Supplementary Information

TDP43 aggregation at ER-exit sites impairs ER-to-Golgi transport

Hongyi Wu[1], Loo Chien Wang[2], Belle M. Sow[1], Damien Leow[3], Jin Zhu[1], Kathryn M. Gallo[4, 5], Kathleen Wilsbach[4, 5], Roshni Gupta[1], Lyle W. Ostrow[4, 5, *], Crystal J. J. Yeo[6-9, *], Radoslaw M. Sobota[2, *], Rong Li[1, 10, 11, *]

[1] Mechanobiology Institute, National University of Singapore (NUS), Singapore

[2] Functional Proteomics Laboratory, SingMass National Laboratory, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore

[3] Department of Anatomy, Yong Loo-Lin School of Medicine, National University of Singapore, Singapore

[4] Department of Neurology, School of Medicine, Johns Hopkins University, USA

[5] Department of Neurology, Lewis Katz School of Medicine, Temple University, USA

[6] National Neuroscience Institute, A*STAR, and Duke-NUS Medical School, Singapore

[7] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

[8] Department of Neurology, Feinberg School of Medicine, Northwestern University, USA

[9] School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, UK

[10] Department of Biological Sciences, National University of Singapore, Singapore

[11] Lead contact: mbihead@nus.edu.sg

* Correspondence

Supplementary Figure Legends

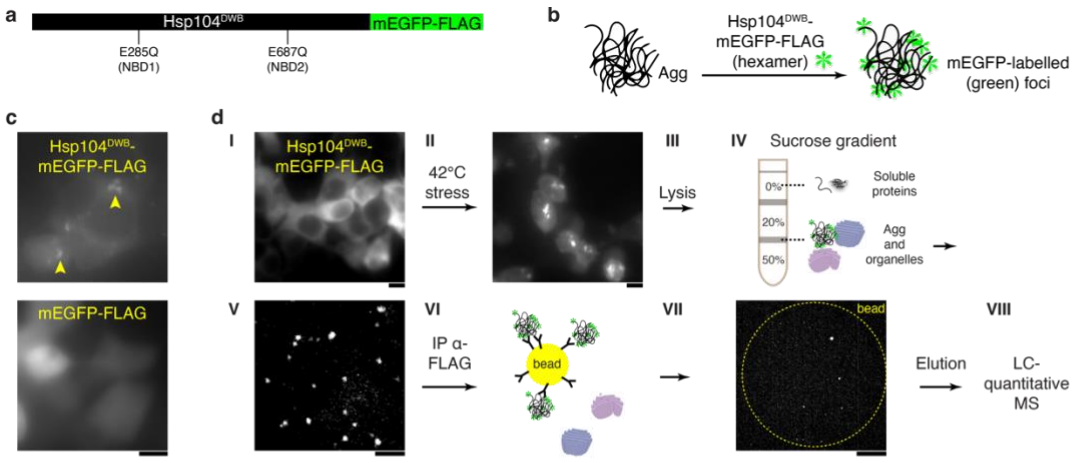
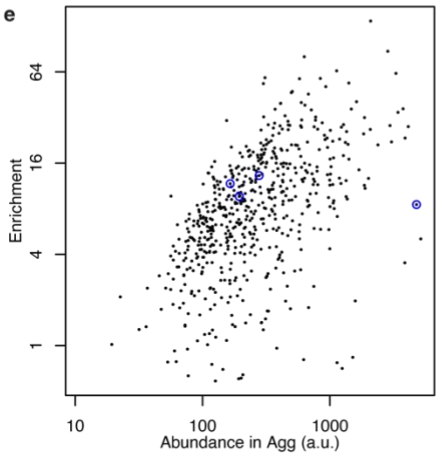


Fig S1



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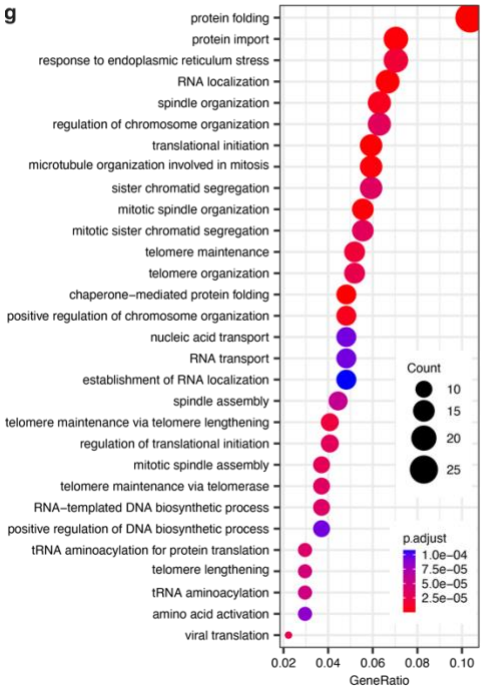
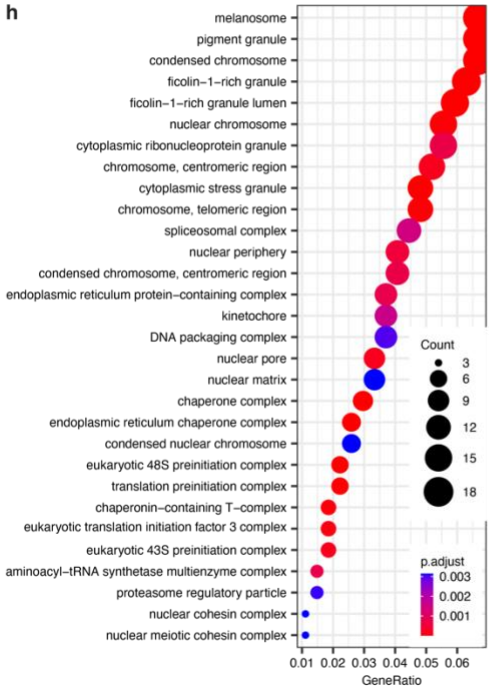
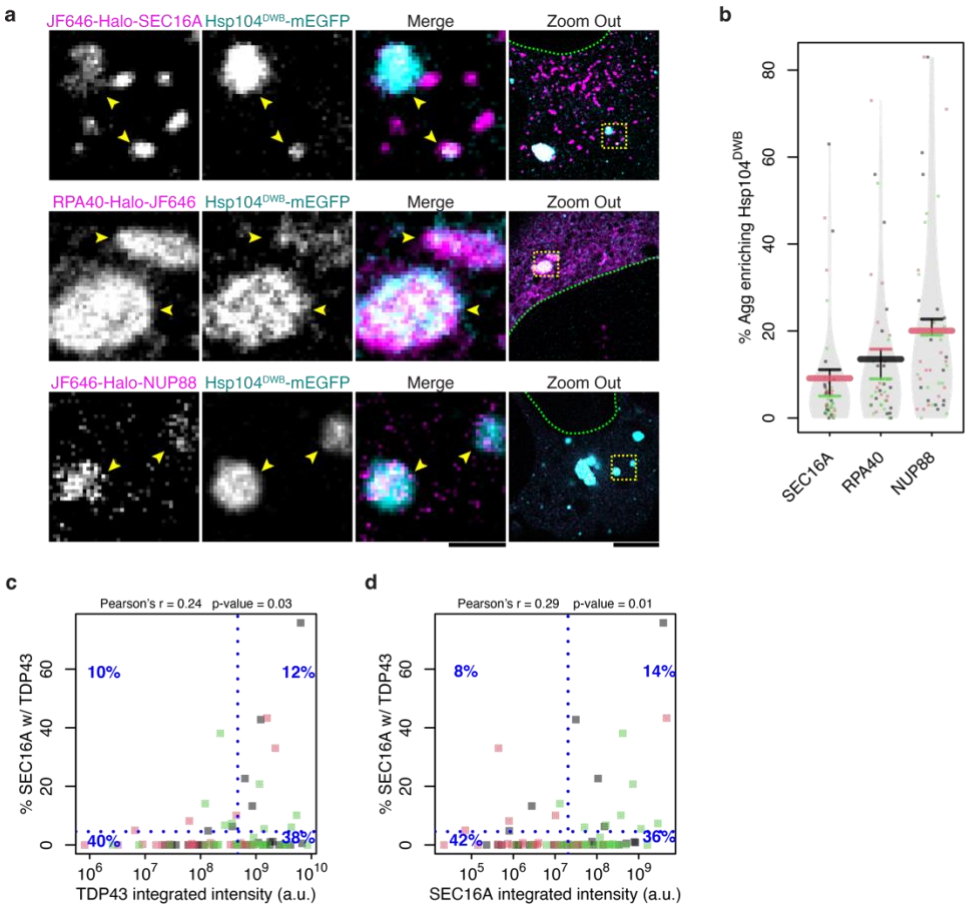
Protein	Abundance in Agg	Enrichment
Hsp104DWB -mEGFP -FLAG	4786	8.53
TDP43	179	Inf
G3BP1	278	13.3
SEC16A	165	11.7
RPA40	100	Inf
NUP88	194	9.65





Supplementary Fig. 1. Hsp104^{DWB} enables affinity-based aggregate purification.

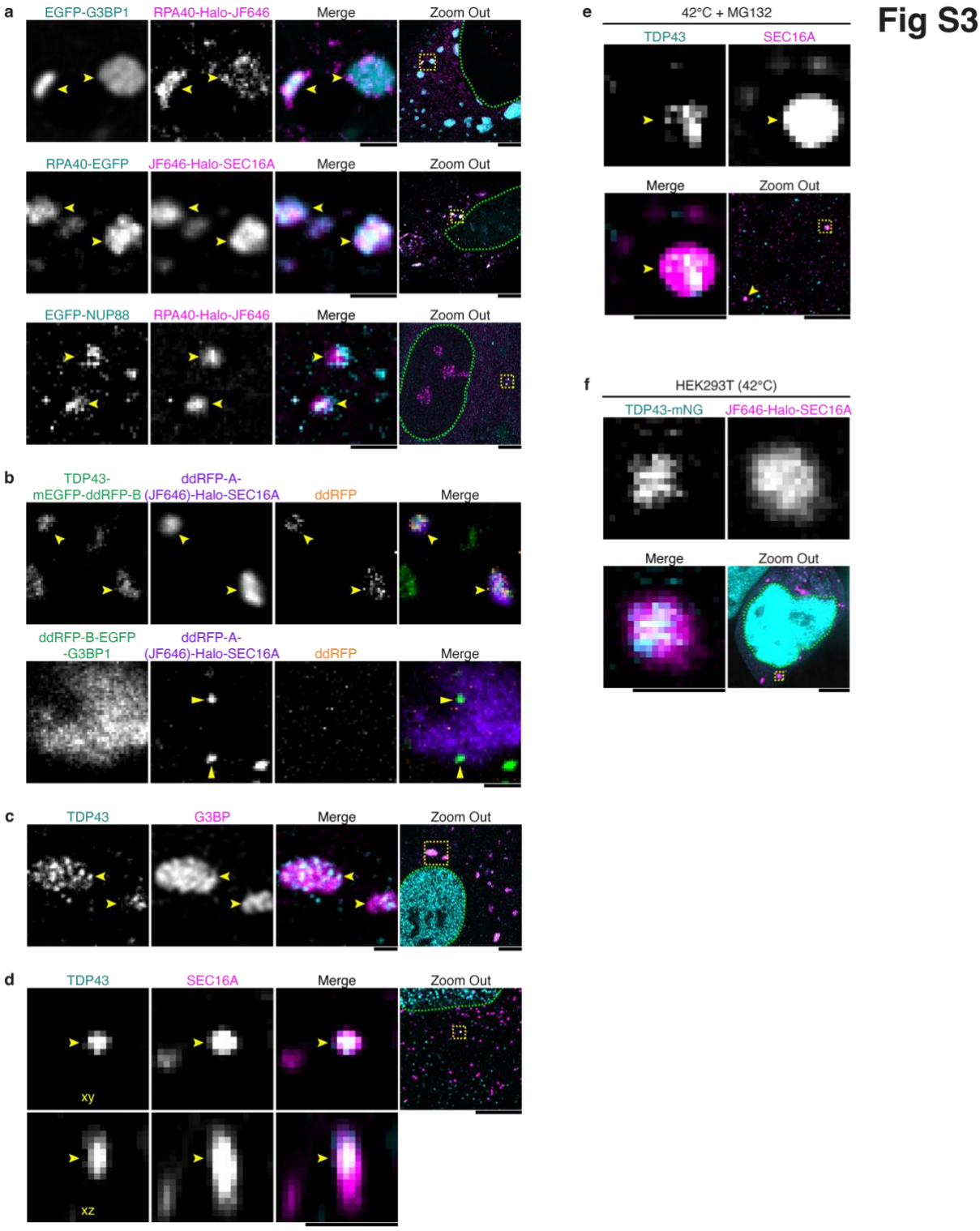
(**a**) Schematic diagram of Hsp104^{DWB}-mEGFP-FLAG showing the locations of double Walker B (DWB) mutations in both nucleotide binding domains (NBDs). (**b**) Working scheme of aggregate labelling. Multiple Hsp104^{DWB}-mEGFP hexamers bind misfolded proteins in aggregates but not release to form bright foci. (**c**) Representative images of Hsp104DWB-mEGFP-FLAG and mEGFP-FLAG expressed in HEK293T cells incubated for 12-16 hr at 42°C. Arrowheads indicate Hsp104^{DWB}-labelled aggregates. Each scale bar represents 10 µm. (**d**) Workflow of protein aggregate purification by sucrose gradient centrifugation and immunoprecipitation. (**I**) HEK293T cells transfected with Hsp104^{DWB}-mEGFP-FLAG were (**II**) heat stressed at 42°C for 12 hr and (**III**) lysed by RIPA buffer. (**IV and V**) The lysate was fractionated by sucrose gradient centrifugation to enrich protein aggregates at the interface between 20% and 50% [w/v] sucrose layers. (**VI and VII**) Then Hsp104^{DWB}-bound aggregates were immunoprecipitated (IP) using anti-FLAG beads and (**VIII**) sent for liquid chromatography (LC)-quantitative mass-spectrometry (MS). Aggregates were imaged by Hsp104^{DWB}-mEGFP-FLAG fluorescence at the various steps. The images in (I) and (II) were captured by a widefield epifluorescence microscope whereas (IV) and (VII) are the max intensity projections of confocal z-stacks. Each scale bar represents 10 µm. The yellow dashed circle in (VII) demarcates an anti-FLAG bead in the transmission light channel. (**e**) Scatter plot showing the abundance of MS-identified proteins in purified aggregates (plotted on a $\log_{10}$ scale) versus fold enrichment in aggregates (*i.e.* ratio between aggregate and IP control, plotted on a $\log_2$ scale). The positions of Hsp104^{DWB}-mEGFP-FLAG and proteins later investigated are circled with values tabulated in (f). a.u.: arbitrary unit. n = 1. (**f**) Table showing the abundance and fold enrichment in aggregate of several proteins investigated in this study. Infinity (Inf) arose as some proteins were detected only in aggregates but not IP control. (**g and h**) GO analysis of the biological processes (g) and subcellular locations (h) of proteins enriched in aggregates. GeneRatio: fraction of aggregate proteins annotated in the respective GO term; Count: number of aggregate proteins annotated in the respective term; p.adjust: adjusted p-value of hypergeometric test.



Supplementary Fig. 2. HSP104^{DWB}-labelled aggregates in HEK293T and correlation analysis of TDP43/SEC16A co-aggregation with expression levels.

(**a**) Representative images of aggregates formed by RPA40, SEC16A and NUP88 and their colocalization with Hsp104^{DWB}. Similar to Fig. 1g, h, but in HEK293T cells stressed at 42°C for 12-16 hr. Arrowheads: Hsp104^{DWB}-labelled aggregates; yellow boxes in "Zoom Out": regions displayed in zoom-in views; green dashes: the nuclei. Scale bars except in "Zoom Out" represent 1 μm, and represent 5 μm in "Zoom Out". (**b**) Quantification of the experiments as in (a) showing the percentage of RPA40, SEC16A and NUP88 aggregates labelled by Hsp104^{DWB} in each cell. n = 45 cells examined over 3 independent experiments. The values of individual cells are plotted as dots, and the mean values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. (**c and d**) Pearson correlation analysis on the relationship between the percentage of SEC16A inclusions that enriched for TDP43 and TDP43 (c) or SEC16A (d) expression. n = 90 cells examined over 3 independent experiments. Each dot represents one cell, and different colors denote different experiments. The blue

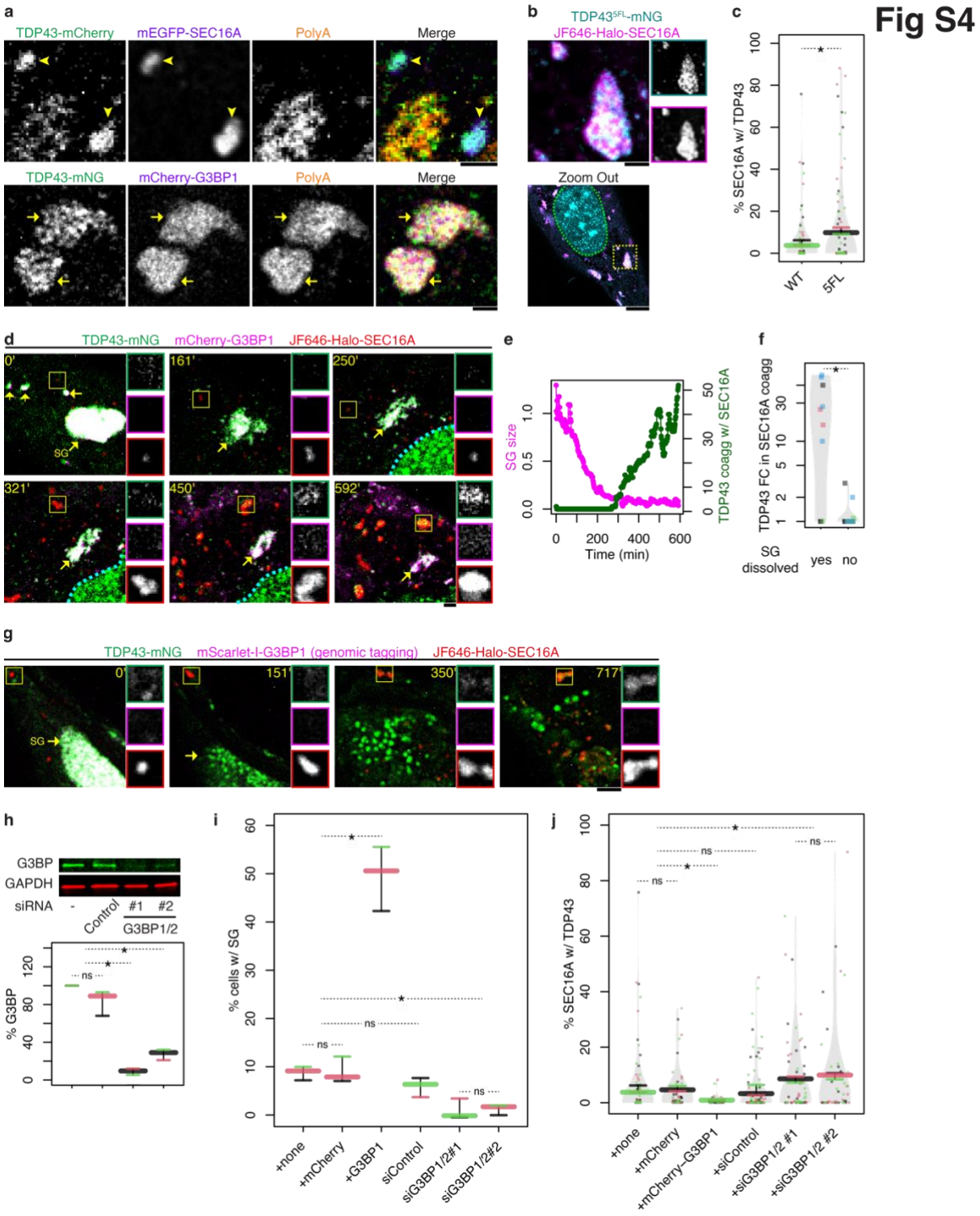
vertical dashes mark the median intensities of TDP43 or SEC16A among cells and the horizontal dashes mark the average co-aggregation level. The percentages of cells that fall into each quadrant are shown.



Supplementary Fig. 3. Proteins co-aggregate with selective partners.

(**a**) Representative images of the colocalization between RPA40 aggregates and aggregates formed by G3BP1, SEC16A or NUP88. Arrowheads: co-aggregates; yellow boxes in “Zoom Out”: regions displayed in zoom-in views; green dashes: the nuclei. The quantification is shown in Fig. 2h. Scale bars except in “Zoom Out”

represent 1 μm, and represent 5 μm in “Zoom Out”. (**b**) Representative images of ddRFP-based protein interaction assays. Displayed are different fluorescence channels of cells forming either coaggs of TDP43-mEGFP-ddRFP/ddRFP-A-Halo-SEC16A or cells with ddRFP-B-EGFP-G3BP1 aggregates and ddRFP-A-Halo-SEC16A aggregates after incubation at 42°C for 12-16 hr. Arrowheads: TDP43/SEC16A coaggs; triangles: SEC16A inclusions without TDP43. Scale bars except in “Zoom Out” represent 1 μm, and represent 5 μm in “Zoom Out”. (**c**) Representative images of the immunofluorescence staining of TDP43 and G3BP (G3BP1/2) in cells forming SGs after incubation at 42°C for 16 hr. Scale bars except in “Zoom Out” represent 1 μm, and represent 5 μm in “Zoom Out”. (**d**) Representative images of the immunofluorescence staining of TDP43 and SEC16A in cells forming coaggs after incubation at 42°C for 12-16 hr. Scale bars except in “Zoom Out” represent 1 μm, and represent 5 μm in “Zoom Out”. (**e**) Representative images of the immunofluorescence staining of TDP43 and SEC16A in cells treated with 10 μM MG132 and incubated for 12-16 hr at 42°C. Scale bars except in “Zoom Out” represent 1 μm, and represent 5 μm in “Zoom Out”. (**f**) Representative images of TDP43/SEC16A coaggs formed in HEK293T cells after incubation for 12-16 hr at 42°C. Scale bars except in “Zoom Out” represent 1 μm, and represent 5 μm in “Zoom Out”.

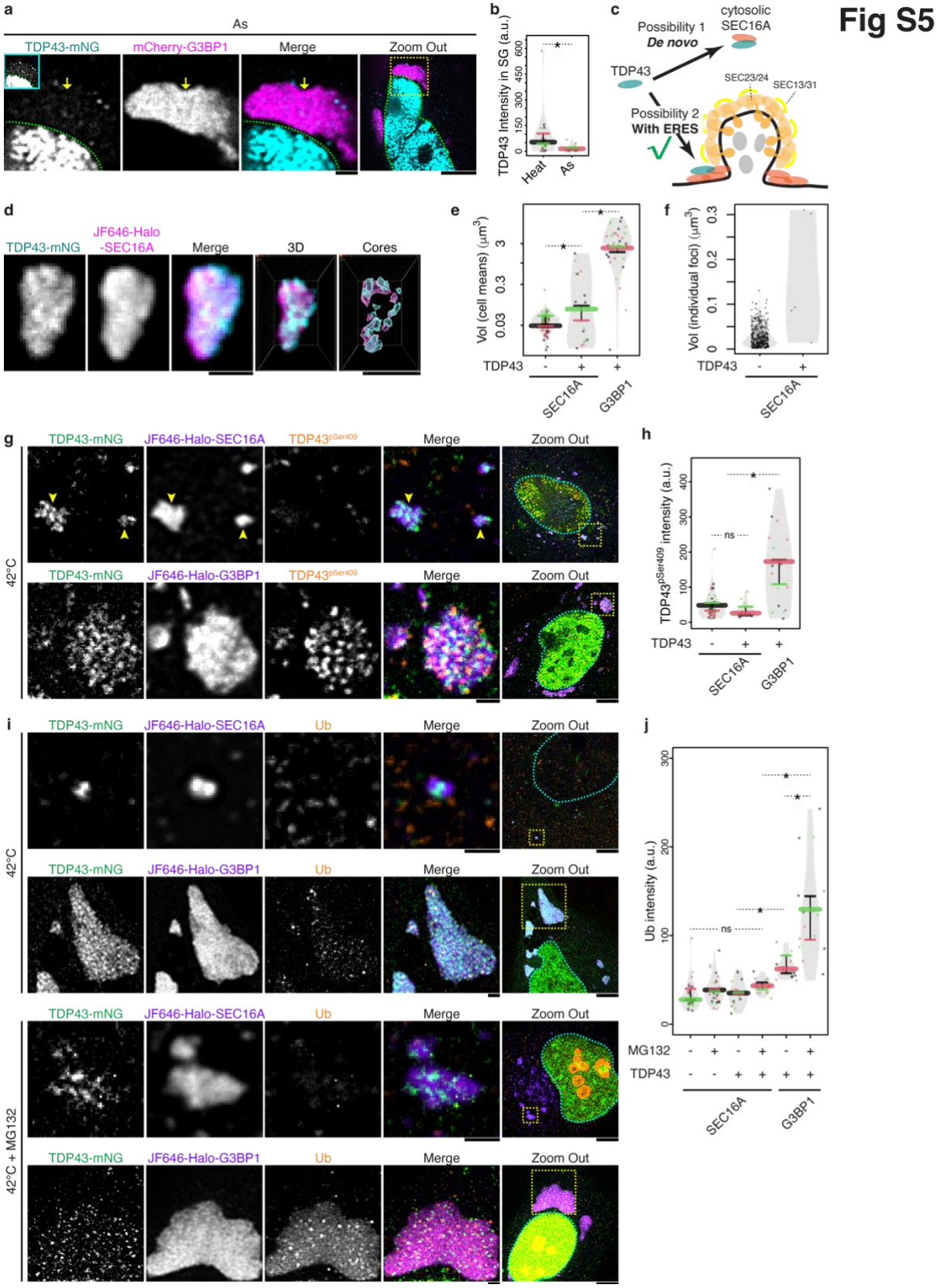


Supplementary Fig. 4. TDP43 partitions dynamically between SGs and co-aggregates with SEC16A.

(**a**) Representative images showing fluorescence in-situ hybridization (FISH) staining of polyA RNAs in cells containing TDP43/SEC16A coaggs (indicated by arrowheads) or SGs (indicated by arrows). PolyA RNAs were probed by oligo(dT)$_{30}$ conjugated to AF647. Each scale bar represents 1 µm. (**b**) Representative images showing SEC16A

co-aggregation with TDP43^{5FL}. In “Zoom out”, the yellow box marks the region shown in zoom-in views, and green dashes demarcate the nucleus. Scale bars except in “Zoom Out” represent 1 μm, and represent 5 μm in “Zoom Out”. (**c**) Quantification of the volume percentage of SEC16A inclusions with wild-type (WT) TDP43 or TDP43^{5FL} aggregation in cells that expressed either construct. n = 45 cells examined over 3 independent experiments. The values of individual cells are plotted as dots, and the mean values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. The asterisk stands for significant (p-value ≤ 0.05) in two-sided t-test. (**d**) Selected frames from a representative timelapse recording of SGs (indicated by arrows) and SEC16A inclusions in a cell shifted to 42°C for 60 min before imaging started. Insets show individual channels of the yellow boxed regions (SEC16A inclusion). The scale bar represents 1 μm. (**e**) Quantification of the experiment in (d) showing the relative SG size and TDP43 intensity in coaggs with SEC16A over time (normalized to their respective initial values). (**f**) Quantification of the experiments as in (d) showing the fold changes (FCs) of TDP43 in SEC16A inclusions at the end of tracking of SG-containing cells, grouped by whether SGs were dissolved to below 10% of the initial size. n = 20 cells examined over 4 independent experiments. The FC of each cell was plotted as a datapoint on $\log_{10}$ scale, and different colors denote different experiments. The asterisk stands for significant (p-value ≤ 0.05) in two-sided t-test. (**g**) Selected frames from a representative timelapse recording of SGs (indicated by arrows) and SEC16A inclusions in a cell shifted to 42°C for 60 min before imaging started. Similar to (d), but G3BP1 is not overexpressed but tagged in-frame by CRISPR/Cas. Insets show individual channels of the yellow boxed regions (SEC16A inclusion). The scale bar represents 1 μm. (**h**) Western blot of G3BP1/2 in cells non-transfected or transfected with non-targeting (control) siRNA or different combinations of siRNAs against G3BP1 and G3BP2. Upper: representative blot; lower: quantification. n = 3 independent experiments. G3BP abundances in each experiment were calculated as percentage relative to that of untreated cells and plotted as horizontal segments. Asterisks and “ns” respectively stand for significant (p-value ≤ 0.05) and non-significant (p-value > 0.05) in two-sided t-tests. (**i and j**) Quantification of the percentage of cells forming SGs (i) and the volume percentage of SEC16A inclusions with TDP43 aggregation (j) after incubation at 42°C for 12-16 hr. Cells were either transfected with TDP43-mNG and Halo-SEC16A only or additionally with mCherry,

mCherry-G3BP1, negative control siRNA, or different combinations of siRNAs against G3BP1 and G3BP2. SGs were stained by using FISH against polyA RNAs as in (a). n = 45 cells examined over 3 independent experiments. The values of individual cells are plotted as dots, and the (mean) values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. Asterisks and “ns” respectively stand for significant (p-value ≤ 0.05) and non-significant (p-value > 0.05) in two-sided t-tests.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As
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mCherry-G3BP1
Merge
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b
TDP43 Intensity in SG (a.u.)
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Possibility 1
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With ERES
SEC23/24
SEC13/31
d
TDP43-mNG
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Vol (cell means) (μm3)
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42°C
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MG132
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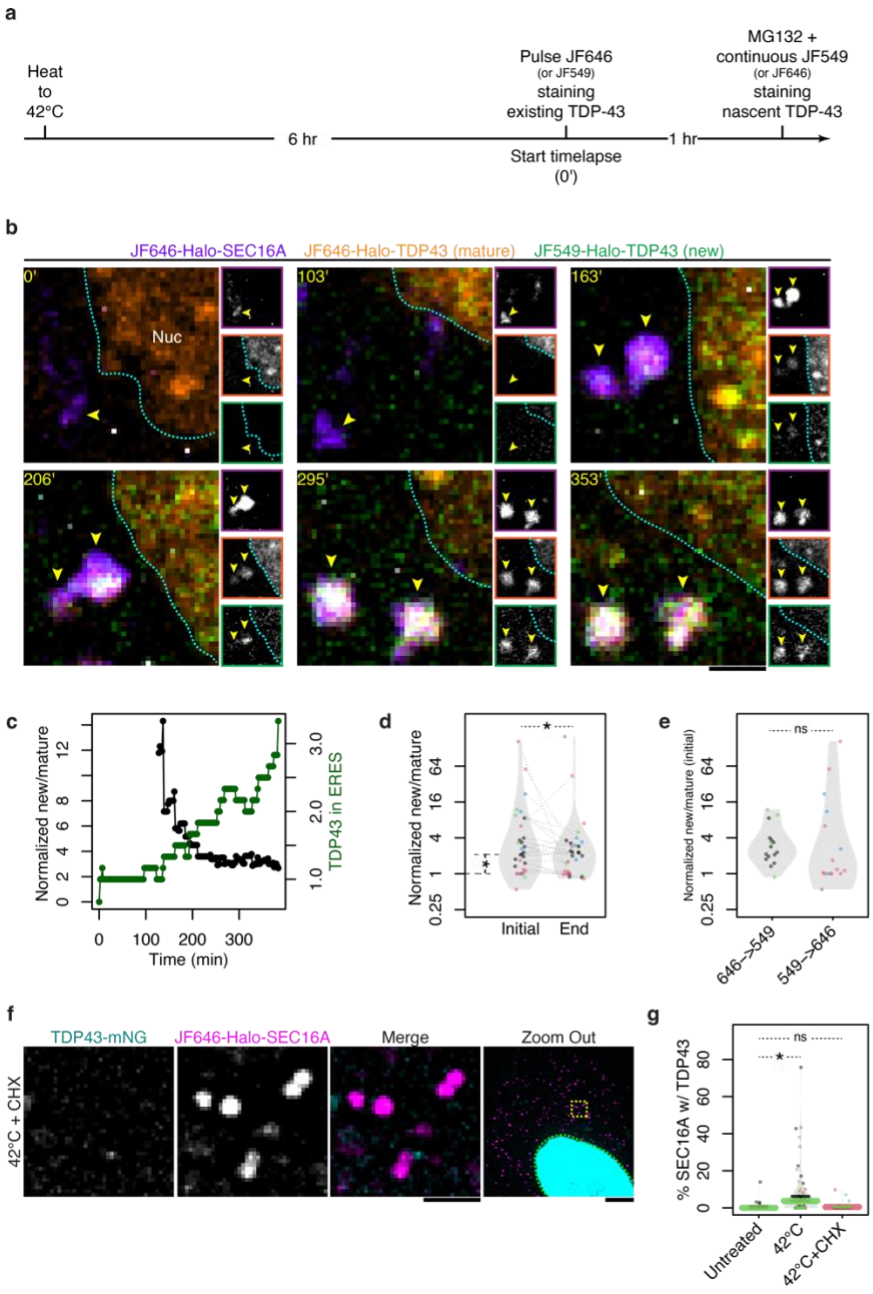
SEC16A
G3BP1

Fig S5

Supplementary Fig. 5. TDP43/SEC16A coaggs are not enriched for Ser409-phosphorylated or ubiquitinated TDP43.

(**a**) Representative images showing that TDP43 co-aggregated with G3BP1 in stress granule (SG) formed after treatment by 0.5 mM sodium arsenite (As) for 1 hr. Arrows indicate SGs; the inset in TDP43-mNG channel shows a contrast-adjusted view; green dashes demarcate the nucleus; in "Zoom out", the yellow box indicates the region shown in zoom-in views. Scale bars except in "Zoom Out" represent 1 μm, and represent 5 μm in "Zoom Out". (**b**) Quantification of the experiments as in (a) and Fig. 2a comparing the abundance of TDP43 in heat versus As-induced SGs. a.u.: arbitrary unit. n = 18 cells examined over 3 independent experiments. The values of individual cells are plotted as dots, and the mean values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. The asterisk stands for significant (p-value ≤ 0.05) in two-sided t-test. (**c**) Schematic diagram showing two possible ways of TDP43/SEC16A coagg formation: *de novo* from cytosolic TDP43 and SEC16A, and TDP43 co-aggregation with ERES. (**d**) Representative images of clusters of TDP43/SEC16A coaggs formed after 12-16 hr of incubation at 42°C. The 3D rendering shows a TDP43/SEC16A cluster viewed from top and "Cores" view annotates high intensity cores of TDP43 or SEC16A within the cluster. Each scale bar represents 1 μm. (**e**) Quantification of the experiments as in Fig. 2a, b comparing the volumes of SEC16A inclusions without TDP43, TDP43/SEC16A coaggs and TDP43-containing SGs. Volumes are plotted on a $\log_{10}$ scale. n = 45 cells examined over 3 independent experiments. The values of individual cells are plotted as dots, and the mean values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. Asterisks and "ns" respectively stand for significant (p-value ≤ 0.05) and non-significant (p-value > 0.05) in two-sided t-tests. (**f**) Quantification of a cell with mild TDP43/SEC16A co-aggregation (Fig. 2b) showing the volumes of individual SEC16A inclusions with or without TDP43. Each data point represents a SEC16A inclusion. (**g**) Representative images showing immunostaining of Ser409-phosphorylated TDP43 ($TDP43^{pSer409}$) in cells containing TDP43/SEC16A coaggs (indicated by arrowheads) or SGs. Scale bars except in "Zoom Out" represent 1 μm, and represent 5 μm in "Zoom Out". (**h**) Quantification of the experiments as in (g) comparing the fluorescence intensity of

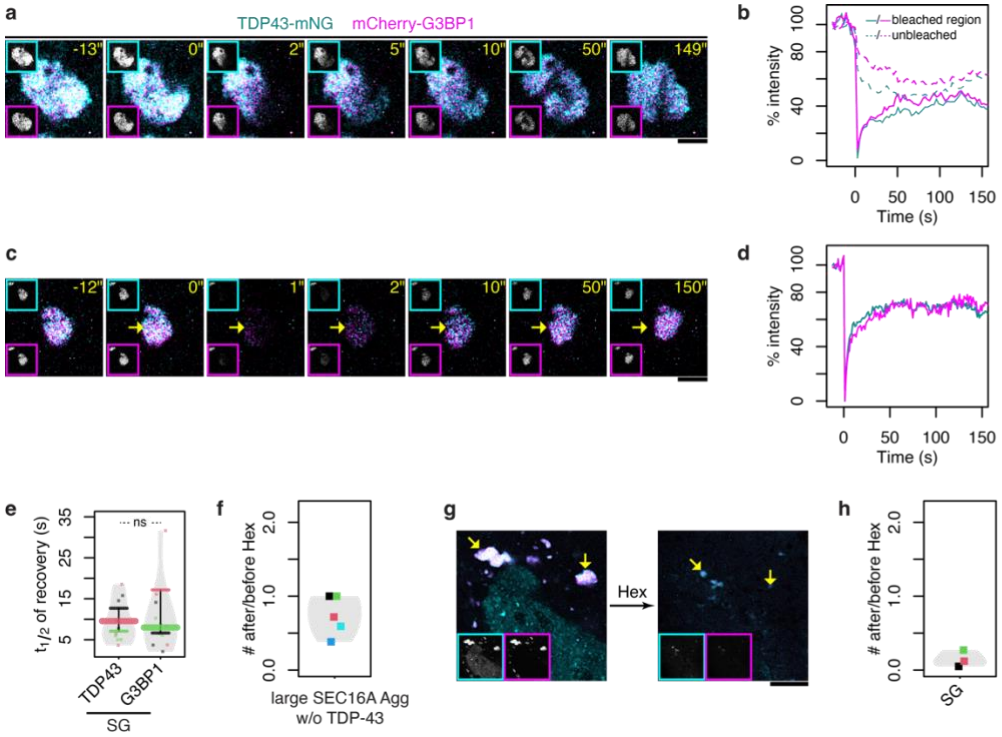
immunostained TDP43^{pSer409} in TDP43/SEC16A coaggs and TDP43-containing SGs. TDP43^{pSer409} intensity in SEC16A inclusions without TDP43 was also quantified to mark the background intensity. n = 15 cells containing each type of aggregates examined over 3 independent experiments. The values of individual cells are plotted as dots, and the mean values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. The asterisks and "ns" respectively stand for significant (p-value ≤ 0.05) and non-significant (p-value > 0.05) in two-sided t-tests. (**i**) Representative images showing immunostaining of ubiquitin (Ub, all forms) in cells forming TDP43/SEC16A coaggs (indicated by arrowheads) or SGs after 12-16 hr of incubation at 42°C, without or with 10 μM MG132 treatment in the last 4 hr. Scale bars except in "Zoom Out" represent 1 μm, and represent 5 μm in "Zoom Out". (**j**) Quantification of the experiments as in (i) comparing the fluorescence intensity of immunostained ubiquitin (Ub) in SEC16A inclusions without TDP43, TDP43/SEC16A coaggs and TDP43-containing SGs. n = 15 cells containing each type of aggregates examined over 3 independent experiments. The values of individual cells are plotted as dots, and the mean values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. Asterisks and "ns" respectively stand for significant (p-value ≤ 0.05) and non-significant (p-value > 0.05) in two-sided t-tests.



Supplementary Fig. 6. TDP43/SEC16A coaggs are ERES enriching nascent TDP43.

(**a**) Workflow of the pulse-chase of Halo-TDP43. Cells pre-stressed at 42°C for 6 hr were pulse-labelled for 30 min with the first dye, JF646 (or JF549), to visualize pre-existing TDP43 followed by dye wash off. Then 1 μM MG132 was used to inhibit TDP43 degradation followed by addition of the second dye, JF549 (or JF646), to continuously label newly synthesized TDP43. The second dye was kept in media. (**b**) Selected frames from a representative Halo-TDP43 pulse-chase. Pre-existing (mature) Halo-TDP43 was pulse-labelled by JF646 (orange) whereas newly synthesized (new)

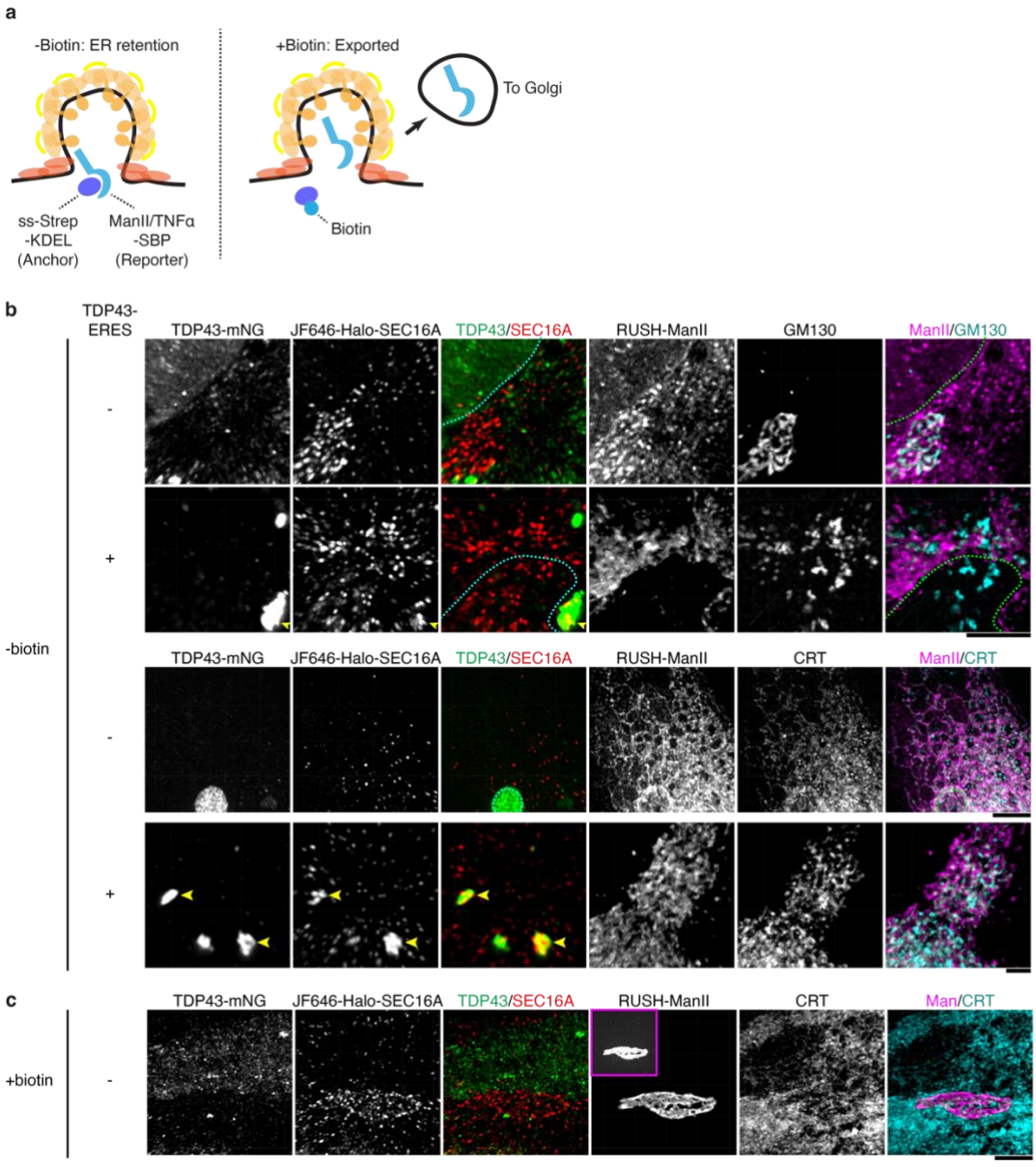
Halo-TDP43 was labelled continuously with JF549 (green). mEGFP-SEC16A (purple) was used to mark ERES. Insets show individual channels. Cyan dashes demarcate the nucleus (Nuc). The scale bar represents 1 μm. (**c**) Quantification of the experiment in (b) showing the ratio between new and mature TDP43 that aggregated in ERES, normalized to the new/mature ratio of the whole cell, over time (black curve). To indicate the time of TDP43 aggregation in ERES, the relative intensity of TDP43 in ERES (normalized to its initial value) was shown (green curve). (**d**) Quantification of the experiments as in (b) showing the normalized new/mature TDP43 ratios upon aggregate formation (initial) and at the end of cell tracking (end), plotted on a $\log_2$ scale. n = 20 cells with TDP43 aggregation in ERES examined over 4 independent experiments. The initial and end ratios of each cell are plotted as data points and, if the initial ratio exceeded 1, linked by gray segments. The asterisk between initial ratios and 1 indicates $p \leq 0.05$ by one sample two-sided t-test, and the asterisk between linked initial and end ratios indicates $p \leq 0.05$ by two-sided paired t-test. (**e**) Quantification of the experiments as in (b) showing the normalized new/mature TDP43 ratios upon aggregate formation, and grouped by the order of dye addition. For each group, n = 10 cells with TDP43 aggregation in ERES examined over 2 independent experiments. “ns” stands for non-significant (p-value > 0.05) in two-sided t-test. (**f**) Representative images of cells incubated at 42°C for 16 hr with cycloheximide (CHX) addition in the last 6 hr. In “Zoom Out”, the yellow box marks the region displayed in zoom-in views, green dashes demarcate the nucleus, and the scale bar represents 5 μm. (**g**) Quantification of the experiments as in (f), Fig. 2b, 3a comparing the volume percentage of SEC16A inclusions with TDP43 aggregation in untreated cells or cells incubated at 42°C for 16 hr, without or with CHX treatment. n = 45 cells examined over 3 independent experiments. The values of individual cells are plotted as dots, and the mean values of cells in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. The asterisk and “ns” respectively stand for significant (p-value ≤ 0.05) and non-significant (p-value > 0.05) in two-sided t-tests.



Supplementary Fig. 7. SGs and SEC16A inclusions without TDP43 are dynamic (liquid-like).

(**a**) Selected frames from a representative timelapse recording of a TDP43/G3BP1 coagg (TDP43-containing SG) after partial photobleaching. Insets show individual channels. The scale bar represents 1 µm. (**b**) Quantification of the experiment in (a) showing the relative intensities of TDP43 (cyan lines) and G3BP1 (magenta lines) in bleached (solid lines) and unbleached regions (dashed lines) over time, normalized to their respective initial intensities. (**c**) Selected frames from a representative timelapse recording of a TDP43/G3BP1 coagg (SG) after photobleaching in its entirety. The position of the SG immediately before photobleaching is indicated by an arrow. The scale bar represents 1 µm. (**d**) Quantification of the experiment in (c) showing the relative intensities of TDP43 (cyan line) and G3BP1 (magenta line) over time, normalized to their respective initial intensities. (**e**) Quantification of full-FRAP experiments as in (c) showing the half time ($t_{1/2}$) of fluorescence recovery for TDP43 and G3BP1 in SGs. n = 15 SGs examined over 3 independent experiments. The values of individual SGs are plotted as dots, and the mean values of SGs in the same experiment as horizontal segments, the median of which is elongated and thickened. Different colors denote different experiments. “ns” stands for non-significant (p-value > 0.05) in two-sided t-test. (**f**) Quantification of the experiments in Fig. 4g showing the

after/before ratios of large ERES (of > 300 nm in radius). n = 50 cells examined over 5 independent experiments. The median values of cells in the same experiments are plotted as dots. (**g**) Representative images showing SGs (indicated by arrows) in a cell before and after 3.5% [v/v] Hex treatment for 15 min. Insets show individual channels. The scale bar represents 5 μm. (**h**) Quantification of the experiments as in (g) showing the after/before ratios of SGs. n = 30 cells examined over 3 independent experiments. The median values of cells in the same experiments are plotted as dots.

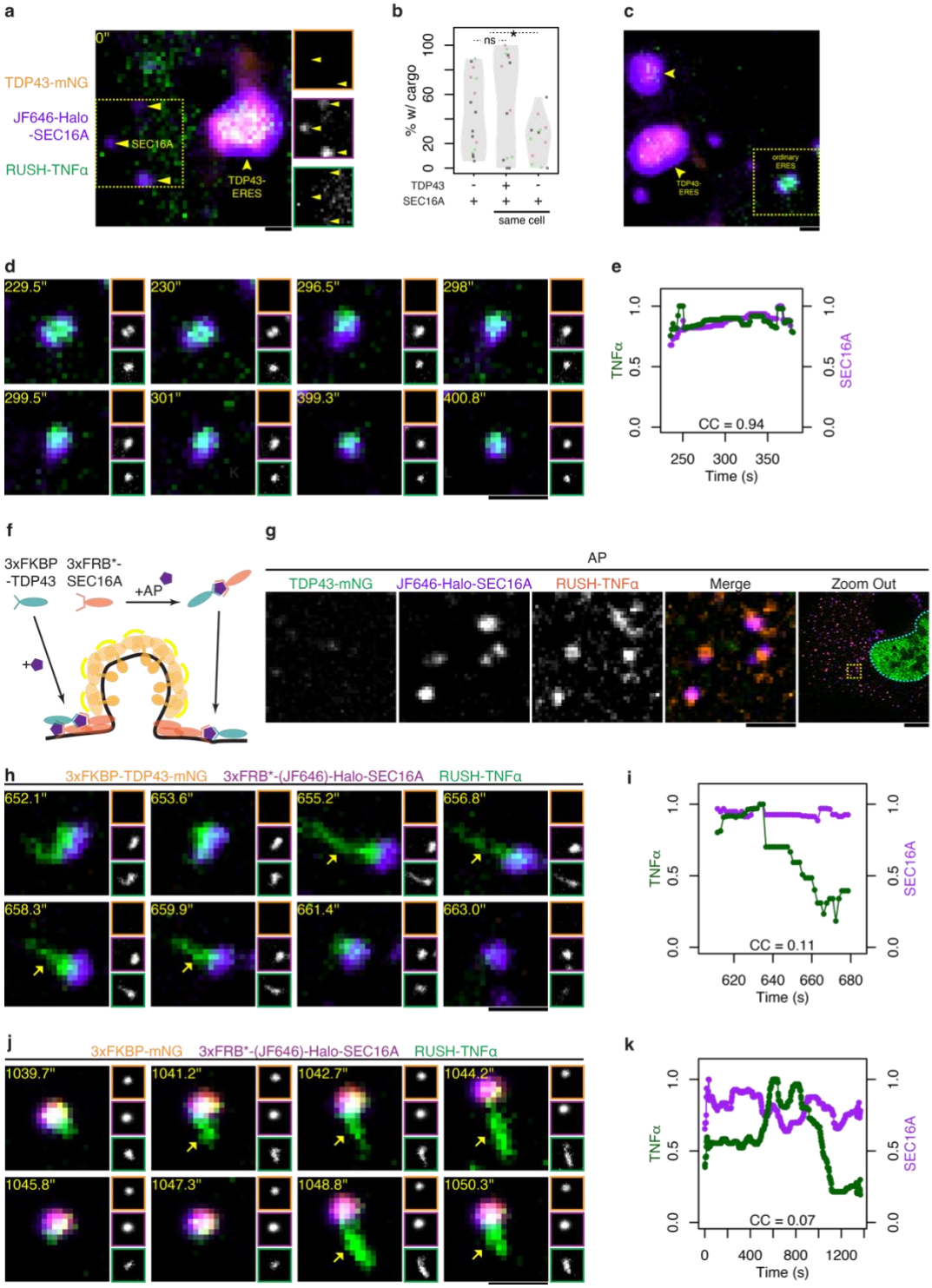


Supplementary Fig. 8. ER-to-Golgi transport of RUSH-ManII in cells without or with TDP43-ERES.

(**a**) Working scheme of RUSH assay for examining ER-to-Golgi protein transport. Left: when cells are cultured in biotin-free media, the RUSH reporter containing streptavidin-binding protein (SBP) is retained in the ER or ERES through binding to the ER anchor ss-streptavidin-KDEL; Right: after biotin supplement, the reporter is released from anchor and subsequently exported to the Golgi. (**b**) Representative images of RUSH assay in cells without or with TDP-ERES. Cells cultured in biotin-free media was imaged with GM130 (Golgi) or Calreticulin (CRT – ER marker) co-stained by immunofluorescence. Arrowheads: TDP43-ERES; cyan or green dashes: the nuclei. The quantification of is shown in (Fig. 5b, c). Each scale bar represents 5 μm. (**c**)

Representative images of RUSH assay in cells without TDP-ERES and supplemented with biotin for 1 hr. CRT was co-stained to visualize the ER. The inset in the ManII channel shows a contrast-adjusted view. The scale bar represents 5 μm.

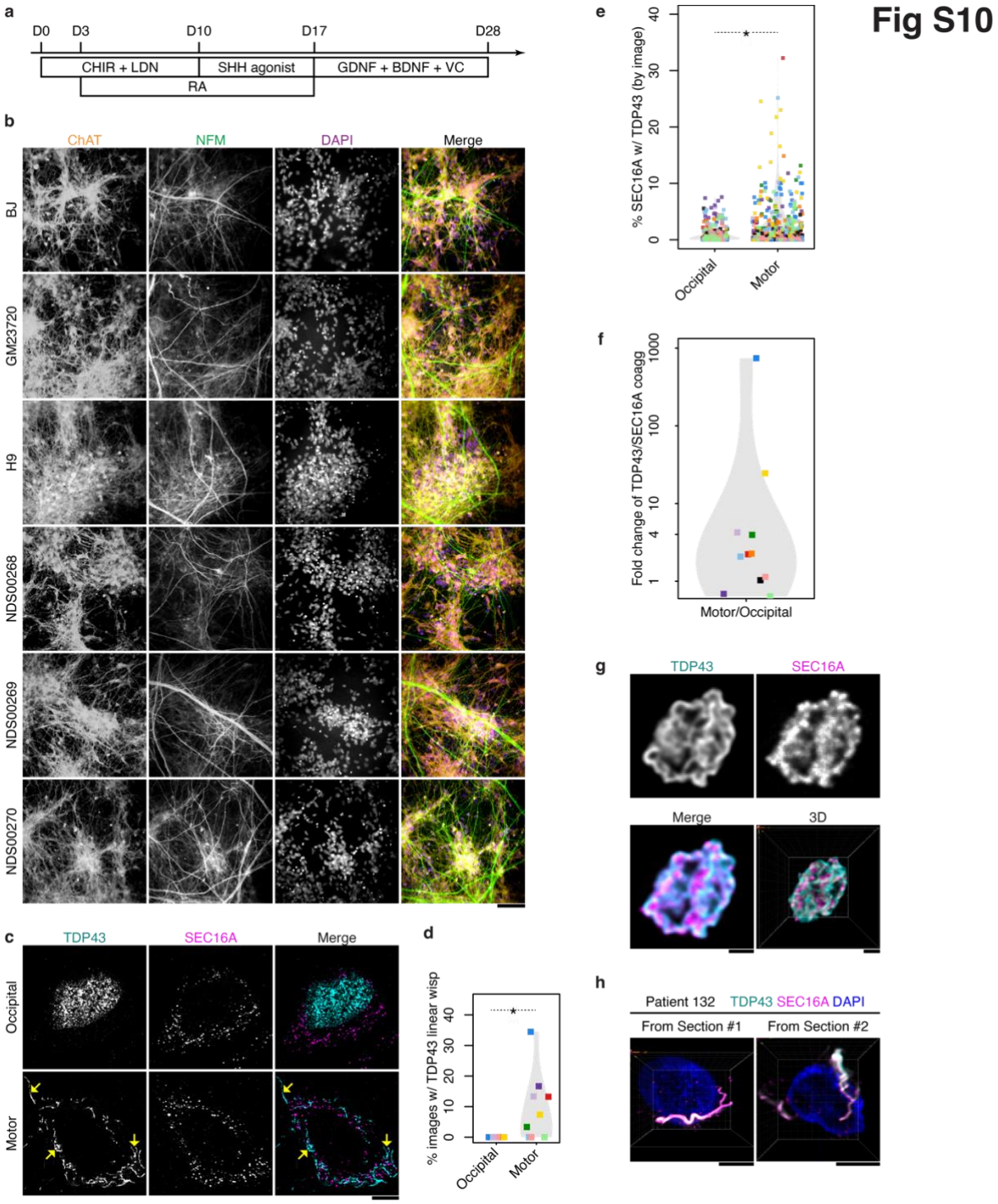
Fig S9



Supplementary Fig. 9. The dominant negative effect of TDP43-ERES over ordinary ERES in the same cell and control experiments for AP21967-induced co-aggregation of TDP43 with ERES.

(**a**) Zoom-out view of the TDP43-ERES (indicated by the arrowhead) in Fig. 5g showing that SEC16A inclusions without TDP43 (indicated by triangles) in its vicinity are devoid of RUSH-TNFα upon biotin addition (0''). Insets show individual channels

of the yellow boxed region. Each scale bar represents 1 μm. (**b**) Quantification of RUSH experiments in Fig. 5e, g showing the percentage of TDP43-ERES and SEC16A inclusions without TDP43 in either the same or different cells that recruited RUSH-TNFα. n = 9 cells examined over 3 independent experiments. Each dot represents one cell, and different colors denote different experiments. The asterisk and "ns" respectively stand for significant (p-value ≤ 0.05) and non-significant (p-value > 0.05) in two-sided t-tests. (**c**) Zoom-out view of an ordinary ERES (yellow boxed region) showing TDP43-ERES nearby (indicated by arrowheads). The scale bar represents 1 μm. The timelapse recording of this ordinary ERES is shown in (d). (**d**) Selected frames of the timelapse recording of the ERES boxed in (c) during RUSH-TNFα assay. The scale bar represents 1 μm. (**e**) Quantification of RUSH-TNFα and SEC16A intensities over time (normalized to their respective max intensities) in the TDP43-ERES tracked in (d), as in Fig. 5f. (**f**) Working scheme of AP21967-induced protein co-aggregation. TDP43 (or mNG control) and SEC16A were respectively tagged with 3xFKBP and 3xFRB*, which can be induced to heterodimerize by both binding to the rapamycin analog AP21967 (AP). As such, AP induced aggregation of 3xFKBP-TDP43 at ERES marked by 3xFRB*-SEC16A. (**g**) Representative images of cells expressing TDP43-mNG, Halo-SEC16A and RUSH-TNFα and treated with AP21967 for 6-10 hr. In "Zoom Out", the yellow box marks the region displayed in zoom-in views, and cyan dashes demarcate the nucleus. Scale bars except in "Zoom Out" represent 1 μm, and represent 5 μm in "Zoom Out". (**h**) Selected frames of a representative timelapse recording of an ERES marked by 3xFRB*-(JF646)-Halo-SEC16A but not 3xFKBP-TDP43-mNG during RUSH-TNFα assay. The cell was not treated with AP21967. Arrows indicate the budding of a tubular transport intermediate. The scale bar represents 1 μm. (**i**) Quantification of RUSH-TNFα and SEC16A intensities over time (normalized to their respective max intensities) in the ERES tracked in (h). (**j**) Selected frames of a representative timelapse recording of an AP-induced mNG-ERES coagg during RUSH-TNFα assay. Arrows indicate the budding of tubular transport intermediates. The scale bar represents 1 μm. (**k**) Quantification of RUSH-TNFα and SEC16A intensities over time (normalized to their respective max intensities) in the mNG-ERES tracked in (j).



Supplementary Fig. 10. Neurons derived from stem cells or in postmortem brain sections of ALS patients.

(**a**) Workflow of induced motor neuron (iMN) production from hESC/hiPSC. CHIR: CHIR99021; LDN: LDN193189; RA: retinoic acid; SHH: Sonic Hedgehog. VC: Vitamin C (L-ascorbic acid). D0: Day 0 of induced differentiation. (**b**) Representative images of the immunofluorescence staining of (cholinergic) neuronal markers ChAT and NFM

in iMNs derived from non-ALS (BJ, GM23720 and H9) and ALS patient (NDS00268, NDS00269 and NDS00270) hESC/hiPSCs. Each scale bar represents 50 μm. (**c**) Representative images of the immunofluorescence staining of TDP43 and SEC16A in the post-mortem sections of the occipital cortex and motor cortex from the same ALS patient. Like Fig. 7c, e but showing the presence of TDP43 linear wisps (fibril-like aggregates indicated by arrows) in the motor neurons. The scale bar represents 5 μm. (**d**) Quantification of the experiments as in (c) comparing the percentage of images containing TDP43 linear wisps in the occipital versus motor cortex of 11 different ALS patients (color-coded). n = 330 z-stacks each taken from the occipital and motor cortex of 11 patients. The values of individual patients are plotted as dots. The asterisk stands for significant (p-value ≤ 0.05) in two-sided t-test. (**e**) Quantification of the experiments as in Fig. 7c showing the volume percentage of SEC16A inclusions containing TDP43 in the occipital cortex versus motor cortex of 11 different ALS patients (color-coded). Similar to Fig. 7d but each dot represents the percentage in one z-stack image (color coded by patient). n = 330 z-stacks each taken from the occipital and motor cortex of 11 patients. The asterisk stands for significant (p-value ≤ 0.05) in two-sided t-test. (**f**) Paired analysis of TDP43/SEC16A coagg fold change comparing motor versus occipital cortex for 11 different ALS patients (color-coded). n = 330 z-stacks each taken from the occipital and motor cortex of 11 patients. Each dot represents the motor/occipital ratio of coagg levels of a patient, plotted on a $\log_{10}$ scale. (**g**) Zoom-in views of the curled TDP43/SEC16A skein in Fig. 7e marked by the arrowhead. Each scale bar represents 1 μm. (**h**) Similar to Fig. 7i, but showing more representative images of intracellular TDP43/SEC16A skein-like aggregates found in the motor cortex of sALS Patient 132. Each scale bar represents 5 μm.