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Supplemental Methods

Approximate estimation of the affinity of GST-RabA and GST-RabB for GTP
Affinities of GST-RabA and GST-RabB for GTP were estimated using a filter binding assay and [γ-^{32}P]-GTP, as described below.

a) Protein purification. To efficiently load GST-Rabs with labeled nucleotide, it is important to ensure that the proteins are in the apo form (Burstein and Macara, 1992). To remove endogenous nucleotides bound to bacterially-expressed GST-Rab proteins, 0.2 ml of glutathione-Sepharose beads containing immobilized GST-Rab fusion proteins or GST (control) were washed, resuspended in 2 ml BB buffer lacking $MgCl_2$ (-Mg^{2+}) and containing 10 mM EDTA and incubated for 30 min at 30ºC. After two additional washes with the same buffer, proteins were eluted with -Mg^{2+} BB containing 25 mM glutathione. Pooled fractions containing the relevant protein (by SDS-PAGE) were passed through 2 ml Zeba Spin Desalting Columns equilibrated with -Mg^{2+} BB. Protein concentrations were accurately determined using 2D-Quant kit (Amershan).

b) GTP binding assays. Binding mixtures containing different concentrations of GST-RabA, GST-RabB or GST were set up in a buffer containing 20 mM Tris-HCl pH 8, 110 mM KCl, 1 mM DTT, 0.1% Triton X-100, 10 nM EDTA and 2.5 nM [γ-^{32}P]-GTP (6000 Ci/mmol) were pre-incubated for 5 min at 30ºC in the absence of Mg^{2+}. After cooling in ice for 5 min, 10 mM $MgCl_2$ was added and the mixtures were incubated in ice for an additional 20 min to further promote nucleotide binding (Burstein and Macara, 1992; Zhang *et al.*, 2000). (Preliminary experiments showed that GTP hydrolysis does not take place under these conditions, even though Mg^{2+} is present). After this second incubation, samples were filtered through pre-wetted nitrocellulose discs (0.45 μm, MF-Millipore) that were washed with 5 ml of ice-cold BB buffer containing 0.1% Triton X-100. The amount of [γ-^{32}P]-GTP that was retained with the proteins was determined using a Wallac Beta counter. For each amount of GST-Rab protein, control assays containing the corresponding amount of GST were set up and the radioactivity retained by these assays was considered as the background.

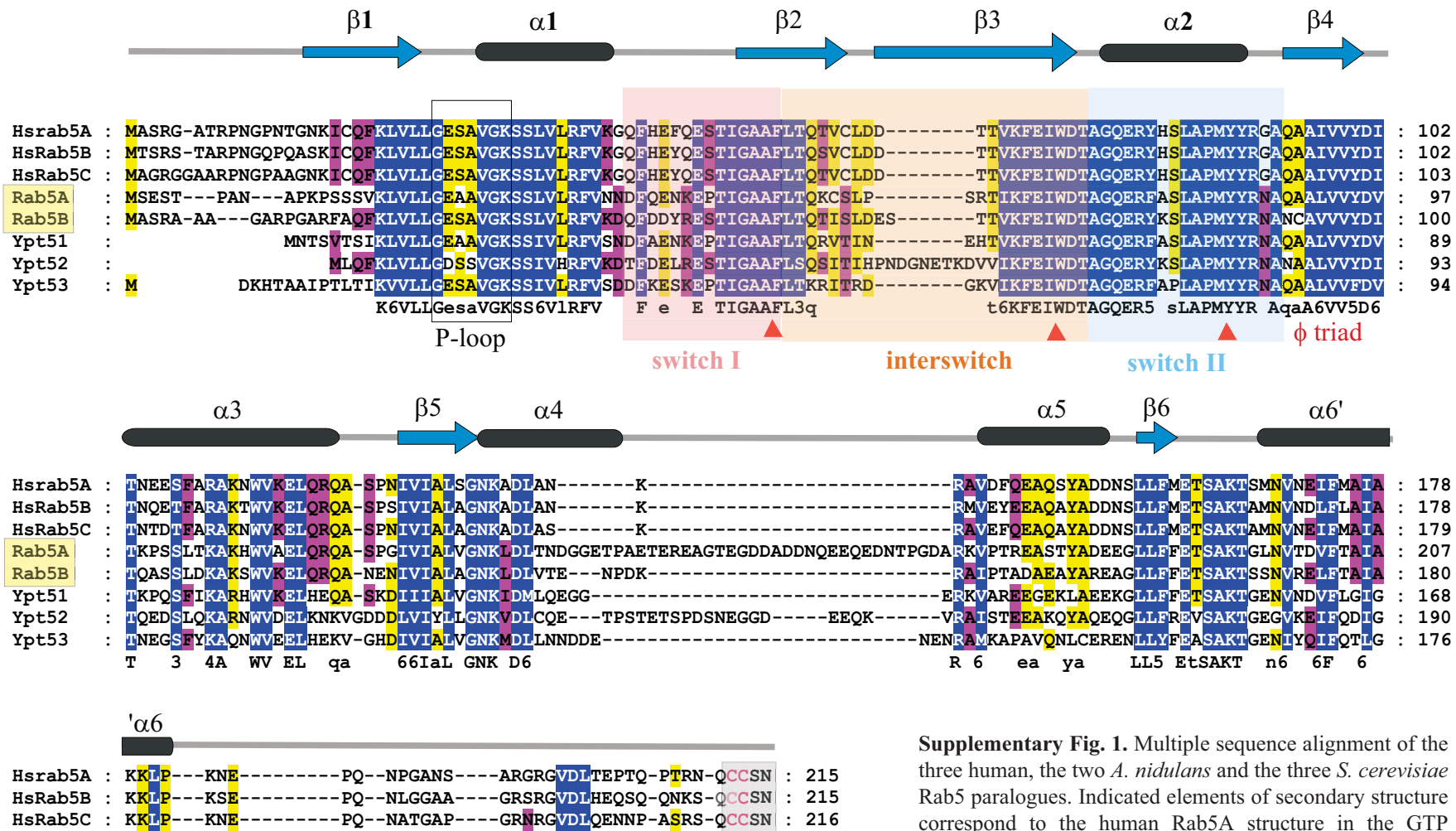
Data were obtained in triplicate and, since the concentration of protein far exceeds that of nucleotide, fitted to the equation $y=ax/(K_d+a)$ (Choo and Klug, 1993), were y corresponds to the concentration of [γ-^{32}P]-GTP complexed with the protein, x corresponds to the initial concentration of GST-Rab and K_d is the apparent dissociation constant. An approximate estimation of the dissociation constant was obtained as the concentration of protein required for half maximal binding. Calculations were made using Sigma Plot 11.00 for Windows (Systat Software Inc.)

Supplemental Methods References

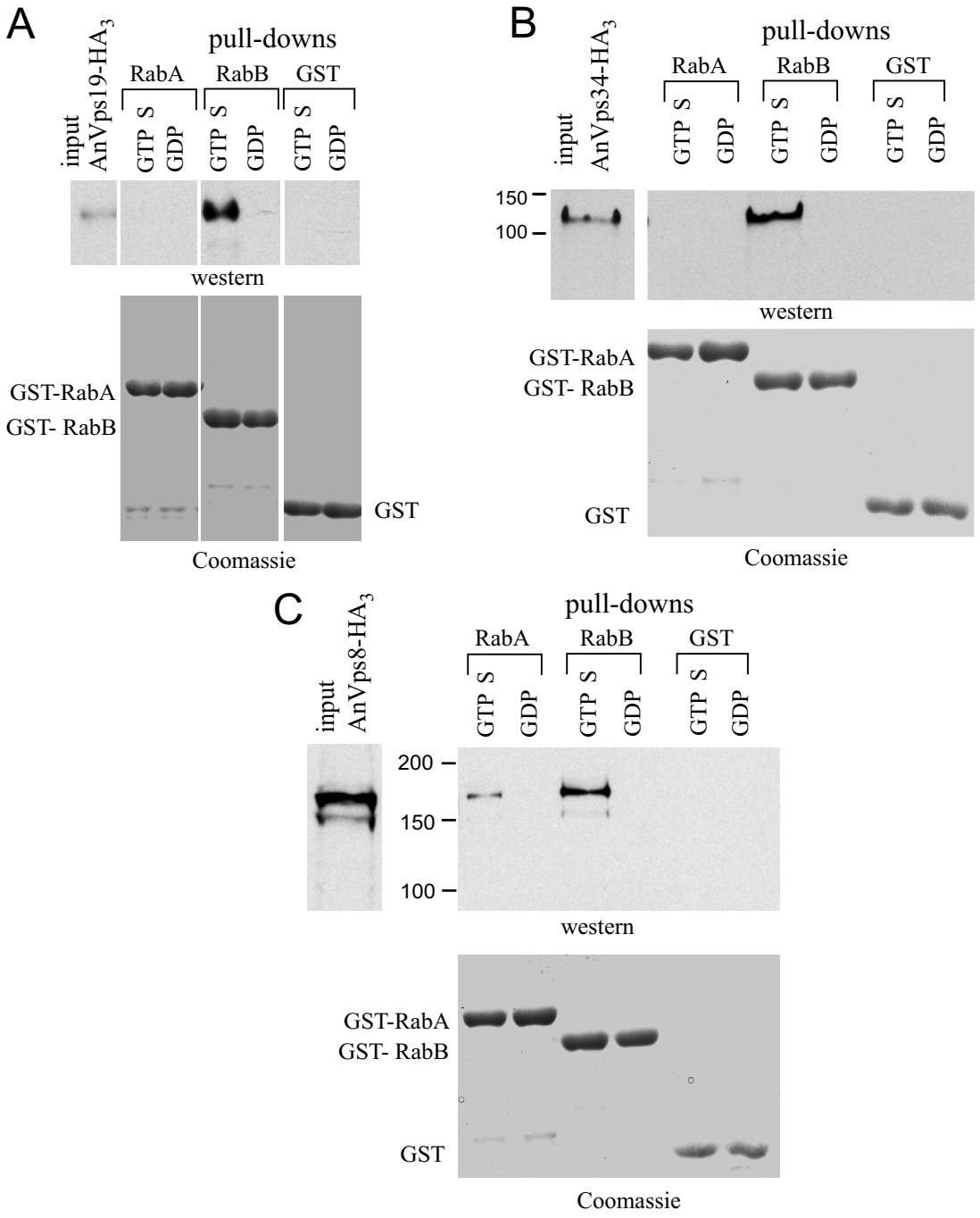
Burstein, E. S., and Macara, I. G. (1992). Interactions of the ras-like protein p25^{rab3A} with Mg^{2+} and guanine nucleotides. Biochem. J. *282*, 387-392.

Choo, Y., and Klug, A. (1993). A role in DNA binding for the linker sequences of the first three zinc fingers of TFIIIA. Nucleic Acids Res. *21*, 3341-3346.

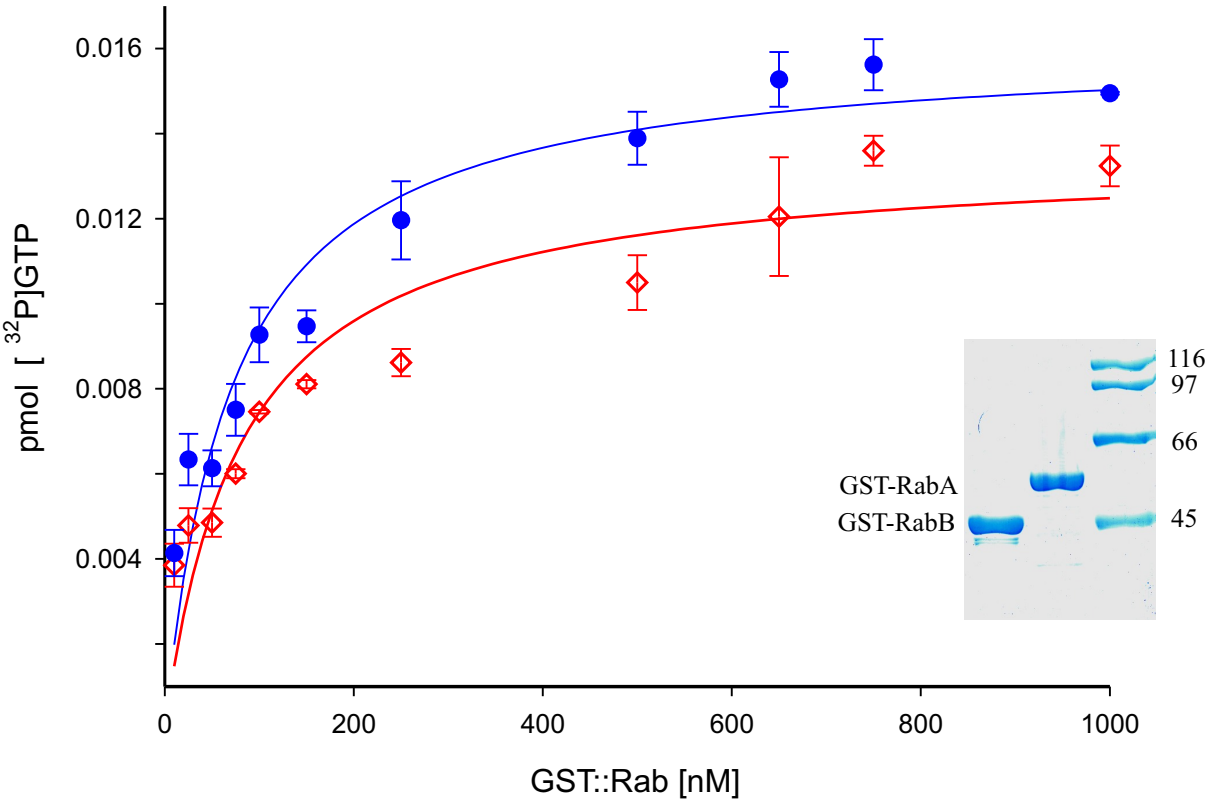
Zhang, B., Zhang, Y., Wang, Z., and Zheng, Y. (2000). The role of Mg^{2+} cofactor in the guanine nucleotide exchange and GTP hydrolysis reactions of Rho family GTP-binding proteins. J. Biol. Chem. *275*, 25299-25307.



Supplementary Fig. 1. Multiple sequence alignment of the three human, the two *A. nidulans* and the three *S. cerevisiae* Rab5 paralogues. Indicated elements of secondary structure correspond to the human Rab5A structure in the GTP conformation (Zhu *et al.*, 2003). The interswitch, switch I and II regions are shaded, as is the C-terminal Cys-containing motif undergoing geranylgeranylation. Also indicated with red triangles are the three highly conserved residues denoted as the hydrophobic triad, which play an important role in receptor recruitment. Note the long insertions sharing sequence similarity which are present between α4 and α5 in *A. nidulans* RabA and *S. cerevisiae* Ypt52p.



Supplementary Fig. 2. Data complementing GST-pull down experiments in Fig. 6 and 10. AnVps19 (panel A) and AnVps34 (panel B), which are pulled-down by GST-RabB but not by GST-RabA, are not pulled-down by GST either. Panel (C) AnVps8 is pulled-down by GST-RabB and to a lesser extent by GST-RabA, but not at all by GST alone. Extracts from cells expressing AnVps19-(HA)3, AnVps34-(HA)3 and AnVps8-(HA)3 at physiological levels were incubated with GST protein baits that were loaded with GTP-γ-S or GDP, as indicated. Proteins co-purifying with the baits on glutathione-Sepharose beads were analyzed by anti-HA western blots. Input lanes contain 10% (AnVps19), 10 % (AnVps34) and 7.5% (AnVps8) of the total material used in the pull-downs.



Supplemental Figure 3. GST-RabA and GST-RabB do not show major differences in GTP binding, as determined by filter binding assays with [γ-^{32}P]-GTP and purified GST-Rabs. The plots shown were used to determine the apparent dissociation constants (K_d) of GTP::GST-RabA (blue) and GTP::GST-RabB (red) complexes. Reaction mixtures containing 2.5 nM [γ-^{32}P]-GTP (6000 Ci/mmol) were incubated with increasing concentrations (10-1000 nM) of the corresponding fusion protein as described under Supplemental Methods. Values are the mean of three independent experiments. Error bars indicate standard error. The apparent K_d values obtained as the concentration of protein at half-maximal binding were 81 nM for GST-RabA and 71 nM for GST-RabB. While the values thus obtained do not intend to represent accurate determinations of the K_d (for example, only the total protein and not the proportion of active protein in the samples is known), these data strongly indicate that GST-RabA and GST-RabB do not show major differences in GTP binding relative to each other.

Supplementary Table I. Strains used in this work

MAD0002	*biA1*	our collection
MAD0005	*wild-type*	our collection
MAD1437	*yA2; argB2::[argB*-alcAp::gfp::rabA]*	Abenza et al, 2009
MAD1739	*pyrG89; pyroA4; nkuAΔ::bar*	H.N.Arst
MAD1740	*wA4; inoB2 nkuAΔ::bar pyroA4; niiA4*	H.N.Arst
MAD2006	*pabaA1 pyrG89*? *wA2; rabAΔ::pyrG^{AF}; pyroA4*	This study
MAD2038	*wA4; pyroA4[pyroA*-rabBp::gfp::rabB] inoB2 nkuAΔ::bar; niiA4*	This study
MAD2050	*agtA::gfp::pyrG^{AF} pabaA1 pyrG89? yA2*	Abenza et al, 2009
MAD2054	*wA4*; *inoB2 nkuAΔ::bar pyroA4[pyroA*-alcA^{p}::mrfp::rabB]; niiA4*	This study
MAD2063	*argB2::[argB*-alcAp::gfp::rabA]; pyroA4[pyroA*-alcAp::mrfp::rabB] nkuA?; niiA4*	This study
MAD2065	*wA4*; *inoB2 nkuAΔ::bar pyroA4::* [*pyroA*-alcA^{p}*:: (*vps27-FYVE*)$_2$::*gfp*]; *niiA4*	This study
MAD2090	*yA2 agtA::gfp::pyrG^{AF} pyrG89?; rabBΔ::pyroA^{AF}; inoB2 pyroA4?*	This study
MAD2095	*agtA::gfp::pyrG^{AF} pabaA1 wA2, rab5BΔ::pyroA^{AF}; pyroA4*	This study
MAD2132	*pabaA1 pyrG89*?; *wA4*; *rabAΔ::pyrG^{AF}*; *inoB2 nkuAΔ::bar pyroA4* [*pyroA*-alcA^{p}*:: (*vps27-FYVE*)$_2$::*gfp*]; *niiA4*	This study
MAD2182	*pyrG89; synA::gfp::pyrGAF; nkuAΔ::bar pyroA4*	This study
MAD2193	*pabaA1; rabBΔ::pyroA^{AF} wA4*;; *inoB2 nkuAΔ::bar pyroA4::* [*pyroA*-alcA^{p}*:: (*vps27-FYVE*)$_2$::*gfp*], *niiA4*	This study
MAD2207	*pyrG89; pyroA4, Δnku::bar; vps19::(HA)3::pyrG^{AF}*	This study
MAD2210	*pyrG89; vps34::(HA)3-pyrG^{AF}; pyroA4, Δnku::bar*	This study
MAD2236	*wa4; rabBΔ::pyroA^{AF}; argB2::[argB*- alcA^{p}::gfp-rabA]; pyroA4?*	This study
MAD2273	*biA1; wA4 rabBΔ::pyroAAF; pyroA4?; pacC900; pantoB100*	This study
MAD2408	*agtA::(HA)3::pyrG^{AF} pyrG89?; pyroA4 nkuAΔ::argB^{AF}?; pantoB100*	This study
MAD2416	*agtA::(HA)3::pyrG^{AF} pyrG89?;rabBΔ::pyroA^{AF}; pyroA4?; pantoB100*	This study
MAD2502	*pyrG89, vps45::(HA)3-pyrG^{AF}; pyroA4, Δnku::bar*	This study
MAD2514	*yA2 pabaA1; argB2::[argB*-alcAp::gfp::rabB^{S31N}]*	This study
MAD2525	*pyrG89; rabAΔ::pyrG^{AF}; nkuAΔ::bar pyroA4*	This study
MAD2526	*pyrG89; wA4 rabBΔ::pyroA^{AF}; pyroA4 nkuAΔ::bar*	This study
MAD2551	*yA2 pabaA1; argB2::[argB*-alcAp::gfp::rabB]*	This study
MAD2679	*pyrG89; pyroA4, Δnku::bar; vps8::(HA)3-pyrG^{AF}*	This study
MAD2685	*rabBΔ::pyroA^{AF}; pyroA4?*	This study
MAD2865	*yA2; pabaA1; argB2::[argB*-alcAp::gfp::rabB^{C216A,C218A}]*	This study
MAD2906	*pyrG89?; rabBΔ::pyroA^{AF}; synA::gfp::pyrG^{AF}; pyroA4? nkuA?*	This study
MAD2912	*pabaA1 yA2 pyrG89?; argB2::[argB*-alcAp::gfp::rabB] rabAΔ::pyrG^{AF}; nkuAΔ::bar*	This study
MAD2931	*pabaA1; rabBΔ::pyroA^{AF}; argB2::[argB*-alcAp::mrfp::rabB]; pyroA4? nkuA?; pacC900?*	This study
MAD2932	*pabaA1; argB2::[argB*-alcAp::mrfp::rabB]; nkuA?; pacC900; pantoB100*	This study
MAD2933	*pabaA1; rabBΔ::pyroA^{AF}; pyroA4?; pantoB100*	This study
MAD3122	*pyrG89, gdiA::(HA)3-pyrG^{AF}; pyroA4; nkuAΔ::bar*	This study

Supplementary Table 2. Plasmids constructed for this work

p1760 pGEX-2T::*rabB*	*rabB* coding region amplified with primers **5´BamHIRabBG** and **3´EcoRIRabB2H** and cloned into pGEX-2T
p1773 pGEX-2T::*rabA*	*rabA* coding region amplified with primers **5´BamHI RabA** and **3´ EcoRI RabA** and cloned into pGEX-2T
p1795 rabB^{p}::GFP-RabB	Constructed by fusion PCR of three fragments: (i) 559 bps of the 5'-UTR of *rabB* were PCR-ed from genomic DNA with primers **JF58** and **JF59**; (ii) The GFP coding region was amplified with primers **Fw5UTRRabBjointGFP** and **RevORFRabBjointGFP**; (iii) The *rabB* (AN3842) ORF was amplified from genomic DNA with primers **FwRabBORF** and **RevpyrojointRabB**. The fusion PCR product was cloned in pGEM-t-Easy to yield p1794. *rabB^{p}::gfp::rabB* was amplified from p1794 with primers **KpnI5UTRRabBFw** and **EcoRIRabBRev** and KpnI/EcoRI-cloned into pgpdA003 (Pantazopoulou and Peñalva, 2009). The resulting plasmid drives GFP-RabB expression under the control of the physiological *rabB* promoter and includes a *gpdA* transcriptional terminator, as well as *pyroA**, which targets integration into the *pyroA* locus.
p1815 pGEX-2T::*rabE^{Ypt31}*	*rabE* coding region amplified with primers 5'BamHI **Ypt31/pGEX** and **3' EcoRI Ypt7/pGEX** and cloned into pGEX-2T
p1816 pGEX-2T::*rabN^{Ypt7}*	*rabN* coding region amplified with primers **5'BamHI Ypt7/pGEX** and **3' EcoRI Ypt7/pGEX** and cloned into pGEX-2T
p1822 alcA^{p}::mRFP-RabB	The RabB ORF was amplified from genomic DNA with primers **FwRabBKpnI** and **RevRabBAgeI** and KpnI/AgeI cloned in pALC^{pyroA} (Calcagno-Pizarelli et al., 2007). *mrfp* was amplified by PCR using primers **Fw RFPKpnI** and **RevRFPKpnI** and attached in frame (KpnI) to the 5'-end of the *rabB* coding sequence. This plasmid contains *pyroA** for *pyroA* targeting. p1822 drives expression of mRFP-RabB under the control of the *alcA^{p}*
p1832 alcA^{p}::[vps27-FYVE]2-GFP	The FYVE domain of AnVps27 (AN2071) was PCR-amplified from genomic DNA (primers **FW KpnFYVE** and **Rev AgeI FYVE**, see oligonucleotide list) and KpnI/AgeI cloned in pALC^{pyroA} (Calcagno-Pizarelli et al., 2007). PCR-amplified GFP (primers **Fw GFP AgeI** and **Rev GFP AgeI** was attached in frame to the FYVE domain C-terminus AgeI site. A second FYVE domain was appended in frame to the N-terminus of the resulting fusion protein as a KpnI fragment (primers **FW KpnFYVE** and **Rev KpnFYVE**). p1832 thus drives expression of [AnVps27-FYVE]2::GFP under the control of the *alcA^{p}*. It carries a mutated *pyroA** allele that targets integration at the *pyroA* locus.
p1885 alcA^{p}::GFP-RabB	The RabB coding sequence was amplified from cDNA with primers **JFA29** and **JFA30** and topo-cloned. After attachment of 5'-BamHI and 3'-EcoRI sites (primers **JFA115** and **JFA116**), the ORF was cloned into pALC1 (Mingot et al., 1999) and the GFP was appended to the RabB N-terminus as a BamHI fragment. pALC1 carries an *argB** allele to target integration to *argB2*.
p1896 alcA^{p}::GFP-RabB^{S21N}	Constructed as above. The missense mutation leading to Ser21Asn substitution predictably locking RabB in the GDP conformation was introduced by site directed mutagenesis with primers **JFA70** and **JFA71**.
p1944 alcA^{p}::GFP-RabB$^{C216A, C218A}$	Constructed as above. The missense mutations leading to Cys216Ala and Cys218Ala substitutions were introduced by site-directed mutagenesis using primers **JFA125** and **JFA126**.

Supplementary Table 3. List of oligonucleotides used in this work

cDNA cloning into pGEX-2T	
rabA	
5´BamHI RabA	GGATCCATGTCTGAATCAACGCCCG
3´ EcoRI RabA	GAATTCTTAACAGGCGCATCCCTC
rabS (ypt7)	
5'BamHI Ypt7/pGEX	AAAGGATCCATGTCATCACGGAAGAAGGTC
3' EcoRI Ypt7/pGEX	AAAGAATTCAGCAGGCGCAACCGTC
rabE (ypt31)	
5'BamHI Ypt31/pGEX	AAAGGATCCATGGCTAACGACGAGTATG
3' BamHI Ypt31/pGEX	AAAGGATCCTTAACAGCATCCACCCTTG
rabB	
5´BamHIRabBG	GGATCCATGGCATCCAGAGCCGC
3´EcoRIRabB2H	GAATTCTAACAATTACACGCTCCGCC
C-terminal (HA)3 tagging	
vps8 (AN0244)	
5´Vps8	GTGGGTATCCTACAGAGCGTCCAGG
3´Vps8	TTCGCTATGTACAGGACAAACCGGAC
5´Vps8TAG	GTGTCCGGTTTGTCCTGTACATAGCGAAGGAGCTGGTGCAGGCGCTGGAGCCGGTGCC
3´Vps8TAG	GTAACATAAAATGCAGATCGCTCATGCCTGTCTGAGAGGAGGCACTGATGCGTGATG
5ÚTRVps8	GCATGAGCGATCTGCATTTTATGTTAC
3ÚTRVps8	CTGACTCCAGACAAATGCCCTGAC
vacA-vps19 (AN3144)	
5´TagVps19	CGAGTCGTTTGCGGTGATGTCTTGAC
3´TagVps19	TGTGTGGTTGCCCATATAGGCACCTTC
5´HAVps19	CTTTGAAGGTGCCTATATGGGCAACCACACAGGAGCTGGTGCAGGCGCTGGAGCCGGTGCC
3´HA-Vps19	GAAGAGAAAAGGATGTGAATATGGAGGGGTGCTGTCTGAGAGGAGGCACTGATGCGTGATG
5´UTR-Vps19	CACCCCTCCATATTCACATCCTTTTCTC
3´UTR-Vps19	GTAGCTTGATTCCCCGAACTCCAGCC
pikA-vps34 (AN4709)	
5´TagVps34	CCAAGGTGAACTTGTCGCCGTGCTC
3´TagVps34	CGCTCTCCATCCCTGGACAAAATCATGAAG
5´TagColVps34	CTTCATGATTTTGTCCAGGGATGGAGAGCGGGAGCTGGTGCAGGCGCTGGAGCCGGTGCC
3´TagcolVps34	GAAAAGTATTATAAATAGGGTTTTGAAAGGCTGTCTGAGAGGAGGCACTGATGCGTGATG
5ÚTRVps34	CCTTTCAAAACCCTATTTATAATACTTTTC
3ÚTRVps34	CTGCTTTCAGGGCTTGCGTTATACTGATAC
vps45 (AN6531)	
5´Vps45	GAGTTACGGGAAATTGTTCTTTCGC
3´Vps45	TCGCCCAATATTCCTCCGAAGCCGTCC
5´Vps45Tag	GGACGGCTTCGGAGGAATATTGGGCGAGGAGCTGGTGCAGGCGCTGGAGCCGGTGCC
3´Vps45Tag	GTAGAGAATGATAAACATAATTGGAAATCTGTCTGAGAGGAGGCACTGATGCGTGATG
5´UTRVps45	ATTTCCAATTATGTTTATCATTCTCTAC
3´UTRVps45	CTCCATGAGTCCCTCAACAGCATAC

gdiA (AN5895)	
3´3UTR-gdi	CAACCACCTCGATCAGTATCTTTAAC
5´3UTR-gdi	ATATCTCAAACCCATATCGGGATTAATATAC
3´gdi	CTCCTGGGCGAGAGTTTGGCCCTC
5´gdi	CCATTGATGGCGCTAACACAGTC
3´gdi-tag	GTATATTAATCCCGATATGGGTTTGAGATATCTGTCTGAGAGGAGGCACTGATGCGTG
5´gdi-tag	GAGAGGGCCAAACTCTCGCCCAGGAGGGAGCTGGTGCAGGCGCTGGAGCCGG
Constructs for fluorescent fusion proteins	
alcAp::[vps27-FYVE]2-GFP	
FW KpnFYVE	GGGGTACCATGCCGGAATGGATCGACTCCGAC
Rev KpnFYVE	GGGGTACCTGAAGTTAGCTTCGCGTAGCAC
Rev AgeI FYVE	GGGACCGGTTGAAGTTAGCTTCGCGTAGCAC
Fw GFP AgeI	GGGACCGGTATGGTGAGCAAGGGCGAGG
Rev GFP AgeI	GGGACCGGTTTACTTGTACAGCTCGTCCA
rabB^{p}::GFP-RabB	
JFA58 5'UTR rabB fw	GGCATCTTCGTCATGATCGTGC
JFA59 5'UTR rabB rev	GATGGGGACGGTTGGACAATGAGC
Fw5UTRRabBjointGFP	GCTCATTGTCCAACCGTCCCCATCATGGTGAGCAAGGGCGAGGAGCTG
RevORFRabBjointGFP	TGCTGCGGCTCTGGATGCCATCTTGTACAGCTCGTCCATGCCGTG
FwRabBORF	ATGGCATCCAGAGCCGCAGGC
RevpyrojointRabB	GTAATCCAGCATCTGATGTCCATCGGCTCTAACAATTACACGCTCCGCCA
KpnI5UTRRabBFw	GGGGTACCCGGAGGGATACTGAATGAGAG
EcoRIRabBRev	GGAATTCGCTCTAACAATTACACGCTCC
alcA^{p}::mRFP-RabB	
FwRabBKpnI	GGGGTACCATGGCATCCAGAGCCGCAGC
RevRabBAgeI	GGGACCGGTCTAACAATTACACGCTCCGC
Fw RFPKpnI	GGGGTACCATGGCCTCCTCCGAGGACGTC
RevRFPKpnI	GGGGTACCGGCGCCGGTGGAGTGGC
alcA^{p}::GFP-RabB wild-type and mutants	
JFA029 5'rabB fw	ATGGCATCCAGAGCCGCAGC
JFA030 3'rabB rev	CTAACAATTACACGCTCCGCC
JFA115 5'BamHI rabB fw	GGATCCATGGCATCCAGAGCCGC
JFA116 3'EcoRI rabB rev	GAATTCTAACAATTACACGCTCCGCC
JFA070 S31N rabB fw	CCTCGGAGAATCTGCCGTAGGAAAGAACTCATTAGTCCTGAG
JFA071 S31N rabB rev	CTCAGGACTAATGAGTTCTTTCCTACGGCAGATTCTCCGAGG
JFA125 C216A C218A rabB fw	CCCAAAGTGGCGGAGCGGCTAATGCTTAGAATTCCAGC
JFA126 C216A C218A rabB rev	GCTGGAATTCTAAGCATTAGCCGCTCCGCCACTTTGGG
Deletion PCRs	
rabB deletion PCR	
JFA058 5'UTR rabB fw	GGCATCTTCGTCATGATCGTGC
JFA059 5'UTR rabB rev	GATGGGGACGGTTGGACAATGAGC
JFA060 3'UTR rabB fw	AGCCTATTCGATCCGTCCG
JFA061 3'UTR rabB rev	GCTCCAGCTCTCGCAAGCC
JFA062 5' delta rabB-pyrGfum	GCTCATTGTCCAACCGTCCCCATCCGATGGACATCAGATGCTGG
JFA063 3' delta rabB-pyrGfum	CGGACGGATCGAATAGGCTGGACAAATGCACAGAACACCC

Anvps8 deletion PCR	
JFA127 5'UTR vps8 fw	CTCGGCGACAAACCAGTCAG
JFA128 5'UTR vps8 rev	CAGCGGTATGTCCGAATGTTC
JFA129 3'UTR vps8 fw	GTTCATATAGGCGTTATTCATCGG
JFA130 3'UTR vps8 rev	GGCCATCTTCCACTTCCTTCG
JFA131 5' delta vps8-pyrGfum	GAACATTCGGACATACCGCTGGTAACGGCCGCCAGTGTGCTG
JFA132 3' delta vps8-pyrGfum	CCGATGAATAACGCCTATATGAACCTGTCTGAGAGGAGGCACTGATGCG

Anvps45 deletion PCR	
JFA136 5'UTR vps45 fw	GTCAAACGCAGGACCGCC
JFA137 5'UTR vps45 rev	GGCGAATGGAAGATTGAAAAATG
JFA138 3'UTR vps45 fw	CGGTGTGCTGGCGTTCATATG
JFA139 3'UTR vps45 rev	GTTTGTCTCAAATTGGTCGCGG
JFA140 delta vps45-pyrG fw	CATTTTTCAATCTTCCATTCGCCGTAACGGCCGCCAGTGTGCTG
JFA141 delta vps45-pyrG rev	CATATGAACGCCAGCACACCGCTGTCTGAGAGGAGGCACTGATGCC

Supplementary Table 4. Proteins identified by MALDI-TOF/TOF

RabB-GTPγS Interacting proteins

Protein name[a]	Accession code[b]	total score[c]	expect[d]	ions score[e]	MW Da theor[f].	pI theor[g].	Matches peptides[h]	Sequence Coverage
AnVps8	XP_657848	127	$1{,}3x10^{-6}$	65	177571	5,52	26	17
AnVps3	XP_658367	99	$6x10^{-5}$	n.d. (**)	126115	5,13	21	20
AnVps18	XP_659870	358	$8{,}3x10^{-31}$	242	109018	5,61	28	31
AnVps11	AN12235.1(*)	80	0.0058	33	107290	5,65	21	20
AnVps16	XP_664515	274	$2{,}1x10^{-22}$	108	87426	5,31	27	37
AnVps33	XP_660022	474	$2{,}1x10^{-42}$	223	72498	6,94	39	56
AnVps45	XP_664135	612	$3{,}3x10^{-56}$	414	67027	6,28	33	45

RabB- and RabA-GDP Interacting protein

Protein name[a]	Accession code[b]	total score[c]	expect[d]	ions score[e]	MW Da theor[f].	pI theor[g].	Matches peptides[h]	Sequence Coverage
AnGdiA	XP_663463	275	$2x10^{-11}$	ND	52499	7,85	19	74

RabA- GTPγS Interacting proteins

Protein name[a]	Accesion code[b]	total score[c]	expect[d]	ions score[e]	MW Da theor[f].	pI theor[g].	Matches peptides[h]	Sequence Coverage
AnVps8	XP_657848	104	$2{,}1x10^{-5}$	52	177571	5,52	20	23
AnVps3	XP_658367	238	$1{,}3x10^{-17}$	117	126115	5,13	33	38
AnVps18	XP_659870	285	$3.3x10^{-25}$	243	109018	5,61	15	23
AnVps11	AN12235.1(*)	174	$2{,}1x10^{-12}$	48	107290	5,65	30	33
AnVps16	XP_664515	157	$2{,}1x10^{-12}$	61	87426	5,48	23	20
AnVps33	XP_660022	138	$8{,}3x10^{-9}$	52	72498	6,94	20	33
AnHsp70	XP_663614	408	$1{,}3x10^{-34}$	277	72328	5,78	27	43

[a]Protein name as shown in 1D SDS-PAGE in Figure 9 .[b]Protein accession code from Swiss-Prot database, with the exception of AnVps11 (*, see below) [c]Mascot score/[d]Expected value. [e]Mascot ion score. [f]Theoretical molecular weight (Da). [g]Theoretical pI. [h]Number of matched peptides (**, see below). [i]Protein sequence coverage for the most probable candidate as provided by Mascot. (*) The amino acid sequence corresponding to this entry (XP_660065) contains an incorrect ~500 residue C-terminal extension due to erroneous prediction of the coding region. The 957 residue AnVps11 protein listed as AN12235.1 in the Broad Institute Aspergillus Database (http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html) is correct and was considered for sequence coverage. (**) Data shown refer all to the same GST-RabB experiment, in which no AnVps3 peptides were sequenced. However, in a second experiment with GST-RabB, we determined the sequence of four peptides (63 residues) matching the AnVps3 sequence from this band, leading to an ions score of 330, and thus unequivocally confirming that this band actually represents AnVps3.