Supplemental figure 1

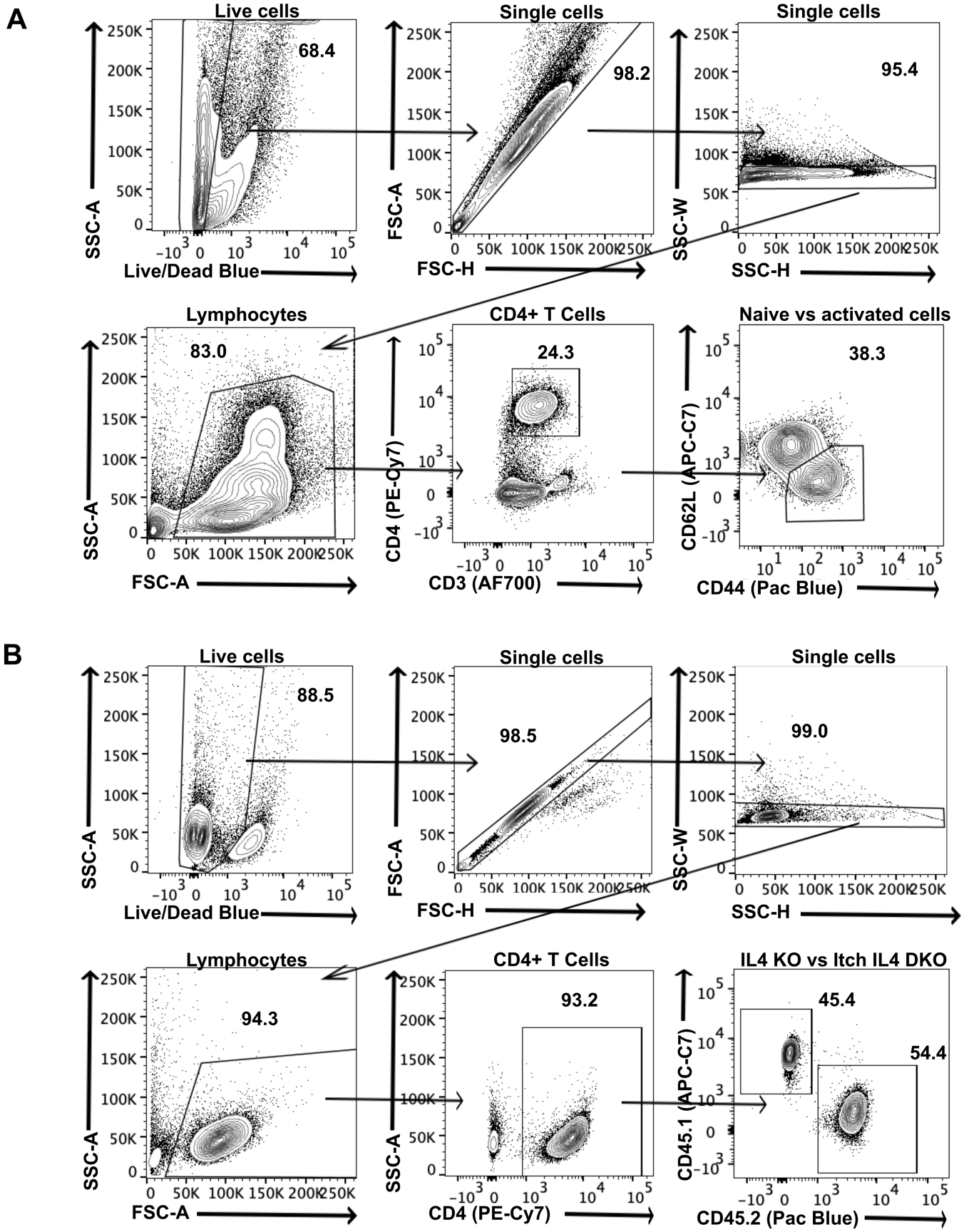

Figure S1: Gating strategy for flow experiments. **A)** All ex vivo experiments were gated on the basis of Live/Dead negative, singlets, lymphocytes (based on low forward and side scatter), and CD3+CD4+ cells. Activated CD4 T cells were defined as CD62L-CD44+ cells, and naïve cells were defined as CD62L+CD44-. The example shown here was a lung sample from one of the mixed chimeras described in Fig 1. The same gating strategy was used in Fig. 2E, replacing APC-Cy7 with APC on CD62L. **B)** *In vitro* cultures were gated on the basis of Live/Dead negative, singlets, lymphocytes (based on forward scatter and side scatter) CD4+ cells, and CD45.1 or CD45.2. The example shown here is a BrdU uptake experiment shown in Fig. 4A. Experiments in Fig. 2 used the same gating strategy, substituting BV786 and PerCP-Cy5.5 for CD45.1 and CD45.2, respectively. Experiments in Fig. 2B were stained with the same panel, but were not gated through Live/Dead blue and SSC-A by FSC-A, so that dead cells could be visualized after the CD45.1 versus CD45.2 gate.

Supplemental figure 2

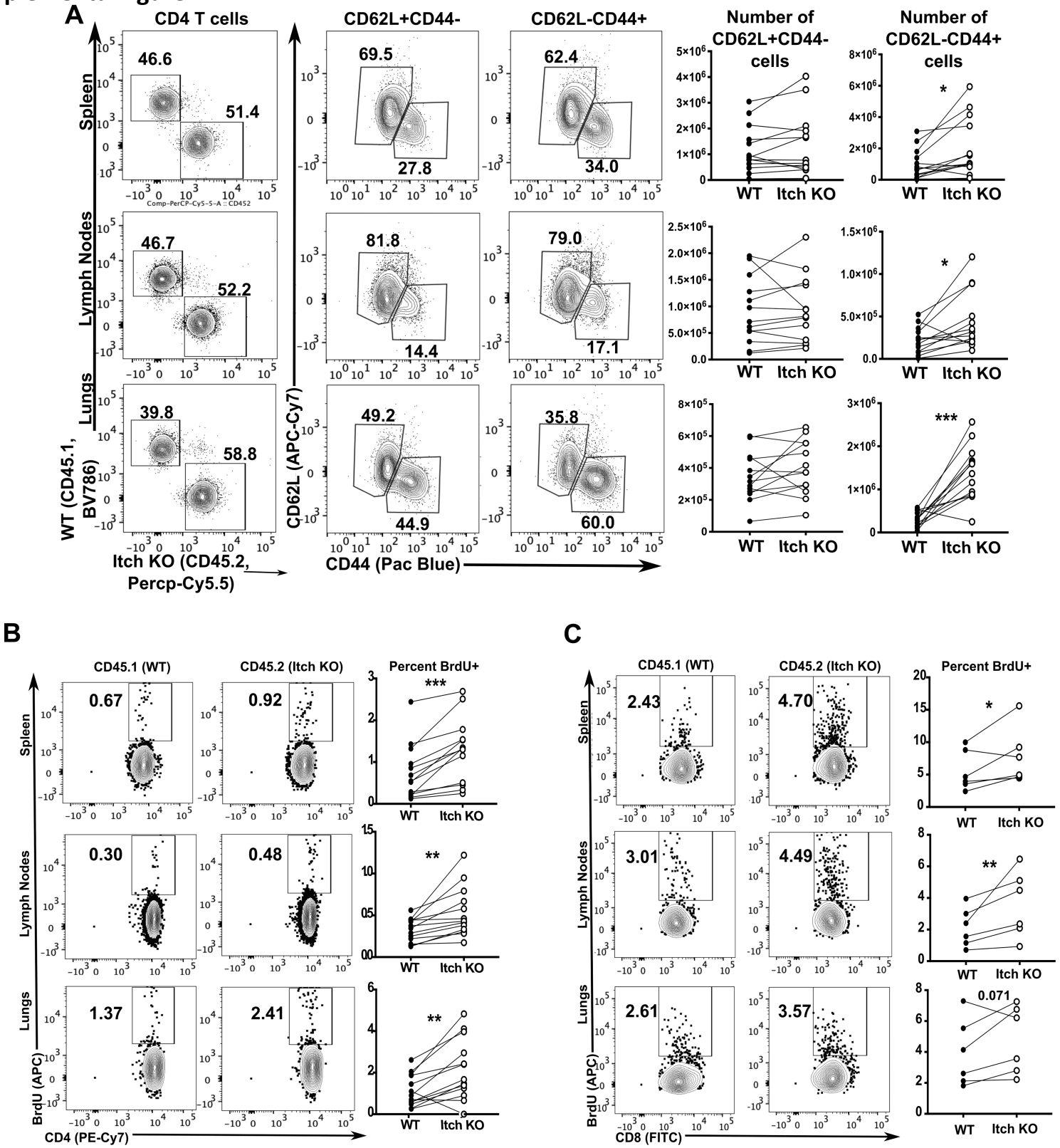

Figure S2: Itch limits activated CD4 T cell numbers and naïve and CD8 T cell proliferation *in vivo.* Bone marrow chimeras were generated as described in Fig, 1. Cells from spleen, lymph nodes and lungs were stained and gated on Live/Dead negative, singlets, lymphocytes (based on low forward and side scatter), and CD3+CD4+ or CD8+, as displayed in Supplemental Fig. 1A. **A)** CD4 T cells were divided into wild type (WT, CD45.1) and Itch deficient (Itch KO, CD45.2) cells. Within each of these populations, naïve (CD62L+CD44-) or activated (CD62L-CD44+) populations were compared. Quantification shows absolute cell numbers of naïve (CD62L+CD44-) and activated (CD62L-CD44+) CD4 T cells of each genotype for each recipient mouse. p-values were determined by paired t-test (paired by recipient mouse). p=0.032 (spleen, activated cells), p=0.035 (lymph nodes, activated cells), p=0.0001 (lungs, activated cells). All paired t-test for wild type versus Itch deficient naïve cells were >0.17. **B)** The percent BrdU positive was analyzed separately for wild type CD45.1 and Itch KO CD45.2 cells. Quantification shows percent of BrdU+ cells within the CD62L+CD44- population for each genotype. p-values were determined by paired t-test (paired by recipient mouse). p=0.0002 (spleen), p=0.0041 (lymph nodes), p=0.0032 (lungs). **C)** Representative flow plots and quantification for the percent of BrdU+ cells within the CD8+CD44+ population for each genotype. p-values were determined by ratio paired t-test (paired by recipient mouse). p=0.042 (spleen), p=0.0076 (lymph nodes), p=0.071 (lungs). n=13 mixed chimeras generated from 2 pairs of bone marrow donors, analyzed over 4 BrdU injection experiments for CD4 T cells. n=6 mixed chimeras generated from 2 pairs of bone marrow donors, analyzed over 2 BrdU injection experiments for CD8 T cells.

Supplemental figure 3

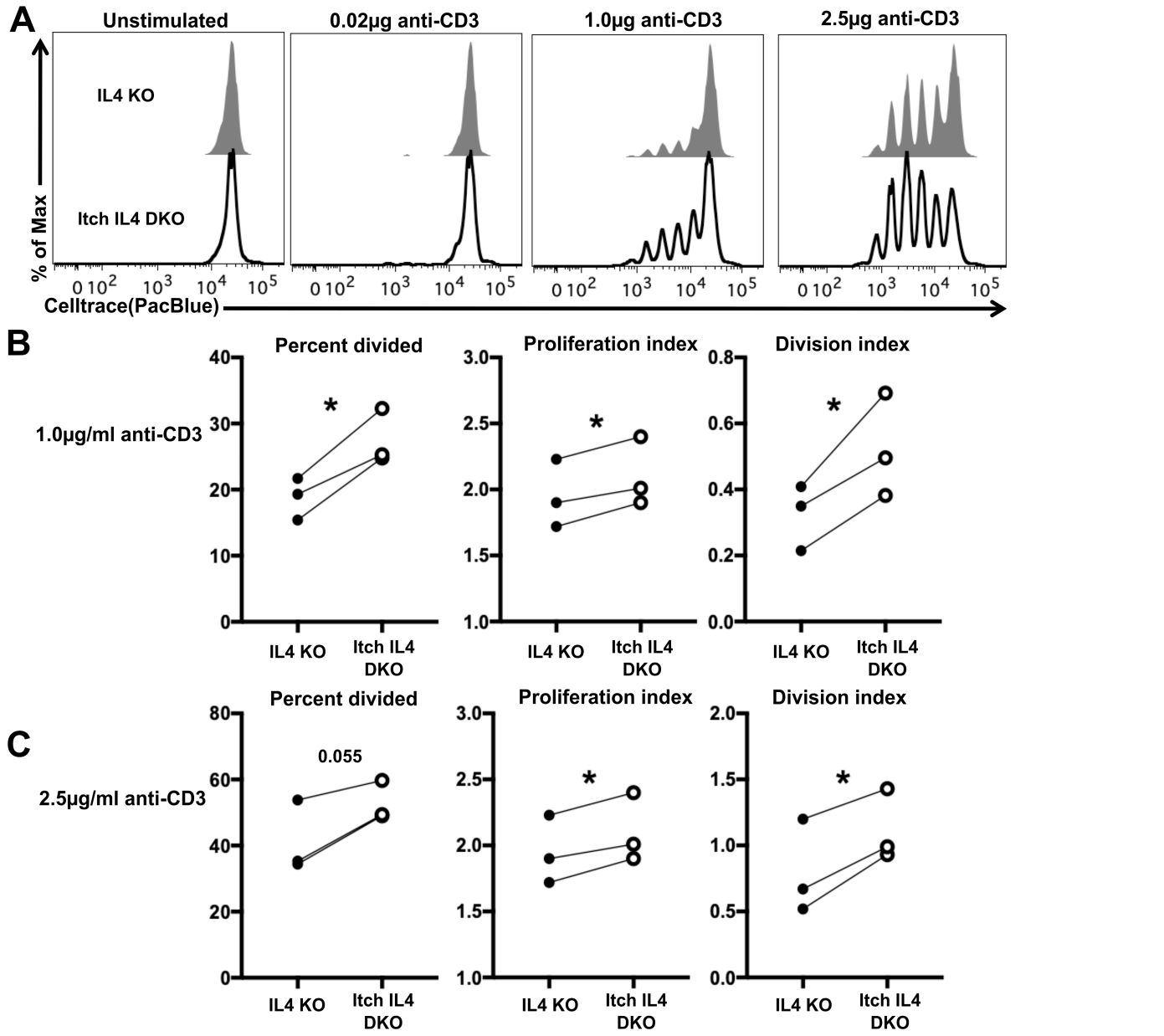

Figure S3: Itch limits CD4 T cell proliferation at sub-maximal levels of TCR stimulation. Naïve CD4 T cells were isolated from IL4 KO CD45.1 and Itch IL4 DKO CD45.2 mice as described in materials and methods. Cells were mixed in 1:1 co-cultures of IL4 and Itch IL4 DKO cells and labeled with Celltrace violet, as described in Fig. 2. Co-cultures were labeled with CellTrace violet and cultured in IL-2-containing media on plates coated with anti-CD3 and anti-CD28 antibodies. Anti-CD28 concentration was held constant at 5ug/ml while anti-CD3 concentration was varied as labeled. CellTrace violet dilution was analyzed on day 3 after plating. Cells were gated on Live/Dead negative, singlets, lymphocytes (based on forward scatter and side scatter) CD4+ cells, and CD45.1 or CD45.2, as displayed in Supplemental Fig. 1B, but using the fluorophores BV786 and PerCP-Cy5.5 for CD45.1 and CD45.2, respectively. **A)** Representative flow plots of Celltrace dilution at different concentrations of anti-CD3 stimulation. **B)** Quantification of percent divided, proliferation index, and division index for the 1ug/ml condition across all experiments. Experiments were analyzed by paired t-test, paired by co-culture. p=0.024 (percent divided), p=0.041 (proliferation index) p=0.043 (division index). **C)** Quantification of percent divided, proliferation index, and division index for the 2.5ug/ml condition across all experiments. Experiments were analyzed by paired t-test, paired by co-culture. p=0.055 (percent divided), p=0.20 (proliferation index), p=0.026 (division index). n=3 biological replicates across 3 independent experiments.

Supplemental figure 4

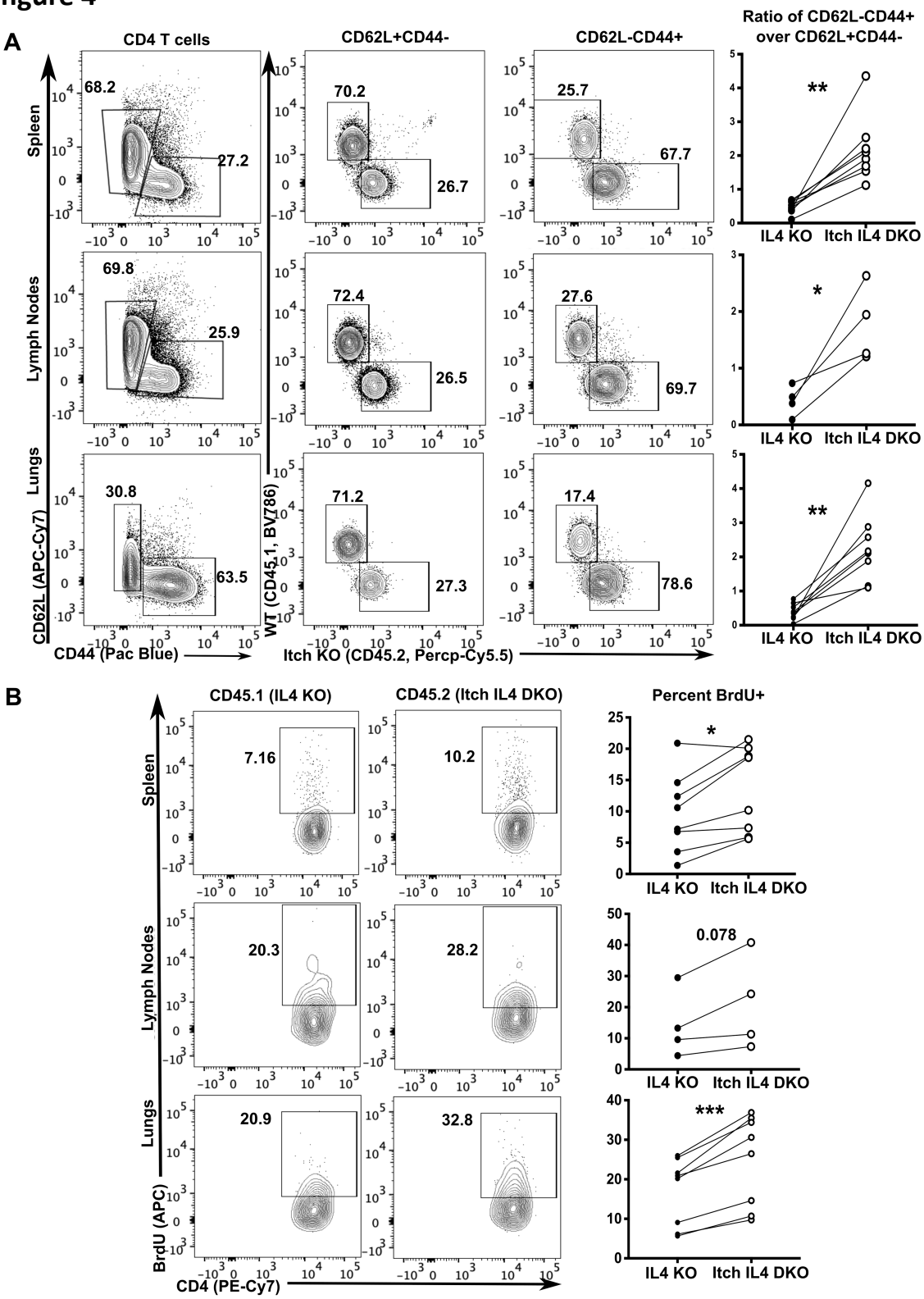

Figure S4: Itch limits CD4 T cell proliferation *in vivo* independently of IL-4. Bone marrow was extracted from IL4 KO CD45.1 and Itch IL4 DKO CD45.2 mice and injected into irradiated Rag-deficient hosts. After reconstitution, mice were injected with BrdU, and 18 hours later, tissues were analyzed by flow cytometry, as described in Fig. 1A. **A)** Cells from spleen, lymph nodes and lungs were stained and gated on Live/Dead negative, singlets, lymphocytes (based on low forward and side scatter), and CD3+CD4+, as displayed in Supplemental Fig. 1A. CD4 T cells were then divided into naïve (CD62L+CD44-) or activated (CD62L-CD44+) populations, and the percent of IL4 KO (CD45.1) was compared to the percent of Itch IL4 DKO (CD45.2) cells. Quantification shows activated IL4 KO cells over naïve IL4 KO cells compared to activated Itch IL4 DKO KO cells over naïve Itch IL4 DKO KO cells. P-values were determined by paired t-test (paired by recipient mouse). p=0.0019 (spleen), p=0.034 (lymph nodes), p=0.0016 (lungs). **B)** Cells from spleen, lymph nodes and lungs were stained and gated on Live/Dead negative, singlets, lymphocytes (based on low forward and side scatter), CD3+CD4+, and CD44+CD62L-, as shown in Supplemental Fig. 1A, representing activated CD4 T cells. The percent BrdU positive was analyzed separately for IL4 KO CD45.1 and Itch IL4 DKO CD45.2 cells. Quantification shows percent of BrdU+ cells within the CD44+CD62L- population for each genotype. P-values were determined by paired t-test (paired by recipient mouse). p=0.0104 (spleen), 0.078 (lymph nodes), 0.0004 (lungs). n=8 recipient mice from 2 different pairs of donors across 4 independent experiments for spleen and lungs; n=4 recipient mice across 2 different pairs of doors between 2 experiments for lymph nodes.

Supplemental figure 5

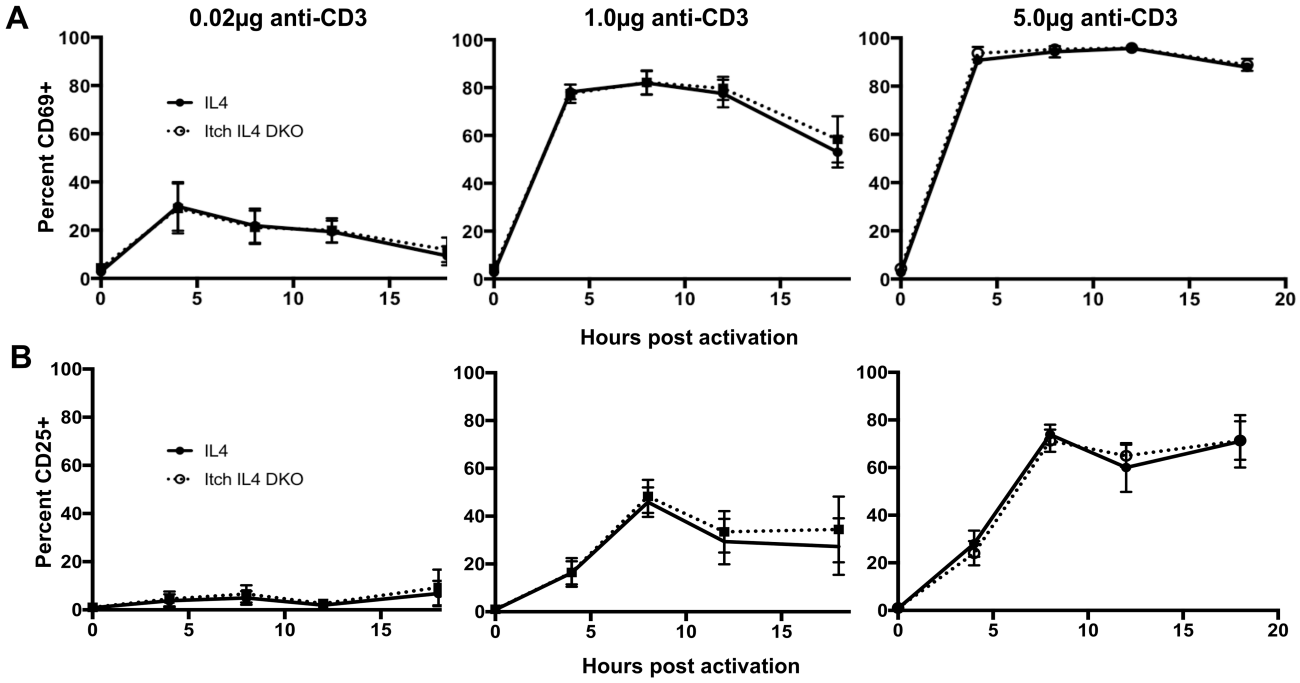

Figure S5: Itch does not affect CD4 T cell exit from quiescence**.** Naïve CD4 T cells were isolated from CD45.1 IL4 KO and CD45.2 Itch IL4 DKO mice and activated in co-culture with plate-bound anti-CD3 at 0.02µg/ml**, 1**µg/ml**,** or 5µg/ml**,** and constant anti-CD28 (5µg/ml) antibodies. Cells were harvested, stained and fixed at the indicated timepoints. Cells shown in **(B)** were cultured in the presence of 50U/ml IL-2. Cells are gated on Live/Dead negative, singlets, lymphocytes (based on low forward and side scatter), and CD4+. Within this population, percent CD69+ (A) or CD25+ (B) was evaluated for each CD45.1 and CD45.2 cells. The gating strategy used is shown in Supplemental Fig. 1B. n=3 biological replicates across 2 independent experiments for **(A);** n=3 biological replicates across 3 independent experiments for (**B**).

Supplemental figure 6

A

Increased in abundance at 0h

Gene name	Log2 FC	P-value
Xpot	0.505288333	0.004176815
Prkar2a	0.507224333	0.047207239
Dapl1	0.5185859	0.009085237
WBP2	0.5229769	0.000418474
Ddx55	0.524588333	0.048636662
Ift27	0.543239	0.005822609
Gvin1	0.568884733	0.009585829
Tgtp2;Tgtp1	0.591271	0.043590121
Dohh	0.591371	0.005448385
Stat1	0.594017333	0.011379827
Chmp7	0.607027667	0.040729459
Mkl2	0.609073333	0.028163405
Slc2a3	0.6759145	0.043589968
Gbp2	0.703300667	0.016207472
Fuom	0.709148667	0.045521976
Pdcl	0.7158945	0.034397086
Cdc123	0.753904	0.045265558
Gng12	0.7645802	0.024465391
Ly75	0.7792533	0.003110886
Mbp	0.833587	0.03939916
Rps6kb1	0.897446833	0.009859392
Scoc	0.9115307	0.002840334
Gbp5	0.989053	0.001750582
Haus7	1.1090125	0.049253862
Gle1	1.2254129	0.016421826
H2afx	1.239724333	0.031245452
Rdh11	1.2873067	0.036701173
Junb	1.489641	0.024246589
Rnpep	1.5966515	9.21E-05
Myod1	1.8261314	0.02063207

B

Increased in abundance at 24h

Gene name	Log2 FC	P-value
Mcfd2	0.504335759	0.023657431
Tgfbi	0.514183825	0.023575476
Hectd3	0.517797692	0.048882053
Bst2	0.525907525	0.027778862
Tjap1	0.537885025	0.032114846
Vps18	0.539032359	0.012768918
Nt5dc1	0.555288159	0.006885019
Mettl14	0.568403725	0.048381399
Cd97	0.653856525	0.015255328
Mrps21	0.705766359	0.006918927
Eea1	0.705854559	0.04349511
Gramd2b	0.713273025	0.009315679
Pcgf6	0.716514025	0.007934505
Hpdl	0.747596359	0.029579053
Ly75	0.752011199	0.002541258
Erc1	0.900367359	0.034348302
Elp5	0.961064992	0.000877234
Mtf1	1.100951359	0.048441496
Commd1	1.105711725	0.044897138
Kif3b	1.664693359	0.038192478
Srpk2	1.671755992	0.001274262
Rnpep	2.008914692	0.000101217
Spc24	2.017432838	0.039987151
Rgcc	2.259764695	0.000959702
Kif27	2.750617839	0.007624066
Lad1	3.461026492	0.003261313

C

Increased in abundance at 48h

Gene name	Log2 FC	P-value
Eef2kmt	0.514800947	0.037653914
Vps39	0.525838281	0.019921796
Ccdc167	0.527530514	0.034867288
Kif16b	0.549733947	0.00182967
Tdg	0.551938814	0.027547493
Vps53	0.573614781	0.035753641
Bst2	0.582734147	0.047828291
Vps37b	0.596757281	0.033989277
Plscr1	0.646000714	0.040066491
F2	0.725757281	0.037721452
Ifi30	0.805667281	0.004313326
Prkab1	0.875698614	0.017559102
Slfn5	1.005873921	0.04342001
Hbb-b1	1.013152947	0.017703993
Atp1a4	1.157067021	0.011833776
Yipf5	1.232318671	0.010982492
Rnpep	1.608016854	0.001219483
Rgcc	2.089190857	0.004952831
Arfrp1	3.152235114	0.002251418
Lad1	3.657150781	0.000639338

D

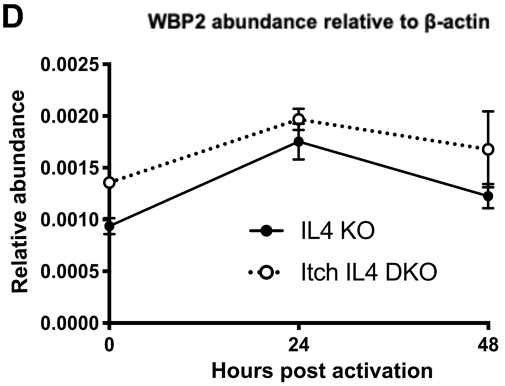

Figure S6: Whole cell proteome analysis reveals potential direct and indirect targets of Itch. **A-C** list proteins identified in the 0-hour timepoint **(A)**, 24-hour timepoint **(B)**, and 48-hour timepoint **(C)** that had at least a log2 fold change (FC) of Itch IL4 DKO over IL4 KO of 0.5 (corresponding to about 1.4 fold change), and a P-value less than 0.05. **D**) WBP2 abundance in each sample, based on intensity values, normalized to β-actin.

Supplemental figure 7

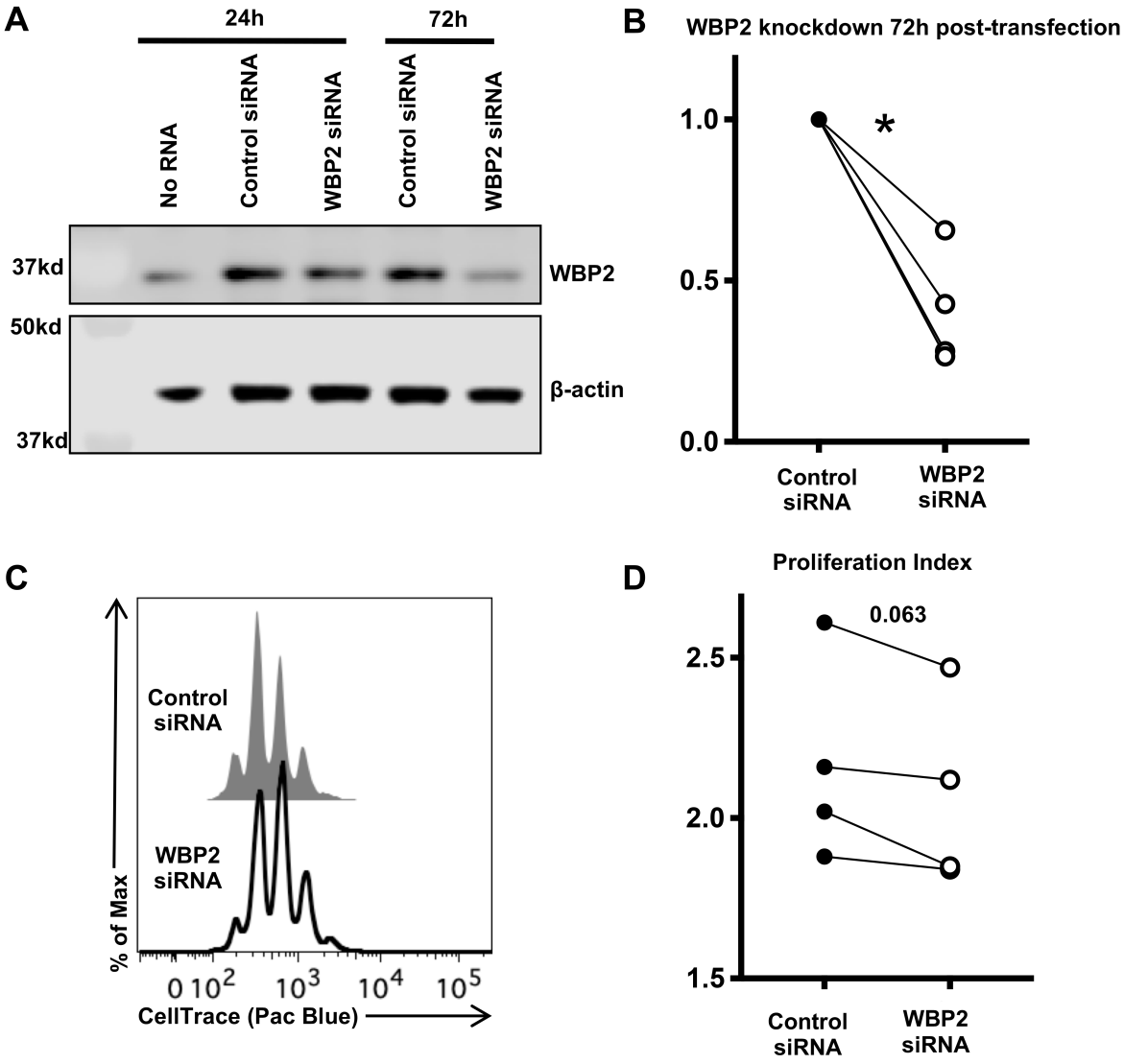

Figure S7: WBP2 promotes CD4 T cell proliferation in Itch deficient cells. Naïve T cells were isolated from Itch IL4 DKO mice, labeled with CellTrace violet and activated using plate-bound anti-CD3 and anti-CD28 in IL-2-containing media for 24 hours, then transfected with either non-targeting control siRNA or siRNA specific for WBP2. **A)** Representative Western showing WBP2 levels in transfected cells. Western blots were probed as described in Fig. 6. Timepoints refer to timing post transfection, which was 24 hours after initial activation. **B)** Quantification of WBP2 protein knockdown. Each sample is normalized to β-actin. Each WBP2-treated timepoint was normalized to its corresponding control siRNA-treated timepoint. p-values were calculated by paired t-test, pairing cells from the same mouse. p=0.0073 (72 hours). **C)** Representative flow analysis of proliferation 72 hours post transfection. Cells were not stained to avoid further stress and cell loss; live lymphocytes were identified on the basis of forward scatter and side scatter as shown in Supplemental Fig. 1. **D)** Quantification of proliferation across all experiments. Proliferation index was calculated using FlowJo software, which determines the number of cells in each peak. p-value was determined by paired t-test, pairing control vs WBP2 siRNA-treated cells from the same mouse. n=4 biological replicates across 3 independent experiments. P=0.063