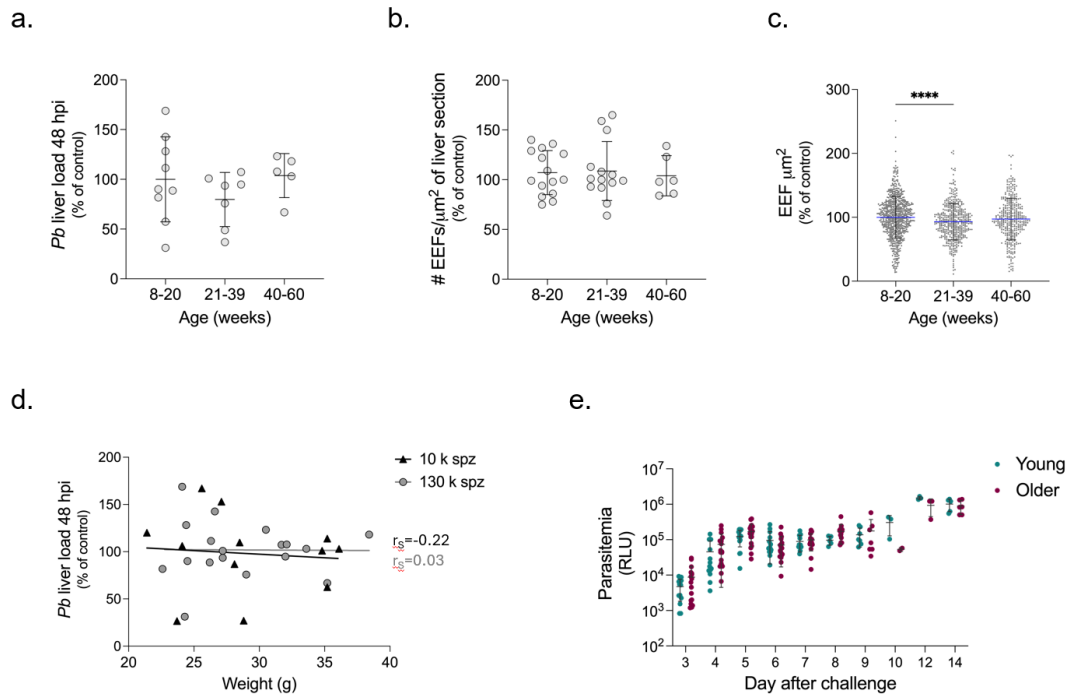
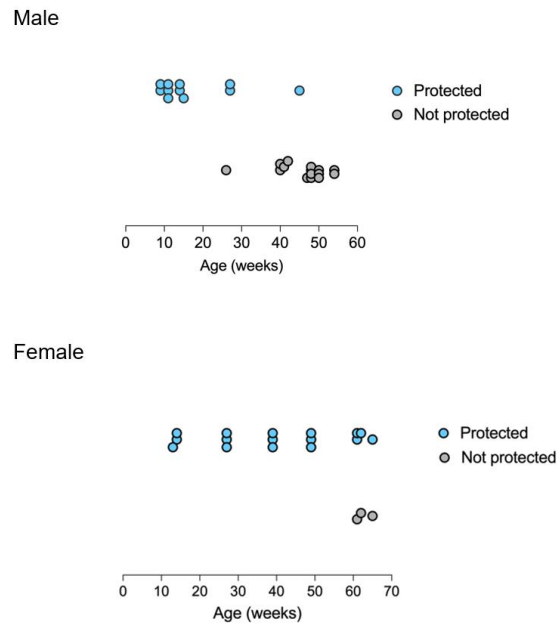
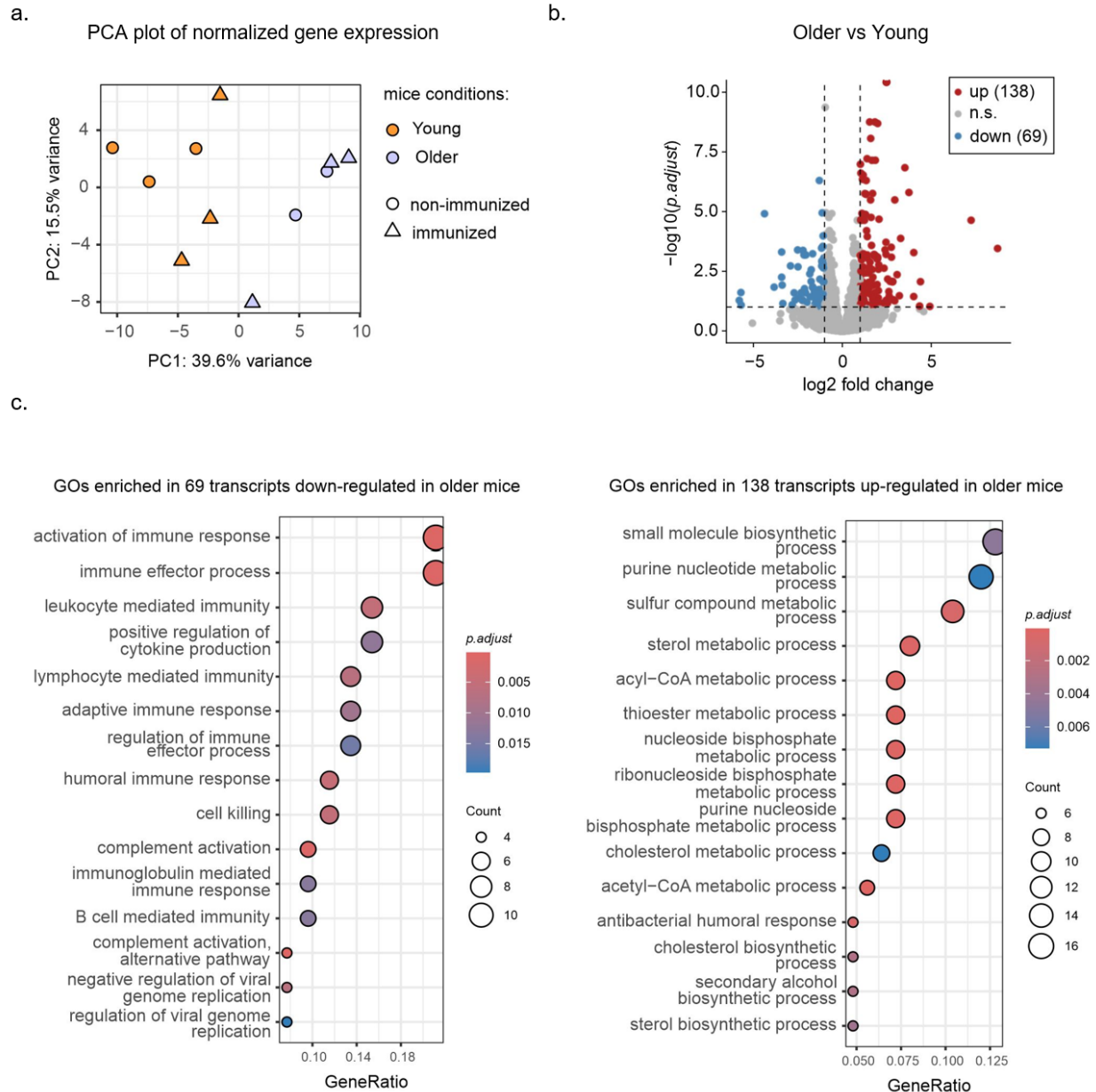




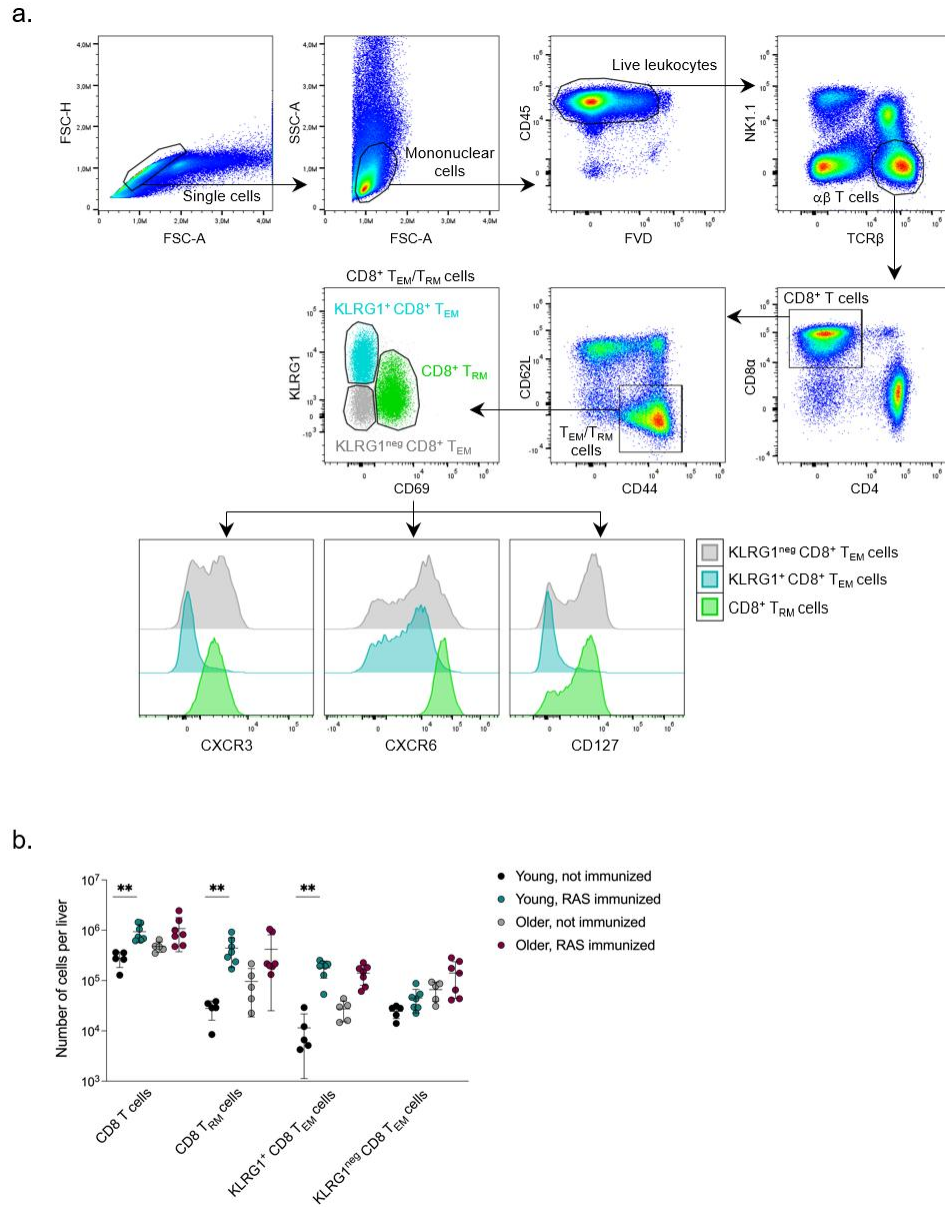
Supplementary Figure 1: Hepatic *Pb* infection and development are largely unaffected by age in mice from 8 to 60 weeks. **a)** *Plasmodium berghei* (*Pb*) liver parasite load 48 hours post-infection (hpi) following intravenous inoculation with 130 k sporozoites (spz) of mice of different ages, presented as age groups of 8-20, 21-39, and 40-60 weeks. Liver burden was quantified by RT-qPCR and normalized to control young mice, as described in Fig. 1 (a), (b). Each symbol represents an individual mouse. **b)** Quantification of exoerythrocytic forms (EEFs) per μm^2 of liver section at 48 hpi in the mice shown in (a), determined by fluorescence microscopy. Values were normalized to the EEF density measured in liver sections of control mice aged 9 to 10 weeks. Each symbol represents a liver section (2-3 liver sections per mouse). **c)** Size of individual EEFs (μm^2) at 48 hpi across age groups. Each dot represents an EEF. Values were normalized to the EEF size measured in liver sections of control mice aged 9 to 10 weeks. **d)** Correlation between mouse body weight and *Pb* liver parasite load at 48 hpi, for infections with 10 k or 130 k spz. Each symbol represents an individual mouse. Data was analyzed using Spearman's rank correlation. Spearman r (r_s) is shown for 10 k (black) or 130 k (gray) inoculum doses (both correlations not significant). **e)** Blood stage parasitemia following infection of young and older non-immunized mice with 10 k *PbLuc* spz. Data are from 14-17 mice per group (each symbol represents a mouse) from 6 independent experiments. Data in (a)-(c) and (e) are presented as mean $\pm$ SD and were compared using the Kruskal-Wallis test with Dunn's multiple comparison post-test (in (e) comparison was only performed between different age groups within the same time-point). ****: $P < 0.0001$.



Supplementary Figure 2: Protection following RAS immunization declines with age in both male and female mice. Male (top) and female (bottom) mice of different ages received three weekly iv immunizations with 10 k *PbRAS* and were challenged iv with 10 k infectious *PbLuc* sporozoites two weeks after the final immunization, as described in Fig. 1c. Protection was defined as absence of blood stage parasitemia 14 days after challenge. Each symbol represents an individual mouse, plotted by age. Blue symbols indicate protected mice and gray symbols indicate non-protected mice.



Supplementary Figure 3: Age-dependent changes in gene expression. a) Principal component analysis (PCA) of log-normalized gene expression showing separation of samples, according to age, young versus older, and immunization status (see Fig. 2). Variance explained by PC1 and PC2 is indicated. b) Differential gene expression analysis between older and young mice. Volcano plot showing log₂ fold change versus -log₁₀(adjusted p-value). Differentially expressed genes (DEGs) were defined as adjusted p-value < 0.1 and absolute log₂ fold change > 1. Numbers of detected upregulated (red) and downregulated (blue) genes are indicated. c) Gene Ontology (GO) enrichment analysis of DEGs. Left, GO terms enriched among genes downregulated in older mice. Right, GO terms enriched among genes upregulated in older mice. Dot size indicates gene count and color indicates adjusted p-value. Top 15 detected categories are indicated.

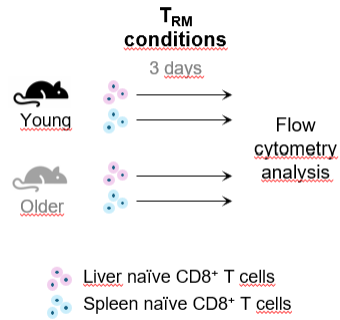


Supplementary Figure 4: Immune responses to RAS immunization in young vs. older mice.

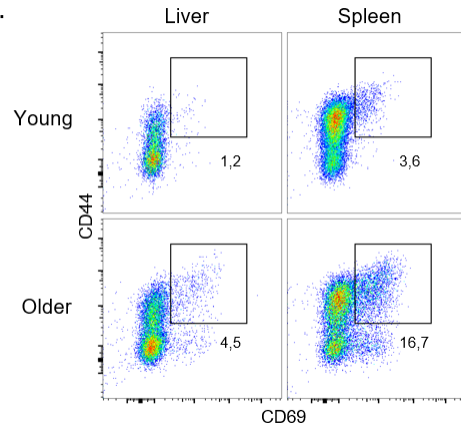
a) Flow cytometry gating strategy used to manually identify and quantify liver CD8⁺ T cell populations in young and older mice, either RAS-immunized or non-immunized. FVD: fixable viability dye. **b)** Absolute numbers of total CD8⁺ T cells, CD8⁺ T_{RM}, KLRG1⁺ T_{EM} cells, or KLRG1^{neg} T_{EM} cells per liver in young and older mice, either RAS-immunized or non-immunized. Data are from 2 independent experiments, and each point represents one mouse. Data are shown as mean ± SD. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's multiple-comparisons test. Pre-specified pairwise comparisons were performed between age groups within each immunization condition (young versus older non-immunized mice and young versus

older immunized mice) and between immunization conditions within each age group (non-immunized versus immunized young mice and non-immunized versus immunized older mice). Only statistically significant differences are indicated: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.

a.

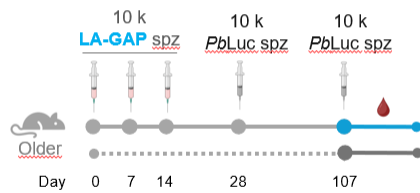


b.

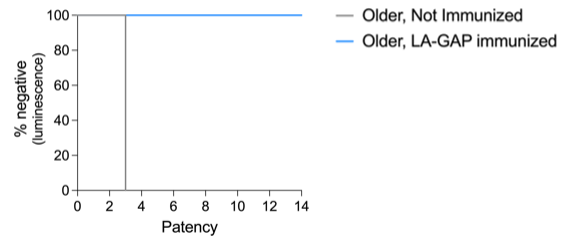


Supplementary Figure 5: Naïve CD8⁺ T cells from older mice retain the capacity to upregulate activation and T_{RM}-associated markers. **a)** Experimental design to assess the ability of naïve CD8⁺ T cells from young and older mice to acquire activation and tissue-residency markers. Naïve CD8⁺ T cells (live single CD45⁺TCRβ⁺NK1.1^{neg}CD8⁺CD4^{neg}CD62L⁺CD44^{neg} cells) were FACS-sorted from the liver or spleen of young or older mice and cultured *in vitro* under T_{RM}-polarizing conditions for 3 days. **b)** Representative flow cytometry plots showing expression of CD44 and CD69 following culture. Numbers indicate the percentage of CD44⁺CD69⁺ cells within the gated population.

a.



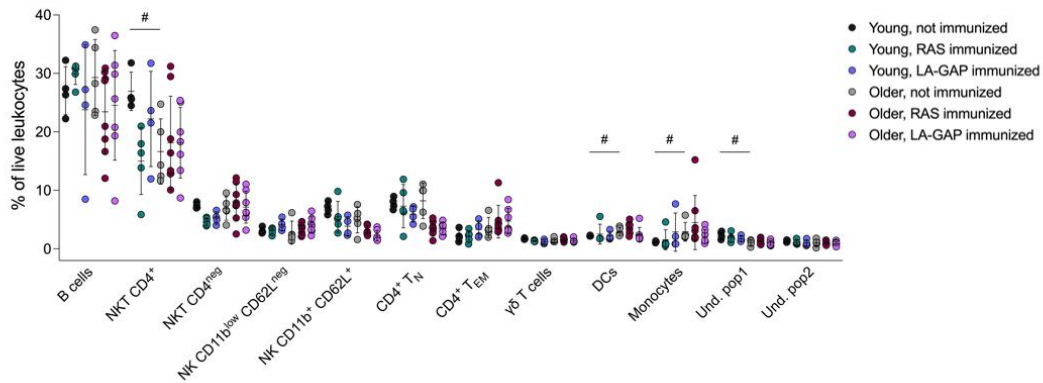
b.



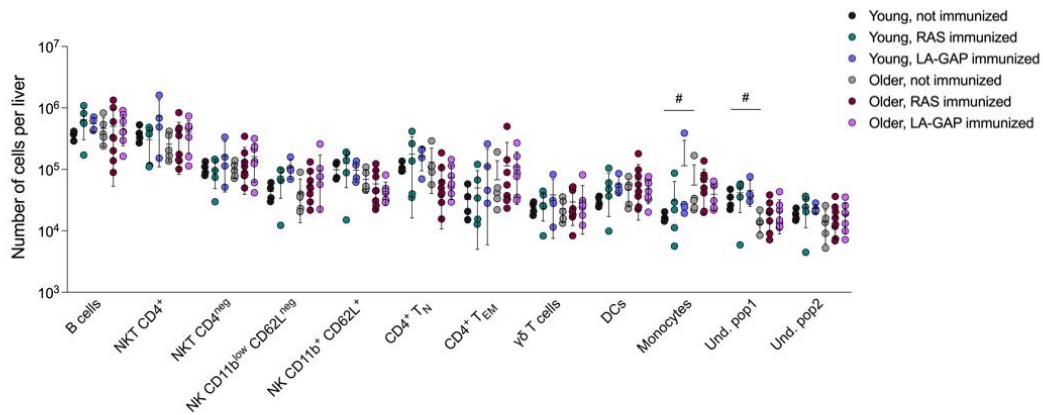
Supplementary Figure 6: LA-GAP vaccination induces durable protection in older mice. a)

Experimental design to evaluate long-term protection following LA-GAP immunization in older mice. Mice were immunized three times at weekly intervals with 10 k LA-GAP sporozoites (spz) and challenged with 10 k *PbLuc* spz. Mice that remained protected were rechallenged approximately 3 months later with the same parasite dose. Blood parasitemia was monitored by luminescence for up to 14 days after challenge. **b)** Kaplan-Meier curve showing the proportion of mice remaining free of blood stage parasitemia following the second challenge. Data are from 3 mice per condition.

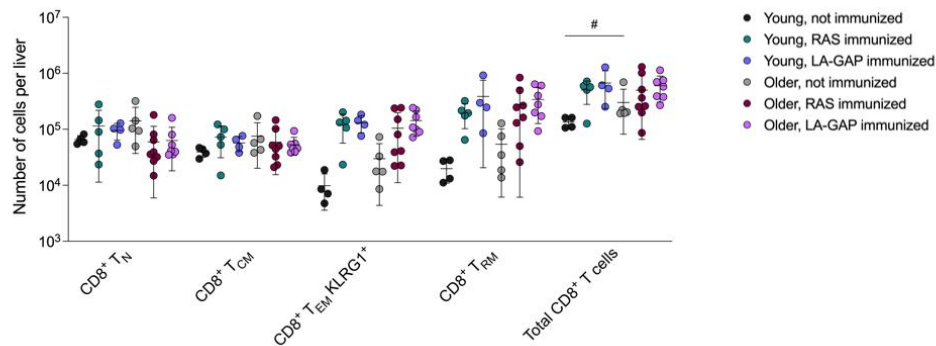
a.



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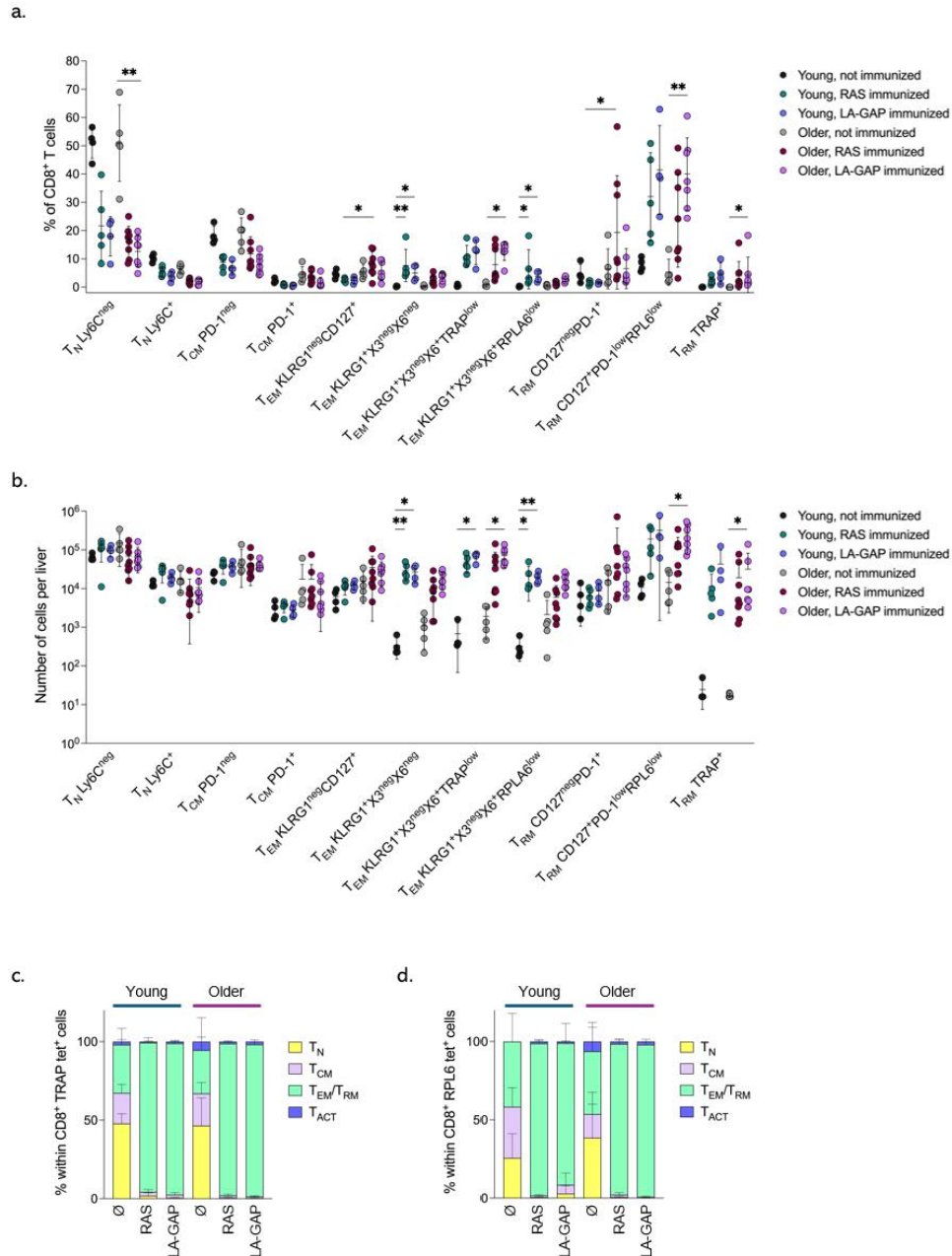


c.



Supplementary Figure 7: Limited quantitative changes, despite enhanced protection, in LA-GAP as compared to RAS immunization in older mice. **a)** Frequencies within live mononuclear leukocytes of the clusters corresponding to non-CD8⁺ T cell populations presented in Fig. 5c. **b)** Absolute numbers of the populations presented in (a). **c)** Absolute numbers of the CD8⁺ T cell populations. Each symbol represents a mouse. Data are representative of 4 independent experiments and are presented as mean $\pm$ SD. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's multiple comparisons test. Pre-specified pairwise comparisons were performed between age groups within each immunization condition (young versus older non-immunized, RAS-immunized, or LA-GAP-immunized mice) and between

immunization conditions within each age group (non-immunized versus RAS-immunized, non-immunized versus LA-GAP-immunized, and RAS-immunized versus LA-GAP-immunized mice). Only statistically significant differences are indicated: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$. Non-immunized young vs older mice were further compared using a Mann-Whitney test: #: $P < 0.05$.



Supplementary Figure 8: Analysis of liver CD8⁺ T cell subsets and antigen-specific CD8⁺ T cell composition following RAS and LA-GAP immunization. Frequencies within liver CD8⁺ T cells (**a**) and number of cells per liver (**b**) of the clusters presented in Fig. 6 (c), (d). Each symbol represents a mouse. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's multiple-comparisons test. Pre-specified pairwise comparisons were performed between age groups within each immunization condition (young versus older non-immunized, RAS-immunized, or LA-GAP-immunized mice) and between immunization conditions within each age group (non-immunized versus RAS-immunized, non-immunized versus LA-GAP-immunized, and RAS-immunized versus LA-GAP-immunized mice). Only statistically significant differences are indicated: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$. Frequency, within TRAP-

specific (**c**) and RPL6-specific (**d**) tetramer-positive (tet⁺) CD8⁺ T cells, of naïve (T_N; CD62L⁺CD44^{neg}), central memory (T_{CM}; CD62L⁺CD44⁺), effector/effector memory or resident memory (T_{EM}/T_{RM}; CD62L^{neg}CD44⁺), or activated (CD62L^{neg}CD44^{neg}) cells. Data are from 4-8 mice and are presented as mean ± SD.