



**Figure S2, related to Figure 2.**

**(A) Antigen density-dependent activation of HER2 CAR** – Conventional HER2 CAR T cells were stimulated with beads coated with BSA, low or high densities of HER2 ectodomain (Low HER2 and High HER2) at indicated beads to CAR-T cells ratio for expression of CD69 and PD-1 at day 3 following stimulation. **(B) Cytotoxic and expansion properties of RB-340-1 cellular components** – Non-transduced (NT), conventional HER2 CAR, cRB-340-1 and RB-340-1 T cells were exposed for three days to FaDu cell line at 1:20 effector to target ratio. Different proportion of CD4<sup>+</sup>/CD8<sup>+</sup> T cells were tested for cytotoxic activity (upper panel) and expansion (lower panel). The numbers in the headline refer to the proportion of CD4<sup>+</sup> T cells (%) present in each group over total number of T cells. **(C) RB-340-1 specificity of gene regulation** – Primary T cells transduced with LdCK plus HER2-TEV constructs including either PD-1sg, TIM-3g or both were tested for expression of the respective target genes five days after stimulation with FaDu cells to induce the expression of the two checkpoints. **(D) Kinetics of gene-expression regulation by RB-340-1** – RB-340-1 (red line) and cRB-340-1 (black line) were stimulated with HER2-coated (filled shapes) or BSA-coated (empty shapes) beads and the expression of PD-1, TIM-3 and CD69 was followed in CD8<sup>+</sup> T cells at baseline and 48 and 72 hours after stimulation.