Suppl Figure S1

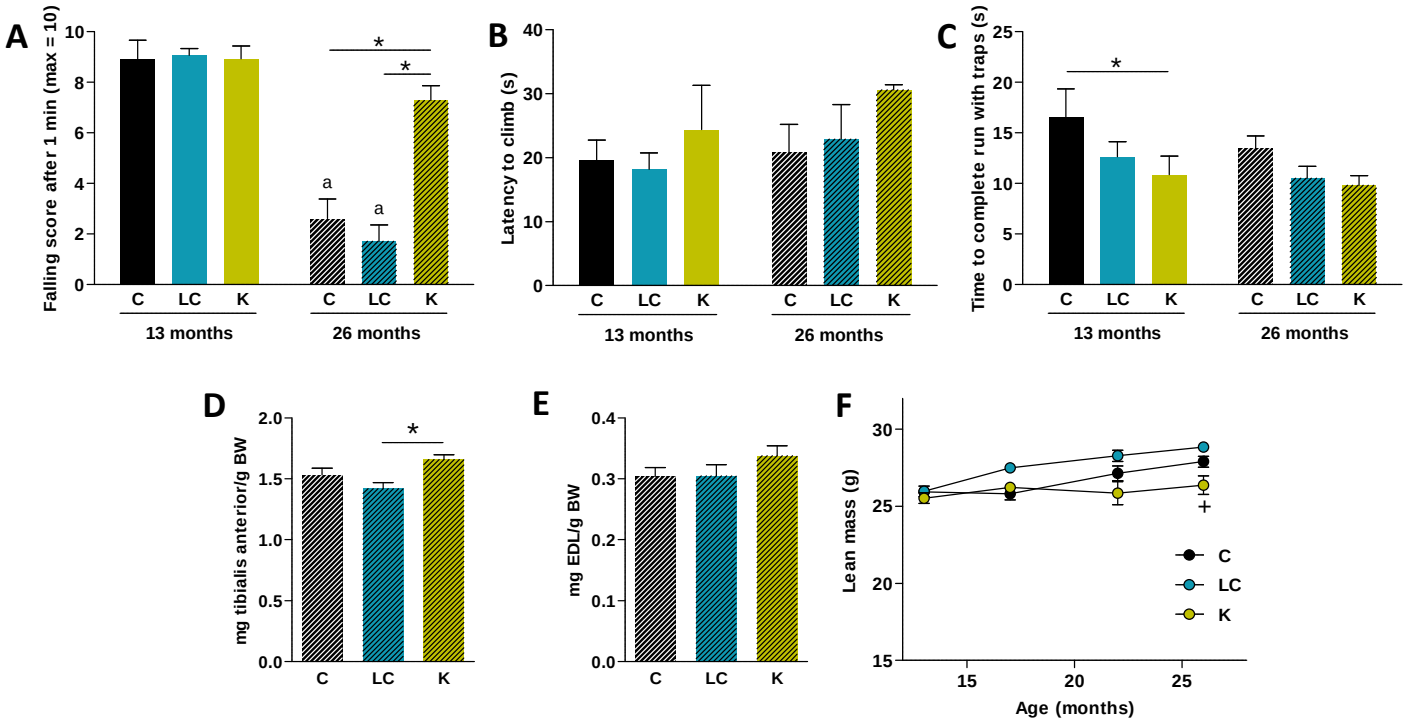

Figure S1. Physical performance, behavior and physiology in male mice fed low-carbohydrate diets. Related to Figures 1 and 2.
(A) Hanging wire test: falling score after 1 min, maximum = 10. (B) Rearing test: latency to climb or time until the first rearing behavior event is observed. (C) Locotronic test: time to complete a run when 3 traps are included in the test.
Relative muscle mass of (D) tibialis anterior and (E) extensor digitorum longus after 14 months of dietary interventions. (F) Lean mass from 1 to 14 months of dietary interventions (n = 15); + indicates lower lean mass in the ketogenic group compared to the rest of diets.
* indicates a difference ($p < 0.05$) between diets.
[a] indicates a difference ($p < 0.05$) between 13-mo and 26-mo for the same diet.

Suppl Figure S2

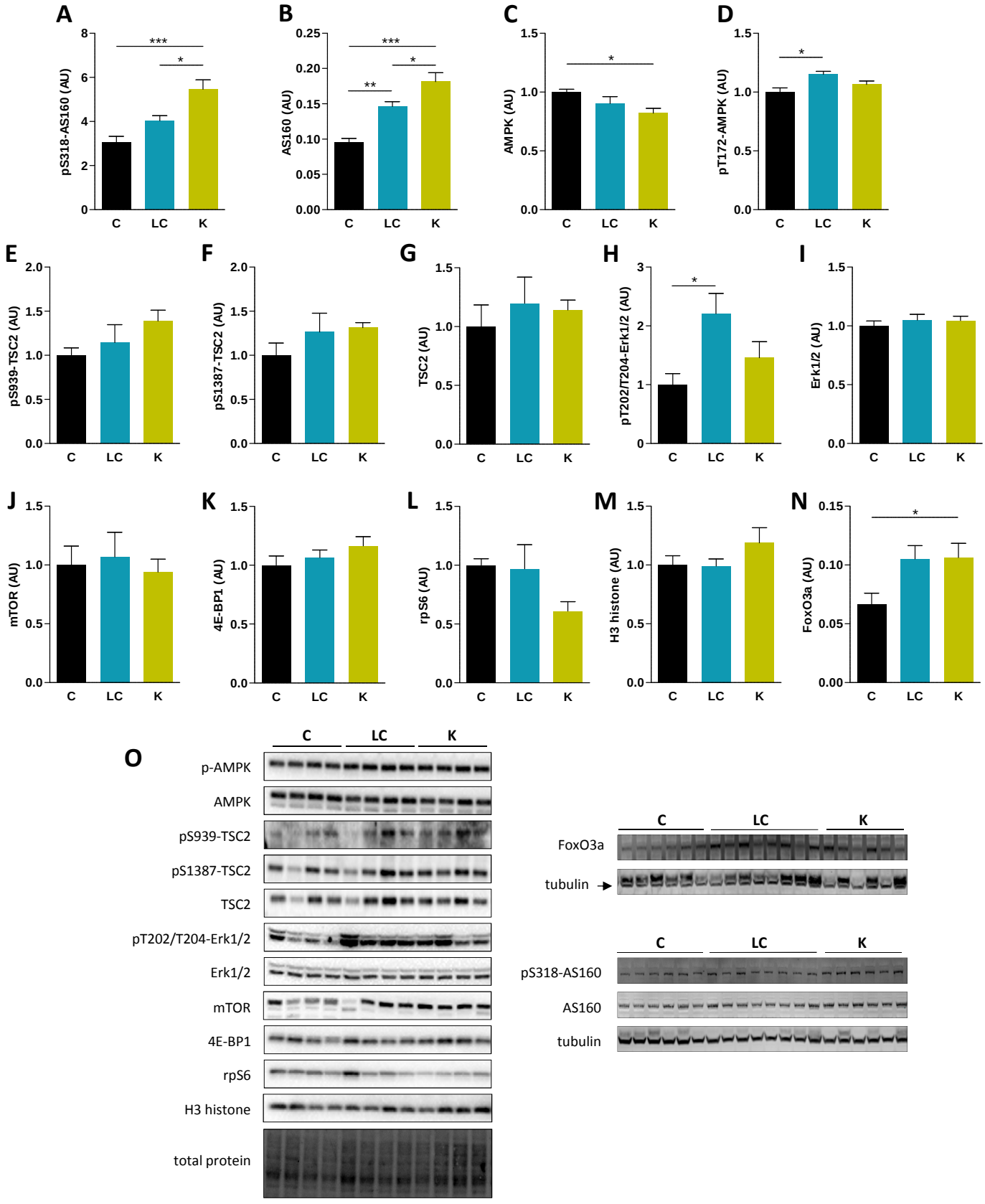

Figure S2. Alterations in nutrient sensing pathways in the liver of male mice after 1 month of diet. Related to Figures 2 and 3.
Quantification of (A) p-AS160, (B) AS160, (C) AMPK, (D) p-AMPK, (E) pS939-TSC2, (F) p1387-TSC2, (G) TSC2, (H) p-Erk1/2, (I) Erk1/2, (J) mTOR, (K) 4E-BP1, (L) rpS6, (M) H3 histone and (N) FoxO3a protein levels after analysis by western blot (n = 4-8). (O) Representative blots are shown for each of the quantified proteins. A representative loading control is shown for those blots with n = 4, corresponding to the p-AMPK and AMPK gel.
* indicates a difference ($p < 0.05$) between diets.

Suppl Figure S3

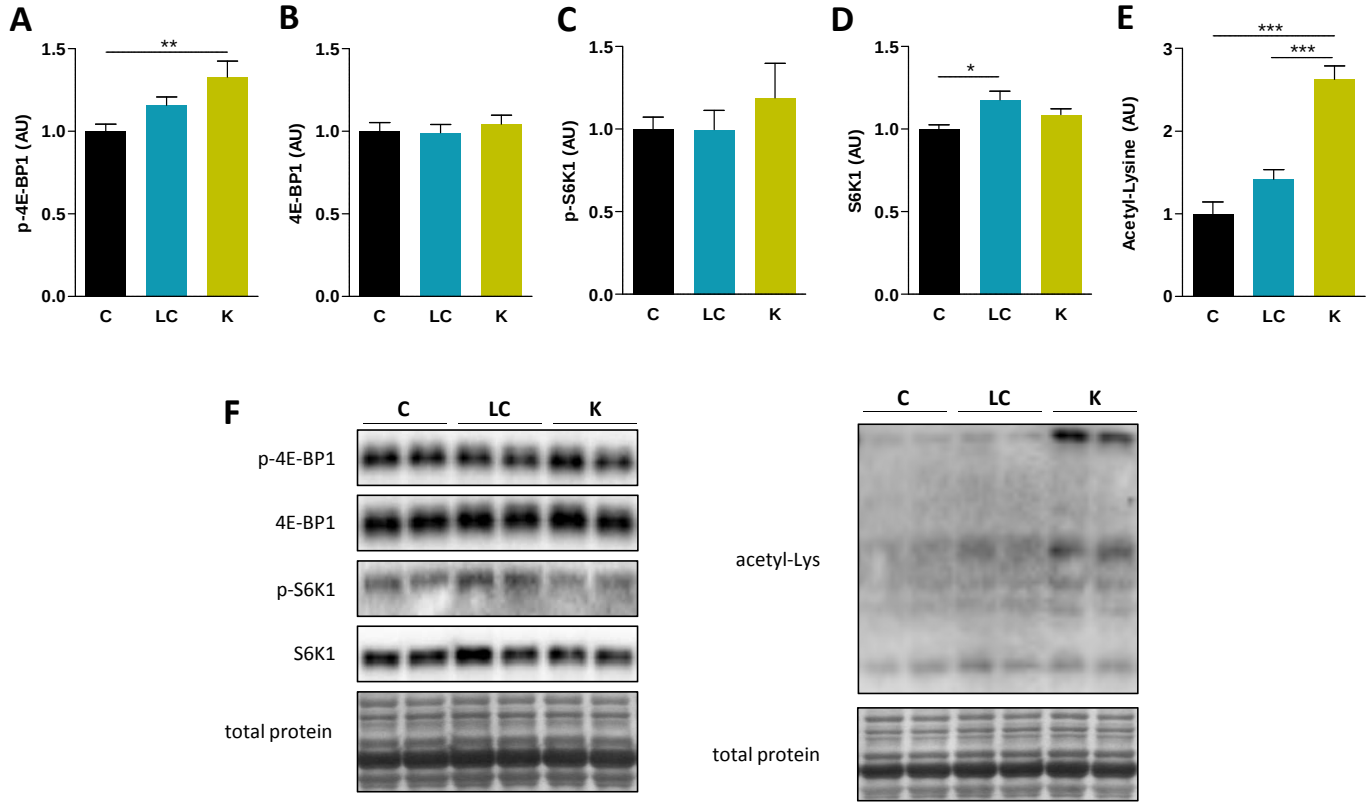

Figure S3. Alterations in mTORC1 signaling and protein acetylation in the skeletal muscle of male mice after 1 month of diet. Related to Figure 3.

Quantification of (A) p-4E-BP1, (B) 4E-BP1, (C) p-S6K, (D) S6K and (E) acetyl-Lysine protein levels. Representative lanes and loading controls are shown (n = 6). Loading controls are shown, corresponding to the 4E-BP1 and S6K gel and the protein acetylation gel.
* indicates a difference ($p < 0.05$) between diets.

Table S1: pathology at time of death in male C57BL/6 mice consuming a ketogenic, LC or control diet. Related to Figure 1. § Indicates differences with CT-KT ($p < 0.1$). **‡** Indicates differences with CT-LC ($p < 0.1$). **#** Indicates differences with LC-KT ($p < 0.1$). n =10. EMH = extramedullary hematopoiesis. Italic font is used for specific tumor types.

	diet		
	C	LC	K
Kidney			
Chronic nephropathy	10	10	10
Tubular mineralization	8	8	9
Glomerular amyloidosis	1	0	1
Histiocytic sarcoma	0	1	0
Pleomorphic lymphoma	0	1	0
Mast cell tumor	0	1	0
Hemangiosarcoma	0	1	0
Neoplasia total	0	4‡	0#
Pancreas			
Exocrine atrophy	1	3	2
Lymphocytic pancreatitis	1	1	2
Spleen			
EMH	7	7	7
Lymphoid depletion	9§	7	4
Angiosarcoma	0	1	1
Pleomorphic lymphoma	0	1	1
Histiocytic sarcoma	4§	4	0#
Neoplasia total	4	6	2
Liver			
Ito cell hyperplasia	8	8	8
Lipogranulomas	10	10	10
Hepatic infarction	0	1	1
EMH	5	4	5
Oval cell hyperplasia	2	0	2
Lipidosis	3	5	5
Kupffer cell hyperplasia	10	10	10
Biliary hyperplasia	4	3	2
Telangiectasis	3	3	4
Lobular atrophy	10	10	10
Histiocytic sarcoma	6§	4	1
Pleomorphic lymphoma	0	1	1
Mast cell tumor	0	1	0
Hepatic adenoma	1	0	0
Neoplasia total	7§	6	2
Lung/trachea			
Hemosiderosis	3	4	2
Pneumonia/pleuritis	2	2	3
Perivascular lymphoplasmacytic infiltrates	4	6	6

	diet		
	C	LC	K
Lung/trachea (cont.)			
Pulmonary adenocarcinoma	1	1	0
Mast cell tumor	0	0	0
Histiocytic sarcoma	3	1	0
Pulmonary adenoma	3	0	1
Neoplasia total	6	4	2
Heart/great vessels			
Myocardial hypertrophy	7	2‡	4
Atrial dilation	0	0	2
Myocardial degeneration	10§	8	6
Great vessel mineralization	2	0	0
Interstitial fibrosis	0	0	2
Ventricular dilation	0	2	3
Myocardial mineralization	4	0‡	1
Thymus			
Atrophy	7	5	5
Stomach			
Hyperkeratosis	5	6	6
Ulceration	8	9	6
Luminal hemorrhage	9	9	7
Brain			
Thalamic mineralization	1	0	2
Lower urinary tract			
Protein in bladder lumen	0	2	1
Dilated bladder	1	0	2
Lymphocytic cystitis	0	2	0
Necrotizing cystitis	2	0	0
Reproductive tract			
Necrotizing prostatitis	2	0	2
Testicular atrophy	6	3	5
Seminal vesicular dilation	7	10	4
Seminal vasculitis	2	0	1
Neoplasia (in any organ)			
Histiocytic sarcoma	6§	6#	1
Other cancer/tumor	4	5	4
Any kind of tumor	8	9#	4

Table S2: influence of diet and age on serum markers of lipid metabolism and inflammation. Related to Figure 2. Values that do not contain the same superscript letter differ ($p < 0.05$) between diets within an age group; values that contain * indicate a difference ($p < 0.05$) between ages in a given diet group. ‡ For FGF21, n = 6 at 13 months and n = 4 at 26 months of age.

	13 months			26 months		
	Control n=8	Low-carb n=8	Ketogenic n=8	Control n=6	Low-carb n=4	Ketogenic n=8
Triglycerides (mg/dl)	77.1±8.6	68.6±7.5	64.0±3.1	39.3±3.5*	42.3±6.5*	42.8±5.7
Total cholesterol (mg/dl)	206.8±8.6	199.4±9.8	199.5±21.1	158.9±37.2	155.6±40.2	192.7±38.3
HDL-C (mg/dl)	160.5±9.2	166.5±8.0	170.0±19.4	114.6±22.8	120.5±34.0	159.5±32.7
LDL-C+VLDL-C (mg/dl)	46.3±2.8	32.9±5.7	29.5±3.0	44.3±15.0	35.0±7.1	33.2±7.6
Free fatty acids (mEq/l)	0.51±0.03	0.47±0.03	0.44±0.03	0.34±0.06^{b}	0.57±0.20^{a}	0.32±0.02^{b}
FGF21 (ng/ml) ‡	1.21±0.37	0.51±0.49	3.04±4.22	3.02±4.24	0.39±0.20	1.25±1.55
IL-6 (pg/ml)	29.1±6.3	12.6±1.7	15.5±2.0	311.2±243.8	220.3±122.3	64.9±19.3
CXCL1 (pg/ml)	99.2±6.7	100.7±4.4	95.6±5.5	282.4±79.5*	238.5±118.1	167.7±27.9
TNF-α (pg/ml)	13.1±1.6	14.3±1.3	17.4±2.0	34.0±10.5*	26.8±11.4	23.4±2.9

Table S3: influence of diet and age on average 24 hour energy expenditure (EE), respiratory quotient (RQ) and physical activity. Related to Figure 2. BW, body weight; LM, lean mass; EE is expressed as EE kJ/mouse/h and EE_{BW} and EE_{LM} (kJ normalized by BW and LM). Values that do not contain the same superscript letter differ between diets within an age group; values that contain * indicate a difference between ages in a given diet group; Bonferroni corrected p values are provided. X-TOT = number of x-axis infrared (IR) beam breaks; X-AMB = number of consecutive x-axis IR beam breaks; Z-TOT = number of z-axis IR beam breaks

	13 months			26 months			p value	
	Control n=7	Low-carb n=7	Ketogenic n=6	Control n=4	Low-carb n=6	Ketogenic n=5	Diet	Age
RQ	0.90±0.01[a]	0.77±0.01[b]	0.72±0.01[c]	0.90±0.01[a]	0.77±0.01[b]	0.73±0.01[c]	<0.001	0.918
EE (kJ/hr)	1.88±1.04	1.98±0.06	2.00±0.05	1.66±0.12	1.80±0.07	1.77±0.05	0.139	<0.001
EE_{BW} (kJ/g/hr)	0.062± 0.001	0.065± 0.003	0.065± 0.002	0.055± 0.002*	0.054± 0.002*	0.061± 0.003	0.063	<0.001
EE_{LM} (kJ/g/hr)	0.083 ±0.002	0.086 ±0.004	0.089 ±0.002	0.064 ±0.002*	0.063 ±0.002*	0.070 ±0.003*	0.140	<0.001
X-TOT Light	19.7±4.3	19.1±3.2	22.1±4.7	27.8±4.3	29.5±5.3	35.0±4.7	0.519	0.008
X-TOT Dark	23.6±3.4[a]	34.3±5.0[a,b]	42.0±6.8[b]	28.5±9.7	32.8±2.8	32.4±2.0	0.121	0.628
X-AMB Light	6.7±2.2	6.9±1.7	6.9±2.5	8.9±2.4	9.6±3.1	9.6±1.5	0.970	0.203
X-AMB Dark	8.0±1.3[a]	15.0±2.5[a,b]	18.1±4.5[b]	9.7±4.1	11.2±1.6	9.3±1.6	0.200	0.119
Z-TOT Light	1.8±0.8	2.9±1.4	1.8±1.0	4.8±2.2	2.8±0.8	3.8±1.2	0.894	0.123
Z-TOT Dark	2.2±0. 5	6.5±1.9	6.3±1.8	6.0±2.2	4.3±0.9	4.4±1.0	0.629	0.938