Supplementary Information for

B cell-derived IL-27 promotes control of persistent LCMV infection

Isaraphorn Pratumchai[1,2], Jaroslav Zak[1], Zhe Huang[1], Booki Min[3], Michael B. A. Oldstone[1,*] & John R. Teijaro[1,*]

[1]Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA 92037, USA

[2]Department of Immunology, Leiden University Medical Center, Leiden, 2333 ZA, The Netherlands

[3]Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

***Correspondence to:**
Michael B. A. Oldstone
Email: mbaobo@scripps.edu

John R. Teijaro
Email: teijaro@scripps.edu
Tel: 858 784 7397
Fax: 858 784 9981

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- **Figures S1-S4**
- **SI References**

Supplementary Methods

Details of reagents and resources

Reagents and resources	Source	Identifier
Antibodies		
PE/Cyanine7 anti-mouse CD8a Antibody	Biolegend	Cat# 100722
PerCP/Cyanine5.5 anti-mouse CD4 Antibody	Biolegend	Cat#116012
APC anti-mouse/human CD44 Antibody	Biolegend	Cat# 103012
FITC anti-mouse CD279 (PD-1) Antibody	Biolegend	Cat# 135214
Brilliant Violet 421™ anti-mouse IFN-γ Antibody	Biolegend	Cat# 505830
PE anti-mouse TNF-α Antibody	Biolegend	Cat#506306
Alexa Fluor® 647 anti-mouse IL-27 p28 Antibody	Biolegend	Cat#516904
PE anti-mouse IL-27 p28 Antibody	Biolegend	Cat#516908
PE/Cyanine7 anti-mouse IL-27 p28 Antibody	Biolegend	Cat#516910
Brilliant Violet 421™ anti-mouse CD138 (Syndecan-1) Antibody	Biolegend	Cat#142508
Brilliant Violet 421™ anti-mouse CD185 (CXCR5) Antibody	Biolegend	Cat#145512

PE anti-mouse CD185 (CXCR5) Antibody	Biolegend	Cat#145504
Recombinant Mouse IL-6 (carrier-free)	Biolegend	Cat#575702
Purified anti-mouse CD40 Antibody	Biolegend	Cat#102802
PE-conjugated anti-mouse EBI3 Antibody	R&D Systems	Cat#IC18341P
Alexa Fluor® 647 AffiniPure $F(ab')_2$ Fragment Goat Anti-Human IgG, Fcγ fragment specific	Jackson Immuno Research Labs	Cat#109-606-170
BV421 mouse anti-Bcl6 clone K112-91	BD	Cat#563363
PE Rat Anti-Mouse IL-27ra Clone 2918 (RUO)	BD	Cat#564337
Alexa Fluor® 647 Mouse Anti-Stat1 (pY701)	BD	Cat#612597
PE Mouse Anti-Stat3 (pY705)	BD	Cat#612569
TotalSeq B0301 Hashtag 1 antibody	Biolegend	155831
TotalSeq B0302 Hashtag 2 antibody	Biolegend	155833
TotalSeq B0303 Hashtag 3 antibody	Biolegend	155835
TotalSeq B0304 Hashtag 4 antibody	Biolegend	155837
TotalSeq B0305 Hashtag 5 antibody	Biolegend	155839
TotalSeq B0306 Hashtag 6 antibody	Biolegend	155841
TotalSeq B0307 Hashtag 7 antibody	Biolegend	155843
TotalSeq B0308 Hashtag 8 antibody	Biolegend	155845
Chemical, Peptides, and Recombinant Proteins		
Protein Transport Inhibitor (Containing Brefeldin A) BD GolgiPlug™	BD	Cat#555029
eBioscience™ Cell Stimulation Cocktail (plus protein transport inhibitors) (500X)	Thermo Fisher Scientific	Cat#00-4975-93
eBio Fix/Perm Diluent	Thermo	Cat#00-5223-56

	Fisher Scientific	
eBioscience™ Fixation/Permeabilization Concentrate	Thermo Fisher Scientific	Cat#00-5123-43
Recombinant Mouse IL-27 (NS0-expressed) Protein	R&D Systems	Cat# 2799-ML-010
Recombinant Mouse IL-21 R Fc Chimera Protein, CF	R&D Systems	596-MR-100
Standard lipopolysaccharide from *E. coli* K12 strain; TLR4 ligand	Invivogen	tlrl-eklps
Experimental Models: Organisms/Strains		
Mb1-Cre	Jackson Laboratory	Cat#20505
granzyme-B-Cre	Jackson Laboratory	Cat#003734
CD4-Cre	Jackson Laboratory	Cat# 022071
STAT1	Jackson Laboratory	Cat# 012606
$Stat3^{f/f}$	Jackson Laboratory	Cat#016923
SMARTA	Jackson Laboratory	Cat#030450
Ebi3	Jackson Laboratory	Cat# 008691
XCR1	Reiken	Cat# RBRC09929
IL-27eGFP	University of Colorado Anschutz	Ross.Kedl@cuanschutz.edu
$IL\text{-}27ra^{flox/flox}$	Northwestern University	booki.min@northwestern.edu
$IL\text{-}27p28^{flox/flox}$	UCSD	lil034@ucsd.edu
Software and Algorithms		
GraphPad Prism 7	GraphPad Software	https://www.graphpad.com/
FlowJo 10.4.2	Tree Star	https://www.flowjo.com/
Cellranger 4.0.0	10X Genomics	https://support.10xgenomics.com/single-cell-gene-

		expression/software/pipelines/latest/what-is-cell-ranger
Seurat 4.0.3	Satija lab	(1)

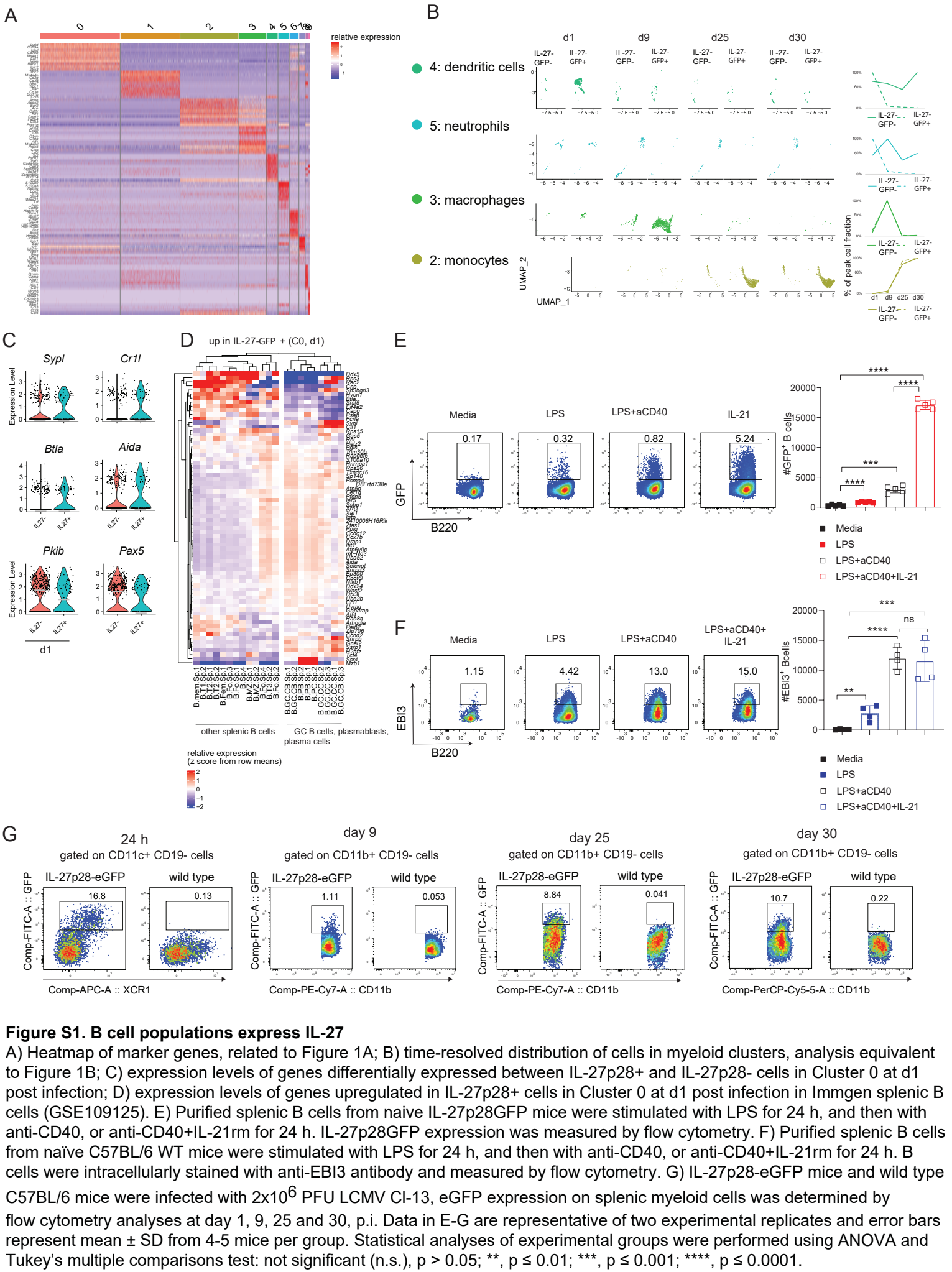

Figure S1. B cell populations express IL-27

A) Heatmap of marker genes, related to Figure 1A; B) time-resolved distribution of cells in myeloid clusters, analysis equivalent to Figure 1B; C) expression levels of genes differentially expressed between IL-27p28+ and IL-27p28- cells in Cluster 0 at d1 post infection; D) expression levels of genes upregulated in IL-27p28+ cells in Cluster 0 at d1 post infection in Immgen splenic B cells (GSE109125). E) Purified splenic B cells from naive IL-27p28GFP mice were stimulated with LPS for 24 h, and then with anti-CD40, or anti-CD40+IL-21rm for 24 h. IL-27p28GFP expression was measured by flow cytometry. F) Purified splenic B cells from naïve C57BL/6 WT mice were stimulated with LPS for 24 h, and then with anti-CD40, or anti-CD40+IL-21rm for 24 h. B cells were intracellularly stained with anti-EBI3 antibody and measured by flow cytometry. G) IL-27p28-eGFP mice and wild type C57BL/6 mice were infected with $2x10^6$ PFU LCMV Cl-13, eGFP expression on splenic myeloid cells was determined by flow cytometry analyses at day 1, 9, 25 and 30, p.i. Data in E-G are representative of two experimental replicates and error bars represent mean ± SD from 4-5 mice per group. Statistical analyses of experimental groups were performed using ANOVA and Tukey's multiple comparisons test: not significant (n.s.), $p > 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

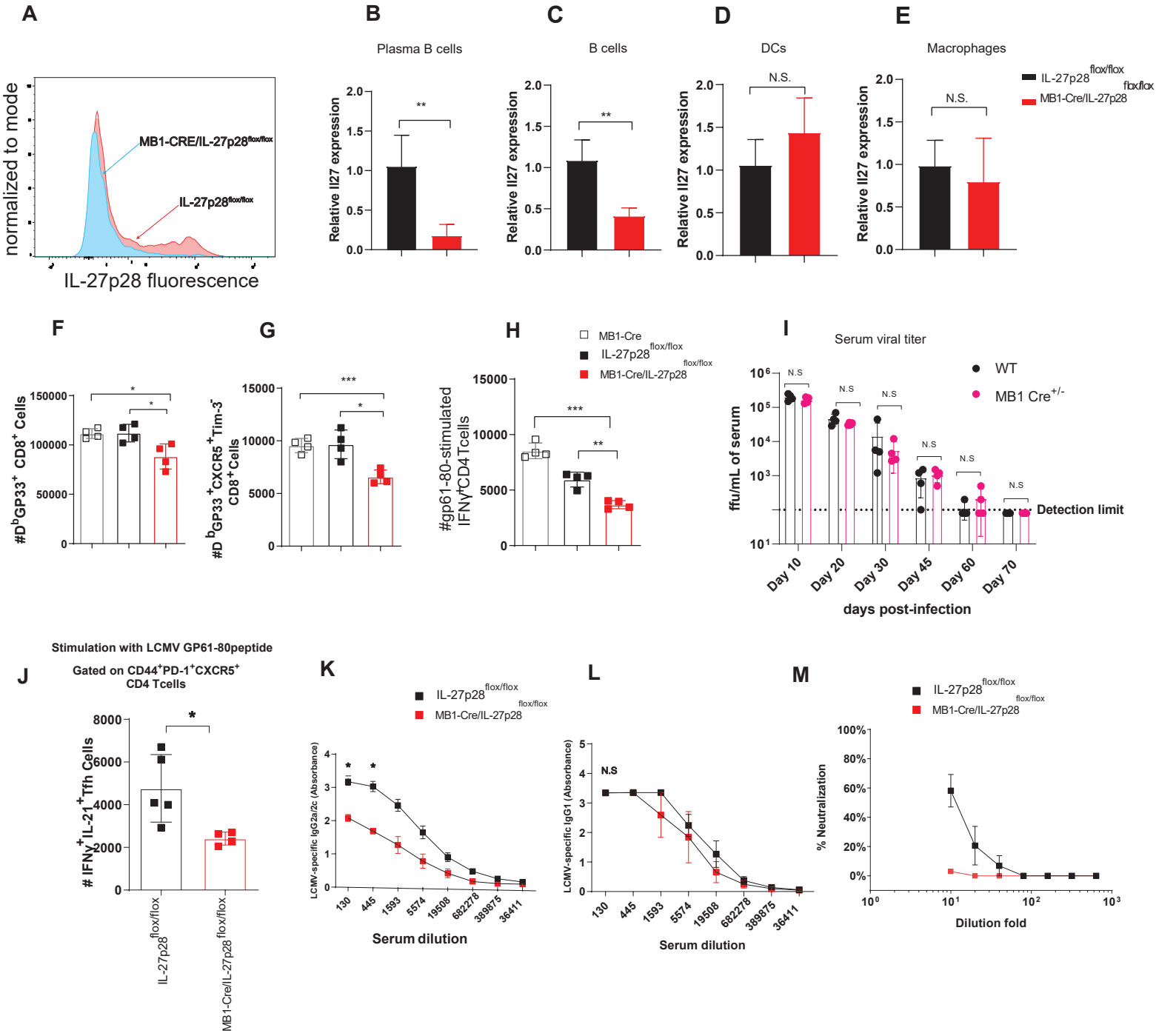

Figure S2. IL-27p28 expressing B cells are essential for control of persistent LCMV infection

Mice with B cell-specific IL-27p28 deletion were generated by crossing IL-27p28$^{flox/flox}$ mice to MB1-cre+/- Mice. IL-27p28$^{flox/flox}$ and MB1-Cre/ IL-27p28$^{flox/flox}$ mice were infected with 2x10^6 PFU LCMV Cl-13. At day 9 p.i. A) IL-27p28 express was determined by intracellular FACS staining in MB1-Cre/ IL-27p28$^{flox/flox}$ and IL-27p28$^{flox/flox}$ mice., B-E) Relative *Il27* mRNA expression in sorted CD138+ plasma cells, B cells, DCs, and macrophages from MB1-Cre/ IL-27p28$^{flox/flox}$ and IL-27p28$^{flox/flox}$ mice was determined by qPCR. (F-I) MB1 cre+/- mice were infected with 2x10^6 PFU LCMV Cl-13 together with IL-27p28$^{flox/flox}$ and MB1-cre+/- Mice. IL-27p28$^{flox/flox}$ and MB1-Cre/ IL-27p28$^{flox/flox}$ mice. Splenocytes were analyzed by flow cytometry to determine total number of F) H2-Db GP33-41 virus-specific CD8 T cells, G) virus-specific stem-like CXCR5+Tim3- CD8 T cells, H) IFN-γ-producing GP61-80 LCMV-specific CD4 T cells. I) Serum viral loads of MB1 cre+/- and WT mice were determined at different time points p.i. J) At day 40 p.i. splenocytes from LCMV Cl-13 infected from MB1-Cre/ IL-27p28$^{flox/flox}$ and IL-27p28$^{flox/flox}$ mice were analyzed by flow cytometry to determine the number of IL-21+IFN-γ+ Tfh cells after GP61-80 peptide stimulation. At day 130, serum LCMV-specific K) IgG2a/2c and L) IgG1 antibodies were determined by ELISA. M) Level of serum LCMV-neutralizing antibodies at day 70 p.i. was determined by LCMV Cl-13 neutralization assay. Data are representative of two to three experimental replicates and error bars represent mean ± SD (panel B-I) or mean ± SEM (K-M) from 4-5 mice per group. Statistical analyses of experimental groups were performed using Student's two-tailed t test (B-H), two-way ANOVA (K-M) or Mann-Whitney U test (I): not significant (n.s.), $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

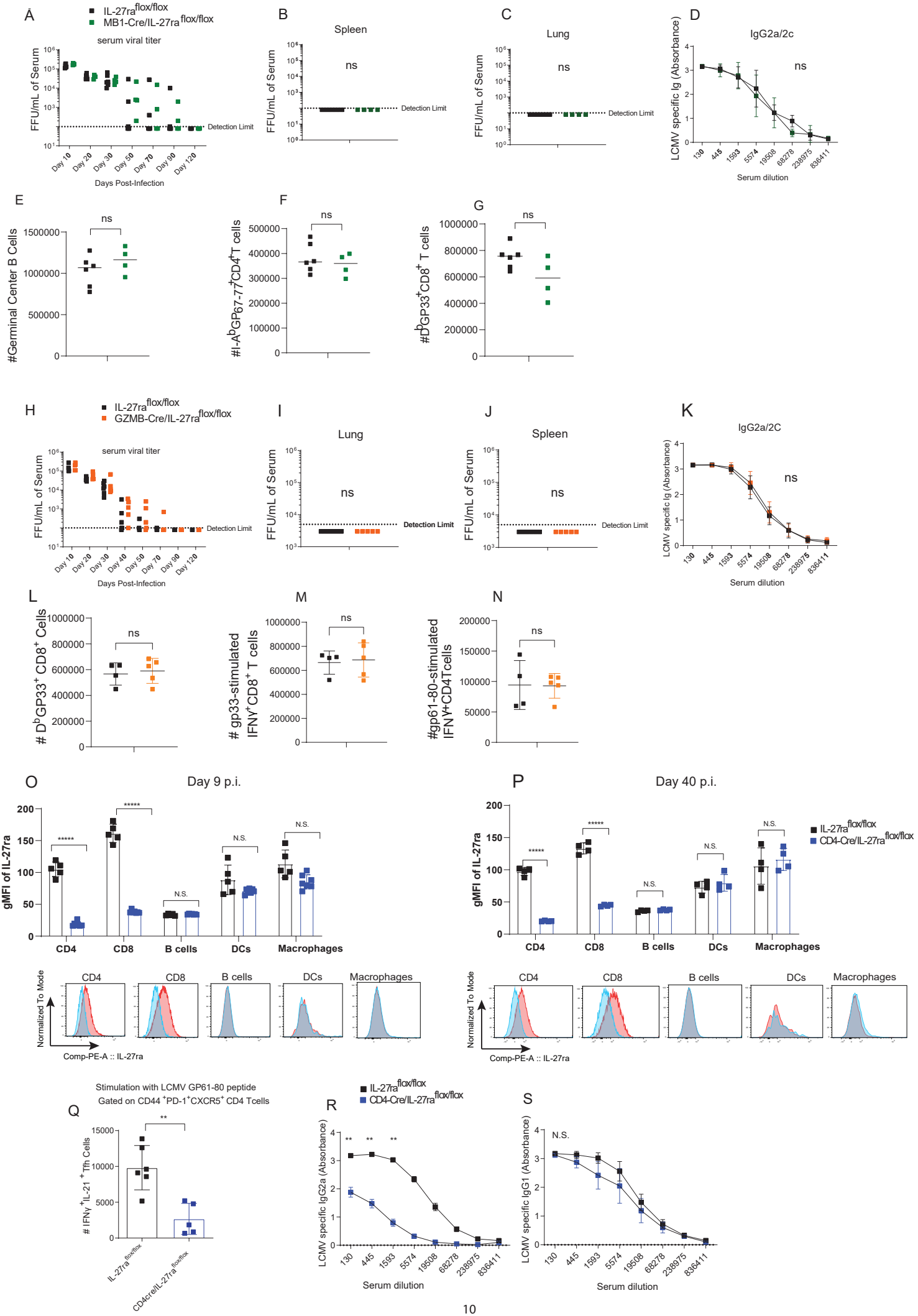
A
IL-27ra flox/flox
MB1-Cre/IL-27ra flox/flox
serum viral titer
FFU/mL of Serum
Detection Limit
Day 10
Day 20
Day 30
Day 50
Day 70
Day 90
Day 120
Days Post-Infection
B
Spleen
ns
C
Lung
D
IgG2a/2c
LCMV specific Ig (Absorbance)
Serum dilution
E
#Germinal Center B Cells
F
#I-Ab GP67-77+CD4+T cells
G
#DbGP33+CD8+ T cells
H
GZMB-Cre/IL-27ra flox/flox
I
J
K
IgG2a/2C
L
DbGP33+ CD8+ Cells
M
gp33-stimulated IFNγ+CD8+ T cells
N
#gp61-80-stimulated IFNγ+CD4Tcells
O
Day 9 p.i.
gMFI of IL-27ra
CD4
CD8
B cells
DCs
Macrophages
N.S.
Normalized To Mode
Comp-PE-A :: IL-27ra
P
Day 40 p.i.
CD4-Cre/IL-27ra flox/flox
Q
Stimulation with LCMV GP61-80 peptide
Gated on CD44 +PD-1+CXCR5+ CD4 Tcells
IFNγ+IL-21+ Tfh Cells
CD4cre/IL-27ra flox/flox
R
LCMV specific IgG2a (Absorbance)
S
LCMV specific IgG1 (Absorbance)

Figure S3. B cells and Granzyme B+ T cells are not required for control of persistent LCMV infection
(A-G) IL-27ra$^{flox/flox}$ (WT) and MB1-Cre/ IL-27ra$^{flox/flox}$ mice (B cell specific IL-27ra KO) were infected with 2x10^6 PFU LCMV Cl-13: A) serum viral loads were determined at different time points p.i. At day 120 p.i. viral loads were measured in B) spleen and C) lung. D) LCMV-specific IgG2a/2c antibody was measured in the serum by ELISA. At day 9 p.i., splenocytes were analyzed by flow cytometry to determine numbers of E) germinal center B cells, F) virus-specific CD4 T cells and G) virus GP33-specific CD8 T cells.
(H-N) IL-27ra$^{flox/flox}$ (WT) and GZMB-Cre/ IL-27ra$^{flox/flox}$ (activated T cell specific IL-27ra KO) were infected with 2x10^6 PFU LCMV Cl-13. At day 120 p.i. H) serum viral loads were determined at different time points p.i. At day 120 p.i. viral loads were measured in I) lung and J) spleen. K) LCMV-specific IgG2a/2c antibody was measured in the serum. At day 9 p.i., splenocytes were analyzed by flow cytometry to determine numbers of L) virus-specific CD8 T cells, M) IFN-γ-producing GP33-41 LCMV-specific CD8 T cells, and N) IFN-γ-producing GP61-80 virus-specific CD4 T cells.
(O-S) IL-27ra$^{flox/flox}$ and CD4-Cre/ IL-27ra$^{flox/flox}$ were infected with 2x10^6 PFU LCMV Cl-13. Splenocytes were stained with IL-27ra antibody and flow cytometry analysis was performed to determine the expression of IL-27ra in CD8 T cells, CD4 T cells, B cells, DCs and macrophages at O) day 9 and P) day 40 p.i. Q) At day 40 p.i. splenocytes were analyzed by flow cytometry to determine the number of IL-21+IFN-γ+-producing Tfh cells after GP61-80 peptide stimulation. R) At day 120, serum LCMV-specific IgG2a/2c and S) IgG1 antibody were determined by ELISA. Data are representative of two to three experimental replicates and error bars represent mean ± SD (panel B-C, E-G, I-J, L-Q) or mean ± SEM (D, K,R-S) from 4-5 mice per group. Statistical analyses of experimental groups were performed using Student's two-tailed t test (E-F, L-N), one-way ANOVA (O-P) or Mann-Whitney U test (B-D, I-K): not significant (n.s.), $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

A

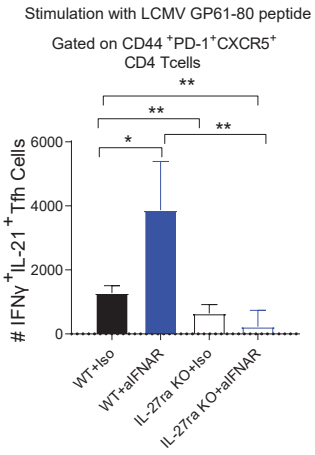

Figure S4. IL-27R signaling is required for anti-IFNAR immunotherapy to promote IL-21 production by Tfh cells
C57BL/6 WT and IL-27ra KO mice were treated with isotype control or anti-IFNAR1 antibody 1 day prior to LCMV-Cl13 infection. At day 40 p.i. splenocytes were analyzed by flow cytometry to determine A) the number of IL-21+IFN-γ+ Tfh cells after GP61-80 peptide stimulation. Data are representative of two to three experimental replicates and error bars represent mean ± SD. Statistical analyses of experimental groups were performed using one-way ANOVA and Tukey's post-test: not significant (n.s.), $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$.

SI References

1. Y. Hao *et al.*, Integrated analysis of multimodal single-cell data. *Cell* **184**, 3573-3587.e3529 (2021).