Table S1. Yeast strains used in this study

Strain	Relevant Genotype[a]	Source/Comments
HPY210[#]	*MAT***a** *his3-Δ200 leu2-Δ1 lys2-801 trp1Δ63 ura3-52*	(SINGH *et al.* 2008)
BY4741[@]	*MAT***a** *his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0*	Resgen Deletion Collection (GE Dharmacon)
YSC1053[@]	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 yfg::kanMX4* (Yeast *MAT***a** collection for 5154 nonessential genes)	Resgen Deletion Collection (GE Dharmacon)
HY-VNL[@]	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0'X'-VN::URA3* (Yeast haploid VN fusion library)	(SUNG *et al.* 2013)
EGY48	*MAT***a** *his3 trp1 ura3 LexA$_{op(x6)}$-LEU2*	(GYURIS *et al.* 1993)
HPY657[@]	*MATα his3-Δ1 leu2-Δ0 ura3-Δ0 lys2Δ0 rho5Δ::kanMX4*	Resgen Deletion Collection (GE Dharmacon)
HPY720[#]	*MAT***a** *rho5Δ::kanMX4*	(SINGH *et al.* 2008)
HPY999[#]	*MAT***a** *rho5^{G12V}::URA3::rho5Δ::kanMX4*	(SINGH *et al.* 2008)
HPY1690[#]	*MAT***a** *aim27Δ::kanMX4*	This study[b]
HPY1691[#]	*MAT***a** *isu1Δ::kanMX4*	This study[b]
HPY1692[#]	*MAT***a** *aim19Δ::kanMX4*	This study[b]
HPY1693[#]	*MAT***a** *atg21Δ::kanMX4*	This study[b]
HPY1694[#]	*MAT***a** *mss1Δ::kanMX4*	This study[b]
HPY1695[#]	*MAT***a** *msp1Δ::kanMX4*	This study[b]
HPY1696[#]	*MAT***a** *mso1Δ::kanMX4*	This study[b]
HPY1697[#]	*MAT***a** *cwp1Δ::kanMX4*	This study[b]
HPY1698[#]	*MAT***a** *paf1Δ::kanMX4*	This study[b]
HPY1699[#]	*MAT***a** *kin3Δ::kanMX4*	This study[b]
HPY1700[#]	*MAT***a** *cnb1Δ::kanMX4*	This study[b]
HPY1706[#]	*MAT***a** *oca1Δ::kanMX4*	This study[b]
HPY1707[#]	*MAT***a** *yor378wΔ::kanMX4*	This study[b]
HPY1708[#]	*MAT***a** *oye3Δ::kanMX4*	This study[b]
HPY1709[#]	*MAT***a** *ras1Δ::kanMX4*	This study[b]
HPY1742[#]	*MAT***a** *tma19Δ::kanMX4*	This study[b]
HPY1778[#]	*MAT***a** *ste24Δ::kanMX4*	This study[b]
HPY1830[#]	*MAT***a** *lsp1Δ::kanMX4*	This study[b]
HPY1831[#]	*MAT***a** *nce102Δ::kanMX4*	This study[b]
HPY1841[#]	*MAT***a** *dse1Δ::kanMX4*	This study[b]
HPY1842[#]	*MAT***a** *dse2Δ::kanMX4*	This study[b]

HPY1843[#]	*MAT***a** *ktr1Δ::kanMX4*	This study[b]
HPY1909[#]	*MAT***a** *glo2Δ::kanMX4*	This study[b]
HPY1910[#]	*MAT***a** *hxk2Δ::kanMX4*	This study[b]
HPY1911[#]	*MAT***a** *hxt3Δ::kanMX4*	This study[b]
HPY1912[#]	*MAT***a** *hxt1Δ::kanMX4*	This study[b]
HPY1913[#]	*MAT***a** *sur7Δ::kanMX4*	This study[b]
HPY1914[#]	*MAT***a** *rpl8aΔ::kanMX4*	This study[b]
HPY1915[#]	*MAT***a** *him1Δ::kanMX4*	This study[b]
HPY1929[#]	*MAT***a** *yor227wΔ::kanMX4*	This study[b]
HPY1930[#]	*MAT***a** *hxt5Δ::kanMX4*	This study[b]
HPY1931[#]	*MAT***a** *rgt1Δ::kanMX4*	This study[b]
HPY1934[#]	*MAT***a** *vph1Δ::kanMX4*	This study[b]
HPY2024[#]	*MAT***a** *eds1Δ::kanMX4*	This study[b]
HPY2025[#]	*MAT***a** *bna1Δ::kanMX4*	This study[b]
HPY2026[#]	*MAT***a** *ybl095wΔ::kanMX4*	This study[b]
HPY2027[#]	*MAT***a** *ynl134cΔ::kanMX4*	This study[b]
HPY2028[#]	*MAT***a** *rtn1Δ::kanMX4*	This study[b]
HPY2029[#]	*MAT***a** *yox1Δ::kanMX4*	This study[b]
HPY2030[#]	*MAT***a** *gal7Δ::kanMX4*	This study[b]
HPY2031[#]	*MAT***a** *mf(α)2Δ::kanMX4*	This study[b]
HPY2033[#]	*MAT***a** *arf1Δ::kanMX4*	This study[b]
HPY2034[#]	*MAT***a** *ygl159wΔ::kanMX4*	This study[b]
HPY2035[#]	*MAT***a** *pml1Δ::kanMX4*	This study[b]
HPY2037[#]	*MAT***a** *ylr001cΔ::kanMX4*	This study[b]
HPY2041[#]	*MAT***a** *dbf20Δ::kanMX4*	This study[b]
HPY2042[#]	*MAT***a** *cat2Δ::kanMX4*	This study[b]
HPY2044[#]	*MAT***a** *sli1Δ::kanMX4*	This study[b]
HPY2045[#]	*MAT***a** *mei4Δ::kanMX4*	This study[b]
HPY2048[#]	*MAT***a** *spo1Δ::kanMX4*	This study[b]
HPY2049[#]	*MAT***a** *tom70Δ::kanMX4*	This study[b]
HPY2050[#]	*MAT***a** *smi1Δ::kanMX4*	This study[b]
HPY2051[#]	*MAT***a** *rma1Δ::kanMX4*	This study[b]
HPY2052[#]	*MAT***a** *suc2Δ::kanMX4*	This study[b]
HPY2068[#]	*MAT***a** *uip3Δ::kanMX4*	This study[b]
HPY2069[#]	*MAT***a** *msl1Δ::kanMX4*	This study[b]
HPY2071[#]	*MAT***a** *yjr056cΔ::kanMX4*	This study[b]
HPY2072[#]	*MAT***a** *rpl41bΔ::kanMX4*	This study[b]

HPY2073[#]	*MAT***a** *nmd2Δ::kanMX4*	This study[b]
HPY2074[#]	*MAT***a** *yor036cΔ::kanMX4*	This study[b]
HPY2076[#]	*MAT***a** *rpl16AΔ::kanMX4*	This study[b]
HPY2084[#]	*MAT***a** *she2Δ::kanMX4*	This study[b]
HPY2085[#]	*MAT***a** *met1Δ::kanMX4*	This study[b]
HPY2086[#]	*MAT***a** *imp2Δ::kanMX4*	This study[b]
HPY2087[#]	*MAT***a** *cdc10Δ::kanMX4*	This study[b]
HPY2110[#]	*MAT***a** *kgd1Δ::kanMX4*	This study[b]
HPY2043[#]	*MAT***a** *mns1Δ::kanMX4*	This study[b]
KY151[#]	*MAT***a** *yca1Δ::kanMX4*	This study[b]
KY152[#]	*MAT***a** *aif1Δ::kanMX4*	This study[b]
KY153[#]	*MAT***a** *ste20Δ::kanMX4*	This study[b]
KY154[#]	*MAT***a** *nuc1Δ::kanMX4*	This study[b]
HY1029[@]	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 His3MX6::pRPL7B-VC-RHO5*	This study[c]
HPY1631[@]	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rho5Δ::kanMX4:RHO5-YFP^{C}-URA3*	This study[d]
HPY1630[@]	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rho5Δ::kanMX4:rho5^{G12V}-YFP^{C}-URA3*	This study[d]
HPY1637[@]	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 rho5Δ::kanMX4:rho5^{K16N}-YFP^{C}-URA3*	This study[d]
HPY3309[@]	*MAT***a***/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 met15Δ0/MET15 RHO5/rho5Δ::kanMX4:rho5^{K16N}-YFP^{C}-URA3 ATG21-VN::URA3/ATG21*	This study[e]
HPY3310[@]	*MAT***a***/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 met15Δ0/MET15 RHO5/rho5Δ::kanMX4:RHO5-YFP^{C}-URA3 ATG21-VN::URA3/ATG21*	This study[e]
HPY3338[@]	*MAT***a***/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 met15Δ0/MET15 RHO5/rho5Δ::kanMX4:rho5^{G12V}-YFP^{C}-URA3 ATG21-VN::URA3/ATG21*	This study[e]

[a] Strains marked with [#] are isogenic to HPY210, which was derived from YEF473 (BI AND PRINGLE 1996; SINGH *et al.* 2008), except indicated; and the background of the strains marked with [@] is S288C.
[b] Each yfg::kanMX4 from YSC1053[@] (that has been confirmed by genomic PCR) was amplified by PCR using genomic DNA from each strain. The PCR product was used to delete each gene in HPY210 background by one-step gene disruption (ROTHSTEIN 1991). The resulting deletion strains were confirmed by genomic PCR.
[c] *RHO5* was tagged with VC by a PCR-based N-terminal-targeting method (SUNG AND HUH 2007).

[d] Plasmid pRS306-YFP^{C}-RHO5 (pHP1501), pRS306-YFP^{C}-rho5^{G12V} (pHP1506), and pRS306-YFP^{C}-rho5^{K16N} (pHP1511) (SINGH *et al.* 2008) were digested with *Bsi*WI, and each linearized plasmid was integrated into the *RHO5* locus in HPY657.
[e] The *ATG21-VN* strain (from the VN library) was mated with each *YFP^{C}-RHO5* strain.

Table S2. Plasmids used in this study

Plasmid Name	Description	Source/Comments
pHP1280	pRS426-RHO5 (2μ, *URA3*)	(SINGH *et al.* 2008)
pHP1281	pRS426-rho5^{G12V} (2μ, *URA3*)	(SINGH *et al.* 2008)
pHP1282	pRS426-rho5^{K16N} (2μ, *URA3*)	(SINGH *et al.* 2008)
pEG202	*ADH1* promoter-LexA DNA binding domain (2μ, *HIS3*)	(GYURIS *et al.* 1993)
pHP1382	pEG202-RHO5^{C328S}	(SINGH *et al.* 2008)
pHP1386	pEG202-rho5$^{G12V, C328S}$	(SINGH *et al.* 2008)
pHP1387	pEG202-rho5$^{K16N, C328S}$	(SINGH *et al.* 2008)
pJG4-5	*GAL1* promoter-activation domain (2μ, *TRP1*)	(GYURIS *et al.* 1993)
pHP2256	pJG4-5-ATG21	This study[a]

[a] pJG4-5-ATG21 was constructed by gap repair as follows: The *ATG21*-encoding DNA sequence plus 40 bp upstream of *EcoR*I site and 40 bp downstream of *Xho*I site in pJG4-5 vector sequence was amplified using a primer pair of oATG214 (CTACCCTTATGATGTGCCAGATTATGCCTCTCCCGAATTC ATGAAAGTATTACAATTCAATC; *EcoR*I recognition site underlined) and oATG215 (TTGACCAAA CCTCTGGCGAAGAAGTCCAAAGCTTCTCGAGTTATGTAAATTTATT ATTTTTAG; *Xho*I recognition site underlined). *EcoR*I/*Xho*I-digested pJG4-5 vector DNA and the PCR product were used for yeast transformation. Plasmid DNAs were recovered from the TRP^{+} transformants and verified.

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