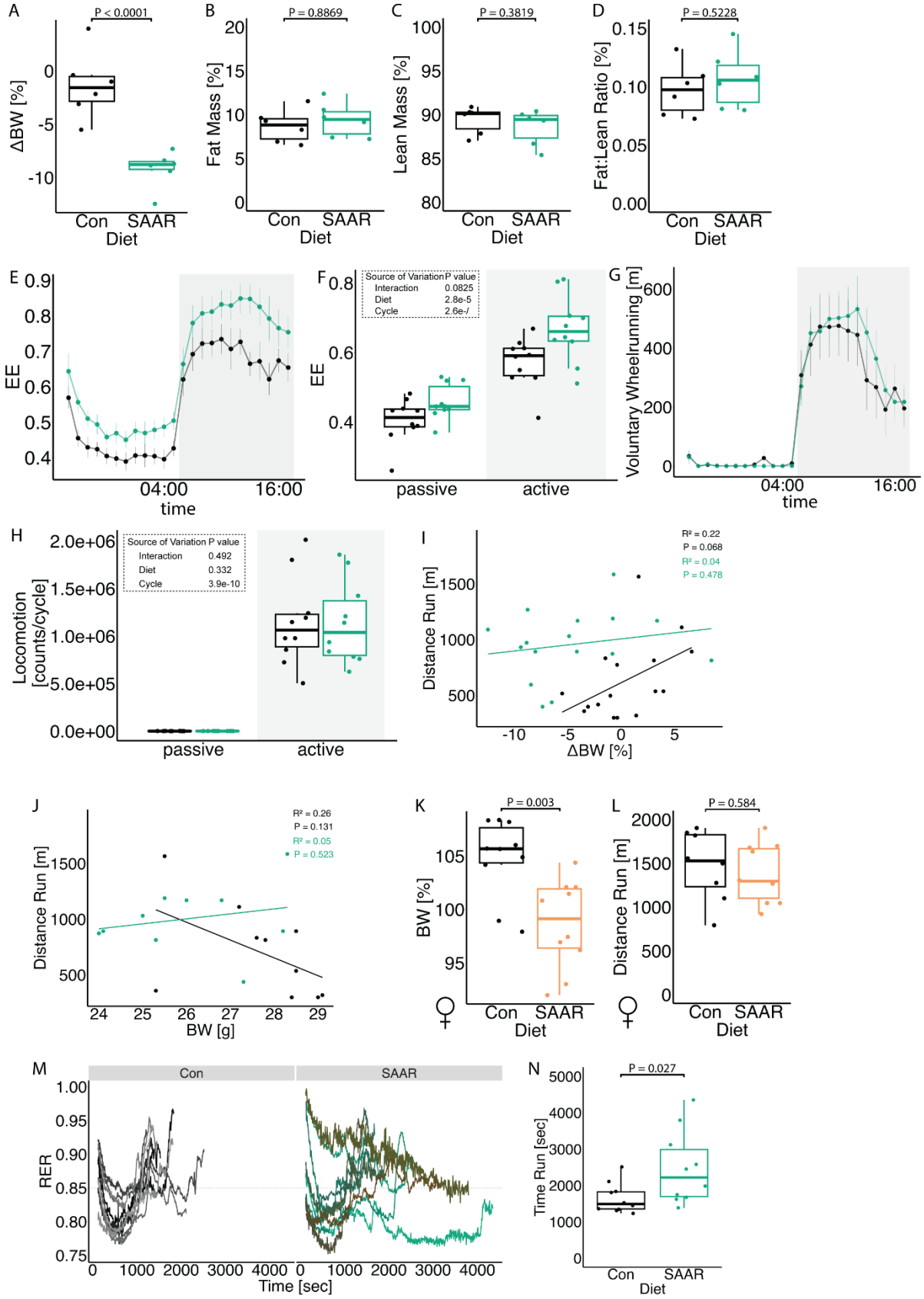
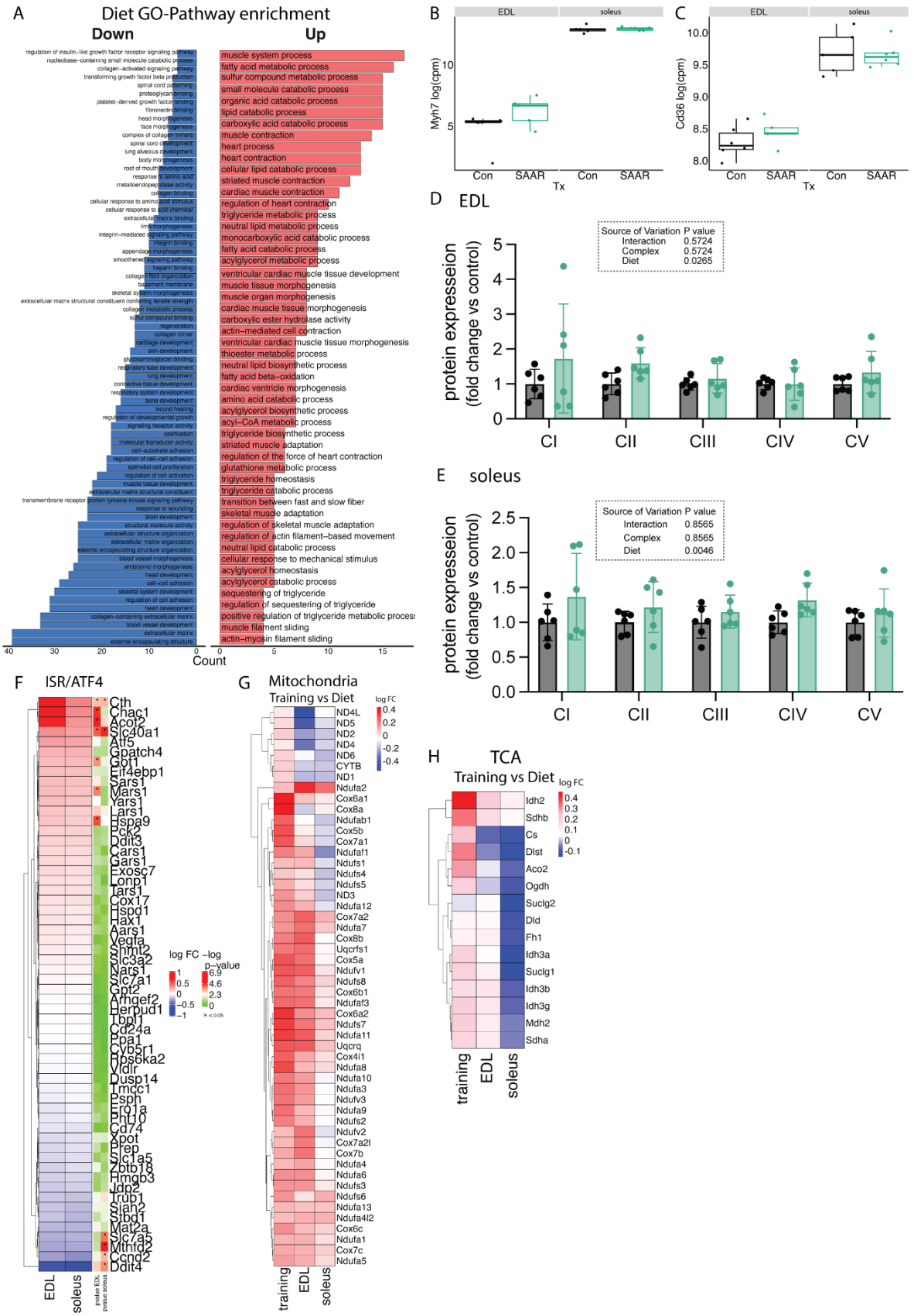
Supplemental Figures



Supplemental Figure 1 Short-term SAAR induces shifts in metabolism and increases endurance exercise capacity in young, sedentary male mice

A. Percent change in body weight (n = 6) of male mice given *ad libitum* access to sulfur amino acid restricted (SAAR) versus control (Con) diet for seven days.
B. Percent fat mass of total body weight measured using ECHO MRI (n = 6) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
C. Percent lean mass of total body weight measured using ECHO MRI (n = 6) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
D. Fat:lean mass ratio calculated from A and B (n = 6) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
E. Percent change in body weight (n = 8) of female mice given *ad libitum* access to SAAR versus Con diet after seven days on the diet.
F. Distance ran during a one-time maximal endurance test (n = 8) of female mice given *ad libitum* access to SAAR versus Con diet.
G. Sable systems indirect calorimetry measurements of energy expenditure (kcal, EE) over a 24 h period (n = 10) of mice given *ad libitum* access to SAAR versus Con diet for seven days and
H. the average EE during a 12 h–12 h light–dark cycle (n = 10) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
I. Sable systems indirect calorimetry measurements of sum of beam breaks (counts/cycle, Locomotion) over a 24 h period (n = 10) of male mice given *ad libitum* access to SAAR versus Con diet for seven days.
J. Sable systems indirect calorimetry measurements of voluntary wheel running behavior over a 24 h period (n = 10) of male mice given *ad libitum* access to SAAR versus Con diet for seven days.
K. Linear regression showing the relationship between relative change in body weight versus distance ran (n = 16) of male mice given *ad libitum* access to SAAR versus Con diet on day seven. R^2 coefficient was calculated using Pearson's method.
L. Linear regression showing the relationship between absolute body weight versus distance ran (n = 16) of male mice given *ad libitum* access to SAAR versus Con diet on day seven. R^2 coefficient was calculated using Pearson's method.
M. RER trajectory over time in seconds during a one-time maximal endurance test on metabolic treadmills (Harvard Apparatus) (n = 10) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
N. Time ran in seconds during a one-time maximal endurance test measured on metabolic treadmills (n = 10) of male mice given *ad libitum* access to SAAR versus Con diet.

All data is shown as mean and error bars indicate SD unless otherwise noted; p values indicate the significance of the difference by Student's t test or two-way ANOVA with Sidak's multiple comparisons test between diets or diet and cycle (indirect calorimetry); significance is determined by a p value of $p < 0.05$. For linear regressions r squared Pearson's coefficient was calculated.



Supplemental Figure 2 Transcriptomics across muscle depots reveal metabolic shift from glycolytic toward oxidative

892 A. Pathway enrichment analysis comparing main effects of diet from bulkRNA
893 sequencing (n = 6) of male mice given *ad libitum* access to sulfur amino acid restricted
894 (SAAR) versus control (Con) diet on day seven showing all significantly increased or
895 decreased pathways.
896 B. Normalized count values of Myh7 in EDL and soleus from bulkRNA sequencing (n =
897 6) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
898 C. Normalized count values of Cd36 in EDL and soleus from bulkRNA sequencing (n =
899 6) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
900 D. Quantification of relative protein abundance normalized to vinculin of all five complexes
901 of the electron transport chain from blots shown in figure 2E of both EDL and
902 E. soleus (n = 5).
903 F. Fold changes of transcripts associated with known dietary SAAR and integrated stress
904 response (ISR) target genes (Torrence et al., 2021) after SAAR when compared to
905 Con.
906 G. Fold changes after Training (Furrer et al., 2023) and SAAR when compared to Con of
907 specific genes associated with mitochondrial matrix.
908 H. Fold changes after Training (Furrer et al., 2023) and SAAR when compared to Con of
909 specific genes associated with TCA cycle as identified in figure 2E.

910 All data is shown as mean and error bars indicate SD unless otherwise noted; p values indicate
911 the significance of the difference by Student's t test or two-way ANOVA with Sidak's multiple
912 comparisons test between diets or diet and complexes; significance is determined by a p value
913 of $p < 0.05$.

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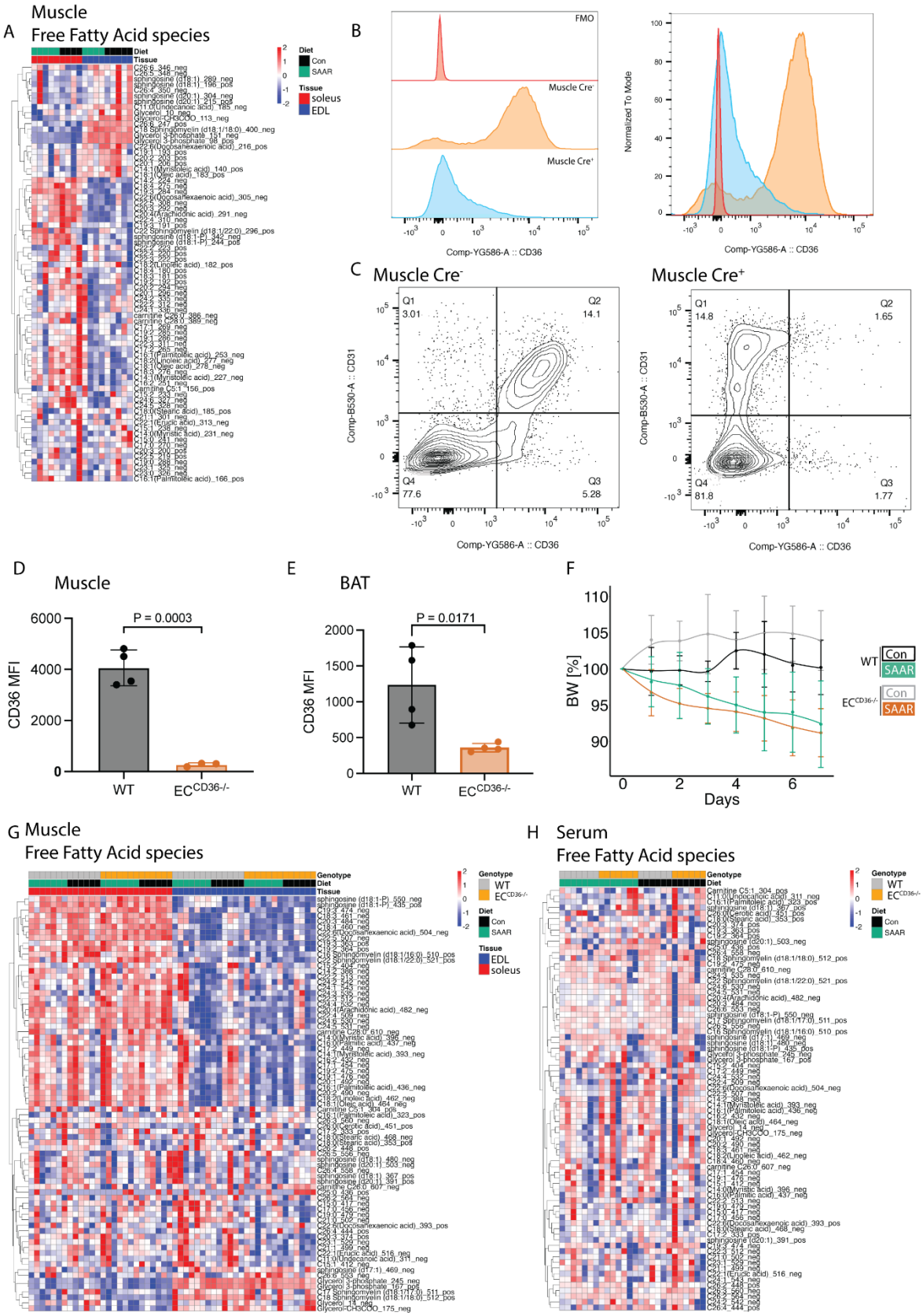
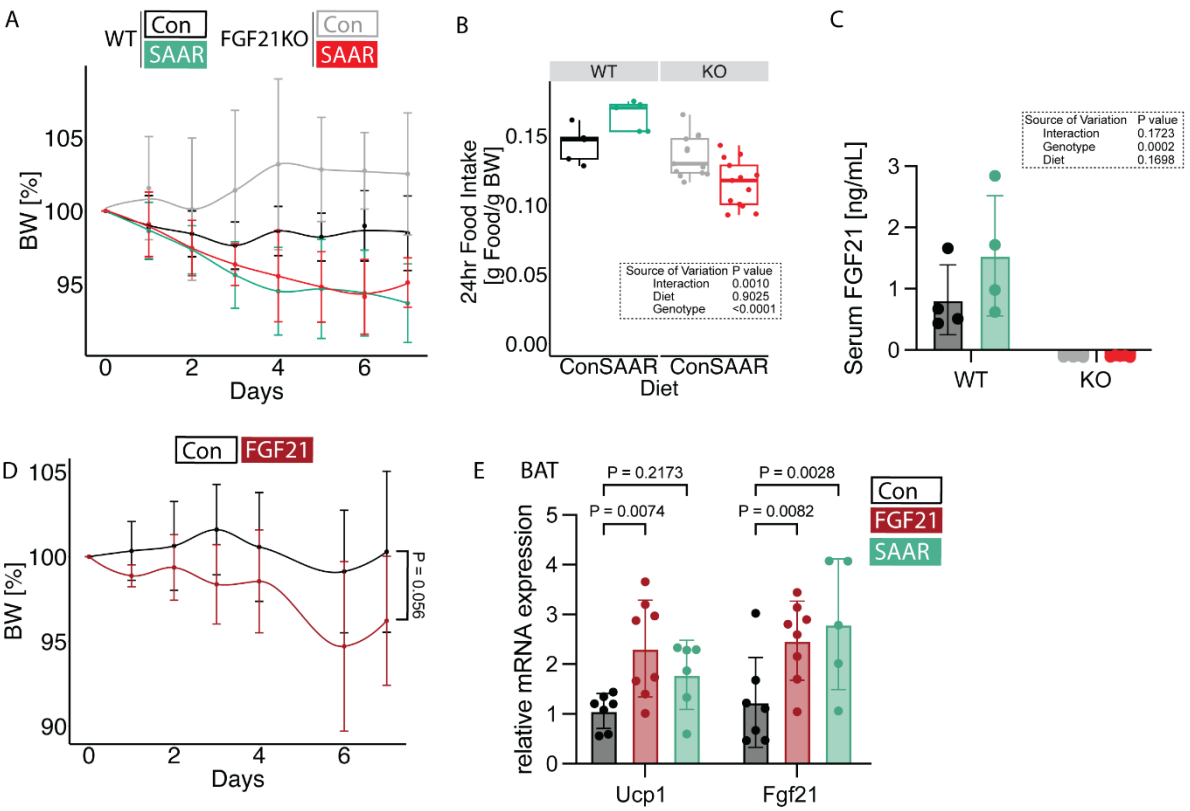


Supplemental Figure 3 SAAR increases muscle lipid flux without altering lipid pool sizes

A. Heatmap of differentially abundant free fatty acid species in muscles (n= 3-4) of male mice given *ad libitum* access to sulfur amino acid restricted (SAAR) versus control (Con) diet for seven days.
B. Representative histograms of $CD36^{+}$ endothelial cells (EC) in the muscle and
C. gating strategy for CD36 positive EC ($CD45^{-}$, $CD31^{+}$, $CD36^{+}$) isolated from muscle of male WT (Cre^{-}) and $EC^{CD36-/-}$ (Cre^{+}) mice.
D. $EC^{CD36-/-}$ KO efficiency was confirmed by FACS analysis of $CD31^{+}/CD36^{+}$ MFI in muscle or
E. brown adipose tissue (BAT) (n = 4) of male WT or $EC^{CD36-/-}$ mice.
F. Daily body weight trajectories shown in percent of starting body weight (n = 8/group) over time of male WT and $EC^{CD36-/-}$ mice given *ad libitum* access to SAAR versus Con diet for seven days.
G. Heatmap of differentially abundant free fatty acid species in muscles of male WT or $EC^{CD36-/-}$ mice (n = 5) fed a Con or SAAR diet for seven days.
H. Heatmap of differentially abundant free fatty acid species in serum of male WT or $EC^{CD36-/-}$ mice (n = 5) fed a Con or SAAR diet for seven days.

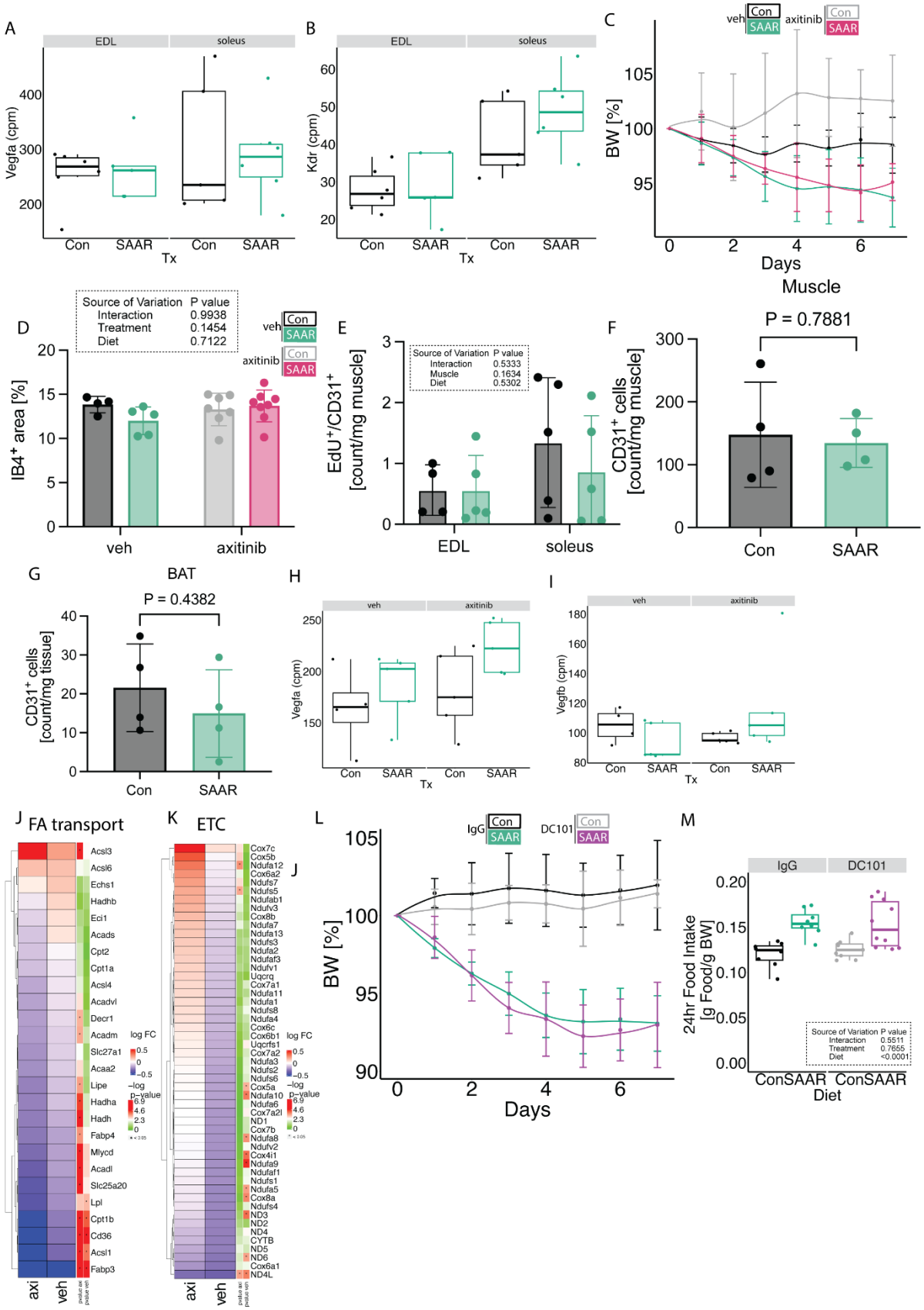
All data is shown as mean and error bars indicate SD unless otherwise noted; p values indicate the significance of the difference by Student's t test between diets, or two-way ANOVA with Sidak's multiple comparisons test between diets and muscle or genotype; significance is determined by a p value of $p < 0.05$.



Supplemental Figure 4 FGF21 is dispensable for running phenotype after SAAR in male mice

A. Daily body weight trajectories shown in percent of starting body weight (n = 4-10) over time, of male WT or FGF21KO mice given *ad libitum* access to sulfur amino acid restricted (SAAR) versus control (Con) diet for seven days.
B. Food intake expressed as grams of food eaten per gram of mouse body weight within a 24 hr period (n = 4-10) of male WT or FGF21KO mice given *ad libitum* access to SAAR versus Con diet for seven days.
C. Serum FGF21 concentrations of male WT or FGF21 KO mice given *ad libitum* access to SAAR versus Con diet for seven days determined using an ELISA.
D. Daily body weight trajectories over time shown in percent when compared to starting body weight (n = 8) of NaCl or recombinant FGF21 treated male mice for seven days.
E. Fgf21 and Ucp1 mRNA levels in brown adipose tissue (BAT) of male mice given *ad libitum* access to SAAR versus Con diet or mice treated with recombinant FGF21 for seven days

All data is shown as mean and error bars indicate SD unless otherwise noted; p values indicate the significance of the difference by Student's t test between diets, or two-way ANOVA with Sidak's multiple comparisons test between diets and genotype; significance is determined by a p value of $p < 0.05$.



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963 **Supplemental Figure 5 Inhibition of VEGFR signaling prevents endurance exercise**
964 **phenotype without induction of angiogenesis**

A. Normalized count values of Vegfa in EDL and soleus from bulkRNA sequencing (n = 6) of male mice given *ad libitum* access to sulfur amino acid restricted (SAAR) versus control (Con) diet on day seven.
B. Normalized count values of Kdr in EDL and soleus from bulkRNA sequencing (n = 6) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
C. Daily body weight trajectories shown in percent of starting body weight (n = 10) over time of male mice given *ad libitum* access to SAAR versus Con diet for seven days, treated with either vehicle (veh) or axtinib by oral gavage.
D. Quantification of $IB4^+$ area of EDL muscle of male mice fed a Con or SAAR for seven days treated with veh or axitinib (n = 5-8).
E. Cell counts of $EdU^+/CD31^+$ double positive cells per mg tissue in muscle from male mice given *ad libitum* access to SAAR versus Con diet for seven days and injected with EdU to label cell proliferation, determined by flow cytometry.
F. Cell counts of $CD31^+$ positive cells per mg tissue in muscle or
G. brown adipose tissue (BAT) or of male mice given *ad libitum* access to SAAR versus Con diet for seven days, determined by flow cytometry.
H. Normalized count values of Vegfa in EDL treated with veh or axitinib from bulkRNA sequencing (n = 5) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
I. Normalized count values of Kdr in EDL treated with veh or axitinib from bulkRNA sequencing (n = 5) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
J. Fold changes of transcripts associated with fatty acid (FA) catabolism and transport as identified in supplementary figure 2A in both EDL treated with veh or axitinib after bulkRNA sequencing (n = 5) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
K. Fold changes of transcripts associated with electron transport chain (ETC) associated genes in EDL treated with veh or axitinib after bulkRNA sequencing (n = 6) of male mice given *ad libitum* access to SAAR versus Con diet on day seven.
L. Body weight trajectory over time, shown as percent of starting body weight (n = 8-10) of male mice given *ad libitum* access to SAAR versus Con diet for seven days treated with IgG or DC101 via i.p. injection every other day.
M. Food intake expressed as grams of food per gram of body weight per mouse within a 24 hr period (n = 8-10) of male mice given *ad libitum* access to SAAR versus Con diet treated with IgG or DC101 via i.p. injection every other day on day seven.

All data is shown as mean and error bars indicate SD unless otherwise noted; p values indicate the significance of the difference by Student's t test between diets, or two-way ANOVA with Sidak's multiple comparisons test between diets and muscle or treatment; significance is determined by a p value of $p < 0.05$.

A17101101-03

Formulated by
Research Diets, Inc
12/14/2022

Product #	A17101101	A17101102	A17101103
	Control	Mid Methionine	Low Methionine
Ingredient			
Casein, Lactic	0	0	0
L-Cystine	0	0	0
L-Isoleucine	7.6	7.6	7.6
L-Leucine	15.8	15.8	15.8
L-Lysine	13.2	13.2	13.2
L-Methionine	4.5	1.8	1.2
L-Phenylalanine	8.4	8.4	8.4
L-Threonine	7.2	7.2	7.2
L-Tryptophan	2.1	2.1	2.1
L-Valine	9.3	9.3	9.3
L-Histidine-HCl-H2O	4.6	4.6	4.6
L-Alanine	5.1	5.1	5.1
L-Arginine	6	6	6
L-Aspartic Acid	12.1	12.1	12.1
L-Glutamic Acid	38.2	38.2	38.2
Glycine	3	3	3
L-Proline	17.8	17.8	17.8
L-Serine	10	10	10
L-Tyrosine	9.2	9.2	9.2
Total L-Amino Acids	*174.1*	*171.4*	*170.8*
Corn Starch	506.2	506.2	506.2
Maltodextrin 10	125	125	125
Sucrose	73.6	76.3	76.9
Cellulose, BW200	50	50	50
Soybean Oil	25	25	25
Lard	20	20	20
Mineral Mix S10026	10	10	10
DiCalcium Phosphate	13	13	13
Calcium Carbonate	5.5	5.5	5.5
Potassium Citrate, 1 H2O	16.5	16.5	16.5
Sodium Bicarbonate	7.5	7.5	7.5
Vitamin Mix V10001	10	10	10
Choline Bitartrate	2	2	2
FD&C Blue Dye #1	0	0.05	0
FD&C Yellow Dye #5	0.05	0	0
FD&C Red Dye #40	0	0	0.05
Total	**1038.450**	**1038.450**	**1038.450**
gm			
Protein	174.1	171.4	170.8
Carbohydrate	714.8	717.5	718.1
Fat	45.0	45.0	45.0
Fiber	50.0	50.0	50.0
gm%			
Protein	16.8	16.5	16.4
Carbohydrate	68.8	69.1	69.2
Fat	4.3	4.3	4.3
Fiber	4.8	4.8	4.8
kcal			
Protein	696	686	683
Carbohydrate	2859	2870	2872
Fat	405	405	405
Total	3961	3961	3961
kcal%			
Protein	18	17	17
Carbohydrate	72	72	73
Fat	10	10	10
Total	100	100	100
kcal / gm	3.8	3.8	3.8

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1316 **Supplemental Table 7 macronutrient composition of the control and SAAR diets used**