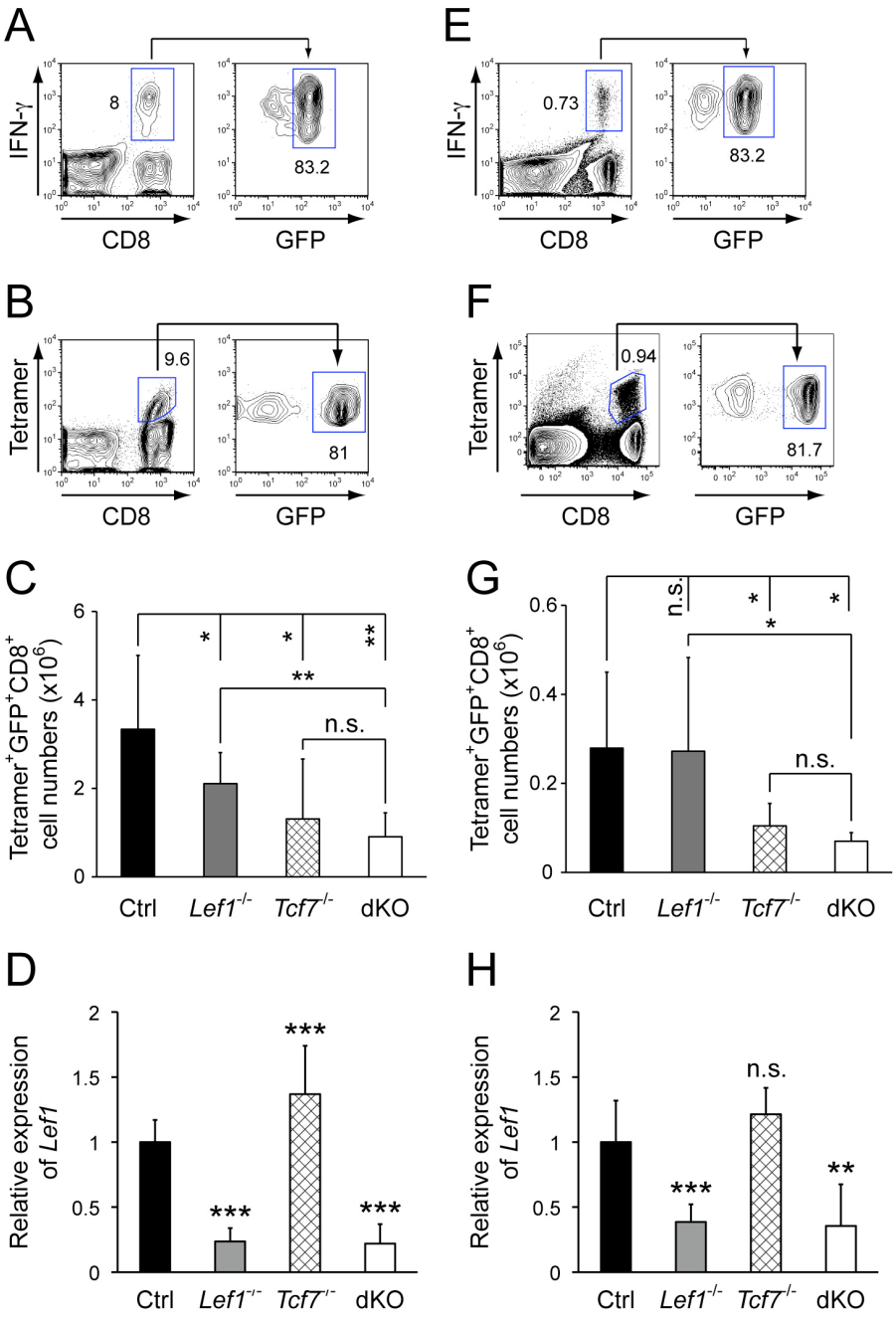
Zhou and Xue. Supplemental Figure S1



Supplemental Figure S1. Detecting antigen-specific CD8$^+$ T cells and assessing *Lef1* excision efficiency at the effector and memory phases.

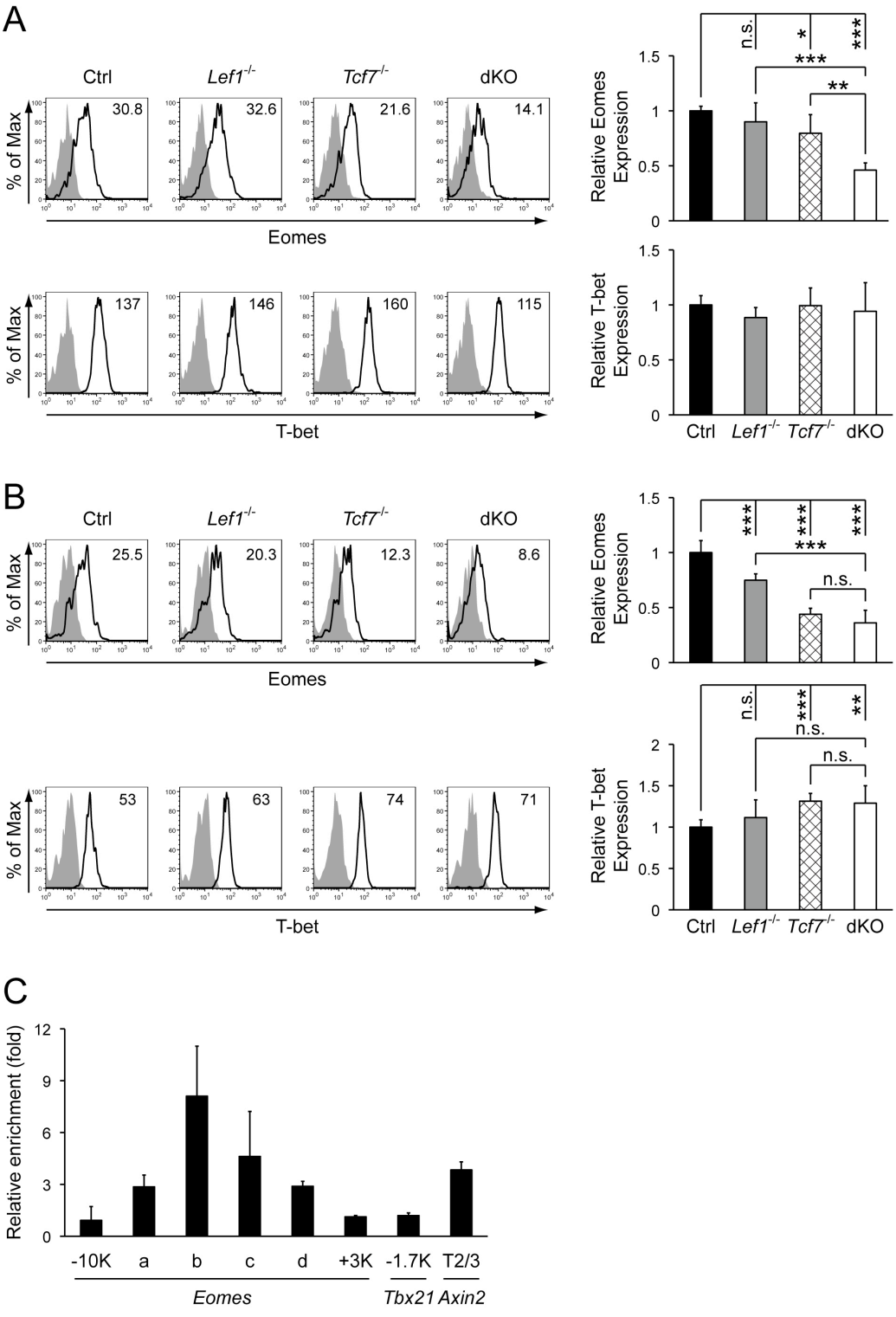
(A) and (E) Detection of Ova-specific CD8$^+$ T cells with peptide-stimulated IFN-γ production. Mice harboring the Gzmb-Cre and Rosa26-EGFP alleles were infected with *actA*$^-$ LM-Ova. To identify Ova-specific T cells with Cre-mediated excision of the floxed *Lef1* sequences, the IFN-γ$^+$CD8$^+$ cells were further fractionated based on GFP reporter expression. IFN-γ$^+$GFP$^+$CD8$^+$ T cells thus identified are defined as Ova-specific CD8$^+$ T cells in this study. The gating strategy is shown in representative contour plots for effector (A, day 7 post-infection) and memory (E, day 35 post-infection) CD8$^+$ T cells in the spleen.

(B) and (F) Detection of Ova-specific $CD8^+$ T cells with SIINFEKL-MHC I tetramer. The tetramer-positive $CD8^+$ T cells were analyzed for GFP expression to identify those with Cre-mediated deletion of the floxed *Lef1* alleles. Detection of the $tetramer^+GFP^+CD8^+$ T cells was used as an alternative means to define Ova-specific $CD8^+$ T cells in this study. The gating strategy is shown in representative contour plots for effector (B, day 7 post-infection) and memory (F, day 35 post-infection) $CD8^+$ T cells in the spleen.

(C) and (G) Numbers of Ova-specific effector and memory $CD8^+$ T cells in the spleen as detected by the tetramer. Mice were infected with LM-Ova, and $tetramer^+GFP^+CD8^+$ T cells were detected and enumerated in the spleen. $CD8^+$ effectors (C) were measured on day 7 post-infection ($n \geq 6$), and memory $CD8^+$ T cells (G) were on day 35 post-infection ($n = 5$). *, $p<0.05$; **, $p<0.01$; n.s., not statistically significant.

(D) and (H) Efficiency of *Lef1* excision in effector and memory $CD8^+$ T cells. Effector (D) and memory (G) $CD8^+$ T cells were detected as in (C) and (G), respectively, and purified by cell sorting. The expression of *Lef1* was assessed by quantitative RT-PCR. The primer sequences are: 5'-tgagtgcacgctaaaggaga (identical to cDNA sequence transcribed from exon 8, which is excised in $Lef1^{-/-}$ or dKO antigen-experienced $CD8^+$ T cells) and 5'-ctgaccagcctggataaagc. Data are pooled results from 2-3 independent experiments ($n \geq 6$ for D, and $n \geq 3$ for H). **, $p<0.01$, ***, $p < 0.001$; n.s., not statistically significant.

Zhou and Xue. Supplemental Figure S2



Supplemental Figure S2. Both TCF-1 and LEF-1 contribute to positive regulation of Eomes in effector and memory CD8$^+$ T cells.

(A) Detection of Eomes and T-bet in CD8$^+$ effectors. On day 7 post-infection, the expression of Eomes or T-bet was determined on IFN-γ^+CD8$^+$ T cells.

(B) Detection of Eomes and T-bet in memory $CD8^+$ T cells. On day 35 post-infection, the expression of Eomes or T-bet was determined on IFN-γ^+CD8^+ T cells.

For both (A) and (B), representative histograms are shown, with shaded area denoting isotype control staining. To quantify the protein expression levels, mean fluorescence intensity (MFI) was determined for Eomes or T-bet antibody staining as well as isotype control staining. The difference in MFI (ΔMFI) was then calculated, as shown in the upper-right corner of each representative histogram. In each experiment, the average of ΔMFI values in control cells was arbitrarily set as 1, and all the ΔMFI values were then normalized to this average value. Cumulative data are pooled from 3 independent experiments and shown as bar graphs on the right in each panel. $n \geq 4$ for (A) and $n \geq 6$ for (B).*, $p<0.05$; **, $p<0.01$; ***, $p < 0.001$; n.s., not statistically significant.

It is of note that due to harsh fixation and permeabilization conditions in the intranuclear staining, the GFP fluorescence was quenched and as a result, the Eomes and T-bet expression was assessed on all IFN-γ^+CD8^+ effector or memory T cells, without discriminating the GFP^+ (*Lef1*-excised) *vs*. GFP^- cells. This may lead to underestimation of the reduced expression of Eomes caused by LEF-1 deficiency. This may also explain the lack of more evident reduction of Eomes in dKO memory $CD8^+$ T cells compared with *Tcf7*$^{-/-}$ cells (B).

(C) LEF-1 and TCF-1 directly bind to the same regulatory regions in the *Eomes* gene locus. Splenic $CD8^+$ T cells were isolated from WT mice by positive selection. The cells were cross-linked and sonicated to generate chromatin fragments, followed by immunoprecipitation with an LEF-1 antibody or IgG control. Enriched LEF-1 binding at indicated locations was assessed by quantitative PCR. We previously showed that TCF-1 is associated with four upstream regulatory regions (marked as "a-d", within the "–3.6 kb to –0.8 kb" regulatory sequences) in the *Eomes* gene (Ref. 14). The –10 kb and + 3 kb regions in *Eomes*, and –1.7 kb region in the *Tbx21* gene (encoding T-bet) were used as negative controls. *Axin2* is a known Wnt target genes, and its T2/3 region was used as a positive control. The PCR-detected signals for each test region were first normalized to an irrelevant region in the *Rag2* gene in the same ChIP sample. The value thus obtained in the LEF-1 ChIP sample was then normalized to that in control IgG sample to calculate the relative enrichment for each test region. Data are pooled results from 3 independent experiments where each sample was measured in duplicates or triplicates. All primer sequences are in Ref. 14.