Supplemental Data

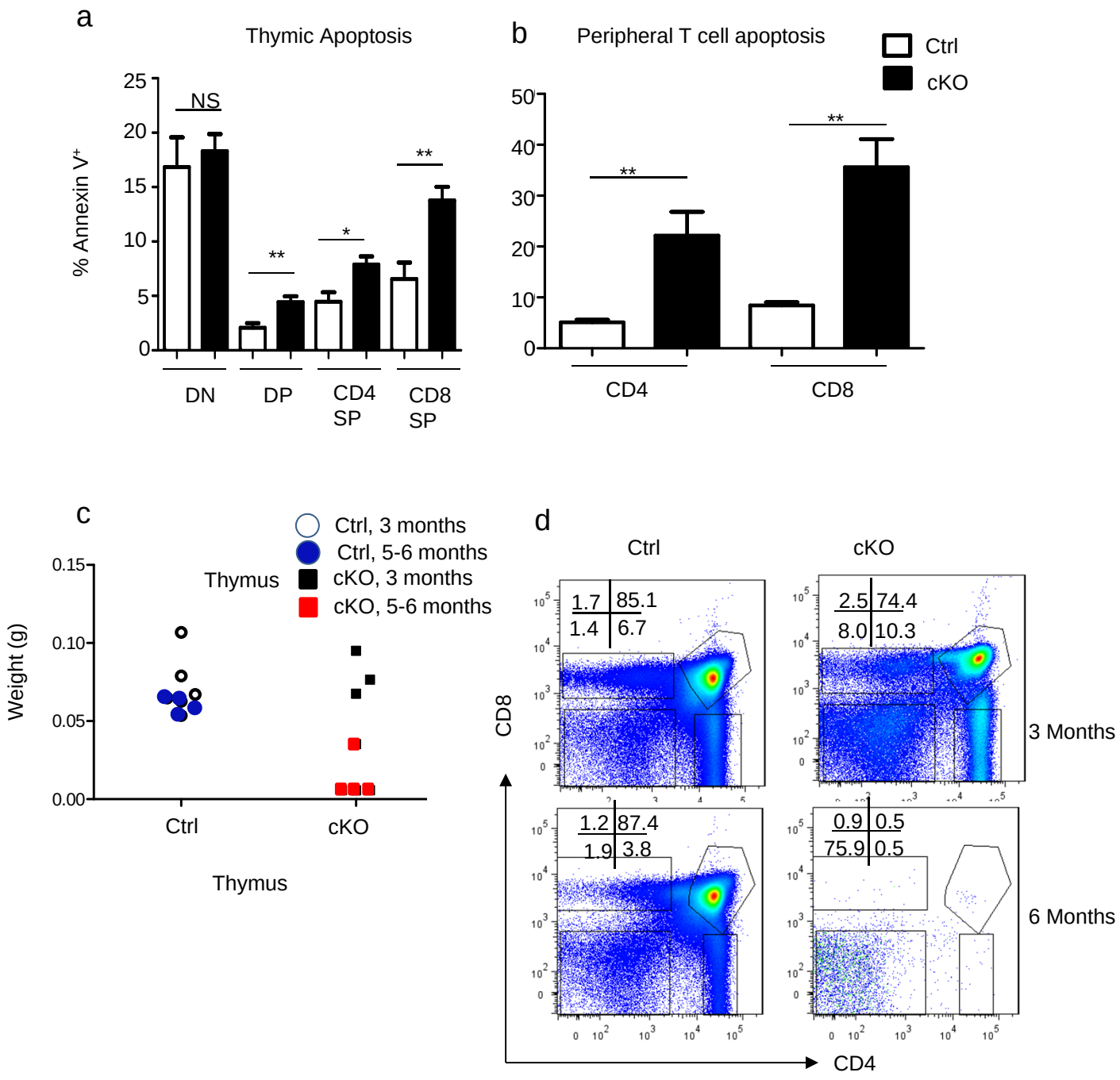


Fig. S1. (a and b) Apc deletion increases apoptosis in mature thymocytes, but apoptosis is less severe than that in peripheral T cells. Ctrl (open bars) and cKO (filled bars) thymocytes (a) and splenocytes (b) were stained with anti-CD4 and anti-CD8 mAbs in conjunction of Annexin V. The data shown was summarized from two independent experiments involving 6 mice at 6-8 weeks of age in each group. (c and d) Progressive loss of thymic cellularity in old cKO mice. c. Thymic weight of 3 or 6 month old control (open circles for three months old and filled blue circles for elder mice) or cKO (closed squares). In red, are cKO mice that are older than 3 months, while the black closed squares are mice that are 3 months old. n=7. d. Thymi of 3-6 month old control or cKO were stained with CD4 and CD8. Shown are representative profiles of two cKO mice sacrificed at 3 and 6 months, respectively. Both cKO mice have developed prolapses.

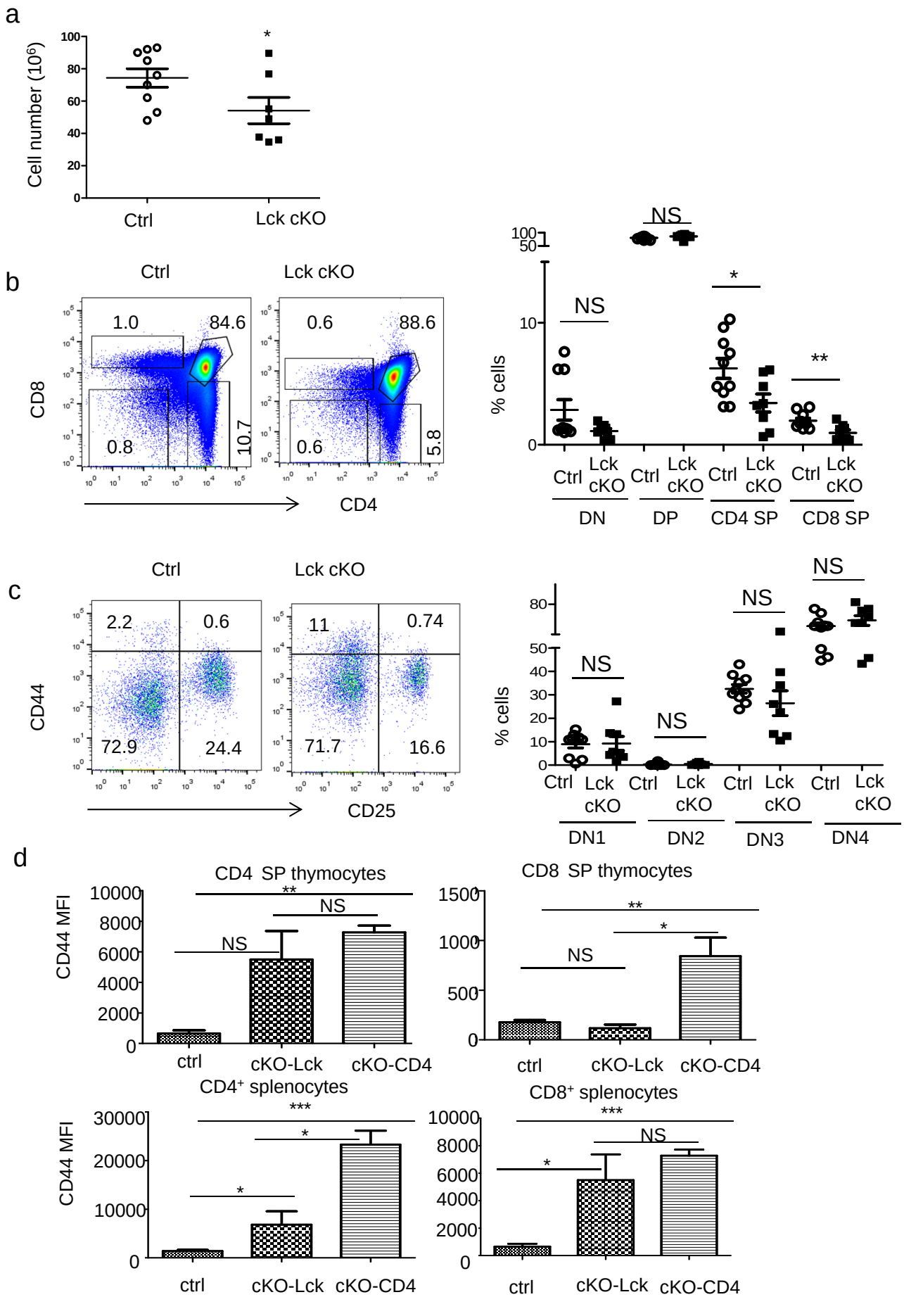

a
Cell number (10^6)
Ctrl
Lck cKO
b
Ctrl
Lck cKO
CD8
CD4
1.0
84.6
0.8
10.7
0.6
88.6
0.6
5.8
% cells
NS
DN
DP
CD4 SP
CD8 SP
c
CD44
CD25
2.2
0.6
72.9
24.4
11
0.74
71.7
16.6
DN1
DN2
DN3
DN4
d
CD4 SP thymocytes
CD8 SP thymocytes
CD44 MFI
ctrl
cKO-Lck
cKO-CD4
CD4⁺ splenocytes
CD8⁺ splenocytes

Fig. S2. Subtle thymocyte developmental defects caused by Lck-Cre-driven deletion of the Apc exon 14 in 6-8 week old mice. a. Thymic cellularity of *Apc*$^{fl/fl}$*;Lck-Cre*$^{-}$ (Ctrl) and the *Apc*$^{fl/fl}$*;Lck-Cre*$^{+}$ (Lck-cKO) mice. n=8. b. Thymocyte development based on distribution of CD4 and CD8 markers. Representative profiles are shown on the left, while summary data are presented on the right. n=9. c. Thymocyte development based on distribution of CD44 and CD25 markers among the CD4^{-}CD8^{-} thymocytes. Representative profiles are shown on the left, while summarized data is presented on the right. n=9. Note that the overall levels of CD44 are elevated among the DN4 subsets in the Lck cKO thymocytes. This is to be expected, as CD44 is a hallmark of Wnt signaling target gene. d. Summarized MFI expression, from one experiment, of CD44 in SP thymocytes and splenic T cells in Lck-cKO and CD-cKO. n=3. This experiment was repeated twice.

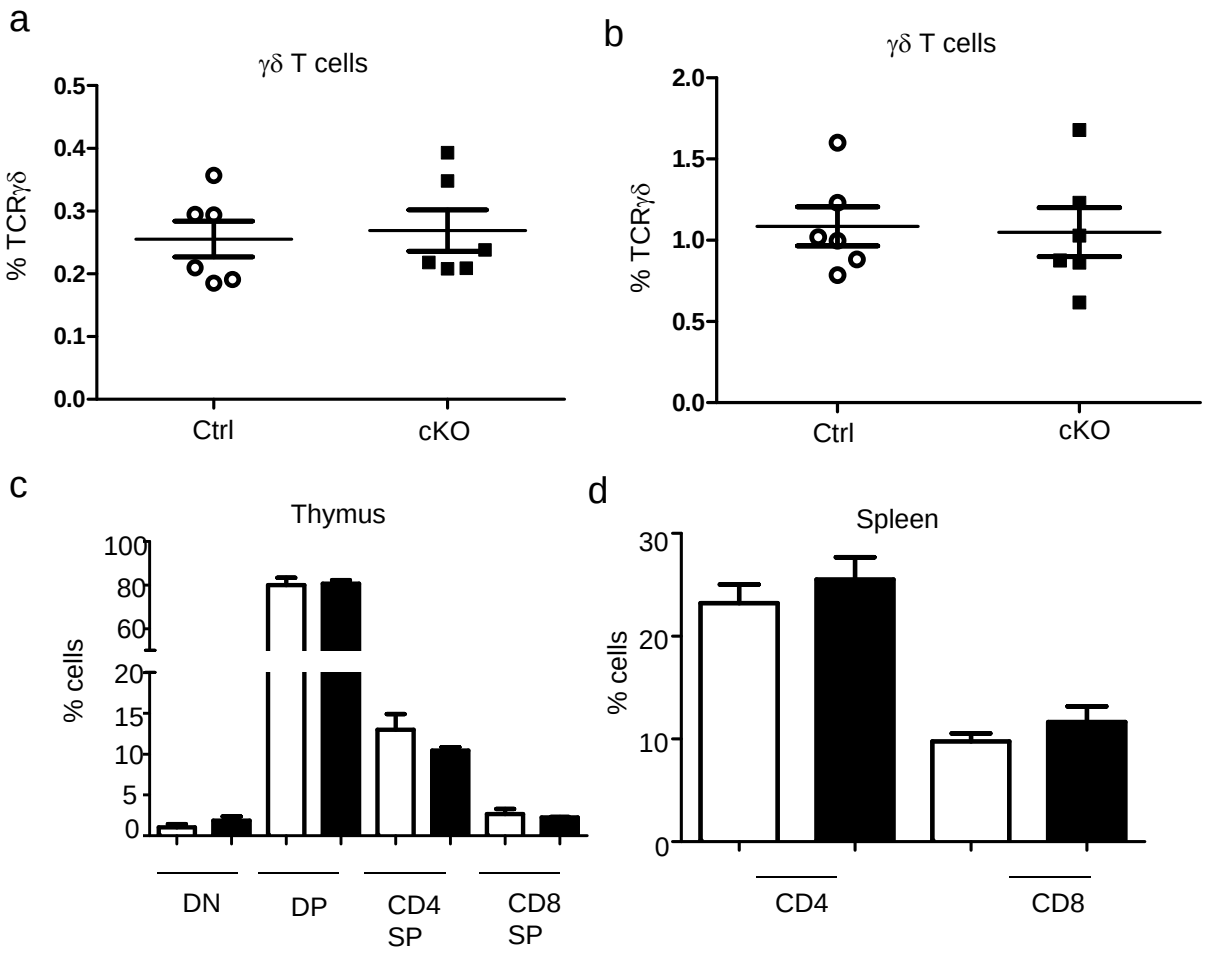
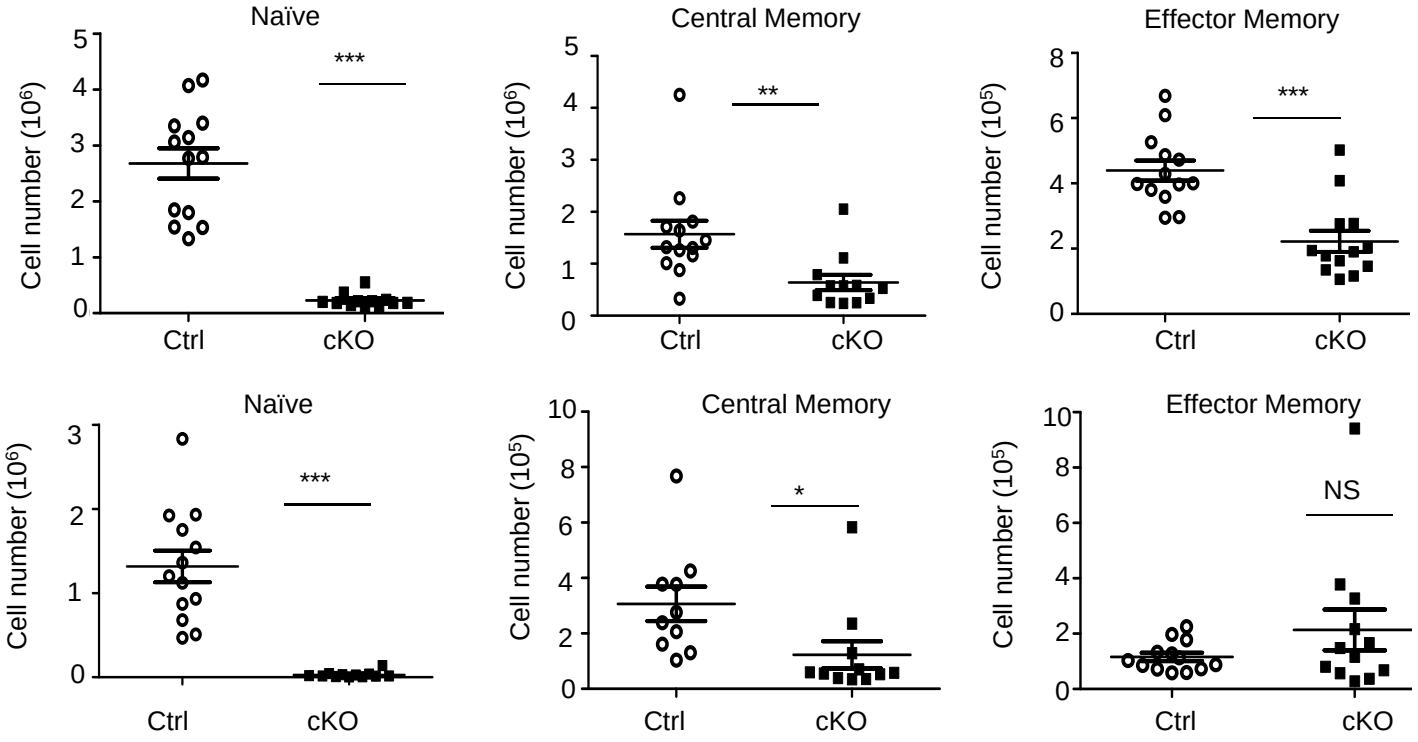
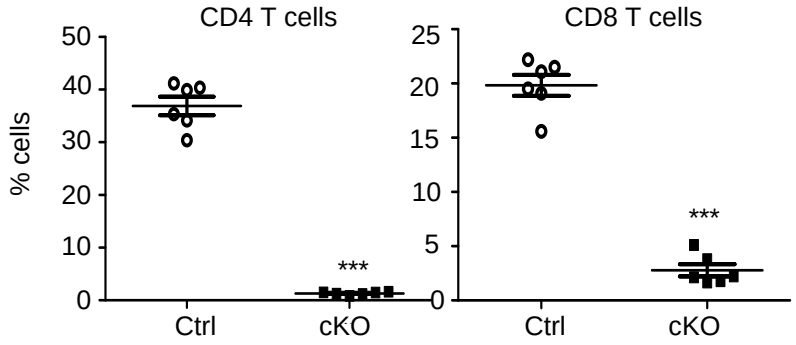

a
γδ T cells
% TCRγδ
0.5
0.4
0.3
0.2
0.1
0.0
Ctrl
cKO
b
γδ T cells
% TCRγδ
2.0
1.5
1.0
0.5
0.0
Ctrl
cKO
c
Thymus
% cells
100
80
60
20
15
10
5
0
DN
DP
CD4 SP
CD8 SP
d
Spleen
% cells
30
20
10
0
CD4
CD8

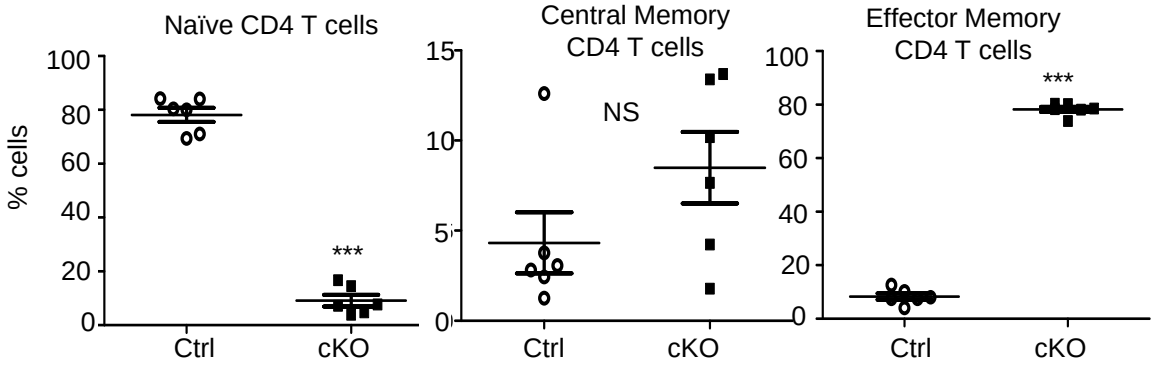
Fig. S3. (a and b) *Apc* deletion by *CD4-Cre* does not cause alteration in γδ T cells lineages. Ctrl (open circles) and cKO (filled squares) thymocytes (a) and splenocytes (b) were stained with anti-TCRβ and anti-TCRγδ complex mAbs. The data is shown as the mean and SEM of % γδ T cells, summarized from two independent experiments involving 6 mice at 6-8 weeks of ages in each group. (c and d) In mixed bone marrow chimeras, *Apc* deletion by *CD4-Cre* in donor-type T cells does not affect the development and survival of recipient-type T cells. c. % WT $CD45.1^+$ recipient-type cells developed in the presence of either Ctrl (open bars) or cKO (filled bars) donor-type ($CD45.2^+$) cells. d. As in c, except spleen cells were analyzed. The data is shown as the mean and SEM, summarized from 3 independent experiments, n=10.


Naïve
Central Memory
Effector Memory
Cell number (10^6)
Cell number (10^5)
Ctrl
cKO
***
**
*
NS

c


CD4 T cells
CD8 T cells
% cells
Ctrl
cKO
***

d


Naïve CD4 T cells
Central Memory CD4 T cells
Effector Memory CD4 T cells
% cells
Ctrl
cKO
***
NS

e

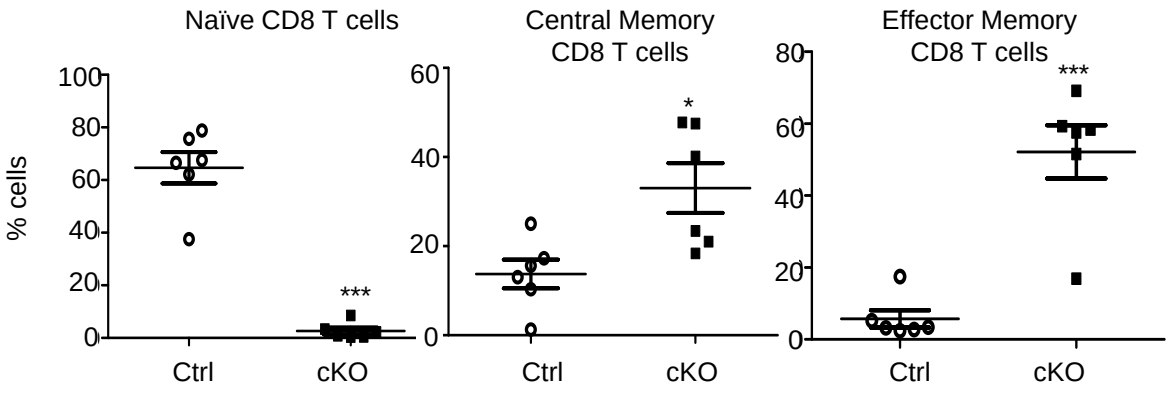

Naïve CD8 T cells
Central Memory CD8 T cells
Effector Memory CD8 T cells
% cells
Ctrl
cKO
***
*

Fig. S4. (a and b) *Apc* deletion by *CD4-Cre* reduces T cell cellularity in the spleen. a. Numbers of naïve, central memory, and effector memory CD4 T cells from the spleen in 6-8 week old mice, n=12. b. Numbers of naïve, central memory, and effector memory CD8 T cells from the spleen in 6-8 week old mice. n=12. (c to e) *Apc* deletion by *CD4-Cre* reduces T cell % in the lymph nodes. c. Reduction of % of CD4 and CD8 T cells. d. Reduced naïve CD4 T cells but increased central ($CD44^{hi}CD62^{hi}$) and effector ($CD44^{hi}CD62^{lo}$) memory CD4 T cells. e. Reduced naïve CD8 T cells but increased central ($CD44^{hi}CD62^{hi}$) and effector ($CD44^{hi}CD62^{lo}$) memory CD8 T cells. The data shown is summarized from two independent experiments, each involving 6 mice of 6-8 week old.