

Figure S1. CD166 expression was significantly increased in osteosarcoma tumor tissues when compared with the adjacent tissues of patients from GEO dataset E-MEXP-3628 (\*\* $P < 0.01$ ).

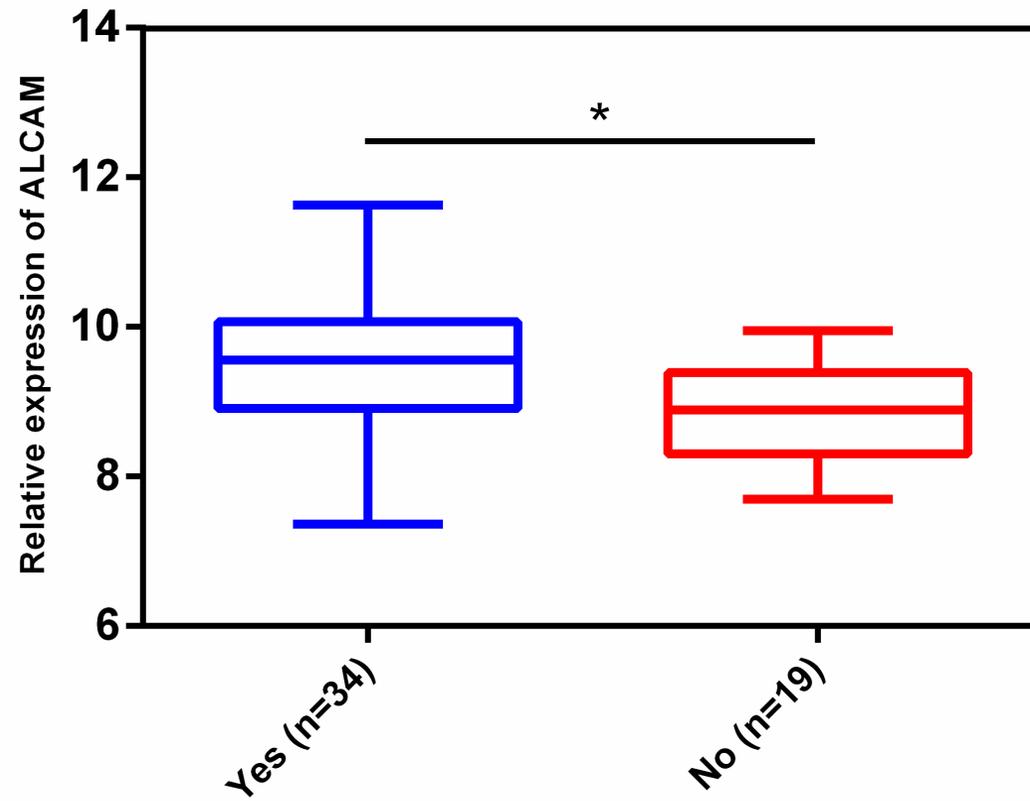


Figure S2. Osteosarcoma patients who developed metastases in five years had significantly higher expression of CD166 than the ones without metastases. Data from ArrayExpress (Access id: GSE21257), \* $P < 0.05$ .

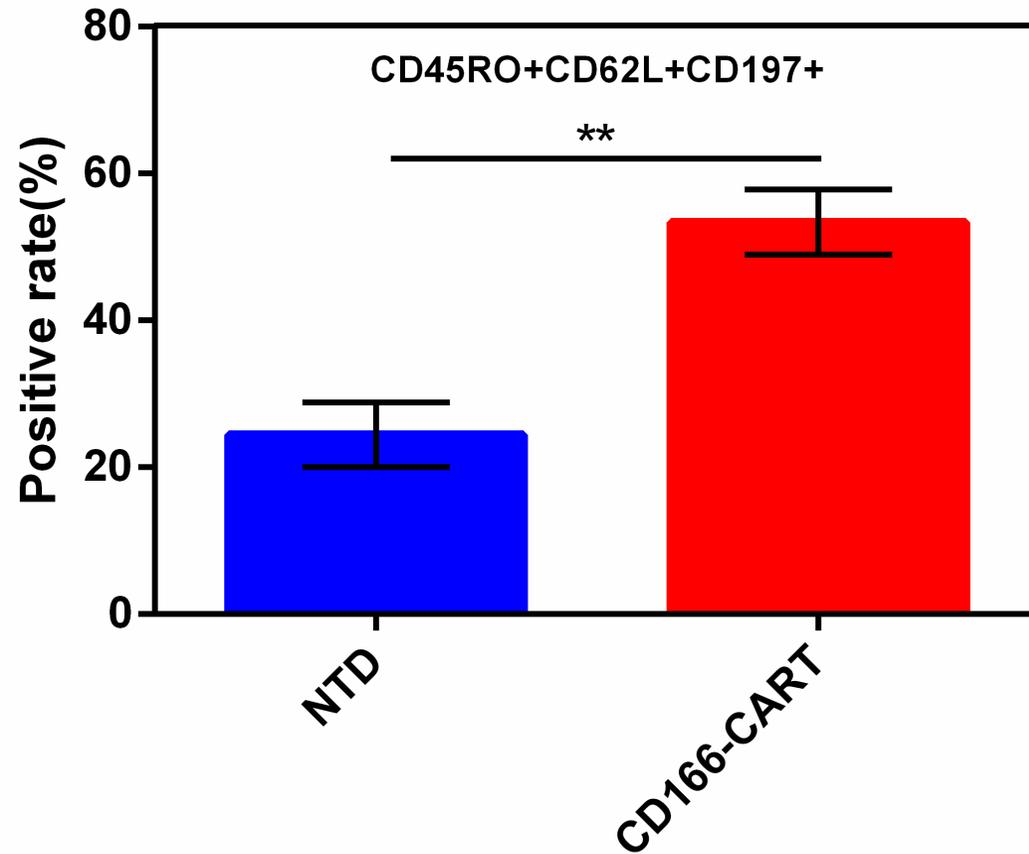


Figure S3. Central memory T phenotypic features of CD166.BB $\zeta$  CAR-T and non-transduced T cells were evaluated by FACS analysis. Mean positive rates  $\pm$  SD from three different T cell lines are shown (\*\* $P < 0.01$ ).



Figure S4. The orthotopic osteosarcoma from different groups were excised and imaged.

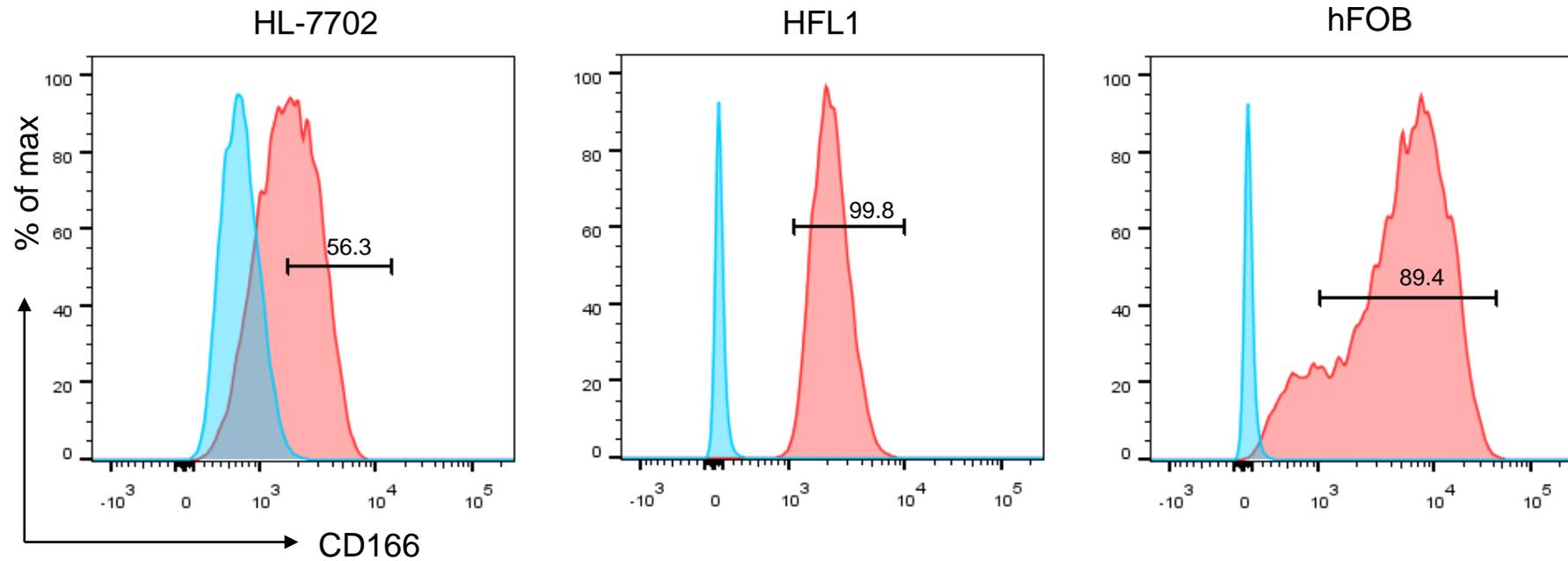


Figure S5. Expression level of CD166 antigen on three normal human cell lines (red histograms). A CD166 isotype antibody was used as a negative control for the detection of CD166 expression (blue histograms). Percentage of positive cells are detailed in the histograms.