Supplemental Figures and Table

Differential response of skeletal muscles to mTORC1 signaling during atrophy and hypertrophy

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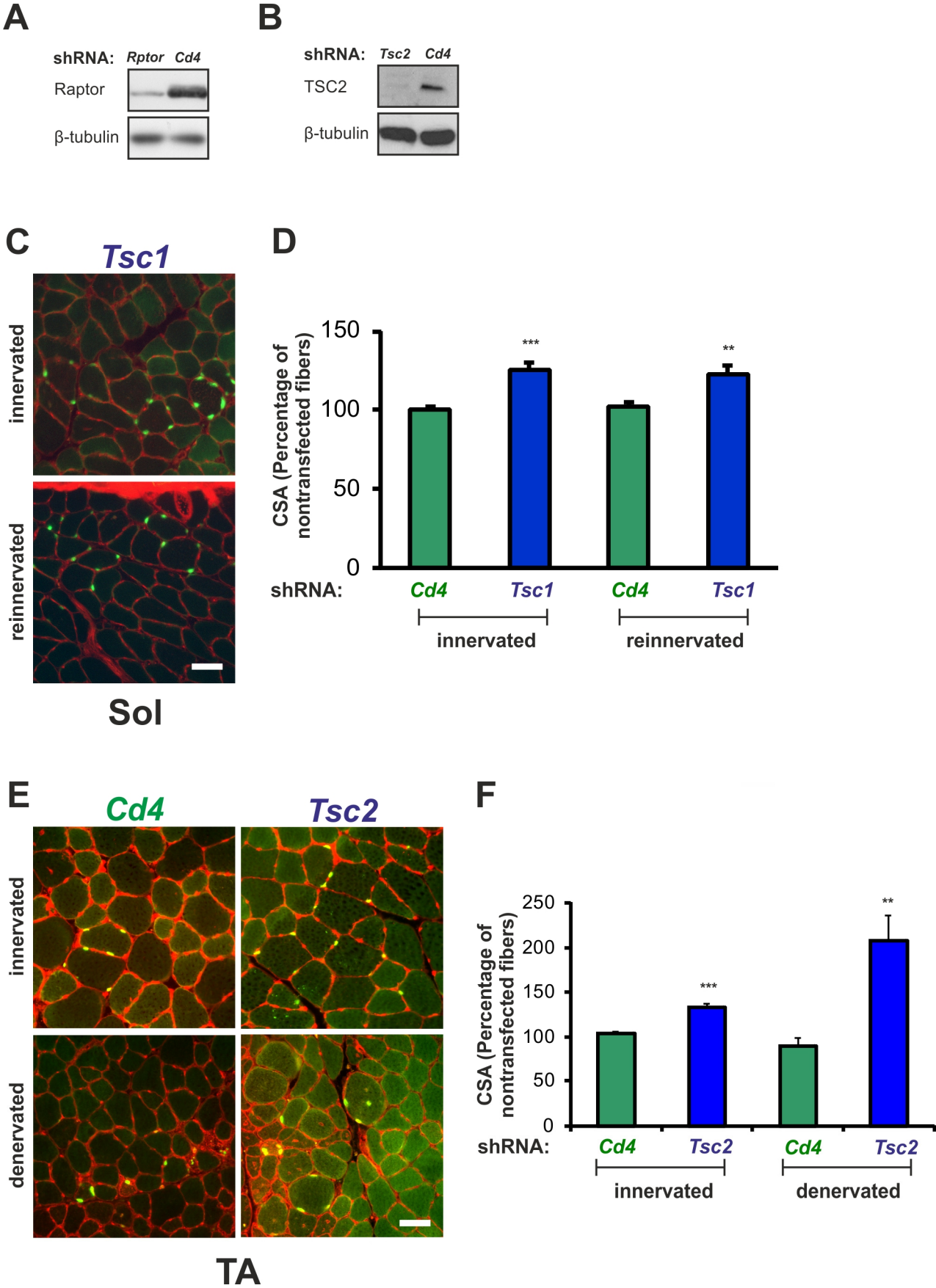5 Supplemental Figures

1 Supplemental Table

Figure S1

Figure S1. **Knockdown of TSC1 or TSC2 in results in muscle hypertrophy**

(**A**) Western blot analysis of COS cells that were co-transfected with an expression construct encoding raptor and a construct encoding shRNA against *rptor* or *Cd4*. (**B**) Myoblasts infected with an adenovirus encoding shRNA against *Tsc2* or *Cd4*. Knockdown of expressed protein was determined using indicated antibodies. Equal amounts of protein were loaded as indicated by the expression of β-tubulin. (**C-F**) *Soleus* (**C** and **D**) and TA (**E** and **F**) muscles were electroporated with cDNA constructs encoding shRNA against *Tsc1*, *Tsc2* or *Cd4*. After four to six weeks, muscle fiber size was determined by staining mid-belly cross-sections with Alexa-594-labeled wheat germ agglutinin (red). Electroporated muscle fibers were identified by the expression of nuclear-localized GFP (green). Muscles analyzed were innervated, reinnervated or denervated as indicated. Scale bar = 50 µm. (**D** and **F**) Quantifications of cross-sectional area (CSA) of muscle fibers in each paradigm are given relative to CSA of neighboring, non-electroporated fibers. Bars represent mean ± SEM (N = 3 mice). P-values are ***$p < 0.001$; **$p < 0.01$; p-values were determined by student's t-test.
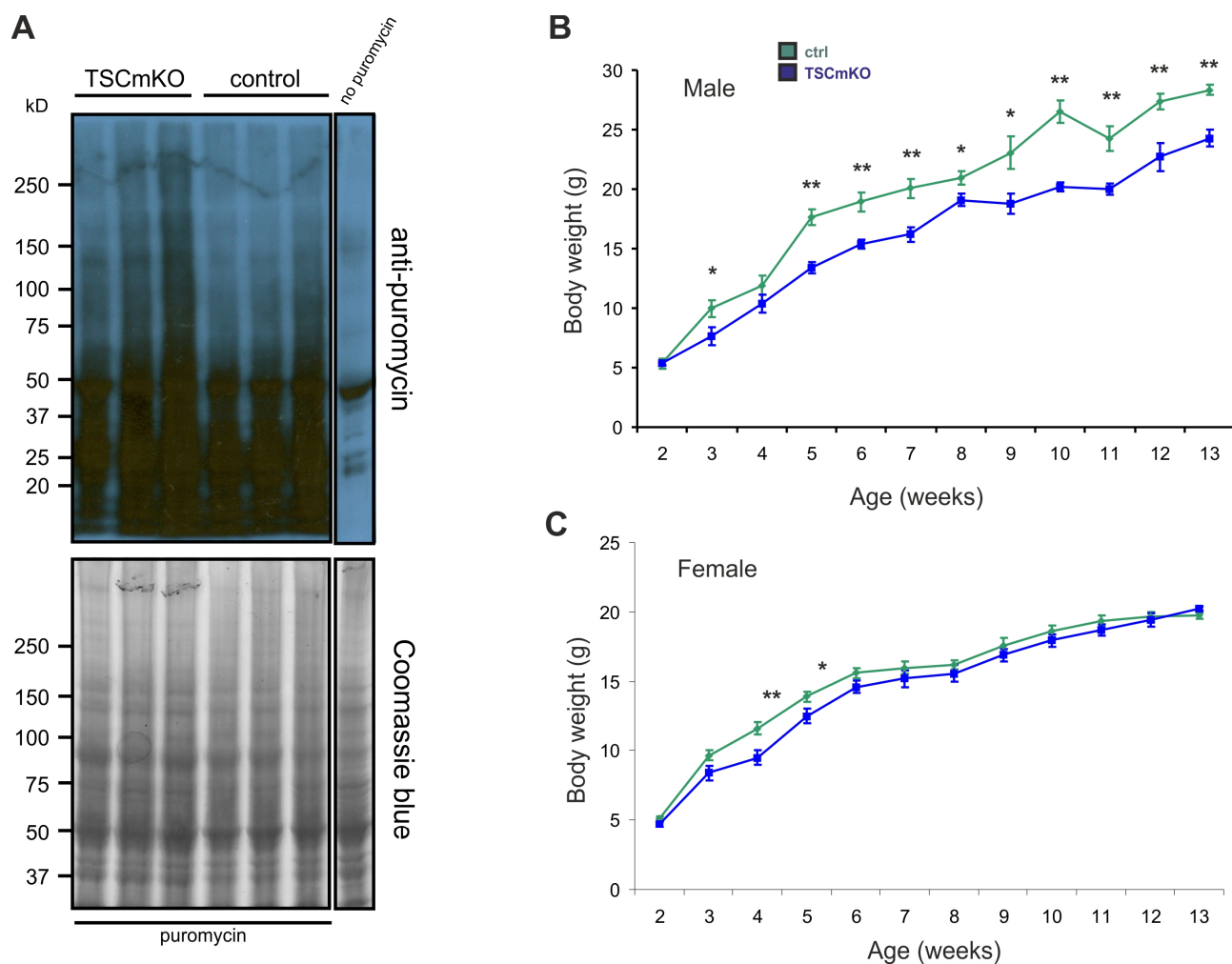
Figure S2

Figure S2. **Increased protein synthesis and lower weight gain in TSCmKO mice**
(**A**) Western blot analysis of puromycin incorporation in total protein extract of EDL muscle from 60 day-old TSCmKO and control mice injected with puromycin. Sham-injected mice show no specific bands demonstrating the specificity of the anti-puromycin antibody (rightmost lane). Coomassie Blue staining of the gel (bottom part) demonstrates equal protein loading. (**B, C**) Body weight of male (**B**) and female (**C**) TSCmKO and control mice (N = 4 - 35 mice for each genotype and gender). Values represent mean ± SEM. P-values are **p < 0.01; *p < 0.05 as determined by student's t-test.
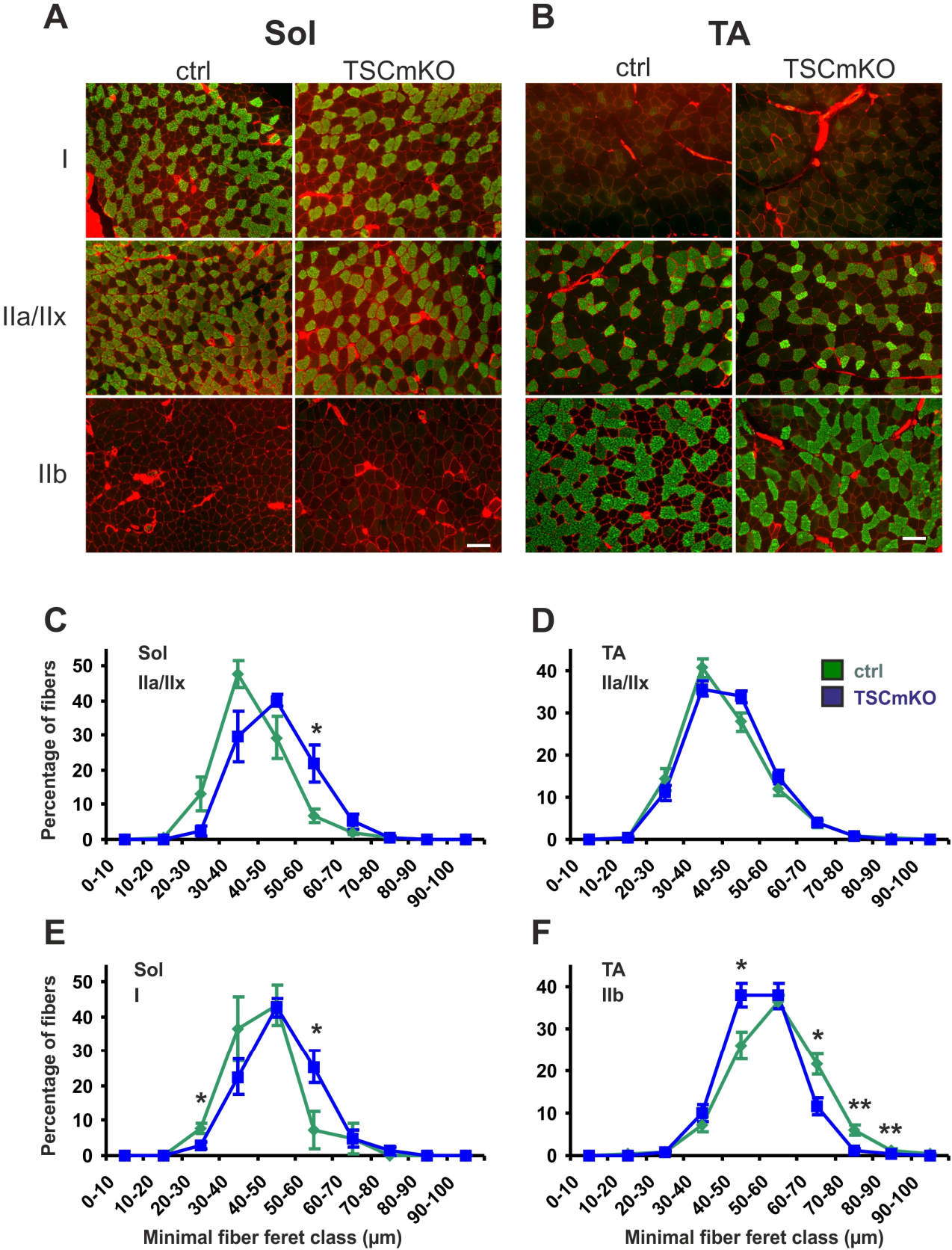
Figure S3

Figure S3. **Quantification of muscle fiber size of individual fiber types**

(**A, B**) Representative images of *soleus* (**A**) and TA (**B**) muscles stained for fiber subtype as indicated. (**C** - **F**) Fiber size distribution of indicated fiber subtype of *soleus* (**C** and **E**) and TA (**D** and **F**) muscles. Note that all the fibers in *soleus* muscle are bigger in TSCmKO than in control mice. In contrast, type IIb fibers in TA muscle are smaller in TSCmKO than in control mice. N = 4 mice in each genotype. Values represent mean ± SEM. P-values are **$p < 0.01$; *$p < 0.05$; determined by student's t-test.
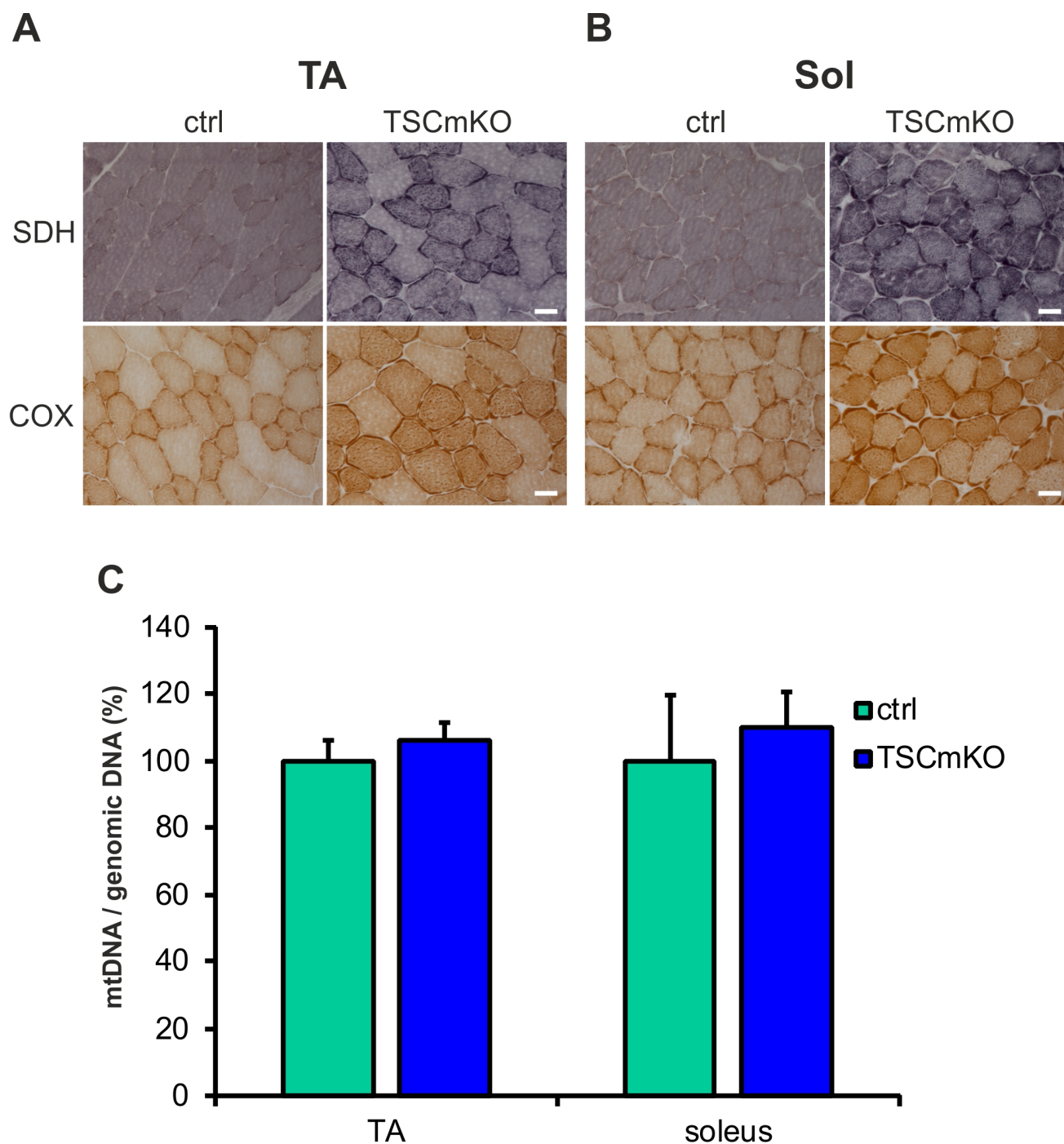
Figure S4

A

TA

B

Sol

C

Figure S4. **Muscles of TSCmKO mice are more oxidative**

(**A** and **B**) SDH and COX staining of TA (**A**) and *soleus* (**B**) muscles of 90 day-old control (ctrl) and TSCmKO mice. Both muscles of TSCmKO mice are more oxidative (N = 4 mice). Scale bar = 50 μm. (**C**) Quantification of the copy number of mitochondrial DNA (mtDNA) in TA and *soleus* muscle of 90 day-old ctrl and TSCmKO mice (N = 3 mice) relative to the amount of genomic DNA. Data represent mean ± SD; N = 4 ctrl and N = 3 TSCmKO mice.
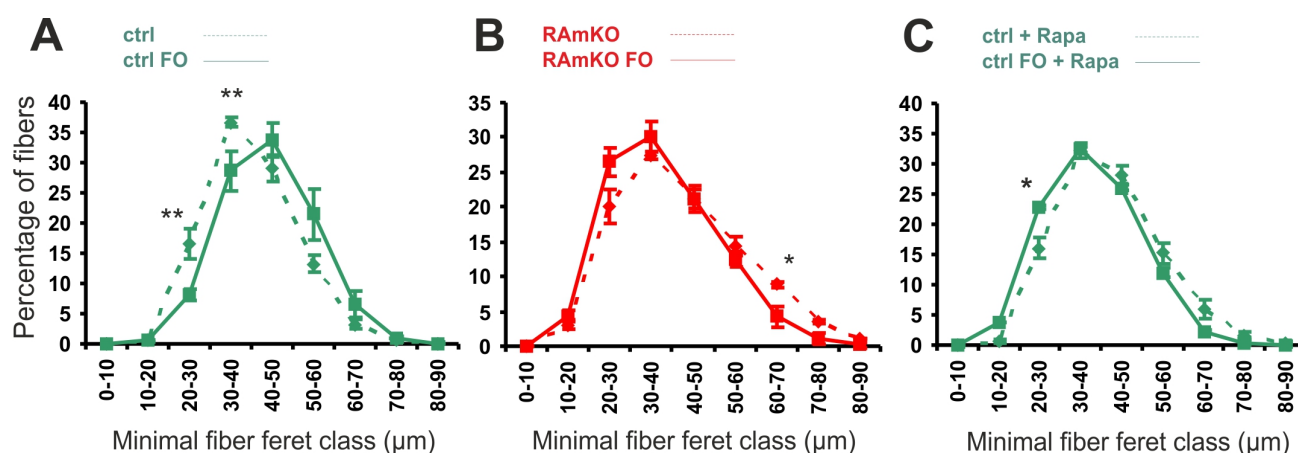
Figure S5

Figure S5. **Increase in muscle fiber size requires mTORC1**

(**A, B**) Fiber size distribution of *plantaris* muscle, which was functionally overloaded (FO) for 7 days (solid lines) and of non-overloaded, contralateral muscle (dashed lines). While the fiber size distribution is clearly shifted toward bigger fibers in control mice (**A**), no shift was seen in RAmKO mice (**B**). (**C**) Daily application of rapamycin prevented muscle fiber growth in *plantaris* muscle of control mice by overloading for 28 days (N = 4 mice in all experiments). Values represent mean ± SEM. P-values are **p < 0.01; *p < 0.05; student's t-test was used to calculate p-values.

Table S1

			soleus			TA		
			ctrl	**TSCmKO**	*p*-value	**ctrl**	**TSCmKO**	*p*-value
Fiber type	total	fiber number	854 ± 50	848 ± 35	0.956	2898 ± 129	2886 ± 200	0.862
		fiber feret (µm)	40.01 ± 1.59	45.67 ± 1.55	0.044	47.42 ± 0.82	47.05 ± 0.99	0.784
	I	fiber number (%)	38.53 ± 1.62	41.95 ± 3.95	0.453	n.d.	n.d.	n.d.
		fiber feret (µm)	41.06 ± 1.82	46.02 ± 1.47	0.078	n.d.	n.d.	n.d.
	IIa	fiber number (%)	55.67 ± 3.27	59.91 ± 1.23	0.270	12.14 ± 3.67	10.60 ± 1.25	0.705
		fiber feret (µm)	37.68 ± 1.62	44.62 ± 1.92	0.033	34.37 ± 1.96	35.19 ± 0.67	0.705
	IIx	fiber number (%)	8.01 ± 2.26	6.89 ± 1.43	0.690	44.66 ± 3.61	35.69 ± 1.50	0.062
		fiber feret (µm)	44.96 ± 2.58	46.58 ± 1.79	0.627	41.75 ± 0.96	43.53 ± 0.96	0.238
	IIb	fiber number (%)	n.d.	n.d.	n.d.	53.29 ± 2.93	66.70 ± 2.66	0.015
		fiber feret (µm)	n.d.	n.d.	n.d.	54.48 ± 1.20	50.29 ± 1.01	0.037

Table S1. **Characterization of muscles from TSCmKO mice**

The number of fibers and their mean feret were determined according to fiber types, in *soleus* and TA muscles from 90 day-old TSCmKO and control (ctrl) mice. n.d.: not detected. Values are mean ± SEM; p-values were determined by student's t-test.