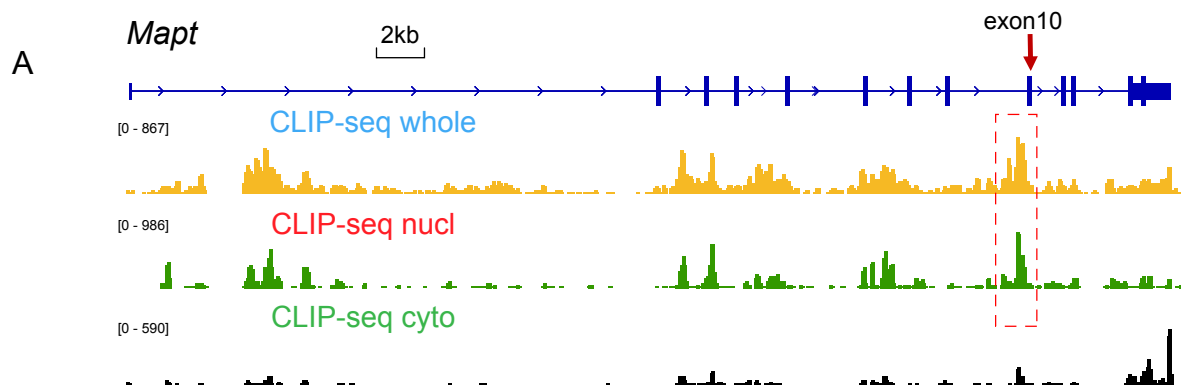


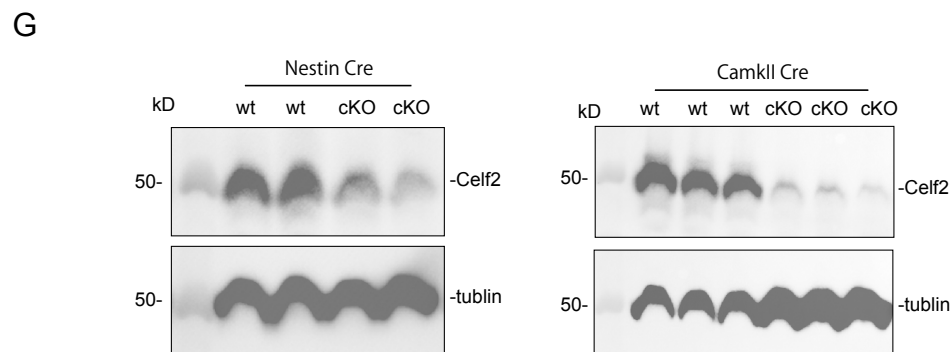
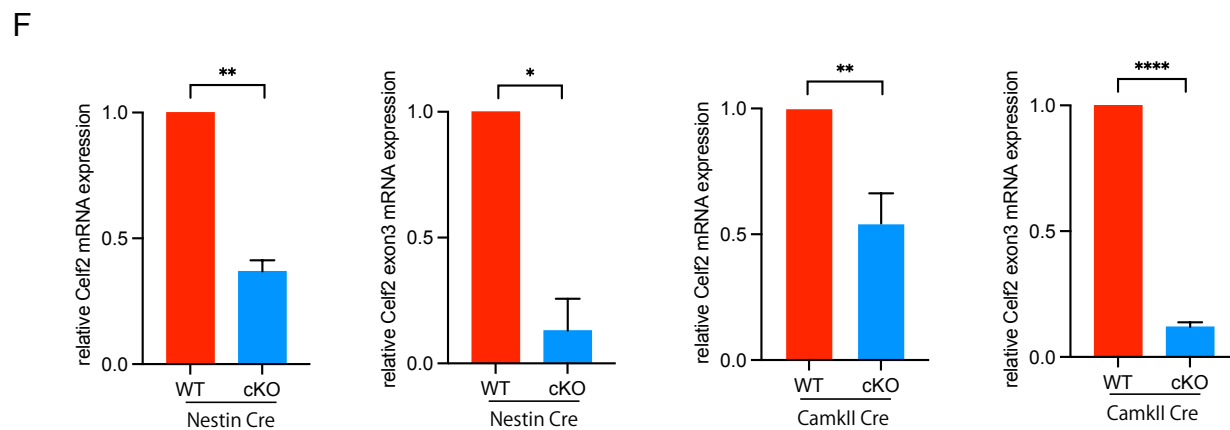
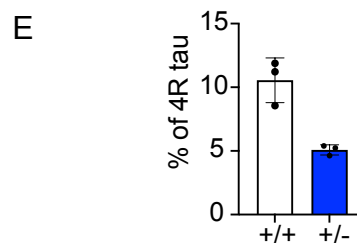
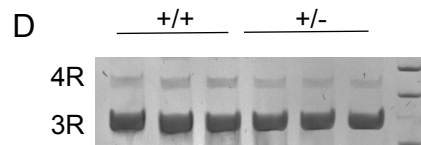
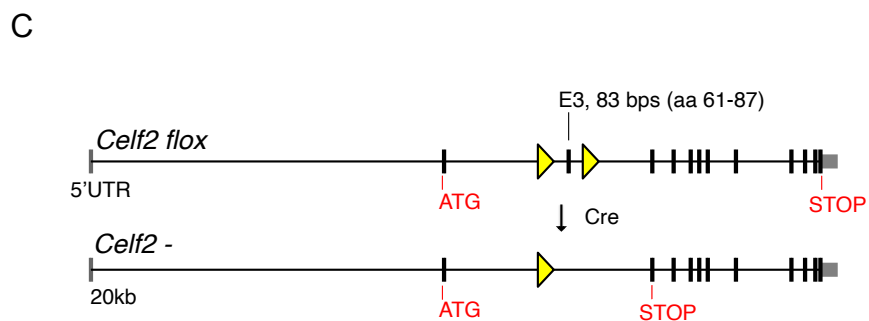


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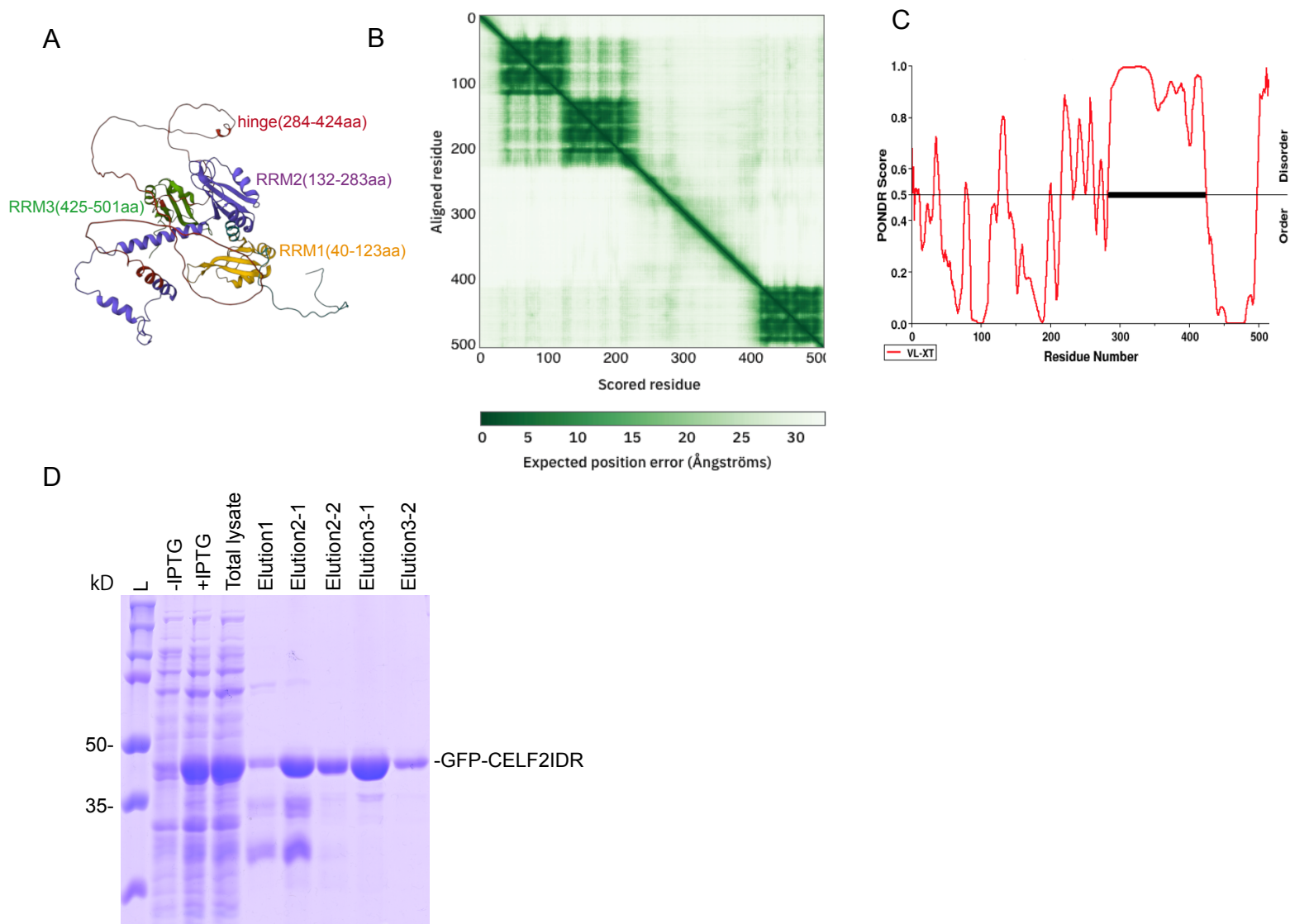
CELF2 binding motif: (UG)n and UGUU

Binding peak sequence (mm9, chr 11, 104179002 – 104179227):

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 CC**TG**AGCCATCCCACCAGCTCAGCTCAAGACAATC**TG**GTT**TGA**AGCCTTTAGCCT**TG**CCACTCCCCAGCCT**TG**GA
 CGCTCTT**TGTG**ATAAGGT**TG**CCT**TG**CAC**TGGTGTG**TC**TGTTTG**GATT**TGTGTTG**ATCTTTCTCTCATCACT**TG**AC

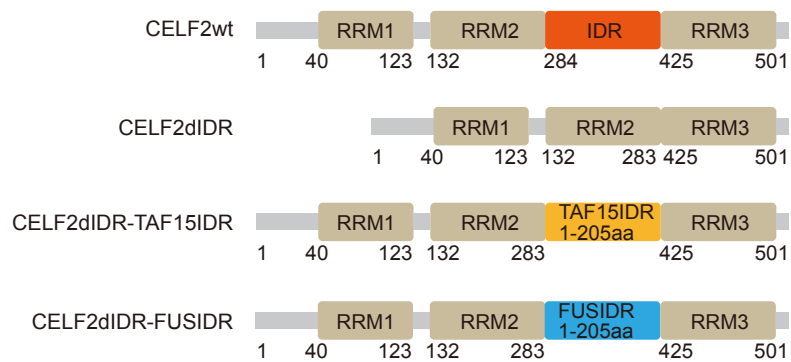


Supplementary Figure. 1. The binding sites of CELF2 on *Mapt* mRNA and validation of *Celf2* cKO mouse. (A) Genome browser view of CLIP-seq peaks on the *Mapt* gene locus. CLIP-seq was performed using mouse N2A cells and the CLIP-seq reads were aligned to mouse genome (mm9). CLIP-seq results from whole cell lysate, cytoplasmic fraction, and nuclear fraction show that CELF2 binds to 3'-UTR of *Mapt* mRNA in the cytoplasm, and binds to the intron 5'-to the alternatively spliced exon 10 in the nucleus. (B) Sequence of the CELF2 binding peak located in the intron immediately upstream of the alternatively spliced exon 10 of *Mapt*. The peak region is enriched for canonical CELF2-binding motifs, including UG repeats and UGUU sequences. (C) Schematic illustration showing the *Celf2* cKO allele (flox allele). Exon 3 is flanked by two *flox* elements, allowing for exon 3 deletion upon *Cre* expression. (D) RT-PCR showing that one allele of *Celf2* loss is sufficient to impact tau splicing. Brain tissues from *Celf2* KO and wildtype newborn mice were used to generate cDNA for RT-PCR to detect the alternative splicing of *Mapt* exon 10. (E) Quantification of RT-PCR results in (D). $n=3$, $**P = 0.0063$, unpaired t test. (F) RT-qPCR showing that *Celf2* cKO brains have reduced *Celf2* mRNA expression. qRT-PCR was done using hippocampal and cortical tissues of *Nest-CreER;Celf2^{flox/flox}* (4 weeks old), *CamKII-CreER;Celf2^{flox/flox}* (2 months old), and littermate control mice. Two sets of PCR primers were used, one set to amplify 3' end of *Celf2* mRNA and the other set to amplify exon3 specifically. *Celf2* transcript was detected in cKO brains as *Celf2* is only deleted in some cells. to perform RT-qPCR and measure total *Celf2* mRNA and *Celf2* mRNA containing exon 3. (G) Western blot showing that *Celf2* cKO brains have reduced *Celf2* protein expression. Western blot was done using hippocampal and cortical tissues of *Nest-CreER;Celf2^{flox/flox}* (4 weeks old), *CamKII-CreER;Celf2^{flox/flox}* (2 months old), and littermate control mice.

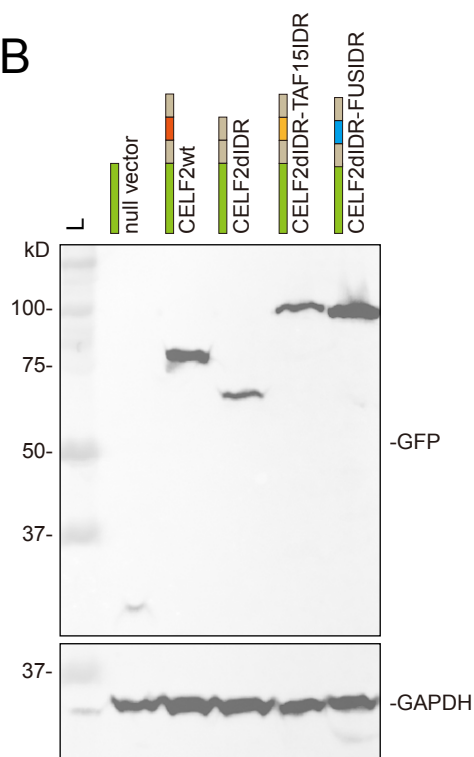


Supplementary Figure 2. CELF2 hinge domain is a low complexity domain. **(A)** Structrue prediction of full-length CELF2 protein by AlphaFold (<https://alphafold.ebi.ac.uk/>). The hinge domain (284- 424) does not exhibit a defined structure. **(B)** Predicted aligned error (PAE) plot for CELF2 protein indicating inter-domain accuracy. The shade of green shows expected distance error, with dark green indicating low error and light green indicating high error. **(C)** Plot of intrinsic protein disorder probability for human CELF2 protein using PrDos (<http://prdos.hgc.jp/cgi-bin/top.cgi>) showing that the hinge domain has high disorder score. **(D)** Coomassie staining of GFP-CELF2IDR (hinge domain) from indicated protein samples. Prior to protein purification, we compared cell lysates from cells without and with IPTG induction. We observed an induced band between 35 KDa and 50 KDa, which matched the size of His-GFP-CELF2NTD (43 KDa). Total lysate from protein purification experiment, flow through and elutes from Ni⁺ columns were also examined.

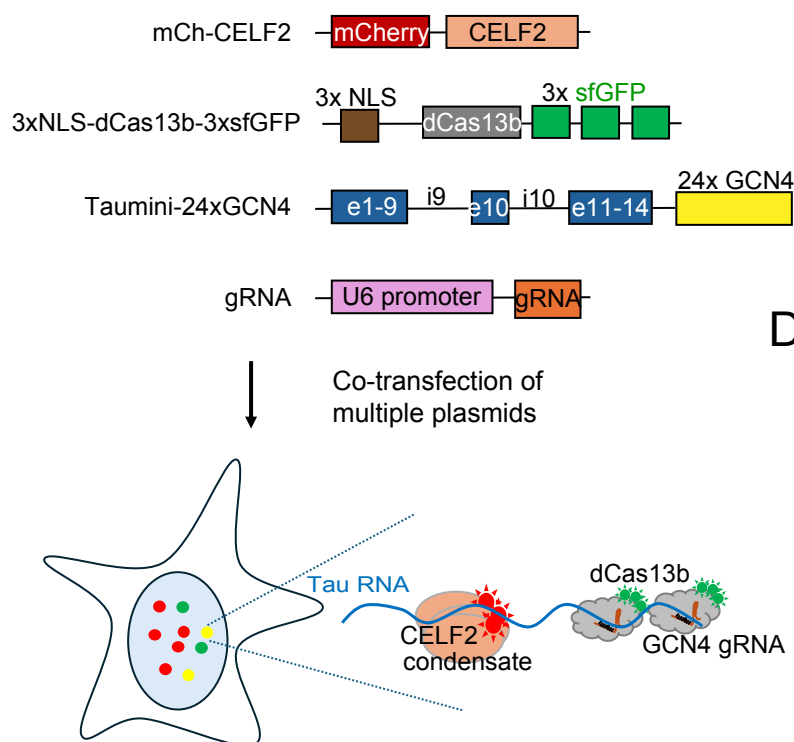
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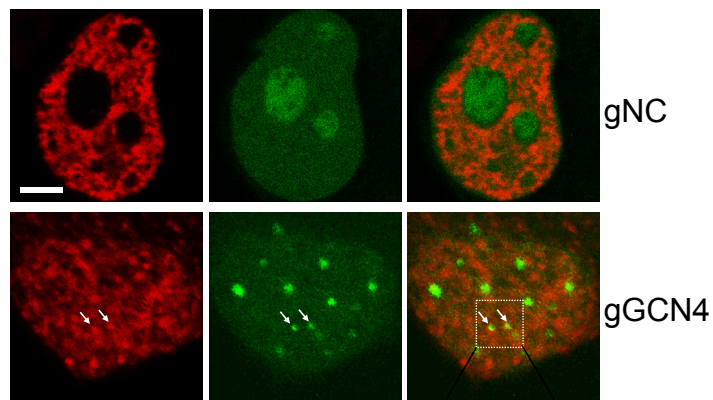
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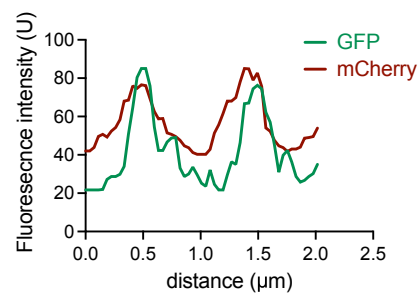
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D

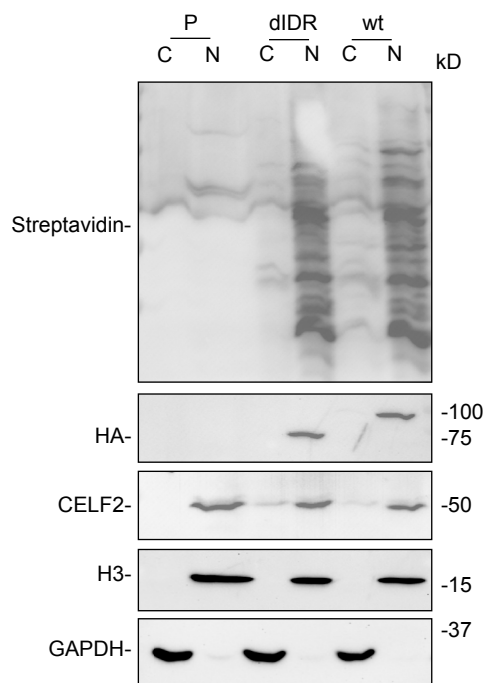


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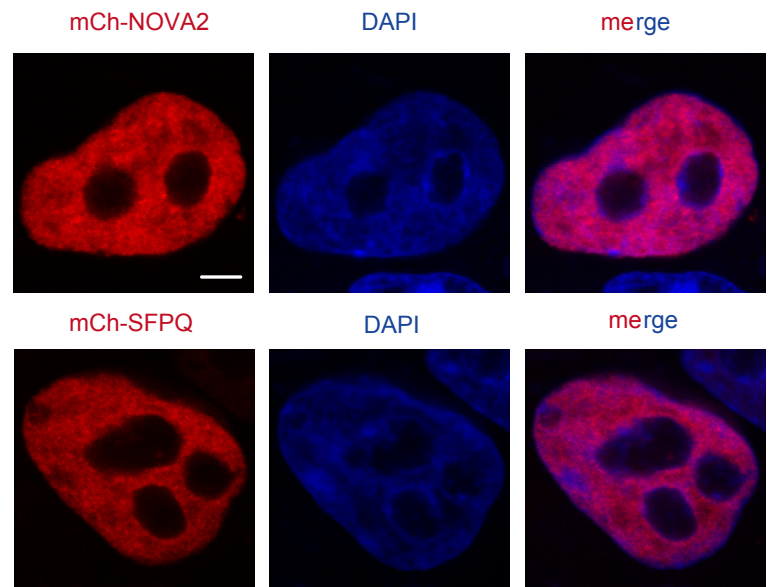


Supplementary Figure.3. Replacing CELF2 IDR with IDRs of TAF15 and FUS. (A) Schematic diagram of the GFP-CELF2 mutant fusion protein constructs. (B) Indicated deletion and fusion proteins were expressed in cells to confirm that their expression. Cells were harvested 48 hours post-transfection for total protein extraction, followed by Western blotting using anti-GFP antibodies to detect their expression. (C) Schematic illustration of the experimental design using CRISPR-based RNA imaging to show colocalization of CELF2 condensates and tau mRNA. (D) Representative confocal images of 293T cells co-transfected with the plasmids indicated in (E). GFP-labeled tau RNA foci were only detected in cells co-transfected with GCN4 gRNA (bottom panels) but not in those co-transfected with the negative control gRNA (gNC) (top panels). (E) Line scan to show the colocalization of GFP foci (tau mRNA) and mCherry foci (CELF2 condensates).

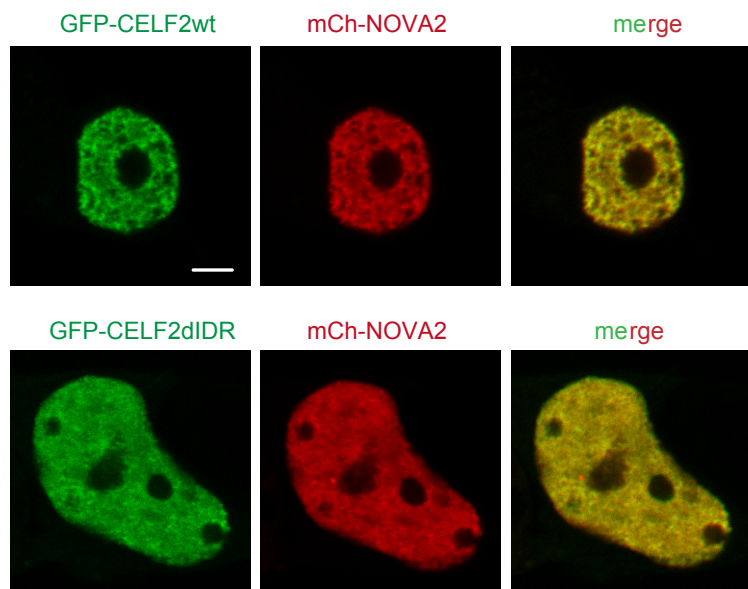
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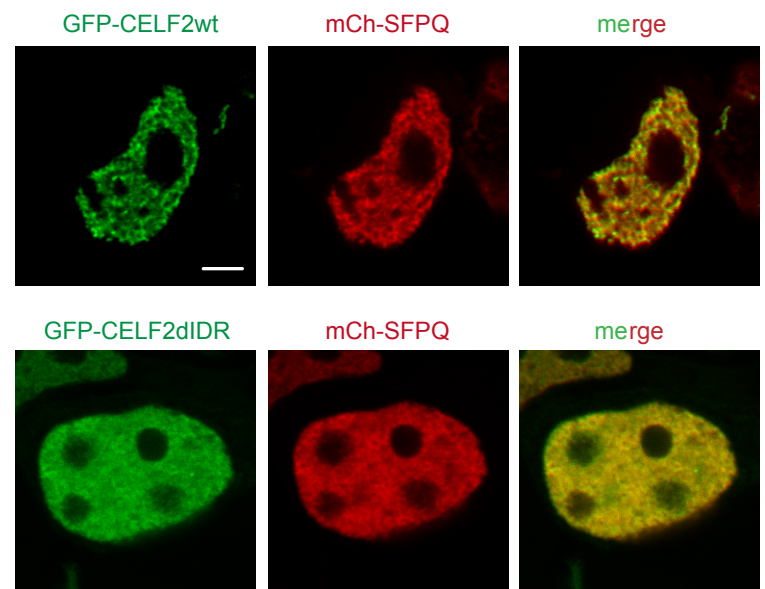
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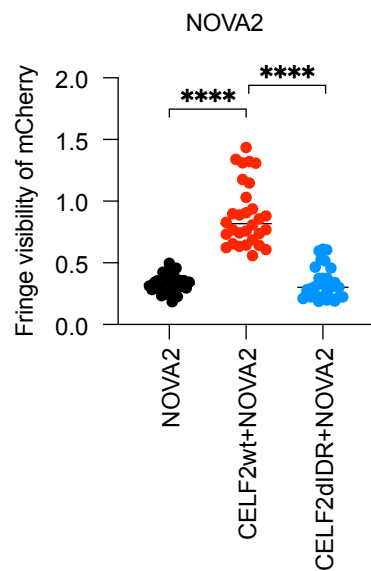
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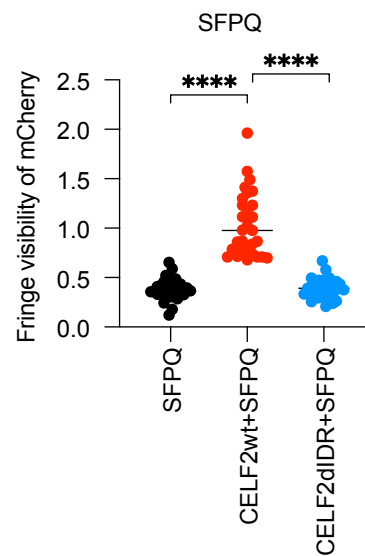
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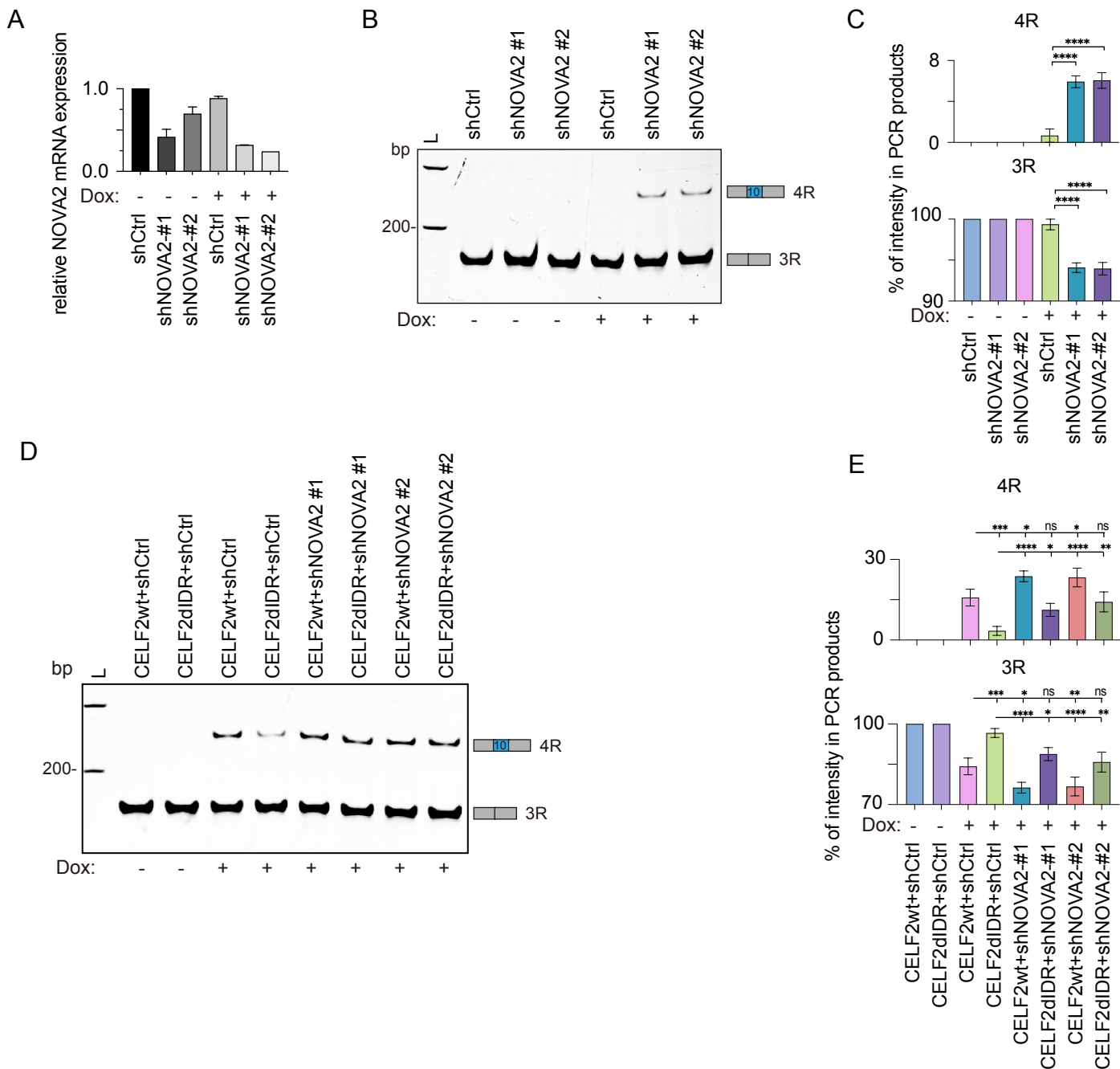
D



F



Supplementary Figure 4. TurboID-identified cofactors of CELF2 colocalize with CELF2 in nuclear assemblies and the colocalization is dependent on CELF2 IDR. **(A)** Western blots confirming the inducible expression and *in vivo* proximity biotinylation in the established tet-on stable SH-SY5Y cell lines for CELF2 TurboID (wt and Δ IDR). The fractionation of nuclear fractions and loading amount were confirmed with Western blots for Histone H3 (nucleus-specific marker). The doxycycline-induced HA-BirA*-CELF2 fusion protein expression was detected by antibodies recognizing CELF2 and HA respectively. Various proteins biotinylated by HA-BirA*-CELF2 were detected using streptavidin-HRP blot. **(B)** Representative images of mCherry-NOVA2 and mCherry-SFPQ in 293 cells, which have low endogenous CELF2 expression. mCherry-NOVA2 and mCherry-SFPQ displayed weakly punctuated expression pattern in the nucleus. **(C-D)** Representative images of 293 cells co-transfected with mCherry-NOVA2 and GFP-CELF2 (wt, Δ IDR) and quantification of fringe visibility of mCherry+ condensates. When co-expressed with GFP-CELF2wt (but not GFP-CELF2 Δ IDR), mCherry-NOVA2 exhibited a more punctate pattern than when expressed alone. This suggests that NOVA2 might be recruited to CELF2 condensates through CELF2 IDR. **(E-F)** Representative images of 293 cells co-transfected with mCherry-SFPQ and GFP-CELF2 (wt, Δ IDR) and quantification of fringe visibility of mCherry+ condensates. When co-expressed with GFP-CELF2wt (but not GFP-CELF2 Δ IDR), mCherry-SFPQ exhibited a more punctate pattern than when expressed alone. Scale bar: 5 μ m. Statistics: one-way ANOVA, ****P < 0.0001.

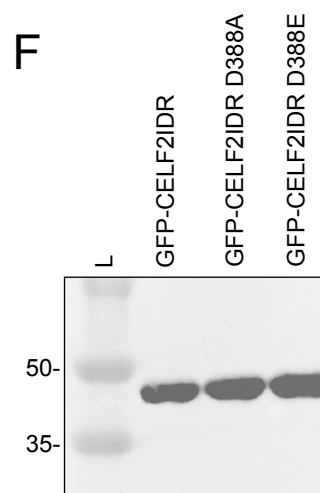
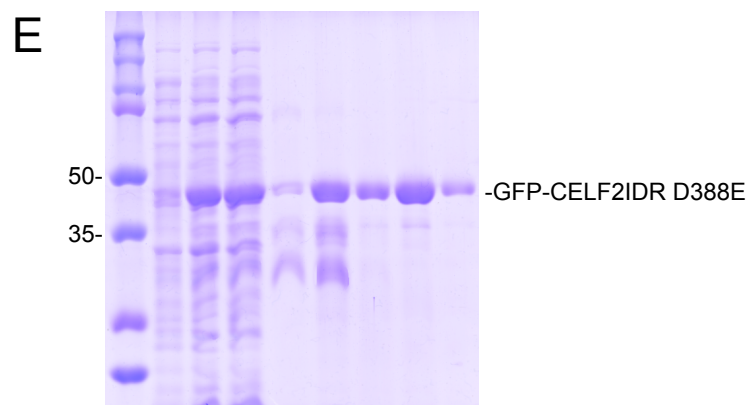
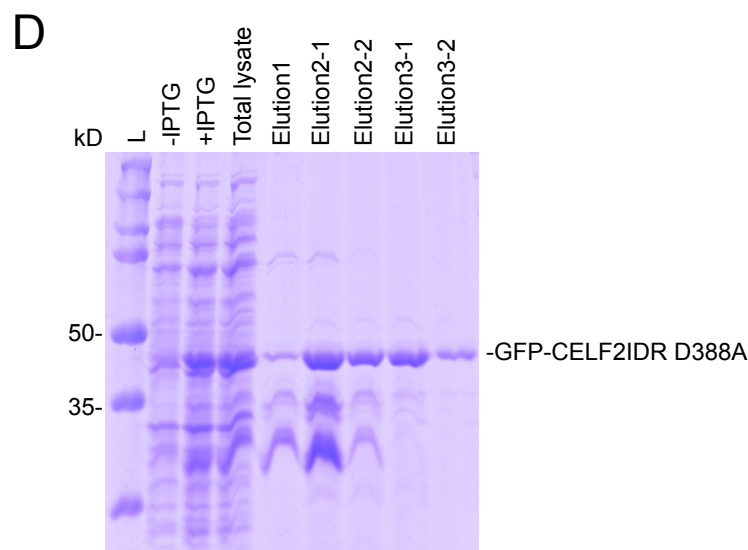
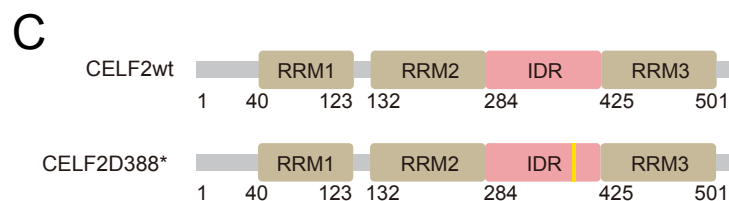
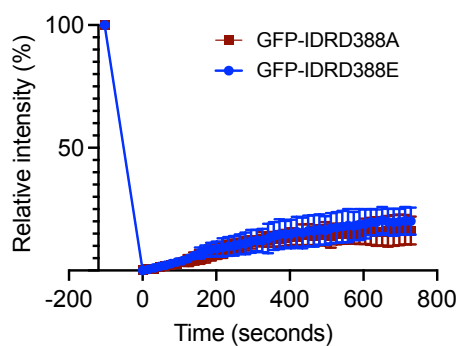
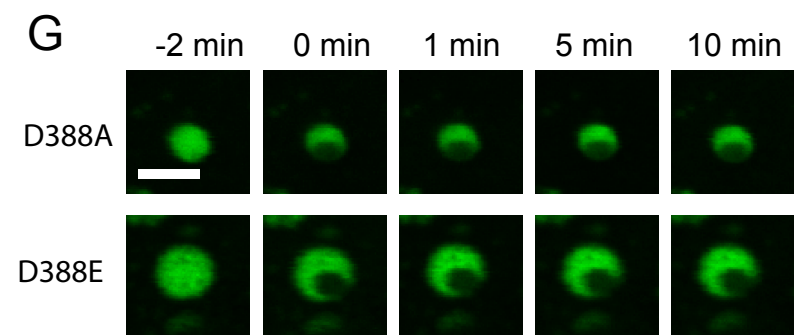
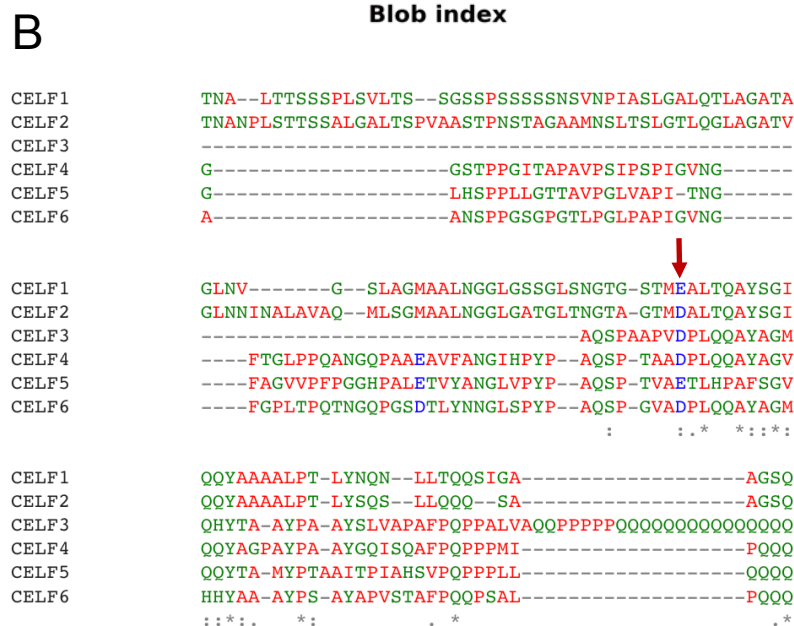
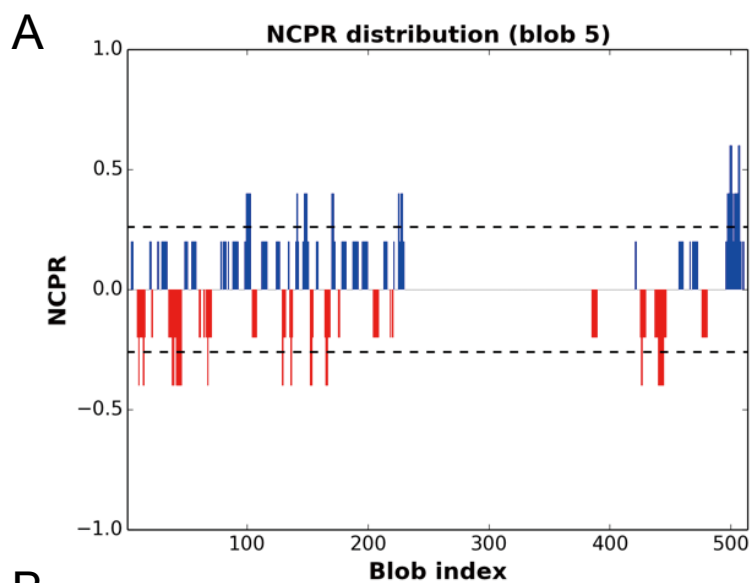


Supplementary Figure 5. CELF2 and NOVA2 have opposing roles in regulating tau exon 10 splicing.

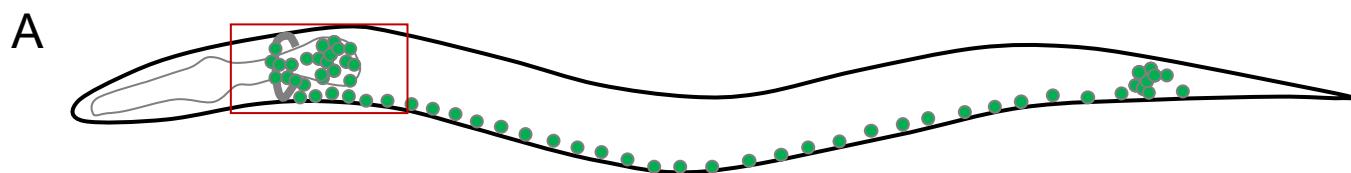
(A) RT-qPCR showing the knockdown efficiency of shNOVA2. Established stable SH-SY5Y cell lines with doxycycline-inducible expression of shNOVA2 were used to assess the efficiency of shRNA targeting NOVA2. Cells were treated with 100 ng/mL Doxycycline for 72 hours, followed by RNA extraction and RT-qPCR.

(B-C) Representative gel image and quantification of 4R and 3R tau transcripts in SH-SY5Y cells with NOVA2 knockdown (KD). Established stable SH-SY5Y cell lines with doxycycline-inducible expression of shNOVA2 were used to assess the alternative splicing of MAPT exon 10. Cells were treated with 100 ng/mL Doxycycline for 72 hours, followed by RNA extraction and RT-PCR. NOVA2 KD led to enhanced 4R tau expression, mirroring the effect of CELF2 overexpression. **(D-E)** Representative gel image and quantification of 4R and 3R tau transcripts in SH-SY5Y cells with CELF2 overexpression and/or NOVA2 KD. Established stable SH-SY5Y cell lines with doxycycline-inducible expression of CELF2 and/or shNOVA2 were used to assess the alternative splicing of MAPT exon 10. Simultaneously overexpressing CELF2 and knocking down NOVA2 resulted in further enhanced 4R tau expression compared to single manipulation alone.

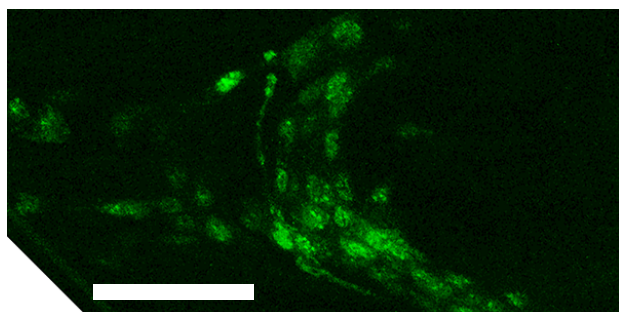
Statistical analysis: one-way ANOVA, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.



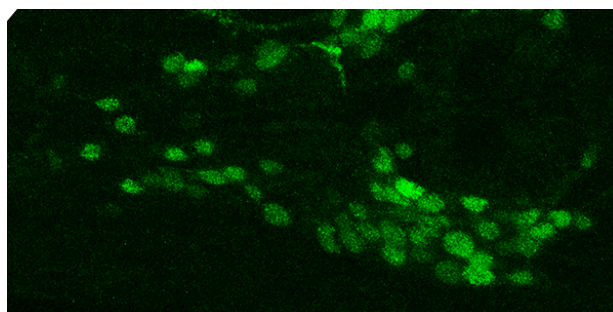
Supplementary Figure 6. A conserved negatively charged residue within CELF2IDR is critical for CELF2 clustering and function. (A) Net charge per residue (NCPR) for CELF2. There is only one charged residue (D388) within the entire CELF2IDR (hinge domain). **(B)** Protein alignment of CELF hinge domains of the six CELF family members showing that the negatively charged residue (either D or E) within the hinge domain is conserved in all six CELF proteins. **(C)** Schematic illustration of CELF2wt and CELF2D388 mutation. **(D-E)** Coomassie staining of GFP-CELF2IDRD388A and GFP-CELF2IDRD388E from indicated protein samples. Prior to protein purification, we compared cell lysates from cells without and with IPTG induction. Total lysate from protein purification experiment, flow through and elutes from Ni²⁺ columns were also examined. **(F)** Western blot using anti-CELF2 antibody (recognizing the C-terminus of hinge domain) to confirm that the purified proteins are correct. **(G)** Representative images and quantification of relative fluorescence intensity in the FRAP assay, demonstrating slow and limited fluorescence recovery. GFP-CELF2IDR(D388A) and GFP-CELF2IDR(D388E) droplets were formed in droplet formation buffer containing 10% PEG-8000 at a protein concentration of 10 μ M.



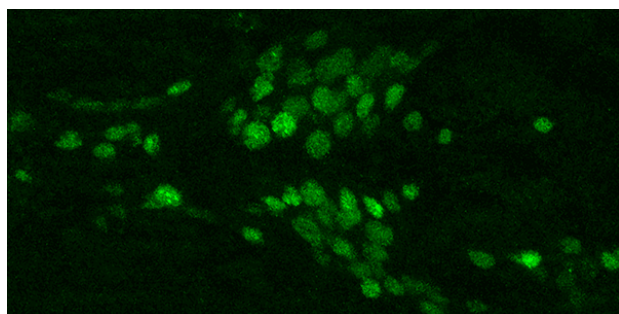
B GFP-CEL2WT



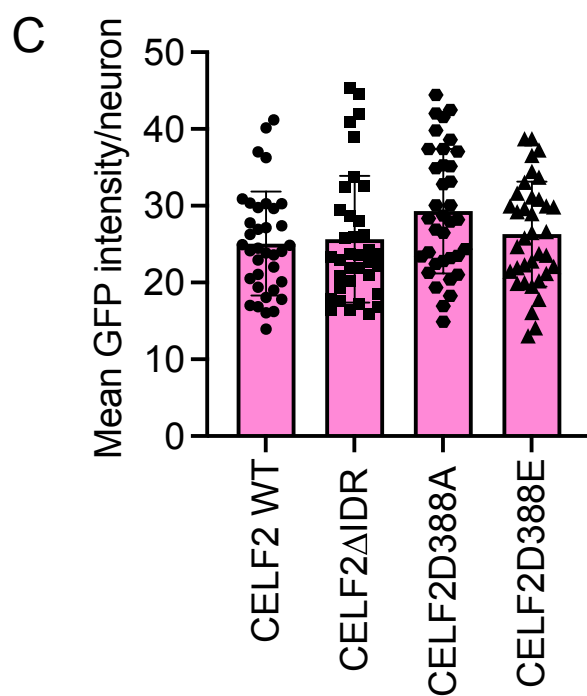
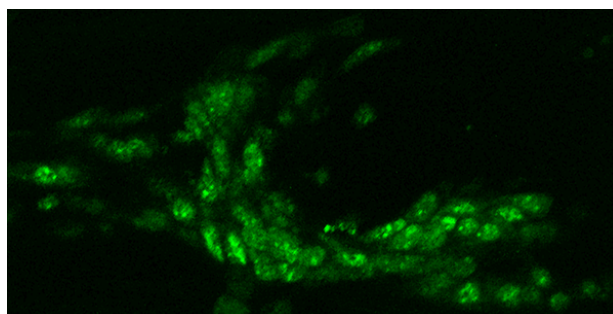
GFP-CEL2 Δ IDR



GFP-CEL2D388A



GFP-CEL2D388E



Supplementary Figure 7. CELF2 variants are expressed at similar levels in transgenic *C. elegans*. **(A)** Schematic illustration of the head ganglion in *C. elegans*. The region outlined in red indicates the area shown in panel B. **(B)** Representative images of head ganglion neurons expressing GFP-tagged CELF2 proteins (wild-type and indicated mutant variants) in transgenic *C. elegans* expressing Prgef-1-GFP-CELF2. All CELF2 variants are expressed at comparable levels in neuronal nuclei. Scale bar: 20 μ m. **(C)** Quantification of mean GFP fluorescence intensity. GFP intensity was measured in 36 nuclei from 6 independent animals per genotype group (6 neurons per animal) and plotted for each condition. No statistically significant differences were observed among the four genotype groups.

Supplementary Table 1: Key resources table

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CELF2 antibody (1H2)	Santa Cruz Biotechnology	sc-47731
GFP antibody	Roche	11814460
GAPDH antibody	Santa Cruz Biotechnology	SC-25778
alpha-tubulin antibody	Sigma	T9026-2ml
Donkey-anti-mouse-HRP	Jackson Immuno	715-035-150
Donkey-anti-rabbit-HRP	Jackson Immuno	711-035-152
Histone H3 antibody	Santa Cruz Biotechnology	SC-10809
Bacterial strains		
E. coli	CGC	OP50
E. coli	ThermoFisher	DE3
E. coli	ThermoFisher	DH5a
E. coli	ThermoFisher	DB3.1
Chemicals, peptides, and recombinant proteins		
Ni-NTA Resin	Thermo Fisher	88221
PEG-8000	Sigma	P2139
1, 6 - Hexanediol	Sigma	240117
cOmplete™ Protease Inhibitor cocktail	Roche	11873580001
Critical commercial assays		
Pierce™ BCA Protein Assay Kit	Thermo Scientific	23227
Gibson Assembly kit	New England Biolabs	E2611
Gateway® LR Clonase™ II	Invitrogen	11791-100

Experimental models: Organisms/strains		
Mouse: Celf2 cKO	Previous work ¹	
Mouse: CamKII-Cre	JAX	
Mouse: Nestin-Cre	JAX	
Oligonucleotides		
qRT-PCR primer (mouse Celf2 exon3)-F 5'-gccacaagatgaacggagct-3'	Integrated DNA Technologies	
qRT-PCR primer (mouse Celf2 exon3)-R 5'-gggcctcaagtgcagctttt-3'	Integrated DNA Technologies	
qRT-PCR primer (mouse Celf2 exon11)-F 5'-GTATGGCGGCTCTGAATGGA-3'	Integrated DNA Technologies	
qRT-PCR primer (mouse Celf2)-R 5'-TCAGTAAGGTTTGCTGTCGT-3'	Integrated DNA Technologies	
qRT-PCR primer (mouse Gapdh)-F 5'-gtgaggccggtgctgagtat -3'	Integrated DNA Technologies	
qRT-PCR primer (mouse Gapdh)-R 5'-cttttgctccaccctcaa -3'	Integrated DNA Technologies	
RT-PCR primer (mouse Mapt)-F 5'-gagcccaagaaggtggcagt -3'	Integrated DNA Technologies	
RT-PCR primer (mouse Mapt)-R 5'-ggatgtccctaacgagcca-3'	Integrated DNA Technologies	
qRT-PCR primer (human GAPDH)-F 5'-aacatcatccctgccttactgg-3'	Integrated DNA Technologies	
qRT-PCR primer (human GAPDH)-R 5'-gttttctagacggcaggtcagg-3'	Integrated DNA Technologies	
qRT-PCR primer (human NOVA2)-F 5'-tcccacttgccaacttctg-3'	Integrated DNA Technologies	

qRT-PCR primer (human NOVA2)-R 5'-ctcggagataagcgacaccc-3'	Integrated DNA Technologies	
RT-PCR primer (human MAPT)-F 5'- ctccaaatcaggggatcgca -3'	Integrated DNA Technologies	
RT-PCR primer (human MAPT)-R 5'-ccttgctcaggtcaactggttt-3'	Integrated DNA Technologies	
Recombinant DNA		
pCMV-Cry2-mCherry-CELF2	This work	
pCMV-Cry2-mCherry-CELF2IDR	This work	
pET-GFP-CELF2IDR	This work	
pET-GFP-CELF2IDR(D388E)	This work	
pET-GFP-CELF2IDR(D388A)	This work	
EYFP-LacI-CELF2	This work	
EYFP-LacI-CELF2 Δ IDR	This work	
mCherry-CELF2	This work	
EGFP-CELF2	This work	
EGFP-CELF2 Δ IDR	This work	
EGFP-CELF2 Δ IDR-TAF15IDR	This work	
EGFP-CELF2 Δ IDR-FUS15IDR	This work	
EGFP-CELF2(D388A)		
EGFP-CELF2(D388E)		
RetroX_TurboID-CELF2	This work	
RetroX_TurboID-CELF-dIDR	This work	
EYFP-LacI-NOVA2	This work	
EYFP-LacI-SFPQ	This work	
EYFP-LacI-HNRNPM	This work	

Software and algorithms		
Fiji		https://imagej.net/ software/fiji/
Prism	GraphPad	https://www.graph pad. com/scientificsoftware/

Fig S1G

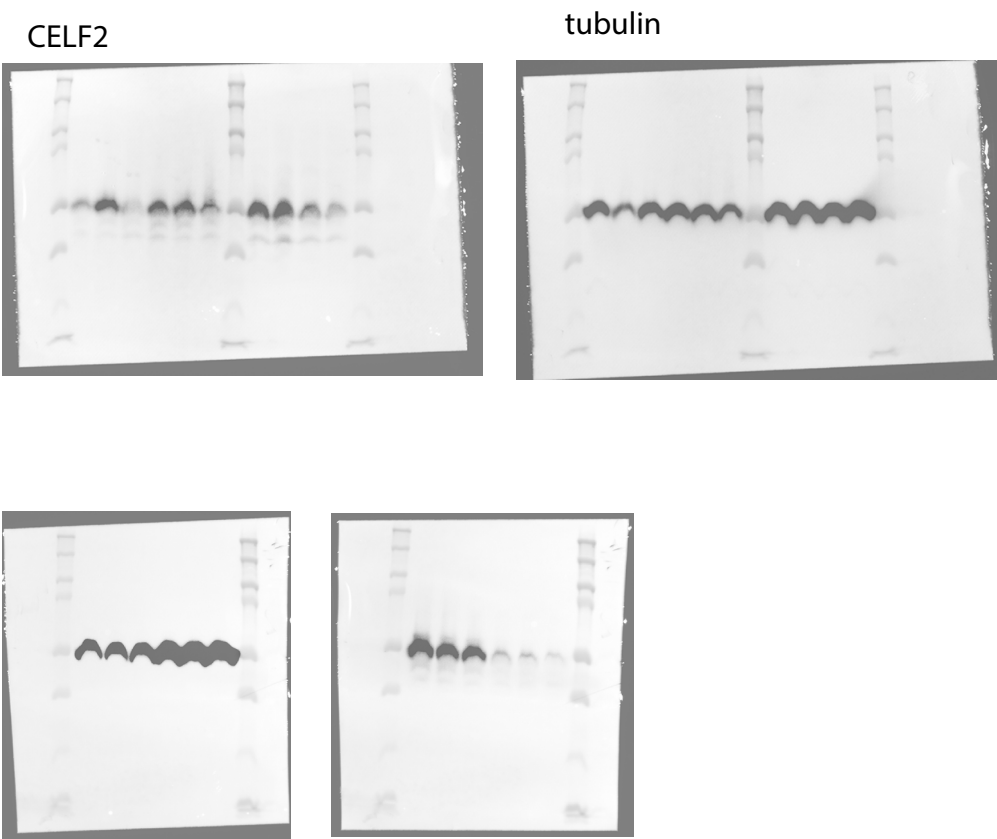


Fig S3B-GFP

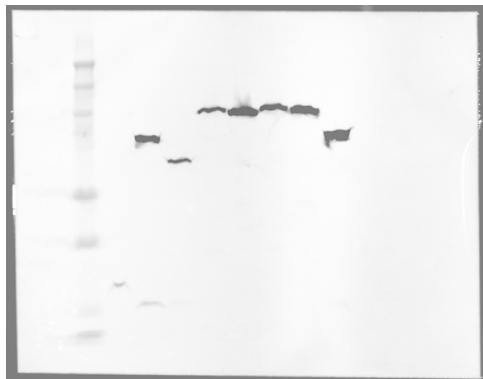


Fig S3B-GAPDH

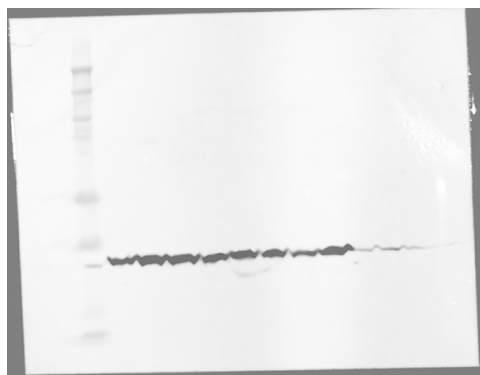


Fig S4A-Streptavidin

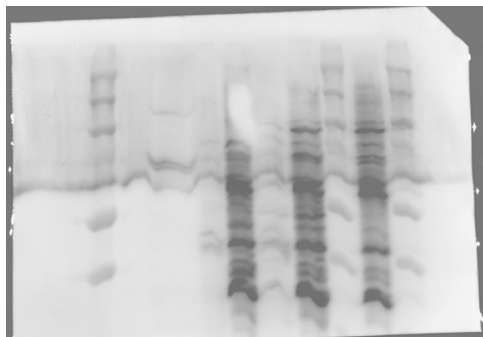


Fig S4A-HA

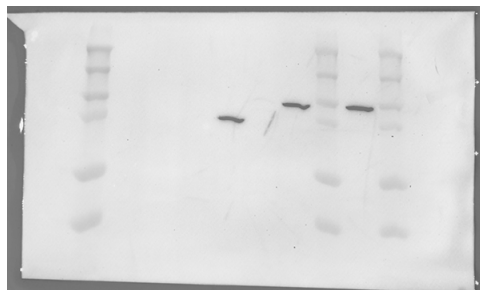


Fig S4A-CELF2

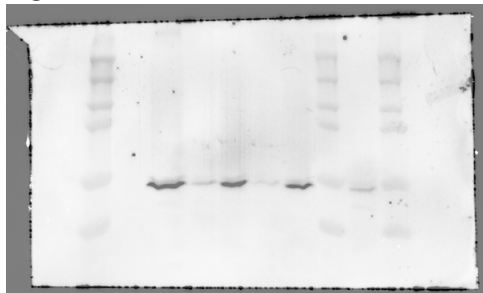


Fig S4A-Histone3

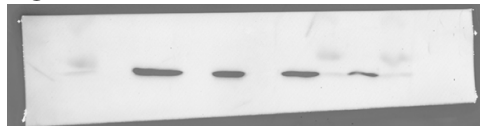


Fig S4A-GAPDH

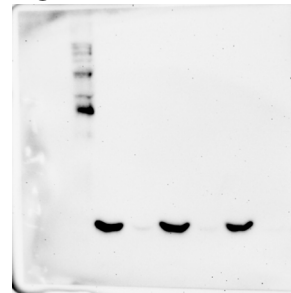


Fig S5B

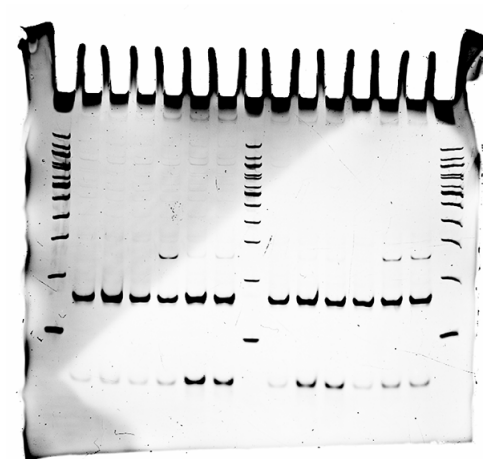


Fig S5D

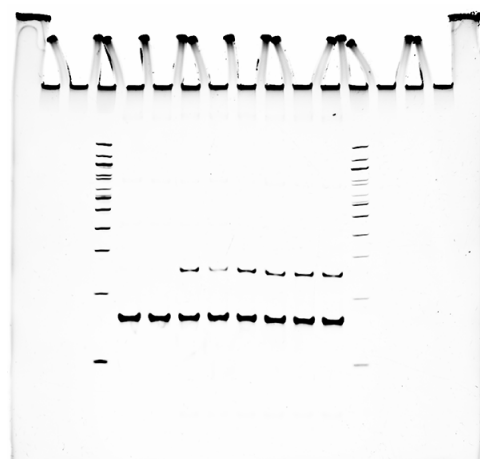


Fig S6F

