Supplementary Materials

Phenylbutyrate Therapy for Pyruvate Dehydrogenase Complex Deficiency and Lactic Acidosis

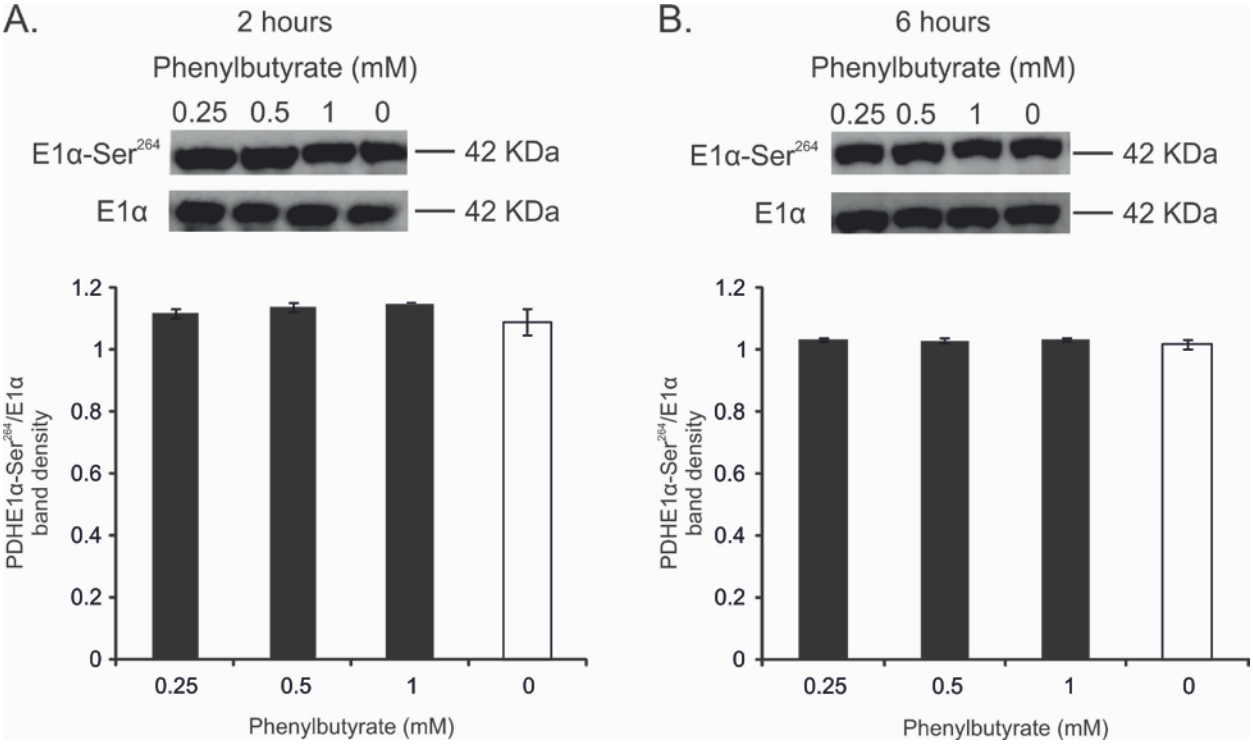

Fig. S1. PDHC phosphorylation after phenylbutyrate incubation for 2 and 6 hours in human wild-type fibroblasts. Western blotting of BA1054 fibroblasts treated with 0.25 mM, 0.5 mM, or 1 mM of phenylbutyrate or untreated, for 2 hours (**A**) or 6 hours (**B**). The average band intensities of phosphorylated E1 normalized for total E1 from two independent experiments are shown.

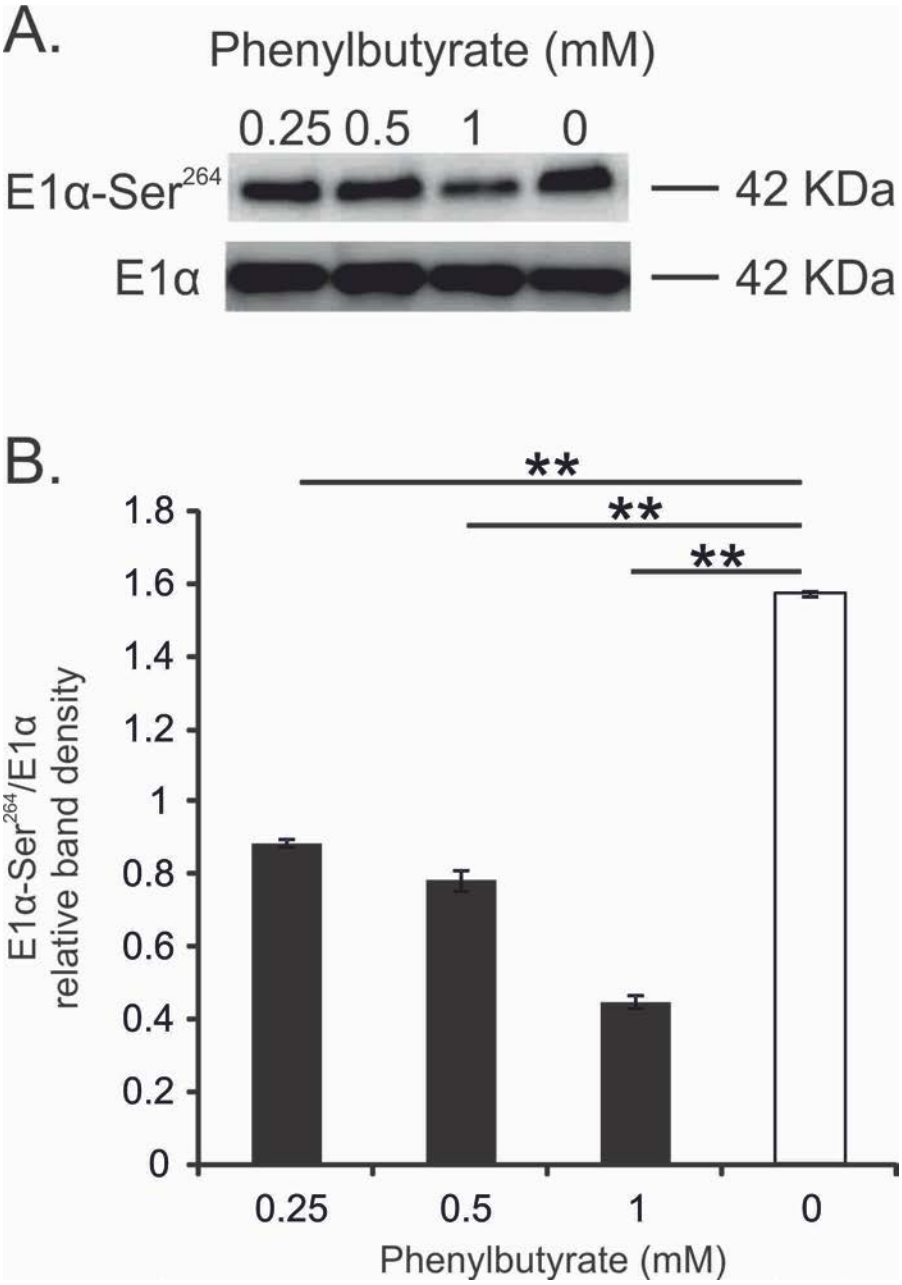

Fig. S2. PDHC phosphorylation after drug treatment of human wild-type fibroblasts. A. Western blotting of BA1020 fibroblasts treated with 0.25 mM, 0.5 mM, or 1 mM of phenylbutyrate for 24 hours or untreated. The images shown in (**A**) are representative of two independent experiments. **B.** The average band intensities of phosphorylated E1 normalized for total E1 from two independent experiments are shown. **: $p<0.01$.

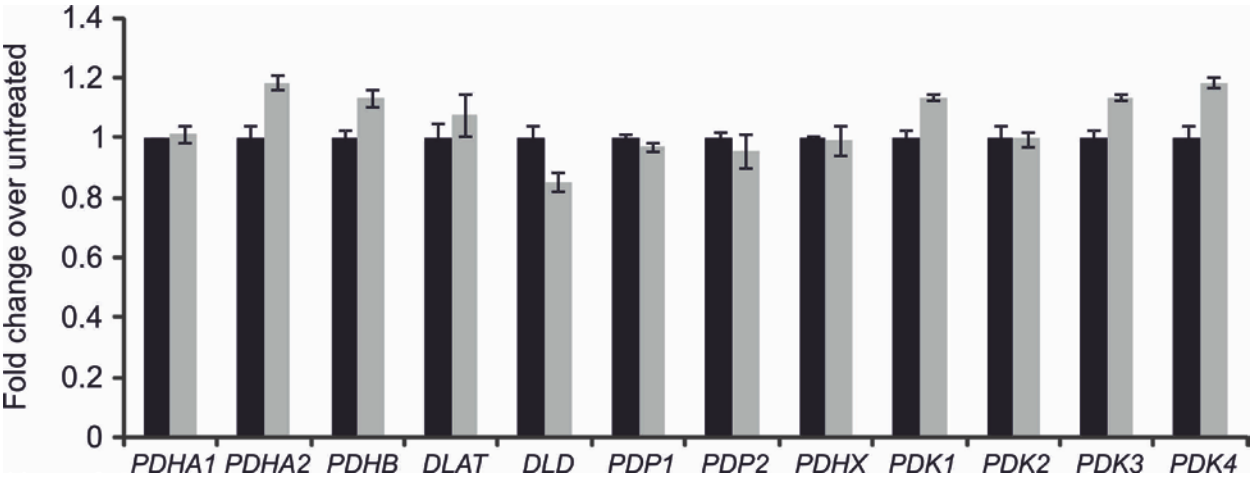

Fig. S3. **Expression of PDHC subunits and regulatory enzymes in fibroblasts.** RNA concentrations of the subunits (*PDHA1*, *PDHA2*, *PDHB*, *DLAT*, *DLD*, *PDHX*), kinases (*PDK1*, *PDK2*, *PDK3*, *PDK4*), and phosphatases (*PDP1* and *PDP2*) of PDHC in untreated (black bars) vs. phenylbutyrate-treated (gray bars) wild-type fibroblasts (n=3 per group). Means ± standard deviations are shown.

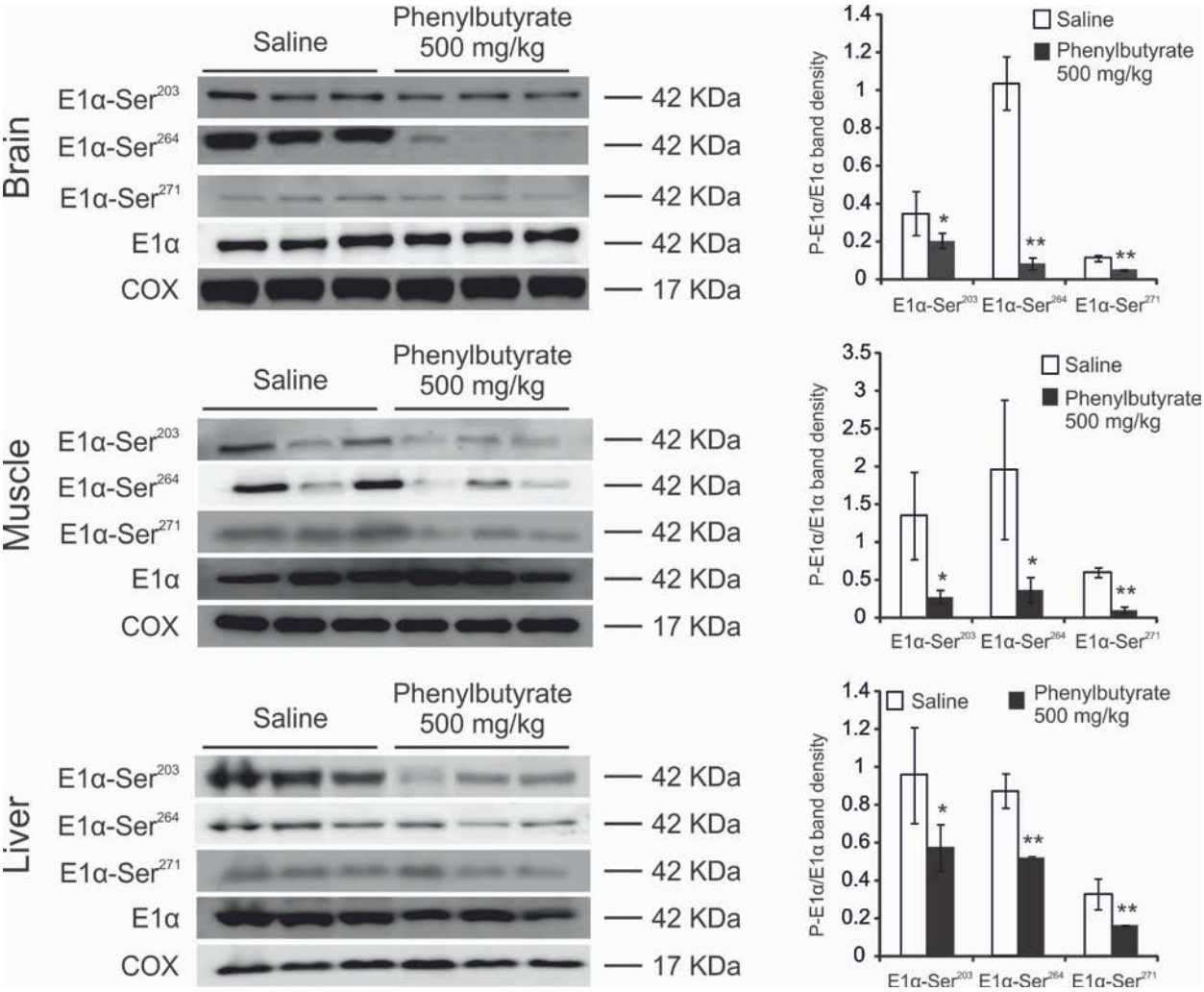

Fig. S4. PDHC phosphorylation status in mouse tissues. Western blot analyses of brain, muscle, and liver mitochondrial extract were performed with antibodies against the phosphorylated form of E1α. Three representative mice treated with 500 mg/kg/day of phenylbutyrate and another three treated with saline are shown. Each lane corresponds to the brain, muscle, or liver mitochondrial extract from an independent mouse. The phosphorylated form of E1α is significantly reduced in phenylbutyrate-treated mice as compared to the saline-treated mice. The amount of total E1 protein and COX proteins was similar in the two groups.

The graphs show densitometric quantification of n=5 mice from each treatment group. Means ± standard deviations are shown. *$p<0.05$; **$p<0.01$.

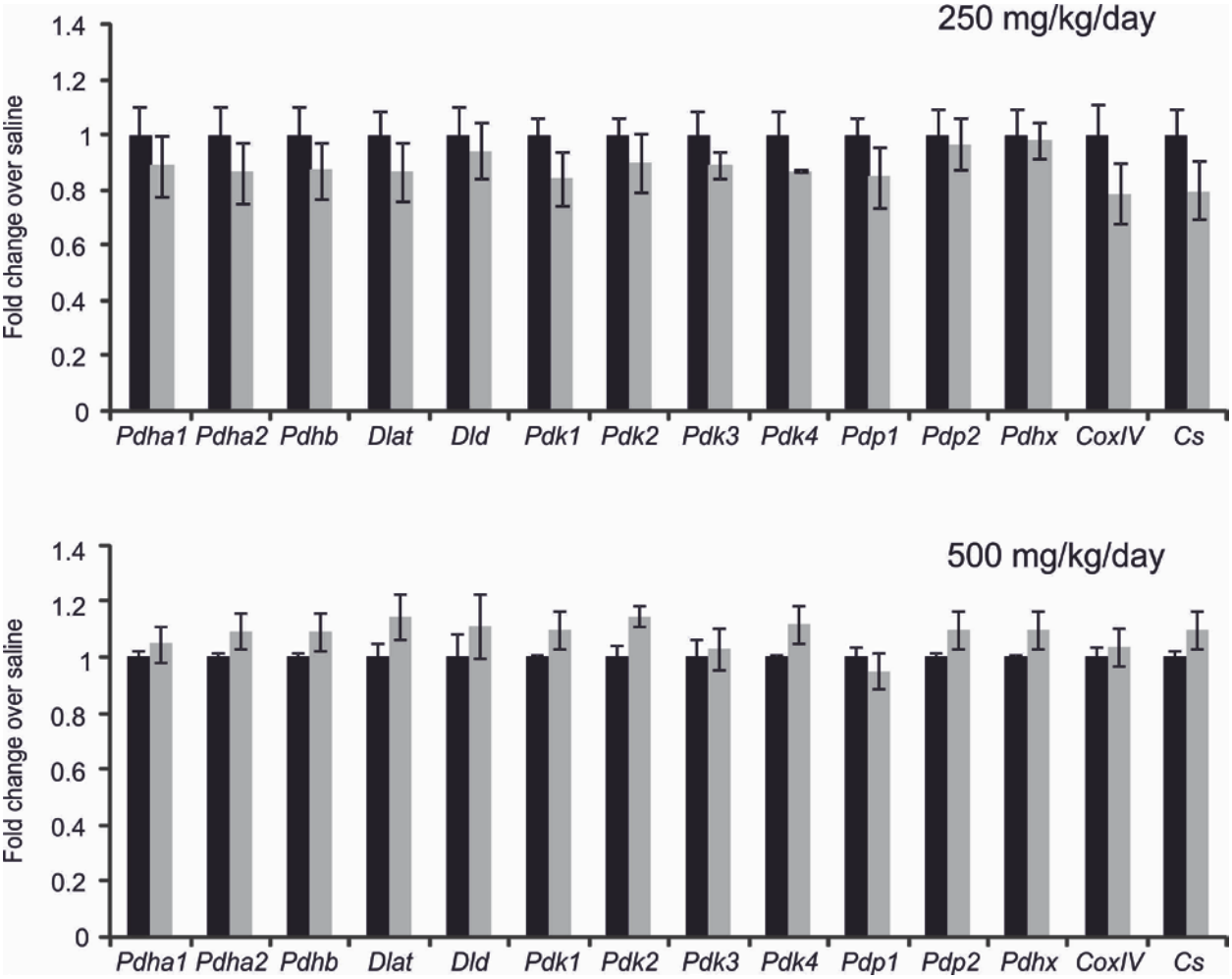

Fig. S5. Expression of PDHC subunits and regulatory enzymes in brain. RNA concentrations of the subunits (*Pdha1*, *Pdha2*, *Pdhb*, *Dlat*, *Dld*, *Pdhx*), kinases (*Pdk1*, *Pdk2*, *Pdk3*, *Pdk4*), and phosphatases (*Pdp1* and *Pdp2*) of PDHC, *CoxIV*, and citrate synthase (*Cs*) in the brains of mice treated with 250 or 500 mg/kg/day of phenylbutyrate or with saline (n=5 per group). Means ± standard deviations are shown.

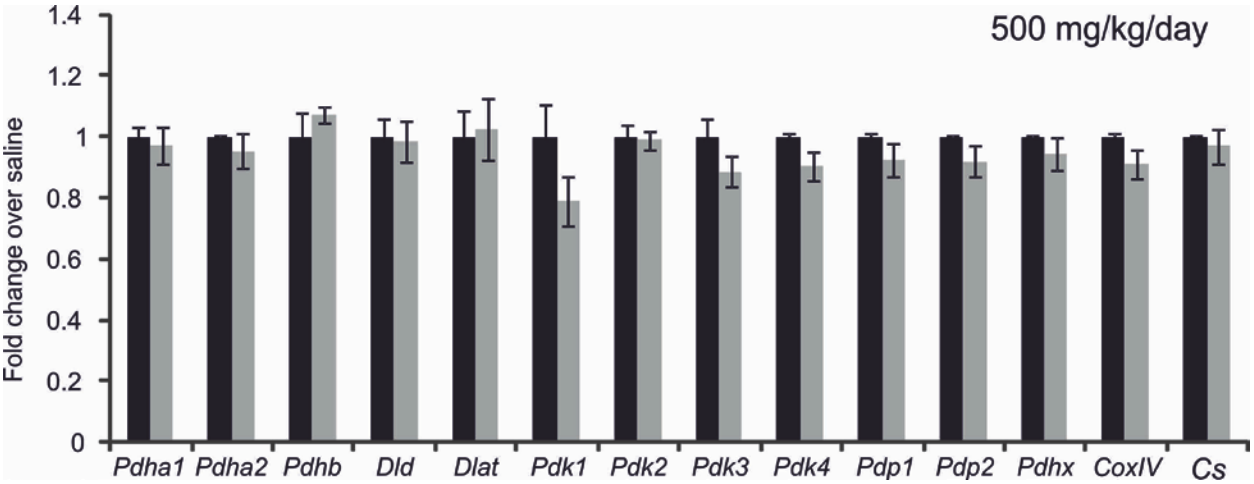

Fig. S6. Expression of PDHC subunits and regulatory enzymes in muscle. RNA concentrations of the subunits (*Pdha1*, *Pdha2*, *Pdhb*, *Dlat*, *Dld*, *Pdhx*), kinases (*Pdk1*, *Pdk2*, *Pdk3*, *Pdk4*), and phosphatases (*Pdp1* and *Pdp2*) of PDHC, *CoxIV*, and citrate synthase (*Cs*) in muscles of mice treated with 500 mg/kg/day of phenylbutyrate or with saline (n=5 per group). Means ± standard deviations are shown.

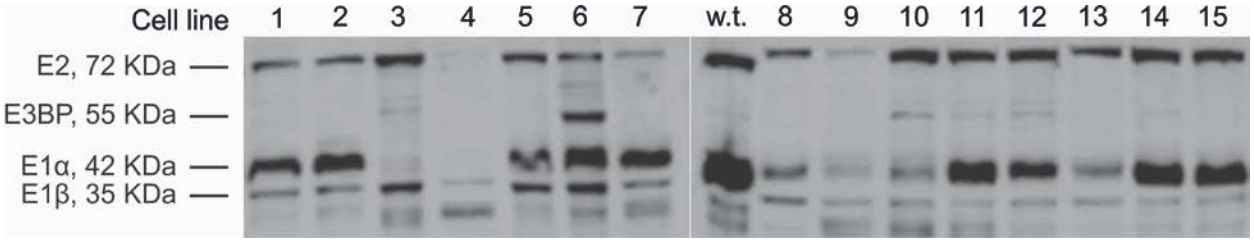

Figure S7. Western blot for PDHC subunits in PDHC-deficient cells. The expression of E1α, E1β, E2, and E3BP was assessed in PDHC-deficient patient cells using a cocktail of antibodies against the components of the complex. Cell line 3 harboring missense mutation p.R349H and cell line 4 with nonsense mutation p.R275* mutation showed little or no expression of the E1α protein and failed to respond to phenylbutyrate.

Supplementary Table 1. Serum concentrations of phenylbutyrate and phenylacetate in mice

	Phenylacetate (µmol/l)	Phenylbutyrate (µmol/l)
Saline	Undetectable	Undetectable
250 mg/kg/day	Undetectable	Undetectable
500 mg/kg/day	5.26±2.3	0.9±2.0

Supplementary Table 2. Primers for real time PCR in human fibroblast RNA

Primer	Sequence
PDHA1_F	CGCAGAGCTTACAGGACGAA
PDHA1_R	CCATTGCCCCCGTAGAAGT
PDHA2_F	GGCGGAGGGGCTTAAATACT
PDHA2_R	AAACCGCGAATGAATTTCTG
PDHB_F	GGGGCATACAAGGTTAGTCG
PDHB_R	ATTCCAGCAAAGCCCATCTC
PDK1_F	CCGCTCTCCATGAAGCAGTT
PDK1_R	TTGCCGCAGAAACATAAATGAG
PDK2_F	GATCCAGCAATGCCTGTGAG
PDK2_R	CGGGAAGCAGGTTGATCTC
PDK3_F	CAAGCAGATCGAGCGCTACTC
PDK3_R	CGAAGTCCAGGAATTGTTTGATG
PDK4_F	CCCGAGAGGTGGAGCATTT
PDK4_F	GCATTTTCTGAACCAAAGTCCAGTA
DLAT_F	TCCAACTCCCCAGCCTTTAG
DLAT_R	GCAAGAGGGCTAACAAACACC
DLD_F	AAAATGCAGAGCTGGAGTCG
DLD_R	TGCAGAAAGTCCCTGTAGGC
PDP1_F	CTGCCACTGTTCTCTGATGC
PDP1_R	CATGCAGTGCCATAGATCCTG
PDP2_F	GCAAATGTGTTCCTTCAGCA
PDP2_R	CCGCAGTTGTGGCACTGT
PDHX_F	CGGGGTGATCCCATTAAGAT
PDHX_R	AATGCATCTCCAGCACTCAC

Supplementary Table 3. Primers for real time PCR in RNA from mouse tissues

Primer	Sequence
Pdk1_F	TCCTGTCACCAGCCAAAATG
Pdk1_R	CCACCGAACAATAAGGAGTGC
Pdk2_F	TGGTGCAGAGCTGGTATGTC
Pdk2_R	GGCATCTGTGAACTGGCTTAG
Pdk3_F	CCTGGACTTCGGAAGGGATA
Pdk3_R	CTCATGGTGTTAGCCAGTCG
Pdk4_F	CAGCTGGTGAAGAGCTGGTA
Pdk4_R	CTCTGACAGGGCTTTCTGGT
Pdha1_F	AAGATGCTTGCCGCTGTATC
Pdha1_R	ATTTGCAAAATTACGGGAAGC
Pdha2_F	GTTGTGCCTCGCGTTTCTC
Pdha2_R	CCTCTGAGAGCTGGCTTTTG
Pdhb_F	GGAGGGAATTGAATGTGAGG
Pdhb_R	CCACAGTCACGAGATGATTTG
Pdhx_F	AGCAAGTTGGAGGTGGTTTC
Pdhx_R	GTTCCCTTGCTCCATCGTAG
Dlat_F	AAGTTGGCAGCAGAGAAAGG
Dlat_R	GGCACAAAAGAGTCAATGTCC
Dld_F	TCATGGCCTACAAGGAGTTTC
Dld_R	ATATCCTCCAGGGCCAGAAC
Pdp1_F	GGAGAAGTGCAGCAACCTG
Pdp1_R	ACTGACTGCCTGGGAACAAG
Pdp2_F	ACGAGGATACGAGGCTGAAA
Pdp2_R	CGATTCCTTGCAGAATTGAAG
CoxIV_F	GTGGCAGAATGTTGGCTTC
CoxIV_R	TTCACAACACTCCCATGTGC
Cs_F	CTCTCCCTTCGGTCCCTTC

Cs_R	CGAGGCAGGATGAGTTCTTG
$\beta_2$microglobulin_F	TGGTGCTTGTCTCACTGACC
$\beta_2$microglobulin_R	GTATGTTCGGCTTCCCATTC