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#### **Supplemental Information**

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#### **Postnatal Maturation of Neuromuscular Junctions in Mice**

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# Splicing factor SRSF1 is essential for satellite cell proliferation and postnatal maturation of neuromuscular junctions in mice

Yuguo Liu<sup>1#</sup>, Yangjun Luo<sup>1#</sup>, Lei Shen<sup>2#</sup>, Ruochen Guo<sup>1</sup>, Zheng Zhan<sup>1</sup>, Ningyang Yuan<sup>1</sup>, Rula Sha<sup>1</sup>, Wenju Qian<sup>1</sup>, Zhenzhen Wang<sup>1</sup>, Zhiqin Xie<sup>1</sup>, and Wenwu Wu<sup>3\*</sup>, Ying Feng<sup>1,3\*</sup>

## **Supplemental Figures**



Figure S1 I Splicing factors could be induced to be highly expressed upon satellite cell activation, additional data in support of figure 1. Analysis of published microarray data derived from quiescent satellite cells, activated satellite cells and non-muscle satellite cells. Red stands for high expression while green for low expression.



Figure S2 I Immunostaining confirmed that FACS-purified cells have satellite cell identity, additional data in support of figure 1B. FACS-isolated cells were plated in Matrigel coated dishes and cultured in the proliferating medium. Cells were fixed and stained with antibodies indicated after 48h culture. Scale bar is 50  $\mu$ m (n=3).



**Figure S3 I Characteristics of MKO mice compared to controls, additional data in support of figure 2.** (A) Diagram showing the tissue-specific knockout strategy. SRSF1<sup>f/f</sup> mice were crossed with MyoD<sup>cre</sup> mice to generate skeletal muscle specific SRSF1 knockout mice (MKO). (B) Blood glucose and serum triglyceride (TG) levels were compared in between control and MKO mice at P28 (n=3). (C) Western blot analysis of SRSF1 proteins in different muscles. Protein samples were prepared from indicated muscles from control and MKO mice at P8 or P28. Tubulin was used as loading control (n=3). (D) Single EDL myofibers were prepared from control or MKO mice at P23 and cultured in vitro. Fibers were labeled with DAPI. Comparison of relative fiber width and the number of myonuclei per fiber between control and MKO mice was shown on the right. Scale bar is 100 µm (n=3).



Figure S4 I No obvious inflammation and fibrosis were observed in MKO mice, additional data to support figure 2D and 2E. (A) GAS muscles were cross sectioned and stained with CD68 antibody (green) and laminin (grey). Nucleus were labeled with DAPI (n=3). (B) GAS and DIA muscles were cross sectioned and stained with fibronectin antibody (green). Scale bar is 100  $\mu$ m (n=3).



Hindlimb (P1)

DIA (P1)

**Figure S5 I The number of MyoD<sup>+</sup> cells was significantly decreased in the MKO mice, additional data in support of figure 3A.** Hindlimb and DIA muscles from P1 control and MKO mice was prepared and stained for MyoD (red), and merged images with DAPI (blue) and laminin (grey) were shown on the right. Scale bar is 50 µm (n=3).



primary myoblasts (p6)

Figure S6 I SRSF1 promoted expression of Fgfr1op2-L isoform, which was required for proliferation of myoblasts, additional data to support figure 4. (A) Schematic diagram of *Fgfr1op2* variants including or lacking alternative E4. (B) RT-PCR analysis of total RNA isolated from primary myoblasts transiently transfected with siSRSF1, siFgfr1op2-L or control siRNA for 2 days (n=3). (C) EdU labeling of primary myoblasts. Primary myoblasts were cultured in growth medium, followed by transfection with indicated siRNAs for 2 days. Cells were then labeled with EdU for another 24 hrs, fixed and stained with EdU and DAPI. Quantification of EdU positive nuclei was shown on the right. Scale bar is 50  $\mu$ m. Error bars depicted mean  $\pm$  SEM (n=3).

## Table S1: Antibodies used in this study that relate to figures 1, 2, 3,5,6

Antibody	Company	Cat.	Dillution
Myf5, C-20	Santa Cruz	sc-302	1:200
Myod, C-20	Santa Cruz	sc-304	1:200
PAX7, Concentrate 0.1 ml	DSHB	Pax7	1:200
SRSF1	Novus	MABE163	1:100
α-Bungarotoxin_Atto-488	Alomone	B-100-AG	1:100
Synaptophysin	ThermoFisher	PA11043	1:100
Laminin-α2	ENZO	ALX-804-190	1:300
α7-integrin-APC	Novus	FAB3518A	FACS
Sca-1-PE	BD Pharminge	562059	FACS
CD31-PerCP-Cy™5.5	BD Pharminge	562861	FACS
CD45-PerCP-Cy™5.5	BD Pharminge	550994	FACS
CD11b-PerCP-Cy™5.5	BD Pharminge	550993	FACS
Gapdh	Abways Technology	AB0037	WB