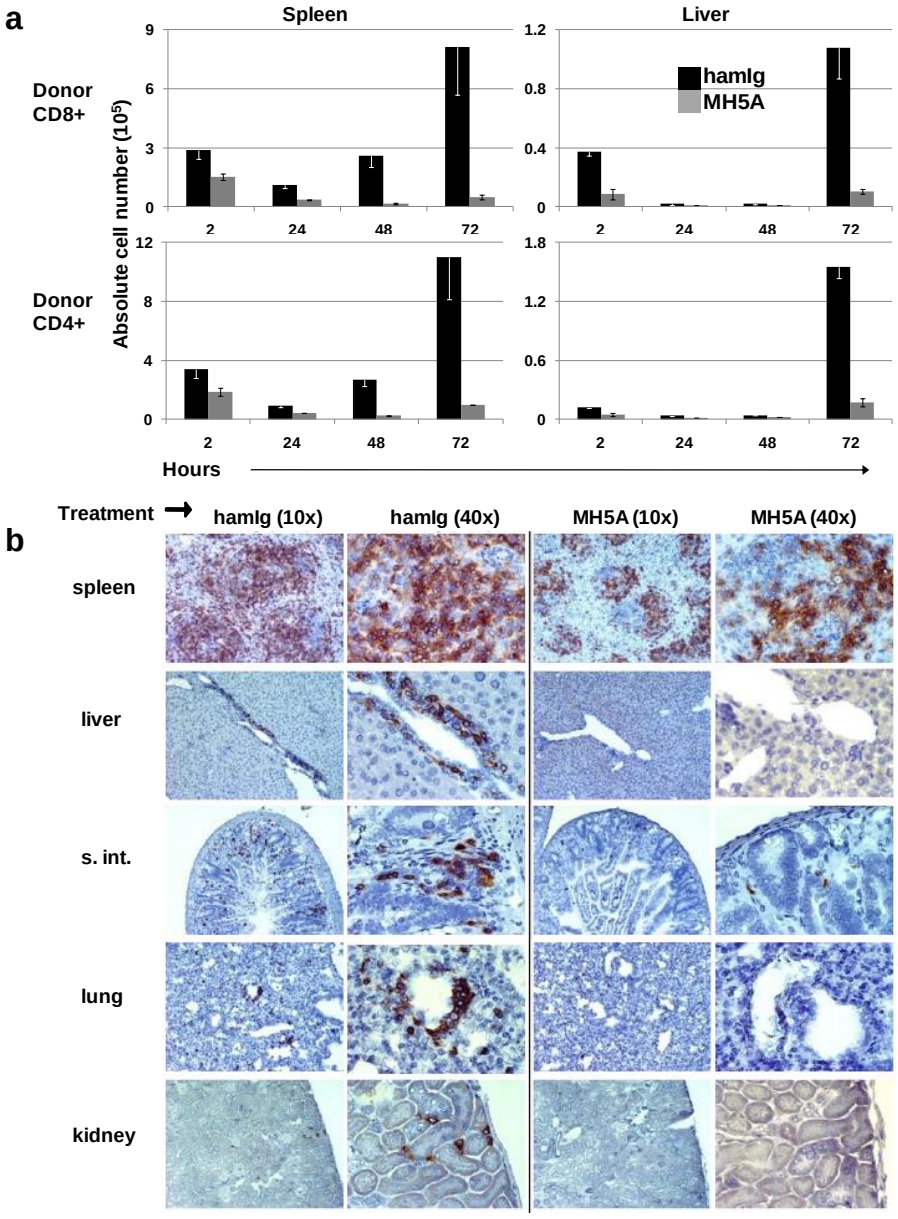
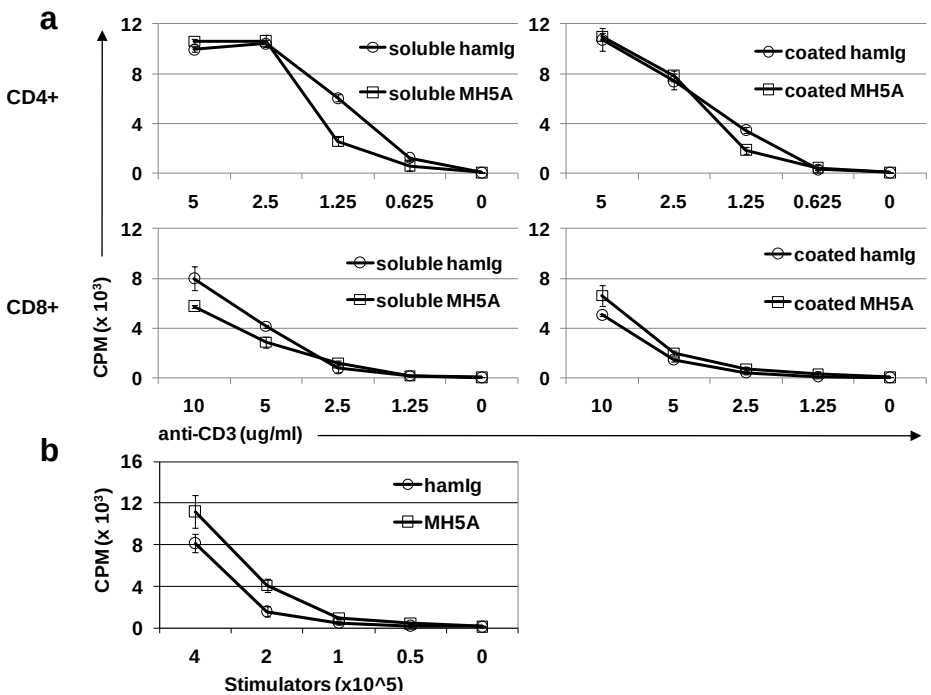
Supplemental Figure 1.



Supplemental Figure 1. MH5A treatment alters early phase donor T cell accumulation and expansion in target organs in acute GVHD. a) Lethally irradiated BDF1 mice received 5 x 106 T-cell-depleted bone marrow cells + 3 x 106 pan T cells from B6 mice and 200 ug of control hamster Ig (hamIg) or anti-PD-1H (clone MH5A) on day 0. Splenocytes and liver lymphocytes were isolated and analyzed at time points indicated. Total cells were counted and analyzed by flow cytometry to determine absolute numbers of donor CD8+ and CD4+ T cells. b) Lethally irradiated BDF1 (Thy1.2+) recipients were adoptively transferred with B6 (Thy1.1+) congenic T cells with control hamster Ig (hamIg) or anti-PD-1H mAb (clone MH5A). Spleen, liver, small intestine, lung, and kidney were isolated 72 hours after the adoptive transfer. Organs from aGVHD mice were placed into optimal cutting temperature compound (OCT) (Ted Pella; Redding CA) and immediately frozen in liquid nitrogen. Tissues were subsequently fixed in acetone and stained with 5 ug/ml biotin labeled thy1.1 mAb clone OX-7 (BD Pharmingen) using Vectastain ABC system (Vector labs, Burlingame, CA).

Supplemental Figure 2.



Supplemental Figure 2. MH5A has no significant effect on T cell proliferation in vitro. a) CD4+ and CD8+ MACS purified T cells stimulated in 96 well plates for 72 hours with titrated doses of anti-CD3 and either soluble or pre-coated (5 ug/ml) control hamster IgG or MH5A. b) Mixed lymphocyte reaction. Balb/c lymph node responder cells ($4x10^5$) were stimulated for 72 hours with titrated doses of irradiated C57BL/6 splenocytes in the presences of control hamster IgG or MH5A. Methyl-^{3}H-thymidine added for the final 8 hours of incubation in all experiments.