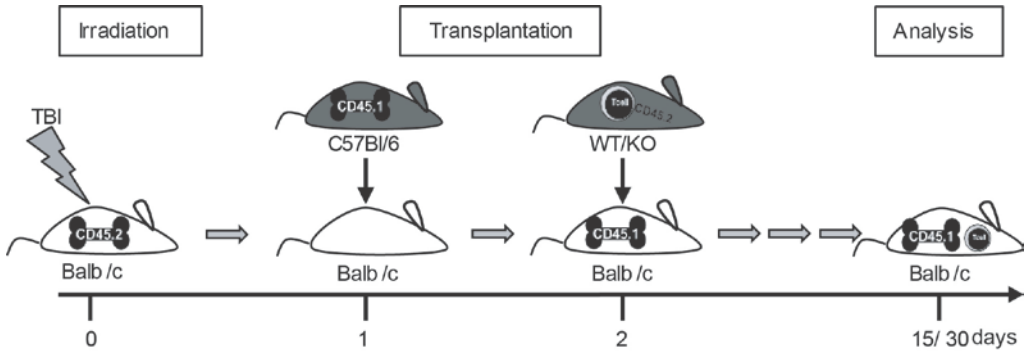
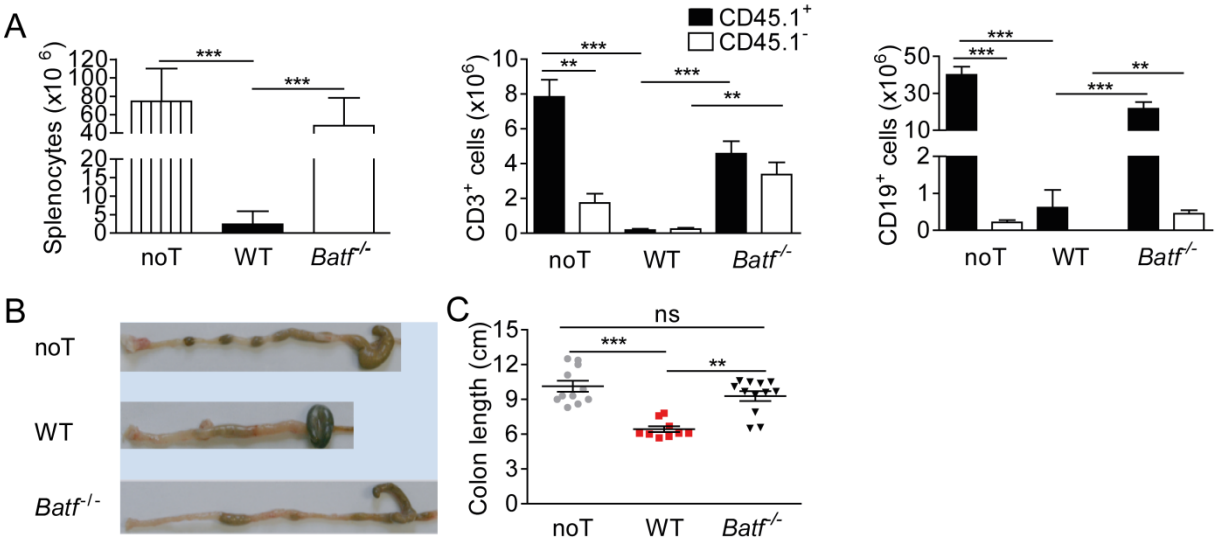
Supplementary Figure 1



Supplementary Figure 1

Schematic outline of the induction protocol of MHC full-mismatched GVHD.

Female Balb/c mice were conditioned by total body irradiation (TBI) with 8 Gy at d0 followed by allogeneic T cell-depleted CD45.1$^+$ B6.SJL WT BMT at d1. Allo-reactive C57Bl/6 CD45.2$^+$CD3$^+$ T cells isolated from WT or gene-deficient donor mice (knockout, KO) were adoptively transferred at d2 as described in the online method section. Further analyses were performed at consecutive days as indicated in the figure legends.

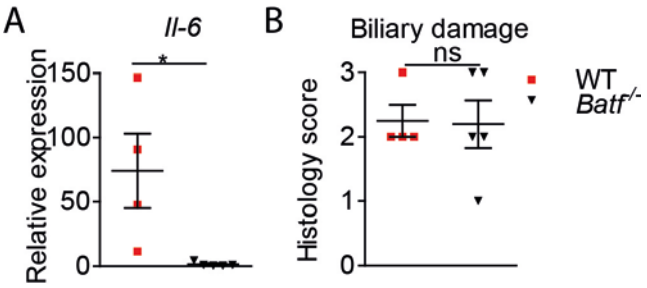


Supplementary Figure 2

Allogeneic Batf$^{-/-}$ *donor T cells protect against hampered T and B cell reconstitution in murine GVHD*

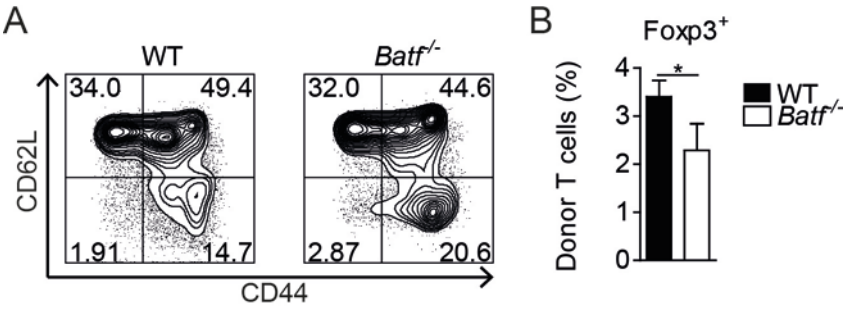
(A) At d30 after allogeneic BMT and donor T cell transfer (C57Bl/6 in Balb/c), splenic reconstitution of BM derived CD45.1^{+} total cells, CD3^{+} T cells and CD19^{+}CD3^{-} B cells were assessed by flow cytometry. Bar graphs represent pooled mean values ± SEM of at least noT (n=14), WT (n=13) and *Batf*$^{-/-}$ (n=15) individual mice derived from three to four independent experiments. One-way ANOVA test with Dunn´s multiple comparisons posttest was used for statistical analyses. (B, C) Assessment of the shortening of colon lengths ex vivo at d30 of GVHD-prone mice transferred with indicated genotypes. Representative images (B) and dot plot graphs (C) representing mean values of colon lengths in cm ± SEM of noT (n=11), WT (n=9) and *Batf*$^{-/-}$ (n=12) individual mice derived from three independent experiments are shown. One-way ANOVA test with Dunn's multiple comparisons posttest was used for statistical analyses. Bar graphs represent mean values ± SEM. (A-D) ns= not significant; *p<.05; **p<.01; ***p<.001; ****p<0.0001.

Supplementary Figure 3



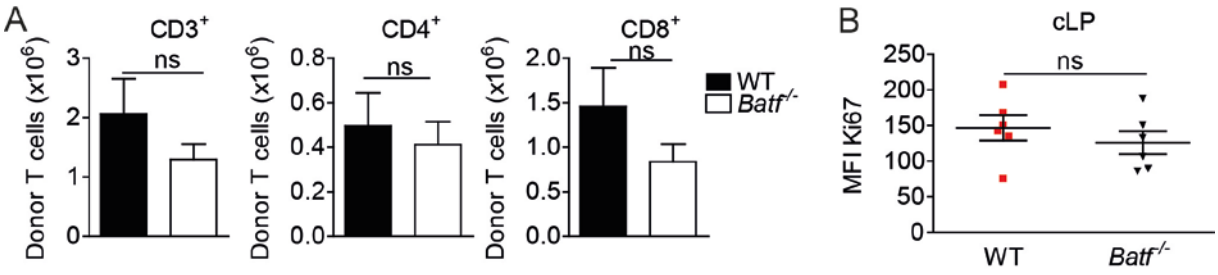
Supplementary Figure 3
Upregulation of IL-6 expression within colonic tissues but not biliary duct damage in mice suffering from (miHA) mismatched GVHD depend on T cell intrinsic BATF expression
(A) WT and *Batf*$^{-/-}$ CD3^{+}C57Bl/6 donor T cells were transferred into irradiated H-2b^{+} Balb.b mice after transplantation of T cell-depleted CD45.1^{+}B6.SJL WT bone marrow. *Il-6* transcript levels expressed within colon tissues was determined by qPCR during established GVHD-associated colitis at d28. Gene expression levels represent normalized, relative fold of expression compared to the expression that was detected in colon tissue of mice receiving T cell-depleted BM only (not shown). (B) Histopathological assessment of GVHD-associated liver lesions are shown as scatter blots summarizing the histological assessment of biliary damage in mice treated as described in (A). Data represent mean values ± SEM. Unpaired Student's t-*test (two-sided)* (E) was used to test for statistical significance. ns= not significant; *p<.05; **p<.01; ***p<.001; ****p<0.0001.

Supplementary Figure 4



Supplementary Figure 4
Batf$^{-/-}$ *donor T cells do not contain higher frequencies of memory T cells prior transfer and do not predominately differentiate into regulatory T cells after transfer in GVHD-prone mice*
(A) Splenic WT and *Batf*$^{-/-}$ CD4^{+} C57Bl/6 donor T cells were analyzed for CD44 and CD62L surface expression. Representative contour blots are displayed derived from one experiment with n=5 mice/ genotype. In (B), lethally irradiated Balb/c mice received allogeneic T cell-depleted CD45.1^{+}B6.SJL WT BM and allo-reactive C57Bl/6 WT or *Batf*$^{-/-}$ donor CD3^{+} T cells. 28d later cLP cell fractions were isolated and the frequencies of CD45.2^{+}CD4^{+}CD3^{+}FoxP3^{+} donor-derived regulatory cLP T cells were determined by flow cytometry. Data represent mean values ± SEM of at least 8 mice for each genotype derived from 3 independent experiments. Unpaired Student's t-*test (two-sided)* was used to test for statistical significance. ns= not significant; *p<.05; **p<.01.

Supplementary Figure 5

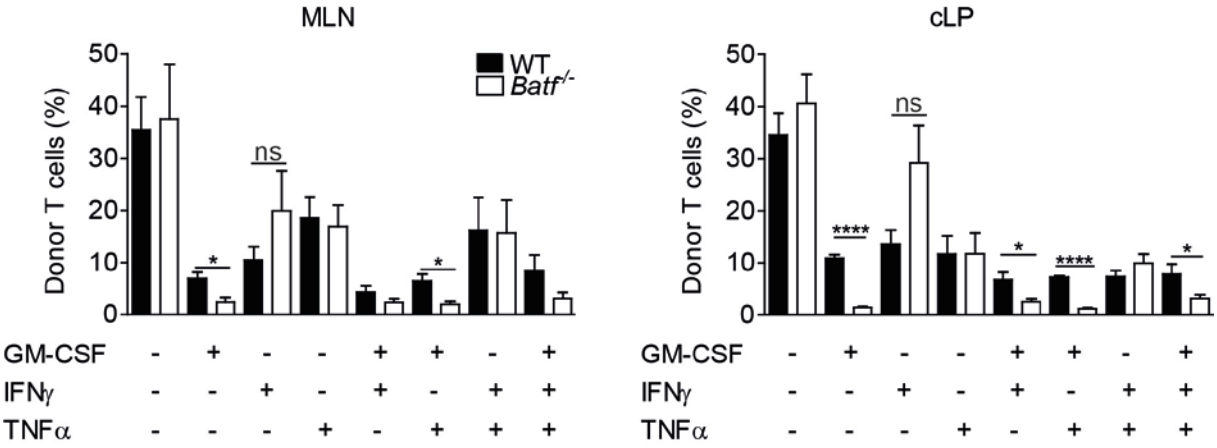


Supplementary Figure 5

Both the pool of MLN donor T cell subsets and Ki67$^+$ cLP T cells are not regulated by BATF

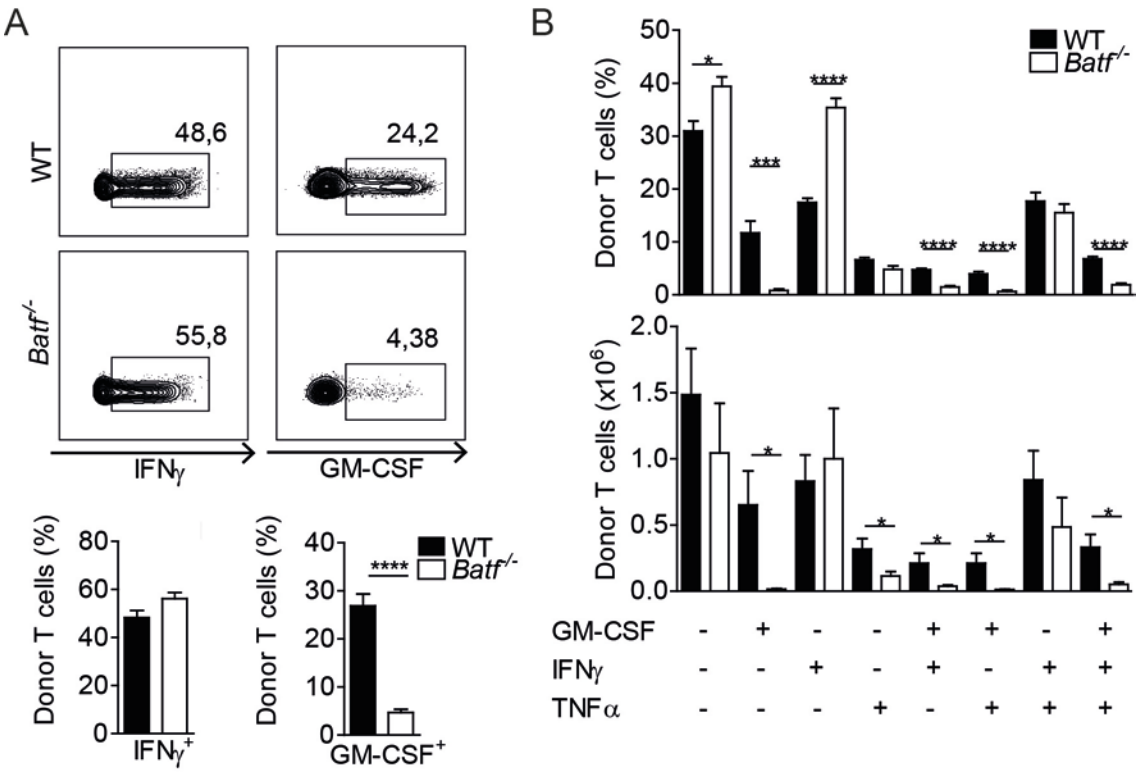
(A-B) WT (black bars) and *Batf*$^{-/-}$ (white bars) CD3$^+$C57Bl/6 donor T cells were transferred into irradiated Balb/c mice after transplantation of T cell-depleted CD45.1$^+$B6.SJL WT BM. At d12, the absolute pool of MLN-resident H-2d$^-$CD45.1$^-$CD3$^+$, CD4$^+$ and CD8$^+$ donor T cells resp. was calculated by flow cytometric analyses. Data represent mean values ± SEM of n=8 WT and n=7 *Batf*$^{-/-}$ individual mice. In (B) mice treated as in (A) were euthanized at d15, cLP cell fractions were isolated and then quantitative flow cytometry analyses of the median fluorescence intensities (MFI) of Ki67 expression levels of H-2d$^-$CD45.2$^+$CD4$^+$Ki67$^+$ donor T cells were performed. Data represent mean values ± SEM of n=6 individual mice/ genotype. Unpaired Student's t-*test (two-sided)* was used to test for statistical significance. ns= not significant.

Supplementary Figure 6



Supplementary Figure 6
BATF deficiency affects multiple subsets of GM-CSF$^+$CD4$^+$ donor T cells in GVHD-prone mice
WT (black bars) and *Batf*$^{-/-}$ (white bars) CD3$^+$ C57Bl/6 donor T cells were transferred into irradiated Balb/c mice after transplantation of T cell-depleted CD45.1$^+$B6.SJL WT BM (C57Bl/6 into Balb/c). At d15, MLN- (left) and cLP-resident (right) H-2d$^-$CD45.2$^+$CD4$^+$ donor T cells were simultaneously evaluated for GM-CSF, IFNγ and TNFα cytokine expression employing intracellular cytokine staining and flow cytometric analyses. Data represent mean values ± SEM of n=6 individual *Batf*$^{-/-}$ mice/ genotype individual mice. Unpaired Student's t-*test (two-sided)* was used to test for statistical significance. ns= not significant; *p<.05; **p<.01; ***p<.001; ****p<0.0001.

Supplementary Figure 7

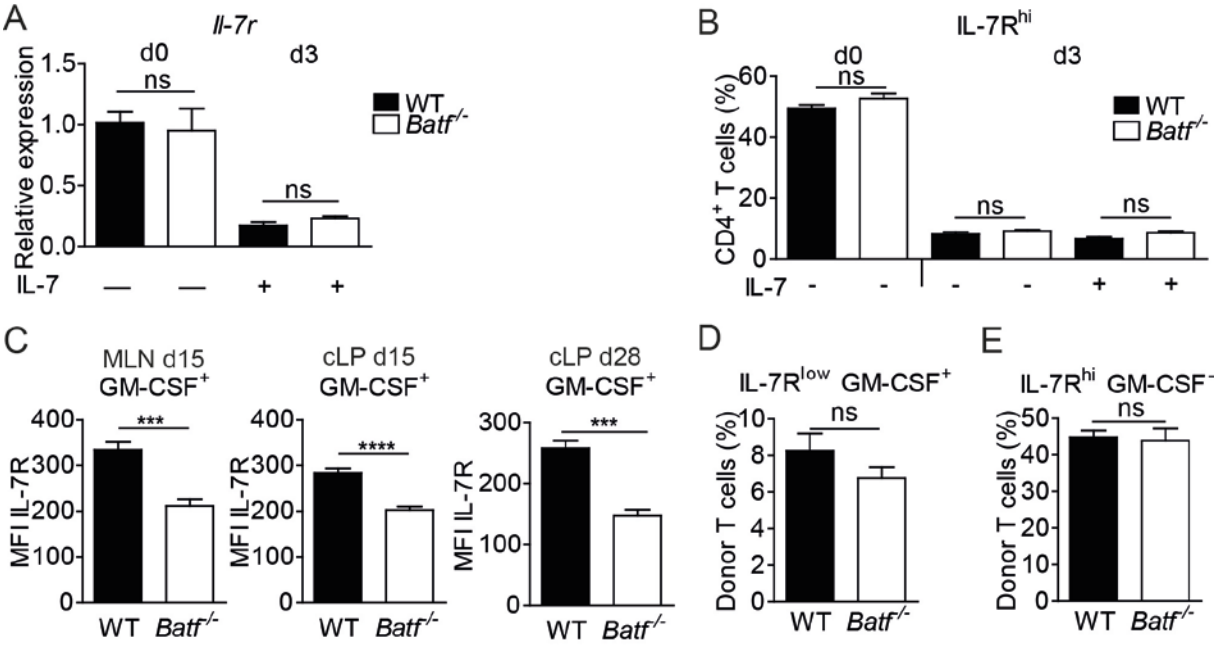


Supplementary Figure 7

GM-CSF$^+$CD4$^+$ donor T cell pool is equally BATF-dependent in the context of a (miHA)-mismatched GVHD model

(A-B) Transfer of allogeneic WT (black bars) and *Batf*$^{-/-}$ (white bars) CD3$^+$ C57Bl/6 donor T cells into irradiated H-2b$^+$ Balb.b mice after transplantation of T cell-depleted CD45.1$^+$B6.SJL WT BM. At d27, cLP-resident CD45.2$^+$CD3$^+$CD4$^+$ donor T cells were simultaneously evaluated for GM-CSF, IFNγ and TNFα cytokine expression profiles employing intracellular cytokine staining and flow cytometric analyses. Data in (A) display representative contour blots (top and middle rows) and bar graphs (bottom row) summarize mean values ± SEM of relative frequencies of IFNγ$^+$ and GM-CSF$^+$ cells within cLP-resident CD45.2$^+$CD3$^+$CD4$^+$ donor T cells consisting of n=4-5 individual mice/ genotype. In (B) GM-CSF, IFNγ and TNFα cytokine expression were simultaneously assessed by intracellular cytokine staining and flow cytometric analyses. Data are displayed as relative frequencies (top row) or absolute numbers (bottom row). Unpaired Student's t-*test (two-sided)* was used to test for statistical significance. ns= not significant; *p<.05; **p<.01; ***p<.001; ****p<0.0001.

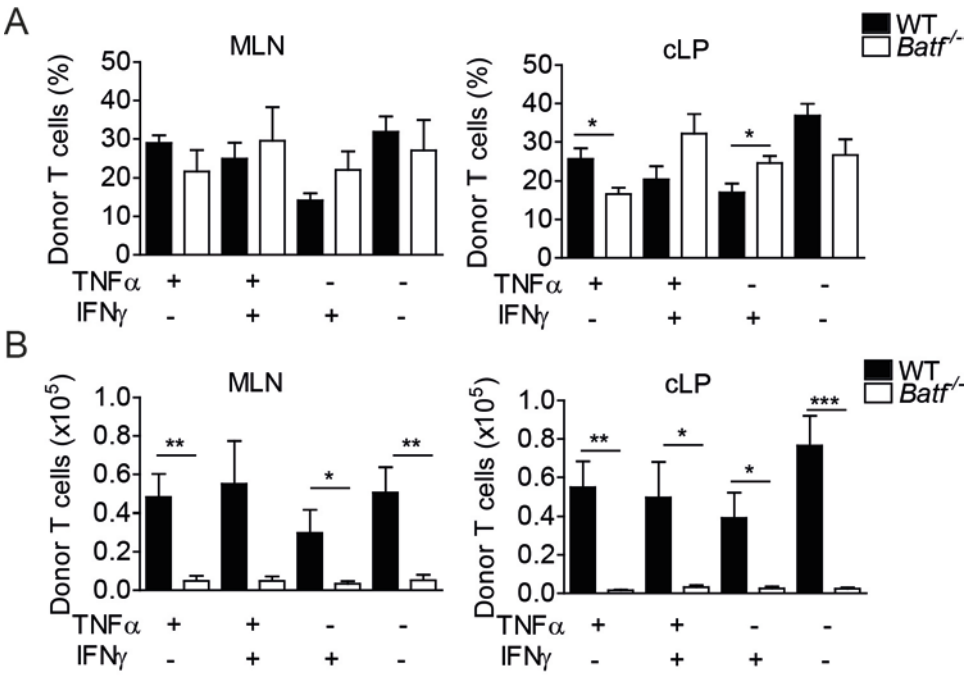
Supplementary Figure 8



Supplementary Figure 8

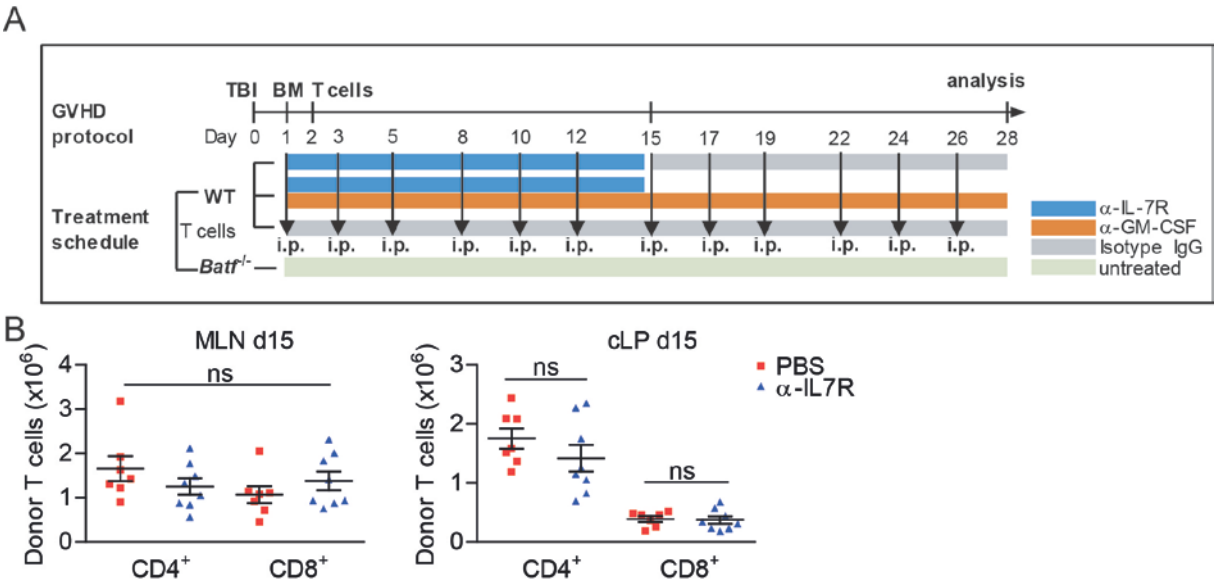
(A, B) IL-7R gene (A) and protein (B) expression levels of naïve and T cell receptor activated *Batf*$^{+/+}$ and *Batf*$^{-/-}$ CD4^{+} T cells cultured for 3d in the presence or absence of IL-7 were assessed by qPCR and flow cytometry resp. (A) For qPCR analyses, gene expression levels at indicated time points are displayed as normalized, relative fold of expression in comparison to levels measurable in freshly isolated, naïve CD4^{+} T cells (d0, IL-7R). Gene expression levels within naïve CD4^{+} WT T cells were arbitrarily set to 1. Bar graphs display mean values ± SEM of pooled data of two independent experiments and display (A) without (-)IL-7 (WT: n=5; *Batf*$^{-/-}$: n=3) and at d3 with (+)IL-7 (WT: n=3 and *Batf*$^{-/-}$: n=2) individual mice; in (B) without (-)IL-7 (WT: n=13 and *Batf*$^{-/-}$: n=13) and at d3 without (-)IL-7 (WT: n=10 and *Batf*$^{-/-}$: n=8) and with (+)IL-7 (WT: n=10 and *Batf*$^{-/-}$: n=8) individual mice are displayed. (C) Quantitative analyses of the median fluorescence intensities (MFI) of IL-7R expression levels of colonic GM-CSF^{+}CD45.2^{+}CD4^{+} MLN and cLP donor T cells 15d and 28d resp. after GVHD induction (C57Bl/6 into Balb/c) by adoptive transfer of C57Bl/6 WT (black) and *Batf*$^{-/-}$ (white) CD3^{+} T cells according to the gating as outlined in Figure 8C. Mean values ± SEM of n=6 WT and n=4 *Batf*$^{-/-}$ individual mice derived from one representative of two independent experiments are shown. (D, E) Relative frequencies of IL-7R$^{-/low}$GM-CSF^{+} (D) and IL-7R^{hi}GM-CSF^{-} (E) donor-derived T cells within the colonic LP CD45.2^{+}CD3^{+}CD4^{+} T cell compartment at d28 after GVHD induction as described in (C) were assessed by intracellular cytokine staining (FACS) ex vivo. IL-7R$^{-/low}$GM-CSF^{+} (D) and IL-7R^{hi}GM-CSF^{-} (E) donor-derived T cell subpopulations are defined according to the gating shown in Figure 8C. Bar graphs represent mean values ± SEM with WT (n=7) and *Batf*$^{-/-}$ (n=4) individual mice derived from one representative out of two independent experiments. One-way ANOVA test with Bonferroni`s multiple comparisons posttest (A-B) and unpaired Student's t-*test (two-sided)* (C-E) were used for statistical analyses. ns= not significant; *p<.05; **p<.01; ***p<.001; ****p<0.0001.

Supplementary Figure 9



Supplementary Figure 9
BATF-dependent IL-7R^{hi}GM-CSF^{+}CD4^{+}T cells display a pro-inflammatory cytokine expression profile
(A-B) WT (black bars) and *Batf*$^{-/-}$ (white bars) CD3^{+}C57Bl/6 donor T cells were transferred into irradiated Balb/c mice after transplantation of T cell-depleted CD45.1^{+} B6.SJL WT BM at d15. MLN- (left) and cLP-resident (right) IL-7R^{hi}GM-CSF^{+} subset within H-2d^{-}CD45.2^{+}CD4^{+} donor T cells was simultaneously evaluated for IFNγ and TNFα cytokine expression employing combined intracellular cytokine and surface staining flow cytometric technologies. In (A) relative frequencies and in (B) absolute numbers are displayed. Data represent mean values ± SEM of n=6 individual mice/ genotype. Unpaired Student's t-*test (two-sided)* was used to test for statistical significance. ns= not significant; *p<.05; **p<.01; ***p<.001; ****p<0.0001.

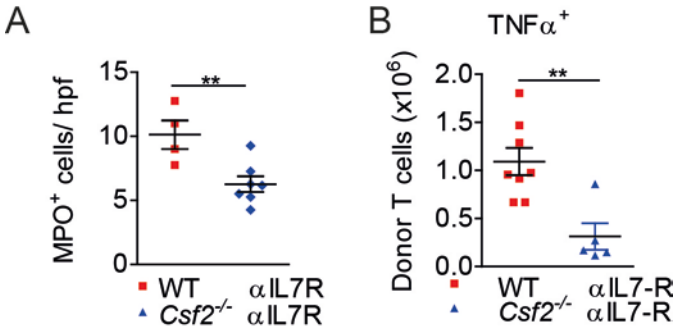
Supplementary Figure 10



Supplementary Figure 10

(A) Schematic illustration of the therapeutic interventions applied to GVHD-prone mice following adoptive transfer of allogeneic WT or Batf$^{-/-}$ CD3^{+} T cells (C57Bl/6 into Balb/c). As indicated, transient anti-IL-7R antibody-mediated blockade alone or in combination with a continuously given anti-GM-CSF blocking antibody was applied to WT T cell receiving mice while *Batf*$^{-/-}$ T cell receiving mice served as untreated controls (untreated). Mice transiently treated with anti-IL-7R antibody (blue) alone received starting at d15 equivalent amounts of isotype IgG control antibody (grey) indicated by the discontinued blue line. As a control, one group was treated with the isotype IgG control antibody alone throughout the experiment. Antibody preparations were given i.p. 3x/ week starting at the day of allogeneic T cell transfer until the treatment regimen was terminated as indicated. (B) Flow cytometric quantification of absolute numbers of CD4^{+} and CD8^{+} T cells within the H2d^{-}CD45.2^{+} MLN and cLP resp. donor T cell compartment 15d after GVHD induction (C57Bl/6 into Balb/c) in WT donor T cell receiving mice treated either with 300µg IL-7R blocking antibodies (blue triangles) or just vehicle (PBS, red squares) 3x/ week. Data represent mean values ± SEM of n=7 vehicle-treated and n=8 anti-IL-7R antibody–treated WT mice. One-way ANOVA test with Bonferroni's multiple comparisons posttest was used to test for statistical significance. ns= not significant.

Supplementary Figure 11



Supplementary Figure 11
IL-7R blockade in the absence of T cell-intrinsic GM-CSF reduces the colonic influx of pro-inflammatory immune cells
(A) Histopathological cross-sections from the colon of GVHD-prone mice following adoptive transfer of allogeneic WT (red squares) or *Csf2*$^{-/-}$ (blue diamonds) CD3^{+} T cells (C57Bl/6 into Balb/c) and systemic anti-IL-7R blockade 3x/week until d15 as described in Figure 10A were assessed for the presence of MPO^{+} cells 28d after GVHD induction. Data represent mean values ± SEM from WT (n=4) and *Csf2*$^{-/-}$ (n=7) individual mice derived from one experiments. (B) At d28, cLP-resident CD45.2^{+}CD3^{+}CD4^{+} donor T cells from indicated mice and described as in (A) were evaluated for TNFα cytokine expression by flow cytometry. Absolute numbers are shown and data represent mean values ± SEM displaying WT (n=7) and *Csf2*$^{-/-}$ (n=6) individual mice. Unpaired Student's t-*test (two-sided)* was used to test for statistical significance. ns= not significant; *p<.05; **p<.01.