Supplemental Figure 1. Microarray and Gene Set Enrichment Analysis (GSEA) for BCAA oxidation enzymes in adipose tissue of AG4OX and AG4KO mice.

Total RNA from epididymal adipose tissue was extracted using the RNeasy Mini Kit from Qiagen from three mice from each of four genotypes: aP2-Cre transgenic littermates (controls for AG4KO mice), AG4KO mice; FVB littermates (controls for AG4OX) and AG4OX. RNA from each mouse was hybridized on an Affymetrix MG-U74-A.v2 Genechip microarray. Genome-wide expression analysis of the microarray data was performed using gene set enrichment analysis (GSEA) software version 2.05 available from the Broad Institute (http://www.broadinstitute.org/gsea/downloads.jsp) {Subramanian, 2005 #59}. The version of the gene set database used was s2.mgu74av2.gmt. The gene set comprising the BCAA oxidation enzymes in this gene set database is entitled: "MAP00280_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION."

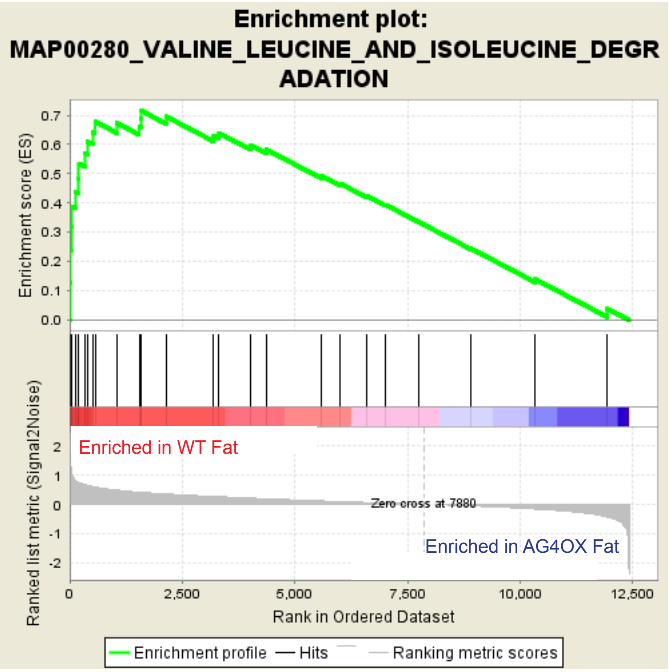
The analysis demonstrated the coordinate down-regulation of BCAA enzymes in AG4OX (Supplmental Figure 1A) adipose tissue and the coordinate upregulation of BCAA enzymes in AG4KO (Supplmental Figure 1B) adipose tissue. In the top panel of figures 1A and 1B, the Affymetrix Probe number, Gene Symbols, and Gene Names are listed for each gene included in BCAA oxidation gene set. 'Rank in Gene List' refers to the position of the gene in the ranked list of all genes present in our expression dataset. The 'Rank Metric Score' is the signal to noise ratio for each gene used to position the gene in the ranked list. The GSEA algorithm calculates an enrichment score reflecting the degree to which the genes included in a gene set are overrepresented at the top or bottom of the ranked list of all genes present in the expression dataset. The 'Running ES' is the enrichment score for this set at this point in the ranked list of genes. The final enrichment score for a set is the maximum running ES, or the maximum deviation from zero encountered for that set (as illustrated in the lower panel). A positive ES indicates gene set enrichment at the top of the ranked list; a negative ES indicates gene set enrichment at the bottom of the ranked list.

The Bottom panel of figures 1A and 1B provides a graphical representation of the 'Running ES' (top portion), the location of the BCAA genes in the ranked set of all genes (middle portion), and the distribution of the rank metric score across all genes present in the expression dataset (bottom portion).

Supplemental Figure 1A

Gene Set Enrichment Analysis of BCAA Oxidation Enzymes in AG4OX Adipose Tissue

AFFYMETRIX PROBE	GENE SYMBOL	GENE NAME	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES
92845_at	OXCT1	3-oxoacid CoA transferase 1	4	1.91	0.13
94940_at	MCCC1	methylcrotonoyl-Coenzyme A carboxylase 1 (alpha)	12	1.64	0.24
94325_at	HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	30	1.23	0.32
104153_at	IVD	isovaleryl coenzyme A dehydrogenase	52	1.04	0.39
161997_f_at	NULL	NULL	129	0.83	0.44
96058_s_at	ALDH2	aldehyde dehydrogenase 2, mitochondrial	196	0.77	0.48
96057_at	ALDH2	aldehyde dehydrogenase 2, mitochondrial	204	0.76	0.54
99571_at	ACAA1B	acetyl-Coenzyme A acyltransferase 1B	346	0.68	0.57
160128_at	PCCB	propionyl Coenzyme A carboxylase, beta polypeptide	402	0.65	0.61
160482_at	ACAA1A	acetyl-Coenzyme A acyltransferase 1A	523	0.61	0.64
95426_at	ECHS1	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	561	0.60	0.68
97316_at	EHHADH	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	1041	0.50	0.67
102302_at	BCKDHB	branched chain ketoacid dehydrogenase E1, beta polypeptide	1539	0.43	0.66
94324_f_at	HMGCL	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	1584	0.42	0.69
102004_at	ACAA1B	acetyl-Coenzyme A acyltransferase 1B	1595	0.42	0.71
92581_at	ACADM	acyl-Coenzyme A dehydrogenase, medium chain	2141	0.37	0.70
92478_at	STAG1	stromal antigen 1	3193	0.28	0.63
99613_at	MUT	methylmalonyl-Coenzyme A mutase	3315	0.28	0.64
161970_f_at	HMGCL	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	4025	0.22	0.60
103401_at	ACADS	acyl-Coenzyme A dehydrogenase, short chain	4371	0.20	0.58
161401_f_at	ALDH3A2	aldehyde dehydrogenase family 3, subfamily A2	5592	0.13	0.49
92590_at	HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	5997	0.11	0.47
161469_r_at	ACADS	acyl-Coenzyme A dehydrogenase, short chain	6589	0.07	0.42
99559_at	ALDH3A2	aldehyde dehydrogenase family 3, subfamily A2	7013	0.05	0.39
104011_at	AOX1	aldehyde oxidase 1	7743	0.01	0.34
96913_at	HADHB /// LOC623031	droxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	8895	-0.04	0.25
161956_at	ALDH1A1	aldehyde dehydrogenase family 1, subfamily A1	10340	-0.15	0.14
100068_at	ALDH1A1	aldehyde dehydrogenase family 1, subfamily A1	11938	-0.42	0.04



Supplemental Figure 1B

Gene Set Enrichment Analysis of BCAA Oxidation Enzymes in AG4KO Adipose Tissue

AFFYMETRIX PROBE	GENE SYMBOL	GENE NAME	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES
161970_f_at	HMGCL	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	880	0.36	-0.04
161469_r_at	ACADS	acyl-Coenzyme A dehydrogenase, short chain	3240	0.12	-0.22
161401_f_at	ALDH3A2	aldehyde dehydrogenase family 3, subfamily A2	3900	0.07	-0.27
100068_at	ALDH1A1	aldehyde dehydrogenase family 1, subfamily A1	4349	0.05	-0.30
92478_at	STAG1	stromal antigen 1	5902	-0.04	-0.42
96058_s_at	ALDH2	aldehyde dehydrogenase 2, mitochondrial	7006	-0.10	-0.50
161997_f_at	NULL	NULL	7457	-0.12	-0.52
96057_at	ALDH2	aldehyde dehydrogenase 2, mitochondrial	7848	-0.14	-0.54
161956_at	ALDH1A1	aldehyde dehydrogenase family 1, subfamily A1	7952	-0.15	-0.54
102302_at	BCKDHB	branched chain ketoacid dehydrogenase E1, beta polypeptide	8824	-0.21	-0.59
94324_f_at	HMGCL	3-hydroxy-3-methylglutaryl-Coenzyme A lyase	9430	-0.25	-0.62
160482_at	ACAA1A	acetyl-Coenzyme A acyltransferase 1A	9805	-0.28	-0.62
99559_at	ALDH3A2	aldehyde dehydrogenase family 3, subfamily A2	9870	-0.28	-0.60
95426_at	ECHS1	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	10632	-0.35	-0.63
103401_at	ACADS	acyl-Coenzyme A dehydrogenase, short chain	11380	-0.45	-0.66
96913_at	HADHB /// LOC623031	droxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	11587	-0.49	-0.63
99571_at	ACAA1B	acetyl-Coenzyme A acyltransferase 1B	11690	-0.51	-0.59
160128_at	PCCB	propionyl Coenzyme A carboxylase, beta polypeptide	11724	-0.52	-0.55
92590_at	HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	11885	-0.58	-0.51
94940_at	MCCC1	methylcrotonoyl-Coenzyme A carboxylase 1 (alpha)	12027	-0.63	-0.47
94325_at	HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	12064	-0.65	-0.42
97316_at	EHHADH	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	12084	-0.66	-0.36
99613_at	MUT	methylmalonyl-Coenzyme A mutase	12090	-0.66	-0.30
92581_at	ACADM	acyl-Coenzyme A dehydrogenase, medium chain	12123	-0.68	-0.25
92845_at	OXCT1	3-oxoacid CoA transferase 1	12150	-0.69	-0.19
104153_at	IVD	isovaleryl coenzyme A dehydrogenase	12216	-0.75	-0.13
102004_at	ACAA1B	acetyl-Coenzyme A acyltransferase 1B	12242	-0.77	-0.06
104011_at	AOX1	aldehyde oxidase 1	12377	-0.94	0.01