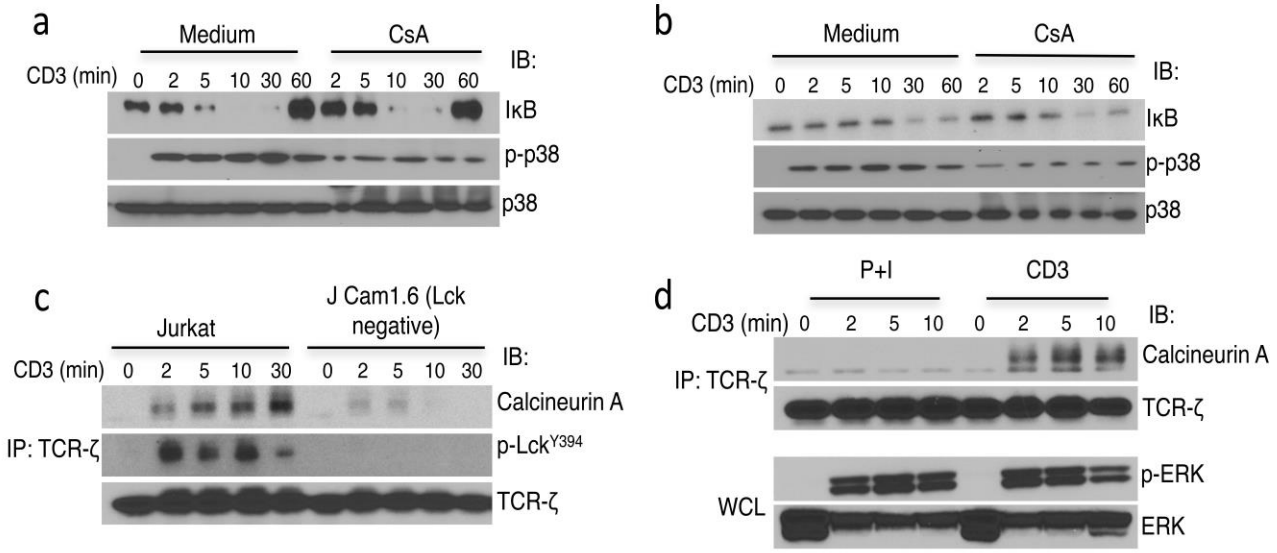
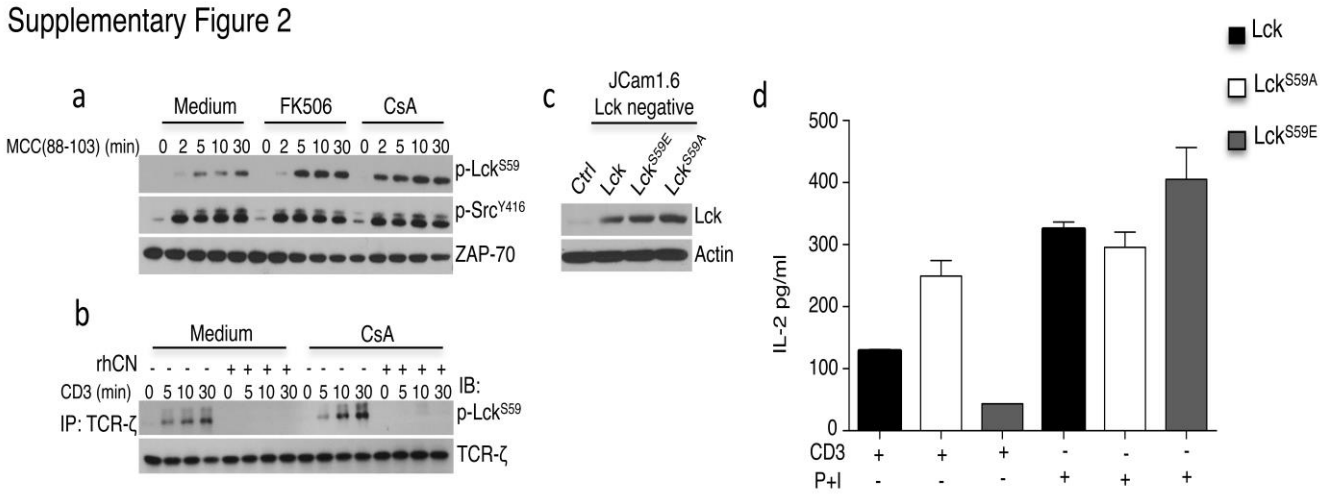
Supplementary Figure 1



Supplementary Figure 1

Recruitment of calcineurin is TCR specific and requires Lck.

Immunoblot analysis of **(a)** Jurkat and **(b**) human $CD4^+$ T cells left unactivated or activated with OKT3 for the indicated times in the presence of medium or CsA (above lanes) followed by probing for phosphorylated protiens (right margin). Immunoblot analysis of Immunoprecipitates of TCR-ζ from Jurkat and Lck-deficient JCam1.6 cells **(c)** activated for different times (aove lanes) followed by reprobing with anti-TCR-ζ to ensure equal loading . Immunoprecipitation and immunoblotting with anti-TCR of Jurkat cells **(d)** activated with either PMA and ionomycin (P+I) or anti-CD3 (OKT3) for the indicated times. Data are representative of 3 independent experiments with similar results.

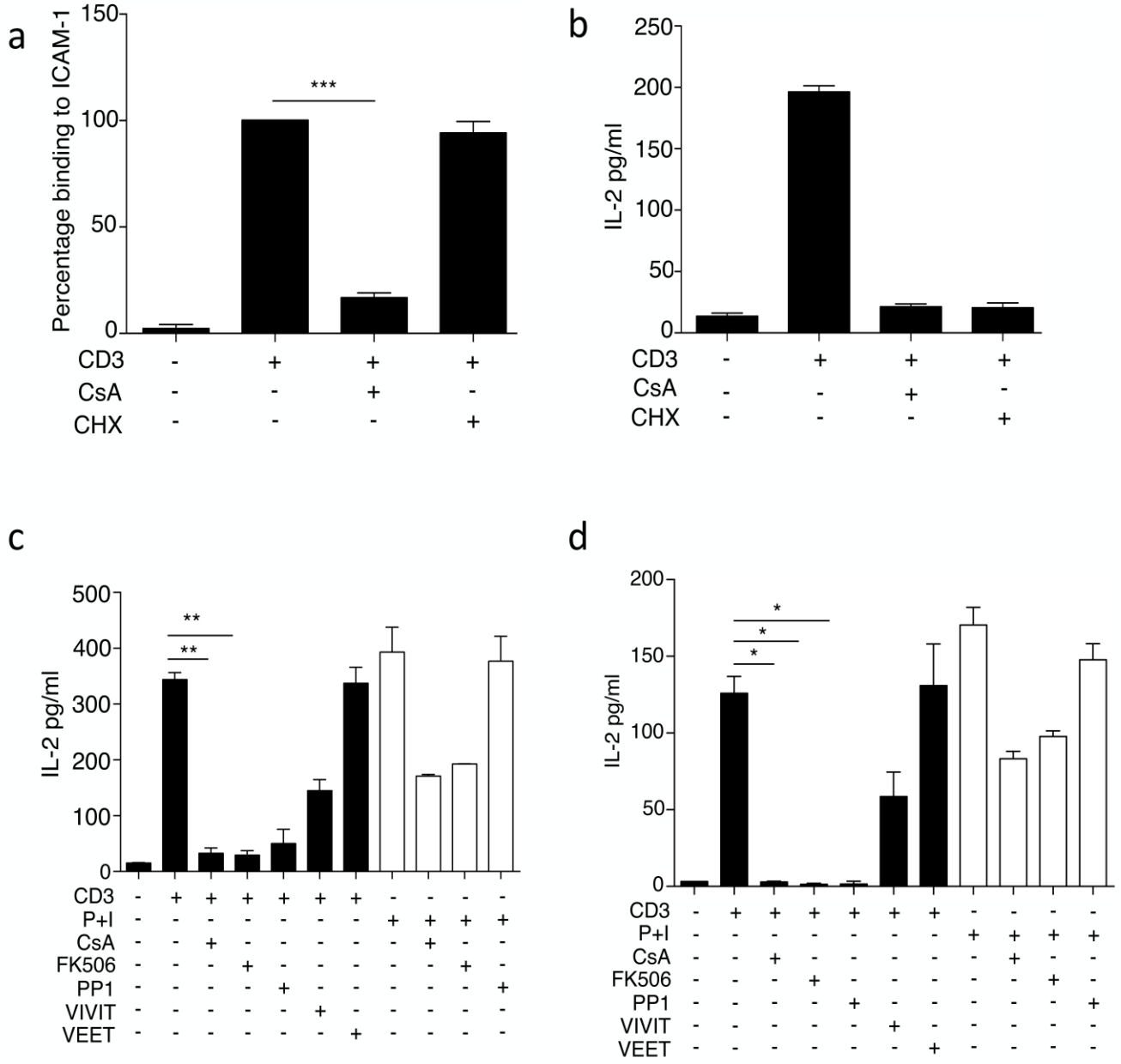


Supplementary Figure 2

Calcineurin dephosphorylates the inhibitory pLck^{S59}.

Immunoblot analysis of **(a)** Lymph node cells from AND TCR-transgenic mice activated with MCC-pulsed APC's (I-E^{k} expressing DCEK) for the indicated times (above lanes) in the presence of medium (-) and CsA or FK506 (+). Immunoprecipitation with anti-TCR-ζ and *in vitro* phosphatase assay of TCR complexes from Jurkat cells **(b)** activated with OKT3 for the different times (above lanes) in the presence of medium (-) or CsA (+), using human recombinant calcineurin (rhCN) for 30 min at 30°C. Western blot analysis of Lck negative JCam1.6 cells **(c)** retrovirally transduced with cDNA encoding Lck, Lck^{S59A} and Lck^{S59E}. Data are representative of 3 independent experiments. ELISA for IL-2 secretion after 12 h in Lck-negative JCam1.6 cells **(d)** stably expressing Lck, Lck^{S59A}, or Lck^{S59E} and activated with anti-CD3 + anti-CD28 or PMA + ionomycin (P+I). The data represent 2 independent experiments (mean ± SD).

Supplementary Figure 3



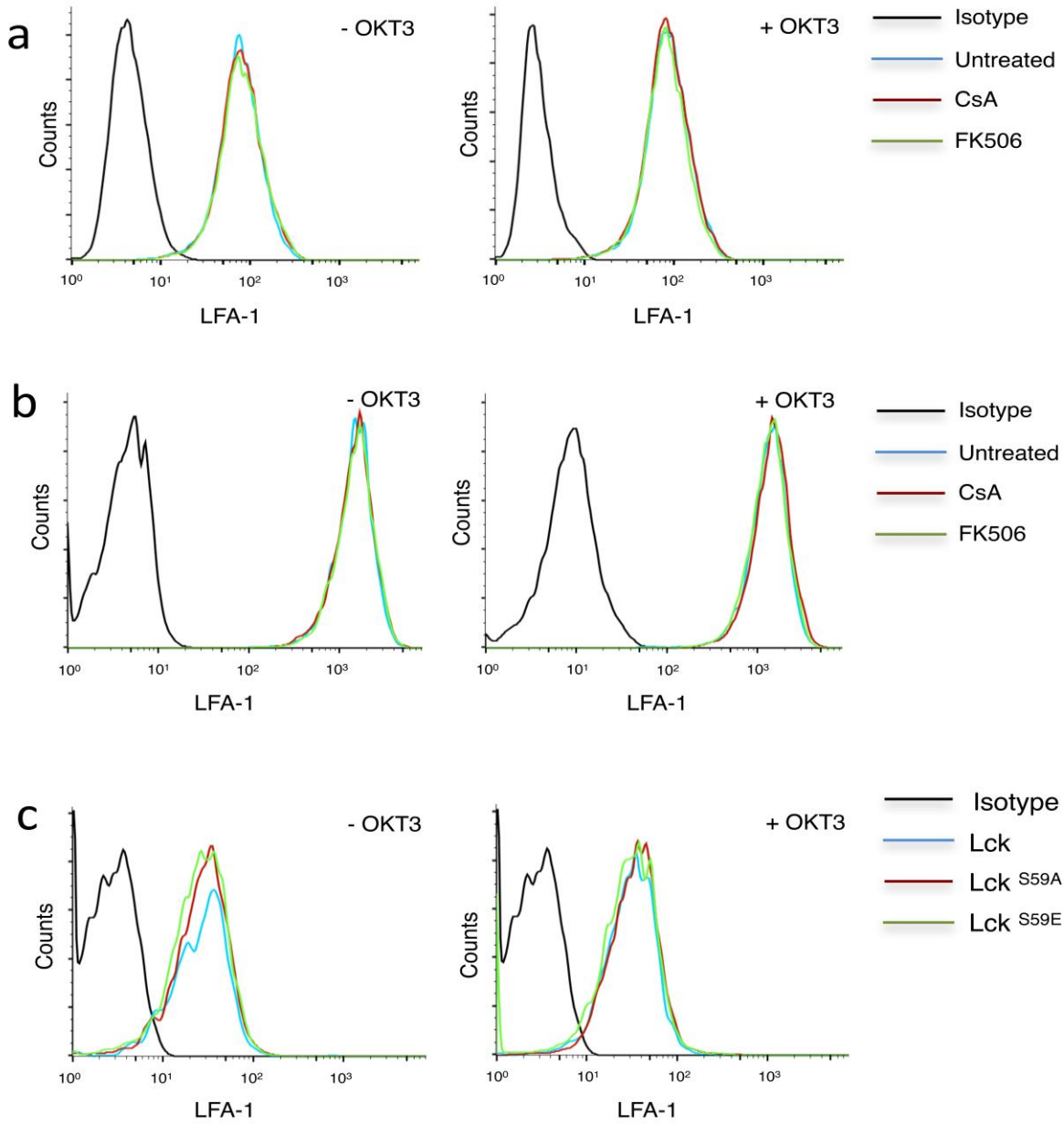
Supplementary Figure 3

Effect of inhibitors on cell adhesion and IL-2 secretion,

Cell quantification (trypan blue exclusion) of Jurkat cells **(a)** activated with OKT3 for 30 min and dropped on ICAM-1-coated wells in the presence of medium (-), CsA (+) or cyclohexamide (+) (bottom rows). Percentage binding calculated for each with respect to the activated sample, taken as 100%. The data are representative of 3 independent experiments (mean ± SEM; *** P value < 0.005; paired *t*-test). Cytokine analysis using ELISA in Jurkat cells **(b)** activated anti-CD3 and anti-CD28 for 8 h. The data are representative of 3 independent experiments (mean ± SEM). Analysis of IL-2 using

ELISA in Jurkat cells at 8 h **(c)** and human $CD4^+$ T cells at 16 h **(d)** activated with anti-TCR Ab or PMA+Ionomycin (P+I) in the presence of the drugs CsA, FK506, PP1, VIVIT and VEET (bottom lanes). The data represent the mean ± SD from 2 independent experiments (* P value < 0.05, ** P value < 0.01, paired *t*-test).

Supplementary Figure 4



Supplementary Figure 4

LFA-1 expression on cells treated with drugs.

Flow cytometry of **(a)** Jurkat, **(b)** human $CD4^+$ T cells and **(c)** Lck-negative J Cam1.6 cells expressing Lck, Lck^{S59A} and Lck^{S59E} left unactivated or activated with anti-CD3 for 30 min with mediun (-), FK506 or CsA (+). The black solid line represents isotype control.