Supplementary Materials

DCTN1 binds to TDP-43 and regulates TDP-43 aggregation

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Supplementary data inventory

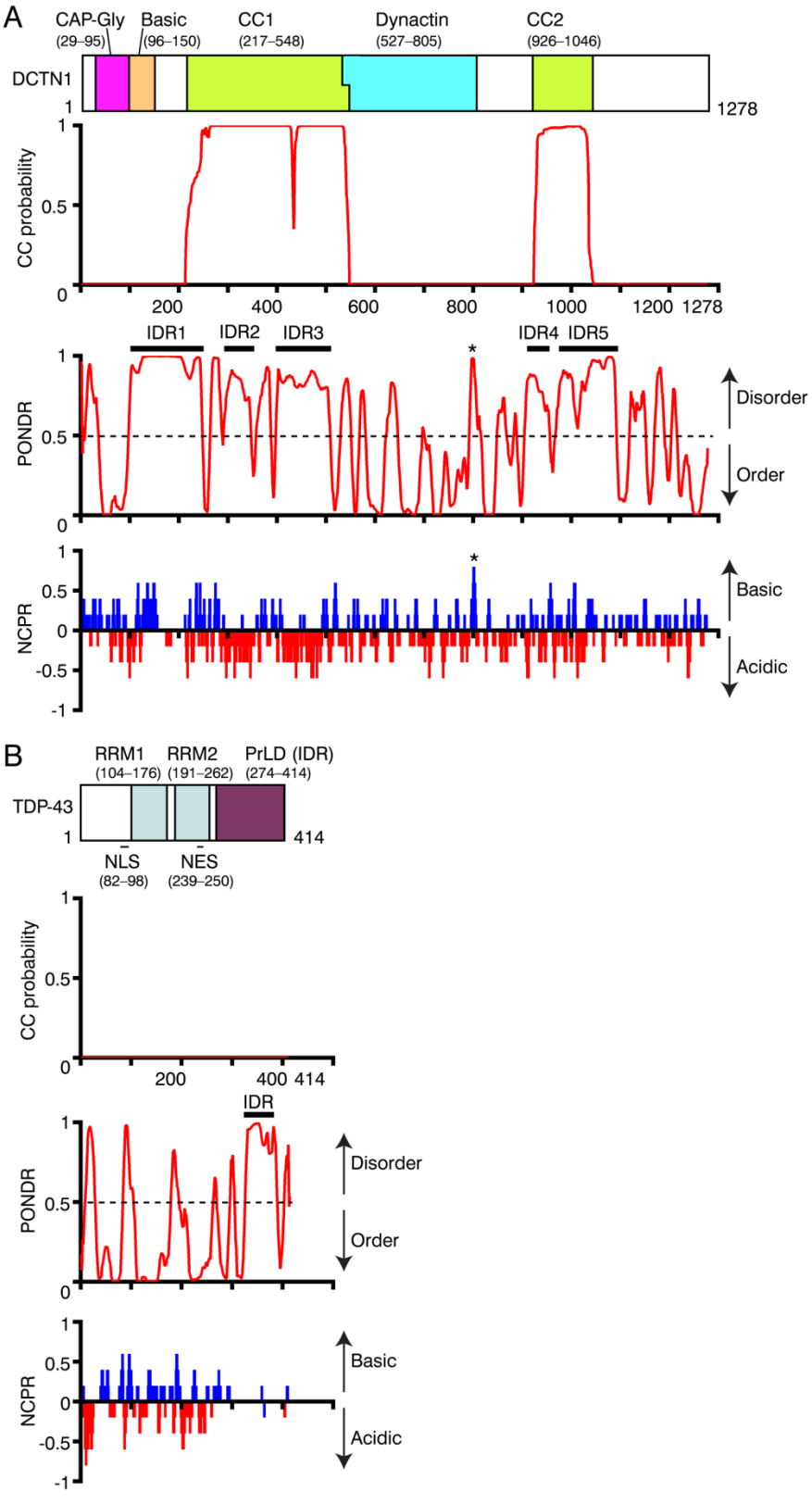
A
CAP-Gly
(29–95)
Basic
(96–150)
CC1
(217–548)
Dynactin
(527–805)
CC2
(926–1046)
DCTN1
1
1278
CC probability
1
0.5
0
200
400
600
800
1000
1200
1278
IDR1
IDR2
IDR3
IDR4
IDR5
PONDR
Disorder
Order
NCPR
Basic
Acidic
-0.5
-1
B
RRM1
(104–176)
RRM2
(191–262)
PrLD (IDR)
(274–414)
TDP-43
414
NLS
(82–98)
NES
(239–250)
IDR

Figure S1. Bioinformatics analyses on DCTN1 and TDP43. (**A**) Analyses on DCTN1. Results of coiled-coil (CC) prediction by Multicoil2, disordered domain prediction by PONDR (predictor of natural disordered regions), and NCPR (net charge per residue; 5-amino acid window) analyses are shown. Black bars in the PONDR graph show predicted intrinsically disordered regions (IDRs). In NCPR, charged residues are shown in blue (basic: Arg and Lys) and red (acidic: Asp and Glu). (**B**) Analyses on TDP-43. Results of CC prediction, PONDR, and NCPR are presented.

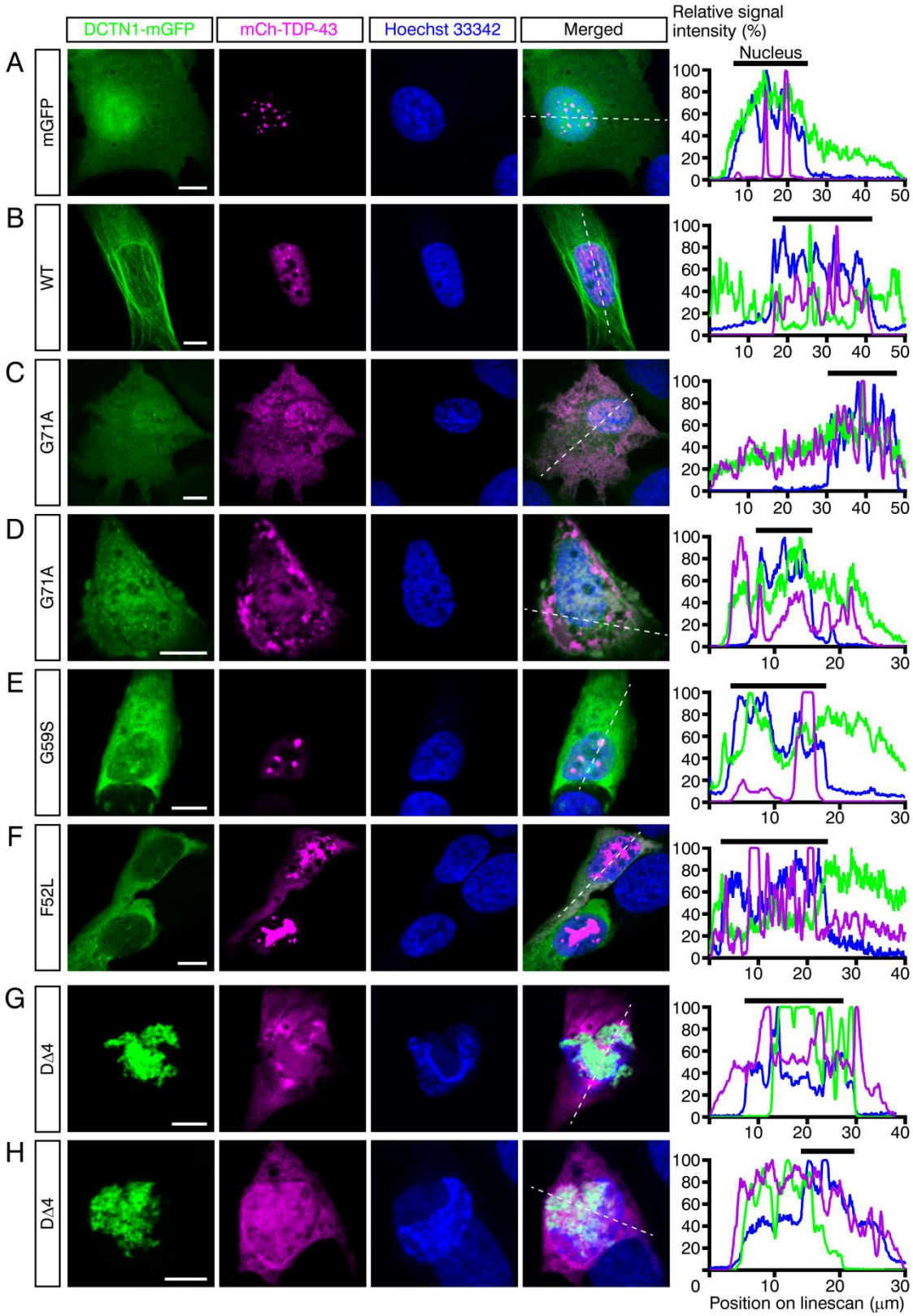
DCTN1-mGFP
mCh-TDP-43
Hoechst 33342
Merged
Relative signal intensity (%)
Nucleus
A
mGFP
B
WT
C
G71A
D
G71A
E
G59S
F
F52L
G
DΔ4
H
DΔ4
Position on linescan (μm)

Figure S2. Effects of missense mutant DCTN1 and DΔ4 on TDP-43 mislocalization and aggregation. Maximum-intensity projections of deconvoluted z-stack confocal images of U2OS cells that co-expressed mGFP (**A**), wild-type (WT) (**B**) or mutant (**C-H**) DCTN1-mGFP and mCherry-tagged WT TDP-43 are shown. The transfected cells were cultured under non-stressed conditions, fixed three days after transfection, and subjected to confocal microscopy. In (F), two representative cellular phenotypes of DCTN1^{F52L}-mGFP- and mCherry-TDP-43-coexpressing cells are shown: nuclear aggregation and ubiquitous cytoplasmic distribution of TDP-43. Scale bars, 10 μm. The right graphs show linescans of the cells along the white broken lines.

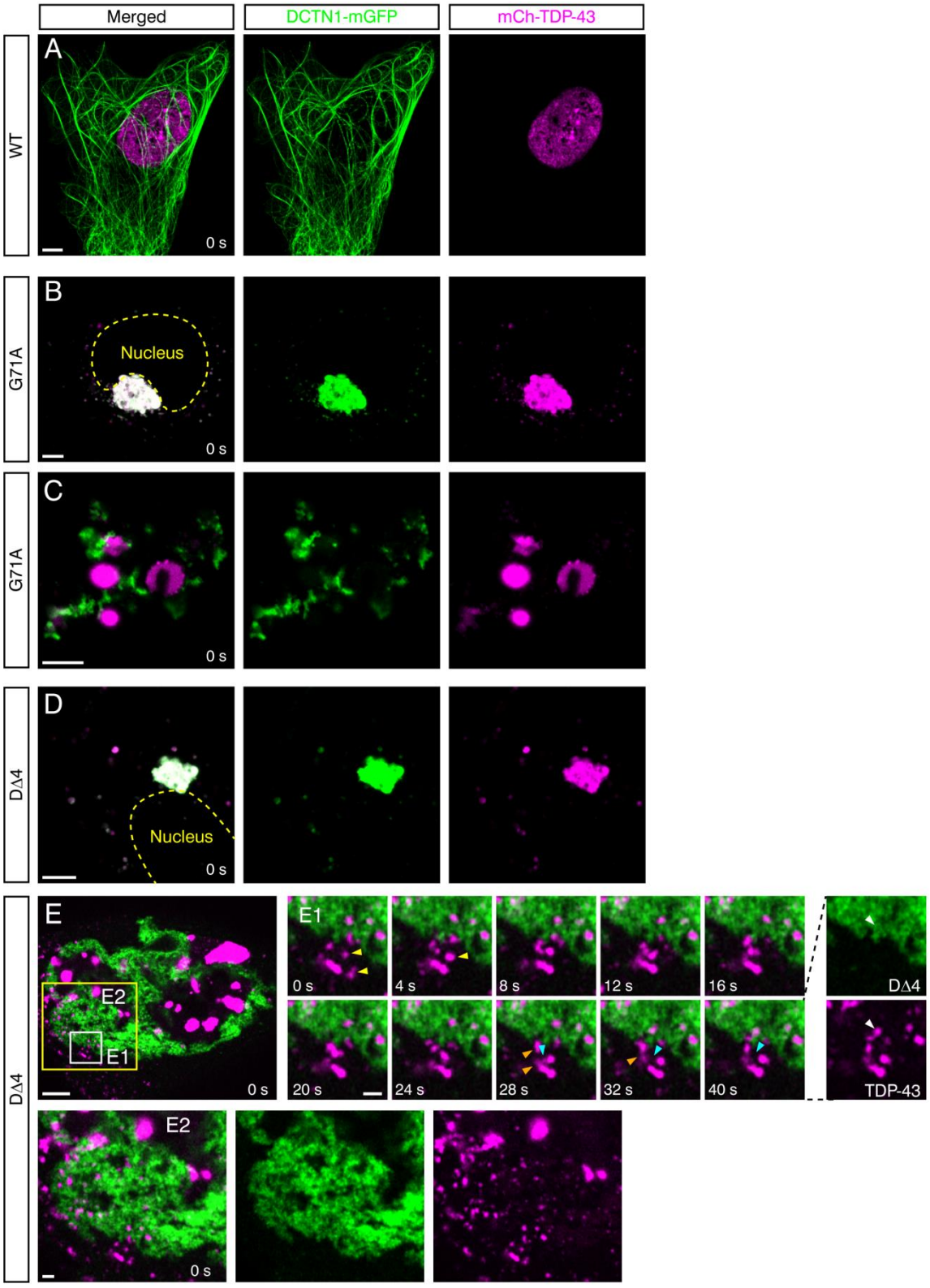
Merged
DCTN1-mGFP
mCh-TDP-43
WT
A
0 s
G71A
B
Nucleus
0 s
G71A
C
0 s
DΔ4
D
Nucleus
0 s
DΔ4
E
E2
E1
0 s
E1
0 s
4 s
8 s
12 s
16 s
20 s
24 s
28 s
32 s
40 s
DΔ4
TDP-43
E2
0 s

Figure S3. Super-resolution live-cell imaging of dynamics of wild-type (WT) or mutant DCTN1-mGFP (p.G71A or DΔ4) and mCherry-TDP-43 (WT) in U2OS cells. (**A**) A snap shot of U2OS cells co-expressing WT DCTN1-mGFP (shown in green) and mCherry-TDP-43 (WT; purple). (**B,C**) Snap shots of aggregates of mutant DCTN1 (p.G71A)-mGFP and mCherry-TDP-43 (WT). In (C), the nucleus is located above the top-left corner. (**D,E**) Snap shots of aggregates of mutant DCTN1 (DΔ4)-mGFP and mCherry-TDP-43 (WT). E1: time-lapse imaging of the white-boxed area in (E) for 40 s. mCherry- TDP-43 aggregates dynamically undergo remodeling through repeated contacts among neighboring aggregates of DCTN1 (DΔ4)-mGFP and mCherry-TDP-43, as well as fission/fusion cycles. Two fusion processes between mCherry-TDP-43 aggregates are marked with yellow and orange arrowheads, while a fission process of mCherry-TDP-43 aggregates is marked with cyan arrowheads (before and after the event). An obviously found colocalization of DCTN1 with TDP-43 is marked by white arrowheads (the right panels). E2: High-power views of the yellow-boxed area in (E). Scale bars, 5 μm (A-E) and 1 μm (E1 and E2). See Movie S1–S6.

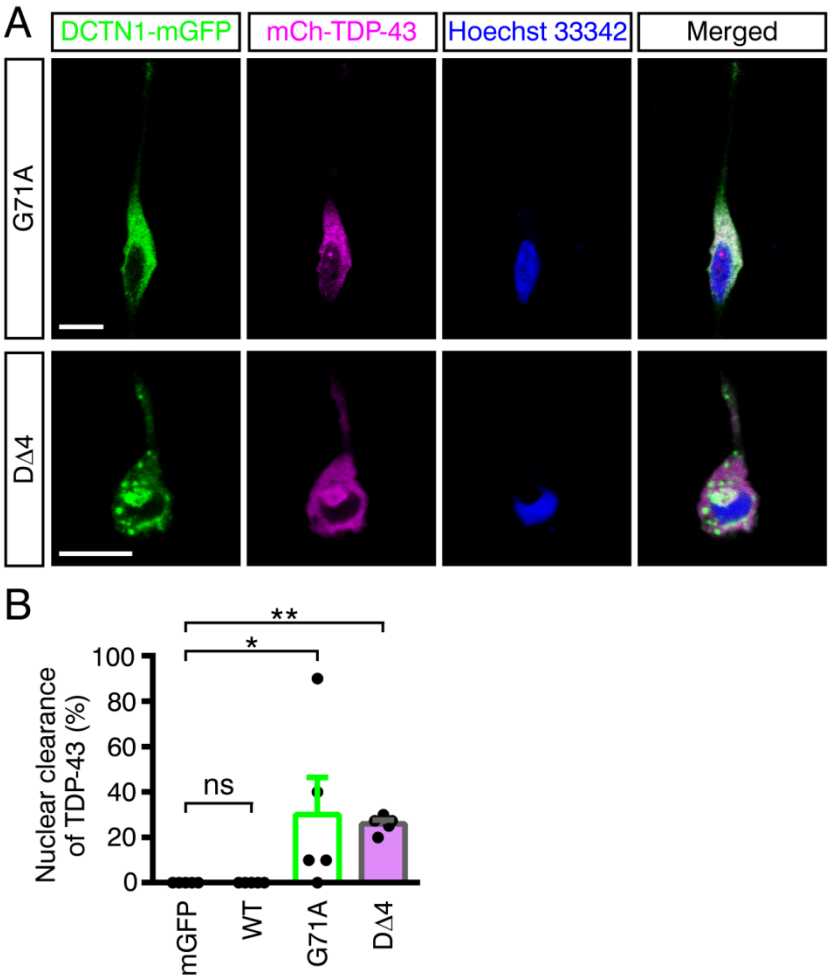

Figure S4. Nuclear clearance of TDP-43 in iPSC-derived neurons co-expressing mutant DCTN1 and wild-type TDP-43. (**A**) Examples of nuclear clearance of mCherry-TDP-43 in mutant DCTN1-mGFP (p.G71A or DΔ4)-coexpressing neurons. Single-section confocal images of deconvoluted z-stacks are shown. Scale bars, 10 μm. (**B**) Quantitative analysis of nuclear clearance of TDP-43. n = 5 experiments (10–12 cells per experimental group). * $p < 0.05$; ** $p < 0.01$ by two-tailed Mann-Whitney test. ns: not significant.

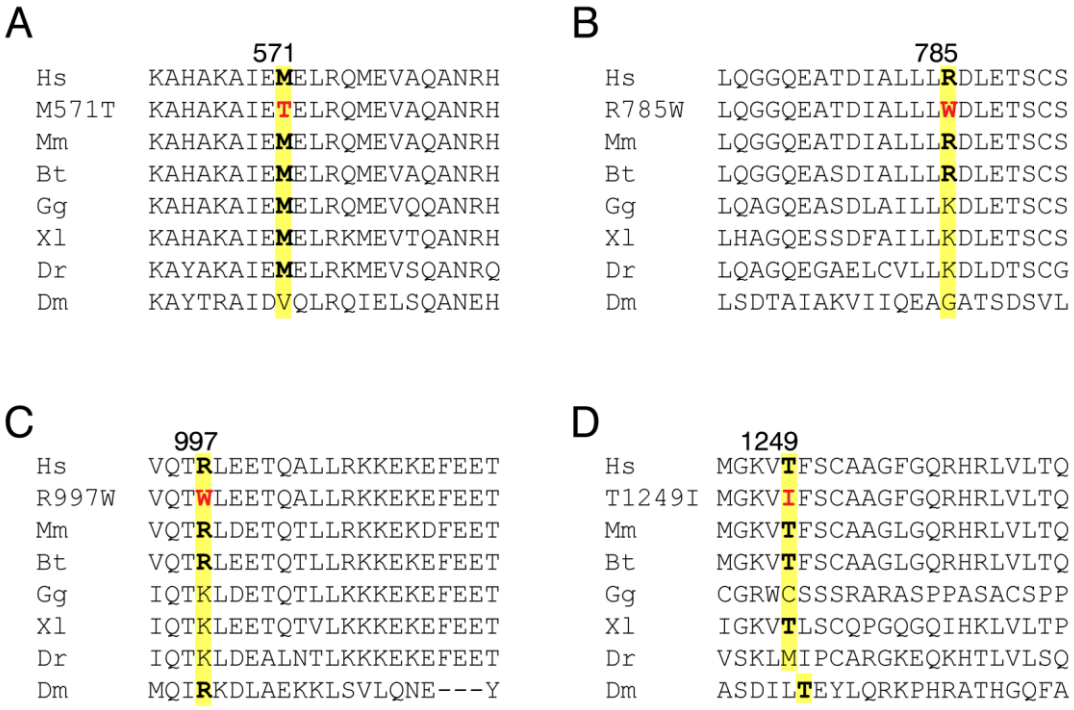

Figure S5. Amino acid sequence conservation in the dynactin domain and C-terminal region of DCTN1 across several animal species. (**A-D**) Four segregated sites of missense mutations from patients with ALS are shown; (A) p.M571T, (B) p.R785W, (C) p.R997W, and (D) p.T1249I. The positions of putative ALS-linked mutations are highlighted in bold. Hs: *Homo sapiens*; Mm: *Mus musculus*; Bt: *Bos Taurus*; Gg: *Gallus gallus*; Xl: *Xenopus laevis*; *Dr: Danio rerio*; Dm: *Drosophila melanogaster*.

Movie S1. A representative case of super-resolution live-cell imaging of a U2OS cell co-expressing DCTN1-mGFP (wild-type [WT]; shown in green) and mCherry-TDP-43 (WT; purple). The example is from Figure S3A. Images were taken every 6 s, and are shown at 3 frames per second.

Movie S2. Super-resolution live-cell imaging of a U2OS cell co-expressing DCTN1-mGFP (p.G71A; shown in green) and mCherry-TDP-43 (WT; purple). The example is from Figure S3B. A large cytoplasmic aggregate, near the nucleus, in which DCTN1^{G71A}-mGFP and mCherry-TDP-43 colocalized, was found. Images were taken every 6 s, and are shown at 3 frames per second.

Movie S3. Super-resolution live-cell imaging of a U2OS cell co-expressing DCTN1-mGFP (p.G71A; shown in green) and mCherry-TDP-43 (WT; purple). The example is from Figure S3C. Cytoplasmic DCTN1^{G71A} aggregates partially colocalized with or repeatedly contacted those of TDP-43. Images were taken every 6 s, and are shown at 3 frames per second.

Movie S4. Super-resolution live-cell imaging of a U2OS cell co-expressing DCTN1-mGFP (DΔ4; shown in green) and mCherry-TDP-43 (WT; purple). The example is from Figure

S3D. A large cytoplasmic aggregate in which DΔ4 and TDP-43 colocalized was observed. Images were taken every 4 s, and are shown at 3 frames per second.

Movie S5. Super-resolution live-cell imaging of a U2OS cell co-expressing DCTN1-mGFP (DΔ4; shown in green) and mCherry-TDP-43 (WT; purple). A whole-cell view; the same cells as that shown in Figure S3E was live-imaged. Aggregates of mCherry-TDP-43 interacted with massive aggregates of DΔ4-mGFP. Images were taken every 4 s, and are shown at 3 frames per second.

Movie S6. Super-resolution live-cell imaging of a U2OS cell co-expressing DCTN1-mGFP (DΔ4; shown in green) and mCherry-TDP-43 (WT; purple). A higher-power view of the cell presented in Movie S5. Microaggregates of mCherry-TDP-43 repeatedly contacted the aggregates of neighboring aggregates of mCherry-TDP-43 and those of DCTN1 (DΔ4), and underwent cycles of fission and fusion, via a liquid-liquid phase separation (LLPS)-mediated process. mCherry-TDP-43 aggregates frequently appeared to grow in such a manner. Images were taken every 4 s, and are shown at 1.5 frames per second. See Figure S3E (E1).