

Pathogenic *UNC13A* variants cause a neurodevelopmental syndrome by impairing synaptic function

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Table of contents

1. Supplementary Table 1: Plasmid and primer sequence information
2. Supplementary Table 2: Antibody information
3. Supplementary Note 1: Sequence information for *C. elegans* strains
4. Supplementary Note 2: Additional funding acknowledgements

Supplementary Table 1: Plasmid and primer sequence information

| Plasmid and primer sequence used to generate the mutation | Domain | Corresponding variation in the Human UNC13A | Figure |
|--|--------------|---|---------------------------|
| f(syn)wrbn- <i>Unc13a</i> ^{WT} -GFP | - | - | Figs. 2-6, ExD Figs. 4, 8 |
| f(syn)wrbn- <i>Unc13a</i> ^{E52K} -GFP 5'-GCTGGGAGCAGGACTTCATGTTTAAGATCAACCGCC -3' | C2A | E52K | Fig. 2 |
| f(syn)wrbn- <i>Unc13a</i> ^{R201H} -GFP 5'-CAGTGATTATCATAGTGAGACGA -3' | IDR | R202H | Figs. 2, 3, 6, ExD Fig. 8 |
| f(syn)wrbn- <i>Unc13a</i> ^{C600F} -GFP 5'-GCATGCGCTGCACCGAGTTCGGCGTTAAG -3' | C1 | C587F | Figs. 2, 5, 6 |
| f(syn)wrbn- <i>Unc13a</i> ^{R812Q} -GFP 5'-GTCGGGCGCCATTCAGCTTCACATCAGTG -3' | C2B | R799Q | Fig. 2, ExD Fig. 4 |
| f(syn)wrbn- <i>Unc13a</i> ^{G821D} -GFP 5'-CAGTGTGGAGATCAAAGACGAGGAGAAGGTGGCACC -3' | UNC13A hinge | G808D | Figs. 2, 4, 6 |
| f(syn)wrbn- <i>Unc13a</i> ^{N1026S} -GFP 5'-CCTACGAGTACATCTTCAGCAACTGTCATGAGCTCTA -3' | MUN | N1013S | Fig. 2, ExD Fig. 4 |
| f(syn)-NLS.GFP-P2A- <i>Unc13a</i> ^{WT} -Flag | - | - | Fig. 3 g-m |
| pEGFP-N1-NLS.GFP-P2A- <i>Unc13a</i> ^{WT} -Flag | | | ExD Fig. 5 |
| pEGFP-N1-NLS.GFP-P2A- <i>Unc13a</i> ^{T22M} -Flag 5'-AAGTTCAACATGTACGTGACG-3' | C2A | T22M | ExD Fig. 5 |
| f(syn)-NLS.GFP-P2A- <i>Unc13a</i> ^{E52K} -Flag | C2A | E52K | Fig. 3 g-m |
| pEGFP-N1-NLS.GFP-P2A- <i>Unc13a</i> ^{E52K} -Flag | C2A | E52K | ExD Fig. 5 |
| pEGFP-N1-NLS.GFP-P2A- <i>Unc13a</i> ^{G183S} -Flag 5'-CTTTGGCTGGAGTGAACAGAATG -3' | IDR | G183S | ExD Fig. 5 |
| pEGFP-N1-NLS.GFP-P2A- <i>Unc13a</i> ^{R201H} -Flag | IDR | R202H | ExD Fig. 5 |
| pEGFP-N1-NLS.GFP-P2A- <i>Unc13a</i> ^{E246K} -Flag 5'-CATGCACAGCTATAAAGAGTTCTCTGAG-3' | IDR | E247K | ExD Fig. 5 |
| pEGFP-N1-NLS.GFP-P2A- <i>Unc13a</i> ^{C600F} -Flag | IDR | C587F | ExD Fig. 5 |

Supplementary Table 2: Antibody information

The following antibodies were used in this study:

Primary and secondary antibodies for immunostaining (Fig.2)

| Antibody | Source | Dilution | RRID | Identifier |
|--------------------------------|-------------------|----------|------------|------------|
| Mouse monoclonal GFP | Merck Millipore | 1:250 | AB_94936 | MAB3580 |
| Rabbit polyclonal VGLUT1 | Synaptic Systems | 1:1000 | AB_887877 | 135 302 |
| Guinea pig polyclonal Shank2 | Synaptic Systems | 1:250 | AB_2619861 | 162 204 |
| Chicken polyclonal MAP2 | Novus Biologicals | 1:1000 | AB_2138178 | NB300-213 |
| Goat anti-Mouse Alexa 488 | Thermo Fisher | 1:2000 | AB_2534088 | A11029 |
| Goat anti-Rabbit Alexa 633 | Thermo Fisher | 1:2000 | AB_141419 | A21071 |
| Goat anti-Guinea Pig Alexa 568 | Abcam | 1:2000 | AB_2864763 | Ab175714 |
| Goat anti-Chicken Alexa 405 | Abcam | 1:1000 | AB_2890171 | Ab175674 |

Primary and secondary antibodies for Western Blot analysis (Extended Fig. 5):

| Antibody | Source | Dilution | RRID | Identifier |
|--|------------------------|----------|------------|---------------|
| Polyclonal rabbit anti-FLAG | Sigma-Aldrich | 1:2000 | AB_439687 | F7425 |
| Monoclonal mouse anti- Green Fluorescent Protein (1E4) | Enzo Life Sciences | 1:1000 | | ADI-SAB-500-E |
| Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) | Jackson immunoResearch | 1:5000 | AB_2307392 | 115-035-146 |
| Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) | Jackson immunoResearch | 1:30000 | AB_2307391 | 111-035-144 |

Supplementary Note 1: Sequence information for *C. elegans* strains

Sequence information (Bold= mutation; Underline = RE)

| Strain | <i>unc-13</i> allele | Description | Human UNC13A | <i>C. elegans</i> UNC-13 |
|---------|----------------------|---------------------|--------------|--------------------------|
| JSD1433 | <i>nu641</i> | C-terminal mScarlet | - | - |
| JSD1381 | <i>tau45</i> | Hinge proline | P814L | P956L |
| JSD1422 | <i>tau48 nu641</i> | Hinge glycine | G808C | G950C |
| JSD1440 | <i>tau64 nu641</i> | C1 domain cysteine | C587F | C729F |

unc-13(tau45)

crRNA: CATGTATACTGAACATGATA

AAATTGACTCGTGAATCTGATGATTTCTTGGGACAAACAGTAATTGAAGTTCGAACTTTA
TCTGGTGAAATGGATGTCTGGTATAATCTTGAAAAGAGAACTGATAAATCTGCTGTATCC
GGAGCAATTCGATTGCATATCAATGTTGAAATCAAGGGAGAAGAGAAGCTAGCATTGTA
TCATGTTCAAGTATACATGTCTTCATGAACATCTTTTTGCTGCTCATTGTGTAGA

unc-13(tau48 nu641)

crRNA: GCATATCAATGTTGAAATCA

AAATCTGCTGTATCCGGAGCAATTCGATTGCATATCAATGTTGAAATCAAGT**GC**GAAGA
GAAGCTTGCACCGTATCATGTTCAAGTATACATGTCTTCATGAACATCTTTTTGCTGCTCA
T

unc-13(tau64 nu641)

Two crRNAs used:

crRNA 1: CGAAGGATTATTGTGGGGAT

crRNA 2: TCCATGTTTCGTCGACTTCT

TTCCAAACACCCACGTTTTGTTACGAATGCGAAGGATTATTGTGGGGATTaGCTAGACA
AGGATTGCGATGTACTCAGT**t**TCAAGTGAAAGTTCACGATAAATGTCGTGAATTGCTCAG
CGCTGATTGTCTgCAgAGAGCAGCtGAGAAGTCGACGAAACATGGAGAAGCTGATAGAA
CACAGTCACTTGTC

Co-CRISPR, *unc-58 (L428F)*

crRNA: ATCCACGCACATGGTCACTA

ATAGCCGAGTTAGGAAACAAATTTTTCTTTCAGGTT**TTCT**CAGTAGTGACCATGTGCGTG
GATCTTGCGTCCACACA

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