

Supplemental information

**Quantitative RNA imaging in single live cells
reveals age-dependent asymmetric inheritance**

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Supplementary information

Table S1. Related to all Figures. List of *S. cerevisiae* strains used in the study.

#	Name	Genotype	Origin	Figures
1	Y7092	Mat α ; <i>can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0</i>	Boone lab	1C&D, 2C&D, 3A-F, 4B, S1B, S2A&B&C, S4C&D&E, S5A-H
2	IKY1	Y7092; <i>pRS406 (2μOri-ACT1pr-mCHERRY-1xdiSPINACH-CYC1term-URA3)</i>	This study	1C&D, S1B-D
3	IKY2	Y7092; <i>pRS406 (2μOri-ACT1pr-mCHERRY-2xdiSPINACH-CYC1term-URA3)</i>	This study	1C&D, S1B-D
4	IKY3	Y7092; <i>pRS406 (2μOri-ACT1pr-mCHERRY-4xdiSPINACH-CYC1term-URA3)</i>	This study	1C&D, S1B-D, S2A
5	IKY4	Y7092; <i>pRS406 (2μOri-ACT1pr-mCHERRY-8xdiSPINACH-CYC1term-URA3)</i>	This study	1A&C&D, 2A&B, 3A&B&G, S1B-F, S2C, S3, S4A&B&F&G
6	IKY5	Y7092; <i>pRS406 (2μOri-ACT1pr-mCHERRY-16xdiSPINACH-CYC1term-URA3)</i>	This study	1C&D, S1B
7	IKY6	Y7092; <i>pRS406 (2μOri-ACT1pr-mCHERRY-CYC1term-URA3)</i>	This study	S1C&D
8	IKY7	Y7092; <i>pRS406 (2μOri-ACT1pr-mCHERRY-12xMANGOIII-CYC1term-URA3)</i>	This study	S2B
9	IKY8	Y7092; <i>pdr5Δ0; pRS406 (2μOri-ACT1pr-mCHERRY-12xMANGOIII-CYC1term-URA3)</i>	This study	S2B
10	IKY9	Y7092; <i>eno2::ENO2-8xdiSPINACH</i>	This study	2C&D, 3A&B, 6B&C, 7B&C, S3, S6A-D, S7A-L
11	IKY10	Y7092; <i>htb2::HTB2-8xdiSPINACH</i>	This study	2C&D, 3C&D&H, S3

12	IKY11	Y7092; <i>ura3::HHO1pr-mCHERRY-8xdiSPINACH-CYC1term-URA3</i>	This study	2C&D, S3, S4C
13	IKY12	Y7092; <i>pRS406 (2μOri-SUT509pr-SUT509-8xdiSPINACH-CYC1term-URA3)</i>	This study	3E&F
14	IKY13	Y7092; <i>eno2::ENO2-8xdiSPINACH ura3::NAB2NLS-2mCHERRY-URA3</i>	This study	4B&C, S5A-E
15	IKY14	Y7092; <i>eno2::ENO2-8xdiSPINACH ura3::NAB2NLS-2xmCHERRY-URA3 she2::LOXP-LEU2-LOXP</i>	This study	4B&C, 6D, S5A-E
16	IKY15	Y7092; <i>eno2::ENO2-8xdiSPINACH ura3::NAB2NLS-2xmCHERRY-URA3 she3::LOXP-LEU2-LOXP</i>	This study	4B&C
17	IKY16	Y7092; <i>eno2::ENO2-8xdiSPINACH ura3::NAB2NLS-2xmCHERRY-URA3 myo4::LOXP-LEU2-LOXP</i>	This study	4B&C
18	IKY17	Y7092; <i>ADE2 met17::LexA-ER-AD-TF-MET17 whi5::LexApr-WHI5-ADH1term-LEU2-URA3</i>	This study	7B&C
19	IKY18	Y7092; <i>eno2::ENO2-8xdiSPINACH ADE2 met17::LexA-ER-AD-TF-MET17 whi5::LexApr-WHI5-ADH1term-LEU2-URA3</i>	This study	7B&C
20	IKY19	Y7092; <i>eno2::ENO2-8xdiSPINACH she2::SHE2-mCHERRY</i>	This study	6E
21	IKY20	Y7092; <i>eno2::ENO2-8xdiSPINACH sir2::NatMX</i>	This study	7D
22	IKY21	Y7092; <i>pdc1::PDC1-8xdiSPINACH</i>	This study	5A&B

23	IKY22	Y7092; <i>tef1::TEF1-8xdiSPINACH</i>	This study	5C&D
24	IKY23	Y7092; <i>tef1::TEF1-8xdiSPINACH</i> <i>she2::NatMX</i>	This study	5C&D
25	IKY24	Y7092; <i>tef1::TEF1-8xdiSPINACH</i> <i>myo4::NatMX</i>	This study	5C&D
26	IKY25	Y7092; <i>eno2::ENO2-mCHERRY</i>	This study	S6E&F
27	IKY26	Y7092; <i>eno2::ENO2-mCHERRY</i> <i>she2:: LOXP-LEU2-LOXP</i>	This study	S6E&F
28	IKY27	Y7092; <i>eno2::ENO2-sfGFP</i>	This study	S6G&H
29	IKY28	Y7092; <i>eno2::ENO2-sfGFP</i> <i>she2:: NatMX</i>	This study	S6G&H

Table S2. Related to Star Methods and all Figures. Aptamer tag sequences.

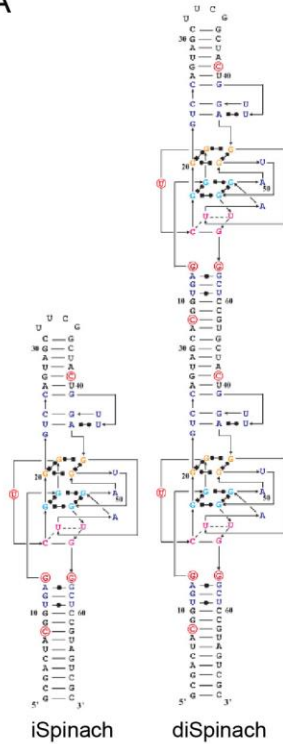
Tag	Sequence
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2xdiSpinach (260 bp)	GCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGT AGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGC GAATCTTCTCTGTCTATCGC GACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAG AGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGC
4xdiSpinach (540 bp)	GCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGT AGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGC GAATCTTCTCTGTCTATCGC GACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAG AGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCTGTTTTCGAAATTACCCTTGCGA CTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAG TGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCCAAGCAGACTCATACTAGATGCGAC TACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGT GTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGC
8xdiSpinach (1100 bp)	GCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGT AGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGC GAATCTTCTCTGTCTATCGC GACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAG AGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCTGTTTTCGAAATTACCCTTGCGA CTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAG TGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCCAAGCAGACTCATACTAGATGCGAC TACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGT GTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCCGAGCATTCTATCACGTCGGCGACT ACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGTG TGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCGTAGATGAGCGCAGGGACACGCGACT ACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGTG TGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCATGCCCTAAGAACCTCTCGGCGACTAC

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16xdiSpinach (2240 bp)	GCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGT AGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCGAATCTTCTCTGTCTATCGC GACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAG AGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCTGTTTTCGAAATTACCCTTTGCGA CTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAG TGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCCAAGCAGACTCATACTAGATGCGAC TACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGT GTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCCGAGCATTCTATCACGTCGGCGACT ACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGTG TGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCGTAGATGAGCGCAGGGACACGCGACT ACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGTG TGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCATGCCCTAAGAACCTCTCGGCGACTAC GGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGTGTG GGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCTCCTGTCACATCATAATCGTGCGACTACGG TGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAGTGTGGG CTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCATCAGATCCACTAGTGGAAGCTTATCGATACC GTCGACATGCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTAC TGTTGAGTAGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCGAATCTTCTCTG TCTATCGCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGT TGAGTAGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCTGTTTTCGAAATTACCC TTTGCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGA GTAGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCCAAGCAGACTCATACTAGA TGCGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGT AGAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCCGAGCATTCTATCACGTCGG

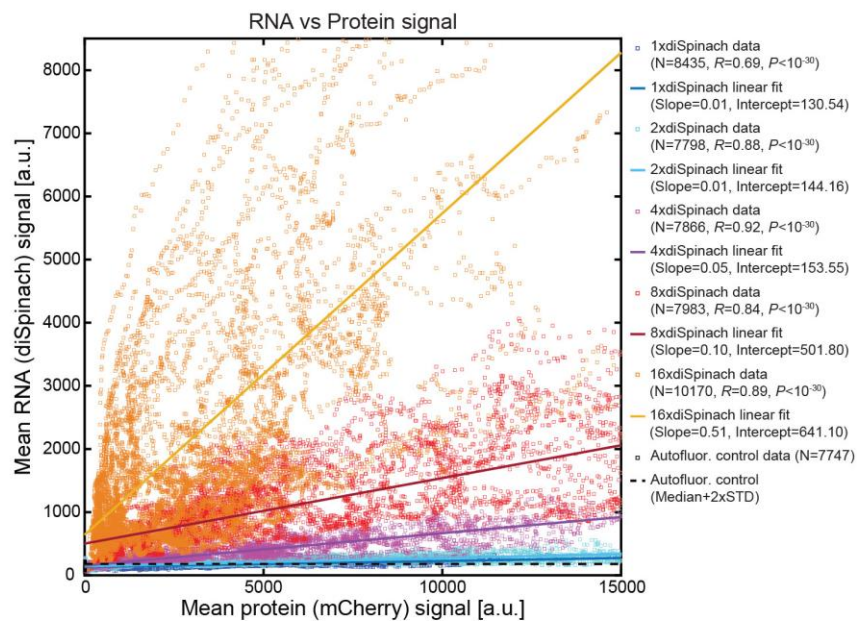
	<p>CGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTA</p> <p>GAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCGTAGATGAGCGCAGGGACACG</p> <p>CGACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTA</p> <p>GAGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCATGCCCTAAGAACCTCTCGGC</p> <p>GACTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAG</p> <p>AGTGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGCTCCTGTCACATCATAATCGTGCGA</p> <p>CTACGGTGAGGGTCGGGTCCAGTGACGGTGAGGGTCGGGTCCAGTAGCTTCGGCTACTGTTGAGTAGAG</p> <p>TGTGGGCTCCGTGCACTGTTGAGTAGAGTGTGGGCTCCGTAGTCGC</p>
<p>12xMango III</p> <p>(607 bp)</p>	<p>GGCACGTACGAAGGAAGGATTGGTATGTGGTATATTCGTACGTGCCTTTTGGCACGTACGAAGGAAGGAT</p> <p>TGGTATGTGGTATATTCGTACGTGCCTTTTGGCACGTACGAAGGAAGGATTGGTATGTGGTATATTCGTAC</p> <p>GTGCCTTTTGGCACGTACGAAGGAAGGATTGGTATGTGGTATATTCGTACGTGCCTTTTGGCACGTACGA</p> <p>AGGAAGGATTGGTATGTGGTATATTCGTACGTGCCTTTTGGCACGTACGAAGGAAGGATTGGTATGTGGT</p> <p>ATATTCGTACGTGCCTTTTGGCACGTACGAAGGAAGGATTGGTATGTGGTATATTCGTACGTGCCTTTTGG</p> <p>CACGTACGAAGGAAGGATTGGTATGTGGTATATTCGTACGTGCCTTTTGGCACGTACGAAGGAAGGATTG</p> <p>GTATGTGGTATATTCGTACGTGCCTTTTGGCACGTACGAAGGAAGGATTGGTATGTGGTATATTCGTACGT</p> <p>GCCTTTTTGGCACGTACGAAGGAAGGATTGGTATGTGGTATATTCGTACGTGCCTTTTGGCACGTACGAAG</p> <p>GAAGGATTGGTATGTGGTATATTCGTACGTGCC</p>

Supplementary figures:

A



B



C

2 μ Ori expression plasmids:

ACT1pr-mCHERRY-1xdiSPINACH
 ACT1pr-mCHERRY-2xdiSPINACH
 ACT1pr-mCHERRY-4xdiSPINACH
 ACT1pr-mCHERRY-8xdiSPINACH
 ACT1pr-mCHERRY



Probe: mCHERRY

D

2 μ Ori expression plasmids:

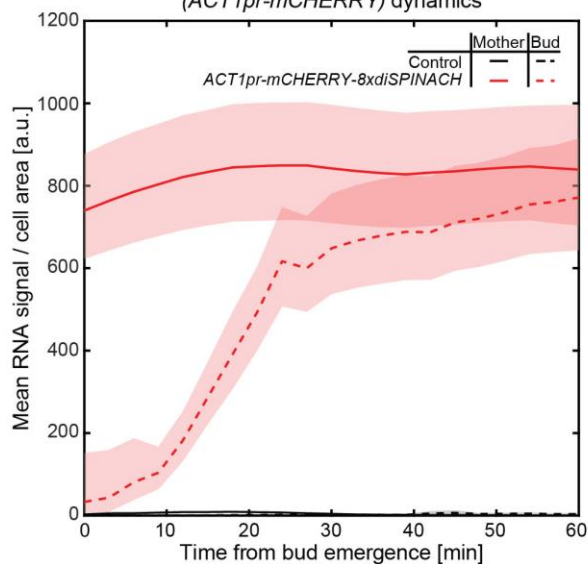
ACT1pr-mCHERRY-1xdiSPINACH
 ACT1pr-mCHERRY-2xdiSPINACH
 ACT1pr-mCHERRY-4xdiSPINACH
 ACT1pr-mCHERRY-8xdiSPINACH
 ACT1pr-mCHERRY



Probe: diSPINACH

E

Mother vs Bud diSpinach signal
 (ACT1pr-mCHERRY) dynamics



F

Bud / Mother diSpinach signal
 (ACT1pr-mCHERRY) ratio

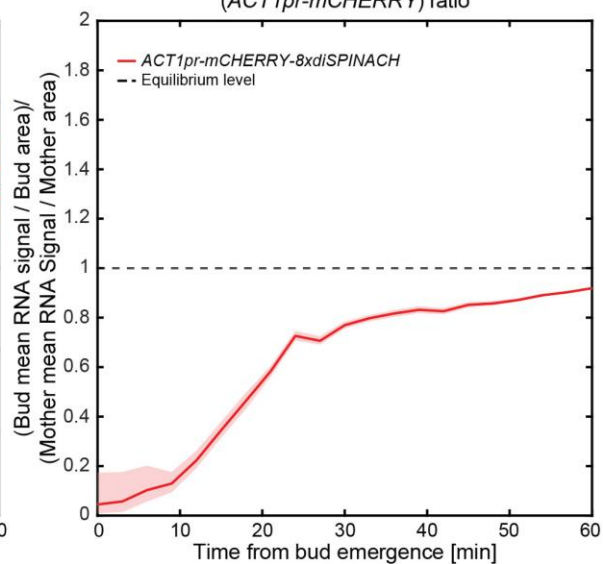


Figure S1. Related to Figures 1, 5. RNA aptamer diSpinach structure, optimization of diSpinach repeats number, northern blot for diSpinach repeats and dynamics of RNA transport into buds for *ACT1pr-mCHERRY-8xdSPINACH* cells. (A) Side-by-side comparison of RNA aptamer iSpinach and diSpinach secondary structures. (B) RNA signal (sum of intracellular pixel intensities in the diSpinach channel) normalized by cell area and plotted against the protein signal (sum of intracellular pixel intensities in the mCherry channel) normalized by cell area for individual cells carrying a 2 μ Ori expression plasmids with *ACT1pr-mCHERRY-1/2/4/8/16xdSPINACH* as well as the autofluorescence control (no expression plasmid). Linear fits are shown for all strains except the control. The dashed black line denotes the median signal plus two standard deviations obtained for the autofluorescence control. Pearson correlation coefficient was used. (C,D) Northern blot on RNA isolated from strains expressing *ACT1pr-mCHERRY-0/1/2/4/8xdSPINACH* from 2 μ Ori expression plasmids. 5 μ g of total RNA was loaded for each sample and 25 ng of radioactively-labeled probes against *mCHERRY* (C) or *diSPINACH* (D) were used per membrane/blot for hybridization. Shown are autoradiograms. (E) Dynamics of mean diSpinach signal (*ACT1pr-mCHERRY*) in mothers and buds (solid and dashed lines) normalized by corresponding cell area, aligned at bud emergence and shown until the median cytokinesis time point (+ 60 min). Cells expressing *ACT1pr-mCHERRY 8xdSPINACH* from 2 μ Ori expression plasmids (N=115 cell cycles) shown in red. The autofluorescence control (N=90 cell cycles) is shown in black. Note that the very low signal in early buds is most likely due to the thresholding in the quantitative analysis. (F) Ratios of the normalized mean signal intensities for buds and mothers plotted in (E). Dashed line shows the ratio at which mean RNA (diSpinach) signals in buds and mothers are equal. Mothers and buds were counted as separate cells from bud emergence. Ribbons denote 95% confidence intervals as determined from 50000 bootstrap samples. (B,E,F) Cells were grown and imaged for 10 hrs in SCD medium with 50 μ M DHFBI-1T.

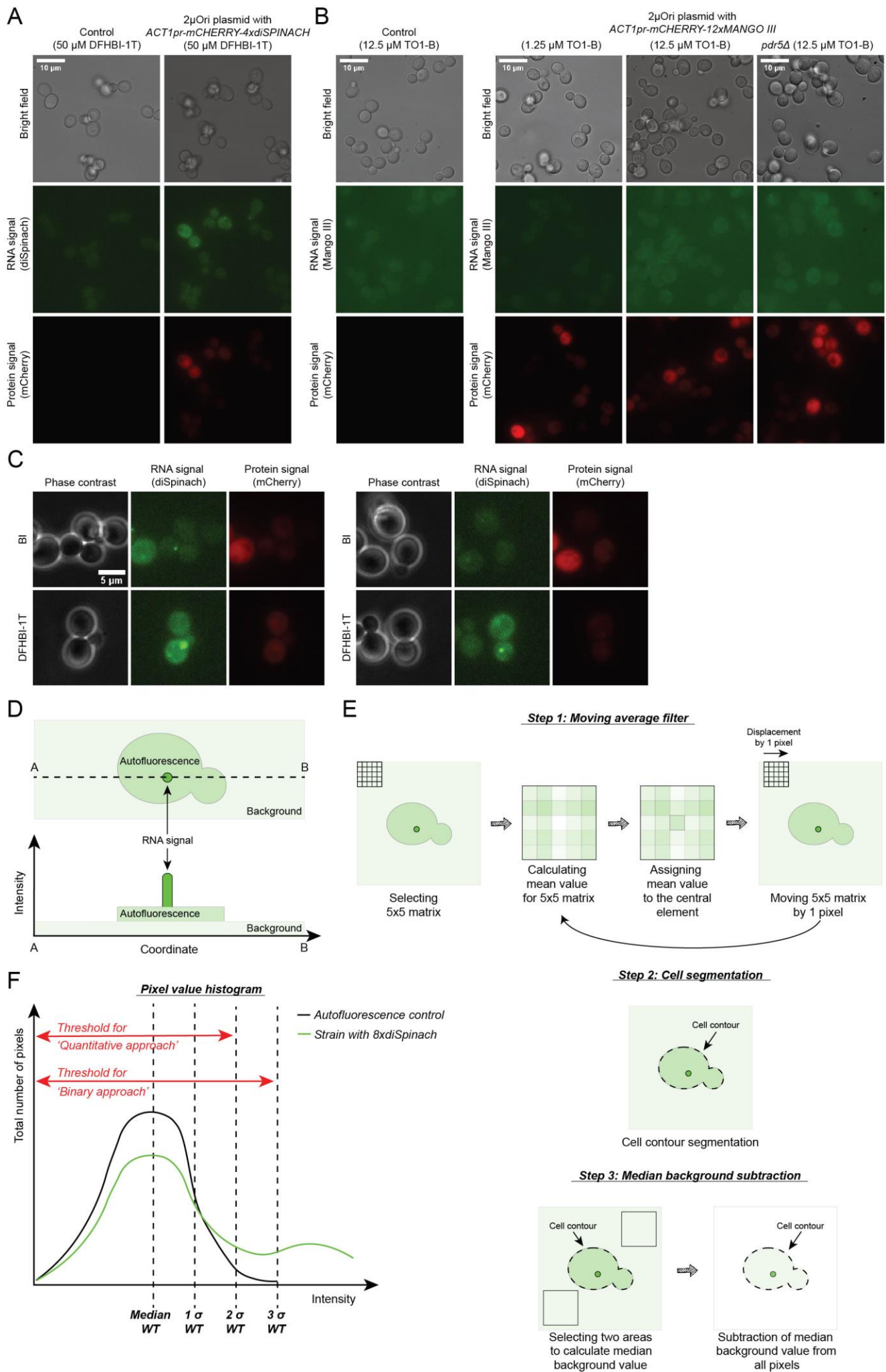


Figure S2. Related to Star Methods and Figures 1, 2, 3. Comparison of 4xdiSpinach and 12xMango III tags, performance of DHFBI1-1T vs BI for 8xdiSpinach tag and schematic illustrating image processing and signal thresholding. (A) Representative raw images for the autofluorescence control and the strain carrying a 2 μ Ori plasmid with *ACT1pr-mCHERRY-4xdiSPINACH*. Cells were grown in SCD and 40 min prior to imaging, 50 μ M DFHBI-1T was added. (B) Representative raw images for the autofluorescence control, the strain carrying a 2 μ Ori plasmid with *ACT1pr-mCHERRY-12xMANGO III*, and a *pdr5 Δ* strain carrying a 2 μ Ori plasmid with *ACT1pr-mCHERRY-12xMANGO III*. Cells were grown in SCD and 40 min prior to imaging 1.25 or 12.5 μ M TO1-B was added. (C) Representative raw images for the strain carrying a 2 μ Ori plasmid with *ACT1pr-mCHERRY-8xdiSPINACH* grown in SCD and 40 min prior to imaging supplemented with 50 μ M of DFHBI-1T or BI. For each channel, all images were acquired using the same settings. (D) Schematic representation of different fluorescence levels detected in the RNA channel during live-cell imaging. (E) Image processing pipeline. (F) Threshold selection for two different data analysis approaches (quantitative and binary) used in this study. For details see Star methods.

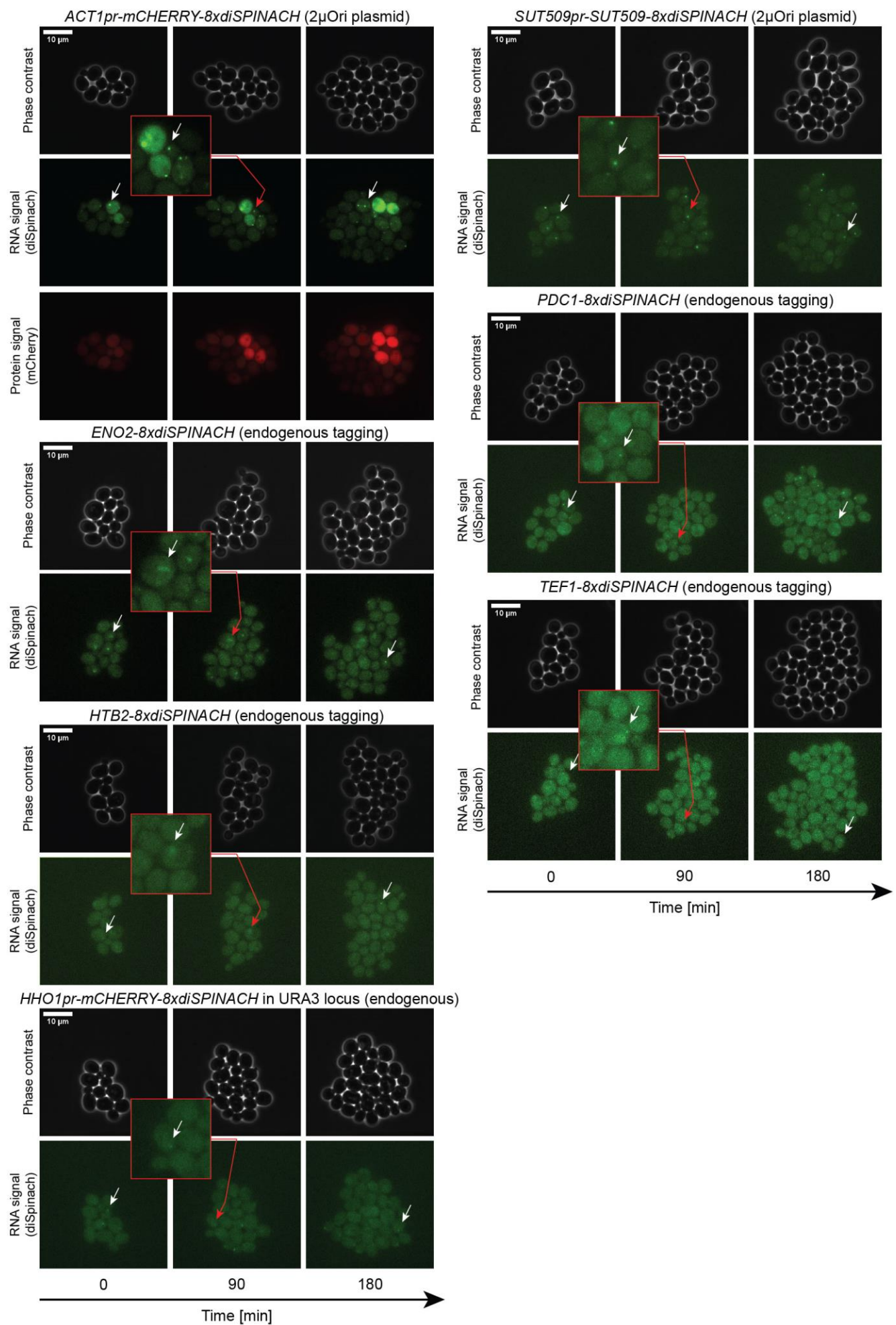


Figure S3. Related to Figures 1, 2, 3, 4, 5. Representative images of indicated strains acquired over 3 hrs of time-lapse experiments. Images acquired in phase contrast, RNA (diSpinach), and protein (only for *ACT1pr-mCHERRY-8xdSPINACH* on 2 μ Ori plasmid strain) channels for the indicated strains. All images shown were acquired using the same settings (see Star methods). White arrows highlight characteristic diSpinach signals. Red arrows denote regions of zoom-in. Scale bars denote 10 μ m. No image processing was applied for the images shown.

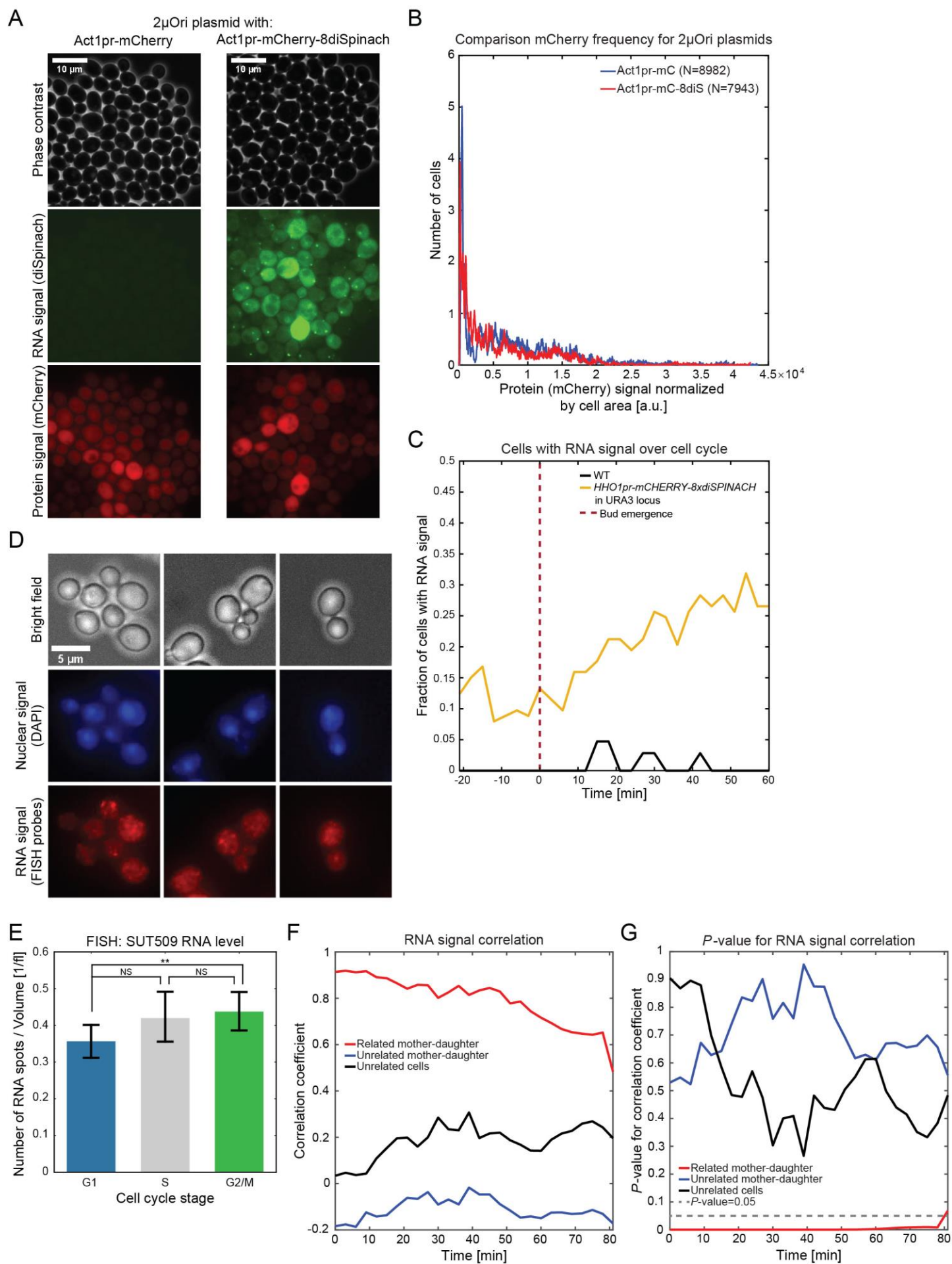


Figure S4. Related to Figures 2, 3. Comparison of *ACT1pr-mCHERRY* expression from 2 μ Ori plasmids with and without 8xdiSpinach tag, cell-cycle dynamics of RNA levels, quantification of SUT509 ncRNA using a smFISH protocol as well as correlation of RNA (diSpinach) signal between related and unrelated cells. (A) Side-by-side comparison of images of cells carrying an expression plasmid with ACT1pr-mCherry with the 8xdiSpinach tag or without shown for 3 channels: phase contrast, diSpinach, and mCherry (corresponding to protein). (B) Plot showing the frequency of mCherry expression from both types of expression plasmids: blue line – without any tag (N=8982 cells), red line – with the 8xdiSpinach tag (N=7943 cells). (C) Application of the binary approach to assess a cell-cycle dependence of RNA (diSpinach) levels in a qualitative manner for the strain with *HHO1pr-mCHERRY-8xdiSPINACH* integrated into the *URA3* locus. Fraction of cells with detectable RNA signal for *HHO1pr-mCHERRY-8xdiSPINACH* (N=113 cell cycles) is plotted as a function of time. Autofluorescence control is shown in black (N=106 cell cycles). All data were aligned by bud emergence and shown from -21 min (corresponding to the median beginning of G1 phase) to +60 min (corresponding to the median time of cytokinesis). Mothers and their buds were counted as one cell before cytokinesis. (D) Representative images of maximum Z-projections for all 3 channels (Bright field, DAPI, smFISH probes) for cells without 8xdiSpinach tag (Y7092 strain) in cells grown in SCD without uracil. (E) Number of RNA spots detected in G1 (N=259 cells), S (N=189 cells) and G2/M (N=131 cells) phases of the cell cycle normalized to cell volume. ** - *P*-value <0.02, NS – not significant (Wilcoxon test). (F,G) 8xdiSpinach-tagged mCherry RNA was expressed from the *ACT1* promoter on a 2 μ Ori plasmid. Plotted in (F) are Pearson correlation coefficients between related mother-daughter pairs (N=15 cell pairs, red), random mother-daughter pairs (N=14 cell pairs, blue), and unrelated cell pairs including both mothers and daughters (N=15 cell pairs, black), as well as the corresponding *P*-values (G) over 81 minutes (median duration of the cell cycle) starting from the time of cytokinesis of mothers. (A,B,C,F,G) Cells were grown and imaged for 10 hrs in SCD medium with 50 μ M DHFBI-1T.

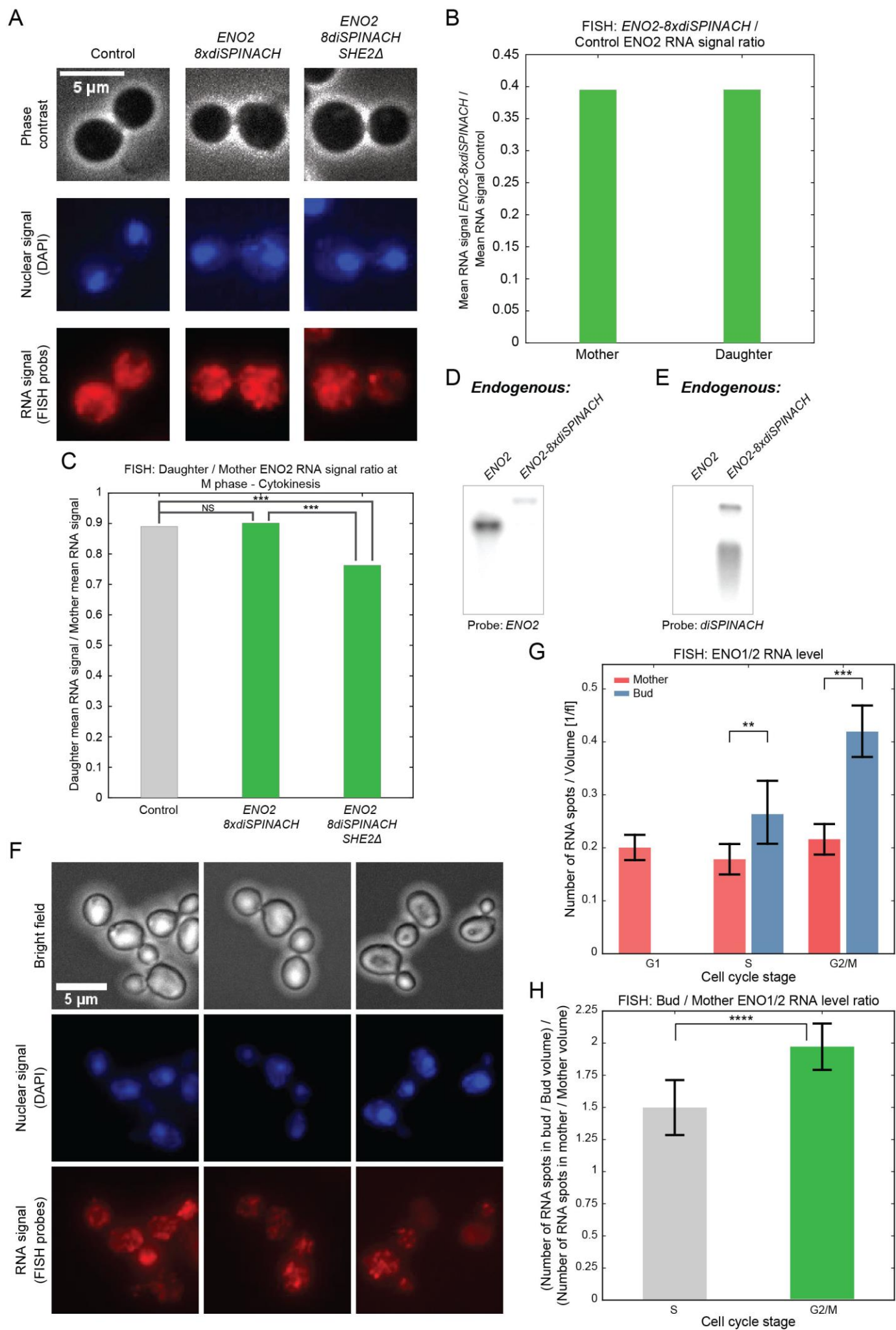


Figure S5. Related to Figure 4. Results of FISH experiments and northern blot results for ENO2 RNA, quantification of ENO1/2 RNA using a smFISH protocol. (A) Representative phase contrast, DAPI staining, and ENO2 RNA images in M phase for control cells, cells with endogenously tagged *ENO2* with *8xdiSPINACH* (WT cells), and cells with additional deletion of *SHE2*. All cells were grown in SCD. (B) Ratios of the mean ENO2 RNA signal of *ENO2-8xdiSPINACH* cells to the mean ENO2 RNA signal of control cells plotted separately for mothers and daughters. (C) Ratios of the normalized mean signal intensities for daughters and mothers: control cells (N=115 cells), *ENO2-8xdiSPINACH* cells (N=156 cells), *ENO2-8xdiSPINACH-she2Δ* cells (N=148 cells). Standard errors are smaller than 0.008 [a.u.] and thus not visible. *** $P < 10^{-11}$ (Wilcoxon test). NS – not significant. (D,E) Northern blot on RNA isolated from strains with and without 8xdiSpinach tag on the endogenous ENO2 RNA. 5 μg of total RNA was loaded for each sample and 25 ng of radioactively-labeled probes against *ENO2* (D) or *diSPINACH* (E) were used per membrane/blot for hybridization. Shown are autoradiograms. (F) Representative images of maximum Z-projections for all 3 channels (Bright field, DAPI, smFISH probes) acquired using a smFISH protocol for ENO1/2 RNA for cells without 8xdiSpinach tag (Y7092 strain) grown in SCGE medium. (G) Number of RNA spots detected in G1 (Mother N=574 cells), S (Mother, Bud N=396 cells) and G2/M (Mother, Bud N=313 cells) phases of the cell cycle normalized to mother/bud volume. ** - P -value $< 10^{-4}$, *** – P -value $< 10^{-5}$ (Wilcoxon test). (H) Ratio of ENO1/2 RNA levels in the bud and mother in S and G2/M phases. **** – P -value $< 10^{-30}$ (Wilcoxon test). Note that, due to the high sequence similarity of *ENO1* and *ENO2*, we expect that the Stellaris probes are visualizing both paralogs.

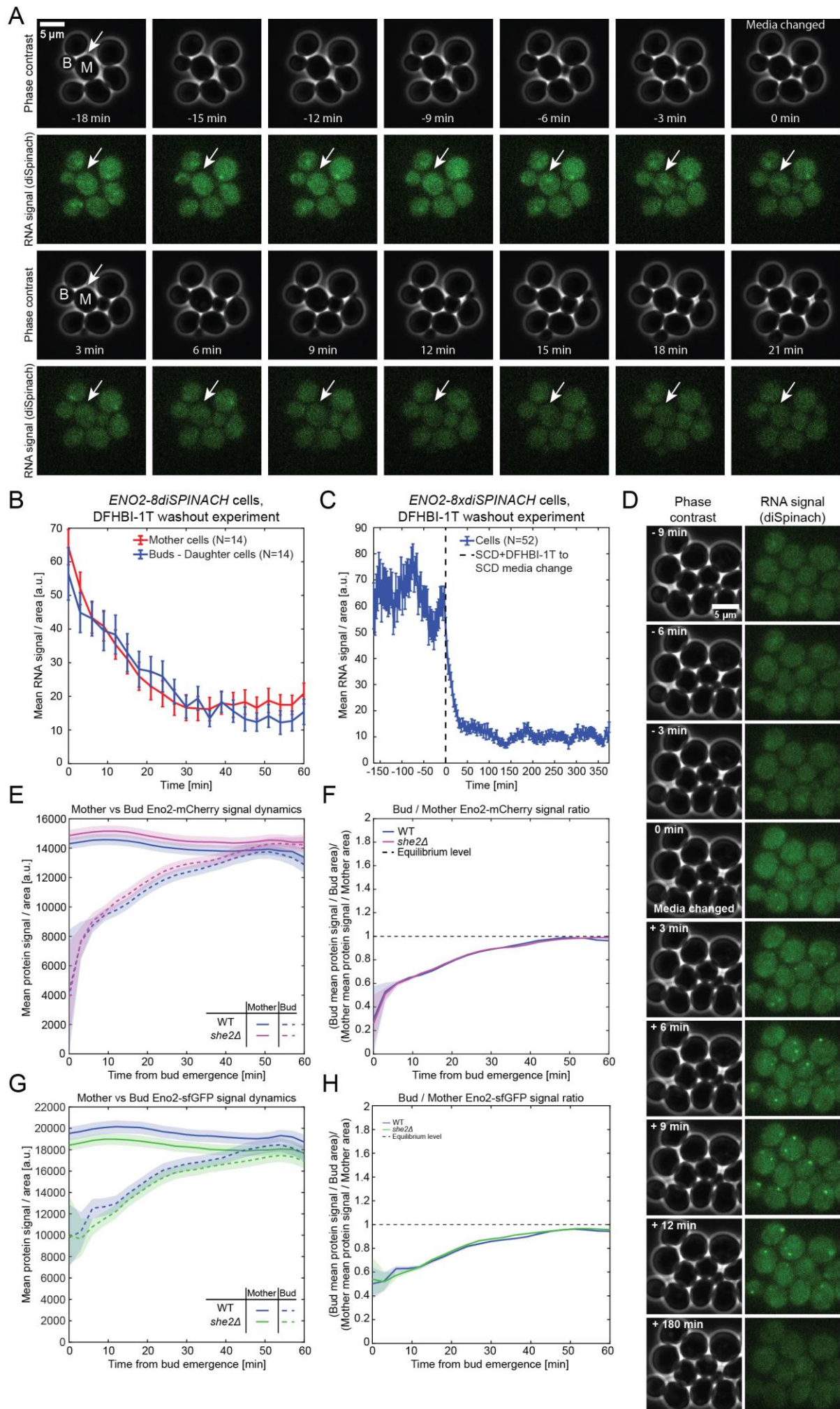


Figure S6. Related to Figure 4. Assessing DFHBI-1T fluorophore availability during washout experiments, ENO2 diSpinach RNA localization upon glucose withdrawal and dynamics of Eno2 protein signal from bud emergence to cytokinesis. (A) Representative time series images of diSpinach signal starting 18 minutes before removal of DFHBI-1T from the media and up to 21 minutes after it. White arrows indicate a budding cell. (B) Dynamics of the mean signal in diSpinach channel plotted starting from the media change time point and shown separately for mother (red line, N=14 track cells) and bud/daughter cells (blue line, N=14 cell tracks). (C) Evolution of the mean signal in diSpinach channel over time during the washout experiment (N=52 cell tracks). Start of washout is indicated by the dotted line. Error bars represent standard errors. (A,B,C) Cells were grown in the custom microfluidic device in SCD with 50 μ M DFHBI-1T for 3 hrs and then in SCD for 7 hrs. (D) Representative time series images of diSpinach (*ENO2*) localization starting 9 minutes before glucose withdrawal and up to 180 minutes after it. Cells were grown in the custom microfluidic device in SCD with 50 μ M DFHBI-1T for 3 hrs and then in SC (no glucose) with 50 μ M DFHBI-1T for 7 hrs. (E,F) Mean Eno2-mCherry or Eno2-sfGFP (protein) signal normalized by the cell area plotted separately for mothers and buds from bud emergence until median cytokinesis time (60 minutes). (E) The bold blue line and the dashed blue line represent the mother cells' Eno2-mCherry signal (N=179 cell cycles) and buds' protein Eno2-mCherry signal (N=179 cell cycles) in WT cells. The bold magenta line and the dashed magenta line represent the mother cells' Eno2-mCherry signal (N=179 cell cycles) and buds' Eno2-mCherry signal (N=179 cell cycles) in *she2 Δ* cells. (G) The bold blue line and the dashed blue line represent the mother cells' Eno2-sfGFP signal (N=140 cell cycles) and buds' protein Eno2-sfGFP signal (N=140 cell cycles) in WT cells. The bold green line and the dashed green line represent the mother cells' Eno2-sfGFP signal (N=137 cell cycles) and buds' Eno2-sfGFP signal (N=137 cell cycles) in *she2 Δ* cells. (F,H) Plots showing ratio of mean bud/mother Eno2-mCherry or Eno2-sfGFP signals. The ratio of Eno2 protein signal in WT cells is shown in blue (F,H), in *she2 Δ* cells shown in magenta (F) or green (H). The dashed line indicates the ratios at which the mother and bud signals are equal. The ribbons show 95% confidence intervals, determined by 50000 bootstrap values. (E,F,G,H) The cells were grown in SC + 2% glucose media with 50 μ M DFHBI-1T.

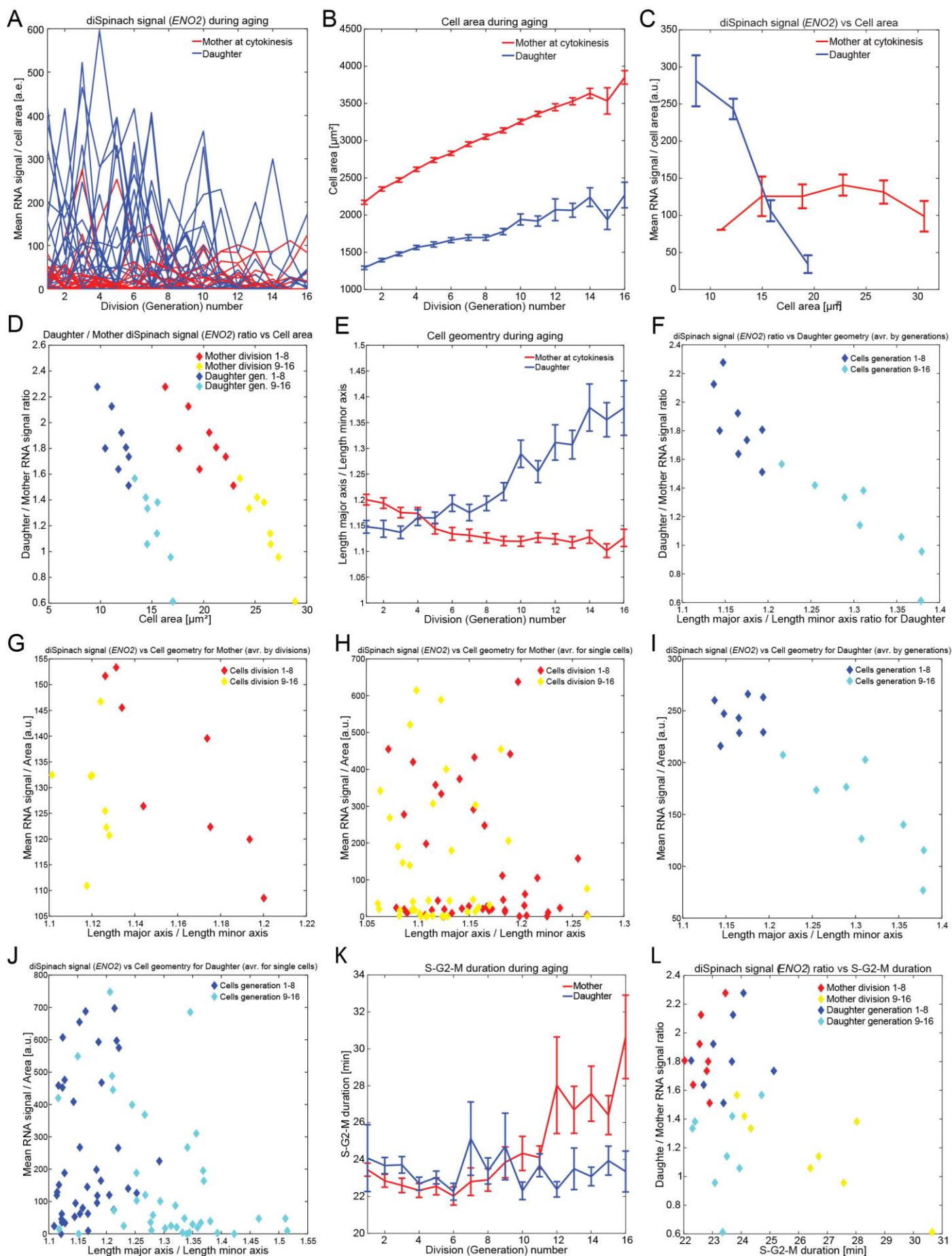


Figure S7. Related to Figures 6, 7. Individual cell tracks indicate loss of asymmetric diSpinach signal (*ENO2*) inheritance during replicative aging as well as the impact of cell size, cell geometry and cycle duration during replicative aging on asymmetric diSpinach signal (*ENO2*) segregation. (A) Randomly selected individual mothers (N=20 cell tracks) are shown in red and individual daughters (N=20 cell tracks) in blue. Plotted is the diSpinach signal (*ENO2*) normalized to the corresponding cell area of mother/daughter cells over multiple divisions/generations. (B) Mean cell area over divisions/generations plotted separately for mother cells (red) and newly born daughter cells (blue). (C) Mean diSpinach signal (*ENO2*) per cell area in mothers at cytokinesis (N=646 cells with different division numbers, red) and their daughters (N=646 cells of different generations, blue) is shown as a function of cell area. (D) Mean Daughter/Mother diSpinach signal (*ENO2*) ratio plotted against cell area for mother cells (red, yellow) and daughter cells (blue, cyan) averaged by divisions/generations. Pearson correlation between mean Daughter/Mother diSpinach signal (*ENO2*) ratio and mother cell area is $R = -0.94$ ($P \sim 10^{-7}$). (E) Mean cell geometry ratio (length of major axis over the length of minor axis) over multiple divisions/generations plotted separately for mother cells (red) and daughter cells (blue). (F) Change of diSpinach (*ENO2*) Daughter/Mother signal ratio plotted against the daughter geometry ratio and averaged by generations (Pearson correlation $R = -0.93$, $P < 10^{-6}$). (G,H,I,J) Mean diSpinach signal (*ENO2*) plotted against geometry ratio for mother (G,H) and daughter (I,J) cells. In (G,I) each diamond corresponds to the respective average of one particular division/generation calculated by taking into account all cells found within that division/generation. In (H,J) each diamond corresponds to the average for one single cell for divisions/generations 1 to 8 and 9 to 16. (K) Mean duration of S-G2-M phase over multiple divisions/generation plotted separately for mother cells (red) and freshly born daughter cells (blue). (L) Mean Daughter/Mother diSpinach signal (*ENO2*) ratio plotted against mean duration of S-G2-M phase for mother cells (red, yellow) and freshly born daughter cells (blue, cyan) averaged by divisions/generations 1-8 and 9-16. Pearson correlation between mean Daughter/Mother diSpinach signal (*ENO2*) ratio and mother S-G2-M phase duration is $R = -0.84$ ($P < 10^{-4}$), while for daughter S-G2-M phase duration is $R = 0.17$ ($P = 0.53$). 40 cell tracks were used in (B,D,E-L). Whiskers on (B,C,E,K) show standard errors. Cells were grown in SCD medium with 50 μ M DHFBI-1T and imaged for up to 72 hrs.