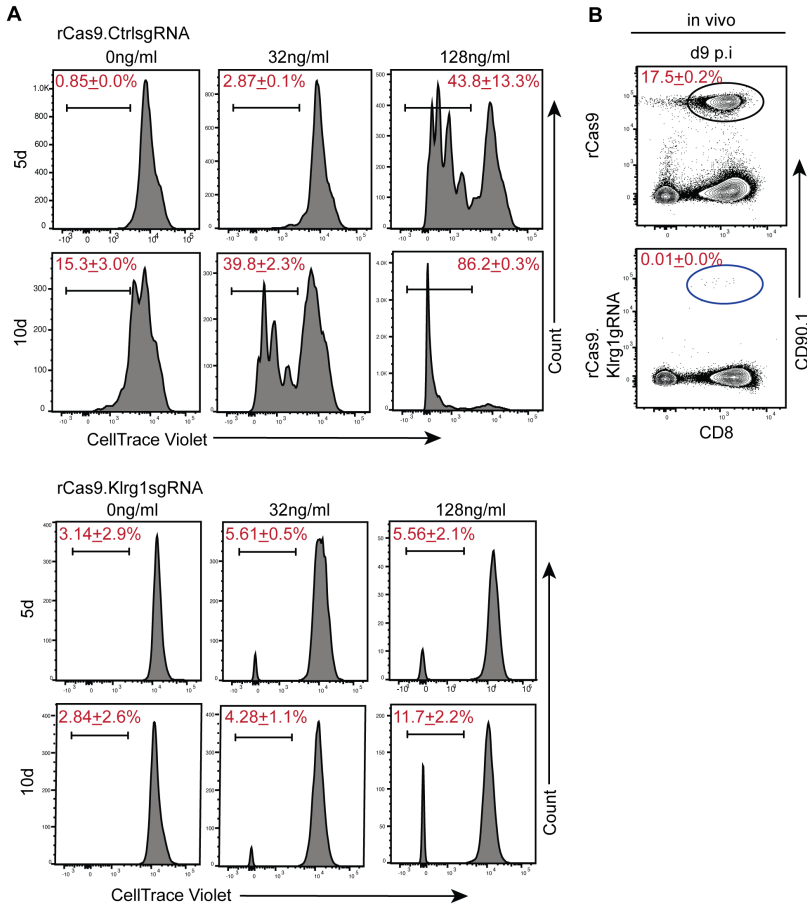
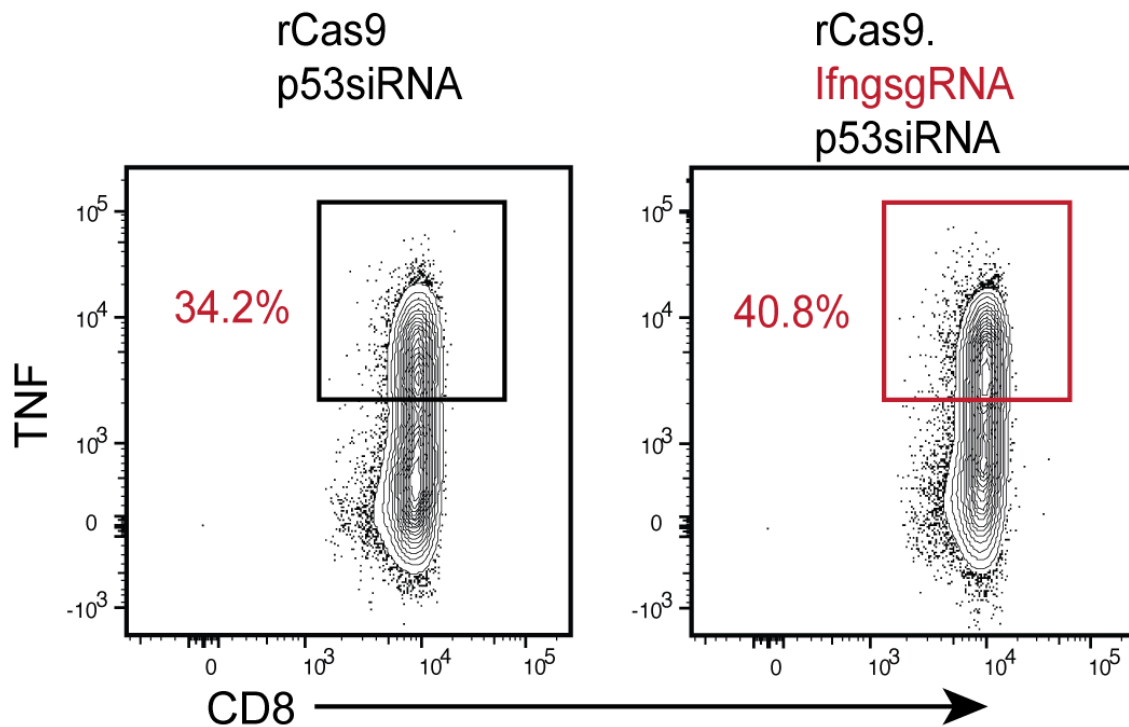


Supplemental Figure 1. Editing genes using CRISPR/Cas9 prevents antigen-driven expansion of memory CD8 T cells. (A) Representative flow-plot depicting frequencies of transfected P14 TCR transgenic (CD90.1⁺; transfected with eGFP-encoded pX458) or endogenous (CD90.1⁻) CD8 T cells in recipient mice 9 days after LCMV challenge. Red histogram indicates the fraction of CD90.1 gated P14 cells that express eGFP. Grey histogram represents the endogenous CD8 T cells in the same recipient. (B-E) Representative flow-plots depicting frequencies of P14 TCR transgenic (CD90.1⁺) CD8 T cells transfected with the indicated constructs and adoptively transferred into recipient B6 mice; 9 days after LCMV challenge, as in Figure 1A. Numbers inset represent the frequency of CD90.1 P14 populations presented as mean ± SEM from at least 2 independent experiments with ≥ 3 recipient mice per group.



Supplemental Figure 2. CRISPR/Cas9 induced DNA damage prevents proliferation of CD8 T cells *in vitro* or *in vivo*

(A) Flow histograms depicting the proliferation of OT-IeGFP CD8 T cells transfected with the indicated reagents, determined by CellTrace Violet dilution at the specified days post transfection, when co-cultured with indicated concentrations of IL-15 and 50ng/ml IL-7. Inset numbers represent the frequencies of expanded cells determined by cell-trace violet dilution, presented as mean ± s.e.m, summarized from 2 experiments. (B) Memory OT-IeGFP memory CD8 T cells were transfected with the indicated reagents and cultured *in vitro* for 10 days before adoptive transfer into recipient B6 mice. Flow plots depict the frequencies of the OT-IeGFP CD8 T CD8 T cells in these recipients challenged with *Lm*-Ova, d9 p.i. Data represent at least 2 separate experiments, with 4 mice/ group.



Supplemental Figure 3. CRISPR/Cas9 mediated loss of IFN γ expression does not alter TNF expression in primary memory CD8 T cells

Flow plots showing the frequency of TNF expressing OT-I TCR transgenic (CD90.1⁺) CD8 T cells transfected with indicated reagents, obtained from recipient B6 mice at d9 p.i, with *Lm-ova* and stimulated with PMA/Ionomycin. Data presented on the representative sample depicted in Figure 3B and is from one of three independent experiments (n > 3 mice per group).