Mutations or amplification of the MET proto-oncogene are involved in the pathogenesis of several tumours\(^1\)–\(^4\), which rely on the constitutive engagement of this pathway for their growth and survival\(^1\)–\(^9\). However, \textit{MET} is expressed not only by cancer cells but also by tumour-associated stromal cells, although its precise role in this compartment is not well characterized\(^1\)–\(^11\). Here we show that \textit{MET} is induced by neutrophils during chronic bowel inflammation (preceding CRC formation), neutrophils, suggests a potential 'Achilles' heel' of \textit{MET}-targeted therapies, in cancer and supports the rationale for evaluating anti-MET drugs in certain inflammatory diseases.

To ensure \textit{Met} deletion in the immune system only, we took advantage of the Tie2:Cre deleter that excises floxed genes in both bone marrow and endothelial cells\(^1\), and we reconstituted lethally irradiated C57BL/6 wild-type (WT) mice with bone marrow cells from Tie2:\textit{Met}fl/fl (WT) or Tie2:\textit{Met}fl/fl\(^6\) (knockout (KO)) mice (Extended Data Fig. 2a, 2b). Thus, \textit{Met} deletion in immune cells favours cancer growth and metastasis. After systemic administration of a MET kinase inhibitor, we prove that the therapeutic benefit of \textit{MET} targeting in cancer cells is partly countered by the pro-tumoural effect arising from \textit{MET} blockade in neutrophils. Our work identifies an unprecedented role of \textit{MET} in neutrophils, suggests a potential 'Achilles' heel' of \textit{MET}-targeted therapies in cancer, and supports the rationale for evaluating anti-MET drugs in certain inflammatory diseases.

To extend our findings to other tumour types, we proved that \textit{Met} deletion in the haematopoietic system increased the growth of (1) orthotopic T241 fibrosarcomas and B16F10 melanomas, (2) spontaneous mammary tumours in MMTV-PyMT\(^{12}\) transgenic mice, (3) H-Ras(G12V)- and c-Myc-driven hepatocellular carcinomas (HCCs), and (4) chemically induced colorectal cancers (CRCs) (Fig. 1a–j and Extended Data Fig. 3a, b). Furthermore, lung colonization of B16F10 melanoma cells (from either the primary tumour or after cancer cell intravenous injection) and of MMTV-PyMT\(^{12}\) breast tumours was boosted in \textit{Met} KO chimaeras (Fig. 2k, l and Extended Data Fig. 4c, d). In sum, \textit{Met} deficiency in neutrophils promotes the progression of different (HGF-secreting) tumours.

Systemic treatment of WT mice carrying B16F10 melanomas (which are dependent on MET\(^{10}\)) with three different MET tyrosine-kinase inhibitors, including Metoestatin, abates tumour growth and metastasis. After systemic treatment of WT mice carrying B16F10 melanomas (which are dependent on MET\(^{10}\)) with three different MET tyrosine-kinase inhibitors, including Metoestatin, abates tumour growth and metastasis. After systemic treatment of WT mice carrying B16F10 melanomas (which are dependent on MET\(^{10}\)) with three different MET tyrosine-kinase inhibitors, including Metoestatin, abates tumour growth and metastasis. After systemic treatment of WT mice carrying B16F10 melanomas (which are dependent on MET\(^{10}\)) with three different MET tyrosine-kinase inhibitors, including Metoestatin, abates tumour growth and metastasis.
inhibitors (PF-04217903, INCB28060 and JNI-38877605), strongly reduced TAN recruitment (Extended Data Fig. 5q). We then compared MET silencing in cancer cells versus systemic MET inhibition. Systemic administration of PF-04217903 decreased the weight and volume of B16F10 melanomas by 36% and 54%, respectively. Instead, approach15–17, we found that TNF-α and lipopolysaccharide (LPS) (but not IL-1β, HGF, or hypoxia) induced MET expression in pre-activated endothelium (Fig. 3i–k). TNF-α-mediated MET induction required TNFR1 and subsequent nuclear factor (NF)-κB activation (Fig. 3l–m). The TNF-α protein was not able to trigger either MET phosphorylation or HGF release in neutrophils (Extended Data Fig. 6b–e).

Silencing of endothelial-cell-borne TNF-α (which is 250-fold increased upon stimulation with IL-1β; Extended Data Fig. 6i), knock-out of neutrophil-borne TNFR1 (but not of TNFR2), and pharmacological blockade of TNF-α with the TNF-α-trap Enbrel, prevented MET induction in mouse or human neutrophils upon co-culture with activated endothelial cells or exposure to TCM/CCM (Fig. 3l and Extended Data Fig. 6j–m). Finally, systemic administration of Enbrel in LLC-tumour-bearing mice diminished MET expression in neutrophils as well, resulting in their reduced recruitment to the tumour (Fig. 3n, i). Although MET is scarcely expressed in naive neutrophils, it is strongly induced by inflammatory stimuli.

Mechanistically, impaired TAN accumulation after Met inactivation was not due to cell death as assessed in LLC tumours and in culture, both at baseline and under LPS stimulation, with or without HGF (Extended Data Fig. 7a–c), but rather to a defect in neutrophil recruitment from the blood. Indeed, also in the case of acute inflammation, Met KO neutrophils displayed reduced exudation to the skin or to the peritoneal cavity (Fig. 4a–d and Extended Data Fig. 8a, b). Macrophage and lymphocyte recruitment did not change (Fig. 4d and Extended Data Fig. 8c, d). Vice versa, recombinant HGF recruited WT neutrophils inside subcutaneous air pouches with similar efficacy to the neutrophil chemoattractant CXCL1 (Fig. 4e and Extended Data Fig. 8e). In contrast, Met KO neutrophils did not migrate towards HGF, although their response to CXCL1 was preserved (Fig. 4e and Extended Data Fig. 8e). Mirroring this approach, an anti-HGF blocking antibody18 prevented neutrophil infiltration to tumours and inflamed skin (Fig. 4f).

We then tested the relevance of MET for neutrophil migration. Stimulation of WT neutrophils with HGF promoted their adhesion and chemotaxis through an activated endothelium whereas Met KO
neutrophils (displaying an 85% reduction in MET protein levels compared to WT; Extended Data Fig. 2a) completely lost this response (Fig. 4g, h and Extended Data Fig. 8f). In line with this, TCM (containing 2.6 ± 0.3 ng ml⁻¹ HGF) promoted transendothelial migration of WT neutrophils, but its effect was 43% lower on Met KO neutrophils (Fig. 4i). Upon HGF neutralization, WT neutrophils responded to TCM only partially, as did Met KO neutrophils (Fig. 4i). Neither HGF nor TCM influenced neutrophil behaviour on non-activated endothelial cells or bare membranes (Extended Data Fig. 8f–i). Hence, HGF-mediated MET activation is required for neutrophil transendothelial migration to the inflammatory site.

Once migrated inside the tumour, N1 or N2 neutrophils can inhibit or favour tumour progression, respectively³⁸. Among the N1 and N2 genes, only the expression of the N1 marker inducible nitric oxide synthase (Nos2, also known as iNos) was lower in Met KO versus WT TANs but similar in macrophages (Fig. 4j and Extended Data Fig. 8j). Compared to WT→WT mice, tumours harvested from KO→WT mice displayed reduced nitric oxide (NO) production and 3-nitrotyrosine (3NT) formation, a sign of NO-mediated cell damage (Fig. 4k–n). In vitro, Met KO TANs had lower cancer-cell-killing capacity than WT TANs; iNOS inhibition by N⁴-monomethyl-L-arginine (L-NMMA) blunted this difference (Fig. 4o and Extended Data Fig. 8j).
**Figure 3** Met expression in neutrophils is induced by tumour-derived soluble factors. a, b, Quantitative polymerase chain reaction with reverse transcription (qRT–PCR) (a) and fluorescence-activated cell sorting (FACS) analysis for MET in blood neutrophils from tumour (TM)-free or LLC-tumour-bearing mice and in TANs. c, qRT–PCR for MET in human neutrophils from lung cancer versus healthy tissue. n = 4 patients. d, e, MET expression by qRT–PCR (d) and western blot (e) in circulating neutrophils from tumour-free mice after co-culture with unstimulated (HUVEC/NS) or IL-1α-pre-stimulated (HUVEC/IL) human umbilical vein endothelial cells (HUVECs), or after stimulation with TCM or CCM. f, g, qRT–PCR (f) or western blot (g) for MET in circulating human neutrophils after stimulation with A549-CCM. h, Western blot for MET in mouse and human neutrophils after LPS or TNF-α stimulation. i, qRT–PCR for Met in WT, TNFR1 KO or TNFR2 KO mouse neutrophils after TNF-α stimulation. j, k, qRT–PCR (j) and western blot (k) for MET in mouse neutrophils after TNF-2 stimulation with or without NF-κB inhibitor. l, qRT–PCR for Met in mouse neutrophils co-cultured with HUVEC/IL or stimulated with TCM in the presence or absence of Enbrel. m, n, FACS for MET in TANs (m) and immunohistochemistry for Ly6G (n) in LLC tumours after Enbrel. Data combine two independent experiments; total mice, 5 per condition. All data in a, b, d, f, i, l are representative of two independent experiments using four biological replicates per condition per experiment. All western blots were repeated three times on independent biological replicates. Full western blot images are shown in Supplementary Fig. 1. Loading control in e, h displays tubulin or actin according to Supplementary Fig. 1. *P < 0.05; †P < 0.05 versus mock (i, l) or versus untreated (j). Graphs show mean ± s.e.m.

**Figure 4** MET is required for neutrophil transendothelial migration and cytotoxicity. a–c, Neutrophil quantification (a) and 12-Otetradecanoylphorbol-13-acetate (TPA)-painted ear skin (c, d). Data combine two independent experiments; total mice: 10 per condition for vehicle; 14 per condition for TPA. d, FACS analysis for Ly6G+ neutrophils or F4/80+ macrophages on peritoneal lavages after zymosan-induced peritonitis. Data are representative of two independent experiments using 4 mice per condition per experiment. e, FACS analysis for neutrophil recruitment towards HGF or CXC1L1 in air pouch assays. Data combine three independent experiments; total mice: 10 per condition. f, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. g, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. h, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. i, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. j, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. k, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. l, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. m, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. n, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. o, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. p, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. q, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. r, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. s, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. t, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. u, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. v, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. w, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. x, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. y, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. z, Ly6G infiltration in LLC tumours or in TPA-painted ear skin after anti-HGF. Data combine two independent experiments; total mice: 10 per condition. **Graphs show mean ± s.e.m.**
HGF-stimulated WT but not Met KO neutrophils displayed enhanced NO release and cytotoxicity, which was ablated by L-NMMA (Extended Data Fig. 8k, l).

We then hypothesized that HGF/MET pathway is key for anti-tumoural neutrophils only. Neutrophil depletion in WT→WT chimerae did not affect LLC tumour growth, implying that in this tumour model anti-tumoural and pro-tumoural neutrophils are in balance (Fig. 4p–r). The same treatment in KO micea did not affect LLC tumour growth, implying that in this tumoural neutrophils only. Neutrophil depletion in WT (Extended Data Fig. 8k, l).

NO release and cytotoxicity, which was abated by L-NMMA.

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Supplementary Information is available in the online version of the paper.

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Author Contributions V.F. performed experimental design, all experiments, data acquisition and interpretation. G.D.C. performed in vitro assays and measured tumour experiments. M.D.M. performed ELISA assays, and designed and performed cloning strategies. J.S. performed all the bone marrow transplantations and in vivo tumour experiments. A.R.T. and S.W. performed neutrophil isolations and peritonitis assays. Z.G. provided the Mrp8 promoter. S.C. performed tumour experiments and skin rash assays in vivo. E.W. provided clinical samples. H.P. provided data interpretation on histological stainings and FACS, data acquisition and interpretation. M.M. performed experimental design, data analysis, conducted scientific direction and wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.M. (massimiliano.mazzone@vib-kuleuven.be) or A.C. (andrea.casazza@vib-kuleuven.be).
METHODS

**Animals.** The Met-floxed mice, a gift from S. S. Thorgeirsson, were backcrossed in a C57BL/6 background. The Tie2-Cre and MMTV-PyMT transgenic lines were obtained from our mouse facility. The Mrp8:Cre mice were a gift from C. A. Lowell for purity by FACS according to the manufacturer’s protocol. 1

**Cell lines.** Murine Lewis lung carcinoma cells (LLC), melanoma B16F10, and human non-small-cell lung carcinoma A549 cells were obtained from the American Type Culture Collection (ATCC); the murine pancreatic tumour cell line Panc02 and the murine fibrosarcoma cell line T241 were gifts from U. Cavallaro and L. Claesson-Welsh, respectively. LLC, B16F10, A549 and T241 cells were cultured in DMEM (Gibco) supplemented with 2 mM glutamine, 100 units ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and containing 10% FBS (DMEM 10% FBS). Panc02 cells were cultured in RPMI (Gibco) supplemented with 2 mM glutamine, 100 units ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and containing 10% FBS. Panc02 cell cultures were verified using LookOut Mycoplasma PCR Kit (Sigma) and MycoAlert Mycoplasma Detection Kit plus Assay Control (Lonza). Panc02 and T241 cells were both authenticated by Idexx Bioresearch. All cells were passaged in the laboratory for no longer than 6 months after receipt. Supplementary Table 1 lists the sequences of all the shRNA constructs used in this study.

**Bone marrow transplantation and blood cell counts.** Recipient 6-week-old female mice were lethally irradiated (9.5 Gy) and then intravenously injected with 10⁶ bone marrow cells from WT or KO mice 16 h later. Experiments were initiated 5 weeks after bone marrow reconstitution. Blood cell count was determined using a haemocytometer on peripheral blood collected by retro-orbital bleeding.

**Haematopoietic stem/progenitor cell transduction.** For MET overexpression or reconstitution in WT or Met KO neutrophils, respectively, lineage-negative haematopoietic stem/progenitor cells (HSPCs) were enriched with the mouse haematopoietic progenitor enrichment kit (Stem Cell Technologies) and checked for purity by FACS according to the manufacturer’s protocol. 1 × 10⁶ cells ml⁻¹ were pre-stimulated for 5 h with stem span serum-free medium (Stem Cell Technologies) supplemented with IL-3 (20 ng ml⁻¹), SCF (100 ng ml⁻¹), TPO (100 ng ml⁻¹) and FLT3-L (100 ng ml⁻¹) (Promega), and transduced with 1 × 10⁸ transducing units (TU) ml⁻¹ of a lentiviral vector expressing mouse Met under the promoter of the human gene S100A8 (Mrp8:Met), which has been engineered for neutrophil-specific transcriptional targeting, or an empty vector (Mrp8:Empty) as control. Briefly, the promoter driving Met expression in neutrophils only corresponds to a 3.6 kb DNA fragment encompassing the natural 5’ and 3’ regulatory regions but deleted of its exon coding sequences. Hence, Met is under the control of the 5’ and 3’ untranslated regions of the human S100A8 gene and contains c-Myc and c-Fos enhancer cis-regulatory sequences present in the surrogate DNA fragment. Ten hours after the first viral transduction, cells received a second round of their respective lentivector, 7 h later 1 × 10⁶ cells were injected via tail vein in lethally irradiated C57BL/6 recipient mice. A fraction of transduced HSPCs were cultured and collected after 9 days to measure the number of integrated vector copies per cell genome (vector copy number (VCN)) by qPCR using custom TaqMan assays specific for HIV gag sequences (Applied Biosystems), as previously described.

**Human colon cancer models.** Human colon cancer models include a chemically induced colorectal cancer model, body-weight-matched mice received one intraperitoneal injection of 10 mg kg⁻¹ of azoxymethane (AOM) followed by 3 cycles of 7 days of 1.5% (cycle I) or 1.7% (cycle II–III) dextran sodium sulphate (DSS) in drinking water, starting from the day of AOM injection. After 160 days, the colon was collected and prepared for histological evaluation with the ‘Swiss roll’ technique. For the oncogene-driven hepaticcellular carcinoma model, mice received a 1:1 molar ratio (3 µg total DNA) of piggyBac transposons encoding c-Myc and H-Ras oncogenes, driven by the PGK promoter, together with the hyperactive piggyBac transposase-encoding plasmid. DNA solutions containing transposons/transposase plasmids were diluted in 2 ml of Ringer’s solution and hydrodynamically delivered in 7 s through the tail vein. Mice were killed 24 weeks after the hydrodynamic injection.

**Lung colonization assay.** In the experimental metastasis assays, 0.5 × 10⁶ B16F10 cells were injected in the tail vein and lungs were collected after 12 days. To quantify pulmonary seeding, lungs were homogenized in Trizol (Ambion) and RNA was purified with the RNeasy Mini kit (Ambion) according to manufacturer’s instructions. The expression of the melanocyte-specific gene Slc02B was measured as a readout of lung colonization by qRT–PCR following reverse transcription to complementary DNA with the QuantiTect Reverse Transcription kit (Qiagen).

**Mice treatments.** To induce chronic colitis, mice received 3 cycles of 7 days of 1.5% (cycle I) or 1.7% (cycle II–III) DSS in drinking water; 2 weeks after the last DSS cycle, the colon was collected and prepared for histological evaluation as described earlier. For in vivo MET inhibition, B16F10 tumour-bearing mice received 40 mg kg⁻¹ PF-04217903 (AbMole Bioscience) or the corresponding vehicle (0.5% methylcellulose in saline) via oral gavage every day once a day starting from day 2 after tumour injection and twice a day from day 11 until the end of the experiment; alternatively mice were treated with 50 mg kg⁻¹ Enbrel (AbMole Bioscience) or 20 mg kg⁻¹ IN-38877605 (Selleckchem).

**Tumour models.** 2 × 10⁶ LLC or 1 × 10⁶ B16F10 cells were injected subcutaneously while 2 × 10⁶ T241 cells were injected intradermally in a volume of 200 µl PBS. Tumour volumes were measured three times a week with a calliper. At end stage, tumours were weighed and collected for histological examination or FACS analysis. MMTV-PyMT spontaneous breast tumours were measured 10 weeks after birth (6 weeks after bone marrow transplantation), three times a week, and mice were killed at week 16. Lung metastases were contrasted by intratracheal injection of a 15% India ink solution, by H&E staining on lung paraffin sections, or detected by qRT–PCR for the melanoma-specific gene S100B in the models involving B16F10 cells. For orthotopic pancreatic tumour growth, mice were anaesthetized with isoflurane, the stomach exteriorized via abdominal midline incision, and 1 × 10⁶ Panc02 tumour cells in 30 µl PBS were injected into the head of the pancreas using a 29-gauge needle. A successful intrapancreatic injection of tumour cells was identified by the appearance of a fluid bleb without intraperitoneal leakage. Mice displaying peritoneal leakage were immediately killed and excluded from the analysis. At day 12, primary tumours were removed and weighed. Enlarged lymph nodes were counted under a stereoscopic microscope. For the chemically induced colorectal cancer model, body-weight-matched mice received one intraperitoneal injection of 10 mg kg⁻¹ of azoxymethane (AOM) followed by 3 cycles of 7 days of 1.5% (cycle I) or 1.7% (cycle II–III) dextran sodium sulphate (DSS) in drinking water, starting from the day of AOM injection. After 160 days, the colon was collected and prepared for histological evaluation with the ‘Swiss roll’ technique. For the oncogene-driven hepatocellular carcinoma model, mice received a 1:1 molar ratio (3 µg total DNA) of piggyBac transposons encoding c-Myc and H-Ras oncogenes, driven by the PGK promoter, together with the hyperactive piggyBac transposase-encoding plasmid. DNA solutions containing transposons/transposase plasmids were diluted in 2 ml of Ringer’s solution and hydrodynamically delivered in 7 s through the tail vein. Mice were killed 24 weeks after the hydrodynamic injection.
Air pouch assay. To create subcutaneous air pouches, bone marrow transplanted chimaeric mice or Mpr8p;Mep8p\textsuperscript{G230E} or Mpr8p;Mep8p\textsuperscript{K563E} mice were injected with 3 ml of sterile air by dorsal subcutaneous injection with a butterfly 23 G needle on day 0 and on day 3. On day 6, 200 ng per mouse of murine CXCL1 or HGF dissolved in 0.5 ml PBS-Heparin (15 U ml\textsuperscript{-1}) or PBS-Heparin (15 U ml\textsuperscript{-1}) as control, were injected in the newly formed dorsal camera. After 4 h, inflammatory cells were harvested by washing the pouch with 5 ml of PBS. Cells were stained for Ly6G (1A8), washed and resuspended in PBS 0.1% BSA with unlabelled counting beads and quantified for CFU-GM.

Mouse white blood cell isolation. Blood was collected from the retro-orbital vein in 10% heparin. For the isolation of white blood cells (WBCs), the blood was diluted in 1.25% dextran in saline to allow the sedimentation of red blood cells (RBCs). After 30 min, the erythrocyte-poor upper layer was collected and washed in PBS 0.1% BSA. The remaining RBCs were lysed in a hypotonic solution of 0.2% NaCl for 30 s and brought in isotonic condition with 1.6% NaCl and 0.1% glucose. WBCs were washed in PBS 0.1% BSA, counted and resuspended accordingly with the experimental setting.

Mouse blood neutrophil isolation. Blood was collected from the retro-orbital vein in 10% heparin and diluted in an equal volume of PBS 0.5% BSA. Up to 5 ml of diluted blood was layered on top of a discontinuous gradient of Histopaque 1119 (4 ml) and Histopaque 1077 (5 ml) from Sigma. The gradient was centrifuged for 30 min at 700 g with the brake off. The neutrophil layer between the Histopaque 1077 and 1119 was collected and washed in PBS 0.5% BSA. RBC lysis was performed as described earlier. Neutrophils were washed in PBS 0.5% BSA, counted and resuspended according to the experimental condition. Alternatively, blood was sedimented in a saline solution containing 1.25% dextran and neutrophils were negatively selected with magnetic beads\textsuperscript{30}. Neutrophil purity, as assessed by the hemocytometer, was always higher than 93%.

Bone marrow neutrophil and mononuclear cell isolation. To reach reasonable amounts of prototols, all the described protocols for analyses in mice were performed on neutrophils isolated from bone marrows. Mice were killed by cervical dislocation. Femurs and tibias were collected in cold sterile Hank balanced salt solution (HBSS; Invitrogen) and flushed with HBSS 0.25% BSA. Cells were layered on top of a discontinuous gradient of Percoll 81%, 62%, 55%, freshly prepared and centrifuged for 30 min at 2000 g with the brake off. Monocytes were collected at the interface between the bone marrow cells and the layer of Percoll 55%, whereas neutrophils were collected at the interface between Percoll 55% and 62%. Cells were washed in HBSS 0.25% BSA and RBC lysis was performed as described earlier. Neutrophils (or monocytes) were washed again, counted and resuspended according to the experimental setting. Neutrophil (or monocyte) purity, as assessed using a haemocytometer, was higher than 87%.

FACS analysis and flow sorting of blood or tumour-associated cells. Blood was collected in 10% heparin, incubated for 15 min with Mouse BD Fc Block (2.4G2, BD Pharmingen) 1:100 and stained for 20 min at room temperature. After RBC lysis, cells were washed and resuspended in FACS buffer (PBS containing 2% FBS and 2mM EDTA). Tumours were minced in RPMI medium containing 0.1% collagenase type I and 0.2% dispase type I (Gibco) for 30 min at 37°C and passed through a 70 and 40 μm cell strainer. After RBC lysis, cells were resuspended in FACS buffer (PBS containing 2% FBS and 2mM EDTA) and counted. The myeloid population, enriched using CD11b-conjugated magnetic beads (MACS, Milteny Biotech) and separated through a magnetic column (MACS, Milteny Biotech), was stained with anti-CD66b (G10F5, BD Pharmingen, 1:100) for 20 min at 4°C and sorted with FACS Aria I (BD Bioscience). Cells were counted and resuspended in RLT buffer (Qiagen) for RNA extraction.

Endothelial cell isolation. Lungs were collected and a single-cell suspension was obtained as described earlier. Endothelial cells were obtained by performing a negative selection for CD45 (30F-11) and F4/80 (CI:A3-1) followed by a positive selection for CD31 (MEC 13.3) by using magnetic beads (Dynabeads, Invitrogen) according to the manufacturer’s protocol.

Peritoneal macrophages. Five milliliters of sterile PBS were injected in the peritoneum of anaesthetized mice and collected after 3 min. Cells were centrifuged, washed and cultured overnight.

TCM and LLC (or A549) CCM preparation. Two grams of end-stage LLC tumour explanted from WT mice were chopped and incubated at 37°C in 7 ml of DMEM (supplemented with 2 mM glutamine, 100 units ml\textsuperscript{-1} penicillin/100 μg ml\textsuperscript{-1} streptomycin) FBS-free (DMEM 0% FBS). 5 × 10\textsuperscript{5} LLC (or A549) were seeded in a 6-multwell plate in DMEM 10% FBS and incubated at 37°C. Medium alone (DMEM 0% FBS or DMEM 10% FBS, respectively) was used to prepare mock controls. After 72 h, the medium was filtered, supplemented with 2 mM glutamine and 20 mM HEPES and stored at −20°C. TCM and mock medium (DMEM 0% FBS) were diluted 1:5 in DMEM 10% FBS; CCM and mock medium (DMEM 10% FBS) were diluted 4:5 in DMEM FBS-free.

qRT–PCR. For mRNA analysis, 1 × 10\textsuperscript{6} or 3 × 10\textsuperscript{5} mouse or human blood neutrophils, respectively, were incubated in normoxic (21% oxygen) or hypoxic (1% oxygen) conditions, or stimulated with TCM (plus 50 μg ml\textsuperscript{-1} Embrel or human IgG where indicated), CCM, A549-CCM, 100 ng ml\textsuperscript{-1} of murine or human TNF-α, 50 ng ml\textsuperscript{-1} LPS, or mock medium in a 96-multwell plate for 4 h at 37°C. For NF-kB inhibition, 0.18 × 10\textsuperscript{6} neutrophils were pre-treated with 10 μM 6-amino-4-(4-phenoxyphenethylamino) quinazoline (Calbiochem) for 1 h at 37°C and stimulated with 100 ng ml\textsuperscript{-1} of murine TNF-α for 1 h at 37°C. 2 × 10\textsuperscript{5} HUVECs were seeded in a 24-multwell plate coated with 0.1% gelatin and stimulated with 5 ng ml\textsuperscript{-1} IL-1α in DMEM 10% FBS for 4 h at 37°C. Cells were washed in PBS, collected in RLT buffer (Qiagen) and kept at −80°C. RNA was extracted with the RNeasy Micro kit (Qiagen) according to the manufacturer’s instructions. Reverse transcription to cDNA was performed using the SuperScript III First Strand DNA Synthesis Kit (Life Technologies) according to the manufacturer’s protocol. Pre-made assays were purchased for Applied Biosystem, except for Nos2 that was provided by IDT, cDNA, primer/probe mix and TaqMan Fast Universal PCR Master Mix were prepared in a volume of 10 μl according to manufacturer’s instructions (Applied Biosystems). Samples were loaded into an optical 96-well Fast Thermal Cycling plate (Applied Biosystems) and qRT–PCR were performed using an ABI Prism 7500 Fast Real-Time PCR System (Applied Biosystems).

ELISA. To quantify plasma, intra-tumoural, tumour-released (TCM) and neutrophil-released HGF, a murine HGF ELISA kit (R&D) was used according to manufacturer’s protocol. Blood was collected from tumour-free or tumour-bearing mice and plasma was prepared according to manufacturer’s instruction. TCM was prepared as described earlier. Tumour proteins were extracted in Extraction Buffer (20 M Tris HCl, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 5 mM EDTA). 0.2 × 10\textsuperscript{6} neutrophils were cultured for 20 h in DMEM complete in

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presence or absence of 100 ng ml⁻¹ of murine TNF-α. Medium was collected, spun down and supernatant stored at -80 °C until use. Mock medium was used as negative control. For phospho-MET quantification, 6 × 10⁴ mouse blood neutrophils isolated from tumour-bearing mice were cultured in the presence or absence of 100 ng ml⁻¹ of murine TNF-α; 11 h later, cells were stimulated or not with 100 ng ml⁻¹ of mouse HGF in the presence of 1 mM orthovanadate for 3 min at 37 °C, washed in PBS supplemented with 1 mM orthovanadate and 1× phosphoStop (Roche) and lysed in Extraction Buffer supplemented with 1 mM orthovanadate, 2× phosphoStop and 1 mM protease inhibitor cocktail (Roche) for 20 min at 4 °C. After clearance, samples were quantified and the same amount of proteins was used for MET and phospho-MET detection using a sandwich ELISA. Briefly, 96-well microtitre plates (MaxiSorp, Nunc) were coated with 1 μg ml⁻¹ of anti-mouse MET antibody (AF527, R&D Systems) overnight at 4 °C and then incubated for 2 h at room temperature in blocking buffer (PBS, 0.1% Tween-20, 6% non-fat dry milk). The same amount of proteins per sample was diluted in blocking buffer and incubated for 2 h at room temperature on the ELISA plate. After 6 washes in PBS 0.1% Tween-20, samples were incubated for 2 h at room temperature with the mouse anti-MET (3D4, Invitrogen) or the mouse anti-phosphorytosine (4G10, Merck Millipore) antibodies diluted 1:500 in blocking buffer, then washed 6 times in PBS 0.1% Tween-20, and incubated with goat anti-mouse immunoglobulins conjugated to horseradish peroxidase (sc-2031, Santa Cruz Biotechnology) diluted 1:500 in blocking buffer for 2 h at room temperature. Signals were developed by 15 min incubation with the 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution (Promega). After stopping the reaction with H₂SO₄, absorbance was measured at 450 nm and corrected for 630 nm with a spectrophotometer.

Western blot. To assess MET deletion, bone marrow cells, neutrophils, peritoneal macrophages, monocytes (all cultured overnight in TCM) or endothelial cells were lysed in hot Laemmli buffer (2.5% SDS, 25% Tris-HCl pH 6.8) for 10 min at 96 °C, sonicated, cleared and quantified. Alternatively, 2 × 10⁶ bone-marrow-derived neutrophils from WT mice were stimulated with TCM, CCM, 100 ng ml⁻¹ of murine TNF-α (or mock medium 0% FBS or 10% FBS as control) for 20 h at 37 °C. For the co-culture with HUVECs, a monolayer of HUVECs was stimulated for 4 h with 5 ng ml⁻¹ IL-1α at 37 °C, and washed before neutrophil seeding. After 20 h of stimulation, neutrophils were collected using Cell Dissociation Buffer Enzyme Free PBS-Based (Gibco). Cells were washed in PBS, lysed in 15 μl of a protease inhibitor mixture and incubated for 15 min on ice. The protease inhibitor mixture was obtained by dissolving one tablet of Complete Mini protease inhibitor mixture (Roche) in 5 ml of PBS with 2 mM diisopropyl fluorophosphate (DFP; Acros Organics). After addition of an equal amount of 2× SDS sample buffer supplemented with 4% 2-mercaptoethanol, the lysates were boiled for 15 min and kept at ~80 °C until use. NF-κB inhibition was achieved by pre-treating 7 × 10⁶ neutrophils with 10 μM 6-amino-4-(4-phenoxyphenylethylamino) quinazoline (Calbiochem) for 1 h at 37 °C, cells were then stimulated with murine TNF-α (100 ng ml⁻¹) for 5 h before lysis. Human MET was assessed by stimulating 3 × 10⁶ blood neutrophils isolated from the blood of healthy volunteers with A549-CCM, 100 ng ml⁻¹ human TNF-α, 50 ng ml⁻¹ LPS (or mock medium 10% FBS as control) for 20 h. Cells were sonicated with 2.7 mM DFP for 15 min at 4 °C, collected and washed in PBS supplemented with 2.7 mM DFP and Complete Mini protease inhibitor 1×, and lysed in hot Laemmli buffer at 96 °C for 10 min. Cell lysates were sonicated, cleared and quantified. 6× loading buffer was added before loading on the gel.

The following primary antibodies were used: mouse anti-mouse Met (3D4, Invitrogen), mouse anti-mouse β-actin (1-19, Santa Cruz), mouse anti-vinculin (hVIN-1, Sigma), rabbit anti-human MET (D1C2, Cell Signaling), horseradish peroxidase (HRP)-conjugated anti-β-tubulin (Abcam). The following secondary antibodies were used: HRP-conjugated goat anti-mouse and HRP-conjugated goat anti-rabbit (Santa Cruz). Signal was visualized by Enhanced Chemiluminescent Reagents (ECL; Invitrogen) or West Femto by Thermofisher, followed by analysis with an Olympus BX41 microscope and Cell Sense imaging software or a Zeiss Axioscope microscope with KS500 image analysis software. The morphometric analysis was performed by acquiring 4–6 fields per section on five independent sections (at a distance of 40 μm in depth during sectioning) from the same biological tissue sample. The values in the graphs represent the average of the means of at least five samples and the standard error indicates the variability among the different samples.

Adhesion assay. 4 × 10⁴ HUVECs were seeded in M199 complete in a 96-well multwell plate, previously coated with 0.1% gelatin. After 12 h, HUVECs were stimulated with 5 ng ml⁻¹ IL-1α in DMEM 10% FBS at 37 °C. After 4 h the endothelial monolayer was gently washed and 2.5 × 10⁵ WBCs were isolated indistinctly from Tie2;Metw/w mice and Tie2;Met(-/-) mice or from WT→WT and KO→WT transplanted mice, were seeded on top of the endothelial monolayer, while mock medium, TCM (with or without 3 mg ml⁻¹ anti-HGF antibody AF-2207; R&D)⁵⁻⁻⁶ or 50 ng ml⁻¹ murine HGF was added in the bottom. After 2 h at 37 °C, transmigrated cells were collected from the bottom chambers and from the lower side of the filter with cold PBS 0.5% EDTA. Cells were stained and Ly6G + cells were quantified as described earlier. In the migration assays neutrophils purified from the blood of tumour-bearing mice or directly from the tumours themselves, were co-cultured with the cancer cells in DMEM 2% FBS for 4 h at 37 °C, with or without 100 ng ml⁻¹ mouse HGF or 1 mM L-NMMA (Sigma). After washing, adherent cells were lysed in 0.2% Triton, 1 mM dithiothreitol (DTT). Luciferase signal was revealed with a microplate luminometer.

The use of shMET was thought to prevent any possible confounding activity of MET on cancer cell survival and thus to restrict the effect of HGF to neutrophils only.

Histology and immunostainings. To obtain serial 7-μm-thick sections, tissue samples were immediately frozen in OCT compound or fixed in 2% PFA overnight at 4 °C, dehydrated and embedded in paraffin. Paraffin slides were first rehydrated to proceed further with antigen retrieval in citrate solution (DAKO). Cryosections were thawed in water and fixed in 100% methanol. If necessary, 0.3% H₂O₂ was added to methanol to block endogenous peroxidase. The sections were blocked with the appropriate serum (DAKO) and incubated overnight with the following antibodies: rat anti-CD45 (30F-11, BD Pharmingen) 1:100, rat anti-Ly6G (1A8, BD Pharmingen) 1:100, rat anti-CD31 (MEC 13.3, BD Pharmingen) 1:200, rabbit anti-ITF-TC (Serotec) 1:200, goat anti-phosphohistidine H3 (Cell Signalling) 1:100, rat anti-F4/80 (CL3-1, Serotec) 1:100, mouse anti-NK1.1-biotin (PK136, BD Pharmingen) 1:200, rabbit anti-CD45R (RA3-6B2, BD Pharmingen) 1:100, rat anti-CD4 (H129.9, BD Pharmingen) 1:100, rat anti-CD8 (53-6.72, BioXCell) 1:100, hamster anti-CD11c biotin (N418, eBioscience) 1:100, mouse anti-3-nitrotyrosin (HM.11 Santa Cruz) 1:200. Appropriate secondary antibodies were used: Alexa-488 or Alexa-648-conjugated secondary antibodies (Molecular Probes) 1:200, HRP-labelled antibodies (DAKO) 1:100, Biotin-labelled antibodies (Jackson ImmunoResearch) 1:100. When necessary, tyramide signalling amplification (Perkin Elmer, Life Sciences) was performed according to the manufacturer’s instructions. Whenever sections were stained in fluorescence, ProLong Gold mounting medium with DAPI (Invitrogen) was used. Otherwise, 3,3′-diaminobenzidine was used as a detection method followed by Harris’ haematoxylin counterstaining, dehydration and mounting with DPX. Apoptotic cells were detected by the TUNEL method, using the AptoTag peroxidase in situ apoptosis detection kit (Millipore) according to the manufacturer’s instructions. For the double staining TUNEL and Ly6G, TUNEL staining was performed as described earlier, followed by Ly6G staining by using the Vectastain ABC kit (Vector Laboratories) according to the manufacturer’s instructions. Tumour necrosis and lung metastasis were evaluated by H&E staining. Necrotic area was defined as the area including necrotic cancer cells, inflammatory cells and stromal cells, compared to the total area of the field. Necrotic cells display a more glassy homogenous appearance in the cytoplasm with increased eosinophilia, while the nuclear changes are reflected by karyolysis, pyknosis and karyorrhexis. Alternatively, the necrotic tissue was visualized by autofluorescence as previously described. Microscopic analysis was performed with an Olympus BX41 microscope and Cell Sense imaging software or a Zeiss Axioscope microscope with KS500 image analysis software. The morphometric analysis was performed by acquiring 4–6 fields per section on five independent sections (at a distance of 40 μm in depth during sectioning) from the same biological tissue sample. The values in the graphs represent the average of the means of at least five samples and the standard error indicates the variability among the different samples.

Hypoxia assessment and tumour perfusion. Tumour hypoxia was detected by injection of 60 mg kg⁻¹ pimonidazole into tumour-bearing mice 1 h before tumour harvesting. To detect the formation of pimonidazole adducts, tumour cryosections were immunostained with pimonidazole-1-Mabi (Hypoxpyrine kit, Chemicon) following the manufacturer’s instructions. Perfused tumour vessels were counted on tumour cryosections from mice injected intravenously with 0.05 mg fluorescein isothiocyanate (FITC)-conjugated lectin (Lycopersicon esculentum; Vector Laboratories).
Tumour-derived nitric oxide production. LLC tumours were collected 8 days after injection, cut in pieces of about 5 x 5 mm, weighted and incubated at 37 °C in a 24-multicell plate with 800 μl of DMEM. After 24 h, the media was collected, centrifuged to remove cell debris, and NO levels were measured using the Griess reagent system kit (Promega).

Statistics. Data entry and all analyses were performed in a blinded fashion. All statistical analyses were performed using GraphPad Prism software. Statistical significance was calculated by two-tailed unpaired t-test on two experimental conditions or two-way analysis of variance (ANOVA) when repeated measures were compared, with P < 0.05 considered statistically significant. Data were tested for normality using the D'Agostino–Pearson omnibus test (for n > 8) or the Kolmogorov–Smirnov test (for n ≤ 8) and variation within each experimental group was assessed. Detection of mathematical outliers was performed using the Grubbs’ test in GraphPad. Sample sizes for all experiments were chosen based on previous experiences. Independent experiments were pooled and analysed together whenever possible as detailed in figure legends. All graphs show mean values ± s.e.m.

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Extended Data Figure 1 | Scheme illustrating the role of MET in neutrophils. During cancer or infections, the release of cytokines such as IL-1 at the inflammatory site will promote the expression of TNF-α on the endothelium and the surrounding tissue. When circulating neutrophils encounter the activated endothelium, TNF-α will unleash NF-κB through binding to TNFR1, which in turn will induce MET expression on the neutrophil surface. HGF, also released and proteolytically activated at the site of inflammation, will bind to MET and stimulate the firm adhesion of neutrophils to the endothelium, probably via integrin engagement, and thus neutrophil diapedesis. Once extravasated, the HGF/MET pathway will still function on neutrophils by reinforcing their cytotoxic response through the induction of iNOS and NO production, ultimately favouring a bactericidal and tumoricidal neutrophil phenotype.
**Tumour apoptosis**

- b: TUNEL+ cells / mm²
  - WT→WT KO→WT

- c: TUNEL
  - WT→WT KO→WT

- d: TUNEL
  - WT→WT KO→WT

**Tumour necrosis**

- f: Necrotic area (% of total)
  - H&E Autofluorescence

- g: Necrotic area (% of total)
  - H&E Autofluorescence

**Tumour proliferation**

- k: pHH3+ area (% of total cells)
  - WT→WT KO→WT

- l: pHH3
  - WT→WT KO→WT

- m: pHH3
  - WT→WT KO→WT

**Vessel area**

- o: CD31+ area (% of total)
  - WT→WT KO→WT WT→KO

- p: CD31+ area (% of total)
  - WT→WT KO→WT WT→KO

- q: CD31+ area (% of total)
  - WT→WT KO→WT WT→KO

**Tumour hypoxia**

- r: CD31+ vessels / mm²
  - WT→WT KO→WT WT→KO

**Tumour growth**

- s: Tumour volume (mm³)
  - WT→WT KO→WT

- t: Tumour weight (g)
  - WT→WT KO→WT

- u: Metastatic nodules
  - WT→WT KO→WT

- v: Lung metastasis
  - WT→WT KO→WT

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Extended Data Figure 2 | Met deletion in immune cells, but not in endothelial cells, fosters tumour growth. a, MET expression in total bone marrow (BM) cells, endothelial cells (EC) and neutrophils harvested from Met floxed mice intercrossed with the Tie2:Cre deleter thus generating Tie2;Met^fl/fl (KO) or Tie2;Met^wt/wt (WT) mice. Western blots are representative of three repetitions on independent biological replicates. Western blot images have been cropped for presentation. Full scan images are shown in Supplementary Fig. 1.

b–d, Quantification (b) and representative images of tumour sections’ TdT-mediated dUTP nick end labelling (TUNEL) stainings (c, d) from subcutaneous end-stage LLC tumours in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 10 mice per condition.

e, FACS quantification of AnnexinV^+ 7AAD^- early apoptotic tumour cells in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 8 mice per condition.

f–j, Tumour necrosis quantification in WT→WT and KO→WT mice (f), assessed by histological evaluation of H&E-stained tumour sections (g, h) and by measurement of autofluorescent tumour areas (i, j); yellow dotted lines demarcate necrosis. Data combine two independent experiments; total n = 10 mice per condition.

k–m, Quantification (k) and representative images of tumour sections stained for the proliferation marker pHH3 (l, m) from subcutaneous end-stage LLC tumours in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 10 mice per condition.

n, FACS quantification of BrdU^+ proliferating tumour cells in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 10 mice per condition.

o–r, CD31^+ vessel area (o), vessel density (p), lectin perfusion (q) and hypoxic (Pimo^-) area (r) in LLC subcutaneous tumours from KO→WT mice (in which the haematopoietic/immune system is knocked out for Met) or WT→KO mice (in which endothelial cells only are knocked out for Met) compared to control WT→WT mice. Data in o–r combine two independent experiments; total n: WT→WT, 12; KO→WT, 8; WT→KO, 8.

s–u, Subcutaneous LLC tumour growth (s), weight (t) and lung metastases (u) in Tie2;Met^fl/fl compared to Tie2;Met^wt/wt mice. Data combine two independent experiments; total n: Tie2;Met^wt/wt, 12; Tie2;Met^fl/fl, 10.

v, Subcutaneous LLC tumour growth in endothelial-cell-specific Met KO (WT→KO) and control (WT→WT) mice. Data combine two independent experiments; total n = 8 per condition. *P < 0.05 versus WT→WT (b, e, f, k, n), versus Tie2;Met^wt/wt (s–u). Scale bars: 50 μm (c, d, l, m); 100 μm (g–j). All graphs show mean ± s.e.m.
Extended Data Figure 3 | Circulating and tumour-infiltrating immune cells upon Met deletion. a–c, FACS analysis showing percentages of circulating monocytes (a), lymphocytes (b), neutrophils (c), eosinophils (d) and basophils (e) in tumour-free or in LLC-tumour-bearing WT→WT and KO→WT mice. Data combine two independent experiments; total n = 8 mice per condition. f, Quantification of LLC tumour sections stained for the pan-leukocyte marker CD45, the macrophage marker F4/80, the NK marker NK1.1, the B lymphocyte marker CD45R, the T helper cell marker CD4, the cytotoxic T cell marker CD8 and the dendritic cell marker CD11c (with exclusion of F4/80\(^{+}\) area) in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 8 mice per condition. g, h, FACS quantification for tumour-associated CD45\(^{+}\) leukocytes (g) or CD45\(^{+}\) IgE\(^{+}\) CD49b\(^{+}\) CD4\(^{+}\) CD45R\(^{+}\) basophils and CD45\(^{+}\) CD11b\(^{+}\) SgIgE\(^{+}\) Ly6C\(^{int}\) F4/80\(^{+}\) MHCII\(^{+}\) eosinophils (h) in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 8 mice per condition. i, j, FACS quantification (i) and gating strategy (j) for tumour-associated neutrophils selected from the main tumour cell population negative for 7AAD staining; tumour-associated neutrophils were then gated as CD11b and Ly6G double-positive cells. Data combine two independent experiments; total n = 8 mice per condition. k, Ly6G\(^{+}\) tumour infiltration at day 9, day 13 and day 19 after LLC subcutaneous tumour injection in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 8 mice per condition. l, Morphometric quantification of leukocytes and macrophages on CD45- and F4/80-stained lung sections, respectively, from LLC-tumour-bearing WT→WT or KO→WT mice. Data combine two independent experiments; total n = 8 mice per condition. m, FACS quantification of CD11b\(^{+}\) Ly6G\(^{+}\) neutrophils and CD11b\(^{+}\) F4/80\(^{+}\) macrophages infiltrating metastatic lungs from LLC-tumour-bearing WT→WT or KO→WT mice. Data combine two independent experiments; total n = 8 mice per condition. *P < 0.05 versus WT→WT (f, g, i–m); †P < 0.05 versus tumour free (a–d). All graphs show mean ± s.e.m.
Extended Data Figure 4 | MET in neutrophils is required for their anti-tumour activity.  

a, b, Western blot analysis (a) and relative densitometric analysis (b) for MET expression in bone marrow neutrophils and monocytes upon reconstitution of WT recipient mice by WT or KO HSPCs transduced in vitro with an empty vector (Mrp8:Empty) or a vector expressing Met under the neutrophil-specific promoter Mrp8 (Mrp8:Met); tubulin was used as loading control. Western blots are representative of three repetitions on independent biological samples where each sample is the pool of neutrophils or monocytes isolated from three mice. Densitometric analysis was performed on these three western blots. A.U., arbitrary units.

c, FACS analysis for green fluorescent protein (GFP) in circulating Ly6G⁺ neutrophils or CD115⁺ monocytes, harvested from the neutrophil-specific Mrp8:Cre line carrying separate expression of GFP because of an internal ribosome entry site (IRES) downstream the Mrp8-driven cre gene. Data combine two independent experiments; total n = 10 mice per condition.

d, MET expression in neutrophils, monocytes and macrophages harvested from Mrp8;Met⁺/⁺ or Mrp8;Met⁻/⁻ mice. Western blots are representative of three repetitions on independent biological replicates. e, FACS analysis for CD11b⁺ Ly6G⁺ neutrophils in subcutaneous LLC tumours from Mrp8;Met⁺/⁺ or Mrp8;Met⁻/⁻. Data combine two independent experiments; total n: Mrp8;Met⁺/⁺, 10; Mrp8;Met⁻/⁻, 11. Western blot images in a, d have been cropped for presentation. Full scan images are shown in Supplementary Fig. 1.

*P < 0.05 versus Mrp8:Empty WT→WT (b), versus Mrp8;Met⁺/⁺ (e); †P < 0.05 versus Mrp8:Empty WT→WT; ‡P < 0.05 versus Mrp8:Empty KO→WT. All graphs show mean ± s.e.m.
**a** Tumour burden (B16F10)

**b** Tumour burden (PyMT)

**c** Metastatic index (PyMT)

**d** Tumour-associated neutrophils (T241)

**e** Tumour-associated neutrophils (PyMT)

**f** Colon shrinkage

**g** Healthy Colitis

**h** Neutrophil infiltration (colitis)

**i** Macrophage infiltration (colitis)

**j** Tumour burden (Panc02)

**k** Mesenteric metastasis (Panc02)

**l** Tumour-associated neutrophils (Panc02)

**m** HGF plasma levels

**n** HGF tumour levels

**o** HGF plasma levels

**p** HGF tumour levels

**q** Tumour-associated neutrophils (B16F10)

**r** Scrambled siRNA

**s** VINCULIN

**t** B16F10
Extended Data Figure 5 | Pharmacological and genetic inhibition of MET prevents the recruitment of anti-tumoural neutrophils to several neoplastic tissues and inflammatory sites. a, Tumour weight of subcutaneous B16F10 melanomas in WT→WT and KO→WT mice. Data combine two independent experiments; total n: WT→WT, 8; KO→WT, 9. b, c, Total tumour weight (b) and metastatic index (c) in MMTV-PyMT mice reconstituted with WT or Met KO bone marrow cells before tumour appearance (WT→PyMT and KO→PyMT mice, respectively). Data combine three independent experiments; total n: WT→PyMT, 13; KO→PyMT, 16. d, e, FACS quantification for CD11b+Ly6G+ neutrophils in T241 tumours harvested from WT→WT or KO→WT mice (d) or in in breast tumours spontaneously grown in WT→PyMT and KO→PyMT mice (e). Data combine two independent experiments; total n = 10 mice per condition (d) or total n = 8 mice per condition (e). f–i, Length measurement (f) and representative image (g) of the colon, as well as quantification of neutrophils (h) and macrophages (i) on bowel sections, from WT→WT and KO→WT mice upon induction of chronic colitis compared to healthy control. Data combine two independent experiments; total n: healthy, 5; WT→WT, 12; KO→WT, 15. j, k, Tumour weight (j) and metastatic mesenteric lymph nodes (k) 12 days after orthotopic injection of pancreatic Panc02 cancer cells in WT→WT and KO→WT mice. Data combine two independent experiments; total n = 12 per condition. l, Histological quantification of Ly6G+ infiltrates in Panc02 pancreatic tumours harvested from WT→WT and KO→WT mice. Data combine two independent experiments; total n = 12 mice per condition. m, Quantification of plasma HGF in tumour (TM)-free mice, in subcutaneous LLC or orthotopic Panc02 tumour-bearing mice. Data combine two independent experiments; total n: tumour free, 10; LLC, 10; Panc02, 8 biological replicates. n, Quantification of HGF in subcutaneous LLC or orthotopic Panc02 tumours. Data combine two independent experiments; total n: LLC, 10; Panc02, 8 biological replicates. o, p, Quantification of HGF in plasma (o) or in subcutaneous LLC tumours (p) from tumour-bearing WT→WT and KO→WT mice. Data are representative of two independent experiments using 5 mice per condition per experiment. q, Quantification of Ly6G+ area on sections from B16F10 melanomas grown in C57BL/6 WT mice, daily treated with PF-04217903, INCB28060, JNJ-38877605, or vehicle as control. Data combine two independent experiments; total n: vehicle, 14; PF-04217903, 9; INCB28060, 6; JNJ-38877605, 4. r, Western blot analysis for MET in B16F10 melanoma cells after transduction with a lentiviral vector encoding scrambled or mouse shMET under a constitutive promoter; vinculin was used as loading control. Western blot analysis is representative of three independent repetitions. Western blot images have been cropped for presentation. Full scan images are shown in Supplementary Fig. 1. *P < 0.05 versus WT→WT (a, d, h), versus WT→PyMT (b, e), versus LLC (m, n), versus vehicle (q); †P < 0.05 versus healthy (f, h, i), versus tumour free (m). Scale bar: 10 mm (g). All graphs show mean ± s.e.m.
Extended Data Figure 6 | HGF is required for MET activation upon induction by TNF-α. a, Gating strategy related to Fig. 3b to quantify MET expression in blood neutrophils from LLC-tumour (TM)-bearing mice and in TANs, where live cells were first gated as CD11b-positive cells; this population was finally gated for Ly6G and MET to identify MET-expressing Ly6G\(^+\) neutrophils. b, c, qRT–PCR for MET in mouse (b) and human (c) neutrophils after LPS or TNF-α stimulation. Data are representative of three independent experiments using four biological replicates per condition per experiment.

d, e, qRT–PCR for MET expression in mouse (d) or human (e) neutrophils cultured in normoxia (21% O\(_2\)) or hypoxia (1% O\(_2\)). Data combine two independent experiments; total n = 8 biological replicates per condition.

f, g, ELISA for total MET (f) and phospho-MET (g) from mouse neutrophils stimulated for 3 min with mock medium or HGF after an overnight incubation with or without TNF-α. Data combine three independent experiments; total n = 6 biological replicates per condition.

h, HGF release by neutrophils stimulated with mock medium or TNF-α after 20 h in culture. Data combine two independent experiments; total n = 6 biological replicates per condition.

i, qRT–PCR for TNFA in HUVECs upon stimulation with IL-1α compared to mock medium. Data combine two independent experiments; total n = 4 biological replicates per condition.

j, qRT–PCR for Met in mouse neutrophils co-cultured with HUVEC/NS or HUVEC/IL transduced with shTNFA or scramble as control. Data are representative of three independent experiments in which three different shRNA sequences were used; total n = 4 biological replicates per condition per experiment.

k, l, qRT–PCR for Met in WT, TNFR1 KO or TNRF2 KO neutrophils upon co-culture with HUVEC/NS or HUVEC/IL (k), or after stimulation with conditioned medium (TCM) from LLC tumours (l). Data are representative of two independent experiments using four biological replicates per condition per experiment.

m, qRT–PCR for MET in human neutrophils stimulated with A549-CCM in the presence or absence of Enbrel or human IgG as control. Data are representative of two independent experiments using four biological replicates per condition per experiment.

*P < 0.05 versus mock (b, c, i), versus TNF-α alone (g), versus HUVEC/NS (j), versus WT (k, l), versus A549-CCM (m); †P < 0.05 versus untreated or HGF alone (f, g), versus HUVEC/NS (k), versus mock (l, m). Graph shows mean ± s.e.m.
Extended Data Figure 7 | Met deletion in neutrophils does not affect apoptosis. a, b, Gating strategy of apoptotic WT (a) and Met KO (b) neutrophils in LLC tumours where single-cell suspensions were first gated for physical parameters and then for CD11b and Ly6G to identify neutrophils as double-positive cells; this population was finally gated for AnnexinV and 7AAD: AnnexinV^+ 7AAD^- cells display early apoptotic neutrophils whereas AnnexinV^- 7AAD^- cells display late apoptotic neutrophils. c, Quantification of apoptotic WT and Met KO tumour-associated neutrophils measured by FACS. Data combine two independent experiments; total n = 7 mice per condition. d, Quantification of apoptotic WT and Met KO neutrophils on LLC tumour sections by immunohistochemistry. Data combine two independent experiments; total n: WT→WT, 7; KO→WT, 6. e, FACS analysis for AnnexinV and 7AAD of WT or KO neutrophils incubated for 10 h in the presence or absence of LPS and HGF, alone or in combination. Data combine two independent experiments; total n = 6 biological replicates per condition. †P < 0.05 versus untreated or HGF alone. Graph shows mean ± s.e.m.
Extended Data Figure 8 | MET affects neither neutrophil basal migration nor polarization but it is required for neutrophil recruitment and cytotoxicity. a, Quantification of Ly6G staining in ear sections upon phorbol ester (TPA)-induced cutaneous rash in Mrp8;Metwt/wt and Mrp8;Mefl/fl mice. Data combine two independent experiments; total n = 8 mice per condition. b, FACS analysis on peritoneal lavages for Ly6G+ neutrophils or F4/80+ macrophages in Mrp8;Metwt/wt and Mrp8;Mefl/fl mice 4 h after intraperitoneal injection of sterile zymosan A. Data are representative of two independent experiments using 5 mice per condition per experiment. c, d, Quantification of F4/80 (c) and CD3 (d) stainings in ear sections at baseline and upon TPA-induced cutaneous rash. Data combine two independent experiments; total n: WT control (CTRL), 22; KO→WT CTRL, 15; WT→WT TPA, 23; KO→WT TPA, 15 (c); or total n = 8 mice per condition (d). e, FACS quantification of Mrp8;Metwt/wt and Mrp8;Mefl/fl neutrophils recruited into subcutaneous air pouches in response to HGF, CXCL1 or PBS. Data combine two independent experiments; total n = 6 mice per condition. f, FACS quantification of WT neutrophil adhesion to quiescent HUVECs (HUVEC/NS) or activated HUVECs (HUVEC/IL) in the presence or absence of HGF. Data are representative of two independent experiments using four biological replicates per condition per experiment. g, h, FACS quantification of WT and Met KO neutrophils migrated through a bare porous filter (that is, in the absence of HUVECs) towards HGF (g) or tumour conditioned medium (TCM) (h). Data are representative of two independent experiments using three biological replicates per condition per experiment. i, Gene expression profile for N1 and N2 markers in neutrophils sorted from LLC tumours grown in WT→WT or KO→WT mice. Data are representative of three independent experiments using 4 mice per condition per experiment. j, Cytotoxicity of WT and KO tumour-associated neutrophils against T241 cells in the absence or presence of the NO synthase inhibitor L-NMMA. Data are representative of three independent experiments using three biological replicates per condition per experiment. k, FACS quantification of DAF-FM-positive circulating neutrophils after co-culture with LLC cancer cells as a readout of NO production in the absence or presence of HGF. Data are representative of four independent experiments using three biological replicates per condition per experiment. l, Quantification of LLC cancer cell killing by WT and KO neutrophils (isolated from the blood of tumour-bearing mice), stimulated with HGF alone or in the presence of L-NMMA. Data are representative of two independent experiments using n = 12 biological replicates per condition per experiment. m, Blood neutrophils in WT→WT and KO→WT mice treated with neutrophil-depleting Ly6G antibody or rat IgG as control. Data combine two independent experiments; total n = 16 per condition. *P < 0.05 versus Mrp8;Metwt/wt (a, b), versus Mrp8;Metwt/wt + HGF (e), versus HUVEC/NS (f), versus WT→WT untreated (j), versus WT→WT + HGF (k,l); †P < 0.05 versus CTRL (c, d), versus PBS (e), versus mock (f, h), versus WT→WT untreated (j–l), versus IgG (m); ‡P < 0.05 versus WT→WT + HGF (l). All graphs show mean ± s.e.m.
## Extended Data Table 1 | Blood count in Tie2;*Met*<sup>wt/wt</sup> or Tie2;*Met*<sup>fl/fl</sup> tumour-free mice

| Tumour free | Tie2;*Met*<sup>wt/wt</sup> | Tie2;*Met*<sup>fl/fl</sup> |
|-------------|-----------------|-----------------|
| WBC (k/µl)  | 5.68±1.44       | 5.55±1.29       |
| NEU (%)     | 23.03±5.45      | 29.67±7.88      |
| LYM (%)     | 69.72±6.46      | 72.03±4.89      |
| MON (%)     | 1.24±0.37       | 2.86±1.15       |
| EOS (%)     | 0.12±0.05       | 0.17±0.12       |
| BAS (%)     | 3.38±1.32       | 4.47±2.1        |
| RBC (M/µL)  | 5.21±1.91       | 4.89±1.52       |
| HCT (%)     | 71.3±3.43       | 60.2±13.38      |
| MCHC (g/dl) | 15.83±2.65      | 18.3±0.26       |
| PLT (K/µL)  | 439.73±26.64    | 508±55.79       |

The values show the haematological parameters (mean ± s.e.m.) in tumour-free Tie2;*Met*<sup>wt/wt</sup> and Tie2;*Met*<sup>fl/fl</sup> mice. Data combine two independent experiments; total n = 10 per condition. BAS, basophil; EOS, eosinophil; HCT, haematocrit; LYM, lymphocyte; MCHC, mean cell haemoglobin concentration; MON, monocyte; NEU, neutrophil; PLT, platelet; RBC, red blood cell; WBC, white blood cell.
Extended Data Table 2 | Blood count in WT→WT and KO→WT tumour-free or tumour-bearing mice

| Tumour free          | WT→WT       | KO→WT       |
|----------------------|-------------|-------------|
| WBC (k/µL)           | 10.03±2.05  | 8.66±0.93   |
| NEU (%)              | 9.18±2.1    | 10.3±3.07   |
| LYM (%)              | 85.2±2.91   | 83.94±3.46  |
| MON (%)              | 1.41±0.48   | 1.3±0.4     |
| EOS (%)              | 0.44±0.17   | 0.25±0.05   |
| BAS (%)              | 3.77±0.68   | 4.21±0.18   |
| RBC (M/µL)           | 8.21±0.54   | 9.3±0.21    |
| HCT (%)              | 59.86±3.52  | 70.16±1.67  |
| MCHC (g/dl)          | 18.63±0.61  | 13.4±0.16   |
| PLT (K/µL)           | 589.26±134.65 | 758.4±50.63 |

| Tumour bearing       | WT→WT       | KO→WT       |
|----------------------|-------------|-------------|
| WBC (k/µL)           | 7.97±0.63   | 9.12±1.22   |
| NEU (%)              | 44.3±0.37   | 53.71±7.23  |
| LYM (%)              | 27.29±8.33  | 33.96±2.52  |
| MON (%)              | 1.79±0.75   | 1.9±0.64    |
| EOS (%)              | 0.26±0.03   | 0.45±0.1    |
| BAS (%)              | 1.94±0.53   | 1.84±0.57   |
| RBC (M/µL)           | 5.5±0.54    | 6.83±0.46   |
| HCT (%)              | 42.13±2.93  | 49.43±3.47  |
| MCHC (g/dl)          | 18.27±0.5   | 19.24±0.05  |
| PLT (K/µL)           | 566.17±109.48 | 805.5±88.19 |

The values show the haematological parameters (mean ± s.e.m.) in tumour-free or LLC-tumour-bearing (21 days) WT→WT and KO→WT chimaeric mice. Data combine two independent experiments; total n = 10 per condition. BAS, basophil; EOS, eosinophil; HCT, haematocrit; LYM, lymphocyte; MCHC, mean cell haemoglobin concentration; MON, monocyte; NEU, neutrophil; PLT, platelet; RBC, red blood cell; WBC, white blood cell.