

Supplemental material

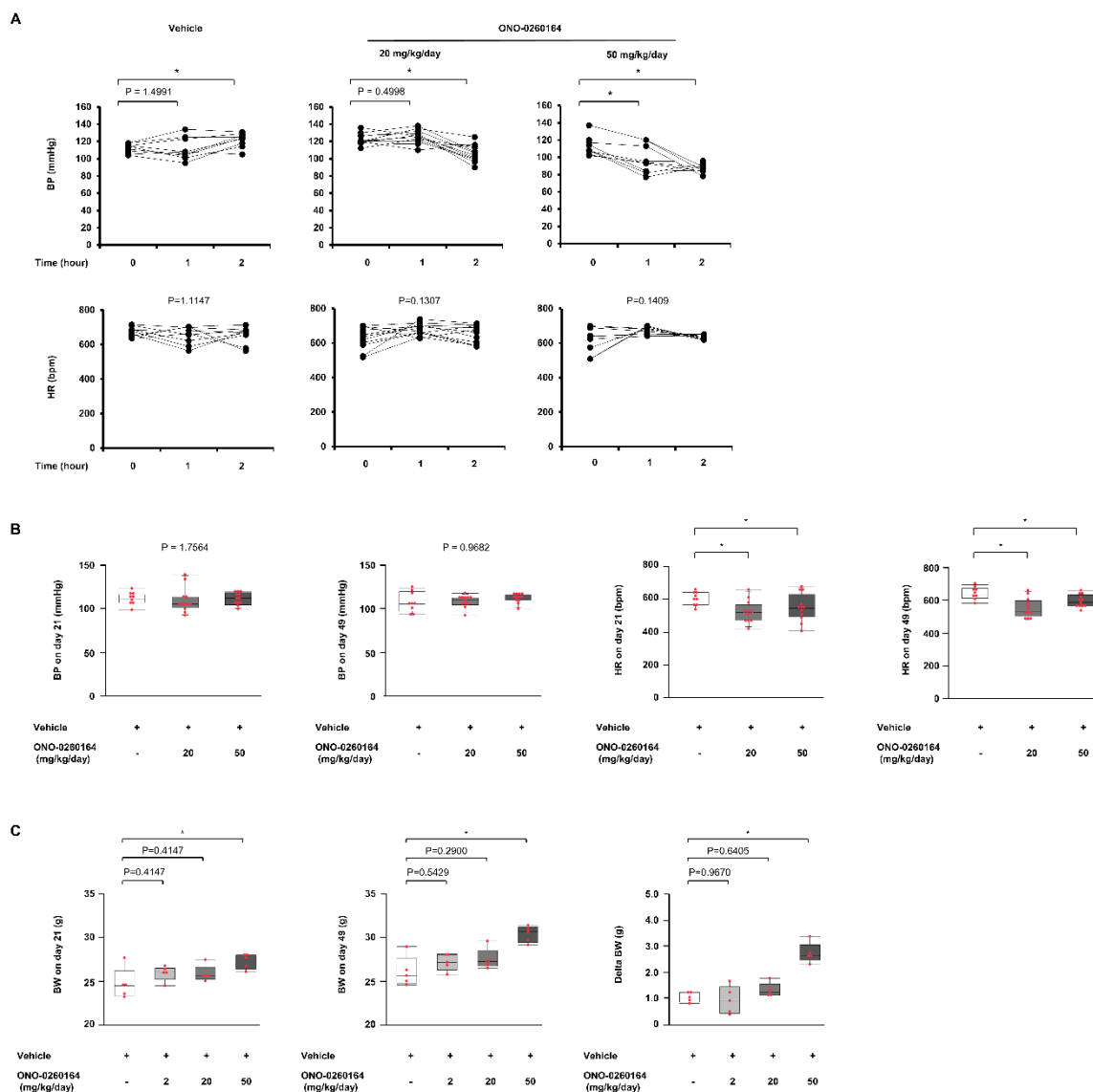
**Prostaglandin-E2 Receptor-4 Stimulant Rescues Cardiac Malfunction during
Myocarditis and Protects the Heart from Adverse Ventricular Remodeling after
Myocarditis**

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Content

Supplementary Figures and legends: 1-5

Supplementary Tables: 1-2

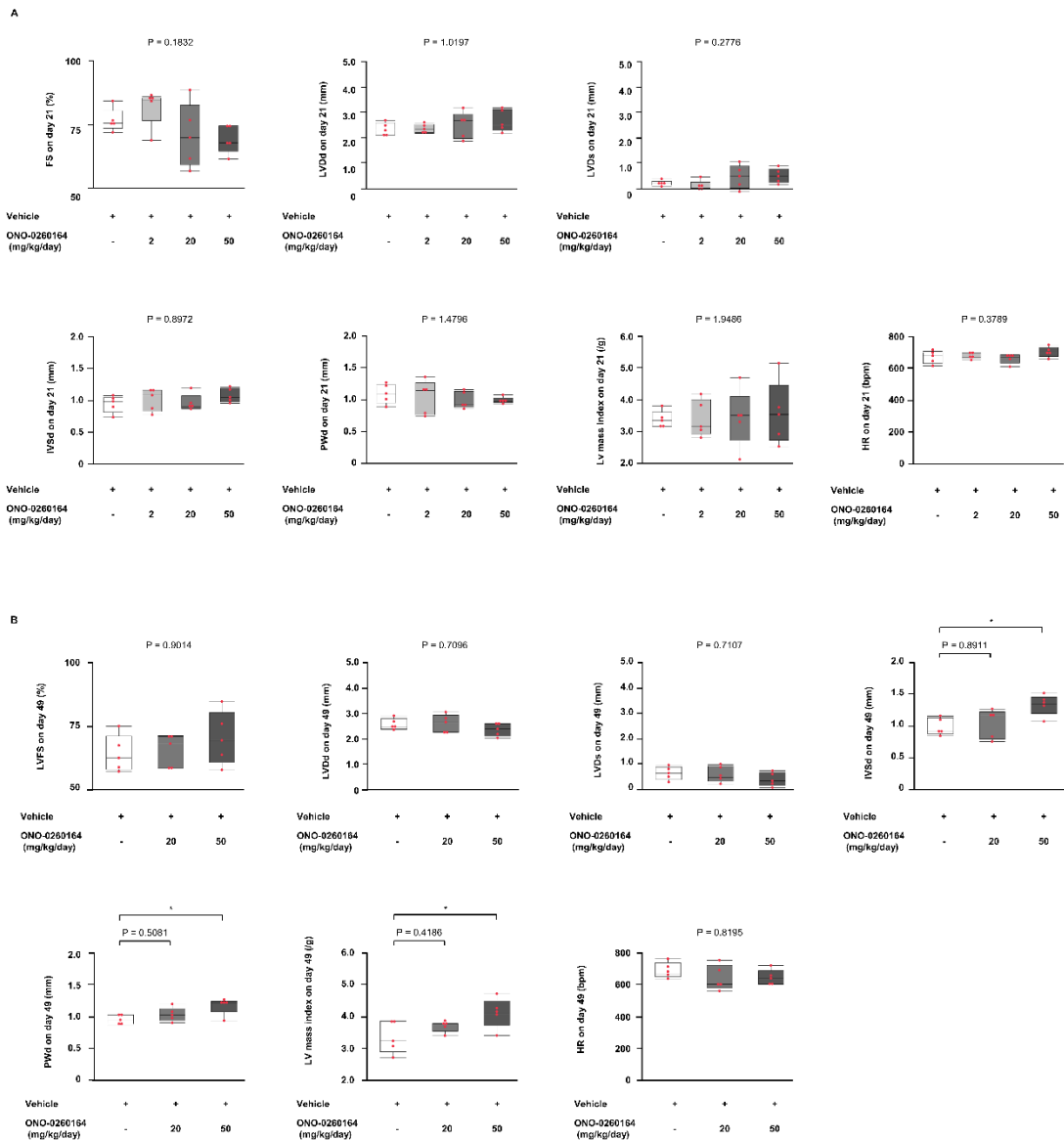


Supplementary Figure 2. Effects of ONO-0260164, a selective prostaglandin-E2 receptor-4 (EP4) agonist on healthy mice.

(A) Systolic blood pressure (BP) and heart rate (HR) after a single administration of vehicle (n=9) or 20 mg/kg (n=12) or 50 mg/kg (n=9) of ONO-0260164. The BP was significantly increased 2 hours after a single administration of vehicle compared with before administration (121 ± 2.7 mmHg vs. 112 ± 1.7 mmHg, $P = 0.0009$, respectively). Conversely, BP was decreased 1 or 2 hours after a single administration of ONO-0260164 (20 or 50

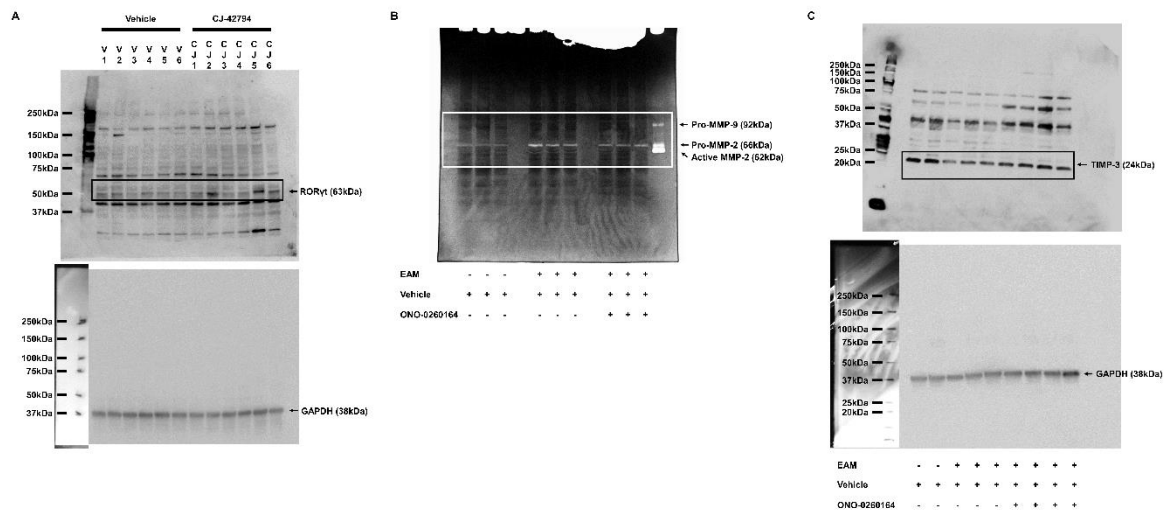
mg/kg) compared with before the administration (20 mg/kg: [2 hours] 106 ± 2.9 mmHg vs. 123 ± 1.8 mmHg, $P < 0.0001$; 50 mg/kg: [1 hour] 98 ± 4.7 mmHg vs. 113 ± 3.1 mmHg, $P = 0.0009$, [2 hours] 87 ± 1.5 mmHg vs. 113 ± 3.1 mmHg, $P < 0.0001$; respectively), while the HR did not change among before and 1 and 2 hours after the administration of vehicle or ONO-0260164 (vehicle: 673 ± 8 bpm vs. 653 ± 15 bpm vs. 654 ± 14 bpm, ANOVA $P=1.1147$; 20 mg/kg of ONO-0260164: 630 ± 18 bpm vs. 678 ± 10 bpm vs. 653 ± 15 bpm, ANOVA $P=0.1307$; 50 mg/kg of ONO-0260164: 619 ± 22 bpm vs. 673 ± 6 bpm vs. 633 ± 3 bpm, ANOVA $P=0.1409$; respectively). **(B)** BP and HR in healthy mice treated daily with vehicle alone ($n=9$) or 20 mg/kg ($n=12$) or 50 mg/kg ($n=12$) of ONO-0260164 daily. ONO-0260164 did not affect BP on days 21 and 49 (vehicle alone vs. 20 mg/kg/day vs. 50 mg/kg/day: [day 21] 114 ± 2.2 mmHg vs. 112 ± 3.9 mmHg vs. 114 ± 1.9 mmHg, ANOVA $P=1.7564$; [day 49] 110 ± 3.5 mmHg vs. 111 ± 1.8 mmHg vs. 114 ± 1.3 mmHg, ANOVA $P=0.9682$; respectively). However, the HR was significantly reduced in 20 mg/kg/day or 50 mg/kg/day of ONO-0260164 compared with in vehicle alone (day 21: 541 ± 18 bpm or 564 ± 23 bpm vs. 614 ± 12 bpm, $P = 0.0021$ or $P = 0.0330$; day 49: 568 ± 16 bpm or 609 ± 10 bpm vs. 652 ± 11 bpm, $P < 0.0001$ or $P = 0.0068$; respectively). **(C)** Body weight (BW) in healthy mice treated daily with vehicle alone ($n=5$) or with 2 mg/kg ($n=5$), 20 mg/kg ($n=5$), or 50 mg/kg ($n=5$) of ONO-0260164 daily. The BWs on days 21 and 49 were significantly increased in the 50 mg/kg/day group of ONO-0260164 compared to the vehicle only group (day 21: 27.8 ± 0.4 g vs. $25.4 \pm$

0.8 g, $P = 0.0138$; day 49: 30.7 ± 0.4 g vs. 26.7 ± 0.7 g, $P = 0.0003$; respectively), but not in the 2 or 20 mg/kg/day group (day 21: 26.5 ± 0.3 g, $P = 0.4147$ or 26.5 ± 0.4 g, $P = 0.4147$ vs. the vehicle only group; day 49: 27.7 ± 0.4 g, $P = 0.5429$ or 28.1 ± 0.5 g, $P = 0.2900$ vs. the vehicle only group; respectively). Furthermore, the change in BW from day 21 to day 49 was significantly increased in the 50 mg/kg/day group of ONO-0260164 compared to the vehicle only group (2.9 ± 0.2 g vs. 1.3 ± 0.1 g, $P < 0.0001$; respectively), but not in the 2 or 20 mg/kg/day groups of ONO-0260164 (1.2 ± 0.2 g, $P = 0.9670$; 1.6 ± 0.1 g, $P = 0.6405$; vs. the vehicle only group; respectively). $P^* < 0.05$ (vs. before administration or vehicle alone). Data from different time points or different treatments were analyzed with 2-way ANOVA followed by the Bonferroni-Dunn post hoc testing method.



and LV mass index (LVMI) did not show any significant differences among vehicle, the 2 mg/kg/day group, the 20 mg/kg/day group, and the 50 mg/kg/day group (LVFS: 78.5 ± 1.9 % vs. 83.2 ± 3.0 % vs. 72.9 ± 5.3 % vs. 71.4 ± 2.3 %, ANOVA $P = 0.1832$; LVDd: 2.5 ± 0.1 mm vs. 2.5 ± 0.1 mm vs. 2.6 ± 0.2 mm vs. 2.8 ± 0.2 mm, ANOVA $P = 1.0197$; LVDs: 0.53 ± 0.04 mm vs. 0.43 ± 0.08 mm vs. 0.76 ± 0.19 mm vs. 0.80 ± 0.12 mm, ANOVA $P = 0.2776$; IVSd: 1.0 ± 0.06 mm vs. 1.1 ± 0.07 mm vs. 1.0 ± 0.06 mm vs. 1.1 ± 0.05 mm, ANOVA $P = 0.8972$; PWd: 1.2 ± 0.06 mm vs. 1.1 ± 0.11 mm vs. 1.1 ± 0.06 mm vs. 1.1 ± 0.02 mm, ANOVA $P = 1.4796$; LVMI: 3.6 ± 0.1 /g vs. 3.6 ± 0.2 /g vs. 3.6 ± 0.4 /g vs. 3.7 ± 0.4 /g, ANOVA $P = 1.9486$; HR: 676 ± 16 bpm vs. 685 ± 8 bpm vs. 669 ± 13 bpm vs. 708 ± 14 bpm, ANOVA $P = 0.3789$; respectively). **(B)** Echocardiographic findings on day 49 in healthy mice treated with vehicle alone or with 20 mg/kg or 50 mg/kg of ONO-0260164 ($n=5$ / each). LVFS, LVDd, LVDs, and HR did not show any significant differences among vehicle alone, 20 mg/kg/day, and 50 mg/kg/day (LVFS: 66.4 ± 3.0 % vs. 67.6 ± 2.7 % vs. 72.2 ± 4.4 %, ANOVA $P = 0.9014$; LVDd: 2.7 ± 0.1 mm vs. 2.8 ± 0.1 mm vs. 2.5 ± 0.1 mm, ANOVA $P = 0.7096$; LVDs: 0.9 ± 0.1 mm vs. 0.9 ± 0.1 mm vs. 0.7 ± 0.1 mm, ANOVA $P = 0.7107$; HR: 697 ± 21 bpm vs. 652 ± 33 bpm vs. 656 ± 20 bpm, ANOVA $P = 0.8195$; respectively). However, IVSd, PWd, and LVMI were significantly increased in 50 mg/kg/day group of ONO-0260164 compared with in vehicle only group (IVSd: 1.4 ± 0.07 mm vs. 1.1 ± 0.06 mm, $P = 0.0277$; PWd: 1.2 ± 0.05 mm vs. 1.0 ± 0.03 mm, $P = 0.0212$; LVMI: 4.2 ± 0.2 /g vs. 3.5 ± 0.2 /g, $P = 0.0282$; respectively). These

parameters did not show any significant differences between 20 mg/kg/day and vehicle alone (IVSd: 1.1 ± 0.1 mm, $P = 0.8911$; PWd: 1.1 ± 0.05 mm, $P = 0.5081$; LVMI: 3.8 ± 0.1 /g, $P = 0.4186$; respectively). $P^* < 0.05$ (vs. vehicle alone). Data from different treatments were analyzed with 2-way ANOVA followed by the Bonferroni-Dunn post hoc testing method.



Supplementary Figure 4. Full-length western blots and gel.

(A) Western blots of the EAM inducible Th17-specific master transcription factor, retinoic acid receptor-related orphan nuclear receptor (ROR γ t) and GAPDH with protein markers. (B) Gelatin zymography gel of matrix metalloproteinase (MMP)-2 and -9 with the proteolytic bands of 62, 66, and 92kDa corresponding to the active form of MMP-2, Pro-MMP-2, and Pro-MMP-9, respectively. (C) Western blots of the tissue inhibitor of metalloproteinase (TIMP)-3 and GAPDH with protein markers.

Supplementary Table 1. Echocardiographic data on day 14 in experimental autoimmune myocarditis (EAM) mice

	non-EAM (n=10)	EAM (n=15)	P value
Parameters			
LVFS (%)	72.7 ± 1.4 [69.5-75.8]	50.0 ± 3.9 [41.7-58.3]	< 0.0001
LVDs (mm)	0.71 ± 0.02 [0.66-0.77]	1.43 ± 0.20 [1.00-1.87]	< 0.0001
LVDd (mm)	2.52 ± 0.02 [2.47-2.57]	2.69 ± 0.16 [2.35-3.04]	0.8027
HR (bpm)	690.1 ± 13.4 [659.8-720.4]	644.4 ± 15.1 [612.2-676.7]	0.0345
BP (mmHg)	118.9 ± 1.3 [115.8-121.9]	90.3 ± 1.6 [87.0-93.6]	0.0004

Data are mean±SEM [95% confidence interval]. LVFS: Left ventricular fractional shortening;

LVDs: LV end-systolic dimension; LVDd: LV end-diastolic dimension; HR: Heart rate; BP;

systolic blood pressure. P values were calculated with nonparametric 2-tailed Mann-Whitney

U test.

Supplementary Table 2. Sequences of Primers used for RT-PCR

Gene	Sense	Antisense
Col1a1	CCTCAGGGTATTGCTGGACAAC	CAGAAGGACCTTGTTTGCCAGG
Col3a1	GACCAAAGGTGATGCTGGACAG	CAAGACCTCGTGCTCCAGTTAG
MMP-2	ACGATGATGACCGGAAGT	GTGTAGATCGGGGCCATC
MMP-9	CAGACGTGGGTCGATTCC	TCATCGATCATGTCTCGC
MT1-MMP	CCCTAGGCCTGGAACATTCT	TTTGGGCTTATCTGGGACAG
TIMP-1	CCCAGAAATCAACGAGA	TGGGACTTGTGGGCATA
TIMP-2	ACGCTTAGCATCACCCAGAAG	TGGGACAGCGAGTGATCTTG
TIMP-3	ACACGGAAGCCTCTGAAA	TGGAGGTCACAAAACAAGG
TIMP-4	CACCCTCAGCAGCACATCTG	GGCCGGAACCTTCTCACT
GAPDH	ACTCCACTCACGGCAAATTCA	GGTCTCGCTCCTGGAAGATG

Col1a1: collagen type I, alpha 1, Col3a1: collagen type III, alpha 1, MMP: matrix metalloproteinase, MT1-MMP: membrane type 1 MMP, TIMP: tissue inhibitors of metalloproteinase.