

Supplementary Information

Trans-presentation of IL-15 modulates STAT5 activation and Bcl-6 expression in T_H1 cells

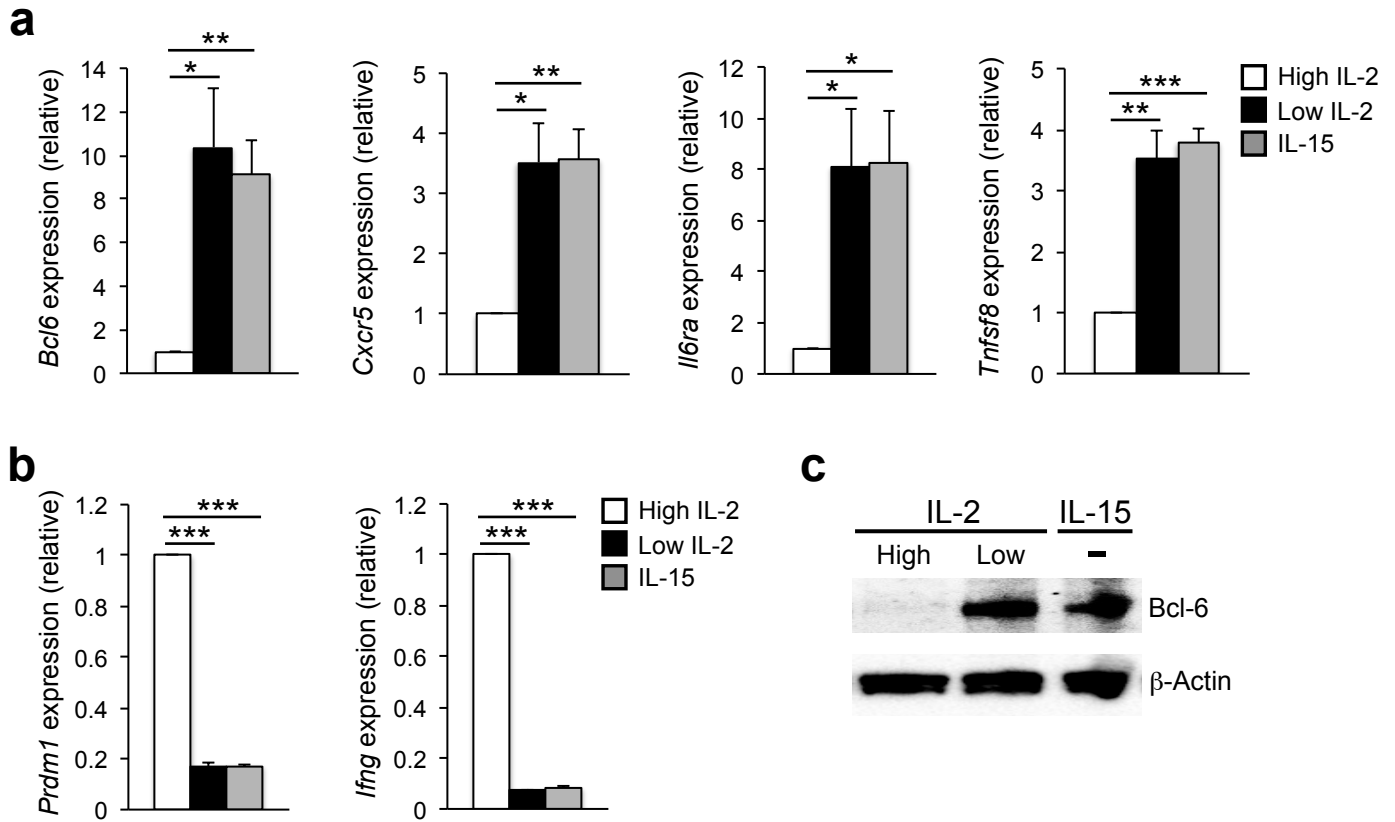
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Supplementary Figure S1

Supplementary Figure S2

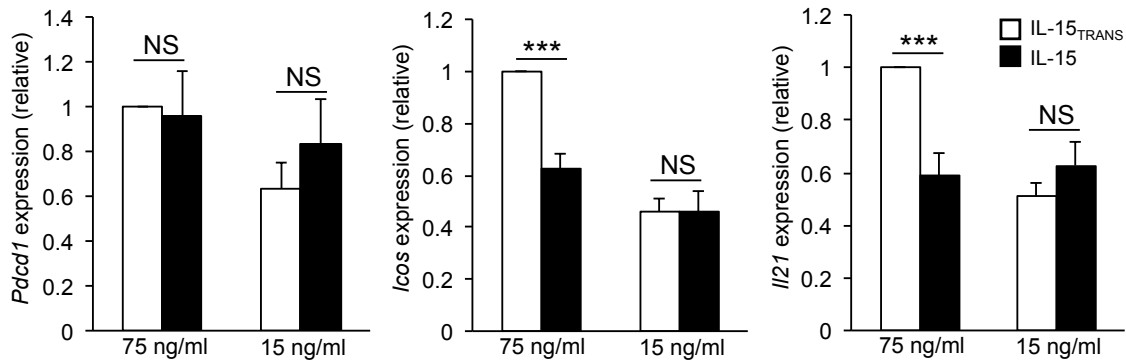
Supplementary Table S1

Supplementary Table S2



Supplementary Figure S1. T_H1 cells maintain flexibility with a T_{FH}-like profile after extended culture in T_H1 conditions.

Primary CD4⁺ T cells were isolated from C57BL/6 mice and stimulated on plate-bound α CD3 and α CD28 for 3 days under T_H1-polarizing conditions. Cells were then split and cultured for additional 2 days under T_H1-skewing conditions. On day 5, cells were split and cultured with either high IL-2 (250 U/ml), low IL-2 (10 U/ml), or IL-15 (15 ng/ml). (a,b) Two days following cytokine treatment, RNA was isolated to determine transcript levels for the indicated T_{FH} and T_H1-associated genes. Samples were normalized to *Rps18* as a control. Data are presented relative to the high IL-2-treated sample. Three independent experiments were performed with the error bars representing SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (unpaired Student's *t*-test). (c) An immunoblot analysis was performed to examine the effect of IL-2 and IL-15 signaling on Bcl-6 expression. β -Actin was monitored to ensure equal protein loading. For (c), the image is representative of three independent experiments.



Supplementary Figure 2. Expression of notable T_{FH} genes is independent of IL-15 signaling. Primary CD4⁺ T cells were cultured as described in Figure 2. RNA was isolated to determine transcript levels for *Pdcd1*, *Icos*, and *Il21*. Samples were normalized to *Rps18* as a control and the data are represented relative to the IL-15_{TRANS} (75 ng/ml) sample. Six independent experiments were performed with the error bars representing SEM. ****P* < 0.001 (unpaired Student's *t*-test).

Supplementary Table S1. Primer sequences

qRT-PCR primer sequences

Gene	Species	Sequence (5' to 3')
<i>Rps18</i>	mouse	fwd: GGAGAACTCACGGAGGATGAG rev: CGCAGCTTGTTGTCTAGACCG
<i>Bcl6</i>	mouse	fwd: CCAACCTGAAGACCCACACTC rev: GCGCAGATGGCTCTTCAGAGTC
<i>Cxcr5</i>	mouse	fwd: GTACCTAGCCATCGTCCATGC rev: GTGCACTGTGGTAAGGAGTCG
<i>IL6ra</i>	mouse	fwd: CCACATAGTGTCACTGTGCG rev: GGTATCGAAGCTGGAAGTGC
<i>Tnfsf8</i>	mouse	fwd: GCAGCTACTTCTACCTCAGCAC rev: GTGCCATCTTCGTTCCATGACAG
<i>Prdm1</i>	mouse	fwd: CTTGTGTGGTATTGTCCGGGAC rev: CACGCTGTA CTCTCTTTGG
<i>lfng</i>	mouse	fwd: CTACCTTCTTCAGCAACAGC rev: GCTCATTGAATGCTTGGCGC
<i>Sh2d1a</i>	mouse	fwd: CTGGATGGAAGCTATCTGCTG rev: CAGGTGCTGTCTCGGCACTCC
<i>IL12rb1</i>	mouse	fwd: CACGACTCGGCTCCTCATGGAC rev: TCTCAACGCAGCAGCCATCACC
<i>IL12rb2</i>	mouse	fwd: CTTGGACGGCATCAGTGTCTGC rev: GACCTGGTGAGGAGCCAGCAAC
<i>Tbx21</i>	mouse	fwd: GTCCAAGTTCAACCAGCACC rev: GTTGGTGAGCTTTAGCTTCC
<i>Pdcd1</i>	mouse	fwd: CGTCCCTCAGTCAAGAGGAG rev: GTCCCTAGAAGTGCCCAACA
<i>Icos</i>	mouse	fwd: CTCACCAAGACCAAGGGAAGC rev: CCACAACGAAAGCTGCACACC
<i>IL21</i>	mouse	fwd: TGGATCCTGAACTTCTATCAGCTCC rev: AGGCAGCCTCCTCCTGAGC

Supplementary Table S2. Antibodies

Primary antibodies

Antibody	Dilution/Concentration	Species	Clone	Catalog#	Source
β -actin:HRP	1:10,000 (WB)	mouse	2D1D10	A00730	Genscript
Bcl-6	1:500 (WB)	mouse	K112-91	561520	BD Biosciences
Blimp-1	1:500 (WB)	rabbit		A01647	Genscript
STAT5 (C-17)	1:5000 (WB)	rabbit		sc-835X	Santa Cruz
pSTAT5 (pY694)	1:5000 (WB)	mouse	47/Stat5(pY694)	611964	BD Biosciences
GAPDH (FL-335)	1:2500 (WB)	rabbit		sc-25778	Santa Cruz
STAT4 (C-20)	1:2500 (WB)	rabbit		sc-486	Santa Cruz
pSTAT4 (pTyr693)	1:1000 (WB)	rabbit		5267	Cell Signalling