

Supplemental Data

Signal Transducer and Activator of Transcription 4

Is Required for the Transcription Factor T-bet

to Promote T Helper 1 Cell Fate Determination

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Table S1

Transcription factor requirements for Th1 specific expression

Gene	Protein	Stat4-dependency	T-bet-dependency
<i>Ifng</i>	IFN γ	(Kaplan et al., 1996; Thierfelder et al., 1996)	(Szabo et al., 2002)
<i>Lta</i>	Lymphotoxin- α	This report	This report
<i>Xcl1</i>	Lymphotoxin	(Zhang et al., 2000)	This report
<i>Hlx1</i>	Hlx	This report	(Martins et al., 2005)
<i>Etv5</i>	ERM	(Ouyang et al., 1999)	This report
<i>Egr2</i>	Egr2	This report	This report
<i>Egr3</i>	Egr3	This report	This report
<i>Runx3</i>	Runx3	This report	(Djuretic et al., 2007)
<u><i>Twist1</i></u>	Twist1	(Niesner et al., 2008)	(Niesner et al., 2008)
<i>Il12rb2</i>	IL-12R β 2	(Lawless et al., 2000)	(Usui et al., 2006)
<i>Il18r1</i>	IL-18R α	(Lawless et al., 2000)	This report
<i>Il18rap</i>	IL-18R β	(Nakahira et al., 2001)	This report
<i>Ccr5</i>	CCR5	(Iwasaki et al., 2001)	(Lord et al., 2005) – this report
<i>Cxcr3</i>	CXCR3	(Nakajima et al., 2002)	(Lord et al., 2005)
<i>Fut7</i>	FucTVII	(White et al., 2001)	(Underhill et al., 2005)
<i>St3gal6</i>	ST3GalVI	(Underhill et al., 2005)	(Underhill et al., 2005)
<i>Gcnt1</i>	C2GlcNacT	(White et al., 2001)	(Underhill et al., 2005)
<u><i>Furin</i></u>	Furin	(Pesu et al., 2006)	This report

Gene-dependent on Stat4 and T-bet

Gene-dependent on T-bet

Gene-dependent on Stat4

Table S1 References

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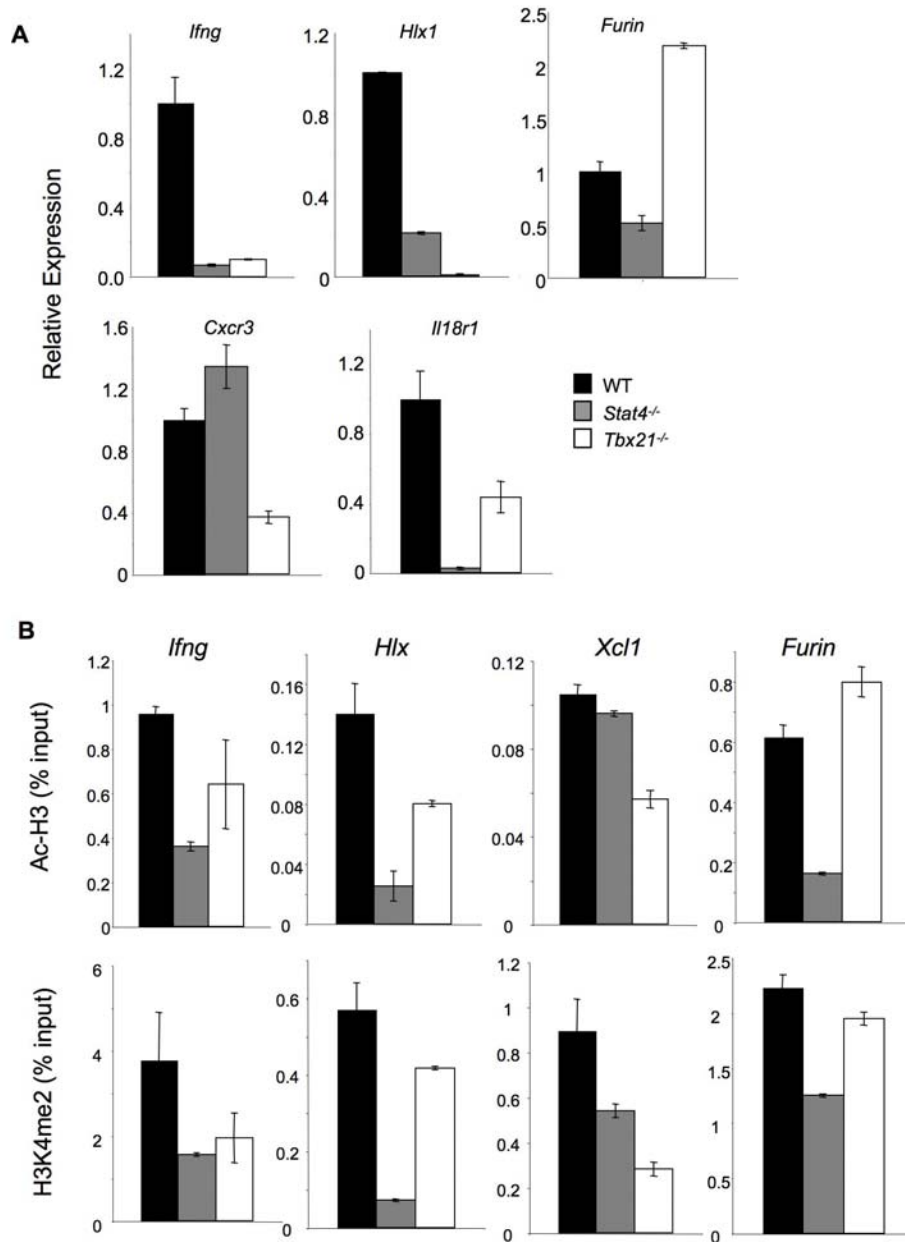
Table S2

Primers used for ChIP experiments

Locus	Primer sets
Ifng promoter (Yu et al, 2007)	ACCCCAAATGGTGTGAAGTAAAA CCCACCTGTGCCATTCTTGT TGCTTTCAGAGAATCC
Ifng +20 (Chang and Aune, 2007)	GGCCTGATTCACACCCA CCCAAGAAGAAGCAGAAATCAA
Ifng 40609 (Schoenborn et al, 2007)	AGCAATGCATTACAAAGACAGAGC GCATGATGTTCTAGAGTGGAGAGC
Xcl1 promoter	GAAGAATGTGGAGCAGACTCCCCAGGAG CATCATTGCAAAGACTTTCGGTGATTCC
Il4 promoter	CTTCAACCTAGCCCAGAACC GTAGGGTTGCCACTGGCTCT
Cd4 intron	AGCCACTGAACCACAAGGGTCGCTTAG ACACCCTCCTCTGTTGCCTAGCTTCG
Hlx1 promoter (CNS 3)	GGAATGACAGCTCCGAATAAAACT TCACCAGAGCTGCTCCAAGA ATTTGTGGTCTGCCCTCCTCCTGGC
Furin promoter (Yu et al, 2007)	GAAAGGCTGGCAGGAGAAGA TAGCCAGACCCCTGAAGGC TGTGCCTGGGTTGC
Il18r1 promoter (Yu et al, 2007)	TGAGATCCAGGCAGGAGAACTC AGAGCTCCACAGTTCCCAGAAC CTCTATCGCCTCAGCG

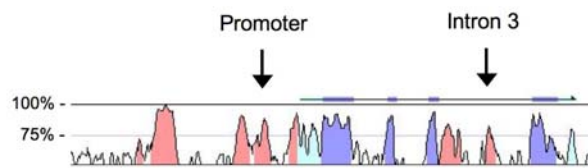
Where two primers are indicated, analysis was performed by SYBR green. Where three primers are indicated, analysis was performed as TaqMan assay (top primer-6FAM-labeled reporter, middle primer-reverse quencher, bottom primer-TaqMan probe). Primers used in previous reports are noted with the appropriate reference.

Figure S1



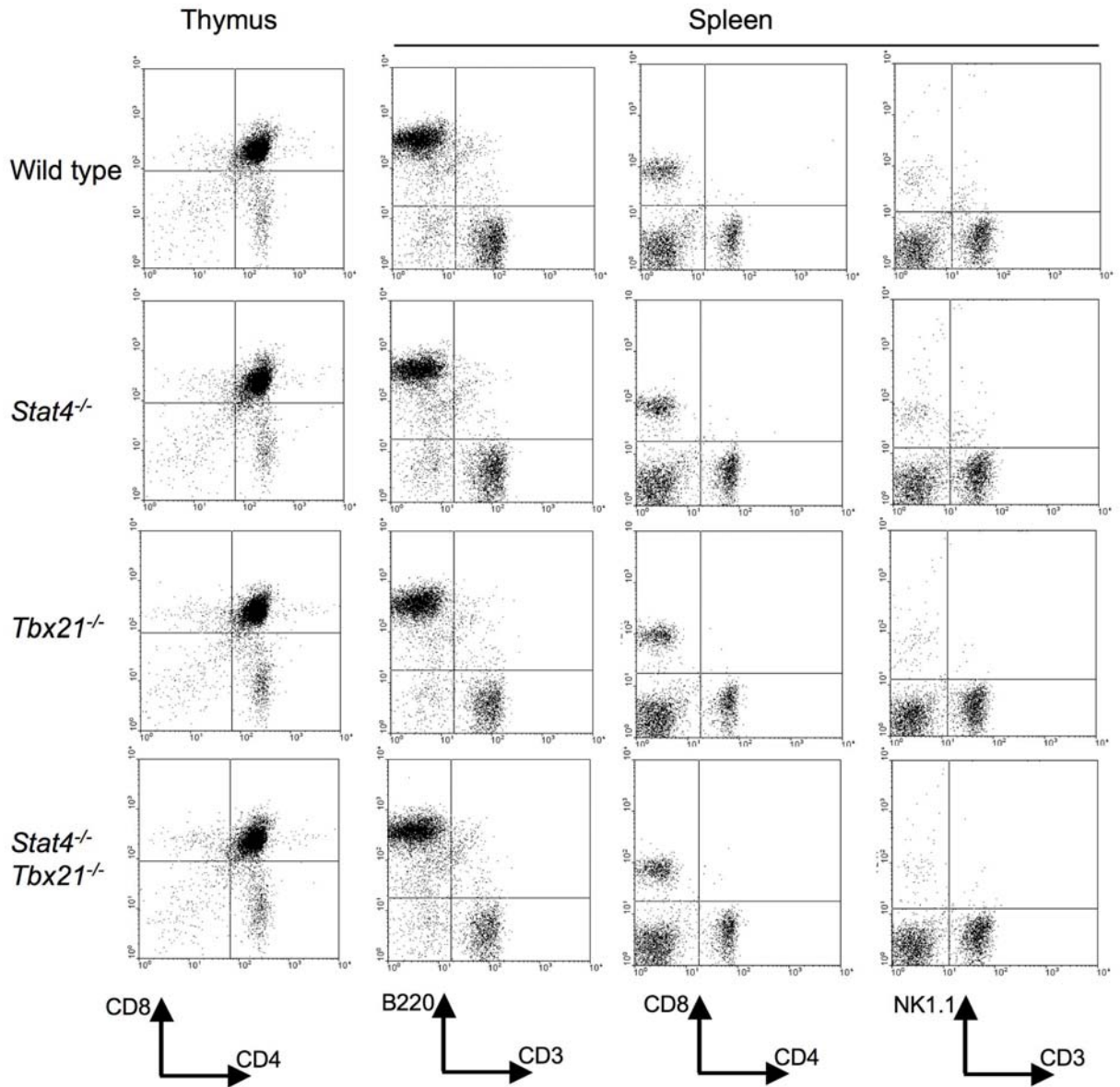
Supplementary Fig 1. Analysis of naïve cells. Naïve (CD4⁺ CD62L⁺) T cells from wild type, Stat4^{-/-} and T-bet-deficient mice were differentiated in Th1 priming conditions. A, Gene expression was analyzed as described in Figure 1. B, Chromatin modifications were analyzed as described in Figures 4 and 5.

Figure S2



Supplementary Fig 2. VISTA analysis of the Hlx1 gene. Purple regions are coding exons. Light blue regions are untranslated exons. Pink regions are conserved non-coding regions. Arrows indicate primers used in this study for ChIP analysis.

Figure S3



Supplementary Fig 3. Flow cytometric analysis of lymphocyte populations in *Stat4*^{-/-}, T-bet- and double-deficient mice. Single cell suspensions of the indicated tissues were stained with the indicated antibodies. All lymphocyte populations appear normal except the previously documented decrease in NK and NKT cells in mice lacking T-bet. This deficiency is not further affected by simultaneous deficiency in T-bet and *Stat4*.