Supplementary Figure 1

A Antibody binding to human HER2
- 4D5-IgG2A
- Trastuzumab

EC50 = 49.12
EC50 = 50.23

B Anti-Trastuzumab antibodies after prolonged treatment
- Control
- Trastuzumab

C MAPK/ERK pathway

MAPK/ERK pathway
- 4D5-IgG2A
- Trastuzumab

E HER2+ BC proliferation in vitro
- KPL-4
- SKBR3

D AP-1/c-Jun Pathway

AP-1/c-Jun Pathway
- 4D5-IgG2A
- Trastuzumab

Figure S1

(A) Cell-based ELISA assay to determine 4D5 and Trastuzumab binding efficiency to human HER2 expressed on NMUMG cell lines. EC50 for each binding assay were calculated using non-linear regression curve fit, Assymetric Sigmoidal model in Graphpad Prism software. **(B)** Immune responses responses against Trastuzumab (a human antibody) in mice were assessed in Trastuzumab-treated mice (I.P. injection 200 µg) after 2 weeks post injection. ELISA assays using Trastuzumab as antigen were performed to determine anti-Trastuzumab responses in mouse serum. **(C-D)** HER2 signaling assays were performed using 293T cells stably transduced with dox-inducible HER2Δ16. Cells were treated with dox and transfected with luciferase reporter constructs for (C) MAPK/ERK or (D) AP-1/c-JUN pathways activation. 4D5 and Trastuzumab were added at titrated concentrations to inhibit HER2 signaling. The HER2-Tyrosine kinase inhibitor Lapatinib were used as positive assay control at the highest possible dose (500nM) without inducing cell toxicity. **(E)** Trastuzumab effect on human HER2+ breast cancer growth (KPL4 and SKBR3 cells) in vitro were assessed by MTT assays 3 days post Trastuzumab treatment.

Supplementary Figure 2

A

CD11b+ Myeloid cells — Neutrophils — Monocytes — NK cells — CD4+ T cells — CD8+ T cells
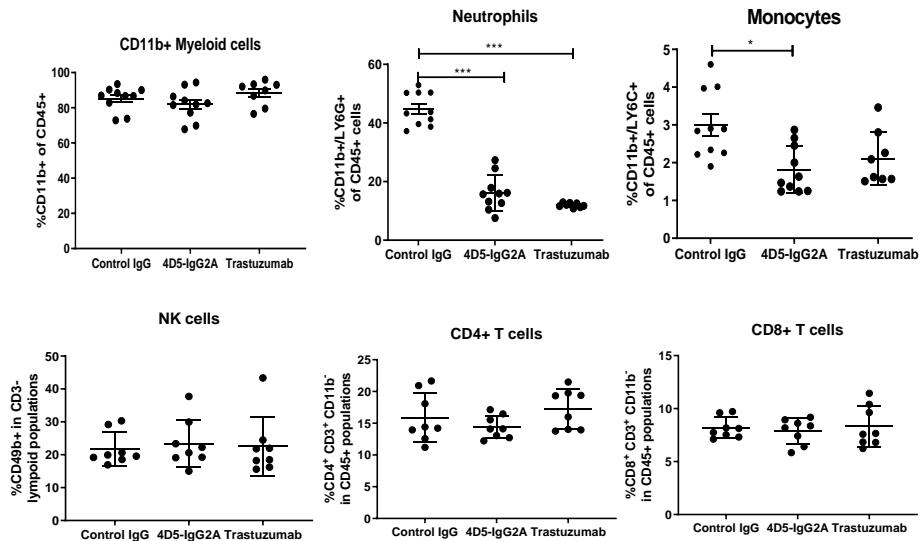
B HER2 specific adaptive T cell responses

C HER2 transgenic mice Adaptive T cell responses

D NK92-mFcGR3
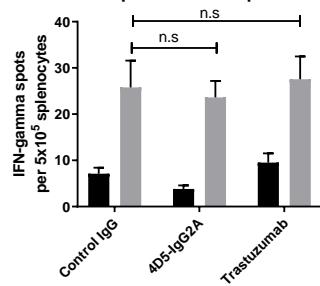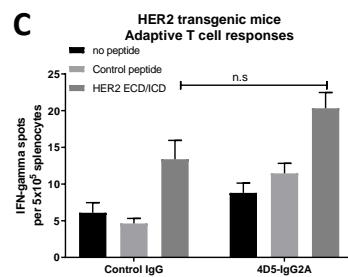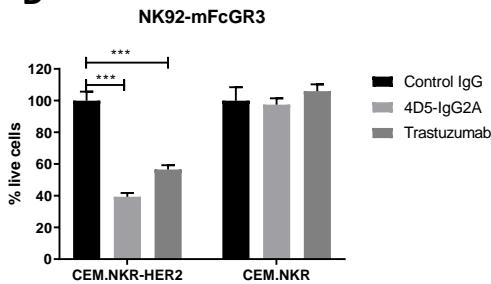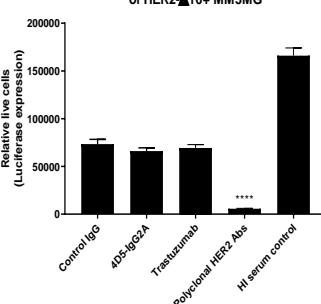
E Complement-dependent-cytotoxicity of HER2-Δ16+ MM3MG — Complement-dependent-cytotoxicity of parental MM3MG
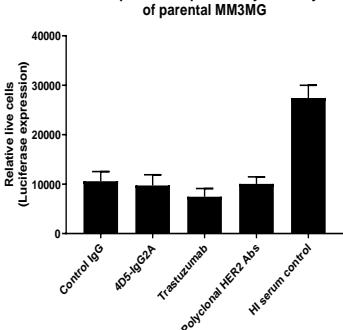
Figure S2

(A) Tumors in Figure 1A were harvested, processed into single cell suspensions, and tumor infiltrating immune cell populations (NK cells, CD4+ T cells and CD8+ T cells) were analyzed by FACS. **(B-C)** Anti-tumor specific T cell responses as measured by IFNγ ELISPOT against human HER2 peptides using mouse splenocytes from **(B)** MM3MG-HER2Δ16 orthotopic model or **(C)** HER2 transgenic model (described in Figure 5A). **(D)** In vitro NK cell mediated ADCC assay were performed using NK.92 expressing mouse FCGR3 as effector cells and CEM.NKR expressing HER2 and luciferase as target cells. Results showed both Trastuzumab and 4D5 treatment enhanced NK-mediated ADCC in vitro. Mean ±SEM, biological replicates *n* = 4, two-sided t-test, ***P < 0.001. **(E)** In vitro Complement-dependent-cytotoxicity (CDC) assay were performed using 25% human serum treatment (4 hours) on MM3MG-HER2Δ16 lines expressing luciferase. Results showed neither Trastuzumab or 4D5-IgG2A mAbs could enhance complement-mediated tumor cell killing. Mean ±SEM, biological replicates *n* = 4, One-Way ANOVA with Tukey's multiple comparisons

Supplementary Figure 3

A

Systemic macrophages

B

Tumor Associated Macrophages

C

MM3MG-HER2Δ16 growth in SCID-Beige

D

Systemic neutrophils

E

Tumor Associated Neutrophils
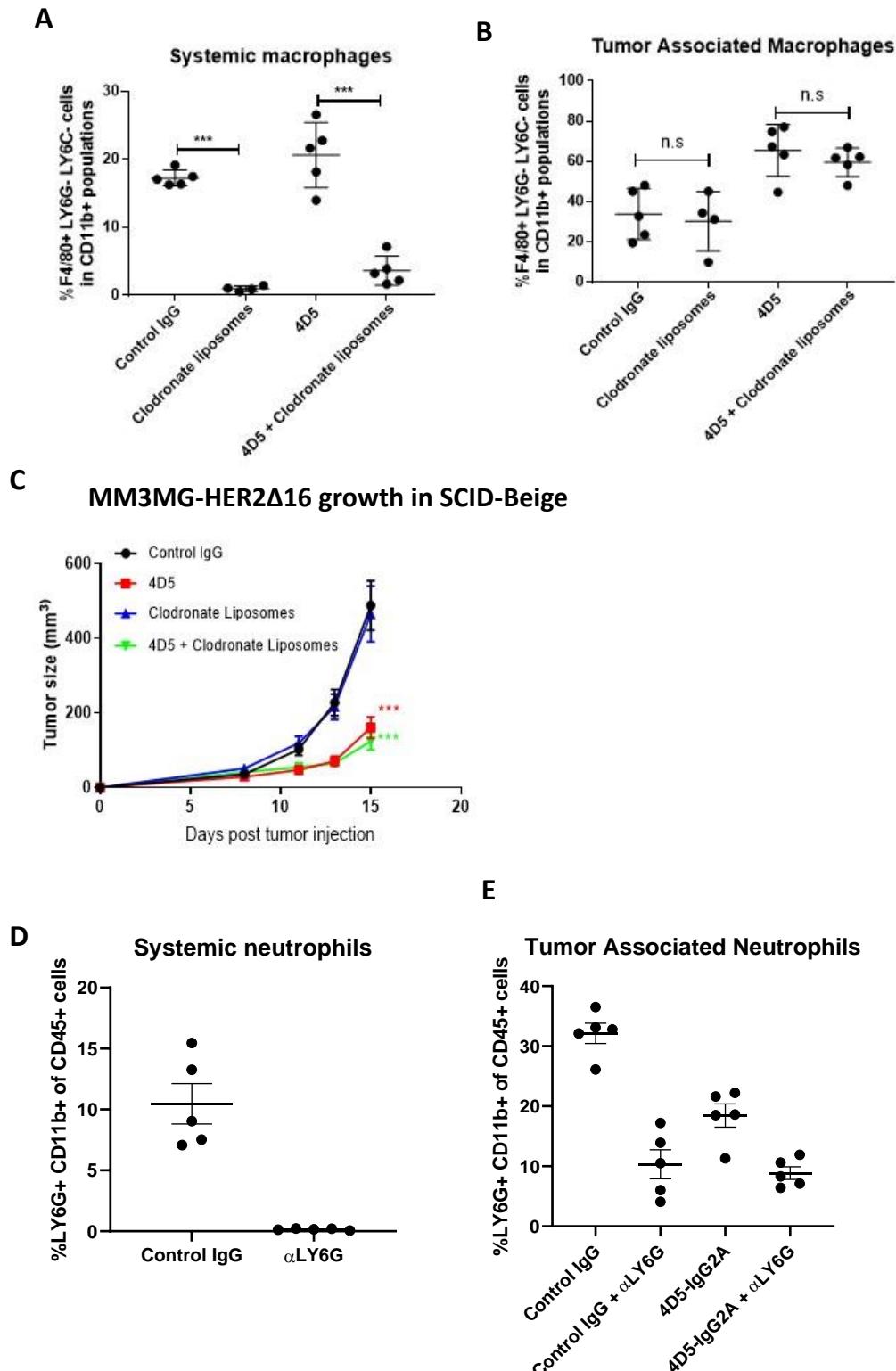
Figure S3

Clodronate Liposomes injections were used to deplete macrophages in SCID-beige mice before implantation of HER2+ MM3MG tumor (100μL/mice, 2x/week). **(A-B)** Macrophages in spleen (A) and tumor (B) were analyzed by FACS. Mean ±SEM, n = 5, One-way ANOVA test, ***P < 0.001. **(C)** Tumor growth were measured over time. Mean ±SEM, n = 5, Two-way ANOVA test with Tukey's multiple comparisons, ***P < 0.001. **(D-E)** Anti-Ly6G antibody were used to deplete neutrophils (biweekly I.P, 300μg/mice). FACS analysis showing neutrophils in spleen (D) and in tumor (E).

Supplementary Figure 4
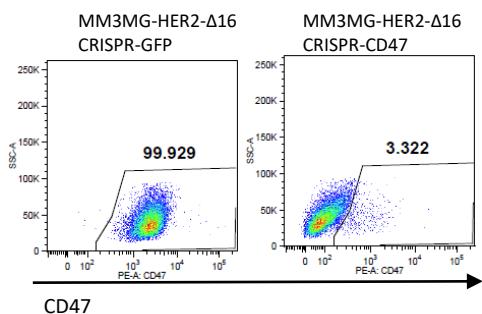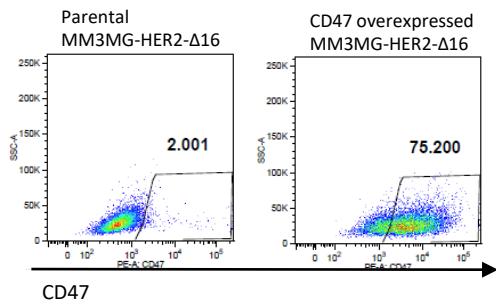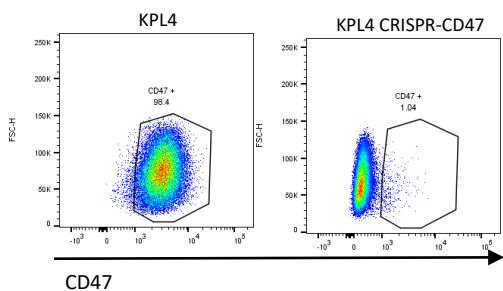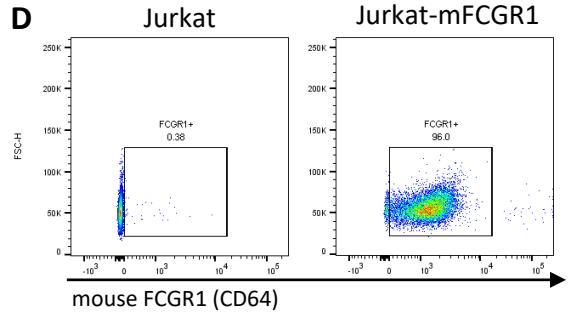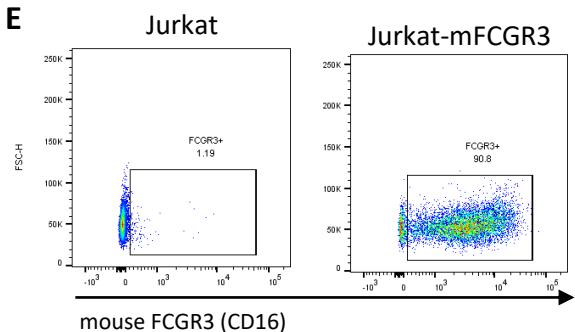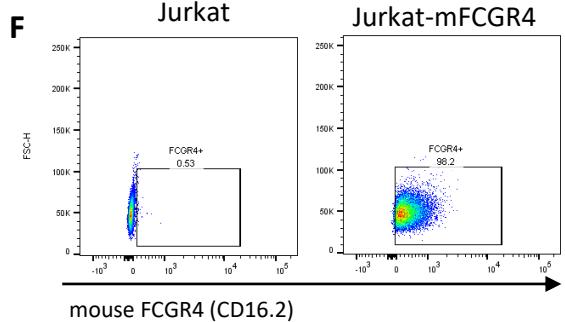
Figure S4

Flow cytometry confirmations of **(A)** CD47 knock-out in MM3MG-HER2-Δ16. **(B)** CD47 overexpression in MM3MG-HER2-Δ16. **(C)** CD47 knock-out in KPL4. **(D)** mouse FCGR1 expression in Jurkat-NFAT-LUC. **(E)** mouse FCGR3 expression in Jurkat-NFAT-LUC. **(F)** mouse FCGR4 expression in Jurkat-NFAT-LUC.

# Supplementary Figure 5
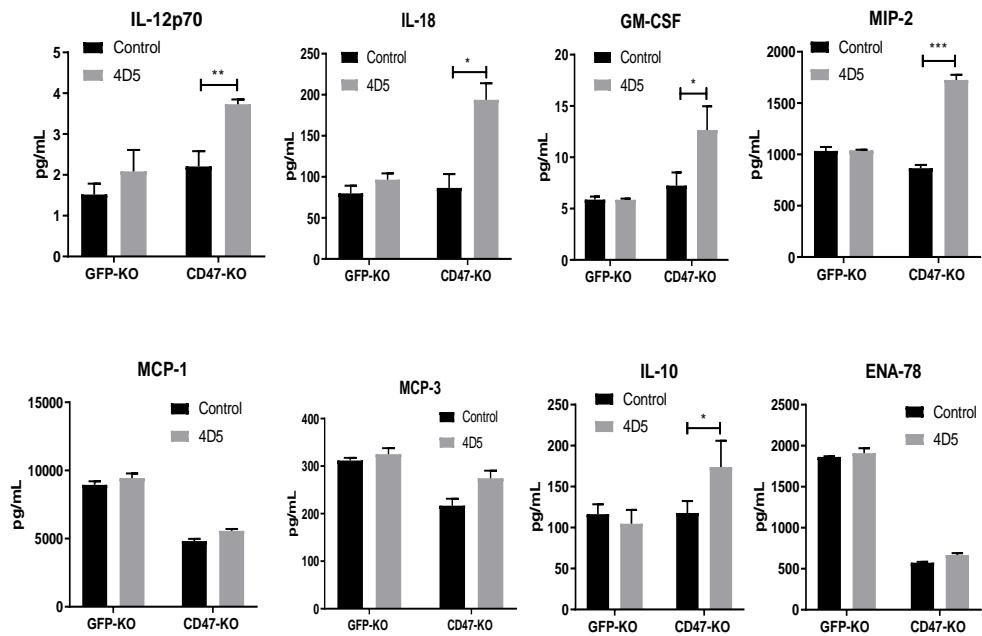
Figure S5
Secreted cytokines and chemokines by macrophages from co-culture experiment with HER2+ BC and antibodies were analyzed using the Luminex platform. Supplementary to Figure 3B

Supplementary Figure 6

A
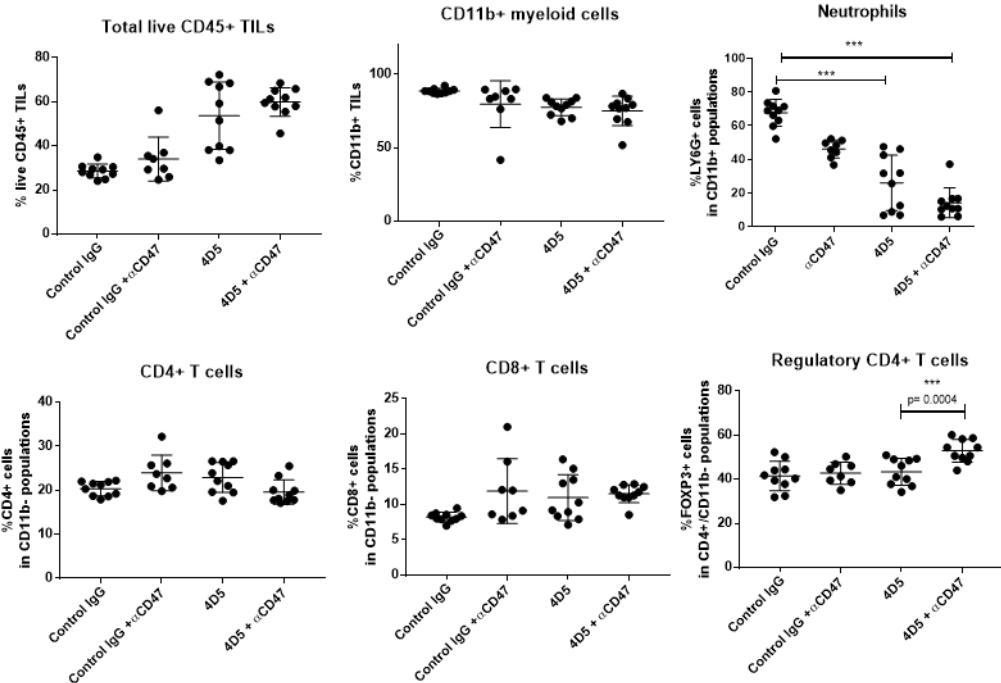
B
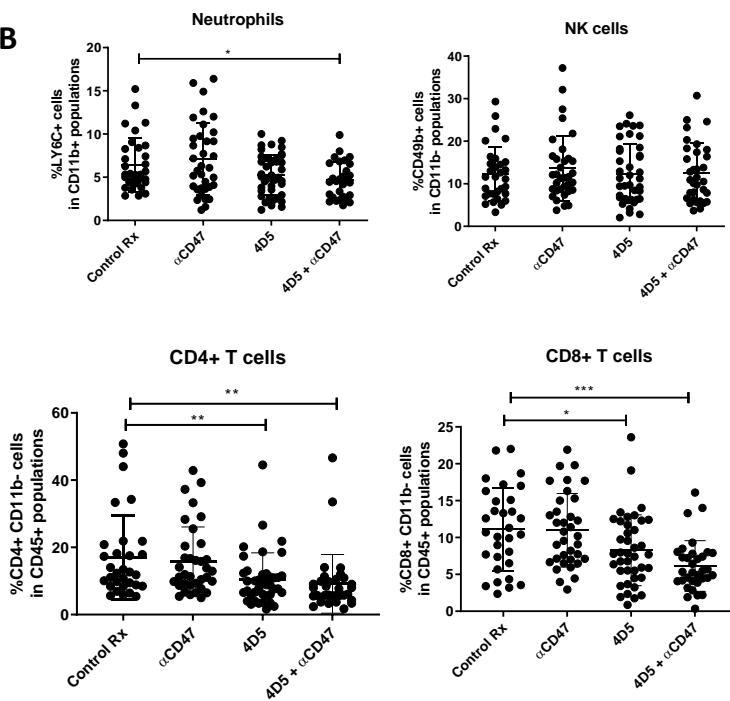
Figure S6
(A) Additional FACS analysis of immune cell populations in the orthotopic HER2+ tumors from experiment shown in Figure 4A. Mean ±SEM, *n* = 8-10. **(B)** Additional FACS analysis of immune cell populations in the HER2 transgenic tumors from experiment shown in Figure 5. Mean ±SEM, Control IgG n=23, αCD47 n=27, 4D5 n=38, 4D5+ αCD47 n=32. (**A** and **B**) One-way ANOVA test with Tukey's multiple comparisons, *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Figure 7

A

B
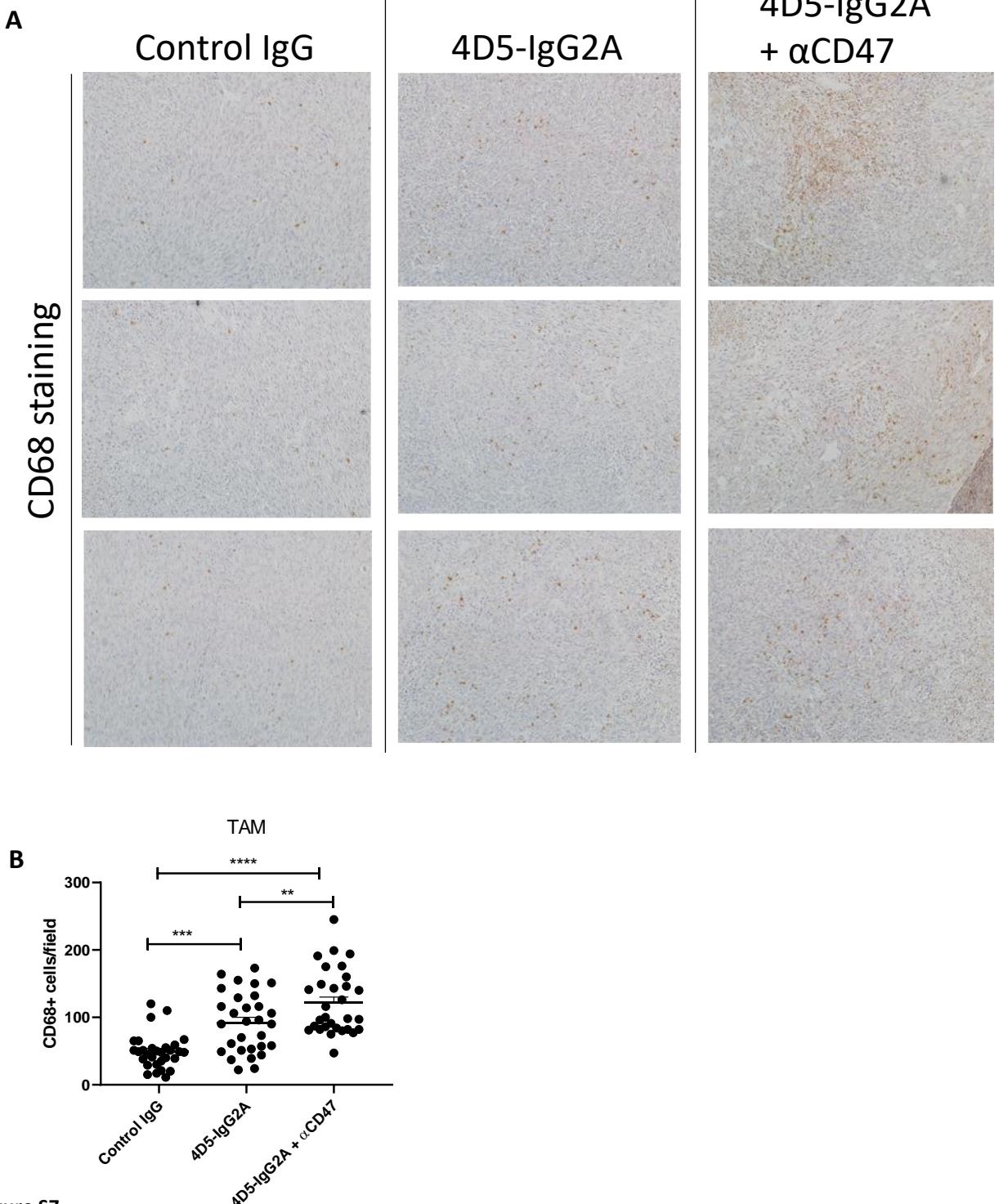
Figure S7
(A) Immunohistochemistry staining of CD68 of paraffin-embedded tumor samples derived from therapy experiments described in Figure 4A. Representative images of tumors from each treatment groups are shown. Original magnification = 20x. **(B)** Summary of CD68+ staining quantifications. n = 30. One-way ANOVA test with Tukey's multiple comparisons. All data represent mean ±SEM, **P<0.01, ***P < 0.001, ****P<0.0001.

Supplementary Table S1

Treatment	% Cd8+ cells in Immune compartment	% Ifng+ cells in Cd8+ cells	% Gzmb+ cells in Cd8+ cells
Control	12.55 %	3.87 %	16.02 %
αCD47	19.7 %	9.9 %	19.57 %
4D5-IgG2A	11.18 %	11 %	23.42 %
4D5-IgG2A + αCD47	3.74 %	14.66 %	41.88 %

Table S1
Single-Cell RNA-seq analysis of total CD8+ T cell frequency in tumor and percentage of CD8+ T cells expressing cytotoxic markers (*Ifng* and *Gzmb*). Data shows the mean of replicates in each treatment group.

Supplementary Methods:

RT-qPCR analysis of sorted macrophages

KPL4 xenografts were processed into single cell suspensions as described above, and tumor associated macrophages were sorted by FACS (Live CD45+ CD11b+ Gr1- and F4/80+). RNA were isolated from sorted macrophages using RNeasy Mini Kit (Qiagen) and cDNA were generated using "All-in-One cDNA Synthesis Supermix (Biotool B24403). RT-qPCR were performed using 2X SYBR Green qPCR Master Mix (Biotool B21202).

In vitro CDC Assay

Complement-dependent cytotoxicity (CDC) assay – MM3MG-HER2Δ16 or MM3MG cells expressing luciferase were incubated with 2 µg/mL of anti-HER2 antibodies for 1 hour at 37 ^{0}C. After incubation, human or rabbit serum (non heat-inactivated) were added to culture to a final concentration of 25% serum. After 4 hours, cells were lysed and viability were assessed by luciferase expression. Heat inactivated serum was used as negative control. A combination of different HER2-targeting antibodies were used as positive control, as this will greatly increase antibody-mediated CDC activity (unpublished results).

HER2 signaling assays

HEK 293T cells stably expressing doxycycline-inducible HER2Δ16 were transfected (lipofectamine 2000) with luciferase reporter constructs (5µg of DNA in 2x10^6 cells) for MAPK/ERK or AP-1/c-JUN pathways activation. Reporter constructs were originated from Cignal Reporter Assay Kit (336841, Qiagen). 12 hours after transfection and dox treatment, cells were treated with of 4D5 or Trastuzumab or lapatinib (Kinase inhibitor of HER2 signaling

as assay positive control) at the concentrations as indicated in the results. HER2 signaling activity were analyzed by luciferase readout of MAPK/ERK and AP-1/c-JUN pathway reporters. Non-induced (no dox treatment) cells were used as negative control.

ELISPOT assay

Mouse splenocytes were harvested by mashing whole spleens into single cells through a 40 µm filter. Red blood cells were lysed for 15 minutes using RBC lysis buffer (Sigma R7757). Live Splenocytes were then counted using the Muse® Cell Analyzer. For adaptive T cell response analysis, we used the mouse IFN-γ ELISPOT (MABTECH 3321-2H) with manufacturer's protocol. Briefly, 500,000 splenocytes were incubated in RPMI-1640 medium (Invitrogen) with 10% fetal bovine serum for 24 hours with peptides at a final concentration of 1 µg/mL. For HER2-specific responses, 169 peptides spanning the extracellular domain of HER2 protein were used. We used irrelevant HIV-1 Gag peptides (1 µg/mL, JPT, Germany) as control peptides. PMA (50 ng/ml) and Ionomycin (1 µg/ml) (Sigma) were used as positive controls.

Library preparation for Single Cell RNA-Seq

Tumors from treated transgenic mice were harvested and processed into single cell suspension using Mouse Tumor Dissociation Kit (Miltenyi, 130-096-730) following manufacturer's protocol with recommendations for 10X Genomics platform use (10X genomic manual, CG000147). Single cell suspensions from tumors were treated with red blood cells lysing buffer (Sigma R7757) for 5 minutes, and stained with "Fixable Far Red Dead Cell Stain Kit" (L10120). Live singlets (single cells) from tumor suspension were sorted by FACS and counted using hemocytometer. To generate 10X Genomics libraries, we used Chromium Single Cell 5' Library Construction Kit (PN-1000020) following manufacturer's protocol. A targeted cell recovery of

4000 cells was used for each tumor sample. Generated cDNA libraries were quality checked on Agilent Bioanalyzer 2100 and submitted to MedGenome Inc for sequencing on NovaSeq S4 instrument.