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Supplemental Information

Repeated Antigen Exposure Extends the Durability of Influenza-Specific Lung-Resident Memory $CD8^+$ T Cells and Heterosubtypic Immunity

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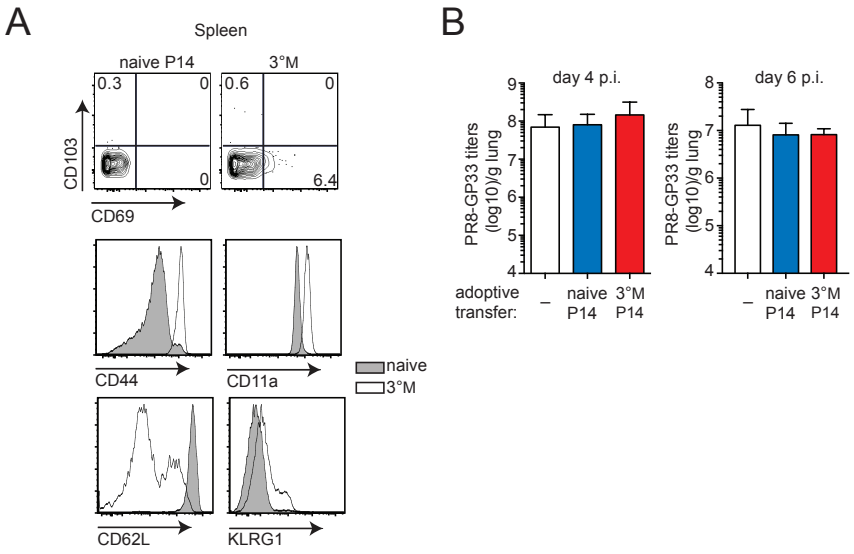

Figure S1: Characterization of adoptively transferred cells and their impact on PR8-GP33 infection. Related to figure 1.

(A) Phenotypic characterization of spleen-derived naive and 3°M P14 used for adoptive transfer and generation of 1°M and 4°M responses. (B) Naive C57Bl/6 recipients were seeded with 10^4 naive or 10^5 3°M P14 cells. 24h later these mice and naive mice were IN infected with PR8-GP33. PR8-GP33 virus titers measured at d4 and d6 p.i. in lungs of mice that received no P14 transfer (white), naive P14 transfer (blue) or 3°M P14 transfer (red). *n*=3-5 mice/group. Representative of 2 independent experiments. Error bars represents mean±SD. No significant differences, Kruskal-Wallis test.

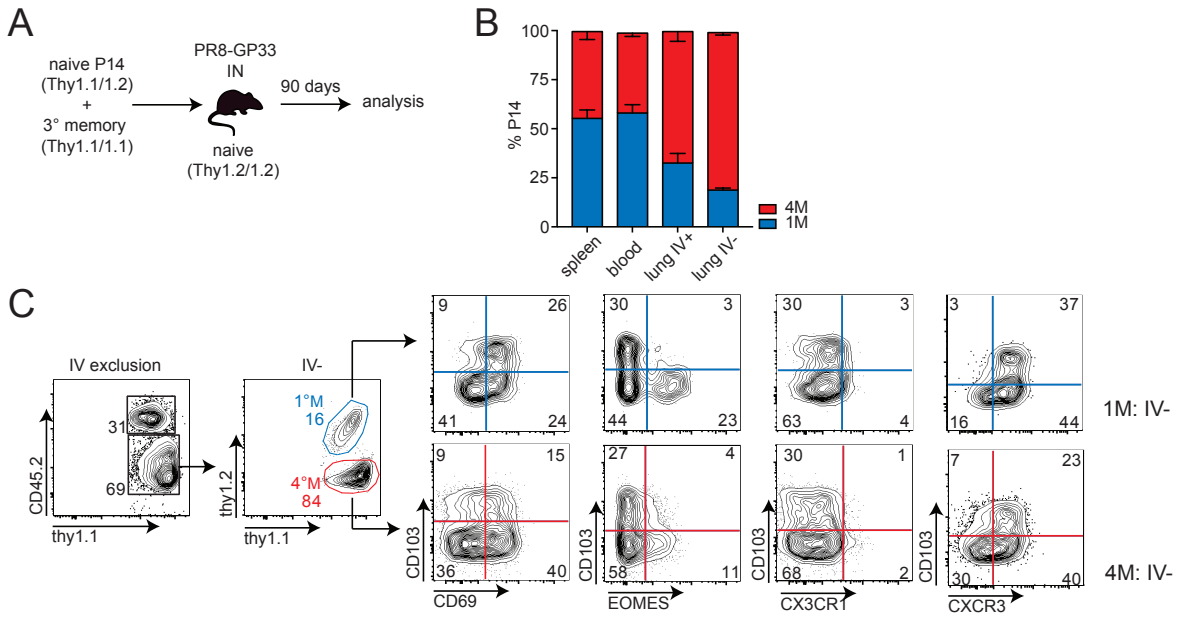

Figure S2: Tissue distribution and phenotypic characterization of 1°M and 4°M P14 cells. Related to figure 1. (A) Naive Thy1.2/1.2 C57Bl/6 mice were seeded with a mixture of 10^4 naive Thy1.1/1.2 and 10^5 3°M P14 cells. 24h later mice were IN infected with PR8-GP33. Mice were analyzed 90 days p.i. (B) Distribution of 1°M and 4°M P14 cells in various tissue compartments expressed as a % of total P14. *n*=3 mice/group. Representative of 2 independent experiments. Error bars represent mean±SD. Two-way ANOVA with Tukey's multiple comparison test. Statistic summary: 1°M – blue (spleen vs bld ns; spleen vs lung IV^+ **p=0.0011; spleen vs lung IV^- ****p<0.0001; blood vs lung IV^+ ***p=0.003; blood vs lung IV^- ****p<0.0001; lung IV^+ vs lung IV^- ns); 4°M – red (spleen vs blood ns; spleen vs lung IV^+ **p=0.0011; spleen vs lung IV^- ****p<0.0001; blood vs lung IV^+ ***p=0.0002; blood vs lung IV^- ****p<0.0001; lung IV^+ vs lung IV^- ns). (C) Representative plots of phenotypic characterization of lung residing IV^- 1°M (blue) and 4°M (red) P14 cells.

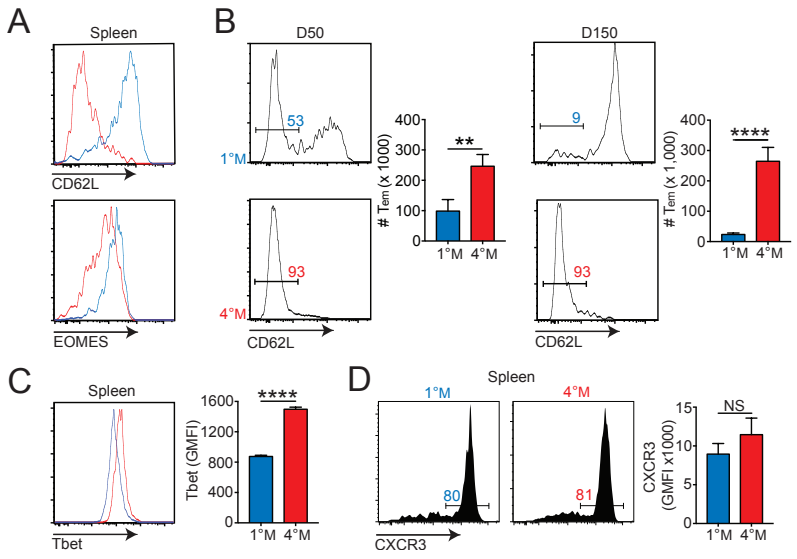

Figure S3: Changes in T_{em}-defining and lung-homing properties of circulating 1°M and 4°M P14 cells. Related to figure 3.

(A) Expression of CD62L by spleen-derived 1°M (blue) and 4°M (red) P14 cells (representative histograms, left) and numbers of $CD62L^{lo}$ T_{em} cells (cumulative bar graphs, right) at D50 and D150 p.i. *n*=4 mice/group. Representative of 2 independent experiments. Error bars represent mean±SD. **p=0.0016, ****p<0.0001, unpaired t test. (B) Representative histograms of expression of CD62L and EOMES by spleen-derived 1°M (blue) and 4°M (red) P14 cells 7 months p.i. (C) Expression of Tbet and (D) CXCR3 by spleen-derived 1°M (blue) and 4°M (red) P14 cells at D150 p.i. Representative histograms (left); cumulative data (right). *n*=4 mice/group. Representative of 2 independent experiments. Error bars represent mean±SD. ****p<0.0001, unpaired t test.