

1 **Supplementary Information**

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3 Muscle-specific deletion of BDK amplifies loss of myofibrillar protein during protein undernutrition

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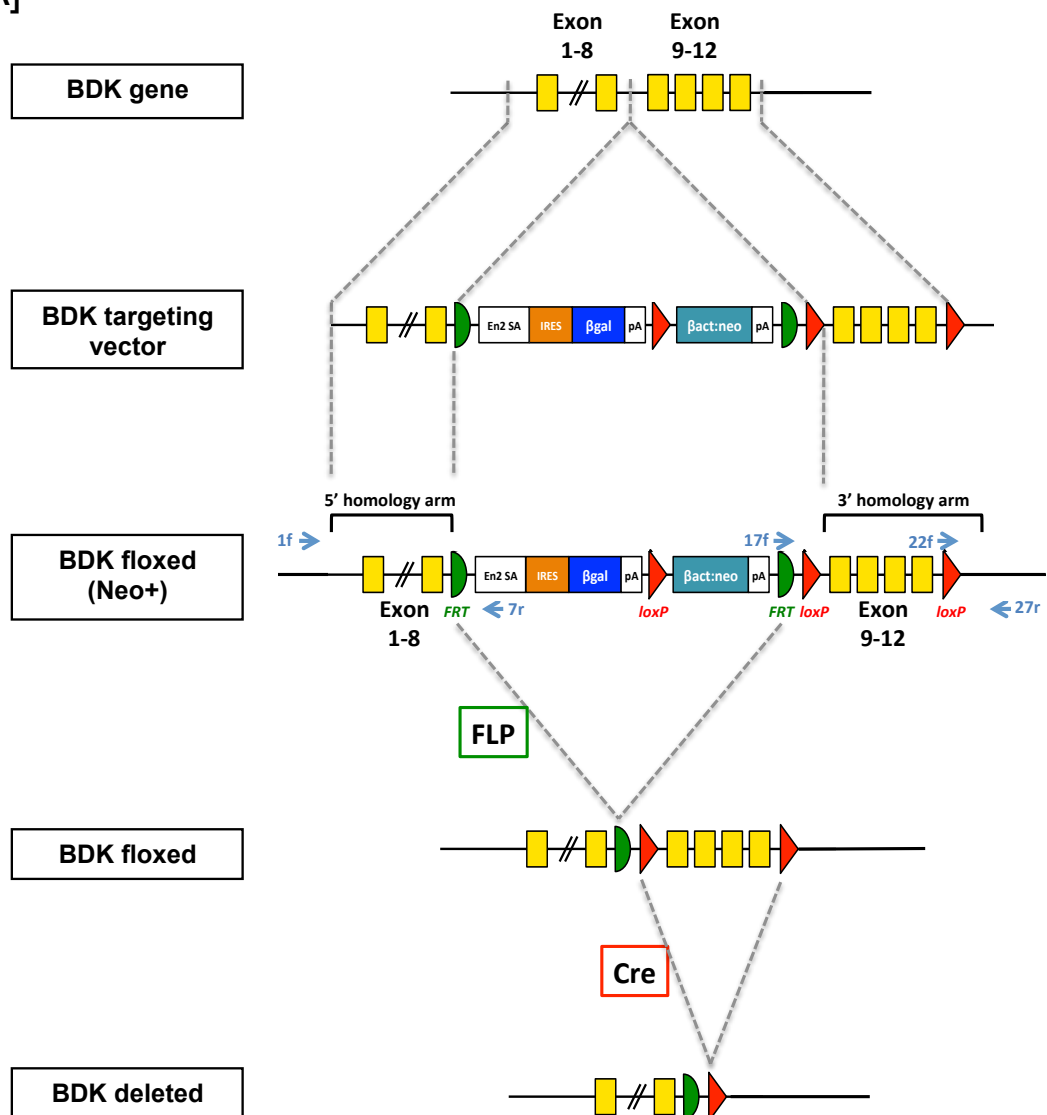
5 Takuya Ishikawa, Yasuyuki Kitaura, Yoshihiro Kadota, Yukako Morishita, Miki Ota, Fumiya

6 Yamanaka, Minjun Xu, Masato Ikawa, Naokazu Inoue, Fuminori Kawano, Naoya Nakai, Taro

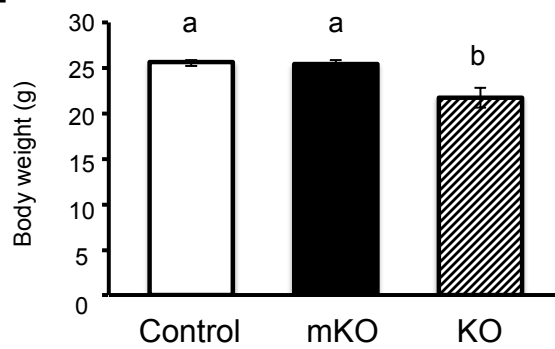
7 Murakami, Shinji Miura, Yukino Hatazawa, Yasutomi Kamei, Yoshiharu Shimomura

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[A]



[B]



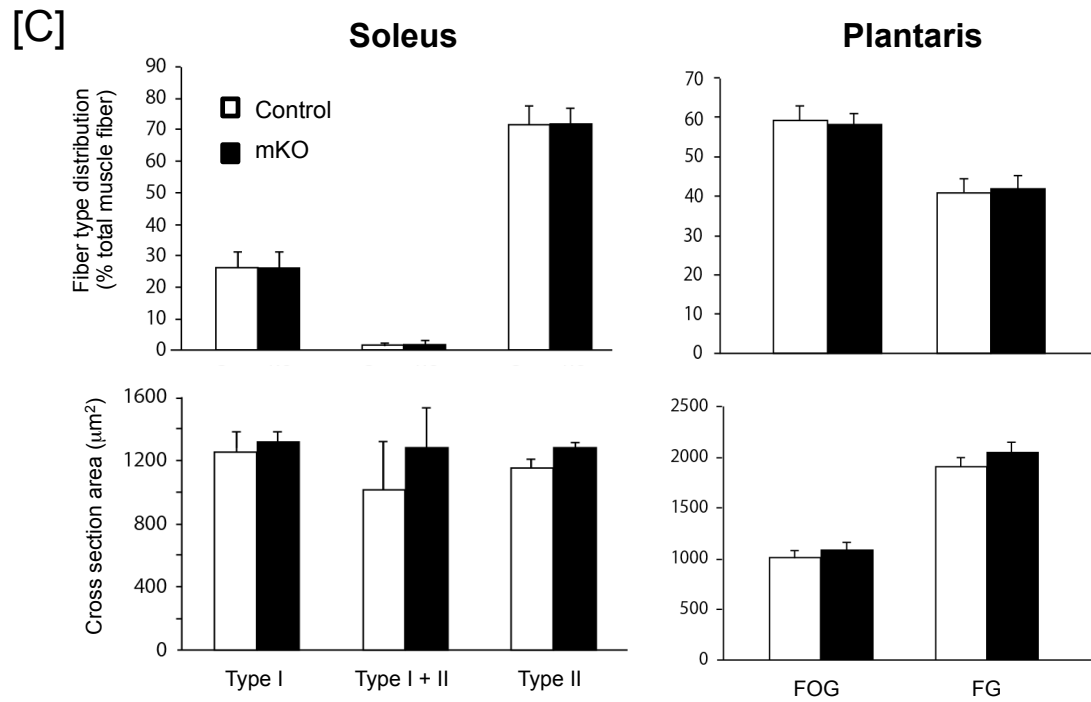
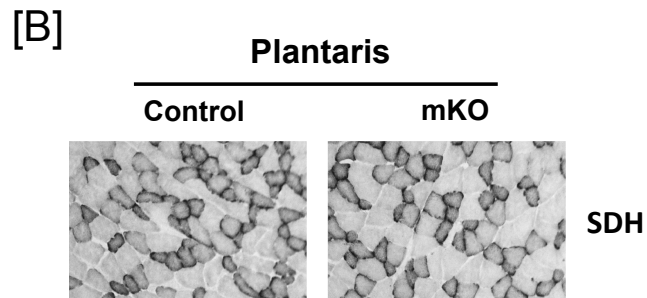
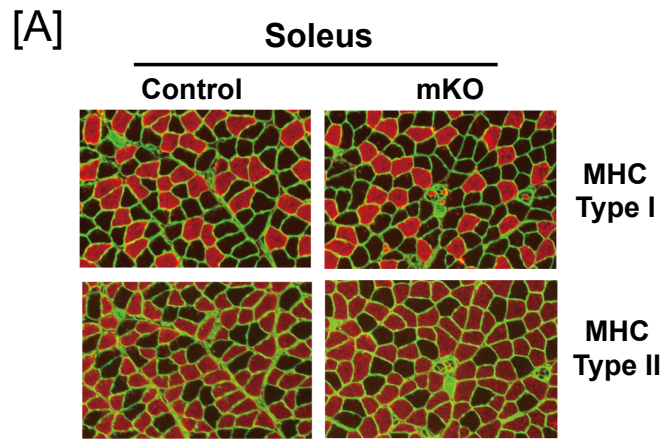
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11 **Supplemental Figure S1. Generating BDK targeted mice**

12 [A] The targeting vector for the *BDK* allele. Schematic of the targeting vector for *BDK* allele with a
13 *FRT*-flanked β gal/neo cassette integrated upstream of exon 9 and the floxed exons 9-12. En2SA,
14 splicing acceptor site; IRES, internal ribosomal entry site; β gal, β -galactosidase; β act:neo,
15 β -actin-promotor followed by neomycin-resistant gene; pA, poly A sequence. The arrows show
16 primers for PCR for genotyping. [B] Body weight of control, BDK-mKO and BDK-KO mice fed
17 CE-2 (25% protein) at 10 weeks. Values are presented as mean \pm SE (n=8-12). Means without
18 common letters are significantly different ($P < 0.05$ by the Tukey-Kramer test).

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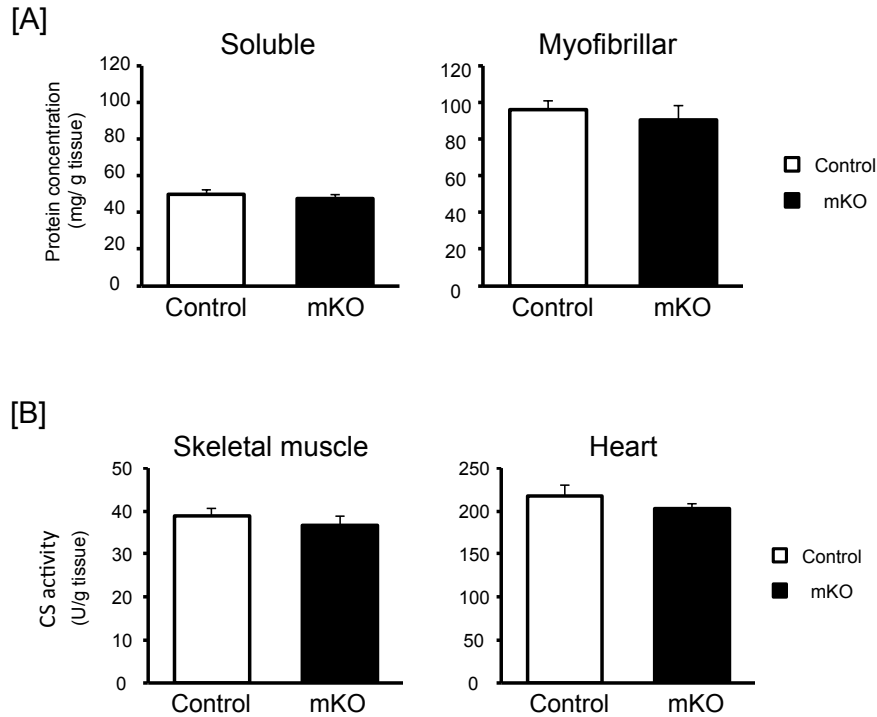
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23 **Supplemental Figure S2. Muscle fiber characteristics of control and BDK-mKO mice.**

24 [A] Tissue cross-sections immunohistochemically stained with type I or II myosin heavy chain
25 (MHC) (red) and laminin (green) to outline myofibers of soleus muscle from control and BDK-mKO
26 mice fed CE-2 (25% protein). [B] Tissue cross-sections stained for succinate dehydrogenase (SDH)
27 activity (dark gray/black) of plantaris muscle from control and BDK-mKO mice fed CE-2. [C] Fiber
28 type distribution and cross-sectional area of Type I, I+II or II fibers of soleus muscle in [A], and fast
29 twitch oxidative glycolytic (FOG) or fast twitch glycolytic (FG) fibers of plantaris muscle in [B].
30 Values are presented as mean \pm SE (n=3).



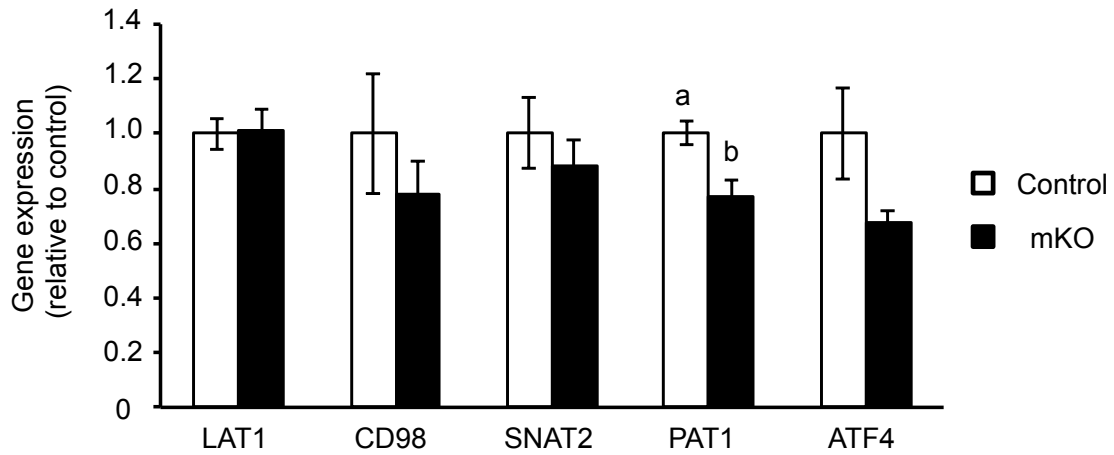
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33 **Supplemental Figure S3. Skeletal muscle protein concentrations and mitochondria enzyme**
 34 **activities in control and BDK-mKO mice.**

35 **[A]** The soluble and myofibrillar protein concentrations in skeletal muscle of 8h-fasted 10-week-old
 36 control and BDK-mKO mice fed CE-2 (25% protein). The protein concentrations are expressed as
 37 mg/g of tissue weight for skeletal muscle (gastrocnemius and plantaris muscle). **[B]** The activity of
 38 citrate synthase of skeletal muscle and heart in 10-week-old control and BDK-mKO mice fed CE-2.
 39 Values are presented as mean \pm SE (n=10-12).

40



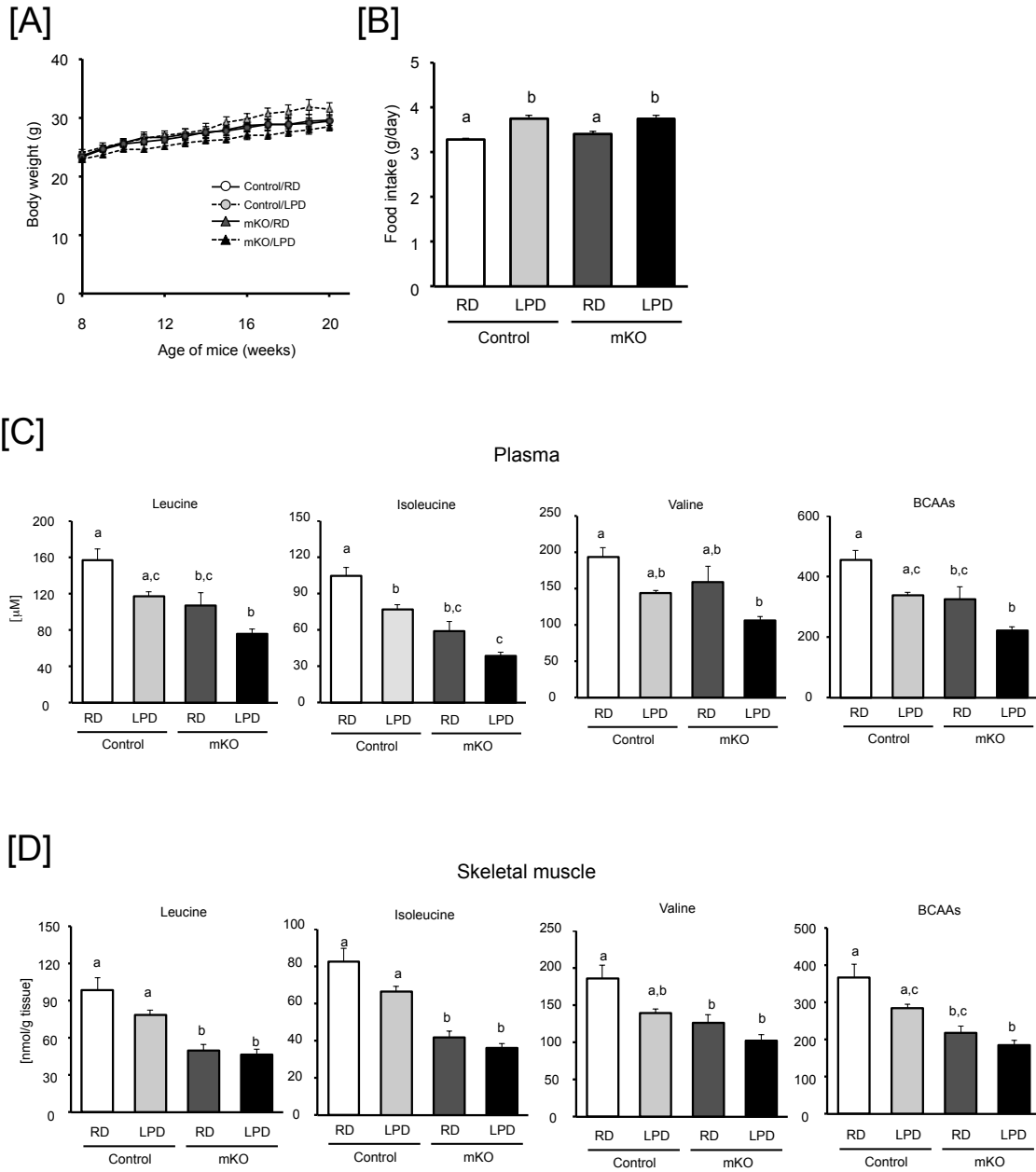
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43 **Supplemental Figure S4. The expression of mRNA encoding amino acid transporters in**
 44 **skeletal muscle from control and BDK-mKO mice.**

45 The gene expression of system L amino acid transporters (LAT1 and CD98), system A amino acid
 46 transporter (SNAT2), proton-assisted amino acid transporter (PAT1) and ATF4 in skeletal muscle of
 47 8h-fasted BDK-mKO mice fed CE-2 (25% protein) are expressed relative to those from control mice.

48 Values are presented as mean ± SE (n=4). Means without common letters are significantly different
 49 (P<0.05 by Student's t test).



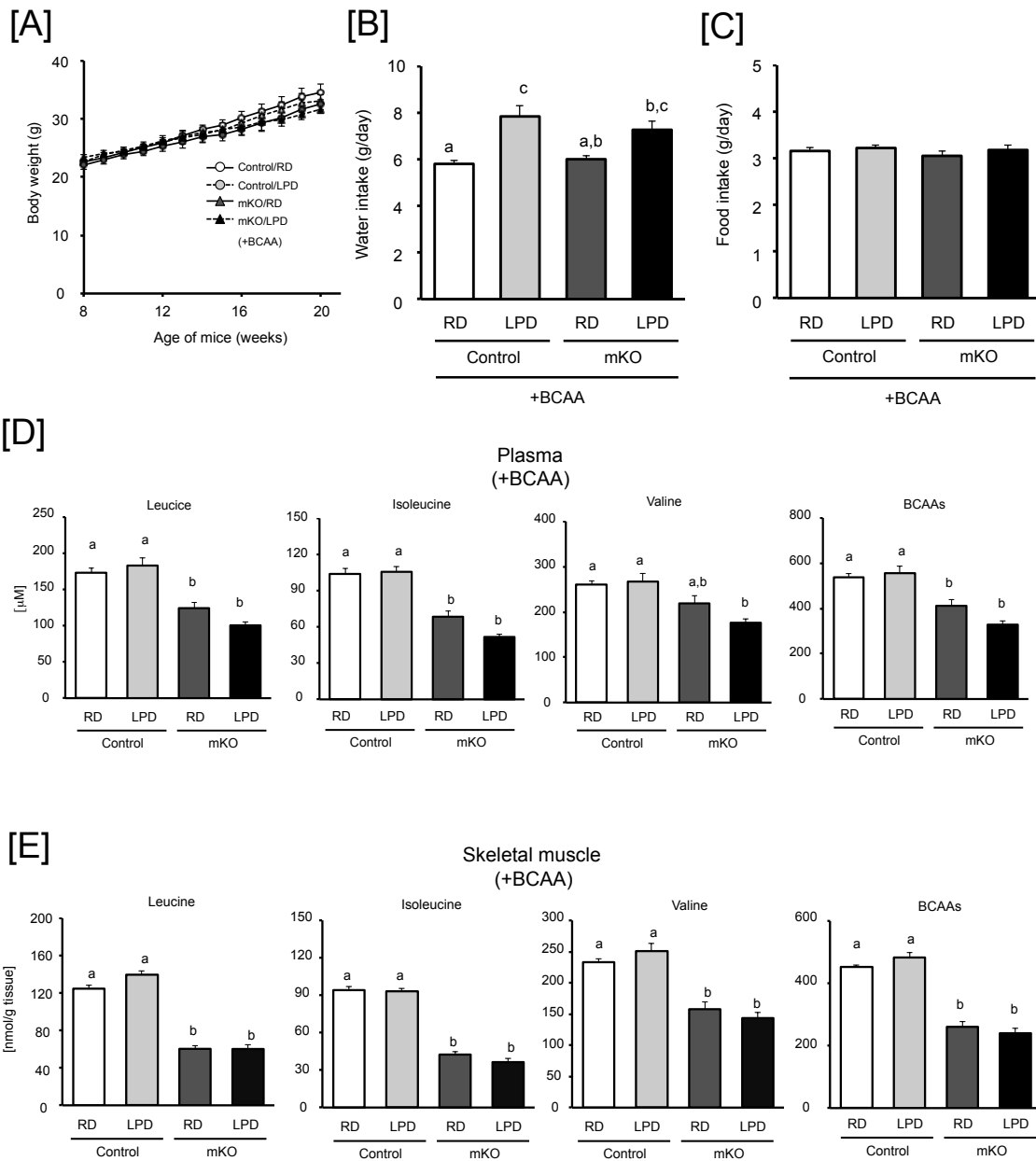
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52 **Supplemental Figure S5. Growth curves, food intake and BCAA concentrations of control and**
 53 **BDK-mKO mice fed RD or LPD for 12 weeks.**

54 Growth curves [A], food intake [B], concentrations of leucine, isoleucine, valine and BCAAs (2h
 55 fasted) in plasma [C] and skeletal muscle [D] of control and mKO mice fed regular protein diet (RD,
 56 20% protein) and low protein diet (LPD, 8% protein). Values are presented as mean ± SE (n=4-7).
 57 Means without common letters are significantly different (P<0.05 by the Tukey-Kramer test).

58



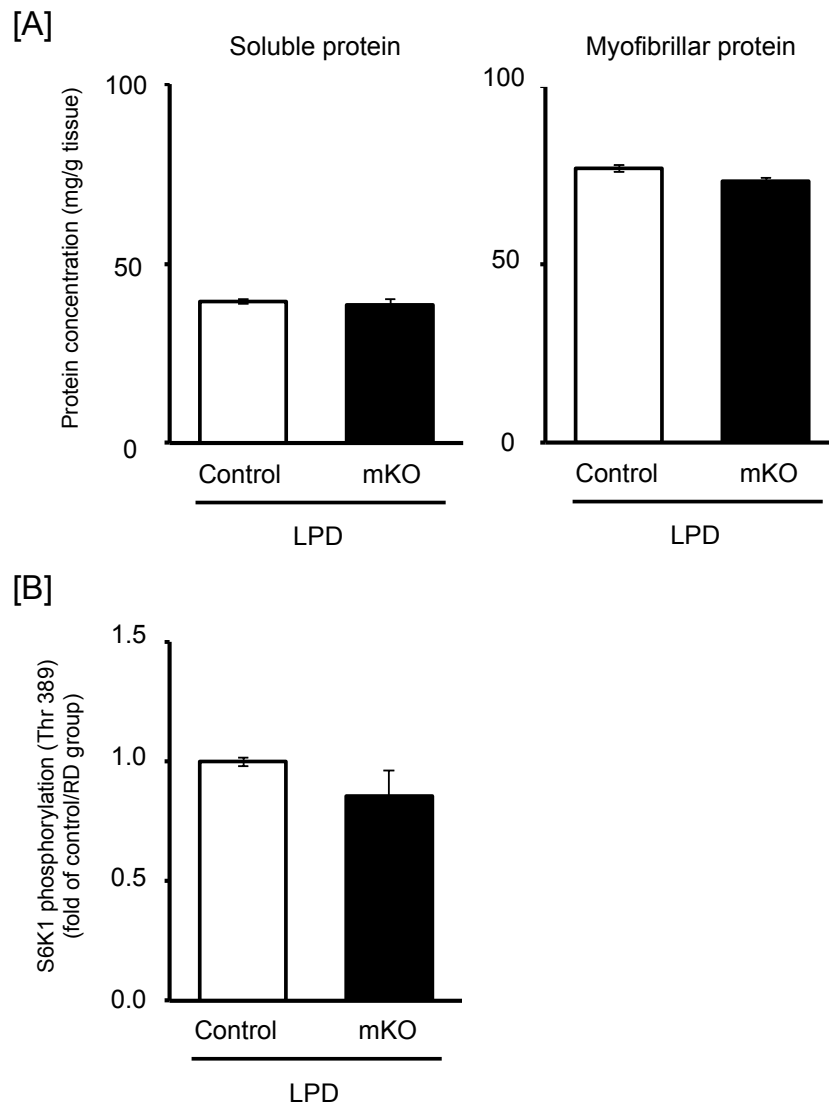
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61 **Supplemental Figure S6. Growth curves, food and water intake of control and mKO mice fed**
 62 **RD or LPD for 12 weeks with BCAA supplemented water.**

63 Growth curves [A], water intake [B], food intake [C] and concentrations of leucine, isoleucine,
 64 valine and BCAAs (2h-fasted) in plasma [D] and skeletal muscle [E] of control and mKO mice fed
 65 RD (20% protein) or LPD (8% protein) with BCAA supplemented water. Values are presented as
 66 mean ± SE (n=5-8). Means without common letters are significantly different (P<0.05 by the
 67 Tukey-Kramer test).

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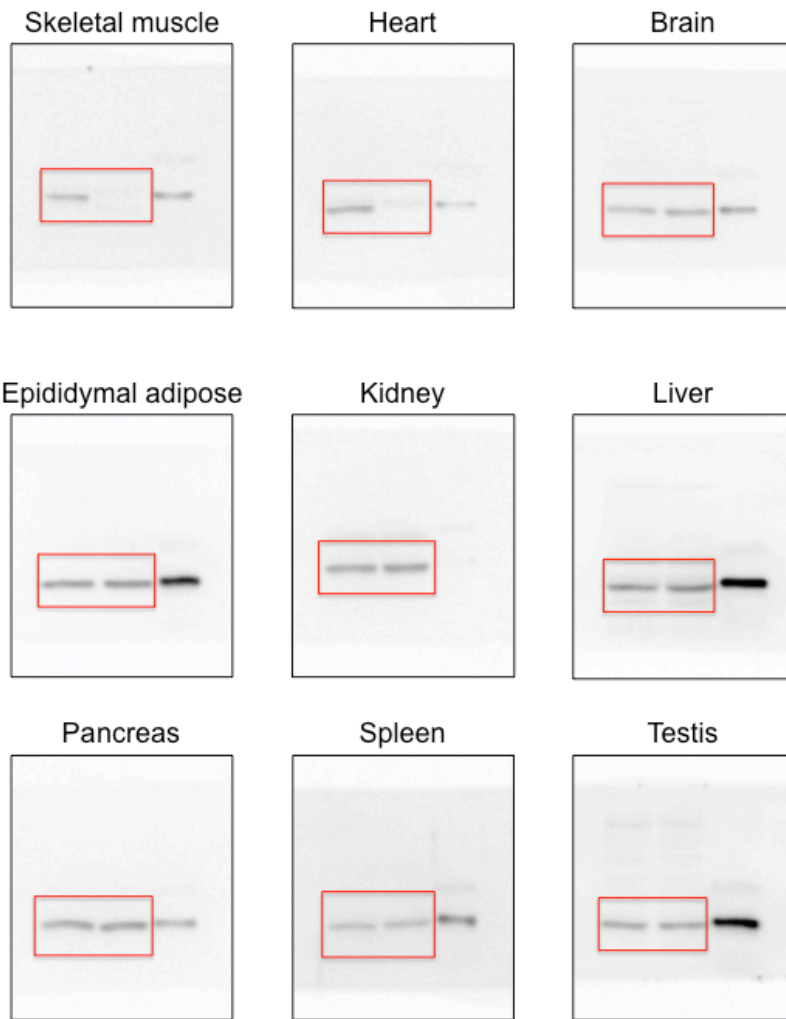


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70 **Supplemental Figure S7. Skeletal muscle protein concentrations and mTORC1 activity in**
 71 **control and BDK-mKO mice fed RD or LPD for 4 weeks.**

72 **[A]** Soluble and myofibrillar protein concentrations in skeletal muscle of 2 h-fasted control and
 73 BDK-mKO mice fed RD (20% protein) or LPD (8% protein) for 4 weeks. The protein
 74 concentrations are expressed as mg/g of tissue weight for skeletal muscle (gastrocnemius and
 75 plantaris muscle). **[B]** The activity of mTORC1 in skeletal muscle of 2 h-fasted control and
 76 BDK-mKO mice fed RD or LPD for 4 weeks. The phosphorylated levels of S6K1 and 4E-BP1 are
 77 expressed as in Fig. 2. The data are presented relative to the mean values of the control/RD group.
 78 Typical images of western blots are shown above each bar. Values are presented as mean \pm SE
 79 (n=4-7).

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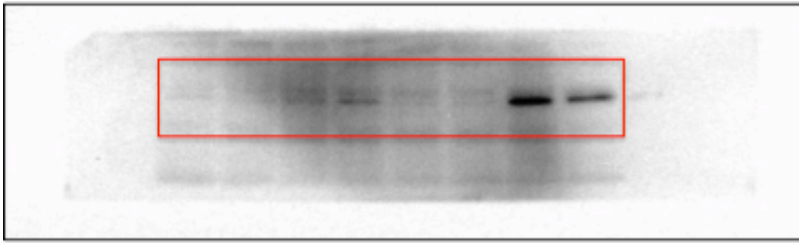
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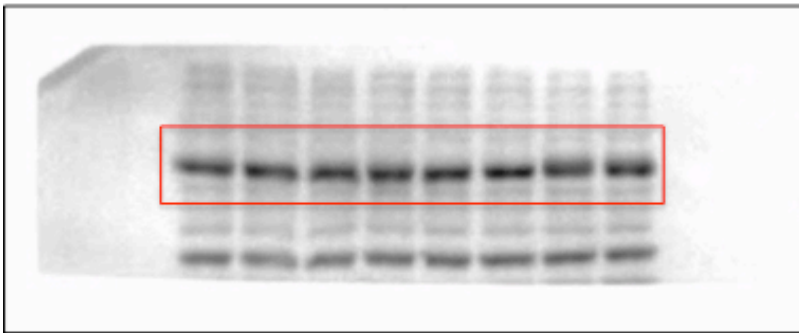
83 **Supplementary Figure S8. Full-length gels/blots of Figure 1 [A].**

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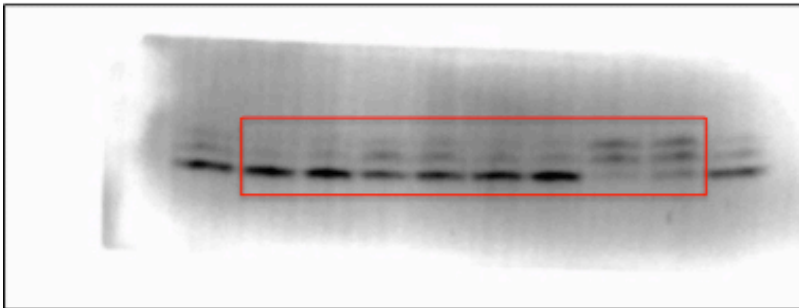
P-S6K1



S6K1



4E-BP1



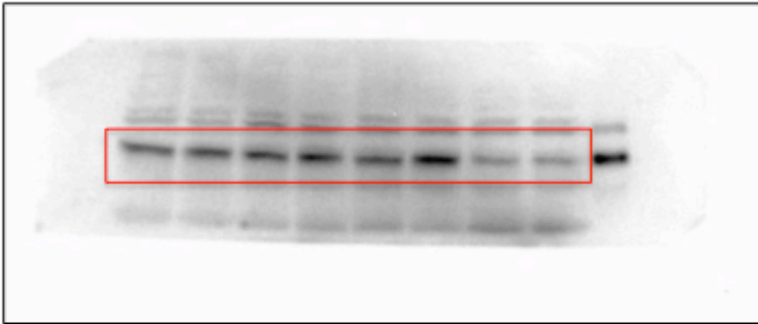
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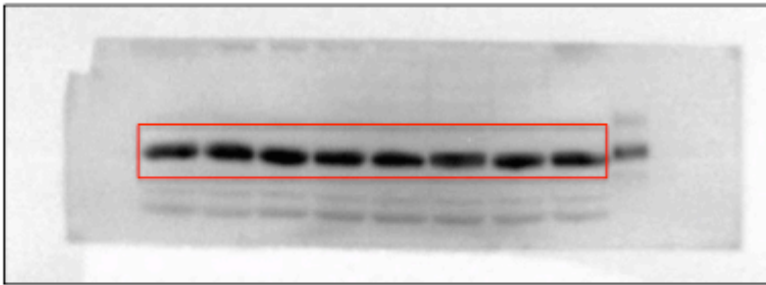
87 **Supplementary Figure S9. Full-length gels/blots of Figure 2.**

88

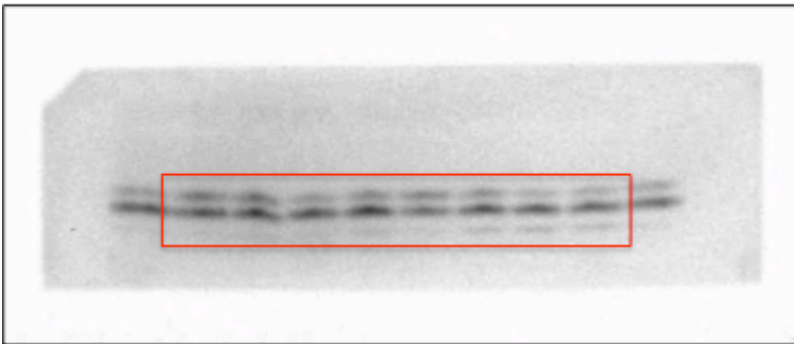
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S6K1



4E-BP1



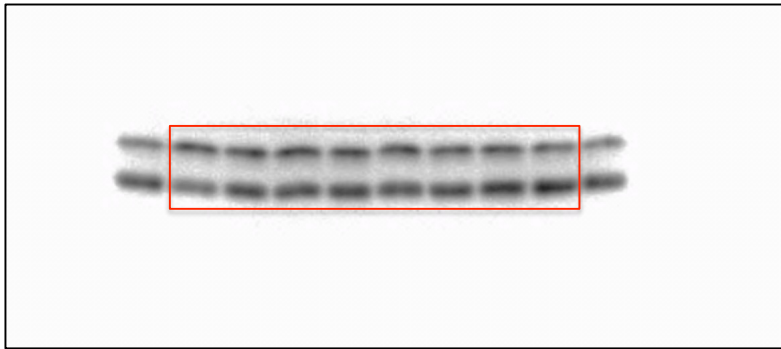
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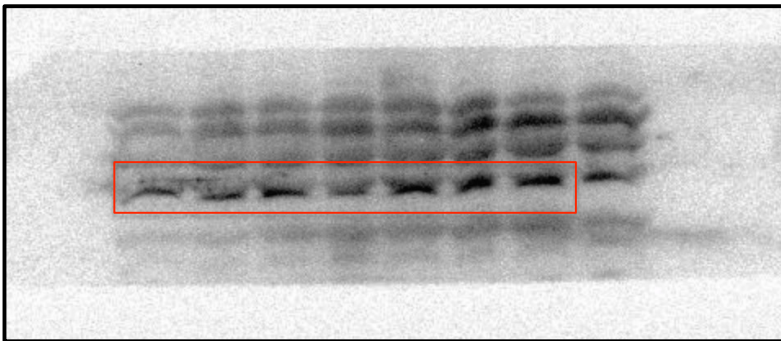
91 **Supplementary Figure S10. Full-length gels/blots of Figure 3[A] and [B].**

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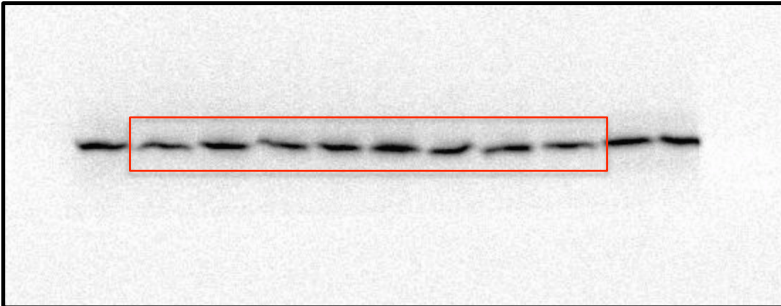
LC3



P-eIF2 α



eIF2 α



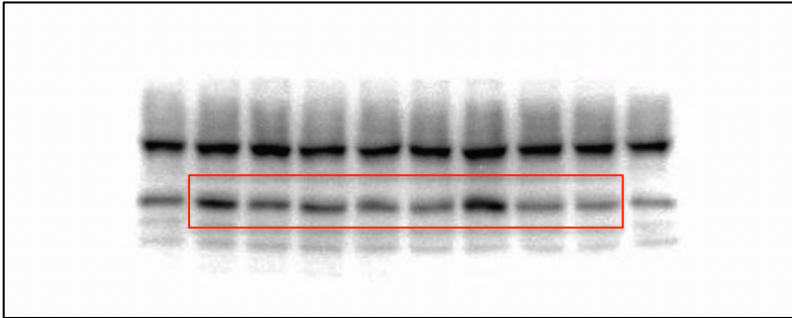
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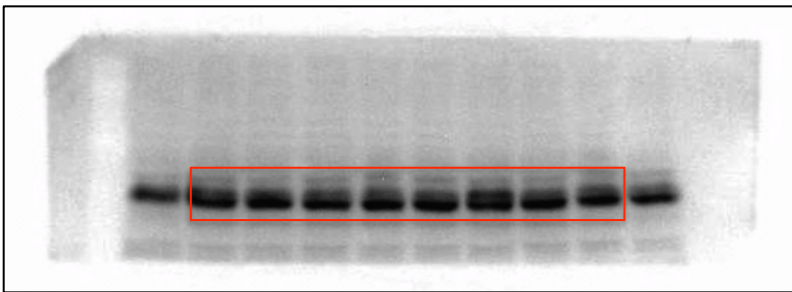
95 **Supplementary Figure S11. Full-length gels/blots of Figure 3[C] and [D].**

96

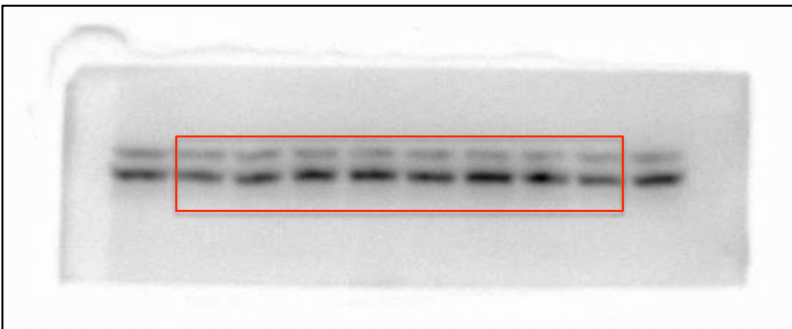
P-S6K1



S6K1



4E-BP1



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98

99 **Supplementary Figure S12. Full-length gels/blots of Figure 4.**

100

101 **Supplemental Table S1. List of genes up- or down-regulated in BDK-mKO mice by**
 102 **microarray.** Listed genes were up-regulated more than 2-fold or down-regulated less than half in
 103 extensor digitorum longus (EDL) and soleus from BDK-mKO mice compared to control mice fed
 104 CE-2 (25% protein) at freely-fed state.
 105

EDL

GeneSymbol	Description	mKO/Control
3300002I08Rik	Mus musculus RIKEN cDNA 3300002I08 gene (3300002I08Rik), mRNA [NM_027017]	8.77
Acyp2	Mus musculus acylphosphatase 2, muscle type (Acyp2), mRNA [NM_029344]	2.24
Angptl4	Mus musculus angiopoietin-like 4 (Angptl4), mRNA [NM_020581]	0.39
Egr1	Mus musculus early growth response 1 (Egr1), mRNA [NM_007913]	0.33
Zfp36l3	Mus musculus zinc finger protein 36, C3H type-like 3 (Zfp36l3), mRNA [NM_001009549]	0.29
Hat1	Mus musculus histone aminotransferase 1 (Hat1), mRNA [NM_026115]	0.22
Bckdk	Mus musculus branched chain ketoacid dehydrogenase kinase (Bckdk), mRNA [NM_009739]	0.03

Soleus

GeneSymbol	Description	mKO/Control
3300002I08Rik	Mus musculus RIKEN cDNA 3300002I08 gene (3300002I08Rik), mRNA [NM_027017]	3.75
Angptl4	Mus musculus angiopoietin-like 4 (Angptl4), mRNA [NM_020581]	0.40
Egr1	Mus musculus early growth response 1 (Egr1), mRNA [NM_007913]	0.35
Bckdk	Mus musculus branched chain ketoacid dehydrogenase kinase (Bckdk), mRNA [NM_009739]	0.07

106

107

108 **Supplemental Table S2. The concentrations of leucine, isoleucine, valine and total BCAAs in**
 109 **the plasma and skeletal muscle of control and BDK-mKO mice fed RD (20% protein) at 1 h**
 110 **after bolus ingestion of water or BCAAs.**

111 The concentrations are expressed as μM for plasma or nmol/g tissue for skeletal muscle. Values are
 112 presented as mean \pm SE (n=4-6). Means without common letters are significantly different ($P < 0.05$
 113 by the Tukey-Kramer test).

114

115 Plasma

	Control/water	Control/BCAAs	BDK-mKO/water	BDK-mKO/BCAAs
Leucine	183 \pm 19 ^a	1433 \pm 61 ^b	119 \pm 8 ^a	1203 \pm 74 ^c
Isoleucine	117 \pm 11 ^a	720 \pm 32 ^b	64 \pm 5 ^a	551 \pm 31 ^c
Valine	194 \pm 19 ^a	1674 \pm 41 ^b	148 \pm 10 ^a	1418 \pm 62 ^c
BCAAs	494 \pm 48 ^a	3827 \pm 128 ^b	330 \pm 23 ^a	3172 \pm 166 ^c

116

117 Skeletal muscle

	Control/water	Control/BCAAs	BDK-mKO/water	BDK-mKO/BCAAs
Leucine	159 \pm 28 ^a	1122 \pm 37 ^b	98 \pm 6 ^a	899 \pm 37 ^c
Isoleucine	125 \pm 19 ^a	589 \pm 20 ^b	73 \pm 4 ^a	450 \pm 19 ^c
Valine	183 \pm 34 ^a	1234 \pm 59 ^b	141 \pm 9 ^a	1073 \pm 34 ^b
BCAAs	467 \pm 81 ^a	2944 \pm 116 ^b	312 \pm 19 ^a	2422 \pm 90 ^b

118

119 **Supplemental Table S3. Skeletal muscle weights of control and BDK-mKO mice fed RD (20%**
 120 **protein) or LPD (8% protein) for 12 weeks.**

121 Weights of the Muscle R and Muscle L correspond to the sum of the gastrocnemius, plantaris and
 122 soleus, and the sum of gastrocnemius and plantaris, respectively. Values are presented as mean \pm SE
 123 (n=4-7).

124

125

	Relative weight [g/100 g BW]							
	Control/RD		Control/LPD		BDK-mKO/RD		BDK-mKO/LPD	
Muscle L	0.54	\pm 0.03	0.50	\pm 0.01	0.55	\pm 0.01	0.56	\pm 0.02
Muscle R	0.58	\pm 0.01	0.55	\pm 0.02	0.59	\pm 0.01	0.58	\pm 0.01
Soleus	0.03	\pm 0.00	0.03	\pm 0.00	0.03	\pm 0.00	0.03	\pm 0.00

126

127

128

129 **Supplemental Table S4 Skeletal muscle weights of control and BDK-mKO mice fed RD (20%**
 130 **protein) or LPD (8% protein) for 12 weeks with BCAA supplemented water.**

131 Weights of the Muscle R and Muscle L correspond to the sum of the gastrocnemius, plantaris and
 132 soleus, and the sum of gastrocnemius and plantaris, respectively. Values are presented as mean \pm SE
 133 (n=5-8).

134

	Relative weight [g/100 g BW]							
	Control/RD		Control/LPD		BDK-mKO/RD		BDK-mKO/LPD	
Muscle L	0.47	\pm 0.01	0.49	\pm 0.02	0.50	\pm 0.02	0.53	\pm 0.02
Muscle R	0.51	\pm 0.01	0.53	\pm 0.02	0.53	\pm 0.02	0.56	\pm 0.02
Soleus	0.03	\pm 0.00	0.03	\pm 0.00	0.03	\pm 0.00	0.03	\pm 0.00

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138 **Supplemental Table S5. The concentrations of amino acids other than BCAAs in the plasma**
 139 **and skeletal muscle of 2 h-fasted control and BDK-mKO mice fed RD (20% protein) or LPD**
 140 **(8% protein) for 12 weeks.**

141 The concentrations are expressed as μM for plasma or nmol/g tissue for skeletal muscle. Values are
 142 presented as mean \pm SE (n=5-8). Means without common letters are significantly different ($P < 0.05$)
 143 by the Tukey-Kramer test).

144

145 **Plasma**

	Control/RD	Control/LPD	BDK-mKO/RD	BDK-mKO/LPD
Alanine	465 \pm 74	527 \pm 46	521 \pm 50	582 \pm 31
Arginine	90 \pm 6	102 \pm 2	85 \pm 8	95 \pm 5
Asparagine	103 \pm 15	126 \pm 7	107 \pm 10	120 \pm 5
Aspartic acid	21 \pm 3 ^{a,b}	21 \pm 1 ^{a,b}	19 \pm 2 ^a	28 \pm 2 ^b
Glutamic acid	55 \pm 7	44 \pm 6	54 \pm 8	51 \pm 5
Glutamine	515 \pm 27 ^{a,b}	518 \pm 15 ^{a,b}	486 \pm 21 ^a	590 \pm 16 ^b
Glycine	255 \pm 15	273 \pm 15	257 \pm 15	241 \pm 8
Histidine	59 \pm 7	42 \pm 2	56 \pm 4	55 \pm 6
Lysine	286 \pm 24	257 \pm 7	315 \pm 30	292 \pm 9
Methionine	84 \pm 12	70 \pm 7	81 \pm 12	63 \pm 8
Phenylalanine	74 \pm 7	65 \pm 3	71 \pm 3	61 \pm 4
Serine	150 \pm 15	173 \pm 9	149 \pm 15	184 \pm 6
Threonine	208 \pm 20	236 \pm 13	213 \pm 25	267 \pm 8
Tyrosine	120 \pm 10	121 \pm 8	126 \pm 9	124 \pm 7

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149 **Skeletal muscle**

	Control/RD	Control/LPD	BDK-mKO/RD	BDK-mKO/LPD
Alanine	2589 ± 232 ^{a,c}	2382 ± 127 ^a	3035 ± 126 ^c	3386 ± 103 ^b
Arginine	240 ± 27 ^a	260 ± 15 ^a	287 ± 37 ^{a,b}	392 ± 38 ^b
Asparagine	180 ± 17	191 ± 12	191 ± 13	225 ± 11
Aspartic acid	881 ± 45 ^{a,b}	772 ± 30 ^a	882 ± 58 ^{a,b}	991 ± 35 ^b
Glutamic acid	648 ± 45 ^{a,b}	510 ± 51 ^a	697 ± 61 ^{a,b}	780 ± 60 ^b
Glutamine	1530 ± 116 ^a	1392 ± 88 ^a	1557 ± 97 ^a	2096 ± 73 ^b
Glycine	2277 ± 188	2674 ± 180	2295 ± 128	2640 ± 101
Histidine	121 ± 32 ^a	148 ± 6 ^a	173 ± 8 ^a	218 ± 8 ^b
Lysine	701 ± 202	814 ± 66	1294 ± 216	1451 ± 168
Methionine	70 ± 7	62 ± 3	70 ± 6	61 ± 2
Phenylalanine	50 ± 8	42 ± 2	46 ± 4	44 ± 3
Serine	327 ± 37 ^a	380 ± 23 ^a	349 ± 23 ^a	517 ± 27 ^b
Threonine	387 ± 51 ^a	408 ± 22 ^a	429 ± 38 ^a	627 ± 35 ^b
Tyrosine	99 ± 10	101 ± 9	115 ± 5	121 ± 3

150

151

152 Supplemental Table S6. Experimental diets

153

Name	Regular Diet (RD)		Low Protein Diet (LPD)	
Product #	D12450J		D06011002	
	g%	kcal%	g%	kcal%
Protein	19	20	8	8
Carbohydrate	67	70	79	82
Fat	4	10	4	10
Total		100		100
kcal/g	3.8		3.8	
Ingredient	g	kcal	g	kcal
Casein	200	800	79.8	319
L-Cystine	3	12	1.2	5
Corn Starch	506.2	2025	628.2	2513
Maltodextrin	125	500	125	500
Sucrose	68.8	275	68.8	275
Cellulose, BW200	50	0	50	0
Soybean Oil	25	225	25	225
Lard	20	180	20	180
Mineral Mix S10026	10	0	10	0
DiCalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate, 1 H ₂ O	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
Total	1055	4057	1055	4057

154