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

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Cancer-associated systemic inflammation is strongly linked to poor disease outcome in patients with cancer1,2. For most human epithelial tumour types, high systemic neutrophil-to-lymphocyte ratios are associated with poor overall survival3, and experimental studies have demonstrated a causal relationship between neutrophils and metastasis4,5. However, the cancer-cell-intrinsic mechanisms that dictate the substantial heterogeneity in systemic neutrophilic inflammation between tumour-bearing hosts are largely unresolved. Here, using a panel of 16 distinct genetically engineered mouse models for breast cancer, we uncover a role for cancer-cell-intrinsic p53 as a key regulator of pro-metastatic neutrophils. Mechanistically, loss of p53 in cancer cells induced the secretion of WNT ligands that stimulate tumour-associated macrophages to produce IL-1β, thus driving systemic inflammation. Pharmacological and genetic blockade of WNT secretion in p33-null cancer cells reverses macrophage production of IL-1β and subsequent neutrophilic inflammation, resulting in reduced metastasis formation. Collectively, we demonstrate a mechanistic link between the loss of p53 in cancer cells, secretion of WNT ligands and systemic neutrophilia that potentiates metastatic progression. These insights illustrate the importance of the genetic makeup of breast tumours in dictating pro-metastatic systemic inflammation, and set the stage for personalized immune intervention strategies for patients with cancer.

To determine how pro-metastatic systemic inflammation is influenced by genetic aberrations in tumours, we studied 16 genetically engineered mouse models (GEMMs) for breast cancer carrying different tissue-specific mutations. These GEMMs represent most subtypes of human breast cancer, including ductal and lobular carcinoma, oestrogen receptor-positive (luminal A), HER2+, triple-negative and basal-like breast cancer. Because we and others have demonstrated that neutrophils expand systemically and promote metastasis5–10, we evaluated circulating levels of neutrophils as a marker for systemic inflammation in mammary tumour-bearing mice with end-stage disease. As expected, most tumour-bearing mice displayed an increase in circulating neutrophils compared with non-tumour-bearing wild-type animals (Fig. 1a). Like the inter-patient heterogeneity in systemic inflammation in human breast cancer11, we observed a striking variability in the extent of neutrophilia between the different tumour-bearing GEMMs (Fig. 1a, Extended Data Fig. 1a). We found that the models exhibiting high neutrophil expansion displayed a subset of neutrophils expressing the stem-cell marker cKIT (Fig. 1b), indicative of an immature neutrophil phenotype5. We subsequently searched for commonalities and differences among the 16 GEMMs with regards to high versus low levels of systemic neutrophils. Notably, mice bearing tumours with a p53 deletion exhibited the most pronounced levels of circulating neutrophils (Fig. 1a). The difference in the magnitude of systemic inflammation between p53-proficient and p53-null tumours was even more apparent when focusing on cKIT+ neutrophils (Fig. 1b).

In mouse models for colorectal, pancreatic, prostate and endometrial cancer, p53 mutation or loss leads to recruitment and activation of immune cells in the primary tumour microenvironment12–16. To study the association between p53 status of the tumour and systemic inflammation, we separated the 16 GEMMs based on the presence or absence of homozygously floxed Trp53 alleles and compared the levels of circulating neutrophils and the proportion of cKIT-expressing neutrophils. This analysis confirmed a statistically significant difference between mice bearing p53-proficient and p53-null tumours (Fig. 1c, d).

We previously demonstrated that expansion of neutrophils in mammary tumour-bearing Krt14 (K14)-cre;Cdhl1F/F;Trp53F/F (KEP) mice is driven by an inflammatory pathway that involves CCL2, IL-1β, IL-17A and granulocyte colony-stimulating factor (G-CSF)17. We found that serum levels of CCL2, IL-1β and G-CSF correlated with p53 loss in primary tumours in the 16 GEMMs (Fig. 1e–h). Principal component analysis of these systemic immune parameters further demonstrated that systemic inflammation correlated with the p53 status of the tumour (Fig. 1i).

To provide evidence for a causal relationship between p53 loss in mammmary tumours and neutrophilia, we derived cancer cell lines from two independent p53-proficient tumour models—Wap-cre;Cdhl1F/F;Akt1E17K (WEA)18 and Wap-cre;Cdhl1F/F;Pik3caE545K (WEP). Using CRISPR-Cas9-mediated gene disruption, we targeted Trp53, which resulted in an inability to increase p21 levels after irradiation (Extended Data Fig. 2a, b, c). We orthotopically transplanted WEA;Trp53+/+ and WEP;Trp53+/+ cells, and matched WEA;Trp53−/− and WEP;Trp53−/− cells into syngeneic wild-type mice (Fig. 2a). Although the loss of p53 conferred a proliferation advantage in vitro, growth kinetics in vivo were similar between p53-proficient and p53-deficient tumours for both cell lines (Extended Data Fig. 2c–g). Consistent with our findings in the GEMM panel, we observed increased expansion of neutrophils, including cKIT+ neutrophils, in the circulation and lungs of mice bearing WEA;Trp53−/− and WEP;Trp53−/− tumours, when compared with mice bearing size-matched p53-proficient tumours (Fig. 2b–d, Extended Data Fig. 2h, i). In addition, mice with WEA;Trp53−/−, but not WEP;Trp53−/−, tumours presented with splenomegaly when compared with Trp53+/+ controls (Extended Data Fig. 2j)—a phenomenon often observed in inflammation and cancer19. These data reveal that the loss of p53 in breast cancer cells is a central driving event of cancer-induced systemic neutrophilic inflammation.

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LETTER

https://doi.org/10.1038/s41586-019-1450-6

538 | NATURE | VOL 572 | 22 AUGUST 2019
Fig. 1 | Loss of p53 in mammary cancer cells correlates with systemic neutrophilic inflammation. a, b, Flow cytometry analysis of frequency of CD11b+Ly6G+Ly6C+ neutrophils (a) and proportion of cKIT+ neutrophils (b) as determined by flow cytometry analysis on blood of breast cancer GEMMs at end stage (cumulative tumour volume 1,500 mm3) and non-tumour-bearing (wild-type; 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). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, compared to wild-type control by two-tailed one-way analysis of variance (ANOVA) with Tukey’s multiple-testing correction. c, Total neutrophil frequencies (c) and neutrophil frequencies (d) in circulation of all Trp53+/+ (n = 28) and Trp53–/– (n = 46) tumour-bearing mice, combined from a and b. e–h, CCL2 levels (n = 17 Trp53+/+, n = 22 Trp53–/–) (e), IL-1β levels (n = 18 Trp53+/+, n = 21 Trp53–/–) (f), IL-17A levels (n = 24 Trp53+/+, n = 30 Trp53–/–) (g) and G-CSF levels (n = 22 Trp53+/+, n = 33 Trp53–/–) (h) in serum of GEMMs at end stage based on p53 status. P values in c–h determined by two-tailed Mann–Whitney U-test. i, Principal component analysis of data depicted in a–h (13 out of 16 GEMMs). Each symbol represents one mouse. Circles contour 40% of group-specific Gaussian probability distributions of sample scores. All data are mean ± s.e.m.

Because we observed cKIT+ immature neutrophils in p53-null tumour-bearing mice (Figs 1d, 2d), we next investigated whether haematopoiensis was altered. In mice bearing WEA;Trp53–/– tumours, the frequencies of Lin−Sca1+ cKIT+ cells, common myeloid progenitors, CD11b+Ly6C0 pro-myelocytes and mature neutrophils were increased in the bone marrow at the expense of megakaryocyte and erythrocyte progenitors, when compared to WEA;Trp53+/+ tumour-bearing mice (Extended Data Fig. 3a–c). This effect on cell proportions was not reflected in the total cell counts, possibly owing to a slight depletion of total bone marrow cell numbers in WEA;Trp53–/– tumour-bearing mice (Extended Data Fig. 3d).

Previously, we reported that macrophage-derived IL-13 in the tumour microenvironment triggers systemic neutrophil expansion in KEP mice3. Because serum levels of IL-13 correlated with p53 status (Fig. 1f), we proposed that loss of p53 changes the secretome of cancer cells, stimulating IL-13 production from tumour-associated macrophages (TAMs) and setting off a systemic inflammatory cascade. Indeed, in vitro exposure of bone marrow-derived macrophages (BMDMs) to conditioned medium from WEA;Trp53–/– or WEA;Trp53+/+ cancer cells differentially affected their phenotype (Extended Data Fig. 4a). Notably, conditioned medium from WEA;Trp53+/+ and WEP;Trp53–/– cells strongly induced IILb mRNA expression in cultured BMDMs compared with conditioned medium from matched Trp53+/+ controls (Fig. 2e). In agreement with our mouse data, human monocyte-derived macrophages (MDMs) cultured with tumour conditioned medium of TP53–/– MCF-7 human breast cancer cells displayed increased CD206 and CD163 expression compared with human MDMs cultured with conditioned medium of TP53+/+ MCF-7 cells compared with TP53+/+ controls (Extended Data Fig. 4d). These data indicate that cancer-cell-intrinsic p53 status dictates the crosstalk between cancer cells and macrophages in a paracrine fashion, resulting in an altered macrophage phenotype and IL-13 production. We also observed increased levels of IILb mRNA expression in breast tumours of The Cancer Genome Atlas (TCGA) with mutations in TP53 compared with wild-type TP53 tumours (Fig. 2f), suggesting similar p53-dependent activation of IL-13 signalling in human breast cancer.

To identify which factor(s) in p53-null tumours mediate TAM activation and subsequent systemic inflammation, we performed RNA sequencing on mammary tumours of 12 different GEMMs (7 p53-null models, 5 p53-proficient models; 145 tumours in total). The p53-deficient tumours differed substantially from p53-proficient tumours in terms of gene expression, regardless of any additional genetic aberrations, demonstrating a dominant effect of p53 loss on the global transcriptome (Extended Data Fig. 5a). Interestingly, the most significantly changed pathways in p53-deficient tumours pertained to adaptive immune phenotypes (Fig. 3a). Although neutrophil and TAM numbers were altered intratumourally, the composition of CD8+, CD4+ or FOXP3+ T cells did not correlate with p53 status (Extended Data Fig. 5b).
Fig. 5b–g, suggesting that the distinct transcriptome profiles are not due to a p53-dependent effect on the composition of the adaptive immune landscape.

From the Gene Ontology analysis, we selected genes that encode secreted factors that could potentially influence TAMs. One of the upregulated pathways in p53-null tumours included WNT and β-catenin signalling (Fig. 3a). WNT signalling is linked to the production of IL-1β in acute arthritis, as well as immune and stromal signalling in cancer. Using a WNT and β-catenin signalling gene signature, we found that p53-null GEMM tumours clustered separately from

Fig. 3 | p53-null tumours display activated WNT signalling. a. The top 10 most significantly differentially activated pathways as determined by Ingenuity Pathway Analysis, comparing Trp53−/− (n = 77) with Trp53+/+ (n = 68) GEMM tumours of 12 different models. Also indicated is the WNT signalling pathway. b. The log-transformed fold change in expression of Wnt1, Wnt6 and Wnt7a in Trp53−/− (n = 77) GEMM tumours compared with Trp53+/+ (n = 68) tumours. c. Western blot analysis of bulk tumours showing non-phosphorylated (non-P) β-catenin (active), porcupine, WNT1, WNT6 and WNT7a (blue indicates Trp53−/− tumours; red indicates Trp53+/+ tumours). Representative of two independent experiments. For uncropped images, see Supplementary.
Because deletion of p53 increases WNT ligand expression, we performed chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) in three independent WEA and WEP cell lines. p53 binding was observed at the \( \text{Cdkn1a} \) (p21) locus (Extended Data Fig. 7a), whereas we did not find p53 binding at the \( \text{Wnt1} \), \( \text{Wnt6} \) or \( \text{Wnt7a} \) loci (Extended Data Fig. 7b), suggesting that p53 regulates their expression indirectly. Because p53 has been described to control Wnt1 expression by activating microRNA-34a (miR-34a)\(^\text{24}\), we wondered whether this microRNA may be involved in the regulation of \( \text{Wnt1} \), \( \text{Wnt6} \) and \( \text{Wnt7a} \). Indeed, we observed p53 chromatin binding at the miR-34a locus in all cell lines (Extended Data Fig. 7c). Overexpression of miR-34a in WEA;\( \text{Trp53}^{-/-} \) cells resulted in a significant reduction of WNT ligand expression (Extended Data Fig. 7d). These data suggest that wild-type p53 negatively regulates the expression of \( \text{Wnt1} \), \( \text{Wnt6} \) and \( \text{Wnt7a} \) via miR-34a.

We then assessed the role of cancer-cell-derived WNT ligands on IL-1\( \beta \) production by macrophages. We treated WEA cells with LGK974—which inhibits porcupine (encoded by \( \text{Porcn} \)) in a WNT-specific acyltransferase that regulates WNT ligand secretion\(^\text{25}\)—and added conditioned medium to macrophages. LGK974 reduced the WEA;\( \text{Trp53}^{-/-} \) cell-derived IL1\( \beta \) expression by macrophages (Fig. 4a). We also depleted \( \text{Porcn} \) in WEA;\( \text{Trp53}^{-/-} \) cells using short hairpin RNAs (shRNAs), which resulted in reduced macrophage expression of IL1\( \beta \), consistent with pharmacological inhibition of porcupine (Fig. 4a).

These data confirm a causal relationship between WNT ligand secretion by p53-deficient cancer cells and IL-1\( \beta \) expression in macrophages.
Frizzled receptors, Fzd7 and Fzd9, were upregulated in the p53-null tumours compared with p53-proficient tumours (Extended Data Fig. 8a). Similarly, expression of Fzd7 and Fzd9 was increased in mutant TP53 human breast tumours compared with wild-type TP53 tumours (Extended Data Fig. 8b). We then used small interfering RNAs (siRNAs) to knockdown both Fzd7 and Fzd9 in BMDCMs (Extended Data Fig. 8c), which prevented induction of Il1b by WEA;Trp53−/− cells (Extended Data Fig. 8d), demonstrating that Fzd7 and Fzd9 are involved in WNT-induced activation of macrophages in vitro.

We next assessed whether the production of WNT ligands by p53-deficient cancer cells drives systemic inflammation. We treated tumour-bearing KEP mice with LGK974 for five consecutive days, and this led to a reduction in the levels of total neutrophils and cKIT+ neutrophils in blood and lungs when compared with vehicle-treated KEP mice (Fig. 4b, Extended Data Fig. 9a). In addition, IL-17A-producing γδ T cells—the key cell type responding to IL-1β that drives neutrophil accumulation and consequently metastasis—were reduced in the lungs of LGK974-treated KEP mice (Extended Data Fig. 9b), indicating that γδ T cell activation upstream of pro-metastatic neutrophil accumulation depends on WNT signalling. Similarly, long-term treatment of KEP mice with LGK974 blocked neutrophil expansion over time (Extended Data Fig. 9c). To exclude the possibility that the observed reduction in inflammation is a result of targeting non-tumour cells by LGK974, we orthotopically transplanted WEA;Trp53−/−;shPorcn cell lines and matched WEA;Trp53−/−;shControl cells into wild-type mice. Analysis of size-matched end-stage tumours revealed an incomplete reduction of Porcn expression (Extended Data Fig. 9d). Although we cannot formally exclude the possibility that non-cancer cells contribute to the residual Porcn expression, expression levels of Porcn in the tumours correlated with levels of circulating neutrophils, cKIT+ neutrophils and Il1b expression (Extended Data Fig. 9e–g). Moreover, knockdown of Porcn prevented splenomegaly (Extended Data Fig. 9h). Collectively, these data confirm the causal link between WNT secretion triggered by p53-deficient mammary tumours and systemic inflammation.

Because the γδ T cell–neutrophil axis promotes metastasis and these cells are regulated by WNT ligands, we proposed that LGK974 treatment may present a viable therapeutic strategy to inhibit metastasis of p53-null mammary tumours. To test this, we treated KEP tumour-bearing mice with LGK974 or vehicle, after which we surgically removed the primary tumour and assessed metastatic progression. LGK974 treatment may present a viable therapeutic strategy to inhibit metastasis triggered by p53-deficient mammary tumours and systemic inflammation.

In summary, we show that the status of p53 is an important driver of systemic pro-metastatic inflammation in breast cancer (Extended Data Fig. 9k) and that targeting WNT signalling may represent a promising therapeutic modality for patients with p53-deficient breast tumours. Together with recent literature on the importance of canonical driver mutations in shaping the local immune composition of primary tumours,26 our findings shed light on the poorly understood inter-patient heterogeneity in the systemic composition and function of immune cells. Mechanistic understanding of the intricate interactions between cancer-cell-intrinsic genetic events and the immune landscape provides a basis for the design of personalized immune intervention strategies for patients with cancer.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1450-6.

Received: 15 February 2018; Accepted: 26 June 2019;
Published online 31 July 2019.

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METHODS

Mice. All animal experiments were approved by the Animal Ethics Committee of the Netherlands Cancer Institute and performed in accordance with institutional, national and European guidelines for Animal Care and Use. The generation and characterization of the mouse models has been described27–34 (and unpublished observations). The following mouse models were used in this study: keratin 14 (K14-cre;Cdh1F/F;Trp53F/F, Wap-cre;Cdh1F/F;En1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F, Wap-cre;Cdh1F/F;En1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F, Wap-cre;Cdh1F/F;En1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F, Wap-cre;Cdh1F/F;En1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F, Wap-cre;Cdh1F/F;En1F/F;Trp53F/F), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F, Wap-cre;Cdh1F/F;En1F/F;Trp53F/F, Myc), keratin 14 (K14-cre;Cdh1F/F;Trp53F/F, Wap-cre;Cdh1F/F;En1F/F;Trp53F/F, Myc, CD16/32 expression). Cells were stained with conjugated antibodies for 30 min at 4 °C, except for bone marrow (to allow assessment of cell purities of >95% CD11b+ M, Selleck Chemicals) for 24 h and harvested for RNA and/or protein isolation. Conditioned medium was obtained by culturing tumor cells at equal confluency in empty DMEM overnight. Cell growth kinetics in vitro were analysed using the Incucyte System (Essen BioScience).

RNA isolation and qRT–PCR. RNA was isolated using either TRIzol or a Qiagen RNeasy column followed by treatment with DNase I (Invitrogen). RNA quality was checked by running 1 μg of RNA on a 1% Agilent RNA 6000 Nano bioanalyzer. RNA from CDNA was generated from 1 μg of total RNA using an AMV reverse transcriptase using Oligo(dT) primers (Invitrogen). cDNA (20 ng per well) was analysed by SYBR green real-time PCR with 500 nM primers using a LightCycler 480 thermocycler (Roche). A typical experiment was performed in triplicate. Real-time PCR data were calculated using the 2−ΔΔCt method.

Protein isolation and western blotting. Protein lysates of cells and tissue were prepared using RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 2 mM EDTA) complemented with protease and phosphatase inhibitors (Roche) and protein concentration was quantified using the BCA protein assay kit (Pierce). Protein lysate was loaded onto NuPAGE 4–12% Bis-Tris gradient gels (Invitrogen) and transferred onto Trans-Blot Turbo Mini or Midi Nitrocellulose membranes (BioRad) using Trans-Blot Turbo Transfer System (BioRad). Membranes were blocked in 10% Western Blotting Reagent (Roche) or 3% BSA for 1 h at room temperature. Primary antibody incubation was performed overnight at 4 °C. Membranes were washed using TBS and Tween 20 (TBS-T) and subjected to secondary fluorochrome-conjugated antibodies for 1 h at room temperature and protein was detected using Odyssey CLX imaging system and processed using ImageJ software 1.48v. Antibodies are listed in Supplementary Table 2.

Immunohistochemistry. Immunohistochemical analyses were performed by the Animal Pathology faculty at the Netherlands Cancer Institute. Formalin-fixed tissues were processed, sectioned and stained as described38. In brief, tissues were fixed for 24 h in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin (H&E) for histopathological evaluation. H&E slides were digitally processed using the Aperio ScanScope (Aperio). For immunohistochemical analysis, 5-μm paraffin sections were cut, deparaffinized and antigen retrieval methods were listed in Supplementary Table 1. Quantitative analysis of cell abundance was performed by counting cells in five high-power (×40) fields of view (FOV) per tissue by two independent researchers. Samples were visualized with a BX43 upright microscope (Olympus).
and images were acquired in bright field using cellsSens Entry software (Olympus). To score pulmonary metastasis, single lung sections were stained for cytokeratin-8 and metastatic nodules were counted by two independent researchers. Stained tissue slides were digitally processed using the Aperio ScanScope. Brightness and contrast for representative images were adjusted equally among groups.

Cytokine analyses. Quantification of cytokine and chemokine levels in serum was performed using BD Cytometric Bead Array for CCL2, IL-1β, IL-17A and G-CSF according to manufacturer’s instructions and analysed on a Beckman Coulter CyAn ADP flow cytometer with Summit software. Data analyses were performed using FlowJo Software version 9.9.

Crispr–Cas9-mediated gene disruption. For knockdown of mouse Trp53, p53-deficient tumour cell lines were transfected with lentivector (provided by F. Zhang (Addgene plasmid 52961)16) containing sgRNA targeting exon 4 (sgRNA1: 5′-TGCTAGCTTGGACATGCTGA-3′) and sgRNA2: 5′-AGTGAAGGCAAGAGGCTGCTC-3′. For knockdown of human TP53, MCF-7 tumour cell lines were transfected with lentivector (provided by F. Zhang) containing sgRNA targeting either exon 4 (sgRNA1: 5′-CACTTGTGATATGGAGCTG-3′) or exon 2 (sgRNA2: 5′-TCGAGCCTAGGCTGCTAGCTG-3′). Cloning of sgRNAs in lentivirus was performed as described37 and sgRNA sequences were designed using the online CRISPR Design tool (http://crispr.mit.edu), of which the two highest scoring sequences were chosen. All vectors were validated by Sanger sequencing. After selection of transfected cells, polyclonal cell lines were used for all subsequent experiments. To determine knockdown efficiency, genomic DNA from cell lines was isolated using Viagen DirectPCR Lysis reagent (Cell) supplemented with 200 µg ml−1 proteinase K after transfection and puromycin selection. Trp53 target region was amplified using PCR with the following primers: forward 5′-GGGAGCTCGTGTGGTCTGACA-3′ and reverse 5′-CCACGTCCCTGGGAGAGTG-3′. The human TP53 target region was amplified using PCR with the following primers: FW1 5′-CAGACCTG CCTTCCGGGTACAC-3′ for sgRNA1, FW2 5′-TGGGAGGTTGGAAGTGGCTCC-3′ for sgRNA2, and RV 5′-CAGTCGACAGAGGACAGACAGC-3′. PCR products were run on 1% agarose gel, purified using the Illustra GEX PCR DNA and Gel Band Purification Kit (Sigma), and subjected to Sanger sequencing using their respective forward primers. Genome editing efficiency was quantified using the Tracking of Indels by Decomposition (TIDE) algorithm as described (http://tide.nki.nl)38.

ShRNA- and siRNA-mediated knockdown of genes. Vectors for shRNAs were collected from the TRC library. To allow stable expression of shRNAs, HEK293T cells (provided by T.N.S.) were transfected with the pLKO.1 lentiviral vector encoding shRNAs, pPAX packaging vector and VSV-G envelope vector. Five independent shRNA clones were used for each experiment. Virus was collected at day 4 and 5 and viral titres were determined using the Abo qPCR lentivirus titration kit (LV900). Cell lines were subsequently transduced and selected using puromycin. Expression efficacy was determined by RT–qPCR compared to non-targeting controls. The shRNA clone used for Porcn knockdown in all experiments after assessment of knockdown efficiency contained following hairpin sequence: 5′-CAACCTTTCTATGCTGCTAT-3′ (shPorcn-1) or 5′-CCCATGTCTTATGTTGATA-3′ (shPorcn-4). For in vivo experiments, shPorcn-4 was used. To silence Fzd receptors, BMDMs were transfected with the following siRNA pools (control siRNA (sc-37007), Fzd7 (sc-39991) and Fzd9 (sc-39995), Santa Cruz Biotechnology), according to the manufacturer’s instructions. In brief, BMDMs were differentiated as described above, and 24 h before exposure to tumour conditioned medium and BMDMs were suspended in transfection medium and incubated with indicated siRNA pools. After 6 h at 37 °C, 2× RPMI medium was added (RPMI, 20% serum, 200 µl ml−1 penicillin, 200 mg ml−1 streptomycin and 20 µg ml−1 recombinant M-CSF) and BMDMs were further cultured overnight. After 24 h, the medium was replaced by tumour conditioned medium for 24 h, after which gene expression was assessed.

Chip–seq analysis. Chip–seq analysis was performed as previously described39. In brief, cell lines from Wap-cre:Cdh1+/−, Akt+/− and Wap-cre:Cdh1+/+Pik3caH1047E) (Pik3caG1047E) tumours (3 cell lines from 3 independent mouse tumours per genotype) were fixed in 1% formaldehyde, crosslinked and processed for sonication. Subsequently, 5 µg of p53 antibody (Supplementary Table 1) and 50 µl of protein G magnetic beads (Invitrogen) were used for each Chip. Eluted DNA was sequenced using the Illumina Hiseq 2500 analyser (using 65 bp reads) and aligned to the Mus musculus mm10 reference genome. Peak calling over input control was performed using the online CRISPR Design tool (http://crispr.mit.edu) for and identification of TP53 target genes. Library size normalization was performed using Ingenuity Pathway Analysis software (QIAGEN), analysing differentially expressed genes with P < 0.05. The selected genes were shown in a heat map of read counts that were normalized to 10 million reads per sample. For Hallmark pathway analysis of mouse transcriptomes, raw read counts were normalized by trimmed means of M-values computed using the function calcNormFactors (edgeR v.3.20.540), from which counts per million (CPM)- normalized gene expression values were computed for plotting purposes using the same R package. CPM values were subsequently transformed as \( \log_2(1 + x) \).

Heat map columns (containing samples) were ordered according to average linkage (UPGMA) hierarchical sample clustering based on pearson correlation distances between the expression values of displayed genes. Heat map rows (containing genes) were ordered according to gene expression fold difference between Trp53+/− and Trp53−/− samples. The R language for statistical computing was used (v.3.4.2) for gene expression normalization and heat map generation. Pathway enrichment analysis of Trp53+/− and Trp53−/− tumours was performed using Ingenuity Pathway Analysis software (QIAGEN), analysing differentially expressed genes with P < 0.05.

TCGA analysis. To obtain a comprehensive view on the cellular processes affected by p53 deficiency in human breast cancer, we performed a gene set enrichment analysis (GSEA) using a 50 hallmark gene sets (Liberson)47 on the TCGA breast cancer (BRCA) cohort. First, we classified p53 deficiency based on mutational status. DNA sequence variant calling (MAF-file) for the BRCA cohort were downloaded from the 21 August 2015 release of the Broad TCGA genome data analysis centre standard run (http://gdc.cancer.gov/). We used two classification schemes to assign p53 deficiency to each sample: one based on TP53 mutation status in all samples, and one based on TP53 mutation status in patients with any kind of TP53 mutation. We applied two classification methods. In the first classification (labeled ‘any TP53 mutation’), patients with a dominant-negative TP53 mutation as annotated using the IARC TP53 mutation database48 (release 18, matched on protein effect of the mutation) were labelled as p53 deficient, as well as patients with gain-of-stop, stop-lost or frameshifting mutations (n = 161). One sample had a trans-activating mutation and was excluded from the analysis. The remaining samples were labelled as p53 proficient (n = 793).

Next, TCGA RNA sequencing data were downloaded from the Broad TCGA genome data analysis centre 1 November 2015 release of the standard runs. We ran a GSEA on the 50 Hallmark gene set using the flexgsea-r R package (https://github.com/KNI-CBB/flexgsea-r) on the read counts normalized with limma voom with the span parameter set to 0.549. Within each permutation of the sample labels, genes were ranked for association with p53 proficiency using the moderated t-statistic from the limma empirical Bayes function (ebayes) ran on the result of LimFit(). Reported FDR-values were obtained from the flexgsea-r output.

Single gene associations with TP53 status in human breast tumours of the TCGA BRCA cohort and correlation coefficients between WNT-related genes and TP53 status (mutant versus wild type) were analysed using R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl/) and visualized using GraphPad Prism version 7.

Statistics and reproducibility. Data analyses were performed using GraphPad Prism (version 7). The statistical tests used are described in figure legends. All tests were performed two-tailed. P < 0.05 was considered statistically significant. All western blot and RT–qPCR analyses were independently repeated more than twice. Sample sizes were based on previous experiments45,46 or determined using G*Power software (version 3.1.1). To exclude bias towards one particular GEMM in gene set enrichment analyses for Fig. 5, we first tested the same analyses on the average of the neutrophil levels and serum cytokine values per model. This demonstrated the same correlations between the assessed values and p53 status of the tumour, thus excluding bias towards one or several particular models. Principal
component analysis was performed using the pcomp-function in R (version 3.4.2), both centering and scaling the input data before applying dimensionality reduction.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**
The RNA-sequencing data have been deposited in the Gene Expression Omnibus (GEO, NCBI) repository under accession number GSE112665. All other data are found in the Source Data, Supplementary Information or available from the authors on reasonable request.

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**Acknowledgements**
Research in the De Visser laboratory is funded by a European Research Council Consolidator award (ERC InflaMet 615300), the Netherlands Organization for Scientific Research (NWO-VICI 91819616), Oncode Institute, the Dutch Cancer Society (KWF10083; KWF10623) and the Beug Foundation for Metastasis Research. K.E.d.V. is an EMBO Young Investigator. Research in the Jonkers laboratory is funded by ERC Synergy grant 319661. We thank members of the De Visser and Jonkers laboratories and R. Mezzadra for fruitful discussion during the preparation of the manuscript. We thank G. van Tellingen, the Mouse Clinic for Cancer and Aging (MCCA) intervention Unit, flow cytometry facility, mouse transgenic facility, genomics core facility, animal laboratory facility and animal pathology facility of the Netherlands Cancer Institute for technical assistance.

**Author contributions**
M.D.W., S.B.C., J.J. and K.E.d.V. conceived the ideas and designed the experiments. M.D.W., S.B.C., D.E.M.D. and M.H.v.M. performed the animal experiments, flow cytometry, RT–qPCR, serum analyses, western blot, immunohistochemistry and other experiments and analysed the data. C.-S.H., K.V., A.P.D., E.S. and R.d.K.-G. provided technical support and performed animal experiments. M.H.v.M., L.H., S.M.K. and J.J. generated mouse models. M.D.W. and R.d.K.-G. performed mouse intervention experiments. I.v.d.H. generated the GEMM-derived cell lines. S.P., M.D.W. and W.Z. performed and analysed the ChiP–seq experiments. M.S., I.d.R., M.D.W., L.F.A.W. and T.N.S. performed the bioinformatics analyses on mouse and human RNA-sequencing datasets. M.D.W., S.B.C. and K.E.d.V. wrote the paper and prepared the figures, with input from all authors.

**Competing interests**
M.D.W., S.B.C., D.E.M.D. and M.H.v.M. performed the animal experiments, flow cytometry, RT–qPCR, serum analyses, western blot, immunohistochemistry and other experiments and analysed the data. C.-S.H., K.V., A.P.D., E.S. and R.d.K.-G. provided technical support and performed animal experiments. M.H.v.M., L.H., S.M.K. and J.J. generated mouse models. M.D.W. and R.d.K.-G. performed mouse intervention experiments. I.v.d.H. generated the GEMM-derived cell lines. S.P., M.D.W. and W.Z. performed and analysed the ChiP-seq experiments. M.S., I.d.R., M.D.W., L.F.A.W. and T.N.S. performed the bioinformatics analyses on mouse and human RNA-sequencing datasets. M.D.W., S.B.C. and K.E.d.V. wrote the paper and prepared the figures, with input from all authors.

**Additional information**
**Supplementary information** is available for this paper at https://doi.org/10.1038/s41586-019-1450-6.

**Correspondence and requests for materials** should be addressed to J.J. or K.E.d.V.

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Extended Data Fig. 1 | Neutrophil expansion in p53-deficient tumour-bearing GEMMs. a, Representative plots of flow cytometry analysis on blood of end-stage (cumulative tumour size 1,500 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 wild-type mice). Quantification and statistical analysis of these data are found in Fig. 1a, b.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | CRISPR–Cas9-mediated gene disruption of Trp53 in WEA and WEP cancer cell lines. **a**, Insertion and deletion (indel) spectrum of bulk Wap-cre;Cdh1F/F;Akt1E17K (WEA) cancer cell lines after transfection with two individual sgRNAs against Trp53 and puromycin selection, as determined by the TIDE algorithm and compared to the sequence of target region of control cells. *P* values associated with the estimated abundance of each indel are calculated by a two-tailed *t*-test of the variance–covariance matrix of the s.e.m. **b**, Western blot analysis showing p53 levels of control and p53-knockout 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. Blot is representative of two independent experiments. For uncropped images, see Supplementary Fig. 1. **c**, In vitro growth kinetics of orthotopically transplanted WEA;Trp53<sup>+/+</sup> (n = 4 mice) and WEA;Trp53<sup>−/−</sup> (n = 6) cancer cell lines, with *t* = 0 being the first day tumours were palpable. **d**, Indel spectrum of bulk Wap-cre;Cdh1F/F;Pik3caE545K (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-knockout cells, as determined by IncuCyte (n = 7 technical replicates per group). **g**, In vivo growth kinetics of orthotopically transplanted WEP;Trp53<sup>+/+</sup> (n = 5) and WEP;Trp53<sup>−/−</sup> (n = 5) cell lines, with *t* = 0 being the first day tumours were palpable. **h**, Gating strategy to identify circulating neutrophils and their cKIT expression. **i**, Gating strategy to identify neutrophils in the lung. **j**, Representative images of spleens from mice bearing WEA;Trp53<sup>+/+</sup> and WEA;Trp53<sup>−/−</sup> tumours and quantification of spleen area (length × width) at end stage (tumour volume 1,500 mm<sup>3</sup>) of mice bearing p53-proficient (n = 4) and p53-deficient WEA (n = 6) and WEP (n = 5 per group) tumours. All data are mean ± s.e.m. *P* values were determined by area under the curve (AUC) analysis followed by two-tailed Welch’s *t*-test (c, d, f, g) or two-tailed Mann–Whitney *U*-test (j). ns, not significant.
Extended Data Fig. 3 | Haematopoiesis in p53-null tumour-bearing mice is skewed towards the development of neutrophils. a, Schematic representation of neutrophil development in the bone marrow. b, Gating strategy of neutrophil progenitor populations in the bone marrow. Dot plot indicates the cKIT expression levels in promyelocytes compared with mature neutrophils (n = 20 mice). MFI, median fluorescence intensity. c, Frequency of bone marrow progenitor populations in mice bearing end-stage Wap-cre;Cdhr1^{F/F};Akt1^{E17K};Trp53^{+/+} (n = 9) and Wap-cre;Cdhr1^{F/F};Akt1^{E17K};Trp53^{-/-} (n = 11) tumours, as determined by flow cytometry. d, Total live cells and total live progenitor population numbers per hindleg of mice bearing WEA;Trp53^{+/+} and WEA;Trp53^{-/-} tumours (n = 5 per group). All data are ± s.e.m. P values are determined by two-tailed Mann–Whitney U-test. LSK, Lin^−Sca1^− cKIT^+, which contain the LT-HSC (long-term haematopoietic stem cells), ST-HSC (short-term haematopoietic stem cells) and MPP (multipotent progenitors). CMP, common myeloid progenitors; GMP, granulocytic and monocytic progenitors; MEP, megakaryocyte and erythrocyte progenitors.
Extended Data Fig. 4 | Macrophages are differentially activated by Trp53−/− mouse and human breast cancer cell lines. a, Expression of CCR2, CCR6, CD206, CSF-1R, CXCR4 and MHC-II on live CD11b+F4/80+BMDMs after exposure to control medium or conditioned medium (CM) of Wap-cre;CdhlF/F;Akt1E17K;Trp53+/+ or Wap-cre;CdhlF/F;Akt1E17K;Trp53−/− cell lines, as determined by flow cytometry (n = 4 biological replicates per group). b, TIDE analysis of bulk MCF-7 cells after transfection with TP53-targeting sgRNAs and puromycin selection. For subsequent experiments, sgRNA1 was used. c, Expression of CD206, CD163 and HLA-DR on human CD11b+CD14+CD68+ monocyte-derived macrophages (MDMs) after exposure to conditioned medium of TP53+/+ MCF-7 or TP53−/− (sgRNA1) MCF-7 cancer cells (n = 3 biological replicates per group). d, RT-qPCR analysis showing IL1B expression in human CD11b+CD14+CD68+ MDMs after exposure to control medium (n = 4 biological replicates) conditioned medium of TP53+/+ MCF-7 or TP53−/− MCF-7 cancer cells (n = 5 biological replicates per group). Data are normalized to normal medium control. Plots are representative of three separate experiments and average of two technical replicates. All data are mean ± s.e.m. P values were determined by two-tailed one-way ANOVA with Tukey’s multiple-testing correction.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Transcriptome profile and composition of the local tumour immune landscape in breast cancer GEMMs.

a, Unsupervised clustering of the top 200 most differentially expressed genes (P < 0.01, log-transformed fold change >3 or <−3) in mammary GEMM tumours as determined by RNA sequencing (n = 145 tumours). Red bars indicate Trp53/+ tumours, blue bars indicate Trp53−/− tumours. Full tumour genotype is displayed in legend and shown by indicated colours. b, Number of Ly6G+ neutrophils in the tumour (n = 1, 4, 10, 2, 4, 3, 6, 13, 4, 22, 4 and 5 mice, top to bottom). c, Macrophage score as indicative of F4/80+ macrophage abundance in the tumour (n = 2, 2, 4, 4, 2, 3, 5, 4, 9, 5 and 4 mice, top to bottom). d, Number 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). 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 bottom). f, Number of FOXP3+ regulatory T cells in the tumour (n = 3, 2, 5, 5, 7, 3, 5, 4, 4 and 5 mice, top to bottom). g, Ratio of CD8/FOXP3 cells in the tumour (n = 3, 2, 5, 5, 7, 3, 7, 2, 5, 4, 4 and 5 mice, top to bottom). All data are the mean of five microscopic fields of view (FOV) per mouse as determined by immunohistochemistry. Inserts show data combined according to p53 status of the tumour. Each symbol represents an individual mouse. All data are mean ± s.e.m. P values are determined by two-tailed one-way ANOVA with FDR multiple-testing correction (a) or two-tailed Mann–Whitney U-test (b–g).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | WNT-related gene activation correlates with loss of p53 in mouse and human breast tumours. a, b, Heat maps showing that $Trp53^{-/-}$ (KO) GEMM tumours ($n = 77$) cluster away from $Trp53^{+/-}$ (WT) tumours ($n = 68$) based on analysis of the Hallmark p53 pathway (represents positive control) (a) and analysis of the Hallmark WNT and β-catenin pathway (b). Analysis was performed on all tumours of Extended Data Fig. 5a. c, The log-transformed fold change in expression of genes involved in WNT signalling ($P < 0.05$) in $Trp53^{-/-}$ ($n = 77$) and $Trp53^{+/-}$ ($n = 68$) GEMM tumours depicted in Extended Data Fig. 5a. Black bars indicate genes that positively regulate or are generally increased with active WNT signalling. Red bars indicate genes that negatively regulate or are downregulated with active WNT signalling. d, Gene set enrichment analysis (GSEA) for Hallmark pathways in TCGA wild-type $TP53$ breast tumours ($n = 643$) versus mutant $TP53$ ($n = 351$) human tumours (any TP53 mutation) or TP53 loss (based on the IARC TP53 database; see Methods). Normalized enrichment score is shown with the FDR indicated. e, Correlation coefficient ($R$) of all genes involved in WNT signalling that correlate significantly ($P < 0.05$) with mutant $TP53$ ($n = 351$) versus wild-type $TP53$ ($n = 643$) in TCGA breast tumours. Black bars indicate genes that positively regulate or are generally increased with active WNT signalling. Red bars indicate genes that negatively regulate or are downregulated with active WNT signalling. $P$ values were determined by two-tailed ANOVA with FDR multiple-testing correction (c, e).
Extended Data Fig. 7 | p53 does not bind the regulatory regions of WNT ligands directly. a, ChIP–seq profile of p53 binding to DNA demonstrating enrichment on the Cdkn1a (p21) locus in Trp53<sup>+/−</sup> WEA and WEP cell lines (three cell lines from three independent tumours per GEMM). b, Absence of p53 binding to Wnt1, Wnt6 or Wnt7a loci. c, Enrichment of p53 on the miR-34a (miR-34a) locus. d, RT–qPCR analysis of Wnt ligand expression in WEA;Trp53<sup>+/−</sup> and WEA;Trp53<sup>−/−</sup> cell lines after overexpression (OE) of miR-34a in WEA;Trp53<sup>−/−</sup> cells (n = 3 technical replicates per group). Plots are representative of three separate experiments with three technical replicates. All data are mean ± s.e.m. P values were determined by two-tailed one-way ANOVA with Tukey multiple-testing correction (d).
Extended Data Fig. 8 | Macrophages are activated by Trp53−/− cancer cells via FZD7 and FZD9 receptors in vitro. a, The log2-transformed fold change in expression of WNT receptors Fzd7 and Fzd9 in bulk tumours comparing Trp53−/− (n = 77) and Trp53+/+ (n = 68) GEMM tumours using RNA-sequencing analysis. b, Expression of FZD7 and FZD9 in TP53 wild-type (n = 643) and TP53 mutant (n = 351) human breast tumours of the TCGA dataset. c, Silencing of Fzd7 and Fzd9 in BMDMs after transfection with siRNA pools against both receptors, as determined by RT–qPCR (n = 6 biological replicates per group). d, Expression of Il1b in BMDMs after exposure to conditioned medium of Trp53+/+ and Trp53−/− Wap-cre;Cdht1F/F;Akt1E17K cell lines (n = 6 biological replicates per group), as determined by RT–qPCR. Where indicated, BMDMs were transfected with control siRNA or Fzd7 and Fzd9 siRNA pools. Data in a, c, d are mean ± s.e.m. Box plots are as described in Fig. 3e. P values were determined by two-tailed one-way ANOVA with FDR multiple-testing correction (a), two-tailed Mann–Whitney U-test (b) or two-tailed one-way ANOVA with Tukey multiple-testing correction (d).
Extended Data Fig. 9 | See next page for caption.
Pharmacological and genetic targeting of PORCN in p53-deficient tumours reduces systemic inflammation.

a, Total and cKIT+ neutrophil frequencies in lungs of vehicle-treated (n = 7) or LGK974-treated (n = 4) K14-cre;Cdh1F/F;Trp53F/F (KEP) mice using indicated 5-day short-term treatment schedule. Representative flow cytometry plots are shown. b, Frequency of IL-17A-producing γδ T cells in lungs of vehicle-treated (n = 6) or LGK974-treated (n = 4) KEP mice. Representative flow cytometry plots are shown. c, Kinetics of circulating neutrophils in vehicle- or LGK974-treated KEP mice using indicated long-term treatment schedule, shown as frequency at indicated tumour volumes (n = 8 per group). d, RT–qPCR analysis of Porcn expression in end-stage bulk tumour (n = 5 per group). Data are normalized to control shRNA (shControl) and represents an average of two technical replicates. e, Correlation of total neutrophil levels in the circulation with the expression of Porcn in WEA;Trp53−/−;shControl and WEA;Trp53−/−;shPorcn whole tumour lysate (n = 5 per group). f, Correlation of cKIT+ neutrophil levels in circulation with expression of Porcn in WEA;Trp53−/−;shControl and WEA;Trp53−/−;shPorcn whole tumour lysate (n = 5 per group). g, Correlation of Porcn expression and Il1b expression in bulk WEA;Trp53−/−;shControl (blue) and WEA;Trp53−/−;shPorcn tumours (grey) (n = 5 per group). Data represent an average of two technical replicates. h, Spleen area in mice with WEA;Trp53−/−;shControl (blue) and WEA;Trp53−/−;shPorcn tumours (grey) tumours at end stage (n = 5 per group). i, Growth kinetics of orthotopically transplanted KEP mammary tumours, treated with vehicle (n = 12) or LGK974 (n = 15). Each line represents an individual mouse. j, Growth kinetics of orthotopically injected Trp53+/− and Trp53−/− WEP cells, treated with vehicle or LGK974. Each line represents an individual mouse (n = 9 per group). k, Schematic representation of the findings of this study: loss of p53 in breast cancer cells triggers secretion of WNT ligands to activate tumour-associated macrophages. This stimulates systemic expansion and activation of neutrophils, which we have previously shown to be immunosuppressive, thus driving metastasis. All data are mean ± s.e.m. P values are determined by two-tailed Mann–Whitney U-test (a–d, h), linear regression analysis (e–g) and area under the curve of average growth curves, followed by two-tailed Welch’s t-test (i, j).
Reporting Summary

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- Clearly defined error bars
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Software and code

- Data collection:
  - Flow cytometry data: data acquisition was performed on BD LSRII flow cytometer using Diva software (BD Biosciences) or Beckman Coulter CyAn ADP flow cytometer with Summit software.
  - For RNAseq of murine tumours: sequenced using on the Illumina Hiseq2000/Hiseq2500 Machine.
  - For IHC: for individual images, cellSens Entry software (Olympus). For digital processing, slides were scanned using the Aperio ScanScope (Aperio, Vista, CA).
  - Cell growth kinetics in vitro were analysed using the IncuCyte System (Essen BioScience).
  - For RT-qPCR: LightCycler 480 thermocycler (Roche).
  - For western blot: Odyssey CLx imaging system.
Data analysis

Statistical analyses were performed using GraphPad Prism 7/8 (GraphPad Software Inc., La Jolla, CA).
Flow cytometry data analysis was performed using FlowJo software version 9.9.
For images: ImageJ software 1.48v
For the mouse RNA-Seq/human TCGA analysis: R software version 3.4.2, Tophat2 (Tophat version 2.1.0 / Bowtie version 1.0.0), edgeR R package version 3.20.5, biomart R package, NMF R-package version 0.20.6, Ingenuity Pathway analysis version 01-06 (QIAGEN), flexsear-R package and R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl/) were used.
Principal component analysis was performed using the prcomp-function in R (version 3.4.2)
For ChIP-sequencing: Easeq software was used for visualisation.
For power calculations and group size determination: G*Power software (version 3.1).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA sequencing datasets have been deposited in the Gene Expression Omnibus (GEO, NCBI) repository under accession number GSE112665.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample size was based on previous experience with the mouse models (Coffelt et al., 2015; Doornebal et al., 2013) or otherwise determined using G*Power software version 3.1.
For immunohistochemistry, flow cytometry and RT-qPCR experiments, ≥ 3 biological replicates were used.
For the intervention experiment assessing metastasis, ≥ 9 mice were used.
To exclude bias towards one particular GEMM in the analyses for Figure 1, we have performed the same analyses on the average of the neutrophil levels and serum cytokine values per model. This demonstrated the same correlations between the assessed values and p53 status of the tumour, thus excluding bias towards one or several particular models.

Data exclusions
Mice with skin tumours (in the models in which Keratin-14 (K14) drives expression of Cre recombinase, skin tumours can arise, since K14 is also expressed in the skin) were excluded from the study, based on assessment of H&E stainings of the tumour.
For orthotopic transplantation of cancer cell lines, samples were excluded when not properly transplanted in the mammary fat pad (e.g. in the skin adjacent to the mammary gland).
For metastasis scoring in the KEP-tumour based metastasis model, mice that died because of reasons not related to metastasis were excluded (surgery-related or local recurrance of the primary tumour after surgery). In the WEP cell line-based metastasis model, samples were excluded when smaller tumours, due to their invasive behaviour, grew through the skin before the end of the experiment (1200 - 1500 mm^3 tumour size), to allow all tumours sufficient time to metastasize.
For RT-PCR analysis, samples were run in technical duplicate and if the difference between the Ct values of the duplo was bigger than 1 cycle, samples were discarded.
Exclusion criteria were pre-determined before the experiments.

Replication
In vitro experiments were repeated in at least 2 - 3 independent experiments and showed comparable results between experiments.

Randomization
For intervention studies, mice were randomly distributed over the two treatment arms when tumours reached the size indicated in the figures. The first animal was assigned randomly in the treatment or control group, after which each subsequent animal was placed in the next group.

Blinding
Tumour measurements and post mortem analyses were performed in a blinded fashion. Assessment of IHC counts were performed by 2 or more independent researchers in a blinded fashion.
Materials & experimental systems

| n/a | Involved in the study |
|-----|------------------------|
|     | Unique biological materials |
|     | Antibodies |
|     | Eukaryotic cell lines |
|     | Palaeontology |
|     | Animals and other organisms |
|     | Human research participants |

Methods

| n/a | Involved in the study |
|-----|------------------------|
|     | ChIP-seq |
|     | Flow cytometry |
|     | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

Biological samples derived from the mouse model are available upon reasonable request and after signing of a standard MTA.

Antibodies

Antibodies used

For a list of used antibodies, also see Supplemental Table 1.

- Flow cytometry:
  - CD45 eFluor 605NC : eBioscience/ThermoFisher 93-0451 1:100
  - CD45 BUV395 30-F11 BD Bioscienes 564279 1:200
  - CD11b BV650 M1/70 BioLegend 101239 1:400
  - CD11b APC M1/70 eBioscience/ThermoFisher 17-0112-81 1:400
  - CD11b APC-eFluor 780 M1/70 eBioscience/ThermoFisher 47-0112-82 1:400
  - Ly6G APC IA8 eBioscience/ThermoFisher 17-9668-82 1:400
  - Ly6G FITC IA8 BD Bioscienes 561105 1:400
  - Ly6C eFluor 450 HK1.4 eBioscience/ThermoFisher 48-5932-82 1:400
  - F4/80 APC-eFluor 780 MB8 eBioscience/ThermoFisher 47-4801-82 1:400
  - F4/80 APC BM8 eBioscience/ThermoFisher 17-4801-82 1:400
  - CD4 APC-eFluor 780 GK1.5 eBioscience/ThermoFisher 47-0041-82 1:200
  - CD8 APC-eFluor 780 53-6.7 eBioscience/ThermoFisher 47-0081-82 1:400
  - Gamma delta-TCR FITC GL3 BD Biosciences 563177 1:400
  - CD19 APC-eFluor 780 eBio1D3 eBioscience/ThermoFisher 47-0193-82 1:200
  - CD34 eFluor 450 RAM34 eBioscience/ThermoFisher 48-0341-82 1:100
  - CD16/32 PerCP-eFluor 710 93 eBioscience/ThermoFisher 46-0161-82 1:200
  - Sca-1 PE D7 eBioscience/ThermoFisher 12-5981-82 1:100
  - CD45R APC-eFluor 780 RA3-6B2 eBioscience/ThermoFisher 47-0981-82 1:100
  - p53 1C12 Cell Signalling 2524 1:1000
  - p21 5X118 BD Bioscienes 564341 1:1000
  - Wnt7a Polyclonal Novus Biologicals NBP2-20913 1:1000
  - Porcupine Polyclonal Novus Biologicals NBP1-79322 1:1000
  - Non-phospho-β-catenin - 8E7 EMD Millipore 05-665 1:1000
  - β-actin D6A6 Cell Signalling 8457 1:5000

- Western blotting:
  - CD45 eFluor 605NC : eBioscience/ThermoFisher 93-0451 1:100
  - CD44 BUV737 : eBioscience/ThermoFisher 47-0472-82 1:100
  - CD44 BUV737 : eBioscience/ThermoFisher 47-0472-82 1:100
  - p53 1C12 Cell Signalling 2524 1:1000
  - Wnt7a Polyclonal Novus Biologicals NBP2-20913 1:1000
  - β-actin D6A6 Cell Signalling 8457 1:5000
Validation

Antibodies for flow cytometry were validated for target species (mouse or human) using FMO or isotype controls where necessary. Antibodies used for IHC were validated for mouse by the Pathology facilities at the NKI.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Mouse cell lines were generated in house. MCF-7 and HEK293T cells were provided by the Schumacher lab (Netherlands Cancer Institute).

Authentication

Mouse tumour-derived cell lines were checked for purity using genotyping. MCF-7 cells were not authenticated, but the TP53-WT and TP53-KO cells were made from the same parental line.

Mycoplasma contamination

Cells were routinely tested for mycoplasma contamination and only negative cells were used.

Commonly misidentified lines

No commonly misidentified lines were used in this study.

Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The following mouse models were used in this study: Keratin14 [K14]-cre;CdhlF/F;Trp53F/F, K14cre;Trp53F/F, K14cre;Brca1F/F;Trp53F/F, Whey Acidic Protein (Wap)-cre;Trp53F/F, Wap-cre;Brca1F/F;Trp53F/F, Wap-cre;Brca1F/F;Trp53F/F;Col1a1invCAG-Met-IRE6-Luc+/ (Wap-cre;Brca1F/F;Trp53F/F;Met), Wap-cre;Brca1F/F;Trp53F/F;Col1a1invCAG-Myc-IRE6-Luc+/ (Wap-cre;Brca1F/F;My6), Wap-cre;Brca1F/F;Trp53F/F;Col1a1invCAG-Mycb2-IRE6-Luc+/ (Wap-cre;Brca1F/F;Myb2), Wap-cre;Trp53F/F;Col1a1invCAG-ESR1-IRE6-Luc+/ (Wap-cre;Trp53F/F;HA-ER1), Wap-cre;CdhlF/F;Col1a1invCAG-Akt67k-IRE6-Luc+/ (Wap-cre;CdhlF/F;Akt67k), Wap-cre;CdhlF/F;Col1a1invCAG-Pik3ca645K-IRE6-Luc+/ (Wap-cre;CdhlF/F;Pik3ca645K), Wap-cre;CdhlF/F;Col1a1invCAG-Fgf21x15-IRE6-Luc+/ (Wap-cre;CdhlF/F;Fgf21x15), Wap-cre;CdhlF/F;Col1a1invCAG-Fgf21x15-IRE6-Luc+/ (Wap-cre;CdhlF/F;Fgf21x15), Wap-cre;CdhlF/F;T2/Onc;Rosa26Lox65Blox71+/ (Wap-cre;CdhlF/F;SB), Wap-cre;Map3k1F/F;PtenF/F, Mouse mammary tumour virus LTR (MMTV)-NeuT40. All mouse models were on FVB/N background, except MMTV-NeuT and Wap-cre;CdhlF/F;SB, which were on Balb/c and a mixed genetic (C57BL/6J and FVB/N) background, respectively. Mice developed tumours between 3 and 18 months of age. For tumour inoculation experiments, FVB/N mice were used of 8 - 10 weeks of age. All used mice were female. All materials were derived from one or more of the above models.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field-collected samples.

ChIP-seq

Data deposition

☑️ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

Files in database submission

See above.

Genome browser session

[Genome browser session](https://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=sprekovic&hgS_otherUserSessionName=p53%20mm10)
Methodology

Replicates
Two cell lines, derived from spontaneous mouse tumours, Wap-cre;Cdh1F/F;Akt-E17K and Wap-cre;Cdh1F/F;Pik3ca-E545K, were used. Of each of these lines, three cell lines from three individual mouse tumours were used as biological replicates.

Sequencing depth
Number of reads per sample were approximately between 1.0x10^7 - 2.0x10^7. Details of sequencing metric (total number of reads and number of unmapped reads) per sample are available upon request. Single-end, 65-bp reads were used.

Antibodies
p53 antibody (clone 1C12, #2524, Lot 13, Cell Signalling) was used for ChIP experiments.

Peak calling parameters
Peak calling over input control was performed using and MACS 2.0 peak caller (callpeak -t P1.bam -c InputP1.bam -f BAM -g mm -n P1tumor -B -q 0.01).

Data quality
Quality control measurements including count in peaks for all the samples, plots indicating all the called peaks and their respective fold enrichments and q-values, Venn diagrams showing the overlap of peaks are available upon request.

Software
Data was visualized using Easeq software.

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Flow cytometry analysis was performed as previously described6. Briefly, tissues were collected in ice-cold PBS and blood was collected in tubes containing heparin. Tumours and lungs were mechanically chopped using a McIlwain tissue chopper (Mickle Laboratory Engineering). Tumours were digested for 1 hour (h) at 37°C in 3 mg/mL collagenase type A (Roche) and 25 μg/mL DNase (Sigma) in serum-free DMEM medium. Lungs were digested for 30 minutes (min) at 37°C in 100 μg/mL Liberase TM (Roche). Enzyme reactions were stopped by addition of cold DMEM/8% Fetal Calf Serum (FCS) and suspensions were dispersed through a 70 μm cell strainer. Bone marrow was collected from the tibia and femurs of both hind legs and flushed using RPMI/8% FCS through a 70 μm cell strainer. Single-cell suspensions were treated with NH4Cl erythrocyte lysis buffer. Before staining, cell suspensions were subjected to Fc receptor blocking (rat anti-mouse CD16/32, BD Biosciences) for 15 min at 4°C, except for bone marrow (to allow assessment of CD16/32 expression). Cells were stained with conjugated antibodies for 30 min at 4°C in the dark in PBS/0.5% BSA. In the dark, 7AAD (1:20; eBioscience) or Fixable Viability Dye eFluor 780 (1:1000; eBioscience) was added to exclude dead cells. For intracellular cytokine staining, single-cell suspensions were treated in IMDM containing 8% FCS, 100 IU/mL penicillin, 100 mg/mL streptomycin, 0.5% β-mercaptoethanol, 50 ng/ml PMA, 1 nM ionomycin and Golgi-Plug (1:1,000; BD Biosciences) for 3h at 37°C. Surface antigens were stained first, followed by fixation and permeabilization using the Cytofix/Cytoperm kit (BD Biosciences) and staining of intracellular proteins.

Instrument
All experiments were performed using a BD LSR II flow cytometer using Diva software or the Beckman Coulter CyAn ADP flow cytometer using Summit software.

Software
The software used to collect data was Diva software (BD Biosciences) and data analysis was performed using FlowJo software version 9.9.

Cell population abundance
No sorting of cells was performed.

Gating strategy
The morphologic gate (FSC/SSC) was used to included all cells and excluding debris. Doublets (using FSC-H/FSC-A and SSC-H/SSC-A) and dead cells were excluded. Immune cells were then gated based on their specific markers, indicated in relevant figures. Gating strategies are shown in Extended Data Fig. 2 and 3.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.