Supplemental Materials

ZNF281 enhances cardiac reprogramming by modulating cardiac and inflammatory gene expression

Huanyu Zhou, Maria Gabriela Morales, Hisayuki Hashimoto, Matthew E. Dickson, Kunhua Song, Wenduo Ye, Min S. Kim, Hanspeter Niederstrasser, Zhaoning Wang, Beibei Chen, Bruce A. Posner, Rhonda Bassel-Duby and Eric N. Olson

Supplemental Table S1. Genes included in human retroviral ORF cDNA library.

Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol	Gene Symbol
AATF	*BMP8A*	*CEBPE*	*CTNNB1*	*ESR2*	*GDF3*	*HOXA5*	*IL17D*
ADIPOQ	*BRPF1*	*CEBPG*	*CUX1*	*ESRRA*	*GDF6*	*HOXA6*	*IL17F*
ADNP	*BRPF3*	*CERS1*	*CX3CL1*	*ETS1*	*GIN1*	*HOXA7*	*IL18*
AEBP1	*BUD31*	*CERS2*	*CXCL10*	*ETS2*	*GLIS3*	*HOXB1*	*IL19*
AFF4	*C17ORF77*	*CERS4*	*CXCL11*	*ETV3*	*GMEB1*	*HOXB13*	*IL1A*
AHR	*C1QTNF4*	*CFL2*	*CXCL12*	*ETV7*	*GPBP1*	*HOXB5*	*IL1B*
AIMP1	*C21ORF66*	*CHIA*	*CXCL13*	*FAM3B*	*GPER*	*HOXB6*	*IL1F3*
ALS2CR8	*CBFA2T2*	*CIR1*	*CXCL14*	*FAM3D*	*GPI*	*HOXB7*	*IL1F5*
ALX1	*CBFA2T3*	*CITED1*	*CXCL16*	*FASLG*	*GREM1*	*HOXB9*	*IL1F6*
ARGFX	*CBFB*	*CITED2*	*CXCL3*	*FBLN1*	*GREM2*	*HOXC4*	*IL1F7*
ARID3A	*CBL*	*CKLF*	*CXCL5*	*FEV*	*GRN*	*HOXC5*	*IL1F8*
ARNT	*CBX5*	*CLCF1*	*CXCL6*	*FL1*	*GTF2H2*	*HOXC6*	*IL2*
ARNT2	*CBX6*	*CMTM1*	*CXCL9*	*FLI1*	*GTF2H2C*	*HOXC8*	*IL20*
ASCL1	*CBX7*	*CMTM2*	*DBT*	*FLT3LG*	*GTF2H3*	*HOXD1*	*IL22*
ASCL2	*CBX8*	*CMTM3*	*DLX1*	*FOS*	*GTF2H4*	*HOXD10*	*IL23A*
ASXL1	*CCDC104*	*CMTM4*	*DLX3*	*FOSB*	*GTF2I*	*HOXD3*	*IL24*
ATAD3B	*CCDC69*	*CMTM5*	*DLX4*	*FOSL1*	*GTF2IRD1*	*HOXD4*	*IL25*
ATF2	*CCL1*	*CMTM6*	*DLX6*	*FOSL2*	*HAT1*	*HOXD8*	*IL26*
ATF4	*CCL11*	*CMTM7*	*DMBX1*	*FOXA1*	*HBM*	*HOXP*	*IL27*
ATF5	*CCL13*	*CMTM8*	*DMRT1*	*FOXA2*	*HCFC1*	*HSAL2*	*IL28B*
ATF6	*CCL14*	*CNBP*	*DPPA4*	*FOXA3*	*HDAC1*	*HSF1*	*IL31*
ATF6B	*CCL15*	*CNOT7*	*DRAP1*	*FOXD4*	*HDAC3*	*HSFY2*	*IL32*
ATF7	*CCL17*	*CNTF*	*DRGX*	*FOXD4L6*	*HDAC4*	*ID1*	*IL33*
ATOH1	*CCL18*	*CNTNAP3*	*E2F2*	*FOXJ1*	*HDAC7*	*IFNA10*	*IL34*
AURKA	*CCL2*	*COBLL1*	*E2F3*	*FOXJ2*	*HDAC8*	*IFNA13*	*IL36G*
AURKB	*CCL21*	*CPA3*	*E2F6*	*FOXL1*	*HES1*	*IFNA14*	*IL4*
AURKC	*CCL22*	*CPB2*	*E2F7*	*FOXL2*	*HES6*	*IFNA16*	*IL6*
BARX1	*CCL23*	*CREB1*	*E2F8*	*FOXM1*	*HESX1*	*IFNA17*	*IL7*
BATF	*CCL24*	*CREB3*	*E4F1*	*FOXN3*	*HEYL*	*IFNA2*	*IL8*
BATF2	*CCL25*	*CREB3L2*	*EBF1*	*FOXO3*	*HHEX*	*IFNA21*	*IL9*
BATF3	*CCL26*	*CREB3L4*	*EBI3*	*FOXP1*	*HIF3A*	*IFNA4*	*ILF10*
BCL6	*CCL27*	*CREB5*	*EDF1*	*FOXP3*	*HINFP*	*IFNA5*	*ING2*
BLZF1	*CCL28*	*CREBL2*	*EDN1*	*FOXP4*	*HIRA*	*IFNA6*	*ING3*
BMP1	*CCL3*	*CREBRF*	*EGR2*	*FUBP1*	*HLF*	*IFNA7*	*INHA*
BMP10	*CCL3L3*	*CREM*	*EHF*	*GABPA*	*HLX*	*IFNA8*	*INHBB*
BMP2	*CCL4*	*CRHR2*	*ELF1*	*GABPB1*	*HLX1*	*IFNE*	*IRF1*
BMP3	*CCL4L1*	*CRLF1*	*ELF2*	*GAS7*	*HMG20B*	*IFNG*	*IRF2*
BMP5	*CCL5*	*CRX*	*ELF4*	*GATA1*	*HMGA1*	*IFNW1*	*IRF3*
BMP8A	*CCL7*	*CSDA*	*ELF5*	*GATA2*	*HMGB*	*IKZF1*	*IRF4*
BRPF1	*CCL8*	*CSF1*	*ELK1*	*GATA3*	*HMGB1*	*IKZF3*	*IRF5*
BRPF3	*CD2*	*CSF2*	*ELK3*	*GATA5*	*HMGB2*	*IL10*	*IRF6*
BUD31	*CD200R1*	*CSF3*	*ELK4*	*GATAD1*	*HNF1A*	*IL11*	*IRF8*
C17ORF77	*CD40LG*	*CSRNP3*	*EMB*	*GATAD2A*	*HNF1B*	*IL12B*	*IRF9*
C1QTNF4	*CD99P1*	*CSRP2BP*	*END1*	*GATAD2B*	*HNF4A*	*IL13*	*IRX2*
C21ORF66	*CDYL*	*CTBP1*	*ERF*	*GBX2*	*HNRNPAB*	*IL15*	*IRX3*
CBFA2T2	*CDYL2*	*CTBP2*	*ERG*	*GDF10*	*HOPX*	*IL16*	*IRX6*
CBFA2T3	*CEBPB*	*CTCF*	*ESAM*	*GDF15*	*HOXA1*	*IL17A*	*ISL1*
BMP8A	*CEBPD*	*CTF1*	*ESR1*	*GDF2*	*HOXA11*	*IL17C*	*ISL2*

JDP2	*MIF*	*NR1D2*	*POU4F3*	*SIX5*	*TCEAL1*	*TSC22D4*	*ZNF189*
JUN	*MITA3*	*NR1H3*	*POU6F1*	*SLC30A9*	*TCF12*	*TSLP*	*ZNF19*
JUNB	*MITF*	*NR1H3*	*PPARA*	*SLURP1*	*TCF19*	*TULP4*	*ZNF192*
KAT5	*MLX*	*NR1I2*	*PPARG*	*SMAD1*	*TCF25*	*TWIST2*	*ZNF207*
KAT7	*MLXIPL*	*NR1I3*	*PPBP*	*SMAD2*	*TCF7*	*UBP1*	*ZNF207*
KAY7	*MMP14*	*NR2C2*	*PRDM1*	*SMAD3*	*TCF7L2*	*UNC50*	*ZNF213*
KCNIP2	*MPLKIP*	*NR2E1*	*PRMT5*	*SMAD4*	*TEAD2*	*USF1*	*ZNF215*
KCNMB3	*MPO*	*NR2E3*	*PRMT6*	*SMAD6*	*TEAD3*	*USF2*	*ZNF232*
KDM1	*MSC*	*NR2F1*	*PROP1*	*SNAI2*	*TEAD4*	*USP16*	*ZNF24*
KDM1A	*MSL3*	*NR2F2*	*PRRX1*	*SNAPC2*	*TEF*	*USP21*	*ZNF256*
KITLG	*MSTN*	*NR3C1*	*PRRX2*	*SNAPC5*	*TFAP2C*	*VAX1*	*ZNF263*
KLF1	*MSX2*	*NR4A1*	*PTGER3*	*SOX10*	*TFAP2E*	*VAX2*	*ZNF268*
KLF10	*MTA1*	*NR4A2*	*PTH*	*SOX13*	*TFCP2*	*VDR*	*ZNF274*
KLF11	*MTA2*	*NR5A1*	*PTTG1*	*SOX2*	*TFCP2L1*	*VEGFA*	*ZNF277*
KLF12	*MXD1*	*NR5A2*	*RARB*	*SOX5*	*TFDP1*	*VENTX*	*ZNF281*
KLF3	*MYB*	*NR6A1*	*RARG*	*SOX6*	*TFDP2*	*VSX2*	*ZNF287*
KLF4	*MYBL2*	*NRDB2*	*RAX2*	*SOX7*	*TFE3*	*WNT1*	*ZNF34*
L3MBTL1	*MYC*	*NRG1*	*RBCK*	*SOX8*	*TFEB*	*WNT2*	*ZNF354A*
L3MBTL4	*MYCL*	*NXPE4*	*RBPJ*	*SOX9*	*TFEC*	*WNT5A*	*ZNF37A*
LAMTOR5	*MYCN*	*OLIG2*	*RBPJL*	*SP140*	*TGFB2*	*WT1*	*ZNF394*
LASS6	*MYF6*	*OSM*	*RCAN1*	*SPDEF*	*TGIF1*	*XBP1*	*ZNF396*
LBX2	*MYOG*	*OTP*	*RELA*	*SPI1*	*TGIF2*	*XCL1*	*ZNF397*
LCOR	*MYT1*	*OTX1*	*RELB*	*SPIB*	*TGIF2LX*	*XCL2*	*ZNF41*
LEF1	*MZF1*	*OTX2*	*RENIN*	*SPINK13*	*THAPB*	*YBX1*	*ZNF444*
LEFTY1	*NAMPT*	*PA2G4*	*RFX3*	*SPP1*	*THRA*	*YEATS4*	*ZNF446*
LEFTY2	*NANOGP8*	*PARS2*	*RFX5*	*SREBF1*	*THRB*	*YY1*	*ZNF449*
LHX2	*NDOR1*	*PAX4*	*RFXANK*	*SRF*	*TLE4*	*ZBTB18*	*ZNF483*
LHX4	*NDP*	*PAX5*	*RFXAP*	*SRY*	*TLX2*	*ZBTB25*	*ZNF496*
LHX6	*NEK6*	*PAX6*	*RHOXF1*	*STAT1*	*TLX3*	*ZBTB48*	*ZNF639*
LHX9	*NEUROD1*	*PAX8*	*RHOXF2*	*STAT3*	*TMEM8C*	*ZEB2*	*ZNF69*
LIF	*NEUROD2*	*PBX2*	*RNF4*	*STAT5*	*TMOD1*	*ZFP29*	*ZNF71*
LMO4	*NEUROG3*	*PCGF6*	*RORB*	*STAT5A*	*TNF*	*ZFP36L1*	*ZNF75D*
LMX1A	*NFATC1*	*PF4*	*RTP3*	*STAT5B*	*TNFRSF11B*	*ZFP36L2*	*ZNF83*
LMX1B	*NFATC3*	*PFDN1*	*RUNX1T1*	*STAT6*	*TNFSF13*	*ZFP42*	*ZNF85*
LRRC8B	*NFE2*	*PGBD1*	*RUNX3*	*SUPT4H1*	*TNFSF13B*	*ZFP69B*	*ZRANB2*
LTA	*NFE2L1*	*PGRMC2*	*RXRA*	*SUV420H1*	*TNFSF15*	*ZHX2*	*ZSCAN1*
LTB	*NFE2L3*	*PHC2*	*RXRB*	*TADA2A*	*TNFSF18*	*ZHX3*	*ZSCAN12*
MAFB	*NFIC*	*PHF5A*	*RXRG*	*TADA3*	*TNFSF4*	*ZIC3*	*ZSCAN16*
MAFF	*NFIL3*	*PHF6*	*SALL2*	*TADA3L*	*TNFSF8*	*ZKSCAN3*	*ZSCAN18*
MAFG	*NFKB1*	*PHF7*	*SATB2*	*TAF10*	*TNLG8A*	*ZKSCAN4*	*ZSCAN20*
MARCH5	*NFYB*	*PHOX2A*	*SCG2*	*TAF12*	*TP53*	*ZKSCAN5*	*ZSCAN21*
MARCKS5	*NFYC*	*PHTF1*	*SCGB3A1*	*TAF5L*	*TP63*	*ZKSCAN7*	*ZSCAN25*
MAX	*NKX2-1*	*PITX1*	*SCMH1*	*TAF6*	*TRAF3IP1*	*ZKSCAN8*	*ZSCAN26*
MBD1	*NKX2-3*	*PITX2*	*SECTM1*	*TAF7*	*TRIM22*	*ZNF131*	*ZSCAN31*
MDS1	*NKX2-5*	*PKNOX1*	*SETD3*	*TARDBP*	*TRIM25*	*ZNF133*	*ZSCAN4*
MECOM	*NKX2-8*	*PKNOX2*	*SETD4*	*TBX2*	*TRIM27*	*ZNF134*	*ZSCAN5A*
MECP2	*NME2*	*PLAGL2*	*SETD8*	*TBX20*	*TRIM28*	*ZNF140*	*ZSCAN9*
MEF2A	*NOLC1*	*POLD4*	*SETDB1*	*TBX21*	*TRIM29*	*ZNF165*	
MEF2D	*NPAS2*	*POU1F1*	*SHOX*	*TBX22*	*TRIM58*	*ZNF169*	
MEIS2	*NR0B1*	*POU2F1*	*SHOX2*	*TBX3*	*TSC22D2*	*ZNF174*	
MEOX1	*NR0B2*	*POU2F2*	*SIX1*	*TBX5*	*TSC22D3*	*ZNF175*	
MICB	*NR1D1*	*POU3F2*	*SIX2*	*TBX6*	*TSC22D3*	*ZNF18*	

Supplemental Table S2. Activators and inhibitors identified by screen from the human retroviral ORF cDNA library.

Activators					
Gene Symbol	**cTnT Z-score**	**αMHC Z-score**	**Gene Symbol**	**cTnT Z-score**	**αMHC Z-score**
PHF7	9.7	7.4	*GTF2IRD1*	2.5	7.2
ZNF281	7.0	2.9	*ATF4*	2.4	0.3
LTA	6.3	0.0	*CD200R1*	2.3	2.4
RNF4	4.6	3.1	*HMGB2*	2.3	1.7
SMAD2	4.5	1.8	*CXCL10*	2.3	0.2
TP53	4.4	1.1	*AFF4*	2.2	2.0
ASCL1	3.9	0.0	*ATF2*	2.2	1.2
MSX2	3.8	2.9	*PPARA*	2.2	2.2
MTA1	3.8	1.7	*KITLG*	2.1	1.6
SHOX2	3.6	1.6	*NFYC*	2.0	0.0
NXPE4	3.6	0.3	*SPP1*	2.0	1.4
TAF6	3.5	4.0	*CMTM3*	1.9	2.6
HHEX	3.4	1.1	*TRIM28*	1.9	2.2
TFDP1	3.3	3.4	*ZSCAN26*	1.6	2.0
SIX2	3.3	0.0	*VENTX*	1.5	2.8
MYBL2	3.3	2.9	*AHR*	1.3	2.0
NR6A1	3.2	1.7	*IFNA16*	1.3	2.1
TCF19	3.2	1.7	*IL18*	1.2	2.2
PAX4	3.2	0.7	*IFNA2*	1.1	2.8
NANOGP8	3.1	1.4	*HLF*	0.6	2.9
PLAGL2	3.0	1.5	*SOX6*	0.5	3.3
CREM	2.9	0.3	*MAX*	0.5	2.2
ELF1	2.8	1.8	*IL10*	0.3	2.3
BATF	2.7	0.6	*MEF2D*	0.2	2.0
Inhibitors					
Gene Symbol	**cTnT Z-score**	**αMHC Z-score**	**Gene Symbol**	**cTnT Z-score**	**αMHC Z-score**
HOXC4	-4.2	-5.6	*NFE2*	-2.8	-2.1
OLIG2	-4.1	-5.4	*ZNF397*	-2.7	-3.9
JUN	-4.0	-4.1	*USF2*	-2.7	-4.8
LHX4	-3.8	-3.3	*HNF4A*	-2.7	-4.5
HOXD4	-3.6	-4.0	*NR5A1*	-2.6	-4.7
TLE4	-3.5	-4.3	*IL2*	-2.6	-3.7
POU2F1	-3.4	-4.9	*FUBP1*	-2.5	-2.9
HOXB9	-3.3	-3.7	*IRF4*	-2.5	-3.1
DLX3	-3.2	-4.0	*CEBPE*	-2.3	-3.1
FOXA3	-3.1	-4.3	*NR4A1*	-2.3	-3.8
FOSL1	-3.0	0.4	*AIMP1*	-2.2	-2.7
SRF	-3.0	-1.5	*PAX6*	-2.2	-3.2
PHOX2A	-3.0	-2.6	*TLX3*	-2.2	-2.4
ZFP36L1	-3.0	-1.2	*SPDEF*	-2.1	-2.6
SOX10	-2.9	-2.8	*FOS*	-2.1	0.3
HOXD10	-2.9	-4.2	*SOX9*	-2.1	-2.0
LHX9	-2.8	-3.4	*TGIF2*	-2.1	-3.6
DBT	-2.8	-5.0	*ZHX2*	-2.1	-1.7
NR3C1	-2.0	-0.6	*IKZF3*	-1.3	-2.1
CTF1	-2.0	-1.8	*E4F1*	-1.2	-2.3
TFEB	-2.0	-1.0	*FL1*	-1.2	-2.1
PITX2	-2.0	-2.7	*ERG*	-1.2	-2.1

SOX7	-2.0	-2.4	*HOXD3*	-1.2	-2.2
HNF1B	-1.9	-2.2	*IL1A*	-1.2	-2.2
SNAI2	-1.9	-3.7	*PITX1*	-1.2	-2.7
FASLG	-1.9	-4.6	*NEUROD1*	-1.1	-2.2
HOXA6	-1.9	-2.2	*ZBTB48*	-1.1	-2.5
OTX1	-1.8	-4.3	*TFAP2E*	-1.1	-2.2
FAM3B	-1.8	-2.2	*OTP*	-1.1	-3.8
NKX2-5	-1.8	-2.8	*SOX8*	-1.1	-2.8
ESR1	-1.8	-5.0	*MECP2*	-1.1	-3.9
HOXB6	-1.7	-2.3	*CREB5*	-1.1	-2.0
DLX4	-1.7	-3.9	*FOXA2*	-1.0	-3.8
TBX2	-1.7	-2.8	*PBX2*	-1.0	-3.4
HOXC6	-1.6	-2.9	*DLX1*	-1.0	-3.8
NR4A2	-1.6	-5.3	*NKX2-1*	-1.0	-3.9
RHOXF1	-1.6	-3.9	*PAX5*	-1.0	-2.1
TFCP2L1	-1.6	-3.3	*FOXL2*	-0.9	-2.3
HOXC5	-1.6	-2.7	*POU3F2*	-0.9	-3.1
DLX6	-1.5	-2.5	*TBX3*	-0.9	-3.6
MEOX1	-1.5	-3.6	*FEV*	-0.9	-2.9
CX3CL1	-1.5	-2.5	*MBD1*	-0.9	-3.3
IL11	-1.5	-2.2	*TGIF1*	-0.8	-2.6
HOXA11	-1.5	-2.3	*ZNF639*	-0.8	-2.2
HOXD8	-1.5	-2.5	*PRDM1*	-0.8	-3.8
SPIB	-1.5	-2.5	*POU4F3*	-0.8	-2.0
ETS1	-1.4	-4.2	*EGR2*	-0.7	-5.3
TEAD3	-1.4	-2.6	*KLF3*	-0.7	-2.6
HOXB5	-1.4	-2.0	*FAM3D*	-0.6	-2.7
DMRT1	-1.4	-2.4	*MIF*	-0.6	-2.6
HNF1A	-1.4	-2.3	*ARGFX*	-0.4	-3.5
TNFSF4	-1.4	-2.2	*CMTM2*	-0.4	-2.4
RORB	-1.4	-2.2	*PRRX2*	0.0	-2.1
ZIC3	-1.4	-2.6	*BMP2*	0.5	-3.2
IL26	-1.4	-3.1	*CXCL13*	0.6	-2.0
CSRNP3	-1.4	-2.9	*ATOH1*	1.2	-2.2
TARDBP	-1.4	-2.5	*PTGER3*	1.2	-2.3
ELF4	-1.3	-2.1	*GDF2*	1.3	-3.0
HOXB13	-1.3	-2.3	*PPARG*	1.6	-4.4
CEBPB	-1.3	-2.1	*CXCL5*	2.5	-3.8
NR2E1	-1.3	-4.1	*NR0B2*	2.8	-2.2
SMAD3	-1.3	-2.1	*CRX*	7.5	-3.6
ALX1	-1.3	-3.2	*NR2F1*	9.4	-2.8
NFIC	-1.3	-2.0	*OTX2*	9.5	-2.3
FOXA1	-1.3	-2.1			

Genes with Z-scores of αMHC-GFP or cTnT expression ≥2 were defined as activators.
Genes with Z-scores of αMHC or cTnT expression ≤-2 were defined as inhibitors.

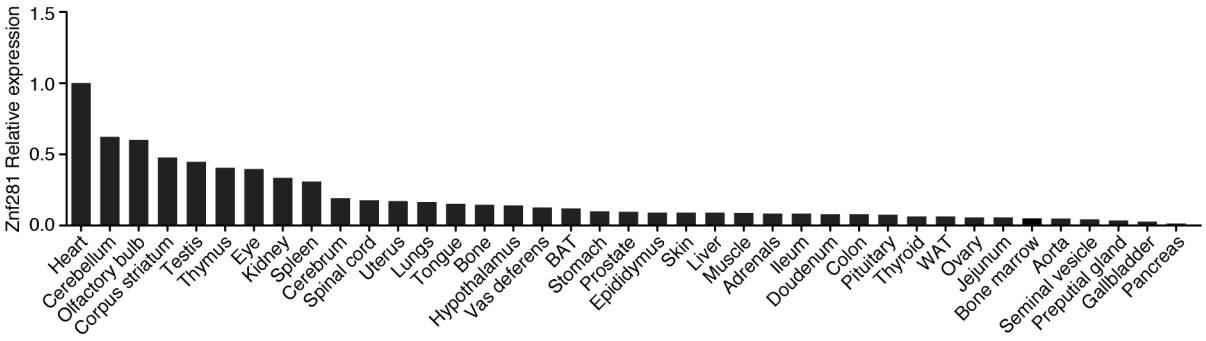
Supplemental Table S3. q-PCR primer list

Gene	Forward (5'-3')	Reverse (5'-3')
Myh6	GCCCAGTACCTCCGAAAGTC	GCCTTAACATACTCCTCCTTGTC
Actc1	CGGACAATTTCACGTTCAGCA	CTGGATTCTGGCGATGGTGTA
Nppa	GCTTCCAGGCCATATTGGAG	GGGGGCATGACCTCATCTT
Col1a2	TCGTGCCTAGCAACATGCC	TTTGTCAGAATACTGAGCAGCAA
Sox9	AGTACCCGCATCTGCACAAC	ACGAAGGGTCTCTTCTCGCT
IL6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Ptgs1	GATTGTACTCGCACGGGCTAC	GGATAAGGTTGGACCGCACT
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

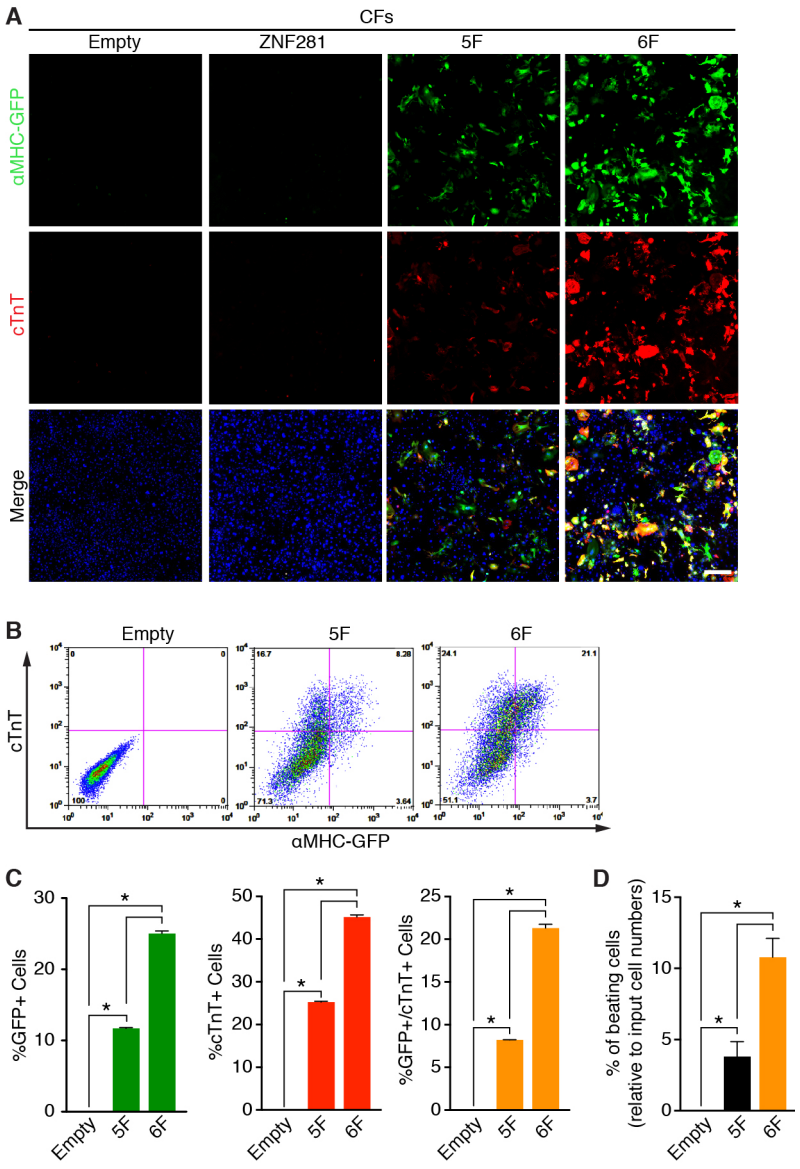
Supplemental Table S4. RNA-seq and ChIP-seq deposited data

Experiment	SRA accession number
GATA4_6F (ChIP-seq)	SAMN07312820
GATA4_6F_minus ZNF281(ChIP-seq)	SAMN07312821
ZNF281_6F (ChIP-seq)	SAMN07312822
ZNF281_6F_minus GATA4(ChIP-seq)	SAMN07312823
IgG control (ChIP-seq)	SAMN07312824
AGHMT_plus_E_Rep1 (RNA-seq)	SAMN07312825
AGHMT_plus_E_Rep2 (RNA-seq)	SAMN07312826
AGHMT_plus_E_Rep3 (RNA-seq)	SAMN07312827
AGHMT_plus_ZNF281_Rep1 (RNA-seq)	SAMN07312828
AGHMT_plus_ZNF281_Rep2 (RNA-seq)	SAMN07312829
AGHMT_plus_ZNF281_Rep3 (RNA-seq)	SAMN07312830

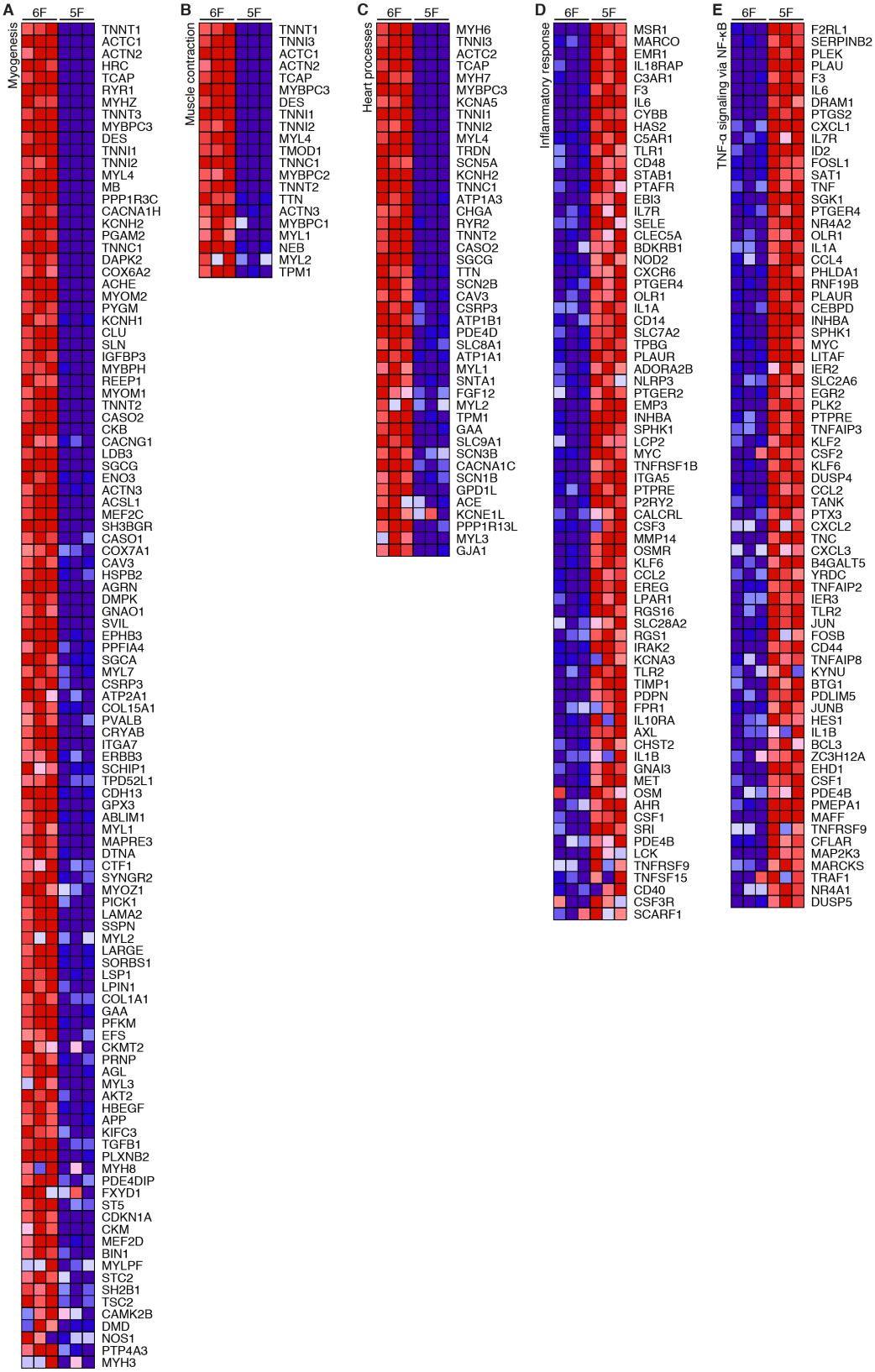
Supplemental Figures



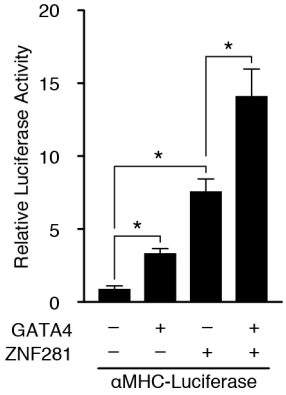
Supplemental Figure S1. ZNF281 mRNA expression profile. Transcript levels of ZNF281 in different tissues isolated from adult wild type C57BL6 mice, as determined with quantitative PCR.



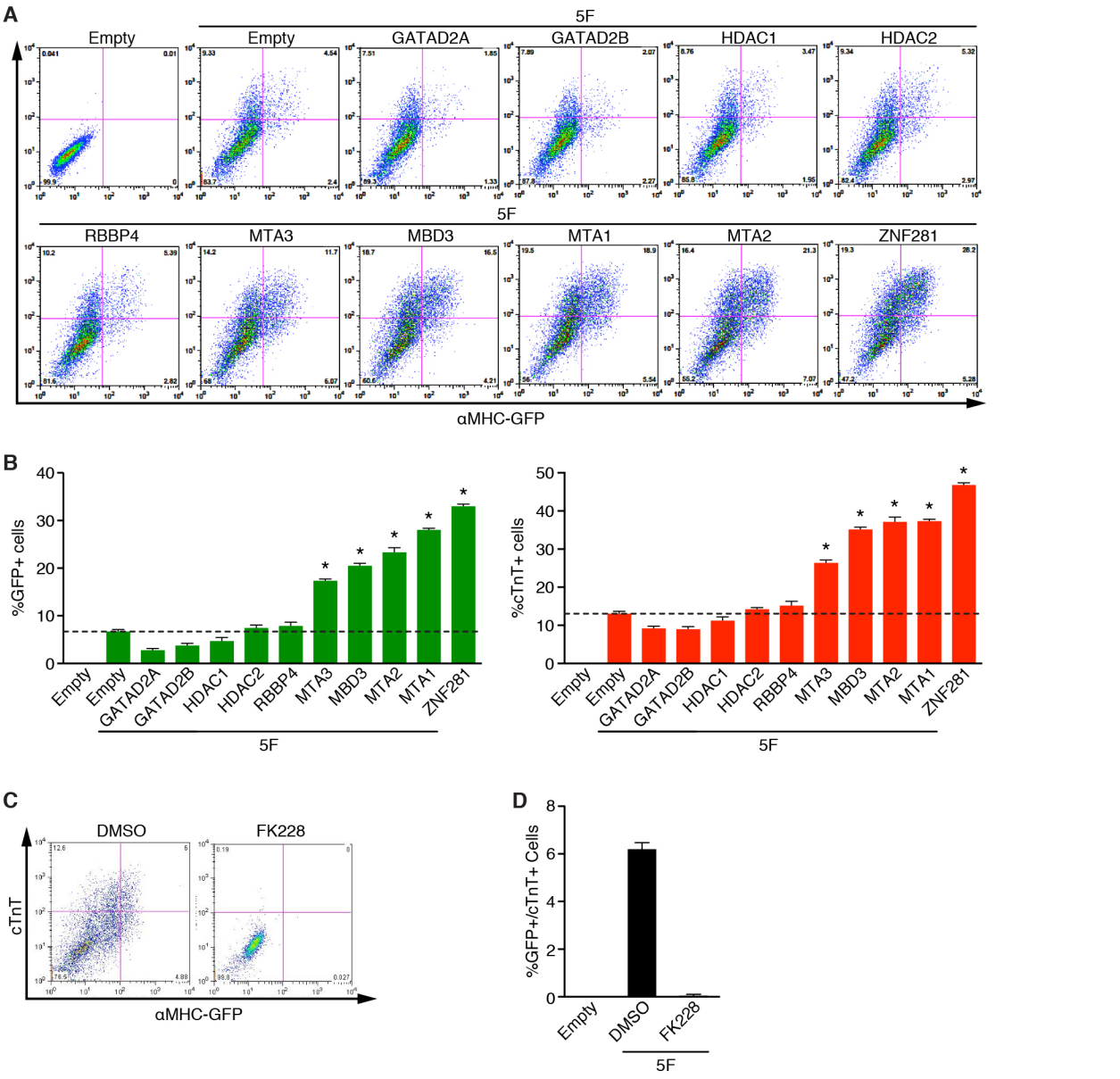
Supplemental Figure S2. ZNF281 enhances cardiac reprogramming of adult cardiac fibroblasts. (A) Immunocytochemistry images of adult αMHC-GFP transgenic CFs 7 days post-infection with Empty, ZNF281, 5F, or 6F (5F+ZNF281) retroviruses. αMHC-GFP (green), cTnT (red), Hoechst (blue). Scale bars: 500 μm. (B-C) Representative flow cytometry plot (B) and analyses (C) of αMHC-GFP+ and cTnT+ CFs 7 days post-infection with Empty, 5F, or 6F retrovirus. * P<0.05. (D) Percentage of beating cells, relative to the number of input cells after 4 weeks post-infection with Empty, ZNF281, 5F, or 6F (5F+ZNF281) retroviruses.


A
6F
5F
Myogenesis
TNNT1
ACTC1
ACTN2
HRC
TCAP
RYR1
MYHZ
TNNT3
MYBPC3
DES
TNNI1
TNNI2
MYL4
MB
PPP1R3C
CACNA1H
KCNH2
PGAM2
TNNC1
DAPK2
COX6A2
ACHE
MYOM2
PYGM
KCNH1
CLU
SLN
IGFBP3
MYBPH
REEP1
MYOM1
TNNT2
CASQ2
CKB
CACNG1
LDB3
SGCG
ENO3
ACTN3
ACSL1
MEF2C
SH3BGR
CASQ1
COX7A1
CAV3
HSPB2
AGRN
DMPK
GNAO1
SVIL
EPHB3
PPFIA4
SGCA
MYL7
CSRP3
ATP2A1
COL15A1
PVALB
CRYAB
ITGA7
ERBB3
SCHIP1
TPD52L1
CDH13
GPX3
ABLIM1
MYL1
MAPRE3
DTNA
CTF1
SYNGR2
MYOZ1
PICK1
LAMA2
SSPN
MYL2
LARGE
SORBS1
LSP1
LPIN1
COL1A1
GAA
PFKM
EFS
CKMT2
PRNP
AGL
MYL3
AKT2
HBEGF
APP
KIFC3
TGFB1
PLXNB2
MYH8
PDE4DIP
FXYD1
ST5
CDKN1A
CKM
MEF2D
BIN1
MYLPF
STC2
SH2B1
TSC2
CAMK2B
DMD
NOS1
PTP4A3
MYH3
B
6F
5F
Muscle contraction
TNNT1
TNNI3
ACTC1
ACTN2
TCAP
MYBPC3
DES
TNNI1
TNNI2
MYL4
TMOD1
TNNC1
MYBPC2
TNNT2
TTN
ACTN3
MYBPC1
MYL1
NEB
MYL2
TPM1
C
6F
5F
Heart processes
MYH6
TNNI3
ACTC2
TCAP
MYH7
MYBPC3
KCNA5
TNNI1
TNNI2
MYL4
TRDN
SCN5A
KCNH2
TNNC1
ATP1A3
CHGA
RYR2
TNNT2
CASQ2
SGCG
TTN
SCN2B
CAV3
CSRP3
ATP1B1
PDE4D
SLC8A1
ATP1A1
MYL1
SNTA1
FGF12
MYL2
TPM1
GAA
SLC9A1
SCN3B
CACNA1C
SCN1B
GPD1L
ACE
KCNE1L
PPP1R13L
MYL3
GJA1
D
6F
5F
Inflammatory response
MSR1
MARCO
EMR1
IL18RAP
C3AR1
F3
IL6
CYBB
HAS2
C5AR1
TLR1
CD48
STAB1
PTAFR
EBI3
IL7R
SELE
CLEC5A
BDKRB1
NOD2
CXCR6
PTGER4
OLR1
IL1A
CD14
SLC7A2
TPBG
PLAUR
ADORA2B
NLRP3
PTGER2
EMP3
INHBA
SPHK1
LCP2
MYC
TNFRSF1B
ITGA5
PTPRE
P2RY2
CALCRL
CSF3
MMP14
OSMR
KLF6
CCL2
EREG
LPAR1
RGS16
SLC28A2
RGS1
IRAK2
KCNA3
TLR2
TIMP1
PDPN
FPR1
IL10RA
AXL
CHST2
IL1B
GNAI3
MET
OSM
AHR
CSF1
SRI
PDE4B
LCK
TNFRSF9
TNFSF15
CD40
CSF3R
SCARF1
E
6F
5F
TNF-α signaling via NF-κB
F2RL1
SERPINB2
PLEK
PLAU
F3
IL6
DRAM1
PTGS2
CXCL1
IL7R
ID2
FOSL1
SAT1
TNF
SGK1
PTGER4
NR4A2
OLR1
IL1A
CCL4
PHLDA1
RNF19B
PLAUR
CEBPD
INHBA
SPHK1
MYC
LITAF
IER2
SLC2A6
EGR2
PLK2
PTPRE
TNFAIP3
KLF2
CSF2
KLF6
DUSP4
CCL2
TANK
PTX3
CXCL2
TNC
CXCL3
B4GALT5
YRDC
TNFAIP2
IER3
TLR2
JUN
FOSB
CD44
TNFAIP8
KYNU
BTG1
PDLIM5
JUNB
HES1
IL1B
BCL3
ZC3H12A
EHD1
CSF1
PDE4B
PMEPA1
MAFF
TNFRSF9
CFLAR
MAP2K3
MARCKS
TRAF1
NR4A1
DUSP5

Supplemental Figure S3. Gene set enrichment analyses of induced by ZNF281 in cardiac reprogramming. (A-E) Heat maps of the indicated gene-set were generated with the genes that constitute the core enrichment group.



Supplemental Figure S4. Luciferase reporter assays were performed using HEK293 cells transfected with equal amounts of ZNF281 and/or GATA4 expression plasmids, as indicated, along with αMHC-luciferase reporter plasmid. * $P<0.05$.



Supplemental Figure S5. ZNF281 represses the inflammatory response through the NuRD complex. (A) Representative flow cytometry plot of TTFs 7 days post-infection with Empty, 5F plus Empty, ZNF281 or each individual NuRD complex subunit retroviruses. (B) Quantification of αMHC-GFP+ and cTnT+ cells from flow cytometry plots from A. (C) Representative flow cytometry plot of TTFs 7 days post-infection with Empty, 5F plus DMSO and 5F plus FK228. (D) Quantification of αMHC-GFP+ and cTnT+ cells from flow cytometry plots from C.