

**Supplemental Figure 1: Antigen-specific gene gun DNA vaccination induces high frequency**

**IFN $\gamma$  T cell responses, but only select antigens are protective. (A)** IFN $\gamma$ -producing cells

following control (ggNTC empty vector) versus CSP-specific plasmid vaccination 10 days after boosting as determined by restimulation for 5 h with CSP-peptide (4 h with brefeldin A) and staining for CD3<sup>+</sup>B220<sup>-</sup>CD4<sup>-</sup>CD8<sup>+</sup>IFN $\gamma$ <sup>+</sup> (Welch's t-test). **(B)** CSP tetramer-stained, activated

(CD44<sup>hi</sup>CD62L<sup>lo</sup>) CD8<sup>+</sup> T cells as in panel A (Welch's t-test). **(C)** IFN $\gamma$ -based ELISPOT of CSP-, MDH- and L3-specific CTL frequency six days after DNA booster. Percent calculated from spot forming units per million splenocytes. **(D)** Results of IVIS-based protection studies

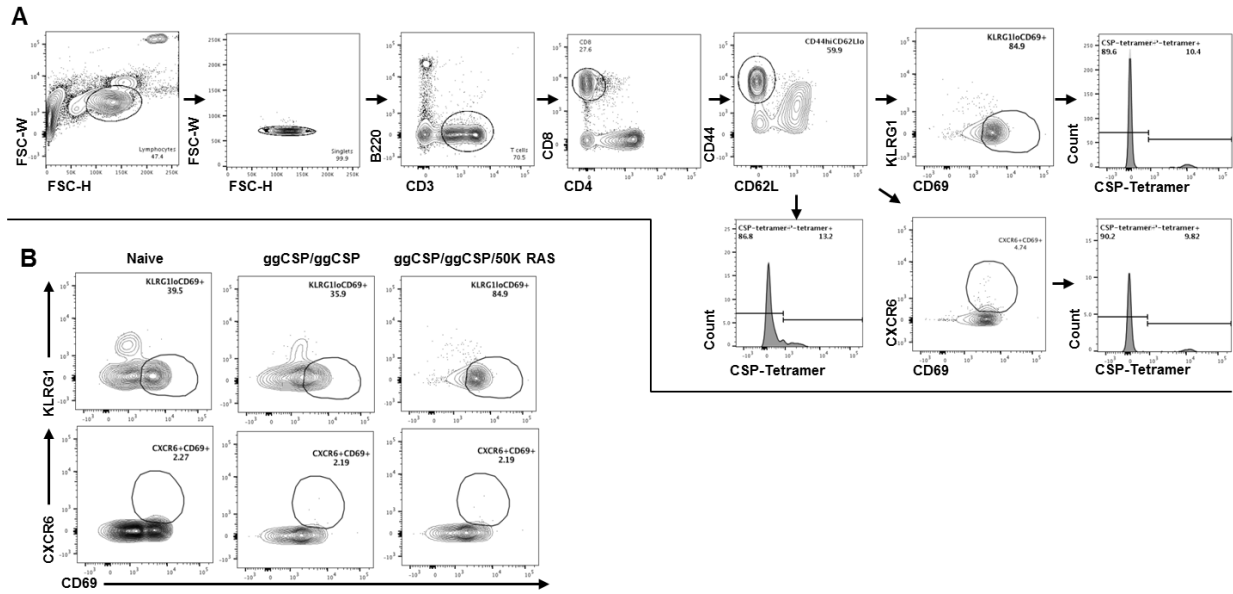
using 2x10<sup>4</sup> luc-expressing purified Py spz administered five days after the last immunization as in Panel C for the indicated vaccine antigen. \*p<0.05, \*\*p<0.01. +Cluster priming for the first

ggCSP vaccination only. All error bars are standard deviation of the mean.

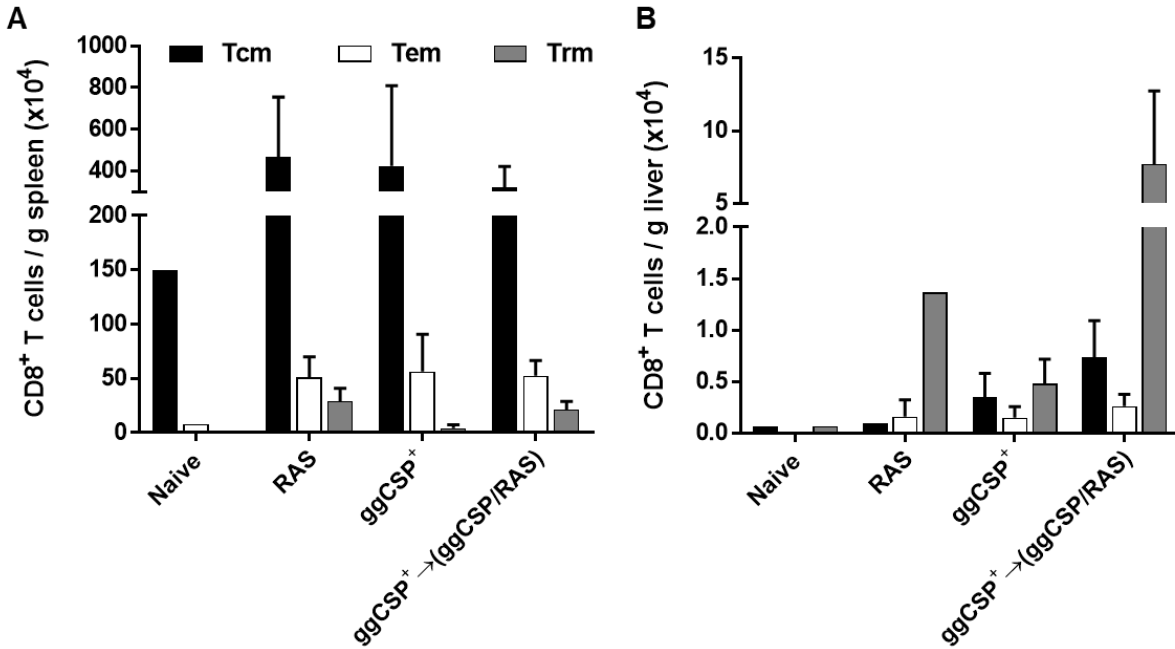
in Panel C for the indicated vaccine antigen. \*p<0.05, \*\*p<0.01. +Cluster priming for the first

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**Supplemental Figure 2. Analysis of CD69<sup>+</sup> liver Trm cells can be achieved by either KLRG1 or CXCR6 markers. (A) Representative plots of flow cytometry panel for liver Trm cells. (B) Representative plots for naïve, ggCSP cluster prime-only, and a ggCSP cluster prime with ggCSP-plus-RAS boost for liver lymphocytes.**



**Supplemental Figure 3. Memory CD8<sup>+</sup> T cell subsets for ggCSP-only, RAS-only and prime-and-trap regimens.** T effector memory (Tem), T central memory (Tcm), and Trm cell population profiles gated on CD8<sup>+</sup> and CD44<sup>hi</sup> for CD62L x CD69. Tem = CD69<sup>-</sup>CD62L<sup>lo</sup>; Tcm = CD69<sup>+</sup>CD62L<sup>hi</sup>; Trm = CD69<sup>+</sup>CD62L<sup>lo</sup>. **(A)** Tcm predominant response in the spleen. **(B)** Trm predominant response in the liver, especially with prime-and-trap regimen. <sup>+</sup>Cluster priming for the first ggCSP vaccination only. All error bars are standard deviation of the mean.