# Supplemental Materials

***Molecular Biology of the Cell***

Sørensen et al.

*Supplementary material*

# The P5A ATPase Spf1p is stimulated by phosphatidylinositol 4-phosphate and influences cellular sterol homeostasis

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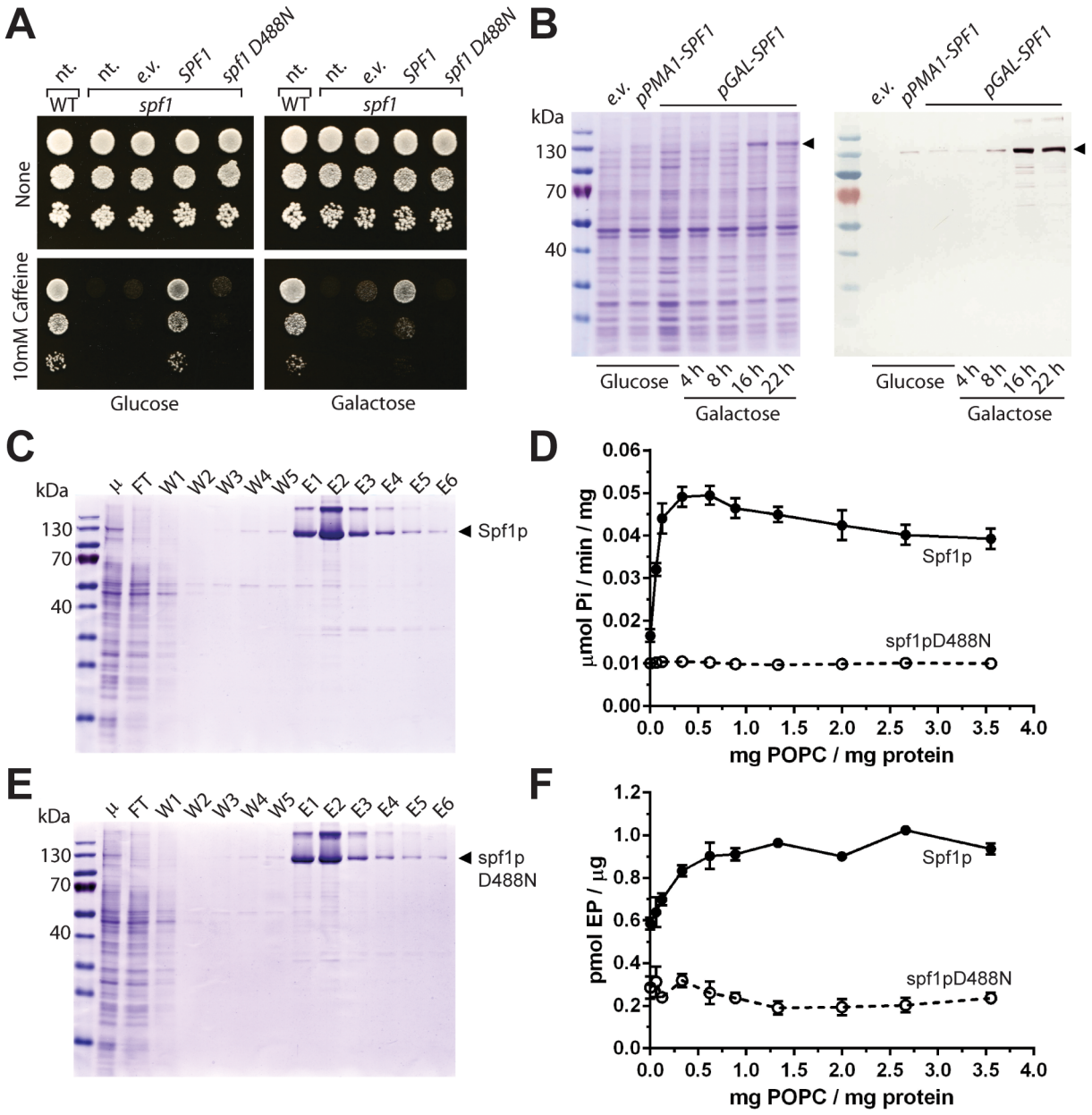
## Supplementary Figures

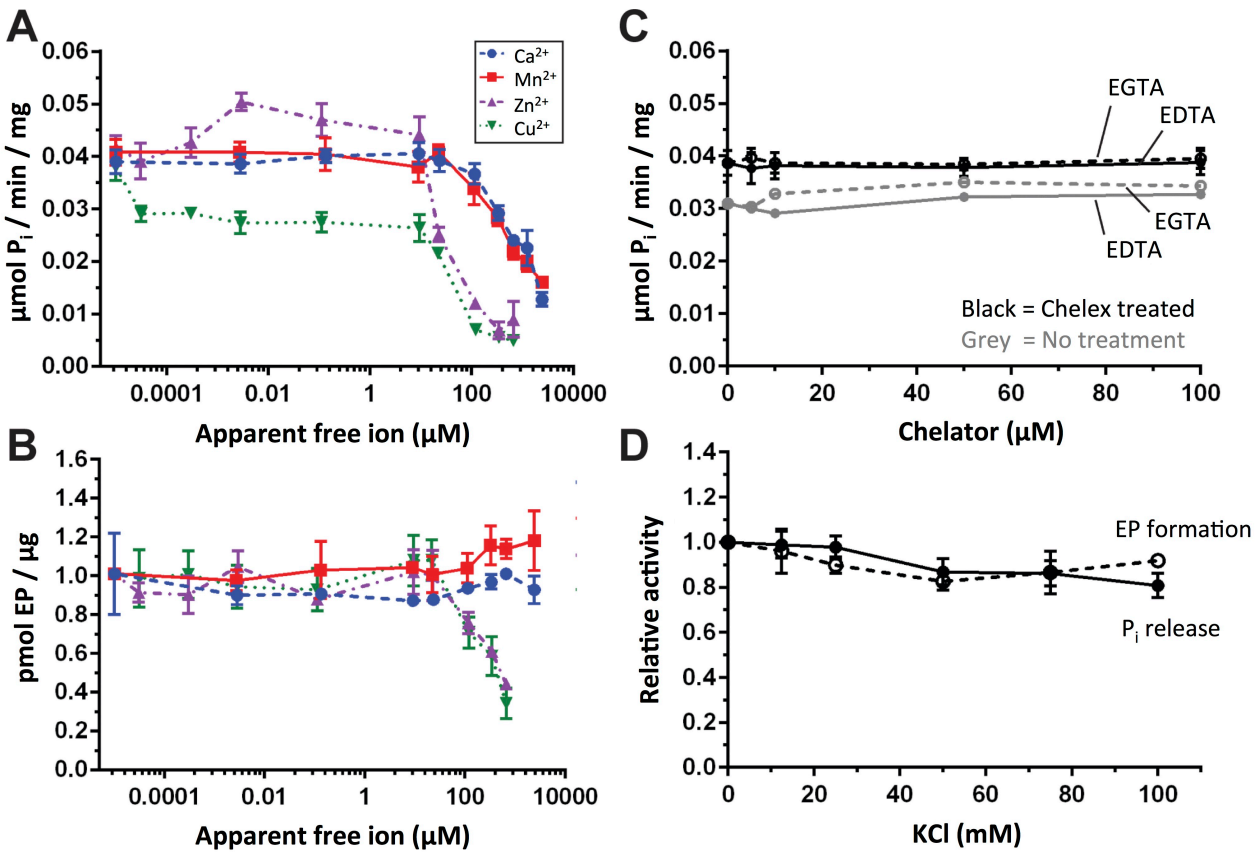
### Supplementary Figure 1




**Supplementary Figure 1 – Expression and purification of Spf1p. (**A) Expression of *SPF1* from a leaky *GAL1* promoter rescues caffeine-induced growth inhibition of *spf1* cells. A catalytically inactive D488N mutant is unable to complement *spf1*. nt. – not transformed, e.v. – empty vector control. (B) SDS-PAGE showing galactose-induced expression of Spf1p-FLAG-RGS10xHis protein (marked by an arrowhead) in *spf1* cells. (C,D) SDS-PAGE showing the purification of (C)

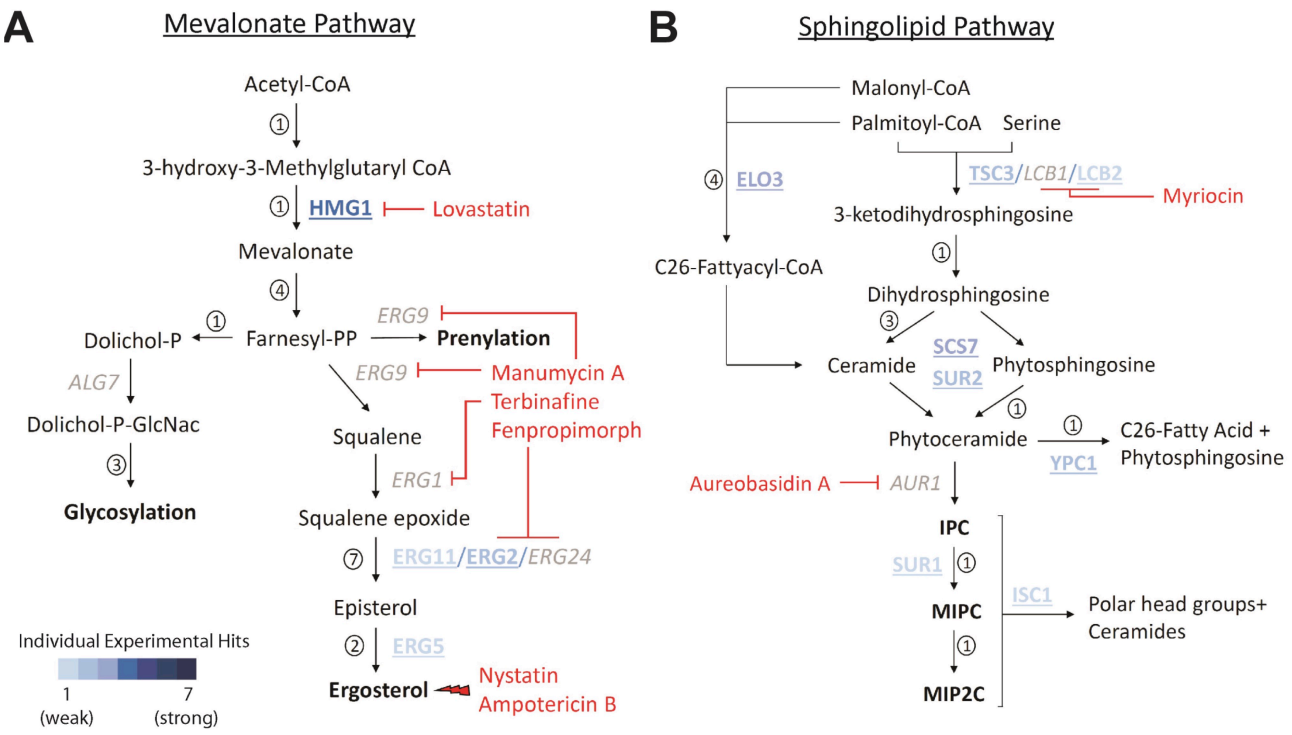
Spf1p and (D) Spf1p D488N. μ - microsomal fraction, FT – flow-through, W – wash, E - eluate. (E) ATP hydrolytic activity of purified Spf1p, but not Spf1p D488N, can be induced by the addition of POPC. (F) A higher level of spontaneous phosphorylation from [$\gamma^{32}$P]-ATP can also be observed after the addition of POPC to Spf1p, but not Spf1p D488N. For all data, error bars represent s.e.m.; $n$ = 3 biological replicates.

**Supplementary Figure 2**



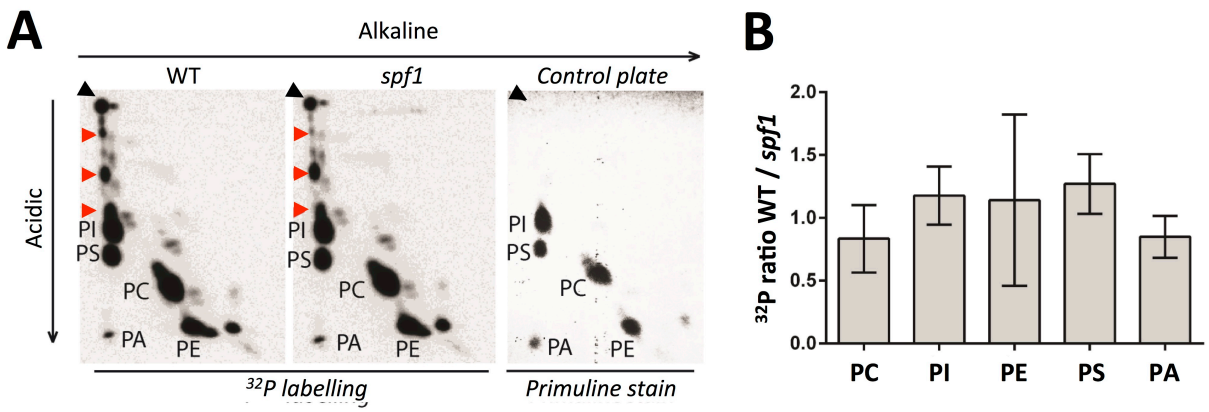

**Supplementary Figure 2 – Effect of cations on ATPase activity and steady-state phosphorylation levels of Spf1p.** POPC-reactivated Spf1p was assayed for ATPase activity or steady-state phosphorylation levels in the presence of EDTA and EGTA (50 µM unless otherwise indicated) and increasing concentrations of the indicated metal cations. (A) Cations previously proposed to be ligands of Spf1p do not stimulate Spf1p ATPase activity. (B) The same cations do not stimulate steady state phosphorylation levels of Spf1p. (C) Chelation of possible trace metals does not influence Spf1p ATPase activity. Increasing concentrations of either EGTA or EDTA were added to the ATPase assay, with or without pretreating the ATPase buffer with Chelex resin to further chelate divalent metal ions. (D) $K^+$ ions do not stimulate ATP hydrolytic activity or change phosphoenzyme formation of Spf1p. Ionic strength was kept constant with NaCl and activity is noted relative to buffer without KCl (but with 100 mM NaCl). For all data, error bars represent s.e.m.; $n = 3$ biological replicates.

**Supplementary Figure 3**




**Supplementary Figure 3 – Genetic and drug interactions in lipid synthesis pathways of *spf1* cells.** The mevalonate pathway (A) and the sphingolipid pathway (B) both contain genes encoding enzymes that were previously shown to genetically interact with SPF1 (underscored). The strength of each interaction is indicated by the intensity of the blue bar, corresponding to individual experimental hits found in previous studies (Supplemental Table S2). The strongest interactors are OPI3 (7 hits), HMG1 (4 hits), SAC1 (4 hits), SCS7 (3 hits), and ELO3 (3 hits). Numbers in circles indicate enzymatic steps not shown. Red lines indicate enzymatic inhibitors that inhibit the growth of *spf1* cells. Lightning bolt indicates increased sensitivity towards sterol binding antibiotics.

**Supplementary Figure 4**




**Supplementary Figure 4 – Quantification of $^{32}$P-labeled phospholipids in wild-type and *spf1* cells.** (A) Total lipid extracts from a similar amount of wild-type and *spf1* cells labeled with $^{32}$P were diluted to 10 nCi and separated in two dimensions on TLC plates using an acidic and an alkaline solvent (see Materials and Methods). Twenty-eight spots labeled with $^{32}$P could be visualized after exposure. For comparison, a control plate was loaded with the indicated lipid standards, developed and stained with primuline. Black arrows indicate loading spots. A representative image for all ($n$ = 3) biological replicates is shown. (B) The molar content of $^{32}$P in each spot represented in A was quantified using a $^{32}$P standard. Deletion of *SPF1* results in a slight but significant change in three spots, which we were unable to identify by mass spectrometry (indicated by red arrowheads in A). However, deletion of *SPF1* had little effect on the total levels of the major glycerophospholipids, as indicated by the quantified ratio of spots corresponding to those from extracts of wild-type and *spf1* cells ($n$ = 3 biological replicates).

## Supplementary Tables

**Supplementary Table S1 – Cell characteristics of wild-type and *spf1* cells**. The two strains have comparable viability and cell count per $OD_{600}$ unit (determined in a NucleoCounter NC3000 using propidium iodide staining for nonviable cells following the manufacturer's instructions) and have similar average cell sizes (determined by microscopy; see Materials and Methods). However, the *spf1* strain has a significantly lower growth rate at the log phase across all temperatures examined (determined on liquid cultures in YPD medium) and contains a significantly higher energy content, reflecting accumulation of chemical energy within the cell (determined by calorimetry; see Materials and Methods). *n* = biological replicate (# denotes a technical replicate), S.D. = standard deviation. *P* = *P* values determined by two-tailed t-tests (# denotes two-tailed Welch t-test). *n.s.* = not significant. Stars indicate significance.

| | WT | S.D. | *n* | *spf1* | S.D. | *n* | *P* | significance |
|---|---|---|---|---|---|---|---|---|
| **Cells pr. $OD_{600}$** | *3.50E+07* | *±2.04+06* | *3* | *3.68E+07* | *±0.66+05* | *3* | *P = 0.2332* | *n.s.* |
| **Viability** | *97.60%* | *-* | *3#* | *95.40%* | *-* | *3#* | *-* | *-* |
| **Growth rate 20°C ($h^{-1}$)** | *0.41* | *±0.03* | *3* | *0.29* | *±0.03* | *3* | *P = 0.0080* | **** |
| **Growth rate 25°C ($h^{-1}$)** | *0.75* | *±0.05* | *3* | *0.59* | *±0.01* | *3* | *P = 0.0043* | **** |
| **Growth rate 30°C ($h^{-1}$)** | *0.77* | *±0.03* | *3* | *0.60* | *±0.02* | *3* | *P = 0.0018* | **** |
| **Growth rate 40°C ($h^{-1}$)** | *0.47* | *±0.02* | *3* | *0.15* | *±0.02* | *3* | *P < 0.0001* | ****** |
| **Cell size (μM)** | *4.35* | *±0.64#* | *54#* | *4.34* | *±0.80* | *64#* | *P = 0.9117#* | *n.s.* |
| **Energy (J/g cells)** | *19320* | *±45.5* | *3* | *20050* | *±14.4* | *3* | *P < 0.0001* | ****** |

**Supplementary Table S2 – Genes encoding proteins involved in lipid synthesis and trafficking, which have been shown to interact with *SPF1* in screening studies.** Twenty genes have been reported in genetic interactions (1 lethal, 15 negative, and 4 positive), nine genes in chemical-genetic (CG) interactions, and two gene products (those of *SAC1* and *SCS2*) have been reported to physically interact with Spf1p. Experimental hits (Exp. hits) indicate independent experiments showing the observed interaction. Protein localization indicates the localization of the gene product. LP = lipid particle, PM = plasma membrane, ER = endoplasmic reticulum, and C = cytoplasm.

| ***Gene*** | ***Lipid species*** | ***Enzymatic activity*** | ***Protein*** | ***Interaction*** | ***Effect*** | ***Exp. hits*** | ***References*** |
|---|---|---|---|---|---|---|---|
| **OPI3** | Phospholipids | Unsaturated phospholipid methyltransferase | PM | Genetic | Negative | 7 | Schuldiner et al. (2005) |
| **DGK1** | Phospholipids | Diacylglycerol kinase | ER | Genetic | Negative | 4 | Schuldiner et al. (2005) |
| **SAC1** | Phospholipids | Phosphatidylinositol phosphate phosphatase | ER/Golgi | Genetic and Physical | Negative / - | 4 | Manford et al. (2012) |
| **ARV1** | Sterols | Transport of glycosylphosphatidylinositol | ER/Golgi | Genetic | Negative | 4 | Schuldiner et al. (2005) |
| **HMG1** | sterols | Hydroxymethylglutaryl-CoA reductase | ER | Genetic and CG | Negative | 4 | Schuldiner et al. (2005) Cronin et al. (2002) |
| **SCS7** | Sphingolipids | Sphingolipid alpha-hydroxylase | ER/V | Genetic | Negative | 3 | Schuldiner et al. (2005) |
| **ELO3** | Sphingolipids / Glycerolipids | Elongase | ER | Genetic | Negative | 3 | Costanzo et al. (2010) |
| **TGL3** | Glycerolipids | Triacylglycerol lipase / lysophosphatidylethanolamine acyltransferase | LP | Genetic | Positive | 2 | Schuldiner et al. (2005) |
| **PSD1** | Phospholipids | Phosphatidylserine decarboxylase | M | Genetic | Negative | 2 | Hoppins et al. (2011) |
| **ZAP1** | Phospholipids | Zinc-regulated transcription factor | N | Genetic | Phenotypic Suppression | 2 | Jonikas et al. (2009) |
| **SUR2** | Sphingolipids | Sphinganine C4-hydroxylase | ER | Genetic | Negative | 2 | Schuldiner et al. (2005) |
| **YPC1** | Sphingolipids | Alkaline ceramidase / ceramide synthase | ER | Genetic | Positive | 2 | Schuldiner et al. (2005) |
| **TSC3** | Sphingolipids | None (Stimulator of serine palmitoyltransferases (Lcb1p, Lcb2p)) | ER/C | Genetic | Negative | 2 | Schuldiner et al. (2005) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **ERG2** | sterols | C-8 sterol isomerase | ER/V | Genetic and CG | Phenotypic Suppression | 2 | Jonikas et al. (2009), Kapitzky et al (2010), This study |
| **SCT1** | Glycerolipids | Glycerol 3-phosphate/dihydroxyacetone phosphate acyltransferase | ER | Genetic | Negative | 1 | Costanzo et al. (2010) |
| **SCS2** | Phospholipids | None (integral ER membrane VAP protein) | ER | Physical | - | 1 | Manford et al. (2012) |
| **LCB2** | Sphingolipids | Serine palmitoyltransferase | ER | Genetic and CG | Negative | 1 | This study |
| **SUR1** | Sphingolipids | Mannosylinositol phosphorylceramidesynthase | Golgi | Genetic | Negative | 1 | Hoppins et al. (2011) |
| **ISC1** | Sphingolipids | Inositol phosphosphingolipid phospholipase | M | Genetic | Negative | 1 | Collins et al. (2007) |
| **ERG11** | Sterols | Lanosterol 14-alpha-demethylase | ER | Genetic | Lethal | 1 | Parsons et al. (2004) |
| **ERG5** | sterols | C-22 sterol desaturase | ER | Genetic | Negative | 1 | Costanzo et al. (2010) |
| **ALG7** | Glycolipids | UDP-N-acetyl-glucosamine-1-P transferase | ER | CG | Negative | - | Suzuki (2001), This study |
| **LCB1** | Sphingolipids | Serine palmitoyltransferase | ER | CG | Negative | - | This study |
| **AUR1** | Sphingolipids | Phosphatidylinositol / ceramide phosphoinositol transferase | Golgi | CG | Negative | - | Cohen et al. (2013), This study |
| **ERG9** | Sterols | Farnesyl-diphosphate farnesyl transferase | ER/V | CG | Negative | - | This study |
| **ERG1** | Sterols | Squalene epoxidase | ER/LP | CG | Negative | - | Cohen et al. (2013), This study |
| **ERG24** | Sterols | C-14 sterol reductase | ER/V | CG | Negative | - | Kapitzky et al (2010), This study |

**Supplementary Table S3 – Yeast strains used in this study.** Yeast strains used and generated in this study. *spf1/sac1* cells were generated by crossing *spf1* cells[*] with *sac1* cells[$]; individual crosses are shown. ZHY709 cells[&] were used for cloning of pre-pro-alpha GFP constructs.

| Background | Strain name | Genotype | Source |
|---|---|---|---|
| BY4741 | WT | *MAT*a: *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0* | Euroscarf |
| BY4741 | *spf1* | *MAT*a: *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0;yel039w::kanMX4* | Euroscarf |
| BY4742[*] | *spf1* | *MAT*α: *his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; yel039w::HIS3* | This study |
| BY4741 | *ypk9* | *MAT*a; *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yor291w::HIS3* | Gitler *et al.* 2009 |
| BY4741[$] | *sac1* | *MAT*a: *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0;ykl212w::KanMX4* | Euroscarf |
| | *spf1/sac1 (#1)* | *MAT*α: *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yel039w::HIS3; ykl212w::KanMX4* | This study |
| | *spf1/sac1 (#2)* | *MAT*a: *his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; yel039w::HIS3; ykl212w::KanMX4* | This study |
| | *spf1/sac1 (#3)* | *MAT*a: *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yel039w::HIS3; ykl212w::KanMX4* | This study |
| ZHY709[&] | *dnf1/dnf2/drs2* | *MAT*a: *his3Δ1; leu2Δ0; ura3Δ0; met15Δ0; dnfΔ; dnfΔ; drs2::LEU2* | Huang and Shusta, 2005 |
| W303 | *hem1/aus1/ pdr11* | *MATa*: *ade2Δ1; his3Δ11,15; leu2Δ3,112; trp1Δ1; ura3Δ1, can1Δ100; hem1Δ::LEU2; pdr11Δ::loxP; aus1Δ::loxP-HIS5Sp-loxP* | Marek *et al.,* 2014 |
| BY4741 | *SPF1-GFP/ SAC1-RFP* | *MAT*a: *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yel039w::SPF1-GFP; ykl212w::SAC1-RFP* | This study |
| BY4741 | *SPF1-GFP/ SEC61-RFP* | *MAT*a: *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yel039w::SPF1-GFP; ylr378c::SEC61-RFP* | This study |
| BY4741 | *Osh1* | *MAT*a*; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; YAR042w::kanMX4* | Euroscarf |
| BY4741 | *Osh2* | *MAT*a*; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; YDL019c::kanMX4* | Euroscarf |
| BY4741 | *Osh3* | *MAT*a*; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; YHR073w::kanMX4* | Euroscarf |
| BY4741 | *Osh4* | *MAT*a*; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; YPL145c::kanMX4* | Euroscarf |
| BY4741 | *Osh5* | *MAT*a*; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; YOR237w::kanMX4* | Euroscarf |
| BY4741 | *Osh6* | *MAT*a*; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; YKR003w::kanMX4* | Euroscarf |

**Supplementary Table S4 – Plasmids used in this study.**

| **Plasmid** | **Reference** |
|---|---|
| pRS316:sec-yEGFP | Huang and Shusta, 2005 |
| pRS316:cyto-yEGFP | This study |
| pRS423 | López-Marqués *et al.,* 2012 |
| yEP351:pPMA1-SPF1-FLAG/RGS10xhis | This study |
| pRS423:pGAL1-SPF1-FLAG/RGS10xhis | This study |
| pRS423:pGAL1-SPF1-D488N-FLAG/RGS10xhis | This study |
| p122:pGAL-ERG6-RFP | Marek *et al.,* 2014 |
| pRS424:GFP-2xPH(Osh2) | Stefan *et al.,* 2011 |
| pBS-ΔSPF1:URA3 | This study |

**Supplementary Table S5 – Accession numbers of the 171 P-type ATPase protein sequences used for the phylogenetic analysis presented in Figure 1** (see Materials and Methods). Full sequence accession numbers from KEGG or NCBI are noted. In Figure 1, the sequence labels are shortened to include only the three-letter organism identifier followed by the digits in the accession numbers.

| Species | Full KEGG accession number (*NCBI accession number) |
|---|---|
| *Caenorhabditis elegans (cel)* | cel:CELE_C10C6.6; cel:CELE_K07E3.7; cel:CELE_Y59H11AR.2; cel:CELE_W08D2.5; cel:CELE_C02E7.1; cel:CELE_C09H5.2; cel:CELE_B0365.3; cel:CELE_ZK256.1; cel:CELE_K11D9.2; cel:CELE_C01G12.8; cel:CELE_F36H2.1; cel:CELE_Y49E10.11; cel:CELE_T24H7.5; cel:CELE_F02C9.3; cel:CELE_W09D10.2; cel:CELE_H06H21.10; cel:CELE_R05C11.3; cel:CELE_Y67D8C.10; cel:CELE_Y76A2A.2; cel:CELE_W09C2.3 |
| *Drosophila melanogaster (dme)* | dme:Dmel_CG6230; dme:Dmel_CG32000; dme:Dmel_CG32451; dme:Dmel_CG5670; dme:Dmel_CG45760; dme:Dmel_CG42321; dme:Dmel_CG3725; dme:Dmel_CG33298; dme:Dmel_CG4301; dme:Dmel_CG42314; dme:Dmel_CG9981; dme:Dmel_CG1886; dme:Dmel_CG14741; dme:Dmel_CG31729 |
| *Homo sapiens (hsa)* | hsa:57130; hsa:79572; hsa:344905; hsa:23400; hsa:84239; hsa:487; hsa:489; hsa:476; hsa:477; hsa:479; hsa:480; hsa:488; hsa:478; hsa:495; hsa:27032; hsa:9914; hsa:490; hsa:492; hsa:23250; hsa:491; hsa:10396; hsa:51761; hsa:23200; hsa:57198; hsa:79895; hsa:57205; hsa:493; hsa:538; hsa:23120; hsa:540; hsa:286410; hsa:374868; hsa:148229; hsa:5205; hsa:10079; hsa:57194 |
| *Sulfolobus solfataricus (sau)* | sso:SSO2651; sso:SSO2896 |
| *Methanococcoides methylutens (mem)* | *WP_048193110.1; *WP_048193070.1; *WP_048205438.1; *WP_048206222.1; *WP_081955762.1; *KGK98552.1; *WP_048195962.1; *WP_052721400.1; *WP_048205268.1; *WP_048205866.1; *WP_048194506.1 |
| *Escherichia coli (eco)* | eco:b4242; eco:b0697; eco:b0484; eco:b3469 |
| *Staphylococcus aureus (sau)* | sau:SA0070; sau:SA2344; sau:SA1880 |
| *Nostoc punctiforme (npu)* | npu:Npun_R6170; npu:Npun_R5980; npu:Npun_R1441; npu:Npun_R4017; npu:Npun_R0509; npu:Npun_R0301; npu:Npun_F0719; npu:Npun_R1694 |
| *Saccharomyces cerevisiae (sce)* | sce:YEL031W; sce:YOR291W; sce:YGL167C; sce:YDR040C; sce:YDR039C; sce:YDR038C; sce:YGL006W; sce:YAL026C; sce:YER166W; sce:YIL048W; sce:YGL008C; sce:YPL036W; sce:YDR093W; sce:YMR162C; sce:YDR270W; sce:YBR295W |
| *Arabidopsis thaliana (ath)* | ath:AT5G23630; ath:AT1G10130; ath:AT3G57330; ath:AT5G44240; ath:AT1G80660; ath:AT5G57350; ath:AT3G63380; ath:AT1G17260; ath:AT3G22910; ath:AT3G42640; ath:AT4G37640; ath:AT1G07670; ath:AT4G29900; ath:AT1G07810; ath:AT2G24520; ath:AT3G47950; ath:AT4G30190; ath:AT2G07560; ath:AT5G62670; ath:AT2G18960; ath:AT2G41560; ath:AT3G60330; ath:AT1G27770; ath:AT3G21180; ath:AT5G57110; ath:AT1G59820; ath:AT2G22950; ath:AT4G00900; ath:AT4G11730; ath:AT3G25610; ath:AT1G26130; ath:AT1G68710; ath:AT3G27870; ath:AT1G72700; ath:AT3G13900; ath:AT1G54280; ath:AT1G13210; ath:AT1G17500; ath:AT1G63440; ath:AT5G21930; ath:AT5G44790; ath:AT4G37270; ath:AT4G30110; ath:AT5G04930; ath:AT4G33520; ath:AT2G19110 |

| | |
|---|---|
| *Nannochloropsis gaditana (nga)* | ngd:NGA_0576820; ngd:NGA_0502700; ngd:NGA_0599100; ngd:NGA_0635410; ngd:NGA_0722800; *EWM26586.1; *EWM22623.1; *EWM29580.1; *EWM24559.1; *EWM25428.1; *EWM27560.1 |