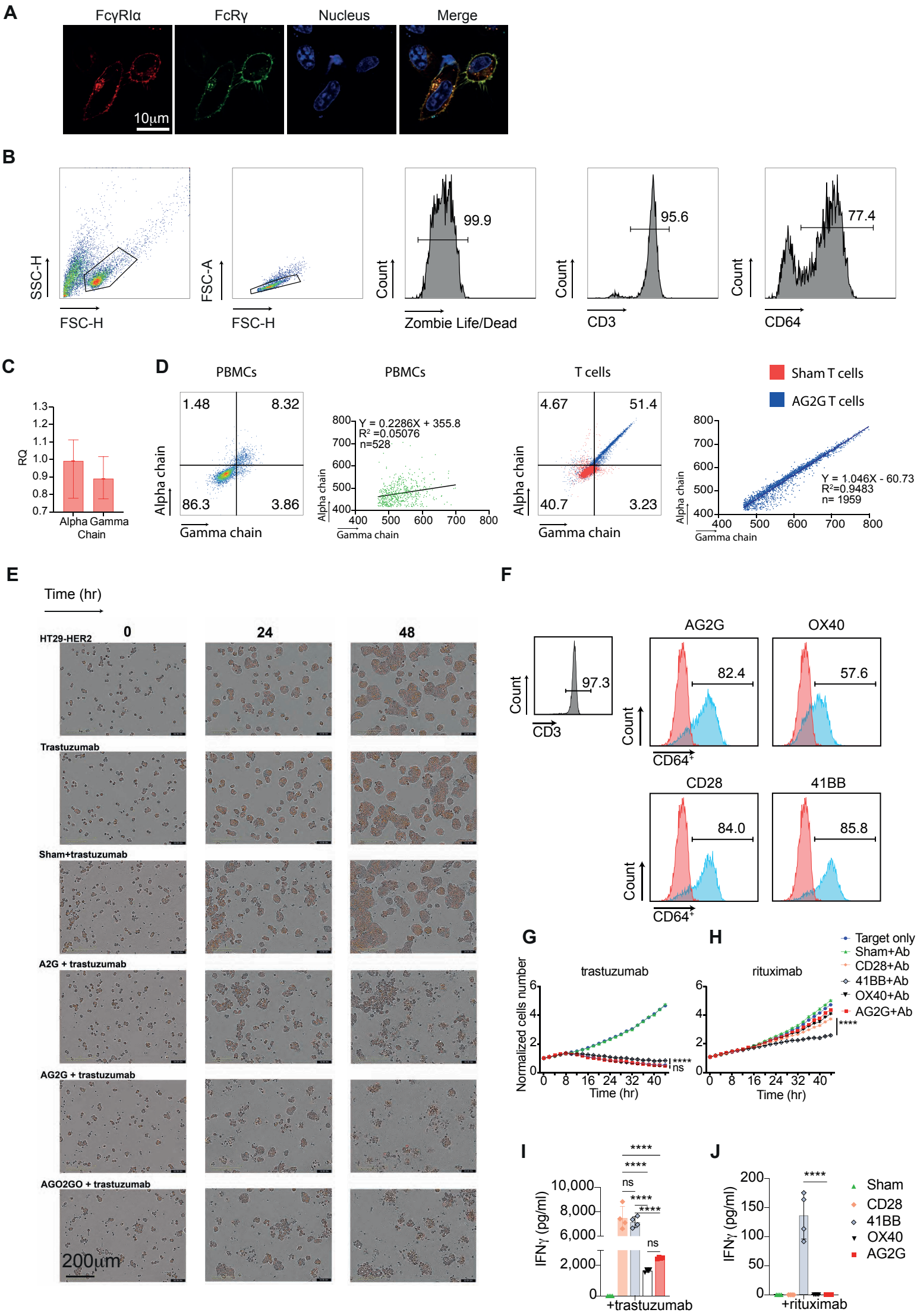
Table 1

Cell line		A431	A549	HCC1954	HEK293-FT	HEK293-GP2	HeLa	HT-29	JIMT-1	NCI-H1299	NCI-N87	SK-BR-3
Marker	AMEL	X	X,Y	X	X	X	X	X	X	X	X,Y	X
	D3S1358	14	16	15,16	15,17	15,17	15,18	15,17	18	17	14	17
	D1S1656	16,17	17,18.3	15,16	15,17.3	15,17.3	12,15	15,16	11	12,15	16	11,17.3
	D2S441	11.3,15	10,13	10,11	11,15	11,15	10,11	11	11.3,14	11,13	11,12	13,14
	D10S1248	13,15	13,16	14,15	14	14	13,15	14,16	16	13,17	13	14,15
	D13S317	9,13	11	8,9	12,14	12,14	12,13.3	11,12	8,13	12	8,11	11,12
	Penta E	12,13	7,11	12,16	7,15	7,15	7,17	14,16	7,19	11	5	10,11
	D16S539	12,14	11,12	9,11	9,13	9,13	9,10	11,12	11	12,13	9,13	9
	D18S51	13,17	14,17	14,18	17,18	17,18	16	13	10	16	17	10,13
	D2S1338	17	24	17,26	19	19	17	19,23	24	23,24	23,24	20,25
	CSF1PO	11,12	10,12	10	7,12	12	9,10	11,12	12,13	12	8,12	12
	Penta D	9,11	9	9,12	9	9,10	8,15	11,13	9,12	13	12	9,12
	TH01	9	8,9.3	7	7,9.3	7,9.3	7	6,9	7	6,9.3	9	8,9
	vWA	15,17	14	18,19	16,19	16,19	16,18	17,19	18	16,18	15,16	17
	D21S11	28,30	29	28,32.2	30.2	28,30.2	27,28	29,30	27,31.2	32.2	30	30,30.2
	D7S820	10	8,11	10,11	11	11	8,12	10	12	10	10,11	9,12
	D5S818	12,13	11	11	8,9	8,9	11,12	11,12	12,13,14	11	12,13	9,12
	TPOX	11	8,11	8,9	11	11	8,12	8,9	8	8	9,11	8,11
	DYS391	-	10	-	-	-	-	-	-	-	10	-
	D8S1179	13	13,14	12,15	12,14	12,14	12,13	10,16	10,13	10,13	14	11,12
	D12S391	18,23	18	22	19,21	19,21	20,25	18.3,21	17	21,22	16,21	18,20
	D19S433	15,15.2	13	13,15.2	15,18	18	13,14	14	12,13	14	14,14.2	14
	FGA	20	23	22,23	23	23	18,21	20,22	21,23	20	20,21	20
	D22S1045	15,16	15	11,15	16	16	16,17	16	11,16	15,16	14,15	15
Cellosaurus version compared		42	42	42	19	39	41	42	37	40	42	38
Cellosaurus no.		CVCL_0037	CVCL_0023	CVCL_1259	CVCL_6911	CVCL_0045 (Parental cell line)	CVCL_0030	CVCL_0320	CVCL_2077	CVCL_0060	CVCL_1603	CVCL_0033
Deviation		D2S1338 LOH	24 out of the 24 available loci	TH01 LOH	D13S317 - additional allele with lower intensity	D18S51 - additional allele. CSF1PO and D7S820 LOH	23 out of the 23 available loci	23 out of the 23 available loci	18 out of the 18 available loci	D12S391 - additional allele	D8S1179 LOH	D8S1179 - additional allel with lower intensith
Conclusion		Authentic	Authentic	Authentic	Authentic	Authentic	Authentic	Authentic	Authentic	Authentic	Authentic	Authentic

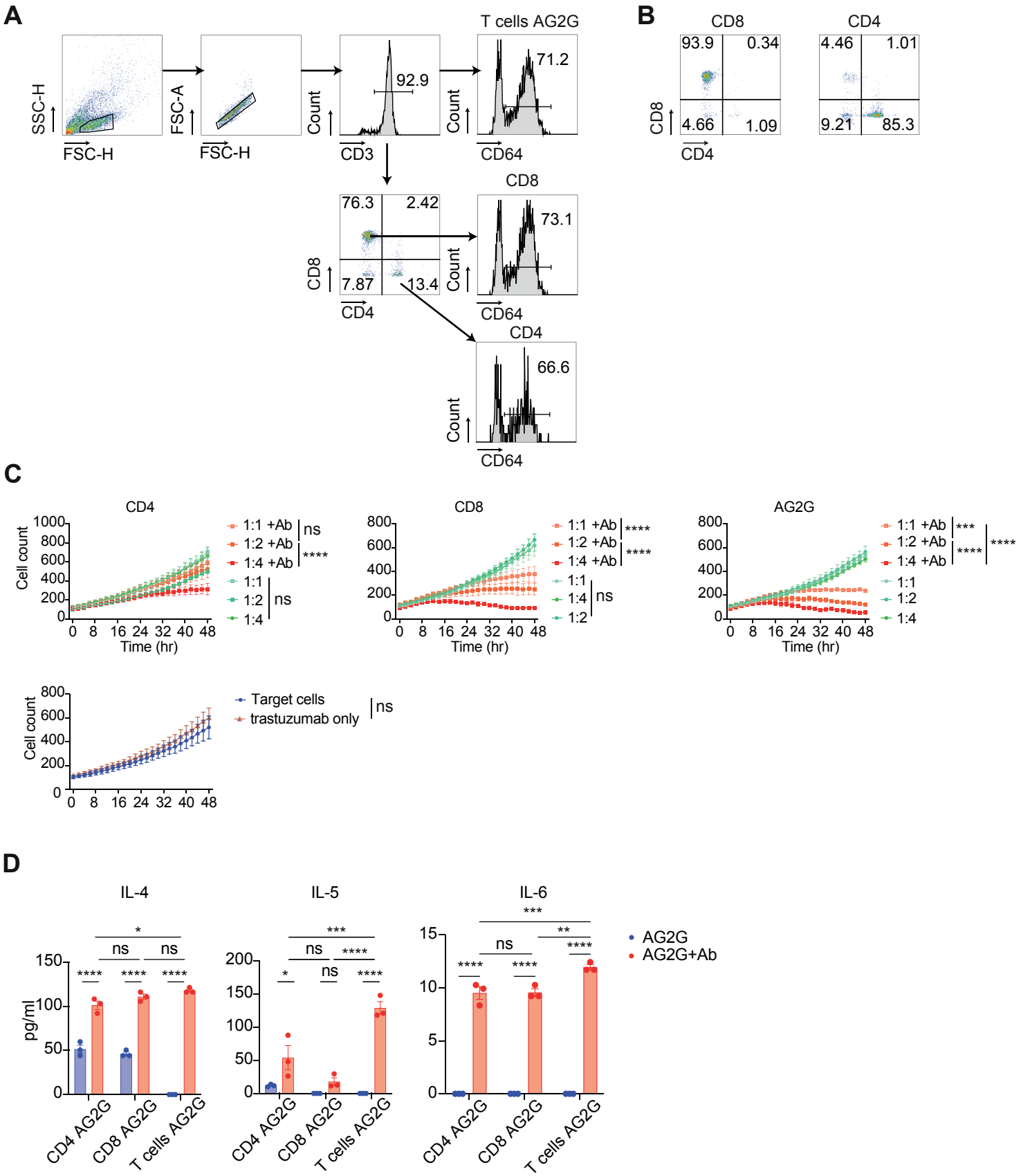
Table 2

Metal	Extracellular markers	Intracellular markers	Clone	Vendor
111Cd	CD45.1		A20	Biolegend
112Cd	CD45.2		104	Biolegend
114Cd	CD8		53-6.7	Biolegend
115In	CD3		145-2C11	Biolegend
116Cd	CD140b (PDGFR beta)		APB5	Biolegend
142Nd	Tim-3		RMT3-23	Biolegend
143Nd	CD86		GL-1	Biolegend
144Nd	F4/80		BM8	Biolegend
145Nd	CD4		RM4-5	Biolegend
147Sm	Ly6c		HK1.4	Biolegend
148Nd	CD11b		M1/70	Fluidigm
149Sm	CD19		6D5	Biolegend
150Nd	Ly6g		1A8	Biolegend
151Eu	CD206		C068C2	Biolegend
152Sm	TCRyd		UC7-13D5	Biolegend
153Eu		pStat-1 (Y701)	58D6	Fluidigm
154Sm	CD11c		N418	Biolegend
155Gd	IgM		RMM-1	Biolegend
156Gd	CD25		3C7	Biolegend
157Gd	CD40		HM40-3	Biolegend
158Gd	PD-1 CD279		RMP1-30	Biolegend
159Tb		RORg(t)	B2D	Fluidigm
160Gd		T-bet	4B10	Biolegend
161Dy	CX3CR1		SA011F11	Biolegend
162Dy	CD64		X54-5/7.1	Biolegend
163Dy	CD127		A7R34	Biolegend
164Dy	Sca-1		D7	Fluidigm
165Ho		FoxP3	FJK-16s	Fluidigm
166Er	SiglecF		238023	RnD systems
167Er		GATA3	TWAJ	Fluidigm
168Er	CD115		AFS98	Biolegend
169Tm	TCRb		H57-597	Fluidigm
170Er	CD62L		MEL-14	Biolegend
171Yb	CD44		IM7	Biolegend
172Yb	CD184		L276F12	Biolegend
173Yb	CD69		H1.2F3	Biolegend
174Yb	I-A/I-E		M5/114.15.2	Biolegend
175Lu	IgD		11-26c.2a	Biolegend
176Yb	LAG3		C9B7W	Biolegend



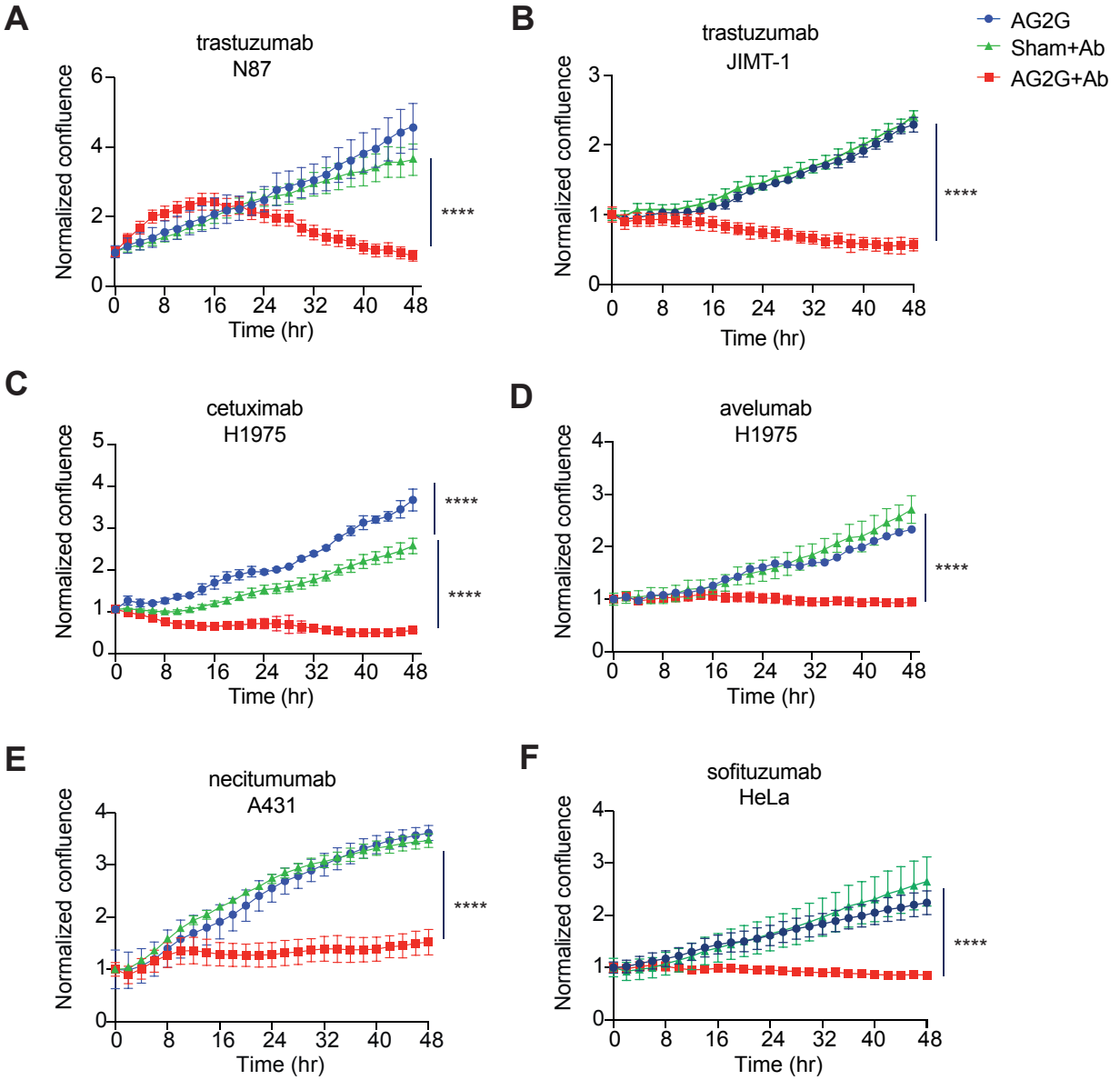
Supplemental Figure S1. The cytotoxic activity and cytokine secretion of T cells expressing altered version of FcγRI.

A. Confocal images of HeLa cells co-transfected with FcγRI alpha chain-mCherry and FcRγ chain-EGFP. Magnitude x400. **B.** Flow cytometry analysis of AG2G-transduced T cells. **C.** Real-Time PCR relative quantification of alpha and gamma chain parts of AG2G coding sequence from T cells transduced with AG2G. **D.** Flow cytometry analysis of PBMCs, Sham-T cells and AG2G-expressing T cells for the FcR alpha and gamma chains expression (left), and linear regression analysis of the double positive cells (right). Analysis was performed by simple linear regression (GraphPad prism 9.4.1) **E.** Representative images showing HT29 HER2 expressing cells, imaged and counted in a live imaging system after 48 h of incubation with different constructs expressing T cells with 1:1 E:T ratio. **F.** Flow cytometry analysis of T cells transduced with AG2G, AG2G with OX40 addition, CD28 addition and 4-1BB addition fusion receptors. **G.** Mean numbers of HT29 cells expressing HER2 following incubation with AG2G, AG2G with OX40 addition, CD28 addition and 4-1BB addition fusion receptors -expressing T cells in combination with trastuzumab over 44 h (n=4, 4:1 E:T ratio, 12 ug/ml trastuzumab). **H.** Mean numbers of HT29 cells expressing HER2 following incubation with AG2G, AG2G with OX40 addition, CD28 addition and 4-1BB addition fusion receptors -expressing T cells in combination with the irrelevant antibody rituximab over 44 h (n=4, 4:1 E:T ratio, 12 μg/ml trastuzumab). **I.** IFNγ levels from supernatants of cytotoxic assay (described in **G**). **J.** IFNγ levels from supernatants of cytotoxic assay (described in **H**). Statistical significance was calculated using two-way ANOVA with Tukey's correction (**G** and **H**) or ordinary one way ANOVA with Tukey's correction (**I** and **J**). **** denotes p<0.0001. Error bars represent



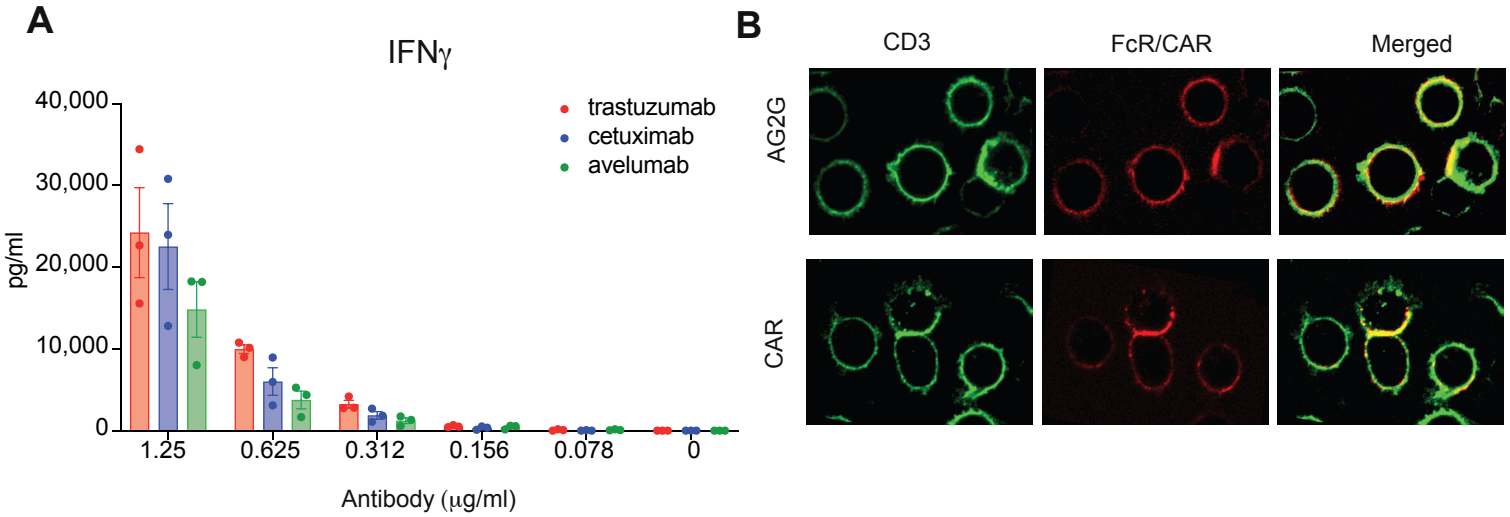
Supplemental Figure S2. The activation, cytotoxicity, and characterization of AG2G-expressing T cells.

A. Flow cytometry analysis of AG2G-expressing cells, 6 days following retrovirus transduction. **B.** Flow cytometry analysis of AG2G-expressing cells, following magnetic separation of CD8 and CD4 T cell populations. **C.** Mean numbers of HT29 cells expressing HER2 following incubation with different E:T ratios of AG2G-expressing T cells that were isolated for their CD4 (left) or CD8 (middle) population or with no isolation (right). **D.** Cytokine levels measured from supernatants of 48 h culture of 4:1 E:T ratios of isolated CD4, isolated CD8, or AG2G-expressing T cells with HT29 cells expressing HER2 and 30 ug/ml trastuzumab.

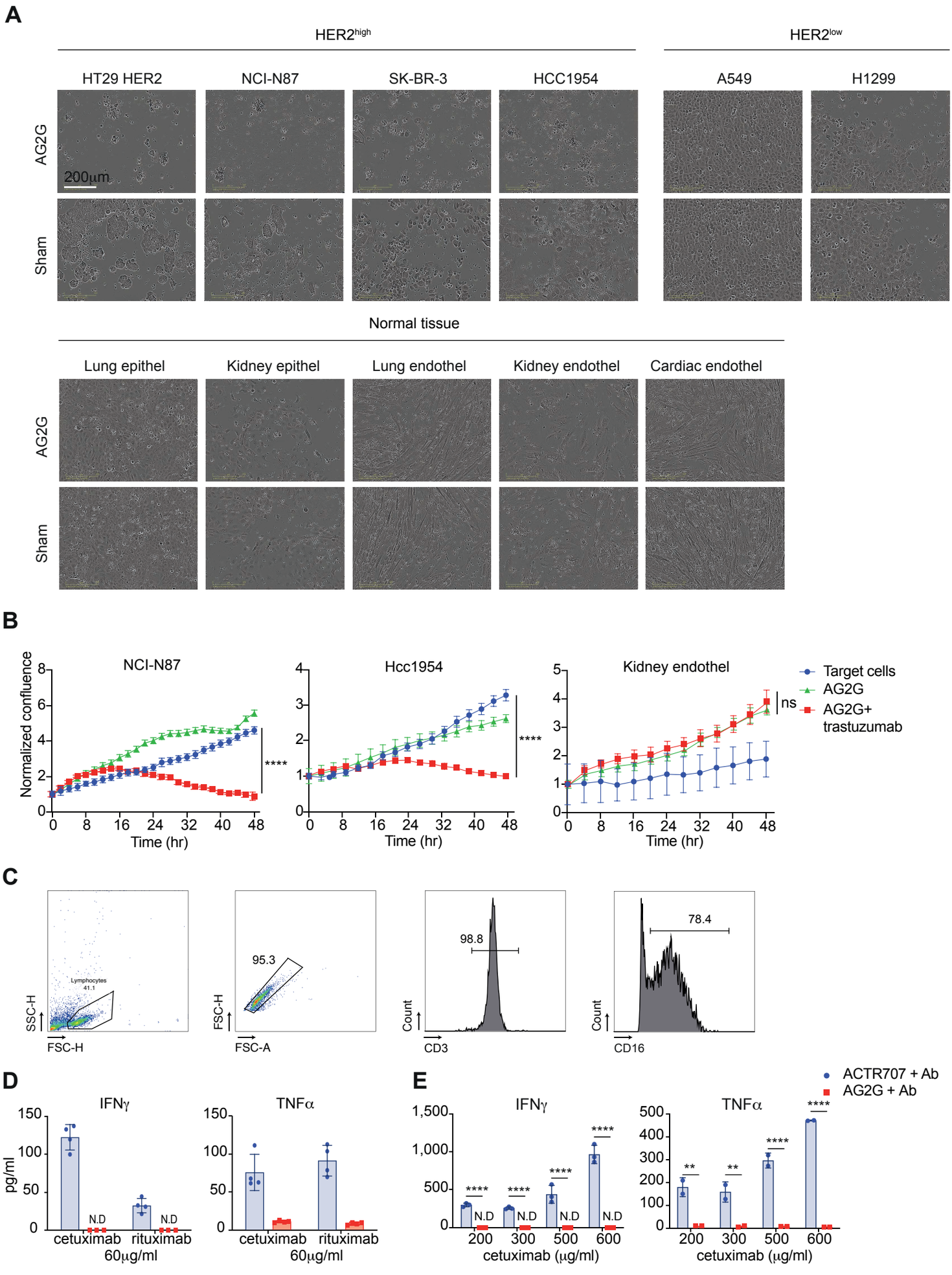


Supplemental Figure S3. AG2G-expressing T cells exhibit cytotoxic activity against different targets once combined with a variety of tumor targeting antibodies.

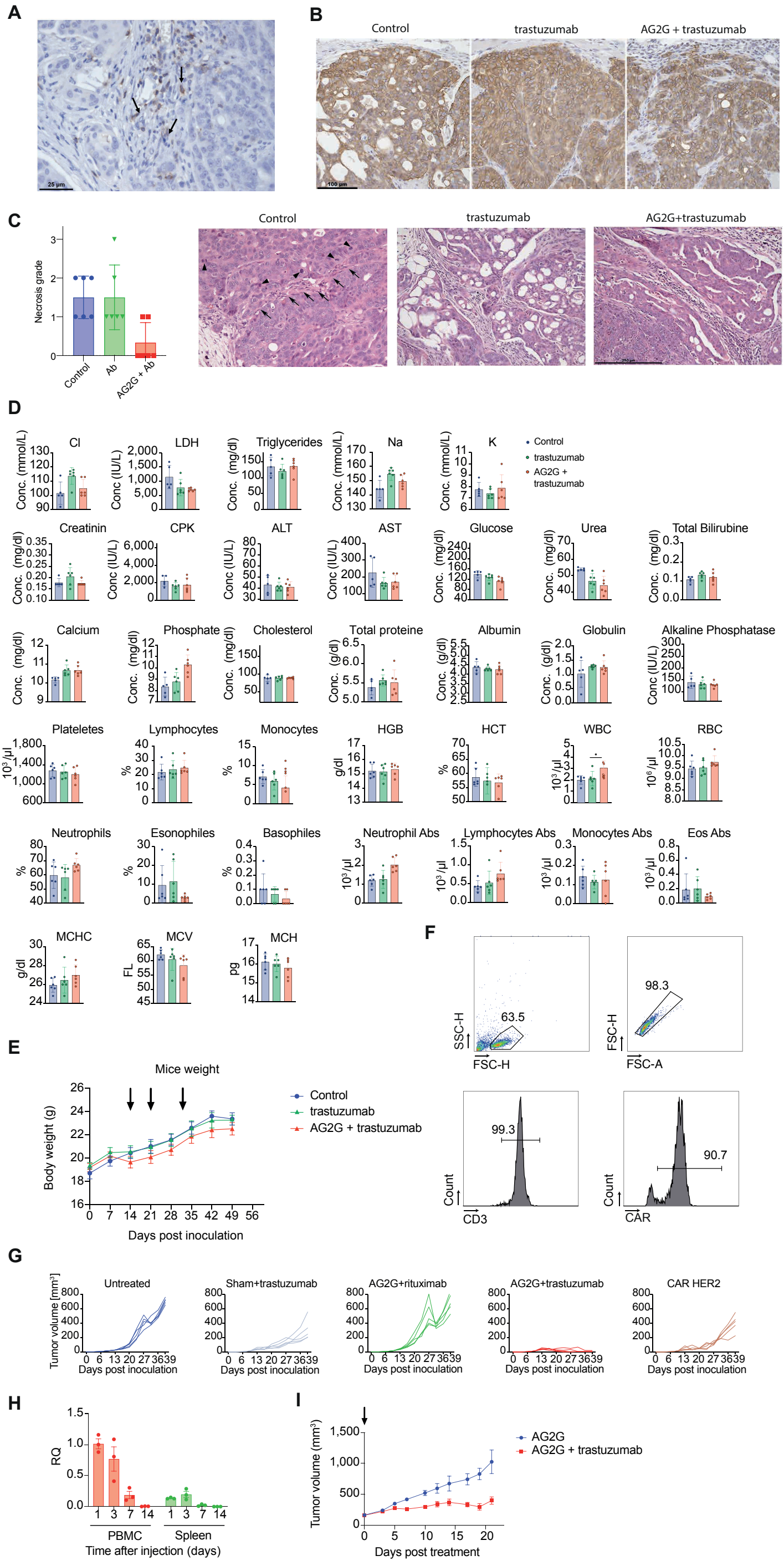
Confluence analysis of tumor cells over 48 h of co-culture in IncuCyte imager with non-transduced T cells (Sham) with targeting antibody or AG2G-expressing cells with or without targeting antibody. **A.** NCI-N87 cells targeted with anti-HER2 trastuzumab (4:1 E:T ratio, n= 4, 30 µg/ml antibody). **B.** JIMT-1 cells targeted with anti-HER2 trastuzumab (4:1 E:T ratio, n= 4, 30 µg/ml antibody). **C.** NCI-H1975 cells targeted with anti-EGFR cetuximab (4:1 E:T ratio, n=3, 60 µg/ml antibody). **D.** NCI-H1975 cells targeted with anti-PD-L1 avelumab (4:1 E:T ratio, n=3, 60 µg/ml antibody). **E.** A431 cells targeted with anti-EGFR necitumumab (4:1 E:T ratio, n=3, 30 µg/ml antibody). **F.** HeLa cells targeted with anti-MUC16 sofituzumab (4:1 E:T ratio, n=4, 30 µg/ml antibody). Statistical significance was calculated using ANOVA with Tukey's correction for multiple comparisons. *** denotes $p<0.001$, **** denotes $p<0.0001$. Error bars represent standard error.



Supplemental Figure S4. AG2G-expressing T cells differentiate between cells expressing high and low antigen levels, are more specific, and less exhausted, compared to classic CAR-T cells.
A. IFNγ levels from 24 h supernatants of 10^5 AG2G-expressing T cells incubated on ELISA plate pre-coated overnight with the indicated concentration of either trastuzumab, cetuximab or avelumab. **B.** Confocal images of T cells transduced with AG2G stained with anti-CD64 and anti-CD3 or T cells transduced with CAR-HER2, stained with PE-labeled soluble HER2 and anti-CD3. Magnitude x400.



Supplemental Figure S5. Human AG2G-expressing T cells exert tumor specific cytotoxicity while sparing normal cells.
A. Representative images of 48 h co-culture sham-T cells or AG2G-expressing T cells with trastuzumab and tumor cells or tissue cells described in Fig 4.**A-B. B.** Percent confluence was normalized to initial percent confluence in each well and plotted over 48 hours in 2 hour intervals. Target cells (NCI-N87, Hcc1954, Kidney endothel) were co cultured with effector cells in E:T 4:1 ratio. Groups with trastuzumab were supplemented with 6µg/ml transtuzumab at time 0 (n=4). Statistical significance was calculated using two-way ANOVA with Sidak correction for multiple comparisons. For NCI-N87, AG2G+Ab and AG2G data sets are also shown in Fig. S3A. **C.** Flow cytometry analysis of T cells transduced with ACTR707 (FcγRIII-CD28-CD3ζ). **D.** IFNγ and TNFα levels from supernatants of T cells transduced with AG2G or ACTR707 (FcγRIII-CD28-CD3ζ) transduced cells, after 48h co-culture with HT-29 HER2-expressing cells, with 60 µg/ml of irrelevant antibodies (4:1 E:T ratio, n=4). **E.** IFNγ and TNFα levels from supernatants of T cells transduced with AG2G or ACTR707 (FcγRIII-CD28-CD3ζ) transduced cells, after 48h co-culture with HT-29 HER2-expressing cells, with increasing concentrations of irrelevant antibody, cetuximab.

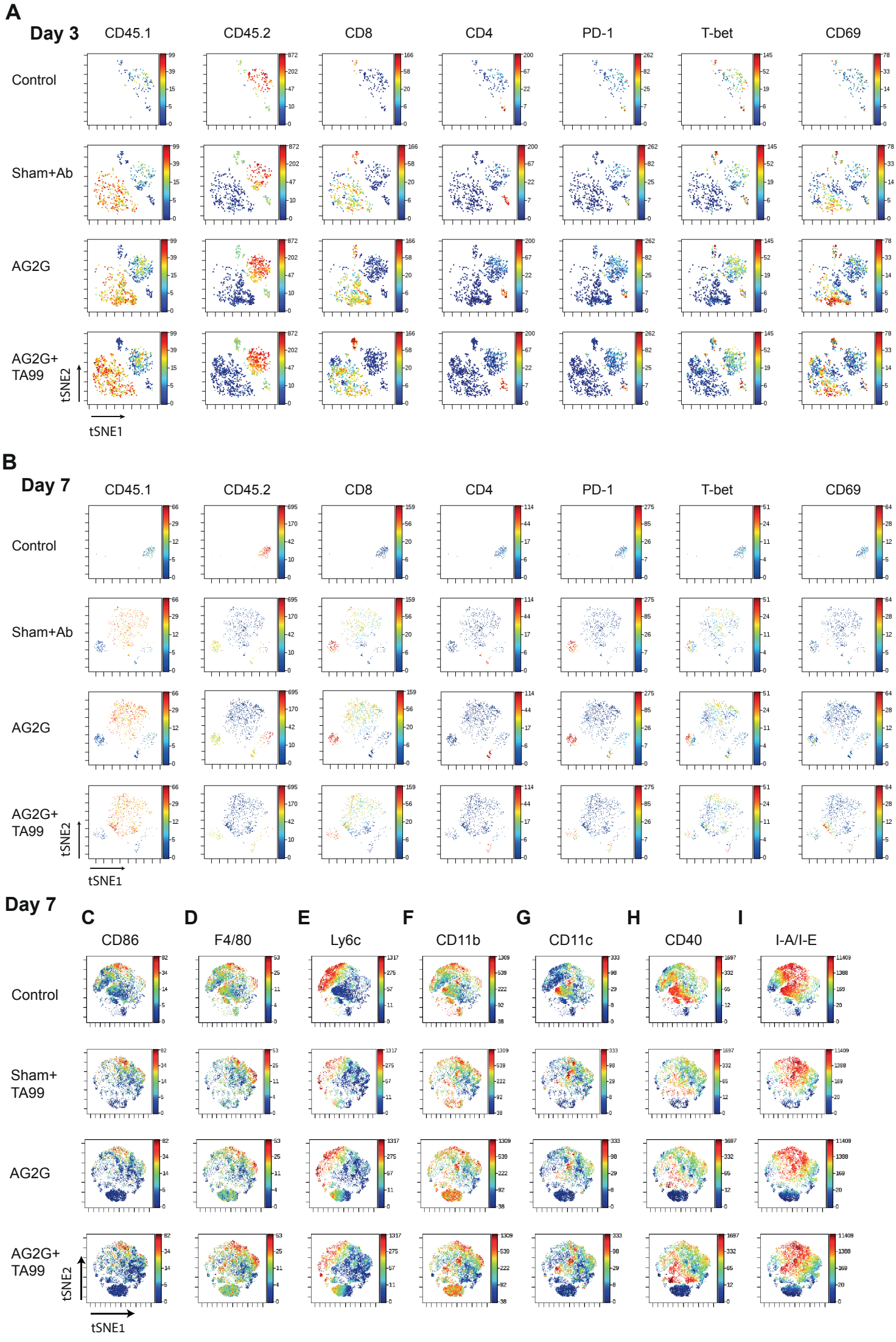


Supplemental Figure S6. Systemic administration of AG2G-expressing T cells in combination with trastuzumab eradicates HER2-expressing tumor cells in vivo.

A. Representative image of one tumor from AG2G+trastuzumab group stained with anti-human CD3 antibody (examples of CD3 stained cells are marked with arrows). Scale bar is 25µm. **B.** Representative images of one tumor from each treatment group stained with anti-human HER2 antibody. **C.** Necrosis was evaluated in HE stained sections according to the necrotic area in relation to the total tumor area and was graded as follows: Grade 0 – 0-10%; Grade 1 – 10-25%; Grade 2 – 25-50%; Grade 3 – 50-75%; Grade 4 - >75%; Results of the necrosis evaluation are summarized in a bar graph, Error bars stand for standard error. Medium power (objective x20) microphotograph of HE stained section of tumors representing tumor morphology. Arrowheads point to mitotic cells; Arrows delineate connective tissue septum. **D.** Blood cell count and chemistry of NSG mice at endpoint (42 days post treatment). **E.** Body weight graph of NSG mice experiment. Administration of treatments are marked with arrows. **F.** Flow cytometry analysis of CAR-HER2 transduced T cells with anti-CD3-FITC and protein-L-PE. **G.** Single mice tumor size graphs of NSG mice experiment described in Fig. 5**F. H.** Relative quantification by qPCR of human SolidT DNA sequence in spleen and PBMCs of naïve NSG mice at four time point (1,3,7 and 14 days) after introduction of $5x10^6$ AG2G T cells i.v. (n=3). Quantity was compared to mouse beta actin DNA by delta-delta Ct method. **I.** Tumor size graph of NSG mice described in Fig. 5**G.**

Table 3

Group	Sample	Brain	Lung	Heart	Kidney	Spleen	Liver
Control	9094	-	-	-	-	-	-
	9094	-	-	-	-	-	-
	9109	-	-	-	-	-	-
	9111	-	-	-	-	-	-
	9115	-	-	-	-	-	-
	9154	-	-	-	-	large calcified focus	-
Trastuzumab	9097	-	-	-	-	-	-
	9099	-	-	-	-	-	-
	9100	-	-	-	-	-	-
	9101	-	-	-	-	-	-
	9110	-	-	-	-	-	-
	9116	-	-	-	-	-	-
AG2G+Trastuzumab	9096	-	-	-	-	-	-
	9098	-	-	-	-	-	-
	9107	-	-	-	-	-	-
	9112	-	-	-	-	-	-
	9113	-	-	-	-	-	-


A
Day 3
CD45.1
CD45.2
CD8
CD4
PD-1
T-bet
CD69
Control
Sham+Ab
AG2G
AG2G+
TA99
tSNE2
tSNE1
B
Day 7
CD45.1
CD45.2
CD8
CD4
PD-1
T-bet
CD69
Control
Sham+Ab
AG2G
AG2G+
TA99
tSNE2
tSNE1
Day 7
C
CD86
D
F4/80
E
Ly6c
F
CD11b
G
CD11c
H
CD40
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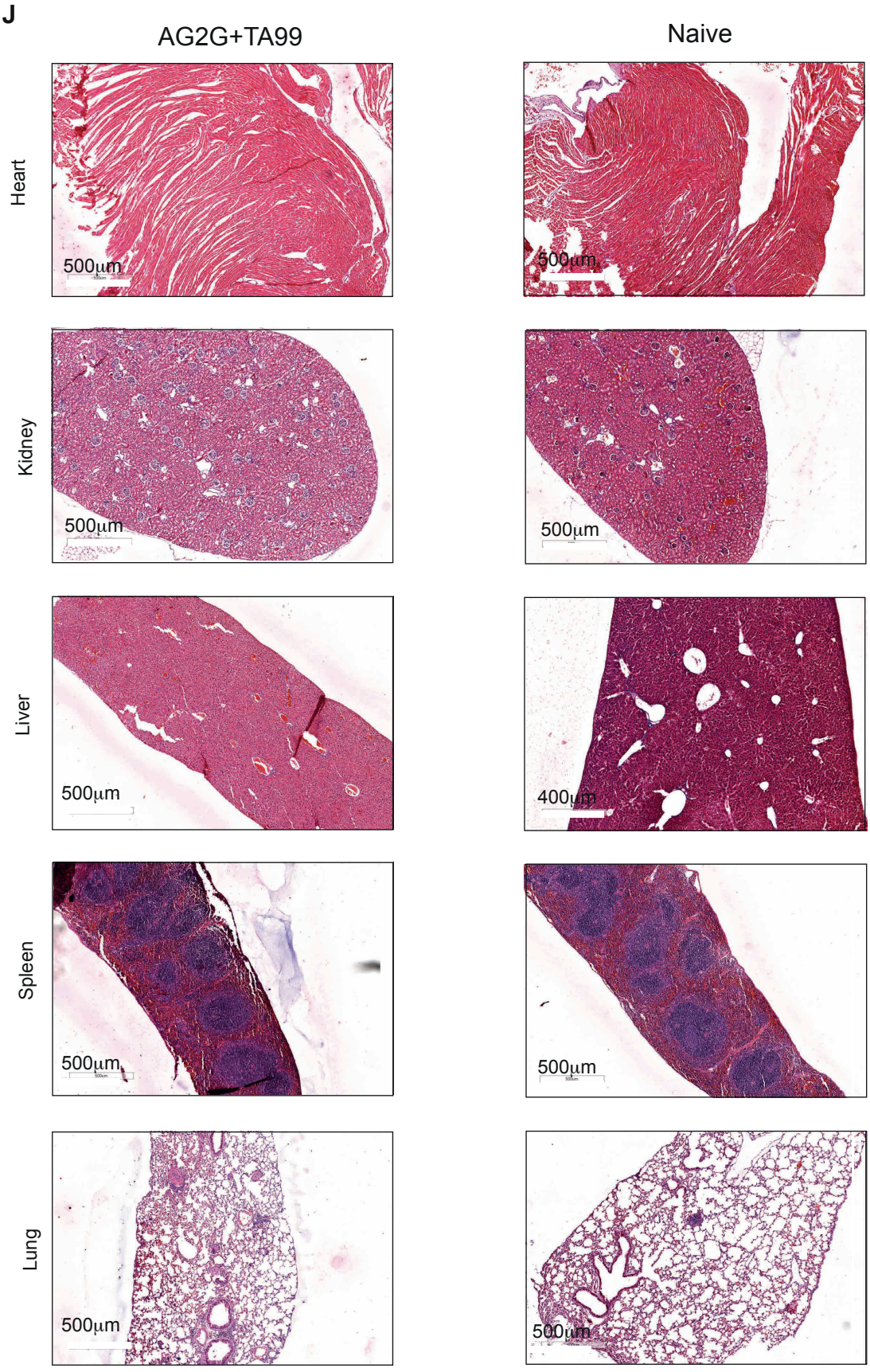
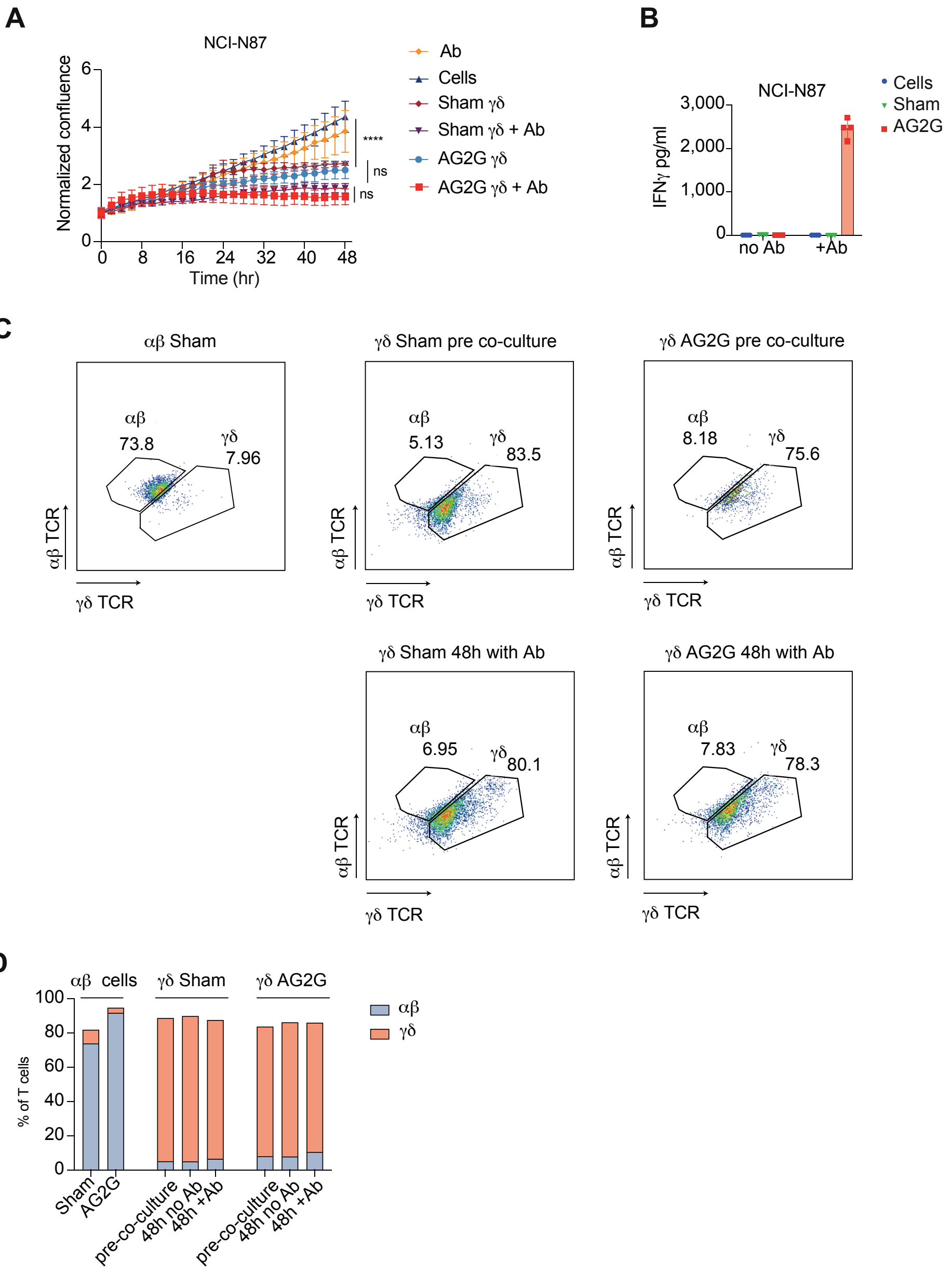
I-A/I-E
Control
Sham+
TA99
AG2G
AG2G+
TA99
tSNE2
tSNE1



Supplemental Figure S7. CyTOF analysis of systemic administration of tumor-binding antibodies and AG2G-expressing T cells.

A-B. tSNE plots of CD3e+/TCRb+ donor and CD3e+/TCRb+ host infiltrating T cells in B16F10 tumors 3 days (**A**) and 7 days (**B**) post treatment with AG2G-expressing or mock transfected T cells with or without TA99 targeting antibody or control untreated mice. Heat map for each column represent the relative expression of the antigen in the title in a scale from low (Blue) to high (Red). **C-I.** High dimensional tSNE plots of expression of selected proteins on tumor-infiltrating myeloid cells CD45+/CD11b+ cells. each column displays a color heat map of the following antigens: **C.** CD86+, **D.** F4/80+, **E.** Ly6c+, **F.** CD11b+, **G.** CD11c+, **H.** CD40+, **I.** I-A/I-E. rows from top to bottom are control, Sham+TA99, AG2G and AG2G+ TA99 respectively for each panel. **J.** Histology of organs from Fig. 6**B**.



Supplemental Figure S8. Retroviral transduction of γδ-T cells with AG2G endows them with anti-tumor ADCC.

A. Confluence analysis of NCI-N87 tumor cells over 48 h of co-culture in IncuCyte imager with non-transduced γδ-T cells (Sham) with targeting antibody or AG2G-expressing γδ-T cells with or without targeting antibody (n=3, 4:1 E:T ratio, 30 µg/ml antibody). **B.** IFNγ levels from supernatants of γδ-T cells after 48h co-culture with NCI-N87 described in **A. C.** Flow cytometer analysis of γδ-T cells before cytotoxicity assay, and following cytotoxic 48 h co-culture with NCI-N87 target cells and trastuzumab (described in **A**). Cells were stained with anti-γδ-TCR-BV605 and γδ-TCR-PE-Cy7. γδ-TCR-T cells served as control. **D.** Summary of percentage of population gated γδ-TCR -T cells and γδ-TCR T cells population in flow cytometer analysis (described in **C**) before and after cytotoxic assay co-culture described in **A.**