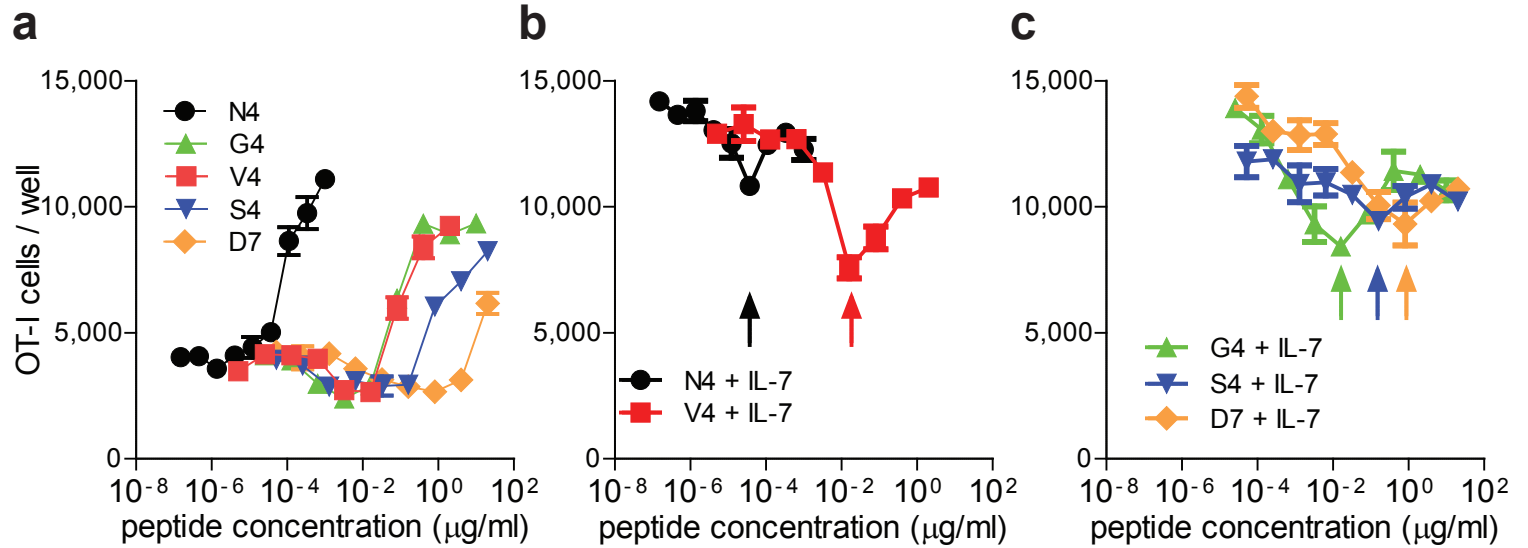
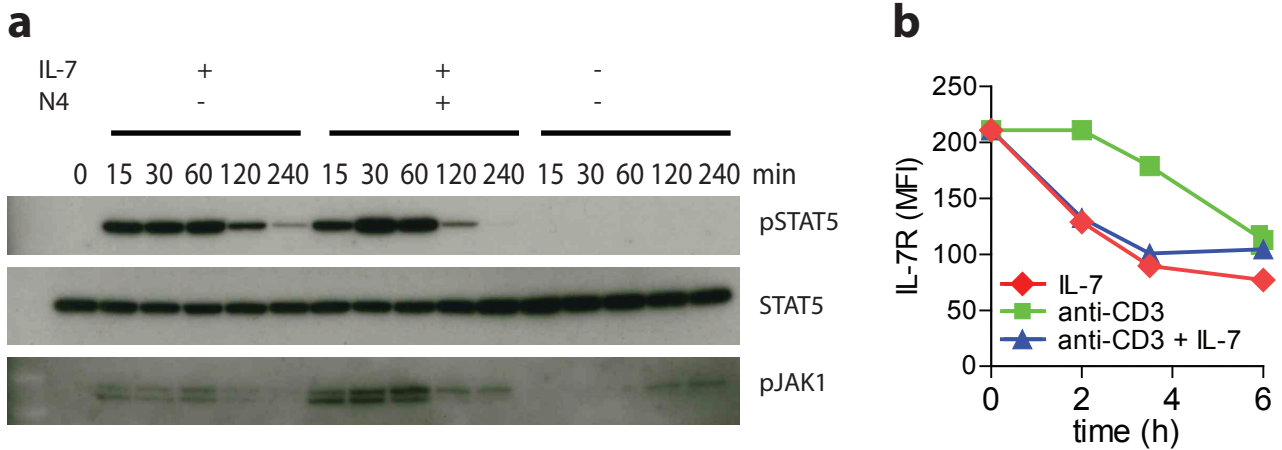


Supplementary Figure S1



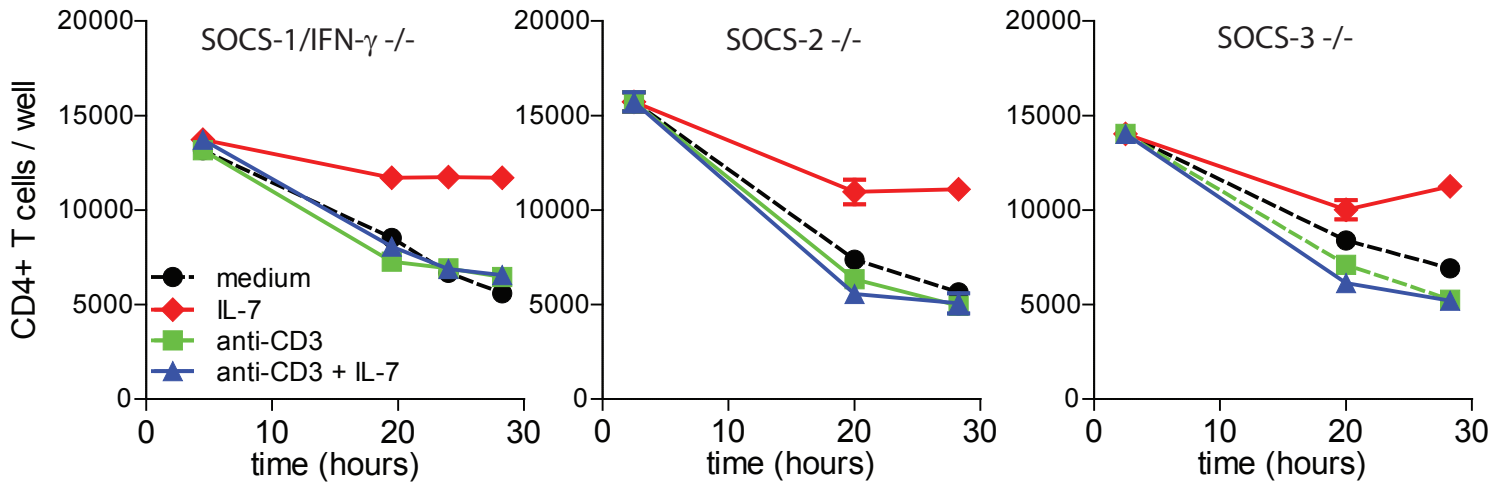
Supplementary Figure S1: Different affinity peptides reveal a relationship between stimulation strength and the switch in survival program. Total viable OT-I CD8⁺ T cells per well were measured 20 h after stimulation with peptide variants N4, V4, G4, S4, D7 (amino acid replacement at position four or seven of the wild type peptide SIINFEKL (N4)) at increasing concentrations in (a) medium or (b, c) with 1 ng/mL IL-7. Arrows indicate loss of survival in lower range of peptide efficacy. Data are presented as means ± s.e.m. of triplicate cultures.

Supplementary Figure S2



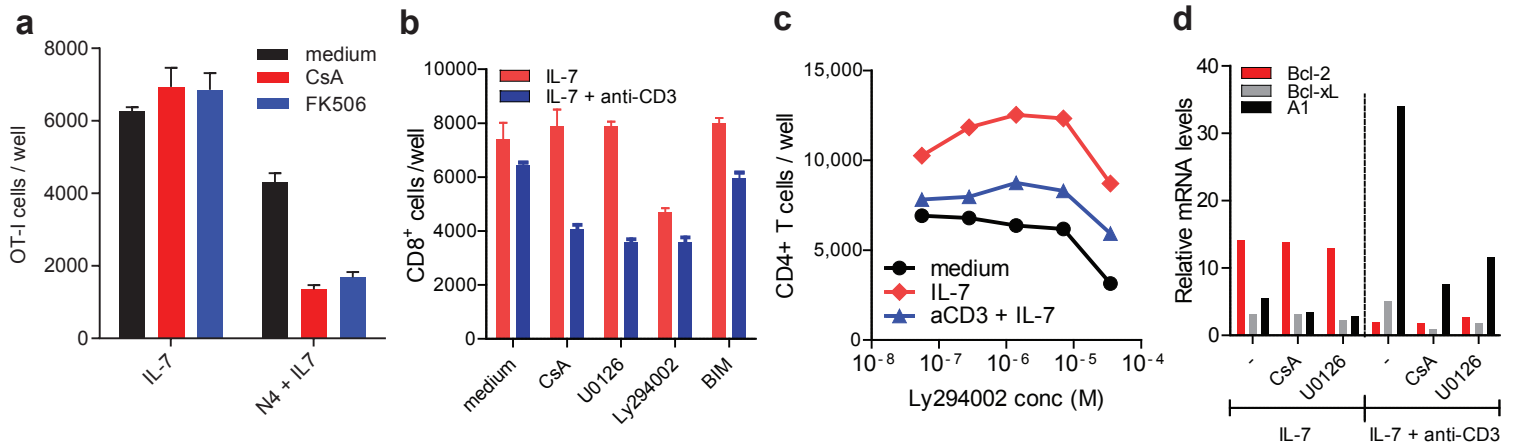
Supplementary Figure S2: TCR ligation inhibits phosphorylation of STAT5 but not Jak1 or IL-7R expression. (a) Western blot analysis of total STAT5 protein and phosphorylation of Tyr694/699 STAT5 and of Tyr1022/1023 Jak1 in OT-1 CD8⁺ T cell cultured for the indicated times in medium alone, with 10ng/mL IL-7 or with 10ng/mL IL-7 and 0.01 μ g/mL SIINFEKL peptide (N4). **(b)** Time course of IL-7R expression in purified CD4⁺ T cells from C57BL/6 mice after culture in 1ng/mL IL-7 or stimulation with plate bound anti-CD3 expressed as mean fluorescence intensity. Data are presented as means \pm s.e.m. of triplicate cultures.

Supplementary Figure S3



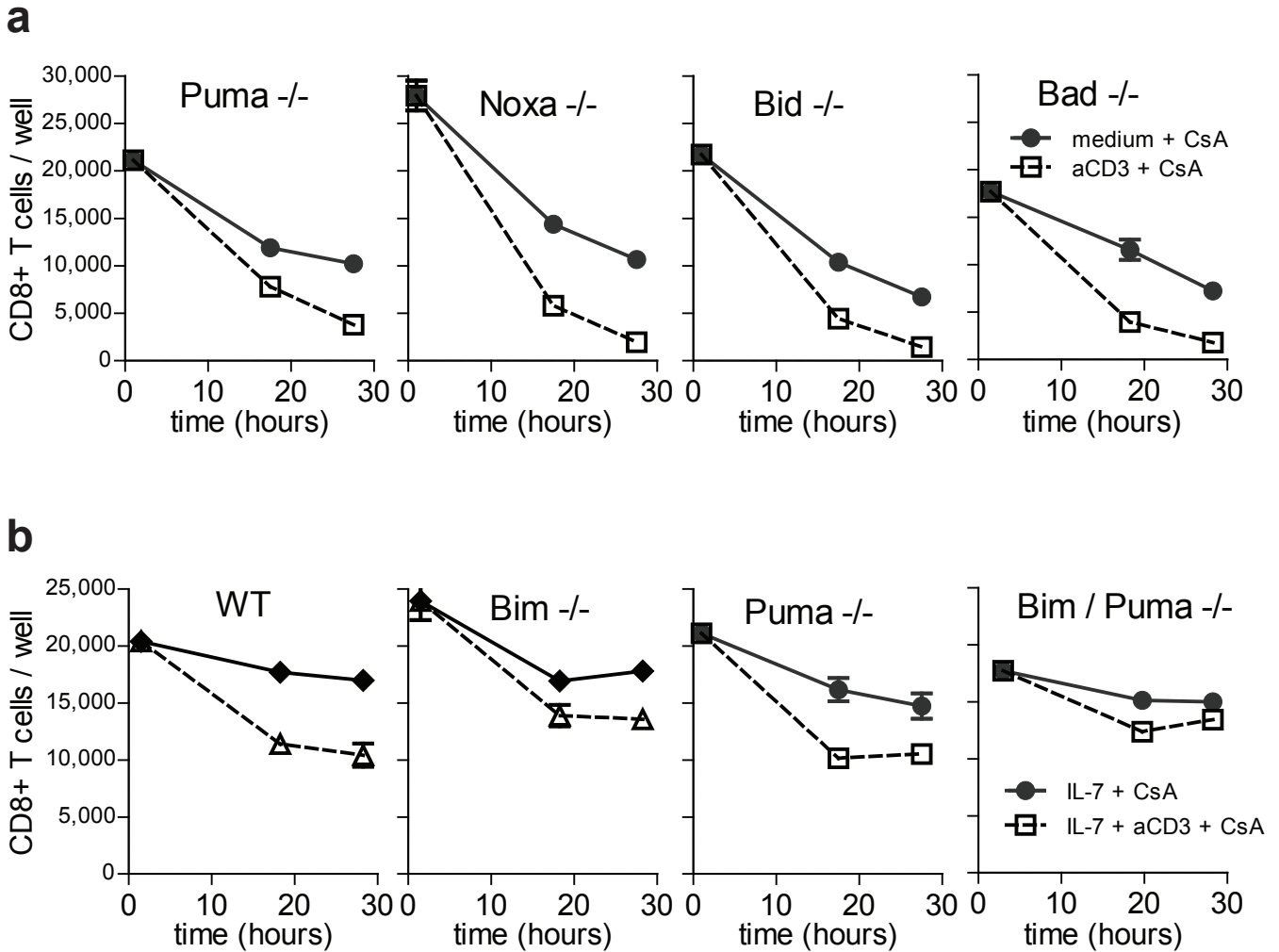
Supplementary Figure S3: Deficiency in SOCS proteins does not prevent inhibition of IL-7 mediated survival through TCR stimulation. Effect of loss of SOCS proteins on cell survival of CD4⁺ T cells after TCR ligation. CD4⁺ T cells from C57BL/6-SOCS-1/IFN γ , -SOCS-2 or -SOCS-3 knock out mice were cultured in medium or on CD3 antibody coated plates with or without 1ng/mL IL-7. Total viable cells were measured at indicated time points. Data are presented as means \pm s.e.m. of triplicate cultures.

Supplementary Figure S4



Supplementary Figure S4: Effect of inhibitors of calcineurin, PKC, PI3K or MEK1/2 on T cell survival before division. (a) CD8⁺ T cells from OT-I TCR tg mice were cultured for 20h with 10ng/mL IL-7 and without or without 0.1µg/mL N4 peptide. Calcineurin inhibitors CsA (1µg/mL) or FK506 (10ng/mL) were added at the start of the culture. Data are presented as means ± s.e.m. of triplicate cultures. (b) CD8⁺ T cells from C57BL/6 mice were cultured with 1ng/mL IL-7 without TCR ligation or on CD3 antibody coated plates in the presence of inhibitors of calcineurin (CsA, 0.5 µg/mL), MEK1/2 (U0126, 10 µM), PI3K (Ly294002, 50 µM) or PKC (Bisindolylmaleimide, (BIM) 0.1 µM). Total viable cells were measured at 18 h of culture. Data are presented as means ± s.e.m. of triplicate cultures. (c) Effect of PI3K inhibitor Ly294002 on survival of T cells. CD4⁺ T cells from C57BL/6 mice were cultured with 1ng/mL IL-7 without TCR ligation or on CD3 antibody coated plates in the presence of Ly294002 at increasing concentrations (56nM to 32µM). Total viable cells were measured at 28 h of culture. Data are presented as means ± s.e.m. of triplicate cultures. (d) Relative expression levels of *bcl-2*, *A1* and *bcl-xL* mRNA in CD8⁺ T cells measured by qPCR. CD8⁺ T cells from C57BL/6 mice were cultured for 6 h with 10ng/mL IL-7 without TCR ligation or on CD3 antibody coated plates in the presence or absence of CsA (2 µg/mL) or U0126 (25µM). Data are expressed as expression relative to freshly isolated uncultured cells.

Supplementary Figure S5



Supplementary Figure S5: Effect of loss of pro-apoptotic Bcl-2 proteins Puma, Noxa, Bid, Bad or Bim on the survival of T cells after TCR ligation. (a) CD8⁺ T cells from C57BL/6-Puma^{-/-}, Noxa^{-/-}, Bid^{-/-} or Bad^{-/-} mice were cultured in medium or on CD3 antibody coated plates in the presence of 1 μ g/mL CsA. Total viable cell were measured at indicated time points. (b) CD8⁺ T cells from C57BL/6-WT Bim^{-/-}, Puma^{-/-}, or Bim/Puma^{-/-} mice were cultured in medium with 1ng/mL IL-7 without TCR ligation or on CD3 antibody coated plates in the presence of 1 μ g/mL CsA. Total viable cell were measured at indicated time points. (a,b) Data are presented as means \pm s.e.m. of triplicate cultures.

Supplementary Table S1: Recovery of OT-I CD8⁺ T cells expressed as percentage of recovery from saline treated non immunised mice.

	saline	FK506	N4	N4 + FK506
Exp 1 12 h	100	106.0	22.7	9.0
Exp 1 17 h	100	69.6	18.2	6.5
Exp 2 19 h	100	90.1	12.4	4.1

CTV labelled OT-I CD8⁺ T cells were adoptively transferred into wild type C57BL/6 mice. A day later FK506 treated groups began a course of 2.5 mg/kg FK506 every 6 h. Peptide stimulated groups received 10 µg N4 1 h (**Exp 1**) or 2 h (**Exp 2**) after FK506 treatments had begun. OT-I cell were identified as CD8⁺, LY5.2⁺, Vα2⁺, CTV⁺.

Supplementary Table S2: Recovery of OT-I CD8⁺ T cells expressed as percent of OT-I CD8⁺ T cells to total CD8⁺ V α 2⁺ population.

	saline	FK506	N4	N4 + FK506
Exp 1 12 h	2.4	3.1	0.5	0.1
Exp 1 17 h	3.5	3.6	0.8	0.3
Exp 2 19 h	5.1	4.6	0.5	0.2

CTV labelled OT-I CD8⁺ T cells were adoptively transferred into wild type C57BL/6 mice. A day later FK506 treated groups began a course of 2.5 mg/kg FK506 every 6 h. Peptide stimulated groups received 10 μ g N4 1 h (**Exp 1**) or 2 h (**Exp 2**) after FK506 treatments had begun. OT-I cells were identified as CD8⁺, LY5.2⁺, V α 2⁺, CTV⁺.