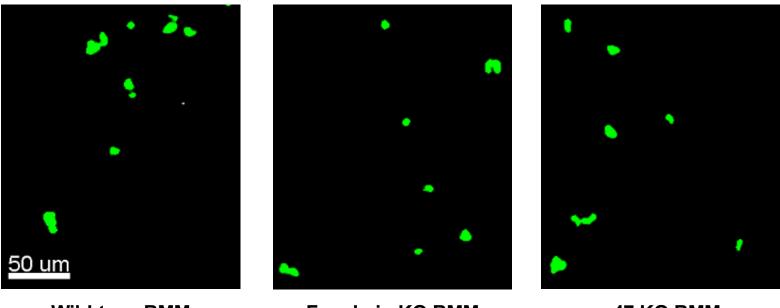


CD8-depleted Transplant Recipients

Supplemental Figure 1. C57BL/6 and Fc γ chain KO recipient mice produce similar alloantibody isotypes following hepatocyte transplantation. C57BL/6 (wild-type; WT) and Fc γ chain KO mice (both H-2^b) were transplanted with FVB/N (H-2^q) hepatocytes (2x10⁶ cells). On day 14 posttransplant, recipient serum was analyzed for alloantibody isotypes (anti-IgG1, anti-IgG2b, anti-IgG2c, anti-IgG3; Bio-Rad Laboratories, Hercules, CA; as previously described in Zimmerer et al., 2014 AJT pages 2491-9). Both WT (n=5) and Fc γ chain KO (n=4) recipients exhibit predominantly IgG1 (WT titer= 725±56, Fc γ chain KO titer= 555±37; p<0.0001 for both compared to naïve control) alloantibody isotype and lesser amounts of IgG3 (WT titer= 37±3, Fc γ chain KO= 58±8; p<0.002 for both compared to naïve control). Naïve control serum represented by the dashed line.

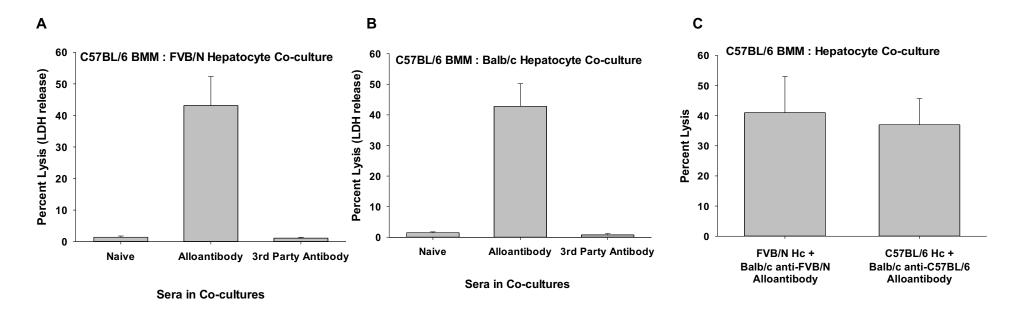


Wild-type BMM

Fcγ chain KO BMM

p47 KO BMM

Supplemental Figure 2. Localization of CFSE-stained Bone Marrow Macrophages (BMM) in the liver. In order to investigate the localization of WT, $Fc\gamma$ chain KO, and p47 KO BMM to the host liver, we adoptively transferred 5 million CFSE-stained BMM from each group into the tail vein of $Fc\gamma$ chain KO recipients on day 14 posttransplant (n=2 for all conditions). Two days after intravenous injection, mice were euthanized and host livers were excised and analyzed by the Olympus FV1000 MPE Multiphoton Laser Scanning Confocal microscope (Olympus, Center Valley, PA). Representative images (25x magnification and analyzed at 840 nm) show that BMM from all groups (WT, $Fc\gamma$ chain KO, and p47 KO mice) traffic to the host liver. Raw imaging data were analyzed using Imaris image analysis software (Bitplane, Zurich, Switzerland) with a Median filter for noise reduction. No fluorescent cells were observed in livers from control mice without adoptive transfer of CFSE-stained BMM (not shown).



Supplemental Figure 3. Antibody-dependent, macrophage-mediated hepatocytotoxicity is strictly dependent on co-incubation with allosera. FVB/N (H-2^q), Balb/c (H-2^d), and C57BL/6 (H-2^b) hepatocytes were isolated and cultured *in vitro*. Allogeneic hepatocytes were incubated with alloserum from FVB/N, Balb/c, or C57BL/6 recipients (day 14). C57BL/6 bone marrow macrophages (BMM) were activated by pretreatment with IFN-γ (2.5 ng/mL; 18 hours). BMM and hepatocytes were co-cultured for 8 hours and cytotoxicity detected by LDH release in the culture supernatant. A,B) C57BL/6 mice (H-2^b) were transplanted with FVB/N (H-2^q) or Balb/c (H-2^d) hepatocytes and sera was collected from primed mice as a source of allosera or "third-party" sera. C57BL/B BMM mediated hepatocytotoxicity equally well when co-cultured with FVB/N or Balb/c (H-2^b) hepatocytes and sera was collected from primed mice as a source of allosera was collected from primed mice as a source of allosera was collected from primed mice as a source of allosera was collected from primed mice as a source of allosera was collected from primed mice as a source of allosera was collected from primed mice as a source of allosera. C57BL/6 hepatocytes incubated with allosera generated in Balb/c recipients.