## Description of Additional Supplementary Files

**File name Supplementary Data 1**
**Description: MS data.** List of proteins identified in stress-induced aggregates and their Gene Ontology (GO) enrichment. (**Sheet 1**) The abundance of proteins identified by mass-spectrometry in the immunoprecipitate (IP) of aggregate-enriched sucrose fraction versus IP control. (**Sheet 2**) GO analysis of proteins enriched in aggregate by more than 8.5-fold compared with IP control showing the Biological Processes (BP) in which these proteins belong. 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. (**Sheet 3**) GO analysis of proteins enriched in aggregate. Similar to (**Sheet 2**) but shows the Cellular Components (CC) where these proteins reside.

**File name: Supplementary Data 2**
**Description**: **Plasmids, RNAi, CRISPR and antibodies.** Plasmids, DsiRNAs, CRISPR crRNA and donor, and antibodies used this this study. (**Sheet 1**) Plasmids. Transfection amounts of different plasmids were optimized for 35 mm glass-bottom dishes and using Lipofectamine 3000. Plasmids generated in this study and their maps and sequences will be submitted to Addgene and also available on request. (**Sheet 2**) The sense and antisense sequences of DsiRNAs against G3BP1 and G3BP2 and negative control DsiRNA (IDT NC1) which has no activity in human cells. The prefix "r" before A/U/C/G indicates ribonucleotide residues. (**Sheet 3**) crRNAs targeting G3BP1 used in CRISPR-mediated genome editing. (**Sheet 4**) Donors for homology-directed repair (HDR) for mScarlet-I-G3BP1 CRISPR knock-in, synthesized as a DNA duplex (IDT). (**Sheet 5**) Antibodies used in this study. Ig: immunoglobin.

**File name: Supplementary Data 3**
**Description: Demographic data of ALS postmortem samples.** The demographic data of postmortem samples of ALS patients used in this study. DOD: diagnosis of disease; M: Male; F: Female; R color code: color coding (in R programming language) used in Fig. 7d, f, g, Supplementary Fig. 10d-f. To protect the identifies of these patients, Age at DOD and Age at onset are provided as ranges.

**File name: Supplementary Movie 1**
**Description: TDP43 partitions dynamically between SGs and co-aggregates with SEC16A.** A representative movie of SGs and SEC16A inclusions in a cell shifted to 42°C for 60 min before recording started. Green: TDP43-mNG; Magenta: mCherry-G3BP1; Red: JF646-Halo-SEC16A. Annotated key frames and quantification are shown in Supplementary Fig. 4d and e, respectively.

**File name: Supplementary Movie 2**
**Description: TDP43 aggregates at ERES.** A representative movie of ERES in a cell shifted to 42°C for 60 min before recording started. Cyan: TDP43-mNG; Magenta: JF646-Halo-SEC16A. Annotated key frames and quantification are shown in Fig. 3c and d, respectively.

**File name: Supplementary Movie 3**
**Description: TDP43-ERES remain ER-associated.** A representative movie of the movement of TDP43-ERES and SEC16A inclusions without TDP43 relative to the ER network. Green: TDP43-mNG; Purple: JF646-Halo-SEC16A; Orange: ss-mCherry-KDEL (ER). An annotated frame and quantification are shown in Fig. 3i and j, respectively.

**File name: Supplementary Movie 4**
**Description: ERES prefer nascent over mature TDP43.** A representative movie showing ERES and nucleus in a cell that was pre-stressed at 42°C for 6 hr and supplemented with 1 μM MG132 just before imaging started. Pre-existing (mature) Halo-TDP43 was pulse-labelled by JF646 (orange) while newly synthesized (new) Halo-TDP43 was labelled continuously with JF549 (green), and mEGFP-SEC16A (purple) was used to mark ERES. The workflow is illustrated in Supplementary Fig. 6a. Annotated key frames and quantification are shown in Supplementary Fig. 6b and c, respectively.

**File name: Supplementary Movie 5**
**Description: Partial-FRAP of TDP43-ERES.** A representative movie of a TDP43-ERES coagg after photobleaching in part by laser. Cyan: TDP43-mCherry; Magenta: mEGFP-SEC16A. Annotated key frames and quantification are shown in Fig. 4a and b, respectively.

**File name: Supplementary Movie 6**
**Description: Partial-FRAP of SG.** A representative movie of a TDP43-containing SG after photobleaching in part by laser. Cyan: TDP43-mNG; Magenta: mCherry-G3BP1. Annotated key frames and quantification are shown in Supplementary Fig. 7a and b, respectively.

**File name: Supplementary Movie 7**
**Description: Full-FRAP of TDP43-ERES.** A representative movie of a TDP43-ERES coagg after photobleaching in its entirety by laser. Cyan: TDP43-mCherry; Magenta: mEGFP-SEC16A. Annotated key frames and quantification are shown in Fig. 4d and e, respectively.

**File name: Supplementary Movie 8**
**Description: Full-FRAP of SG.** A representative movie of a TDP43-containing SG after photobleaching in its entirety by laser. Cyan: TDP43-mNG; Magenta: mCherry-G3BP1. Annotated key frames and quantification are shown in Supplementary Fig. 7c and d, respectively.

**File name: Supplementary Movie 9**
**Description: 1,6-Hexanediol induces TDP43/SEC16A co-aggregates.** A movie showing a cell forming TDP43/SEC16A coaggs during 1,6-hexanediol (Hex) treatment. Cyan: TDP43-mCherry; Magenta: mEGFP-SEC16A. Hex was added between 7 min and 9 min. The 3D projection (top view) of an z stack is shown.

**File name: Supplementary Movie 10**
**Description: ERES without TDP43 export RUSH-TNFα.** A representative movie of ERES without TDP43 exporting RUSH-TNFα after biotin supplement (at 0 sec). Orange: TDP43-mNG; Purple: JF646-Halo-SEC16A; Green: RUSH-TNFα. SEC16A inclusions were identified by ilastik (magenta contours) and tracked using Trackmate. Only the trajectory of the ERES shown in Fig. 5e, f is displayed with color coding for time (blue: track start; red: track end). A tubule-like transport intermediate formed and released around 526 sec.

**File name: Supplementary Movie 11**
**Description: TDP43-ERES trap RUSH-TNFα.** A representative movie of TDP43-ERES not exporting RUSH-TNFα after biotin supplement (at 0 sec). Orange: TDP43-mNG; Purple: JF646-Halo-SEC16A; Green: RUSH-TNFα. SEC16A inclusions were identified by ilastik (magenta contours) and tracked

using Trackmate. Only the trajectory of the ERES shown in Fig. 5g, h is displayed with color coding for time (blue: track start; red: track end).

**File name: Supplementary Movie 12**
**Description: ERES in the vicinity of TDP43-ERES trap RUSH TNFα.** A representative movie of ERES without TDP43 but in the vicinity of TDP43-ERES not exporting RUSH-TNFα after biotin supplement (at 0 sec). Orange: TDP43-mNG; Purple: JF646-Halo-SEC16A; Green: RUSH-TNFα. SEC16A inclusions were identified by ilastik (magenta contours) and tracked using Trackmate. Only the trajectory of the ERES shown in Supplementary Fig. 9d, e is displayed with color coding for time (blue: track start; red: track end).

**File name: Supplementary Movie 13**
**Description: AP21967-induced TDP43-ERES trap RUSH-TNFα.** A representative movie of AP21967-induced TDP43-ERES not exporting RUSH-TNFα after biotin supplement (at 0 sec). Orange: 3xFKBP-TDP43-mNG; Purple: 3x-FRB*-(JF646)-Halo-SEC16A; Green: RUSH-TNFα. 3x-FRB*-(JF646)-Halo-SEC16A inclusions were identified by ilastik (magenta contours) and tracked using Trackmate. Only the trajectory of the ERES shown in Fig. 6e, f is displayed with color coding for time (blue: track start; red: track end).