



Supplementary Figure 3. Impact of *EGR2* knockout on different T-cell subsets and CD28-based CAR T-cells. **A**, Naive, central memory, effector memory, and effector T-cells were magnetically isolated. PSMA CAR-T cells with *AAVS1* and *EGR2* knockout (KO) were then generated from the T-cell subsets and restimulation experiments were conducted to examine the development of exhaustion and memory. **B**, Expression of exhaustion markers (TIM3⁺LAG3⁺), **C**, cytotoxicity and **D**, expression of early memory markers (CD45RO⁺CD27⁺) after chronic CAR stimulation. **(E-H)** *EGR2* was deleted in PSMA-CD28 based CAR T-cells, and the immunophenotype of CAR T-cells was assessed during the restimulation assay. **E**, Expression of exhaustion markers (TIM3⁺LAG3⁺), **F**, cytotoxicity, **G**, expression of early memory markers (CD45RO⁺CD27⁺) after chronic CAR stimulation and **H**, CAR T-cell expansion capacity during restimulation. All experiments were conducted using T-cells from two independent healthy donors, and panels **(A-H)** are representative data from one donor. (Mann-Whitney test, $n = 4$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns.: not significant.