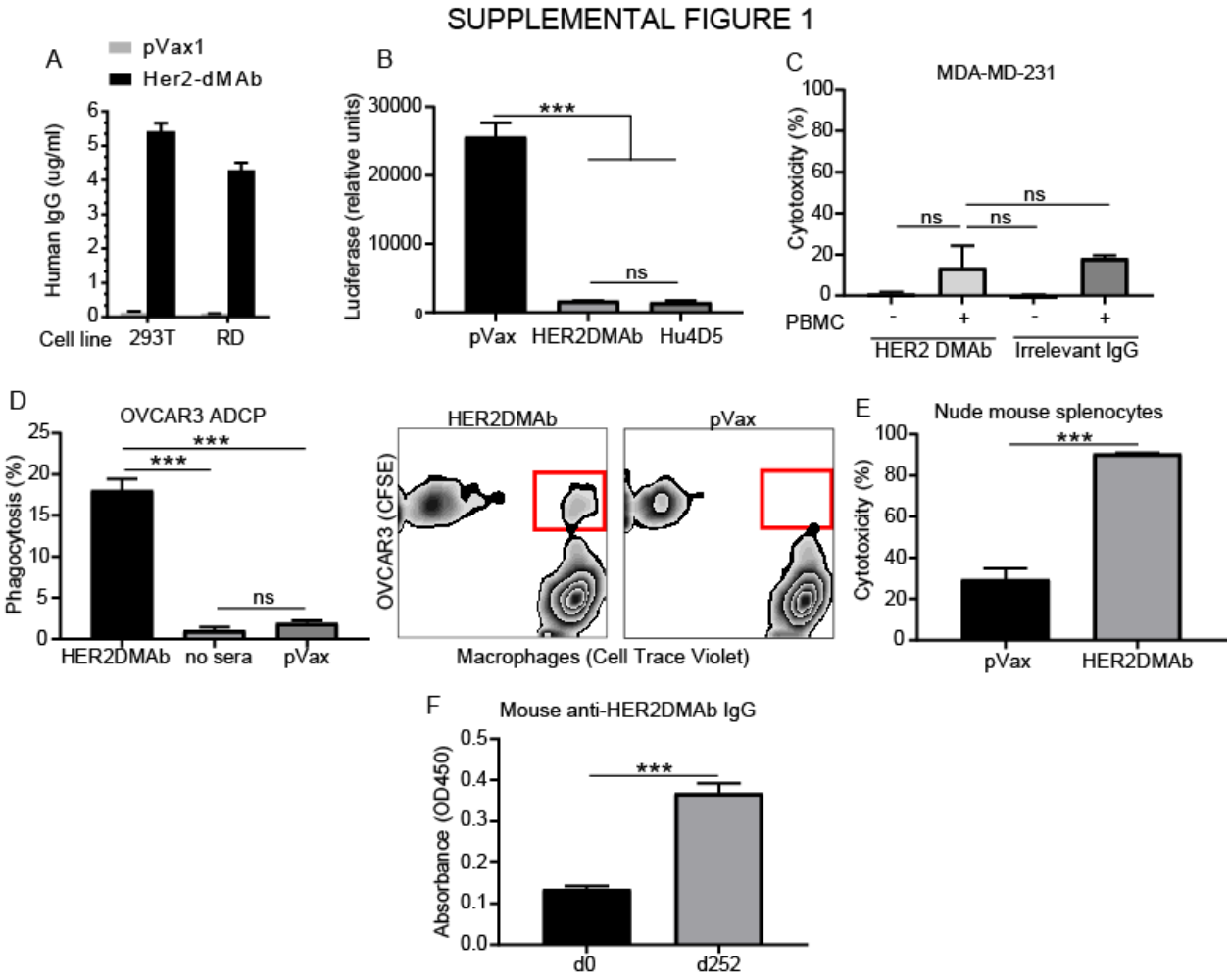
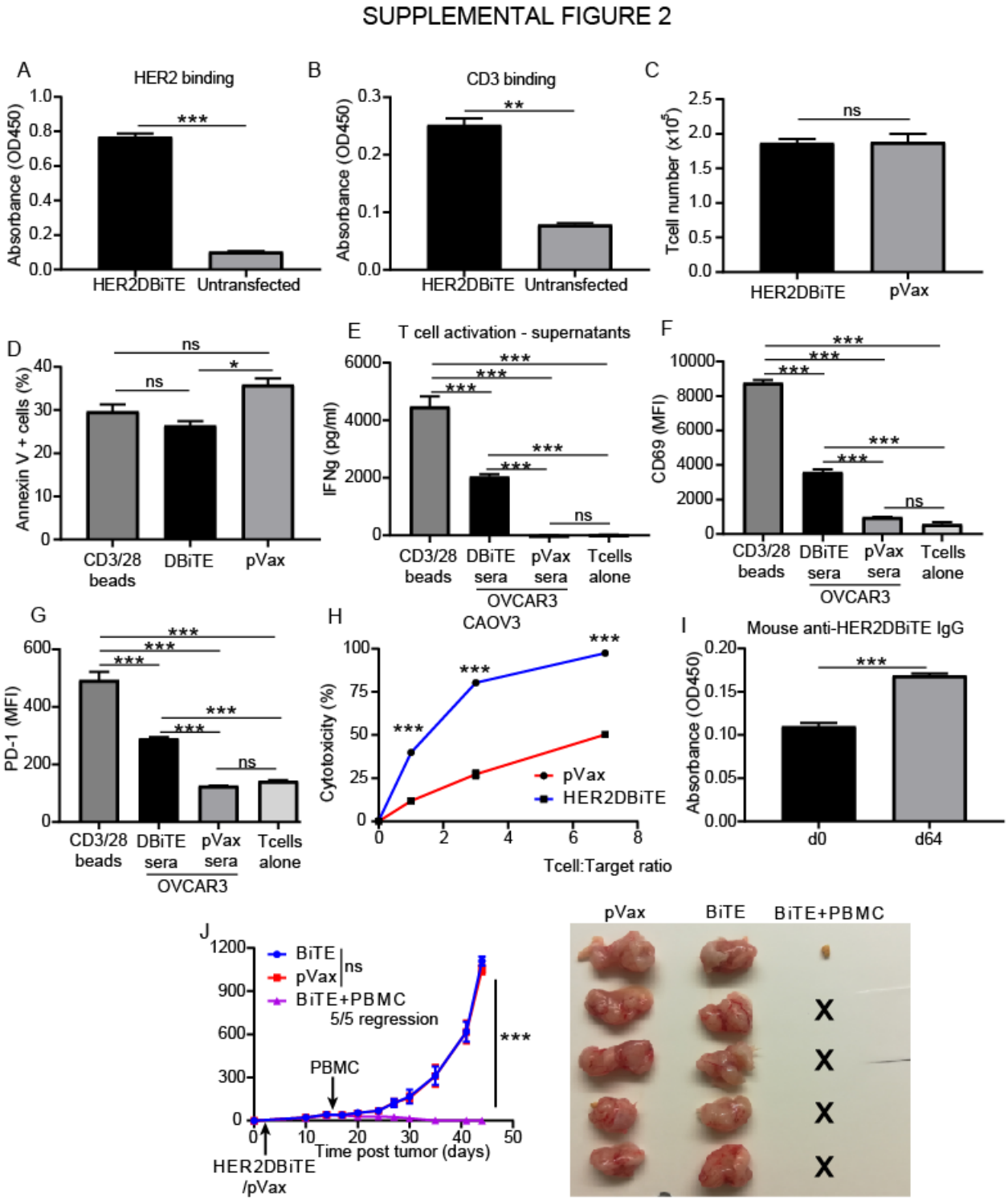


Supplemental Figure 1. In vitro expression and anti-tumor activity of HER2 DNA encoded monoclonal antibodies (DMAb). (**A**) Expression levels of HER2DMAb quantified from the supernatant of 293T or RD cells 48h after DNA transfection (n=3/group). (**B**) In vitro cytotoxicity resulting from culture of human PBMC (0.5 millions) with OVCAR3-luciferase (10,000) cells in the presence of HER2DMAb or pVax sera or Hu4D5 antibody as positive control (triplicates). (**C**) In vitro cytotoxicity resulting from coculture of human PBMC (0.5 millions) with HER2 negative cell line MDA-MD-231 (10,000) cells in the presence of sera from HER2DMAb or pVax injected mice (triplicates). (**D**) Percentage of OVCAR3 cells phagocytosed by macrophages in the presence of HER3DMAb, pVax sera or no added sera and representative flow cytometry plots (triplicates). (**E**) In vitro cytotoxicity resulting from coculture of splenocytes from Nu/J mice (0.5 millions) with OVCAR3 (10,000) cells in the presence of sera from HER2DMAb or pVax injected mice (triplicates). (**F**) Mouse anti-HER2DMAb IgG at days 0 and 252 in Nu/J sera (triplicates). ANOVA. T-test. ***p<0.001. ns: not significant.



Supplemental Figure 2. Binding, cytotoxicity, activation and in vivo effectiveness of HER2DBiTE. (**A**) HER2DBiTE binding to recombinant HER2 protein measured by binding ELISA (triplicates). (**B**) HER2DBiTE binding to recombinant CD3 protein measured by binding ELISA (triplicates). (**C**) Number of T cells present in wells 24 hours after coincubation of T cells with OVCAR3 in the presence of HER2DBiTE or pVax sera (triplicates). (**D**) Presence of

apoptotic (Annexin V+) cells 5 days after activation of T cells with HER2DBIiTE or pVax sera in the presence of OVCAR3 cells. Anti-CD3/anti-CD28 beads were used as positive control (triplicates). (**E**) T cell activation measured as IFNg in the supernatant of T cells cultured for 24 hours in the presence of HER2DBIiTE or pVax sera and OVCAR3 cells. Anti-CD3/anti-CD28 beads were used as positive control, T cells alone as negative control (triplicates). (**F**) T cell activation measured as expression of CD69 in T cells cultured for 72 hours in the presence of HER2DBIiTE or pVax sera and OVCAR3 cells. Anti-CD3/anti-CD28 beads were used as positive control, T cells alone as negative control (trilpicates). (**G**) T cell activation measured as expression of PD-1 in T cells cultured for 72 hours in the presence of HER2DBIiTE or pVax sera and OVCAR3 cells. Anti-CD3/anti-CD28 beads were used as positive control, T cells alone as negative control (triplicates). (**H**) In vitro cytotoxicity resulting from coculture of T cells with OVCAR3 cells at different ratios in the presence of sera from HER2DBiTE or pVax mice (2 independent experiments in triplicate). (**I**) Mouse anti-HER2DBiTE IgG at days 0 and 64 in Nu/J sera (trilpicates). (**J**) Average growth curve of OVCAR3 tumors grafted into NSG mice treated with HER2DBiTE or empty vector without PBMC and HER2DBiTE with PBMC and image of tumors (n=5 mice per group; X denotes no tumor (full rejection)). T-test, ANOVA, Two-way ANOVA. *p<0.05, **p<0.01 ***p<0.001. ns: not significant.

SUPPLEMENTAL METHODS

In vitro DMAb expression

We plated 1 million 293T cells in each chamber of a 6-well plate. The following day we transfected 1 µg of HER2DMAb plasmid with Lipofectamin 2000 (Invitrogen). We collected the supernatant 48 hours post transfection.

Flow cytometry

Anti-human antibodies used were directly fluorochrome conjugated. We used: HER2 (24D2) and CD45 (HI30), CD3 (HIT3A), CD69 (FN50), PD-1 (EH12.2H7), secondary anti-human IgG APC (polyclonal) all from Biolegend. Live/dead exclusion was done with 7AAD (Invitrogen) and Annexin V (Biolegend).

Immunoblotting

Protein extraction, denaturation and western blotting were performed as previously described (25). Membranes were blotted with polyclonal anti-human IgG (H+L) (Bethyl) and anti-β-actin (a5441, Sigma-Aldrich), anti-Akt (#9272, Cell Signaling), anti-phophoAkt (Ser473, Cell Signaling). Images were captured with ImageQuantLAS 4000 (GE Healthcare Life Sciences).

For signaling blockade experiments 200,000 OVCAR3 cells were plated in a 6-well plate and starved overnight with serum free media. On the next day, 10ug of purified HER2DMAb or PBS were added to the appropriate wells for 1h followed by 10ng/ml of HRG (Peprotech) for 30 minutes.

DMAb quantification ELISA

We coated ELISA plates with 1ug/ml of goat anti-human IgG-Fc fragment antibody (Bethyl) overnight at 4°C. The following day, we blocked with PBST-10%FBS for 1 hour at room temperature, washed, incubated for 1 hour at room temperature with the samples diluted in PBST-1%FBS, washed, and incubated at room temperature with HRP conjugated goat anti-human kappa light chain antibody (Bethyl). After 1h incubation we developed with SIGMAFAST OPD (Sigma Aldrich) and read at 450nm. The standard curve was generated using purified human IgG/Kappa (Bethyl).

HER2 Binding ELISA (DMAb)

We coated ELISA plates with 1ug/ml of human HER2 recombinant protein (abcam) overnight at 4ºC. We blocked with PBST-10%FBS for 1h. As primary antibody we used sera from HER2DMAb expressing mice or controls (electroporated with empty pVax plasmid) at different dilutions. We incubated at room temperature for 1h. Secondary antibody was a goat anti-human

IgG Fc HRP conjugated (Bethyl). After 1h incubation we developed with SIGMAFAST OPD (Sigma Aldrich) and read at 450nm.

CD3 and HER2 binding ELISA (DBiTE)

We coated ELISA plates with 1ug/ml of human HER2 recombinant protein (abcam) or human CD3 epsilon (Acrobiosystems) overnight at 4ºC. We blocked with PBST-10%FBS for 1h. As primary antibody we used sera from HER2DBiTE expressing mice or controls (electroporated with empty pVax plasmid). We incubated at room temperature for 1h. Secondary antibody was a Goat anti-human IgG H+L HRP conjugated (Bethyl). After 1h incubation we developed with SIGMAFAST OPD (Sigma Aldrich) and read at 450nm.

Detection of anti-HER2DMAb and HER2DBiTE antibodies

ELISA plates were coated with 1ug/ml of purified HER2DMAb or HER2DBiTE overnight at 4°C. The following day, plates were blocked with PBST-10%FBS for 1 hour at room temperature, washed, incubated for 1 hour at room temperature with the samples diluted in PBST-1%FBS, washed, and incubated at room temperature with HRP conjugated goat anti-mouse IgG antibody (Abcam). After 1h incubation, plates were developed with SIGMAFAST OPD (Sigma Aldrich).

Detection of T cell activation and apoptosis by HER2DBiTE

We plated 96-well plates with 5,000 OVCAR3 overnight at 4ºC. The following day, we added sera from HER2DBiTE expressing mice or pVax controls (1:20 dilution in PBS, 100ul) and 50,000 T cells and incubated them at 37`C. 24h later we took supernatant for IFNg ELISA and added fresh supernatant. After 72h we performed flow cytometry to measure T cell apoptosis and activation (CD3, CD69, PD-1, Annexin V). For cell counts, we plated 5,000 OVCAR3 with 100,000 T cells

and counted live T cell numbers using dead cell exclusion dye Trypan Blue (ThermoFisher) and Countess II automated cell counter (ThermoFisher).

Interferon gamma ELISA

Determination of human interferon gamma from supernatants was performed using Human IFNg ELISA MAX (Biolegend) following manufacturer's instructions.

In vitro cytotoxicity

We plated 10,000 OVCAR3 cells per well in a 96-well plate and 18 hours later we coincubated them for 5 hours with 500,000 human PBMC from a healthy donor (provided by the University of Pennsylvania Human Immunology Core) or 500,000 splenocytes from nude mice in the presence or absence of HER2DMAb. After 4 hours we collected the supernatant, trypsinized the cells, stained them for 7AAD (Invitrogen), Annexin V (Biolegend) and anti-human CD45 (Biolegend) and performed a flow cytometry-based cytotoxicity assay as described previously(20). Alternatively, we used OVCAR3 or MDA-MB-231 expressing luciferase and after coculture measured luciferase expression. For BiTE killing assay we incubated 10,000 OVCAR3-luciferase or CAOV3-luciferase cells with different ratios of T cells for 5h, washed with PBS, lysed the cells and measured luciferase expression.

Antibody dependent cellular phagocytosis

We differentiated macrophages from human monocytes by plating 1million monocytes per T25 with 50ng/ml of human M-CSF (Peprotech). We changed media with cytokines at days 3 and 6. On day 6 we trypsinized the macrophages, stained them with cell trace violet (Invitrogen) according to manufactuer's instuction and plated 50,000/well in a 96-well plate and lefT them with 20ng/ml M-CSF overnight. On day 7 we stained OVCAR3 cells with CFSE (Invitrogen) and plated

10,000 OVCAR3 cells on the wells with macrophages with HER2DMAb or pVax sera. 24 hours later we trypsinized the cells, and performed flow cytometry. We measured phagocytosis as double positive stained cells(29).

Immunofluorescence

Mouse tumors were frozen in OCT (TissueTek), and frozen sections cut. Slides were then fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Sections were blocked using 5% normal goat serum followed by staining with HER2DMAb antibodies and anti-human AF488 conjugated secondary (Invitrogen).

Slides were viewed using the Leica TCS SP5 II confocal microscope and the LAS software (Leica).