Supplementary information

Table S1. Antibodies used for flow cytometry and tissue/cell staining

Antibody/reagent	Clone	Concentration (µg/ml)	Supplier (Cat#)
Biotin anti-F4/80	REA126	FC (3.75)	Mitenyi Biotec 130-116-514
PerCP/Cy5.5 anti-CD45	30-F11	FC (1)	Biolegend 103132
FITC anti-CD3ε	145-2C11	FC (2.5)	Biolegend 100306
FITC anti-CD11b	M1/70	FC (2.5)	Biolegend 101206
FITC anti-CD11c	N418	FC (2.5)	Biolegend 117306
FITC anti-CD19	6D5	FC (2.5)	Biolegend 115506
FITC anti-CD49b	DX5	FC (2.5)	Biolegend 108906
FITC anti-F4/80	BM8	FC (2.5)	Biolegend 123108
FITC anti-FcεRI	MAR-1	FC (2.5)	Biolegend 134306
eFlour 450 anti-Thy1.2	53-2.1	FC (4)	Thermo Fisher 48-0902-82
PE anti-ST2	U29-93	FC (5)	BD 566311
PE anti-RELMα	DS8RELM	FC (1)	Thermo Fisher 12-5441-80
PE/Cy7 anti-CD8a	53-6.7	FC (1)	Biolegend 100722
PE/Cy7 Streptavidin	N/A	FC (2)	Thermo Fisher 25-4317-82
APC anti-KLRG1	2F1	FC (2)	Biolegend 138411
APC anti-SiglecF	S17007L	FC (2)	Biolegend 155507
APC anti-CD11c	N418	FC (1)	Biolegend 117310
APC anti-NKp46	29A1.4	FC (1)	Biolegend 137607
Alexa Fluor 647 anti-GATA3	L50-823	FC (1:5)	BD 560068
APC/Cy7 anti-CD4	GK1.5	FC (1)	Biolegend 100413
APC/Cy7 anti-CD19	6D5	FC (1)	Biolegend 115529
APC/Cy7 anti-CD45	30-F11	FC (1)	BD 557659
BV421 anti-CD86	GL-1	FC (2)	Biolegend 105032
BV421 anti-GATA3	16E10A23	FC (1:20)	Biolegend 653814
BV421 anti-SiglecF	S17007L	FC (2)	Biolegend 155509
Fixable Viability Dye eFluor 506	N/A	FC (1:250)	Thermo Fisher 65-0866-14
Fixable Viability Dye eFluor 780	N/A	FC (1:250)	Thermo Fisher 65-0865-14
Anti-Cardiac Troponin I	4C2	IF (1:200)	Abcam, ab10231
Alexa Fluor 647 WGA	N/A	IF (1:200)	W32466,
Biotin-Isolectin B4	N/A	IF (1:100)	Invitrogen, I21414
Anti-IL-33	N/A	IF (1:200)	R&D Systems, AF3626
Anti-Phospho-Histone H2AX (Ser139)	20E3	IF (1:200)	Cell Signaling, 9718
Anti-Vimentin	EPR3776	IF (1:200)	Abcam, ab92547
Anti-Periostin	N/A	IHC (1:200)	Sino Biological, 50450-RP02
Anti-BMP7	4E7	Neu (1.0)	Arigo, ARG56924

Table S2. Primers used for qRT-PCR.

Gene	Species	Sequence (5'-3')
IL5	Mouse	Forward: TCAGGGGCTAGACATACTGAAG
		Reverse: CCAAGGAACTCTTGCAGGTAAT
IL13	Mouse	Forward: CCTGGCTCTTGCTTGCCTT
		Reverse: GGTCTTGTGTGATGTTGCTCA
Areg	Mouse	Forward: GCAGATACATCGAGAACCTGG
		Reverse: CTGCAATCTTGGATAGGTCCTTG
Hbegf	Mouse	Forward: TTTGGAGAGTCCTTTGCAGA
		Reverse: TGTGACAATGAGATTCCTTGTG
Bmp7	Mouse	Forward: GATTTCAGCCTGGACAACGAG
		Reverse: GGGCAACCCTAAGATGGACAG
IL10	Mouse	Forward: GCTCTTACTGACTGGCATGAG
		Reverse: CGCAGCTCTAGGAGCATGTG
Csf3	Mouse	Forward: CCTGGAGCAAGTGAGGAAGA
		Reverse: CAGCTTGTAGGTGGCACACA
Bcl2	Mouse	Forward: CCTGTGGATGACTGAGTACC
		Reverse: GAGACAGCCAGGAGAAATCA
Bax	Mouse	Forward: GTTTCATCCAGGATCGAGCAG
		Reverse: CATCTTCTTCCAGATGGTGA
Arg1	Mouse	Forward: CCAGAAGAATGGAAGAGTCAGTGT
		Reverse: GCAGATATGCAGGGAGTCACC
Ym1	Mouse	Forward: CAAGTTGAAGGCTCAGTGGCTC
		Reverse: CAAATCATTGTGTAAAGCTCCTCTC
Postn	Mouse	Forward: GAACGAATCATTACAGGTCC
		Reverse: GGAGACCTCTTTTTGCAAGA
Tnc	Mouse	Forward: GCTACTGCCAGGCATCTTTC
		Reverse: GAAGCTCCCACTGGACTCTG
Timp1	Mouse	Forward: GAGAAACCAGCTTGGAACCAG
		Reverse: GGGGCCATCATGGTATCTGC
Col1a2	Mouse	Forward: ACTCAGCCACCCAGAGTGGAA
		Reverse: TTGACAGGTTGGGCCTGGA
Col3a1	Mouse	Forward: GCACAGCAGTCCAACGTAGA
		Reverse: GCACAGCAGTCCAACGTAGA
Lox	Mouse	Forward: TCTTCTGCTGCGTGACAACC
		Reverse: GAGAAACCAGCTTGGAACCAG

Table S3. Reagents used in this study

Reagent	Supplier (Cat#)
FBS	Gibco (10437-028)
DMEM/F12	Gibco (11330-032)
10X Trypsin-EDTA (0.5%)	Gibco (15400-054)
Collagenase Type II	Worthington (LS004177)
Penicillin/Streptomycin	Gibco (15140122)
L-Ascorbic acid	Sigma (A4544)
Mouse recombinant IL-33	BioLegend (580502)
Human recombinant TGF-β	PeproTech (100-21C)
High-Capacity RNA-to-cDNA™ Kit	Applied Biosystems (4387406)
TRIzol™ Reagent	Invitrogen™ (15596026)
Quick-RNA 96 Kit	ZYMO (R1052)
Quantinova SYBR Green PCR Kit	Qiagen (208054)
Galetin	Sigma (G9136)
TUNEL (In Situ Cell Death Detection Kit, Fluorescein)	Roche (11684795910)
Picro Sirius Red Stain Kit	Abcam (ab150681)
DL-Isoproterenol Hemisulfate	Santa Cruz (sc-294398)
Mouse IL-5 ELSIA	R&D Systems (DY405-05)
Mouse IL-13 ELISA	R&D Systems (DY413-05)
Mouse IL-10 ELISA	R&D Systems (DY417-05)
Mouse HB-EGF ELISA	R&D Systems (DY8239-05)
Mouse Amphiregulin ELISA	R&D Systems (DY-989)
Mouse G-CSF ELISA	R&D Systems (DY414-05)
Mouse BMP-7 ELISA	Invitrogen (# EMBMP7)
MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel - Immunology Multiplex Assay	Merck (MCYTOMAG-70K)

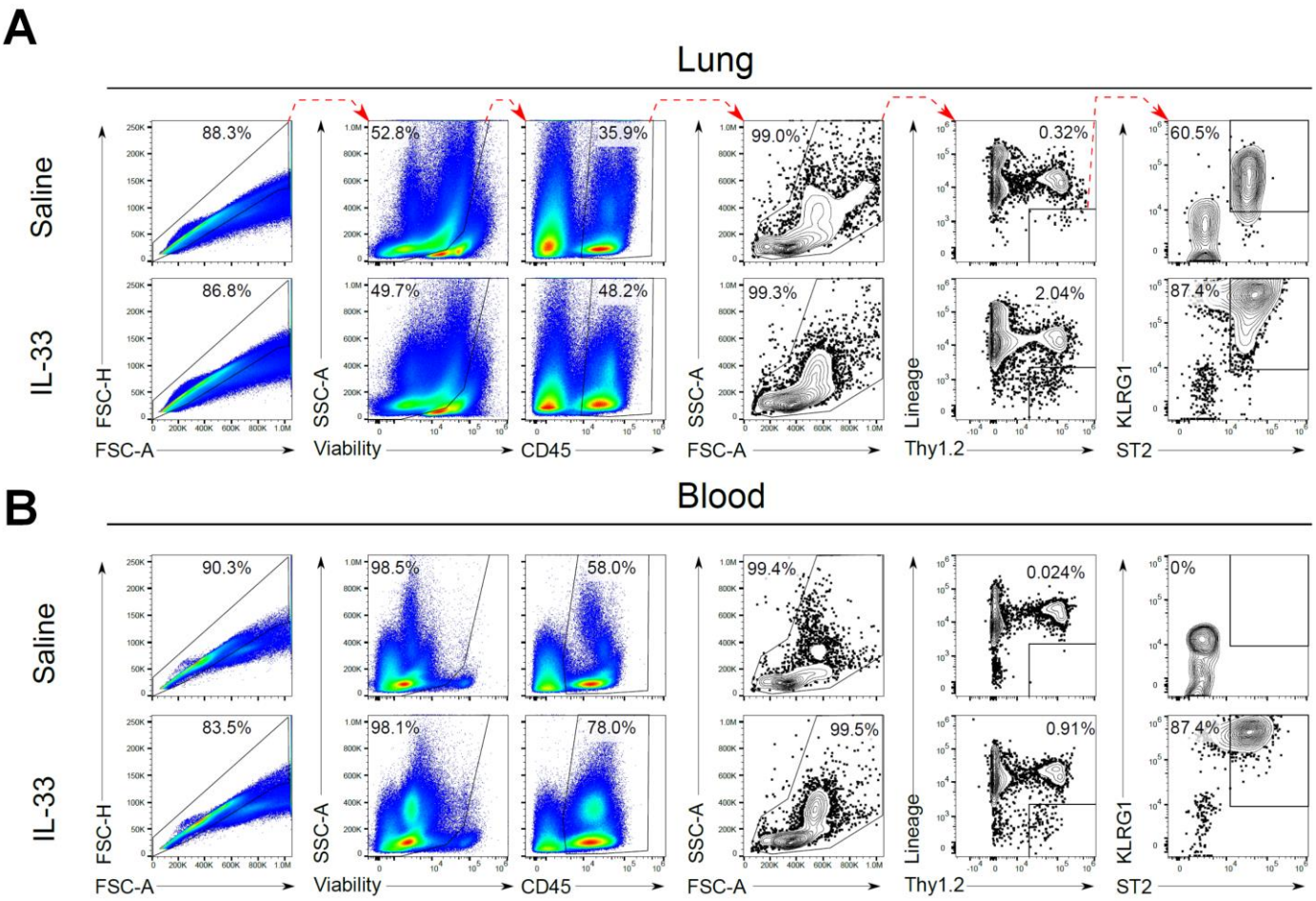

Figure S1. IL-33 increases the cardiac ILC2 population via ST2. Wild-type C57BL/6 mice were treated with saline or IL-33 (2 μg mouse^{-1}day^{-1} for 5 days). **(A-B)** The lung tissues and peripheral blood from the mice were harvested for flow cytometry analysis of the cell surface markers.

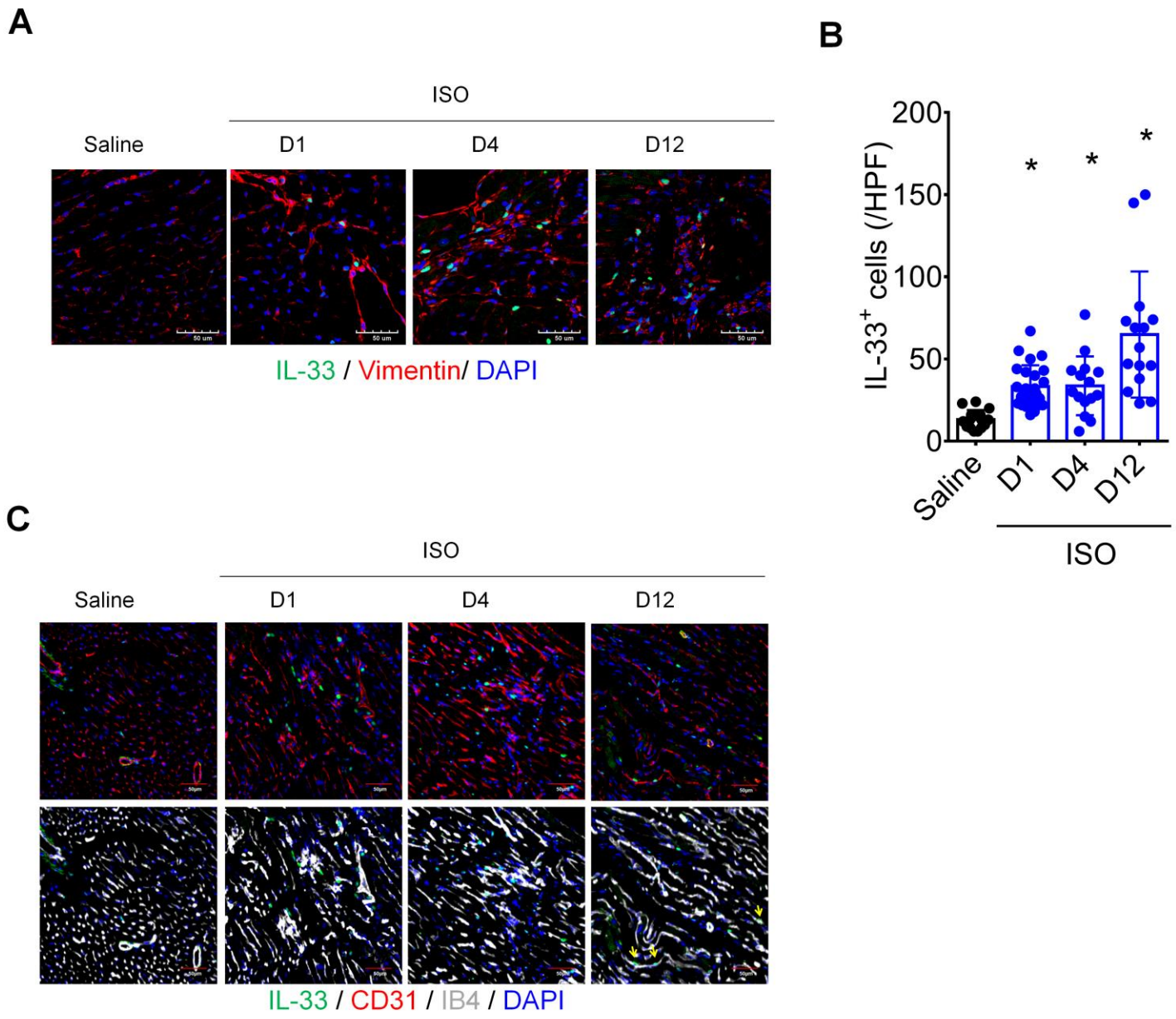

Figure S2. IL-33 expression is upregulated following isoproterenol (ISO)-induced cardiac injury. BALB/cByJ mice (n=3 in each group) were subcutaneously administered with ISO (60 mg/kg) for 3 days. The heart tissues were collected on day 1, 4, and 12 after the last ISO treatment. **(A)** Representative images of immunofluorescence staining for IL-33 (green), Vimentin (red), and nuclei (blue). **(B)** Quantification of IL-33$^+$ cells per high-power field (200X magnification). **(C)** Representative images of immunofluorescence staining for IL-33 (green), CD31 (red), isolectin IB4 (IB4) (gray), and nuclei (blue).

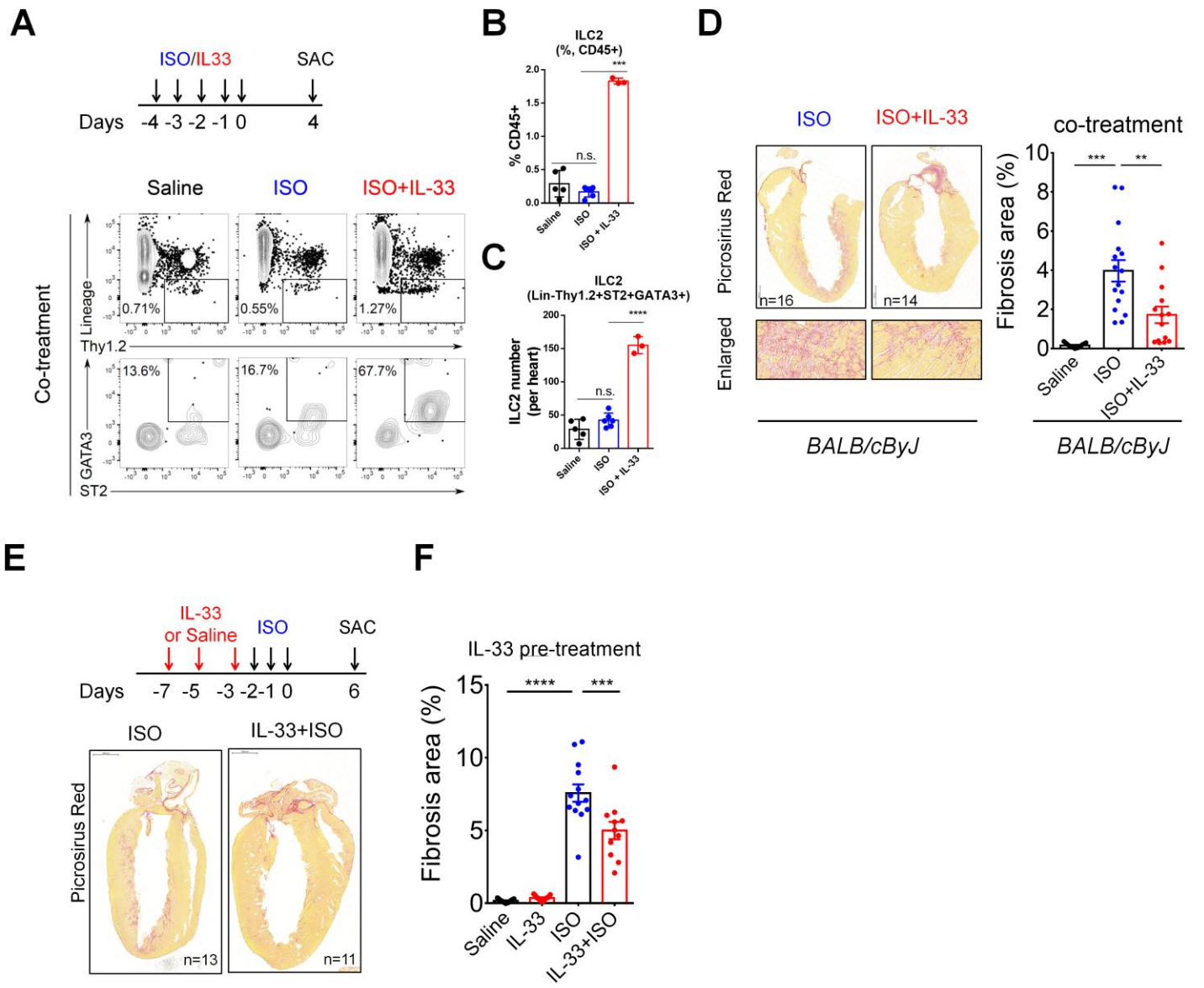

Figure S3. Co-treatment or pre-treatment of IL-33 reduces ISO-induced cardiac fibrosis. (A) BALB/cByJ mice were subcutaneously administered with saline, isoproterenol (ISO), or ISO+ IL-33 for 5 days. On day 4 after the last ISO injection, mice were euthanized (SAC), and cardiac tissues were collected for histological analysis. Flow cytometry analysis of the $CD45^{+}Lin^{-}Thy1.2^{+}GATA3^{+}$ ILC2 population in the hearts. **(B)** Frequency of ILC2 among $CD45^{+}$ cells. **(C)** Cell number of ILC2s per heart. **(D)** Picrosirius red staining. Quantification of the cardiac fibrosis area. Scale bar = 100 μm. **(E-F)** BALB/cByJ mice were pre-treated with intraperitoneally administered saline or IL-33 (0.5 μg/mouse) for a total of 3 doses. Then the mice were subcutaneously administered saline or ISO (60 mg kg^{-1} day^{-1}) for 3 days. The cardiac tissues were collected for histological analysis on day 6 after the last ISO injection (SAC). **(E)** Picrosirius red staining for fibrosis area. **(F)** Quantification of cardiac fibrosis area. ***$P < 0.001$, ****P<0.0001 by one-way ANOVA followed by the Bonferroni multiple comparison post-hoc test. All values are means ± SD. Each dot indicates a biological replicate.

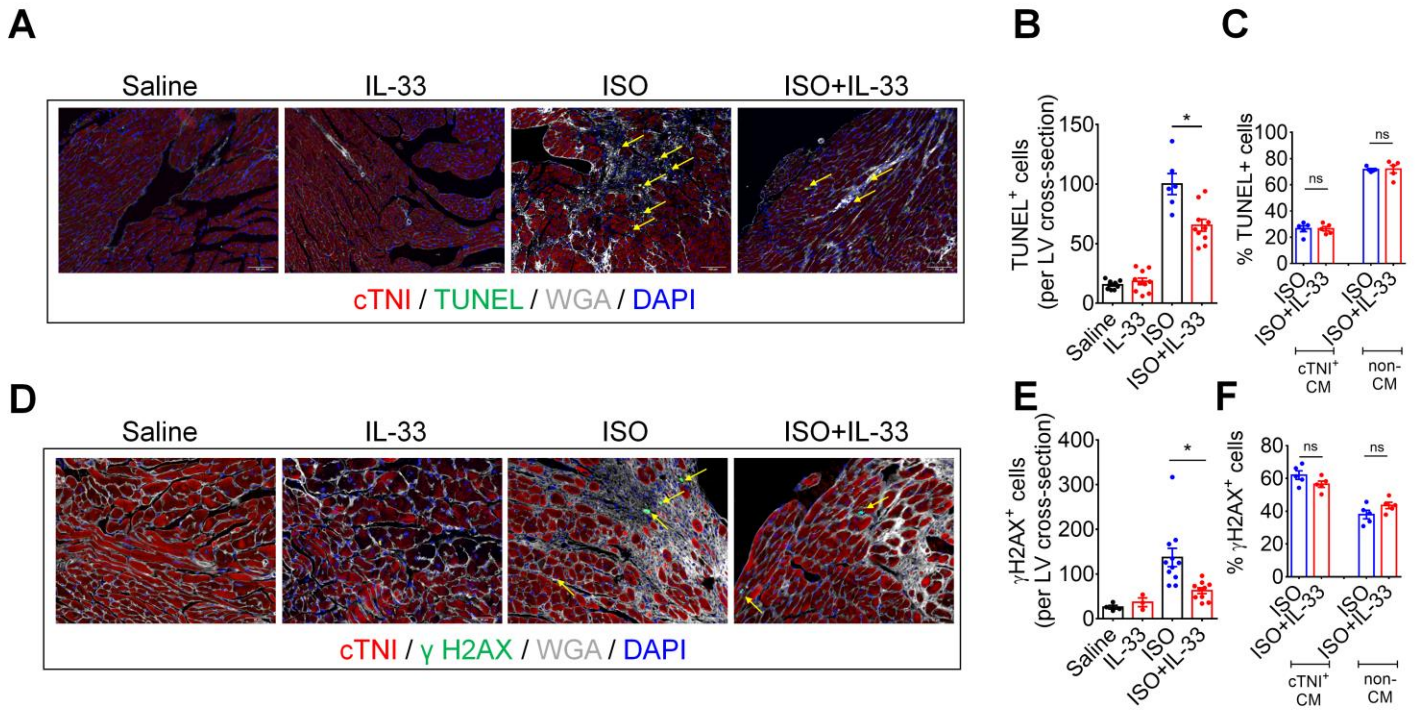

Figure S4. IL-33 treatment ameliorates ISO-induced cell death. (A) Representative images of immunofluorescent staining and **(B)** quantification of TUNEL$^+$ cells in the left ventricle region. Yellow arrowheads indicate respectively labeled cells. Number of positive stained cells in the left ventricle region was quantified in each heart cross-section (8–10 immunofluorescent images at 200X magnification). **(C)** Percentages of cTNI$^+$TUNEL$^+$ CMs and cTNI$^-$TUNEL$^+$ non-CMs among the total TUNEL$^+$ cells. **(D)** Representative images of immunofluorescent staining and **(E)** quantification of γH2AX$^+$ cells in the left ventricle region. Yellow arrowheads indicate respectively labeled cells. Number of positive stained cells in the left ventricle region was quantified in each heart cross-section (8–10 immunofluorescent images at 200X magnification). **(F)** Percentages of cTNI$^+$γH2AX$^+$ CMs and cTNI$^-$γH2AX$^+$ non-CMs among the total γH2AX$^+$ cells. ns, no significance; *P < 0.05 by one-way ANOVA followed by the Bonferroni multiple comparison post-hoc test. All values are means ± SD. Each dot indicates a biological replicate.

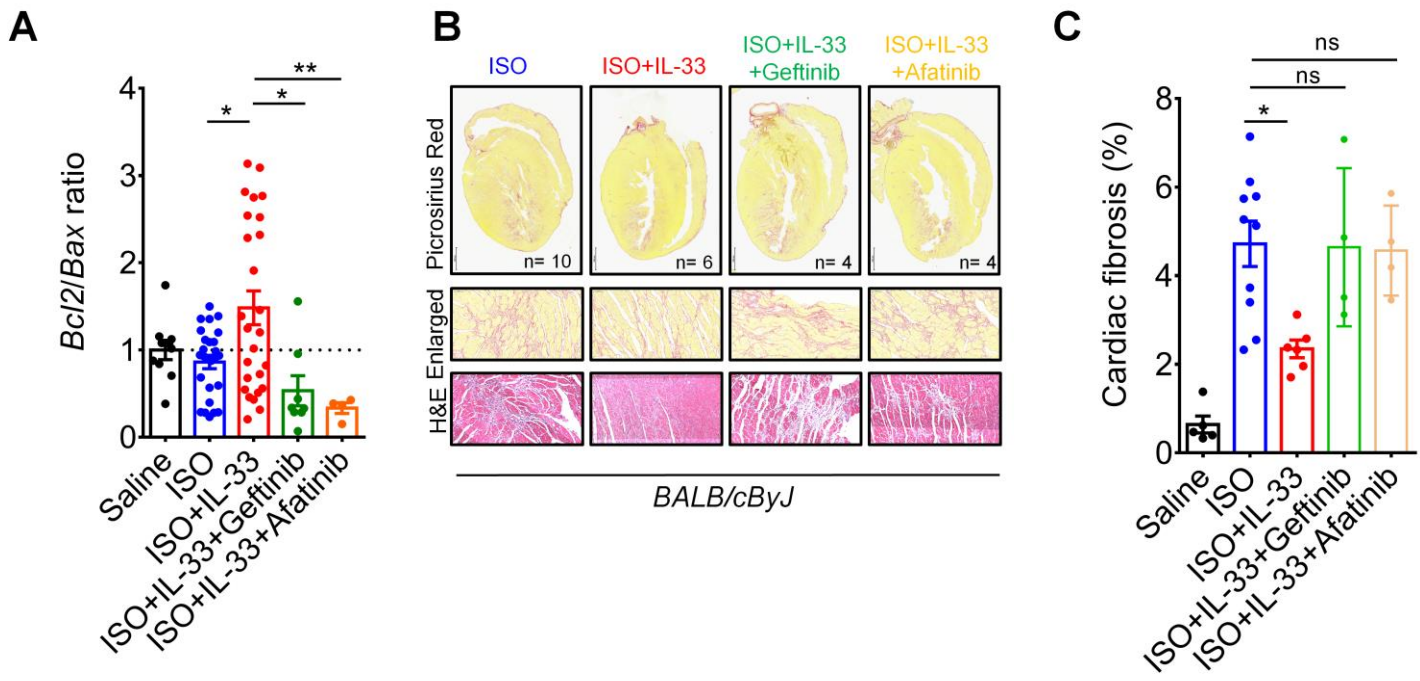

Figure S5. EGFR Signaling is required for optimal function of IL-33 to mitigate cardiac fibrosis. The mice were intraperitoneally administered with Saline, IL-33 (0.5 μg/mouse), IL-33 (0.5 μg) + Geftinib (10 mg/kg), or IL-33 (0.5 μg) + Afatinib (10 mg/kg) on day 3, 5, and 7. **(A)** Ratio of *Bcl2* versus *Bax* gene expression levels in the cardiac tissues. **P* < 0.05, ***P* < 0.01 by one-way ANOVA followed by the Bonferroni multiple comparison post-hoc test. The fold increase represents relative gene expression compared with saline-treated controls. **(B)** The cardiac tissues were collected for Picrosirius red staining of the fibrotic area. **(C)** Quantification of the fibrosis area in the heart sections. **P* < 0.05. ns, not significant by one-way ANOVA followed by the Bonferroni multiple comparison post-hoc test. All values are means ± SD. Each dot indicates a biological replicate.

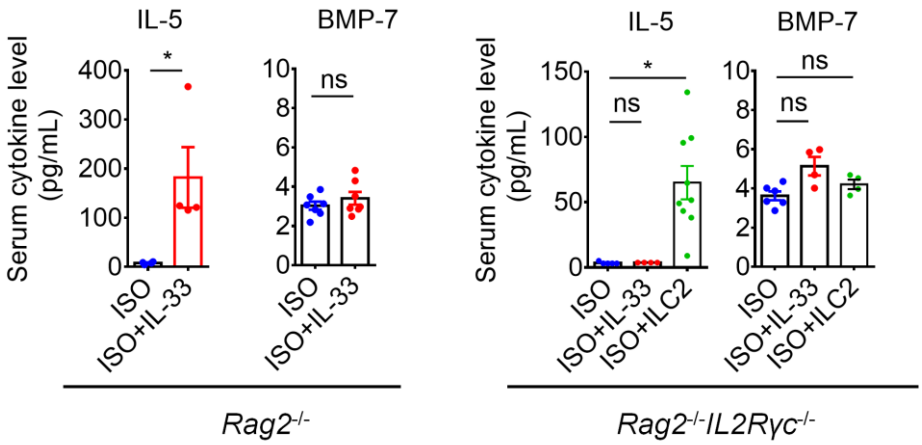

Figure S6. ILC2 contribute to IL-33-induced circulating IL-5. *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$*IL2Rγc*$^{-/-}$ mice were subcutaneously administered with isoproterenol (ISO; 30 mg/kg) for 3 days and intraperitoneally administered with saline or IL-33 (0.5 μg/mouse) on days 3, 5, and 7. The sera were collected on day 10 for ELISA analysis of IL-5 and BMP-7 levels. ns, not significant by one-way ANOVA followed by the Bonferroni multiple comparison post-hoc test.