

Supplemental Legends

Table 1: GVHD clinical score.

Clinical GVHD scores at time point after allo-HCT (n=6-17 per group, pooled from 2 experiments). Mean values of clinical GVHD score \pm standard error of mean (SEM) are given. Differences in clinical GVHD scores between C3H.sw \rightarrow B6 and C3H.sw \rightarrow *Batf3*^{-/-} groups were statistically evaluated using Mann-Whitney test. **p<0.01.

Table 2: GVHD Histopathological score on day 14.

B6WT and *Batf3*^{-/-} animals received 10Gy on day -1 and 0.5x10⁶ CD8⁺T cells along with 5x10⁶ TCD-BM cells from either syngeneic B6 or allogeneic C3H.sw donors and analyzed histopathological GVHD scores of GI tract (small and large intestine and liver on day 14 (n=5-9 per group, pooled from 2 experiments). *p<0.05, syngeneic B6 \rightarrow B6 vs allogeneic C3H.sw \rightarrow B6 and C3H.sw \rightarrow *Batf3*^{-/-} animals.

Figure 1: Serum cytokines on day 14.

B6WT and *Batf3*^{-/-} animals received 10Gy on day -1 and 0.5x10⁶ CD8⁺T cells along with 5x10⁶ TCD-BM cells from either syngeneic B6 or allogeneic C3H.sw donors. Serum levels of IFN- γ (a) and IL-17A (b) on day 21 (n=4-7 per group, pooled from 2 experiments). (The bars represent mean \pm standard deviation. N.S denotes no significant differences.

Figure 2: Spleen DCs from *Batf3*^{-/-} animals show impaired cross-presentation.

CD8⁺ T cell proliferation. (a) Representative figure of CFSE-labeled splenic CD8⁺ T cells from B6 mice were incubated with spleen CD11c⁺ DCs from either B6 (left) or *Batf3*^{-/-} (right) animals and irradiated class I^{-/-} splenocytes and MBL-2 tumor lysate for 4 days. (b) The proliferation of CD8⁺ T cells stimulated by *Batf3*^{-/-} DCs were significantly decreased (n=3). Data are representative of two independent experiments and bars represent as the mean ± standard deviation. (c) Representative figure of spleen CD11c⁺CD8⁺DCs. (right) WT, (middle) *Batf3*^{-/-} (6x backcrossed), (left) *Batf3*^{-/-} (10xbackcrossed). The number showed the percentage of CD8⁺DCs in total CD11c⁺DCs population. (d) The summary of the percentage of spleen CD11c⁺CD8⁺DCs in WT, *Batf3*^{-/-} (6x backcrossed) and *Batf3*^{-/-} (10xbackcrossed) animals.

Figure 3: TLR3 expression on host hematopoietic APCs are critical for GvT

In order to test GvT responses, both B6 WT and *Tlr3*^{-/-} animals received 10Gy on day -1 and 0.5x10⁶ CD8⁺ T cells along with 5x10⁶ TCD-BM from either syngeneic B6 or allogeneic MHC match, miHA mismatched C3H.sw donors and added syngeneic syngeneic 2x10⁴ MBL-2 tumor cells at the time of BMT. (a) Combined tumor mortality data from two independent experiments showed. n=6-11 per group. p=0.01 when allo-B6 WT animals compared with allo-*Tlr3*^{-/-} animals. To examine that the impact of TLR3 deficiency only on host APC without other confounding effects, we firstly generated [WT-B6→B6 Ly5.2] and [*Tlr3*^{-/-} B6→ B6 Ly5.2] chimeras. After four months later, these animals were used as recipients in MHC matched, multiple minor mismatched allo-BMT. They received 9Gy and 0.5x10⁶ CD8⁺ T cells along with 5x10⁶ TCD-BM from either syngeneic B6 or allogeneic C3H.sw donors. (b) Clinical GVHD score (n=5-12 per group, pooled from 2 experiments). N.S means not significant differences. (c.d) Serum levels of

IFN- γ (c) and IL-17A (d) on day 14 after allo-BMT (n=6-12 per group, pooled from 2 experiments). (e) Photon flux/animal. [*Tlr3*^{-/-} B6 \rightarrow B6 Ly5.2] animals showed high luciferase activity on day 28 after allo-BMT. * p<0.05, allo [WT-B6 \rightarrow B6 Ly5.2] vs allo [*Tlr3*^{-/-} B6 \rightarrow B6 Ly5.2]. Data are representative of two independent experiments.

Figure 4: Stimulation of TLR3 on host hematopoietic APCs enhances GvT

BMT and tumor induction were performed in the [WT-B6 \rightarrow B6Ly5.2] and [*Tlr3*^{-/-}B6 \rightarrow B6Ly5.2] animals as in Methods. The recipient animals received TLR3 stimulant poly IC or the diluent control and analyzed for GvHD and tumor free survival. Photon flux/animal. [WT-B6 \rightarrow B6 Ly5.2] animals with poly I:C showed less luciferase activity after allo-BMT. *p<0.05, allo [WT-B6 \rightarrow B6 Ly5.2] with polyI:C vs allo[WT-B6 \rightarrow B6] with PBS and allo[*Tlr3*^{-/-} B6 \rightarrow B6 Ly5.2] with PBS.

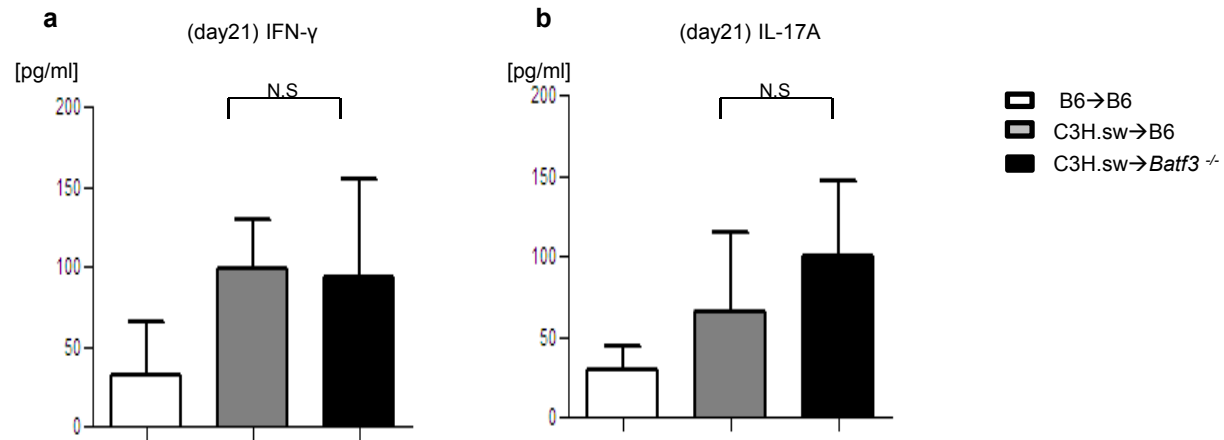
Supplemental Tables 1: Clinical scores of GVHD Severity are similar in *Batf3*^{-/-} and WT recipients.

Day	C3H.sw→B6	C3H.sw→ <i>Batf3</i> ^{-/-}	P value
7	2.18±0.22 (n=11)	2±0.24 (n=17)	P=0.44
14	1.86±0.29 (n=11)	1.25±0.16 (n=16)	P=0.06
21	3.32±0.48 (n=11)	1.81±0.22 (n=16)	P=0.005**
28	3.33±0.36 (n=9)	2.47±0.33 (n=15)	P=0.11
35	4.07±0.68 (n=7)	2.54±0.43 (n=14)	P=0.08
42	3.58±0.80 (n=6)	2.69±0.46 (n=13)	P=0.37
49	4.00±0.91 (n=5)	2.25±0.52 (n=10)	P=0.09
56	3.00±0.58 (n=4)	1.89±0.38 (n=9)	P=0.10
63	2.75±0.43 (n=4)	2.00±0.35 (n=9)	P=0.18
70	2.63±0.38 (n=4)	2.06±0.29 (n=9)	P=0.34

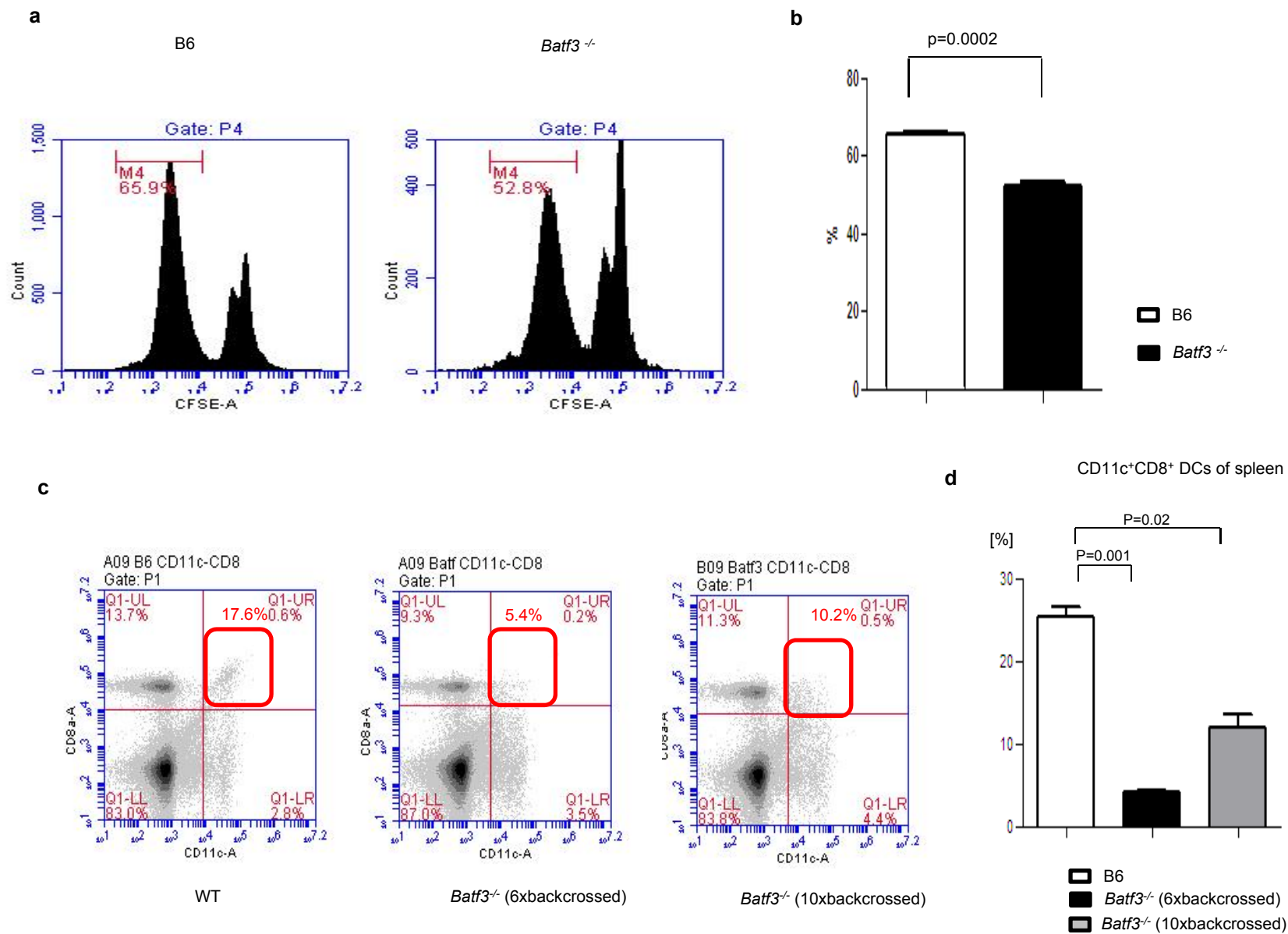
Supplemental Table 2: Histopathological scores are similar in *Batf3*^{-/-} and WT animals

GVHD model	GI tract scores	Liver scores	
B6→B6 (n=6)	7.7±1.8*	8.3±1.8*	
C3H.sw→B6 (n=9)	20.0±3.3	16.8±1.6	
C3H.sw→ <i>Batf3</i> ^{-/-} (n=9)	22.0±1.9	20.9±1.4	*p<0.05

Supplemental figure 1

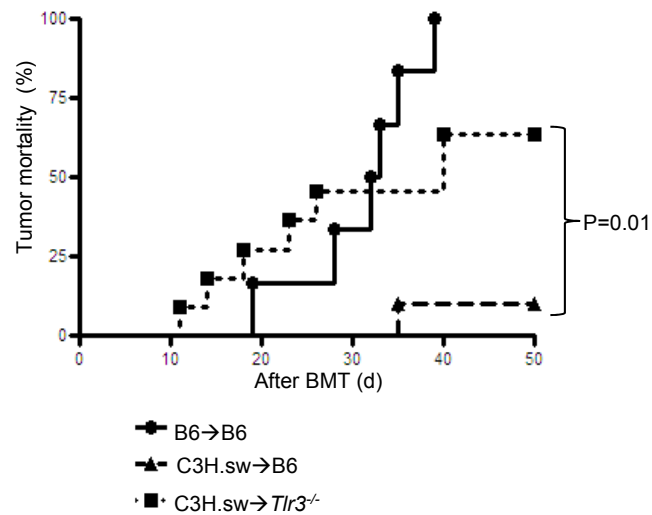


Supplemental figure 2

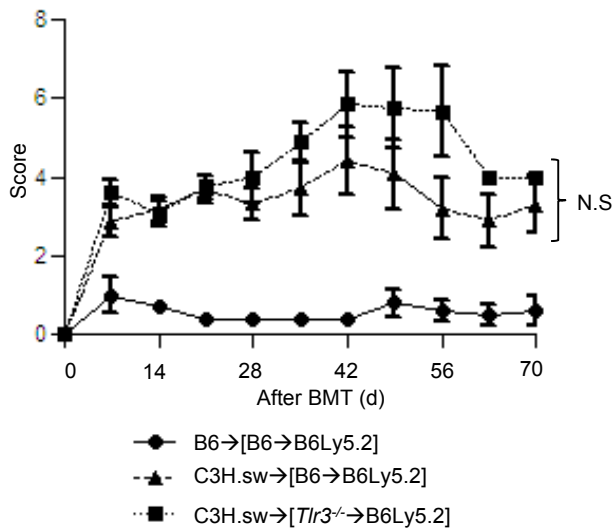


Supplemental Figure 3

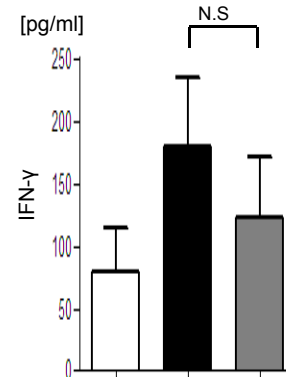
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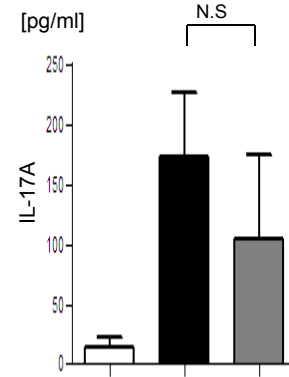
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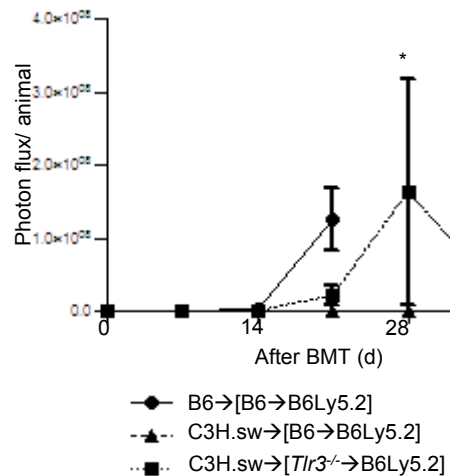
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d



e



Supplemental Figure 4

a

