Supplemental Figures, Legends and Tables for:

EGFR-mediated Macrophage Activation Promotes Colitis-associated Tumorigenesis

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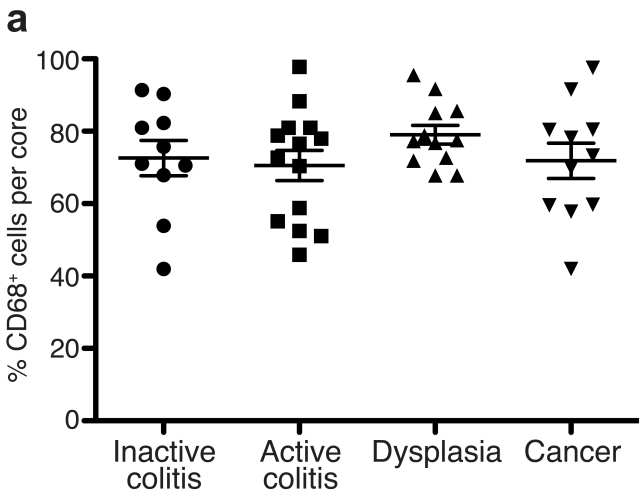
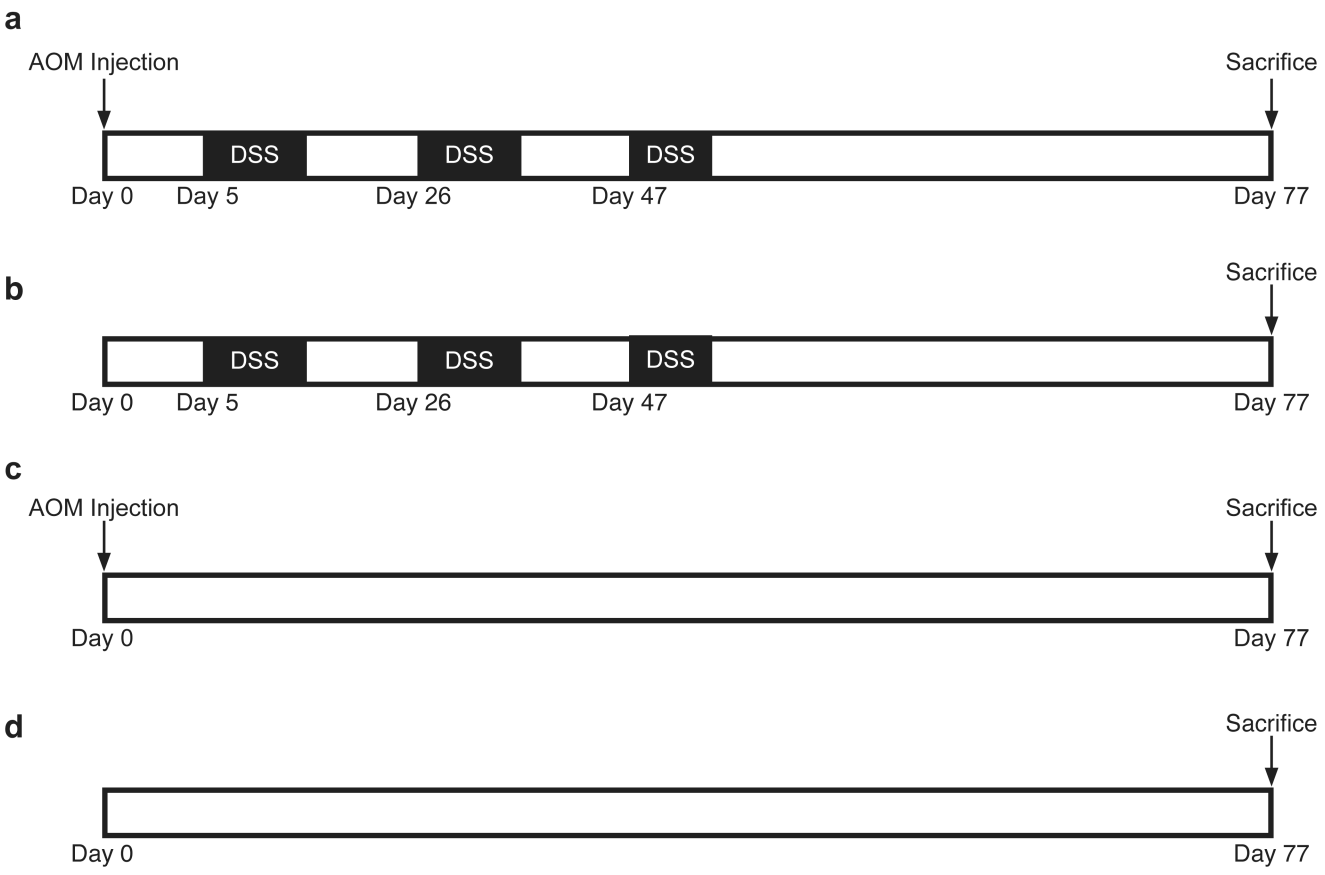
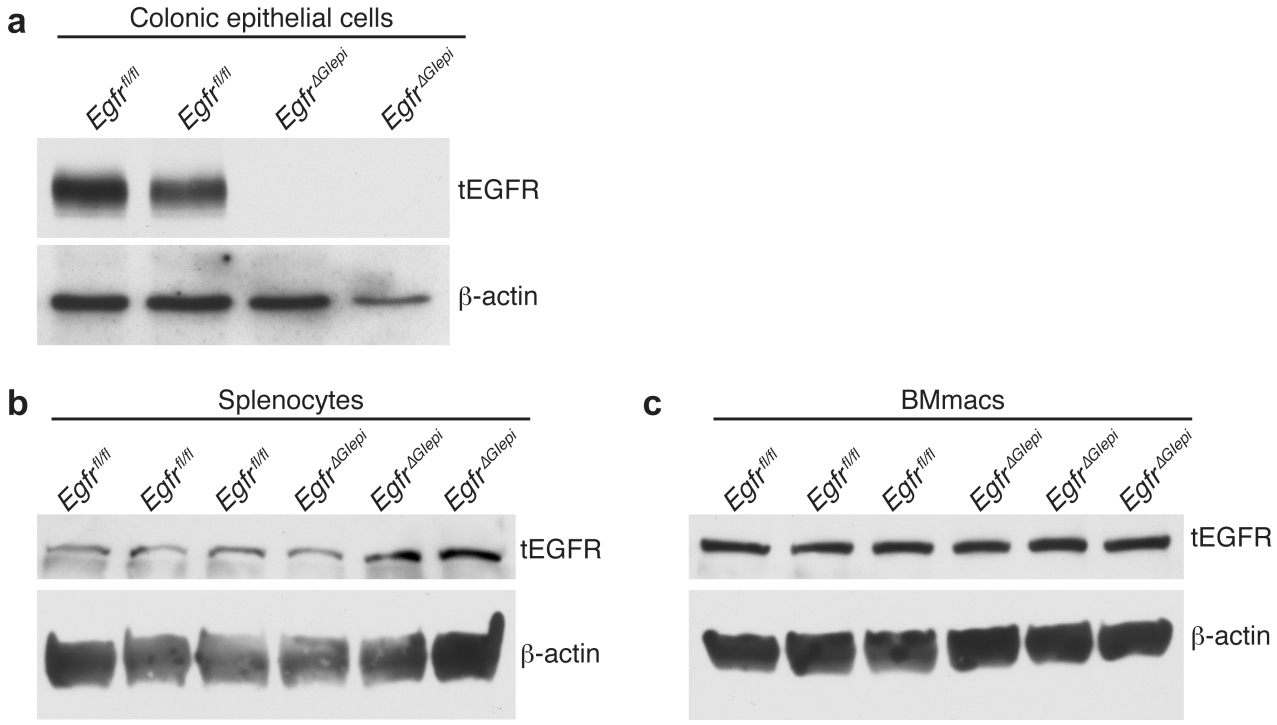


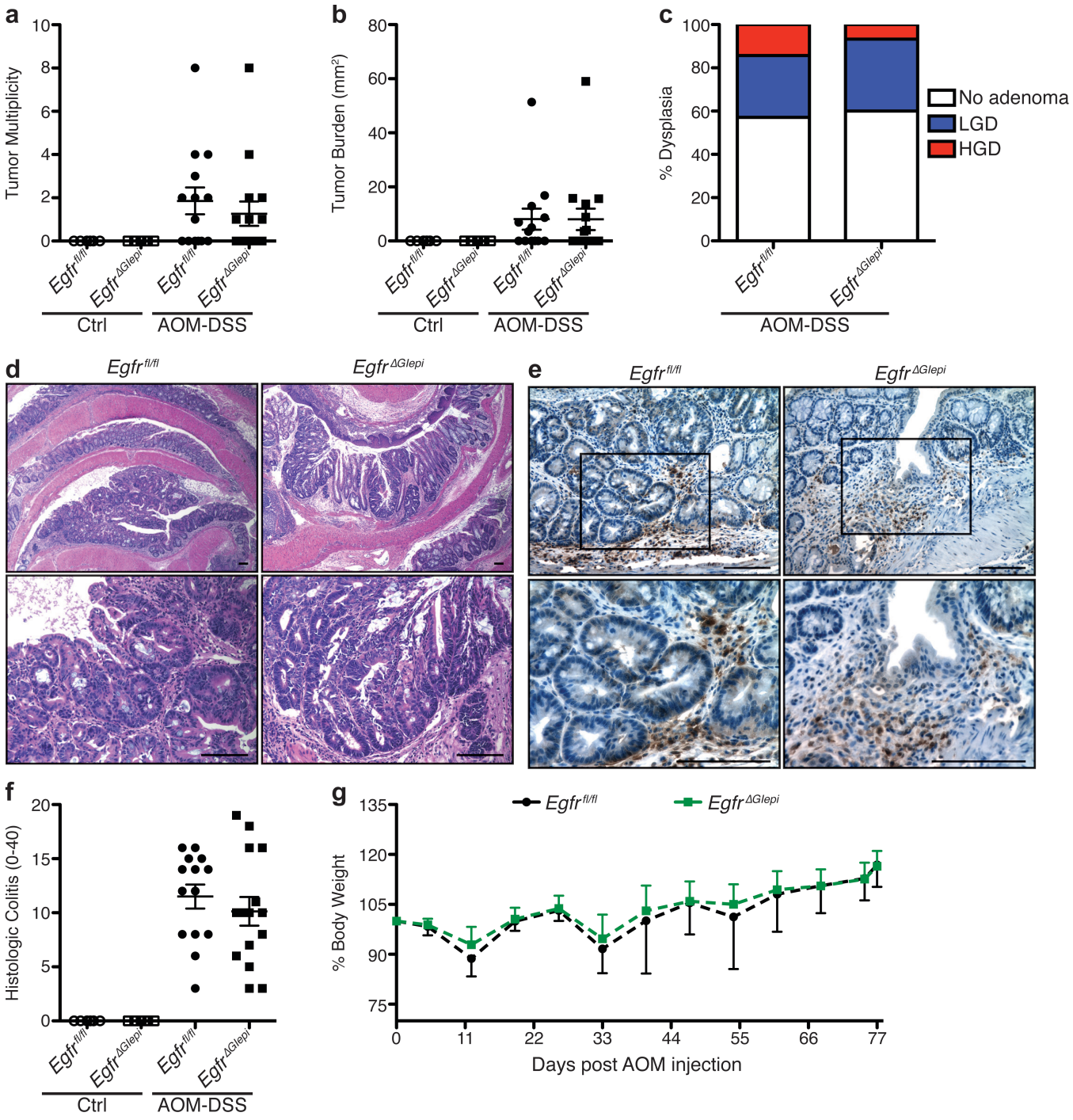
Supplementary Figure 1. Percentages of macrophages in human CAC TMA. (a) Quantification of the percentage of $CD68^+$ cells among the total number of nuclei in each individual core in the TMA. For (a-c), n = 10 inactive colitis (normal or quiescent histology) samples, 14 active colitis (mild, moderate or severe histology) samples, 12 dysplasia samples, and 11 colorectal cancer samples.



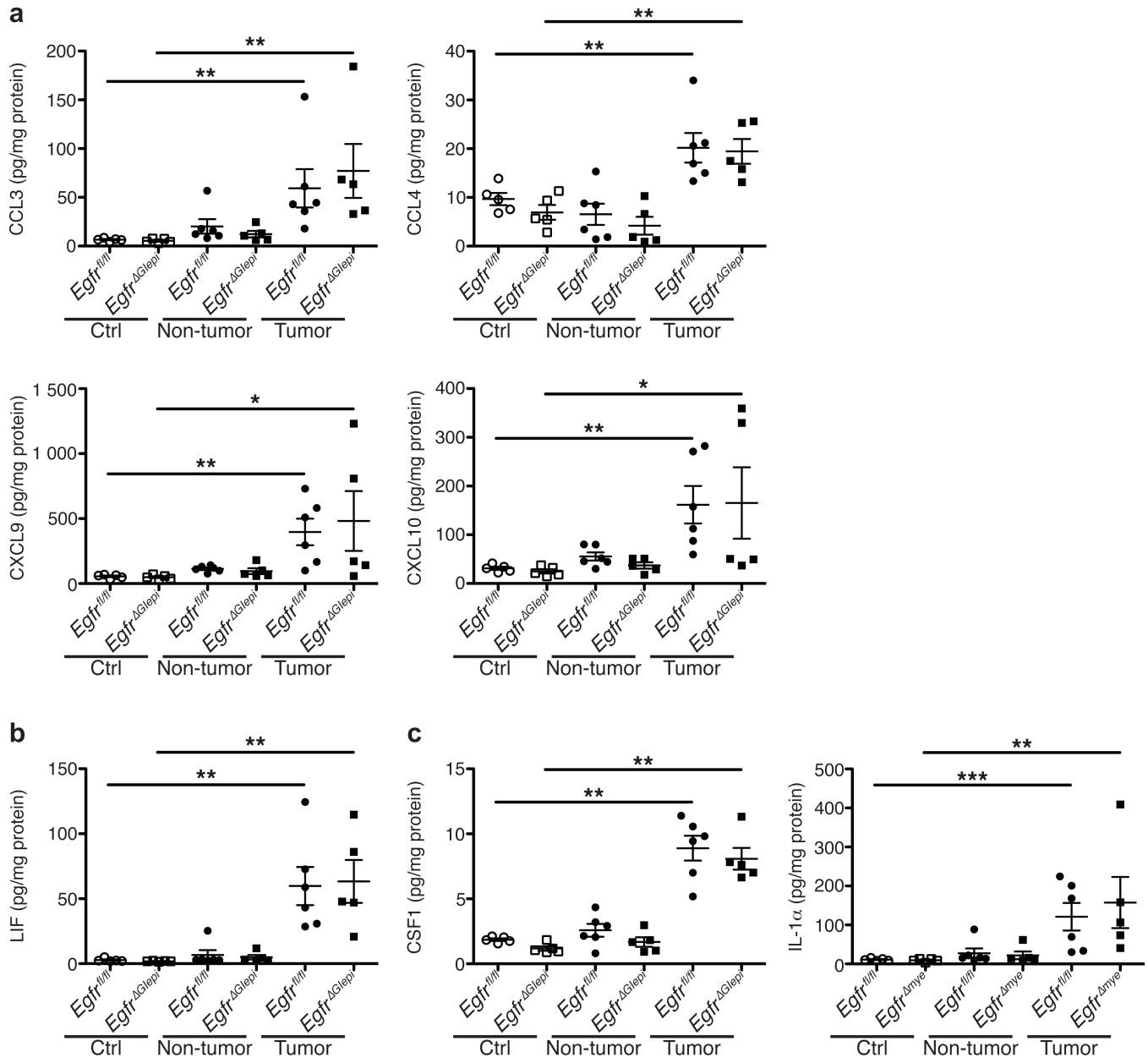
Supplementary Figure 2. Schematic of the AOM-DSS protocol utilized in these studies. (a) AOM-DSS group: Mice were injected with 12.5 mg/kg AOM on Day 0. Animals then received 3 cycles of 4% DSS beginning on Day 5 (for 5 days), Day 26 (for 5 days), and Day 47 (for 4 days). Mice were sacrificed on Day 77 post-AOM injection. (b) DSS only group: Mice did not receive an AOM injection, but received 3 cycles of 4% DSS as in the AOM-DSS group. Mice were sacrificed on Day 77. (c) AOM only group: Mice were injected with 12.5 mg/kg AOM on Day 0 and were then maintained on normal drinking water throughout the protocol. Mice were sacrificed on Day 77 post-AOM injection. (d) Control group: Mice did not receive an AOM injection nor did mice receive DSS in their drinking water. Mice were sacrificed on Day 77.



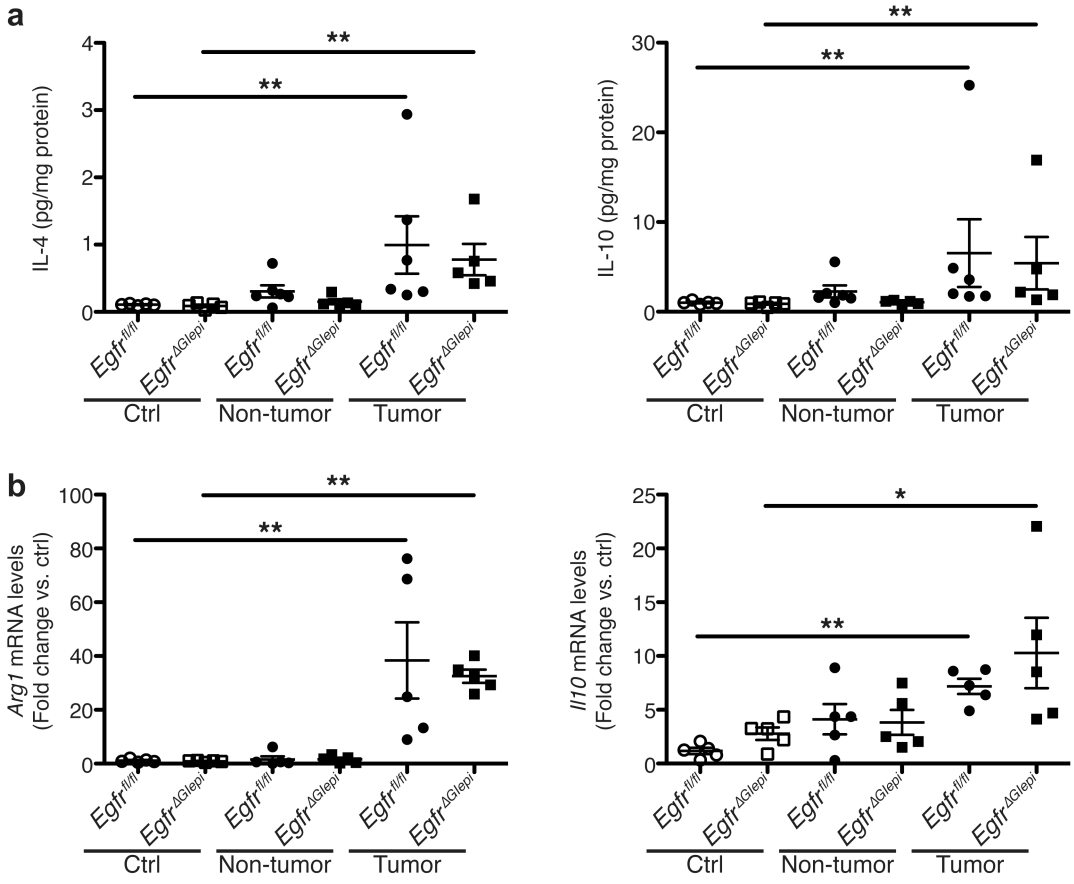
Supplementary Figure 3. *Egfr*$^{\Delta GIepi}$ colonic epithelial cells demonstrated tEGFR knockout, but splenocytes and bone marrow-derived macrophages did not. (a) Representative Western blot of tEGFR levels in colonic epithelial cells from naïve *Egfr*$^{fl/fl}$ *and Egfr*$^{\Delta GIepi}$ mice. (b) Representative Western blot of tEGFR levels in splenocytes from naïve *Egfr*$^{fl/fl}$ *and Egfr*$^{\Delta GIepi}$ mice. (c) Representative Western blot of tEGFR levels in bone marrow-derived macrophages (BMmacs) from naïve *Egfr*$^{fl/fl}$ *and Egfr*$^{\Delta GIepi}$ mice.



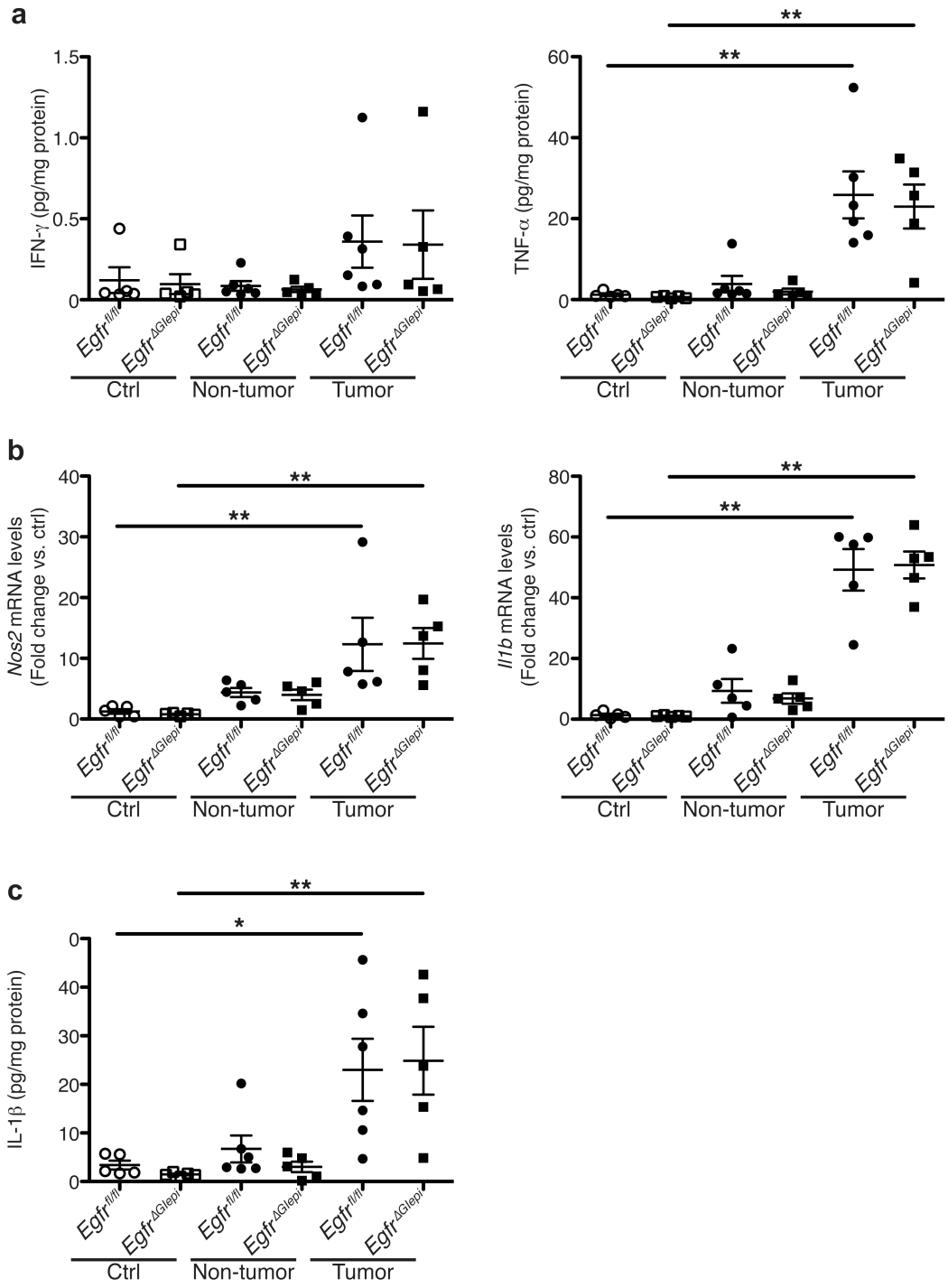
Supplementary Figure 4. *Egfr*$^{\Delta GIepi}$ mice did not demonstrate altered tumorigenesis compared to *Egfr*$^{fl/fl}$ mice. (a) Tumor multiplicity was assessed by gross visual inspection, utilizing a dissecting microscope. (b) Tumor burden was determined by the addition of the calculated area of each identified tumor, as assessed with an electronic caliper for both length and width. (c) Percentage of cases with either no adenoma, low-grade dysplasia (LGD), and high-grade dysplasia (HGD) was determined by a gastrointestinal pathologist (M.K.W.) in a blinded manner. (d) Representative H&E-stained images from AOM-DSS-treated mice. Scale bars = 100 μm. (e) Representative immunoperoxidase images of pEGFR Y1068 from mice in (a-f). Scale bars = 50 μm. *n* = 6 mice per genotype assessed. (f) Histologic colitis was determined by M.K.W. in a blinded manner. (g) Percentage of initial body weight was assessed at indicated time points. In (a-c) and (f-g), *n* = 6 control and 14-15 AOM-DSS treated mice per genotype.



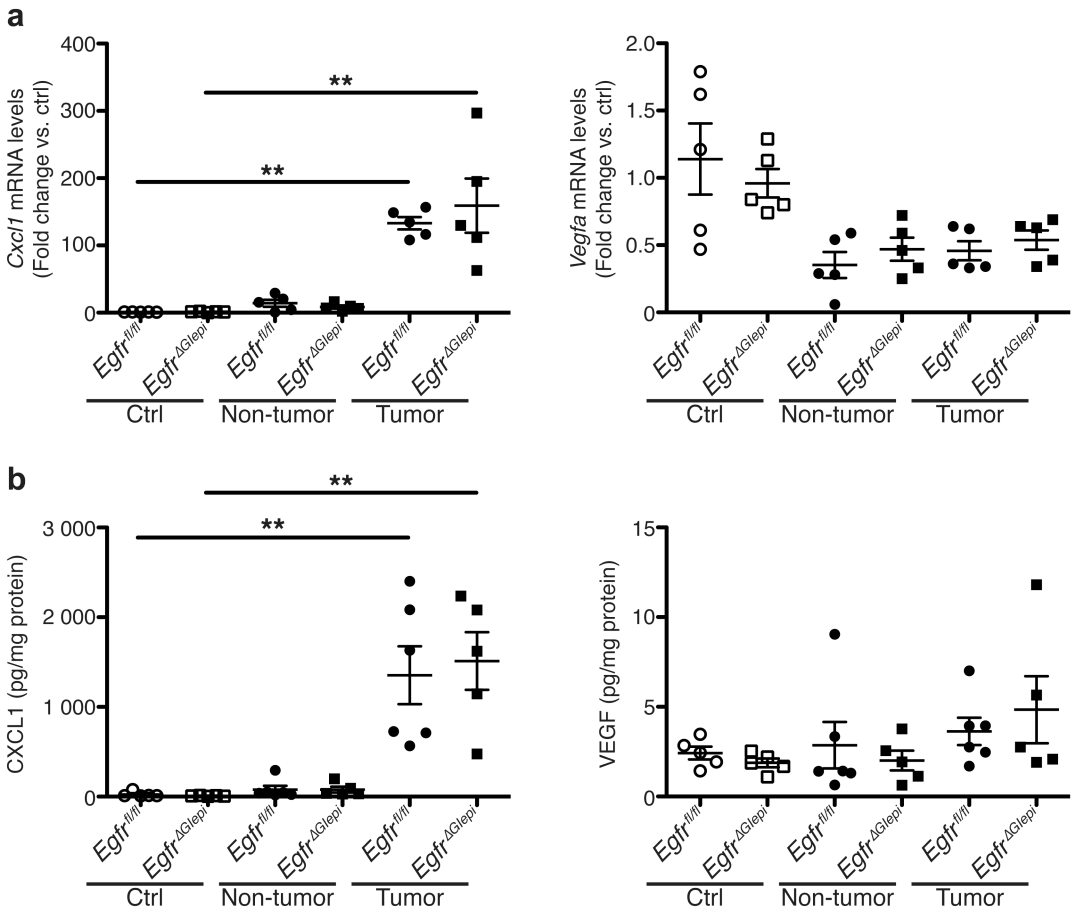
Supplementary Figure 5. *Egfr*$^{\Delta Glepi}$ mice demonstrate no alterations in cytokine and chemokine production within tumors. (a) Protein levels of the general C-C motif and C-X-C motif chemokines CCL3 (MIP-1α), CCL4 (MIP-1β), CXCL9 (MIG), and CXCL10 (IP-10) were assessed by Luminex Multiplex Array from colonic tissues 77 days post-AOM injection. (b) Protein levels of the pleiotropic cytokine, LIF, were assessed by Luminex Multiplex Array from colonic tissues 77 days post-AOM injection. (c) Protein levels of cytokines produced by activated macrophages, CSF1 (M-CSF) and IL-1α were assessed by Luminex Multiplex Array from colonic tissues 77 days post-AOM injection. In all panels, $n = 5$ control tissues and 5-6 tumors with paired non-tumor area per genotype. In all panels, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ by one-way ANOVA with Kruskal-Wallis test, followed by Mann-Whitney *U* test.



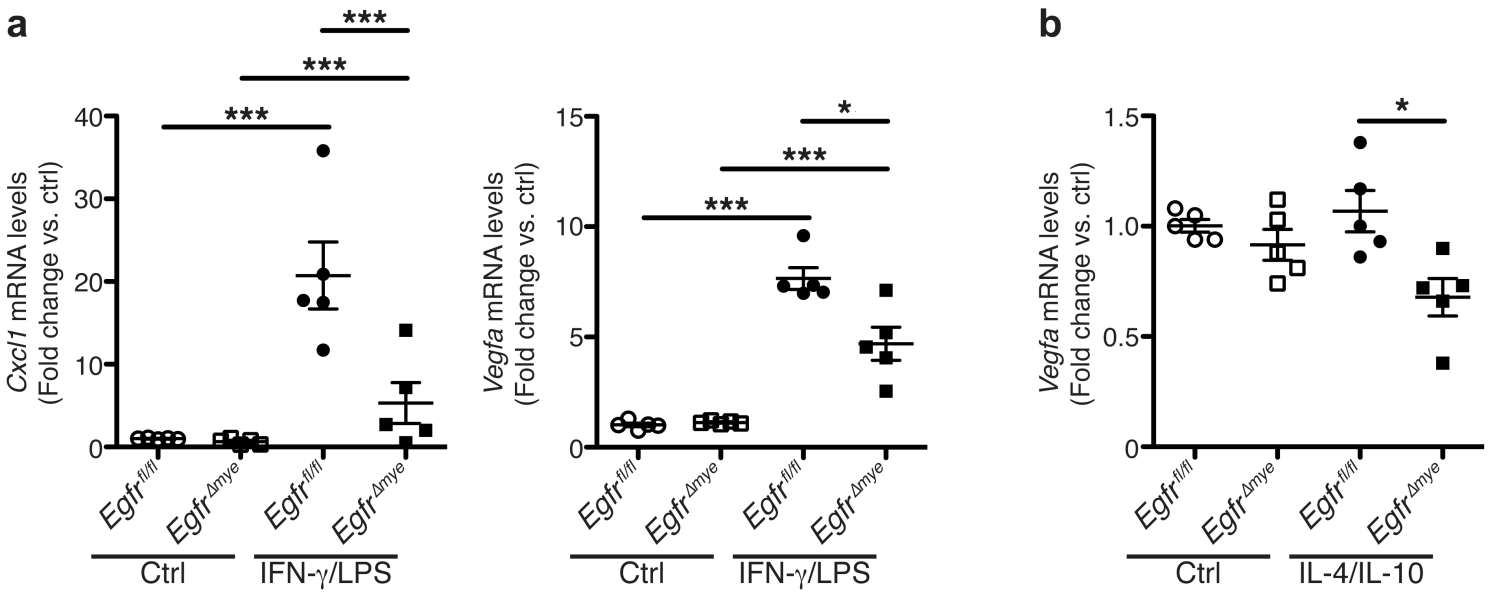
Supplementary Figure 6 *Egfr*$^{\Delta GIepi}$ mice demonstrate no alterations in M2 macrophage activation during colon tumorigenesis. (a) Protein levels of M2 stimuli, IL-4 and IL10, were assessed by Luminex Multiplex Array from colonic tissues 77 days post-AOM injection. $n = 5$ control tissues and 5-6 tumors with paired non-tumor area per genotype. (b) mRNA levels of M2 markers, *Arg1* and *Il10*, were assessed by qRT-PCR from colonic tissues 77 days post-AOM injection. $n = 5$ control tissues and 5 tumors with paired non-tumor area per genotype. In all panels, $^{*}P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA with Kruskal-Wallis test, followed by Mann-Whitney *U* test.



Supplementary Figure 7. *Egfr*$^{\Delta Glepi}$ mice demonstrate no alterations in M1 macrophage activation during colon tumorigenesis. (a) Protein levels of M1 stimuli, IFN-γ and TNF-α, were assessed by Luminex Multiplex Array from colonic tissues 77 days post-AOM injection. *n* = 5 control tissues and 5-6 tumors with paired non-tumor area per genotype. (b) mRNA levels of M1 markers, *Nos2* and *Il1b*, were assessed by qRT-PCR from colonic tissues 77 d post-AOM injection. *n* = 5 control tissues and 5 tumors with paired non-tumor area per genotype. (c) Protein levels of M1 marker, IL-1β, were assessed by Luminex Multiplex Array from colonic tissues 77 days post-AOM injection. *n* = 5 control tissues and 5-6 tumors with paired non-tumor area per genotype. In all panels, $^{*}P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA with Kruskal-Wallis test, followed by Mann-Whitney *U* test.



Supplementary Figure 8. *Egfr*$^{\Delta Glepi}$ mice demonstrate no alterations in pro-angiogenic chemokine/cytokine production during colon tumorigenesis. (a) mRNA levels of the pro-angiogenic chemokine, *Cxcl1*, and the pro-angiogenic cytokine, *Vegfa*, were assessed by qRT-PCR from colonic tissues 77 days post-AOM injection. n = 5 control tissues and 5 tumors with paired non-tumor area per genotype. (b) Protein levels of CXCL1 and VEGF were assessed by Luminex Multiplex Array from colonic tissues 77 days post-AOM injection. n = 5 control tissues and 5-6 tumors with paired non-tumor area per genotype. In all panels, $^{*}P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA with Kruskal-Wallis test, followed by Mann-Whitney U test.



Supplementary Figure 9. *Egfr*$^{\Delta mye}$ BMmacs demonstrate significant alterations in *Cxcl1* and *Vegfa* mRNA levels during M1 or M2 macrophage activation. (a) mRNA levels of the pro-angiogenic chemokine, *Cxcl1*, and the pro-angiogenic cytokine, *Vegfa*, were assessed by qRT-PCR in BMmacs 24 h post-stimulation with IFN-γ (200 U/mL) and LPS (10 ng/mL). $n = 5$ biological replicates per genotype. (b) mRNA levels of the pro-angiogenic cytokine, *Vegfa*, were assessed by qRT-PCR in BMmacs 24 h post-stimulation with IL-4 (10 ng/mL) and IL-10 (10 ng/mL). $n = 5$ biological replicates per genotype. In all panels, $^{*}P < 0.05$, $^{***}P < 0.001$ by one-way ANOVA with Newman-Keuls post-test.

Analyte	Concentration of Analyte (pg/mg protein); Mean ± S.E.M.					
	Egfr$^{fl/fl}$			*Egfr*$^{\Delta mye}$		
	Control	Non-tumor	AOM-DSS	Control	Non-tumor	AOM-DSS
CCL2	3.87 ± 0.40	6.85 ± 1.55	113.5 ± 20.32***	4.03 ± 1.39	1.36 ± 0.88	83.90 ± 17.56##
CCL5	7.08 ± 1.43	8.15 ± 2.22	2.62 ± 0.45	5.04 ± 0.66	3.06 ± 0.61	0.80 ± 0.11
CCL11	109.6 ± 48.44	130.8 ± 27.15	145.2 ± 36.29	134.6 ± 27.05	84.97 ± 15.02	113.5 ± 44.59
CSF2	0.21 ± 0.88	0.36 ± 0.15	15.65 ± 4.24***	0.16 ± 0.68	0.20 ± 0.01	11.06 ± 2.81#
CSF3	2.71 ± 0.92	34.29 ± 23.53	277.1 ± 108.6**	4.45 ± 0.66	15.15 ± 3.15	366.2 ± 120.0##
CXCL2	4.66 ± 0.54	47.66 ± 13.03	2492 ± 507.3***	4.15 ± 0.94	58.25 ± 27.15	1478 ± 340.1###
CXCL5	0.04 ± 0.01	15.83 ± 14.49	117.7 ± 56.67**	0.04 ± 0.01	0.08 ± 0.01	10.38 ± 6.65
IL-2	1.15 ± 0.21	1.30 ± 0.31	1.85 ± 0.55	0.92 ± 0.15	1.94 ± 0.16	0.81 ± 0.16§
IL-5	0.95 ± 0.28	0.84 ± 0.35	0.80 ± 0.26	0.76 ± 0.27	0.42 ± 0.08	0.25 ± 0.07
IL-6	1.47 ± 0.68	3.77 ± 0.95	548.4 ± 234.2***	1.33 ± 0.48	5.94 ± 1.74	465.9 ± 119.7##
IL-7	2.14 ± 0.20	2.34 ± 0.33	4.19 ± 0.52*	2.20 ± 0.15	3.01 ± 0.41	1.22 ± 0.39§§§
IL-9	32.41 ± 8.75	41.05 ± 9.63	72.04 ± 19.40	22.84 ± 8.14	39.38 ± 4.70	24.90 ± 2.02§§
IL-12B	2.09 ± 0.30	1.59 ± 0.40	1.92 ± 0.44	1.19 ± 0.25	2.68 ± 0.15	1.77 ± 0.26
IL-15	7.60 ± 1.40	5.86 ± 2.03	3.97 ± 1.06	6.32 ± 0.72	3.55 ± 0.94	1.84 ± 0.51
IL-17	0.64 ± 0.16	1.79 ± 0.43	24.74 ± 5.71***	0.45 ± 0.06	1.29 ± 0.45	18.89 ± 3.26##
Not detected: IL-3, IL-12A						

Supplementary Table 1. Luminex analytes that did not demonstrate significant differences in colonic tissues from *Egfr*$^{fl/fl}$ and *Egfr*$^{\Delta mye}$ mice. A total of 32 distinct analytes were assessed in colonic tissues from control mice and from tumor and adjacent non-tumor tissues from mice treated with AOM-DSS. Listed are the analytes that were not significantly induced during AOM-DSS treatment, were not relevant to subsequent analyses in the study or demonstrated no significant differences between genotypes. n = 5 control and 6-9 non-tumor/tumor pairs per genotype. *P<0.05, **P<0.01, and ***P< 0.001 versus *Egfr*$^{fl/fl}$ control. #P<0.05, ##P<0.01, and ###P< 0.001 versus *Egfr*$^{\Delta mye}$ control. §P<0.05, §§P<0.01, and §§§P< 0.001 versus *Egfr*$^{fl/fl}$ AOM-DSS by one-way ANOVA with Kruskal-Wallis test, followed by Mann-Whitney U test.

Analyte	Concentration of Analyte (pg/mg protein); Mean ± S.E.M.					
	Egfr$^{fl/fl}$			*Egfr*$^{\Delta Glepi}$		
	Control	**Non-tumor**	**AOM-DSS**	**Control**	**Non-tumor**	**AOM-DSS**
CCL2	9.67 ± 0.99	17.28 ± 2.81	110.7 ± 28.54**	4.705 ± 0.90	13.82 ± 4.94	144.2 ± 42.02##
CCL5	15.08 ± 1.27	6.39 ± 0.95	4.75 ± 1.86	9.68 ± 1.47	5.39 ± 1.33	4.57 ± 2.30
CCL11	136.9 ± 23.32	196.8 ± 22.02	202.4 ± 32.14	219.8 ± 26.30	360.5 ± 100.7	504.0 ± 125.3
CSF2	1.30 ± 0.13	0.95 ± 0.42	19.19 ± 7.13**	0.31 ± 0.11	0.45 ± 0.23	24.77 ± 8.90##
CSF3	6.86 ± 4.27	14.93 ± 3.66	254.3 ± 126.3**	1.98 ± 0.32	15.16 ± 5.50	262.8 ± 160.5##
CXCL2	26.10 ± 16.47	102.6 ± 87.36	1952 ± 727.4***	4.54 ± 0.92	77.22 ± 53.77	2186 ± 625.3##
CXCL5	2.39 ± 0.19	5.95 ± 2.06	155.3 ± 34.67**	2.28 ± 0.36	4.46 ± 1.14	116.9 ± 46.21##
IL-2	0.72 ± 0.07	2.99 ± 2.17	7.32 ± 5.58	0.71 ± 0.11	1.38 ± 0.68	12.13 ± 10.35
IL-5	0.62 ± 0.10	0.46 ± 0.06	1.01 ± 0.17	0.54 ± 0.15	0.47 ± 0.12	0.83 ± 0.19
IL-6	6.15 ± 5.10	4.81 ± 1.16	490.3 ± 275.2*	0.67 ± 0.09	8.56 ± 3.45	909.9 ± 517.9##
IL-7	2.27 ± 0.20	2.72 ± 0.39	3.06 ± 0.27	1.85 ± 0.13	3.62 ± 1.53	2.53 ± 0.28
IL-9	26.01 ± 3.37	43.58 ± 9.70	72.24 ± 13.17*	21.40 ± 4.24	29.28 ± 6.35	91.55 ± 25.81##
IL-12B	1.12 ± 0.41	0.16 ± 0.05	0.50 ± 0.31	0.39 ± 0.16	0.12 ± 0.03	0.62 ± 0.39
IL-13	0.25 ± 0.20	1.14 ± 0.46	1.07 ± 0.28	0.06 ± 0.02	1.22 ± 0.21	1.36 ± 0.14
IL-15	7.28 ± 0.27	2.58 ± 0.50	3.72 ± 0.67	5.67 ± 1.17	2.15 ± 0.75	2.56 ± 0.67
IL-17	0.85 ± 0.43	2.89 ± 0.51	18.02 ± 3.36**	0.35 ± 0.09	1.57 ± 0.66	11.70 ± 4.83##
Not detected: IL-3, IL-12A						

Supplementary Table 2. Luminex analytes that did not demonstrate significant differences in colonic tissues from *Egfr*$^{fl/fl}$ and *Egfr*$^{\Delta Glepi}$ mice. A total of 32 distinct analytes were assessed in colonic tissues from control mice and from tumor and adjacent non-tumor tissue from mice treated with AOM-DSS. Listed are the analytes that were not significantly induced during AOM-DSS treatment or demonstrated few or no significant differences between genotypes. *n* = 5 control and 6-9 non-tumor/tumor pairs per genotype. **P*<0.05, ***P*<0.01, and ****P*< 0.001 versus *Egfr*$^{fl/fl}$ control. ##*P*<0.01 versus *Egfr*$^{\Delta mye}$ control by one-way ANOVA with Kruskal-Wallis test, followed by Mann-Whitney *U* test.

Species	Target	Sequence
Mouse	*β-actin*	F: CCAGAGCAAGAGAGGTATCC
		R: CTGTGGTGGTGAAGCTGTAG
Mouse	*Nos2*	F: CACCTTGGAGTTCACCCAGT
		R: ACCACTCGTACTTGGGATGC
Mouse	*Tnfa*	F: CTGTGAAGGGAATGGGTGTT
		R: GGTCACTGTCCCAGCATCTT
Mouse	*Il1b*	F: ACCTGCTGGTGTGTGACGTTCC
		R: GGGTCCGACAGCACGAGGCT
Mouse	*Arg1*	F: AAGAAAAGGCCGATTCACCT
		R: CACCTCCTCTGCTGTCTTCC
Mouse	*Chil3*	F: ACTTTGATGGCCTCAACCTG
		R: AATGATTCCTGCTCCTGTGG
Mouse	*Il10*	F: CCAAGCCTTATCGGAAATGA
		R: TCACTCTTCACCTGCTCCAC
Mouse	*Cxcl1*	F: GCTGGGATTCACCTCAAGAA
		R: CTTGGGGACACCTTTTAGCA
Mouse	*Vegfa*	F: GAGGATGTCCTCACTCGGATG
		R: GTCGTGTTTCTGGAAGTGAGCAA

Supplementary Table 3. List of primers used for qRT-PCR.

Antibody	Dilution	Application	Source (Location)
Rabbit polyclonal anti-pEGFR Y1068	Pre-diluted	IHC-P IF	Biocare Medical (Concord, CA) Cat. No. API 300
Rabbit polyclonal anti-tEGFR	1:3,000 1:100	WB IF	Cell Signaling (Danvers, MA) Cat. No. 2232
Rat monoclonal anti-CD31	1:100	IHC-P	Dianova (Hamburg Germany) Cat. No. DIA 310
Mouse monoclonal anti-β-actin	1:10,000	WB	Sigma-Aldrich (St. Louis, MO) Cat. No. A1978
Goat anti-mouse IgG, HRP labeled	1:30,000	WB	Jackson ImmunoResearch (St. Louis, MO) Cat. No. 115-035-003
Goat anti-rabbit IgG, HRP labeled	1:3,000	WB	Jackson ImmunoResearch (St. Louis, MO) Cat. No. 115-035-003
Mouse monoclonal anti-CD68	Pre-diluted	IF	Biocare Medical (Concord, CA) Cat. No. PM033AA
Goat anti-mouse IgG, Alexa555	1:500	IF	Life Technologies (Carlsbad, CA) Cat. No. A31570
Goat anti-rabbit IgG, Alexa488	1:500	IF	Jackson ImmunoResearch (St. Louis, MO) Cat. No. 111-095-003
Rabbit HRP Polymer	Pre-diluted	IF/IHC-P*	Biocare Medical (Concord, CA) Cat. No. RHRP520
Donkey anti-HRP, FITC	1:400	IF	Jackson ImmunoResearch (St. Louis, MO) Cat. No. 123-545-021
Rabbit anti-rat IgG, biotinylated	1:200	IHC-P**	Vector Laboratories (Burlingame, CA) Cat. No. BA-4000
Goat anti-rabbit IgG, biotinylated	1:400	IHC-P***	Vector Laboratories (Burlingame, CA) Cat. No. BA-1000
Rabbit polyclonal anti-CD68	1:200	IHC-P	Boster Biological Technology (Pleasanton, CA) Cat. No. PA-1518
Rabbit polyclonal anti-MPO	Pre-diluted	IHC-P	Biocare Medical (Concord, CA) Cat. No. PP-023-AA
Rabbit monoclonal anti-CD3	1:250	IHC-P	Abcam (Cambridge, MA) Cat. No. ab16669
Rat monoclonal anti-CD45R	1:30,000	IHC-P	BD Pharmingen (San Jose, CA) Cat. No. 553084
Rabbit polyclonal anti-pSTAT6 Y941	1:100	IHC-P	Lifespan Biosciences, Inc. (Seattle, WA) Cat. No. LS-C117487

Supplementary Table 4. List of all antibodies used for this study, including the dilution, application and company/catalog number from which the antibodies were purchased. WB = western blotting, IF = immunofluorescence, IHC-P = immunohistochemistry-immunoperoxidase. * = used for CD68, MPO, and pSTAT6 IHC-P. ** = used for CD45R and CD31 IHC-P. *** = used for CD3 IHC-P.

Figure	Panel	*P* value
1	b	= 0.002
1	c	= 0.008
2	a	< 0.0001
2	b	< 0.0001
2	d	< 0.0001
3	a	CCL3: = 0.0005 CCL4: = 0.0003 CXCL9: = 0.0003 CXCL10: = 0.0011
3	b	< 0.0001
3	c	CSF1: = 0.0003 IL-1α: = 0.0011
5	a	IFN-γ: = 0.0079 TNF-α: < 0.0001
5	b	*Nos2*: < 0.0001 *Il1b*: < 0.0001
5	c	= 0.0004
6	a	IL-4: = 0.002 IL-10: = 0.04 IL-13: = 0.0049
6	b	*Arg1*: < 0.0001 *Il10*: = 0.0032
7	a	*Cxcl1*: < 0.0001 *Vegfa*: = 0.05
7	b	CXCL1: < 0.0001 VEGF: = 0.0095

Figure	Panel	*P* value
S5	a	CCL3: = 0.0005 CCL4: = 0.0003 CXCL9: = 0.0003 CXCL10: = 0.0011
S5	b	< 0.0001
S5	c	CSF1: = 0.0003 IL-1α: = 0.0011
S6	a	IL-4: = 0.0202 IL-10: = 0.05
S6	b	*Arg1*: 0.0012 *Il10*: = 0.0049
S7	a	< 0.0001
S7	b	*Nos2*: = 0.0002 *Il1b*: = 0.0002
S7	c	= 0.0004
S8	a	= 0.0002
S8	b	< 0.0001

Supplementary Table 5. List of all *P* values derived from Kruskal-Wallis testing. S = supplementary figure.