

Supplementary information

Seipin forms a flexible cage at lipid droplet formation sites

In the format provided by the authors and unedited

Supplementary Information

Seipin forms a flexible cage at lipid droplet formation sites

Henning Arlt^{1,2,3}, Xuewu Sui^{1,2}, Brayden Folger⁴, Carson Adams⁵, Xiao Chen⁴, Roman Remme⁶, Fred A. Hamprecht⁶, Frank DiMaio⁵, Maofu Liao², Joel M. Goodman^{4,*}, Robert V. Farese, Jr.^{1,2,7,*} & Tobias C. Walther^{1,2,3,7,*}

¹Department of Molecular Metabolism, Harvard T. H. Chan School of Public Health, Boston, MA, 02115 USA

²Department of Cell Biology, Harvard Medical School, Boston, MA, 02115 USA

³Howard Hughes Medical Institute, Boston, MA, 02115 USA

⁴Department of Pharmacology, University of Texas Southwestern Medical School, Dallas, TX 75390-9041, USA

⁵Department of Biochemistry and Institute of Protein Design, University of Washington, Seattle, WA, 98195 USA

⁶Heidelberg Collaborative for Image Processing, Interdisciplinary Center for Scientific Computing, Heidelberg University, 69120 Heidelberg, Germany

⁷Broad Institute of Harvard and MIT, Cambridge, MA, 02124 USA

* These authors contributed equally

Correspondence should be addressed to Joel M. Goodman (Joel.Goodman@UTSouthwestern.edu), Robert Farese, Jr. (robert@hsph.harvard.edu) and Tobias C. Walther (twalther@hsph.harvard.edu)

Table S1. Plasmids used in this study

| Name | Description | Source |
|---------------------------|--|------------|
| pSK ⁻ -NAT-PGK | | this study |
| pBMF1 | <i>SEI1pr-SEI1-13xmyc::HIS3</i> | this study |
| pHA144 | pET28a <i>SEI1(47-235) R178A-6xHis</i> | this study |
| pHA147 | pET28a <i>SEI1(47-235)-6xHis</i> | this study |
| pHA234 | pRS416 <i>ADHpr-GFP</i> | this study |
| pHA236 | pRS416 <i>ADHpr-SEI1 (S33I Y37A Y41A)-GFP (Patch1)</i> | this study |
| pHA238 | pRS416 <i>ADHpr-SEI1 (S33I Y37A Y41A M240G Y248I F255R I259K)-GFP (Patch1+2)</i> | this study |
| pHA240 | pRS416 <i>ADHpr-SEI1(M240G Y248I F255R I259K)-EGFP (Patch2)</i> | this study |
| pHA246 | pRS416 <i>ADHpr-SEI1-GFP</i> | this study |
| pHA247 | pRS416 <i>ADHpr-SEI1 (R178A)-GFP</i> | this study |
| pHA249 | pRS416 <i>ADHpr-SEI1(Q114A)-EGFP</i> | this study |
| pHA250 | pRS416 <i>ADHpr-SEI1(E172A)-EGFP</i> | this study |
| pHA251 | pRS416 <i>ADHpr-SEI1(E172A R178A)-EGFP</i> | this study |
| pHA252 | pRS416 <i>ADHpr-SEI1(Q114A E172A R178A)-EGFP</i> | this study |
| pHA253 | pRS416 <i>ADHpr-SEI1(Q114A E172A)-EGFP</i> | this study |
| pHA254 | pRS416 <i>ADHpr-SEI1(Q114A R178A)-EGFP</i> | this study |
| pHA279 | pRS416 <i>ADH1pr-dm seipin-EGFP</i> | this study |
| pHA406 | pRS416 <i>ADHpr-SEI1 Δ169-173 -GFP</i> | this study |
| pHA407 | pRS416 <i>ADHpr-SEI (169,172,173 to A) -GFP</i> | this study |
| pHA410 | pRS416 <i>ADHpr-human seipin isoform 2 (1-300 aa) -GFP</i> | this study |
| pHA412 | pRS416 <i>ADHpr-dm lumenal- yeast TM-GFP</i> | this study |
| pHA413 | pRS416 <i>ADHpr-human seipin-yeast TM-GFP</i> | this study |
| pHA418 | pRS416 <i>ADHpr-yeast lumenal-human TM-GFP</i> | this study |
| pHA432 | pRS416 <i>ADHpr-SEI1 (167-185 7xA)-GFP</i> | this study |
| pHA433 | pRS416 <i>ADHpr-SEI1 (Δ167-174)-GFP</i> | this study |
| pHA444 | pRS416 <i>ADHpr-yeast lumenal-dm TM-GFP</i> | this study |
| pHA445 | pRS416 <i>ADHpr-SEI1 (shuffled-switch)-GFP</i> | this study |
| pHA446 | pRS416 <i>ADHpr-SEI1 (Δ-switch)-GFP</i> | this study |
| pHA523 | pRS416 <i>ADHpr-SEI1 (C260L S266L T269I)-GFP</i> | this study |
| pHA525 | pRS416 <i>ADHpr-SEI1 shuffled TMD N-GFP</i> | this study |
| pHA526 | pRS416 <i>ADHpr-SEI1 shuffled TMD C-GFP</i> | this study |
| pHA527 | pRS416 <i>ADHpr-SEI1 shuffled TMD N+C-GFP</i> | this study |
| pHA528 | pRS416 <i>ADHpr-SEI1 TMD N FIT2-GFP</i> | this study |
| pHA529 | pRS416 <i>ADHpr-SEI1 TMD C FIT2-GFP</i> | this study |
| pHA530 | pRS416 <i>ADHpr-SEI1 TMD N+C FIT2-GFP</i> | this study |
| pTW185 | ssHDEL-MARS:: <i>LEU2</i> | |

Italic font indicates gene or promoter information.

Table S2. Yeast *S. cerevisiae* strains used in this study

| Name | Genotype | Source |
|----------------------------|---|------------|
| BY4741 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i> | Euroscarf |
| HAY60 | BY4741; <i>kanMX::GALpr-FLD1-3xFLAG-TEV-ProtA::hygR</i> <i>natNT2::GAL1pr-LDB16</i> | this study |
| TWY2143 | BY4741; <i>sei1::kanMX</i> | Euroscarf |
| W303-1A | <i>MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15</i> | 35 |
| W303-1A <i>sei1Δ</i> | W303-1A; <i>sei1::hygR</i> | 47 |
| W303-1A <i>sei1Δ pln1Δ</i> | W303-1A; <i>sei1::hygR pln1::HIS3MX6</i> | 47 |
| W303-1A <i>pln1Δ</i> | W303-1A; <i>pln1::hygR</i> | 47 |
| <i>SEI1-myc</i> | W303-1A <i>pln1Δ</i> ; <i>SEI1::13xmyc-HIS3</i> | this study |
| <i>ldb16Δ SEI1-myc</i> | W303-1A <i>pln1Δ</i> ; <i>ldb16:kanMX:natNT1::PGK1pr- SEI1::13xmyc-HIS3</i> | this study |
| R178A | W303-1A <i>pln1Δ</i> ; <i>SEI1 (R178A)::13xmyc-HIS3</i> | this study |
| Q114A | W303-1A <i>pln1Δ</i> ; <i>SEI1 (Q114A)::13xmyc-HIS3</i> | this study |
| E172A | W303-1A <i>pln1Δ</i> ; <i>SEI1 (E172A)::13xmyc-HIS3</i> | this study |
| Q114A R178A | W303-1A <i>pln1Δ</i> ; <i>SEI1 (Q114A R178A)::13xmyc-HIS3</i> | this study |
| E172A R178A | W303-1A <i>pln1Δ</i> ; <i>SEI1 (E172A R178A)::13xmyc-HIS3</i> | this study |
| Q114A E172A | W303-1A <i>pln1Δ</i> ; <i>SEI1 (Q114A E172A)::13xmyc-HIS3</i> | this study |
| Q114A E172A R178A | W303-1A <i>pln1Δ</i> ; <i>SEI1 (Q114A E172A R178A)::13xmyc-HIS3</i> | this study |
| PGK1pr-R178A | W303-1A <i>pln1Δ</i> ; <i>natNT1::PGK1pr-SEI1 (R178A)::13xmyc-HIS3</i> | this study |
| R178A PGK1pr-LDB16 | W303-1A <i>pln1Δ</i> ; <i>natNT1::PGK1pr-SEI1 (R178A)::13xmyc-HIS3</i> | this study |
| Patch1 | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (Patch1)-13xmyc::HIS3</i> | this study |
| Patch1 R178A | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (Patch1 R178A)-13xmyc::HIS3</i> | this study |
| Patch2 | W303-1A <i>pln1Δ</i> ; <i>hygR PGK1pr-SEI1 (Patch2)-13xmyc::HIS3</i> | this study |
| Patch2 R178A | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (Patch2 R178A)-13xmyc::HIS3</i> | this study |
| Patch1+2 | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (Patch1+2)-13xmyc::HIS3</i> | this study |
| Patch1+2 R178A | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (Patch1+2 R178A)-13xmyc::HIS3</i> | this study |
| Sei1 (TM-N-FIT2) | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (TMD-N)-13xmyc::HIS3</i> | this study |
| Sei1 (TM-C-FIT2) | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (TMD-C)-13xmyc::HIS3</i> | this study |
| Sei1 (TM-NC-FIT2) | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (TMD-N+C)-13xmyc::HIS3</i> | this study |
| Sei1 (shuffled TM-N) | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (shTMD-N)-13xmyc::HIS3</i> | this study |
| Sei1 (shuffled TM-C) | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (shTMD-C)-13xmyc::HIS3</i> | this study |
| Sei1 (shuffled TM-NC) | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (shTMD-N+C)-13xmyc::HIS3</i> | this study |
| C260L S266L T269I | W303-1A <i>pln1Δ</i> ; <i>PGK1pr-SEI1 (C260L S266L T269I)-13xmyc::HIS3</i> | this study |
| Δ-switch | W303-1A <i>pln1Δ</i> ; <i>hygR natNT1::PGK1pr-SEI1 (Δ-switch)-</i> | this study |
| shuffled-switch | W303-1A <i>pln1Δ</i> ; <i>natNT1::PGK1pr-SEI1 (shuffled-switch)-13xmyc</i> <i>::HIS3</i> | this study |
| 169,172,173 to A | W303-1A <i>pln1Δ</i> ; <i>natNT1::PGK1pr-SEI1 (3xA)::13xmyc-HIS3</i> | this study |
| 167-185 7xA | W303-1A <i>pln1Δ</i> ; <i>natNT1::PGK1pr-SEI1 (7xA)::13xmyc-HIS3</i> | this study |
| Δ169-173 | W303-1A <i>pln1Δ</i> ; <i>natNT1::PGK1pr-SEI1 (Δ169-173)::13xmyc-HIS3</i> | this study |
| Δ167-174 | W303-1A <i>pln1Δ</i> ; <i>natNT1::PGK1pr-SEI1 (Δ167-174)::13xmyc-</i> <i>HIS3</i> | this study |

Italic font indicates gene or promoter information.