

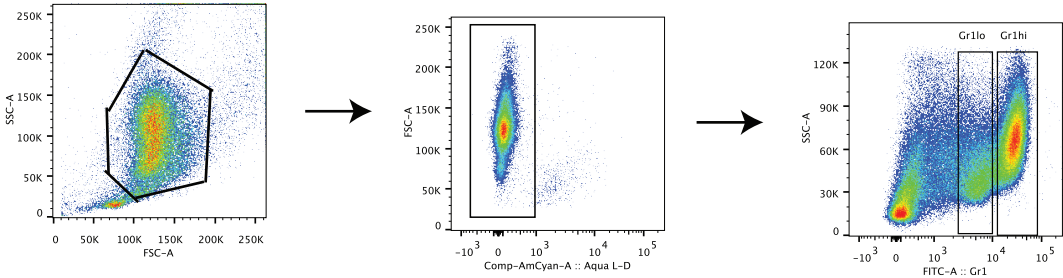
Supplementary Figures

Schmid et al.

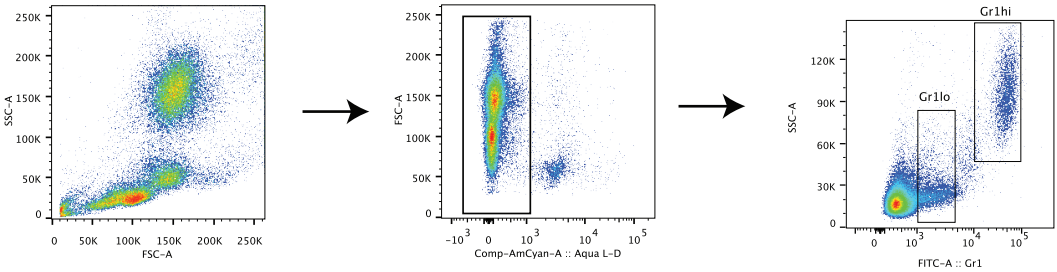
Integrin CD11b activation drives anti-tumor innate immunity

Supplementary Figure 1

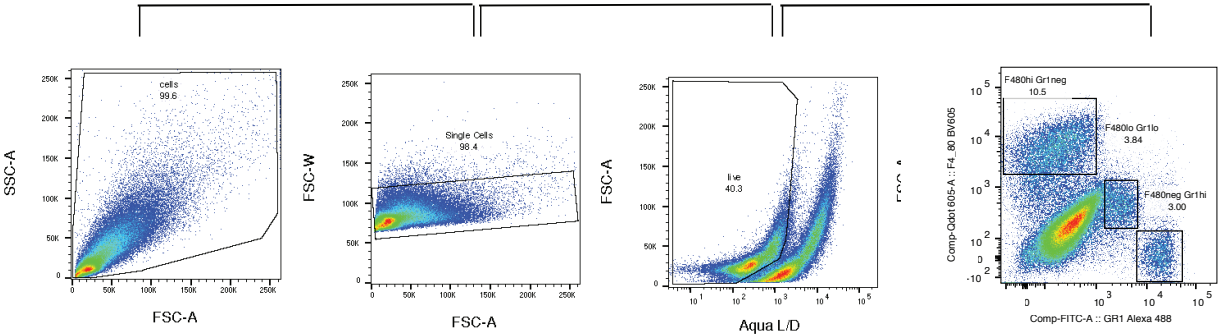
Bone Marrow



Peripheral Blood



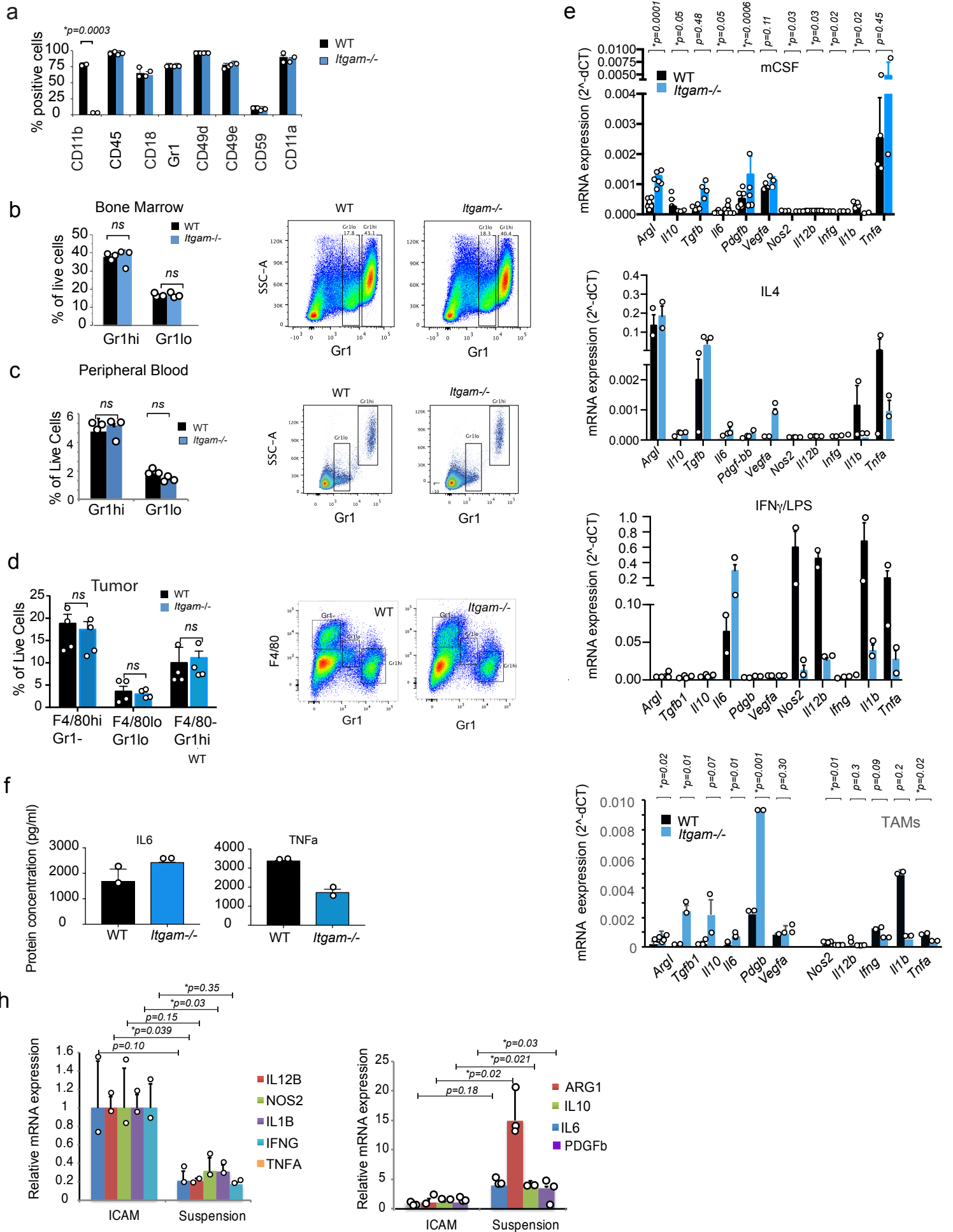
Tumor



Supplementary Figure 1: Schematic of Immune profiles determined by flow cytometry

Flow cytometry analysis schematics for myeloid cells in bone marrow, peripheral blood and tumor from WT and *Itgam*^{-/-} mice.

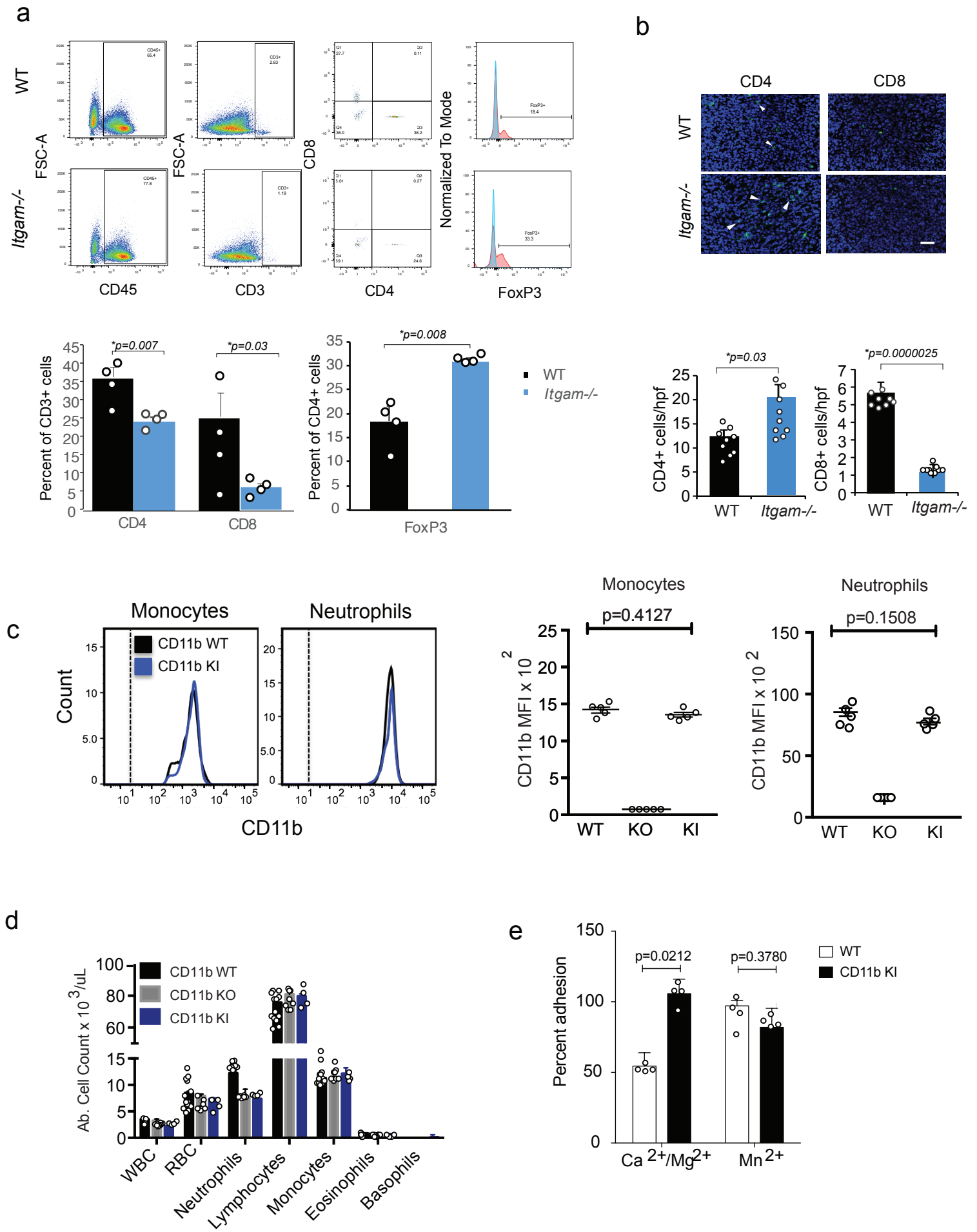
Supplementary Figure 2



Supplementary Figure 2: Immune profiles of normal and tumor tissues of *Itgam*^{-/-} mice

(a) Expression of immune cell markers and integrins in BM derived leukocytes from WT (black bars) and *Itgam*^{-/-} (blue bars) mice (n=4). (b-c) Graphs and FACs profiles indicating proportions of Gr1^{hi} granulocytes and Gr1^{lo} monocytes in (b) bone marrow and (c) peripheral blood in WT (black bars) and *Itgam*^{-/-} (blue bars) mice. (c-d) Graphs and FACs profiles indicating proportions of Gr1^{neg} F/480^{hi} macrophages, Gr1^{lo}F/480^{hi} monocytes and Gr1^{hi}F/480^{neg} granulocytes in LLC tumors from WT (black bars) and *Itgam*^{-/-} (blue bars) mice. (e) mRNA expression of pro- and anti-inflammatory factors in basal, IL-4 and IFN γ /LPS-stimulated WT (black bars) and *Itgam*^{-/-} (blue bars) in vitro cultured BM macrophages (n=4). (f) Concentrations (pg/ml) of IL-6 and TNF α secreted by WT (black bars) and *Itgam*^{-/-} (blue bars) BMDM. (g) mRNA expression of pro- and anti-inflammatory factors in WT (black bars) and *Itgam*^{-/-} (blue bars) TAMs from LLC tumors (n=3-4). (h) Relative mRNA expression of immune response genes in human macrophages adherent on ICAM-1 or maintained in suspension (n=3). Error bars indicate sem. "n" indicates biological replicates. *p (<0.05) indicates statistical significance by Student's t-test. Source data are provided as a Source Data file.

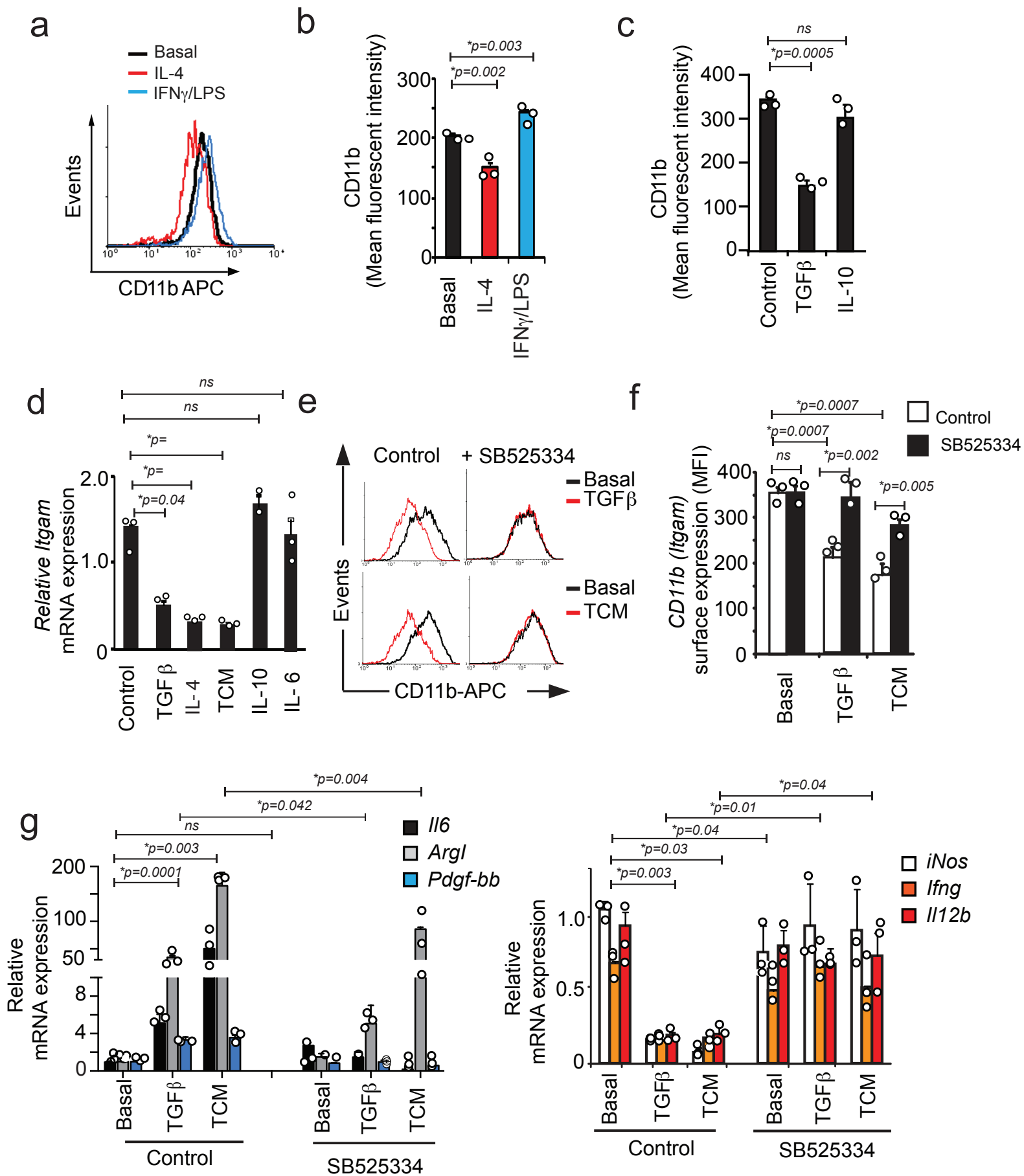
Supplementary Figure 3



Supplementary Figure 3: Tumor derived cytokines suppress CD11b expression and promote immune suppressive macrophage phenotype

(a) Representative FACS profiles and quantification of CD4⁺, CD8⁺ and CD4⁺CD25⁺FoxP3⁺ T cells in WT (black bars) and *Itgam*^{-/-} (blue bars) tumors (n=4). (b) Images and quantification of CD4 and CD8 immunostaining in LLC tumors from WT (black bars) and *Itgam*^{-/-} (blue bars) mice (n=10). In images, bar indicates 50µm. (c) Representative FACS profiles of CD11b expression on neutrophils and monocytes from peripheral blood of *Itgam*^{-/-} (KO) and *Itgam* I332G knockin (KI) mice; quantification of mean fluorescent intensities of CD11b on neutrophils and monocytes from peripheral blood of WT, *Itgam*^{-/-} (KO) and *Itgam* I332G knockin (KI) mice (n=5). (d) Absolute numbers of white blood cells, red blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils in WT (black bars, n=13) *Itgam*^{-/-} (KO) (grey bars, n=8) and *Itgam* I332G knockin (KI) (blue bars, n=4) mice. (e) Percent adhesion to ICAM-coated surfaces of bone marrow derived macrophages from WT (white bars) and *Itgam* I332G knockin mice (black bars) in the presence of physiological concentration of Ca²⁺ and Mg²⁺ or in the presence of Mn²⁺ (n=4). Bar on micrographs indicates x 50 µm. n= number of biological replicates. *p (< 0.05) indicates statistical significance determined by Student's t-test (Supplementary Figure 3a-3b) and Mann-Whitney t-test (Supplementary Figure 3c-3e). Source data are provided as a Source Data file.

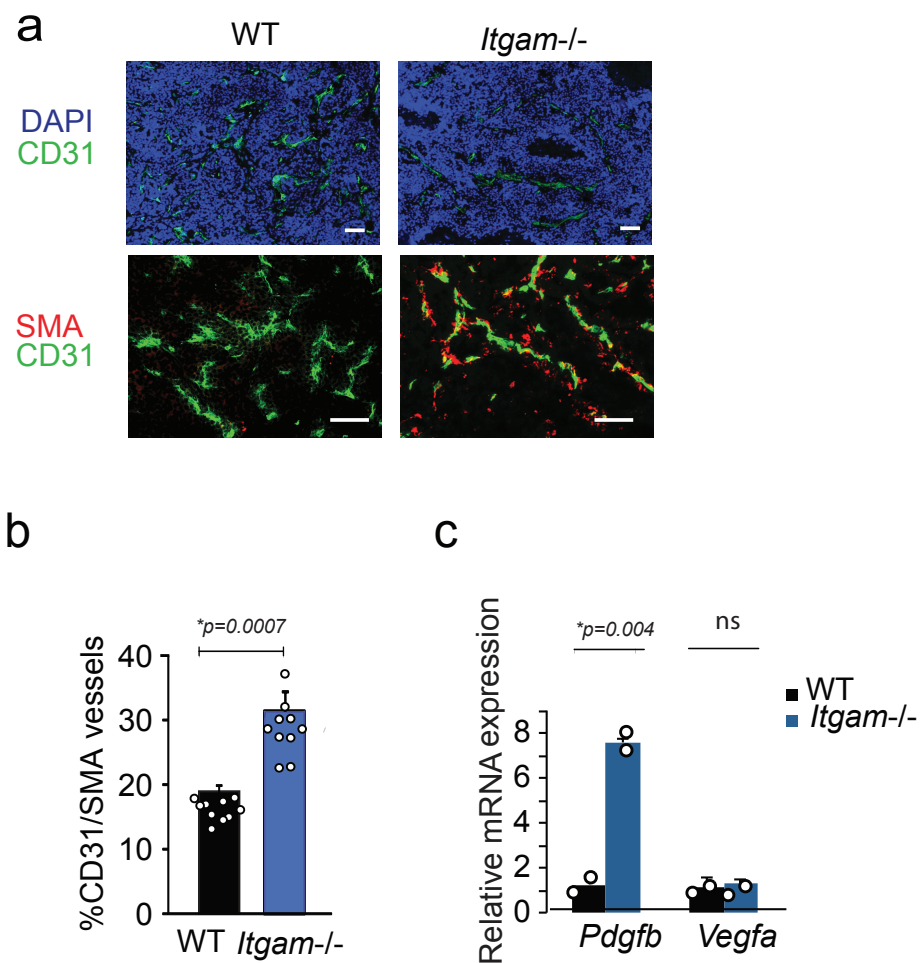
Supplementary Figure 4



Supplementary Figure 4: Tumor derived cytokines suppress CD11b expression and promote immune suppressive macrophage phenotype

(a) FACS profile of CD11b expression levels in bone marrow derived macrophages stimulated with LPS/IFN γ (blue lines), IL-4 (red lines), or basal media (black lines) (n=3). (b) Quantification of CD11b mean fluorescence intensities (MFI) in bone marrow derived macrophages stimulated with LPS/IFN γ (blue bars), IL-4 (red bars), or basal (black bars) media (n=3). (c) Quantification of CD11b MFI in bone marrow derived macrophages stimulated with MCSF (Control), TGF β , or IL10 (n=3). (d) *Cd11b* mRNA expression in WT bone marrow derived macrophages stimulated with TGF β , IL-4, tumor conditioned medium (TCM), IL-10 or IL-6 (n=3). (e) FACS profiles of CD11b expression levels in bone marrow derived macrophages stimulated with basal media (black lines), TCM (red lines, lower panels) or TGF β (red lines, lower panels) in the absence (Control, left panels) or presence (+SB525334, right panels) of the TGF β R1 inhibitor SB525334 (n=3). (f) Quantification of CD11b MFI in bone marrow derived macrophages stimulated with MCSF (basal) or TGF β in the absence (control) or presence of the TGF β R1 inhibitor SB525334 (n=3). (g) Relative mRNA expression of WT and *Cd11b*^{-/-} bone marrow derived macrophages stimulated with mCSF (basal), TGF β or tumor conditioned medium (TCM) in the presence and absence of the TGF β R1 inhibitor (SB525334) (n=3). Error bars indicate sem. "n" indicates biological replicates. *p (< 0.05) indicates statistical significance by Anova with Tukey's post-hoc testing (Supplementary Figure 4b-g). Source data are provided as a Source Data file.

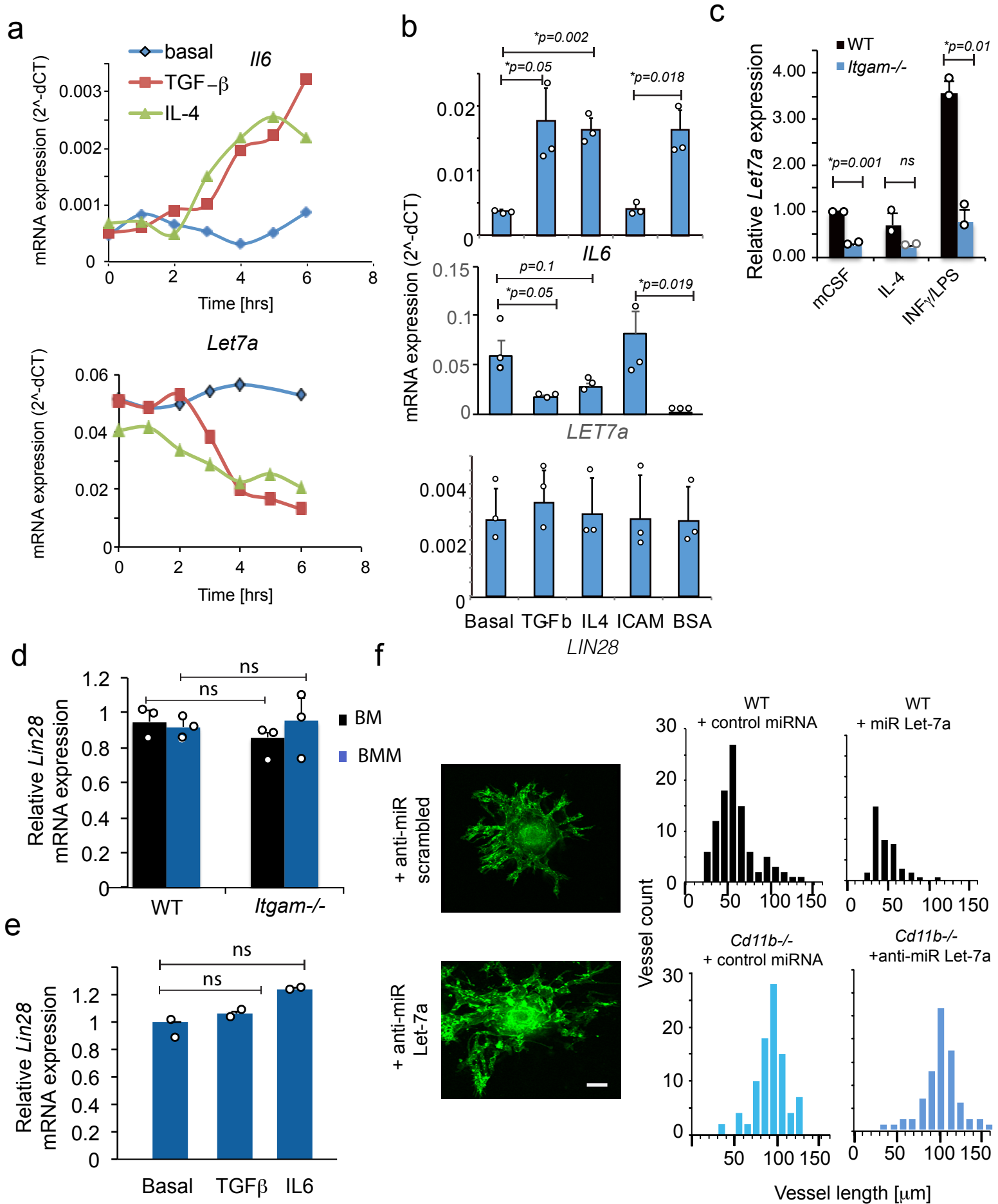
Supplementary Figure 5



Supplementary Figure 5: CD11b loss promotes vascular normalization

(a) Images of CD31/DAPI and CD31/SMA immunofluorescence staining of mammary tumors from PyMT WT and *Itgam*^{-/-} animals. Bar indicates 50 μ m. (b) Percentage of CD31/SMA⁺ vessels in WT (black bars) and *Itgam*^{-/-} (blue bars) tissues from a (n=10). (c) Relative mRNA expression of *Pdgfb* and *Vegfa* in mammary tumors from WT (black bars) and CD11b^{-/-} (blue bars) *PyMT*⁺ animals (n=4). Error bars indicate sem. "n" indicates biological replicates. *p (<0.05) indicates statistical significance by Student's t-test (Supplementary Figure 5b-c). Source data are provided as a Source Data file.

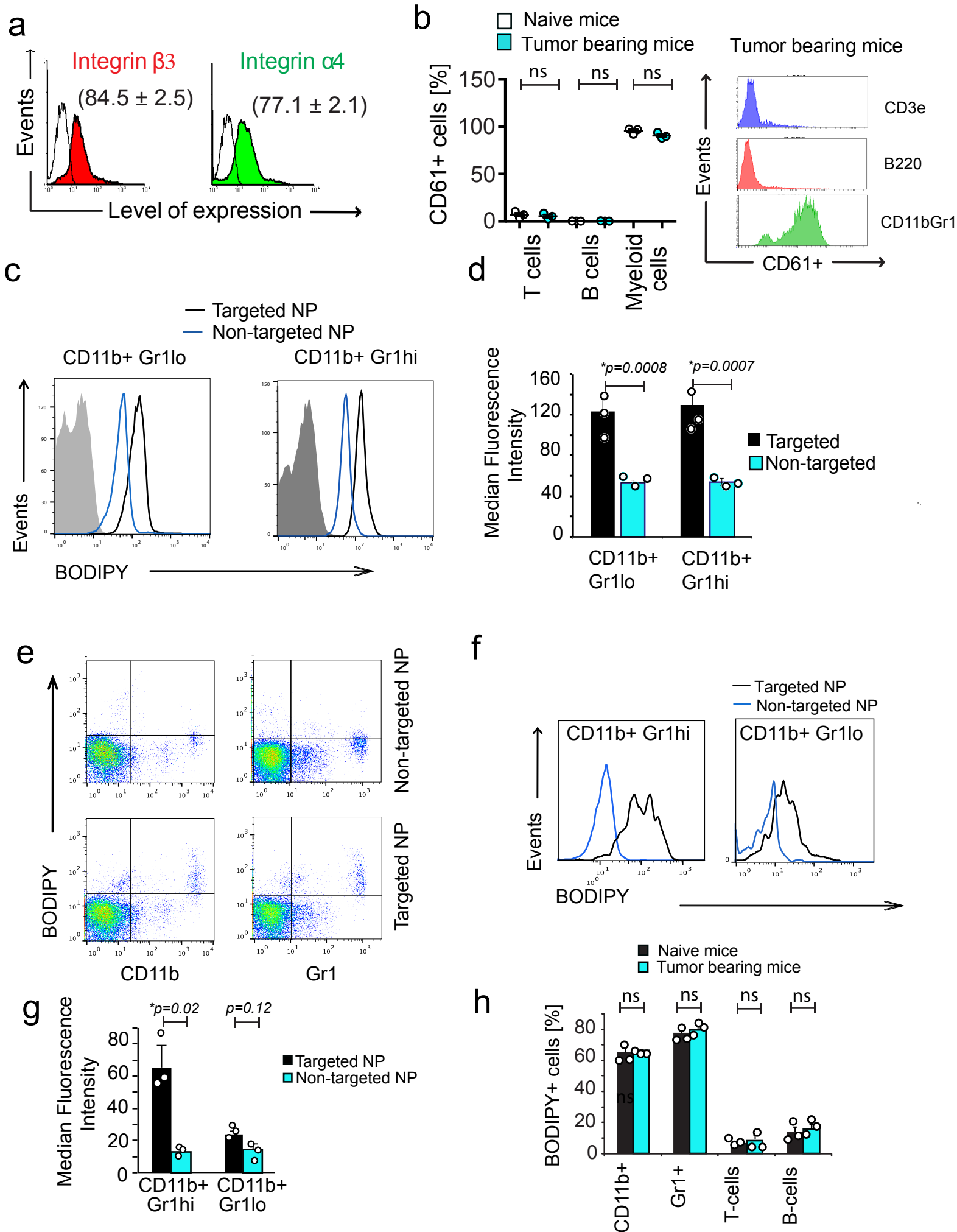
Supplementary Figure 6



Supplementary Figure 6: Let 7a expression and role in macrophages

(a) Time course of expression of *Il6* mRNA (upper panel) or miRNA *Let7a* (lower panel) in mCSF-1 (basal, blue lines), TGF β (red lines) or IL-4 (green lines) stimulated murine bone marrow derived macrophages in vitro (n=2). (b) Relative expression of *IL6*, *LET7a* and *LIN28* expression in human bone marrow derived macrophages treated with mCSF (basal), TGF β or IL-4 or adherent to ICAM-1 vs in suspension (BSA) (n=3). (c) Relative expression levels of *Let7a* in mCSF-1 (basal), IL-4 or IFN γ /LPS stimulated WT (black bars) or *Itgam*^{-/-} (blue bars) murine bone marrow derived macrophages in vitro (n=2). (d) Relative expression levels of Lin28 in total murine bone marrow myeloid cells (BM, black bars) or bone marrow derived murine macrophages (BMMs, blue bars) (n=3). (e) Relative expression levels of Lin28 in BMMs that were treated with basal medium, TGF β or IL-6 (n=3). (f) Endothelial cells and vascular smooth muscle cells attached to microcarrier beads were cultured in fibrin gels containing WT or *Itgam*^{-/-} BMMs transduced with control miRNA, miRNA *Let7a*, or anti-miRNA *Let7a*. Left: Images of representative anti-miR Let-7A and control-treated cultures. Right: Histograms of the lengths of CD31⁺ positive vessels in cultures containing WT (black bars) or *Itgam*^{-/-} (blue bars) BMMs (n=10). Error bars indicate sem. "n" indicate biological replicates. *p (< 0.05) indicates statistical significance by Student's t-test for panels Supplementary Figure 6c, d, e. Anova with Tukey post-hoc testing for panel Supplementary Figure 6b. Source data are provided as a Source Data file.

Supplementary Figure 7

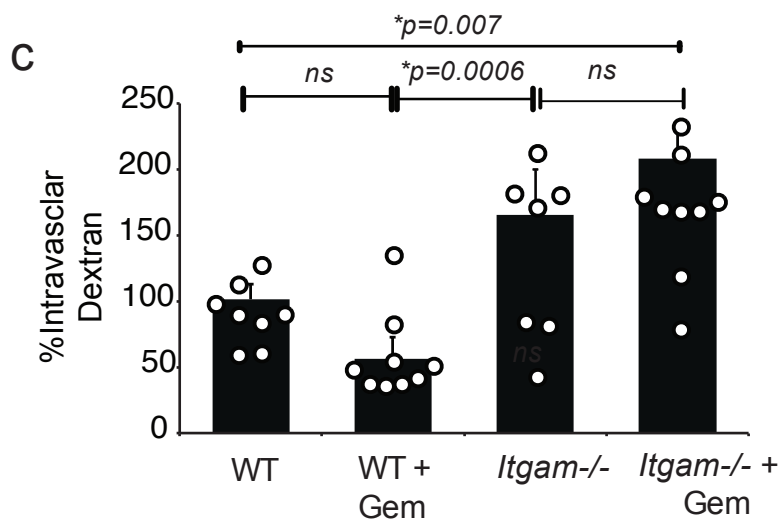
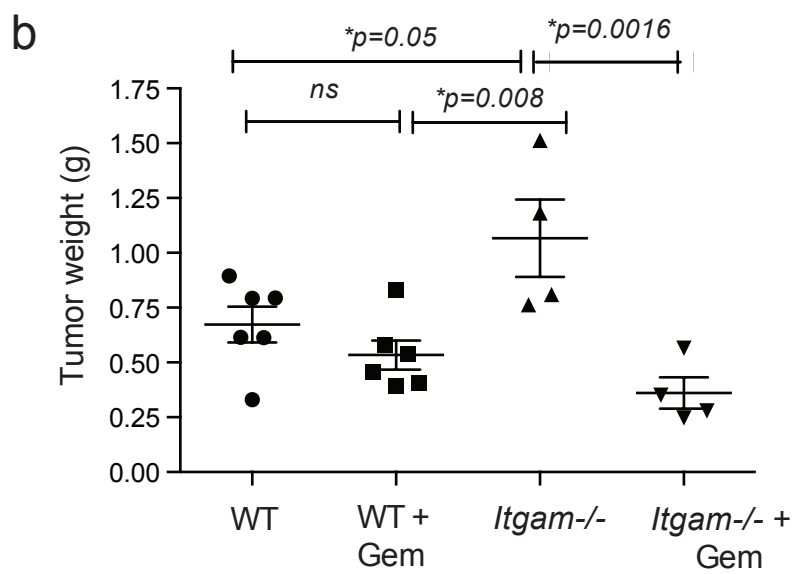
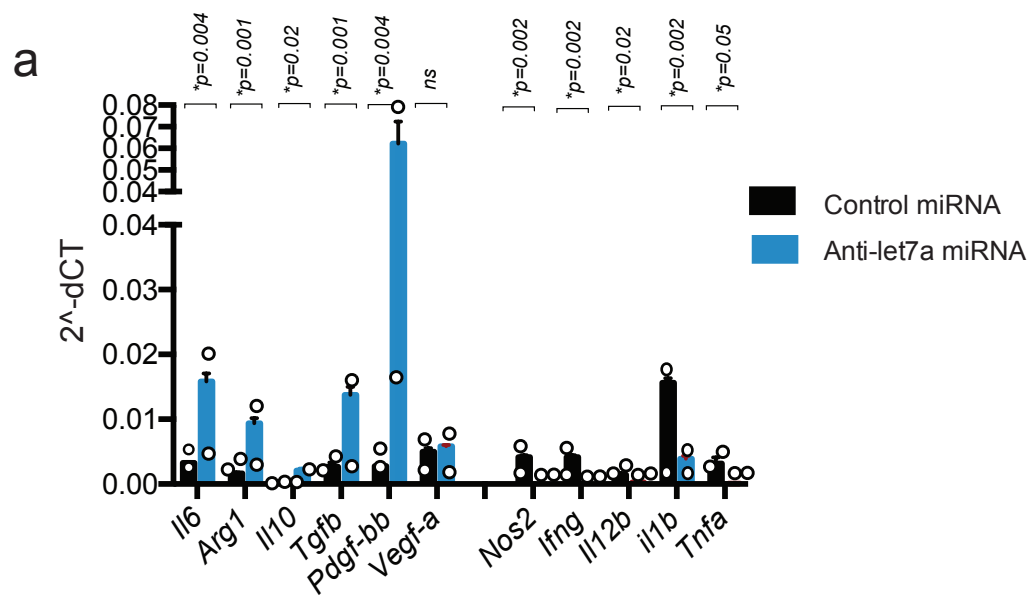


Supplementary Figure 7: RGD-coated nanoparticles target circulating and tumor associated myeloid cells.

(a) Representative flow cytometric profiles of control IgG (black line), anti-integrin $\alpha 4$ (green filled) and anti-integrin $\beta 3$ (red filled) antibody immunostained murine peripheral blood CD11b⁺ myeloid cells. **(b)** Left, graph of percent integrin $\beta 3$ (CD61) positive T, B and myeloid cells in circulation in naive (white bars) and LLC tumor bearing mice (cyan bars) (n=3). Right, representative histograms illustrating CD11b expression in these populations. **(c)** Representative BODIPY⁺ flow cytometric profiles of CD11b⁺Gr1^{lo} monocytes and CD11b⁺Gr1^{hi} granulocytes after in vitro administration of RGD-targeted (black lines) or non-targeted (blue lines) BODIPY-nanoparticles, compared to untreated cells (filled grey histogram). **(d)** Quantification of mean BODIPY⁺ fluorescence intensity induced in CD11b⁺Gr1^{lo} monocytes and CD11b⁺Gr1^{hi} granulocytes by incubation with RGD-targeted (black bars) or non-targeted (cyan bars) BODIPY-nanoparticles from **c** (n=3). **(e)** Representative flow cytometric profiles of BODIPY⁺ CD11b⁺ and Gr1⁺ peripheral blood mononuclear cells from tumor-bearing mice 2h after administration of RGD-targeted or non-targeted BODIPY-nanoparticles (n=3). **(f)** Histograms of relative levels of BODIPY uptake by RGD-targeted (black lines) or non-targeted (blue lines) BODIPY-nanoparticles in circulating CD11b⁺Gr1^{lo} monocytes and CD11b⁺Gr1^{hi} granulocytes from **e**. **(g)** Mean Fluorescence Intensity of RGD-targeted (black bars) or non-targeted (cyan bars) of BODIPY-nanoparticles in CD11b⁺Gr1^{lo} monocytes and CD11b⁺Gr1^{hi} granulocytes from **e** (n=3). **(h)** Quantification of percent BODIPY⁺ CD11b⁺myeloid cells, Gr1⁺myeloid cells, T cells and B cells in peripheral blood 2h after administration of RGD-targeted nanoparticles to naive (black bars) or tumor bearing

(cyan bars) animals (n=3). Error bars indicate sem. "n" indicates biological replicates. *p (< 0.05) indicates statistical significance by Student's t-test (Supplementary Figure 7d,7g,7h). Source data are provided as a Source Data file.

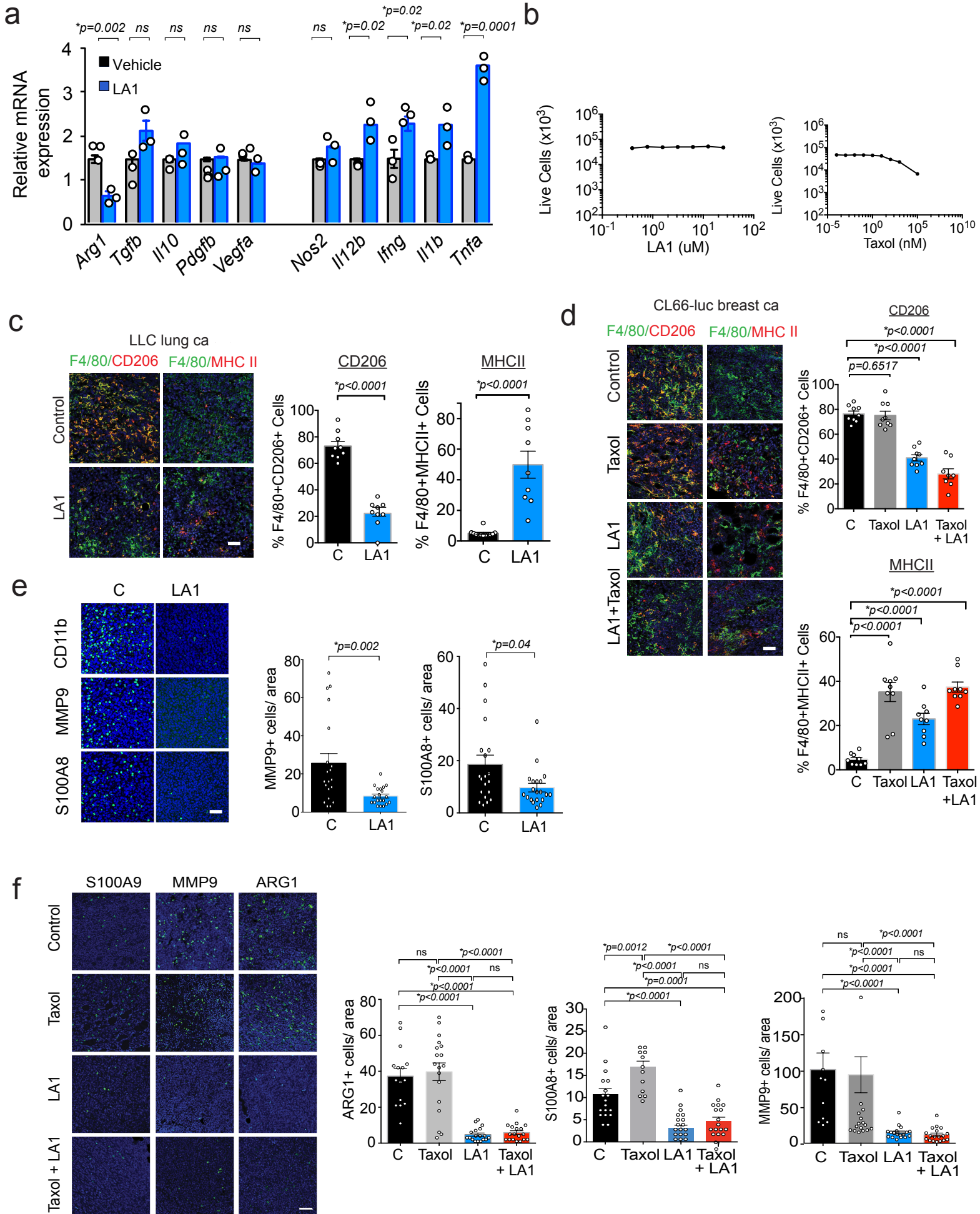
Supplementary Figure 8



Supplementary Figure 8: Effect of Let7a and CD11b inhibition on tumors

(a) mRNA expression of inflammatory factors in sorted macrophages from LLC tumor-bearing mice treated with control miRNA (black bars) or anti-miR Let7a (blue bars)(n=3). **(b)** Weight of LLC tumors from WT and *Itgam*^{-/-} mice treated with or without gemcitabine (n=4-6). **(c)** Percent perfusion (% intravascular FITC-dextran) in mice from (n=8 fields). **b.** Error bars indicate sem. "n" indicates biological replicates. *p (< 0.05) indicates statistical significance by Student's t-test for Supplementary Figure 8a and Anova with Tukey's post-hoc testing for Supplementary Figure 8b and 8c. Source data are provided as a Source Data file.

Supplementary Figure 9

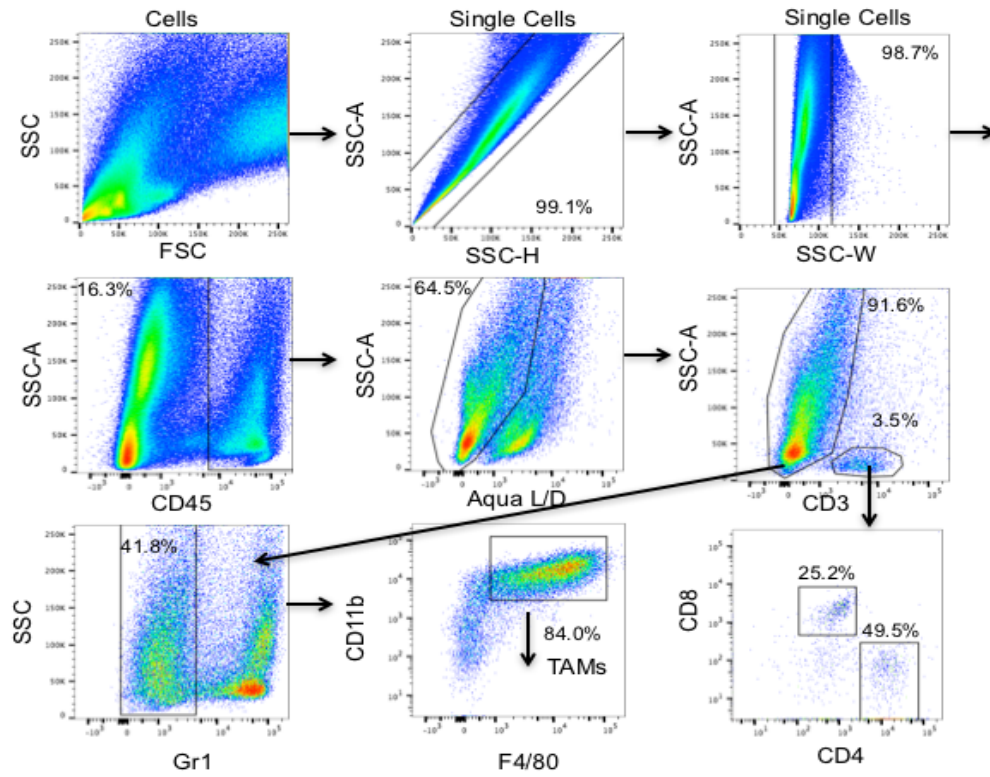


Supplementary Figure 9: Effects of LA1 on lung tumor progression

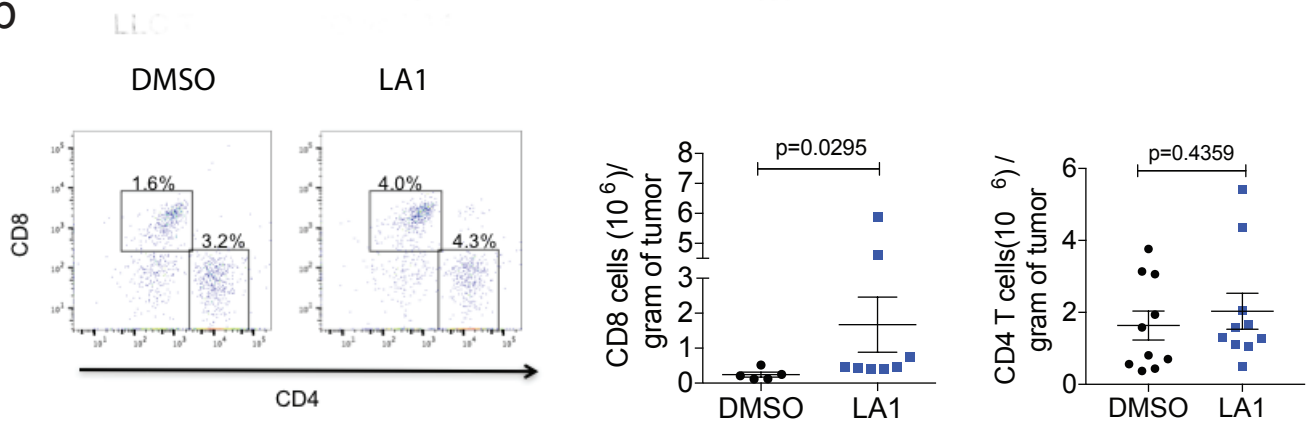
(a) mRNA expression of inflammatory cytokines in vehicle (grey bars) or LA1 (blue bars) treated IFN γ +LPS stimulated bone marrow derived murine macrophages (n=3). (b) Effect of LA1 (left) or taxol (right) on CL66-Luc breast tumor cell survival in vitro (n=3). (c) Images and quantification of CD206+ F4/80+ and MHC-II+ F4/80+ expression in LA1 (blue bars) and vehicle (black bars) -treated LLC tumors (n=5). (d) Images and quantification of CD206+F4/80+ and MHC-II+F4/80+ expression in vehicle (black bars), taxol (grey bars), LA1 (blue bars) and taxol + LA1 (red bars) -treated CL66-Luc tumors (n=5). (e) Images and quantification of CD11b, S100A9 and MMP9 expression in LA1 (blue bars) and vehicle (black bars) -treated LLC tumors (n=5). (f) Images of and quantification of LA1 (blue bars), taxol (grey bars), taxol + LA1 (red bars) and vehicle-treated (black bars) CL66-Luc tumor cryosections immunostained to detect S100A9, MMP9, and Arg1. Error bars indicate sem. "n" indicated biological replicates. *p (< 0.05) indicates statistical significance determined by Student's t-test for Supplementary Figure 9a, c, and e, and by Anova with Tukey's post-hoc testing for Supplementary Figure 9d and f. Source data are provided as a Source Data file.

Supplementary Figure 10

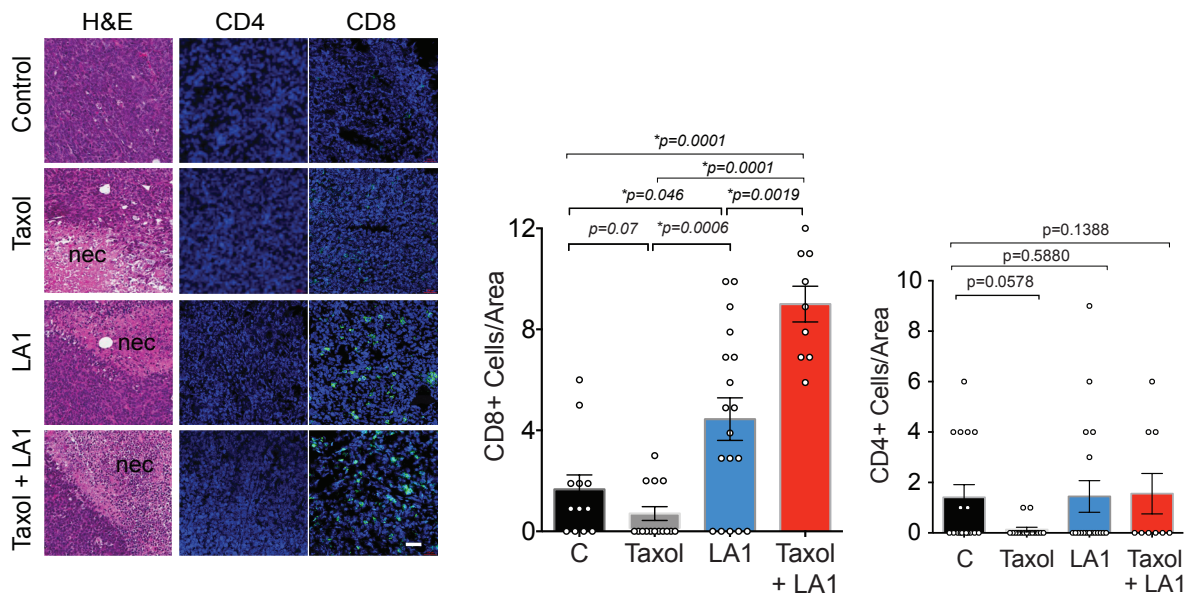
a



b



c



Supplementary Figure 10: Effects of LA1 on breast tumor progression

(a) FACS gating scheme for analysis of T cells in LA1 treated tumors. (b) Representative FACS profiles and quantification of CD4⁺ and CD8⁺ T cells in LA1 (blue dots, n=10) and vehicle (DMSO) treated (black dots, n=10) LLC tumors. (c) Images of and quantification of cryosections of CL66-Luc tumors from vehicle- (black bars), taxol (grey bars), LA1 (blue bars) and LA1 + taxol (red bars) treated tumors that were immunostained to detect CD4 and CD8 (n=8-16). Error bars indicate sem. "n" indicated biological replicates. Bar indicates 50 μ m. *p (< 0.05) indicates statistical significance as determined by Student's t-test for Supplementary Figure 10b and by Anova with Tukey's post-hoc testing for Supplementary Figure 10c. Source data are provided as a Source Data file.