

Fig. S1: Intravital imaging of Arg1⁺ macrophages in non-tumor tissue. Arg1-eYFP (green) mouse organs were resected after i.v. administration of macrophage (Cascade Blue dextran; Blue) and vascular (rhodamine lectin; red) imaging agents. Representative confocal images of (A) liver, (B) lung, (C) spleen, and (D) heart.

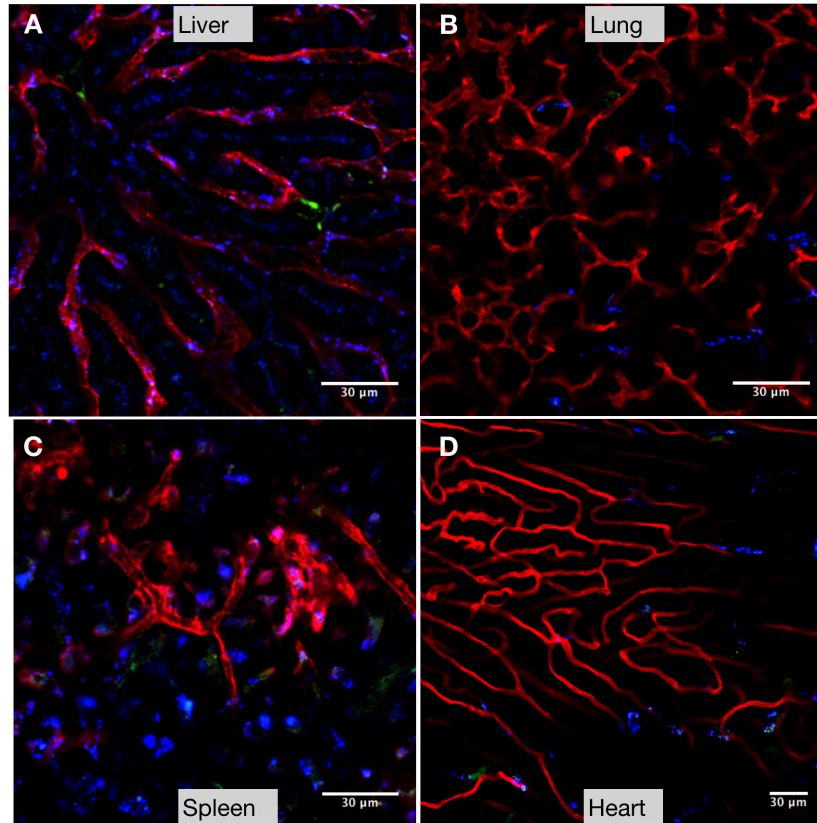


Fig. S2: Flow cytometric analysis of Arg1 expression in tumor immune cells. **A.** FACS plots showing gating of MC38 TAM (CD45+ Ly6G- CD90- F4/80+) from Arg1-eYFP mice analyzed by flow cytometry. **B.** Gating of Arg1+ immune cells into sub-clusters to compare the percentage of Arg1+ TAM (F4/80+) to other immune populations. Values represent mean \pm S.E.M. for 4 MC38 tumors plotted individually as grey circles.

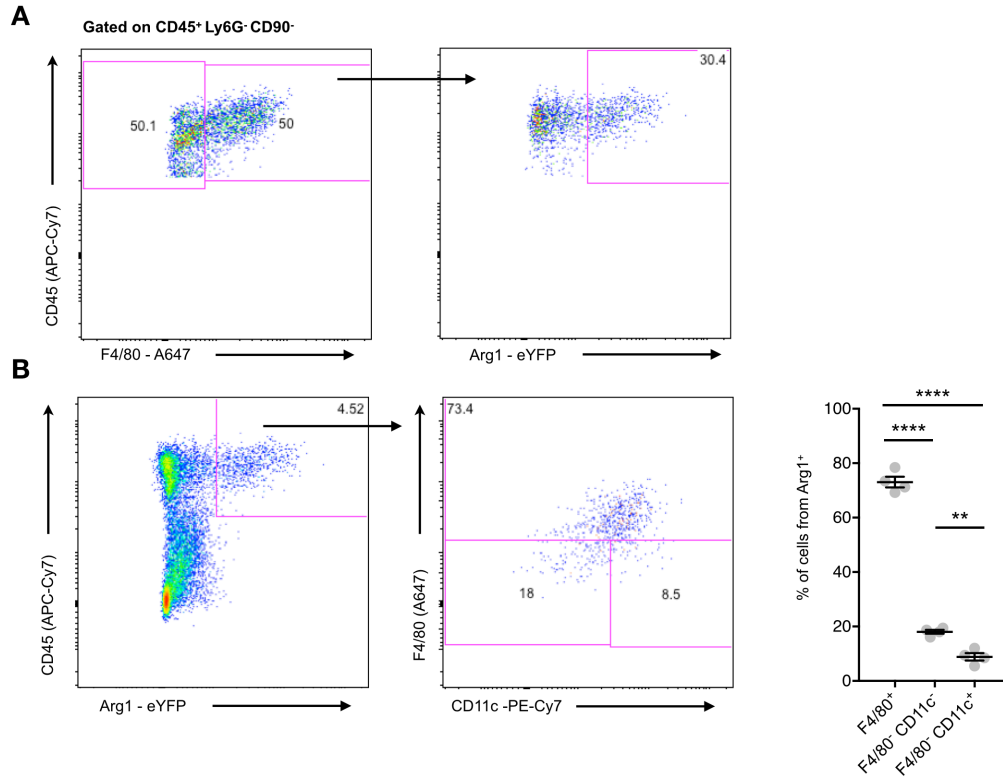


Fig. S3: Image analysis of Arg1 cells defined by motility. Bar graphs showing (A) motility coefficient and (B) instantaneous velocity calculated for Arg1+ macrophages (n = 54) in MC38 tumor core or periphery. ****P < 0.0001.

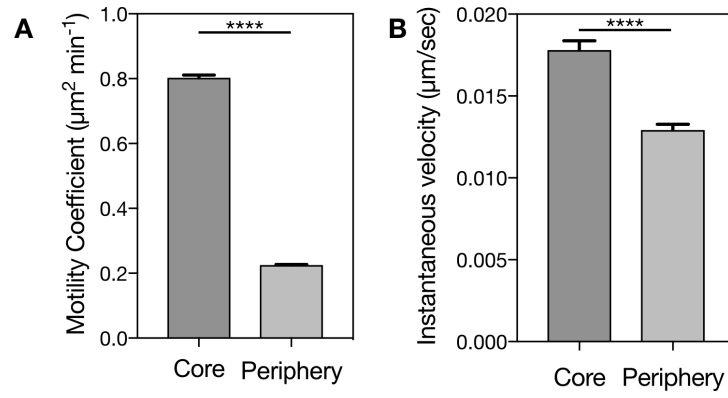


Fig. S4: Intravital imaging of EL4 mouse lymphoma model. **A.** Low resolution intravital microscopy images of Arg1⁺ macrophages (green) in EL4-H2B-mApple (red) tumors implanted in Arg1-eYFP reporter mice. The tumor margin is demarcated with a white solid line. **B.** Representative high resolution intravital microscopy image of EL4 (red) tumor cells at the tumor margin showing Arg1⁺ macrophage (green) phenotypes. **C.** Arg1⁺ macrophage shape analysis performed at the tumor core and periphery. Values represent mean \pm SEM for 2 mice. ****P < 0.0001 (Student's t test).

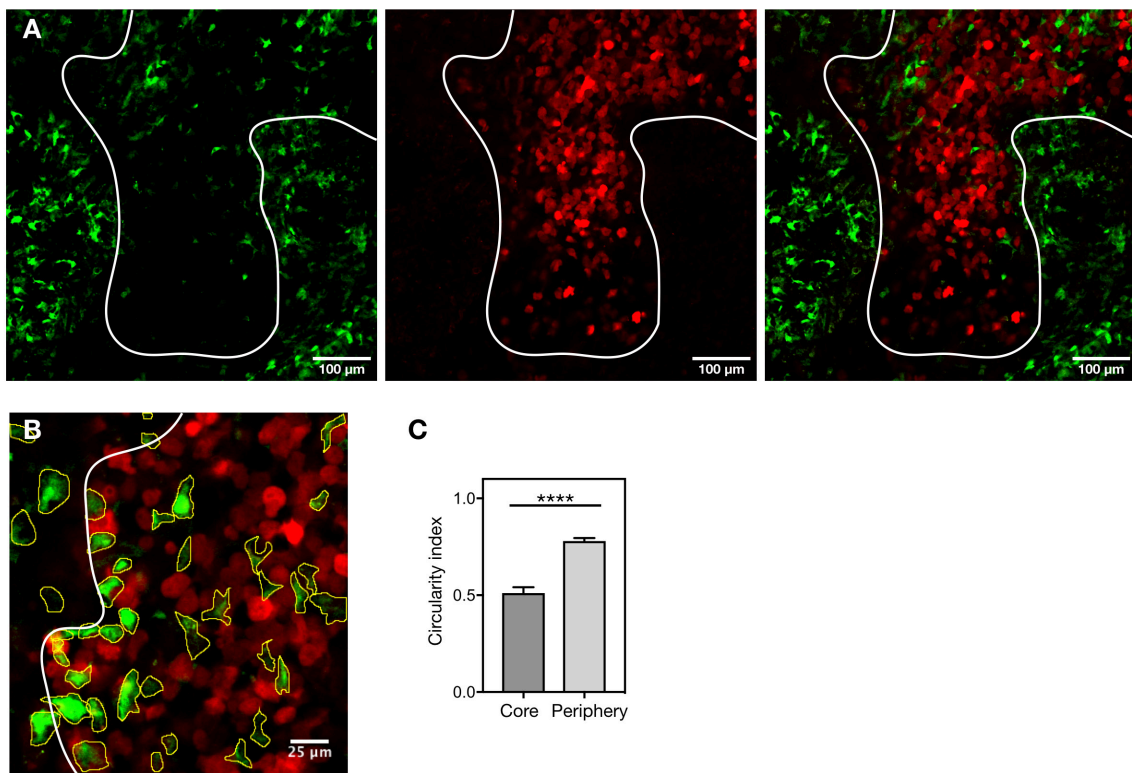


Fig. S5: In situ imaging of Arg1 expression in an orthotopic melanoma model. **A.** A representative confocal image of B16-H2B-mApple (red) tumor cells and Arg1-eYFP TAM (green) at the core of a surgically removed tumor from the flank of a reporter mouse. Yellow ROIs overlaid to highlight shape phenotype. **B.** Peripheral Arg1-eYFP cells at the tumor margin (solid white line) with yellow ROIs overlaid to highlight shape phenotype. **C.** Plotted mean circularity index of Arg1-eYFP⁺ TAM at the tumor core and periphery. Values represent mean \pm SEM for 4 mouse tumors. ****P < 0.0001 (Student's t test).

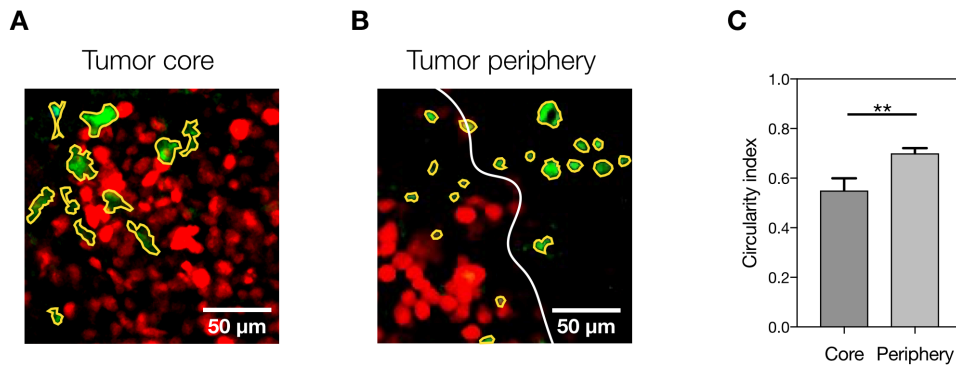


Fig. S6: In situ imaging of Arg1 phenotypes in MC38 tumors independent of dorsal skin-fold model. **A.** A representative microscopy image showing the core region of an intradermal MC38-H2B-mApple tumor surgically-resected from an Arg1-eYFP mouse. Yellow outlines representing ROIs from thresholded Arg1+ cells were used to plot Arg1 expression (eYFP mean fluorescence intensity) vs. circularity index. **B.** A representative image from the same tumor at the periphery (margin demarcated by solid white line) with a positive trend shown on a plot of Arg1 expression vs. circularity index. **C.** Mean Arg1 expression at the core and periphery of 4 independent MC38 tumors. Values represent mean \pm SEM. **** $P < 0.0001$ (Student's t test).

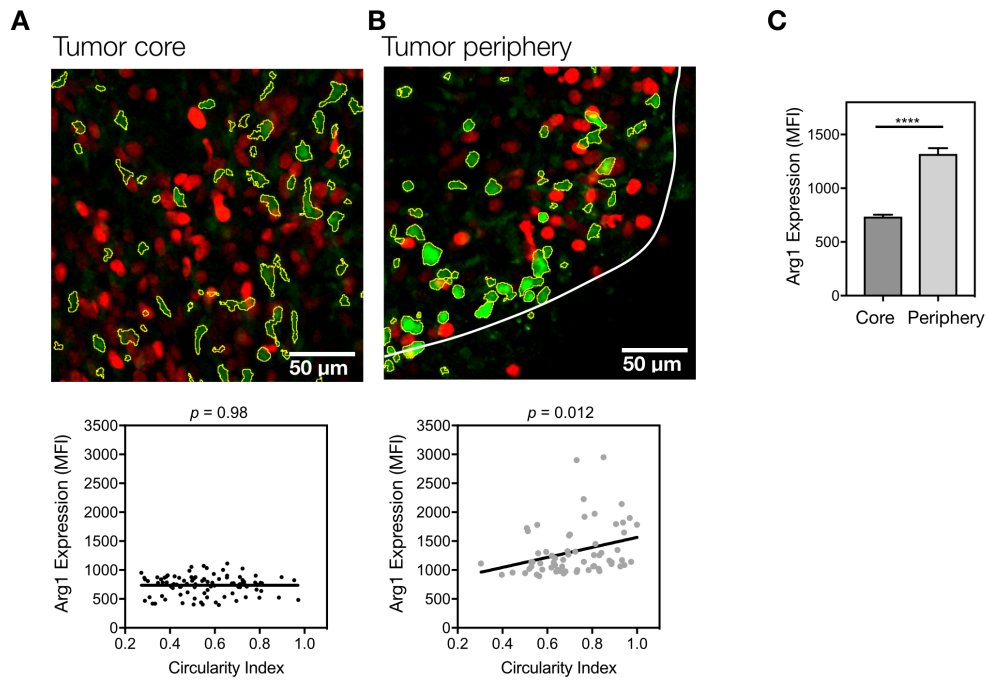


Fig. S7: scRNA seq for putative macrophage gene markers. tSNE plots showing expression of each indicated gene across all CD45+ immune cells queried in an MC38 tumor.

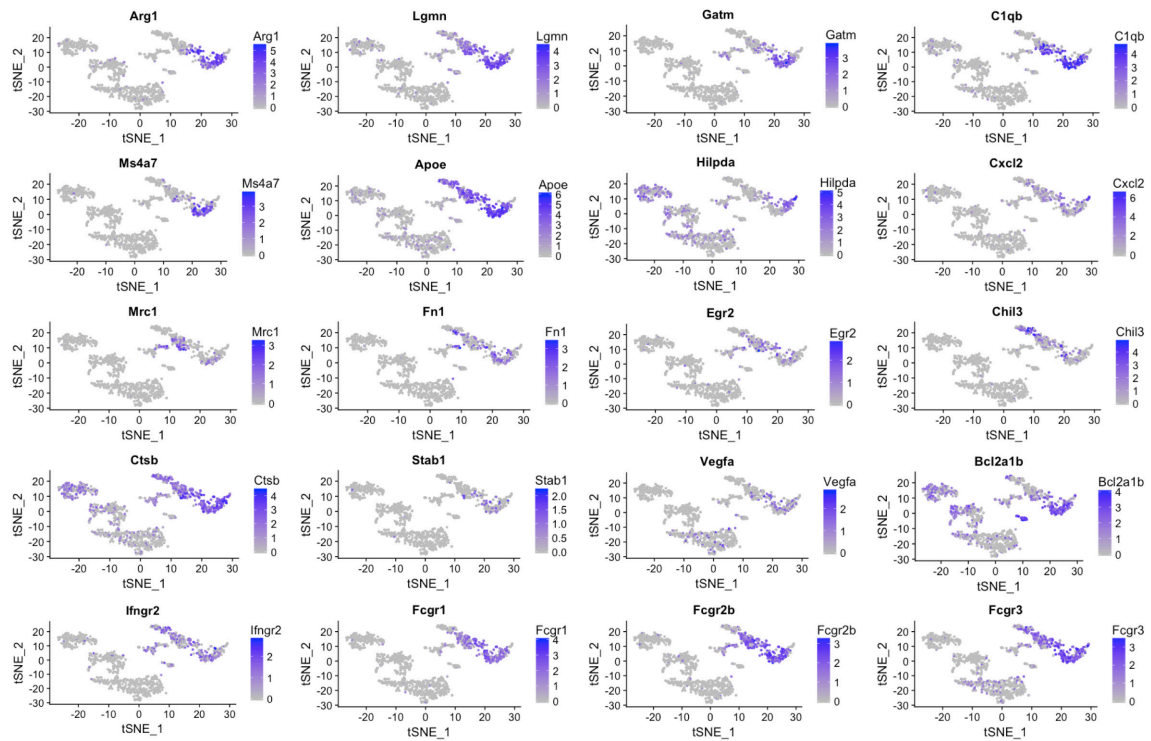


Fig. S8: scRNA seq identification of Arg1A and Arg1B discriminating genes. (A) Violin plots showing probability distribution of Arg1, Arg1A-specific genes (Gbp2b, Bst1), and genes that discriminate Arg1A from Arg1B (Ccl6, Ccl9, F13a1). **(B)** Violin plots showing Arg1B-specific genes (Ms4a7, Sgk1, Pmepa1) and genes that discriminate Arg1B from Arg1A (Cd72, Cxcl16, Rgs1). Values were obtained from untreated MC38 tumor tissue pre-sorted on CD45+ cells.

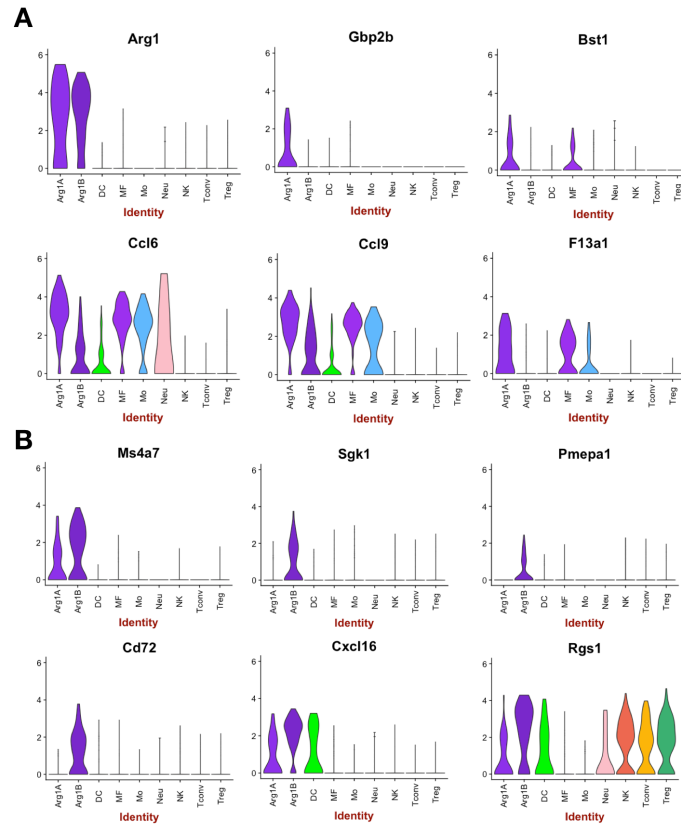


Fig. S9: Arg1A gene expression and shape by histological staining. **A.** Staining for the Arg1A-specific gene product GBP1 (green) in histological sections prepared from MC38-H2B-mApple (red) tumor tissue co-stained with DAPI (blue) for cell nuclei identification. **B.** Average perinuclear expression of GBP1 quantified across 3 tumors. Values represent mean \pm SEM. ****P < 0.0001 (Student's t test).

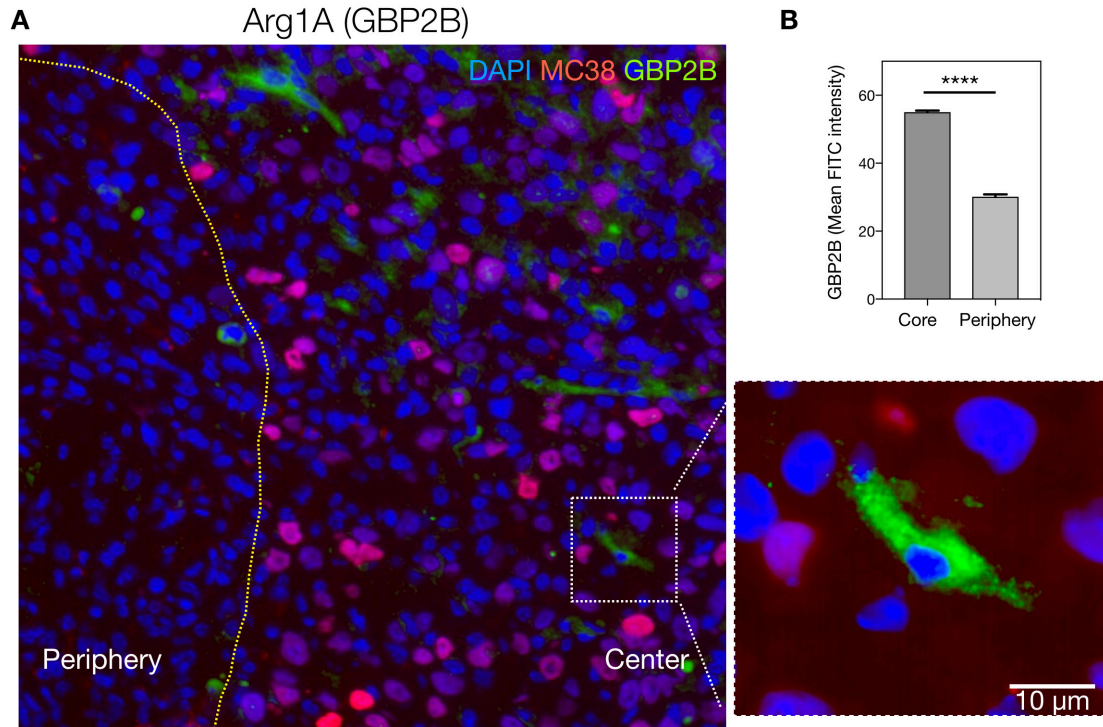


Fig. S10: Gene enrichment analysis of macrophage populations. A. Differential gene expression from MC38 tumor scRNA seq data showing common pathways shared among genes enriched in the Arg1A cluster relative to the Arg1B cluster. **B.** Differential gene expression from scRNA seq data showing common pathways shared among genes enriched in MC38 tumor macrophages after treatment with aPD-1. GO Function terms are plotted against their Fisher Exact P-Value and colored according to pathways of interest. Black bars indicate “other” pathways.

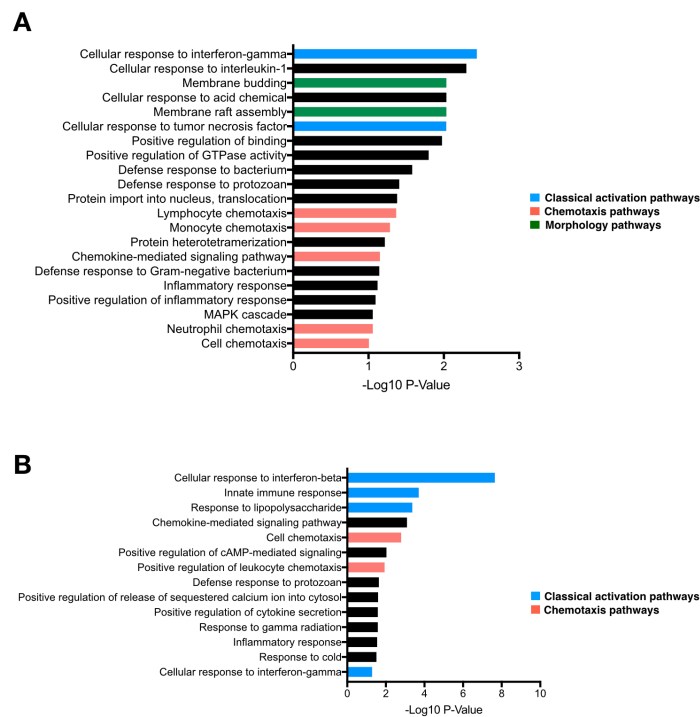


Fig. S11: Response of Arg1+ macrophages to immunotherapy. **A.** Intravital microscopy time lapse imaging showing the change in Arg1-eYFP macrophages (green) in MC38-H2B-mApple (red) tumors treated with aPD-1. Pacific Blue-dextran nanoparticle injected i.v. for TAM labeling (blue). **B.** Plot showing Arg1-eYFP expression quantified in the tumor core before and 72 hours after aPD-1 administration. **C.** Plot showing Arg1+ TAM circularity index at the tumor core. **D.** Plot showing Arg1+ TAM circularity index at the tumor periphery. Values represent mean \pm SEM. ****P < 0.0001.

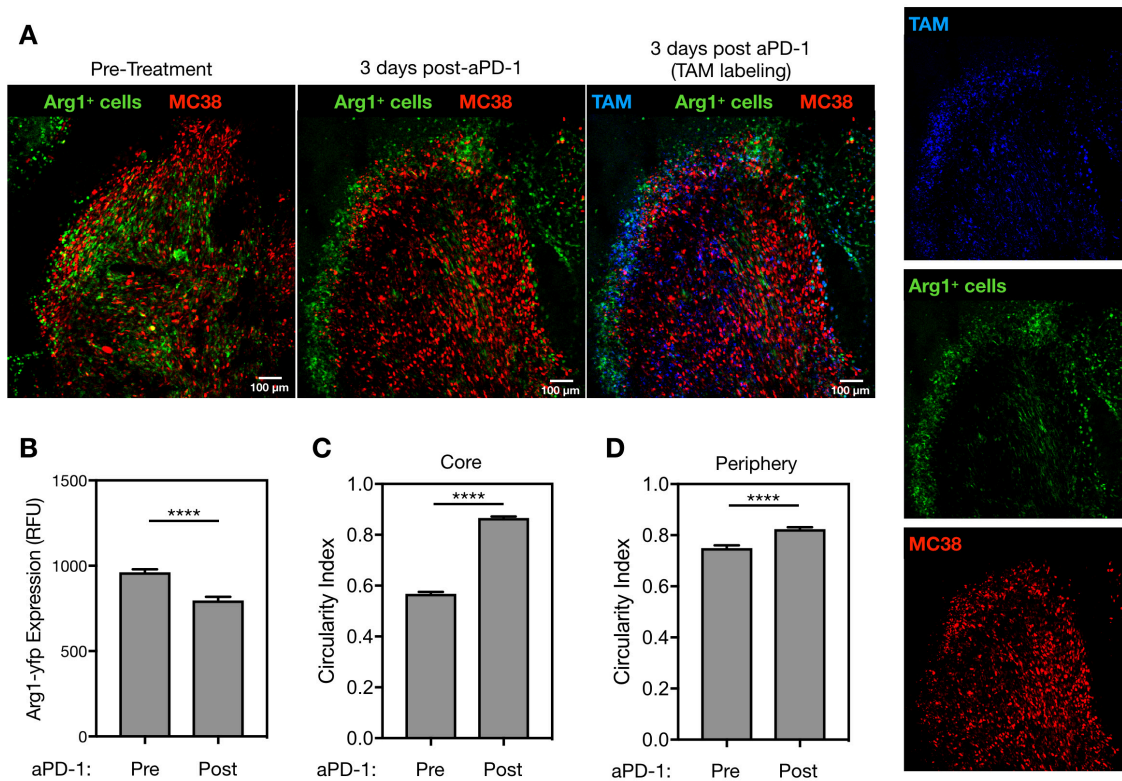
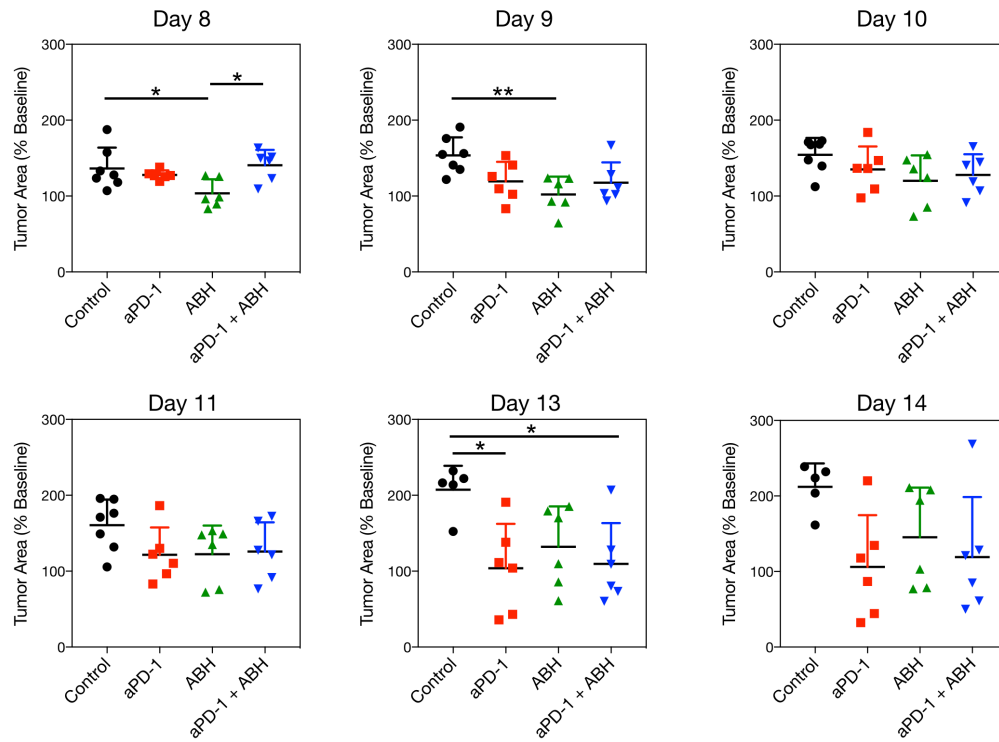


Fig. S12: Statistical analysis of MC38 tumor growth studies. Dot plots of MC38 tumors in C57BL/6 mice treated 8-12 days prior with vehicle control, aPD-1, ABH, or aPD-1+ABH combination. Values are presented as mean \pm S.E.M. *P < 0.05, **P < 0.01.



Movie 1: Arg1+ macrophage morphological heterogeneity. 3D rendering of intravital microscopy Z-stacks acquired near the margin of an MC38-H2B-mApple tumor in an Arg1-eYFP reporter mouse. Tumor cell nuclei (red), Arg1+ macrophages (green). Dimensions of the field of view are 85 by 85 by 30 μm^3 .

Movie 2: Arg1+ macrophage motility revealed by in vivo time lapse microscopy. Z-projected in vivo confocal microscopy movies acquired from Arg1-eYFP (green) reporter mice injected with Pacific Blue dextran (blue) for vascular labeling. MC38-H2B-mApple cells (red) shown at the tumor core.

Movie 3: Arg1+ macrophage speed during observation. The instantaneous speed of Arg1+ macrophages observed using in vivo confocal microscopy of Arg1-eYFP reporter mice. Regions were selected in the core of MC38-H2B-mApple tumors.