

Supplemental Figure 1. Titration of MH5A dose in GVHD. In the B6 to lethally irradiated BDF1 model of GVHD, MH5A was administered at the indicated dose at the time of adoptive transfer (day 0). Mice were monitored for survival.



7-AAD



were intraperitoneally injected with 300 ug of MH5A or control Ab and splenocytes were harvested 72 hours later and counted and stained for various cell subsets for flow cytometry analysis. Absolute cell numbers were calculated by multiplying total viable cell counts with percent of total/100 as determined by flow cytometry (cell count x (% total/100)). 3 mice were analyzed per group. (E) An in vitro complement-mediated lysis assay was performed using CD4+ T cells isolated from the spleen of C57BL/6 wt mouse. CD4+ T cells expressing PD-1H were incubated with MH5A, control hamster IgG or CD4 mAb (clone GK1.5) as a positive control for 30 minutes on ice, followed by the addition of baby-rabbit complement (10:1 dilution) and incubation at 37 degrees C for 15 minutes. Cells were then stained with 7-AAD for 5-10 minutes and immediately analyzed by flow cytometry to determine cell viability.

were examined at each time point

repeated three times. (D) Wt B6 mice

Supplemental Figure 3.



Supplemental Figure 3. Treg cell expansion and viability are similar in control and MH5A treated mice. In the Balb/c to lethally irradiated B6 GVHD model, splenocytes were isolated on days 10, 15 and 20 and analyzed by flow cytometry. CD4+FoxP3+ donor T cells were stained for the proliferation marker Ki67 and for viability with a fixable viability marker prior to intracellular staining for FoxP3 and Ki67.