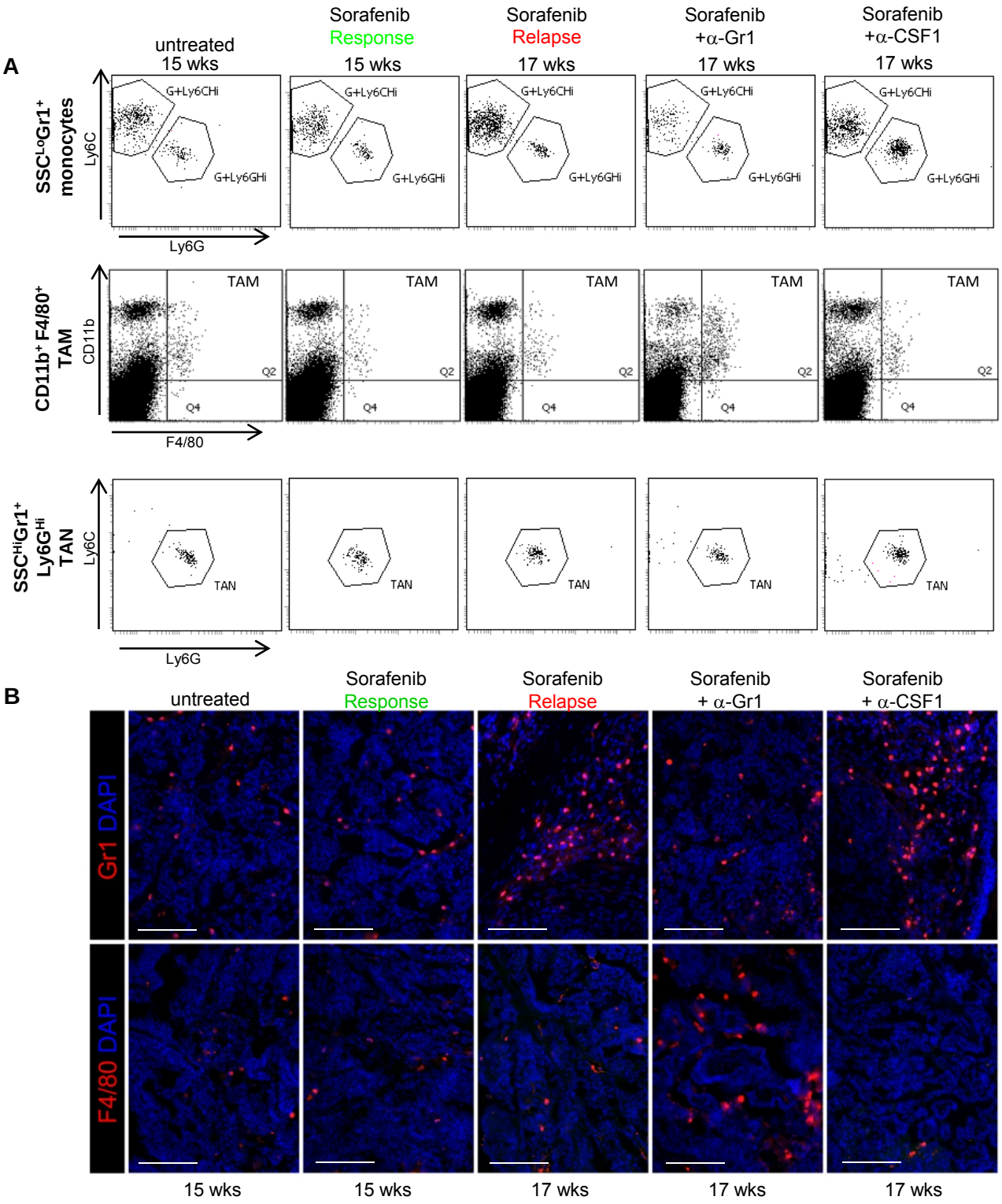

Figure S1. Flow cytometry and immunofluorescent staining of myeloid cells in Rip1Tag2 tumors, Related to Figure 1

(A) Myeloid cell composition in Rip1Tag2 tumors treated with either sorafenib alone, sorafenib plus anti-Gr1, or sorafenib plus anti-CSF1. (B) Immunfluorescent staining Gr1-expressing cells and F4/80$^+$ TAM in treated and untreated Rip1Tag2 tumors.

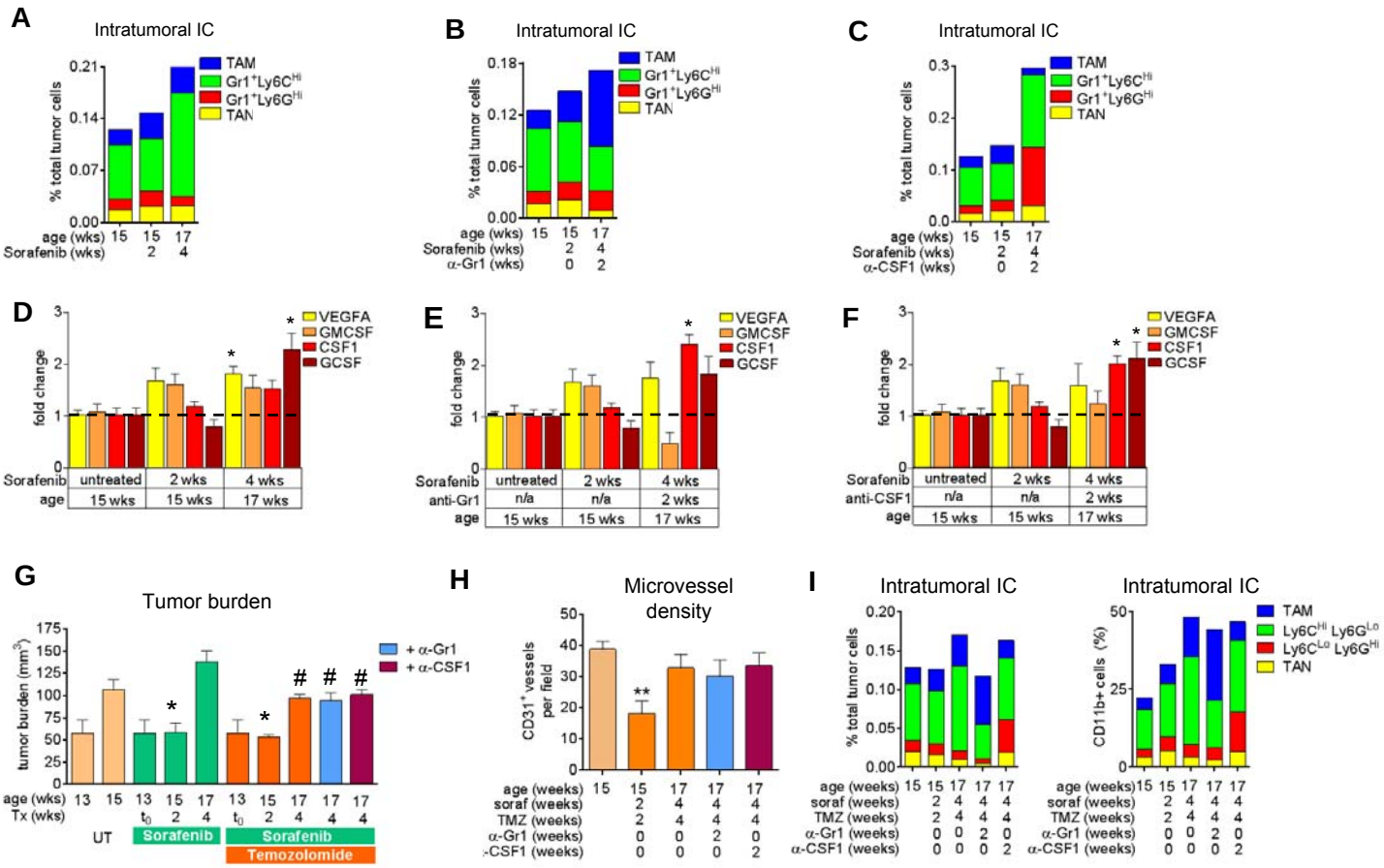

Figure S2. Oscillating resistance in Rip1Tag2 mice, and oscillating resistance in the context of chemotherapy, Related to Figure 1 and Discussion

(A-C) FACS-analysis of myeloid cell composition in Rip1Tag2 tumors treated with either sorafenib alone (A), plus anti-Gr1, or plus anti-CSF1 (C). P<0.05 for relapse versus response phase $Gr1^{+}Ly6C^{Hi}$ cells, TAMs in sorafenib relapse tumors versus sorafenib plus anti-Gr1, and for $Gr1^{+}Ly6G^{Hi}$ in sorafenib plus anti-Gr1 versus sorafenib plus anti-CSF1. (D-F) QPCR-based expression analyses of factors that regulate myeloid homoestasis in Rip1Tag2 tumors. *p<0.05 versus untreated. (G-I) Effect of combining temozolomide (TMZ) with sorafenib and immune cell-depleting antibodies on Rip1Tag2 tumor burden (mean±SEM) (J), microvessel density (mean±SEM) (K), and myeloid cell composition (L), normalized to total tumor (right panel) or total $CD11b^{+}$ content (left panel). IC=immune cells. Untreated and sorafenib-treated data in (J) are from Figure. For tumor burden, *p<0.05 versus untreated (UT) 15 weeks; *p≤0.05 versus 4 weeks sorafenib (reflecting 17 weeks of age). For microvessel density, **p<0.005 versus 15 weeks untreated. For myeloid cell composition, p≤0.05 for $Gr1^{+}Ly6C^{Hi}$ monocytes and TAN from four versus two week sorafenib/temozolomide (TMZ), for $Gr1^{+}Ly6C^{Hi}$ and $Gr1^{+}Ly6G^{Hi}$ monocytes, TAN, and TAM from four week sorafenib/temozolomide versus four week sorafenib/temozolomide plus either anti-Gr1 or anti-CSF1.

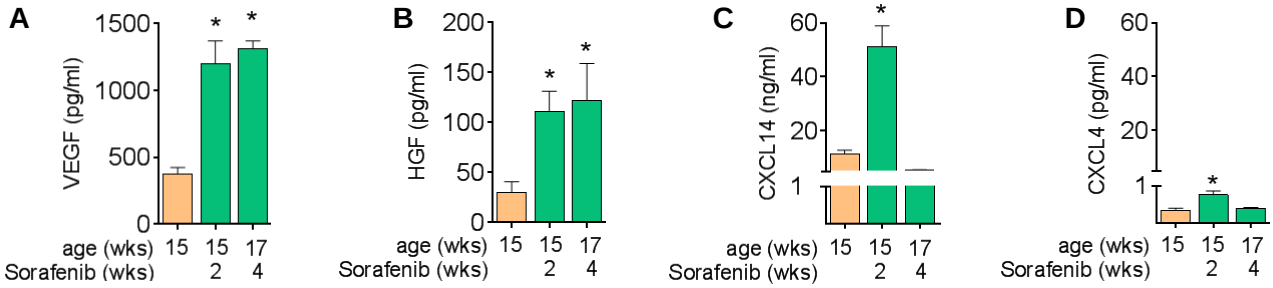

Figure S3. Quantitative analysis of secreted factors in RT2 tumors via multiplex sandwich ELISA, Related to Figure 2

(A-D) Protein concentrations of factors simultaneously detected in tumor lysates taken from RT2 mice treated as indicated. (A) VEGF; *p<0.05 versus untreated. (B) HGF; *p<0.05 versus untreated. (C) CXCL14; p*<0.05 versus untreated and four weeks sorafenib treatment. (D) CXCL4; *p<0.05 versus untreated and four weeks sorafenib treatment.

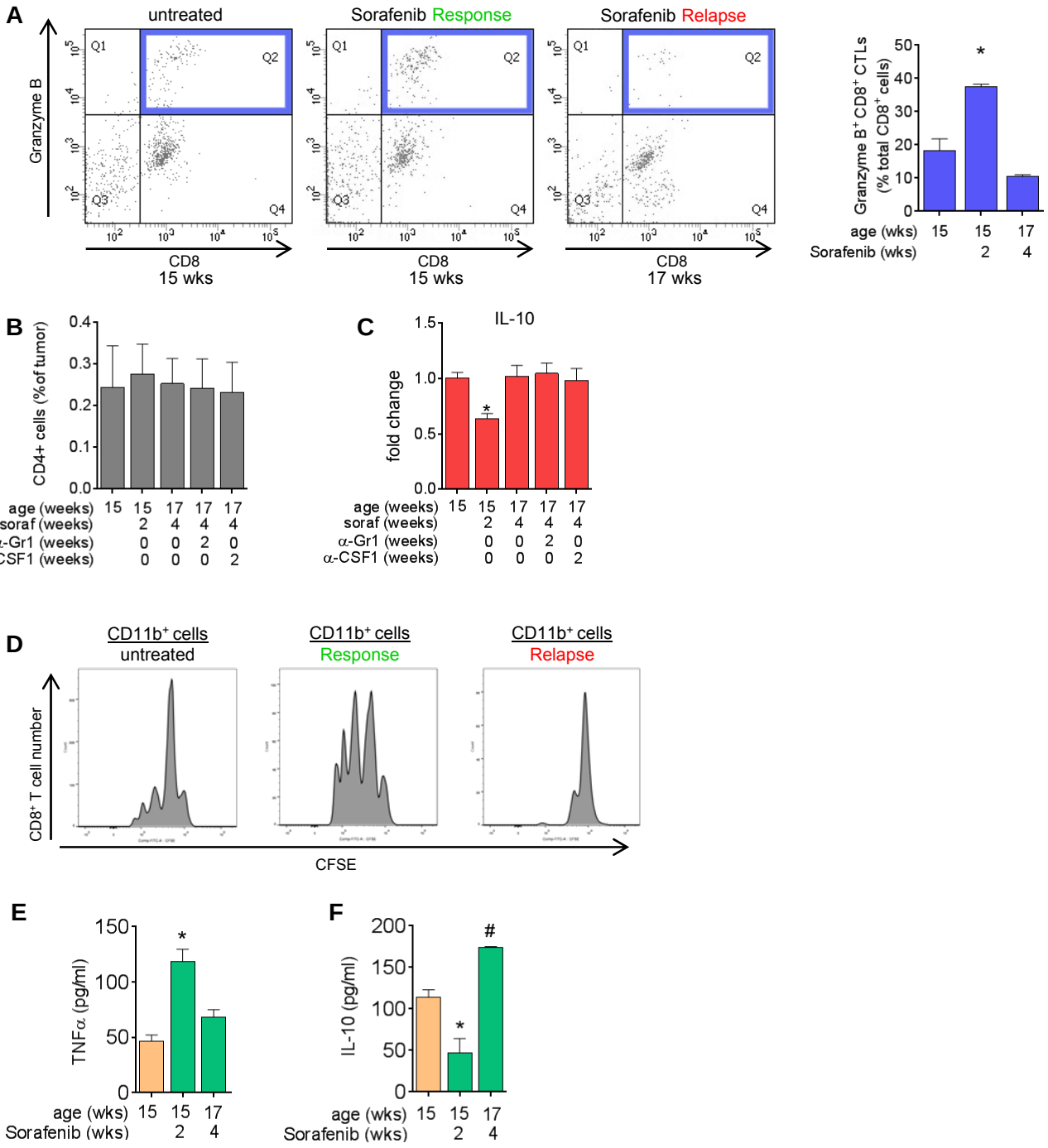

Figure S4. Rip1Tag2 tumors responding to sorafenib treatment exhibit enhanced anti-tumor immunity while resistant tumors exhibit an immune-suppressive phenotype, Related to Figure 4

(A) Granzyme B content in intratumoral CD8$^+$ T cells from untreated, sorafenib response (two week treatment) and sorafenib relapse (four week treatment). Quantitation of Granzyme B$^+$ CD8$^+$ T cells versus total CD8$^+$ T cells is presented in the far-right panel. (B) FACS-analysis of CD4$^+$ T cell-content of Rip1Tag2 tumors (mean±SEM). (C) QPCR analysis of IL-10 expression in CD4$^+$ T cells FACS-sorted from Rip1Tag2 tumors (mean±SEM). *p=0.05 versus 15 weeks untreated. (D) CFSE proliferation assay of naiive CFSE-labeled CD8$^+$ T cells cocultured with CD11b$^+$ cells isolated from untreated, sorafenib response, or sorafenib relapse phase Rip1Tag2 tumors. (E and F) Protein concentrations of factors simultaneously detected in tumor lysates taken from RT2 mice treated as indicated. TNFα; p*<0.05 versus untreated and four weeks sorafenib treatment. IL-10; *p<0.05 versus untreated and four weeks sorafenib treatment, #p<0.05 versus untreated, two weeks sorafenib treatment.

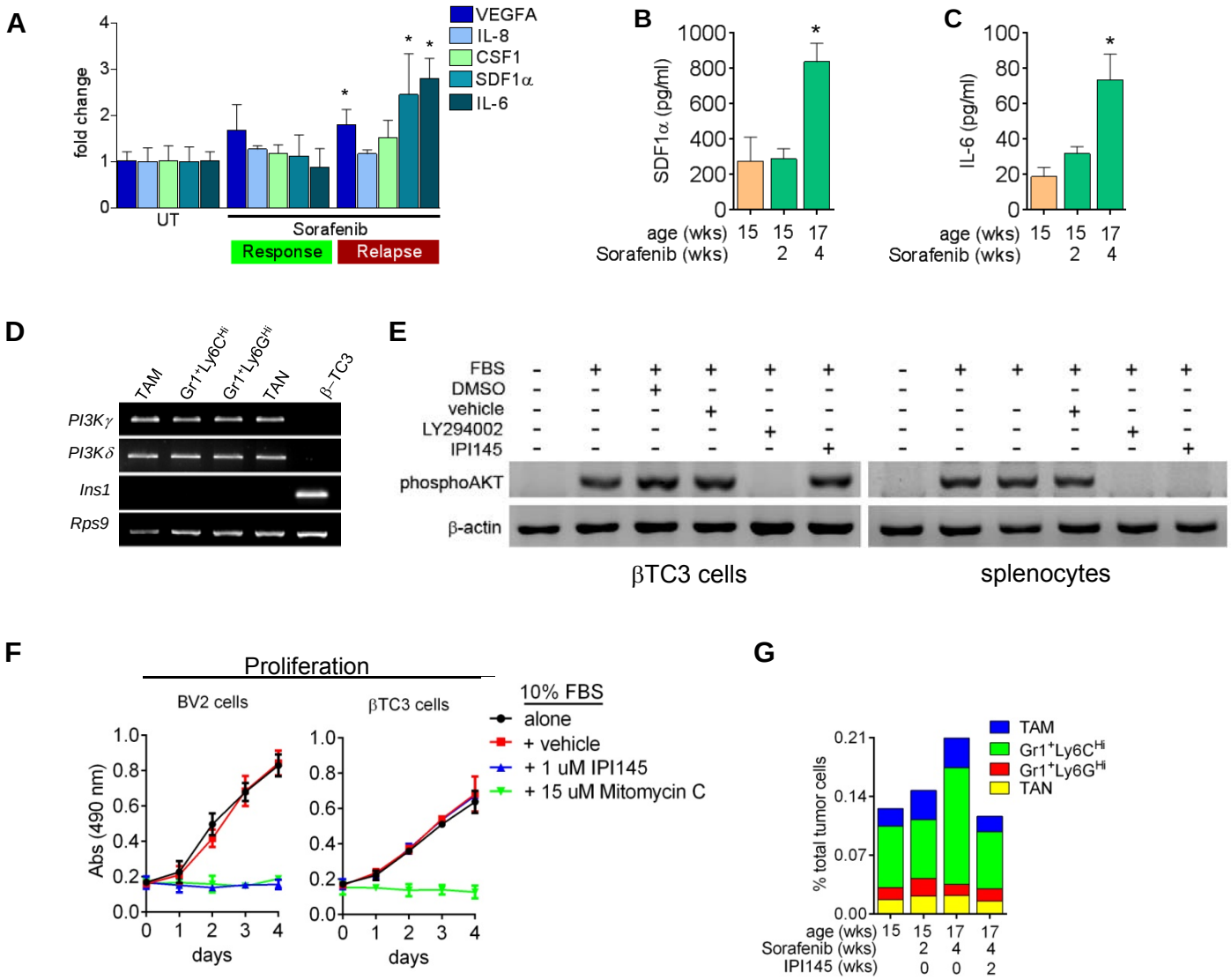

Figure S5. Rip1Tag2 tumors do not respond to IPI145, Related to Figure 5

(A) QPCR-based expression analyses of Rip1Tag2 tumors for factors previously demonstrated to induce myeloid cell PI3K activation. *p≤ 0.05 versus untreated (UT). (B and C) Protein concentrations of factors simultaneously detected in tumor lysates taken from RT2 mice treated as indicated. *p<0.05 versus untreated, two weeks sorafenib treatment. (D) RTPCR of cDNA generated from Rip1Tag2 β-TC3 cells or FACS-sorted myeloid cells from Rip1Tag2 tumors. (E) PI3K signaling in β-TC3 cells versus CD11b^{+} splenocytes. Serum starved cells were treated overnight with 50 uM LY294002 or its vehicle DMSO, or with 500 nM IPI145, or its vehicle (PEG400/Tween). (F) MTS assay showing proliferation of BV2 cells and βTC3 cells in response to the 10% FBS plus the indicated treatment. (G) FACS-analysis of myeloid cell composition of tumors treated with sorafenib combined with IPI145 compared to untreated and sorafenib-treated tumors from Figure S2C. P≤0.05 for Gr1^{+}Ly6C^{Hi} and Gr1^{+}Ly6G^{Hi} monocytes and TAN from four week sorafenib alone versus four week sorafenib plus IPI145.

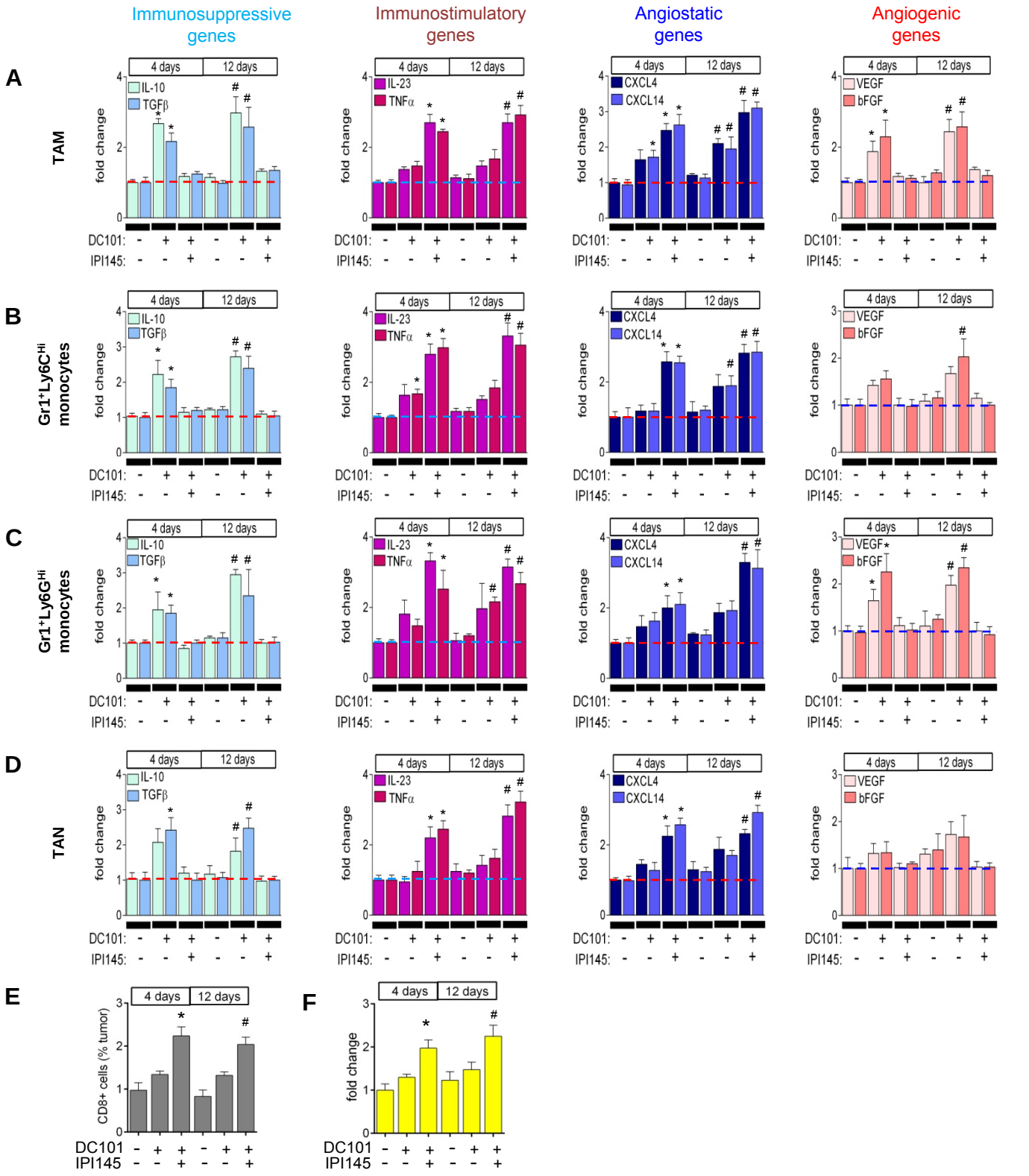

Figure S6. IPI145 skews myeloid cell polarization and angiogenic potential in PyMT tumors, Related to Figure 7

(A-D) QPCR-based expression analyses of FACS-sorted TAMs (A), Gr1^{+} Ly6C^{Hi} monocytes (B) and Gr1^{+} Ly6G^{Hi} monocytes (C), and TANs (D) from PyMT tumors of mice untreated or treated with DC101 with or without IPI145 (mean±SEM). (E) FACS-analysis of CD8^{+} CTLs from tumors of treated and untreated mice (mean±SEM). (H) QPCR expression analysis of *Perforin* in FACS-sorted CD8^{+} CTLs (mean±SEM). *p< 0.05 versus four day control; #p< 0.05 versus 12 day control. Dotted lines indicate baseline gene expression in untreated samples.

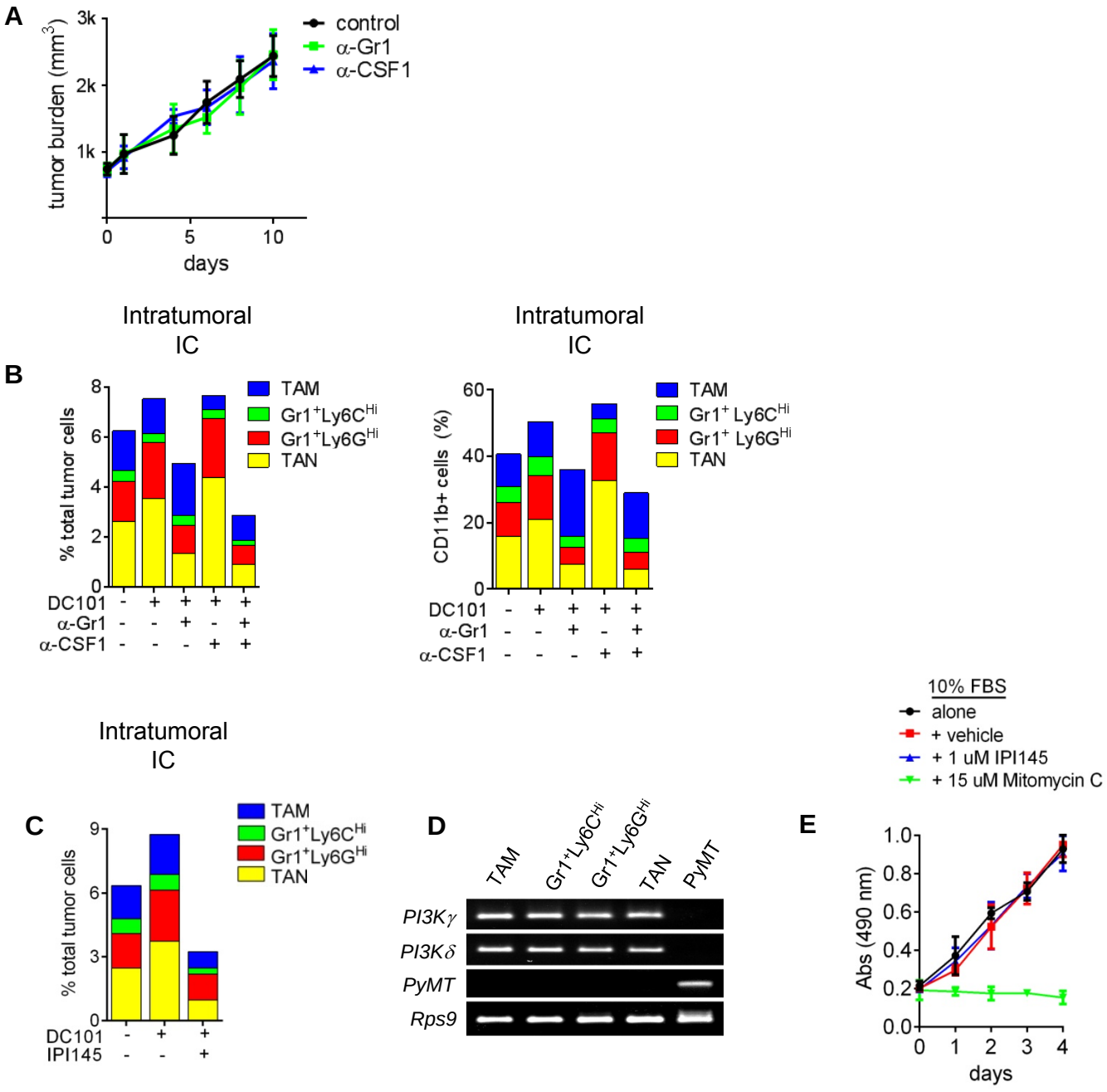

Figure S7. Depletion of myeloid cells enhances the efficacy of DC101 but causes leukopenia in mice bearing PyMT tumors, Related to Figure 7

(A) Orthotopic PyMT tumors treated with myeloid cell-depleting antibodies as indicated. (B) Myeloid cell composition of 12 day tumors. P< 0.05 for TAM, Gr1^{+}Ly6G^{Hi} monocytes, and TAN from DC101 alone versus DC101 plus anti-Gr1/anti-CSF1. (C) Myeloid cell composition of blood from mice at 12 days. P<0.05 for TAM, Gr1^{+}Ly6G^{Hi} and Gr1^{+}Ly6C^{Hi} monocytes, and TAN from DC101 versus DC101 plus IPI145. (D) RTPCR of cDNA generated from PyMT tumor cells or FACS-sorted myeloid cells from orthotopic PyMT tumors. (E) MTT assay of PyMT cells.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animal studies

Heterozygous C57BL/6 Rip1Tag2 (Tg(RIP1-Tag)2Dh) mice were enrolled onto 30 mg/kg sorafenib therapy beginning at 13 weeks of age. Sorafenib was administered orally five days a week for either two weeks (response phase) or four weeks (relapse phase). Anti-Gr1 clone RB6-8C5 was administered at 10 mg/kg three times a week beginning at 15 weeks of age, at the onset of relapse to Sorafenib, while anti-CSF1 clone 5A1 was administered at every five days at 1 mg per mouse for the first dose and 0.5 mg per mouse for subsequent doses beginning at 15 weeks of age. Both anti-Gr1 and anti-CSF1 were administered by intraperitoneal injection. IPI145 (Active Biochem) was administered seven days a week at 10 mg/kg/day beginning at 15 weeks of age via oral gavage. For experiments assessing the effect of chemotherapy on immune cell oscillations, TMZ was administered to Rip1TAg2 mice five days a week via intraperitoneal injection at 0.5 mg/kg beginning simultaneously with sorafenib at 13 weeks of age, while antibody therapy was initiated at 15 weeks of age. For isotype control (clone LTF-2) and anti-CD8a (clone 2.43) + sorafenib combinations, mice were were injected i.p with 0.5 mg IgG per mouse for 3 consecutive days before start of sorafenib treatment, followed by weekly injections of antibodies (1 mg). For anti-CXCL14, mice were dosed 3 times per week at 0.5 mg per mouse i.p. concurrent with sorafenib treatment.

For survival studies, IPI145 treatment was initiated at 13 weeks of age. Tumor burden of each Rip1-Tag2 mouse was equal to the sum of each individual tumor per mouse. For Rip1Tag2 tumor burden analysis, the following number of mice per group were used: untreated 13 weeks n=7, 14 weeks n=19, 15 weeks n=56; sorafenib one week (reflecting 14 weeks of age) n=6, two weeks (reflecting 15 weeks of age) n=36, three weeks (reflecting 15 weeks of age) n=15, four weeks (reflecting 17 weeks of age) n=53; sorafenib + anti-Gr1 one week (reflecting 16 weeks of age) n=9, sorafenib + anti-Gr1 two weeks (reflecting 17 weeks of age) n=69; sorafenib + anti-

CSF1 one week (reflecting 16 weeks of age) n=9, sorafenib + anti-CSF1 two weeks (reflecting 17 weeks of age) n=32; sorafenib + combined anti-Gr1/anti-CSF1 one week (reflecting 16 weeks of age) n=8, sorafenib + combined anti-Gr1/anti-CSF1 two weeks (reflecting 17 weeks of age) n=30; sorafenib + IPI145 two weeks (reflecting 17 weeks of age) n=8; sorafenib + anti-CXCL14 n=5; sorafenib; + anti-CD8a n=5, sorafenib + isotype control n=5.

Orthotopic breast cancer experiments were performed using a cell line generated from a primary FVB MMTV- PyMT mammary tumor (Zena Werb). 100,000 PyMT cells were implanted into the mammary fat pads of FVB female mice, then allowed to establish until they reached 5 mm in diameter. Mice were then dosed with DC101 at 1 mg/mouse twice a week via intraperitoneal injection. For cohorts treated with both DC101 plus myeloid cell-depleting antibodies, anti-Gr1 or anti-CSF1 was initiated simultaneously with DC101. For IPI145 experiments, IPI145 was initiated two days before starting DC101 treatment. Tumor burden for both PyMT and Rip1Tag2 tumors was measured using the formula to approximate the volume of an ellipsoid: 0.52 X $(\text{width})^2$ X (length).

FACS

Tumors were harvested from PBS-perfused mice then mechanically dissociated before incubating in digest buffer (1% BSA with, 12,500 units of collagenase II, 12,500 units collagenase IV, 40 units DNAse I, and 1X penicillin/streptomycin in PBS) at 37^{o}C for 13 minutes with gentle agitation. Digests were stopped using Automacs buffer (Miltenyi), and dissociated tumors were passed through a 70 um-pore size mesh before washing in PBS. Cell pellets were treated with Pharmlyse (BD) to remove red blood cells, then washed several times with 1% BSA in PBS. Cells were then treated with Fc-block and stained with pacific blue-conjugated anti-CD45 (eBioscience 57-0451), allophycocyanine-conjugated anti-CD11b (eBioscience 17-0112-82), fluorescein isothiocyanate-conjugated anti-F4/80 (eBioscience 11-4801-81), allophycocyanine-Cy7-conjugated anti-Ly6C (BD Pharmingen 560596), peridinin chlorophyll-

Cy5.5-conjugated anti-Ly6G (BD Pharmingen 560602), phycoerythrin-conjugated anti-Gr1 (BD Pharmingen 553128), phycoerythrin-Cy7-conjugated anti-CD4 (BioLegend 1100527), and eFluor650NC-conjugated anti-CD8 (eBioscience 95-0081-41). Samples were then washed and sorted using a FACS ARIA III and FACS DIVA software (BD). TAMs were sorted as $CD45^{+}$ $CD11b^{+}$ $F4/80^{+}$ $Gr1^{-}$, TANs were SSC^{high} $CD45^{+}$ $CD11b^{high}$ $Gr1^{high}$, immature $Gr1^{+}Ly6G^{High}$ cells as SSC^{low} $CD45^{+}$ $CD11b^{high}$ $Gr1^{high}$ $Ly6C^{low}$ $Ly6G^{high}$, and immature $Gr1^{+}Ly6C^{high}$ cells were SSC^{low} $CD45^{+}$ $CD11b^{high}$ $Gr1^{high}$ $Ly6C^{high}$ $Ly6G^{low}$. For blood analyses, 100 ul of blood was treated with Fc-block then stained with the above antibodies. Red blood cells were then lysed in BD Pharm Lyse (BD Biosciences), and samples were washed several times in 1% BSA in PBS before analysis. Compensation was performed using singly-stained beads (Molecular Probes). Data was analyzed using FlowJo software (Treestar).

Immunostaining

Staining was performed on either frozen or paraffin-embedded tissue sections. To prepare frozen tissue sections, excised pancreata or tumors were fixed in paraformaldehyde, then washed in PBS. Tumors were then washed in PBS and sunk in 30% sucrose before being frozen embedded in OCT (Tissue-tek). To prepare paraffin-embedded sections, excised pancreata or tumors were fixed in paraformaldehyde then washed in 70% ethanol in water before processing and embedding. Paraffin-embedded tissue sections were rehydrated then unmasked using citrate buffer under high heat and pressure. Mouse tissues were stained using anti-CD31 (BioLegend 102501), anti-CD11b (BioLegend 101213), and anti-phospho-S6 (Cell Signaling 4858) primary antibodies, while paraffin human tissues were stained using anti-CD45 (BioLegend 304001) and anti-phospho-S6 primary antibodies (Cell Signaling 4858). Staining was visualized using fluorophore-conjugated secondary antibodies with an Observer Z1 microscope (Zeiss). Images were captured using an AxioCam MRM camera (Zeiss) and analyzed using Axiovision software (Zeiss). For microvessel density, at least 8 representative

images were analyzed per mouse from at least 10 mice per group for Rip1Tag2 studies, or 7 mice per group for PyMT studies, and taken using an objective with 20X magnification. For phospho-S6/CD11b, images were taken using an objective with 40X magnification. For phospho-S6/CD45 staining in human sections, 10 representative images per tumor were analyzed and taken using an objective with 40X magnification.

CFSE proliferation assay

$CD8^+$ T cells were FACS-sorted from spleens of naïve 5 week old C57BL/6 mice. Cells were then washed in AutoMacs buffer (Miltenyi) and, using the CellTrace CFSE Cell Proliferation Kit (Life Technologies), pulsed with 10 um carboxyfluorescein succinimidyl ester and washed in RPMI1645 media with 10% FBS. Cells were seeded at 10,000 cells per well in a 96 well plate in 100 ul volume. To stimulate proliferation, anti-CD3/anti-CD28 coated beads (Invitrogen) were added to cells at a 1:2 cell:bead ratio. Macrophages, $Gr1^+Ly6G^{high}$, and $Gr1^+Ly6C^{high}$ monocytes were FACS-sorted from spleens of 15 week old C57BL/6 Rip1-Tag2 mice (untreated or treated 2 weeks with Sorafenib), and 17 week old RT2 mice (treated 4 weeks with Sorafenib), and CD11b+ cells were isolated from tumors. Cells were then added at a 1:3 $CD8^+$T cell:myeloid cell ratio and cultured for 7 days. CFSE levels were then assessed in $CD8^+$ cells by flow cytometry using a FACS ARIA III and FACS DIVA software (BD).

Cell Culture and western blot analysis

$CD11b^+$ splenocytes were isolated using anti-CD11b-conjugated magnetic MicroBeads and a MACS separator (Miltenyi). Cells were washed and suspended in serum free RPMI1645 media, then seeded at 100,000 cells per well in a 6 well plate. Similarly, βTC3 cells were plated at 100,000 cells per well. Cells were serum-starved overnight at 37^{o}C then stimulated with serum in the presence or absence of 50 uM LY294002, DMSO (LY294002 vehicle), 500 nM IPI145, or IPI145 vehicle (PEG400/Tween). Cells were cultured overnight then subjected to lysis and

western blot analysis using antibodies against phospho-AKT (Cell Signaling 4060) and β-actin (Cell Signaling 4970). For *in vitro* proliferation studies, cells were plated in triplicate, serum starved overnight, then incubated in the presence of 10% FBS medium. Viable cells were assessed daily using the CellTiter 96 Aqueous assay (Promega).

Aortic Slice assay

Aortas were harvested from 42-day old male C57/BL6 mice and sliced into 1 mm long fragments. These were singly embedded into 100 ul 1ug/ml type I collagen in 1X DMEM (Corning) in 16-well chamber slides (Lab-Tek). Collagen-embedded slices were then allowed at 37^{o}C for 60 minutes to allow collagen polymerization. 75 ul of assay medium (2% FBS-supplemented OptiMem medium (UCSF Cell Culture Facility) containing antibiotics and antimycotics) was then added to the top each collagen plug. Endothelial cell migration into the collagen matrix was assessed by immunofluorescent staining of CD31 after 7 days. To test the effects of CD11b+ cells on aortic-endothelial cell migration, CD11b+ cells were sorted from tumors of 4 untreated, 5 two-week sorafenib treated (response phase), or 5 four-week sorafenib treated (relapse phase) RT2 mice, were suspended at 10,000 cells per 100 ul medium, then lysed via sonication. The aqueous portion of each CD11b+ cell lysate was then separated and used as assay medium, thus 75 ul of medium was equivalent to 7,500 CD11b+ cells. Each lysate was assayed with aorta from two mice, each in duplicate.

Quantitative multiplex ELISA

RT2 PNET were homogenized using mechanical disruption and sonication. The aqeuous portion of each lysate was brought to 500 ug/ml in nondenaturing buffer containing Triton detergent and protease inhibitors (Cell Lysis Buffer; Cell Signaling). Lysates representing 3 mice per treatment condition were assessed, and 100 ul of each tumor lysate was analyzed for

VEGF, HGF, CXCL14, CXCL4, TNFα, IL-10, SDF1α, and IL-6 using a multiplex ELISA-based system (Quantibody Array; RayBiotech).

gene	symbol (*Mus musculus*)	forward primer (5'-3')	reverse primers (5'-3')
VEGFA	Vegfa	ATG TGA ACA AGC CAA GGC GGT G	CTG CTC TCT TGG GTC CAC TGG
CXCL10	Cxcl10	TCC CTC TCG CAA GGA C	TTG GCT AAA CGC TTT CAT
IL-6	Il6	GAG GAT ACC ACT CCC AAC AGA CC	AAG TGC ATC ATC GTT GTT CAT ACA
CCL17	Ccl17	ACC ATG AGG TCA CTT CAG AGC T	AAT GGC CCC TTT GAA GTA ATC C
CXCL4	Pf4	TGC ACT TAA GAG CCC TAG ACC CAT	AGA TCT CCA TCG CTT TCTT CGG GA
IL-1	Il1a	TTG ACG GAC CCC AAA AGA TG	AGA AGG TGC TCA TGT CCT CA
IL-12	Il2b	AGT CCC TTT GGT CCA GTG TG	AGC AGT AGC AGT TCC CCT GA
IL-23	Il23a	CCA GCG GGA CAT ATG AAT CT	AGG CTC CCC TTT GAA GAT GT
CCL11	Ccl11	GAA TCA CCA ACA ACA GAT GCA C	ATC CTG GAC CCA CTT CTT CTT
Arg1	Arg1	AAG AAA AGG CCG ATT CAC CT	CAC CTC CTC TGC TGT CTT CC
CXCL1	Cxcl1	CTG GGA TTC ACC TCA AGA ACA TC	CAG GGT CAA GGC AAG CCT C
Mmp9	Mmp9	GCC CTG GAA CTC ACA CGA CA	TTG GAA ACT CAC ACG CCA GAA G
bFGF	Fgf2	GGC TGC TGG CTT CTA AGT GT	CCG TTT TGG ATC CGA GTT TA
Perforin	Prf1	CGC CTA CCT CAG GCT TAT CTC	CCT CGA CAG TCA GGC AGT C
MMR	Mrc1	CAA GGA AGG TTG GCA TTT GT	CCT TTC AGT CCT TTG CAA GC
CXCl11	Cxcl11	AGG AAG GTC ACA GCC ATA GC	CGA TCT CTG CCA TTT TGA CG
CXCL14	Cxcl14	GAA GAT GGT TAT CGT CAC CAC C	CGT TCC AGG CAT TGT ACC ACT
IL-4	Il4	CAT CGG CAT TTT GAA CGA GGT CA	CTT ATC GAT GAA TCA GGC ATC G
CXCL2	Cxcl2	CCA ACC ACC AGG CTA CAG G	GCG TCA CAC TCA AGC TCT G
IL-10	Il10	CAG GGA TCT TAG CTA ACG GAA A	GCT CAG TGA ATA AAT AGA ATG GGA AC
TNFa	Tnf	CGG AGT CCG GGC AGG T	GCT GGG TAG AGA ATG GAT GAA CA
CCL22	Ccl22	GTG GCT CTC GTC CTT CTT GC	GGA CAG TTT ATG GAG TAG CTT
HGF	Hgf	ATC CAC GAT GTT CAT GAG AG	GCT GAC TGC ATT TCT CAT TC
CXCL9	Cxcl9	GGA GTT CGA GGA ACC CTA GTG	GGG ATT TGT AGT GGA TCG TGC
p47	Ncf1	GTT CTC AGC CCA ACA ATA CAA GA	GTG GAC GGG TCG ATG TCA
Mmp7	Mmp7	AGG AAG CTG GAG ATG TGA GC	TCT GCA TTT CCT TGA GGT TG
IL-8	Cxcl5	TGC CCT ACG GTG GAA GTC ATA	TGC ATT CCG CTT AGC TTT CTT T
Plgf	Pgf	GTG TGC CGA TAA AGA CAG CCA	GAA ATG TGG ATC CCG ATT GG
PI3Kγ	Pik3cg	CTT TAG AGT TCC CTA TGA TCC CT	TTT CGT TGG ATA GGA CTG TGG
iNOS	Nos2	ACA TCG ACC CGT CCA CAG TAT	CAG AGG GGT AGG CTT GTC TC
TGFβ1	Tgfb1	CGG GTC TAC TAT GCT AAA GAG GTC AC	TTT CTC ATA GAT GGC GTT GTT GC
GMCSF	Cfs2	GGC CTT GGA AGC ATG TAG AGG	GGA GAA CTC GTT AGA GAC GAC TT
GCSF	Csf3	GCT GCT GCT GTG GCA AAG T	AGC CTG ACA GTG ACC AGG
CSF1	Csf1	ATG AGC AGG AGT ATT GCC AAG G	TCC ATT CCC AAT CAT GTG GCT A
PyMT	PyMT	ACA TGC CAA TGG AGG ATC TG	GCA AAT CCC GAA GAA TCA GAC
L19	Rpl19	CTG GAT GAG AAG GAT GAG GAT C	GGA TGT GCT CCA TGA GGA TG

Table S1. Primers used for qPCR-based expression analyses.