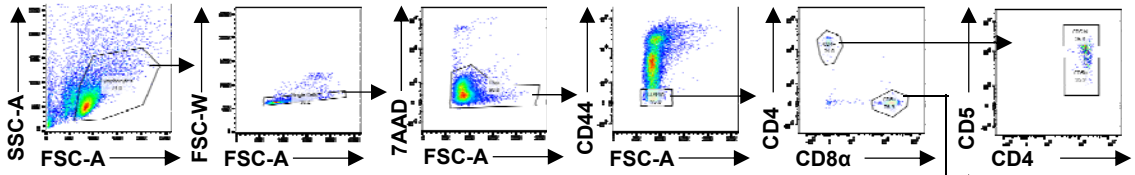
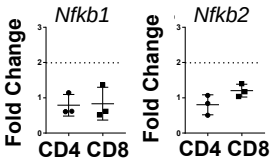
SI Appendix Figure S1

A



B

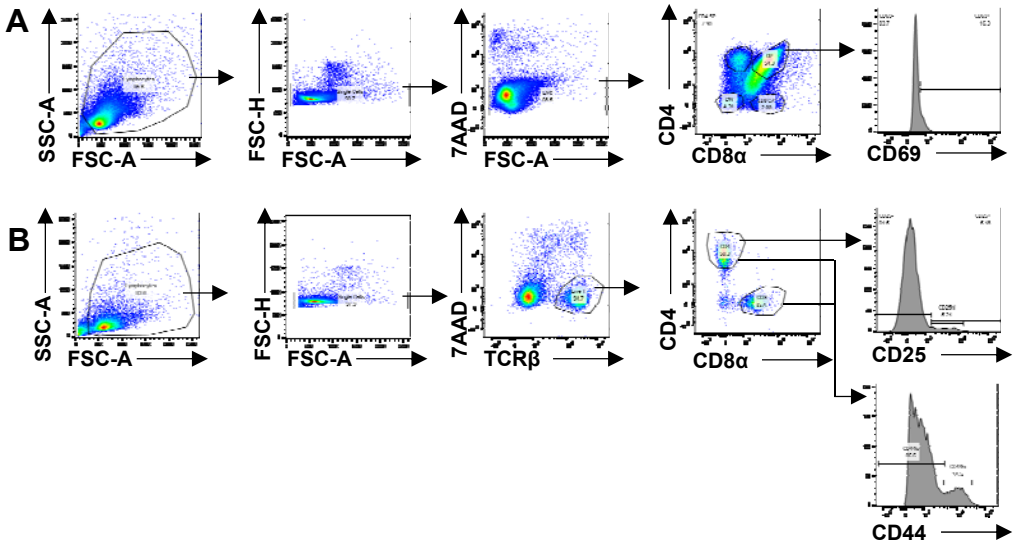


C

CD4:CD5lo vs hi	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Cd5	46521.99	-2.41	0.07	-32.19	2.45E-227	1.84E-223
Nfkb1	23416.20	-0.15	0.06	-2.37	0.017884996	0.218935017
Nfkbia	26346.78	0.06	0.21	0.27	0.783588692	1
Rela	12974.57	0.05	0.15	0.31	0.760287785	1
Nfkbib	3753.16	0.05	0.06	0.87	0.383883222	0.955049738
Ikbkb	27088.15	0.07	0.08	0.88	0.380134906	0.951405574
Chuk	6020.10	0.00	0.13	0.04	0.970329382	1
Nfkb2	8815.96	0.16	0.17	0.98	0.328588202	0.930298216
Relb	5202.47	-0.08	0.13	0.60	0.546680546	0.999464978

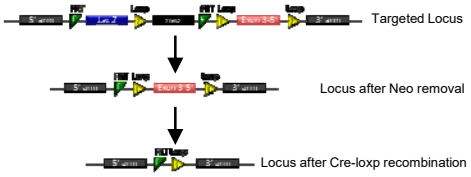
CD8: CD5lo vs hi	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Cd5	46521.99	-1.30	0.07	-17.35	2.00E-67	1.47E-63
Nfkb1	23416.20	-0.06	0.06	0.95	0.340057601	0.652899859
Nfkbib	3753.16	0.02	0.06	0.31	0.756061527	0.911582885
Nfkbia	26346.78	0.27	0.21	1.30	0.194513475	0.492214303
Rela	12974.57	0.17	0.15	1.09	0.276705834	0.592229245
Ikbkb	27088.15	0.18	0.08	2.19	0.028471523	0.151758882
Chuk	6020.10	0.04	0.13	0.29	0.774605829	0.921294884
Nfkb2	8815.96	0.08	0.16	0.47	0.635654019	0.851817538
Relb	5202.47	-0.08	0.13	0.58	0.559642441	0.808232267

SI Appendix Figure S2

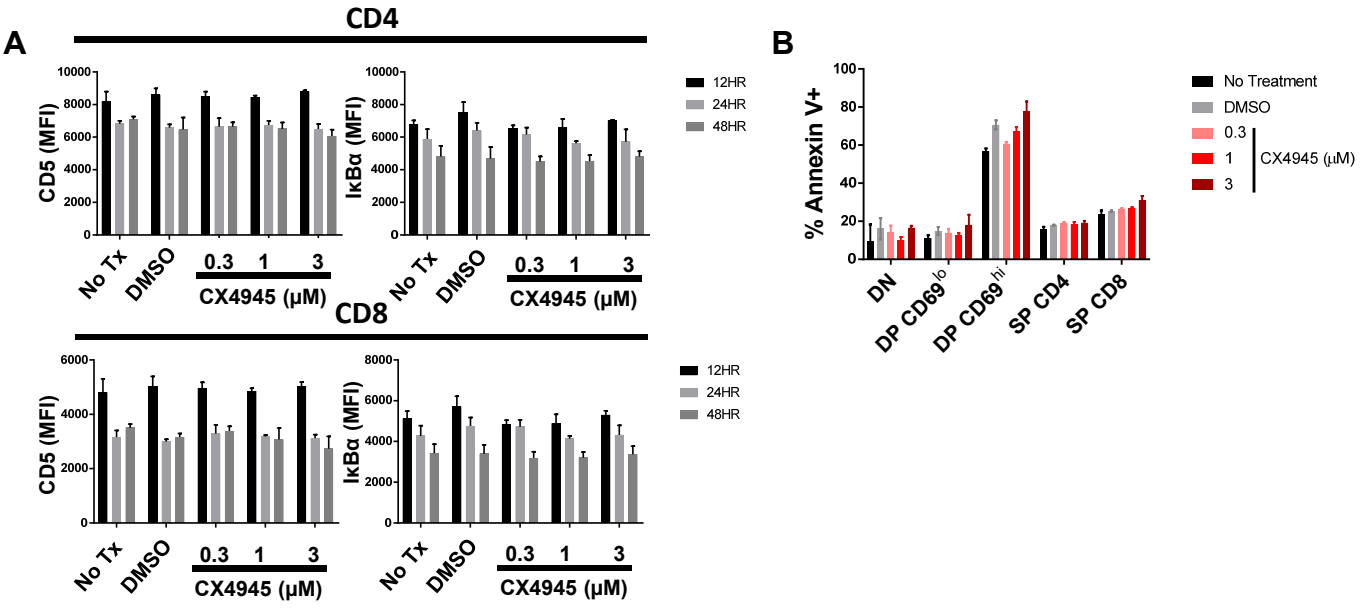


SI Appendix Figure S3

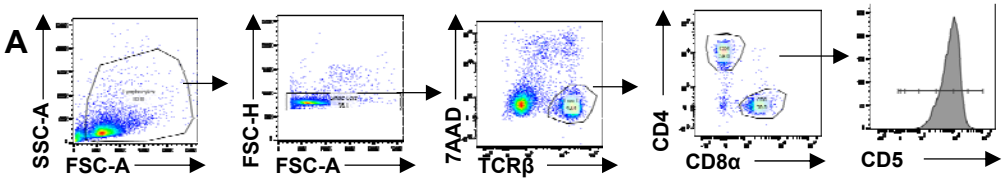
A



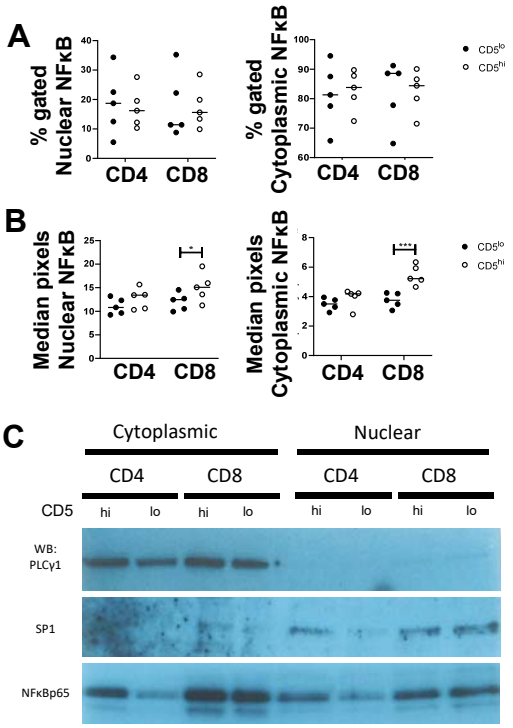
SI Appendix Figure S4



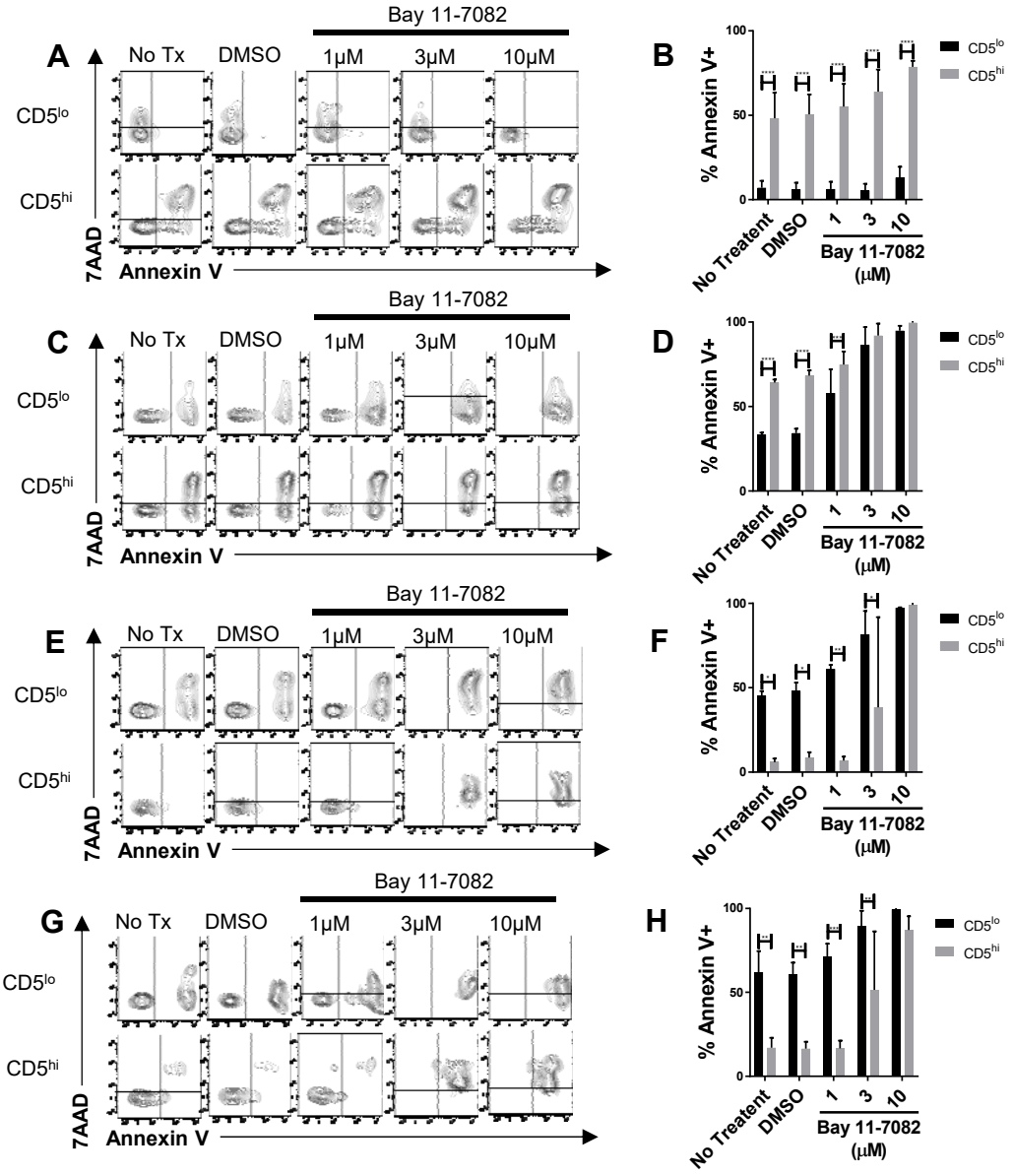
SI Appendix Figure S5



SI Appendix Figure S6



SI Appendix Figure S7



1. SI Appendix Figure S1. Sort gating strategy for western blot or qPCR analysis.
    A. Wild-type B6 lymph nodes and splenocytes were homogenized into a single cell suspension. Cells were blocked and stained with anti-CD4, anti-CD8, anti-CD44, anti-CD5, and 7AAD. Cells were sorted using the strategy depicted and then analyzed via western blot or used to harvest RNA for qPCR analysis.
    B. RT-qPCR analysis of sorted $CD5^{hi}$ and $CD5^{lo}$ CD4 and CD8 T cells as collected by gates shown in A.
    C. Tables of RNA-Seq analysis of of NFκB pathway genes in sorted $CD5^{hi}$ and $CD5^{lo}$ subsets of CD4 and CD8 T cells.

2. SI Appendix Figure S2. Gating strategy for flow cytometric analysis of thymocytes and peripheral T cells.
    A. Gating strategy for thymocyte analysis.
    B. Gating strategy for peripheral T cell analysis.

3. SI Appendix Figure S3. Schematic of CD5-floxed mice with the CAG-CreERT2 transgene.
    A. Diagram showing the excision of CD5 gene before and after tamoxifen administration.

4. SI Appendix Figure S4. Casein kinase 2 inhibition does not impact expression of CD5 or IκBα and does not impact thymocyte survival.
    A. Pooled lymph node and spleens were homogenized into a single cell suspension and cultured ex vivo with varying concentrations of CX4945 and harvested at the indicated time points. Cells were analyzed for expression of CD5 and IκBα within CD4 and CD8 T cell populations (n = 3 from 1 independent experiment).

B. Thymocytes were cultured ex vivo for 15 hours with varying concentrations of CX4945. The frequency of Annexin V^+ cells within each population is shown (n = 3 from 1 independent experiment).

5. SI Appendix Figure S5. Binning strategy for WT and CD5-Tg analysis.
   A. Binning strategy for wild-type and CD5 transgenic (CD5-Tg) analysis as displayed in Figure 4G. Bins for CD5 expression were based on the following gMFI values : 0 – 100 (Bin 1), 100 – 300 (Bin 2), 300 – 1000 (Bin 3), 1000 – 3000 (Bin 4), 3000 – 10000 (Bin 5), 10000 – 30000 (Bin6), 30000 – 100000 (Bin 7).

6. SI Appendix Figure S6. Amnis ImageStream analysis and western blot analysis of NFκB localization in resting peripheral T cells
   A. Western blot analysis of sorted CD4 and CD8 B6 lymphocytes gated on 20% $CD5^{hi}$ and $CD5^{lo}$. Cells were fractionated into cytoplasmic and nuclear fractions and probed for PLCγ1 (cytoplasmic normalization), SP1 (nuclear normalization), and NFκB p65. (n = 1 from 1 independent experiment)
   B. Percentage, median pixel values, and gMFI values of nuclear and cytoplasmic NFκB p65 in resting peripheral T cells. (n = 5 from 1 independent experiment)

Significance is indicated as follows: $p<0.05$ = * ; $p<0.01$ = II ; $p<0.001$ = *** ; $p<0.0001$ = ****. Anything not marked is not statistically significant.

7. SI Appendix Figure S7. Inhibition of NFκB ablates the survival advantage of $CD5^{hi}$ cells
   A. Thymocytes from B6 mice were cultured ex vivo for 15 hours with or without varying Bay11-7082 doses. Cells were stained and gated as in Supplementary Figure 2, together with Annexin V and 7AAD to assess cell

death. Representative flow plots are shown for the double-negative population.

B. The frequencies of Annexin V+ cells within the top and bottom 20% of CD5 expressers of the double-negative population are shown (n = 3 from 1 independent experiment).
C. Thymocytes from B6 mice were cultured ex vivo for 15 hours with or without varying Bay11-7082 doses. Cells were stained and gated as in Supplementary Figure 2, together with Annexin V and 7AAD to assess cell death. Representative flow plots are shown for the double-positive CD69lo population.
D. The frequencies of Annexin V+ cells within the top and bottom 20% of CD5 expressers of the double-positive CD69lo population are shown (n = 3 from 1 independent experiment).
E. Thymocytes from B6 mice were cultured ex vivo for 15 hours with or without varying Bay11-7082 doses. Cells were stained and gated as in Supplementary Figure 2, together with Annexin V and 7AAD to assess cell death. Representative flow plots are shown for the single-positive CD4 population.
F. The frequencies of Annexin V+ cells within the top and bottom 20% of CD5 expressers of the single-positive CD4 population are shown (n = 3 from 1 independent experiment).
G. Thymocytes from B6 mice were cultured ex vivo for 15 hours with or without varying Bay11-7082 doses. Cells were stained and gated as in Supplementary Figure 2, together with Annexin V and 7AAD to assess cell death. Representative flow plots are shown for the single-positive CD8 population.
H. The frequencies of Annexin V+ cells within the top and bottom 20% of CD5 expressers of the single-positive CD8 population are shown (n = 3 from 1 independent experiment).

Table 1. Antibodies used for flow cytometry staining.

Antibody	Fluorochrome	Manufacturer	Catalog #	Clone
B220	APC-Cy7	Biolegend	103224	RA3.6B2
B220	PE	BD Pharmingen	553090	RA3-6B2
CD11b	APC	eBioscience	17-0112-83	M1/70
CD11b	PE	eBioscience	12-0112-81	M1/70
CD11c	APC	Biolegend	117309	N418
CD11c	PE	eBioscience	12-0114-83	N418
CD127	APC	eBioscience	17-1271-82	A7R34
CD19	APC	eBioscience	17-0193-82	eBio1D3
CD25	BV650	Biolegend	102037	PC61
CD25	APC-Cy7	BD Pharmingen	557658	PC61
CD4	BV605	Biolegend	100451	GK1.5
CD4	APC-Cy7	BD Pharmingen	552051	GK1.5
CD4	PE	BD Pharmingen	553730	GK1.5
CD4	PE	eBioscience	12-0042-83	RM4-5
CD44	FITC	Biolegend	103006	IM7
CD45.1	FITC	Biolegend	110706	A20
CD45.1	APC-Cy7	Biolegend	110716	A20
CD45.1	APC	Biolegend	110714	A20
CD45.2	PE-Cy5.5	Invitrogen	35-0454-82	104
CD5	eFluro450	eBioscience	48-0051-82	53-7.3
CD5	PE-Cy7	eBioscience	25-0051-81	53-7.3
CD62L	PE-Cy7	Biolegend	104418	MEL-14
CD69	PerCP-Cy5.5	BD Pharmingen	551113	H1.2F3
CD69	FITC	BD Pharmingen	553236	H1.2F3
CD69	eFluor450	eBioscience	48-0691-82	H1.2F3
CD8α	APC-Cy7	BD Pharmingen	557654	53-6.7
CD8α	FITC	Biolegend	100706	53-6.7
CD8α	PerCP-Cy5.5	BD Pharmingen	551162	53-6.7
CD8α	FITC	eBioscience	11-0081-85	56-6.7
CD8α	APC-eFluro780	eBioscience	47-0081-82	53-6.7
CD8α	BV650	BD OptiBuild	740552	H35-17.2
CD8α	BB515	BD Pharmingen	564422	53-6.7

CD8α	APC	eBioscience	17-0081-81	53-6.7
CD8α	PE-Cy7	eBioscience	25-0081-81	53-6.7
IκBα	PE	Invitrogen	12-9036-41	MFRDTRK
IκBα	APC	Invitrogen	17-9036-42	MFRDTRK
LAT	APC	eBioscience	17-9967-42	LAT.10-17
NFκB p65	AF647	CST	8801S	D14E12
NK1.1	APC	eBioscience	17-5941-82	PK136
NK1.1	PE	Biolegend	108708	PK136
PLCγ	PE	BD Phosflow	558575	10/PLCg
TCRβ	PE-Cy7	Biolegend	109222	H57-597
TCRβ	FITC	BD Pharmingen	553171	H57-597
Vβ3	PE	BD Pharmingen	553209	KJ25
Zap70	FITC	eBioscience	11-6695-82	1E7.2