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	tgggtcatgtgggtttagtg	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	ctagtttttttttggagtgggag	162_SN1

Supplementary Table 1: Primers used in this study

AG255	caataatccaaataaatcaagagac	162_SN2
AG256	GACCTGCAGCGTACGAAGCTTCgatatgtgtgatgctatggttg	162_SN3
AG212	CTCGAATTCATCGATGATATCAGAggaccataaaataatagttgtgctt	162_SN4
AG213	agtttttcgtcatcgctcgt	162_SN5
AG156	ctttctcttttctatttctcacttttc	162_SN6
AG171	GAAGAAGAAGAAGAAGAGGCTC	Abp1_Tag_Cntrl
AG172	CGAAAAGGACGAAGATAATG	Abp1_Tag_F
AG173	GAAGCACCTGCGCCAGCACCCTCATTCAAGACAACATAGTTAGC	Abp1_Tag_R
AG174	CTCGAATTCATCGATGATATCAGAGATAAATTGGTGCTATAGCCAG	Abp1_SN4
AG175	GTTGCCACAGAAGTTTTAC	Abp1_SN5
AG176	TTCAACAAAACATTTAGGAGATTAC	Abp1_SN6
AG075	catttcggaatagtaccacttg	167_SN6
AG169	aaa CTGCAGggtgctggcgcaggtgcttctGTTTCAAAAGGTGAAGAAGATAA	yEmRFP_PstI_F
	TAT	
AG170	tGGCGCGCCTTATTTATATAATTCATCCATACCACC	yEmRFP_AscI_R
AG157	TCGACCCCGGGCGCGCCTAGATCTGCCATGGCCCTGCAGAA	pPC_MCS-Asc-Nco-F
AG158	CTAGTTCTGCAGGGCCATGGCAGATCTAGGCGCGCCCGGGG	pPC_MCS-Asc-Nco-R
	TCGAGCGCTGAACAAAAATTGATTTCTGAAGAGGATTTGTCTGGGTC	pPC_MYC-F
AG159	GACCCCGGG	
	CGCGCCCGGGGTCGACCCAGACAAATCCTCTTCAGAAATCAATTTTT	pPC_MYC-R
AG160	GTTCAGCGC	
	TCGAGCGCTTATCCTTATGATGTTCCAGATTATGCTTCTGGGTCGAC	pPC_HA-F
AG161	CCCGGG	
	CGCGCCCGGGGTCGACCCAGAAGCATAATCTGGAACATCATAAGGAT	pPC_HA-R
AG162	AAGCGC	
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	GTGTTTTCGTTTCTATTAGGAG	Rvs167_2_SN1
AG106	CTCACAGGTTTCTGCAGTATAC	Rvs167_2_SN2
AG107	GACCTGCAGCGTACGAAGCTTCCAATCGGTAAGATGATATATAT	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	167-3∆∆	162 ΔΔ & 167-3ΔΔ	161ΔΔ & 162ΔΔ	167ΔΔ & 167-3 ΔΔ
	CONDITION	CNC				10/88			10, 565	10222	10, 5 88
1	YPD_16°C		+++	+++	+++	+++	+++	++++(b)	++++(b)	+++	+++
2	YPD_28°C		++++	++++	++++	++++	++++	++++	++++	++++	++++
3	YPD_37°C		++++(y)	++++(y)	++++(h)	++++(h)	++++(y)	++++(y)	++++(y)	++++(y)	++++(h)
4	YPD_42°C		++++	++++	-	-	++++	++++	++++	++++	-
5	YPD_EDTA	0.75M	++++	++++	++++	+++	++++	++++(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	++++	-	-	-	++++	+++	++++	-	-
16	VDD Hamas 7.2	mg/ml									
10	VPD Heres 9.5	150mM	++++	++++	++++	++++	++++	++++(D)	++++(D)	++++	++++
17	VPD_REPES 0.5	100/	++++	++++	+++++	++++	++++	++++(D)	++++(D)	++++	++++
18	YPD_FCS	10%	++++(y)	++++(y)	++++(n)	++++(n)	++++	++++	++++	++++(n)	++++(y)
19	Spider_28%		++++	++++	+++	++++	++++	++++	++++	++++	++++
20	Spider_3/%	200mM	++++(y)	++++(y)	++++(n)	++++(n)	++++(y)	++++(y)	++++(y)	+++++(y)	++++(n)
21	YPD_LICI	300mM	++++	-	-	-	++++	++++	++++	-	-
22	YPD_Sorbitol	1.5M								-	-
23	YPD_Nacl	0.5M	++++	++++	++++	++++	++++	++++(b)	++++(b)	+++++	+++++
24	YPD_NaCl	IM	++++	++++	++++	++++	++++	++++(b)	++++(b)	+++++	+++++
25	YPD_NaCl	1.5M	+++	-	-	-	+++	++++(b)	++++(b)	-	-
26	SD_28%C		++++	++++	+++++	++++	++++	++++	++++	+++++	++++
27	SD_20°C_pH2.1		++++	++++	++++	++++	++++	++++	++++	++++	++++
20	SD_20%C_PH2.0		++++	++++	++++	++++	++++	++++	++++	++++	++++
29	SD_20°C		++++	++++	++++	++++				++++	++++
30	SD_20%C_pH2.1		++++	++++	++++(s)	++++				++++	++++
31	SD_20%C_pH2.0		++++	++++	++++(s)	++++				++++	++++
32	SD_16°C		++	++	++	++	+++	+++	+++	++	++
33	SD_16°C_pH2.1		++	+	+	+	++	+++	+++	+	+
34	SD_16°C_pH2.6		++++	++	+	+	+++	+++(D)	+++(D)	+	+
35	3D_10°C		+	+	+	+		-	-	+	+
30	YNB_W/0(NH4)2504		-	-	-	-	-	-	-	-	-
5/	+NH4Cl		++++	++++	++++	++++	++++	****	++++	****	++++
38	YNB w/o(NH4)2SO4 +Ornithine	15mM	++++	++++	++++	++++	++++	++++	++++	++++	++++
39	YNBw/o(NH4)2SO4	15mM	++++	++++	++++	++++	++++	++++	++++	++++	++++
40	YNB w/o(NH4)2SO4	40mM	++++	++++	++++	++++	++++	++++	++++	++++	++++
41	YNB_28°C		-	-	-	-	-	-	-	-	-
42	YNB_Mannitol	2%	++++	++++	++++	++++	++++	++++	++++	++++	++++
43	YNB_Maltose	2%	++++	++++	++++	+++	++++	++++	++++	++++	++++
44	YNB_Galactose	2%	++++	++++	++++	++++	++++	++++	++++	++++	++++
45	YNB_Ethanol	1	++++	++++	++++	++++	++++	++++	++++	++++	++++
46	SD_Canavanine	0.5 ug/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 OD₆₀₀ 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).

	HELIX 0		-0	HELIX 1
CaRVS167-3	WVVDQLKNNIVLISSNI	GTHIKDGYKYTES	EFKNISYNIKDTVT	FHTRDYDKDDELVDIY
ScRVS167	MSFKGFTKAVSRAPOSF	ROKFKMGEOTEDP	VYEDAER	REOELE
CaRVS167	MSFKGF <mark>K</mark> KGVLRAPQTM	RÕKFNMGEĨTQDA	VYLDAER	RFKEIE
AgRVS167	MSFKG <mark>lg</mark> kavvraposi	KORFNVGEOTSDV	VYADAER	RFKDLE
SpHOB1	MSWKGFTKALARTPQTL	RSKFNVGEITKDP	IYEDAGR	RFKSLE
-			HELIX 1	
CaRVS167-3	YHDIKOSVSGLKYITSO	NHH I SKSFLPHVL	SLNIKIIKGFINLI	GQDSLRED-KINEYYQ
ScRVS167	QETKKLSEESKRYSTAV	NGMLTH		QIGFAKSMEEIFK
CaRVS167	METKKLSEESKKYFNAV	NGMLDE		– – – QIDFAKAVAEIYK
AgRVS167	TETOKLGTDAKRYFAAV	NGMLQH		OISFAQAMEEIFK
SpHOB1	TEAKKLAEDAKKYTDAI	NGLLNH		QIGFADACIEIYK
-			HELIX 2	
CaRVS167-3	EFDSWQATQEICHVH	REMOFLNESINE	ELNNYLITVENIKF	DENNWEAYDESLKYR
ScRVS167	PISGKMSDPNATIPEDN	PQGIE	ASEQYRAIVAELQE	TLKPDLALVEEKIVTP
CaRVS167	PISGRLSDPSATVPEDN	POGIE	ASESYQAVVKDLKD	TLKPDLELIEKRIVEP
AgRVS167	PISGVMSDPTAVLPEDN	PKGIE	ASEQYRSIVNELQS	VLSPDLELIEDKVVQP
SpHOB1	PISGRASDPESYEQEGN	AEGIE	AAEAYKEIVYDLQK	NLASEMDVINTRIVNP
_	F	ELIX 2		HELIX 3
CaRVS167-3	IDEMKKYLKKTLKLIKK	RNMKRTEQDHLHR	KIEKLNQKAIPLDE	KDNDKLEKLETTYQTV
ScRVS167	CQELLKIITYIRKMATK	RNHK <mark>KLDLDRH</mark> LN	TYNKHEKKE - PTA	KDEERLYKAQAQVEVA
CaRVS167	AQELLKIIQAIRKMSVK	RDHK <mark>Q</mark> LDLDRHKR	NFSKYESKKE-RTV	K D E E K <mark>M F S A Q A E V E I</mark> A
AgRVS167	VQDLLKIIGTVRKMATK	RNHK <mark>K</mark> LDLDR <mark>R</mark> LN	THRKYESKRE-RSP	KDDEKLYKAEA <mark>ELQ</mark> VA
SpHOB1	TGELLKIVKDVDKLLLK	RDHKQLDYDRHRS	SFKKLQEKKD-KSL	KDEKKLYEAETAFEQS
		HELIX 3		
CaRVS167-3	DAAFNSLNKKCIQL LPN	IVSFLDEFVENIT	KMILCQQLETFKKL	TDTFQYFTEFYGLIKE
ScRVS167	Q QEYDYYNDLLK <mark>TQ</mark> LPI	LF <mark>SL</mark> EAEFVKPLF	VSFYMQLNIFYTL	YNRLODMKIPYFDLN-
CaRVS167	QQEYDYYNDLLK <mark>NELP</mark> V	LFQMQSDFIKPLF	VSFYYMQLNIFYTL	YTR <mark>ME-LKIPYFDL</mark> S-
AgRVS167	RQEYDYYNDLLKTELPQ	LF <mark>ALQAEFVR</mark> PLF	VSFYYMQLNIFYTL	HNA <mark>MQ</mark> DLR <mark>IPYFDL</mark> A-
SpHOB1	SQEYEYYNEMLKEELPK	LF <mark>AL</mark> AQSFIAPLF	QGFYYMQLNVYY VL	YEK <mark>MSHCEIQYFD</mark> FN-
		HELIX 3		
CaRVS167-3	NSPKSYTEIIDDWELNS	TATRL <mark>QIESL</mark> LTI	V <u>H</u> E	К
ScRVS167	SDIVESYIAKK	GNVEEQ <mark>TDA-LTI</mark>	THFKLGY	SK
CaRVS167	TDIVEAYTAKK	GNIEEQTDS-IGI	THFKV	G
AgRVS167	SDIVARFEAKR	GNVEEQADA-LTI	THFRVGYSRNKLEL	TKRRYGSEASPSPTAT
SpHOB1	TDILESYERRR	GDVKDRAEA-LTI	TKFKTAKPT	– – – – YKRPGMGPGGKD

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

(<u>http://tcoffee.crg.cat/apps/tcoffee/do:regular</u>) 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*.

CaRVS167-3	MVVDOLKNNIVSISSNIGTHIKDGYKYTESEFKNISYNIEDTVTFHTRDYDKDDELV
CdRVS167-3	MVVEOLKNNIVSISSNIGTHIKDGYKYTESEFKNISYNIKDTVTFHTRDYNKDDELI
ClRVS167-3	MNKEKLOKNLANVGTNIOYAGSSLGSSLKHKLTGH-KEHDAESEMI
CtRVS167-3	MVADNLKNNITOFGSSIGTHIKDGYKYTGNELTHLSYNIKDTVTFHKRDYDKDDELL
DhRVS167-3	MSVDKIKNNLGVFGSSVKDTLTHH-KOFNNDDELI
SSRVS167-3	MSVGKLKKGLTEFGSSVKDTVVSVSDTVTTVRIH-RDYDKDDELI
CaRVS167-3	EIYYHDIKQSVSGLKYITSQNHHLSKSFLPHVLGSNIKIIKGFINLIGQDSLRFDKINEY
CdRVS167-3	EIYYHDIKQSISGLKYITSQNHHLCKSFLPHVLSSNIKIIKGFINLIGQDSLRFDKINEY
ClRVS167-3	DQCDHDIKQLIKGLQYLKKQVSRLAAKHWPDLFKMSLKTAKVLIVLGEKSLTFKGIDEF
CtRVS167-3	QVYKHDIKQAISGLKYIVSQTHHLAKSFLPHVLSTNIKIVKNFISLIGPNSLYFKDINKY
DhRVS167-3	SHYKHDIKKAISALKFVDKQTRKIGSSHWPHLLKSNIKMGELFIKLIGADSLOFDGIEEY
SSRVS167-3	EHYKHDISKAKSGLSYIASQQKKMASSHWGKLFKLNIRIVEHFIQLGTDSLSFKGIEDY
CaRVS167-3	YQEFDSWQATQEIPHVHPKEMQFLNESINEELNNYLITVENLKFDLENNWEAYDESLKYR
CdRVS167-3	YQEFDLWQATQEIPHVHPKEMQFLNESINEELSNYLVTVENLKFDLENNWEAYDESLKYR
ClRVS167-3	YDRFDALQSSAESFVVHPKEKQFFVPSLQQELFNYLATMAQLSERFVTLGETHAKKVGAK
CtRVS167-3	FEAFDEWQATQEIPHIHPKEMQFLNDTINEELYNYLITVENLKIELESEWEVHDESTKLR
DhRVS167-3	YSDFDKWQAEQEIPMIHPKERQLVISGVNKEMDHYMMSMEKLRIRVTSEWEFYSKSVHIR
SsRVS167-3	YHDFDKFQATEEIPMVHPKERQFLIESVHSELVNYMSSLQQGKFKITQDWDIHEKSLKLR
CaRVS167-3	IDEMKKYLKKTLKLIKKRNMKRTEQDHLHRKIEKLNQKAIPLDEKDNDKLEKLETTYQTV
CdRVS167-3	IDEMKKYLKKALKLIKKRNMKRTEQDHLHRKIEKLNQKAIPLDEKDNAKLEKLETTYQTV
ClRVS167-3	VDPIVRHIKHVRHAIAARNKQRAKCDKLQWKTDKLAKKS-SLSDKESREFEELERRLDFE
CtRVS167-3	IGEMVKYLKKSLKLLSKRERHRTDQDHLHRKIEKINQKTTPLDEKEQRKLDKLQEESEQE
DhRVS167-3	VKEMVGYLNDLLKLIKKRNSKKSNYDNIHKKINKIMKKTTPLDAKEQKQLNKLDEELKES
SSRVS167-3	ITEMNKHISDTLKLIKKRNKKKTNYIKTEHKISKLMKKTAPLETKEQDQLNTLESSLKEE
CaRVS167-3	DAAFNSLNKKCI-LLPNIVSFLDEFVENITKMILCQQLETFKKLTDTFQYFTEFYGLIKE
CdRVS167-3	DAAFNSLNKRCVELLPNIVSFLDEFVENITKMILCQQLETYKKLTDTFQYFTEFYGLIAE
ClRVS167-3	TGIYSNIHSRLKTVIPEFLALADEFVEKLTAWFICHQYQLYKEMNSALEYYATFYGLLAK
CtRVS167-3	DDKFDKLNKKCLDLLPNVVALLDEFVESVTILFLHQQIDLYQTUSKSFDHFAVFYGMLKT
DhRVS167-3	SIVFNKLDEKLKSILPHALTFLDEFVENLTKLTLCKQLDAYEDIKHTLMNYATFHGFLDT
SSRVS167-3	EKEYTKINDKLKSILPHVISFLDEFVENITKIILCKQVETYKEIAQMLDYFCTFHGFLDT
CaRVS167-3	NSPKSYTEIIDDWESNSTATRLQIESLLTIVHEK
CdRVS167-3	IPQSYTEIIDDWESNSTATRLQIESLLTIVHDK
ClRVS167-3	SADEDDPSGLLAQSYNKIIEDWESQATPVRLQTESFLSVIYNK
CtRVS167-3	DISYQQIIDQWEANSTTTRLQIESFITIIHNK
DhRVS167-3	ESEDNIQTYEAITNQWETLITPTRLRIESFISFIHEK
SsRVS167-3	SGDP-HNKIQSYEDIISKWEEATTPTRLQIESFISIIYDK

Supplementary Figure 2. Multiple sequence alignment of Rvs167-3

orthologs. Sequences were aligned using the tcoffee program (<u>http://tcoffee.crg.cat/apps/tcoffee/do</u>: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. 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 H₂O₂) 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.

Α.

Number of spotted cells

BAIT	PREY	HIS ⁺	HIS ⁻	HIS ⁻ 10mM 3AT HIS ⁻ 2	0mM 3AT	ADE ⁻
Ca167	Sc167		🕒 🔍 👷 🕓		n de l	•••
empty	empty	• • * *	0 1 F .	le Rick de Barge		: 김 영 관리
Ca161 (+H0)	Ca167-2 (+H0)	• • •				
Ca161 (+H0)	Ca167-2 (-H0)	• • •				
Ca162 (+H0)	Ca167-2 (+H0)	• • • •	🕒 🔍 🚓 ·		난 그 동방	
Ca162 (+H0)	Ca167-2 (-H0)	• • *	• • •	🕒 🌒 🕸 🔶		• • • •
Ca167-2 (+H0)	Ca161 (+H0)	• • •	19 A.		· · · ·	
Ca167-2 (-H0)	Ca161 (+H0)	🛛 🖏 🎋 🔆				
Ca167-2 (+H0)	Ca162 (+H0)	• • •	• • • -	••••	9 i -	🗢 48 og 🚬
Ca167-2 (-H0)	Ca162 (+H0)	• • \$	·• 💿 🕸 📡	• • • •	👼 🎋 🕂	🔵 🥮 🔐 . j
empty	Ca167-2 (+H0)	 Section 1 				
empty	Ca167-2 (-H0)	🕒 🔍 👷 🍐				
Ca161 (+H0)	empty	🔴 🕲 🔅 🎐				
Ca162 (+H0)	empty	• • •				
Ca167-2 (+H0)	empty	• • • •	P. 1			
Ca167-2 (-H0)	empty	🌻 🔹 🕸 👎	• * *			
empty	Ca161 (+H0)	• • -	141 201 - E			
empty	Ca162 (+H0)	* <i>7</i> 🔮 🍽	. • The state	그렇다는 것 그는 것 같이 있는 것 같은 것 같이 없다.		

B.

Number of spotted cells

BAIT	PREY	HIS ⁺	HIS ⁻	HIS ⁻ 20mM 3AT	ADE
empty Ca161 (FL) empty Ca167 (+H0) empty	Ca161 (FL) empty Ca167 (+H0) empty Ca167 (-H0)	• • • • • • • • • • • • • • • • • • •	0 0 . ● . ● . •		
empty Ca167-3 (+H0) Ca167-3 (-H0) empty Ca162 (FL)	Ca167-3 (+H0) empty empty Ca162 (FL) empty	 • •<	• • • • 2 • • •		

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 H₀) 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. 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. 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 (5.1μ M) is not substantially increased as compared to low protein concentration (1.7μ M) (see also Figure 7B). Experiment was carried out as described in the legend to 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 midlogarithmic 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: 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

- 1. **Zhang C, Konopka JB**. 2010. A photostable green fluorescent protein variant for analysis of protein localization in Candida albicans. Eukaryotic Cell **9**:224–226.
- 2. Strijbis K, van Roermund CWT, Hardy GP, van den Burg J, Bloem K, de Haan J, van Vlies N, Wanders RJA, Vaz FM, Distel B. 2009. Identification and characterization of a complete carnitine biosynthesis pathway in Candida albicans. The FASEB Journal **23**:2349–2359.
- 3. **Homann OR, Dea J, Noble SM, Johnson AD**. 2009. A Phenotypic Profile of the Candida albicans Regulatory Network. PLoS Genet **5**:e1000783.
- 4. **Gautier R**, **Douguet D**, **Antonny B**, **Drin G**. 2008. HELIQUEST: a web server to screen sequences with specific alpha-helical properties. Bioinformatics 24:2101–2102.