Potential Role of miRNAs in Pediatric HF

Analysis of potential miRNA targets was based on changes in miRNA expression shown in Figure 1B. Differences in fold change are consistent with changes observed in adult miRNA expression [1].Interestingly, miR-133a does not change in response to treatment, but expression of miR-133b is increased in PDEi-treated patients. It is likely that miR-133a and miR-133b have similar targets since they have identical seed sequences. Up-regulation of miR-133a is protective in the setting of cardiac pathology, preventing the transition to a dilated phenotype [2]. It is possible that up-regulation of miR-133b in PDEi-treated patients correlates with a beneficial response to treatment. miR-125a/b is induced in early hypertrophic growth [3], and although expression of this miRNA was not significantly increased by array, it was increased by RT-PCR in HF patients. Expression of miR-27b is also increased in PDEi-treated patients. An increase in miR-27b expression results in increased levels of β-myosin heavy chain (MyHC) [4], but it is also anti-hypertrophic in cardiomyocytes [5]. Expression levels of miR-204 were increased in PDEi-treated patients. Down-regulation of miR-204 in rats is associated with autophagy [6], and up-regulation of miR-204 reduces disease severity in pulmonary hypertension [7], and may correlate with the beneficial response to treatment in PDEi-treated IDC patients. Conversely to what was observed by array, miR-146a was up-regulated in PDEi-treated patients. Up-regulation of miR-146a is mediated by IL-1β, and miR-146a is involved in orchestrating inflammation [8]. This suggests that inflammation may be an important aspect of pediatric IDC. Over-expression of miR-195 is involved in cardiac hypertrophy and failure [2]. Although expression of this miRNA was not significantly increased by array, it was by RT-PCR, and it may be involved in the

hypertrophic response. Although expression of miR-7 and miR-223 changed significantly by array, this was not observed by RT-PCR. Expression of miR-7, miR-223 and miR-146a was very low by array which may have prevented an accurate measurement. Expression of miR-21 consistently changes in adult human and animal heart failure models [2]. However, we failed to observe changes in in its expression by array or RT-PCR. miR-21 is involved in fibrosis which is rare in pediatric HF [9], and could be related to the lack of changes in miR-21 expression. Expression of the contractile-related miRNAs, miR-208a, miR-208b and miR-499 was also analyzed. Expression of miR-208a, 208b and miR-499 is increased in adult human HF [10], however, this has not been shown in all studies. A recent study has shown that down-regulation of miR-208a is beneficial in a heart disease model [11], and it is interesting that miR-208a is not increased in pediatric IDC patients. miR-1 can be up- or down-regulated in adult HF, and studies have shown that up-regulation of miR-1 can be protective in a hypertrophic setting [2]. Interestingly, miR-1 is up-regulated only in treated patients. Finally, we had previously shown that miR-486-5p is down-regulated in adult HF by miRNA array [1]. We were not able to confirm down-regulation of miR-486-5p by RT-PCR in adult HF patients (data not shown). However, expression of miR-486-5p is significantly decreased in PDEi-treated pediatric IDC patients. Not much is known about this miRNA in myocytes. However, it is known to target FOXO1, which promotes cardiomyocyte survival [12], and the histone deacetylase Sirt1, which may increase cells proliferation [13], suggesting that down-regulation of miR-486-5p may be involved in promoting cellular proliferation, and could contribute to the beneficial clinical effects of PDEi treatment in children with IDC.

References:

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[9] Sato H, Shiraishi I, Takamatsu T, Hamaoka K. Detection of TUNEL-positive cardiomyocytes and c-kit-positive progenitor cells in children with congenital heart disease. J Mol Cell Cardiol. 2007;43:254-61.
[10] Satoh M, Minami Y, Takahashi Y, Tabuchi T, Nakamura M. Expression of microRNA-208 is associated with adverse clinical outcomes in human dilated cardiomyopathy. J Card Fail. 2010;16:404-10.
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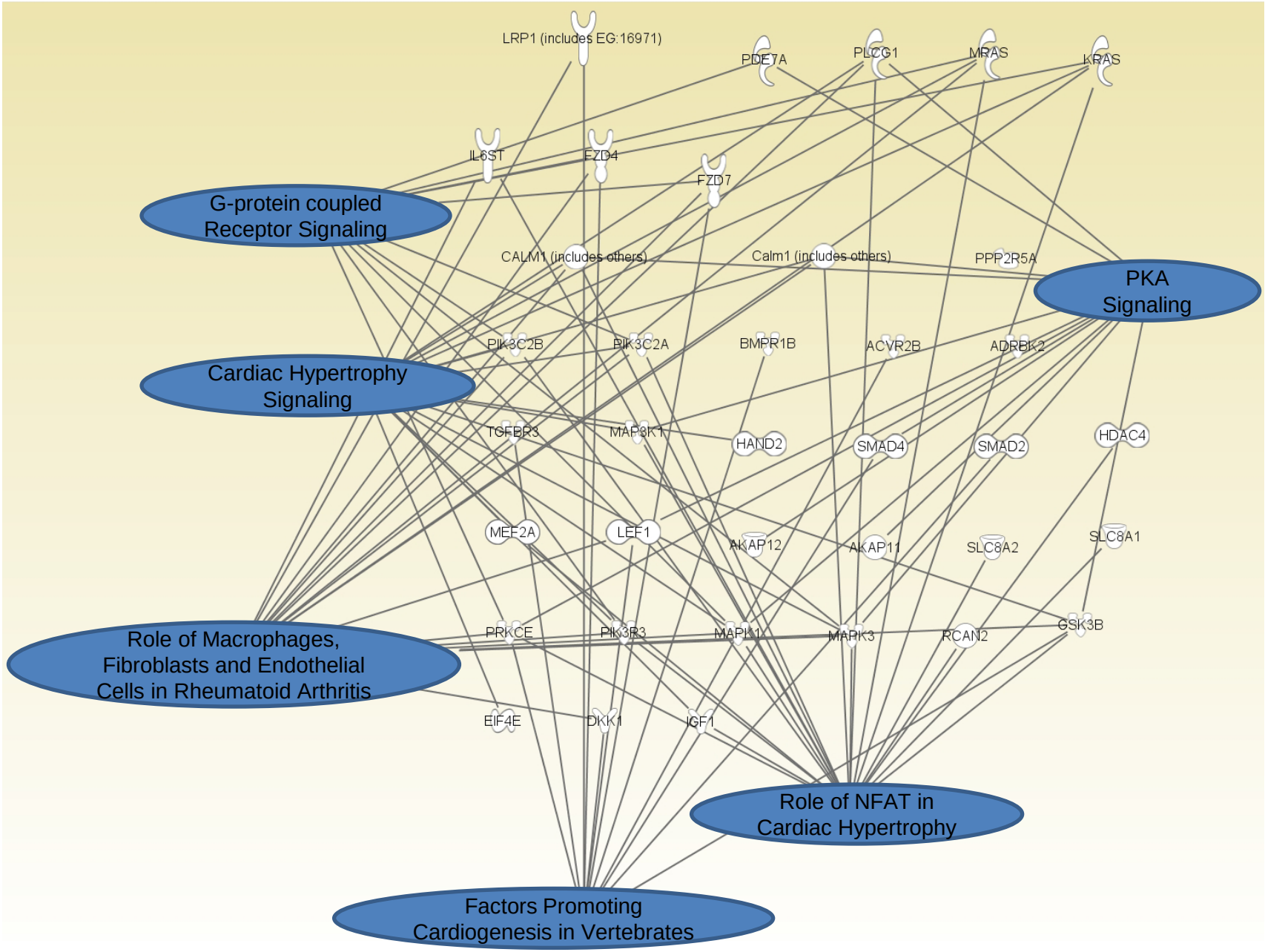
[12] Xu J, Li R, Workeneh B, Dong Y, Wang X, Hu Z. Transcription factor FoxO1, the dominant mediator of muscle wasting in chronic kidney disease, is inhibited by microRNA-486. Kidney Int. 2012.
[13] Kim YJ, Hwang SH, Lee SY, Shin KK, Cho HH, Bae YC, et al. miR-486-5p Induces Replicative Senescence of Human Adipose Tissue-Derived Mesenchymal Stem Cells and Its Expression Is Controlled by High Glucose. Stem Cells Dev. 2012;21:1749-60.

Figure Legends:

Figure S1: Pathway analysis for putative mRNA targets of miRNAs that change in PDEi-treated IDC patients. (A) miR-1, (B) miR-27b, (C) miR-133b, (D) miR-146a, (E) miR-204, (F) miR-486-5p.

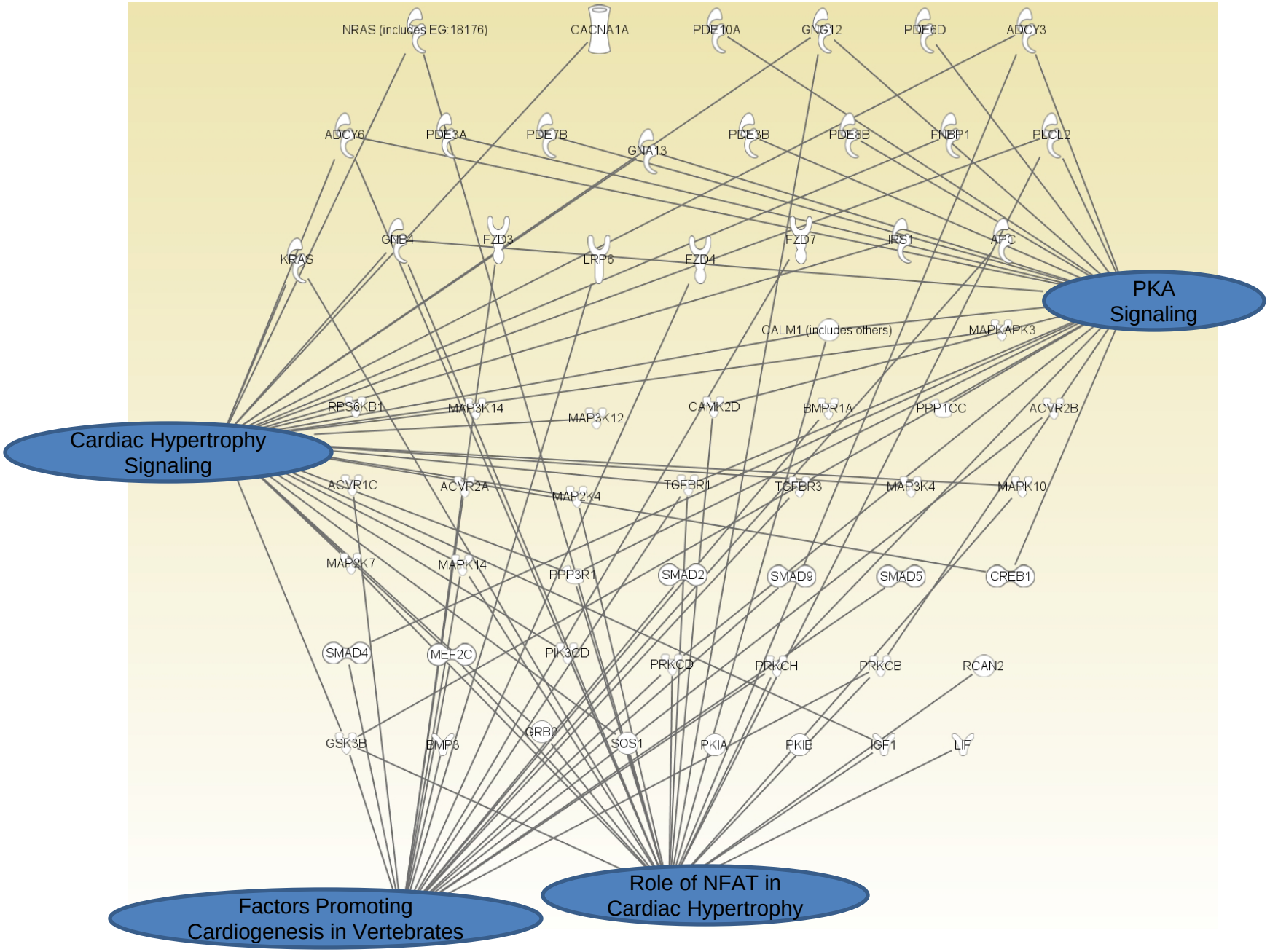
Table S1: Patient Subject Characteristic and Analysis

Table S2: Common putative targets regulated by a minimum of three PDEi-up-regulated miRNAs


LRP1 (includes EG:16971)
PDE7A
PLCG1
MRAS
KRAS
IL6ST
EZD4
FZD7
G-protein coupled Receptor Signaling
CALM1 (includes others)
Calm1 (includes others)
PPP2R5A
PKA Signaling
PIK3C2B
PIK3C2A
BMPR1B
ACVR2B
ADRBK2
Cardiac Hypertrophy Signaling
TGFBR3
MAP3K1
HAND2
SMAD4
SMAD2
HDAC4
MEF2A
LEF1
AKAP12
AKAP11
SLC8A2
SLC8A1
PRKCE
PIK3R3
MAPK11
MAPK3
RCAN2
GSK3B
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis
EIF4E
DKK1
IGF1
Role of NFAT in Cardiac Hypertrophy
Factors Promoting Cardiogenesis in Vertebrates

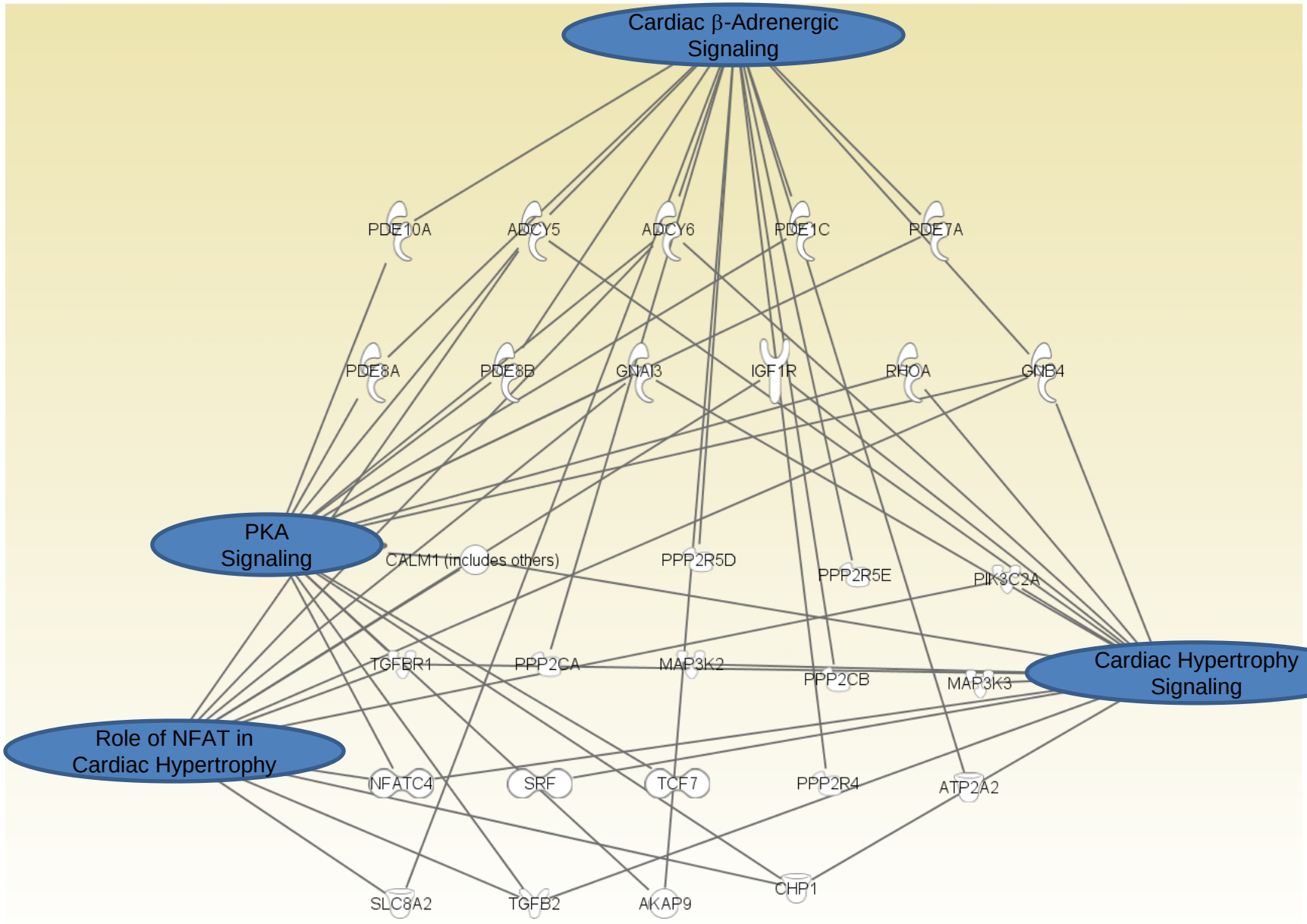
A.

Figure S1


NRAS (includes EG:18176)
CACNA1A
PDE10A
GNG12
PDE6D
ADCY3
ADCY6
PDE3A
PDE7B
GNA13
PDE3B
PDE8B
FNBP1
PLCL2
KRAS
GNB4
FZD3
LRP6
FZD4
FZD7
IRS1
APC
PKA Signaling
CALM1 (includes others)
MAPKAPK3
RPS6KB1
MAP3K14
MAP3K12
CAMK2D
BMPR1A
PPP1CC
ACVR2B
Cardiac Hypertrophy Signaling
ACVR1C
ACVR2A
MAP2K4
TGFBR1
TGFBR3
MAP3K4
MAPK10
MAP2K7
MAPK14
PPP3R1
SMAD2
SMAD9
SMAD5
CREB1
SMAD4
MEF2C
PIK3CD
PRKCD
PRKCH
PRKCB
RCAN2
GSK3B
BMP8
GRB2
SOS1
PKIA
PKIB
IGF1
LIF
Factors Promoting Cardiogenesis in Vertebrates
Role of NFAT in Cardiac Hypertrophy

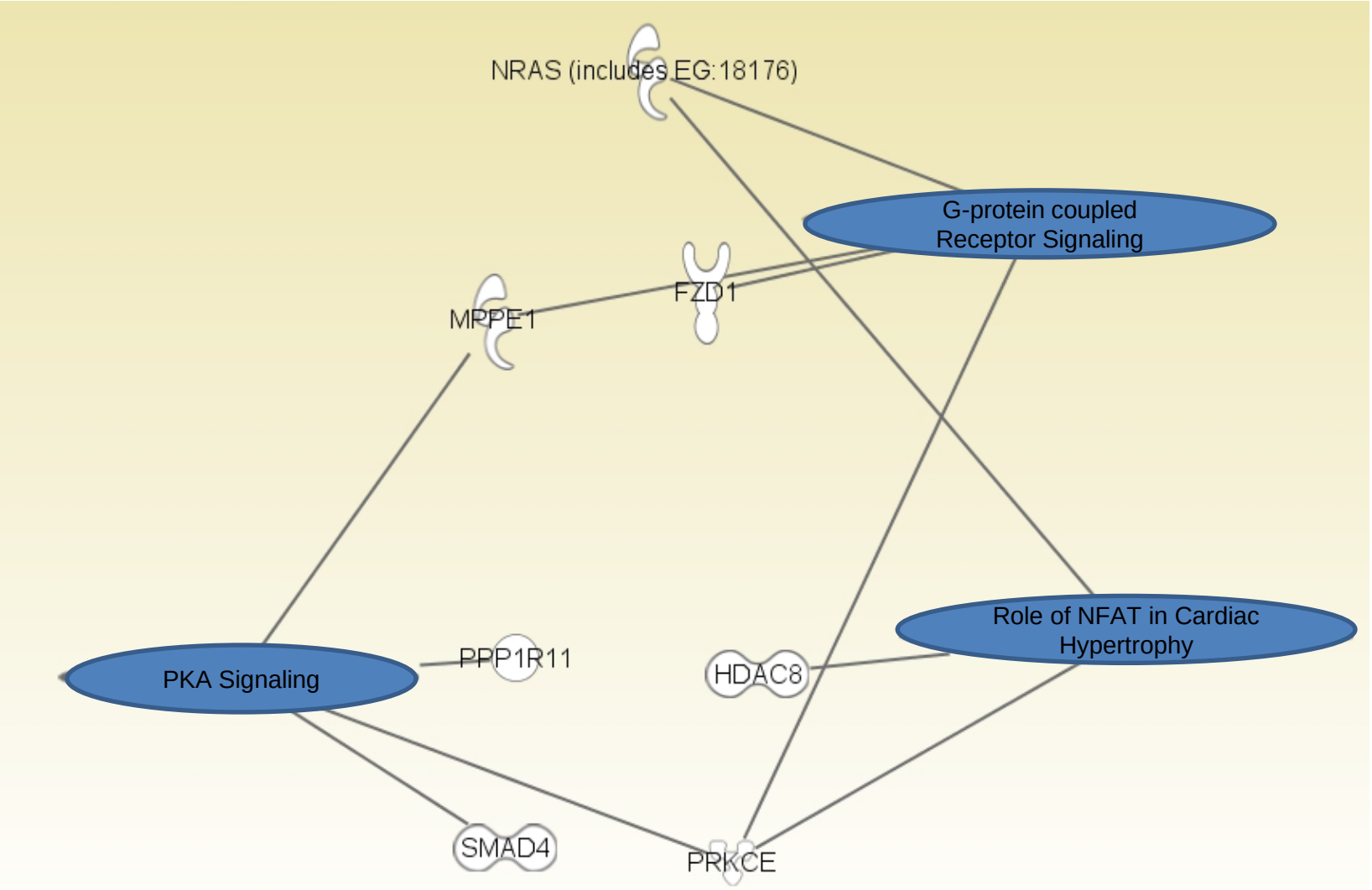
B.

Figure S1


Cardiac β-Adrenergic Signaling
PDE10A
ADCY5
ADCY6
PDE1C
PDE7A
PDE8A
PDE8B
GNAI3
IGF1R
RHOA
GNB4
PKA Signaling
CALM1 (includes others)
PPP2R5D
PPP2R5E
PIK3C2A
TGFBR1
PPP2CA
MAP3K2
PPP2CB
MAP3K3
Cardiac Hypertrophy Signaling
Role of NFAT in Cardiac Hypertrophy
NFATC4
SRF
TCF7
PPP2R4
ATP2A2
SLC8A2
TGFB2
AKAP9
CHP1

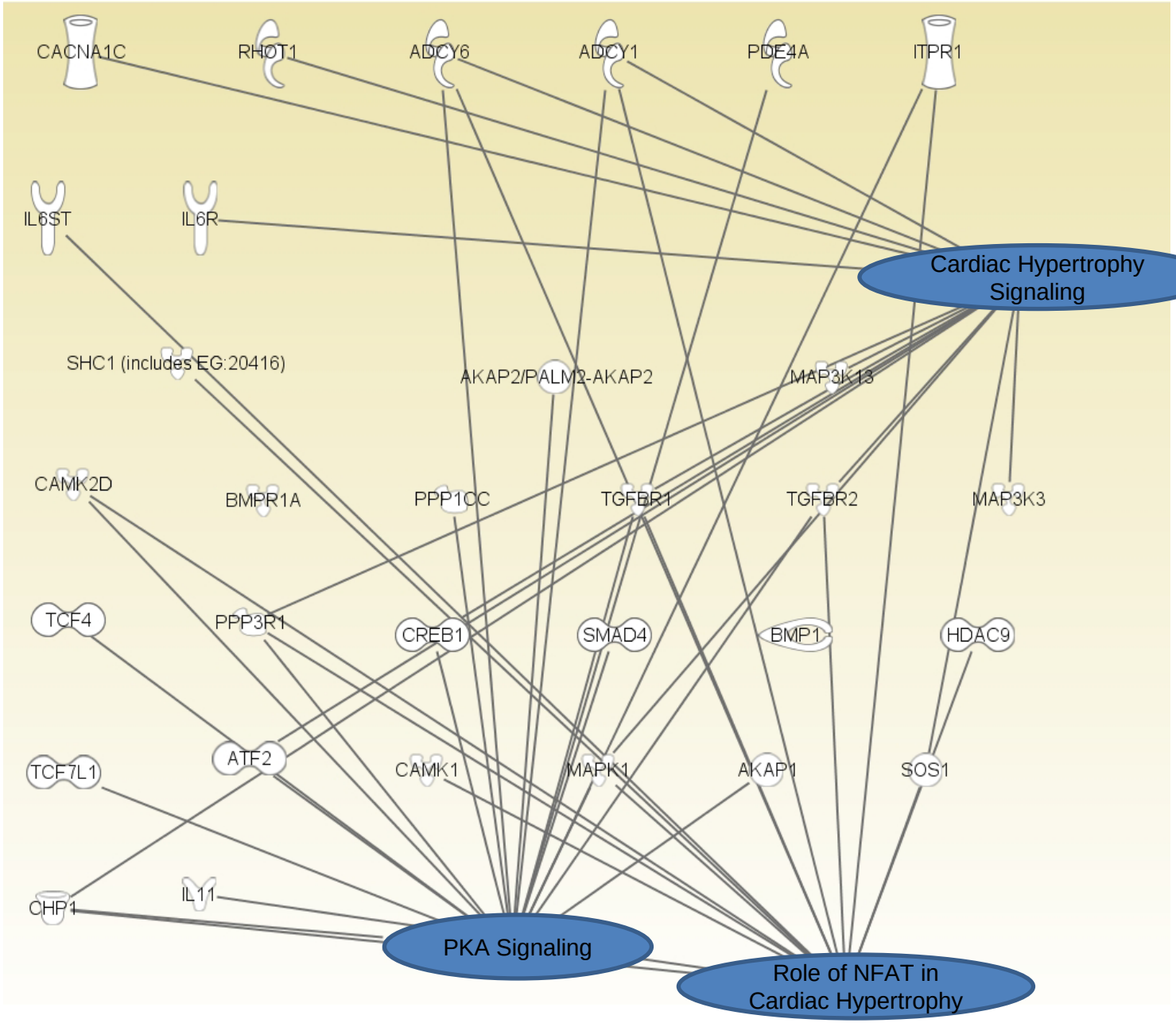
C.

Figure S1


NRAS (includes EG:18176)
G-protein coupled Receptor Signaling
FZD1
MPPE1
Role of NFAT in Cardiac Hypertrophy
PPP1R11
PKA Signaling
HDAC8
SMAD4
PRKCE

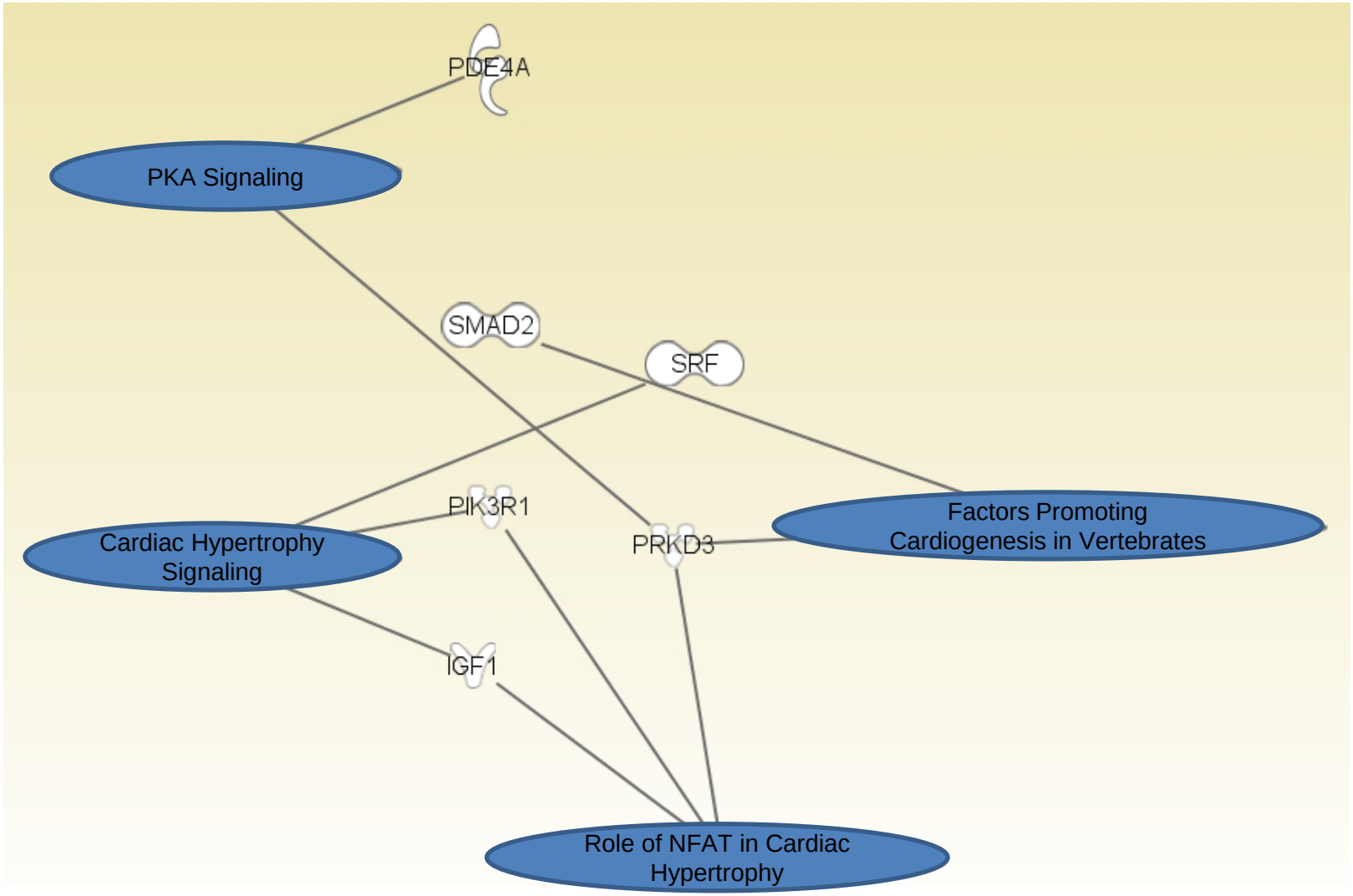
D.

Figure S1


CACNA1C
RHOT1
ADCY6
ADCY1
PDE4A
ITPR1
IL6ST
IL6R
Cardiac Hypertrophy Signaling
SHC1 (includes EG:20416)
AKAP2/PALM2-AKAP2
MAP3K13
CAMK2D
BMPR1A
PPP1CC
TGFBR1
TGFBR2
MAP3K3
TCF4
PPP3R1
CREB1
SMAD4
BMP1
HDAC9
ATF2
TCF7L1
CAMK1
MAPK1
AKAP1
SOS1
CHP1
IL11
PKA Signaling
Role of NFAT in Cardiac Hypertrophy

E.

Figure S1


PDE4A
PKA Signaling
SMAD2
SRF
PIK3R1
Cardiac Hypertrophy Signaling
PRKD3
Factors Promoting Cardiogenesis in Vertebrates
IGF1
Role of NFAT in Cardiac Hypertrophy

F.

Figure S1

ID	Sex	Race	Ethinicity	Group	Age at tissue collection	EF/FS	Inotrope	Digoxin	ACEI	Beta Blocker	Diuretic	PDEi	miRNA array	miRNA RT-PCR
1	F	NA	NA	NF	1	EF 49%	X							X
2	M	NA	NA	NF	1	NA	NA						X	X
3	F	NA	NA	NF	2	EF 27%	X							X
4	F	NA	NA	NF	3	EF 57%	X							X
5	M	NA	NA	NF	7	EF 63%	X						X	X
6	M	NA	NA	NF	7	NA	NA						X	X
7	F	NA	NA	NF	8	NA	NA							X
8	F	NA	NA	NF	9	EF 61%	X				X			X
9	M	NA	NA	NF	9	NA	X			X				X
10	M	NA	NA	NF	10	NA	NA							X
11	F	NA	NA	NF	11	NA	X							X
12	M	NA	NA	NF	12	EF 39%							X	X
13	M	NA	NA	NF	13	EF 50%	X			X				X
14	M	NA	NA	NF	14	NA	NA						X	
15	F	White	NH	IDC	0.3	EF 21%	X		X		X	X		X
16	F	NA	NA	IDC	0.8	EF 35%		X	X		X			X
17	M	White	H	IDC	1	EF 17%	X		X		X	X		X
18	F	White	H	IDC	2	EF 41%	X	X	X		X	X		X
19	M	White	NH	IDC	3	EF 15%			X		X	X	X	X
20	M	Asian	NH	IDC	3	EF 14%	X	X	X		X	X	X	X
21	M	Asian	NH	IDC	3	EF 13%	X		X		X		X	X
22	F	White	H	IDC	3	FS16%	X	X	X	X	X	X		X
23	F	White	NH	IDC	4	EF 32%		X	X		X			X
24	F	Asian	NH	IDC	4	EF 13%			X		X			X
25	M	White	H	IDC	4	EF 15%	X	X	X	X	X	X	X	X
26	M	White	NH	IDC	5	FS 16%			X					X
27	F	Asian	NH	IDC	9	UK	X					X	X	X
28	F	White	H	IDC	10	FS 22%	X	X	X	X	X	X		X
29	F	White	H	IDC	11	EF 15%			X		X			X
30	F	White	NH	IDC	12	EF 25%	X				X	X		X
31	F	White	NH	IDC	3.8	EF 32%	X	X	X	X	X	X		X
32	F	White	H	IDC	12	EF 25%	X		X		X			X

Table S1

Putative Targets	miRNAs
Smad4	miR-1, miR-146a, miR-204, miR-27b
Calmodulin	miR-1, miR-133b, miR-27b
TGFβ receptor 1	miR-133b, miR-204, miR-27b
TGFβ receptor 3	miR-133b, miR-204, miR-27b
Adenylyl Cyclase 6	miR-133b, miR-204, miR-27

Table S2