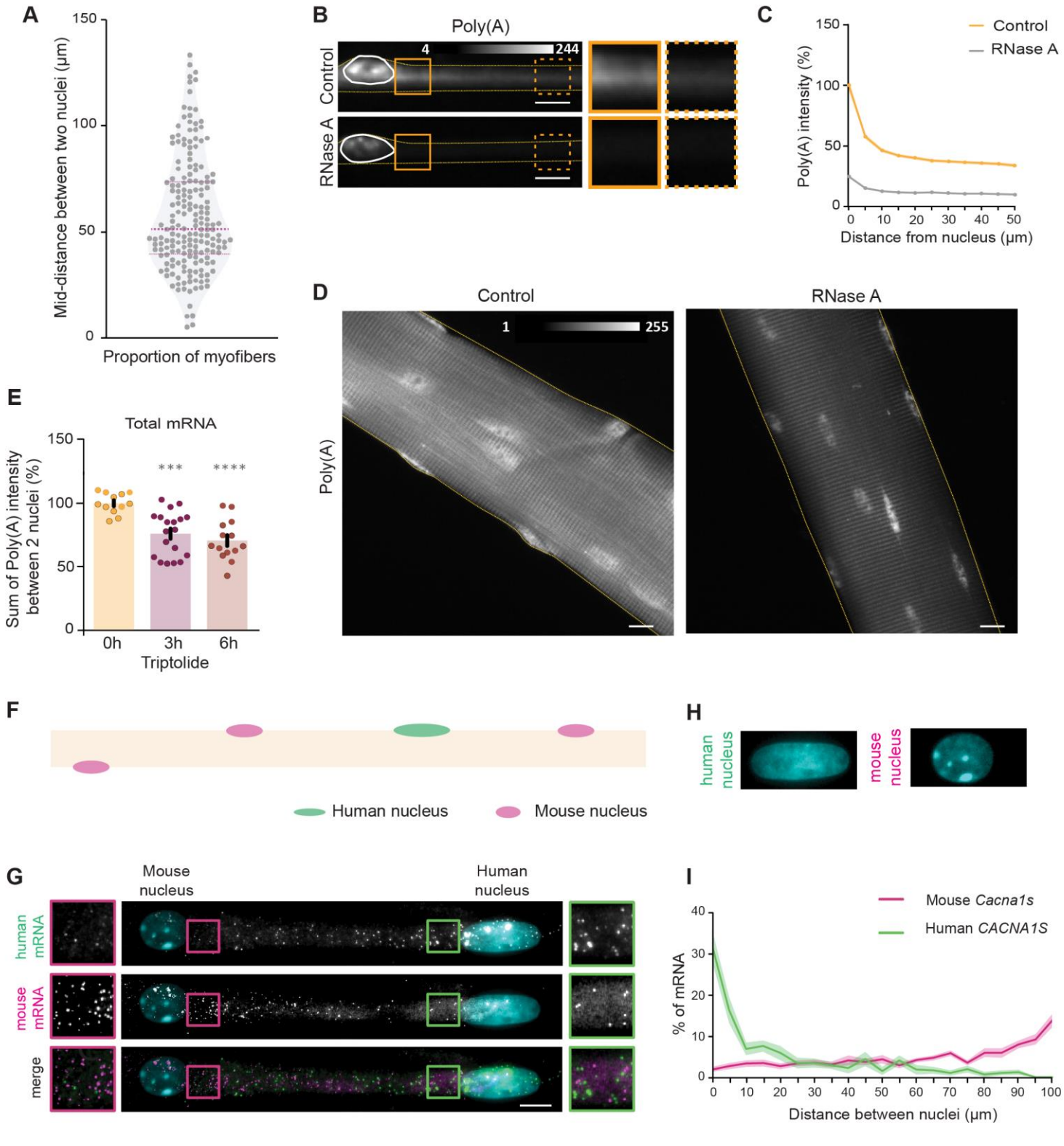
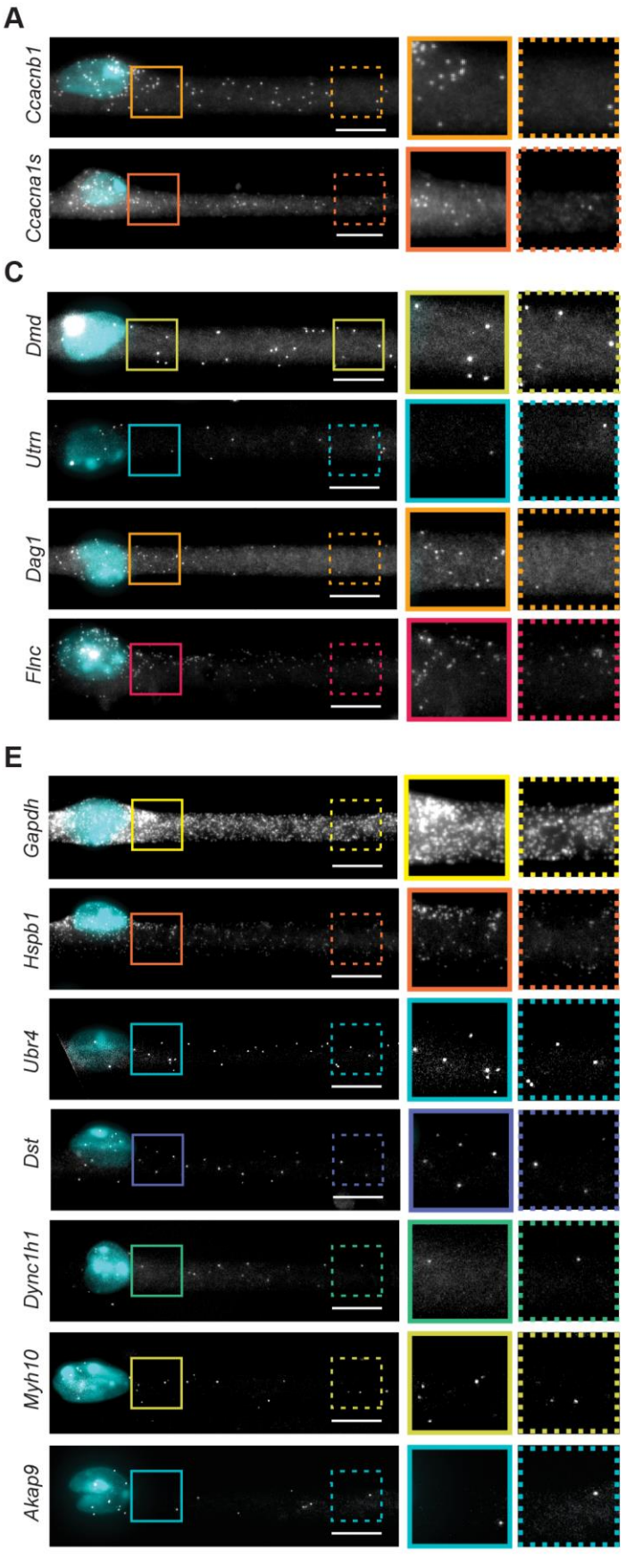


Fig. S1. Relative to Fig. 1.

(A) Violin plot representing the distribution of the mid-distance between two adjacent nuclei in *in vitro* myofibers. The width of the shaded area represents the proportion of myofibers by the mid-distance between two nuclei. Dotted lines represent the median and the interquartile range. The distance between two adjacent nuclei was measured in 188 cells from more than 3 independent experiments. (B) Representative FISH image of total mRNA in myofibers treated for 1h with RNase A. (C) Quantification of total mRNA distribution in RNase A-treated myofibers. Mean ± SEM of 11 (control) and 11 (RNase treated) cell segments in 2 independent experiments. (D) Representative FISH image of total mRNA in isolated fibers treated for 1h with RNase A. (E) Average of total poly(A) mRNA intensity in SUM projections of segments between two adjacent nuclei in myofibers treated for 3h and 6h with triptolide. Mean ± SEM of 12, 19 and 14 cells from 2 independent experiments. (F) Schematic representation of the species mosaic experiment. (G) smFISH image of human and mouse Cacna1s mRNA in a segment of a mouse myofiber containing a single human nucleus. (H) Human and mouse nuclei stained with DAPI. (I) Quantification of the percentage of human and mouse Cacna1s mRNA from the mouse nucleus to the human nucleus. Mean ± SEM of 7 (human mRNA) and 13 (mouse mRNA) cell segments. Shade indicates SEM. (***$p < 0.001$, ****$p < 0.0001$. One-way ANOVA with Tukey's multiple comparisons test. smFISH images are maximum intensity projections. Scale bars: 10 um.)



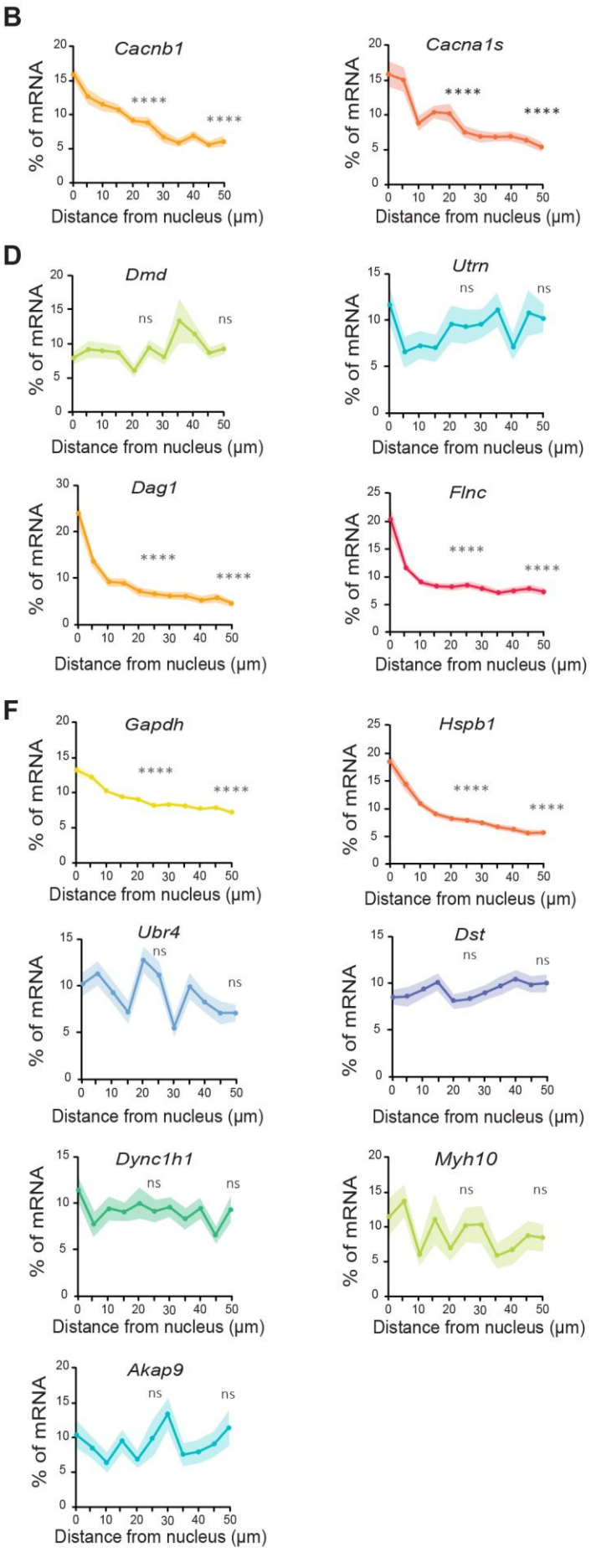


Fig. S2. Relative to Fig. 2. (A, C, E) Representative smFISH images of the distribution of *Cacnb1* and *Cacna1s (A), Dmd, Utrn, Dag1* and *Flnc (C)* and *Gapdh, Hspb1, Ubr4, Dst, Dync1h1, Myh10* and *Akap9* (E) mRNAs. 1.5x magnification of the perinuclear region (undashed) and 50 um away from the nucleus (dashed) on the right. (B, D, F) Quantification of mRNA amount relative to the distance to the nucleus (5 um bins), normalized to total mRNA counts in each cell segment. Statistical significance presented at 25 and 50 um comparing with 0 um. Mean ± SEM of 41 *(Cacnb1), 34 (Cacna1s),* 29 *(Dmd), (Utrn), (Dag1), (Flnc),* 45 (*Gapdh), Hspb1, Ubr4, Dst, Dync1h1, Myh10* and *Akap9* cell segments from 3 to 6 independent experiments. (ns $p > 0.05$, ****$p < 0.0001$, one-way ANOVA with Tukey's multiple comparisons test. Shade indicates SEM. smFISH images are maximum intensity projections. Scale bars: 10 um.)

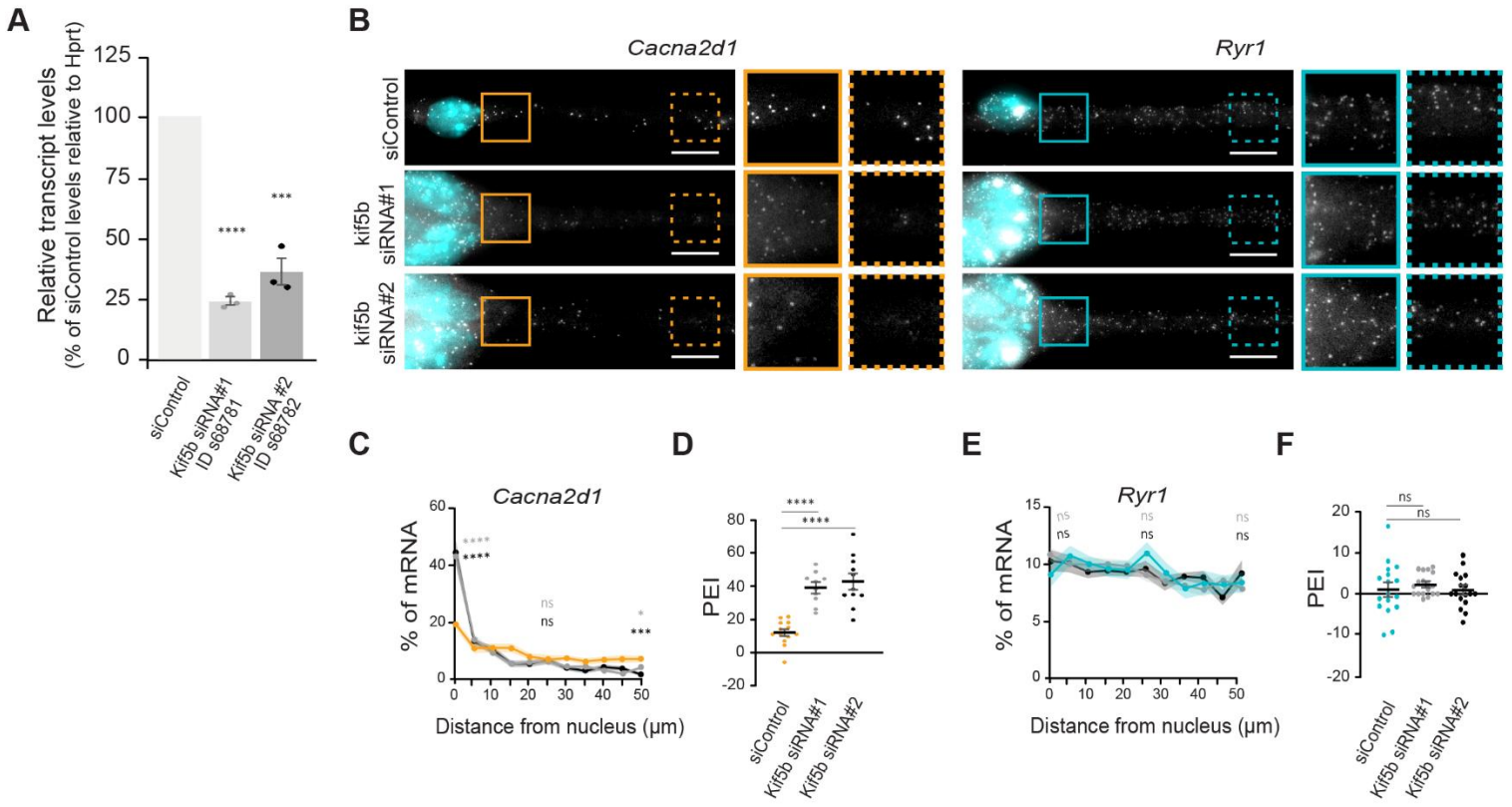


Fig. S3. Relative to Fig. 2. (A) Quantification of Kif5b mRNA amount (normalized to Hprt) measured by RT-qPCR in myofibers transfected with Kif5b siRNAs #1 (s68781) and #2 (s68782) relative to control. (B) Representative smFISH image of the distribution of *Cacna2d1* and *Ryr1* mRNAs in control and Kif5b depleted myofibers. Kif5b depletion results in myofibers that exhibit nuclei aggregation. 1.5x magnification of the perinuclear region (undashed) and 50 um away from the nuclei (dashed) on the right. (C, E) Quantification of *Cacna2d1* and *Ryr1* distribution in control and Kif5b depleted myofibers. (D, F) PEI of *Cacna2d1* and *Ryr1* in control and kif5b depleted myofibers. (ns $p > 0.05$, *$p < 0.05$, ***p < 0.001, ****$p < 0.0001$, one-way ANOVA with Tukey's multiple comparisons test. Shade indicates SEM. smFISH images are maximum intensity projections. Scale bars: 10 um.)

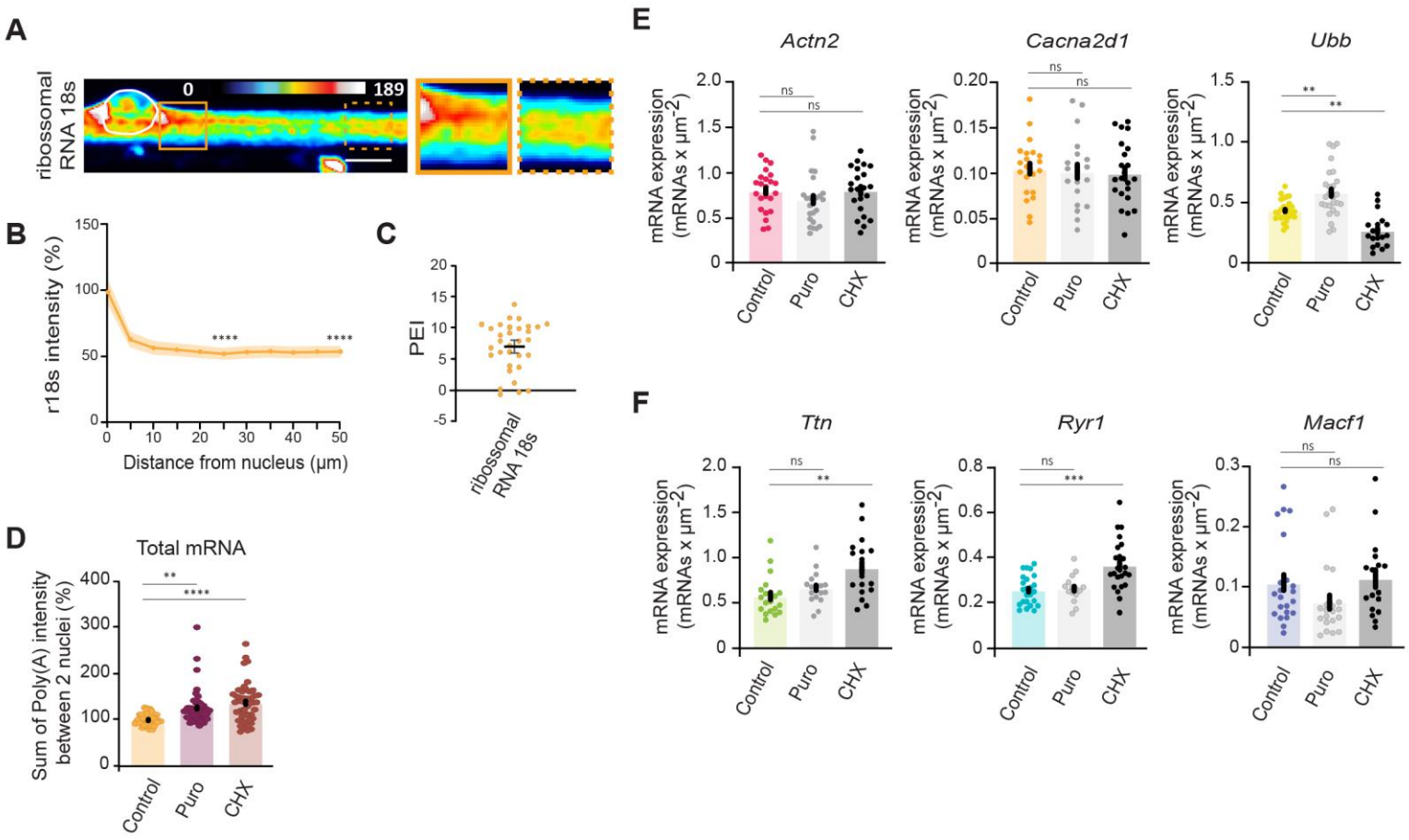


Fig. S4. Relative to Fig. 4. (A) Representative FISH image of 18S ribosomal RNA. (B) Quantification of 18S ribosomal RNA distribution. (C) PEI of 18S ribosomal RNA. (D) Quantification of total poly(A) mRNA intensity in segments between two adjacent nuclei in myofibers treated for 6h with puromycin and cycloheximide. Mean ± SEM of 40, 48 and 47 cell segments. (E) Quantification of *Actn2, Cacna2d1* and *Ubb* mRNA amount measured as number of mRNAs per um^2 by smFISH in segments of myofibers between two adjacent nuclei in control, cycloheximide and puromycin-treated myofibers. Mean ± SEM of 23, 24 and 22 (*Actn2*), 21, 19 and 22 (*Cacna2d1*) and 26, 26 and 19 (*Ubb*) cells from 3 independent experiments. (F) Quantification of *Ttn, Ryr1* and *Macf1* mRNA amount measured as number of mRNAs per um^2 by smFISH in segments of myofibers between two adjacent nuclei in control and cycloheximide and puromycin-treated myofibers. Mean ± SEM of 19, 17 and 18 (*Ttn),* 24, 15 and 23 (*Ryr1*) and 22, 20 and 17 (*Macf1*) cells from 3 independent experiments. (ns $p > 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, one-way ANOVA with Tukey's multiple comparisons test. Shade indicates SEM. smFISH images are maximum intensity projections. Scale bars: 10 um.)

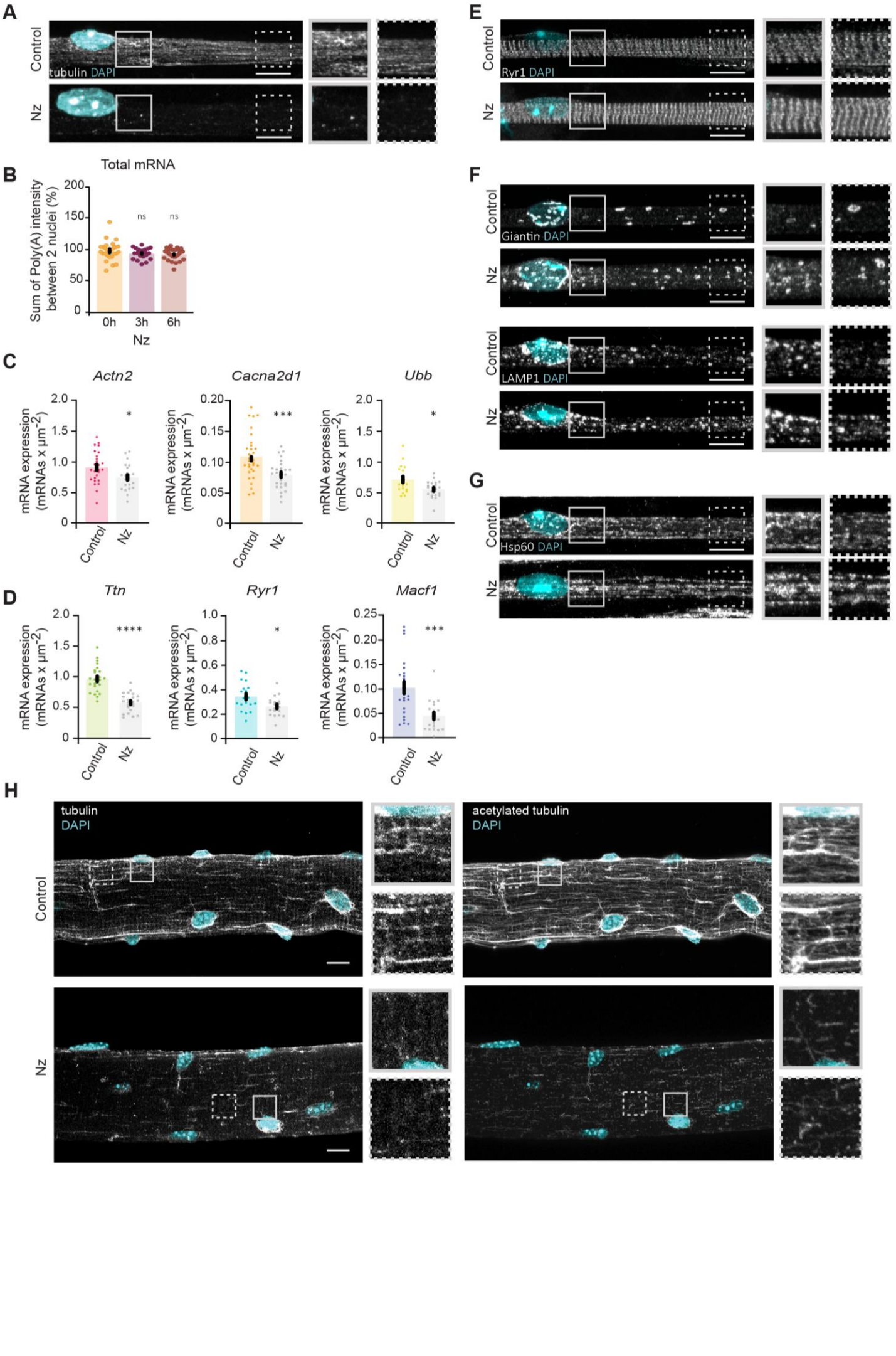


Fig. S5. Relative to Figs 5 and 6. (A) Representative image of microtubules in control and myofibers treated with nocodazole for 6h stained for tubulin. 1.5x magnification of the highlighted region on the right. (B) Quantification of total poly(A) mRNA intensity in segments between two adjacent nuclei in myofibers treated for 3h and 6h with nocodazole. Mean ± SEM of 21-28 cells from 3 independent experiments. (C) Quantification of *Actn2, Cacna2d1* and *Ubb* mRNA amount measured as number of mRNAs um^{-2} by smFISH in segments of myofibers between two adjacent nuclei in control and nocodazole-treated myofibers. Mean ± SEM of 27 and 22 (*Actn2*), 31 and 29 (*Cacna2d1*) and 19 and 20 (*Ubb*) cells from 3 independent experiments. (D) Quantification of *Ttn, Ryr1* and *Macf1* mRNA amount measured as number of mRNAs um^{-2} by smFISH in segments of myofibers between two adjacent nuclei in control and nocodazole-treated myofibers. Mean ± SEM of 23 and 23 (*Ttn),* 21 and 19 (*Ryr1*) and 24 and 20 (*Macf1*) cells from 3 independent experiments. (E-G) Representative images of (E) sarcoplasmic reticulum (Ryr1), (F) golgi (Giantin) and lysosomes/late-endosomes (LAMP1) and (G) mitochondria (Hsp60) in control and nocodazole-treated myofibers. (H) Representative image of microtubules in control and isolated fiber treated with nocodazole for 9h, stained for detyrosinated and acetylated tubulin. 3x magnification of the highlighted regions on the right. (ns $p > 0.05$, *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$. One-way ANOVA with Tukey's multiple comparisons test and student's *t* test for comparisons between 2 experimental conditions. 95% confidence interval. Images are maximum intensity projections. Scale bars: 10 um.)

Table S1. Summary table of the main characteristics of the cohort of transcripts analyzed and their encoded proteins. Predictive mRNA sizes were obtained from the National Center for Biotechnology Information (NCBI) database and correspond to the longer isoform. Protein mass was estimated using the formula: Weight (kDa) = number of aminoacids x 110 / 1000.

mRNA	PEI ± SEM	Expression ± SEM (mRNA.um^{-2})	Size (bp)	NCBI accession	Protein	Weight (kDa)	Type	Function /Localization	Muscle specific
Dag1	19.63 ± 2.6	2.6 ± 0.15	5591	NM_010017.4	Dystroglycan	98	TM	Sarcolemma	No
Scn4a	18.99 ± 3.1	3.1 ± 0.04	6598	NM_133199.2	Sodium channel protein type 4 subunit alpha	202	TM	Triads	Yes
Ubb	15.48 ± 1.4	1.4 ± 1.97	1513	NM_011664.4	Ubiquitin B	34	Cyt	Protein degradation	No
Jph1	13.28 ± 2.1	2.1 ± 0.07	4809	NM_020604.2	Junctophilin-1	73	TM	Triads	Yes
Flnc	13.06 ± 3.1	3.1 ± 0.37	9167	NM_001081185.2	Filamin-C	300	Cyt	Sarcolemma	Yes
Hspb1	12.66 ± 1.5	1.5 ± 0.49	913	NM_013560.2	Heat shock protein beta-1	23	Cyt	Stress response	No
Cacna1s	10.81 ± 1.9	1.9 ± 0.09	6341	NM_001081023.2	Calcium Voltage-Gated Channel Subunit α1S	204	TM	T-Tubule	Yes
Cacnb1	10.09 ± 1.2	1.2 ± 0.08	3524	NM_001282977.1	Calcium Voltage-Gated Channel Auxiliary Subunit β1	71	Cyt	T-Tubule	Yes
Cacna2d1	9.55 ± 2.5	2.5 ± 0.10	7472	NM_001110843.1	Calcium Voltage-Gated Channel Auxiliary Subunit α2δ1	121	TM	T-Tubule	Yes
Gapdh	6.47 ± 1.1	1.1 ± 1.40	1296	NM_001289726.1	Glyceraldehyde 3-phosphate Dehydrogenase	40	Cyt	Glycolysis	No
Actn2	5.25 ± 1.0	1.0 ± 0.79	3091	NM_033268.4	α-actinin 2	98	Cyt	Sarcomere	Yes
Obscn	3.17 ± 2.7	2.7 ± 0.04	24175	NM_001171512.2	Obscurin	884	Cyt	Sarcomere	Yes
Ubr4	3.08 ± 1.4	1.4 ± 0.04	15901	NM_001160319.1	E3 ubiquitin-protein ligase UBR4	570	Cyt	Protein degradation	No
Myh10	2.91 ± 3.4	3.4 ± 0.02	7783	NM_175260.2	Myosin-10	217	Cyt	Cytoskeleton	No
Macf1	2.39 ± 1.1	1.1 ± 0.12	23495	NM_001199136	Microtubule actin crosslinking factor 1	809	Cyt	Cytoskeleton	No
Dync1h1	2.04 ± 2.0	2.0 ± 0.05	14398	NM_030238.2	Cytoplasmic dynein 1 heavy chain 1	511	Cyt	Cytoskeleton	No
Ttn	1.63 ± 0.7	0.7 ± 0.92	101674	NM_011652.3	Titin	3681	Cyt	Sarcomere	Yes
Utrn	1.59 ± 2.5	2.5 ± 0.03	12382	NM_011682.4	Utrophin	42	Cyt	Costamere	No
Ryr1	0.74 ± 1.7	1.7 ± 0.20	15362	NM_009109.2	Ryanodine receptor 1	554	TM	Sarcoplasmic Reticulum	Yes
Dmd	-1.41 ± 1.3	1.3 ± 0.06	13852	NM_007868.6	Dystrophin	404	Cyt	Costamere	Yes
Dst	-1.52 ± 1.3	1.3 ± 0.09	24271	NM_001276764.1	Dystonin	849	Cyt	Cytoskeleton	No
Neb	-3.04 ± 0.7	0.7 ± 0.72	22489	NM_010889.1	Nebulin	787	Cyt	Sarcomere	Yes
Akap9	-3.21 ± 3.4	3.4 ± 0.02	12105	NM_194462.2	A-kinase anchor protein 9	416	Cyt	MT nucleation	No

Table S2. smFISH probe sequences (5’ to 3’).

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