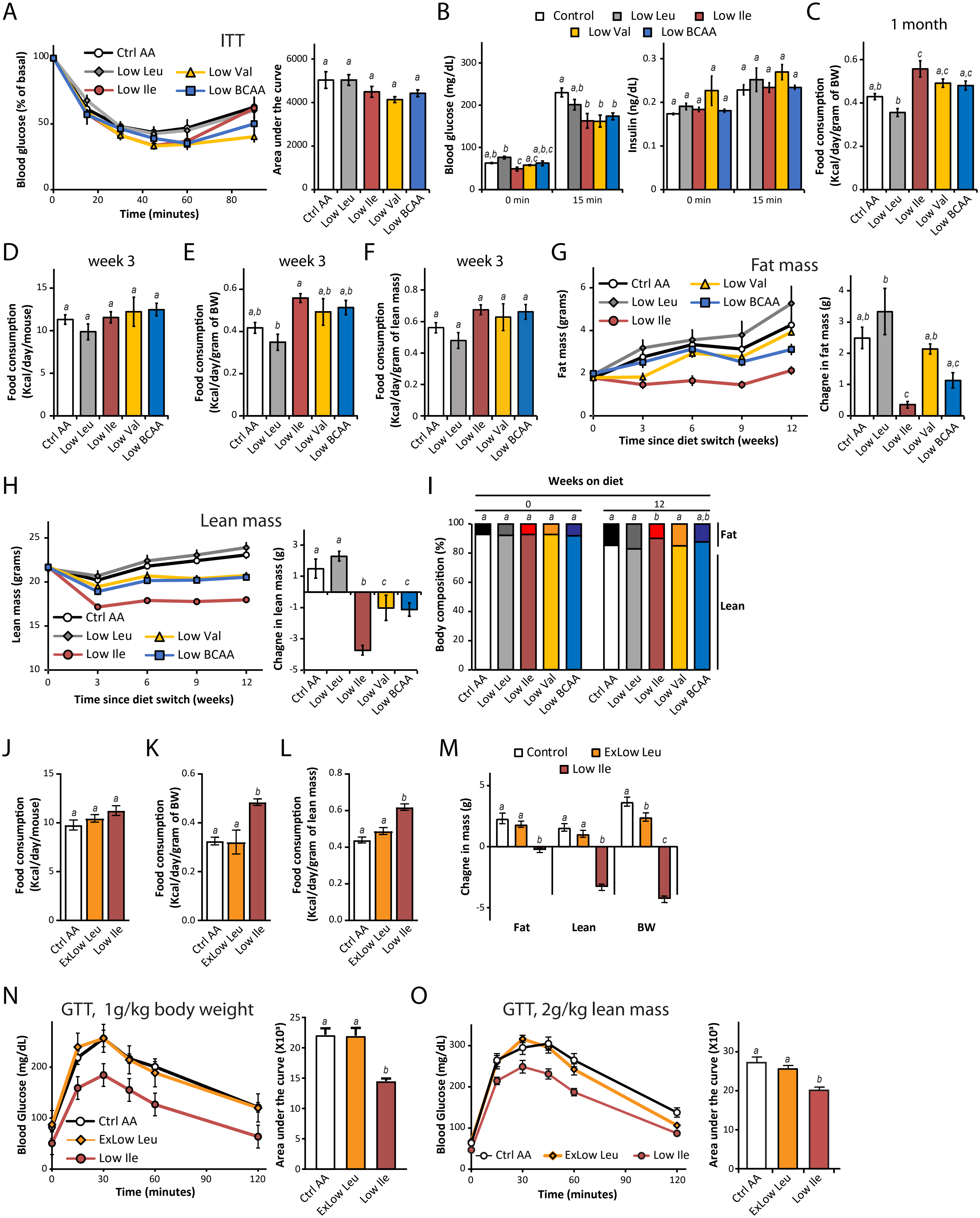
Figure S1



A
ITT
Ctrl AA
Low Leu
Low Ile
Low Val
Low BCAA
Blood glucose (% of basal)
Time (minutes)
Area under the curve
B
Control
Low Leu
Low Ile
Low Val
Low BCAA
Blood glucose (mg/dL)
Insulin (ng/dL)
0 min
15 min
C
1 month
Food consumption (Kcal/day/gram of BW)
D
week 3
Food consumption (Kcal/day/mouse)
E
week 3
Food consumption (Kcal/day/gram of BW)
F
week 3
Food consumption (Kcal/day/gram of lean mass)
G
Fat mass
Fat mass (grams)
Time since diet switch (weeks)
Chagne in fat mass (g)
H
Lean mass
Lean mass (grams)
Time since diet switch (weeks)
Chagne in lean mass (g)
I
Weeks on diet
Body composition (%)
Fat
Lean
J
Food consumption (Kcal/day/mouse)
K
Food consumption (Kcal/day/gram of BW)
L
Food consumption (Kcal/day/gram of lean mass)
M
Control
ExLow Leu
Low Ile
Chagne in mass (g)
Fat
Lean
BW
N
GTT, 1g/kg body weight
Blood Glucose (mg/dL)
Time (minutes)
Ctrl AA
ExLow Leu
Low Ile
Area under the curve (X10³)
O
GTT, 2g/kg lean mass
Blood Glucose (mg/dL)
Time (minutes)
Area under the curve (X10³)

Supplemental Figure Legends

Figure S1, related to Figure 1. Dietary restriction of isoleucine or valine, but not leucine, improves metabolic health.

(A) Insulin tolerance test of mice after 4 weeks on the indicated diets (n = 9/group). (B) Fasting and glucose-stimulated glucose and insulin levels in mice after 6 weeks on the indicated diets (n=3-4/group). (C) Average food consumption during the first month on the indicated diets normalized by body weight. (D-F) Food consumption per mouse (D), normalized by body weight (E), or by lean mass (F) of mice during the third week on the indicated diets (n=3 cages of 3 animals each/group). (G) Fat mass and change in fat mass of mice after 12 weeks on the indicated diets (n=8-9/group). (H) Lean mass and change in lean mass of mice after 12 weeks on the indicated diets (n = 8-9/group). (I) Change in body composition over 12-week period (n=8-9/group). (J-L) Food consumption per mouse (J), normalized by body weight (K) or by lean mass (L) of mice fed on Ctrl AA, ExLow Leu and Low Ile diets after three weeks of diet feeding (n = 11-12/group). (M) Change in body composition of mice fed on Ctrl AA, ExLow Leu and Low Ile diets (n=11-12/group). (N) Glucose tolerance test (1 g/kg of body weight) of mice after 3 weeks feeding on Ctrl AA, ExLow Leu and Low Ile diets (n=12/group). (O) Glucose tolerance test (2 g/kg of lean mass) of mice after 4 weeks feeding on Ctrl AA, ExLow Leu and Low Ile diets (n = 11-12/group). (A-O) Significance was calculated using Tukey-Kramer test following ANOVA. Means with different letters are statistically different from one another, $p<0.05$. Data are represented as mean ± SEM.

Figure S2

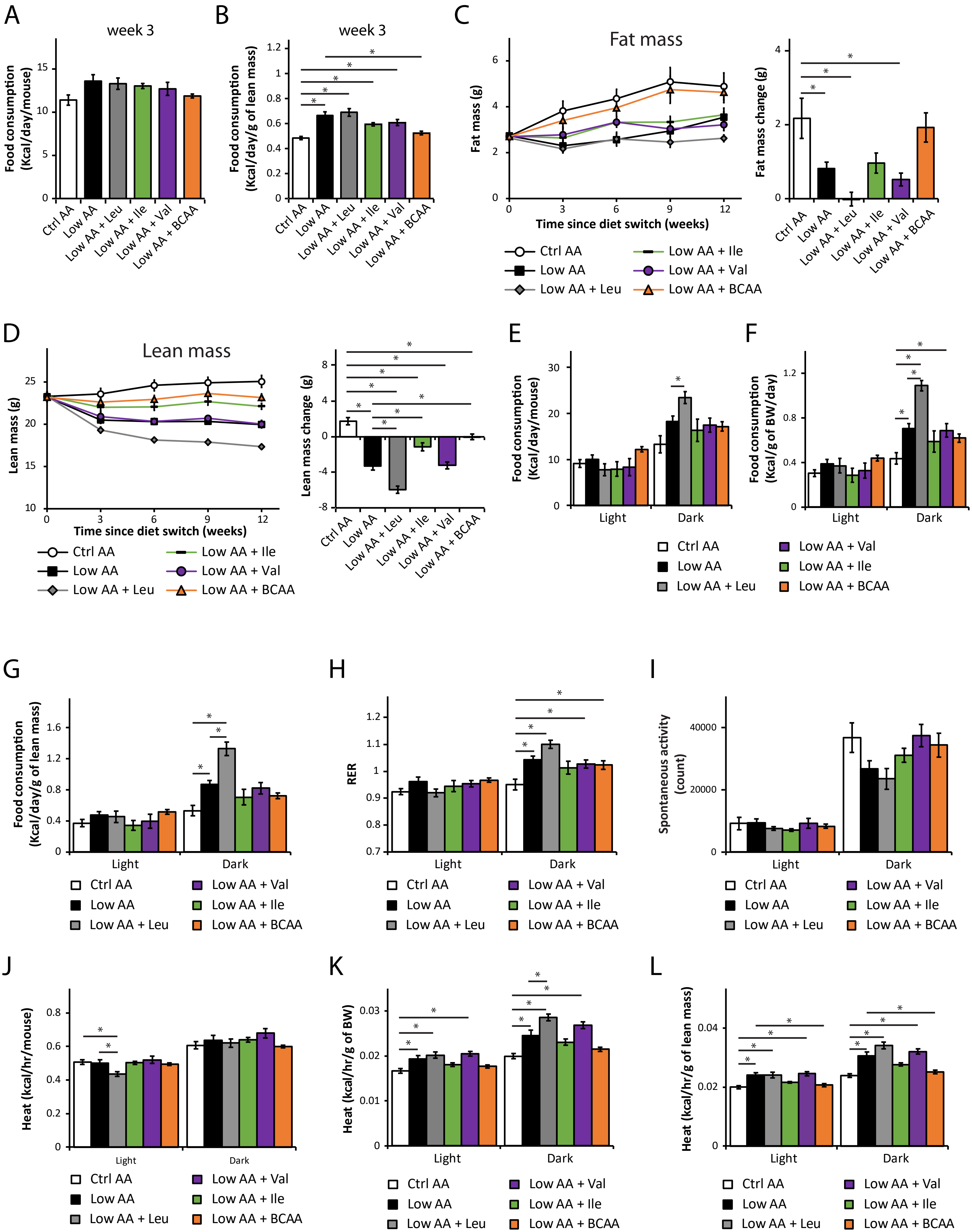
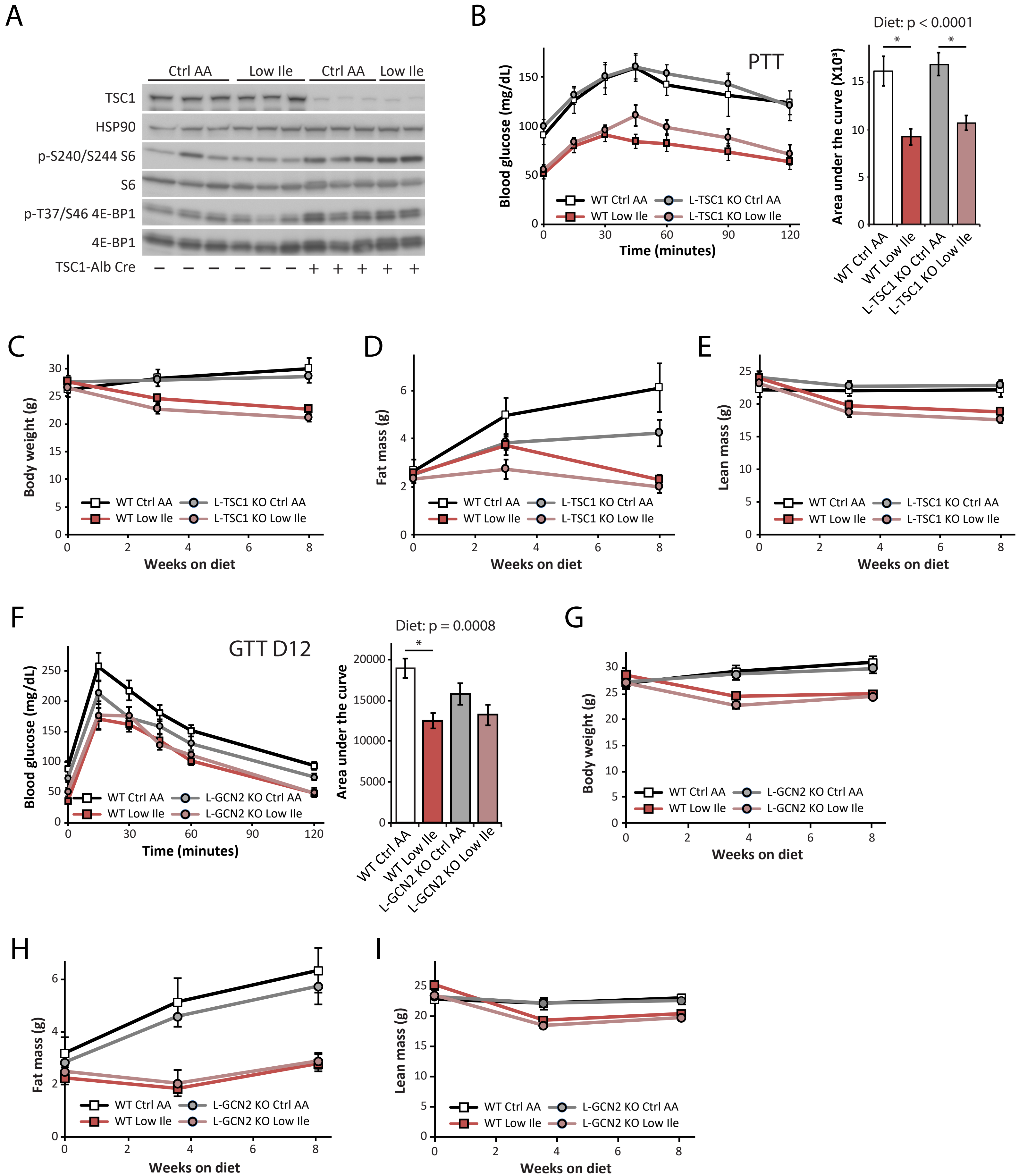

A
week 3
Food consumption (Kcal/day/mouse)
Ctrl AA
Low AA
Low AA + Leu
Low AA + Ile
Low AA + Val
Low AA + BCAA
B
week 3
Food consumption (Kcal/day/g of lean mass)
C
Fat mass
Fat mass (g)
Time since diet switch (weeks)
Fat mass change (g)
D
Lean mass
Lean mass (g)
Time since diet switch (weeks)
Lean mass change (g)
E
Food consumption (Kcal/day/mouse)
Light
Dark
F
Food consumption (Kcal/g of BW/day)
G
Food consumption (Kcal/day/g of lean mass)
H
RER
I
Spontaneous activity (count)
J
Heat (kcal/hr/mouse)
K
Heat (kcal/hr/g of BW)
L
Heat (kcal/hr/g of lean mass)

Figure S2 related to Figure 2. Replenishing isoleucine attenuates the metabolic effects of PR. (A-B) Food consumption per mouse (A) and normalized by lean mass (B) during the third week on the indicated diets. (C) Fat mass and change in fat mass of mice after 12 weeks on the indicated diets. (D) Lean mass and change in lean mass of mice after 12 weeks on the indicated diets. (A-D) n=4 cages of 3 animals each/group (A-B) or n=11-12/group (C-D); *$p<0.05$, Sidak's test post ANOVA, comparing all groups to the Ctrl AA and Low AA diet groups. (E-K) Food consumption per mouse (E), food consumption normalized by body weight (F), food consumption normalized by lean mass (G), RER (H), spontaneous activity (I), energy expenditure (heat) production per mouse (J), energy expenditure normalized by body weight (K) and energy expenditure normalized by lean mass (L) of mice after 7 weeks on the indicated diets were assessed by metabolic chambers. (E-L) n=4-8/group, *$p < 0.05$, Sidak's test post ANOVA, comparing all groups to the Ctrl AA and Low AA diet groups. Data are represented as mean ± SEM.

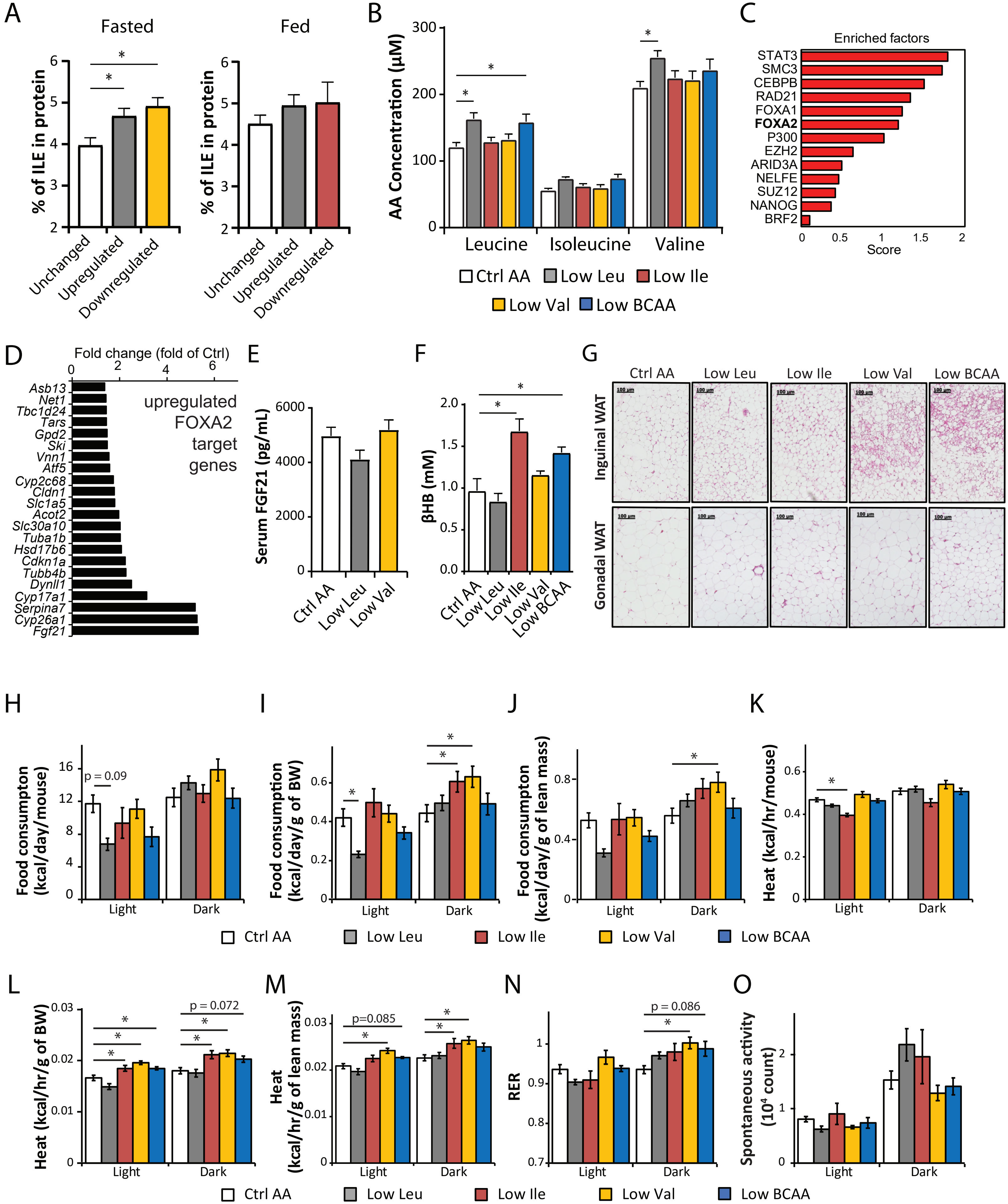
Figure S3



A
Ctrl AA
Low Ile
Ctrl AA
Low Ile
TSC1
HSP90
p-S240/S244 S6
S6
p-T37/S46 4E-BP1
4E-BP1
TSC1-Alb Cre
– – – – – – + + + + +
B
PTT
Blood glucose (mg/dL)
Time (minutes)
WT Ctrl AA
L-TSC1 KO Ctrl AA
WT Low Ile
L-TSC1 KO Low Ile
Diet: p < 0.0001
Area under the curve (X10³)
WT Ctrl AA
WT Low Ile
L-TSC1 KO Ctrl AA
L-TSC1 KO Low Ile
C
Body weight (g)
Weeks on diet
D
Fat mass (g)
Weeks on diet
E
Lean mass (g)
Weeks on diet
F
GTT D12
Blood glucose (mg/dL)
Time (minutes)
WT Ctrl AA
L-GCN2 KO Ctrl AA
WT Low Ile
L-GCN2 KO Low Ile
Diet: p = 0.0008
Area under the curve
WT Ctrl AA
WT Low Ile
L-GCN2 KO Ctrl AA
L-GCN2 KO Low Ile
G
Body weight (g)
Weeks on diet
H
Fat mass (g)
Weeks on diet
I
Lean mass (g)
Weeks on diet

Figure S3, Related to Figure 3. The metabolic effects of isoleucine restriction are independent of hepatic mTORC1 and GCN2 activity. (A) Western blot analysis of mTORC1 signaling in the livers of fasted WT and L-TSC1 KO mice fed the indicated diets for 8 weeks. (B) Pyruvate tolerance test in WT and L-TSC1 KO mice fed the indicated diets for 4 weeks (n=7-8/group; for area under the curve, statistics for the overall effects of genotype, diet, and the interaction represent the p value from a two-way ANOVA, *p<0.05, from a Sidak's post-test examining the effect of parameters identified as significant in the two-way ANOVA). (C-E) Body weight (C), fat mass (D), and lean mass (E) of WT and L-TSC1 KO mice on the indicated diets (n=7-8/group). (F) Glucose tolerance test in WT and L-GCN2 KO mice fed the indicated diets for 12 days (n = 6-7/group; for area under the curve, statistics for the overall effects of genotype, diet, and the interaction represent the p value from a two-way ANOVA, *p<0.05, from a Sidak's post-test examining the effect of parameters identified as significant in the two-way ANOVA). (G-I) Body weight (G), fat mass (H), and lean mass (I) of WT and L-GCN2 KO mice on the indicated diets (n=6-7/group). Data are represented as mean ± SEM.

Figure S4



A
Fasted
Fed
% of ILE in protein
Unchanged
Upregulated
Downregulated
B
AA Concentration (µM)
Leucine
Isoleucine
Valine
Ctrl AA
Low Leu
Low Ile
Low Val
Low BCAA
C
Enriched factors
STAT3
SMC3
CEBPB
RAD21
FOXA1
FOXA2
P300
EZH2
ARID3A
NELFE
SUZ12
NANOG
BRF2
Score
D
Fold change (fold of Ctrl)
upregulated FOXA2 target genes
Asb13
Net1
Tbc1d24
Tars
Gpd2
Ski
Vnn1
Atf5
Cyp2c68
Cldn1
Slc1a5
Acot2
Slc30a10
Tuba1b
Hsd17b6
Cdkn1a
Tubb4b
Dynll1
Cyp17a1
Serpina7
Cyp26a1
Fgf21
E
Serum FGF21 (pg/mL)
F
βHB (mM)
G
Inguinal WAT
Gonadal WAT
H
Food consumpton (kcal/day/mouse)
p = 0.09
Light
Dark
I
Food consumption (kcal/day/g of BW)
J
Food consumpton (kcal/day/g of lean mass)
K
Heat (kcal/hr/mouse)
L
Heat (kcal/hr/g of BW)
p = 0.072
M
Heat (kcal/hr/g of lean mass)
p=0.085
N
RER
p = 0.086
O
Spontaneous activity (10⁴ count)

Figure S4, related to Figures 4 and 5. Isoleucine restriction reprograms hepatic metabolism.

(A) Isoleucine content of the proteins encoded by the 100 genes most significantly upregulated or downregulated by a Low Ile diet relative to the proteins encoded by the 100 genes least altered by a Low Ile diet (*$p<0.05$, Tukey's test following ANOVA). (B) Levels of each BCAA in mice fed the indicated individual BCAA restricted mice after three weeks feeding on the indicated diets following an overnight fast (n = 9/group, significance was calculated using Dunnett's test following ANOVA, *$p<0.05$). (C) Significantly enriched transcription factors predicted to mediate the differential gene expression in the liver of fasted Low Ile fed mice. (D) FOXA2 target genes significantly upregulated by Low Ile feeding from RNA-seq data. (E) Plasma FGF21 level in Low Leu and Low Val fed mice after three weeks feeding on the indicated diets (n = 8/group, no significance detected using ANOVA). (F) Plasma βHB concentration in plasma of mice fed the indicated diets after an overnight fast (n=4-5/group, significance was calculated using Dunnett's test following ANOVA, *$p<0.05$). (G) Representative images of H&E stained inguinal white adipose tissue (iWAT) and gonadal white adipose tissue (gWAT) of mice after 12 weeks feeding on the indicated diets. Scale bar = 100 μm. n=5-6/group. (H-O) Food consumption per mouse (H), food consumption normalized by body weight (I), food consumption normalized by lean mass (J), energy expenditure (heat) production per mouse (K), energy expenditure normalized by body weight (L), energy expenditure normalized by lean mass (M), RER (N), and spontaneous activity (O) of mice after 6 weeks on the indicated diets were assessed by metabolic chambers (n = 4-8/group; significance was calculated using Dunnett's test following ANOVA, *$p < 0.05$). (G, L) Ctrl AA and Low Ile data are duplicated from Figure 5 to allow comparison. Data are represented as mean ± SEM.

Figure S5

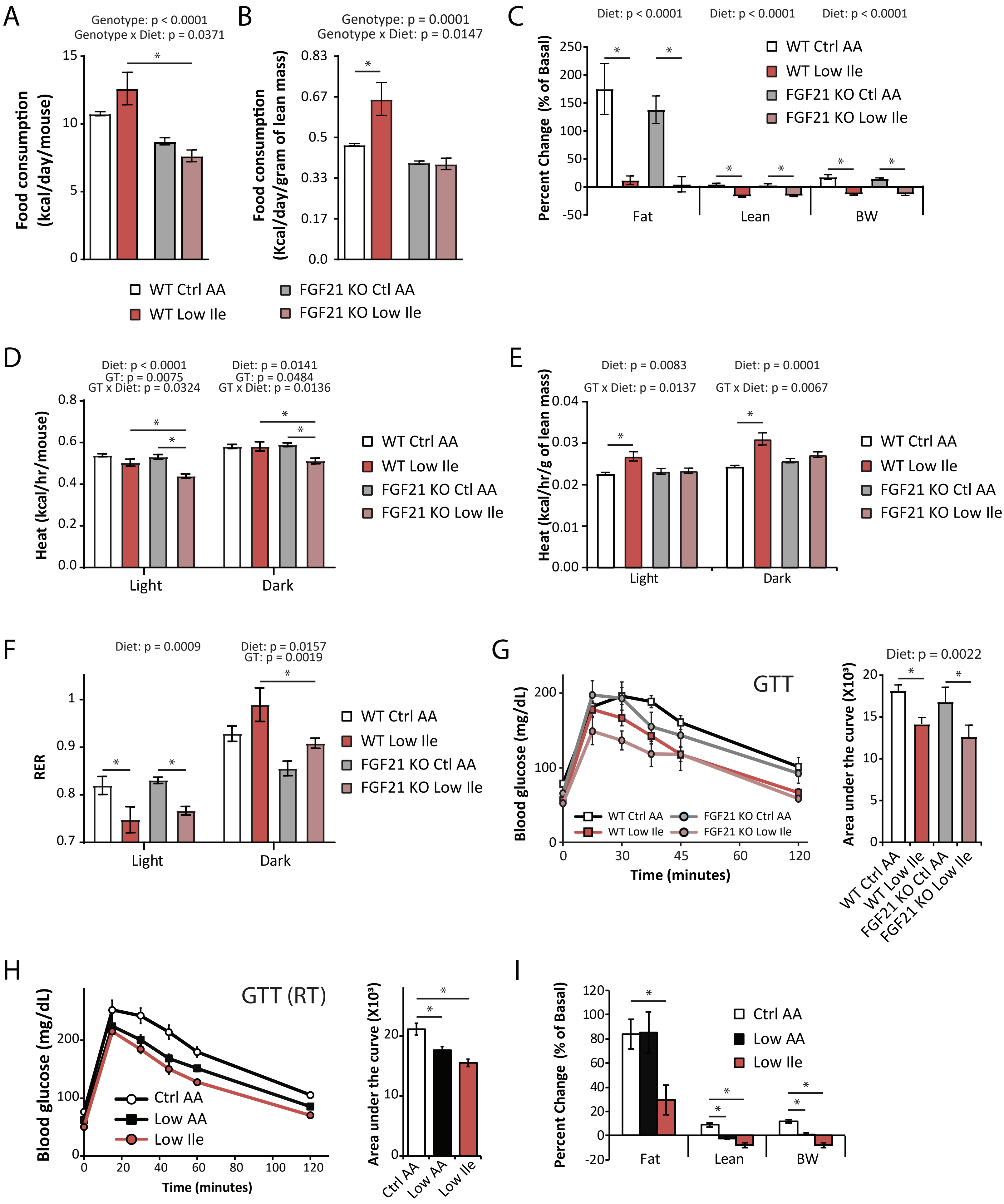
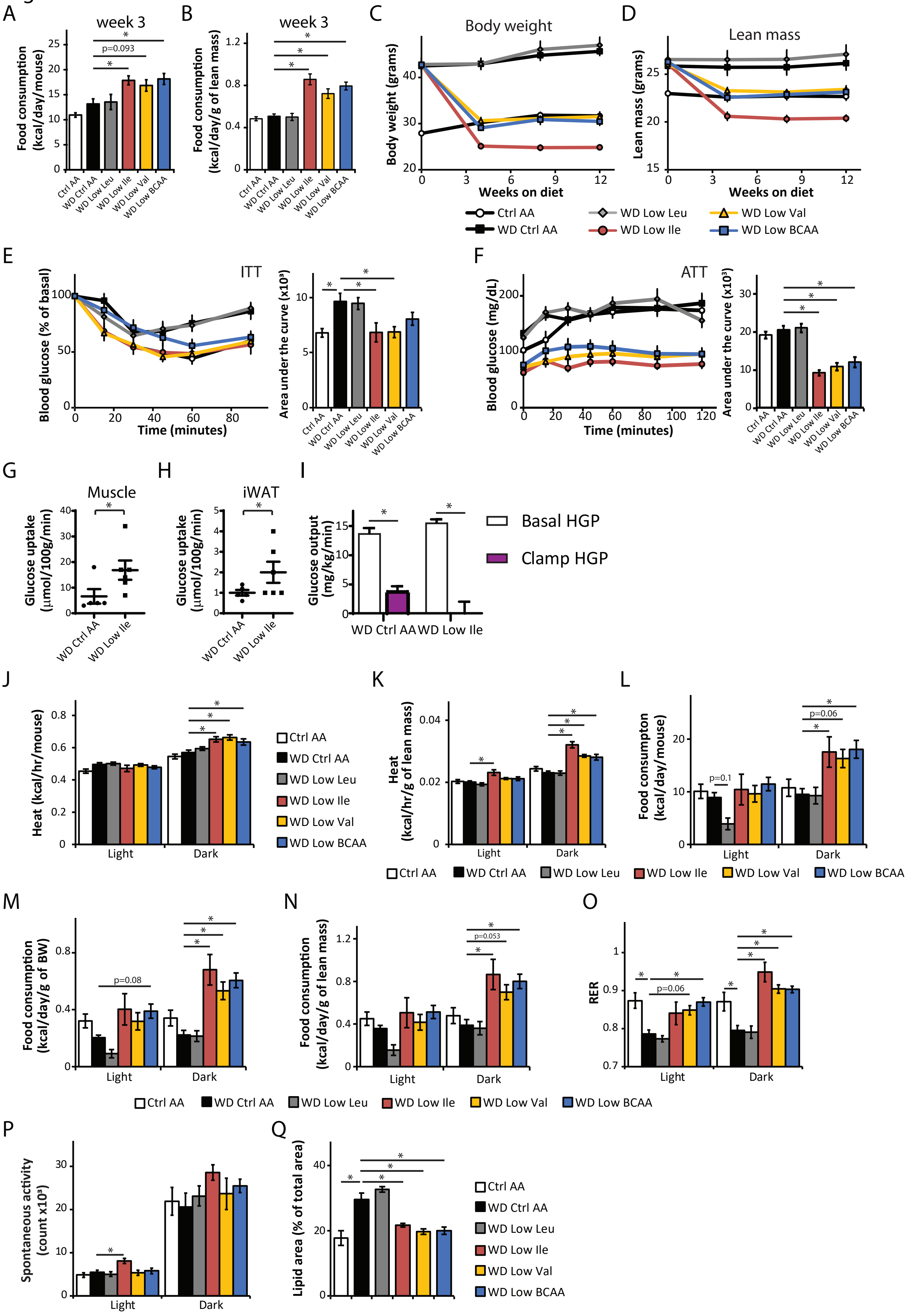

A
Genotype: p < 0.0001
Genotype x Diet: p = 0.0371
Food consumption (kcal/day/mouse)
B
Genotype: p = 0.0001
Genotype x Diet: p = 0.0147
Food consumption (Kcal/day/gram of lean mass)
WT Ctrl AA
WT Low Ile
FGF21 KO Ctl AA
FGF21 KO Low Ile
C
Diet: p < 0.0001
Diet: p < 0.0001
Diet: p < 0.0001
Percent Change (% of Basal)
Fat
Lean
BW
WT Ctrl AA
WT Low Ile
FGF21 KO Ctl AA
FGF21 KO Low Ile
D
Diet: p < 0.0001
GT: p = 0.0075
GT x Diet: p = 0.0324
Diet: p = 0.0141
GT: p = 0.0484
GT x Diet: p = 0.0136
Heat (kcal/hr/mouse)
Light
Dark
WT Ctrl AA
WT Low Ile
FGF21 KO Ctl AA
FGF21 KO Low Ile
E
Diet: p = 0.0083
GT x Diet: p = 0.0137
Diet: p = 0.0001
GT x Diet: p = 0.0067
Heat (kcal/hr/g of lean mass)
Light
Dark
WT Ctrl AA
WT Low Ile
FGF21 KO Ctl AA
FGF21 KO Low Ile
F
Diet: p = 0.0009
Diet: p = 0.0157
GT: p = 0.0019
RER
Light
Dark
WT Ctrl AA
WT Low Ile
FGF21 KO Ctl AA
FGF21 KO Low Ile
G
GTT
Blood glucose (mg/dL)
Time (minutes)
WT Ctrl AA
WT Low Ile
FGF21 KO Ctrl AA
FGF21 KO Low Ile
Diet: p = 0.0022
Area under the curve (X10³)
WT Ctrl AA
WT Low Ile
FGF21 KO Ctl AA
FGF21 KO Low Ile
H
GTT (RT)
Blood glucose (mg/dL)
Time (minutes)
Ctrl AA
Low AA
Low Ile
Area under the curve (X10³)
Ctrl AA
Low AA
Low Ile
I
Percent Change (% of Basal)
Fat
Lean
BW
Ctrl AA
Low AA
Low Ile

Figure S5, related to Figure 5. Reducing dietary isoleucine induces the FGF21-UCP1 axis and promotes energy expenditure. (A-B) Food consumption per mouse (A) or normalized by lean mass (B) measured after three weeks of feeding the indicated diets to wild-type and FGF21 KO mice. (C) Percent change in fat mass, lean mass, and body weight. (A-C) $n = 6$-9/group; the overall effect of genotype (GT), diet, and the interaction represent the p-value from a two-way ANOVA conducted separately for the light and dark cycles; *$p < 0.05$, Sidak's test post two-way ANOVA. (D-F) Energy expenditure (heat) per mouse (D), energy expenditure normalized by lean mass (E), and RER (F) measured using metabolic chambers after twelve weeks of diet feeding to wild-type and FGF21 KO mice ($n = 6$-9/group; the overall effect of genotype (GT), diet, and the interaction represent the p-value from a two-way ANOVA conducted separately for the light and dark cycles; *$p < 0.05$, Sidak's test post two-way ANOVA). (G) Glucose tolerance test from wild-type and FGF21 KO mice fed the indicated diets for 3 weeks ($n=6$-12/group; the overall effect of genotype (GT), diet, and the interaction represent the p-value from a two-way ANOVA; *$p < 0.05$, Sidak's test post two-way ANOVA). (H and I) Glucose tolerance test (H) and change in body composition (I) of mice on the indicated diets housed at room temperature (RT, 23°C) ($n = 12$/group; significance was calculated using Sidak's test following ANOVA, *$p < 0.05$). Data are represented as mean ± SEM.

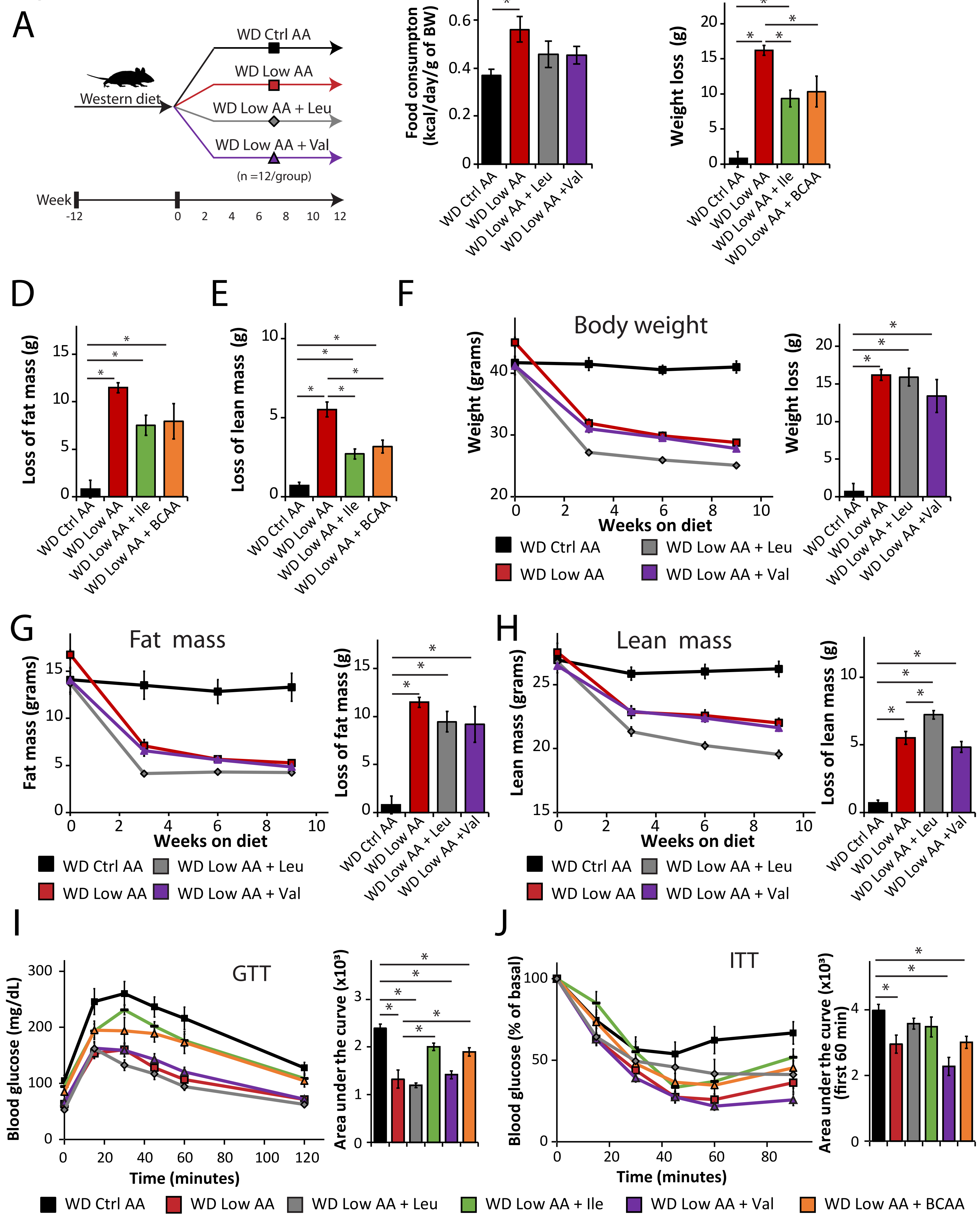
Figure S6



A
week 3
Food consumption (kcal/day/mouse)
p=0.093
Ctrl AA
WD Ctrl AA
WD Low Leu
WD Low Ile
WD Low Val
WD Low BCAA
B
week 3
Food consumption (kcal/day/g of lean mass)
C
Body weight
Body weight (grams)
Weeks on diet
D
Lean mass
Lean mass (grams)
Weeks on diet
E
ITT
Blood glucose (% of basal)
Time (minutes)
Area under the curve (x10³)
F
ATT
Blood glucose (mg/dL)
Time (minutes)
Area under the curve (x10³)
G
Muscle
Glucose uptake (μmol/100g/min)
H
iWAT
Glucose uptake (μmol/100g/min)
I
Glucose output (mg/kg/min)
WD Ctrl AA
WD Low Ile
Basal HGP
Clamp HGP
J
Heat (kcal/hr/mouse)
Light
Dark
K
Heat (kcal/hr/g of lean mass)
L
Food consumpton (kcal/day/mouse)
p=0.1
p=0.06
M
Food consumption (kcal/day/g of BW)
p=0.08
N
Food consumption (kcal/day/g of lean mass)
p=0.053
O
RER
p=0.06
P
Spontaneous activity (count x10³)
Q
Lipid area (% of total area)

Figure S6, related to Figure 6. Restricting dietary isoleucine or valine improves metabolic health of diet-induced obese mice. (A-B) Average food consumption per mouse (A) or normalized by lean mass (B) of mice during the third week on the indicated diets (n=5-6 cages of 2 animals each/group; significance was calculated using Dunnett's test following ANOVA, means of different diet groups were compared to that of WD Ctrl AA diet group. *$p<0.05$). (C-D) Body weight (C) and lean mass (D) of mice on the indicated diets (n=10/group). (E-F) Insulin (E) and alanine (F) tolerance tests of mice after 4 or 5 weeks, respectively, of indicated diet feeding (n=10/group; significance was calculated using Dunnett's test following ANOVA, means of different diet groups were compared to that of WD Ctrl AA diet group. *$p<0.05$). (G-I) Glucose uptake into muscle (G) and iWAT (H), and basal and clamp hepatic gluconeogenesis (I) were determined during a hyperinsulinemic-euglycemic clamp in diet-induced obese mice switched to either a WD Ctrl AA diet or a WD Low Ile diet for 3 weeks. Each symbol represents a single animal, n=5-6/group. Significance was calculated using 1-tailed Student's t-test, *$p<0.05$. (J-P) Energy expenditure (heat) per mouse (J), energy expenditure normalized by lean mass (K), Food consumption per mouse (L), food consumption normalized by body weight (M), food consumption normalized by lean mass (N), respiratory exchange ratio (O), and spontaneous activity (P) of mice after 6 weeks on the indicated diets. n=5-8/group. (Q) Quantification of lipid droplet area in Figure 6I. n=4/group. (J-Q) Significance was calculated using Dunnett's test following ANOVA, (J-P) conducted separately for the light and dark cycles; means of different diet groups were compared to that of WD Ctrl AA diet group; *$p < 0.05$. Data are represented as mean ± SEM.

Figure S7



A
WD Ctrl AA
WD Low AA
WD Low AA + Leu
WD Low AA + Val
Western diet
(n =12/group)
Week
-12
0
2
4
6
8
10
12
B
Food consumpton (kcal/day/g of BW)
C
Weight loss (g)
D
Loss of fat mass (g)
E
Loss of lean mass (g)
F
Body weight
Weight (grams)
Weeks on diet
Weight loss (g)
G
Fat mass
Fat mass (grams)
Loss of fat mass (g)
H
Lean mass
Lean mass (grams)
Loss of lean mass (g)
I
GTT
Blood glucose (mg/dL)
Time (minutes)
Area under the curve (x10³)
J
ITT
Blood glucose (% of basal)
Area under the curve (x10³) (first 60 min)
WD Ctrl AA
WD Low AA
WD Low AA + Leu
WD Low AA + Ile
WD Low AA + Val
WD Low AA + BCAA

Figure S7, related to Figure 7. Increased isoleucine intake attenuates the beneficial effects of PR on obesity and is associated with higher BMI in human.

(A) Experimental scheme. 6-week-old male C57BL/6J mice were fed a western diet (WD) for 12 weeks and then switched to the indicated diets. (B) Average food consumption during the first month on the indicated diets normalized by body weight (n=6 cages of 2 animals each/group; *$p<0.05$, Sidak's test post ANOVA; each group compared to the WD Ctrl AA and WD Low AA groups.). (C–E) Loss of body weight (C), fat mass (D), and lean mass (E) of mice consuming WD Ctrl AA, WD Low AA, WD Low AA + Ile, and WD Low AA + BCAA diets. (C-E) n=11-12/group; significance was calculated using Sidak's test following ANOVA. Means of different diet groups were compared to that of WD Ctrl AA and WD Low AA diet groups. *$p < 0.05$. (F–I) Physiological impact of addback of Leu and Valine to a WD Low AA diet. Body weight (F), fat mass (G), lean mass (H), and glucose tolerance (I) of mice consuming the indicated diets (n=11-12/group; significance was calculated using Sidak's test following ANOVA. Means of different diet groups were compared to that of WD Ctrl AA and WD Low AA diet groups. *$p < 0.05$). (J) Insulin tolerance of mice consuming the indicated diets (n=10-12/group; significance was calculated using Sidak's test following ANOVA. Means of different diet groups were compared to that of WD Ctrl AA diet group. *$p < 0.05$). (B-J) WD Ctrl AA, WD Low AA, WD Low AA + Ile and WD Low AA + BCAA data are duplicated from Figure 7 to allow comparison. Data are represented as mean ± SEM.

Supplemental Table Legends

Supplemental Table 1, related to Figures 1, 3, 4, and 5. Diet composition of diets used to investigate the effect of reduced levels of individual BCAAs in the context of a normal calorie diet.

Supplemental Table 2, related to Figure 2. Diet composition of diets used to investigate the effect of supplementation of individual BCAAs to the Low AA diet in the context of a normal calorie diet.

Supplemental Table 4, related to Figure 6. Diet composition of diets used to investigate the effect of reduced levels of individual BCAAs in the context of a WD.

Supplemental Table 5, related to Figure 7. Diet composition of diets used to investigate the effect of supplementation of individual BCAAs to the Low AA diet in the context of a WD.

Supplemental Table 6, related to Figure 7. Associations between leucine, isoleucine and valine consumption and BMI in population-based samples; SHOW population demographics

Supplemental Table 7, related to Figure 5 and Star Methods. Sequences of primers used in the study.

Table S1

Amino Acid Defined Diets	Ctrl AA	Low AA	Low non-BCAA	Low BCAA	Low Leu	Low Ile	Low Val	ExLow Leu
Teklad Diet name	21% Protein	7% Protein	2/3 EAA, BCAA no adj	BCAA 2/3 Adj, 21% Protein	Leu 2/3 Adj, 21% Protein	Ile 2/3 Adj, 21% Protein	Val 2/3 Adj, 21% Protein	Leu 83.5% Adj, 21% Protein
Teklad Diet number	TD.140711	TD.140712	TD.150663	TD.150662	TD.160736	TD.160734	TD.160735	TD.200006
Color	Red	Blue	Black	Orange	Green	Orange	Black	Blue
Formula	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**
L-Alanine	9.38	3.05	20	11.8183	10.8334	9.8267	9.9183	10.46
L-Arginine	6.3	2.05	2.05	6.3	6.3	6.3	6.3	6.3
L-Asparagine	20.58	6.7	20	22.388	21.6577	20.9113	20.9792	22.95
L-Aspartic Acid	20.58	6.7	30	24.2237	22.7518	21.2475	21.3844	22.95
L-Cysteine	7.2	2.34	2.34	7.2	7.2	7.2	7.2	7.2
L-Glutamic Acid	28.97	9.43	36.4377	32.9963	31.37	29.7077	29.859	32.31
L-Glutamine	33.77	11	24.5324	35.7873	34.9725	34.1395	34.2154	37.66
Glycine	2.96	0.96	14.7389	5.0141	4.1844	3.3363	3.4135	3.3
L-Histidine HCl, monohydrate	4.6	1.5	1.5	4.6	4.6	4.6	4.6	4.6
L-Isoleucine	7.8	2.54	7.8	2.54	7.8	2.54	7.8	7.8
L-Leucine	25.4	8.27	25.4	8.27	8.27	25.4	25.4	4.2
L-Lysine HCl	20.38	6.64	6.64	20.38	20.38	20.38	20.38	20.38
L-Methionine	6.7	2.18	2.18	6.7	6.7	6.7	6.7	6.7
L-Phenylalanine	6.6	2.15	2.15	6.6	6.6	6.6	6.6	6.6
L-Proline	7.41	2.41	20	10.5596	9.2874	7.987	8.1054	8.265
L-Serine	7.41	2.41	20	10.2855	9.124	7.9368	8.0448	8.265
L-Threonine	9.7	3.16	3.16	9.7	9.7	9.7	9.7	9.7
L-Tryptophan	3.4	1.1	1.1	3.4	3.4	3.4	3.4	3.4
L-Tyrosine	6.9	2.25	2.25	6.9	6.9	6.9	6.9	6.9
L-Valine	8.4	2.735	8.4	2.735	8.4	8.4	2.74	8.4
Sucrose	291.248	291.248	291.248	291.248	291.248	291.248	291.248	291.248
Corn Starch	150.0	232.4325	146.8805	153.0211	152.0044	150.6136	150.4	153.05
Maltodextrin	150.0	232.4325	146.8805	153.0211	152.0044	150.6136	150.4	15305
Anhydrous Milkfat	0.0							
Cholesterol	0.0							
Corn Oil	52.0	52.0	52.0	52.0	52.0	52.0	52.0	52
Olive Oil	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29
Cellulose	30.0	30	30	30	30	30	30	30
Mineral Mix, AIN-93M-MX (94049)	35.0	35	35	35	35	35	35	35
Calcium Phosphate $Ca(H_2PO_4)_2 \cdot H_2O$	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
Vitamin Mix, Teklad (40060)	10.0	10	10	10	10	10	10	10
TBHQ, antioxidant	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012
Food Coloring	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
% kcal from								
Protein (based on N x 6.25)	22	7.1	22.1	21.9	21.9	22	22	21.9
Carbohydrates	59.4	74.4	59.1	59.6	59.5	59.4	59.4	59.6
Fat	18.6	18.5	18.8	18.5	18.6	18.6	18.6	18.5
Kcal/g	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9

Table S2

Amino Acid Defined Diets	Ctrl AA	Low AA	Low AA + Leu	Low AA + Ile	Low AA + Val	Low AA + BCAA
Teklad Diet name	21% Protein	7% Protein	Low AA Diet w/ 25.4g/kg Leu	Low AA Diet w/ 7.8g/kg Ile	Low AA Diet w/ 8.4g/kg Val	Low AA Diet w/ High BCAA
Teklad Diet number	TD.140711	TD.140712	TD.170671	TD.170670	TD.170672	TD.160794
Color	Red	Blue	Green	Yellow	Black	Orange
Formula	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**
L-Alanine	9.38	3.05	3.05	3.05	3.05	3.05
L-Arginine	6.3	2.05	2.05	2.05	2.05	2.05
L-Asparagine	20.58	6.7	6.7	6.7	6.7	6.7
L-Aspartic Acid	20.58	6.7	6.7	6.7	6.7	6.7
L-Cysteine	7.2	2.34	2.34	2.34	2.34	2.34
L-Glutamic Acid	28.97	9.43	9.43	9.43	9.43	9.43
L-Glutamine	33.77	11	11	11	11	11
Glycine	2.96	0.96	0.96	0.96	0.96	0.96
L-Histidine HCl, monohydrate	4.6	1.5	1.5	1.5	1.5	1.5
L-Isoleucine	7.8	2.54	2.54	7.8	2.54	7.8
L-Leucine	25.4	8.27	25.4	8.27	8.27	25.4
L-Lysine HCl	20.38	6.64	6.64	6.64	6.64	6.64
L-Methionine	6.7	2.18	2.18	2.18	2.18	2.18
L-Phenylalanine	6.6	2.15	2.15	2.15	2.15	2.15
L-Proline	7.41	2.41	2.41	2.41	2.41	2.41
L-Serine	7.41	2.41	2.41	2.41	2.41	2.41
L-Threonine	9.7	3.16	3.16	3.16	3.16	3.16
L-Tryptophan	3.4	1.1	1.1	1.1	1.1	1.1
L-Tyrosine	6.9	2.25	2.25	2.25	2.25	2.25
L-Valine	8.4	2.735	2.735	2.735	8.4	8.4
Sucrose	291.248	291.248	291.248	291.248	291.248	291.248
Corn Starch	150.0	232.4325	223.8675	229.8025	229.6	218.405
Maltodextrin	150.0	232.4325	223.8675	229.8025	229.6	218.405
Anhydrous Milkfat	0.0		0	0	0	0
Cholesterol	0.0		0	0	0	0
Corn Oil	52.0	52.0	52.0	52.0	52.0	52.0
Olive Oil	29.0	29.0	29.0	29.0	29.0	29.0
Cellulose	30.0	30	30	30	30	30
Mineral Mix, AIN-93M-MX (94049)	35.0	35	35	35	35	35
Calcium Phosphate $Ca(H_2PO_4)_2 \cdot H_2O$	8.2	8.2	8.2	8.2	8.2	8.2
Vitamin Mix, Teklad (40060)	10.0	10	10	10	10	10
TBHQ, antioxidant	0.012	0.012	0.012	0.012	0.012	0.012
Food Coloring	0.1	0.1	0.1	0.1	0.1	0.1
% kcal from						
Protein (based on N x 6.25)	22	7.1	8.3	7.5	7.5	9.1
Carbohydrates	59.4	74.4	73.1	74	73.9	72.3
Fat	18.6	18.5	18.6	18.5	18.5	18.6
Kcal/g	3.9	3.9	3.9	3.9	3.9	3.9

Table S4

Amino Acid Defined Diets	Ctrl AA	WD Ctrl AA	WD Low Leu	WD Low Ile	WD Low Val	WD Low BCAA
Teklad Diet name	21% Protein	AA Adj Calories, 21% Milkfat, 0.2% Chol	Leu 2/3 Adj, 21% Milkfat, 0.2% Chol	Ile 2/3 Adj, 21% Milkfat, 0.2% Chol	Val 2/3 Adj, 21% Milkfat, 0.2% Chol	BCAA 2/3 Adj, 42% Fat, 21% Protein
Teklad Diet number	TD.140711	TD.160186	TD.170483	TD.170484	TD.170485	TD.160188
Color	Red	Aqua	Purple	Red	Brown	Black
Formula	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**
L-Alanine	9.38	9.38	10.8334	9.8267	9.9183	11.8183
L-Arginine	6.3	6.3	6.3	6.3	6.3	6.3
L-Asparagine	20.58	20.58	21.6577	20.9113	20.9792	22.388
L-Aspartic Acid	20.58	20.58	22.7518	21.2475	21.3844	24.2237
L-Cysteine	7.2	7.2	7.2	7.2	7.2	7.2
L-Glutamic Acid	28.97	28.97	31.37	29.7077	29.859	32.9963
L-Glutamine	33.77	33.77	34.9725	34.1395	34.2154	35.7873
Glycine	2.96	2.96	4.1844	3.3363	3.4135	5.0141
L-Histidine HCl, monohydrate	4.6	4.6	4.6	4.6	4.6	4.6
L-Isoleucine	7.8	7.8	7.8	2.54	7.8	2.54
L-Leucine	25.4	25.4	8.27	25.4	25.4	8.27
L-Lysine HCl	20.38	20.38	20.38	20.38	20.38	20.38
L-Methionine	6.7	6.7	6.7	6.7	6.7	6.7
L-Phenylalanine	6.6	6.6	6.6	6.6	6.6	6.6
L-Proline	7.41	7.41	9.2874	7.987	8.1054	10.5596
L-Serine	7.41	7.41	9.124	7.9368	8.0448	10.2855
L-Threonine	9.7	9.7	9.7	9.7	9.7	9.7
L-Tryptophan	3.4	3.4	3.4	3.4	3.4	3.4
L-Tyrosine	6.9	6.9	6.9	6.9	6.9	6.9
L-Valine	8.4	8.4	8.7	8.4	2.735	2.735
Sucrose	291.248	341.46	341.46	341.46	341.46	341.46
Corn Starch	150.0	49.63	51.6344	50.2436	50.0325	52.6511
Maltodextrin	150.0	49.63	51.6344	50.2436	50.0325	52.6511
Anhydrous Milkfat	0.0	210	210	210	210	210
Cholesterol	0.0	1.5	1.5	1.5	1.5	1.5
Corn Oil	52.0	0.0	0.0	0.0	0.0	0.0
Olive Oil	29.0	0.0	0.0	0.0	0.0	0.0
Cellulose	30.0	50	50	50	50	50
Mineral Mix, AIN-93M-MX (94049)	35.0	35	35	35	35	35
Calcium Phosphate $Ca(H_2PO_4)_2 \cdot H_2O$	8.2	8.2	8.2	8.2	8.2	8.2
Vitamin Mix, Teklad (40060)	10.0	10	10	10	10	10
TBHQ, antioxidant	0.012	0.04	0.04	0.04	0.04	0.04
Food Coloring	0.1	0.1	0.1	0.1	0.1	0.1
% kcal from						
Protein (based on N x 6.25)	22	20.7	18.9	19	19	20.2
Carbohydrates	59.4	38.5	39.5	39.3	39.3	39
Fat	18.6	40.9	41.6	41.7	41.7	40.9
Kcal/g	3.9	4.6	4.5	4.5	4.5	4.6

Table S5

Amino Acid Defined Diets	Ctrl AA	WD Ctrl AA	WD Low AA	WD Low AA + Leu	WD Low AA + Ile	WD Low AA + Val	WD Low AA + BCAA
Teklad Diet name	21% Protein	AA Adj Calories, 21% Milkfat, 0.2% Chol	AA Adj Calories, 7% Protein, 21% Milkfat	Low AA Western (2.54% Leu)	Low AA Western (0.78% Ile)	Low AA Western (0.84% Val)	BCAA Adj Low AA Western
Teklad Diet number	TD.140711	TD.160186	TD.160187	TD.171011	TD.171012	TD.171013	TD.171014
Color	Red	Aqua	Orange	Yellow	Pink	Purple	Brown
Formula	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**	**g/kg**
L-Alanine	9.38	9.38	3.05	3.05	3.05	3.05	3.05
L-Arginine	6.3	6.3	2.05	2.05	2.05	2.05	2.05
L-Asparagine	20.58	20.58	6.7	6.7	6.7	6.7	6.7
L-Aspartic Acid	20.58	20.58	6.7	6.7	6.7	6.7	6.7
L-Cysteine	7.2	7.2	2.34	2.34	2.34	2.34	2.34
L-Glutamic Acid	28.97	28.97	9.43	9.43	9.43	9.43	9.43
L-Glutamine	33.77	33.77	11	11	11	11	11
Glycine	2.96	2.96	0.96	0.96	0.96	0.96	0.96
L-Histidine HCl, monohydrate	4.6	4.6	1.5	1.5	1.5	1.5	1.5
L-Isoleucine	7.8	7.8	2.54	2.54	7.8	2.54	7.8
L-Leucine	25.4	25.4	8.27	25.4	8.27	8.27	25.4
L-Lysine HCl	20.38	20.38	6.64	6.64	6.64	6.64	6.64
L-Methionine	6.7	6.7	2.18	2.18	2.18	2.18	2.18
L-Phenylalanine	6.6	6.6	2.15	2.15	2.15	2.15	2.15
L-Proline	7.41	7.41	2.41	2.41	2.41	2.41	2.41
L-Serine	7.41	7.41	2.41	2.41	2.41	2.41	2.41
L-Threonine	9.7	9.7	3.16	3.16	3.16	3.16	3.16
L-Tryptophan	3.4	3.4	1.1	1.1	1.1	1.1	1.1
L-Tyrosine	6.9	6.9	2.25	2.25	2.25	2.25	2.25
L-Valine	8.4	8.4	2.735	2.735	2.735	8.4	8.4
Sucrose	291.248	341.46	341.46	341.46	341.46	341.46	341.46
Corn Starch	150.0	49.63	132.0625	123.4975	129.4325	129.23	118.035
Maltodextrin	150.0	49.63	132.0625	123.4975	129.1325	129.23	118.035
Anhydrous Milkfat	0.0	210	210	210	210	210	210
Cholesterol	0.0	1.5	1.5	1.5	1.5	1.5	1.5
Corn Oil	52.0	0.0	0.0	0.0	0.0	0.0	0.0
Olive Oil	29.0	0.0	0.0	0.0	0.0	0.0	0.0
Cellulose	30.0	50	50	50	50	50	50
Mineral Mix, AIN-93M-MX (94049)	35.0	35	35	35	35	35	35
Calcium Phosphate $Ca(H_2PO_4)_2 \cdot H_2O$	8.2	8.2	8.2	8.2	8.2	8.2	8.2
Vitamin Mix, Teklad (40060)	10.0	10	10	10	10	10	10
TBHQ, antioxidant	0.012	0.04	0.04	0.04	0.04	0.04	0.04
Food Coloring	0.1	0.1	0.1	0.1	0.1	0.1	0.1
% kcal from							
Protein (based on N x 6.25)	22	20.7	6.8	7.2	6.5	6.5	7.9
Carbohydrates	59.4	38.5	52	51.2	52	52	50.4
Fat	18.6	40.9	41.2	41.6	41.5	41.5	41.7
Kcal/g	3.9	4.6	4.6	4.5	4.6	4.6	4.5

Table S6: Associations between leucine, isoleucine and valine consumption and BMI in population-based samples; SHOW population demographics.

% of Total Protein from Amino Acid of Interest (Grams of Protein from Amino Acid of Interest/Total Grams of Protein)	Association between Increased percent of diet from amino acid and body mass index+		
	Estimate	Standard Error	p-value
Isoleucine	2.46	1.23	0.046*
Valine	1.14	1.06	0.283
Leucine	1.06	0.64	0.098

*p-value less than .05; +regression models adjusted for age, gender, education, household income, saturated fat intake, total calories per day consumed & physical activity

SHOW Demographics	
Age, mean (SD)	53.6 (16.4)
male, n (%)	333 (42.2)
BMI, mean (SD)	30.6 (7.7)
BMI categories:	
<25, n (%)	189 (25.1)
25<30, n (%)	226 (28.6)
≥ 30, n (%)	365 (46.2)
Education:	
< high school, n (%)	53 (6.72)
HS Diploma/GED, n (%)	307 (38.9)
> high school, n (%)	429 (54.4)
Race	
Non-Hispanic White, n (%)	628 (79.7)
Black/African American, n (%)	101 (12.8)
Hispanic, n (%)	30 (3.8)
Other, n (%)	29 (3.7)

Table S7

Gene name	Forward sequence	Reverse sequence
Acc1	AAGGCTATGTGAAGGATG	CTGTCTGAAGAGGTTAGG
Acc2	AGTCTTCCGTGCCTTTGTAC	TTCTGCAAACTCATCCCTCG
Actb	ACCTTCTACAATGAGCTGCG	CTGGATGGCTACGTACATGG
Atgl	ATATCCCACTTTAGCTCCAAGG	CAAGTTGTCTGAAATGCCGC
Cidea	GAATAGCCAGAGTCACCTTCG	AGCAGATTCCTTAACACGGC
Elolv3	ATGCAACCCTATGACTTCGAG	ACGATGAGCAACAGATAGACG
Fasn	CCCCTCTGTTAATTGGCTCC	TTGTGGAAGTGCAGGTTAGG
Fgf21	CAAATCCTGGGTGTCAAAGC	CATGGGCTTCAGACTGGTAC
Lipe1	CTGAGATTGAGGTGCTGTCG	CAAGGGAGGTGAGATGGTAAC
Ucp1	GCATTCAGAGGCAAATCAGC	GCCACACCTCCAGTCATTAAG