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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 therapy<sup>1-7</sup>. 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 cancer<sup>8</sup>, 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- $\beta$ 1 administration<sup>9,10</sup> 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<sup>2,4-7,11,12</sup>. 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*)<sup>13-15</sup> 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 function<sup>16-27</sup>. The  
79 therapeutic anti-Netrin-1 blocking antibody NP137 is currently tested in clinical trial for the  
80 treatment of cancer patients<sup>8</sup>.

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<sup>3,6,7,15,28-30</sup>. 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 model<sup>15</sup>.  
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 EMT<sup>31-34</sup>, 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<sup>1,4-6,11,12,35</sup>. 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<sup>2,36</sup>. 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<sup>37,38</sup> 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<sup>+</sup> 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*<sup>+</sup>/*Krt14*<sup>+</sup>/*Vim*<sup>-</sup>), early hybrid EMT (*Epcam*<sup>-</sup>/*Krt14*<sup>+</sup>/*Vim*<sup>+</sup>), late hybrid EMT  
172 (*Epcam*<sup>-</sup>/*Krt14*<sup>-</sup>/*Krt8*<sup>+</sup>/*Vim*<sup>+</sup>/*Pdgfra*<sup>-</sup>), and late EMT (*Epcam*<sup>-</sup>/*Krt14*<sup>-</sup>, *Krt8*<sup>-</sup>/*Vim*<sup>+</sup>/*Pdgfra*<sup>+</sup>)  
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 reported<sup>15</sup>. NP137 administration increased the

183 epithelial state (*Epcam*<sup>+</sup>/*Krt14*<sup>+</sup>) and inhibited the occurrence of late EMT state (*Krt14*<sup>-</sup>/*Krt8*<sup>-</sup>  
184 */Vim*<sup>+</sup>) blocking EMT progression at the hybrid EMT state (*Krt8*<sup>+</sup>/*Vim*<sup>+</sup>) (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 genesets<sup>39</sup> 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 <sup>16-27</sup>. 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 Unc5b<sup>40</sup>. 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<sup>12,39,41</sup> 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  $\beta$ 1 administration<sup>9,10</sup>. A549 cells were treated with recombinant TGF- $\beta$ 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 administered 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  $\mu$ 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  $\mu$ 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 1<sup>st</sup> 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  $\mu$ 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 MSigDB<sup>37</sup> 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, 25<sup>th</sup> and 75<sup>th</sup> 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, 25<sup>th</sup> and 75<sup>th</sup> 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  $\mu$ 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> cells 6 days after plating  
438 of 100% EPCAM<sup>+</sup> 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  $\mu$ 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<sup>12,39,41</sup> 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- $\beta$ 1 treatment (n = 3, scale bars, 20  $\mu$ 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- $\beta$ 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- $\beta$ 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  $\mu$ 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

464 **References**

- 465 1 Ye, X. & Weinberg, R. A. Epithelial-Mesenchymal Plasticity: A Central Regulator of  
466 Cancer Progression. *Trends Cell Biol* **25**, 675-686, doi:10.1016/j.tcb.2015.07.012  
467 (2015).
- 468 2 Shibue, T. & Weinberg, R. A. EMT, CSCs, and drug resistance: the mechanistic link and  
469 clinical implications. *Nat Rev Clin Oncol* **14**, 611-629, doi:10.1038/nrclinonc.2017.44  
470 (2017).
- 471 3 Lambert, A. W. & Weinberg, R. A. Linking EMT programmes to normal and neoplastic  
472 epithelial stem cells. *Nat Rev Cancer* **21**, 325-338, doi:10.1038/s41568-021-00332-6  
473 (2021).
- 474 4 Puisieux, A., Brabletz, T. & Caramel, J. Oncogenic roles of EMT-inducing transcription  
475 factors. *Nat Cell Biol* **16**, 488-494, doi:10.1038/ncb2976 (2014).
- 476 5 Brabletz, S., Schuhwerk, H., Brabletz, T. & Stemmler, M. P. Dynamic EMT: a multi-tool  
477 for tumor progression. *EMBO J* **40**, e108647, doi:10.15252/emboj.2021108647 (2021).
- 478 6 Pastushenko, I. & Blanpain, C. EMT Transition States during Tumor Progression and  
479 Metastasis. *Trends Cell Biol* **29**, 212-226, doi:10.1016/j.tcb.2018.12.001 (2019).
- 480 7 Nieto, M. A., Huang, R. Y., Jackson, R. A. & Thiery, J. P. Emt: 2016. *Cell* **166**, 21-45,  
481 doi:10.1016/j.cell.2016.06.028 (2016).
- 482 8 <https://clinicaltrials.gov/ct2/show/NCT02977195>.
- 483 9 Kasai, H., Allen, J. T., Mason, R. M., Kamimura, T. & Zhang, Z. TGF-beta1 induces human  
484 alveolar epithelial to mesenchymal cell transition (EMT). *Respir Res* **6**, 56,  
485 doi:10.1186/1465-9921-6-56 (2005).
- 486 10 Kim, J. H. *et al.* Transforming growth factor beta1 induces epithelial-to-mesenchymal  
487 transition of A549 cells. *J Korean Med Sci* **22**, 898-904, doi:10.3346/jkms.2007.22.5.898  
488 (2007).
- 489 11 Voon, D. C., Huang, R. Y., Jackson, R. A. & Thiery, J. P. The EMT spectrum and  
490 therapeutic opportunities. *Mol Oncol* **11**, 878-891, doi:10.1002/1878-0261.12082  
491 (2017).
- 492 12 Tan, T. Z. *et al.* Epithelial-mesenchymal transition spectrum quantification and its  
493 efficacy in deciphering survival and drug responses of cancer patients. *EMBO Mol Med*  
494 **6**, 1279-1293, doi:10.15252/emmm.201404208 (2014).
- 495 13 Lapouge, G. *et al.* Skin squamous cell carcinoma propagating cells increase with  
496 tumour progression and invasiveness. *EMBO J* **31**, 4563-4575,  
497 doi:10.1038/emboj.2012.312 (2012).
- 498 14 Latil, M. *et al.* Cell-Type-Specific Chromatin States Differentially Prime Squamous Cell  
499 Carcinoma Tumor-Initiating Cells for Epithelial to Mesenchymal Transition. *Cell Stem*  
500 *Cell* **20**, 191-204 e195, doi:10.1016/j.stem.2016.10.018 (2017).
- 501 15 Pastushenko, I. *et al.* Identification of the tumour transition states occurring during  
502 EMT. *Nature* **556**, 463-468, doi:10.1038/s41586-018-0040-3 (2018).
- 503 16 Paradisi, A. *et al.* Combining chemotherapeutic agents and netrin-1 interference  
504 potentiates cancer cell death. *EMBO Mol Med* **5**, 1821-1834,  
505 doi:10.1002/emmm.201302654 (2013).
- 506 17 Paradisi, A. *et al.* Netrin-1 up-regulation in inflammatory bowel diseases is required for  
507 colorectal cancer progression. *Proc Natl Acad Sci U S A* **106**, 17146-17151,  
508 doi:10.1073/pnas.0901767106 (2009).

- 509 18 Paradisi, A. *et al.* NF-kappaB regulates netrin-1 expression and affects the conditional  
510 tumor suppressive activity of the netrin-1 receptors. *Gastroenterology* **135**, 1248-  
511 1257, doi:10.1053/j.gastro.2008.06.080 (2008).
- 512 19 Fitamant, J. *et al.* Netrin-1 expression confers a selective advantage for tumor cell  
513 survival in metastatic breast cancer. *Proc Natl Acad Sci U S A* **105**, 4850-4855,  
514 doi:10.1073/pnas.0709810105 (2008).
- 515 20 Delloye-Bourgeois, C. *et al.* Netrin-1 acts as a survival factor for aggressive  
516 neuroblastoma. *J Exp Med* **206**, 833-847, doi:10.1084/jem.20082299 (2009).
- 517 21 Sung, P. J. *et al.* Cancer-Associated Fibroblasts Produce Netrin-1 to Control Cancer Cell  
518 Plasticity. *Cancer Res* **79**, 3651-3661, doi:10.1158/0008-5472.CAN-18-2952 (2019).
- 519 22 Park, K. W. *et al.* The axonal attractant Netrin-1 is an angiogenic factor. *Proc Natl Acad Sci U S A* **101**, 16210-16215, doi:10.1073/pnas.0405984101 (2004).
- 520  
521 23 Arakawa, H. Netrin-1 and its receptors in tumorigenesis. *Nat Rev Cancer* **4**, 978-987,  
522 doi:10.1038/nrc1504 (2004).
- 523 24 Brisset, M., Grandin, M., Bernet, A., Mehlen, P. & Hollande, F. Dependence receptors:  
524 new targets for cancer therapy. *EMBO Mol Med* **13**, e14495,  
525 doi:10.15252/emmm.202114495 (2021).
- 526 25 Hao, W. *et al.* The pan-cancer landscape of netrin family reveals potential oncogenic  
527 biomarkers. *Sci Rep* **10**, 5224, doi:10.1038/s41598-020-62117-5 (2020).
- 528 26 Dumartin, L. *et al.* Netrin-1 mediates early events in pancreatic adenocarcinoma  
529 progression, acting on tumor and endothelial cells. *Gastroenterology* **138**, 1595-1606,  
530 1606 e1591-1598, doi:10.1053/j.gastro.2009.12.061 (2010).
- 531 27 Kefeli, U. *et al.* Netrin-1 in cancer: Potential biomarker and therapeutic target? *Tumour*  
532 *Biol* **39**, 1010428317698388, doi:10.1177/1010428317698388 (2017).
- 533 28 Haerinck, J. & Berx, G. Partial EMT takes the lead in cancer metastasis. *Dev Cell* **56**,  
534 3174-3176, doi:10.1016/j.devcel.2021.11.012 (2021).
- 535 29 Simeonov, K. P. *et al.* Single-cell lineage tracing of metastatic cancer reveals selection  
536 of hybrid EMT states. *Cancer Cell* **39**, 1150-1162 e1159,  
537 doi:10.1016/j.ccell.2021.05.005 (2021).
- 538 30 Yang, J. *et al.* Guidelines and definitions for research on epithelial-mesenchymal  
539 transition. *Nat Rev Mol Cell Biol* **21**, 341-352, doi:10.1038/s41580-020-0237-9 (2020).
- 540 31 Jin, X. *et al.* Netrin-1 interference potentiates epithelial-to-mesenchymal transition  
541 through the PI3K/AKT pathway under the hypoxic microenvironment conditions of  
542 non-small cell lung cancer. *Int J Oncol* **54**, 1457-1465, doi:10.3892/ijo.2019.4716  
543 (2019).
- 544 32 Zhang, X. *et al.* Netrin-1 elicits metastatic potential of non-small cell lung carcinoma  
545 cell by enhancing cell invasion, migration and vasculogenic mimicry via EMT induction.  
546 *Cancer Gene Ther* **25**, 18-26, doi:10.1038/s41417-017-0008-8 (2018).
- 547 33 Yan, W. *et al.* Netrin-1 induces epithelial-mesenchymal transition and promotes  
548 hepatocellular carcinoma invasiveness. *Dig Dis Sci* **59**, 1213-1221,  
549 doi:10.1007/s10620-013-3016-z (2014).
- 550 34 Han, P. *et al.* Netrin-1 promotes cell migration and invasion by down-regulation of  
551 BVES expression in human hepatocellular carcinoma. *Am J Cancer Res* **5**, 1396-1409  
552 (2015).
- 553 35 Revenco, T. *et al.* Context Dependency of Epithelial-to-Mesenchymal Transition for  
554 Metastasis. *Cell Rep* **29**, 1458-1468 e1453, doi:10.1016/j.celrep.2019.09.081 (2019).

555 36 DeConti, R. C. Chemotherapy of squamous cell carcinoma of the skin. *Semin Oncol* **39**,  
556 145-149, doi:10.1053/j.seminoncol.2012.01.002 (2012).

557 37 Khansur, T. & Kennedy, A. Cisplatin and 5-fluorouracil for advanced locoregional and  
558 metastatic squamous cell carcinoma of the skin. *Cancer* **67**, 2030-2032,  
559 doi:10.1002/1097-0142(19910415)67:8<2030::aid-cncr2820670803>3.0.co;2-k  
560 (1991).

561 38 Chen, Q. Y. *et al.* miR-206 regulates cisplatin resistance and EMT in human lung  
562 adenocarcinoma cells partly by targeting MET. *Oncotarget* **7**, 24510-24526,  
563 doi:10.18632/oncotarget.8229 (2016).

564 39 Liberzon, A. *et al.* The Molecular Signatures Database (MSigDB) hallmark gene set  
565 collection. *Cell Syst* **1**, 417-425, doi:10.1016/j.cels.2015.12.004 (2015).

566 40 Grandin, M. *et al.* Structural Decoding of the Netrin-1/UNC5 Interaction and its  
567 Therapeutical Implications in Cancers. *Cancer Cell* **29**, 173-185,  
568 doi:10.1016/j.ccell.2016.01.001 (2016).

569 41 Mak, M. P. *et al.* A Patient-Derived, Pan-Cancer EMT Signature Identifies Global  
570 Molecular Alterations and Immune Target Enrichment Following Epithelial-to-  
571 Mesenchymal Transition. *Clin Cancer Res* **22**, 609-620, doi:10.1158/1078-0432.CCR-  
572 15-0876 (2016).

573 42 Srinivas, S. *et al.* Cre reporter strains produced by targeted insertion of EYFP and ECFP  
574 into the ROSA26 locus. *BMC Dev Biol* **1**, 4, doi:10.1186/1471-213x-1-4 (2001).

575 43 Barker, N. *et al.* Identification of stem cells in small intestine and colon by marker gene  
576 *Lgr5*. *Nature* **449**, 1003-1007, doi:10.1038/nature06196 (2007).

577 44 Tuveson, D. A. *et al.* Endogenous oncogenic K-ras(G12D) stimulates proliferation and  
578 widespread neoplastic and developmental defects. *Cancer Cell* **5**, 375-387,  
579 doi:10.1016/s1535-6108(04)00085-6 (2004).

580 45 Jonkers, J. *et al.* Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional  
581 mouse model for breast cancer. *Nat Genet* **29**, 418-425, doi:10.1038/ng747 (2001).

582 46 Boussouar, A. *et al.* Netrin-1 and Its Receptor DCC Are Causally Implicated in  
583 Melanoma Progression. *Cancer Res* **80**, 747-756, doi:10.1158/0008-5472.CAN-18-1590  
584 (2020).

585 47 Satija, R., Farrell, J. A., Gennert, D., Schier, A. F. & Regev, A. Spatial reconstruction of  
586 single-cell gene expression data. *Nat Biotechnol* **33**, 495-502, doi:10.1038/nbt.3192  
587 (2015).

588 48 Traag, V. A., Waltman, L. & van Eck, N. J. From Louvain to Leiden: guaranteeing well-  
589 connected communities. *Sci Rep* **9**, 5233, doi:10.1038/s41598-019-41695-z (2019).

590 49 Zappia, L. & Oshlack, A. Clustering trees: a visualization for evaluating clusterings at  
591 multiple resolutions. *Gigascience* **7**, doi:10.1093/gigascience/giy083 (2018).

592 50 Korsunsky, I. *et al.* Fast, sensitive and accurate integration of single-cell data with  
593 Harmony. *Nat Methods* **16**, 1289-1296, doi:10.1038/s41592-019-0619-0 (2019).

594 51 Franzen, O., Gan, L. M. & Bjorkegren, J. L. M. PanglaoDB: a web server for exploration  
595 of mouse and human single-cell RNA sequencing data. *Database (Oxford)* **2019**,  
596 doi:10.1093/database/baz046 (2019).

597 52 Buttner, M., Ostner, J., Muller, C. L., Theis, F. J. & Schubert, B. scCODA is a Bayesian  
598 model for compositional single-cell data analysis. *Nat Commun* **12**, 6876,  
599 doi:10.1038/s41467-021-27150-6 (2021).



- 600 53 Elyada, E. *et al.* Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma  
601 Reveals Antigen-Presenting Cancer-Associated Fibroblasts. *Cancer Discov* **9**, 1102-  
602 1123, doi:10.1158/2159-8290.CD-19-0094 (2019).
- 603 54 Trapnell, C. *et al.* The dynamics and regulators of cell fate decisions are revealed by  
604 pseudotemporal ordering of single cells. *Nat Biotechnol* **32**, 381-386,  
605 doi:10.1038/nbt.2859 (2014).
- 606 55 Saelens, W., Cannoodt, R., Todorov, H. & Saey, Y. A comparison of single-cell  
607 trajectory inference methods. *Nat Biotechnol* **37**, 547-554, doi:10.1038/s41587-019-  
608 0071-9 (2019).
- 609 56 Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for  
610 interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* **102**, 15545-  
611 15550, doi:10.1073/pnas.0506580102 (2005).

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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 (2cm<sup>3</sup>) 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 mice<sup>42</sup>, Lgr5CreER mice<sup>43</sup>, KRasLSL-G12D mice<sup>44</sup> and p53fl/fl mice<sup>45</sup> were  
641 imported from the NCI mouse repository and Jackson Laboratories. NOD/SCID/Il2R $\gamma$  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 France<sup>46</sup>.

647

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

651 The model Lgr5CreER/ KrasLSL-G12D/p53fl/fl/Rosa26-YFP<sup>+/+</sup> (LKPR) is a combination of  
652 a crossing of genetically modified mice: Rosa26-YFP mice<sup>42</sup>, the Lgr5CreER mice<sup>43</sup>,  
653 KrasLSL\_G12D mice<sup>44</sup> and p53fl/fl4 mice<sup>45</sup> and has been developed in our laboratory<sup>13</sup>.  
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 described<sup>13</sup> 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<sup>LSL-</sup>  
667 G12D/p53<sup>fl/fl</sup>/Rosa26LSL-YFP<sup>+/+</sup>/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 promoter<sup>46</sup>.

### 671 *Cell culture*

672 FACS isolated EPCAM<sup>+</sup> and EPCAM<sup>-</sup> 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- $\beta$ 1<sup>10</sup>. 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- $\beta$ 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% CO<sub>2</sub>.

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% CO<sub>2</sub>. Invasion experiments were performed using 16-  
697 well integrated Boyden chamber RTCA CIM plates (ref 5665817001 Agilent). Wells were  
698 prepped by depositing 190 $\mu$ l of cell free culture medium (5% FBS) in lower chambers and 20 $\mu$ 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% CO<sub>2</sub>) 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 $\mu$ 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% CO<sub>2</sub>)  
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<sup>LSL-G12D</sup>/p53<sup>fl/fl</sup>/Rosa26-YFP<sup>+/+</sup>* 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<sup>-</sup>). Different  
754 tumour cell subpopulations were defined in EPCAM<sup>+</sup> and EPCAM<sup>-</sup> 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<sup>+</sup> 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- $\mu$ m cell trainer. Living single YFP<sup>+</sup>/ EPCAM<sup>+</sup> 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<sup>-</sup> cells subpopulation was resuspended in 50  $\mu$ l PBS and injected into the tail  
770 vein of NOD/SCID/Il2R $\gamma$ -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  $\mu$ m) based on YFP<sup>+</sup> (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 ( $5 \times 10^6$ ) suspended in 100 $\mu$ L of PBS in the flank.  
784 Tumors were allowed to grow for 15 days. Once tumors reached a volume of 100mm<sup>3</sup>, 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 $\mu$ 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 (mm<sup>3</sup>) = (D x d<sup>2</sup>) / 2 (mm<sup>3</sup>).

791

792 A549 tumour cells have been pre-treated *in vitro* with TGF- $\beta$ 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 ( $1 \times 10^6$  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 – ccctgctatacacggagatg, *hCDHI* Fw –  
888 cccgggacaacgtttatt, *hCDHI*– Rv gctggctcaagtcaaagtcc, *hHOOK1* Fw –  
889 tgctgctgagattatgccagtga, *hHOOK1* Rv - tcagcctctgctcagttccagtz, *hMUC1* Fw -  
890 gccaggatctgtggtgtaca at, *hMUC1* Rv – tgtctccaggctgtggacattgat, *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 separately<sup>47</sup> (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 clustering<sup>48</sup> 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 clustree<sup>49</sup> 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 Harmony<sup>50</sup> (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 PanglaoDB<sup>51</sup> 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 algorithm<sup>52</sup> implemented in the pertpy 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.<sup>53</sup> 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*<sup>+</sup>, *Krt14*<sup>+</sup>, *Vim*<sup>-</sup>), early hybrid (*Epcam*<sup>-</sup>, *Krt14*<sup>+</sup>, *Vim*<sup>+</sup>), late hybrid  
973 (*Epcam*<sup>-</sup>, *Krt14*<sup>-</sup>, *Krt8*<sup>+</sup>, *Vim*<sup>+</sup>, *Pdgfra*<sup>-</sup>) or full EMT (*Epcam*<sup>-</sup>, *Krt14*<sup>-</sup>, *Krt8*<sup>-</sup>, *Vim*<sup>+</sup>, *Pdgfra*<sup>+</sup>)

974 based on the expression of *Epcam*, *Krt14*, *Krt8*, *Vim*, *Pdgfra*. The mouse MSigDB hallmark  
975 gene sets and the AUCCell R package<sup>39</sup> (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 monocle<sup>54</sup> (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 dynplot<sup>55</sup> 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 ssGSEA<sup>56</sup> on the genes from the  
1004 “HALLMARK\_EPITHELIAL\_MESENCHYMAL\_TRANSITION” signature, as obtained  
1005 from MSigDB<sup>39</sup> while the “Thiery” signature was computed similarly using the genes from<sup>12</sup>.  
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*<sup>G12D</sup>, *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<sup>-</sup> 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<sup>-</sup> 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<sup>+</sup>/EPCAM<sup>+</sup> and  
1027 EPCAM<sup>-</sup> tumour cells. **e**, Drawing showing the experimental strategy of NP137 administration  
1028 after Tamoxifen induction in *Lgr5CreER/Kras*<sup>LSL-G12D</sup>/*p53*<sup>fl/fl</sup>/*Rosa26-YFP*<sup>+/+</sup> 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  $\mu$ m).

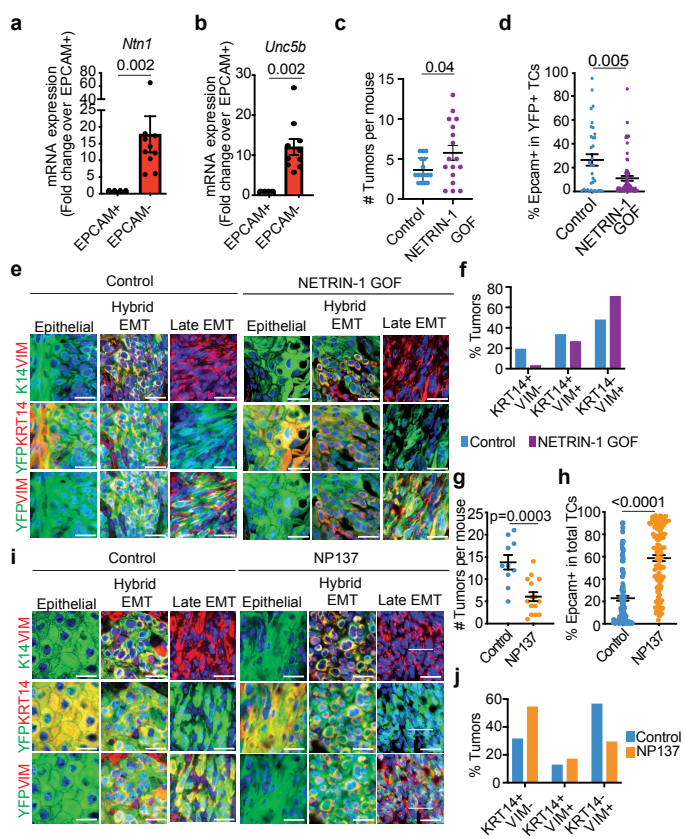
1071 **Extended data 5. Expression of markers of the different EMT states in control and**  
1072 **NP137-treated skin SCCs. a, b**, UMAP plots coloured by normalized gene expression values  
1073 for the indicated genes in the control (**a**) and treated samples (**b**). Gene expression values are  
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  $\mu$ m, scale  
1087 bars in b, 20  $\mu$ m).

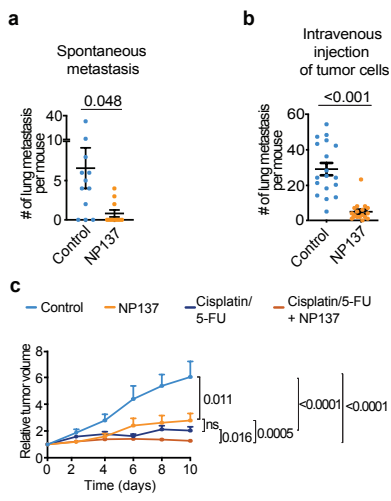
1088

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

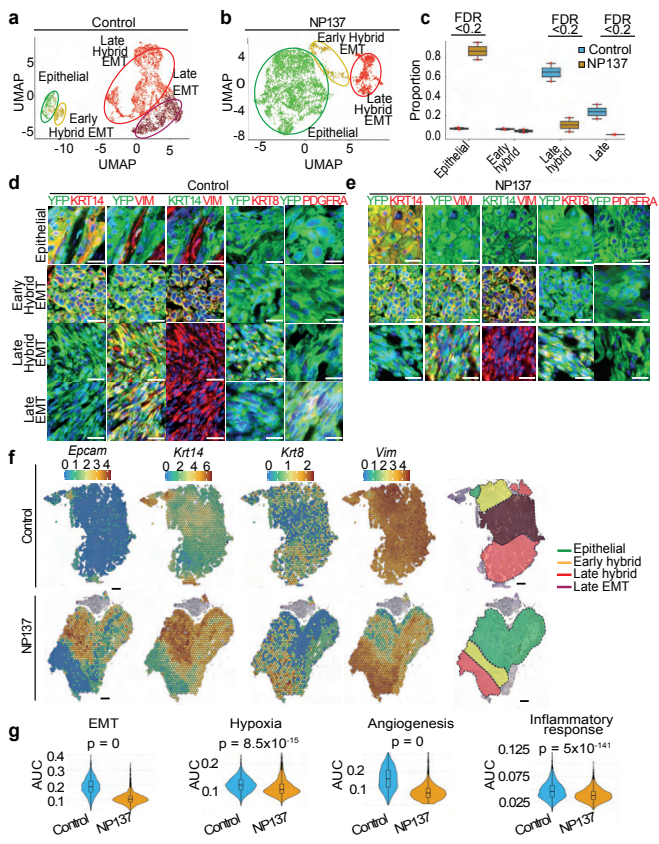
1091 endometrial carcinoma cells grafted in nude mice treated with either control (n=9) or NP137  
1092 (n=9) (mean  $\pm$  s.e.m., 2-way ANOVA). **b**, Relative mRNA expression of epithelial markers  
1093 *CDH1*, *MUC1* and *HOOK1* by qRT-PCR in Ishikawa human cells grafted in nude mice treated  
1094 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

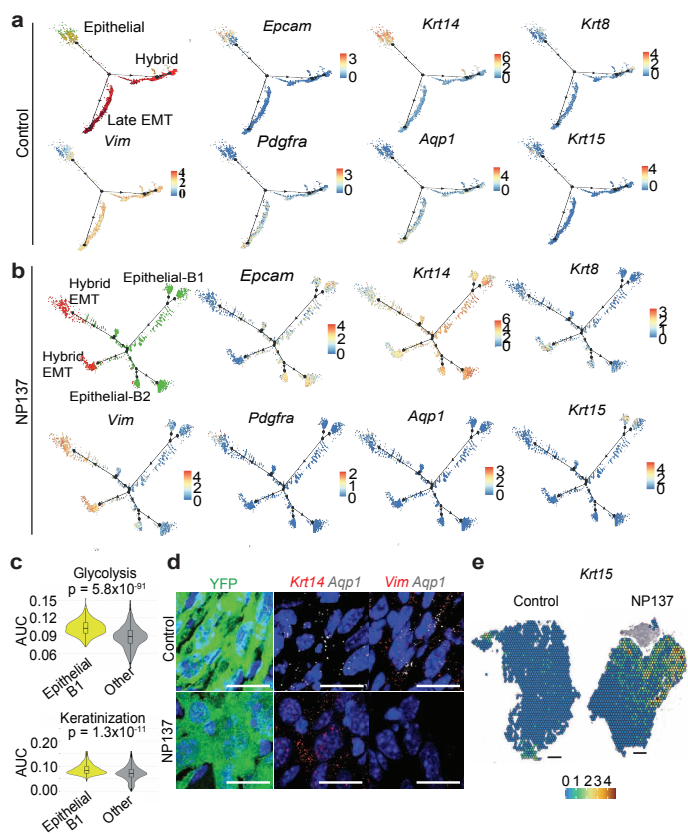


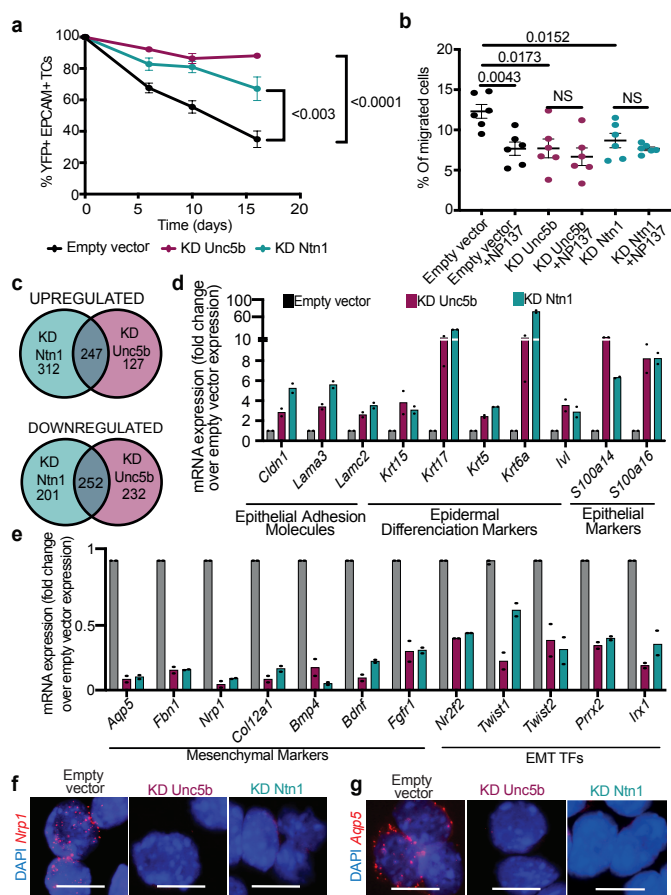
Lengrand et al Fig. 2



Lengrand et al Fig. 3







Lengrand et al Fig. 5

