Supplemental Figures

Supplemental Figure 1: Tumor-infiltrating CD8$^+$ T cells can express FcγRI. **A**. *Relative intensity of various markers superimposed on UMAP projection.* **B.** *Representative FACS analysis of FcγRI in T cells in the tumor and DLN of Yummer1.7 tumor-bearing mice.* **C.** *Relative intensity of CD64 (FCγR1α) superimposed on UMAP projection from tumors and metastatic DLN and normal LN of patients with head and neck squamous cell carcinoma (HNSCC) (n=10).* **D.** *Abundance of CD11b high (also CD64 high) CD8$^+$ T cells across patients.* **E.** *Representative FACS analysis of FcγRI-expressing T cells from freshly resected human melanoma.* **F.** *Immunofluorescence of FcγRI in histological sections from patient with chronic tonsilitis.* **G.** *Representative FACS analysis of FcγRI-expressing T cells from freshly resected tonsils of patients suffering from chronic tonsilitis and blood of COVID-19 recovered patients. Experiments were repeated at least three independent times. Statistical significance was calculated using ANOVA with Tukey's correction for multiple comparisons (** denotes p<0.001, **** denotes p<0.00001). Error bars represent standard error. Scale bars = 20 μm.*

Supplemental Figure 2: FCγRI expressing T cells exist in inflammatory tissue **A**. *Comparison of expression of selected genes across T cell clusters. Dot size represents percentage of cells expressing the gene, dot color represents mean log normalized expression.* **B**. *Percentage of T cells with any expression of FCGR1A across diseases.* **C**. *Immunofluorescence of FcγRI on CD8+ T cells from day 10 B16F10 incubated with monocytes and IFNg for 7 days.* **D**. *FcγRI expression on naïve splenic CD8+ T cells cultured ex vivo with monocytes and cytokines (n=4).* **E**. *Representative Lattice-SIM of CD8$^+$ T cells co-cultured with monocytes.* **F**. *Representative*

*Lattice-SIM on sorted Fc□RI-expressing CD8$^+$ T cells immune synapse with NCI-N87 cancer cells in the presence of trastuzumab. Experiments were repeated at least three independent times. Statistical significance was calculated using ANOVA with Tukey's correction for multiple comparisons (** denotes p<0.001, **** denotes p<0.00001). Error bars represent standard error. Scale bars = 20 μm.*

Supplemental Figure 3: Activation of T cells expressing modified FcγRI induces IFN and TNF signaling. **A**. *Representative TIRF imaging of transduced T cells interaction with Her2 on SLB through intermediate antibodies.* **B**. *Representative SIM microscopy of AG2G transduced T cells incubated for 20 min with trastuzumab.* **C.** *Schematic illustration of AGG2GG construct on a cell membrane.* **D.** *Mean levels of IFNg in the supernatants of transduced human T incubated 48 hours with antibody -conjugated beads (n=3).* **E.** *Hierarchal clustering of differentially expressed genes in transduced T cells (n=4).* **F.** *Gene Set Enrichment Analysis of activated vs. not activated transduced T cells.* **G.** *KEGG analyses on differentially expressed cytokine-cytokine receptor interactions in activated vs. not activated AG2G transduced T cells.* **H.** *Normalized fluorescents intensity change in the synapse between AG2G transduced T cells and Tumor cells in the presence of relevant antibody (Cetuximab), irrelevant antibody (Trastuzumab) and no antibody (n=18).* **I.** *Mean levels of IFNγ in the supernatants of transduced human T cells incubated 48 hours with fixed antibody or Her2 antigen (n=4).* **J**. *Normalized HT29 cells confluence following incubation with trastuzumab (n=4). Statistical significance was calculated using ANOVA with Tukey's correction*

*for multiple comparisons (** denotes p<0.001, **** denotes p<0.00001). Error bars represent standard error.*

Supplemental Figure 4: Free circulating IgG are recycled in transduced CD8+ T cells endosomes without activating FcγRI signaling. **A.** *Mean levels TNFα in the supernatants of AG2G transduced T cells and CAR based FcγR incubated with various concentrations of free antibody (n=4).* **B.** *Normalized HT29 cells confluence following incubation with Sham transduced T cells in combination with trastuzumab (n=4)* **C.** *Normalized HT29 cells confluence following incubation with trastuzumab (n=4)* **D.** *Mean fluorescence intensity of stained transduced human T cells following wash of FITC-IgG1 (n=3).* **E.** *Mean percentages of transduced T cells that bind FITC-labeled IgG1 overtime (n=5).* **F.** *Representative confocal images of T cells expressing FcR-based CAR pulsed with FITC-labeled IgG1.* **G.** *Representative SIM microscopy of A2G transduced T cells incubated for 20 min with trastuzumab.* **H.** *Immunofluorescence following wash of FITC-IgG1 and loading of PE-IgG1. Experiments were repeated at least three independent times. Statistical significance was calculated using ANOVA with Tukey's correction for multiple comparisons (** denotes p<0.001, **** denotes p<0.00001). Error bars represent standard error.*

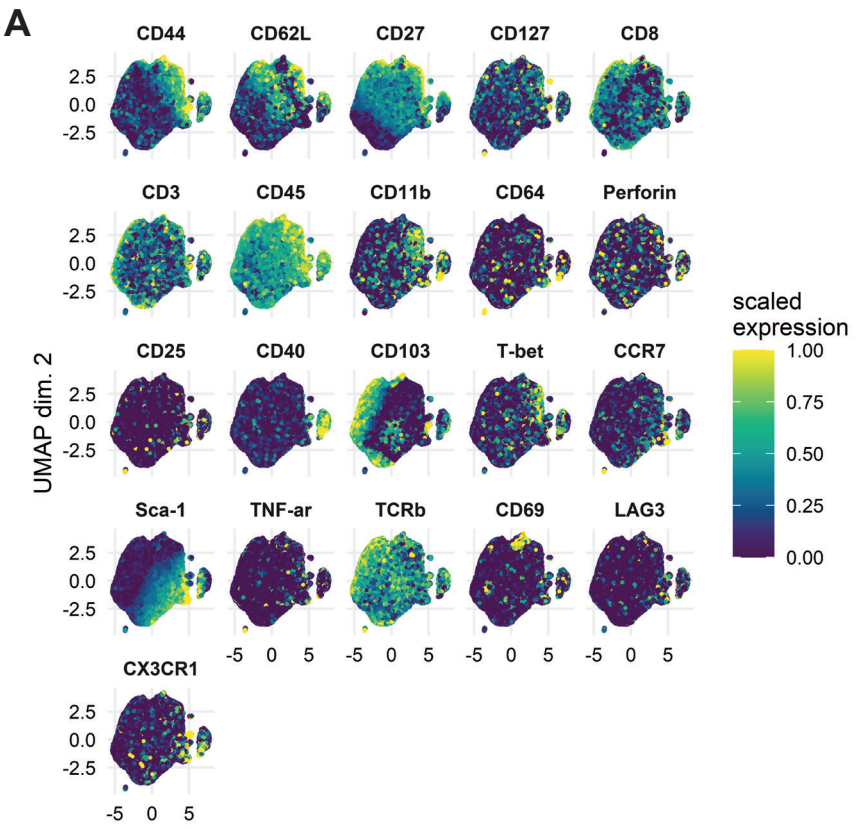
Supplemental Figure 5: High dose of transduced T cell injection controls tumor growth. **A.** *NCI-N87 tumor volume in individual NSG mice following various treatment with AG2G and Sham transduced T cells in combination with trastuzumab (n=5).* **B-C.** *Mean levels of LDH (***B***) and Granzyme B (* **C***) in the serum of NCI-N87 tumor bearing mice following treatments (n=6). D.*

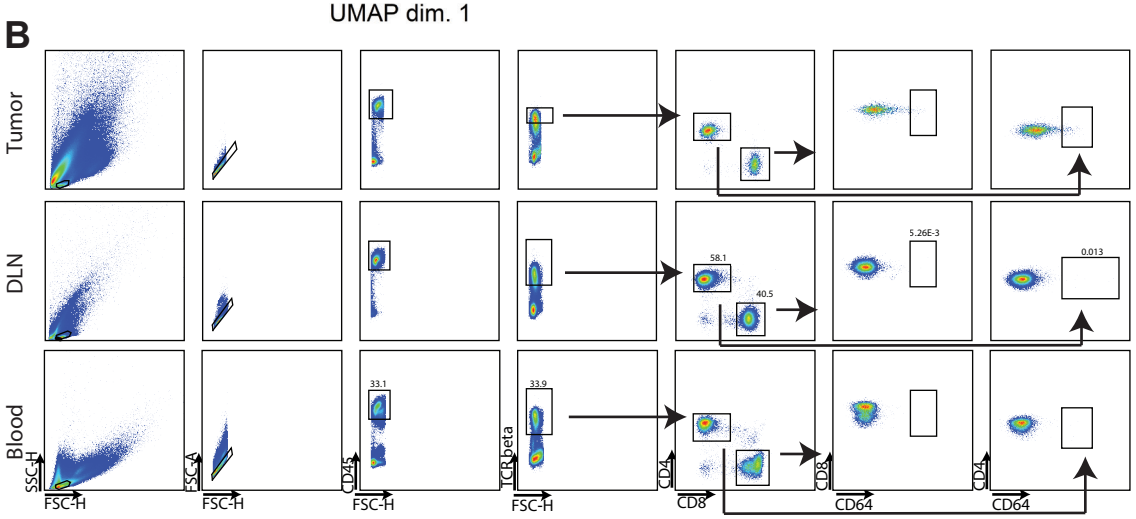
*Mean levels of Granzyme B on day 50 in the tumor sites of NCI-N87 tumor bearing mice (n=6). Statistical significance was calculated using ANOVA with Tukey's correction for multiple comparisons (** denotes p<0.001, **** denotes p<0.00001). Error bars represent standard error.*

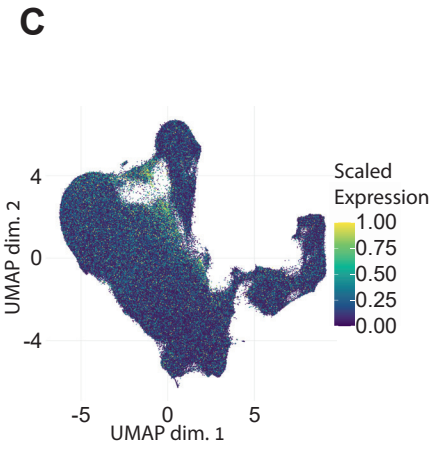
Supplemental Figure 6: AG2G transduced T cells exhibit weak adhesion with basement membrane which enables infiltration to the tumor core. **A.** *Representative SIM microscopy of AG2G, CAR-Her2 and Sham transduced T cells on glass.* **B.** *Correlation of mean expression of ITGAM and FCGR3A across T cell clusters. R2 and P as well as the trend line were calculated using linear regression.* **C.** *Graphic representation of the coating protocol described in the Materials and Methods section.* **D.** *Matrigel immunofluorescence staining with anti-Nidogen (Ab1) and Alexa Fluor 488-conjugated (Ab2). Representative single-photon fluorescence scans of Silica beads prior to Matrigel coating (bead location is marked with a dashed circle), Partial and uniform coating of the beads, accompanied by a graphic representation of the photon count profile with respect to the bead center.* **E.** *Mean fluorescence intensity of the edge of the bead.* **F**. *Representative bright field image of AG2G transduced T-cell and trapped bead prior to interaction.* **g.** *Representative confocal microscopy of AG2G transduced T-cell during contact with a Matrigel-coated bead.* **H-I.** *CDF of the unbinding force for AG2G (H) and Sham (I) transduced T cells in the presence (black) and absence (colored) of 15 mM EDTA. Data in every CFD contains 5 cells with up to 10 interactions per cell (AG2G: n = 50; Sham: n = 43 for both conditions).* **J.** *Probability of interaction profile of transduced T cell with Matrigel coated beads. Five independent experiments were performed for each cell type, with color-coded symbols of the same*

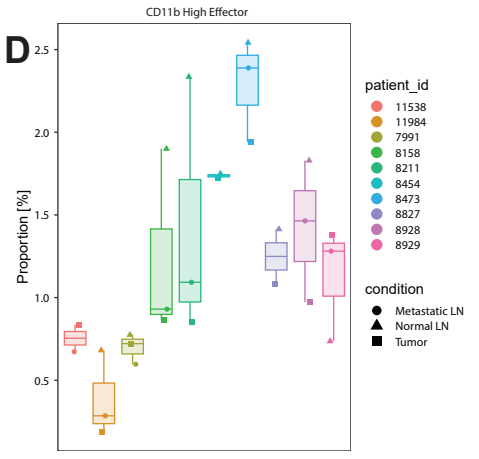
*color showing the mean probability for the experiment (n=4 per experiment). Experiments were repeated at least three independent times. Statistical significance was calculated using ANOVA with Tukey's correction for multiple comparisons (** denotes p<0.001, **** denotes p<0.00001). Error bars represent standard error.*

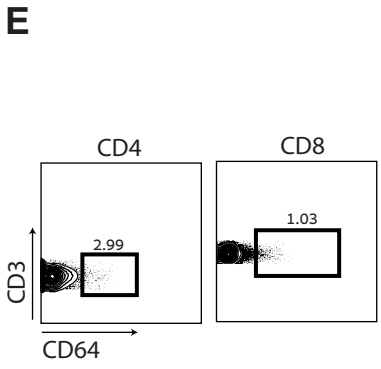
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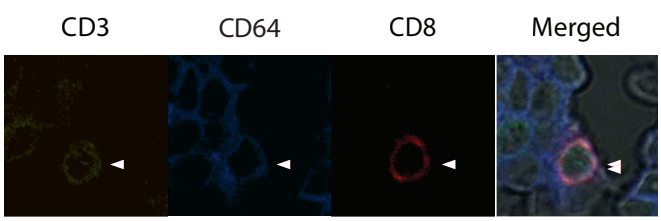


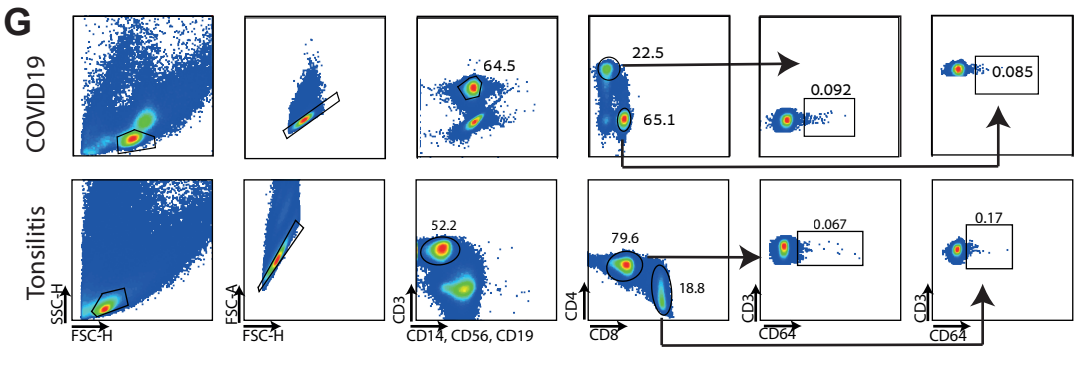




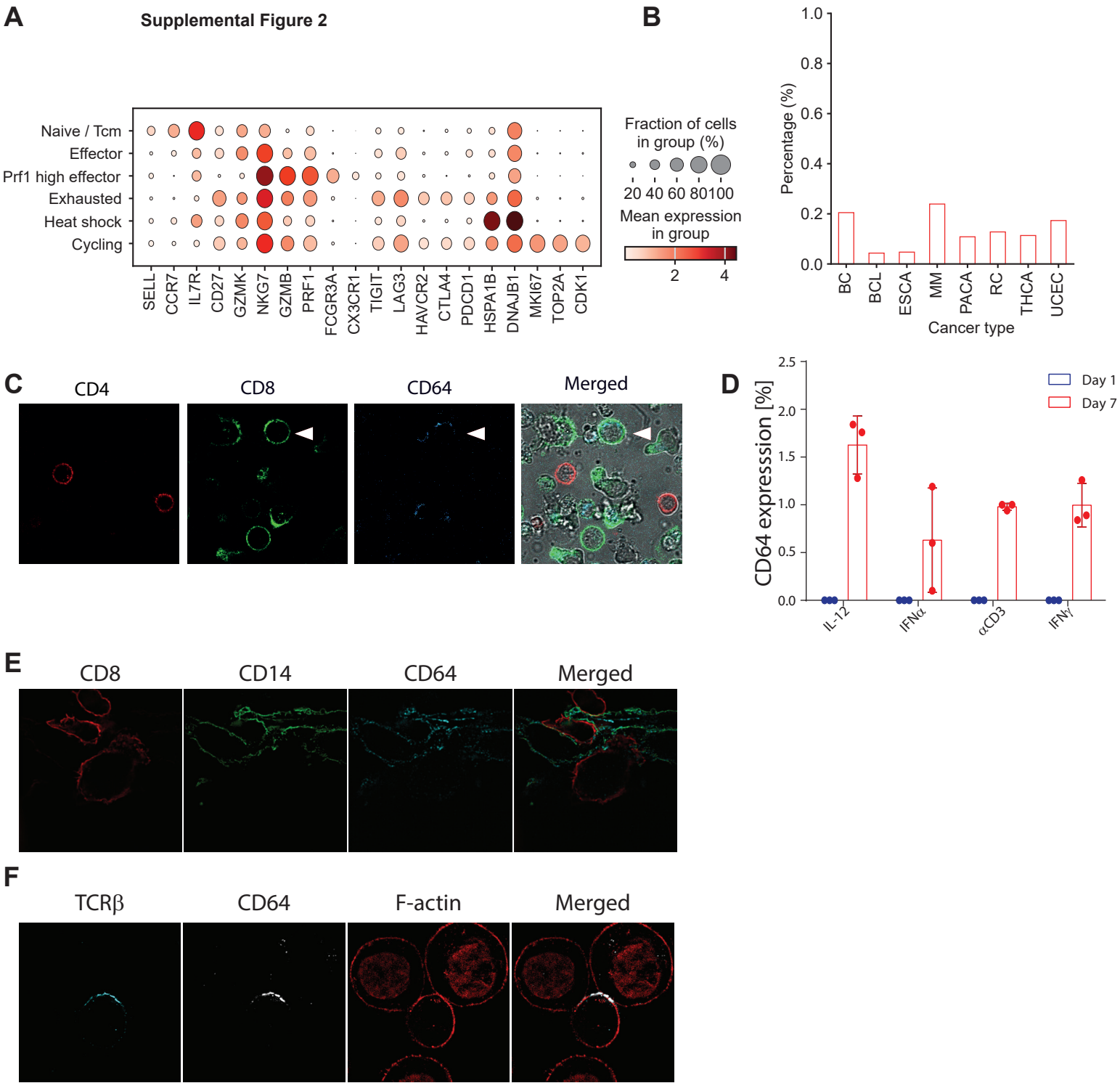




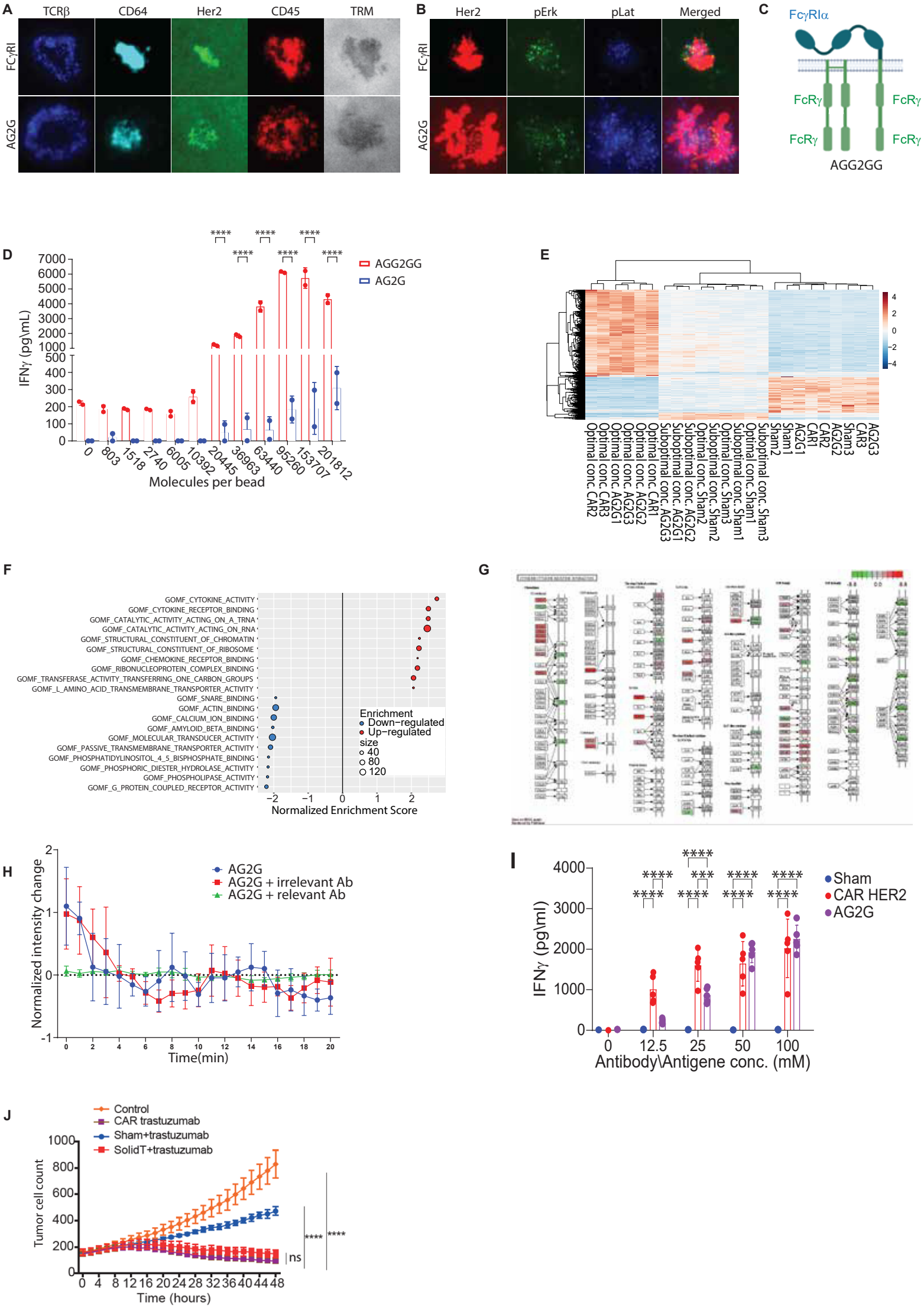




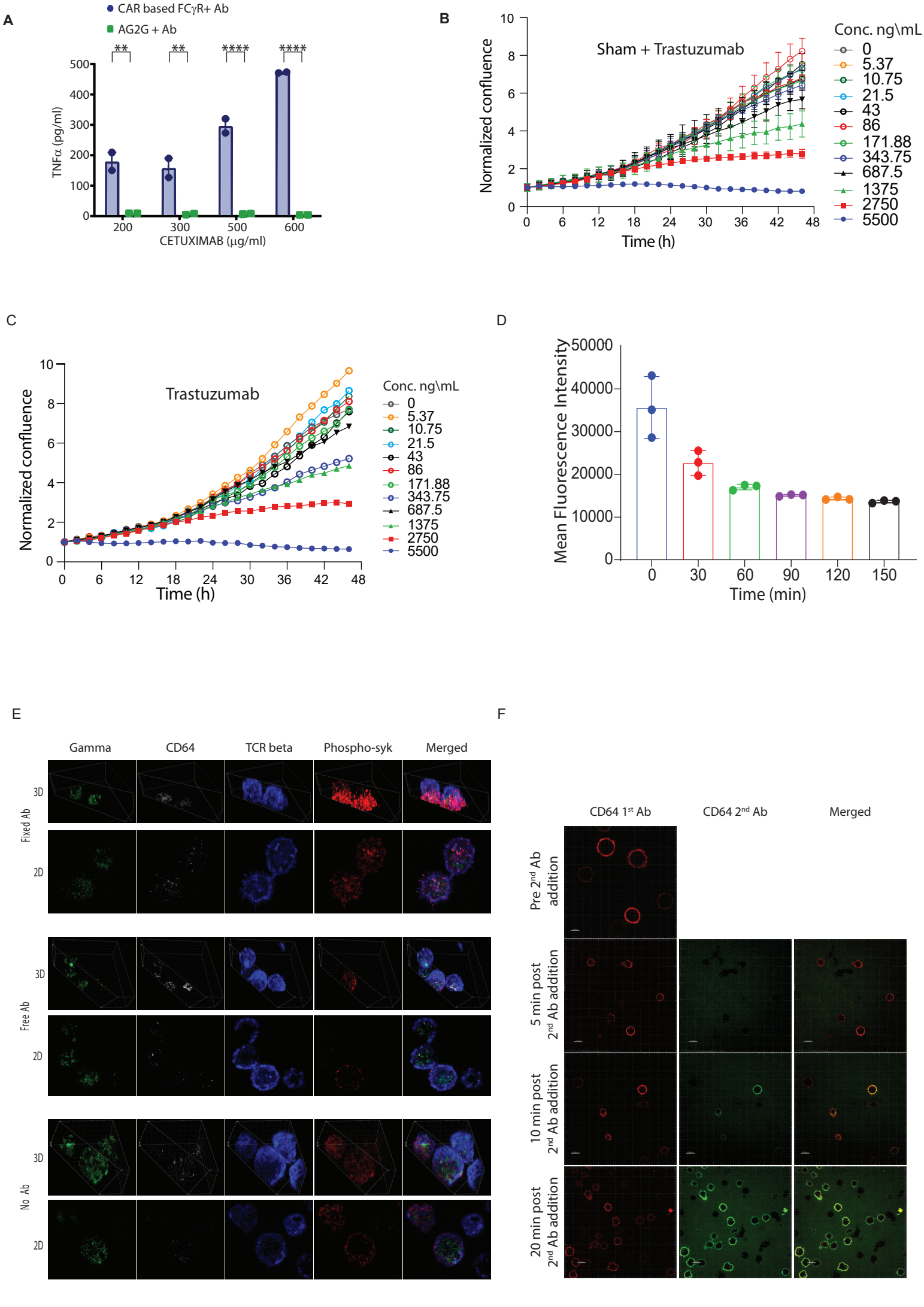
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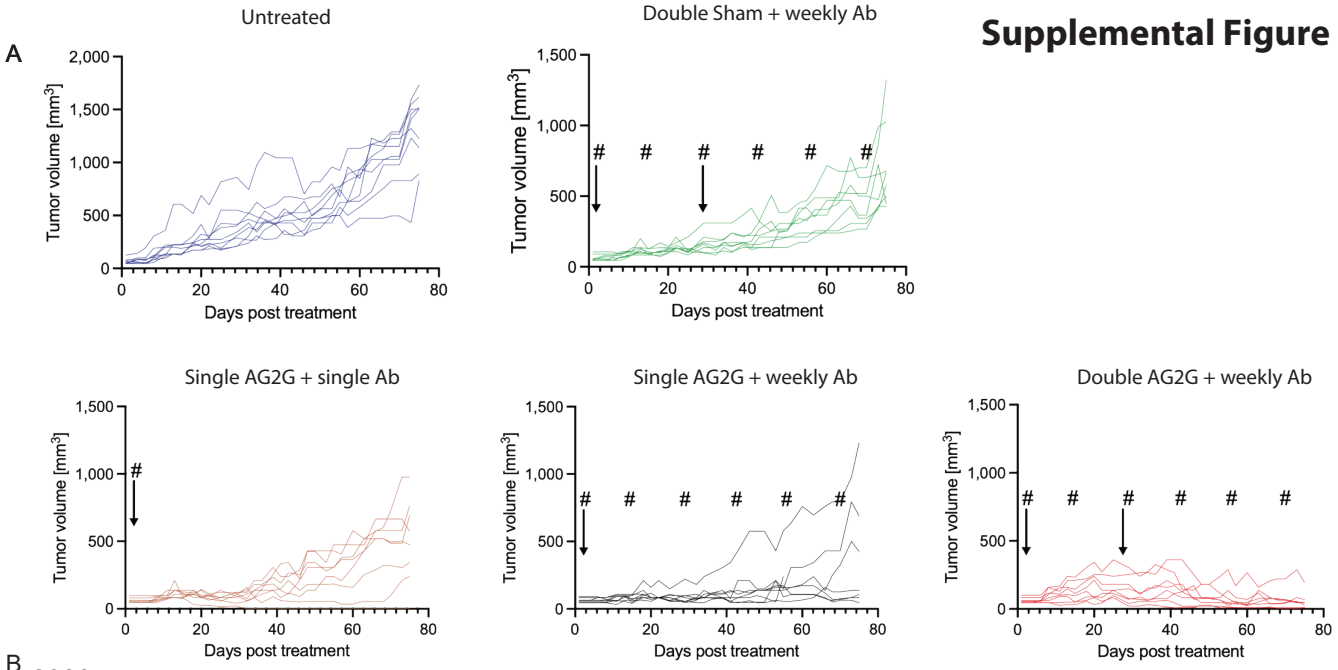
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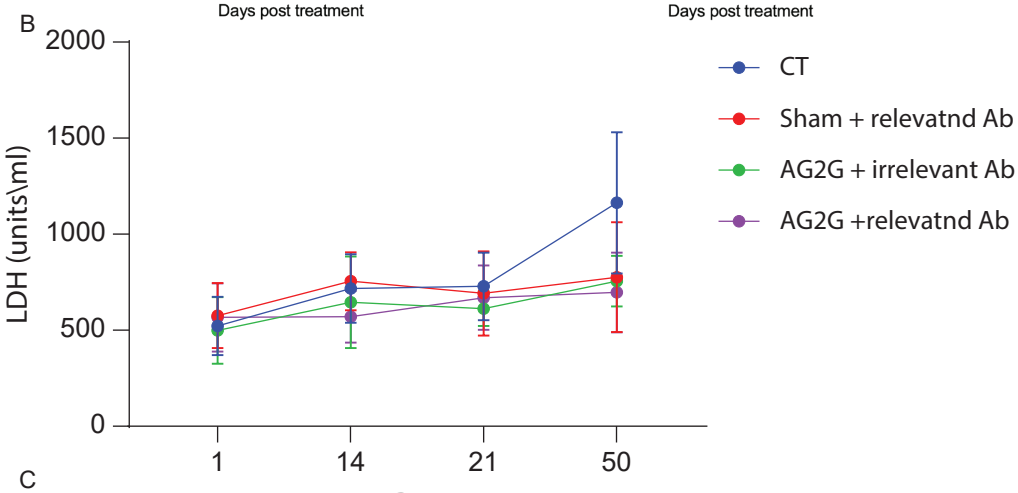


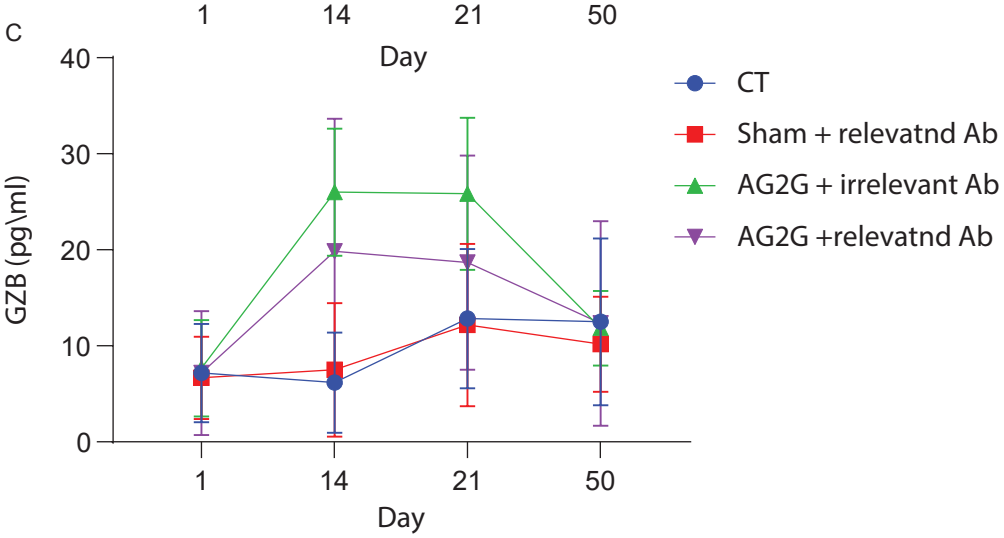
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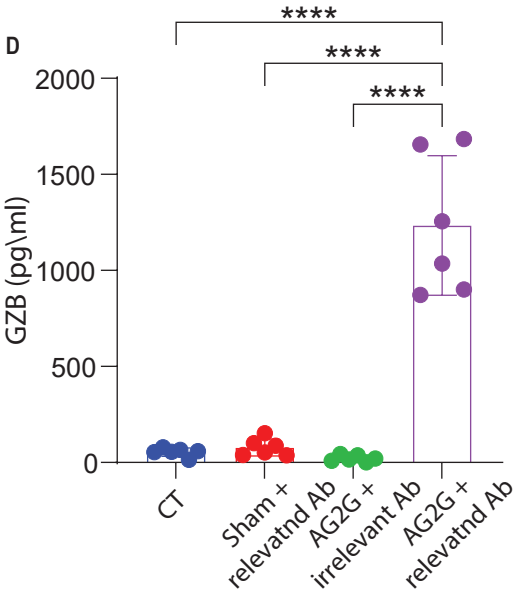


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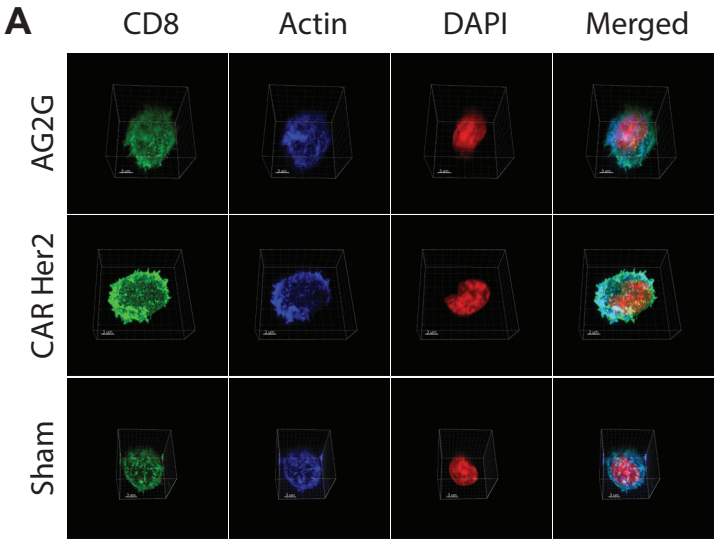


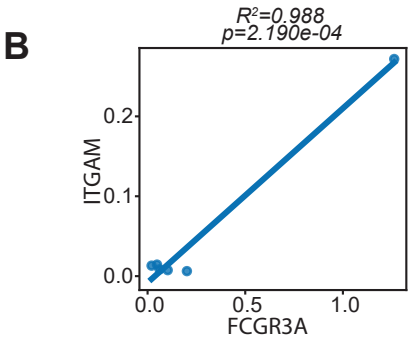


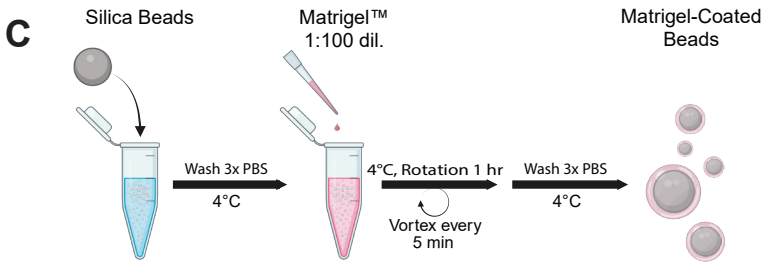


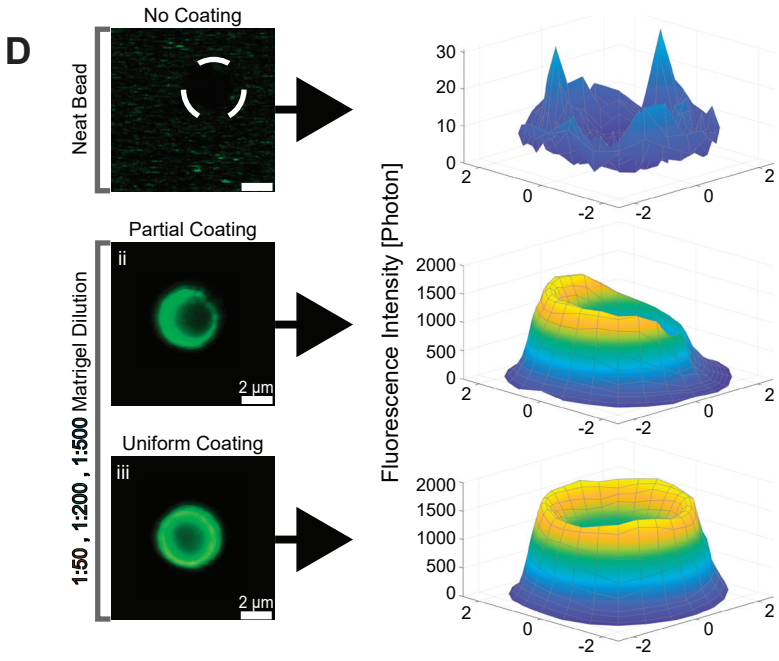


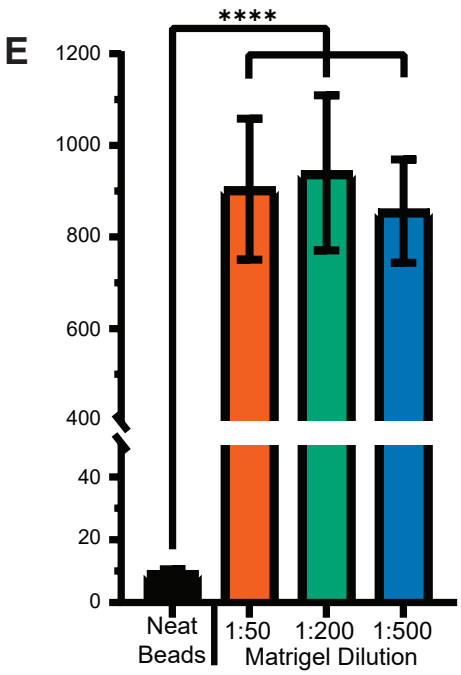
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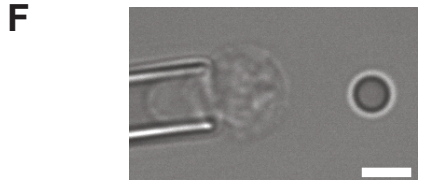


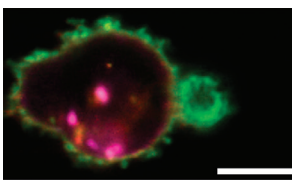


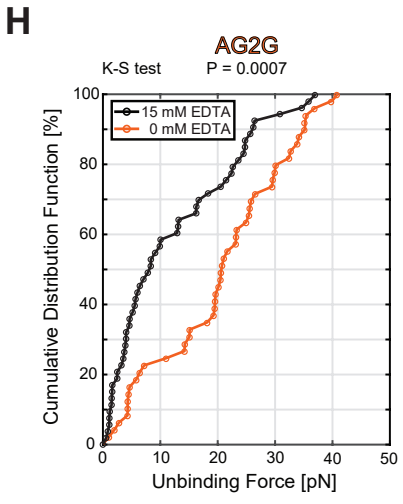


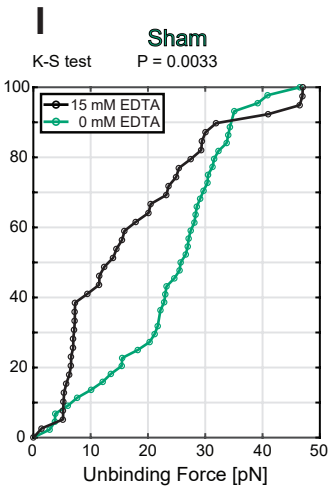


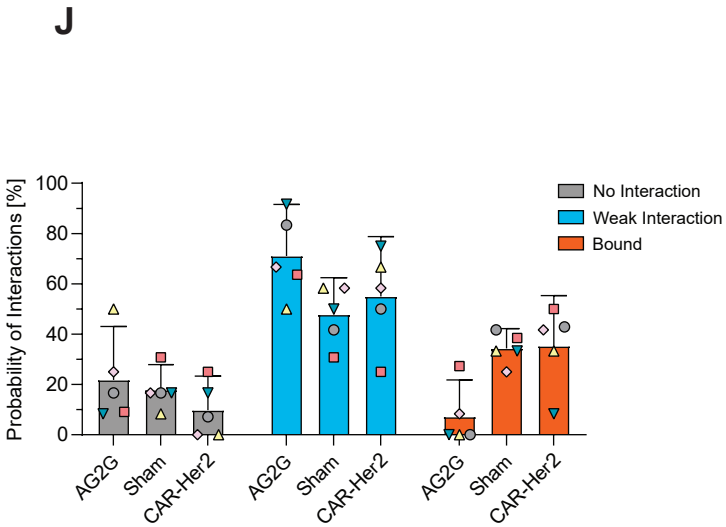












Movie Legend

Supplementary movie 1: Representative live imaging by Incucyte of Her2 expressin HT29 tumor cells with AG2G transduced T cells in combination with trastuzumab. Human T cells were co-cultured with target cells (HT29-HER2) at 4:1 ratio. Tumor cell killing is visible.

Supplementary movie 2: Representative live imaging by Incucyte of Her2 expressing HT29 tumor cells with AG2G transduced T cells. Human T cells were co-cultured with target cells (HT29-HER2) at 4:1 ratio. No tumor cell killing is visible.

Supplementary movie 3: Representative live imaging by Incucyte of Her2 expressin HT29 tumor cells with CAR-Her2 transduced T cells. Human T cells were co-cultured with target cells (HT29-HER2) at 4:1 ratio. Tumor cell killing is visible.

Supplementary movie 4: Representative live imaging by Incucyte of primary adult kidney epithelial cells with AG2G transduced T cells in combination with trastuzumab. Human T cells were co-cultured with target cells (primary adult kidney epithelial cells) at 4:1 ratio. No epithelial cell killing is visible.

Supplementary movie 5: Representative live imaging by Incucyte of primary adult kidney epithelial cells with AG2G transduced T. Human T cells were co-cultured with target cells (primary adult kidney epithelial cells) at 4:1 ratio. No epithelial cell killing is visible.

Supplementary movie 6: Representative live imaging by Incucyte of primary adult kidney epithelial cells with CAR-Her2 transduced T cells. Human T cells were co-cultured with target cells (primary adult kidney epithelial cells) at 4:1 ratio. Epithelial cell killing is visible.

Supplementary movie 7: Representative live imaging by Incucyte of Her2 expressin HT29 tumor cells with CAR based FCγR transduced T cells in combination with trastuzumab. Human T cells were co-cultured with target cells (HT29-HER2) at 4:1 ratio. Tumor cell killing is visible.

Supplementary movie 8: Representative live imaging by Incucyte of primary adult kidney epithelial cells with CAR based FCγR transduced T cells in combination with trastuzumab. Human T cells were co-cultured with target cells (primary adult kidney epithelial cells) at 4:1 ratio. Epithelial cell killing is visible

Supplementary movie 9: Representative SIM microscopy of AG2G transduced human T cells with stained lysosomes incubated with fixed antibody 1 hour following addition of FITC-IgG1.

Supplementary movie 10: Representative SIM microscopy of AG2G transduced human T cells with stained lysosomes incubated with fixed antibody 2 hour following addition of FITC-IgG1.

Supplementary movie 11: Immunofluorescence of AG2G transduced T cells following wash of FITC-IgG1 5 minutes post PE-IgG1 addition. Cells were incubated for 30 minutes with FITC-IgG1 antibodies then washed with PBS and resuspended in medium with PE-IgG1. The movie was taken f5 minutes post PE-IgG1 addition.

Supplementary movie 12: Immunofluorescence of transduced T cells following wash of FITC-IgG1 20 minutes post PE-IgG1 addition. Cells were incubated for 30 minutes with FITC-IgG1 antibodies then washed with PBS and resuspended in medium with PE-IgG1. The movie was taken f5 minutes post PE-IgG1 addition.

Supplementary movie 13: Bright field of AG2G transduced T cells migration on Matrigel towards 100ng\ml CCL5. 15μ Slide $I^{0.4}$ luer (80176, Ibidi) were covered with 1:2 Matrigel

Matrix (356231, corning) in PBS 5×10^4 transduced T cells were then added to one side of the slide and 100ng\ml CCL5 to the other side. Videos were taken after 1 hour incubation with 5 minutes interval between frames.

Supplementary movie 14: Bright field of Sham transduced T cells migration on Matrigel towards 100ng\ml CCL5. 15µ Slide $I^{0.4}$ luer (80176, Ibidi) were covered with 1:2 Matrigel Matrix (356231, corning) in PBS 5×10^4 transduced T cells were then added to one side of the slide and 100ng\ml CCL5 to the other side. Videos were taken after 1 hour incubation with 5 minutes interval between frames.

Supplementary movie 15: Fluorescence imaging of AG2G transduced T cell aspirated with a micropipette exhibiting no interaction with Matrigel coated bead (Green – CD8, Red – CD64, Magenta – F-actin). Scale bars is 5 µm. T-cell was aspirated with a micropipette, then an optically trapped Matrigel-coated bead was brought into contact with the cell for 30 seconds and pulled away at a constant speed (0.8 µm/s).

Supplementary movie 16: Fluorescence imaging of AG2G transduced T cell aspirated with a micropipette exhibiting weak interaction with Matrigel coated bead (Green – CD8, Red – CD64, Magenta – F-actin). Scale bars is 5 µm. T-cell was aspirated with a micropipette, then an optically trapped Matrigel-coated bead was brought into contact with the cell for 30 seconds and pulled away at a constant speed (0.8 µm/s).

Supplementary movie 17: Fluorescence imaging of CAR Her2 transduced T cell aspirated with a micropipette exhibiting strong interaction with Matrigel coated bead (Green – CD8, Red – CAR Her2, Magenta – F-actin). Scale bars is 5 µm. T-cell was aspirated with a micropipette, then an optically trapped Matrigel-coated bead was brought into contact with the cell for 30 seconds and pulled away at a constant speed (0.8 µm/s).

Supplementary movie 18: Bright field imaging of Sham transduced T cell aspirated with a micropipette exhibiting strong interaction with Matrigel coated. Scale bars is 5 µm. T-cell was aspirated with a micropipette, then an optically trapped Matrigel-coated bead was brought into contact with the cell for 30 seconds and pulled away at a constant speed (0.8 µm/s).