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Supplementary Materials for

The systemic response to surgery triggers the outgrowth of distant immune-controlled tumors in mouse models of dormancy

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Materials and Methods

Cell lines and culture. D2A1 murine mammary cancer cells (Balb/c background) were a gift from Fred Miller and were transduced with GFP as previously reported *(56)*. D2A1-GFP cells were cultured in DMEM supplemented with 5% calf serum, 5% heat-inactivated fetal bovine serum (IFS), non-essential amino acids, penicillin (100 U/mL), and streptomycin (100 mg/mL). B16 cells and corresponding B16 cells overexpressing GM-CSF (B16-GM cells) were a gift from Glenn Dranoff and were cultured in DMEM supplemented with 10% fetal bovine serum and penicillin/streptomycin. 293T cells (ATCC) were cultured in DMEM supplemented with 10% IFS, and 0.1X penicillin (10 U/mL) and streptomycin (10 mg/mL).

Animals. Balb/c, C57BL/6, C57BL/6-GFP, and NOD/SCID mice were purchased from The Jackson Laboratory and subsequently bred in house. Balb/c and C57BL/6-GFP mice were crossed to generate F1 offspring with and without germline expression of GFP. Female mice aged 8-12 weeks were used for all experiments. Mice were handled according to MIT Committee on Animal Care protocol 1014-109-17. During all surgical procedures, mice were anesthetized with isofluorane, and buprenorphine (0.1 mg/kg) was administered as a peri- and post-operative analgesic.

Surgical wounding via sponge implantation. Sterile polyvinyl-acetate sponges (9 x 15 mm; Ivalon) were cut in half and moistened with sterile PBS prior to subcutaneous implantation. One, two, or three half-sponges were implanted subcutaneously through a 1 cm cutaneous incision located in the center of the back or on the left flank, depending on the experiment. Sponges were placed on one or both flanks, or between the shoulder blades, as illustrated in Fig. 2C and Fig 3A. Control, unwounded mice were anesthetized and prepared for surgery in parallel with wounded mice, but no incision was made in the control mice. When used as an anti-inflammatory agent, meloxicam (2 mg/kg) was injected subcutaneously 2 hours prior to wounding or mock treatment, followed by injections at 1.5 mg/kg every 12 hours for three days. In such experiments, mice were treated with meloxicam in addition to, rather than instead of buprenorphine.

Tumor formation in mice. D2A1-GFP cells were injected into syngeneic Balb/c mice or into C57BL/6:Balb/c F1 mice, either as orthotopic injections into a mammary fat pad or as subcutaneous injections. B16 cells were injected subcutaneously into C57BL6 mice. For orthotopic injections, a 1 cm vertical cutaneous incision was made in the center of the abdomen, and the skin was retracted to allow injection into mammary fat pad #4. D2A1-GFP cells ($5x10^4$, $1x10^5$, or $2x10^5$ per mouse) were injected as a suspension in 20 uL of PBS. For experiments using D2A1-GFP-sgLuciferase and D2A1-GFP-sgCCL2 cells (see below), $1x10^6$ cells were injected per mouse. For subcutaneous injections, $5x10^5$ D2A1-GFP cells (in 100 uL PBS) or $2.5x10^5$ B16 cells [in 100 uL of Hank's buffered saline solution (HBSS)] were injected in the right flank of recipient mice.

Tumor measurements. Tumor size was determined by measuring the tumor axis length in three orthogonal dimensions. Tumor diameter was reported as either the median axis length or as the geometric mean of the length of all axes. A tumor-bearing mouse was defined as having a tumor of diameter ≥ 2mm in diameter. This threshold was introduced due to the high frequency with which pockets of immune infiltrate or an enlarged lymph node remained after complete rejection of D2A1-GFP. Upon dissection, masses with a diameter < 2 mm typically contained no tumor cells, whereas masses ≥ 2mm always contained GFP^+ tumor cells. Thus, a threshold of 2 mm enabled us to distinguish tumor-bearing mice from mice whose tumors had been rejected. Surgical wounding via sponge implantation was performed one week before or one week after the injection of tumor cells, as described for individual experiments.

***In vivo* antibody-mediated cell depletion and receptor neutralization.** All neutralizing antibodies were purchased from Bio-X-Cell. Antibodies were administered twice weekly as intraperitoneal injections (200 ug per injection; ~10 mg/kg). Antibody administration was initiated according to timelines described below and continued for the duration of the experiment. $CD8^+$ T cells were depleted using anti-CD8 antibodies (clone YTS169.4; isotype-control clone LTF-2), with treatment beginning one day prior to the injection of tumor cells. Neutrophils were depleted using anti-Ly6G antibodies (clone 1A8; isotype-control clone 2A3), with treatment beginning seven days after the injection of tumor cells, when small tumors (~2 mm) had been established. PD-1

blockade was performed using anti-PD1 antibodies (clone RMP1-14; isotype-control clone 2A3), with treatment beginning three days after the injection of tumor cells.

Tail vein injection and quantification of tumor nodules in lungs. For intravenous injection, D2A1-GFP cells were resuspended to a density of $5x10^6$ cells/mL or $1x10^7$ cells/mL in PBS, and 100 uL ($5x10^5$ or $1x10^6$ cells) were injected into the tail vein of Balb/c mice such that cells would lodge in the pulmonary capillary bed. The mice were euthanized 14-17 days after injection of D2A1-GFP cells. The lungs were removed by dissection, and tumor nodules (GFP^+) within the lungs were visualized on a fluorescence dissecting microscope. Nodules that could be detected at 1.6X magnification were counted as macroscopic metastases.

Vaccination with B16-GVAX. Vaccination of C57BL/6 mice with B16-GM cells has been previously reported (*30*). Briefly, B16-GM cells were resuspended in HBSS at a density of $10x10^6$ cells/mL and irradiated with 35 Gy. Following irradiation, 100 uL of the cell suspension ($1x10^6$ cells) was injected subcutaneously on top of the sternum. Prophylactic vaccination was administered as a single dose, 7 days prior to the injection of tumorigenic B16 cells. For therapeutic vaccination, two doses of vaccine were administered: the first dose was concurrent with the injection of tumorigenic B16 cells, and the second dose was administered two days later. Surgical wounding via the implantation of a subcutaneous sponge was performed 7 days after the injection of B16 cells. For these experiments, B16 tumor cells were injected on the right flank, and the sponge was implanted on the left flank, thereby avoiding local interactions between the tumor cells, the wound site, and the site of vaccination.

Immunofluorescence staining. Immediately after dissection, tissues were fixed overnight in 10 percent neutral-buffered formalin. Fixed tissues were embedded in paraffin and sections were cut at a thickness of 5 µm. Prior to staining, paraffin was removed by incubation with Histo-Clear (National Diagnostics), and sections were rehydrated through a series of ethanol washes (100%, 95%, 70%, 35% ethanol, twice each) followed by washing with water. Antigen retrieval was performed by boiling in sodium citrate (pH 6.0), and the samples

were blocked with 3%horse serum in PBS. Sections were incubated with primary antibodies overnight at 4ºC. After washing with PBS, sections were then stained with secondary antibodies (if necessary) for 1 h at room temperature. Samples were counterstained with ProLong® Gold Antifade mounting medium with DAPI (Thermo Fisher). αSMA staining was performed with a FITC-conjugated primary antibody (clone 1A4, Sigma). F4/80 staining was performed using an unconjugated primary antibody (clone BM8, Thermo Fisher) and HRP-linked anti-rat secondary antibody (Vector Labs). For F4/80 staining, HRP-based signal amplification was performed using the TSA Plus Cy3 reagent (PerkinElmer). For these samples, endogenous peroxidase activity was quenched with 0.3%hydrogen peroxide in 10%methanol prior blocking with horse serum.

Flow cytometry. Cells were isolated for analysis by flow cytometry as follows: To collect circulating immune cells, mice were euthanized by carbon dioxide asphyxiation. Blood was immediately isolated by cardiac puncture and collected in EDTA tubes (Sarstedt). To purify white blood cells, 350 μL of whole blood was added to 3.5 mL homemade ACK red blood cell lysis buffer for 2 minutes at room temperature, followed by quenching with 35 mL of cold PBS containing 1% IFS (PBS-IFS). To collect sponge-infiltrating cells, sponges were removed from mice and treated with collagenase, hyaluronidase, and DNase I to digest deposited matrix and liberate cellular contents. Sponges were then placed in PBS and squeezed with forceps to physically dislodge cells and push them into suspension. To isolate tumor-infiltrating cells, tumors were dissected and then digested with collagenase and hyaluronidase at 37 ºC for 45 minutes. Cell suspensions were diluted in cold PBS and passed successively through 70 um and 40 um cell strainers. Subsequent steps were consistent across all sample types and were performed at 4 ºC in PBS-IFS. After centrifugation of the cells isolated above, cells were incubated with anti-CD16/CD32 for 5 minutes to block Fcγ receptors on the surface of immune cells. Samples were then incubated with the appropriate antibody cocktail or single-antibody control solution for 25 minutes (Supplementary Table 1), followed by the addition of DAPI (1 μg/mL; Sigma) for 5 minutes. After being washed and resuspended in PBS-IFS, samples were run on a custom LSR Fortessa (BD Biosciences).

Detection of circulating cytokines. Mice were euthanized by carbon dioxide asphyxiation. Blood was immediately isolated by cardiac puncture and collected in heparin tubes (Sarstedt). Blood plasma was isolated by centrifugation of whole blood to pellet blood cells (2000 x *g*, 5 minutes). Samples were analyzed using ELISA kits for detection of CCL2, G-CSF, and IL-6 (all from R&D Systems), according to the manufacturer's instructions.

***Ex vivo* immune activation.** Tumor-draining and contralateral inguinal lymph nodes were isolated by dissection from mice bearing orthotopic D2A1-GFP tumors or from tumor-free mice. Lymph nodes were pressed through a 40 um cell strainer to liberate leukocytes into cold RPMI. Cells were washed once, and ten percent of cells from each lymph node were plated into each of three wells of a 96-well plate (technical triplicates). To each well was added 1000 D2A1-GFP cells that had been irradiated (35 Gy). Lymph node and tumor cells were co-cultured in RPMI supplemented with 10% IFS, non-essential amino acids, penicillin (100 U/mL), streptomycin (100 mg/mL), and IL-2. Negative control wells contained irradiated tumor cells but no lymph node cells. After 48 hours, conditioned medium was isolated and centrifuged to remove cellular debris and cells in suspension. Immune activity was determined by the detection of interferon-gamma (IFNγ) in the medium using an IFNγ ELISA kit (BD Biosciences) according to the manufacturer's instructions.

Generation of lentiviral sgRNA constructs for CRISPR-Cas9-mediated gene disruption. Sequences for sgRNAs targeting mCCL2 were designed using the Broad Institute web tool (http://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design). Cloning into the pLentiCRISPRv2 plasmid, containing an sgRNA cloning site and a Cas9 expression cassette, was performed according to a published protocol (http://www.genome-engineering.org/crispr/). Briefly, forward and reverse oligonucleotides were designed to contain a 5' CACC-overhang on the forward oligo, and a 5' AAAC-overhang on the reverse oligos. Complementary oligonucleotides (Integrated DNA Technologies) were annealed using a PCR cycler. The pLentiCRISPRv2 plasmid was digested with Esp3I restriction endonuclease (Thermo), and the annealed oligonucleotides were ligated into the plasmid using Quick Ligase (New England Biolabs).

Production of lentivirus and infection of D2A1-GFP cells. For each viral construct, $3.5x10^6$ 293T cells were seeded in a 10 cm tissue culture dish and were transfected approximately 30 hours later. Plasmid DNA (0.3 ug VSV-G, 3 ug psPax2, 3 ug pLentiCRISPRv2) was incubated with 18 uL Xtremegene9 in OptiMEM for 25 minutes at RT and then added dropwise to a dish of 293T cells. After 20 hours, transfection medium was removed and replaced with viral harvest medium (DMEM supplemented with 20% IFS and penicillin/streptomycin). Lentivirus-containing conditioned medium was collected 24 hours later and passed through a 0.45 um filter to remove cellular debris. For infection of D2A1-GFP cells, lentivirus-containing medium was diluted 10-fold in D2A1 culture medium containing 6 ug/mL protamine sulfate (Sigma) and added to D2A1-GFP cells at approximately 50% confluence. 24 hours after infection, D2A1-GFP cells were selected in puromycin (1 ug/mL) for 72 hours. One week after infection, cells were injected into Balb/c mice, and knock out was confirmed in parallel by assaying CCL2 present in conditioned medium. For this assay, $9.5x10^4$ cells were seeded in 6 well plates (3 wells per cell line). Conditioned medium was collected 72 hours later and passed through a 0.45 um filter. CCL2 levels were determined by ELISA.

***In vitro* proliferation assay.** To compare the proliferation of D2A1-GFP-sgLuciferase and D2A1-GFP-sgCCL2 cell lines, each cell line was seeded in 6-well dishes at $3x10^4$ cells per well, 6 wells per cell line. After 96 hours, cells were trypsinized and counted on a ViCell (Beckman Coulter).

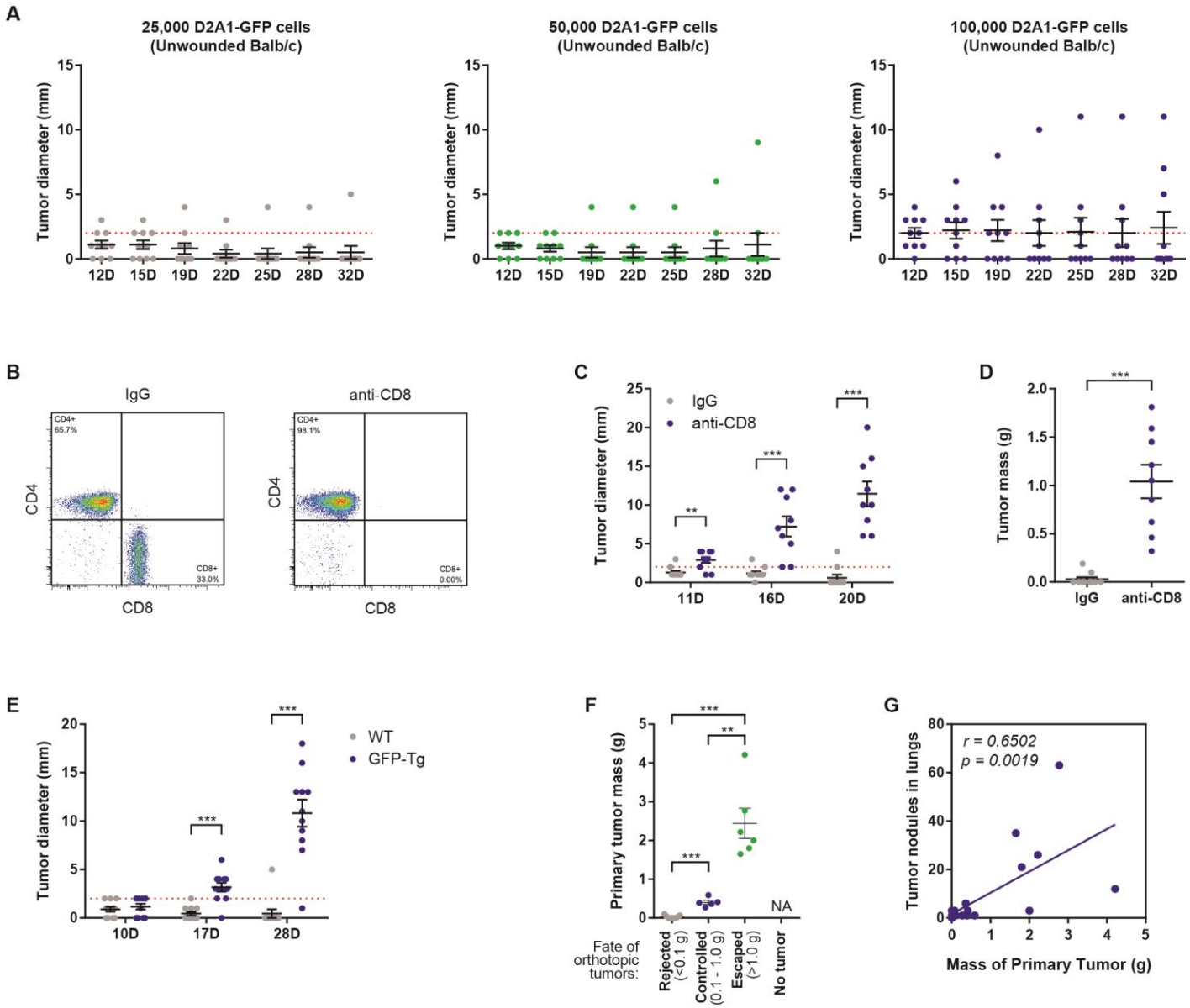

Fig. S1. The outgrowth of D2A1-GFP tumors in Balb/c mice is restricted by a GFP-specific CD8$^+$ T cell response. (**A**) Tumor diameter as a function of time for D2A1-GFP cells ($2.5x10^4$, $5x10^4$, or $1x10^5$) injected orthotopically into a mammary fat pad (N = 10 per group). Data correspond to tumor incidence in Fig. 1B. The dashed red line indicates tumor diameter of 2 mm, the threshold for tumor incidence. (**B**) Representative flow cytometry plots of circulating immune cells in Balb/c mice treated with control IgG or anti-CD8 antibodies. CD4 and CD8 staining of CD45$^+$CD3$^+$ cells is shown. (**C**) Diameter of individual tumors as a function of time and (**D**) final tumor mass after the orthotopic injection of $1x10^5$ D2A1-GFP cells into Balb/c mice treated with control IgG or anti-CD8 antibodies (N = 9-10 per group). Data correspond to tumor data in Fig. 1C. (**E**)

Diameter of individual tumors as a function of time after the orthotopic injection of $1x10^5$ D2A1-GFP cells into wild-type or GFP-transgenic Balb/c:C57BL/6-F1 mice (N = 11 per group). Data correspond to tumor data in Fig. 1D. (**F**) Mass of D2A1-GFP tumors in the mammary fat pad of Balb/c mice in which mice were re-challenged with D2A1-GFP cells introduced via the tail vein. Tumor mass was determined at the termination of the experiment, when lung nodules were counted. Corresponding data for lung nodules is presented in Fig. 1G (N = 3-6 per group). (**G**) Correlation between the number of D2A1-GFP lung nodules and the final mass of D2A1-GFP tumors in the mammary fat pad, comprising data presented in Fig. 1G and Fig. S1F. For all panels, data are plotted as mean ± SEM. P-values were calculated using the Mann-Whitney test (** $p<0.005$, *** $p<0.0005$).

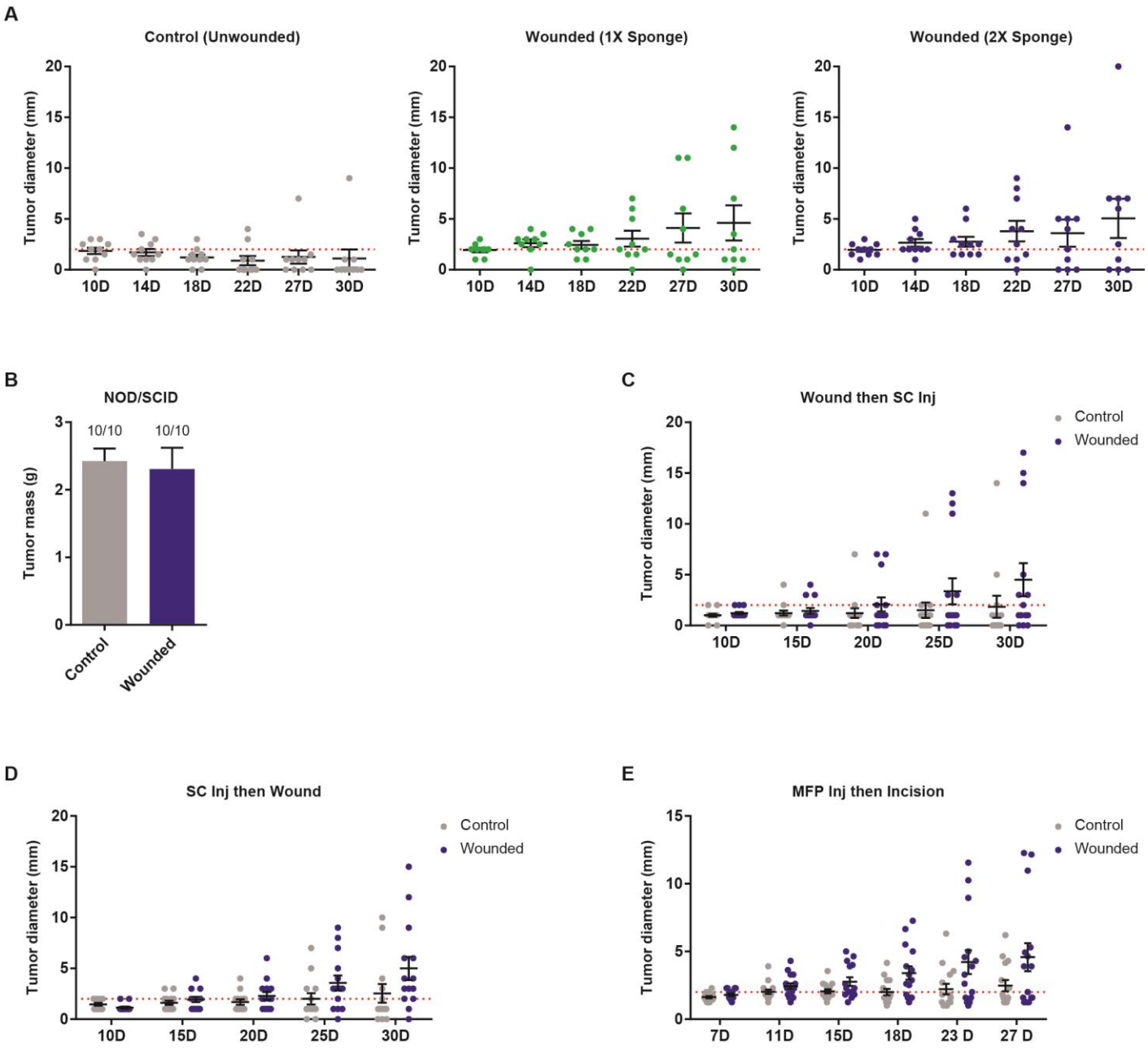

Fig. S2. Surgical wounding triggers the outgrowth of tumor cells at distant anatomical sites. (**A**) Diameter of individual tumors after the orthotopic injection of $1x10^5$ D2A1-GFP cells into control Balb/c mice or into Balb/c mice surgically wounded by sponge implantation at one or two distant sites. Data correspond to tumor data plotted in Fig. 3B,C (N = 9-10 per group). (**B**) Mass of tumors after the orthotopic injection of $1x10^5$ D2A1-GFP cells into control NOD/SCID mice or into NOD/SCID mice surgically wounded by sponge implantation at two distant sites (N = 10 per group). Tumor incidence is indicated above each bar. (**C** to **E**) The diameter of individual tumors as a function of time for three experiments in which D2A1-GFP tumor cells and

surgical wounds were introduced at contralateral sites: (**C**) mice were wounded by sponge implantation prior to the subcutaneous injection of $5x10^5$ cancer cells; (**D**) mice were wounded by sponge implantation after the subcutaneous injection of $5x10^5$ cancer cells; and (**E**) mice were wounded by a cutaneous incision after the orthotopic injection of $2x10^5$ cancer cells into a mammary fat pad. Data correspond to tumor incidence presented in Fig. 3D-F (N = 12-14 per group in each experiment). For all panels, data are plotted as mean ± SEM.

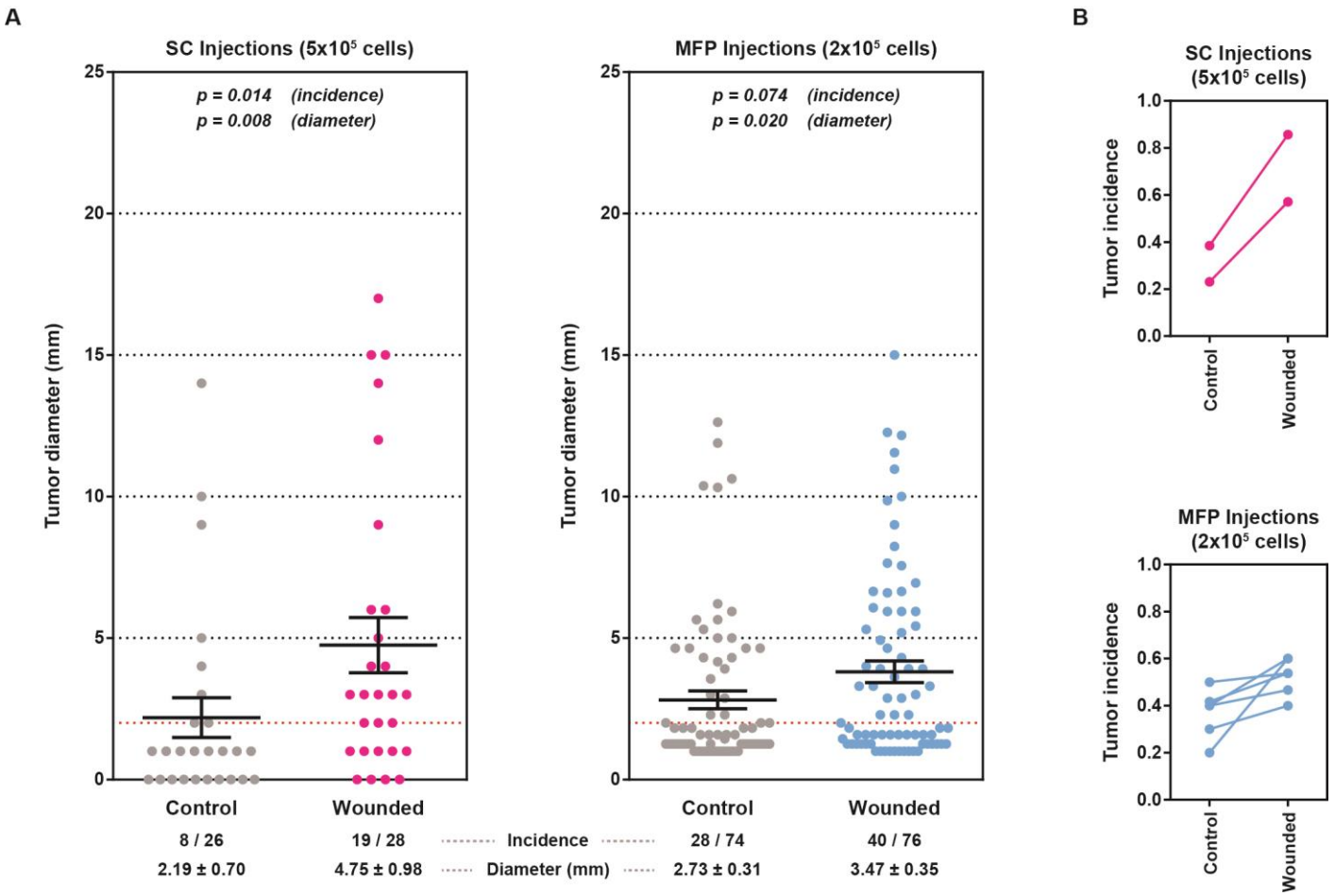

Fig. S3. Meta-analyses demonstrate that surgical wounding promotes the outgrowth of distantly implanted tumor cells. (**A**) Meta-analysis of tumor diameter and tumor incidence for experiments in which D2A1-GFP cells were injected into unwounded Balb/c mice or into mice surgically wounded at distant anatomical sites. Data are shown for experiments in which $5x10^5$ D2A1-GFP cells were injected subcutaneously (*left*) and for experiments in which $2x10^5$ D2A1-GFP cells were injected orthotopically into a mammary fat pad (*right*). (**B**) Linkage plots of tumor incidence for experiments in which D2A1-GFP cells were injected into unwounded mice or into mice surgically wounded at distant anatomical sites. Datasets are the same as in (**A**). For all panels, data are plotted as mean ± SEM. P-values were calculated using Fisher's exact test (**G**).

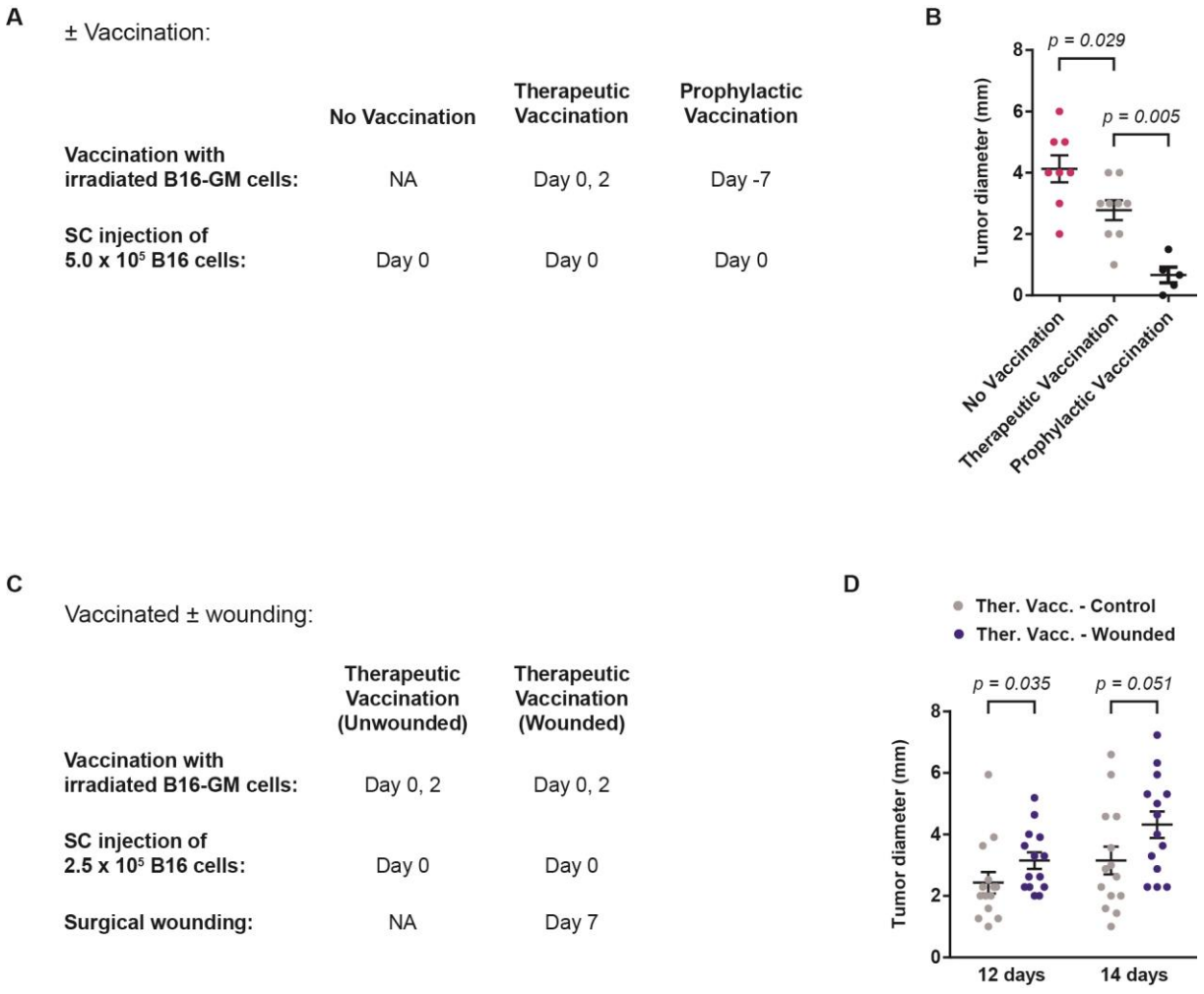

Fig. S4. Surgical wounding overcomes the effect of a tumor vaccine to promote the growth of distant B16 tumors. (**A** and **B**) Use of the B16-GVAX vaccine to restrict the growth of B16 tumors. (**A**) Outline of vaccine schedule for therapeutic vaccination to slow tumor growth, and prophylactic vaccination to prevent B16 tumor growth in C57BL/6 mice. (**B**) Diameter of subcutaneous B16 tumors in control and vaccinated mice, 14 days after the injection of tumor cells (no vaccination: N = 8; therapeutic vaccination: N = 9; prophylactic vaccination: N = 5). (**C** and **D**) Surgical wounding of mice vaccinated using the B16-GVAX system. (**C**) Outline of the schedule for therapeutic vaccination, B16 cell injection, and surgical wounding of C57BL/6 mice. (**D**) Diameter of subcutaneous B16 tumors in vaccinated (± wounding) C57BL/6 mice, 12 and 14 days after the injection of tumor cells. (N = 14 for both control and wounded mice). For all panels, data are plotted as mean ± SEM. P-values were calculated using the Mann-Whitney test.

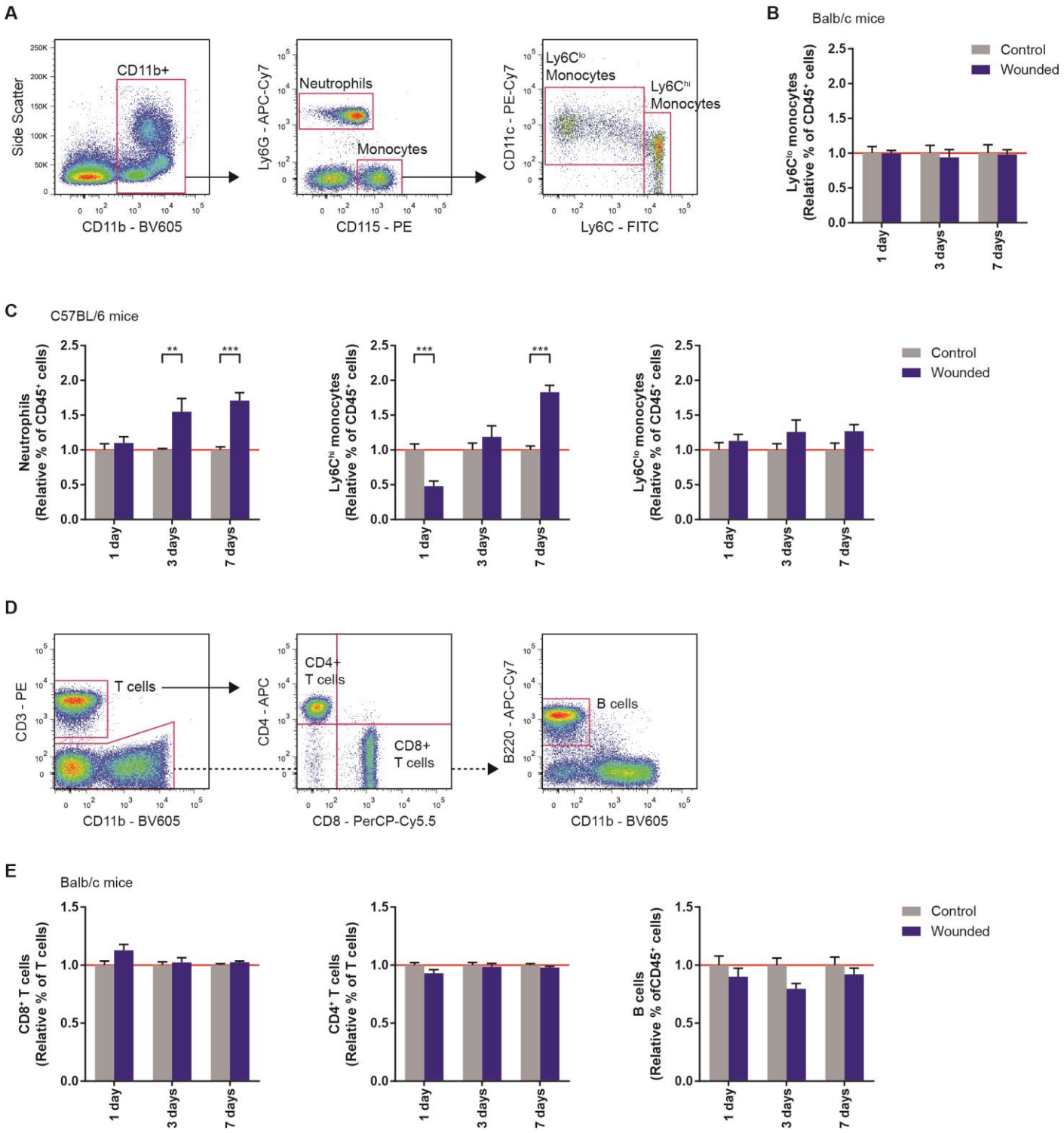

Fig. S5. Surgical wounding triggers a systemic inflammatory response characterized by the mobilization of inflammatory myeloid cells into the circulation. (**A**) Schematic for the gating of myeloid cell populations. (**B**) Relative proportions of circulating non-inflammatory ($Ly6C^{lo}$) monocytes in wounded and control Balb/c mice. Blood was collected 1, 3, and 7 days after surgical wounding, and the proportion of each cell type in the

circulation was determined as a percentage of $CD45^+$ leukocytes. For graphical display, values were normalized to control mice on each collection day (N = 4-6 per group). (**C**) Relative proportions of circulating myeloid cells in wounded and control C57BL/6 mice. Blood was collected and analyzed as described above for Balb/c mice (N = 5-6 per group). (**D**) Schematic for the gating of lymphoid cell populations. (**E**) Relative proportions of circulating $CD8^+$ T cells, $CD4^+$ T cells, and B cells in wounded and control Balb/c mice (N = 4-6 per group). For all panels, data are plotted as mean ± SEM. P-values were calculated using Student's *t*-test (** $p<0.005$, *** $p<0.0005$).

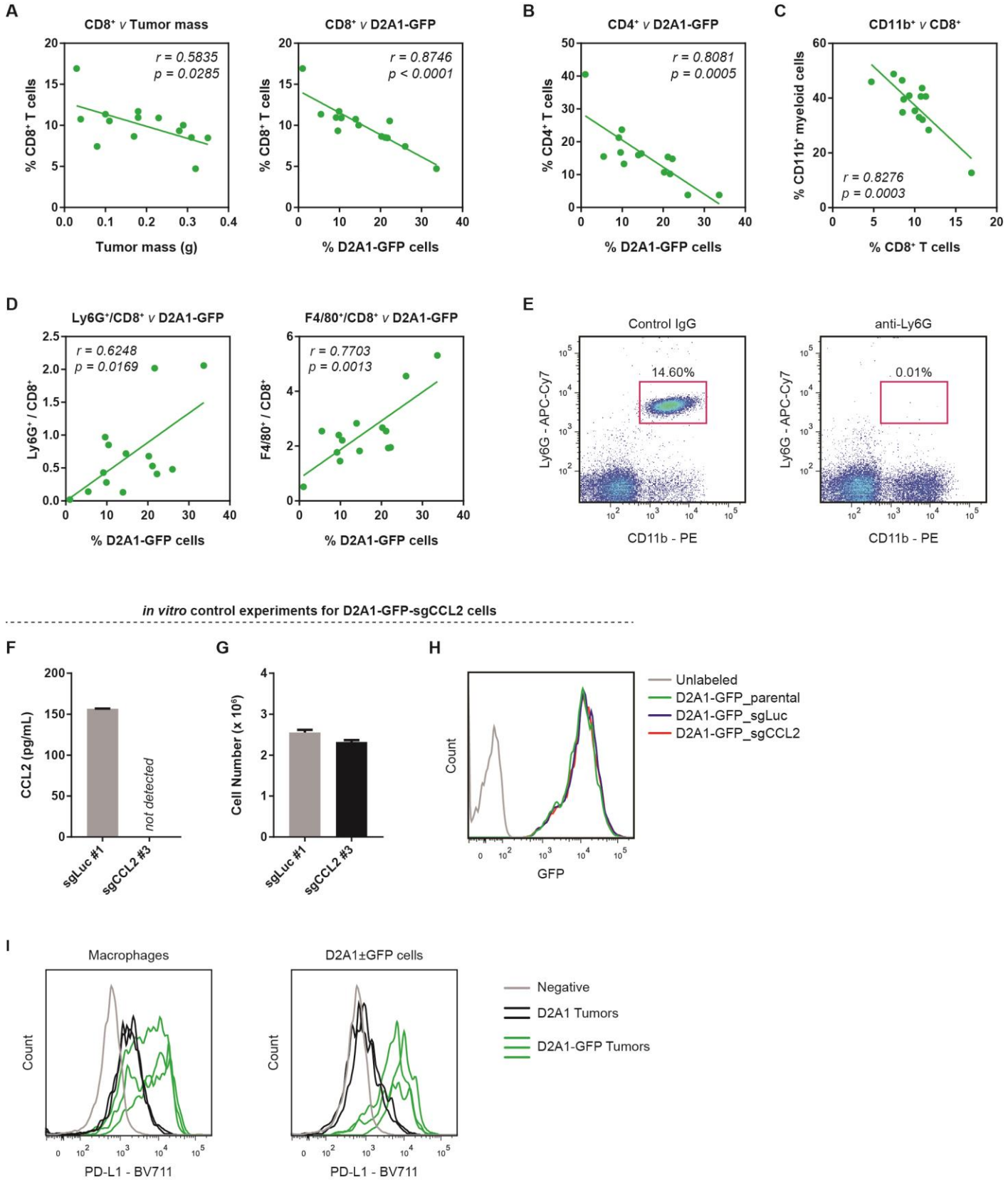

Fig. S6. Myeloid cells infiltrate D2A1-GFP tumors and promote tumor growth. (**A** to **D**) Analysis of tumor-infiltrating leukocytes, 17 days after the orthotopic injection of $1x10^5$ D2A1-GFP cells into unwounded

Balb/c mice (N = 14). (**A**) Correlation between the percentage of $CD8^+$ T cells and the mass of those tumors (*left*) or the percentage of D2A1-GFP cells (*right*) within those tumors. (**B**) Correlation between the percentage of $CD4^+$ T cells and the percentage of D2A1-GFP cells within tumors. (**C**) Correlation between the percentage of myeloid cells ($CD11b^+$) and the percentage of $CD8^+$ T cells within tumors. (**D**) Correlation between the neutrophil-to-$CD8^+$ T cell ratio (*left*) and the macrophage-to-$CD8^+$ T cell ratio (*right*) and the percentage of D2A1-GFP cells within tumors. (**E**) Representative flow cytometry plots of circulating leukocytes ($CD45^+$ cells) in the blood of mice treated with isotype-control or anti-Ly6G antibodies. Neutrophils ($CD11b^+Ly6G^+$) are contained within the pink gate. (**F**) CCL2 in the conditioned medium generated by D2A1-GFP-sgLuciferase and D2A1-GFP-sgCCL2 cells, as detected by ELISA (N = 3). (**G**) Proliferation of D2A1-GFP-sgLuciferase and D2A1-GFP-sgCCL2 cells. Cell number was determined after 4 days of *in vitro* culture (N = 6). (**H**) Expression of GFP in parental D2A1-GFP cells, sgLuciferase and sgCCL2 derivatives, as determined by flow cytometry. (**I**) Flow cytometry histograms indicating the expression of PD-L1 on the surface of macrophages (*left*) and cancer cells (*right*) within tumors formed 14 days after the orthotopic injection of $1x10^5$ unlabeled D2A1 cells (black lines) or D2A1-GFP cells (green lines) into unwounded Balb/c mice. For all panels, data are plotted as mean ± SEM.

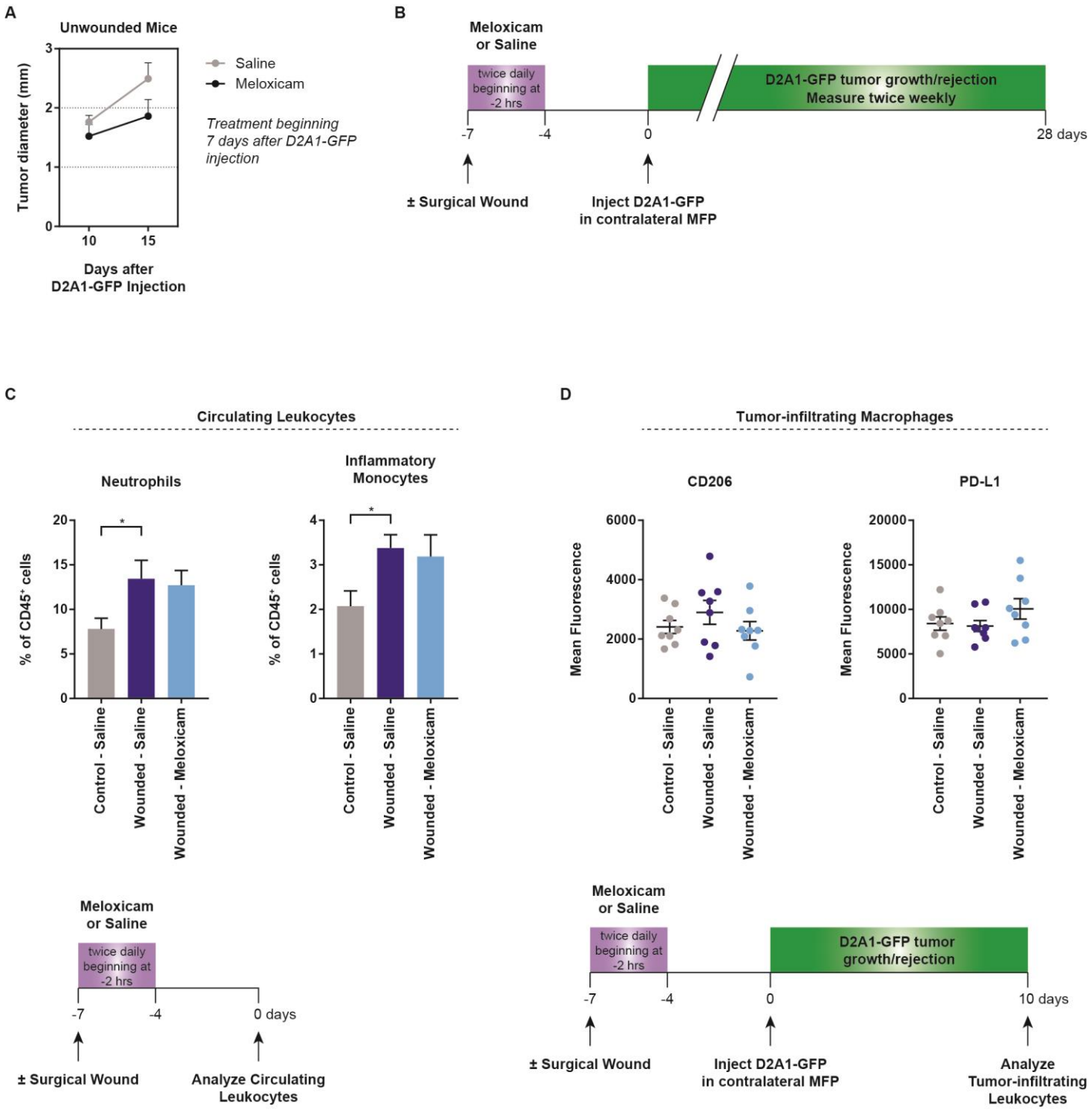

Fig. S7. NSAIDs alter the polarization of tumor-infiltrating macrophages. (**A**) Diameter of tumors in unwounded Balb/c mice treated with saline or meloxicam beginning 7 days after the injection of $1x10^5$ D2A1-GFP cells (N = 15 mice per group). (**B**) Schematic illustrating the timeline for orthotopic injection of $1x10^5$ D2A1-GFP cells into previously unwounded or wounded Balb/c mice that were treated peri- and post-operatively with saline or meloxicam. Tumor size is plotted in Fig. 4G. (**C**) (*top*) Levels of circulating

neutrophils and inflammatory monocytes in control and wounded mice that were treated peri- and post-operatively with either saline or meloxicam (N = 6-8 per group). (*bottom*) Schematic illustrating the timeline of the experiment. (**D**) (*top*) Expression of CD206 and PD-L1 on the surface of macrophages within D2A1-GFP tumors, as determined by flow cytometry. For these experiments, $1x10^5$ D2A1-GFP cells were orthotopically injected into control and wounded mice that were treated peri- and post-operatively with either saline or meloxicam (N = 8 per group). (*bottom*) Schematic illustrating the timeline of the experiment. For all panels, data are plotted as mean ± SEM. P-values were calculated using Student's *t*-test (* $p<0.05$).

Table S1. Components of flow cytometry antibody cocktails.

Myeloid Cell Antibody Cocktail – for circulating cells:

Marker	Fluorophore	Clone	Dilution	Source
α-CD16/CD32	NA	2.4G2	1:100	BD Pharmingen
α-CD45	eFluor-450	30-F11	1:100	eBioscience
α-CD11b	BV605	M1/70	1:100	BioLegend
α-Ly6C	FITC	AL-21	1:200	BD Pharmingen
α-CD115	PE	AFS98	1:50	eBioscience
α-CD3ε	APC	145-2C11	1:100	BD Pharmingen
α-B220	APC	RA3-6B2	1:100	BD Pharmingen
α-CD11c	PE-Cy7	N418	1:100	eBioscience
α-Ly6G	APC-Cy7	1A8	1:200	BD Pharmingen

Lymphoid Cell Antibody Cocktail – for circulating cells:

Marker	Fluorophore	Clone	Dilution	Source
α-CD16/CD32	NA	2.4G2	1:100	BD Pharmingen
α-CD11c	V450	HL3	1:100	BD Horizon
α-CD11b	BV605	M1/70	1:100	BioLegend
α-CD8α	PerCP-Cy5.5	53-6.7	1:200	eBioscience
α-CD3ε	PE	145-2C11	1:100	eBioscience
α-CD4	APC	RM4-5	1:100	eBioscience
α-CD25	PE-Cy7	PC61.5	1:100	eBioscience
α-B220	APC-Cy7	RA3-6B2	1:100	BD Pharmingen

Additional Antibodies Used – for sponge-infiltrating cells:

Marker	Fluorophore	Clone	Dilution	Source
α-CD11b	PE	M1/70	1:200	BD Pharmingen
α-F4/80	PE-Cy7	BM8	1:200	eBioscience
α-Ly6G	Alexa-700	1A8	1:200	eBioscience

Table S1, continued

Myeloid Cell Antibody Cocktail – for tumor-infiltrating cells:

Marker	Fluorophore	Clone	Dilution	Source
α-CD16/CD32	NA	2.4G2	1:100	BD Pharmingen
α-CD45	PE	30-F11	1:100	eBioscience
α-CD11b	BV605	M1/70	1:100	BioLegend
α-CD206	APC	C068C2	1:100	BioLegend
α-PD-L1	BV711	MIH5	1:100	BD Horizon
α-F4/80	PE-Cy7	BM8	1:100	eBioscience
α-Ly6G	APC-Cy7	1A8	1:200	BD Pharmingen