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Supplementary Materials for

Histone deacetylases 1 and 2 silence cryptic transcription to promote mitochondrial function during cardiogenesis

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This PDF file includes:

Supplementary Materials

Materials and Methods

Mitochondrial DNA (mtDNA)/Nuclear DNA (nDNA) Quantification

Primers for mtDNA/nDNA Quantification		
Target	**Forward (5' – 3')**	**Reverse (5' – 3')**
mMito	CTAGAAACCCCGAAACCAAA	CCAGCTATCACCAAGCTCGT
nDNA	ATGGGAAGCCGAACATACTG	CAGTCTCAGTGGGGGTGAAT

Primers taken from: Malik, Afshan N., Anna Czajka, and Phil Cunningham. 2016. "Accurate Quantification of Mouse Mitochondrial DNA without Co-Amplification of Nuclear Mitochondrial Insertion Sequences." *Mitochondrion* 29 (July): 59–64.

Rapid Amplification of cDNA Ends (RACE)

Primers for 5'RACE	
Target	**Reverse ((5' – 3'))**
CS (Exon 5)	GGCTGCAACGCACGGCAGCTTGGCA
Ndufb9 (Exon 4)	TGGGCCGTTCCCGAGGTCTGGTCACA

Chromatin Immunoprecipitation Quantitative PCR (ChIP-qPCR)

Primers for ChIP-qPCR Analysis		
Target	**Forward (5' – 3')**	**Reverse (5' – 3')**
CS TSS^{C}	CCATTTTGGGCCAATGGTCG	GGGGAAGGGTAGACAAACCG
CS TSS^{A}	AAGTGCCACACTGTAAGCCT	GGACAGCCTGAGTGAGTTTGA
Ndufb9 TSS^{C}	GAGGCTCACGACCAAGACAA	GGAACCGGAAGGACGAGC
Ndufb9 TSS^{A1}	TGCACCGTGCTGTTACTGAT	ACCTCATCAACTTCAGGCCG
Ndufb9 TSS^{A2}	AGCAGCCCCTTGAGTTTTGT	GCTTTCTCAGAGGGATGCCA

Quantitative Reverse Transcription PCR (RT-qPCR)

Primers for Early/Late Exon Quantification		
Target	**Forward (5' – 3')**	**Reverse (5' – 3')**
Gapdh	ATGTTCCAGTATGACTCCACTCACG	GAAGACACCAGTAGACTCCACGACA
CS Early	TTGGGAGCCAAGAACTCATCCTG	TCATGTCCACAGTGATCTGGC
CS Late 6-7	ACAGTGAAAGCAACTTCGCC	TCAATGGCTCCGATACTGCTG
Ndufb9 Early	GAAGGTGCTGCGGCTGTATAA	TACCGGTACTTGTCCCTGTGG
Ndufb9 Late	GGCATCCCTCTGAGAAAGCA	GCTTAACCTCCCGATCCCAG

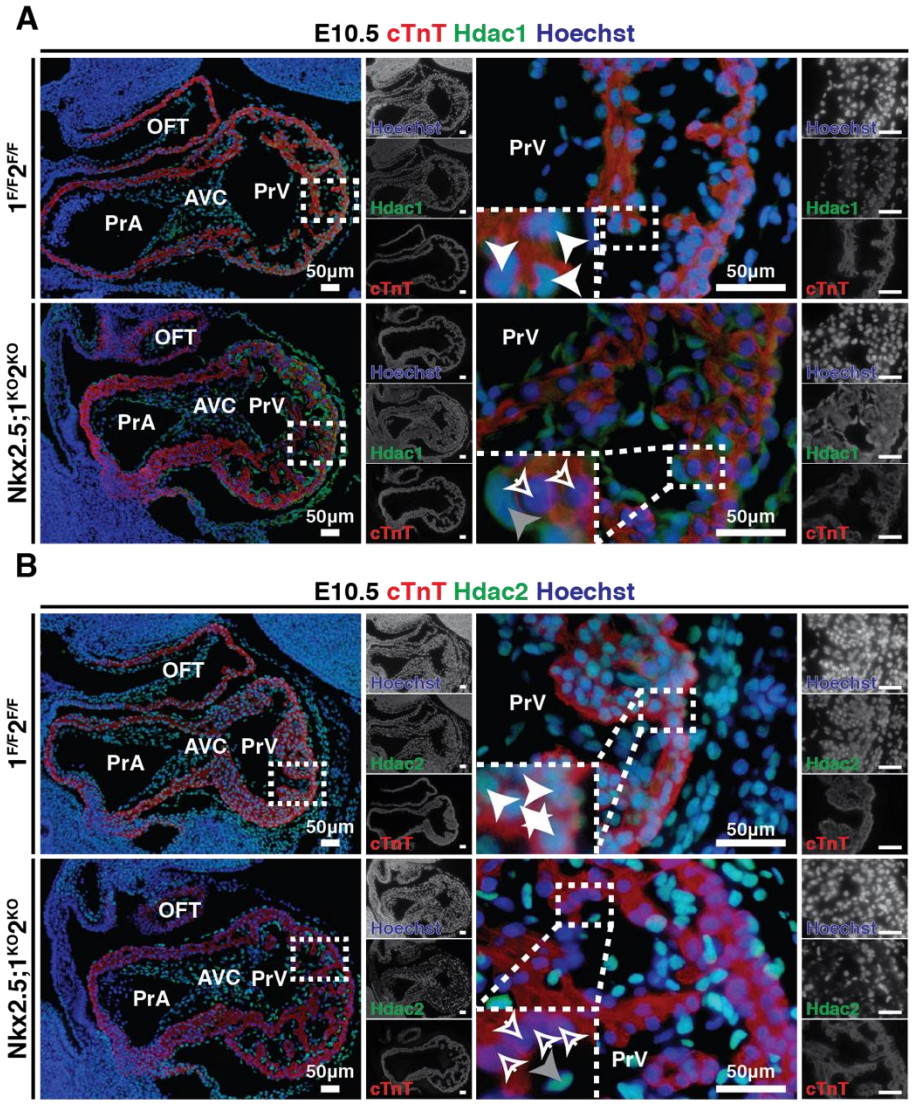

Fig. S1. Complete loss of Hdac1 and Hdac2 in primitive heart tube cardiomyocytes by E10.5.

(A-B) Hdac1 (A) and Hdac2 (B) staining on *Nkx2.5;1^{KO}2^{KO}* and *1^{FF}2^{FF}* E10.5 sagittal sections at AVC level with cardiac troponin (cTnT) and Hoechst nuclear counterstain (solid white arrows, Hdac1^{+} or Hdac2^{+} cardiomyocytes; hollow white arrows, Hdac1^{-} or Hdac1^{-} cardiomyocytes; gray arrows, Hdac1^{+}/cTnT^{-} or Hdac2^{+}/cTnT^{-} cells). Grayscale images are unedited. OFT, outflow tract; PrA, primitive atria; AVC, atrioventricular canal; PrV, primitive ventricle.

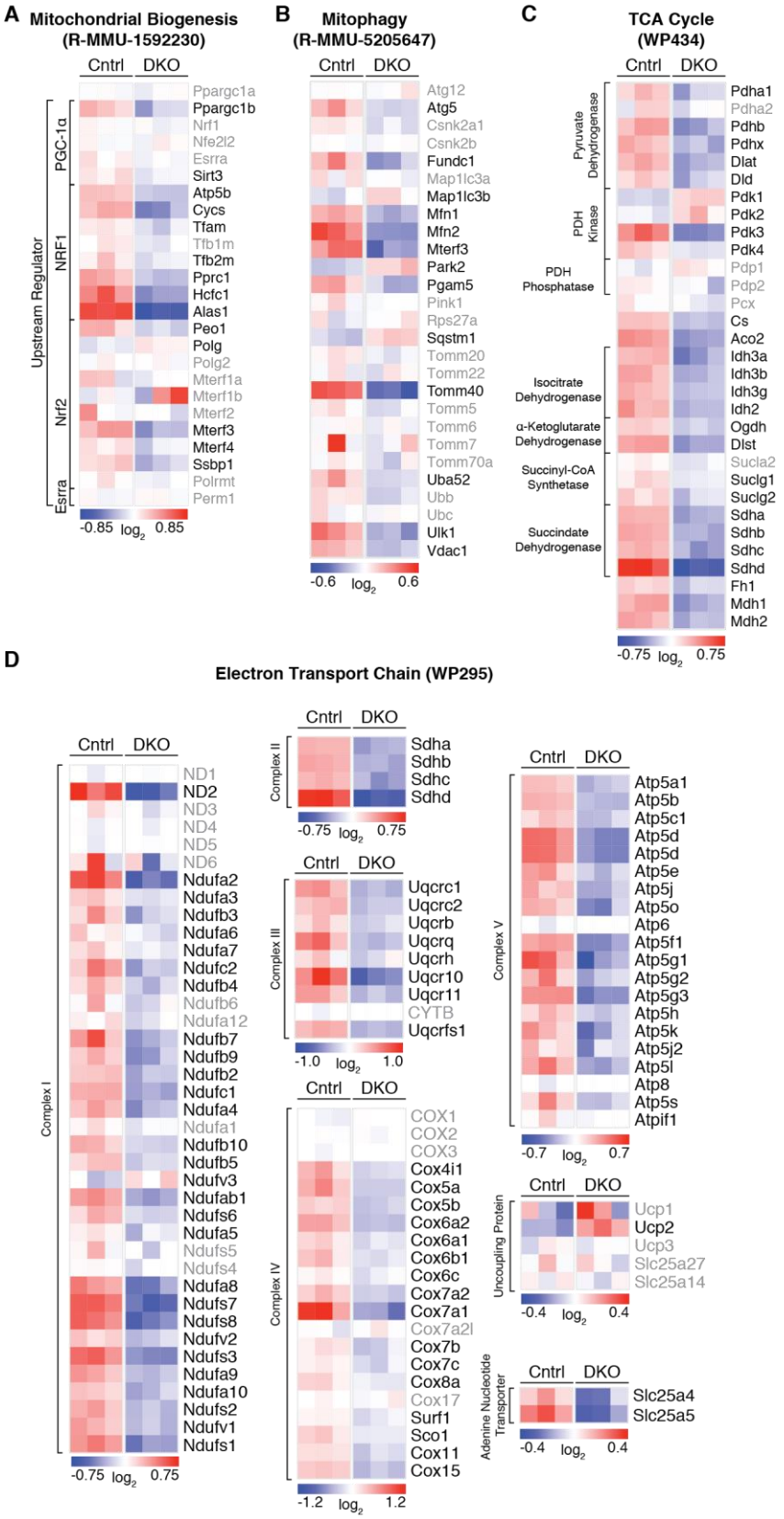
A
Mitochondrial Biogenesis
(R-MMU-1592230)
Cntrl
DKO
Upstream Regulator
PGC-1α
NRF1
Nrf2
Esrra
Ppargc1a
Ppargc1b
Nrf1
Nfe2l2
Esrra
Sirt3
Atp5b
Cycs
Tfam
Tfb1m
Tfb2m
Pprc1
Hcfc1
Alas1
Peo1
Polg
Polg2
Mterf1a
Mterf1b
Mterf2
Mterf3
Mterf4
Ssbp1
Polrmt
Perm1
-0.85 log2 0.85
B
Mitophagy
(R-MMU-5205647)
Cntrl
DKO
Atg12
Atg5
Csnk2a1
Csnk2b
Fundc1
Map1lc3a
Map1lc3b
Mfn1
Mfn2
Mterf3
Park2
Pgam5
Pink1
Rps27a
Sqstm1
Tomm20
Tomm22
Tomm40
Tomm5
Tomm6
Tomm7
Tomm70a
Uba52
Ubb
Ubc
Ulk1
Vdac1
-0.6 log2 0.6
C
TCA Cycle
(WP434)
Cntrl
DKO
Pyruvate Dehydrogenase
PDH Kinase
PDH Phosphatase
Isocitrate Dehydrogenase
α-Ketoglutarate Dehydrogenase
Succinyl-CoA Synthetase
Succindate Dehydrogenase
Pdha1
Pdha2
Pdhb
Pdhx
Dlat
Dld
Pdk1
Pdk2
Pdk3
Pdk4
Pdp1
Pdp2
Pcx
Cs
Aco2
Idh3a
Idh3b
Idh3g
Idh2
Ogdh
Dlst
Sucla2
Suclg1
Suclg2
Sdha
Sdhb
Sdhc
Sdhd
Fh1
Mdh1
Mdh2
-0.75 log2 0.75
D
Electron Transport Chain (WP295)
Cntrl
DKO
Complex I
ND1
ND2
ND3
ND4
ND5
ND6
Ndufa2
Ndufa3
Ndufb3
Ndufa6
Ndufa7
Ndufc2
Ndufb4
Ndufb6
Ndufa12
Ndufb7
Ndufb9
Ndufb2
Ndufc1
Ndufa4
Ndufa1
Ndufb10
Ndufb5
Ndufv3
Ndufab1
Ndufs6
Ndufa5
Ndufs5
Ndufs4
Ndufa8
Ndufs7
Ndufs8
Ndufv2
Ndufs3
Ndufa9
Ndufa10
Ndufs2
Ndufv1
Ndufs1
-0.75 log2 0.75
Complex II
Sdha
Sdhb
Sdhc
Sdhd
-0.75 log2 0.75
Complex III
Uqcrc1
Uqcrc2
Uqcrb
Uqcrq
Uqcrh
Uqcr10
Uqcr11
CYTB
Uqcrfs1
-1.0 log2 1.0
Complex IV
COX1
COX2
COX3
Cox4i1
Cox5a
Cox5b
Cox6a2
Cox6a1
Cox6b1
Cox6c
Cox7a2
Cox7a1
Cox7a2l
Cox7b
Cox7c
Cox8a
Cox17
Surf1
Sco1
Cox11
Cox15
-1.2 log2 1.2
Complex V
Atp5a1
Atp5b
Atp5c1
Atp5d
Atp5d
Atp5e
Atp5j
Atp5o
Atp6
Atp5f1
Atp5g1
Atp5g2
Atp5g3
Atp5h
Atp5k
Atp5j2
Atp5l
Atp8
Atp5s
Atpif1
-0.7 log2 0.7
Uncoupling Protein
Ucp1
Ucp2
Ucp3
Slc25a27
Slc25a14
-0.4 log2 0.4
Adenine Nucleotide Transporter
Slc25a4
Slc25a5
-0.4 log2 0.4

Fig. S2. Loss of Hdac1/Hdac2 causes downregulation of metabolic and cardiac gene programs without concordant upstream changes.

(A) Heatmap of Mitochondrial Biogenesis Reactome gene set (R-MMU-1592230). (B) Heatmap of Mitophagy Reactome gene set (R-MMU-5205647). (C) Heatmap of Tricarboxylic Acid Cycle (TCA Cycle) WikiPathways gene set (WP434). (D) Heatmap of Electron Transport Chain WikiPathways gene set (WP295) separated by individual chain components/complexes. Black text, significantly changed (FDR<0.05); gray text, not significantly changed (FDR>0.05).

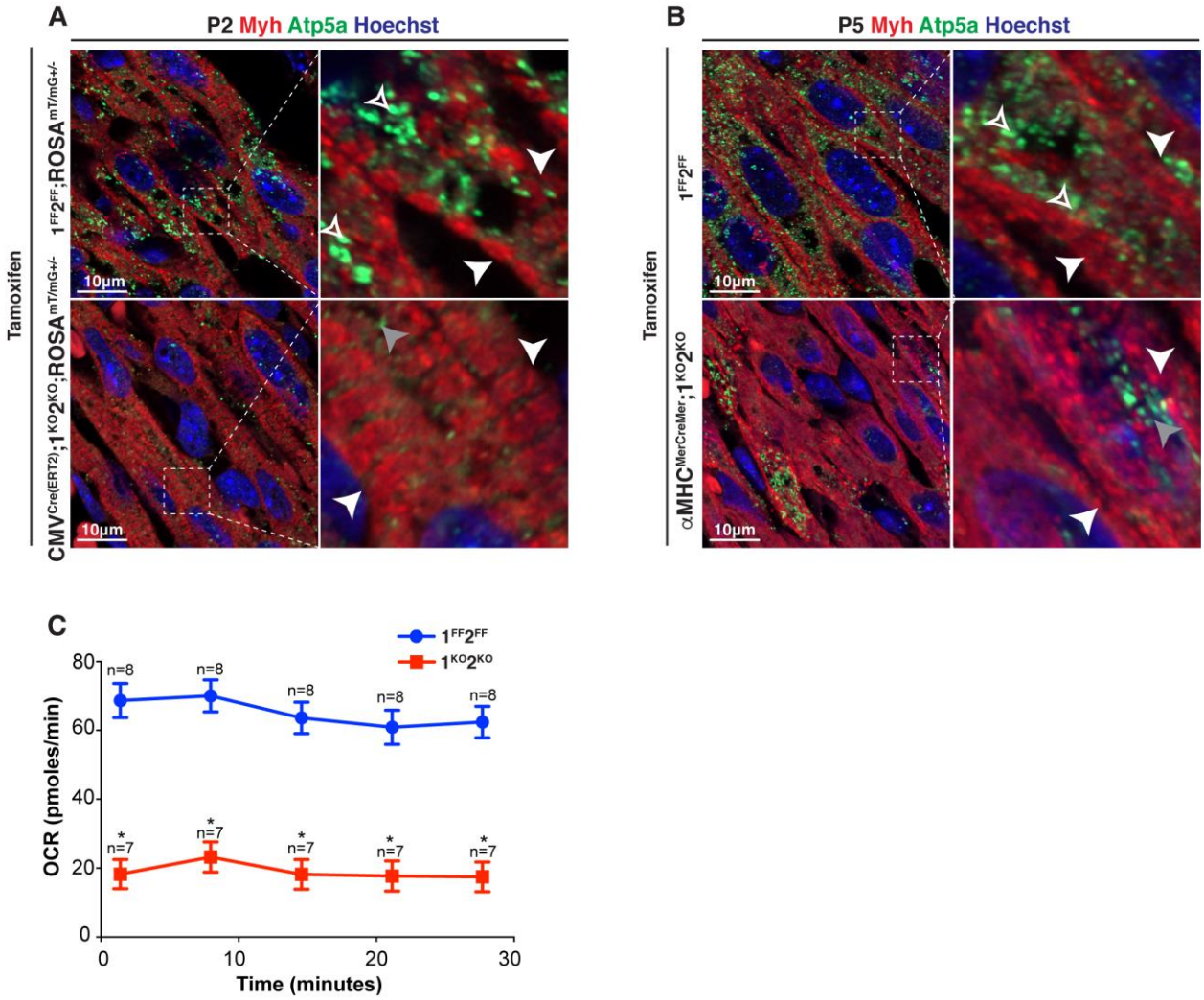

Fig. S3. Inducible loss of Hdac1 and Hdac2 in postnatal hearts lead to downregulation of critical mitochondrial protein and reduced oxygen consumption.

(A-B) Atp5a (Green) and Myh (Red) co-staining on tamoxifen treated *CMV*$^{Cre(ERT2)}$;*1*^{KO}*2*^{KO};*ROSA*$^{mTmG+/-}$ (A), *aMHC*^{MerCreMer};*1*^{KO}*2*^{KO} (B), and *1*^{FF}*2*^{FF} (A-B) P2 (A) and P5 (B) heart frontal sections with Hoechst nuclear counterstain. (solid white arrows, Myh^{+} contractile network; hollow white arrows, Atp5a^{+} cardiomyocytes; gray arrows, reduced Atp5a expression in cardiomyocytes). (C) Oxygen consumption rate (OCR) at multiple time points in neonatal P2 cardiomyocytes derived from P0 *1*^{FF}*2*^{FF} hearts infected with control virus (*1*^{FF}*2*^{FF}) or with Cre-expressing virus (*1*^{KO}*2*^{KO}). Data represent the mean ± SEM. *P<0.00003. Statistical significance determined using the Holm-Sidak method, with alpha = 0.05.

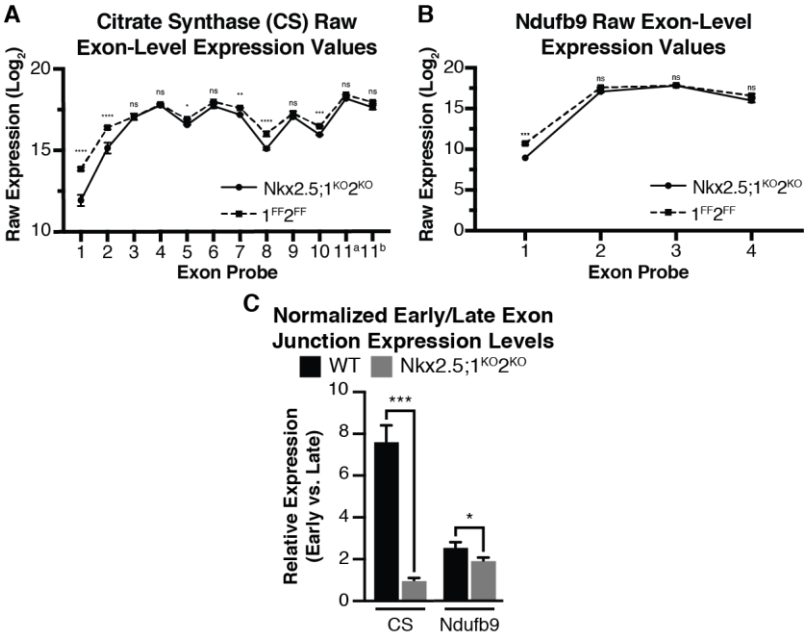

Fig. S4. Loss of Hdac1/Hdac2 causes downregulation of metabolic and cardiac gene programs without concordant upstream changes.

(A-B) Raw exon-level expression values (log_2) for citrate synthase (*CS*, A) and *Ndufb9* (B) in *Nkx2.5;1^{KO}2^{KO}* and *1^{FF}2^{FF}* E10.5 PHTs. (C) RT-qPCR showing the relative abundance of *CS* or *Ndufb9* transcripts spanning an early exon junction (*CS* – Exon 1-2; *Ndufb9* – Exon 1-2) compared to late exon junction (*CS* – Exon 6-7; *Ndufb9* – Exon 3-4) in *Nkx2.5;1^{KO}2^{KO}* and *1^{++}2^{++}* E10.5 PHTs.

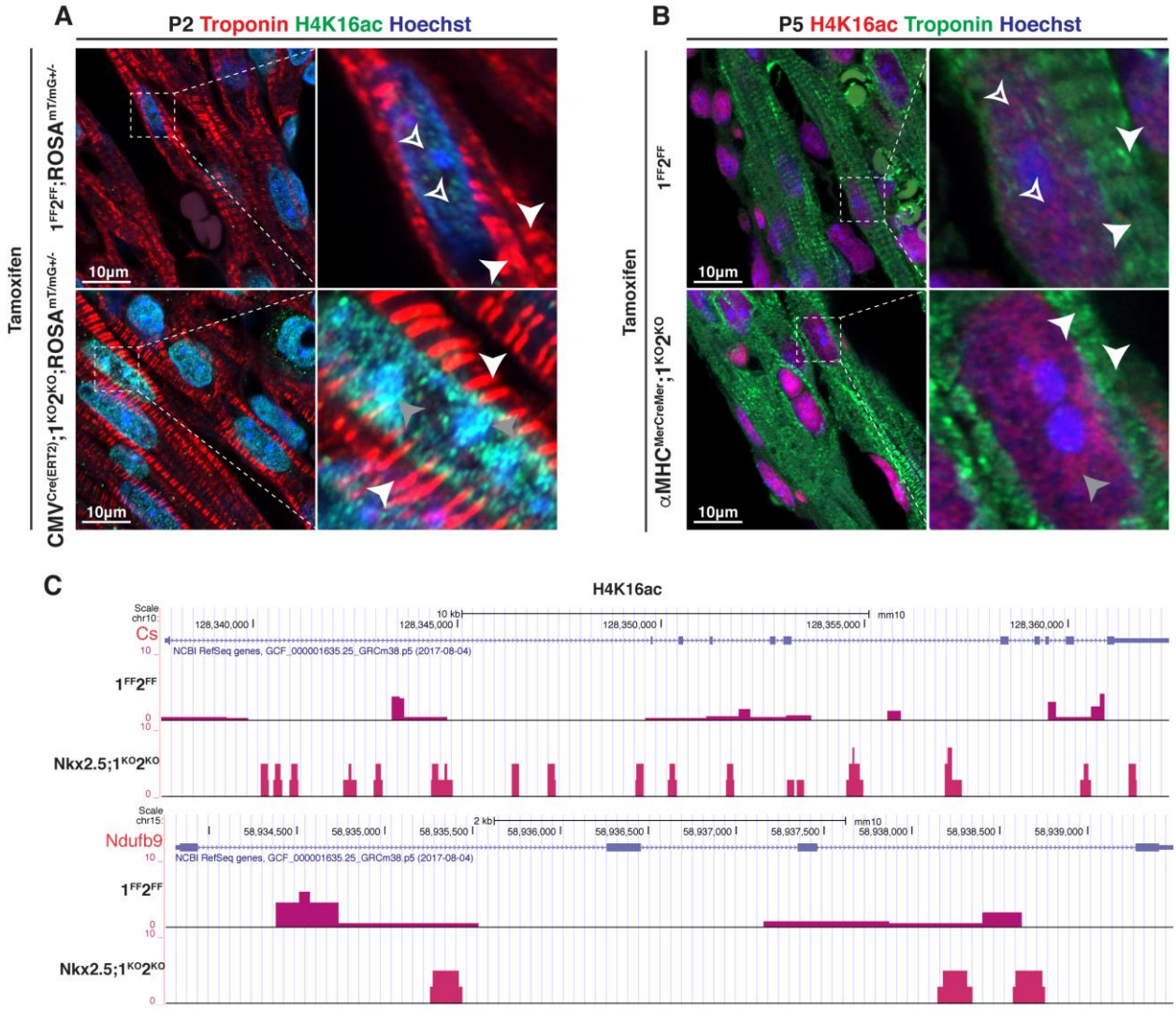

Fig. S5. Inducible loss of Hdac1 and Hdac2 in postnatal hearts leads to increased H4K16ac. (A-B) H4K16 and Troponin co-staining on tamoxifen treated *CMV*$^{Cre(ERT2)}$*;1*^{KO}*2*^{KO}*;ROSA*$^{mTmG+/-}$ (A), *αMHC*^{MerCreMer}*;1*^{KO}*2*^{KO} (B), and *1*^{FF}*2*^{FF} (A-B) P2 (A) and P5 (B) heart frontal sections with Hoechst nuclear counterstain. (solid white arrows, Troponin^{+} cardiomyocytes; hollow white arrows, H4K16ac^{+} cardiomyocytes; gray arrows, increased H4K16ac foci in cardiomyocytes). (C) Browser tracks of *Cs* (top) and *Ndufb9* (bottom) showing H4K16ac enrichment in *1*^{FF}*2*^{FF} and *Nkx2.5;1*^{KO}*2*^{KO} E10.5 primitive heart tubes.