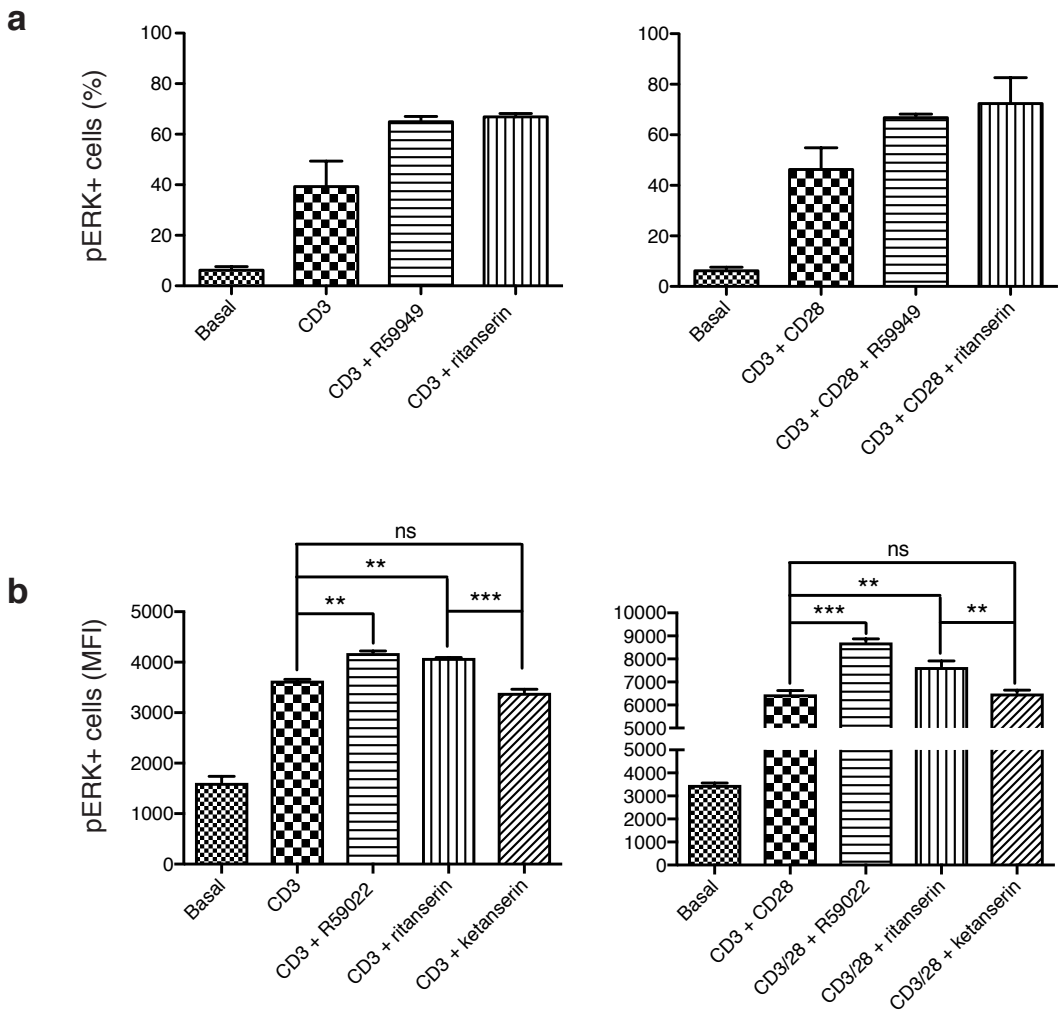
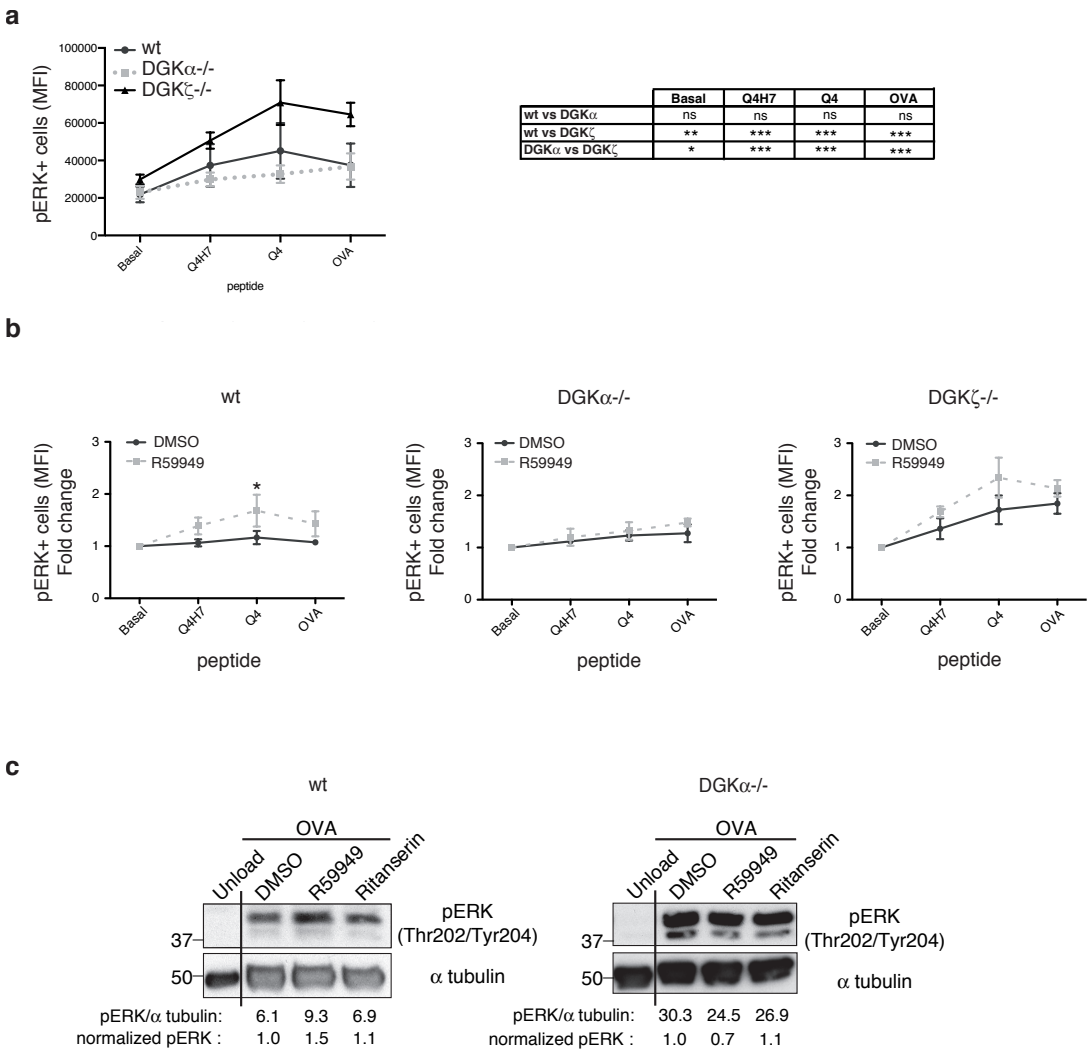
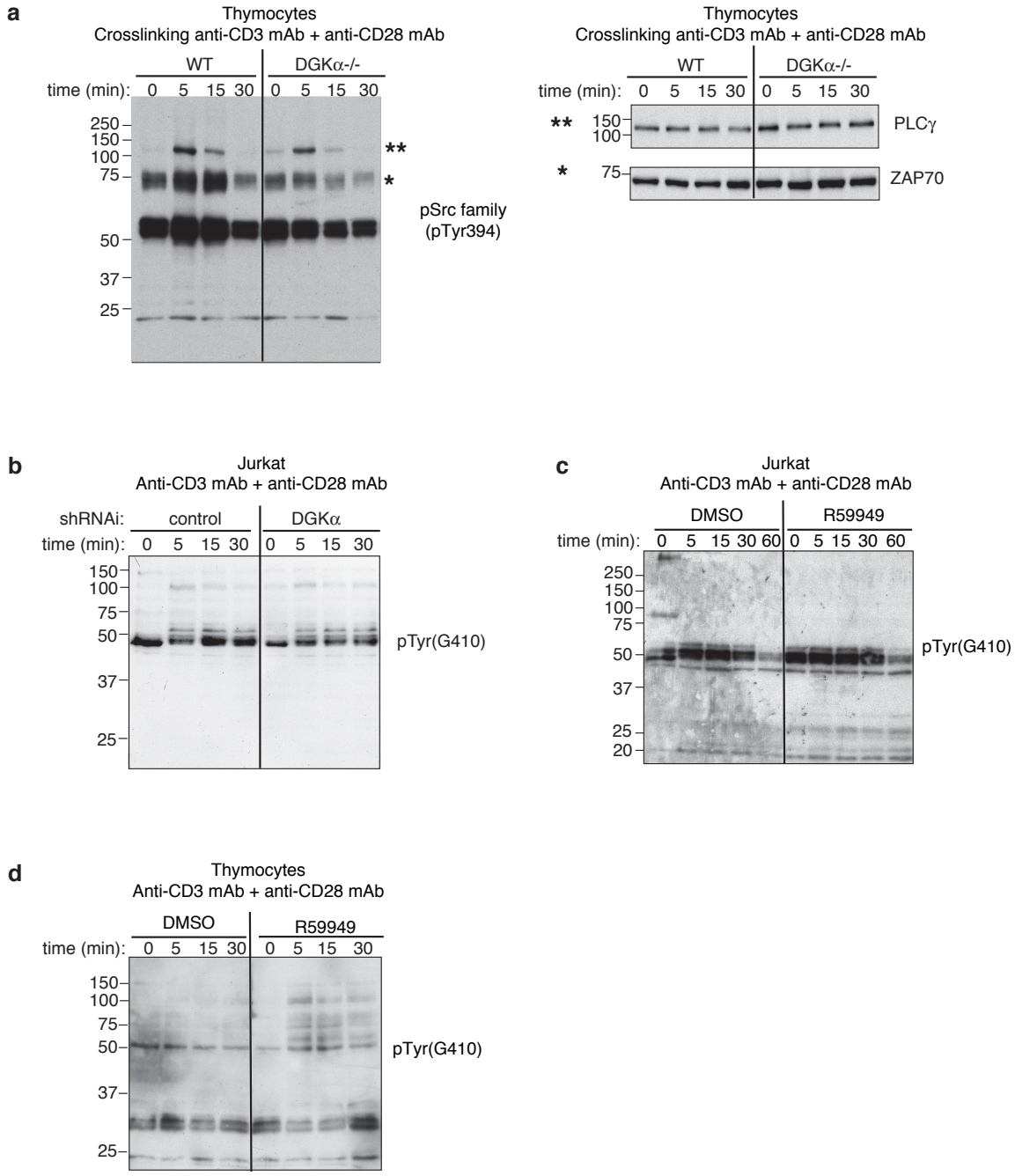


Supplementary Figure 1. DGKα negatively controls ERK phosphorylation downstream of TCR and is specifically targeted by R59949 and ritanserin, but not by ketanserin. Jurkat cells were stimulated with anti-CD3 or -CD3/CD28 mAb (15 min), alone or with the indicated drug. pERK was determined by flow cytometry analysis. **a**, Percentage of pERK$^+$ cells. **b**, MFI of pERK. Results from three independent experiments are shown. Data were analyzed using one-way ANOVA and Bonferroni post-test. *** $p < 0.001$; ** $p < 0.01$.

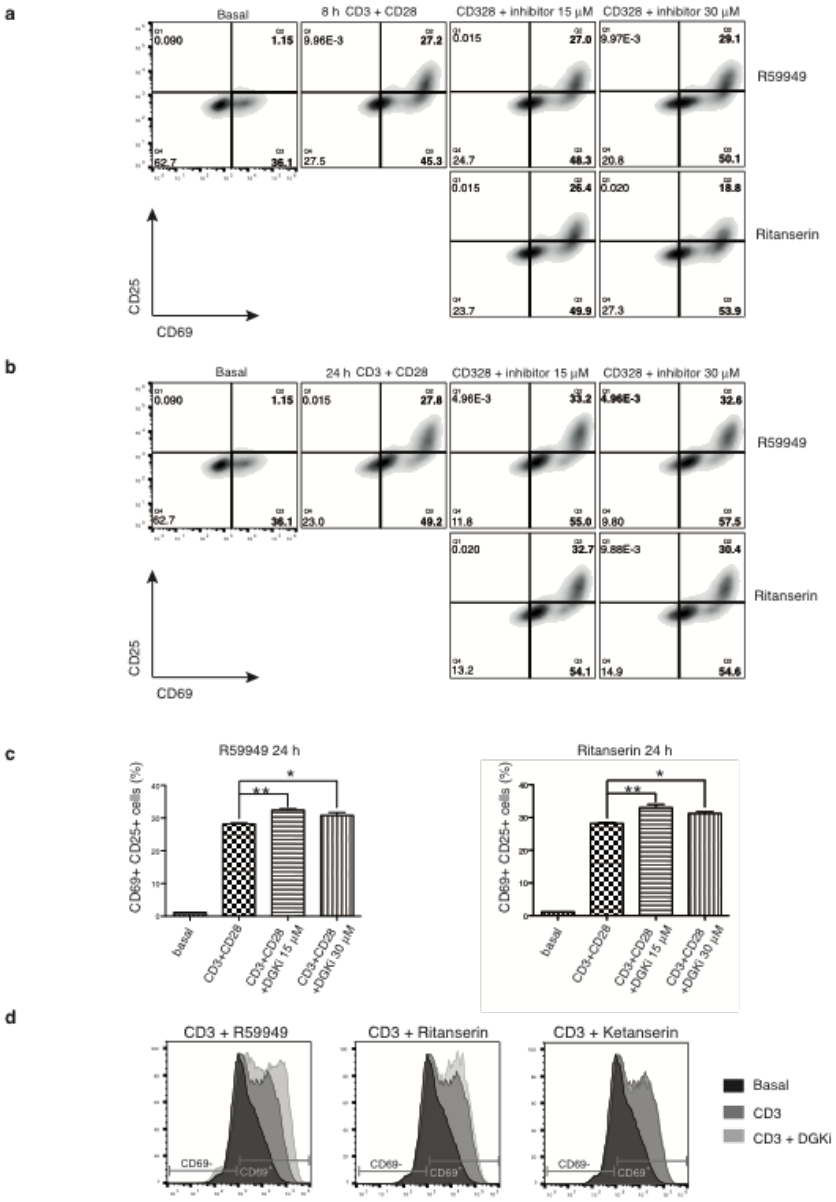


	Basal	Q4H7	Q4	OVA
wt vs DGKα	ns	ns	ns	ns
wt vs DGKζ	**	***	***	***
DGKα vs DGKζ	*	***	***	***

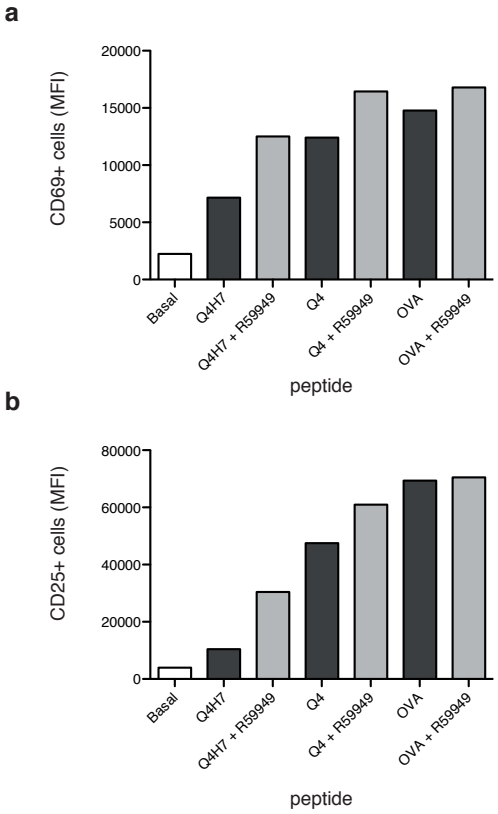
Supplementary Figure 2. DGK negatively controls ERK phosphorylation downstream of TCR. **a**, Spleen cells from wt or DGK-deficient OT-I transgenic mice were loaded with indicated peptides, alone or with R59949. ERK1/2 phosphorylation and CD44 expression were determined in the $CD8^+$ T subset by flow cytometry. MFI of pERK in the $CD44^{low}$ population of samples without DGKi. **b**, Values for the MFI of pERK in the $CD44^{low}$ population were normalized to basal conditions to determine the DGKi effect in each genotype. Results summarize the results of two experiments, each with 3 mice/genotype. Data were analyzed using two-way ANOVA and Bonferroni post-test. Statistical results of the comparisons in a) are summarized in the table. Significant differences are indicated in b). **c**, $CD8^+$ cells from OT-I wt or DGK$\alpha^{-/-}$ mice were differentiated to CTL. Cells were stimulated with EL-4 cells, unloaded or loaded with the cognate OVA peptide (40 min), alone or with R59949 or ritanserin. ERK1/2 phosphorylation was evaluated using phospho-specific antibodies and corrected to α-tubulin values. Values for pERK/α-tubulin ratios and normalized values (basal condition = 1.0) are shown beneath the blots.



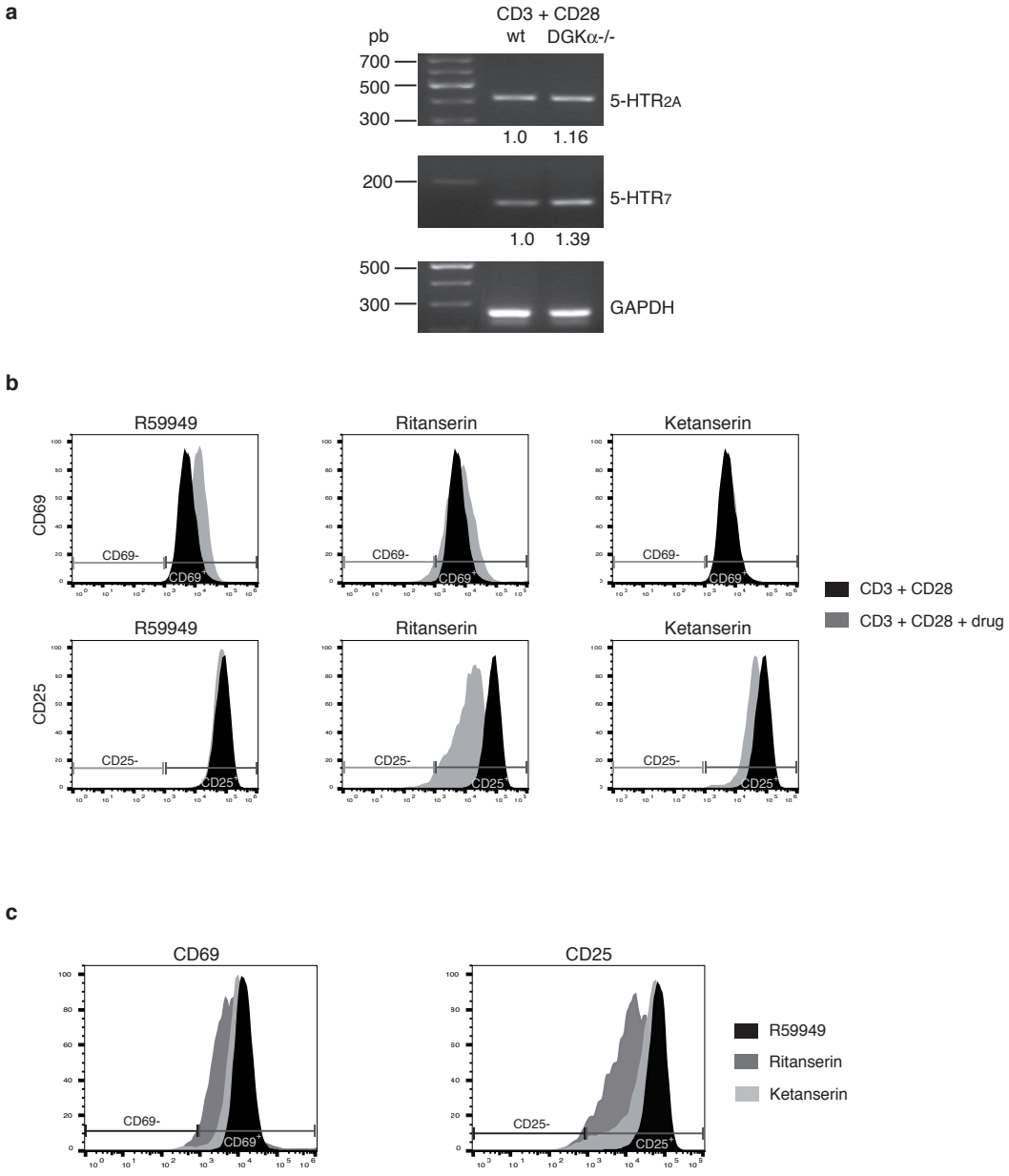
Supplementary Figure 3. Effects of DGKα on p-Tyr signaling. **a,** Complete and overexposed profiles of the blot in Fig. 3a with the anti-pSrc-family Ab. The Ab recognized proteins in addition to Lck, indicated with * and ** (left). PLCγ and ZAP-70 were analyzed in a second blot of the same samples (right). **b-d,** The total phosphotyrosine profile of Jurkat or thymus cells in Fig. 3 was analyzed by western blot. **b**, Control and silenced Jurkat cells. **c**, Jurkat cells or **d**, thymus cells, vehicle- or R59949-treated. Results shown for a representative experiment (*n* = 3).



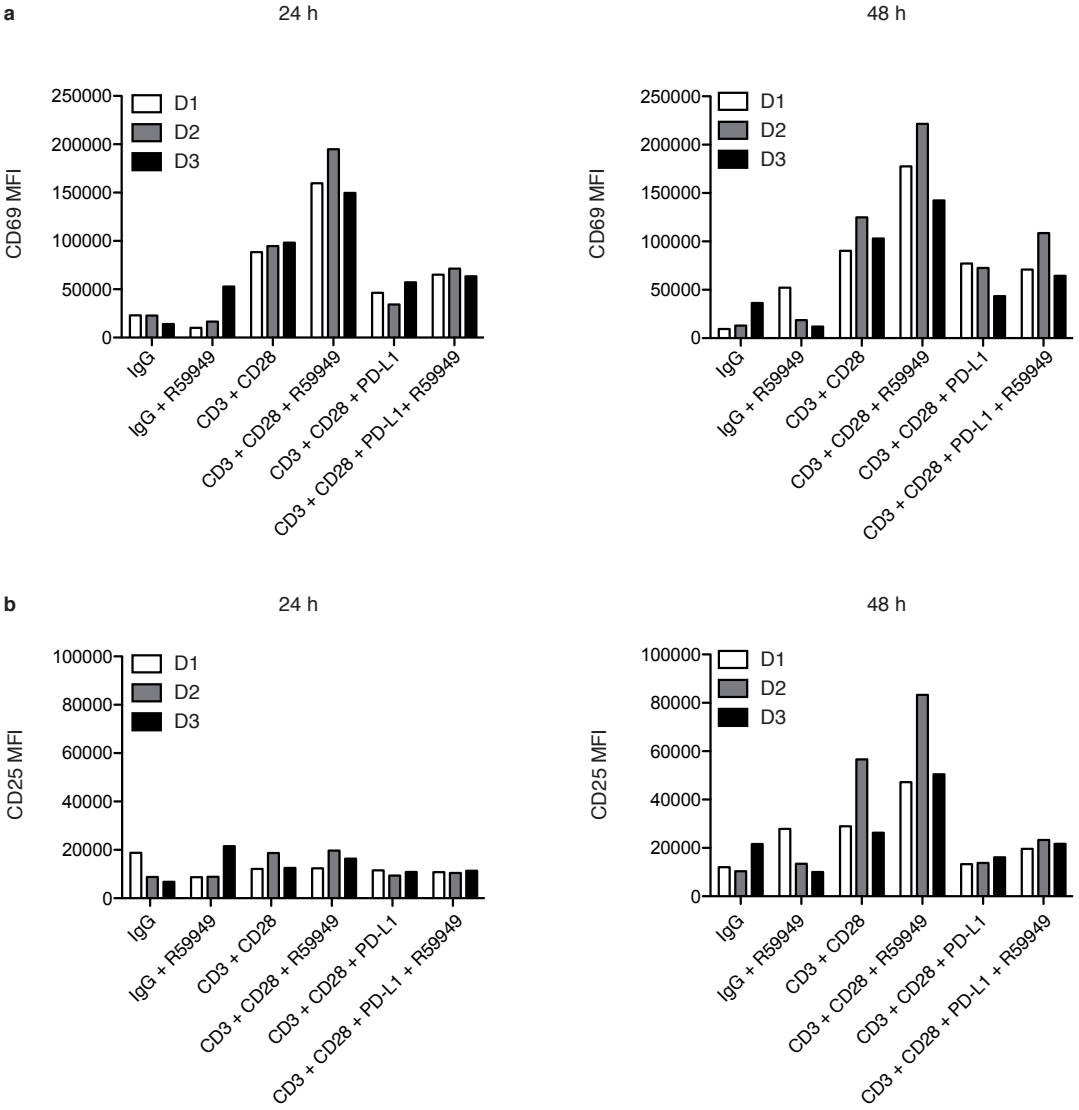
Supplementary Figure 4. R59949 and ritanserin enhanced CD69 and CD25 expression in Jurkat cells. Jurkat T cells were stimulated with anti-CD3/CD28, alone or with 15 or 30 µM R59949 or ritanserin. CD69 and CD25 expression were analyzed by flow cytometry. Biparametric dot plots of CD25 *vs* CD69 after **a**, 8 h, and **b**, 24 h. One representative experiment is shown of at least 3 performed. **c**, Percentage of CD69 and CD25 double-positive cells after 24 h treatment. Results shown for a representative experiment, with three replicates, of at least three independent series of experiments with similar results. Data were analyzed using one-way ANOVA and Bonferroni post-test. ** $p < 0.01$; * $p < 0.05$. **d**, R59949 and ritanserin, but not ketanserin, enhanced the number of $CD69^+$ cells. Jurkat T cells were stimulated with anti-CD3, alone or with R59949, ritanserin or ketanserin (6 h). CD69 expression was analyzed by flow cytometry. The percentage of $CD69^+$ cells is shown. Results shown for a representative experiment ($n = 3$).



Supplementary Figure 5. R59949 enhances CD69 and CD25 expression after TCR stimulation in spleen $CD8^+$ T cells from OT-I transgenic mice. The cells were stimulated with the indicated peptide, alone or with R59949 (24 h); the activation state was evaluated by measuring **a**, CD69 and **b**, CD25 expression. Results shown for a representative experiment of at least two independent series of experiments with similar results.



Supplementary Figure 6. R59949 and ritanserin have distinct effects in naïve T cell activation. **a**, 5-HTR expression in activated mouse T cells. Naïve lymph node cells from C57BL/6J wt and DGK$\alpha^{-/-}$ mice were stimulated with anti-CD3/CD28 mAb (48 h) and 5-HT_{2A} and 5-HT_7 expression were analyzed by RT-PCR. Expression of each 5-HTR was corrected to that of GAPDH and normalized to values for wt. **b**, Spleen T cells were activated with anti-CD3/CD28 mAb, alone or with the indicated drug (48 h). Cell surface CD69 and CD25 expression were analyzed by flow cytometry in $CD8^+$ T cells. The percentage of $CD69^+$ or $CD25^+$ cells in untreated and drug-treated conditions is shown in each histogram. **c**, as in b), using lymph node T cells. The percentage of $CD69^+$ or $CD25^+$ cells with each different drug treatment is shown in each histogram. Results are representative of three experiments with similar results.



Supplementary Figure 7. DGKα inhibition increases CD69 and CD25 expression after TCR costimulation or the PD-1/PD-L1 immune checkpoint. PBMC from three healthy donors (D1-3) were purified with anti-CD8-coupled beads and stimulated with IgG, anti-CD3/CD28 or anti-CD3/CD28 + PD-L1 for indicated times, alone or with R59949. CD69 and CD25 induction were analyzed by flow cytometry. **a**, MFI of CD69 and **b**, CD25 in the double-positive cells.