Appendix

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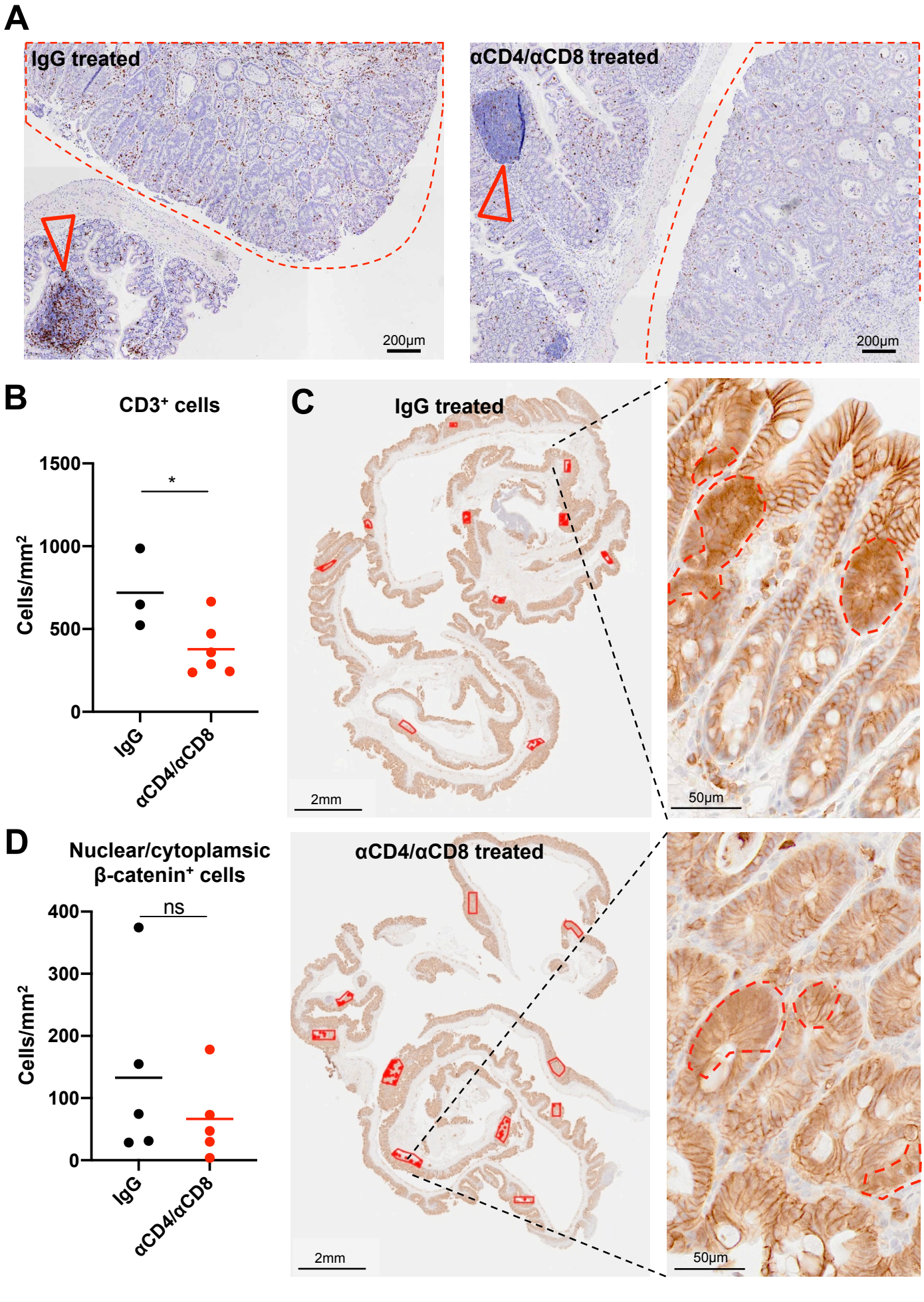

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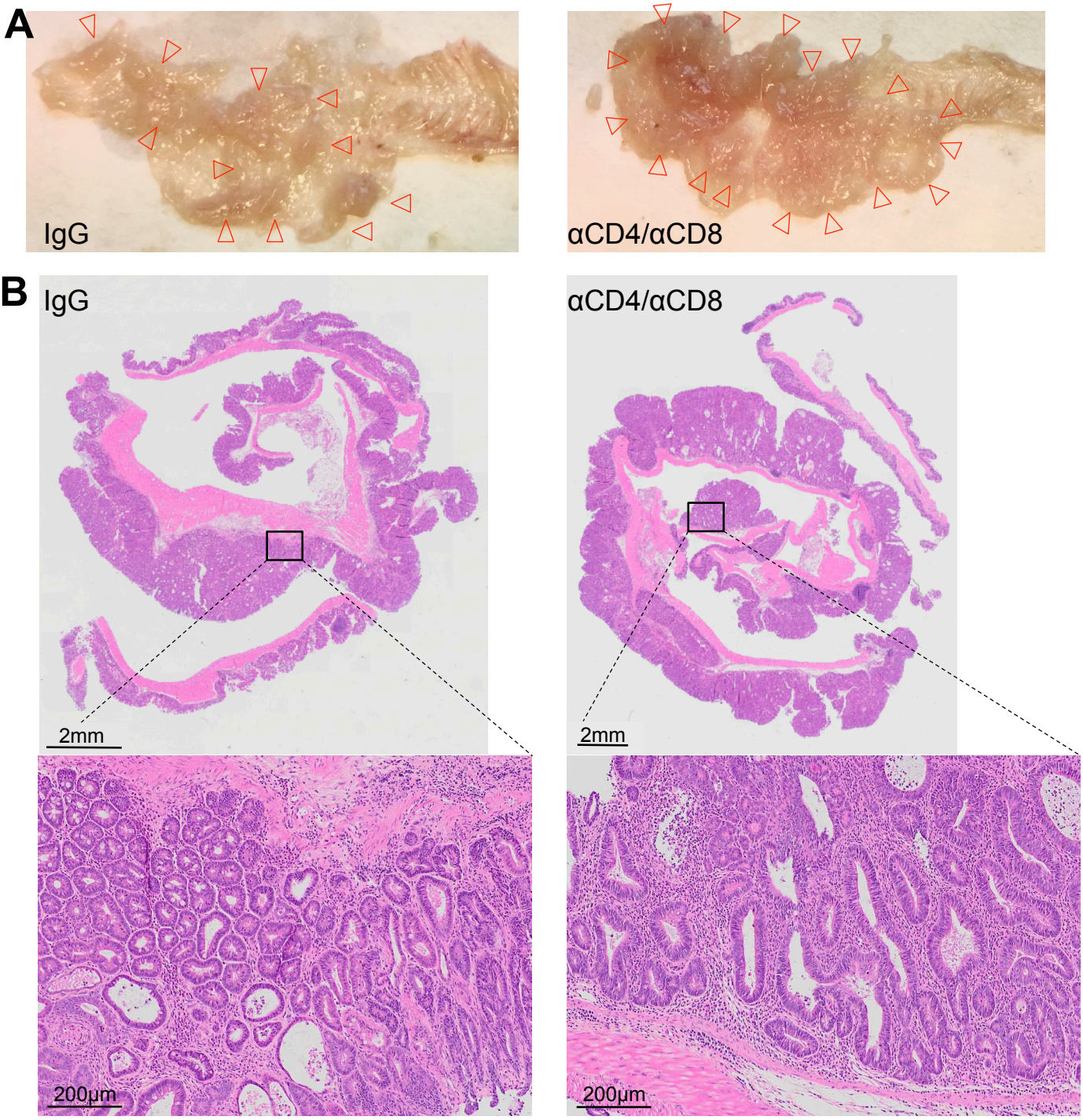

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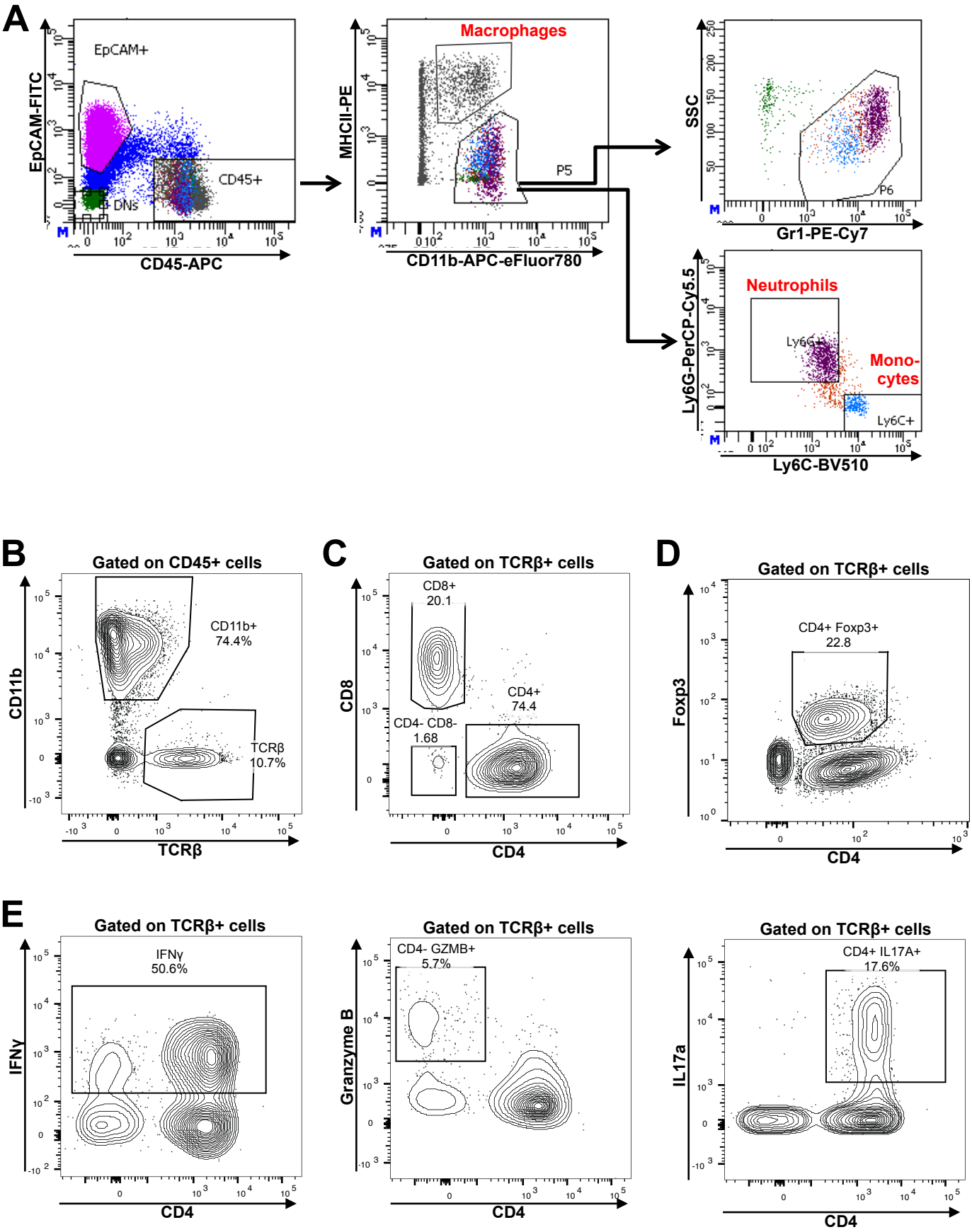

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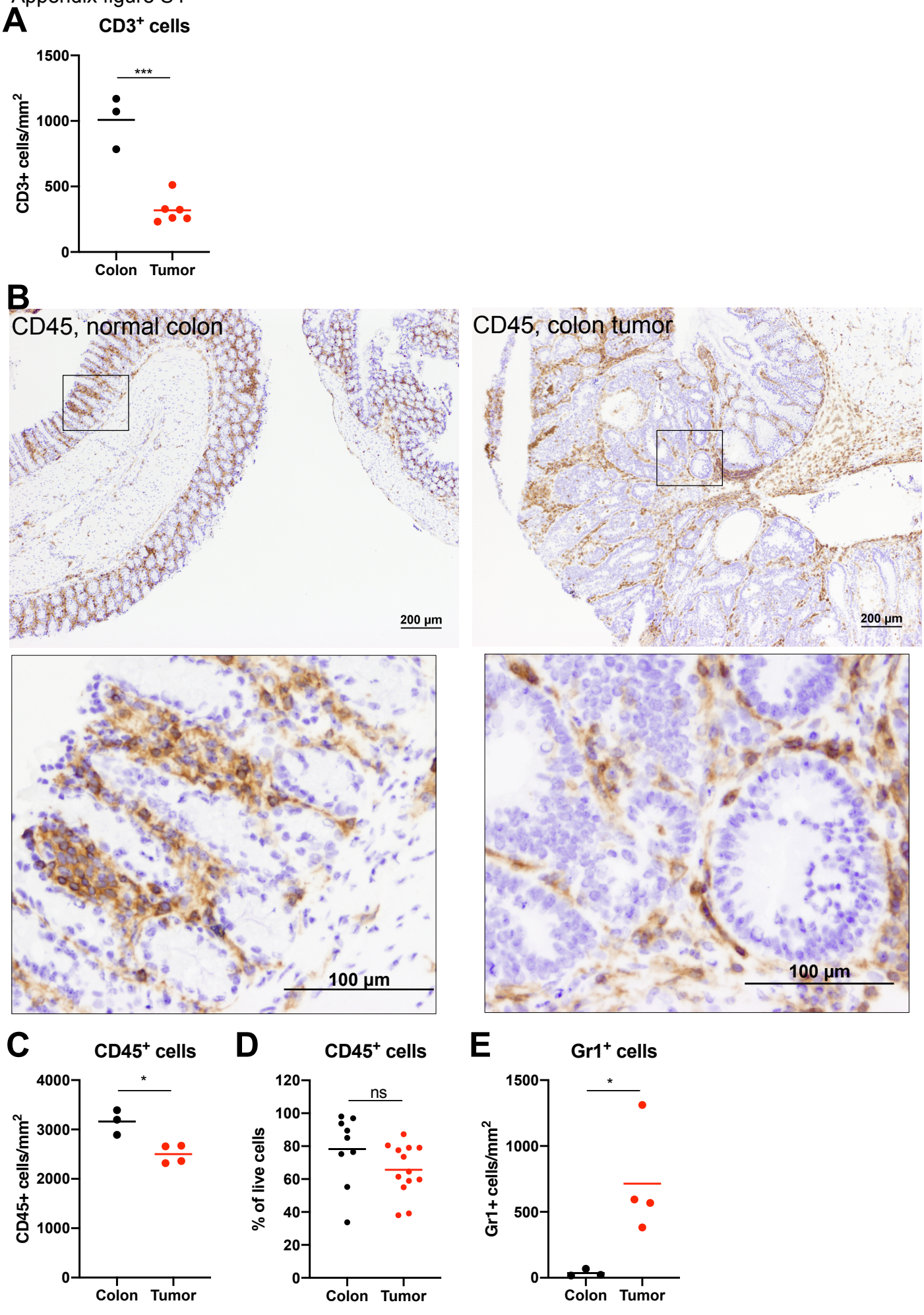

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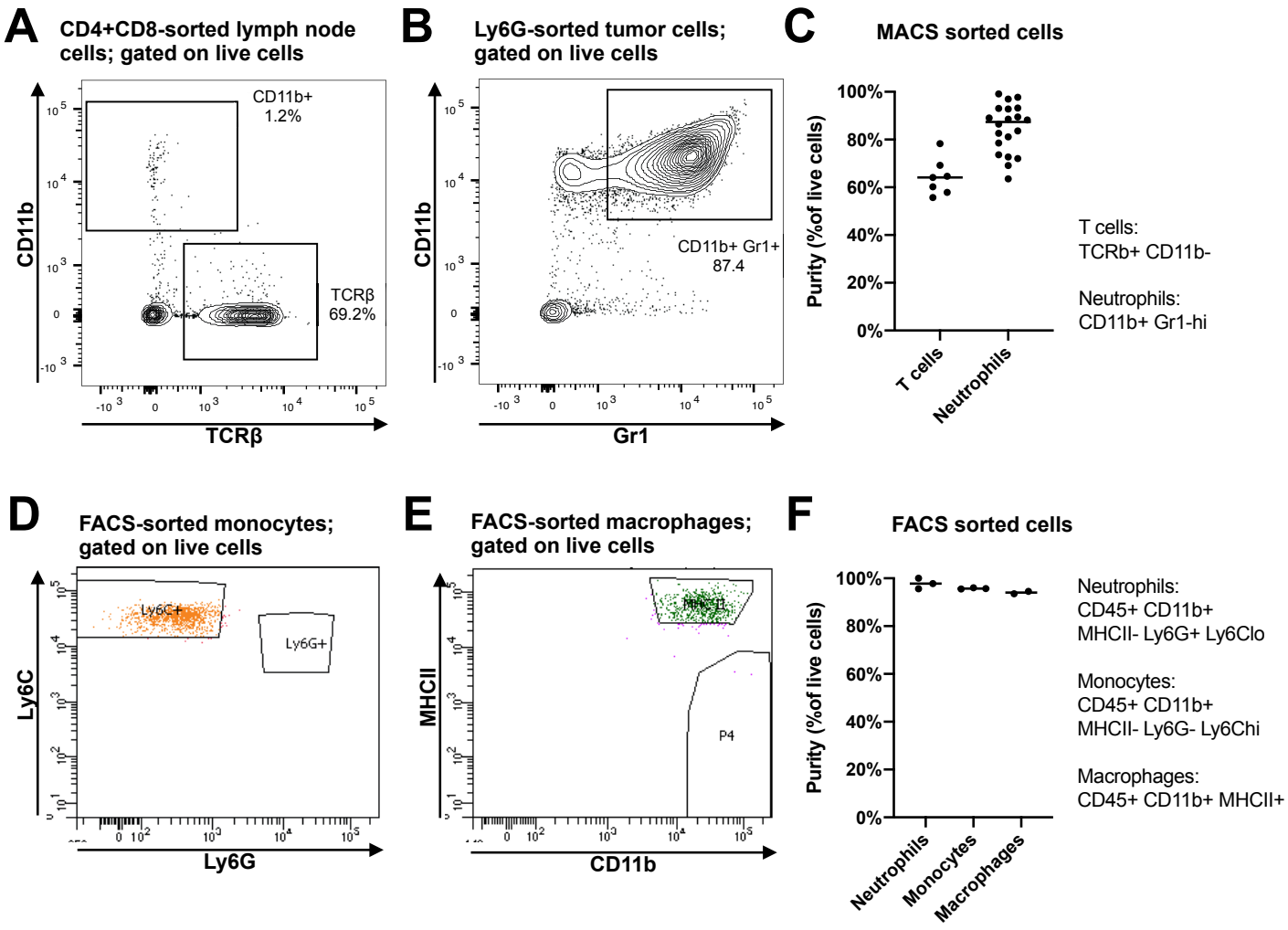

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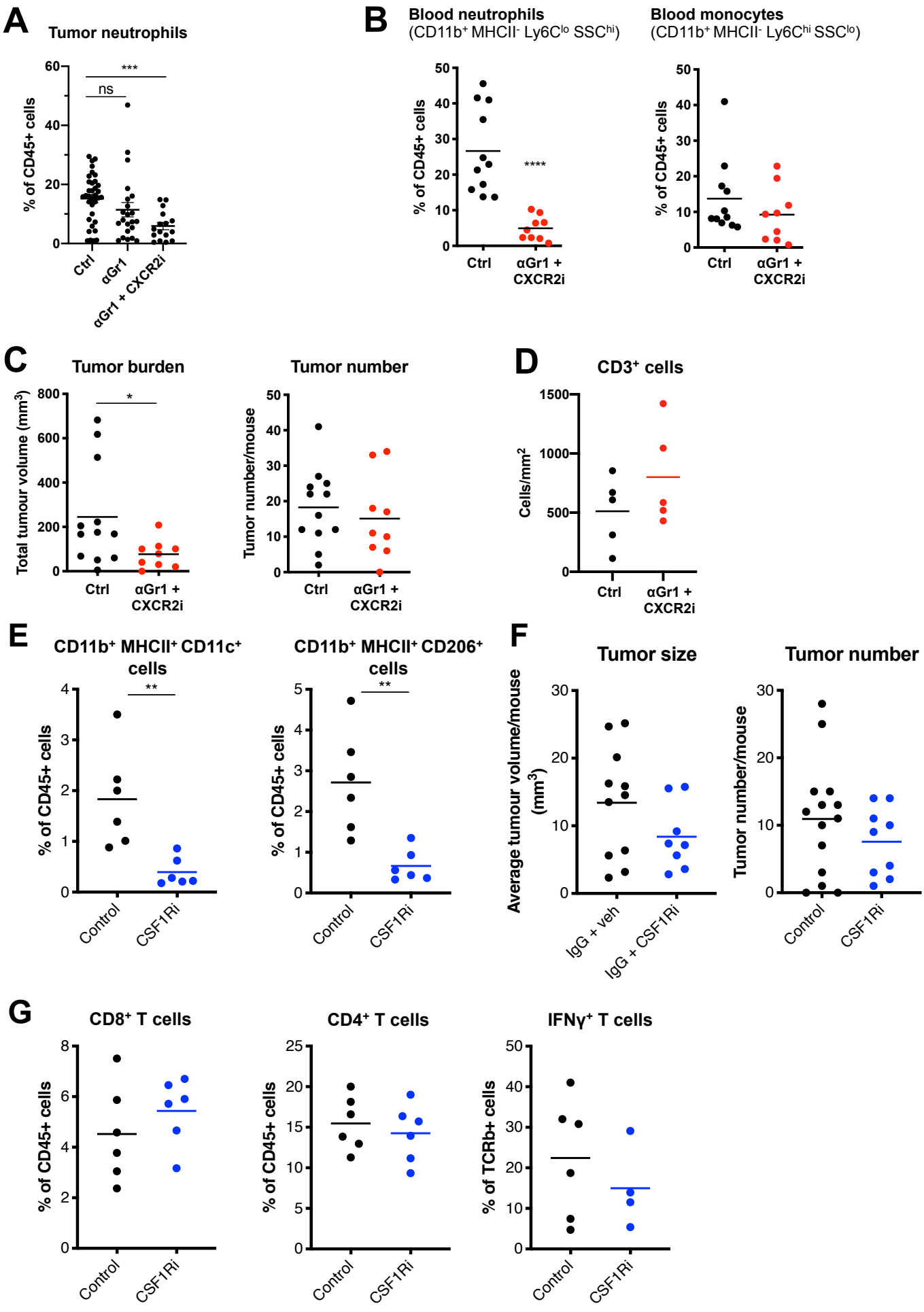

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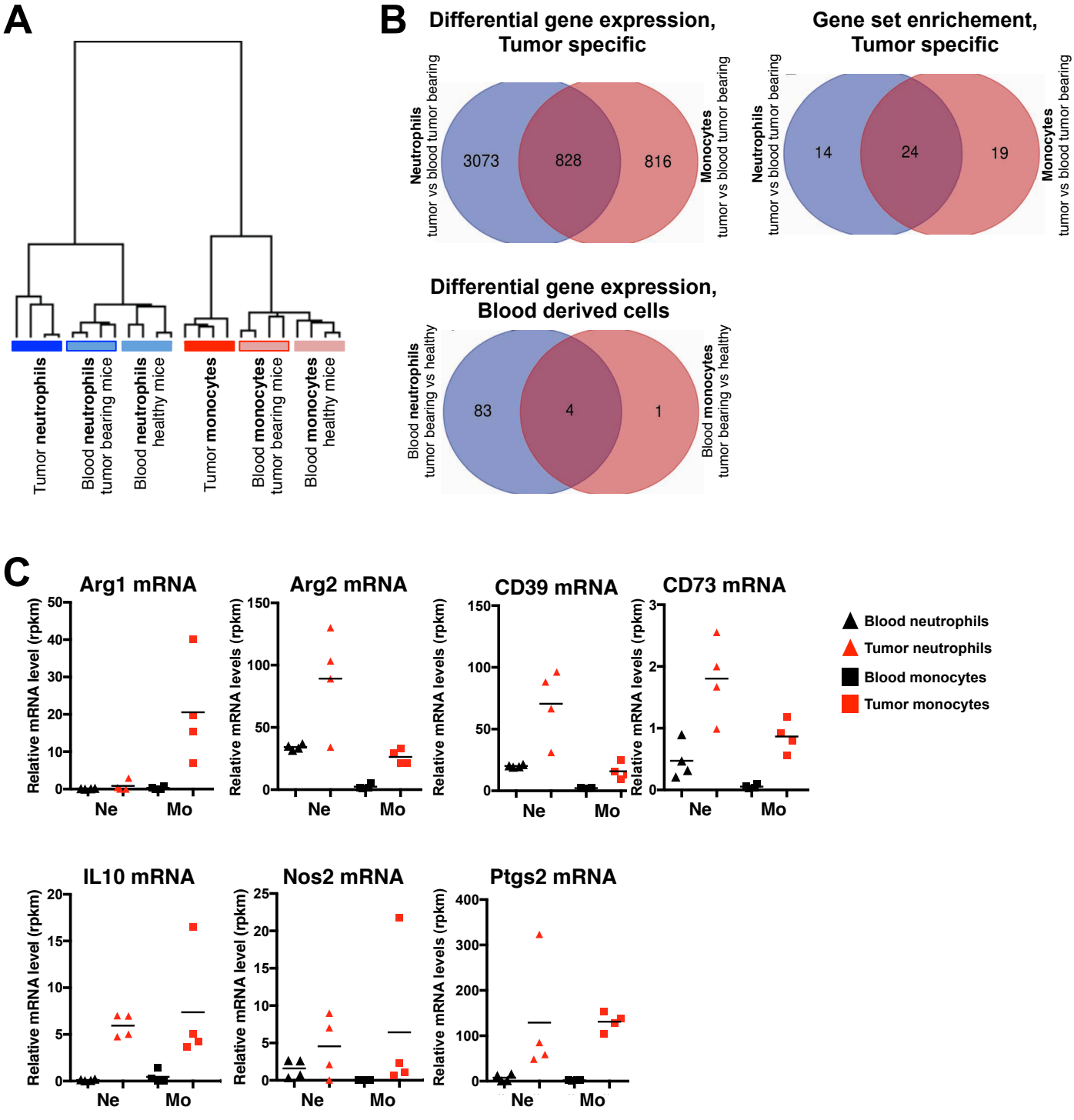

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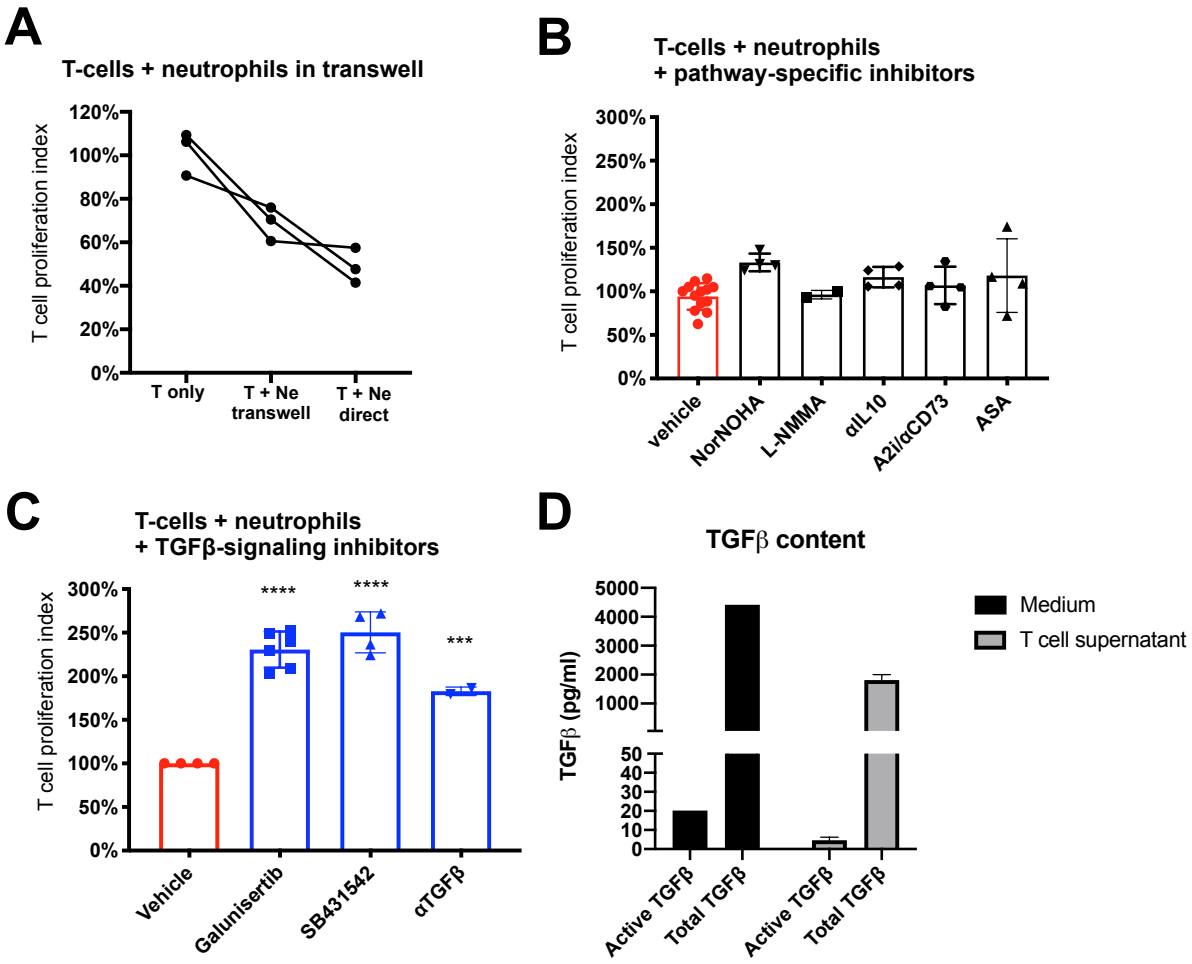

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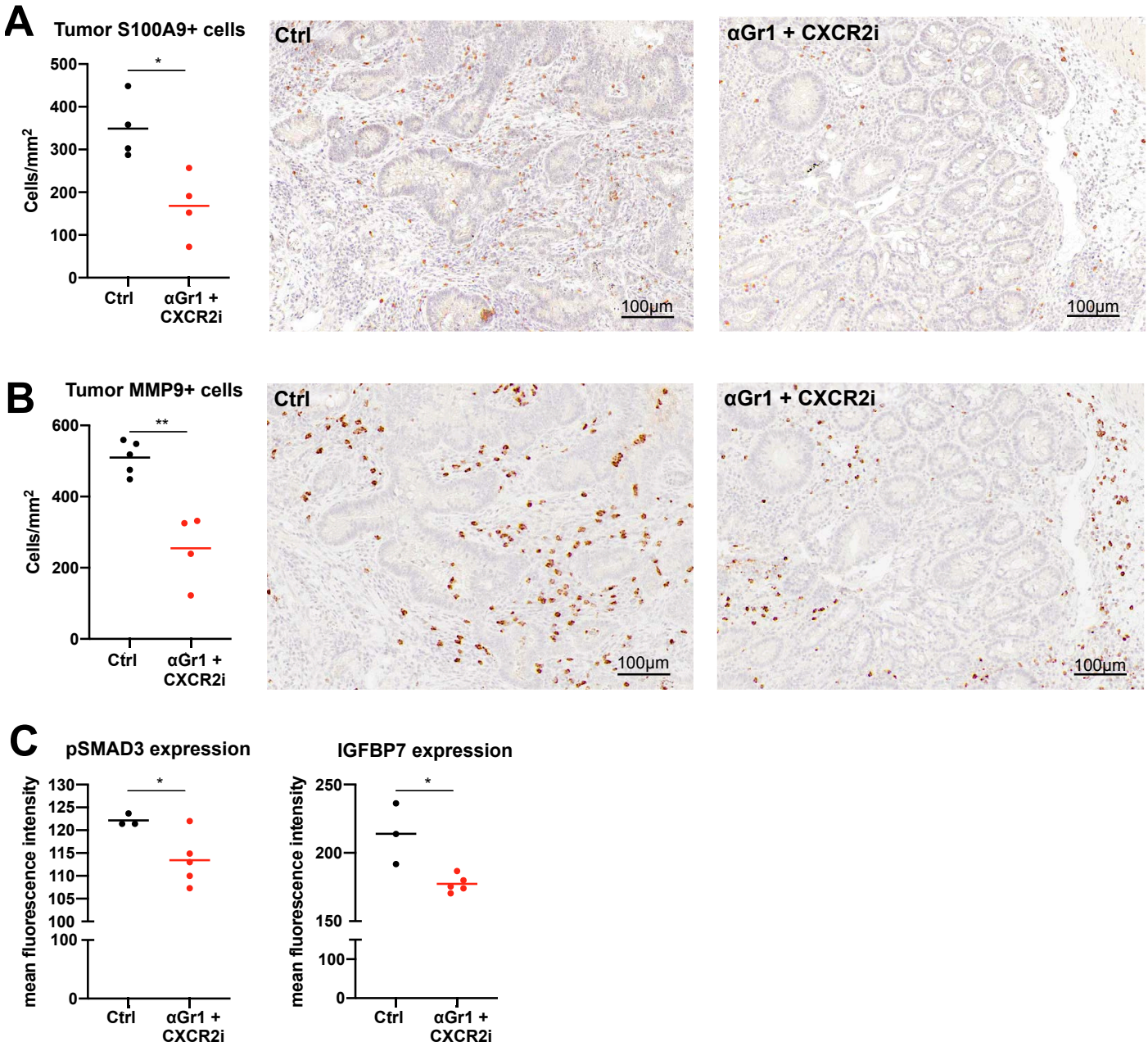

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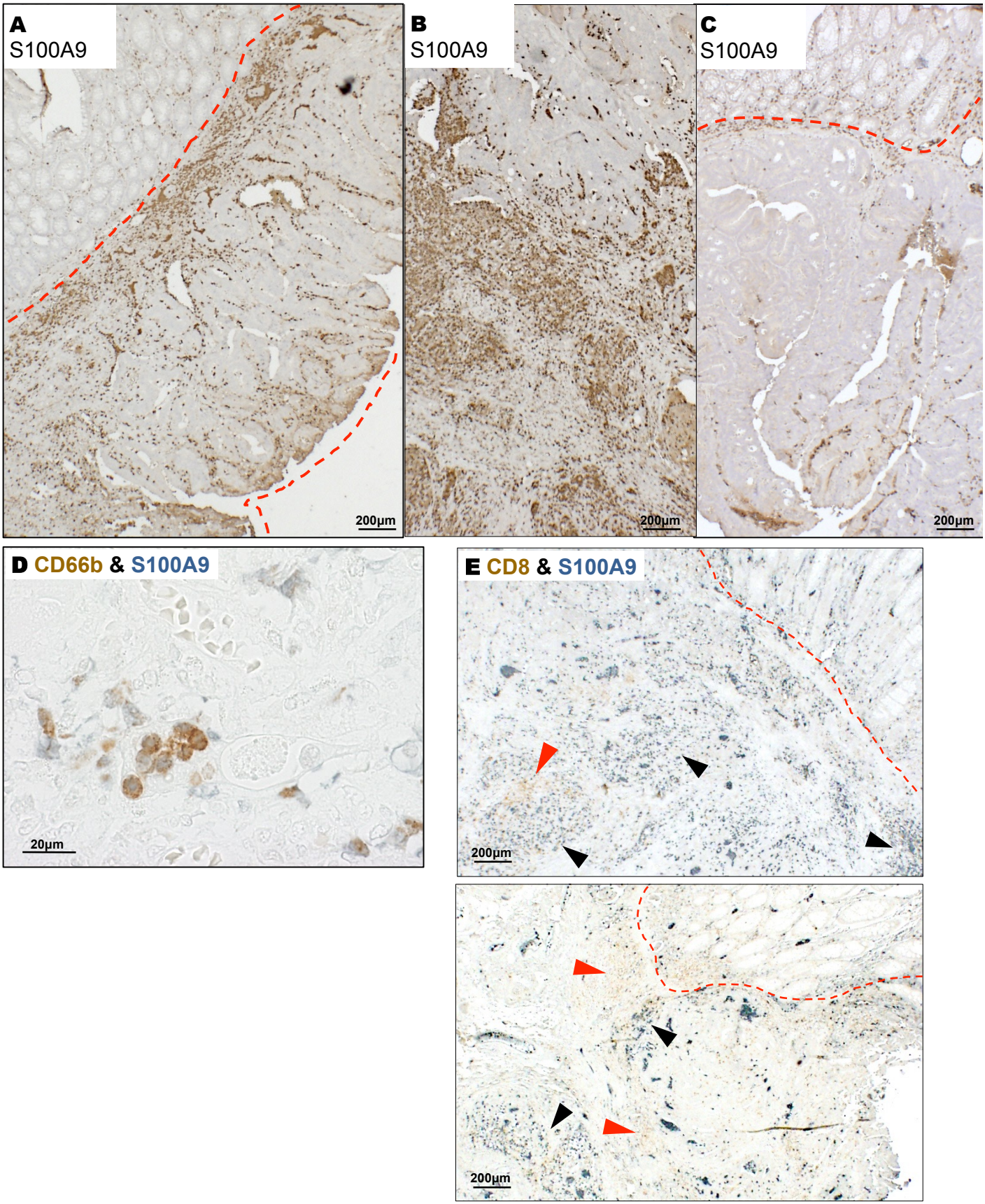

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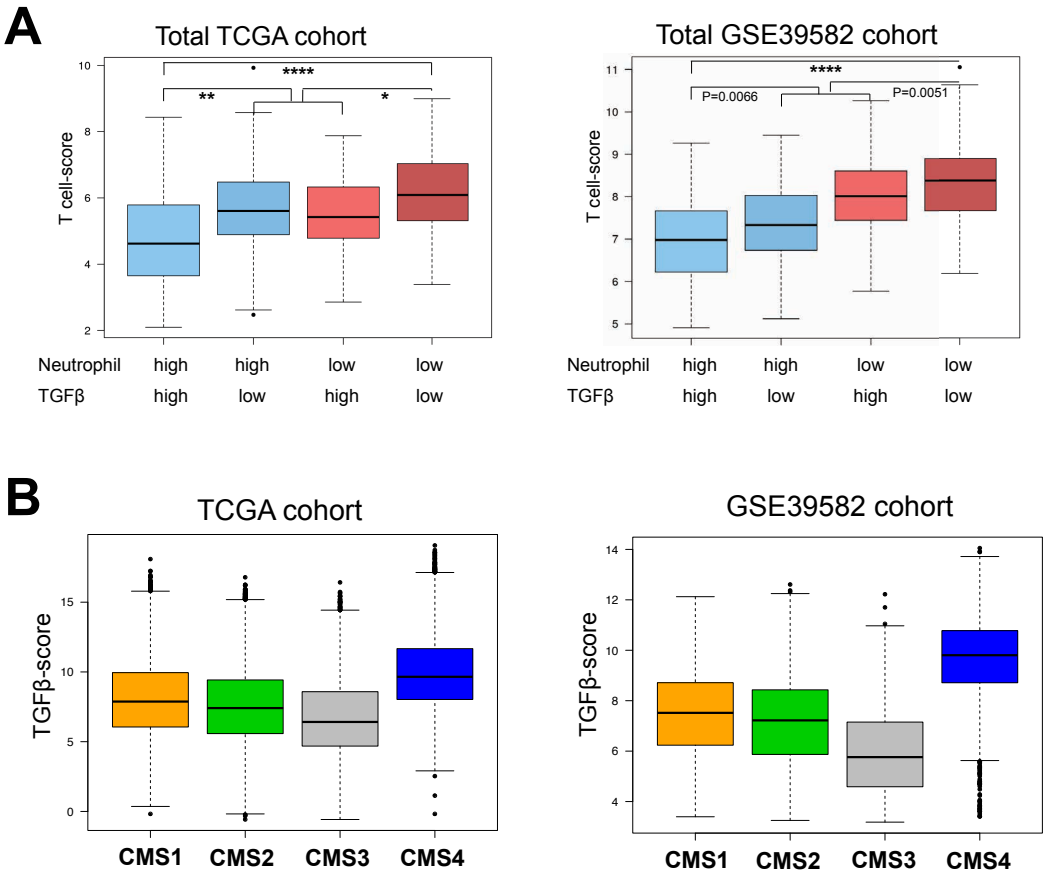

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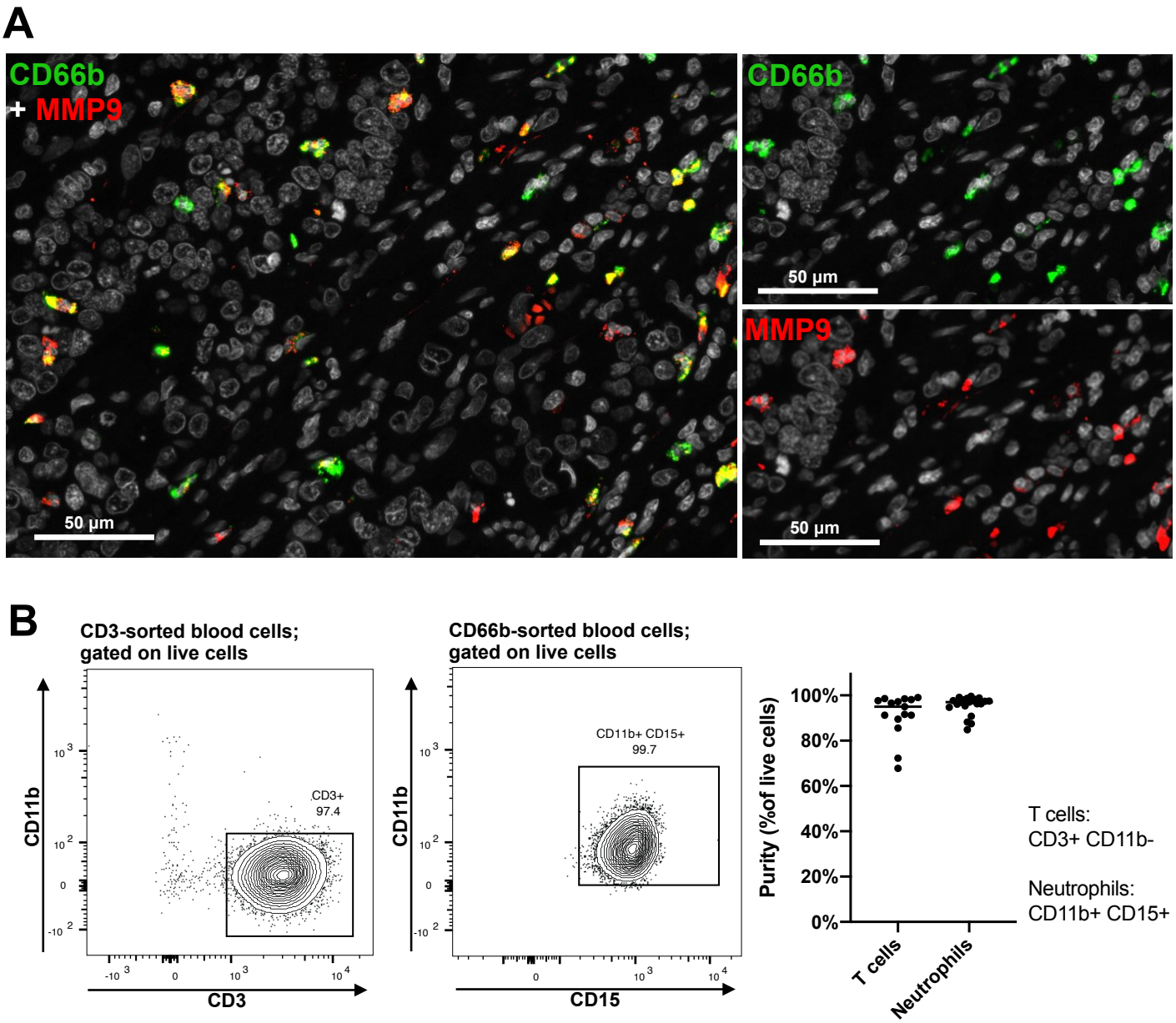

Appendix figure legends

Figure S1. Effect of anti-CD4/anti-CD8 treatment on mouse colon adenoma T cell infiltration and on Tamoxifen-induced CreERT recombinase activity.

A and B, $Apc^{fl/fl\text{-}Cdx2CreERT2}$ mice were treated with Tamoxifen and one day post treatment injected with anti-CD4 plus anti-CD8 neutralizing antibodies (αCD4/αCD8) or IgG control twice a week for six weeks. Colons were then excised and tissue sections were stained with anti-CD3 antibody and $CD3^+$ cells were quantified. A, Representative anti-CD3 immunostaining on sections derived from IgG treated (left panel) and αCD4/αCD8 treated (right panel) mice. Dashed lines outline tumor tissue. Arrowheads indicate tertiary lymphoid structures in adjacent benign colon mucosa. B, Quantification of $CD3^+$ cells in sections of tumors derived from IgG- (n=3) and αCD4/αCD8-treated (n=6) mice. At least five arbitrarily selected areas per section were scored.

C and D, $Apc^{fl/fl\text{-}Cdx2CreERT2}$ mice were treated with Tamoxifen and one day post treatment injected with anti-CD4 plus anti-CD8 neutralizing antibodies (αCD4/αCD8) or IgG control twice a week for five days. Colons were then excised and tissue sections were stained with anti-β-catenin antibody and cells with detectable nuclear β-catenin were quantified. C, Representative anti-β-catenin immunostaining on sections derived from IgG-(upper panel) and αCD4/αCD8-treated (lower panel) mice. Right panels are higher magnifications of indicated areas in left panels. Red delineated areas in left panels were the quantified areas on those sections. Red dashed lines in right panels outline cells with increased nuclear and cytoplasmic β-catenin staining. D, Quantification of nuclear and cytoplasmic β-$catenin^+$ cells in sections of tumors derived from IgG- (n=5) and αCD4/αCD8-treated (n=5) mice. Ten arbitrarily selected areas per section were scored.

Data information: In (B and D), statistical analysis was performed by unpaired two-tailed Student's t-tests. *: $p<0.05$; ***: $p<0.001$. Exact p-values are provided in Appendix table S4.

Figure S2. Effect of anti-CD4/anti-CD8 treatment on mouse colon adenoma phenotype.

Apc$^{fl/fl\text{-}Cdx2CreERT2}$ mice were treated with Tamoxifen and one day post treatment injected with anti-CD4 plus anti-CD8 neutralizing antibodies (αCD4/αCD8), anti-CD8 neutralizing antibody alone (αCD8) or IgG control twice a week for five to six weeks.

A, Dissected and cleaned cecum and proximal colon of *Apc*$^{fl/fl\text{-}Cdx2CreERT2}$ mice at the end of indicated treatments. Red arrowheads outline tumor containing tissue.

B, Cross sections of cecum and proximal colon of mice at the end of indicated treatments, stained with hematoxylin/eosin. Lower panels are higher magnifications of indicated areas in upper panels. Tumors in both IgG and αCD4/αCD8 treated mice displayed with an adenomatous, non-invasive phenotype.

C and D, Comparison of αCD8 treatment to αCD4/αCD8 treatment. C, Relative numbers of $CD8^+$ $TCR\beta^+$ cells determined by FACS in blood (left panel) and tumors (right panel) in mice at the end of indicated treatments (IgG: n=11; αCD8: n=7). D, Number (left panel) and average tumor size (right panel) per mouse at the end of indicated treatments (Pooled data from two independent experiments; IgG: n=18; αCD8: n=9; αCD4/αCD8: n=16).

Data information: In (C) and (D), each dot represents an individual mouse and exact group sizes are mentioned in brackets above. Statistical analysis was performed by

unpaired two-tailed Student's t-tests. **: p<0.01; ****: p<0.0001. Exact p-values are provided in Appendix table S4.

Figure S3. FACS gating strategy to identify and sort individual myeloid cell and T cell populations.

A, Gating strategy to identify and sort EpCAM^{+} CD45^{-} epithelial cells, EpCAM^{-} CD45^{-} stromal cells, CD45^{+} CD11b^{+} MHCII^{+} macrophages, CD45^{+} CD11b^{+} MHCII^{-} Ly6G^{+} Ly6C^{low} neutrophils and CD45^{+} CD11b^{+} MHCII^{-} Ly6G^{-} Ly6C^{high} monocytes in tumor derived single cell suspension.

B, Gating strategy to identify TCRβ^{+} cells among CD45^{+} cells in tumor or blood derived single cell suspension.

C, Gating strategy to identify CD4^{+} and CD8^{+} T cell subsets among CD45^{+} TCRβ^{+} cells in tumor or blood derived single cell suspension.

D, Gating strategy to identify Foxp3^{+}CD4^{+} regulatory T cells among CD45^{+} TCRβ^{+} cells in tumor derived single cell suspension.

E, Gating strategy to identify IFNγ^{+} (left panel), CD4^{-} Granzyme B^{+} (middle panel) and CD4^{+} IL17a^{+} (right panel) effector T cell subsets among CD45^{+} TCRβ^{+} cells in tumor derived single cell suspension.

Figure S4. Quantification of CD3^{+} T cells, CD45^{+} hematopoietic cells and Gr1^{+} myeloid cells in mouse normal colon and colon adenomas by immunohistochemistry.

Colons of healthy *Apc*$^{fl/fl}$ (Colon) or tumor bearing (Tumor) *Apc*$^{fl/fl\text{-}Cdx2CreERT2}$ mice 10-14 weeks after Tamoxifen treatment.

A, Quantification of $CD3^+$ cells using immunohistochemistry. Colon: n=3; Tumor: n=6.

B, Immunohistochemical staining for $CD45^+$ on representative sections of healthy normal colon (left panels) or colon tumor (right panels). Lower panels are higher magnifications of indicated areas in upper panels.

C, Quantification of $CD45^+$ cells using immunohistochemistry. Colon: n=3; Tumor: n=4.

D, Quantification of $CD45^+$ cells using FACS analysis described in Appendix Figure S3A. Colon: n=9; Tumor: n=13.

E, Quantification of $Gr1^+$ cells using immunohistochemistry. Colon: n=3; Tumor: n=4.

Data information: In (A) and (C-E), each dot represents an individual mouse. Statistical analysis was performed by unpaired two-tailed Student's t-tests. *: $p<0.05$; ***: $p<0.001$. Exact p-values are provided in Appendix table S4.

Figure S5. Purity control of mouse T cells and neutrophils used for *in vitro* experiments.

A - C, Purity of tumor-derived MACS sorted T cells and neutrophils used for *in vitro* co-culture assays**.** A, Representative purity test using TCRβ and CD11b FACS analysis on anti-CD4/anti-CD8 isolated lymph node cells. B, Representative purity test using Gr1 and CD11b FACS analysis on anti-Ly6G isolated tumor cells. C, Quantification of the purity of isolated $TCRb^+$ $CD11b^-$ cells and $CD11b^+$ $Gr1^+$ cells. Each dot represents an individually isolated sample. T cells: n=7; neutrophils: n=20.

D - F, Purity of tumor-derived FACS sorted neutrophils, monocytes and macrophages for *in vitro* co-culture assays and for cell type specific gene expression analysis. The gating strategy used is described in Appendix Fig. S3A. D,

Representative purity test on $CD45^+$ $CD11b^+$ $MHCII^-$ $Ly6G^-$ $Ly6C^{high}$ monocytes. E, Representative purity test on $CD45^+$ $CD11b^+$ $MHCII^+$ macrophages F, Quantification of the purity of isolated neutrophils, monocytes and macrophages. Each dot represents an individually isolated sample. Neutrophils: n=3; monocytes: n=3, macrophages: n=2.

Figure S6. Effect of treatment of mice with anti-Gr1-neutralizing antibody, CXCR2-inhibitor or CSF1R-inhibitor on neutrophils, macrophages and colon tumor formation.

A, Comparison of the effect of anti-Gr1-neutralizing antibody treatment (αGr1; n=22) to the combined αGr1 plus CXCR2-inhibitor (αGr1 + CXCR2i; n=16) on neutrophil tumor infiltration. Ctrl: n=35

B - D, Effect of αGr1 + CXCR2i treatment on blood neutrophil content and blood monocyte content determined by FACS analysis (B; Ctrl: n=11, aGr1 + CXCR2i: n=9), on total tumor burden and tumor number per mouse (C; Ctrl: n=12, aGr1 + CXCR2i: n=9) and on tumor $CD3^+$ cell content determined by immunohistochemistry (D; Ctrl: n=5, aGr1 + CXCR2i: n=5).

E - G, Effect of CSF1R-inhibitor (CSF1Ri) treatment on tumor M1 macrophages and M2 macrophages ($CD11b^+$ $MHCII^+$ $CD206^+$) content (E; Control: n=6, CSF1Ri: n=6**)**, on average tumor size and tumor number per mouse (F; Control: n=11, CSF1Ri: n=9) and on tumor $CD8^+$, $CD4^+$ and $IFN\gamma^+$ T cell content determined by FACS analysis (G; Control: n=6, CSF1Ri: n=6).

Data information: In (A – G), each dot represents an individual mouse and exact group sizes are mentioned in brackets above. Statistical analysis was performed by

unpaired two-tailed Student's t-tests. *: p<0.05; **: p<0.01; ***: p<0.001. Exact p-values are provided in Appendix table S4.

Figure S7. Expression of T cell suppressive pathway genes in mouse tumor neutrophils and monocytes.

RNA-sequencing analysis of neutrophils and monocytes isolated from colon tumors, blood of tumor-bearing mice or blood of healthy mice.

A, Unsupervised clustering of 1000 highest overall expressed genes.

B, Overlap of localization-specific gene expression pattern between neutrophils (blue) and monocytes (red). Differential gene expression was evaluated for tumor-derived cells compared to blood-derived cells (upper panels) and for cells derived from blood of tumor bearing mice compared to cells derived from blood of healthy mice (lower panel).

C, mRNA levels of indicated genes that have previously been described to be involved in myeloid cell mediated T cell suppression. Shown are mRNA levels in neutrophils (Ne; triangles) and monocytes (Mo; squares), derived from blood (black) or tumor (red), as reads per kilobase of transcript, per million mapped reads (rpkm); n=4 for all groups.

Figure S8. *In vitro* activity of T cell-suppressive pathways in mouse tumor neutrophils and monocytes.

In vitro co-culture of activated T cells with tumor-derived neutrophils.

A, Culture of T cells alone (T only), together with neutrophils plated in a transwell insert (T + Ne transwell) or together with neutrophils in direct contact (T & Ne direct).

Each line represents a culture established with neutrophils from an individual mouse. T cell proliferation index is numbers of proliferated T cells after three days of indicated co-culture condition relative to the number of proliferated T cells when cultured alone; n=3.

B and C, Proliferation of T cells in co-culture with neutrophils and treated with the arginase inhibitor NorNOHA (n=4), the iNOS inhibitor L-NMMA (n=2), an IL10 neutralizing antibody (αIL10; n=4), the adenosine receptor 2A inhibitor SCH58261 (A2i; n=4) and a CD73 neutralizing antibody (αCD73; n=4) or the Ptgs2 inhibitor acetylsalicylic acid (ASA; n=4), the TGFBR inhibitors Galunsertib (n=6) and SB431542 (n=4) or TGFβ neutralizing antibody (αTGFβ; n=2). T cell proliferation index is numbers of proliferated T cells after three days of indicated treatment as relative to the number of proliferated T cells when treated with vehicle.

D, Protein levels of free active TGFβ1 and of total TGFβ1 in T cell culture medium (n= 1) and T cell culture supernatants (n=4) determined by ELISA.

Data information: In (A – C), each dot represents a measurement on an individually derived sample and exact group sizes are mentioned in brackets above. In (C), statistical analysis performed by using one-way ANOVA with Dunnett's multiple comparison tests. ***: $p<0.001$; ****: $p<0.001$. Exact p-values are provided in Appendix table S4.

Figure S9. Quantification of S100A9$^+$ cells, MMP9$^+$ cells and protein levels of pSMAD3$^+$ and IGFBP7$^+$ in tumors of neutrophil-depleted mice.

Eight weeks after Tamoxifen treatment of *Apc*$^{fl/fl\text{-}Cdx2CreERT2}$ mice, animals were treated with anti-Gr1 antibody plus CXCR2 inhibitor (αGr1 + CXCR2i; n=4) or with IgG plus DMSO control (Ctrl; n=4) for one to three weeks. Tumor sections were then analyzed

for specific marker expression using immunohistochemistry (A and B) and immunofluorescence (C).

A, Quantification (left panel) and immunostaining of representative tumor sections (middle and right panel) for S100A9+ cells.

B, Quantification (left panel) and immunostaining of representative tumor sections (middle and right panel) for $MMP9^+$ cells. Ctrl: n=5, αGr1 + CXCR2i: n=4.

C, Quantification of pSMAD3 (left panel) and IGFBP7 (right panel) staining intensity per positive cell. Each dot represents an individual mouse. Ctrl: n=3, αGr1 + CXCR2i: n=5.

Data information: In (A – C), at least five arbitrarily selected areas per section were scored. Statistical analysis was performed by unpaired two-tailed Student's t-tests. *: $p<0.05$; **: $p<0.01$. Exact p-values are provided in Appendix table S4.

Figure S10. Neutrophil infiltration in human colon tumors.

Immunostainings for neutrophils and $CD8^+$ T cells on representative human CRC sections.

A - C, Anti-S100A9 immunostainings described in table S1. A, Sample with high $S100A9^+$ cell infiltration at tumor border and in tumor center. B, Sample with high $S100A9^+$ cell infiltration in tumor center. C, Sample with high $S100A9^+$ cell infiltration at tumor border and in benign tissue, but not in tumor center.

D, Co-localization of immunostainings for neutrophil markers S100A9 (brown) and CD66b (grey-blue).

E, Section co-stained for the T cell marker CD8 (brown) and the neutrophil marker S100A9 (grey-blue). Upper and lower panels depict two areas on the same section.

Black arrowheads indicate foci of $S100A9^+$ cells and red arrowheads indicate foci of $CD8^+$ cells.

A – C and E, Red dashed lines indicate tumor border.

Figure S11. Comparison of gene expression signatures indicative of T cells, neutrophils and TGFβ signaling in CRC and colon adenoma datasets.

Gene expression signatures and scores specific for either T cells, TGFβ signaling or neutrophils were generated as described in material and methods.

A**,** T cell score in categories of CRC samples according to neutrophil and TGFβ scores.

B, TGFβ scores in categorization of CRC samples according CMS subtypes. Boxes are lower and upper quartiles with median as solid lines; horizontal lines define minimum and maximum; dots define outliers.

Data information: In (A), p-values were calculated with Mann-Whitney test. *: $p<1x10^{-3}$; **: $p<1x10^{-6}$; ***: $p<1x10^{-8}$; **** $p<1x10^{-10}$. Exact p-values are provided in Appendix table S4.

Figure S12. MMP9 expression by human neutrophils and purity of isolated human neutrophils and T cells.

A, Co-immunostaining for MMP9 (red) and CD66b (green) with DAPI counterstaining on a representative human CRC section. Left panel is merged channels for CD66b- and MMP9-specific fluorescent staining. Right panels show a region of left panel with individual channels for CD66b- and MMP9-specific fluorescence.

B, Purity of CRC patient and healthy volunteer derived blood T cells and neutrophils used for in vitro co-culture assays. T cell purity was tested with CD3 plus CD11b FACS analysis on anti-CD3 MACS-isolated cells. Neutrophil purity was tested using CD11b plus CD15 FACS analysis on anti-CD66b MACS isolated cells. Each dot represents an individual human sample. T cells: n=15; Neutrophils: n=21.

Appendix Table S1

Information on cell marker panels used to analyze and sort individual cell types

Species	Cell type	Marker MACS	Marker FACS
Mouse	T cell	CD4 & CD8	CD45+ EpCAM- TCRb+
Mouse	Neutrophil	Ly6G	CD45+ EpCAM- CD11b+ MHCII- Ly6C-low Ly6G+
Mouse	Monocyte	not done	CD45+ EpCAM- CD11b+ MHCII- Ly6C-high Ly6G-
Mouse	Macrophage	not done	CD45+ EpCAM- CD11b+ MHCII+
Mouse	Epithelial cell	not done	CD45- EpCAM+
Mouse	Stromal cell	not done	CD45- EpCAM-
Human	T cell	CD3	CD45+ CD3+
Human	Neutrophil	CD66b	CD45+ CD11b+ HLA-DR- CD14- CD15+ CD66b+

Appendix Table S2

Gene signatures indicative for the presence of neutrophils, T cells or TGFβ signaling. Individual gene signatures for TCGA and GSE39582 were generated as described in Material and Methods

Cohort	TCGA					
Samples	CMS1-4			CMS4		
Signature type	Neutrophils	T cells	TGFβ	Neutrophils	T cells	TGFβ
Signature genes	AQP9	CCL5	ADAM12	AQP9	ABCD2	ADAM12
	CSF3R	CD2	ADAMTS16	CXCL5	CCL5	ADAMTS16
	CXCL5	CD27	AEBP1	CXCR1	CD2	AEBP1
	CXCR1	CD3D	ANTXR1	CXCR2	CD226	APCDD1L
	CXCR2	CD3E	AOC3	FCAR	CD27	ARSI
	FCAR	CD3G	ASPN	FCGR3B	CD38	C1QTNF3
	FCGR3B	CD7	BGN	FPR2	CD3D	C5orf46
	FPR2	CD8A	BNC2	IL1B	CD3E	CALB2
	PROK2	CD96	CCDC8	IL24	CD3G	CERCAM
	S100A8	CIITA	CCDC80	MEFV	CD40LG	CHST6
		CRTAM	COL10A1	MMP1	CD8A	CIDEC
		CTSW	COL11A1	MMP3	CD96	CILP
		CXCL9	COL1A1	PROK2	CXCL9	CNTN1
		CXCR6	COL1A2	S100A12	CXCR2P1	COL1A1
		FASLG	COL3A1	S100A8	CXCR6	COL1A2
		FCRL6	COL8A1	SERPINB2	EOMES	COL3A1
		GBP4	COL8A2		FASLG	COMP
		GBP5	CTHRC1		FCRL5	CPXM1
		GNLY	CYS1		GBP4	EPYC
		GPR171	EFEMP1		GBP5	FABP4
		GPR174	EVC		GZMA	FBLN2
		GZMA	FBLN1		GZMH	FNDC1
		GZMH	FBLN2		GZMK	FST
		GZMK	FBN1		IRF4	GAS1
		HLA-DOA	FIBIN		ITK	GDF6
		IDO1	FNDC1		JAKMIP1	GPIHBP1
		IFNG	GLI3		KLRB1	HTRA1
		IL12RB1	HMCN1		KLRK1	IGFL1
		IL21R	HTRA3		LAX1	IGFL2
		IL2RB	ISLR		LY9	KLK5
		ITGAL	ITGA11		MAP4K1	MFAP5
		ITK	ITGBL1		NKG7	MMP23A
		JAKMIP1	LUM		P2RY10	NPR3
		KLRK1	MRGPRF		PDCD1	OMD
		LAG3	MSRB3		PLA2G2D	PCDHGB5
		MAP4K1	NAP1L3		PTPRCAP	PDGFRL
		NKG7	NTM		PYHIN1	PTH1R
		P2RY10	P4HA3		RASGRP1	SCARF2
		PDCD1	PDLIM3		SH2D1A	SFRP1
		PYHIN1	PTGER3		SIRPG	SFRP2
		SH2D1A	SCARF2		SLA2	SFRP4
		SIRPG	SFRP4		SLAMF1	SNCG
		SLA2	SGCD		SLAMF7	SPON1
		SLAMF7	SPOCK1		TBX21	SSC5D
		TBX21	SSC5D		THEMIS	TGFB3
		THEMIS	ST6GALNAC5		TIGIT	THBS2
		TIGIT	SULF1		TRAT1	WISP2
		TRAT1	TAGLN		UBASH3A	
		UBASH3A	THBS2			
		ZNF683				

Cohort	GSE39582					
Samples	CMS1-4			CMS4		
Signature type	Neutrophils	T cells	TGFβ	Neutrophils	T cells	TGFβ
Signature genes	AQP9 BCL2A1 CCL3 CHI3L1 CXCL1 CXCL2 CXCL3 CXCL5 CXCL6 CXCL8 DMBT1 FCGR3B FPR1 G0S2 HCAR3 IL1B IL1RN IL6 LYZ MMP1 MMP3 PLEK PPBP PROK2 PTGS2 REG1A REG1B REG3A S100A12 S100A8 S100A9 SRGN TNFAIP6 TREM1	APOBEC3G C1QA C1QB CCL5 CCR5 CD2 CD247 CD27 CD274 CD3D CD52 CD8A CXCL10 CXCL13 CXCL9 GBP1 GBP4 GBP5 GIMAP6 GNLY GPR171 GZMA GZMH GZMK HLA-DMA HLA-DMB HLA-DPB1 HLA-DRB1 IDO1 IKZF1 IL18BP IRF1 ITGAL ITK NCKAP1L NKG7 PRF1 PSMB9 RARRES3 RASGRP1 SLAMF7 STAT1 TAP1 TRAC TRAF3IP3 TRBC1 UBE2L6 WARS	ACTG2 ANTXR1 ASPN COL10A1 COL11A1 COL1A1 COL1A2 COL8A1 DCN EFEMP1 FBLN1 FNDC1 GAS1 GREM1 MAB21L2 MGP MSRB3 MYL9 PCDH7 PLN POSTN SFRP2 SFRP4 SPOCK1 SULF1 SYNPO2 TAGLN THBS2 TNS1 VCAN	AQP9 BCL2A1 CCL3 CHI3L1 CXCL1 CXCL3 CXCL5 CXCL6 CXCL8 FCGR3B FPR1 G0S2 HCAR3 IL1B IL1RN IL24 IL6 MCEMP1 MMP1 MMP3 PROK2 S100A12 S100A8 S100A9 TFPI2 TREM1	AIM2 APOBEC3G BTN3A3 C1QB CCL5 CCR5 CD2 CD247 CD27 CD37 CD38 CD3D CD48 CD52 CD8A CXCL13 CXCL9 GATA3 GBP4 GBP5 GNLY GPR171 GZMA GZMB GZMH GZMK HLA-DMA HLA-DOA IDO1 IKZF1 IL10RA IL2RB IRF1 ITGAL ITK LAMP3 NKG7 NLRC3 PSMB9 RASGRP1 SLAMF7 STAT1 TAGAP TRAC TRAF3IP3 TRBC1	ADAM12 ASPN COL10A1 COL11A1 COL12A1 COL1A1 COL1A2 COL5A1 COL5A2 COL8A1 COL8A2 COLEC12 COMP DCN DPYSL3 EPYC FBLN1 FNDC1 GAS1 GLT8D2 GXYLT2 HOPX LOX LOXL1 MFAP5 MXRA5 POSTN RGS4 SFRP2 SFRP4 SPOCK1 SPON1 TWIST1 VCAN VGLL3 ZFPM2

Appendix table S3

FACS

Antigen	Clone	Reactivity	Supplier	Cat. number	Lot number	Dilution
CD11b	M1/70	Human, mouse	Biolegend	101241	B235643	1/400
CD14	HCD14	Human	Biolegend	325603	B211976	1/100
CD15	HI98	Human	Biolegend	301907	7068674	18264
CD206	C068C2	Mouse	Biolegend	141711	B230155	1/50
CD3	HIT3a	Human	Biolegend	300317	B241860	1/100
CD4	GK1.5	Mouse	in house	not applicable	not applicable	1/800
CD45	HI30	Human	Biolegend	304041	B242281	1/100
CD45	M1/89	Mouse	in house	not applicable	not applicable	1/400
CD66b	G10F5	Human	Biolegend	305105	B221033	1/200
CD8	YTS169.4	Mouse	in house	not applicable	not applicable	1/1600
EpCAM	G8.8	Mouse	Biolegend	118207	B199448	1/100
FOXP3	236A/E7	Human, Monkey	eBioscience	14-4777-80	E04269-1631	1/100
Gr1	RB6-8C5	Mouse	in house	not applicable	not applicable	1/800
HLA-DR	L243	Human	Biolegend	307635	B223586	1/50
IFNg	XMG1.2	Mouse	Tonbo	20-7311-U02	C711111716202	1/100
Ly6C	HK1.4	Mouse	Biolegend	128013	B236102	1/400
Ly6G	1A8	Mouse	Biolegend	127615	B218783	1/100
MHCII	M5/114.15.2	Mouse	Biolegend	107603	B189443	1/800

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Antigen	Clone	Reactivity	Supplier	Cat. number	Lot number	Dilution
CD3	HIT3a	Human	Biolegend	300303	B230221	1/100
CD66b	G10F5	Human	Biolegend	305120	B215969	1/100
Ly6G	1A8	Mouse	Biolegend	B205345	B205345	1/100

IHC-Paraffin

Antigen	Clone	Reactivity	Supplier	Cat. number	Lot number	Dilution
CD3	Sp7	Human, Mouse, Ra	Abcam	ab16669		1/100
CD8	C8/144B	Human	eBioscience	14-0085	E15903-103	1/200
CD66b	G10F5	Human	Biolegend	305102	B197492	1/600
MMP9	5G3	Human	Thermo	MA5-15886	TI2443744	1/400
S100A9	Polyclonal	Human, Mouse, Ra	Novus	NB110-8972	A-2	1/5000
MMP9	Polyclonal	Mouse	Abcam	ab38898	Gift of D Hanahan (EPFL)	1/400

IHC-Frozen

Antigen	Clone	Reactivity	Supplier	Cat. number	Lot number	Dilution
CD45	30-F11	Mouse	BD Bioscier	5505539		1/200
Gr1	RB6-8C5	Mouse	in house	not applicable	not applicable	1/400

***In vivo* neutralisation**

Antigen	Clone	Reactivity	Supplier	Cat. number	Lot number	Dilution
CD4	YTS191.1	Mouse	in house	not applicable	not applicable	10 mg/kg body weight
CD8	YTS169.4	Mouse	in house	not applicable	not applicable	10 mg/kg body weight
Gr1	RB6-8C5	Mouse	in house	not applicable	not applicable	20 mg/kg body weight

***In vitro* neutralisation**

Antigen	Clone	Reactivity	Supplier	Cat. number	Lot number	Dilution
CD73	TY/23	Mouse	BioXcell	BE0209	616317J1	2 µg/ml
IL10	JES5-2A5	Mouse	Biolegend	504903	B178143	2 µg/ml
Tgfb1	11D1	Mouse	BioXcell	BE0057	642917J1	2 µg/ml

Appendix table S4

Figure	Panel	exact p-value
1	B	Blood TCRbeta+ cells: p=0.00000006; Tumor TCRb+ cells: p=0.0155
1	C	Tumor burden: p=0.0272; Tumor number: p=0.0174
2	A	Colon vs Tumor: p=0.0000009
2	C	Colon vs Tumor: p=0.00007727
2	E	Colon vs Tumor: p=0.00002965
2	F	Colon vs Tumor: p=0.00042612
2	H	Colon vs Tumor: p=0.0005
2	I	Colon vs Tumor: p=0.0303
2	K	Colon vs Tumor: p=0.0367
3	A	T cells + neutrophils vs T only 1:5: p=0.0434
3	A	T cells + neutrophils vs T only 1:2: p=0.0011
3	A	T cells + neutrophils vs T only 1:1: p=0.0026
3	A	T cells + macrophages vs T only 1:1: p=0.0145
3	C	Neutrophils IgG+DMSO vs aGr1+CXCR21: p=0.0167
3	D	Tumor size IgG+DMSO vs aGr1+CXCR21: p=0.0075
3	F	Tumor Tregs IgG+DMSO vs aGr1+CXCR21: p=0.0073
3	G	Tumor activated T cells IgG+DMSO vs aGr1+CXCR21: p=0.0040
3	G	Tumor Th17 T cells IgG+DMSO vs aGr1+CXCR21: p=0.0014
4	E	T+Ne+TGFBRi vs T+Ne: p=0.00024829
4	F	T+Ne vs T only: p=0.0376; T+Ne+TGFBRi vs T+Ne: p=0.0349
4	G	T+Ne+MMPi vs T+Ne: p=0.0038
4	H	T+Ne+TGFBRi vs T+Ne: p=0.0011
4	H	T+Ne+TGFBRi+MMPi vs T+Ne: p=0.0009
4	H	T+Ne+TGFBRi+MMPi vs T+Ne+TGFBRi: p=0.0033
5	C	pSMAD3+ cells MMP2/9i vs Vehicle: p=0.0021. pSMAD3 intensity/cell MMP2/9i vs Vehicle: p=0.0013
5	E	Tumor size TGFBRi vs Vehicle: p=0.0450. Tumor size MMP2/9i vs Vehicle: p=0.0280
6	C	S100A9+ cells Tumor center vs tumor border: p=0.0078. S100A9+ cells Tumor border vs adjacent benign: p=0.0097.
6	C	CD8+ cells Tumor border vs adjacent benign: p=0.0261. S100A9+ cells Tumor center vs adjacent benign: p=0.0024.
6	E	TCGA cohort; neutrophil-high/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=3.8e-8
6	E	TCGA cohort; neutrophil-high/TGFbeta-high vs Neutrophil-high/TGFbeta-low p=1.9e-5
6	E	TCGA cohort; neutrophil-low/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=0.00024
6	E	GSE39582 cohort; neutrophil-high/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=2.3e-9
6	E	GSE39582 cohort; neutrophil-high/TGFbeta-high vs Neutrophil-high/TGFbeta-low p=6.4e-9
6	E	GSE39582 cohort; neutrophil-low/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=0.00048
6	F	T cells + neutrophils vs T only 1:8: p=0.02325821
6	F	T cells + neutrophils vs T only 1:4: p=0.00954330
6	F	T cells + neutrophils vs T only 1:2: p<0.00000001
6	F	T cells + neutrophils vs T only 1:1: p<0.00000001
6	F	T cells + neutrophils vs T only 2:1: p<0.00000001
6	G	T+Ne+TGFBRi vs T+Ne: p=0.0204
EV1	A	Colon vs Tumor: p=0.0009
EV1	E	Activated T cells Colon vs Tumor: p=0.0023
EV1	E	CTLs Colon vs Tumor: p=0.0423
EV2	A	aT+aNe vs Ctrl: p=0.0018
EV2	B	aNe vs Ctrl: p=0.0119. aT+aNe vs Ctrl: p=0.0011
EV2	C	aNe vs Ctrl: p=0.0486. aT+aNe vs aNe: p=0.0021
S1	B	IgG vs aCD4/aCD8: p=0.0383
S2	C	Blood CD8+ T cells: p=0.000064. Tumor CD8+ T cells: p=0.0057
S2	D	Tumor number Ctrl vs aCD4/aCD8: p=0.0043
S4	A	Colon vs Tumor: p=0.0002
S4	C	Colon vs Tumor: p=0.0106
S4	E	Colon vs Tumor: p=0.0383
S6	A	Tumor neutrophils Ctrl vs aGr1+CXCR2i: p=0.0001
S6	B	Blood neutrophils Ctrl vs aGr1+CXCR2i: p=0.000058
S6	C	Tumor burden Ctrl vs aGr1+CXCR2i: p=0.0312
S6	E	CD11b+ MHCII+ CD11c+ cells Control vs CSF1Ri: p=0.0059. CD11b+ MHCII+ CD206+ cells Control vs CSF1Ri: p=0.0035.
S8	C	Galunisertib vs Vehicle: p<0.0001. SB431542 vs Vehicle: <0.0001. aTgfb vs Vehicle: p=0.000502
S9	A	Tumor S100A9+ cells Ctrl vs aGr1+CXCR2i: p=0.0144
S9	B	Tumor MMP9+ cells Ctrl vs aGr1+CXCR2i: p=0.0013
S9	C	pSMAD3 expression Ctrl vs aGr1+CXCR2i: p=0.0417. IGFBP7 expression Ctrl vs aGr1+CXCR2i: p=0.0109
S11	A	TCGA cohort; neutrophil-high/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=1.1e-15
S11	A	TCGA cohort; neutrophil-high/TGFbeta-high vs Neutrophil-high/TGFbeta-low p=2.2e-9
S11	A	TCGA cohort; neutrophil-low/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=7.7e-7
S11	A	GSE39582 cohort; neutrophil-high/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=2.2e-16
S11	A	GSE39582 cohort; neutrophil-high/TGFbeta-high vs Neutrophil-high/TGFbeta-low p=0.0074
S11	A	GSE39582 cohort; neutrophil-low/TGFbeta-high vs Neutrophil-low/TGFbeta-low p=0.17