Supplemental Materials

Group 2 innate lymphoid cells protect mouse heart from myocardial infarction injury via interleukin 5, eosinophils, and dendritic cells

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Supplemental Figure S1. Sirius red and immunofluorescent staining to detect collagen-I (red and orange) and collagen-III (green) (A) and IL33 expression (B) in the infarct, border, and remote regions of infarcted hearts from \( \text{Il7r}^{\text{Cre}+/+} \) and \( \text{Rora}^{-/-}\text{Il7r}^{\text{Cre}+/+} \) mice at 28 days post-MI. Scale: 200 \( \mu m \) in A and 50 \( \mu m \) in B. Data are mean±SEM. \( n=9-10 \) mice per group in A and \( n=5 \) mice per group in B, non-parametric Mann-Whitney \( U \) test followed by Bonferroni correction.
Supplemental Figure S2. ILC2 deficiency aggravates cardiac dysfunction at 1 day post-MI. A. Representative LV M-mode echocardiogram images of \( \text{Il}7\text{r}^{+/+} \) and \( \text{Rora}^{fl/fl}\text{Il}7\text{r}^{Cre/+} \) mice post-MI or sham as indicated (time stamp: 100 ms, scale: 1.7 mm). B. Cardiac functions post-MI or sham in different groups of mice. C/D. Infarct thickness, infarct size ratio and representative images (scale: 1.50 mm), BW/HW, and BW/TL post-MI or sham in different groups of mice. E/F. Sirius red staining was used to quantify collagen-I (red and orange) and collagen-III (green) in infarct, media, and remote regions from \( \text{Il}7\text{r}^{Cre/+} \) and \( \text{Rora}^{fl/fl}\text{Il}7\text{r}^{Cre/+} \) mice post-MI. Representative images are shown in C and F (scale: 200 µm). Data are mean±SEM. n=7~10 mice per sham group, n=15~20 mice per MI group, one-way ANOVA test followed by a post hoc Tukey’s test (B and D), or non-parametric Mann-Whitney \( U \) test followed by Bonferroni correction (E).
Supplemental Figure S3. ILC2 deficiency aggravates cardiac dysfunction at 7 days post-MI. A. Representative LV M-mode echocardiogram images of Il7r<sup>Cre</sup>/+ and Rora<sup>fl/fl</sup>Il7r<sup>Cre</sup>/+ mice post-MI or sham as indicated (time stamp: 100 ms, scale: 1.7 mm). B. Cardiac functions post-MI or sham in different groups of mice. C/D. Infarct thickness, infarct size ratio and representative images (scale: 1.50 mm), BW/HW, and BW/TL post-MI or sham in different groups of mice. E/F. Sirius red staining was used to quantify collagen-I (red and orange) and collagen-III (green) in infarct, media, and remote regions from Il7r<sup>Cre</sup>/+ and Rora<sup>fl/fl</sup>Il7r<sup>Cre</sup>/+ mice post-MI. Representative images are shown in C and F (scale: 200 µm). Data are mean±SEM. n=7~10 mice per sham group, n=15~20 mice per MI group, one-way ANOVA test followed by a post hoc Tukey’s test (B and D), or non-parametric Mann-Whitney U test followed by Bonferroni correction (E).
**Supplemental Figure S4.** FACS quantification of spleen immune cells in $Il7r^{Cre/+}$ and $Rora^{fl/fl}Il7r^{Cre/+}$ mice at 28 days post-MI. CD45$^+$CD11b$^+$Gr-1$^+$ neutrophils, CD45$^+$CD11b$^+$Ly6C$^{hi}$ monocytes, CD45$^+$CD11b$^+$Ly6C$^{lo}$ monocytes, CD45$^+$CD11c$^+$MHC-II$^+$ dendritic cells, CD45$^+$CD4$^+$ and CD45$^+$CD8$^+$ T cells from $Il7r^{Cre/+}$ and $Rora^{fl/fl}Il7r^{Cre/+}$ mice. Representative FACS images are shown to the left. Data are mean±SEM. $n=5–7$ mice per group, non-parametric Mann-Whitney $U$ test followed by Bonferroni correction.
Supplemental Figure S5. Sirius red and immunofluorescent staining to detect collagen-I (red and orange) and collagen-III (green) (A) and IL33 expression (B) in the infarct, border, and remote regions of infarcted hearts from ICOS-T mice treated with or without DTX at 28 days post-MI. Scale: 200 µm in A and 50 µm in B. Data are mean±SEM. n=8 mice per group in A and n=5 mice per group in B, non-parametric Mann-Whitney U test followed by Bonferroni correction.
Supplemental Figure S6. FACS quantification of splenic immune cells in ICOS-T mice with different treatments as indicated at 28 days post-MI. CD45^+CD11b^+Gr-1^+ neutrophils, CD45^+CD11b^+Ly6C^hi^ monocytes, CD45^+CD11b^+Ly6C^lo^ monocytes, CD45^+CD11c^-MHC-II^- dendritic cells, CD45^+CD11b^-Siglec-F^-EOS, and CD45^+CD4^- and CD45^+CD8^- T cells. Representative FACS images are shown to the left. Data are mean±SEM. n=3~6 mice per group, one-way ANOVA test followed by a post hoc Tukey’s test.
Supplemental Figure S7. Donor DC from WT mice protects cardiac functions in Rora<sup>β<sub>2</sub>Il7r<sub>Cre<i>+</i></sub></sup> mice at 7 and 28 days post-MI. A. Representative LV M-mode echocardiogram images (time stamp: 100 ms, scale: 1.7 mm). B. Cardiac functions and heart rates. C/D. Infarct thickness, infarct size ratio, HW/BW, and HW/TL. Representative H&E staining images are shown in C. Scale: 1.50 mm. E/F. Sirius red staining of collagen-I (red and orange) and collagen-III (green) in infarct, media, and remote regions from Rora<sup>β<sub>2</sub>Il7r<sub>Cre<i>+</i></sub></sup> mice at 7 days post-MI. G/H. Sirius red staining of collagen-I and collagen-III in infarct, media, and remote regions from Rora<sup>β<sub>2</sub>Il7r<sub>Cre<i>+</i></sub></sup> mice at 28 days post-MI. Representative images are shown in E and G (scale: 200 µm). Data are mean±SEM. n=8 mice per group, non-parametric Mann-Whitney U test followed by Bonferroni correction.
**Supplemental Figure S8.** ILC2 do not affect cardiomyocyte and lymphocyte death. 

**A.** Immunoblot detected Bcl-2 expression in adult mouse cardiomyocytes treated with ILC2 lysates (equivalent to 0, 10^3, 10^4 and 5x10^4 ILC2/ml) with or without H_2O_2 (100 µM) for 4 hrs. Representative immunobLOTS are shown to the left. n=3-4 independent experiments.

**B/C.** FACS analysis of mouse cardiomyocyte and lymphocyte apoptosis. Cardiomyocytes (B) and splenic lymphocytes (C) were treated with ILC2 lysates (equivalent to 0, 2x10^3, 2x10^4 ILC2/ml) with or without H_2O_2 (100 µM) for 4 hrs. Representative FACS images are shown on the left. n=3 independent experiments. Data are mean±SEM, one-way ANOVA test followed by a post hoc Tukey’s test.
Supplemental Figure S9. Immunofluorescent staining, confocal imaging, and quantification of myocardial border region IL4 and IL5 levels in Il7r<sup>Cre<sup>+/+</sup></sup> and Rora<sup>fl/fl</sup>Il7r<sup>Cre<sup>+/+</sup></sup> mice at 1, 7, and 28 days post-MI. A. IL4. B. IL5. Scale: 50 µm. Data are mean±SEM. n=5 mice per group, non-parametric Mann-Whitney U test followed by Bonferroni correction.
Supplemental Figure S10. Immunofluorescent double staining, confocal imaging, and quantification of myocardial border region Th1 and Th2 cells in \( \text{Il7r}^{+/+} \) and \( \text{Rora}^{+/+}\text{Il7r}^{+/+} \) mice at 1, 7, and 28 days post-MI. A. CD4\(^{+}\)IFN-\(\gamma\)^+ Th1 cells. B. CD4\(^{+}\)IL4\(^{+}\) Th2 cells and Th1/Th2 ratio. Scale: 50 \(\mu\)m. Data are mean±SEM. \(n=5\) mice per group, non-parametric Mann-Whitney \(U\) test followed by Bonferroni correction.
Supplemental Figure S11. ILC2 do not affect cardiac fibroblast Smad signaling. Immunoblots detected p-Smad2/3 and total Smad2/3 in mouse cardiac fibroblasts treated with ILC2 lysates (equivalent to 0, 10^3, 10^4 and 2x10^4 ILC2/ml) with or without TGF-β (10 ng/ml) for 30 min. Representative immunoblots are shown to the left. n=5-7 independent experiments. Data are mean±SEM, one-way ANOVA test followed by a post hoc Tukey’s test.