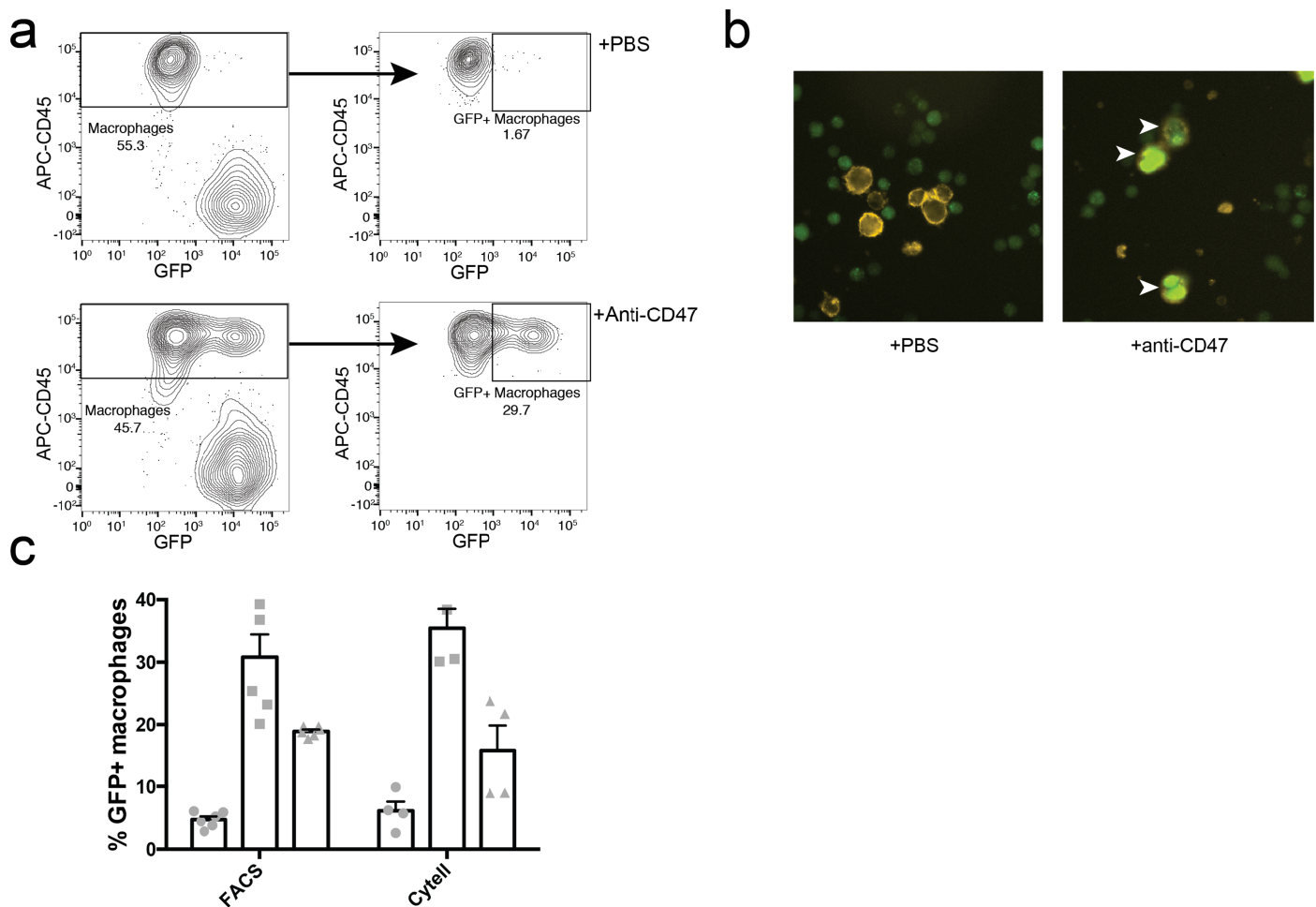


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Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy

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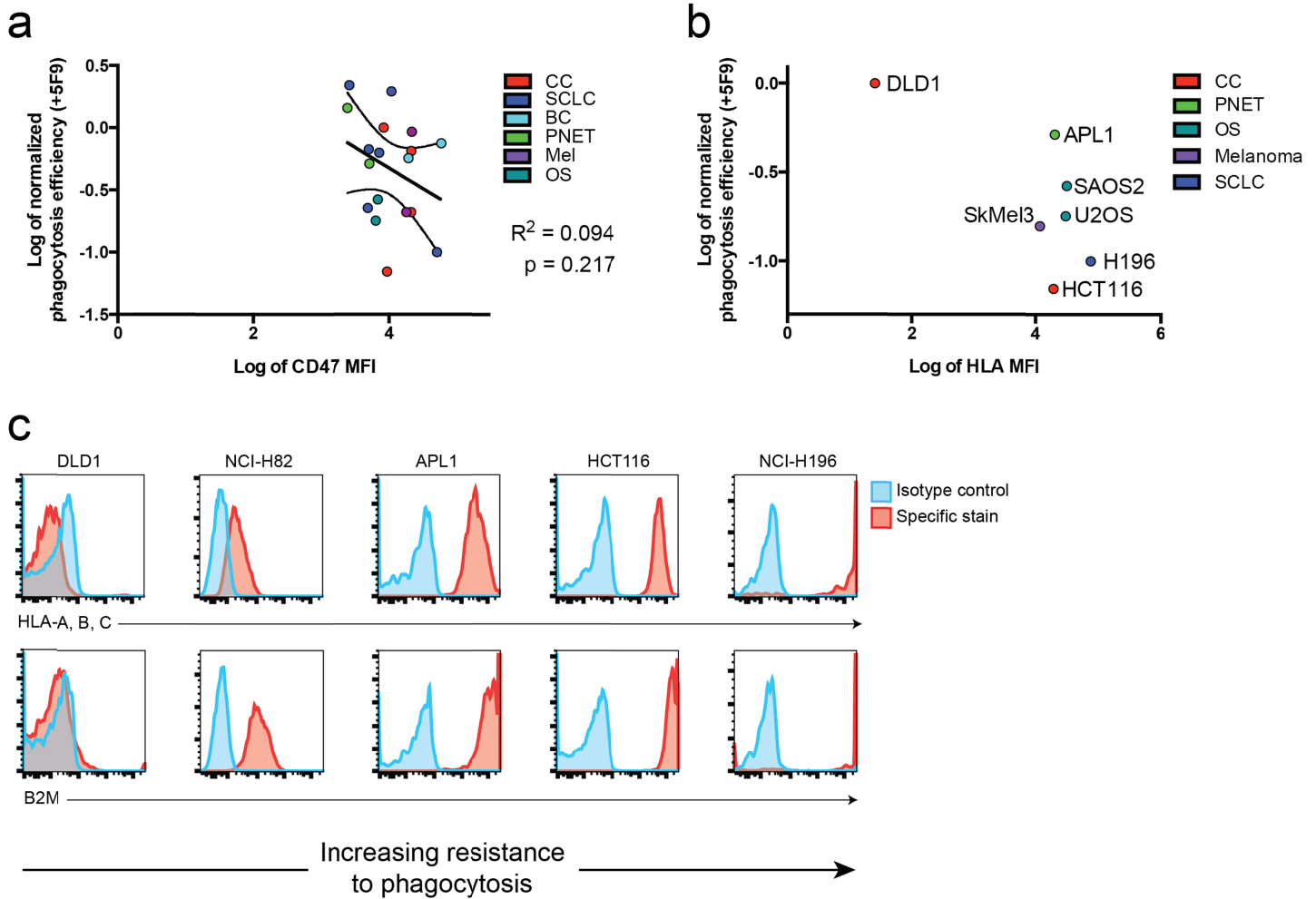
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Supplementary Figure 1

Validation of flow cytometry-based in vitro phagocytosis assay.

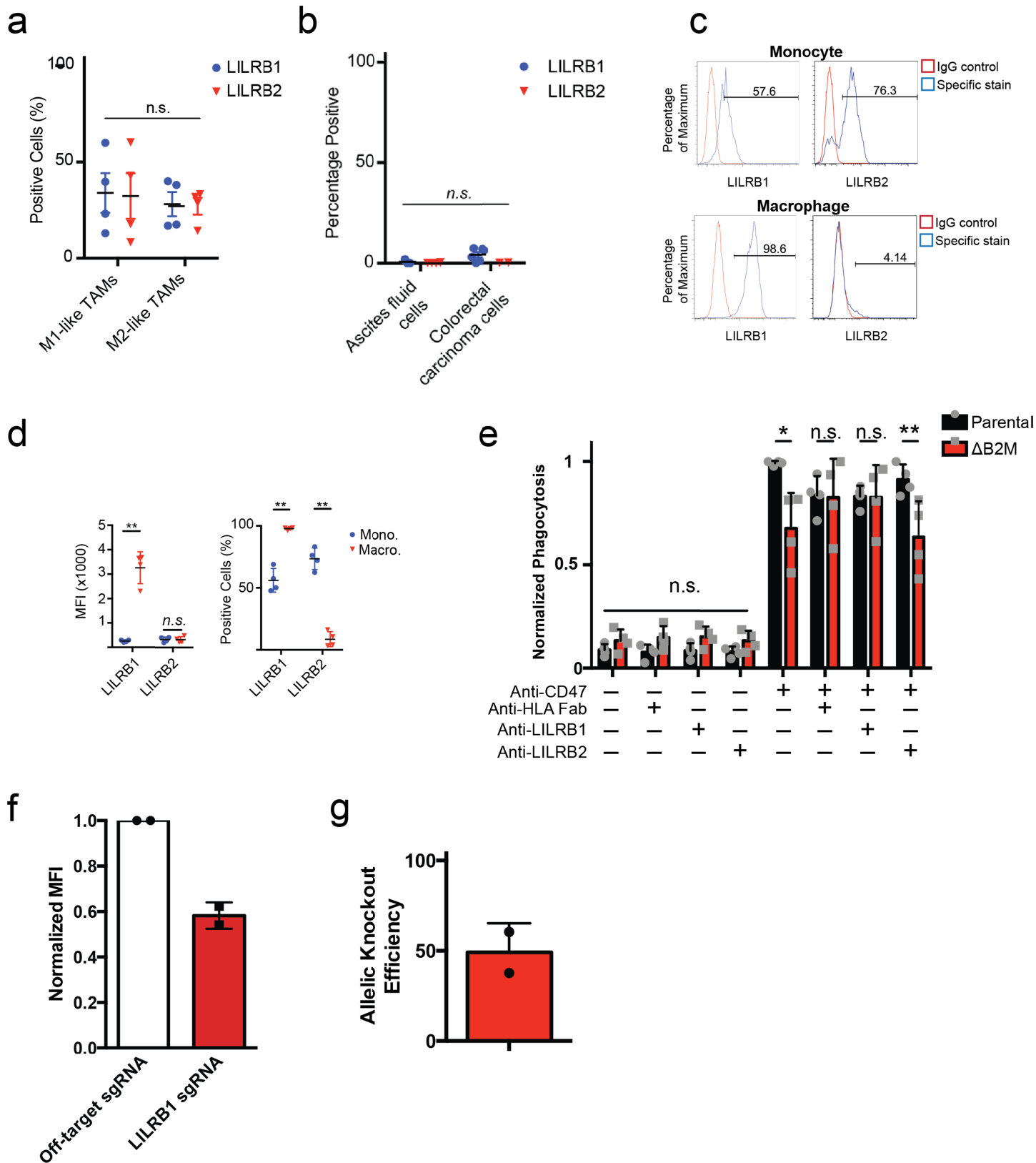
(a) Representative flow cytometry plots of *in vitro* phagocytosis assay. Macrophages are defined as CD45⁺ events, and tumor cells as GFP⁺ events. CD45⁺, GFP⁺ events correspond to macrophages that have phagocytosed target cells. **(b)** Representative images from an automated microscopy-based approach for quantifying in vitro phagocytosis. Using a custom program on the GE Cytell, we defined human macrophages based on staining with anti-CD45-PE antibody (yellow), and tumor cells based on GFP expression (green). Phagocytic events are defined based on overlap of these two signals (right panel, white arrowheads). **(c)** Comparison of FACS-based quantification (left) or automated microscopy-based quantification (right) of DLD1 phagocytosis under conditions of PBS treatment, treatment with the anti-CD47 antibody Hu5F9-G4, or treatment with the anti-EGFR antibody Cetuximab.



Supplementary Figure 2

Analysis of CD47 and MHC class I component expression in cell lines.

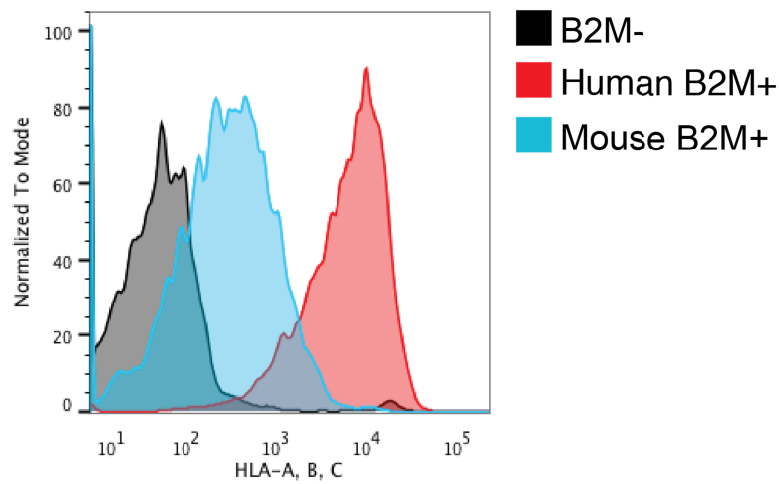
(a) Log-transformed scatterplot of normalized phagocytic efficiency upon treatment with the anti-CD47 antibody Hu5F9-G4 (Y axis) plotted against surface expression of CD47, as measured by FACS analysis with the anti-human mouse monoclonal antibody B6H12 (X axis). There is no significant relationship between these two parameters, $R^2=0.094$, $p=0.217$. **(b)** Histogram plots of HLA-A, B, C (top) and β 2M expression (bottom) for two colon cancer cell lines: DLD1 (red) and HCT116 (magenta); two small cell lung cancer lines, NCI-H82 (blue), and NCI-H196 (purple); and a pancreatic neuroendocrine tumor APL1 (green), as measured by the BioLegend LegendScreen FACS-based antibody array system. Isotype control stain is indicated in blue, while specific stain is indicated in red. **(c)** Log-scale scatterplot of phagocytic efficiency in a panel of cell lines upon treatment with the anti-CD47 antibody Hu5F9-G4 (Y axis), plotted against surface expression of HLA-A, B, C as measured by FACS analysis with a pan-HLA-A, B, C binding antibody (X axis). The data is a subset of that shown in Figure 1b, in order to highlight the cell lines used for the experiment shown in Figure 2g.



Supplementary Figure 3

Analysis of the expression and function of LILRB1 and LILRB2 in macrophages.

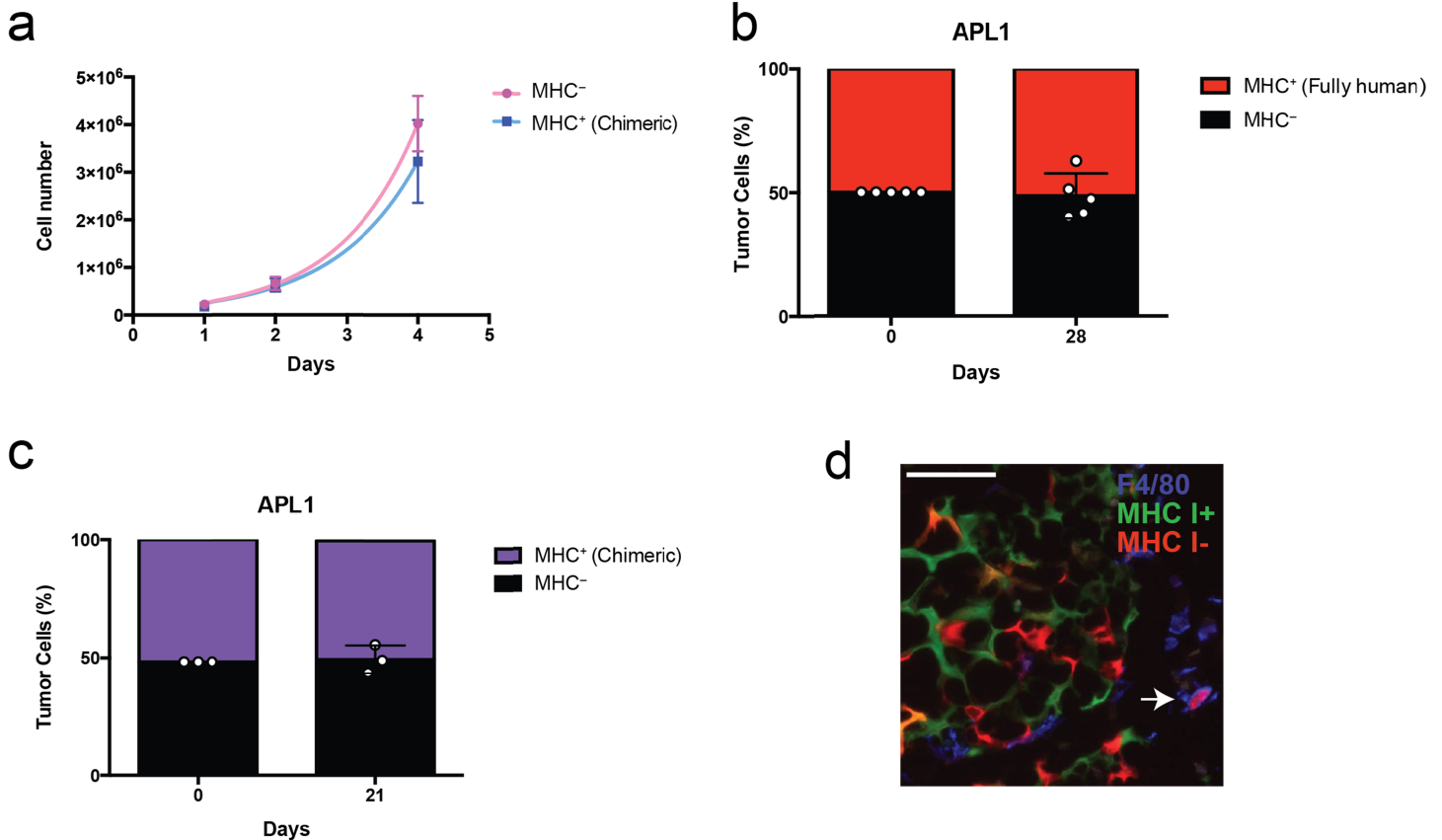
(a) Flow cytometry-based measurement of the percentage of LILRB1 (blue) or LILRB2 (red) positive, non-immune cells from six samples of primary ascites fluid macrophages, and four samples of primary human colorectal carcinoma samples, as determined by fluorescence minus one controls. n.s. not significant, 2-way ANOVA with multiple comparisons correction. **(b)** Flow cytometry-based measurement of the percentage of LILRB1 (blue) or LILRB2 (red) positive tumor associated macrophages from four independent primary human colorectal carcinoma samples, as determined by fluorescence minus one controls. n.s. not significant, 2-way ANOVA with multiple comparisons correction. **(c)** Representative histogram plots from FACS analysis of LILRB1 and LILRB2 expression on primary human CD14⁺ peripheral blood monocytes (left), and day 7 *ex vivo* cultured macrophages derived from the same donor (right). Specific staining is indicated in blue; IgG control is indicated in red. **(d)** LILRB1 and LILRB2 expression levels, as measured by mean fluorescence intensity (MFI, left panel) or percent positive cells (right panel), in 4 pairs of primary monocytes (red) and *ex vivo* cultured macrophages from the same donors (blue). n.s., not significant. *** $p < 0.001$, 2-way ANOVA with multiple comparisons correction. **(e)** Flow cytometry-based measurement of phagocytosis by donor-derived macrophages of MHC I⁻ DLD1 (black) and MHC I⁺ DLD1 (red). Values are normalized to the highest level of phagocytosis observed in a given experimental replicate. Error bars represent s.d. of assays performed with $n=8$ biological replicates. * $p < 0.01$, ** $p < 0.001$, 2-way ANOVA with multiple comparisons correction.



Supplementary Figure 4

Mouse $\beta 2M$ does not efficiently form stable MHC class I complexes with human HLA α -chains.

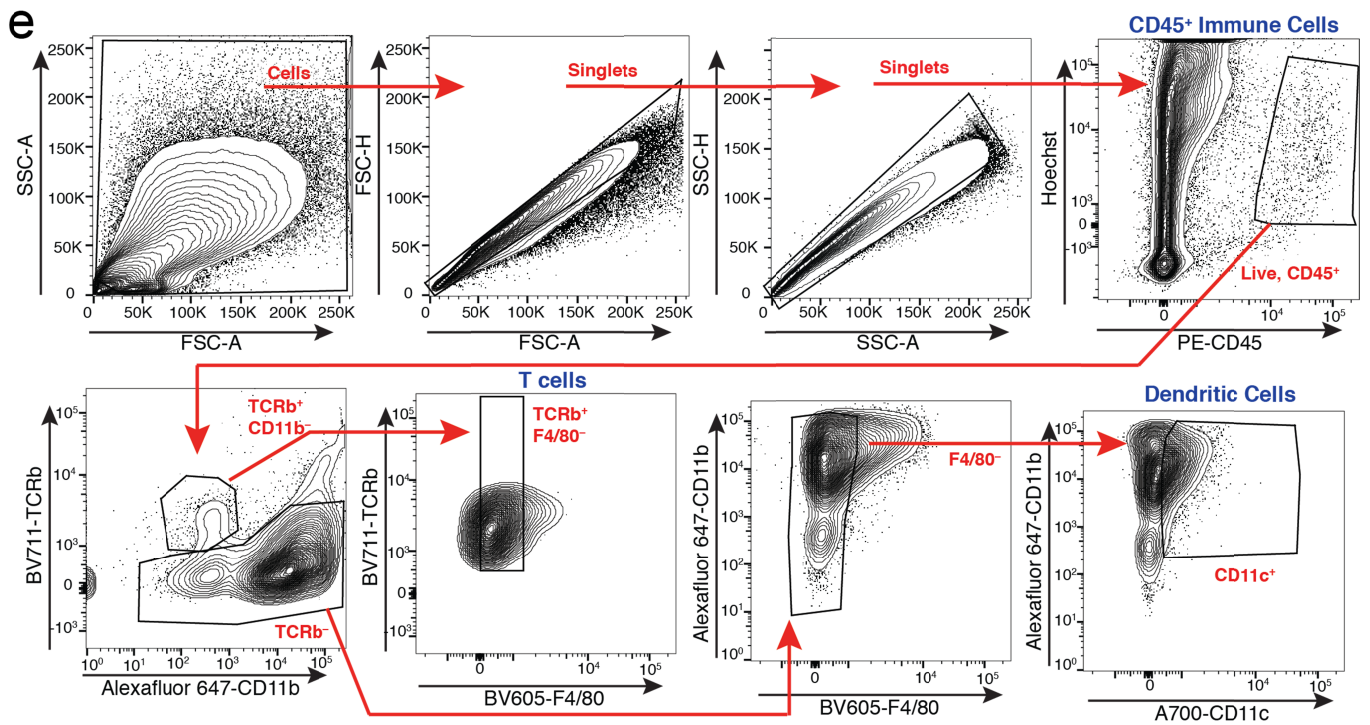
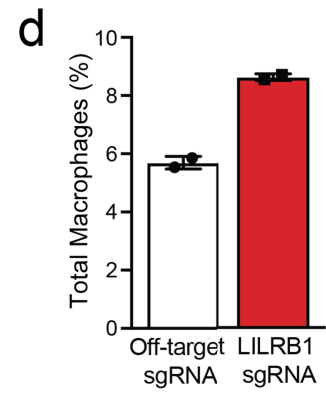
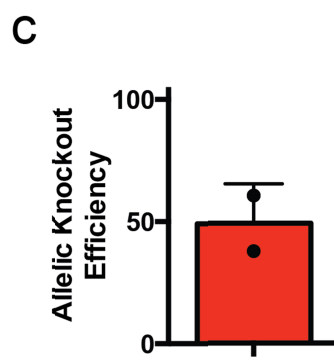
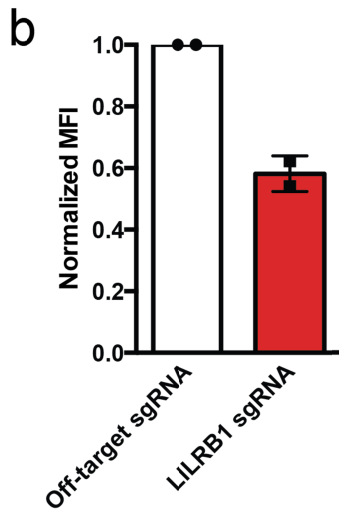
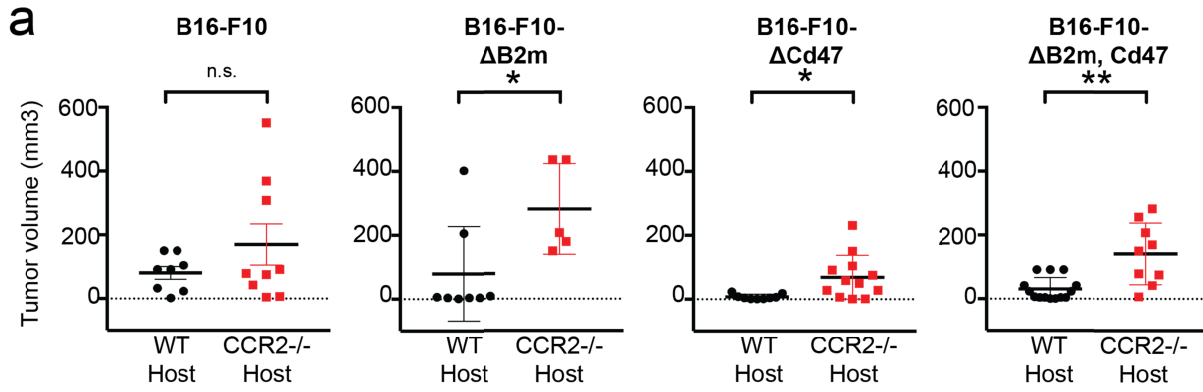
FACS histogram of parental DLD1 cells (black), DLD1 cells reconstituted with a human $\beta 2M$ transgene (red), or with a mouse $\beta 2M$ transgene (blue). While fully human $\beta 2M$ expression facilitates robust surface MHC class I expression, as detected by the pan-HLA antibody W6/32, expression of mouse $\beta 2M$ facilitates only low levels of surface MHC class I.



Supplementary Figure 5

Growth kinetics and immunofluorescence of engineered cell lines.

(a) Growth curve of MHC I⁻ DLD1 (pink) and chimeric MHC I⁺ DLD1 (blue), as measured by cell counting at 1, 2, and 4 days. Error bars represent s.d. of n=3 replicates. **(b)** Flow cytometry analysis of MHC class I expression in a 50-50 *in vitro* co-culture of MHC I⁻ APL1 and MHC I⁺ APL1. Error bars represent s.d. of n=3 replicates. **(c)** Flow cytometry-based quantification of the percentage of fully human MHC class I expression in a 50-50 *in vitro* co-culture of MHC I⁻ APL1 and MHC I⁺ APL1 cells pre-engraftment and at 28 days post engraftment into NSG mice. Error bars are s.d. of n=5 mice. **(d)** Fluorescence microscopy of sections taken from mixed MHC I⁺ and MHC I⁻ APL1 tumors injected subcutaneously into the flanks of NSG mice. Chimeric MHC class I⁺ cells are marked by GFP expression, while MHC I⁻ cells are marked by RFP expression. Mouse macrophages are visualized by F4/80 staining (blue). A high degree of macrophage infiltration into the tumor is evident, and macrophages can be observed in the act of phagocytosis (white arrowhead). Scale bar represents 200μm.



Supplementary Figure 6

Dynamics of tumor-infiltrating macrophages.

(a) Tumor volume of B16-F10 variants 15 days post-engraftment into immunocompetent C57Bl/6 wild type mice (black) or CCR2^{-/-} mice (red). n.s. not significant. * $p < 0.05$, *** $p < 0.001$, Student's t test. **(b)** FACS-based measurement of LILRB1 expression in primary human macrophages electroporated with either off-target sgRNA (black) or LILRB1-targeting sgRNA, as determined by MFI. MFI values are normalized to the highest value for each experimental replicate. Error bars represent the standard deviation of assays performed with $n=2$ macrophage donors. **(c)** Frequency of allelic deletion of LILRB1 in primary human macrophages, as measured by TIDE analysis software, $n=2$ macrophage donors. **(d)** Percentage of total macrophages with an M1-like phenotype in macrophages modified with off-target sgRNA (black) or LILRB1-targeting sgRNA (red) in $n=2$ macrophage donors. **(e)** Gating strategy to define infiltrating immune cells within dissociated B16-F10 mouse tumors.