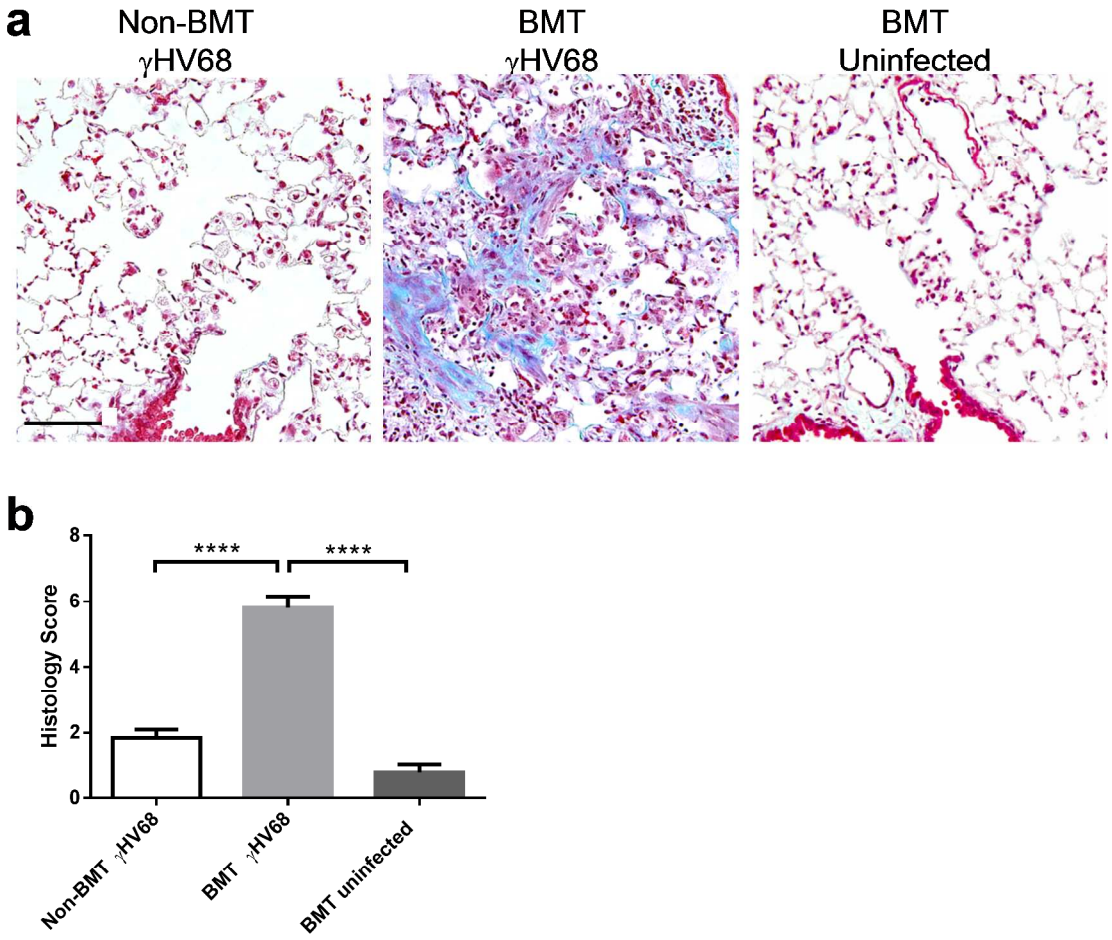
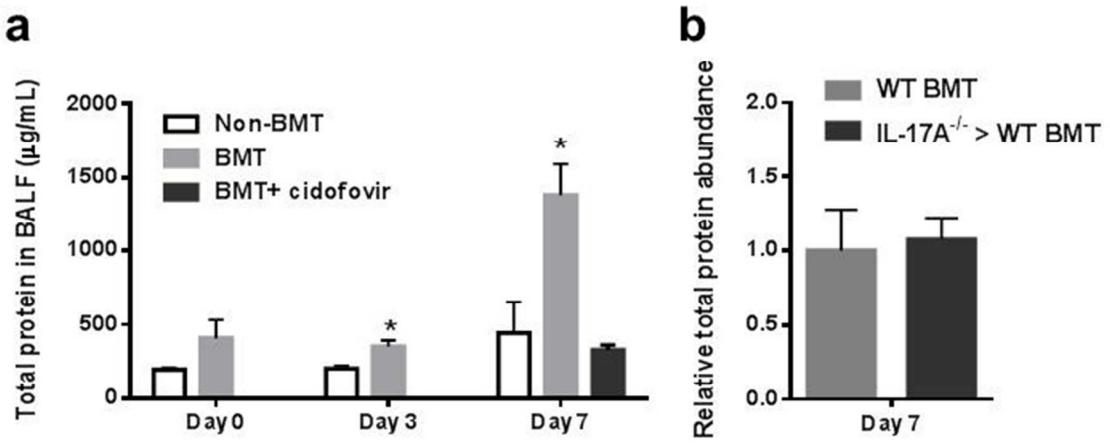
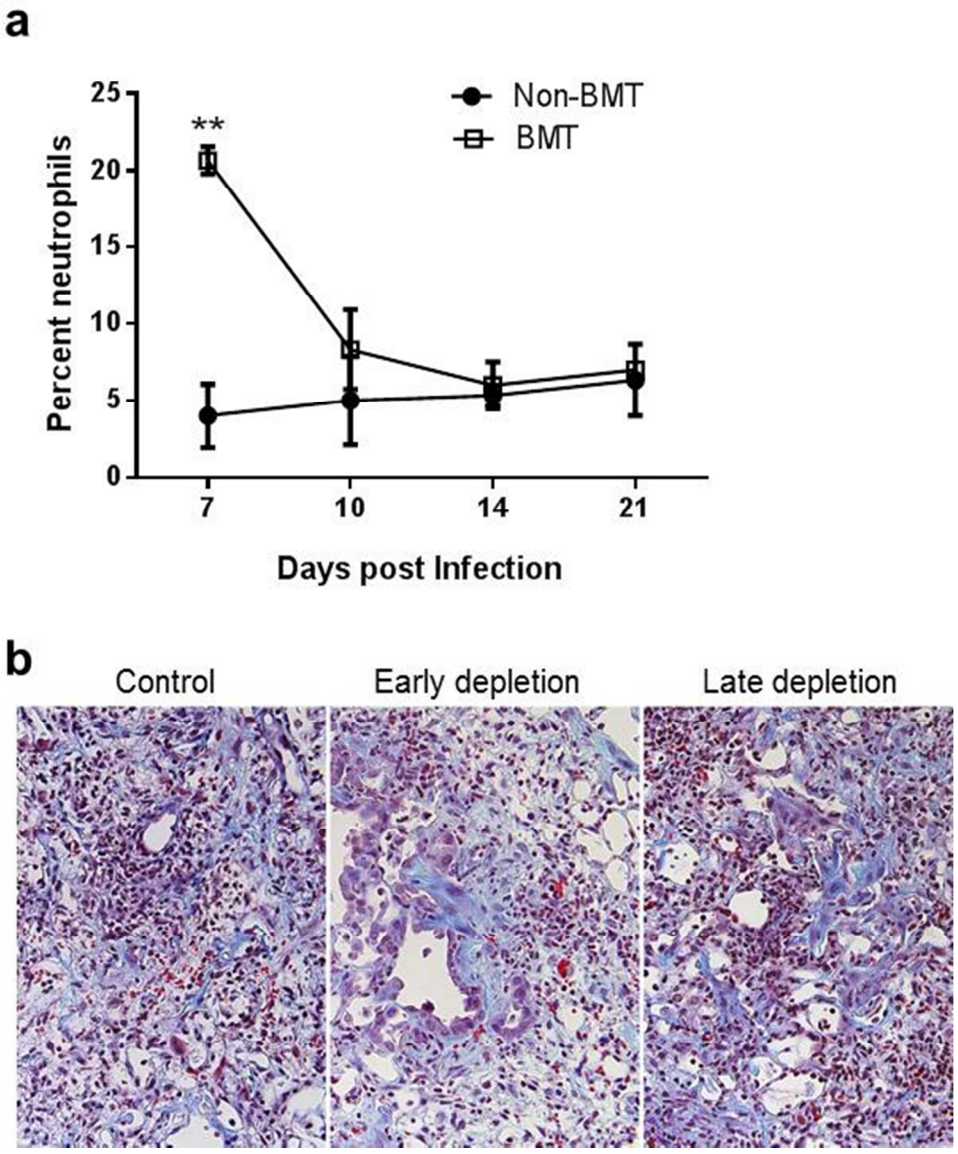
Supplemental Data



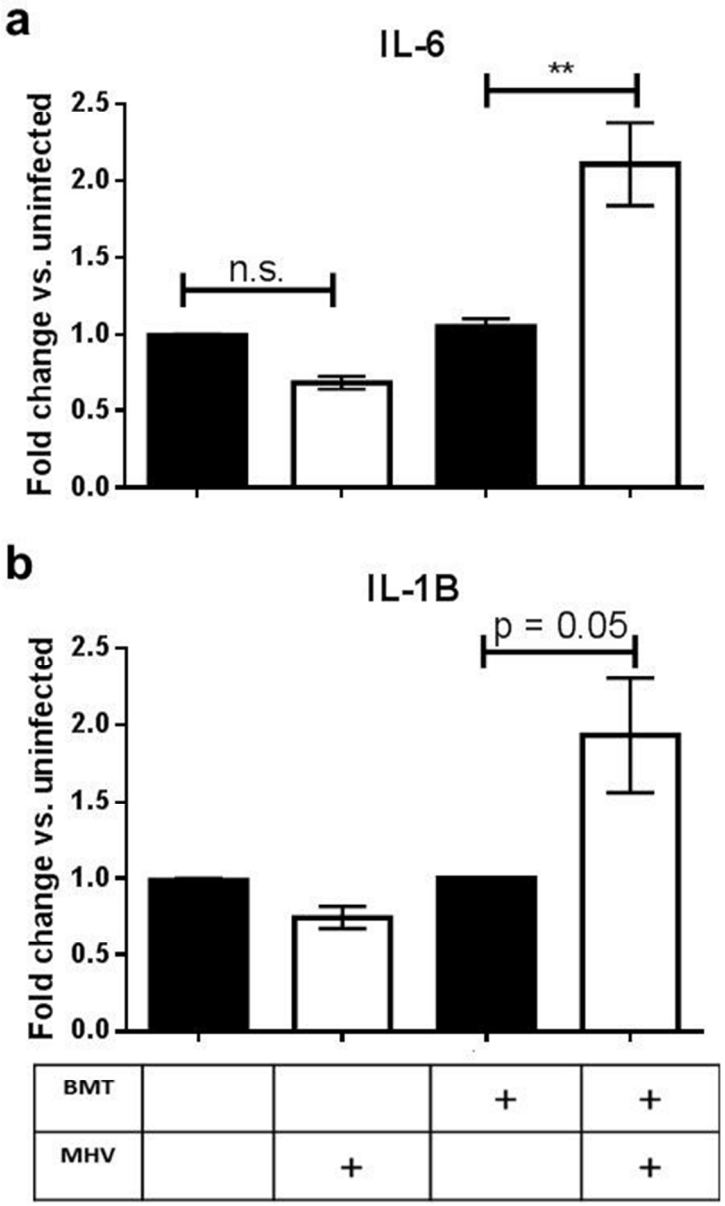
Supplemental Figure 1. BMT mice infected with γHV-68 develop pulmonary fibrosis by 21dpi. (a) Representative lung sections from infected BMT or non-BMT mice at 21 dpi or age-matched uninfected BMT mice stained with Masson's trichrome (bars = 100 μm, same magnification for the images). Blue staining represents deposition of collagen. Histology is representative of at least n = 10 mice/group. (b) Average histology scores of lung sections (mean + SEM, at least n = 10 mice/group). **** $P < 0.0001$. Lung sections were scored for severity of fibrosis, perivascular inflammation, peripheral inflammation, and presence or absence of foamy alveolar macrophages and intra-alveolar fibrin.



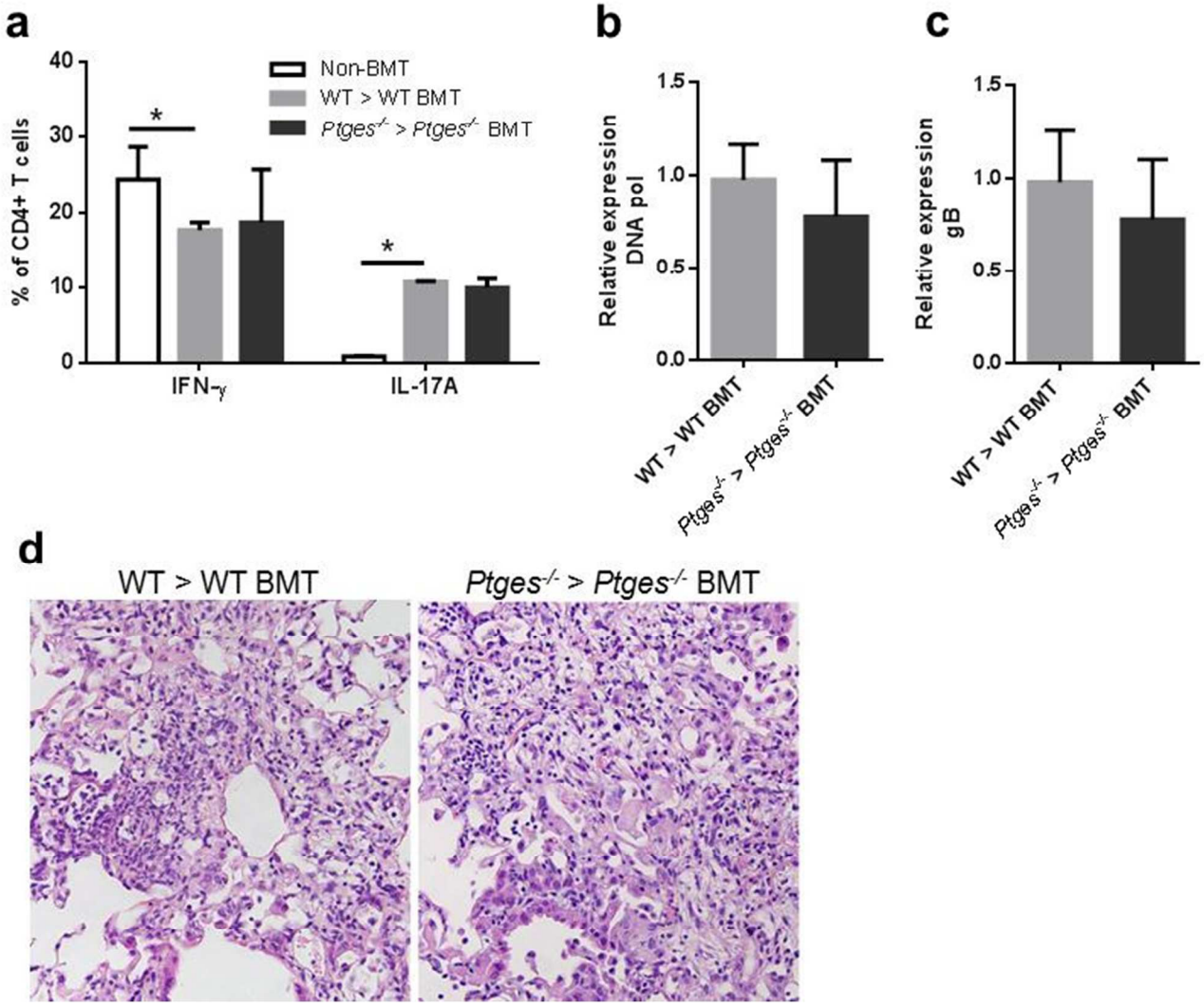
Supplemental Figure 2. Increased lung injury in BMT mice infected with γHV-68. (a) Total protein levels in bronchoalveolar lavage fluid (BALF) as indication of lung injury post viral infection. Syngeneic BMT C57BL/6J mice or age-matched non-BMT mice were infected with 5 x 10^4 pfu γHV-68 intranasally at 5 weeks post-BMT. BALF was harvested by instilling 1 ml PBS into the lung and removing fluid via suction at designated time points. The BALF was centrifuged to remove cells and then total protein concentration was determined using Pierce BCA protein assay kit (Life Technologies). Cidofovir (Mylan, Institutional LLC) was administered subcutaneously at a dose of 25 mg/kg of body weight every other day starting one day after viral infection. Mean ± SEM, n = 4 mice per group, * $P < 0.05$. (b) Comparison of protein abundance in BALF of mice transplanted with WT bone barrow and in BALF of mice transplanted with IL-17A$^{-/-}$ bone barrow. BALF was collected at 7 days post infection (dpi). Mean ± SEM, n = 5 mice per group.



Supplemental Figure 3. Neutrophils are dispensable for development of pneumonitis and fibrosis post-γHV-68 infection in BMT mice. (a) The percent of neutrophils in lungs determined by differential staining of collagenase digested lungs from non-BMT control or BMT mice at 7, 10, 14 or 21 dpi with γHV-68. Mean ± SEM, n = 4 mice per group, ** $P < 0.01$. Data are representative of two independent experiments. (b) Representative Masson's trichrome staining of lung sections from anti-Ly-6G antibody clone 1A8 (BioXcell, West Lebanon, NH) treated BMT mice at 21 dpi. The blue staining represents deposition of collagen. The anti-Ly-6G antibody is neutrophil specific and can deplete neutrophils when i.p. injecedt at a dose of 500 µg/mouse[1, 2]. BMT mice received anti-Ly-6G antibody treatment either at 1, 4 and 8 dpi (early depletion), or at 11, 14 and 17 dpi (late depletion). N = 2 mice per group.



Supplemental Figure 4. The expression of pro-Th17 cytokines in BMT lung APCs is dependent on γHV-68 infection. Lung CD11c+ APCs were enriched from uninfected non-BMT or BMT mice and were stimulated with or without 1 MOI virus *ex vivo* for 24 hours (MHV=γHV-68). Relative expression of mRNA of IL-6 (a) and IL-1β (b) in lung APCs was determined by RT-PCR (mean ± SEM, n = 3); **p<0.01.



Supplemental Figure 5. PGE_2 is not required for the skewing of Th17 differentiation nor for the pathology outcomes for γHV-68 infection in BMT mice. (a) Percent of CD4+ cells that express IFN-γ or IL-17A. Single cell suspensions were prepared by collagenase digestion of whole lungs of non-BMT control, WT > WT BMT or *Ptges*$^{-/-}$ > *Ptges*$^{-/-}$ BMT mice at 7 dpi with γHV-68. Cells were then stimulated with PMA and ionomycin and analyzed by flow cytometry. $CD45^+$ $CD4^+$ cells were gated for analysis. Mice with mPGES-1 deficiency [*Ptges*$^{-/-}$ specifically] lack PGE_2[3, 4]. Mean ± SEM, n = 3 mice per group. * P <0.05. (b) and (c) γHV-68 lytic replication in BMT or non-BMT mice at designated time points post-infection as measured by relative mRNA abundance for viral DNA polymerase (a) and envelope glycoprotein gB (b). Mean ±SEM, n = 4. (d) Representative Hematoxylin and Eosin staining of lung sections from BMT mice receiving bone marrow from WT or *Ptges*$^{-/-}$ mice. Representative of n = 5 mice per group.

References

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