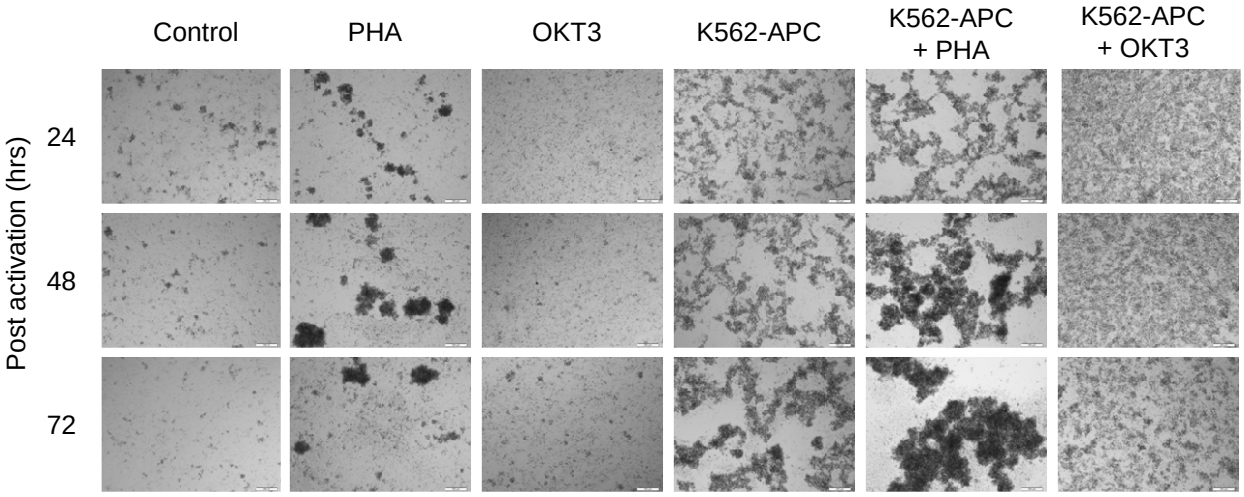
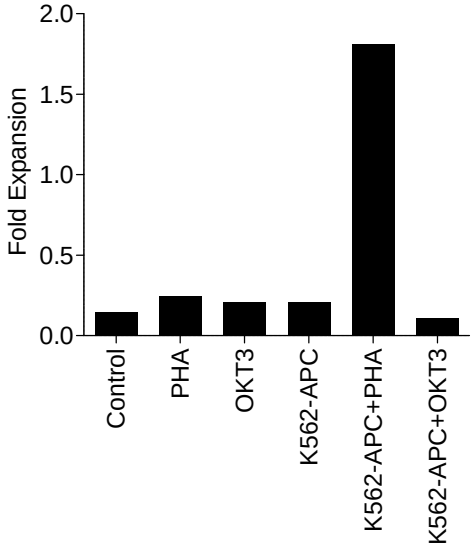
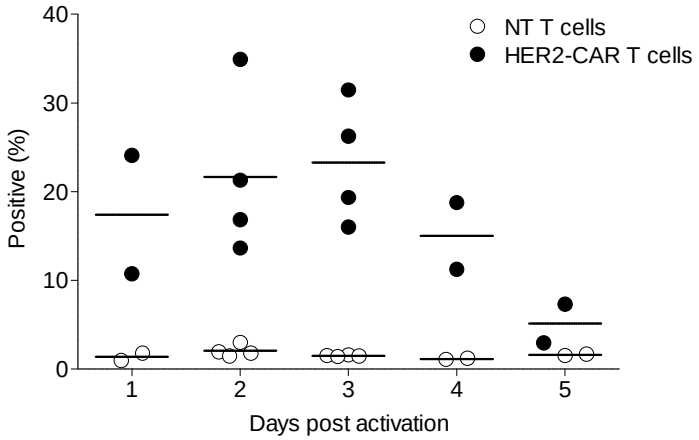
A



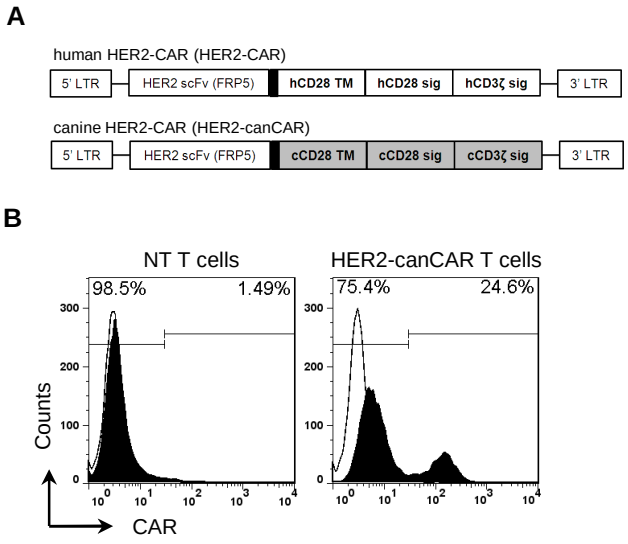
B



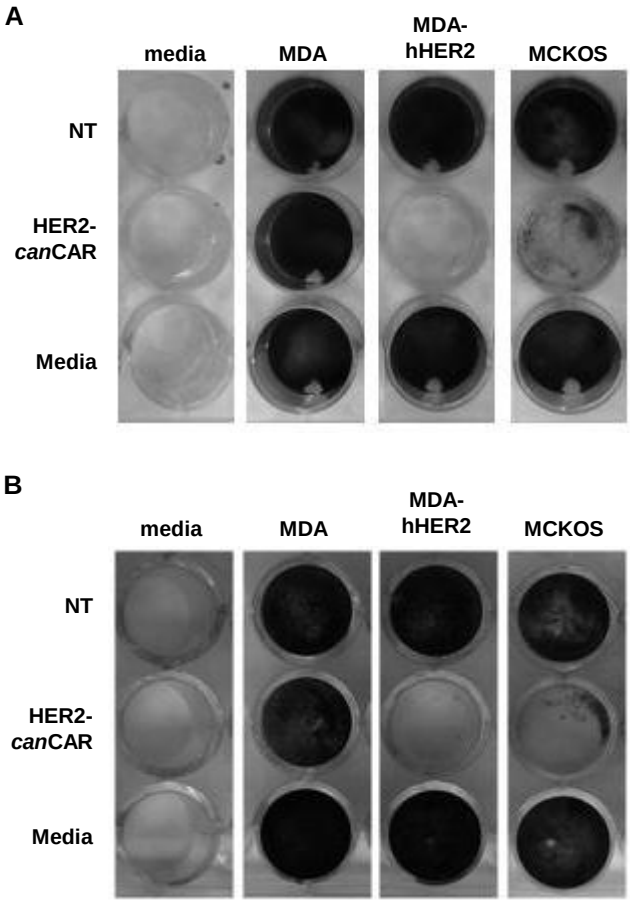
Supplemental Figure 1: Activation of canine T cells. Canine PBMCs were stimulated with plate bound OKT3 (1μg/ml), PHA (5μg/ml), γ-irradiated (100 Gy) K562-APCs (2:1 T-cell:K562-APC ratio), PHA plus K562-APCs, or OKT3 plus K562-APCs in the presence of IL21 (30ng/ml). Unstimulated PBMCs served as controls. Cells were fed with IL21 (30ng/ml) on day 3 and counted on day 7. **(A)** Pictures of cells were taken every 24 hrs for the first 3 days using a bright field microscope (40x magnification). **(B)** Expansion on day 7. One representative experiment in shown.



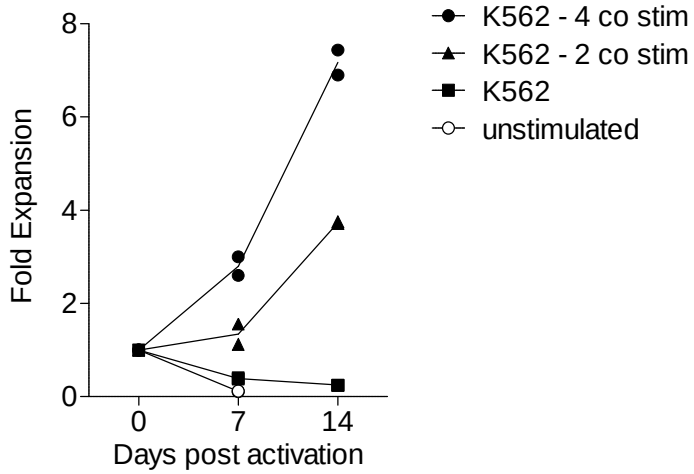
Supplemental Figure 2: Transduction of canine T cells with retroviral vectors encoding HER2-CAR. Canine PMBCs were activated with PHA and K562-APCS and transduced on day 1 through 5 after activation with HER2-CAR retroviral vectors (n=4). Transduction efficiency was determined on day 7.



Supplemental Figure 3: HER2-canCARs with canine transmembrane and signaling domains is functional in canine T-cells. (A) Scheme of human and canine HER2-CAR. **(B)** FACS analysis for HER2-canCAR expression of non-transduced (NT) and transduced (HER2-canCAR) T cells. Open line: Isotype Control; Filled in line: CAR expression.



Supplemental Figure 4: Canine T cells expressing HER2-*can*CAR have cytolytic activity *in vitro.* Canine PBMCs from a healthy dog (A) and a dog with osteosarcoma (B) were expanded *ex vivo,* transduced to express HER2-*can*CAR and then used in a co-culture assay with MDA (human, HER2-), MDA-hHER2 (human, HER2+) and MCKOS (canine, HER2+) at a 2:1 effector-to-target ratio in a 24-well plate. Crystal violet staining was done to assess lytic function of HER2-*can*CAR T cells 24 hours later.



Supplemental Figure 5: Requirement of co-stimulation for the ex vivo expansion of canine T cells. Canine PBMC were activated in the presence of IL21 with PHA in the absence or presence of unmodified K562 (K652), K562-APCs expressing human CD80 and 41BBL (K562 - 2 co-stim) or K562-APCs expressing human CD80, CD83, CD86 and 41BBL (K562 - 4 co-stim) at a 2:1 T-cell:K562 ratio. Cells were counted and re-stimulated on day 7 in the presence of IL21 and IL2 (n=2).