Supplemental Methods

Animals

All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH). The Institutional Animal Care & Use Committee at the Medical University of South Carolina approved all animal procedures. Rag1-/- and C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, ME). All mice used were on a C57BL/6 background, male, age 8 -12wks. Mice were kept on a 12 hour light/dark cycle, fed a pellet diet and given water ad libitum. For surgical procedures mice were anesthetized with of a ketamine cocktail (ketamine (13 mg/ml), xylazine (2.6 mg/ml), and acepromazine (0.15 mg/ml)) injected i.p. at 5ml/kg.

Liver Function Assay

Serum levels of alanine aminotransferase (ALT) were used as an indication of liver function. Serum was collected by centrifugation of whole blood at 2000 rpm for 10 minutes at 4° C and levels of ALT were determined using analytical kits from either Pointe Scientific INC (Canton MI) or Sigma-Aldrich (St. Louis, MO).

Tissue Histology

Liver histology was assessed on 4-μm paraffin sections fixed in 10% formalin and stained with H&E. Histological score was determined in a blinded fashion on a semi-quantitative scale ranging from 0 (no damage) - 4(total necrotic destruction of liver). Necrotic damage was characterized by increased eosinophilia, karyolysis, vacuolization, and loss of normal hepatic architecture [12].

Assessment of liver regeneration

Mice were injected with BrdU (50 mg/kg i.p.) 2 hours prior to sacrifice, and incorporation of BrdU was visualized by immunohistochemistry using an anti-BrdU antibody (AbCam) as described [13]. Restitution of liver weight is expressed as percentage of regenerated liver mass relative to total liver weight, and was calculated as previously described [14]. Mitotic index was calculated by tallying the precentage of hepatocytes undergoing mitosis in 10 HPF on tissue sections stained with H&E.

Immunohistochemistry and immunofluorescence

Paraffin or frozen sections were cut at 4μm for IHC and IF respectively. IHC sections were deparaffinized and antigen retrieval was performed using proteinase K (Vector Labs, Burlingame, CA). Frozen sections were fixed in acetone for 6 minutes and then air dried and equilibrated in PBS. C3d deposition was detected using a goat anti-mouse C3d (1:20, R&D Systems, Minneapolis, MN), and IgM binding in mouse and human tissues was detected using a goat anti-mouse IgM (1:50, Sigma-Aldrich) and rabbit anti-human IgM (1:1000, Sigma-Aldrich) or anti-mouse IgM-FITC for IF (1:50, Millipore), human endothelial cells were detected using anti-human CD31-FITC (1:25, Abcam), annexin IV on human liver biopsies was detected using mouse B4 mAb (5 μg/ml, prepared as described above). For IHC primary antibodies were detected using goat-IMMpress or rabbit-IMMpress kits (Vector Labs). For IF primary antibodies were detected using anti-goat IgG AlexaFluor-555 conjugate (1:200, Invitrogen, Carlsbad, CA) or anti-mouse IgM AlexaFluor-555 conjugate (1:200, Invitrogen).

Human sample analysis

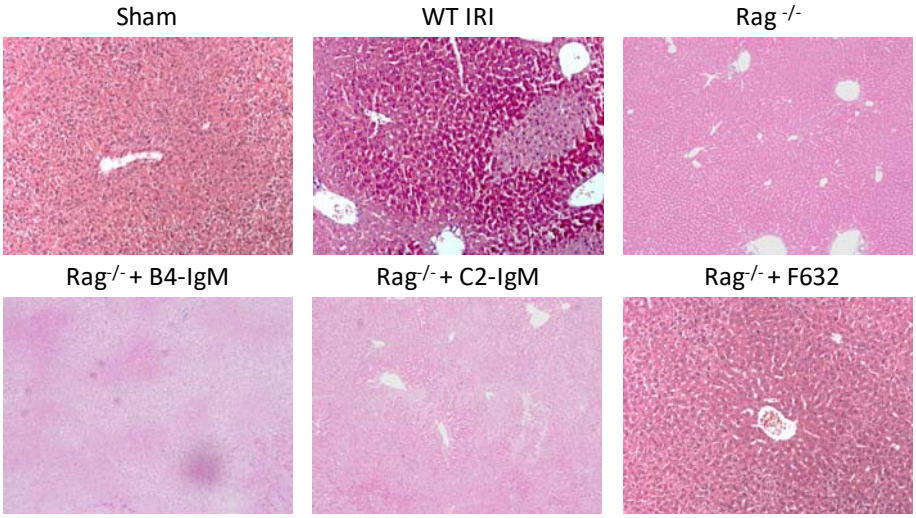
All tissue samples and sera were collected as part of an approved institutional IRB. For analysis of B4 mAb binding to human samples, liver biopsies were obtained from ischemic human donor livers prior to transplantation, and “normal” liver samples were taken from areas of normal pathology following resection of hepatic hemangioma. All

samples were stored at -80 ^{0}C until analyzed. Samples were cut into 8 μM sections, washed and permeabilized prior to blocking with serum-free protein block. Sections were then incubated with B4 mAb or control F632 mAb (5 ng/ul), washed, and incubated with HRP-conjugated anti-mouse IgM (1:200, Sigma-Aldrich). Signal was developed using DAB. All sections were imaged on an Olympus BX61 Microscope Light microscope with Visiopharm Acquisition Software. Serum samples were analyzed as described below. Data on donor and recipeint organs were part of our transplant and resection databases, and analysis was a part of our institutional approved IRB**.**

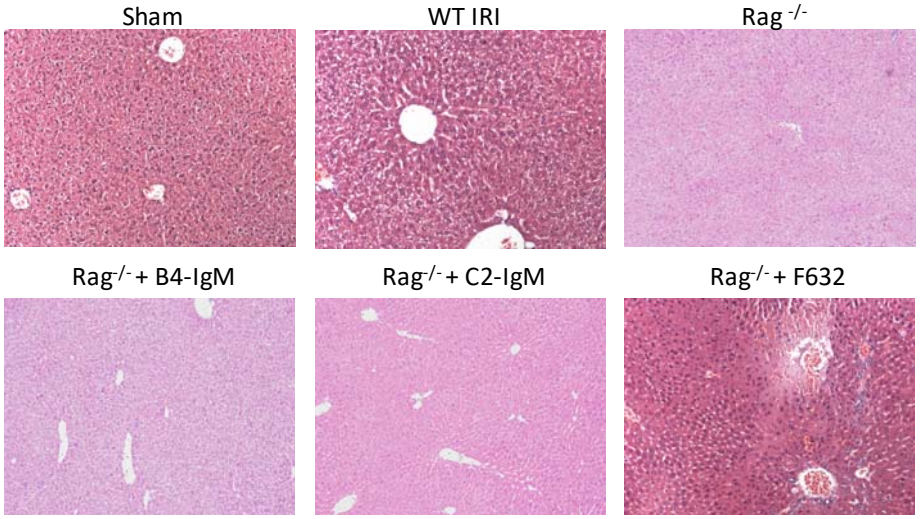
Biodistribution study

A B4 scFv biodistribution study was performed as we have previously described [13]. Briefly, B4 scFv was radiolabeled with ^{125}Iodine (New England Nuclear Corp.) using Peirce Iodination Reagent according to manufactures instructions (Thermo Scientific), and 5 mCi was used to label 100 ug B4scFv. Rag1-/- mice were injected via the tail vein with 2 ug radiolabeled protein immediately following IR, 70% Phx or sham surgery, and animals sacrificed 6 h post surgery. Blood was removed by cardiac puncture and the animals were perfused with PBS before the heart, brain, liver, intestine, lung, kidney, and spleen were removed. Tissues were rinsed with PBS, shredded, weighed and then radioactivity was measured with a Hewlett-Packard 5780 γ counter. Results were recorded as μCi/g of tissue.

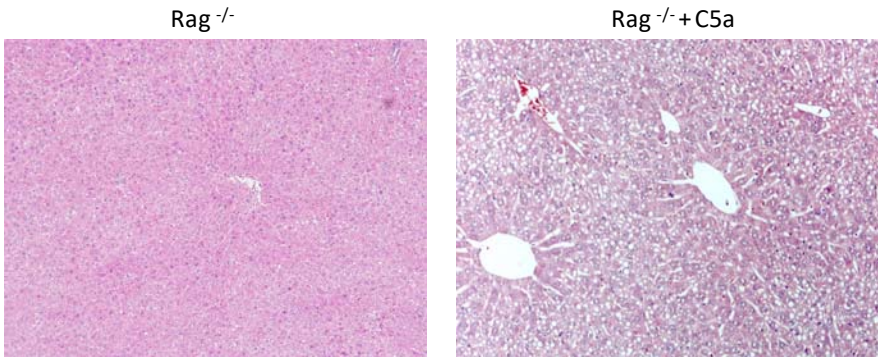
Supplementary figures



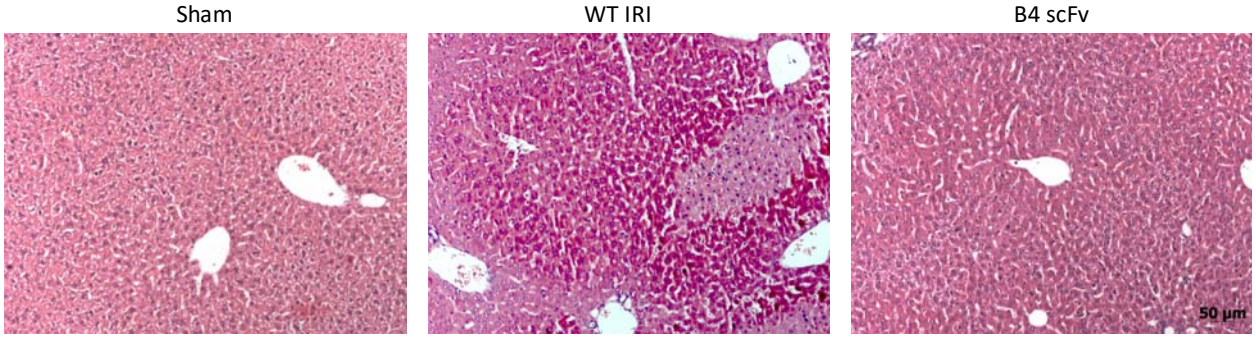
Supplementary Figure 1. Representative H&E stained sections for histological quantification of necrosis and injury, as reported in manuscript figure 1A (IgM antibodies reconstitute hepatic ischemia reperfusion injury in Rag1-/- mice). Sham, Rag1-/- and Rag1-/- +F632 show normal liver structure with no obvious signs of injury in portal acinus zone 1 hepatocytes or hepatocytes near the central portal triad and central veins. WT and Rag1-/- mice reconstituted with B4 or C2 IgM showed marked degeneration with pale hematoxylin and eosin staining of hepatocytes in zones 1, 2, and 3, particularly in zone 2. Magnification x10.



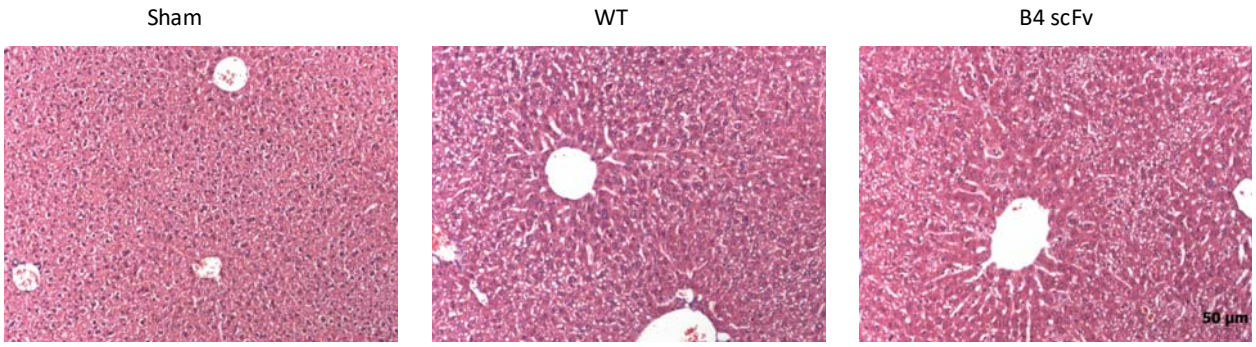
Supplementary Figure 2. Representative H&E stained sections for histological quantification of necrosis and injury, as reported in manuscript figure 2A (IgM antibodies are sufficient to stimulate regeneration in Rag1-/- following 70% partial hepatectomy). Magnification x10.



Supplementary Figure 3. Representative H&E stained sections for histological quantification of necrosis and injury, as reported in manuscript figure 2F (IgM antibodies are sufficient to stimulate regeneration in Rag1-/- following 70% partial hepatectomy). Magnification x10.



Supplementary Figure 4. Representative H&E stained sections for histological quantification of necrosis and injury, as reported in manuscript figure 5A (Treatment with B4 scFv limits injury following hepatic ischemia reperfusion injury and does not alter liver recovery or regeneration following 70% partial hepatectomy in wild type mice). Magnification x10.



Supplementary Figure 5. Representative H&E stained sections for histological quantification of necrosis and injury, as reported in manuscript figure 5C (Treatment with B4 scFv limits injury following hepatic ischemia reperfusion injury and does not alter liver recovery or regeneration following 70% partial hepatectomy in wild type mice). Magnification x10.

Table 1. Donor, ischemia and analysis characteristics

ID	Gender	Age	Ischemia Time	ALT Levels (at donation)	IgM (Intensity/pixel)	C3d (Intensity/pixel)
1	male	56	4 hrs 47 min	36 u/l	16.13	13.33
2	male	69	7 hrs 40 min	15 u/l	26.68	23.24
3	male	48	10 hrs 2 min	19 u/l	38.85	17.19

Supplementary Table 1