1 **Hepatic stellate cells suppress NK cell sustained breast cancer dormancy**

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24 **The persistence of undetectable disseminated tumour cells (DTCs) after primary**
25 **tumour resection poses a major challenge to effective cancer treatment[1-3]. These**
26 **enduring dormant DTCs are seeds of future metastases, and the mechanisms that**
27 **switch them from dormancy to outgrowth require definition. Because cancer dormancy**
28 **provides a singular therapeutic window to prevent metastatic disease, a comprehensive**
29 **understanding of the distribution, composition and dynamics of dormant DTC**
30 **reservoirs is imperative. Here we reveal tissue-specific microenvironments that restrain**
31 **or allow progression of breast cancer in the liver, a frequent site of metastasis[4] and**
32 **often associated with patients' cause of death[5]. Using mouse models, we show that the**
33 **dormant milieu features a selective rise in natural killer (NK) cells. Adjuvant**
34 **interleukin-15-based immunotherapy ensures an abundant NK cell pool that sustains**
35 **dormancy through interferon-γ signalling, preventing hepatic metastases and**
36 **prolonging survival. Exit from dormancy follows a dramatic contraction of the NK cell**
37 **compartment and concurrent accumulation of activated hepatic stellate cells (aHSCs).**
38 **Our proteomics studies on liver co-cultures implicate the chemokine CXCL12 secreted**
39 **by aHSCs in inducing NK cell quiescence via its cognate receptor CXCR4. Expression**
40 **of CXCL12 and aHSC abundance are closely correlated in patients with liver**
41 **metastases. Our data identify the interplay between NK cells and aHSCs as a master**
42 **switch of cancer dormancy, and suggest that therapies aimed at normalizing the NK cell**
43 **pool might succeed in preventing metastatic outgrowth.**

44

45   In many cancer patients, metastatic disease surfaces long after successful treatment of the
46 primary tumour. The detection of DTCs in the bone marrow of patients with no evidence of
47 metastatic disease[6,7], combined with the establishment of their prognostic significance[8-10],
48 demonstrates that a population of cancer cells disseminating from the primary tumour may
49 survive systemic therapy and initiate future metastases. The ability of persistent DTCs to
50 enter dormancy[11,12] accounts for the unpredictable timing of metastasis and is a main barrier
51 to long-lasting cure. What tips the balance from dormancy to outgrowth remains unclear. The
52 fact that dormant DTCs bear multiple genetic abnormalities, and yet are often unable to
53 reinitiate growth for a long time, argues that the microenvironment within distant sites is the
54 dominant force in timing metastatic progression[13]. This opens an exciting possibility of
55 restoring homeostatic mechanisms that normally contribute to the chronicity of cancer as a
56 new strategy for treating metastatic disease by preventing dormant DTC reservoirs from ever
57 awakening. Deriving and implementing such microenvironmental-targeted therapies urges an

58   understanding of the nature of tissue-specific switches that leverage DTC outgrowth, which is

59   currently lacking.

60

Coexistence of dormant DTCs and metastases

62   To determine which microenvironmental shifts control DTC outgrowth, we used a

63   spontaneous metastasis model of breast cancer that yields both dormant disease (that is, DTC

64   reservoirs remaining mostly quiescent)[14] and metastases[15]. We engineered MDA-MB-231

65   cells to co-express luciferase tdTomato and a mutant reporter of p27 (mVenus-p27K⁻) that

66   identifies quiescent cells[16], implanted them into mammary glands of NOD-*Prkdc^{scid}* (NOD

67   scid) immune-compromised mice, resected the primary tumours and quantified DTCs and

68   stroma in distant tissues 6 weeks post-resection (Fig. 1a, b). DTCs were found at different

69   frequencies in each tissue (Fig. 1c), and laid preferentially quiescent in the liver (Fig. 1d).

70   Analysis of the DTC distribution pattern within individual livers revealed the coexistence of

71   distinct disease stages: the few areas with metastases mostly harboured cycling DTCs,

72   whereas the majority of the organ was apparently disease-free but still sheltered dormant

73   reservoirs in the form of scattered essentially quiescent single cells or small clusters (with

74   less than 10 cells) (Fig. 1e).

75

The size of the NK cell pool is determinant

77   To explore whether the distinct DTC fates stem from intra-organ microenvironmental

78   differences, we performed global transcriptomic profiling on hepatic milieus corresponding

79   to dormancy and metastasis, which we retrieved by splitting livers from different animals into

80   smaller parts and isolating their constituent cell populations (stroma, quiescent and cycling

81   DTCs) by fluorescence-activated cell sorting (Fig. 1f). Not surprisingly, cycling DTCs were

82   enriched in cell division-associated transcripts, whereas quiescent cells featured Wnt and

83   retinoic acid signalling previously associated with cellular dormancy[17-19], reassuring the

84   robustness of the DTC reporter (Extended Data Fig. 1a–d). Most strikingly, we found that

85   stromal cells clustered on the basis of disease stage (Extended Data Fig. 1e), and genes

86   upregulated in dormancy samples were associated with host defence processes and NK cell-

87   mediated response (Fig. 1g, Extended Data Fig. 1f, g). Notably, the transcript abundance of

88   many NK cell marker genes was substantially higher in dormancy stroma (Extended Data

89   Fig. 1h, i, Supplementary Table 1), and converged in a distinct NK cell gene expression

90   signature (Extended Data Fig. 1j). This prompted a more thorough examination of viable

91   immune cell populations in each hepatic milieu. We discovered that NK cells were the only

92   immune cell type increased in dormancy milieus compared to tumour-free livers, both in

93   percentage and number of cells (Fig. 1h left, Extended Data Fig. 2a). In contrast, metastases

94   had many fewer NK cells and were populated rather with myeloid-derived subsets previously

95   described to support lung[20-22] and liver [21,23,24] metastasis. The long-reported immunogenicity

96   of heterologous genes[25,26] prevented us from using our DTC reporter in immune-competent

97   hosts without selecting for immune-evading clones. Consequently, we chose to orthotopically

98   implant non-engineered murine dormant 4T07 or metastatic 4T1 mammary cancer cells into

99   immune-competent BALB/c mice (Extended Data Fig. 2b–d), and recapitulated the sole

100 enrichment of NK cells in dormancy milieus even in presence of an intact immune system

101 (Fig. 1h right, Extended Data Fig. 2e, f). The fact that both conventional (cNK) and liver-

102 resident (LrNK) NK cells decrease during metastatic progression, but only the latter slightly

103 increase in dormancy milieus, suggests that NK cell subset diversity might influence

104 dormancy (Extended Data Fig. 3).

105   Given the substantial differences in NK cell abundance observed between dormancy and

106 metastasis milieus, we reasoned that the pool of NK cells encountered by DTCs may play a

107 decisive part in their awakening. To test this possibility, we evaluated the impact of adjuvant

108 therapies modulating the NK cell compartment on DTC persistence and resumption of

109 growth in two metastatic models of breast cancer (Fig. 1i). Depletion of NK cells with an

110 anti-asialo-GM1 antibody resulted in heightened DTC outgrowth in multiple organs

111 (Extended Data Fig. 4a, b), and particularly escalated liver metastases, as shown by more and

112 larger metastatic foci (Fig. 1j, Extended Data Fig. 4c, d). Remarkably, sustained shortage of

113 NK cells even reactivated dormant 4T07 DTCs in the liver (Extended Data Fig. 4e–g).

114 Conversely, expanding the NK cell pool using interleukin-15 (IL-15) greatly reduced the

115 overall metastatic burden (Extended Data Fig. 4a, b) and completely prevented hepatic

116 metastases (Fig. 1j, Extended Data Fig. 4c, d), but spared the dormant DTC reservoir (Fig.

117 1k, Extended Data Fig. 4h). Because IL-15 can also stimulate T cells[27], and some cancer cells

118 express the receptor for IL-15 (IL-15Rα) (Extended Data Fig. 4i, j), we investigated whether

119 those cells could respond to IL-15 in our models. Our observations that IL-15 neither skew

120 cancer cells towards quiescence when treated *in vitro* (Extended Data Fig. 4k), nor altered the

121 frequency and activation of T cells in the liver (Extended Data Fig. 4l–n), argue that IL-15

122 contributes to dormancy rather via NK cell expansion. Importantly, the boost of NK cells

123 translated into significantly extended animal survival (Fig. 1l), confirming that continued

124   functional capacity of the overall NK cell pool equates to hepatic metastasis prevention and

125   survival benefit.

126

127   **NK cells sustain dormancy through IFN-γ**

128   How NK cells sustain cancer dormancy required elucidation. As quiescent DTCs

129   survived NK cell surveillance, we explored whether those are intrinsically resistant to NK

130   cell recognition and killing as previously proposed[28]. Surprisingly, profiling of quiescent and

131   cycling DTCs from mouse livers showed mostly similar repertoires of NK cell activating and

132   inhibitory ligands (Extended Data Fig. 5a, b), except for increased transcript abundance of

133   the NK activators *ULBP2* and *ICAM1* in quiescent DTCs, and *MICB* in cycling DTCs. Also,

134   when co-cultured with NK cells, cycling and quiescent populations were equally sensitive to

135   cytotoxicity (Extended Data Fig. 5c, d), hinting that NK cell-mediated dormancy is not

136   rooted on DTC inherent resistance to killing. To uncover the fingerprints of NK cells

137   enforcing dormancy, we performed global transcriptomic profiling on NK cells sorted from

138   different hepatic milieus (Fig. 2a). Compared to tumour-free milieus, NK cells from

139   dormancy samples featured innate immunity and inflammatory signalling (Extended Data

140   Fig. 6a, b), reflecting an immune response against DTCs. Conversely, cell division-associated

141   transcripts were diminished in NK cells from metastasis (Extended Data Fig. 6c, d),

142   corroborating our observations that metastatic outgrowth requires contraction of the NK cell

143   compartment. Furthermore, dormancy NK cells contrasted from those of metastasis by

144   upregulating many cytokine-encoding pathways (Fig. 2b, Extended Data Fig. 6e,

145   Supplementary Table 2), including TNF-α and IFN-γ, which are prominent in activated NK

146   cells and induce tumour cell growth arrest[29]. To probe whether these cytokines also trigger

147   DTC dormancy, we immune-profiled different liver milieus, and validated the increased

148   frequency of IFN-γ⁺, but not of TNF-α⁺, NK cells in dormancy compared to tumour-free and

149   metastasis samples, both in immune-compromised and immune-competent models (Fig. 2c,

150   Extended Data Fig. 6f). Remarkably, IFN-γ response was also upregulated in quiescent DTCs

151   themselves (Extended Data Fig. 6g), as evidenced by significantly higher transcript

152   abundance of many members of the pathway (Extended Data Fig. 6h). To elucidate the direct

153   effects of IFN-γ on DTC quiescence, we generated co-cultures of hepatocytes and sparsely

154   seeded cancer cells (mimicking *in vivo* DTC seeding in the liver), and treated them with

155   recombinant IFN-γ (Fig. 2d). Consistent with the mouse studies, exogenous IFN-γ

156   substantially increased the fraction of quiescent DTCs only in presence of hepatocytes in the

157 liver-like milieus (Fig. 2e, f). These results suggest that NK cells control dormant DTCs via

158 IFN-γ-induced quiescence.

159

160 **Liver injury stalls NK cell expansion**

161 Understanding what eventually disrupts homeostasis within the NK cell compartment

162 became our priority. Mining our transcriptomic data from metastatic stroma, we noticed a

163 remarkable upregulation of collagen formation and muscle contraction processes (Extended

164 Data Fig. 1g), with particular abundance of transcripts encoding markers of activated hepatic

165 stellate cells (aHSC) (Fig. 3a, Extended Data Fig.7 a, b, Supplementary Table 1), which are

166 central drivers of fibrosis that often precedes cancer onset[30]. The prominent accumulation of

167 alpha smooth muscle actin-positive (αSMA^{+}) cells and collagen deposition found only in

168 metastasis (Fig. 3b–d) validated the emergence of aHSCs as requirement for disease

169 progression. Moreover, while absent from NK cell-rich livers of IL-15-treated animals,

170 aHSCs expanded upon NK cell depletion (Extended Data Fig. 3c). This led us to query

171 whether activation of HSCs perturbs the size of the NK cell pool. Using an established model

172 of HSC activation by treatment with carbon tetrachloride (CCl_4)[31] (Fig. 3e, Extended Data

173 Fig. 8a), we found that accumulation of aHSCs specifically increased liver metastasis (Fig.

174 3f, Extended Data Fig. 7d, e), and paralleled a severe drop in NK cell frequency (Fig. 3g). Of

175 note, livers from tumour-free animals treated with CCl_4 showed a similar reduction in NK

176 cells upon persistent HSC activation (Extended Data Fig. 8b), which suggests that aHSCs

177 subvert NK cell-mediated immunity even in the absence of DTCs. In both contexts, the

178 observed shrinkage in NK cells stemmed from a lack of proliferation (Fig. 3h, Extended Data

179 Fig. 8c). These data hinted that accumulated aHSCs could suppress NK cell-sustained cancer

180 dormancy by stalling the expansion of the NK cell pool.

181

182 **CXCL12 suppresses NK cell proliferation**

183 To determine how aHSCs suppress NK cell proliferation, we turned to culture systems,

184 where the isolated effect of aHSCs could be tested in the absence of the many cell types

185 present in the liver. Considering the importance of HSC-mediated paracrine signalling in

186 liver physiology and disease[32], we hypothesized that the suppressive effect of aHSCs on NK

187 cell proliferation is driven by secreted factors. We generated all-human liver co-cultures

188 composed of immortalized hepatocytes and aHSCs either alone or mixed at a ratio of 20:1

189 (reflecting *in vivo* proportions), and exposed NK cells to conditioned medium from these

190   cultures (Fig. 4a). We found that conditioned medium from cultures harbouring aHSCs

191   significantly reduced NK cell proliferation rate (Fig. 4b). To identify what factors mediate the

192   functional activity of aHSCs on NK cells, we performed global proteomics on conditioned

193   medium from cultures of aHSC or hepatocytes alone. The most upregulated protein in the

194   secretome of aHSCs was the chemokine CXCL12 (also known as SDF1) (Fig. 4c,

195   Supplementary Table 3), which has been previously implicated in hematopoietic transit[33] and

196   homing of cancer cells to metastasis-prone tissues via its receptor CXCR4[34]. In addition to its

197   chemoattractant effects, CXCL12 maintains the quiescent hematopoietic stem cell pool in the

198   bone marrow[35], a mechanism repurposed by breast cancer cells to remain dormant in this

199   hideout[36]. We posited that CXCL12 secreted by aHSCs may also impose quiescence on NK

200   cells in the liver. After confirming the expression of the cognate receptor CXCR4 both in

201   human liver NK cells and in the surrogate line used for the co-culture experiments (Extended

202   Data Fig. 9a, b), we assessed the impact of CXCL12 on NK cell cycle, and found that it

203   nearly doubled the percentage of G_0/G_1 resting cells (Extended Data Fig. 9c). Cell death was

204   comparable among treatments (Extended Data Fig. 9c), indicating that CXCL12 does not

205   compromise NK cell viability. Similarly, CXCL12 limited the proliferation of blood-derived

206   NK cells isolated from healthy donors and breast cancer patients with liver metastases

207   (Extended Data Fig. 9d–f). Of note, priming NK cells with IL-15 rescued the inhibitory

208   effects of CXCL12, reinforcing the potential for that cytokine to restore NK cell function.

209   The necessary and sufficient effect of CXCL12 on aHSC-induced NK cell quiescence

210   was verified in cells directly isolated from mouse livers (Extended Data Fig. 9g). Culturing

211   NK cells with conditioned medium from aHSCs yielded more cells arrested in G_0/G_1, while

212   adding a function-blocking antibody against CXCL12 neutralized its proliferation inhibitory

213   effect (Fig. 4d). Consistent with these observations, we found that liver metastatic milieus,

214   which are abundant in aHSCs and CXCL12, harboured many more quiescent NK cells than

215   dormant and non-tumour counterparts (Extended Data Fig. 9h), and almost all of CXCR4^{+}

216   NK cells were resting (Extended Data Fig. 9i). Collectively, these data show that CXCL12-

217   CXCR4 signalling mediates aHSC-induced quiescence on NK cells in the liver, thereby

218   tipping the scales from dormancy to outgrowth.

219   Because CXCL12 also stimulates primary breast tumour growth directly[37], we sought to

220   determine whether aHSC-secreted CXCL12 furthers metastatic outgrowth via paracrine

221   stimulation of CXCR4 on DTCs. To test this possibility, we used co-cultures of hepatocytes

222   and sparsely seeded cancer cells, and modulated the CXCL12-CXCR4 interactions in this

223   liver-like milieu (Extended Data Fig. 10a). Treatment with aHSC-derived conditioned

224   medium or recombinant CXCL12 alone increased cancer cell number, whereas preventing

225   CXCL12 binding to CXCR4 or specific inhibition of the latter restrained CXCL12-induced

226   proliferation (Extended Data Fig. 10b). Therefore, while CXCL12-CXCR4 signalling induces

227   NK cell quiescence, it also fuels DTC proliferation.

228   Our findings that CXCL12 acts both on NK cells and DTCs raised the question of which

229   effect is rate limiting for metastasis. To address this, we generated 4T1 cells KO for CXCR4

230   through CRISPR/Cas9 genome editing (Extended Data Fig. 10c, d) and assessed their

231   metastatic potential when orthotopically injected in immune-competent BALB/c mice

232   (Extended Data Fig. 10e). Although slightly less than WT counterparts, 4T1.CXCR4_KO

233   cells still seeded liver metastases, which expanded by aHSC-mediated loss of NK cell

234   function (Extended Data Fig. 10f–h). Thus, whereas CXCR4 expression on cancer cells

235   confers them a proliferative advantage, it is not required for DTC outgrowth. Together with

236   our observations that adjuvant IL-15 immunotherapy maintains dormancy even of CXCR4-

237   expressing DTCs (Fig 1j–l), these data support the concept that NK cell abundance is the

238   limiting factor in DTC awakening.

239   We extended our study to human patient samples to assess the degree to which the

240   aHSC-NK cell axis relates to disease progression. We analysed paired metastases and normal

241   adjacent liver tissue biopsies from a cohort of breast cancer patients. Similar to the mouse

242   studies, aHSCs accumulated in metastasis and were inversely correlated with NK cell

243   abundance (Extended Data Fig. 11a, b). Opposing frequency of these two cell types was also

244   found in patients with liver disease but no metastasis (Extended Data Fig. 11c, d), which

245   supports the notion that impaired NK cell homeostasis driven by HSC activation is the

246   convergence point in the development of chronic liver disease and metastasis. Transcriptome

247   analysis of published data on liver metastases from colorectal cancer[38] revealed an analogous

248   inverse correlation between NK cell and aHSC gene signatures (Extended Data Fig. 11e–h),

249   expanding the importance of this interplay to progression of other types of cancer. Finally,

250   querying a larger patient cohort[39], we found that *CXCL12* expression correlated with the

251   accumulation of aHSCs in liver metastases from different types of cancer (Extended Data

252   Fig. 11i). Because of the difficulty in obtaining samples and detect single DTCs in patient

253   livers that are asymptomatic, whether a similar aHSC-induced NK cell quiescence

254   mechanism might awaken dormant cancer in patients is still warranted.

255

Discussion

The impact of NK cells on metastatic disease is well accepted[40]. The conventional assumption has been that cancer cells evade NK cell surveillance by altering the surface repertoire of inhibitory and activating signals that NK cells use to distinguish malignant from healthy cells. This tumour-intrinsic immune evasion tactic has been recently proposed to control cancer dormancy; accordingly, some DTCs enter self-imposed quiescence, downregulate NK cell activators, and escape NK cell-mediated cytotoxicity[28]. Here we show an alternative cytostatic IFN-γ-mediated mechanism of NK cell immunity essential to control breast cancer dormancy (Fig. 4e, left). Without excluding the possibility for cytotoxic effects from NK cells *in vivo*, our data argue against those being differential towards proliferating DTCs, and support another view that entails the size of the NK cell pool and IFN-γ availability within liver sub-microenvironments as determinants of dormancy and metastatic outgrowth.

Our findings have direct implications on the understanding of the equilibrium phase of the cancer immunoediting postulate[41], whereby IFN-γ, interleukin-12, interleukin-23 and T cell-mediated cytotoxicity ensure a match of cancer cell death and proliferation that results in stable tumour mass dormancy. By recognizing that IFN-γ-driven NK cell cytostatic effects hold breast DTCs dormant, our study reveals that the equilibrium phase goes beyond classical killing functions of adaptive immunity, and that the entire spectrum of NK cell functional outputs needs to be considered. Conceivably, differences in effector cell types and mechanisms of dormancy reflect tumour type, time and organ specificities, or simply result from dynamically interconnected processes as DTCs evolve through immunoediting. Nonetheless, IFN-γ strikes as common gatekeeper of the equilibrium state, whether by driving tumour mass dormancy through T cell-mediated cytotoxicity[42], or directly inducing DTC quiescence as shown here for breast cancer and also recently suggested for multiple myeloma[43]. Despite being a strong predictor of treatment success[44], the pleiotropic nature of IFN-γ and its failure to improve patient outcome in multiple advanced stage cancers[45] has undermined its continued application in the clinics. The mechanism we uncovered here reroutes the importance of IFN-γ to much earlier stages of disease, and encourages its therapeutic use for establishing the non-permissive cytokine milieu necessary to limit DTC emergence from dormancy.

Another key finding of our work is that a stromal response hampers NK cell-mediated immunity and triggers the switch from dormancy to liver metastasis. Fibrotic injury, here steered by aHSCs, is a long-standing disruptor of tissue homeostasis[46], and can itself

290 reactivate dormant DTCs in lungs[47]. Mechanistically, we show that aHSCs orchestrate this

291 process through secretion of the chemokine CXCL12, which retains and renders NK cells

292 quiescent, suppressing immune surveillance and licensing DTC re-emergence (Fig. 4e, right).

293 Because aHSC frequency changes upon NK cell modulation, HSC activation might also be

294 downstream of NK cells, suggesting a feedback loop navigating liver homeostasis. The

295 immune-inhibitory effect of CXCL12 resembles that of cancer-associated fibroblasts in

296 pancreatic cancer, where this chemokine was reported to cause T cell exclusion from tumour

297 areas[48-50]. It is possible that, while inducing NK cell quiescence, CXCL12 also excludes both

298 NK and T cells from the vicinity of DTCs, ensuring low lymphocyte numbers permissive of

299 metastasis. Our findings revive clinical interest on the use of CXCR4 inhibitors in the

300 treatment of cancer patients, but repurposed to earlier stages to prevent progression of

301 dormant disease. Because the size of the NK cell pool can itself determine metastatic

302 outgrowth, a decrease of NK cells in apparently disease-free cancer patients might identify

303 patients at risk of recurrence who would benefit from CXCR4 inhibition therapy. Our study

304 highlights that DTC dormancy is achieved by preserving tissue homeostasis, particularly

305 immune and fibroblast homeostasis, and disruptions of tissue physiology present DTCs with

306 an opportunity for reactivation. In patients, this is reflected by the invariable decline in host

307 defence during ageing, but also the increased risk that recurrent infections and lifestyle injury

308 triggers (such as alcohol, obesity and smoking) bring to the sprouting of site-specific

309 metastases. We envision adjuvant NK cell immunotherapy as a means to preserve tissue

310 homeostasis and prevent metastatic disease.

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441

442   **Figure Legends**

443

444   **Fig. 1 | NK cells sustain breast cancer dormancy in the liver.**

445   **a**, Experimental design for retrieving cycling and quiescent breast DTCs and surrounding

446   stroma from distant tissues. MDA-MB-231 cells co-expressing Luciferase.tdTomato and

447   mVenus-p27K$^-$ were injected into the mammary gland of NOD scid female mice, resulting

448   tumours were resected, and DTCs and stroma were sorted from distant sites. **b**,

449   Representative flow cytometry analysis for quantifying cycling (Tomato$^+$mVenus^{neg}) and

450   quiescent (Tomato$^+$mVenus$^+$) DTCs and stroma (Tomato^{neg}mVenus^{neg}). **c-d**, Quantification

451   of DTCs relative to stroma (**c**) and cycling relative to quiescent DTCs (**d**) in lung (Lg, $n =$

452   10), liver (Li, $n = 10$), bone marrow (BoMa, $n = 6$), spleen (Sp, $n = 6$) and kidney (Kid, $n =$

453   6). Mean ± s.d. **e**, DTC distribution within the liver. Top: tile scans of entire liver lobe and

454   full Z-depth (~50 μm) show that dormancy and metastasis coexist within the same liver.

455   Bottom: examples of quiescent DTCs (corresponding to (i)–(iii) from top image), and one

456   metastasis (iv). Scale bars, 2 mm (top), 20 μm (bottom). **f**, Images and DTC quantification in

457   dormancy (i) and metastasis (ii) liver parts ($n = 17$ dormancy, $n = 12$ metastases; mean ± s.d.;

458   three independent experiments). **g**, Gene set enrichment analysis (GSEA) comparing gene

459   expression data from dormancy and metastasis stroma ($n = 17$ dormancy, $n = 12$ metastases;

460   one-tailed comparisons of the empirical enrichment score (ES) of a gene set to a null

461   distribution of ESs derived from permuting the gene set, adjusted for multiple hypotheses

462   testing, i.e., false discovery rate, FDR). **h**, Flow cytometry quantification of liver NK cell

463   frequency. Left: liver parts from MDA-MB-231 model ($n = 11$ no tumour, $n = 17$ dormancy,

464    $n = 20$ metastases; three independent experiments). Right: liver parts from dormant 4T07 and

465   metastatic 4T1 models in BALB/c ($n = 10$ no tumour, $n = 10$ 4T07, $n = 10$ 4T1; two

466   independent experiments; mean ± s.d., two-tailed nonparametric Kruskal-Wallis test with

467   Dunn's multiple comparison post hoc). **i**, Experimental design for querying the impact of NK

468   cell modulation on metastasis. **j**, Representative Tomato- and H&E-stained livers from

469   MDA-MB-231 and 4T1 models, respectively. Arrows and coloured lines indicate metastases.

470   Scale bar, 2 mm. **k**, IL-15 treatment spares Ki67^{neg} quiescent DTCs. Scale bar, 20 μm. **l**,

471   Adjuvant IL-15 administration extends animal survival in the MDA-MB-231 model ($n = 10$

472   IgG, $n = 10$ anti-GM1, $n = 10$ PBS, and $n = 10$ IL-15; two independent experiments; two-

473   tailed log-rank test).

474

475 **Fig. 2 | NK cells sustain dormancy through IFN-γ.**

476 **a**, Experimental design for retrieving NK cells from tumour-free, dormancy and metastasis

477 liver parts from the MDA-MB-231 model. **b**, GSEA comparing gene expression data from

478 dormancy and metastasis liver NK cells (*n* = 17 dormancy, *n* = 7 metastases; one-tailed

479 comparisons of the ES of a gene set to a null distribution of ESs derived from permuting the

480 gene set, adjusted for multiple hypotheses testing (i.e., FDR). **c**, Flow cytometry

481 quantification of liver IFN-γ$^+$ NK cells. Left: liver parts from the MDA-MB-231 model (*n* =

482 6 no tumour, *n* = 12 dormancy, *n* = 9 metastases; two independent experiments). Right: livers

483 from dormant 4T07 and metastatic 4T1 models (*n* = 12 no tumour, *n* = 12 4T07, *n* = 12 4T1;

484 two independent experiments; mean ± s.d.; two-tailed nonparametric Kruskal-Wallis test with

485 Dunn's multiple comparison post hoc). **d**, Experimental strategy to determine whether IFN-γ

486 induces DTC quiescence. **e–f**, Representative micrographs (**e**) and quantification (**f**) of the

487 fraction of quiescent cells per field of view (FOV) upon treatment with IFN-γ or PBS. Cells

488 expressing only Tomato were used to set background green fluorescence. Scale bar, 50 μm.

489 Arrows point to quiescent cells (16 FOV per sample; three independent experiments; two-

490 tailed nonparametric Kruskal-Wallis test with Dunn's multiple comparison post hoc. Solid

491 and dashed horizontal lines on violin plots depict median and upper and lower quartiles,

492 respectively.

493

494 **Fig. 3 | aHSCs steer NK cell depletion and promote liver metastasis.**

495 **a**, Heatmap depicting the hierarchical clustering of standard score-normalized (z-score)

496 expression level of aHSC markers across stromal samples from the MDA-MB-231 model (*n*

497 = 12 metastases, *n* = 17 dormancy). **b–d**, Representative micrographs (**b**) and quantification

498 of α-SMA$^+$ aHSCs (**c**) and collagen deposition (**d**) in liver parts corresponding to distinct

499 disease stages (*n* = 11 no tumour, *n* = 17 dormancy, *n* = 20 metastases; 5 FOV per sample;

500 two independent experiments; mean ± s.d.; two-tailed nonparametric Kruskal-Wallis test with

501 Dunn's multiple comparison post hoc). Arrows indicate HSCs. Scale bar, 30 μm. **e**,

502 Experimental design for assessing the impact of HSC activation on NK cells and metastatic

503 burden. **f**, Quantification of metastatic foci in livers of oil and CCl$_4$-treated animals,

504 normalized to liver lobe area (*n* = 10 oil, *n* = 16 CCl$_4$; two independent experiments). **g-h**,

505 Flow cytometry quantification of NK cell frequency (**g**) and proliferation (**h**) in livers of oil

506 and CCl$_4$-treated animals (*n* = 10 oil, *n* = 16 CCl$_4$; two independent experiments). In **f–h**,

507 mean ± s.d.; two-tailed nonparametric Mann-Whitney U test.

508

509 **Fig. 4 | CXCL12 mediates hepatic stellate cell-induced quiescence on NK cells.**

510 **a**, Experimental design for testing the influence of the aHSC secretome on NK cell

511 proliferation. NK-92 cells were exposed to conditioned medium (CM) from liver co-cultures

512 composed of hepatocytes (Heps) and aHSCs either alone or mixed, and proliferation assessed

513 by EdU incorporation. **b**, NK-92 cell proliferation decreased upon exposure to CM from

514 aHSC-containing cultures ($n = 5$ independent experiments). **c**, Proteomic analysis of aHSCs

515 and Heps secretome ($n = 3$ CM_aHSCs, $n = 3$ CM_Heps, normalized by $n = 3$ control growth

516 medium; Bayes-moderated *t*-statistics, multiple testing correction using two-tailed

517 Benjamini-Hochberg). **d**, CXCL12 is necessary and sufficient on aHSC-induced NK cell

518 quiescence ($n = 5$ independent experiments). In **b** and **d**, mean ± s.d.; nonparametric two-

519 tailed Kruskal-Wallis test with Dunn's multiple comparison post hoc. **e**, Model summarizing

520 the interplay of NK cells and HSCs as a master switch of cancer dormancy. Increased levels

521 of IL-15 induce NK cell proliferation, ensuring an abundant NK cell pool that controls

522 dormant DTCs via IFN-γ-induced quiescence. Liver injury results in HSC activation and

523 secretion of CXCL12, which retains and renders NK cells quiescent via its cognate receptor

524 CXCR4. This NK cell-inhibitory function of CXCL12 adds to its canonical effect as inducer

525 of DTC proliferation, and contributes to metastatic outgrowth. LSEC, liver sinusoidal

526 endothelial cells.

527

528 **Methods**

529 **Animal studies.** Female NOD scid and BALB/c mice were acquired from Janvier Labs or

530 bred in barrier animal facilities of the Friedrich Miescher Institute for Biomedical Research

531 and the Department of Biomedicine, and all animal work was performed in accordance with

532 Swiss national guidelines on animal welfare and the regulations of the cantonal veterinary

533 office of Basel-Stadt, under permits #2159 and #2256. Mice were maintained in a light-,

534 humidity- and temperature-controlled environment (light–dark cycle with light from 7am to

535 5pm, with a gradual change from light to dark, 45–65% humidity and 21–25 °C temperature).

536 Mice were allowed to acclimatize for a minimum of seven days before each experiment.

537   To model spontaneous dissemination of breast cancer in immune-compromised mice, $1 \times$

538 10^6 Luc2-tdTomato.mVenus-p27K$^-$ MDA-MB-231 cells were injected into the inguinal

539 mammary gland of 7–8-week-old female NOD scid female mice in 50 μl of growth factor-

540 reduced Matrigel:PBS (1:1). Tumours were resected at a final volume of ~500 mm^3 ~4 weeks

541 later. Bioluminescence imaging was performed on an IVIS Lumina XR (Caliper

542 LifeSciences) one week after resection following intraperitoneal delivery of 100 μl D-

543 Luciferin (15 mg ml^{-1}, Biosynth) to confirm successful resection. Any mice with residual

544 bioluminescence signals were excluded from future study. To model spontaneous

545 dissemination of breast cancer in immune-competent mice, 1×10^6 4T07 and 4T1 cells were

546 injected into the inguinal mammary gland of 7–8-week-old female BALB/c mice in 50 μl of

547 growth factor-reduced Matrigel:PBS (1:1), and resulting tumours were resected at a final

548 volume of ~500 mm^3 ~3 weeks later.

549   Depletion and stimulation of NK cells was achieved by intraperitoneal injection of 75 μl

550 of anti-Asialo-GM1 (75 μg, Thermo Fisher Scientific) or recombinant IL-15 (10 μg, Thermo

551 Fisher Scientific) (Supplementary Table 4), respectively, diluted in PBS every 5 days starting

552 right after tumour resection and thereafter throughout the experiments. Control mice received

553 the same amount of isotype control (75 μg, rabbit IgG, Thermo Fisher Scientific) or PBS.

554 Different mice cohorts were used to quantify metastatic burden and measure overall survival.

555   To activate hepatic stellate cells, mice were treated with 50 μl of CCl$_4$ (0.2 μL g^{-1},

556 Sigma-Aldrich) diluted in olive oil (1:10) and delivered intraperitoneally every 4 days

557 starting right after tumour resection and for 6 weeks thereafter. Control mice received the

558 same amount of olive oil.

559   Upon animal sacrifice, organs were collected as follows: livers were perfused with PBS

560 via the inferior vena cava, and immediately dissected into individual lobes; lungs were

561 collected and inflated with PBS; femurs and tibia were cleared of connective tissue, cut at

562   each end, and flushed with PBS and a 26G insulin syringe until becoming pale; spleen and

563   kidney were collected in PBS.

564

565   **Cell culture and reagents.** Human hepatocytes immortalized by SV40 large T-antigen were

566   obtained commercially (ATCC #PTA-5565) and cultured in William's E Medium with

567   glutamax supplemented with 5% FBS. LX-2 human activated hepatic stellate cells

568   immortalized by SV40 large T-antigen were obtained commercially (Millipore) and grown in

569   high glucose DMEM supplemented with 10% FBS. NK-92 cells were provided by the DSMZ

570   and grown in alpha-MEM supplemented with 12.5% FBS, 12.5 % horse serum, 2 mM L-

571   glutamine and 250U ml^{-1} IL-2 (Novoprotein). MDA-MB-231, 4T1 and HEK293T cells were

572   purchased from ATCC and grown in high glucose DMEM supplemented with 10% FBS.

573   4T07 cells were acquired from Karmanos Cancer Institute, Wayne State University, and

574   propagated in high glucose DMEM supplemented with 10% newborn calf serum, 2 mM L-

575   glutamine and 1 mM mixed nonessential amino acids. Cell line identity was confirmed using

576   short tandem repeat (STR) fingerprinting; all cell lines were routinely tested for mycoplasma

577   contamination.

578

579   **Human samples.** A series of 69 formalin-fixed paraffin-embedded human liver biopsy

580   sections from breast cancer patients was obtained from the biobank of pathology University

581   Hospital of Basel, Switzerland. All samples were pathologically confirmed. Human

582   peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation

583   using Histopaque-1077 (Sigma) from buffy coats obtained from 6 healthy donors (Blood

584   Bank, University Hospital Basel, Switzerland) and blood samples from 6 breast cancer

585   patients with liver metastases (Oncology, University Hospital Basel, Switzerland). The

586   ethical protocol for the study was accepted by the Ethikkommission beider Basel (EKBB).

587   All individuals provided informed consent for tissue and blood donation, and personal

588   information about the identity of the donors was not accessible to the researchers.

589

590   **Isolation of human blood NK cells.** NK cells from PBMCs were isolated by MACS-based

591   sorting using a human NK cell isolation kit (Miltenyi) according the manufacturer's protocol.

592   Purity of sorted populations (CD3^{neg}CD335^{+}) was tested by flow cytometry on a BD LSR

593   Fortessa Cell analyser (BD Biosciences).

594

595   **Isolation of DTCs and stroma from murine livers.** Liver tissues were minced with two

596   parallel razor blades, and enzymatically digested in a mixture of collagenase-hyaluronidase in

597   DMEM (Stemcell Technologies) for 2 h at 37 °C. To remove cell clumps and undissociated

598   tissue, the suspension was strained through a 100-μm filter, treated with red blood cell lysis

599   buffer (Sigma) for 5 min at RT, and finally resuspended in PBS with 2% FBS.

600

601   **Isolation of HSCs and liver immune cells.** Liver tissues were minced with two parallel

602   razor blades, and gently shaken for 10 min at 37 °C in a mixture of 100 U ml^{-1} collagenase

603   type IV (Thermo Fisher), 5U DNase I (Thermo Fisher) and 2% FBS in PBS. To remove cell

604   clumps and undissociated tissue, the suspension was strained through a 70-μm filter, treated

605   with ice-cold red blood cell lysis buffer (Sigma) by vortexing for 10 s, centrifuged at 580 g

606   for 10 min, and resuspended in flow buffer (PBS supplemented with 50mM EDTA and 2%

607   FBS). For analysis of immune cell subsets, this cell suspension was further stained for

608   surface and intracellular markers. For isolating NK cells for co-culture experiments, this cell

609   suspension was further purified by MACS-based sorting using a mouse NK cell isolation kit

610   (Miltenyi) according the manufacturer's protocol. Purity of sorted populations

611   (CD3^{neg}CD335^{+}) was tested by flow cytometry on a BD LSR Fortessa Cell analyser (BD

612   Biosciences). For NK cell sequencing, cell suspensions were pre-enriched in immune cells by

613   MACS-based sorting with mouse CD45 microbeads (Miltenyi) according the manufacturer's

614   protocol. For isolation of HSCs, the cell suspension was mixed with 33% Percoll (Sigma) and

615   centrifuged at 1130 g for 30 min (without brake). After centrifugation, HSCs located in the

616   interface were collected and washed once in flow buffer. Purity was determined by UV flow

617   analysis of retinoid-positive cells on a BD LSR Fortessa Cell analyser (BD Biosciences).

618   HSCs were set in culture in DMEM supplemented with 10% newborn calf serum, and

619   maintained for 7 days until becoming activated.

620

621   **Proliferation of murine NK cells in response to treatment with aHSC conditioned**

622   **medium (CM).** Isolated liver NK cells were seeded at a density of 0.5×10^{5} cells per well in

623   96-well plates, in the presence of 48 h-collected CM from aHSCs (pre-activated for 7 days),

624   and cultured for 18 h. For proliferation analysis, parallel cultures incorporated EdU (5 μM,

625   Thermo Fisher) for 1 h, and EdU-replaced DNA was detected using the Click-iT EdU Flow

626   Cytometry kit (Thermo Fisher) as per manufacturer's instructions. Staining was combined

627   with a dead cell indicator (DAPI, 2 μg ml^{-1}), and samples were analysed on a CytoFLEX

628   flow cytometer (Beckman Coulter) using the CytExpert Software (Beckman Coulter, v2.3).

629

630   **Flow cytometry analysis of liver immune cell populations.** Cell suspensions were strained

631   over 40-μm filters, and the total viable cell number was determined using an automated cell

632   viability analyser. A total of 1×10^6 cells per liver were stained with anti-mouse CD16/CD32

633   Fc block (1:100, BD Biosciences) in flow buffer for 20 min on ice, and subsequently, with

634   appropriate antibodies for 30 min at 4 °C (details listed in Supplementary Table 4) and DAPI

635   (2 μg ml^{-1}). For intracellular cytokine detection, cells were stimulated with PMA (50 ng ml^{-1})

636   and ionomycin (500 ng ml^{-1}) in the presence of 1x Brefeldin A (eBioscience) at 37 °C for 4 h.

637   For intracellular staining, samples were stained for viability with Zombie NIR fixable

638   viability   dye   (1:1000,   Biolegend),   then   fixed   and   permeabilized   using   a

639   Fixation/Permeabilization Concentrate set (eBioscience), and finally stained with antibodies

640   for 30 min at RT (Biolegend). After staining, cells were washed, resuspended in flow buffer,

641   and analysed on a BD LSR Fortessa Cell analyser (BD Biosciences) using the BD FACS

642   Diva Software (BD Biosciences, v7). All samples were analysed by gating on viable cells

643   followed by exclusion of duplets (for details on gating strategy please refer to Supplementary

644   information, SI Fig. 2). All results show integrated fluorescence area on a biexponential

645   scale. The absolute number of each immune subset was calculated by normalizing its

646   percentage within total live immune cells to the total cell number per gram of tissue.

647

648   **NK proliferation assay.** Sorted NK cells from healthy donor and breast cancer patients were

649   seeded at a density of 0.5×10^6 cells ml^{-1} per well in 12-well plates and cultured during 16 h

650   at 37 °C in the presence of 200U ml^{-1} rh-IL-2 (Novoprotein) and 100 ng/ml rh-IL-15

651   (Peprotech). Cells were labelled with cell trace violet (5 μM, Thermo), transferred to a 96-

652   well U bottom plate at a density of 0.05×10^6 cells per well, and cultured in the presence of

653   200U ml^{-1} rh-IL-2 (Novoprotein) and different concentrations of rCXCL12. Treatments were

654   refreshed every other day, and cells were cultured for 4.5 days. Upon harvest, cells were

655   stained with a Zombie NIR fixable viability dye (1:1000, Biolegend) and anti-CD3, fixed and

656   permeabilized using a Fixation/Permeabilization Concentrate set (eBioscience). Samples

657   were acquired on a CytoFLEX flow cytometer (Beckman Coulter) using the CytExpert

658   Software (Beckman Coulter, v2.3), and analysed with FlowJo (BD Biosciences, v10.5.3).

659

660   **NK cell-mediated cytotoxicity assay.** Sorted NK cells from healthy donor PBMCs or mouse

661   livers were seeded at a density of 0.02×10^6 cells per well in 96-well U bottom plates and

662   cultured during 16 h at 37 °C in the presence of 20 ng/ml rh-IL-15 (Peprotech) or 1000U ml^{-1}

663   rh-IL-2 (Novoprotein). Murine NK cells were cultured with Luc2-tdTomato.mVenus-p27K$^-$-

664   expressing 4T07 or 4T1 cells, and human NK cells were cultured with Luc2-

665   tdTomato.mVenus-p27K$^-$-expressing MDA-MB-231 cells at different E:T ratios for 5 h at 37

666   °C. NK cells cultured without target cells were used as spontaneous cell death control.

667   Following incubation, cells were washed and resuspended in PBS supplemented with 50mM

668   EDTA and 2% FBS. Shortly before sample acquisition, cells were stained with a dead-cell

669   indicator (DAPI, 2 µg ml^{-1}), and then acquired on a CytoFLEX flow cytometer (Beckman

670   Coulter) using the CytExpert Software (Beckman Coulter, v2.3) and analysed with FlowJo

671   (BD Biosciences, v10.5.3). Specific NK cell killing was measured for cycling and quiescent

672   cancer cells as follows:

$$\% \ specific \ lysis = \% \ DAPI^+(dead) \ targets - \% \ spontaneous \ DAPI^+ \ targets$$

673

674   **Generation of lentivirus and transduced lines.** Lentivirus were generated by co-

675   transfection of sub-confluent HEK293T cells with 2 µg each of VSVG and packaging vector

676   (third-generation packaging system[52]) and 4 µg plasmid DNA of interest (pCDH-EF1-

677   mVenus-p27K$^-$ or pFU-Luc2-tdTomato[53]) in DMEM containing a 3:1 (µl µg^{-1}) ratio of

678   FuGENE HD (Promega):total plasmid DNA. HEK293T medium was changed to growth

679   medium 24 h after transfection and lentivirus was collected 48 h later. MDA-MB-231, 4T1

680   and 4T07 cells were transduced at a multiplicity of infection (MOI) of 5 using MISSION

681   ExpressMag Beads (Sigma) according to the manufacturer's instructions. Stable Luc2-

682   tdTomato- and Luc2-tdTomato.mVenus-p27K$^-$-expressing MDA-MB-231, 4T1 and 4T07

683   cells were generated by sequential infection with pFU-Luc2-tdTomato lentivirus, selection

684   with 200 µg ml^{-1} zeocin, lentiviral transduction with pCDH-EF1-mVenus-p27K$^-$ and

685   selection with 2 µg ml^{-1} puromycin.

686

687   **Cloning.** The pMXs-IRES-puro/mVenus-p27K$^-$ vector was a gift from T. Oki and T.

688   Kitamura (University of Tokyo, Japan; [16]). The mVenus-p27K$^-$ gene sequence was removed

689   from this vector and placed within the pCDH-EF1 lentiviral vector (CD527A-1, System

690   Biosciences) using traditional blunt end cloning. The pFU-Luc2-Tomato vector was a gift

691   from A. Bottos and N.E. Hynes (Friedrich Miescher Institute, Switzerland).

692

693   **CRISPR mediated knockout of CXCR4.** Two Alt-R CRISPR-Cas9 crRNA targeting *Cxcr4*
694   and one targeting an intergenic region were used (details in Supplementary Table 5). RNA
695   complexes were formed together with Alt-R CRISPR-Cas9 tracrRNA (IDT) in equimolar
696   concentrations, heated for 5 min at 95 °C, and then added to Cas9-NLS protein (qb3
697   Berkeley) in a 1.1 to 1 ratio. 2×10^6 4T1 cells were electroporated with the prepared RNPs
698   using the 4D-Nucleofector X Kit L (Lonza, V4XC-1012, program CH125). Media was
699   exchanged 24 h after electroporation, and live cells were single-cell sorted in 96-well plates
700   48 h later. Media was exchanged every 2-3 days with sterile filtered media containing 50 %
701   conditioned media from parental cells, 25 % FCS and 25 % normal growth media. Clonal
702   derived cells were genotyped (details in Supplementary Table 5), and four clones each of
703   intergenic and *Cxcr4* knockout genotypes were pooled together.

704

705   **Fluorescence-activated cell sorting.** Cell suspensions were filtered twice through 40-μm
706   filters to obtain single cells, and fluorescence-activated cell sorting was carried out with a BD
707   FACSAria III sorter (BD Biosciences) using a 70-μm nozzle. Single cells were gated on the
708   basis of their forward and side-scatter profiles, and pulse width was used to exclude doublets.
709   Dead cells ($DAPI^{bright}$) were gated out. Different cell populations were isolated on the basis of
710   expression of Tomato and mVenus as cycling DTCs ($Tomato^+mVenus^{neg}$), quiescent DTCs
711   ($Tomato^+mVenus^+$) and stroma ($Tomato^{neg}mVenus^{neg}$). NK cells were isolated on the basis of
712     $CD3^{neg}CD335^+$ expression.

713

714   **mRNA preparation and sequencing.** *Sequencing of bulk liver milieus.* Isolated cells were
715   sorted in the extraction buffer of the RNA purification kit (Norgen Biotek Corporation) and
716   mRNA was isolated using the manufacturer's protocol. RNA was depleted of rRNA using the
717   Ribo-Zero rRNA removal kit (Epicentre) and the column was purified with the RNA Clean &
718   Concentrator kit (Zymo Research). RNA integrity was measured on an Agilent 2100
719   Bioanalyzer using RNA Pico reagents (Agilent). The library was prepared using the
720   ScriptSeq v2 RNA-seq Library preparation kit (Epicentre). Library quality was measured on
721   an Agilent 2100 Bioanalyzer for product size and concentration. Single-end libraries were
722   sequenced by an Illumina HiSeq 2500 (50-nt read length).

723   *Sequencing of bulk NK cells.* Isolated cells were sorted in the lysis buffer of the
724   NucleoSpin RNA Plus XS kit for RNA purification (Macherey Nagel) and mRNA was
725   isolated using the manufacturer's protocol. RNA was depleted of rRNA using the RiboGone
726   kit (Takara). Libraries were prepared using the SMART-Seq Stranded Kit in combination

727 with the SMARTer RNA Unique Dual Index Kit (Takara), and quality-checked on the

728 Fragment Analyzer (Advanced Analytical). Single-end libraries were sequenced on the

729 Illumina Novaseq 6000 S1 (100-nt read length).

730

731 **Computational analyses.** *mRNA sequencing differential expression analysis.* Sequenced

732 reads were subjected to 3′ adapter trimming

733 (GATCGGAAGAGCACACGTCTGAACTCCAGTCAC for liver milieus and

734 AGATCGGAAGAGCACACGTCTGAACTCCAGTCA for NK cells) and quality control

735 (reads shorter than 20 nucleotides or for which over 10% of the nucleotides had a PHRED

736 quality score <20 were discarded). Filtered reads from mouse stroma/NK cells and human

737 DTCs were mapped to the mouse transcriptome based on genome assembly mm10 and

738 transcript annotations from RefSeq (June, 2015) and the human transcriptome based on

739 genome assembly hg38 and transcript annotations from Ensembl (June, 2015), respectively.

740 Mapping was performed with segemehl v0.1.7-411[54] allowing a minimum mapping accuracy

741 of 90%. Transcript counts were calculated based on uniquely mapped reads and used for

742 differential expression analysis with DESeq2 v1.26.0[55] using R v3.6.3. The large number of

743 uniquely mapped reads to mRNAs (~4-18 and ~9-17 million for mouse and human samples,

744 respectively) allowed a robust quantification of genome-wide transcript abundances.

745 Upregulation and downregulation of mRNAs was considered significant when FDR<0.01

746 (i.e., two-tailed Wald tests with Benjamin Hochberg multiple test correction).

747 *aHSC, NK cell, cNK and LrNK gene signatures*. We used a previously published[51] 5-

748 gene aHSC signature (including *Col1a2*, *Col1a1*, *Col3a1*, *Acta2* and *Tagln*) and a 20-gene

749 NK cell signature (comprising *Ccl3*, *Alox5ap*, *Cd69*, *Nkg7*, *Eomes*, *Klrd1*, *Cd160*, *Cd7*,

750 *Klrc1*, *Txk*, *Xcl1*, *Ncr1*, *Il2rb*, *Prf1*, *Gzmk*, *Klrb1a*, *Klrb1b*, *Klrb1f*, *Ifitm1* and *Itga1*) to probe

751 the correlations between these cell types. We also used a published[56] 9-gene cNK cell

752 signature (*Itga1*, *Itga2*, *Eomes*, *Cd200r1*, *Trail*, *Gzma*, *Cd107a*, *Prf1* and *Gzmk*) and 11-gene

753 LrNK signature (*Itga1*, *Itga2*, *Eomes*, *Cd200r1*, *Trail*, *Tbx21*, *Lag3*, *Pdcd1lg2*, *Cd274*, *Cd39*

754 and *Cd73*) to distinguish between conventional and liver resident NK cells, respectively.

755 *Analysis of RNA sequencing from human samples.* Gene expression for different genes

756 across human liver cell-types was retrieved from a published single cell RNA sequencing

757 dataset on 8444 cells from 5 human livers[51] following instructions provided by the authors at

758 https://github.com/BaderLab/HumanLiver. Published microarray data on 18 liver metastases

759 from colon cancer and 5 normal liver samples[38] were retrieved from the Gene Expression

760 Omnibus (GEO) database (accession number GSE14297) already normalized. Analysis of

761   134 liver metastases from different types of cancer was carried out on gene expression data

762   obtained from a previously published dataset[39] (https://met500.path.med.umich.edu/).

763   *Gene set enrichment analysis*. The tool GSEA v3.0 [build: 0160]

764   (http://software.broadinstitute.org/gsea/index.jsp)[57] was used to calculate the enrichment of

765   gene sets derived from the BioCarta/Reactome/KEGG pathway databases, Gene Ontology:

766   Biological Processes, and the Hallmark collection (v6.2 from all gene sets). Enrichment

767   scores (ES) were determined using a pre-ranked gene list based on mRNA fold-changes, and

768   *P*-values were estimated by one-tailed comparisons of the empirical ES of a gene set relative

769   to a null distribution of ESs derived from permuting the gene set 1,000 times, and then

770   adjusted for multiple hypotheses testing[57].

771

772   **Proliferation and apoptosis of NK-92 cells in response to CXCL12 treatment.** NK-92

773   cells were seeded at a density of 0.5×10^6 cells per well in 24-well culture plates, and

774   cultured in the presence of recombinant CXCL12 (0.1 μg ml^{-1}, Thermo Fisher) for 2 h. For

775   proliferation analysis, parallel cultures incorporated EdU (5 μM, Thermo Fisher) for 1 h, and

776   EdU-replaced DNA was detected using the Click-iT EdU Flow Cytometry kit (Thermo

777   Fisher) as per manufacturer's instructions. For apoptosis analysis, cultures were stained with

778   annexin V (1:20, Biolegend) according to the manufacturer's instructions. In both assays,

779   staining was combined with a dead-cell indicator (DAPI, 2 μg ml^{-1}), and samples were

780   acquired on a BD LSR Fortessa Cell analyser (BD Biosciences) using BD FACS Diva

781   Software (BD Biosciences, v7), and analysed with FlowJo (BD Biosciences, v10.5.3).

782

783   **Proliferation of NK-92 cells in response to treatment with conditioned medium (CM).**

784   NK-92 cells were seeded at a density of 0.25×10^6 cells ml^{-1} per well in 12-well plates, in the

785   presence of 48 h-collected CM from NK-92 cells or liver co-cultures composed of

786   immortalized hepatocytes (Heps) and aHSCs either alone or mixed in reflective proportions

787   (at a ratio of 20:1), and cultured for 18 h. For proliferation analysis, parallel cultures

788   incorporated EdU (5 μM, Thermo Fisher) for 1 h, and EdU-replaced DNA was detected using

789   the Click-iT EdU Flow Cytometry kit (Thermo Fisher) as per manufacturer's instructions.

790   Staining was combined with a dead cell indicator (DAPI, 2 μg ml^{-1}), and samples were

791   acquired on a BD LSR Fortessa Cell analyser (BD Biosciences) using BD FACS Diva

792   Software (BD Biosciences, v7), and then analysed with FlowJo (BD Biosciences, v10.5.3).

793

794 **Liver-like cultures with cancer cells.** A layer of immortalized hepatocytes was seeded and

795 grown close to confluence during 2 days. For IFN-γ experiments, hepatocytes were labelled

796 with cell trace violet (5 μM, Thermo) and seeded at a density of 6.5×10^4 cells per well in

797 96-well plates. For CXCL14-CXCR4 modulation experiments, unlabelled hepatocytes were

798 seeded at a density of 4×10^5 cells per well in 24-well plates. Luc2-tdTomato.mVenus-p27K$^-$

799 -expressing 4T07 or 4T1 cells and Luc2-tdTomato-expressing MDA-MB-231 or 4T1 cells

800 were seeded on top of confluent hepatocyte layers at a sufficiently low number (i.e., at a

801 density of 500 cells per 96-well or 1000 cells per 24-well) to recapitulate DTC seeding in the

802 liver without causing an immediate burden on the hepatic tissue. Treatments started 1 h after

803 cancer cell seeding, and were refreshed every other day until the end of experiments. For

804 CXCL12-CXCR4 experiments, cultures were harvested at day 7, stained with a dead-cell

805 indicator (DAPI, 2 μg ml^{-1}), and analysed on a BD LSR Fortessa Cell analyser (BD

806 Biosciences). For IFN-γ experiments, cultures were imaged at day 6 with the CQ1 Benchtop

807 High-Content Analysis System (Yokogawa). Each well was scanned using three laser lines

808 and a 0.4 NA ×10 air objective to visualize hepatocytes and quantify cycling and quiescent

809 cancer cells over 16 fields of view (FOV) covering almost the entire well. Images of selected

810 FOV were acquired using a 0.95 NA ×40 air objective. Cancer cells were quantified in

811 scanned images using Fiji v2.0.0-rc-69/1.52n. The Tomato channel was automatically

812 thresholded to create a binary image, cancer cells were masked, and all particles with a size

813 ≥120 pixels were counted using the function "Analyze Particles". This output was overlapped

814 with the GFP channel and median intensities (red and green) and cell area were collected for

815 each single cell. Measurements were confirmed with manual counts of one FOV from each

816 condition to ensure accuracy.

817

818 **Immunofluorescent staining.** Immediately after collection, each liver lobe was embedded in

819 $22 \times 22 \times 20$ mm embedding moulds in OCT compound, and flash frozen. 50 μm serial tissue

820 sections were generated with a Leica CM3050 S cryostat (Leica Microsystems). Sections

821 were thawed and placed into 4% PFA for 20 min at RT for fixation. Tissues were then rinsed

822 extensively with PBS and permeabilized with 0.5% TritonX-100 in PBS for 20 min at RT.

823 After extensive rinsing, tissues were blocked for 1 h at RT in 10% normal goat serum with

824 0.5% Triton X-100 in PBS under constant gentle agitation, and then stained with a

825 combination of chicken anti-GFP and rabbit anti-RFP overnight (4 °C) in blocking buffer

826 (details regarding these antibodies are provided in Supplementary Table 4). The following

827   day, tissues were washed 3 times with PBS, and then counterstained in blocking buffer

828   containing secondary antibodies (Supplementary Table 4) and DAPI (2 μg ml^{-1}).

829   Liver lobes were imaged on a Zeiss LSM710 confocal microscope and scanned using

830   three laser lines and a 0.3 NA M27 ×10 air objective to quantify DTC number over the entire

831   section depth (50 μm). Images of scattered DTCs and metastases were acquired using a 0.6

832   NA Corr M27 ×40 air objective.

833

834   **Quantification of primary tumour volume, DTCs and metastases in mice.** *Mammary*

835   *tumour measurements*. Tumours were measured *in vivo* and *ex vivo* using digital calipers to

836   measure the width (W) and length (L) of the tumour. Volume (V) was calculated using the

837   equation $V = 0.5\ LW^2$.

838   *Quantification of scattered quiescent DTCs in liver lobes*. To assess the impact of

839   modulating the NK cell compartment on the distribution of scattered quiescent DTCs in the

840   liver, DTCs were quantified in tile-scanned images using Fiji (v2.0.0-rc-69/1.52n). The GFP

841   channel (illustrating quiescent mVenus^{+} DTCs) was thresholded to remove all background

842   and create a binary image. DTCs were masked, and all particles with a size ≥50 μm^2 and

843   circularity between 0.5 and 1.00 were counted using the function "Analyze Particles". The

844   obtained values were normalized to the total liver area imaged. Measurements were

845   confirmed with manual counts of one liver from each treated group to ensure accuracy.

846   *Assessment of liver metastases*. Metastases (defined as metastatic foci of ≥10 cells) were

847   quantified in tile-scanned images of Tomato- and H&E-stained livers. Metastatic burden was

848   determined by counting the frequency of metastases (i.e., the number of metastatic foci

849   present in a given liver area) and measuring the percentage of area they cover (i.e., the sum of

850   the area occupied by all metastases scored in a given liver area) using the "Tissue Classifier

851   Add-on" in Halo (Indica Labs).

852

853   **Immunohistochemistry.** *Processing of mouse livers.* Upon collection, mouse tissues were

854   fixed overnight (4 °C) in 4% paraformaldehyde (PFA):PBS solution, transferred to 70%

855   Ethanol, and then banked in paraffin.

856   *Staining and quantification of aHSCs in mouse livers.* Sections of 3 μm were cut and

857   stained simultaneously for GFP and α-SMA (antibody details provided in Supplementary

858   Table 4) using a Ventana DiscoveryXT instrument (Roche Diagnostics) following the RUO

859   Universal HQ procedure and the Discovery Purple kit. Briefly, slides were subjected to heat-

860   induced antigen retrieval and then incubated with rabbit anti-α-SMA for 32 min at 37 °C.

861   After washing, anti-rabbit HQ (Roche Diagnostics) was applied for 20 min at 37 °C, and

862   detected with anti-rabbit HQ HRP (Roche Diagnostics) for 20 min at 37 °C. A step of

863   antibody denaturation preceded staining with rabbit anti-GFP for 60 min at 37 °C. After

864   washing, Discovery purple chromogen was applied for 32 min at 37 °C, and finally slides

865   were counterstained with hematoxylin II and bluing reagent for 8 min each. GFP and α-SMA

866   double-stained livers were imaged on a Nikon Ti2 twin widefield microscope with a 0.95 NA

867   ×40 air objective, and images were quantified using Fiji (v2.0.0-rc-69/1.52n). To quantify α-

868   SMA$^+$ aHSCs, we converted the images to grayscale, segmented the α-SMA staining using

869   the "Threshold" function, masked all α-SMA$^+$ cells and counted all particles with circularity

870   between 0 and 1.00 using the function "Analyze Particles".

871   *Staining and quantification of collagen deposition in mouse livers.* Sections of 3 μm

872   were stained with a celestin blue-hemalum sequence of 7 min each. After quick washing in

873   water, slides were immersed in hydrochloric acid-ethanol 0.2% for up to 10 min until

874   becoming blue. Slides were then incubated in 0.1% picro Sirius red for 30 min, washed in

875   water, dehydrated in absolute ethanol, transferred to xylene and finally mounted. Sirius red-

876   stained livers were imaged on a Nikon Ti2 twin widefield microscope with a 0.95 NA ×40 air

877   objective, and images were quantified using Fiji (v2.0.0-rc-69/1.52n). To quantify collagen

878   deposition, we converted the images to grayscale, segmented the red-stained collagen using

879   thresholding, and measured the area fraction occupied by the thresholded area.

880   *Staining and quantification of aHSCs and NK cells in human liver biopsies.* Staining

881   against α-SMA was performed on 3 μm sections using a Ventana DiscoveryXT instrument

882   (Roche Diagnostics) following the RUO Universal HQ procedure. After heat-induced antigen

883   retrieval, slides were incubated with rabbit anti-α-SMA for 32 min at 37 °C, followed by

884   labelling with anti-rabbit HQ (Roche Diagnostics) for 20 min at 37 °C, and detection with

885   anti-rabbit HQ HRP (Roche Diagnostics) for 20 min at 37 °C. Finally, slides were

886   counterstained with hematoxylin II and bluing reagent for 8 min each. For double staining of

887   CD3 and CD57 (antibody details provided in Supplementary Table 4), a BOND-III fully

888   automated stainer and BOND kits (Leica) were used. Slides were pre-treated with EDTA

889   buffer for 20 min at 100 °C, stained with rabbit anti-CD3 for 15 min at RT, and then with the

890   BOND Polymer Refine Red Detection (Leica) kit. After CD3 staining, mouse anti-CD57 was

891   applied for 15 min at RT, and revealed with the BOND Polymer Refine Detection kit (Leica).

892   Counterstaining was performed with hematoxylin for 5 min. Stained slides were digitally

893   processed using the Ultra Fast Scanner (Philips, v1.6.1.11) with a 0.75 NA Plan Apo ×40 air

894   objective. Immunoreactivity of α-SMA was graded for perisinusoidal areas in function of the

895 percentage of α-SMA$^+$ aHSCs, and scored as 0 (no positive cells), 1 (<33% positive cells), 2

896 (34-66% positive cells) and 3 (>67% positive cells). For statistical analysis, we compared

897 cases with no α-SMA$^+$ cells (i.e., scored 0) with all the positive ones (i.e., scored 1, 2 or 3).

898 Immunoreactivity of combined CD3 and CD57 was classified according to the abundance of

899 CD3^{neg}CD57$^+$ NK cells, and scored as – (no positive cells), + (<5 positive cells), 2+ (5-10

900 positive cells) and 3+ (>10 positive cells). For statistical analysis, we compared cases with

901 none or very few NK cells (i.e., scored – or +) with cases with some or many positive NK

902 cells (i.e., scored 2+ or 3+).

903

904 **Analysis of the secretome from liver cell cultures via liquid chromatography–tandem**

905 **mass spectrometry (LC–MS/MS).** *Sample Preparation*. 8 ml of conditioned media of

906 hepatocytes and activated hepatic stellate cells grown for 48 h were collected and the proteins

907 were precipitated by adding 100% TCA (1:4, v:v, TCA:sample, Sigma, 250 g mixed with

908 113.5 ml of water). In brief, after incubation for 10 min at 4 °C, samples were centrifuged at

909 20,000 × *g* for 10 min and the supernatant removed. The protein pellet was washed twice

910 with ice cold acetone, then spun for 10 min at 20,000 × *g*, and dried at RT for 10 minutes.

911 Subsequently, proteins were dissolved in 2 M urea, 10 mM TCEP, 100 mM Tris, pH 8.5 by

912 shaking at 1,400 rpm at 25 °C in a Thermomixer (Eppendorf), and then sonicated for twenty

913 cycles (30 sec on, 30 sec off, Bioruptor, Dianode). Proteins were then reduced at 37 °C for 1

914 h, and the total protein amount was determined by BCA assay (Thermo Fisher Scientific)

915 according the manufacturer's instructions. The proteins were alkylated in 15 mM

916 chloroacetamide for 30 min at 37 °C, and then digested using sequencing-grade modified

917 trypsin (1:50, w/w, trypsin:protein, Promega) overnight at 37 °C. After digestion, the samples

918 were supplemented with TFA to a final concentration of 1%. Peptides were cleaned up using

919 iST Cartridges (Phoenix, PreOmics, Martinsried) following the manufacturer's instructions.

920 After drying the samples under vacuum, the peptides were re-suspended in 0.1% aqueous

921 formic acid solution at a concentration of 0.5 mg ml^{-1}. 0.5 μg of peptides of each sample were

922 subjected to LC-MS analysis using a dual pressure LTQ-Orbitrap Elite mass spectrometer

923 connected to an electrospray ion source (both Thermo Fisher Scientific) as recently

924 described[58] and a custom-made column heater set to 60 °C.

925   Peptide separation was carried out using an EASY nLC-1000 system (Thermo Fisher

926 Scientific) equipped with a RP-HPLC column (75 μm × 30 cm) packed in-house with C18

927 resin (ReproSil-Pur C18–AQ, 1.9 μm resin; Dr. Maisch GmbH, Ammerbuch-Entringen)

928 using a linear gradient from 95% solvent A (0.1% formic acid, 99.9% water) and 5% solvent

929 B (80% acetonitrile, 0.1% formic acid, 19.9% water) to 10% solvent B over 5 min to 35%

930 solvent B over 45 min to 50% B over 10 min to 95% solvent B over 2 min and 95% solvent B

931 over 18 min at a flow rate of 0.2 µl min^{-1}.

932 The data acquisition mode was set to obtain one high resolution MS scan in the FT part

933 of the mass spectrometer at a resolution of 240,000 full width at half maximum (at 400 m/z,

934 MS1) followed by MS/MS (MS2) scans in the linear ion trap of the 20 most intense MS

935 signals. The charged state screening modus was enabled to exclude unassigned and singly

936 charged ions and the dynamic exclusion duration was set to 30 sec. The ion accumulation

937 time was set to 300 ms (MS1) and 50 ms (MS2). MS1 and MS2 scans were acquired at a

938 target setting of 1E6 ions and 10,000 ions, respectively. The collision energy was set to 35%,

939 and one microscan was acquired for each spectrum.

940 *Label-free quantification*. The generated raw files were imported into the Progenesis QI

941 software (Nonlinear Dynamics, v2.0) and analysed using the default parameter settings.

942 MS/MS-data were exported directly from Progenesis QI in mgf format and searched against a

943 decoy database with forward and reverse sequences of the predicted proteome from homo

944 sapiens including common contaminants like keratins (download date: 5/5/2015, total of

945 41,158 entries) using MASCOT (Matrix Science, v2.4.1). The search criteria were set as

946 follows: full tryptic specificity was required (cleavage after lysine or arginine residues); 3

947 missed cleavages were allowed; carbamidomethylation (C) was set as fixed modification;

948 oxidation (M) as variable modification. The mass tolerance was set to 10 ppm for precursor

949 ions and 0.02 Da for fragment ions. Results from the database search were imported into

950 Progenesis QI and the final peptide measurement list containing the peak areas of all

951 identified peptides, respectively, was exported. This list was further processed and analysed

952 using our in-house developed SafeQuant R script[59]. The peptide and protein false discovery

953 rate (FDR) was set to 1% using the number of reverse hits in the dataset.

954

955 **Western Blotting**. Cells were lysed in RIPA buffer (50 mM Tris-HCl pH 8, 150 mM NaCl,

956 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with 1x protease inhibitor

957 cocktail (Complete Mini, Roche), 0.2 mM sodium orthovanadate, 20 mM sodium fluoride

958 and 1 mM phenylmethylsulfonyl fluoride. Proteins (80 µg) were resolved in a 10%

959 polyacrylamide gel, and transferred to a PVDF membrane (Immobilon-P, Millipore) at 100 V

960 for 65 min. The membrane was blocked for nonspecific binding with 5% BSA in TBS-T for 1

961 h at room temperature, and then probed with primary antibodies overnight at 4°C and with

962 HRP-conjugated or IRDye 680RD secondary antibodies (GE Healthcare and LI-COR) for 1 h

963 at room temperature. Blots were visualized with ECL detection and Vilber fusion imaging

964 systems, and chemiluminescent signal was captured with a LI-COR Odyssey CLx imager.

965

966 **Statistics and reproducibility.** Statistical analyses were conducted using GraphPad Prism

967 (GraphPad Software, v8). Comparisons of two groups were performed using two-tailed

968 nonparametric Mann–Whitney U tests. Comparisons of more than two groups were

969 performed using two-tailed nonparametric Kruskal-Wallis tests with Dunn's multiple

970 comparison post hoc. Please refer to figure legends for individual *n* and *P* values and the

971 specific statistical test (or tests) employed. Data represent mean ± s.d. unless stated

972 otherwise. Experiments were repeated independently, with similar results obtained. Overall

973 survival curves were computed by the Kaplan–Meier method and compared using two-tailed

974 log-rank test. Contingency tables and the Fisher's exact test were used to estimate the

975 distribution and correlation between aHSCs and NK cells in liver biopsies from breast cancer

976 patients.

977

978 **Reporting Summary.** Further information on research design is available in the Nature

979 Research Reporting Summary linked to this article.

980

981 **Data availability**

982 All mass spectrometry raw data files have been deposited to the ProteomeXchange

983 Consortium via the PRIDE[60] partner repository with the dataset identifier PXD015426. The

984 mRNA sequencing data have been deposited in the Sequence Read Archive (SRA) database

985 under BioProject accession number PRJNA576660. Source Data are provided for all figures

986 and extended data figures.

987

988 **Code availability**

989 The source code to replicate genomics and image analysis presented in this study is available

990 from Zenodo at https://doi.org/10.5281/zenodo.4570079.

991

992 **Methods References**

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1022 **Main Text Statements**

1023

1024 **Acknowledgements**

1045

1046 **Author contributions**

1047 A.L.C. conceived the study, conducted experiments, analysed and interpreted data, and wrote
1048 the manuscript. J.C.G. contributed to experimental design, and conducted all analyses and
1049 data interpretation related to mRNA sequencing. P.A.d.M. designed and performed
1050 experiments related to CRISPR mediated knockout of Cxcr4, and assisted with animal
1051 experiments. D.D.S. performed many experiments involving flow cytometry, and analysed
1052 and interpreted resulting data. M.P.T. helped design and perform experiments related to NK
1053 cell-mediated cytotoxicity. R.O. assisted with animal experiments. S.B. performed
1054 experiments to harmonize nutritional requirements and assemble different cell types in the
1055 liver co-cultures. A.S. conducted proteomics experiments, and analysed and interpreted

1056 resulting data. K.M. performed immunohistochemistry and analysed NK cell frequency on

1057 liver biopsies. K.V. conducted image acquisitions on the CQ1 Benchtop High-Content

1058 Analysis System. L.T. provided patient materials and assisted in analysing HSC frequency on

1059 liver biopsies. A.Z., M.V., W.P.W., and C.K. provided patient material. M.B.-A. conceived

1060 the study. All authors read and provided feedback on the manuscript.

1061

1062 **Competing interests**

1063 A.L.C., P.A.d.M., M.P.T., R.O., A.S., K.M., K.V., L.T., M.V. and C.K. declare no competing

1064 interests. J.C.G. and D.D.S. are employees of F. Hoffmann-La Roche. S.B. is an employee of

1065 Novartis. A.Z. received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme,

1066 Hoffmann–La Roche, NBE Therapeutics, Secarna, ACM Pharma, and Hookipa. A.Z.

1067 maintains non-commercial research agreements with Hoffmann–La Roche. A.Z. maintains

1068 further non-commercial research agreements with NBE Therapeutics, Secarna, ACM

1069 Pharma, Hookipa, and BeyondSpring. M.B.-A. owns equities in, and receives laboratory

1070 support and compensation from Novartis, and serves as consultant for Basilea.

1071

1072 **C**orrespondence and requests for materials** should be addressed to A.L.C. and M.B.-A.

1073 **Extended Data Figure Legends**

1074

1075 **Extended Data Fig. 1 | Expression profiling of breast DTCs and stroma from dormant**

1076 **and metastatic milieus reveals the determinants of progression of breast cancer in the**

1077 **liver.**

1078 **a**, Principal component analysis (PCA) of cycling and quiescent DTCs in the MDA-MB-231

1079 model. Transcriptional profiles cluster on the basis of cell cycle state. The dots in the plot

1080 represent DTCs isolated from different liver parts ($n = 11$ cycling, $n = 13$ quiescent; data

1081 combine three independent experiments). **b**, Scatter plot of mRNA expression levels (library

1082 normalized mRNA counts) in cycling and quiescent DTCs. Shown are mean expression

1083 values for each transcript in each cell cycle state ($n = 11$ cycling, $n = 13$ quiescent). mRNAs

1084 significantly upregulated or downregulated (|log2 fold change| > 1 and false discovery rate,

1085 FDR<0.01) in cycling DTCs are shown in red and blue, respectively. The dashed line

1086 indicates equal abundances in the two different conditions. **c–d**, Gene set enrichment analysis

1087 (GSEA) comparing gene expression data from cycling and quiescent DTCs. **e**, PCA of

1088 dormancy and metastasis stroma. Transcriptional profiles cluster on the basis of disease

1089 stage. The dots in the plot represent stroma isolated from different liver parts ($n = 17$

1090 dormancy, $n = 12$ metastases). **f**, Scatter plot of mRNA expression levels (library normalized

1091 mRNA counts) in metastasis and dormancy stroma. Shown are mean expression values for

1092 each transcript in each stroma ($n = 12$ metastases, $n = 17$ dormancy). mRNAs significantly

1093 upregulated or downregulated (|log2 fold change| > 1 and false discovery rate, FDR<0.01) in

1094 metastasis stroma are shown in red and blue, respectively. The dashed line indicates equal

1095 abundances in the two different conditions. **g**, GSEA comparing gene expression data from

1096 metastasis and dormancy liver stroma. **h**, Heatmap depicting the hierarchical clustering of

1097 standard score-normalized (z-score) expression level of NK markers[51] across stroma ($n = 12$

1098 metastases, $n = 17$ dormancy). **i**, Mean ± s.e.m. mRNA fold-changes (log2) of NK cell

1099 markers in metastases ($n = 12$) compared to dormancy ($n = 17$) samples. Multiple test

1100 corrected *P*-values for two-tailed Wald tests comparing fold-changes between metastasis and

1101 dormancy samples are depicted above each dot (* $P < 0.05$, *** $P < 0.001$). **j**, Violin plot

1102 showing the distribution of the median standard-score normalized (z-score) expression level

1103 of NK cell markers across metastasis and dormancy stroma ($n = 12$ metastases, $n = 17$

1104 dormancy). Solid and dashed horizontal lines depict the median and the upper and lower

1105 quartiles, respectively. Shown is the *P*-value for the two-tailed nonparametric Mann-Whitney

1106 U test. In **c**, **d** and **g**, *P*-values were calculated by one-tailed comparisons of the empirical

1107   enrichment score (ES) of a gene set to a null distribution of ESs derived from permuting the

1108   gene set, and then adjusted for multiple hypotheses testing (i.e., FDR).

1109

1110   **Extended Data Fig. 2 | NK cells are specifically enriched in liver dormancy milieus.**

1111   **a**, Flow cytometry quantification of the frequency (top) and number (bottom) of different

1112   immune cell subsets in liver parts isolated from the MDA-MB-231 model ($n = 11$ no tumour,

1113 $n = 17$ dormancy, $n = 20$ metastases; data combine three independent experiments). **b–c**,

1114   Histological characterization of the dormant 4T07 (**b**) and metastatic 4T1 (**c**) models. Left:

1115   Representative H&E-stained liver lobes. Scale bar, 2 mm. Right: Examples (corresponding to

1116   (i)–(vi) from the left images) of scattered Ki67^{neg} quiescent DTCs (indicated by arrows), and

1117   liver metastases (surrounded by a dashed coloured line). Scale bar, 30 μm. **d**, Quantification

1118   of metastatic foci in livers of 4T07 and 4T1 models, normalized to the liver lobe area

1119   analysed ($n = 10$ 4T07, $n = 10$ 4T1; mean ± s.d.; two-tailed nonparametric Mann-Whitney U

1120   test). **e**, Flow cytometry quantification of the frequency (top) and number (bottom) of

1121   different immune cell populations in livers from dormant 4T07 and metastatic 4T1 models (n

1122 $= 10$ no tumour, $n = 10$ 4T07, $n = 10$ 4T1; data combine two independent experiments). **f**,

1123   Flow cytometry quantification of the frequency of NK and T cells, as well as T cell activated

1124   populations, in liver sub-microenvironments from the metastatic 4T1 model ($n = 10$ no

1125   tumour, $n = 10$ dormancy, $n = 10$ metastasis; data combine two independent experiments). In

1126   **a**, **e** and **f**, mean ± s.d.; two-tailed nonparametric Kruskal-Wallis test with Dunn's multiple

1127   comparison post hoc.

1128

1129   **Extended Data Fig. 3 | Both cNK and LrNK cells decrease during metastatic**

1130   **progression.**

1131   **a**, NK cell, but not cNK or LrNK, gene signature alone can reliably distinguish dormancy and

1132   metastasis in liver. Violin plots show the distribution of the median standard-score

1133   normalized (z-score) expression level of NK cell (left), cNK (middle) and LrNK (right)

1134   markers across stroma ($n = 12$ metastases, $n = 17$ dormancy). Solid and dashed horizontal

1135   lines depict the median and the upper and lower quartiles, respectively. Shown is the *P*-value

1136   for the two-tailed nonparametric Mann-Whitney U test. **b-c**, cNK and LrNK cells are

1137   similarly represented within the NK compartment across different hepatic milieus. Flow

1138   cytometry quantification of the number per g of liver (**b**) or the frequency within the NK

1139   compartment (**c**) of cNK cells (CD49b^{+}CD49a^{neg}TRAIL^{neg}) and LrNK cells

1140   (CD49b^{neg}CD49a^{+}TRAIL^{+}) in liver parts isolated from the MDA-MB-231 model ($n = 6$ no

1141   tumour, $n = 12$ dormancy, $n = 9$ metastases; data combine two independent experiments;

1142   mean ± s.d.; two-tailed nonparametric Kruskal-Wallis test with Dunn's multiple comparison

1143   post hoc).

1144

1145   **Extended Data Fig. 4 | Normalizing the NK cell pool prevents hepatic metastases.**

1146   **a**, Flow cytometry quantification of NK cell frequency in IgG-, anti-GM1-, PBS- and IL-15-

1147   treated animals. Left: MDA-MB-231 model ($n = 8$ IgG, $n = 10$ anti-GM1, $n = 8$ PBS, and $n =$

1148   10 IL-15; data combine two independent experiments). Right: 4T1 model ($n = 4$ IgG, $n = 5$

1149   anti-GM1, $n = 5$ PBS, and $n = 5$ IL-15). **b**, Bioluminescence imaging 10 weeks after MDA-

1150   MB-231 tumour resection. **c–d**, Quantification of metastatic foci (**c**) and metastatic area (**d**)

1151   in livers of IgG-, anti-GM1-, PBS- and IL-15-treated animals, normalized to the liver lobe

1152   area analysed. Left: MDA-MB-231 model ($n = 8$ IgG, $n = 10$ anti-GM1, $n = 8$ PBS, and $n =$

1153   10 IL-15; data combine two independent experiments). Right: 4T1 model in BALB/c mice (n

1154 $= 5$ IgG, $n = 10$ anti-GM1, $n = 6$ PBS, and $n = 10$ IL-15). **e**, Experimental design for querying

1155   the impact of NK cell depletion on the 4T07 model. **f**, Sustained NK cell depletion reactivates

1156   dormant 4T07 DTCs in the liver. Arrows indicate single Ki67^{neg} quiescent DTCs. Coloured

1157   line delineates a metastasis. Scale bar, 30 μm. **g**, Quantification of liver metastatic upon NK

1158   cell depletion in the 4T07 model, normalized to the liver lobe area analysed ($n = 10$ IgG, $n =$

1159   10 anti-GM1). **h**, Quantification of scattered quiescent DTCs in livers of IgG-, anti-GM1-,

1160   PBS- and IL-15-treated animals, normalized to the liver lobe area analysed ($n = 8$ IgG, $n = 10$

1161   anti-GM1, $n = 8$ PBS, and $n = 10$ IL-15; data combine two independent experiments). **i**,

1162   Expression of IL-15Rα in MDA-MB-231, 4T07 and 4T1 cells assessed by Western blotting.

1163   ERK2 was used as loading control. For gel source data, see Supplementary Information, SI

1164   Fig. 1 ($n = 3$ experiments). **j**, Histogram of IL-15Rα measured by antibody-based staining

1165   and flow cytometry on MDA-MB-231, 4T07 and 4T1 cells. **k**, Quantification of relative

1166   percentages of quiescent (Tomato^{+}mVenus^{+}) and cycling (Tomato^{+}mVenus^{neg}) cancer cells

1167   upon 24h of IL-15 treatment shows no effect on cell population ratios ($n = 3$ independent

1168   experiments). **l–n**, Flow cytometry quantification of T cell frequency (**l**) and activation (**m**, **n**)

1169   in livers from 4T1-injected mice treated with PBS or IL-15 ($n = 5$ PBS, $n = 5$ IL-15). In **a**, **c**,

1170   **d**, **g**, **h** and **k–n**, mean ± s.d. are shown, and *P*-values were calculated using two-tailed

1171   nonparametric Mann-Whitney U test.

1172

1173   **Extended Data Fig. 5 | Quiescent DTCs are not intrinsically resistant to NK cell**

1174   **recognition and killing.**

1175　**a–b**, Mean ± s.e.m. mRNA fold-changes (log2) of NK cell activating (**a**) and inhibitory (**b**)

1176　ligands in cycling (*n* = 11) compared to quiescent DTCs (*n* = 13). Multiple test corrected *P*-

1177　values for two-tailed Wald tests comparing fold-changes between cycling and quiescent

1178　DTCs are depicted above each dot (**P < 0.05, ** *P* < 0.01, *** *P* < 0.001). **c**, Schematic of

1179　experiment to test the sensitivity of cycling and quiescent DTCs to NK cell-mediated

1180　cytotoxicity. Human MDA-MB-231 or mouse 4T07 and 4T1 cancer cells co-expressing

1181　Tomato and mVenus-p27K⁻ were co-cultured with human blood- or mouse liver-derived NK

1182　cells, and assayed for cytolysis. MACS, magnetic-activated sorting. FACS, fluorescence-

1183　activated cell sorting. **d**, NK cells kill DTCs regardless of their cell cycle stage. The

1184　percentage of specifically killed cycling and quiescent cancer cells was calculated for

1185　different effector:target (E:T) ratios. For 4T07 and 4T1, *n* = 3 pooled mice per experiment,

1186　data combine three independent experiments; for MDA-MB-231, *n* = 4 healthy donors; mean

1187　± s.d.; two-tailed nonparametric Mann-Whitney U test.

1188

1189　**Extended Data Fig. 6 | Transcriptional landscape of NK cells from dormant, metastatic**

1190　**and tumour-free liver milieus.**

1191　**a–b**, GSEA comparing gene expression data from dormancy and tumour-free liver NK cells

1192　(*n* = 10 no tumour, *n* = 17 dormancy). **c-d**, GSEA comparing gene expression data from

1193　metastasis and tumour-free liver NK cells (*n* = 10 no tumour, *n* = 7 metastases). **e**, GSEA

1194　comparing gene expression data from metastasis and dormancy liver NK cells (*n* = 17

1195　dormancy, *n* = 7 metastases). In **a–e**, one-tailed comparisons of the ES of a gene set to a null

1196　distribution of ESs derived from permuting the gene set, and then adjusted for multiple

1197　hypotheses testing (i.e., FDR). **f**, Flow cytometry quantification of liver TNF-α⁺ NK cells.

1198　Left: liver parts from the MDA-MB-231 model (*n* = 6 no tumour, *n* = 12 dormancy, *n* = 9

1199　metastases; data combine two independent experiments). Right: livers from dormant 4T07

1200　and metastatic 4T1 models (*n* = 12 no tumour, *n* = 12 4T07, *n* = 12 4T1; data combine two

1201　independent experiments; mean ± s.d.; two-tailed nonparametric Mann-Whitney U test.). **g**,

1202　GSEA of the Hallmark "IFN-γ response" pathway (*n* = 11 cycling, *n* = 13 quiescent). NES,

1203　Normalized Enrichment Score. FDR, False Discovery Rate. **h**, Mean ± s.e.m. mRNA fold-

1204　changes (log2) of members of the IFN-γ signalling pathway in cycling (*n* = 11) compared to

1205　quiescent DTCs (*n* = 13). Multiple test corrected *P*-values for two-tailed Wald tests

1206　comparing fold-changes between cycling and quiescent DTCs are depicted above each dot (*

1207　*P* < 0.05, ** *P* < 0.01, *** *P* < 0.001).

1208

1209 **Extended Data Fig. 7 | aHSCs steer NK cell depletion and promote liver metastasis.**

1210 **a**, Mean ± s.e.m. mRNA fold-changes (log2) of aHSC markers in metastasis (n = 12)

1211 compared to dormancy (n = 17). Multiple test corrected P-values for two-tailed Wald tests

1212 comparing fold-changes between metastasis and dormancy are depicted above each dot (* P

1213 < 0.05, *** P < 0.001). **b**, Violin plot showing the distribution of the median z-score

1214 expression level of aHSC markers across liver stroma (n = 12 metastases, n = 17 dormancy).

1215 Solid and dashed horizontal lines depict the median and the upper and lower quartiles,

1216 respectively; two-tailed nonparametric Mann-Whitney U test. **c**, Quantification of α-SMA$^+$

1217 aHSCs upon NK cell modulation. Left: MDA-MB-231 model (n = 8 IgG, n = 10 anti-GM1, n

1218 = 8 PBS, and n = 10 IL-15; data combine two independent experiments). Right: 4T1 model (n

1219 = 5 IgG, n = 10 anti-GM1, n = 6 PBS, and n = 10 IL-15). **d**, Bioluminescence imaging 6

1220 weeks after tumour resection. **e**, Quantification of bioluminescence shows no changes in lung

1221 metastatic burden (n = 10 oil, n = 16 CCl$_4$; data combine two independent experiments). In **c**

1222 and **e**, mean ± s.d.; two-tailed nonparametric Mann-Whitney U test.

1223

1224 **Extended Data Fig. 8 | Activation of HSCs shrinks the NK cell compartment even in the**

1225 **absence of tumour cells.**

1226 **a**, Representative micrographs of α-SMA$^+$ aHSCs and collagen deposition in livers from non-

1227 tumour-bearing NOD scid animals treated with oil or CCl$_4$ for 1 or 6 weeks. Scale bar, 30

1228 μm. **b-c**, Flow cytometry quantification of NK cell frequency (**b**) and proliferation (**c**) in

1229 livers from non-tumour-bearing NOD scid animals treated with oil or CCl$_4$ for 1 or 6 weeks

1230 (for each time point, n = 10 oil, n = 10 CCl$_4$; data combine two independent experiments;

1231 mean ± s.d.; two-tailed nonparametric Mann-Whitney U test).

1232

1233 **Extended Data Fig. 9 | CXCL12 limits the proliferation of NK cells from healthy donors**

1234 **and breast cancer patients with liver metastases.**

1235 **a**, t-SNE plot showing the relative expression of CXCR4 on different liver cell types based

1236 on 8444 human liver cells previously sequenced[51]. Each dot represents a single cell, and cells

1237 are coloured from lowest (yellow) to highest (purple) expression. **b**, Histogram of CXCR4

1238 measured by flow cytometry on human NK-92 cells. **c**, Exogenous CXCL12 increases the

1239 number of G$_0$/G$_1$ resting NK-92 cells, but it has no effect on NK cell viability (n = 5

1240 independent experiments; mean ± s.d.; two-tailed nonparametric Kruskal-Wallis test with

1241 Dunn's multiple comparison post hoc). **d**, Schematic of experiments to test the effect of

1242 CXCL12 on blood-derived NK cells purified from healthy donors and breast cancer patients

1243 with liver metastases. NK cells labelled with cell trace violet (CTV) were primed with IL-2

1244 and IL-15, and then expanded with IL-2 in the presence of CXCL12 alone or combined with

1245 IL-15 until assessed for division profile. **e**, Representative histogram of the NK cell division

1246 profile of a healthy donor. **f**, Quantification of the division index (i.e., the average number of

1247 cell divisions a cell has undergone) of blood-derived NK cells from healthy donors (left, $n =$

1248 6) and breast cancer patients with liver metastases (right, $n = 6$) upon treatment with

1249 CXCL12 alone or combined with IL-15. C1-C3 correspond to different concentrations of

1250 recombinant CXCL12 (C1 = 0.02μg ml^{-1}, C2 = 0.2μg ml^{-1} and C3 = 2μg ml^{-1}). Mean ± s.d.;

1251 two-tailed nonparametric Kruskal-Wallis test with Dunn's multiple comparison post hoc. **g**,

1252 Experimental schematic to probe the effect of aHSC-secreted CXCL12 on liver NK cells.

1253 Mouse NK cells were treated with CM from liver-derived aHSCs in the presence of a

1254 function-blocking antibody against CXCL12 or a control IgG, and G_0/G_1 resting cells were

1255 quantified after EdU incorporation. **h**, Flow cytometry quantification of quiescent Ki67^{neg}

1256 NK cells in mouse liver milieus ($n = 6$ no tumour, $n = 12$ dormancy, $n = 9$ metastases; data

1257 combine two independent experiments; mean ± s.d.; nonparametric two-tailed Kruskal-Wallis

1258 test with Dunn's multiple comparison post hoc). **i**, Proliferation of CXCR4$^+$ NK cells from

1259 metastatic milieus ($n = 9$ metastases; mean ± s.d.; nonparametric Mann-Whitney U test).

1260

1261 **Extended Data Fig. 10 | CXCR4 expression confers DTCs a proliferative advantage but**

1262 **is not required for outgrowth.**

1263 **a**, Experimental design for testing the influence of aHSC-secreted CXCL12 on cancer cell

1264 proliferation. Co-cultures of hepatocytes and sparsely seeded cancer cells were exposed to

1265 recombinant CXCL12 protein or conditioned medium (CM) from aHSCs alone or in

1266 combination with either anti-CXCL12, anti-CXCR4, control IgG or a CXCR4 inhibitor, and

1267 cancer cell number was analysed by flow cytometry. **b**, Quantification of cancer cell number

1268 in different liver-like milieus shows that CXCL12-CXCR4 signalling induces cancer cell

1269 proliferation (for each cell line, $n = 5$ independent experiments). **c**, Scheme of *Cxcr4* sites

1270 targeted by sgRNAs to generate 4T1.CXCR4_KO cells. **d**, Genotyping of clonal derived cells

1271 obtained through CRISPR-Cas9 targeting of *Cxcr4*. Coloured lanes represent clones selected

1272 and pooled as 4T1.CXCR4_WT and 4T1.CXCR4_KO lines ($n = 1$ PCR per clone; selected

1273 clones were also confirmed by sequencing). bp, base pair. **e**, Experimental design for

1274 assessing the requirement of CXCR4 for liver metastasis. **f**, Representative H&E-stained

1275   livers from 4T1.CXCR4_WT and 4T1.CXCR4_KO lines injected in BALB/c

1276   immunocompetent mice. Arrows and coloured lines indicate metastases. Scale bar, 2 mm. **g**,

1277   Quantification of liver metastatic foci in livers of Oil- and CCl_4-treated animals, normalized

1278   to the liver lobe area analysed (n = 6 WT Oil, n = 9 WT CCl_4, n = 8 KO Oil, and n = 11 KO

1279 CCl_4). **h**, Quantification of metastatic area in livers of Oil- and CCl_4-treated animals,

1280   normalized to the liver lobe area analysed (n = 6 WT Oil, n = 9 WT CCl_4, n = 8 KO Oil, and

1281 n = 11 KO CCl_4). In **b**, **g** and **h**, mean ± s.d.; two-tailed nonparametric Mann-Whitney U test.

1282

1283   **Extended Data Fig. 11 | Activated hepatic stellate cells and CXCL12 accumulate in**

1284   **patients with liver metastases.**

1285   **a**, Staining of NK cells (CD3^{neg}CD57^{+}) and aHSCs (α-SMA^{+}) in paired metastases and

1286   normal adjacent tissues in liver biopsies from breast cancer patients. Arrows indicate HSCs

1287   (top) and NK cells (bottom). Scale bar, 30 μm. **b**, Correlation between aHSCs and NK cells

1288   in paired metastases and normal adjacent tissues in liver biopsies from breast cancer patients

1289   (n = 34 paired biopsies; Fisher's exact test). **c**, Staining of NK cells (CD3^{neg}CD57^{+}) and

1290   aHSCs (α-SMA^{+}) in liver biopsies from breast cancer patients with chronic liver disease but

1291   no metastases. Arrows indicate HSCs (top) and NK cells (bottom). Scale bar, 30 μm. **d**,

1292   Correlation between aHSCs and NK cells in liver biopsies from breast cancer patients with

1293   chronic liver disease but no metastases (n = 35 biopsies; Fisher's exact test). **e**, Heatmap

1294   depicting the hierarchical clustering of standard score-normalized (z-score) expression level

1295   of aHSC markers[51] across normal and metastatic liver samples from colon cancer patients[38]

1296   (n = 5 normal livers, n = 18 liver metastases). **f**, Violin plot showing the distribution of the

1297   median standard-score normalized (z-score) expression level of aHSC markers across human

1298   normal livers (n = 5) and liver metastases (n = 18). Solid and dashed horizontal lines depict

1299   the median and the upper and lower quartiles, respectively. Shown is the *P*-value for the two-

1300   sided nonparametric Mann-Whitney U test. **g**, Heatmap depicting the hierarchical clustering

1301   of standard score-normalized (z-score) expression level of NK cell markers[51] across normal

1302   livers (n = 5) and liver metastases (n = 18) from colon cancer patients[38]. **h**, Violin plot

1303   showing the distribution of the median standard-score normalized (z-score) expression level

1304   of NK markers across human normal livers (n = 5) and liver metastases (n = 18). Solid and

1305   dashed horizontal lines depict the median and the upper and lower quartiles, respectively.

1306   Two-sided nonparametric Mann-Whitney U test. **i**, Scatter plot of median standard-score

1307   normalized (z-score) expression level of HSC markers and *CXCL12* expression across human

1308   liver metastases (n = 134). Shown is also the Pearson correlation coefficient (R) and

1309   respective *P*-value. The dashed line indicates the linear regression between the two estimates.

1310   FPKM, fragments per kilobase per million mapped reads.

1311

a

Ubi — Luc2 — tdTomato

EF1 — mVenus — p27K⁻

DTC live tracker
Cycling vs Quiescent

Cancer cell injection → 4 wks → Tumour resection → 6 wks → DTCs and stroma in distant tissues

b

DTCs / Quiescent DTCs / Stroma

Tomato vs mVenus-p27K⁻

c Cells (%) — DTCs / Stroma — Lg, Liv, BoMa, Sp, Kid

d Cells (%) — Quiescent DTCs / Cycling DTCs — Lg, Liv, BoMa, Sp, Kid

e

DNA / mVenus-p27K⁻ / Tomato

(i) (ii) (iii) (iv)

f

(i) Dormancy / (ii) Metastasis

Cycling DTCs / Quiescent DTCs

Cells (%) — Quiescent / Cycling — Dormancy, Metastasis

g GSEA Dormancy stroma

IFN-γ response
IL-12 pathway
Cell killing
NK immunity
Peroxisome
ROS pathway
IFN-γ production
NK cells pathway
Bile acid metabolism
NK cytotoxicity

-log10(FDR)

h

NOD scid / BALB/c

% NK cells of immune cells in liver

No tumour / Dormancy / Metastasis — P = 0.0006, P = 0.03
No Tumour / 4T07 / 4T1 — P = 0.008, P = 0.02

i

Cancer cell injection → Tumour resection → Quantification of metastasis
2.5 → 10 wks

MDA-231
4T1

IgG, anti-GM1, PBS or IL-15

j MDA-231 / 4T1 — IgG, Anti-GM1, PBS, IL-15

k 4T1 + IL-15 — H&E / Ki67

l Overall survival (%)

IgG / Anti-GM1 / PBS / IL-15

P < 0.001

Days after tumor resection

a Cancer cell injection → 4 wks → Tumour resection → 6 wks → Isolation of livers

Metastasis / Dormancy → No tumour → FACS → NK_Metastasis / NK_Dormancy / NK_No tumour

b GSEA Dormancy NK cells

- G2/M checkpoint
- Inflammatory response
- TNF-α signaling via NF-κB
- IL-6/JAK/STAT3 signaling
- IFN-γ response
- IFN-α response
- Mitotic spindle
- Glycolysis
- Protein secretion
- mTORC1 signaling

-log10(FDR)

c

NOD scid mice
% IFN-γ⁺ NK cells in liver
P = 0.006
P = 0.03
No tumour / Dormancy / Metastasis

BALB/c mice
P = 0.02
P = 0.008
No tumour / 4T07 / 4T1

d Hepatocytes → 2 days → Tomato mVenus-p27K⁻ cancer cells → 1 h → IFN-γ → 4 days → Quantification of: Quiescent cells / Cycling cells

e

Hepatocytes + 4T07 Tomato mVenus — PBS / IFN-γ
Hepatocytes + 4T1 Tomato mVenus — PBS / IFN-γ

f

4T07
Fraction of quiescent cells per FOV
P = 0.0002
Untreated / PBS / IFN-γ / FBS starvation / Tomato only

4T1
P < 0.0001
Untreated / PBS / IFN-γ / FBS starvation / Tomato only

a

Col3a1
Col1a1
Col1a2
Acta2
Tagln

Gene expression (z-score)
-2 -1 0 1 2

Metastasis
Dormancy

b

α-SMA mVenus · Collagen

No tumour

Dormancy

Metastasis

c

α-SMA⁺ cells per FOV

P < 0.0001
P < 0.0001
NS

No tumour · Dormancy · Metastasis

d

Collagen deposition (% of area per FOV)

P < 0.0001
P < 0.0001
NS

No tumour · Dormancy · Metastasis

e

Cancer cell injection
Tumour resection
Quantification of NKs & metastatic burden

MDA-231

2.5 4   6   10 wks
CCl₄ or oil

f

Metastatic foci (per 100 mm³ liver)

P = 0.02

Oil · CCl₄

g

% NK cells of immune cells in liver

P = 0.0003

Oil · CCl₄

h

% Ki67⁺ liver NK cells

P = 0.0001

Oil · CCl₄

b

%EdU⁺ NK-92 cells

$P = 0.005$
$P = 0.02$
NS
NS

Control, CM_NK cells, CM_Heps, CM_Heps+aHSCs, CM_aHSCs

c

$-\log_{10}$ Adj. P

MMP9
SEMA5A
CXCL12
SCARF1
ARPC4
SFRP1

$\log_2$ CM_Heps/CM_aHSCs

d

%G₀/G₁ resting NK cells

NS
$P = 0.02$
$P = 0.02$

Control, CM_aHSCs, CM_aHSCs+IgG, CM_aHSCs+CXCL12Ab

e

Dormancy

NK cell — NK cell proliferation
LSEC
IL-15
Quiescent HSC
IFN-γ
Hepatocyte — Quiescent DTC

Metastasis

NK cell — NK cell quiescence
CXCR4
LSEC
CXCL12
Activated HSC
Tissue injury
CXCR4
Hepatocyte — Proliferating DTCs