Supplementary Materials and Methods

Animals

C57Bl/6J (stock#000664), ΔdblGATA1(on C57Bl/6J background, stock#005653), Il-4$^{-/-}$(on C57Bl/6J background, stock#002253), Il-4/GFP-enhanced transcript (on BALB/c background, 4Get, stock#004190), Alb-Cre (on C57Bl/6J background, stock#035593), and Clec4f-Cre (on C57Bl/6J background, stock#033296), BALB/cJ (stock# 000651) and Il-4rα$^{-/-}$(on BALB/c background, stock#003514) mice were purchased from the Jackson Laboratory. Breeders of PHIL[1], iPHIL[2], Il-4/13$^{fl/fl}$eoCre mice (backcrossed to C57Bl/6J at least 10 generations) [3] were obtained from Dr. Elizabeth Jacobsen (Mayo Clinic Arizona). Il-4rα$^{flox/flox}$ mice [4] were generated by Dr. Frank Brombacher (University of Cape Town, South Africa) and provided by Dr. Brain Kim (Washington University School of Medicine). The Hb-egf$^{flox/flox}$ mice were generated by Dr. Eisuke Mekada (Osaka University, Japan) and provided by Dr. Raymond Harris (Vanderbilt University). Colonies of Il-4rα$^{fl/fl}$Alb-Cre$^{+/-}$, Il-4rα$^{fl/fl}$Clec4f-Cre$^{+/-}$, Hb-egf$^{fl/fl}$Clec4f-Cre$^{+/-}$ mice were generated and maintained in the University of Texas Health Science Center at Houston (UTHealth) animal facility. All experiments were performed according to the guidelines of the institutional animal care and use committee (IACUC) at UTHealth.

Hepatic ischemia-reperfusion (IR) surgery and animal experiments

Hepatic IR or sham surgeries were performed in 10- to 12-week-old male mice as previously reported[5]. Mice were anesthetized by intraperitoneal (i.p.) injection of sodium pentobarbital (60 mg/kg) and then positioned on their back on a heated surgery table to maintain body temperature. A midline laparotomy was made and an atraumatic clip was used to block blood flow to the left lateral and median lobes of the liver. After 60 minutes (mins) of partial hepatic ischemia, the clip was removed to start hepatic reperfusion. For the sham group, the same steps were followed without vascular blockage. Throughout the procedure, the surgical site for the mice was draped with warm saline-soaked gauze. Mice were sacrificed at various post-reperfusion time points to collect blood and ischemic lobes of the liver tissues for further analysis.

For eosinophil depletion, iPHIL mice and WT littermates were i.p. injected with diphtheria toxin (DT; 15 ng/g) 16h prior to (1st dose) and 6h after (2nd dose) hepatic IR surgery. For eosinophil adoptive transfer, 10x10^6 bone marrow-derived eosinophils (bmEos) were injected intravenously (i.v.) into recipient mice at 24h after liver ischemia surgery. Control mice were injected with saline. For IL-4 or IL-13 neutralization, mice were i.p. injected with anti-mouse IL-4 antibody (10

μg/mouse, clone: 11B11, BioLegend) or anti-mouse IL-13 antibody (10 μg/mouse, AF-413-NA, R&D Systems) on days 1 and 3 after liver ischemia surgery. Control mice were injected with IgG.

For exogenous IL-4 treatment, mice were i.p. injected with IL-4 complex (IL-4c), which consisted of recombinant IL-4 (5 μg, Peprotech) complexed with anti-IL-4 antibody (25μg, clone11b11, BioXcell)[6, 7, 8] or Phosphate Buffered Saline (PBS) control on days 1 and 3 after liver ischemia surgery. For exogenous IL-13 treatment, mice were i.p. injected with IL-13 complex (IL-13c), which consisted of recombinant IL-13 (5 μg, PeproTech) complexed with anti-IL-13 antibody (25 μg, clone: eBio13A, eBioscience)[8] or PBS control on days 1 and 3 after liver ischemia surgery. For recombinant mouse (rm) HB-EGF treatment, mice were i.p. injected with rmHB-EGF (Sigma-Aldrich) or PBS on days 1 and 3 after IR surgery.

Ex vivo culturing of mouse bone marrow-derived eosinophils (bmEos) and bone marrow-derived macrophages (BMDM)

The *ex vivo* culturing of mouse bmEos was performed as reported previously[9]. Bone marrow cells were collected from the femurs of mice and cultured at $5x10^6$/mL in RPMI 1640 (Corning Cellgro) containing 20% fetal bovine serum (Corning), penicillin/streptomycin (100x, Corning Cellgro), 2 mM glutamine (Invitrogen), 25 mM HEPES, 1x non-essential amino acids, 1 mM sodium pyruvate (Gibco), 50 μM β-mercaptoethanol (Sigma-Aldrich) and supplemented with 100 ng/mL stem-cell factor (SCF, PeproTech) and 100 ng/mL FLT3-Ligand (FLT3-L, PeproTech) from days 0 to 4. On days 4 and 9, the cells were washed and cultured in fresh medium containing 10 ng/mL recombinant mouse interleukin-5 (IL-5, PeproTech). On day 14, the cells were collected and used for adoptive transfer or cell culture experiments.

To generate BMDM, bone marrow cells were collected from the femurs of mice and cultured at a density of $3x10^6$/mL in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco) containing 20% fetal bovine serum (GenDEPOT), penicillin/streptomycin (100x, Corning Cellgro), 100 mM Sodium Pyruvate (Corning Cellgro), 50 μM β-mercaptoethanol (Sigma-Aldrich) and 10% L929-conditioned medium for 7 days. The cells were then collected for *in vitro* stimulation by recombinant IL-4 (20ng/ml, Peprotech) for 6h or 24h.

Assessments of liver injury and repair

Serum concentrations of alanine transaminase (ALT) and aspartate aminotransferase (AST) were detected using diagnostic assay kits from Teco Diagnostics following the manufacturer's protocols. Liver tissue sections from ischemic lobes were fixed in 10% formalin overnight, embedded in

paraffin, and cut into 5 μm sections. Liver sections were stained with hematoxylin and eosin (H&E) for the examination of necrotic areas. Further, the degree of liver injury was evaluated based on the Suzuki's criteria and graded according to a scale of 0-4[10]. 0, none congestion, none vacuolization and none necrosis; 1, minimal congestion, minimal vacuolization and single-cell necrosis; 2, mild congestion, mild vacuolization and <30% necrosis; 3, moderate congestion, moderate vacuolization and 30-40% necrosis; 4, severe congestion, severe vacuolization and >60% necrosis. IHC staining was performed using paraffin-embedded sections to determine Ki67 expression in the liver. Briefly, endogenous peroxidases were inactivated by 3% hydrogen peroxide. Nonspecific antigen binding was blocked using 2.5% horse serum. Rabbit monoclonal Ki67 antibody (#ab16667, Abcam) was used. After overnight incubation, the slides were incubated with a secondary antibody (HRP-Polymer, Vector laboratories) for 30 mins, followed by washing and staining with 3,3'-diaminobenzidine (DAB, Vector laboratories). The quantification of $Ki67^+$ proliferating hepatocytes and the detection of PCNA in liver tissues by immunoblotting were performed to determine the extents of liver regeneration and recovery after IR injury.

Isolation of liver non-parenchymal cells (NPCs)

Liver NPCs were isolated following a previously established method[9]. Liver tissues were perfused *in situ* with a perfusion buffer (1x *Hank's Balanced Salt Solution,* HBSS), followed by a digestion buffer (1x HBSS supplemented with 0.04% Collagenase type I, Sigma-Aldrich). Once digested, the liver was disrupted in an anti-coagulant-citrate-dextrose solution and the cells passed through a 70μm cell strainer. After filtering, cells were further purified using 35% Percoll (Sigma-Aldrich). The remaining red blood cells were neutralized in Ammonium-Chloride-Potassium (ACK) lysing buffer, which consists of NH_4Cl (150 mM), $KHCO_3$ (10 mM), and Na_2EDTA (0.1 mM) set to a pH range of 7.2-7.4.

Flow cytometry analysis

Cell surface staining was performed by incubating cells ($1x10^6$/per tube) with antibodies for 30 mins at 4°C after blocking with anti-CD16/CD32 (#101302, Biolegend). Dead cells were excluded by staining with blue fluorescent reactive dye (1:200, Invitrogen, #2176884). Flurochrome-conjugated antibodies against CD45 (1:200, clone 30-F11), CD11b (1:200, clone M1/70), Siglec-F (1:200, clone E50-2440), CCR3 (1:200, clone JO73E5), CD3 (1:200, clone 17A2), CD124 (1:200, IL-4Rα, clone mIL4R-M1), Clec4f (1:200, clone 3E3F9), IL-13 (1:100, clone eBio13A), CD45.1 (1:200, clone A20), CD31 (1:200, clone MEC13.3) and F4/80 (1:200, clone BM8) were purchased from eBioscience or BD Biosciences or Biolegend. For IL-13 intracellular staining,

NPCs were treated with 500 ng/mL ionomycin (Sigma-Aldrich) in the presence of GolgiPlug (BD) for 4h before staining with anti-IL-13 antibody using a Fixation & Permeabilization Kit (Invitrogen). The cells were analyzed using a CytoFLEX LX flow cytometer (Beckman Coulter) and the data was analyzed with FlowJo v10.7.

RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was purified from liver tissues or culture cells using Pure-Link RNA Mini Kit (Invitrogen) according to the manufacturer's protocol, and 1 μg RNA was reverse transcribed into cDNA using an iScript cDNA Synthesis Kit (Bio-Rad). Relative quantitative gene expression was measured with SYBR Green PCR Supermix from GenDEPOT. 18S rRNA was used as an internal standard. The primers used for qRT-PCR are listed in *Table S1*.

Western blotting

Liver tissues were homogenized in RIPA buffer (Thermo Fisher Scientific) containing protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Protein concentrations were quantified with a BCA protein assay kit (Thermo Fisher Scientific). At least 30 μg of total protein from each sample were loaded and separated by gel electrophoresis and then transferred to nitrocellulose membranes. After blocking, membranes were incubated with primary antibodies at 4°C overnight. The primary antibodies recognizing PCNA (1:1000, #2586), phosphorylated-EGFR (1:1000, #3777), and total-EGFR (1:1000, #4267, Cell Signaling Technology) were purchased from Cell Signaling Technology. β-actin (1:8000, #A3854) was purchased from Sigma-Aldrich. The next day, the membranes were washed and incubated with HRP-conjugated secondary antibodies (anti-Rabbit, #7074, 1:5000, 1:5000, Cell Signaling Technology) or (anti-mouse, #7076, 1:5000, Cell Signaling Technology) at room temperature for 1 h. Proteins were visualized with the ECL chemiluminescent kit (ECL-plus, Thermo Scientific). Detection and quantification of protein bands were performed using a ChemiDoc Imaging System with ImageLab Software (BioRad Laboratories).

Enzyme-linked immunosorbent assay (ELISA)

BMDM from BALB/c wild-type mice or Il-4r$\alpha^{-/-}$mice were treated with or without IL-4 (20 ng/ml, Peprotech) for 24h at 37°C. The supernatants were then harvested to detect HB-EGF protein by ELISA (#DY8239-05, R&D systems) according to the manufacturer’s protocols.

Statistical analysis

All statistical analyses and graphing were conducted with GraphPad Prism version 10.1.2 (GraphPad Software Inc.). Results were presented as mean ± SEM. For all comparisons in which there were two groups of values, a two-tailed unpaired Student's t-test with Welch's correction was performed after demonstrating that the data follow a normal distribution by the Shapiro-Wilk normality test. One-way ANOVA was used to compare values obtained from three or more groups with one independent variable, followed by Tukey's test. To compare groups with two independent variables, two-way ANOVA was used followed by Tukey's test. Differences in values were considered significant at $p<0.05$. All experiments were repeated a minimum of three times.

Reference:

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Supplemental figures:

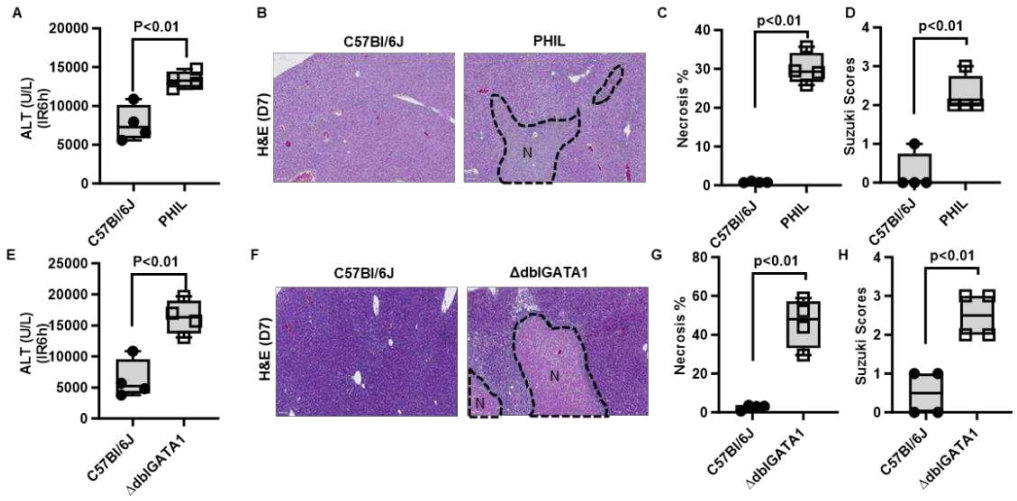

Fig. S1. Liver repair after IR injury is impaired in eosinophil-deficient mice. (A-D) Male PHIL mice and WT littermates were subjected to hepatic IR surgery (n=4/group). (E-H) Male ΔdblGATA-1 and WT C57Bl/6J mice were subjected to hepatic IR surgery (n=4/group). All mice were sacrificed on day 7 after IR surgery. (A, E) Serum ALT levels at 6h after IR surgery. (B, C and F, G) Liver necrosis (N, outlined areas) was evaluated and quantified on day 7 after IR surgery. (D, H) Liver pathology was assessed by using the Suzuki's scoring system on day 7 after IR surgery. Two-tailed unpaired Student's t-test with Welch's correction was performed in A, C, D, E, G, and H.

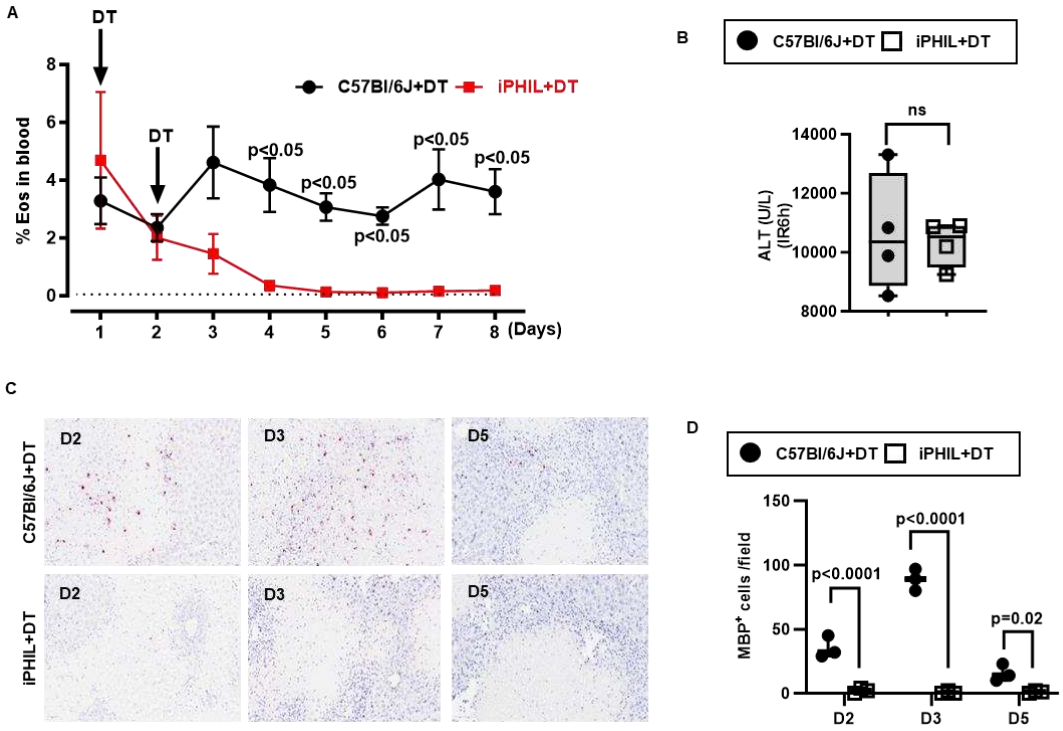

Fig. S2. Depletion of eosinophils in iPHIL mice. (A) Male iPHIL mice and WT littermates (C57Bl/6J) were injected i.p. with DT (5ng/g mouse) on day 1 and day 2. Blood eosinophils were analyzed by flow cytometry from day 1 to day 8 (n=3/group). (B) Male iPHIL mice and WT littermates were subjected to hepatic IR surgery. Mice were administered (i.p.) the first dose of DT at 16h prior to IR surgery and the second dose at 6h after surgery. Serum ALT levels were measured at 6h after IR surgery (n=4/group). (C-D) iPHIL mice and WT littermates were i.p. injected with DT at 16h prior to and 6h after hepatic IR surgery. Mice were sacrificed on days 2, 3, and 5 after IR surgery. IHC staining for eosinophils by anti-MBP antibody and the numbers of MBP^+ cells quantified (n=3/group). Two-tailed unpaired Student's t-test with Welch's correction was performed in A, B and D.

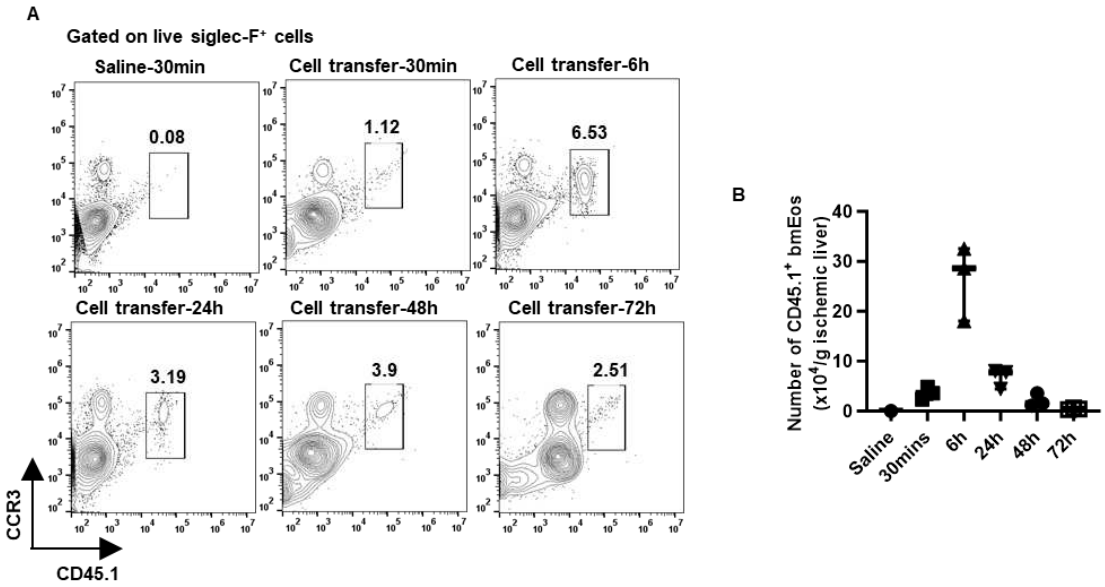

Fig. S3. Detection of bmEos in the liver after adoptive transfer. C57Bl/6J (CD45.2) mice were subjected to hepatic IR surgery and after 1 day injected with bmEos ($10x10^6$) obtained from B6-CD45.1 mice. Control mice were injected with saline. The numbers of transferred bmEos ($CD45.1^+$ $SiglecF^+CCR3^+$) in the liver of recipient mice were measured by flow cytometry at 6h, 24h, 48h, and 72h after adoptive transfer of the cells (n=3/group).

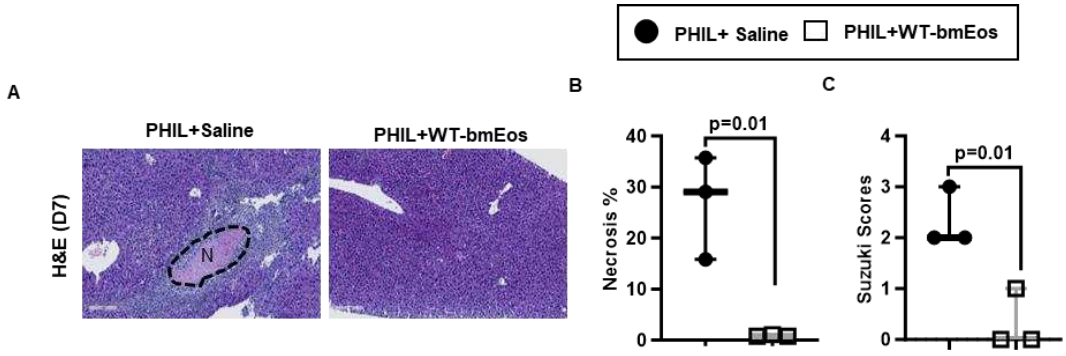

Fig. S4. Adoptive transfer of WT eosinophils to PHIL mice restores liver repair after IR injury. Male PHIL mice were subjected to hepatic IR surgery and after 1 day injected with WT-bmEos ($10x10^6$). Control mice were injected with saline. All mice were sacrificed on day 7 after IR surgery (n=3/group). (A, B) Liver necrosis (N, outlined areas) was evaluated and quantified. (C) Liver pathology was assessed by using the Suzuki's scoring system. Two-tailed unpaired Student's t-test with Welch's correction was performed in B and C.

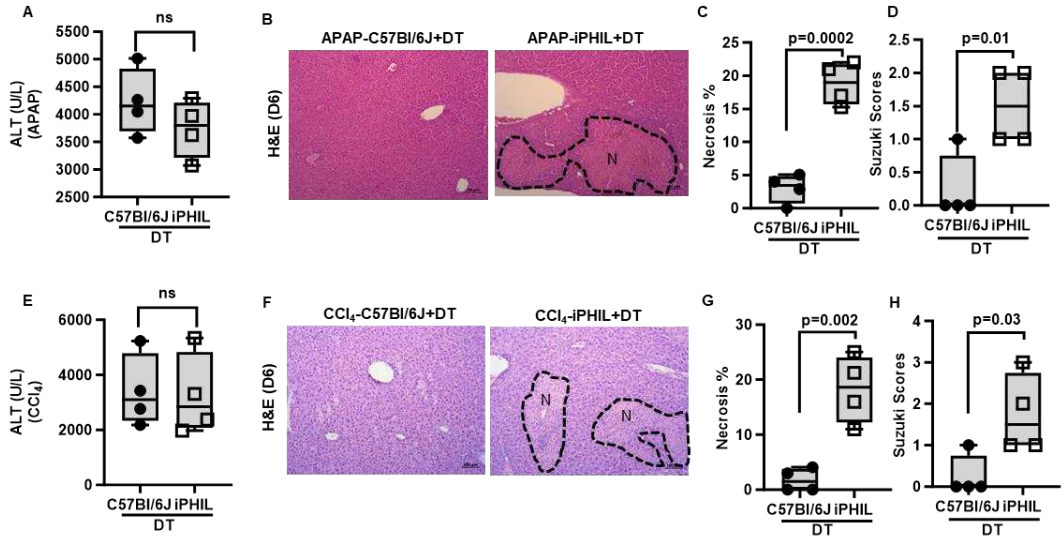

Fig. S5. Liver repair is delayed in iPHIL mice after APAP and CCl_4 treatment. (A-H) Male iPHIL mice and their WT littermates were i.p. injected with APAP or CCl_4. For APAP treatment, mice were fasted overnight before injected intraperitoneally i.p. with APAP. Mice were treated with 210 mg/kg of APAP. For CCl_4 treatment, a 1:4 dilution of 1 ml/kg CCl_4 in corn oil was i.p. injected to mice. All mice were administered (i.p.) the first dose of DT at 16h prior to APAP or CCl_4 treatment. They were then injected with a second dose of DT at 8h after APAP injection or 12h after CCl_4 injection. All mice were sacrificed on day 6 (n=4/group). (A, E) Serum ALT levels at 8h after APAP treatment and 12h after CCl_4 treatment. (B, C and F, G) Liver necrosis (N, outlined areas) was evaluated and quantified. (D, H) Liver pathology was assessed by using the Suzuki's scoring system. Two-tailed unpaired Student's t-test with Welch's correction was performed in A, C, D, E, G, and H.

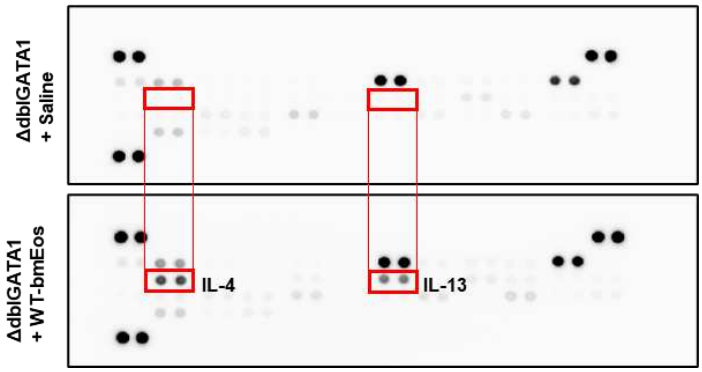

Fig. S6. Adoptive transfer of WT bmEos to ΔdblGATA-1 mice increased hepatic levels of IL-4 and IL-13. 6 Male ΔdblGATA-1 were subjected to hepatic IR surgery. After 24h, half of the mice were i.v. injected with bmEos ($10x10^6$) and the other half injected with saline as control. Mice were sacrificed after 3 days, and the expression levels of 40 cytokines in the liver samples were measured by using a Proteome Profiler Mouse Cytokine Array Kit. Data are representative of three independent experiments.

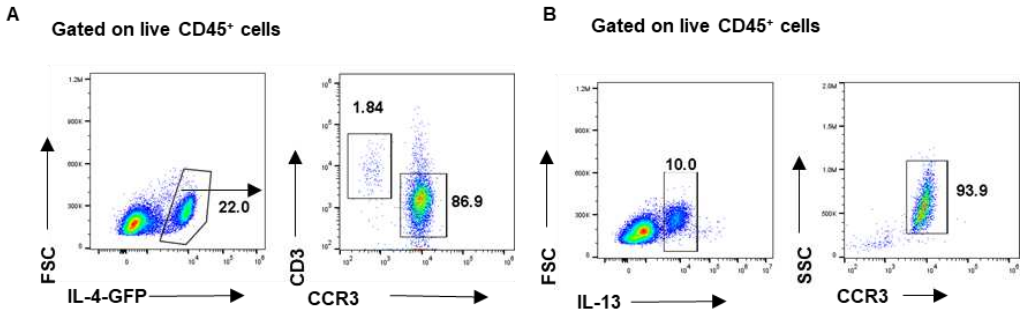

Fig. S7. IL-4 and IL-13 production by hepatic eosinophils during liver IR injury. (A, B) Male 4Get mice were subjected to hepatic IR injury and sacrificed after 3 days. Liver NPCs were isolated and stained intracellularly for IL-13. The IL-4-GFP$^+$ cells and IL-13$^+$ cells were gated. The proportions of IL-4$^+$ or IL-13$^+$ cells that were eosinophils (CCR3$^+$) are shown. Data are representative of three independent experiments.

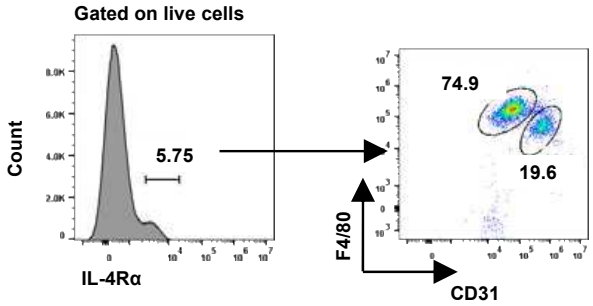

Fig. S8. Liver macrophages express IL-4Rα. Liver NPCs were isolated from naïve C57Bl/6J mice and analyzed by flow cytometry. IL-4Rα$^+$ cells were gated and identified as macrophages (F4/80$^+$, 74.9%) and LSECs (CD31$^+$, 19.6%). Data are representative of three independent experiments.

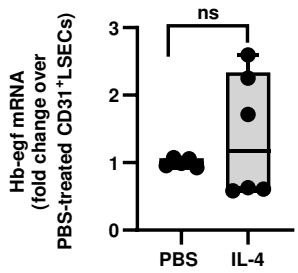

Fig.S9. IL-4 does not induce Hb-egf mRNA expression in liver sinusoidal endothelial cells (LSECs). LSECs were purified from naïve C57Bl/6J mice by magnetic-associated cell sorting (MACS) using anti-CD31 antibody. The cells were stimulated with IL-4 (10ng/ml) or PBS as the control for 6h (n=5-6/group). The mRNA levels of Hb-egf were measured by q-PCR. A two-tailed unpaired Student's t-test with Welch's correction was performed.

Table S1: Primer sequences for qRT-PCR

Gene Name (Symbol)	**Forward Sequence**	**Reverse Sequence**
Mouse Hb-egf	CGGGGAGTGCAGATACCTG	TTCTCCACTGGTAGAGTCAGC
Mouse Areg	GGTCTTAGGCTCAGGCCATTA	CGCTTATGGTGGAAACCTCTC
Mouse Tgf-α	CACTCTGGGTACGTGGGTG	CACAGGTGATAATGAGGACAGC
Mouse Btc	AATTCTCCACTGTGTGGTAGCA	GGTTTTCACTTTCTGTCTAGGGG
Mouse Ereg	CTGCCTCTTGGGTCTTGACG	GCGGTACAGTTATCCTCGGATTC
Mouse Epgn	GGGGGTTCTGATAGCAGTCTG	TCGGTGTTGTTAAATGTCCAGTT
Mouse Egf	AGAGCATCTCTCGGATTGACC	CCCGTTAAGGAAAACTCTTAGCA
Mouse 18s	ACGGAAGGGCACCACCAGGA	CACCACCACCCACGGAATCG