

Supplementary Information

**Designer Membraneless Organelles Sequester Native Factors for Control of Cell Behavior**

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**This PDF file includes:**

Supplementary Table 1

Supplementary Table 2

Supplementary Video Legends

Supplementary References

**Supplemental Files**

Supplementary Video 1 (.avi)

Supplementary Video 2 (.avi)

Supplementary Video 3 (.avi)

**Supplementary Table 1**

<b>Strain Number</b>	<b>Genotype</b>	<b>Source</b>
YEF473	Wildtype. Mata, <i>his3 leu2 lys2 trp1 ura3</i>	Gift from Erfei Bi
yMV96	Mata, <i>GAL1-SZ1-RGG-GFP::URA3, leu2 his3 lys2 trp1</i>	This study
yMV97	Mata, <i>GAL1-SZ1-RGG-GFP-RGG::URA3, leu2 his3 lys2 trp1</i>	This study
yMV98	Mata, <i>GAL1-SZ1-RGG-GFP-RGG-RGG::URA3, leu2 his3 lys2 trp1</i>	This study
yMV100	Mata, <i>GAL1-SZ1-RGG-GFP-RGG-RGG::URA3, MET17-mScarlet-SZ2::LEU2 his3 lys2 trp1</i>	This study
yMV101	Mata, <i>GAL1-TsCC(A)-RGG-GFP-RGG-RGG::URA3 his3 leu2 lys2 trp1</i>	This study
yMV102	Mata, <i>GAL1-TsCC(A)-RGG-GFP-RGG-RGG::URA3, MET17-mScarlet-TsCC(B)::LEU2 his3 lys2 trp1</i>	This study
yMV48	Mata, <i>CDC24-TsCC(B)::KANMX6 his3 leu2 lys2 trp1 ura3</i>	This study
yMV65	Mata, <i>CDC24-mScarlet-TsCC(B)::KANMX6 his3 leu2 lys2 trp1 ura3</i>	This study
yMV103	Mata, <i>GAL1-TsCC(A)-RGG-GFP-RGG-RGG::URA3, CDC24-TsCC(B)::KANMX6 his3 leu2 lys2 trp1</i>	This study
yMV105	Mata, <i>GAL1-RGG-GFP-RGG-RGG::URA3, CDC24-mScarlet-TsCC(B)::KANMX6 his3 leu2 lys2 trp1</i>	This study
yMV104	Mata, <i>GAL1-RGG-GFP-RGG-RGG::URA3, CDC24-mScarlet-TsCC(B)::KANMX6 his3 leu2 lys2 trp1</i>	This study
yMV121	Mata, <i>CDC5-mScarlet-TsCC(B)::KANMX6 his3 leu2 lys2 trp1 ura3</i>	This study
yMV122	Mata, <i>GAL1-TsCC(A)-RGG-GFP-RGG-RGG::URA3, CDC5-mScarlet-TsCC(B)::KANMX6 his3 leu2 lys2 trp1</i>	This study
yMV135	Mata, <i>CDC24-PhoCI-TsCC(B)::KANMX6 his3 leu2 lys2 trp1 ura3</i>	This study
yMV136	Mata, <i>GAL1-TsCC(A)-RGG-GFP-RGG-RGG::URA3, CDC24-PhoCI-TsCC(B)::KANMX6 his3 leu2 lys2 trp1</i>	This study

yMV140	Mata, <i>tor1-1 fpr1Δ::KANMX6, FRB-RGG-GFP-RGG-RGG::URA3, MET17-mScarlet-FKBP::LEU2 his3Δ1 met15Δ0</i>	This study
yMV142	Mata, <i>tor1-1 fpr1Δ::KANMX6, FRB-RGG-GFP-RGG-RGG::URA3, CDC24-mScarlet-FKBP::HIS3 leu2Δ0 met15Δ0</i>	This study
yMV147	Mata, <i>BNR1ΔDAD-mScarlet-TsCC(B)::HIS3 bni1Δ::TRP1 leu2 lys2 ura3</i>	This study
yMV149	Mata, <i>GAL1-TsCC(A)-RGG-GFP-RGG-RGG::URA3, BNR1ΔDAD-mScarlet-TsCC(B)::HIS3 bni1Δ::TRP1 leu2 lys2</i>	This study
yMV150	Mata, <i>GAL1-TsCCA-PhoCl2f-RGG-GFP-RGG-RGG::URA3, CDC24-mScarlet-TsCC(B)::KANMX6 his3 leu2 lys2 trp1</i>	

**Supplementary Table 2**

<b>Number</b>	<b>Plasmid</b>	<b>Source</b>
pMV5	Yiplac211- proGAL1--SZ1-RGG-GFP::URA3	This study
pMV6	Yiplac211- proGAL1--SZ1-RGG-GFP-RGG::URA3	This study
pMV7	Yiplac211- proGAL1-SZ1-RGG-GFP-RGG-RGG::URA3	This study
pMV8	Yiplac211-proGAL1-TsCC(A)-RGG-GFP-RGG-RGG::URA3	This study
pMV9	Yiplac128-proMET17-mScarlet-SZ2::LEU2	This study
pMV10	Yiplac128-proMET17-mScarlet-TsCC(B)::LEU2	This study
pMV32	pfa6a-mScarlet-TsCC(B)::KANMX6	This study
pMV33	pfa6a-mScarlet-TsCC(B)::HIS3	This study
pMV34	Yiplac211-proGAL1-FRB-RGG-GFP-RGG-RGG::URA3	This study
pMV35	Yiplac128-proMET17-mScarlet-FKBP::HIS3	This study
pMV36	pfa6a-mScarlet-FKBP::HIS3	This study
pMV41	pfa6a-PhoCI-TsCC(B)::KANMX6	This study
pJH2972	pCas9::URA3	Anand et al., 2017 <sup>2</sup>
pMV68	pCas9 Bnr1-DAD gRNA::URA3	This study
pMV69	pUC19 RAC1 5'-mCherry-TsCC(B)-Neo <sup>R</sup> - RAC1 3'	This study
pMV70	pUC19 ERK1 5'-mCherry-TsCC(B)-Neo <sup>R</sup> - ERK1 3'	This study

pMV71	pCas9-Guide RAC1 gRNA	This study
pMV72	pCas9-Guide ERK1 gRNA	This study
pMV73	pUC19 PAR6 5'-mCherry-TsCC(B)-Neo <sup>R</sup> - ERK1 3'	This study
pMV74	pCas9-Guide PAR6 gRNA	This study
pMV75	Yiplac211-proGAL1-TsCC(A)-PhoCI2f-RGG-GFP-RGG-RGG::URA3	This study

**Supplementary Video 1.**

Rapamycin-induced client recruitment to preformed condensates in cells. Green: FRB-(RGG)<sub>3</sub> GFP-tagged scaffold. Red: exogenous client, mScarlet-TsCC(B).

**Supplementary Video 2.**

Inducible expression of scaffold disrupts native cortical localization of Cdc24, sequestering it to newly formed synthetic condensates. Green: TsCC(A)-(RGG)<sub>3</sub> GFP-tagged scaffold. Red: native client, Cdc24-mScarlet-TsCC(B).

**Supplementary Video 3.**

Induced condensate sequestration of endogenous client, Cdc24, upon rapamycin addition. Green: FRB-(RGG)<sub>3</sub> GFP-tagged scaffold. Red: native client, Cdc24-mScarlet-FKBP.

## Supplementary References

1. Schuster, B.S. *et al.* Controllable protein phase separation and modular recruitment to form responsive membraneless organelles. *Nat Commun* **9**, 2985 (2018).
2. Anand, R., Memisoglu, G. & Haber, J. Cas9-mediated gene editing in *Saccharomyces cerevisiae*. *Protocol Exchange* (2017).