Supplemental Methods

Cloning of plasmids

Standard methods of recombinant DNA manipulation were used. See Supplemental table I for a list of plasmids used in this study.

Plasmids for Bimolecular fluorescence complementation assays

For expression of Rsr1 and its mutant proteins fused to YFP^N, the *Not*I fragment (carrying the GFP portion) of pRS304-GFP-RSR1 (pHP808), pRS304-GFP-rsr1$^{K260-264S}$ (pHP1065), pRS304-GFP-RSR1^{G12V} (pHP841) and pRS304-GFP-RSR1^{K16N} (pHP842) (Park *et al.*, 2002) was replaced with the *Not*I fragment carrying the YFP^N fragment from pRS304-TRR1-YFP^N (pHP1514) (Singh *et al.*, 2008), resulting in pRS304-YFP^N-RSR1 (pHP1598), pRS304-YFP^N-rsr1$^{K260-264S}$ (pHP1614), pRS304-YFP^N-RSR1^{G12V} (pHP1619) and pRS304-YFP^N-RSR1^{K16N} (pHP1679), respectively. Similarly, the GFP portion was replaced with the DNA fragment encoding YFP^C from pRS306-RHO5-YFP^C (pHP1501) (Singh *et al.*, 2008), resulting in pRS304-YFP^C-RSR1 (pHP1612), pRS304-YFP^C-rsr1$^{K260-264S}$ (pHP1613), pRS304-YFP^C-RSR1^{G12V} (pHP1674) and pRS304-YFP^C-RSR1^{K16N} (pHP1678). To express YFP^C-Rsr1 from a CEN plasmid, the *Not*I fragment (carrying the GFP portion) of YCp50-GFP-RSR1 (pHP761) was replaced with the *Not*I fragment carrying the YFP^C fragment from pRS306-RHO5-YFP^C, resulting in YCp-YFP^C-RSR1 (pHP1599). Both YFP^N and YFP^C were fused to the N terminus of Rsr1 and its mutants to avoid any possible interference with their C-terminal modification. A control plasmid pHP1730 (YCp50-YFP^C) was generated from YCp50-YFP^C-RSR1 (pHP1599) by deleting the 1.1 kb-SnaBI fragment, resulting in expression of YFP^C fused to the first 30 residues of Rsr1 from the *RSR1* promoter.

The polybasic region (PBR) mutants of *RSR1*

Mutations in the polybasic region (PBR) of *RSR1* were introduced by PCR-based site-directed mutagenesis. The plasmid pRS304-GFP-rsr1-8$^{K260-261S}$ (pHP1040) was generated by PCR using the plasmid pRS304-GFP-RSR1 (pHP808) as a template and a primer pair of oBUD132 (5'-GAATTAGCGCTACTTCACAACAGAGCTCAAAGAAGAAAAACGCTTCCAC-3') and oBUD133 (5'-GTGGAAGCGTTTTTCTTCTTTGAGCTCTGTTGTGAAGTAGCGCTAATTC-3'). Similarly, pRS304-GFP-rsr1-9$^{K263-264S}$ (pHP1041) was generated using a primer pair of oBUD134 (5'-CTTCACAACAAAAGAAAAAGAGCTCAAACGCTTCCACTTGCACTATTC-3') and oBUD135 (5'-GAATAGTGCAAGTGGAAGCGTTTGAGCTCTTTTTCTTTTGTTGTGAAG-3').

The integrating plasmid pRS304-rsr1-7$^{K260-264S}$ (pHP1373) was generated from pRS304-GFP-rsr1-7$^{K260-264S}$ (pHP1065) (Park *et al.*, 2002) by deleting the *Not*I fragment carrying the GFP moiety. Other integrating plasmids pRS304-rsr1-8$^{K260-261S}$ (pHP1747) and pRS304-rsr1-9$^{K263-264S}$ (pHP1748) were generated similarly from pHP1040 and pHP1041. To generate an integrating plasmid carrying *rsr1-7$^{K260-264S}$* with the *LEU2* marker, the 1.58 kb *Sal*I-*Sac*I fragment (carrying *rsr1$^{K260-264S}$*) from pHP1373 was inserted into pRS305, resulting in pRS305-rsr1-7$^{K260-264S}$ (pHP1634).

To construct multicopy plasmids carrying the *rsr1-8$^{K260-261S}$* and *rsr1-9$^{K263-264S}$* alleles and the *URA3* marker, the 1.09 kb *Bsu36*I-*Sal*I fragment of YEplac195-RSR1 (pHP1118) (Kozminski *et al.*, 2003) was replaced with the *Bsu36*I-*Sal*I fragment from pHP1040 and pHP1041, resulting in pHP1755 and pHP1753, respectively.

To construct multicopy plasmids carrying the *rsr1*$^{G12V,\ K260\text{-}264S}$ and *rsr1*$^{K16N,\ K260\text{-}264S}$ alleles with URA3 marker, the 1.09 kb *Bsu*36I-*Sal*I fragments of YEplac195 *rsr1*^{G12V} (pHP1125) and YEplac195 *rsr1*^{K16N} (pHP1126) (Kozminski *et al.*, 2003) were replaced with the *Bsu*36I-*Sal*I fragment from pHP1065, resulting in pHP1771 and pHP1772, respectively.

Plasmids for Rsr1 protein purification

GST-Rsr1 was expressed in *E. coli* using the plasmid pRS4 (pHP593) (Holden *et al.*, 1991). To express GST-rsr1-7$^{K260\text{-}264S}$ in yeast, the 1.09 kb *Bsu36*I-*Sal*I fragment of pRD56-RSR1 (pHP594) (Park *et al.*, 1993) was replaced with *Bsu36*I-*Sal*I fragment from pHP1065, resulting in pRD56-rsr1-7$^{K260\text{-}264S}$ (pHP1630). To express GST-rsr1-7$^{K260\text{-}264S}$ in *E. coli*, the 1.4 kb *BamH*I-*Sal*I fragment from pHP1630 was then cloned into pGEX2*vector, resulting in pGEX2*-rsr1-7$^{K260\text{-}264S}$ (pHP1651).

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Supplemental Table I. Plasmids used in this study

Plasmid	Description	Source/Comments[@]
pRS304	*TRP1* (integrative)	(Sikorski and Hieter, 1989)
pRS305	*LEU2* (integrative)	(Sikorski and Hieter, 1989)
YEplac195	*URA3* (high copy)	(Gietz and Sugino, 1988)
pHP501	pUC18-bud5Δ::URA3	(Chant *et al.*, 1991)
pHP529	pUC19-bud2Δ::LEU2	(Park *et al.*, 1993)
pHP582	pRS425-RSR1-HA	(Park *et al.*, 1993)
pHP808	pRS304-GFP-RSR1	(Park *et al.*, 2002)
pHP841	pRS304-GFP-RSR1^{G12V}	(Park *et al.*, 2002)
pHP842	pRS304-GFP-RSR1^{K16N}	(Park *et al.*, 2002)
pHP1065	pRS304-GFP-rsr1-7$^{K260-264S}$	(Park *et al.*, 2002)
pHP1501	pRS306-RHO5-YFP^{C}	(Singh *et al.*, 2008)
pHP1514	pRS304-TRR1-YFP^{N}	(Singh *et al.*, 2008)
pHP1598	pRS304-YFP^{N}-RSR1	Derived from pHP808; This study
pHP1612	pRS304-YFP^{C}-RSR1	Derived from pHP808; This study
pHP1614	pRS304-YFP^{N}-rsr1-7$^{K260-264S}$	Derived from pHP1065; This study
pHP1613	pRS304-YFP^{C}-rsr1-7$^{K260-264S}$	Derived from pHP1065; This study
pHP1619	pRS304-YFP^{N}-RSR1^{G12V}	Derived from pHP841; This study
pHP1674	pRS304-YFP^{C}-RSR1^{G12V}	Derived from pHP841; This study
pHP1679	pRS304-YFP^{N}-RSR1^{K16N}	Derived from pHP842; This study
pHP1678	pRS304-YFP^{C}-RSR1^{K16N}	Derived from pHP842; This study
pHP761	YCp50-GFP-RSR1	(Park *et al.*, 2002)
pHP1599	YCp50-YFP^{C}-RSR1	Derived from pHP761; This study
pHP1730	YCp50-YFP^{C}-rsr1Δ	Derived from pHP1599; This study
pHP1373	pRS304-rsr1-7$^{K260-264S}$	Deleted GFP from pHP1065; This study
pHP1634	pRS305-rsr1-7$^{K260-264S}$	This study
pHP1040	pRS304-GFP-rsr1-8$^{K260-261S}$	This study
pHP1041	pRS304-GFP-rsr1-9$^{K263-264S}$	(Park *et al.*, 2002)
pHP1747	pRS304-rsr1-8$^{K260-261S}$	This study
pHP1748	pRS304-rsr1-9$^{K263-264S}$	This study
pHP1118	YEplac195-RSR1	(Kozminski *et al.*, 2003)
pHP1123	YEplac195-rsr1-7$^{K260-264S}$	(Kozminski *et al.*, 2003)
pHP1125	YEplac195-RSR1^{G12V}	(Kozminski *et al.*, 2003)
pHP1126	YEplac195- RSR1^{K16N}	(Kozminski *et al.*, 2003)
pHP1755	YEplac195- rsr1-8$^{K260-261S}$	This study

pHP1753	YEplac195- rsr1-9$^{K263\text{-}264S}$	This study
pHP1771	YEplac195- RSR1$^{G12V,\ K260\text{-}264S}$	This study
pHP1772	YEplac195- RSR1$^{K16N,\ K260\text{-}264S}$	This study
pHP635	pGEX2*	A *E. coli* GST fusion plasmid (Amp^{R})
pHP593	pRS4-RSR1	(Holden *et al.*, 1991)
pHP594	pRD56-RSR1	GALp-GST-RSR1, CEN, URA3 (Park *et al.*, 1993)
pHP1630	pRD56-rsr1-7	This study
pHP1651	pGEX2*-rsr1-7$^{K260\text{-}264S}$	This study
pHP1593	pKTUB1-CFP [URA3]	From Marie-Noelle Simon (Bailly *et al.*, 2003)

[@] For details of cloning procedure, see Supplemental Methods.

Figure Legends

Suppl. Fig. S1. Analyses of the pixel intensity of YFP-Rsr1 in the cells marked in Figure 3. Localization of YFP-Rsr1 to the mother-bud neck was analyzed in wild type (HPY1632), *bud5Δ* (HPY1668) and *bud2Δ* (HPY1664). Pixel intensities along the indicated lines of the 2D images (maximum projection) are shown (in arbitrary unit) after subtracting the background. A cell (a) undergoes nuclear division, whereas cells (b - d) have completed nuclear division (see Figure 3). A blue arrow and arrowheads mark the position of the mother-bud neck; and grey arrows mark the position of the plasma membrane. About 2~2.5 fold higher fluorescence intensity was observed at the mother-bud (compared to the overall plasma membrane) in wild-type and *bud2Δ* cells after anaphase.

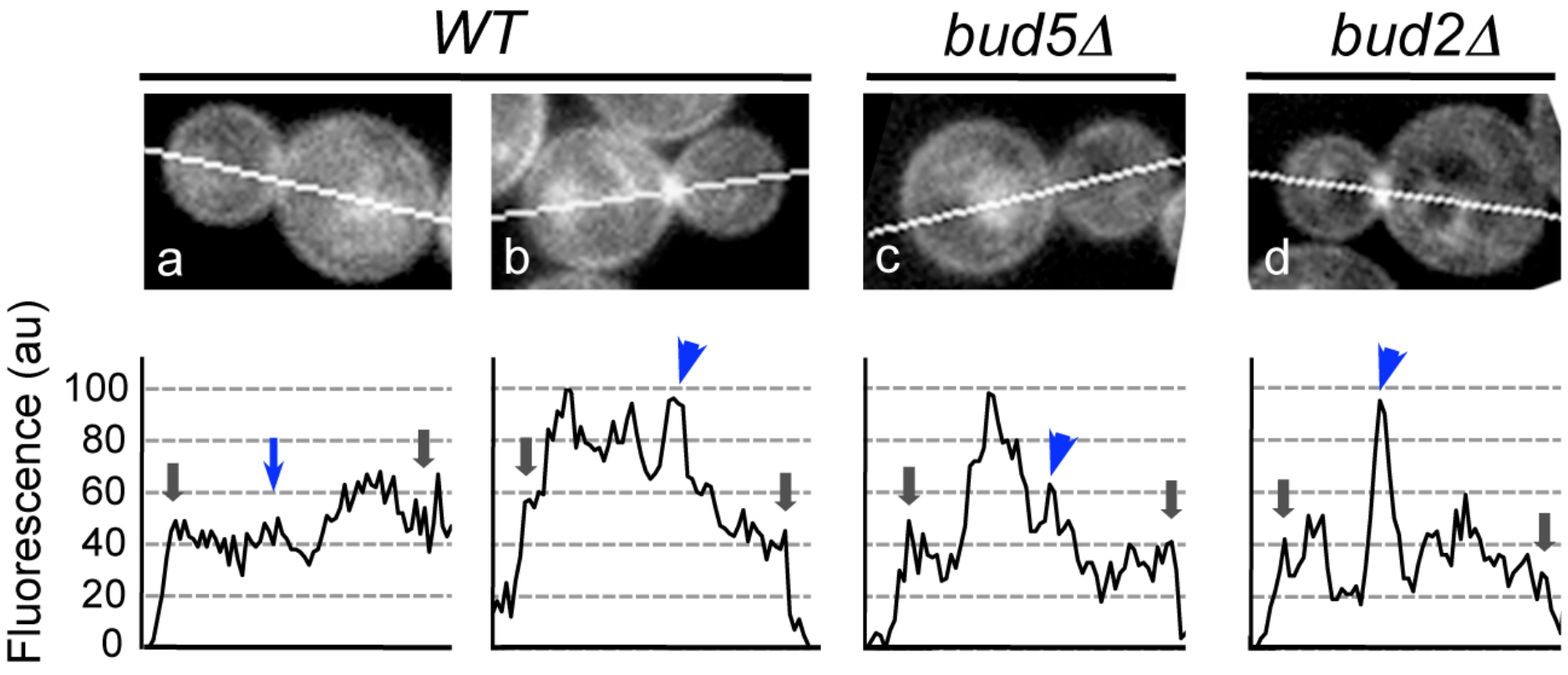
Suppl. Fig. S2. The steady-state levels of GFP-Rsr1 and GFP fusions of the Rsr1 mutant proteins at 25°C, 30°C and 37°C.

Strains expressing GFP-Rsr1 (HPY401), GFP-Rsr1-7 (HPY621), GFP-Rsr1-8 (HPY588), and GFP-Rsr1-9 (HPY589) were grown at the indicated temperatures. Each GFP fusion was expressed from the *RSR1* locus on the chromosome. Approximately equal amounts of the total protein were loaded on each lane. The GFP fusion proteins were detected with polyclonal antibodies against GFP (top panel). An asterisk marks a non-specific cross-reacting band (lower panel) that was used as a loading control. The level of GFP-Rsr1-9 was higher than wild type and other Rsr1 mutant proteins at all temperatures tested. This is likely to be caused by the multiple insertion events of the plasmid on the chromosome.

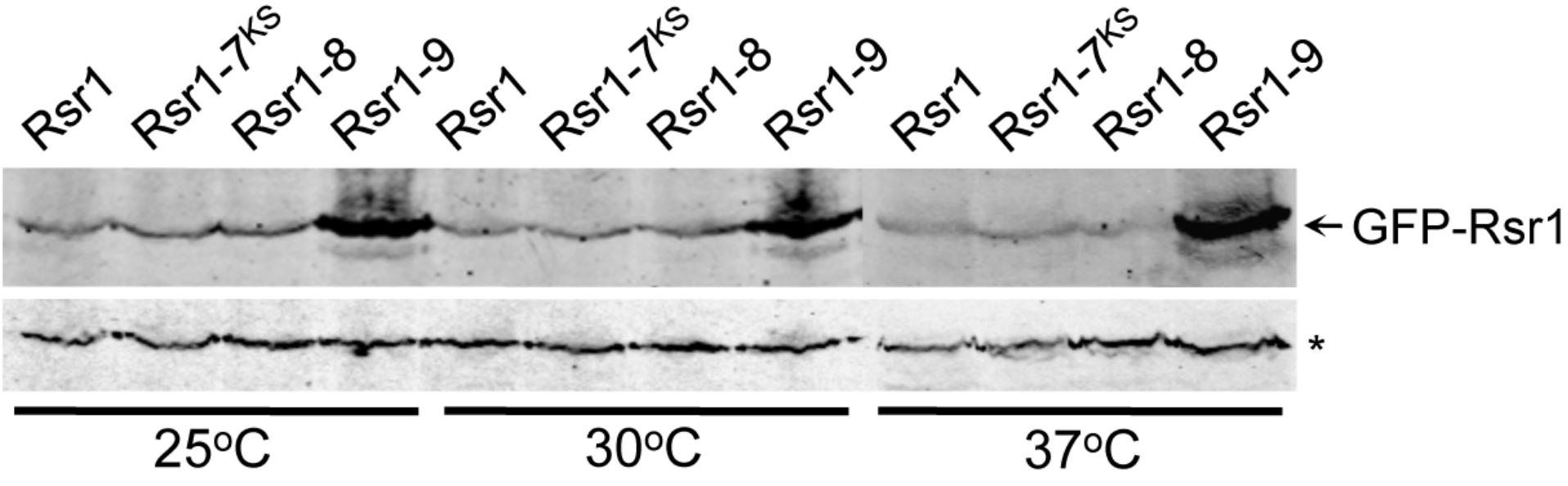
Suppl. Fig. S3. The fluorescence intensity of YFP-Rsr1 and YFP-Rsr1^{K16N} are about the same at 25°C and 30°C.

A. A single Z section of cells (HPY402), which express YFP-Rsr1 from the *RSR1* locus, is shown after deconvolution. Cell were grown at 25°C (a) and 30°C (b). Pixel intensity along the line is shown below after subtracting the background. Bar, 5 μm.

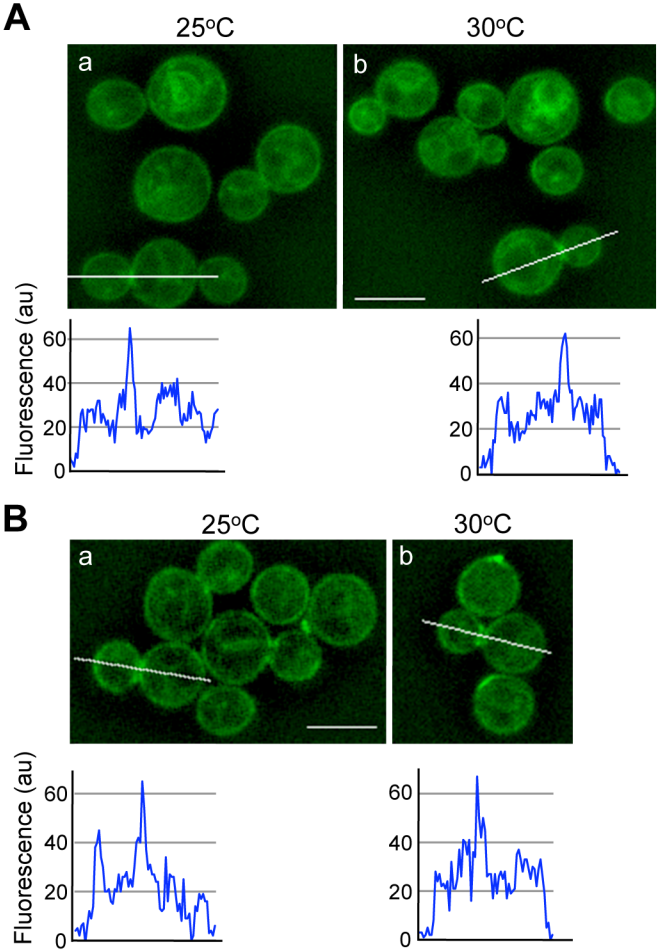
B. A single Z section of cells (HPY423) expressing YFP-Rsr1^{K16N} from the *RSR1* locus is shown as in A. Bar, 5 μm.



Supplemental Fig S1



Supplemental Fig S2



Supplemental Fig S3