

## **Supplementary methods**

### **HER2 expression and TDB binding to HER2-transfected CHO cells**

HER2 expressing CHO cells were stained 48h after transfection with indicated therapeutic antibodies and with 1:200 Alexa 647 Goat Anti-Human IgG, Fcy Fragment (Jackson ImmunoResearch #109-606-098) in FACS buffer and incubated on ice 30 min. Cells were washed 2x with FACS buffer, stained with propidium iodine (PI), and analyzed using FACS Caliber (BD Biosciences).

### **Cynomolgus monkey immunophenotyping and cytokine measurement**

Immunophenotyping was conducted on whole blood samples collected via a peripheral vein into sodium heparin tubes. Lymphocytes (CD4 and CD8) and T cell activation markers (CD69) were quantified using specific antibodies against the marker antigens. BD TruCount™ tubes were used for quantification of lymphocyte absolute counts in whole blood samples. Tissue absolute cell counts were determined by Guava® cytometry system. BD TruCount™ tubes were used in combination with CD45 labeling for real time quantification of lymphocyte absolute counts in whole blood samples. The lymphocyte subset percentages were taken from the whole blood analysis and applied to the total numbers of lymphocytes.

Cytokine analysis was conducted on plasma samples collected via a peripheral vein into potassium EDTA tubes. Cytokine concentrations were determined using a bead-based multiplex sandwich immunoassay with microplates analyzed by a Luminex detection system (Softmax® Pro GxP version 5.0.1).

Supplementary figures and tables

Figure S1

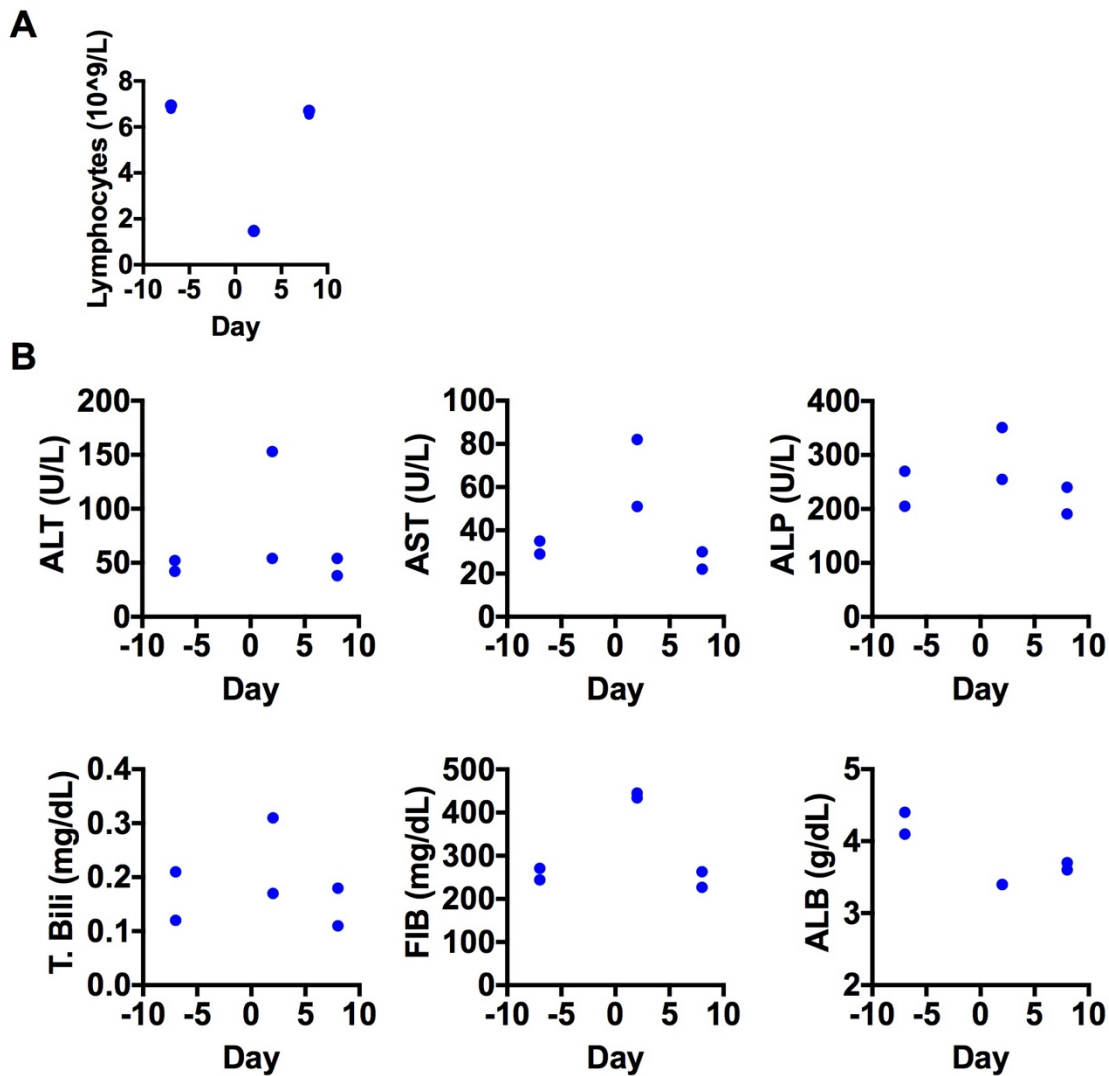
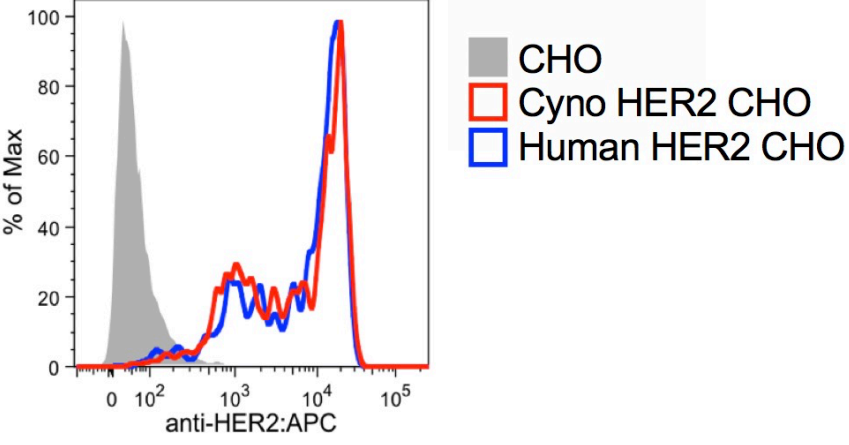


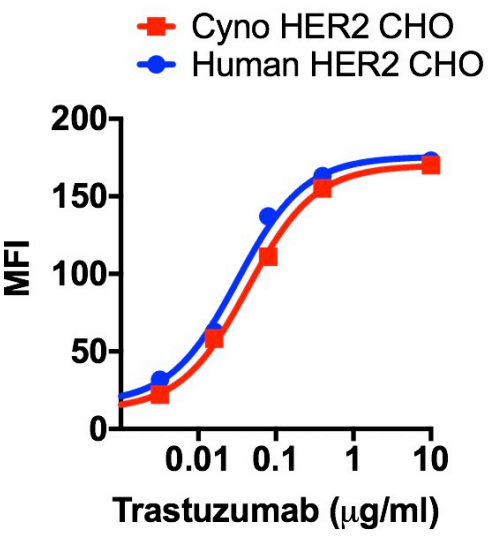
Figure S1. Clinical chemistry parameters in cynomolgus monkeys (N=2) treated with a single dose of HER2-TDB 1 at 0.5 mg/kg IV.

# Figure S2

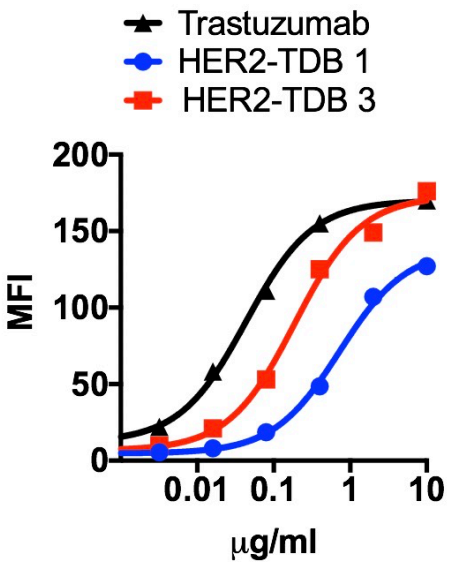
**A**



**B**



**C**



**Figure S2. Binding of antibodies to cells that express cynomolgus monkey HER2 (A-B)** stable CHO cell lines expressing human (blue) or cynomolgus monkey (red) HER2 were tested for **(A)** HER2 expression and **(B)** Trastuzumab binding using flow cytometry. **(C)** Relative binding of trastuzumab (black), HER2-TDB 3 (red) and HER2-TDB 1 (blue) on cynomolgus monkey HER2 expressing CHO cells. N=1

Figure S3

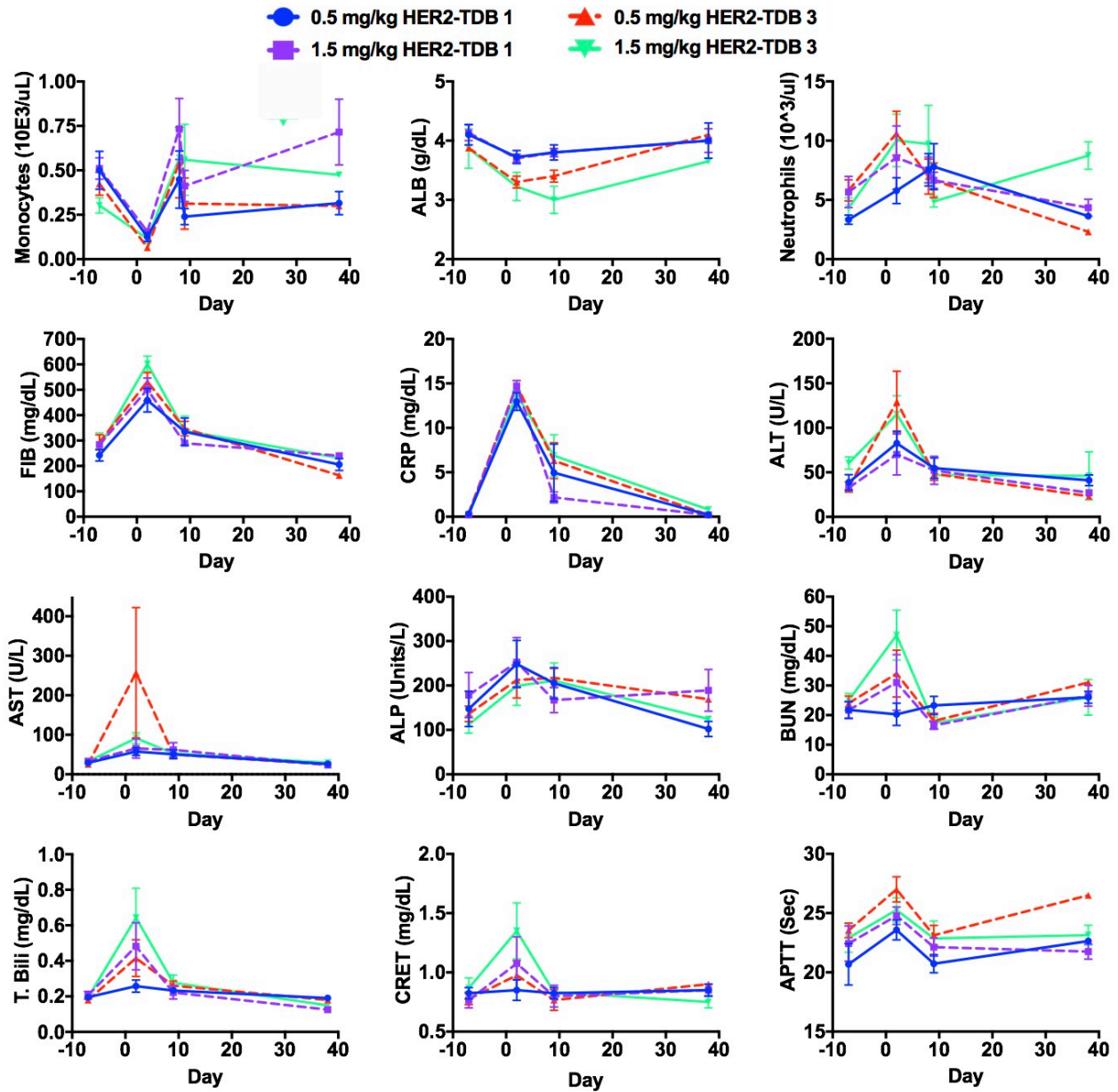


Figure S3. Clinical chemistry parameters in cynomolgus monkeys treated with HER2-TDB 1 or HER2-TDB 3 at dose levels of 0.5 and 1.5 mg/kg (IV) on Day 1 and Day 8. Data presented as mean  $\pm$  SEM; N=4 or 3 for each group, except for the recovery timepoint (N=2).

**Table S1. Toxicokinetic (TK) parameters obtained following a 1-hour IV infusion administration of HER2-TDB 3 in Female Cynomolgus Monkeys**

For TK data calculations, Study Day 1 was converted to TK Day 0 to indicate the start of dosing. Therefore, Study Days 1, 2, 3, etc. will be referred to as TK Days 0, 1, 2 etc. Mean values were reported where  $N \geq 2$  and ( $\pm$ ) standard deviation (SD) values were reported where  $N \geq 3$ . Nominal sample collection times and nominal doses were used in the TK data analysis. All concentrations that were below lower limit of quantification (LLOQ) were interpreted to be missing for graphical representation and equated to 0  $\mu\text{g/mL}$  for estimations of TK parameters  $C_{\text{max}}$ : maximum observed serum concentration post end of infusion;  $\text{AUC}_{0-2}$ : area under the serum concentration-time curve from time zero (TK Day 0) to TK Day 2;  $\text{AUC}_{0-7}$ : area under the serum concentration-time curve from time zero (TK Day 0) to TK Day 7;  $\text{AUC}_{7-9}$ : area under the serum concentration-time curve from TK Day 7 to TK Day 9;  $\text{AUC}_{7-14}$ : area under the serum concentration-time curve from TK Day 7 to TK Day 14;  $\text{AUC}_{\text{inf}}$ : area under the serum concentration-time curve from time 0 (TK Day 0) extrapolated to infinity.

Treatment	Post End of Infusion of First Dose			Post End of Infusion of Last Dose			
	$C_{\text{max}}$ ( $\mu\text{g/mL}$ )	$\text{AUC}_{0-2}$ ( $\text{day} \cdot \mu\text{g/mL}$ )	$\text{AUC}_{0-7}$ ( $\text{day} \cdot \mu\text{g/mL}$ )	$C_{\text{max}}$ ( $\mu\text{g/mL}$ )	$\text{AUC}_{7-9}$ ( $\text{day} \cdot \mu\text{g/mL}$ )	$\text{AUC}_{7-14}$ ( $\text{day} \cdot \mu\text{g/mL}$ )	$\text{AUC}_{\text{inf}}$ ( $\text{day} \cdot \mu\text{g/mL}$ )
Group 1A* 0.2, 0.2 and 0.4 mg/kg dosing on TK days 0, 1 and 7, (N = 1)	4.25	4.59	8.66	11.8	9.31	10.6	19.1
Group 1B* 0.1, 0.2 and 0.4 mg/kg dosing on TK days 0, 1 and 7, (N = 2)	1.45	2.29	6.01	11.8	9.67	12.0	18.6
Group 2 0.5 and 0.5mg/kg dosing on TK days 0 and 7 (N = 4)	$15.2 \pm 3.20$	$14.8 \pm 3.19$	$20.9 \pm 6.25$	$12.7 \pm 2.39$	$12.8 \pm 3.29$	$18.6 \pm 2.48$	$39.9 \pm 8.57^a$

Note: The first sample collection time-point was 15 minutes after the first dose and 2 hours after the last dose.

<sup>a</sup> early euthanasia (n=1) on TK day 2

Figure 1C unedited Western blot

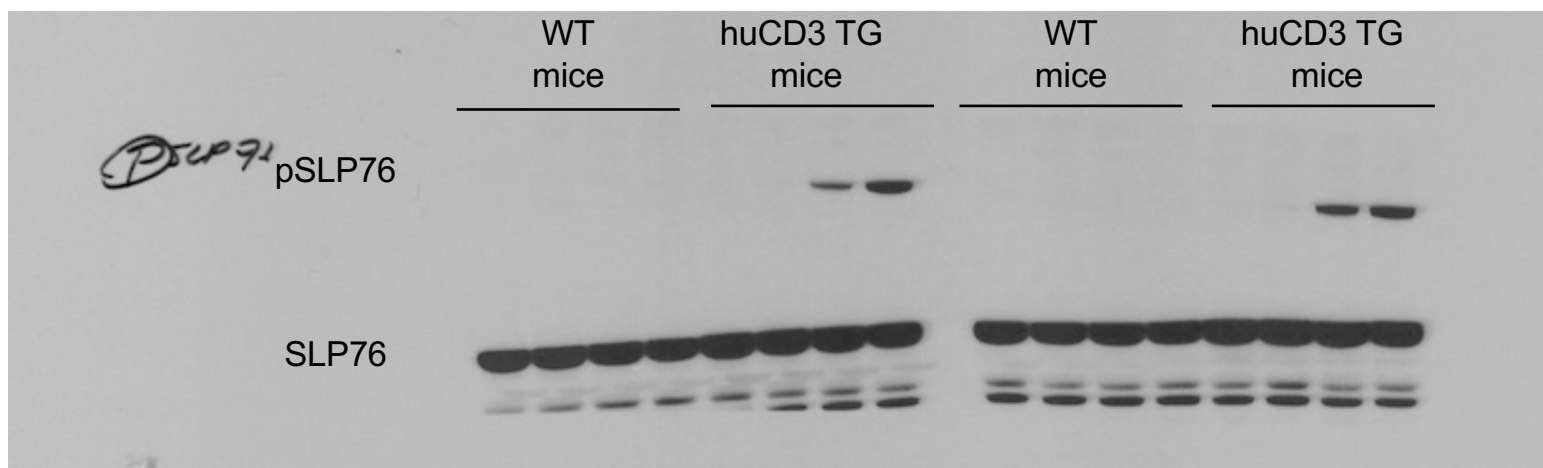


Figure 7A unedited Western blot

