

Supplementary Information

Eosinophils improve cardiac function after myocardial infarction

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Supplementary Tables

Supplementary Table 1. Baseline information of patient with (Yes) and without (No) respective variables.

Variable	Yes		No		P*
	N	EOS (x10 ⁹ /L) Mean (SD)	N	EOS (x10 ⁹ /L) Mean (SD)	
Age ≥70 years	1626	0.213 (0.148)	3903	0.211 (0.157)	0.651
Current or former smoker	3708	0.218 (0.162)	1796	0.198 (0.135)	<0.001
Hypertension	2553	0.213 (0.141)	2977	0.210 (0.165)	0.415
Diabetes Mellitus	633	0.239 (0.178)	4897	0.208 (0.151)	<0.001
Comorbidity					
Previous stroke	411	0.219 (0.174)	5119	0.211 (0.153)	0.278
Previous AMI	345	0.261 (0.261)	5185	0.208 (0.144)	<0.001
Previous peripheral artery disease	138	0.215 (0.123)	5392	0.211 (0.155)	0.751
Abdominal aortic aneurysm	113	0.211 (0.123)	5417	0.211 (0.155)	0.905
Atrial fibrillation	775				
Previous coronary intervention	434	0.242 (0.237)	5096	0.209 (0.145)	<0.001
Heart valve replacement	71	0.226 (0.148)	5459	0.211 (0.154)	0.431
Chronic pulmonary obstructive disease	404	0.259 (0.209)	5126	0.207 (0.148)	<0.001
Medication					
Use of low dose aspirin	1257	0.229 (0.192)	4273	0.206 (0.141)	<0.001
Use of warfarin	397	0.212 (0.159)	5133	0.211 (0.154)	0.957
Use of Statin	1500	0.226 (0.183)	4030	0.206 (0.141)	<0.001
Use of ACE inhibitor or AT2-antagonists	1900	0.217 (0.163)	3630	0.208 (0.149)	0.061
Use of calcium blocker	1047	0.213 (0.138)	4433	0.211 (0.158)	0.737
Use of loop diuretics	251	0.250 (0.183)	5279	0.209 (0.152)	<0.001
Use of beta-agonist	173	0.295 (0.232)	5357	0.209 (0.150)	<0.001
Use of inhalation glucocorticoid	306	0.278 (0.291)	5224	0.207 (0.141)	<0.001
Use of oral glucocorticoid	104	0.195 (0.193)	5426	0.215 (0.153)	0.292
Use of NSAID	182	0.213 (0.161)	5348	0.211 (0.154)	0.862
Symptoms					
Chest pain	934	0.235 (0.210)	4596	0.207 (0.14)	<0.001
Dyspnoea	990	0.234 (0.206)	4530	0.206 (0.140)	<0.001
Body mass index					
<18.5	18	0.180 (0.149)			
18.5-25	1198	0.200 (0.148)			
25-30	2638	0.213 (0.167)			
>30	1665	0.216 (0.136)			0.025
Mobility score (Eur-Qol-5D)					
No problems	4640	0.208 (0.150)			
Some problems (Eur-Qol-5D)	880	0.230 (0.176)			
Bed bound	4	0.230 (0.111)			0.001
NYHA classification score					
NYHA class 1	4871	0.207 (0.150)			
NYHA class 2	456	0.239 (0.173)			
NYHA class 3	176	0.250 (0.189)			
NYHA class 4	12	0.366 (0.361)			<0.001

Abbreviations: NSAID–nonsteroidal anti-inflammatory drug; NYHA– The New York Heart Association. *Student t test.

Supplementary Table 2. Multivariate logistic regression analysis of previous AMI dependent variables.

Dependent variable	B	S.E.	P	OR	95% C.I.	
					Lower	Upper
EOS count (x10 ⁹ /L)	0.988	0.357	0.006	2.685	1.335	5.401
BMI group	0.180	0.091	0.049	1.197	1.001	1.432
Former of current smoking	0.363	0.157	0.020	1.438	1.058	1.954
COPD	0.051	0.206	0.804	1.052	0.703	1.575
Diabetes mellitus	-0.163	0.160	0.306	0.849	0.621	1.161
Use of low dose aspirin	2.773	0.182	0.000	16.005	11.193	22.886
Use of statin	1.638	0.161	0.000	5.145	3.755	7.050
Constant	-6.004	0.299	0.000	0.002		

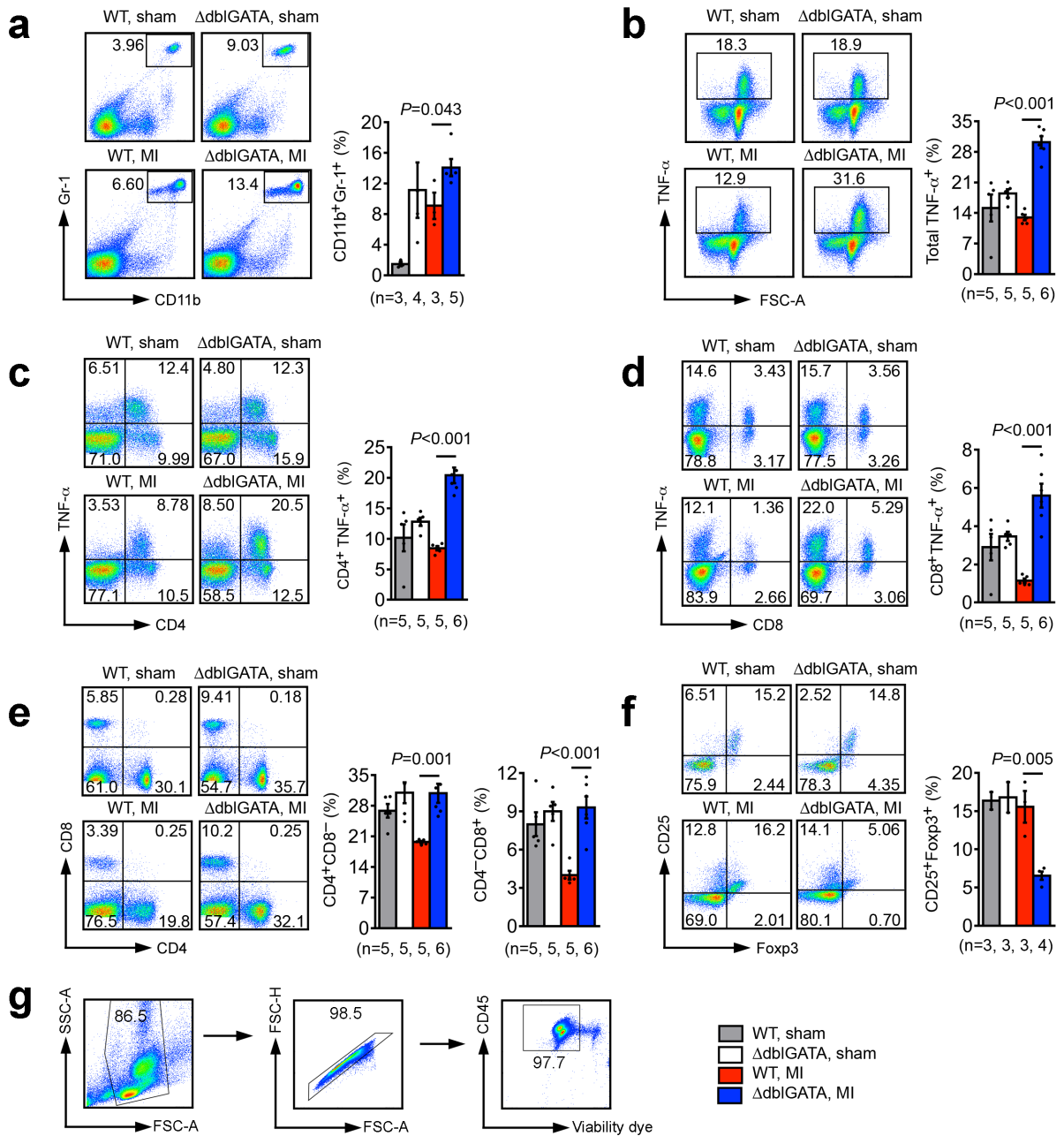
Supplementary Table 3. Multivariate logistic regression analysis of NYHA classification dependent variables.

Dependent variable	Unstandardized coefficient		P	95% C.I. for B		Correlations	
	B	Std error		Lower	Upper	Zero-Order	Partial
(Constant)	-0.100	0.020	0.000	-0.139	-0.061		
EOS count (x10 ⁹ /L)	0.102	0.036	0.005	0.030	0.173	0.082	0.038
BMI group	0.062	0.008	0.000	0.046	0.077	0.115	0.105
Former or current smoking	0.037	0.012	0.003	0.013	0.060	0.108	0.041
COPD	0.636	0.022	0.000	0.593	0.679	0.382	0.366
Diabetes mellitus	0.067	0.018	0.000	0.030	0.103	0.101	0.049
Use of low dose aspirin	0.089	0.015	0.000	0.059	0.118	0.142	0.080
Use of statin	0.018	0.015	0.204	-0.010	0.047	0.103	0.017

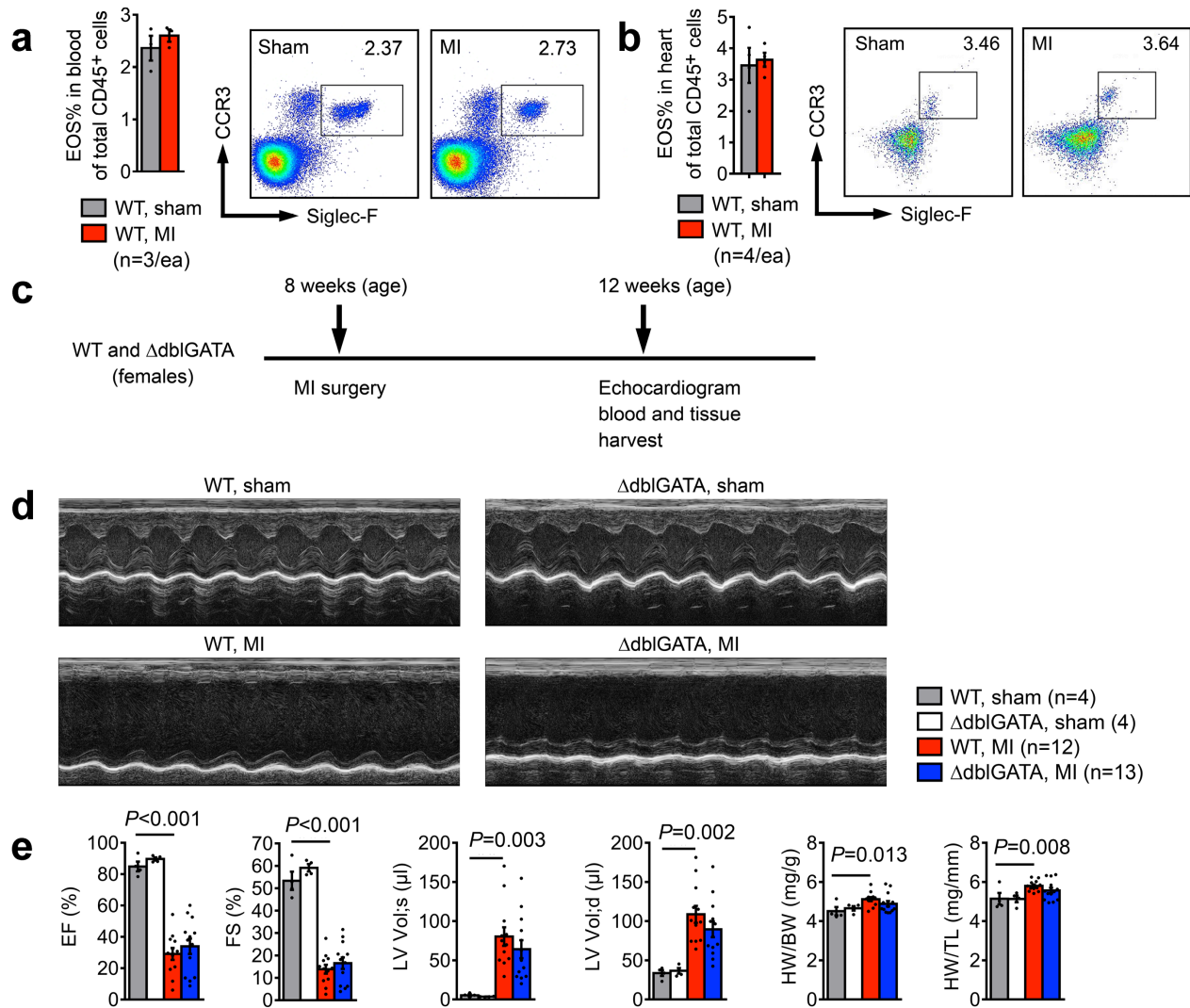
Supplementary Table 4. RT-PCR primer sequences.

Gene	Forward primers	Reverse primers
<i>β-actin</i>	5'-GTGACGTTGACATCCGTAAAGA-3'	5'-GCCGGACTCATCGTACTCC-3'
<i>Icam1</i>	5'-GTGATGCTCAGGTATCCATCCA-3'	5'-CACAGTTCTCAAAGCACAGCG-3'
<i>Vcam1</i>	5'-AGTTGGGGATTTCGGTTGTTCT-3'	5'-CCCCTCATTCTTACCACCC-3'
<i>E-selectin</i>	5'-ATGCCTCGCGCTTTCTCTC-3'	5'-GTAGTCCCGCTGACAGTATGC-3'
<i>Ccr3</i>	5'-TCGAGCCCCGAACGTGACT-3'	5'-CCTCTGGATAGCGAGGACTG-3'
<i>Eotaxin</i>	5'-GAATCACCAACAACAGATGCA-3'	5'-ATCCTGGACCCACTTCTTCTT-3'
<i>Eotaxin-2</i>	5'-TCTTGCTGCACGTCCTTTATT-3'	5'-GCATCCAGTTTTTGTATGTGCC-3'
<i>Eotaxin-3</i>	5'-TTCTTCGATTTGGGTCTCCTTG-3'	5'-GTGCAGCTCTTGTCGGTGAA-3'
<i>Rantes</i>	5'-TTTGCCTACCTCTCCCTCG-3'	5'-CGACTGCAAGATTGGAGCACT-3'
<i>Mip1α</i>	5'-TGTACCATGACACTCTGCAAC-3'	5'-CAACGATGAATTGGCGTGGAA-3'
<i>Mip1β</i>	5'-TTCCTGCTGTTTCTCTTACACCT-3'	5'-CTGTCTGCCTCTTTTGGTCAG-3'
<i>Mcp1</i>	5'-TTAAAAACCTGGATCGGAACCAA-3'	5'-GCATTAGCTTCAGATTTACGGGT-3'
<i>Mcp2</i>	5'-CTGGGCCAGATAAGGCTCC-3'	5'-CATGGGGCACTGGATATTGTT-3'
<i>Mcp3</i>	5'-CCTCTGGATAGCGAGGACTG-3'	5'-ACACCGACTACTGGTGATCCT-3'
<i>Cxcl1</i>	5'-ACTGCACCCAAACCGAAGTC-3'	5'-TGGGGACACCTTTTAGCATCTT-3'
<i>Cxcl2</i>	5'-CCAACCACCAGGCTACAGG-3'	5'-GCGTCACACTCAAGCTCTG-3'
<i>Cxcl3</i>	5'-CAGCCACACTCCAGCCTA-3'	5'-CACAACAGCCCCTGTAGC-3'
<i>Cxcl5</i>	5'-GTTCCATCTCGCCATTCATGC-3'	5'-GCGGCTATGACTGAGGAAGG-3'
<i>Cxcl7</i>	5'-CTCAGACCTACATCGTCCTGC-3'	5'-GTGGCTATCACTTCCACATCAG-3'
<i>Collagen1</i>	5'-GCTCCTCTTAGGGGCCACT-3'	5'-ATTGGGGACCCTTAGGCCAT-3'
<i>Collagen3</i>	5'-CCTGGCTCAAATGGCTCAC-3'	5'-GACCTCGTGTTCCGGGTAT-3'

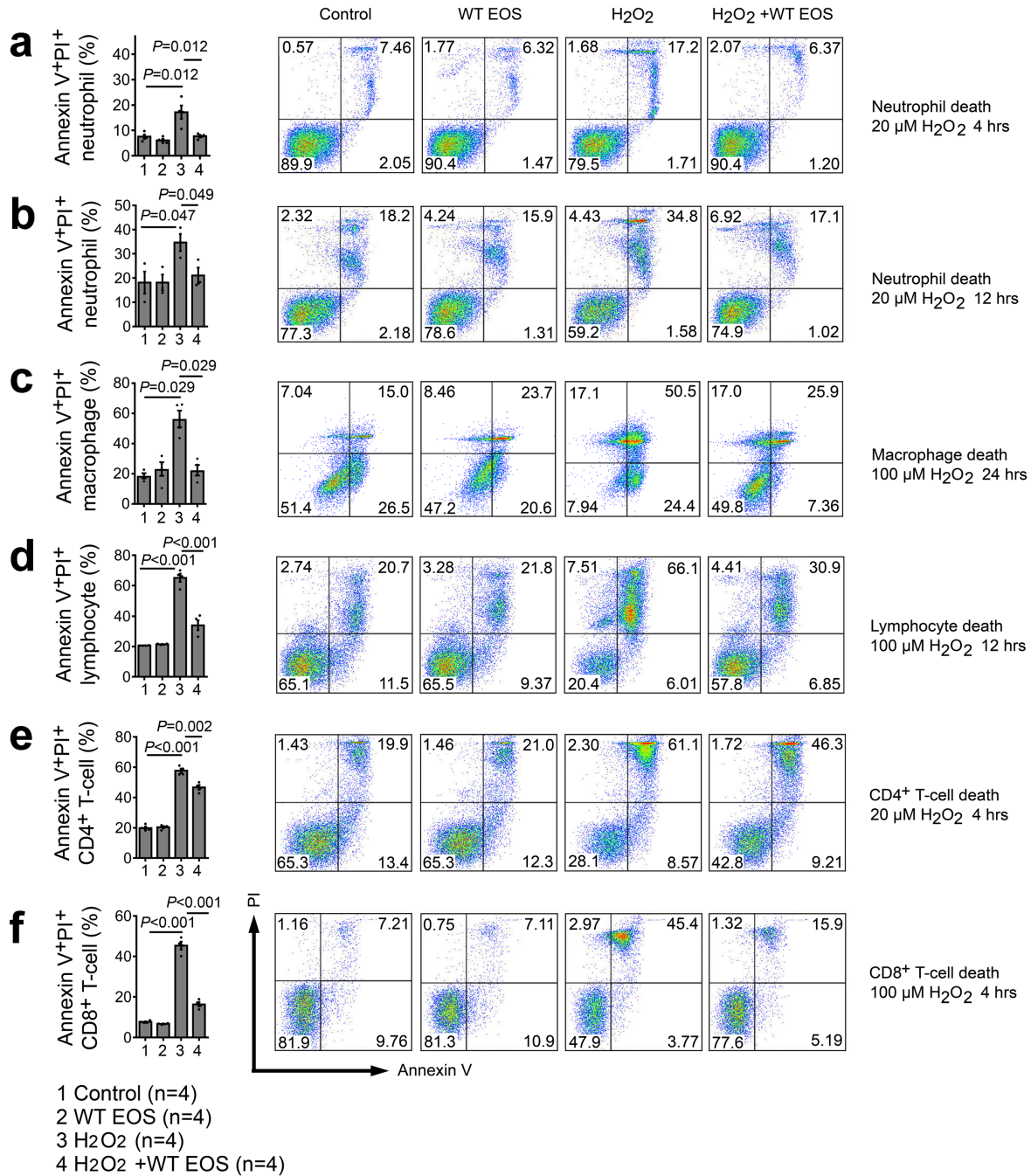
Supplementary Figures



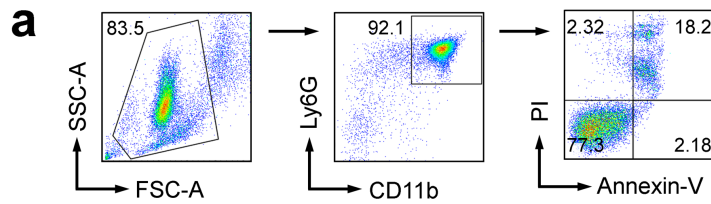
Supplementary Fig. 1. FACS analysis of splenic CD45⁺ immune cells from different groups of mice as indicated at 1-month post-MI. **a.** CD11b⁺Gr-1⁺ neutrophils. **b.** Total TNF- α ⁺ cells. **c.** CD4⁺TNF- α ⁺ T cells. **d.** CD8⁺TNF- α ⁺ T cells. **e.** CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells. **f.** CD4⁺CD25⁺Foxp3⁺ Tregs. **g.** CD45⁺ immune cell FACS gating strategy. Representative FACS images are shown to the left of each panel. Data are mean $\pm$ SEM. The number of mice in each group and *P* values are indicated, one-way ANOVA test.



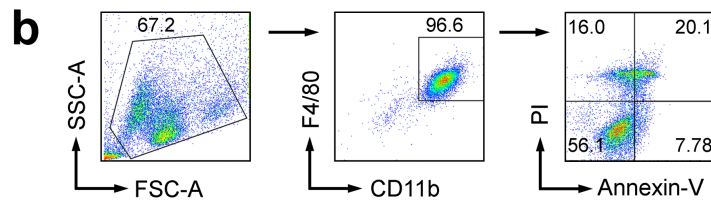
Supplementary Fig. 2. EOS deficiency does not affect cardiac functions post-MI in female mice. **a/b.** FACS analysis of blood (**a**) and heart (**b**) EOS in female mice at 1day post-MI or sham. FACS gating strategy is shown in **Fig. 1c** in the main text. **c.** MI surgery scheme. **d.** Representative LV M-mode echocardiography images at 1-month post-MI. **e.** Cardiac functions at 1-month post-MI: EF, FS, LV Vol;d, LV Vol;s, HW/BW, and HW/TL of different mice as indicated. Data are mean $\pm$ SEM. The number of mice in each group and *P* values are indicated, non-parametric Mann-Whitney *U* test (**a/b**) or one-way ANOVA test (**e**).



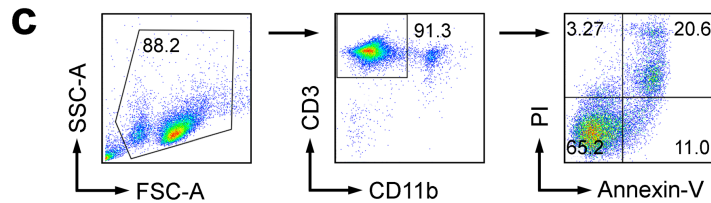
Supplementary Fig. 3. WT EOS activity in protecting immune cells from apoptosis. FACS detected H₂O₂-induced Annexin V⁺PI⁺ late apoptotic neutrophils (**a/b**), macrophages (**c**), splenic lymphocytes (**d**), CD4⁺ T cells (**e**), and CD8⁺ T cells (**f**) after cells were treated with or without 20 μ M H₂O₂ and WT EOS lysate equivalent to 10⁶ EOS/ml at indicated hrs. Data are mean $\pm$ SEM. The number of mice in each group and *P* values are indicated, one-way ANOVA test.



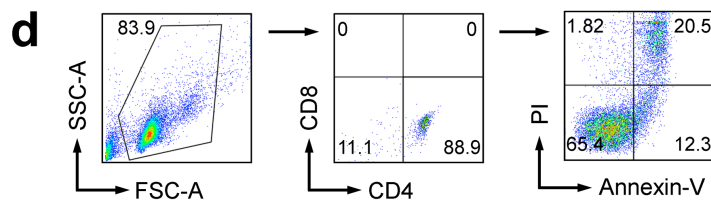
Bone-marrow neutrophil apoptosis gating strategy for Fig. S3a and S3b.



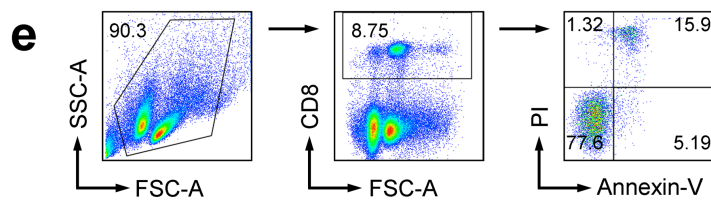
Macrophage apoptosis gating strategy for Fig. S3c.



Lymphocyte apoptosis gating strategy for Fig. S3d.

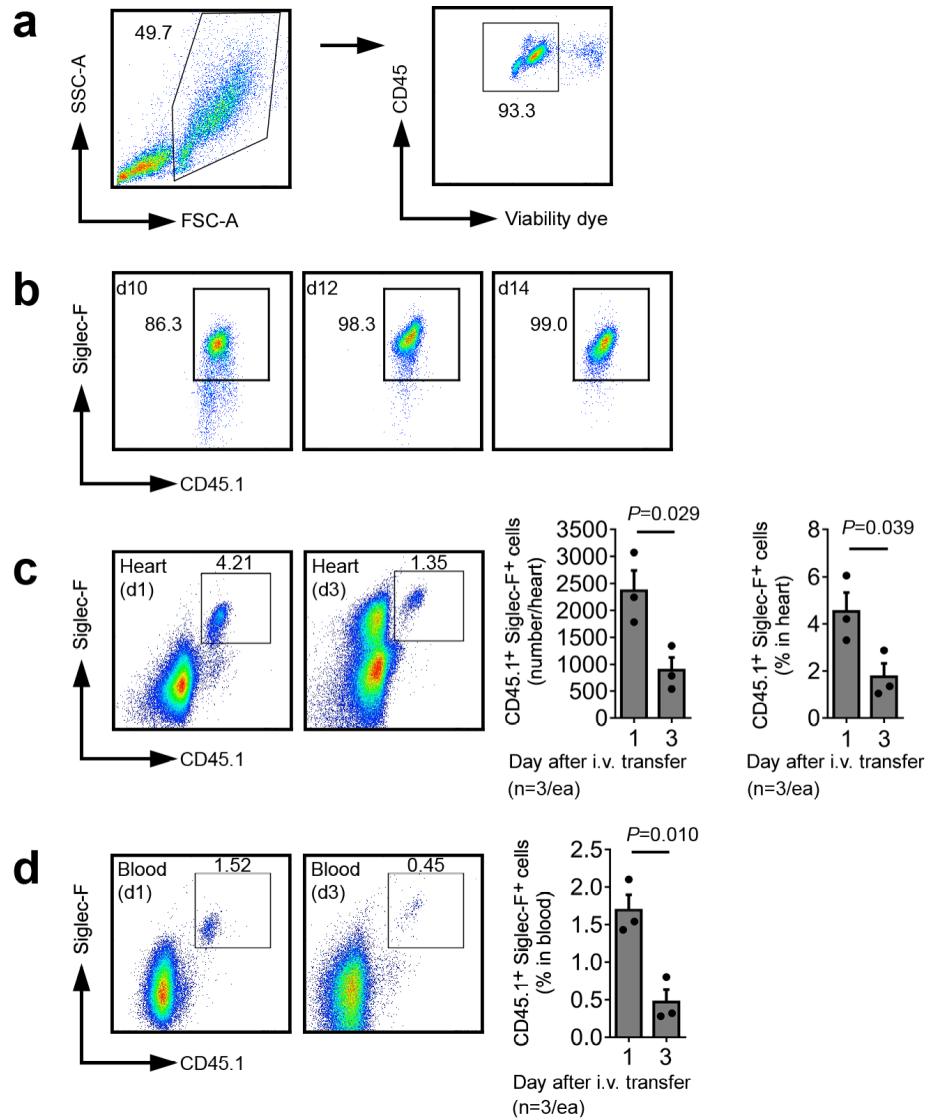


CD4⁺ T-cell apoptosis gating strategy for Fig. S3e.

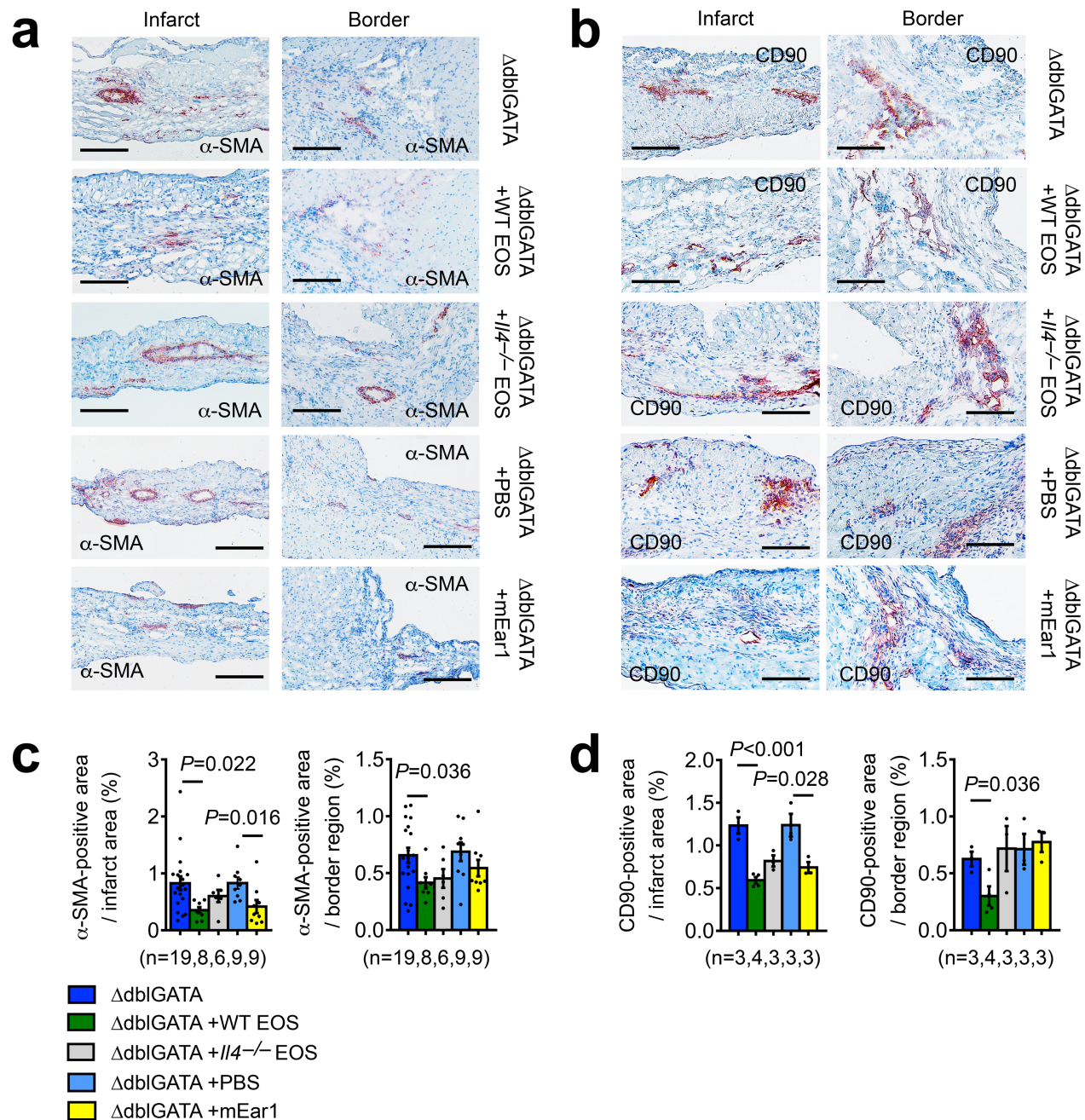


CD8⁺ T-cell apoptosis gating strategy for Fig. S3f.

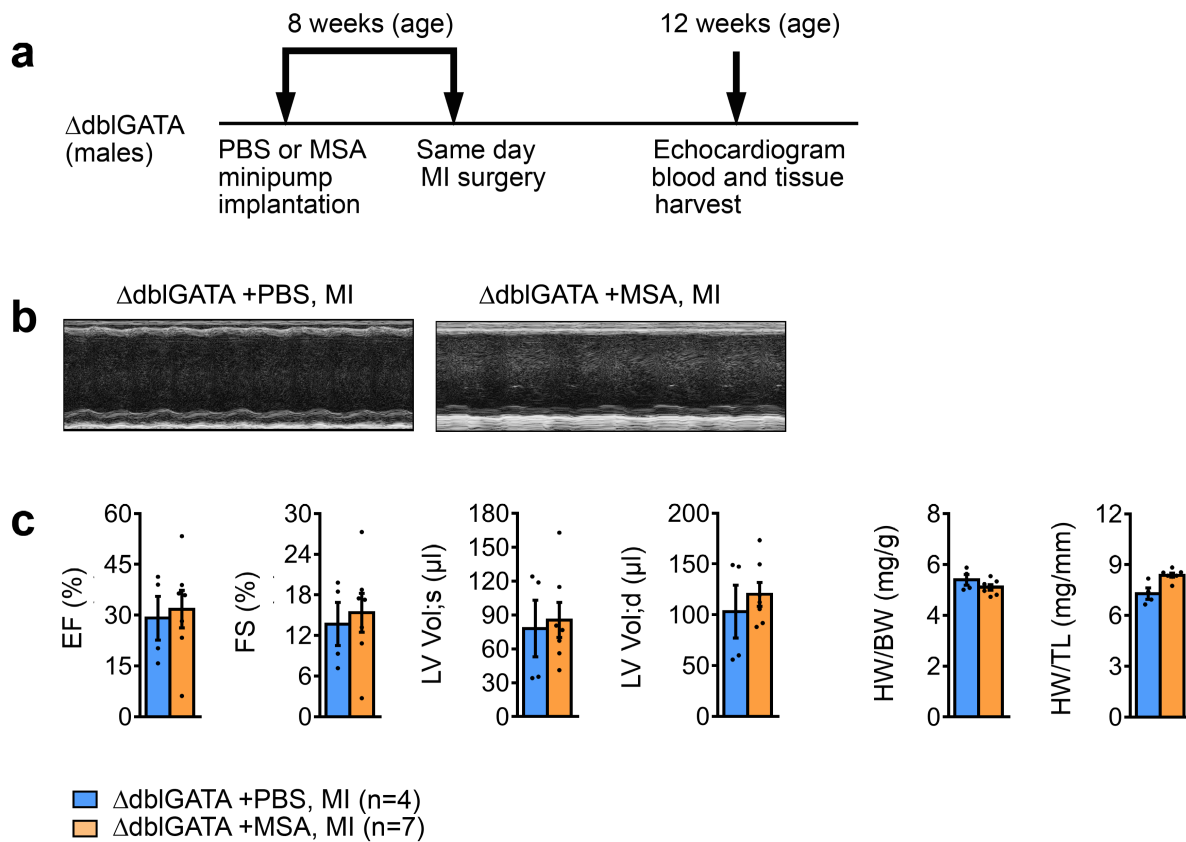
Supplementary Fig. 4. FACS gating strategy. Gating strategies for apoptotic neutrophils (**a/b**), macrophages (**c**), splenic lymphocytes (**d**), CD4⁺ T cells (**e**), and CD8⁺ T cells (**f**) corresponding to the results from **Supplementary Fig. 3**.



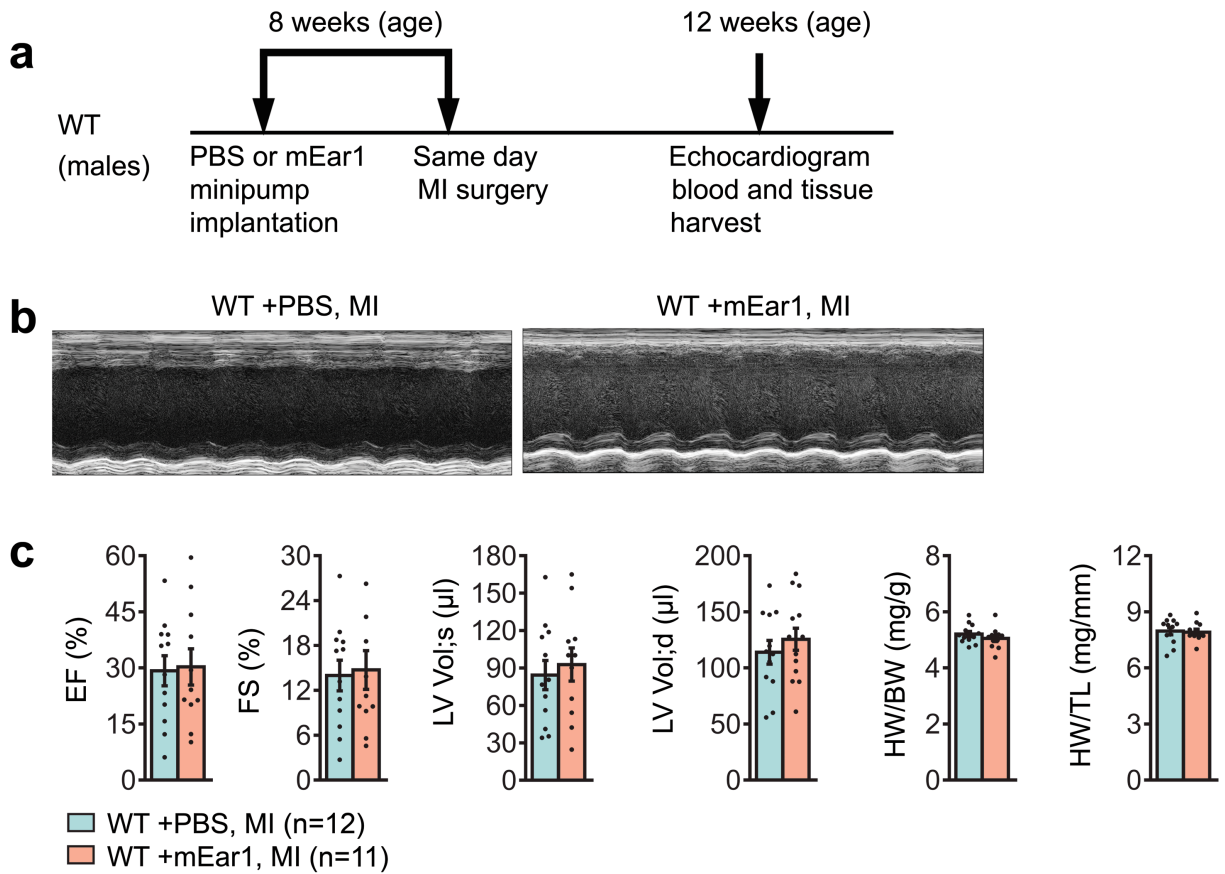
Supplementary Fig. 5. CD45.1⁺Siglec-F⁺ EOS preparation and adoptive transfer. **a.** FACS gating strategy for purified bone marrow-derived CD45.1⁺Siglec-F⁺ EOS. **b.** FACS determined the purity of bone marrow-derived CD45.1⁺Siglec-F⁺ EOS at 10, 12 and 14 days after differentiation. **c.** FACS determined donor CD45.1⁺Siglec-F⁺ EOS in heart at 1 and 3 days after i.v. adoptive transfer. **d.** FACS determined donor CD45.1⁺Siglec-F⁺ EOS in blood at 1 and 3 days after i.v. adoptive transfer. FACS gating strategy for heart and blood CD45.1⁺Siglec-F⁺ EOS is shown in Fig. 3h from the main text. Data are mean±SEM. The number of mice in each group and *P* values are indicated, non-parametric Mann-Whitney *U* test (**c/d**).



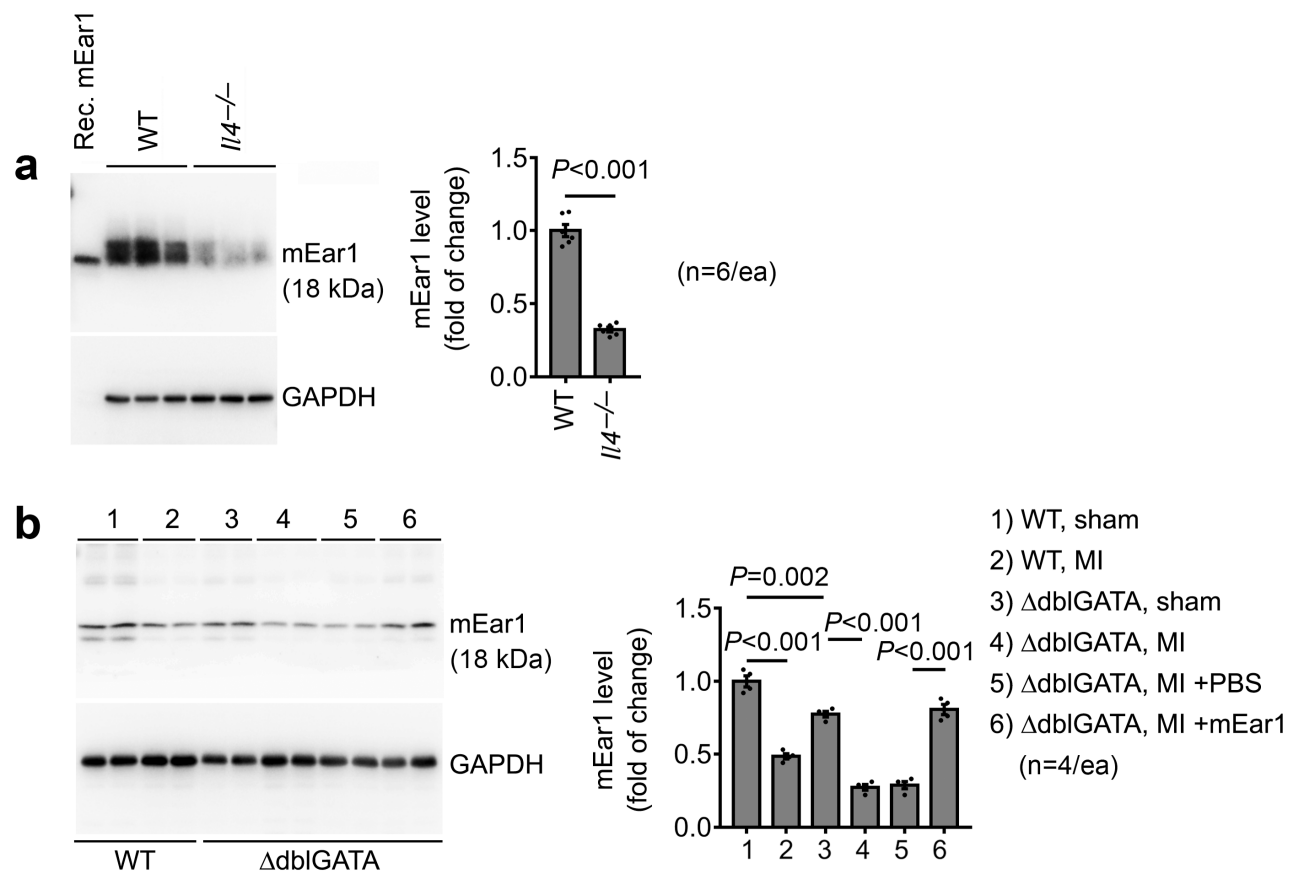
Supplementary Fig. 6. Infarct and border α -SMA- and CD90-positive areas in $\Delta\text{dblGATA}$ mice at 1-month post-MI. **a/b.** Representative images of α -SMA and CD90 staining in the infarct and border regions. **c/d.** Quantifications of α -SMA- and CD90-positive areas in the infarct and border regions from $\Delta\text{dblGATA}$ mice and those received adoptive transfers of WT EOS, $\text{I/4}^{-/-}$ EOS, PBS, or mEar1 (1 $\mu\text{g/day}$ by minipump) at 1-month post-MI. Scale bar: 200 μm . Data are mean $\pm$ SEM. The number of mice in each group and P values are indicated, one-way ANOVA test (**c/d**).



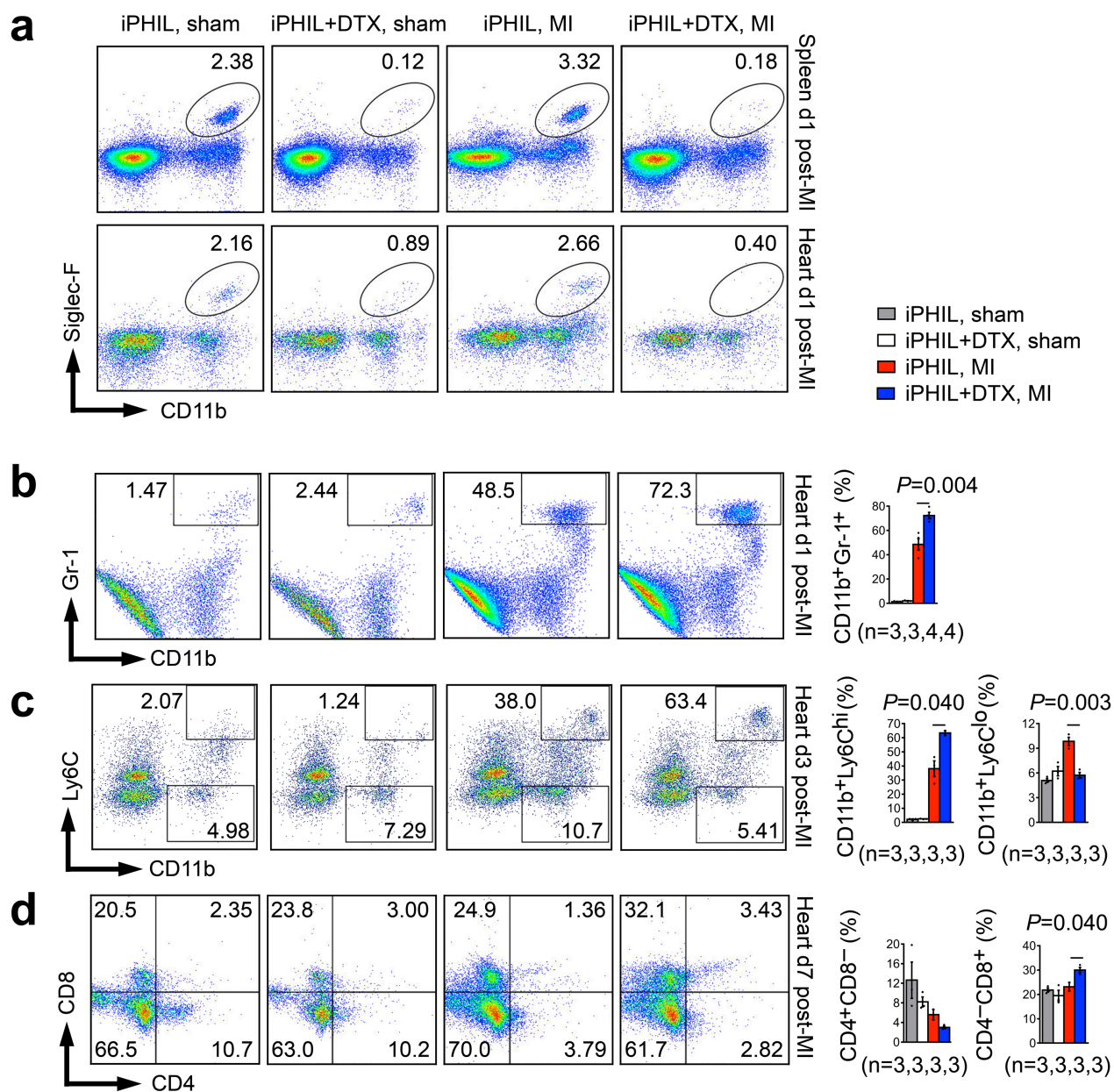
Supplementary Fig. 7. Mouse serum albumin activity in $\Delta\text{dblGATA}$ mice post-MI. **a.** MI surgery scheme. **b.** Representative LV M-mode echocardiography images at 1-month post-MI of $\Delta\text{dblGATA}$ mice received PBS or MSA (1 $\mu\text{g}/\text{day}$ by minipump). **c.** EF, FS, LV end diastolic volume, LV end systolic volume, HW/BW, and HW/TL in $\Delta\text{dblGATA}$ mice received PBS or MSA as indicated. Data are mean $\pm$ SEM. The numbers of mice in each group are indicated in the parenthesis. Non-parametric Mann-Whitney U test was used to test the statistical significance between the groups



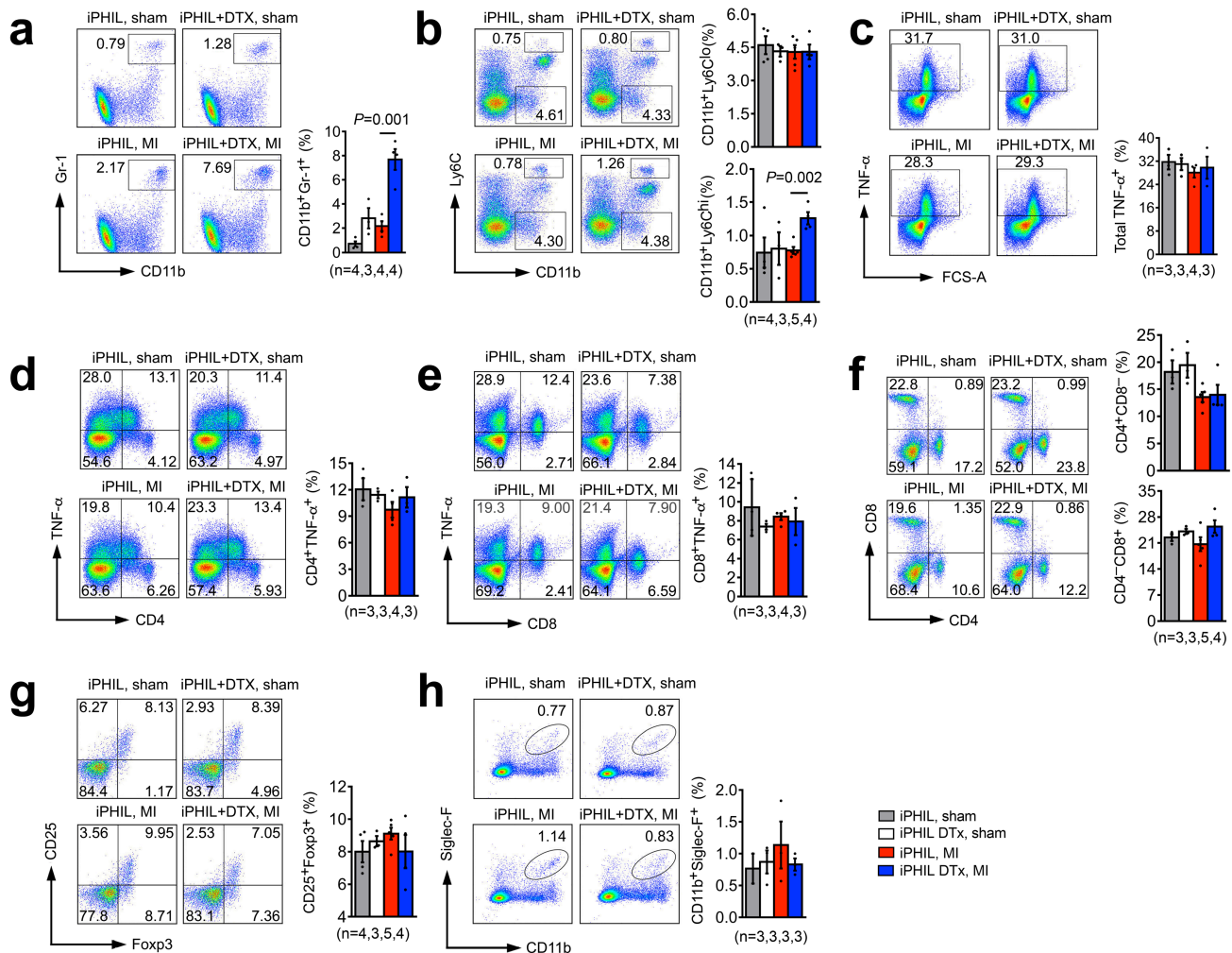
Supplementary Fig. 8. Exogenous mEar1 activity in post-MI cardiac function in WT male mice. **a.** MI surgery scheme. **b.** Representative LV M-mode echocardiography images at 1-month post-MI of male WT mice received PBS or mEar1 (1 μ g/day by minipump). **c.** EF, FS, LV Vol;d, LV Vol;s, HW/BW, and HW/TL in WT mice received PBS or mEar1 as indicated. Data are mean $\pm$ SEM. The numbers of mice in each group are indicated in the parenthesis. Non-parametric Mann-Whitney *U* test was used to test the statistical significance between the groups.



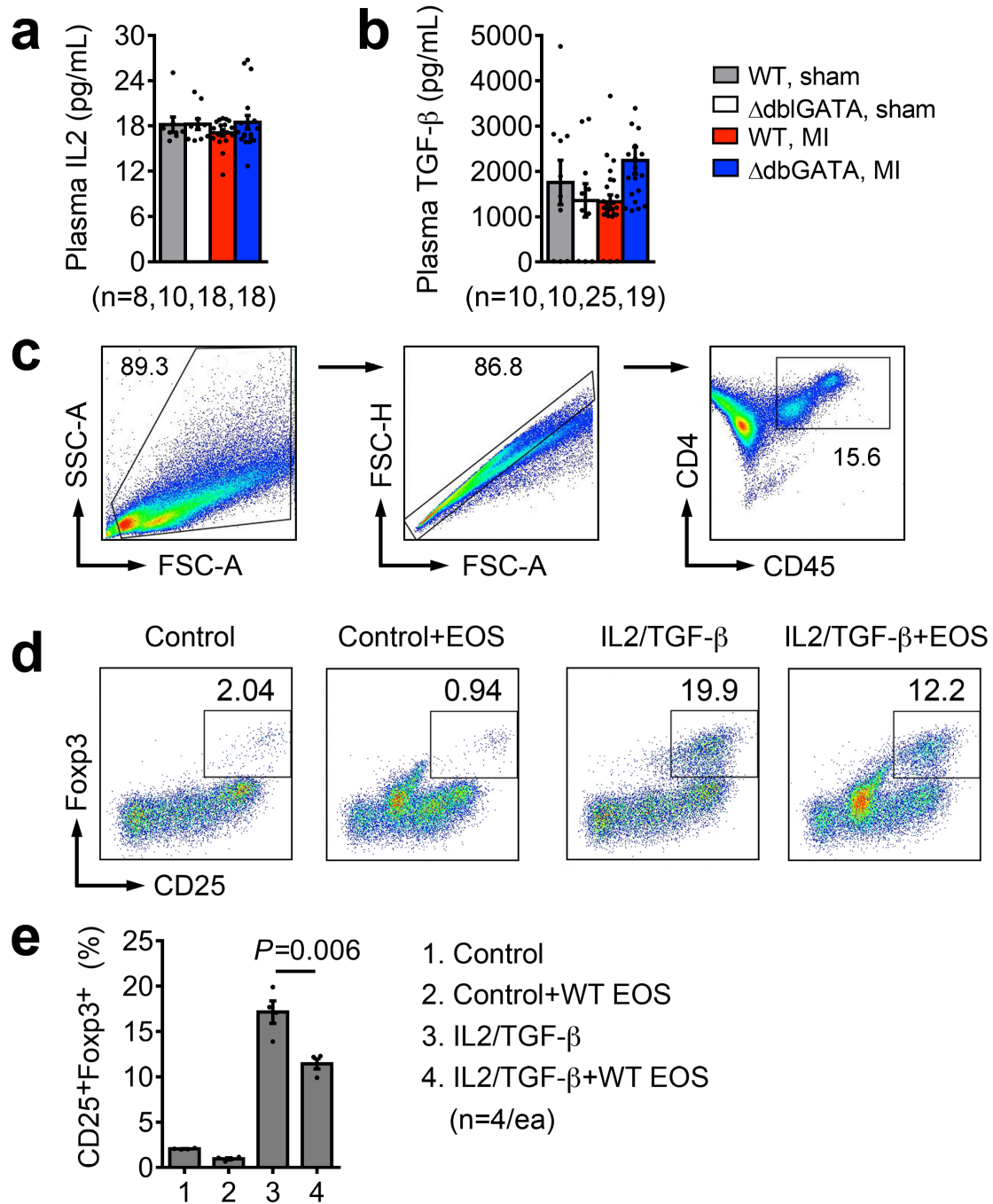
Supplementary Fig. 9. Immunoblot analysis of mEar1 expression in WT and $Il4^{-/-}$ EOS (**a**, recombinant mouse mEar1 was used as immunoblot size control) and in heart tissues from 1-month post-MI of different mice as indicated (**b**). Data are mean $\pm$ SEM. The number of mice in each group and P values are indicated, non-parametric Mann-Whitney U test (**a**) or one-way ANOVA test (**b**).



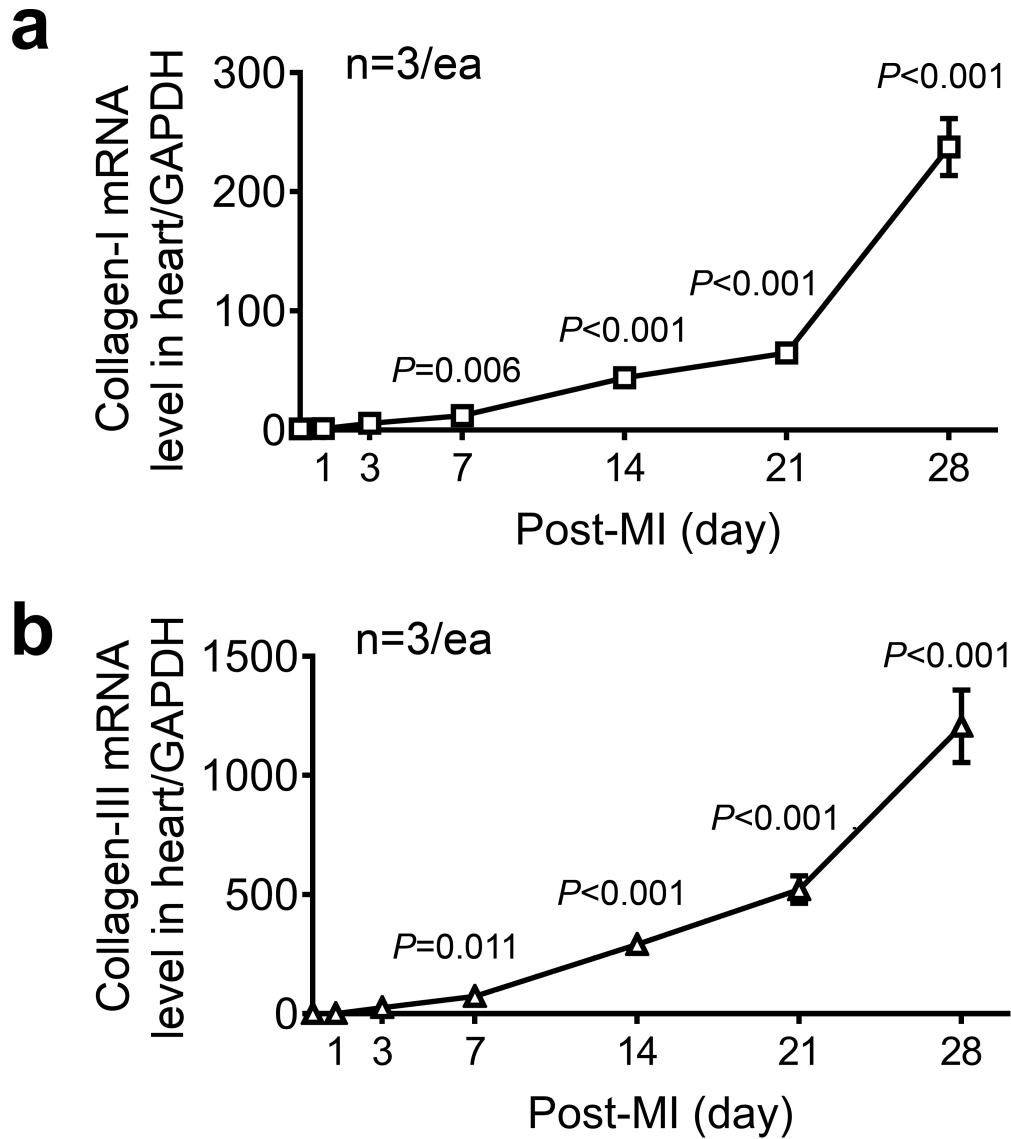
Supplementary Fig. 10. Acute heart CD45⁺ immune cell changes post-MI in iPHIL mice with or without DTX-mediated EOS depletion. **a.** FACS ensured efficient EOS depletion in spleen and heart at 1-day post-MI. FACS detected CD11b⁺Gr-1⁺ neutrophils at 1-day post-MI or sham (**b**), CD11b⁺Ly6C^{hi} and CD11b⁺Ly6C^{lo} monocytes at 3-day post-MI or sham (**c**), and CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells at 7-day post-MI or sham (**d**) operation as indicated. Representative FACS images are shown to the left. Gating strategy is shown in **Fig. 3h** from the main text. Data are mean±SEM. The number of mice in each group and *P* values are indicated, one-way ANOVA test (**b-d**).



Supplementary Fig. 11. FACS analysis of splenic CD45⁺ immune cells from different groups of mice as indicated at 1-month post-MI. **a.** CD11b⁺Gr-1⁺ neutrophils. **b.** CD11b⁺Ly6C^{hi} and CD11b⁺Ly6C^{lo} monocytes. **c.** Total TNF- α ⁺ cells. **d.** CD4⁺TNF- α ⁺ T cells. **e.** CD8⁺TNF- α ⁺ T cells. **f.** CD4⁺CD8⁻ and CD4⁺CD8⁺ T cells. **g.** CD4⁺CD25⁺Foxp3⁺ Tregs. **h.** CD11b⁺Siglec-F⁺ EOS. Representative FACS images are shown to the left of each panel. FACS gating strategy is the same as in **Supplementary Fig. 1g**. Data are mean $\pm$ SEM. The number of mice in each group and *P* values are indicated, one-way ANOVA test.



Supplementary Fig. 12. EOS reduce Treg cell differentiation. Plasma IL2 (**a**) and TGF- β (**b**) levels in WT and Δ dblGATA mice at 1-month post-MI. **c.** FACS gating strategy. **d/e.** Representative images and quantification of WT EOS activity in IL2 and TGF- β -induced CD45⁺CD4⁺CD25⁺Foxp3⁺ Treg cell differentiation. Data are mean $\pm$ SEM. The number of mice in each group (**a/b**), the numbers of experiments in each treatment (**e**), and P value are indicated, one-way ANOVA test (**a/b, e**).



Supplementary Fig. 13. Mouse heart collagen expression post-MI. RT-PCR determined myocardium collagen-I (**a**) and collagen-III (**b**) mRNA levels in WT mice at different days post-MI. Data are mean±SEM. The number of mice in each group and *P* values compared with baseline collagen expression levels (0 day) are indicated, one-way ANOVA test (**a/b**).