Supplemental Materials and Methods

Generation of mice with the conditionally targeted Dlgh1 gene. The conditional *Dlgh1* knockout strategy was based on our previously published strategy to generate the *Dlgh1* null allele (1). In brief, to delete a 700 bp fragment containing exon 4, we constructed a targeting vector modified by the addition of two loxP sites flanking exon 4. The original Sal I site in the 5'arm was removed by digestion followed by klenow treatment and religation. A new Sal I site was introduced by PCR-mediated mutagenesis 290 bp upstream of exon 4, followed by the introduction of a polylinker containing a loxP sequence and SacI and EcoRV sites, which was cloned into the new SalI site. The 5'arm (5'loxP) was cloned into the pLNTK3'arm vector (1) to generate the final 20.5 kb targeting construct (pLNTK loxP) including a neo cassette flanked by two other loxP sites. F1BS ES cells were cultured, electroporated, selected, and screened as previously described (1). Positive targeted clones were transiently transfected with the Cre recombinase expression vector pMC-CreN (2). Cells were genotyped by southern blotting and clones with Cre-mediated deletions of the neo^r gene were used to generate chimeric mice for germline transmission. The offspring were bred with lck-Cre transgenic mice, and Cre-mediated deletion was confirmed by PCR and western blotting with anti-DLGH1 antibodies.

Migration Assays. $1x10^6$ splenocytes were assayed for migration across 6.5mm diameter, 3 μM pore size Transwell culture inserts (Costar). Migration occurred over 3 hours in DMEM-10% FBS with the bottom chamber supplemented with the indicated concentrations of SDF-1α (Peprotech). Migrated cells were stained with antibodies to CD4 and counted with a FACSscan for 60 seconds. The percent migration was defined

as the number of $CD4^+$ cells in the bottom chamber (counted for 60 seconds) divided by the number of cells (counted for 60 seconds) in the 100% input well ($1x10^6$ cells plated in well without transwell insert).

Intracellular Cytokine Assays. $2x10^6$ lymph node T cells were stimulated with anti-CD3 and anti-CD28 antibodies for 6 hours with monensin (2 μM) added to the cell culture for the final 4 hours. Cells were surface stained with antibodies against CD4 and CD8, fixed with 4% paraformaldehyde, and permeabilized in PBS/0.5% saponin before staining with antibodies against IFN-γ and IL-2.

NFAT Translocation Assays. T cell blasts were generated by stimulating lymph node cells with anti-CD3 and anti-CD28 for three days followed by rh-IL-2 for one additional day. Cells were rested in serum free media for 2-6 hours before stimulation on cover slips coated with poly-l-lysine (untreated) or anti-CD3 and anti-CD28. Cells were fixed, stained with anti-NFATc1 (Santa Cruz), and scored. Cells containing predominantly nuclear NFAT were scored positive for translocation, whereas cells demonstrating primarily nuclear NFAT were scored as negative.

Supplemental References:

1. **Mahoney, Z. X., B. Sammut, R. J. Xavier, J. Cunningham, G. Go, K. L. Brim, T. S. Stappenbeck, J. H. Miner, and W. Swat.** 2006. Discs-large homolog 1 regulates smooth muscle orientation in the mouse ureter. Proc Natl Acad Sci U S A **103:**19872-7.

2. **Sleckman, B. P., C. G. Bardon, R. Ferrini, L. Davidson, and F. W. Alt.** 1997. Function of the TCR alpha enhancer in alphabeta and gammadelta T cells. Immunity **7:**505-15.

Supplemental Figure 1. Fetal thymus development in *Dlgh1*-deficient embryos. Single cell suspensions of E14-E18 thymocytes were counted, stained with the indicated fluorescent antibody conjugates, and analyzed by flow cytometry as described in the Materials and Methods. Data shown is from one representative experiment, (n >5).

Supplemental Figure 2. Generation of mice with conditionally-deleted *Dlgh1.* (A) Schematic of the strategy used to generate the conditional *Dlgh1* allele. (B) F1BS ES cells were transfected with the targeting constuct and screened by Southern blotting. (C) Positively targeted ES cell clones were transiently transfected with the Cre-vector pMC-CreN and screened for deletion of the neo^r gene. (D) Tail DNA was analyzed by Southern blotting to detect the floxed allele. (E) The Cre-mediated deletion of *Dlgh1* was confirmed by PCR analysis of DNA isolated from lymph node T cells and (F) by western blotting with anti-DLGH1 antibodies.

Supplemental Figure 3. Chemokine-induced migration with *Dlgh1*-deficient T lymphocytes. The migration of *Dlgh1*-sufficient and -deficient splenocytes to the indicated concentrations of SDF-1α were analyzed using transwell assays. Data is presented as the mean percentage of migrating $CD4^+$ cells from n=3 experiments, ±SD.

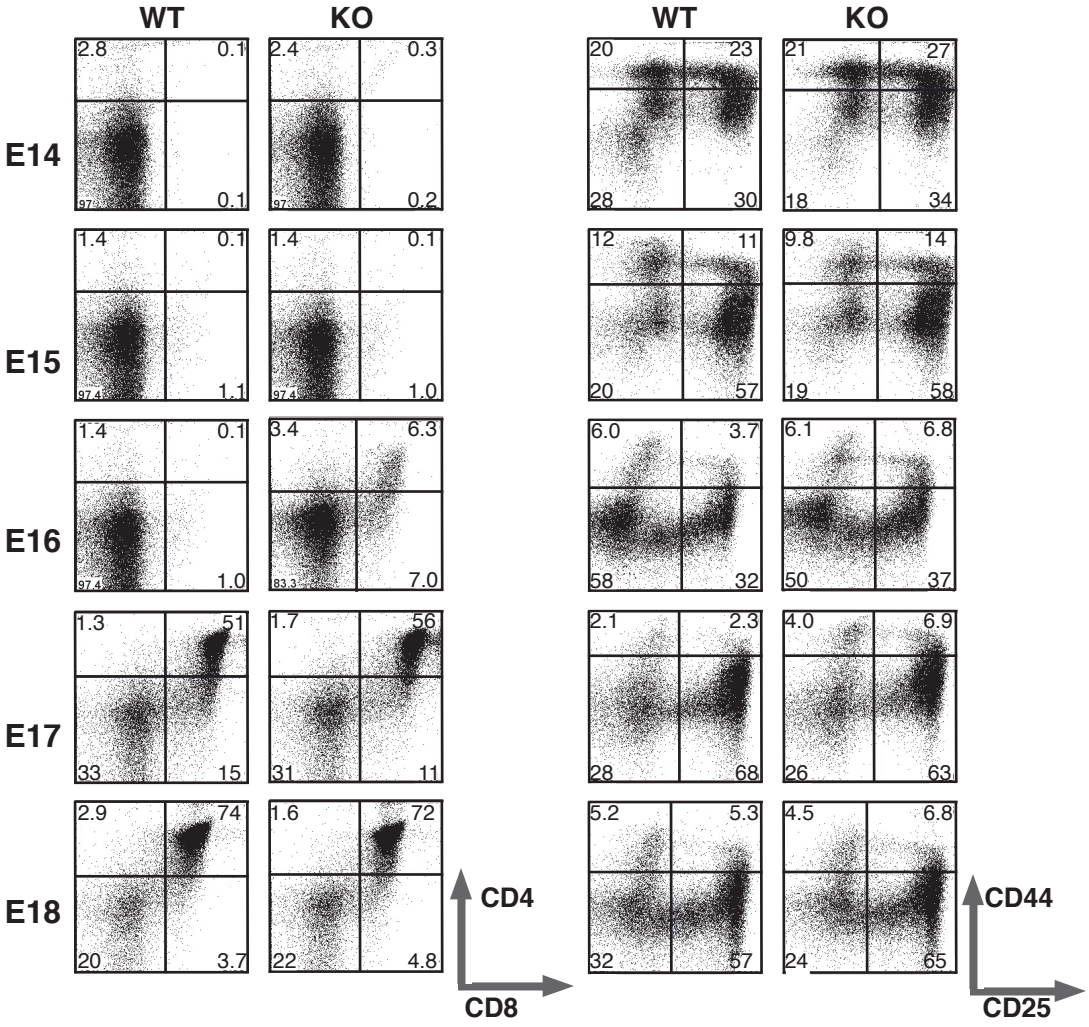
Supplemental Figure 4. DLGH1 is not required for TCR-induced NFAT activation, or p38 kinase activation. (A) T cell blasts from WT ($Dlgh1^{f/f}$ LckCre-) and KO ($Dlgh1^{f/f}$ LckCre+) mice were scored for NFAT translocation. Shown are representative images from three independent experiments. At least 75 cells per condition were scored.

(B) B cell-depleted lymph node T cells from WT (Dlgh1$^{f/f}$ LckCre-) and KO (Dlgh1$^{f/f}$ LckCre+) mice were stimulated for the indicated time points with streptavidin dynabeads (Dynal) coated with biotinylated anti-CD3 and anti-CD28. After stimulation, cell lysates were made and separated by PAGE for analysis of p38 phosphorylation by western blot.

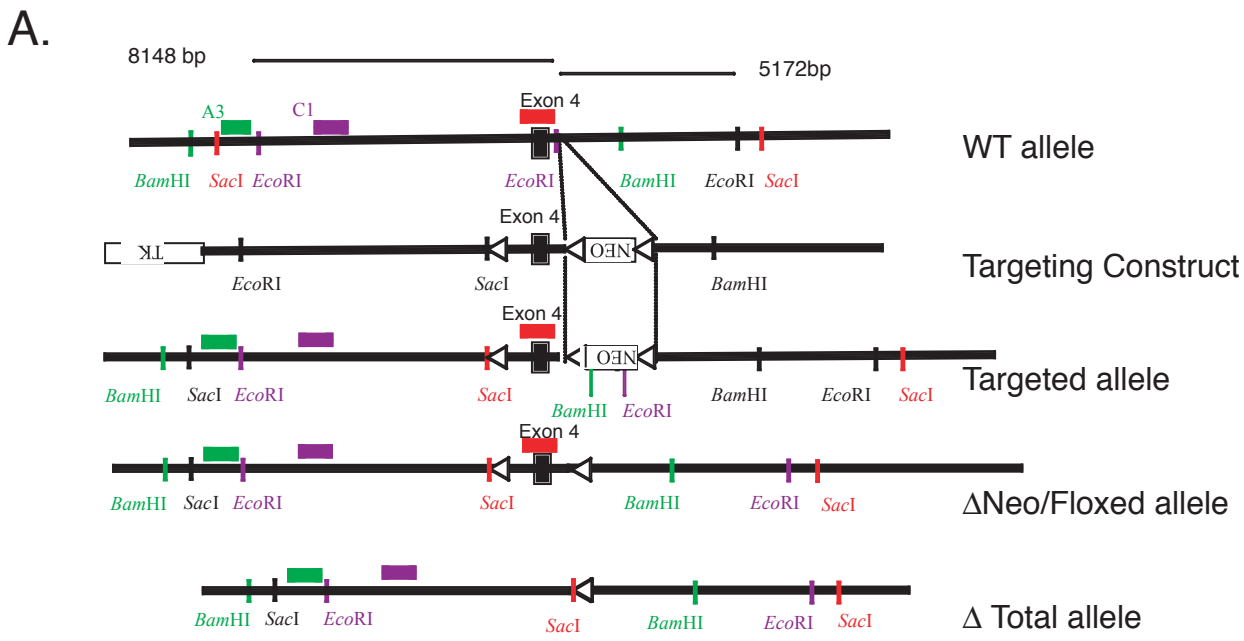
Supplemental Figure 5. Induction of CD69 and CD25 between WT and KO T cells. Lymph node cells were stimulated with 1 μg/mL anti-CD3 for the indicated times and then stained with antibodies against CD4, CD8, CD25, and CD69. Show are the mean percent positive cells from the indicated population, ±SD. Results are from n=4 experiments.

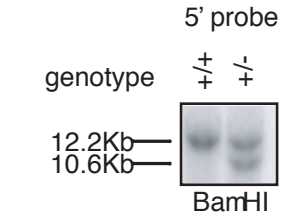
Supplemental Figure 6. Normal IL-2 production by *Dlgh1*-deficient T lymphocytes. Lymph node cells were stimulated with 1 μg/mL anti-CD3+ 1 μg/mL anti-CD28 for 6 hours and then processed for intracellular staining of IL-2 and IFN-γ. Shown is one representative experiment, n=3, all with similar results.

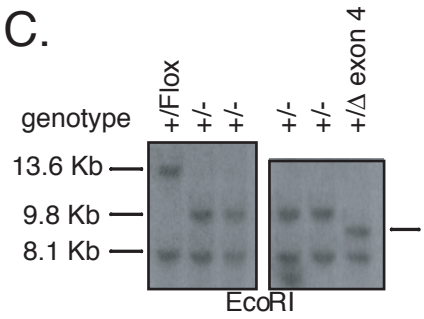
Supplemental Figure 1

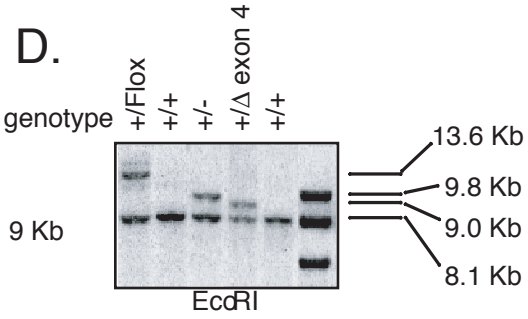


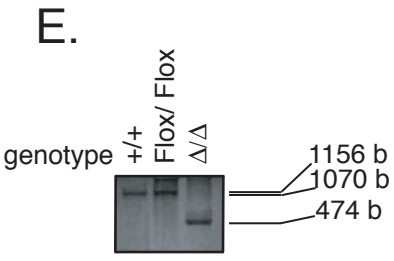
Supplemental Figure 2

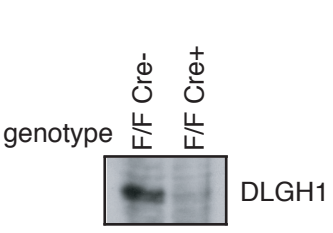




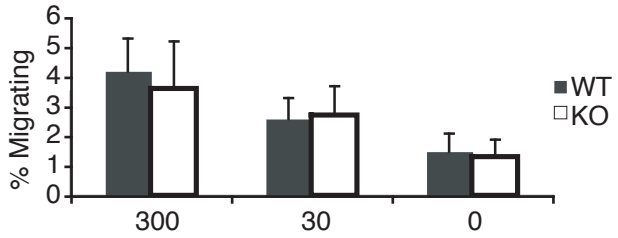






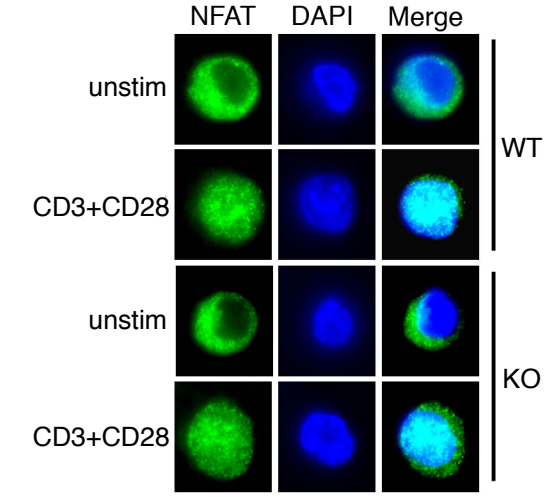


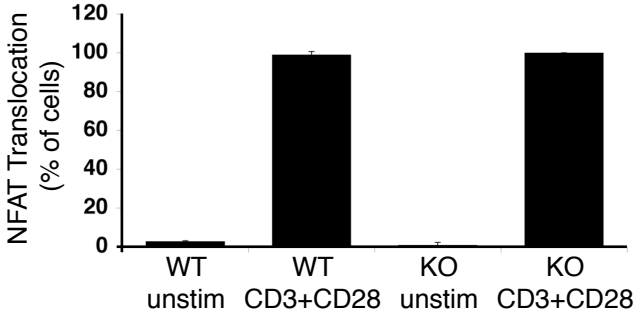
Supplemental Figure 3



Supplemental Figure 4

A.





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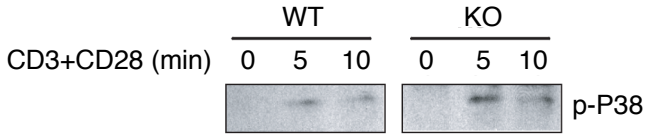
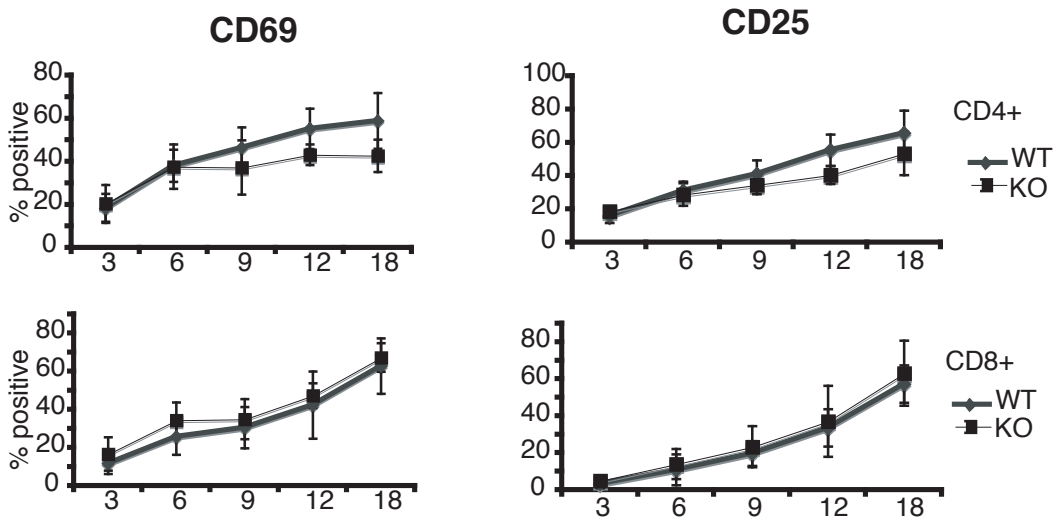


Supplemental Figure 5



Supplemental Figure 6