Legends for Supplemental Figures

Supplemental figure 1. EPA and DHA reduce the TGF-β1-induced Smad-responsive promoter activity. (A) Left ventricular mRNA expression of PAI-1 analyzed by real-time PCR 3 days and 7 days after TAC. Data are presented as mean ± SEM (n=5 per group). Data were analyzed by two-way ANOVA with Tukey's post-hoc test. (B) Cardiac fibroblasts were transfected with VSV-g pseudotyped lentivirus particles expressing the firefly luciferase gene under the control of a CMV promoter and tandem repeats of the Smad transcriptional response element (TRE). TGF-β1 (1 ng/ml) increased the luciferase activity in transfected cardiac fibroblasts; 24hrs pretreatment of DHA and EPA reduced TGF-β1-induced increase of luciferase activity. Data are presented as mean ± SEM (n=5-6 per group). Data were analyzed by one-way ANOVA with Tukey's post-hoc test.

Supplemental Figure 2. Proposed anti-fibrotic mechanism of ω-3 PUFAs in cardiac fibroblast.

Supplemental Figure 3. EPA and DHA do not block TGF-b1-induced activation of ERK1/2. (A) Phosphorylated ERK1/2 (Thr202/Tyr204) and total ERK1/2 were detected by Western blot in cultured cardiac fibroblasts treated for 24 hr with EPA or DHA (10 μM) and for an additional 1hr of TGF-β1 (1 ng/ml) with fatty acids. (B) Quantification of Phosphorylated ERK1/2 relative to total ERK1/2. Data are presented as mean ± SEM (n=5 per group).Data were analyzed by one-way ANOVA with Tukey's post-hoc test.

Supplemental Table 1: **Fatty acid profiles from red blood cells**[a].

Fatty acid[b]	Structure	Control diet		Fish oil diet	
		Sham (n=6)	TAC (n=9)	Sham (n=6)	TAC (n=9)
Myristic acid	C14:0	0.26 ± 0.03	0.25 ± 0.01	0.31 ± 0.01 [A]	0.29 ± 0.01 [C]
Palmitic acid	C16:0	29.3 ± 0.20	29.7 ± 0.27	31.6 ± 0.44 [A]	32.4 ± 0.28 [C]
Palmitoleic acid	C16:1n7	0.83 ± 0.08	0.84 ± 0.06	0.79 ± 0.04	0.79 ± 0.03
trans Palmitoleic acid	C16:1n7t	0.05 ± 0.00	0.06 ± 0.00	0.07 ± 0.01 [A]	0.08 ± 0.00 [C]
Stearic acid	C18:0	12.8 ± 0.43	13.3 ± 0.24	12.4 ± 0.10	11.5 ± 0.13 [B,C]
Oleic acid	C18:1n9	14.1 ± 0.40	15.3 ± 0.34 [A]	13.3 ± 0.10	13.7 ± 0.14 [C]
trans Oleic acid	C18:1t	0.19 ± 0.02	0.17± 0.01	0.18 ± 0.02	0.15 ± 0.01
Linoleic acid (n-6)	C18:2n6	11.1 ± 0.53	10.5 ± 0.34	10.2 ± 0.12	10.3 ± 0.16
trans Linoleic acid (n-6)	C18:2n6t	0.24 ± 0.02	0.28 ± 0.02	0.32 ± 0.04	0.17 ± 0.02 [B,C]
α-Linolenic acid (n-3)	C18:3n3	0.05 ± 0.01	0.05 ± 0.00	0.04 ± 0.01 [A]	0.03 ± 0.00 [C]
γ-Linolenic acid (n-6)	C18:3n6	0.08 ± 0.01	0.09 ± 0.00	0.10 ± 0.01	0.13 ± 0.01 [B,C]
Eicosenoic acid	C20:1n9	0.48 ± 0.01	0.52 ± 0.01	0.45 ± 0.02	0.38 ± 0.01 [B,C]
Eicosadienoic acid (n-6)	C20:2n6	0.30 ± 0.02	0.28 ± 0.01	0.20 ± 0.01 [A]	0.18 ± 0.00 [C]
Eicosatrienoic acid (n-6)	C20:3n6	1.26 ± 0.08	1.37 ± 0.04	1.13 ± 0.05	1.06 ± 0.02 [C]
Arachidonic acid (AA, n-6)	C20:4n6	19.3 ± 0.63	18.4 ± 0.34	10.6 ± 0.29 [A]	10.4 ± 0.14 [C]
Eicosapentaenoic acid (EPA, n-3)	C20:5n3	0.05 ± 0.01	0.08 ± 0.04	3.59 ± 0.14 [A]	3.64 ± 0.11 [C]
Docosatetraenoic aicd (n-6)	C22:4n6	2.17 ± 0.10	2.26 ± 0.06	0.51 ± 0.00 [A]	0.50 ± 0.01 [C]
Docosapentaenoic acid (n-3)	C22:5n3	0.42 ± 0.06	0.26 ± 0.02 [A]	2.16 ± 0.02 [A]	2.14 ± 0.03 [C]
Docosapentaenoic acid (n-6)	C22:5n6	2.17 ± 0.24	2.61 ± 0.06 [A]	0.24 ± 0.01 [A]	0.28 ± 0.00 [C]
Docosahexaenoic acid (DHA, n-3)	C22:6n3	4.41 ± 0.22	3.45 ± 0.11 [A]	11.5 ± 0.09 [A]	11.6 ± 0.11 [C]
Lignoceric acid	C24:0	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.22 ± 0.01 [B,C]
Nervonic acid	C24:1n9	0.15 ± 0.01	0.12 ± 0.01	0.15 ± 0.02	0.17 ± 0.01 [C]
Composite Indices					
Omega-3 Content (DHA+EPA)		4.45 ± 0.23	3.54 ± 0.13 [A]	15.1 ± 0.11 [A]	15.2 ± 0.17 [C]
Total Saturated		42.6 ± 0.42	43.4 ± 0.40	44.5 ± 0.40 [A]	44.3 ± 0.29
Total Monounsaturated		15.8 ± 0.43	17.0 ± 0.40 [A]	15.0 ± 0.14	15.3 ± 0.14 [C]
n-3 Polyunsaturated		4.93 ± 0.29	3.84 ± 0.15 [A]	17.3 ± 0.11 [A]	17.4 ± 0.19 [C]
n-6 Polyunsaturated		36.7 ± 0.64	35.7 ± 0.36	23.2 ± 0.31 [A]	23.0 ± 0.26 [C]
n-3 : n-6		0.135 ± 0.008	0.108 ± 0.005	0.745 ± 0.011 [A]	0.756 ± 0.011 [C]
EPA : AA		0.003 ± 0.001	0.004 ± 0.002	0.342 ± 0.021 [A]	0.350 ± 0.011 [C]
EPA : DHA		0.010 ± 0.002	0.022 ± 0.009	0.313 ± 0.014 [A]	0.314 ± 0.009 [C]

[A] $P<0.05$ verus Control diet/sham; [B] $P<0.05$ verus Fish oil diet/sham; [C] $P<0.05$ verus Control diet/TAC. All measurements are means ± SEM, and significance was determined using the two-way ANOVA with post tukey test.

[a] Reported as percent of total fatty acid.

[b] Most common name given. Omega-9, omega-6 and omega-3 FAs are indicated as n9, n6, and n3, respectively

Supplemental Table 2: **Fatty acid profiles from LV tissue**[a].

Fatty acid[b]	Structure	Control diet		Fish oil diet	
		Sham (n=6)	TAC (n=9)	Sham (n=6)	TAC (n=9)
Myristic acid	C14:0	0.23 ± 0.02	0.23 ± 0.01	0.31 ± 0.02 [A]	0.30 ± 0.03 [C]
Palmitic acid	C16:0	13.5 ± 0.21	13.7 ± 0.10	15.0 ± 0.26 [A]	14.9 ± 0.20 [C]
Palmitoleic acid	C16:1n7	0.50 ± 0.07	0.56 ± 0.04	0.65 ± 0.07	0.66 ± 0.06
trans Palmitoleic acid	C16:1n7t	0.03 ± 0.00	0.03 ± 0.00	0.037 ± 0.002 [A]	0.044 ± 0.001 [B,C]
Stearic acid	C18:0	16.6 ± 0.39	17.0 ± 0.15	16.0 ± 0.27	16.2 ± 0.25 [C]
Oleic acid	C18:1n9	12.2 ± 0.76	12.9 ± 0.47	9.99 ± 0.46 [A]	10.6 ± 0.35 [C]
trans Oleic acid	C18:1t	0.17 ± 0.01	0.16± 0.00	0.15 ± 0.00 [A]	0.14 ± 0.00 [C]
Linoleic acid (n-6)	C18:2n6	17.4 ± 0. 51	16.6 ± 0.33	11.7 ± 0.14 [A]	12.4 ± 0.45 [C]
trans Linoleic acid (n-6)	C18:2n6t	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
α-Linolenic acid (n-3)	C18:3n3	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
γ-Linolenic acid (n-6)	C18:3n6	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.01 [B,C]
Eicosenoic acid	C20:1n9	0.58 ± 0.04	0.57 ± 0.02	0.45 ± 0.02 [A]	0.46 ± 0.03 [C]
Eicosadienoic acid (n-6)	C20:2n6	0.33 ± 0.01	0.30 ± 0.01 [A]	0.24 ± 0.01 [A]	0.25 ± 0.01 [C]
Eicosatrienoic acid (n-6)	C20:3n6	0.75 ± 0.08	0.80 ± 0.02	0.56 ± 0.01 [A]	0.54 ± 0.01 [C]
Arachidonic acid (AA, n-6)	C20:4n6	10.2 ± 0.15	10.6 ± 0.15	3.85 ± 0.08 [A]	3.83 ± 0.16 [C]
Eicosapentaenoic acid (EPA, n-3)	C20:5n3	0.01 ± 0.00	0.01 ± 0.00	0.49 ± 0.02 [A]	0.47 ± 0.03 [C]
Docosatetraenoic aicd (n-6)	C22:4n6	1.18 ± 0.09	1.21 ± 0.03	0.13 ± 0.01 [A]	0.13 ± 0.00 [C]
Docosapentaenoic acid (n-3)	C22:5n3	0.60 ± 0.04	0.55 ± 0.07	2.38 ± 0.04 [A]	2.37 ± 0.06 [C]
Docosapentaenoic acid (n-6)	C22:5n6	9.84 ± 1.05	12.3 ± 0.25 [A]	0.42 ± 0.01 [A]	0.41 ± 0.01 [C]
Docosahexaenoic acid (DHA, n-3)	C22:6n3	15.5 ± 0.94	12.3 ± 0.59 [A]	37.5 ± 0.61 [A]	36.0 ± 0.59 [C]
Lignoceric acid	C24:0	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.00 [B,C]
Nervonic acid	C24:1n9	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.11 ± 0.01 [B,C]
Composite Indices					
Omega-3 Content (DHA+EPA)		15.5 ± 0.94	12.3 ± 0.59 [A]	37.9 ± 0.60 [A]	36.5 ± 0.59 [C]
Total Saturated		30.4 ± 0.41	31.0 ± 0.20	31.3 ± 0.16 [A]	31.4 ± 0.17
Total Monounsaturated		1.33 ± 0.08	1.39 ± 0.04	1.36 ± 0.07	1.42 ± 0.07
n-3 Polyunsaturated		16.2 ± 0.96	12.9 ± 0.66 [A]	40.3 ± 0.58 [A]	38.9 ± 0.64 [C]
n-6 Polyunsaturated		39.8 ± 0.67	41.9 ± 0.36 [A]	17.0 ± 0.16 [A]	17.6 ± 0.48 [C]
n-3 : n-6		0.408 ± 0.030	0.309 ± 0.018	2.371 ± 0.040 [A]	2.227 ± 0.092 [C]
EPA : AA		0.001 ± 0.000	0.001 ± 0.000	0.124 ± 0.006 [A]	0.125 ± 0.010 [C]
EPA : DHA		0.000 ± 0.000	0.001 ± 0.000	0.013 ± 0.001 [A]	0.013 ± 0.001 [C]

[A] $P<0.05$ verus Control diet/sham; [B] $P<0.05$ verus Fish oil diet/sham; [C] $P<0.05$ verus Control diet/TAC. All measurements are means ± SEM, and significance was determined using the two-way ANOVA with post tukey test.

[a] Reported as percent of total fatty acid.

[b] Most common name given. Omega-9, omega-6 and omega-3 FAs are indicated as n9, n6, and n3, respectively

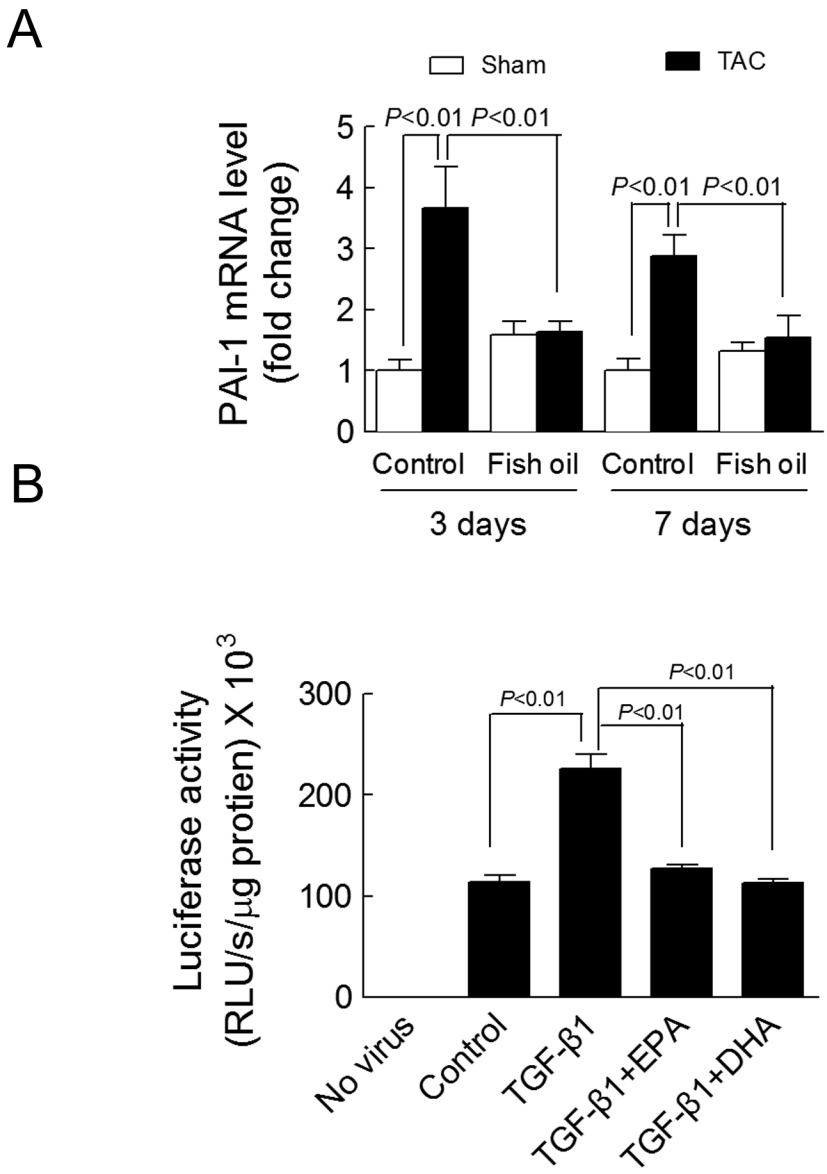
Supplemental Table 3: Echocardiographic and hemodynamic assessment of cardiac structure and function after 4 weeks of TAC.

	Control diet		Fish oil diet	
	Sham	**TAC**	**Sham**	**TAC**
	n=6	**n=9**	**n=6**	**n=9**
BW (g)	26 ± 0.5	27 ± 0.5	26 ± 0.5	26 ± 0.4
HW (mg)	133 ± 7	197 ± 12[A]	118 ± 2	160 ± 10[B,C]
HW/BW (mg/g)	5.1 ± 0.2	7.3 ± 0.4[A]	4.5 ± 0.1	6.0 ± 0.3[B,C]
Echocardiography	**n=6**	**n=9**	**n=6**	**n=9**
HR	610 ± 19	608 ± 22	640 ± 9	641 ± 10
IVSth (mm)	0.87 ± 0.03	1.21 ± 0.04[A]	0.88 ± 0.02	1.11 ± 0.05[B]
LVPWth (mm)	0.86 ± 0.01	1.27 ± 0.06[A]	0.86 ± 0.01	1.05 ± 0.03[B,C]
LVEDD (mm)	3.37 ± 0.08	3.79 ± 0.19[A]	3.31± 0.06	3.66 ± 0.09
LVESD (mm)	1.87 ± 0.11	2.76 ± 0.28[A]	1.62 ± 0.12	2.01 ± 0.11[C]
FS(%)	45 ± 2	28 ± 4[A]	51 ± 3	45 ± 3[C]
Hemodynamics	**n=6**	**n=8**	**n=6**	**n=8**
ESP (mmHg)	110 ± 2	186 ± 7[A]	118 ± 6	206 ± 9[B]
EDP (mmHg)	1.8 ± 1.6	13.4 ± 3.5[A]	2.0 ± 0.7	2.5 ± 0.9[C]
dP/dT max (mmHg/sec)	11979 ± 753	8485 ± 538[A]	11450 ± 897	12144 ± 631[C]
dP/dT min (mmHg/sec)	-11380 ± 1101	-8340 ± 549[A]	-11038 ± 764	-11088 ± 654[C]
Tau_w	3.9 ± 0.9	8.5 ±1.6[A]	7.0 ± 1.9	4.2 ± 0.9[C]
PG(mmHg)		67.2 ± 8.8		76.4 ± 12.0

[A]*P*<0.05 verus Control diet/sham; [B]*P*<0.05 verus Fish oil diet/sham; [C]*P*<0.05 verus Control diet/TAC. All measurements are means ± SEM, and significance was determined using the two-way ANOVA with post tukey test. BW, body weight; HW, heart weight; IVSth, intraventricular septal thickness; LVPWth, left ventricular posterior wall thickness; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; FS, fractional shortening; HR, heart rate; ESP, end-systolic pressure; EDP, end-diastolic pressure; PG, pressure gradient.

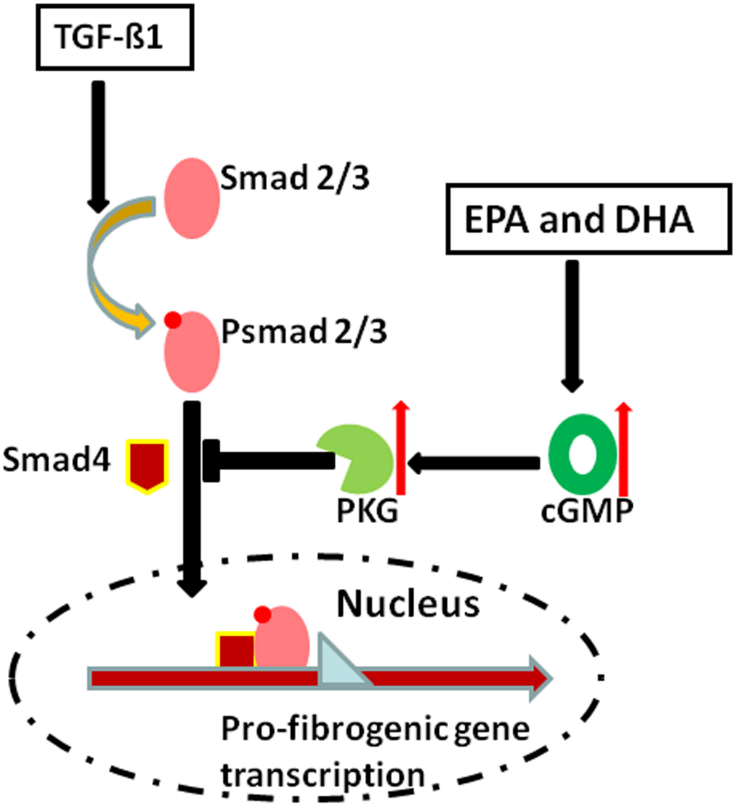
Supplemental figure 1

EPA and DHA reduce the TGF-β1-induced Smad-responsive promoter activity



Supplemental Figure 2.

Proposed anti-fibrotic mechanism of ω-3 PUFAs in cardiac fibroblast



Supplemental Figure 3.

EPA and DHA do not block TGF-β1-induced activation of ERK1/2