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Appendix

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This file is a(n) Appendix of:

Dendritic cell-mediated responses to secreted *Cryptosporidium* effectors
promote parasite-specific CD8+ T cell responses

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Byerly, Jessica H; Smith, Eleanor J; Buenconsejo, Gracyn Y [and 4 more]

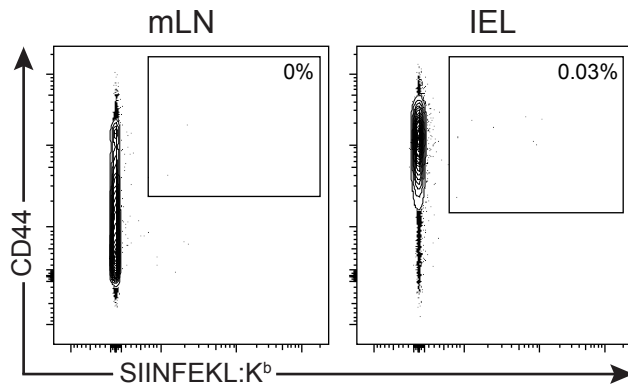
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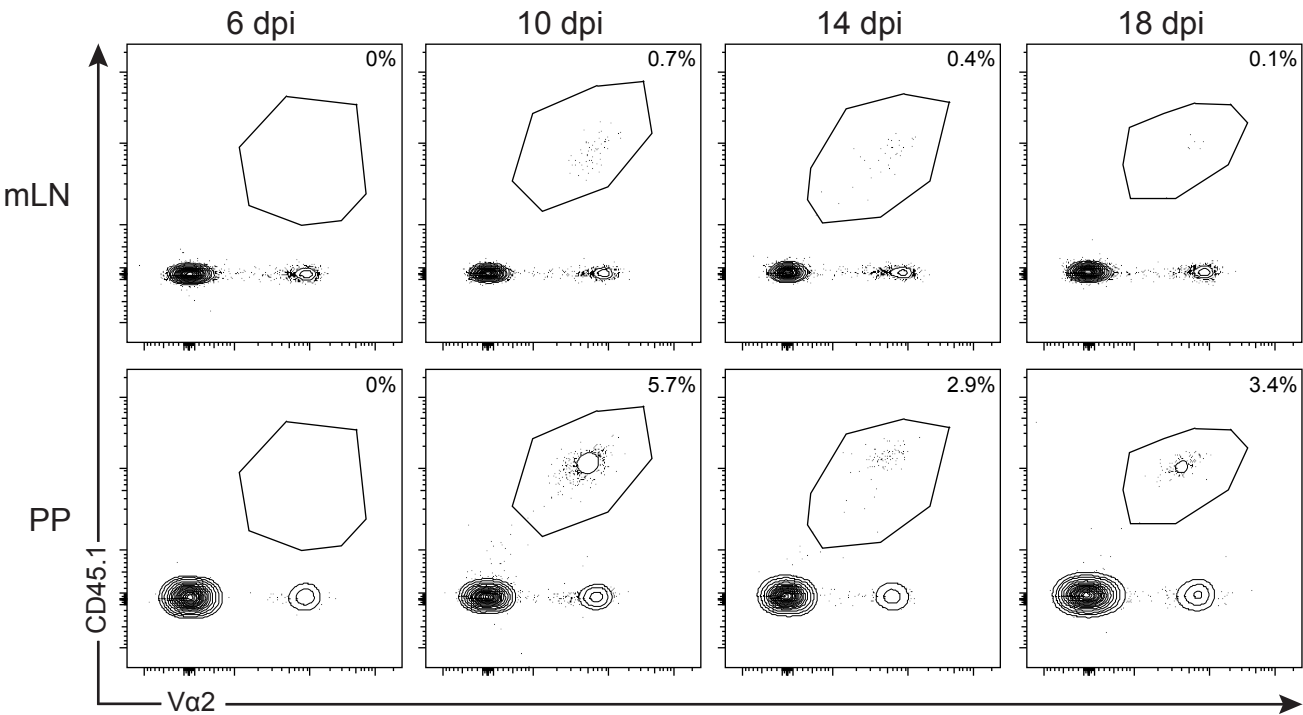
[10.1016/j.mucimm.2024.03.003](https://doi.org/10.1016/j.mucimm.2024.03.003)

Supplemental Figure 1.



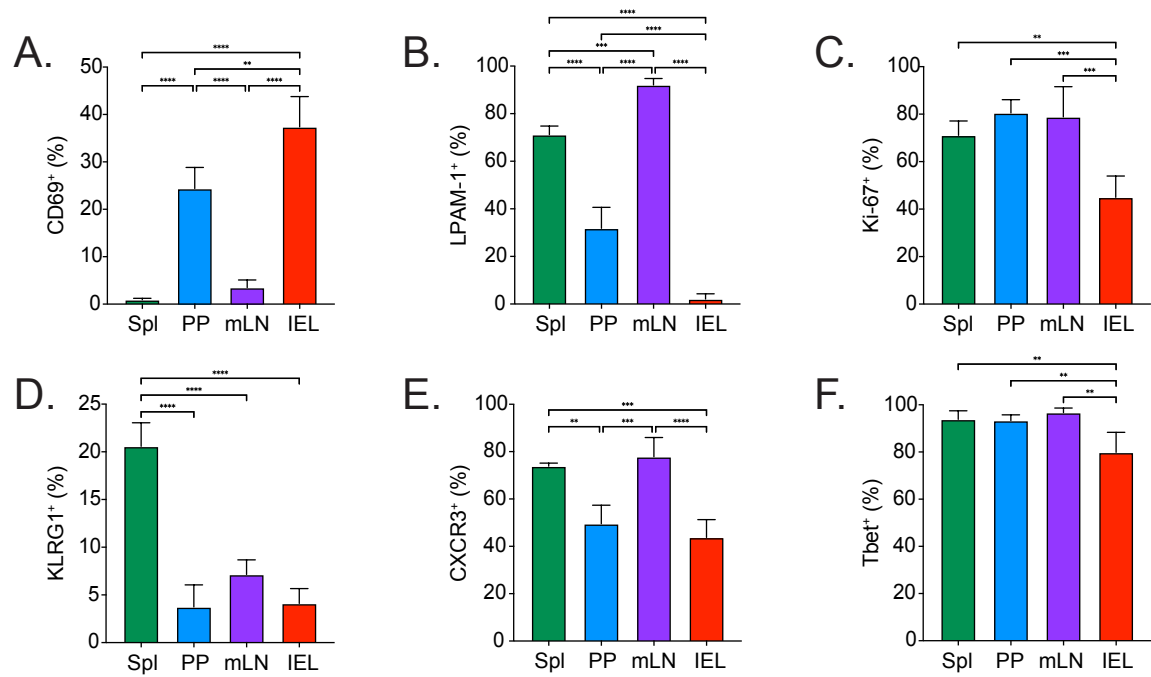
Supplemental Figure 1. (Refers to Figure 1). Representative flow plots display SIINFEKL:K^b CD8⁺ T cells in mLN and IEL from one uninfected mouse in experiments performed in Fig. 1D.

Supplemental Figure 2.



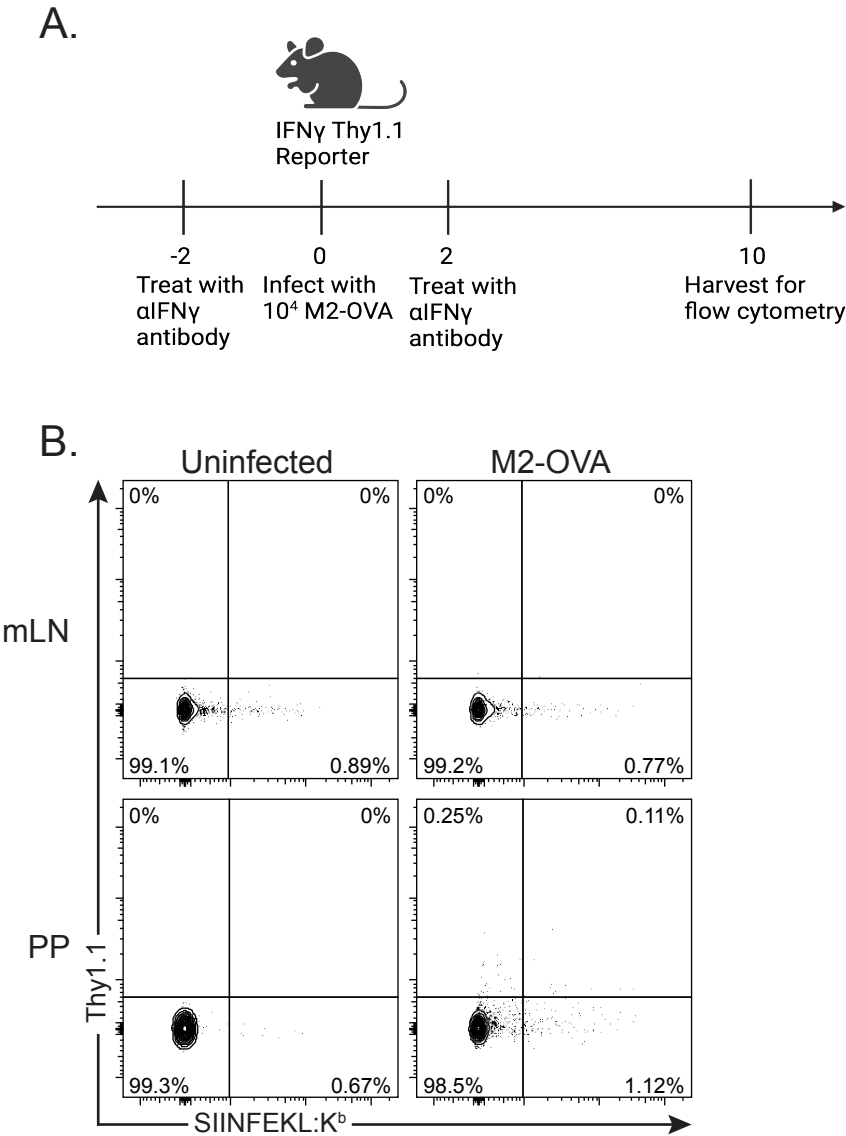
Supplemental Figure 2. (Refers to Figure 2). Representative flow plots display OT-I cells in mLN and PP from experiments performed in Fig. 2A/B.

Supplemental Figure 3.



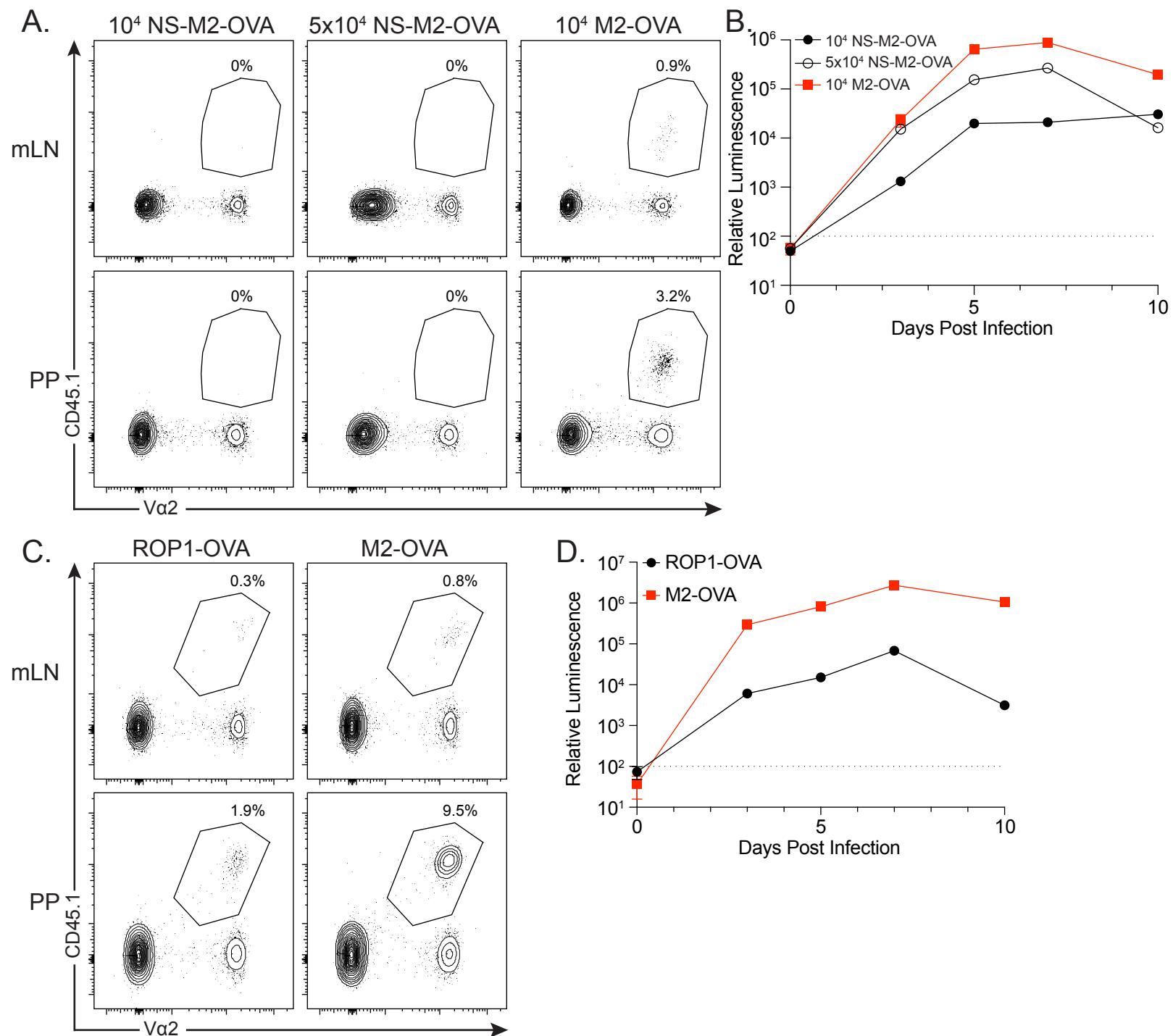
Supplemental Figure 3. (Refers to Figure 3). **A-F**. Summary bar graphs of the percentages of OT-I cells that express the given parameter from Fig. 3D-I.

Supplemental Figure 4.



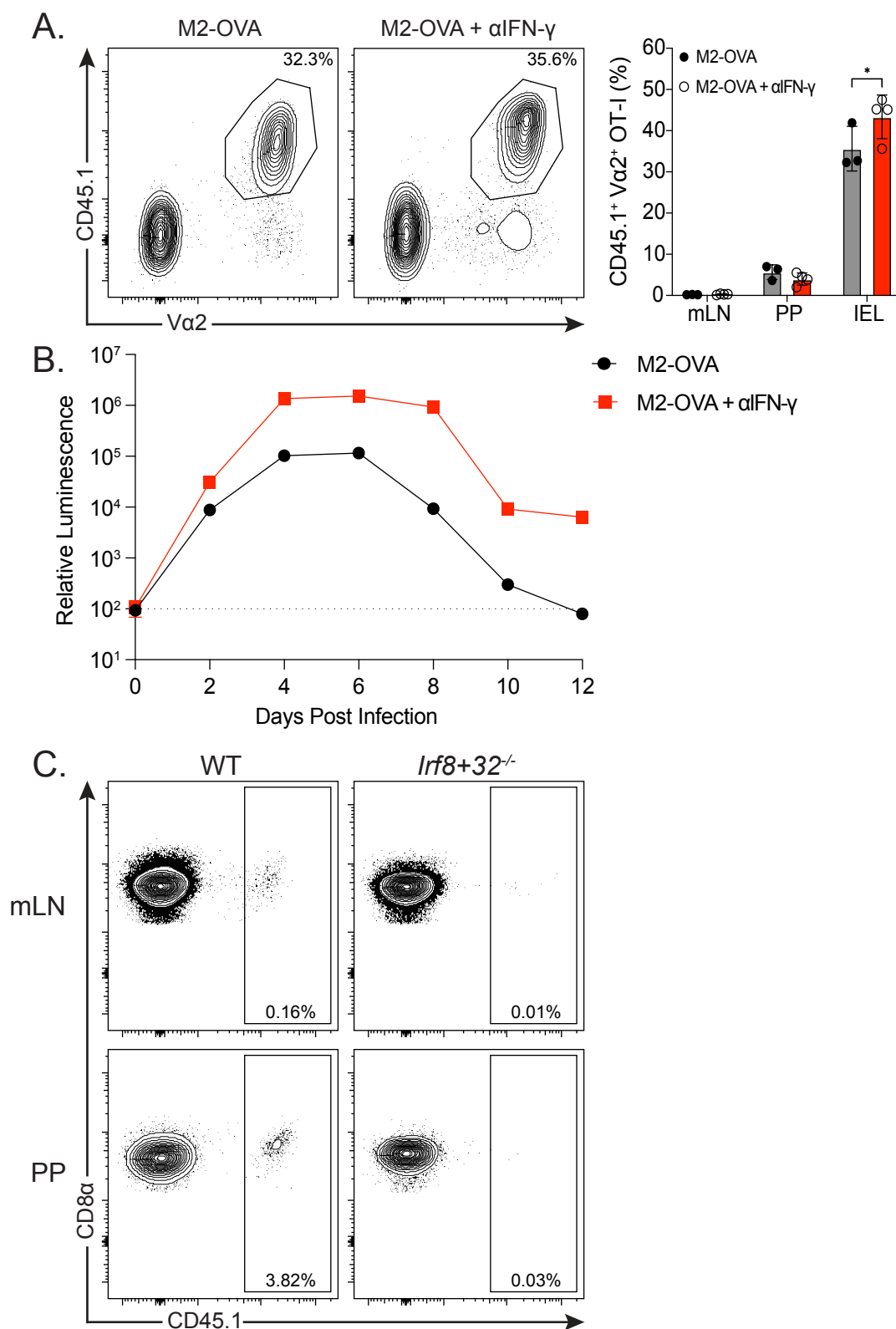
Supplemental Figure 4. (Refers to Figure 4). **A.** Experimental timeline for experiments performed in Fig. 4A created with BioRender.com. **B.** Representative flow plots show CD8⁺ T cellThy1.1 and SIINFEKL:K^b expression in mLN and PP from experiments performed in Fig. 4A.

Supplemental Figure 5.



Supplemental Figure 5. (Refers to Figure 5). **A.** Representative flow plots show OT-I cells in mLN and PP from experiments in Fig. 5C. **B.** Parasite burdens (relative luminescence) over time from mice infected in Fig. 5C. **C.** Representative flow plots show OT-I cells in mLN and PP from experiments in Fig. 5D. **D.** Parasite burdens (relative luminescence) over time from mice infected in Fig. 5D.

Supplemental Figure 6.



Supplemental Figure 6. (Refers to Figure 6). **A.** WT C57BL/6J mice were left untreated or treated with 1 mg/mouse αIFN-γ 2 days prior to infection and 2 days post infection with 10^4 M2-OVA. mLN, PP, and IEL were harvested 12 dpi for flow cytometry. Representative flow plots show OT-I cells in IEL, gated on Singlets, Live, CD19⁻, NK1.1⁻, CD90.2⁺, CD4⁻, CD8α⁺, CD45.1⁺ Va2⁺. Summary bar graphs from one experiment of percentages of OT-I cells. N=3-4 mice/group for 2 independent experiments. **B.** Parasite burdens from experiments performed in **A.** **C.** Representative flow plots show OT-I cells in mLN and PP from experiments in Fig. 6B.

Supplemental Table 1. List of Primers for plasmid construction							
Use	Sequence						
Linearizing p3XHA_nLuc-Neo for SIINFEKL insertion	taaccgggatgcatctca						
	ggcataatctggaacatcgtaagg						
SIINFEKL sequence for Gibson assembly	ccttacgatgttcagattatgccagtataatcaacttgaaaaactg						
	tgaagatgcatccgggttacagttttcaaagtgattatact						
Repair template amplification M2-OVA	aaaaagaaaaagagaagagggggaaaaagatgatcttagaggactattaagaggattatccgggtcaagtaagacatactacacgacttagagaaaattggaagtgaggacgggaattc						
	taattatctgttaataacacatttgaatagttatacttcacataaccaaattaagataaaaaagaaaaacttaatcgatactatcctacacg						
M2 targeting guide	gttgaggattatccgggtcaagta						
	aaactacttgaaccggataatcct						
Repair template amplification ROP1-OVA	tataaatcacctgaattccgaaaatggaagtggaggacgggaattc						
	gaatttgggtattttctcgtctcaccgcgtttaactgattggtacta						
ROP1 targeting guide	gttgactaaaaataattgttcaa						
	aaactgaaacaattatttttagtc						
Linearizing p3XHA_nLuc-Neo_M2 for tdTom insertion	aattggctcgtggcgtgtagga						
	gaagaattcgtcaagaagacgatagaag						
tdTom amplification for Gibson assembly	gccttctatcgtctcttgacgaattctcggtgagggcaggggtagattgtgacttgcggtgacgttgaggagaaccccgcccgatggtagtaagggcgag						
	tcacttatacagctcatccatgc						
Repair template NS-M2-OVA	tagctttttgccacagcgacaaatagtttgatttcagtaagttatcaccatagctgcgccaaattttgc						
	tccagtactatgctatggttgagaacagacttaagggaaatttattgatggggaaactaaatactactgaaattcgggt						
TK targeting guide for NS-M2-OVA insertion	gttgaaggagtaataactatttagca						
	aaactgctaataagtatttacttc						



The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item	Recommendation	Section/line number, or reason for not reporting
Study design	1 For each experiment, provide brief details of study design including: <ul style="list-style-type: none"> a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals). 	Results Figure legends
Sample size	2 a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	Figure legends
Inclusion and exclusion criteria	3 a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i> . If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of <i>n</i> in each experimental group.	Figure legends
Randomisation	4 a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Not applicable
Blinding	5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Not applicable
Outcome measures	6 a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Results
Statistical methods	7 a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods
Experimental animals	8 a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: <ul style="list-style-type: none"> a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures). 	Methods Results
Results	10 For each experiment conducted, including independent replications, report: <ul style="list-style-type: none"> a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval. 	Results Figure legends