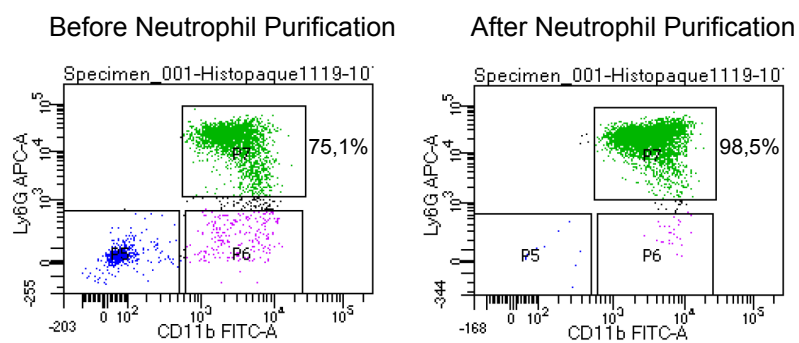
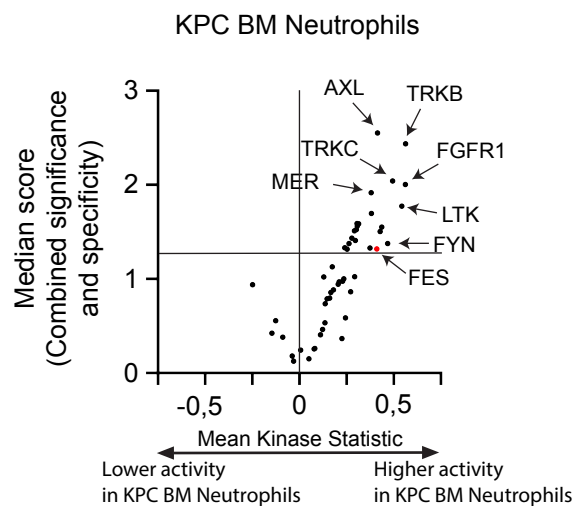


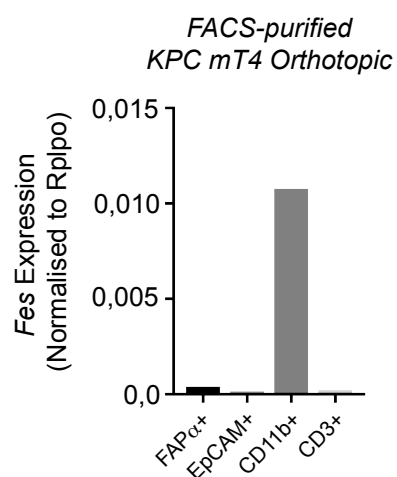
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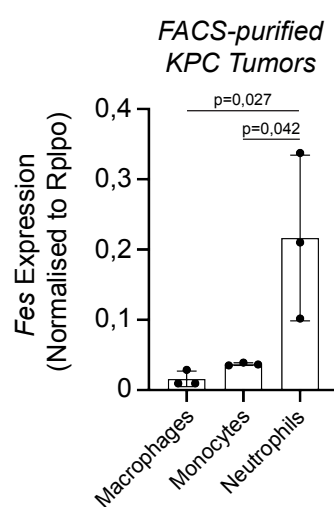
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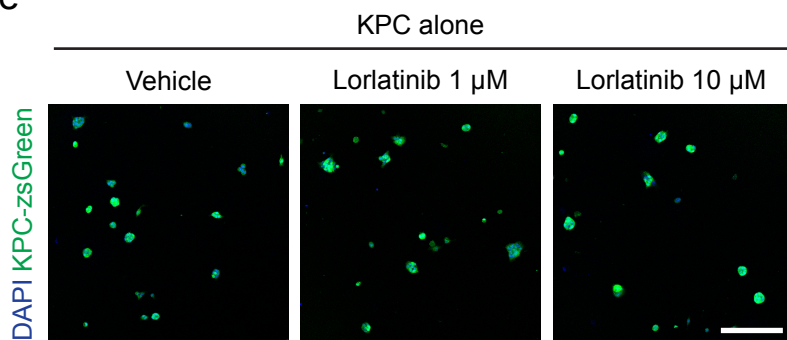
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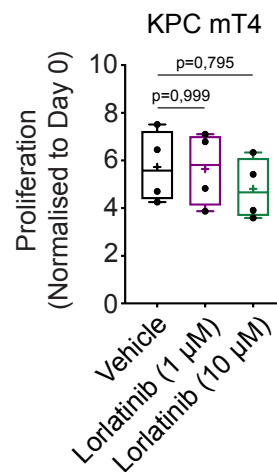
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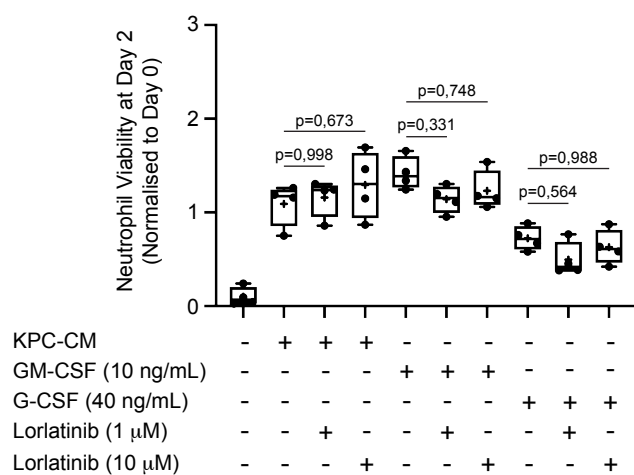
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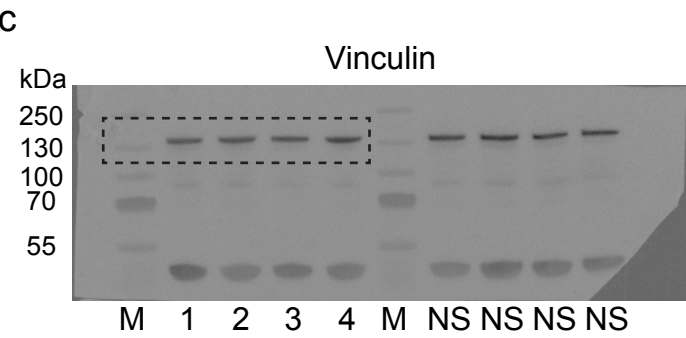
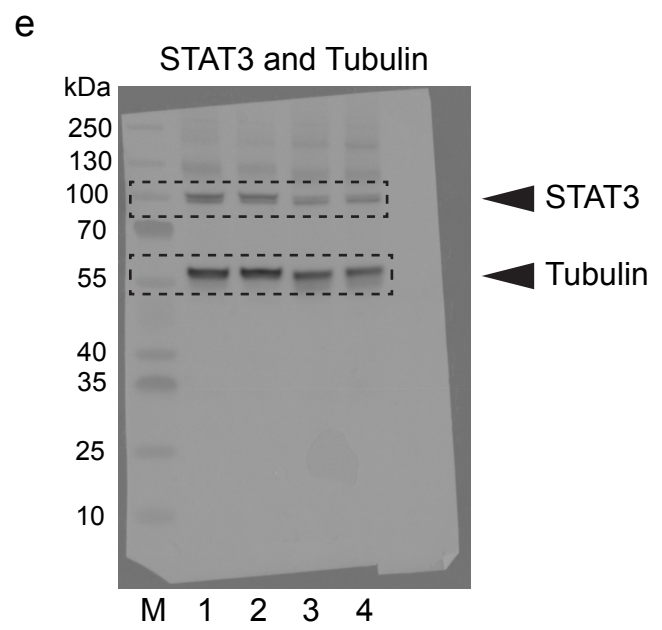
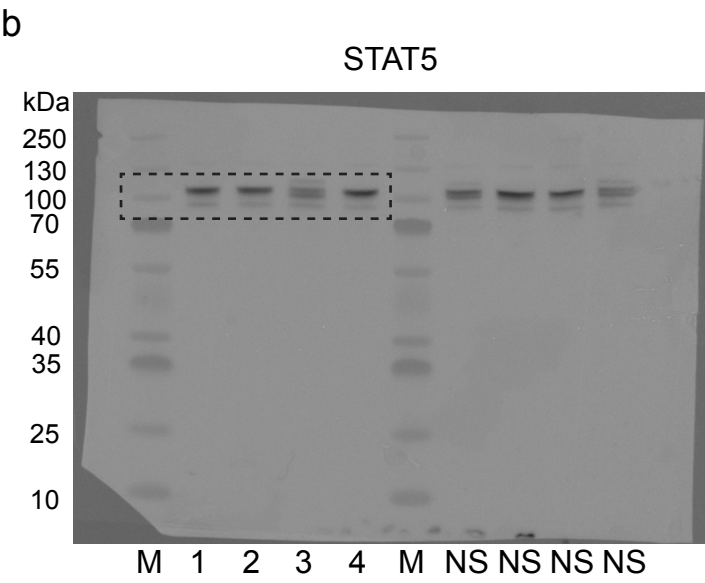
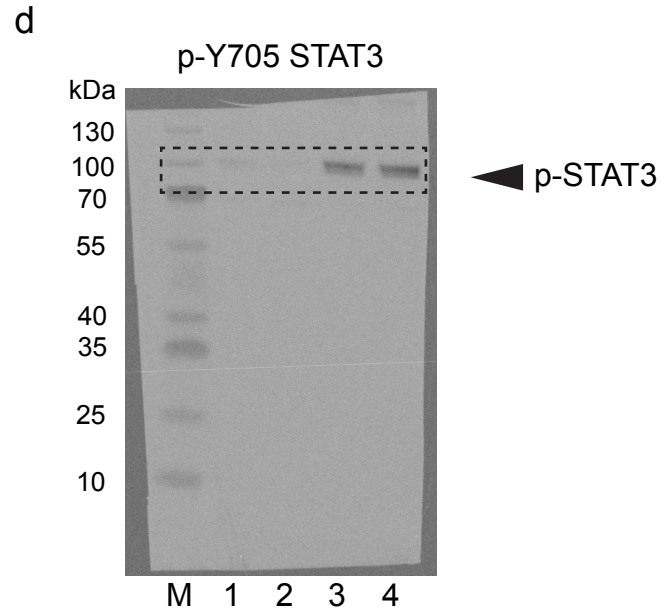
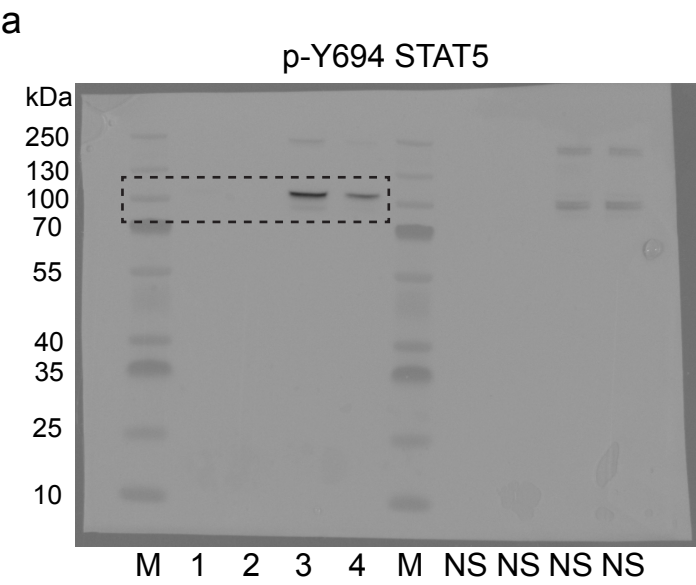


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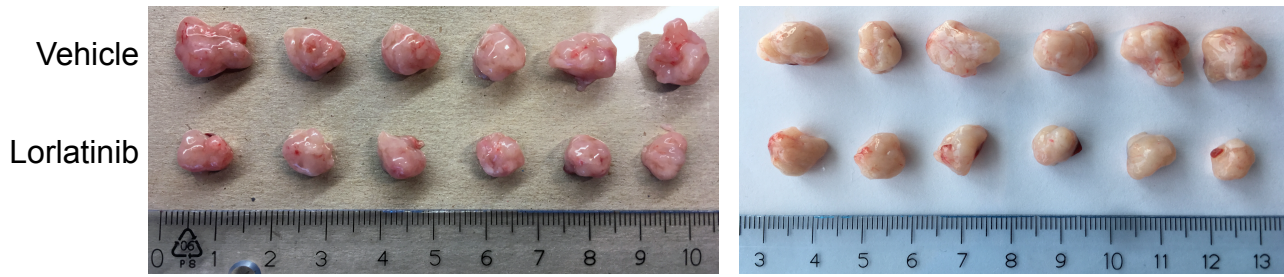
Supplementary figure 1. *Fes* is mainly expressed by neutrophils in the pancreatic tumor microenvironment.

(a) Representative flow cytometry staining of BM-derived neutrophils before and after purification with magnetic beads. Purity of the neutrophil isolation was assessed by staining with antibodies against CD45, CD11b and Ly6G (n = 2 mice; representative of 1 independent experiment). 98,5% of recovered viable (SYTOX^{neg}) cells were CD45⁺ CD11b⁺ Ly6G⁺ neutrophils after purification with magnetic beads. (b) Volcano plots of the kinase activity prediction based on bait peptide phosphorylation of PTK chip arrays with protein lysate from BM neutrophils isolated from tumor-bearing KPC mice or tumor-free control mice (data show results with three independent samples from each group). (c) Relative expression of *Fes* by qPCR in FACS-sorted cell populations from an orthotopic PDAC tumor (FAP α ⁺ fibroblasts, EpCAM⁺ epithelial cells, CD11b⁺ myeloid cells or CD3⁺ T cells) (cells were sorted from 1 tumor). (d) Relative expression of *Fes* by qPCR in FACS-sorted cell populations from PDAC tumors in the KPC mouse model (CD11b⁺F4/80⁺ macrophages, CD11b⁺ F4/80^{neg} Ly6C⁺ Ly6G^{neg} monocytes or CD11b⁺ F4/80^{neg} Ly6C^{low} Ly6G⁺ neutrophils) (n = 3 mice, data are from one experiment). (e) Representative immunofluorescence images and quantification of KPC-zsGreen cells without neutrophils in the presence of vehicle or lorlatinib in 2 days of culture. Quantification is shown in Fig. 1e. Five FOV were acquired per condition from each experiment and used for quantification of zsGreen⁺ cells (n = 3 independent experiments). (f) Quantification of proliferation of KPC mT4 cells in the presence of lorlatinib or vehicle for two days. The relative proliferation was normalized to the measurement of cells seeded on the same plates before treatment with vehicle or lorlatinib (n = 4 independent experiments). (g) Quantification of neutrophil viability after 2 days of stimulation with KPC-CM, 10 ng/ml GM-CSF or 40 ng/mL G-CSF in the presence of vehicle or lorlatinib (n = 4 independent experiments)). Scale bar represents 200 μ m. Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers); bar graphs represent mean and standard deviation; hypothesis testing performed using two-way ANOVA with (d, f) Bonferroni's or (g) Sidak's method for multiple comparisons.

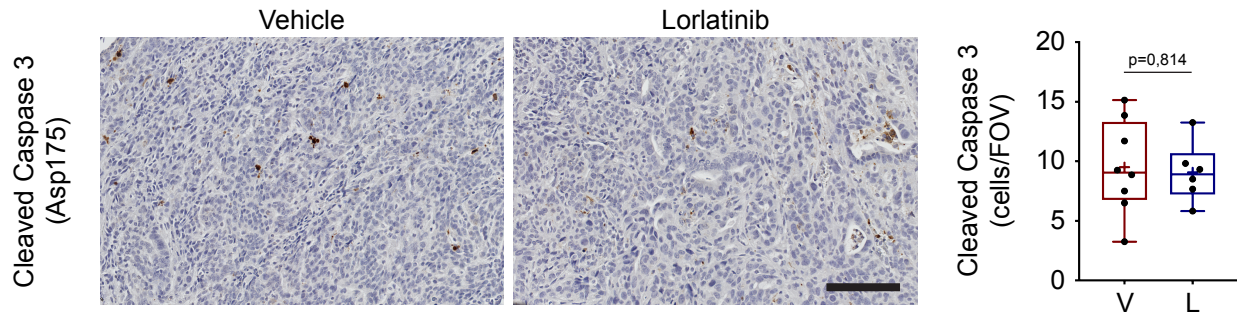


Supplementary figure 2. Full scans of immunoblots from figure 1c. Dashed boxes indicate cropped regions displayed in Figure 1c. Arrows indicate the expected band position for each protein or phosphorylation site probed. M, molecular weight markers; NS, non-relevant sample; 1, Neutrophils treated with control medium and vehicle for 1 hour; 2, Neutrophils treated with control medium and 10 μ M lorlatinib for 1 hour; 3, Neutrophils treated with KPC-conditioned medium and vehicle for 1 hour; 4, Neutrophils treated with KPC-conditioned medium and 10 μ M lorlatinib for 1 hour.

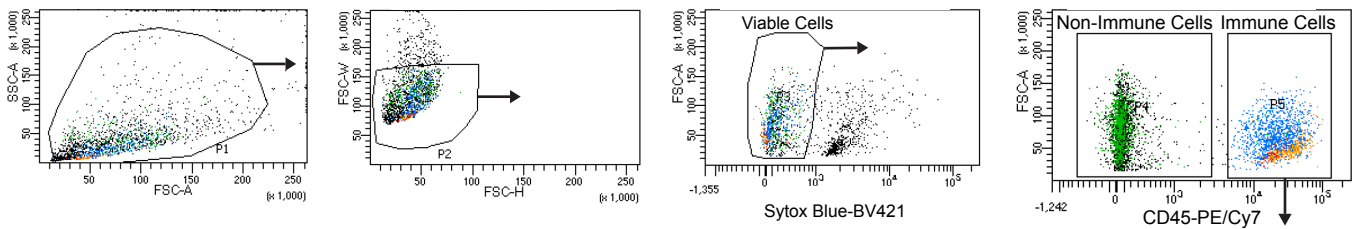
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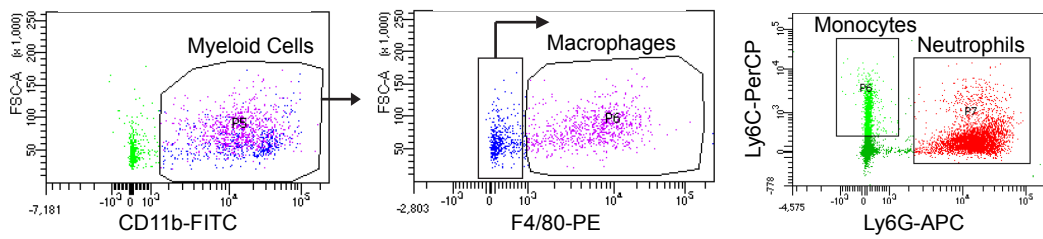


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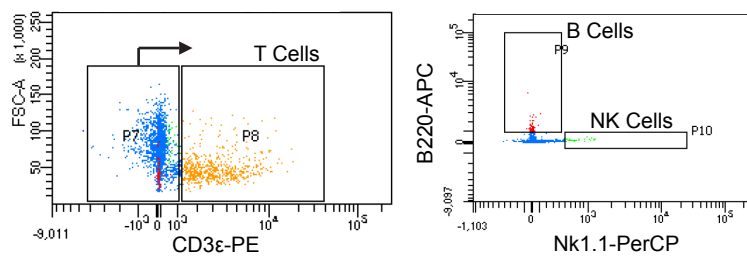
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Macrophages, Monocytes and Neutrophils (Gated on CD45+)

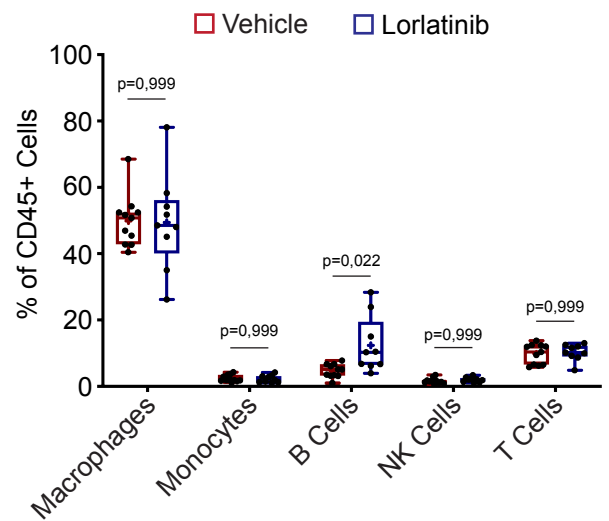


e

T Cells, B cells and NK cells (Gated on CD45+)



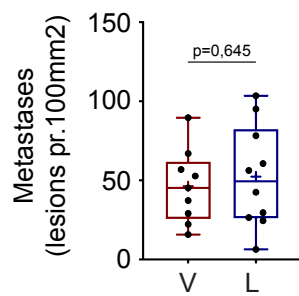
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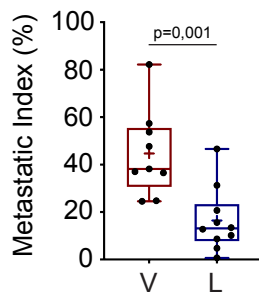
Supplementary figure 3. Lorlatinib treatment attenuates PDAC tumor progression. (a)

Representative photographs of orthotopic PDAC tumors at day 28 from vehicle- or lorlatinib-treated mice. Images are from two independent experiments. (b) Representative IHC images and quantification of cleaved caspase 3 (Asp175)-positive cells from in orthotopic PDAC tumors from vehicle- and lorlatinib-treated mice (n = 8 mice, vehicle; n = 6 mice, lorlatinib; data are from two experiments). Six images were acquired per mouse and used for quantification of cleaved caspase 3⁺ cells. (c-e) Representative flow cytometry gating strategy for quantification of the cellular composition of the TME from orthotopic PDAC tumors. (c) Representative flow cytometry dot plots showing the gating strategy used to identify hematopoietic cells (CD45⁺). Only viable (SYTOX^{neg}) cells were used for analysis. (d) Representative flow cytometry dot plots showing gating strategy used to identify macrophages (CD11b⁺F4/80⁺), neutrophils (CD11b⁺F4/80^{neg}Ly6G⁺) and inflammatory monocytes (CD11b⁺F4/80^{neg}Ly6G^{neg}Ly6C⁺) from the hematopoietic cell population (CD45⁺). (e) Representative flow cytometry dot plots showing gating strategy used to identify B cells (B220⁺), T cells (CD3⁺) and NK cells (CD3^{neg}B220^{neg} NK1.1⁺) from hematopoietic cells (CD45⁺). (f) Flow cytometry analysis of immune cells in PDAC tissue from mice bearing orthotopic tumors treated for 14 days with vehicle or lorlatinib. The composition of intratumoural immune cells is shown as a percentage of CD45⁺ cells (n = 11 mice, vehicle; n = 9 mice, lorlatinib; data are from two experiments). Scale bar represents 100μm (b). Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers); hypothesis testing performed using unpaired two-sided Student's t test (b) or two-way ANOVA with Tukey's method for multiple comparisons (f).

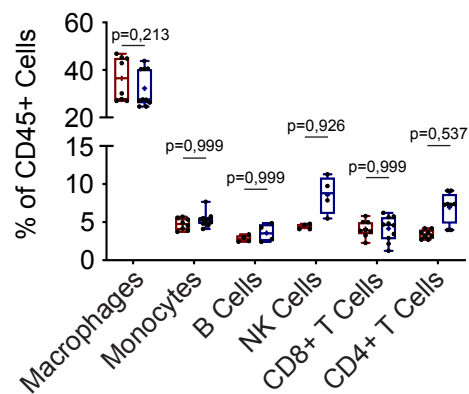
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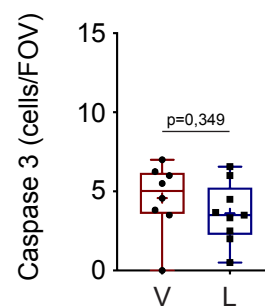
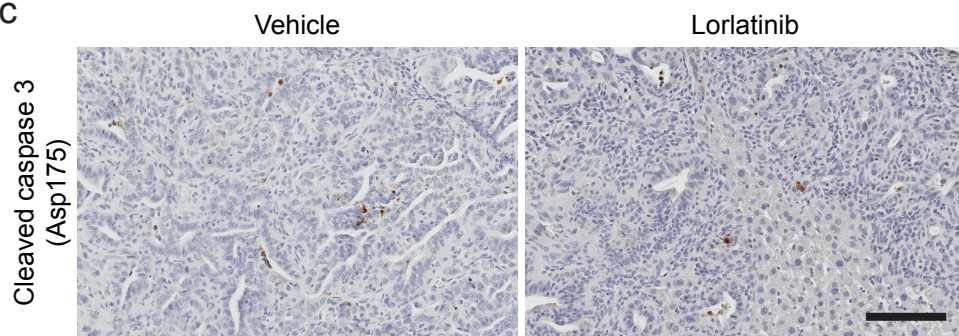
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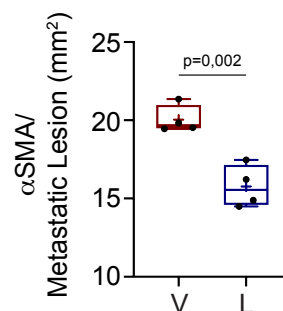
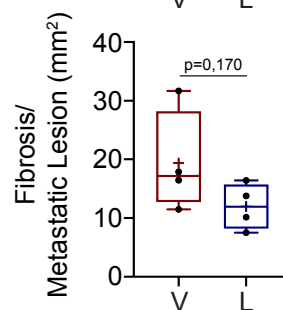
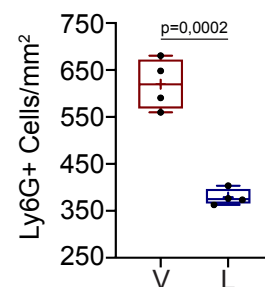
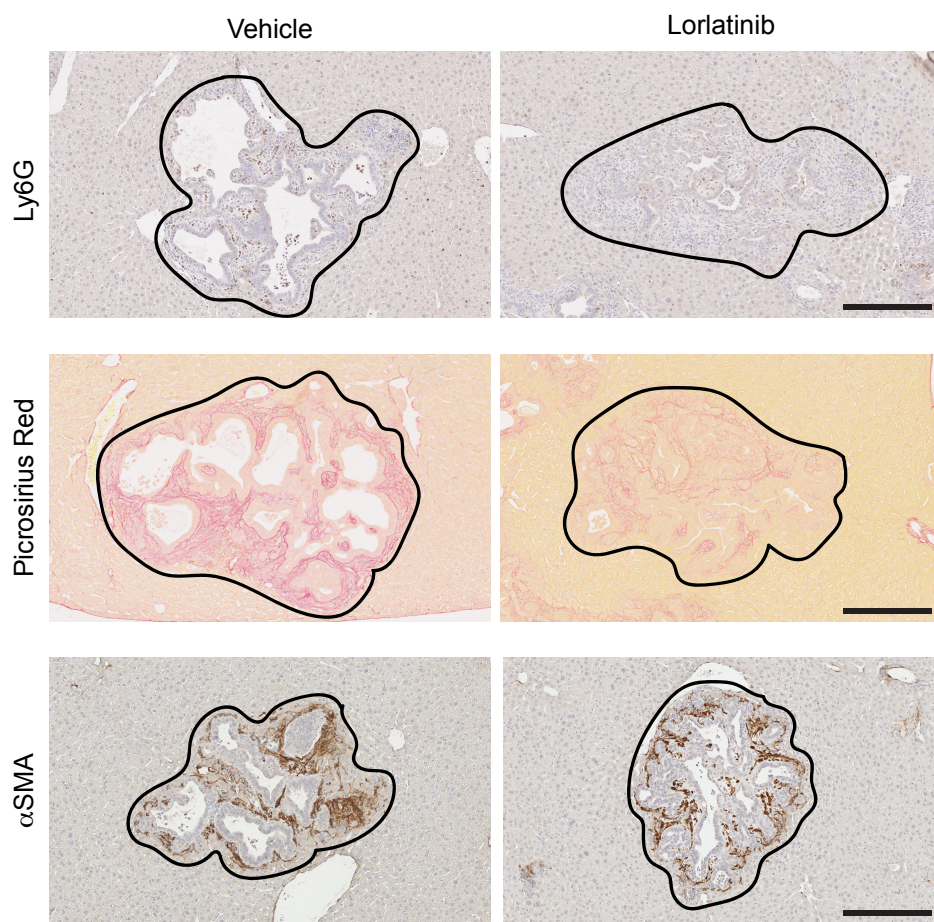
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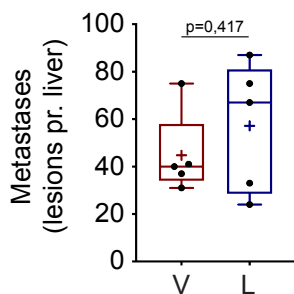
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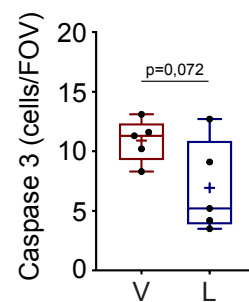
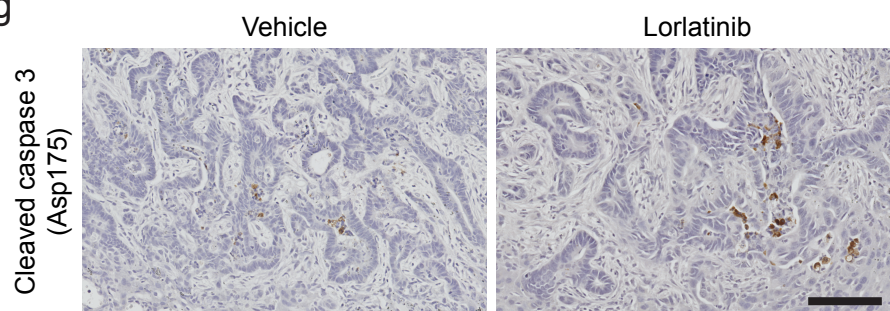
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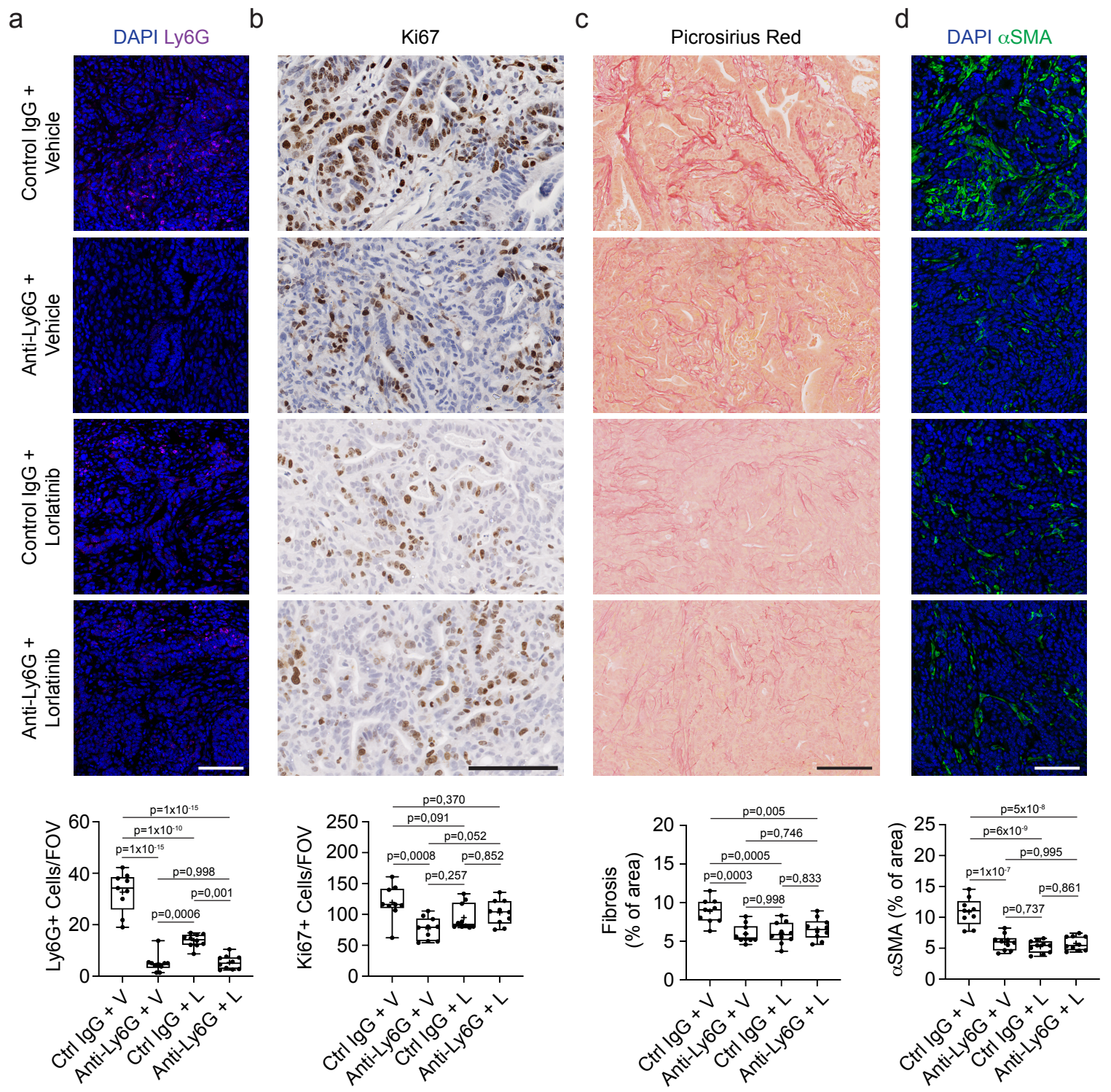
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g

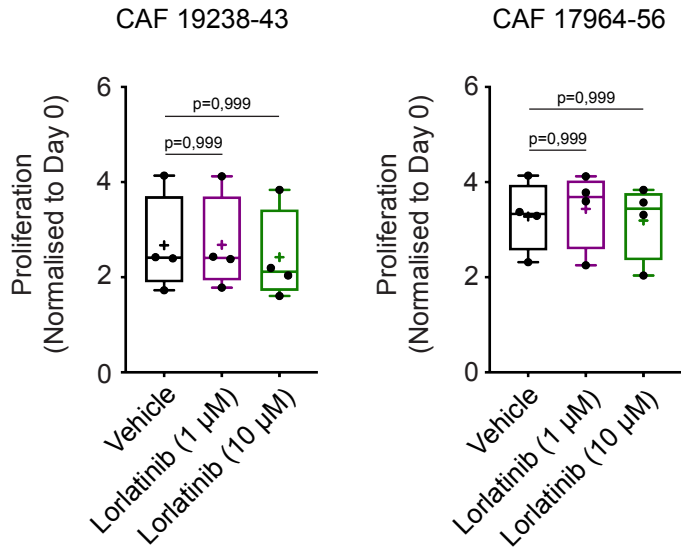


Supplementary figure 4. Lorlatinib reduces the hepatic metastatic burden in mouse models of pancreatic and colorectal cancer metastasis. Hepatic metastatic burden three weeks after intrasplenic injection of KPC mT4 cells. Mice received daily treatment with vehicle or lorlatinib (5mg/kg) starting 7 days after injection for further 14 days. Metastatic burden was determined three weeks after injection by hematoxylin and eosin staining. (a) Number of hepatic metastatic lesions per 100 mm² (n = 9 mice, vehicle; n = 10 mice, lorlatinib; data are from two independent experiments). (b) Metastatic index calculated as the percentage of metastatic lesions that covers each liver section (n = 9 mice, vehicle; n = 10 mice, lorlatinib; data are from two independent experiments). (c) Representative images of cleaved caspase 3 (Asp175) IHC images of hepatic metastatic tissue from intrasplenic injection of KPC mT4 cells (n = 8 mice, vehicle; n = 9 mice, lorlatinib; data are from two experiments). (d) Flow cytometry analysis of immune cells in hepatic metastases shown as a percentage of CD45⁺ cells (n = 8 mice, vehicle; n = 10 mice, lorlatinib; data are from two independent experiments except B and NK cells where n = 4 mice per condition from one experiment). (e) Representative images and quantification of IHC staining for neutrophils (Ly6G), fibrosis (picrosirius red) and fibroblasts (αSMA) in size-matched hepatic metastases (n = 4 mice per condition; data are from one experiment). Eight images were acquired per IHC staining per mouse and used for quantification as a percentage of the total area. (f) Number of metastatic lesions per liver after intrasplenic injection of CRC cells followed by treatment with vehicle control or lorlatinib (n = 5 mice per group; data are from one experiment). (g) Representative cleaved caspase 3 (Asp175) IHC images of hepatic metastatic tissue from intrasplenic injection of CRC organoids followed by treatment with vehicle control or lorlatinib (n = 5 mice per condition; data are from one experiment). Ten images were acquired per mouse and used for quantification of cleaved caspase 3⁺ cells. Scale bar represents 100 μm (c, g) or 250 μm (e). Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers); hypothesis testing performed using unpaired two-sided Student's t test (a-c, f-g) or two-way ANOVA with Tukey's method for multiple comparisons (d).

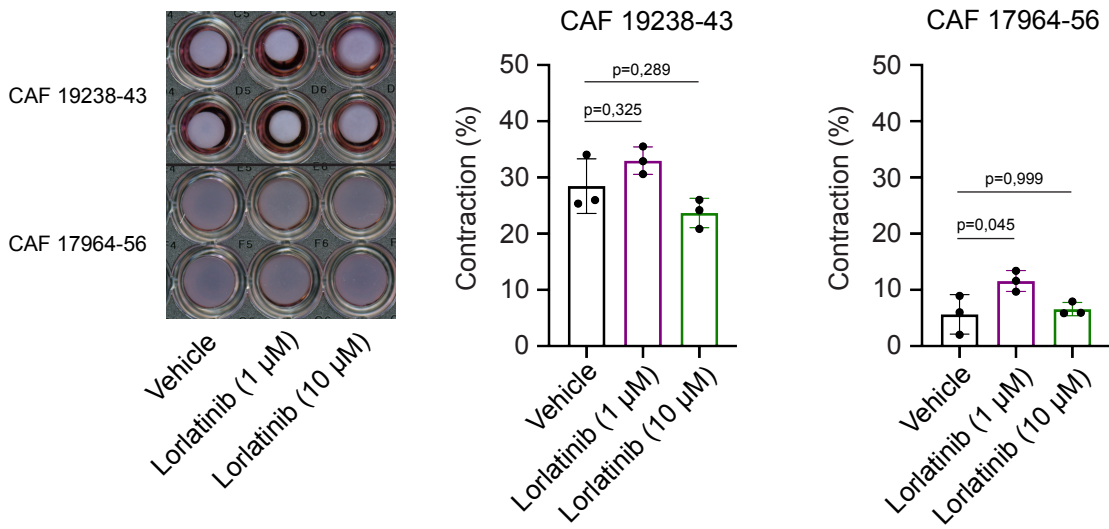


Supplementary figure 5. (a-d) Representative images and quantification of (a) neutrophils (Ly6G⁺), (b) Ki67⁺ cells, (c) fibrosis (picrosirius red) and (d) fibroblasts (α SMA⁺) in tumors from mice treated with a combination of vehicle or lorlatinib and control IgG or anti-Ly6G. Five (Ly6G and α SMA) or eight (Ki67 and picrosirius red) images were acquired per mouse and used for quantifications (n = 9 mice, vehicle + control IgG; n = 10 mice, all other conditions; data are from two experiments). Scale bar represents 100 μ m (a, b, d) or 250 μ m (c). Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers); hypothesis testing performed using two-way ANOVA with Tukey's method for multiple comparisons.

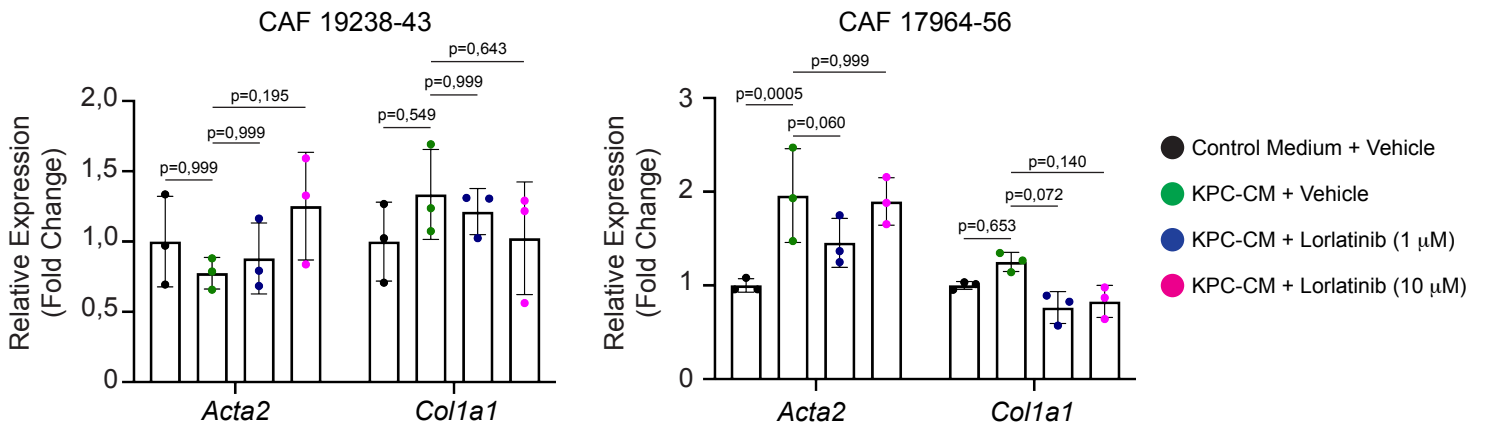
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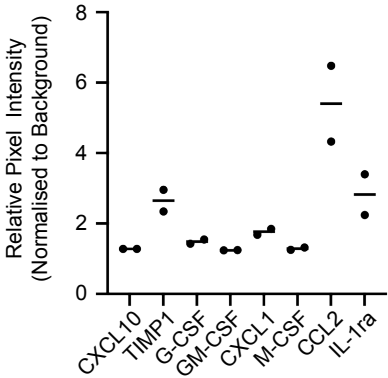
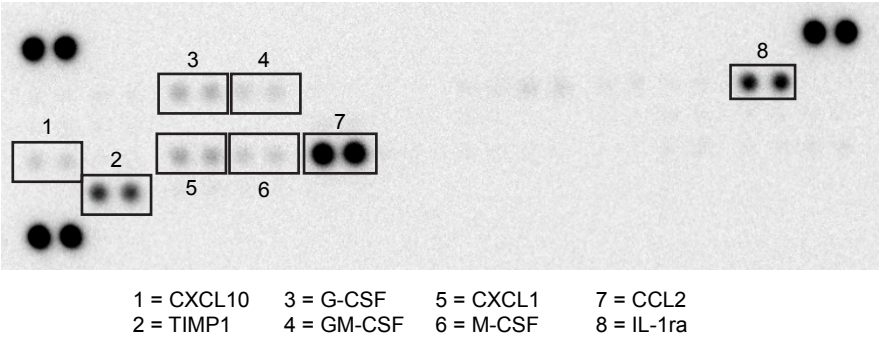


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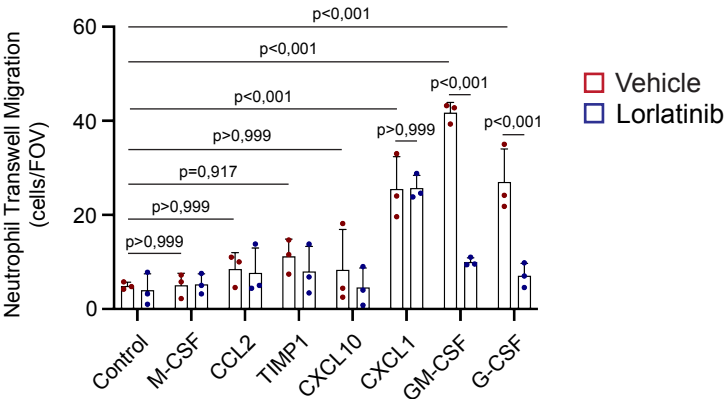


Supplementary figure 6. Lorlatinib does not inhibit the proliferation or activity of cancer-associated fibroblasts (CAFs). (a) Quantification of proliferation of KPC-derived CAF cell lines 19238-43 and 17964-56 in the presence of lorlatinib or vehicle for two days. The relative proliferation was normalized to the measurement of cells seeded on the same plates before treatment with vehicle or lorlatinib (n = 4 independent experiments). (b) Representative images and quantification of CAF-mediated force-contraction of extracellular matrix gels consisting 4 mg/ml collagen I. CAFs were embedded in gels in the presence of lorlatinib or vehicle and analyzed after three days (n = 3 independent experiments). (c) Quantification of *Acta2* and *Colla1* expression by qPCR in CAFs stimulated with KPC-CM in the presence of vehicle or lorlatinib (n = 3 independent experiments). Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers); bar charts show mean and standard deviation from independent experiments with each dot representing the mean of six technical replicates from 1 experiment; hypothesis testing performed using two-way ANOVA with Bonferroni's method for multiple comparisons.

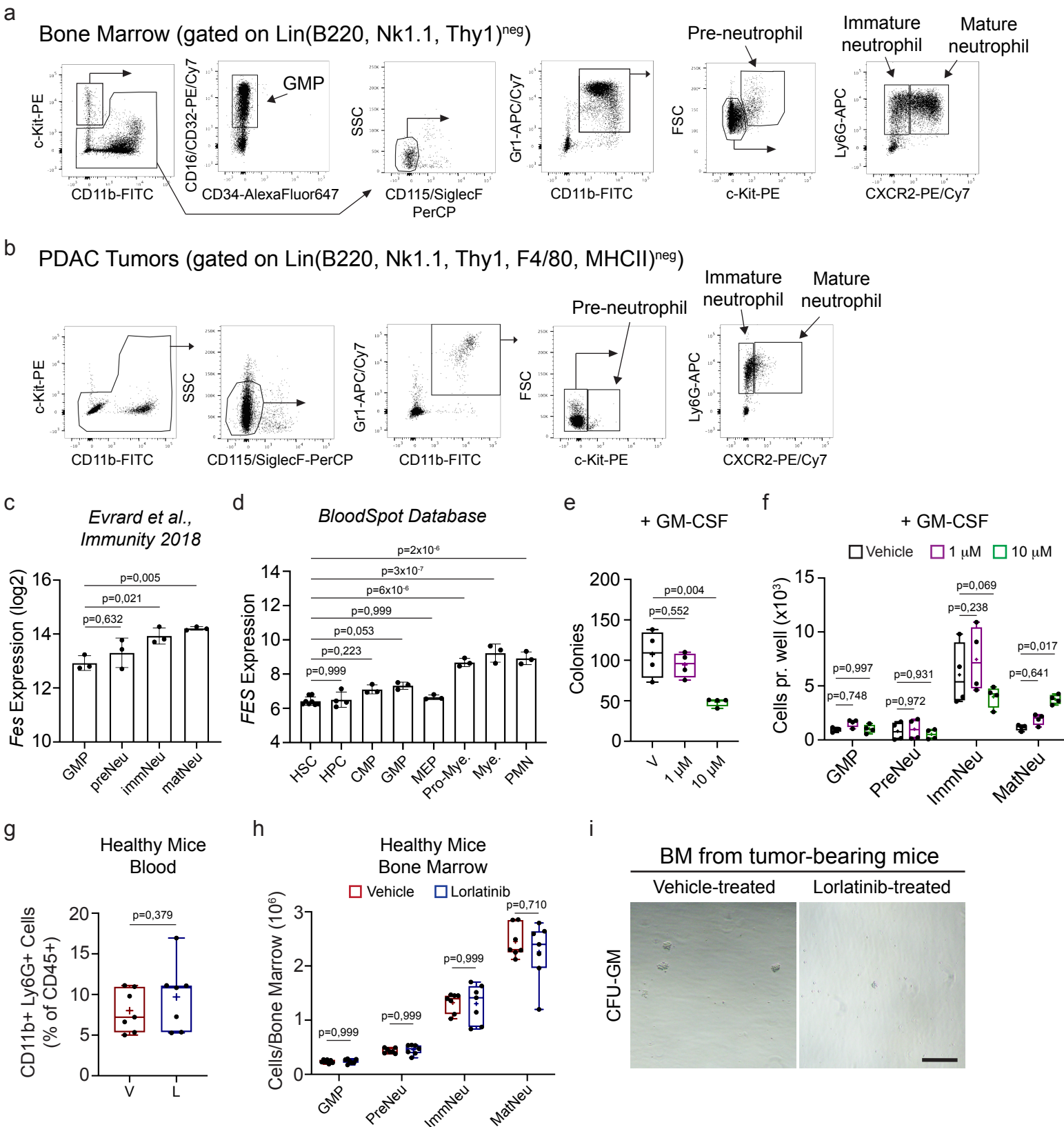
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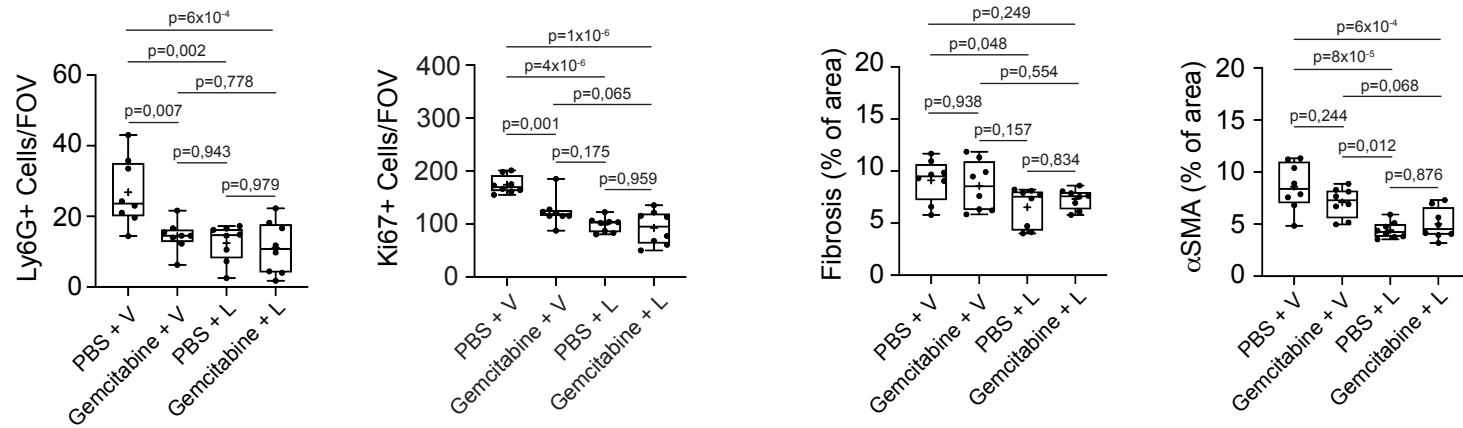
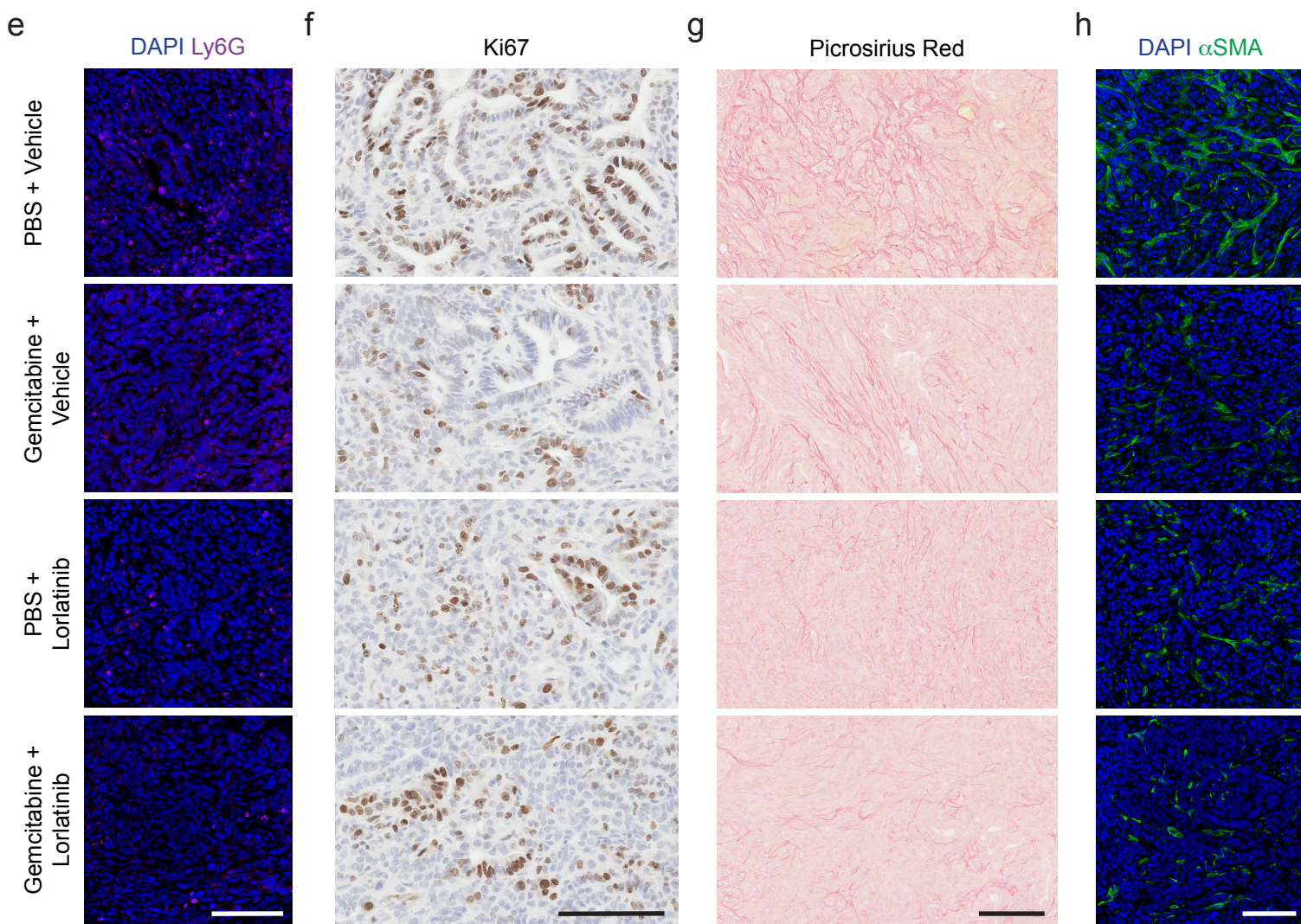
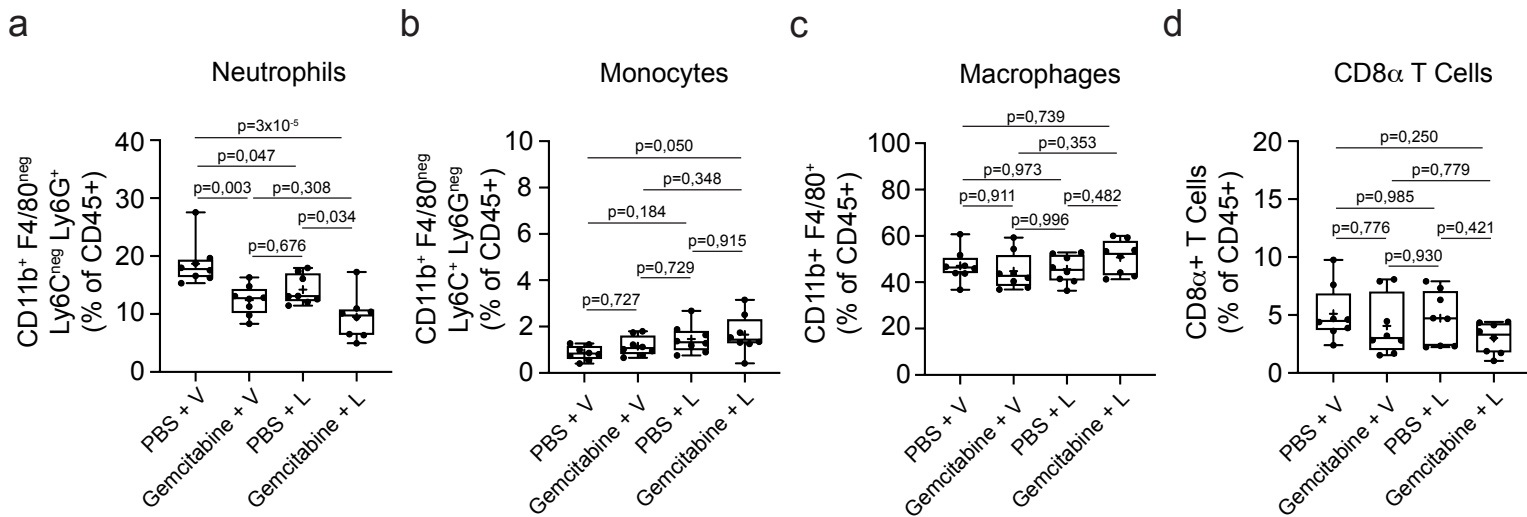
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Supplementary figure 7. Lorlatinib suppresses neutrophil migration in response to G-CSF and GM-CSF. (a) Representative image of the cytokine array and quantification of cytokines in conditioned medium from KPC mT4 cells (n=2 independent experiments). (b) Transwell migration of neutrophils towards recombinant cytokines and chemokines identified in (a) in the presence of vehicle or 1 μ M lorlatinib. Neutrophils were stimulated with 10ng/ml of CXCL10, G-CSF GM-CSF, M-CSF or CCL2, 20ng/mL of CXCL1 or 100ng/mL of TIMP-1 (n = 3 independent experiments). Lines show mean; Bar charts show mean and standard deviation from independent experiments with each dot representing the mean of five field of views (FOVs) from each experiment; hypothesis testing performed using two-way ANOVA with Bonferroni's method for multiple comparisons.



Supplementary figure 8. Lorlatinib suppresses neutrophil development. (a) Representative flow cytometry gating strategy for granulocyte-macrophage progenitors (GMP) and neutrophil subsets (pre-, immature and mature neutrophils) in the BM of tumor-bearing mice treated with vehicle or lorlatinib. (b) Representative gating strategy of neutrophil subsets in PDAC tumors from mice treated with vehicle or lorlatinib. (c) *Fes* expression in FACS-sorted murine BM-derived GMPs or neutrophil subsets from Evrard et al., Immunity 2018 (ref.1) (n = 3 mice per group). (d) *FES* expression in subsets of human hematopoietic cells from the BM of healthy donors in the BloodSpot database (ref. 2) (n = 8 biologically independent samples, HSC; n = 4 biologically independent samples, HPC; n = 3 biologically independent samples per group, remaining groups). Cell populations are labelled. HSC, hematopoietic stem cells; HPC, hematopoietic progenitor cells; CMP, common myeloid progenitor; GMP, granulocyte-monocyte progenitor; MEP, megakaryocyte–erythroid progenitor; pro-Mye., promyelocyte; Mye., myelocyte; PMN, polymorphonuclear neutrophils. (e, f) Number of colonies from CFU-GM assay (e) and flow cytometry analysis (f) of neutrophils after culture in semi-solid methylcellulose medium containing recombinant cytokines (SCF, IL-3 and IL-6) for 3 days in the presence of recombinant GM-CSF and lorlatinib or vehicle (n = 4 independent experiments). (g) Flow cytometry analysis of neutrophils (CD45⁺ CD11b⁺ Ly6G⁺) in blood samples from healthy (tumor-free) mice treated with lorlatinib or vehicle for 14 days (n = 7 mice per group; data are from two independent experiments). (h) Flow cytometry analysis of GMPs and neutrophil subsets in BM from healthy (tumor-free) mice treated for 14 days with lorlatinib or vehicle (n = 7 mice per group; data are from two independent experiments). (i) Representative images of *colony forming unit-granulocyte macrophage* (CFU-GM) assay (from Fig. 3k) with BM cells isolated from mice with orthotopic PDAC tumors after treatment with vehicle or lorlatinib. Scale bar represents 200 μ m. Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers); bar charts show mean and standard deviation from independent biological replicates; hypothesis testing performed using unpaired two-sided Student's t test (g) or two-way ANOVA with Tukey's (c,e,f) or Sidak's (d) method for multiple comparisons.



Supplementary figure 9. Combination of lorlatinib with gemcitabine does not further improve the therapeutic response to chemotherapy. (a-d) Flow cytometry analysis of (a) neutrophils, (b) monocytes, (c) macrophages or (d) CD8⁺ T cells in tumors from mice treated with a combination of vehicle or lorlatinib and PBS or gemcitabine (n = 8 mice per condition; data are from two independent experiments). (e-h) Representative images and quantification of (e) neutrophils (Ly6G⁺), (f) Ki67⁺ cells, (g) fibrosis (picosirius red) and (h) fibroblasts (α SMA⁺) in tumors from mice treated with a combination of vehicle or lorlatinib and PBS or gemcitabine. Five (Ly6G and α SMA) or eight (Ki67 and picosirius red) images were acquired per mouse and used for quantifications (n = 8 mice per condition; data are from two independent experiments). Scale bars represent 100 μ m (e, f, h) or 250 μ m (g). Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers); hypothesis testing performed using two-way ANOVA with Tukey's method for multiple comparisons.

Supplementary Table 1.**Antibodies for flow cytometry**

Name	Host	Clone	Company	Cat. No.	Dilution
PE/Cy7 anti-mouse CD45	Rat	30-F11	Biolegend	103114	1:100 (FC)
PE anti-mouse/human CD11b	Rat	M1/70	Biolegend	101208	1:100 (FC)
FITC anti-mouse/human CD11b	Rat	M1/70	Biolegend	101206	1:100 (FC)
PE anti-mouse F4/80	Rat	BM8	Biolegend	123110	1:100 (FC)
APC anti-mouse F4/80	Rat	BM8	Biolegend	122116	1:100 (FC)
PerCP anti-mouse Ly-6C	Rat	HK1.4	Biolegend	128028	1:100 (FC)
APC anti-mouse Ly6G	Rat	1A8	Biolegend	127614	1:100 (FC)
PE anti-mouse CD3e	Armenian Hamster	145-2C11	Biolegend	100308	1:100 (FC)
PerCP anti-mouse CD4	Rat	RM4-5	Biolegend	100537	1:100 (FC)
APC anti-mouse CD8	Rat	53-6.7	Biolegend	100712	1:100 (FC)
APC anti-mouse/human CD45R/B220	Rat	RA3-6B2	Biolegend	103212	1:100 (FC)
PerCP anti-mouse Nk-1.1	Mouse	PK136	Biolegend	108726	1:100 (FC)
Brilliant Violet 421™ anti-mouse/human CD45R/B220	Rat	RA3-6B2	Biolegend	103239	1:100 (FC)
Brilliant Violet 421™ anti-mouse NK-1.1	Rat	PK136	Biolegend	108731	1:100 (FC)
Brilliant Violet 421™ anti-mouse CD90.2 (Thy-1.2)	Rat	53-2.1	Biolegend	140327	1:100 (FC)
Brilliant Violet 421™ anti-mouse F4/80	Rat	BM8	Biolegend	123131	1:100 (FC)
Brilliant Violet 421™ anti-mouse I-A/I-E	Rat	M5/114.15.2	Biolegend	107631	1:100 (FC)
PE anti-mouse CD117 (c-Kit)	Rat	2B8	Biolegend	105808	1:100 (FC)
PerCP/Cyanine5.5 anti-mouse CD115 (CSF-1R)	Rat	AFS98	Biolegend	135526	1:100 (FC)
BD Pharmingen™ PerCP-Cy™5.5 Rat Anti-Mouse Siglec-F	Rat	E50-2440	BD Pharmingen	565526	1:100 (FC)
APC/Cyanine7 anti-mouse Ly-6G/Ly-6C (Gr-1)	Rat	RB6-8C5	Biolegend	108424	1:100 (FC)

PE/Cy7 anti-mouse CD182 (CXCR2)	Rat	SA044G4	Biolegend	149316	1:100 (FC)
Brilliant Violet 711™ anti-mouse CD184 (CXCR4)	Rat	L276F12	Biolegend	146517	1:100 (FC)
PE/Cy7 anti-mouse CD16/32	Rat	S17011E	Biolegend	156609	1:100 (FC)
Alexa Fluor® 647 Rat anti-Mouse CD34	Rat	RAM34	BD Pharmingen	560233	1:100 (FC)
APC/Cyanine7 anti-mouse CD69	Hamster	H1.2F3	Biolegend	104525	1:100 (FC)
FITC anti-mouse/human CD44	Rat	IM7	Biolegend	103022	1:100 (FC)

Supplementary Table 2.**Antibodies for immunohistochemistry, immunofluorescence & western blotting**

Name	Host	Clone	Company	Cat. No.	Dilution
F4/80	Rat	BM8	ThermoFisher Scientific	14-4801-85	1:100 (IF)
Ly6G	Rat	1A8	Biolegend	127602	1:50 (IF)
α SMA	Rabbit		Abcam	ab5694	1:200 (IF), 1:1000 (IHC)
CD8a	Rabbit	D4W2Z	Cell Signaling	98941	1:200 (IF)
Ki67	Rabbit	D3B5	Cell Signaling	12202	1:100 (IHC)
Cleaved Caspase 3 (Asp175)	Rabbit		Cell Signaling	9661	1:100 (IHC)
p-tyr705 STAT3	Rabbit	D3A7	Cell Signaling	9145	1:1000 (WB)
STAT3	Rabbit	C-20	Santa Cruz	sc-482	1:1000 (WB)
p-tyr694 STAT5	Rabbit	C71E5	Cell Signaling	9314	1:1000 (WB)
STAT5	Rabbit	D206Y	Cell Signaling	94205	1:1000 (WB)
Vinculin	Mouse	hVIN-1	Sigma	V9131	1:200 (WB)
Tubulin	Mouse	DM1A	Sigma	T6199	1:1000 (WB)
Anti-Rabbit IgG H&L (Alexa Fluor® 488)	Goat		Abcam	ab150077	1:500 (IF)
Anti-Rat IgG H&L (Alexa Fluor® 555)	Goat		Abcam	ab150158	1:500 (IF)
Anti-rabbit HRP	Goat		Dako	G-21234	1:1000 (WB)
Anti-mouse HRP	Rabbit		Dako	P0260	1:1000 (WB)
Dako EnVision+ System- HRP Labelled Polymer Anti-mouse			Dako	K4001	
Dako EnVision+ System- HRP Labelled Polymer Anti-Rabbit			Dako	K4003	

Supplementary References

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2. Bagger, F. O., Kinalis, S. & Rapin, N. BloodSpot: A database of healthy and malignant haematopoiesis updated with purified and single cell mRNA sequencing profiles. *Nucleic Acids Res.* **47**, D881–D885 (2019).