Figure S1. Deletion of SLP-76 does not lead to lymphopenia
(A) cHET and cKO mice were bled longitudinally, and peripheral blood lymphocytes were isolated and stained for multicolor flow cytometry. $CD8^+$ T cells were gated and frequencies were normalized to number of cells per one million lymphocytes as described in the Materials and Methods. (B–D) Numbers of $CD8^+CD44^{lo}$, total $CD8^+$ and $CD8^+CD44^{hi}$ T cells lacking YFP expression were similarly calculated from longitudinal bleeds. (E) Total spleen numbers were calculated from cell counts at 6, 10 and 48 weeks post deletion, compiled from four independent infections. (F) $CD8^+$ cellularity in the spleen was calculated from multicolor flow cytometry data acquired on LSR II at 6, 10 and 48 weeks post deletion. Each point represents a single mouse. Mean and standard deviation are shown.

Figure S2. SLP-76 loss does not affect Bcl-2 expression
Peripheral blood lymphocytes were assessed for Bcl-2 protein expression by intracellular flow cytometry. Samples were gated on $CD8^+$, $CD8^+$H-2D^b:$GP33^+$ and $CD8^+$H-2Db$^+$$YFP^+$ cells. The mean fluorescence intensity (MFI) of each gated sample was measured using FlowJo software. Mean and standard deviation from 4 cHET and 4 cKO mice are shown.

Figure S3. Conversion to CD8 central memory in the spleen is altered 10 weeks after deletion of SLP-76
cHET and cKO mice were sacrificed at 10 weeks post deletion, and spleens were harvested. Splenocytes were immunophenotyped using multicolor flow cytometry. Representative histograms are gated on $CD8^+$H-2D^b:$GP33^+YFP^+$ splenocytes. Numbers in the upper right hand corner show the relative percentage of cells in the $CD62L^{hi}$ gate as indicated.

Figure S4. SLP-76 is required for $CD8^+$ memory recall responses
Expansion of LCMV-specific T cells following rechallenge. Forty-eight weeks post deletion, mice were re-challenged with LCMV clone 13 (Cl13) infection and expansion of AgSp memory cells was assessed in peripheral blood and spleen. (A) Numbers of total CD8 (left panel), total $CD8^+CD44^{hi}$ (center panel) and $CD8^+$H-2D^b:$GP33^+$ cells (right panel) from peripheral blood were calculated prior to rechallenge and at days 3 and 5 p.i. Each data point shows mean and standard deviation from two mice. (B) Splenocyte numbers of YFP^+ and YFP^-CD8^+H-2D^b:$GP33^+$ cells in the spleen were compiled from unchallenged mice at 6, 10 and 48 weeks (Cl13−) and from mice 5 days after re-challenged with Clone 13 (Cl13+). Numbers of YFP^+H-2D^b:$GP33^+$ are depicted as the white portions of the bars, and YFP^-H-2D^b:$GP33^+$ populations are the gray portions of each bar. The overall height of each column represents total H-2D^b:GP33 cell numbers. Cl13-numbers are averaged from 6 cHET and 9 cKO mice from four independent infections. Cl13+ averages are representative of 2 cHET and 2 cKO mice.

Figure S5. Deletion of SLP-76 results in abnormal memory differentiation
cHET and cKO mice were bled longitudinally following deletion of SLP-76 during the contraction phase. Longitudinal quantitation of $CD8^+$H-2D^b:$GP33^+YFP^+$ (A) $CXCR3^{hi}$ and Bb) $CD127^{hi}CD62L^{hi}$ frequency from cHET (black) versus cKO (grey) peripheral blood lymphocytes. Mean and standard deviations are a composite from two independent experiments with a total of 10 mice. Asterisks indicate time points with statistically significant differences ($p<0.05$).

Figure S1

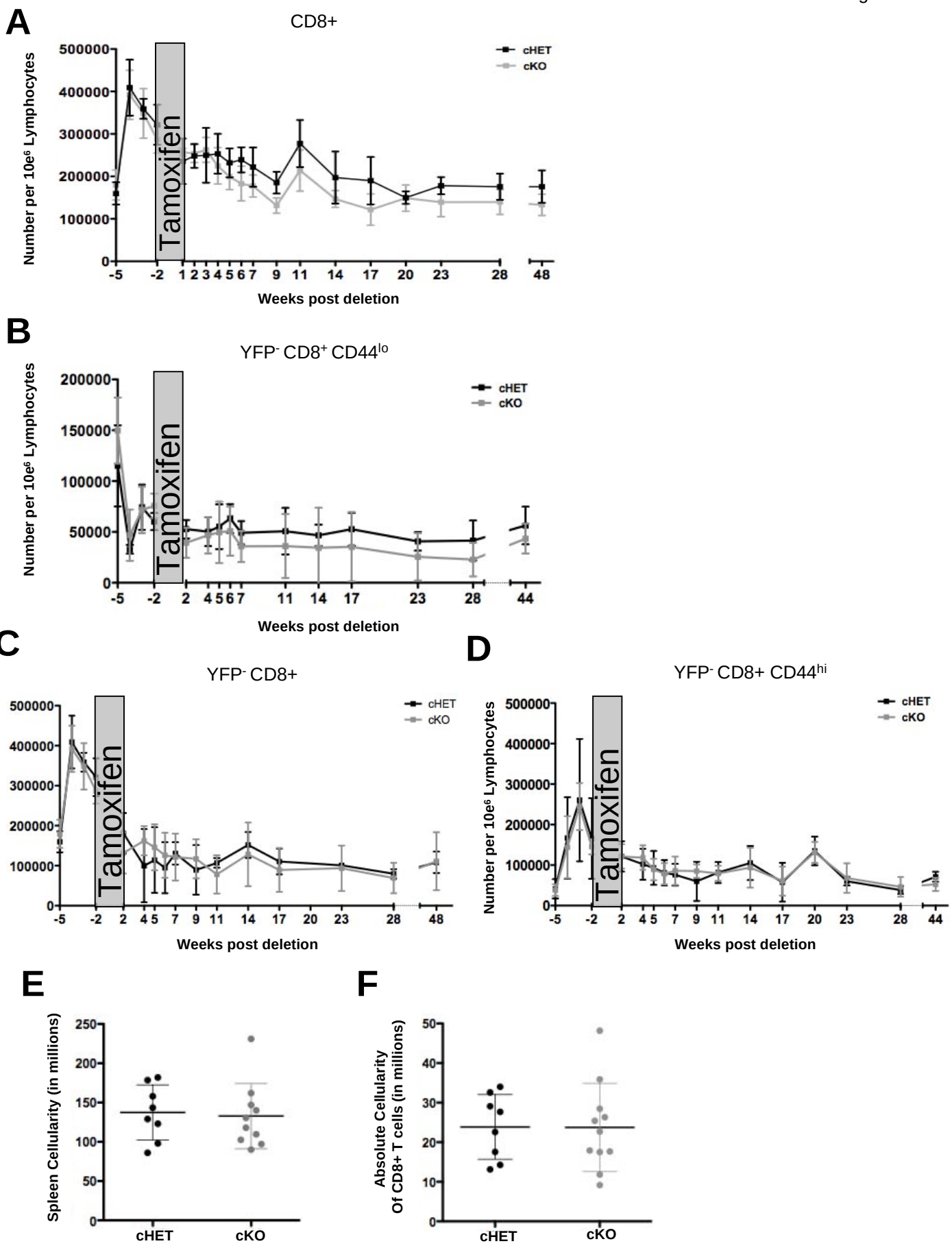

A
CD8+
Number per 10e6 Lymphocytes
Tamoxifen
cHET
cKO
Weeks post deletion
B
YFP- CD8+ CD44lo
Number per 10e6 Lymphocytes
Tamoxifen
cHET
cKO
Weeks post deletion
C
YFP- CD8+
Number per 10e6 Lymphocytes
Tamoxifen
cHET
cKO
Weeks post deletion
D
YFP- CD8+ CD44hi
Number per 10e6 Lymphocytes
Tamoxifen
cHET
cKO
Weeks post deletion
E
Spleen Cellularity (in millions)
cHET
cKO
F
Absolute Cellularity
Of CD8+ T cells (in millions)
cHET
cKO

Figure S2

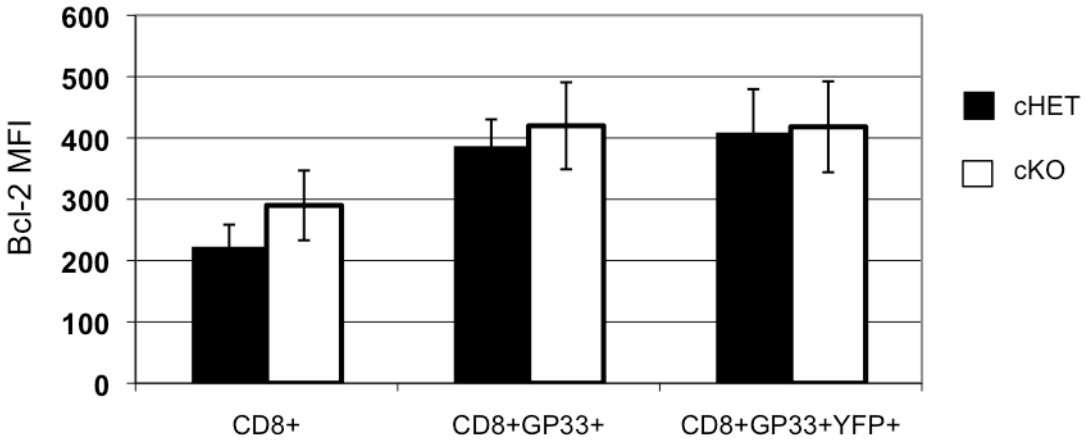

600
500
400
300
200
100
0
Bcl-2 MFI
CD8+
CD8+GP33+
CD8+GP33+YFP+
cHET
cKO

Figure S3

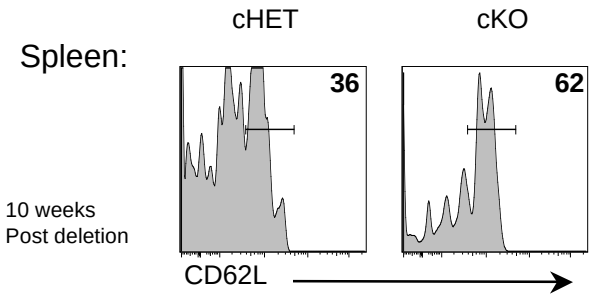


Figure S4

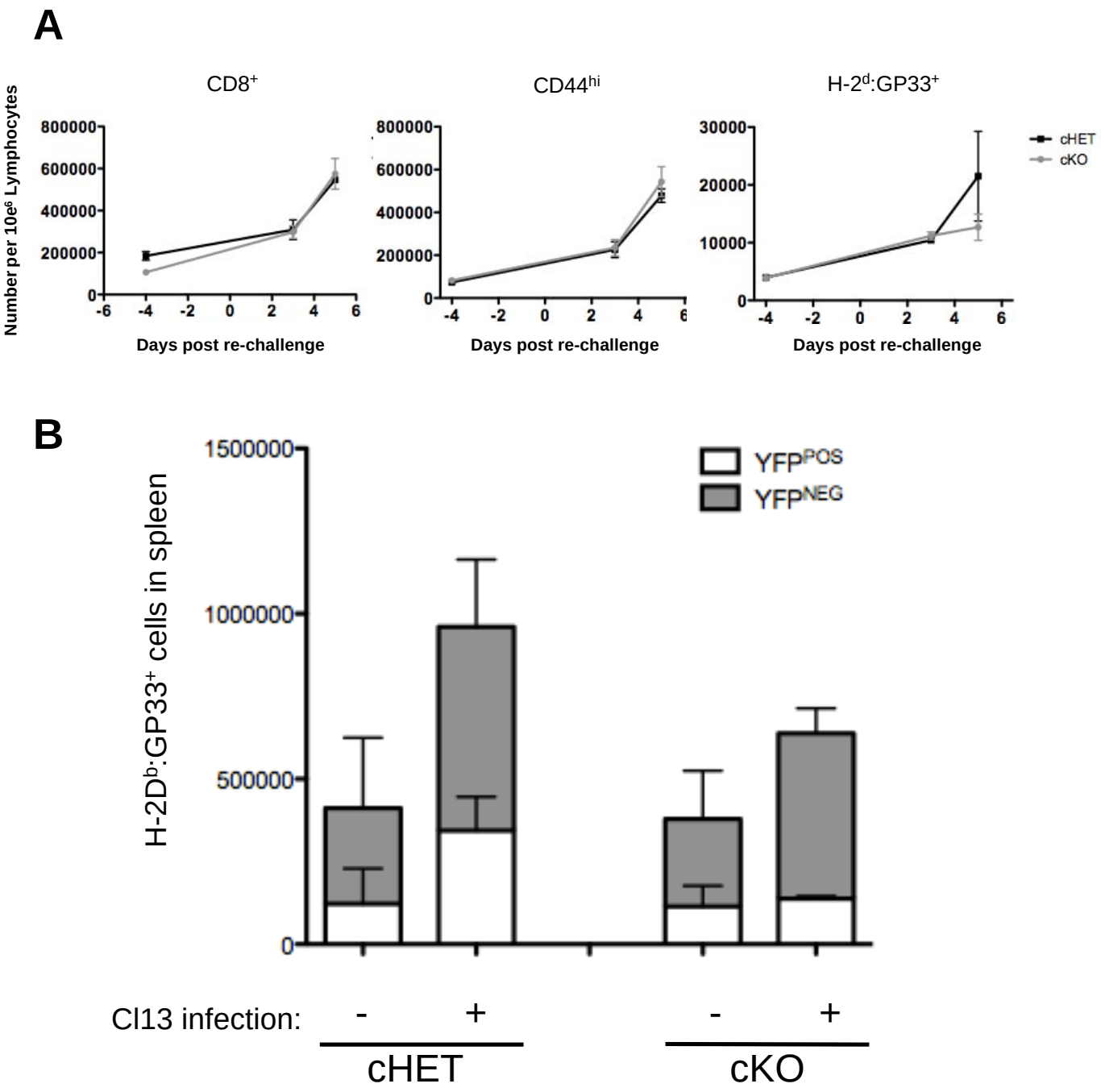
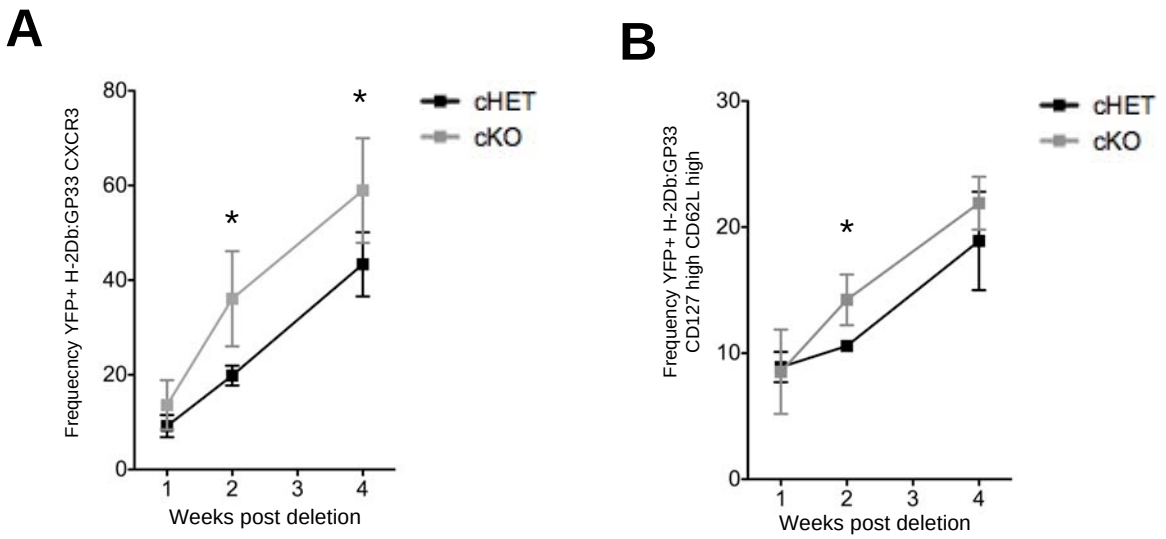

A
CD8+
CD44hi
H-2d:GP33+
Number per 10e6 Lymphocytes
800000
600000
400000
200000
0
30000
20000
10000
-6
-4
-2
0
2
4
6
Days post re-challenge
cHET
cKO
B
H-2Db:GP33+ cells in spleen
1500000
1000000
500000
0
YFPPOS
YFPNEG
Cl13 infection:
-
+
-
+
cHET
cKO

Figure S5


A
B
cHET
cKO
Frequecncy YFP+ H-2Db:GP33 CXCR3
Weeks post deletion
Frequency YFP+ H-2Db:GP33 CD127 high CD62L high
Weeks post deletion
*
*
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