SUPPLEMENTARY MATERIALS AND METHODS

Two-hybrid screen

To ensure that the mod5p bait was successfully translocated to the nucleus and not targeted to the plasma membrane, the C-terminal prenylation motif was removed. The *mod5* cDNA sequence was cloned into the two-hybrid LexA bait vector *pBTM116* (Vojtek and Cooper, 1993) and transformed into the *S. cerevisiae* two-hybrid test strain L40 (Hollenberg et al., 1995). 2 x 10^6 prey plasmids from a meiotic *S. pombe* library were screened for *mod5*-interacting clones. 10 of the 11 clones obtained showed strong interaction with *mod5*, while the 11th showed only a weak interaction and contained a fragment of the gene encoding the 14-3-3 protein rad24p (Bridges and Moorhead, 2004; Ford et al., 1994). The *mod5* internal deletions additionally lacking the C-terminal prenylation sequence, *mod5:156-255* and *mod5:206-255,* were cloned into the *pBTM116-1* vector to test their ability to interact with tea3p in *S. cerevisiae* L40. We were unable to clone *mod5Δ1-55* into *pBTM116-1*. Semi-quantitative assays of betagalactosidase expression in two-hybrid strains expressing combinations of tea1p, mod5p and tea3p were carried out using 2-nitrophenol-β-D-galactopyranoside (ONPG; Sigma) as a substrate.

Biochemical experiments

GST pulldowns from fission yeast extracts: 400-500 mg of frozen cell pellets were ground with a mortar and pestle under liquid nitrogen and extracts prepared in 20 mM NaHEPES pH 7.5, 50 mM KAcetate, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 1% Triton X-100. Samples were incubated with 25 μl GSH agarose beads (Sigma, UK) for 3 h at 5°C. All samples were separated on 8% SDS PAGE and subjected to western blotting.

In vitro transcription and translation: Fragments of tea1p and tea3p were amplified by a 2 step PCR protocol to include the T7 promoter at the 5' end, and three haemagglutinin tags at the 3' end, and expressed by *in vitro* translation according to the manufacturer's instructions (TnT® T7 Quick for PCR DNA, Promega, Southampton, UK). GST-mod5p(28-447) and GST-GFP were purified from bacteria following standard protocols, and *in vitro* binding assays were performed as described (Samejima et al., 2005).

Construction of *mod5* internal deletion mutants

Initially, DNA fragments 5' and 3' of the desired deletion were amplified by PCR to include a common amino-acid linker sequence GGSAGSA that would replace the deleted amino acids. Pairs of PCR fragments were then combined and used as mega-primers in a second round of PCR to produce the final mod5p deletion sequences. Each deletion fragment retained the C-terminal prenylation signal to ensure correct membrane-targeting, and was cloned 3' to the *nmt81* promoter fused to GFP in pKM074 (*leu1+* in pBluescript). The resulting plasmids were integrated into the *leu1* locus of *mod5*Δ cells. The expression level of each mod5p internal deletion protein in EMM liquid media was confirmed by western blot with anti-GFP antibodies and adjusted to wild-type levels by the addition of 150 nM thiamine. Deletions of amino acids 156-205 and 206-255 at the endogenous *mod5+* locus were constructed by first inserting the *ura4+* gene between nucleotides 580 and 680 of *mod5*, and then replacing *ura4+* under 5-fluoroorotic acid (5-FOA) selection with PCR generated fragments of *mod5* lacking the sequence encoding amino acids 156-205 or 206-255.

Construction of mCherry and tdTomato tagging plasmids

To construct plasmids for PCR-mediated tagging of yeast genes with mCherry (mCh) and tdimerTomato (tdT) (Shaner et al.,, 2004), we followed a strategy to make these identical to the tagging plasmids of Bahler et al. (1998), except for the tag itself. The mCh and tdT open reading frames were amplified by PCR, using pRSETB-mCh and pRSETB-tdT plasmids as templates, kindly provided by Dr. R. Tsien, HHMI, UCSD. The PCR products were cloned into pGEM-T Easy (Promega), sequenced and subcloned into PacI and AscI sites of the the appropriate C-terminal tagging vectors (Bahler et al., 1998; Goldstein and McCusker, 1999; Kim et al., 2005). N-terminal tagging vectors were made in the same manner as by Bahler *et al.*, by excising PacI/BglI fragments containing the mCh and tdT sequences from the C-terminal tagging plasmids and subcloning these fragments into PacI/BamHI sites of pFA6a-kanMX6-P3nmt1, pFA6a-kanMX6-P41nmt1 and pFA6a-kanMX6-P81nmt1. Our plasmid nomenclature therefore follows that of Bahler et al. (1998).

A single point mutation was identified in all tdT plasmids constructed, and we subsequently determined that this was also in the parent plasmid pRSETB-tdT. Nucleotide 1244 of the tdT coding sequence was expected to be A but found to be G, converting CAC (His) into CGC (Arg). However, the corresponding bases in the first half of the tdT coding sequence are correct. As far as we know, this mutation is unlikely to affect the properties of the protein.

In order to make a junction that is more compatible for protein fusions, Shaner et al. (2004) added seven amino acids of GFP sequence to both N- and C-termini of both mCherry and tdTomato, and the codons used differ from those used in the GFP-tagging plasmid pFA6a-GFP(S65T)-kanMX6 of Bahler et al. (1998) This does not affect the primer sequence for PCR amplification of cassettes used in C-terminal tagging (i.e.,

plasmids pKS390-pKS393); for these one can use the sequences of Bahler et al. (1998), which hybridize to the linker. However, for N-terminal tagging cassettes (pKS394-pKS399), a different primer sequence is required. The final codons of mCh and tdT are (C) GGC ATG GAC GAG CTG TAC AAG TAA, which encode the amino acids GMDELYK-stop. Therefore, the reverse primer for gene tagging should be: 5'-(gene specific sequence)-CTT GTA CAG CTC GTC CAT GCC (G)-3' (The final G is optional).

Summary of new tagging plasmids, nomenclature following Bahler et al. (1998):

Plasmid	**Description**	**Reference**
pKS390	*pFA6a-mCherry-kanMX6*	This study
pKS391	*pFA6a-mCherry-natMX6*	This study
pKS392	*pFA6a-tdTomato-kanMX6*	This study
pKS393	*pFA6a-tdTomato-natMX6*	This study
pKS394	*pFA6a-kanMX6-P3nmt1-mCherry*	This study
pKS395	*pFA6a-kanMX6-P41nmt1-mCherry*	This study
pKS396	*pFA6a-kanMX6-P81nmt1-mCherry*	This study
pKS397	*pFA6a-kanMX6-P3nmt1-tdTomato*	This study
pKS398	*pFA6a-kanMX6-P41nmt1-tdTomato*	This study
pKS399	*pFA6a-kanMX6-P81nmt1-tdTomato*	This study

Microscopy

For still images of tea3p-GFP, Z-series of 6 sections with 0.5μm spacing were acquired using the appropriate neutral density filters, and for timelapse movie analysis of tea3p-GFP Z-series of 5 sections with 0.5 μm spacing were collected at 15 s intervals (with neutral density filters). For time lapse Z-series acquisition of cells doubly labelled with tea3p-mCh and either tea1p-GFP or GFP-atb2p, Z-series of 4 sections at 0.6 μm spacing were collected with exposure times of 1200 ms for mCh and 800 ms for mCh, at 15 s intervals (with neutral density filters)

SUPPLEMENTARY TABLES

Supplementary Table 1: Plasmids used in this study

Plasmid	Description	Reference
pKS183	*pBTM116*	(Vojtek and Cooper, 1993)
pKS185	*pBTM116-1*	(Vojtek and Cooper, 1993)
pKS135	*pGAD424*	(Bartel et al., 1993)
pKS156	*pAA*	(Chang et al., 1994)
pKS182	*mod5aa1-519* in *pBMT116*	This study
pKS148	*tea3aa739-1125* in *pGAD424*	This study
pKS173	*tea3aa513-1125* in *pGAD424*	This study
pKS383	*mod5aa1-519* in *pGAD424*	This study
pKS384	*mod5aa1-519* in *pAA*	This study
pKS385	*tea1aa1-500* in *pBTM116*	This study
pKS21	*GFP* in *pGEX-4T1*	Laboratory stock
pKS181	*mod5aa28-447* in *pGEX-4T3*	This study
pKS235	*tea1aa1-1147* in *pGEM-T*	This study
pKS186	*tea3aa1-1125* in *pBS-KS*	This study
pKS212	*mod5Δ56-105* in *pBTM116-1*	This study
pKS199	*mod5Δ106-155* in *pBTM116-1*	This study
pKS254	*mod5Δ156-205* in *pBTM116-1*	This study
pKS256	*mod5Δ206-255* in *pBTM116-1*	This study
pKS202	*mod5Δ256-305* in *pBTM116-1*	This study
pKS203	*mod5Δ306-355* in *pBTM116-1*	This study
pKS204	*mod5Δ356-405* in *pBTM116-1*	This study
pKS205	*mod5Δ406-455* in *pBTM116-1*	This study
pKS233	*mod5aa156-205* in *pBMM116-1*	This study
pKS237	*mod5aa156-255* in *pBTM116-1*	This study
pKS190	*nmt81GFP* in *pKM074*	From H. Ohkura
pKS136	*mod5Δ1-55* in *pKS190*	This study
pKS137	*mod5Δ56-105* in *pKS190*	This study
pKS138	*mod5Δ106-155* in *pKS190*	This study
pKS247	*mod5Δ156-205* in *pKS190*	This study
pKS249	*mod5Δ206-255* in *pKS190*	This study
pKS141	*mod5Δ256-305* in *pKS190*	This study
pKS142	*mod5Δ306-355* in *pKS190*	This study
pKS143	*mod5Δ356-405* in *pKS190*	This study
pKS144	*mod5Δ406-455* in pKS190	This study
pKS145	*mod5Δ456-519* in pKS190	This study
pKS146	*mod5aa1-522* in *pKS190*	This study
pKS386	*pRSETB-mCherry*	Shaner et al., 2004
pKS387	*pRSETB-tdTomato*	Shaner et al., 2004

Supplementary Table 2: Yeast strains used in this study

	***S. pombe* strains**	
Strain	**Genotype**	**Reference**
KS136	*h- tea1Δ::ura4+ ade6-M210 ura4-D18*	(Mata and Nurse, 1997)
KS515	*h+ ade6-M216 leu1-32 ura4-D18*	Lab strain
KS564	*h- orb2-34 ade6-M216 leu1-32 ura4-D18*	This study
KS706	*h- kanMX6:nmt81GFPmod5 ade6-M210 leu1-32 ura4-D18*	(Snaith and Sawin, 2003)
KS753	*h- mod5Δ::kanMX6 tea1Δ::ura4+ ade6-M210 ura4-D18*	(Snaith and Sawin, 2003)
KS780	*h+ mod5Δ::kanMX6 ade6-M216 leu1-32 ura4-D18*	(Snaith and Sawin, 2003)
KS781	*h- tip1Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
KS1066	*h+ p[nmt81GFPmod5Δ1-55:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1069	*h+ p[nmt81GFPmod5Δ56-105:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1071	*h+ p[nmt81GFPmod5Δ106-155:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1075	*h+ p[nmt81GFPmod5Δ206-255:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1076	*h+ p[nmt81GFPmod5Δ256-305:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1078	*h+ p[nmt81GFPmod5Δ306-355:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1080	*h+ p[nmt81GFPmod5Δ356-405:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1082	*h+ p[nmt81GFPmod5Δ406-455:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1086	*h+ p[nmt81GFPmod5 1-522:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M216 ura4-D18*	This study
KS1098	*h+ kanMX6:nmt41GSTmod5 ade6-M210 leu1-32 ura4-D18*	This study
KS1141	*h+ p[nmt81GFPmod5Δ1-55:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1143	*h- p[nmt81GFPmod5Δ56-105:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1144	*h+ p[nmt81GFPmod5Δ106-155:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1145	*h+ p[nmt81GFPmod5Δ156-205:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1147	*h+ p[nmt81GFPmod5Δ206-255:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1148	*h+ p[nmt81GFPmod5Δ256-305:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1149	*h+ p[nmt81GFPmod5Δ306-355:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study

KS1150	*h+ p[nmt81GFPmod5Δ356-405:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1151	*h+ p[nmt81GFPmod5Δ406-455:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1153	*h+ p[nmt81GFPmod5 1-522:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1160	*h- tea3Δ::kanMX6 leu1-32 ura4-D18*	(Arellano et al., 2002)
KS1161	*h+ tea3GFP:kanMX6 leu1-32 ura4-D18*	(Arellano et al., 2002)
KS1171	*h- kanMX6:nmt81GFPmod5 tea3Δ::his3+ ade6-M210 his3-D1 leu1-32 ura4-D18*	This study
KS1222	*h+ mod5Δ::kanMX6 tea3GFP:kanMX6 ade-6-M210 leu1-32 ura4-D18*	This study
KS1224	*h- tea3GFP:kanMX6 tea1Δ::ura4+ ade6-M210 leu1-32 ura4-D18*	This study
KS1264	*h- tea3Δ::kanMX6 tea1Δ::ura4+ ade6-M210 leu1-32 ura4-D18*	This study
KS1267	*h+ mod5Δ::kanMX6 tea3Δ::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS1310	*h+ mod5Δ::kanMX6 tip1Δ::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS1419	*h- tea1Δ200::kanMX6 ade6-M216 leu1-32 ura4-D18*	(Behrens and Nurse, 2002)
KS1443	*h+ kanMX6:nmt81GFPmod5 tea1Δ200::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS1690	*h+ mod5Δ::kanMX6 p[nmt81GFPmod5Δ156-205:leu1+]:leu1-32 ade6-M216 ura4-D18*	This study
KS1749	*h+ kanMX6:nmt41GSTmod5 tea3Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
KS1753	*h+ kanMX6:nmt41GSTmod5 tea3GFP:kanMX6 tea1Δ::ura4+ ade6-M16 leu1-32 ura4-D18*	This study
KS1891	*h+ mod5Δ156-205 ade6-M216 leu1-32 ura4-D18*	This study
KS1892	*h+ mod5Δ206-255 ade6-M216 leu1-32 ura4-D18*	This study
KS1927	*h+ kanMX6:nmt41GSTmod5Δ156-205 ade6-M216 leu1-32 ura4-D18*	This study
KS1928	*h+ kanMX6:nmt41GSTmod5Δ206-255 ade6-M216 leu1-32 ura4-D18*	This study
KS1929	*h+ tea3HA:kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS1947	*h- mod5Δ::kanMX6 tea3HA:kanMX6 ade6-M216 ura4-D18*	This study
KS2048	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ1-55:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2049	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ56-105:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2050	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ106-155:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2051	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ156-205:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2052	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ206-255:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2053	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ256-305:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study

KS2054	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ306-355:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2055	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ356-405:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2056	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5Δ406-455:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2058	*h+ tip1Δ::kanMX6 p[nmt81GFPmod5 1-522:leu1+]:leu1-32 mod5Δ::kanMX6 ade6-M210 ura4-D18*	This study
KS2150	*h- tea3GFP:kanMX6 tea1Δ200::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS2157	*h+ tea3GFP:kanMX6 mod5Δ156-205 ade6-M216 leu1-32 ura4-D18*	This study
KS2158	*h+ tea3GFP:kanMX6 mod5Δ206-255 ade6-M216 leu1-32 ura4-D18*	This study
KS2159	*h+ tea3HA:kanMX6 tea1Δ200::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS2183	*h- tea3GFP:kanMX6 bud6Δ::kanMX6 leu1-32 ura4-D18*	This study
KS2208	*h- kanMX6:nmt41GSTmod5 tea1Δ200::kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS2223	*h- tea3GFP:kanMX6 tea1Δ::ura4+ mod5Δ::kanMX6 ade6-M210 leu1-32 ura4-D18*	This study
KS2526	*h+ tea3-mCherry:natMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS2534	*h- tea3-mCherry:natMX6 tea1GFP:kanMX6 mod5Δ::natMX6 ade6-M210 leu1-32 ura4-D18*	This study
KS2535	*h- natMX6:nmt41GSTmod5 tea3HA:kanMX6 tea1Δ200:kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS2536	*h+ tea3Δ501-1125HA:kanMX6 ura4-D18 leu1-32 ade6-M216*	This study
KS2537	*h+ tea3Δ703-1125HA:kanMX6 ura4-D18 leu1-32 ade6-M216*	This study
KS2538	*h+ tea3Δ901-1125HA:kanMX6 ura4-D18 leu1-32 ade6-M216*	This study
KS2569	*h- tea3-mCherry:natMX6 kanMX:nmt41GFPabt2 ade6-M216 leu1-32 ura4-D18*	This study
KS2574	*h- tea3-mCherry:natMX6 tea1GFP:kanMX6 ade6-M216 leu1-32 ura4-D18*	This study
KS2576	*h+ tea3-mCherry:natMX6 kanMX6:nmt41GFPatb2 mod5Δ::natMX6 ade6-M210 leu1-32 ura4-D18*	This study

***S. cerevisiae* strain**

L40	*MAT**a** his3Δ200 trp-901 leu2-3112 ade2 lys2-801am URA3(LexAop)$_8$-lacZ LYS2(lexAop)$_4$-HIS3*	(Hollenberg et al., 1995)

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SUPPLEMENTARY MOVIE LEGENDS:

Supplementary Movie 1:
Wild-type cell expressing tea3p-GFP. Timelapse images were acquired as described in Supplementary Materials and Methods, with cells imaged every 15 seconds. A total of 5 minutes of real-time is shown. Note the lack of signficiant movement of tea3p-GFP particles in the cytoplasm.

Supplementary Movie 2:
*mod5*Δ cells expressing tea3p-GFP. Timelapse images were acquired as described in Supplementary Materials and Methods, with cells imaged every 15 seconds. A total of 2.5 minutes of real-time is shown. Note two separate particles of tea3p-GFP moving in linear fashion to opposte cell tips.

Supplementary Movie 3:
*mod5*Δ *tea1*Δ cells expressing tea3p-GFP. Timelapse images were acquired as described in Supplementary Materials and Methods, with cells imaged every 15 seconds. A total of 2.5 minutes of real-time is shown. Note the reduced movement of tea3p-GFP particles relative to Supplementary Movie 2.