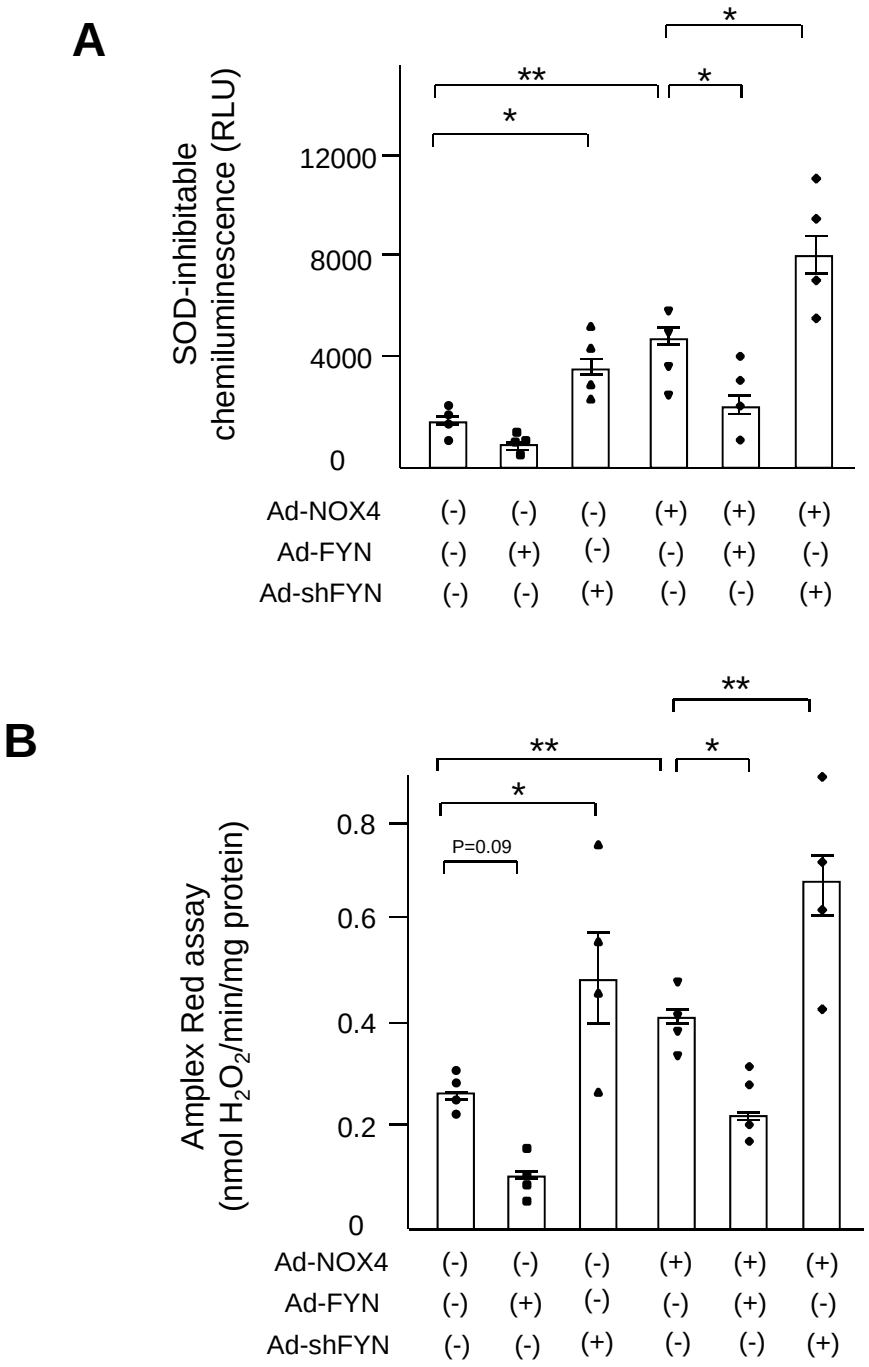
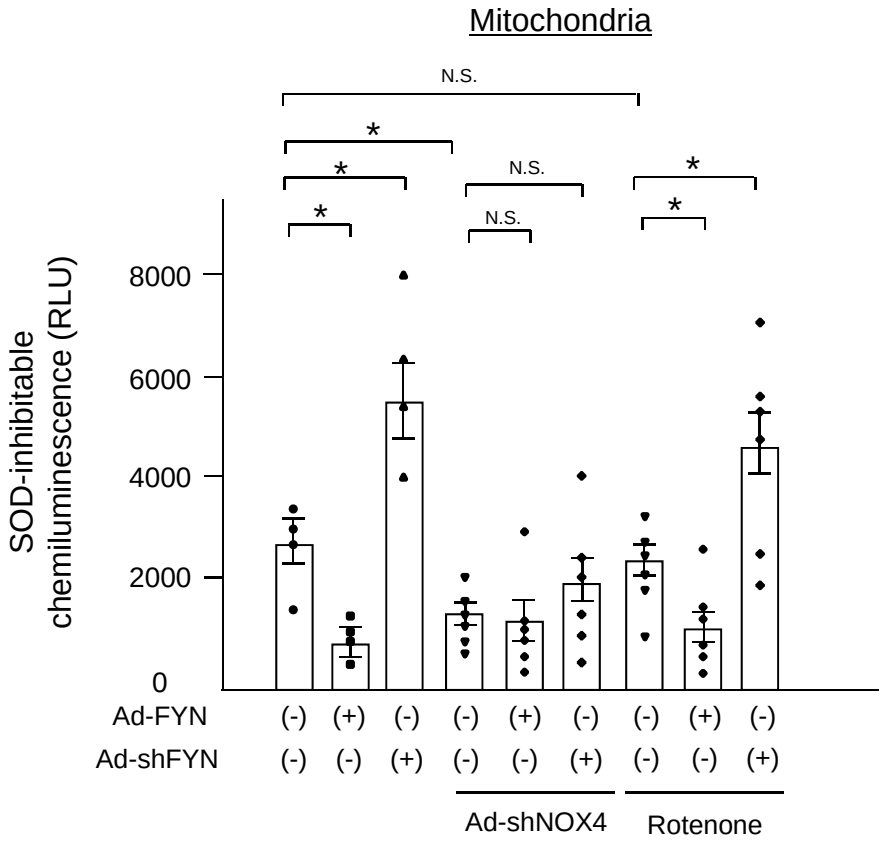
Supplemental Figure 1



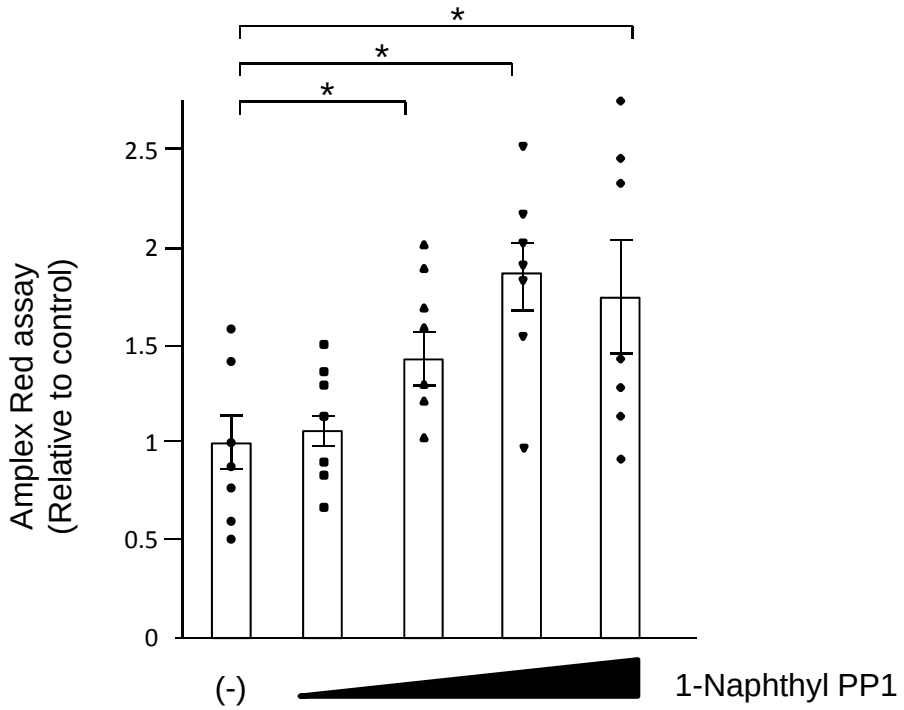
Supplemental Figure 1 A. NADH-dependent O_2^- release was measured by the lucigenin method. The SOD-inhibitable component of O_2^- release from the mitochondrial fraction in CMs transduced with indicated adenoviruses was determined (n=4). **B.** H_2O_2 content in mitochondria was measured by Amplex Red assay (n=4). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *****P<0.05, ******P<0.01.

Supplemental Figure 2



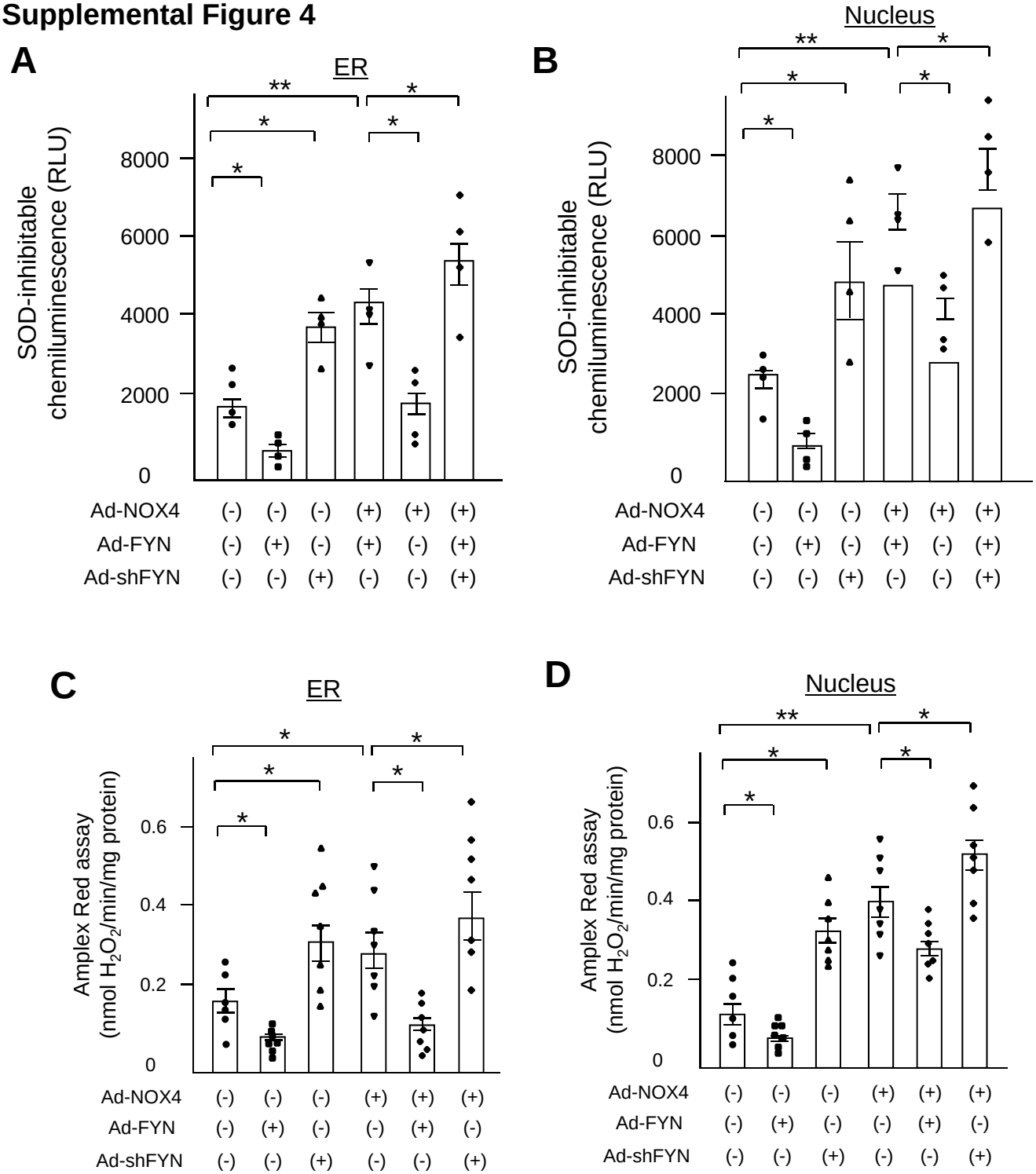
Supplemental Figure 2 NADPH-dependent O_2^- release was measured by the lucigenin method. The SOD-inhibitable component of O_2^- release from the mitochondrial fraction in CMs transduced with indicated adenoviruses was determined (n=4-6). Rotenone (10 nM) was added to mitochondrial fractions 5 min before the addition of NADPH. Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *P<0.05.

Supplemental Figure 3



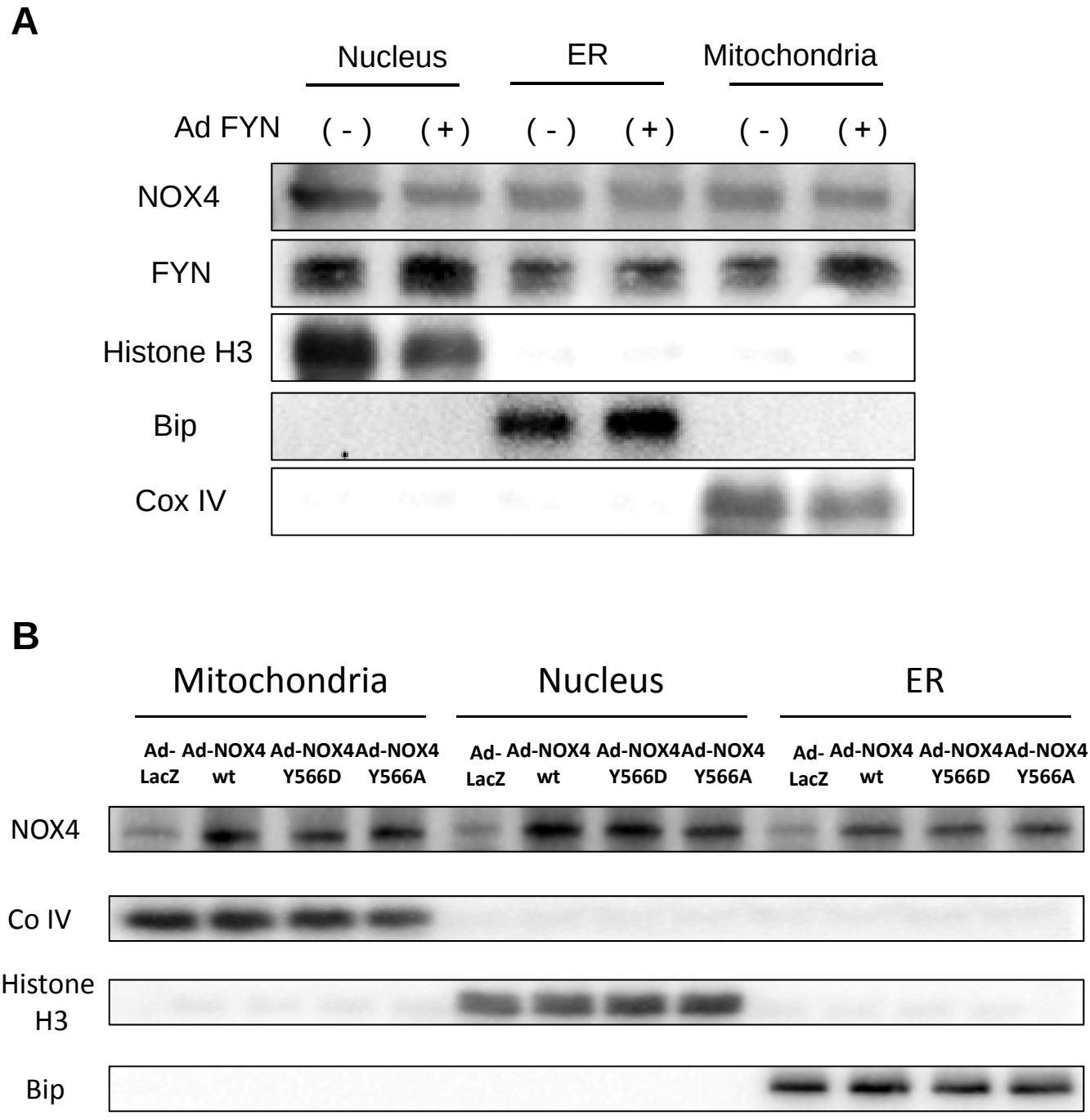
Supplemental Figure 3 Cardiomyocytes were treated with a Fyn inhibitor (1-Naphthyl PP1, 0, 0.3, 1, 3, and 10 μM) for 30 minutes. H_2O_2 production was examined with Amplex Red assays (n=7). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *****P<0.05.

Supplemental Figure 4



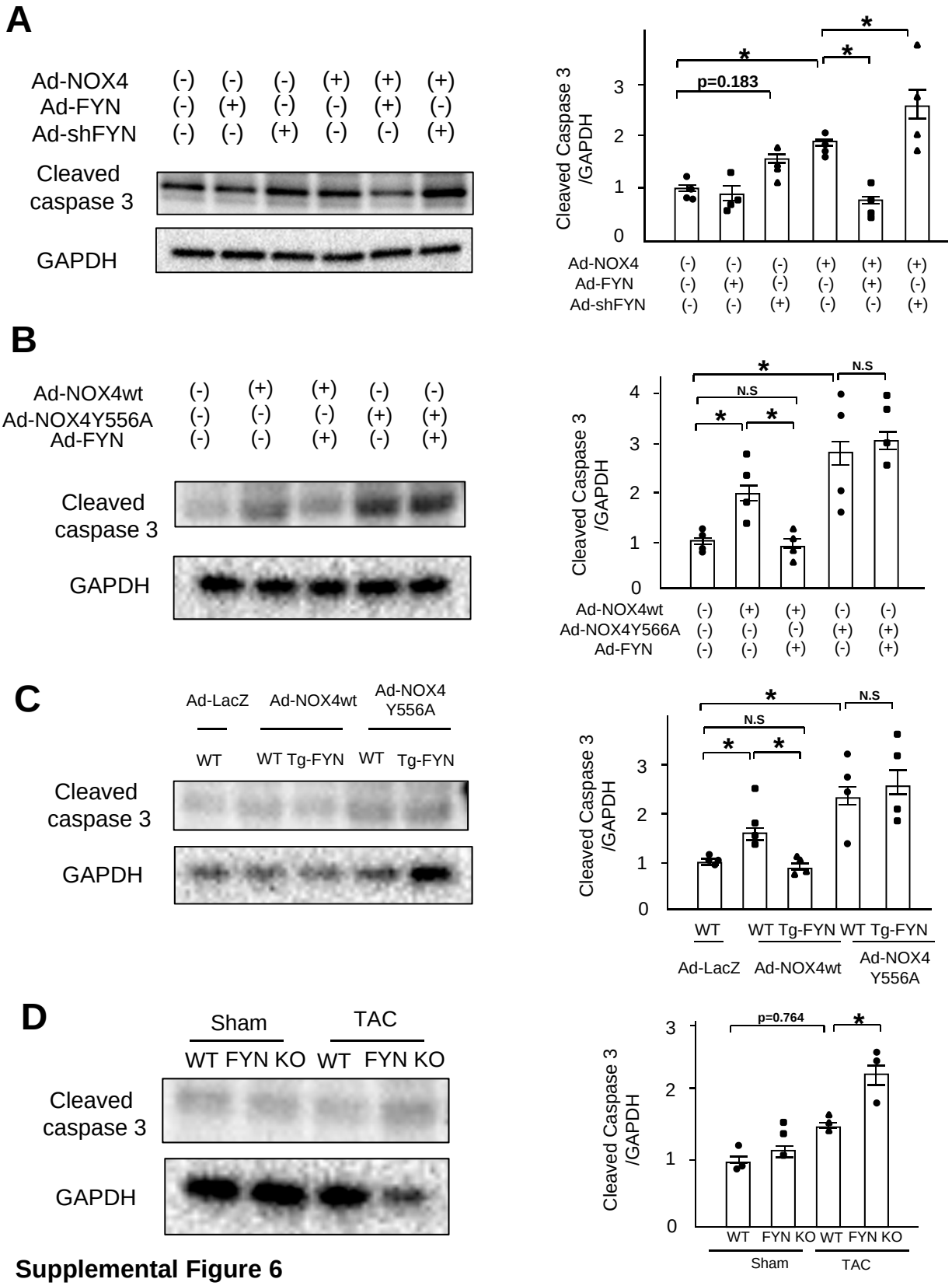
Supplemental Figure 4 NADPH-dependent O_2^- release was measured by the lucigenin method. **A, B.** The SOD-inhibitable component of O_2^- release from ER fraction (**A**) and nuclear fraction (**B**) in CMs transduced with indicated adenoviruses was determined (n=4). **C, D.** H_2O_2 content in ER fraction (**C**) and nuclear fraction (**D**) was measured by Amplex Red assay (n=6-7). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *****P<0.05, ******P<0.01

Supplemental Figure 5



Supplemental Figure 5 **A.** Protein levels of NOX4, FYN, Histone H3, Bip, and COXIV in nucleus, ER, and mitochondria in CMs transduced with indicated adenovirus were determined. The experiment was conducted 3 times. **B.** Protein levels of NOX4, Histone H3, Bip, and COXIV in nucleus, ER, and mitochondria in CMs transduced with indicated adenovirus were determined. The experiment was conducted 3 times.

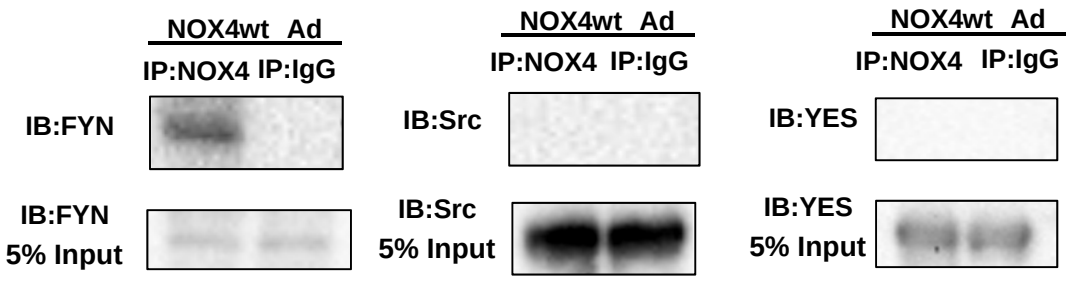
Supplemental Figure 6



Supplemental Figure 6

A, B. Protein levels of cleaved caspase3 and GAPDH in cultured neonatal rat CMs transduced with indicated adenoviruses. **C, D.** Protein levels of cleaved caspase3 and GAPDH in the heart from indicated mice. Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *****P<0.05

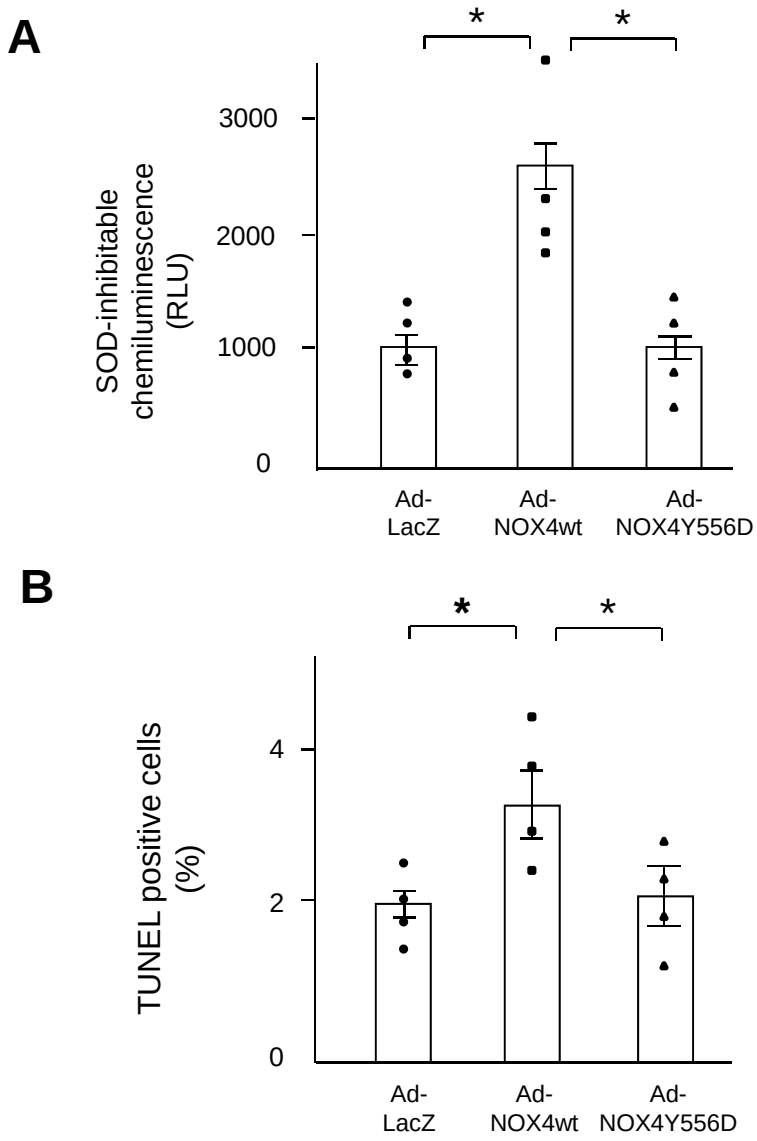
Supplemental Figure 7



Supplemental Figure 7

Co-immunoprecipitation assays using lysates of CMs transduced with Ad-NOX4wt. After immunoprecipitation (IP) with control IgG or anti-NOX4 antibody, immunoblotting for FYN, Src, or YES was performed. Immunoblots of input controls (5% lysates) are also shown. The experiment was conducted 3 times.

Supplemental Figure 8

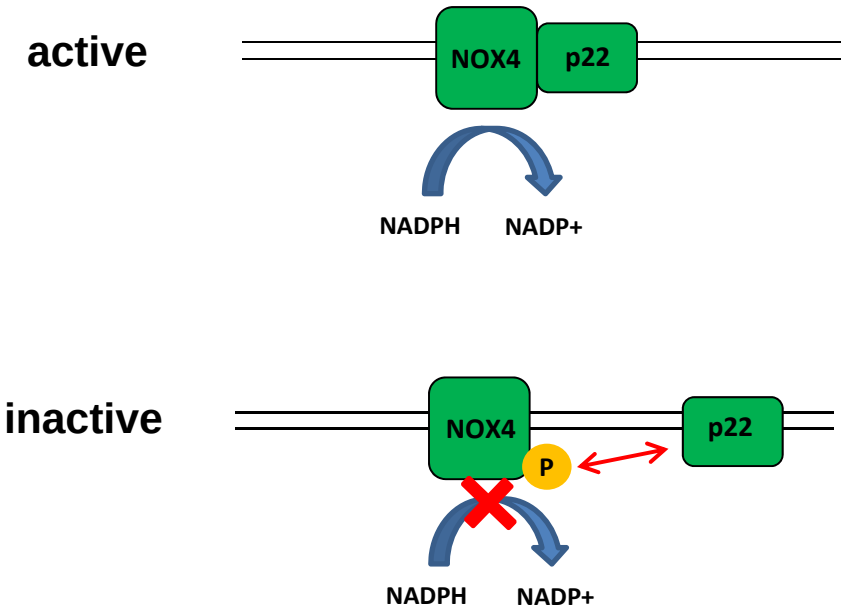


Supplemental Figure 8
A. NADPH-dependent O2- release was measured by the lucigenin method. The SOD-inhibitable component of O2- release from the mitochondrial fraction in CMs transduced with indicated adenoviruses was determined (n=4). **B.** Apoptosis in CMs transduced with indicated adenoviruses was evaluated with TUNEL staining (n=4). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *P<0.05

Supplemental Figure 9

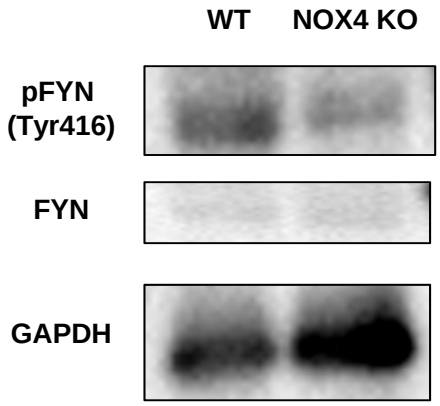
A

B



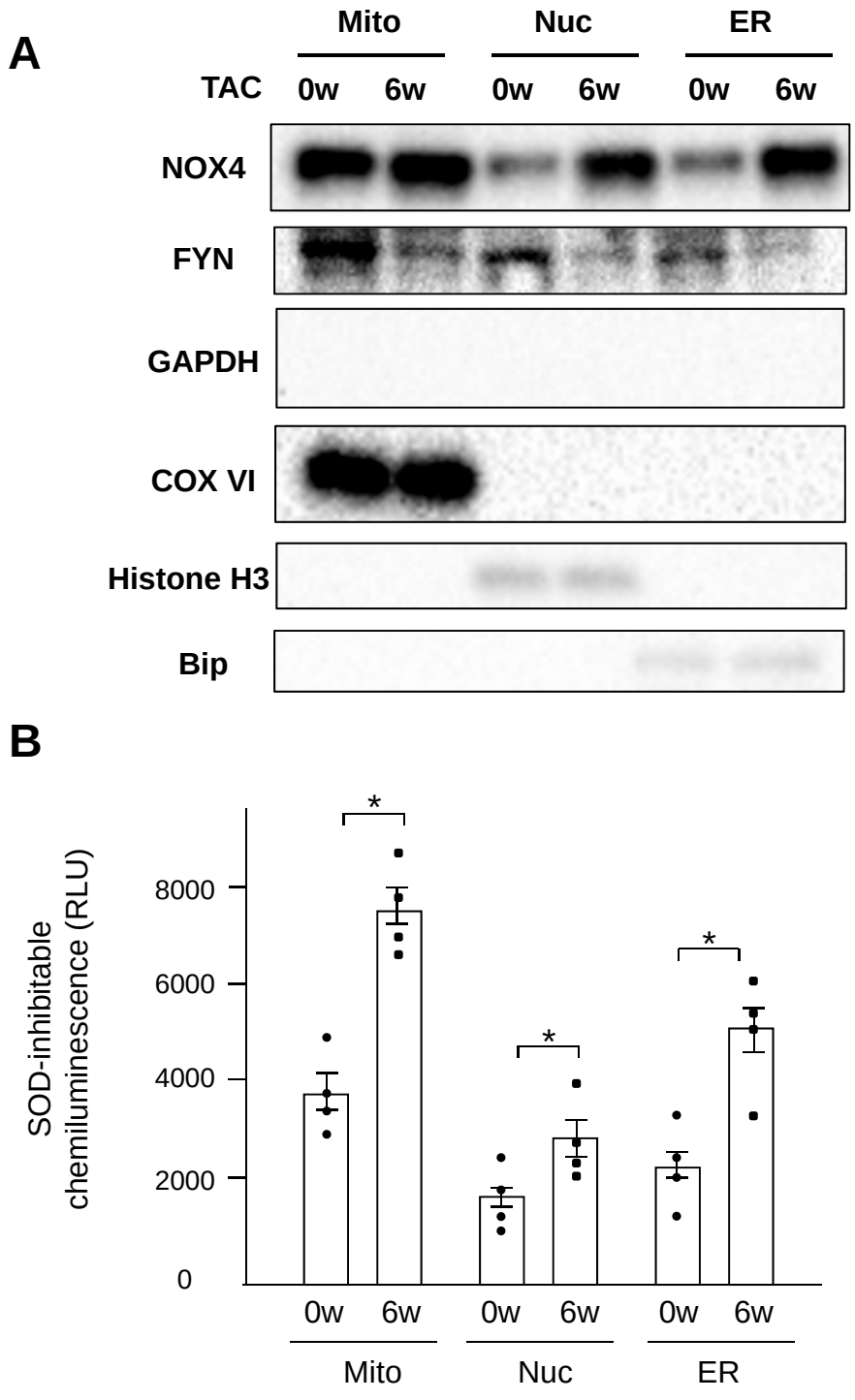
Supplemental Figure 9 p22^{phox} has negatively charged amino acids in its N-terminus. a. The N terminal amino acid sequence of p22^{phox}. **b.** A schematic representation of our hypothesis as to how Y566 phosphorylation of NOX4 inhibits Nox4.

Supplemental Figure 10



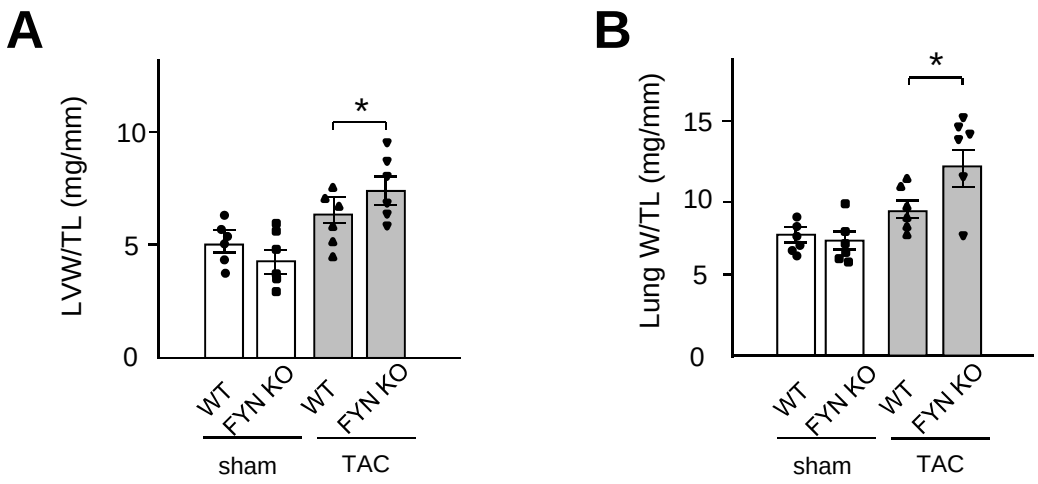
Supplemental Figure 10
phosphorylated FYN, total FYN, and GAPDH in LV after in WT and NOX4 KO mice. After immunoprecipitation (IP) with an anti-FYN antibody, immunoblot analyses (IB) for phospho-Src (S416) were performed to detect FYN phosphorylated at the tyrosine in the activation loop of the kinase domain (pFYN). The experiment was conducted 3 times.

Supplemental Figure 11



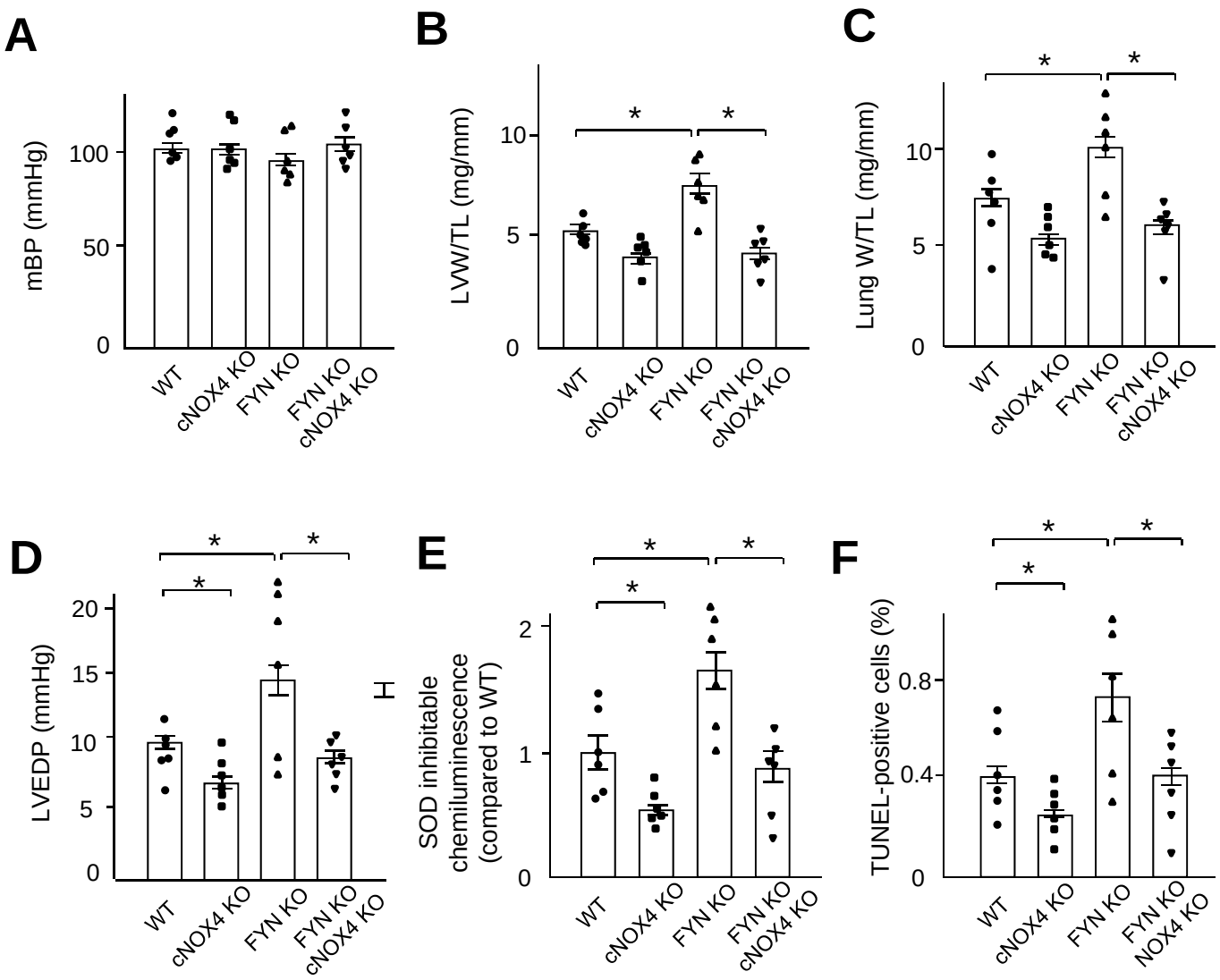
Supplemental Figure 11 A. Expression levels of NOX4, FYN, GAPDH, COX IV, Histone H3, and Bip in each fraction from the indicated mouse hearts 0 and 6 weeks after TAC operation. The experiment was conducted 3 times. **B.** NADPH-dependent and SOD-inhibitable O_2^- release in each fraction from the indicated mouse hearts was measured by the lucigenin method (n=4). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *****P<0.05.

Supplemental Figure 12



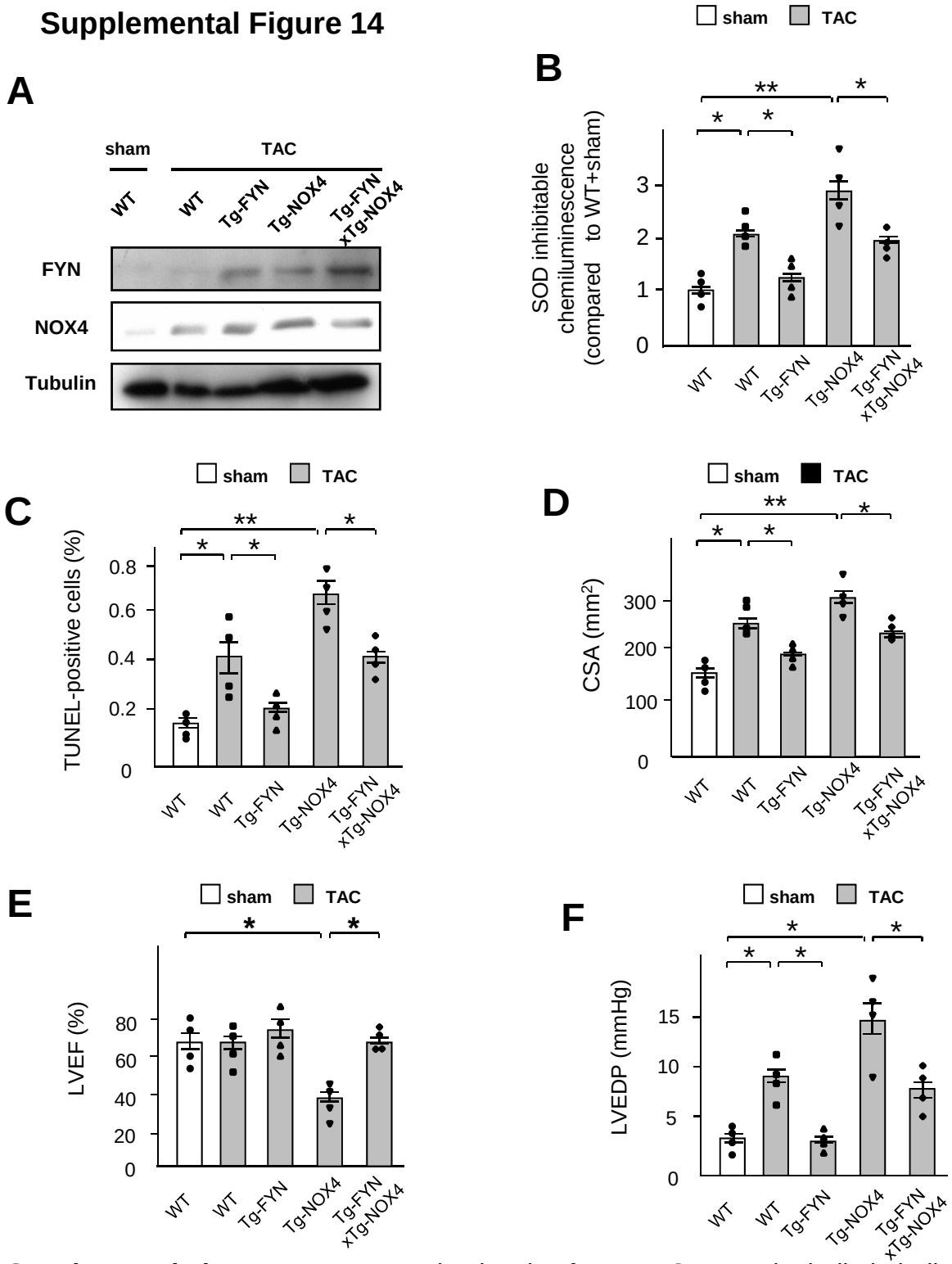
Supplemental Figure 12. Deletion of FYN promotes LV dysfunction and heart failure in response to pressure overload. A, B. LVW/TL, and Lung W/TL were determined in the indicated mice 2 weeks after TAC (n=6). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *P<0.05

Supplemental Figure 13



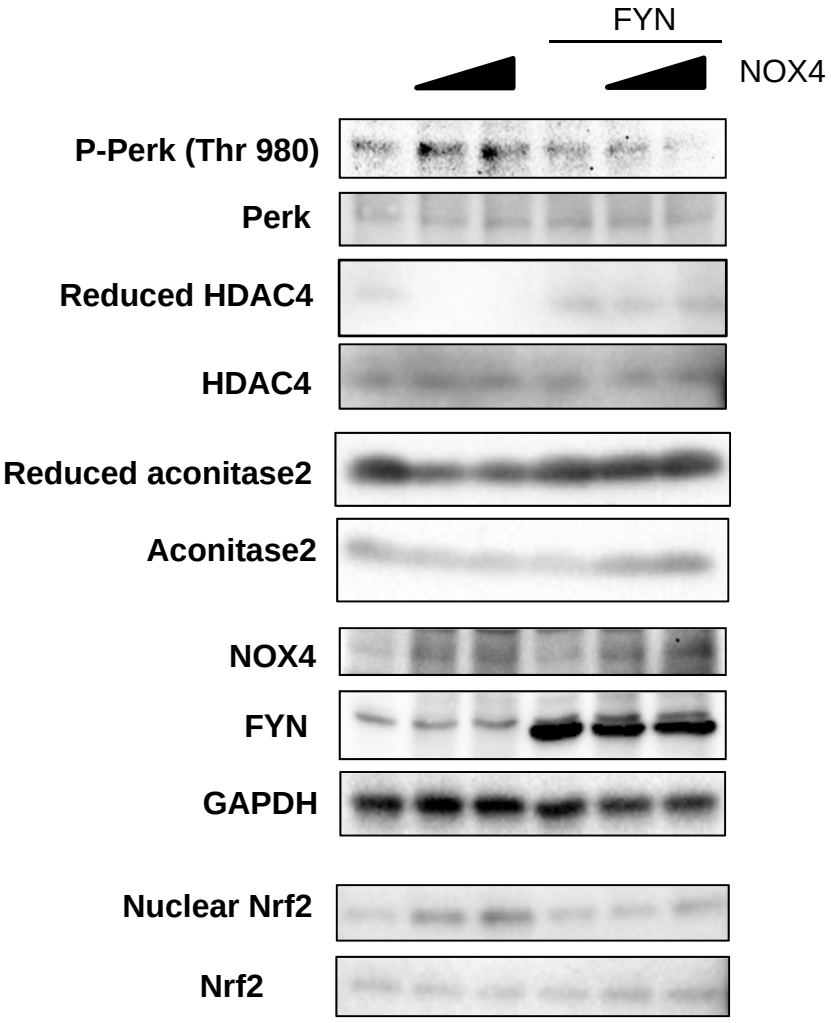
Supplemental Figure 13. Deletion of NOX4 rescues cardiac dysfunction and heart failure in FYN KO mice in response to PO. A, D. Mean BP and LV end-diastolic pressure were evaluated with Millar catheter in the indicated mice 2 weeks after TAC operation (n=6). **B, C.** LVW/TL and Lung W/TL were determined in the indicated mice 2 weeks after TAC operation (n=6). **E.** NADPH-dependent and SOD-inhibitable O_2^- release in mitochondrial fraction from the indicated mouse hearts was measured by the lucigenin method (n=6). **F.** Apoptosis in the indicated mouse hearts was evaluated with TUNEL staining (n=6). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test..
***P<0.05**

Supplemental Figure 14



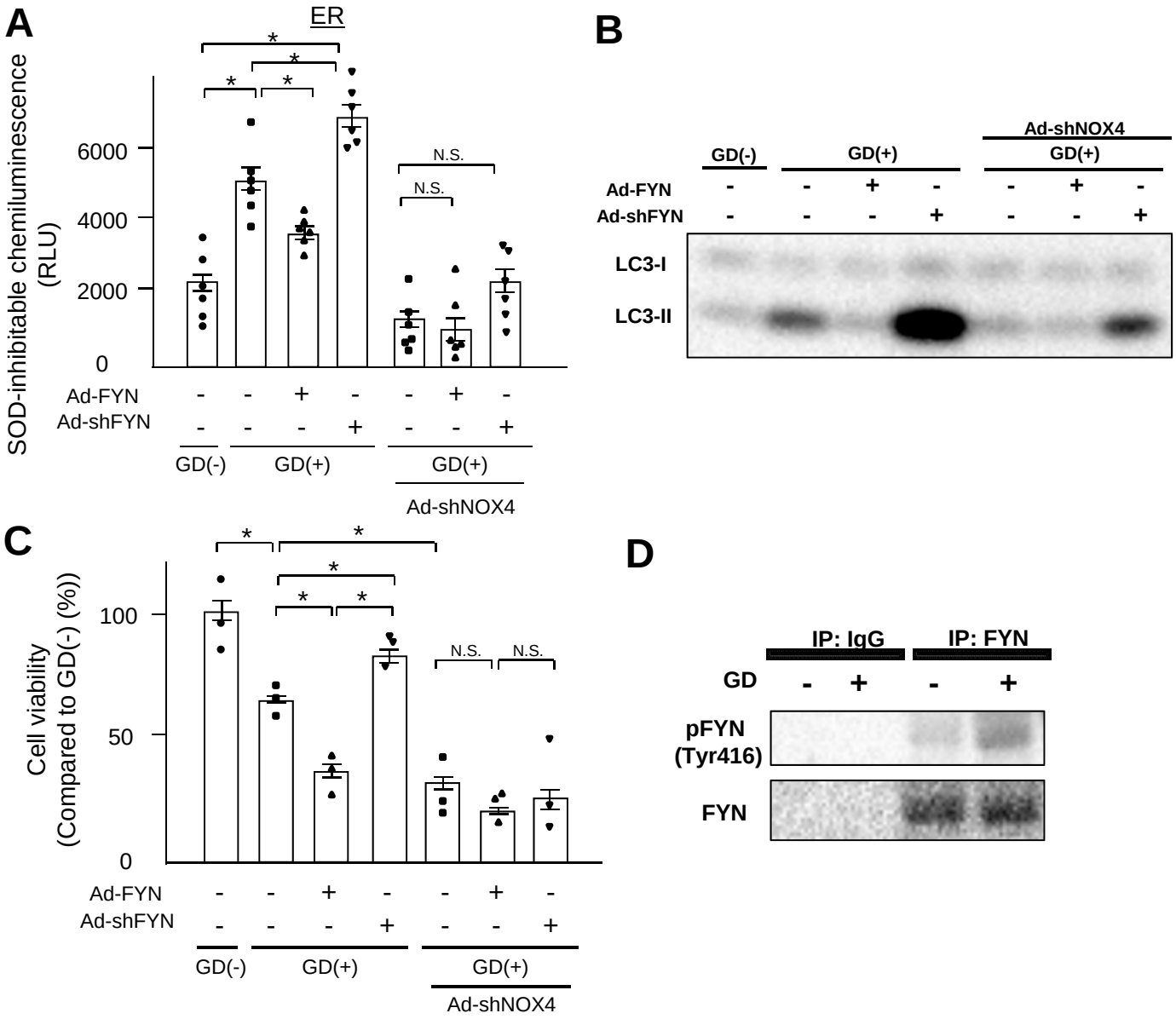
Supplemental Figure 14 A. Expression levels of FYN, NOX4, and tubulin in indicated mouse hearts subjected to either sham or TAC operation. The experiment was conducted 3 times. **B.** NADPH-dependent and SOD-inhibitable O_2^- release in mitochondrial fraction from the indicated mouse hearts was measured by the lucigenin method (n=4). **C.** Apoptosis in the indicated mouse hearts was evaluated with TUNEL staining (n=4). **D.** LV CM cross-sectional area (CSA) evaluated with wheat germ agglutinin staining (n=4). **E.** LVEF was evaluated with echocardiography (n=4). **F.** LVEDP was evaluated with a Millar catheter (n=4). Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *****P<0.05, ******P<0.01.

Supplemental Figure 15



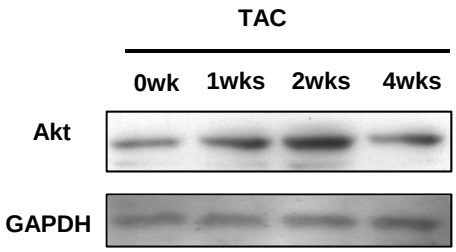
Supplemental Figure 15 Protein levels of phosphorylated Perk at Thr980, Perk, reduced HDAC4, HDAC4, reduced aconitase2, aconitase2, NOX4, FYN, GAPDH, nuclear Nrf2, and Nrf2 in CMs transduced with indicated adenoviruses was determined. The extent of cysteine reduction in HDAC4 and aconitase2 in CMs transduced with indicated adenoviruses was detected by iodoacetamide assay. The experiment was conducted 3 times.

Supplemental Figure 16



Supplemental Figure 16 A. The cells were pre-incubated with the indicated adenovirus for 48 hours and then the cells we cultured with normal or glucose-free medium for 4 hours. NADPH-dependent O_2^- release was measured by the lucigenin method. The SOD-inhibitable component of O_2^- release from the ER fraction in CMs transduced with indicated adenoviruses was determined (n=6). **B.** The cells were pre-incubated with the indicated adenovirus for 48 hours and then the cells were cultured with normal or glucose-free medium for 4 hours. Protein levels of LC3-I and LC3-II in cultured neonatal rat CMs transduced with indicated adenoviruses (n=3). The experiment was conducted 3 times. **C.** The cells were pre-incubated with the indicated adenovirus for 48 hours and then the cells we cultured with normal or glucose-free medium for 24 hours. Viable cell numbers were measured by CTB assays according to the supplier's protocol (n=8). **D**. The cells were cultured with normal or glucose-free medium for 4 hours. The expression levels of phosphorylated FYN and total FYN were evaluated with immunoblotting. After immunoprecipitation (IP) with an anti-Fyn antibody, immunoblot analyses (IB) for phospho-Src (S416) were performed to detect FYN phosphorylated at the tyrosine in the activation loop of the kinase domain (pFYN). GD, glucose deprivation. The experiment was conducted 3 times. Statistical analyses were done by one-way ANOVA followed by a post hoc Fisher's comparison test. *P<0.05.

Supplemental Figure 17



Supplemental Figure 17 Protein levels of Akt and GAPDH in LV after TAC at various time points. The experiment was conducted 3 times.

Supplemental Table 1

Echocardiographic data of WT and FYN KO mice 2 weeks after TAC operation

	WT+Sham n=6	FYN KO+Sham n=6	WT+TAC n=8	FYN KO+TAC n=8
HR, bpm	511±11	517±15	500±15	493±10
LVEDD, mm	3.5±0.1	3.5±0.1	3.2±0.09*[1]	4.0±0.09*[2]
LVESD, mm	2.2±0.1	2.1±0.2	1.8±0.2*[1]	3.3±0.1*[2]
LVEF, %	74.7±5.3	76.7±5.6	79.5±5.2	44.3±4.6*[2]
IVSWT, mm	0.73±0.02	0.74±0.03	1.08±0.04*[1]	1.25±0.05*[2]
PWT, mm	0.72±0.03	0.73±0.03	1.06±0.06*[1]	1.20±0.07*[2]

HR, heart rate; LV, left ventricle; EDD, end-diastolic dimension; ESD, end-systolic dimension; EF, ejection fraction; EDP, end-diastolic pressure; IVSWT, interventricular septal wall thickness; PWT, posterior wall thickness.
Data are mean ± SEM, *[1] P<0.05 vs WT+Sham, *[2] P<0.05 vs WT+TAC.

Supplemental Table 2

Hemodynamic analyses of WT and FYN KO mice 2 weeks after TAC operation

	WT+Sham n=6	FYN KO+Sham n=6	WT+TAC n=8	FYN KO+TAC n=8
HR, bpm	497±18	503±19	494±17	481±18
sBP, mmHg	99±4	100±4	161±6*[1]	156±6*[1]
dBP, mmHg	67±3	64±4	71±5	70±4
mBP, mmHg	77±2	76±3	101±4*[1]	99±4*[1]
Peak LVP, mmHg	102±3	99±4	157±8*[1]	160±5*[1]
LVEDP, mmHg	2.6±0.7	3.0±0.7	9.9±1.0*[1]	15.3±1.9*[2]
+dP/dt, mmHg/s	6733±402	7033±578	7663±566	4825±432*[2]
-dP/dt , mmHg/s	6517±423	6467±786	4875±275*[1]	3625±339*[2]

HR, heart rate; sBP, systolic blood pressure; dBP, diastolic blood pressure; mBP, mean blood pressure; LV, left ventricle; EDP, end-diastolic pressure. Data are mean ± SEM, *[1] P<0.05 vs WT+Sham, *[2] P<0.05 vs WT+TAC.

Supplemental Table 3

Echocardiographic data of WT, cNOX4 KO, FYN KO, and double KO mice 2 weeks after TAC operation

	WT+TAC n=6	cNOX4 KO+TAC n=6	FYN KO+TAC n=6	DKO+TAC n=6
HR, bpm	501±16	489±15	531±20	496±14
LVEDD, mm	3.3±0.06	3.2±0.2	3.8±0.1*[1]	3.4±0.1*[2]
LVESD, mm	2.0±0.1	2.0±0.2	3.1±0.1*[1]	2.3±0.1*[2]
LVEF, %	75.5±4.0	76.7±3.1	44.6±3.6*[1]	68.0±4.0*[2]
IVSWT, mm	1.06±0.04	0.9±0.04*[1]	1.20±0.06*[1]	1.06±0.06*[2]
PWT, mm	0.98±0.12	0.77±0.03*[1]	1.18±0.08 *[1]	1.02±0.06*[2]

DKO, double knock-out (FYNKO-cNOX4KO); HR, heart rate; LV, left ventricle; EDD, end-diastolic dimension; ESD, end-systolic dimension; EF, ejection fraction; EDP, end-diastolic pressure; IVSWT, interventricular septal wall thickness; PWT, posterior wall thickness. Data are mean ± SEM, *[1] P<0.05 vs WT+TAC, *[2] P<0.05 vs FYN KO+TAC.

Supplemental Table 4

Hemodynamic analyses of WT, cNOX4 KO, FYN KO, and double KO mice 2 weeks after TAC operation

	WT+TAC n=6	cNOX4 KO+TAC n=6	FYN KO+TAC n=6	DKO+TAC n=6
HR, bpm	489±26	473±25	463±29	504±18
sBP , mmHg	158±5	163±7	157±5	166±6
dBP, mmHg	72±5	70±3	67±4	75±6
mBP, mmHg	100±2	101±3	98±3	105±5
Peak LVP, mmHg	163±6	157±8	162±10	160±8
LVEDP, mmHg	9.5±0.9	7.1±0.7*[1]	16.2±2.6*[1]	8.7±0.8*[2]
+dP/dt, mmHg/s	7717±620	8133±392	4650±367*[1]	6717±410*[2]
-dP/dt, mmHg/s	4683±304	5883±295*[1]	3383±386*[1]	4717±289*[2]

DKO, double knockout; HR, heart rate; sBP, systolic blood pressure; dBP, diastolic blood pressure; mBP, mean blood pressure; LV, left ventricle; EDP, end-diastolic pressure.

Data are mean ± SEM, *[1] P<0.05 vs WT+TAC, *[2] P<0.05 vs FYN KO+TAC.

Supplemental Table 5

Echocardiographic data of WT, Tg-NOX4, Tg-FYN and bigenic mice 2 weeks after TAC operation

	WT+Sham n=4	WT+TAC n=4	Tg-FYN+TAC n=4	Tg-NOX4+TAC n=4	Bigenic+TAC n=4
HR, bpm	497±25	496±23	551±31	522±15	506±14
LVEDD, mm	3.4±0.09	3.1±0.1	3.4±0.1	3.9±0.1*2	3.5±0.09*3
LVESD, mm	2.3±0.2	2.1±0.2	2.1±0.2	3.2±0.2*2	2.3±0.1*3
LVEF, %	68.3±6.3	66.8±5.0	73.5±6.1	43.5±4.6*2	70.8±3.7*3
IVSWT, mm	0.74±0.04	1.15±0.06*1	0.88±0.08*2	1.30±0.11*2	1.12±0.07*3
PWT, mm	0.73±0.03	1.20±0.04*1	0.89±0.05*2	1.35±0.06*2	1.10±0.07*3

HR, heart rate; LV, left ventricle; EDD, end-diastolic dimension; ESD, end-systolic dimension; EF, ejection fraction; EDP, end-diastolic pressure; IVSWT, interventricular septal wall thickness; PWT, posterior wall thickness. Data are mean ± SEM, *1 P<0.05 vs WT+Sham, *2 P<0.05 vs WT+TAC, *3 P<0.05 vs Tg-NOX4+TAC.

Supplemental Table 6

Hemodynamic analyses of WT, Tg-NOX4, Tg-FYN and bigenic mice 2 weeks after TAC operation

	WT+Sham n=4	WT+TAC n=4	Tg-FYN+TAC n=4	Tg-NOX4+TAC n=4	Bigenic+TAC n=4
HR, bpm	478±10	505±13	493±32	459±37	495±26
sBP, mmHg	97±7	154±5*[1]	151±11*[1]	146±9*[1]	150±6*[1]
dBP, mmHg	68±5	76±4	75±3	75±3	72±7
mBP, mmHg	79±3	102±4*[1]	101±5*[1]	99±5*[1]	98±4*[1]
Peak LVP, mmHg	99±3	159±9*[1]	154±9*[1]	151±11*[1]	164±5*[1]
LVEDP, mmHg	3.3±0.3	9.2±1.3*[1]	3.3±0.3*[2]	14.0±2.4*[2]	8.3±0.9*[3]
+dP/dt, mmHg/s	8425±649	6925±660	7525±390	3513±401*[2]	6550±585*[3]
-dP/dt, mmHg/s	6425±622	4350±340*[1]	6025±487*[2]	3263±626*[2]	5325±309*[3]

HR, heart rate; sBP, systolic blood pressure; dBP, diastolic blood pressure; mBP, mean blood pressure; LV, left ventricle; EDP, end-diastolic pressure. Data are mean ± SEM, *[1] P<0.05 vs WT+Sham, *[2] P<0.05 vs WT+TAC, *[3] P<0.05 vs Tg-NOX4+TAC.