Figure S1

A.

WT   Y145F   Y112/128F

22.6   79.9   27.3   CD8

12.8   26.6   13.1   CD4

CD122

CD44

B.

WT   Y145F   Y112/128F

5.55	15.4	3.89	59	5.77	22.2	CD8
67.1	11.9	12.2	24.9	62.5	9.56	

0.201	1.88	0.825	6.41	0.342	3.39	CD4
89.8	8.11	67.6	25.1	83	13.3	

IFNγ

CD44

C.

CD8   CD4

CD44lo

CD44hi

FL5/FL4

WT
Y145F
Y112/128F

100 200 300   100 200 300

CD3, coreceptor
crosslinking
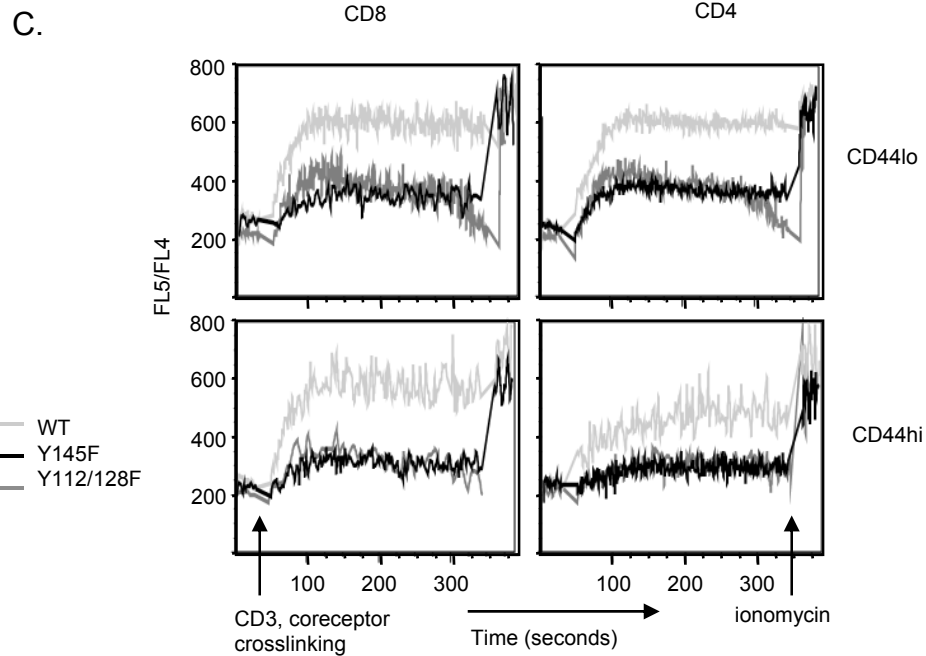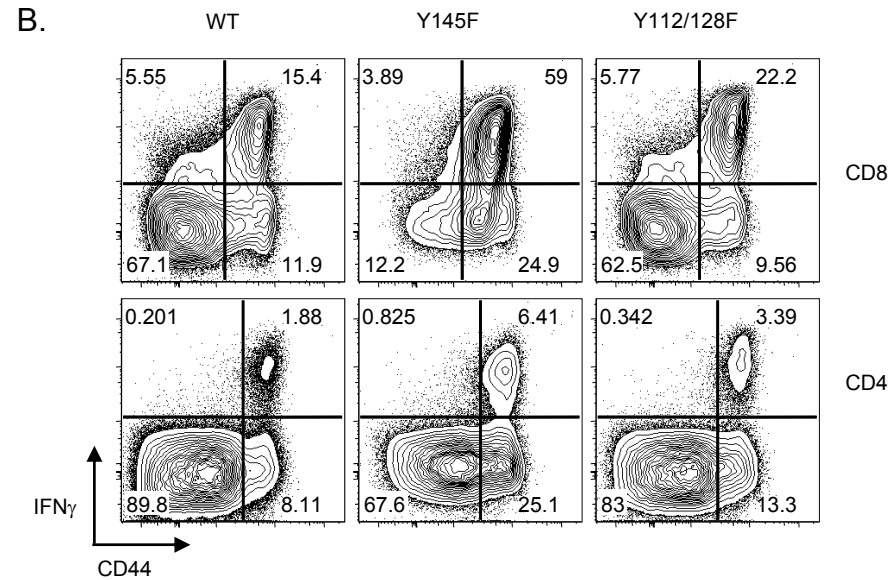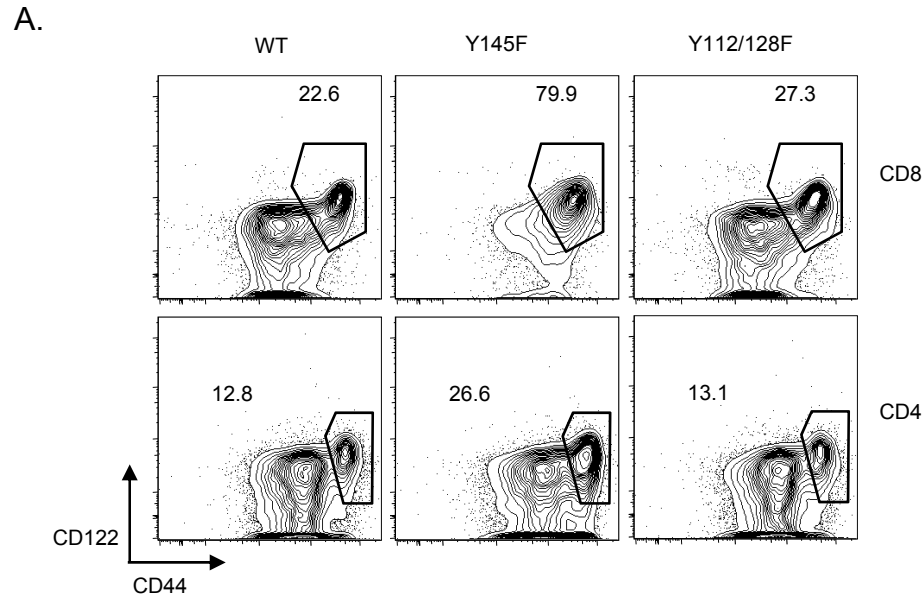
Time (seconds)

ionomycin

Figure S1. *Y145F KI mice show a preponderance of ILLs.* a) Contour plots show CD44 and CD122 surface expression on splenocytes from WT, Y145F and Y112/128F mice gated on either CD8$^+$ (top panel) or CD4$^+$ (bottom panel) lymphocytes (n=6). Numbers represent the percent of cells in each gate. b) Splenocytes from na•ve WT, Y145F and Y112/128F mice were incubated directly *ex vivo* with PMA and ionomycin for 5 hours. Contour plots show CD44 and IFNγ expression in cells gated on CD8$^+$ or CD4$^+$ lymphocytes (n=3). Numbers indicate percent of cells within each gate. C) Ca^{2+} flux was measured in WT, Y145F and Y112/128F CD4$^+$CD44^{hi}, CD4$^+$CD44^{lo}, CD8$^+$CD44^{hi} and CD8$^+$CD44^{lo} gated lymph node cells by flow cytometry following CD3, CD4 and CD8 cross-linking (n=3).
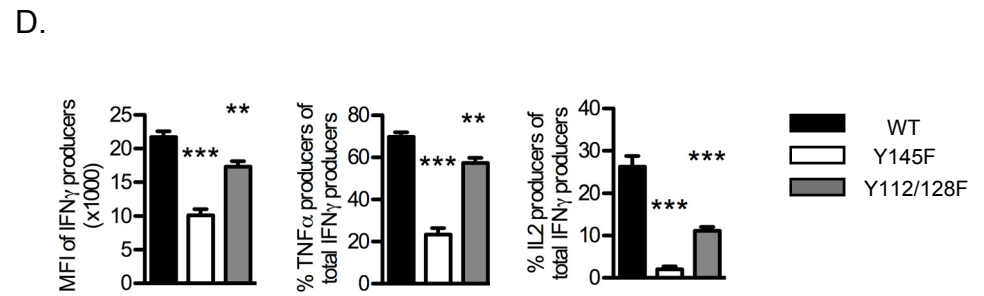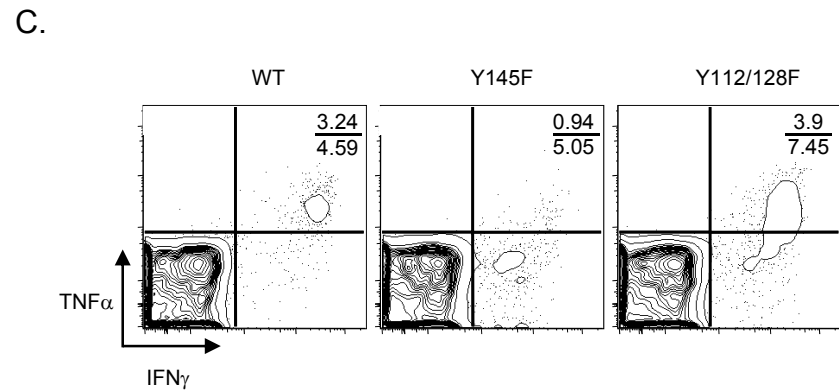
Figure S2

A.

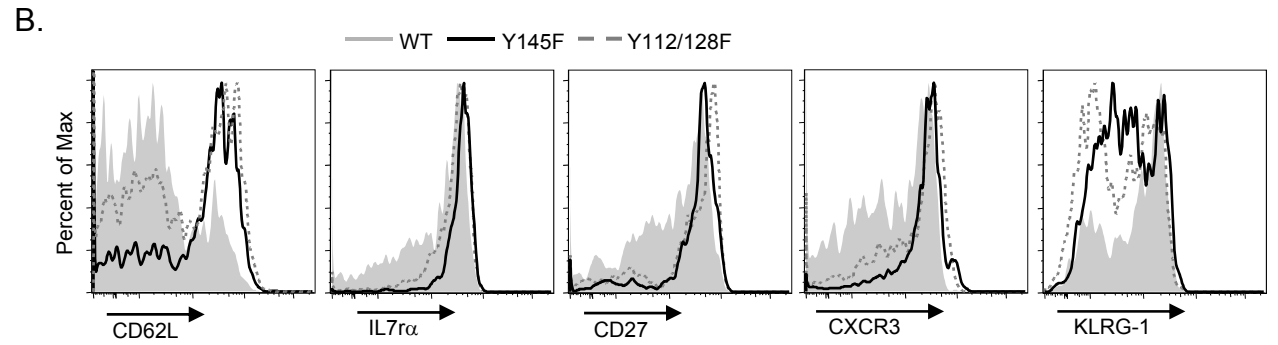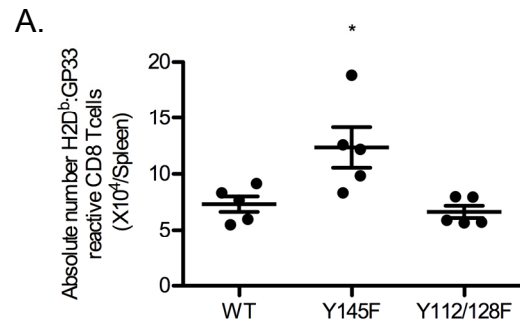

B.

C.

D.

Figure S2. *SLP-76 KI LCMV-specific memory cells persist but show defective effector responses in vitro.* a) Absolute numbers of H2D^b:GP33-reactive splenic CD8$^+$ T cells is shown ± SEM. Each point represents an individual mouse. b) Representative histograms show CD62L, IL7rα, CD27, CXCR3 and KLRG-1 surface expression of H2D^b:GP33$^+$, CD8$^+$, CD44$^+$ splenocytes from WT, Y145F and Y112/128F mice >day 70 p.i. (representative of 4 independent experiments each with 3-5 mice per group). c) Splenocytes from WT, Y145F and Y112/128F mice day >70 p.i. were incubated *ex vivo* with GP33 peptide. Representative contour plots show TNFα and IFNγ expression on CD8$^+$ gated lymphocytes (left panel). Numbers indicate the percentage of CD8$^+$, TNFα IFNγ double producers over the percentage of total CD8$^+$ IFNγ producers. d) The MFI of IFNγ in all CD8$^+$ cells producing IFNγ is shown (left graph). Percent of IFNγ splenocytes that co-produced TNFα (center graph) or IL-2 (right graph) is shown. All graphs show average ±SEM, significant p-values, when present, comparing KI to WT are indicated by asterisks: ***p<0.001, **p=0.001-0.01, *p=0.01-0.05.