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Supplemental Information

The downregulation of IL-18R defines bona fide kidney-resident $CD8^+$ T cells

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Supplemental Figures

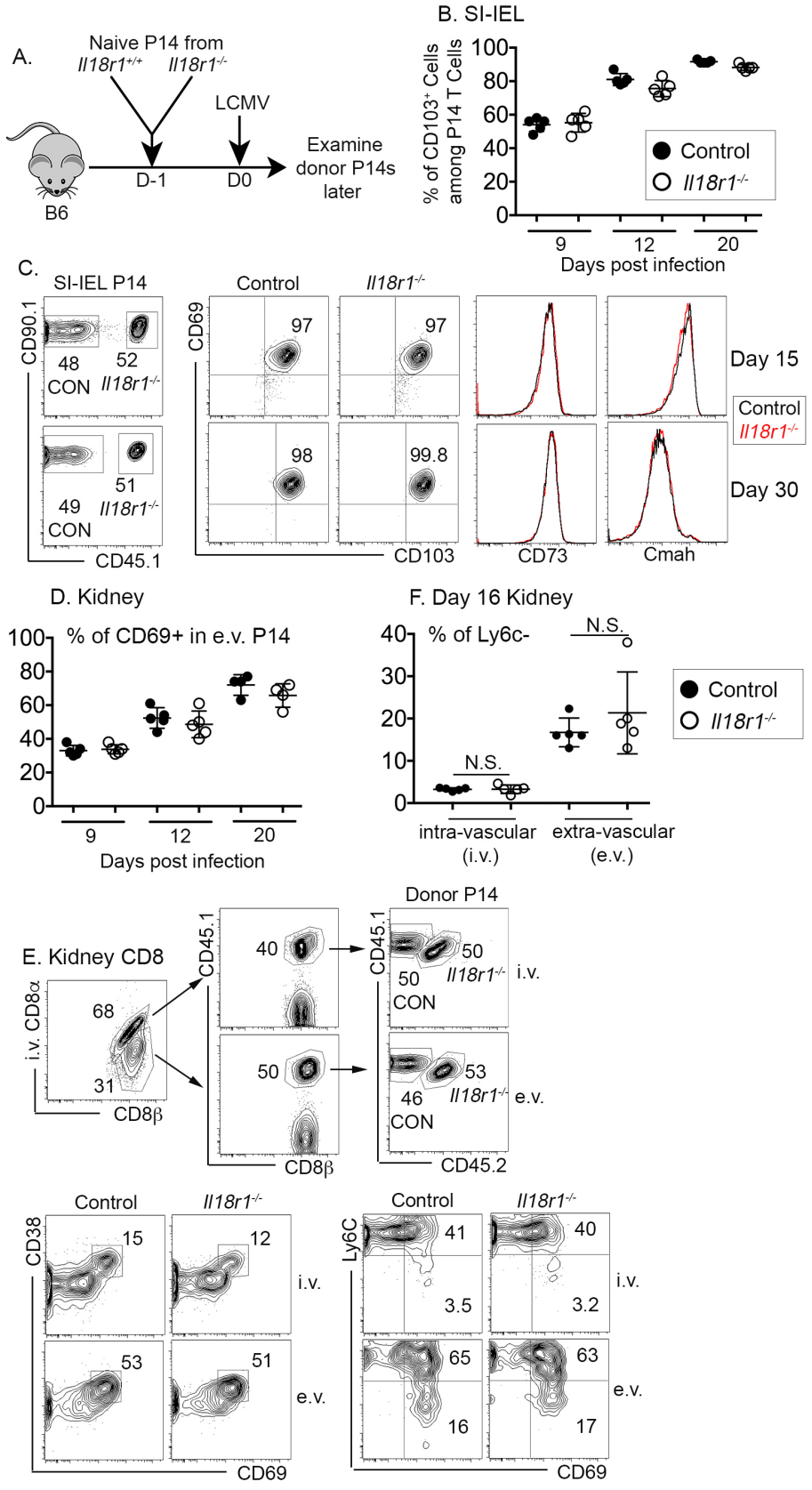

Figure S1. Apparently normal differentiation of Trms in the absence of IL-18R, Related to Figure 1 and Figure 2. (**A**) Experimental design. (**B**) At different time points after infection, the percentage of $CD69^{+}CD103^{+}$ cells among SI-IEL P14 T cells is shown. (**C**) Representative FACS profiles of SI-IEL P14 T cells at day 15 (upper) or day 30 (lower) after infection. (**D**) The percentage of $CD69^{+}$ cells among e.v. kidney P14 T cells is shown. (**E**) Representative FACS profiles of kidney P14 T cells at day 16 post infection. (**F**) The percentage of $Ly6C^{-}$ cells among indicated kidney P14 populations is shown. Each symbol in (**B**), (**D**) and (**F**) represents the results from an individual recipient mouse. Combined results from four independent experiments are shown. No statistical significance was detected at any time points between control and *Il18r1*$^{-/-}$ cells by Student *t*-test.

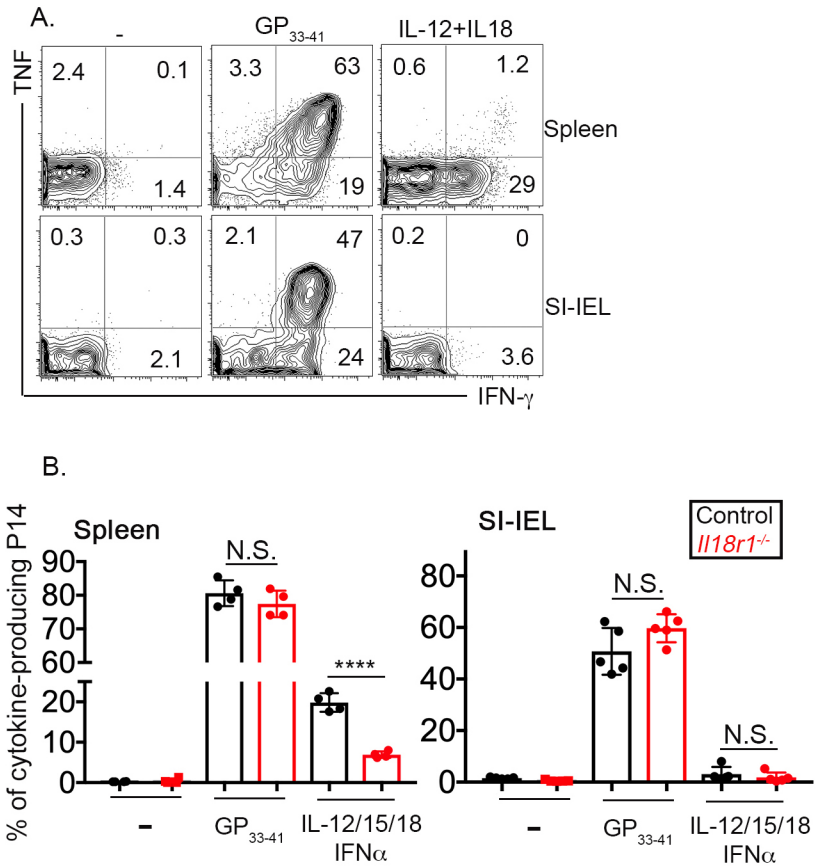

Figure S2. Downregulation of IL-18R is associated with the loss of response to bystander inflammation, Related to Figure 2. (**A**) Same experimental setup as in Figure 1 and 2. Day 30 post infection, splenic P14 and SI-IEL P14 were isolated and cultured for 4 hours ex vivo stimulation in the presence of Golgi STOP. Cytokine production was determined by intracellular FACS staining. Representative FACS profiles of pre-gated live P14 T cells are shown (n=5). (**B**) Same experimental setup as in Figure S1. Day 30 post infection, splenic and SI-IEL P14 were isolated and subjected to ex vivo stimulation and IFN-γ production was measured by intracellular FACS staining. Each symbol represents the results from an individual mouse. N.S., not significant and ****, p<0.0001 by Student *t*-test.

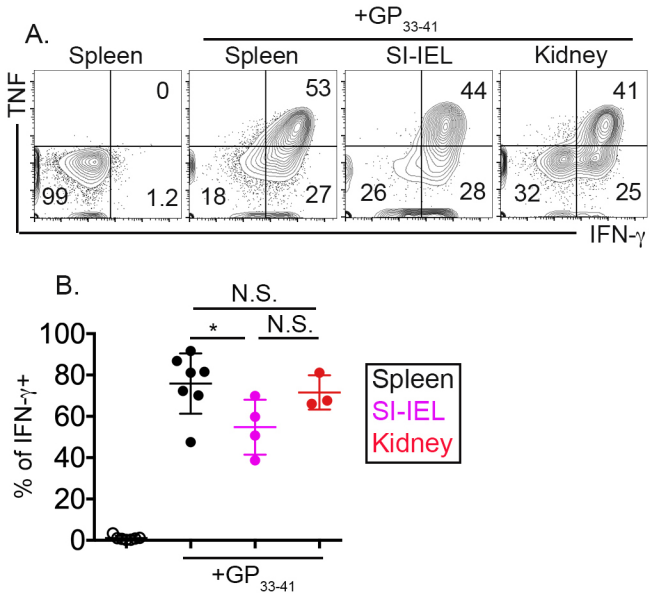

Figure S3. In vitro recall response of Trm, Related to Figure 3. Same experimental setup as in Figure 1. Day 30 post infection, P14 T cells were isolated and cultured with GP_{33-41} peptide in the presence of Golgi STOP for 4 hours. Cytokine production was measured by intracellular FACS staining. (**A**) Representative FACS profiles of pre-gated live P14 T cells are shown. (**B**) The percentage of IFN-γ^+ P14 T cells. Each symbol in (B) represents the results from an individual mouse. N.S., not significant and *, $p<0.05$ by One-way ANOVA with Tukey multi comparison post-test. Pooled results from 3 independent experiments are shown in (B).

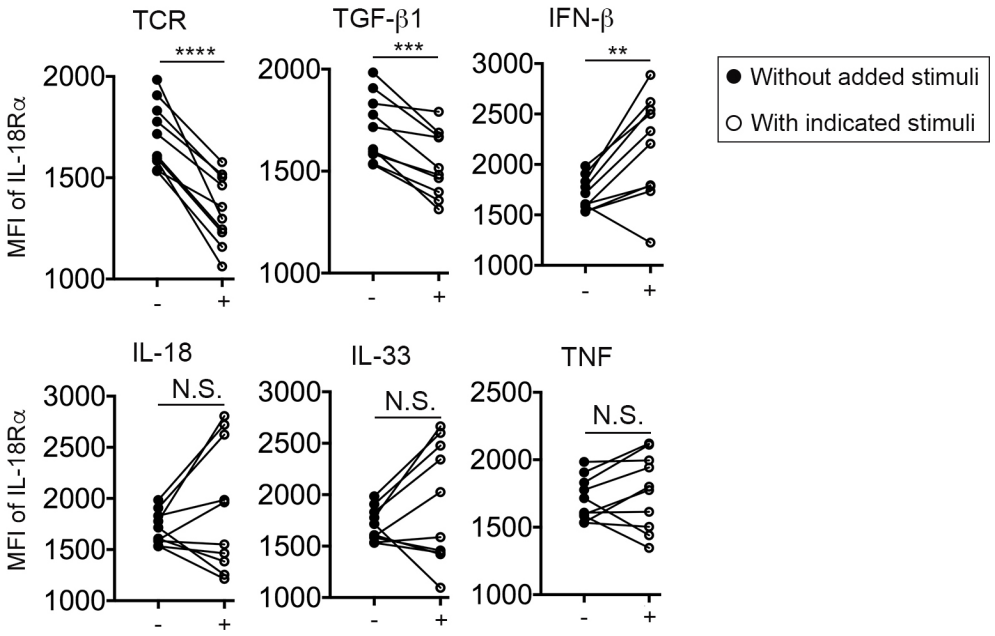

Figure S4. TGF-β and Type I IFN impact IL-18R expression of early effector T cells, Related to Figure 5. Naïve P14 T cells were adoptively transferred into B6 recipients followed by LCMV Arm infection. Day 4.5 post infection, splenic P14 T cells were isolated and cultured overnight with indicated stimuli: TCR (1μg/ml GP_{33-41}), 50ng/ml hTGF-β1, 20ng/ml IFN-β, 20ng/ml IL-18, 20ng/ml IL-33 and 20ng/ml TNF. All conditions include 5ng/ml IL-2 to keep effector T cell alive. MFI of IL-18Rα on live P14 T cells were measured by FACS. Each pairs of symbol represents the results from an individual recipient mouse. Pooled results from 3 independent experiments. N.S., not significant, **, $p<0.01$, ***, $p<0.001$ and ****, $p<0.0001$ by paired Student *t*-test.

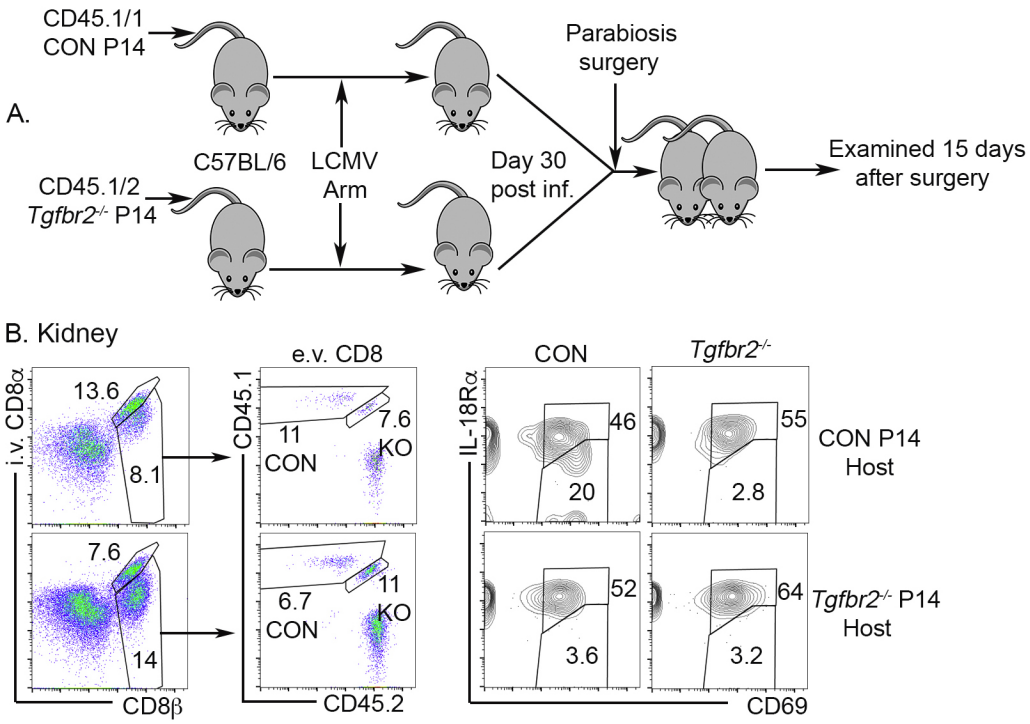

Figure S5. TGF-β is required for the differentiation of IL-18R^{lo} kidney-resident memory T cells, Related to Figure 5. (A) Experimental design. Naïve WT control and *Tgfbr2*$^{-/-}$ P14 T cells with distinct congenic markers were separately transferred into B6 mice followed by LCMV Arm infection. D30 p.i., pairs of mice carrying WT control and *Tgfbr2*$^{-/-}$ P14s were surgically connected and examined 15 days later. (**B**) Representative FACS profiles of kidney CD8^{+} T cells derived from the same parabiosis pair (n=4). Upper row, kidney sample from control P14 host; lower row, kidney sample from *Tgfbr2*$^{-/-}$ P14 host.

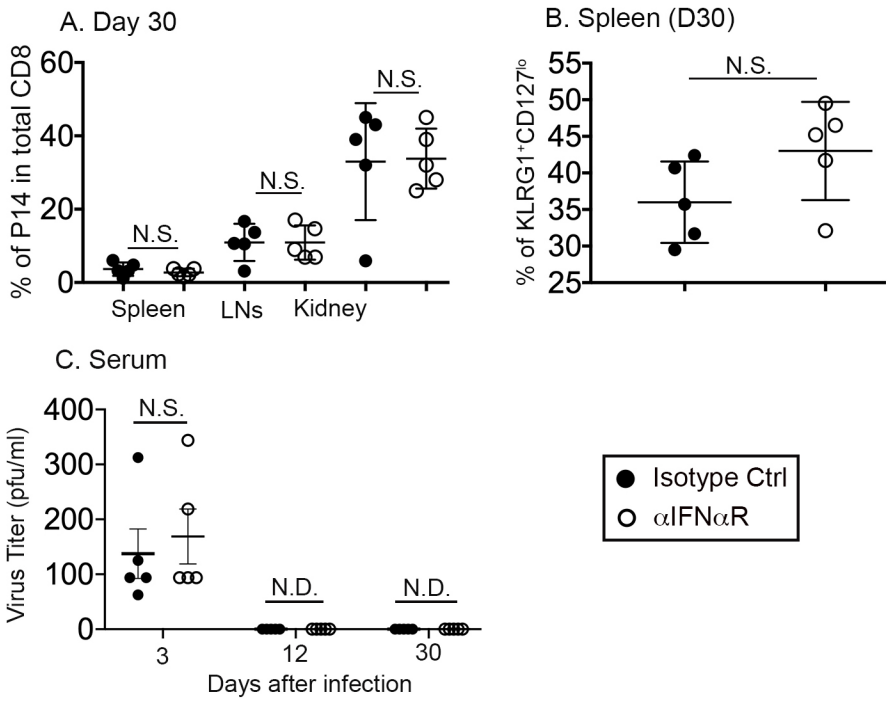

Figure S6. Type I IFN blockade at day 4 and day 7 does not impact CD8$^+$ T cell expansion and viral clearance, Related to Figure 5. Similar experimental setup to **Figure 5D**. (**A**) The percentage of donor P14 T cells in spleen, lymph nodes and kidney. (**B**) The percentage of KLRG1$^+$ cells in splenic P14. (**C**) Viral titer in the serum. Each symbol represents the results from an individual recipient. N.D., not detectable. N.S., not significant by Student *t*-test.

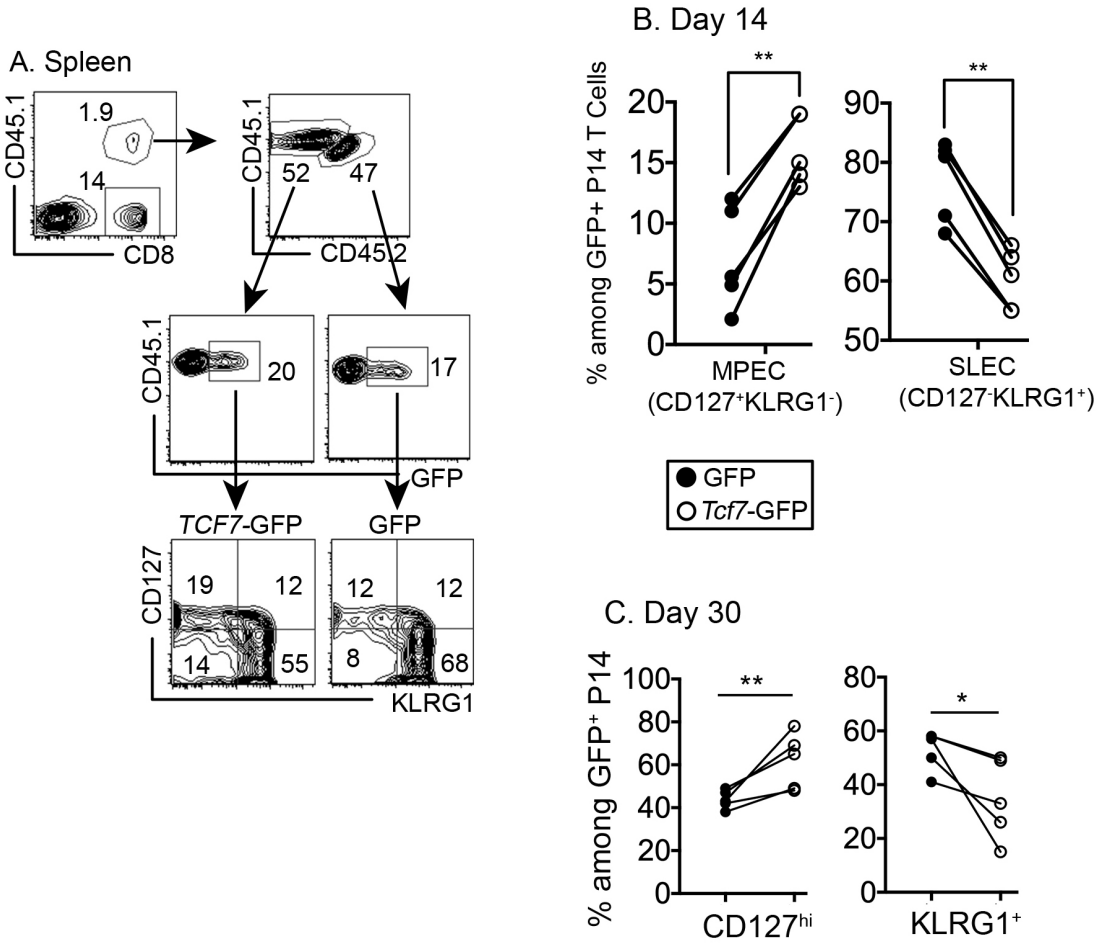

Figure S7. Overexpression of Tcf-1 promotes memory precursor differentiation in the spleen, Related to Figure 6. Similar experimental setup to **Figure 6**. FACS gating strategy is shown in (**A**). (B) Day 14 or (**C**) day 30 post infection, the percentage of KLRG1$^-$CD127$^+$ cells (**left**) and KLRG1$^+$CD127$^-$ cells (**right**) among GFP$^+$ P14 T cells are shown. Each pair of symbols represents the results from an individual recipient. *, $p<0.05$ and **, $p<0.01$ by paired Student *t*-test. Pooled results from 2 independent experiments are shown.

Transparent Methods

Ethics Statement.

All the animal handling and experimental procedures performed have adhered strictly to the general guidelines of Animal Welfare Act (AWA), AAALAC and IACUC. An Animal Care Protocol has been approved by UT Health San Antonio IACUC (protocol# 20150014AR, PI: Nu Zhang). All infectious agents used have been approved by UT Health San Antonio Institutional Biosafety Committee (protocol# 14-09-6080, PI: Nu Zhang).

Mice, Viruses and Bacteria.

Tgfbr2$^{f/f}$ dLck-cre mice were as described before (Zhang and Bevan, 2012, 2013). *Il18r1*$^{-/-}$ (stock no. 004131), and C57BL/6 (stock no. 000664) mice were obtained from The Jackson Laboratory and a colony of D^b-GP_{33-41} TCR transgenic (P14) mice was maintained at our specific pathogen-free animal facilities at the University of Texas Health Science Center at San Antonio (San Antonio, Texas). All recipient mice were used at 6 to 12 wk of age. Both male and female mice were used in the current project. No influence of gender or age was identified. All experiments were done in accordance with the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee guidelines. Mice were infected i.p. by $2x10^5$ pfu LCMV Arm. Viruses were grown and quantified as described (Ahmed et al., 1984).

Naïve T Cell Isolation and Adoptive Transfer.

Naïve $CD8^+$ T cells were isolated from pooled spleen and lymph nodes using MojoSort™ mouse CD8 T cell isolation kit (Biolegend) following manufacturer's instruction. During the first step of biotin antibody cocktail incubation, biotin-αCD44 (IM7, Biolegend) was added to label and deplete effector and memory T cells. Isolated naïve $CD8^+$ T cells were numerated, 1:1 mixed when indicated, 10^4 cells adoptively transferred into each sex-matched unmanipulated B6 recipient via an i.v. route.

Intra-vascular Labeling of $CD8^+$ T Cells.

3μg biotin-αCD8α (53-6.7, Tonbo Biosciences) was injected i.v. 5 mins before euthanasia. After lymphocyte isolation, fluorescence labeled streptavidin (Thermo Fisher) was used during surface staining to identify blood-borne $CD8^+$ T cells.

Lymphocyte Isolation from the Kidney, SG and SI-IEL.

Lymphocyte isolation procedures have been described before (Liao, 2020; Ma et al., 2017; Zhang and Bevan, 2013). Briefly, kidney and SG was minced and digested with 1mg/ml collagenase B (Roche) in RPMI 1640/2% FCS at 37°C for 45 mins with gentle shaking. Digested tissues were further mashed and washed with RPMI 1640/10% FCS. For IEL isolation, small pieces of the small intestine were stirred at 800 rpm for 20 min in HBSS buffer containing 1mM dithiothreitol and 10% FCS at 37°C. Both digested kidney and SG, and released IEL were further purified by density gradient centrifugation with PBS-balanced 44% and 67% Percoll (GE Healthcare).

Parabiosis Surgery.

Parabiosis surgery was performed according to a published protocol (Kamran et al., 2013). Briefly, mice were anesthetized and shaved along opposite lateral flanks. Skin was thoroughly cleaned. Longitudinal skin incisions were performed on the shaved sides of each mouse. The skin of the two animals was connected with 5-0 VICRYL suture. Additional 3-0 sutures were placed through the olecranon and knee joints to secure the legs.

Ex vivo Culture of Splenic Effector P14 T Cells.

Day 4.5 post LCMV Arm infection, total splenocytes containing P14 T cells were cultured in complete RPMI with 5ng/ml IL-2 (Tonbo) and one of the following stimuli: 1μg/ml GP_{33-41} (AnaSpec), 50ng/ml hTGF-β1, 20ng/ml IFN-β, 20ng/ml IL-18, 20ng/ml IL-33 and 20ng/ml TNF. All tested

cytokines were purchased from Biolegend. 12-16 hours later, the expression of IL-18R on live P14 T cells were determined by flow cytometry.

Retrovirus Transduction.

Tcf7 (p45)-GFP (MSCV vector) was a gift from Dr. Haihui Xue. Helper plasmid pCL-Eco was a gift from Dr. Inder Verma (Addgene plasmid#12371). *Tcf7*-GFP and pCL-Eco were co-transfected into 293T cells by FuGENE 6 (Promega). Retrovirus was harvested 48 hours after transfection and used freshly. Naïve P14 T cells were stimulated with 10nM GP_{33-41} peptide (AnaSpec) plus soluble 1μg/ml αCD28 (E18, Biolegend) in the presence of 5ng/ml IL-2 (eBioscience) overnight. Activated P14 T cells were spin infected with retrovirus at 3,000rpm 30°C for 1.5 hours in the presence of 8μg/ml polybrene (Sigma) and 5ng/ml IL-2. After spin infection, P14 T cells were incubated with retrovirus for another hour at 37°C. After extensive wash, $2x10^5$ P14 T cells were adoptively transferred into each B6 recipient followed by LCMV Arm infection.

RNA-seq Analysis.

Day 12 after infection, pooled kidney P14 T cells from 10-15 recipient mice were FACS sorted into $CD69^-$, $CD69^+IL\text{-}18R^{hi}$ and $CD69^+IL\text{-}18R^{lo}$ cells. Total RNA was extracted from sorted cells using a Quick-RNA Miniprep kit from Zymo Research. Sequencing library was constructed according to Illumina TruSeq Total RNA Sample Preparation Guide (RS-122-2201). Each library was barcoded and then pooled for cluster generation and sequencing run with 50bp single-end sequencing protocol on an Illumina HiSeq 3000 platform. Original RNA-seq results can be accessed by GSE111801.

Antibodies and Flow Cytometry.

For in vivo type I IFN blocking, 1mg anti-IFNAR-1 (MAR1-5A3, BioXcell) was given i.p. on day 4 and day 7 post LCMV infection. For flow cytometry, single cell suspension from spleen, kidney and gut IEL was incubated with FcR blocker (clone 2.4G2, generated in the lab). Cells were typically stained with fluorescence labeled streptavidin (Thermo Fisher), IL-18Rα (P3TUNYA, eBioscience), CD8β (H35-17.2, eBioscience), CD45.1 (A20, Tonbo), CD45.2 (104, Tonbo), CD11b (M1/70, Tonbo) and the following antibodies from BioLegend, CD127 (A7R34), KLRG1 (2F1/KLRG1), CD69 (H1.2F3), CD16/32(2.4G2), CD73 (TY/11.8), CD103 (2E7), CD90.1 (OX-7), CXCR3 (CXCR3-173), Ly6C (HK1.4), CD38 (90) and CXCR4 (L276F12). Cmah activity was determined by an anti-Neu5Gc antibody kit from Biolegend. Fixable Viability Dye eFluor 506 (eBioscience) or Ghost Dye™ Violet 510 (Tonbo) was used to identify live cells. For Tcf-1 staining, surface stained cells were treated by True-Nuclear TF Buffer Set (Biolegend) and stained with anti-Tcf-1 (C63D9, Cell Signaling). Washed and fixed samples were analyzed by BD LSRII or BD FACSCelesta, and analyzed by FlowJO (TreeStar) software.

In vivo IFN-γ Production During Reactivation.

After 30 days of LCMV infection, we re-challenged the mice with 32μg GP_{33-41} peptide/mouse (Genscript) via an i.v. route together with 250μg Brefeldin A (B6542, Sigma) in 200μl PBS 4 hours before euthanasia similar to a previous report (Sega et al., 2014). During the lymphocyte isolation procedure of kidney, 5μg/ml Brefeldin A was added to the digestion buffer and density gradient centrifugation buffer. Freshly isolated lymphocytes were surface stained, fixed, permeablized and intracellular stained by anti-IFN-γ antibody (XMG1.2, Biolegend) and anti-granzyme B (NGZB, Invitrogen).

Ex vivo Stimulation to Detect Cytokine Production.

Fresh isolated lymphocytes from various tissues were cultured in complete RPMI in the presence of Golgi STOP with or without different stimuli for 4 hours. Stimuli used in current study include 1μM GP_{33-41}, 20ng/ml IL-12+20ng/ml IL-18 or 20ng/ml IL-12+20ng/ml IL-18+20ng/ml IL-15+ 20ng/ml IFN-α. All recombinant mouse cytokines were purchased from Biolegend. Stimulated cells were surface stained, fixed, permeablized and intracellular stained by anti-IFN-γ antibody (XMG1.2, Biolegend) and anti-TNF antibody (MP6-XT22, Biolegend). Ghost Dye™ Violet 510 (Tonbo) was

used to identify live cells. Washed samples were analyzed by BD FACSCelesta, and analyzed by FlowJO (TreeStar) software.

Statistic Analysis.

P value was calculated by two-tail paired or unpaired Student *t*-test, One-way ANOVA using Prism 7 software.

Supplemental References

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