

Supplemental Figures

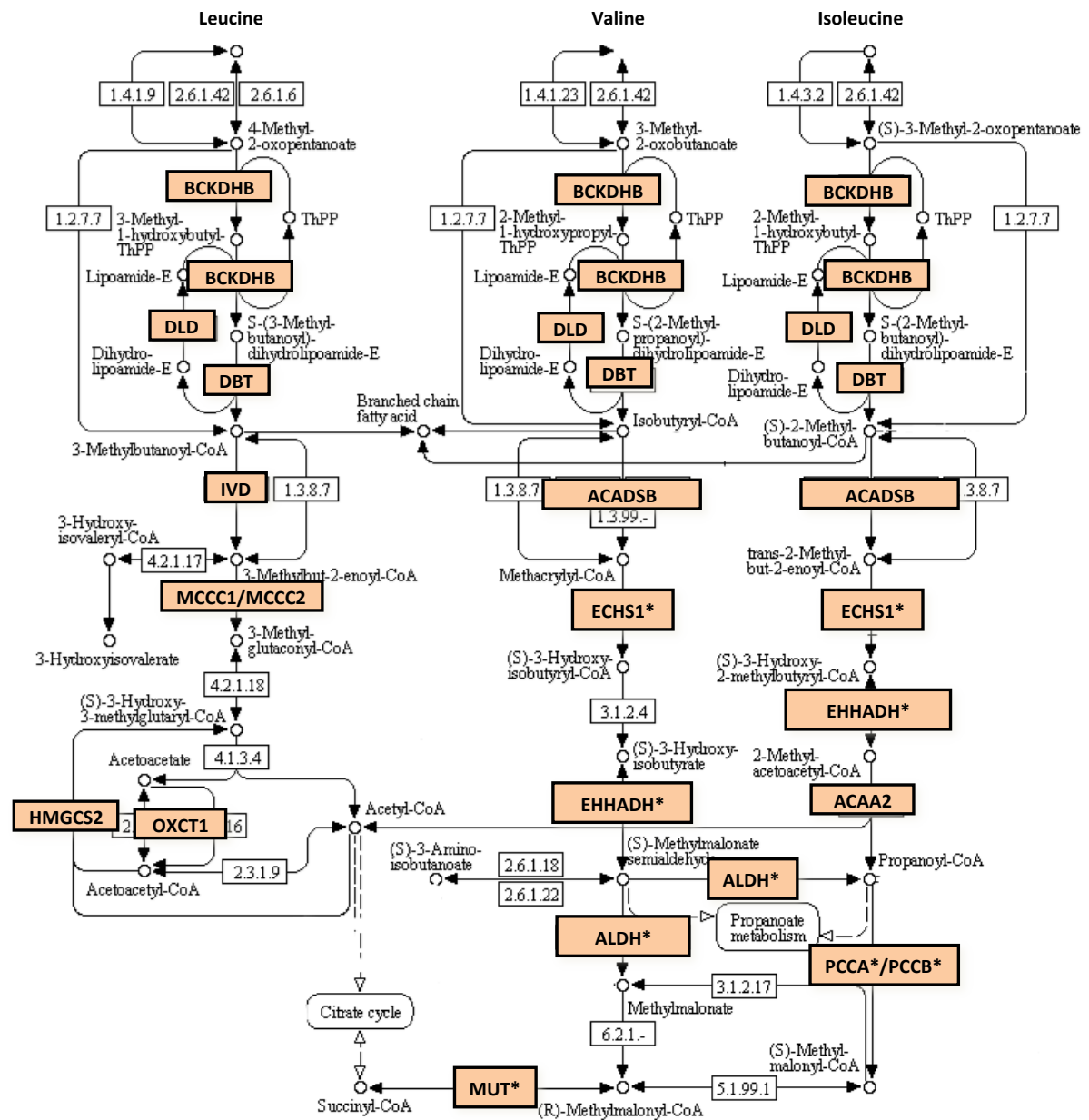


Figure S1. Insulin resistance is associated with a general downregulation of the BCAA degradation pathway. GSEA identified the BCAA degradation pathway as downregulated with insulin resistance; proximal, middle, and distal genes in the pathway contributed to the phenotype (orange boxes). * indicates genes that also contributed to downregulation of the Propanoate Metabolism Pathway. Adapted from KEGG Pathway (<http://www.genome.jp/kegg/pathway.html>).

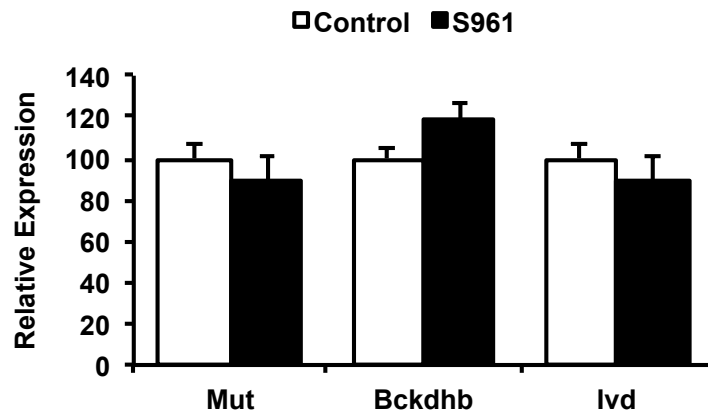


Figure S2. Muscle BCAA gene expression is not decreased in mice made insulin resistant by S961-infusion. C57BL/6J mice were treated with 10 nmol S961 for 1 week, and mRNA levels in gastrocnemius muscle were analyzed by RT-PCR (n=5). Data are mean \pm SEM.

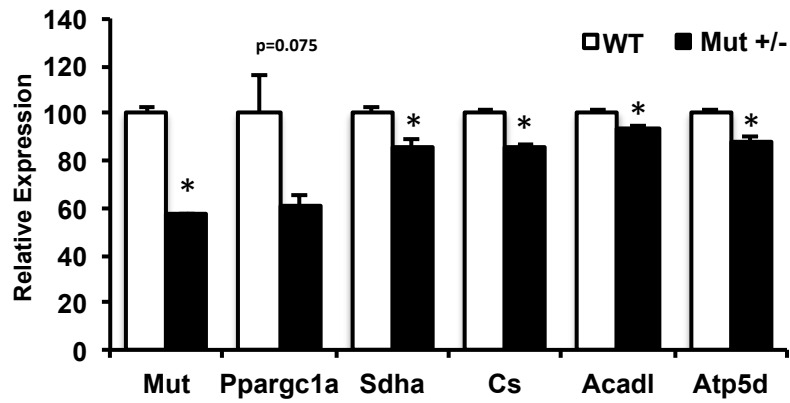
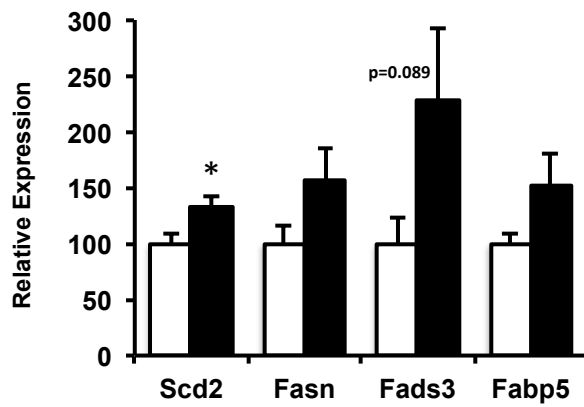
A**B**

Figure S3. *Mut*^{+/-} mice show differential muscle gene expression patterns. Gene expression from skeletal muscle of wild type (Wt, white bars) and *Mut*^{+/-} mice (black bars) was determined by microarray analysis (n=5); genes involved in fatty acid oxidation (**A**) and lipid synthesis (**B**) are shown. Data are expressed relative to the mean of the Wt group. *, p<0.05 (t-test).

BCAA Levels

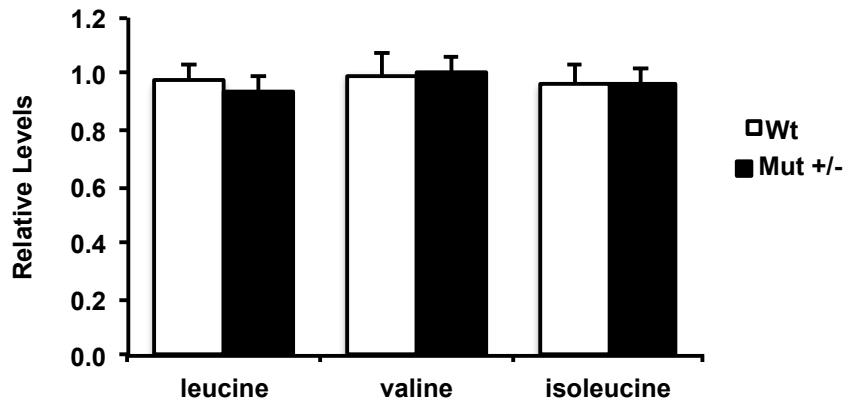


Figure S4. Muscle branched-chain amino acid levels in the *Mut*^{+/-} mice. Relative levels in skeletal muscle of wild-type (white bars, n=8) and *Mut*^{+/-} mice (black bars, n=8). Data are mean \pm SEM.

Supplemental Tables

PATHWAY NAME	p-val	FDR q-val
Classic Pathway	0	0.08
Radiation Sensitivity	0	0.11
Complement Pathway	0	0.13
Complement Activation Classical	0	0.14
Cytotoxic T-Lymphocyte Pathway	0	0.15
Glycolysis Pathway	0.02	0.15
B-Cell Pathway	0.03	0.16
B Lymphocyte Pathway	0	0.16
GPCR Class A Rhodopsin-Like 2	0	0.16
Antigen-Dependent B-Cell Pathway	0	0.17
T-Cell Apoptosis Pathway	0	0.20
St Type I Interferon Pathway	0.02	0.23
Lectin Pathway	0	0.23
CR Cytoskeleton	0	0.23

Table S1. Pathways inversely correlated with insulin sensitivity ($\log S_I$), identified using gene set enrichment analysis (GSEA). Pathways showing an FDR $q < 0.25$ are shown.

Probe ID	Gene Symbol	Correlation Coefficient	Correlation p val
202960_s_at	MUT	0.53	0.0001
221588_x_at	ALDH6A1	0.44	0.0014
205355_at	ACADSB	0.40	0.0047
231919_at	DBT	0.38	0.0061
213321_at	BCKDHB	0.35	0.0136
230426_at	DLD	0.33	0.0193
204290_s_at	ALDH6A1	0.33	0.0196
205371_s_at	DBT	0.33	0.0217
209095_at	DLD	0.32	0.0230
216958_s_at	IVD	0.31	0.0279
221590_s_at	ALDH6A1	0.30	0.0342
205222_at	EHHADH	0.30	0.0343
218440_at	MCCC1	0.30	0.0349
1559239_s_at	ACAT1	0.29	0.0425
221589_s_at	ALDH6A1	0.28	0.0513
226030_at	ACADSB	0.28	0.0548
212694_s_at	PCCB	0.28	0.0554
202054_s_at	ALDH3A2	0.27	0.0563
202959_at	MUT	0.27	0.0587
210544_s_at	ALDH3A2	0.26	0.0734

Table S2. Values for coefficients of correlation of expression of BCAA metabolism genes with log S_i. Probes with a correlation coefficient greater than 0.25 are shown.

	Insulin Sensitive	Insulin Resistant
Subjects	n = 10	n = 13
Age (years)	36.3 ± 3.2	40.5 ± 3.2
Females	7 (70%)	7 (54%)
BMI (kg/m²)	24.7 ± 1.4	29.7 ± 1.3 ^a
Fasting Glucose (mmol/l)	4.92 ± 0.11	5.23 ± 0.13
Fasting Insulin (pmol/l)	30.0 ± 3.6	70.8 ± 9.6 ^c
Triglycerides (mmol/l)	0.69 ± 0.12	1.18 ± 0.10 ^b
Cholesterol (mmol/l)	1.92 ± 0.13	1.97 ± 0.08
HDL-Cholesterol (mmol/l)	0.64 ± 0.04	0.49 ± 0.04
LDL-Cholesterol (mmol/l)	1.08 ± 0.18	1.35 ± 0.08
S_I (IVGTT)	7.60 ± 0.61	2.50 ± 0.36 ^d
M (mg/kg/min)	8.24 ± 0.68	6.11 ± 0.50 ^a

Table S3. Clinical characteristics of the subset of participants for which skeletal muscle metabolomic analysis was performed (due to availability of adequate muscle biopsy material in these individuals). Participants were categorized as insulin sensitive or resistant based on S_I values above or below the median value of 4.78 for a larger population of normoglycemic individuals at the Joslin Diabetes Center. M, glucose metabolism rate. Data are mean ± S.E. a, p<0.05 b, p<0.01 c, p<0.005 d, p<0.001

Metabolite	Spearman ρ	p val
X - 13424	0.70	0.011
X - 13465	0.69	0.007
X - 12984	0.66	0.004
X - 11437	0.66	0.003
X - 13227	0.66	0.014
N-acetylmethionine	0.65	0.032
fumarate	0.63	0.002
X - 4795	0.62	0.030
7-beta-hydroxycholesterol	0.61	0.015
asparagine	0.60	0.003
X - 13470	0.60	0.029
X - 11859	0.58	0.019
X - 5215	0.57	0.011
cholesterol	0.57	0.007
X - 13463	0.55	0.042
squalene	0.54	0.039
X - 13093	0.50	0.020
alpha-ketoglutarate	0.49	0.046
4-methyl-2-oxopentanoate (KIC)	0.49	0.025
X - 10419	0.49	0.022
stachydrine	0.48	0.022
3-methyl-2-oxovalerate (KMV)	0.48	0.044
pantothenate	0.44	0.034
X - 12422	0.44	0.046
choline	0.44	0.048
nicotinamide	-0.45	0.045
isoleucine	-0.53	0.013
X - 13450	-0.90	0.037

Table S4. Spearman correlation of individual metabolite levels with $\log S_1$. Metabolites with correlation p val < 0.05 are shown.

Data File S1. Metabolite levels in IS and IR subjects.

See Excel Spreadsheet.