

**Supplementary Table 1: Strains, materials and reagents**

	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Anti-FLAG-Peroxidase	Sigma-Aldrich	Cat# H7425
Anti-Protein A	Sigma-Aldrich	Cat# P3775
Anti-HA	Abcam	Cat# ab18181
RPL5 Antibody	Cell Signaling Technology	Cat# 14568
<b>Chaetomium thermophilum strains</b>		
		ID in Hurt Lab spores collection
<i>C. thermophilum</i> : Erg1::P <sub>ACTIN</sub> -pA-TEV-FLAG- <i>ctRPF2</i> (CTHT_0061110)	This study	<i>ctRpf2</i> -FtpA
<i>C. thermophilum</i> : Erg1::P <sub>ACTIN</sub> -pA-TEV-FLAG- <i>ctRRS1</i> (CTHT_0057260)	This study	<i>ctRrs1</i> -FtpA
<i>C. thermophilum</i> : Erg1::P <sub>ACTIN</sub> -pA-TEV-FLAG- <i>ctSYO1</i> (CTHT_0033460)	This study	<i>ctSyo1</i> -FtpA
<b>Yeast strains</b>		
		ID in Hurt Lab yeast collection
<b>w303</b> wild type ( <i>S. cerevisiae</i> )	1	Y-3609
<b><i>syo1Δ</i></b> : w303: SYO1::natNT2	2	Y-3833
<b><i>syo1Δ</i>, <i>rpl5Δ</i> shuffle strain</b> : w303: SYO1::natNT2; RPL5::His3MX6, pRS316-scUL18	2	Y-4575
<b><i>rpf2Δ</i> shuffle strain</b> : w303: RPF2::natNT2; pRS316-RPF2	This study	Y-5697
<b><i>rrs1Δ</i> shuffle strain</b> : w303: RRS1::natNT2, pRS316-RRS1	This study	Y-5693
<b><i>syo1Δ</i>, <i>rpf2Δ</i> shuffle strain</b> : w303: SYO1::natNT2, RPF2::His3MX6, pRS316-RPF2	This study	Y-6381
<b>Nsa1-FtpA</b> : w303: NSA1-FtpA:natNT2	This study	Y-6382
<b>Nsa3-FtpA</b> : w303: NSA3-FtpA:natNT2	This study	Y-6383
<b>Yvh1-FtpA</b> : w303: YVH1-FtpA:klURA3	This study	Y-5560
<b>Nsa1-FtpA, GAL::HA-Rpf2</b> : w303: NSA1-FtpA:natNT2, His3MX6:P <sub>GAL</sub> -HA-RPF2	This study	Y-6384
<b>Nsa1-FtpA, GAL::HA-Rrs1</b> : w303: NSA1-FtpA:natNT2, His3MX6:P <sub>GAL</sub> -HA-RRS1	This study	Y-6385

<b>Nsa1-FtpA, GAL::HA-uL18:</b> w303: NSA1-FtpA:natNT2, His3MX6:P <sub>GAL</sub> -HA-UL18	This study	Y-6386
<b>Nsa3-FtpA, GAL::HA-Rpf2:</b> w303: NSA3-FtpA:natNT2, His3MX6:P <sub>GAL</sub> -HA-RPF2	This study	Y-6387
<b>Nsa3-FtpA, GAL::HA-Rrs1:</b> w303: NSA3-FtpA:natNT2, His3MX6:P <sub>GAL</sub> -HA-RRS1	This study	Y-6388
<b>Nsa3-FtpA, GAL::HA-uL18:</b> w303: NSA3-FtpA:natNT2, His3MX6:P <sub>GAL</sub> -HA-UL18	This study	Y-6389
<b>GAL::HA-Rpf2, GAL:HA-Rrs1:</b> w303: His3MX6:P <sub>GAL</sub> -HA-RPF2, His3MX6:P <sub>GAL</sub> -HA-RRS1	This study	Y-6390
<b>PJ69-4:</b> Strain for yeast two-hybrid assays	<sup>3</sup>	Y-4969
<b>Plasmids</b>		ID in Hurt Lab plasmid collection
Expression plasmid as negative control <b>YEplac112</b> <i>2μ replication origin, TRP1 marker</i>	4	#550
Expression plasmid as negative control <b>YEplac181</b> <i>2μ replication origin, LEU2 marker</i>	4	#552
Expression plasmid as negative control <b>YEplac195</b> <i>2μ replication origin, URA3 marker</i>	4	#551
Expression plasmid as negative control <b>YCplac22</b> <i>CEN4 (centromer vector), TRP1 marker</i>	4	#547
Expression plasmid as negative control <b>YCplac111</b> <i>CEN4 (centromer vector), LEU2 marker</i>	4	#4549
Co-expression of <b>ctRpf2</b> and <b>FLAG-ctRrs1:</b> pYEplac112-ctRPF2-P <sub>GAL1/10</sub> -FLAG-ctRRS1	This study	#6884
Expression of <b>pA-TEV-ctSyo1:</b> pYEplac181-P <sub>GAL1/10</sub> -ProteinA-TEV-ctSYO1	This study	#6885
Co-expression of <b>scRpf2</b> and <b>FLAG-scRrs1:</b> pYEplac112-scRpf2-P <sub>GAL1/10</sub> -FLAG-scRRs1	This study	#6886
Expression of <b>pA-TEV-scSyo1:</b> pYEplac181-P <sub>GAL1/10</sub> -ProteinA-TEV-scSyo1	This study	#6887
Plasmid for co-expression of <b>scuL18</b> and <b>scuL5:</b> pYEplac195-scUL18-P <sub>GAL1/10</sub> -scUL5	This study	#6888
Plasmid for co-expression of <b>scuL18</b> and <b>pA-TEV-scuL5:</b> pYEplac195-scUL18-P <sub>GAL1/10</sub> -ProteinA-TEV-scUL5	This study	#6889
Plasmid for expression of <b>scSyo1:</b> pYEplac181-P <sub>GAL1/10</sub> -scSYO1	This study	#6890
Plasmid for co-expression of <b>scRpf2ΔC</b> and <b>FLAG-scRrs1ΔC:</b> pYEplac112-scRPF2(1-252aa)-P <sub>GAL1/10</sub> -FLAG-scRRS1(1-108 aa)	This study	#6891
Plasmid for co-expression of <b>scuL18-HA</b> and <b>scuL5-3xGFP:</b> pYEplac195-scUL18-HA-P <sub>GAL1/10</sub> -scUL5-(3x)yeGFP	This study	#6892
Plasmid for expression of <b>pA-TEV-Syo1-HA:</b> pYEplac181-P <sub>GAL1/10</sub> -ProteinA-TEV-scSYO1-HA	This study	#6893
Plasmid for co-expression of <b>scRpf2-HA</b> and <b>FLAG-HA-scRrs1:</b> pYEplac112-scRPF2-HA-P <sub>GAL1/10</sub> -FLAG-HA-scRRS1	This study	#6894
Plasmid for co-expression of <b>scuL5-HA</b> and <b>scuL18-HA:</b> pYCplac195-scUL5-HA-P <sub>GAL1/10</sub> -scUL18-HA	This study	#6895

Plasmid for co-expression of <b>hsRrs1</b> and <b>FLAG-hsRpf2</b> : pYCplac112- <i>hsRRS1</i> -P <sub>GAL1/10</sub> -FLAG- <i>hsRPF2</i>	This study	#6896
Plasmid for co-expression of <b>hsuL18</b> and <b>pA-TEV-hsuL5</b> : pYCplac181- <i>hsUL18</i> -P <sub>GAL1/10</sub> -ProteinA-TEV- <i>hsUL5</i>	This study	#6897
Plasmid for expression of <b>hsSyo1</b> : pYCplac195-PGAL1/10-HEATR3	This study	P #6898
Plasmid for expression of <b>scRpf2-FtpA</b> : pYCplac111-P <sub>RPF2</sub> - <i>scRPF2</i> -FLAG-TEV-ProteinA	This study	#5785
Plasmid for expression of <b>hsRpf2-FtpA</b> : pYCplac111-P <sub>RPF2</sub> - <i>hsRPF2</i> -FLAG-TEV-ProteinA	This study	#6899
Plasmid for expression of <b>scRpf2-FtpA</b> ( <i>in vivo</i> binding assay): pYCplac111-P <sub>RPF2</sub> - <i>scRPF2</i> -FLAG-TEV-ProteinA	This study	#5785
Plasmid for expression of <b>scRpf2ΔC-FtpA</b> ( <i>in vivo</i> binding assay): pYCplac111-P <sub>RPF2</sub> - <i>scRPF2</i> (1-252 aa)-FLAG-TEV-ProteinA	This study	#5805
Plasmid for expression of <b>scRrs1</b> ( <i>in vivo</i> binding assay): pYCplac22-P <sub>RRS1</sub> - <i>scRRS1</i>	This study	#6900
Plasmid for expression of <b>scRrs1ΔC</b> ( <i>in vivo</i> binding assay): pYCplac22-P <sub>RRS1</sub> - <i>scRRS1</i> (1-108 aa)	This study	#6901
Plasmid for expression of <b>scRpf2</b> (complementation assays): pYCplac111-P <sub>RPF2</sub> - <i>scRPF2</i>	This study	#6902
Plasmid for expression of <b>scRpf2ΔC</b> (complementation assays): pYCplac111-P <sub>RPF2</sub> - <i>scRPF2</i> (1-252 aa)	This study	#6903
Plasmid for expression of <b>scRrs1</b> (complementation assays): pYCplac111-P <sub>RRS1</sub> - <i>scRRS1</i>	This study	#5749
Plasmid for expression of <b>scRrs1ΔC</b> (complementation assays): pYCplac111-P <sub>RRS1</sub> - <i>scRRS1</i> (1-108 aa)	This study	#6904
Plasmid for expression of <b>hsRpf2</b> (complementation assays): pYCplac111-P <sub>RPF2</sub> - <i>hsRPF2</i>	This study	#6905
Plasmid for expression of <b>ctRpf2</b> (complementation assays): pYCplac111-P <sub>RPF2</sub> - <i>ctRPF2</i>	This study	#6906
Plasmid for expression of <b>hsRrs1</b> (complementation assays): pYCplac111-P <sub>RRS1</sub> - <i>hsRRS1</i>	This study	#6907
Plasmid for expression of <b>ctRrs1</b> (complementation assays): pYCplac111-P <sub>RRS1</sub> - <i>ctRRS1</i>	This study	#6908
Plasmid for expression of <b>scRpf2</b> (over-expression assays): pYEplac112-P <sub>GAL1/10</sub> - <i>scRPF2</i>	This study	#6909
Plasmid for expression of <b>scRpf2ΔC</b> (over-expression assays): pYEplac112-P <sub>GAL1/10</sub> - <i>scRPF2</i> (1-252 aa)	This study	#6910
Plasmid for expression of <b>scRrs1</b> (over-expression assays): pYEplac112-P <sub>GAL1/10</sub> - <i>scRRS1</i>	This study	#5815
Plasmid for expression of <b>scRrs1ΔC</b> (over-expression assays): pYEplac112-P <sub>GAL1/10</sub> - <i>scRRS1</i> (1-108 aa)	This study	#5816
Plasmid for <b>25S rRNA(H81-H87)</b> <i>in vitro</i> synthesis (EMSA assays): pBlueScript-P <sub>T7</sub> -25SrRNA(H81-H87)	This study	#6911
Plasmid for <b>Phe-tRNA</b> <i>in vitro</i> synthesis (EMSA assays): pBlueScript-P <sub>T7</sub> -tRNA <sup>PHE</sup>	This study	#512
Plasmid for expression of <b>scuL18</b> (yeast growth analysis): pYCplac111-P <sub>UL18</sub> - <i>scUL18</i>	This study	#6912
Plasmid for expression of <b>scuL18 169G&gt;S</b> (yeast growth analysis): pYCplac111-P <sub>UL18</sub> - <i>scUL18</i> (169 G>S)	This study	#6913

Plasmid for expression of <b>scSyo1</b> (yeast growth analysis): pYCplac22-P <sub>SYO1</sub> -scSYO1	This study	#6914
Plasmid for expression of <b>scSyo1-A</b> (yeast growth analysis): pYCplac22-P <sub>SYO1</sub> -scSYO1 (118 K>E)	This study	#6915
Plasmid for expression of <b>scSyo1-B</b> (yeast growth analysis): pYCplac22-P <sub>SYO1</sub> -scSYO1 (74K>E, 76K>E)	This study	#6916
Plasmid for expression of <b>scSyo1-AB</b> (yeast growth analysis): pYCplac22-P <sub>SYO1</sub> -scSYO1 (74K>E, 76K>E, 118 K>E)	This study	#6917
Plasmid for expression of <b>FLAG-scuL18</b> : pYEplac112-P <sub>GAL1/10</sub> -FLAG-scuL18	This study	#6918
Plasmid for expression of <b>FLAG-scuL18 169G&gt;S</b> : pYEplac112-P <sub>GAL1/10</sub> -FLAG-scuL18 (169G>S)	This study	#6919
Plasmid for expression of <b>pA-TEV-scSyo1-AB</b> : pYEplac181-P <sub>GAL1/10</sub> -ProteinA-TEV-scSYO1 (74K>E, 76K>E, 118 K>E)	This study	#6920
Plasmid for expression of <b>hsSyo1-FLAG</b> : pYEplac181-P <sub>GAL1/10</sub> -MDM2-FLAG	This study	#6921
Plasmid for co-expression of <b>hsuL5</b> and <b>hsuL18-TEV-pA</b> : pYEplac112-hsUL5-P <sub>GAL1/10</sub> -hsUL18-TEV-ProteinA	This study	#6922
Plasmid for expression of <b>hsuL18-TEV-pA (1-20 aa)</b> : pYEplac112-P <sub>GAL1/10</sub> -hsUL18(1-20 aa)-TEV-ProteinA	This study	#6936
Plasmid for expression of <b>hsuL18-TEV-pA (1-30 aa)</b> : pYEplac112-P <sub>GAL1/10</sub> -hsUL18(1-30 aa)-TEV-ProteinA	This study	#6923
Plasmid for expression of <b>hsuL18-TEV-pA (1-41 aa)</b> : pYEplac112-P <sub>GAL1/10</sub> -hsUL18(1-41 aa)-TEV-ProteinA	This study	#6924
Plasmid for expression of <b>hsuL18-TEV-pA (21-297 aa)</b> : pYEplac112-P <sub>GAL1/10</sub> -hsUL18(21-297 aa)-TEV-ProteinA	This study	#6925
Plasmid for expression of <b>hsuL18-TEV-pA (31-297 aa)</b> : pYEplac112-P <sub>GAL1/10</sub> -hsUL18(31-297 aa)-TEV-ProteinA	This study	#6926
Plasmid for expression of <b>hsuL18-TEV-pA (42-297 aa)</b> : pYEplac112-P <sub>GAL1/10</sub> -hsUL18(42-297 aa)-TEV-ProteinA	This study	#6927
Yeast two-hybrid control plasmid <b>pGADT7-SV40</b> : LEU2 marker, T7 promoter, SV40-AD (Activation Domain)	<sup>5</sup>	#3724
Plasmid for expression of <b>hsuL18</b> (Y2H assay): pGADT7-hsUL18-AD	This study	#6928
Plasmid for expression of <b>hsuL18 (1-20 aa)</b> (Y2H assay): pGADT7-hsUL18(1-20 aa)-AD	This study	#6929
Plasmid for expression of <b>hsuL18 (1-30 aa)</b> (Y2H assay): pGADT7-hsUL18(1-30 aa)-AD	This study	#6930
Plasmid for expression of <b>hsuL18 (1-41 aa)</b> (Y2H assay): pGADT7-hsUL18(1-41 aa)-AD	This study	#6931
Plasmid for expression of <b>hsuL18 (21-297 aa)</b> (Y2H assay): pGADT7-hsUL18(21-297 aa)-AD	This study	#6932
Plasmid for expression of <b>hsuL18 (31-297 aa)</b> (Y2H assay): pGADT7-hsUL18(31-297 aa)-AD	This study	#6933
Plasmid for expression of <b>hsuL18 (22-297 aa)</b> (Y2H assay): pGADT7-hsUL18(42-297 aa)-AD	This study	#6934
Yeast two-hybrid control plasmid <b>pGBKT7-p53</b> : TRP1 marker, T7 promoter, p53-BD (Binding Domain)	<sup>6</sup>	#3467

Plasmid for expression of <b>MDM2</b> (Y2H assay): pGBKT7-MDM2-BD	This study	#6935
<b>Reagents and chemicals</b>		
3X FLAG® Peptide lyophilized powder	Sigma-Aldrich	Cat# F3290
TEV protease	Sigma-Aldrich	Cat# T4455
SIGMAFAST™ Protease inhibitor tablets for general use	Sigma-Aldrich	Cat# S8820
[alpha-P32] UTP, 3000 Ci/mmol, 10 mCi/ml	Hartman Analytic	Cat# SRP-210
IGEPAL® CA-630	Sigma-Aldrich	Cat# I8896
DSS-H12/D12	Creative Molecules	Cat# 001S
Coomassie Brilliant Blue R-250	Thermo Fisher Scientific	Cat# 20278
SYBR®Green II RNA gel stain	Sigma-Aldrich	Cat# S9305
ANTI-FLAG® M2 Affinity Gel	Sigma-Aldrich	Cat# A2220
IgG Sepharose® 6 Fast Flow	Sigma-Aldrich	Cat# GE17-0969-01
HiScribe-T7 High Yield RNA Synthesis Kit	NEB	Cat# E2040S
NuPAGE™ 4 to 12%, Bis-Tris	Thermo Fisher Scientific	Cat# NP0322PK2, Cat# WG1403A
<b>Oligonucleotides</b>		
<i>S. cerevisiae</i> 5S rRNA probe: 5'-CTACTCGGTCAGGCTC-3'	This study	N/A
<i>C. thermophilum</i> 5S rRNA probe: 5'-TCAGTGGCTTGTCTATGG-3'	This study	N/A
<b>Software and algorithms</b>		
MaxQuant software v1.6.17.0. <a href="https://www.maxquant.org">https://www.maxquant.org</a>	7	N/A
xQuest <a href="http://proteomics.ethz.ch">http://proteomics.ethz.ch</a>	8	N/A
xProphet <a href="http://proteomics.ethz.ch">http://proteomics.ethz.ch</a>	9	N/A

xiNet <a href="http://crosslinkviewer.org/">http://crosslinkviewer.org/</a>	10	N/A
Xlink Analyzer <a href="https://www.embl-hamburg.de/XlinkAnalyzer/XlinkAnalyzer.html">https://www.embl-hamburg.de/XlinkAnalyzer/XlinkAnalyzer.html</a>	11	N/A
UCSF Chimera <a href="https://www.cgl.ucsf.edu/chimera/">https://www.cgl.ucsf.edu/chimera/</a>	12	N/A
UCSF ChimeraX <a href="https://www.cgl.ucsf.edu/chimerax/">https://www.cgl.ucsf.edu/chimerax/</a>	13	N/A
EMAN2 <a href="https://blake.bcm.edu/emanwiki/EMAN2">https://blake.bcm.edu/emanwiki/EMAN2</a>	14	N/A
IMAGIC-4D <a href="https://www.imagescience.de/imagick-4d.html">https://www.imagescience.de/imagick-4d.html</a>	15	N/A
Gctf <a href="https://www2.mrc-lmb.cam.ac.uk/research/locally-developed-software/zhang-software/">https://www2.mrc-lmb.cam.ac.uk/research/locally-developed-software/zhang-software/</a>	16	N/A
CTFFIND4 <a href="https://grigoriefflab.umassmed.edu/ctffind4">https://grigoriefflab.umassmed.edu/ctffind4</a>	17	N/A
CrYOLO <a href="https://cryolo.readthedocs.io/en/stable/">https://cryolo.readthedocs.io/en/stable/</a>	18	N/A
cryoSPARC <a href="https://cryosparc.com/">https://cryosparc.com/</a>	19	N/A
Relion 3.1 <a href="https://relion.readthedocs.io/en/latest/">https://relion.readthedocs.io/en/latest/</a>	20,21	N/A
SWISS-MODEL <a href="https://swissmodel.expasy.org">https://swissmodel.expasy.org</a>	22	N/A
Coot <a href="https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/">https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/</a>	23	N/A
Phenix <a href="http://www.phenix-online.org/">http://www.phenix-online.org/</a>	24,25	N/A

## Supplemental references

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