EXTENDED SUPPLEMENTAL METHODS

Primer List

Col3a1
5′-CTGTAACATGGAAACTGGGGAAA-3′
5′-CCATAGCTGAACTGAAAACCACC-3′
Bnp
5′-GAGGTCACTCCTATCCTCTGG-3′
5′-GCCATTTCCTCCGACTTTTCT-3′
Anf
5′-GCTTCCAGGCCATATTGGAG-3′
5′-GGGGGCATGACCTCATCTT-3′
Frda
5′-ATGGCGTGCTCACCATTAAG-3′
5′-GGCCAA TGAAGACAAGTCCA-3′
Sirt3
5′-GCTTCCAGGCCATATTGGAG-3′
5′-GGGGGCATGACCTCATCTT-3′
36B4/Rplp; internal control as recommended by (1).
5’-TGGAAGTCCAACTACTTCCTCAA-3’
5’-ATCTGCTGCATCTGCTTGGAG-3’

Invasive Cardiac Function Assessment

In vivo pressure-volume (P-V) analysis was performed as previously described (2). Briefly, after bilateral vagotomy, the chest was opened and the pericardium was dissected to expose the heart. A 7-0-suture ligature was placed around the transverse aorta to manipulate loading conditions. A 1.4-Fr pressure-conductance catheter (Millar Instruments, Houston, TX) was inserted retroaortically into the LV to record hemodynamics. Baseline hemodynamic parameters were obtained once the catheter recordings had achieved steady state, usually 3-5 minutes following conductance catheter placement. Cardiac contractility and compliance measures were established by generating a series of PV loops with decreasing preload, generated by gently pulling on the suture to transiently constrict the inferior vena cava. Subsequently, parallel conductance (Vp) was determined by 10 μl injection of 15% saline into the right jugular vein to establish the parallel conductance of the blood pool. The derived Vp was used to correct the P-V loop data. Data were recorded digitally at 1,000 Hz and analyzed with pressure volume analysis software (PVAN data analysis software version 3.3; Millar Instruments) as previously described (2). Mice that died after receiving anesthesia or became hypotensive during the course of the protocol, suggesting a surgical complication were excluded.

Tissue Isolation and Western blot analysis

Mouse hearts were homogenized (40–50 strokes) in 15mL of ice-cold homogenization buffer (320 mM sucrose, 50 mM KH2PO4 [pH 7.4], 10 mM Tris-HCl [pH 7.4], and 1 mM EDTA) in the presence of protease inhibitors, phosphatase inhibitors (Roche), and deacetylase inhibitors (2.5 mM Trichostatin A, 5 mM nicotinamide, and 5 mM sodium butyrate) using glass homogenizers. Crude mitochondria were isolated fresh hearts by differential centrifugation.

Homogenates were centrifuged at 1,600 rpm for 10 min at 4 °C. The supernatant was centrifuged again at 1,600 rpm for 10 min at 4 °C. The supernatant subsequently was centrifuged at 10,000 x *g* for 10 min at 4 °C. The pellet was collected, rinsed with 1 ml homogenization buffer, and centrifuged at 10,000 x *g* again for 10 min at 4 °C. The pellet was resuspended in 100 ml 20 mM HEPES (pH 7.4), 150 mM NaCl, and 1% Triton X-100 with protease, phosphatase, and deacetylase inhibitors. Western blot analysis was performed with 20-30 µg protein per lane. Membranes were incubated in primary antibody either overnight at 4 °C or at room temperature for 1–2 hr with rocking. Primary antibodies were purchased from the following companies: Acetylated-lysine, CST 9441; SIRT3 (D22A3), CST 5490; pAMPKa (Th172), CST 2535P; AMPKa, CST 2603P; Total OXPHOS, MitoSciences ab110413; pPDH (S293), CST Calbiochem AP1062; PDH E1A Abcam 1101416; GLUT1, CST 12939; GLUT4, Abcam ab654-250; GAPDH, CST 97166. Primary antibodies were used at a concentration of 1:1,000. Blots were washed with TBST and then incubated for 1 hr with rocking in Li-Cor secondary antibodies used at a concentration of 1:10,000 in TBST. Western blots were developed using a Li-Cor Odyssey CLx. Blots were quantified using the Li-Cor software.

Metabolite extraction for Metabolomics

Flash frozen hearts were pulverized and weighed (10 mg). Metabolite extraction was performed (500 µL solvent/10 mg of tissue) as described in previous study (3). The supernatant was transferred to a new Eppendorf tube and dried in vacuum concentrator at room temperature. The dry pellets were reconstituted into 30 µL (per 3 mg tissue) sample solvent (water:methanol:acetonitrile, 2:1:1, v/v) and 3 µL was further analyzed by liquid chromatography-mass spectrometry (LC-MS).

Metabolomics LC-MS method

Ultimate 3000 UHPLC (Dionex) is coupled to Q Exactive Plus-Mass spectrometer (QE-MS, Thermo Scientific) for metabolite profiling. A hydrophilic interaction chromatography method (HILIC) employing an Xbridge amide column (100 x 2.1 mm i.d., 3.5 µm; Waters) is used for polar metabolite separation. Detailed LC method was described previously (4) except that mobile phase A was replaced with water containing 5 mM ammonium acetate (pH 6.8). The QE-MS is equipped with a HESI probe with related parameters set as below: heater temperature, 120 °C; sheath gas, 30; auxiliary gas, 10; sweep gas, 3; spray voltage, 3.0 kV for the positive mode and 2.5 kV for the negative mode; capillary temperature, 320 °C; S-lens, 55; scan range (*m/z*): 70 to 900 for pos mode (1.31 to 12.5 min) and neg mode (1.31 to 6.6 min) and 100 to 1000 for neg mode (6.61 to 12.5 min); resolution: 70000; automated gain control (AGC), 3×10^6 ions. Customized mass calibration was performed before data acquisition.

Metabolomics data analysis

LC-MS peak extraction and integration were performed using commercial available software Sieve 2.2 (Thermo Scientific). The peak area was used to represent the relative abundance of each metabolite in different samples. The missing values were handled as described in previous study (4).

Proteomic Materials

Protease (Complete mini EDTA-free) inhibitor tablets (Roche). 6-plex Tandem Mass Tags

(TMT) were purchased from Thermo Fisher Scientific. Sequencing Grade Modified Trypsin was purchased from Promega. Lysyl Endopeptidase (LysC) was purchased from Wako Chemicals. tC18 SEP-PAK SPE columns were purchased from Waters. PTMScan® Acetyl-lysine motif sepharose-conjugated antibodies were purchased from Cell Signaling Technologies.

Tissue harvest, mitochondrial extraction, protein digestion, and peptide labeling for TMT proteomic experiment

Hearts were harvested from wildtype or FXNKO mice from three age cohorts (age cohorts: 13-, 8- and 5-weeks of age; n = 3/cohort/genotype; 18 total). Hearts were quickly rinsed in ice-cold PBS and homogenize in 10mL of STE lysis buffer (320mM sucrose, 50mM KH_2PO_4, 10mM Tris (pH 7.4), and 1mM EDTA) containing protease inhibitors (1uM leupeptin, 1uM pefabloc, 1uM bebstatin, and 1uM pepstatin A). Homogenization involved glass mortar and pestle, 30-40 passes within 3-5 minutes on ice. Mitochondria were crudely isolated from hearts by differential centrifugation performed at 4°C: 1) 1,600 RPM for 10 minutes, 2) supernatant was passed through surgical gauze to remove excess fat and transferred into clean 50mL conical tube (pellet was discarded), 3) strained supernatant was centrifuged at 10,000 x g for 10 minutes, 4) supernatant was discarded and 5mL of STE was gently added to the pellet, swirled to remove excess fat and decanted, 5) 1mL of STE was added, pellet gently loosened from sides of conical tube, 6) pellets were centrifuged at 7,000 x g for 10 minutes, supernatant was aspirated, 1mL of STE added, pellet gently loosened, and centrifuged at 7,000 x g for 10mins, 7) supernatant was aspirated, pellet loosened with 1mL of STE and centrifuged at 3000x g for 10 minutes, 9) mitochondrial pellets were snap frozen in liquid nitrogen and stored at -80°C.

Pellets were re-suspended in 500 µL of ice-cold 8M Urea Lysis Buffer (8 M urea in 50 mM Tris, pH 8.0, 40 mM NaCl, 2 mM MgCl2, 1x cOmplete mini EDTA-free protease inhibitor tablet, 10 mM Nicotinamide, 10 µM TSA). Re-suspended pellets were solubilized by sonication with a probe sonicator on ice (3 x 10 sec pulses), incubation at 37C for 30 minutes with internment vortexing, and subjected to centrifugation at 10,000 x g for 10 min. Protein content was quantified by BCA assay and equal amount of protein (156ug, adjusted to 520ug/mL with 8M Urea buffer). Samples were reduced with 5 mM DTT at 37°C for 30 min, cooled to RT, alkylated with 15 mM iodoacetimide for 30 min in the dark, and unreacted iodoacetimide quenched with DTT at a final concentration of 15 mM for 10 min at RT. The samples were digested with LysC (100:1, w/w, protein:enzyme) for 4 hrs at 37°C. Following dilution to 1.5 M urea with 50 mM Tris pH 8.0, 5 mM $CaCl_2$, the samples were further digested with trypsin (50:1 w/w, protein:enzyme) overnight at 37°C.

The samples were acidified to 0.5% TFA and centrifuged at 4000 x g for 10 min at 4 °C to pellet insoluble material. The supernatant containing soluble peptides was desalted by solid phase extraction (SPE) with a Waters 50 mg tC18 SEP-PAK SPE column per the manufacturer's instructions and eluted once with 500 µL 25% acetonitrile/0.1% TFA and twice with 500 µl 50% acetonitrile/0.1% TFA. The 1.5 ml eluate was frozen and dried in a speed vac. For each time-point assessed, all samples were re-suspended in 100uL of 200mM triethylammonium bicarbonate (TEAB), mixed with a unique 6-plex Tandem Mass Tag (TMT) reagent (0.8mg re-suspended in 50uL 100% acetinitrile)—one 6-plex set per age group—and shaken for 4 hours at room temperature. After samples were quenched with 08uL 50% hydroxylamine and shaken

for 15 additional minutes at room temperature, all six samples were combined for each time-point, frozen, and dried in a speed vacuum. The mixture for each time-point was re-suspended in 1mL 0.5% TFA and subjected to SPE again with a Waters 100mg tC18 SEP-PAK SPE column as described above. Each eluate was vortexed and split into one aliquot containing ~5% of the total peptide mixture (46.8ug) and a second aliquot containing ~95% (889.2 ug). Both aliquots for each-time-point were frozen and dried in a speed vacuum. The 46ug aliquot of the "input" material was saved at -80°C for quantification of unmodified peptides. The 889.2ug aliquots were used for acetyl enrichment as described below.

Acetylpeptide enrichment for TMT proteomic experiment

For each time-point, the 889 ug aliquot was resuspended in 1.4 mL 1X IAP Buffer and subjected to immunoprecipitation (IP) with PTMScan AcetylLysine Motif IAP Beads on a rotator overnight at 4°C. The next day, the antibody-peptide complexes were pelleted via centrifugation at 2000 x g for 30 seconds and the precipitate was washed 2 times in 1mL of IP buffer and 3 times in 1mL water (centrifuging each time). Acetylpeptides were eluted in 55 µL of 0.1% TFA for 10 minutes followed by a quick wash in 50 µL of 0.1% TFA and the supernatants (2000 x g, 30 sec) from each elution were combined. The eluate was acidified to 0.5% TFA (and brought to a 1 mL volume), desalted on a 50 mg tC18 SEP-PAK SPE column and eluted as described above. The eluate was frozen on dry ice and dried in a speed vac. All samples were submitted to the Duke University School of Medicine Proteomics Core facility for analysis by nLC-MS/MS described below.

Nano-LC-MS/MS for TMT proteomic experiment

All samples were subjected to *nano*LC-MS/MS analysis using a nano-Acquity UPLC system (Waters) coupled to a *Q Exactive Plus* Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) via a nanoelectrospray ionization source. Prior to injection, the acetylpeptide samples was re-suspended in 12uL 1% TFA/2% acetonitrile and were analyzed with technical duplicates runs, with 4uL of sample injected. For each injection, the samples was first trapped in a Symmetry C16 20mm x 180mm trapping column (5uL/min at 99.89/0.1 v/v water/acetonitrile, after which the analytical separation was performed over a 90 minute gradient (flow rate of 400 nanoliters/minute) of 3 to 30% acetonitrile using a 1.7um Acquity BEH130 C18 75um x 250mm column (Waters Corp.), with a column temperature of 55°C. MS^1 (precursor ions) was performed at 70,000 resolution, with an AGC target of $1x10^6$ ions and a maximum injection time of 60ms. MS^2 spectra (product ions) were collected by data-dependent acquisition (DDA) of the top 20 most abundant precursor ions with a charge greater than 1 and less than 8 per MS^1 scan, with dynamic exclusion enabled for a window of 30 seconds. Precursor ions were filtered with a 1.2m/*z* isolation window and fragmented with a normalized collision energy of 30. MS^2 scans were performed at 17,500 resolution, with an AGC target of $1x10^5$ ions and a maximum injection time of 60ms. The input material fractions were analyzed in singlicate, with 2uL injections, run as above but with the gradient lengthened to 180 minutes.

Data processing for TMT proteomic experiment

Raw LC-MS/MS data have been deposited to the ProteomeXchange Consortium via the PRIDE (5) partner repository with the dataset identifier PXD006754. Raw LC-MS/MS data were processed in Proteome discoverer v2.1 with service pack 1 (PD2.1, SP1, Thermo Fisher

Scientific), using both the Sequest HT and MS Amanda search engines. Data were searched against the UniProt mouse complete proteome database of reviewed (Swiss-Prot) and unreviewed (TrEMBL) proteins, which consisted of 49,838 sequences on the date of download (10/19/2016). Default search parameters included oxidation (15.995 Da on M) as a variable modification and carbamidomethyl (57.021 Da on C) and TMTplex (229.163 Da on peptide N-term and K). To assess labeling efficiency as a quality control measure, the input fraction was re-searched with N-terminal TMT6plex as a variable modification, confirming N-terminal labeling of 97.47%, 97.76% and 98.08% of all PSMs at the 5-, 8- and 13-week time-point, respectively. Acetyl runs added acetylation (42.011 Da on K) and changed TMT to a variable modification on K (remaining fixed on peptide N-term). Data were searched with a 10 ppm precursor mass and 0.02 Da product ion tolerance. The maximum number of missed cleavages was set to a default value of 2 (but changed to 3 for acetyl runs) and enzyme specificity was trypsin (full). Considering each data type (acetyl and input) separately, peptide spectral matches (PSMs) from each search algorithm were filtered to a 1% false discovery rate (FDR) using the percolator node of PD2.1. For acetyl data, site localization probabilities were determined for all modifications using the ptmRS algorithm. PSMs were grouped to unique peptides while maintaining a 1% FDR at the peptide level and using a 95% site localization threshold for acetyl modifications. Peptides from all samples (acetyl and input) were grouped to proteins together using the rules of strict parsimony and proteins were filtered to 1% FDR using the Protein FDR Validator node of PD2.1. Reporter ion intensities for all PSMs having co-isolation interference below 0.5 (50% of the ion current in the isolation window) and average reporter S/N>1 for all reporter ions were summed together at the peptide group and protein level, but keeping quantification for each data type (acetyl, input) and each time-point (5-, 8-, 13-weeks) separate. Peptides shared between protein groups were excluded from protein quantitation calculations.

Statistical analysis for TMT proteomic experiment

Protein and peptide groups tabs in the PD2.1 results were exported as tab delimited .txt. files, opened in Microsoft EXCEL, and analyzed as described previously (6). First, peptide group reporter intensities for each peptide group in the input material were summed together for each TMT channel within a given time-point (5-, 8-, and 13-weeks), each channel's sum was divided by the average of all channels' sums, resulting in channel-specific loading control normalization factors to correct for any deviation from equal protein/peptide input into the six sample comparison. Reporter intensities for peptide groups from both the acetyl fractions and for proteins from the input fraction were divided by the loading control normalization factors for each respective TMT channel represented in a given time-point. Analyzing the acetylpeptide and protein datasets separately, all loading control-normalized TMT reporter intensities were converted to $\log_2$ space, and the average value from the six samples was subtracted from each sample-specific measurement to normalize the relative measurements to the mean. For the wild-type and FXNKO comparison at each time-point (n=3/genotype), condition average, standard deviation, p-value (p, two-tailed student's t-test, assuming equal variance), and adjusted p-value ($P_{adjusted}$, Benjamini Hochberg FDR correction) were calculated (Benjamini and Hochberg, 1995; Lesack and Naugler, 2011). For protein-level quantification, only Master Proteins—or the most statistically significant protein representing a group of parsimonious proteins containing common peptides identified at 1% FDR were used for quantitative comparison. PTM level measurements (acetyl) were calculated both alone (referred to as

abundance) and with normalization to any change in the corresponding Master Protein (referred to as *relative occupancy*), calculated by subtracting Log_2 Master Protein values from PTM-containing peptide quantitation values on a sample-specific basis.

REFERENCES

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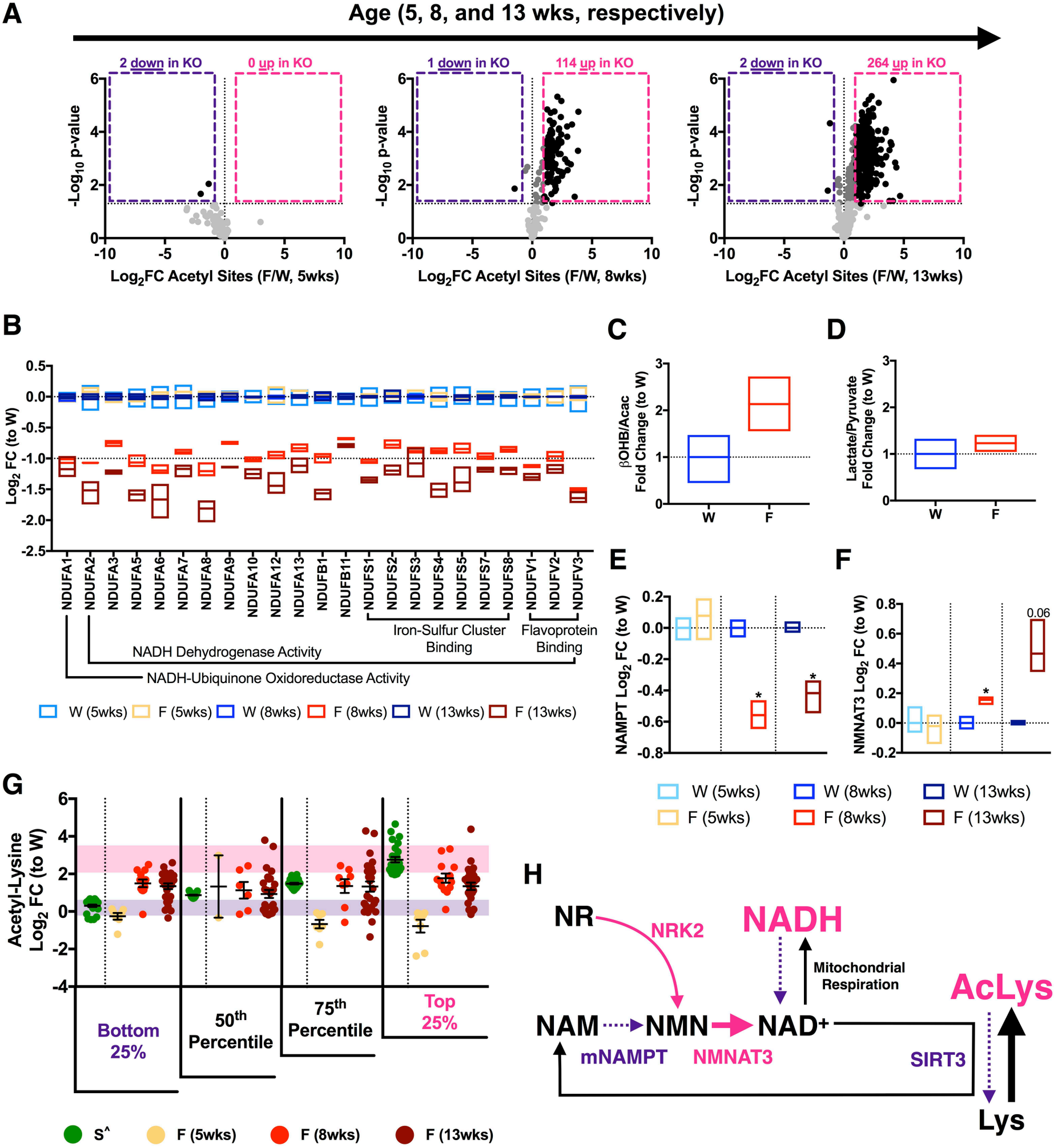
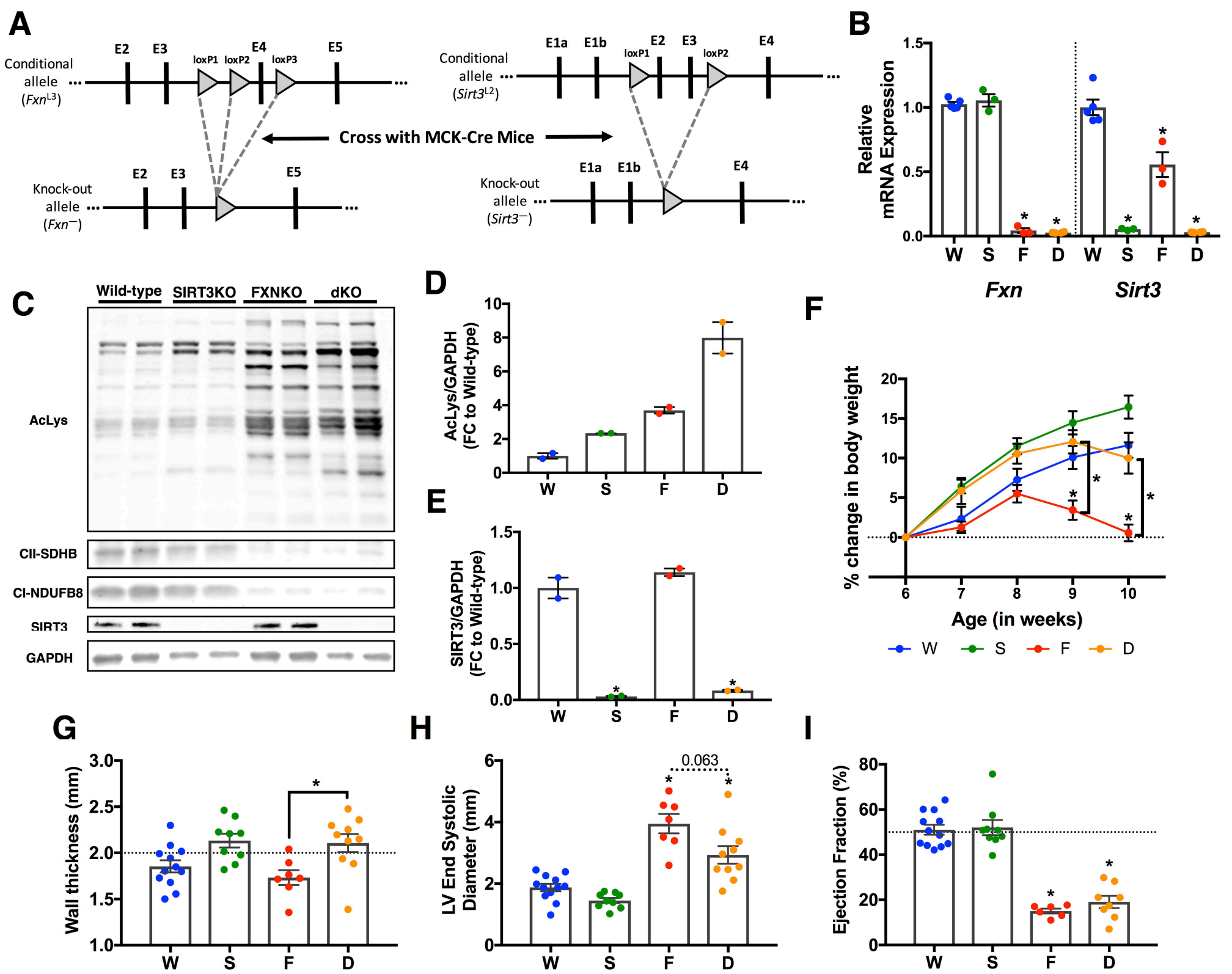
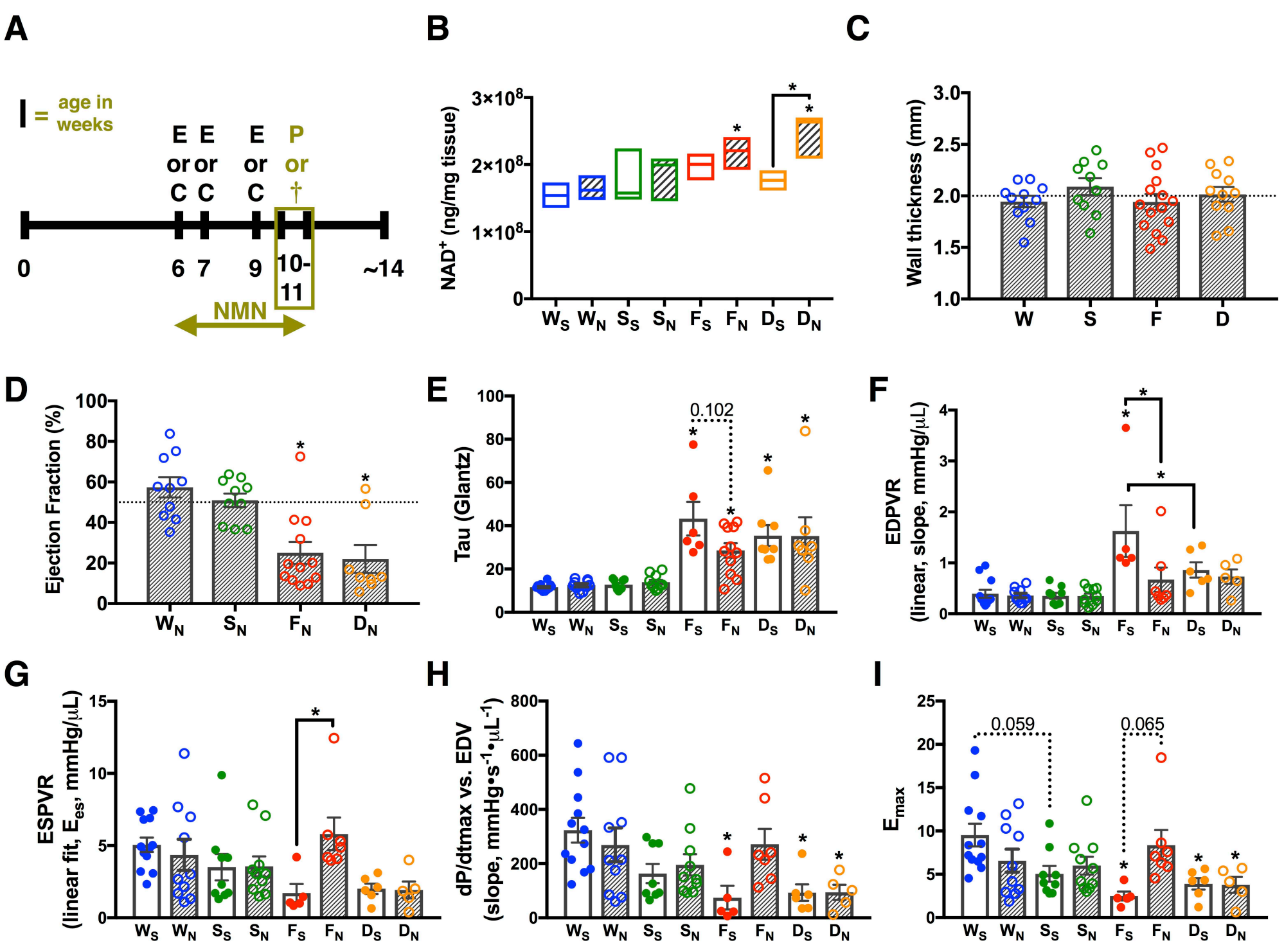
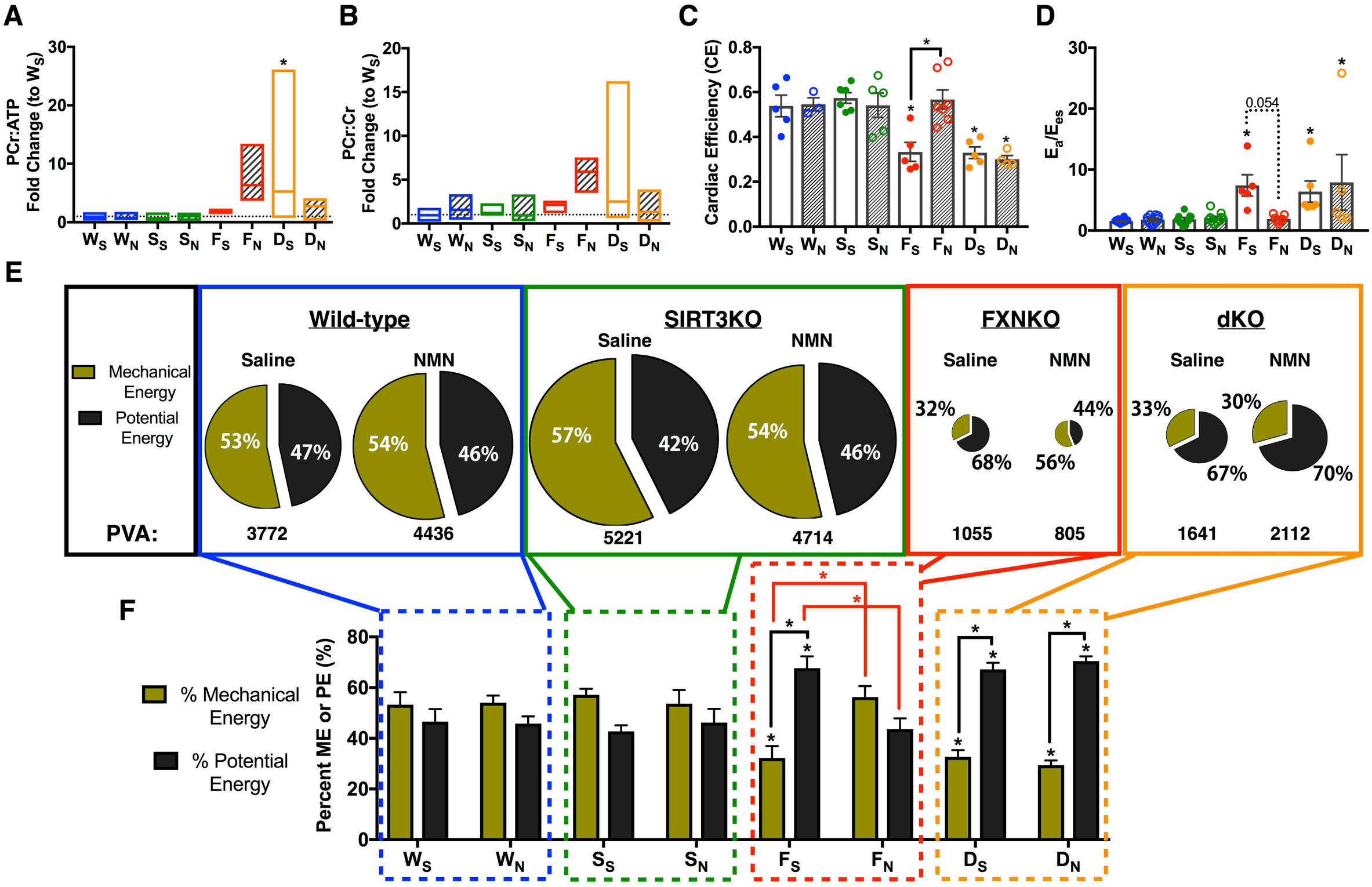
6. McDonnell E et al. Lipids Reprogram Metabolism to Become a Major Carbon Source for Histone Acetylation. *Cell Rep* 2016;17(6):1463–1472.


A
Age (5, 8, and 13 wks, respectively)
2 down in KO
0 up in KO
1 down in KO
114 up in KO
2 down in KO
264 up in KO
-Log10 p-value
Log2FC Acetyl Sites (F/W, 5wks)
Log2FC Acetyl Sites (F/W, 8wks)
Log2FC Acetyl Sites (F/W, 13wks)
B
Log2 FC (to W)
NDUFA1
NDUFA2
NDUFA3
NDUFA5
NDUFA6
NDUFA7
NDUFA8
NDUFA9
NDUFA10
NDUFA12
NDUFA13
NDUFB1
NDUFB11
NDUFS1
NDUFS2
NDUFS3
NDUFS4
NDUFS5
NDUFS7
NDUFS8
NDUFV1
NDUFV2
NDUFV3
Iron-Sulfur Cluster Binding
Flavoprotein Binding
NADH Dehydrogenase Activity
NADH-Ubiquinone Oxidoreductase Activity
W (5wks)
F (5wks)
W (8wks)
F (8wks)
W (13wks)
F (13wks)
C
βOHB/Acac Fold Change (to W)
W
F
D
Lactate/Pyruvate Fold Change (to W)
W
F
E
NAMPT Log2 FC (to W)
*
*
F
NMNAT3 Log2 FC (to W)
0.06
*
W (5wks)
F (5wks)
W (8wks)
F (8wks)
W (13wks)
F (13wks)
G
Acetyl-Lysine Log2 FC (to W)
Bottom 25%
50th Percentile
75th Percentile
Top 25%
S^
F (5wks)
F (8wks)
F (13wks)
H
NR
NRK2
NADH
Mitochondrial Respiration
AcLys
NAM
NMN
NAD+
mNAMPT
NMNAT3
SIRT3
Lys


A
Conditional allele (Fxn^{L3})
E2
E3
loxP1
loxP2
E4
loxP3
E5
Cross with MCK-Cre Mice
Knock-out allele (Fxn^{-})
Conditional allele ($Sirt3^{L2}$)
E1a
E1b
loxP1
E2
E3
loxP2
E4
Knock-out allele ($Sirt3^{-}$)
B
Relative mRNA Expression
W
S
F
D
Fxn
Sirt3
C
Wild-type
SIRT3KO
FXNKO
dKO
AcLys
CII-SDHB
CI-NDUFB8
SIRT3
GAPDH
D
AcLys/GAPDH (FC to Wild-type)
E
SIRT3/GAPDH (FC to Wild-type)
F
% change in body weight
Age (in weeks)
G
Wall thickness (mm)
H
LV End Systolic Diameter (mm)
0.063
I
Ejection Fraction (%)


A
I = age in weeks
E or C
E or C
E or C
P or †
0
6
7
9
10-11
~14
NMN
B
NAD+ (ng/mg tissue)
3×10^8
2×10^8
1×10^8
0
WS WN SS SN FS FN DS DN
C
Wall thickness (mm)
3.0
2.5
2.0
1.5
1.0
W S F D
D
Ejection Fraction (%)
100
80
60
40
20
0
WN SN FN DN
E
Tau (Glantz)
0.102
WS WN SS SN FS FN DS DN
F
EDPVR (linear, slope, mmHg/μL)
WS WN SS SN FS FN DS DN
G
ESPVR (linear fit, Ees, mmHg/μL)
WS WN SS SN FS FN DS DN
H
dP/dtmax vs. EDV (slope, mmHg•s-1•μL-1)
800
600
400
200
0
WS WN SS SN FS FN DS DN
I
Emax
0.059
0.065
25
20
15
10
5
0
WS WN SS SN FS FN DS DN


A
PCr:ATP
Fold Change (to WS)
B
PCr:Cr
Fold Change (to WS)
C
Cardiac Efficiency (CE)
D
Ea/Ees
0.054
WS WN SS SN FS FN DS DN
E
Mechanical Energy
Potential Energy
PVA:
Wild-type
Saline
NMN
53%
47%
54%
46%
3772
4436
SIRT3KO
57%
42%
54%
46%
5221
4714
FXNKO
32%
68%
44%
56%
1055
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dKO
33%
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30%
70%
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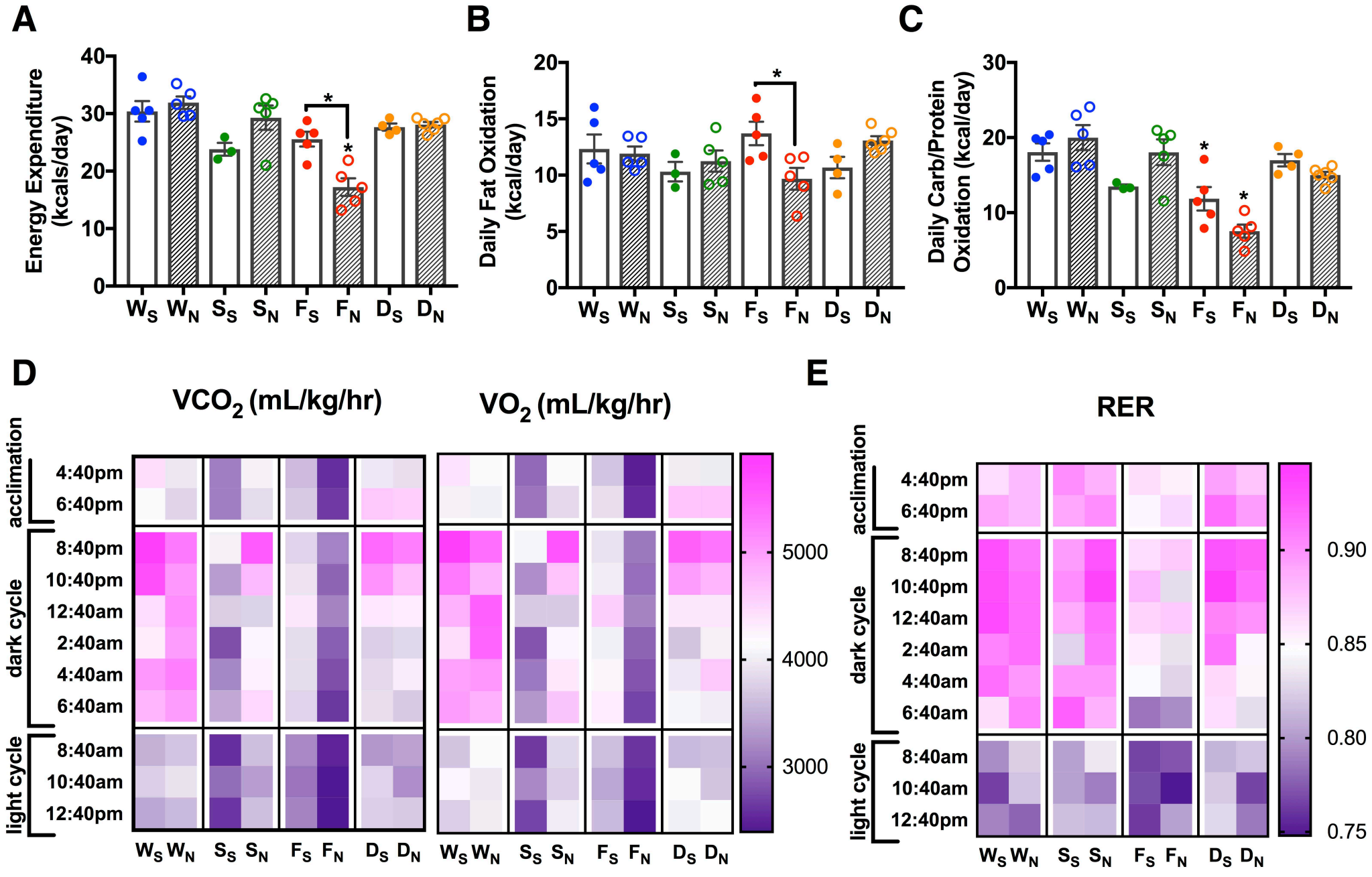
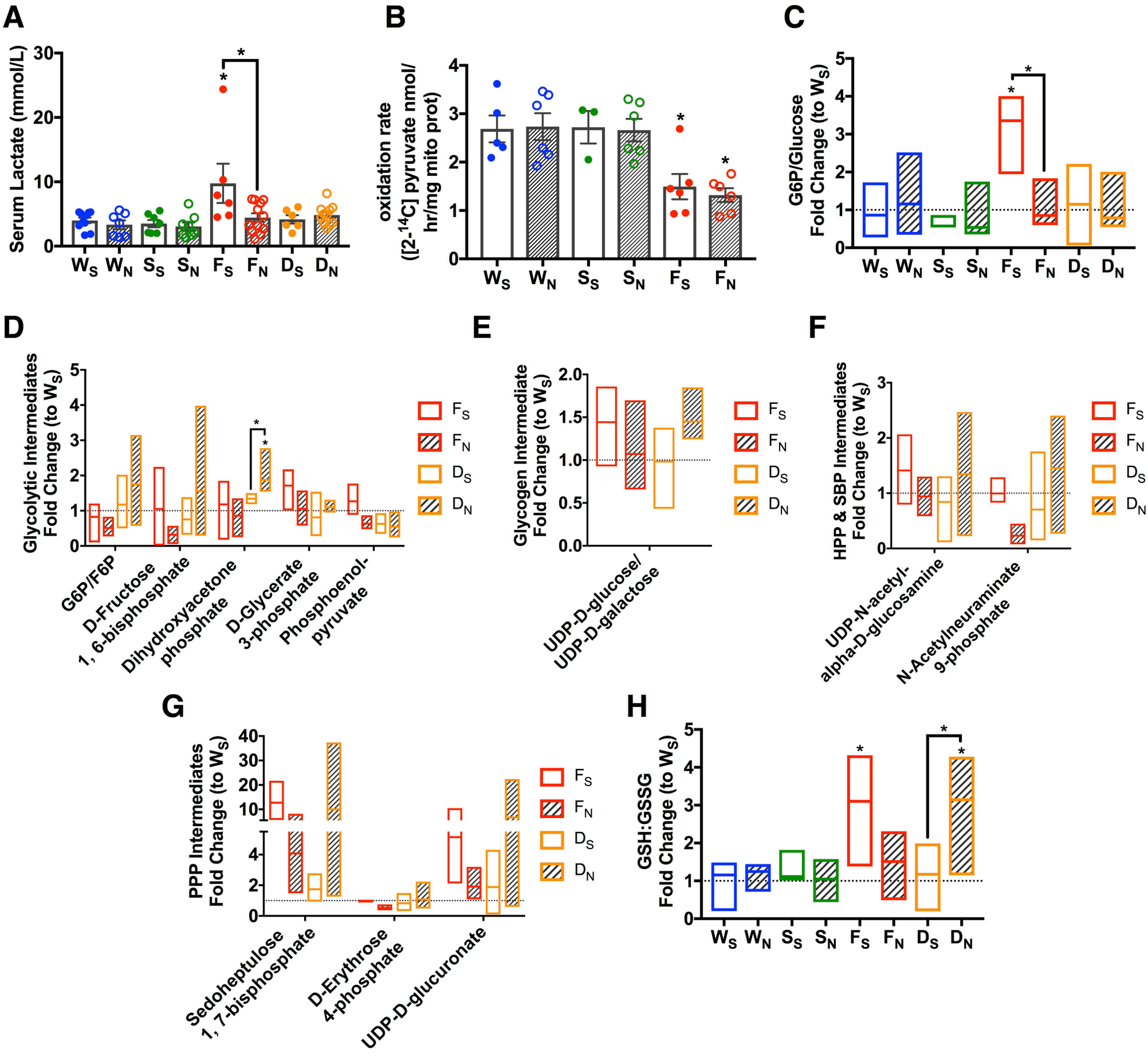
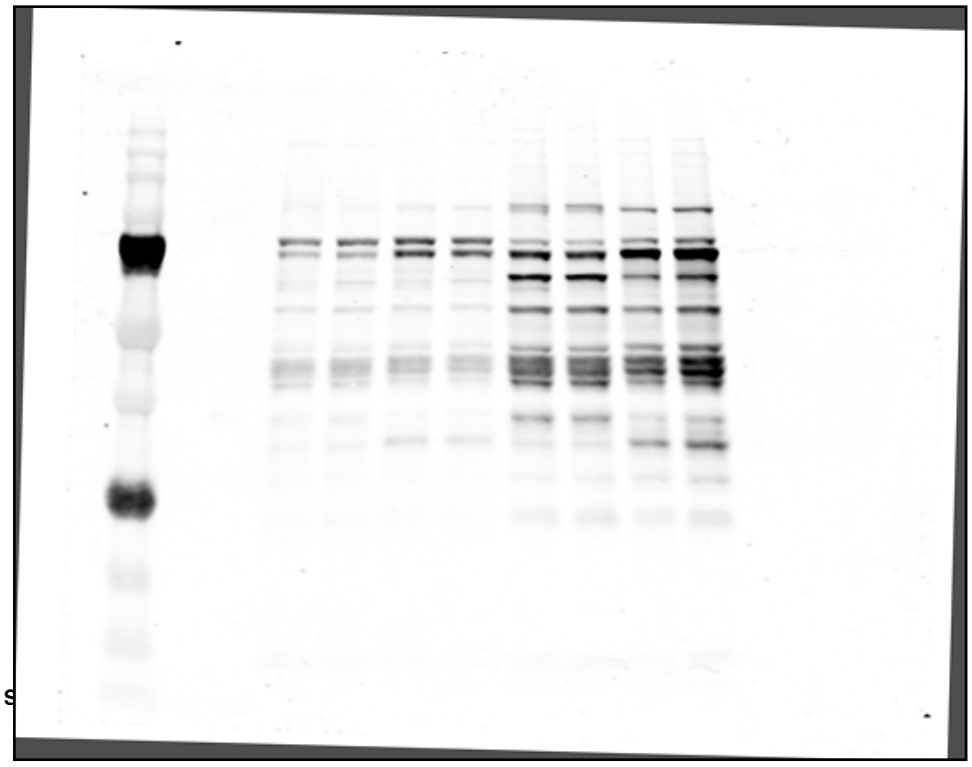
2112
F
% Mechanical Energy
% Potential Energy
Percent ME or PE (%)


A
Energy Expenditure (kcals/day)
40
30
20
10
0
WS WN SS SN FS FN DS DN
B
Daily Fat Oxidation (kcal/day)
20
15
10
5
0
C
Daily Carb/Protein Oxidation (kcal/day)
30
20
10
0
D
VCO2 (mL/kg/hr)
VO2 (mL/kg/hr)
acclimation
dark cycle
light cycle
4:40pm
6:40pm
8:40pm
10:40pm
12:40am
2:40am
4:40am
6:40am
8:40am
10:40am
12:40pm
5000
4000
3000
E
RER
0.90
0.85
0.80
0.75

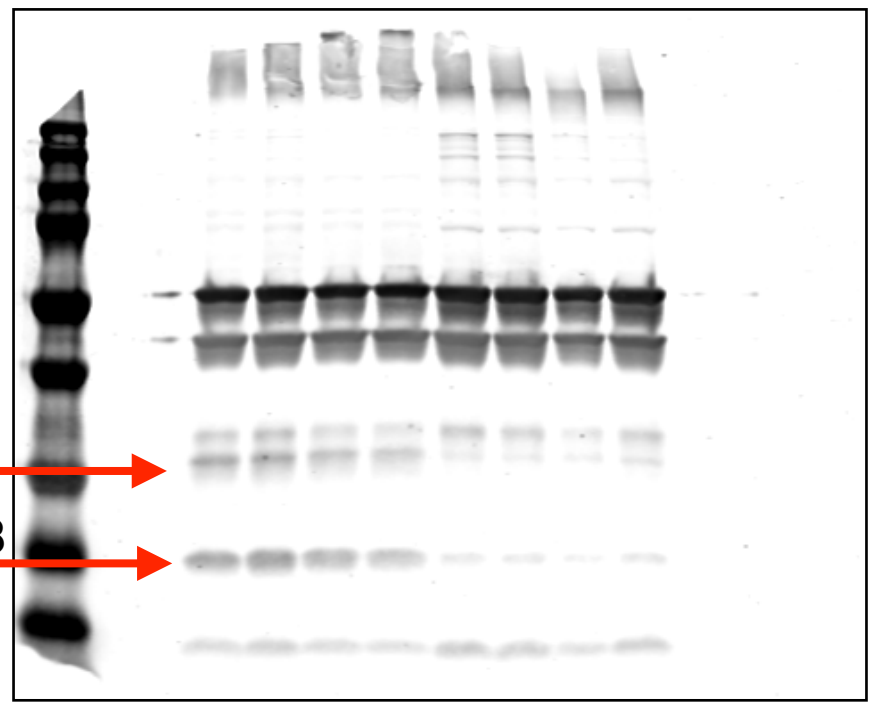

A
Serum Lactate (mmol/L)
30
20
10
0
WS WN SS SN FS FN DS DN
B
oxidation rate ([2-14C] pyruvate nmol/ hr/mg mito prot)
4
3
2
1
0
WS WN SS SN FS FN
C
G6P/Glucose Fold Change (to WS)
5
4
3
2
1
0
WS WN SS SN FS FN DS DN
D
Glycolytic Intermediates Fold Change (to WS)
5
4
3
2
1
0
G6P/F6P
D-Fructose 1, 6-bisphosphate
Dihydroxyacetone phosphate
D-Glycerate 3-phosphate
Phosphoenol-pyruvate
FS
FN
DS
DN
E
Glycogen Intermediate Fold Change (to WS)
2.0
1.5
1.0
0.5
0.0
UDP-D-glucose/ UDP-D-galactose
FS
FN
DS
DN
F
HPP & SBP Intermediates Fold Change (to WS)
3
2
1
0
UDP-N-acetyl-alpha-D-glucosamine
N-Acetylneuraminate 9-phosphate
FS
FN
DS
DN
G
PPP Intermediates Fold Change (to WS)
40
30
20
10
4
2
0
Sedoheptulose 1, 7-bisphosphate
D-Erythrose 4-phosphate
UDP-D-glucuronate
FS
FN
DS
DN
H
GSH:GSSG Fold Change (to WS)
5
4
3
2
1
0
WS WN SS SN FS FN DS DN

Wild-type SIRT3KO FXNKO dKO

AcLys



Full Unedited Gel for Figure 2C

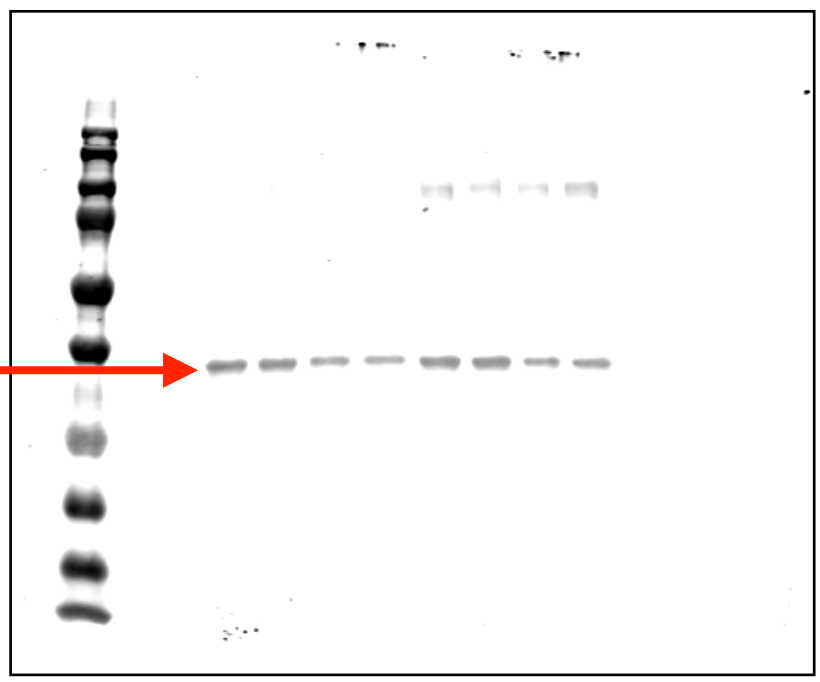


CII-SDHB

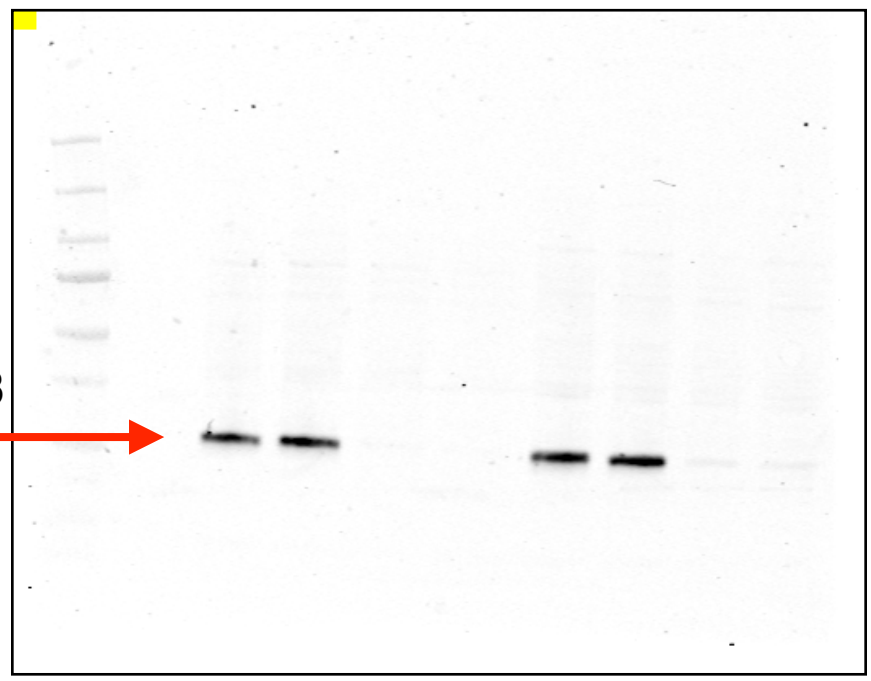
CI-NDUFB8

START: second lane from ladder

1-2: Wild-type
3-4: SIRT3KO
5-6: FXNKO
7-8: dKO

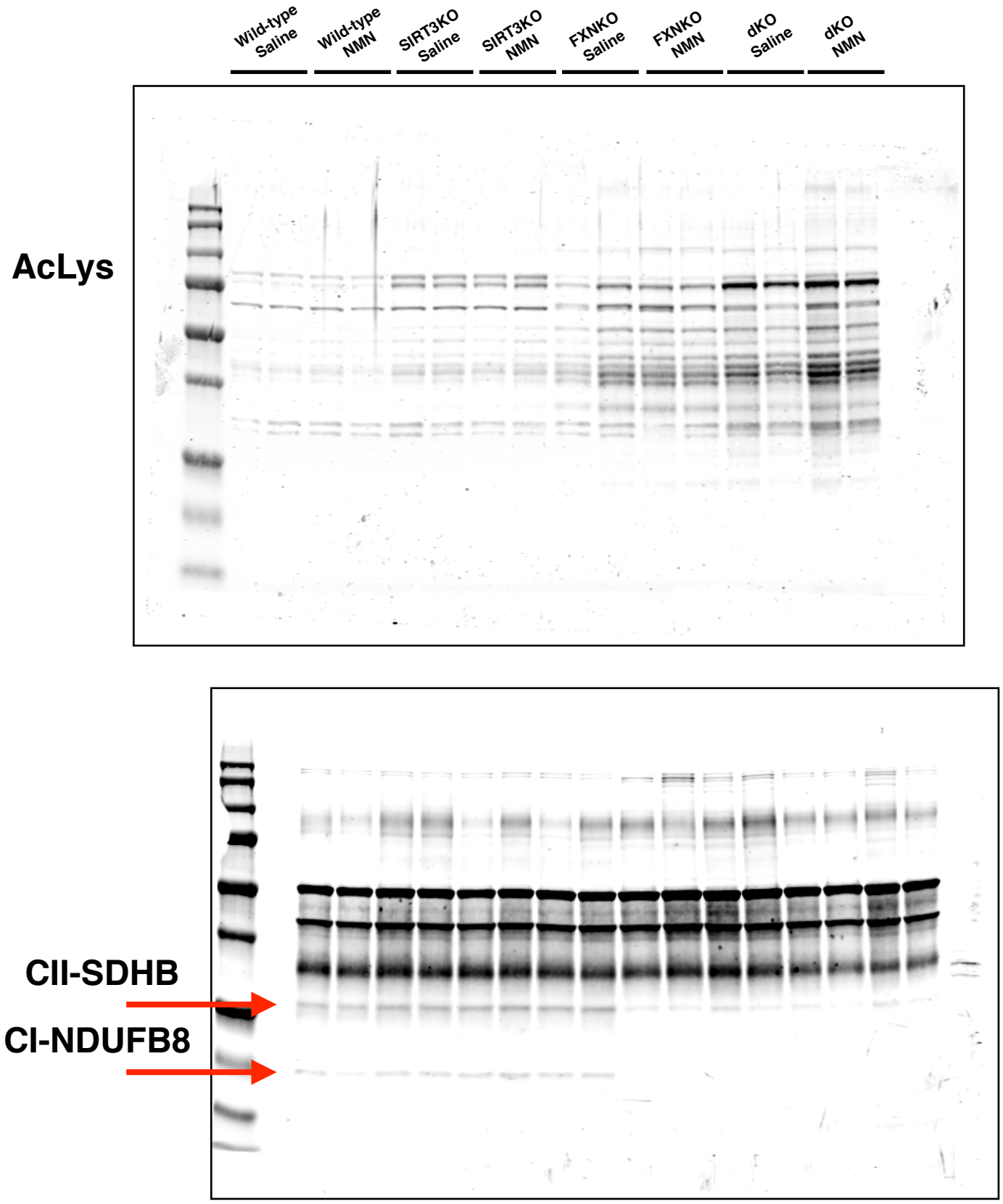


GAPDH



SIRT3

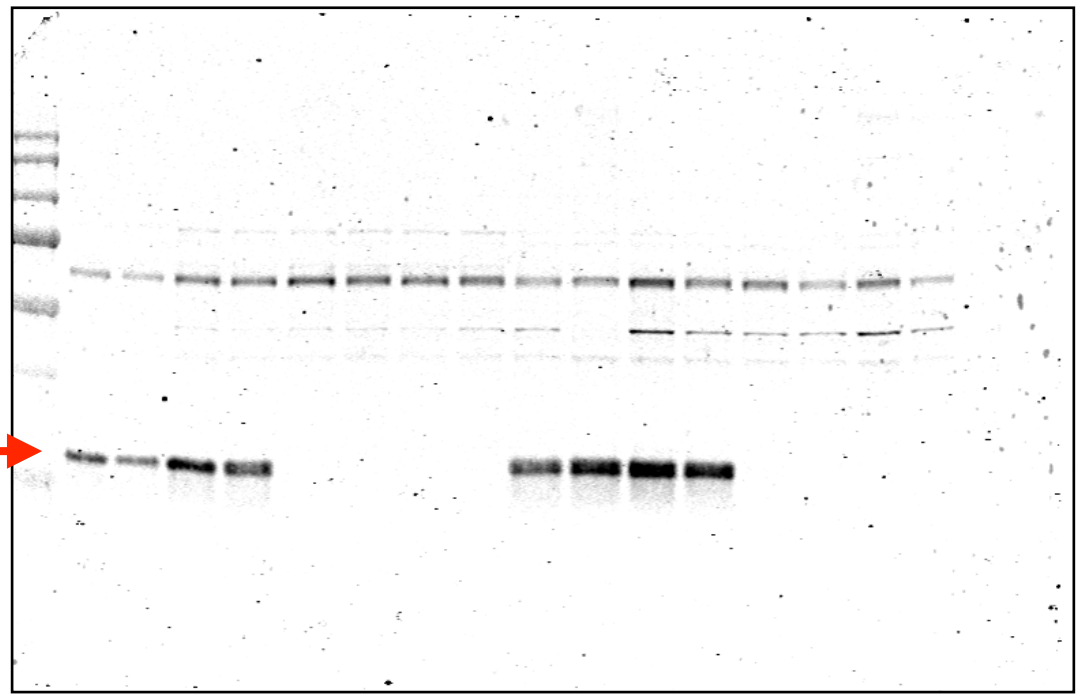
Full Unedited Gel for Supplemental Figure 2B (PART 1)



1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN

Wild-type Saline | Wild-type NMN | SIRT3KO Saline | SIRT3KO NMN | FXNKO Saline | FXNKO NMN | dKO Saline | dKO NMN

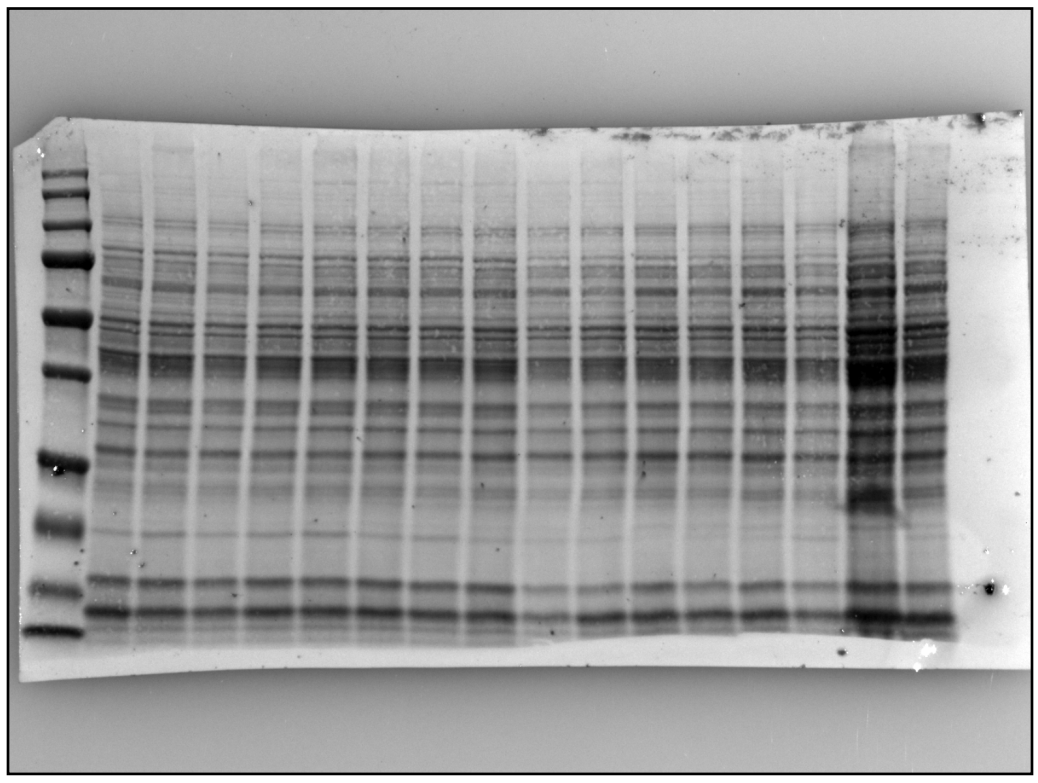
SIRT3



Full Unedited Gel for Supplemental Figure 2B (PART 2)

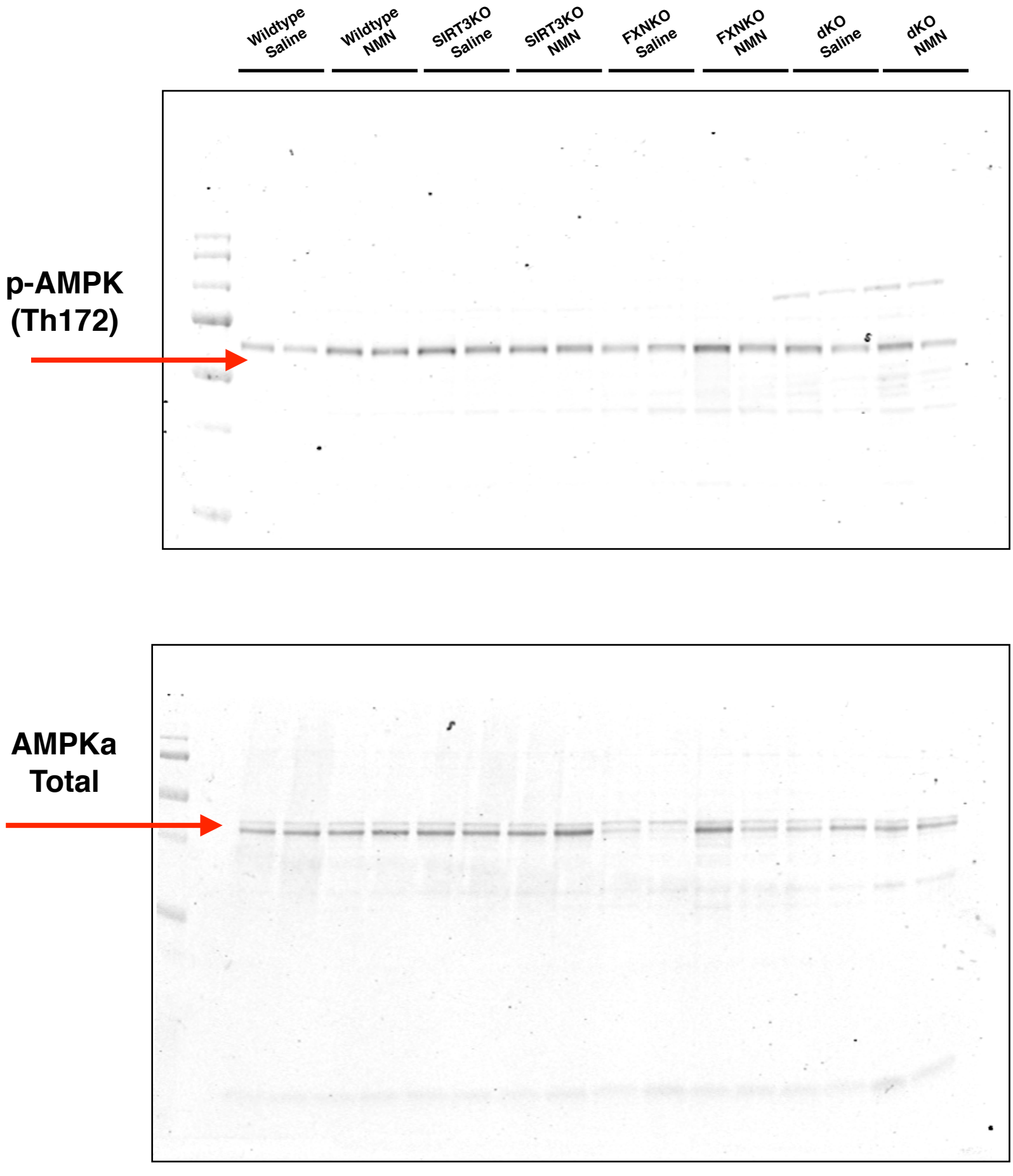
1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN

Total Mitochondrial Protein Stain



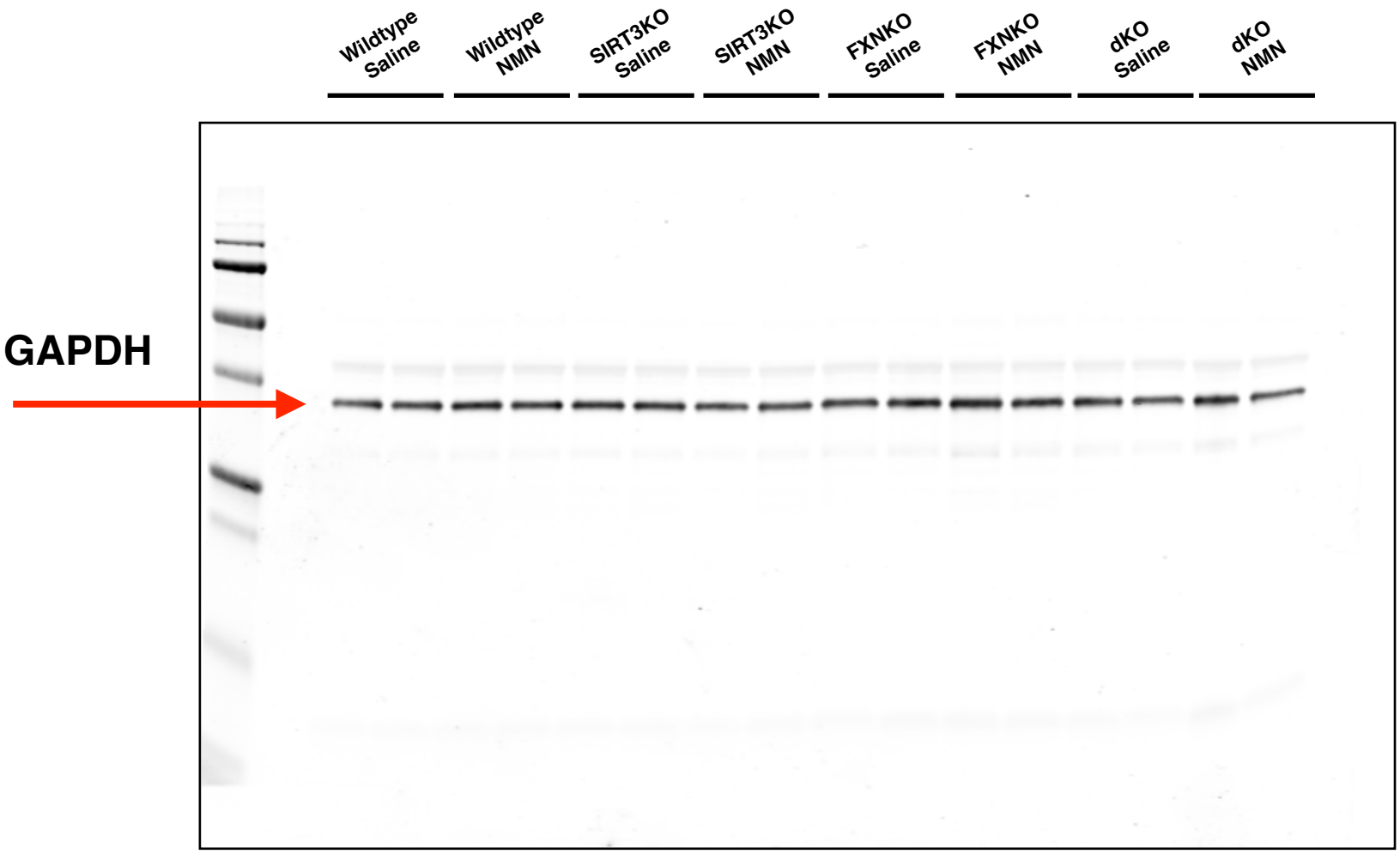
Full Unedited Gel for Supplemental Figure 3A (PART 1)

1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN



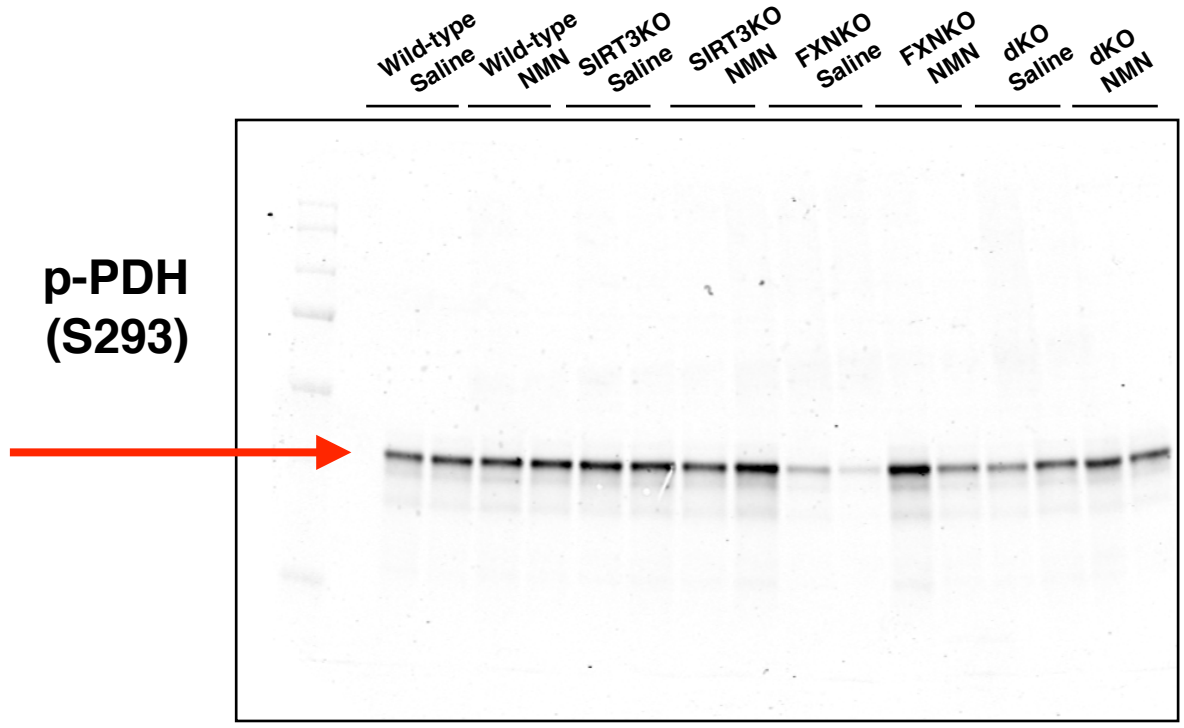
Full Unedited Gel for Supplemental Figure 3A (PART 2)

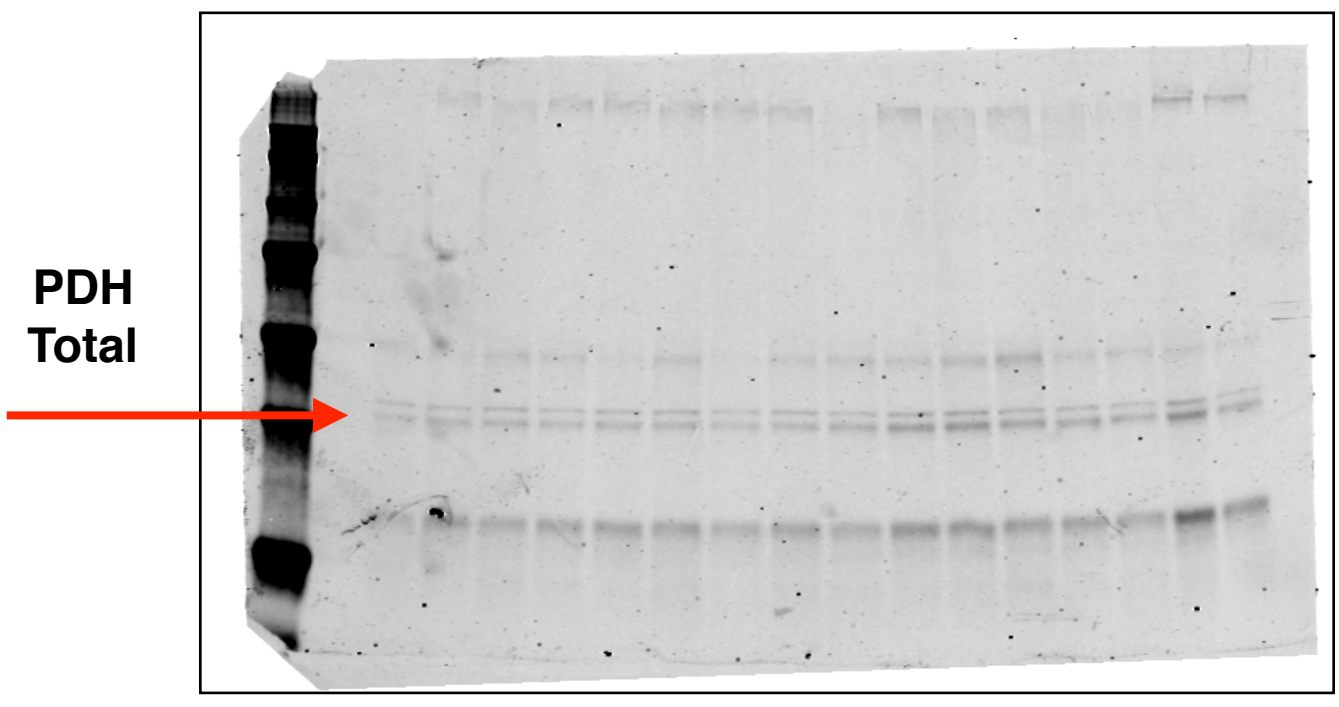
1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN

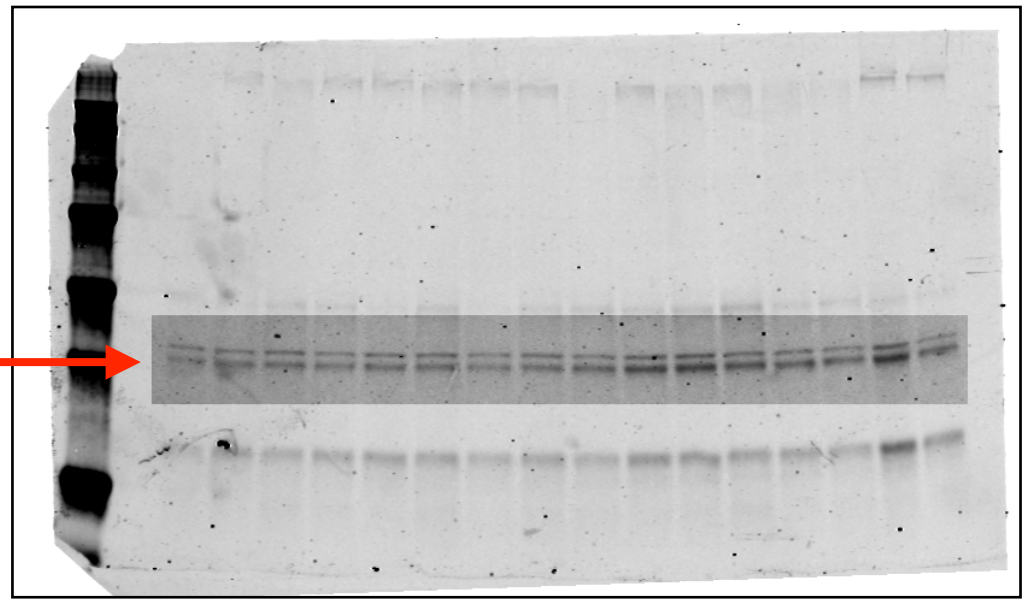


Full Unedited Gel for Supplemental Figure 5A (PART 1)

1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN

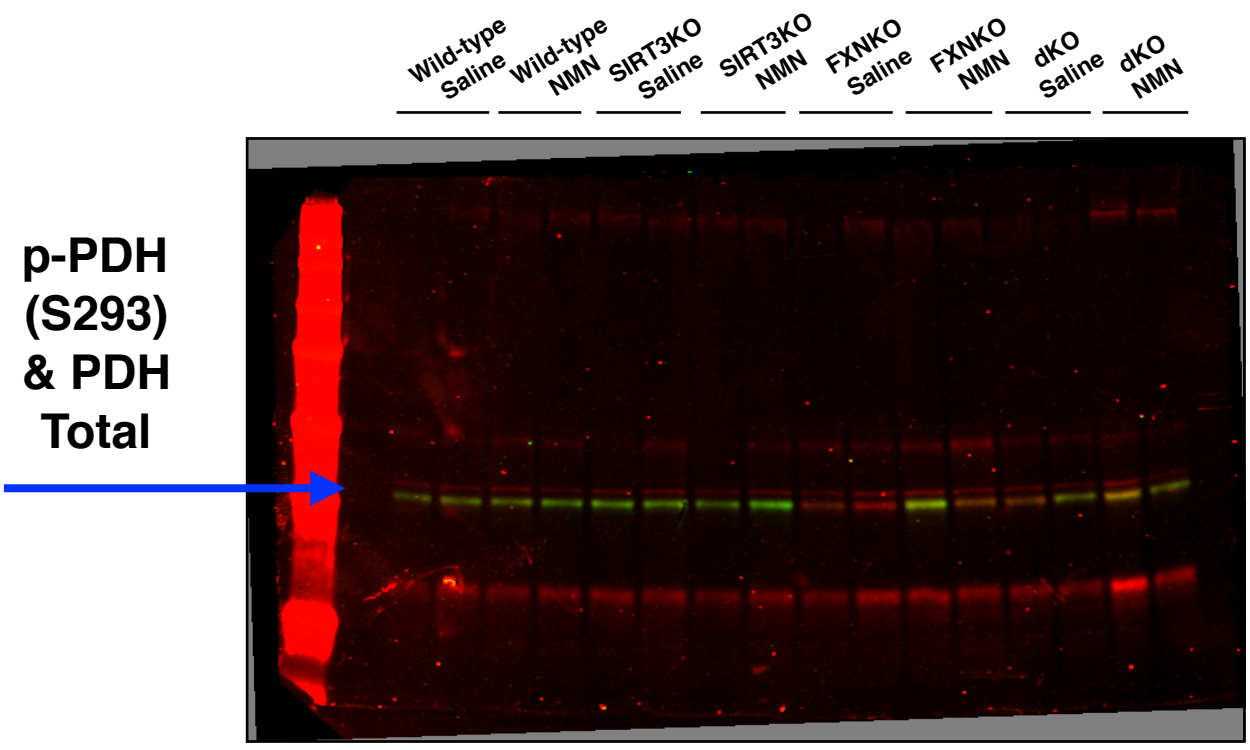


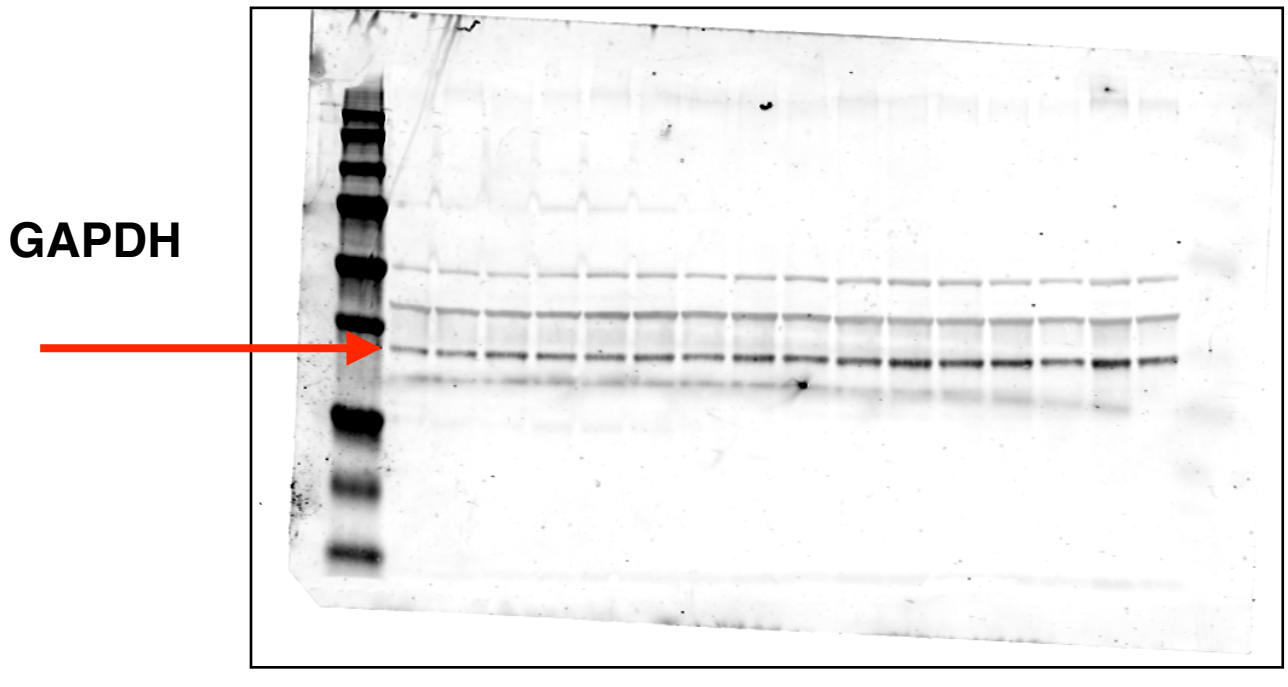




Full Unedited Gel for Supplemental Figure 5A (PART 2)

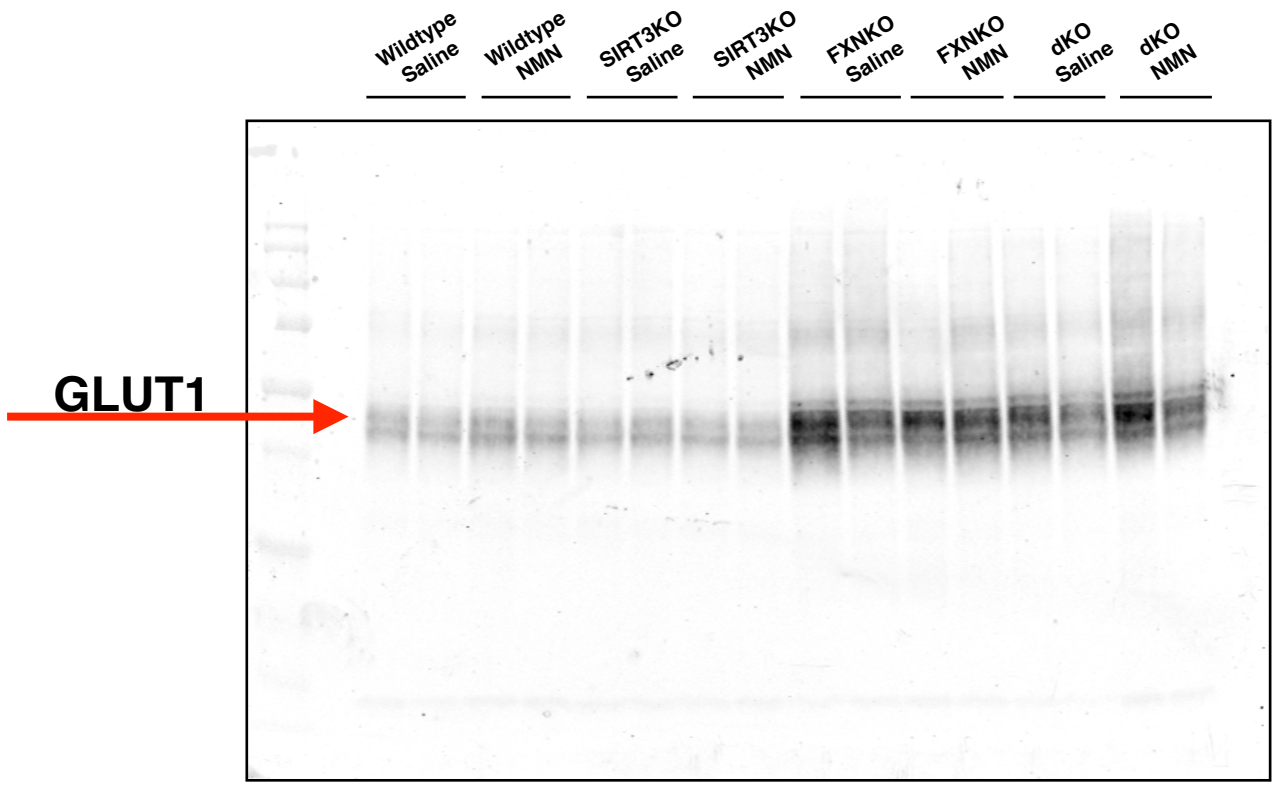
1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN

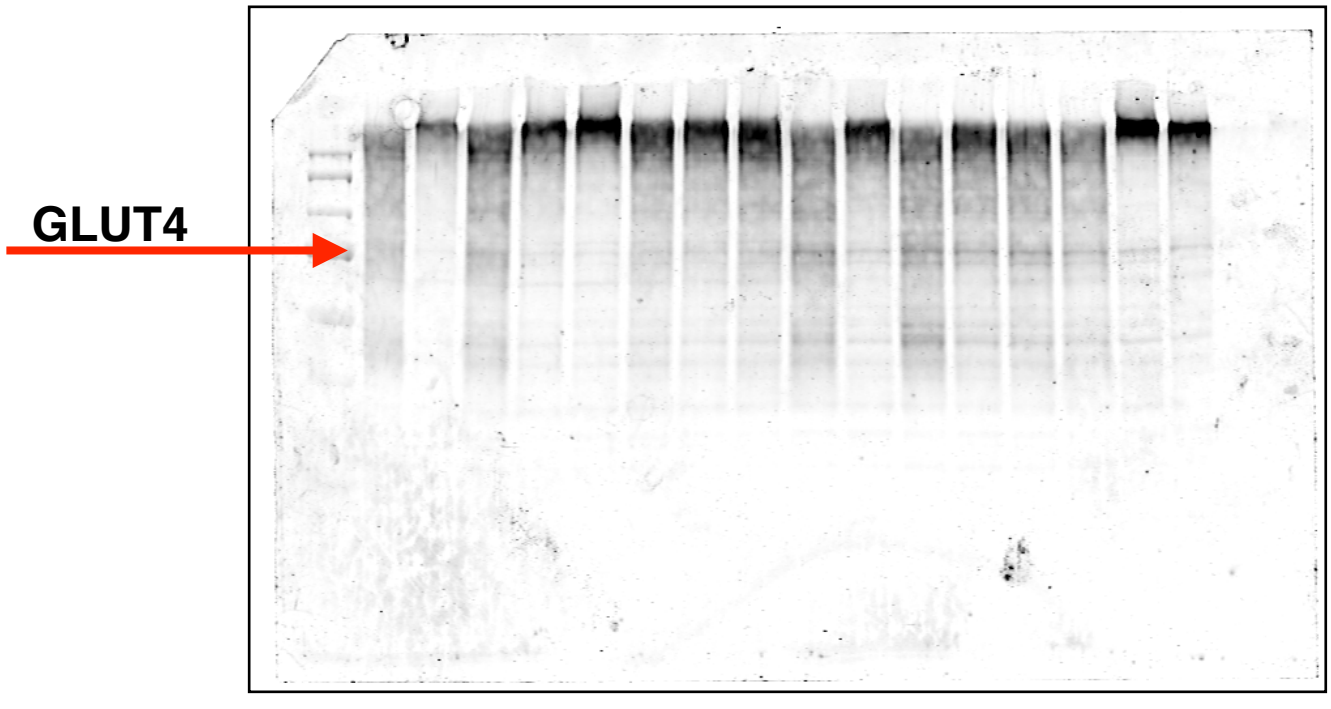




Full Unedited Gel for Supplemental Figure 5C (PART 1)

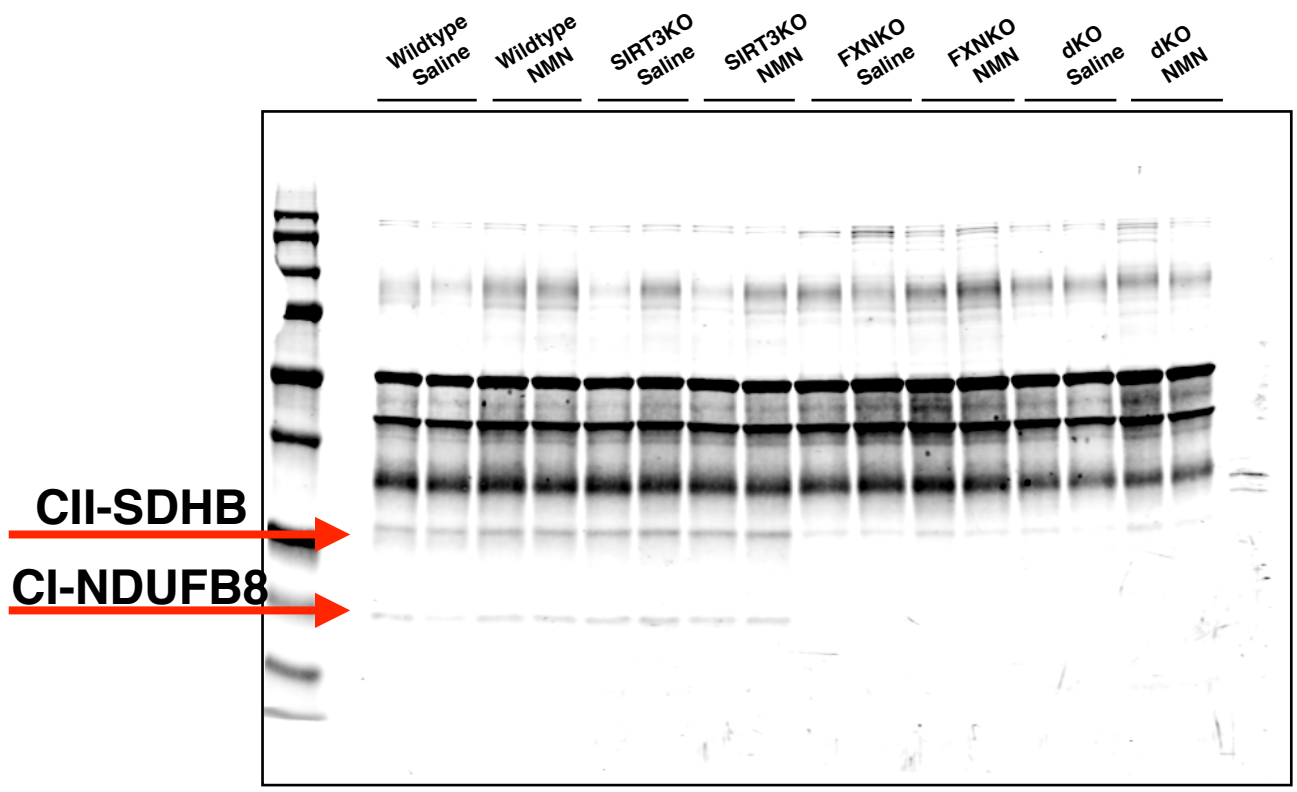
1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN

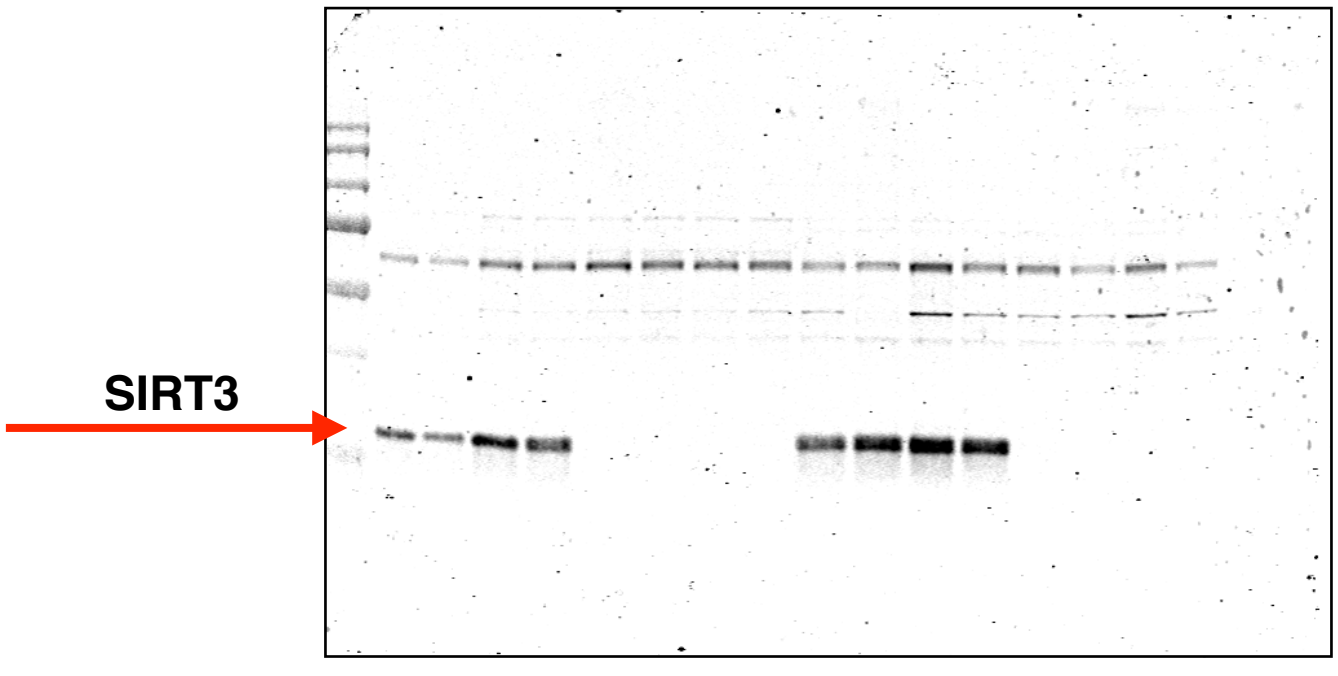




Full Unedited Gel for Supplemental Figure 5C (PART 2)

1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN





Full Unedited Gel for Supplemental Figure 5C (PART 3)

1-2: Wild-type-Saline
3-4: Wild-type-NMN
5-6: SIRT3KO-Saline
7-8: SIRT3KO-NMN
9-10: FXNKO-Saline
11-12: FXNKO-NMN
13-14: dKO-Saline
15-16: dKO-NMN