

Figure S1. RT-qPCR of OTUD6A deficient PMs after viruses infection in vitro. (a) mRNA expression of *Ifit2* and *Ifna5* in WT or *Otud6a*^{-/-} mouse-derived PMs infected with Sev (0.1 MOI) for 8h were assessed by RT-qPCR. (b) Peritoneal macrophages were obtained from wild-type and *Otud6a*^{-/-} mice, and the cells were infected with Sev (0.1 MOI) virus for 0, 4, 8, and 12 h. Cellular *Ifna4* and *Ifnβ* mRNA expression levels were detected by qPCR. (c) Peritoneal macrophages were obtained from wild-type and *Otud6a*^{-/-} mice, and the cells were infected with Sev (0.1 MOI) virus for 8 h, Cellular *Il6*, *Tnf-α* and *Il2* mRNA expression levels were detected by qPCR. (d) qPCR was performed to detect the expression of *Ifnβ* in WT and *Otud6a*^{-/-} PM cells when the cells were in the absence of bacterial infection. (a-d) Student's t-test; Significance levels are noted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$; ns, not significant. All values are presented as mean \pm SEM.

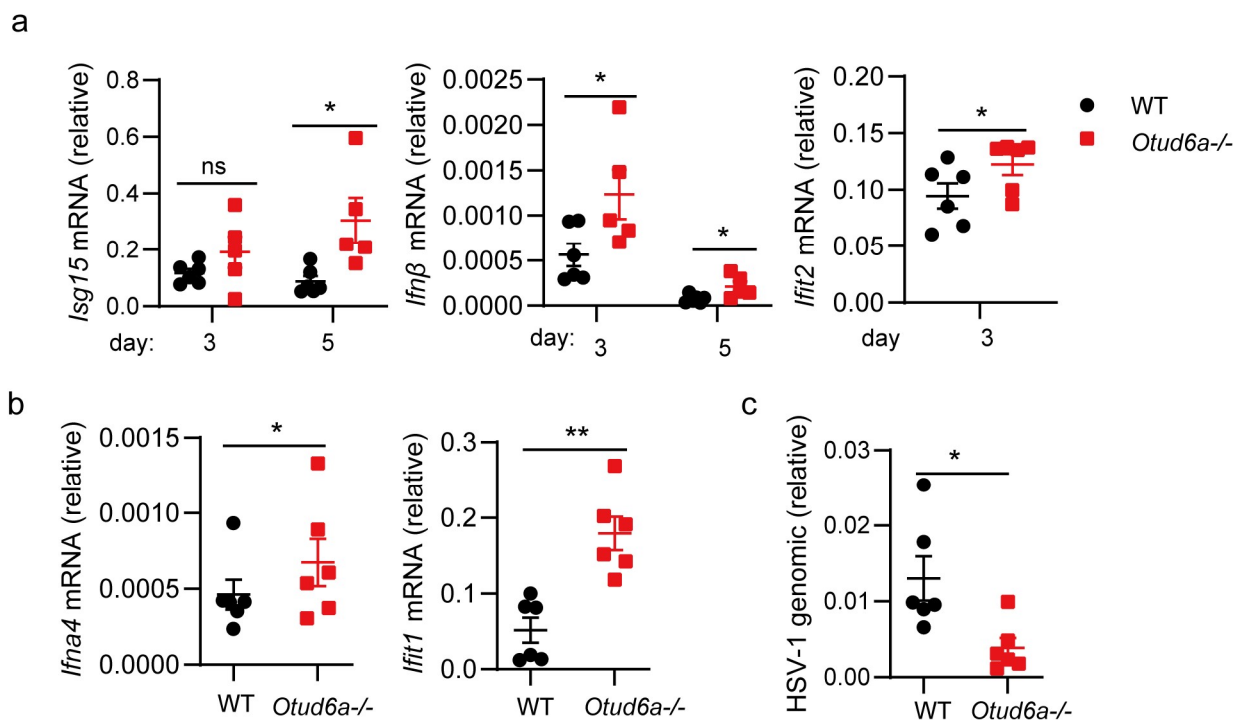


Figure S2. OTUD6A deficiency increases innate immunity in vivo and in vitro. **(a)** On the third and fifth day of HSV-1 infection in mice, total mRNA was extracted from blood and the mRNA levels of *Isg15*, *Ifnβ* and *Ifit2* were measured by qPCR, respectively. **(b)** On the third day of VSV infection in mice, total mRNA was extracted from blood and the mRNA levels of *Ifna4* and *Ifit1* were measured by qPCR. **(c)** On the third day of HSV-1 infection in mice, total virus load was measured by RT-qPCR. **(a,b)** Student's t-test; Significance levels are noted as * $p < 0.05$, ** $p < 0.01$; All values are presented as mean \pm SEM.

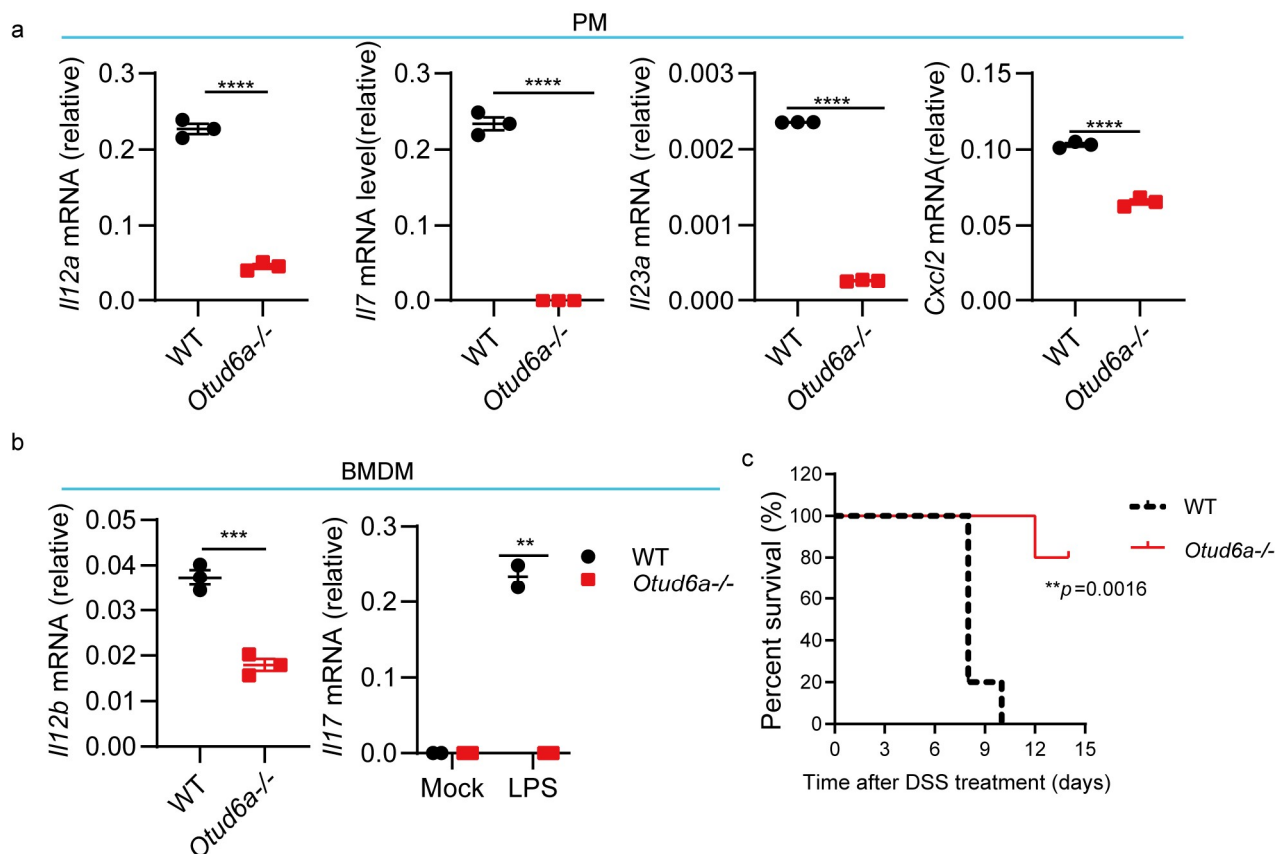


Figure S3. OTUD6A deficiency attenuates inflammatory response in vitro and in vivo. **(a)** Peritoneal macrophages were obtained from wild-type and *Otud6a*^{-/-} mice, stimulated with 1 µg/mL LPS for 6 h. Total mRNA was extracted and mRNA levels of *Il12a*, *Il7*, *Il23a* and *Ccl2* genes were measured by RT-qPCR. **(b)** Bone marrow-derived macrophages were obtained from wild-type and *Otud6a*^{-/-} mice, stimulated with 1 µg/mL LPS for 6 h. Total mRNA was extracted and mRNA levels of *Il12b* and *Il17* genes were measured by RT-qPCR. **(c)** Wild-type mice and *Otud6a*^{-/-} mice (*n* = 5) were fed with 2% DSS water for one week and then replaced with normal water, and the mortality of each group was recorded. **(a,b)** Student's t-test; **(c)** Log-rank test; Significance levels are noted as ***p* < 0.01, ****p* < 0.001 and *****p* < 0.0001 ; ns, not significant. All values are presented as mean ± SEM.