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 °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HER2-targeting antibodies were used as positive control, as this will greatly increase antibody-mediated CDC activity (unpublished results).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 singlet (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.