Online Methods

Generation of *Batf*$^{-/-}$ mice. Murine *Batf* exons 1–2 were deleted by homologous recombination via a targeting vector constructed in pLNTK[31] using a 1 kb genomic fragment (left arm) upstream of the *Batf* exon 1 and a 3.6 kb genomic fragment (right arm) downstream of exon 2. The left arm was generated by PCR from genomic DNA with the use of the following oligonucleotides: left arm forward (5'-ATTA**CTCGAG**TGAAACAAACAGGCAGTCGCAGTG) and left arm reverse (5'-ATTA**CTCGAG**CCTACTACCTTTCAGGGCTACTGC). The right arm was generated by PCR with the use of the following oligonucleotides: right arm forward (5'-ATTA**GTCGAC**GCATTCTTCATGGTCCTTAGCCTTGG) and right arm reverse (5'-ATTA**GTCGAC**CAGAGAATGAGAAATGTTGGAGG). EDJ22 embryonic stem cells were transfected with linearized targeting vector and targeted clones were identified by Southern blot analysis using probes A and B located 5' to the left arm and 3' to the right arm respectively. Probe A was generated using the oligonucleotides 5'-CAACTGGGTCTGAGTCAAGAGGT and 5'-CGTAGCCGCTGATTGTTTTAGAAC to generate a 531bp product. Probe B was generated using the oligonucleotides 5'-ACAGCTTGAACTTCAGAGCCCTCC and 5'-CACATTTAAGTCACAATAACACTGC to generate a 772bp product. The neomycin resistance cassette was deleted from successfully targeted clones by *in vitro* treatment with Adeno-Cre virus (gift from Dr. Barry Sleckman, Washington University, St. Louis, MO) and targeted clones with successful neo deletion were identified by Southern blot using probes A and B (Supplementary Fig. 2a and b). Blastocyst injections were performed with two distinct recombinant clones each of which generated germline

transmission of the targeted *Batf* allele. Male chimeras were crossed with 129SvEv females to establish *Batf* mutants on the pure 129SvEv genetic background. All experiments were performed with mice harboring the neo-deleted mutant allele. Homozygous mice were obtained by intercrossing heterozygous siblings and littermates were used as controls in most experiments. For some experiments 129SvEv wild type mice purchased from Taconic served as controls. For experiments with DO11.10 transgenic Batf$^{-/-}$ mice, mice were crossed to BALB/c mice for at least 5 generations and littermates were used as control.

For the generation of transgenic mice, *Batf* cDNA was cloned from CD4^{+} T cell mRNA using primers 5'-GGAAGATTAGAACCATGCCTC and 5'-AGAAGGTCAGGGCTGGAAG and subcloned into the GFP-RV retrovirus[32]. An N-terminal FLAG tag was introduced by Quick Change Mutagenesis kit (Stratagene) using the primers 5'-

GGACTACAAAGACGATGACGACAAGCCTCACAGCTCCGACAGCA and 5'-CTTGTCGTCATCGTCTTTGTAGTCCATGGTTCTAATCTTCCAGATC. The underlined sequence indicates nucleotides used to introduce the FLAG-tag. The FLAG-tagged *Batf* was cloned into the CD2 microinjection cassette[33] via blunt end strategy into Sma1 digested CD2 microinjection cassette. Transgene expression in CD4^{+} T cells was tested by anti-FLAG western blot. CD2-N-FLAG-*Batf* transgenic mice were crossed to C57BL/6 and BALB/c mice for at least 5 generations. Transgene-negative littermates were used as control mice. Mice were bred and maintained at the animal facilities at Washington University in St. Louis. All animal experiments were approved by the Animal Studies Committee at Washington University.

Visualization of lymph nodes. To visualize superficial inguinal lymph nodes mice were injected with 50μl of 1% Evans Blue dye solution into each hind foot pad. After 1.5 hours mice were sacrificed and lymph nodes were visualized using a dissecting microscope[34].

Western Blot analysis. To test for residual Batf protein expression, total splenocytes from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ 129SvEv mice were stimulated with anti-CD3 for 3 days under T_H17 conditions. Cells were then lysed in RIPA buffer, electrophoresed on 15% polyacrylamide gels, transferred to nitrocellulose and analyzed by Western Blot with rabbit anti-murine Batf polyclonal serum and HRP-conjugated anti-rabbit Ig antibody (Jackson ImmunoResearch). Affinity purified rabbit anti-murine Batf polyclonal serum (Brookwood Biomedical; Birmingham, AL) was generated by immunization with full length recombinant Batf protein. Equal protein loading was assessed by subsequent immunoblotting with antibody to β-actin (Santa Cruz Biotechnology) and HRP conjugated anti-mouse antibody (Jackson ImmunoResearch).

For analysis of Batf protein expression in naïve $CD4^+$ T cells, magnetically purified $CD4^+$ T cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ 129SvEv mice were isolated. Equal cell numbers were lysed in RIPA buffer and subjected to Western Blot analysis as described above.

For analysis of Batf expression in T_H2 cells, magnetically purified $CD4^+$ T cells from Batf$^{+/+}$ and Batf$^{-/-}$ mice were activated with anti-CD3/CD28 in the presence of IL-4, anti-IL-12 (Tosh), and anti-IFN-γ (H22). On day 4 cells were left unstimulated or stimulated with PMA/ionomycin for 4 hrs. Cells were collected by centrifugation, washed with PBS, and resuspended (100e^6 cells/ml) in Affymetrix Chip lysis buffer (10mM Tris pH 7.5, 10mM NaCl, 3mM $MgCl_2$, 0.5% IGEPAL, with protease inhibitors (PMSF,

aprotinin, leupeptin)). After 5 min at 4°C, nuclei were collected by centrifugation (800 rcf for 3 min 4°C) and lysed in RIPA ($100e^6$ cell equivalents/ml) with protease inhibitors. Nuclear lysates were centrifuged for 10 min 4°C 15000 rcf, diluted with an equal volume of 2x SDS-PAGE sample buffer containing 2-ME and extracts from equal cell numbers were subjected to Western Blot analysis using rabbit anti-murine Batf polyclonal serum. Equal protein loading was assessed by subsequent immunoblotting with antibody to Lamin B (Santa Cruz Biotechnology) and HRP conjugated anti-goat Ig (Jackson ImmunoResearch).

Immunohistochemistry. $CD4^+$ T cells from CD2-N-FLAG-*Batf* transgenic mice were isolated by magnetic separation and either left untreated or stimulated with PMA/ionomycin for 4h. Cells were then allowed to settle on poly-L-lysine treated slides, fixed with 4% Formaldehyde, permeabilized with 0.25% TritonX100 and were stained with an anti-FLAG antibody (M2, Sigma Aldrich) according to the manufacturer's recommendations. A goat anti-mouse AF-488 (Invitrogen) antibody was to detect anti-FLAG staining. For analysis of cellular localization of Batf in T_H2 cells, DO11.10 $CD4^+$ T cells from CD2-N-FLAG-*Batf* transgenic mice were isolated and differentiated with OVA and APC under T_H2 conditions for 7 days. On day 7 cells were either left untreated or stimulated with PMA/ionomycin for 4h. Cells were stained with anti-FLAG antibody as described above. Cells were also stained with anti-CD4APC antibody (BDBiosciences). Confocal images were obtained with the Olympus FV1000 microscope and software using a 60x oil objective. The pinhole was set to 110μm. The excitation/emission settings used for DAPI, Alexa 488 and Alexa 633 were 405/461nm, 488/520nm and 635/668nm respectively.

Additional methods can be found in the Supplementary Information.

Methods Reference List

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