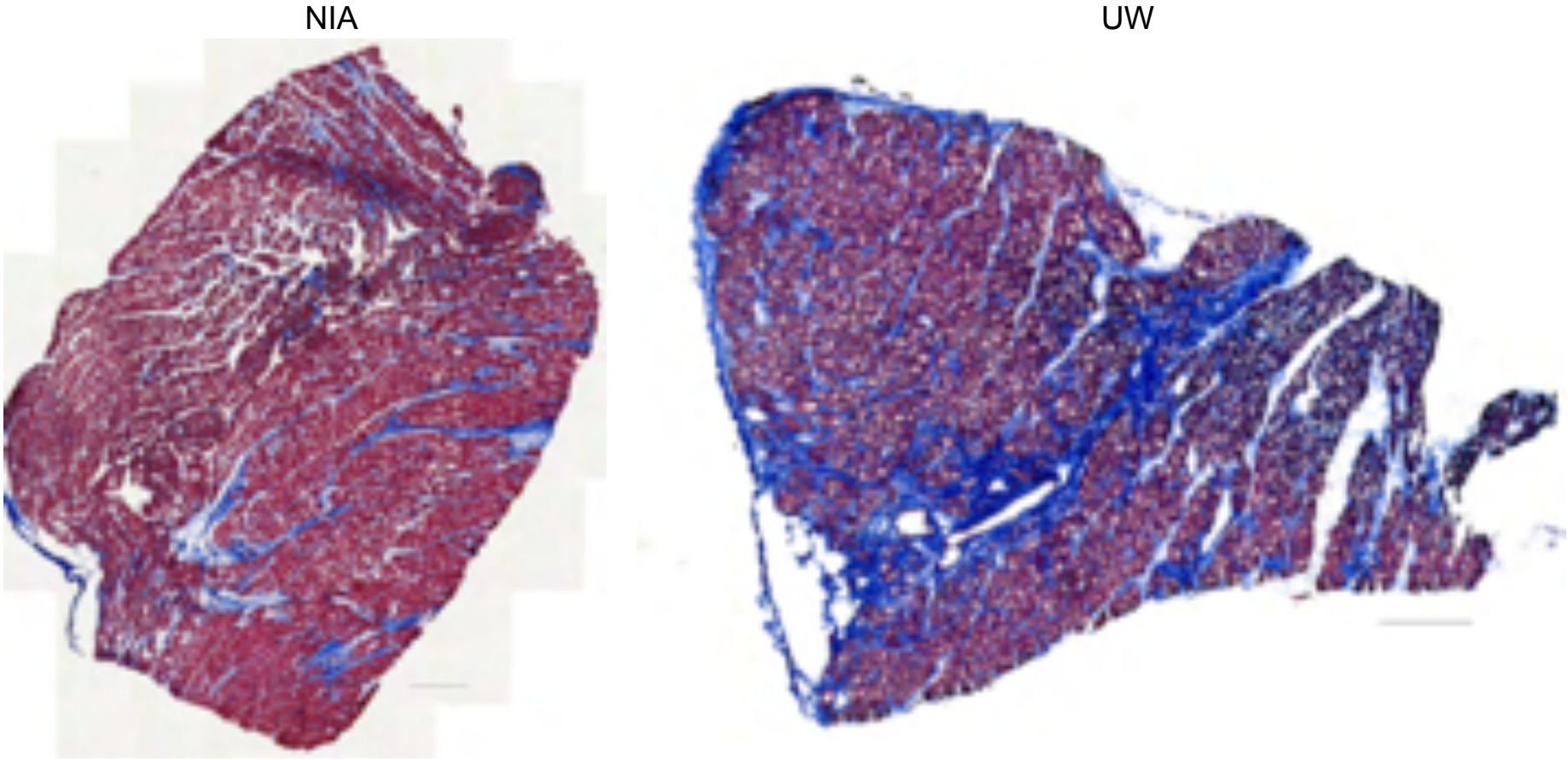
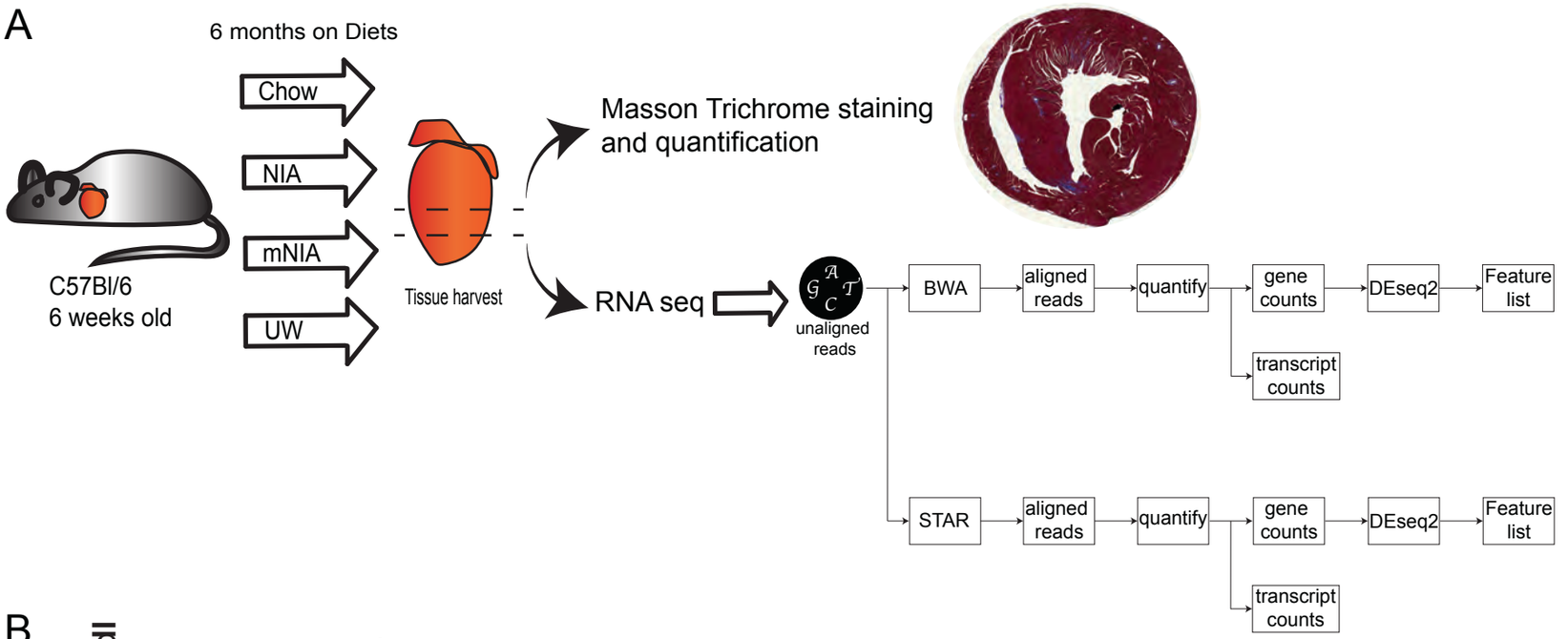
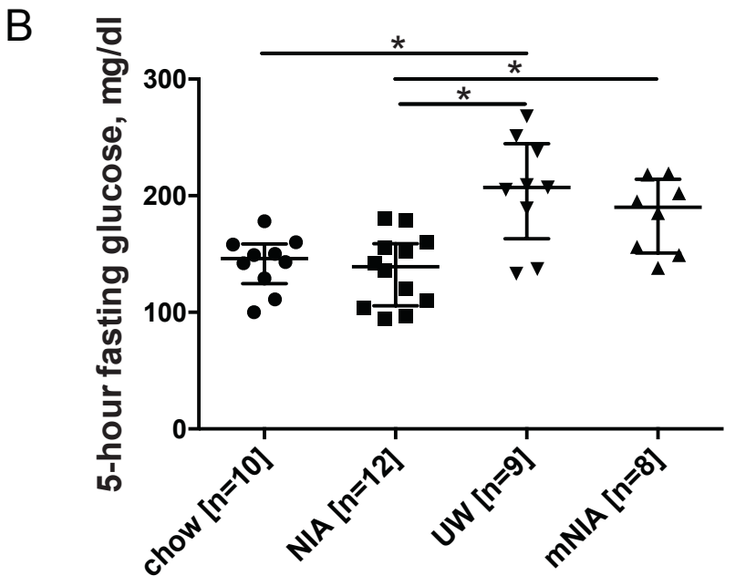
Figure S1

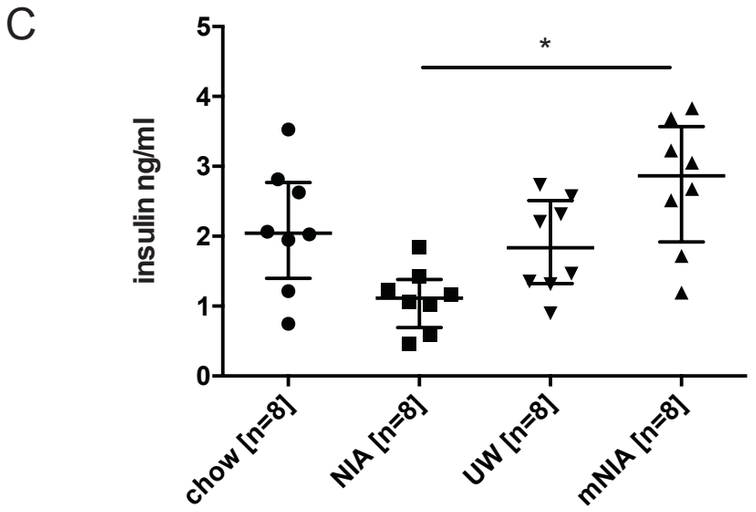


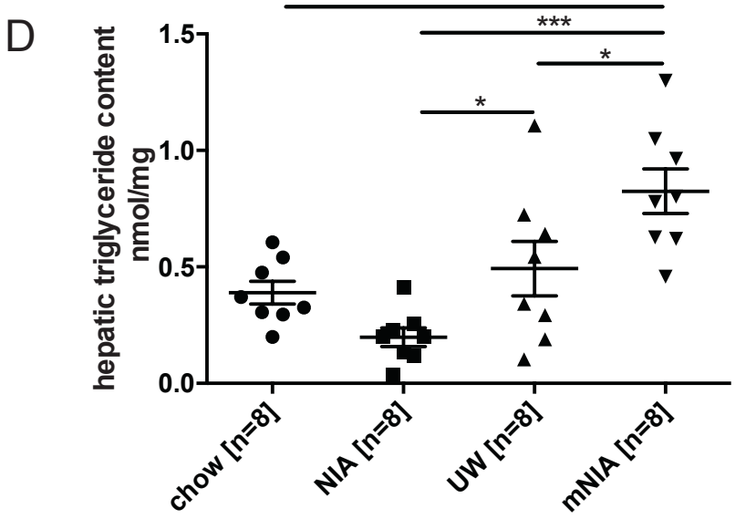
Supplementary Figure 1. Representative images of whole Masson Trichrome stained left ventricle tissues from an NIA and UW NHP from the longitudinal CR study cohorts, scale bar: 1000μm

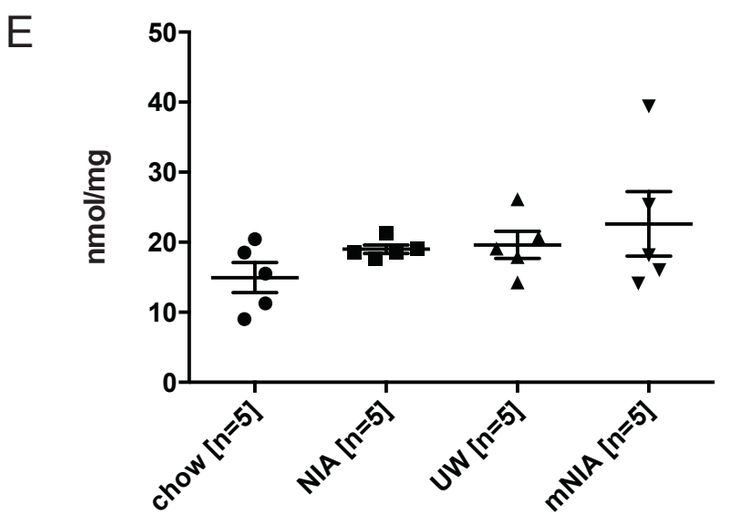
Figure S2


A
6 months on Diets
Chow
NIA
mNIA
UW
C57Bl/6
6 weeks old
Tissue harvest
Masson Trichrome staining
and quantification
RNA seq
unaligned
reads
BWA
aligned
reads
quantify
gene
counts
DEseq2
Feature
list
transcript
counts
STAR
aligned
reads
quantify
gene
counts
DEseq2
Feature
list
transcript
counts


B
5-hour fasting glucose, mg/dl
300
200
100
0
*
*
*
chow [n=10]
NIA [n=12]
UW [n=9]
mNIA [n=8]


C
insulin ng/ml
5
4
3
2
1
0
*
chow [n=8]
NIA [n=8]
UW [n=8]
mNIA [n=8]


D
hepatic triglyceride content
nmol/mg
1.5
1.0
0.5
0.0
**
***
*
*
chow [n=8]
NIA [n=8]
UW [n=8]
mNIA [n=8]


E
nmol/mg
50
40
30
20
10
0
chow [n=5]
NIA [n=5]
UW [n=5]
mNIA [n=5]

Supplementary Figure 2:

A: Experimental setup for the investigation of long-term high sucrose consumption on cardiac fibrosis in mice.

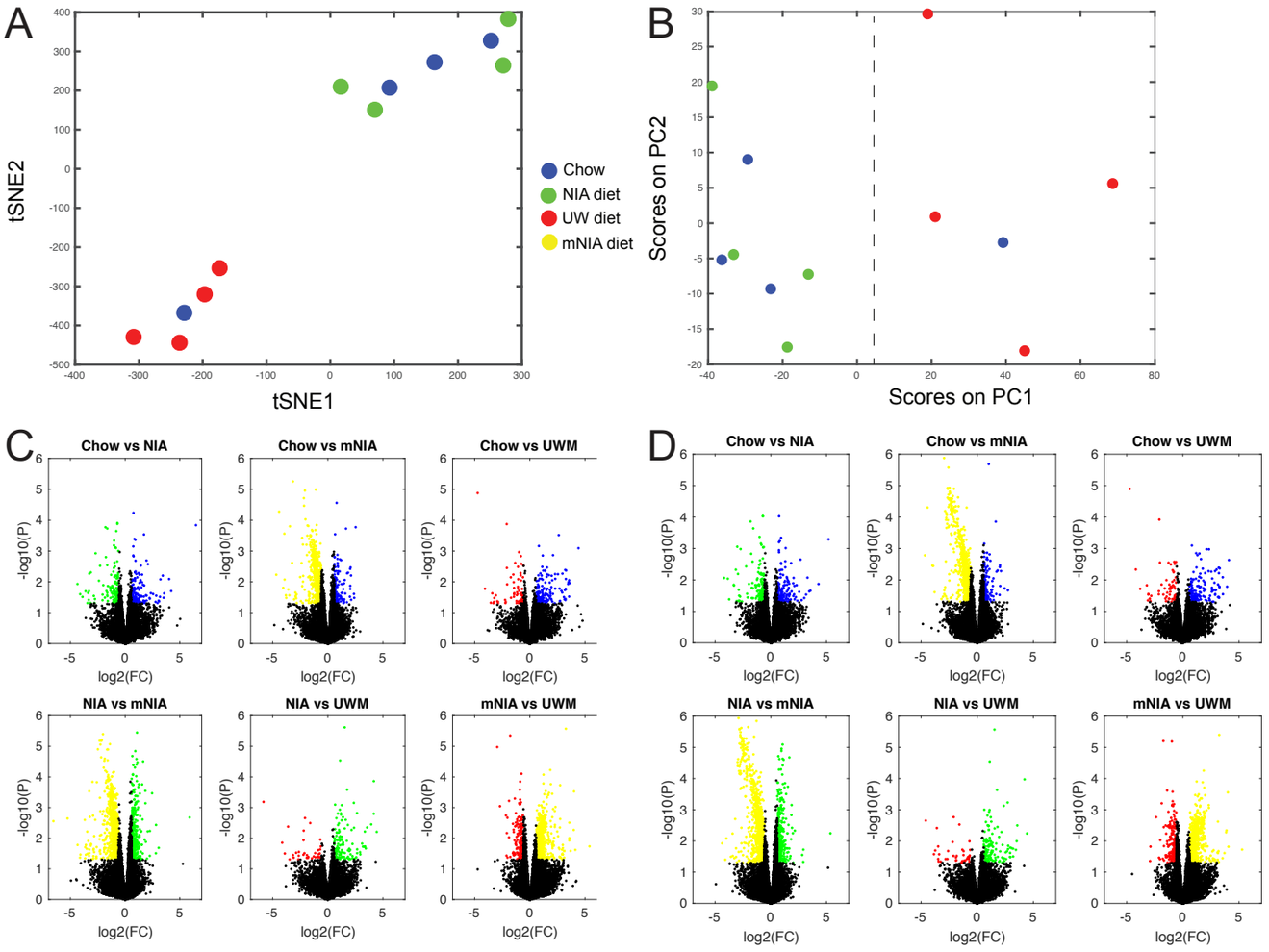
B: Five-hour fasting glucose levels after 6 months on chow, NIA, UW and mNIA diets overall $p<0.01$ Kruskal-Wallis test. In the figure panel, pairwise significance / lack of significance is indicated based on p value from pair-wise Mann-Whitney U-tests with appropriate Bonferroni corrections to adjust for multiple comparisons, $*p<0.05$. Scatter plots to represent the data are shown with median and interquartile range.

C: Five-hour fasting plasma insulin after 6 months on diet, overall $p<0.05$, pairwise significance is indicated based on post-hoc Mann Whitney U-test with Bonferroni multiple testing correction, $*p<0.05$. Scatter plots to represent the data are shown with median and interquartile range.

D: Hepatic triglyceride content after 6 months on diet, overall $p<0.0001$, one-way ANOVA, pairwise significance is indicated based on post-hoc two-tailed t-test with Bonferroni multiple testing correction, $*p<0.05$, $**p<0.01$, $***p<0.001$. Scatter plots to represent the data are shown with mean and SEM.

E: Plasma triglyceride content after 6 months on diet, overall p=non-significant, one-way ANOVA, pairwise p=non-significant based on post-hoc two-tailed t-test with Bonferroni multiple testing correction. Scatter plots to represent the data are shown with mean and SEM.

Figure S3


A
tSNE2
tSNE1
Chow
NIA diet
UW diet
mNIA diet
B
Scores on PC2
Scores on PC1
C
Chow vs NIA
Chow vs mNIA
Chow vs UWM
NIA vs mNIA
NIA vs UWM
mNIA vs UWM
-log10(P)
log2(FC)
D
Chow vs NIA
Chow vs mNIA
Chow vs UWM
NIA vs mNIA
NIA vs UWM
mNIA vs UWM
-log10(P)
log2(FC)

Supplementary Figure 3:

A: Technical replicate of t-SNE visualization of global transcriptomic profiles of hearts from mice fed chow, NIA and UW diets

B: Three-way principal component analysis of chow, NIA and UW diets.

C: Volcano plots illustrating genes that are differentially expressed between each pair of diets using an alternate alignment algorithm – STAR.

D: Volcano plots illustrating genes (instead of transcripts as in Fig. 3C) that are differentially expressed between each pair of diets

Supplementary Table 1: Test statistics of age at death for NIA and UW NHPs analyzed in this study

Comparison	*p* value (pair-wise Mann-Whitney test with Bonferroni multiple testing correction)
NIA control vs. UW control	0.1191
NIA CR vs. UW CR	~1 (non-significant)
NIA vs. UW	0.4134

Supplementary Table 2: Enrichment of significantly differentially expressed genes across KEGG pathways

Higher in mNIA compared to chow

Pathway	Enrichment	P value	FDR	Genes involved
Cardiac muscle contraction	11.4	0.0004	0.115	Cox6b1, Cox7a1, Tpm1, Uqcrb
Alzheimer's disease	6.4	0.0001	0.15	Cox6b1, Cox7a1, Grin2a, Gsk3b, Uqcrb

Higher in mNIA compared to NIA

Pathway	Enrichment	P value	FDR	Genes involved
Oxidative phosphorylation	12.5	< 0.00001	< 0.00001	Cox6b1, Atp5g1, Atp5k, Cox17, Cox5b, Cox6a2, Cox6c, Cox7a1, Cox7a2, Cox7c, Cox8b, Ndufa2, Ndufa4, Ndufs4, Cox7a2l, Ndufb6, Atp5l, Atp5o, Ndufa1, Atp5j2, Ndufs5, Ndufb5, Ndufa3, Ndufb9, Ndufc1, Ndufa12, Ndufa7, Ndufb3, Uqcrh, Uqcr11, Atp5e, Ndufa6, Ndufb8, Uqcrb, Ndufa5, Ndufb10, Ndufa8, Ndufa11, Atp5h, Ndufv2, Ndufs7
Non-alcoholic fatty liver disease	9.4	< 0.00001	< 0.00001	Cox6b1, Cox5b, Cox6a2, Cox6c, Cox7a1, Cox7a2, Cox7c, Cox8b, Cycs, Ndufa2, Ndufa4, Ndufs4, Cox7a2l, Ndufb6, Ndufa1, Gsk3b, Ndufs5, Ndufb5, Ndufa3, Ndufb9, Ndufc1, Ndufa12, Ndufa7, Ndufb3, Uqcrh, Uqcr11, Ndufa6, Ndufb8, Uqcrb, Ndufa5, Ndufb10, Ndufa8, Ndufa11, Ndufv2, Ndufs7
Alzheimer's disease	9.4	< 0.00001	< 0.00001	Cox6b1, Atp5g1, Cox5b, Cox6a2, Cox6c, Cox7a1, Cox7a2, Cox7c, Cox8b, Cycs, Grin2a, Ndufa2, Ndufa4, Ndufs4, Cox7a2l, Ndufb6, Atp5o, Ndufa1, Gsk3b, Ndufs5, Ndufb5, Ndufa3, Ndufb9, Ndufc1, Ndufa12, Ndufa7, Ndufb3, Uqcrh, Uqcr11, Atp5e, Ndufa6, Ndufb8, Uqcrb, Ndufa5, Ndufb10, Ndufa8, Ndufa11, Atp5h, Ndufv2, Ndufs7
Parkinson's disease	11.1	< 0.00001	< 0.00001	Cox6b1, Atp5g1, Cox5b, Cox6a2, Cox6c, Cox7a1, Cox7a2, Cox7c, Cox8b, Cycs, Ndufa2, Ndufa4, Ndufs4, Cox7a2l, Vdac3, Ndufb6, Atp5o, Ndufa1, Ndufs5, Ndufb5, Ndufa3, Ndufb9, Ndufc1, Ndufa12, Ndufa7, Ndufb3, Uqcrh, Uqcr11, Atp5e, Ndufa6, Ndufb8, Uqcrb, Ndufa5, Ndufb10, Ndufa8, Ndufa11, Atp5h, Ndufv2, Ndufs7
Huntington's disease	8.7	< 0.00001	< 0.00001	Cox6b1, Atp5g1, Cox5b, Cox6a2, Cox6c, Cox7a1, Cox7a2, Cox7c, Cox8b, Cycs, Ndufa2, Ndufa4, Ndufs4, Cox7a2l, Vdac3, Ndufb6, Polr2h, Atp5o, Ndufa1, Ndufs5, Ndufb5, Ndufa3, Ndufb9, Ndufc1, Ndufa12, Ndufa7, Polr2l, Ndufb3, Uqcrh, Uqcr11, Atp5e, Ndufa6, Ndufb8, Uqcrb, Ndufa5, Ndufb10, Ndufa8, Ndufa11, Atp5h, Ndufv2, Ndufs7,
Cardiac muscle contraction	7.4	< 0.00001	< 0.00001	Cox6b1, Cox5b, Cox6a2, Cox6c, Cox7a1, Cox7a2, Cox7c, Cox8b, Myl2, Cox7a2l, Tnni3, Uqcrh, Uqcr11, Uqcrb
Spliceosome	4	0.00002	0.0007	Magoh, Snrpb2, Snrpd1, Snrpe, U2af1l4, Sf3b6, Lsm7, Lsm5, Zmat2, Snrnp27, Snrpd3, Ppil1, Snrpf
Protein export	8.2	0.00007	0.0023	Sec61g, Srp14, Srp9, Srp19, Immp1l, Immp2l
Metabolic pathways	1.6	0.0003	0.0089	Cox6b1, Prdx6, Atp5g1, Atp5k, Comt, Coq7, Cox17, Cox5b, Cox6a2, Cox6c, Cox7c, Cox8b, Cycs, Ldha, Ndufa2, Ndufa4, Ndufs4, Ndufb6, Ogdhl, Polr2h, Galntl6, Atp5l, Atp5o, Ndufa1, Atp5j2, Ndufs5, Ndufb5, Ndufa3, Ndufb9, Ndufc1, Ndufa12, Ndufa7, Polr2l, Ndufb3, Uqcrh, Crls1, Uqcr11, Atp5e, Ndufa6, Ndufb8, Uqcrb, Akr1b10, Ndufa5, Ndufb10, Ndufa8, Nadk2, Ndufa11, Kdsr, Atp5h, Ndufv2, Ndufs7
Proteasome	4.6	0.0045	0.12	Psmb1, Psme1, Shfm1, Psmb2, Pomp

Higher in NIA compared to mNIA

Pathway	Enrichment	P value	FDR	Genes involved
AMPK signaling pathway	5.5	0.0003	0.047	Acacb, Acaca, Eef2, Fasn, Ppp2r5d, Foxo3, Rptor
Fatty acid biosynthesis	21.7	0.0003	0.047	Acacb, Acaca, Fasn

Supplementary Table 3: Genes downstream of significantly differentially expressed transcription factors (TFs) and transcription factors (TFs) upstream of significantly differentially expressed genes

Upstream TF	Downstream gene(s) with significantly higher expression in mNIA compared to chow
AR	KLKB1
ARNTL	BHLHE41
CLOCK	BHLHE41
CTCF	PAX6
EGR1	ITGB6
EMX2	PAX6
ETV2	PLAU
ETV4	PLAU
FOS	PLAU
HMGA2	IGF2BP2
JUN	PLAU
JUNB	PLAU
KAT5	PAX6
KLF12	TFAP2A
MEIS1	PAX6
MEIS2	PAX6
MITF	PAX6
MXI1	IFT20
MYC	NFIA
NCOR2	KLKB1
NEUROD1	PAX6
NEUROD6	YBX1
NFKB1	IGF2BP2
PAX2	PAX6
PAX3	POU3F2
PAX4	PAX6
PBX1	PAX6
PBX2	PAX6
PPARA	GSK3B
PYGO2	PAX6
RELA	IGF2BP2
SIM1	POU3F2
SIX3	PAX6
SMAD3	PLAU
SP1	CLDN19
TRP53	GSK3B
VAX2	PAX6
ZFP513	PAX6

Upstream TF	Downstream gene(s) with significantly higher expression in mNIA compared to NIA
AATF	BAK1;TPT1
ANKRD1	MYL2
AR	KLKB1
ARNTL	BHLHE41
BRCA1	TPT1
CITED2	WNT4
CLOCK	BHLHE41
CREB1	LDHA
CREBBP	LDHA
CTCF	PAX6
CTNNB1	WNT4
E2F1	TPT1
EGR1	ITGB6;WNT4
EMX2	PAX6
EP300	LDHA;WNT4
ETV2	PLAU
ETV4	PLAU
FOS	PLAU
GFI1	BAK1
HIF1A	FAM162A
HMGA2	IGF2BP2
ID2	TPT1
IRF4	ZBTB20
JUN	PLAU
JUNB	PLAU
KAT5	TPT1;PAX6
KLF12	TFAP2A
MEIS1	PAX6
MEIS2	PAX6
MITF	PAX6
MTF1	SELENOW
MXI1	IFT20
MYC	NFIA;WNT4
MYEF2	MYL2
MYOD1	SELENOW;MYL2
NCOR2	KLKB1
NEUROD1	PAX6
NFE2	HBB-BS;HBB-B1
NFE2L2	COX17
NFKB1	XIAP;IGF2BP2
NKX2-5	MYL2
NR5A1	WNT4
NRF1	COX17
PAX2	PAX6
PAX3	POU3F2
PAX4	PAX6
PAX5	PRDX6
PBX1	PAX6
PBX2	PAX6
PITX2	TPT1
POU2AF1	LGALS1
POU5F1	TPT1
PPARA	GSK3B
PPARG	DBI
PYGO2	PAX6
RELA	IGF2BP2;XIAP
SIM1	POU3F2
SIX2	WNT4
SIX3	PAX6
SKIL	TPT1
SMAD3	PLAU;TPT1
SOX11	WNT4
SP1	RDX6;CLDN19;COX17;TPT1
TCF3	MYL2;TPT1
TGFB1I1	TPT1
TRP53	3B;BAK1;RAP2B;TPT1
TRP73	TPT1
VAX2	PAX6
WT1	WNT4
ZBTB33	ZFP131
ZFP513	PAX6

Downstream gene	Upstream TF(s) with significantly higher expression in mNIA compared to chow
ABCB1A	YBX1
ALDH3A1	PAX6
APOE	TFAP2A
ATOH7	PAX6
AVP	YBX1
BCL9	PAX6
BCL9L	PAX6
BMI1	YBX1
CDK6	PAX6
CDKN1B	PAX6
CEBPA	TFAP2A
CRYAA	PAX6
CRYAB	PAX6
DKK3	PAX6
DMD	TFAP2A
EGFR	YBX1
EOMES	KDM6B
FAM89B	NFIA
FEZF2	RNF2
FGF15	PAX6
G6PC2	PAX6
GABRA6	NFIA
GAL3ST1	CTCFL
GCG	PAX6
GCK	PAX6
GFAP	NFIA
GNRH1	POU3F2
HGF	NFIA
HOXA7	TFAP2A
HOXA9	TFAP2A
HOXB4	RNF2
HOXB6	RNF2
HOXB8	RNF2
HOXD4	PAX6
ITPR1	TFAP2A
KCTD13	TFAP2A
LHCGR	TFAP2A
MAB21L2	PAX6
MAF	PAX6
MAFA	PAX6
MEIS1	TFAP2A
MET	PAX6
MIA	YBX1
MMP9	PAX6
NES	POU3F2
NEUROG1	PAX6
NEUROG2	PAX6
NONO	YBX1
NR2E1	PAX6
ODF2	PAX6
OTX2	POU3F2
PAX2	PAX6
PAX3	POU3F2
PCSK1	PAX6
PCSK2	PAX6
PDX1	PAX6
PLAGL1	PAX6
RLBP1	PAX6
SCNN1A	RNF2
SFRP2	PAX6
SHH	PAX6
SHROOM3	PAX6
SIX3	PAX6
SLC2A3	YBX1
SLC2A4	NFIA
SOST	PAX6
SOX2	PAX6;RNF2
SP8	PAX6
TCF4	PAX6
TGFB2	PAX6
TLR4	PAX6
TRIM11	PAX6
TRP53	PAX6
TRP63	RNF2
VEGFA	TFAP2A
VSX2	PAX6
WNT7B	PAX6
ZFP42	RNF2

Downstream gene	Upstream TF(s) with significantly higher expression in mNIA compared to chow
ALDH3A1	PAX6
APOE	TFAP2A
ATOH7	PAX6
BCL9	PAX6
BCL9L	PAX6
CDK6	PAX6
CDKN1B	PAX6
CEBPA	TFAP2A
CRYAA	PAX6
CRYAB	PAX6
DKK3	PAX6
DMD	TFAP2A
EOMES	KDM6B
FAM89B	NFIA
FGF15	PAX6
G6PC2	PAX6
GABRA6	NFIA
GAL3ST1	CTCFL
GCG	PAX6
GCK	PAX6
GFAP	NFIA
GNRH1	POU3F2
HGF	NFIA
HOXA7	TFAP2A
HOXA9	TFAP2A
HOXD4	PAX6
ITPR1	TFAP2A
KCTD13	TFAP2A
LHCGR	TFAP2A
MAB21L2	PAX6
MAF	PAX6
MAFA	PAX6
MEIS1	TFAP2A
MET	PAX6
MMP9	PAX6
NES	POU3F2
NEUROG1	PAX6
NEUROG2	PAX6
NR2E1	PAX6
ODF2	PAX6
OTX2	POU3F2
PAX2	PAX6
PAX3	POU3F2
PCSK1	PAX6
PCSK2	PAX6
PDX1	PAX6
PLAGL1	PAX6
PRM1	GTF2A1
RLBP1	PAX6
SFRP2	PAX6
SHH	PAX6
SHROOM3	PAX6
SIX3	PAX6
SLC2A4	NFIA
SOST	PAX6
SOX2	PAX6
SOX9	ZBTB20
SP8	PAX6
TCF4	PAX6
TGFB2	PAX6
TLR4	PAX6
TNP1	GTF2A1
TRIM11	PAX6
TRP53	PAX6
VEGFA	TFAP2A
VSX2	PAX6
WNT7B	PAX6