Supplemental Materials

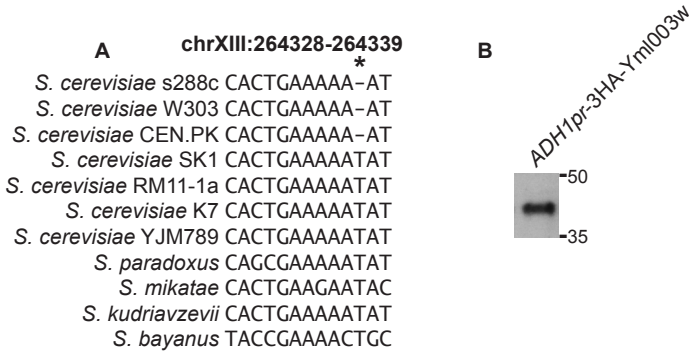
Molecular Biology of the Cell

Bean et al.

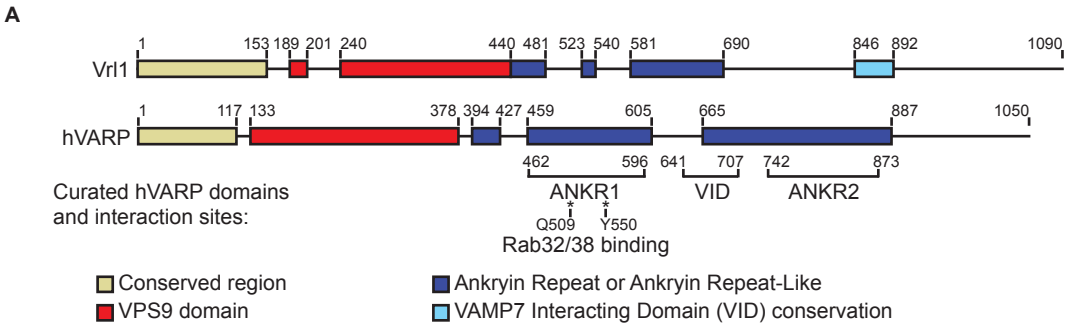
SUPPLEMENTARY FIGURE 1: There is a deletion present in lab *Saccharomyces cerevisiae* strains in the gene *YML003W* that is absent from other *S. cerevisiae* and truncates the normal gene product. (A) An alignment of YML003W in laboratory/wild *S. cerevisiae* strains and other related fungi. (B) Cells expressing *ADH1pr*-3HA-Yml003w were lysed, resolved by 10% SDS-PAGE and immunoblotted with anti-HA. Expected size was roughly 40 kDa. HA, hemagglutinin.

SUPPLEMENTARY FIGURE 2: Conservation of the hVARP N-terminal domain and the VAMP7-interacting domain. (A) Schematic of Vrl1 and hVARP with curated domains (Tamura *et al.*, 2011) including Ankyrin repeat domains (ANKR), the VAMP7 interacting domain (VID) and key Rab32/38-interacting residues. (B) An alignment of the N-terminus of hVARP/Vrl1 shows broad conservation. (C) Partial conservation of the VAMP7-interacting domain.

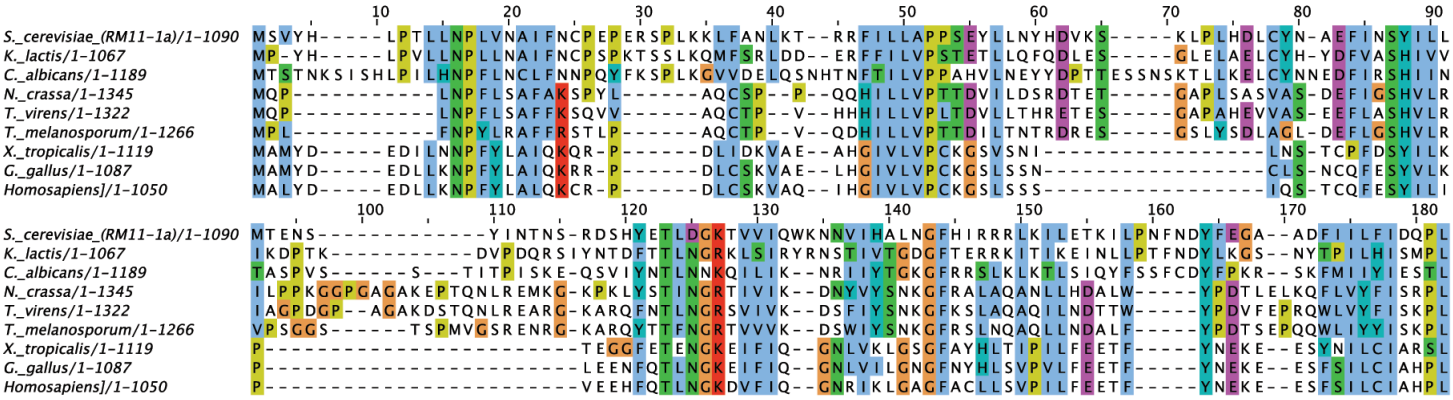
SUPPLEMENTARY FIGURE 3: All NubG iMYTH and GFP-*MUK1* vectors were expressed. (A) iMYTH strains expressing different Nub-HA-tagged GEFs were lysed and immunoblotted with anti-HA to determine relative expression levels. (B) By immunoblotting with anti-HA, strains with *ADH1pr*-GFP-Muk1 vectors were found to express GFP-Muk1 at similar levels. HA, hemagglutinin.



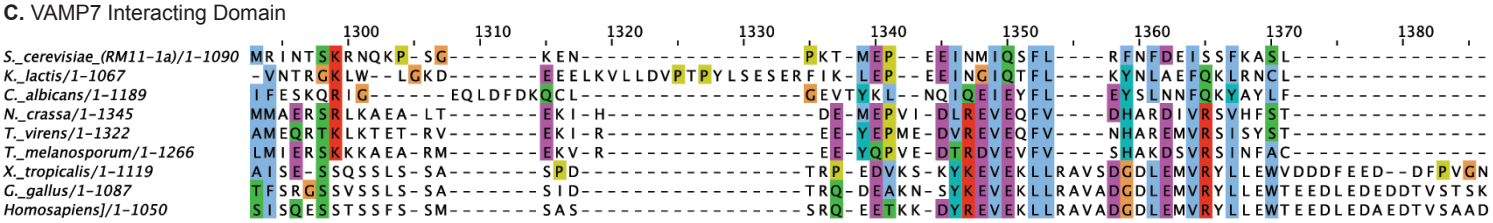
SUPPLEMENTARY FIGURE 1



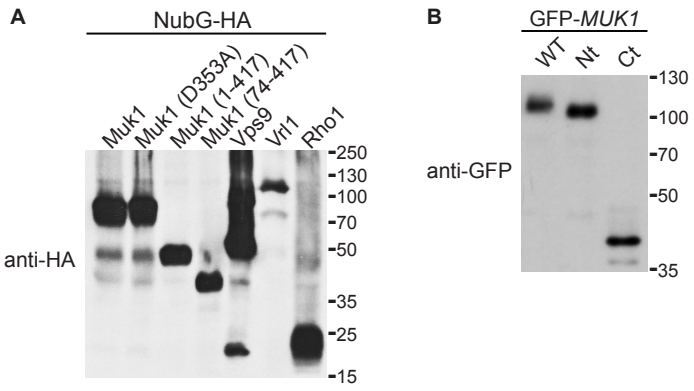
B. N-terminal Conservation



C. VAMP7 Interacting Domain



SUPPLEMENTARY FIGURE 2



SUPPLEMENTARY FIGURE 3

Supplementary Table 1. Strains used in this work

Strain ID	Genotype	Source or Reference
BY4741	*MAT*a *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	O'shea Lab
Vps5-TAP	*BY4741 VPS5-TAP::HIS3*	Yeast Tap-tagged fusion library (Ghaemmaghami *et al.*, 2003)
Vps17-TAP	*BY4741 VPS17-TAP::HIS3*	Yeast Tap-tagged fusion library
Vps26-TAP	*BY4741 VPS26-TAP::HIS3*	Yeast Tap-tagged fusion library
Vps29-TAP	*BY4741 VPS29-TAP::HIS3*	Yeast Tap-tagged fusion library
Vps35-TAP	*BY4741 VPS35-TAP::HIS3*	Yeast Tap-tagged fusion library
BBY203	*BY4741 VPS35-GFP::KAN*	This Study
BBY204	*BY4741 NatNT2::ADH1-3HA-VPS9*	This Study
BBY207	*BY4741 NatNT2::ADH1-3HA-VPS9 VPS35-GFP::KAN*	This Study
BBY208	*BY4741 NatNT2::ADH1-3HA-MUK1 VPS35-GFP::KAN*	This Study
CUY4412	*MATα VPS26-GFP::KANMX4 his3D200 leu2D0 lys2D0 met15D0 ura3D0*	Balderhaar *et al.* 2010
BBY210	*CUY4412 muk1Δ::NAT*	This Study
BBY211	*CUY4412 vps9Δ::HIS3*	This Study
BBY212	*CUY4412 muk1Δ::NATR vps9Δ::HIS3*	This Study
BBY177	*BY4741 muk1Δ::KANR*	This Study
BBY201	*BY4741 vps9Δ::HIS3*	This Study
BBY182	*BY4741 muk1Δ::KANR vps9Δ::HIS3*	This Study
BBY422	*BY4741 NatNT2::ADH1-3HA-YML003W*	This Study
MDY956	*CUY4412 vps21Δ::NAT*	This Study
BBY312	*CUY4412 vps21Δ::NAT ypt52Δ::HPH*	This Study
NMY51	*his3Δ200 trp1-901 leu2-3,112 LYS2::(lexAop)4-HIS3 ura3::(lexAop)8-lacZ ade2::(lexAop)8-ADE2 GAL4*	Snider *et al.* 2010
AO445	*NMY51 VPS5-Cub-LexA-VP16-KANMX*	This Study
AO447	*NMY51 VPS35-Cub-LexA-VP16-KANMX*	This Study
AO464	*NMY51 VPS26-Cub-LexA-VP16-KANMX*	This Study
AO468	*NMY51 Artificial Bait-Cub-LexA-VP16-KANMX*	Snider *et al.* 2010
BBY327	*BY4741 VPS26-tDimer2::URA NatNT2::ADHpr-yeGFP-MUK1*	This Study
BBY216	*BY4741 NatNT2::ADHpr-yeGFP-VPS9*	This Study
BBY322	*BY4741 NatNT2::ADHpr-yeGFP-VPS9 vps35Δ::HPH*	This Study
BBY217	*BY4741 NatNT2::ADHpr-yeGFP-MUK1*	This Study
BBY324	*BY4741 NatNT2::ADHpr-yeGFP-MUK1 vps35Δ::HPH*	This Study
BBY413	*BY4741* VPS34-GFP+::*NAT*	This Study
BBY414	*BY4741 muk1Δ::KANR VPS34-GFP+::NAT*	This Study
BBY420	*BY4741 vps9Δ::HIS3* VPS34-GFP+::*NAT*	This Study
BBY421	*BY4741 muk1Δ::KANR vps9Δ::HIS3* VPS34-GFP+::*NAT*	This Study
BBY416	*BY4741 vps21Δ::NATR ypt52Δ::HPH* VPS34-GFP+::*HIS3*	This Study

Supplementary Table 2. Plasmids used in this work.

Plasmid ID	Description	Source or Reference
pMuk1-HA	*pGAL1pr-MUK1-HA::URA3*	MORF collection (Gelperin et al., 2005)
pCS11	*pSNF7-RFP::LEU2*(CEN)	Conibear Lab
pRS415	p*LEU2*(CEN)	ATCC#87520
pRS416	*pURA3*(CEN)	ATCC#87521
pTS48	p*VPS9::URA3*(2μ)	Stevens Lab
pMUK1(2μ)	p*MUK1::LEU2*(2μ)	MoBY ORF collection (Ho *et al.* 2006)
pMD120	p*MUK1pr-3HA-MUK1::URA3*(CEN)	This Study (Conibear Lab)
pBB12	p*MUK1pr-3HA-MUK1(D353A)::URA3*(CEN)	This Study (Conibear Lab)
pBB33	pADH*1pr-3HA-MUK1::URA3*(CEN)	This Study (Conibear Lab)
pBB34	pADH*1pr-3HA-MUK1(D353A)::URA3*(CEN)	This Study (Conibear Lab)
pMD121	*pMUK1pr-GFP-MUK1::URA3*(CEN)	This Study (Conibear Lab)
pMT1	*pADH1pr-GFP-MUK1::URA3*(CEN)	This Study (Conibear Lab)
pBB14	*pADH1pr-GFP-MUK1(D353A)::URA3*(CEN)	This Study (Conibear Lab)
pMT2	*pADH1pr-GFP-MUK1(503-612)::URA3*(CEN)	This Study (Conibear Lab)
pBB15	*pADH1pr-GFP-MUK1(1-575)::URA*(CEN)	This Study (Conibear Lab)
pBB23	*pADH1pr-VRL1::URA3*(CEN)	This Study (Conibear Lab)
pBB33	*pVRL1pr-3HA-VRL1::URA3*(CEN)	This Study (Conibear Lab)
pBB28	*pVRL1pr-3HA-VRL1(D373A)::URA3*(CEN)	This Study (Conibear Lab)
pBB32	*pADH1pr-GFP-VRL1::URA3*(CEN)	This Study (Conibear Lab)
pAO538	p*CYC1pr-NubG-HA-MUK1(1-417t)::TRP1*(2μ)	This Study (Stagljar Lab)
pAO542	p*CYC1pr-NubG-HA-MUK1(74-417t)::TRP1*(2μ)	This Study (Stagljar Lab)
pA0531	p*CYC1pr-NubG-HA-MUK1(D353A)::TRP1*(2μ)	This Study (Stagljar Lab)
pAO470	p*CYC1pr-NubG-HA-MUK1::TRP1*(2μ)	This Study (Stagljar Lab)
pBB9	p*CYC1pr-NubG-HA-VPS9::TRP1*(2μ)	This Study (Conibear Lab)
pBB24	p*CYC1pr-NubG-HA-VRL1::TRP1*(2μ)	This Study (Conibear Lab)
pPR3N-NubG-Rho1	p*CYC1pr-NubG-HA-RHO1::TRP1*(2μ)	Stagljar Lab
pPR3N-NubI-Rho1	p*CYC1pr-NubI-RHO1::TRP1*(2μ)	Stagljar Lab
pBB21	*pGFP-FYVE(EEA1)::LEU2*(2μ)	This Study (Conibear Lab)
pSRG92	*p-myc-VPS21::URA3*(2μ)	Stevens Lab
pSRG93	*p-myc-VPS21(Q66L)::URA3*(2μ)	Stevens Lab