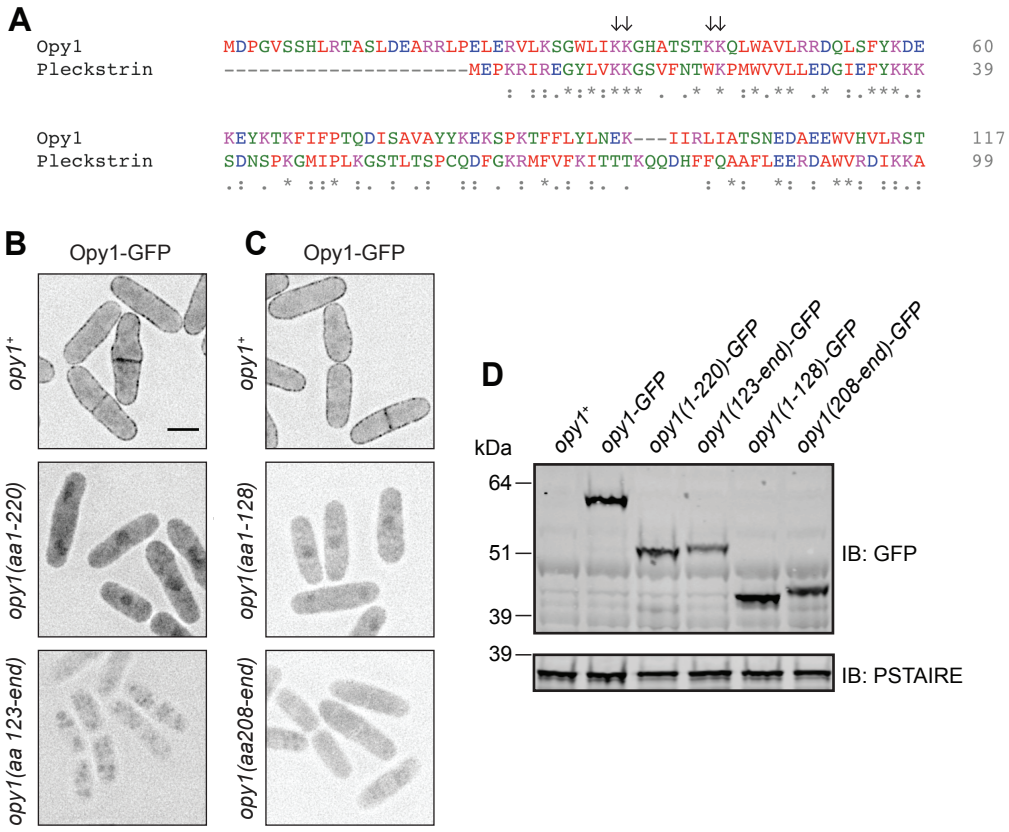
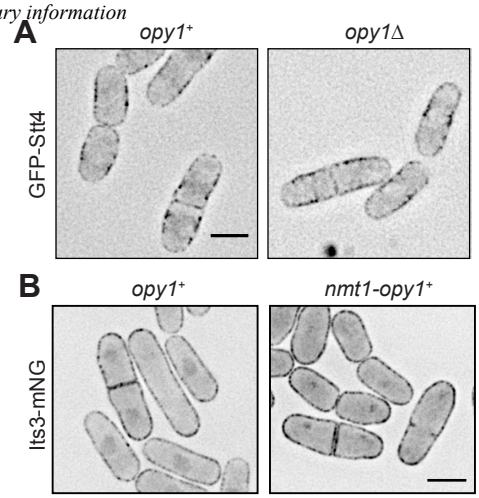
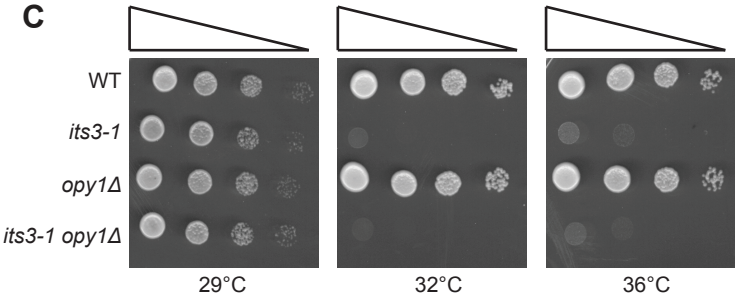
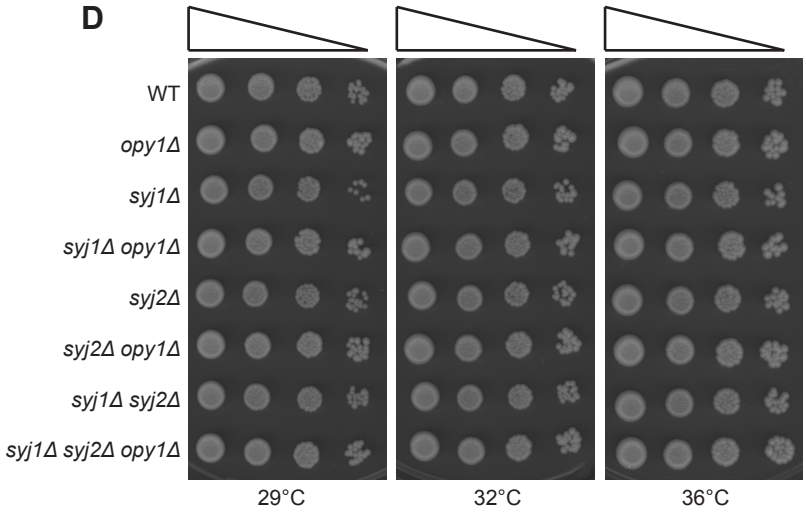


Figure S1. (A) Sequence alignment of residues 1-99 of H. sapiens pleckstrin and residues 1-117 of S. pombe Opy1 generated by Clustal Omega (Madeira et al., 2019). Asterisks denote residues that are conserved; colons denote groups with strongly similar qualities; periods denote groups with weakly similar qualities. Red = small + hydrophobic residues; blue = acidic residues; magenta = basic residues; green = hydroxyl + sulfhydryl + amine + G residues. Arrows indicate the Opy1 residues implicated in PI(4,5)P2 binding (Harlan et al., 1995). (B-C) Representative live-cell images of Opy1-GFP in indicated genotypes. Scale bar = 5 µm. (D) Western blot of the indicated GFP-fusions, IB = immunoblot.







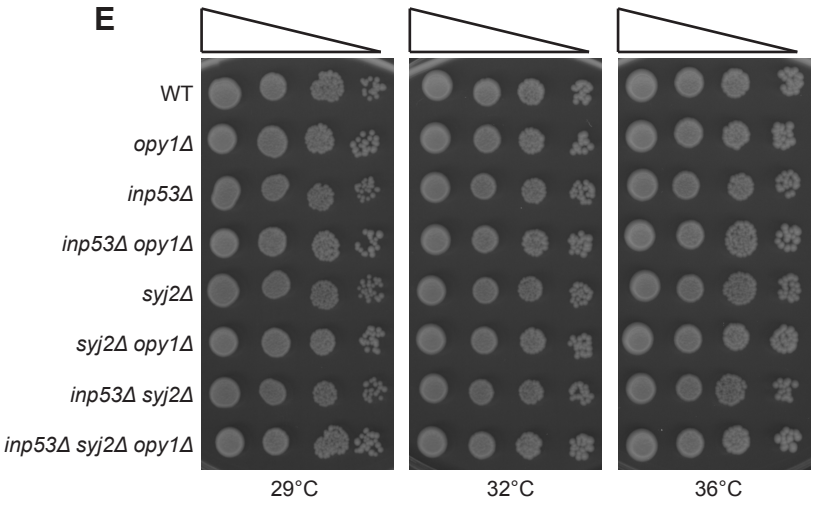


Figure S2. (A) Representative live-cell images of GFP-Stt4 in cells of the indicated genotypes. Scale bar = 5 µm. (B) Representative images of Its3-mNG in wild type (opy1+) or in cells over-producing opy1 from the nmt1 promoter (nmt1-opy1+) after 24 hours in media lacking thiamine. Scale bar = 5 µm. (C-E) Growth assay of indicated strains at the indicated temperatures on YE.

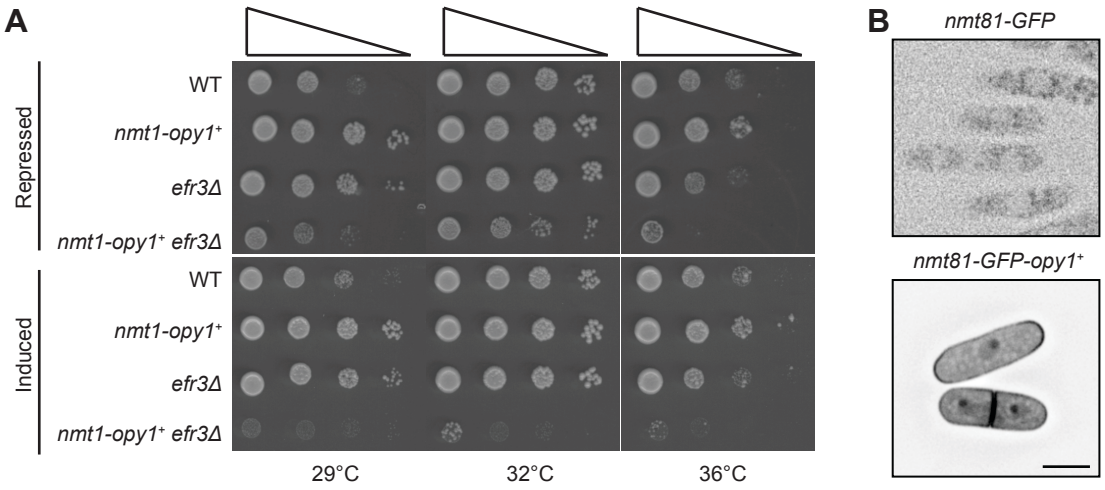


Figure S3. (A) Growth assay of indicated strains at the indicated temperatures on plates containing (repressed) or lacking (induced) thiamine. (B) Representative images showing localization of overexpressed GFP-Opy1. Prior to imaging, overexpression was induced in cells carrying either empty vector (nmt81-GFP) or a GFP-Opy1 overexpression vector (nmt81-GFP-opy1+) for 24 hours in media lacking thiamine. Images are not scaled identically due to the difference in signal. Scale bar = 5 µm.

Table S1. *S. pombe* strains used in this study

Strain number	Genotype	Source
KGY19319	*opy1*-GFP:*kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY19335	*opy1(aa1-220)*-GFP:*kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY19412	*opy1(123-end)*-GFP:*kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY19413	*opy1(aa1-128)*-GFP:*kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY19414	*opy1(aa208-end)*-GFP:*kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY2810-2	*opy1(K34N K35N K42N K43N)*-GFP:*kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY243-2	*its3*-mNG:*hyg*^{R} *ade6*-M210 *leu1*-32 *ura4*-D18 h-	This study
KGY473-2	*its3*-mNG:*hyg*^{R} *opy1::kan*^{R} *ade6*-M210 *leu1*-32 *ura4*-D18 h+	This study
KGY446-2	*its3*-mNG:*hyg*^{R} *efr3::kan*^{R} *ade6*-M210 *leu1*-32 *ura4*-D18 h+	This study
KGY510-2	*its3*-mNG:*hyg*^{R} *nmt1-opy1:ura ade6*-M210 *ura4*-x *leu1*-32 h-	This study
KGY18661	GFP-*stt4 ade6*-704 *leu1*-32 h-	Lab stock
KGY178-2	GFP-*stt4 opy1::kan*^{R} *ade6*-x *leu1*-32 *ura4*-D18 h-	This study
KGY167-2	*opy1*-TAP:*kanR ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY19217	GFP-P4C$_{SidC}$:*leu*$^+$ *ade6*-M210 *leu1*-32 *ura4*-D18 h+	Lab stock
KGY3119-2	GFP-P4C$_{SidC}$:*leu*$^+$ *nmt1-opy1:ura leu1*-32 *ura4*-x h+	This study
KGY2255-2	GFP-PH$_{Akt}$:*leu*$^+$ *ptn1::kan*^{R} *ade6*-M210 *leu1*-32 *ura4*-D18 h-	Lab stock
KGY3193-2	GFP-PH$_{Akt}$:*leu*$^+$ *ptn1::kan*^{R} *nmt1-opy1:ura ade6*-M210 *leu1*-32 *ura4*-x h-	This study
KGY269-2	GFP-2xPH$_{Plc}$:*leu*$^+$ *ade6*-M210 *leu1*-32 *ura4*-D18 h-	Lab stock
KGY3192-2	GFP-2xPH$_{Plc}$:*leu*$^+$ *nmt1-opy1:ura ade6*-M210 *leu1*-32 *ura4-x* h-	This study
KGY246	*ade6*-M210 *ura4*-D18 *leu1*-32 h-	Lab stock
KGY3117-2	*nmt1-opy1:ura+ ade6*-M210 *ura4*-294 *leu1*-32 h+	This study
KGY18056	*efr3::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	Lab stock
KGY1120-2	*nmt1-opy1:ura+ efr3::kan*^{R} *ade6*-M210 *ura4*-x *leu1*-32 h-	This study
KGY1898-2	*opy1::ura+ ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY796-2	*syj1::kan*^{R} *opy1::ura+ ade6*-M210 *ura4*-D18 *leu1*-32 h+	This study

KGY872-2	*syj2::kan*^{R} *opy1::ura+ ade6*-M210 *ura4*-D18 *leu1*-32 h+	This study
KGY1977-2	*opy1::ura+ syj1::kan*^{R} *syj2::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY2184-2	*opy1::ura+ inp53::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h+	This study
KGY2495-3	*opy1::ura+ inp53::kan*^{R} *syj2::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	This study
KGY1951-2	*inp53::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h+	Lab stock
KGY2685-2	*inp53::kan*^{R} *syj2::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h-	Lab stock
KGY1905-2	*syj2::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h+	Lab stock
KGY2328-2	*syj1::kan*^{R} *syj2::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h+	Lab stock
KGY18196	*syj1::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h+	Lab stock
KGY1004-2	*opy1::kan*^{R} *ade6*-M210 *ura4*-D18 *leu1*-32 h+	Lab stock
KGY6369	*its3-1 ade6*-M21x *ura4*-D18 *leu1*-32 h-	Lab stock
KGY593-2	*its3-1 opy1::kan*^{R} *ade6*-M21x *ura4*-D18 *leu1*-32 h-	This study

Table S2. Proteins identified by LC-MS/MS in Opy1-TAP. Proteins identified by LC-MS/MS in Opy1-TAP. Shown in the table are the ORF numbers (ID), protein names and their descriptions. Also indicated are the Total Spectral Counts (TSC), the percent of the protein's sequence that was identified (coverage) and the predicted molecular weight (MW) of each protein identified. Only proteins with a minimum of two spectral counts are shown and proteins identified in mock TAPs are listed at the end of the table in grey-shaded text.

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References

Harlan, J. E., Yoon, H. S., Hajduk, P. J. and Fesik, S. W. (1995). Structural characterization of the interaction between a pleckstrin homology domain and phosphatidylinositol 4, 5-bisphosphate. *Biochemistry* **34**, 9859-9864.

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D. et al. (2019). The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic acids research* **47**, W636-W641.