

Supplementary figure and table legends:

Figure S1. Acute LCMV infection course in C57BL/6/J mice. (A) Schematic representation of LCMV-Doc infection and time points of organ collection. (B) Body weight during the course of an acute LCMV infection. (C) Viral loads were assessed in spleen, lung and liver from infected mice by FFA. Data shown are the mean $\pm$ SEM from 4–6 mice/group in one representative experiment. Three independent experiments were performed. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Figure S2. Diminished vaccine-specific cellular response in vaccine-draining lymph nodes of LCMV-recovered mice. (A) Schematic representation of vaccine regimen in naive mice and LCMV-recovered mice at days 30 and 60 p.i. Vaccine-specific cellular responses were analysed 14 days after vaccination boost by ELISpot in both draining and non-draining lymph nodes from vaccinated mice (B). ICS from vaccine draining lymph nodes was performed to assess IFN γ and TNF α production and degranulation (CD107a/b) in CD8+ (C) and CD4+ (D) T cells, shown as frequencies and absolute numbers. Data represent the mean $\pm$ SEM from 3–6 mice/group in one representative experiment. Three independent experiments were performed. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Figure S3. Effector memory CD8+ and CD4+ T cells were the main affected vaccine-specific T cell subset in the spleen of LCMV-recovered mice. Vaccine-specific cytokine production (IFN γ and TNF α) and degranulation (CD107a/b) were evaluated in different T cell subpopulations from splenocytes of vaccinated naive mice and LCMV-recovered mice vaccinated at days 30 and 60 p.i.. Analyses were performed on T cell subsets defined as naive (CD44-, CD62+), effector memory (CD44+ CD62L-) and central memory (CD44+ CD62L+) within CD8+ (A) and CD4+ (B) T cell populations. Results are presented as both percentages and absolute cell numbers. Data shown represent the mean $\pm$ SEM from 4–6 mice/group in one representative experiment. Three independent experiments were performed. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Figure S4. Effector memory CD8+ and CD4+ T cells were the main affected vaccine-specific T cell subset in the vaccine-draining lymph nodes of LCMV-recovered mice. Vaccine-specific cytokines production (IFN γ and TNF α) and degranulation (CD107a/b) were evaluated by ICS from vaccine-draining lymph nodes of naive mice and LCMV-recovered mice vaccinated at day 30 and 60 p.i. Analyses were performed across T cell subsets defined as naive (CD44-, CD62+), effector memory (CD44+ CD62L-) and central memory (CD44+ CD62L+) T cells within the CD8+ (A)

and CD4⁺ (B) T cell compartments. Results are presented as both percentages and absolute cell numbers. Data shown represent the mean $\pm$ SEM from 4–6 mice/group in one representative experiment. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Figure S5. Immune and stromal cell subset characterization in LCMV-recovered mice. Flow cytometry analysis of splenic immune populations from naive mice and LCMV-recovered mice at day 30 and 60 p.i.. Absolute numbers of T cell subsets (A), B cell subsets (B), macrophage populations (C) and dendritic cells subsets (D) are shown. (E) Absolute numbers of different stromal cell subsets: mesothelial cells, glial cells, T reticular cells (TRC), follicular dendritic cells (FDC), marginal reticular cells (MRC) and red pulp reticular cells (RPRC). Data shown represent the mean $\pm$ SEM from 4–6 mice/group in one representative experiment. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

Figure S6. Quality control assessment of splenic transcriptomic data across time points. Given the limited sample size ($n=2$ per time point), consistency between biological replicates was assessed using Pearson correlations of gene expression between replicate 1 and replicate 2 at each timepoint. Pearson correlation coefficients and associated P-values are displayed in the figure.

Figure S7. Physiological functions such as blood filtration and erythropoiesis homeostasis were impaired in LCMV-recovered mice. Given the extensive splenic changes observed and the indications of downregulated heme pathways revealed by spleen transcriptome analysis, we investigated its blood filtration capacity and erythropoiesis homeostasis. To address this, erythroid precursors and Howell-Jolly bodies (HJB) were analysed from peripheral blood. Under normal conditions, erythroid precursors in the bone marrow give rise to mature erythrocytes that lack nuclei. However, under stress or inflammatory conditions, erythropoiesis can also take place in the spleen (Paulson et al., 2020). HJB are nuclear DNA remnants in erythrocytes that are typically removed from bloodstream by a healthy spleen. Their presence in circulation serves as a marker for asplenia or compromised splenic function (Hoss et al., 2018). For the respective analysis, peripheral blood samples were stained with the erythrocyte marker Ter-119 and the DNA dye Syto13 and characterised by flow cytometry with imaging reconstruction capabilities (BD FACSDiscover S8 Cell Sorter). (A) Mature erythrocytes are Ter-119-positive and Syto13-negative, HJB are Ter-119-positive and Syto13-intermediate while erythroid precursors are strongly positive for both markers. (B) To confirm the erythroid precursor's identity of the Ter-119-positive Syto13^{high} population, CD71 antibody (marker for transferrin receptor) was included to the staining panel. Within the Ter-119-positive Syto13^{high} population, three erythroid progenitor

populations were distinguished: basophilic erythroblasts (EryA; Ter-119-positive, CD71-positive and FSC^{high}), polychromatic erythroblasts (EryB; Ter-119-positive, CD71-positive and FSC^{low}) and orthochromatic erythroblasts or reticulocytes (Ter-119-positive, CD71 negative and FSC^{low}). (C) Reconstructed images show the intensity and localisation of Syto13 staining (green) from the different populations and support the gating strategy used. LCMV-recovered mice at day 30 p.i. had an increased frequency of HJB (D), albeit not significant, and a significantly increased frequency of erythroid precursors compared to naïve mice (E). EP – Erythroid precursors; HJB – Howell Jolly Bodies; ME – Mature erythrocytes. Data shown represent the mean $\pm$ SEM from 4–6 mice/group in one representative experiment. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons (* $p < 0.05$).

Table S1. Upregulated gene expression in LCMV-infected mice relative to naïve mice. Log2 fold change values for genes upregulated in LCMV-infected mice (day 1-9 p.i.; log2FC>1.5) relative to naïve mice, corresponding to the heatmap shown in Figure 3B.

Table S2. Result of the enrichment analysis of upregulated genes in LCMV-infected mice. Over-representation analyses of upregulated genes was performed using the enricher function of the clusterProfiler R package. An FDR-adjusted p-value <0.01 was applied. This table complements Figure 3C, where only pathways containing more than 15 genes were selected.