	WT sedentary (ng/ml) (n=8-10)	WT trained (ng/ml) (n=9-11)	S6K1KO sedentary (ng/ml) (n=9-11)	S6K1KO trained (ng/ml) (n=9-11)
Insulin	10.29±1.92	5.86±1.33	**5.77±1.03**$^{\#}$	4.42±0.98
Leptin	25.07±7.86	17.62±4.43	9.22±2.61	11.24±4.16
Resistin	246.4±31.33	184.57±27.57	263.0±46.49	319.8±62.5
Adiponectin	12912±405.7	12928±478.5	**9011±748.3**$^{\#\#\#\#}$	**9931±536.3**$^{\#\#}$
PAI-1	3.51±0.5	**1.66±0.22****	2.7±0.36	**3.03±0.38**$^{\#}$
Ghrelin	12.56±1.34	**6.02±1.24****	12.48±1.49	**13.91±1.52**$^{\#\#\#}$
GIP	0.76±0.09	0.74±0.13	0.72±0.08	0.67±0.07
GLP-1	OOR	OOR	OOR	OOR
Glucagon	0.36±0.05	0.30±0.03	0.37±0.04	0.33±0.03
Corticosterone	74.46±11.45	**135.65±15.57****	82.01±13.8	**54.36±9.84**$^{\#\#\#}$

	WT sedentary (n=9-10)	WT trained (n=10-11)	S6K1KO sedentary (n=10-11)	S6K1KO trained (n=10-11)
Lactate (nmol/µl)	5.05±0.7	3.58±0.32	3.74±0.45	3.28±0.31
TAG (nmol/µl)	0.32±0.03	0.34±0.02	0.31±0.02	0.30±0.01
NEFA (mmol/L)	0.93±0.04	**1.20±0.05*****	1.02±0.06	1.15±0.05
Cholesterol (nmol/µl)	0.60±0.06	**0.91±0.07****	0.67±0.06	**0.53±0.04**$^{\#\#\#\#}$
Total Ketone Bodies (µmol/L)	528.3±45.04	**887.3±54.06******	815.0±54.81$^{\#\#\#}$	**1024.0±64.0***

	WT sedentary [mg/dl] (n=10)	WT exercised [mg/dl] (n=10)	S6K1KO sedentary [mg/dl] (n=10)	S6K1KO exercised [mg/dl] (n=10)
Blood Glucose (mg/dL)	230.1±11.2	206.8±3.7	232.6±15.1	**182.0±12.8****

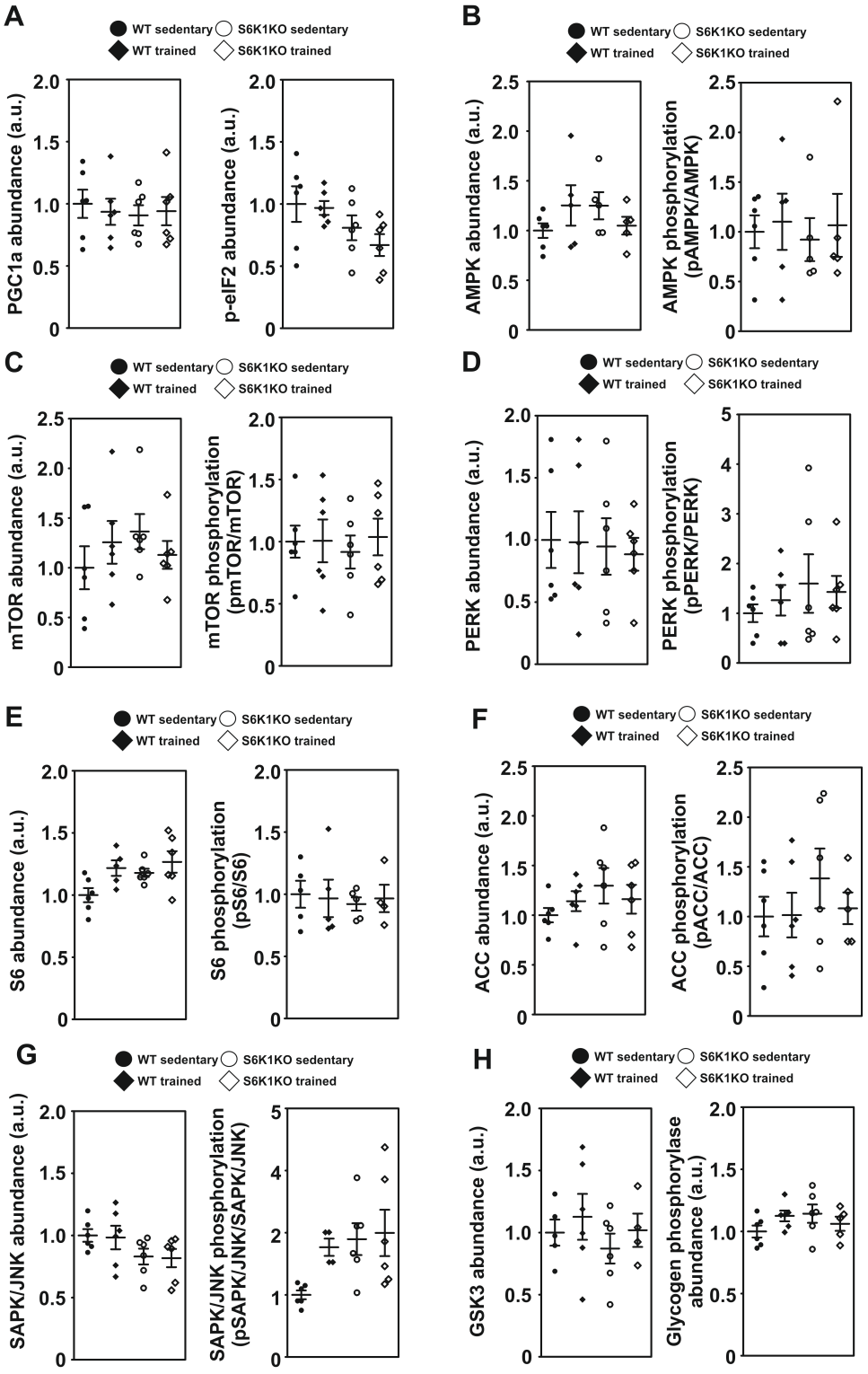
Supplementary Table 1 – Plasma parameters after 4 weeks of chronic endurance exercise training. Data presented as mean ± SEM, Statistical analyses were performed by two-way ANOVA with Bonferroni post-test, sedentary vs. exercised: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; WT vs. S6K1KO: $^{\#}$p<0.05 $^{\#\#}$p<0.01 $^{\#\#\#}$p<0.001 $^{\#\#\#\#}$p<0.0001.

Gene	Primer Sequence	
Atf4	Forward:	5'- GAGCTTCCTGAACAGCGAAGTG -3'
	Reverse:	5'- TGGCCACCTCCAGATAGTCATC -3'
Cat	Forward:	5'- AGCGACCAGATGAAGCAGTG -3'
	Reverse:	5'- TCCGCTCTCTGTCAAAGTGTG -3'
Il1	Forward:	5'- TCGCTCAGGGTCACAAGAAA -3'
	Reverse:	5'- CATCAGAGGCAAGGAGGAAAAC -3'
Nadphox	Forward:	5'- TTGGGTCAGCACTGGCTCTG -3'
	Reverse:	5'- TGGCGGTGTGCAGTGCTATC -3'
Sod1	Forward:	5'- AACCAGTTGTGTTGTCAGGAC -3'
	Reverse:	5'- CCACCATGTTTCTTAGAGTGAGG -3'
Sod2	Forward:	5'- CAGACCTGCCTTACGACTATGG -3'
	Reverse:	5'- CAGACCTGCCTTACGACTATGG -3'
***Xbp1* (spliced)**	Forward:	5'- GAGTCCGCAGCAGGTG -3'
	Reverse:	5'- GTGTCAGAGTCCATGGGA -3'
***Xbp1* (unspliced)**	Forward:	5'- TGAGAACCAGGAGTTAAGAACACGC -3'
	Reverse:	5'- TTCTGGGTAGACCTCTGGGAGTTCC -3'

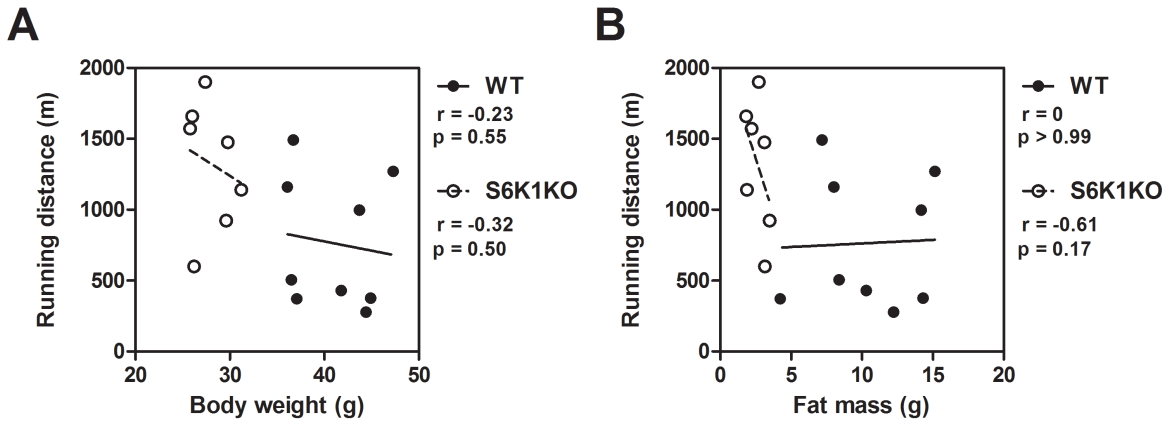
Supplementary Table 2 – Primer Sequences for SYBR Green Real-Time PCR. *Atf4*= activating transcription factor 4, *Cat*= Catalase, *Il1*= Interleukin-1, *Nadphox*= NADPH oxidase, *Sod1*= Superoxide dismutase [Cu-Zn], *Sod2*= Superoxide dismutase [Mn], mitochondrial, *Xbp1*= X-box-binding protein 1.

Target protein	Provider	
ACC	Cell Signaling	#3662
AMPK	Cell Signaling	#2532
GAPDH	Cell Signaling	#2118
GS	Cell Signaling	#3893
GSK3	Cell Signaling	#9338
mTOR	Cell Signaling	#2972
PCK1	Abcam	Ab87340
PCK2	Cell Signaling	#6924
PERK	Cell Signaling	#3192
PGC1α	Abcam	Ab72230
phospho(Ser240/244)-S6	Cell Signaling	#5364
phospho(Ser2448)-mTOR	Cell Signaling	#2971
phospho(Ser51)-eIF2α	Cell Signaling	#9721
phospho(Ser641)-GS	Cell Signaling	#3891
phospho(Ser97)-ACC	Cell Signaling	#3661
phospho(Thr183/Tyr185)-SAPK/JNK	Cell Signaling	#4668
phospho(Thr980)-PERK	Cell Signaling	#3179
phospho(Thr172)-AMPK	Cell Signaling	#2531
PYGB	Santa Cruz	Sc-46347
S6	Cell Signaling	#2217
S6K1	Cell Signaling	#9202
SAPK/JNK	Cell Signaling	#9252

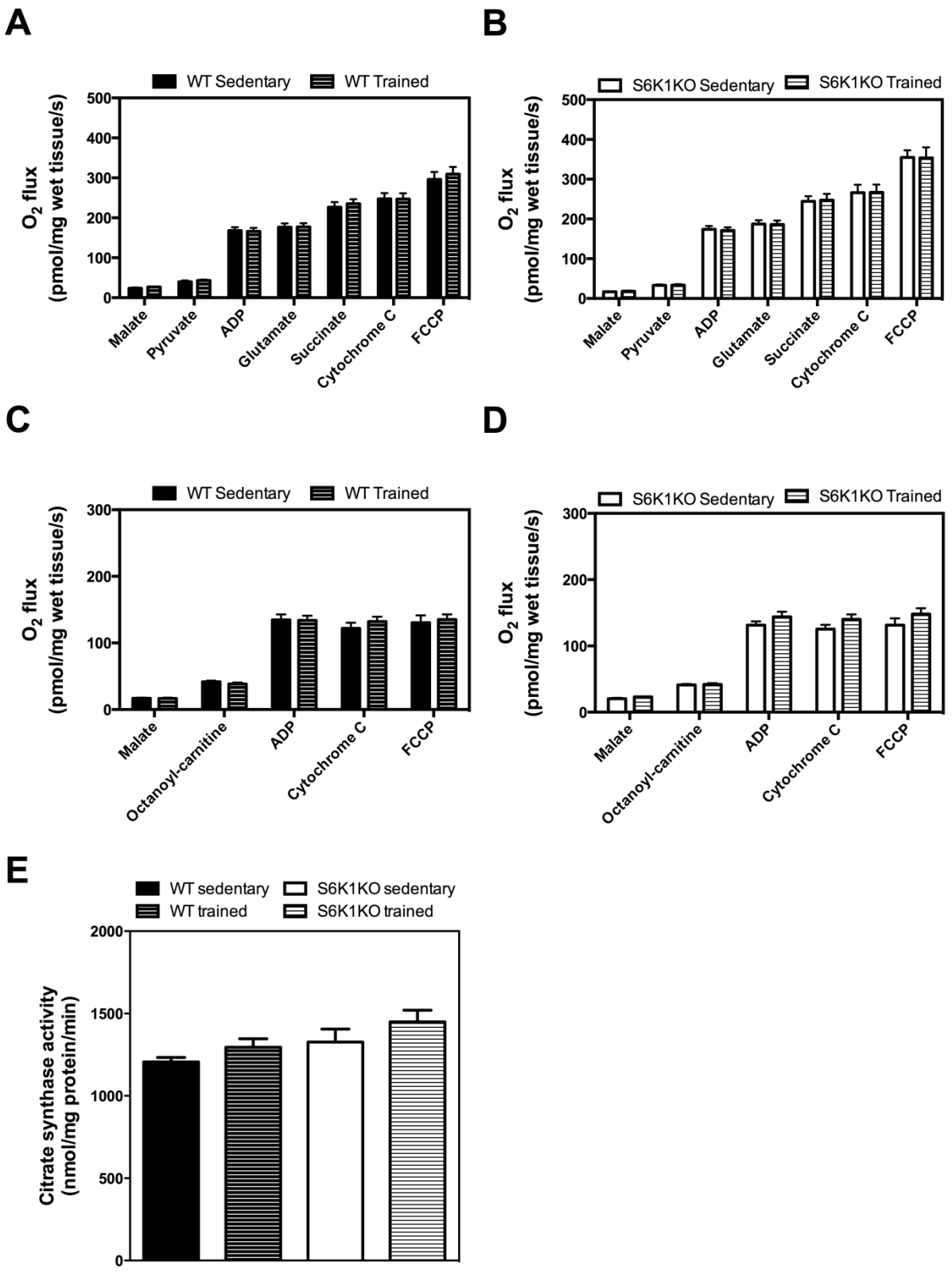
Supplementary Table 3 – Primary antibodies for Western Blot analyses ACC, Acetyl-CoA carboxylase, AMPK, 5'-AMP-activated protein kinase, GAPDH, Glyceraldehyde-3-phosphate dehydrogenase, GS, Glycogen Synthase, GSK3, Glycogen synthase kinase-3, mTOR, mammalian target of rapamycin, PCK1, cytosolic phosphoenolpyruvate carboxykinase, PCK2, mitochondrial phosphoenolpyruvate carboxykinase, PERK, PRKR-like endoplasmic reticulum kinase, PGC1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, eIF2α, eukaryotic translation initiation factor 2-alpha, PYGB, Glycogen Phosphorylase, S6, ribosomal protein S6, S6K1, ribosomal protein S6 kinase-1, SAPK/JNK, C-Jun-amino-terminal kinase.



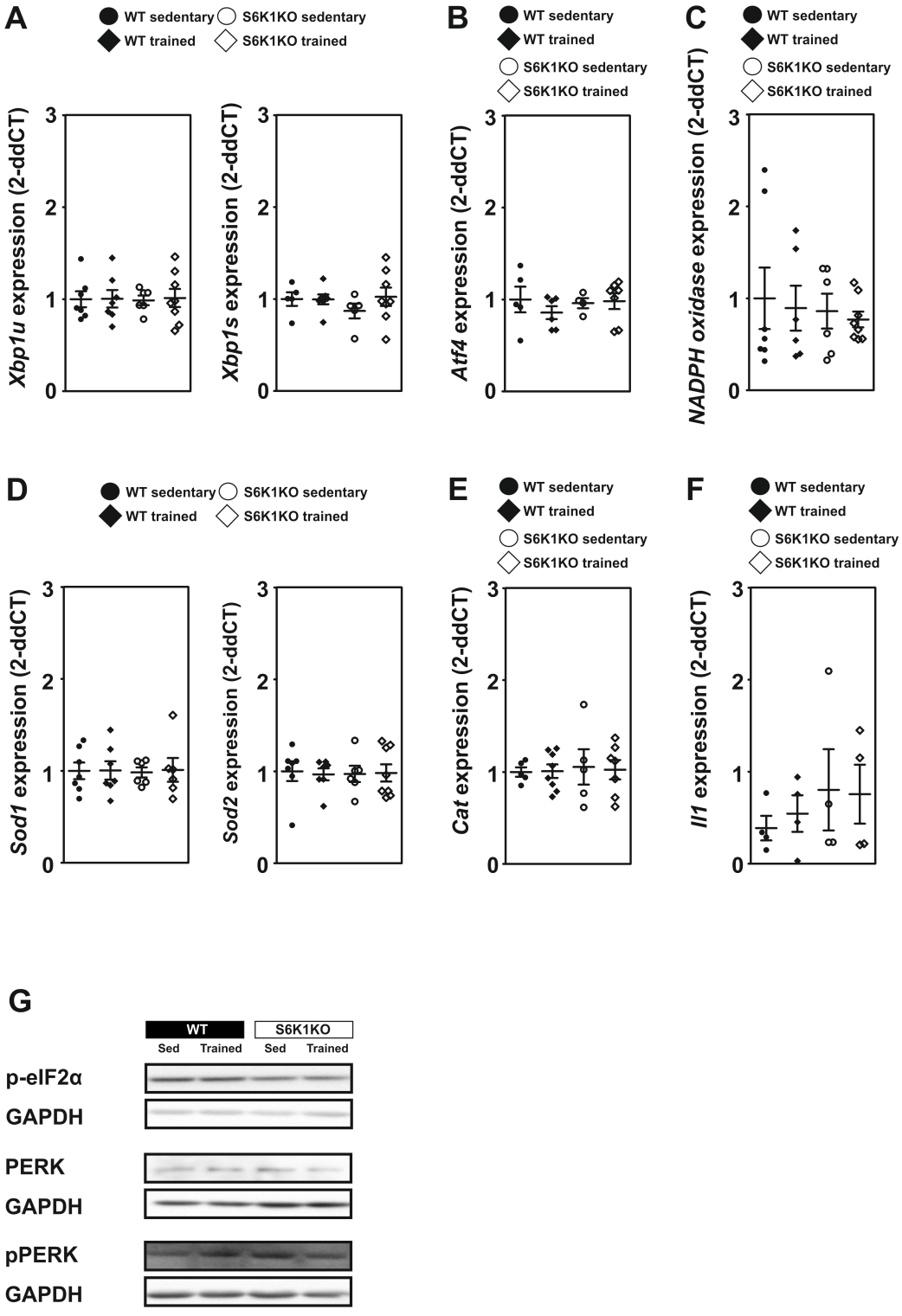
Supplementary Figure S1 – Effect of exercise and S6K1 knockout on abundance of proteins involved in skeletal muscle energy metabolism. Protein abundance was determined by Western Blot analysis and results were normalized by GAPDH abundance and WT sedentary controls. (A) PGC1α and p-EIF2α, (B) (phosphor-)AMPK, (C) (phospho-)mTOR, (D) (phospho-)PERK, (E) (phosphor-)S6, (F) (phospho-)(ACC), (G) (phospho-)SAPK/JNK in skeletal muscle and (H) GSK3 in liver. Data presented as mean ± SEM (n=6-8), Statistical analyses were performed by two-way ANOVA with Bonferroni post-test.



Supplementary Figure S2 – Correlation of body weight, body fat and running endurance. (A) Correlation of body weight (A) and body fat (B) with maximal running distance of WT and S6K1KO mice. Statistical analysis was performed using nonparametric Spearman correlation analysis. (n=7-9).



Supplementary Figure S3 – Mitochondrial oxidative capacity and citrate synthase activity in the soleus muscle of sedentary and trained mice WT and S6K1KO genotype. Oxygen flux was analyzed at different respiratory states using the substrates of tricarboxylic acid (TCA) cycle (A, B) as well as β–oxidation (C, D). Mitochondrial density was assessed from the citrate synthase activity (E). Data are presented as mean ± SEM (n=8-10), Statistical analyses were performed by two-way ANOVA with Bonferroni post-test, sedentary vs. exercised: *p<0.05.



Supplementary Figure S4 – Transcriptional markers for ER-stress are not altered through the ablation of *S6k1* nor due to chronic endurance exercise. Gene expression analysis was assessed via quantitative PCR (qPCR) and results were normalized by gene expression of β-actin and the wild type sedentary group. *(A)* Gene expression of spliced (*Xbp1u*) and unspliced (*Xbp1s*) form of *Xbp1*, *(B) Atf4*, (C) *Nadphox (D) Sod1* and *Sod2*, *(E)* Cat and *(F)* Interleukin-1. Data presented as mean ± SEM. *(G)* Representative Western Blots of phospho-eIF2α and (phospho-)PERK in red part of gastrocnemius muscle. Data presented as mean ± SEM (n=6-8), Statistical analyses were performed by two-way ANOVA with Bonferroni post-test.