

**Figure S1. B cells promote memory-fated CD8 T cell responses to vaccination and infection. (A)** Representative flow cytometry gating, pre-gated on single-cell lymphocytes. (**B-C**) Eight days after LCMV-Armstrong infection, spleens were assed for (**B**) the number of NP396 (top) or GP276 (bottom) tetramer+ cells, and (**C**) the percentage of CD8 T cells positive for tetramer. (**D**) Representative CD127 x granzyme B staining for GP33 tetramer+ cells. (**E-F**) The percentage of GP33 tetramer+ cells (**E**) expressing high CD127 and (**F**) positive for granzyme B. (**G**) The CD127 gMFI of CD127high cells. (**H**) The granzyme B gMFI of granzyme B+ cells.

## **Supplementary Table 1: Human subject demographics**

Characteristic	Healthy control	CAR T therapy	MS anti-α4 integrin	MS anti- CD20
Median age, years (range)	40 (27-68)	50 (6-79)	46 (25-66)	49 (30-72)
Female sex, n (%)	18 (94.7)	2 (25)	14 (74)	17 (85)
Median BMI, (range)	27 (20-37)	25 (13-18)	29 (20-44)	28 (19-62)
Therapy, n (%)				
Natalizumab			19 (100)	
Rituximab				11 (55)
Ocrelizumab				9 (45)
anti-CD19 CAR		8 (100)		
Median time on therapy, months, (range)		9 (3-15)	82 (15-143)	25 (5-90)
Primary disease, n (%)				
Relapsing-remitting MS			18 (95)	20 (100)
Secondary-progressive MS			1 (5)	
B cell acute lymphoblastic leukemia (B-ALL)		3 (37.5)		
Diffuse large B-cell lymphoma (DLBCL)		2 (25)		
Follicular lymphoma (FL)		2 (25)		
Transformed lymphoma (TL)		1 (12.5)		



Figure S2. B cells shape naïve CD8 T cell programming, promoting FOXO1-mediated homeostasis in mice and humans. (A) GSEA was performed using the GO pathway gene sets. Depicted are the top 10 data sets based on combined score. (B) Heat maps for the top four gene sets, where the differentially-expressed genes associated with the Rho and Ras protein signal transduction gene sets were combined into one heat map. (C) Flow cytometry staining of CD8 T cells from WT and MD4 mice. Representative histograms are shown for CD44<sup>low</sup> naïve (top panels) and for CD44<sup>high</sup> memory and virtual memory (middle panels) cells. Plots of the gMFI (x 10^3, 3, 3, 2, 3, 3, 3, 4, respectively) are shown below for 4-5 mice per group. Data shown are means  $\pm$  SEM, representative of  $\ge 2$  experiments. Significance was defined by two-way ANOVA with the Holm-Šídák's multiple comparisons test, where \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001. (D) Naïve CD8 T cells from the spleens of CAR- or control-treated mice from Figure 1 were analyzed for levels of Foxo1 and CD127. Data are means  $\pm$  SEM, from 2 experiments combined. (E) Gating strategy. (F) Representative CD19 staining for all 4 patient groups. g, Summarized results showing CD19+ cells as a percentage of total live PBMCs for each subject. Data shown are means  $\pm$  SEM, where n = 19, 8, 20, and 19, respectively.



**Figure S3.** Naïve CD8 T cells from B cell-restricted hosts exhibit normal proliferative capacity, but defective survival. (A) Representative flow cytometry gating, pre-gated on live, CD8 T cells in unmanipulated WT or MD4 mice. Arrows indicate population hierarchies. (B) Total splenic cell numbers for the given CD8 T cell subsets. (C) gMFI of CD127, and d, gMFI of CD122 for the cell subsets. Data shown are means ± SEM, from 2 experiments combined.



**Figure S4. The B cell environment in which a naïve CD8 T cell develops has significant consequences on its response to vaccination.** (**A-D**) Four million purified, congenically distinct polyclonal CD8 T cells from WT mice were transferred IV into WT or MD4 recipients. One month later, mice were vaccinated with the combined adjuvant protein subunit vaccine. After 7 days, spleens were analyzed by flow cytometry. (**A**) Experimental schematic. (**B**) Percentage of tetramer+ cells expressing high levels of CD127. (**C**) The gMFI of CD127 staining on tetramer+ CD127<sup>high</sup> cells. (**D**) The gMFI of CD122 staining on tetramer+ cells. (**E**) The percentage of tetramer+ cells within CD45.1/1 transferred CD8 T cells (left columns) or CD45.2/2 endogenous CD8 T cells (right columns). Data shown are means ± SEM, n = 5 mice per group. Experiment using polyclonal, non-Tg CD8 T cells was performed once.



Figure S5. B cells limit effector CD8 T cell expansion following mRNA lipid nano-particle vaccination, preserving memory pool. (A) Mice were given either a primary mRNA LNP vaccination only (left) or a primary vaccine followed by a booster 30 days later (right). Spleens were assessed for the number of tetramer+ CD127<sup>high</sup> cells and tetramer+ CD127<sup>low</sup> cells. (B) Gating strategy for analysis of Ag-specific CD8 T cells using publicly available data from Bar-Or and colleagues. (C) AIM+ cells as a percent of non-naïve CD8 T cells at 25-30 days post vaccination boost. (D) UMAP visualizations of all cells sequenced split by CITE-seq antibody or gene, for the identification and exclusion of CD4 T cells, MAIT cells, and  $\gamma\delta$ T cells. (E) UMAP visualization showing the clusters selected for downstream, conventional CD8 T cell analyses.



**Figure S6. FOXO1 haploinsufficient CD8 T cells closely resemble those deprived of B cell help.** Purified CD8 T cells from WT, *E8I-Cre Foxo1<sup>WT/FL</sup>*, and MD4 mice were plated in vitro with or without 5 ng/ml of human IL-7. The number of live cells counted compared to the number of cells plated is displayed for days 0, 2, and 7. Data shown are combined from 2 separate experiments, where each n-value equals the average of all the replicates from one experiment.