SUPPLEMENTAL MATERIAL

Supplemental Methods

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

For left atrial and differentiated cardiomyocyte knockdown samples, 12.5 µl of the TaqMan® gene expression master mix (Applied Biosystems), 1.25 µl of the custom designed PANCR or PITX2c primer/probe set (Supplemental Table 1, obtained from IDT) and the primer limited cardiac actin (ACTC1) primer/probe mix (assay number Hs00606316_m1 from Applied Biosystems) were mixed to create a master mix to be added to each sample. For the time course of H9 cell differentiation into cardiomyocytes and tissue array comparison, a similar qRT-PCR assay was done, but normalized to cyclophilin A (PPIA, assay number Hs04194521_s1 from Applied Biosystems) instead of ACTC1, since the latter gene is not expressed in undifferentiated H9 cells or most other tissues. This 15 µl master mix was pipetted into individual wells of a 96-well working plate. 10 µl of the diluted cDNA was added to each well. 5 µl of this working mixture was pipetted in triplicate to a 384-well assay plate. Real time PCR was performed in a Bio-RAD CRX thermocycler that had been calibrated for FAM and VIC fluorescent probes. Thermal cycling was performed with a hot-start at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds.

RNAseq and analysis

RNAseq library preparation and sequencing for differentiated H9 cardiomyocytes was completed at the University of Chicago using the Illumina HiSeq platform. The 100 bp paired-end reads were aligned to hg19 using STAR aligner [1] and the Ensembl 71 transcript annotation.[2] Read counts were summed using htseq-counts. Gene ontology searches for the genes regulated by *PANCR* or *PITX2c* knockdown was performed using DAVID.[3, 4] Differential gene expression analyses after *PANCR* and *PITX2c* knockdown was performed using the

edgeR[5] package in R/Stats. Small RNAseq of differentiated H9 cardiomyocytes was performed at the Cleveland Clinic on the Illumina MySeq platform. Sequences were aligned to the genome using STAR aligner[1] and the transcript counts were determined using FeatureCounts.[6] miRNA families were collapsed to a single miRNA family identifier using the miRbase annotations, thus discarding sequences that were not annotated miRNAs. Differential miRNA gene expression was determined using DEXSeq software.[7] False discovery rates were determined by the Benjamini Hochberg method[8] in the DEXSeq package.

References:

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Supplemental Table 1: PANCR TaqMan primers/probe and siRNA sequences

Identifier	Sequence
PANCR Forward Primer	5' AAT TCT CCA TAG GAC TGC ATG AG-3'
PANCR Reverse Primer	5'-CAC CTC GGT TCC ACT CAA C-3'
TaqMan Probe	5'-/56-FAM/CGG TTG TCT /ZEN/TCT CCC AGA ATG AGT GA/3IABkFQ/-3'
PANCR siRNA sense	5’ CGG UUC CAC UCA ACC GAU UUU 3’
PANCR siRNA antisense	5’ AAU CGG UUG AGU GGA ACC GUU 3’

* FAM fluorophore with internal ZEN and 3’ IOWA BLACK FQ quencher modification

Supplemental table 2. Top 20 differentially expressed genes after PANCR knockdown, by fold change.

Gene symbol	Chromosome	log_2FC^*	logCPM†	P value‡
SERPINA7	X	5.86	-1.91	2.19E-04
FGFBP2	4	4.87	-1.52	5.99E-04
PLG	6	3.44	-0.68	6.53E-03
PRODH2	19	-2.89	-1.25	2.27E-04
EMX1	2	-2.80	-1.46	4.45E-03
ENSG00000233639	2	2.69	-1.47	7.71E-02
ENSG00000248362	5	2.69	-1.07	2.53E-05
ANGPT4	20	-2.65	-0.01	1.51E-03
ITIH1	3	2.65	-0.96	4.40E-02
OR51E2	11	-2.58	-0.40	1.82E-03
FABP1	2	2.53	-1.40	1.23E-02
SCNN1G	16	-2.47	2.03	1.18E-02
RPSAP56	16	2.47	-0.60	1.61E-02
CD300E	17	-2.41	-1.81	3.69E-02
ENSG00000258123	12	2.41	-1.76	1.26E-04
SST	3	-2.37	0.80	5.04E-03
PHOX2A	11	2.29	-1.67	1.26E-03
ENSG00000261286	16	2.29	-1.47	5.31E-04
OR51E1	11	-2.28	-1.10	1.99E-02
ST3GAL6-AS1	3	2.28	-1.65	9.34E-04

*, Log2 fold change relative to scramble siRNA
†, log read counts per million
‡, t-test p-value determined by edgeR software comparing PANCR knockdown vs. scrambled siRNA

Supplemental table 3. Top 20 differentially expressed genes after PITX2c knockdown, by fold change

Gene symbol	Chromosome	log_2FC*	logCPM†	P value‡
CST1	20	8.44	-1.414	1.94E-05
KRT6A	12	-4.47	-1.270	9.97E-03
EOMES	3	3.28	-0.845	8.54E-03
SERPINA7	X	3.16	-1.911	7.22E-02
CHRDL2	11	-3.15	0.790	1.43E-04
OR51E1	11	-3.02	-1.097	3.15E-03
PLG	6	2.92	-0.677	2.01E-02
LINC00461	5	-2.90	1.675	1.31E-01
CD300E	17	-2.60	-1.812	2.65E-02
ENSG00000228741	13	-2.43	-1.481	3.60E-03
CER1	9	2.42	-0.178	2.24E-03
SCGB3A2	5	2.40	-1.901	1.30E-03
TMPRSS3	21	2.39	-1.656	1.32E-03
SST	3	-2.38	0.800	4.83E-03
ENSG00000261286	16	2.35	-1.470	3.73E-04
FAM179A	2	-2.30	-1.695	9.55E-03
ANGPT4	20	-2.30	-0.009	5.12E-03
POU5F1	6	2.30	-1.371	1.48E-01
HOXB8	17	-2.29	-0.967	5.38E-03
CMKLR1	12	-2.26	2.585	1.00E-03

*, Log2 fold change relative to scramble siRNA
†, log read counts per million
‡, t-test p-value determined by edgeR software comparing PITX2c knockdown vs. scrambled siRNA

Supplemental table 4. Top 20 differentially expressed miRNA families after PANCR knockdown, by p-value

miR family	Read counts*	log_2FC†	P value‡	FDR§
MIR423	4481	1.42	3.28E-10	4.41E-08
MIR501	169	1.68	1.34E-09	1.35E-07
MIR500A	1313	1.64	1.65E-08	1.17E-06
MIR191	31516	-0.94	1.75E-08	1.17E-06
MIR589	154	1.19	3.99E-08	2.29E-06
MIR126	2116	-1.10	1.38E-07	6.96E-06
MIR29A	44	1.86	2.57E-07	1.15E-05
MIR151B	124	1.14	1.26E-06	4.61E-05
MIR99A	1116	-1.22	2.10E-06	6.50E-05
MIR140	1003	0.80	3.24E-06	9.33E-05
MIR720	4	3.94	7.60E-06	2.04E-04
MIR542	320	-0.69	1.81E-05	4.47E-04
MIR143	307561	1.14	1.88E-05	4.47E-04
MIR490	1767	-0.58	3.01E-05	6.74E-04
MIR532	1593	1.02	1.12E-04	2.35E-03
MIR194-1	176	-0.96	1.17E-04	2.35E-03
MIR301A	1896	-1.03	1.29E-04	2.48E-03
MIR194-2	186	-0.93	1.52E-04	2.71E-03
MIR30B	2873	-0.55	1.54E-04	2.71E-03

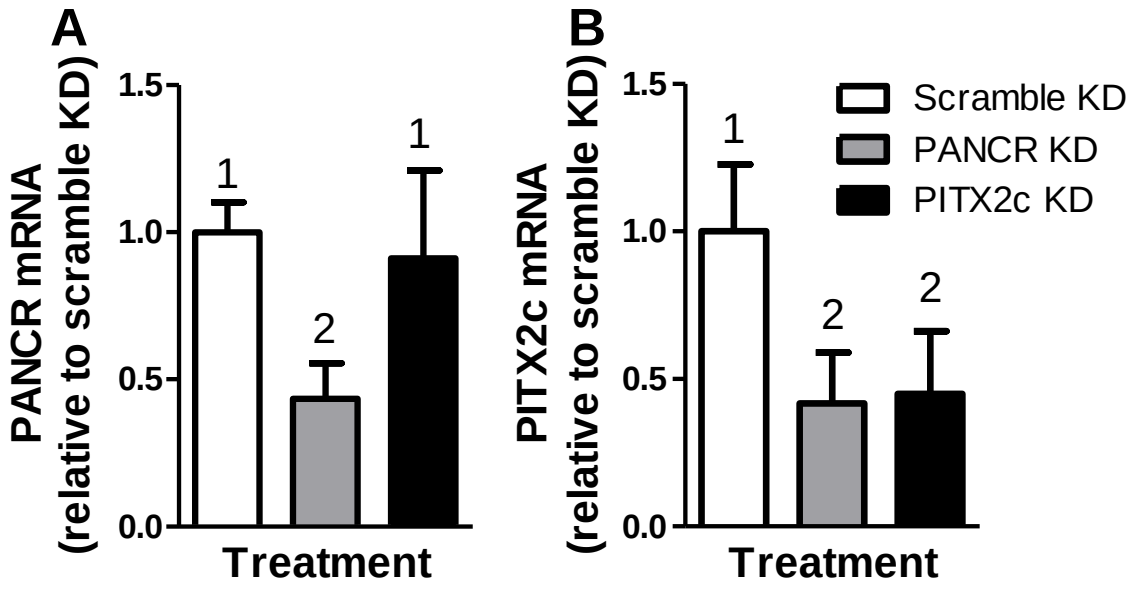
*, Read counts per group, quantile normalized
†, Log2 fold change relative to scramble siRNA
‡, t-test p-value determined by DESseq software comparing PANCR knockdown vs. scrambled siRNA.
§, false discovery rate for testing multiple miRNA families (type II error) implemented in DEXSeq [6, 7].

Supplemental table 5. Top 20 differentially expressed miRNA families after PITX2c knockdown, by p-value

miR family	Read counts*	log_2FC†	P value‡	FDR§
MIR301B	933	-1.23	2.34E-05	1.90E-03
MIR302A	566	-0.74	8.81E-05	3.53E-03
MIR328	178	-0.80	9.77E-05	3.53E-03
MIR941-1	33	2.03	5.90E-05	3.53E-03
MIRLET7F1	1494	0.86	9.23E-05	3.53E-03
MIRLET7F2	1519	0.86	9.24E-05	3.53E-03
MIR301A	1896	-1.04	1.11E-04	3.59E-03
MIR126	2116	-0.75	3.14E-04	8.50E-03
MIR143	307561	0.96	3.03E-04	8.50E-03
MIR30B	2873	-0.52	3.57E-04	8.93E-03
MIRLET7G	476	0.85	4.85E-04	1.13E-02
MIR182	44768	1.37	7.92E-04	1.72E-02
MIR140	1003	0.57	8.51E-04	1.73E-02
MIR302D	279	-0.77	1.36E-03	2.32E-02
MIR589	154	0.69	1.35E-03	2.32E-02
MIR222	1099	0.89	1.74E-03	2.84E-02
MIR191	31516	-0.52	2.03E-03	3.15E-02
MIRLET7I	159	0.88	2.47E-03	3.65E-02
MIR423	4481	0.68	2.76E-03	3.80E-02

*, Read counts per group, quantile normalized.
†, Log2 fold change relative to scramble siRNA.
‡, t-test p-value determined by DESseq software comparing PITX2c knockdown vs. scrambled siRNA.
§, false discovery rate for testing multiple miRNA families (type II error) implemented in DEXSeq [6, 7].

Supplemental Figure 1



Supplemental Figure 1: siRNA knockdown of PANCR and PITX2c in differentiated cardiomyocytes. qPCR was used to measure PANCR (A) and PITX2c (B) expression levels after indicated siRNA treatments (N = 4 $\pm$ SD, different numbers above bars indicate $p < 0.05$ by ANOVA with Newman-Keuls posttest). ACTC1 qPCR was used for internal normalization, the $2^{-\Delta\Delta CT}$ method was used for adjusting values to a linear scale, and expression was then shown relative to expression in the scramble KD control.