Figure S1

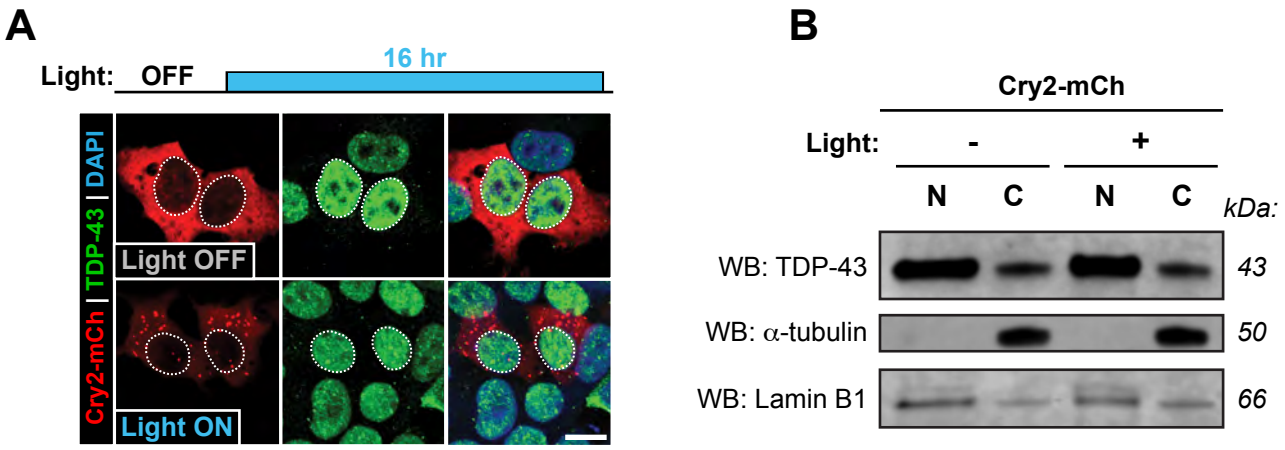
A
Light: OFF
16 hr
DAPI | TDP-43 | Cry2-mCh
Light OFF
Light ON
B
Cry2-mCh
Light: - +
N C N C
kDa:
WB: TDP-43
43
WB: α-tubulin
50
WB: Lamin B1
66

Figure S1. Chronic blue light stimulation alone does not induce mislocalization of endogenous TDP-43, Related to Figure 1.
(A-B) HEK293 cells expressing Cry2olig-mCh were exposed to chronic blue light stimulation (16h, ~0.3 mW/cm^2, 465 nm) and analyzed for mislocalization of endogenous TDP-43. (A) Cells exposed to chronic blue light stimulation (bottom) or darkness (top) both show no cytoplasmic mislocalization of endogenous TDP-43 (green) by immunofluorescence. Cell nuclei are circled. (B) Cell lysates were collected following chronic blue light stimulation (lanes 3-4) or darkness (lanes 1-2) and separated into nuclear (N; lanes 1, 3) and cytoplasmic (C; lanes 2, 4) fractions. Western blot analysis of endogenous TDP-43 shows no cytoplasmic mislocalization of TDP-43 with or without blue light stimulation. Scale bar = 10 μm.

Figure S2

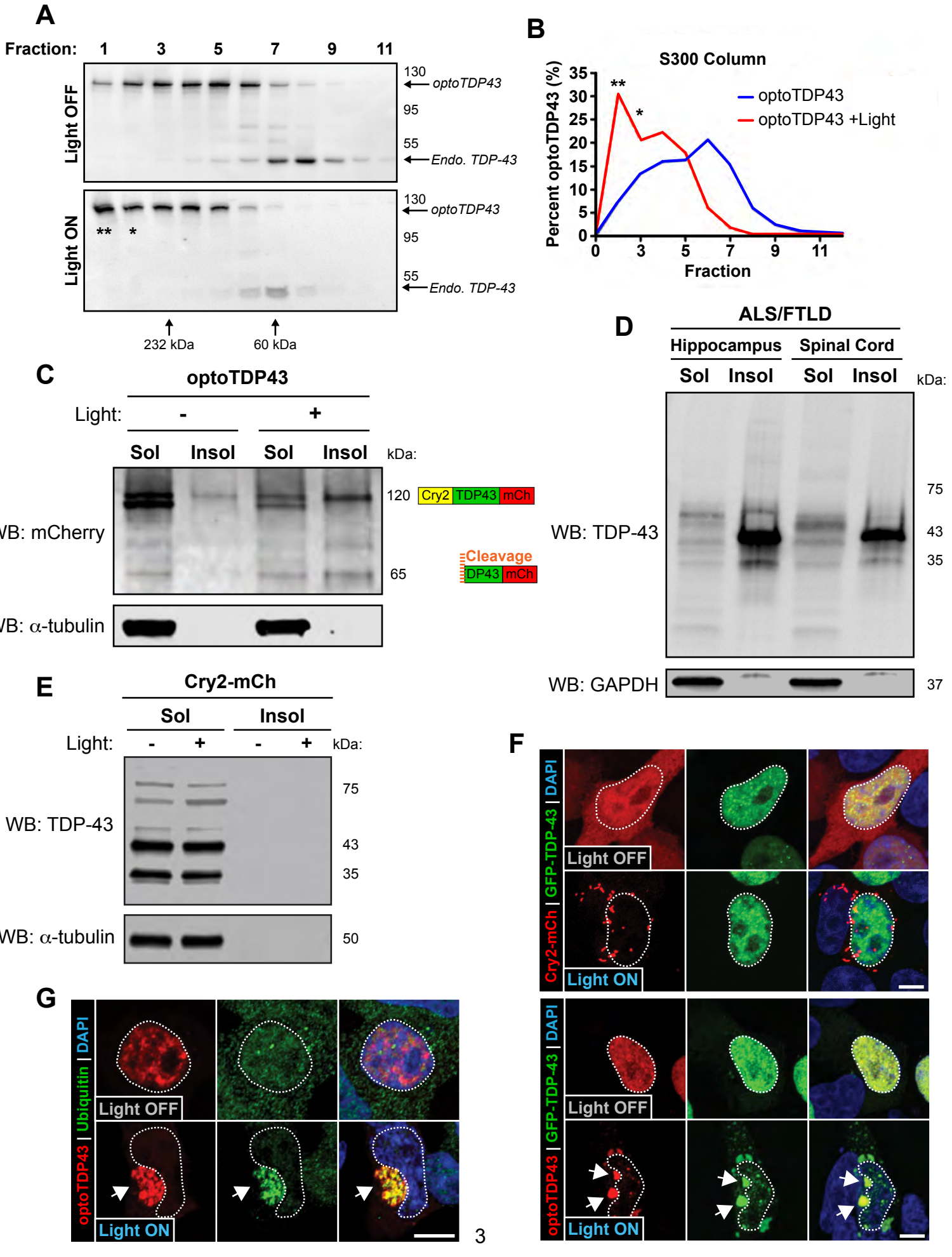
A
Fraction: 1 3 5 7 9 11
Light OFF
Light ON
130
optoTDP43
95
55
Endo. TDP-43
**
*
232 kDa
60 kDa
B
S300 Column
Percent optoTDP43 (%)
optoTDP43
optoTDP43 +Light
Fraction
C
optoTDP43
Light: - +
Sol Insol Sol Insol
kDa:
WB: mCherry
120
Cry2 TDP43 mCh
65
Cleavage
DP43 mCh
WB: α-tubulin
D
ALS/FTLD
Hippocampus
Spinal Cord
Sol Insol Sol Insol
kDa:
WB: TDP-43
75
43
35
WB: GAPDH
37
E
Cry2-mCh
Sol Insol
Light: - + - +
kDa:
WB: TDP-43
75
43
35
WB: α-tubulin
50
F
Light OFF
Light ON
Cry2-mCh | GFP-TDP-43 | DAPI
optoTDP43 | GFP-TDP-43 | DAPI
G
Light OFF
Light ON
optoTDP43 | Ubiquitin | DAPI

Figure S2. Chronic blue light stimulation promotes the formation of high-molecular-weight optoTDP43 oligomers and recruitment of non-optogenetic TDP-43 to inclusions, Related to Figure 1.

(A-B) Cell lysates from HEK293 cells expressing optoTDP43 were collected and analyzed by size exclusion chromatography. Samples were collected from cells either exposed to stimulation with blue light (16h, ~0.3mW/cm^2, 465 nm, bottom) or kept in darkness (top). The void volume of the column was determined with blue dextran (2000 kDa). Column fractions were subjected to SDS-PAGE and western blot analysis for TDP-43. The molecular weight standards are indicated to the right of the representative blots. The elution peak of column size standards (232 kDa and 60 kDa) are indicated by arrows at the bottom of the gel. (B) optoTDP43 proteins exposed to blue light stimulation demonstrate a shift towards higher-molecular weight species (fractions 1-3, indicated by asterisks), indicating light-induced oligomerization. (C) Lysates from optoTDP43-expressing cells exposed to chronic blue light treatment (16h, ~0.3mW/cm^2, 465 nm) or darkness were collected and separated into detergent-soluble (lanes 1, 3) and RIPA-insoluble, urea-soluble (lanes 2, 4) fractions. Western blot analysis of mCherry proteins shows an enhanced shift of full-length and n-terminal cleaved optoTDP43 products in the detergent-insoluble fraction. (D) ALS/FTLD patient tissue from the hippocampus and spinal cord was separated into detergent-soluble (lanes 1, 3) and RIPA-insoluble, urea-soluble (lanes 2, 4) fractions prior to western blot analysis of TDP-43. (E) Cell lysates collected from Cry2-mCh-expressing cells following chronic blue light stimulation (lanes 2, 4) or darkness (lanes 1, 3) were separated into detergent-soluble (lanes 1, 2) and RIPA-insoluble, urea-soluble (lanes 3, 4) fractions. Western blot analysis probing for endogenous TDP-43 shows no recruitment of TDP-43 to the insoluble fraction with or without chronic blue light stimulation. (F) To confirm the ability of optoTDP43 to recruit non-optogenetic TDP-43 species into light-induced inclusions, HEK293 cells were co-transfected with either optoTDP43 (bottom) or the photoreceptor-only control Cry2olig-mCh (top) and EGFP-TDP43. optoTDP43-expressing cells exposed to chronic blue light stimulation (16h, ~0.3mW/cm^2, 465 nm) show co-localization of light-induced inclusions with EGFP-TDP43 (indicated by arrows). Light-induced Cry2olig-mCh clusters show no co-localization with EGFP-TDP43 signal, indicating a TDP-43:TDP-43 interaction-dependence of recruitment to induced optoTDP43 inclusions. Cell nuclei are circled. Scale bar = 10 μm. (G) Cells expressing optoTDP43 were exposed to chronic blue light stimulation (16h, ~0.3mW/cm^2, 465 nm, bottom) or darkness (top) and immunostained for ubiquitin (green). Light-induced optoTDP43 inclusions strongly co-localize with ubiquitin signal (indicated by arrows). Cell nuclei are circled. Scale bar = 10 μm.

Figure S3

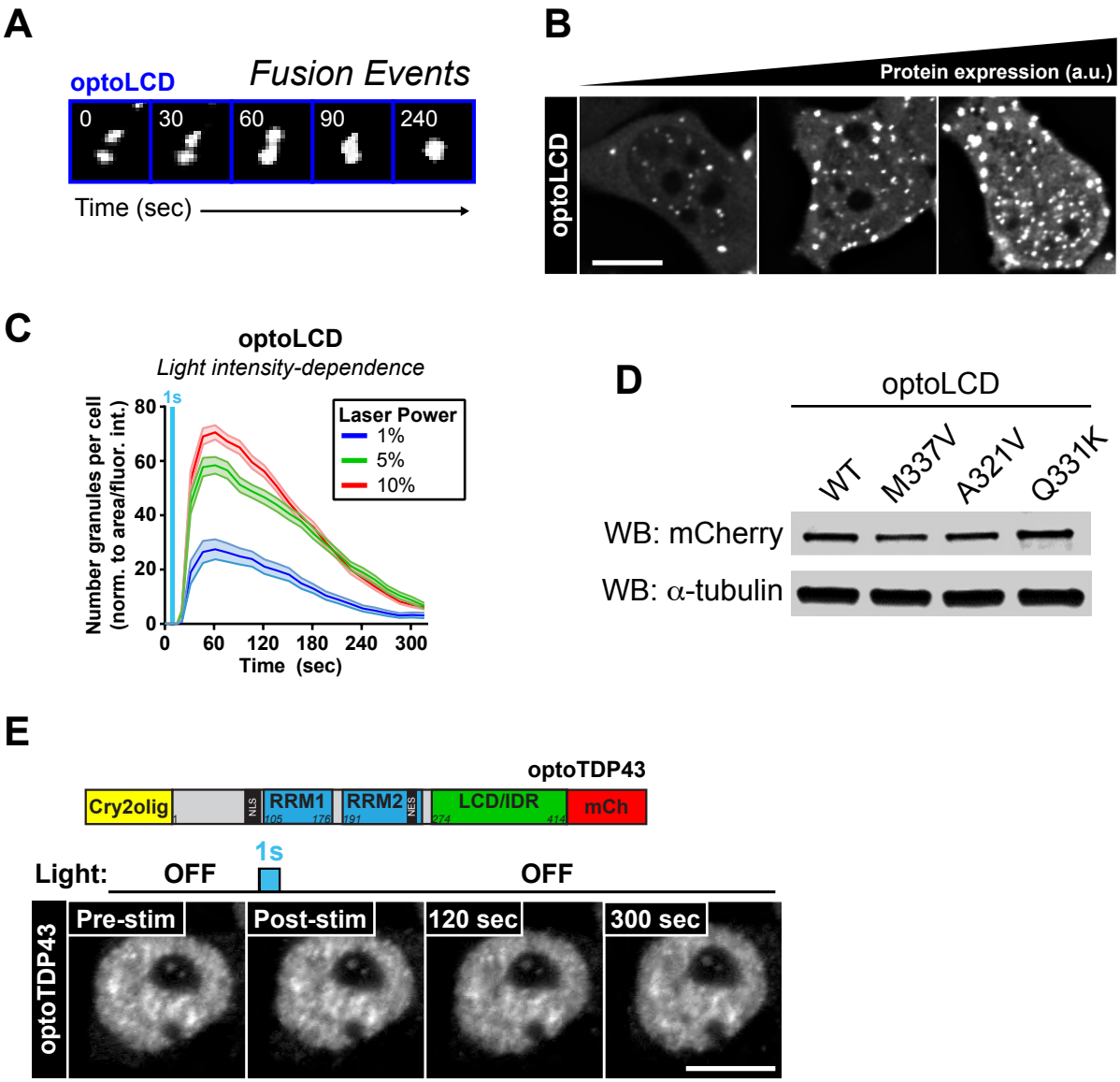
A
optoLCD
Fusion Events
0
30
60
90
240
Time (sec)
B
Protein expression (a.u.)
optoLCD
C
optoLCD
Light intensity-dependence
Number granules per cell (norm. to area/fluor. int.)
1s
Laser Power
1%
5%
10%
Time (sec)
D
optoLCD
WT
M337V
A321V
Q331K
WB: mCherry
WB: α-tubulin
E
optoTDP43
Cry2olig
NLS
RRM1
RRM2
NES
LCD/IDR
mCh
Light:
OFF
1s
OFF
optoTDP43
Pre-stim
Post-stim
120 sec
300 sec

Figure S3. optoLCD granules display properties of phase-separated droplets and full-length optoTDP43 does not form optoDroplets, Related to Figure 2.
(A) Representative images of individual optoLCD granules showing fusion events following acute blue light stimulation (1 sec, 1% laser power, 488 nm). optoLCD granules underwent fusion and subsequent relaxation upon granule:granule contact, suggesting a liquid-like state of light-induced LCD granules. (B) Representative images of optoLCD particle formation following acute blue light stimulation (1 sec, 1% laser power, 488 nm) in cells expressing increasing concentrations of optoLCD proteins (as determined by relative fluorescence intensity). (C) Cells expressing optoLCD were exposed to acute blue light stimulation of increasing laser intensity (1 sec, 1-10% as indicated, 488 nm) and normalized granule number per cell was tracked over time. optoLCD proteins displayed enhanced phase transition responses following acute blue light stimulation of increasing light input, indicating a tunable property of light-induced LCD phase transitions as a function of activated photoreceptor molecules. n = 25-67 cells per laser setting. (D) Western blot analysis of WT and mutant optoLCD protein expression levels. (E) Full-length optoTDP43 was expressed in HEK293 cells exposed to acute blue light stimulation (1 sec, 10% laser power, 488 nm) to determine whether full-length TDP-43 could undergo LIPS. No optoTDP43 granule formation was observed in response to acute blue light stimulation. Data shown are mean +/- S.E.M. Scale bars = 10 μm.

Figure S4

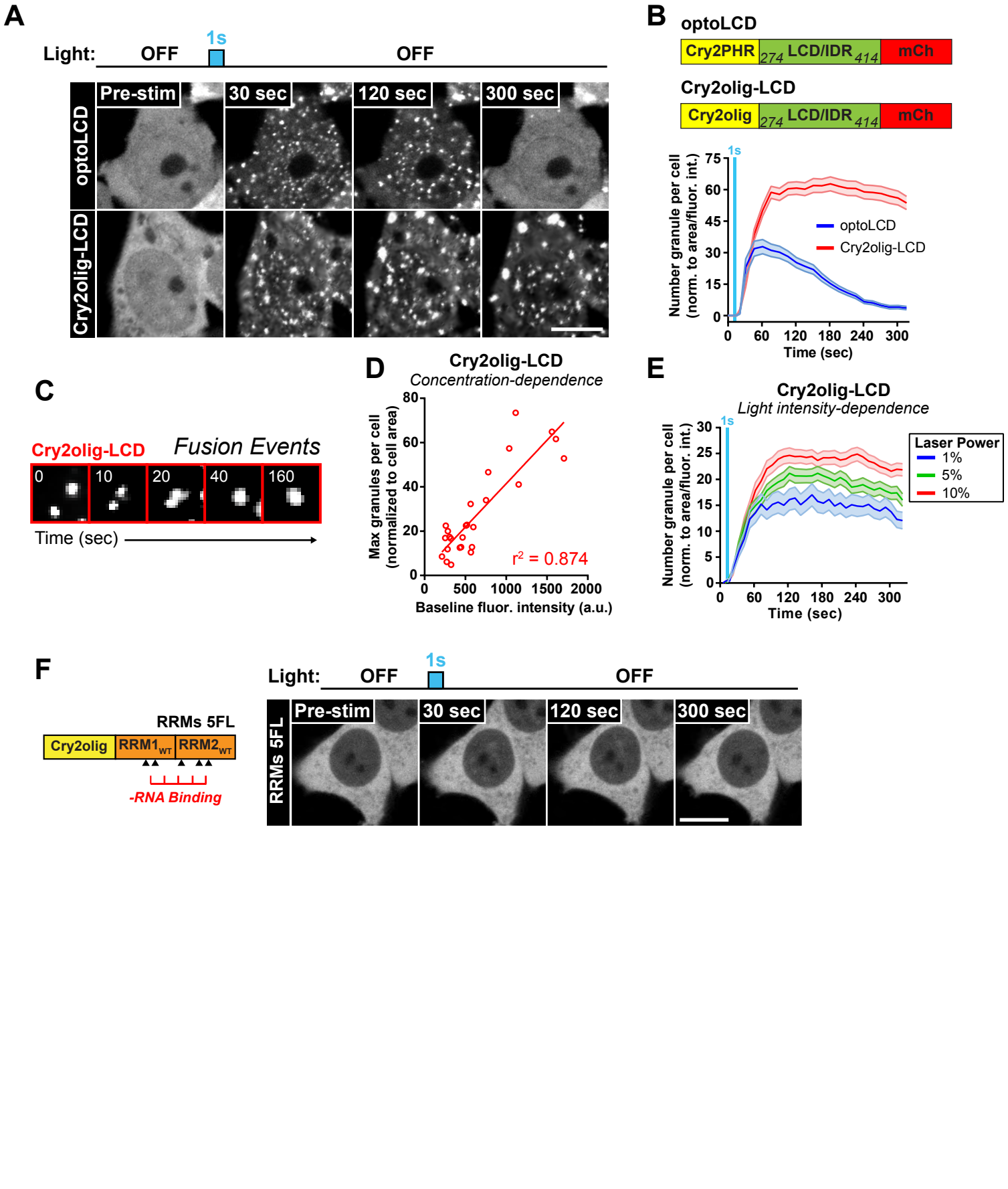
A
Light:
OFF
1s
OFF
Pre-stim
30 sec
120 sec
300 sec
optoLCD
Cry2olig-LCD
B
optoLCD
Cry2PHR
274
LCD/IDR
414
mCh
Cry2olig-LCD
Cry2olig
274
LCD/IDR
414
mCh
Number granule per cell (norm. to area/fluor. int.)
Time (sec)
optoLCD
Cry2olig-LCD
C
Cry2olig-LCD
Fusion Events
0
10
20
40
160
Time (sec)
D
Cry2olig-LCD
Concentration-dependence
Max granules per cell (normalized to cell area)
Baseline fluor. intensity (a.u.)
r2 = 0.874
E
Cry2olig-LCD
Light intensity-dependence
Number granule per cell (norm. to area/fluor. int.)
Time (sec)
Laser Power
1%
5%
10%
F
RRMs 5FL
Cry2olig
RRM1WT
RRM2WT
-RNA Binding
Light:
OFF
1s
OFF
RRMs 5FL
Pre-stim
30 sec
120 sec
300 sec

Figure S4. Cry2olig-LCD confers an enhanced phase separation response to light, Related to Figure 3.
(A-B) HEK293 cells expressing either optoLCD (top) or the Cry2olig modified photoreceptive domain fused to the TDP-43 LCD (Cry2olig-LCD, bottom) were subjected to acute blue light stimulation (1 sec, 1% laser power, 488 nm) and granule formation was tracked over time. (B) Quantification of normalized granules per cell over time shows an enhanced phase transition response to light when the TDP-43 LCD is fused to the Cry2olig domain versus the WT Cry2PHR domain. *n* = 25-48 cells per construct. Data shown are mean +/- S.E.M. (C) Representative images of individual Cry2olig-LCD granules undergoing fusion events following acute blue light stimulation (1 sec, 1% laser power, 488 nm). As was observed with optoLCD droplets, Cry2olig-LCD granules underwent fusion and relaxation upon granule:granule contact. (D) A Pearson's correlation was executed to analyze the protein concentration-dependence of Cry2olig-LCD phase transitions in response to acute blue light stimulation (1 sec, 10% laser power, 488 nm). Baseline fluorescence intensity was plotted against maximum granule number per cell during the imaging period. Data points shown are representative of individual cells. *n* = 26 cells. (E) Cry2olig-LCD-expressing cells were exposed to acute blue light stimulation of increasing laser intensity (1 sec, 1-10% as indicated, 488 nm) and normalized granule number per cell was quantified post-stimulation. Again, an enhanced phase separation response was observed following blue light stimulation of increasing intensity. *n* = 50-81 cells per laser setting. (F) Cells expressing a protein containing the TDP-43 RRMs with RNA-binding mutations fused to the Cry2olig photoreceptor (RRMs 5FL) were exposed to acute blue light stimulation (1 sec, 10% laser power, 488nm). No droplet formation is observed following light stimulation, suggesting the TDP-43 LCD is necessary for LIPS behavior. Data shown are mean +/- S.E.M. Scale bars = 10 μm.

Figure S5

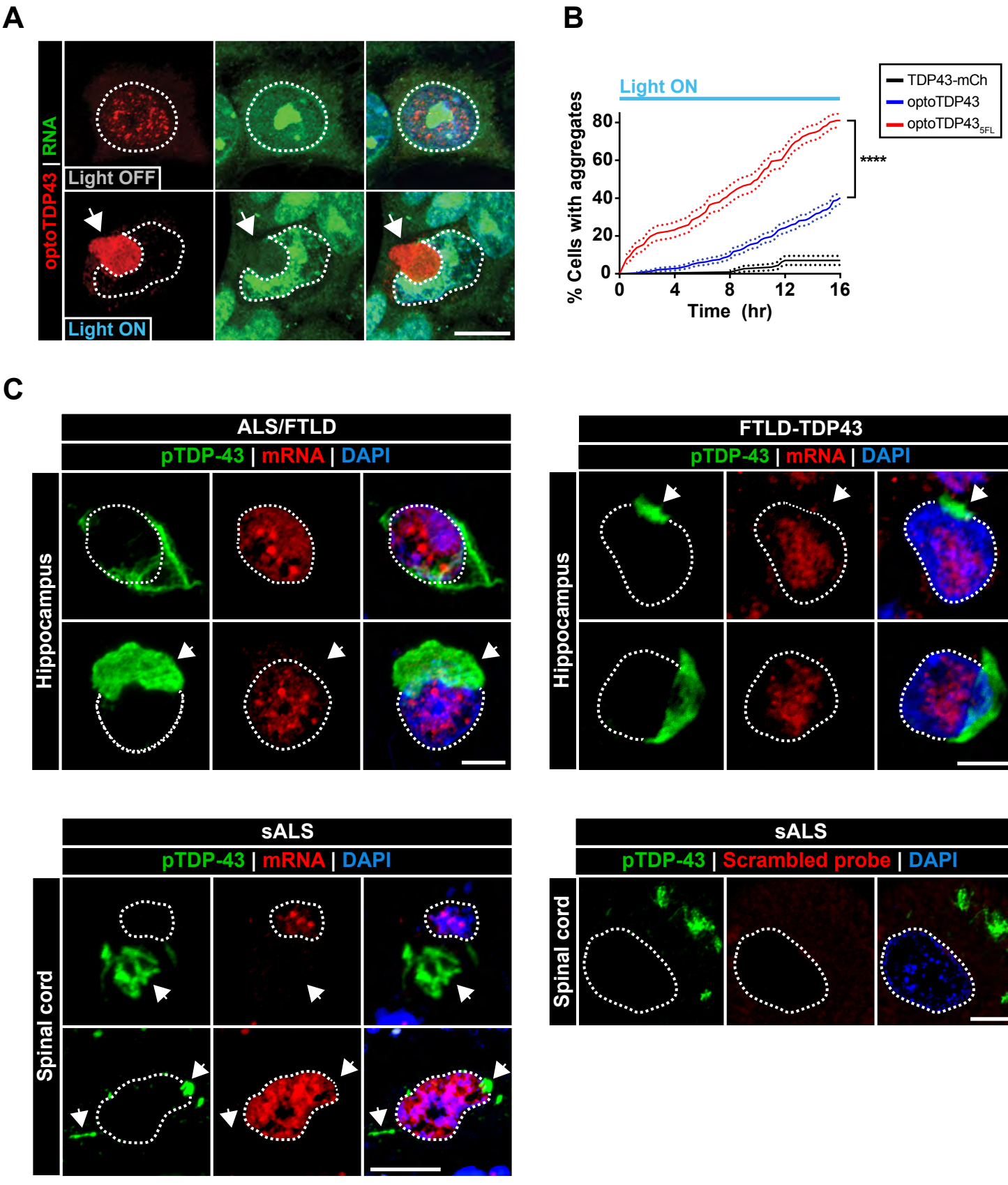
A
optoTDP43 | RNA
Light OFF
Light ON
B
Light ON
TDP43-mCh
optoTDP43
optoTDP43 5FL
% Cells with aggregates
Time (hr)
****
C
ALS/FTLD
pTDP-43 | mRNA | DAPI
Hippocampus
FTLD-TDP43
pTDP-43 | mRNA | DAPI
Hippocampus
sALS
pTDP-43 | mRNA | DAPI
Spinal cord
sALS
pTDP-43 | Scrambled probe | DAPI
Spinal cord

Figure S5. TDP-43 inclusions in patient tissue and optoTDP43 system show an absence of RNA, Related to Figure 3.

(A) optoTDP43 was expressed in HEK293 cells that were exposed to chronic blue light stimulation (16h, ~0.3mW/cm^2, 465 nm) to induce optoTDP43 inclusion formation. Cells were then fixed in ice-cold methanol and stained with SYTO RNAselect non-selective RNA dye to determine whether optoTDP43 inclusions contained any RNA species, in addition to mRNA. optoTDP43 inclusions do not appear to co-stain with SYTO RNAselect dye, suggesting there are no RNA species contained within inclusions. (B) HEK293 cells expressing WT optoTDP43 (blue), RNA-binding-deficient optoTDP43 5FL (red), or non-optogenetic TDP43-mCh (black) were chronically stimulated with blue light (~0.3mW/cm^2, 465 nm) and simultaneously imaged over time in an automated microscopy screen to assess whether RNA-binding affects full-length TDP-43 inclusion formation. Quantification of percentage of cells with inclusions over time shows a significantly increased rate of light-induced inclusion formation with reduced RNA-binding efficiency (optoTDP43 5FL, red). n = 113-239 cells per construct. (C) Additional representative images of mRNA FISH analysis of ALS/FTLD patient tissue. Hippocampal and spinal cord sections from two FTLD cases (*C9ORF72*-FTLD, top left; FTLD-TDP43, top right) and one ALS case (sporadic ALS, bottom left) were examined by immunohistochemistry and RNA FISH. In all cases, no co-localization was observed between mRNA and pTDP-43 signal. Data shown are mean +/- S.E.M. ****, $p < .0001$. Cell nuclei are circled. Scale bars = 10 μm.

Figure S6

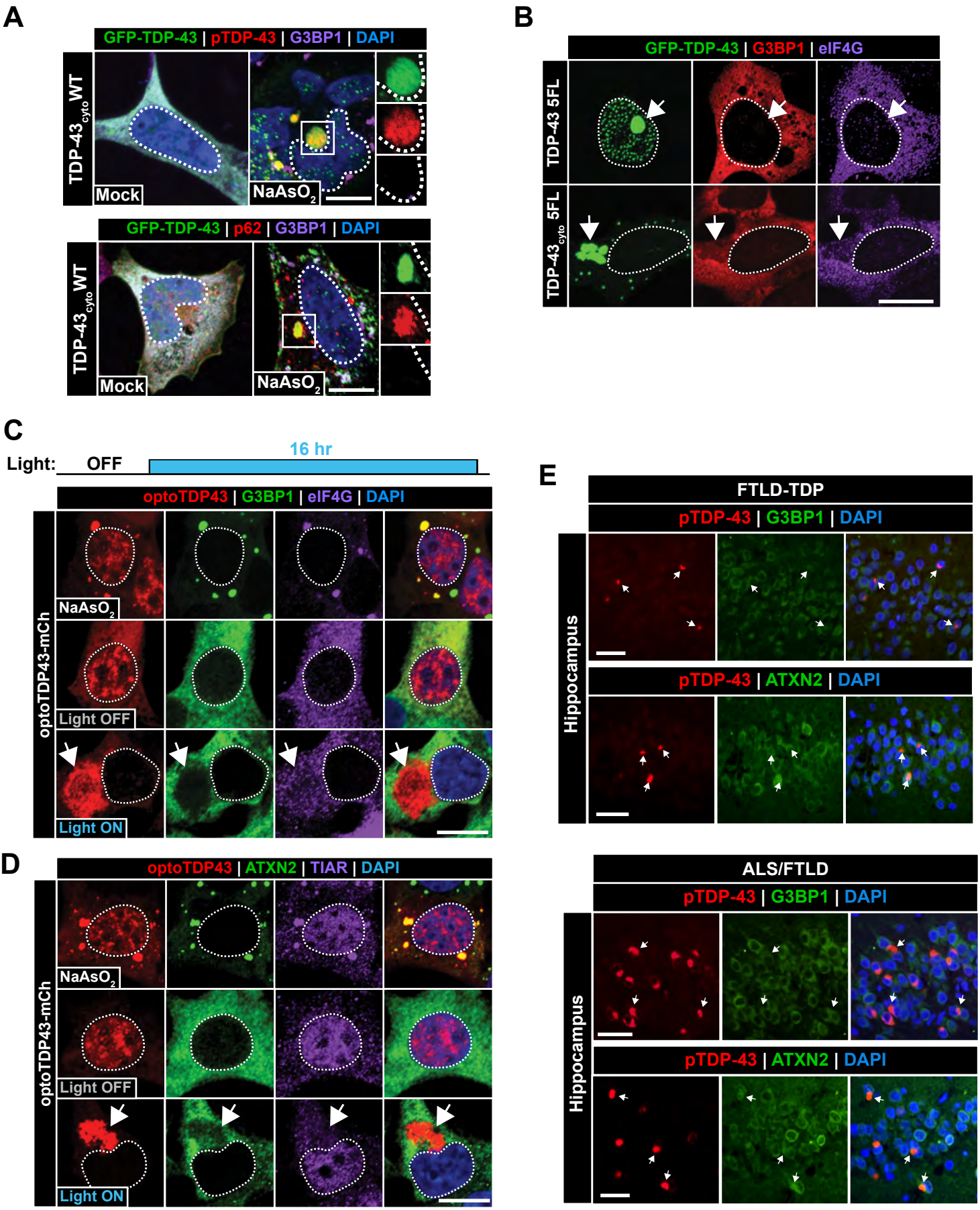
A
GFP-TDP-43 | pTDP-43 | G3BP1 | DAPI
TDP-43cyto WT
Mock
NaAsO2
GFP-TDP-43 | p62 | G3BP1 | DAPI
TDP-43cyto WT
Mock
NaAsO2
B
GFP-TDP-43 | G3BP1 | eIF4G
TDP-43 5FL
TDP-43cyto 5FL
C
Light:
OFF
16 hr
optoTDP43 | G3BP1 | eIF4G | DAPI
optoTDP43-mCh
NaAsO2
Light OFF
Light ON
D
optoTDP43 | ATXN2 | TIAR | DAPI
optoTDP43-mCh
NaAsO2
Light OFF
Light ON
E
FTLD-TDP
pTDP-43 | G3BP1 | DAPI
Hippocampus
pTDP-43 | ATXN2 | DAPI
ALS/FTLD
pTDP-43 | G3BP1 | DAPI
Hippocampus
pTDP-43 | ATXN2 | DAPI

Figure S6. RNA-deficient TDP-43 inclusions do not co-localize with stress granule components, Related to Figure 5.
(A) HEK293 cells expressing EGFP-TDP43cyto were immunostained for hyperphosphorylated TDP-43 (top row, red) or p62 (bottom row, red) and G3BP1 (purple) following sodium arsenite treatment. (B) Representative images of cells containing TDP-43 5FL and TDP-43cyto 5FL inclusions immunostained for stress granule proteins G3BP1 (red) and eIF4G (purple).
(C-D) HEK293 cells expressing optoTDP43 were subjected to sodium arsenite treatment (top row), chronic blue light stimulation (16 hr, ~0.3 mW/cm^2, 465 nm) (bottom row), or darkness (middle row) and immunostained for known stress granule components G3BP1 (green) and eIF4G (purple) (C) or ATXN2 (green) and TIAR (purple) (D). Cells treated with sodium arsenite (top row) show that optoTDP43 can be recruited to stress granules through the activation of endogenous cellular stress pathways. However, optoTDP43 inclusions (indicated by arrows) induced with blue light stimulation do not co-localize with any of the tested stress granule markers. Scale bars = 10 μm. Cell nuclei are circled. (E) Representative images of neuropathological examination of TDP-43 inclusions and SG component proteins (G3BP1, top rows; ATXN2, bottom rows) in FTLD-TDP and ALS/FTLD hippocampal sections. Arrows indicate lack of co-localization between SG components and TDP-43 inclusions. Scale bars = 50 μm.

Figure S7

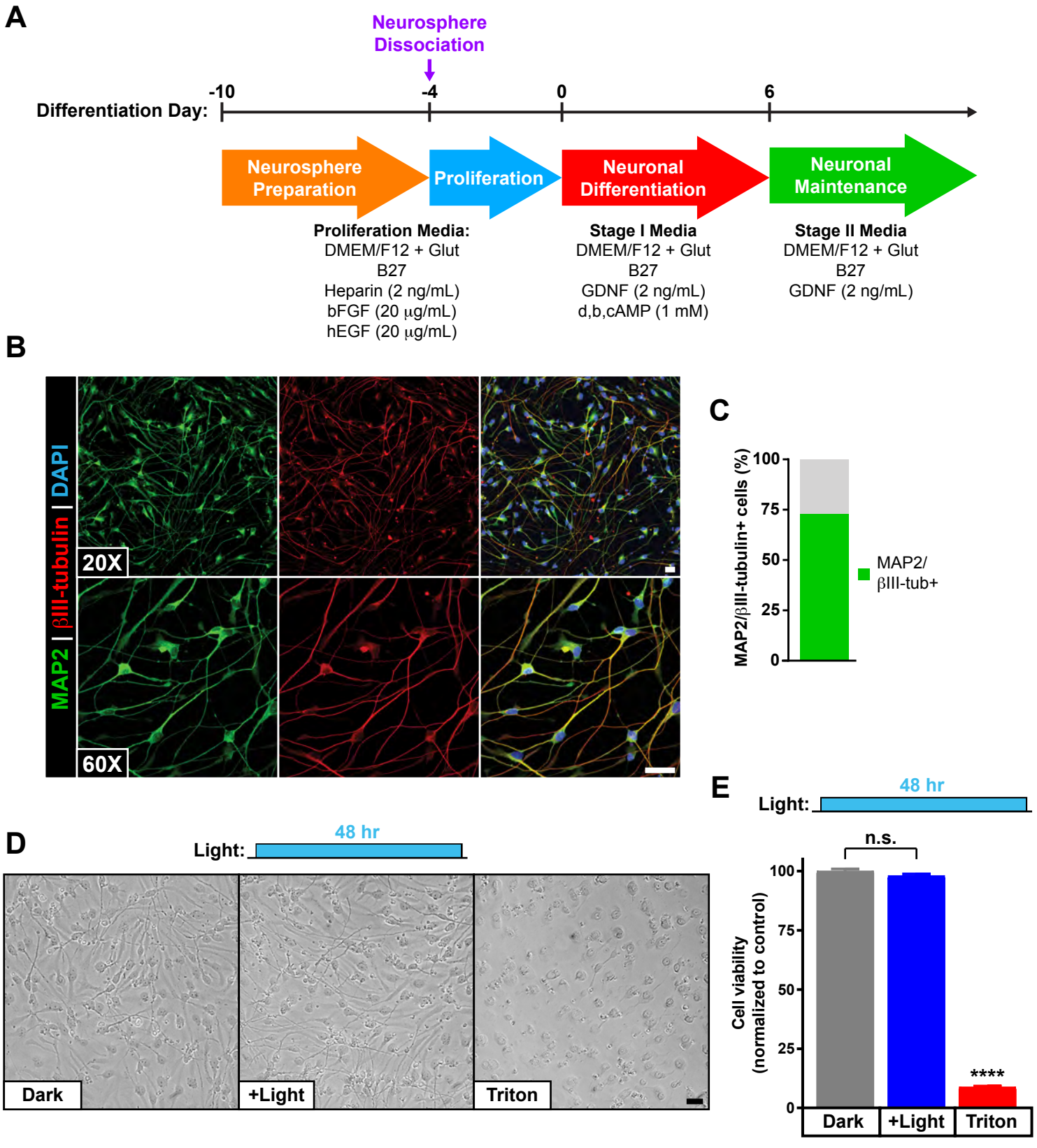
A
Neurosphere Dissociation
Differentiation Day:
-10
-4
0
6
Neurosphere Preparation
Proliferation
Neuronal Differentiation
Neuronal Maintenance
Proliferation Media:
DMEM/F12 + Glut
B27
Heparin (2 ng/mL)
bFGF (20 μg/mL)
hEGF (20 μg/mL)
Stage I Media
DMEM/F12 + Glut
B27
GDNF (2 ng/mL)
d,b,cAMP (1 mM)
Stage II Media
DMEM/F12 + Glut
B27
GDNF (2 ng/mL)
B
MAP2 | βIII-tubulin | DAPI
20X
60X
C
MAP2/βIII-tubulin+ cells (%)
100
75
50
25
0
MAP2/
βIII-tub+
D
Light:
48 hr
Dark
+Light
Triton
E
Light:
48 hr
n.s.
Cell viability
(normalized to control)
100
75
50
25
0
****
Dark
+Light
Triton

Figure S7. ReNcell VM cortical differentiation yields a highly neuronally-enriched culture, Related to Figure 6.
(A) ReNcell VM neuronal differentiation protocol schematic. (B) Representative immunofluorescence images of ReNcell VM neurons (differentiation day 18) following the differentiation protocol outlined in (A). MAP2 (green) and βIII-tubulin (red) are shown at 20X (top) and 60X (bottom) magnification. (C) Quantification of the percentage of MAP2/BIII-tubulin double-labeled cells shows a highly enriched neuronal population following the outlined differentiation protocol. *n* = 260 cells. (D-E) ReNcell neurons were exposed to chronic blue light stimulation (48 hr, ~0.3 mW/cm^2, 465 nm) or darkness prior to examination of neuronal morphology (D) and cell viability, as assessed by measurements of ATP levels (E) (CellTiter-Glo, Promega). No differences in morphology (D) or cell viability (E) were observed between neurons in blue light or darkness conditions. Treatment with 0.1% Triton X-100 served as a positive death control. Scale bars = 20 μm.

Supplementary Table 1. ALS/FTD patient information and scoring, related to Figures 3 and 4

Supplementary Table 2. Primer Sequences and cloning information, related to Star Methods

Movie S1. optoTDP43 chronic stimulation longitudinal imaging, related to Figure 1

Movie S2. optoTDP43 inclusion FRAP analysis, related to Figure 1

Movie S3. Cry2PHR vs optoLCD light induced phase separation, related to Figure 2

Movie S4. optoLCD vs. optoLCD^{M377V}, optoLCD^{Q331K}, and optoLCD^{A321V} repetitive light induced phase separation, related to Figure 2

Movie S5. Lack of optoTDP43 light induced phase separation, related to Figure 3

Movie S6. Cry2olig LCD RRMs and FUS RRM + LCD light induced phase separation, related to Figure 4

Movie S7. Cytoplasmic TDP-43 FRAP within and outside of G3BP+ stress granules, related to Figure 5

Movie S8. optoTDP43 neuronal survival imaging, related to Figure 6

Movie S9. Cry2-mCh neuronal survival imaging, related to Figure 6