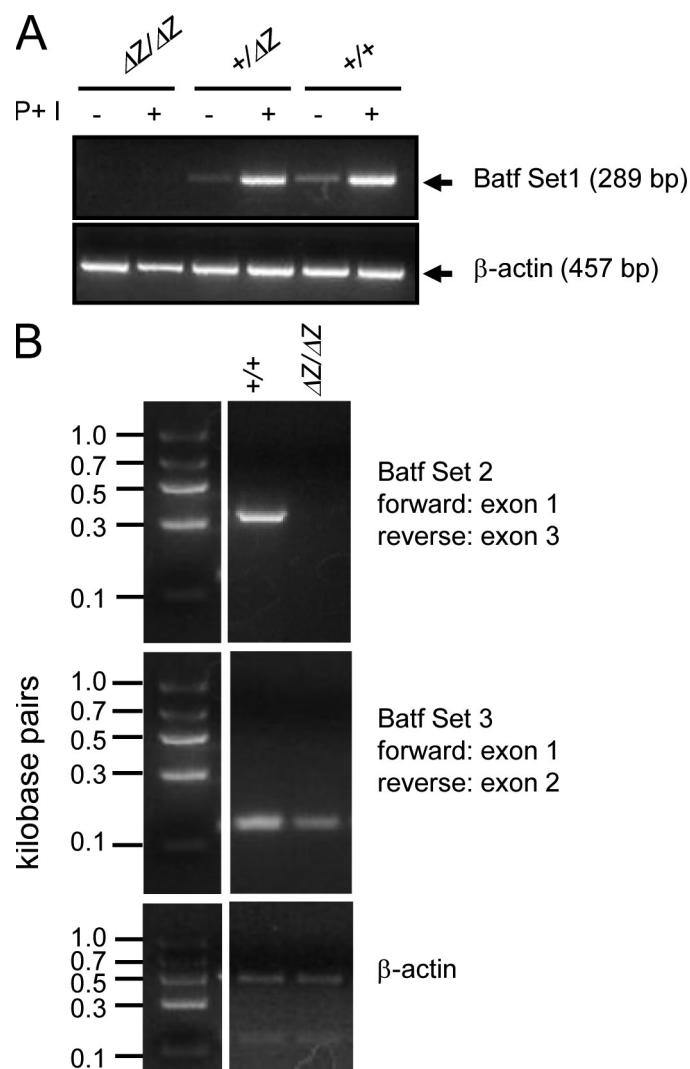
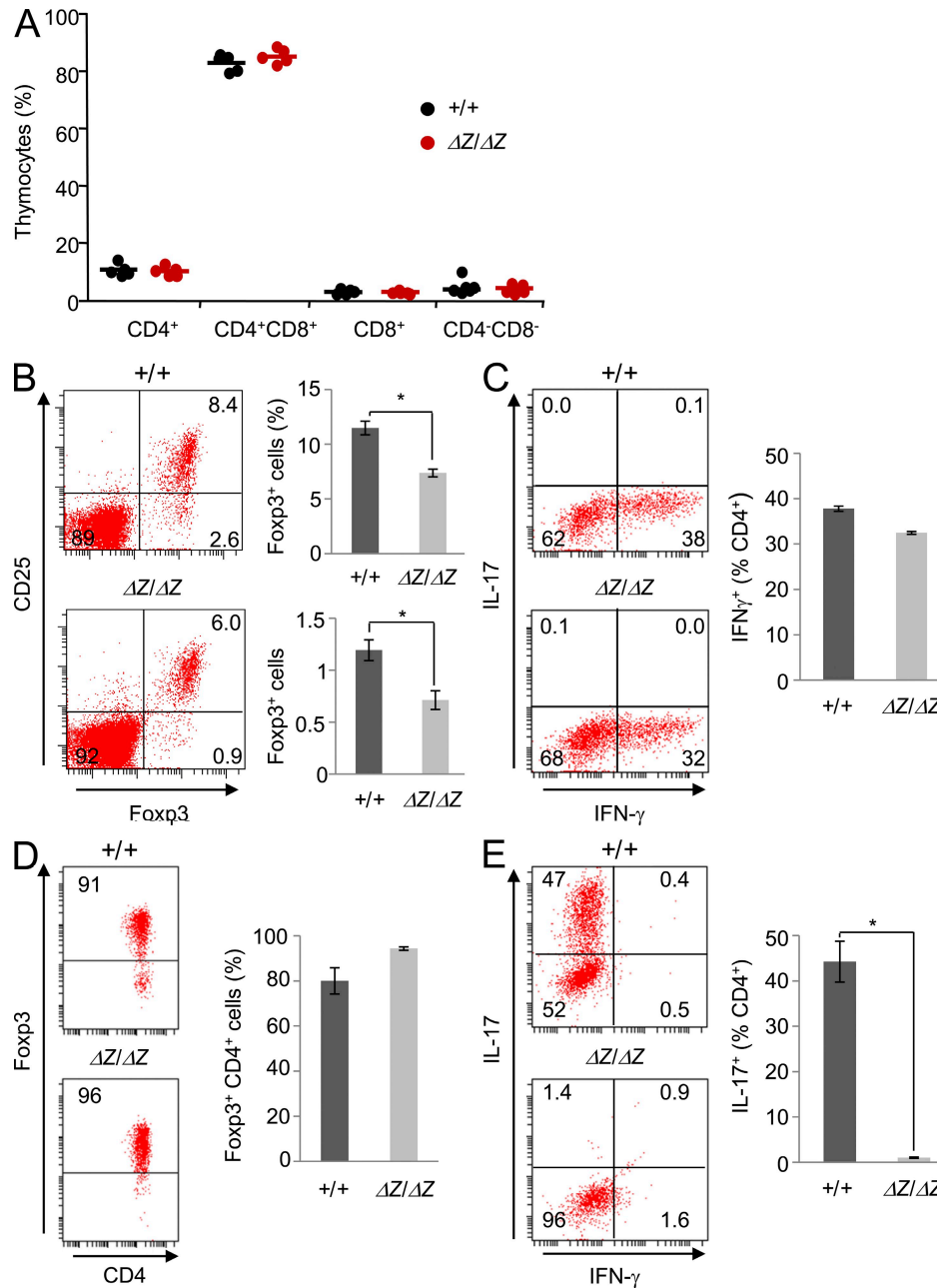


## SUPPLEMENTAL MATERIAL

Betz et al., <http://www.jem.org/cgi/content/full/jem.20091548/DC1>

**Figure S1. Analysis of *Batf* transcripts in *Batf*<sup>ΔZ/ΔZ</sup> mice.** (A) Splenocytes from *Batf*<sup>+/+</sup>, *Batf*<sup>+/ΔZ</sup>, and *Batf*<sup>ΔZ/ΔZ</sup> mice were stimulated for 6 h with 2.5 ng/ml PMA and 125 ng/ml ionomycin (P + I). RNA was isolated and converted to cDNA, and semi-qPCR was performed using *Batf* primer Set 1 (forward, 5'-GATGTGAGGAAAGTTCAGAG-3'; reverse, 5'-GTGGCGAGCTGATGTGAG-3') and *β-actin* primers as a control. A representative gel from more than five independent experiments is shown. (B) Semi-qPCR was performed on splenocyte RNA as in A using *Batf* primer Set 2 (forward, 5'-GCAGTGACTC-CAGCTTCAGC-3'; reverse, 5'-GTGGCGAGCTGATGTGAG-3'), primer Set 3 (forward, 5'-GTTCTGTTTCTCCAGGTCC-3'; reverse, 5'-GAAGGGTGTCGGCTTTCTG-3'), and *β-actin* primers as a control. Representative gels from three independent experiments are shown. M, DNA size marker in kbp.



**Figure S2. Quantifying thymic T cell subsets and peripheral CD4<sup>+</sup> T cell differentiation in *Batf*<sup>ΔZ/ΔZ</sup> mice.** (A) Thymocytes from *Batf*<sup>+/+</sup> and *Batf*<sup>ΔZ/ΔZ</sup> mice were analyzed for the indicated T cell subsets as previously described (Williams et al. 2001. *Eur. J. Immunol.* doi:10.1002/1521-4141(200105)31:5<1620::AID-IMMU1620>3.0.CO;2-3). Individual data from five sex-matched *Batf*<sup>+/+</sup> and *Batf*<sup>ΔZ/ΔZ</sup> littermates are plotted. Horizontal bars indicate mean ( $n = 5$ ). (B) Splenocytes from *Batf*<sup>+/+</sup> and *Batf*<sup>ΔZ/ΔZ</sup> mice were analyzed for Foxp3<sup>+</sup> expression in CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>-</sup> populations (T reg) as previously described (Lee et al. 2007. *J. Immunol.* 178:301–311). A representative plot is shown with data expressed as the percentage of Foxp3<sup>+</sup> cells (top) or as absolute number of T reg cells per spleen (bottom), averaged from three independent experiments ( $n = 3$ ). Error bars indicate SE. \*,  $P < 0.05$ . (C) Naive T cells from *Batf*<sup>ΔZ/ΔZ</sup> and *Batf*<sup>+/+</sup> spleens were cultured for 5–6 d with 5 μg/ml anti-CD3ε, 2 μg/ml anti-CD28, 20 U/ml rhIL-2, 2 ng/ml rmlL-12, 2 ng/ml rmlFN-γ, and 10 μg/ml anti-IL-4 mAb. Cells were stained with anti-IFN-γ and anti-IL-17A mAb and analyzed by flow cytometry. Representative plots are shown along with data averaged from five independent experiments ( $n = 5$ ). Error bars indicate SE. (D) Magnetic separation was used to isolate naive CD4<sup>+</sup>CD62L<sup>+</sup>CD25<sup>-</sup>CD44<sup>-</sup> T cells from *Batf*<sup>+/+</sup> and *Batf*<sup>ΔZ/ΔZ</sup> spleens.  $1 \times 10^5$  cells/well in 96-well plates were cultured for 6 d with 5 μg/ml anti-CD3ε, 2 μg/ml anti-CD28, 100 U/ml rhIL-2, and 5 ng/ml rhTGF-β1 (BioLegend) to induce T reg cells. Differentiation was assessed as in B. A representative plot is shown along with mean data expressed as the percentage of Foxp3<sup>+</sup> cells from four independent cell preparations per genotype ( $n = 4$ ). Error bars indicate SE. (E) Naive T cells from *Batf*<sup>ΔZ/ΔZ</sup> and *Batf*<sup>+/+</sup> spleens were cultured in Th17 conditions (Wang et al. 2009. *Mucosal Immunol.* doi:10.1038/mi.2008.84), stained, and analyzed as in C. Representative plots and mean data averaged from five independent experiments ( $n = 5$ ) are shown. Error bars indicate SE. \*,  $P < 0.05$ .

**Table S1.** qPCR primers

Transcript	Sequence of primers
Batf	Sense, 5'-GTTCTGTTTCTCCAGGTCC-3'; antisense, 5'-GAAGAATCGCATCGCTGC-3'
IL-17A	Sense, 5'-CTCCAGAAGGCCCTCAGACTAC-3'; antisense, 5'-AGCTTCCCTCCGATTGACACAG-3'
IL-21	Sense, 5'-ATCCTGAACCTCTATCAGCTCCAC-3'; antisense, 5'-GCATTAGCTATGTGCTTCTGTTTC-3'
T-bet	Sense, 5'-CAACAACCCCTTTGCCAAG-3'; antisense, 5'-TCCCCAAGCAGTTGACAGT-3'
IL-23R	Sense, 5'-GCCAAGAGAACCATTCCCGA-3'; antisense, 5'-TCAGTGCTACAATCTTCAGAGGAC-3'
Gata-3	Sense, 5'-AGAACCGGCCCTTATCAA-3'; antisense, 5'-AGTTCGCGCAGGATGTCC-3'
IL-4	Sense, 5'-AGATCATCGGCATTTTGAACG-3'; antisense, 5'-TTTGGCACATCCATCTCCG-3'
FoxP3	Sense, 5'-GGCCCTTCTCCAGGACAG-3'; antisense, 5'-GCTGATCATGGCTGGGTTGT-3'
Pax5	Sense, 5'-AGTCTCCAGTGCCGAATG-3'; antisense, 5'-TCCGTGGTGGTGAAGATG-3'
Bcl-6	Sense, 5'-TCGTGAGGTCGTGGAGAAC-3'; antisense, 5'-AGAGAAGAGGAAGGTGCTGAG-3'
Irf4	Sense, 5'-GGACTACAATCGTGAGGAGGAC-3'; antisense, 5'-ACGTACAGGACATTGATATGG-3'
Prdm1	Sense, 5'-AACACGTGGTACAACCCAAAG-3'; antisense, 5'-AGGCTGCAGAGATGGATGTAG-3'
Xbp1s	Sense, 5'-TGAGTCCGAGCAGGT-3'; antisense, 5'-AGACTCTGGGGAAGGACATT-3'
Aicda	Sense, 5'-TGCTACGTGGTGAAGAGGAG-3'; antisense, 5'-TCCCAGTCTGAGATGTAGCG-3'
β-actin	Qiagen QT00095242
Hprt	Sense, 5'-CTCCTCAGACCGCTTTTGC-3'; antisense, 5'-GAGGGTAGGCTGGCCTATAGGCT-3'

The sequences of oligonucleotide primers used for qPCR analysis of the indicated transcripts in T and B cell RNA from *Batf*<sup>+/+</sup> and *Batf*<sup>Δ2/Δ2</sup> mice are listed.

**Table S2.** Antibodies.

Ab	Clone number	Application	Source
Anti-mouse CD4	GK1.5	Flow cytometry	BD
Anti-mouse CD19	6D5	Flow cytometry	BioLegend
Anti-mouse CD8	53-6.7	Flow cytometry	Miltenyi Biotec
Anti-mouse IFN-γ	XMG1.2	Flow cytometry	BioLegend
Anti-mouse IL-4	11B11	Flow cytometry	BioLegend
Anti-mouse IL-17A	TC11-18H10.1	Flow cytometry	BioLegend
Anti-mouse IgM	II/41	Flow cytometry	eBioscience
Anti-mouse IgG1	A85-1	Flow cytometry	BD
Anti-mouse IgE	R35-72	Flow cytometry	BD
Anti-mouse B220	RA3-6B2	Flow cytometry	eBioscience
Anti-mouse CD62L	MEL-14	Flow cytometry	BioLegend
Anti-mouse CXCR5	2G8	Flow cytometry	BD
Anti-mouse CD44	IM7	Flow cytometry	BioLegend
Purified anti-mouse IgM	II/41	ELISA	BD
Biotin anti-mouse IgM	II/41	ELISA	BD
Purified anti-mouse IgG1	A85-3	ELISA	BD
Biotin anti-mouse IgG1	A85-1	ELISA	BD
Purified anti-mouse IgA	C10-1	ELISA	BD
Biotin anti-mouse IgA	C10-1	ELISA	BD
Rat anti-mouse B220	RA3-6B2	IHC	eBioscience
FITC rabbit anti-rat IgG	FI-4000	IHC	Vector Laboratories
Biotin-PNA	B-1075	IHC	Vector Laboratories
Texas Red-avidin	A-2006	IHC	Vector Laboratories
Anti-mouse CD25	PC61.5	Flow Cytometry	eBioscience
Anti-mouse FoxP3	FJK-16s	Flow Cytometry	eBioscience
Goat anti-mouse IgG1	1070-01	IHC, ELISA	SouthernBiotech
Goat anti-mouse IgG2c	1079-01	IHC	SouthernBiotech
Biotin rabbit anti-goat IgG	BA-5000	IHC	Vector

Antibodies used in this study are listed together with clone numbers, commercial source, and experimental application.