Supplemental Experimental Procedures

Animals

The targeting construct harboring mutations in the miR-155 target site in the 3' UTR of the SOCS1 gene was generated using recombineering. For detailed information see the National Cancer Institute recombineering website (http://redrecombineering.ncifcrf.gov/). Mice on a B6 genetic background with the germ-line transmission were bred to Cre deleter mice to remove the neomycin resistance cassette. miR-155^{KO} and C57BL/6J mice were purchased from the Jackson Laboratories. *SOCS1^{fl}* mice and Klra8^{KO} (Ly49H-deficient) mice were kindly provided by A. Yoshimura and S. Vidal, respectively. All the mice were bred and housed in the specific-pathogen-free animal facility at the Memorial Sloan-Kettering Cancer Center and used in accordance with institutional guidelines.

EAE

Mice were immunized s.c. with 100μg MOG_{35-55} emulsified with complete Freund's adjuvant plus 200ng pertussis toxin in 500ul PBS. Animals were monitored 3 times a week for disease symptoms until the experiment terminated, usually 25 days post induction and classified into one of five grades of severity: grade 1 - no tail tone and the tail drops when the mouse is picked up by the base of the tail; grade 2 - tail drop and weakness in the rear limbs, usually evident by an uneven gait; grade 3 - rear limb paralysis; grade 4 - rear limb paralysis and weakness in the forelimbs; grade 5 - moribund. In some experiments, mice were harvested at the peak of the disease (~14 days after EAE induction) followed by FACS analysis.

In Vitro T cell Proliferation and Th17 Polarization Assays

To measure the recall response to MOG antigen, splenic $CD4^+$ T cells from mice subjected to EAE induction were co-cultured with CD11c bead-sorted DCs pulsed with different concentrations of MOG_{35-55} peptides for 3 days. T cell proliferation was

assessed by with ^{3}H-TdR incorporation (cpm) in triplicate during the last 8 hr of culture. For *in vitro* Th17 polarization assay, FACS sorted CD4^{+}CD25^{-}CD62L^{hi} naïve T cells (2x10^{5}) were stimulated with αCD3 (1 μg/ml) in the presence of irradiated T cell-depleted splenocytes (1x10^{6}) as APCs plus TGFβ (2ng/ml) and IL-6 (20ng/ml) for 4 days. IL-17 production was assessed by intracellular FACS analysis.

Quantitative PCR and Gene Expression Profiling Analysis

Total RNA was extracted with TriZOL reagent (Invitrogen) from FACS-purified cells. cDNA was synthesized with Superscript III Reverse Transcriptase (Invitrogen), followed by real-time PCR analysis (SYBR green; Applied Biosystems).

For gene expression profiling analysis, CD45.2^{+} miR-155^{KO} or SOCS1^{KI} or WT OT-1 TCR-tg CD8^{+} T cells or NK cells were adoptively transferred into CD45.1^{+} WT or Ly49H-deficient hosts, respectively, prior to infection with MCMV-OVA or MCMV. OT-1 CD8^{+} T cells or Ly49H^{+} NK cells were FACS-sorted on d4 post infection and poly-A RNA-sequencing was performed using 3 biological replicates for each cell type analyzed using an Illumina HiSeq2500 platform. Genes with FDR < 5% and the absolute value of log2 fold change more than 0.75 under both conditions were considered as similarly regulated in miR-155^{KO} and SOCS1^{KI} cells compared to WT controls, while genes with FDR < 5% in only one condition and absolute value of log2 fold change less than 0.75 in the other condition were considered as miR-155^{KO} specific or SOCS1^{KI} specific. Each type of regulated genes can be further separated into up-regulated and down-regulated categories based on the direction of regulation. RNA-sequencing data are available from NCBI under accession number GSE68511.

Figure S1 (related to Figure 1). Generation of mice harboring mutations in the putative miR-155 target site in the 3' UTR of the SOCS1. Schematic representation of the SOCS1 targeting strategy.

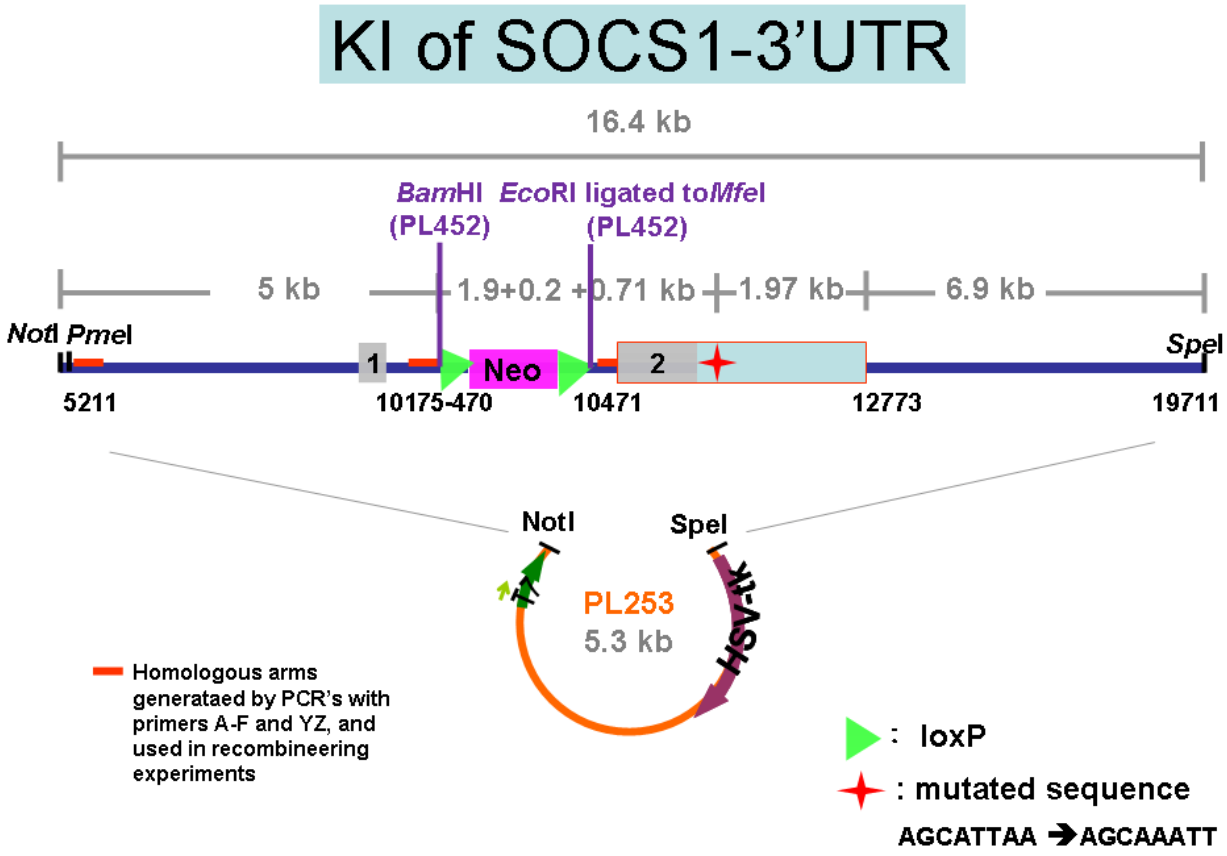

Figure S2 (related to Figure 1). Specific disruption of miR-155-mediated regulation of SOCS1 by mutations in the 3' UTR of the *Socs1* gene. (A) Retroviral miR-155, miR-19 or empty vectors equipped with a GFP reporter were expressed in T cells from SOCS1^{KI}, miR-155^{KO} or WT mice. GFP^{+} cells were sorted 4 days after retroviral transduction and the amounts of SOCS1 were assessed. **(B)** Relative reductions in SOCS1 protein amounts in T cells overexpressed with different miRNAs compared to corresponding T cell populations with empty vector transduction were shown. The data are representative of 2 independent experiments (n = 2).

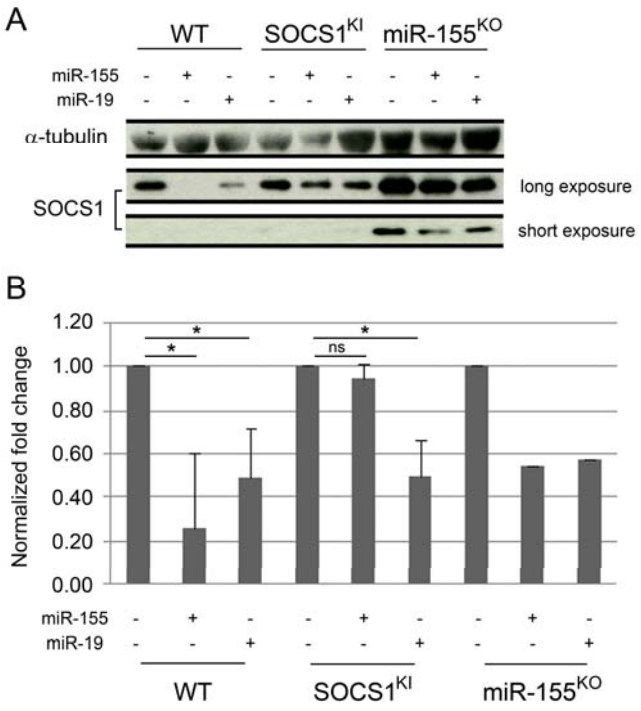

Figure S3 (related to Figure 2). SOCS1 deficiency does not rescue reduced miR-155^{KO} Treg cell numbers. FACS analysis of **(A)** thymus and **(C)** spleen from WT, miR-155^{KO}, *Foxp3^{cre}SOCS1$^{fl/fl}$* (SOCS1^{cKO}) or miR-155^{KO}SOCS1^{cKO} (DKO) mice. **(B,D)** Cellularity of the thymus and spleen and the proportion and absolute numbers of thymis and splenic Foxp3^{+}CD4^{+} T cells in indicated mice are shown. The data are shown as mean ± SD and are representative of 4 independent experiments (n = 5–12).

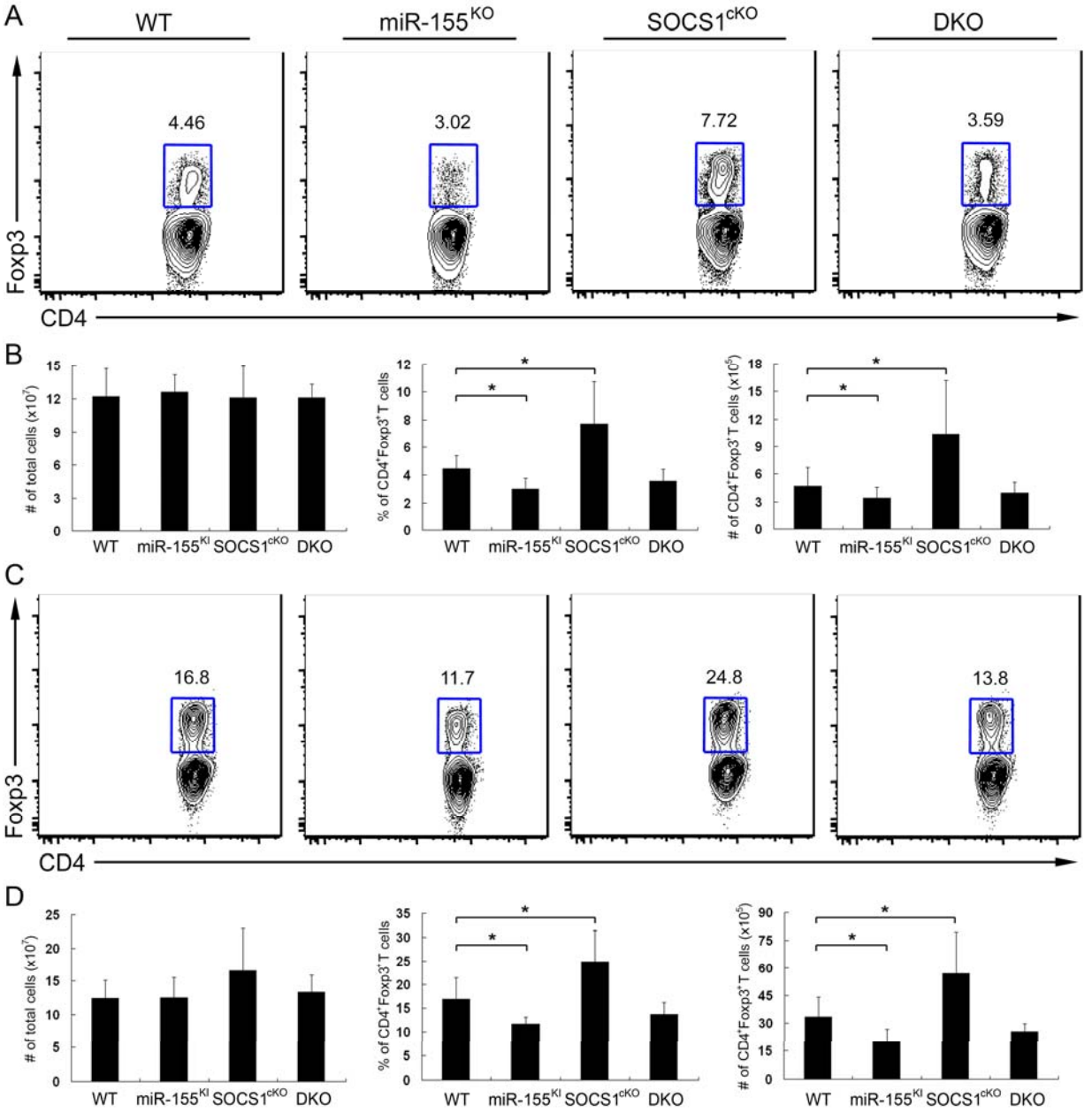

Figure S4 (related to Figure 4). Comparable production of proinflammatory cytokines by SOCS1^{KI} DCs. qPCR analysis of IL-6 expression in CD11c+ DCs isolated from WT, SOCS1^{KI} or miR-155^{KO} mice in the presence or absence of LPS stimulation. The data are shown as mean ± SD and are pooled of 4 independent experiments (n = 7–8).

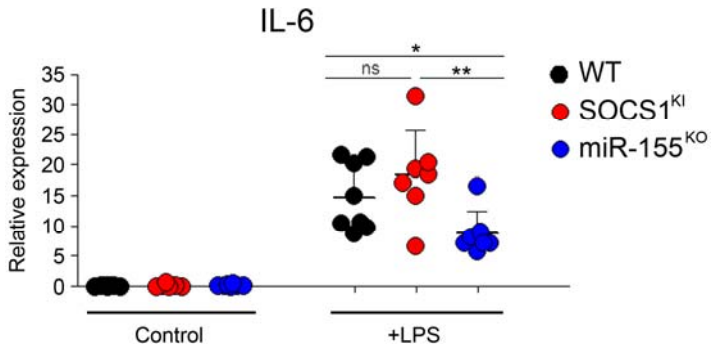

Figure S5 (related to Figure 4). Lack of miR-155-mediated SOCS1 regulation did not diminsh Th17 polarization *in vitro*. $CD4^+CD25^-CD62L^{hi}$ naive T cells isolated from WT, $SOCS1^{KI}$ and $miR\text{-}155^{KO}$ mice were cultured *in vitro* under Th17 polarizing condition. **(A)** FACS analysis and **(B)** frequencies of $IL\text{-}17^+$ in $CD4^+$ T cells were shown. The data are shown as mean ± SD and are representative of three independent experiments (n = 9).

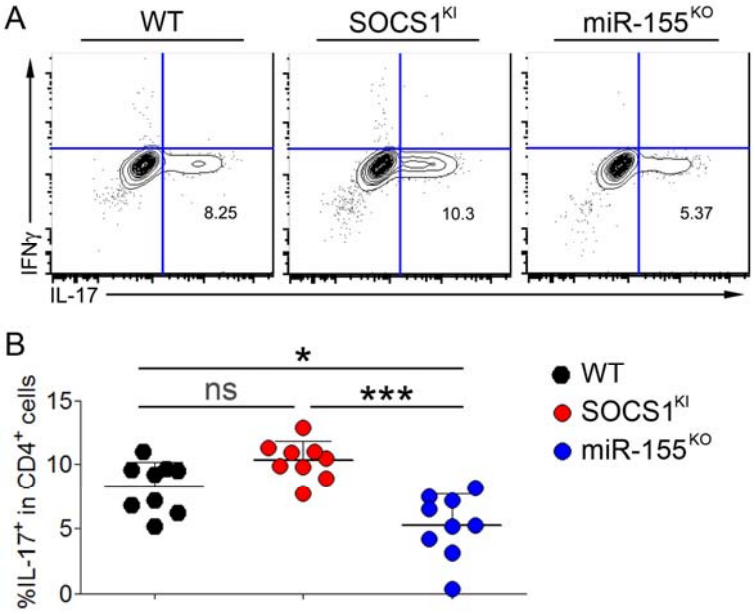

Figure S6 (related to Figure 7). Severe defect of miR155-deficient T cell response in both acute and chronic LCMV infection. CD45.2^{+} miR-155^{KO} or SOCS1^{KI} and CD45.1^{+}/CD45.2^{+} naïve CD8^{+} P14 TCR-tg T cells (10^{4} each) were cotransferred into CD45.1^{+} wt hosts prior to infection with LCMV Armstrong (Arm) or Clone 13. Figure shows representative FACS plots and ratios of P14 T cells on d8 pi.

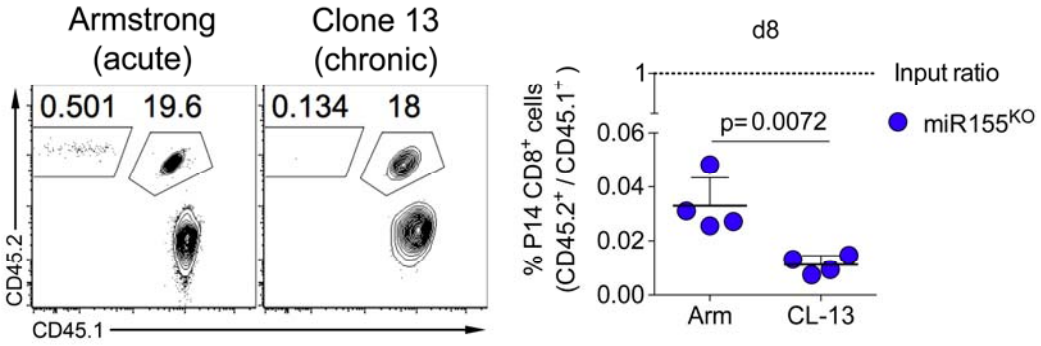

Figure S7 (related to Figure 7)..Unimpeded terminal differentiation and memory inflation of MCMV-specific SOCS1^{KI} T cells. Mixed BM chimeras were infected with MCMV and MCMV-m38 tetramer binding CD8^{+} T cells were analyzed on d40 pi for expression of differentiation markers CD127 and KLRG-1