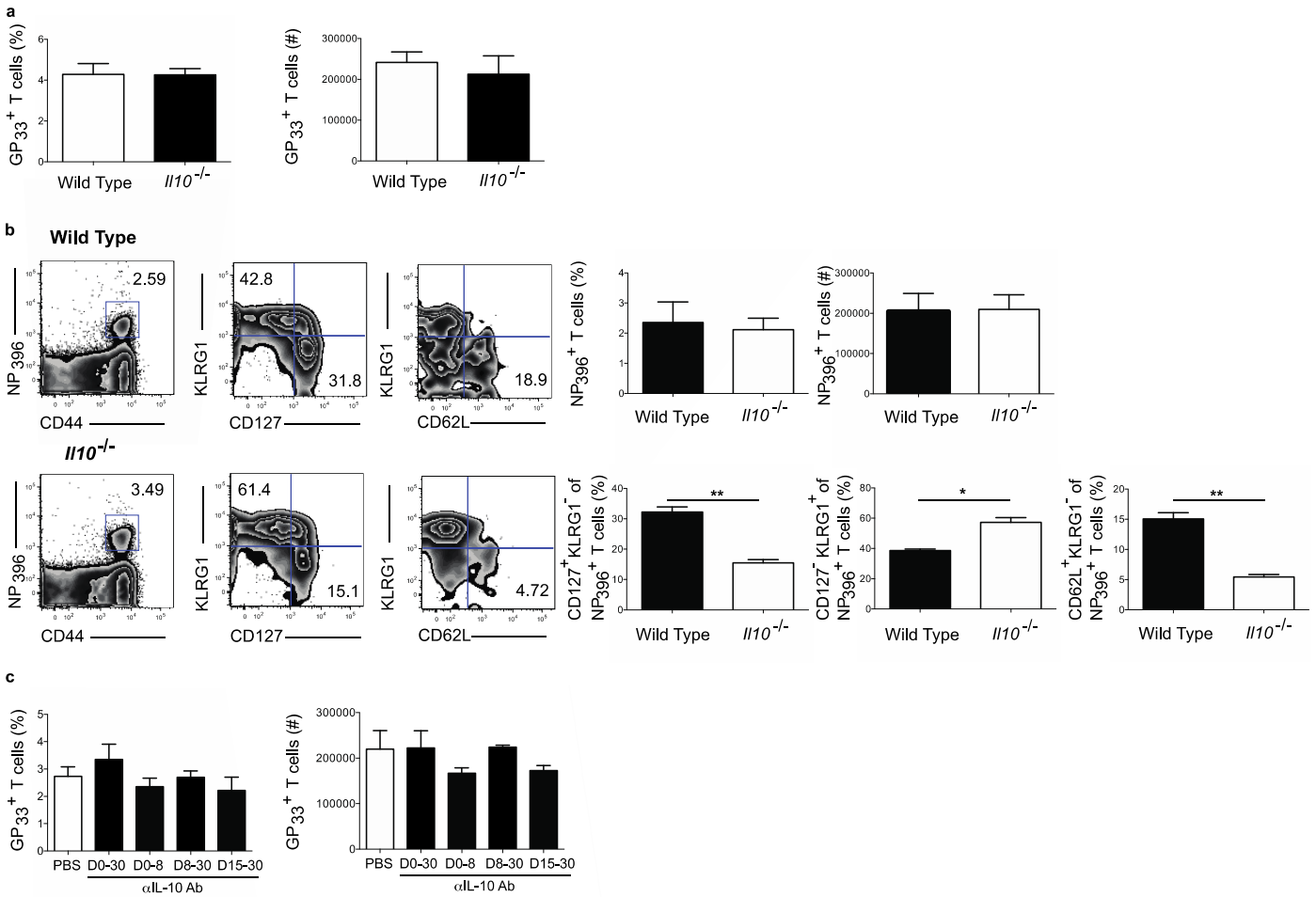
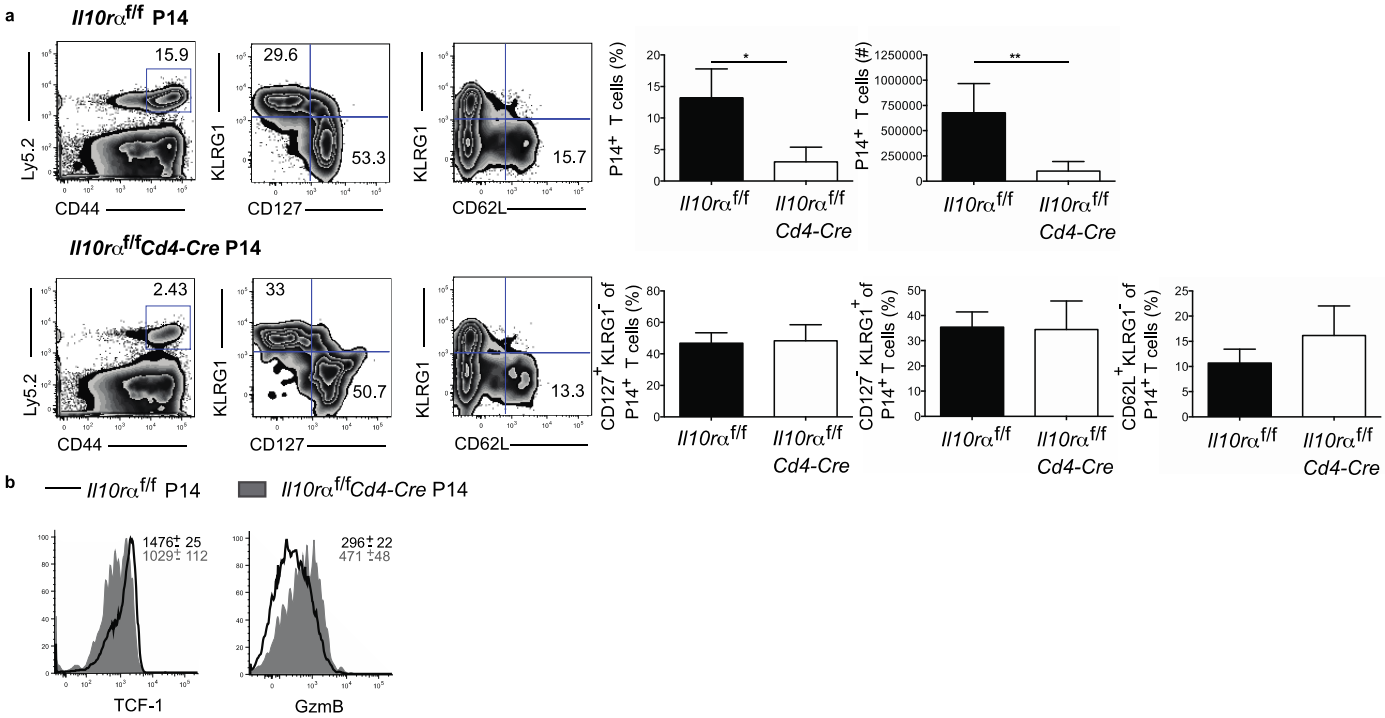


Supplementary Figure 1

IL-10 is required for optimal maturation of memory CD8$^+$ NP396$^+$ T cells.

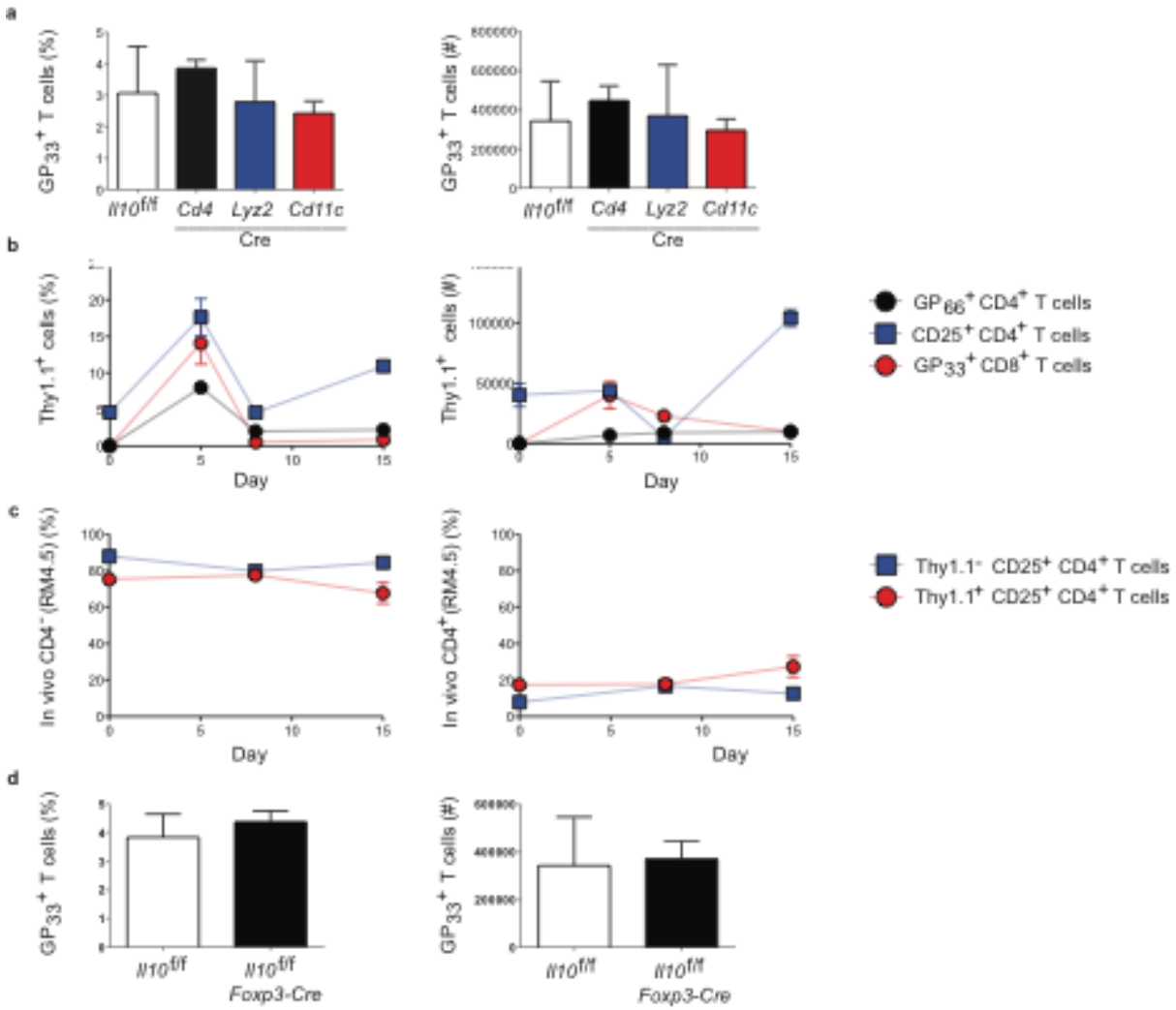
(**a**) Analysis of the virus-specific CD8$^+$ T cell response 60 days post acute LCMV Armstrong infection. The percentages and numbers of GP$_{33}^+$ T cells along with the (**b**) representative plots and analysis of the NP$_{396}^+$ T cell response in the spleen of wild type and *Il10*$^{-/-}$ mice are shown. Percentages and numbers of NP$_{396}^+$ T cells, along with the percentages of CD127$^+$KLRG1$^-$, CD127$^-$KLRG1$^+$, and CD62L$^+$KLRG1$^-$ cells in the NP$_{396}^+$ T cell population are shown. (**c**) Mice were infected with acute LCMV and treated with αIL-10 between days 0-30, 0-8, 8-30, 15-30 or mock injected with PBS. Mice were sacrificed at day 30 and the percentage and numbers of GP$_{33}^+$ T cells determined. Statistical analyses were performed using the unpaired two-tailed Student's *t*-test. (*, $p < 0.01$; **, $p < 0.001$). Data are from one experiment representative of 5 experiments (a, b) or 3 experiments (c) with at least 4 mice per group carried out 45-60 days (a, b) or 30 days (c) following LCMV Armstrong infection (mean and s.e.m).



Supplementary Figure 2

IL-10 is required in a CD8$^+$ T cell–extrinsic and CD8$^+$ T cell–intrinsic manner to allow the maturation and survival of memory CD8$^+$ T cells.

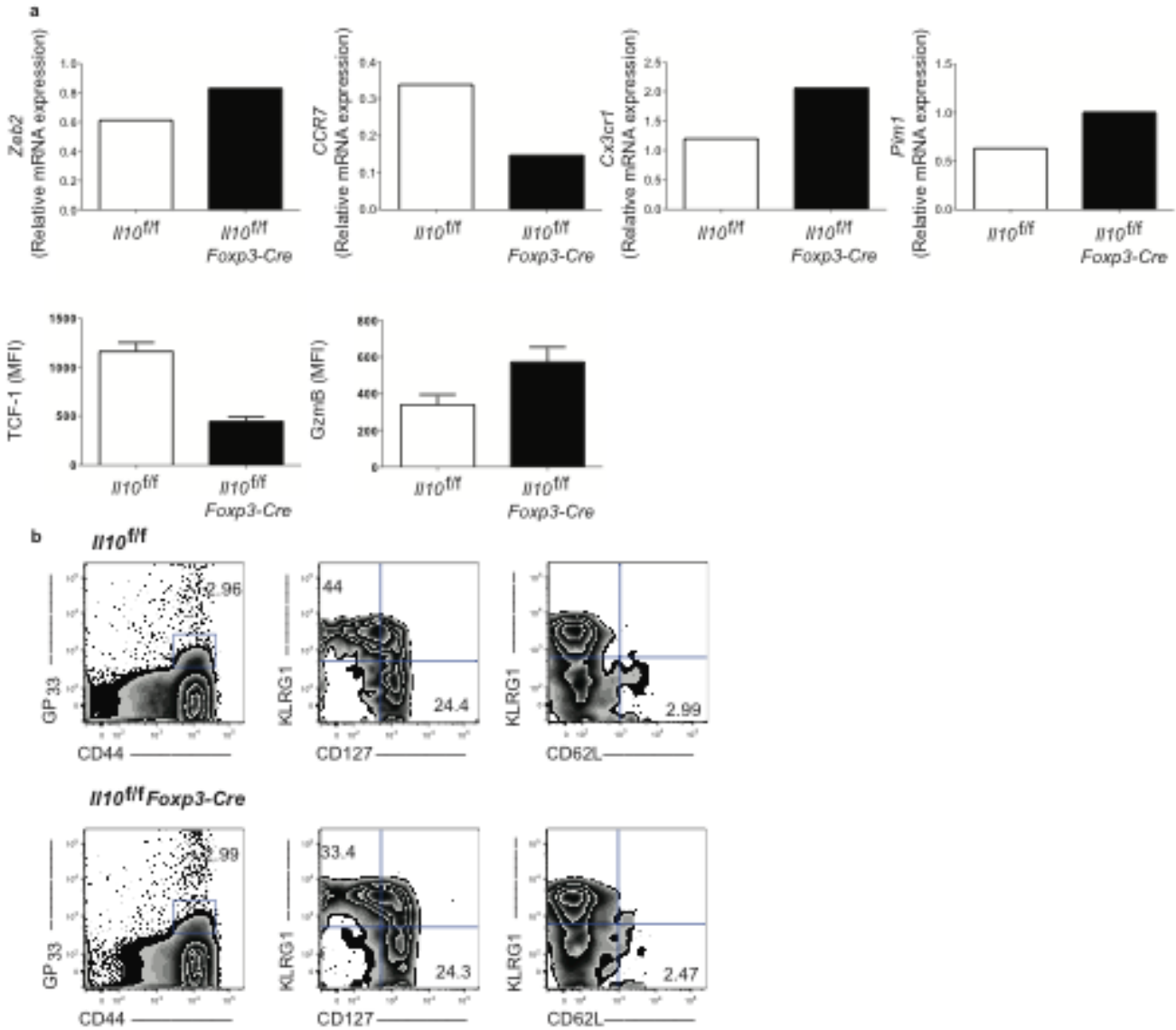
50,000 *Il10ra*$^{f/f}$ or *Il10ra*$^{f/f}$*Cd4*-Cre P14 GP$_{33}^+$ cells were transferred into congenically-mismatched mice one day prior to infection with acute LCMV infection. Analysis of the P14 GP$_{33}^+$ T cell response was carried out 30 days p.i. **(a)** Representative plots and analysis of the P14 GP$_{33}^+$ T cell response. Percentages and numbers of P14 GP$_{33}^+$ T cells, along with the percentages of CD127$^+$KLRG1$^-$, CD127$^-$KLRG1$^+$, and CD62L$^+$KLRG1$^-$ cells in the P14 GP$_{33}^+$ T cell population are shown. (**b**) Representative histograms of GzmB and Tcf1 expression in *Il10ra*$^{f/f}$ (black) or *Il10ra*$^{f/f}$*Cd4*-Cre (gray filled) P14 GP$_{33}^+$ cells. Statistical analyses were performed using the unpaired two-tailed Student's *t*-test. (*, $p < 0.05$; **, $p < 0.01$). Data are from one experiment representative of 2 experiments with 4 mice per group carried out 30 days following LCMV Armstrong infection (mean and s.e.m).



Supplementary Figure 3

CD4$^+$ T$_{reg}$ cells continue to produce IL-10 during the resolution phase of infection.

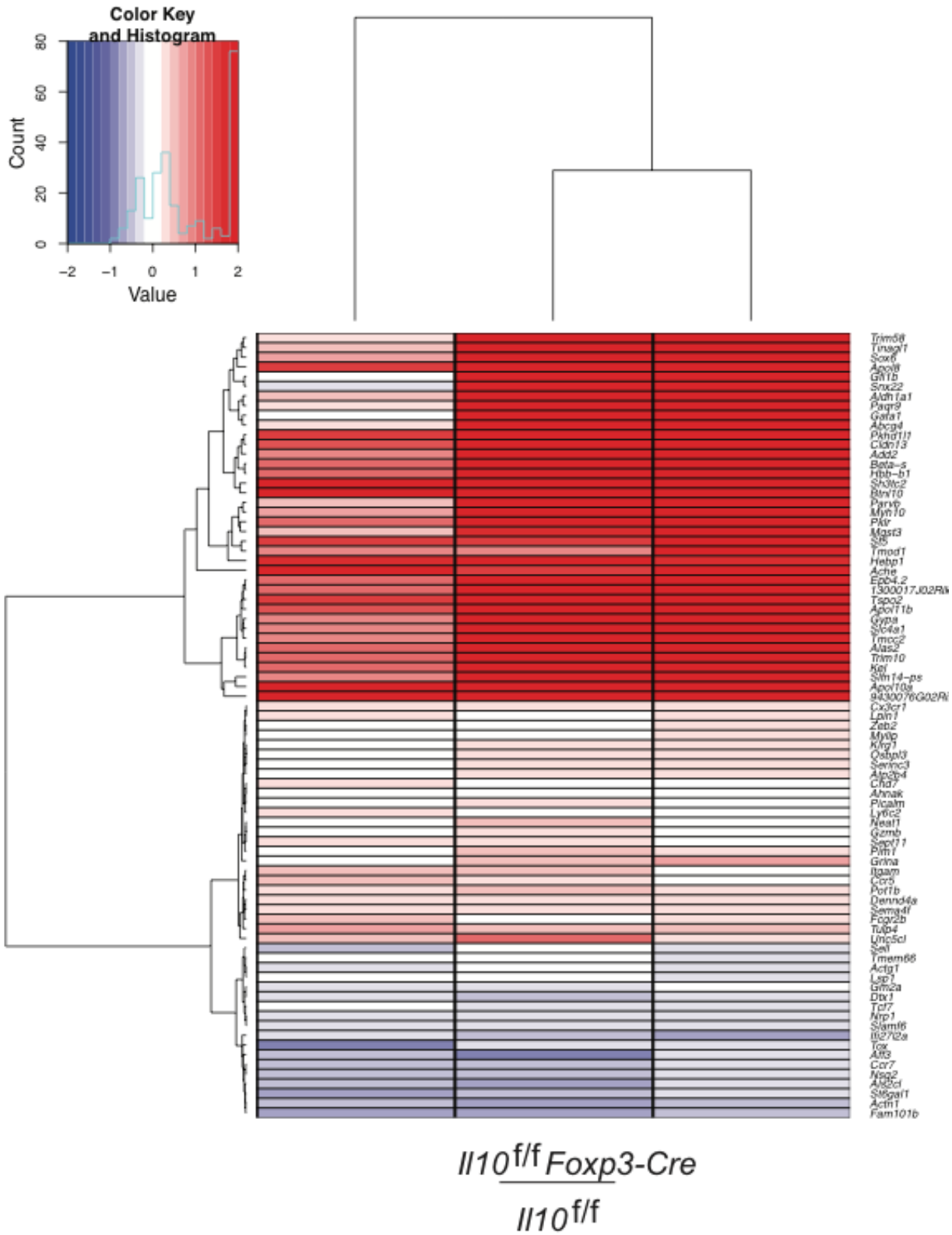
(**a**) Analysis of the GP$_{33}^+$ T cell response 60 days post acute LCMV infection in *Il10*$^{f/f}$, *Il10*$^{f/f}$*Cd4*-Cre, *Il10*$^{f/f}$*Lyz2*-Cre, and *Il10*$^{f/f}$*Cd11c*-Cre mice. Percentages and numbers of GP$_{33}^+$ T cells were determined. **(b)** Analysis of IL-10 production by T cells following acute LCMV infection in IL-10 reporter (10BiT Thy1.1 mice). Percentages and numbers of IL-10-Thy1.1 reporter-positive cells at multiple time points post LCMV infection are shown. (**c**) Intravenously (i.v.) administered anti-CD4 antibody was used to distinguish circulating (red pulp localized) versus resident (white pulp localized) CD4$^+$ T cells. **(d)** Analysis of the GP$_{33}^+$ T cell response 60 days post acute LCMV Armstrong infection. Percentages and numbers of GP$_{33}^+$ T cells in *Il10*$^{f/f}$ and *Il10*$^{f/f}$ *Foxp3*-Cre mice are shown. Data are from one experiment representative of 3 experiments (a, b, c, d) with 3-6 mice per group carried out 45-60 days (a, d), or 0, 8, and 15 days (b, c) following LCMV Armstrong infection (mean and s.e.m).



Supplementary Figure 4

Validation of RNA-seq results.

(**a**) Expression of *Zeb2*, *Ccr7*, *Cx3cr1*, and *Pim1* was determined by qPCR analysis of cDNA isolated from pooled GP_{33}^{+} and NP_{396}^{+} $CD8^{+}$ T cells at 15 days post acute LCMV infection from $Il10^{f/f}$ and $Il10^{f/f}$ *Foxp3*-Cre mice. Expression of TCF-1 and GzmB in GP_{33}^{+} in T cells at 15 days post acute LCMV infection from $Il10^{f/f}$ and $Il10^{f/f}$ *Foxp3*-Cre mice was determined by flow cytometric analysis. (**b**) Representative plots of the GP_{33}^{+} T cell response in $Il10^{f/f}$ and $Il10^{f/f}$ $Foxp3^{Cre}$ mice at day 15 post LCMV infection. Data are from one experiment representative of 3 experiments with 3-5 mice per group carried out 15 days following LCMV Armstrong infection (mean and s.e.m).



$Il10^{f/f}\,Foxp3\text{-}Cre$

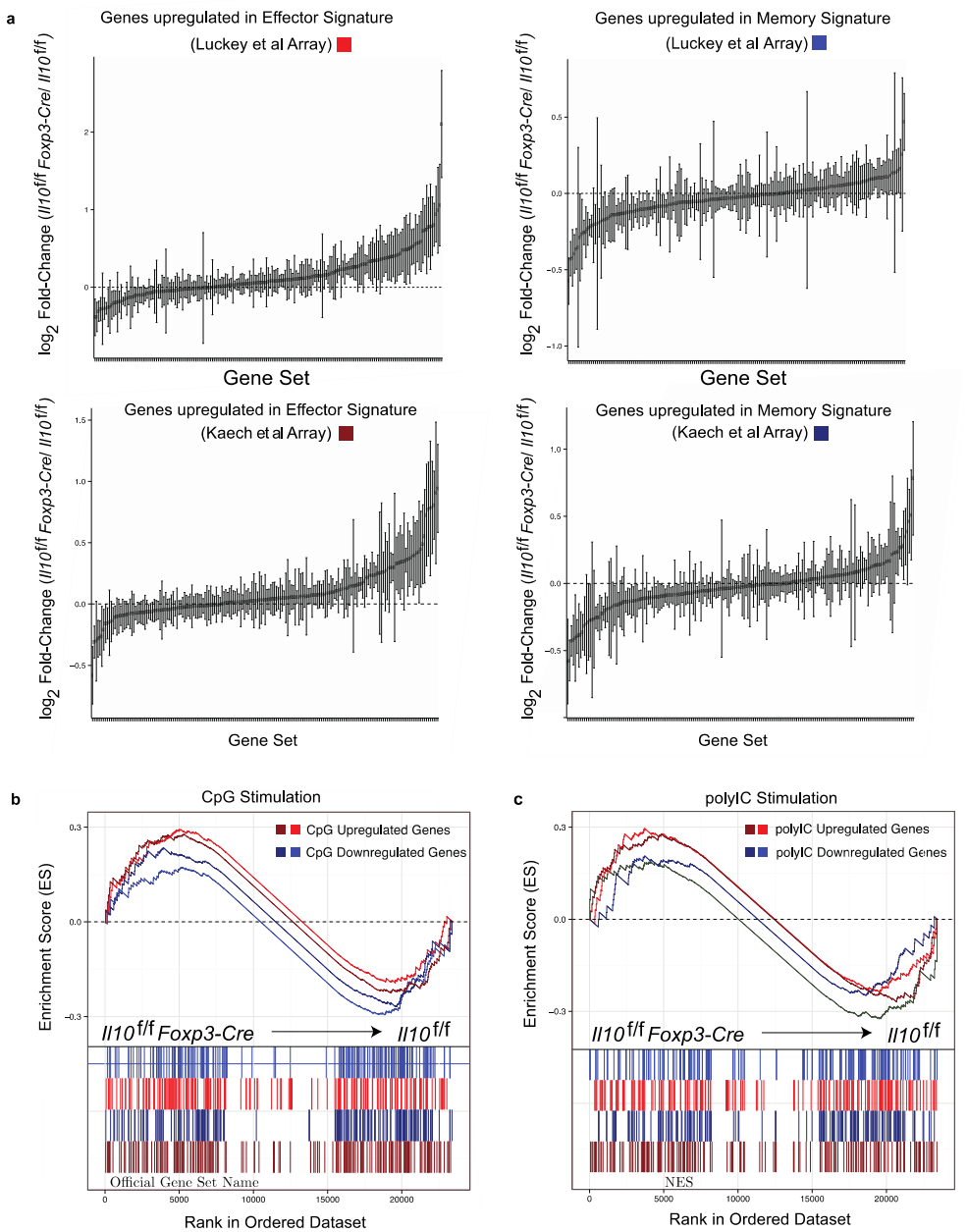
$Il10^{f/f}$

Nature Immunology: doi:10.1038/ni.3224

Supplementary Figure 5

Heat map of differentially expressed genes based on RNA-seq results.

Genes with a p-adjusted value < 0.2 (Benjami-Hochberg) and the corresponding $\log_2$ fold-change in mRNA isolated from pooled GP_{33}^+ and NP_{396}^+ $CD8^+$ T cells at 15 days post acute LCMV infection from *Il10*$^{f/f}$ and *Il10*$^{f/f}$ *Foxp3*-Cre mice.



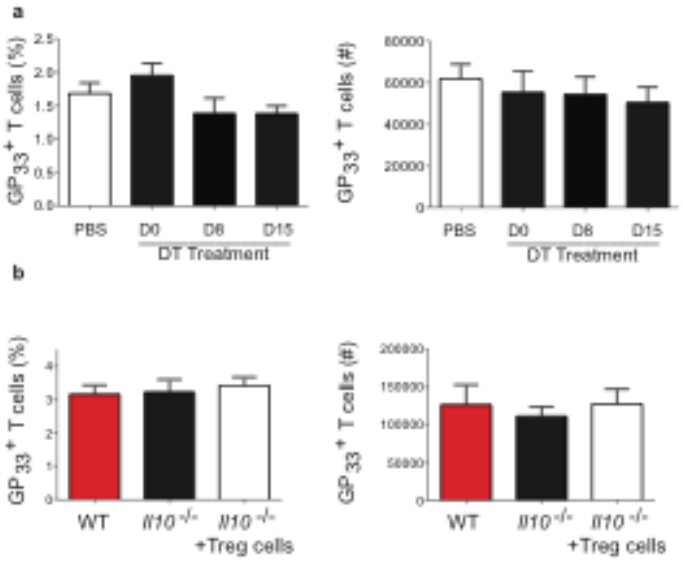
d

5.B	GOLDRATH_EFF_VS_MEMORY_CD8_TCELL_UP	1.41
	GOLDRATH_EFF_VS_MEMORY_CD8_TCELL_DN	-2.04
	KAECH_DAY8_EFF_VS_MEMORY_CD8_TCELL_UP	1.6
	KAECH_DAY8_EFF_VS_MEMORY_CD8_TCELL_DN	-1.56
5.C	GSE15930_NAIVE_VS_24H_IN_VITRO_STIM_INFAB_CD8_TCELL_UP	-1.49
	GSE15930_NAIVE_VS_24H_IN_VITRO_STIM_INFAB_CD8_TCELL_DN	1.36
5.D	GSE15930_STIM_VS_STIM_AND_IL-12_48H_CD8_T_CELL_UP	-1.82
	GSE15930_STIM_VS_STIM_AND_IL-12_48H_CD8_T_CELL_DN	1.41
S6.B	GSE17721_CTRL_VS_CPG_6H_BMDM_UP	-1.46
	GSE17721_CTRL_VS_CPG_6H_BMDM_DN	1.49
	GSE17721_CTRL_VS_CPG_4H_BMDM_UP	-1.34
	GSE17721_CTRL_VS_CPG_4H_BMDM_DN	1.46
S6.C	GSE17721_CTRL_VS_POLYIC_2H_BMDM_UP	-1.63
	GSE17721_CTRL_VS_POLYIC_2H_BMDM_DN	1.73
	GSE17721_CTRL_VS_POLYIC_1H_BMDM_UP	-1.35
	GSE17721_CTRL_VS_POLYIC_1H_BMDM_DN	1.73

Supplementary Figure 6

Virus-specific $CD8^+$ T cells from mice lacking $CD4^+$ T_{reg} cell–derived IL-10 display a robust inflammatory and effector gene signature.

mRNA was isolated from pooled GP_{33}^+ and NP_{396}^+ $CD8^+$ T cells at 15 days post acute LCMV infection from *Il10*$^{f/f}$ and *Il10*$^{f/f}$ *Foxp3*-Cre mice and compared by RNA-seq. **(a)** Gene set plots showing individual $\log_2$ fold-changes of with corresponding standard error based on published effector vs memory gene set [1,2]. Gene Set Enrichment Analysis (GSEA) was performed using gene sets from the Broad MSigDB collection; select significantly enriched gene sets (FDR < 1e-5) are shown with their running Enrichment Score (ES) (line), where members of the gene set appear in the ranked list of genes (barcode), and the signal to noise ranking metric (bar). A positive ES signifies enrichment in the *Il10*$^{f/f}$ *Foxp3*-Cre sample relative to the WT condition of a given gene set; *i.e.*, more highly expressed. (**b**) GSEA results of CpG **(c)** and poly:IC stimulated genes (bottom) were visualized [3,4]. (**d**) Normalized enrichment scores for Gene Set Enrichment Analysis. Normalized enrichment scores (NES) was calculated for select significantly enriched gene sets (FDR < 1e-5). Gene set name, figure GSEA plots shown in, and NES are shown in table.



Supplementary Figure 7

Transfer of IL-10-competent CD4$^+$ T_{reg} cells during the resolution phase of LCMV infection is sufficient to 'rescue' the maturation defect of memory CD8$^+$ T cells in *Il10*$^{-/-}$ mice.

Analysis of the GP_{33}^+ T cell response 60 days p.i. in *Foxp3*$^{GFP\text{-}DTR}$ mice treated with diphtheria toxin at day -1, day 8, or day 15 p.i. or mock injected with PBS. **(a)** Percentage and numbers of GP_{33}^+ T cells are shown. Representative of 3 independent experiments with 3-6 mice per group carried out 45-60 days following LCMV Armstrong infection. **(b)** Analysis of the GP_{33}^+ T cell response 60 days post acute LCMV Armstrong infection in *Il10*$^{-/-}$ mice and *Il10*$^{-/-}$ mice that were administered 3x10^5 *Foxp3*$^+$ CD4$^+$ T cells isolated from coinfected *Foxp3*$^{GFP\text{-}DTR}$ mice at day 8 p.i. Percentage and numbers of GP_{33}^+ T cells are shown. Representative of 2 independent experiments with 3-7 mice per group carried out 60 days following LCMV Armstrong infection. Data are from one experiment representative of 3 experiments (a) or 2 experiments (b) with 3-7 mice per group carried out 45-60 days following LCMV Armstrong infection (mean and s.e.m).

Nature Immunology: doi:10.1038/ni.3224