Online Supplemental Materials

Loss of Fused in sarcoma (FUS) promotes pathological Tau splicing

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Legends for Supplemental Figures and Tables

Supplemental Figure S1: Tau genomic structure and primer design

(A) Exon structure of the MAPT gene. Exons with alternative splicing in the CNS are shown in red. Note that exon 4a is only included in the peripheral nervous system. Exon 6 and 8 are not transcribed in human CNS. (B) Isoform specific primers used for qPCR. Upstream primers for 0N, 1N and 3R isoforms bind to the splice site of exons 1/4, 2/4 and 9/11, respectively. (C) Primers used for pre-mRNA qPCR. Specificity was confirmed by sequencing of the PCR products.

Supplemental Figure S2: FUS knockdown has no effect on cell viability and expression of several synaptic proteins

Hippocampal neurons (DIV2+7) were infected with lentivirus expressing the indicated shRNAs, GFP or HA-tagged FUS. (A) Neuron viability was measured by XTT assay. FUS knockdown and overexpression did not significantly alter cell viability compared to shLuc or GFP, respectively (one-way ANOVA, n=12). Mean +/- SEM are given. (B) Immunoblots with the indicated antibodies show expression of neuronal proteins. n=2 replicates shown. Compare to Figure 1A.

Supplemental Figure S3: TDP-43 knockdown does not significantly affect Tau splicing

Hippocampal neurons (DIV2+7) infected with control (shLuc) or a TDP43-knockdown virus (shTDP-43). Total RNA was analyzed by qPCR for TDP-43, total Tau and Tau isoforms. TDP-43 and total Tau expression was normalized to YHAWZ. Tau isoform expression was normalized to total Tau levels. n=6, mean +/- SEM, Student's t-test: ** $p<0.01$.

Supplemental Figure S4: FUS knockdown in hippocampal neurons does not affect neurite number, axonal branching and cell viability

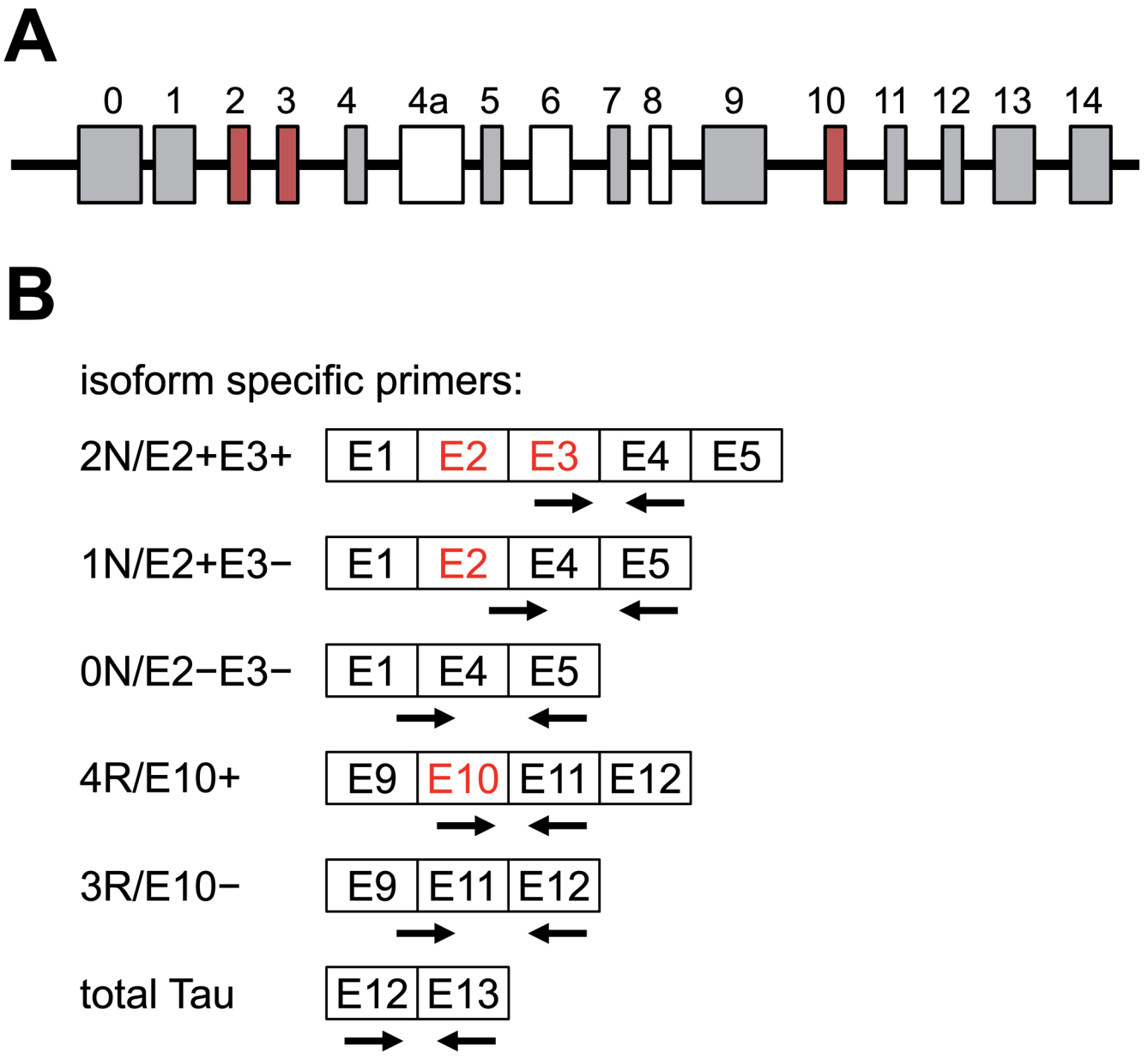
Hippocampal neurons co-transfected with the shLuc or shFUS#2 and EYFP-C1 prior to plating. (A) Immunostaining at day 4 with anti-YFP for transfection control and anti-FUS antibody to confirm the knockdown efficiency. Arrows mark transfected cells. (B) Quantification of the number of neurites (left, n=204 for shLuc, n=235 for shFUS#2) and axonal branch points (right, n=161 for shLuc, n=177 for shFUS#2) measured blinded to the experimental condition. (C) Neuron viability was measured by XTT assay after 14 days in culture. Prolonged FUS knockdown did not significantly alter cell viability (t-test, n=5 independent experiments). Means +/- SEM are given.

Supplemental Figure S5: Tau RNA probes used for crosslinking experiments

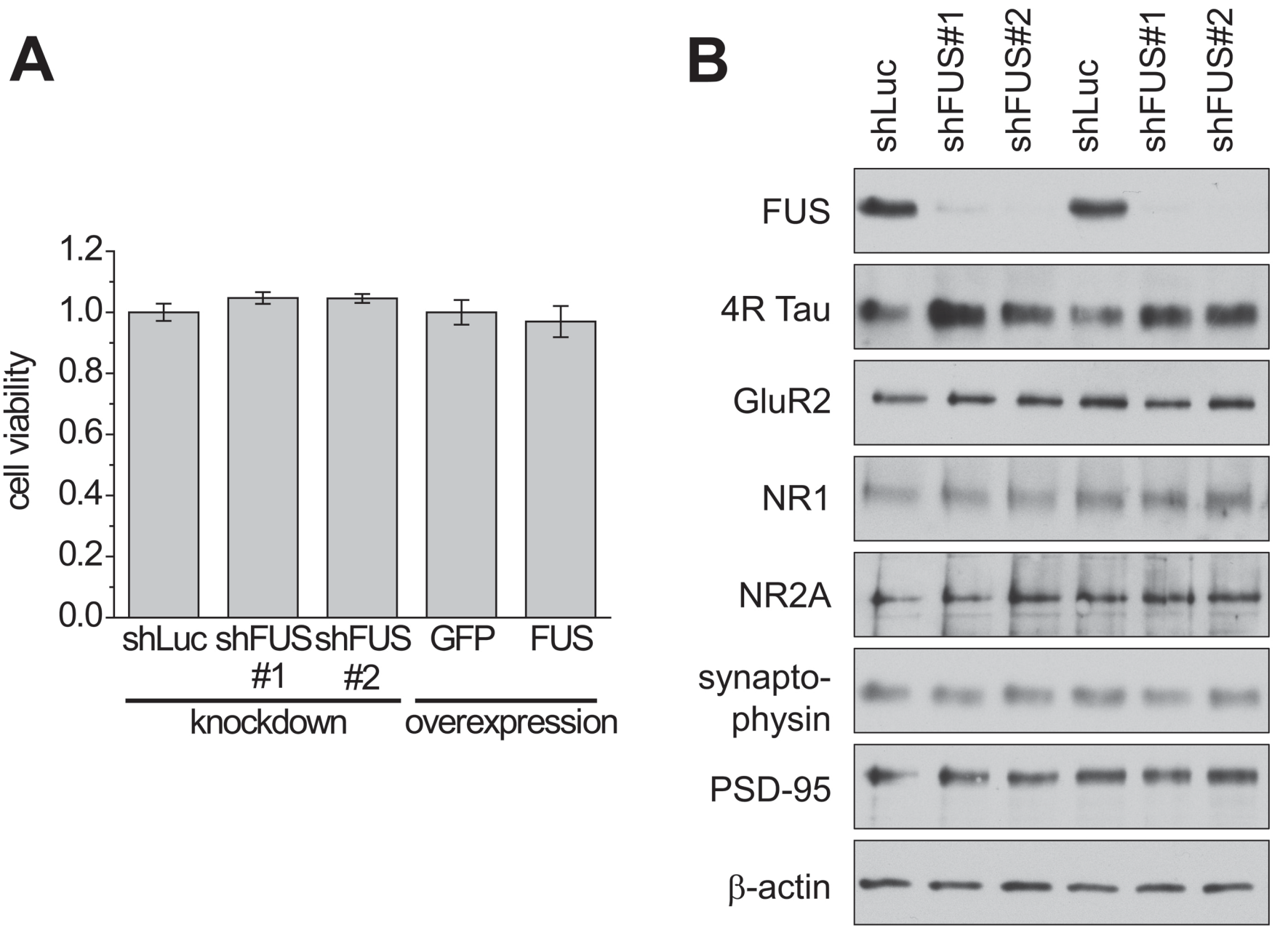
(A) Approximate localization of the RNA probes within the Tau pre-mRNA. FUS-interacting RNA probes are depicted in red. Compare Figure 3C. (B, C, D) ClustalW alignment of mouse, rat and human Tau pre-mRNA for the probes e10, i9-2 and i2-1. Exon 10 spans position 144 to 236 of the alignment in (B).

Supplemental Table S1: DNA oligonucleotides used in this study

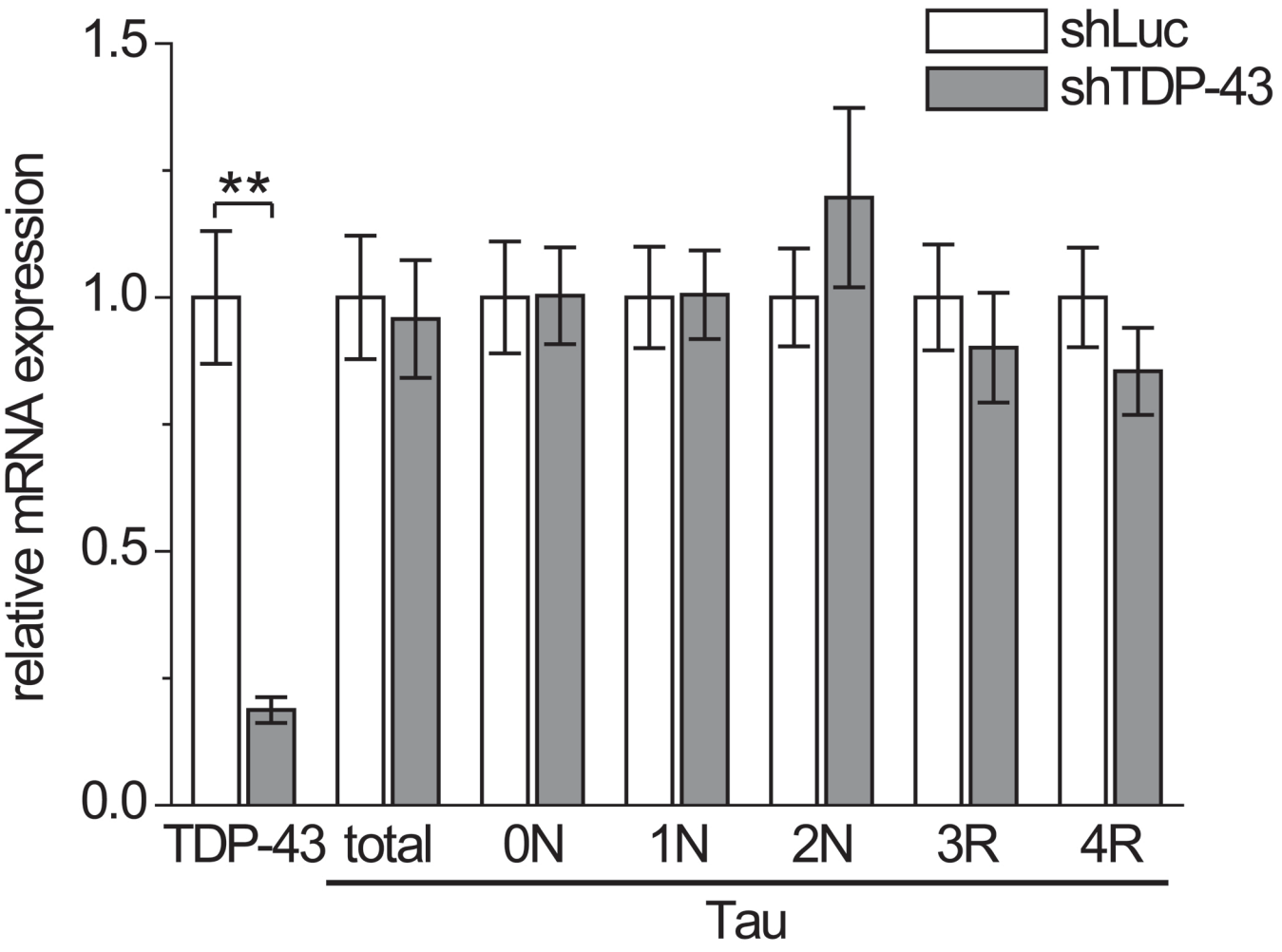
Oligonucleotides used for cloning shRNA and overexpression constructs. Primers used for qPCR are also listed. The second column denotes the originating species or the species that are targeted by the shRNAs or PCR assays (h=human, m=mouse, r=rat).



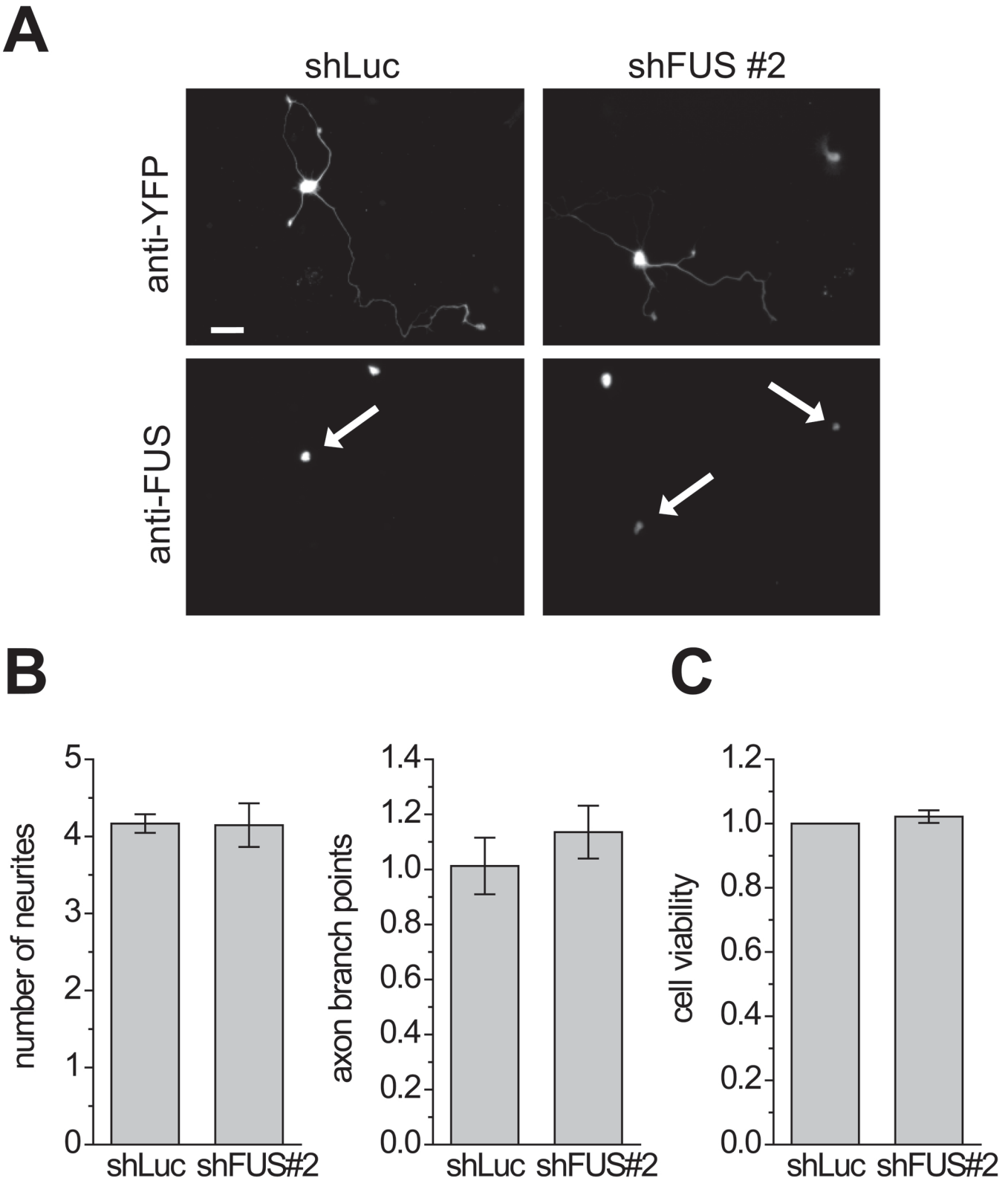
Orozco et al., Supplemental Figure S1



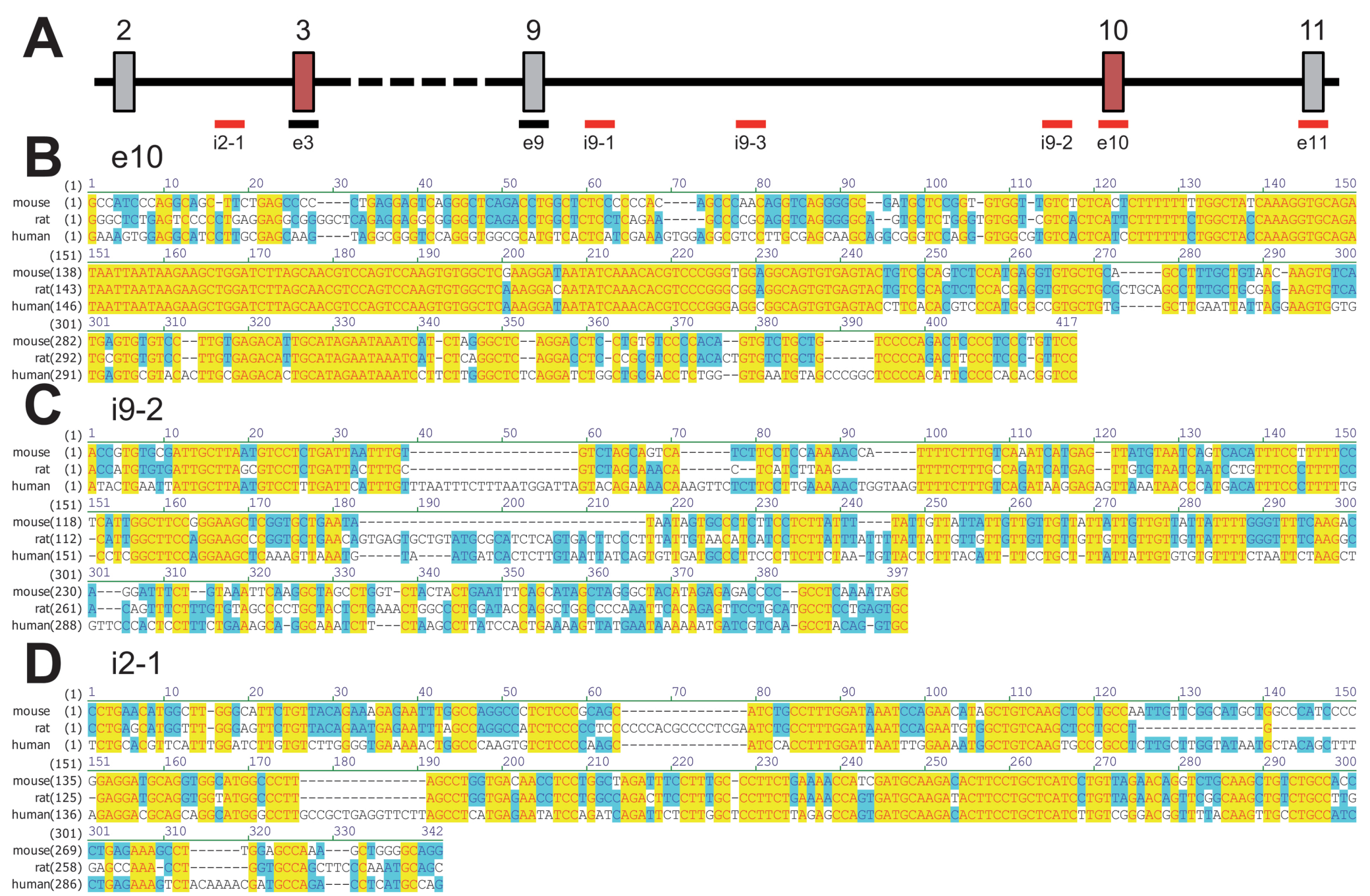
Orozco et al., Supplemental Figure S2



Orozco et al., Supplemental Figure S3



Orozco et al., Supplemental Figure S4



Orozco et al., Supplemental Figure S5

Orozco et al, Supplemental Table S1

shRNA cloning	**species**	**sense**	**antisense**
shFUS#1	m/r	gatccccGTGCAAGGCCTAGGCGAtAttcaagagaTCTCGCCTAGGCCTTGCACtttttggaaa	agcttttccaaaaaGTGCAAGGCCTAGGCGAtAtctcttgaaTCTCGCCTAGGCCTTGCACggg
shFUS#2	m/r	gatccccGGCCTAGGCGAGAATGTTAttcaagagaTAACATTCTCGCCTAGGCCtttttggaaa	agcttttccaaaaaGGCCTAGGCGAGAATGTTAtctcttgaaTAACATTCTCGCCTAGGCCggg
shFUS-hu	h	gatccccGGACAGCAGCAAAGCTATGttcaagagaCATAGCTTTGCTGCTGTCCtttttggaaa	agcttttccaaaaaGGACAGCAGCAAAGCTATGtctcttgaaCATAGCTTTGCTGCTGTCCggg
shTDP-43	m/r	gatccccGTAGATGTCTTCATTCCCAAAttcaagagaTTTGGGAATGAAGACATCTACtttttggaaa	agcttttccaaaaaGTAGATGTCTTCATTCCCAAAtctcttgaaTTTGGGAATGAAGACATCTACggg
HA-FUS		**sense**	**antisense**
5' PCR	h	gaggatccGCGGACATGTACCCATACGACGTC	caattgTcACgTTtTCACCCAGGCCttgcacaaagatgg
3' PCR	h	GGCCTGGGTGAaAAcGTgAcaattgagtctgtggctg	gaGATATCTTAATACGGCCTCTCCCTtCGgTCtTGcCTGTGCTCACCCCTGGAA
mRNA qPCR	**species**	**sense**	**antisense**
total Tau	m/r	tccagtcgaagattggctccttgg	aggtgccgtggagatgtgtccc
0N Tau	r	ccatggcttaaaaGCtgaaga	cctgtcctgtctttgcttacg
1N Tau	r	tgctgaaGCtgaagaagcag	cctgtcctgtctttgcttacg
2N Tau	r	gaagagagagctcccgacaa	cagcttggtcctccatgttc
3R Tau	r	gcggcaaGGtgcaaatagt	acgatttctgctccatggtc
4R Tau	m/r	aagaagctggatcttagcaacg	ctggcttgtgatggatgttc
FUS	m/r	ctggcaagttgaagggtgaggc	gctcgttgctgtcctccacctc
TDP-43	m/r	agtgttgggtctcccctggaaa	acagtcacaccatcgcccatct
YWHAZ	m/r	tgagcagaagacggaaggtgctg	tctgatggggtgtgtcggctgc
GAPDH	m/r	ccgcatcttcttgtgcagtgcc	agactccacgacatactcagcacc
SON	m/r	tcttctcagcttgctctgga	caggcaaaggtccctctatg
pre-mRNA qPCR	**species**	**sense**	**antisense**
Tau exon 10	m	ctccggtgtggttgtctctc	agcacacctcatggagactg
GAPDH exon 4	m	tctgaaatcaacttctttccctta	tgtctccccactgcctacat
Tau minigene	**species**	**sense**	**antisense**
MAPT exon 9-11	m	caatcttcgacctgacattctttaggtctggcatgggcacaggggcagtctgcagCAGGTCCTCCTCCGAGATAAGCTTCTGCTC	gagcaaagtgacctccaagtgtggctcgttagggaacatccatcacaagccaACGCGTTACCCATACGACGTCCCAGACTAC
Tau RNA probes	**species**	**sense**	**antisense**
Tau e3	m	atgGAATTCcactccttgcactgcccctgcat	gatTCTAGAtgcacccgactatgagggtgacct
Tau e9	m	atgGAATTCctcagggactttgcagacgccgc	gatTCTAGAtactggctaggtgggagtctggct
Tau e10	m	atgGAATTCgccatcccaggcagcttctgagc	gatTCTAGAggaacagggaggggagtctgggg
Tau e11	m	atgGAATTCgcagcagctgatgggagccactg	gatTCTAGAagacccaggtccaaggccacaca
Tau i2-1	m	atgGAATTCctgaacatggcttgggcattctgtt	gatTCTAGAcctgccccagctttggctcc
Tau i9-1	m	atgGAATTCaggaatgacagcctgtttctacacct	gatTCTAGAacacctttgaaggagaaagatgaagtctttga
Tau i9-2	m	atgGAATTCaccgtgtgcgattgcttaatgtcct	gatTCTAGATgctattttgaggcggggtctctct
Tau i9-3	m	atgGAATTCaaggtcgatctccaccacaggcaaa	gatTCTAGAccatctcaccagacccaagccctttt