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SUPPLEMENTARY INFORMATION

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Host NLRP6 exacerbates graft-versus-host disease independent of gut microbial composition

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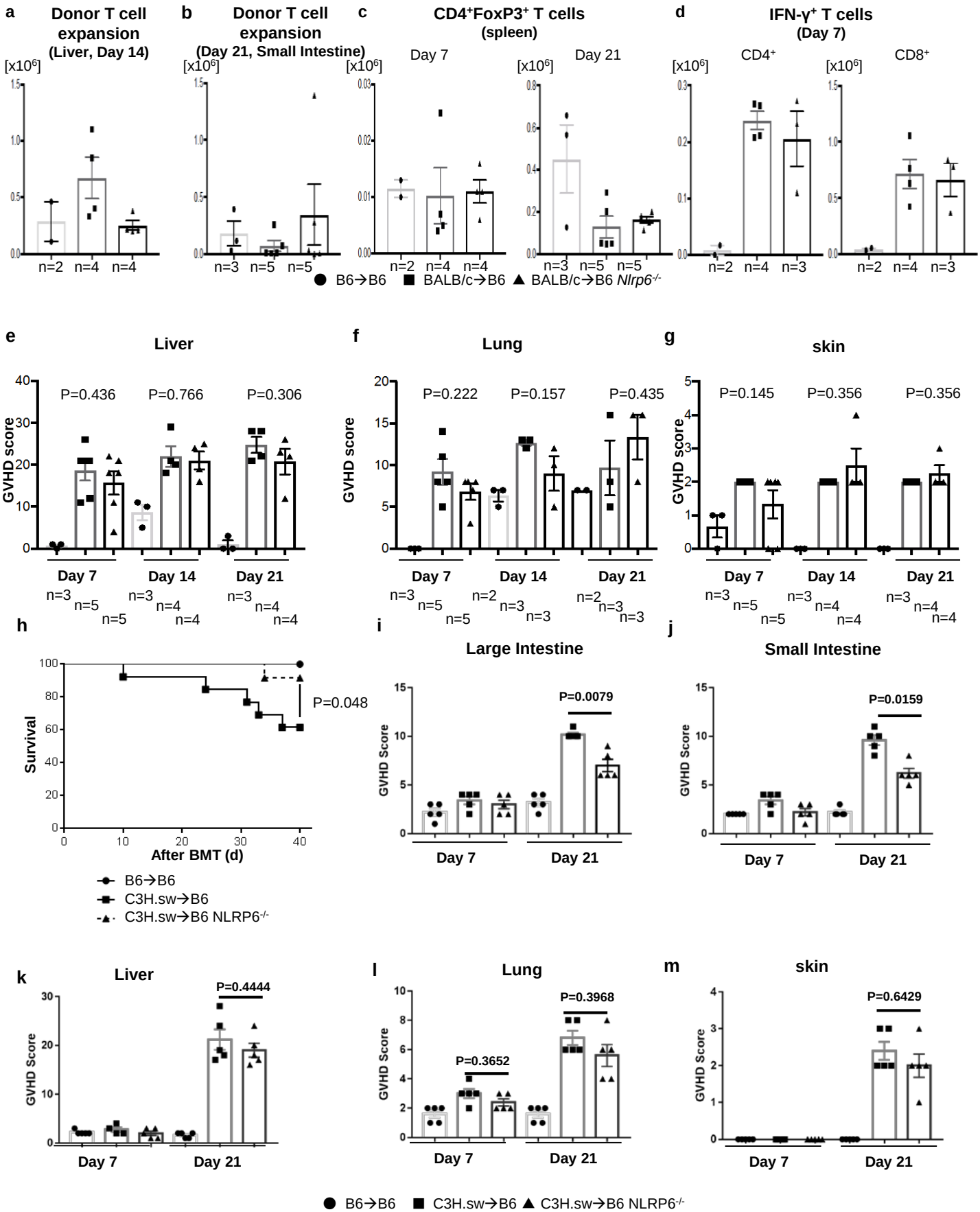
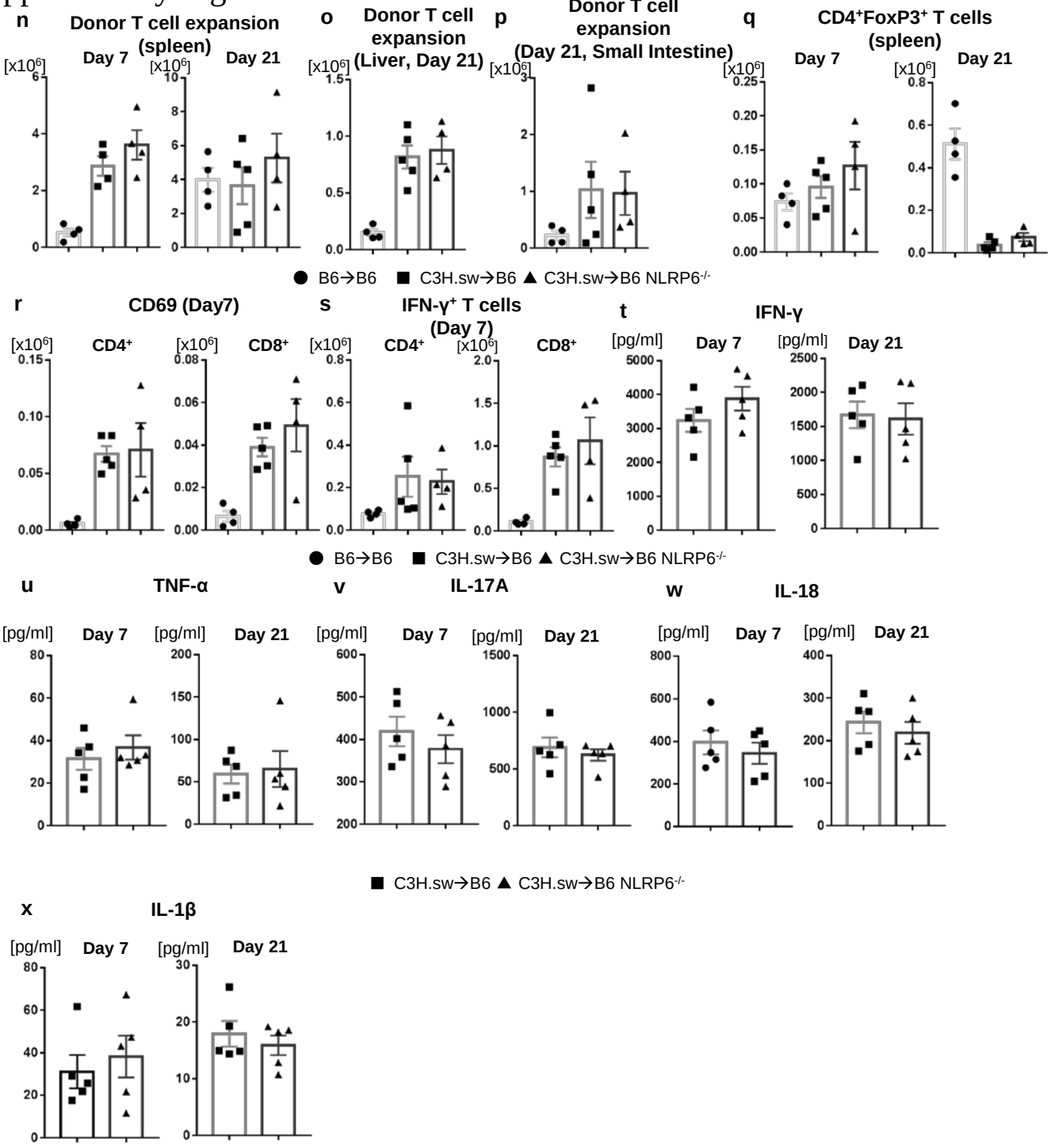
Supplementary Figure 1

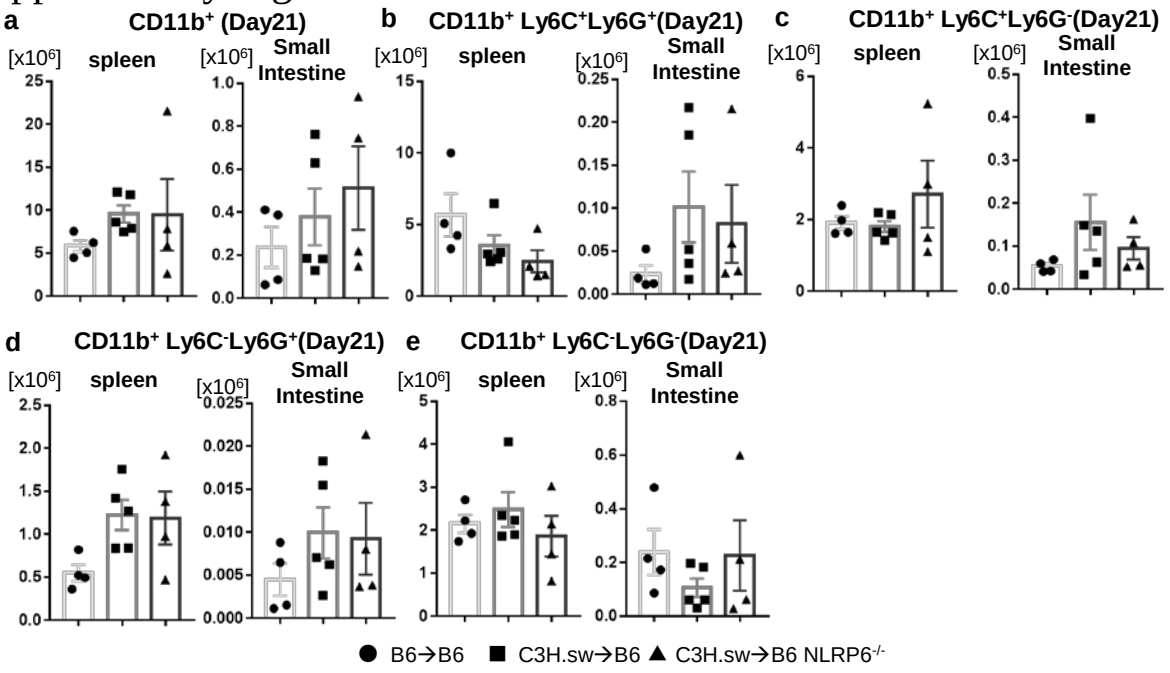


Supplementary Figure 1 Cont



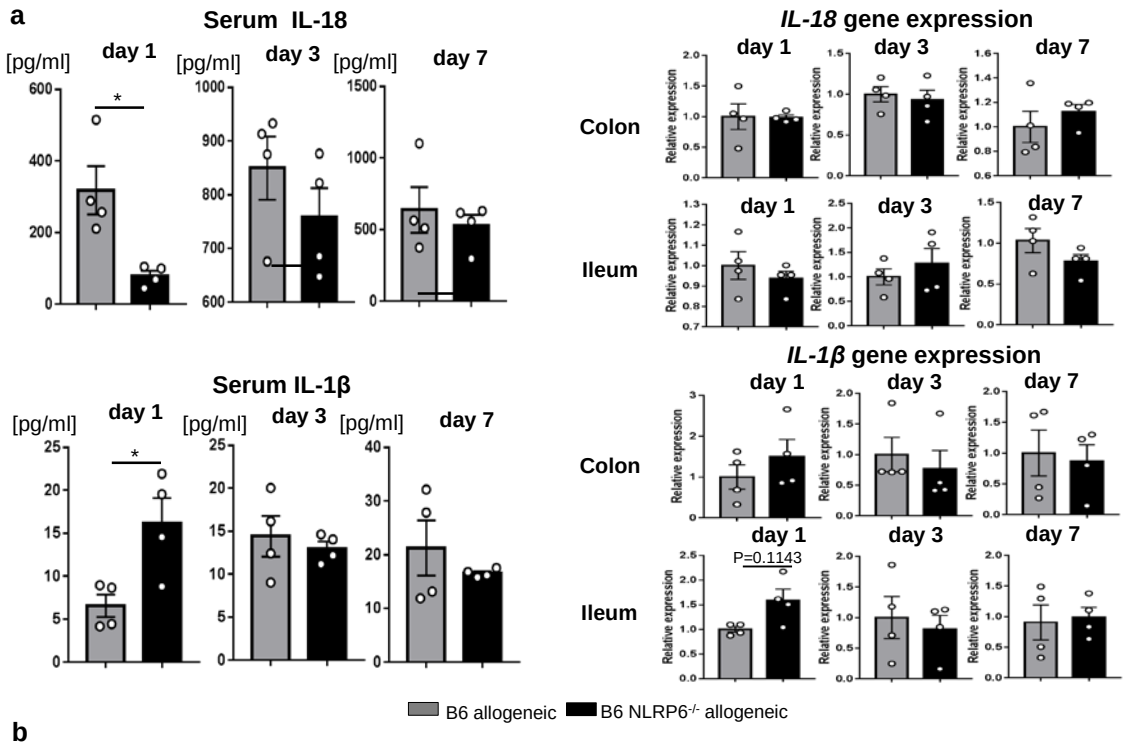
Supplementaey Figure 1: *Nlrp6*$^{-/-}$ animals showed equivalent donor T cell expansions in liver and GI tract to B6 WT animals as well as GVHD histopathological score.
B6 WT and *Nlrp6*$^{-/-}$ animals received 11Gy on day -1 and were transplanted with 3.0x10^6 CD90.2$^+$ splenic T cells along with 5x10^6 TCD-BM from either syngeneic B6 or allogeneic MHC mismatched BALB/c donors. **(a-b)** Donor T cell expansion in liver on day 14, and in small intestine on day 21 after BMT. **(c)** The absolute numbers of donor derived CD4$^+$CD25$^+$Foxp3$^+$T cells in spleen on day 7 and 21 after BMT. **(d)** The absolute numbers of donor derived CD4$^+$ IFN-γ$^+$ and CD8$^+$ IFN-γ$^+$ T cells in spleen on day 7 after BMT BMT. **(e-g)** GVHD histopathological score in liver (e), lung (f) and skin (g) at different time point after BMT. **(h-x)** B6 WT and *Nlrp6*$^{-/-}$ animals were received 11Gy on day -1 and were transplanted with 3.0x10^6 CD90.2$^+$ splenic T cells along with 5x10^6 TCD-BM from either syngeneic B6 or allogeneic C3H.sw donors. (h) Survival of C3H.sw→B6 model (B6→B6:n=5, C3H.sw→B6:n=10, C3H.sw→B6 *Nlrp6*$^{-/-}$:n=13). GVHD histopathological score in large intestine (i), small intestine (j) liver (k), lung (l) and skin (m) at different time point after BMT(n=5, each group). (n) Donor T cell expansion in the spleen on day 7 and 21 after BMT. (o) donor T cell expansion in liver on day 21 and (p) in small intestine on day 21 after BMT. (q) the absolute numbers of donor derived CD4$^+$CD25$^+$Foxp3$^+$T cells in spleen on day 7 and 21 after BMT. (r) CD69$^+$ expression on donor CD4$^+$ and CD8$^+$ T cells in the spleen on day 7 after allo-BMT. (s) the absolute numbers of donor derived CD4$^+$ IFN-γ$^+$ and CD8$^+$ IFN-γ$^+$ T cells in spleen on day 7 after BMT (n-s;B6→B6:n=4, C3H.sw→B6:n=5, C3H.sw→B6 *Nlrp6*$^{-/-}$:n=4). Serum IFN-γ (t), TNF-α (u), IL-17A (v), IL-18 (w) and IL-1β (x) levels on day 7 and 21 after allo-BMT (t-x;C3H.sw→B6:n=5, C3H.sw→B6 *Nlrp6*$^{-/-}$:n=5). Data are analyzed in allo-B6 WT vs allo- *Nlrp6*$^{-/-}$ animals with (a-d, n-x), (e-g, i-m) two-tailed Mann-Whitney *U* test, (h) Wilcoxon test (see Supplementary Table for exact *P* values). The bar shows the mean ± SEM. The representative data are from at least two independent experiments.

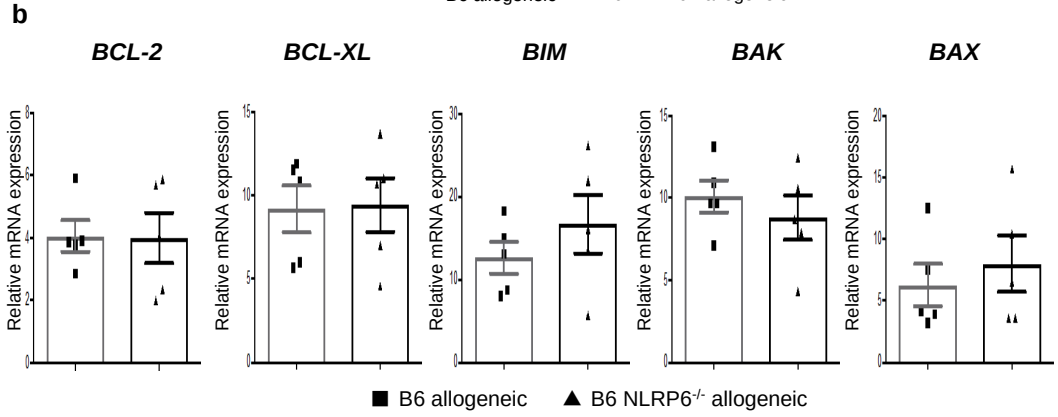
Supplementary Figure 2

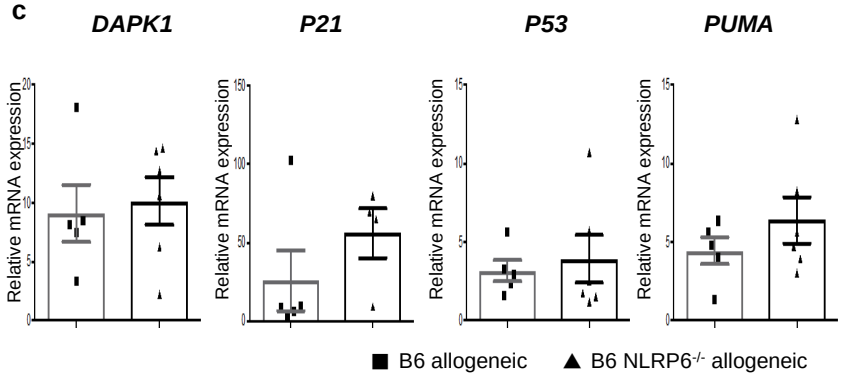


Supplementary Figure 2: *Nlrp6*$^{-/-}$ animals showed similar expansion of donor derived myeloid cells with B6 WT animals in minor mismatch model.
Isolated spleens and intraepithelial lymphocytes from recipients were stained to test the expansion of donor derived myeloid cells on day 21 after BMT. The absolute numbers of (a) total CD11b$^+$ cells, (b) CD11b$^+$Ly6C$^+$Ly6G$^+$ cells, (c) CD11b$^+$Ly6C$^+$Ly6G$^-$ cells, (d) CD11b$^+$Ly6C$^-$Ly6G$^+$ cells, and (e) CD11b$^+$Ly6C$^-$Ly6G$^-$ cells are shown (B6→B6:n=4, C3H.sw→B6:n=5, C3H.sw→B6 *Nlrp6*$^{-/-}$:n=4). Data are analyzed in allo-B6 WT vs allo- *Nlrp6*$^{-/-}$ animals with (a-e) two-tailed unpaired *t*-test (see Supplementary Table for exact *P* values). The bar shows the mean ± SEM. The representative data are from at least two independent experiments.

Supplementary Figure 3



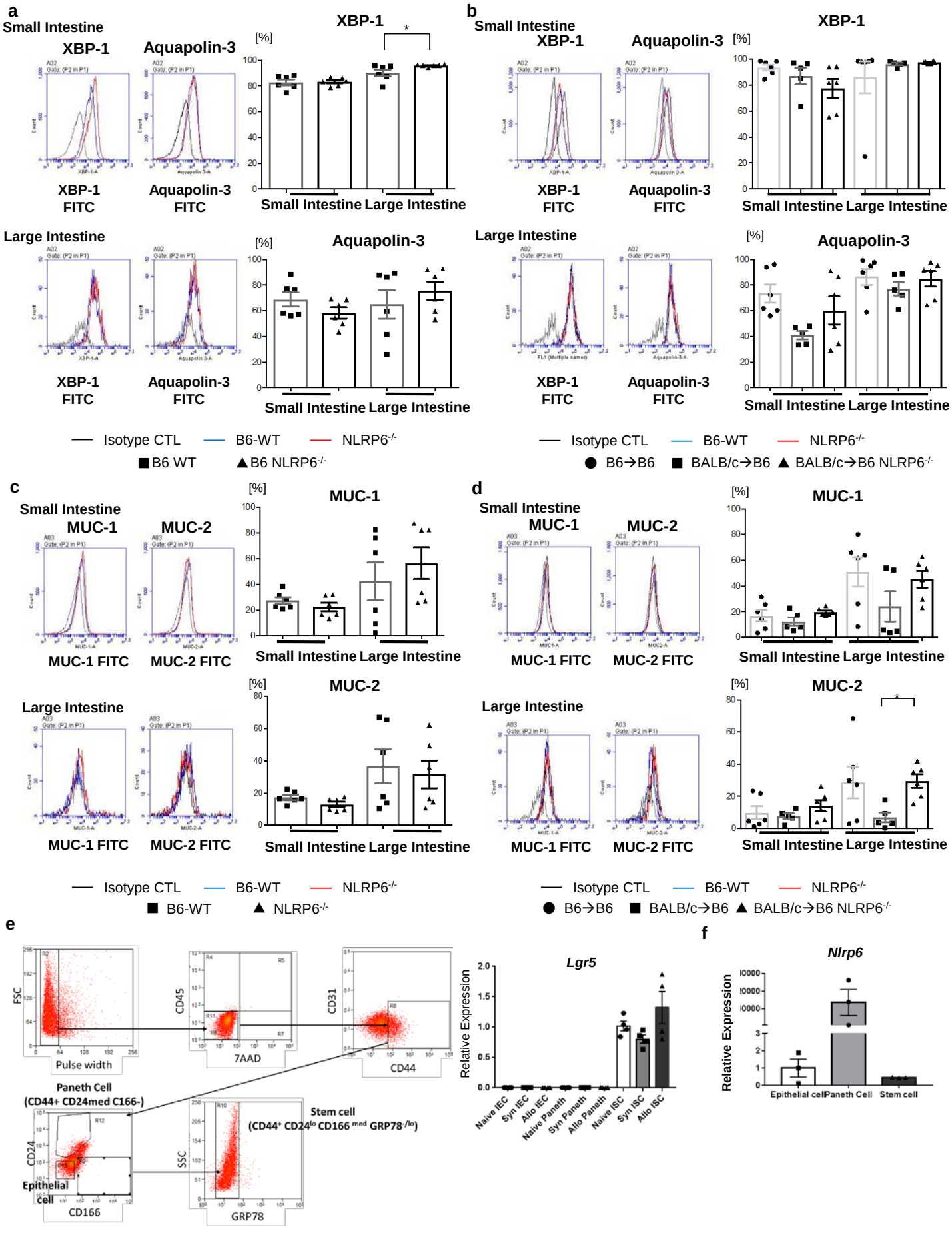




Supplementary Figure 3: Absence of NLRP6 on host non-hematopoietic cells showed equivalent apoptotic related genes to B6-WT animals

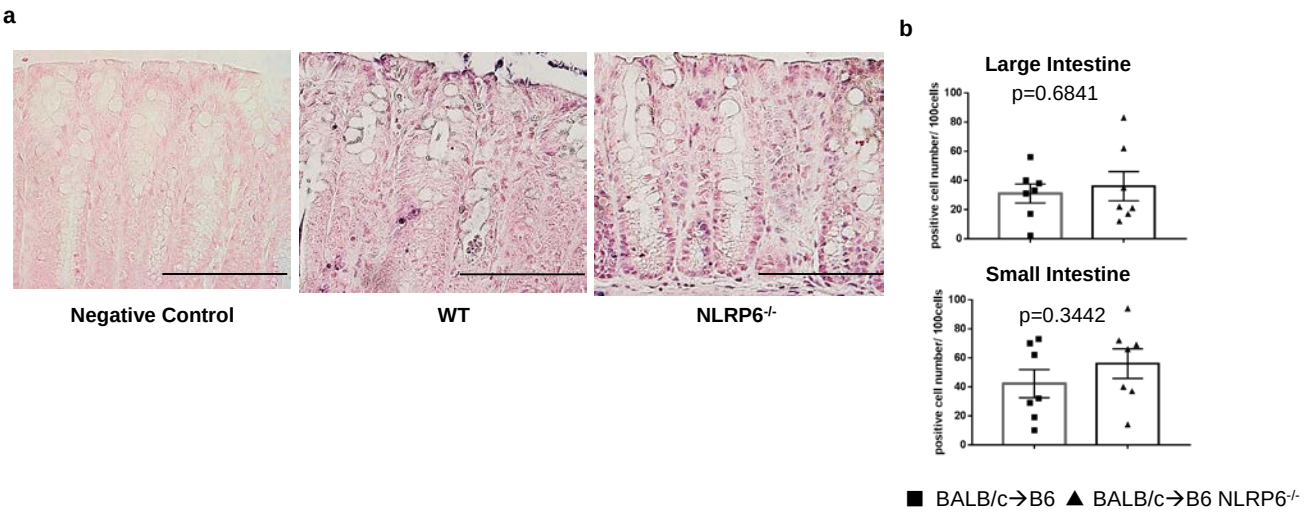
(a) Left; IL-18 and IL-1β levels in serum at day 1, 3 and 7 after allo-BMT. Right; IL-18 and IL-1β gene expression levels in epithelial cells from colon and ileum at day 1, 3 and 7 after BMT(n=4, each group). **(b)**The expression of BCl-2, BCL-XL, BIM, BAX and BAK in the large intestinal epithelial cells was measured by quantitative RT-PCR at day 7 after BMT(n=5, each group). **(c)** The expression of DAPK1, P21, P53, and PUMA in the small intestinal epithelial cells was measured by quantitative RT-PCR at day 7 after allo-BMT(n=5, each group). Data are analyzed in allo-B6 WT vs allo- *Nlrp6*$^{-/-}$ animals with (a gene expression, b, c) two-tailed Mann-Whitney *U* test, (a protein,) two-tailed unpaired *t*-test (see Supplementary Table for exact *P* values). *p<0.05. The bar shows the mean ± SEM. The representative data are from at least two independent experiments.

Supplementary Figure 4



Supplementary Figure 4: The expression of homeostatic related proteins in the small intestine epithelial cells after allo-BMT.
(a) Expression of XBP-1 and AQP-3 in the small intestine and large intestinal epithelium ($CD326^+$ cells) in naïve B6 WT and *Nlrp6*$^{-/-}$ animals. (left) Representative histogram data. (right) Representative data (n=6 per group). **(b)** Expression of XBP-1 and AQP-3 in the small intestine and large intestinal epithelium ($CD326^+$ cells) in transplanted B6 WT and *Nlrp6*$^{-/-}$ animals at day 7 after BMT. (left) Representative histogram data. (right) Representative data. (B6→B6:n=6, BALB/c→B6:n=5, BALB/c→B6 *Nlrp6*$^{-/-}$:n=6). **(c)** Expression of MUC-1 and MUC-2 in the small and large intestinal epithelium ($CD326^+$ cells) in naïve B6 WT and *Nlrp6*$^{-/-}$ animals (n=6 per group). (left) Representative histogram data. (right) Representative data (B6→B6:n=6, BALB/c→B6:n=5, BALB/c→B6 *Nlrp6*$^{-/-}$:n=6). **(d)** Expression of MUC-1, and MUC-2 in the small and large intestinal epithelium ($CD326^+$ cells) in transplanted B6 WT and *Nlrp6*$^{-/-}$ animals at day 7 after BMT. (left) Representative histogram data. (right) Representative data. (B6→B6:n=6, BALB/c→B6:n=5, BALB/c→B6 *Nlrp6*$^{-/-}$:n=6). **(e)** Cell sorting strategy for sequentially gating single cells, live cells, $CD44^+$ cells and cells with different CD24, CD166 and GRP78 expression (left). qRT-PCR analysis of *Lgr5* expression in sorted cells from B6 WT at day 0 and 7 after BMT (right; n=3 per group). **(f)** qRT-PCR analysis of *Nlrp6* expression in sorted cells from naïve B6 WT. (right; n=3 per group). Representative data from two experiments. (a-d) two-tailed unpaired *t*-test, (e) two-tailed Mann-Whitney *U* test, (e, f) Kruskal-Wallis test with Dunn's multiple comparisons test (see Supplementary Table for exact *P* values). The bar shows the mean ± SEM. *p<0.05. The representative data are from at least two independent experiments.

Supplementary Figure 5



Supplementary Figure 5: Absence of NLRP6 on host intestinal epithelial showed equivalent apoptosis after irradiation.
(a) The representative images of TUNEL-positive cells in the large intestine on day 7 after allo-BMT (scale bar, 200 μm). **(b)** The TUNEL-positive cell numbers in intestinal epithelial cells after BMT (n=7 per group). (b) two-tailed unpaired *t* test. The bar shows the mean ± SEM. The representative data are from at least two independent experiments.