Supplementary materials

Type 2 innate lymphoid cells are protective against hepatic ischemia reperfusion injury

Qi Cao, Ruifeng Wang, Zhiguo Niu, Titi Chen, Farhana Azmi, Scott A. Read, Jianwei Chen, Vincent W.S. Lee, Chunze Zhou, Sohel Julovi, Qingsong Huang, Yuan Min Wang[5], Malcolm R. Starkey, Guoping Zheng, Stephen I. Alexander, Jacob George, Yiping Wang, David C.H. Harris

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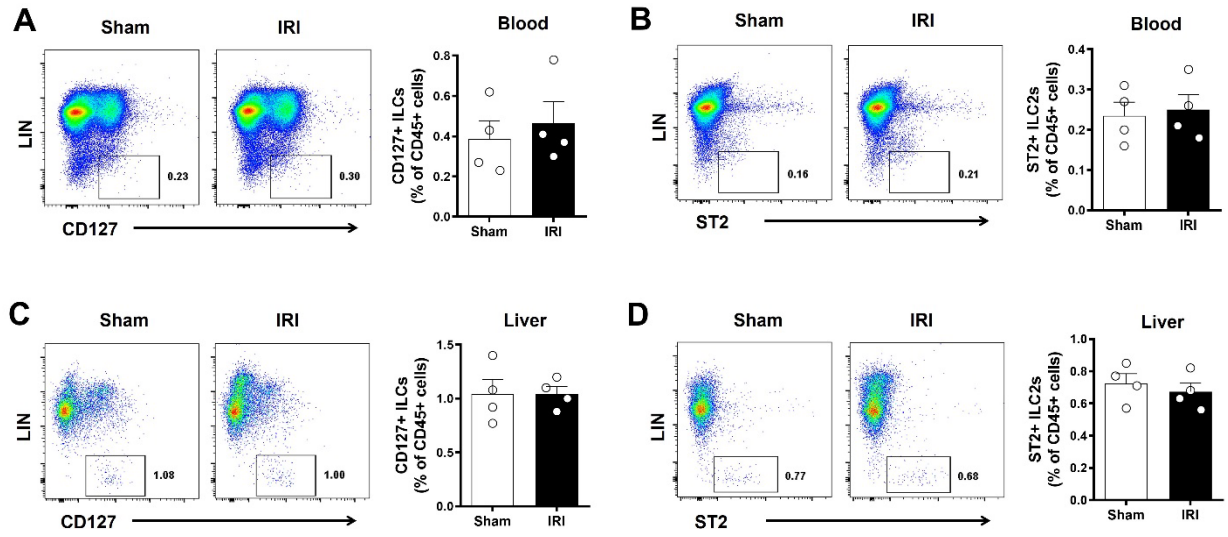

Fig. S1. ILC2 population did not increase in peripheral blood and liver tissue after hepatic IRI. (A, B) Proportion of CD127+ ILCs and ST2+ ILC2s in CD45+ leukocytes in peripheral blood of Sham and IRI mice. (C, D) Proportion of CD127+ ILCs and ST2+ ILC2s in CD45+ leukocytes in the livers of Sham and IRI mice. Data shown are the mean ± SEM (n=4 per group). Statistical significance was assessed using the Student t test.

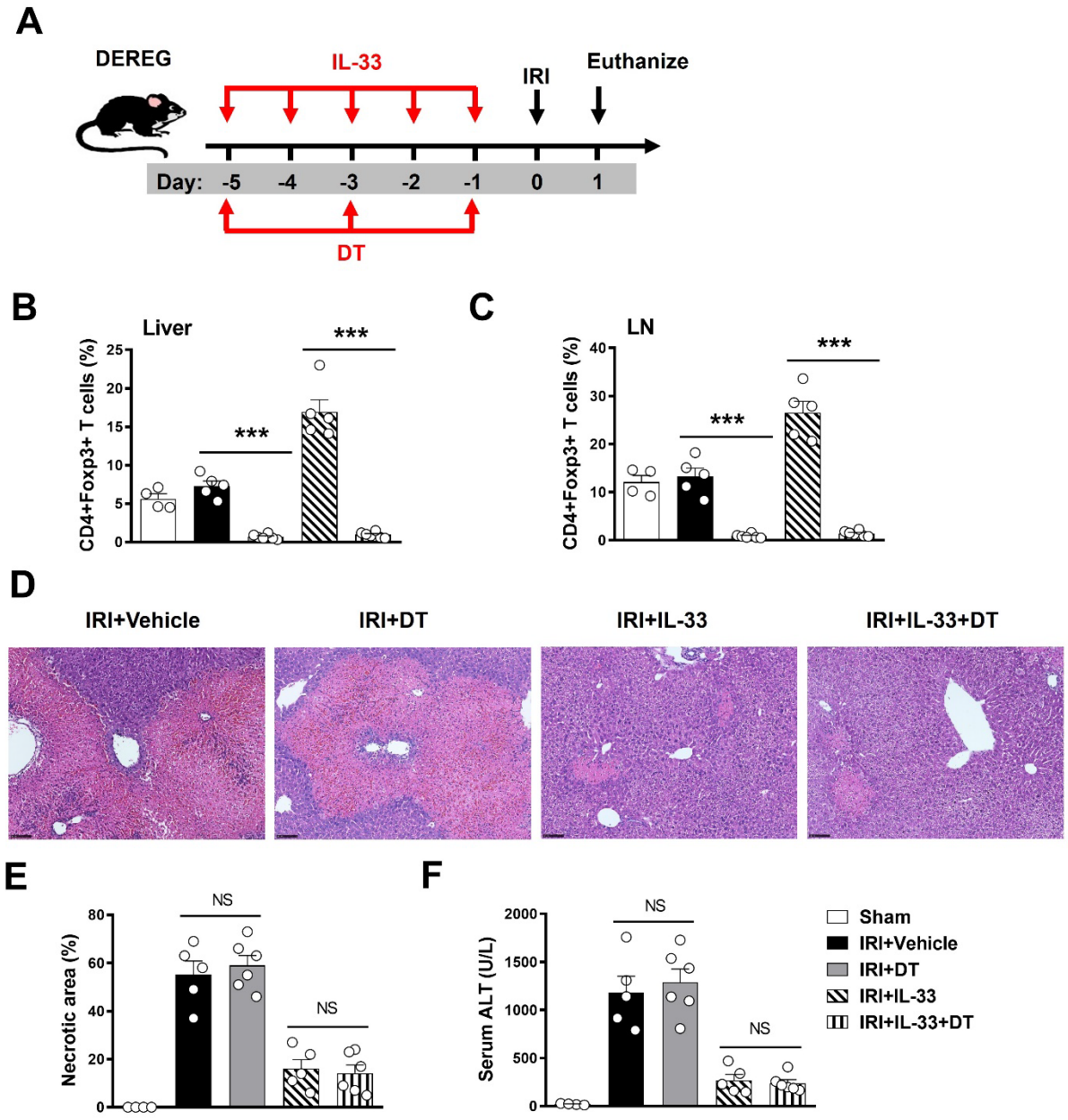

Fig. S2. Tregs did not contribute to IL-33-mediated hepatoprotection in IRI mice.
(A) DEREG C57BL/6 mice were treated with mouse recombinant IL-33 daily for 5 consecutive days, as well as DT at day -5, -3 and -1 before hepatic ischemia. (B, C) Proportion of CD4+Foxp3+ Tregs in the CD4+ T cell compartment from the livers and lymph nodes of Sham, IRI+Vehicle, IRI+DT, IRI+IL-33 or IRI+IL-33+DT mice. (D) Representative H&E-stained sections of livers from mice at one day after IRI. Bar = 200 μm. (E, F) Liver necrosis areas and serum ALT levels were assessed in these mice. Data shown are the mean ± SEM (n=4-6 per group); Statistical significance was assessed using a one-way ANOVA. NS: non significant, ***P<0.001.

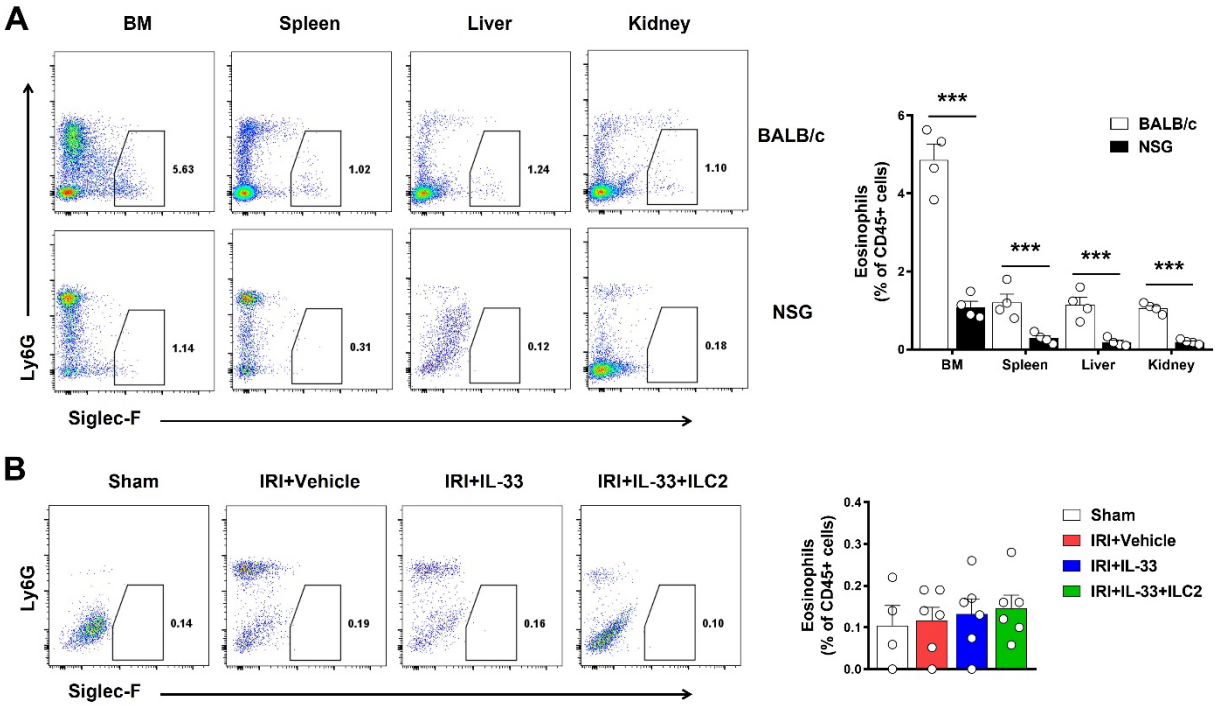

Fig. S3. There was no increase of eosinophils in livers of NSG mice treated with IL-33. (A) Flow cytometric analysis of Siglec-F+ eosinophils in bone marrow (BM), spleen, liver and kidney of normal BALB/c and NSG mice. Data shown are the mean ± SEM (n=4 per group); Statistical significance was assessed using the Student t test. ***P<0.001. (B) NSG mice were administered mouse recombinant IL-33 daily for 5 consecutive days and were injected with ILC2s at day -5 before ischemia. Frequency of Siglec-F+ eosinophils in the CD45+ leukocyte compartment from the liver was assessed in Sham, IRI+Vehicle, IRI+IL-33 or IRI+IL-33+ILC2 mice. Data shown are the mean ± SEM (n=4-6 per group), Statistical significance was assessed using a one-way ANOVA.

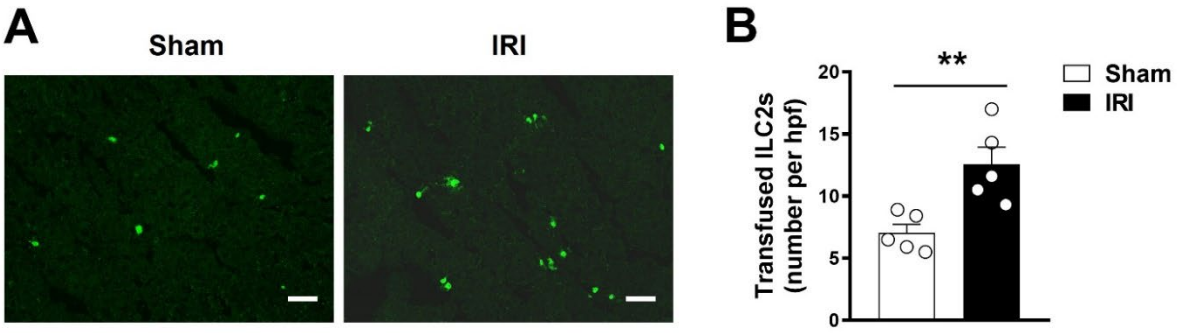

Fig. S4. Transfused mouse ILC2s distributed into liver.
CFSE labeled ILC2 cells were adoptively transferred into C57BL/6 mice one day before ischemia. (A) Transfused CFSE+ ILC2s were observed in Sham liver and IRI liver at 4 hours after IRI. Bar = 100 μm. (B) Numbers of CFSE labeled ILC2 cells in Sham liver and IRI liver were counted. Data shown are the mean ± SEM per high power field (hpf) from each group (n=5 per group). Statistical significance was assessed using the Student t test. **P<0.01.

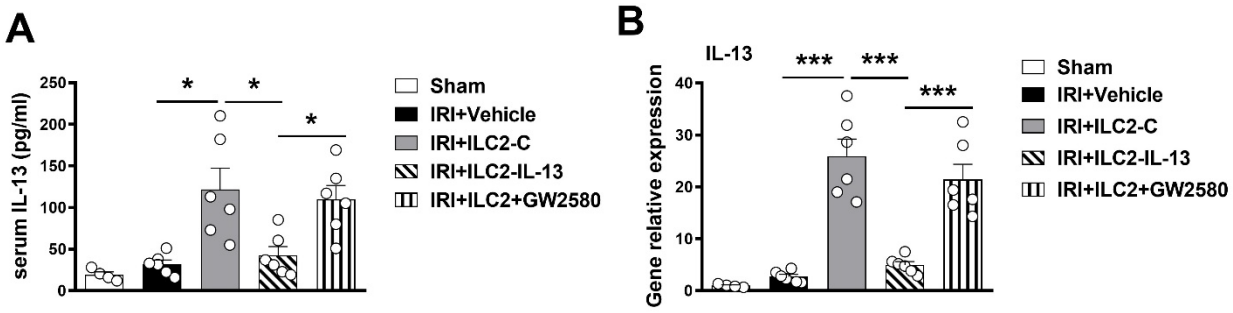

Fig. S5. IL-13 was increased in peripheral blood and liver of IRI mice treated with ILC2s. C57BL/6 mice were treated with transfected ILC2 one day before ischemia, and GW2580 daily for 3 consecutive days before ischemia. IL-13 in the serum (A) and its mRNA expression in the livers (B) from these mice were measured at one day after IRI. Data shown are the mean ± SEM (n=4-6 per group). Statistical significance was assessed using a one-way ANOVA. *P<0.05, ***P<0.001.

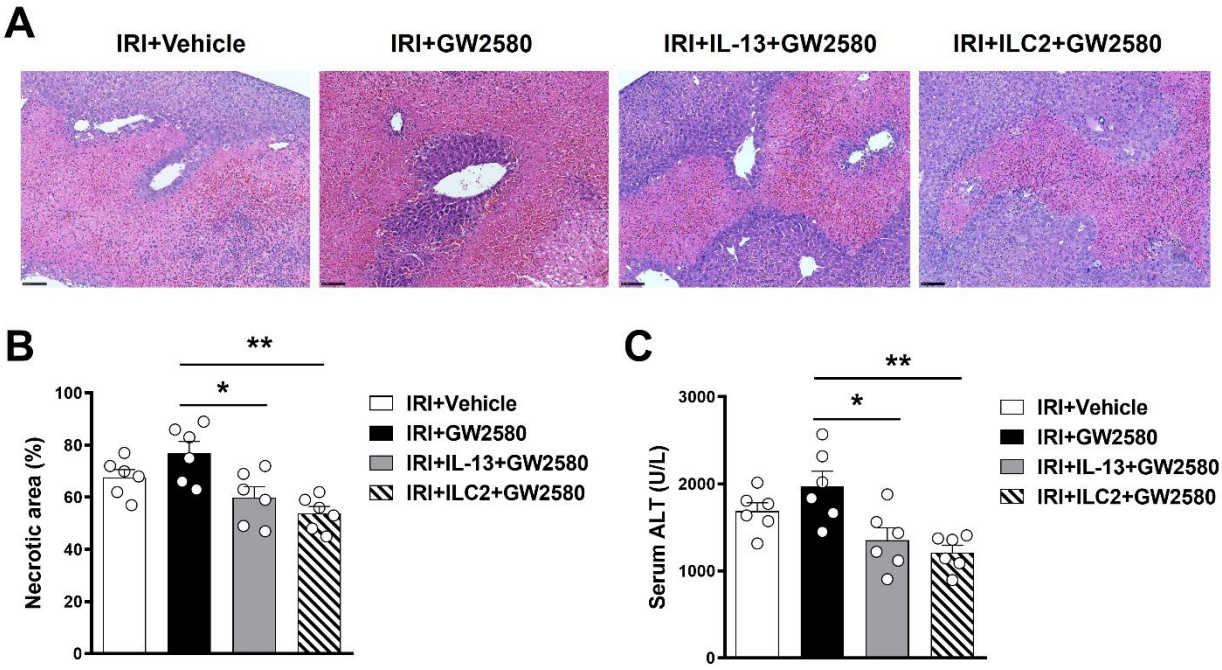

Fig. S6. Macrophages were required for ILC2-mediated hepatoprotection in IRI mice. C57BL/6 mice were treated with ILC2s or IL-13 one day before ischemia, and GW2580 daily for 3 consecutive days before ischemia. (A) Representative H&E-stained sections of livers from mice at one day after IRI. Bar = 200 μm. (B and C) Liver necrosis areas and serum ALT levels were assessed in these mice. Data shown are the mean ± SEM (n=6 per group). Statistical significance was assessed using a one-way ANOVA. *P<0.05, **P<0.01.

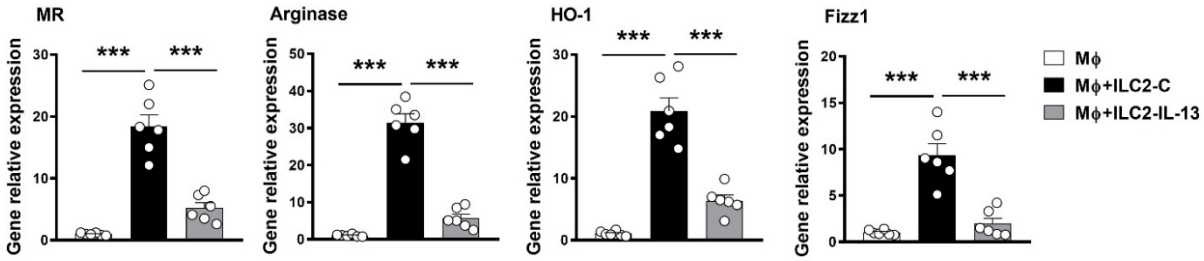

Fig. S7. ILC2 induced M2 macrophage through IL-13 production.
Liver macrophages were cultured with transfected ILC2-C or ILC2-IL-13 for 6 hours. Macrophage phenotype was examined by qPCR. The mRNA expression of M2 macrophage markers (MR, arginase, HO-1 and FIZZ1) was examined by qPCR in liver macrophages. Data shown are the mean ± SEM (n=6 per group). Statistical significance was assessed using a one-way ANOVA. ***P<0.001.

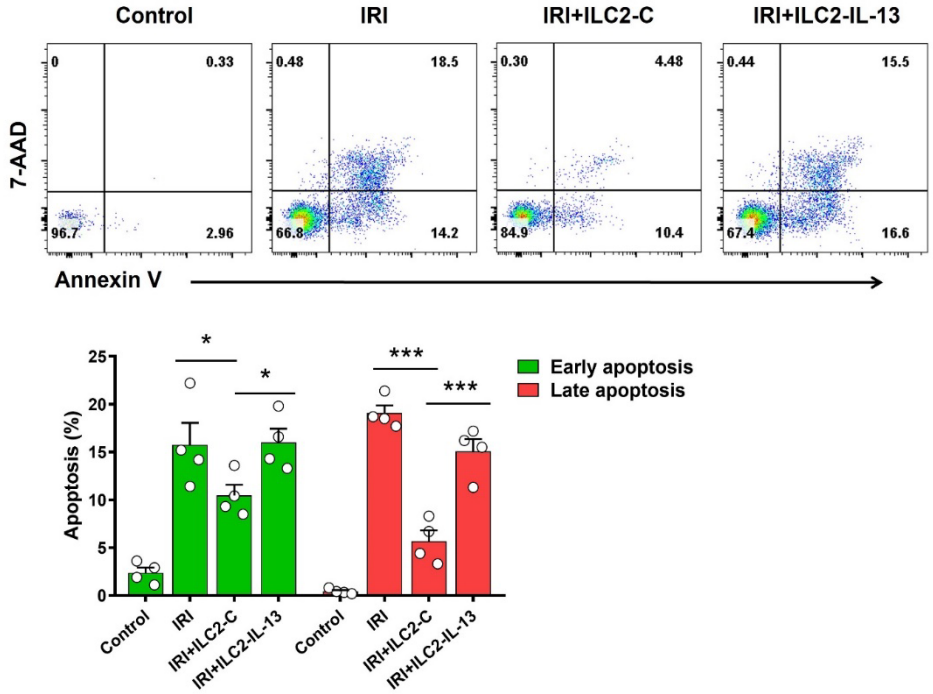

Fig. S8. ILC2s protected against ischemic hepatocyte injury through IL-13 production.
Transfected ILC2 cells (ILC2-C or ILC2-IL-13) were cocultured with ischemic hepatocytes for 12 hours. Hepatocytes were exposed to DMEM/F12 medium alone as the nonischemic control. Frequency of early apoptosis (Annexin-V+7AAD- cells) and late apoptosis (Annexin-V+7AAD+ cells) in hepatocytes were assessed by flow cytometry. Data shown are

the mean ± SEM (n=4 per group). Statistical significance was assessed using a one-way ANOVA. *P<0.05, ***P<0.001.

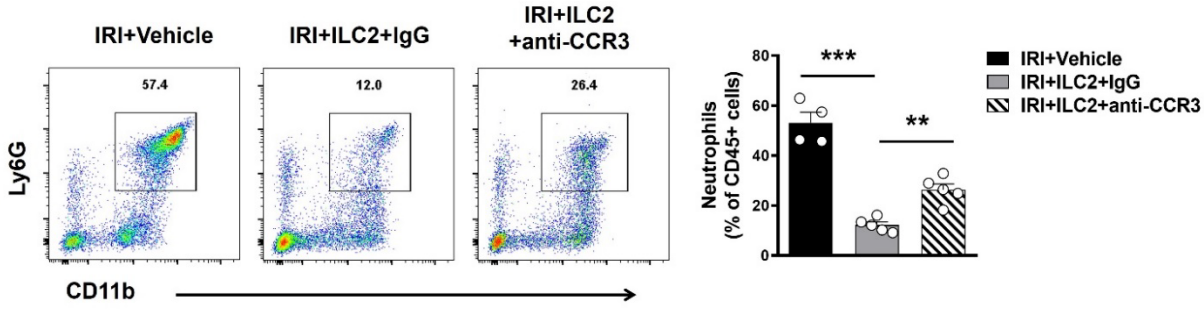

Fig. S9. Eosinophil depletion resulted in an infiltration of neutrophils to the liver.
The infiltration of neutrophils in livers of IRI+Vehicle, IRI+ILC2+IgG or IRI+ILC2+anti-CCR3 mice was assessed by flow cytometry. Data shown are the mean ± SEM (n=4-5 per group). Statistical significance was assessed using a one-way ANOVA. **P<0.01, ***P<0.001.

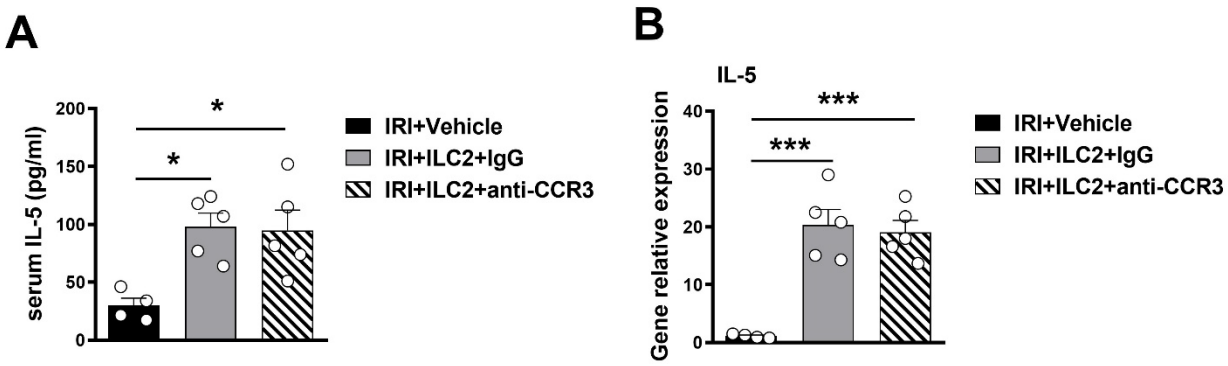

Fig. S10. IL-5 was increased in peripheral blood and liver of IRI mice treated with ILC2s. C57BL/6 mice were treated with ILC2 one day before ischemia, and anti-CCR3 antibody daily for 2 consecutive days before ischemia. IL-5 in the serum (A) and its mRNA expression in the livers (B) from these mice were measured at one day after IRI. Data shown are the mean ± SEM (n=4-5 per group). Statistical significance was assessed using a one-way ANOVA. *P<0.05, ***P<0.001.

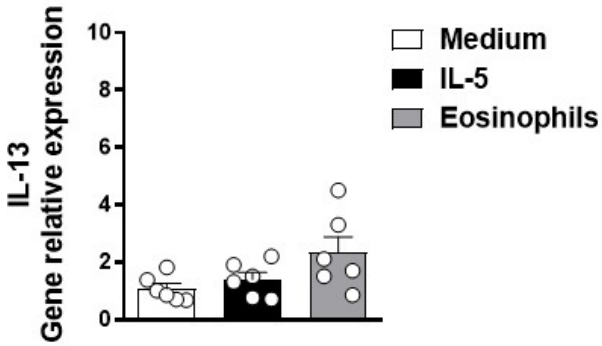

Fig. S11. IL-13 expression was not increased in ILC2s cultured with IL-5 or eosinophils. ILC2s were cultured with IL-5 or eosinophils for 24 hours. The expression of IL-13 in ILC2s was measured by qPCR. Data shown are the mean ± SEM (n=6 per group). Statistical significance was assessed using a one-way ANOVA.

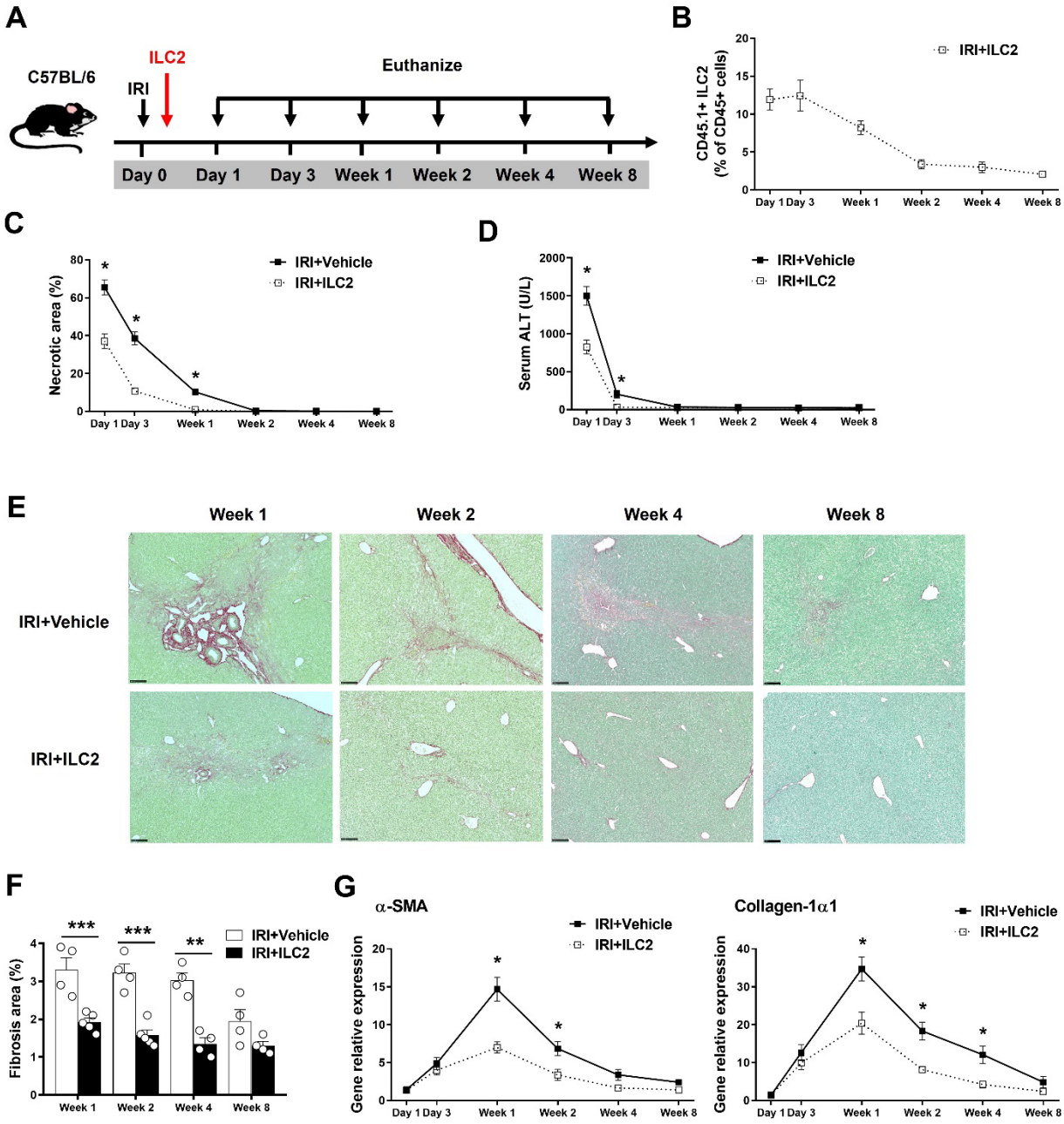

Fig. S12. ILC2 were protective post-IRI. (A) C57BL/6 mice were treated with ILC2s at 6 hours after ischemia. Mice were euthanized at indicated time points. (B) Percentage of CD45.1+ ILC2s in the CD45+ leukocyte compartment from the livers was measured at day 1, day 3, week 1, week 2, week 4 and week 8 after IRI. (C and D) Liver necrosis areas and serum ALT levels were assessed at indicated time points. (E) Liver fibrosis was determined by Sirius red staining. Bar = 200 μm. (F) Liver mRNA expression of α-smooth muscle actin (α-SMA), collagen-1α1 after IRI was measured by qPCR. Data shown are the mean ± SEM (n=4-5 per group). Statistical significance was assessed using the Student t test or ANOVA. *P<0.05, **P<0.01, ***P<0.001.

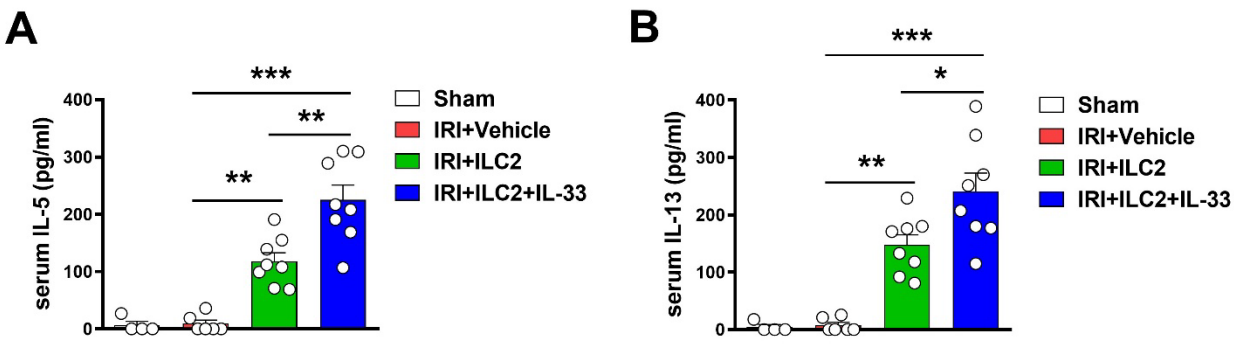

Fig. S13. Human IL-5 and IL-13 were increased in peripheral blood of NSG mice treated with human ILC2s and IL-33. NSG mice were treated with human ILC2 (5x106) one day before IRI or human ILC2s (0.5x106) at day -5 before ischemia followed by administration of human recombinant IL-33 daily for 5 consecutive days. (A and B) Human IL-5 and IL-13 levels in the serum from these mice. Data shown are the mean ± SEM (n=4-8 per group). Statistical significance was assessed using a one-way ANOVA. *P<0.05, **P<0.01, ***P<0.001.

Table S1. Real-time PCR primers

Gene	Forward (5'-3')	Reverse (5'-3')
IL-4	tcaacccccagctagttgtc	tctgtggtgttcttcgttgc
IL-5	aaagagaagtgtggcgaggag	tcaccatggagcagctcag
IL-13	cagcatggtatggagtgtgg	aggctggagaccgtagtgg
Mannose receptor	caaggaaggttggcatttgt	cctttcagtcctttgcaagc
Arginase	agtctggcagttggaagcat	ctggttgtcaggggagtgtt
HO-1	ggtgatggcttccttgtacc	agtgaggcccataccagaag
FIZZ1	tgctgggatgactgctactg	ctgggttctccacctcttca
TNF-α	gctgagctcaaaccctggta	cggactccgcaaagtctaag
IL-1β	tgccacctttgacagtgatg	atgtgctgctgcgagatttg
IL-6	cacaagtccggagaggagac	ttgccattgcacaactcttt
MCP-1	agcaccagccaactctcact	cgttaactgcatctggctga
CXCL1	tggctgggattcacctcaagaaca	tgtggctatgacttcggtttgggt
α-SMA	tgctgacagaggcaccactgaa	cagttgtacgtccagaggcatag
collagen-1α1	cctcagggtattgctggacaac	cagaaggaccttgtttgccagg