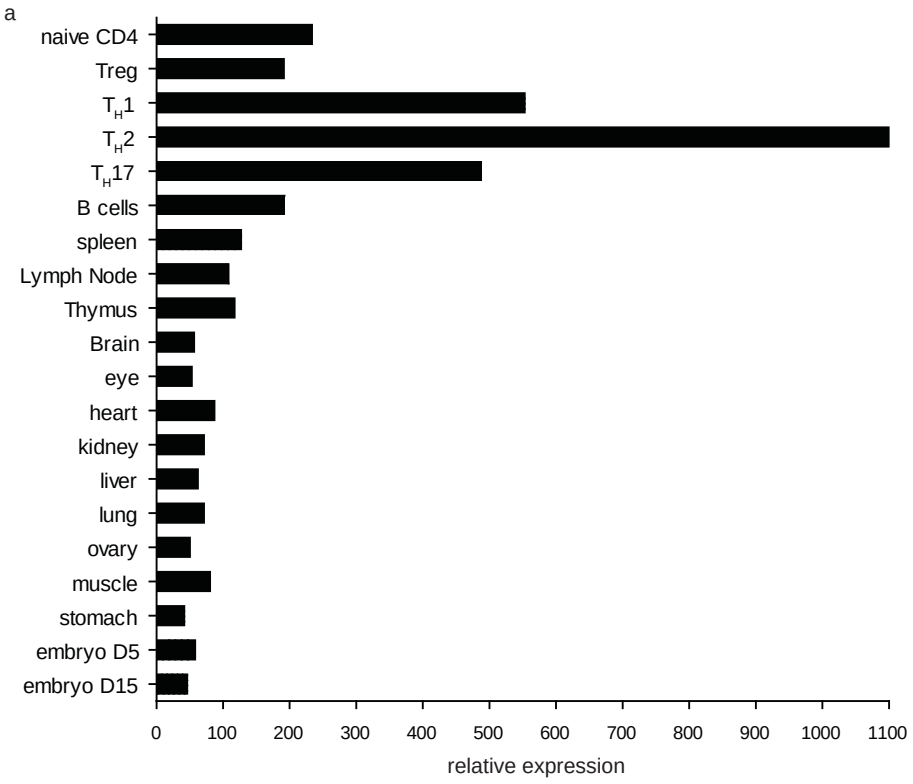
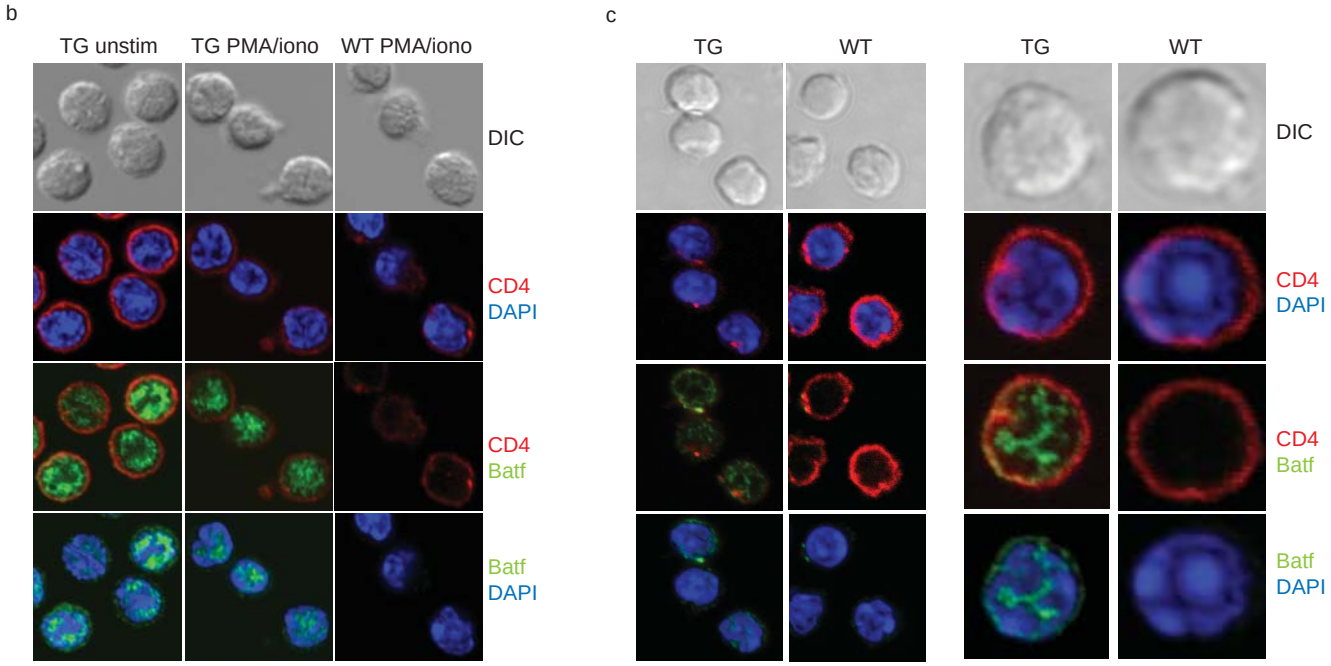
Supplementary Figure 1



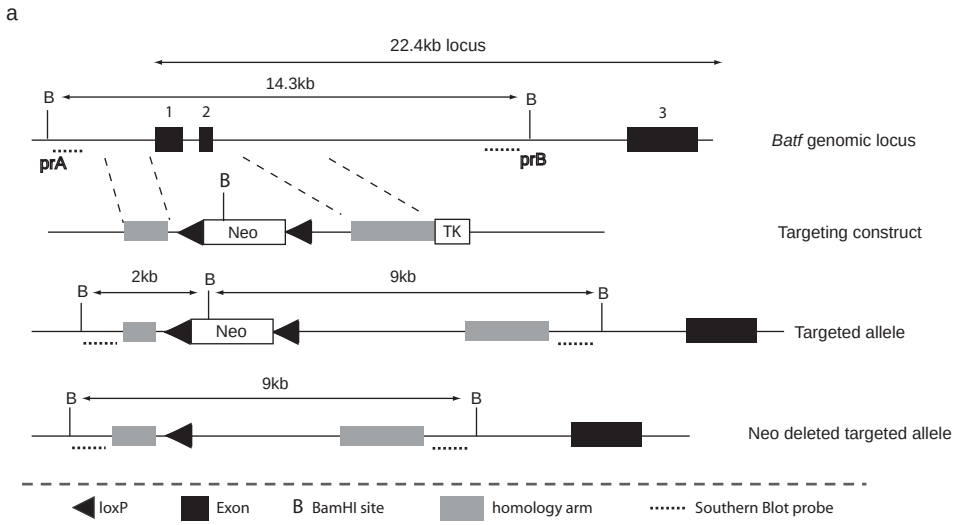


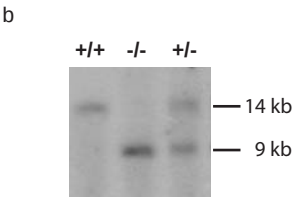
Supplementary Figure 1

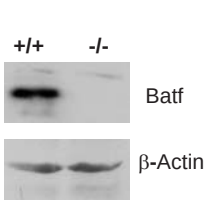
Expression and cellular location of Batf in T cells

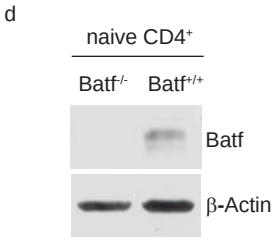
a, The expression profile of *Batf* among the indicated tissues was determined by Affymetrix gene microarray. The data are presented in arbitrary units and reflect normalized and modeled expression values generated using DNA-Chip analyzer (dChip) software. **b, c,** Batf is located in the cytoplasm and nucleus of resting T cells. **b,** DO11.10 $CD4^+$ T cells from CD2-N-FLAG-*Batf* transgenic or littermate control mice were isolated and differentiated with OVA and APCs under T_H2 conditions. On day 7 cells were either left untreated or stimulated with PMA/ionomycin for 4h. Cells were then allowed to settle on to poly-L-lysine treated slides and stained with an anti-FLAG antibody, anti-CD4 antibody and DAPI as a nuclear stain as described in Methods. **c,** Naïve DO11.10 $CD4^+$ T cells from CD2-N-FLAG-*Batf* transgenic or littermate control mice were isolated and stained as in **c**. Data are representative of 2 independent experiments.

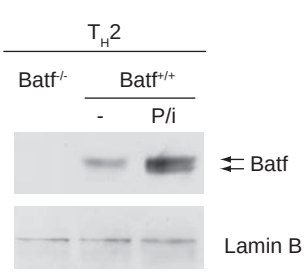
Supplementary Figure 2











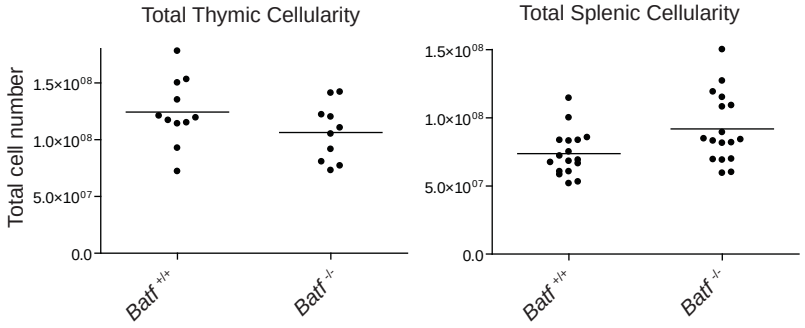
Supplementary Figure 2

Targeting of the *Batf* locus by homologous recombination.

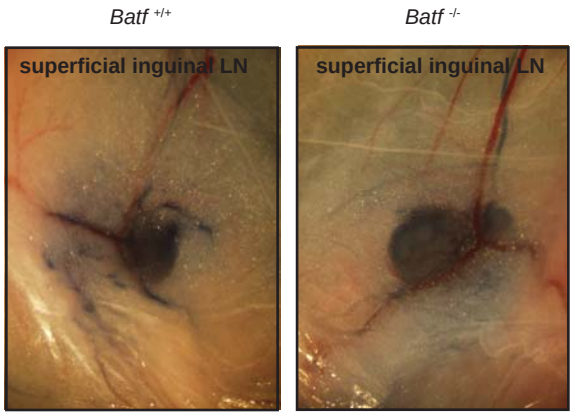
a, The endogenous genomic *Batf* locus, targeting construct and the mutant allele before and after cre-mediated deletion of the neomycin cassette are shown. Restriction enzyme digestion with BamHI of the genomic locus results in a 14.3kb wild type fragment that is detected by Southern Blot probes A and B; in the targeted allele, probe A detects a 2kb and probe B detects a 9kb fragment. In the neomycin-deleted targeted allele, BamHI digestion results in a 9kb fragment that is detected by both the 5' and 3' Southern Blot probes. The neomycin resistance cassette was deleted by in vitro treatment with a cre-expressing Adenovirus. **b,** Southern Blot analysis of targeted *Batf* alleles. Probe A was used to hybridize BamHI digested genomic DNA from the indicated genotypes resulting from $Batf^{+/-}$ intercrosses. **c,** No residual protein expression in $Batf^{-/-}$ mice. Equal cell numbers from total splenocytes activated under T_H17 conditions for 3 days were lysed in RIPA buffer and analyzed by Western Blot using anti-Batf antibody. The blots were stripped and reblotted with an antibody to β-actin to show equal protein loading. **d,** Batf expression in naïve T cells. Magnetically purified $Batf^{+/+}$ and $Batf^{-/-}$ $CD4^+$ T cells were lysed in RIPA buffer. $1.5x10^6$ cell equivalents were subjected to Western Blot analysis. Blots were stripped and reprobed with anti-β-actin to show equal protein loading. **e,** $CD4^+$ T cells from $Batf^{+/+}$ and $Batf^{-/-}$ mice were stimulated with anti-CD3/CD28 under T_H2 conditions for 4 days, left untreated or restimulated with PMA/ionomycin for 4h. Nuclear extracts from $0.5x10^6$ cell equivalents were analyzed for Batf expression by Western Blot. The blots were stripped and reprobed with anti-Lamin B antibody to show equal protein loading. Data are representative of 2 independent experiments.

Supplementary Figure 3

a



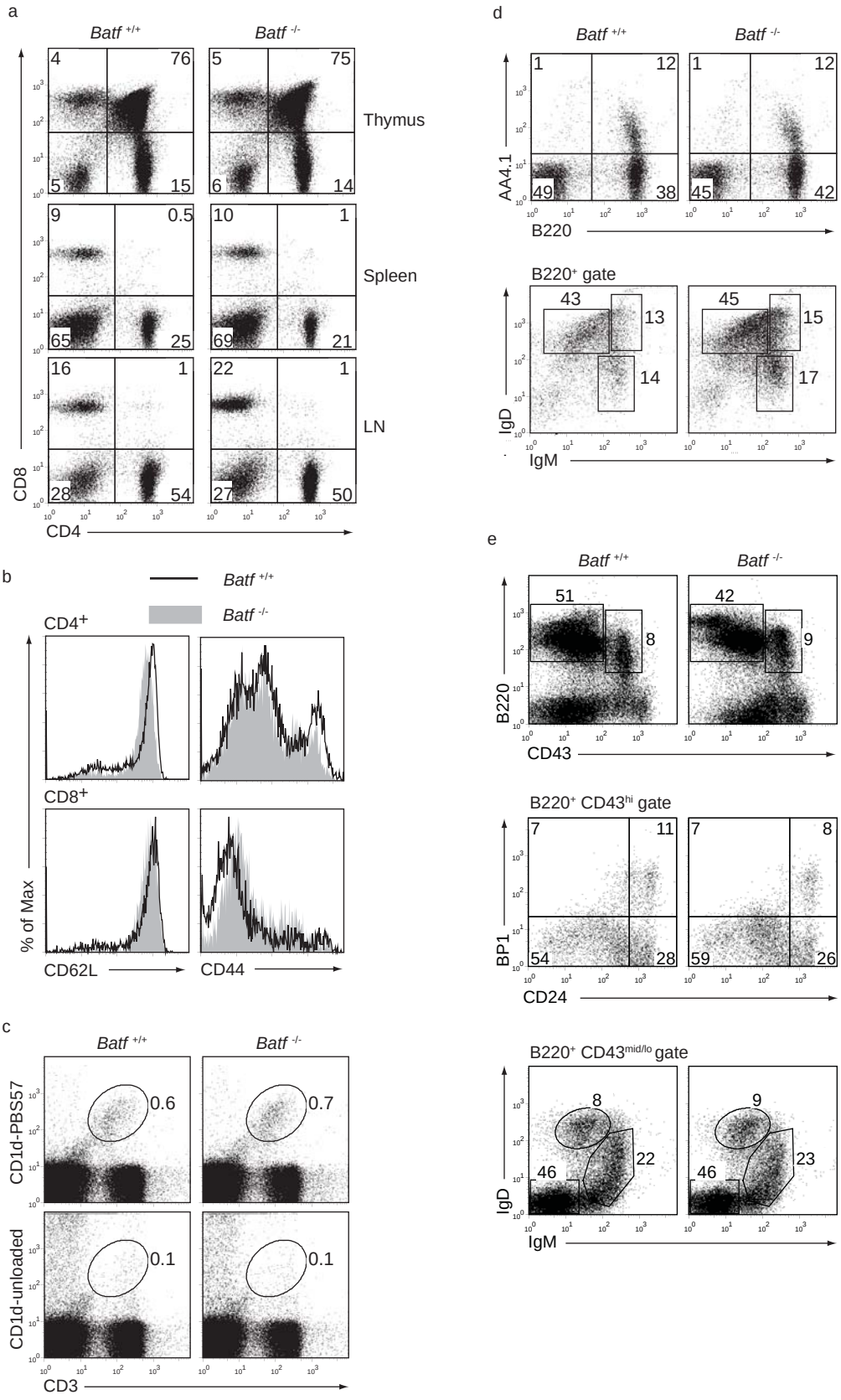
b



Supplementary Figure 3

Thymus, spleen and lymph nodes develop normally in *Batf*$^{-/-}$ mice. a, Total cell numbers of thymus (n=11) and spleen (n=17) from individual 8-10 week old *Batf*$^{+/+}$ *and* *Batf*$^{-/-}$ mice are shown (horizontal bars indicate mean cell numbers). **b,** *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were injected with Evans Blue dye solution into each hind foot pad. After 1.5 hrs, mice were sacrificed and superficial inguinal lymph nodes were visualized using a dissecting microscope[35]. Data are representative of 2 independent experiments.

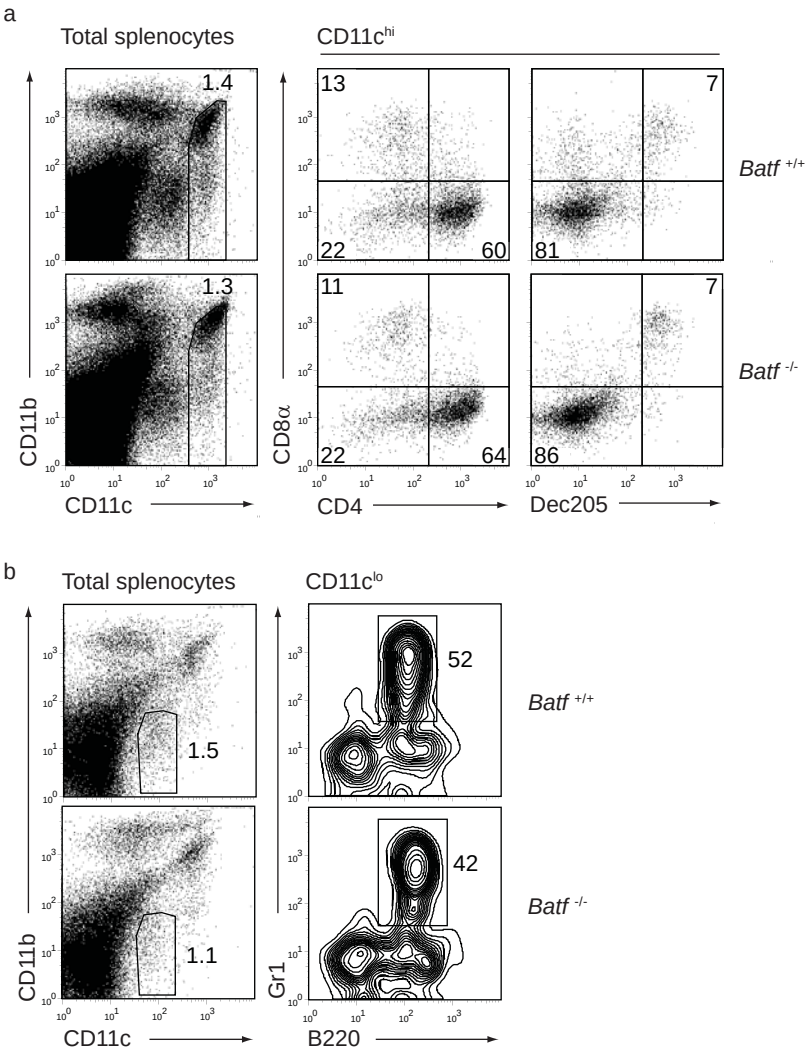
Supplementary Figure 4



Supplementary Figure 4

T and B cell development is normal in *Batf*$^{-/-}$ mice. a, Thymus, spleen and lymph nodes of mice of the indicated genotypes were analyzed for the surface expression of CD4 and CD8 by flow cytometry. The percentages of $CD8^+$, $CD4^+$ and $CD4^+CD8^+$ T cells were similar between *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice. **b,** Splenic $CD4^+$ and $CD8^+$ cells were analyzed for the surface expression of the activation markers CD62L (left panel) and CD44 (right panel) on *Batf*$^{+/+}$ and *Batf*$^{-/-}$ cells. A histogram overlay of surface expression of CD62L and CD44 on *Batf*$^{+/+}$ and *Batf*$^{-/-}$ $CD4^+$ and $CD8^+$ T cells is shown. **c,** Total splenocytes were stained for CD3 in conjunction with unloaded or PBS57-loaded CD1d tetramers. NKT cells are identified as $CD3^+$CD1d-PBS57$^+$. **d,** Total splenocytes were analyzed by staining with antibodies to B220, AA4.1, IgM and IgD. The percentages of immature B cells ($AA4.1^+$ $B220^+$), Transitional 1 ($B220^+IgM^{hi}IgD^{lo}$), Transitional 2 ($B220^+IgM^{hi}$, IgD^{hi}) or mature B cells ($AA4.1^-B220^+$; $B220^+IgM^{lo}IgD^{hi}$) were similar between *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice. **e,** Bone marrow cells were stained for the expression of B220, CD43 and either BP1 and CD24 or IgD and IgM. The percentages of cells included in $B220^+CD43^{hi}$ subsets: BP-1^-CD24^- (Hardy fraction A), BP-1^-CD24^+ (Hardy fraction B), and BP-1^+CD24^+ (Hardy fraction C) were similar between *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice. Also the percentages of $B220^+$ $CD43^-$ subsets; IgM^-IgD^- (Hardy fraction D), IgM^+IgD^{lo} (Hardy fraction E), and $IgM^{lo}IgD^{hi}$ (Hardy fraction F) were similar between *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice. Numbers of all FACS plots indicate percentage of cells in the indicated region or gate. Data are representative of at least 2 independent experiments performed with multiple mice of each genotype.

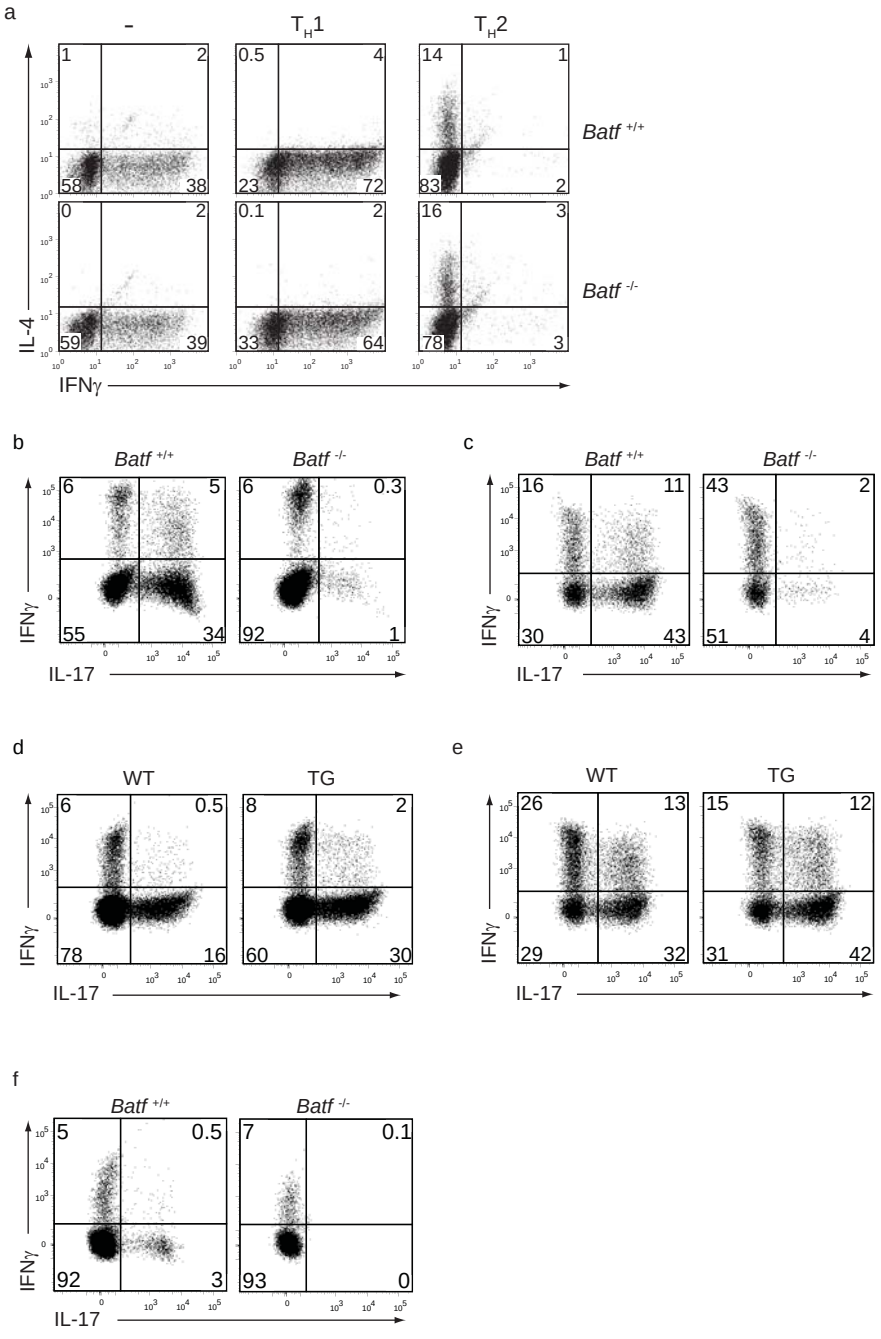
Supplementary Figure 5



Supplementary Figure 5

The development of myeloid cells is grossly normal in *Batf*$^{-/-}$ mice. a, Conventional splenic dendritic cell (cDC) subsets are present at normal ratios in *Batf*$^{-/-}$ mice. Single cell suspensions from collagenase and DNase treated spleens were stained with the indicated antibodies. cDCs were identified as CD11c^{hi} cells and further subdivided into CD4^{+} DCs and CD8^{+} DCs, identified as CD11c^{hi}CD4^{+}CD8^{-} and CD11c^{hi}CD4^{-}CD8α^{+} respectively. CD8^{+} DCs were further identified as CD11c^{hi}CD8α^{+} Dec205^{+}. **b,** Splenic single cell suspensions were prepared as in **a** and stained with antibodies to CD11c, CD11b, Gr1 and B220. Percentages of plasmacytoid dendritic cells, identified as CD11b^{-} CD11c^{lo}B220^{+}Gr1^{+}, were similar between *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice. Numbers for all FACS plots indicate the percentage of live cells in each gate or region. Data are representative of at least 2 independent experiments performed with multiple mice of each genotype.

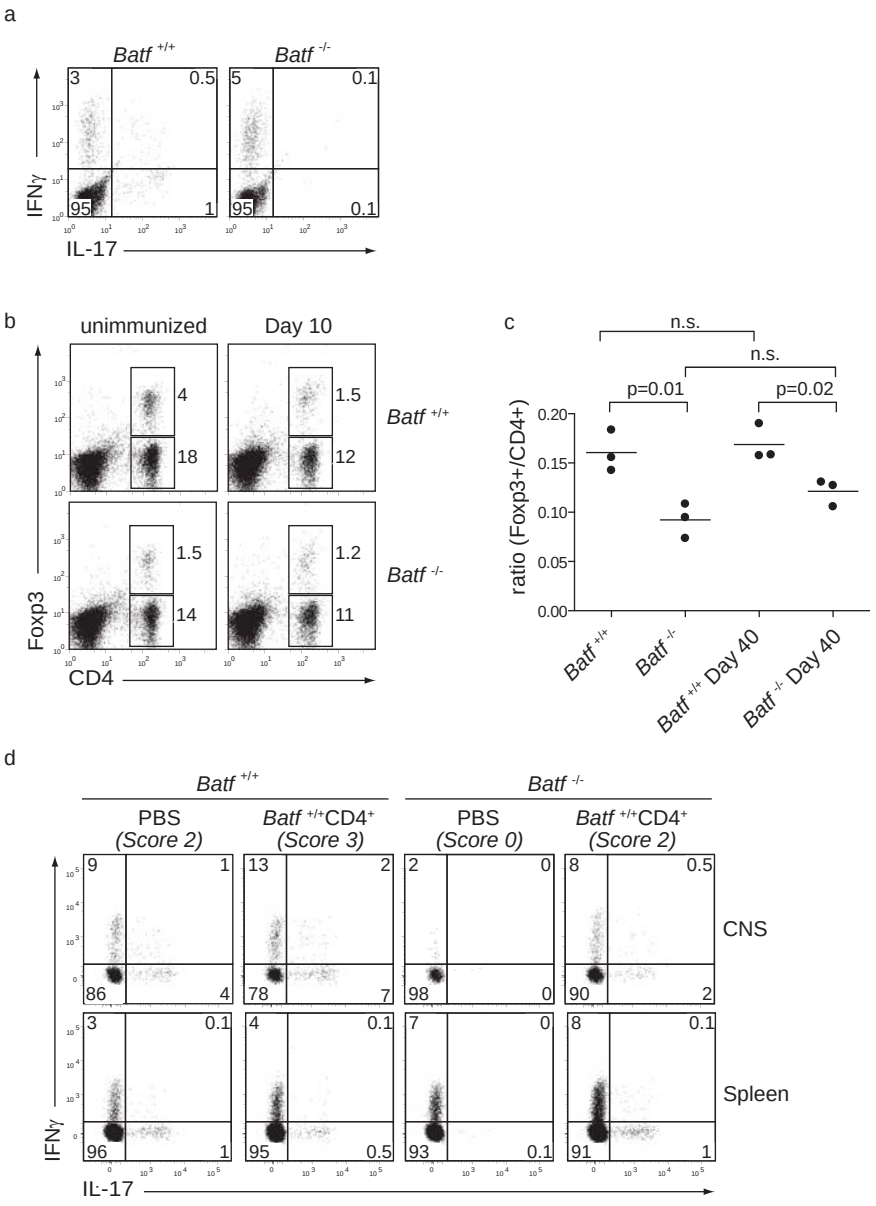
Supplementary Figure 6



Supplementary Figure 6

Batf regulates IL-17 production by $CD4^+$ and $CD8^+$ cells. a, Naïve $CD4^+CD62L^+CD25^-$ T cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice activated with anti-CD3 and anti-CD28 alone or under T_H1 or T_H2 conditions. Cells were restimulated on day 7 with anti-CD3/CD28 for 24h and analyzed for IFN-γ and IL-4 production. **b,** $CD4^+$ T cells from DO11.10 *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were purified by magnetic bead separation and activated with OVA and irradiated APCs under T_H17 conditions. 3 days later, cells were split and allowed to expand for 4 days in the presence of T_H17 inducing cytokines. After 3 rounds of differentiation, cells were restimulated with PMA/ionomycin for 4 hours and analyzed for IFN-γ and IL-17 expression by flow cytometry. **c,** Total splenocytes from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were stimulated under T_H17 conditions for 3 days. Cells were restimulated with PMA/ionomycin and analyzed for IL-17 and IFN-γ expression by intracellular cytokine staining and flow cytometry. Plots are gated on $CD8^+$ cells. **d,** DO11.10 transgenic $CD4^+$ T cells from CD2-N-FLAG-*Batf* transgenic (TG) or transgene-negative (WT) control mice were stimulated with OVA and APC under T_H17 conditions. 3 days later, cells were restimulated with PMA/ionomycin and cytokine production was analyzed by flow cytometry as described in Methods. **e,** Total splenocytes from CD2-N-FLAG-*Batf* transgenic (TG) or transgene-negative (WT) control mice were stimulated and analyzed as in **c**. **f,** Small intestinal lamina propria cells were isolated from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice and stimulated with PMA/ionomycin and stained for IL-17 and IFN-γ production. Plots are gated on $CD4^+$ lymphocytes. Numbers for all FACS plots indicate the percentage of live cells in each indicated gate. Data are representative of at least 2 independent experiments performed with multiple mice of each genotype.

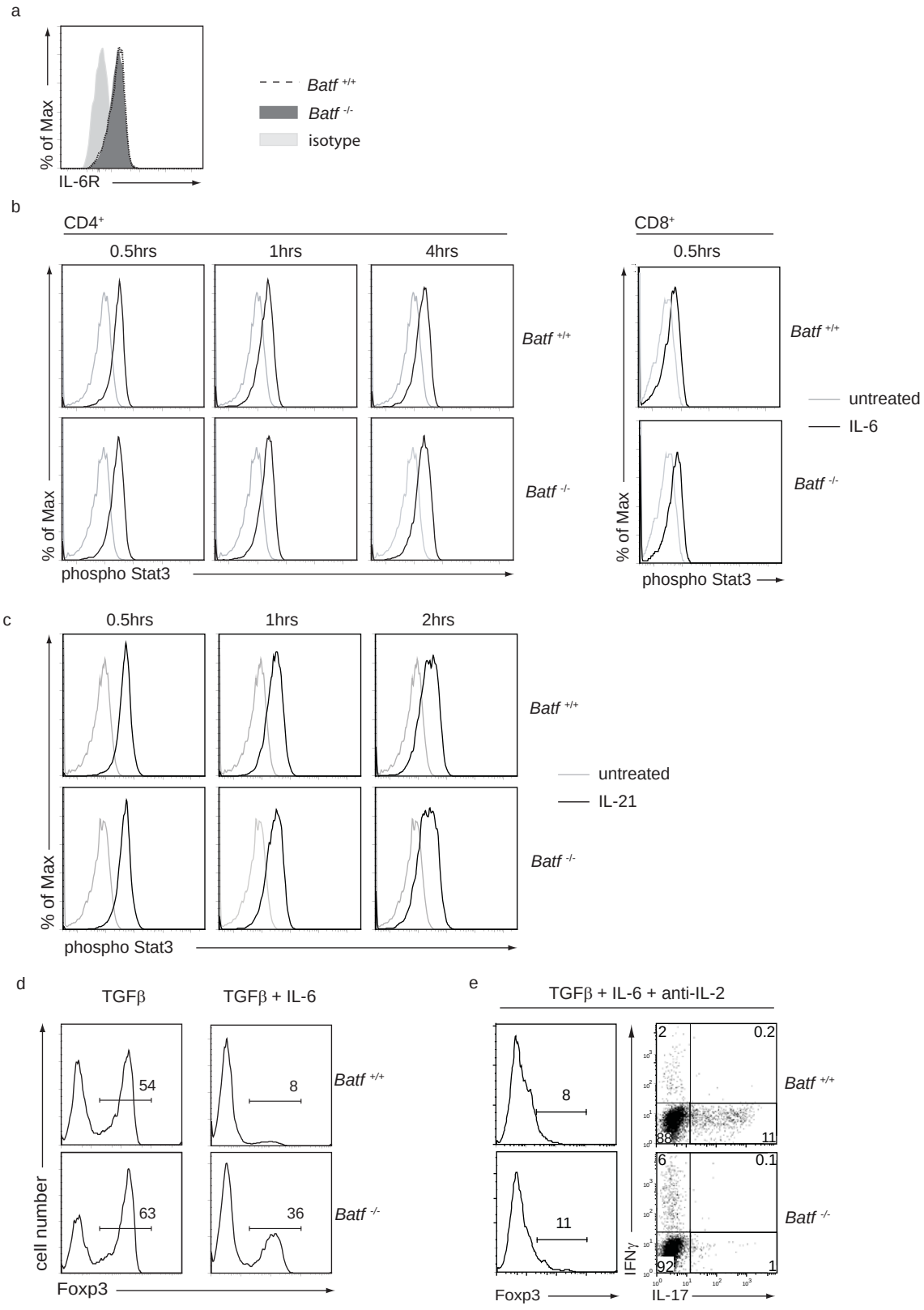
Supplementary Figure 7



Supplementary Figure 7

***Batf*$^{-/-}$ mice are resistant to EAE.** **a,** Total splenocytes were isolated from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice 10 days after EAE induction, stimulated with PMA/ionomycin for 3 hours and analyzed for IL-17 and IFN-γ expression by intracellular cytokine staining. Plots are gated on CD4^{+} cells. **b,** Spleens were isolated from unimmunized *Batf*$^{+/+}$ and *Batf*$^{-/-}$ or from mice 10 days after EAE induction. Total splenocytes were stained for the expression of CD4 and Foxp3 and analyzed by flow cytometry. **c,** Spleens were isolated from unimmunized *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice or from mice 40 days after EAE induction. The abundance of Foxp3^{+} cells is depicted as the ratio of CD4^{+}Foxp3^{+} cells in the total CD4^{+} T cell compartment. **d,** 4 days prior to EAE induction, *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice received either control buffer (PBS) or 1x10^{7} *Batf*$^{+/+}$ CD4^{+} T cells. 40 days after EAE induction splenic and CNS infiltrating lymphocytes were stimulated with PMA/ionomycin for 4h and analyzed for IL-17 and IFN-γ production. Genotypes and whether mice received PBS or CD4^{+} T cells are indicated, disease scores are given in parentheses. FACS plots in **a** and **d** are gated on CD4^{+} cells. FACS plots are representative of 2-3 mice analyzed per group. Numbers for FACS plots indicate percentage of cells in each indicated gate.

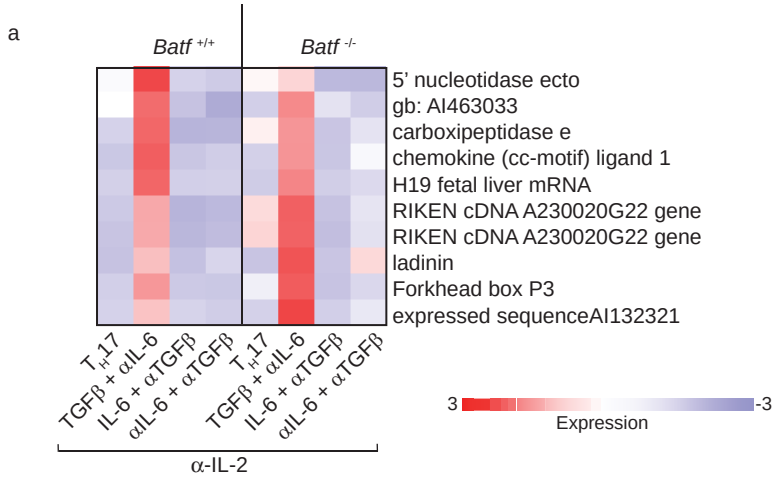
Supplementary Figure 8

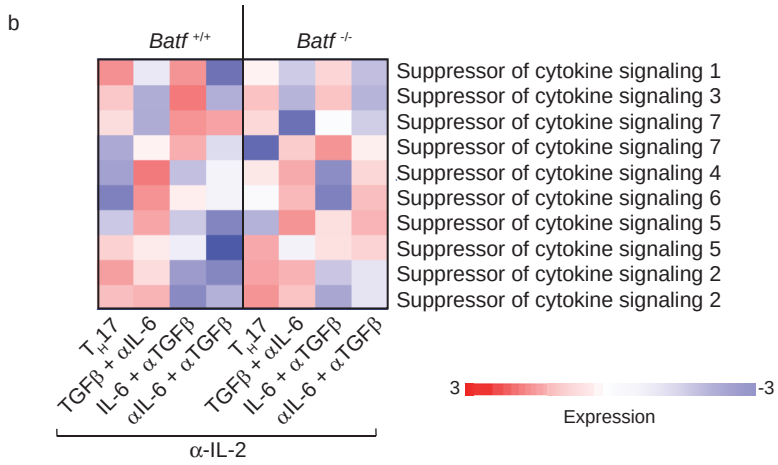


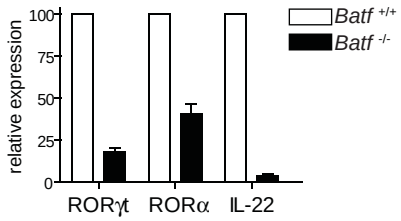
Supplementary Figure 8

Proximal IL-6 receptor signaling is normal in *Batf*$^{-/-}$ T cells. **a,** Splenocytes from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were stained with antibodies to CD4 and IL-6 receptor (IL-6R). A histogram overlay of IL-6R expression on CD4^{+} cells of the indicated genotypes is shown. **b,** Magnetically purified *Batf*$^{+/+}$ and *Batf*$^{-/-}$ CD4^{+} T cells (left) and CD8^{+} T cells (right) were stimulated with anti-CD3/CD28 in the presence of IL-6 for the indicated times and stained with an antibody to phospho-STAT3 (black lines) by intracellular staining as described in Methods. Untreated cells (grey lines) served as a negative control. **c,** Magnetically purified *Batf*$^{+/+}$ and *Batf*$^{-/-}$ CD4^{+} T cells were stimulated with anti-CD3/CD28 in the presence of IL-21 for the indicated times and stained with an antibody to phospho-STAT3 (black lines) by intracellular staining. Untreated cells (grey lines) served as a negative control. **d,** Naïve CD4^{+}CD62L^{+}CD25^{-} T cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were stimulated with TGF-β or TGF-β plus IL-6 for 3 days. Cells were stained for Foxp3 and analyzed by flow cytometry. **e,** Naïve CD4^{+}CD62L^{+}CD25^{-} T cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were stimulated with TGF-β plus IL-6 in the presence of a neutralizing antibody to IL-2 for 3 days. Cells were stained for Foxp3, IL-17 and IFN-γ and analyzed by flow cytometry. Numbers for all FACS plots indicate the percentage of live cells in each indicated gate. Data are representative of at least 2 independent experiments performed with multiple mice of each genotype.

Supplementary Figure 9



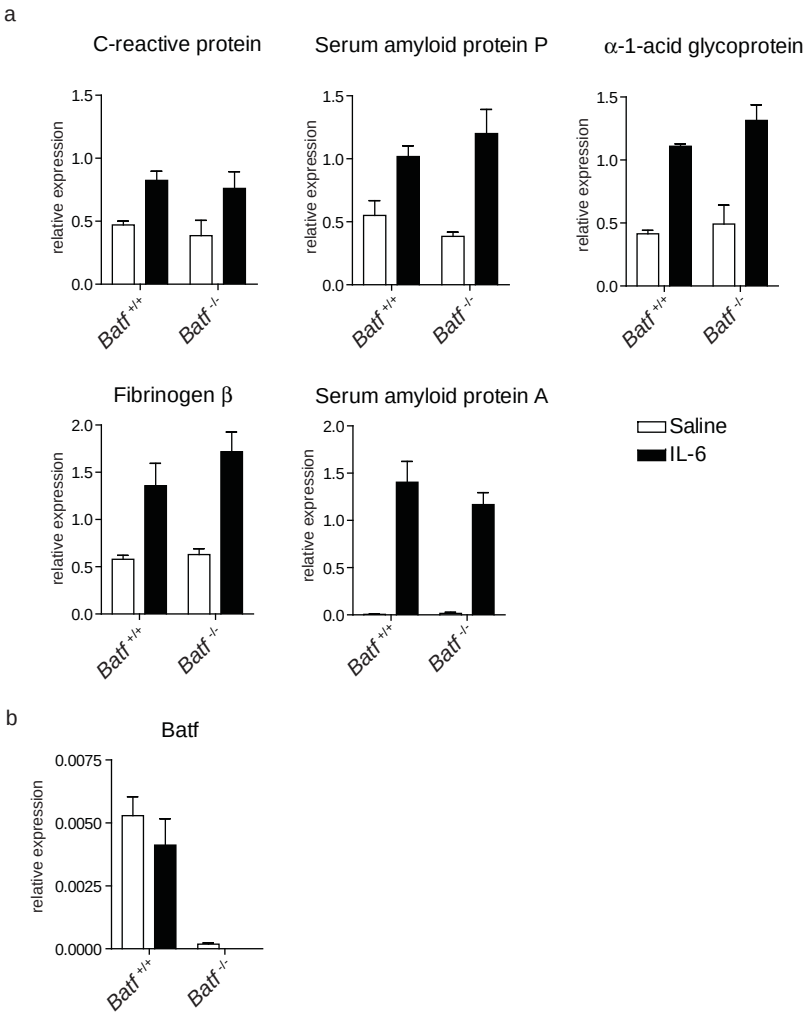




Supplementary Figure 9

Batf does not regulate expression of genes induced by TGF-β alone or regulate SOCS gene expression. a, b, Gene expression microarray analysis of T cells activated with anti-CD3/CD28 for 72h in the presence of the indicated cytokines and antibodies. **a,** A representative heat map of genes at least 5-fold induced by TGF-β compared to neutral conditions in *Batf*$^{+/+}$ T cells is presented. **b,** A representative heat map of the expression of suppressor of cytokine signaling (SOCS) genes in *Batf*$^{+/+}$ and *Batf*$^{-/-}$ T cells is presented. **c,** Relative expression of RORγt, RORα and IL-22 in T cells 72h after activation with anti-CD3/CD28 under T_H17 conditions was assessed by qRT-PCR. Data are normalized to HPRT and presented as percent expression relative to *Batf*$^{+/+}$ cells (mean + s.d. of 3 individual mice).

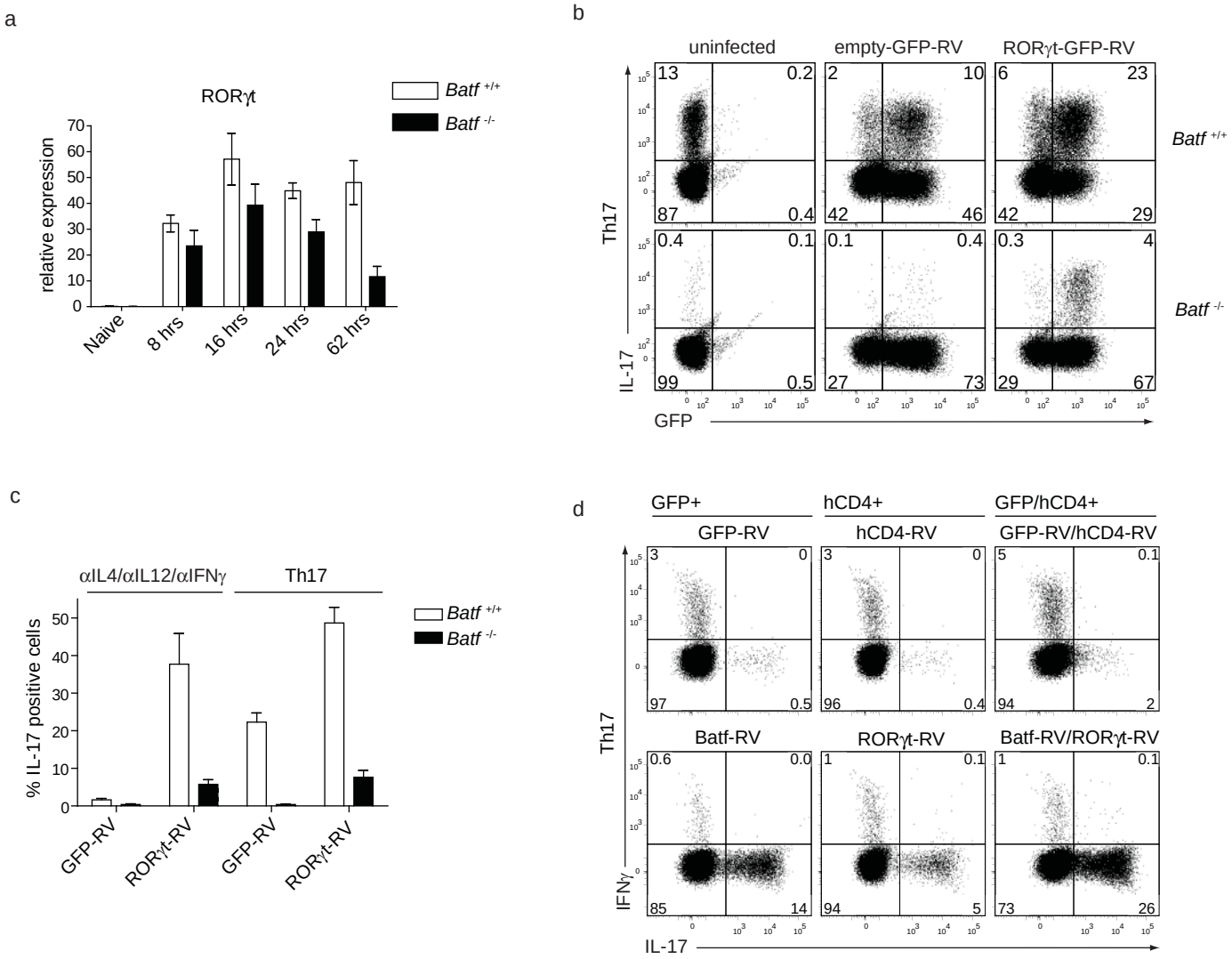
Supplementary Figure 10



Supplementary Figure 10

Several aspects of the IL-6-induced liver acute phase response are normal in *Batf*$^{-/-}$ mice. a, *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were injected intraperitonally with either 0.3ug IL-6 or saline. 4h after injection of mice the expression of the indicated acute phase proteins in liver was assessed by quantitative real time PCR. The relative expression of proteins normalized to HPRT is presented in arbitrary units. **b,** Relative expression of Batf in liver 4h after injection of mice with 0.3ug IL-6 or saline. The relative expression of proteins normalized to HPRT is presented in arbitrary units. Data represent mean + s.d. of 3 individual mice from independent experiments.

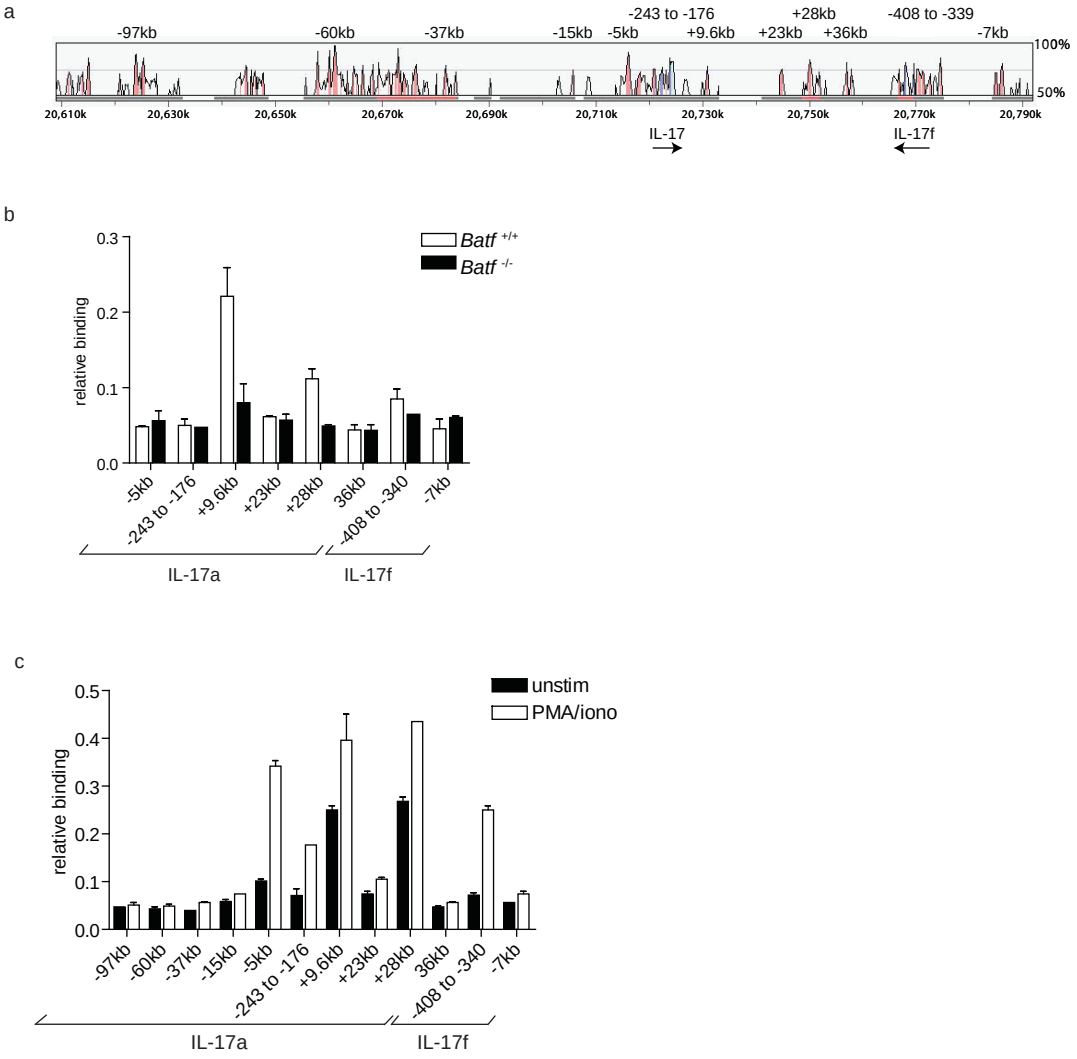
Supplementary Figure 11



Supplementary Figure 11

Retroviral overexpression of RORγt only partially restores IL-17 production in *Batf*$^{-/-}$ T cells. **a,** Naïve CD4^{+}CD62L^{+}CD25^{-} T cells were stimulated with anti-CD3/CD28 under T_H17 conditions for 0, 8, 16, 24 and 62h. Relative expression (normalized to HPRT) of RORγt in *Batf*$^{+/+}$ and *Batf*$^{-/-}$ T cells is depicted (error bars: mean ± s.d. of 3 individual mice). **b,** Magnetically purified CD4^{+} T cells were stimulated with anti-CD3/CD28 under T_H17 conditions and were either left untreated or infected with empty-IRES-GFP-retrovirus (GFP-RV) or RORγt expressing IRES-GFP-retrovirus (RORγt-RV) as described in Methods. Cells were restimulated with PMA/ionomycin for 4h and analyzed for cytokine expression on day 3. **c,** CD4^{+} T cells were stimulated as indicated and infected with retrovirus as in **(b)** and Fig. 3e. The percentage of IL-17 producing cells among stably infected (GFP^{+}) cells is shown (mean + s.d. of three independent experiments). **d,** Dual retroviral overexpression of Batf and RORγt in *Batf*$^{-/-}$ T cells. Magnetically purified *Batf*$^{-/-}$ CD4^{+} T cells were stimulated with anti-CD3/CD28 under T_H17 conditions and either infected with Batf-expressing IRES-GFP-retrovirus (Batf-RV), RORγt-expressing IRES-hCD4-retrovirus (RORγt-RV) or both retroviruses (bottom panel) as described in Methods. As a control, cells were infected with empty-control retroviruses as indicated (top panel). Cells were restimulated with PMA/ionomycin and analyzed for IL-17 and IFN-γ expression on day 3. Data are representative of 2 independent experiments. Representative FACS plots shown are gated as indicated. Numbers represent percentage of cells in each gate or quadrant.

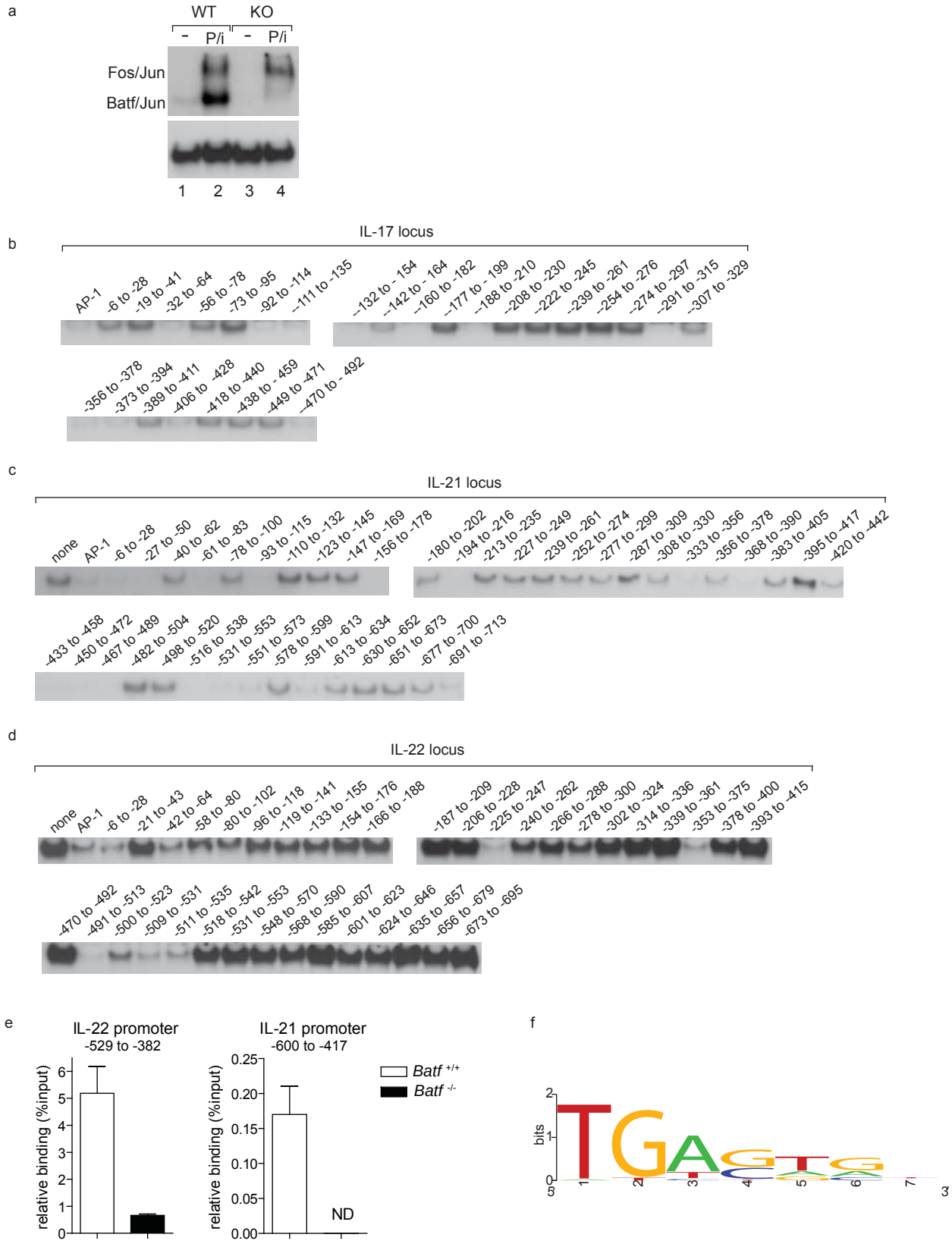
Supplementary Figure 12



Supplementary Figure 12

Batf binds several conserved non-coding regions in the IL-17 locus. a, Vista blot depicting the sequence conservation of the human and mouse IL-17 loci. The locations of primers used for ChIP analysis are indicated. **b,** Magnetically purified $CD4^+$ T cells from *Batf*$^{+/+}$ or *Batf*$^{-/-}$ mice were activated with anti-CD3/CD28 coated beads under T_H17 conditions (IL-6/TGF-β) for 24h, then subjected to ChIP analysis using anti-Batf polyclonal antibody as in Fig. 4b. Data are presented as relative binding based on normalization to unprecipitated input DNA (mean + s.d. of 2 independent experiments). **c,** *Batf*$^{+/+}$ $CD4^+$ T cells from C57Bl/6 mice were stimulated with anti-CD3 and APCs under T_H17 conditions for 5 days. ChIP analysis of T cells before and after PMA/ionomycin stimulation for 4h was performed using anti-*Batf* antibody. The analyzed sites are denoted relative to the ATG for the *Il17a* or *Il17f* genes. Data are presented as relative binding based on normalization to unprecipitated input DNA (mean + s.d. of 2 independent experiments).

Supplementary Figure 13

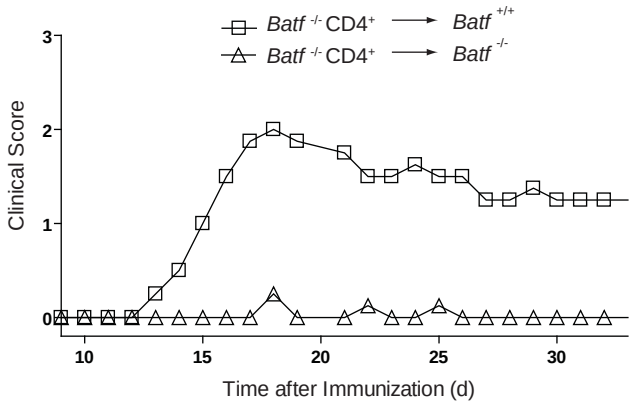


Supplementary Figure 13

Identification of potential Batf binding sites in the IL-17a, IL-21 and IL-22 promoters. a, $CD4^+$ T cells from DO11.10 *Batf*$^{+/+}$ and *Batf*$^{-/-}$ littermates were purified by magnetic bead separation and activated with OVA and irradiated APCs under T_H17 conditions. 3 days later, cells were split and allowed to expand for 4 days in the presence of T_H17 inducing cytokines. On day 7 after initial stimulation cells were either left untreated or restimulated with PMA/ionomycin for 4 hours. Total cell extracts were analyzed for DNA binding ability to a consensus AP-1 probe (AGCTTCGCTTGATGAGTCAGCCG)[36] by electrophoretic mobility shift assay. **b-d,** Identification of potential Batf binding sites in the IL-17, IL-21 and IL-22 promoters. Total splenocytes from *Batf*-transgenic mice were stimulated under T_H17 conditions for 3 days. Total cell extracts were analyzed for DNA binding ability to a consensus AP-1 probe[36] by EMSA as in **a**. Batf containing complexes were identified by supershift with anti-FLAG antibody. Sequences from the IL-17a **(b)**, IL-21 **(c)** and IL-22 **(d)** promoters were used to assess their ability to inhibit formation of Batf containing complexes as described in Methods. Sequences of competitors used are supplied in Supplementary Table 6. **e,** *Batf*$^{+/+}$ and *Batf*$^{-/-}$ $CD4^+$ T cells were stimulated under T_H17 conditions for 5 days. ChIP analysis was performed as above. The analyzed sites are denoted relative to the ATG for the *IL21* or *IL22* genes. Data are presented as relative binding based on normalization to unprecipitated input DNA. **f**, WebLogo[37] presentation of the Batf-binding motif in the IL-17, IL-21 and IL-22 promoters. The size of each nucleotide is proportional to the frequency of its appearance at each position.

Supplementary Figure 14

a



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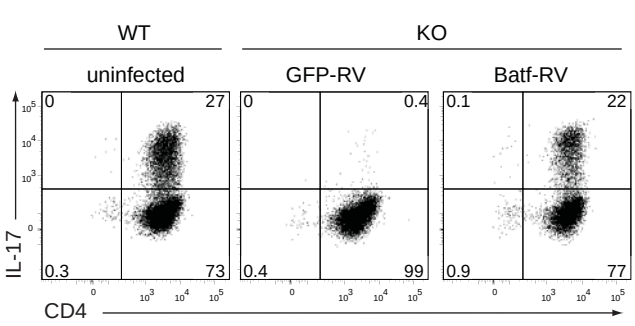

Supplementary Figure 14

Batf $^{-/-}$ **T cell do not protect against EAE.** **a,** *Batf* $^{+/+}$ and *Batf* $^{-/-}$ mice were injected $1x10^7$ *Batf* $^{-/-}$ $CD4^+$ T cells (n=4). Four days later, mice were immunized MOG_{33-35} peptide as described in Methods. Clinical EAE scores (mean +/- s.e.m) representative of two independent experiments are shown. **b,** Magnetically purified $CD4^+$ T cells from wild type (WT) or mice lacking *Batf* (KO) were stimulated with anti-CD3/CD28 under T_H17 conditions and either infected with control virus (GFP-RV) or Batf-expressing IRES-GFP-retrovirus (Batf-RV) as described in Methods. On day 7, cells re-stimulated with anti-CD3/CD28 under T_H17 conditions. After 4 days, cells were restimulated with PMA/ionomycin and analyzed for CD4 and IL-17 expression. Representative FACS plots are gated on GFP^+ cells (KO) or GFP^- cells (wild type uninfected). Numbers represent percentage of cells in each region.

Supplementary Table 1

Transfer of *Batf* $^{+/+}$ CD4^{+} T cells into *Batf* $^{-/-}$ mice restores EAE

Group	Incidence	Mean Max. Score	Mortality	Mean day of onset[‡]
PBS→*Batf* $^{+/+}$	5 of 5 (100%)	3.4 ± 0.7	1of 5 (20%)	12±0.8[¶]
PBS→*Batf* $^{-/-}$	0 of 5 (0%)	0	0 of 13 (0%)	NA
Batf $^{+/+}$CD4^{+}→*Batf* $^{+/+}$	5 of 6 (83%)	3.0 ± 0.6	0 of 6 (0%)	13.6±2.3[§¶]
Batf $^{+/+}$CD4^{+}→*Batf* $^{-/-}$	4 of 6 (66%)	2.4 ± 1.0	2 of 6 (33%)	15.5±1.7[§]

Four days prior to induction of EAE mice were injected with 1x10^{7} CD4^{+}*Batf* $^{+/+}$ T cells or control buffer (PBS) as indicated. The mice were monitored for disease development as described in Methods. Mean maximum score of disease was calculated and is presented ± s.e.m. Only animals positive for disease are included in the analysis. [§] not significant (p=0.215). [¶]not significant (p=0.232). NA, not applicable.

Supplementary Table 5

Primers	Chr. 1 location	5'FAM 3' BHQ1 Probes	Chr. 1 location
IL17a -97 **(-97kb)**			
5' AAATGTGAGCCCCAGATCGA 3'	20,623,606-20,623,625	CTGCTGCTGTCCCAGGCACAGTTG	20,623,627-20,623,650
5' GGGACATTTTTTCCACCATGA 3'	20,623,652-20,623,672		
IL17a -60 **(-60kb**			
5' TTGTCCCCTGGCTGTTCCT 3'	20,661,177-20,661,247	CCTTATCCAGCTGTCTTTTTCTCT	20,661.249-20,661,272
5' GGGCTCCCCAAAAATTCACA 3'	20,661,274-20,661,293		
IL17a -37 **(-37kb)**			
5' GTCCCTCTGTTGTTTCCAAGGAT 3'	20,683,616-20,683,638	TCATTGAGTCCTTCCAGCAGAGATTTCAGG	20,683,640-20,683,669
5' GCCATTTCAGCCACTGTGAA 3'	20,683,671-20,683,690		
IL17a -15 **(-15kb)**			
5' TGGCAAATGTTTTGTCAACCA 3'	20,705,507-20,705,527	TTCCTCGATTGCTGTCTACTCATC	20,705,529-20,705,552
5' CATGCAGCCTCTGCTTGAGA 3'	20,705,554-20,705,573		
IL17a -5 **(-5kb)**			
5' CGATACTTTTCAGTGACATCCGTTT 3'	20,715,852-20,715,876	ACTTGAAACCCAGTCAGTTGCTGACCTTGA	20,715,879-20,715,908
5' TGCTGACTTCATCTGATACCCTTAGA 3'	20,715,910-20,715,935		
IL17a promoter **(-243 to -176)**			
5' GAACTTCTGCCCTTCCCATCT 3'	20,720,800-20,720,820	CCTTCGAGACAGATGTTGCCCGTCA	20,720,822-20,720,846
5' CAGCACAGAACCACCCCTTT 3'	20,720,848-20,720,867		
IL17a +9.6 **(+9.6kb)**			
5' ATTTAGGGCACAGGTGACATGA 3'	20,730,688-20,730,709	TGGTTCTCAAAGCATAAACCTCATTC	20,730,711-20,730,736
5' CCACTTCCCCGACCTCACTA 3'	20,730,738-20,730,757		
IL17a +23 **(+23kb)**			
5' CAAATCCGTGTGCCTTCTGTT 3'	20,744,816-20,744,836	CTGCAGTGAGGAAGATGTTTCCAATGAGG	20,744,838-20,744,866
5' AGGTTGACTTCGTCCCTGTGA 3'	20,744,870-20,744,890		
IL17a +28 **(+28kb)**			
5' GTGGCCTACTTCAGGCAGATG 3'	20,749,994-20,750,014	TGAGAAGCCAGCGTCGGGTCC	20,750,016-20,750,036
5' GGAGCCGATGAGAAGCATTC 3'	20,750,039-20,750,058		
IL17a +36 **(+36kb)**			
5' AGATAATGTATCACACAGCCCTGAAG 3'	20,757,551-20,757,576	AGCCAGTGCCTTAATCCATTGGG	20,757,578-20,757,600
5' CATGGTTGTGAAGTTGGTGAGATG 3'	20,757,602-20,757,625		
IL17f promoter **(-408 to -340)**			
5' ACTGCATGACCCGAAAGCA 3'	20,774,671-20,774,688	AACCCACACGCAGAGCATGACAAGAG	20,774,643-20,774,669
5' TTTAATTCCCCCACAAAGCAA 3'	20,774,620-20,774,640		
IL17f -7 **(-7kb)**			
5' TTCCCTTTTCTGCCTTGCA 3'	20,782,972-20,782,990	ACGAAGCACAGGGCTGGGCC	20,782,996-20,783,015
5' TGTGTAACACGCAGAGTGGAATG 3'	20,783,017-20,783,039		

	Chr. 3 location
IL21 promoter **(-529 to -382)**	
5' GCATAGTCATCACCCCATAAA 3'	37,131,996-37,132,016
5' TCAGAGAAGTAAACACAAACAC 3'	37,131,869-37,131,890

	Chr. 10 location
IL22 promoter **(-600 to -417)**	
5' GCACAGAATATAGGACACGGGT	117,641,447-117,641,468
5' ACACAGTTTTCAAAGAAAGCCA	117,641,609 117,641,630

Supplementary Table 6

IL-17a promoter Oligos	Sequence 5' to 3'
33-1-top-IL17a	GCACCCAGCACCAGCTGATCAGGACGCG
33-1-bot-IL17a	GTTTGCGCGTCCTGATCAGCTGGTGCTG
46-14-top-IL17a	ACGAGGCACAAGTGCACCCAGCACCAGC
46-14-bot-IL17a	GATCAGCTGGTGCTGGGTGCACTTGTGC
69-37-top-IL17a	GCACTACTCTTCATCCACCTCACACGAG
69-37-bot-IL17a	TGTGCCTCGTGTGAGGTGGATGAAGAGT
83-51-top-IL17a	AAAGAGAGAAAGGAGCACTACTCTTCAT
83-51-bot-IL17a	GGTGGATGAAGAGTAGTGCTCCTTTCTC
100-68-top-IL17a	GTAGTAAAACCGTATAAAAAGAGAGAAA
100-68-bot-IL17a	GCTCCTTTCTCTCTTTTTATACGGTTTT
119-87-top-IL17a	ACGTAAGTGACCACAGAGGTAGTAAAA
119-87-bot-IL17a	TACGGTTTTACTACCTCTGTGGTCACT
140-106-top-IL17a	GTCACCCCCCAACCCACTCTTGACGTAAGT
140-106-bot-IL17a	TGGTCACTTACGTCAAGAGTGGGTTGGGGG
159-127-top-IL17a	GAATCTTTACTCAAATGGTGTCACCCCC
159-127-bot-IL17a	GGTTGGGGGGTGACACCATTTGAGTAAA
169-137-top-IL17a	TTTGAGGATGGAATCTTTACTCAAATGG
169-137-bot-IL17a	TGACACCATTTGAGTAAAGATTCCATCC
187-155-top-IL17a	GGTTCTGTGCTGACCTCATTTGAGGATG
187-155-bot-IL17a	GATTCCATCCTCAAATGAGGTCAGCACA
204-172-top-IL17a	GCCCGTCATAAAGGGGTGGTTCTGTGCT
204-172-bot-IL17a	AGGTCAGCACAGAACCACCCCTTTATGA
215-183-top-IL17a	AGACAGATGTTGCCCGTCATAAAGGGGT
215-183-bot-IL17a	GAACCACCCCTTTATGACGGGCAACATC
235-203-top-IL17a	GCCCTTCCCATCTACCTTCGAGACAGAT
235-203-bot-IL17a	GCAACATCTGTCTCGAAGGTAGATGGGA
250-217-top-IL17a	GCATAGTGAACTTCTGCCCTTCCCATCTA
250-217-bot-IL17a	GAAGGTAGATGGGAAGGGCAGAAGTTCAC
266-234-top-IL17a	GAAGTCATGCTTCTTTGCATAGTGAACT
266-234-bot-IL17a	GCAGAAGTTCACTATGCAAAGAAGCATG
281-249-top-IL17a	CTGTTCAGCTCCCAAGAAGTCATGCTTC
281-249-bot-IL17a	GCAAAGAAGCATGACTTCTTGGGAGCTG
302-269-top-IL17a	CTGAATCACAGCAAAGCATCTCTGTTCAG
302-269-bot-IL17a	GGGAGCTGAACAGAGATGCTTTGCTGTGA
320-286-top-IL17a	GTCCATACACACATGATACTGAATCACAGC
320-286-bot-IL17a	GCTTTGCTGTGATTCAGTATCATGTGTGTA

334-302-top-IL17a	GCAGCTTCAGATATGTCCATACACACAT
334-302-bot-IL17a	GTATCATGTGTGTATGGACATATCTGAA
349-317-top-IL17a	GAGCCCAGCTCTGCAGCAGCTTCAGATA
349-317-bot-IL17a	GGACATATCTGAAGCTGCTGCAGAGCTG
370-337-top-IL17a	GACTCACAAACCATTACTATGGAGCCCAG
370-337-bot-IL17a	CAGAGCTGGGCTCCATAGTAATGGTTTGT
383-351-top-IL17a	GAGACTGTCAAGAGACTCACAAACCATT
383-351-bot-IL17a	ATAGTAATGGTTTGTGAGTCTCTTGACA
400-368-top-IL17a	AAAGTGTGTGTCACTAGGAGACTGTCAA
400-368-bot-IL17a	GTCTCTTGACAGTCTCCTAGTGACACAC
416-384-top-IL17a	GATCAAGTCAAAATTCAAAGTGTGTGTC
416-384-bot-IL17a	CTAGTGACACACACTTTGAATTTTGACT
433-401-top-IL17a	GGTAGAAAAGTGAGAAAGATCAAGTCAA
433-401-bot-IL17a	GAATTTTGACTTGATCTTTCTCACTTTT
445-413-top-IL17a	GCCAGGGAATTTGGTAGAAAAGTGAGAA
445-413-bot-IL17a	GATCTTTCTCACTTTTCTACCAAATTCC
464-432-top-IL17a	GGGCAAGGGATGCTCTCTAGCCAGGGAA
464-432-bot-IL17a	GCAAATTCCCTGGCTAGAGAGCATCCCT
476-44-top-IL17a	GTGGGTTTCTTTGGGCAAGGGATGCTCT
476-44-bot-IL17a	GCTAGAGAGCATCCCTTGCCCAAAGAAA
497-465-top-IL17a	GTTTACATACTAAGACATTGAGTGGGTT
497-465-bot-IL17a	AAAGAAACCCACTCAATGTCTTAGTATG

IL-21 promoter Oligos	**Sequence 5' to 3'**
33-1-top-IL21	GTCATCAGCTCCTGGAGACTCAGTTCTG
33-1-bottom-IL21	GCCACCAGAACTGAGTCTCCAGGAGCTG
55-22-top-IL21	GTGAGAACCAGACCAAGGCCCTGTCATCA
55-22-bottom-IL21	GGAGCTGATGACAGGGCCTTGGTCTGGTT
67-35-top-IL21	AGTCAGGTTGAAGTGAGAACCAGACCAA
67-35-bottom-IL21	GGGCCTTGGTCTGGTTCTCACTTCAACC
88-56-top-IL21	TAGCGACAACCTGTGCACAGTCAGGT
88-56-bottom-IL21	GTTCAACCTGACTGTGCACAGGTTGT
105-73-top-IL21	GATGAATAAATAGGTAGCCGTAGCGACA
105-73-bottom-IL21	CAGGTTGTCGCTACGGCTACCTATTTAT
120-88-top-IL21	GGCCTCTTCTTGAGGGATGAATAAATAG
120-88-bottom-IL21	GCTACCTATTTATTCATCCCTCAAGAAG
137-105-top-IL21	CTGCAATGGGAGGGCTTGGCCTCTTCTT

137-105-bottom-IL21	GCCTCAAGAAGAGGCCAAGCCCTCCCAT
150-118-top-IL21	AAAGATTTCCAGGCTGCAATGGGAGGGC
150-118-bottom-IL21	GCCAAGCCCTCCCATTGCAGCCTGGAAA
174-142-top-IL21	GTTACTCACACTCATCCACTATACAAAG
174-142-bottom-IL21	GAAATCTTTGTATAGTGGATGAGTGTGA
183-151-top-IL21	GAAAAACGAGTTACTCACACTCATCCAC
183-151-bottom-IL21	GTATAGTGGATGAGTGTGAGTAACTCGT
207-175-top-IL21	CACGTACACCTAGCCAATGGAAAAGAAA
207-175-bottom-IL21	TCGTTTTTCTTTTCCATTGGCTAGGTGT
221-189-top-IL21	TGCCCCCACACGCACACGTACACCTAGC
221-189-bottom-IL21	CATTGGCTAGGTGTACGTGTGCGTGTGG
240-208-top-IL21	TGTGGACTCTATCCATCCCTGCCCCCAC
240-208-bottom-IL21	TGCGTGTGGGGGCAGGGATGGATAGAGT
254-222-top-IL21	GATGGGGCACATTTTGTGGACTCTATCC
254-222-bottom-IL21	GGGATGGATAGAGTCCACAAAATGTGCC
266-234-top-IL21	GTCTAAGATGCAGATGGGGCACATTTTG
266-234-bottom-IL21	GTCCACAAAATGTGCCCCATCTGCATCT
279-247-top-IL21	GTCTCTTTTTCCTGTCTAAGATGCAGAT
279-247-bottom-IL21	GCCCCATCTGCATCTTAGACAGGAAAAA
304-272-top-IL21	GCTGAAAACTGGAATTCACCCATGTGTC
304-272-bottom-IL21	AAAGAGACACATGGGTGAATTCCAGTTT
314-282-top-IL21	CTTGGTGAATGCTGAAAACTGGAATTCA
314-282-bottom-IL21	ATGGGTGAATTCCAGTTTTCAGCATTCA
334-303-top-IL21	GACACACACACACACACACACCTTGGTG
334-303-bottom-IL21	GCATTCACCAAGGTGTGTGTGTGTGTGTG
361-328-top-IL21	GCCACACACACACACACACACACACACA
361-328-bottom-IL21	GTGTGTGTGTGTGTGTGTGTGTGTGTGT
383-351-top-IL21	GAAATCTGACGGTGCCTCCTGTGCCACA
383-351-bottom-IL21	GTGTGTGTGGCACAGGAGGCACCGTCAG
395-363-top-IL21	GTTTACTTCTCTGAAATCTGACGGTGCC
395-363-bottom-IL21	CAGGAGGCACCGTCAGATTTCAGAGAAG
410-378-top-IL21	GATCAAAGTGTTTGTGTTTACTTCTCTG
410-378-bottom-IL21	GATTTCAGAGAAGTAAACACAAACACTT
422-390-top-IL21	TGCAGAGCAAAAGATCAAAGTGTTTGTG
422-390-bottom-IL21	GTAAACACAAACACTTTGATCTTTTGCT
447-415-top-IL21	GACAAACCAGGTGAGGTGCCAGGGATGC
447-415-bottom-IL21	GCTCTGCATCCCTGGCACCTCACCTGGT

463-429-top-IL21	GCCTTTATGACTGTCAGACAAACCAGGTGA
463-429-bottom-IL21	GCACCTCACCTGGTTTGTCTGACAGTCATA
476-445-top-IL21	GTCATTGCAGAAGTGCCTTTATGACTGT
476-445-bottom-IL21	GTCTGACAGTCATAAAGGCACTTCTGCA
494-462-top-IL21	GCCATGCCGCTGCTTTACTCATTGCAGA
494-462-bottom-IL21	GCACTTCTGCAATGAGTAAAGCAGCGGC
509-477-top-IL21	AAAGTTCCAATAAAGGCCATGCCGCTGC
509-477-bottom-IL21	GTAAAGCAGCGGCATGGCCTTTATTGGA
525-493-top-IL21	AGTCATCACCCCATAAAAAGTTCCAATA
525-493-bottom-IL21	GCCTTTATTGGAACTTTTTATGGGGTGA
543-511-top-IL21	GGTTCAGTCAAAAAGCATAGTCATCACC
543-511-bottom-IL21	TATGGGGTGATGACTATGCTTTTTGACT
558-526-top-IL21	AATGGAGTACAGGATGGTTCAGTCAAAA
558-526-bottom-IL21	ATGCTTTTTGACTGAACCATCCTGTACT
578-546-top-IL21	GTAACCTCTTCCATCATTGCAATGGAGT
578-546-bottom-IL21	CCTGTACTCCATTGCAATGATGGAAGAG
604-573-top-IL21	GCCCATCATTTAATTCTTCCTAAGAAG
604-573-bottom-IL21	GGTTACTTCTTAGGAAGAATTAAATGA
618-586-top-IL21	AGGTTAGAAAACTAGCCCATCATTTAAT
618-586-bottom-IL21	GAAGAATTAAATGATGGGCTAGTTTTCT
639-607-top-IL21	AGGATCTAAAATACTCTTGCTAGGTTAG
639-607-bottom-IL21	GTTTTCTAACCTAGCAAGAGTATTTTAG
657-625-top-IL21	GCACCCTTACAAAAAGATAAGGATCTAA
657-625-bottom-IL21	GTATTTTAGATCCTTATCTTTTTGTAAG
678-646-top-IL21	TGGAAGCAAATCCTATTTTAACACCCTT
678-646-bottom-IL21	TTTGTAAGGGTGTTAAAATAGGATTTGC
705-672-top-IL21	GCTATTTAAAGATACACTGGTGAAAATTG
705-672-bottom-IL21	GCTTCCAATTTTCACCAGTGTATCTTTAA
718-686-top-IL21	AGGCACCATTAGTGCTATTTAAAGATAC
718-686-bottom-IL21	CCAGTGTATCTTTAAATAGCACTAATGG
736-704-top-IL21	GTTACATAAAGTGTCAGGAGGCACCATT
736-704-bottom-IL21	GCACTAATGGTGCCTCCTGACACTTTAT
754-722-top-IL21	GTATTTACAATCCATATTGTTACATAAA
754-722-bottom-IL21	GACACTTTATGTAACAATATGGATTGTA
775-743-top-IL21	AGTTCATCAAAACTGTTTATTGTATTTA
775-743-bottom-IL21	GATTGTAAATACAATAAACAGTTTTGAT
792-760-top-IL21	GAGCACGCTGTCTACTTAGTTCATCAAA

792-760-bottom-IL21	ACAGTTTTGATGAACTAAGTAGACAGCG

IL-22 promoter oligos	**Sequence 5' to 3'**
33-1-top-IL22	AGTTATCAACTGTTGACACTTGTGCGAT
33-1-bottom-IL22	CAGAGATCGCACAAGTGTCAACAGTTGA
48-16-top-IL22	ACAGGCTCTCCTCTCAGTTATCAACTGT
48-16-bottom-IL22	TGTCAACAGTTGATAACTGAGAGGAGAG
69-37-top-IL22	TTGCCTTTTGCTCTCTCACTAACAGGCT
69-37-bottom-IL22	AGGAGAGCCTGTTAGTGAGAGAGCAAAA
85-53-top-IL22	TGCTCCCCTGATGTTTTTGCCTTTTGCT
85-53-bottom-IL22	GAGAGAGCAAAAGGCAAAAACATCAGGG
107-75-top-IL22	GTACCATGCTACCCGACGAACATGCTCC
107-75-bottom-IL22	TCAGGGGAGCATGTTCGTCGGGTAGCAT
123-91-top-IL22	GACAATCATCTGCTTGGTACCATGCTAC
123-91-bottom-IL22	GTCGGGTAGCATGGTACCAAGCAGATGA
146-114-top-IL22	AGGTAAGCACTCAGACCTCTACAGACAA
146-114-bottom-IL22	GATGATTGTCTGTAGAGGTCTGAGTGCT
160-128-top-IL22	AGAGACACCTAAACAGGTAAGCACTCAG
160-128-bottom-IL22	GAGGTCTGAGTGCTTACCTGTTTAGGTG
181-149-top-IL22	TCTGCCTCTCCCATCACAAGCAGAGACA
181-149-bottom-IL22	TTAGGTGTCTCTGCTTGTGATGGGAGAG
193-161-top-IL22	AAAAGCAGCAACTTCTGCCTCTCCCATC
193-161-bottom-IL22	CTTGTGATGGGAGAGGCAGAAGTTGCTG
214-182-top-IL22	CCTGGTGTCCCGATGGCTATAAAAGCAG
214-182-bottom-IL22	AGTTGCTGCTTTTATAGCCATCGGGACA
233-201-top-IL22	GTCACAATACCAAAAAAACCCTGGTGTC
233-201-bottom-IL22	ATCGGGACACCAGGGTTTTTTTGGTATT
252-220-top-IL22	AATGTCTGATGTCATATCATTCACAATA
252-220-bottom-IL22	TTTGGTATTGTGAATGATATGACATCAG
267-235-top-IL22	GACTGGAAATTAGATAATGTCTGATGTC
267-235-bottom-IL22	GATATGACATCAGACATTATCTAATTTC
293-261-top-IL22	GTGGTTAGGTACTTCTCAGAAGACAGGA
293-261-bottom-IL22	TCCAGTCCTGTCTTCTGAGAAGTACCTA
305-273-top-IL22	TGGCCTCCTATGGTGGTTAGGTACTTCT
305-273-bottom-IL22	TTCTGAGAAGTACCTAACCACCATAGGA
329-297-top-IL22	GGAAGGCTTGGAGGTGGTGTCTTGTGGC
329-297-bottom-IL22	AGGAGGCCACAAGACACCACCTCCAAGC

340-309-top-IL22	GCTCTCAAGGTGGGAAGGCTTGGAGGTG
340-309-bottom-IL22	GACACCACCTCCAAGCCTTCCCACCTTG
366-334-top-IL22	GTGACGTTTTAGGGAAGACTTCCCATCT
366-334-bottom-IL22	TTGAGAGATGGGAAGTCTTCCCTAAAAC
380-348-top-IL22	TGTTGGCCCTCACCGTGACGTTTTAGGG
380-348-bottom-IL22	GTCTTCCCTAAAACGTCACGGTGAGGGC
405-373-top-IL22	CTGGGATTTGTGTGCAAAAGCACCTTGT
405-373-bottom-IL22	GGCCAACAAGGTGCTTTTGCACACAAAT
420-388-top-IL22	GTGTTTAGAAGATTTCTGGGATTTGTGT
420-388-bottom-IL22	TTTGCACACAAATCCCAGAAATCTTCTA
497-465-top-IL22	AATAGCTACGGGAGATCAAAGGCTGCTC
497-465-bottom-IL22	GAGTAGAGCAGCCTTTGATCTCCCGTAG
518-486-top-IL22	CCGTGACCAAAACGCTGACTCAATAGCT
518-486-bottom-IL22	CCCGTAGCTATTGAGTCAGCGTTTTGGT
528-495-top-IL22	GAAAATGAGTCCGTGACCAAAACGCTGAC
528-495-bottom-IL22	ATTGAGTCAGCGTTTTGGTCACGGACTCA
536-504-top-IL22	GTTGGTGGGAAAATGAGTCCGTGACCAA
536-504-bottom-IL22	GCGTTTTGGTCACGGACTCATTTTCCCA
540-506-top-IL22	TGAAGTTGGTGGGAAAATGAGTCCGTGACC
540-506-bottom-IL22	GTTTTGGTCACGGACTCATTTTCCCACCAA
547-513-top-IL22	GAATCTATGAAGTTGGTGGGAAAATGAGTC
547-513-bottom-IL22	TCACGGACTCATTTTCCCACCAACTTCATA
558-527-top-IL22	TAAAGAGATAAGAATCTATGAAGTTGGT
558-527-bottom-IL22	GTCCCACCAACTTCATAGATTCTTATCT
574-543-top-IL22	GTATTTCTGGTCACTTCTAAAGAGATAA
574-543-bottom-IL22	GATTCTTATCTCTTTAGAAGTGACCAGA
595-563-top-IL22	GAATATAGGACACGGGTCTTTTATTTCT
595-563-bottom-IL22	TGACCAGAAATAAAAGACCCGTGTCCTA
612-580-top-IL22	GCTTATTTCAAAGCACAGAATATAGGAC
612-580-bottom-IL22	CCCGTGTCCTATATTCTGTGCTTTGAAA
628-596-top-IL22	CCAAGTTTTCATTATGGCTTATTTCAAA
628-596-bottom-IL22	TGTGCTTTGAAATAAGCCATAATGAAAA
650-619-top-IL22	GATTTTAAAAATTGAAATAATCTCCAAG
650-619-bottom-IL22	GAAAACTTGGAGATTATTTCAATTTTTA
662-630-top-IL22	AGAGATATAATTATTTTAAAAATTGAAA
662-630-bottom-IL22	GATTATTTCAATTTTTAAAATAATTATA
684-652-top-IL22	GGATTCCATATACTAAAAAAATAGAGATA

684-652-bottom-IL22	GATTATATCTCTATTTTTTTAGTATATGG
700-668-top-IL22	AGCTAGTTATAGTTTAGGATTCCATATA
700-668-bottom-IL22	TTTAGTATATGGAATCCTAAACTATAAC

Ap-1 consensus probe[1]	**Sequence 5' to 3'**
Top	AGCTTCGCTTGATGAGTC
Bottom	GCCGACTGAGTAGTTCGC

RORE element[2]	**Sequence 5' to 3'**
Top	GAAAGTTTTCTGACCCACTTTAAATCA
Bottom	CTTTAACTAAATTTCACCCAGTCTTTT

1. Echlin,D.R., Tae,H.J., Mitin,N. & Taparowsky,E.J. B-ATF functions as a negative regulator of AP-1 mediated transcription and blocks cellular transformation by Ras and Fos. *Oncogene* **19**, 1752-1763 (2000).

2. Yang,X.O. *et al.* T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity* **28**, 29-39 (2008).

Supplementary Methods

Flow cytometry

All flow cytometric data was collected on a FACS Calibur or FACS Canto (both BD Biosciences) and analyzed using FloJo analysis software (Tree Star, Inc.).

The following antibodies were purchased from BD Biosciences; anti-CD4-Allophycoerythrin (APC), CD4-Phycoerythrin (PE)/Cy7 (RM4-5), anti-CD8-APC (53-6.7), anti-CD44-APC (IM7), anti-CD62L-PE (Mel14), anti-CD3-APC (145-2C11), anti-IgM-PE (II/41), anti-B220 Fluorescein isothiocyanate (FITC) (RA3-6B2), anti-IL-17-PE (TC11-18H10), anti-IFNγ- PE (XMG1.2), anti-IFNγ-APC, anti-IL-4-APC (11B11), anti-IL-10-APC (JES5-16E3), anti-CD16/32 (2.4G2), anti-CD11c-PE (HL3), anti-CD11b-PECy7 (M1/70)), anti-CD44-APC (1M7), anti-CD25-APC (3C7), anti-phospho Stat3-AlexaFluor 647 (4/P-Stat3), Streptavidin-PeCy7, 7-AAD, AnnexinV-FITC and AnnexinV staining solution.

The following antibodies and solutions were purchased from eBioscience; anti-AA4.1 APC (AA4.1), anti-IgD PE (11-26c), IL-17A-FITC (eBio17B7), anti-Foxp3 (FJK-16s) and Foxp3 staining buffers.

Anti-CD4-FITC and anti-CD8-FITC were purchased from Invitrogen. Anti-Dec205-biotin (MG38) was purchased from Cedarlane.

CD1d-PBS57-PE and CD1d-unloaded-PE tetramers were obtained from the tetramer facility at the NIH.

Anti-IL-22 (RMF 222CK) was purchased from Antigenix.

Isolation of dendritic cells for flow cytometry. Spleens were isolated, cut into small pieces and digested with Collagenase B (Roche) and DNase I (Sigma) for 30 min at 37^{o}C.

Red blood cells were lysed by incubation with Red Blood Cell Lysis Buffer (Sigma) (1 minute at room temperature). Single cell suspensions were prepared by passing digested spleens through 35μm nylon cell strainers (Fisher Scientific) and were stained with antibodies for analysis by flow cytometry.

Isolation of naïve T cells. Splenic single cells suspensions were generated and red blood cells were lysed by incubation with Red Blood Cell Lysis Buffer (Sigma) (1 minute at room temperature). Splenocytes were then negatively depleted of $B220^+$ and $CD8^+$ cells using magnetically labeled beads followed by depletion over LD columns (all Miltenyi Biotec). The depleted fraction was then stained with antibodies to CD4, CD62L and CD25 (all BD Biosciences) and $CD4^+CD62L^+CD25^-$ cells were sorted on a MoFlo cytometer. Sort purity was generally >98%. For some experiments, as indicated, $CD4^+$ T cells were isolated from spleens by incubation with anti-CD4 magnetic beads and selection via LS columns (Miltenyi Biotec) according to the manufacturer's recommendations.

Cell culture. For T cell differentiation assays, sorted naïve $CD4^+$ $CD62L^+CD25^-$ T cells or magnetically purified $CD4^+$ T cells were isolated as indicated. Cells were cultured at $0.5x10^6$ cells/well in 48 well plates containing plate-bound anti-CD3 (from ascites) and soluble anti-CD28 (37.5; BioXcell; 4μg/ml). Cultures were supplemented with anti-IL-4 (11B11; hybridoma supernatant), IFN-γ (Peprotech; 0.1ng/ml) and IL-12 (Genetics Institute; 10U/ml) for T_H1; anti-IFN-γ (H22; BioXcell; 10μg/ml), anti-IL-12 (Tosh; BioXcell; 10μg/ml) and IL-4 (Peprotech; 10ng/ml) for T_H2; anti-IL-4, anti-IL-12, anti-IFNγ, IL-6 (Peprotech; 20ng/ml) and TGF-β (Peprotech; 0.5ng/ml) for T_H17 differentiation. In some experiments, cultures were supplemented with IL-21 (50ng/ml;

all Peprotech), anti-IL-6 (MP5-20F3; eBioscience; 10μg/ml), anti-TGF-β (1D11, R&D Biosystems, 10μg/ml) or anti-IL-2 (JES6-1A12; BioXcell; 10μg/ml) as indicated. For T_H1 and T_H2 conditions and differentiation without addition of cytokines (Supplementary Fig. 6a) cells were restimulated on day 7 with anti-CD3 and anti-CD28. Brefeldin A was added for the last 4 hours of stimulation. Unless otherwise indicated cells differentiated under T_H17 conditions, were restimulated at the indicated time points with Phorbol 12-myristate 13-acetate (PMA) (50ng/ml; Sigma) and ionomycin (1μM; Sigma) for 4 hours in the presence of Brefeldin A (1μg/ml; Epicentre). Cells were then analyzed by intracellular cytokine staining and flow cytometry.

In some experiments, as indicated, magnetically purified $CD4^+$ T cells from DO11.10 transgenic mice were activated with OVA (3μM) and irradiated syngeneic splenocytes in the presence of anti-IL-4, anti-IL-12, anti-IFNγ, IL-6 and TGF-β (1ng/ml) to induce T_H17 differentiation.

To induce T_H17 differentiation in total splenocytes, single cells suspensions from spleens were prepared and red blood cells were lysed. Total splenocytes were activated at $4x10^6$ cells/well in 12 well plates containing plate-bound anti-CD3, anti-IL-4 (hybridoma supernatant), anti-IL-12 (10μg/ml), anti-IFNγ (10μg/ml), IL-6 (20ng/ml) and TGF-β (1ng/ml). Cells were restimulated with PMA and ionomycin for 4h in the presence of Brefeldin A before intracellular cytokine staining and analysis by flow cytometry.

For STAT3-phosphorylation assays magnetically purified $CD4^+$ or $CD8^+$ T cells were stimulated with anti-CD3 and anti-CD28 in the presence of IL-6 or IL-21 (50ng/ml) followed by intracellular staining and analysis by flow cytometry.

ELISA. The concentration of IL-21 in supernatants from $CD4^+$ T cells activated for 3 days under T_H17 conditions was determined by ELISA (R&D Systems) according to the manufacturer's recommendations.

Isolation of Lamina Propria T cells. For isolation of lamina propria T cells, mice were sacrificed; small intestines removed, placed in cold DMEM media (10%FCS) and cleared of Peyer's patches and residual mesenteric fat tissue. Intestines were then opened longitudinally, cleared of contents and cut into 0.5cm pieces. The pieces were washed multiple times in cold media and twice in ice cold Citrate BSA (CB-BSA) buffer followed by two 15 minute incubations in CB-BSA with agitation. After each incubation cells were vortexed to remove epithelial cells. The remaining intestinal pieces were then washed twice with cold media before digestion in media containing 75U/ml Collagenase IV (Sigma) at 37^{o}C for 1 hour. The solution was vortexed at 20 min intervals to detach lymphocytes. After one hour the solution was filtered through a 35μm strainer, the pieces were collected and digested a second time. Supernatants from both digestions were combined, washed once, suspended in the 70% fraction of a percoll gradient and overlaid with 37% and 30% percoll gradient fractions. Lymphocytes were collected at the 70-37% interface, washed once in PBS and stimulated with PMA/ionomycin in the presence of Brefeldin A for 3 hours before cells were stained for extracellular markers and intracellular cytokines.

Induction of EAE and disease scoring. Age and sex matched mice (7-10 weeks old) were immunized subcutaneously with 100μg MOG_{35-55} peptide (Sigma) emulsified in CFA (IFA supplemented with 500μg *Mycobacterium tuberculosis*) on day 0. On days 1 and 3, mice were injected with 300ng Pertussis Toxin (List Biological Laboratories)

intraperitonally (i.p.). Clinical scores were given on a scale of 1-5 as follows: 0, no overt signs of disease; 1, limp tail or hind limb weakness, but not both; 2, limp tail and hind limb weakness; 3, partial hind limb paralysis; 4, complete hind limb paralysis; 5, moribund state or death by EAE. Mice with a score of 4 were given 300 μl saline solution subcutaneously to prevent dehydration. Mice with a score of 5 were euthanized. Some mice died during the course of the experiment. Their clinical score of 5 was included in the analysis for the remainder of the experiment. For T cell transfer experiments, $CD4^+$ T cells were isolated from splenic single cell suspensions by magnetic separation with anti-CD4 magnetic beads and positive selection via LS columns (Miltenyi Biotec). $1x10^7$ MACS purified $CD4^+$ T cells were injected i.p. on day -4 followed by EAE induction on day 0 as described above.

Isolation of CNS lymphocytes. Brain and spinal cords were removed from mice after perfusion with 30ml of saline solution. Single cell suspensions were prepared by dispersion through sterile 35μ nylon cell strainers (Fisher Scientific) and mixed at room temperature for 1h in HBSS containing 0.1% collagenase, 0.1μg/ml TLCK (N-α-tosyl-L-lysine chloromethylketone hydrochloride), and 10μg/ml DNaseI (all Sigma). The resulting suspension was pelleted, resuspended in the 70% fraction of a Percoll gradient and overlaid by additional 37% and 30% layers. The Percoll gradient separation was achieved by centrifugation for 20 min at 2000rpm and lymphocytes were collected at the 70-37% interface. Subsequently cells were activated with PMA and ionomycin for 3-4 hours in the presence of Brefeldin A and intracellular cytokine staining was performed.

Real time PCR. Naïve $CD4^+CD62L^+CD25^-$ T cells were isolated by cell sorting and activated with plate-bound anti-CD3 and soluble anti-CD28 antibodies under T_H17

conditions for 3 days, unless otherwise indicated. Total RNA was isolated from the indicated cells using Quiagen RNeasy Mini Kit and cDNA was synthesized using SuperscriptIII reverse transcriptase (Invitrogen). Real time PCR analysis was performed using ABI SYBR Green master mix according to the manufacturer's instructions on an ABI7000 machine (Applied Biosystems) using the relative standard curve method. The PCR conditions were 2min at 50^{o}C, 10 min at 95^{o}C followed by 40 2-step cycles of 15s at 95^{o}C and 1min at 60^{o}C.

Primers for RORγt (RORγt forward 5'-CGCTGAGAGGGCTTCAC, RORγt reverse 5'-GCAGGAGTAGGCCACATTACA)[38], IL-21 (IL-21 forward 5'-ATCCTGAACTTCTATCAGCTCCAC, IL-21 reverse 5′-GCATTTAGCTATGTGCTTCTGTTTC)[39], IL-22 (IL-22 forward-5' CATGCAGGAGGTGGTACCTT, IL-22 reverse- 5'-CAGACGCAAGCATTTCTCAG)[40], RORα (RORα forward 5'-TCTCCCTGCGCTCTCCGCAC, RORα reverse 5'-TCCACAGATCTTGCATGGA)[41], IRF-4 (IRF-4 forward 5'-GCCCAACAAGCTAGAAAG, IRF-4 reverse: 5'-TCTCTGAGGGTCTGGAAACT) [42] and HPRT as normalization control (HPRT forward 5'-AGCCTAAGATGAGCGCC, HPRT reverse 5'-TTACTAGGCAGATGGCCACA) were used to evaluate relative gene expression.

For analysis of acute phase response proteins, mice were injected intraperitonally with either 0.9% saline solution or IL-6 (0.3μg per mouse) in 0.9% saline solution. Four hours later, total liver RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturer's recommendations. cDNA was synthesized and real time PCR performed as described above. Primers used for serum amyloid protein P (SAP forward: 5'-

TTTCAGAAGCCTTTTGTCAGA and SAP reverse: 5'-AAGGTCACTGTAGGTTCGGA) [43], c-reactive protein (CRP forward: 5'-TTCTGGATTGATGGGAAAAGC and CRP reverse: 5'- AAACATTGGGGCTGAGTGTC)[43], Serum amyloid protein A (SAA forward 5'-TCTCTGGGGCAACATAGTATACCTCTCAT and SAA reverse 5'-TTTATTACCCTCTCCTCCTCAAGCAGTTAC) [44], fibrinogen β (fibß forward: 5'-ATTAGCCAGCTTACCAGGATGGGACCCAC-3', Fibß reverse: 5'-CAGTAGTAT CTGCCGTTTGGATTGGCTGC-3')[45], alpha-1-acid glycoprotein (AGP forward: TCT CTG AAC TCC GAG GGC TG AGP reverse: GAGACAGAATCAAAGTGCACAGGA)[46] and HPRT as normalization control (HPRT forward 5'-AGCCTAAGATGAGCGCC, HPRT reverse 5'-TTACTAGGCAGATGGCCACA) were used to evaluate relative gene expression.

Gene expression profiling. Naïve $CD4^+$ $CD62L^+$ $CD25^-$ T cells and $CD4^+$ $CD62L^+$ $CD25^+$ regulatory T cells were isolated from C57BL/6 mice. Naïve $CD4^+$ $CD62L^+$ $CD25^-$ T cells were differentiated under T_H1 and T_H2 conditions for 7 days. After restimulation with anti-CD3 and anti-CD28 for 24h, T_H1 and T_H2 cells were sorted for IFN-γ and IL-4 production respectively using cytokine secretion assays (Miltenyi Biotec) according to the manufacturer's recommendations. For gene expression profiling of T_H17 cells, naïve $CD4^+$ $CD62L^+$ $CD25^-$ T cells were activated for 3 days with anti-CD3 and anti-CD28 in the presence of anti-IL-4, anti-IL-12, anti-IFNγ, anti-IL-2, IL-6 and TGF-β (0.5ng/ml). For gene expression analysis in *Batf*$^{-/-}$ T cells, naive $CD4^+$ $CD62L^+$ $CD25^-$ T cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were activated for 3 days with anti-CD3 and anti-CD28 in the presence of either anti-IL-4, anti-IL-12, anti-IFNγ, IL-6 and TGF-β (0.5ng/ml); anti-IL-4,

anti-IL-12, anti-IFNγ, IL-6 and anti-TGF-β; anti-IL-4, anti-IL-12, anti-IFNγ, anti-IL-6 and TGF-β or anti-IL-4, anti-IL-12, anti-IFNγ, anti-IL-6 and anti-TGF-β. IL-2 was neutralized in all conditions. Total RNA was isolated from cells using Quiagen RNeasy Mini Kit. Biotinylated antisense cRNA was generated using two cycle target preparation kit (Affymetrix). After fragmentation, cRNA was hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 Arrays. Data were normalized and expression values were modeled using DNA-Chip analyzer (dChip) software (www.dChip.org).

Retroviral infection and analysis. mRNA was isolated from 129SvEv total thymocytes using Quiagen RNAeasy Mini Kit and cDNA was amplified by SuperscriptIII (Invitrogen). Murine RORγt transcript was amplified using primers 5'-CTCGAGGTGTGCTGTCCTGGGCTAC and 5'-CTCGAGGGGAGACGGGTCAGAGGG. Underlined nucleotides indicate XhoI overhangs used to clone RORγt into XhoI digested GFP-RV retrovirus[47] or XhoI digested hCD4-RV[48].

Batf cDNA was cloned from $CD4^+$ T cell mRNA using primers 5'-GGAAGATTAGAACCATGCCTC and 5'-AGAAGGTCAGGGCTGGAAG and subcloned into the GFP-RV retrovirus[47]. An N-terminal FLAG tag was introduced by Quick Change Mutagenesis kit (Stratagene) using the primers 5'-**G**GACTACAAAGACGATGACGACAAGCCTCACAGCTCCGACAGCA and 5'-CTTGTCGTCATCGTCTTTGTAGTCCATGGTTCTAATCTTCCAGATC. The underlined sequence indicates nucleotides used to introduce the FLAG-tag.

The retrovirus based reporter hCD4-pA-GFP-RV[48], in which a cytoplasmic truncated human CD4 (hCD4) marks viral infection and green fluorescence protein (GFP) is used to

report promoter activity has been described previously and was modified as follows to generate hCD4-pA-GFP-RV-IL-17p. The 1021bp promoter region of murine IL-17a was generated by PCR from genomic 129SvEv DNA using primers 5'-AGCTTGAACAGGAGCTATCGGTCC and 5'-AAGCTTGAGGTGGATGAAGAGTAGTGC. Underlined nucleotides indicate overhangs containing HindIII restriction sites used to clone the resulting PCR product into hCD4-pA-GFP-RV.

Retroviral vectors were packaged in Phoenix E cells as described previously[47]. Magnetically purified $CD4^+$ T cells were infected with viral supernatants on days 1 and 2 after activation with anti-CD3 and anti-CD28. 3 days after activation cells were restimulated with PMA/ionomycin in the presence of Brefeldin A and analyzed by intracellular cytokine staining and Flow Cytometry. For the experiments in Figure 4, $CD4^+$ T cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ mice were activated under T_H17 conditions and infected with the IL-17 reporter virus. Stably infected T cells were restimulated with PMA/ionomycin for 4h and examined for GFP expression on day 3 after initial activation.

Statistical Analysis. A Student's unpaired two-tailed t-test was used to indicate statistically significant differences between indicated groups. Differences with a *P* value <0.05 were considered significant.

Electrophoretic mobility shift assays. Whole cell extracts were prepared from total splenocytes activated for 3 days with anti-CD3, TGF-β and IL-6 as described previously[49]. For EMSA analysis the AP-1 consensus probe[36] (top: AGCTTCGCTTGATGAGTC and bottom: GCCGACTGAGTAGTTCGC), RORE

element in CNS2 of the IL-17 gene[41] (top: GAAAGTTTTCTGACCCACTTTAAATCA and bottom: CTTTAACTAAATTTCACCCAGTCTTTT)
and -187 to -155 of the IL-17 promoter (top: GGTTCTGTGCTGACCTCATTTGAGGATG and bottom: AAAAGACTGGGTGAAATTTAGTTAAAG), Eα Y box probe (TCGACATTTTTCTGATTGGTTAAAAGTC)[50] were used after labeling with ^{32}P-dCTP. The probe ($2.5x10^4$cpm per reaction) was used along with 15μg of total cell extracts and 1ug poly diDC as described previously[50].

For competitor-supershift assay, Batf binding to the AP-1 consensus probe[36] was assessed by anti-FLAG supershift. Unlabeled probes from the IL-17a, IL-21 and IL-22 promoters (Supplementary table 4) were used to compete for Batf binding to the AP-1 consensus probe. Single stranded overhangs of the competitor oligos were not filled in. Sequences identified as competitors for Batf binding were used to determine the Batf consensus motif.

For supershift analysis of the EMSA complexes formed on the AP-1 probe, whole cell extracts were prepared as above. 8μg whole cell extracts were incubated for 15min on ice with anti-Batf, anti-Fos (K25), anti-c-Jun (D), anti-c-Jun (N), anti-JunB (C11), anti-JunD (329), anti-ATF-1 (H60) and anti-ATF-3 (C-19) (all Santa Cruz Biotechnology) before $2.5x10^4$cpm of the AP-1 consensus probe was added.

To test whether Batf binding to the AP-1 probe requires stimulation DO11.10 transgenic $CD4^+$ T cells were activated for 3 days with OVA, irradiated APCs, anti-IFN-γ/IL-4/IL12, TGF-β and IL-6, followed by a period of 3 days rest in the presence of TGF-β and IL-6. Cells were left untreated or activated with PMA/ionomycin for 4 hrs before

whole cell extracts were prepared and used in EMSA analysis as described above. Reactions were electrophoresed on 7.5 or 10% bisacrylamide gels to achieve optimal band separation.

CONSENSUS program for determination of Batf binding motif. Sequences of the proximal promoter regions of IL-17, IL-21, and IL-22 identified as competitors for Batf binding in the competitor-supershift EMSA assay were input into CONSENSUS version v6d[51]. Default program parameters were applied, except for searching the reverse complement of the input sequences (c2) and uniform background nucleotide frequencies. The program was searching potential motif lengths from 5 to 15 using the expected frequency statistic (e-value) and the optimal motif length was determined as 7. The corresponding weight matrix, with a sample size adjusted information content of 4.467, was chosen from the final cycle. The enrichment of the binding motif in the input set was verified using PATSER v3e[52]. Using the numerically calculated cutoff score, 38/40 of the input training sequences were identified as containing the motif. The motif is presented as a Weblogo[37] in which the size of each nucleotide is proportional to the frequency of its appearance at each position.

Batf Chromatin immunoprecipitation (ChIP). ChIP was performed as previously described[53] using an affinity purified anti-Batf rabbit polyclonal antibody prepared by Brookwood Biomedical (Birmingham, AL). Briefly, chromatin was prepared from $1x10^7$ $CD4^+$ T cells isolated from C57BL/6 *Batf*$^{+/+}$ mice stimulated under T_H17 polarizing conditions with anti-CD3 (2.5μg/ml) and syngeneic splenic feeder cells, then restimulated or not at the indicated time points with PMA (50ng/ml) and ionomycin (750ng/ml) for 4 h. For experiments assessing early binding of Batf to the DNA $CD4^+$ T

cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ 129SvEv mice were activated with anti-CD3/CD28 coated beads under T_H17 conditions for 24 hours, then processed for ChIP analysis. Immunoprecipitations were performed with 20 μg/ml anti-Batf rabbit polyclonal antibody using the Chromatin Immunoprecipitation (ChIP) Assay Kit from Millipore (Billerica, MA) according to the manufacturer's recommendations. Immunoprecipitated DNA released from cross-linked proteins was quantitated by real-time PCR as previously reported[53], and was normalized to input DNA. Unless otherwise indicated data are presented as mean + s.d from 2 independent experiments. All real-time PCR primers and probes are included in Supplementary table 5. The analyzed sites are denoted relative to the ATG start codons for the *Il17a* or *Il17f* gene.

For ChIP analysis of the IL-21 and IL-22 promoters DO11.10 transgenic $CD4^+$ T cells from *Batf*$^{+/+}$ and *Batf*$^{-/-}$ were stimulated with OVA and APC for 3 days, rested for 3 days before restimulation with PMA/ionomycin for 4h on day 5 and processing for ChIP as described above. Real time PCR analysis was performed using ABI SYBR Green master mix according to the manufacturer's instructions on a Step One Plus (Applied Biosystems) using the relative standard curve method. Results were normalized to input DNA. Sequences of primers used in the analysis are given in Supplementary Table 5.

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