SUPPLEMENTAL MATERIAL

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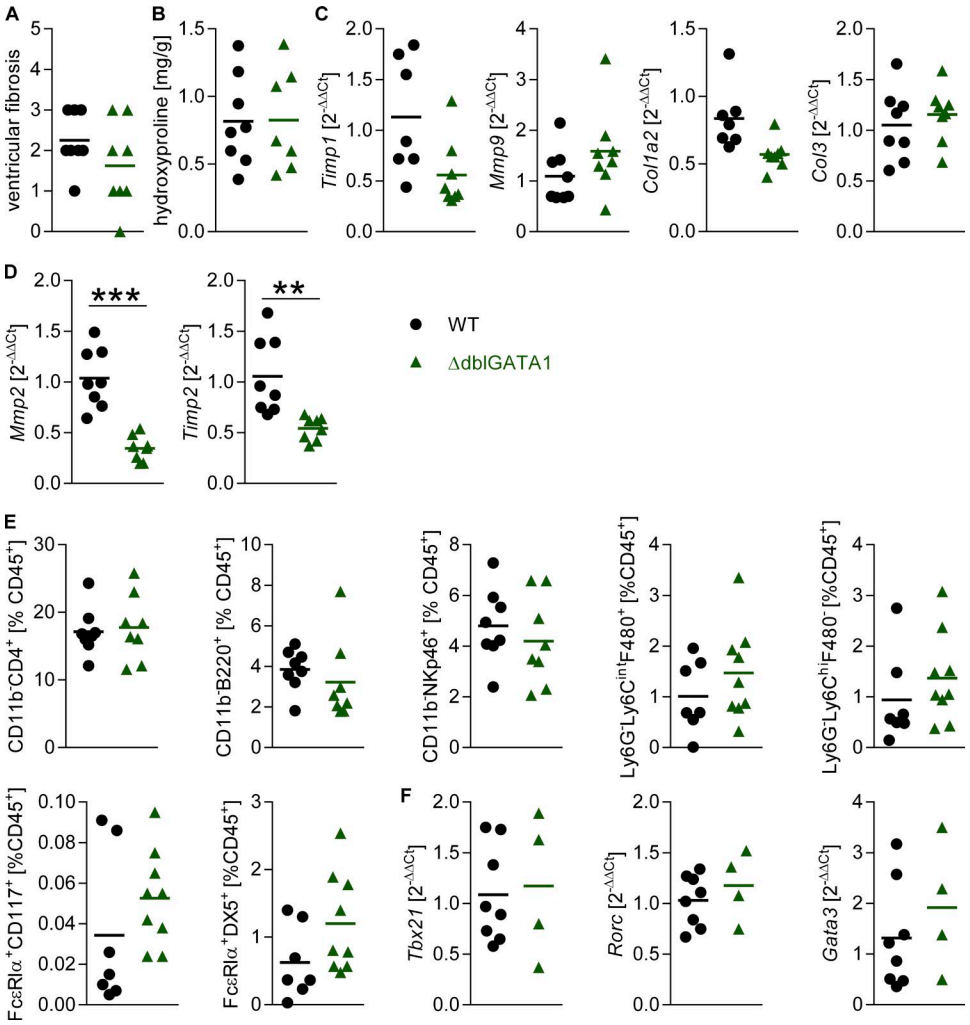

Figure S1. **Protection of ΔdblGATA1 mice from DCMi is accompanied by changes in tissue remodeling–associated genes but not by changes in heart-infiltrating cell populations in EAM.** (A–D) Mice were analyzed at day 45 of EAM. (A and B) The extent of fibrosis was determined by scoring fibrotic areas on histological sections from five experiments (A) and by quantifying the amino acid hydroxyproline in heart samples from two experiments (B). (C and D) Gene expression in heart homogenates was analyzed by quantitative PCR. Data are representative of two to three independent experiments with five to eight mice per group. (E) Frequency of heart infiltrating cells was determined by flow cytometry on day 21 of EAM. Data are combined from different experiments and are representative of one to three independent experiments with five to eight mice per group. Cell types were gated as follows: neutrophils, $CD11b^+Ly6G^+$; T cells, $CD11b^-CD3^+CD4^+$ or $CD8^+$; B cells, $CD11b^-B220^+$; NK cells, $CD11b^-CD3^-NKp46^+$; monocytes/macrophages, $CD11b^+Ly6G^-Ly6C^+$; basophils, $CD11b^+Ly6G^-Ly6C^-Fc\varepsilon RI\alpha^+DX5^+$; and mast cells, $CD11b^+Ly6G^-Ly6C^-Fc\varepsilon RI\alpha^+$ $CD117^+$. (F) Expression of transcription factors was determined by quantitative PCR from heart homogenates from day 21 of EAM and normalized to expression of CD3 and WT controls. Data are representative of two independent experiments with four to eight mice per group. Groups were compared by Mann-Whitney test (A) or Student's *t* test (B–F). **, $P < 0.01$; ***, $P < 0.001$.

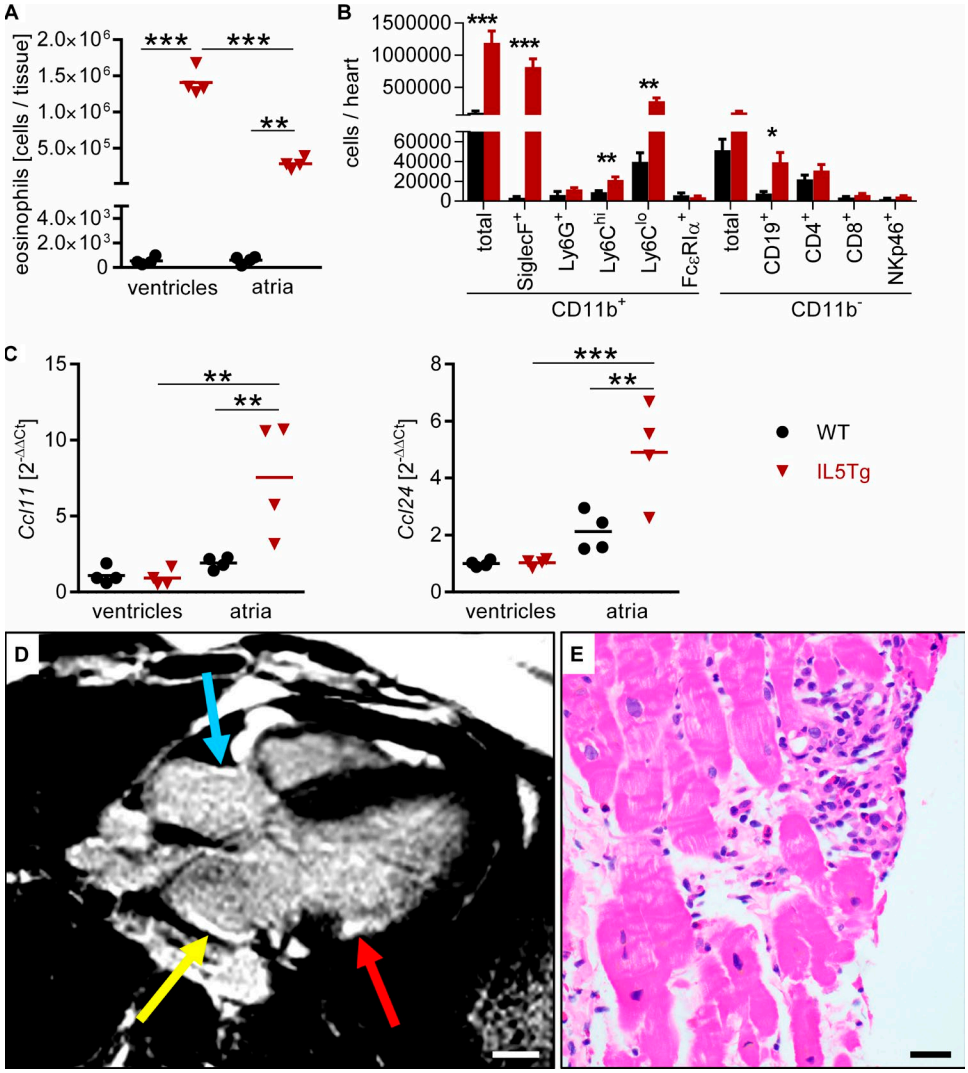

Figure S2. **Atrial inflammation in IL-5Tg mice and patients with eosinophilic myocarditis.** (A–C) Mice were analyzed at day 21 of EAM. (A) Absolute number of eosinophils in ventricles and atria was determined by flow cytometry. Data shown are from one experiment with four mice per group. (B) Average ± SD of total number of different cell populations in whole hearts was determined by flow cytometry. For each population, WT and IL-5Tg mice were compared by Student's *t* test. Data are representative of two independent experiments with five to six mice per group. (C) Gene expression in ventricles and atria was determined by quantitative PCR and normalized to *Gapdh*. Data are representative of two independent experiments with three to four mice per group. (A and C) Groups were compared by one-way ANOVA followed by Tukey's multiple comparison test. (D and E) Eosinophilic myocarditis in a 41-yr-old female with a history of HES. (D) Cardiovascular magnetic resonance (four-chamber view) shows LGE in the right (blue arrow) and the left (yellow arrow) atrial free wall and focal subendocardial LGE of the inferolateral myocardial wall of the left ventricle (red arrow). Bar, 1 cm. (E) H&E-stained endomyocardial biopsy from the right ventricular septum showing eosinophilic infiltration in the myocardium. Bar, 30 µm. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

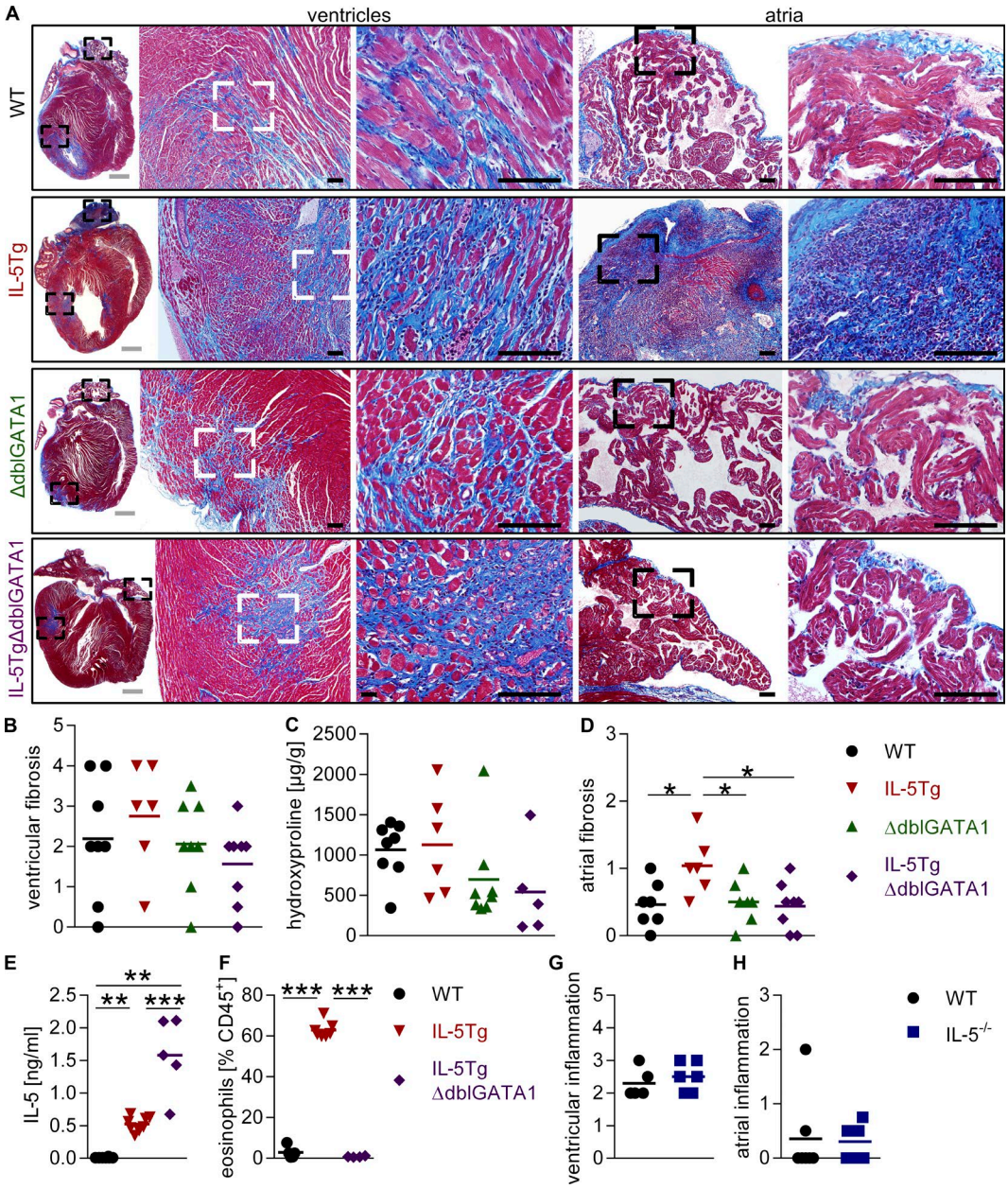

Figure S3. **Eosinophils rather than IL-5 contribute to severe DCMi and atrial inflammation/fibrosis.** (A–D) Mice were analyzed at day 45 of EAM. (A) Heart sections were stained with Masson's trichrome and are shown representatively for all indicated mouse strains. Representative median animals from different experiments are shown. Bars: (gray) 1 mm; (black) 100 µm. (B–D) The extent of fibrosis was determined by scoring fibrotic areas on histological sections (B and D) and by quantifying the amino acid hydroxyproline in heart samples (C). Data are representative of two independent experiments with five to nine mice per group. (E) IL-5 concentration in serum of naive mice was determined by ELISA in one experiment with five to eight mice per group. (F–H) Mice were analyzed at day 21 of EAM. (F) Heart-infiltrating eosinophils were quantified by flow cytometry in one experiment with four to seven mice per group. (G and H) Severity of inflammation was scored on H&E-stained heart sections. (G) Data are representative of two independent experiments with five to six mice per group. (H) Data are pooled from two independent experiments with two to six mice per group. Groups were compared by one-way ANOVA followed by Tukey's multiple comparisons test (B–F) or by Mann Whitney test (G and H). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

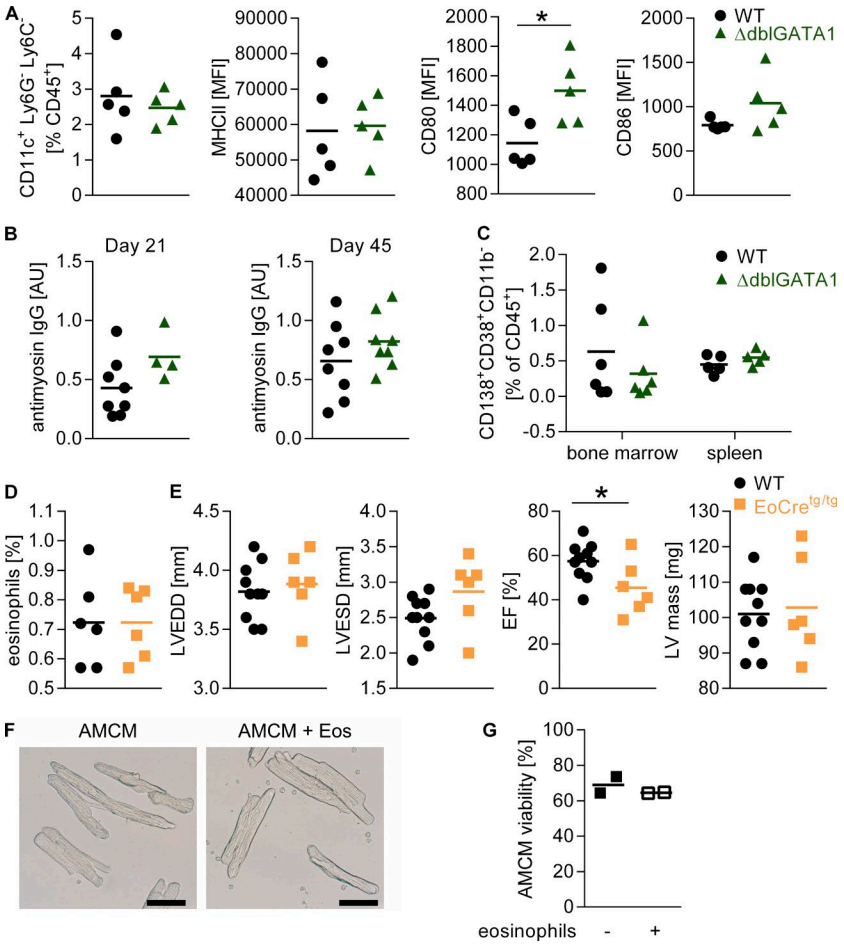

Figure S4. **Eosinophils do not drive DCMi through effects on DCs, antibody production, cytotoxic effects on cardiomyocytes, or eosinophil peroxidase.** (A) DC frequency and expression of co-stimulatory molecules on DCs in the heart at day 10 of EAM was determined by flow cytometry. MFI, mean fluorescence intensity. (B) Myosin-specific IgG was quantified in serum from WT and ΔdblGATA1 mice on days 21 and 45 of EAM. AU, arbitrary units. (C) Plasma cell frequency in WT and ΔdblGATA1 mice on day 21 of EAM. (D) Eosinophil frequency in the blood of WT and EoCre$^{tg/tg}$ mice on day 45 of EAM was determined by flow cytometry. (E) Echocardiography of WT and EoCre$^{tg/tg}$ mice on day 45 of EAM. Mice homozygous for the EoCre transgene are functionally eosinophil peroxidase knockouts. (A–E) Data are from one experiment, and symbols represent individual animals. (F and G) Adult mouse cardiomyocytes (AMCM) from WT mice were cultured in vitro for 20 h with or without eosinophils (Eos) isolated from the blood of IL-5Tg mice. Dead (round, detached) and viable (adherent, rod shaped) adult mouse cardiomyocytes were counted under the microscope. Bars, 50 µm. (G) Viability is depicted. Data are representative of two independent experiments. Groups were compared by Student's *t* test. *, $P < 0.05$.

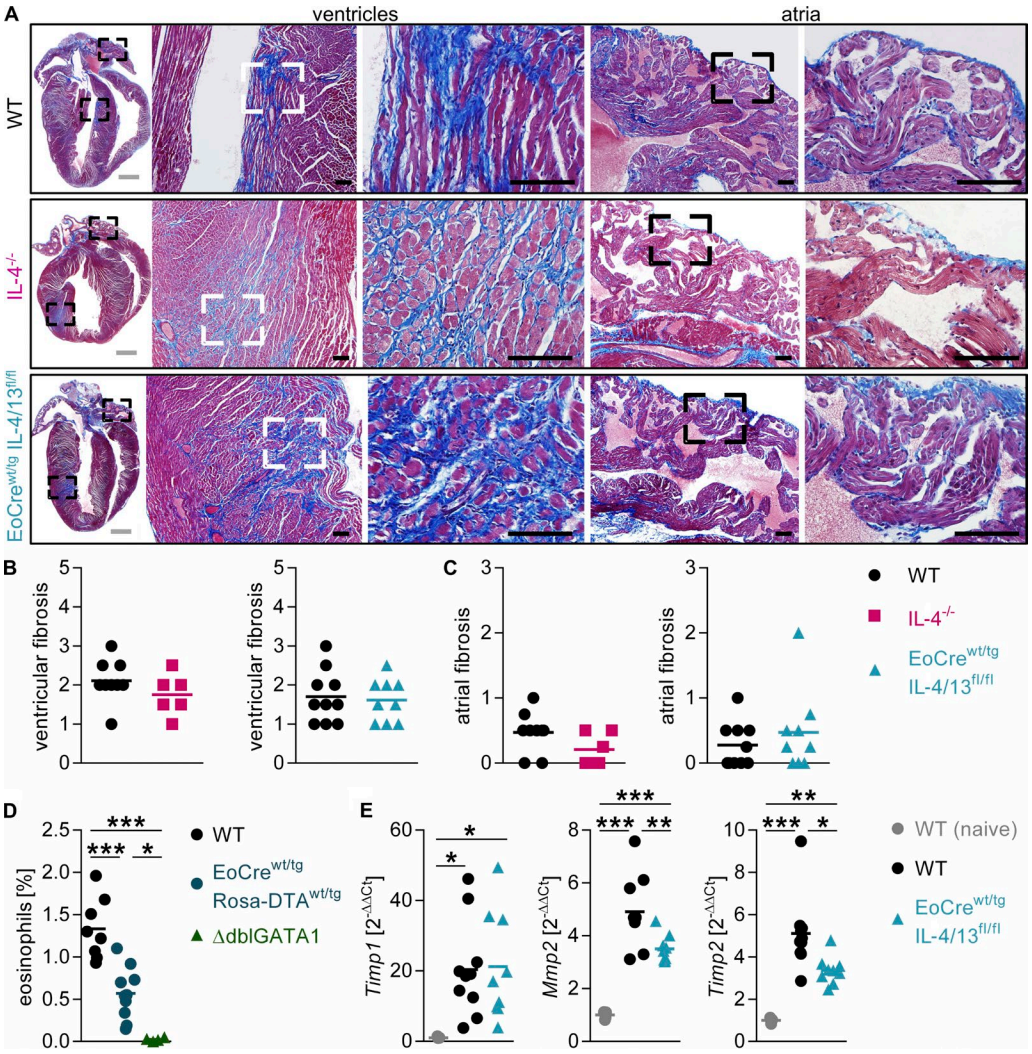

Figure S5. **EoCre$^{wt/tg}$IL-4/13$^{fl/fl}$ mice develop fibrosis to the same extent as WT mice but show altered tissue remodeling similar to ΔdblGATA1 mice.** (A–E) Mice were analyzed at day 45 of EAM. (A) Heart sections were stained with Masson's trichrome and are shown representatively for all indicated mouse strains. Representative median animals from different experiments are shown. Bars: (gray) 1 mm; (black) 100 µm. (B and C) The extent of fibrosis was determined by scoring fibrotic areas on histological sections. Groups were compared by Mann-Whitney test (no significant differences). (D) Eosinophil frequency in blood was determined by flow cytometry. (E) Gene expression in heart homogenates was analyzed by quantitative PCR. (D and E) Groups were compared by one-way ANOVA followed by Tukey's multiple comparisons test. Data are from one experiment, and symbols represent individual animals. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table S1. **Primer sequences**

Gene	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
Ccl11	GAATCACCAACAACAGATGCAC	TCCTGGACCCACTTCTTCTT
Ccl24	TCTTAGGGCCCTTCTTGGTG	AATTCCAGAAAACCGAGTGG
Col1a2	AGCAGGTCCTTGGAAACCTT	AAGGAGTTTCATCTGGCCCT
Col3a1	GTGAACGGGGCGAAGCTGGTT	GCGGCTCCTGGAAGCCCATTTG
Gapdh	TCCTCCTCAGACCGCTTTT	TCTGCTGGAGTCCCCTTG
Gata3	CTCGGGCATTCGTACATGGAA	GGATACCTCTGCACCGTAGC
Hprt	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG
Mmp2	TTTGCTCGGGCCTTAAAAGTAT	CCATCAAACGGGTATCCATCTC
Mmp9	TGCCCATTTCGACGACGAC	GTGCAGGCCGAATAGGAGC
Rorc	ATCCTGTAATGGCTTGTGGG	TCAACCAGCACCAGACAGAG
Tbx21	ATCCTGTAATGGCTTGTGGG	TCAACCAGCACCAGACAGAG
Timp1	GCAACTCGGACCTGGTCATAA	CGGCCCGTGATGAGAAACT
Timp2	CTTCTGCAACTCCGACATCGT	GGGGCATCTTACTGAAGCCTC