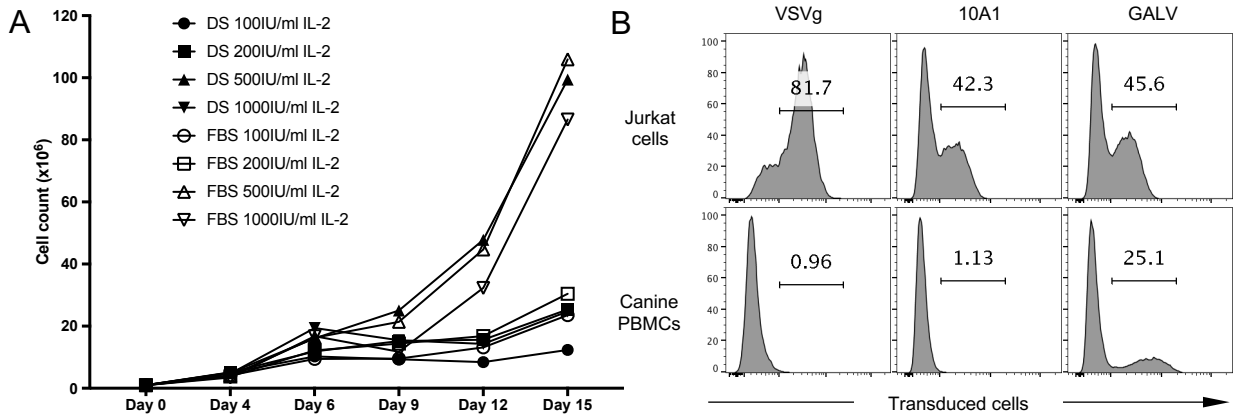
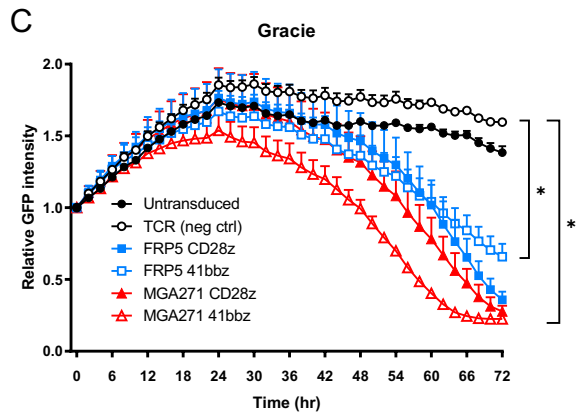
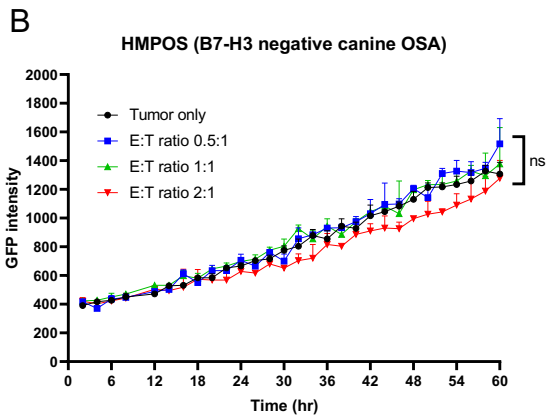
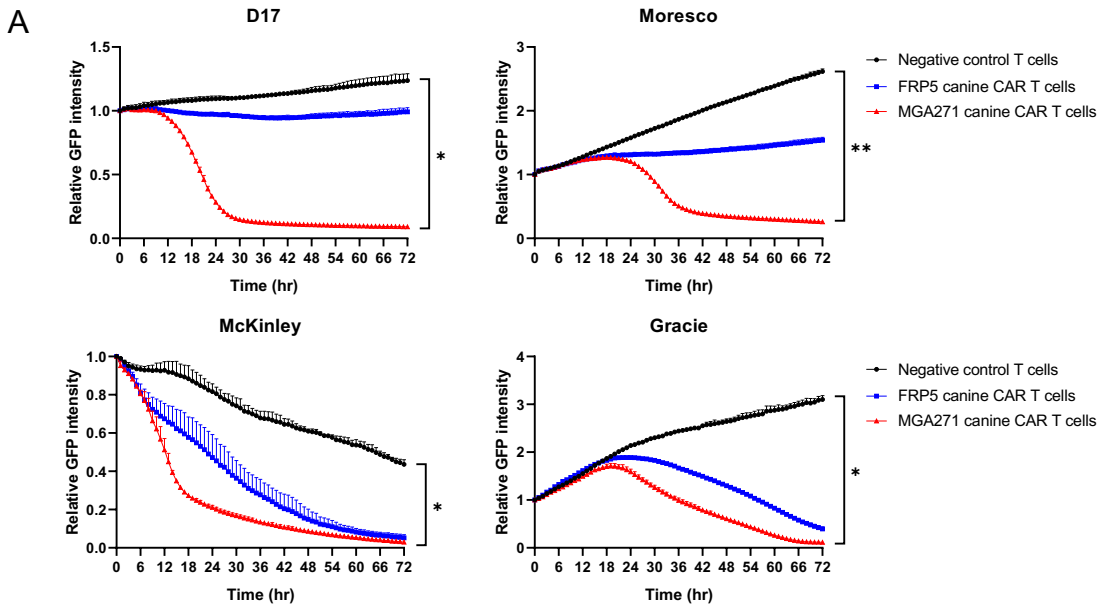


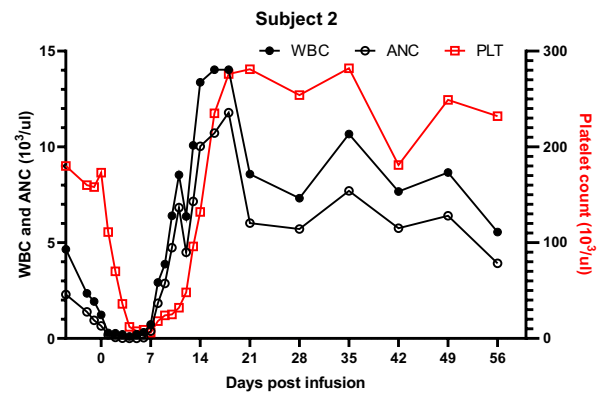
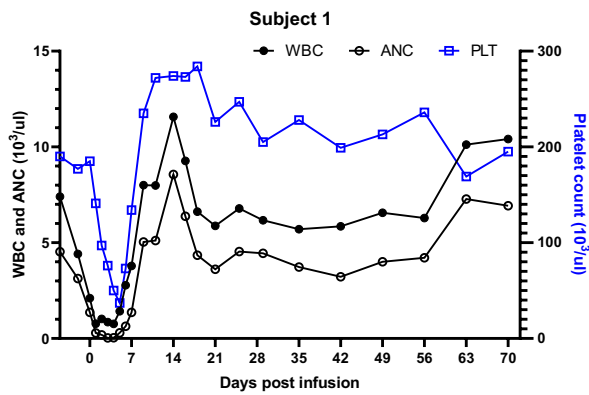
Supplementary Figure S1. Human and canine B7-H3 are highly homologous and are expressed on tumor cells. (A) Amino acid sequences of human B7-H3 and canine B7-H3 were obtained and aligned on the Uniprot website. Pink highlight shows the leader sequences. Yellow highlight shows the transmembrane domains. Full sequences are 93.4% identical. Extracellular domains between the two species have an identity of 95.9%. High level of homology between human and canine B7-H3. (B) Flow cytometry analysis of B7-H3 expression on canine osteosarcoma (OSA) cell lines D17, Moresco, McKinley and Gracie. Human OSA cell lines MG63 and Saos-2 were used as positive control. Human PBMCs were stained at the same time as a negative control.



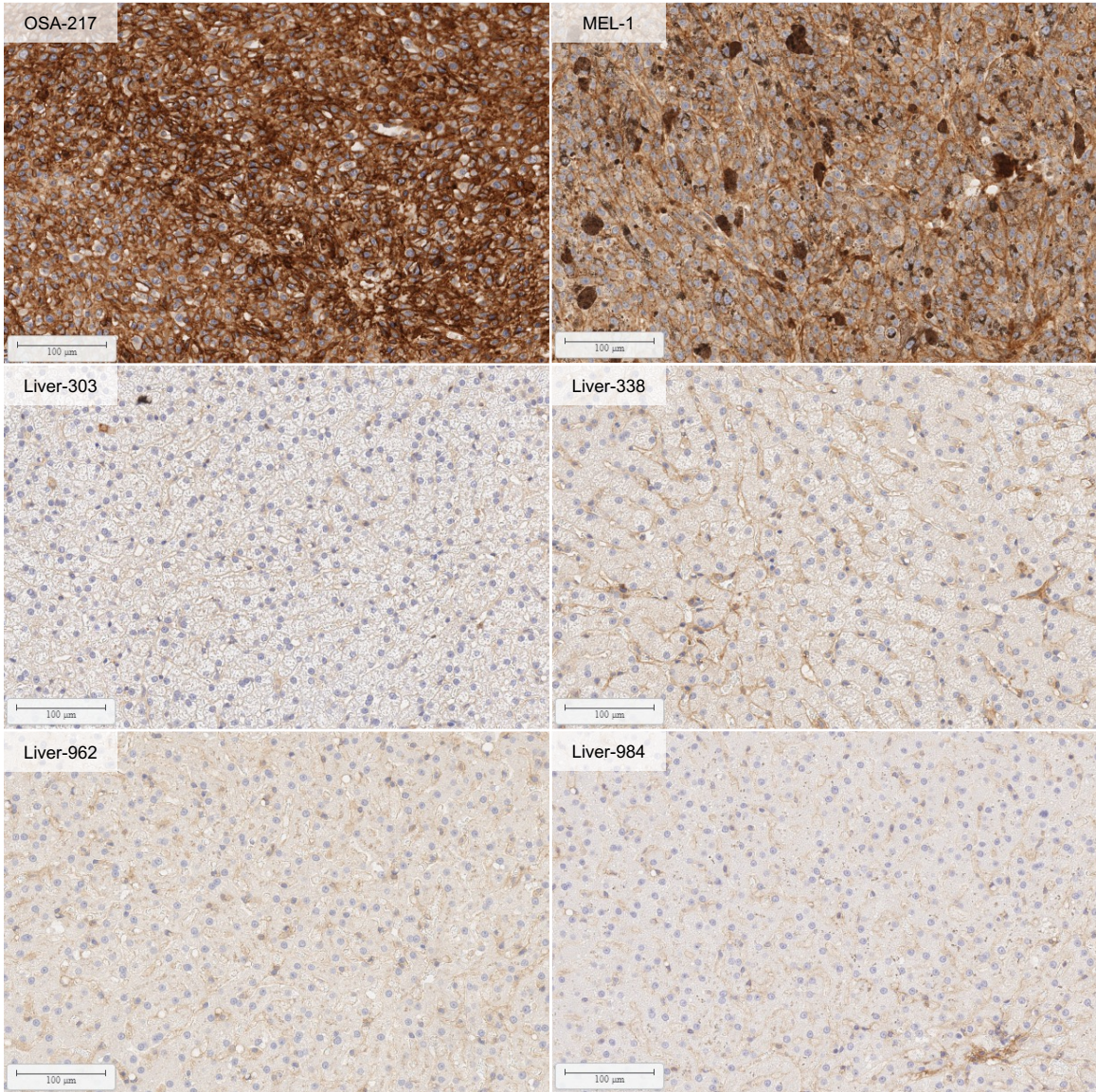
Supplementary Figure S2. Expansion and transduction of canine T cells. (A) Expansion of anti-canine CD3/28 stimulated canine PBMCs in dog serum (DS) or fetal bovine serum (FBS) containing media supplemented with IL2 of different concentrations. (B) Transduction of human Jurkat cells and activated canine PBMCs with a human TCR-encoding retrovirus pseudotyped with VSVg, 10A1 and GALV. TRBV was stained to identify transduced cells. The experiments were done at least twice.



Supplementary Figure S3. MGA271 is specific for canine B7-H3 molecule. MGA271 canine CAR T cells can kill B7-H3 positive canine OSA cells efficiently (A), but not the B7-H3 negative canine OSA cells HMPOS (B). (C) Summary of relative GFP intensity of Gracie spheroids cocultured with different T cells. Samples were measured in duplicate in (A) and triplicate in (B) and (C). Graph shows mean \pm SD. Statistical analysis was performed using the multiple group comparison Holm-Šidák test, * indicates $p < 0.05$, ns: not significant.



Supplementary Figure S4. Longitudinal complete blood count (CBC) results. White blood cells (WBC), absolute neutrophil counts (ANC) and platelets (PLT) were counted throughout the study and depicted in the graph.



Supplementary Figure S5. B7H3 expression on hepatocytes. FFPE liver samples from four healthy canine (303, 338, 962 and 984) were stained for B7H3 by IHC. Two cancer samples OSA-217 and MEL-1 were stained and imaged at the same time as positive controls.