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Loss of p53 triggers Wnt-dependent systemic inflammation to drive breast cancer metastasis

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36

37 **Abstract**

38 Cancer-associated systemic inflammation is strongly linked with poor disease outcome in
39 cancer patients[1,2]. For most human epithelial tumour types, high systemic neutrophil-to-
40 lymphocyte ratios are associated with poor overall survival[3], and experimental studies have
41 demonstrated a causal relationship between neutrophils and metastasis[4,5]. However, the
42 cancer cell-intrinsic mechanisms dictating the substantial heterogeneity in systemic
43 neutrophilic inflammation between tumour-bearing hosts are largely unresolved. Using a panel
44 of 16 distinct genetically engineered mouse models (GEMMs) for breast cancer, we have
45 uncovered a novel role for cancer cell-intrinsic p53 as a key regulator of pro-metastatic
46 neutrophils. Mechanistically, p53 loss in cancer cells induced secretion of Wnt ligands that
47 stimulate IL-1β production by tumour-associated macrophages, which drives systemic
48 inflammation. Pharmacological and genetic blockade of Wnt secretion in p53-null cancer cells
49 reverses IL-1β expression by macrophages and subsequent neutrophilic inflammation,
50 resulting in reduced metastasis formation. Collectively, we demonstrate a novel mechanistic
51 link between loss of p53 in cancer cells, Wnt ligand secretion and systemic neutrophilia that
52 potentiates metastatic progression. These insights illustrate the importance of the genetic
53 makeup of breast tumours in dictating pro-metastatic systemic inflammation, and set the stage
54 for personalized immune intervention strategies for cancer patients.

55

56 **Main text**

57 To determine how pro-metastatic systemic inflammation is influenced by genetic aberrations
58 in tumours, we studied 16 GEMMs for breast cancer carrying different tissue-specific
59 mutations. These GEMMs represent most subtypes of human breast cancer, including ductal
60 and lobular carcinoma, oestrogen receptor-positive (luminal A), $HER2^+$, triple-negative and
61 basal-like breast cancer. Because we and others have demonstrated that neutrophils expand
62 systemically and promote metastasis[5-10], we evaluated circulating neutrophil levels as a marker
63 for systemic inflammation in mammary tumour-bearing mice with end-stage disease. As
64 expected, most tumour-bearing mice displayed an increase in circulating neutrophils as
65 compared to non-tumour-bearing animals (wild-type [WT]) (Fig. 1a). Like the inter-patient
66 heterogeneity in systemic inflammation in human breast cancer[11], we observed a striking
67 variability in the extent of neutrophilia between the different tumour-bearing GEMMs (Fig. 1a,
68 Extended Data Fig. 1a). We found that the models exhibiting high neutrophil expansion
69 displayed a subset of neutrophils expressing the stem cell marker cKIT (Fig. 1b), indicative of
70 an immature neutrophil phenotype[5]. We subsequently searched for commonalities and
71 differences among the 16 GEMMs with regards to high versus low systemic neutrophil levels.
72 Strikingly, mice bearing tumours with a p53 deletion exhibited the most pronounced circulating
73 neutrophil levels (Fig. 1a). The difference in magnitude of systemic inflammation between p53-
74 proficient and p53-null tumours was even more apparent when focusing on $cKIT^+$ neutrophils
75 (Fig. 1b).

76 In mouse models for colorectal, pancreatic, prostate and endometrial cancer, p53
77 mutation or loss leads to recruitment and activation of immune cells in the primary tumour
78 microenvironment[12-16]. To study the association between p53 status of the tumour and
79 systemic inflammation, we separated the 16 GEMMs based on the presence or absence of
80 homozygously floxed *Trp53* alleles and compared the levels of circulating neutrophils and the
81 proportion of cKIT-expressing neutrophils. This analysis confirmed a statistically significant
82 difference between mice bearing p53-proficient and p53-null tumours (Fig. 1c, d).

83 We previously demonstrated that expansion of neutrophils in mammary tumour-bearing
84 *K14-cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* (KEP) mice is driven by an inflammatory pathway involving CCL2,
85 IL-1β, IL-17A and G-CSF[5,17]. We found that serum levels of CCL2, IL-1β and G-CSF correlated
86 with p53 loss in primary tumours in the 16 GEMMs (Fig. 1e–h). Principal component analysis
87 of these systemic immune parameters further demonstrated that systemic inflammation
88 correlated with p53 status of the tumour (Fig. 1i).

89 To provide evidence for a causal relationship between p53-loss in mammary tumours
90 and neutrophilia, we derived cancer cell lines from two independent p53-proficient tumour
91 models, *Wap-cre;Cdh1$^{F/F}$;Akt^{E17K}* (WEA)[18] and *Wap-cre;Cdh1$^{F/F}$;Pik3ca^{E545K}* (WEP). Using
92 CRISPR/Cas9-mediated gene disruption, we targeted *Trp53*, which resulted in an inability to
93 increase p21 levels after irradiation (Extended Data Fig. 2a, b, e). We orthotopically
94 transplanted *WEA;Trp53$^{+/+}$* and *WEP;Trp53$^{+/+}$* cells, and matched *WEA;Trp53$^{-/-}$* and
95 *WEP;Trp53$^{-/-}$* cells into syngeneic WT mice (Fig. 2a). While p53-loss conferred a proliferation
96 advantage *in vitro, in vivo* growth kinetics were similar between p53-proficient and -deficient
97 tumours for both cell lines (Extended Data Fig. 2c–g). Consistent with our findings in the GEMM
98 panel, we observed increased expansion of neutrophils, including cKIT^{+} neutrophils, in the
99 circulation and lungs of mice bearing *WEA;Trp53$^{-/-}$* and *WEP;Trp53$^{-/-}$* tumours, when
100 compared to mice bearing size-matched p53-proficient tumours (Fig. 2b–d, Extended Data
101 Fig. 2h, i). In addition, mice with *WEA;Trp53$^{-/-}$*, but not *WEP;Trp53$^{-/-}$* tumours, presented with
102 splenomegaly when compared to *Trp53$^{+/+}$* controls (Extended Data Fig. 2j), a phenomenon
103 often observed in inflammation and cancer[19]. These data reveal that loss of p53 in breast
104 cancer cells is a central driving event of cancer-induced systemic neutrophilic inflammation.

105 Since we observed cKIT^{+} immature neutrophils in p53-null tumour-bearing mice (Fig.
106 1d, 2d), we next investigated whether haematopoiesis was altered. In mice bearing
107 *WEA;Trp53$^{-/-}$* tumours, frequencies of Lin^{-}Sca1^{+}cKIT^{+} cells (LSKs), common myeloid
108 progenitors (CMPs), CD11b^{+}Ly6G^{low} pro-myelocytes and mature neutrophils were increased
109 in the bone marrow at the expense of megakaryocyte and erythrocyte progenitors (MEPs),
110 when compared to *WEA;Trp53$^{+/+}$* tumour-bearing mice (Extended Data Fig. 3a–c). This effect

111 on cell proportions was not reflected in the total cell counts, possibly due to a slight depletion
112 of total bone marrow cell numbers in *WEA;Trp53*$^{-/-}$ tumour-bearing mice (Extended Data Fig.
113 3d).

114 Previously, we reported that macrophage-derived IL-1β in the tumour
115 microenvironment triggers systemic neutrophil expansion in KEP mice[5]. Since IL-1β serum
116 levels correlated with p53 status (Fig. 1f) we hypothesized that loss of p53 changes the
117 secretome of cancer cells, stimulating IL-1β production from tumour-associated macrophages
118 (TAMs) and setting off a systemic inflammatory cascade. Indeed, *in vitro* exposure of bone
119 marrow-derived macrophages (BMDMs) to conditioned medium (CM) from *WEA;Trp53*$^{-/-}$ or
120 *WEA;Trp53*$^{+/+}$ cancer cells differentially affected their phenotype (Extended Data Fig. 4a).
121 Notably, CM from *WEA;Trp53*$^{-/-}$ and *WEP;Trp53*$^{-/-}$ cells strongly induced *Il1b* mRNA
122 expression in cultured BMDMs as compared to CM from matched *Trp53*$^{+/+}$ controls (Fig. 2e).
123 In agreement with our mouse data, human monocyte-derived macrophages (hMDMs) cultured
124 with tumour CM of *TP53*$^{-/-}$ MCF-7 human breast cancer cells displayed increased CD206 and
125 CD163 expression compared to hMDMs cultured with CM of p53-proficient MCF-7 cells
126 (Extended Data Fig. 4c). We also observed increased *IL1B* expression in hMDMs upon
127 exposure to *TP53*$^{-/-}$ MCF-7 cells compared to *TP53*$^{+/+}$ controls (Extended Data Fig. 4d). These
128 data indicate that cancer cell-intrinsic p53 status dictates the crosstalk between cancer cells
129 and macrophages in a paracrine fashion, resulting in an altered macrophage phenotype and
130 IL-1β production. We also observed elevated levels of *IL1B* mRNA expression in breast
131 tumours of The Cancer Genome Atlas (TCGA) with mutations in *TP53* (*TP53*^{MUT}) compared to
132 *TP53*^{WT} tumours (Fig. 2f), suggesting similar p53-dependent activation of IL-1β signalling in
133 human breast cancer.

134 To identify which factor(s) in p53-null tumours mediate TAM activation and subsequent
135 systemic inflammation, we performed RNA sequencing on mammary tumours of 12 different
136 GEMMs (7 p53-null models, 5 p53-proficient models; 145 tumours in total). The p53-deficient
137 tumours differed substantially from p53-proficient tumours in terms of gene expression,

138 regardless of any additional genetic aberrations, demonstrating a dominant effect of p53-loss
139 on the global transcriptome (Extended Data Fig. 5a). Interestingly, the most significantly
140 changed pathways in p53-deficient tumours pertained to adaptive immune phenotypes (Fig.
141 3a). While neutrophil and TAM numbers were altered intratumourally, the composition of $CD8^+$,
142 $CD4^+$ or $FOXP3^+$ T cells did not correlate with p53-status (Extended Data Fig. 5b–g),
143 suggesting that the distinct transcriptome profiles are not due to a p53-dependent effect on the
144 composition of the adaptive immune landscape.

145 From the gene ontology analysis, we selected genes encoding secreted factors that
146 could potentially influence TAMs. One of the up-regulated pathways in p53-null tumours
147 included WNT/β-catenin signalling (Fig. 3a). WNT signalling is linked to IL-1β production in
148 acute arthritis, as well as immune and stromal signalling in cancer[20-23]. Using a WNT/β-catenin
149 signalling gene signature, we found that p53-null GEMM tumours clustered separately from
150 p53-proficient tumours, indicating an association between p53-loss and WNT-related gene
151 expression (Extended Data Fig. 6a, b). Many WNT-related genes were up-regulated in p53-
152 deficient tumours, including three WNT ligands, *Wnt1*, *Wnt6* and *Wnt7a*, while expression of
153 negative regulators of WNT signalling was decreased (Fig. 3b, Extended Data Fig. 6c).
154 Elevated protein levels of WNT1 and WNT7A were confirmed in a set of independent p53-
155 deficient tumours (Fig. 3c, d). We also found increased expression of non-phosphorylated β-
156 catenin, indicative of activated WNT signalling (Fig. 3c, d). In human breast tumours,
157 expression of *WNT1, WNT6* and *WNT7A* was increased upon aberrant expression of *TP53,*
158 compared to $TP53^{WT}$ tumours (Fig. 3e). We then broadened our analysis of TCGA data to
159 other WNT-related genes and discovered a trend towards enrichment of these genes in *TP53*-
160 mutated tumours (Extended Data Fig. 6d). Additionally, individual WNT-stimulating genes
161 were upregulated, while WNT-inhibiting genes were downregulated in $TP53^{MUT}$ versus $TP53^{WT}$
162 human tumours (Extended Data Fig. 6e), indicating that WNT signalling is activated upon
163 aberrant expression of *TP53*. Using WEA cell lines, we confirmed that WNT1, WNT6 and
164 WNT7A proteins are increased intracellularly in $WEA;Trp53^{-/-}$ cells and secreted, when

165 compared to *WEA;Trp53*$^{+/+}$ cells (Fig. 3f). Collectively, these data indicate cancer cell-
166 autonomous WNT ligand secretion upon loss of p53.

167 Since deletion of p53 increases WNT ligand expression, we hypothesized that wild-
168 type p53 negatively regulates these genes, either directly or indirectly. To determine whether
169 p53 binds the regulatory regions of *Wnt1, Wnt6* and/or *Wnt7a*, we performed chromatin
170 immunoprecipitation-sequencing (ChIP-seq) in 3 independent WEA and WEP cell lines. p53
171 binding was observed at the *Cdkn1a* (p21) locus (Extended Data Fig. 7a), whereas we did not
172 find p53 binding at the *Wnt1, Wnt6* or *Wnt7a* loci (Extended Data Fig. 7b), suggesting that p53
173 regulates their expression indirectly. Since p53 has been described to control *Wnt1* expression
174 by activating microRNA-34a (miR-34a)[24], we wondered whether this microRNA may be
175 involved in the regulation of *Wnt1*, *Wnt6* and *Wnt7a*. Indeed, we observed p53 chromatin
176 binding at the miR-34a locus in all cell lines (Extended Data Fig. 7c). Overexpression of miR-
177 34a in *WEA;Trp53*$^{-/-}$ cells resulted in a significant reduction of WNT ligand expression
178 (Extended Data Fig. 7d). These data suggest that wild-type p53 negatively regulates the
179 expression of *Wnt1, Wnt6* and *Wnt7a* via miR-34a.

180 We then assessed the role of cancer cell-derived WNT ligands on IL-1β production by
181 macrophages. We treated WEA cells with LGK974 – which inhibits Porcupine (*Porcn*), a Wnt-
182 specific acyltransferase that regulates WNT ligand secretion[25] – and added CM to
183 macrophages. LGK974 reduced the *WEA;Trp53*$^{-/-}$ cell-induced *Il1b* expression by
184 macrophages (Fig. 4a). We also depleted *Porcn* in *WEA;Trp53*$^{-/-}$ cells using short hairpin
185 RNAs (shRNA) and knockdown reduced *Il1b* expression by macrophages, consistent with
186 pharmacological Porcupine inhibition (Fig. 4a). These data confirm a causal relationship
187 between WNT ligand secretion by p53-deficient cancer cells and IL-1β expression in
188 macrophages.

189 To identify the receptors involved in the crosstalk between p53-null cancer cells and
190 macrophages, we looked for genes encoding WNT receptors in the GEMM gene expression
191 data. We found that Frizzled receptors, *Fzd7* and *Fzd9,* were up-regulated in the p53-null
192 tumours compared to p53-proficient tumours (Extended Data Fig. 8a). Similarly, *FZD7* and

193 *FZD9* were increased in expression in $TP53^{MUT}$ human breast tumours compared to $TP53^{WT}$
194 tumours (Extended Data Fig. 8b). We then used small interfering RNAs (siRNA) to knockdown
195 both *Fzd7* and *Fzd9* in BMDMs (Extended Data Fig. 8c), which prevented *Il1b* induction by
196 *WEA;Trp53*$^{-/-}$ cells (Extended Data Fig. 8d), demonstrating that that FZD7 and FZD9 are
197 involved in WNT-induced activation of macrophages *in vitro.*

198 We next assessed whether WNT ligand production by p53-deficient cancer cells drives
199 systemic inflammation. We treated tumour-bearing KEP mice with LGK974 for five consecutive
200 days and this led to a reduction in total neutrophils and cKIT^{+} neutrophils in blood and lungs
201 when compared to vehicle-treated KEP mice (Fig. 4b, Extended Data Fig. 9a). Additionally, IL-
202 17A-producing $\gamma\delta$ T cells – the key cell type responding to IL-1β that drive neutrophil
203 accumulation and consequently metastasis[5] – were reduced in the lungs of LGK974-treated
204 KEP mice (Extended Data Fig. 9b), indicating that $\gamma\delta$ T cell activation upstream of pro-
205 metastatic neutrophil accumulation depends on WNT signalling. Similarly, long-term treatment
206 of KEP mice with LGK974 blocked neutrophil expansion over time (Extended data Fig. 9c). To
207 exclude that the observed reduction in inflammation is a result of targeting non-tumour cells
208 by LGK974, we orthotopically transplanted *WEA;Trp53*$^{-/-}$*;shPorcn* cell lines and matched
209 *WEA;Trp53*$^{-/-}$*;shControl* cells into WT mice. Analysis of size-matched end-stage tumours
210 revealed an incomplete reduction of *Porcn* expression (Extended Data Fig. 9d). Although we
211 cannot formally exclude the possibility that non-cancer cells contribute to the residual *Porcn*
212 expression, expression levels of *Porcn* in the tumours correlated with circulating neutrophils,
213 cKIT^{+} neutrophils and *Il1b* expression (Extended Data Fig. 9e–g). Moreover, knockdown of
214 *Porcn* prevented splenomegaly (Extended Data Fig. 9h). Collectively, these data confirm the
215 causal link between WNT secretion triggered by p53-deficient mammary tumours and systemic
216 inflammation.

217 Since the $\gamma\delta$ T cell–neutrophil axis promotes metastasis[4,5] and these cells are regulated
218 by WNT ligands, we hypothesized that LGK974 treatment may present a viable therapeutic
219 strategy to inhibit metastasis of p53-null mammary tumours. To test this, we treated KEP

220 tumour-bearing mice with LGK974 or vehicle, after which we surgically removed the primary
221 tumour and assessed metastatic progression. Strikingly, while Porcupine blockade did not
222 affect primary tumour growth (Extended Data Fig. 9i), pulmonary metastases were reduced
223 (Fig. 4c, d). In an independent metastasis model in which we orthotopically transplanted
224 matched *Trp53*$^{+/+}$ and *Trp53*$^{-/-}$ WEP cell lines, we observed that the absence of p53 increases
225 lung metastasis formation (Fig. 4e, left and right graphs; P=0.0153). We then treated both
226 *WEP;Trp53*$^{+/+}$ and *WEP;Trp53*$^{-/-}$ tumour-bearing mice with LGK974, which failed to influence
227 primary tumour growth (Extended Data Fig. 9j). However, LGK974 treatment reduced
228 metastasis of *WEP;Trp53*$^{-/-}$ tumours, without affecting metastasis of *WEP;Trp53*$^{+/+}$ tumours
229 (Fig. 4e, f). These data show that blocking WNT-induced systemic inflammation impedes
230 metastasis formation of p53-null mammary tumours.
231 In summary, we show that p53 status is an important driver of systemic pro-metastatic
232 inflammation in breast cancer (Extended Data Fig. 9k) and that targeting WNT signalling may
233 represent a promising therapeutic modality for patients with p53-deficient breast tumours.
234 Together with recent literature on the importance of canonical driver mutations in shaping the
235 local immune composition of primary tumours[26], our findings shed light on the poorly
236 understood inter-patient heterogeneity in the systemic composition and function of immune
237 cells. Mechanistic understanding of the intricate interactions between cancer cell-intrinsic
238 genetic events and the immune landscape provides a basis for the design of personalized
239 immune intervention strategies for cancer patients.
240

241 **References**

242 **Figure legends**

243

244 **Figure 1: Loss of p53 in mammary cancer cells correlates with systemic neutrophilic**
245 **inflammation. a.** Flow cytometry analysis of frequency of $CD11b^+Ly6G^+Ly6C^+$ neutrophils and
246 **b.** proportion of $cKIT^+$ neutrophils as determined by flow cytometry analysis on blood of breast
247 cancer GEMMs at end-stage (cumulative tumour volume 1500 mm^3) and non-tumour-bearing
248 (WT) controls (*n*=4, 3, 4, 7, 3, 4, 4, 3, 6, 7, 6, 9, 3, 5, 4, 7 and 7 mice, top to bottom). Asterisks
249 indicate statistically significant differences compared to WT. * $P < 0.05$, ** $P < 0.01$, *** $P <$
250 0.001, **** $P < 0.0001$. **c.** Total neutrophil frequencies and **d.** $cKIT^+$ neutrophil frequencies in
251 circulation of all $Trp53^{+/+}$ (*n*=28) and $Trp53^{-/-}$ (n=46) tumour-bearing mice, combined from **a.**
252 and **b. e.** CCL2 levels (*n*=17 $Trp53^{+/+}$, *n*=22 $Trp53^{-/-}$), **f.** IL-1β levels (*n*=18 $Trp53^{+/+}$, *n*=21
253 $Trp53^{-/-}$), **g.** IL-17A levels (*n*=24 $Trp53^{+/+}$, *n*=30 $Trp53^{-/-}$) and **h.** G-CSF levels (*n*=22 $Trp53^{+/+}$,
254 *n*=33 $Trp53^{-/-}$) in serum of GEMMs at end-stage based on p53 status. **i.** Principal component
255 analysis of data depicted in **a – h** (13 out of 16 GEMMs). Each symbol represents one mouse.
256 Circles contour 40% of group-specific Gaussian probability distributions of sample scores. All
257 data are means ± s.e.m., *P*-values are indicated as determined by two-tailed one-way ANOVA,
258 Tukey's multiple-testing correction (**a, b**) or two-tailed Mann-Whitney U-test (**c – h**).

259

260 **Figure 2. p53 status in mammary tumours dictates immune activation. a.** Experimental
261 setup: cell lines are derived from $Trp53^{+/+}$ tumours (*Wap-cre;Cdh1*$^{F/F}$;*Akt*^{E17K} (WEA) and *Wap-*
262 *cre;Cdh1*$^{F/F}$;*Pik3ca*^{E545K} (WEP)) and p53 is knocked out (KO) using CRISPR/Cas9. KO and
263 control cell lines are orthotopically transplanted into syngeneic mice. **b.** Frequency of total
264 $CD11b^+Ly6G^+Ly6C^+$ neutrophils in circulation and **c.** in lungs, and **d.** frequency of $cKIT^+$
265 neutrophils (% of total neutrophils) in circulation at end-stage (tumour volume 1500 mm^3) of
266 mice with $Trp53^{+/+}$ and $Trp53^{-/-}$ *WEA* and *WEP* tumours, as determined by flow cytometry (*n*=4
267 *WEA;*$Trp53^{+/+}$, *n*=6 *WEA;*$Trp53^{-/-}$, *n*=5 *WEP;*$Trp53^{+/+}$, *n*=5 *WEP;*$Trp53^{-/-}$). **e.** RT-qPCR
268 analysis of the expression of *Il1b* in bone marrow-derived macrophages (BMDM) after
269 exposure to conditioned medium of $Trp53^{+/+}$ and $Trp53^{-/-}$ *WEA* (*n*=4 biological

270 replicates/group) or *WEP* cell lines (*n*=3 biological replicates/group). Plots show representative
271 of 3 independent experiments with 2 technical replicates per biological replicate. **f.** *IL1B*
272 expression in *TP53* wild-type (WT, *n*=643) or *TP53* mutant (MUT, *n*=351) human breast
273 tumours of The Cancer Genome Atlas (TCGA) database. Data in **b** – **e** are means ± s.e.m., **f.**
274 shows 5 – 95 percentile boxplot with median and quartiles indicated. *P*-values are indicated
275 as determined by two-tailed Mann-Whitney U-test (**b, c, d, f**) or two-tailed one-way ANOVA,
276 Tukey's multiple-testing correction (**e**).
277

278 **Figure 3. p53-null tumours display activated Wnt signalling. a.** Top 10 most significantly
279 differentially activated pathways determined by Ingenuity Pathway Analysis, comparing *Trp53*$^{-}$
280 $^{/-}$ (*n*=77) with *Trp53*$^{+/+}$ (*n*=68) GEMM tumours of 12 different models. Also indicated is the Wnt
281 signalling pathway. **b.** Log_2 fold change expression of *Wnt1, Wnt6* and *Wnt7a* in *Trp53*$^{-/-}$ (*n*=77)
282 GEMM tumours compared to *Trp53*$^{+/+}$ (*n*=68) tumours. **c.** Western blot analysis of bulk tumours
283 showing non-phospho(active)-β-catenin, Porcupine, Wnt1, Wnt6 and Wnt7a (blue indicates
284 *Trp53*$^{-/-}$ tumours and red indicates *Trp53*$^{+/+}$ tumours). Representative of two independent
285 experiments. For uncropped images, see Supplemental Fig. 1. **d.** Quantification of **c**
286 (*n*=3/group). **e.** Expression of *WNT1, WNT6* and *WNT7A* in *TP53* wild-type (WT, *n*=643) and
287 *TP53* mutant (MUT, *n*=351) human breast tumours of TCGA breast cancer database. **f.**
288 Western blot analysis on cell lysate and conditioned medium of *Wap-*
289 *cre;Cdh1*$^{F/F}$*;Akt*^{E17K}*;Trp53*$^{+/+}$ (WT) and *Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K}*;Trp53*$^{-/-}$ (KO) cell lines for Wnt
290 ligands. Representative of two independent experiments. **d.** shows mean ± s.e.m., **e** shows 5
291 – 95 percentile boxplot with median and quartiles indicated. *P*-values are indicated as
292 determined by two-tailed one-way ANOVA, FDR multiple-testing correction (**b**) or two-tailed
293 Mann-Whitney U-test (**d, e**).
294

295 **Figure 4. Wnt-induced systemic inflammation promotes metastasis. a.** RT-qPCR analysis
296 of bone marrow-derived macrophages (BMDM) after exposure to control medium or
297 conditioned medium from *Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K}*;Trp53*$^{+/+}$ (WT), *Wap-*

298 *cre;Cdh1*$^{F/F}$*;Akt*^{E17K};*Trp53*$^{-/-}$ (KO) or *Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K};*Trp53*$^{-/-}$ cells transduced with 2
299 independent shRNAs against *Porcn* (KO shPorcn-1 and KO shPorcn-4). Where indicated, cell
300 lines were pre-treated with 1 µM LGK974 (KO + LGK974) (*n*=5 biological replicates/group for
301 WT, WT + LGK974, KO and KO + LGK974, *n*=3 biological replicates for KO shPorcn-1 and
302 KO shPorcn-4). Plots show representative data of 3 separate experiments with 2 technical
303 replicates per biological replicate. **b.** Frequency of total CD11b^{+}Ly6G^{+}Ly6C^{+} neutrophils and
304 cKIT^{+} neutrophils in circulation of *K14cre;Cdh1*$^{F/F}$*;Trp53*$^{F/F}$ (KEP) mice after 5 day LGK974
305 (*n*=4) or vehicle (*n*=7) treatment starting at tumour volume 500 mm^3. **c.** Number of pulmonary
306 metastases after KEP tumour-bearing mice were treated with LGK974 (*n*=15) or vehicle
307 (*n*=12). KEP tumour fragments were orthotopically transplanted in FVB/N mice and treatment
308 was initiated when tumours were 30 – 40 mm^3 and continued until mastectomy. **d.**
309 Representative images of cytokeratin-8 staining of lungs of KEP tumour-bearing mice. Scale
310 bars, 1.9 mm. **e.** Number of pulmonary metastases after orthotopic injection of *Trp53*$^{+/+}$ and
311 *Trp53*$^{-/-}$ *Wap-cre;Cdh1*$^{F/F}$*;Pik3ca*^{E545K} (WEP) cells and treatment with LGK974 or vehicle
312 (*n*=9/group). Treatment was initiated when tumours were 30 – 40 mm^3 and continued until
313 1500 mm^3. **f.** Representative images of cytokeratin-8 staining of lungs of WEP tumour-bearing
314 mice, arrows indicate examples of metastatic nodules. Scale bars, 1.4 mm. All data are means
315 ± s.e.m. *P*-values are indicated as determined by two-tailed one-way ANOVA, Tukey's
316 multiple-testing correction (**a**) or two-tailed Mann-Whitney U-test (**b, c, e**), ns: non-significant.
317

318 **Methods**

319

320 *Mice*

321 All animal experiments were approved by the Animal Ethics Committee of the Netherlands
322 Cancer Institute and performed in accordance with institutional, national and European
323 guidelines for Animal Care and Use. The generation and characterization of the mouse models
324 has been described[27-34] (and unpublished). The following mouse models were used in this
325 study: *Keratin14 (K14)-cre;Cdh1*$^{F/F}$*;Trp53*$^{F/F}$, *K14cre;Trp53*$^{F/F}$, *K14cre;Brca1*$^{F/F}$*;Trp53*$^{F/F}$, *Whey*
326 *Acidic Protein (Wap)-cre;Trp53*$^{F/F}$, *Wap-cre;Brca1*$^{F/F}$*;Trp53*$^{F/F}$, *Wap-*
327 *cre;Brca1*$^{F/F}$*;Trp53*$^{F/F}$*;Col1a1*$^{invCAG\text{-}Met\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Brca1*$^{F/F}$*;Trp53*$^{F/F}$*;Met*), *Wap-*
328 *cre;Brca1*$^{F/F}$*;Trp53*$^{F/F}$*;Col1a1*$^{invCAG\text{-}Myc\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Brca1*$^{F/+}$*;Trp53*$^{F/F}$*;Myc*), *Wap-*
329 *cre;Brca1*$^{F/F}$*;Trp53*$^{F/F}$*;Col1a1*$^{invCAG\text{-}Myb2\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Brca1*$^{F/F}$*;Trp53*$^{F/F}$*;Myb2*), *Wap-*
330 *cre;Trp53*$^{F/F}$*;Col1a1*$^{invCAG\text{-}ESR1\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Trp53*$^{F/F}$*;HA-ESR1*), *Wap-*
331 *cre;Cdh1*$^{F/F}$*;Col1a1*$^{invCAG\text{-}AktE17K\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K}), *Wap-*
332 *cre;Cdh1*$^{F/F}$*;Col1a1*$^{invCAG\text{-}Pik3caE545K\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Cdh1*$^{F/F}$*;Pik3ca*^{E545K}), *Wap-*
333 *cre;Cdh1*$^{F/+}$*;Col1a1*$^{invCAG\text{-}Fgfr2ex1\text{-}15\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Cdh1*$^{F/+}$*;Fgfr2*$^{ex1\text{-}15}$), *Wap-*
334 *cre;Cdh1*$^{F/F}$*;Col1a1*$^{invCAG\text{-}Fgfr2ex1\text{-}15\text{-}IRES\text{-}Luc/+}$ (*Wap-cre;Cdh1*$^{F/F}$*;Fgfr2*$^{ex1\text{-}15}$), *Wap-*
335 *cre;Cdh1*$^{F/F}$*;T2/Onc;Rosa26*$^{Lox66SBLox71/+}$ (*Wap-cre;Cdh1*$^{F/F}$*;SB*), *Wap-cre;Map3k1*$^{F/F}$*;Pten*$^{F/F}$,
336 *Mouse mammary tumour virus LTR (MMTV)-NeuT*. All mouse models were on FVB/N
337 background, except *MMTV-NeuT* and *Wap-cre;Cdh1*$^{F/F}$*;SB*, which were on Balb/c and a mixed
338 genetic (C57BL/6J and FVB/N) background, respectively. Female mice were monitored twice
339 weekly for the onset of spontaneous mammary tumour formation by palpation starting at 6-7
340 weeks of age. The perpendicular tumour diameters of mammary tumours were measured twice
341 per week using a calliper, and tumour volume was calculated using $vol(mm^3) = 0.5(length\ x$
342 $width^2)$. Maximum permitted tumour volumes were 1500 mm^3. Age-matched WT littermates
343 were used as controls. Average systemic total and cKIT$^+$ neutrophil levels in non-tumour-
344 bearing FVB/N and Balb/c mice were similar (data not shown). For orthotopic transplantation
345 experiments, 1x10^6 cells were injected into the right 4th mammary fat pad of WT FVB/N mice

346 (Janvier Labs). For intervention studies targeting Porcupine, *K14cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice
347 were treated daily with LGK974[35] (10 mg/kg, in 10% DMSO/10% Cremophor in PBS) or vehicle
348 (10% DMSO/10% Cremophor in PBS) via oral gavage, starting at matched tumour sizes
349 indicated in the figures. For metastasis experiments, the KEP-based model for spontaneous
350 breast cancer metastasis was used as previously described[36]. Briefly, tumour fragments of
351 *K14cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mice were orthotopically transplanted into FVB/N mice and surgically
352 removed when tumours reached 500 mm^3 in size. In this model, LGK974 treatment was
353 initiated when tumours were 30 – 40 mm^3 in size and continued until mastectomy, after which
354 mice were monitored for signs of metastatic disease. Disease endpoint was defined as mice
355 showing signs of respiratory distress or palpable metastatic nodules in lymph nodes or other
356 organs reaching 1500 mm^3 in size. For metastasis experiments using the *Wap-*
357 *cre;Cdh1$^{F/F}$;Pik3ca^{E545K}* model, matched *Trp53$^{+/+}$* and *Trp53$^{-/-}$* tumour-derived cell lines were
358 orthotopically injected in the mammary fat pad of FVB/N mice (1x10^6 cells) and tumours were
359 allowed to grow out until end stage (1500 mm^3). During this time, tumours spontaneously
360 metastasize to the lungs. LGK974 or vehicle treatment was initiated when tumours were 30 –
361 40 mm^3 and continued until end stage. Orthotopically transplanted WEA tumours did not
362 spontaneously metastasize before the primary tumours reached 1500 mm^3. For intervention
363 studies, mice were randomly distributed over the two treatment arms when tumours reached
364 the indicated size. Tumour measurements and post mortem analyses were performed in a
365 blinded fashion. Mice were kept in individually ventilated cages, and food and water were
366 provided *ad libitum*. The maximal tolerated disease endpoints were not exceeded in any of the
367 experiments.
368

369 *Flow cytometry*

370 Flow cytometry analysis was performed as previously described[5]. Briefly, tissues were
371 collected in ice-cold PBS and blood was collected in tubes containing heparin. Tumours and
372 lungs were mechanically chopped using a McIlwain tissue chopper (Mickle Laboratory
373 Engineering). Tumours were digested for 1 hour (h) at 37°C in 3 mg/mL collagenase type A

374 (Roche) and 25 µg/mL DNase (Sigma) in serum-free DMEM medium. Lungs were digested for
375 30 minutes (min) at 37°C in 100 µg/mL Liberase TM (Roche). Enzyme reactions were stopped
376 by addition of cold DMEM/8% Fetal Calf Serum (FCS) and suspensions were dispersed
377 through a 70 µm cell strainer. Bone marrow was collected from the tibia and femurs of both
378 hind legs and flushed using RPMI/8% FCS through a 70 µm cell strainer. Single-cell
379 suspensions were treated with NH_4Cl erythrocyte lysis buffer. Before staining, cell suspensions
380 were subjected to Fc receptor blocking (rat anti-mouse CD16/32, BD Biosciences) for 15 min
381 at 4°C, except for bone marrow (to allow assessment of CD16/32 expression). Cells were
382 stained with conjugated antibodies for 30 min at 4°C in the dark in PBS/0.5% BSA. 7AAD
383 (1:20; eBioscience/ThermoFisher) or Fixable Viability Dye eFluor 780 (1:1000;
384 eBioscience/ThermoFisher) was added to exclude dead cells. For intracellular cytokine
385 staining, single-cell suspensions were stimulated in IMDM containing 8% FCS, 100 IU/mL
386 penicillin, 100 mg/mL streptomycin, 0.5% β-mercaptoethanol, 50 ng/ml PMA, 1 mM ionomycin
387 and Golgi-Plug (1:1,000; BD Biosciences) for 3h at 37°C. Surface antigens were stained first,
388 followed by fixation and permeabilization using the Cytofix/Cytoperm kit (BD Biosciences) and
389 staining of intracellular proteins. All antibodies used are listed in Extended Data Table 1. All
390 experiments were performed using a BD LSR II flow cytometer using Diva software or the
391 Beckman Coulter CyAn ADP flow cytometer using Summit software. Data analyses were
392 performed using FlowJo Software version 9.9.
393
394 *Cell culture*
395 Mouse cell lines were generated as follows: *Wap-cre;Cdh1$^{F/F}$;Akt^{E17K}* (WEA) and *Wap-*
396 *cre;Cdh1$^{F/F}$;Pik3ca^{E545K}* (WEP) tumour material was collected in ice-cold PBS and
397 mechanically chopped using a McIlwain tissue chopper (Mickle Laboratory Engineering).
398 Tumours were subsequently digested for 30 min at 37°C in 3 mg/mL Collagenase A, 0.1%
399 trypsin and fungizone in DMEM/2% FCS. Enzyme reactions were stopped by addition of
400 DMEM/2% FCS and suspensions were dispersed through a 40 µm cell strainer. Cells were
401 initially cultured in DMEM containing 10% FCS, 100 IU/mL penicillin, 100 mg/mL streptomycin,

402 Insulin, EGF and Cholera toxin. After establishment, mouse cell lines were cultured in DMEM
403 medium supplemented with 8% FCS, 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM
404 L-glutamine. To ensure relatedness to parental GEMM tumours, polyclonal cells were used at
405 low passage number for all experiments. MCF-7 cells were cultured in DMEM medium
406 supplemented with 8% FCS, 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM L-
407 glutamine. For *in vitro* culture of bone marrow-derived macrophages (BMDMs), bone marrow
408 was aseptically collected by flushing tibia and femurs from euthanized WT mice with sterile
409 RPMI/8% FCS. Bone marrow cells were cultured for 7 days in RPMI medium supplemented
410 with 8% FCS, 100 IU/mL penicillin, 100 mg/mL streptomycin and 10 ng/mL recombinant M-
411 CSF (Peprotech). BMDMs were harvested at day 7 and examined for CD11b and F4/80
412 expression by flow cytometry. Consistent purities of >95% CD11b$^+$F4/80$^+$ cells were obtained.
413 For *in vitro* culture of human monocyte-derived macrophages (MDMs), human PBMCs
414 (Sanquin, Amsterdam) were enriched by magnetically activated cell sorting (MACS) using
415 CD14 microbeads (Miltenyi Biotec). CD14$^+$ cells were cultured in RPMI medium supplemented
416 with 8% FCS, 100 IU/mL penicillin, 100 mg/mL streptomycin and 10 ng/mL recombinant M-
417 CSF (Peprotech). MDMs were harvested at day 7 and examined for CD11b, CD14 and CD68
418 expression by flow cytometry. Consistent purities of >95% CD11b$^+$CD14$^+$CD68$^+$ cells were
419 obtained. Where indicated, BMDMs and MDMs were exposed to conditioned medium (CM)
420 from tumour cell lines, in presence or absence of LGK974 (1 μM, Selleck Chemicals) for 24 h
421 and harvested for RNA and/or protein isolation. CM was obtained by culturing tumour cells at
422 equal confluency in empty DMEM overnight. Cell growth kinetics *in vitro* were analysed using
423 the IncuCyte System (Essen BioScience).
424
425 *RNA isolation and quantitative RT-PCR*
426 RNA was isolated using either Trizol or a Qiagen Rneasy column followed by treatment with
427 Dnase I (Invitrogen). RNA quality was confirmed with a 2100 Bioanalyzer from Agilent. RNA
428 was converted to complementary DNA (cDNA) with an AMV reverse transcriptase using
429 Oligo(dT) primers (Invitrogen). cDNA (20 ng per well) was analysed by SYBR green real-time

430 PCR with 500 nM primers using a LightCycler 480 thermocycler (Roche). β-actin and/or
431 GAPDH were used as reference genes. Primer sequences used for each gene are listed in
432 Extended Data Table 2. Fold change in expression was calculated using $2^{-(\Delta Ct.x - average[\Delta Ct.control])}$.
433
434 *Protein isolation and western blotting*
435 Protein lysates of cells and tissue were prepared using RIPA buffer (50 mM Tris-HCl, pH 7.4,
436 150 mM NaCl, 1% NP40, 0.5% DOC, 0.1% SDS, 2 mM EDTA) complemented with protease
437 and phosphatase inhibitors (Roche) and protein concentration was quantified using the BCA
438 protein assay kit (Pierce). Protein lysate was loaded onto NuPAGE 4–12% Bis-Tris gradient
439 gels (Invitrogen) and transferred onto Trans-Blot® Turbo™ Mini or Midi Nitrocellulose
440 membranes (BioRad) using Trans-Blot Turbo Transfer System (BioRad). Membranes were
441 blocked in 10% Western Blot Blocking Reagent (Roche) or 3% BSA for 1 h at room
442 temperature (RT). Primary antibody incubation was performed overnight at 4°C. Membranes
443 were washed using TBS-T and subjected to secondary fluorochrome-conjugated antibodies
444 for 1 h at RT and protein was detected using the Odyssey CLx imaging system and processed
445 using ImageJ software 1.48v. Antibodies are listed in Extended Data Table 1.
446
447 *Immunohistochemistry*
448 Immunohistochemical analyses were performed by the Animal Pathology facility at the
449 Netherlands Cancer Institute. Formalin-fixed tissues were processed, sectioned and stained
450 as described[36]. Briefly, tissues were fixed for 24 h in 10% neutral buffered formalin, embedded
451 in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin (H&E) for
452 histopathological evaluation. H&E slides were digitally processed using the Aperio ScanScope
453 (Aperio, Vista, CA). For immunohistochemical analysis, 5 μm paraffin sections were cut,
454 deparaffinised and stained. Antibodies and antigen retrieval methods are listed in Extended
455 Data Table 1. Quantitative analysis of cell abundance was performed by counting cells in five
456 high-power (x40) fields of view (FOV) per tissue by two independent researchers. Samples
457 were visualized with a BX43 upright microscope (Olympus) and images were acquired in bright

458 field using cellSens Entry software (Olympus). To score pulmonary metastasis, single lung
459 sections were stained for cytokeratin-8 and metastatic nodules were counted by two
460 independent researchers. Stained tissue slides were digitally processed using the Aperio
461 ScanScope. Brightness and contrast for representative images were adjusted equally among
462 groups.
463
464 *Cytokine analyses*
465 Quantification of cytokine and chemokine levels in serum was performed using BD Cytometric
466 Bead Array for CCL2, IL-1β, IL-17A and G-CSF according to manufacturer's instructions and
467 analysed on a Beckman Coulter CyAn ADP flow cytometer with Summit software. Data
468 analyses were performed using FlowJo Software version 9.9.
469
470 *CRISPR/Cas9-mediated gene disruption*
471 For knock-out of murine *Trp53*, p53-proficient tumour cell lines were transfected with
472 lentiCRISPR v2 (provided by Feng Zhang (Addgene plasmid #52961)[37]) containing sgRNA
473 targeting exon 4 (sgRNA1: 5'- TCCGAGTGTCAGGAGCTCCT-3' and sgRNA2: 5'-
474 AGTGAAGCCCTCCGAGTGTC-3'). For knock-out of human *TP53*, MCF-7 tumour cell lines
475 were transfected with lentiCRISPRv2 containing sgRNA targeting either exon 4 (sgRNA1: 5'-
476 CCATTGTTCAATATCGTCCG-3') or exon 2 (sgRNA2: 5'-TCGACGCTAGGATCTGACTG-3').
477 Cloning of sgRNAs in lentiCRISPR was performed as described[37] and sgRNA sequences were
478 designed using the online CRISPR Design tool (http://crispr.mit.edu), of which the two highest
479 scoring sequences were chosen. All vectors were validated by Sanger sequencing. After
480 selection of transfected cells, polyclonal cell lines were used for all subsequent experiments.
481 To determine knock-out efficiency, genomic DNA from cell lines was isolated using Viagen
482 DirectPCR Lysis reagent (Cell) supplemented with 200 µg/mL proteinase K after transfection
483 and puromycin selection. Murine *Trp53* target region was amplified using PCR with the
484 following primers: FW 5'-GGGGACTGCAGGGTCTCAGA-3' and RV 5'-
485 CCACGTCCCCTGGAGAGATG-3'. Human *TP53* target region was amplified using PCR with

486 the following primers: FW1 5'-CAGACTGCCTTCCGGGTCAC-3' for sgRNA1, FW2 5'-
487 TGGGAAGGTTGGAAGTCCCTC-3' for sgRNA2, and RV 5'-
488 CACTGACAGGAAGCCAAAGGG-3'. PCR products were run on 1% agarose gel, purified
489 using the Illustra GFX™ PCR DNA and Gel Band Purification Kit (Sigma), and subjected to
490 Sanger sequencing using their respective FW primers. Genome editing efficiency was
491 quantified using the Tracking of Indels by Decomposition (TIDE) algorithm as described
492 (http://tide.nki.nl)[38].
493
494 *shRNA-* and *siRNA-mediated knock-down of genes*
495 Vectors for shRNAs were collected from the TRC library. To allow stable expression of
496 shRNAs, HEK293T cells were transfected with the pLKO.1 lentiviral vector encoding shRNAs,
497 pPAX packaging vector and VSV-G envelope vector. Five independent shRNA clones were
498 used for each experiment. Virus was harvested at day 4 and 5 and viral titres were determined
499 using the Abm qPCR lentivirus titration kit (LV900). Cells lines were subsequently transduced
500 and selected using puromycin. Knock-down efficiency was determined by RT-qPCR as
501 compared to non-targeting controls. The shRNA clone used for *Porcupine* knock-down in all
502 experiments after assessment of knock-down efficiency contained following hairpin sequence:
503 5'-CAACTTTCTATGCCTGTCAAT-3' (shPorcn-1) or 5'-CCCATGTCTTATTGGTTAAAT-3'
504 (shPorcn-4). For *in vivo* experiments, shPorcn-4 was used. To silence *Fzd* receptors, BMDMs
505 were transfected with the following siRNA pools (control siRNA (sc-37007), *Fzd7* (sc-39991),
506 and *Fzd9* (sc-39995), Santa Cruz Biotechnology), according to manufacturer's instructions.
507 Briefly, BMDMs were differentiated as described above, and 24 h before exposure to tumour
508 CM and BMDMs were suspended in transfection medium and incubated with indicated siRNA
509 pools. After 6 h at 37°C, 2X RPMI medium was added (RPMI, 20% serum, 200 IU/mL penicillin,
510 200 mg/mL streptomycin and 20 ng/mL recombinant M-CSF) and BMDMs were further
511 cultured overnight. After 24 h, the medium was replaced by tumour CM for 24 h, after which
512 gene expression was assessed.
513

514 *Chromatin immunoprecipitation (ChIP)-sequencing*

515 ChiP-seq was performed as previously described[39]. Briefly, cell lines from *Wap-*
516 *cre;Cdh1$^{F/F}$;Akt^{E17K}* and *Wap-cre;Cdh1$^{F/F}$;Pik3ca^{E545K}* tumours (3 cell lines from 3 independent
517 mouse tumours per genotype) were fixed in 1% formaldehyde, crosslinked and processed for
518 sonication. 5 µg of p53 antibody (Extended Data Table 1) and 50 µL of Protein G magnetic
519 beads (Invitrogen) were used for each ChIP. Eluted DNA was sequenced using the Illumina
520 Hiseq 2500 analyser (using 65 bp reads) and aligned to the *Mus musculus* mm10 reference
521 genome. Peak calling over input control was performed using and MACS 2.0 peak caller. Data
522 was visualized using Easeq[40].

523

524 *Overexpression of miR-34a*

525 The MSCV-miR-34a retroviral vector (provided by Lin He (Addgene plasmid #63932)[41]) was
526 transfected in HEK293T cells, together with pGag-Pol and VSV-G vectors to generate
527 retrovirus. Mouse cancer cell lines were exposed to viral supernatant and assessed for
528 expression of Wnt target genes after puromycin selection.

529

530 *RNA sequencing and analysis*

531 Total RNA was extracted from tumours using TRIzol reagent (Ambion Life Technologies)
532 according to the manufacturer's instructions. Samples were equimolar pooled and were single-
533 end sequenced for 51 or 65 base pairs on the Illumina Hiseq2000/Hiseq2500 Machine. The
534 reads were aligned against the mouse transcriptome (mm10) using Tophat2 (Tophat version
535 2.1.0 / Bowtie version 1.0.0) that allows for exon-exon junctions[42,43]. Tophat was guided using
536 a reference genome as well as a reference transcriptome. The reference transcriptome was
537 created using a gene transfer file (GTF) that was downloaded from Ensembl (version 77).
538 Gene counts were generated using a custom script, that functions identically to HTSeq-count[44].
539 Only reads that mapped uniquely to the transcriptome were used for gene expression
540 quantification. While some of the libraries were generated with strand-specific protocols, all
541 samples have been aligned without taking strandedness into account. Next, differential

542 expression analysis was performed using the R package edgeR[45] in combination with the
543 voom[46] method, using raw read counts as input. Library size normalisation was performed
544 during differential expression analysis within the voom function. Genes with *P*-values < 0.05
545 were labelled as differentially expressed. Genes were further filtered for display by requiring
546 them to be protein coding and to have an absolute $\log_2$ fold change ≥ 3 and a *P*-value ≤ 0.01.
547 The selected genes were shown in a heatmap of readcounts that were normalized to 10 million
548 reads per sample.

549 For Hallmark pathway analysis of murine transcriptomes, raw read counts were
550 normalised by trimmed means of M-values computed using the function calcNormFactors
551 (edgeR version 3.20.5[45]), from which CPM-normalized gene expression values were computed
552 for plotting purposes using the same R-package. CPM-values were subsequently transformed
553 as $f(x) = \log_2(x + 1)$. Ensembl77 murine gene identifiers were then converted to homologous
554 human gene identifiers using the biomaRt-R package (server oct2016.archive.ensembl.org).
555 Gene expression heatmaps for hallmark human gene sets obtained from MsigDB[47] were
556 generated using the *aheatmap*-function provided by the NMF R-package (version 0.20.6).
557 Heatmap columns (containing samples) were ordered according to average linkage (UPGMA)
558 hierarchical sample-clustering based on Pearson correlation-distances between the
559 expression values of displayed genes. Heatmap rows (containing genes) were ordered
560 according to gene expression fold difference between *Trp53*$^{-/-}$ and *Trp53*$^{+/+}$ samples. The R
561 language for statistical computing was used (version 3.4.2) for gene expression normalisation
562 and heatmap generation. Pathway enrichment analysis of *Trp53*$^{-/-}$ and *Trp53*$^{+/+}$ tumours was
563 performed using Ingenuity Pathway Analysis software (QIAGEN), analysing differentially
564 expressed genes with $P \leq 0.05$.

565

566 *The Cancer Genome Atlas (TCGA) analysis*

567 To obtain a comprehensive view on the cellular processes affected by p53-deficiency in human
568 breast cancer, we performed a gene set enrichment analyses (GSEA) using a 50 hallmark
569 gene sets (Liberzon)[47] on the TCGA breast cancer (BRCA) cohort. First, we classified p53-

570 deficiency based on mutational status. DNA sequencing variant calls (MAF-file) for the BRCA
571 cohort were downloaded from the 2015-08-21 release of the Broad TCGA genome data
572 analysis centre standard run (http://gdac.broadinstitute.org/runs/stddata). We utilized two
573 classifications for p53-deficiency: in the first classification (labelled 'any *TP53* mutation'),
574 patients with any kind of *TP53* mutation were classified as p53-deficient. In the second
575 classification (labelled 'IARC *TP53* database'), only patients with a dominant negative *TP53*
576 mutation as annotated using the IARC *TP53* mutation database[48] (release 18, matched on
577 protein effect of the mutation) were labelled as p53-deficient, as well as patients with gain-of-
578 stop, stop-lost or frameshifting mutations (*n*=161). One sample had a trans-activating mutation
579 and was excluded from the analysis. The remaining samples were labelled as p53-proficient
580 (*n*=793).

581 Next, TCGA RNA sequencing data were downloaded from the Broad TCGA genome
582 data analysis centre 2015-11-01 release of the standard runs. We ran a gene set enrichment
583 analysis (GSEA) on the 50 Hallmark gene set using the flexgsea-r R package
584 (https://github.com/NKI-CCB/flexgsea-r) on the read counts normalized with limma voom with
585 the span parameter set to 0.5[46]. Within each permutation of the sample labels, genes were
586 ranked for association with p53-proficiency using the moderated *t*-statistic from the limma
587 empirical Bayes function (ebayes() ran on the result of lmFit()). Reported FDR-values were
588 obtained from the flexgsea-r output.

589 Single gene associations with *TP53* status in human breast tumours of the TCGA
590 BRCA cohort and correlation coefficients between WNT-related genes and *TP53* status (MUT
591 vs WT) were analysed using R2 Genomics Analysis and Visualization Platform
592 (http://r2.amc.nl/) and visualized using GraphPad Prism version 7.

593

594 *Statistics and reproducibility*

595 Data analyses were performed using GraphPad Prism (version 7). The statistical tests used
596 are described in figure legends. All tests were performed two-tailed. *P*-values < 0.05 were
597 considered statistically significant. All western blot and RT-qPCR analyses were independently

598 repeated more than twice. Sample sizes were based on previous experiments[5,17,36] or
599 determined using G*Power software (version 3.1). To exclude bias towards one particular
600 GEMM in the analyses for Figure 1, we have performed the same analyses on the average of
601 the neutrophil levels and serum cytokine values per model. This demonstrated the same
602 correlations between the assessed values and p53 status of the tumour, thus excluding bias
603 towards one or several particular models. Principal component analysis was performed using
604 the prcomp-function in R (version 3.4.2), both centering and scaling the input data before
605 applying dimensionality reduction.
606
607 *Reporting summary*
608 Further information on research design is available in the Nature Research Reporting
609 Summary linked to this paper.
610
611 *Data availability statement*
612 The RNA sequencing data have been deposited in the Gene Expression Omnibus (GEO,
613 NCBI) repository under accession number GSE112665. All other data are found in the source
614 data, supplemental information or available from the authors on reasonable request.
615

616 **Methods References**

617

618 **End notes**

619

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630

631 **Author contributions**

632 M.D.W., S.B.C., J.J. and K.E.d.V. conceived the ideas and designed the experiments.
633 M.D.W., S.B.C., D.E.M.D., M.H.v.M., performed the flow cytometry, RT-qPCR, CBA, western
634 blot, immunohistochemical, animal and other experiments. C.H., K.V., A.P.D., E.S. and
635 R.d.K-G. provided technical support and performed animal experiments. M.H.v.M., L.H.,
636 S.M.K. and J.J. generated mouse models. M.D.W. and R.d.K-G. performed mouse
637 intervention experiments. I.v.d.H. generated the GEMM-derived cell lines. S.P., M.D.W. and
638 W.Z. performed and analysed the ChIP-seq experiments. M.D.W., S.B.C., D.E.M.D.
639 M.H.v.M., and K.E.d.V. analysed the data. M.S., I.d.R., M.D.W., L.F.A.W. and T.N.M.S.
640 performed the bioinformatics analyses. M.D.W., S.B.C. and K.E.d.V. wrote the paper and
641 prepared the figures, with input from all authors.

642

643 **Competing interests**

644 M.D.W., S.B.C., D.E.M.D., M.H.v.M., M.S., I.d.R., L.H., S.M.K., S.P., C-S.H. K.V., A.P.D.,
645 R.d.K-G., E.S. I.v.d.H., W.Z. and J.J. report no competing interests. L.F.A.W. reports

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652 Ventures, outside the scope of this work.

653

654 **Materials & Correspondence**

655 Correspondence to Karin E. de Visser and Jos Jonkers

656

Extended Data Figure legends

Extended Data Figure 1. Neutrophil expansion in p53-deficient tumour-bearing GEMMs. **a.** Representative plots of flow cytometry analysis on blood of end-stage (cumulative tumour size 1500 mm^3) mammary tumour-bearing mice. Neutrophils were defined as $CD11b^+Ly6G^+Ly6C^+$. cKIT expression on gated total neutrophils in blood is shown (gating was based on blood of WT mice). Quantification and statistical analysis of these data is found in Fig. 1a, b.

Extended Data Figure 2. CRISPR/Cas9-mediated gene disruption of *Trp53* in *Wap-cre;Cdh1$^{F/F}$;Akt^{E17K}* and *Wap-cre;Cdh1$^{F/F}$;Pik3ca^{E545K}* cancer cell lines. a. Insertion and deletion (indel) spectrum of bulk *Wap-cre;Cdh1$^{F/F}$;Akt^{E17K}* (WEA) cancer cell lines after transfection with 2 individual sgRNAs against *Trp53* and puromycin selection, as determined by the TIDE algorithm and compared to the sequence of target region of control cells. The *P*-value associated with the estimated abundance of each indel is calculated by a two-tailed t-test of the variance–covariance matrix of the standard errors. **b.** Western blot analysis showing p53 levels of control and p53-knockout (KO) WEA cell lines. Inactivation of the p53 pathway is shown by loss of p21 staining after 10 Gy irradiation. KO1 (sgRNA1) resulted in a truncated p53 protein and KO2 (sgRNA2) shows absence of p53 protein. For all subsequent experiments, KO2 was used. Representative of two independent experiments. For uncropped images, see Supplemental Fig. 1. **c.** *In vitro* growth kinetics of WEA control and p53-KO cells, as determined by IncuCyte (*n*=7 technical replicates/group). **d.** *In vivo* growth kinetics of orthotopically transplanted *WEA;Trp53$^{+/+}$* (*n*=4 mice) and *WEA;Trp53$^{-/-}$* (*n*=6) cancer cell lines, with t = 0 being the first day tumours were palpable. **e.** Indel spectrum of bulk *Wap-cre;Cdh1$^{F/F}$;Pik3ca^{E545K}* (WEP) cancer cell lines after transfection with sgRNA2 against *Trp53* and puromycin selection, as determined by the TIDE algorithm. **f.** *In vitro* growth kinetics of *WEP* control and p53-KO cells, as determined by IncuCyte (*n*=7 technical replicates/group). **g.** *In vivo* growth kinetics of orthotopically transplanted *WEP;Trp53$^{+/+}$* (*n*=5) and *WEP;Trp53$^{-/-}$*

685 (*n*=5) cell lines, with t=0 being the first day tumours were palpable. **h.** Gating strategy to identify
686 circulating neutrophils and their cKIT expression. **i.** Gating strategy to identify neutrophils in
687 the lung. **j.** Representative images of spleens from mice bearing *WEA;Trp53*$^{+/+}$ and
688 *WEA*;*Trp53*$^{-/-}$ tumours and quantification of spleen area (length x width) at end-stage (tumour
689 volume 1500 mm^3) of mice bearing p53-proficient (*n*=4) and p53-deficient WEA (*n*=6) and
690 WEP tumours (*n*=5/group). All data are means ± s.e.m. *P*-values are indicated as determined
691 by Area Under the Curve followed by two-tailed Welch's t-test (**c, d, f , g**) or two-tailed Mann-
692 Whitney U-test (**j**), ns: not significant.
693
694 **Extended Data Figure 3. Haematopoiesis in p53-null tumour-bearing mice is skewed**
695 **towards the development of neutrophils. a.** Schematic representation of neutrophil
696 development in the bone marrow. **b.** Gating strategy of neutrophil progenitor populations in the
697 bone marrow. Dot plot indicates the cKIT expression levels (median fluorescence intensity
698 [MFI]) in promyelocytes compared to mature neutrophils (*n*=20 mice). **c.** Frequency of bone
699 marrow progenitor populations in mice bearing end-stage *Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K}*;Trp53*$^{+/+}$
700 (*n*=9) and *Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K}*;Trp53*$^{-/-}$ (*n*=11) tumours, as determined by flow cytometry.
701 **d.** Total live cells and total live progenitor population numbers per hindleg of mice bearing
702 *WEA;Trp53*$^{+/+}$ and *WEA;Trp53*$^{-/-}$ tumours (*n*=5/group). All data are ± s.e.m. *P*-values are
703 indicated as determined by two-tailed Mann-Whitney U-test. Abbreviations: LSK (Lin^{-}
704 Sca1^{+}cKIT^{+}, which contain the LT-HSC (long-term haematopoietic stem cells), ST-HSC (short-
705 term haematopoietic stem cells) and MPP (multipotent progenitors)), CMP (common myeloid
706 progenitors), GMP (granulocytic and monocytic progenitors), MEP (megakaryocyte and
707 erythrocyte progenitors).
708
709 **Extended Data Figure 4. Macrophages are differentially activated by *Trp53*$^{-/-}$ mouse and**
710 **human breast cancer cell lines. a.** Expression (median fluorescence intensity [MFI]) of
711 CCR2, CCR6, CD206, CSF-1R, CXCR4 and MHC-II on live CD11b^{+}F4/80^{+} bone marrow-
712 derived macrophages after exposure to control medium or conditioned medium (CM) of *Wap-*

713 *cre;Cdh1*$^{F/F}$*;Akt*^{E17K}*;Trp53*$^{+/+}$ or *Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K}*;Trp53*$^{-/-}$ cell lines, as determined by
714 flow cytometry (*n*=4 biological replicates/group). **b.** TIDE analysis of bulk MCF-7 cells after
715 transfection with *TP53*-targeting sgRNAs and puromycin selection. For subsequent
716 experiments, sgRNA1 was used. **c.** Expression (MFI) of CD206, CD163 and HLA-DR on
717 human CD11b^{+}CD14^{+}CD68^{+} monocyte-derived macrophages (MDMs) after exposure to CM
718 of *MCF-7;TP53*$^{+/+}$ or *MCF-7;TP53*$^{-/-}$ (sgRNA1) cancer cells (*n*=3 biological replicates/group).
719 **d.** RT-qPCR analysis showing *IL1B* expression in human CD11b^{+}CD14^{+}CD68^{+} MDMs after
720 exposure to control medium (*n*=4 biological replicates) CM of *MCF-7-TP53*$^{+/+}$ or *MCF-7-TP53*$^{-}$
721 $^{/-}$cancer cells (*n*=5 biological replicates/group). Data are normalized to normal medium control.
722 Plots shows representative data of 3 separate experiments and average with 2 technical
723 replicates. All data are means ± s.e.m. *P*-values are indicated as determined by two-tailed one-
724 way ANOVA, Tukey's multiple-testing correction.
725
726 **Extended Data Figure 5. Transcriptome profile and composition of the local tumour**
727 **immune landscape in breast cancer GEMMs. a.** Unsupervised clustering of top 200 most
728 differentially expressed genes ($P < 0.01$, LFC > 3 or < –3) in mammary GEMM tumours as
729 determined by RNA sequencing (*n*=145 tumours). Red bars indicate *Trp53*$^{+/+}$ tumours, blue
730 bars indicate *Trp53*$^{-/-}$ tumours. Full tumour genotype is displayed in legend and shown by
731 indicated colours. **b.** Number of Ly6G^{+} neutrophils in the tumour (*n*=1, 4, 10, 2, 4, 3, 6, 13, 4,
732 22, 4 and 5 mice, top to bottom). **c.** Macrophage score as indicative of F4/80^{+} macrophage
733 abundance in the tumour (*n*=2, 2, 4, 4, 4, 2, 3, 5, 4, 9, 5 and 4 mice, top to bottom). **d.** Number
734 of CD8^{+} cytotoxic T cells in the tumour (*n*=3, 2, 5, 5, 7, 3, 7, 3, 5, 4, 4 and 5 mice, top to bottom).
735 **e.** Number of CD4^{+} T cells in the tumour (*n*=3, 2, 5, 5, 7, 3, 7, 3, 5, 4, 4 and 5 mice, top to
736 bottom). **f.** Number of Foxp3^{+} regulatory T cells in the tumour (*n*=3, 2, 5, 5, 7, 3, 7, 3, 5, 4, 4
737 and 5 mice, top to bottom). **g.** Ratio of CD8/Foxp3 cells in the tumour (*n*=3, 2, 5, 5, 7, 3, 7, 2,
738 5, 4, 4 and 5 mice, top to bottom). All data are means of 5 microscopic fields of view (FOV)
739 per mouse as determined by IHC. Inserts show data combined according to p53 status of the
740 tumour. Each symbol represents an individual mouse. All data are means ± s.e.m. *P*-values

741 are indicated as determined by two-tailed one-way ANOVA, FDR multiple-testing correction
742 (**a**) or two-tailed Mann-Whitney U-test (**b – g**).
743
744 **Extended Data Figure 6. Wnt-related gene activation correlates with loss of p53 in**
745 **mouse and human breast tumours. a.** Heatmap showing that *Trp53*$^{-/-}$ (KO) GEMM tumours
746 (n=77) cluster away from *Trp53*$^{+/+}$ (WT) tumours (n=68) based on analysis of the Hallmark p53
747 pathway (represents positive control) and **b.** analysis of the Hallmark Wnt/β-catenin pathway.
748 Analysis was performed on all tumours of Extended Data Fig. 5a. **c.** Log_2 fold change
749 expression of genes involved in Wnt signalling ($P < 0.05$) in *Trp53*$^{-/-}$ (n=77) and *Trp53*$^{+/+}$ (n=68)
750 GEMM tumours depicted in Extended Data Fig. 5a. Black bars indicate genes that positively
751 regulate, or are generally increased with active Wnt signalling. Red bars indicate genes that
752 negatively regulate, or are down-regulated with active Wnt signalling. **d.** Gene set enrichment
753 analysis (GSEA) for Hallmark pathways in TCGA *TP53*^{WT} breast tumours (*n*=643) vs *TP53*^{MUT}
754 (*n*=351) human tumours (any TP53 mutation) or *TP53* loss (based on the IARC TP53
755 database, see Materials and Methods). Normalized enrichment score is shown with False
756 Discovery Rate (FDR) indicated. **e.** Correlation coefficient (R) of all genes involved in Wnt
757 signalling that correlate significantly ($P < 0.05$) with *TP53*^{MUT} (*n*=351) vs *TP53*^{WT} (*n*=643) in
758 TCGA breast tumours. Black bars indicate genes that positively regulate, or are generally
759 increased with active Wnt signalling. Red bars indicate genes that negatively regulate, or are
760 down-regulated with active Wnt signalling. *P*-values were determined by two-tailed ANOVA
761 with FDR multiple-testing correction (**c, e**).
762
763 **Extended Data Figure 7. p53 does not bind the regulatory regions of Wnt ligands**
764 **directly. a.** Chromatin immunoprecipitation-sequencing (ChIP-seq) profile of p53 binding to
765 DNA demonstrating enrichment on the *Cdkn1a* (p21) locus in *Trp53*$^{+/+}$ *Wap-cre;Cdh1*$^{F/F}$*;Akt*^{E17K}
766 (WEA) and *Wap-cre;Cdh1*$^{F/F}$*;Pik3ca*^{E545K} (WEP) cell lines (3 cell lines from 3 independent
767 tumours per GEMM). **b.** Absence of p53 binding to *Wnt1*, *Wnt6* or *Wnt7a* loci. **c.** Enrichment
768 of p53 on microRNA-34a (*miR-34a*) locus. **d.** RT-qPCR analysis of Wnt ligand expression in

769 *WEA;Trp53*$^{+/+}$ and *WEA;Trp53*$^{-/-}$ cell lines after overexpression (OE) of miR-34a in
770 *WEA;Trp53*$^{-/-}$cells (*n*=3 technical replicates/group). Plots show representative data of 3
771 separate experiments with 3 technical replicates. All data are means ± s.e.m. *P*-values are
772 indicated as determined by two-tailed one-way ANOVA, Tukey multiple-testing correction (**d**).
773
774 **Extended Data Figure 8. Macrophages are activated by *Trp53*$^{-/-}$ cancer cells via Fzd7**
775 **and Fzd9 receptors *in vitro*. a.** Log_2 fold change in expression of Wnt receptors *Fzd7* and
776 *Fzd9* in bulk tumours comparing *Trp53*$^{-/-}$ (*n*=77) and *Trp53*$^{+/+}$ (*n*=68) GEMM tumours using
777 RNA-sequencing. **b.** Expression of *FZD7* and *FZD9* in *TP53* wild-type (WT, *n*=643) and *TP53*
778 mutant (MUT, *n*=351) human breast tumours of TCGA dataset. **c.** Silencing of *Fzd7* and *Fzd9*
779 in bone marrow-derived macrophages (BMDMs) after transfection with siRNA pools against
780 both receptors, as determined by RT-qPCR (*n*=6 biological replicates/group). **d.** Expression of
781 *Il1b* in BMDMs after exposure to conditioned medium of *Trp53*$^{+/+}$ and *Trp53*$^{-/-}$ *Wap-*
782 *cre;Cdh1*$^{F/F}$*;Akt*^{E17K} cell lines (*n*=6 biological replicates/group), as determined by RT-qPCR.
783 Where indicated, BMDMs were transfected with control siRNA or Fzd7/9 siRNA pools. **a, c, d**
784 show means ± s.e.m. **b.** shows 5 – 95 percentile boxplot with median and quartiles indicated.
785 *P*-values are indicated as determined by two-tailed one-way ANOVA, FDR multiple-testing
786 correction (**a**), two-tailed Mann-Whitney U-test (**b**) or two-tailed one-way ANOVA, Tukey
787 multiple-testing correction (**d**).
788
789 **Extended Data Figure 9. Pharmacological and genetic targeting of Porcupine in p53-**
790 **deficient tumours reduces systemic inflammation. a.** Total and cKIT^{+} neutrophil
791 frequencies in lungs of vehicle (*n*=7) or LGK974 (*n*=4)-treated *K14cre;Cdh1*$^{F/F}$*;Trp53*$^{F/F}$ (KEP)
792 mice using indicated 5 day short-term treatment schedule. Representative flow cytometry plots
793 are shown. **b.** Frequency of IL-17A-producing $\gamma\delta$ T cells in lungs of vehicle (*n*=6) or LGK974
794 (*n*=4)-treated KEP mice. Representative flow cytometry plots are shown. **c.** Kinetics of
795 circulating neutrophils in vehicle or LGK974-treated KEP mice using indicated long-term
796 treatment schedule, shown as frequency at indicated tumour volumes (*n*=8/group). **d.** RT-

797 qPCR analysis of *Porcn* expression in end-stage bulk tumour (*n*=5/group). Data are normalized
798 to *shControl* and represents an average of 2 technical replicates. **e.** Correlation of total
799 neutrophil levels in circulation with expression of *Porcn* in *WEA;Trp53*$^{-/-}$*;shControl* and
800 *WEA;Trp53*$^{-/-}$*;shPorcn* whole tumour lysate (*n*=5/group). **f.** Correlation of cKIT^{+} neutrophil
801 levels in circulation with expression of *Porcn* in *WEA;Trp53*$^{-/-}$*;shControl* and *WEA;Trp53*$^{-/-}$
802 *;shPorcn* whole tumour lysate (*n*=5/group). **g.** Correlation of *Porcn* expression and *Il1b*
803 expression in bulk *WEA;Trp53*$^{-/-}$*;shControl* (blue) and *WEA;Trp53*$^{-/-}$*;shPorcn* tumours (grey)
804 (*n*=5/group). Data represent an average of 2 technical replicates. **h.** Spleen area in mice with
805 *WEA;Trp53*$^{-/-}$*;shControl* (blue) and *WEA;Trp53*$^{-/-}$*;shPorcn* tumours (grey) tumours at end-
806 stage (*n*=5/group). **i.** Growth kinetics of orthotopically transplanted KEP mammary tumours,
807 treated with vehicle (*n*=12) or LGK974 (*n*=15). Each line represents an individual mouse. **j.**
808 Growth kinetics of orthotopically injected *Trp53*$^{+/+}$ and *Trp53*$^{-/-}$ *Wap-cre;Cdh1*$^{F/F}$*;Pik3ca*^{E545K}
809 (WEP) cells, treated with vehicle or LGK974. Each line represents an individual mouse
810 (*n*=9/group). **k.** Schematic representation of the findings of this study: loss of p53 in breast
811 cancer cells triggers secretion of Wnt ligands to activate tumour-associated macrophages. This
812 stimulates systemic expansion and activation of neutrophils, which we have previously shown
813 to be immunosuppressive[5], thus driving metastasis. All data are means ± s.e.m. *P*-values are
814 indicated as determined by two-tailed Mann-Whitney U-test (**a – d, h**) and R^2 and *P*-values
815 determined by linear regression analysis (**e – g**).
816

817 **References**

818 1 Diakos, C. I., Charles, K. A., McMillan, D. C. & Clarke, S. J. Cancer-related
819 inflammation and treatment effectiveness. *Lancet Oncol.* **15**, e493-503 (2014).

820 2 McAllister, S. S. & Weinberg, R. A. The tumour-induced systemic environment as a
821 critical regulator of cancer progression and metastasis. *Nat. Cell Biol.* **16**, 717-27
822 (2014).

823 3 Templeton, A. J. *et al.* Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors:
824 a systematic review and meta-analysis. *J. Natl. Cancer Inst.* **106**, dju124 (2014).

825 4 Coffelt, S. B., Wellenstein, M. D. & de Visser, K. E. Neutrophils in cancer: neutral no
826 more. *Nat. Rev. Cancer* **16**, 431–46 (2016).

827 5 Coffelt, S. B. *et al.* IL-17-producing gammadelta T cells and neutrophils conspire to
828 promote breast cancer metastasis. *Nature* **522**, 345–48 (2015).

829 6 Kowanetz, M. *et al.* Granulocyte-colony stimulating factor promotes lung metastasis
830 through mobilization of Ly6G+Ly6C+ granulocytes. *Proc Natl Acad Sci U S A* **107**,
831 21248-55 (2010).

832 7 Bald, T. *et al.* Ultraviolet-radiation-induced inflammation promotes angiotropism and
833 metastasis in melanoma. *Nature* **507**, 109-13 (2014).

834 8 Wculek, S. K. & Malanchi, I. Neutrophils support lung colonization of metastasis-
835 initiating breast cancer cells. *Nature* **528**, 413–17 (2015).

836 9 Park, J. *et al.* Cancer cells induce metastasis-supporting neutrophil extracellular DNA
837 traps. *Sci. Transl. Med.* **8**, 361ra138 (2016).

838 10 Steele, C. W. *et al.* CXCR2 Inhibition Profoundly Suppresses Metastases and
839 Augments Immunotherapy in Pancreatic Ductal Adenocarcinoma. *Cancer Cell* **29**, 832-
840 45 (2016).

841 11 Ethier, J. L., Desautels, D., Templeton, A., Shah, P. S. & Amir, E. Prognostic role of
842 neutrophil-to-lymphocyte ratio in breast cancer: a systematic review and meta-analysis.
843 *Breast Cancer Res.* **19**, 2 (2017).

844 12 Cooks, T. *et al.* Mutant p53 prolongs NF-kappaB activation and promotes chronic
845 inflammation and inflammation-associated colorectal cancer. *Cancer Cell* **23**, 634-46
846 (2013).
847 13 Schwitalla, S. *et al.* Loss of p53 in enterocytes generates an inflammatory
848 microenvironment enabling invasion and lymph node metastasis of carcinogen-
849 induced colorectal tumors. *Cancer Cell* **23**, 93-106 (2013).
850 14 Stodden, G. R. *et al.* Loss of Cdh1 and Trp53 in the uterus induces chronic
851 inflammation with modification of tumor microenvironment. *Oncogene* **34**, 2471-82
852 (2015).
853 15 Wörmann, S. M. *et al.* Loss of P53 Function Activates JAK2-STAT3 Signaling to
854 Promote Pancreatic Tumor Growth, Stroma Modification, and Gemcitabine Resistance
855 in Mice and is Associated With Patient Survival. *Gastroenterology* **151**, 180-93 (2016).
856 16 Bezzi, M. *et al.* Diverse genetic-driven immune landscapes dictate tumor progression
857 through distinct mechanisms. *Nat. Med.* **24**, 165-75 (2018).
858 17 Kersten, K. *et al.* Mammary tumor-derived CCL2 enhances pro-metastatic systemic
859 inflammation through upregulation of IL1beta in tumor-associated macrophages.
860 *Oncoimmunology* **6**, e1334744 (2017).
861 18 Annunziato, S. *et al.* Modeling invasive lobular breast carcinoma by CRISPR/Cas9-
862 mediated somatic genome editing of the mammary gland. *Genes Dev.* **30**, 1470-80
863 (2016).
864 19 Song, X. *et al.* CD11b+/Gr-1+ immature myeloid cells mediate suppression of T cells
865 in mice bearing tumors of IL-1beta-secreting cells. *J Immunol* **175**, 8200-8 (2005).
866 20 Singh, V., Holla, S., Ramachandra, S. G. & Balaji, K. N. WNT-inflammasome signaling
867 mediates NOD2-induced development of acute arthritis in mice. *J. Immunol.* **194**, 3351-
868 60 (2015).
869 21 Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic beta-catenin signalling
870 prevents anti-tumour immunity. *Nature* **523**, 231-5 (2015).

871 22 Avgustinova, A. *et al.* Tumour cell-derived Wnt7a recruits and activates fibroblasts to
872 promote tumour aggressiveness. *Nat. Commun.* **7**, 10305 (2016).
873 23 Luke, J. J., Bao, R., Sweis, R. F., Spranger, S. & Gajewski, T. F. WNT/beta-catenin
874 pathway activation correlates with immune exclusion across human cancers. *Clin.*
875 *Cancer Res.* (2019).
876 24 Kim, N. H. *et al.* p53 and microRNA-34 are suppressors of canonical Wnt signaling.
877 *Sci. Signal.* **4**, ra71 (2011).
878 25 Nusse, R. & Clevers, H. Wnt/beta-Catenin Signaling, Disease, and Emerging
879 Therapeutic Modalities. *Cell* **169**, 985-99 (2017).
880 26 Wellenstein, M. D. & de Visser, K. E. Cancer-Cell-Intrinsic Mechanisms Shaping the
881 Tumor Immune Landscape. *Immunity* **48**, 399–416 (2018).
882 27 Boggio, K., Nicoletti, G., Di Carlo, E., Cavallo, F., Landuzzi, L., Melani, C., Giovarelli,
883 M., Rossi, I., Nanni, P., De Giovanni, C., Bouchard, P., Wolf, S., Modesti, A., Musiani,
884 P., Lollini, P.L., Colombo, M.P., Forni, G. Interleukin 12–mediated Prevention of
885 Spontaneous Mammary Adenocarcinomas in Two Lines of Her-2/neu Transgenic Mice.
886 *J. Exp. Med.* **188**, 589-96 (1998).
887 28 Jonkers, J. *et al.* Synergistic tumor suppressor activity of BRCA2 and p53 in a
888 conditional mouse model for breast cancer. *Nat. Genet.* **29**, 418-25 (2001).
889 29 Derksen, P. W. *et al.* Somatic inactivation of E-cadherin and p53 in mice leads to
890 metastatic lobular mammary carcinoma through induction of anoikis resistance and
891 angiogenesis. *Cancer Cell* **10**, 437-49 (2006).
892 30 Liu, X. *et al.* Somatic loss of BRCA1 and p53 in mice induces mammary tumors with
893 features of human BRCA1-mutated basal-like breast cancer. *Proc Natl Acad Sci U S A*
894 **104**, 12111-6 (2007).
895 31 Henneman, L. *et al.* Selective resistance to the PARP inhibitor olaparib in a mouse
896 model for BRCA1-deficient metaplastic breast cancer. *Proc. Natl. Acad. Sci. U.S.A.*
897 **112**, 8409-14 (2015).

898 32 Huijbers, I. J. *et al.* Using the GEMM-ESC strategy to study gene function in mouse
899 models. *Nat. Protoc.* **10**, 1755-85 (2015).
900 33 Kas, S. M. *et al.* Insertional mutagenesis identifies drivers of a novel oncogenic
901 pathway in invasive lobular breast carcinoma. *Nat. Genet.* **49**, 1219-30 (2017).
902 34 Annunziato, S. *et al.* Comparative oncogenomics identifies combinations of driver
903 genes and drug targets in BRCA1-mutated breast cancer. *Nat. Commun.* **10**, 397
904 (2019).
905 35 Liu, J. *et al.* Targeting Wnt-driven cancer through the inhibition of Porcupine by
906 LGK974. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 20224-9 (2013).
907 36 Doornebal, C. W. *et al.* A preclinical mouse model of invasive lobular breast cancer
908 metastasis. *Cancer Res* **73**, 353-63 (2013).
909 37 Sanjana, N. E., Shalem, O. & Zhang, F. Improved vectors and genome-wide libraries
910 for CRISPR screening. *Nat. Methods* **11**, 783-4 (2014).
911 38 Brinkman, E. K., Chen, T., Amendola, M. & van Steensel, B. Easy quantitative
912 assessment of genome editing by sequence trace decomposition. *Nucleic Acids Res.*
913 **42**, e168 (2014).
914 39 Schmidt, D. *et al.* ChIP-seq: using high-throughput sequencing to discover protein-DNA
915 interactions. *Methods* **48**, 240-8 (2009).
916 40 Lerdrup, M., Johansen, J. V., Agrawal-Singh, S. & Hansen, K. An interactive
917 environment for agile analysis and visualization of ChIP-sequencing data. *Nat. Struct.*
918 *Mol. Biol.* **23**, 349-57 (2016).
919 41 Okada, N. *et al.* A positive feedback between p53 and miR-34 miRNAs mediates tumor
920 suppression. *Genes Dev.* **28**, 438-50 (2014).
921 42 Kim, D. *et al.* TopHat2: accurate alignment of transcriptomes in the presence of
922 insertions, deletions and gene fusions. *Genome Biol.* **14**, R36 (2013).
923 43 Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions with
924 RNA-Seq. *Bioinformatics* **25**, 1105-11 (2009).

925 44 Anders, S., Pyl, P. T. & Huber, W. HTSeq--a Python framework to work with high-
926 throughput sequencing data. *Bioinformatics* **31**, 166-9 (2015).
927 45 Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for
928 differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-
929 40 (2010).
930 46 Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. voom: Precision weights unlock linear
931 model analysis tools for RNA-seq read counts. *Genome Biol.* **15**, R29 (2014).
932 47 Liberzon, A. *et al.* The Molecular Signatures Database (MSigDB) hallmark gene set
933 collection. *Cell Syst.* **1**, 417-25 (2015).
934 48 Bouaoun, L. *et al.* TP53 Variations in Human Cancers: New Lessons from the IARC
935 TP53 Database and Genomics Data. *Hum. Mutat.* **37**, 865-76 (2016).
936