

## Supplemental Tables

**Table 1: U2OS stress granule summary statistics.** Per-replicate median values were computed for each metric, then averaged across replicates within each condition. *P*-values are from two-sided Wilcoxon rank-sum tests on replicate-level medians (control:  $n = 3$  replicates; arsenite:  $n = 4$  replicates).

Metric	Control mean	Arsenite mean	<i>P</i> -value
G3BP1 puncta per cell	1.83	2.50	0.59
G3BP1 fraction condensed	0.0008	0.0059	0.11
G3BP1 condensate index	1.52	1.53	0.86
PABPC1 puncta per cell	1.33	2.50	0.36
PABPC1 fraction condensed	0.0005	0.0022	0.057
PABPC1 condensate index	1.44	1.48	0.11

**Table 2: Yeast temperature series summary.** Per-condition means of key metrics computed across all cells at each temperature. Single replicate per condition; values are descriptive.

Metric	25°C	30°C	32°C	36°C	40°C
<i>n</i> cells	580	266	183	501	164
<i>Condensate metrics</i>					
Sis1 condensate index	1.68	1.61	1.71	1.88	1.67
Sis1 fraction condensed	0.012	0.023	0.018	0.006	0.017
Sis1 puncta/cell	0.41	0.82	1.19	0.20	0.80
Tif6 condensate index	1.85	1.95	1.89	1.87	1.70
Tif6 fraction condensed	0.010	0.018	0.034	0.004	0.028
Tif6 puncta/cell	0.28	0.39	1.39	0.08	2.58
<i>Colocalization</i>					
Pearson <i>R</i> (Tif6 vs Sis1)	0.87	0.85	0.84	0.79	0.71
Pearson <i>R</i> (Tif6 vs Nsr1)	0.84	0.83	0.82	0.72	0.54
Pearson <i>R</i> (Sis1 vs Nsr1)	0.77	0.74	0.73	0.76	0.59
Manders M1 (Tif6 vs Sis1)	1.00	0.79	1.00	0.99	0.66
Manders M1 (Tif6 vs Nsr1)	0.85	0.85	0.78	0.86	0.49
Manders M1 (Sis1 vs Nsr1)	0.82	0.81	0.79	0.86	0.68
<i>Nucleolar proximity</i>					
Sis1 fraction proximal	0.41	0.44	0.23	0.96	0.71
Sis1 mean distance (px)	8.32	8.55	10.82	1.06	4.54
Tif6 fraction proximal	0.56	0.59	0.30	0.85	0.35
Tif6 mean distance (px)	6.28	6.91	10.31	1.69	9.94

**Table 3: cellquant parameters.** Complete parameter settings used for the U2OS mammalian and yeast temperature series analyses. Parameters not listed used default values.

Parameter	U2OS analysis	Yeast analysis
Cell type preset	mammalian	yeast
Cellpose model	cpsam	cpsam
Seg. downsample	3×	1× (none)
Cell diameter (px)	auto	40
Seg. channel	PABPC1	composite
Cell area filter	default	200–5,000 px
Cellprob threshold	0.0	−1.0
Puncta channels	G3BP1, PABPC1	Sis1, Tif6
Puncta compartment	cytosol	whole-cell
LoG sigma	2.0	1.5
Puncta min area (px)	6	3
Puncta max area (px)	10,000	300
Threshold method	Otsu	Otsu
Nuclear req.	1–4 nuclei	none
Nucleus dilation (px)	3	0
Colocalization	no	yes (whole-cell)
Nucleolar proximity	no	yes (Nsr1, 5 px)
Plot style	superplot	trend