SUPPL. FIGURE LEGENDS

Supplemental Fig. S1. Exercise TRAINing attenuates weight gain and helps maintain muscle weight throughout DETRAINing.
A) Endurance exercise TRAIN paradigm in which mice fed a control diet (CD) underwent voluntary wheel running (VWR) or remained sedentary for 4wks. **B)** Body composition of the TRAIN cohort indicating stacked fat (yellow) and lean (pink) mass and body weight (lines, BW) changes. **C)** Weekly BW changes over time of the TRAIN cohort. **D)** Matched changes in fat (left) and lean (right) mass from 0 to 4wks. Inset above indicates a two-way ANOVA (time*exercise) showing significant effects with indicated *p*-values (1 cohort, n=10/group). **E)** Relative muscle weights 2d post exercise of the TRAIN cohort. **F)** Absolute muscle weights 2d post exercise of the TRAIN cohort (1 cohort, n=3/group). **G)** Endurance exercise TRAIN-DETRAIN paradigm in which mice trained for 4wks then detrained for 4wks or remained sedentary for 8wks. **H)** Body composition of the DETRAIN cohort indicating stacked fat (yellow) and lean (pink) mass and BW (lines) changes. **I)** Weekly BW changes from the DETRAIN cohort over 8wks. **J)** Matched changes in fat (left) and lean (right) after 4wks of TRAINing and 4wks of DETRAINing. Inset above indicates a two-way ANOVA (time*exercise) showing significant effects with indicated *p*-values (1 cohort, n=10/group). **K)** Relative muscle weights from the DETRAIN cohort after 4wks of DETRAINing. **L)** Absolute muscle weights from the DETRAIN cohort after 4wks of DETRAINing (1 cohort, n=3/group). **M)** Relative (left) and absolute (right) muscle weights of the Levator Ani (LA) 2d post completion of each cohort (4 cohorts, n=3-6/group). **N)** Relative (left) and absolute (right) muscle weights of the Levator Ani (LA) 1wk post completion of each cohort (4 cohorts, n=6-7/group). Data are presented as means ± SD. Within timepoint comparisons determined by Student t-test; $p<0.1, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplemental Fig. S2. RETRAINing tends to shift fiber distribution towards larger fibers with little impact on protein accretion.
A) Muscle fiber size frequency distribution of all fibers following TRAINing (left), DETRAINing (middle), and RETRAINing (right) (3 independent cohorts, 1 whole cross section per animal, n=5-7/group). **B)** Representative western blot and **C)** analysis of puromycin incorporation (TA) into newly synthesized proteins (3 independent cohorts, n=3/group). **D**) qPCR expression of genes related to protein degradation. Data are presented as means ± SD. Within timepoint comparisons determined by Student t-test; $p<0.1, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplemental Fig. S3. Effect of endurance training, detraining, and retraining on skeletal muscle gene expression.
Gastrocnemius RNA-seq showing the top 10 up- (red) and downregulated (blue) genes (left) and the KEGG enrichment analysis (right) following **A)** 4wks of endurance TRAINing (1 cohort, n=3/group), **B)** 8wk DETRAINing (1 cohort, n=3/group), and **C**) 12wk endurance RETRAINing cycles (1 cohort, n=3/group) in VWR/CD mice, compared to age-matched SED/CD. DEGs adjusted $p< 0.2$. *Tibialis anterior* qPCR expression of **D)** *Pgc-1α* and **E)** epigenetic modifiers *Dnmt1*, *Tet1*, *Tet2*, and *Tet3* (3 independent cohorts, n=6/group). Data are presented as means ± SD. Within timepoint comparisons determined by Student t-test; $p<0.1, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplemental Fig. S4. Exercise TRAINing negates HFD weight gain while increasing muscle growth, even following DETRAINing.

A) Mice fed a HFD and either exposed to 4wks voluntary wheel running (VWR) or static, sedentary cages (TRAIN). **B)** Body composition of the TRAIN cohort indicating stacked fat (yellow) and lean (pink) mass and body weight (BW, lines) changes. **C)** Weekly BW changes over time of the TRAIN cohort. **D)** Matched changed in fat (left) and lean (right) mass from 0 to 4wks from the TRAIN cohort. Inset above indicates a two-way ANOVA (time*exercise) showing significant effects with indicated *p*-values (1 cohort, n=10/group). **E)** Relative muscle weights 2d post exercise of the TRAIN cohort. **F)** Absolute muscle weights 2d post exercise of the TRAIN cohort (1 cohort, n=3/group). **G)** Mice were fed a HFD and completed 8wk TRAIN-DETRAIN with VWR or remained sedentary for 8wks (DETRAIN). **H)** Body composition of the DETRAIN cohort over 8wks indicating stacked fat (yellow) and lean (pink) mass and BW (lines) changes. **I)** Weekly BW changes of the DETRAIN cohort. J) Matched changes in fat (left) and lean (right) after 4wks of TRAINing and 4wks of DETRAINing. Inset above indicates a two-way ANOVA (time*exercise) showing significant effects with indicated *p*-values (1 cohort, n=10/group). **K)** Relative muscle weights from the DETRAIN cohort after 4wks of DETRAINing. **L)** Absolute muscle weights from the DETRAIN cohort after 4wks of DETRAINing (1 cohort, n=3/group). **M)** Relative (left) and absolute (right) muscle weights of the Levator Ani (LA) 2d post completion of each cohort (3 cohorts, n=3-6/group). **N)** Relative (left) and absolute (right) muscle weights of the Levator Ani (LA) 1wk post completion of each cohort (3 cohorts, n=6-7/group). Data are presented as means ± SD. Within timepoint comparisons determined by Student t-test; $p<0.1, * $p<0.05, ** $p<0.01, *** $p<0.001, **** $p<0.0001.

Supplemental Fig. S5. HFD feeding negates any increases in fiber size distribution, while RETRAINing may impact new protein accretion.

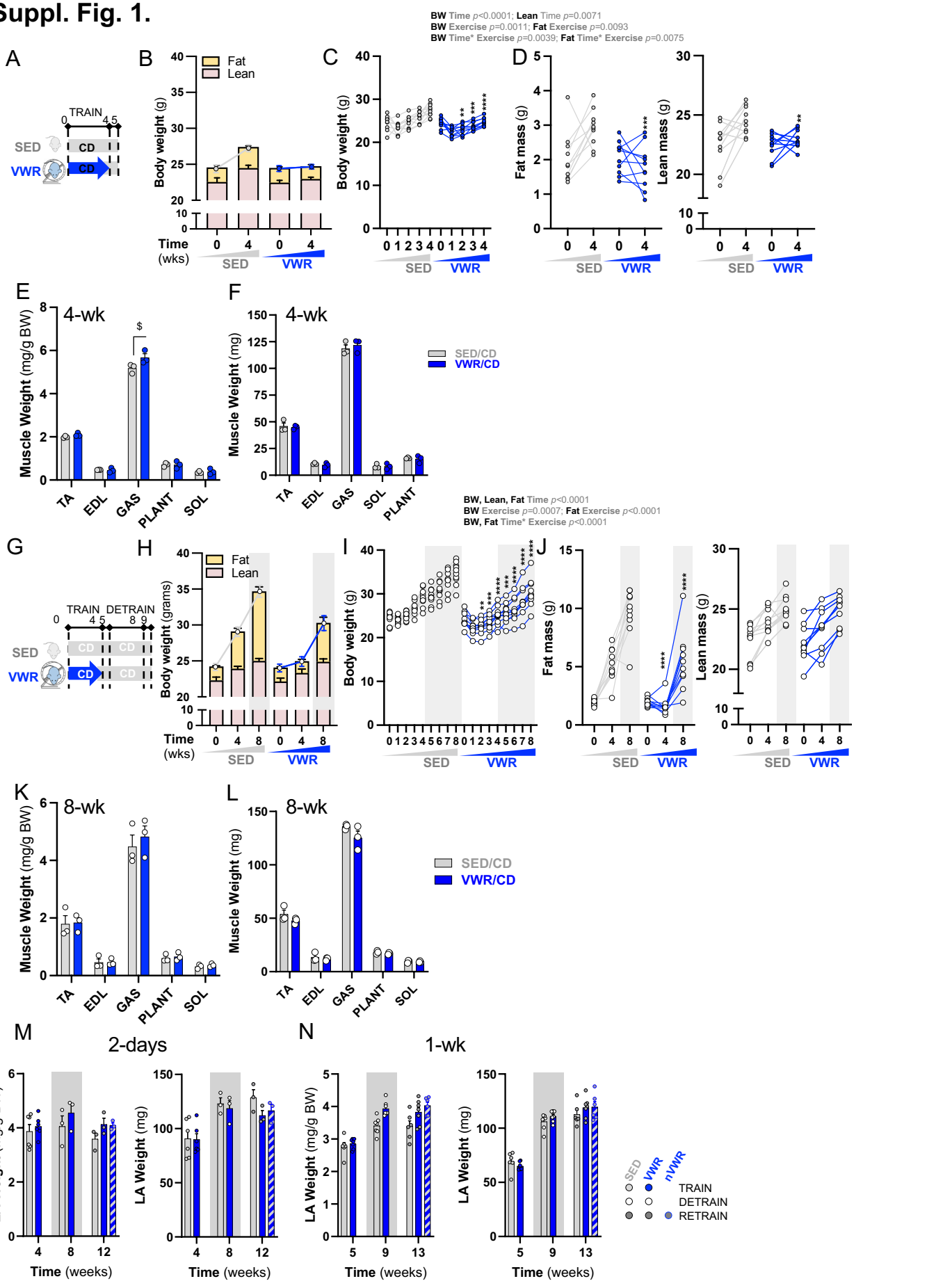
A) Muscle fiber size frequency distribution of all fibers following TRAINing (left), DETRAINing (middle), and RETRAINing (right) (3 independent cohorts, 1 whole cross section per animal, n=6-7/group). **B)** Representative western blot and **C)** analysis of puromycin incorporation (TA) into newly synthesized proteins (3 independent cohorts, n=3/group). **D**) qPCR expression of genes related to protein degradation. Data are presented as means ± SD. Within timepoint comparisons determined by Student t-test; *$p<0.05, **$p<0.01, ***$p<0.001, $$p<0.1.

Supplemental Fig. S6. Impact of high-fat diet and exercise paradigms on skeletal muscle gene expression and pathway adaptations.

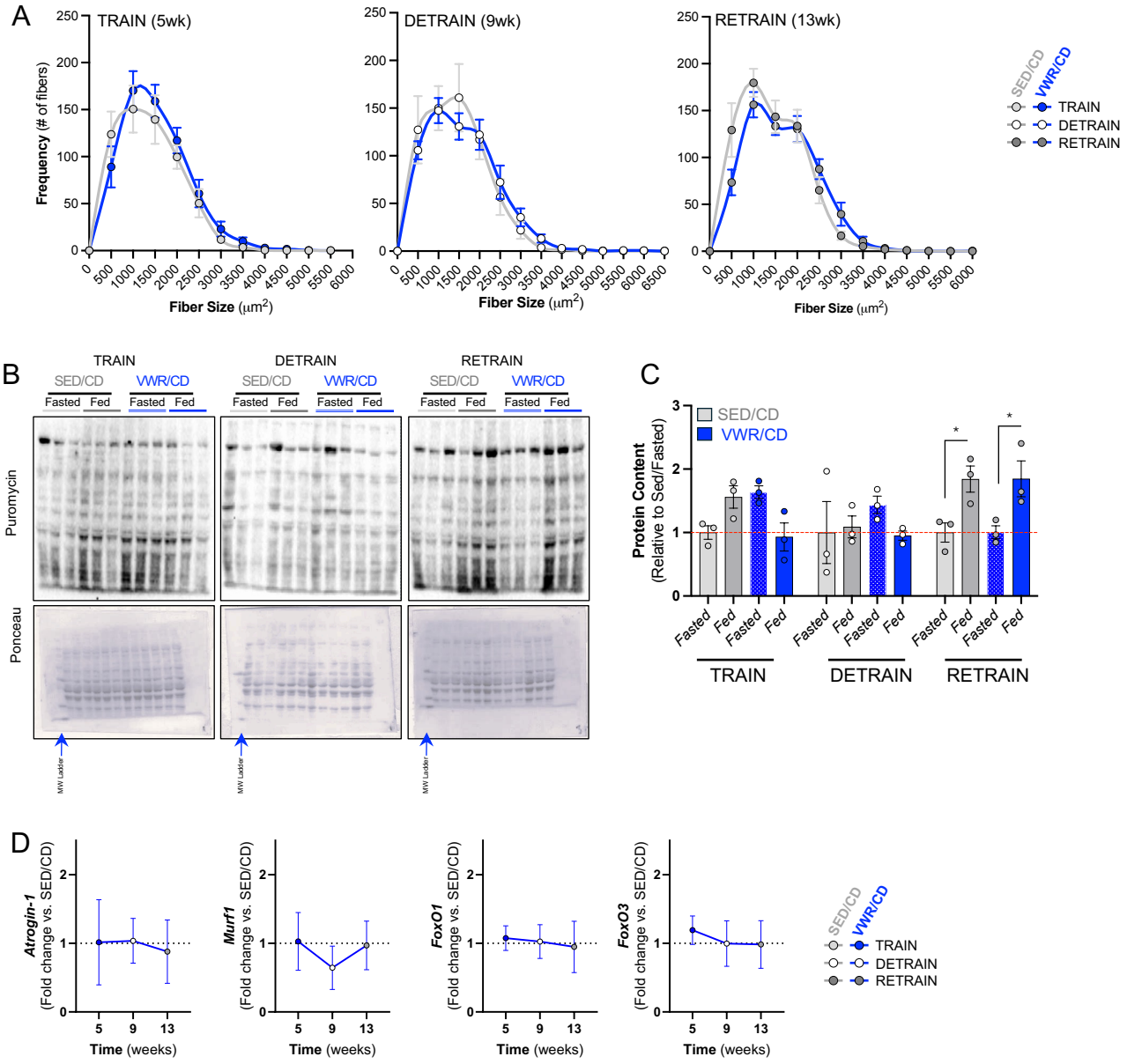
Gastrocnemius muscle RNA-seq showing the top 10 up- (red) and downregulated (blue) genes (left) and the KEGG enrichment analysis (right) comparing **A)** SED/HFD mice vs. SED/CD at 4wks, and **B)** 4wks of endurance TRAINing in VWR/HFD vs. SED/CD mice or, **C)** VWR/HFD vs. SED/HFD mice (1 cohort, n=3/group). Muscle RNA-seq showing the top 10 up- (red) and downregulated (blue) genes (left) and the KEGG enrichment analysis (right) comparing **D)** SED/HFD mice vs. SED/CD at 8wks, and **E)** 8wks of DETRAINing in VWR/HFD vs. SED/CD mice or, **F)** VWR/HFD vs. SED/HFD mice (1 cohort, n=3/group). Muscle RNA-seq showing the top 10 up- (red) and downregulated (blue) genes (left) and the KEGG enrichment analysis (right) comparing **D)** SED/HFD mice vs. SED/CD at 12wks, and **H)** 12wks of endurance RETRAINing in VWR/HFD vs. SED/CD mice or, **I)** VWR/HFD vs. SED/HFD mice (1 cohort, n=3/group). DEGs adjusted $p< 0.2$. *Tibialis anterior* qPCR expression of **J)** *Pgc-1α* and **K)** epigenetic modifiers *Dnmt1*, *Tet1*, *Tet2*, and *Tet3* (3 independent cohorts, n=6/group). Data are presented as means ± SD. Within timepoint comparisons determined by Student t-test; $p<0.1, * $p<0.05, ** $p<0.01, *** $p<0.001, **** $p<0.0001.

Supplemental Fig. S7. RETRAINing optimizes mitochondrial metabolism regardless of diet. Venn Diagram showing DEG overlap from VWR/CD, VWR/HFD, and SED/HFD following **A)** TRAINing, **B)** DETRAINing, and **C)** RETRAINing, compared to SED/CD. Upregulated (top; red, LogFC>1.0) and downregulated genes (bottom; blue, LogFC>1.0). DEGs adjusted $p < 0.2$. **D**) Sum of DEGs from at RETRAINing in both VWR/CD (vs. SED/CD) and VWR/HFD (vs. SED/HFD), and gene-network analysis for shared up- and downregulated genes.

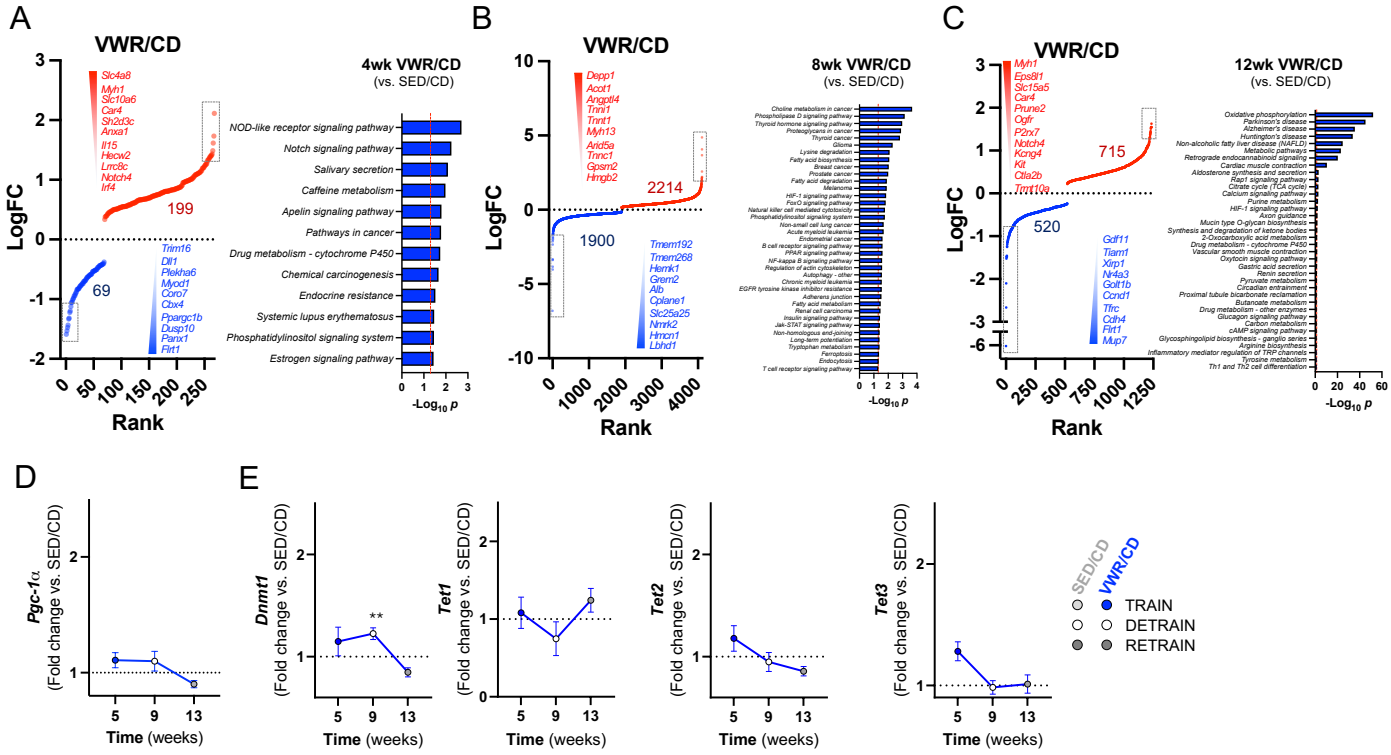
Suppl. Fig. 1.


BW Time p<0.0001; Lean Time p=0.0071
BW Exercise p=0.0011; Fat Exercise p=0.0093
BW Time* Exercise p=0.0039; Fat Time* Exercise p=0.0075
A
TRAIN
SED
VWR
CD
B
Fat
Lean
Body weight (g)
Time (wks)
SED
VWR
C
Body weight (g)
D
Fat mass (g)
Lean mass (g)
E
4-wk
Muscle Weight (mg/g BW)
TA
EDL
GAS
PLANT
SOL
F
4-wk
Muscle Weight (mg)
SED/CD
VWR/CD
BW, Lean, Fat Time p<0.0001
BW Exercise p=0.0007; Fat Exercise p<0.0001
BW, Fat Time* Exercise p<0.0001
G
TRAIN DETRAIN
H
Body weight (grams)
I
Body weight (g)
J
Fat mass (g)
Lean mass (g)
K
8-wk
Muscle Weight (mg/g BW)
L
8-wk
Muscle Weight (mg)
M
2-days
LA Weight (mg/g BW)
LA Weight (mg)
Time (weeks)
N
1-wk
LA Weight (mg/g BW)
LA Weight (mg)
Time (weeks)
SED
VWR
nVWR
TRAIN
DETRAIN
RETRAIN

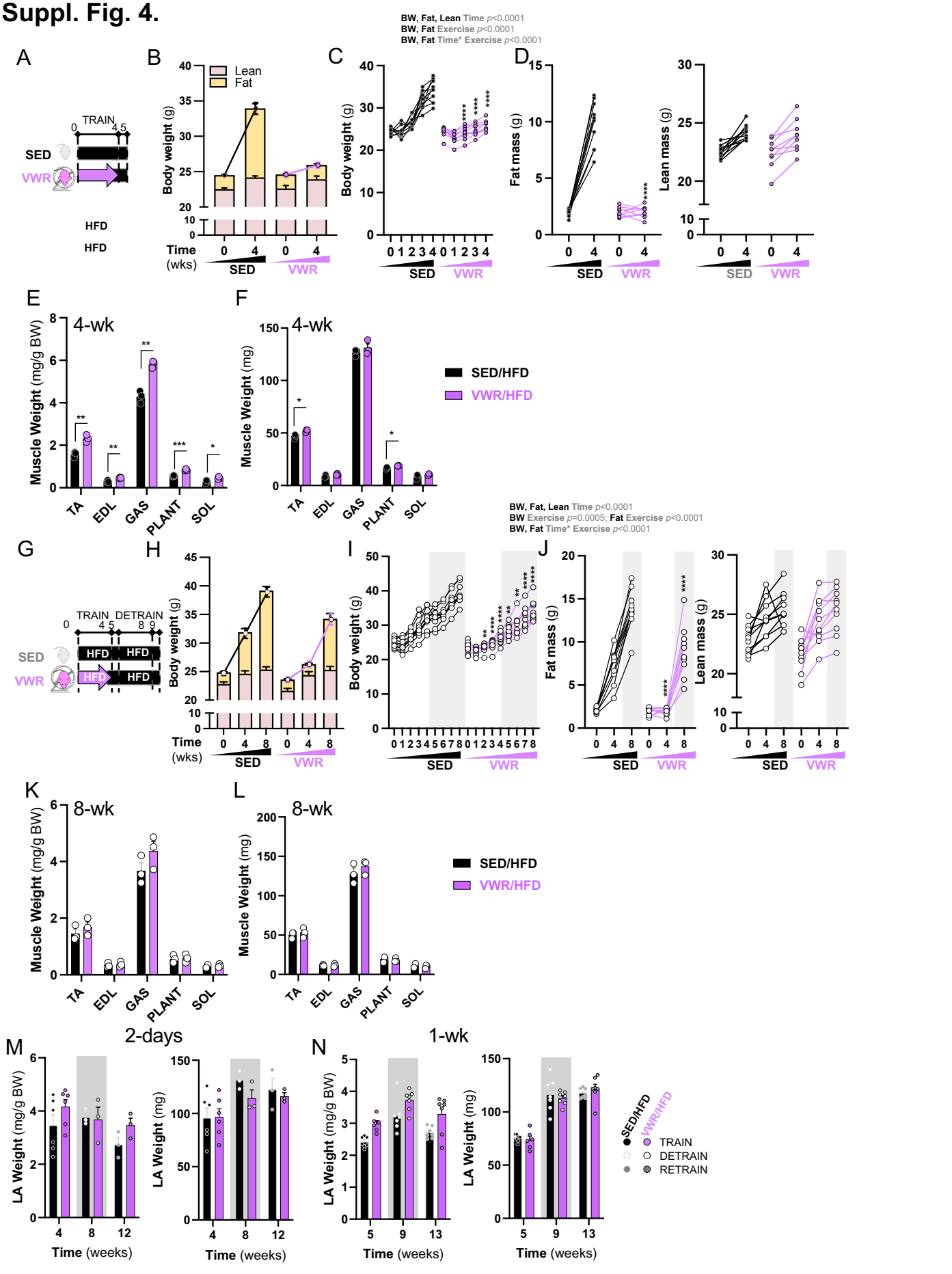
Suppl. Fig. 2.


A
TRAIN (5wk)
DETRAIN (9wk)
RETRAIN (13wk)
Frequency (# of fibers)
Fiber Size (μm²)
SED/CD
VWR/CD
TRAIN
DETRAIN
RETRAIN
B
TRAIN
DETRAIN
RETRAIN
SED/CD
VWR/CD
Fasted
Fed
Puromycin
Ponceau
MW Ladder
C
Protein Content
(Relative to Sed/Fasted)
SED/CD
VWR/CD
Fasted
Fed
TRAIN
DETRAIN
RETRAIN
D
Atrogin-1
Murf1
FoxO1
FoxO3
(Fold change vs. SED/CD)
Time (weeks)
SED/CD
VWR/CD
TRAIN
DETRAIN
RETRAIN

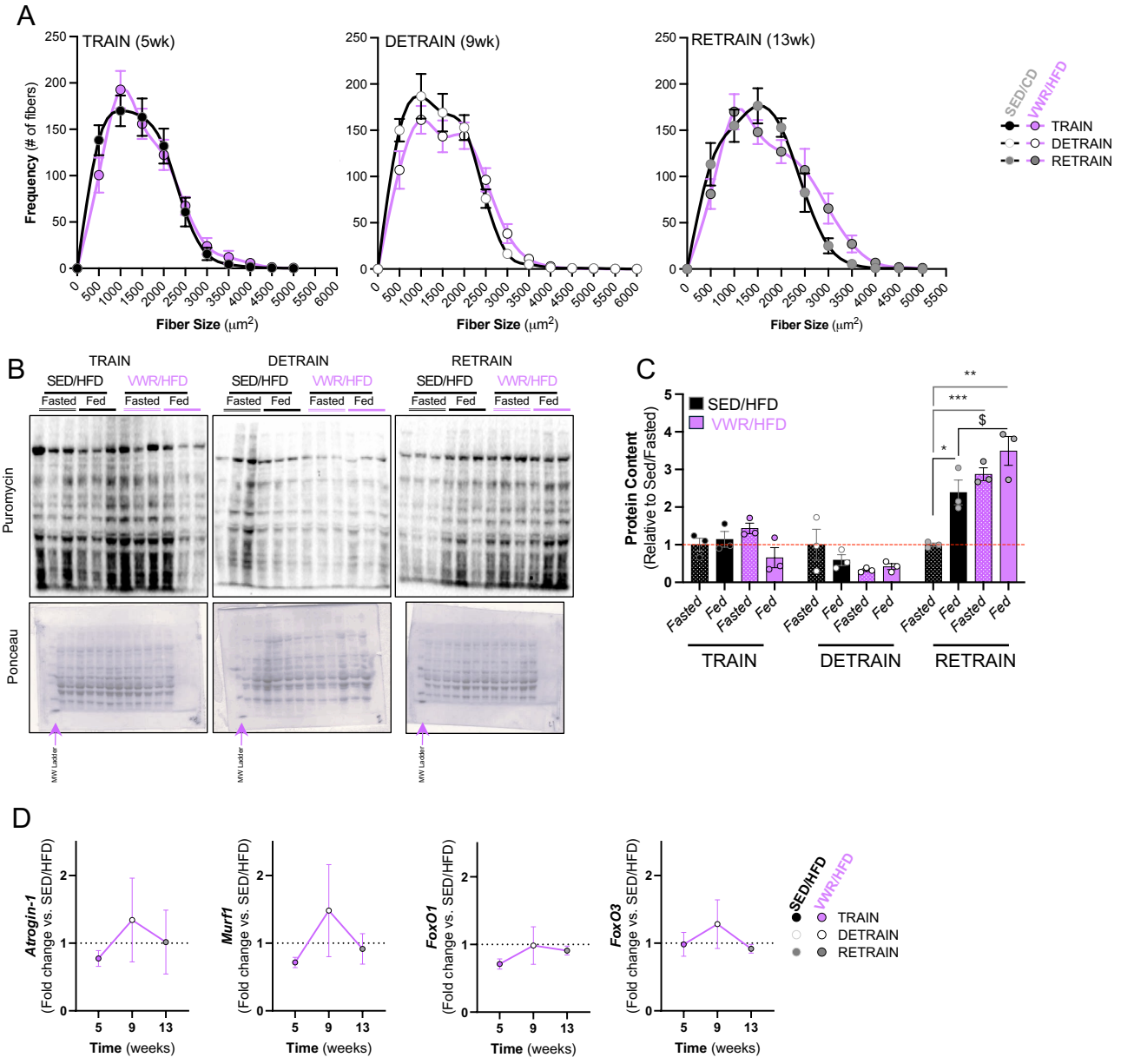
Suppl. Fig. 3.


A
VWR/CD
LogFC
Rank
199
69
4wk VWR/CD
(vs. SED/CD)
NOD-like receptor signaling pathway
Notch signaling pathway
Salivary secretion
Caffeine metabolism
Apelin signaling pathway
Pathways in cancer
Drug metabolism - cytochrome P450
Chemical carcinogenesis
Endocrine resistance
Systemic lupus erythematosus
Phosphatidylinositol signaling system
Estrogen signaling pathway
-Log10 p
B
VWR/CD
LogFC
Rank
2214
1900
8wk VWR/CD
(vs. SED/CD)
-Log10 p
C
VWR/CD
LogFC
Rank
715
520
12wk VWR/CD
(vs. SED/CD)
-Log10 p
D
Pgc-1α
(Fold change vs. SED/CD)
Time (weeks)
E
Dnmt1
Tet1
Tet2
Tet3
(Fold change vs. SED/CD)
Time (weeks)
SED/CD
VWR/CD
TRAIN
DETRAIN
RETRAIN

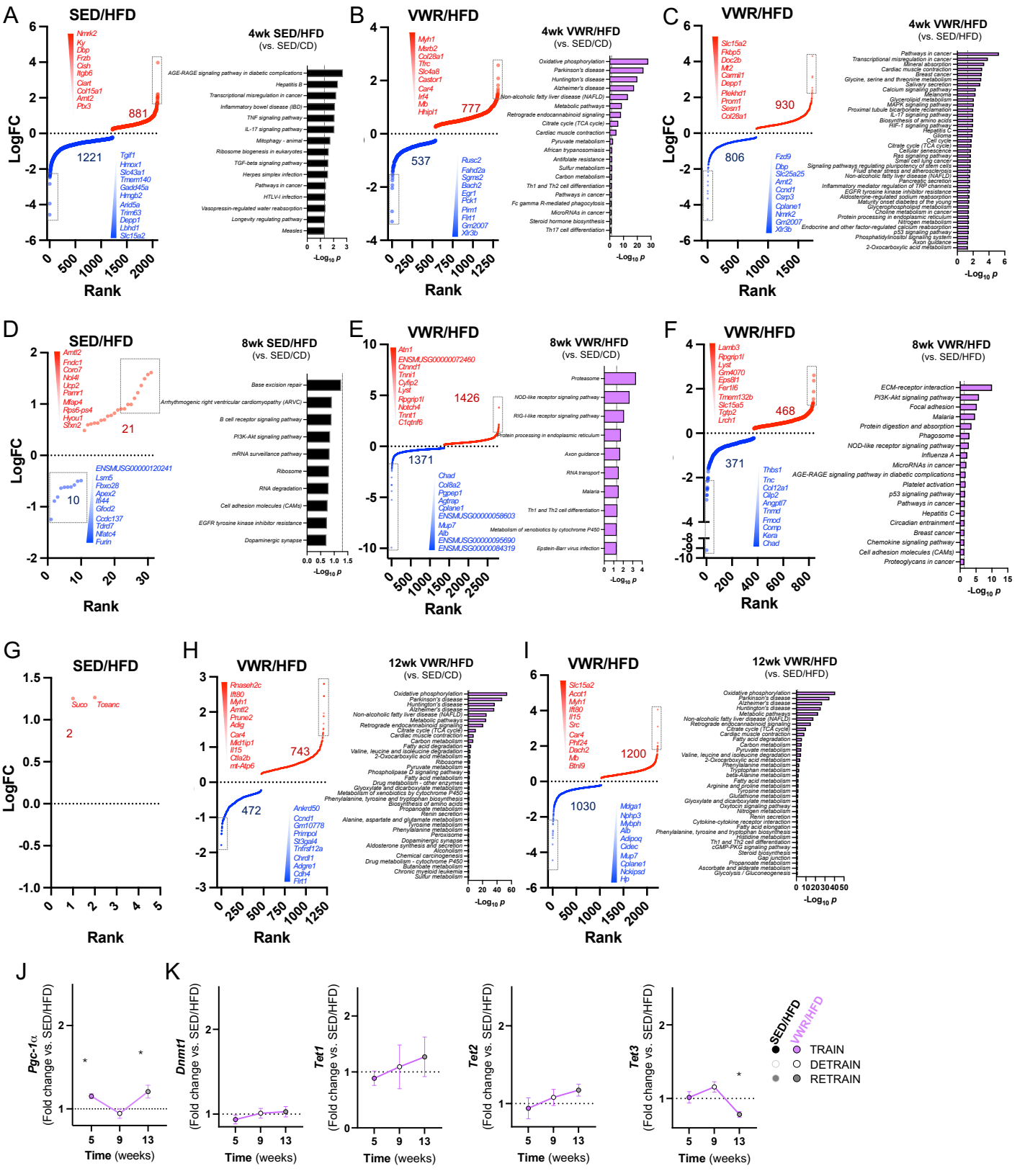
Suppl. Fig. 4.


BW, Fat, Lean Time p<0.0001
BW, Fat Exercise p<0.0001
BW, Fat Time* Exercise p<0.0001
A
B
C
D
TRAIN
SED
VWR
HFD
HFD
Lean
Fat
Body weight (g)
Time (wks)
Fat mass (g)
Lean mass (g)
E
F
4-wk
Muscle Weight (mg/g BW)
Muscle Weight (mg)
TA
EDL
GAS
PLANT
SOL
SED/HFD
VWR/HFD
BW, Fat, Lean Time p<0.0001
BW Exercise p=0.0005; Fat Exercise p<0.0001
BW, Fat Time* Exercise p<0.0001
G
H
I
J
TRAIN DETRAIN
HFD
K
L
8-wk
M
N
2-days
1-wk
LA Weight (mg/g BW)
LA Weight (mg)
Time (weeks)
TRAIN
DETRAIN
RETRAIN

Suppl. Fig. 5.


A
TRAIN (5wk)
DETRAIN (9wk)
RETRAIN (13wk)
Frequency (# of fibers)
Fiber Size (μm²)
SED/CD
VWR/HFD
TRAIN
DETRAIN
RETRAIN
B
TRAIN
DETRAIN
RETRAIN
SED/HFD
VWR/HFD
Fasted
Fed
Puromycin
Ponceau
MW Ladder
C
SED/HFD
VWR/HFD
Protein Content
(Relative to Sed/Fasted)
TRAIN
DETRAIN
RETRAIN
D
Atrogin-1
Murf1
FoxO1
FoxO3
(Fold change vs. SED/HFD)
Time (weeks)
SED/HFD
VWR/HFD
TRAIN
DETRAIN
RETRAIN

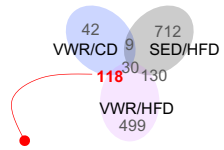
Suppl. Fig. 6.


A
SED/HFD
LogFC
Rank
881
1221
4wk SED/HFD
(vs. SED/CD)
-Log10 p
B
VWR/HFD
777
537
4wk VWR/HFD
(vs. SED/CD)
C
VWR/HFD
930
806
4wk VWR/HFD
(vs. SED/HFD)
D
SED/HFD
21
10
8wk SED/HFD
(vs. SED/CD)
E
VWR/HFD
1426
1371
8wk VWR/HFD
(vs. SED/CD)
F
VWR/HFD
468
371
8wk VWR/HFD
(vs. SED/HFD)
G
SED/HFD
2
H
VWR/HFD
743
472
12wk VWR/HFD
(vs. SED/CD)
I
VWR/HFD
1200
1030
12wk VWR/HFD
(vs. SED/HFD)
J
Pgc-1α
(Fold change vs. SED/HFD)
Time (weeks)
K
Dnmt1
Tet1
Tet2
Tet3
SED/HFD
VWR/HFD
TRAIN
DETRAIN
RETRAIN

Suppl. Fig. 7.

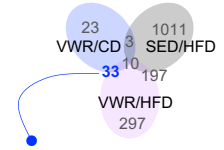
A

Upregulated (4wk)



LogFC>1: *Slc4a8, Myh1, Slc10a6, Car4, Notch4, Irf4, Usp54, Cyp1b1, Plekhg1, Castor1, Rab27a, Csgalnact1, Arhgap25, Ifi205, Mcf2l, Arhgap27, Adgrg1, Igfbp3, Lnx1, Rasgrp2, Scn2b, Dll4, Ccdc141, Xdh, Fmo2, Rsad2, ENSMUSG00000120992, Ehd3, Fbxl17, Kcna5, Kank3, Apobec3, Iqsec2, Btnl9.*

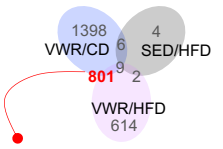
Downregulated (4wk)



LogFC<-1: *Flrt1, Myod1, Dll1, Trim16, Smad3, Cirbp, Rccd1, Plin2.*

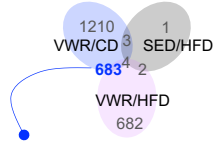
B

Upregulated (8wk)



LogFC>1: *ENSMUSG00000072460, ENSMUSG00000107478, Tnni1, Tnnt1, Tnnc1, Myh7, Car4, Tpm3, ENSMUSG00000106037, Acsf2, P2rx7, Atp2a2, C1qtnf6, Cyfip2, Chn1, Ablim1, Btnl9, Lmod2, Sh2d3c, Cldn15, Myl2, Notch4, Rnf125, Lgi1, ENSMUSG00000099342, Abr, Med17, Pim3, N4bp3, Cyp1b1, Lipe, Cecr2, Aifm2, Impa2, Fmo2, Lnx1, Casc3, Zfp738, Shfl, Baz1a, Rnf144a, Kit, Slc39a14, Cd36, Rab15, Icam2, Lilr4b, Sbds, Spaar, Aldh9a1, Shank3, Cobll1, Wfs1, Fhl2, Rassf9, Tmem132b, Scube2.*

Downregulated (8wk)



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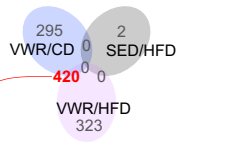
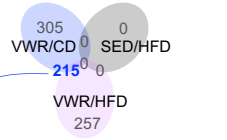
C

Upregulated (12wk)



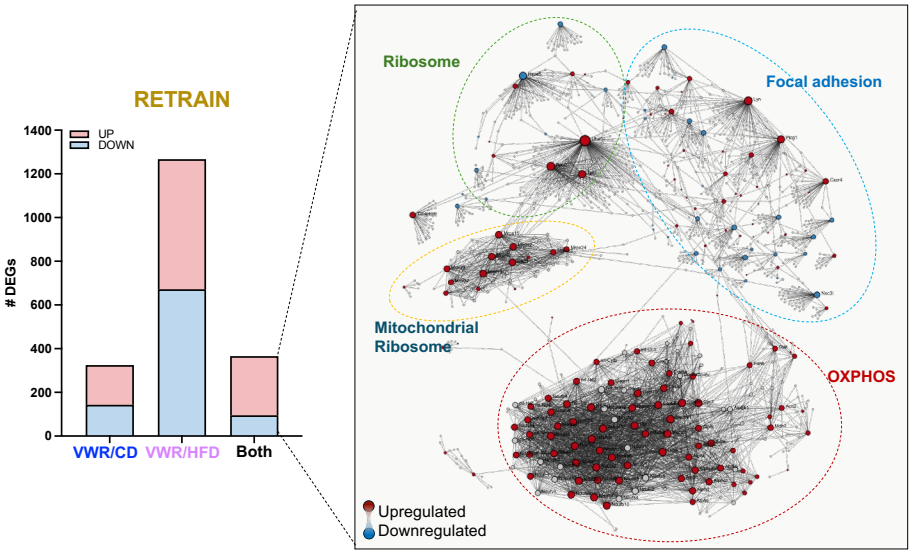
LogFC>1: *Myh1, Slc15a5, Car4, Prune2, ENSMUSG00000102070, Ogfr, ENSMUSG00000101249, Kcng4, Ctla2b, Mid1ip1, ENSMUSG00000089718, Cep85l, Dll4, Ckmt2, Ralgds, Cacna1c, Cldn15, Adh1, Cxcr4, mt-Atp6, Kank3, Il15, ENSMUSG00000097462, mt-Co3, Pde4c, ENSMUSG00000115970, Mtg1, Mb, Iqsec2, Tnfsf10, Btnl9, Uqcc1, ENSMUSG00000100862, Apobec3, Cdh23, C1qtnf9, Xdh, Gstt2, mt-Nd3.*

Downregulated (12wk)



LogFC<-1: *ENSMUSG00000087579, Rasd2, Rbpj, ENSMUSG00000121486, Ankrd1, Xirp1, Nr4a3, ENSMUSG00000097125, Ccnd1, Cdh4, Flrt1.*

D



TABLES

Supplemental Table S1. RT-qPCR primer sequences.

Gene	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
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Dnmt1	GGACAAGGAGAATGCCATGAAGC	TTACTCCGTCCAGTGCCACCAA
Tet1	CCCCAACTCCCACCAAACCC	AAACCCTTGTGGGACGGCTG
Tet2	ACCTGGCTACTGTCATTGCTCC	TGCAGTGACTCCTGAGAATGGC
Tet3	GGCCGATGCAGTAGTGGAG	TGAGCTCTGAGCCTGTCTTGA
Atrogin-1	CTTCTCGACTGCCATCCTGGAT	TCTTTTGGGCGATGCCACTCAG
Murf1	TACCAAGCCTGTGGTCATCCTG	ACGGAAACGACCTCCAGACATG
FoxO1	CTACGAGTGGATGGTGAAGAGC	CCAGTTCCTTCATTCTGCACTCG
FoxO3	CCTACTTCAAGGATAAGGGCGAC	GCCTTCATTCTGAACGCGCATG