Supplementary Table 1: Primers used in this study

Primer	5'-3' sequence	Comments
AG145	GATGAGGAGACTGTGAAACAG	161_Tag_Cntrl
AG207	AGCAGTGCAAAGCCAAAGTT	161_Tag_F
AG147	gaagcacctgcgccagcaccCTTTCCTAAAGAAGTTATACTCAAACC	161_Tag_R
AG231	catgttggatgattttggtgag	161_SN1
AG232	tgggtcatgtgtggtttagtg	161_SN2
AG257	GACCTGCAGCGTACGAAGCTTCgatactcaagctccaggaaaag	161_SN3
AG208	CTCGAATTCATCGATGATATCAGAggcgcattttaattcgacag	161_SN4
AG209	cccaagaaacaaatgggcta	161_SN5
AG150	cacacctacgaccaaattg	161_SN6
AG070	GAGGAACAAACCGATTCTATTG	167_Tag_Cntrl
AG220	CTACCGGTGCCAGCTCTATT	167_Tag_F
AG356	gatatatctggcaagtgggtg	167_SN1
AG357	ctcgtattgtttagccatggtg	167_SN2
AG358	GACCTGCAGCGTACGAAGCTTCcaatctgacacatggtagctc	167_SN3
AG221	CTCGAATTCATCGATGATATCAGAccccacgtaacccaatgtat	167_SN4
AG222	ttggtggtccgttagagaca	167_SN5
AG178	GAAAACCGCATTATTGGATTATG	167-2_Tag_Cntrl
AG214	GGTGAGAGAGCTACCACTTTTGA	167-2_Tag_F
AG180	gaagcacctgcgccagcaccTTTTCCGTTTAATTGCCCTTCC	167-2_Tag_R
AG181	CTCGAATTCATCGATGATATCAGACTTCTAACTATGTTCAATTAATC	167-2_SN4
AG182	caacaataaggcgaagaattcag	167-2_SN5
AG183	GTAAAGGAAATGGTTGCTTCTG	167-2_SN6
AG184	GAAGAAAGATGGTCCTGAATTG	167-3_Tag_Cntrl
AG216	AACCATGATTCATCCGAAGC	167-3_Tag_F
AG217	gaagcacctgcgccagcaccCTCAACATAGTTATTAGGTACAAATCCA	167-3_Tag_R
AG102	CTTACTAATCGTGGTCGTTTG	167-3_SN1
AG103	GTTAAAGAGAAGATCTATGGAGAC	167-3_SN2
AG104	GACCTGCAGCGTACGAAGCTTCCTATCAGTAGTAAATTGAAAGGAGG	167-3_SN3
AG218	CTCGAATTCATCGATGATATCAGAttttggtactgttttctctcttttt	167-3_SN4(2)
AG219	attggagcgcagataggttg	167-3_SN5(2)
AG189	GATTTGGACTTGTTTGGGTATC	167-3_SN6
AG151	CTGTATTAAATCCAATTGCCAG	162_Tag_Cntrl
AG152	CAACAAATATATCACCATCGATG	162_Tag_F
AG211	gaagcacctgcgccagcaccATCTAACCCACTAGTGATGTCTAACTT	162_Tag_R
AG254	ctagttttttttttggagtgggag	162_SN1

AG255	caataatccaaataaatcaagagac	162_SN2
AG256	GACCTGCAGCGTACGAAGCTTCgatatgtgtgatgctatggttg	162_SN3
AG212	CTCGAATTCATCGATGATATCAGAggaccataaaataatagttgtgctt	162_SN4
AG213	agtttttcgtcatcgctcgt	162_SN5
AG156	ctttctctttctatttctcacttttc	162_SN6
AG171	GAAGAAGAAGAAGAAGAGGCTC	Abp1_Tag_Cntrl
AG172	CGAAAAGGACGAAGATAATG	Abp1_Tag_F
AG173	GAAGCACCTGCGCCAGCACCCTCATTCAAGACAACATAGTTAGC	Abp1_Tag_R
AG174	CTCGAATTCATCGATGATATCAGAGATAAATTGGTGCTATAGCCAG	Abp1_SN4
AG175	GTTGCCACACAGAAGTTTTAC	Abp1_SN5
AG176	TTCAACAAAACATTTAGGAGATTAC	Abp1_SN6
AG075	catttcggaatagtaccacttg	167_SN6
AG169	aaaCTGCAGggtgctggcgcaggtgcttctGTTTCAAAAGGTGAAGAAGATAATAT	yEmRFP_PstI_F
AG170	tGGCGCGCCTTATTTATATAATTCATCCATACCACC	yEmRFP_AscI_R
AG157	TCGACCCCGGGCGCGCCTAGATCTGCCATGGCCCTGCAGAA	pPC_MCS-Asc-Nco-F
AG158	CTAGTTCTGCAGGGCCATGGCAGATCTAGGCGCGCCCGGGG	pPC_MCS-Asc-Nco-R
AG159	TCGAGCGCTGAACAAAAATTGATTTCTGAAGAGGATTTGTCTGGGTCGACCCCGGG	pPC_MYC-F
AG160	CGCGCCCGGGGTCGACCCAGACAAATCCTCTTCAGAAATCAATTTTTGTTCAGCGC	pPC_MYC-R
AG161	TCGAGCGCTTATCCTTATGATGTTCCAGATTATGCTTCTGGGTCGACCCCGGG	pPC_HA-F
AG162	CGCGCCCGGGGTCGACCCAGAAGCATAATCTGGAACATCATAAGGATAAGCGC	pPC_HA-R
AG108	GAGAGATCTAACATGTTGAGTTTTAAAGGGTTTACCAAGG	ScRvs167Bar_F1
AG111	CAAGCGGCCGCTCGAGTTAgccaggagctgccgctac	ScRvs167Bar_R3
AG113	GAGAGATCTGCCATGGGATCTTGGGGAGGATTTAAGAAAG	CaRVS161Bar_F1
AG115	CAAGCGGCCGCCGCGGCTACTTTCCTAAAGAAGTTATACTCAA	CaRVS161Bar_R1
AG116	GAGGGATCCATGGGATCATGGATAGGAATTAAAAAAGCT	CaRVS162Bar_F1
AG118	CAAGCGGCCGCTCGAGTCAATCTAACCCACTAGTGATGTC	CaRVS162Bar_R1
AG119	GAGGGATCCATGGTTGTGGATCAATTGAAAAAT	CaNH2Bar_F1
AG120	GAGGGATCCATGGGAACCAGAGATTACGATAAGGATG	CaNH2Bar_F2
AG123	CAAGCGGCCGCTCGAGTTATTTCTCGTGCACAATTGTTAG	CaNH2Bar_R2
AG124	GAGGGATCCATGGGAAGTTGGGAAGGTTTTAAGAAAG	ScRvs161Bar_F1
AG125	CAAGCGGCCGCTCGAGTTATTTTATCCCGAGCGCACA	ScRvs161Bar_R1
AG126	GAGGGATCCATGGGATCATTTAAAGGATTCAAAAAGGG	CaRvs167Bar_F1
AG127	GAGGGATCCATGGGAGAAATCACCCAAGATGC	CaRvs167Bar_F2
AG130	CAAGCGGCCGCCGCGGTTACCCGACTTTGAAATGAGTGA	CaRvs167Bar_R2

AG114	GAGAGATCTGCCATGGGAAAGACTATGGATAAGGACTTTGATG	CaRVS161Bar_F2
AG070	GAGGAACAAACCGATTCTATTG	167_TAPtag_Control
AG075	catttcggaatagtaccacttg	167_SN6
AG226	cagcaccctgcagcgtacTAATTGCACATAATTACCAGGGA	167_Tag_R
AG227	tctgccggtctccctatagttgcattcatcaaatgaagctg	167_SN4(3)
AG228	tctgccggtctccctatagtcatattttagccccacgtaacc	167_SN4(4)
AG105	GTGTTTTTCGTTTCTATTAGGAG	Rvs167_2_SN1
AG106	CTCACAGGTTTCTGCAGTATAC	Rvs167_2_SN2
AG107	GACCTGCAGCGTACGAAGCTTCCAATCGGTAAGATGATATATATCTG	Rvs167_2_SN3
AG215	CTCGAATTCATCGATGATATCAGAgacaatttccttctaactatgttcaa	Rvs167_2_SN4(2)

Supplementary Table 2: Plasmids used in this study

Name	Vector-insert	Purpose	Source
pAG103	pGEMT-easy-Sc161_FL	Cloning facilitation	This study
pAG105	pGEMT-easy-Sc167_BAR	Cloning facilitation	This study
pAG108	pGEMT-easy-Ca161_FL	Cloning facilitation	This study
pAG109	pGEMT-easy-Ca161(-H0)	Cloning facilitation	This study
pAG113	pGEMT-easy-Ca167_BAR(+H0)	Cloning facilitation	This study
pAG114	pGEMT-easy-Ca167_BAR(-H0)	Cloning facilitation	This study
pAG128	pGEMT-easy-Ca162_FL	Cloning facilitation	This study
pAG125	pGEMT-easy-Ca167-3(+H0)	Cloning facilitation	This study
pAG126	pGEMT-easy-Ca167-3(-H0)	Cloning facilitation	This study
pAG202	pPC97mMYC	Y2H_BAIT_plasmid	This study
pAB05	pPC97mMYC-Sc161_FL	Y2H_BAIT_plasmid	This study
pAB07	pPC97mMYC-Ca161_FL	Y2H_BAIT_plasmid	This study
pAB23	pPC97mMYC-Ca167BAR(+H0)	Y2H_BAIT_plasmid	This study
pAB27	pPC97mMYC-Ca167-3BAR(+H0)	Y2H_BAIT_plasmid	This study
pAB28	pPC97mMYC-Ca167-3BAR(-H0)	Y2H_BAIT_plasmid	This study
pAB30	pPC97mMYC-Ca162_FL	Y2H_BAIT_plasmid	This study
pAG201	pPC86mHA	Y2H_PREY_plasmid	This study
pAB10	pPC86mHA-Sc167BAR	Y2H_PREY_plasmid	This study
pAB11	pPC86mHA-Ca161_FL	Y2H_PREY_plasmid	This study
pAB14	pPC86mHA-Ca167BAR(+H0)	Y2H_PREY_plasmid	This study
pAB15	pPC86mHA-Ca167BAR(-H0)	Y2H_PREY_plasmid	This study
pAB18	pPC86mHA-Ca167-3BAR(+H0)	Y2H_PREY_plasmid	This study
pAB21	pPC86mHA-Ca162_FL	Y2H_PREY_plasmid	This study
pAB16	pPC86mHA-Ca167-2BAR(+H0)	Y2H_PREY_plasmid	This study
pAB17	pPC86mHA-Ca167-2BAR(-H0)	Y2H_PREY_plasmid	This study
pAB25	pPC97mMYC-Ca167-2BAR(+H0)	Y2H_BAIT_plasmid	This study
pAB26	pPC97mMYC-Ca167-2BAR(-H0)	Y2H_BAIT_plasmid	This study
pAG259	pETM-41m	Protein expression	This study
pAG265	pETM-41m-Ca167BAR(-H0)	Protein expression	This study
pAG269	pETM-41m-Ca167-3BAR(-H0)	Protein expression	This study
pAG273	pET-21d(+)-Ca161(-H0)	Protein expression	This study
pAG281	pET-21d(+)-Ca162_FL	Protein expression	This study
pAG294	Polycistronic 12 (167/161)	Protein expression	This study
pAG302	Polycistronic 20 (167-3/162)	Protein expression	This study
pSB02	pFA-yEmRFP-CdARG4	C-terminal tagging	This study

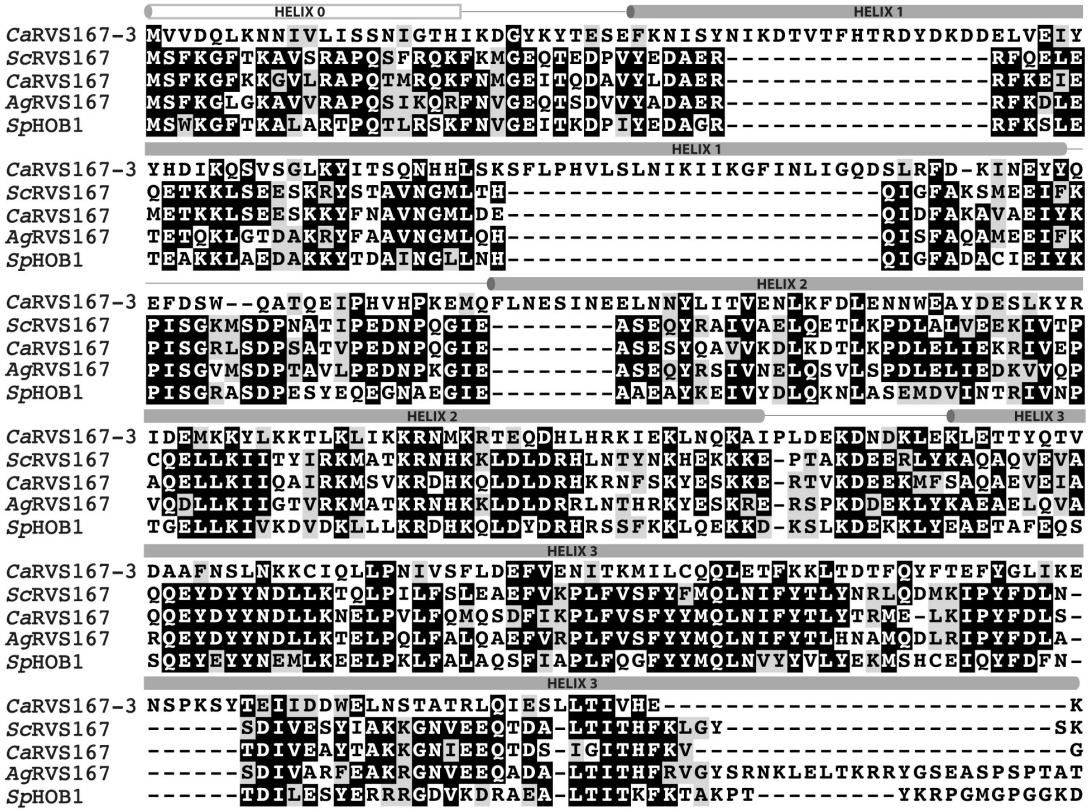
#696	pFA-GFPγ-CdHIS1	C-terminal tagging	(1)
pIS66	pFA-3HA-CdHIS1	C-terminal tagging	This study
pIS67	pFA-3HA-CdARG4	C-terminal tagging	This study
pJB170	pFA-6MYC-CmLEU2	C-terminal tagging	This study
pJB115	pFA-CdHIS1-loxP	HIS1 disruption with loxP-recombination sites	(2)
pJB109	pFA-CdARG4-loxP	ARG4 disruption with loxP-recombination sites	(2)
pJB113	pFA-CmLEU2-loxP	LEU2 disruption with loxP-recombination sites	(2)
pJB116	pFA-CaURA3-loxP	URA3 disruption with loxP-recombination sites	(2)
pJB171	(CaURA3+CmLeu2)	Prototrophy reconstitution	This study
pJB173	(CdARG4+CmLeu2)	Prototrophy reconstitution	This study

Supplementary Table 3: Phenotypic screen results summary

	STRAIN		SN148	161ΔΔ	167ΔΔ	161ΔΔ & 167ΔΔ	162ΔΔ	167-3ΔΔ	162 ΔΔ & 167-3ΔΔ	161ΔΔ & 162ΔΔ	167ΔΔ & 167-3 ΔΔ
	CONDITION	**CNC**									
1	**YPD_16ºC**		+++	+++	+++	+++	+++	++++(b)	++++(b)	+++	+++
2	**YPD_28ºC**		++++	++++	++++	++++	++++	++++	++++	++++	++++
3	**YPD_37ºC**		++++(y)	++++(y)	++++(h)	++++(h)	++++(y)	++++(y)	++++(y)	++++(y)	++++(h)
4	**YPD_42ºC**		++++	++++	-	-	++++	++++	++++	++++	-
5	**YPD_EDTA**	0.75M pH8.0	++++	++++	++++	+++	++++	++++(b)	++++(b)	++++	++++
6	**YPD_Menadione**	80µM	++++	++++	++++	++++	++++	++++(b)	++++(b)	++++	++++
7	**YPD_H2O2**	6.0mM	++++	++	++	++	++++	++++(b)	++++(b)	+++	++
8	**YPD_Caffeine**	15mM	++	++	++	++	++	++	++	++	++
9	**YPD_Rapamycin**	5nM	+	-	+	++	+++	+	+	-	+
10	**YPD_Rapamycin**	2.5nM	++++	+	++	+	++++	++++	++++	+	+
11	**YPD_SDS**	0.04%	+++	-	+++	-	+++	++++	++++	-	++
12	**YDP_CuSO4**	15mM	++++	++++	+++	+++	++++	++++(b)	++++(b)	++++	++++
13	**YPD_ZnSO4**	5 mM	-	-	-	-	-	-	-	-	-
14	**YPD_CFW**	20µM	++++	-	-	-	++++	++++(b)	++++(b)	-	-
15	**YPD_Congo red**	200 mg/ml	++++	-	-	-	++++	+++	++++	-	-
16	**YPD_Hepes 7.3**	150mM	++++	++++	++++	++++	++++	++++(b)	++++(b)	++++	++++
17	**YPD_Hepes 8.5**	150mM	++++	++++	++++	++++	++++	++++(b)	++++(b)	++++	++++
18	**YPD_FCS**	10%	++++(y)	++++(y)	++++(h)	++++(h)	++++	++++	++++	++++(h)	++++(y)
19	**Spider_28ºC**		++++	++++	+++	++++	++++	++++	++++	++++	++++
20	**Spider_37ºC**		++++(y)	++++(y)	++++(h)	++++(h)	++++(y)	++++(y)	++++(y)	++++(y)	++++(h)
21	**YPD_LiCl**	300mM	++++	-	-	-	++++	++++	++++	-	-
22	**YPD_Sorbitol**	1.5M									
23	**YPD_NaCl**	0.5M	++++	++++	++++	++++	++++	++++(b)	++++(b)	++++	++++
24	**YPD_NaCl**	1M	++++	++++	++++	++++	++++	++++(b)	++++(b)	++++	++++
25	**YPD_NaCl**	1.5M	+++	-	-	-	+++	++++(b)	++++(b)	-	-
26	**SD_28ºC**		++++	++++	++++	++++	++++	++++	++++	++++	++++
27	**SD_28ºC_pH2.1**		++++	++++	++++	++++	++++	++++	++++	++++	++++
28	**SD_28ºC_pH2.6**		++++	++++	++++	++++	++++	++++	++++	++++	++++
29	**SD_20ºC**		++++	++++	++++	++++				++++	++++
30	**SD_20ºC_pH2.1**		++++	++++	++++(s)	++++				++++	++++
31	**SD_20ºC_pH2.6**		++++	++++	++++(s)	++++				++++	++++
32	**SD_16ºC**		++	++	++	++	+++	+++	+++	++	++
33	**SD_16ºC_pH2.1**		++	+	+	+	++	+++	+++	+	+
34	**SD_16ºC_pH2.6**		+++	++	+	+	+++	+++(b)	+++(b)	+	+
35	**SD_10ºC**		+	+	+	+				+	+
36	**YNB_w/o(NH4)2SO4**		-	-	-	-	-	-	-	-	-
37	**YNB_w/o(NH4)2SO4 +NH4Cl**		++++	++++	++++	++++	++++	++++	++++	++++	++++
38	**YNB w/o(NH4)2SO4 +Ornithine**	15mM	++++	++++	++++	++++	++++	++++	++++	++++	++++
39	**YNBw/o(NH4)2SO4 +Isoleucine**	15mM	++++	++++	++++	++++	++++	++++	++++	++++	++++
40	**YNB w/o(NH4)2SO4 +Proline**	40mM	++++	++++	++++	++++	++++	++++	++++	++++	++++
41	**YNB_28ºC**		-	-	-	-	-	-	-	-	-
42	**YNB_Mannitol**	2%	++++	++++	++++	++++	++++	++++	++++	++++	++++
43	**YNB_Maltose**	2%	++++	++++	++++	+++	++++	++++	++++	++++	++++
44	**YNB_Galactose**	2%	++++	++++	++++	++++	++++	++++	++++	++++	++++
45	**YNB_Ethanol**		++++	++++	++++	++++	++++	++++	++++	++++	++++
46	**SD_Canavanine**	0.5 µg/ml	++++	+++(s)	+++(s)	++++	++++	++++	++++	+++(s)	++++
47	**SD_Fluconazole**	100µM	++++	+	++++	-	++++	++++	++++	-/+	++
48	**SD_Fluphenazine**	0.3mM	++++	++++(s)	++++	++++(s)	++++	++++	++++	++++(s)	++++
49	**SD_Fluphenazine**	1mM	++++	++++(s)	+++	+++(s)	++++	++++	++++(b)	++++(s)	++++
50	**SD_Fenpropimorph**	2µM	++++	++++	++++	++++	++++	++++(b)	++++(b)	++++	++++
51	**SD_Fenpropimorph**	4µM	++++	++++	++++	++++	++++	++++(b)	++++(b)	++++	++++
52	**SD_BCS**	15mM	++++	++++	++++	++++	++++	++++	++++	++++	++++

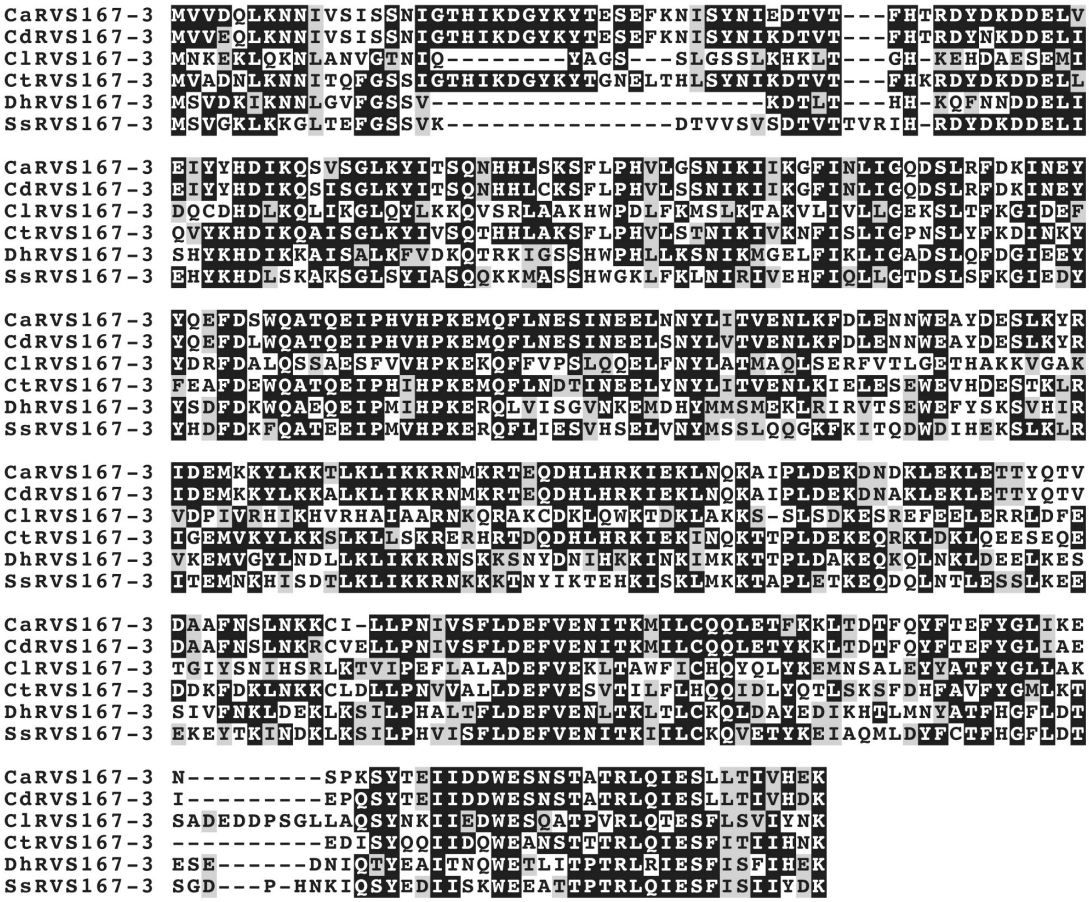
Cells were pre-grown on either rich (YPD) or minimal (YNB/0.3% glucose) medium, washed twice, resuspended to an OD_{600} of 0.2, and serially diluted (1:10). Four micro liters of each dilution was spotted onto agar plates. Growth was scored after 2-5 days incubation at indicated temperatures. -, no growth; +, 1 spot; ++, 2 spots; +++, 3 spots; ++++, 4 spots; (b), bigger colonies compared to wild-type strain; (s), smaller colonies compared to wild-type strain. YNB: Minimal medium containing 0.67% (w/v) Yeast Nitrogen Base with or without ammonium sulphate (as indicated) and 2% of selected carbon source (carbon source is glucose if not indicated otherwise). SDS, Sodium Dodecyl Sulphate; CFW, Calcofluor White; BCS, Bathocuproinedisulfonic acid (copperchelator). Details of the mechanism of action of the used phenotypic media can be found in (3).

Supplementary figure 1



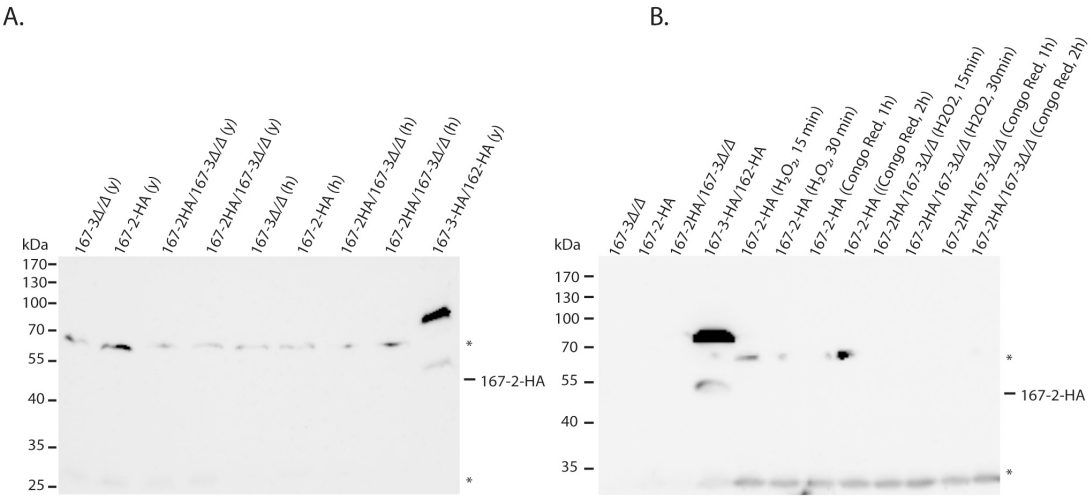
Supplementary Figure 1. Multiple sequence alignment of the BAR domains of *Ca*Rvs167-3 and Rvs167 from different yeast species. Sequences were aligned using the tcoffee program (http://tcoffee.crg.cat/apps/tcoffee/do:regular) and amino acid identities and similarities were highlighted using boxshade (http://mobyle.pasteur.fr/cgibin/portal.py?#forms::boxshade). Residues that are identical in at least 3 proteins are shades black, while those that are similar in at least 3 proteins are shaded grey. Indicated are the predicted α-helices and the N-terminal amphipathic helix (H0). *Ca, Candida albicans*; *Sc*, *Saccharomyces cerevisiae*; *Ag*, *Ashbya gosyppii*; *Sp*, *Schizosaccharomyces pombe*.

Supplementary figure 2



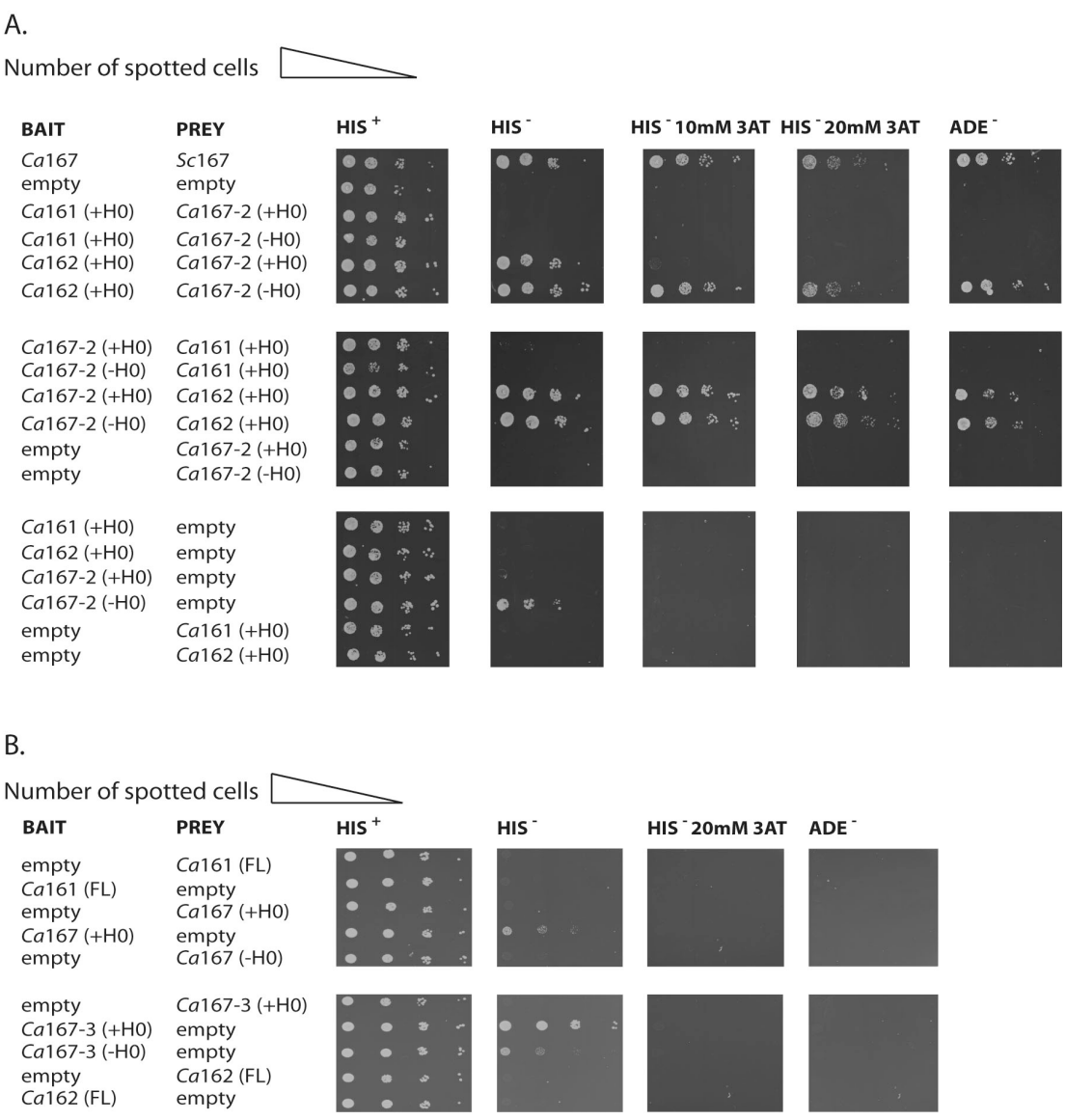
Supplementary Figure 2. Multiple sequence alignment of Rvs167-3 orthologs. Sequences were aligned using the tcoffee program (http://tcoffee.crg.cat/apps/tcoffee/do:regular) and amino acid identities and similarities were highlighted using boxshade (http://mobyle.pasteur.fr/cgibin/portal.py?#forms::boxshade). Residues that are identical in at least 3 proteins are shades black, while those that are similar in at least 3 proteins are shaded grey. *Ca, Candida albicans*; *Cd, Candida dubliniensis; Cl, Candida lusitaniae; Ct, Candida tropicalis; Dh, Debaryomyces hansenii; Ss, Scheffersomyces stipitis;*

Supplementary Figure 3



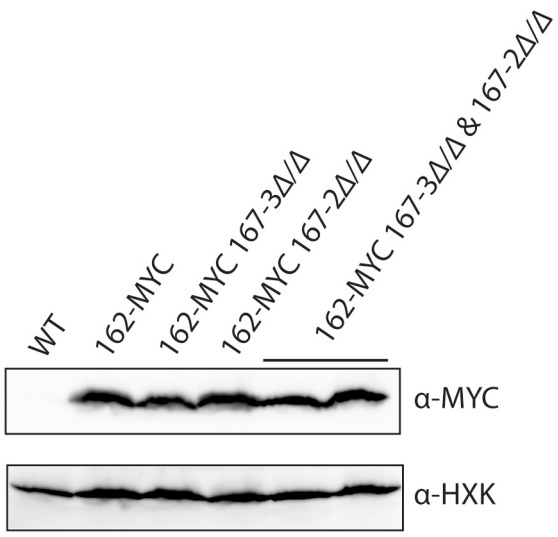
Supplementary Figure 3. Rvs167-2-HA expression. Immunoblot analysis of HA-tagged Rvs167-2 in wild type and *rvs167-3 Δ/Δ* mutant (A). The indicated strains were grown to mid-logarithmic phase in YPD or in YPD+10% FCS to test for protein expression in yeast (y) and hyphal (h) cells, respectively. Cells were then lysed and protein extracts were analyzed by SDS-PAGE and immunoblotting. Blots were probed with antibodies directed against HA. Expected running position of Rvs167-2-HA (calculated molecular weight 45.8kDa) is indicated. (B) Rvs167-2-HA expression under oxidative stress (5 mM H_2O_2) and cell wall stress caused by Congo Red (100µM) in both wild type and *rvs167-3 Δ/Δ* mutant cells. Protein extracts were prepared and analyzed by immunoblotting. Lysates of a double-tagged 167-3-HA/162-HA strain were loaded as positive control. Asterisk (*) indicates cross-reacting band.

Supplementary Figure 4



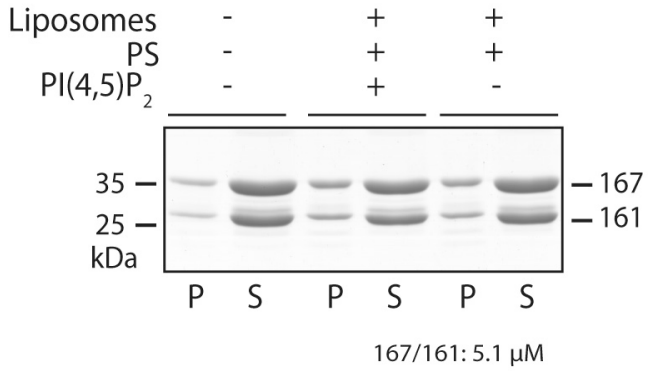
Supplementary Figure 4. Yeast-2-Hybrid control experiments. Serial dilutions of strains co-transformed with the indicated bait and prey constructs were spotted on minimal plates with histidine (His+), without histidine (His$^-$), without histidine and containing 20mM 3-amino-1,2,4-triazole (3AT) and without adenine (Ade$^-$). (A) The BAR domain of Rvs167-2 (without H_0) interacts strongly in both orientations with Rvs162 but not with Rvs161. All Rvs167 paralogs show weak self-activation when present in the bait plasmid as revealed by growth on His$^-$ plates (A, bottom panel and B).

Supplementary Figure 5



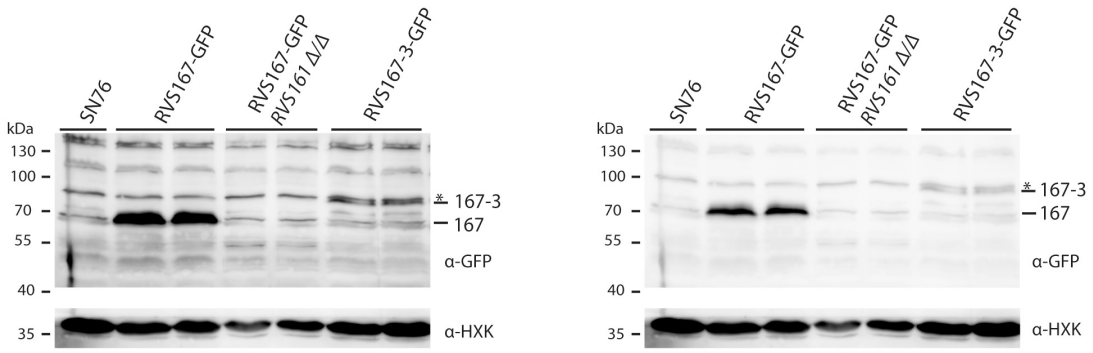
Supplementary Figure 5. Rvs162 levels in the absence of its binding partners. Immunoblot analysis of protein extracts of wild type and *rvs* deletion strains expressing 6MYC tagged Rvs-162 protein. The indicated strains were grown to mid-logarithmic phase in YPD, lysed and protein extracts were analyzed by SDS- PAGE and immunoblotting. Blots were probed with antibodies directed against MYC and hexokinase (HXK) as loading control.

Supplementary Figure 6



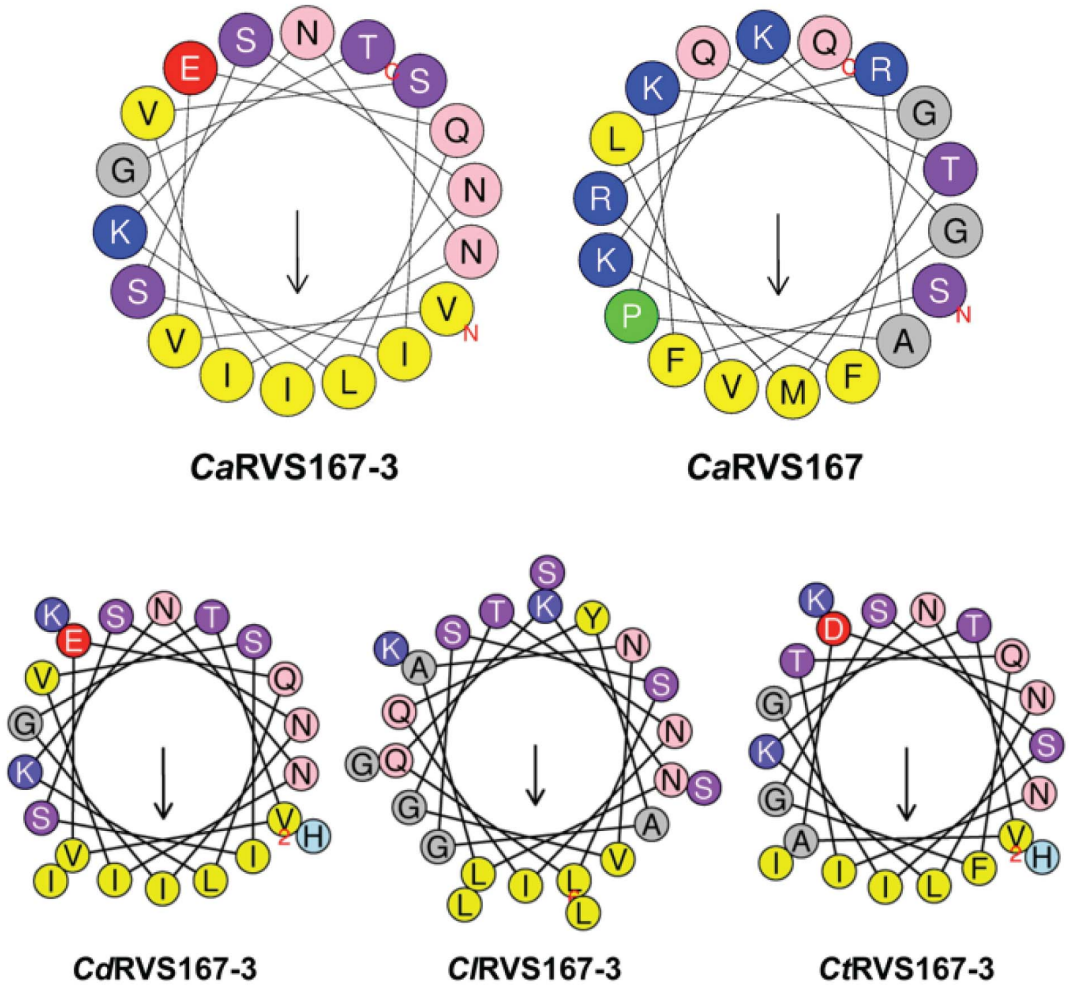
Supplementary Figure 6. The Rvs161/Rvs167 heterodimer bind membranes *in vitro.* Lipid co-sedimentation experiment showing that membrane binding of the purified Rvs161/Rvs167 heterodimer at high protein concentration (5.1 μM) is not substantially increased as compared to low protein concentration (1.7 μM) (see also Figure 7B). Experiment was carried out as described in the legend to Figure 7.

Supplementary Figure 7



Supplementary Figure 7: GFP tagging does not interfere with Rvs167 and Rvs167-3 function. Immunoblot analysis of wild type and *rvs* deletion strains expressing GFP tagged Rvs proteins. The indicated strains were grown to mid-logarithmic phase in YPD, lysed and protein extracts were analyzed by SDS-PAGE and immunoblotting. Blots were probed with antibodies directed against GFP and hexokinase (HXK) as loading control.

Supplementary Figure 8



Supplementary Figure 8: Amphipathic Helixes. Helical-wheel representation of the amphipathic helix of *Ca*Rvs167-3 (residues 2-19) and *Ca*Rvs167 (residues 2-19), and of Rvs167-3 orthologs in *C. dubliniensis* (residues 2-22), *C. lusitaniae* (residues 6-29) and *C. tropicalis* (residues 2-22). Yellow, hydrophobic residues; purple, serine and threonine; blue, basic; red, acidic; pink, asparagine and glutamine; gray, other residues. Arrow denotes the hydrophobic face. Helical wheels were created with HeliQuest (http://heliquest.ipmc.cnrs.fr/) (4)

Supplementary references

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