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Supplemental information

Nicotinamide riboside supplementation confers marginal metabolic benefits in obese mice without remodeling the muscle acetyl-proteome

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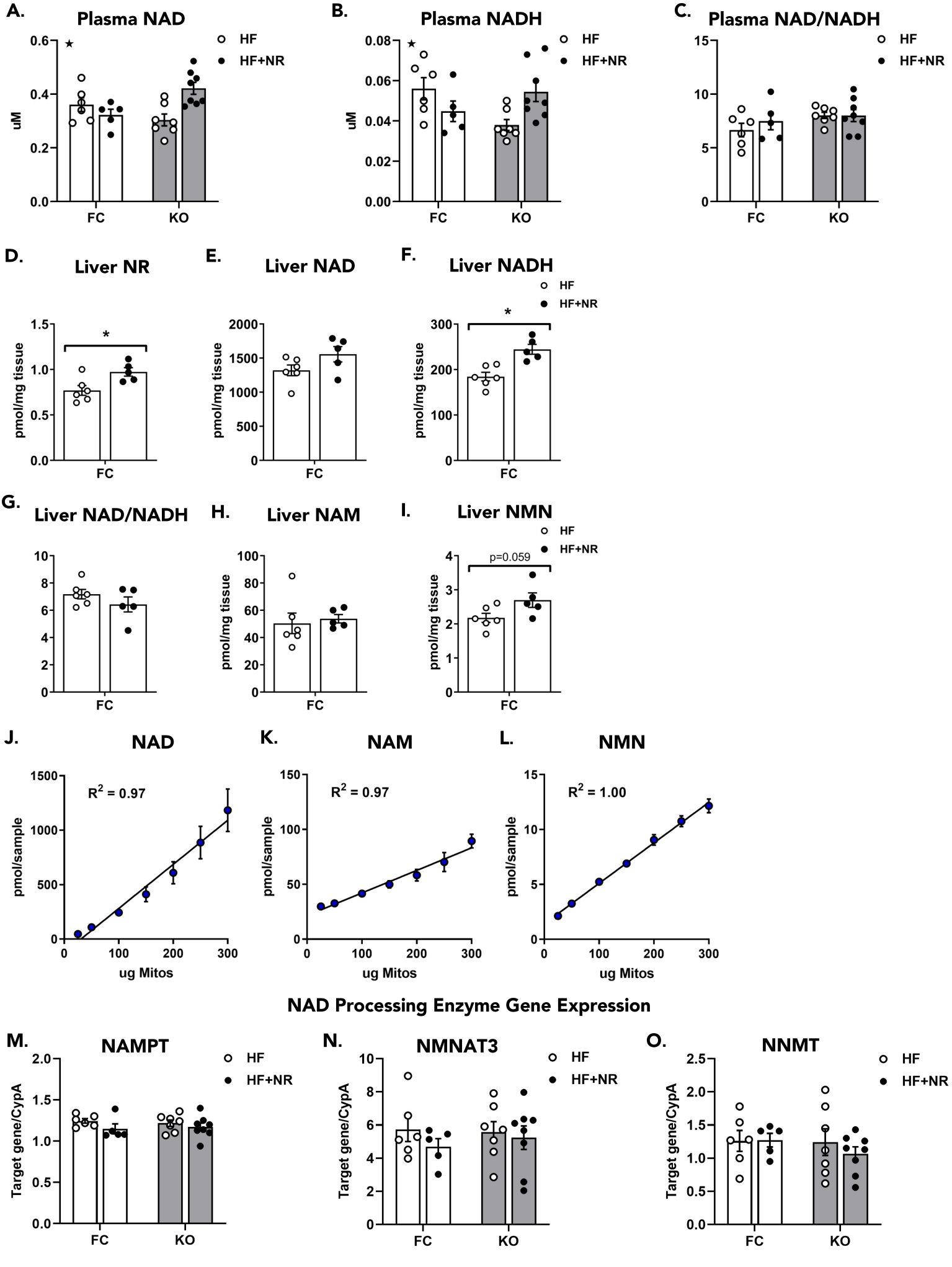
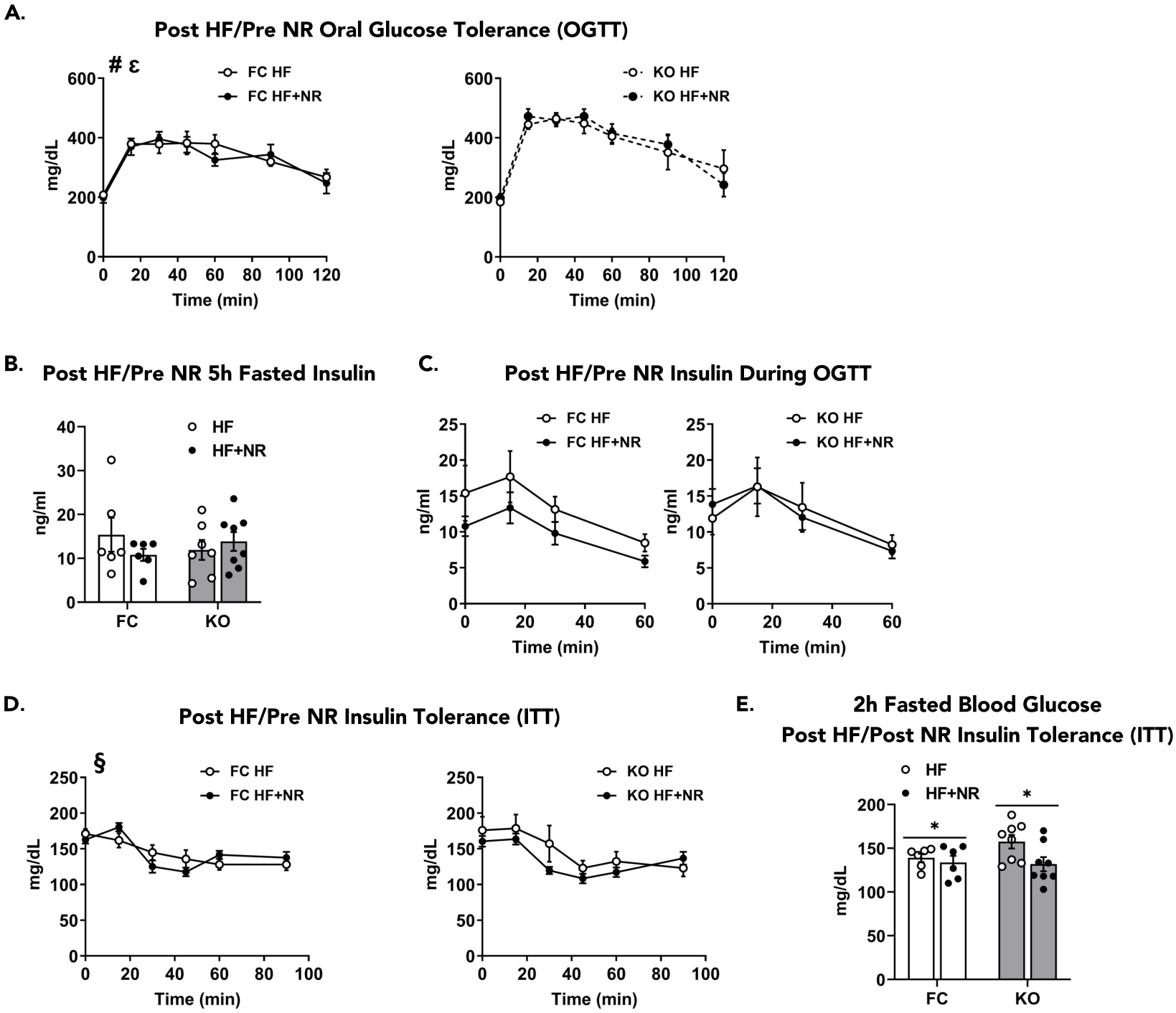
Supplemental Figure 1.



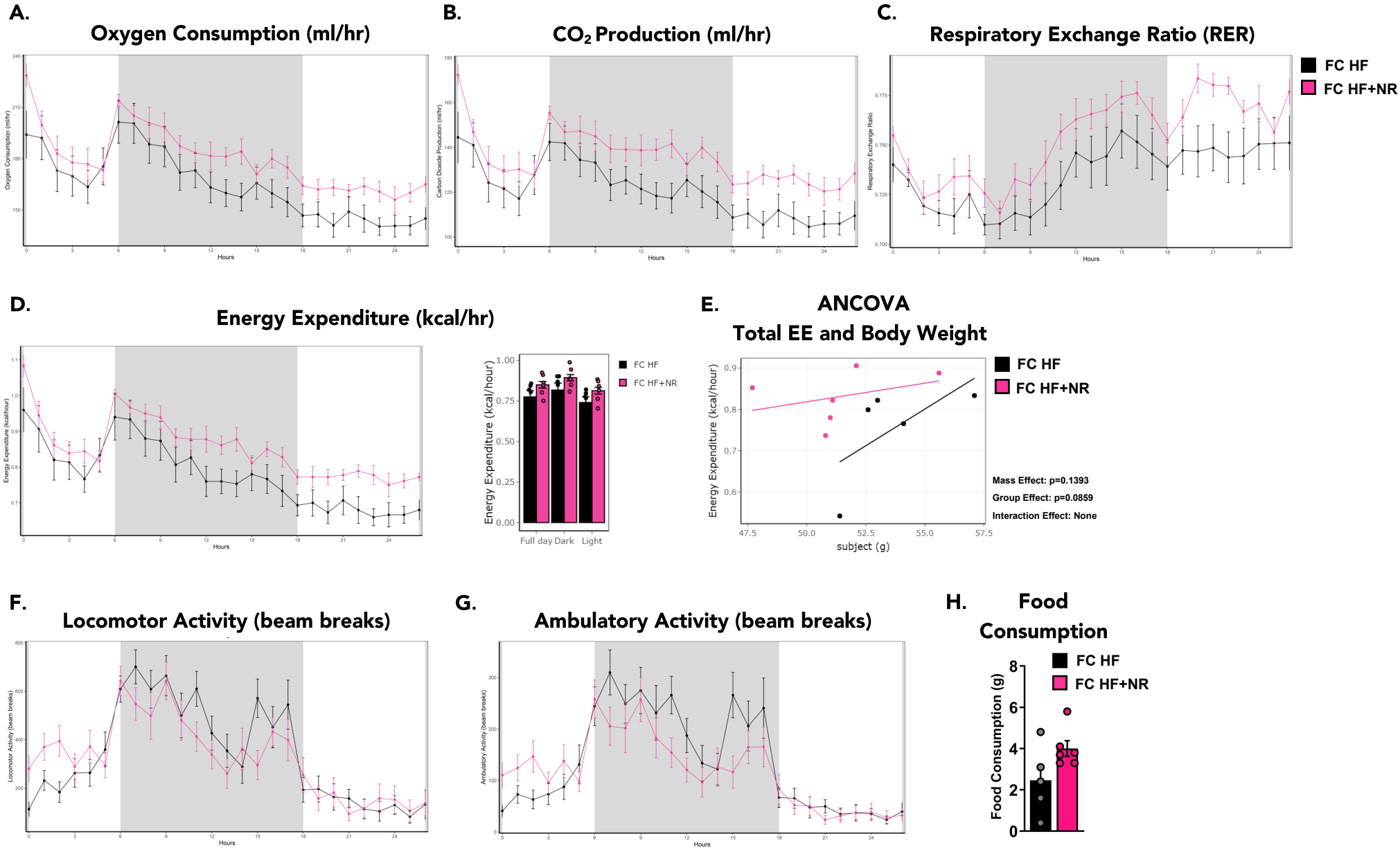
Supplemental Figure 1. Plasma and liver NAD metabolome and skeletal muscle NAD processing enzyme gene expression in HF and HF+NR fed mice. Plasma (**A**) NAD, (**B**) NADH, and (**C**) NAD/NADH ratio in FC and KO HF and HF+NR fed mice. Liver (**D**) NR, (**E**) NAD, (**F**) NADH, (**G**) NAD/NADH ratio, (**H**) NAM, and (**I**) NMN in FC HF and HF+NR fed mice. (**J-L**) LC/MS metabolomics method validation in isolated skeletal muscle mitochondria (**J**) NAD, (**K**) NAM, and (**L**) NMN. (**M-O**) Muscle NAD processing enzyme gene expression. Data are represented as mean ± SEM. (**A-C** and **M-O**) N=5-8 per group. (**J-L**) N=3 per group. (**D-I**) N=5-6 per group. Data in A-C and M-O were analyzed by two-way ANOVA. In Figures A and B, ★ represents an interaction between treatment and genotype. Data in D-I were analyzed by two-tailed Student's t-test and * represents a significant difference between HF and HF+NR fed mice. *P≤0.05. N represents biological replicates. Related to Figure 1.

Supplemental Figure 2.



Supplemental Figure 2. Post HF/Pre NR metabolic phenotyping in FC and KO mice. (**A**) Oral glucose tolerance, (**B**) 5h fasting insulin, (**C**) insulin during the oral glucose tolerance test (OGTT), (**D**) insulin tolerance in HF fed FC and KO mice before randomization to HF or HF+NR diets, and (**E**) ITT 2h fasted blood glucose from HF and HF+NR fed FC and KO mice. Data are represented as mean ± SEM. (**A**-**E**) N=5-8 per group. In A, C, and D, data were analyzed by three-way ANOVA (treatment x genotype x time); and # represents a main effect of genotype, **ε** represents a time x genotype interaction, and **§** represents a time x treatment interaction. Data in B and E were analyzed by two-way ANOVA. In E, * represents a main effect of treatment. *P≤0.05. N represents biological replicates. Related to Figure 2.

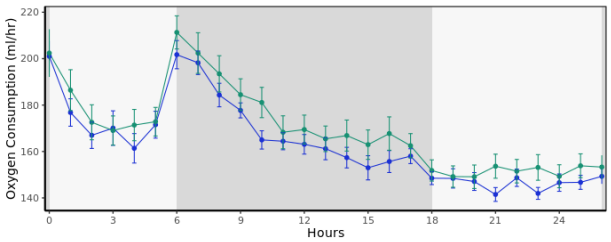
Supplemental Figure 3.



Supplemental Figure 3. NR treatment does not impact energy balance in HF fed FC mice. Energy balance in FC mice fed a HF or HF+NR diet. (**A**) Oxygen consumption. (**B**) CO_2 production. (**C**) Respiratory Exchange Ratio (RER). (**D**) Energy Expenditure (EE). (**E**) ANCOVA analysis (total EE and body weight). (**F**) Locomotor activity. (**G**) Ambulatory activity. (**H**) CLAMS food consumption. Data are represented as mean ± SEM. (**A-H**) N=5-6 per group. Data were analyzed by two-tailed Student's t-test. * represents a significant difference between HF and HF+NR fed mice. *P≤0.05. N represents biological replicates. Related to Figure 2.

Supplemental Figure 4.

A.



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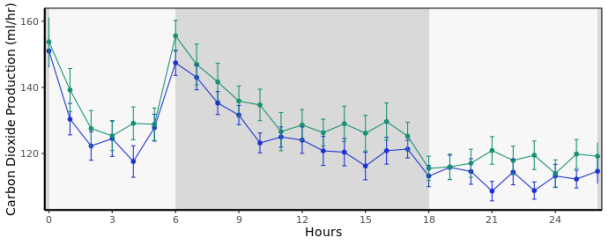

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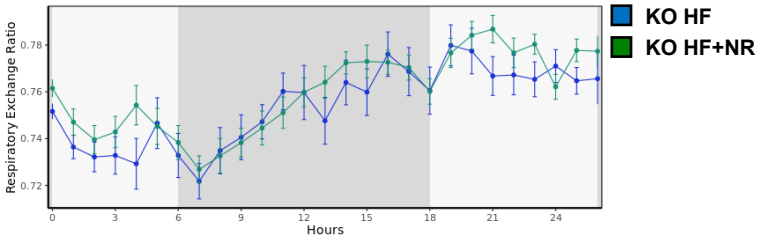

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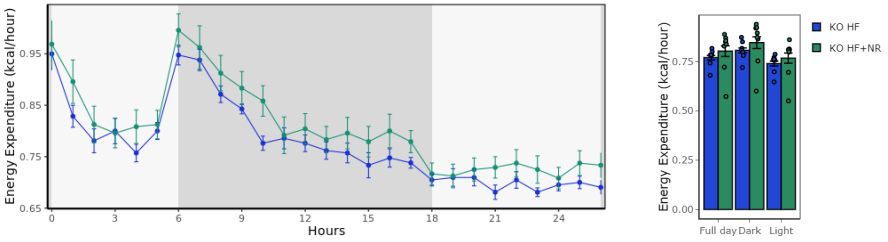

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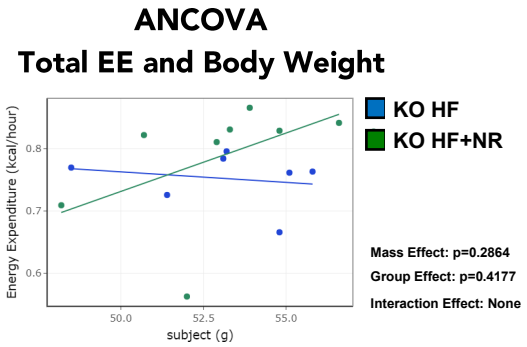

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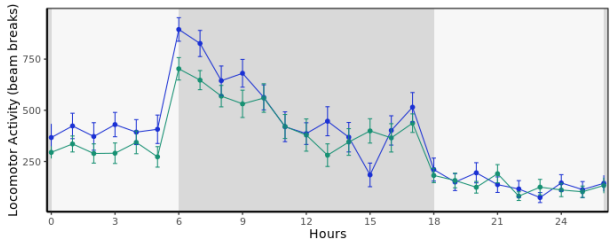

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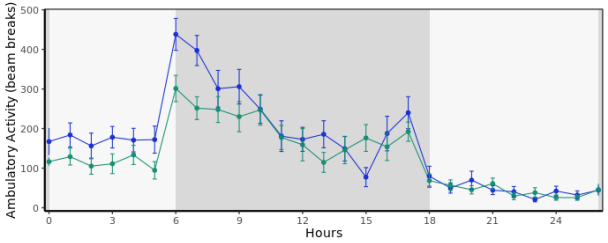

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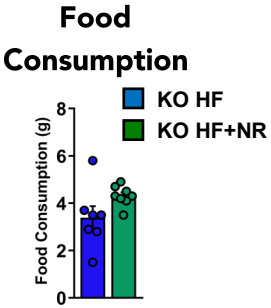
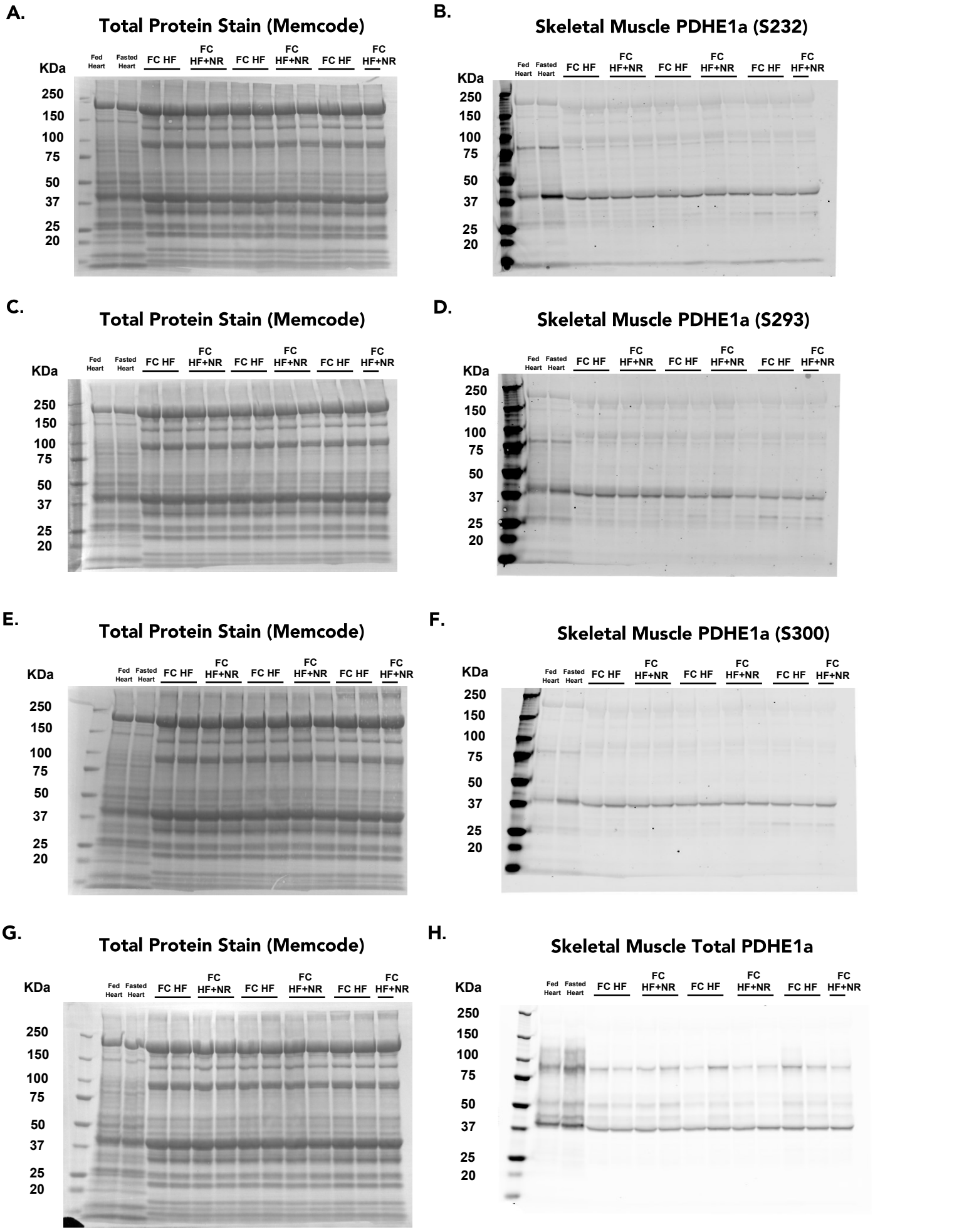


Supplemental Figure 4. NR treatment does not affect energy balance in HF fed KO mice. Energy balance in KO mice fed a HF or HF+NR diet. (**A**) Oxygen consumption. (**B**) CO_2 production. (**C**) Respiratory Exchange Ratio (RER). (**D**) Energy Expenditure (EE). (**E**) ANCOVA analysis (total EE and body weight). (**F**) Locomotor activity. (**G**) Ambulatory activity. (**H**) CLAMS food consumption. Data are represented as mean ± SEM. (**A-H**) N=7-8 per group. Data were analyzed by two-tailed Student's t-test. * represents a significant difference between HF and HF+NR fed mice. N represents biological replicates. Related to Figure 2.

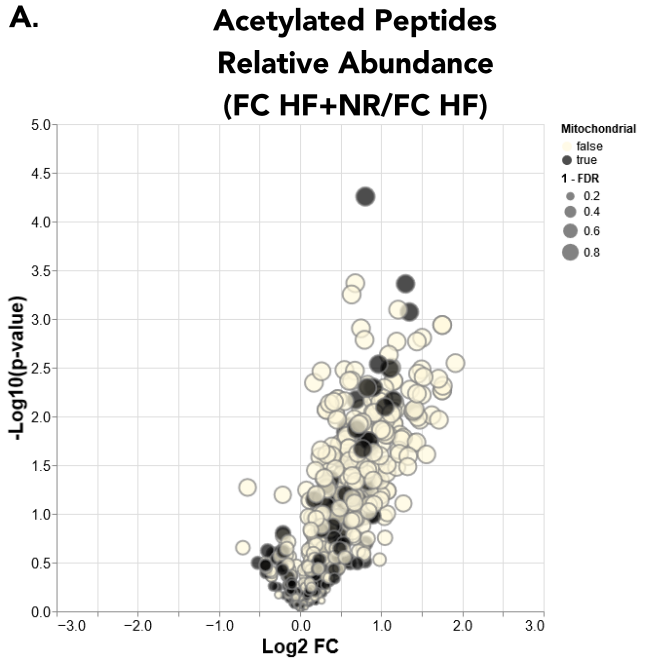
Supplemental Figure 5.



Supplemental Figure 5. Muscle PDHE1a phosphorylation is not different in NR treated HF fed FC mice. Western blot analysis was performed on proteins extracted from tibialis anterior (TA) muscles from FC mice fed a HF or HF+NR diet. (**A**, **C**, **E**, and **G**) Membrane images showing total protein *via* the total protein stain Memcode. Western blots for (**B**) PDHE1a phosphoserine 232 (S232), (**D**) PDHE1a phosphoserine 293 (S239), (**F**) PDHE1a phosphoserine 300 (S300), and (**H**) total PDHE1a. (**A-H**) N=5-6 per group. N represents biological replicates. Related to Figure 3.

Supplemental Figure 6.

A.



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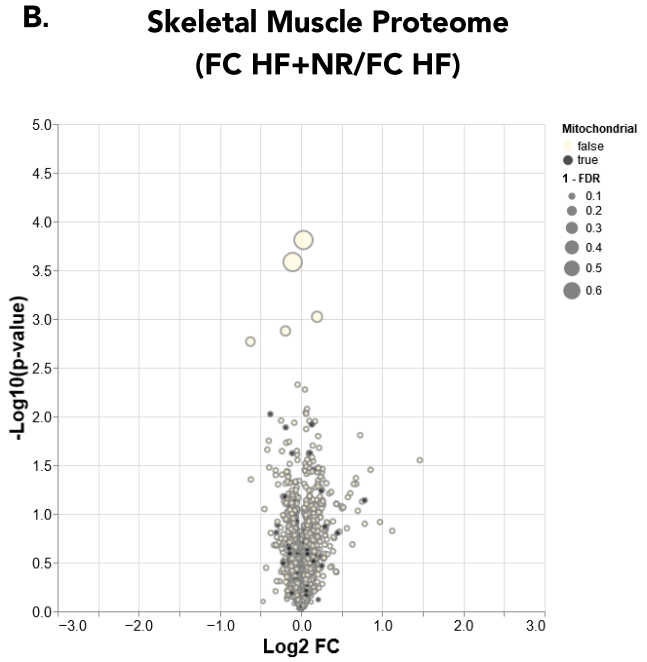

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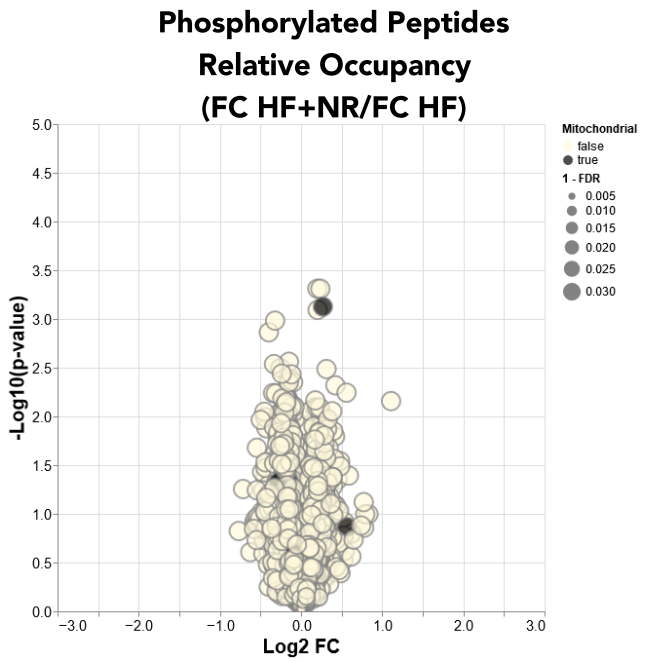

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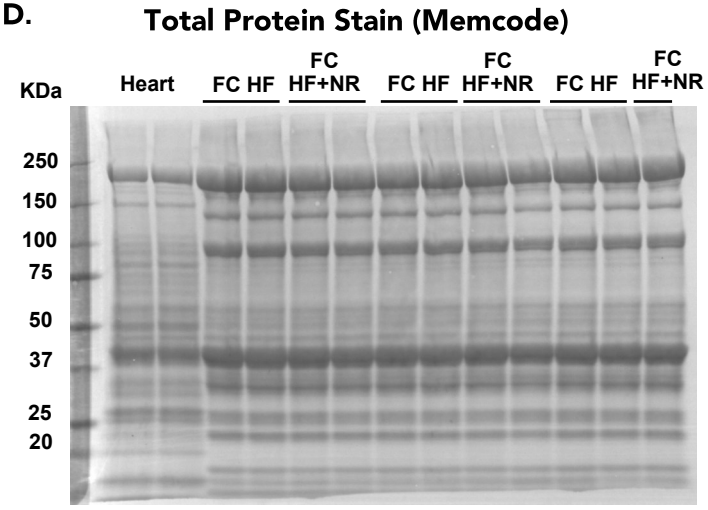

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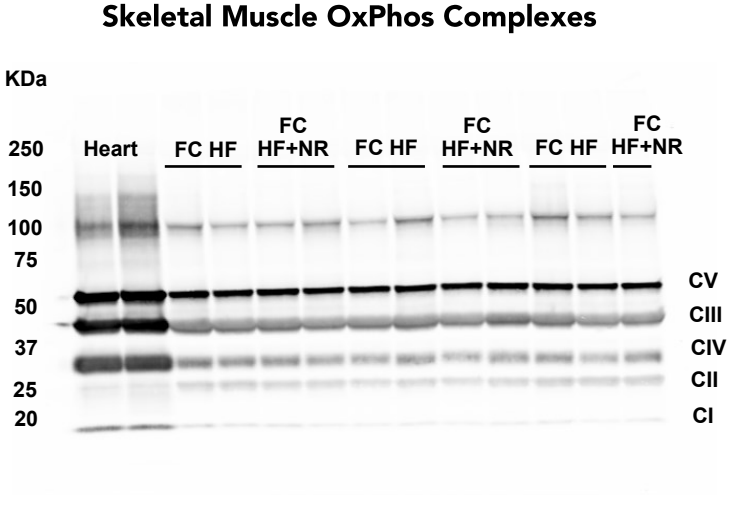
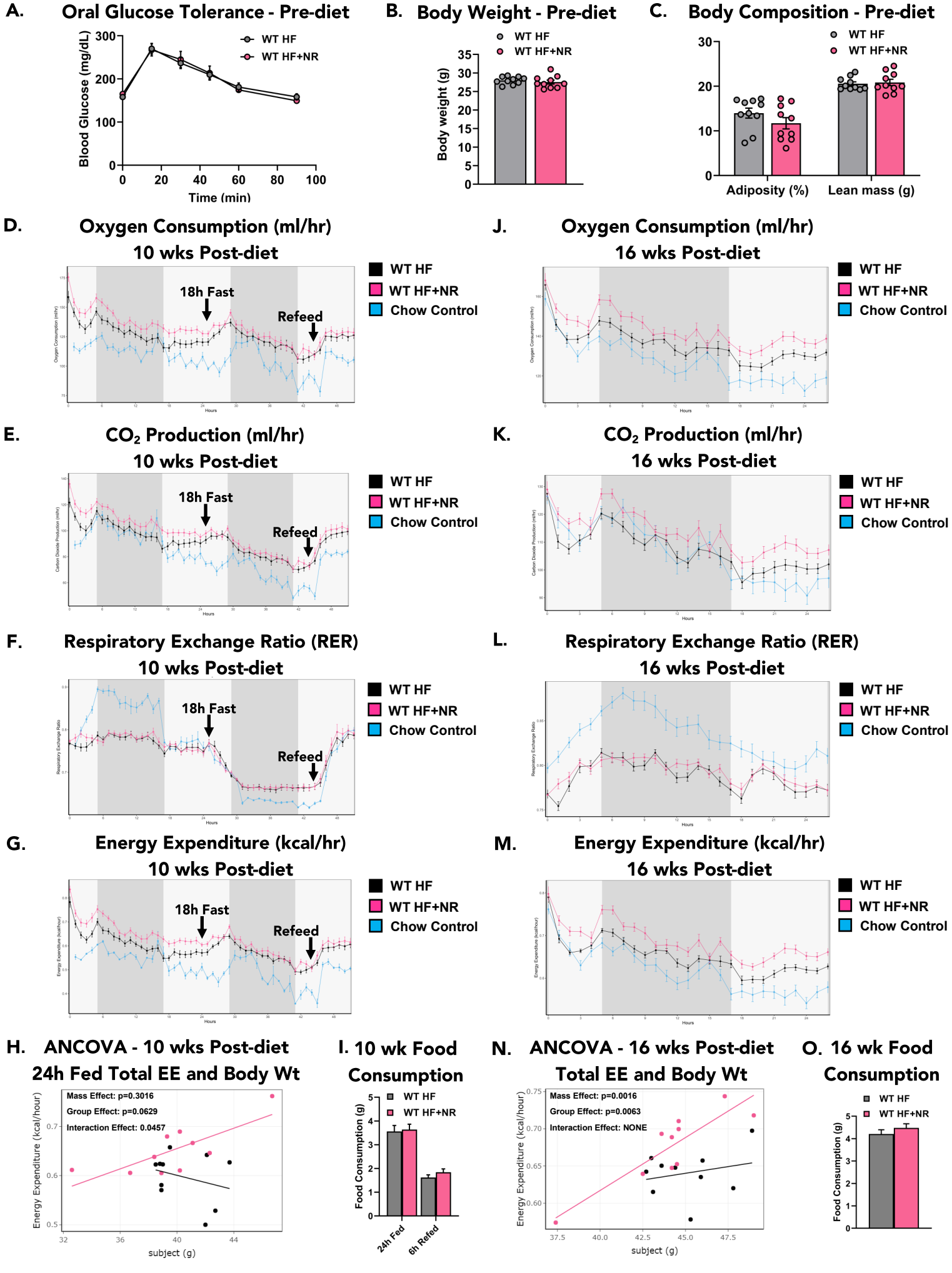


Supplemental Figure 6. NR treatment does not affect the muscle acetylome, proteome, phosphoproteome or protein expression of mitochondrial oxidative phosphorylation (OxPhos) subunits in HF fed FC mice. Volcano plots depicting (**A**) relative abundance of acetyl-peptides, (**B**) relative abundance of proteins, and (**C**) relative occupancy of phospho-peptides identified in quadriceps tissue from HF and HF+NR FC mice. Open and closed dots refer to non-mitochondrial and mitochondrial peptides respectively. (**D-E**) Western blot analysis was performed on proteins extracted from tibialis anterior (TA) muscles from FC mice fed a HF or HF+NR diet. (**D**) Membrane total protein stain (memcode). (**E**) Skeletal muscle mitochondrial oxidative phosphorylation (OxPhos) subunits. (**A-C**) N=5 per group. (**D-E**) N=5-6 per group. N represents biological replicates. Related to Figures 3 and 5.

Supplemental Figure 7.



Supplemental Figure 7. Pre-diet metabolic phenotyping and post-diet energy balance in wild-type C57BL6/NJ mice fed a HF or HF+NR diet. (**A**) Oral glucose tolerance, (**B**) body weight pre-HF and (**C**) body composition pre-HF or HF+NR diet. (**D-I**) HF and HF+NR fed mice were subjected to a fed/18h fast/6h refeed protocol 10 weeks after the start of the HF or HF+NR diets. (**D**) Oxygen consumption. (**E**) Carbon dioxide production. (**F**) Respiratory Exchange Ratio (RER). (**G**) Energy Expenditure (EE). (**H**) ANCOVA analysis for the first 24h fed time period (total EE and body weight). (**I**) CLAMS food consumption. (**J-O**) HF and HF+NR fed mice were subjected to a 24h CLAMS run in the fed state 16 weeks after the start of the HF or HF+NR diets. (**J**) Oxygen consumption. (**K**) Carbon dioxide production. (**L**) Respiratory Exchange Ratio (RER). (**M**) Energy Expenditure (EE). (**N**) ANCOVA analysis (total EE and body weight). (**O**) CLAMS food consumption. Data are represented as mean ± SEM. (**A-C and J-O**) N=10 per group. (**D-I**) N=10 per group for HF and HF+NR mice and N=2 chow mice. N represents biological replicates. Related to Figure 6.