SUPPLEMENTARY FIGURES AND LEGENDS

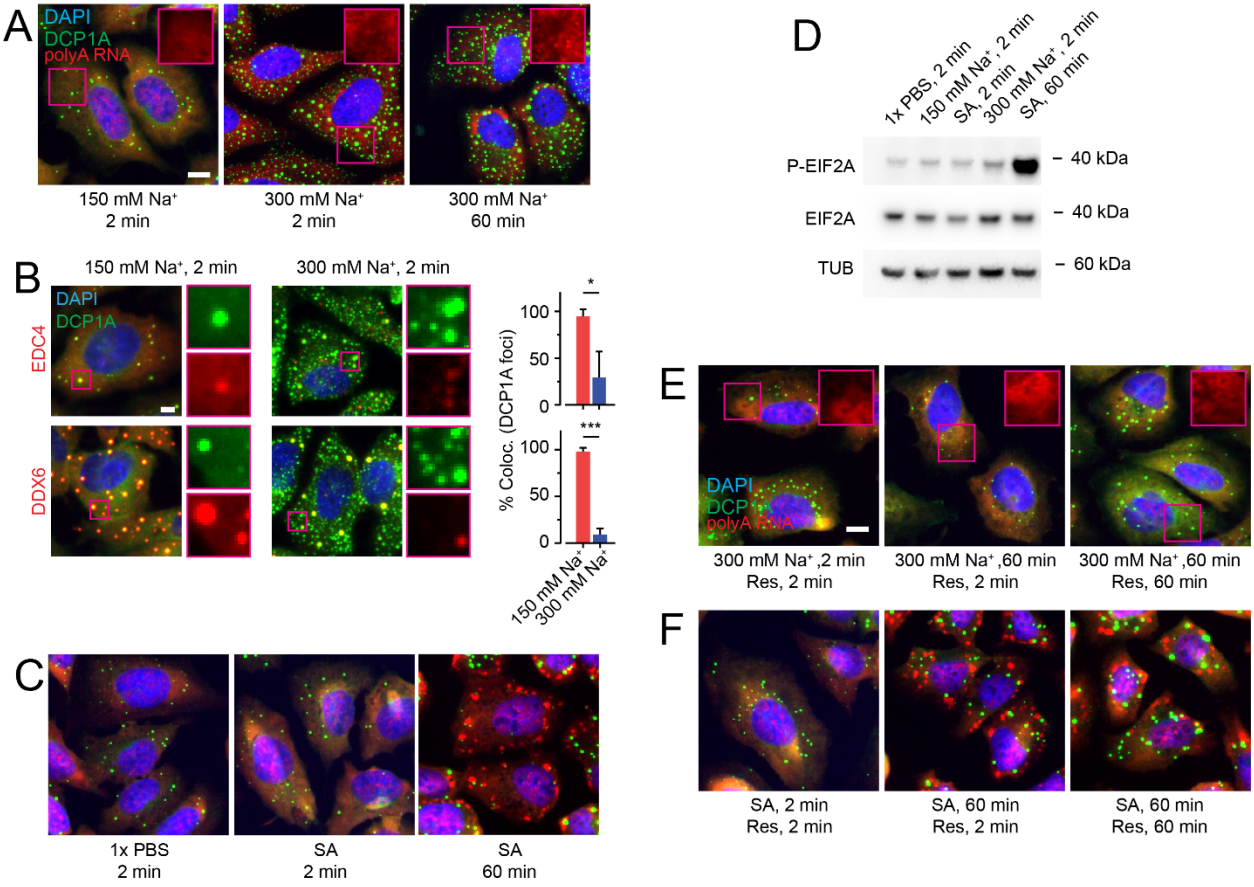

Figure S1. Hypertonicity-induced DCP1A foci are not canonical PBs or SGs. Related to Figure 1. (A) Representative pseudocolored, combined IF – RNA-FISH images of U2-OS cells stained for DAPI (blue), DCP1A (green) and polyA RNA (red) at various time points after isotonic (150 mM Na^+) or hypertonic (300 mM Na^+) medium addition. Scale bar, 10 μm. (B) Representative pseudocolored IF images of U2-OS cells stained for DAPI (blue), DCP1A (green), and EDC4 (top panels, red) or DDX6 (bottom panels, red) as indicated, treated with isotonic (150 mM Na^+) or hypertonic (300 mM Na^+) medium for 2 min. Scale bar, 10 μm. The plots represent the fraction of DCP1A foci colocalizing with EDC4 or DDX6 foci under each condition. n = 3, 50 cells, *$p < 0.01$, ***$p < 0.0001$, significance by two-tailed, unpaired Student's t-test. (C) Representative

pseudocolored, combined IF – RNA-FISH images of U2-OS cells stained for DAPI (blue), DCP1A (green) and polyA RNA (red), and either mock treated with 1x PBS or treated with 0.5 mM SA for the appropriate time points. (D) Western blot of phosphorylated EIF4E (P-EIF4E), EIF4E and tubulin upon various osmotic and SA perturbations. (E-F) Representative pseudocolored, combined IF – RNA-FISH images of U2-OS cells stained for DAPI (blue), DCP1A (green) and polyA RNA (red). Scale bar, 10 μm. (E) Cells were first treated with hypertonic (300 mM Na+) medium for the appropriate time points and then rescued with isotonic (150 mM Na+) medium for various durations. (F) Cells were first treated with 0.5 mM SA for the appropriate time points and then rescued with medium containing 1x PBS for various durations.

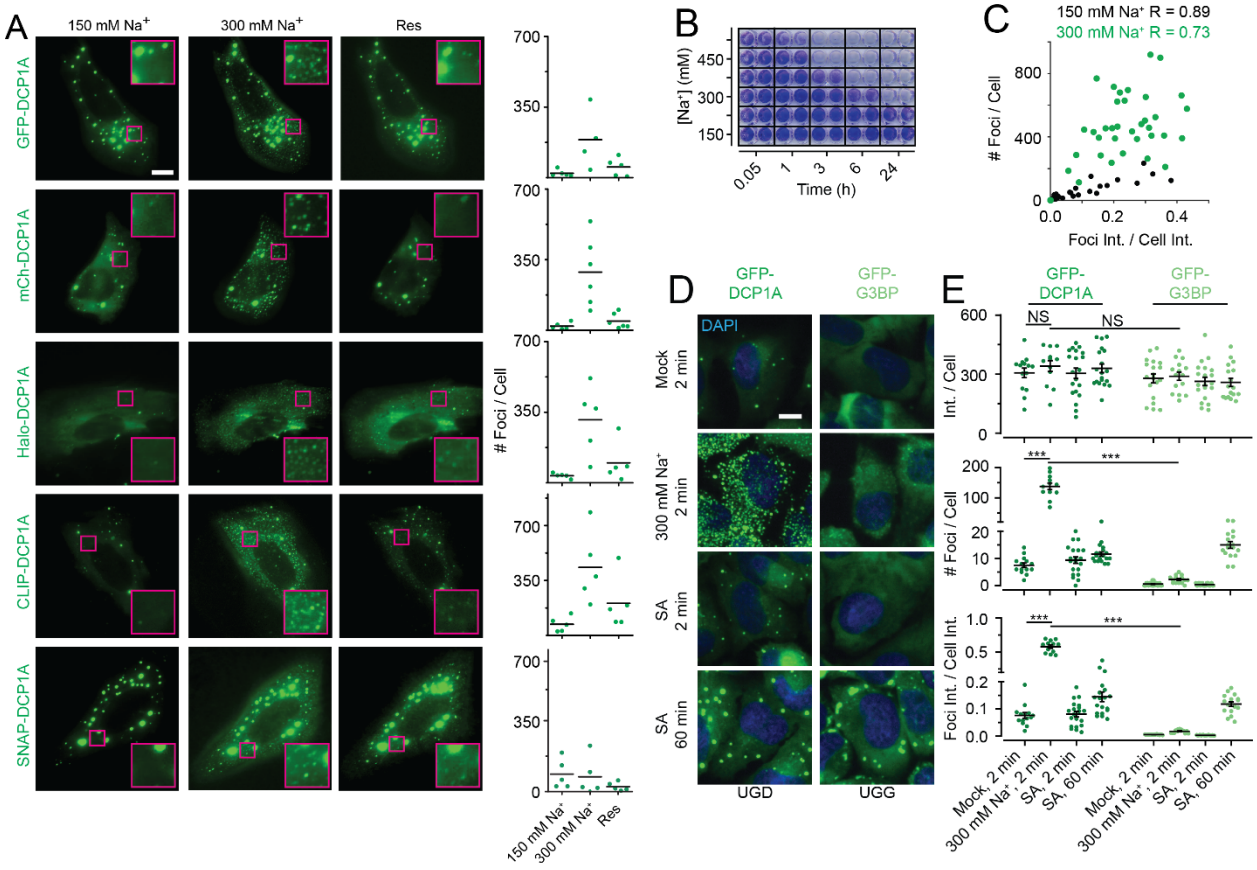

Figure S2. Rapid and reversible condensation of DCP1A does not depend on the fusion tag, does not affect cell viability and is distinct from G3BP condensation. Related to Figure 2. (A) Representative pseudocolored images of U2-OS cells expressing DCP1A fused to different types of fluorescent or fluorogenic tags (green). Cells were treated with isotonic (150 mM Na^+, 2 min) medium, hypertonic (300 mM Na^+, 2 min) medium, or rescued with isotonic medium (2 min) after hypertonic treatment (2 min). Scale bar, 10 μm. Scatter plot of the number of foci per cell for each treatment condition is also shown. n = 2, > 5 cells per sample. (B) Representative image of a 96-well plate probed for cell viability by crystal violet staining across various Na^+ concentrations and multiple time points. n = 3, with technical replicates for each n. (C) Correlation plot of the number of foci per cell against the partition coefficient in the same

cell. R = correlation coefficient. Black data points = 150 mM Na^+ treatment, green data points = 300 mM Na^+ treatment. (D) Representative pseudocolored images of cells stably expressing GFP-DCP1A (UGD, green) or GFP-G3BP (UGG, green) and subjected to osmotic or SA stress for various amounts of time. After fixation, cells were stained with DAPI (blue). Scale bar, 10 μm. (E) Scatter plots of area normalized cell intensity (top), number of foci per cell (middle) and partition coefficient (bottom). Each dot represents a cell. Line, mean, error bars, standard error. n = 3, > 15 cells per condition, NS = not significant, ***$p < 0.0001$, significance by two-tailed, unpaired Student's t-test.

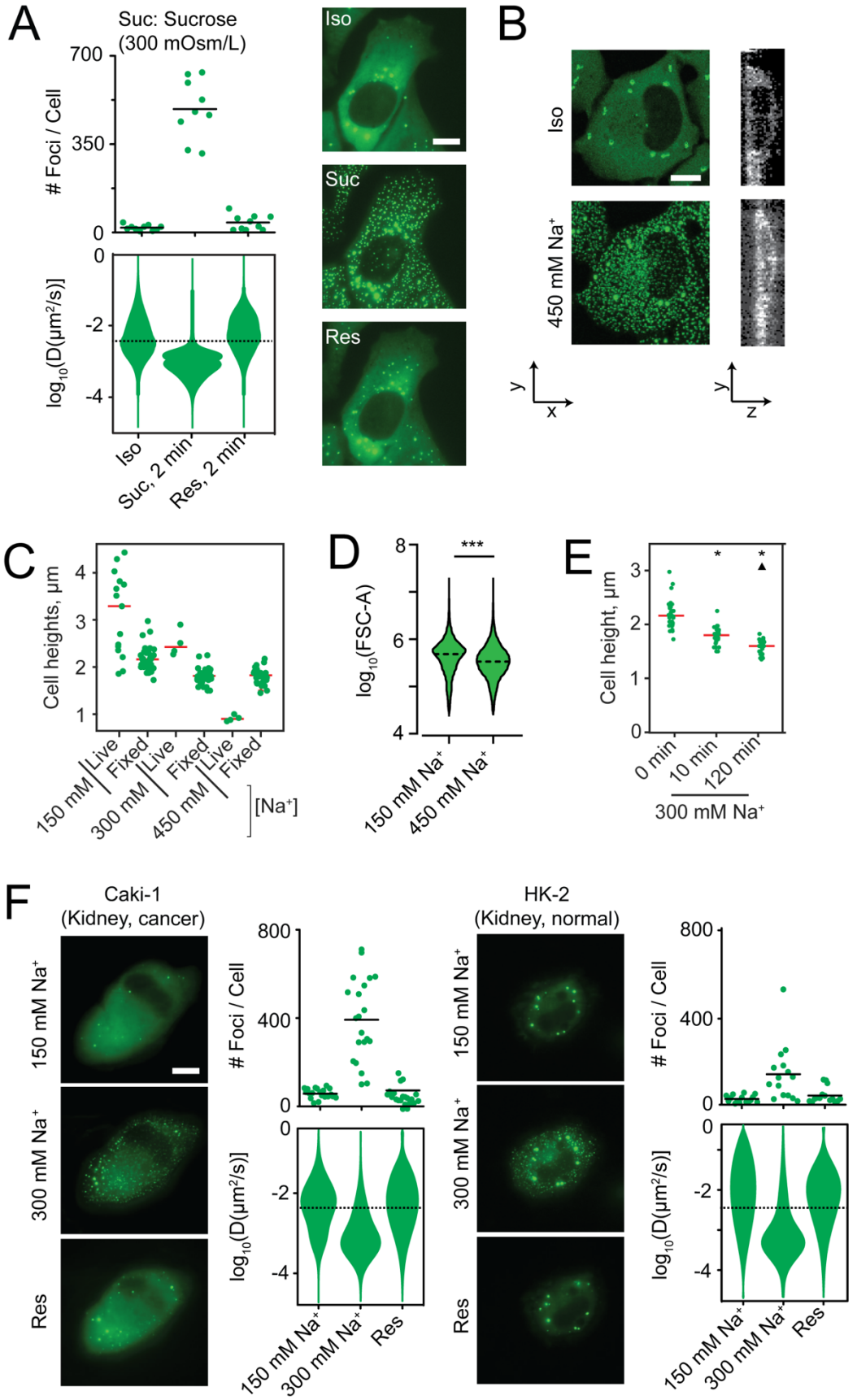
A
Suc: Sucrose
(300 mOsm/L)
Foci / Cell
700
350
0
log10(D(μm²/s))]
0
-2
-4
Iso
Suc, 2 min
Res, 2 min
Iso
Suc
Res
B
Iso
450 mM Na+
y
x
y
z
C
Cell heights, μm
4
3
2
1
Live
Fixed
150 mM
300 mM
450 mM
[Na+]
D
log10(FSC-A)
8
6
4
***
150 mM Na+
450 mM Na+
E
Cell height, μm
3
2
1
0
*
*
0 min
10 min
120 min
300 mM Na+
F
Caki-1
(Kidney, cancer)
HK-2
(Kidney, normal)
150 mM Na+
300 mM Na+
Res
Foci / Cell
800
400
0
log10(D(μm²/s))]
0
-2
-4
150 mM Na+
300 mM Na+
Res

Figure S3. Hyperosmotic phase separation of DCP1A is correlated with cellular compression and is independent of cell type. Related to Figure 3. (A) Scatter plot of the number of foci per cell (top), violin plots of diffusion constants associated with DCP1A foci (bottom). Representative pseudocolored images of UGD cells (GFP, green) were treated with isosmotic (Iso) growth medium, hyperosmotic growth medium containing the non-ionic osmolyte Sucrose (Suc, 2min) or rescued (Res) with isosmotic medium (2 min) after sucrose treatment (2 min). n = 2, > 5 cells per sample. Scale bar, 10 μm. (B) Representative x-y (green) and y-z (gray) projection of a UGD cell from 3-D imaging assay wherein the cell was treated with isotonic (150 mM Na^+) medium or hypertonic (300 mM Na^+) medium. n = 1, 4 cells per sample. Scale bars, 10 μm (x and y) and 5 μm (z). (C) Scatter plots representing cell heights measured by DiI staining and 3-D imaging in live- and fixed-cells. Cell heights were measured from YZ sections of Z-stack images of UGD. n=2, >8 cells per replicate. (D) Violin plots of forward scatter from fixed UGD cells as measured by flow-cytometry. Cells were treated with isotonic (150 mM Na^+) or hypertonic (300 mM Na^+) medium for 2 min. n = 3, > 50,000 events per condition, ***$p < 0.0001$, significance by two-tailed, unpaired Student's t-test. Dotted line, median. (E) Scatter plots representing cell heights measured by DiI staining and 3-D imaging in fixed-cells. Cells were treated with hypertonic (300 mM Na^+) medium prior to fixation. Cell heights were measured from YZ sections of Z-stack images of UGD, n=2, >8 cells per trial. (F) Representative pseudocolored images of Caki-1 or HK-2 cells expressing GFP-DCP1A (green). Cells were treated with isotonic (150 mM Na^+, 2 min) medium, hypertonic (300 mM Na^+, 2 min) medium or rescued with isotonic medium (2 min) after hypertonic treatment (2 min). Scale bar, 10 μm. Scatter plot of the number of foci per cell (top) and

violin plots of diffusion constants associated with DCP1A foci (bottom) for each treatment condition for Caki-1 or HK-2 cells are also shown. n = 2, > 5 cells per sample.

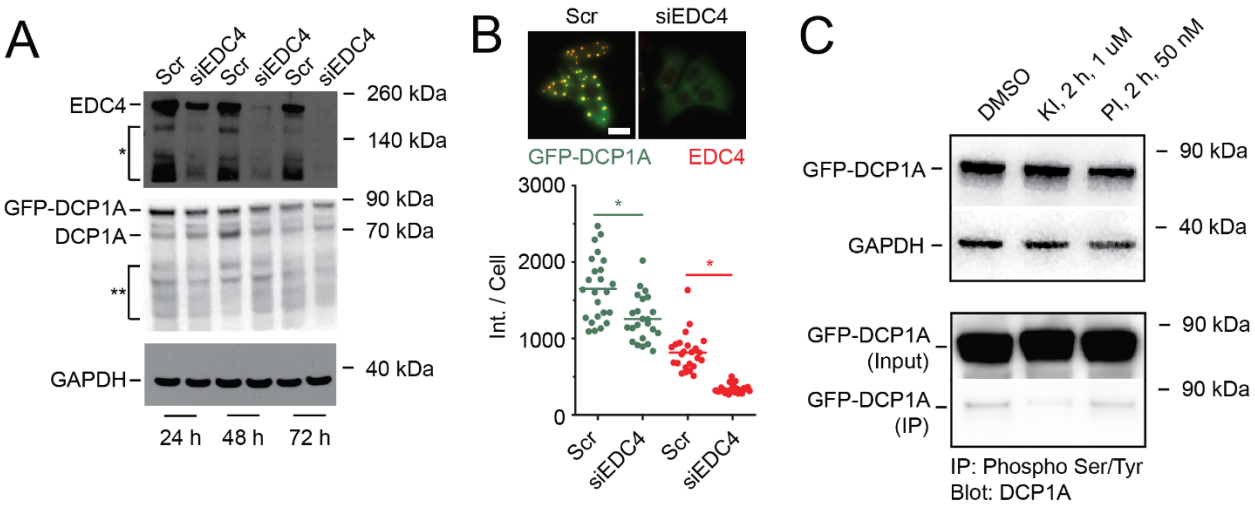

Figure S4. Knockdown of EDC4 results in reduced expression of DCP1A, but kinase inhibitor treatment only reduced DCP1A phosphorylation. Related to Figure 4. (A) Western Blot of EDC4, DCP1A, and GAPDH after various siRNA treatment times (24, 48 and 72 hr post siRNA transfection). Bands labeled with "*" and "**" were detected by EDC4 and DCP1A antibodies respectively and either denote non-specific bands or shorter protein fragments. (B) Representative pseudocolored IF images of UGD cells (top) expressing GFP-DCP1A (green), stained for EDC4 (red). Scale bar, 20 μm. Cells were either transfected with a scrambled siRNA (Scr) or siEDC4 for 48 h. Scatter plot (bottom) of the average intensity of GFP (green) or EDC4 (Cy5, red) per UGD cell transfected with a scrambled siRNA (Scr) or siEDC4 in isotonic conditions. n = 2, > 20 cells per sample, *p ≤ 0.01, by two-tailed, unpaired Student's t-test. (C) Western blot of DCP1A, and GAPDH after various drug perturbations (top). Western blot of DCP1A after immunoprecipitation with phospho-specific antibody, upon various drug perturbations (bottom).

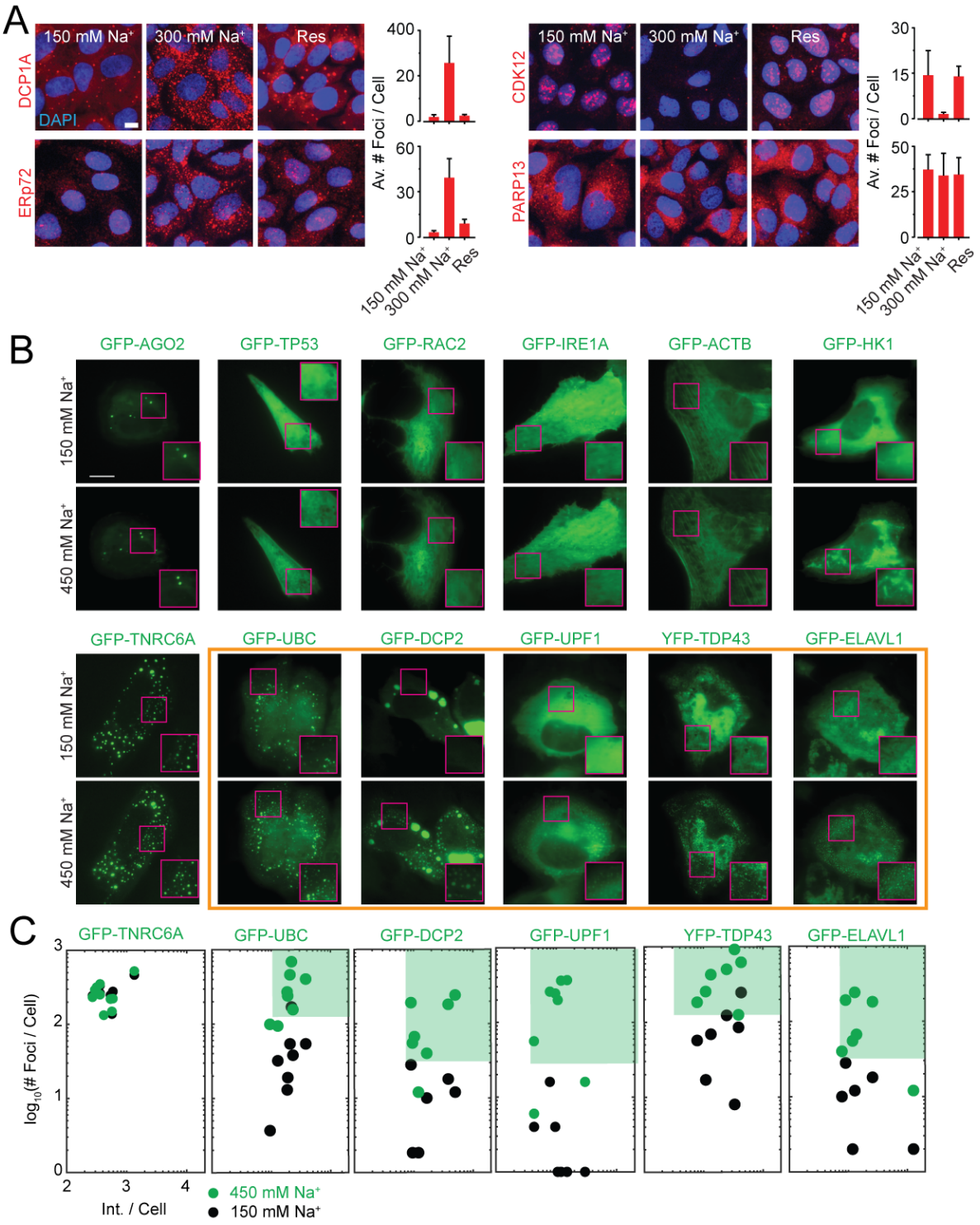

Figure S5. High-thoughput IF and GFP imaging of proteins in U2OS cells. Related to Figure 5. (A) Representative pseudocolored images of U2OS cells stained with the appropriate antibody (red, DCP1A, ERp72, CDK12, PARP13). Cells were treated with isotonic (150 mM Na^+) medium

(2 min), hypertonic (300 mM Na^+) medium (2 min) or rescued (res) with isotonic medium (2 min) after hypertonic treatment (2 min). Scale bar, 10 μm. Quantification of the average number of foci per cell is also depicted. Error bar, standard deviation. n = 3, > 50 cells. (B) Representative pseudocolored images of U2OS cells (GFP, green) transfected with the appropriate GFP-tagged construct and treated with isotonic (150 mM Na^+) medium or hypertonic (300 mM Na^+) medium for 2 min. Scale bar, 10 μm. Inset depicts a zoomed-in area corresponding to a 15 x 15 μm^2 magenta box. Constructs that exhibit HOPS are highlighted in orange. (C) Scatter plot of the number of foci per cell against the area-normalized total cell fluorescence intensity for each GFP-labeled protein. n = 2, > 5 cells per sample. The green contour depicts HOPS.

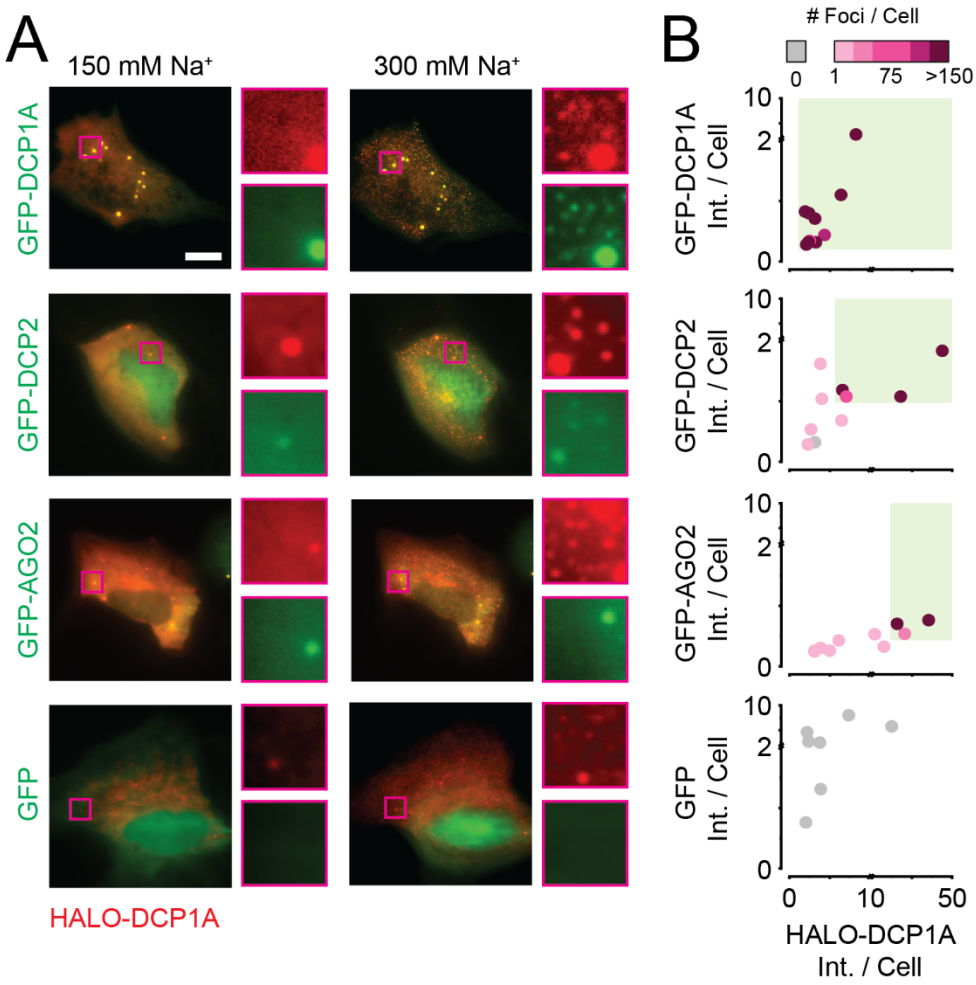

Figure S6. Interactors of DCP1A can exhibit HOPS at high DCP1A concentration. Related to Figure 5. (A) Representative pseudocolored images of U2OS cells (GFP, green, JF646, red) transfected with GFP-tagged DCP1A (positive control), DCP2, AGO2, or GFP (negative control) and treated with isotonic (150 mM Na^+) or hypertonic (300 mM Na^+) medium for 2 min. Scale bar, 10 μm. Insets depict magnified areas corresponding to a 2 x 2 μm^2 box as indicated; n = 2, 5 cells per replicate. (B) Color mapped scatter plots of area normalized Halo-DCP1A fluorescence intensity against area normalized GFP-tagged protein intensity. Each dot represents a cell and its color represents the number of foci within the cell upon hypertonic (300 mM Na^+) treatment. The color scheme is provided on the top of the plot. Green contours depict HOPS conditions, which is defined

by an at least 3-fold increase in the number of foci under hypertonic conditions compared to isotonic controls.

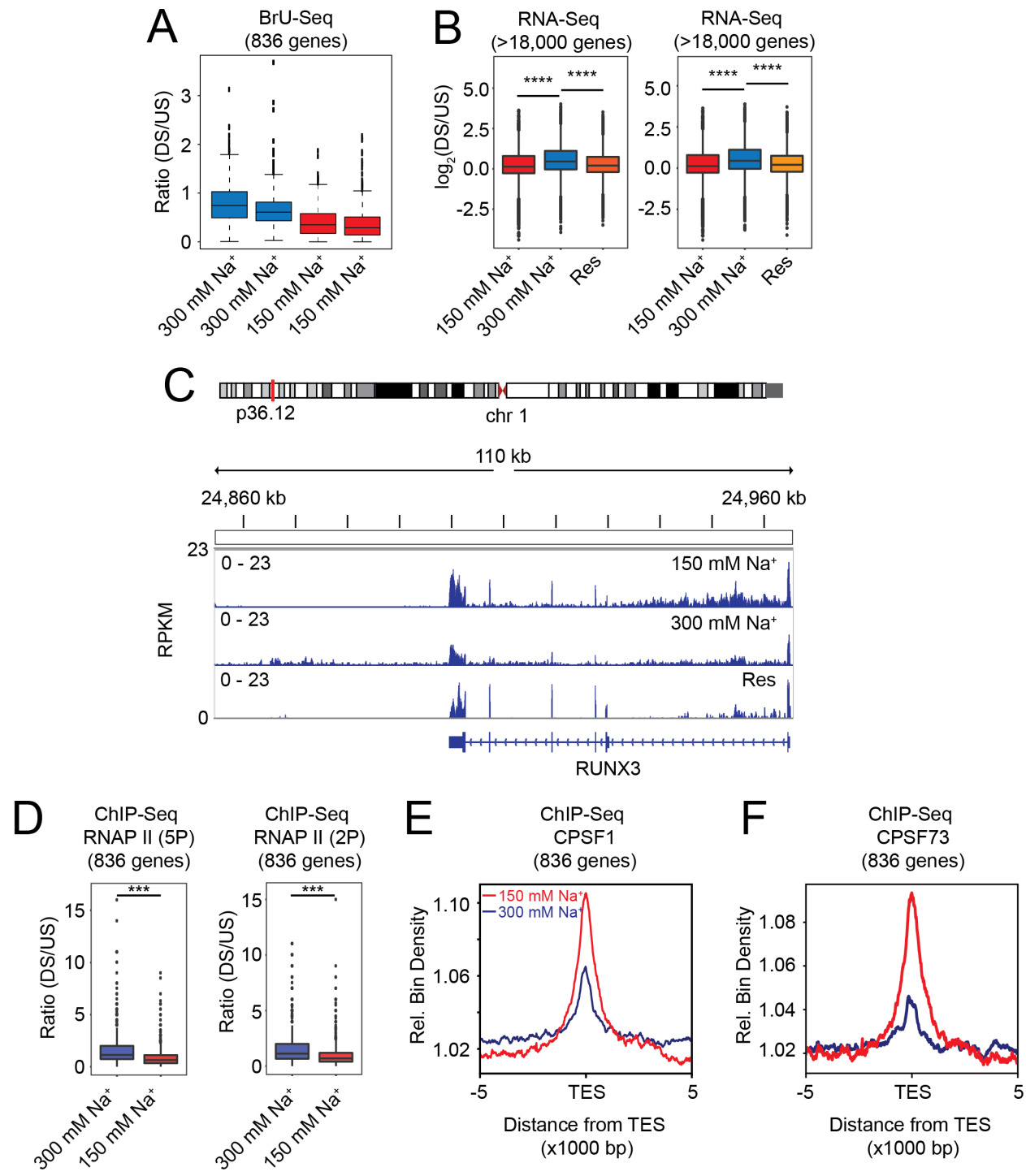

Figure S7. Hyperosmolarity-induced transcript read-through correlates with loss of TES occupancy by CPSFs. Related to Figure 6. (A) Ratio between read counts

downstream (DS) and read-counts upstream (US) of TES for 836 genes assayed by BrU-Seq for each replicate. Cells were treated with isotonic (150 mM Na^+, 30 min) or hypertonic (300 mM Na^+, 30 min) mediums prior to sequencing. (B) DS:US ratio of > 18,000 genes that show transcript read-through in RNA-Seq assays. Cells were treated with isotonic (150 mM Na^+, 4 h) medium, or hypertonic (300 mM Na^+, 4 h) medium, or rescued (Res) with isotonic medium (4 h) after hypertonic treatment (4 h) prior to sequencing. (C) RNA-seq tracks of the RUNX3 locus under isotonic (150 mM Na^+, 4 h) medium, hypertonic (300 mM Na^+, 4 h) medium, or rescued (Res) with isotonic medium (4 h) after hypertonic treatment (4 h) prior to sequencing. (D) Ratio between ChIP-seq read counts downstream (DS) and upstream (US) of TES for 836 genes. Left panel, ChIP-seq with antibody against Serine 5P in C-terminal domain (CTD) of RNA Pol II; right panel, ChIP-seq with antibody against Serine 2P in CTD of RNA Pol II. Cells were treated with isotonic (150 mM Na^+, 30 min) or hypertonic (300 mM Na^+, 30 min) medium prior to ChIP-seq. (E and F) Aggregated ChIP-seq reads of CPSF1 (E) and CPSF73 (F) for 836 genes mapped around the TES. Cells were treated with isotonic (150 mM Na^+, 30 min, red) or hypertonic (300 mM Na^+, 30 min, blue) medium prior to ChIP-seq.