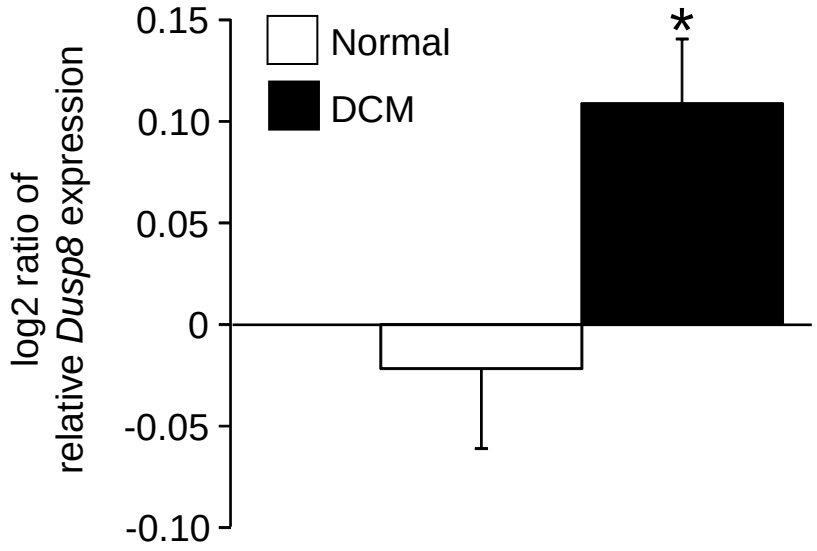
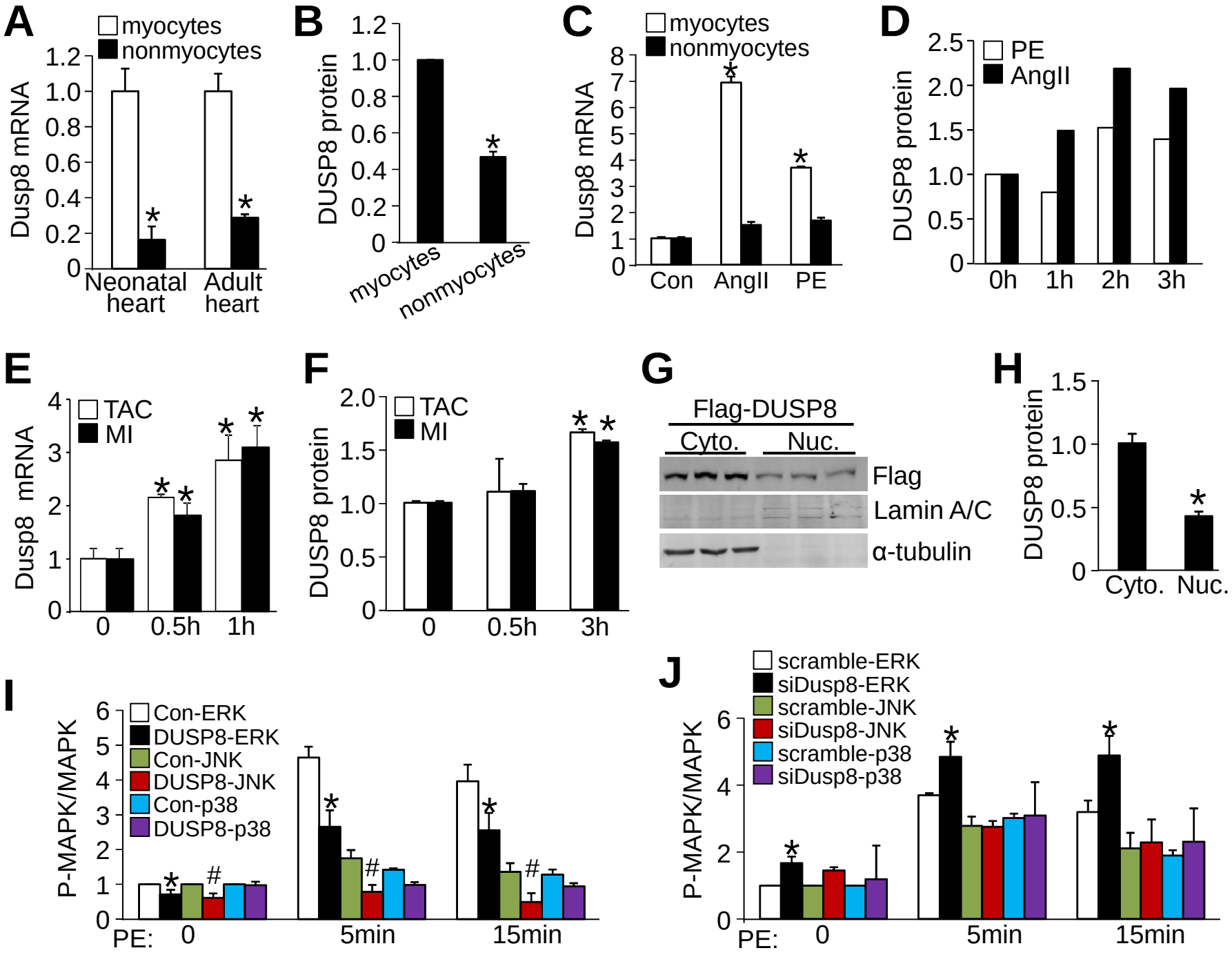
Online Figures and Online Tables for:

DUSP8 regulates cardiac ventricular remodeling by altering ERK1/2 signaling

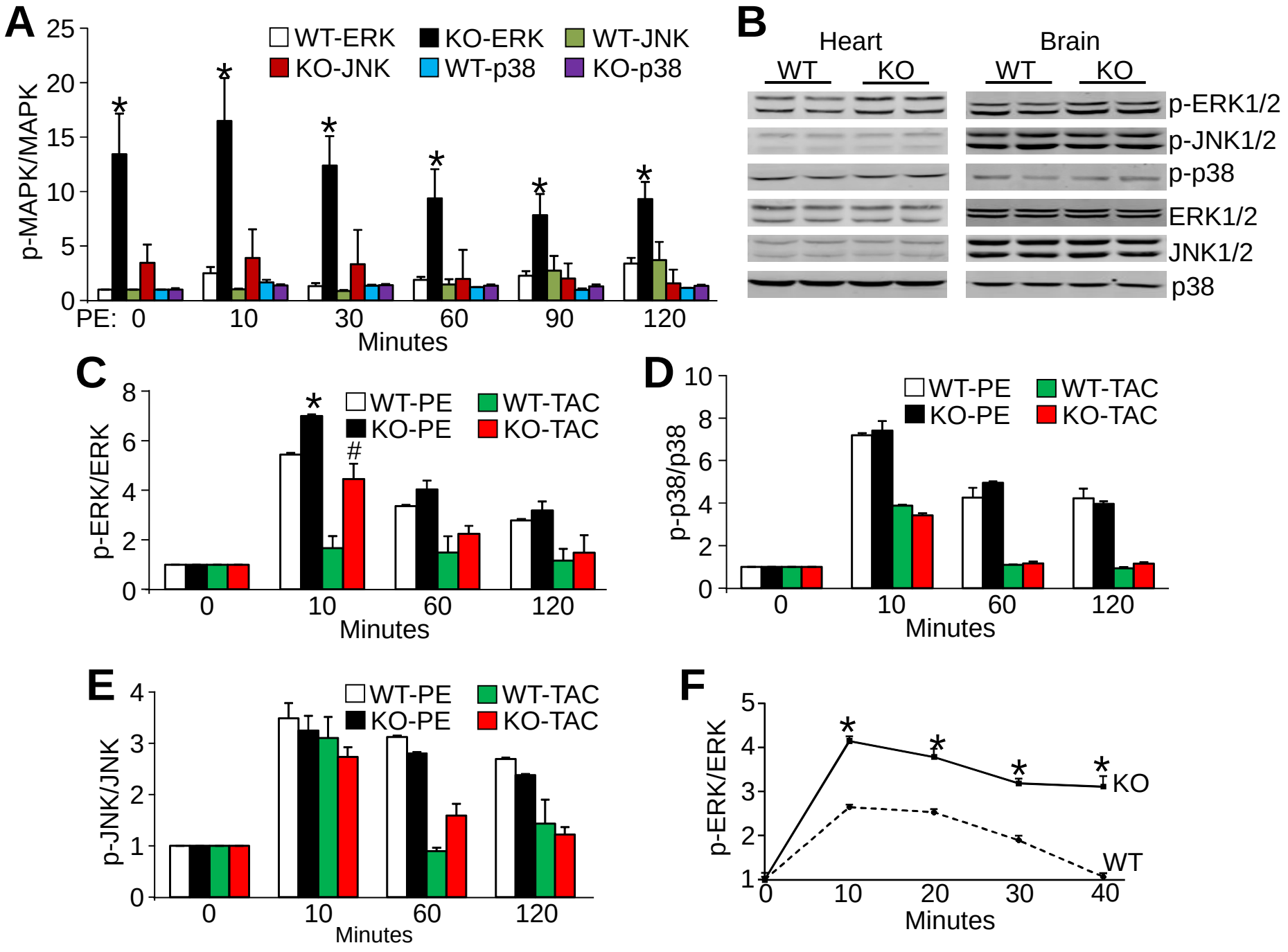
Ruijie Liu, Jop H. van Berlo, Allen J. York, Marjorie Maillet, Ronald J. Vagnozzi, Jeffery D. Molkentin



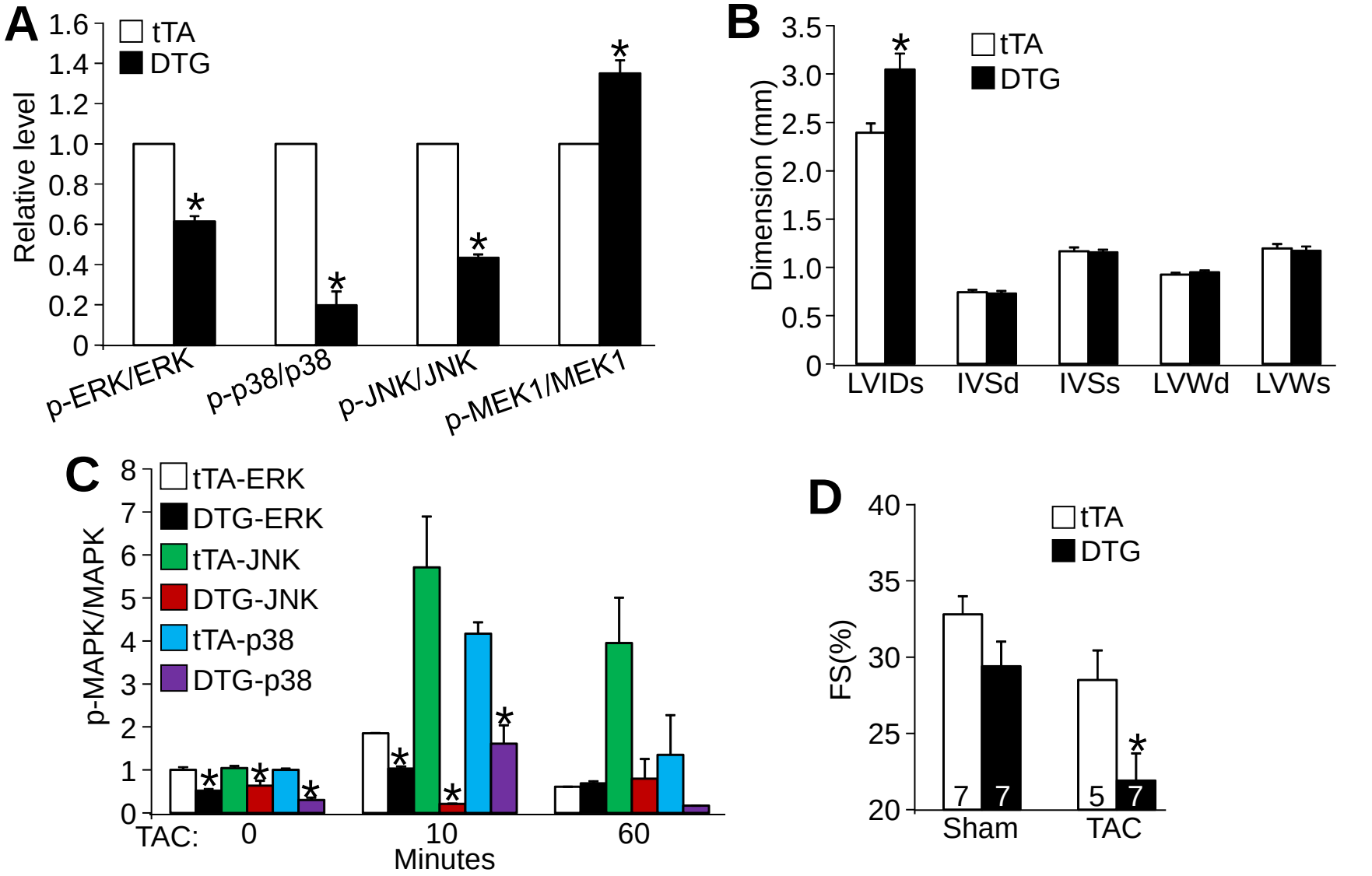
Online Figure I. *Dusp8* mRNA is highly expressed in human dilated cardiomyopathy patients. Data in the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) database contained an elaborate microarray experiment from human heart biopsies (GEO accession GDS2206, reference #32) that showed an increase in *Dusp8* mRNA in patients with dilated cardiomyopathy (DCM). Available data included 15 normal hearts samples and 13 dilated cardiomyopathy samples examined with custom made microarrays to assess mRNA levels. *p<0.05 vs normal.


A
myocytes
nonmyocytes
Dusp8 mRNA
Neonatal heart
Adult heart
B
DUSP8 protein
myocytes
nonmyocytes
C
myocytes
nonmyocytes
Dusp8 mRNA
Con
AngII
PE
D
PE
AngII
DUSP8 protein
0h
1h
2h
3h
E
TAC
MI
Dusp8 mRNA
0
0.5h
1h
F
TAC
MI
DUSP8 protein
0
0.5h
3h
G
Flag-DUSP8
Cyto.
Nuc.
Flag
Lamin A/C
α-tubulin
H
DUSP8 protein
Cyto.
Nuc.
I
Con-ERK
DUSP8-ERK
Con-JNK
DUSP8-JNK
Con-p38
DUSP8-p38
P-MAPK/MAPK
PE:
0
5min
15min
J
scramble-ERK
siDusp8-ERK
scramble-JNK
siDusp8-JNK
scramble-p38
siDusp8-p38
P-MAPK/MAPK
PE:
0
5min
15min

Online Figure II. Expression and localization of DUSP8, and its effect on MAPK signaling. **A**, Real-time PCR analysis of *Dusp8* mRNA expression in myocytes and nonmyocytes isolated from neonatal or adult hearts. * $p<0.05$ vs myocytes. N=4 for each group. **B**, Quantification of DUSP8 protein expression from experiments as shown in Figure 1A. **C**, Real-time PCR analysis of *Dusp8* expression in myocytes and nonmyocytes following AngII (0.1 μmol/L) or PE (10 μmol/L) stimulation for 4 hours. *$p<0.05$ vs control (Con) myocytes. **D**, Quantification of DUSP8 protein induction in response to AngII or PE as shown in Figure. 1B. **E**, Real-time PCR analysis of *Dusp8* mRNA expression in the hearts of mice following TAC or MI for the indicated time periods. *$p<0.05$ vs 0 min sham control for TAC or MI. **F**, Quantification of DUSP8 protein induction in response to TAC or MI as shown in Figure 1C. * $p<0.05$ vs 0 min sham control for TAC or MI. **G**, **H**, Western blot and quantitation of subcellular protein fractions for localization of Flag-DUSP8 in cytoplasm versus nucleus of HEK293 cells transfected with a Flag-*Dusp8* DNA expression plasmid. Lamin A/C and α-tubulin were used as loading controls for nucleus and cytoplasm, respectively. Cyto., cytoplasm; Nuc., nucleus. **I**, Quantification of the indicated phospho-MAPK and total MAPK levels from at least 3 independent experiments as shown in Figure 1D. *$p<0.05$ vs control ERK (Adβgal infected); #$p<0.05$ versus control JNK. **J**, Quantification of the indicated phospho-MAPK levels from at least 3 independent experiments as shown in Figure 1E. *$p<0.05$ vs scramble-ERK.


A
p-MAPK/MAPK
WT-ERK
KO-ERK
WT-JNK
KO-JNK
WT-p38
KO-p38
PE: 0 10 30 60 90 120
Minutes
B
Heart
Brain
WT
KO
WT
KO
p-ERK1/2
p-JNK1/2
p-p38
ERK1/2
JNK1/2
p38
C
p-ERK/ERK
WT-PE
KO-PE
WT-TAC
KO-TAC
Minutes
D
p-p38/p38
WT-PE
KO-PE
WT-TAC
KO-TAC
Minutes
E
p-JNK/JNK
WT-PE
KO-PE
WT-TAC
KO-TAC
Minutes
F
p-ERK/ERK
KO
WT
Minutes

Online Figure III. DUSP8 preferentially dephosphorylates ERK1/2 in vivo. A, Quantitative analysis of phosphorylated MAPKs relative to total MAPKs in *Dusp8* WT and KO MEFs following PE (10 µmol/L) stimulation for indicated time shown in Figure 3A. Data are representative of at least three independent experiments. *p<0.05 vs WT ERK for each time point. **B**, Western blot analysis of MAPK phosphorylation and total MAPK levels in hearts and brains of 2 month-old *Dusp8* WT and KO mice. **C-E**, Quantitative analysis of phosphorylated MAPKs relative to total MAPKs in *Dusp8* WT and KO hearts following PE or TAC stimulation shown in Figure 3C and 3D. N=4 for each group. *p<0.05 vs WT PE; #p<0.05 vs WT TAC. **F**, Quantitative analysis of phosphorylated ERK/total ERK shown in Figure 3E. At least 3 samples were quantified from each group at each time point. *p<0.05 vs WT.



Online Figure IV. Cardiac overexpression of DUSP8 leads to reduced MAPK activity and impaired cardiac function. A, Quantitative analysis of phosphorylated MAPKs relative to total MAPKs in tTA and DTG mice shown in Figure 6D. At least 3 independent heart samples were processed in each group. *p<0.05 vs tTA. **B**, Echocardiographic parameters of hearts from tTA and DTG mice, 6 weeks after Dox removal to induce DUSP8 expression. Abbreviations: LVIDs, left ventricular end-systolic chamber diameter; IVSd and IVSs, intraventricular end-diastolic and end-systolic septal thickness; LVWd and LVWs, left ventricular end-diastolic and end-systolic posterior wall thickness. Five mice were analyzed in each group. * p<0.05 vs tTA. **C**. Quantitative analysis of phosphorylated MAPKs relative to total MAPKs in hearts from tTA and DTG mice following TAC stimulation for the indicated times as shown in Figure 6K. At least 3 independent samples were processed for each. *p<0.05 vs tTA for each respective MAPK following TAC. **D**, Echocardiography assessment of fractional shortening percentage (FS%) from hearts of tTA and DTG mice after 2 weeks of TAC or a sham procedure. Number of mice assessed is shown in the graph. Mice were 9 weeks-old, with 6 weeks no Dox. *p<0.05 vs tTA TAC.

Online Table I. Echocardiographic parameters in *Dusp8* WT and KO mice at baseline

	WT	KO	P value
2 months	N=16	N=12	
LVIDd(mm)	3.62±0.09	3.43±0.06*	0.009
LVIDs(mm)	2.49±0.10	2.43±0.09	0.65
LVWd(mm)	0.92±0.03	0.89±0.02	0.41
LVWs(mm)	1.09±0.03	1.13±0.02	0.28
IVSs(mm)	1.09±0.03	1.23±0.03*	0.001
HR(bpm)	557.32±16.84	541.86±7.09	0.42
6 months	N=12	N=14	
LVIDd(mm)	3.76±0.04	3.48±0.08*	0.04
LVIDs(mm)	2.71±0.08	2.61±0.07	0.47
LVWd(mm)	0.96±0.02	0.93±0.01	0.08
LVWs(mm)	1.15±0.03	1.15±0.02	0.36
IVSs(mm)	1.15±0.03	1.28±0.02*	0.007
HR(bpm)	473.62±11.13	441.56±22.01	0.46

Data are presented in millimeters (mm) as mean±SE. LVIDd and LVIDs: left ventricular end-diastolic and end-systolic chamber diameters; LVWd and LVWs: left ventricular end-diastolic and end-systolic posterior wall thickness; IVSs: end-systolic intraventricular septal thickness. HR: heart rate, which is given in beats per minute (bpm). *$p<0.05$ vs WT.

Online Table II. Echocardiographic parameters in *Dusp8* WT and KO mice after 2 weeks of surgery.

	Sham	AngII/PE	Sham	TAC
WT	N=6	N=8	N=6	N=10
LVIDd(mm)	3.72±0.08	3.99±0.09	3.74±0.07	3.91±0.03
LVIDs(mm)	2.91±0.07	2.88±0.13	2.82±0.05	2.73±0.08
LVWd(mm)	0.92±0.02	0.89±0.03	0.91±0.02	1.00±0.03
LVWs(mm)	1.08±0.03	1.07±0.04	1.16±0.01	1.18±0.03
IVSd(mm)	0.70±0.02	1.07±0.04	0.72±0.01	0.82±0.03
IVSs(mm)	1.09±0.04	1.19±0.04	1.03±0.03	1.21±0.05
HR(bpm)	476±13	503±24	468±33	472±11
KO	N=6	N=8	N=10	N=8
LVIDd(mm)	3.47±0.07#	3.65±0.11#	3.40±0.10*	3.48±0.09*
LVIDs(mm)	2.62±0.06#	2.43±0.08#	2.62±0.06*	2.38±0.07*
LVWd(mm)	0.89±0.02	0.88±0.04	0.93±0.07	1.01±0.02
LVWs(mm)	1.13±0.02	1.16±0.06	1.12±0.06	1.22±0.05
IVSd(mm)	0.80±0.02#	1.16±0.06#	0.85±0.05*	0.93±0.02*
IVSs(mm)	1.23±0.03#	1.14±0.03	1.12±0.03*	1.32±0.03*
HR(bpm)	436±21	476±20	471±20	446±16

Data are presented as millimeters (mm) as the mean±SE. LVIDd and LVIDs: left ventricular end-diastolic and end-systolic chamber diameters; IVSd and IVSs: end-diastolic and end-systolic intraventricular septal thickness. LVWd and LVWs, left ventricular end-diastolic and end-systolic posterior wall thickness. HR: heart rate, which is given in beats per minute (bpm). #$p<0.05$ vs WT Sham-AngII/PE; *$p<0.05$ vs WT Sham-TAC.