SUPPLEMENTAL DATA

FIGURE S1. **Expression of FUS domain constructs containing the WT PY-NLS.**
Immunocytochemistry of transiently transfected HeLa cells expressing HA-tagged FUS-Z, FUS-RZ and FUS-GRZ, respectively. 24 hours after transfection cells were exposed to heat shock (44°C for 1 hour) or left untreated (37°C). Cells were fixed, stained with an HA-specific antibody (green), a TIA-1–specific antibody (red) and a nuclear counterstain (blue) and were analyzed by confocal microscopy. FUS proteins containing the Z-domain with the WT PY-NLS were nuclear both under control conditions and after heat shock. Scale bars: 20 μm.

FIGURE S2. **Heat shock-induced FUS-positive granules are SG.**
(A) To verify that stress-induced FUS-positive granules are bona fide SG, HeLa cells expressing HA-FUS-P525L were treated with cycloheximide (CHX) during heat shock (44°C + CHX) or were allowed to recover from heat shock (44°C + rec) by incubation at 37°C for 1 hour subsequent to heat shock. Afterwards cells were fixed, stained with an HA-specific antibody (green), a TIA-1-specific antibody (red) and a nuclear counterstain (blue) and were analyzed by confocal microscopy. CHX blocks translational elongation and is a well-known inhibitor of SG assembly. Addition of CHX prevented the formation of TIA-1/FUS-positive SG. Moreover, FUS-positive granules disassembled after recovery from heat stress, confirming their SG identity. Scale bars: 20 μm.
(B) SH-SY5Y neuroblastoma cells were transiently transfected with HA-tagged FUS-WT or FUS-P525L. 24 hours post-transfection cells were subjected to heat shock (44°C for 1 hour) or kept at control temperature (37°C). SH-SY5Y cells were fixed, stained with an HA-specific antibody (green), a TIA-1-specific antibody (red) and a nuclear counterstain (blue) and were analyzed using confocal microscopy. Like in HeLa cells, FUS-WT was nuclear with and without stress, while cytoplasmically mislocalized FUS-P525L was recruited into SG upon cellular stress. Scale bars: 20 μm.

FIGURE S3. **Expression of FUS domain constructs.**
Expression levels of HA-FUS constructs were examined by immunoblot. Total cell lysates were prepared in RIPA buffer and after SDS-PAGE were analyzed by immunoblotting with an HA-specific antibody (upper panel). A longer exposure of the same blot shows the weakly expressed R domain (middle panel). Actin served as a loading control (lower panel). Note that HA-tagged Q and Z_{P525L} were not detectable by HA immunoblot.

FIGURE S4. **GFP-tagged FUS-Z_{P525L} but not Q is recruited to SG.**
Since HA-tagged FUS-Q and Z_{P525L} were poorly expressed and not detectable by HA immunoblot, GFP-Q, GFP-Z_{P525L} and full-length GFP-FUS-P525L were expressed in HeLa cells and analyzed for expression and SG recruitment 24 hours post-transfection.
(A) Protein levels in total cell lysates were analyzed by immunoblotting with a GFP-specific antibody (upper panel). β-Actin served as a loading control (lower panel). Black arrowheads indicate GFP-tagged FUS proteins, white arrowhead indicates a degradation product.
(B) 24 hours post-transfection cells were subjected to heat shock (44°C for 1 hour) or left untreated (37°C). Cells were fixed, stained with a TIA-1-specific antibody (red) and a nuclear counterstain (blue) and analyzed by confocal microscopy. GFP-tagged proteins were visualized in the green channel via GFP fluorescence (green). Note that despite the P525L mutation, GFP-Z_{P525L} showed a more prominent nuclear localization than full-length GFP-FUS-P525L. Nevertheless, GFP-Z_{P525L} can be found to overlap with TIA-1, whereas GFP-Q is poorly recruited to SG and remains mainly diffusely distributed in the cytosol. Scale bars = 20 μm.

FIGURE S5. **Heat shock-induced TDP-43-positive granules are SG.**
(A) To verify that stress-induced TDP-43-positive granules are bona fide SG, HeLa cells expressing V5-tagged TDP-43 NLSmut were treated with cycloheximide (CHX) during heat shock (44°C + CHX) or were

allowed to recover from heat shock (44°C + rec) by incubation at 37°C for 1 hour subsequent to heat shock. Subsequently, cells were fixed, stained with a V5-specific antibody (green), a TIA-1-specific antibody (red) and a nuclear counterstain (blue) and analyzed by confocal microscopy. CHX-treated cells show no SG and TDP-43-positive granular structures resolve after recovery from heat shock, showing that the TDP-43-positive granules are indeed SG and not aggregates due to overexpression of TDP-43. Scale bars: 20 µm.
(B) Immunocytochemistry of SH-SY5Y cells transfected with TDP-WT-V5 or TDP-NLSmut-V5. 24 hours post-transfection, cells were subjected to heat shock (44°C for 1 hour) or left untreated (37°C). Cells were fixed, stained with a V5-specific antibody (green), a TIA-1-specific antibody (red) and a nuclear counterstain and analyzed by confocal microscopy. Like in Hela cells, TDP-WT remained nuclear and NLSmut was sequestered into SG upon heat stress. Scale bars: 20 µm.

FIGURE S6. **Percentage of PABP-1-positive TDP-43 inclusions in the spinal cord of ALS-TDP and FTLD-TDP patients.**
(A) Double-label immunofluorescence stainings for pTDP-43 (green) and the SG marker PABP-1 (red) showing colocalization in inclusions in ALS case #2 (I) and ALS case #3 (II). Notice that a subset of pTDP-43 inclusions in the spinal cord in all cases are only labeled for pTDP-43, as shown here for ALS case #1 (III). Nuclei were stained with DAPI (blue). Scale bar = 10 µM.
(B) pTDP-43 and PABP-1-labeled neuronal cytoplasmic inclusions in the anterior horn on double-label immunofluorescence stainings were counted on 1-2 spinal cord sections from each case. 66 % of the pTDP-43-positive inclusions showed co-labeling with the SG marker protein PABP-1.

FIGURE S7. **GFP-tagged TDP-Δ1-173 is not recruited to SG.**
Since V5-tagged TDP-Δ1-173 was poorly expressed and only gave a weak band in V5 immunoblot, GFP-Δ1-173 and full-length GFP-NLSmut were expressed in HeLa cells and analyzed for expression and SG recruitment 24 hours post-transfection.
(A) Protein levels in total cell lysates were analyzed by immunoblotting with a GFP-specific antibody (upper panel). β-Actin served as a loading control (lower panel). Black arrowheads indicate GFP-tagged TDP-43 proteins, white arrowheads indicate degradation products.
(B) 24 hours post-transfection cells were treated with clotrimazole (20 µM for 30 min) or left untreated (control). Cells were fixed, stained with a TIA-1-specific antibody (red) and a nuclear counterstain (blue) and analyzed by confocal microscopy. GFP-tagged proteins were visualized in the green channel via GFP fluorescence (green). Despite high expression levels, GFP-Δ1-173 did not co-localize with TIA-1-positive granules, whereas full-length GFP-NLSmut was readily recruited to SG. Scale bars = 20 µm.

Fig. S1

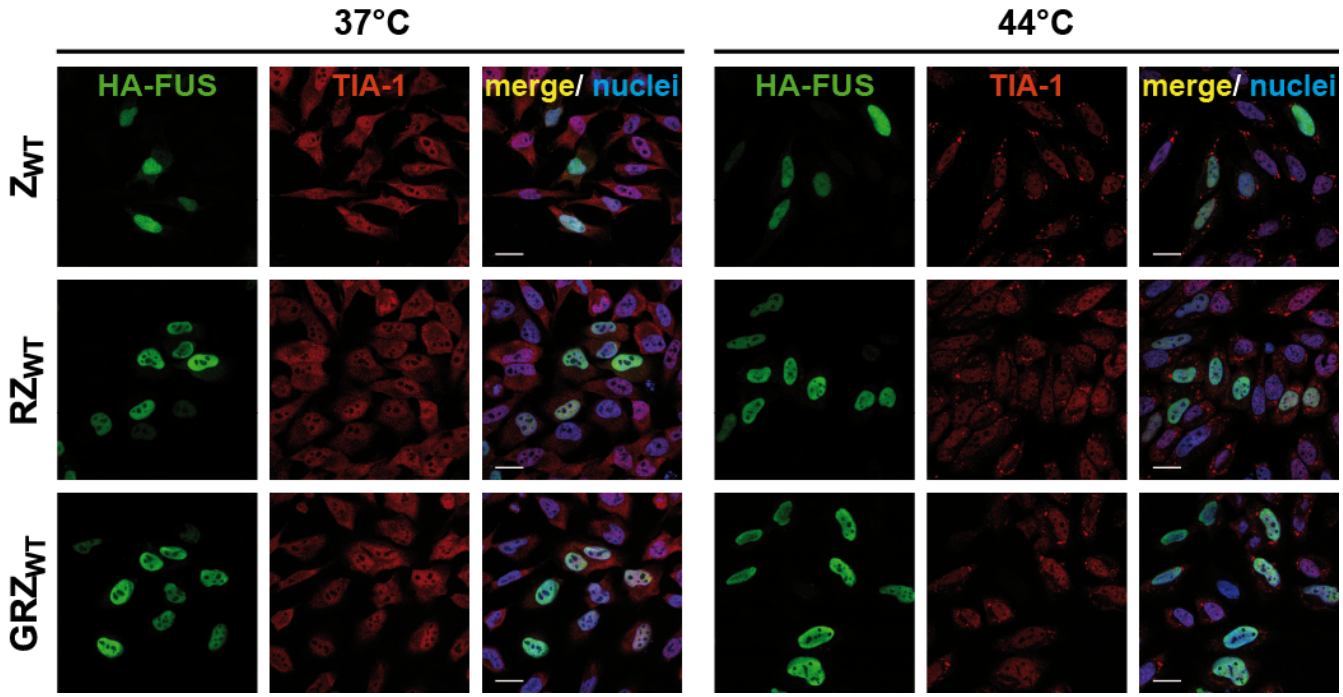

37°C
44°C
HA-FUS
TIA-1
merge/ nuclei
HA-FUS
TIA-1
merge/ nuclei
Z_{WT}
RZ_{WT}
GRZ_{WT}

Fig. S2

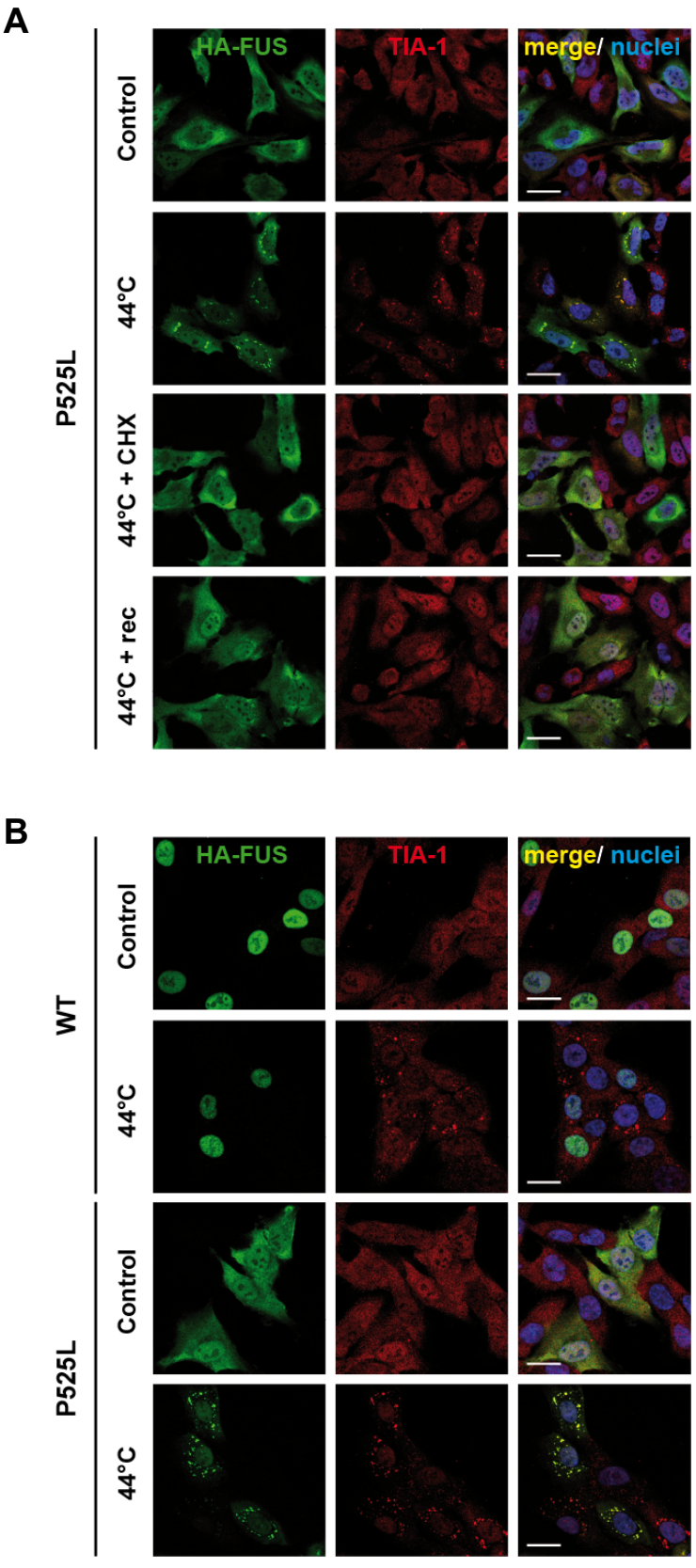

A
HA-FUS
TIA-1
merge/ nuclei
P525L
Control
44°C
44°C + CHX
44°C + rec
B
HA-FUS
TIA-1
merge/ nuclei
WT
Control
44°C
P525L
Control
44°C

Fig. S3

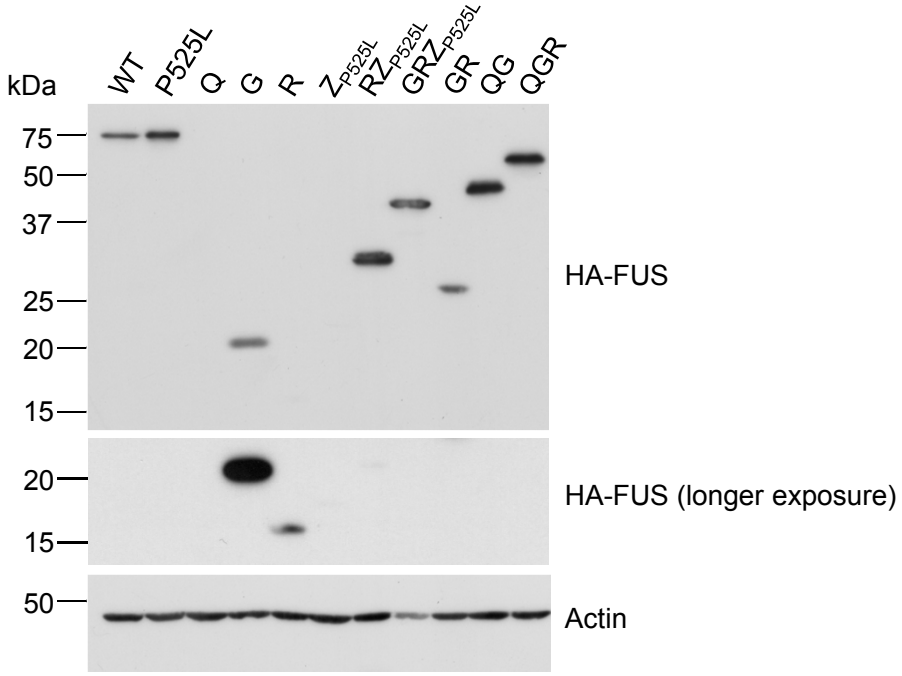

kDa
WT
P525L
Q
G
R
ZP525L
RZP525L
GRZP525L
GR
QG
QGR
75
50
37
25
20
15
HA-FUS
20
15
HA-FUS (longer exposure)
50
Actin

Fig. S4

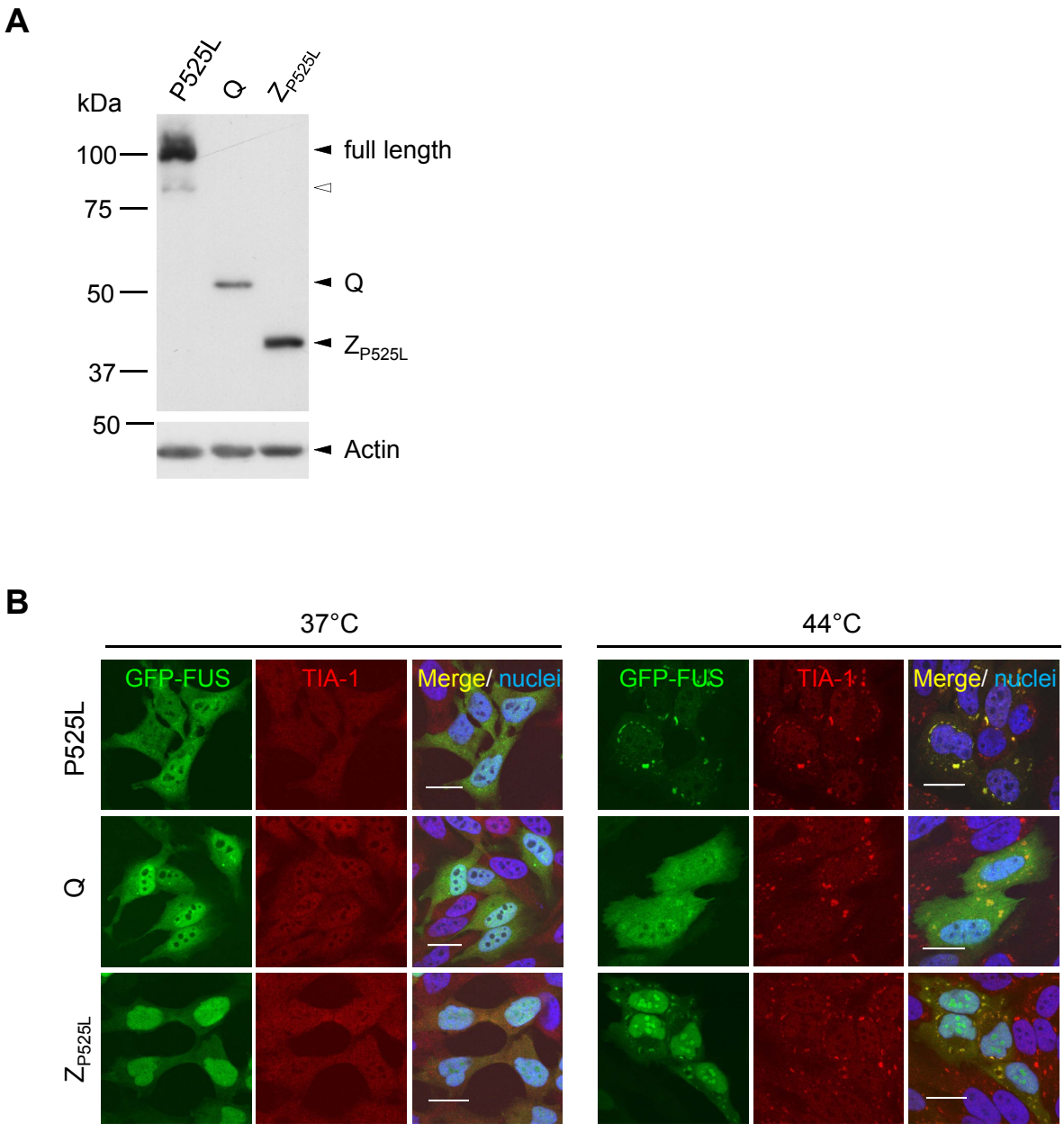

A
P525L
Q
Z_{P525L}
kDa
100
75
50
37
50
full length
Q
Z_{P525L}
Actin
B
37°C
44°C
GFP-FUS
TIA-1
Merge/ nuclei
P525L
Q
Z_{P525L}

Fig. S5

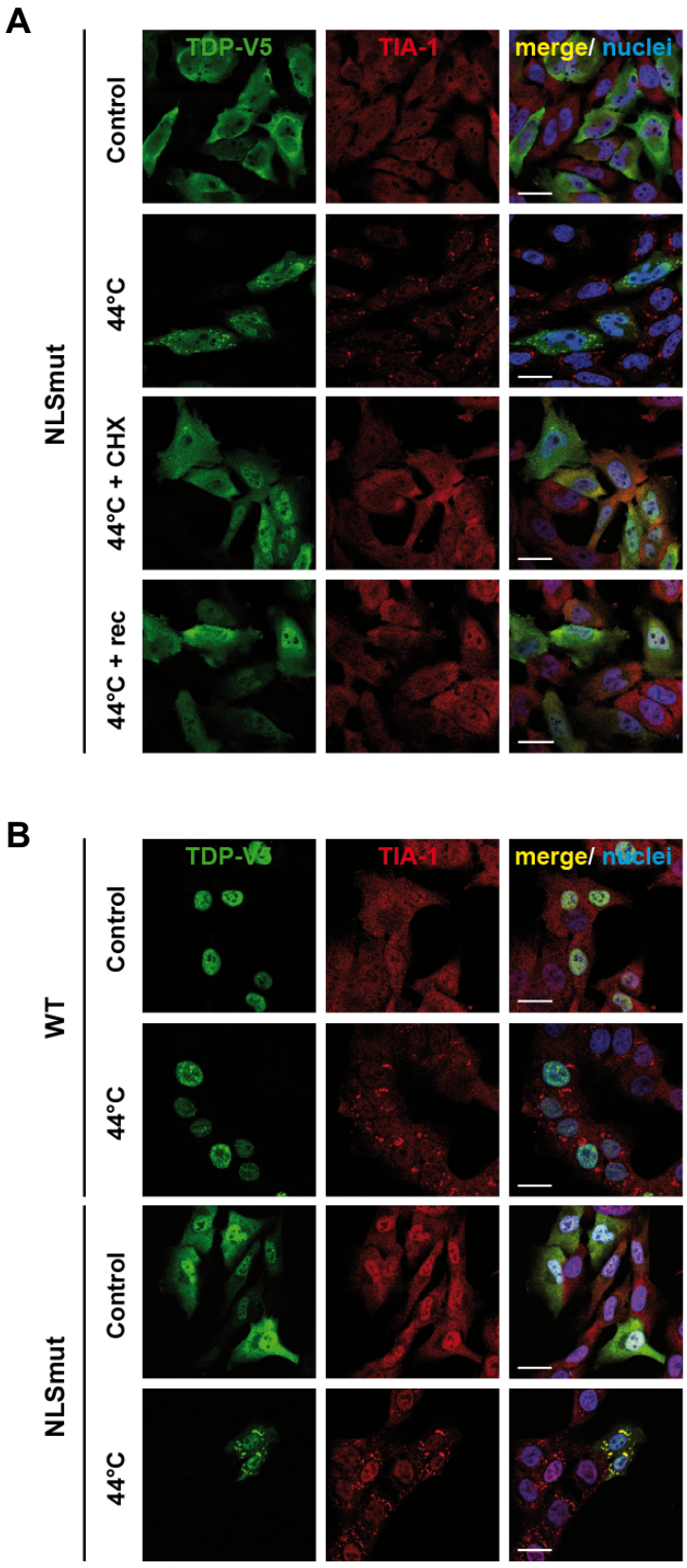
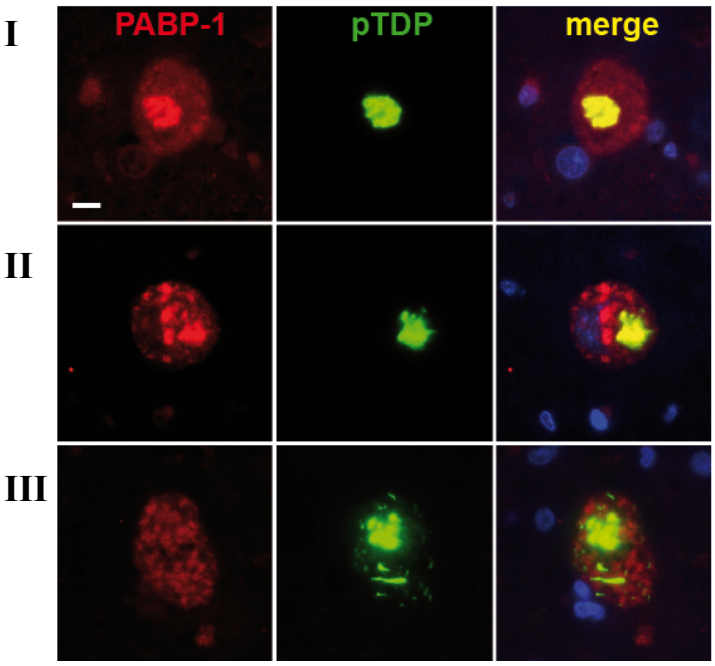

A
NLSmut
Control
44°C
44°C + CHX
44°C + rec
TDP-V5
TIA-1
merge/ nuclei
B
WT
Control
44°C
NLSmut
Control
44°C
TDP-V5
TIA-1
merge/ nuclei

Fig. S6

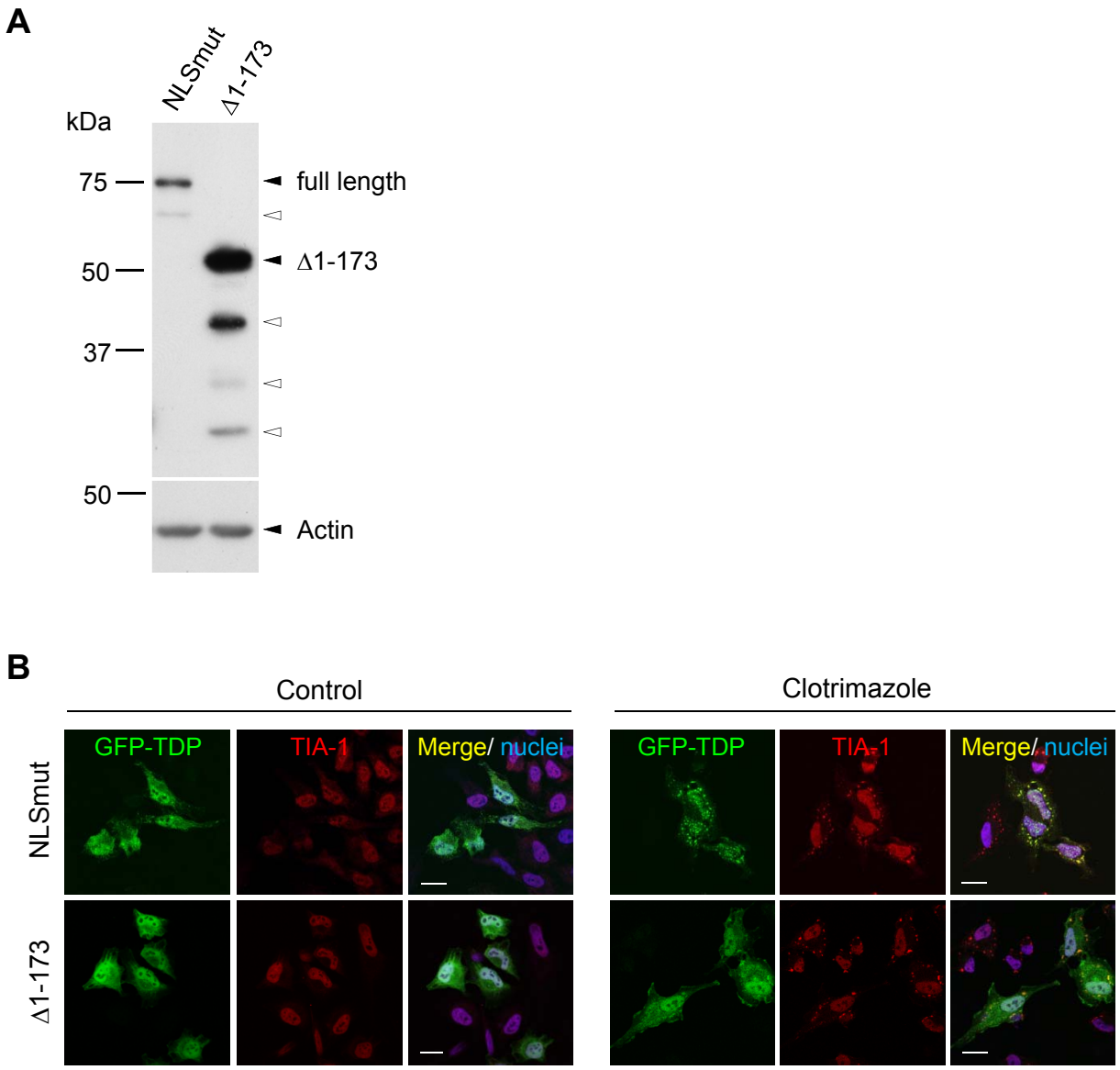
A



B

	Number of pTDP-43 positive NCI in spinal cord	Number of pTDP-43 and PABP1 positive NCI in spinal cord	Percentage pTDP-43 and PABP1 positive NCI in spinal cord
ALS #1	7	4	57.14%
ALS #2	9	7	77.78%
ALS #3	3	2	66.67%
ALS #4	4	3	75.00%
FTLD-TDP #1	11	7	63.64%
FTLD-TDP #2	5	3	60.00%
Total	**39**	**26**	**66.67%**

Fig. S7


A
NLSmut
Δ1-173
kDa
75
50
37
50
full length
Δ1-173
Actin
B
Control
Clotrimazole
GFP-TDP
TIA-1
Merge/ nuclei
NLSmut
Δ1-173