Expanded View Figures

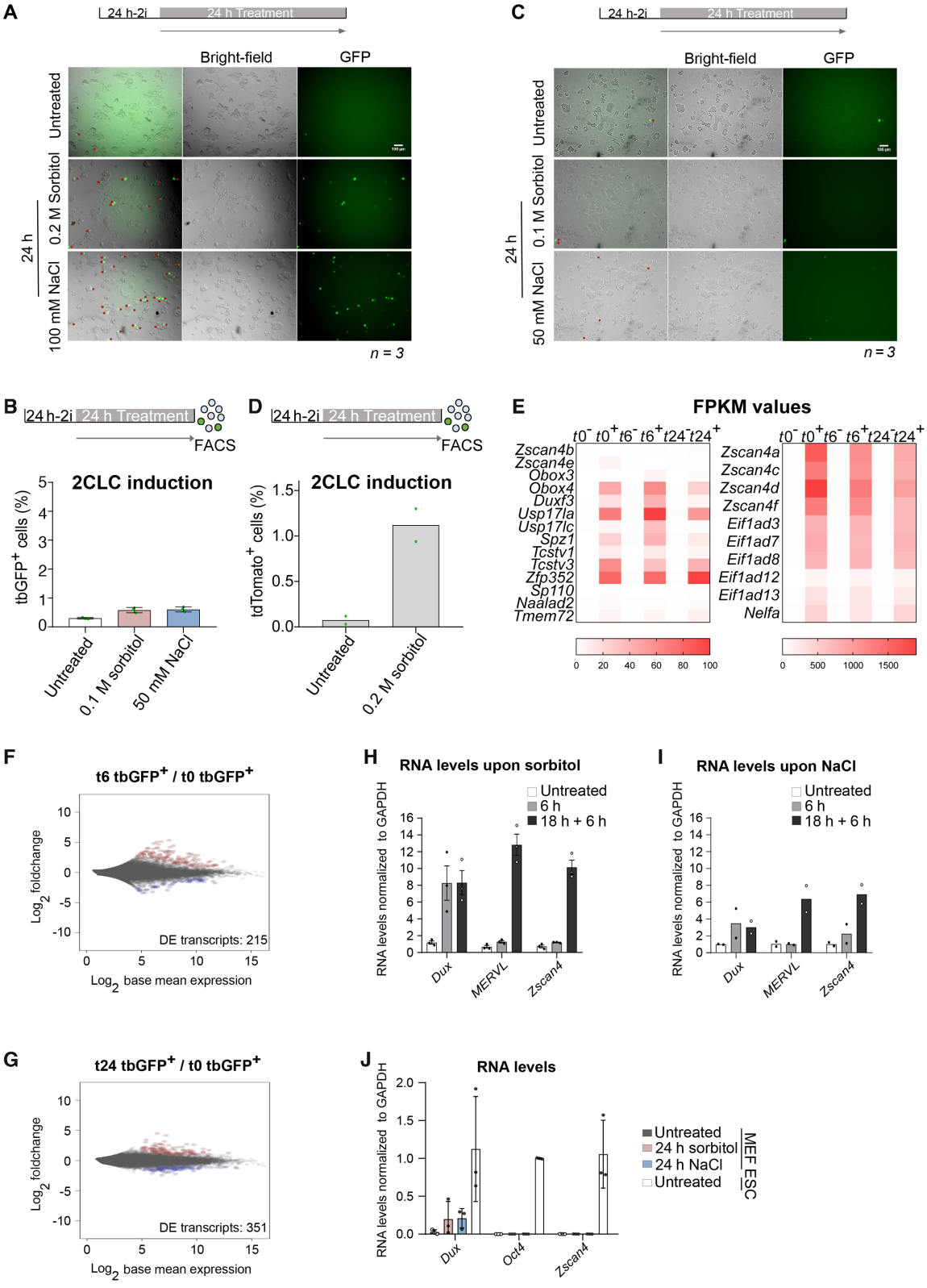

Figure EV1.

Figure EV1. Hyperosmotic stress induces 2CLCs.

A Representative images showing bright-field and GFP fluorescence from ESCs incubated for 24 h with indicated hyperosmotic treatment. Red arrows indicate tbGFP-positive 2CLCs. Scale bar: 100 μm.

B Percentage of 2CLCs obtained by FACS after the indicated sorbitol or NaCl treatments for 24 h. Shown are the mean from two replicates. Individual dots indicate measurement of an independent biological replicate by FACS.

C Representative image showing bright-field and GFP fluorescence from ESCs incubated for 24 h with the indicated hyperosmotic treatment. Red arrows indicate tbGFP-positive 2CLCs. Scale bar: 100 μm.

D Percentage of 2CLCs obtained by FACS after the indicated 0.2 M sorbitol treatment for 24 h. Shown are the mean from two replicates. Individual dots indicate measurement of an independent biological replicate by FACS.

E Heatmap depicting FPKM values of the 25 selected 2CLC markers from Fig 1G for all RNA-sequencing conditions. All genes displayed significative differential expression at t0, t6 and t24 sorbitol treatment in $tbGFP^+$ cells compared to $tbGFP^-$ cells of related time point ($P.adj < 0.01$).

F, G MA plots displaying differentially expressed genes in $tbGFP^+$ from t6 compared to t0 or t24 compared to t0. Significantly DE genes are highlighted in red for up-regulated transcripts and blue for downregulated transcripts ($P.adj < 0.05$; $Log_2FoldChange(tbGFP^+/tbGFP^-) > 1$ or -1).

H, I RT-qPCR analysis of the indicated genes in ESC cultures treated with sorbitol or NaCl for the indicated time. Shown are the mean ± s.d. of three independent replicates for (G) or the mean of two independent replicates for H. Individual dots indicate fold-change measurement for an independent biological replicate by qPCR.

J RT-qPCR of the indicated genes in MEF control (untreated) and treated with sorbitol or NaCl for 24 h and compared with untreated ESCs. *Oct4* is shown as comparison of a gene strongly and specifically expressed in ESCs. Shown are the mean ± s.d. of three independent replicates.

Source data are available online for this figure.

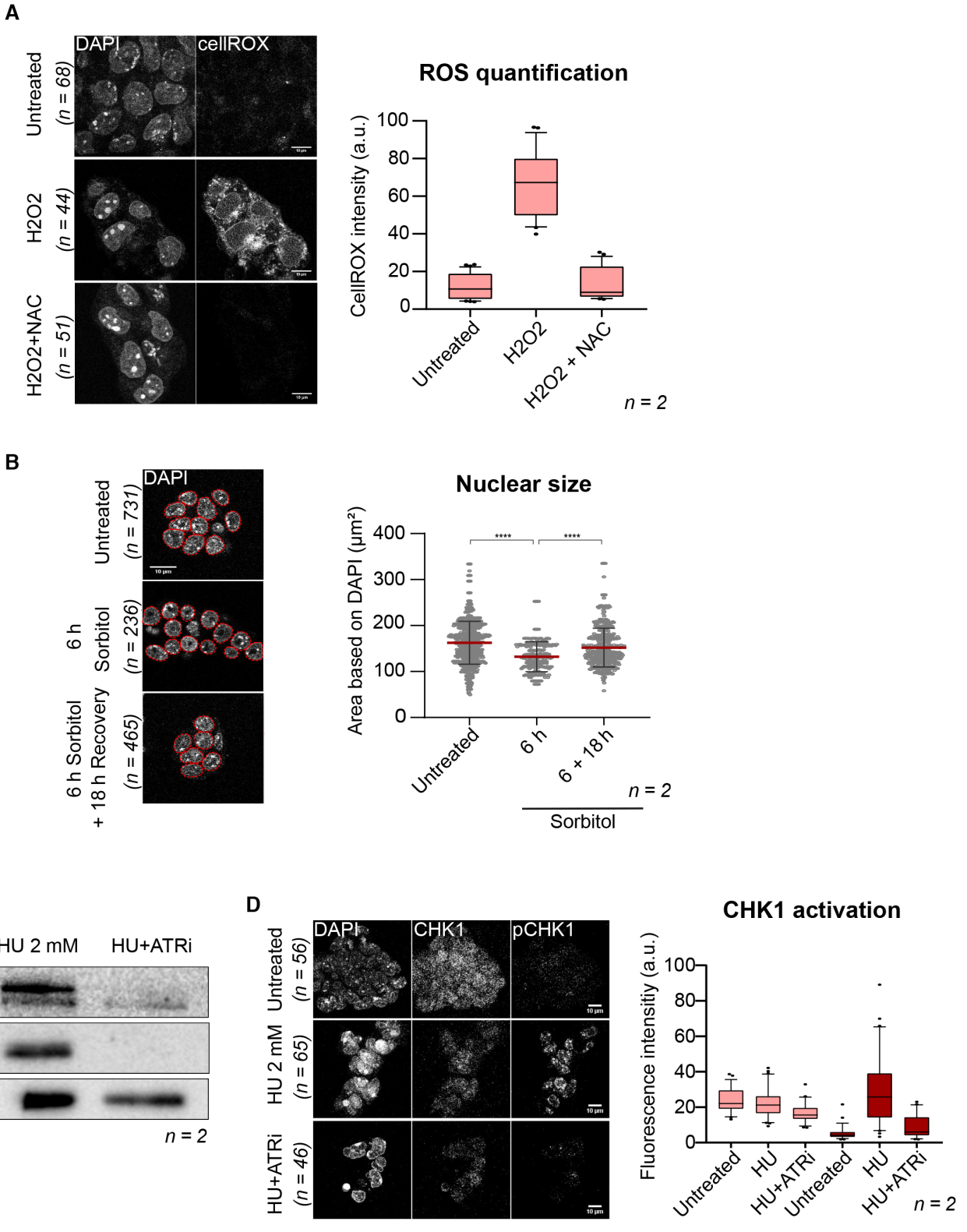
A
DAPI
cellROX
Untreated (n = 68)
H2O2 (n = 44)
H2O2+NAC (n = 51)
ROS quantification
CellROX intensity (a.u.)
100
80
60
40
20
0
Untreated
H2O2
H2O2 + NAC
n = 2
B
DAPI
Untreated (n = 731)
6 h Sorbitol (n = 236)
6 h Sorbitol + 18 h Recovery (n = 465)
Nuclear size
Area based on DAPI (µm²)
400
300
200
100
0
****
****
Untreated
6 h
6 + 18 h
n = 2
Sorbitol
C
HU 2 mM
HU+ATRi
pATR
pCHK1
GAPDH
n = 2
D
DAPI
CHK1
pCHK1
Untreated (n = 56)
HU 2 mM (n = 65)
HU+ATRi (n = 46)
CHK1 activation
Fluorescence intensity (a.u.)
100
80
60
40
20
0
Untreated
HU
HU+ATRi
Untreated
HU
HU+ATRi
n = 2

Figure EV2.

Figure EV2. Hyperosmotic-mediated 2CLC induction is mediated through ROS generation and ATR activation.

A (Left) Representative single confocal z-sections of CellROX-DeepRed fluorescence of ESCs treated with either hydrogen-peroxide (H_2O_2) with or without addition of the NAC ROS scavenger from two biological replicates. Scale bar: 10 μm. (Right) Boxplots showing quantification of CellROX-DeepRed fluorescence intensity from single z-sections. Boxes indicate the range between the first and third quartile, the line indicates the median and the whiskers display the spread from 5 to 95% of the data. Two-tailed Mann–Whitney tests were performed. A total of 68, 44, and 51 nuclei were quantified in untreated, H2O2, H202 + NAC, respectively.

B (Left) DAPI signal of representative single-section of ESCs treated with 0.2 M sorbitol for the indicated time from two biological replicates. Nuclei segmentation is shown in red. Scale bar: 10 μm. (Right) The plot displays median (red line) area ± s.d. of nuclear area as determined by DAPI fluorescence. Each dot represents a single nucleus from 2 biological replicates. Two-tail Mann–Whitney tests were performed. A total of 731, 236, and 465 nuclei were quantified in untreated, 6 h, 6 + 18 h sorbitol, respectively.

C Western blot analysis for the indicated antibodies in ESCs treated with hydroxy-urea (HU) with or without the ATR inhibitor. Shown is one representative image from two independent replicates. p, phosphorylated.

D (Left) Representative single confocal z-section of immunofluorescence for CHK1 and phosphoCHK1 (pCHK1) of ESCs treated with HU with or without ATR inhibitor from two biological replicates. Scale bar: 10 μm. (Right) Quantification of immunofluorescence intensities from single z-sections. A total of 56, 65, and 46 nuclei were quantified in untreated, 2 mM HU, 2 mM HU + ATRi, respectively. Boxes indicate the range between the first and third quartile, the line indicates the median and the whiskers display the spread from 5 to 95% of the data.

Data information: Stars indicate significant differences obtained using indicated statistical tests with *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.
Source data are available online for this figure.