Supplemental Information

Arsenite treatment induces Hsp90 aggregates distinct from conventional stress granules in fission yeast

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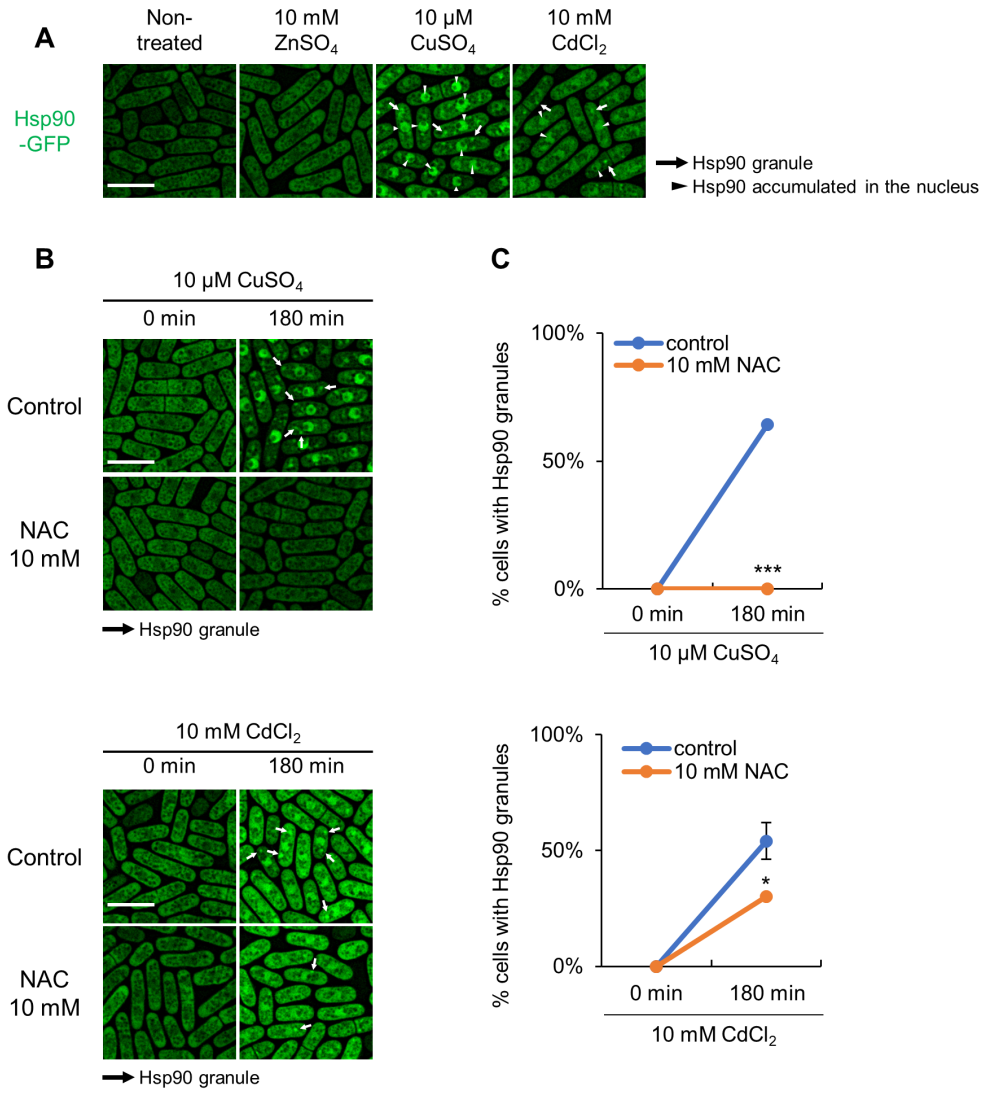

Figure S1: $CuSO_4$ and $CdCl_2$, but not $ZnSO_4$, induce granule formation and nuclear accumulation of Hsp90.

(A) Representative fluorescence images of the cells expressing Hsp90-GFP after treatment with 10 mM $ZnSO_4$, 10 µM $CuSO_4$, or 10 mM $CdCl_2$ for 180 min at 27°C. **(B)** Effect of NAC on granule formation and nuclear accumulation of Hsp90 induced by $CuSO_4$ or $CdCl_2$. **(C)** Percentage of the cells with Hsp90 granule after 180 min treatment with $CuSO_4$ or $CdCl_2$ in the presence or absence of NAC. The graphs show mean ± SE (n=3).

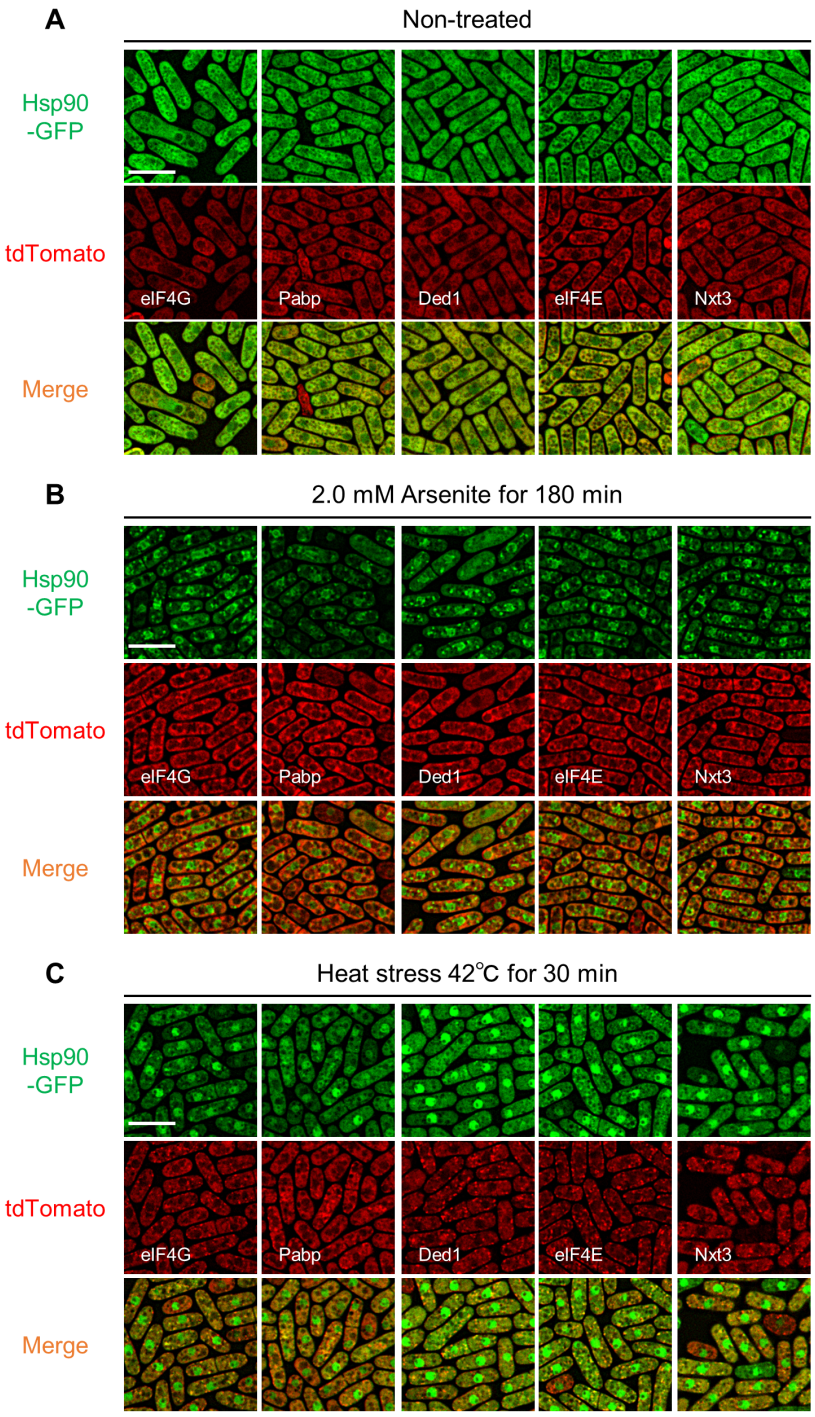

Figure S2: Wide-field fluorescence images corresponding to Figure 2.

Representative fluorescence images of the cells expressing Hsp90-GFP and SG markers tagged with tdTomato under non-stressed condition at 27℃ (A), after 2.0 mM arsenite treatment for 180 min at 27℃ (B), or 42℃ heat stress for 30 min. Scale bars: 10 µm.

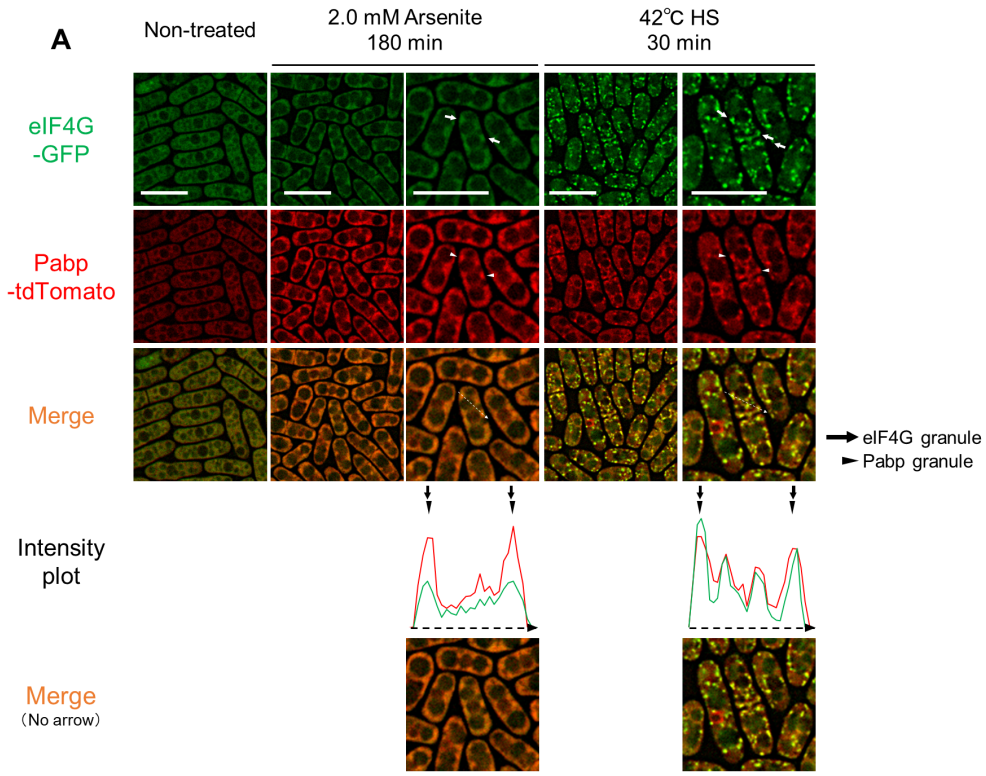

Figure S3: Co-localization analysis of eIF4G and Pabp

Representative images of the cells expressing eIF4G-GFP (green) and Pabp-tdTomato (red) from their endogenous promoter after treatment with the indicated conditions. Arrows and arrowheads indicate representative eIF4G and Pabp granules, respectively. The dashed arrows in the merged images correspond to those below the intensity plot. Scale bars: 10 μm.

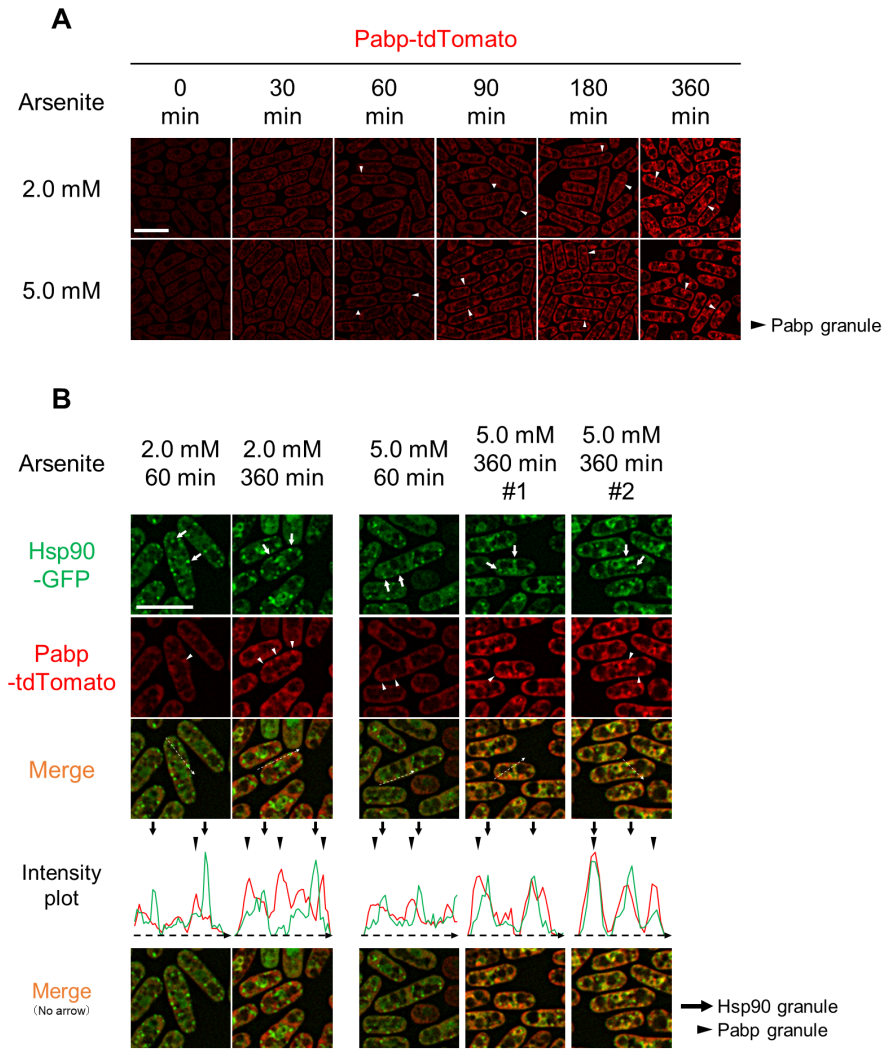

Figure S4: Co-localization analysis of Hsp90 and SGs under more intense arsenite stress conditions.

(A) Representative images of the cells expressing Pabp-tdTomato from its endogenous promoter after 2.0 mM or 5.0 mM arsenite treatment for the indicated times at 27°C. **(B)** Co-localization analysis of Hsp90-GFP (green) and Pabp-tdTomato (red) after 2.0 mM or 5.0 mM arsenite treatment for 60 min and 360 min at 27°C. #1 and #2 are two representative images with different characteristics from the same specimen. Arrows and arrowheads indicate representative Hsp90 granules and SGs, respectively. The dashed arrows in the merged images correspond to those below the intensity plot. Scale bars: 10 μm.

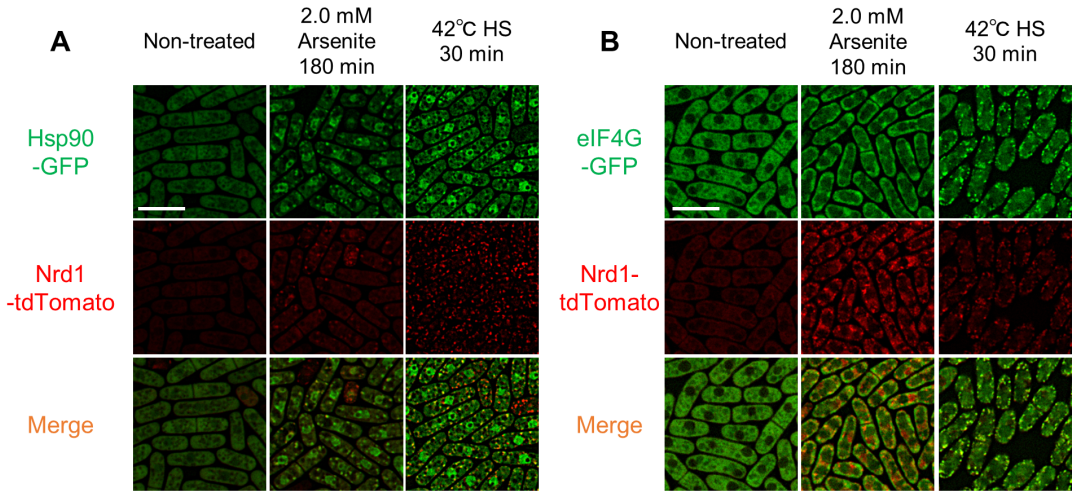

Figure S5: Wide-field fluorescence images corresponding to Figures 3A and 3B. Representative fluorescence images of the cells expressing Nrd1-tdTomato (red) and Hsp90-GFP (green) (A) or eIF4G-GFP (green) (B) treated with the indicated conditions. Scale bars: 10 µm.

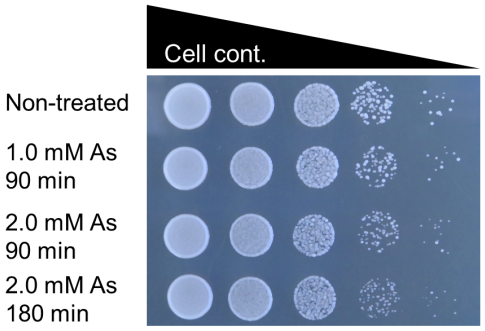

Figure S6: Cell viability after removal of arsenite stress

Cells grown to the mid-log phase were incubated with arsenite at the indicated conditions at 27˚C in the liquid EMM and then washed twice with arsenite-free fresh EMM and adjusted to OD_{660} = 0.6. The cells were then 10-fold serially diluted as indicated (10^{0} to 10^{-4}), and 10 µL were spotted onto EMM plates. Plates were incubated for 4 days at 27°C.

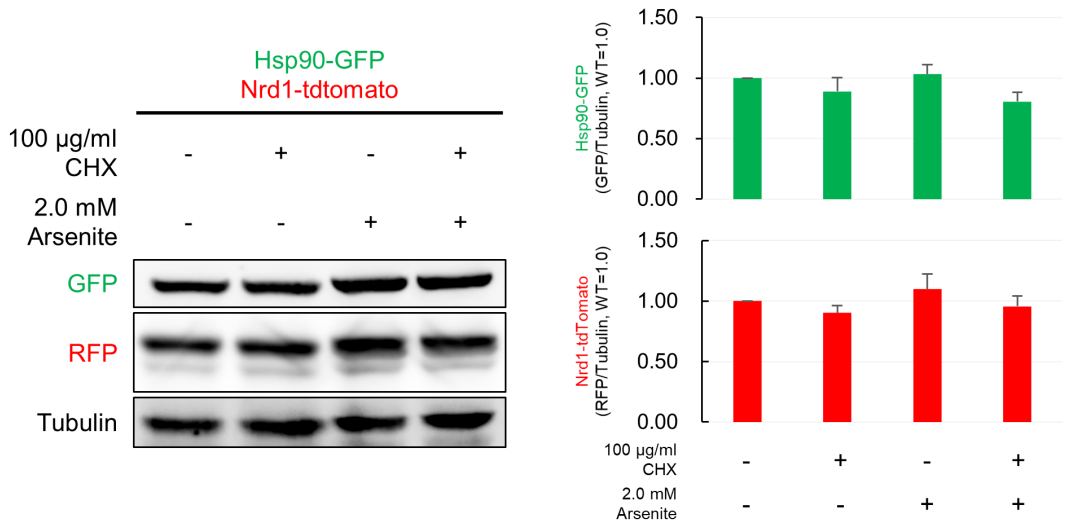

Figure S7: Protein levels of Hsp90-GFP and Nrd1-tdTomato after the CHX and arsenite treatment.

Cells expressing Hsp90-GFP and Nrd1-tdTomato from their endogenous promoters were grown to mid-log phase in EMM and then pre-treated with or without 100 µg/ml CHX for 10 min, and subsequently incubated with or without 2.0 mM arsenite for 120 min at 27°C. The levels of Hsp90 and Nrd1 in the cell lysates were analyzed by western blot using antibodies against GFP and RFP, respectively. Tubulin was detected as the loading control. The blots were quantified by densitometry analysis and the graphs show the mean ± SE (n=3).

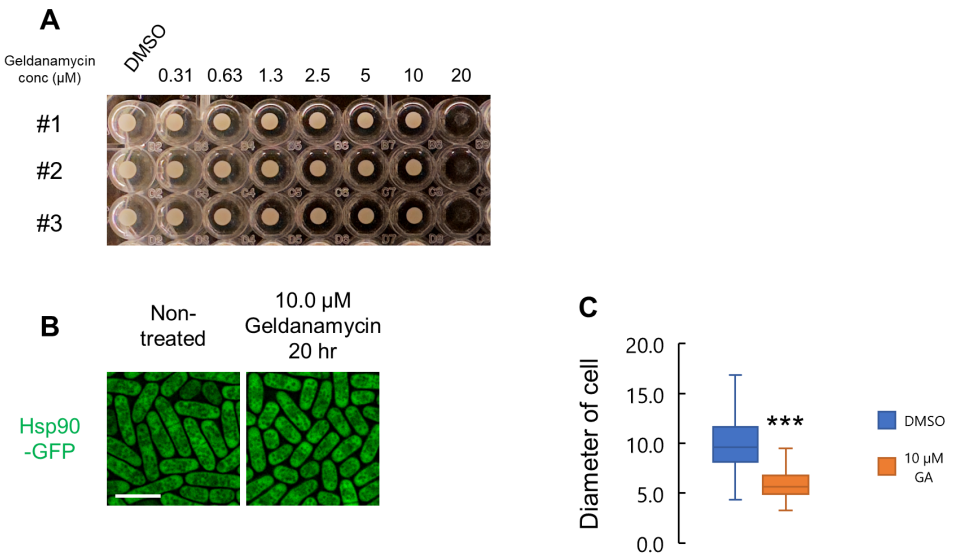

Figure S8: Effect of geldanamycin on cell growth

(A) The minimum inhibitory concentration (MIC) determination of geldanamycin. Cells grown to mid-log phase in the EMM at 27˚C were adjusted to OD_{660} = 0.5 and then diluted 300-fold with the fresh EMM. 200 µl aliquots of the cells were incubated in a 96-well plate with the indicated final concentrations of geldanamycin for 4 days at 27°C. #1-3 are technical triplicates. **(B)** Representative images of the cells expressing Hsp90-GFP treated with or without 10 µM geldanamycin for 20 h at 27°C. **(C)** The length of the cell in Figure S8B. The box signifies the 25-75th percentiles and the median is represented by a short line within the box. The whiskers show the range of data. GA; geldanamycin. P***<0.001; significantly different from each sample by paired Student's t-test. Scale bars: 10 µm.

TABLE S1. Strains and plasmids used in this study.

Strain	**Genotype**	**Reference**
HM123	*h⁻ leu1-32*	Lab stock
SP3000	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6*	[18]
SP3011	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6 pabp⁺-tdTomato::HphMX6*	[18]
SP3020	*h⁺ cut11⁺-mCherry::NatMX6*	[30]
SP3033	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6 cut11⁺-mCherry::NatMX6*	[18]
SP3415	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6 ded1⁺-tdTomato::HphMX6*	This study
SP3419	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6 nxt3⁺-tdTomato::HphMX6*	This study
SP3422	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6 nrd1⁺-tdtomato::HphMX6*	This study
SP3433	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6 eIF4G⁺-tdTomato::HphMX6*	This study
SP3434	*h⁻ leu1-32 hsp90⁺-GFP::KanMX6 eIF4E⁺-tdTomato::HphMX6*	This study
SP3470	*h- leu1-32 ura4-D18 eIF2α-S52A::ura4⁺ hsp90⁺-GFP::KanMX6 nrd1⁺-tdTomato::HphMX6*	This study
SP3486	*h- leu1-32 eIF4G⁺-GFP::KanMX6 pabp⁺-tdTomato::HphMX6*	This study
SP3487	*h- leu1-32 eIF4G⁺-GFP::KanMX6 nrd1⁺-tdTomato::HphMX6*	This study
Plasmid	**Description**	**Reference**
pKB1037	LEU2 marker multi-copy vector	Lab stock

18. Takasaki T, Tomimoto N, Ikehata T, Satoh R, Sugiura R (**2021**). Distinct spatiotemporal distribution of Hsp90 under high-heat and mild-heat stress conditions in fission yeast. **MicroPubl Biol** 2021. doi: 10.17912/micropub.biology.000388

30. Hayashi T, Teruya T, Chaleckis R, Morigasaki S, Yanagida M (**2018**). S-Adenosylmethionine Synthetase Is Required for Cell Growth, Maintenance of G0 Phase, and Termination of Quiescence in Fission Yeast. **iScience** 5: 38-51. doi: 10.1016/j.isci.2018.06.011

TABLE S2. Reagents used in this study.

Reagent	**Source**
$CdCl_2$	06613, Nakacai tesque, Japan
$CuSO_4$	09605, Nakacai tesque, Japan
Cycloheximide	06741, Nakacai tesque, Japan
Geldanamycin	#ant-gl, Invtrogen, U.S.A
N-Acetyl-L-Cysteine	00512, Nakacai tesque, Japan
Sodium arsenite	S7400, Sigma-Aldrich, St. Louis, MO, U.S.A
$ZnSO_4$	37011, Nakacai tesque, Japan