
194 identified in the control tumour, with a trajectory going from epithelial cells towards hybrid
195 EMT and another trajectory going from the epithelial state toward the late full EMT state (Fig.
196 4a). NP137-treated tumour cells were characterized by different lineage trajectories with the
197 absence of a trajectory toward the late EMT state expressing high level of *Aqp1* and the
198 appearance of two new trajectories toward epithelial states (Epithelial-B1 and Epithelial-B2),
199 and two different hybrid EMT trajectories (Fig. 4b). Epithelial-B1, was characterized by high
200 expression of *Krt15*, while gene ontology (GO) terms enrichment analysis of marker genes of
201 cells belonging to Epithelial-B2 revealed an upregulation of glycolysis and a higher
202 keratinization (Fig. 4b, c).

203 *In situ* analysis using RNA-fish and Visium 10x spatial transcriptomic on tumour
204 sections of control and NP137-treated tumours showed a decrease of *Aqp1* expressing cells
205 (Fig. 4d) and an increase of TCs expressing *Krt15* following Netrin-1 inhibition confirming the
206 inhibition of the late EMT trajectory and the increase of epithelial states upon NP137
207 administration (Fig. 4e). Altogether these data reveal that EMT is characterized by the presence

208 of different EMT tumour states with two different differentiation trajectories of epithelial state
209 toward distinct EMT states and that the pharmacological targeting of Netrin-1 inhibits the
210 switch of epithelial into the late EMT state and promotes tumour cell differentiation into
211 epithelial state.

212

213 **Netrin-1/Unc5b axis promotes EMT**

214 Netrin-1 has been proposed to play a pleiotropic role in cancer including a cellular
215 autonomous role in tumour proliferation and apoptosis as well as a non-cellular autonomous
216 mechanism regulating tumour growth by controlling tumour angiogenesis and CAFs functions
217 ¹⁶⁻²⁷. Our single-cell analysis shows that NP137 administration modulates the composition of
218 the tumour microenvironment and the proportion of the different EMT tumour cell states.
219 NP137 has been shown to specifically inhibit the interaction of Netrin-1 with its receptor
220 Unc5b⁴⁰. To assess whether the regulation of EMT and the promotion of pro-epithelial states
221 by NP137 administration is the consequence of a disruption of a paracrine or an autocrine
222 Netrin-1/Unc5b signalling axis that directly regulates EMT states, we studied the impact of
223 genetic knockdown (KD) of *Netrin-1* and *Unc5b* on EMT states in a tumour cell autonomous
224 manner *in vitro* in the absence of the tumour microenvironment. FACS isolated EPCAM+ TCs
225 from primary SCCs from LKPR mice and cultured *in vitro* progressively underwent EMT as
226 shown by the progressive loss of EPCAM expression by TCs (Fig. 5a) with an average of 60%
227 of EPCAM+ TCs that switch to EPCAM- TCs over 2 weeks in culture. Interestingly, both
228 ShRNA KD of *Netrin-1* and *Unc5b*, resulted in a strong decrease of the ability of EPCAM+
229 TCs to switch to the EPCAM- EMT phenotype (Fig. 5a). The inhibition of EMT upon ShRNA
230 KD of *Netrin-1* and *Unc5b* was accompanied by a decrease in cell migration that was not further
231 enhanced by NP137 administration (Fig. 5b).

232

233 To assess the molecular mechanisms by which Netrin-1 and Unc5b signalling regulate
234 EMT, we performed bulk RNA-seq of EPCAM+ TCs from control, *Netrin-1* KD, and *Unc5b*
235 KD TCs cultured *in vitro* 6 days after plating of FACS isolated Epcam+ TCs. We found that
236 more than 50% of the genes downregulated and upregulated following *Unc5b* KD were also
237 differentially regulated by *Netrin-1* KD (Fig. 5c), showing that Netrin-1 and Unc5b regulate a
238 similar signalling pathway and transcriptional program. Consistent with the results of the
239 single-cell RNA-seq following NP137 administration, *Netrin-1* and *Unc5b* KD promote the
240 expression of genes associated with the epithelial state (eg: *Krt14*, *Krt15*, *Involucrin*, *Claudin*)
241 (Fig. 5d) and decrease the expression of genes associated with EMT and promoting EMT (eg:
242 *Aqp5*, *Nrp1*, *Nr2f2*, *Twist1*, *Twist2*) (Fig. 5e-g). Altogether, these data demonstrate that
243 targeting Netrin-1/Unc5b signalling axis in TCs decrease EMT and promote epithelial state in
244 skin SCCs in a cellular autonomous manner independently of the tumour microenvironment.

245

246 **NP137 prevents EMT in human cancer**

247 To assess the human and clinical relevance of our findings, we first assessed the
248 correlations between Netrin-1 expression, Unc5b expression and EMT signature in human non-
249 small cell lung carcinoma (lung SCC and lung adenocarcinoma) and melanoma. We used
250 several human EMT signature previously described^{12,39,41} and the gene expression dataset from
251 the panTCGA version of lung SCC (LUSCC) (484 primary tumors), lung adenocarcinoma
252 (LUAD) (510 primary tumors) and skin cutaneous melanoma (SKCM) (76 primary tumors, 367
253 metastasis). While Netrin1 expression was not strongly associated with the EMT score, there
254 was a very good correlation between the expression of Netrin1 receptor Unc5b and the different
255 EMT scores in these 3 cancers (Fig 6a, b).

256 To assess the functional relevance of these data, we then assessed the impact of blocking
257 Netrin-1/Unc5b signaling on EMT in A549 human lung adenocarcinoma cell line, the most

258 commonly used human cancer cell line that can undergo EMT in a plastic manner upon TGF-
259 β 1 administration^{9,10}. A549 cells were treated with recombinant TGF- β 1 *in vitro* for 3 days,
260 which promoted mesenchymal cell morphology, increased Netrin-1 expression and promoted
261 EMT as shown by the upregulation of Vimentin and downregulation of E-cadherin expression
262 (Fig. 6c, d). We next performed subcutaneous grafting of these A549 cells that underwent EMT
263 cells into immunodeficient mice and administrated anti-Netrin-1 antibody every two days.
264 Interestingly, immunostaining analysis revealed that Netrin-1 inhibition significantly increases
265 the number of TCs expressing high levels of epithelial marker E-cadherin (Fig. 6e, f),
266 demonstrating that Netrin-1 inhibition decreases EMT in human cancer cells *in vivo*. Treatment
267 of a human endometrial adenocarcinoma cell line (Ishikawa cells) with NP137 following their
268 transplantation into immunodeficient mice decreased tumour growth (Extended data 7a) and
269 resulted in an increase in epithelial gene expression (Extended data 7b), further demonstrating
270 that targeting Netrin1 inhibits EMT. Consistent with the decrease of EMT induced by Netrin1
271 inhibition, we found that NP137 administration decreased cell migration *in vitro* (Extended data
272 7c).

273

274 **Discussion**

275 Our study demonstrates that pharmacological targeting of Netrin-1 using NP137, an
276 anti-Netrin-1 blocking monoclonal antibody currently tested in phase II clinical trials for the
277 treatment of different solid tumours is a safe and effective strategy to target EMT in primary
278 mouse and human tumours, decreasing lung metastasis and increasing the response of tumour
279 cells to chemotherapy.

280 Our bioinformatics analysis of single-cell RNA-seq data upon Netrin-1 inhibition
281 combined with *in situ* characterization revealed the molecular mechanisms by which Netrin-1
282 regulates EMT and promotes a late EMT differentiation trajectory. Inhibition of Netrin-1

283 induces a switch in the lineage differentiation of tumour cells that redirect the tumour
284 differentiation toward an epithelial tumour state that is more sensitive to chemotherapy and less
285 prone to give rise to metastasis. Netrin-1 exerts its pro-EMT function by signalling to Unc5b,
286 which regulates positively the expression of a mesenchymal transcriptional program and
287 negatively the expression of genes controlling cell-cell adhesion and promoting the epithelial
288 differentiation program. The sensitization of tumour cells to chemotherapy following NP137
289 administration suggests that the combination of anti-Netrin-1 antibody with might be beneficial
290 for patients with cancer presenting EMT features.

291 We also demonstrated the human relevance of pharmacological targeting EMT
292 following treatment with anti-Netrin-1 blocking antibody in human lung cancer cell line and
293 endometrial adenocarcinoma that presents EMT plasticity, demonstrating that pharmacological
294 inhibition of EMT can be achieved in human cancers *in vivo* in pre-clinical settings.

295

296 Altogether, our study provides the proof of principle that pharmacological targeting of
297 EMT in primary mouse tumour model and human cancers is now possible. These results have
298 important implications for developing new strategies for anti-cancer therapy combining Netrin-
299 1 inhibition in patients with cancer presenting EMT, for the development of novel biomarkers
300 that will help to better stratify the cancer patients that are more likely to respond to anti-Netrin-
301 1 therapy.

302

303

304

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320

321 **AUTHOR CONTRIBUTION**

322 J.L., I.P., S.V. and C.B. designed the experiments and performed data analysis. J.L. and I.P.
323 performed most of biological experiments. S.V. performed most of bioinformatic analysis for
324 single-cell sequencing. N.R., Y.S. and A.S. helped for bioinformatic analysis. J.V.H. helped
325 with 10x single-cell sequencing. R.M.S. helped with RNA-Scope analysis. V.M., A.B., S.S.,
326 S.L., S.G. and S.B. helped with cell culture experiments immunostainings, blocking antibody
327 injection and follow-up of the mice. I.S., J.A., E.Z, C.D. and A.C. performed immunostaining
328 and quantification of EMT in human cancer samples. B.D., M.B and N.B performed biological
329 *in vivo* and *in vitro* experiments on Ishikawa endometrial cell lines. C.S and D.V. performed

330 bioanalysis from TCGA. C.D. performed FACS sorting. T.V. helped and supervised the single-
331 cell data analysis. P.M. and A.B. helped with the design of the experiments, data analysis and
332 provided NP137 antibody. All authors read and approved the final manuscript.

333

334 **COMPETING INTERESTS**

335 Authors declare potential competing interests.

336 J.L., P.M. A.B., B.D., M.B and N.B are employees of NETRIS Pharma, Lyon, France.

337 T.V. is co-inventor on licensed patents WO/2011/157846 (Methods for haplotyping single
338 cells); WO/2014/053664 (High-throughput genotyping by sequencing low amounts of genetic
339 material); WO/2015/028576 (Haplotyping and copy number typing using polymorphic variant
340 allelic frequencies).

341

342 **DATA AVAILABILITY STATEMENT**

343 All raw data sequencing data for mouse RNA-seq, single cell RNA-seq and 10x Visium have
344 been deposited in the gene expression Omnibus with the following accession number:

345 GSE234267

346

347

348 **FIGURE LEGENDS**

349

350 **Figure 1. Targeting Netrin-1 inhibits EMT.** **a, b**, Relative mRNA expression of *Ntn1* (**a**) and
351 *Unc5b* (**b**) in EPCAM+ (n= 4) as compared to EPCAM- (n= 10) tumour cells (TCs) defined by
352 qRT-PCR (data are normalized to *Tbp* gene, mean \pm s.e.m., two-tailed Mann-Whitney U test).
353 **c**, Dot plot showing the number of tumours in control (n= 16) and NETRIN-1 gain of function
354 (n= 17) mice (mean \pm s.e.m., two-tailed t test). **d**, Proportion of EPCAM+ TCs in control (n=
355 34 tumours from n= 16 mice) and NETRIN-1 gain of function mice (n= 70 tumours from n=
356 17 mice) (mean \pm s.e.m., two-tailed t test). **e**, Co-immunostaining of YFP and KRT14 or
357 Vimentin in primary control and NETRIN-1 gain of function tumours (n= 21 tumours from 11
358 control mice and n= 34 tumours from 9 NETRIN-1 gain of function mice) (Scale bars, 20 μ m).
359 **f**, Percentage of tumours presenting epithelial (KRT14+/VIM-), hybrid EMT (KRT14+ and
360 VIM+) and full EMT (KRT14-/VIM+) (n= 21 tumours from n= 11 control mice and n= 34
361 tumours from n= 9 NETRIN-1 gain of function mice). **g**, Dot plot showing the number of
362 tumours in control (n= 10) and NP137 treated (n= 15) mice (mean \pm s.e.m., two-tailed t test).
363 **h**, Proportion of EPCAM+ TCs in control (n= 148 tumours from n= 20 mice) and NP137 -
364 treated (n= 117 tumours from 16 mice) skin SCC mice (mean \pm s.e.m., two-tailed t test). **i**, Co-
365 immunostaining of YFP and KRT14 or Vimentin in control and NP137-treated skin SCC (n=
366 32 tumours from 10 control mice and n= 24 NP137 treated SCCs from 9 mice) (Scale bars, 20
367 μ m). **j**, Percentage of tumours presenting epithelial (KRT14+/VIM-), hybrid EMT (KRT14+
368 and VIM+) and full EMT (KRT14-/VIM+) (n= 32 tumours from 10 control mice and n= 24
369 NP137-treated SCCs from 9 mice).

370

371 **Figure 2. Targeting Netrin-1 reduces metastasis and sensitizes tumour cells to**
372 **chemotherapy in skin SCC.** **a**, Dot plot showing the number of spontaneous lung metastases

373 in control (n= 12) and NP137-treated (n= 11) mice presenting skin SCC (mean \pm s.e.m, two-
374 tailed t test). **b**, Dot plot showing the number of lung metastases arising from the intravenous
375 injection of 1000 EPCAM- TCs (n= 18 mice injected control mice and n= 18 mice injected for
376 NP137-treatment) (mean \pm s.e.m, two-tailed t test). **c**, Relative tumour volume overtime of
377 control tumours (n= 29 from 5 mice) or upon therapy with Cisplatin/5-Fluoracil (5-FU) (n= 58
378 from 8 mice), anti-Netrin-1 antibody (n= 29 from 5 mice) or combination of Cisplatin/5-FU
379 and anti-Netrin-1 (n= 59 from 8 mice) (mean + s.e.m., two-tailed t test). Tumour volumes were
380 normalized to the tumour volume at the 1st day of chemotherapy.

381

382 **Figure 3. Pharmacological inhibition of Netrin-1 inhibits late EMT and promotes**
383 **epithelial tumour states. a, b**, Uniform Manifold Approximation and Projection (UMAP) plots
384 coloured by the EMT states for control (**a**) and NP137-treated YFP+ TCs skin SCC (**b**). The
385 colours represent the different tumour states. **c**, Boxplot depicting the proportion of the different
386 tumour states for the 4 samples in control and NP137-treated conditions. The centre line, top
387 and bottom of the boxplots represent respectively the median, 25th and 75th percentile and
388 whiskers are $1.5 \times$ IQR. Significant proportion changes are indicated by $FDR < 0.2$. **d**, Co-
389 immunostaining of YFP and KRT14, KRT8, Vimentin or PDGFRA in control (left) and anti-
390 Netrin-1 treated skin SCC (right) that define areas with different degrees of EMT (n= 3 control
391 tumours, n= 3 NP137-treated tumours) (Scale bars, 20 μ m). **e**, Spatial transcriptomic using 10x
392 VISIUM was conducted on tumour sections of control and NP137-treated mice. Normalized
393 gene expression values are visually represented as a colour gradient. **f**, Summary of the different
394 areas presenting different tumour states based on the expression of *Epcam*, *Krt14*, *Krt8* and *Vim*
395 (green area highlights epithelial state: *Epcam*+/*Krt14*+/*Vim*-; orange area highlights early
396 hybrid EMT state: *Epcam*-/*Krt14*+/*Vim*+; red area defines late hybrid EMT state: *Epcam*-
397 /*Krt14*-/*Krt8*+/*Vim*+; dark red defines late full EMT state: *Epcam*-/*Krt14*-/*Krt8*-/*Vim*+). **g**,

398 Combined boxplot and violin plot displaying the activity of 4 MSigDB³⁷ hallmark gene sets
399 (Epithelial-to-Mesenchymal Transition, Hypoxia, Angiogenesis and Inflammatory response) in
400 control (n=2) and NP137-treated (n=2) tumours. The “Area Under the Curve” (AUC) value
401 depicts whether the different hallmark gene sets are enriched between control and NP137
402 treated tumours (the center line, top and bottom of the boxplots represent respectively the
403 median, 25th and 75th percentile and whiskers are 1.5 × the interquartile range (IQR), two-sided
404 Wilcoxon rank-sum test with Bonferroni correction).

405

406 **Figure 4. Pharmacological inhibition of Netrin-1 inhibits late EMT promotes epithelial**
407 **differentiation trajectories of tumour cells. a, b,** Pseudotemporal analysis using Monocle2
408 showing lineage trajectories in control skin SCC showing two EMT trajectories (hybrid and
409 full EMT trajectories) **(a)** and in NP137-treated skin SCC showing the absence of the late EMT
410 trajectory and the appearance of new epithelial trajectories **(b)**. Dots represent single cells.
411 Green , orange, red and dark red colors respectively represent the epithelial state, the early
412 hybrid, the late hybrid EMT and the late EMT state. Gene expression is visualized as a colour
413 gradient with blue indicating no expression and red indicating maximum expression. Two new
414 branches were detected in the NP137-treated trajectory, indicated by Epithelial-B1 and
415 Epithelial-B2. **c,** Combined boxplot and violin plot displaying the activity of glycolysis and
416 keratinization gene sets for the TCs belonging to the new epithelial state and other EMT states
417 based on GO terms analysis in NP137-treated tumours (n=2) (the centre line, top and bottom
418 of the boxplots represent respectively the median, 25th and 75th percentile and whiskers are 1.5
419 × IQR, two-sided Wilcoxon rank-sum test with Bonferroni correction). **d,** Immunostaining for
420 YFP and RNA in situ hybridization (RNA-scope) for *Krt14*, *Vim*, or *Aqp1* in control and
421 NP137-treated skin SCCs (n = 2 independent biological replicates , Scale bars, 20 µm). **e,** *Krt15*
422 expression spots using 10x VISIUM spatial transcriptomic on tumour sections. Gene expression

423 values have been normalized in the control and treated sample and are visualized as colour
424 gradient (scale bars, 500 μ m).

425

426 **Figure 5. Netrin-1 and Unc5b knockdown inhibits EMT and promotes epithelial state. a,**
427 EPCAM expression following *in vitro* culture of FACS isolated primary EPCAM⁺ tumour cells
428 transduced with empty vector, *Ntn1* and *Unc5b* ShRNA KD (n= 8 independent replicates for
429 empty vector, n= 6 independent replicates for *Unc5b* KD and n= 3 independent replicates for
430 *Ntn1* KD; mean \pm s.e.m., two-tailed t test) **b,** Percentage of migrated Epcam – LKPR cells
431 quantified with crystal violet staining (n=2 independent replicates, 3 wells per condition;
432 mean \pm s.e.m, two-tailed Mann-Whitney U test). **c,** Venn diagram showing the overlap between
433 the genes upregulated and downregulated in *Ntn1* and *Unc5b* KD cell lines. **d,** mRNA
434 expression of upregulated epithelial genes as defined by RNA-sequencing of EPCAM⁺ cells 6
435 days after plating of 100% EPCAM + TCs (Histograms represent mean, n= 2 for empty vector,
436 *Ntn1* KD and *Unc5b* KD). **e,** mRNA expression of mesenchymal genes downregulated upon
437 *Ntn1* KD and *Unc5b* KD, as defined by RNA-sequencing in EPCAM⁺ cells 6 days after plating
438 of 100% EPCAM⁺ TCs (Histograms represent mean, n= 2). **f, g,** *In situ* hybridization (RNA-
439 scope) for *Nrp1* (**f**) and *Aqp5* (**g**) in empty vector control, *Ntn1* and *Unc5B* KD cell line. (n = 2
440 independent biological replicates, Scale bars, 20 μ m).

441

442 **Figure 6. Anti-Netrin-1 therapy inhibits EMT in human cancer cells. a,** Scatterplots of
443 *NTN1* and *UNC5B* expression versus Hallmark EMT signatures are shown across Lung
444 squamous cell carcinoma (LUSCC) (n= 484 primary tumors), and Lung adenocarcinoma
445 (LUAD) (n= 510 primary tumors) cancer types from TCGA, one dataset per row. Spearman
446 correlations are shown in each panel top left corner. **b,** Barplots with Spearman correlations
447 between *NTN1*, *UNC5B* expression and three EMT signatures^{12,39,41} are shown across lung SCC

448 (LUSCC) (n = 484 primary tumors), lung adenocarcinoma (LUAD) (n = 510 primary tumors),
449 and skin cutaneous melanoma (SKCM) (n = 443 tumors including 76 primary tumors and 367
450 metastases) cancer types from TCGA, as well as 95% confidence intervals (eventually clipped
451 at 0 for low correlations). **c**, Microscopic appearance of A549 NSCLC cell line not treated or
452 after 3 days upon TGF- β 1 treatment (n = 3, scale bars, 20 μ m). **d**, Western blot analysis of
453 Netrin-1, E-cadherin and Vimentin expression in human A549 NSCLC cell line in control
454 conditions or following 3 days of TGF- β 1 treatment. **e**, Co-immunostaining of E-cadherin and
455 Pan-cytokeratin on tumours arising from subcutaneous grafting into immunodeficient mice of
456 A549 NSCLC cell line pre-treated with TGF- β 1 *in vitro* for 6 days. The mice were treated with
457 physiologic serum or NP137 for 25 days and the tumours were collected for histological
458 analysis (scale bars, 20 μ m) **f**, Percentage of Pan-cytokeratin + TCs expressing high levels of
459 epithelial marker E-cadherin in tumours (each dot represents the mean of CDH1 high cells in 4
460 representative areas from each tumour, n= 6 control tumours and n= 6 NP137 treated tumours)
461 (mean \pm s.e.m., two-tailed t test).

462

463

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612

613

614

615 **METHODS**

616

617 ***Compliance with ethical regulations.***

618 Mouse colonies were maintained in a certified animal facility in accordance with the European
619 guidelines. All the experiments were approved by the corresponding ethical committee
620 (Commission d'Etiquette et du Bien-Être Animal CEBEA, Faculty of Medicine, Université Libre
621 de Bruxelles). CEBEA follows the European Convention for the Protection of Vertebrate
622 Animals used for Experimental and other Scientific Purposes (2010/63/UE). The mice were
623 checked every day and were euthanized when the tumour reaches the end-point size (2cm³) or
624 if the tumour was ulcerated (independently of the size) or if the mouse lost > 20% of its initial
625 weight or showed any other sign of distress (based on general health status and spontaneous
626 activity). None of the experiments performed in this study surpassed the size limit of the
627 tumours. All the experiments complied strictly with the protocols approved by ethical
628 committee. The housing conditions of all animals were strictly following the ethical regulations.
629 The room temperature ranged from 20 and 24°C. The relative ambient humidity at the level of
630 mouse cages was 55 per cent +/-10. Each cage was provided with food, water and two types of

631 nesting material. Semi-natural light cycle of 12:12 was used. All the experiments complied
632 strictly with the protocols approved by ethical committee.

633 For subcutaneous grafting using Ishikawa cells, Female Swiss nude mice, 6-weeks old, were
634 purchased from Janvier laboratories (France) and maintained in specific pathogen-free
635 conditions (P-PAC, Lyon, France) and stored in sterilized filter-topped cages. Their care and
636 housing were in accordance with institutional European guidelines as put forth by the CECCAP
637 local Ethical Committee.

638

639 *Mouse strains.*

640 Rosa26-YFP mice⁴², Lgr5CreER mice⁴³, KRasLSL-G12D mice⁴⁴ and p53fl/fl mice⁴⁵ were
641 imported from the NCI mouse repository and Jackson Laboratories. NOD/SCID/Il2Rγ null
642 mice were purchased from Charles River. All mice used in this study were composed of males
643 and females with mixed genetic background. No randomization and no blinding were
644 performed in this study. tgNTN1 (for hNTN1 gain of function) mice was imported from Mehlen
645 Laboratory – Apoptosis, cancer and development, Centre de Recherche en Cancérologie, Lyon,
646 France⁴⁶.

647

648 *Lgr5CreER/ Kras^{LSL-G12D}/p53^{fl/fl}/Rosa26-YFP^{+/+} (LKPR) and Lgr5CreER/ Kras^{LSL-}*
649 *G12D/p53^{fl/fl}/Rosa26LSL-YFP^{+/+}/Rosa26LSL-hNTN1 (LKPRhNTN1) induced skin*
650 *Squamous Cell Carcinoma (SCC).*

651 The model Lgr5CreER/ KrasLSL-G12D/p53fl/fl/Rosa26-YFP+/+ (LKPR) is a combination of
652 a crossing of genetically modified mice: Rosa26-YFP mice⁴², the Lgr5CreER mice⁴³,
653 KrasLSL_G12D mice⁴⁴ and p53fl/fl4 mice⁴⁵ and has been developed in our laboratory¹³.
654 Technically, intraperitoneal administration of Tamoxifen (estrogen analog) (Sigma,

655 Cat#T5648-1G) activates cre recombinase which is merged to oestrogen ERT2 specifically in
656 hair follicle stem cells expressing Lgr5. Irreversible Cre/Lox recombination bring expression
657 of Kras oncogene, loss of p53 suppressor gene, expression of the YFP reporter gene as tool for
658 lineage tracing of tumour cells. Tamoxifen was diluted at 25 mg/ml in sunflower seed oil, 10%
659 EtOH (Sigma). Four daily intraperitoneal (IP) injections of 2.5 mg tamoxifen were administered
660 at P28 as previously described¹³ to LKPR mice. After 7-9 week after Tamoxifen injection
661 tumour appearance and size were detected by daily observation and palpation. Mice were
662 euthanized when tumour size was reached or when mice presented signs of distress (see
663 Compliance with ethical regulations). Skin tumours were measured using a precision calliper.
664 Tumour volumes were measured on the first day of appearance of the tumour and then every
665 week until the death of the animal or every 2 days during chemotherapy assay in combination
666 with Anti-Netrin-1 antibody. To generate the Lgr5CreER/ Kras^{LSL-}
667 G12D/p53^{fl/fl}/Rosa26LSL-YFP^{+/+}/Rosa26LSL-hNTN1 (LKPRhNTN1) model which is the
668 LKPR model with overexpression of Netrin-1 in tumour cells, we crossed the Rosa26-Lox-
669 Stop-Lox(LSL)-Netrin-1 transgenic mice. These mice conditionally overexpress flag-tagged
670 Netrin-1 under the control of a Rosa26 promoter⁴⁶.

671 *Cell culture*

672 FACS isolated EPCAM⁺ and EPCAM⁻ cells were cultured in Modified eagle medium (MEM)
673 (Capricorn scientific, Cat#SP-2002-500ml) supplemented with 10% Foetal bovine serum (FBS)
674 (Serana, Cat#S-FBS-SA-015), 4µg/ml hydrocortisone (Sigma, Cat#H0888) 1%
675 penicillin/streptomycin (100X) (Capricorn scientific, Cat#PS-B), 2mM L-glutamine, 2ml
676 amphotericin B (100X) (Capricorn scientific, Cat#AMP-B) and 500µl T3 (Sigma,
677 Cat#T6397). Cells were washed with Dulbecco's phosphate buffered saline (PBS 1X) detached
678 from the cell culture plate with trypsin (Capricorn scientific, Cat#TRY-2B10).

679 The A549, a human non small cell lung cancer cell line, has been donated by Pr Derynck
680 (University of California San Francisco) and has been used *in vitro* as model in which EMT is
681 plastic and can be induced by TGF- β 1¹⁰. Cells were cultured in DMEM (Gibco, Cat#11965092)
682 with 10% FBS and 1% penicillin/streptavidin. For in EMT induction, cells have been plated,
683 deprived in FBS the day after for 24h, then treated with recombinant TGF- β 1 (Peprotech,
684 Cat#100-21) at 5ug/ml *in vitro* for 3 days or 6 days (treatment every 2 days) in DMEM 1.5%
685 FBS.

686

687 The Ishikawa cell line, a well-differentiated endometrial adenocarcinoma cell line with Netrin-
688 1 and UNC5B expression, was purchased from the American Type Culture Collection (ATCC).
689 They were grown in Minimum Essential Medium (Ozyme Cat#COR10-009-CV),
690 supplemented with 1% of penicillin/streptomycin and 5% FBS at 37°C with saturating humidity
691 and 5% CO₂.

692

693 ***In vitro invasion assays***

694 Regarding invasion assays on Ishikawa cell line, experiments were carried out using the
695 xCELLigence RTCA DP instrument (Agilent, Cat#56665817001) which was placed in a
696 humidified incubator at 37°C and 5% CO₂. Invasion experiments were performed using 16-
697 well integrated Boyden chamber RTCA CIM plates (ref 5665817001 Agilent). Wells were
698 prepped by depositing 190 μ l of cell free culture medium (5% FBS) in lower chambers and 20 μ l
699 of cell free serum free culture medium complemented with 2,5% of Matrigel (Corning) on the
700 porous membranes. CIM plates were placed in the humidified incubator (37°C, 5% CO₂) for 1
701 hour to let the Matrigel polymerize. Ishikawa cells that had been treated with NP137 or its
702 isotypic control NP001 at 20ng/ml for 72h were harvested, suspended in serum free medium,
703 counted, and 150 000 Ishikawa cells were seeded 170 μ l serum free medium complemented

704 with NP137 or its isotypic control NP001 at 20ng/ml in the CIM plates upper chambers. CIM
705 plates were then placed in the xCELLigence RTCA DP instrument and cell invasion was
706 monitored by detection of cells passing through the porous membrane and attaching to the
707 impedance microelectrode in the lower chamber.

708

709 ***In vitro migration assays***

710 Regarding migration assays with Epcam- cells (Primary LKPR mouse skin SCC cell lines),
711 experiments were carried out using transwell migration plates (6.5 mm Transwell with 8.0 µm,
712 Corning, Cat#3422). Cells had been deprived in FBS (0,5%) for 12h before to be plated in
713 migration plate. Wells were prepped by depositing 150µl of cell culture medium (10% FBS) in
714 lower chambers (control or supplemented with NP137 at 20ug/ml) and 50µl serum free culture
715 medium containing 5 000 cells. Plates were placed in the humidified incubator (37°C, 5% CO₂)
716 for 18 hours for migration progression. To detect that had passed through the porous membrane,
717 lower side of transwell has been fixed using fresh PFA 4%, transwells have been washed in
718 PBS and bottom side of transwells had been cleaned. Lower side of membrane have been then
719 stained with 0.2% crystal violet (in 20% methanol) for 20 min. After several washes, passed
720 cells have been counted using reversed microscope.

721

722 ***Lentiviral transfection/transduction using ShRNA.***

723 HEK 293T (Human embryonic kidney 293T) (ATCC) were used as packaging cells. Transfer
724 plasmid pLKO.1-puro, carrying our gene of interest *Unc5b* or *Ntn1* (Sigma), TRC1 as empty
725 vector, PPAX and PMDG packaging plasmids were transfected into the cells with lipofectamine
726 2000 (Thermo Fisher Scientific) using Opti-medium (Capricorn scientific). Cell line of interest
727 (EPCAM+ Primary mouse skin SCC cell lines derived from LKPR) was plated and transduced

728 with the lentiviral ShRNA with additional polybrene (Sigma, Cat#T2-1003G). Puromycin
729 resistance test has been realized after transduction.

730

731 ***Anti-Netrin-1 treatment in vivo on primary LKPR mice***

732 For *in vivo* experiments, to determine the effect of anti-Netrin-1 molecule on primary tumour
733 progression and metastasis, LKPR mice were treated intraperitoneally with anti-Netrin-1
734 monoclonal antibody at 10 mg/kg every two days from 4 weeks after Tamoxifen injection and
735 until the death of animal (see compliance with ethical regulation part). In combination with
736 chemotherapeutics drugs, the 5-Fluoracil (5-FU) has been used at 10mg/kg and Cisplatin at
737 4,4mg/kg once a week for 2 weeks and mice were treated with anti-Netrin-1 antibody every
738 two days.

739

740 ***FACS isolation of tumour cells.***

741 Skin tumours from *Lgr5CreER/Kras^{LSL-G12D}/p53^{fl/fl}/Rosa26-YFP^{+/+}* mice were dissected,
742 rinsed and digested in collagenase type I (Sigma) at 3,5mg/ml in HBSS (Gibco) for 1h at 37°C
743 on a rocking plate protected from the light. Collagenase was blocked by the addition of EDTA
744 (5mM) and then the cells were rinsed 1 time in PBS supplemented with 10% FBS and the cell
745 suspension were filtered through a 70-µm cell trainer (BD Bioscience). For the wash next and
746 antibody incubation, cells were resuspended in PBS supplemented with 2% FBS (facs buffer).
747 For cell sorting, cells were also filtered through a 40-µm cell trainer (BD Bioscience). Cells
748 were incubated with BV711- conjugated anti-EPCAM (rat, clone G8.8, BD Bioscience
749 Cat#563134, dilution 1:100), PE-conjugated anti-CD45 (rat, clone 30-F11, BD, Cat#553081,
750 dilution 1:100) and PE-conjugated anti-CD31 (rat, clone MEC 13.3, BD, Cat#553373, dilution
751 1:100) antibodies for 30 min at 4 °C protected from light. Living single tumour cells were
752 selected by forward and side scatter, doublet discrimination and Hoestch exclusion. Tumour

753 cells were selected by YFP expression and the exclusion of CD45 and CD31 (Lin⁻). Different
754 tumour cell subpopulations were defined in EPCAM⁺ and EPCAM⁻ tumour cells. FACS and
755 analysis were performed using FACSAria and LSRFortessa, using FACSDiva software (v.9.1,
756 BD Bioscience). Sorted cells were collected in culture medium complemented with 50% FBS
757 for *in vivo* vein tail injection experiments and generation of culture cell lines or lysis buffer for
758 RNA extraction from RNeasy micro kit (Qiagen).

759 To analyze EPCAM profile on FACS on EPCAM⁺ LKPR cell line, cells were washed in PBS
760 and detached from the cell culture plate with trypsin. Cells were resuspended and washed with
761 PBS supplemented with 2% FBS (Facs buffer) and incubated with BV711- conjugated anti-
762 EPCAM (rat, clone G8.8, BD Bioscience Cat#563134, dilution 1:200) 30min at room
763 temperature and protected from the light. For cell sorting, cells were washed 2 times in Facs
764 buffer and filtered through a 40- μ m cell trainer. Living single YFP⁺/ EPCAM⁺ tumour cells
765 were selected by forward and side scatter, doublet discrimination and Hoestch exclusion.

766

767 ***Metastasis assay.***

768 Primary tumours from mice were generated as described above and collected. FACS-isolated
769 tumour EPCAM⁻ cells subpopulation was resuspended in 50 μ l PBS and injected into the tail
770 vein of NOD/SCID/Il2R γ -null mice (1000 cells per injection). Mice were treated with NP137
771 every 2 days (10 mg/kg) for 1 month. Mice were killed after the last treatment and lungs were
772 analysed. The number of metastases was quantified on 10 cryosections per lung (separated by
773 100 μ m) based on YFP⁺ (skin SCCs tumours) expression and presented as number (#) of
774 metastases per lung.

775

776

777 ***Tumour transplantation assays.***

778 Female Swiss nude mice, 6-weeks old, were purchased from Janvier laboratories (France) and
779 maintained in specific pathogen-free conditions (P-PAC, Lyon, France) and stored in sterilized
780 filter-topped cages. Their care and housing were in accordance with institutional European
781 guidelines as put forth by the CECCAP local Ethical Committee as previously described
782 (C2EA-15, CLB_2014_001; CLB_2014_012; CECCAPP_CLB_2016_017). Animals were
783 subcutaneously injected with Ishikawa cells (5×10^6) suspended in 100 μ L of PBS in the flank.
784 Tumors were allowed to grow for 15 days. Once tumors reached a volume of 100mm³, mice
785 were stratified into treatment groups with 1 tumor per mouse based on their tumor volume at
786 the start of the experiment, such that the starting tumor volume in each group were uniform.
787 Mice were treated via intravenous injection of 100 μ l of NP137 or its isotypic control NP001
788 diluted in PBS at 20mg/kg for 3 days, then 10mg/kg every 2 days thereafter for 1 month.
789 Tumors were measured every 2 days with calipers. Tumor size was calculated using the
790 formula: tumor volume (mm³) = (D x d²) / 2 (mm³).

791

792 A549 tumour cells have been pre-treated *in vitro* with TGF- β 1 for 6 days (see cell culture
793 section) then collected in Matrigel 50% + PBS 50% to be subcutaneously grafted into
794 NOD/SCID mice (1×10^6 cells for each grafting point). Secondary tumours were detected by
795 palpation every week and their size were monitored until tumour reach 1 cm or when mice
796 presented signs of distress. The mice were killed at the same time and tumours have been
797 collected for histology analyses.

798

799 ***Immunofluorescence stainings.***

800 All stainings were performed on frozen sections. Tumour tissues and lungs were pre-fixed in
801 4% PFA for 2 h at room temperature, rinsed in PBS, incubated overnight in 30% sucrose at 4

802 °C and embedded in OCT (Tissue Tek) for cryopreservation. Tissues were cut into 6-µm
803 sections using a CM3050S cryostat (Leica Microsystems GmbH) and rinsed with PBS three
804 times (5 min). Non-specific antibody binding was blocked with 5% horse serum, 1% BSA and
805 0.2% Triton X-100 for 1 h at room temperature. Primary antibodies were incubated overnight
806 at 4 °C in blocking buffer. Sections were rinsed in PBS three times (5 min) and incubated with
807 secondary antibodies diluted in blocking buffer at 1:400 for 1 h at room temperature. Nuclei
808 were stained with Hoechst (4 mM) and slides were mounted using SafeMount (Labonord).
809 Image acquisition was performed on a Zeiss Axio Imager.M2 fluorescence microscope with a
810 Zeiss Axiocam MRm camera using Axiovision release 4.8 software. Brightness, contrast and
811 picture size were adjusted using Adobe Photoshop.

812

813 *Antibodies for immunostaining.*

814 For Immunofluorescence the following primary antibodies were used: anti-GFP (goat
815 polyclonal, Abcam Cat#ab6673, dilution 1:500), anti-KRT14 (chicken, polyclonal, Thermo
816 Fisher Scientific Cat#MA5-11599, dilution 1:1 000), anti-VIM (rabbit, clone ERP3776 Abcam
817 Cat#ab92547, dilution 1:500), Anti-TROMA/KRT8 (rat, clone 1/28/21, DSHB
818 Cat#AB_531828, dilution 1:1 000), Anti-PDGFRα (rat, clone APA5, ebioscience, Cat#13-
819 1401, dilution 1:500), Anti-CDH1 (mouse, clone 67A4, BD Cat#563570, dilution 1:500) and
820 anti-pan-CK antibody (clone CKAE/1AE3, Dako Belgium, 1:150). The following secondary
821 antibodies were used (dilution 1:400): anti-rabbit, anti-rat, anti- goat, anti-chicken conjugated
822 to rhodamine Red-X (Jackson ImmunoResearch), Cy5 (Jakson ImmunoResearch) or Alexa
823 Fluor-A488 (Invitrogen).

824

825 ***Immunoblot analysis.***

826 Subconfluent cells were washed with cold PBS and lysed in a lysis buffer containing SDS.
827 Lysates were sonicated 5x10min using Bioruptor plus (Diagenode Cat#UCD-300) and cellular
828 debris were pelleted by centrifugation (10,000g 15 minutes at 4°C). Protein quantifications
829 were carried out using the Pierce 660nm Protein assay kit (Thermofisher Cat#22662) with a
830 ionic detergent compatibility reagent (Thermofisher Cat#22663) and measured using Bio-Rad
831 iMark microplate. 30 or 40 µg of protein extracts were loaded onto 4-12% SDS-polyacrylamide
832 gels (Thermo) and blotted onto nitrocellulose sheets. The membranes were then blocked with
833 5% non-fat dried milk + 0,05% BSA for an hour at room temperature and incubated at 4°C
834 overnight with primary antibodies : Anti-NTN1 (rabbit, clone EPR5428, Abcam,
835 Cat#ab126729, dilution 1: 10 000), anti-CDH1 (Mouse, clone 4A2, Cell Signaling Cat#14472,
836 dilution 1:1000), anti-VIM (rabbit, clone ERP3776 Abcam Cat#ab92547, dilution 1:1000) and
837 anti-β-ACTIN (rabbit, Abcam, Cat#ab8227, dilution 1:2000). After three washes with PBS-T,
838 it was followed by incubation 1h incubation with anti-rabbit secondary antibody (anti-Rabbit
839 IgG HRP NA9340V, 1/5 000, Sigma Aldrichfor, cat#Gena9340) at room temperature. After
840 washing in TBS-T, immunoreactive antibody-antigen complexes were visualized with the
841 enhanced chemiluminescence reagents, West Dura or ECL Chemiluminescence System
842 (Pierce). Membranes were imaged using iBright FL1500, Invitrogen.

843

844 ***In situ hybridization/RNA FISH.***

845 All stainings were performed on frozen sections or on cytopsin. Tumour tissues were pre-fixed
846 in 4% paraformaldehyde for 2 h at room temperature, rinsed in PBS, incubated overnight in
847 30% sucrose at 4 °C and embedded in OCT (Tissue Tek) for cryopreservation. Cytopsin cell
848 culture were generated using CellspinI (Tharmac) (20 000 cells for 1 cytopsin). *In situ* protocol

849 was performed according to the manufacturer instructions (Advanced Cell diagnostics). The
850 following mouse probes were used: Mm-Aqp1 Cat#504741-C2. Mm-Nrp1 Cat#471621, Mm-
851 Aqp5 Cat#430021-C2, Mm-Vim Cat#457961-C2 or Mm-Krt14 Cat#422521-C3. The LSM-
852 780 (Carl Zeiss) confocal system and ZEN2012 software were used to acquire and analyse the
853 ISH images.

854

855 ***Bulk RNA sequencing.***

856 RNA quality was evaluated by Bioanalyzer 2100 (Agilent) prior sequencing. Indexed cDNA
857 libraries were obtained using Ovation Solo RNA-seq Systems (NuGen) according to
858 manufacturer's recommendations. The multiplexed libraries (11 pM/18 pM) were loaded onto
859 flow cells and sequences were produced using a HiSeq PE Cluster Kit v4 and TruSeq SBS Kit
860 v3-HS (250 cycles) on a Novaseq 6000 (Illumina). Approximately 20 million paired-end reads
861 per sample were generated and quality checks were performed with FastQC
862 <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>. The adaptor sequences and low-
863 quality regions were trimmed by Trimmomatic. Trimmed reads were mapped against the mouse
864 reference genome (Grcm38/mm10) using STAR software to generate read alignments.
865 Duplicated reads are removed by picard MarkDuplicates. Annotations for Grcm38.87 were
866 obtained from <ftp://ftp.ensembl.org/>. After transcript assembly, gene level counts were obtained
867 using HTseq and normalized to 20 million of aligned reads. Average expression for each gene
868 in the different sample was computed using two biological replicates and fold changes were
869 calculated between control sample (Empty vector) and sample of interest (*Ntn1* KD or *Unc5b*
870 KD). Genes for which all the mean expressions across the sample were lower than 100 read per
871 million mapped reads were considered not expressed and removed from further analysis. Genes
872 having a fold change of expression greater than or equal to 2 were considered up-regulated and
873 those having a fold change of expression lower than or equal to 0.5 were considered down-

874 regulated.

875 ***RNA extraction and real-time PCR.***

876 RNA was extracted from FACS-isolated cells or culture cell lines using RNeasy micro kit
877 (QIAGEN) according to the manufacturer's recommendations. For real-time PCR, after mRNA
878 quantification using Nanodrop1000, the first-strand cDNA was synthesized using Superscript
879 II (Invitrogen) and random hexamers (Roche) in 50 µl final volume. Control of genomic
880 contamination was measured for each sample by performing the same procedure with or
881 without reverse transcriptase. Quantitative PCR assays were performed using 1 ng cDNA as
882 template, SYBRGreen mix (Applied Bioscience) on a Light Cycler 96 (Roche) real-time PCR
883 system. *mTbp* or *hHpvt* housekeeping genes were used for normalization. The following probes
884 were used : *mNtn1* Fw – gcaagctgaagatgaacatga, *mNtn1* Rv – cttgtcggccttcaggat, *mUnc5b* Fw
885 – ttccagctgcacacaacg, *mUnc5b* Rv – gcagagcagagagcatcca, *mTbp* Fw
886 tgtaccgcagcttcaaaatattgtat– , *mTbp* Rv – aatcaacgcagttgtccgtg, *hNTNI* Fw -
887 aaaagtactgcaagaaggactatgc , *hNTNI* Rv – ccctgcttatacacggagatg, *hCDHI* Fw –
888 cccgggacaacgtttatt, *hCDHI*– Rv gctggctcaagtcaaagtcc, *hHOOK1* Fw –
889 tgctgctgagattatgccagtga, *hHOOK1* Rv - tcagcctctgctcagttccagtz, *hMUC1* Fw -
890 gccaggatctgtggtgtaca at, *hMUC1* Rv – tgtctccaggtcgtggacattgat, *hHPRT* Fw –
891 tgaccttgattatatttgcatacc, *hHPRT* Rv - cgagcaagacgttcagtcct

892

893

894 ***Single-cell transcriptomic data analysis.***

895 ***Single cell RNA library preparation and gene expression analysis.***

896 After FACS isolation, living cells from fresh LKPR control and anti-Netrin-1 treated skin SCC
897 tumours were sorted and loaded onto each channel of the Chromium Single Cell 3' microfluidic
898 chips (V2-chemistry, 10X Genomics) and individually barcoded with a 10X Chromium

899 controller according to the manufacturer's recommendations (10X Genomics). RNA from the
900 barcoded cells was reverse transcribed, followed by amplification. The libraries were prepared
901 using the Chromium Single Cell 3' Library Kit (V2-chemistry, 10X Genomics), quantified
902 using a low coverage Illumina NextSeq 550 run and sequenced on an Illumina NovaSeq.
903 Cell Ranger (v3.0.2) was used with the default parameters to demultiplex, align, and annotate
904 the obtained sequencing reads with the 10x Genomics mm10-3.0.0 reference dataset extended
905 with the *Yfp* transgene. The Seurat R package was used to perform further downstream analysis
906 of the gene expression matrices for the treated and control samples separately⁴⁷ (v3.1.5). Only
907 cells passing the following criteria were considered for further analysis: between 600 and 8000
908 uniquely expressed genes and less than 25% of the UMI counts mapping to mitochondrial
909 sequences. For the treated samples (NP137 1 and NP137 2), respectively, 16.926 of the 19.288
910 cells and 14.104 of 17.620 passed quality control. While for the control samples (Control 1 and
911 Control 2), respectively, 10986 of the 12.180 cells and 5248 of the 8327 cells were considered
912 for downstream analysis.

913

914

915 **scRNA-seq clustering leading to cell types.**

916 Default parameters of Seurat were used, unless mentioned otherwise. Before determining the
917 cell-cycle state, the read counts were log-normalized and scaled. The scaled expression data for
918 the 2000 most highly variable genes (HVG) (identified using Seurat's FindVariableGenes
919 function) served as input for principal component analysis (PCA). Following that, the
920 JackStraw methodology implemented in Seurat was used to determine the number of significant
921 principal components (PCs) for Leiden clustering⁴⁸ and UMAP dimensionality reduction
922 (maximum of 30 PCs). Clustering resolutions ranging from 0.1-1.0 with steps of 0.1, were
923 assessed for stability with the clustree⁴⁹ R package (v0.4.2). The lowest stable resolution was

924 chosen for a general overview of the cell types present in both conditions (control: 0.1 & treated:
925 0.2). Following that, the Wilcoxon rank-sum was used to identify marker genes for each
926 subcluster, only reporting genes that were expressed in at least 25% of the cells in the cluster
927 and had an average log-fold change of at least 0.25. Batch integration was performed per
928 condition using Harmony⁵⁰ (v1.0) with standard parameters. Cell type clusters were
929 subsequently annotated using Seurat's module scoring function and cell type specific marker
930 genes obtained from the PanglaoDB⁵¹ database (version of 27/03/2020). Our annotations were
931 further confirmed by plotting the expression of canonical cell markers as *Ptprc* for immune
932 cells, *Pecam* for endothelial cells, *Cd3d* for T cells and *Col6a3* for CAFs. The clusters
933 containing the tumour cells were characterised by expression of the *Yfp* transgene. Afterwards
934 the two datasets, except for the tumor cells, were integrated using Harmony with 30 PCs and
935 standard parameters/ Leiden clustering was subsequently performed in steps of 0.1 for
936 resolutions ranging from 0.1 to 1.0. The clustree R package was then used to determine the
937 lowest stable resolution (0.2). Cell type clusters were then annotated in the same way as
938 previously described. To compare the abundance of the identified cell types between the control
939 and treated conditions, we used the scCODA algorithm⁵² implemented in the perpty v4.0 Python
940 package using the pericytes as the reference cell type and a false discovery rate (FDR) threshold
941 of 0.2.

942

943 **scRNA-seq clustering leading to cell subtypes for CAFs.**

944 After being rescaled, the combined CAFS cluster across the conditions was further
945 subclustered by using the top 2000 HVG as input for PCA analysis. The number of significant
946 PCs for clustering and UMAP calculation, which ranged from 1 to 30, was determined using
947 the JackStraw methodology. Seurat's AddModuleScore was then used to identify clusters with
948 a high enrichment of PanglaoDB-derived marker genes for other cell types using a resolution

949 of 0.4 for the Leiden clustering. Following the removal of these clusters, the previous analysis
950 steps were repeated, and stable clustering resolutions for the treated condition were determined
951 using clustree. A resolution of 0.3 was used for the identification of the final CAF subclusters.
952 Harmony was used with standard parameters to perform batch integration. The mouse MSigDB
953 hallmark gene sets and the AUCell R package (v1.8.0). were used to calculate pathway
954 activities. Clusters were assigned the apCAF, iCAF or myCAF label using Seurat's
955 AddModuleScore function and the signatures obtained from Elyada et al.⁵³ Compositional
956 analysis between the control and treated condition was performed with the scCODA algorithm,
957 implemented in the perpty v4.0 Python package using the “glycolytic CAFs” as the reference
958 and a FDR threshold of 0.2.

959

960 **scRNA-seq clustering leading to EMT states for *Yfp* positive cells and pseudotime**
961 **analysis.**

962

963 For both conditions, all *Yfp* positive cells (defined as at least having two transcripts assigned
964 to the *Yfp* transgene) were subclustered. The top 2000 variable genes were used as input for
965 PCA analysis after the counts were rescaled. The JackStraw methodology was then used to
966 calculate the number of significant PCs in a range of 1 to 30. The lowest stable clustering
967 resolution per condition was determined using clustree (control: 0.1, treated: 0.3). Based on
968 PanglaoDB-derived marker genes, the AddModuleScore was used to score clusters that showed
969 an enrichment of expression signatures related to other cell types. Following the removal of
970 these clusters, the preceding steps were repeated, with clustering resolutions of 0.7 and 0.6 used
971 for the control and treated conditions, respectively. Subsequently the obtained clusters were
972 scored as epithelial (*Epcam*⁺, *Krt14*⁺, *Vim*⁻), early hybrid (*Epcam*⁻, *Krt14*⁺, *Vim*⁺), late hybrid
973 (*Epcam*⁻, *Krt14*⁻, *Krt8*⁺, *Vim*⁺, *Pdgfra*⁻) or full EMT (*Epcam*⁻, *Krt14*⁻, *Krt8*⁻, *Vim*⁺, *Pdgfra*⁺)

974 based on the expression of *Epcam*, *Krt14*, *Krt8*, *Vim*, *Pdgfra*. The mouse MSigDB hallmark
975 gene sets and the AUCCell R package³⁹ (v1.8.0). were used to analyse pathway activities. Before
976 performing the Wilcoxon rank-sum test with Bonferroni correction for multiple testing between
977 conditions, the area under the curve (AUC) distributions were normalised per sample using
978 linear regression. To compare the abundance of the identified cell subtypes and cell cycle
979 distribution per EMT state between the control and treated conditions, the scCODA algorithm
980 of the perpty v4.0 Python package was used with the early hybrid EMT state as reference a
981 FDR threshold of 0.2. Harmony with standard parameters was used for batch integration per
982 condition. Pseudotime ordering of YFP positive cells in the G1 cell-phase was calculated using
983 monocle⁵⁴ (v2.14.0) with the DDRtree (v0.1.5) method for dimensionality reduction on the top
984 2000 HVGs for each condition, controlling for sample effects. The dynplot⁵⁵ R-package
985 (v1.0.2) was used to create the trajectory plots.

986

987 *Visium spatial transcriptomic analysis.*

988 To analyze the spatial distribution and localization of different EMT tumors states previously
989 described by scRNA-seq in Control and anti-Netrin-1 treated skin SCC tumors, we used 10x
990 VISIUM technology for spatial transcriptomic analysis (10X Genomics). FFPE tissue section
991 were places on Visium slides and prepared according to 10x Genomics protocols. After H&E
992 staining, imaging and decrosslinking steps, tissue sections were incubated with human specific
993 probes targeting 10,551 genes (10x genomics, Visium Mouse Transcriptome probe Set v1.0).
994 Probes hybridized on mRNA were captures onto Visium slides and gene Expression library
995 were prepared following the provided protocole and sequences on an illumina Novaseq 6000
996 with 50.000 reads per spot targeted sequencing depth. For each FFPE section, FASTQ files and
997 histology images were processed using 10X Space Ranger v2.0 to obtain matrix associated to

998 each spots. SCutility method was used to perform the analysis. Briefly, filtered matrix were
999 loaded, merged per sample and spot with less than 1000 detected genes were removed.

1000

1001 *EMT score*

1002 Three signatures were used to assess the EMT level. The “Hallmark” signature was computed
1003 using ssGSEA⁵⁶ on the genes from the
1004 “HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION” signature, as obtained
1005 from MSigDB³⁹ while the “Thiery” signature was computed similarly using the genes from¹².
1006 The “Mak” signature was calculated from the gene sets in PMID: 26420858, as the difference
1007 of two signatures: a mesenchymal signature defined as the mean of the mesenchymal genes
1008 expressions and an epithelial signature defined as the mean of the epithelial genes expressions.

1009

1010 *Statistical analysis.*

1011 All statistical analyses are based on biological replicates (n indicated in the text, figures or
1012 figure legends). Multiple unpaired t-test were performed using graphPad Prism version 9.00 for
1013 Mac software. Bar graphs and dot plots were generating with mean +s.e.m by graphPad Prism.
1014 Wilcoxon rank-sum test and Fisher test with Bonferroni correction have been used for multiple
1015 analyses in single cell RNA sequencing.

1016

1017 **Extended data Figure Legends**

1018

1019 **Extended data 1. Strategy to study the impact of Netrin-1 on EMT in mouse skin SCCs.**

1020 **a**, Mouse model of skin SCC allowing the expression of *Kras*^{G12D}, *YFP*, *p53* deletion and
1021 overexpression of human NETRIN-1 in hair follicle stem cells and their progeny using
1022 *Lgr5CreER*. **b**, Relative mRNA expression of *Ntn1* in EPCAM⁻ control (n= 5) and NETRIN-1
1023 gain of function (n= 8) skin SCC defined by qRT-PCR (data are normalized to *Tbp* gene, mean
1024 \pm s.e.m., two tailed Mann-Whitney U test). **c**, Western blot analysis of Netrin-1 expression in
1025 EPCAM⁻ control and NETRIN-1 gain of function skin SCC TCs. **d**, FACS plots showing the
1026 gating strategy used to FACS-isolate or to analyse the proportion of YFP⁺/EPCAM⁺ and
1027 EPCAM⁻ tumour cells. **e**, Drawing showing the experimental strategy of NP137 administration
1028 after Tamoxifen induction in *Lgr5CreER/Kras*^{LSL-G12D}/*p53*^{fl/fl}/*Rosa26-YFP*^{+/+} mice. IP,
1029 intraperitoneal.

1030

1031 **Extended data 2. Single cell analysis of the cellular composition of control and NP137-**

1032 **treated skin SCCs. a, b** Uniform Manifold Approximation and Projection (UMAP) plot for
1033 control (**a**) and NP137 -treated skin SCC (**b**) coloured by the identified cell types. **c, d**, UMAP
1034 plot for control (c) and NP137-treated skin SCC (d) coloured by the sample of origin for each
1035 cell. CAFs, cancer-associated fibroblasts.

1036

1037 **Extended data 3. Annotation of the cell types found by single cell RNA-seq in control and**

1038 **NP137-treated skin SCCs. a**, UMAP plots coloured by normalized *Yfp* and *Epcam* expression
1039 in the control tumours. Gene expression values are visualized as colour gradient with grey
1040 indicating no expression and red indicating the maximum expression. **b**, UMAP plots coloured
1041 by normalized *Yfp* and *Epcam* in NP137- treated samples. **c**, UMAP plots coloured by the

1042 activity of modules containing the mouse-specific marker genes of the different cell types
1043 including CAFs, Macrophages, Neutrophils, Endothelial cells and T cells obtained from the
1044 PanglaoDB database in control samples (left) and anti-Netrin-1 treated samples (right). Module
1045 activity visualized as a colour gradient with blue indicating no expression and yellow indicating
1046 maximum activity. **d**, UMAP plots coloured by normalized *Pdgfra*, *Acta2*, *Pecam1*, *Cd3d*,
1047 *Ptprc*, *Itgam*, *Cd86* and *Cxcr2* expression in the control samples (left) and NP137- treated
1048 samples (right). CAFs, cancer-associated fibroblasts. **e**, UMAP plot coloured by normalized
1049 *Ntn1* expression in control condition.

1050

1051 **Extended data 4. Impact of anti-Netrin antibody administration on the cellular**
1052 **composition of skin SCCs. a, b**, Uniform Manifold Approximation and Projection (UMAP)
1053 plots coloured by the cell type labels obtained from the analysis of the microenvironment for
1054 the integration of all the samples in total and split per sample, respectively. **c**, Boxplot depicting
1055 the proportions of the different cell types for the 4 samples, split by their condition. The
1056 boxplots are coloured by their condition, and the individual measurements are visualized as red
1057 dots. The centre line, top and bottom of the boxplots represent respectively the median, 25th
1058 and 75th percentile and whiskers are $1.5 \times \text{IQR}$. Significant proportion changes are indicated
1059 by $\text{FDR} < 0.2$. **d**, barplot depicting the relative log fold change of the relative abundance of the
1060 different cell types after NP137-treated samples compared to the pericytes. Bars are coloured
1061 according to their cell type. **e, f**, UMAP plot of the CAFs subclustering, coloured by the
1062 identified seven subclusters and the sample the cell originated from, respectively. **g**, Boxplot
1063 depicting the proportions of the different CAF subclusters for the 4 samples, split by their
1064 condition. The boxplots are coloured by their condition, and the individual measurements are
1065 visualized as red dots. The centre line, top and bottom of the boxplots represent respectively
1066 the median, 25th and 75th percentile and whiskers are $1.5 \times \text{IQR}$. **h**, barplot depicting the

1067 relative log fold change of the relative abundance of the different CAF subclusters after NP137-
1068 treatment compared to the glycolysis CAFs subcluster. **i**, Co-immunostaining of YFP and
1069 Vimentin in control (top) (n= 5 tumours) and NP137- treated skin SCC (bottom) (n= 5 tumours)
1070 that defines YFP-/VIM+ CAFs as cells (Scale bars, 20 μ m).

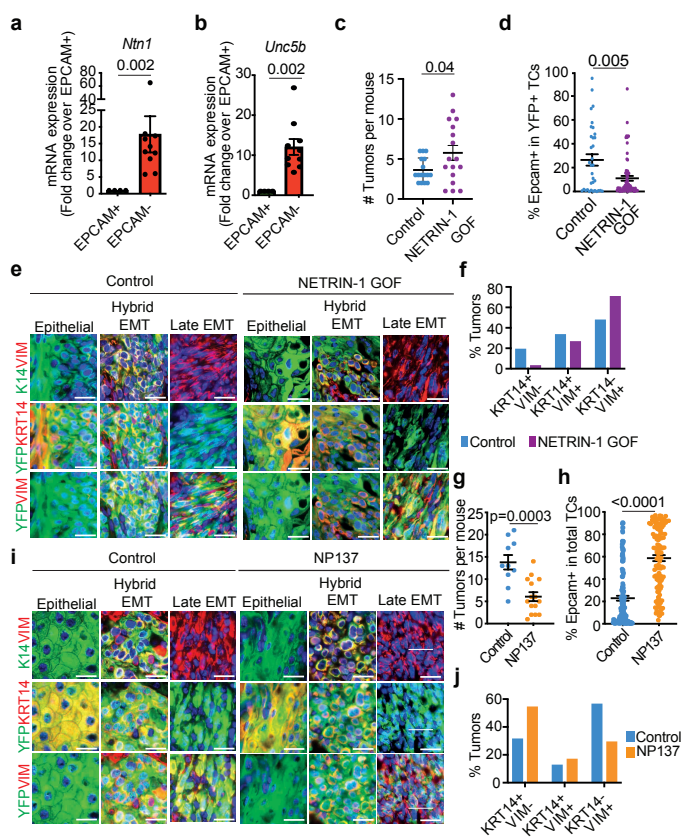
1071 **Extended data 5. Expression of markers of the different EMT states in control and**
1072 **NP137-treated skin SCCs. a, b**, UMAP plots coloured by normalized gene expression values
1073 for the indicated genes in the control (**a**) and treated samples (**b**). Gene expression values are
1074 visualized as colour gradient with grey indicating no expression and red indicating the
1075 maximum expression. Circles represent TCs groups with a different degree of EMT based on
1076 the expression of *Epcam*, *Krt14*, *Krt8*, *Vim*, *Pdgfra* (green: *Epcam*+/*Krt14*+/*Vim*- as epithelial
1077 state; orange: *Epcam*-/*Krt14*+/*Vim*+ as early hybrid EMT state; red: *Epcam*-/*Krt14*-
1078 /*Krt8*+/*Vim*+ as late hybrid EMT state; dark red: *Epcam*-/*Krt14*-/*Krt8*-/*Vim*+ as late full EMT
1079 state expressing *Pdgfra* and *Aqp1*). **c**, Barplot depicting the relative log fold change of the
1080 relative abundance of the different EMT states after NP137-treatment compared to the early
1081 hybrid state. Significant proportion changes are indicated by FDR < 0.2.

1082 **Extended data 6. Histological analysis of the control and NP137-treated tumors. a-d, .**
1083 Haematoxylin and Eosin staining showing the control (n=1) (**a, b**) or NP137-treated (n=1) (**c,**
1084 **d**) tumour skin SCC analysed in Visium spatial transcriptomic method. The annotated areas
1085 represent the EMT states previously defined by the expression of *Epcam*, *Krt14*, *Krt8* and *Vim*
1086 (1: epithelial, 2: early hybrid, 3, late hybrid, 4: full late EMT) (scale bars in a, c, 500 μ m, scale
1087 bars in b, 20 μ m).

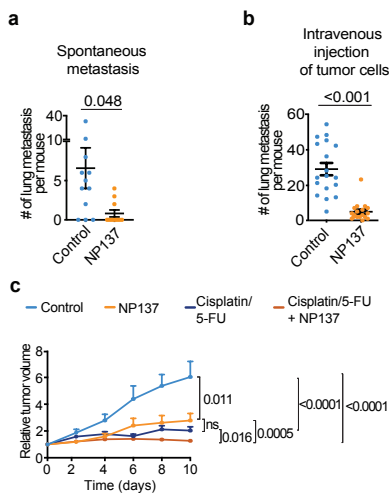
1088

1089 **Extended data 7. Analysis of NP137 treatment on tumour growth, EMT and migration in**
1090 **endometrial human cancer cell line. a**, Tumor growth quantification of human Ishikawa

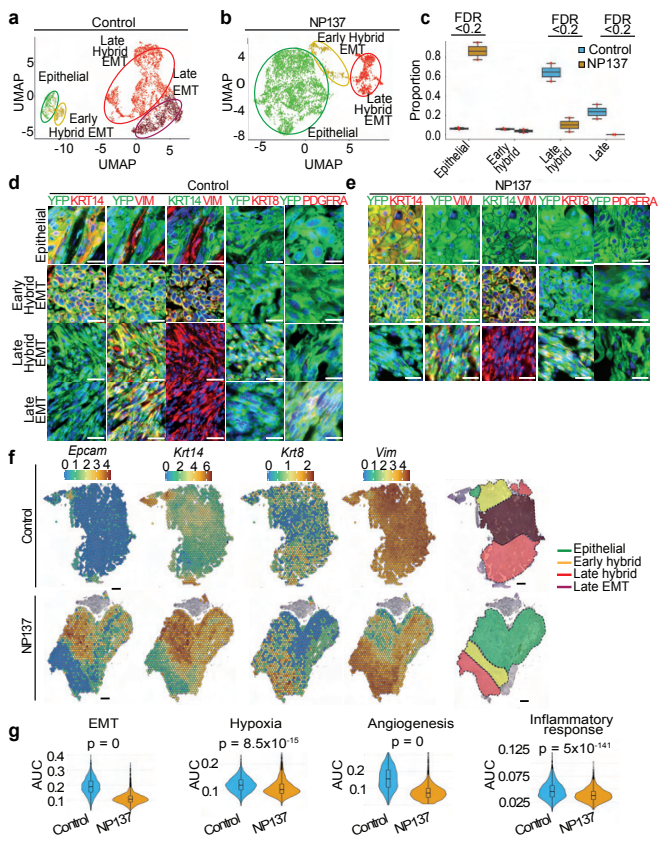
1091 endometrial carcinoma cells grafted in nude mice treated with either control (n=9) or NP137
1092 (n=9) (mean \pm s.e.m., 2-way ANOVA). **b**, Relative mRNA expression of epithelial markers
1093 *CDH1*, *MUC1* and *HOOK1* by qRT-PCR in Ishikawa human cells grafted in nude mice treated
1094 with control (n=7) or NP137 (n=8) (data are normalized to *HPRT* gene, mean \pm s.e.m., two
1095 tailed Mann-Whitney U test). **c**, Percentage of migrated Ishikawa cells treated with NP137
1096 relative to the migration of control condition through serum deprived culture medium
1097 complemented with 2.5% Matrigel between 5 and 24 hours of invasion. (n=3) (mean \pm s.e.m,
1098 two tailed t test).
1099



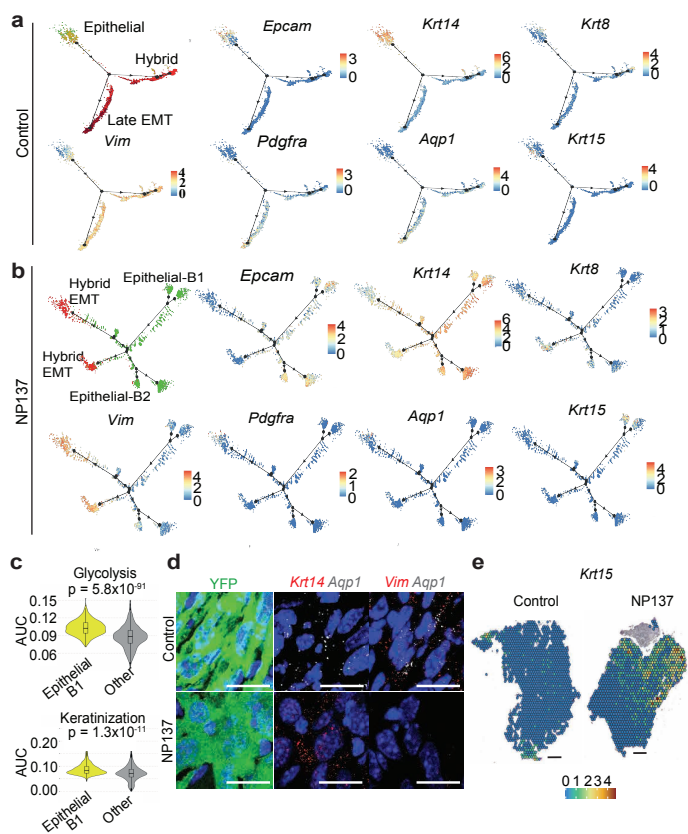
Lengrand et al Fig. 1

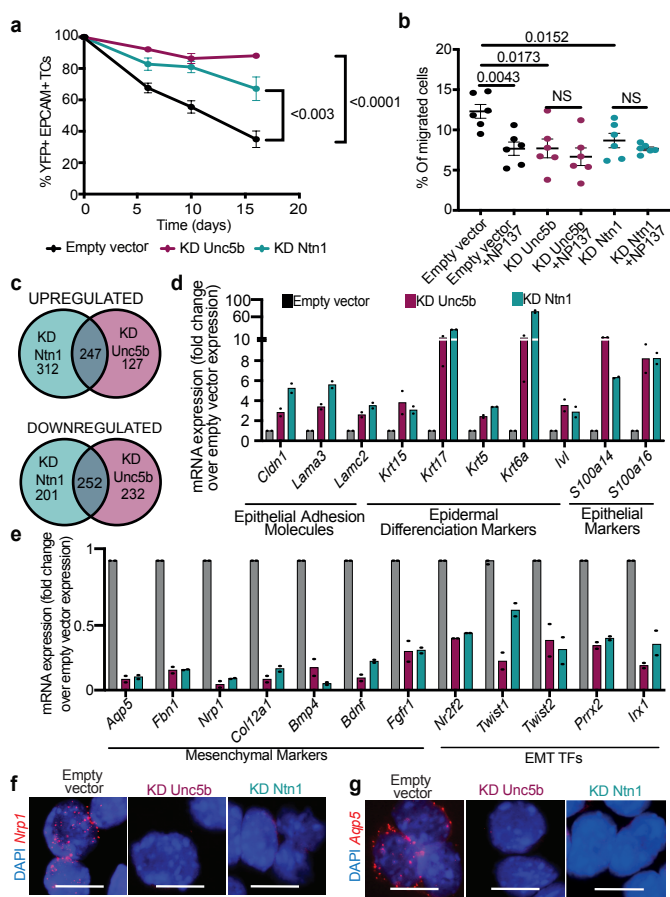


Lengrand et al Fig. 2



Lengrand et al Fig. 3





Lengrand et al Fig. 5

