**Supplementary TABLE 3**

|  |  |  |
| --- | --- | --- |
| **REAGENT or RESOURCE** | **SOURCE** | **IDENTIFIER** |
| **Antibodies** |  |  |
| Rabbit Polyclonal anti-TAP | Open Biosystems | cat# CAB1001 |
| Mouse Monoclonal anti-HA | Sigma | cat# H9658 |
| Mouse Monoclonal anti-polyHistidine | Sigma | cat# H1029 |
| Rabbit Polyclonal anti-Sch9 | Loewith Lab | N/A |
| Mouse Monoclonal anti-P-Sch9S758 | Loewith Lab | N/A |
| Donkey anti-Mouse, IRDye 680 | Li-Cor Biosciences | cat# 926-68072 |
| Donkey anti-Rabbit, IRDye 800 | Li-Cor Biosciences | cat# 926-32213 |
| **Bacterial and Virus Strains** |  |  |
| DH5α Competent Cells | Invitrogen | Cat# 18265017 |
| NEB 10-beta chemically competent E.coli | NEB | Cat# C3019 |
| BL21-Star (DE3) chemically competent E.coli | Invitrogen | Cat# C601003 |
| **Chemicals, Peptides, and Recombinant Proteins** |  |  |
| Rapamycin | LC Laboratories | Cat# R-5000 |
| Ampicillin sodium salt | Applichem | Cat# A0839 |
| Kanamycin sulfate | Axon Lab | Cat# A1493 |
| Bathophenanthrolinedisulfonic acid | Sigma | Cat# 146617 |
| IPTG | Applichem | Cat# A1008 |
| Tryptone Broth | USBiological | Cat# T8770 |
| Yeast Extract | USBiological | Cat# Y2010 |
| Glucose Anhydrous | USBiological | Cat# G3050 |
| Yeast Nitrogen Base w/o AA, Carbohydrate & w/o AS | USBiological | Cat# Y2030 |
| Drop-out Mix Synthetic Minus Uracil | USBiological | Cat# D9535 |
| Drop-out Mix Synthetic Minus Leucine | USBiological | Cat# D9525 |
| Drop-out mix Complete w/o Yeast Nitrogen Base | USBiological | Cat# D9515 |
| Tween 20 | Axon Lab | Cat# A1389 |
| CHAPS | Applichem | Cat# A1099 |
| TERGITOL type NP40 | Sigma | Cat# NP40S |
| Di-sodium hydrogen phosphate anhydrous | ACROS Organics™ | Cat# 20485 |
| Sodium phosphate, monobasic | ACROS Organics™ | Cat# AC389872500 |
| Tris ultrapure | Applichem | Cat# A1086 |
| Sodium chloride | Acros Organics | Cat# 32730 |
| Magnesium chloride anhydrous | Merck Millipore | Cat# 8.14733 |
| Dithiothreitol (DTT) | Axon Lab | Cat# A2948 |
| Set of dATP, dCTP, dGTP, dTTP (dNTP 100mM) | Promega | Cat# U1420 |
| Polyethylene glycol PEG 8000 | Sigma | Cat# P5413 |
| β-Nicotinamide adenine dinucleotide hydrate | Acros Organics | Cat# AC124532500 |
| [Ammonium iron(II) sulfate hexahydrate](https://www.sigmaaldrich.com/CH/en/substance/ammoniumironiisulfatehexahydrate392147783859?context=product) | Sigma | Cat# 215406 |
| MES hydrate | Sigma | Cat# M2933 |
| T5 exonuclease | Tebu bio | Cat# 15E4111K |
| Taq DNA ligase | MC lab | Cat# TDL-100 |
| Phusion® High-Fidelity DNA Polymerase | NEB | Cat# F-530L |
| KAPA HiFi DNA Polymerase | Roche | Cat# 07958846 |
| Glycerol anhydrous | Applichem | Cat# A2926 |
| Imidazole | Applichem | Cat# A1073 |
| PMSF (Phenylmethanesulfonyl fluoride) | Sigma | Cat# 78830 |
| cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets | Roche | Cat# 05056489001 |
| DNase I | Applichem | Cat# A3778 |
| Lysozyme | Sigma | Cat# 62970 |
| Albumin Bovine (BSA) Cohn Fraction V pH7 | USBiological | Cat# A1310 |
| Precision Plus Protein Dual Color Standards | Bio-rad | Cat# 1610374 |
| PageRuLer Plus Prestained Protein Ladder | Pierce | Cat# 26620 |
| ProBlue Safe Stain | Giottotech | Cat# G00PB002 |
| IgG from human serum | Sigma | Cat# I4506 |
| IgG from rabbit | Sigma | Cat# I5006 |
| **Critical Commercial Assays** |  |  |
| NucleoSpin® Plasmid | Macherey-Nagel | Cat# 740499250 |
| Phire Green Hot Start II PCR Master Mix | Thermo Fisher Scientific | Cat# F126L |
| GeneMorphII Random Mutagenesis Kit | Agilent Technologies | Cat# 200550 |
| **Experimental Models: Cell Lines** |  |  |
| *S. cerevisiae*: TB50: JK9-3da MATa leu2-3,112 ura3-52 trp1 his3 rme1 HMLa | [Beck and Hall, 1999](http://www.yeastgenome.org/reference/S000054286/overview) | N/A |
| S. cerevisiae: BY4741:MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* |  |  |
| **Experimental Models: Organisms/Strains** |  |  |
| RL3823: MATa; TB50, *gtr1∆*::TRP1, *gtr2∆*::HPH, *VPH1::mCherry-KanMX*4 | This study | N/A |
| RL3013: MATα; TB50, LEU2::*GFP-TOR1* | Prouteau et al., 2017 | N/A |
| RL3788: TB50, LEU2::*GFP-TOR1, lst4::*KanMX6 | This study | N/A |
| RL3832: TB50, LEU2::*GFP-TOR1, gtr1∆::TRP1, gtr2∆::HPH lst4::*KanMX6 | This study | N/A |
| RL3789: TB50, LEU2::*GFP-TOR1, lst7::*NatMX4 | This study | N/A |
| RL3826: TB50, LEU2::*GFP-TOR1,* sea1*::*KanMX6 | This study | N/A |
| TB50, LEU2*::GFP-TOR1 LST8Q29A* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 LST8H292A* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1∆α21*, 725-736(AG)n | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1HC*, (KOG1(*802-905)*::Raptor(624-653)) | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1∆twix (*807-881) | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1Screen (717-895)* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1∆tack(1068-1086)* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1∆1004-22* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1∆claw (1121-1132)* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1E784A* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 KOG1R895-896A* | This study | N/A |
| TB50, LEU2::GFP-*TOR1 KOG1R1383A* | This study | N/A |
| TB50, LEU2::GFP-*TOR1 KOG1∆1544-end* | This study | N/A |
| TB50, LEU2::GFP-*TOR1326-334(AG)*n | This study | N/A |
| TB50, LEU2::GFP-*TOR1368-370(AG)*n | This study | N/A |
| TB50, LEU2::*GFP-TOR1W1279A* | This study | N/A |
| TB50, LEU2::*GFP-TOR11449/54/56A* | This study | N/A |
| TB50, LEU2::*GFP-TOR1W2203R* | This study | N/A |
| TB50, LEU2::*GFP-TOR1 TOR2337-345(AG)*n | This study | N/A |
| TB50, LEU2::*GFP-TOR1,TOR2379-381(AG)*n | This study | N/A |
| TB50, LEU2:*:GFP-TOR1, TOR2K2053/53F* | This study | N/A |
| TB50, LEU2::*GFP-TOR1, TOR2W2207R* | This study | N/A |
| TB50, LEU2::*GFP-TOR1W2203R, TOR2W2207R* | This study | N/A |
| TB50, LEU2::*GFP-TOR1W2203R, TOR2W2207R, KOG1HC* | This study | N/A |
| RL3821: TB50, *LEU2::GFP-TOR1, ego1Δ*::HIS3 | This study | N/A |
| RL3015: MATa; TB50, LEU2::*GFP-TOR1, gtr1∆::*TRP1*, gtr2∆::*HPH | Prouteau et al., 2017 | N/A |
| TB50, EGO1TEV *, LEU2::GFP-TOR1*, pRS416 | This study | N/A |
| TB50, EGO1TEV *, LEU2::GFP-TOR1*, pRS416: PCTH2-6xHIS-TEV-TCTH2 | This study | N/A |
| SKY596: MATa, BY4741, LEU2::*GFP-TOR1*, *EGO3::3xmCherry-NatMX4* | Ukai et al., 2018 | N/A |
| RL2500: MATα; TB50, *GFP-KOG1* | Prouteau et al., 2017 | N/A |
| RL3867: TB50, GFP-KOG1, *gtr1∆*::TRP1, *gtr2∆*::HPH | Prouteau et al., 2017 | N/A |
| RL3843: TB50, *GFP-KOG1 EGO3::3xmCherry-NatMX4* | This study | N/A |
| RL3872: TB50, *GFP-KOG1 EGO3::3xmCherry-NatMX4* , *gtr1∆*::TRP1, *gtr2∆*::HPH | This study | N/A |
| RL3642: MATα; TB50, *EGO3::GFP-HPH* | This study | N/A |
| RL3786: TB50, EGO3::GFP-HPH*, lst4::*KanMX6 | This study | N/A |
| RL3833: TB50, EGO3::GFP-HPH*, gtr1∆::TRP1, gtr2∆::HPH lst4::*KanMX6 | This study | N/A |
| RL3787: TB50, EGO3::GFP-HPH*, lst7::*NatMX4 | This study | N/A |
| RL3827: TB50, EGO3::GFP-HPH*,* sea1*::*KanMX6 | This study | N/A |
| RL3816: TB50, EGO1TEV *EGO3::GFP-HPH* | This study | N/A |
| RL3824: TB50, *EGO3::GFP-HPH, ego1Δ*::HIS3 | This study | N/A |
| RL3734: TB50, *EGO3::GFP-HPH*, *gtr1∆*::TRP1, gtr2∆::HPH | This study | N/A |
| RL171-2d: MATa; TB50,*KOG1::TAP-HIS3* | Prouteau et al., 2017 | N/A |
| RL174-5b: MATa; TB50, *KOG1::TAP-HIS3 tor1∆::*KanMX6 | This study | N/A |
| RL3692: TB50, *KOG1HC::TAP-KanMX6* | This study | N/A |
| **Oligonucleotides** |  |  |
| See Table S2 for primers used in this study |  |  |
| **Recombinant DNA** |  |  |
| pRS415: *CEN/ARS, LEU2* | [Sikorski and Hieter](https://www.sciencedirect.com/science/article/pii/S096098222031530X#bib73) | N/A |
| pRS416: *CEN/ARS, URA3* | [Sikorski and Hieter](https://www.sciencedirect.com/science/article/pii/S096098222031530X#bib73) | N/A |
| pRS416: P*CTH2-3xHA-TEV-*T*CTH2* | This study | N/A |
| pRS416: P*CTH2-6xHIS-TEV-*T*CTH2* | This study | N/A |
| pRS415: *GTR1* | Binda, 2009 | N/A |
| pRS415: *GTR1Q65L* (expressing Gtr1GTP locked) | Binda, 2009 | N/A |
| pRS415: *GTR1S20L* (expressing Gtr1GDP locked) | Binda, 2009 | N/A |
| pRS416: *GTR2* | Binda, 2010 | N/A |
| pRS416: *GTR2Q66L* (expressing Gtr2GTP locked) | Binda, 2009 | N/A |
| pRS416: *GTR2S23L* (expressing Gtr2GDP locked) | Binda, 2009 | N/A |
| pACYC-T7: *EGO2∆1-21-EGO3-6xHIS -EGO1∆1-111*(codon optimized) | This study | N/A |
| pET-42 w/out tag: *GTR1-GTR2* | This study | N/A |
| pET-42 w/out tag: *GTR1Q65L-GTR2S23L* | This study | N/A |
| pET-42 w/out tag: *GTR1S20L-GTR2Q66L* | This study | N/A |
| **Deposited data** |  |  |
| TOROID cryoEM map (signal subtracted) | This study | D\_1292115620 |
| TOROID cryoEM map (helical reconstruction) | This study | D\_1292115621 |
| TOROID structural model | This study | PDB ID XXXX |
| **Software and Algorithms** |  |  |
| Fiji | National Institutes of Health | https://imagej.net/Fiji/Downloads |
| Imaris | Bitplane | RRID:SCR\_007370 |
| Adobe Photoshop CS4 | Adobe Inc. | N/A |
| Adobe Illustrator CS6 | Adobe Inc. | N/A |
| Microsoft Excel | Microsoft Inc. | N/A |
| ApE | M.W. Davis | https://jorgensen.biology.utah.edu/wayned/ape/ |
| ClustalX | T.J. Gibson, D.G.Higgins | https://clustalx.software.informer.com/2.1/ |
| MotionCor2 | Zheng et al. 2017 | https://emcore.ucsf.edu/ucsf-motioncor2 |
| CTFFIND4 | Rohou & Grigorieff, 2015 | https://grigoriefflab.umassmed.edu/ctffind4 |
| EMAN2 | Tang et al. 2007 | https://blake.bcm.edu/emanwiki/EMAN2 |
| RELION 2.0 | Scheres, 2012 | https://www3.mrc-lmb.cam.ac.uk/relion/index.php/Main\_Page |
| Gctf | Zhang et al. 2016 | https://www2.mrc-lmb.cam.ac.uk/research/locally-developed-software/zhang-software/#gctf |
| cryoSPARC 3.01 | Punjani et al., 2017 | https://cryosparc.com/ |
| DeepEMhancer | Sanchez-Garcia et al., 2020 | https://github.com/rsanchezgarc/deepEMhancer |
| Alphafold | Jumper et al, 2021, Tunyasuvunakool et al., 2021 | https://alphafold.ebi.ac.uk/ |
| PyMol 2.3 | Schrödinger | https://pymol.org/2/ |
| USCF Chimera | Pettersen et al., 2004 | https://www.cgl.ucsf.edu/chimera/ |
| USCF ChimeraX | Pettersen et al., 2021 | https://www.rbvi.ucsf.edu/chimerax/ |
| **Other** |  |  |
| MF-Millipore Membrane, mixed cellulose esters, Hydrophilic, 0.45 µm, 25 mm, white, plain | Merck Millipore | Cat# HAWP04700 |
| Dynabeads™ His-Tag Isolation and Pulldown | Thermo Fisher Scientific | Cat# 10103D |
| Dynabeads™ M-270 Epoxy | Thermo Fisher Scientific | Cat# 14302D |
| Lacey Carbon Film, Copper, 300 mesh | Quantifoil | <https://www.emsdiasum.com/microscopy/products/grids/grids.aspx> |
| Titan Krios G2 | FEI/Thermo Fisher Scientific | N/A |
| K2 Summit Direct Electron Detector | Gatan | N/A |
| AVESTIN EMULSIFLEX C3 | Avestin | Cat# EFC3 |
| HisTrap™ Fast Flow Crude | GE healthcare | Cat# GE11-0004-58 |
| GE AKTA Explorer | Amersham Pharmacia | Cat# 8149-30-0002 |
| Superdex 200 Increase column | GE healthcare | Cat# 28990944 |
| Amicon Ultra-4 Centrifugal filters ultracel 50K | Merck Millipore | Cat# UFC805096 |