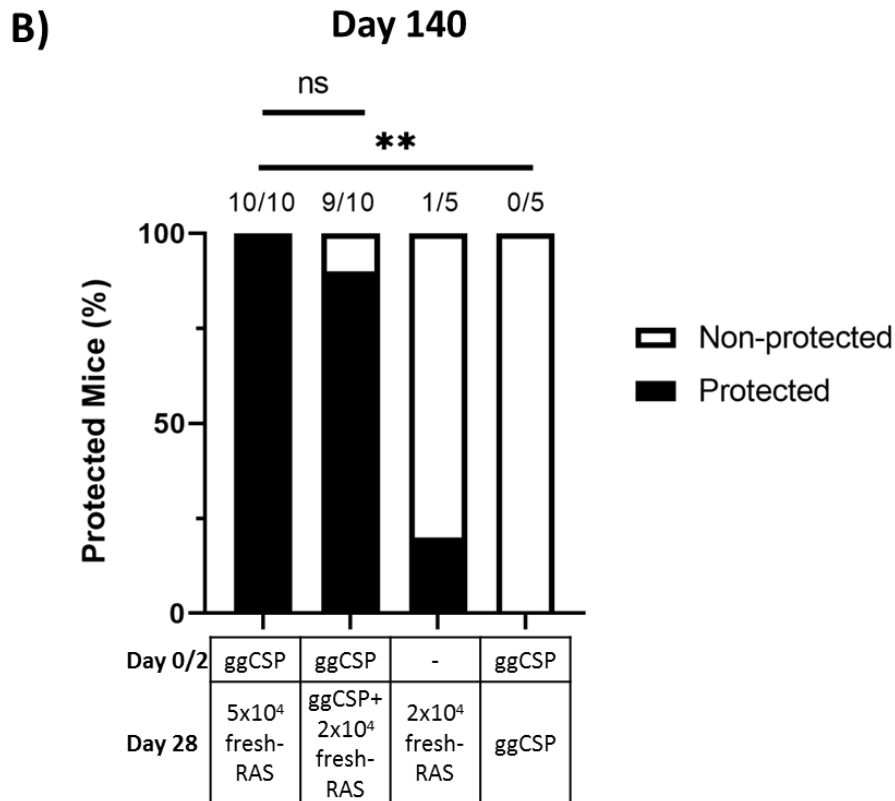
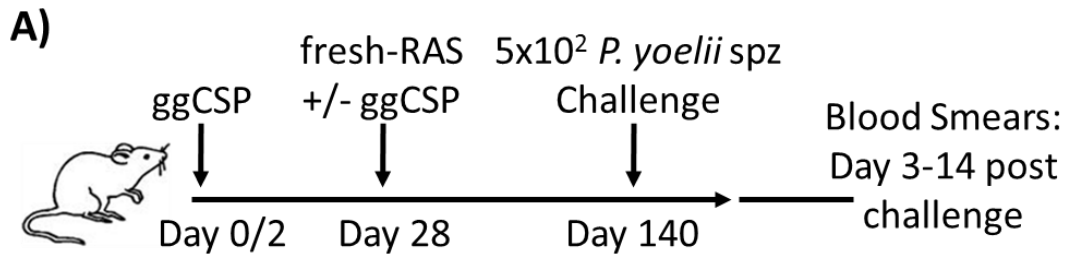
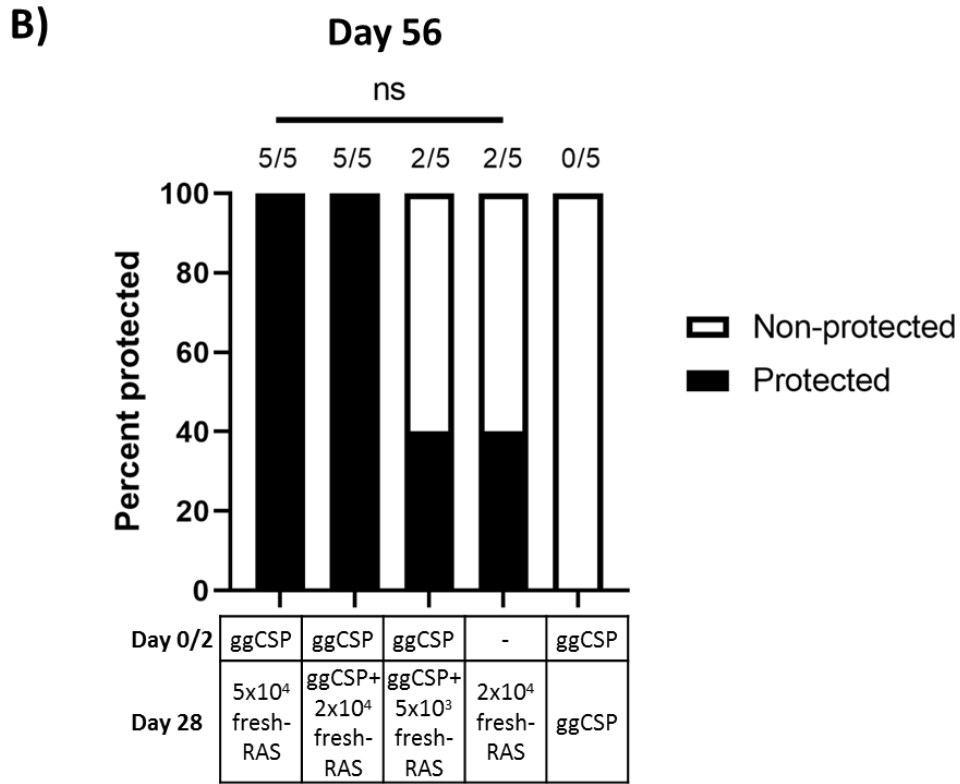
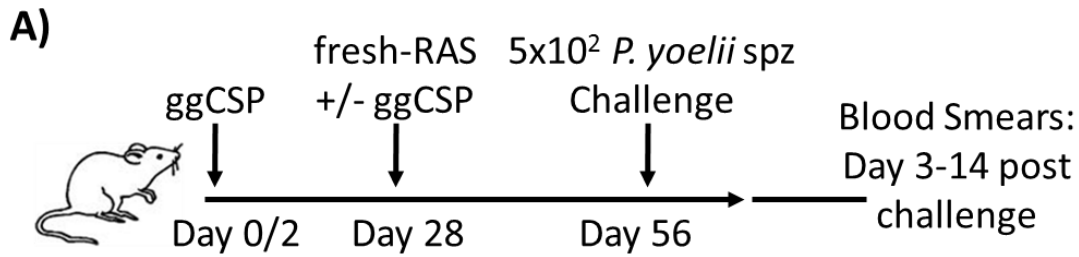


Supplemental Figure 1. Representative gating strategy and identification of PyCSP-specific Trm cells in the liver of a prime-and-trap vaccinated animal. Cells were gated for CD8⁺ T cells (CD3e⁺, B220⁻, CD4⁺), CD44^{hi} by CD62L^{lo}, then assessed by either KLRG1^{lo} by CD69^{hi} or by CXCR6^{hi} by CD69^{hi}. Antigen specificity was then assessed by PyCSP-tetramer (SYVPSAEQI-specific H2-K^d tetramer). Cell count per gram of tissue was calculated based on a known concentration of counting beads per samples to normalize data.



Supplemental Figure 2. DNA priming followed by fresh-RAS trap protects mice from spz challenge at four months: **(A)** Experimental design of prime-and-trap studies. **(B)** Results of protection studies after challenge with 500 wild-type purified *P. yoelii* spz administered four months after trapping. n=5-10 mice from one experiment. Fractions above bars indicate number of animals protected out of total group size. Protection was assessed with thin blood smears on Days 3-14 post challenge. Fisher Exact Test: fresh-RAS vs. all other groups. ns=p>0.05. $p < 0.001$.



Supplemental Figure 3. Dose-dependent protection of prime-and- fresh-RAS trap vaccination: **(A)** Experimental design of prime-and-trap studies. **(B)** Results of protection studies after challenge with 500 wild-type purified *P. yoelii* spz administered four weeks after trapping. n=5 mice from one experiment. Fractions above bars indicate number of animals protected out of total group size. Protection was assessed with thin blood smears on Days 3-14 post challenge. Fisher Exact Test: fresh-RAS vs all other groups. ns= $p > 0.05$. □

Specificity	Clone	Fluorochrome	Vendor	Catalog
CD3e	145-2C11	BUV 395	BD	563565
B220	RA3-6B2	BV711	BioLegend	103255
CD4	GK1.5	Alexa Fluor 700	BioLegend	100429
CD8a	53-6.7	BV421	BD	563898
CD69	H1.2F3	BV510	BD	563030
CD44	IM7	Alexa Fluor 488	BioLegend	103015
CD62L	MEL-14	PE-Cy7	BD	560516
KLRG1	2F1	PerCP-Cy5.5	BioLegend	138418
CXCR6	221002	PE	Fisher Scientific	FAB2145P100
CSP-tetramer	-	APC	NIH	-
APC	-	Streptavidin-APC	Prozyme	PJ27S
Zombie NIR™ Fixable Viability	-	NIR	BioLegend	423105
Fc CD16/32	2.4G2	-	BD	553141
Counting Beads	-	-	Polyscience	18328-5

Supplemental Table 1: Flow cytometry reagents and antibodies that were used to assess liver resident memory cells.