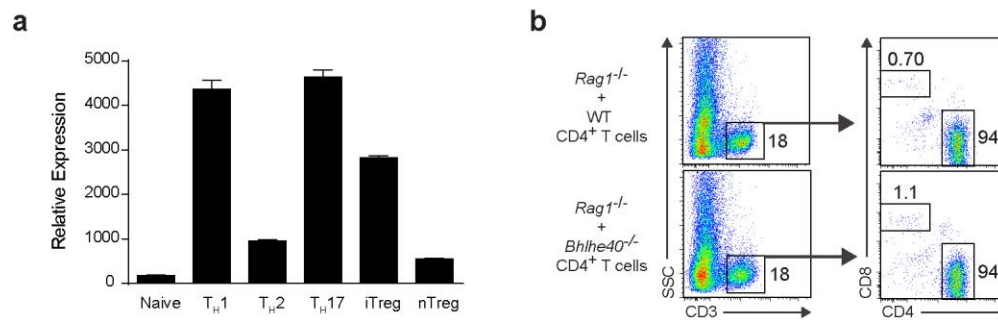


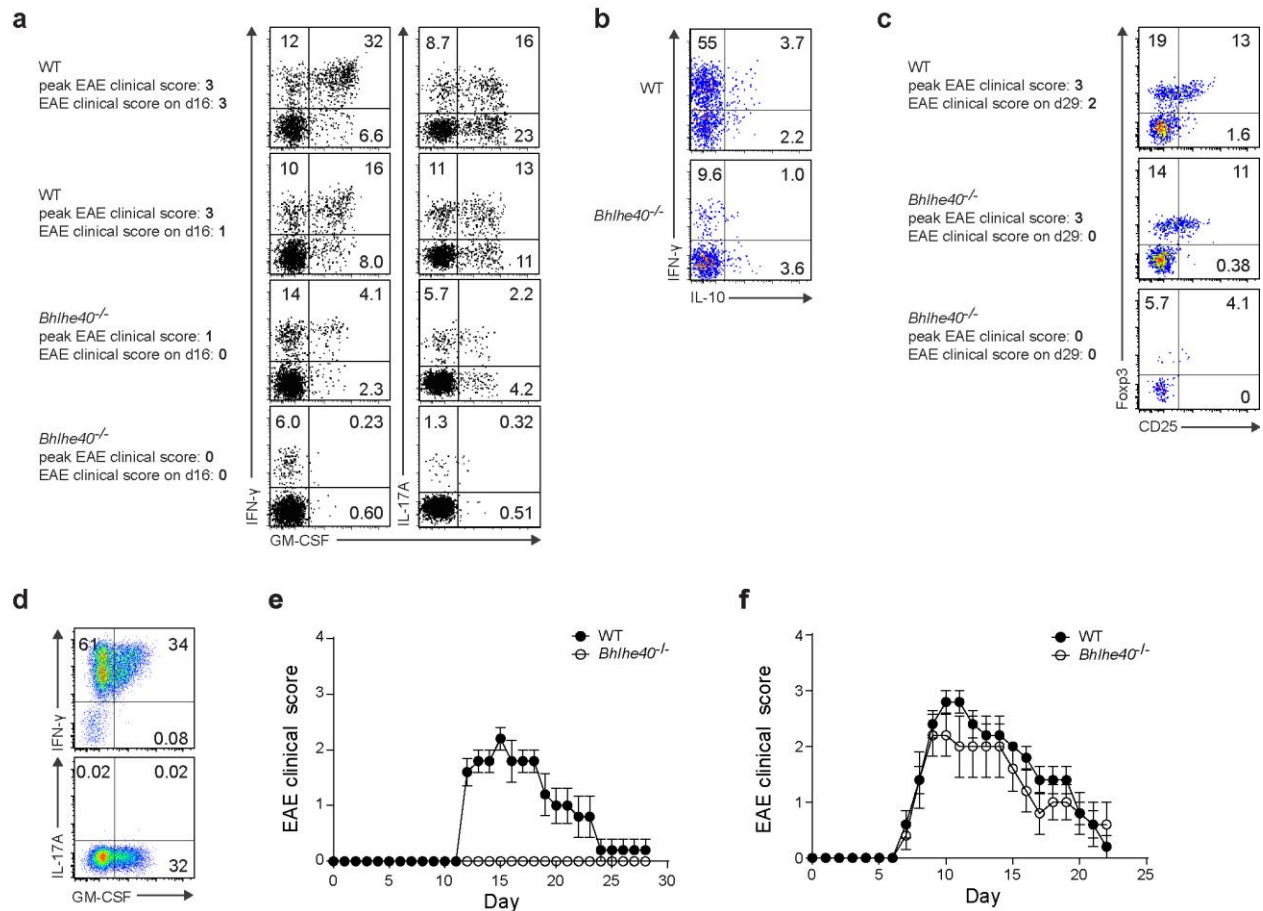
SUPPLEMENTARY FIGURES

Supplementary Figure 1



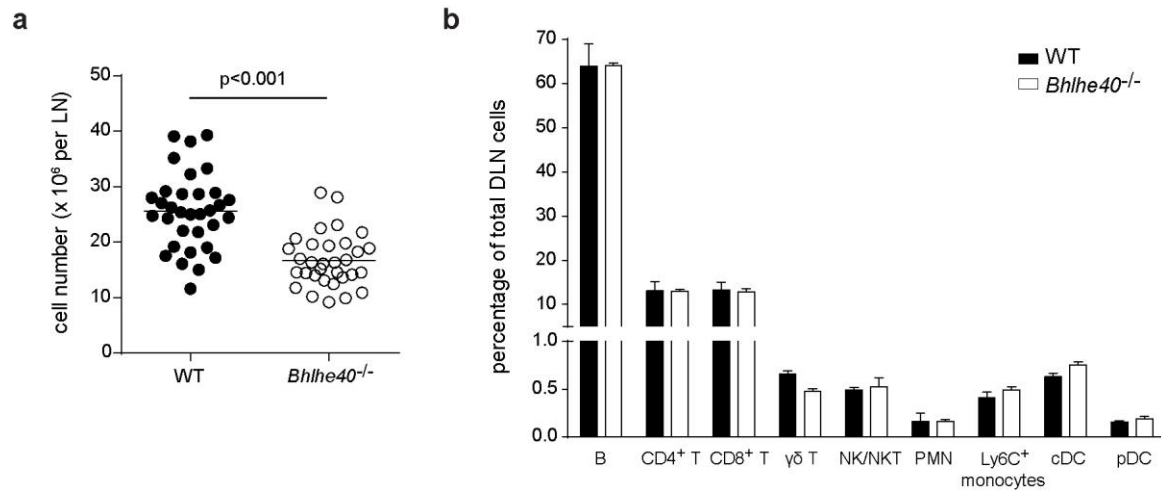
Supplementary Figure 1. Activated T_H cells express *Bhlhe40*. **a**, Relative expression of *Bhlhe40* determined by expression microarrays performed by Wei et al. (GSE14308). For all supplementary figures throughout, error bars show mean \pm s.e.m. **b**, $Rag1^{-/-}$ mice used in Figure 1d were sacrificed at day 38 after CD4⁺ T cell transfer (day 37 following EAE induction). Spleens were analyzed by flow cytometry.

Supplementary Figure 2



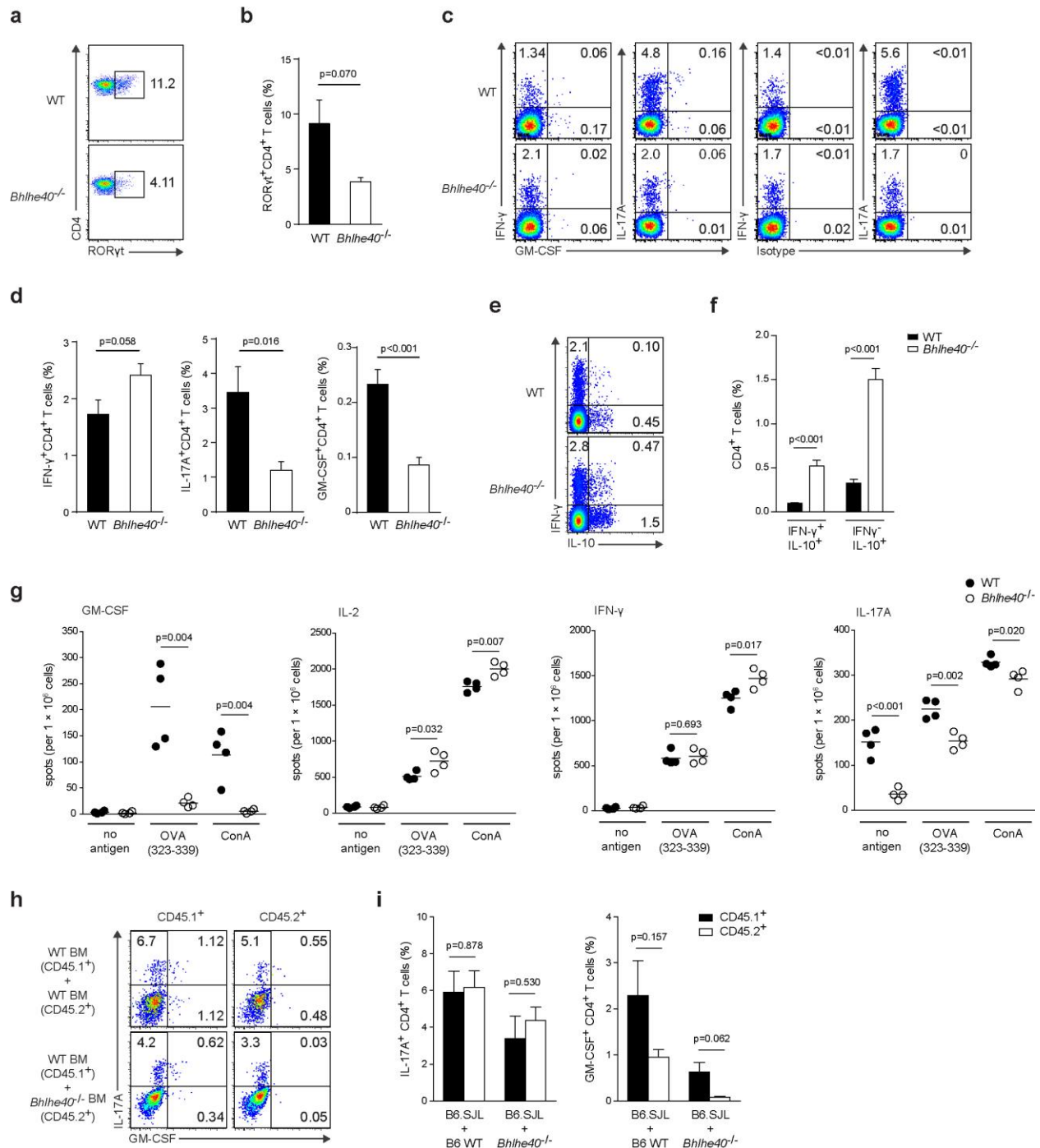
Supplementary Figure 2. CD4⁺ T cells from *Bhlhe40*^{-/-} mice with mild EAE produce low amounts of GM-CSF in the CNS. **a**, Infiltrating CNS cells were prepared from WT and *Bhlhe40*^{-/-} mice on day 16 after EAE induction and ICS was performed. Four mice are shown. The first WT mouse achieved a peak EAE clinical score of 3 on day 11, and maintained this score through day 16. The second WT mouse achieved a peak EAE clinical score of 3 on days 12 and 13, but EAE had resolved to a clinical score of 1 on day 15 and 16. The first *Bhlhe40*^{-/-} mouse showed mild clinical EAE, with a peak clinical score of 1 on days 11 and 12 with resolution to a score of 0 on days 13 through 16. The second *Bhlhe40*^{-/-} mouse showed no evidence of disease through day 16 (clinical score of 0). **b**, Flow cytometry of CD4⁺ T cells in the CNS of WT and *Bhlhe40*^{-/-} mice on day 14 after immunization. Representative ICS for IFN- γ and IL-10. **c**, Flow cytometry of CD4⁺ T cells in the CNS of WT and *Bhlhe40*^{-/-} mice on day 29 after immunization. Representative staining for CD25 and Fop3 to identify Tregs. **d**, ICS of our WT MOG(35-55)-specific T_H1 cell line upon PMA/ionomycin stimulation tested on the day of adoptive transfer. **e**, **f**, Mean clinical scores of EAE in WT or *Bhlhe40*^{-/-} mice after receipt of (e) 5 million or (f) 10 million adoptively transferred MOG(35-55)-specific WT T_H1 cells. Incidence of disease: (e) WT mice 5/5, *Bhlhe40*^{-/-} mice 0/5, (f) WT mice 5/5, *Bhlhe40*^{-/-} mice 5/5.

Supplementary Figure 3



Supplementary Figure 3. Decreased cellularity of DLNs in immunized *Bhlhe40*^{-/-} mice. **a**, WT (n=33) and *Bhlhe40*^{-/-} (n=32) mice were immunized with MOG(35-55)/CFA, and DLNs were collected at day 7. Live cell numbers per DLN were determined. Data are compiled from 10 independent experiments. **b**, Frequencies of the indicated cell types in DLNs from immunized mice at day 7 (n=3 mice per group). Cell types were identified by the following surface markers using flow cytometry: B cells, B220⁺MHC II⁺; $CD4^+$ T cells, B220⁻CD3e⁺CD4⁺CD8⁻; $CD8^+$ T cells, B220⁻CD3e⁺CD4⁻CD8⁺; $\gamma\delta$ T cells, B220⁻CD3e⁺CD4⁻CD8⁻ $\gamma\delta$ TCR⁺; NK/NKT cells, B220⁻NK1.1⁺; Polymorphonuclear leukocytes (PMNs), B220⁻NK1.1⁻Ly6G^{high}CD11b⁺; Ly6C⁺ monocytes, B220⁻NK1.1⁻Ly6G^{neg/low}CD11b⁺Ly6C^{high}; conventional dendritic cells (cDCs), B220⁻NK1.1⁻Ly6G^{neg/low}CD11c^{high}MHC II⁺; plasmacytoid DCs (pDCs), B220⁺CD3e⁻Siglec H⁺.

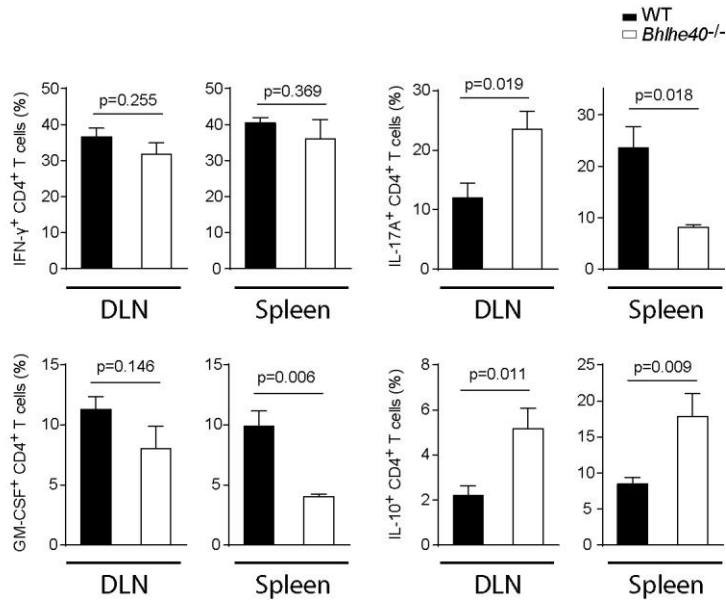
Supplementary Figure 4



Supplementary Figure 4. CD4⁺ T cells require Bhlhe40 for normal cytokine production after immunization. a-f, WT and *Bhlhe40*^{-/-} mice were immunized with MOG(35-55)/CFA, and DLNs were collected at day 7. (a) Representative plots showing intracellular staining for RORγt gated on CD4⁺ T cells. (b) Frequencies of RORγt⁺ CD4⁺ T cells (n=3 per group). (c) Representative plots gated on CD4⁺ T cells, showing ICS data for the indicated cytokines. Data

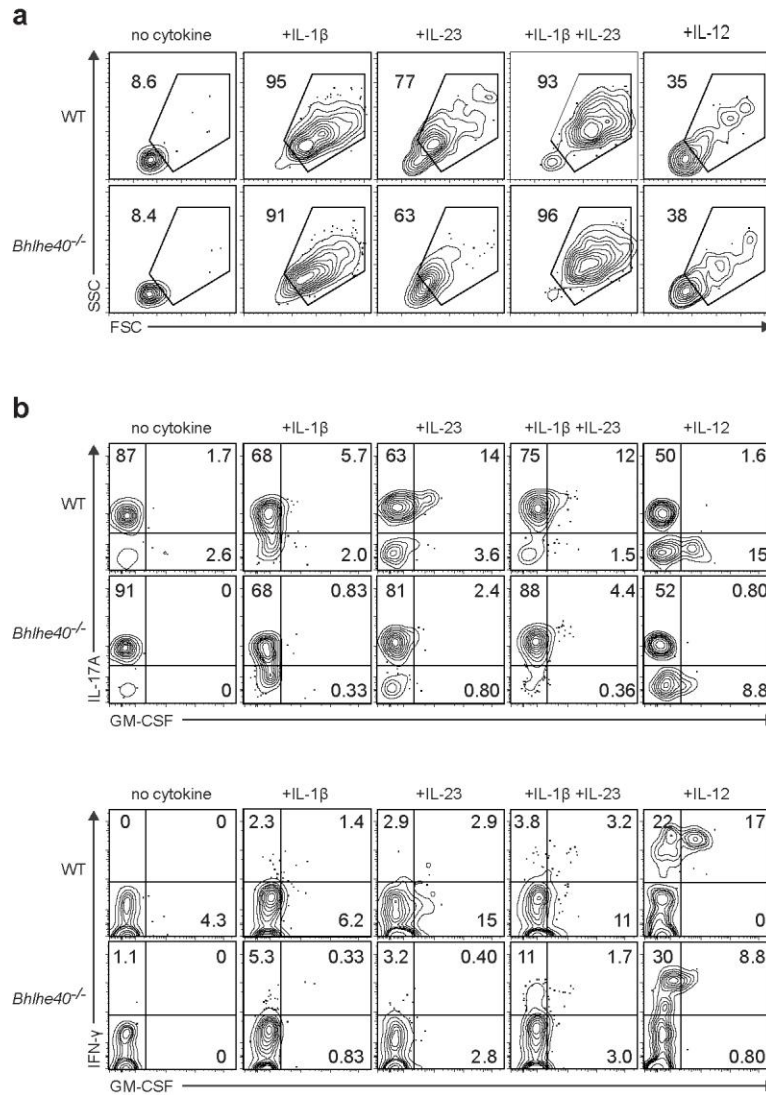
from stains in which a PE-isotype control antibody (anti-KLH) was included in place of PE-anti-GM-CSF are also presented. **(d)** Frequencies of IFN- γ ⁺, IL-17A⁺, and GM-CSF⁺ CD4⁺ T cells (n=6 per group). **(e)** Representative plots gated on CD4⁺ T cells, showing ICS data for IFN- γ and IL-10. **(f)** Frequencies of IFN- γ ⁺IL-10⁺ and IFN- γ ⁻IL-10⁺ CD4⁺ T cells (n=3-4 per group). **(g)**, ELISPOT assays for the quantitation of cells secreting GM-CSF, IL-2, IFN- γ , and IL-17 performed on DLN cells 7 days after immunization of WT and *Bhlhe40*^{-/-} mice with OVA(323-339) (n=4 per group). **(h, i)**, ICS on DLN cells from the indicated MOG(35-55)/CFA-immunized mixed BM chimeric mice (n=3 per group). **(h)** Representative plots gated on CD4⁺CD45.1⁺ or CD4⁺CD45.2⁺ T cells. **(i)** Frequencies of IL-17A⁺ and GM-CSF⁺ CD4⁺ T cells (either CD45.1⁺ or CD45.2⁺).

Supplementary Figure 5



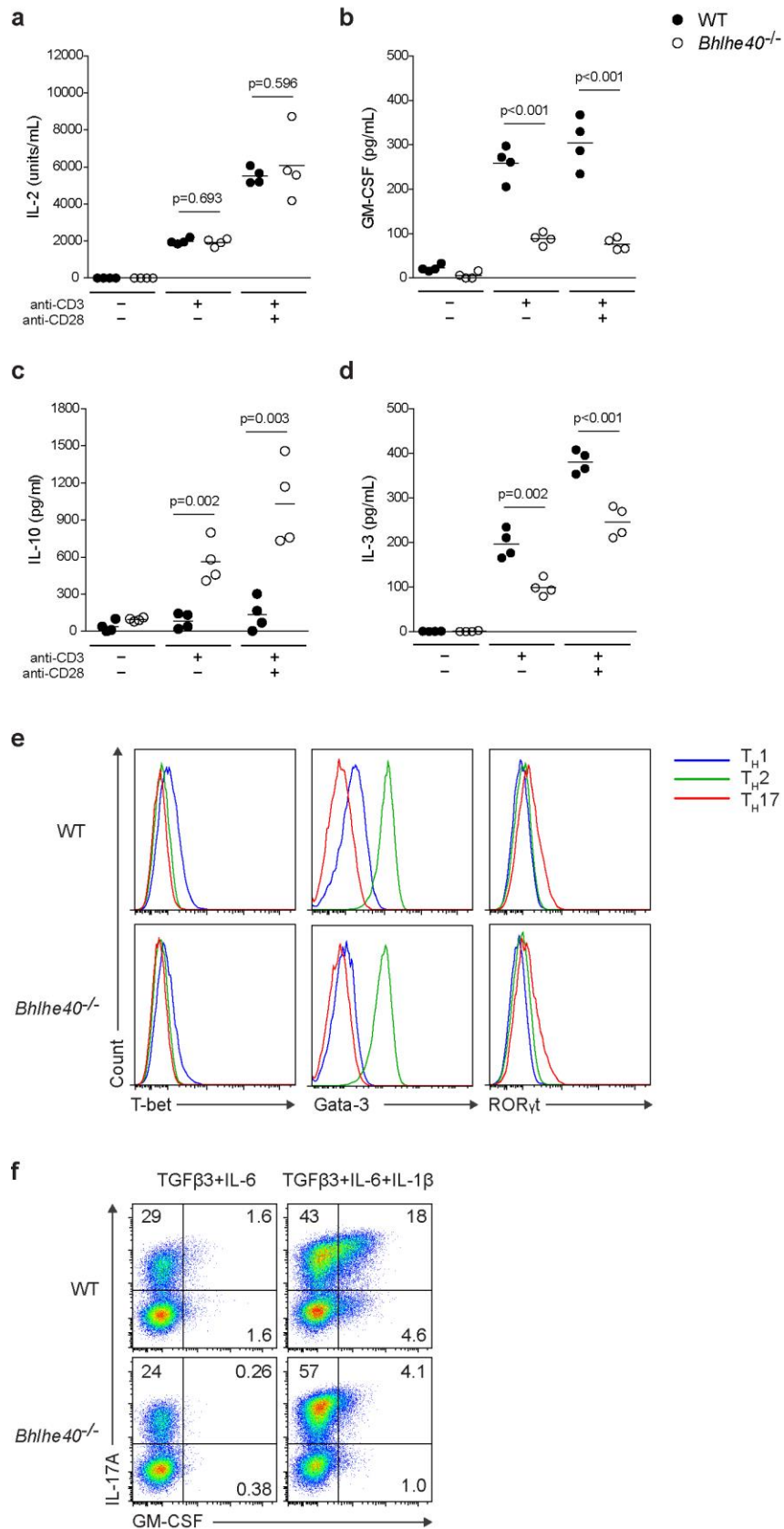
Supplementary Figure 5. *Bhlhe40*^{-/-} CD4⁺ T cells display a cell-intrinsic defect in GM-CSF and IL-10 production after adoptive transfer into *Rag1*^{-/-} recipients. *Rag1*^{-/-} mice were immunized with MOG(35-55)/CFA one day after receiving transfers of 7 million purified WT or *Bhlhe40*^{-/-} CD4⁺ T cells. At day 7, responses in the spleen and DLN were analyzed by ICS for the indicated cytokines, gating on CD4⁺ T cells.

Supplementary Figure 6



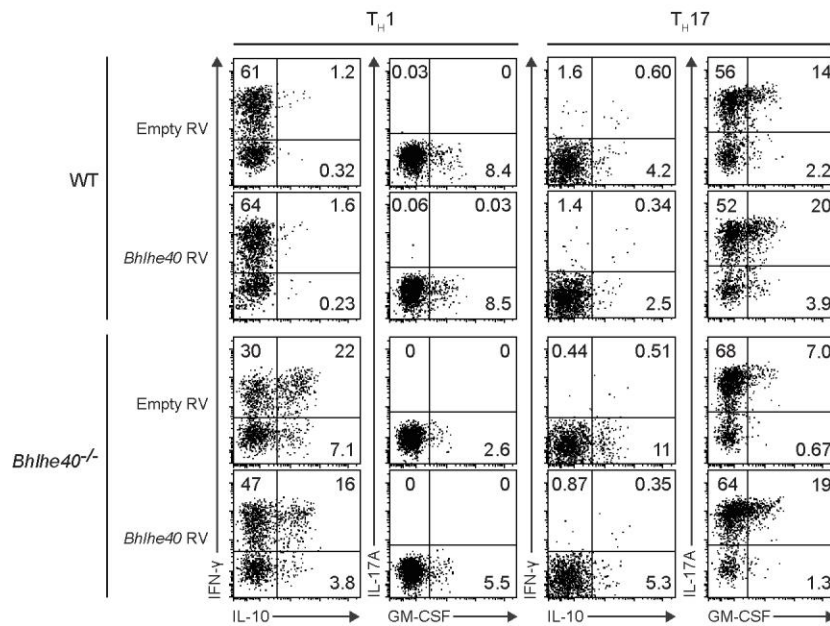
Supplementary Figure 6. $\gamma\delta$ T cells require *Bhlhe40* for GM-CSF production in response to cytokines. **a**, WT and *Bhlhe40*^{-/-} splenocytes were cultured with the indicated cytokines for 3 days in the absence of TCR stimulation. Cells were analyzed by flow cytometry for forward scatter (FSC) and side scatter (SSC) after PMA/ionomycin stimulation. Plots are gated on $\gamma\delta$ T cells. **b**, WT and *Bhlhe40*^{-/-} splenocytes were cultured with the indicated cytokines for 3 days in the absence of TCR stimulation. Cells were stimulated with PMA/ionomycin in the presence of brefeldin A for 4 hours and analyzed for IL-17A, GM-CSF, and IFN- γ by intracellular staining (i.e. our normal ICS protocol). Plots are gated on $\gamma\delta$ T cells.

Supplementary Figure 7



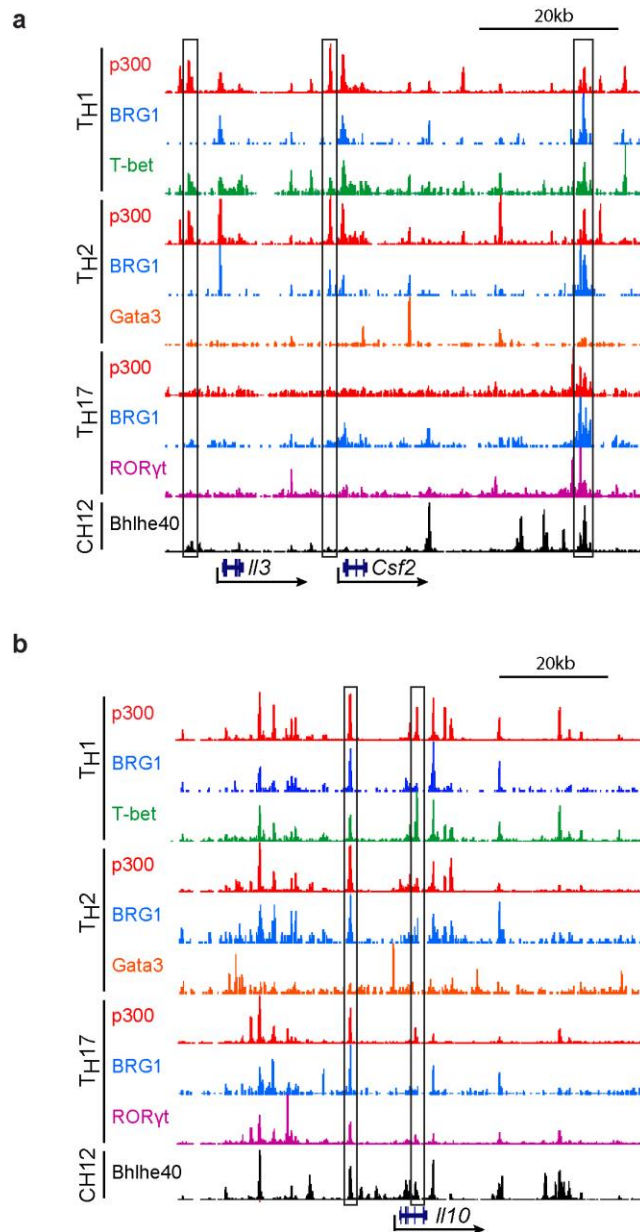
Supplementary Figure 7. Cytokine secretion and transcription factor expression by *Bhlhe40*^{-/-} CD4⁺ T cells. **a-d**, CD4⁺ T cells from WT and *Bhlhe40*^{-/-} mice (n=4 per group) were stimulated for 48 hours with plate-bound anti-CD3 and anti-CD28 antibodies as indicated. **(a)** IL-2, **(b)** GM-CSF, **(c)** IL-10, and **(d)** IL-3 were measured in the supernatant by ELISA. **e**, CD4⁺ T cells from WT and *Bhlhe40*^{-/-} mice (n=2-4 per group) were polarized in T_H1, T_H2, or T_H17 culture conditions for 4 days and intracellularly stained for expression of T-bet, Gata-3, and RORγt. Representative histograms are shown. **f**, CD4⁺ T cells from WT and *Bhlhe40*^{-/-} mice were polarized in T_H17 culture conditions using TGF-β3 for 4 days and intracellularly stained for expression of IL-17A and GM-CSF.

Supplementary Figure 8



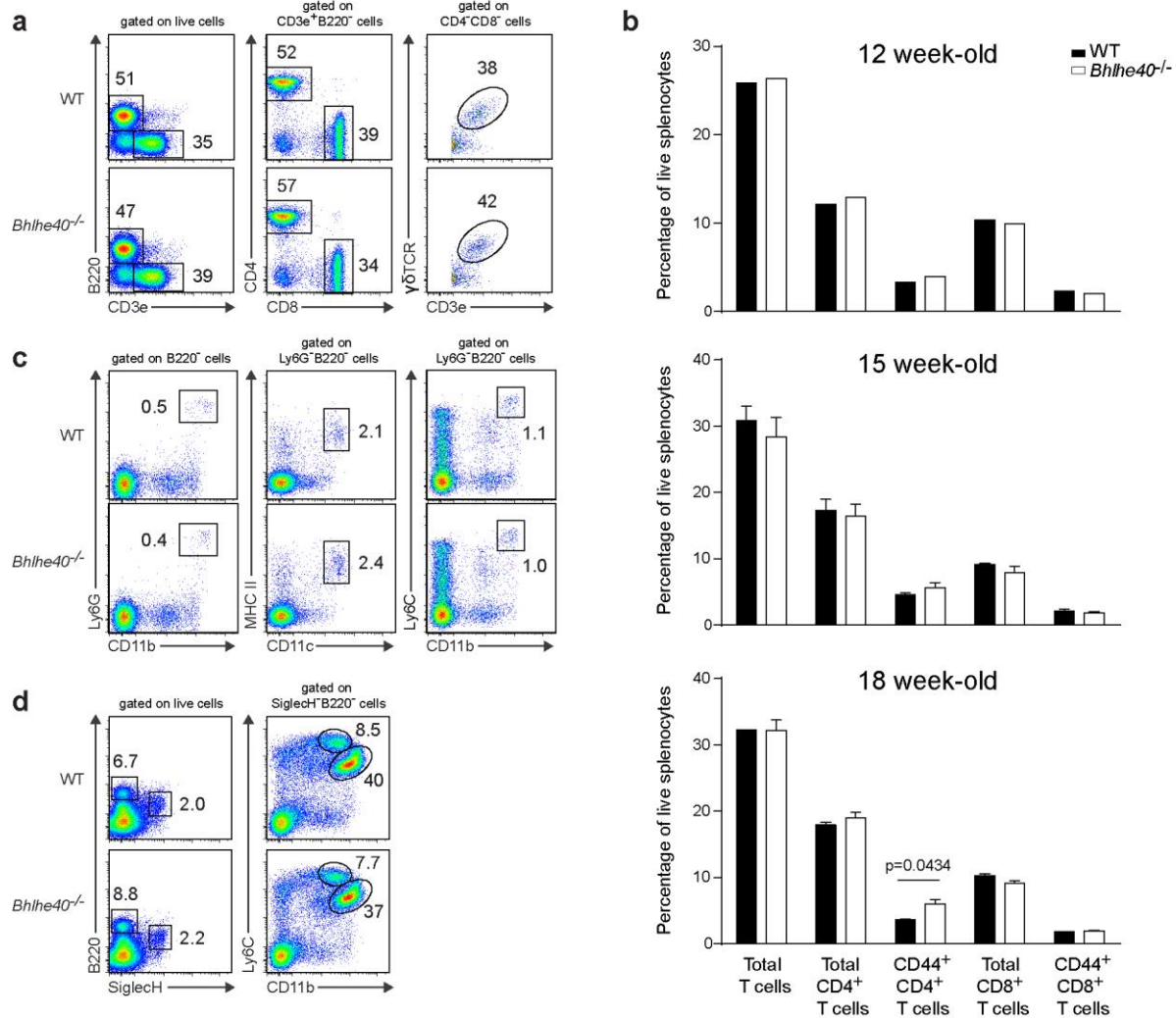
Supplementary Figure 8. Retroviral transduction of *Bhlhe40* corrects the GM-CSF and IL-10 production by *Bhlhe40*^{-/-} T_H cells. ICS on CD4⁺ T cells from WT or *Bhlhe40*^{-/-} mice following T_H1 or T_H17 polarization and retroviral transduction with control empty retrovirus (RV) or *Bhlhe40* RV. Plots are gated on GFP⁺CD4⁺ T cells.

Supplementary Figure 9



Supplementary Figure 9. Bhlhe40 binds multiple sites within the *Il3/Csf2* and *Il10* loci. **a**, **b**, ChIP-Seq binding tracks derived from T_H cells for p300, BRG1, T-bet, Gata3, and RORyt, and from CH12 cells for Bhlhe40 at the (**a**) *Il3/Csf2* and (**b**) *Il10* loci. Boxes indicate regions previously identified as enhancers for *Il3/Csf2* or *Il10*.

Supplementary Figure 10



Supplementary Figure 10. Flow cytometry of splenocytes and bone marrow cells from WT and *Bhlhe40*^{-/-} mice. **a**, Representative flow cytometry on splenocytes from 15 week-old WT and *Bhlhe40*^{-/-} mice showing populations of B cells (B220⁺CD3e⁻), CD4⁺ T cells (B220⁻CD3e⁺CD4⁺CD8⁻), CD8⁺ T cells (B220⁻CD3e⁺CD4⁻CD8⁺), and $\gamma\delta$ T cells (B220⁻CD3e⁺CD4⁻CD8⁻TCR $\gamma\delta$ ⁺). **b**, Percentages of splenic T cell populations in 12 (n=1 mouse per group), 15 (n=3 mice per group), and 18 week-old (n=2 mice per group) WT and *Bhlhe40*^{-/-} mice. **c**, Representative flow cytometry on splenocytes from 15 week-old WT and *Bhlhe40*^{-/-} mice showing populations of PMNs (B220⁻Siglec H⁺Ly6G⁺CD11b⁺), cDCs (B220⁻Siglec H⁺Ly6G⁻CD11c^{high}MHC II⁺), and Ly6C⁺ monocytes (B220⁻Siglec H⁺Ly6G⁻CD11b⁺Ly6C^{high}). **d**, Representative flow cytometry on bone marrow cells from 15 week-old WT and *Bhlhe40*^{-/-} mice showing populations of B cells (B220⁺Siglec H⁻), pDCs (B220⁺Siglec H⁺), PMNs (B220⁻Siglec H⁺CD11b⁺Ly6C^{mid}), and Ly6C⁺ monocytes (B220⁻Siglec H⁺CD11b⁺Ly6C^{high}).