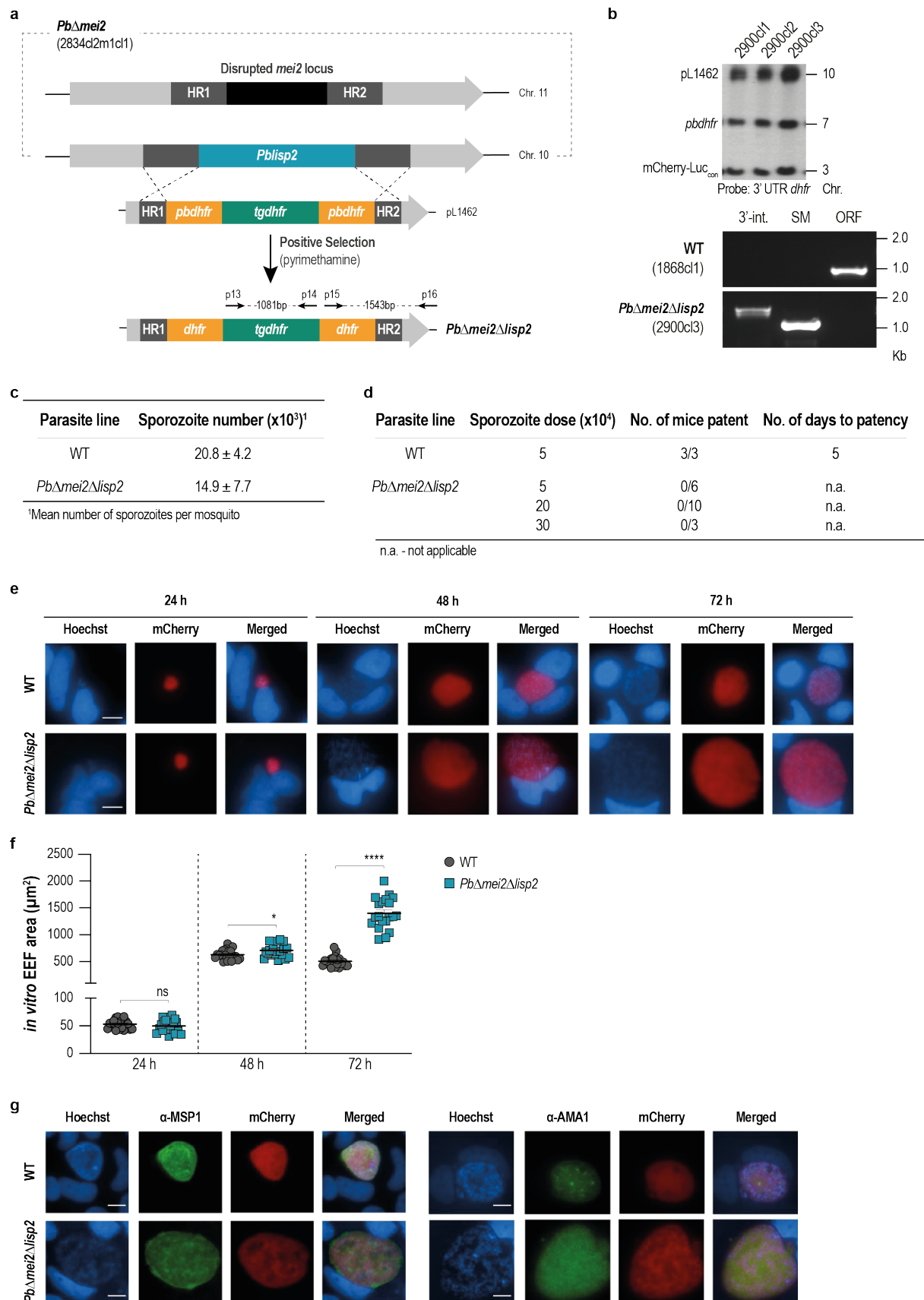


| Primer ID | Gene ID        | Sequence                           | Product (bp) | Description                         |
|-----------|----------------|------------------------------------|--------------|-------------------------------------|
|           |                | TTTGCTGACCTGCTGGATTAC              | N/A          | Forward <i>mhprt</i>                |
|           |                | CAAGACATTCTTTCCAGTTAA<br>AGTTG     | N/A          | Reverse <i>mhprt</i>                |
|           |                | AAGCATTAAATAAAGCGAATA<br>CATCCTTAC | N/A          | Forward <i>pb18S</i>                |
|           |                | GGAGATTGGTTTTGACGTTTA<br>TGTG      | N/A          | Reverse <i>pb18S</i>                |
| p13       |                | AATGAAGCGACGTATCGACC               | 1081         | Forward <i>tgdhfr</i>               |
| p14       |                | TGATGCGTTCCTTGTTGAGG               |              | Reverse <i>tgdhfr</i>               |
| p15       |                | CATATAAACACAAATGATGTT<br>TTTTC     | 1543         | Forward 3' <i>lisp2</i> integration |
| p16       |                | GAAGAAGTATGACCATACGC               |              | Reverse 3' <i>lisp2</i> integration |
|           | PBANKA_1003000 | CAGATTCGGCTTATCCATCTC              | 966          | Forward <i>lisp2</i> ORF            |
|           | PBANKA_1003000 | CACCATTGATTTGTTCTCAC               |              | Reverse <i>lisp2</i> ORF            |

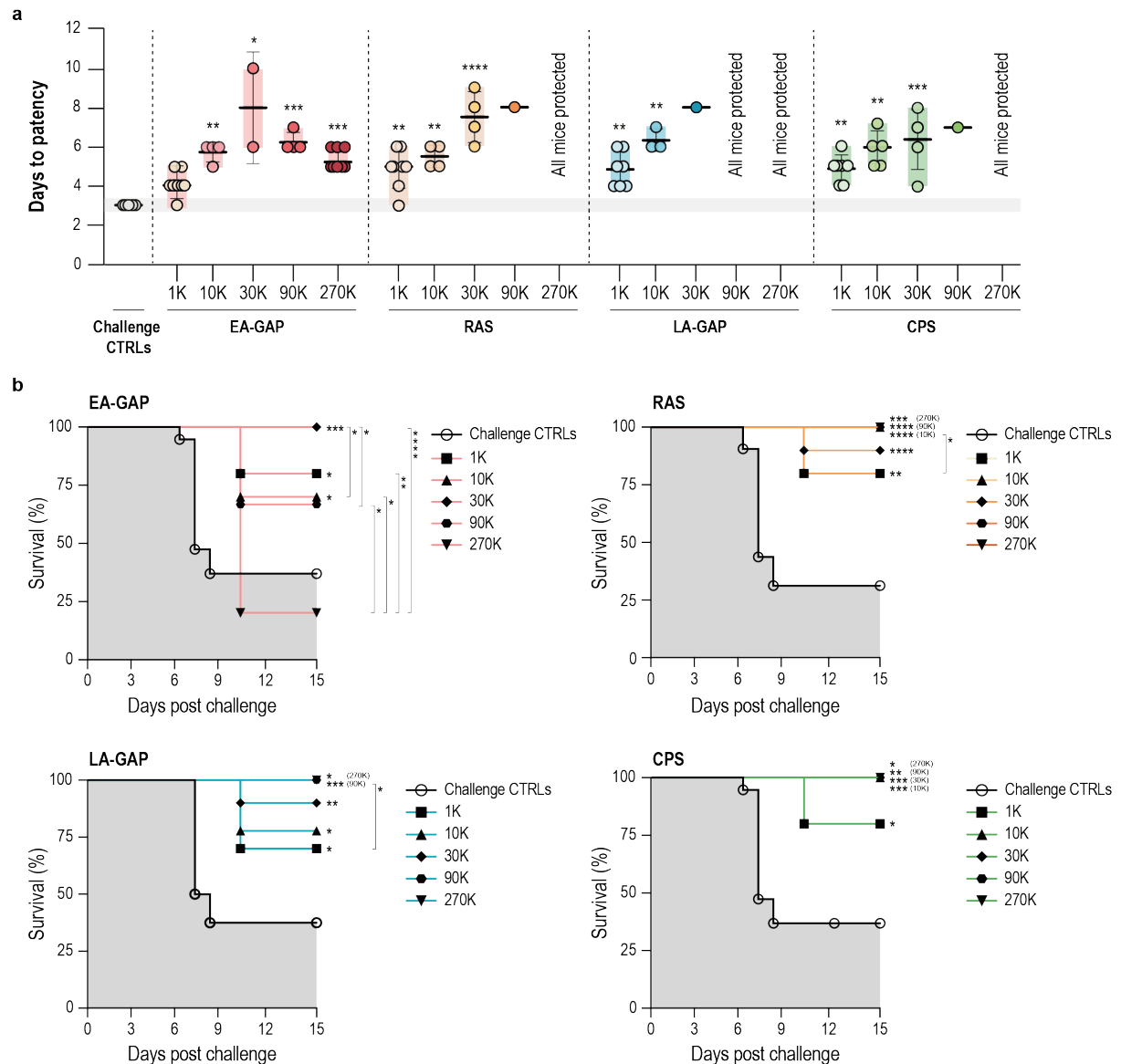
Bp – base pairs; m – mouse; *pb* – *Plasmodium berghei*; *tg* – *Toxoplasma gondii*; N/A – non applicable

**Supplementary Table 1.** List of primers used in this study.



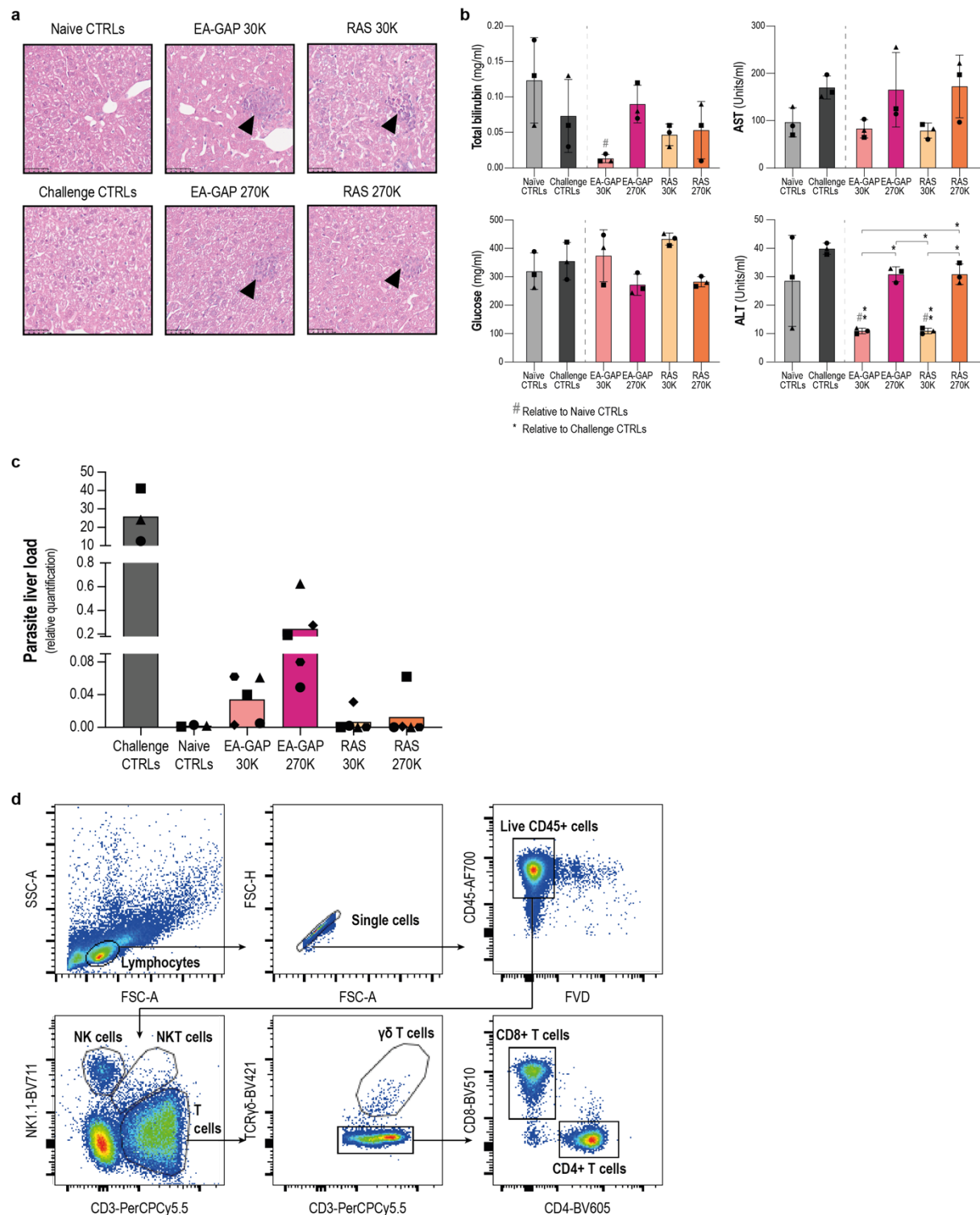
**Supplementary Figure 1: Generation, genotyping, and phenotyping of the double gene-deletion mutant *PbΔmei2Δlisp2*. (a)** Schematic representation of the introduction of the *tgdhfr*

SM cassette into the *Pblisp2* locus of the single gene-deletion mutant *PbΔmei2* (line 2834cl2m1cl1). To delete the *lisp2*, the gene-deletion construct pL1462, which contains the *tgdhfr* flanked by the *pbdhfr* promoter region and the 3'-UTR of *pbdhfr*, was used. This construct integrates into the *lisp2* locus by double cross-over homologous recombination at the *lisp2* homology regions (HR1, HR2). Positive selection with pyrimethamine selects for parasites that have the *lisp2* coding sequence replaced by the SM cassette of the pL1462 construct, resulting in the *PbΔmei2Δlisp* parasite line. Location of primers (p) used for PCR analyses and sizes of PCR products are shown. Details of primers are shown in **Supplementary Table 1**. Chr. – Chromosomes. **(b)** Southern analysis of PFG-separated chromosomes (top) and diagnostic PCR (bottom) confirm correct integration of construct pL1462 in *PbΔmei2* (2834cl2m1cl1) parasite line. PCR shows the presence of the *tgdhfr* SM (primers p13/p14) and 3' integration of *lisp2* PCR (3'-int; primers p15/p16). **(c)** Salivary gland spz numbers in WT- and *PbΔmei2Δlisp2*-infected mosquitoes. **(d)** Blood stage infections in C57BL/6J mice infected through retro-orbital I.V. injection of a single dose of either WT or *PbΔmei2Δlisp2* spz. **(e)** Representative live images of WT and *PbΔmei2Δlisp2* EEFs developing in Huh7 cells at different time-points post infection. EEFs express cytoplasmic mCherry (red) under the control of the constitutive *hsp70* promoter. Nuclei of both the parasites and Huh7 cells were stained with Hoechst (blue). Scale bar: 10 μm. **(f)** EEF area at 24, 48 and 72 hpi of Huh 7 cells with spz of either WT (grey dots) or *PbΔmei2Δlisp2* (blue squares), assessed by live imaging of mCherry-expressing parasites. Data are expressed as mean ± SD and were compared using the unpaired Student's *t*-test (ns, not significant; \*P < 0.05; \*\*\*\*P < 0.0001). **(g)** Representative immunofluorescence microscopy images of WT and *PbΔmei2Δlisp2* parasites expressing merozoite proteins MSP1 and AMA1 at 72 hpi of Huh7 cells. Immunofluorescence staining with anti-MSP1 (green) and anti-AMA1 (green), confirms the expression of both proteins by the LA-GAP. Liver stage parasites express cytoplasmic mCherry (red). Nuclei of both the parasites and Huh7 cells were stained with Hoechst (blue). Scale bar: 10 μm.



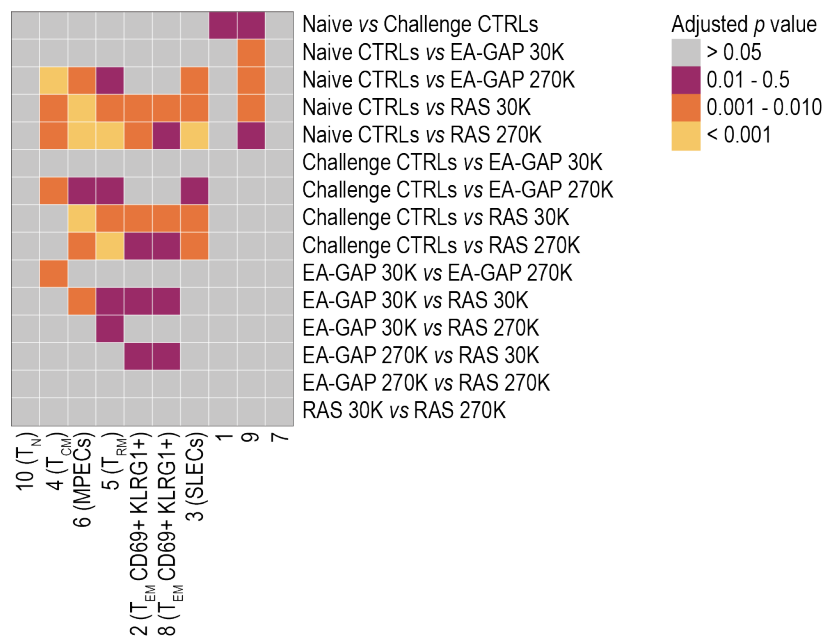
**Supplementary Figure 2:** Prepatent period and protection from experimental cerebral malaria (ECM) of C57BL/6J mice following a P2B immunization with different dosages of the various WSpz formulations. **(a)** Days to patency for challenge (non-immunized) controls (grey circles) and immunized but non-protected mice (coloured circles) are presented as the number of days until the detection of blood stage parasites, as measured through a bioluminescence assay. Data are expressed as mean  $\pm$  SD and were compared using the Kruskal-Wallis test with Dunn's multiple comparison post-test whenever the number of non-protected mice allowed for sufficient statistical power (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ ). **(b)** Kaplan-Meier survival plots showing the percentage of mice which developed ECM for each WSpz dosage and immunization approach (n = 5-20 mice per immunization dosage from 1-4

independent experiments). Statistically significant differences relative challenge controls (\* next to symbols) or between experimental groups (\* next to the lines) were calculated by Log-rank (Mantel-Cox) test. Signs of ECM were monitored daily between days 5 and 10 post challenge.



**Supplementary Figure 3:** Liver pathology and immune population analyses of the spleens and livers of C57BL/6J mice immunized with either 30 or 270K EA-GAP or RAS WSpz following a P2B immunization regimen. **(a)** Representative microphotographs of haematoxylin- and eosin-stained liver sections collected from non-immunized (challenge controls) and immunized mice 48 h after *Pb*-Luci infectious challenge as well as from naïve controls (n = 3-

5 mice per group). Arrows indicate inflammatory cell infiltrates. Scale bar: 50  $\mu$ m. **(b)** Quantification of the levels of metabolites (total bilirubin, glucose, aspartate aminotransferase, AST, alanine aminotransferase, ALT) in the serum collected from non-immunized (challenge controls) and immunized mice 48 h after *Pb*-Luci infectious challenge as well as from naïve controls (n = 3 mice per group). Data are expressed as mean  $\pm$  SD. Statistically significant differences relative to naïve control mice (#), relative to challenge controls (\* above the bars) or between experimental groups (\* above the lines) were assessed through the Kruskal-Wallis test with Dunn's multiple comparison post-test (\*, #P < 0.05; \*\*, ##P < 0.01). **(c)** Parasite liver load 48 h post challenge of C57BL/6J mice immunized either with 30 or 270K EA-GAP or RAS WSpz and compared to the corresponding challenge control mice. Symbols represent the individual values of each mouse and bars indicate the mean. **(d)** Representative gating strategy applied for the flow cytometric analysis of immune populations of the spleen and liver. FVD: Fixable viability dye.



**Supplementary Figure 4:** Clustering analysis of the CD8+ T cell pool in the livers of C57BL/6J mice immunized with either 30 or 270K EA-GAP or RAS WSpz following a P2B immunization regimen. Heatmap of the statistical analysis performed for Fig. 4D on the number of cells within each cluster and in each experimental condition.