Table S1. Oligonucleotides used in this study.

Primer	*Sequence 5'→3'**
VPS21ORFF	TCAGGATCCAGTCAACATCCAGCCCCCGCA
VPS21ORFR-MLUI	TCATCAACGCGTATTACCTTAACTTCAGTCTCC
VPS21DETR	GCAACATGTGCTTCTTGGAGC
YPT52AMPF	TCAGGATCCGAGCTCTGTAGATCTGTGCACGTGACC
YPT52AMPR	TCAGGATCCGGTACCGCTGAAAAAGTTGGTTCTGCC
YPT52AMPF2	TCAGGATCCGAGCTCAATCTTTACAACCATTGTTCG
YPT52DETF	GAAGAGCTTTACCTGTGGACG
YPT52DETR	TTCTTGACAAAACGGTGTACG
YPT52DETR2	GTTGCTTTGATTTACTTGACC
YPT52SEQF	TATATTGTATACCTTCACCTC
YPT52DISF	TTTATCTTAACTTGTGTAATATATTGTATATGTATACCTTCACCTCATCTATTTATTCCAAGT TAAATTTATTTTCGTGGAATTGTGAGCGGATA
YPT52DISR	CTTTGATCAAGTAAAAATAGAAGCTCGAATAATGATTAATGAATAATAATAACTATATCA AATTCTAGGTTTTCCCAGTCACGACGTT
YPT52ORFR-MLUI	TCATCAACGCGTTCAAGTAAAAATAGAAGCTCG
YPT53DISF	AAAAAAAGCGAGTAAGAAAACTGATACAGTGTTAATTTGCATTAGAATCAAATAGTGCTA TTAAATCACAGTGGAATTGTGAGCGGATA
YPT53DISR	TTAACAGCAATATGAGTTAGATGTCTGTCTCGTACGTAGCATGTTTCCTATTTGATCAGAAGT ATCACGAGGTTTTCCCAGTCACGACGTT
YPT53AMPF	TCAGGATCCGAGCTCGAAATCTCAGTATACGAAGGG
YPT53AMPR	TCAGGATCCGGTACCACTTTTCTGACATGACAGTGG
YPT53DETF	CAGTTCAAAAGAAGGCACAGG
YPT53DETF2	ATAGAGATTCGATTGGTAGGG
YPT53DETR2	GTTAGATGTCTGTCTCGTACG
YPT53SEQF	TGCATTAGAATCAAATAGTGC
YPT53ORFF-BAMHI	TCATCAGGATCCAAATAGTGCTATTAAATCACA
YPT53ORFF-CLAI	TCATCAATCGATCAAATAGTGCTATTAAATCAC
YPT53ORFR-PSTI	TCATCACTGCAGCAATGATTCAATTCCTGTGCC
YPT53ORFR-EAGI	TCATCACGGCCGGATTTGTAATGATGATAAAGG
YPT53ORFR-MLUI	TCATCAACGCGTGATTTGTAATGATGATAAAGG
YPT52EAGF	TCCAAGTTAAATTTATTTTCCGGCCGTCGAACAAACAATCTAACCAACG
YPT52EAGR	CGTTGGTTAGATTGTTTGTTCGACGGCCGGAAAATAAATTTAACTTGGA
YPT53EAGF	CAAATAGTGCTATTAAATCACACGGCCGCTGGAAACATCTCACTCATCAA
YPT53EAGR	TTGATGAGTGAGATGTTTCCAGCGGCCGTGTGATTTAATAGCACTATTTG
YPT52S26NF	GGAGAAAGTGCAGTTGGTAAAAATTCTATCGTACACCGTTTTGTC
YPT52S26NR	GACAAAACGGTGTACGATAGAATTTTTACCAACTGCACTTTCTCC
YPT52Q73LF	GAAATATGGGATACTGCAGGACTTGAGCGTTACAAGTCATTGGC
YPT52Q73LR	GCCAATGACTTGTAACGCTCAAGTCCTGCAGTATCCCATATTTC
YPT53T27NF	GGAGATTCTTCCGTGGGAAAAAATTCGCTTGTACACAGATTCAC
YPT53T27NR	GTGAATCTGTGTACAAGCGAATTTTTTCCCACGGAAGAATCTCC
YPT53Q75LF	GATATGGGACACAGCAGGCCTAGAAAGGTACCGTTCATTG
YPT53Q75LR	CAATGAACGGTACCTTTCTAGGCCTGCTGTGTCCCATATC
VPS21S24NF	GGGAGAAGCTGCAGTAGGAAAGAATTCGTTGGTGTTGCGATTTGTGTCC
VPS21S24NR	GGACACAAATCGCAACACCAACGAATTCTTTCCTACTGCAGCTTCTCCC
VPS21Q69LF	GAAATCTGGGACACTGCTGGGTTAGAGCGTTTTGCTTCTCTTGCC
VPS21Q69LR	GGCAAGAGAAGCAAAACGCTCTAACCCAGCAGTGTCCCAGATTTC
VPS21SEQF	AATACTAGTAGTATCACCATC
YPT72T24NF	AGATTCTGGTGTTGGTAAAAATTCACTTATGCAACAATTTG
YPT72T24NR	CAAATTGTTGCATAAGTGAATTTTTACCAACACCAGAATCT
YPT72Q70LF	CAAATCTGGGATACCGCTGGTTTAGAAAGATTTCAAAGTTTAGG

YPT72Q70LR	CCTAAACTTTGAAATCTTTCTAAACCAGCGGTATCCCAGATTTG
YPT72SEQF	GAAGATAATTAAAAGGCTGAC
APS3DISF	AATGATGGTCTTCCGAGACTAATGAAATTCTATACCAAAGTCGATATT CCAACACAGAAATTGCTCTTGCTGTGGAATTGTGAGCGGATA
APS3DISR	TATACTATTTTTGCATAATTCATTTCGTATTTATCGCTTTTACCTACCC CAGAACCCTCTATCTCGAGACTTTCCCAGTCACGACGTT
APS3AMPF2	TCATCAGAGCTCCTTTGCACCAACAATGGGGGC
APS3AMPR	TCATCAGGATCCAGGAAGCAGAAAGGTTGATGC
APS3DETF2	CAAGTGCACCTGTTGATATCTAC
APS3DETR2	GTACTTCCATTGGAGTTGACG
APS3FRAGF	TCAGTCGACTAATCTACTAGATCAGCACAAGAGTG
APS3FRAGR2	TCAACGCGTGGTGTCGATCACCATCCCGCC
ACT1PF	TCATCAGGTACCCCAGCCTCGTTTATAAACTTAGTC
ACT1PF-BAMHI	TCAGGATCCCCAGCCTCGTTTATAATAAACTTAGTC
ACT1PR2	TCATCAGAGCTCTCAGTCGACTTTGAATGATTATATTTTTTTAATATTAATATCGAG
ADH1-3'UTRF	TCATCAGTCGAC*GGTGGT*ATCGAT*GGTGGT*CGGCCG*GGTGGT*ACGCGTGCATGCTAAGCAAAT AGCTAAATTATATACG
ADH1-3'UTRR	TCATCAGAGCTCGAAAACTTGAAACTTGAAAACACC
ADH1-3'UTRR-APAI	TCATCAGGGCCCATCAATGCCAGAGATCAAACC
ARG4DETF	ATCAATTAACACAGAGATACC
ARG4DET2	CCGAGCTTGGCGTAATCATGG
HIS1F1268	CCGCTACTGTCTCTACTTTG
PGUR	GAATACTCAAGCTATGCATCC
URA3INTF	TTAGTGTTACGAATCAATGGC
URA3INTR	CAATTATAAATGTGAAGGGGG
GFPEAGF	TCACGGCCGATGTCTAAAGGTGAAGAATTATTC
GFPEAGR	TCACGGCCGTTTGTACAATTCATCCATACC
GFPSALIF	TCAGTCGACATGTCTAAAGGTGAAGAATTATTC
GFPSEQF	TACTTATCCACTCAATATGCC
GFPDETR2	AACCAAAATTGGGACAACACC
MCHORFF	TCAGTCGACCGGCCGATGGTTTCAAAAGGTGAAGAAG
MCHORFR	TCACGGCCG*ACCACCACC*TTTATATAATTCATCCATACCACC
LUXINTDETF	CTGACCTTTAGTCTTTCCTGC
LUXINTDETR	CAGTAGTACTTGTTGTTGTATCG
MLT1GFPF	ACCTCAAAACTTGTTGAAGAACAAAGACAGTATTTTCTACTCTCTTG CCAAAGAAGGTGGATACATAGATGGTGGTGGTTCTAAAGGTGAAGA ATTATT
MLT1GFPR	TGTAAACTAAAAAAAATATTATTGTATAAATAAAAAAATCACTATAT GAATATATATCGCACCGATATATATCTAGAAGGACCACCTTTGATT
MLT1DETF	GATCTTAGTGTTAGATAGTGG
TETOAMPF	TCAGGTACCTGGACTTCTTCGCCAGAGG
TETOAMPR	TCAGTCGACTTTTCTGAGATAAAGCTGTTTTT
UME6ORFF-SalI	TCATCAGTCGACGTATAAATGATTACCCATATGG
UME6ORFR-MluI	TCAACGCGTAATTCTTAATTCTTAACTTAGCC
HWP1DETF	GACTCCAGCTGGTTCACAACC
HWP1DETR	ATACCAATAATAGCAGCACCG
ACT1DETF	TTGGCTGGTAGAGACTTGACC
ACT1DETR	GTGGTGAACAATGGATGGACC

* Engineered restriction enzyme sites are underlined. Glycine encoding linkers are italicized.

Table S2. Percent identity* and similarity between *S. cerevisiae* and *C. albicans* endosomal GTPases.

	ScVps21p	**ScYpt52p**	**ScYpt53p**	**CaVps21p**	**CaYpt52p**	**CaYpt53p**
ScVps21p	100					
ScYpt52p	46.4(62.8)	100				
ScYpt53p	54.3(70.6)	43.8(57.8)	100			
CaVps21p	66.8(78.2)	45.7(59.1)	50.4(67.2)	100		
CaYpt52p	48.9(63.3)	48.6(60.1)	43.4(58.3)	48.1(59.7)	100	
CaYpt53p	37.6(56.0)	33.9(51.0)	34.6(53.0)	37.3(53.4)	35.1(50.4)	100

* Protein sequences were aligned by using the EMBOSS pairwise align algorithm (http://www.ebi.ac.uk/emboss/).

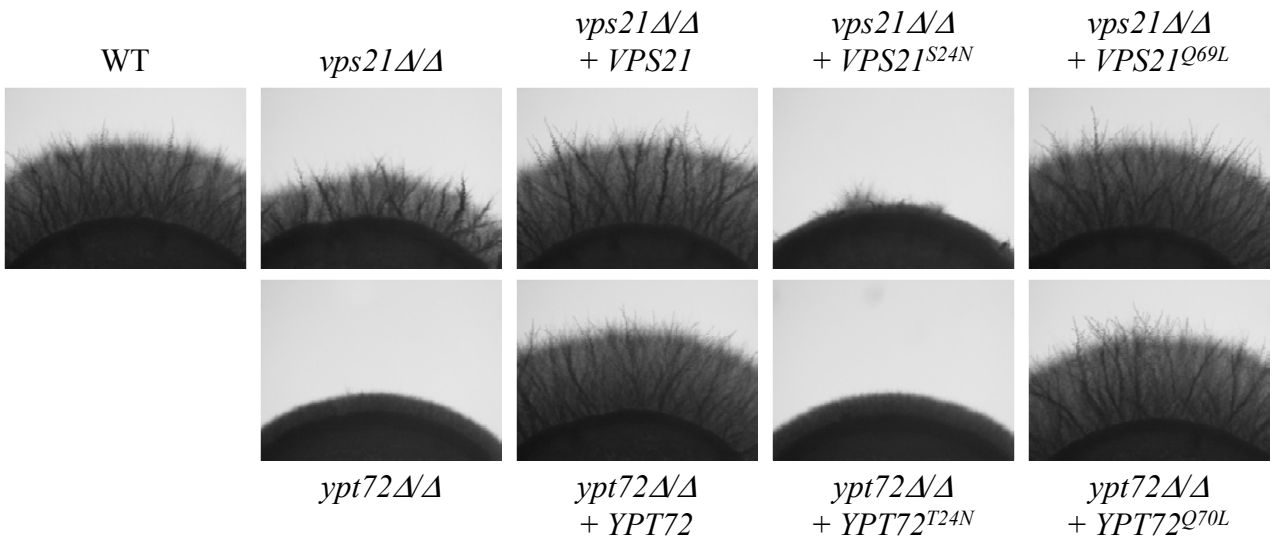


Figure S1. Vps21p and Ypt72p Rab GTPases are required to be in their active conformation to support *C. albicans* hyphal growth. Inactive (*VPS21*^{S24N} and *YPT72*^{T24N}) and active (*VPS21*^{Q69L} and *YPT72*^{Q70L}) conformational mutant alleles of either GTPase were introduced into their respective *vps21*Δ/Δ and *ypt72*Δ/Δ gene deletion strains. Cell suspensions of each strain were applied as spots to M199 agar and incubated at 37ºC for 4 days. Similar results were found on 10% FBS agar at 37ºC (data not shown). WT = YJB6284. Data representative of three independent experiments is shown.

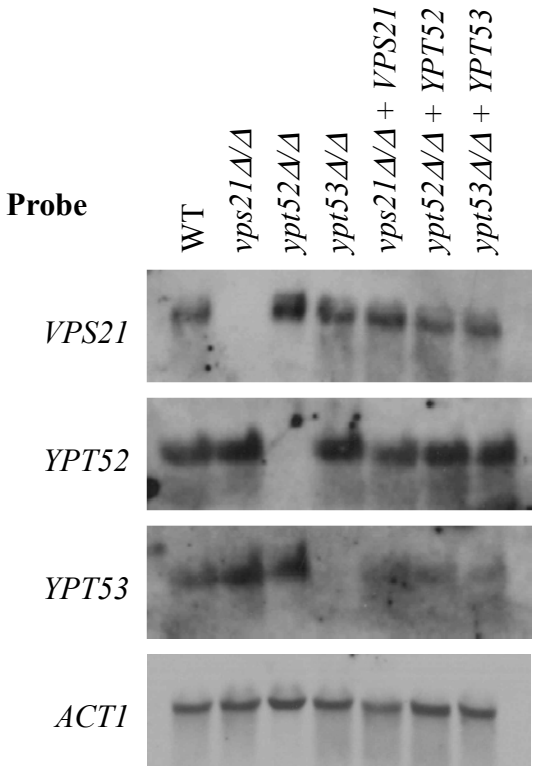


Figure S2. Detection of *VPS21*, *YPT52* and *YPT53* transcripts. RNA was extracted from each strain grown in YPD broth at 30ºC (yeast) overnight, and subject to northern blot analysis with *VPS21*, *YPT52* and *YPT53* specific probes, or an *ACT1* probe as a loading and RNA integrity control. WT = YJB6284. Exposure times with the non-isotypic detection system were 5 minutes for *VPS21*, overnight for *YPT52* and *YPT53*, and 3 minutes for the *ACT1* probe.

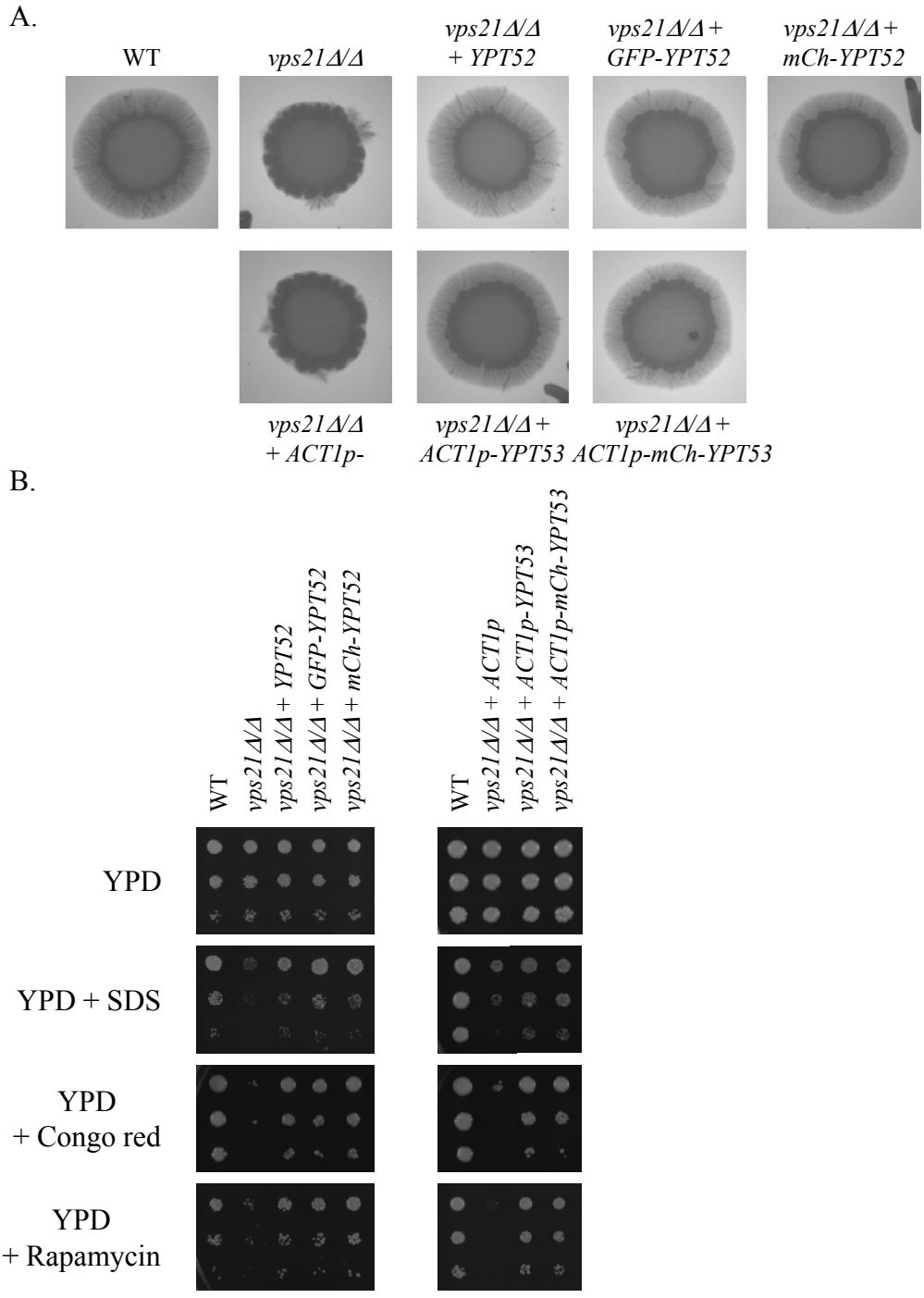


Figure S3. Fluorescent Ypt52p and Ypt53p fusion proteins retain biological function. (A) Cell suspensions of each strain were applied as spots to M199 agar plates and incubated at 37ºC for 5 days. (B) Cell suspensions of each strain were prepared by serial dilution, applied to YPD agar or to YPD agar supplemented with 0.02% SDS, 25 µg/ml congo red or 5 nM rapamycin and incubated at 30ºC for 2 days. WT = YJB6284. Each experiment was performed at least twice with similar results. Data representative of 2 independent experimental replicates is shown.

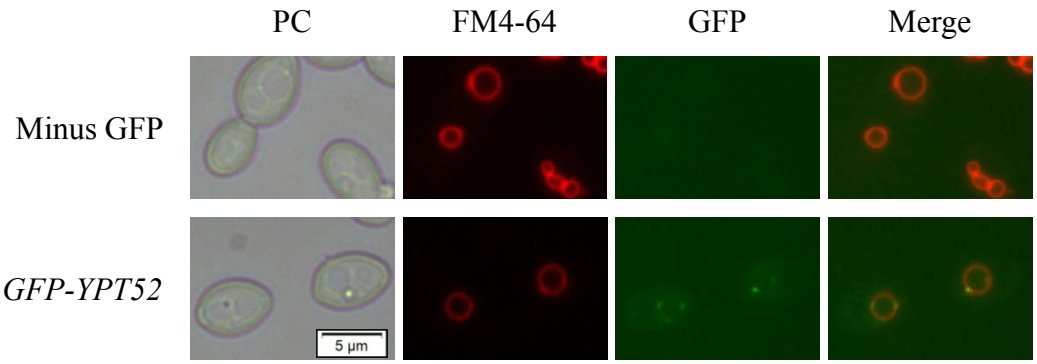


Figure S4. Ypt52p localizes to a sub-vacuolar compartment. A *GFP-YPT52* fusion construct was introduced into the *ypt52*Δ/Δ mutant (transcription of the *GFP-YPT52* fusion was dependent upon *YPT52* 5' and 3' UTR sequences). Each strain was pulse-chase labeled with FM4-64 to label the vacuole (red), and cells imaged using phase contrast (PC) and epifluorescence microscopy with Texas-red and FITC (green) filter sets and 100X objective. Data representative of three independent experimental replicates is shown.

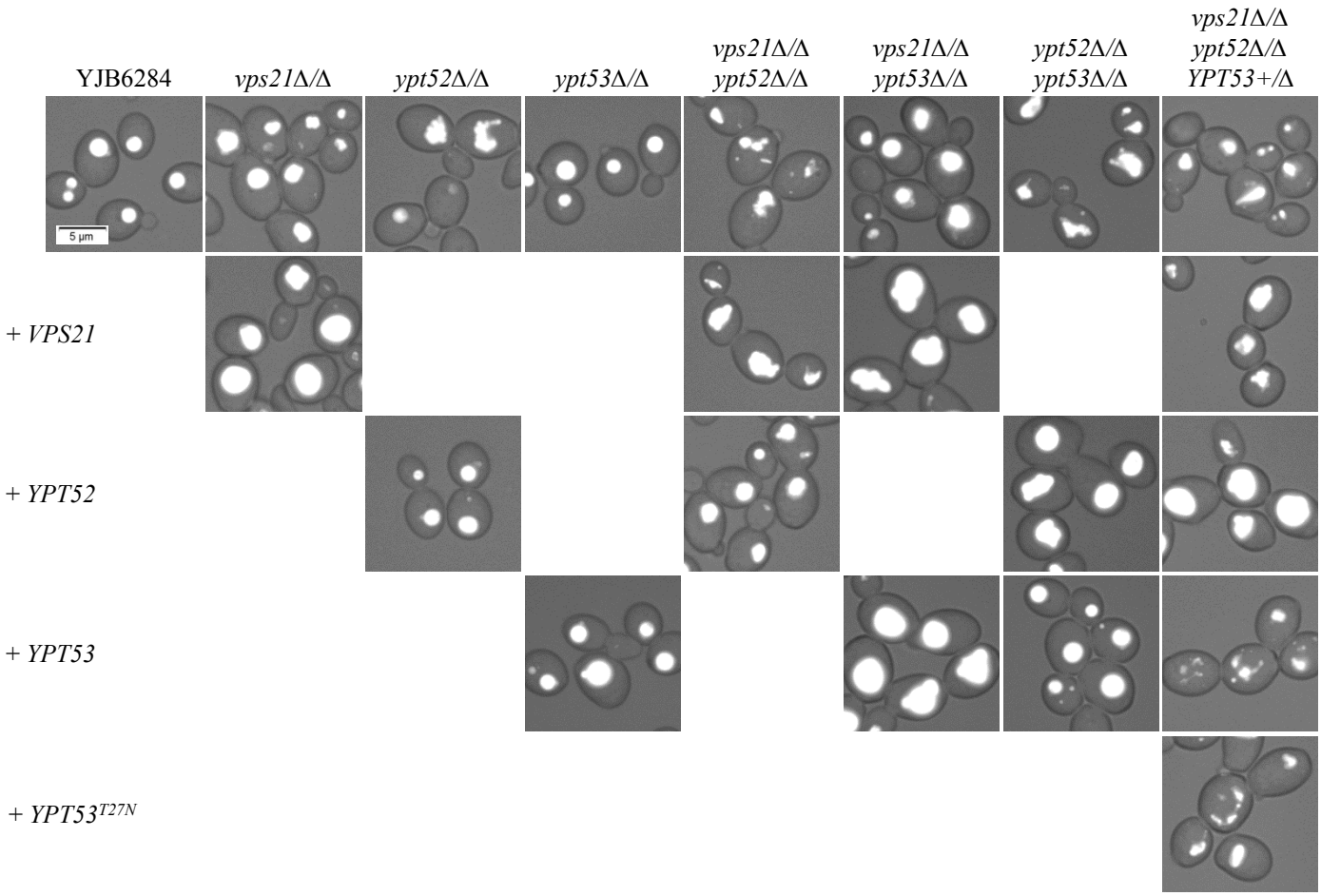
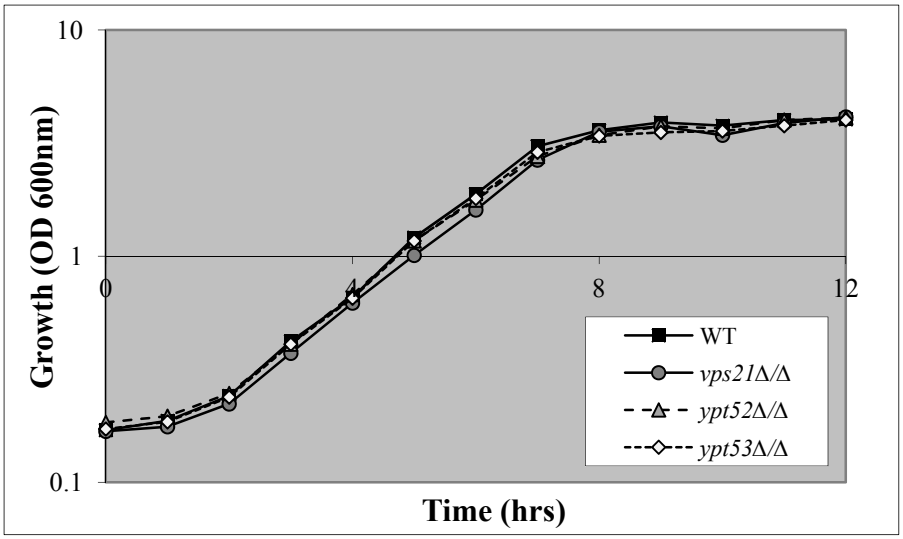


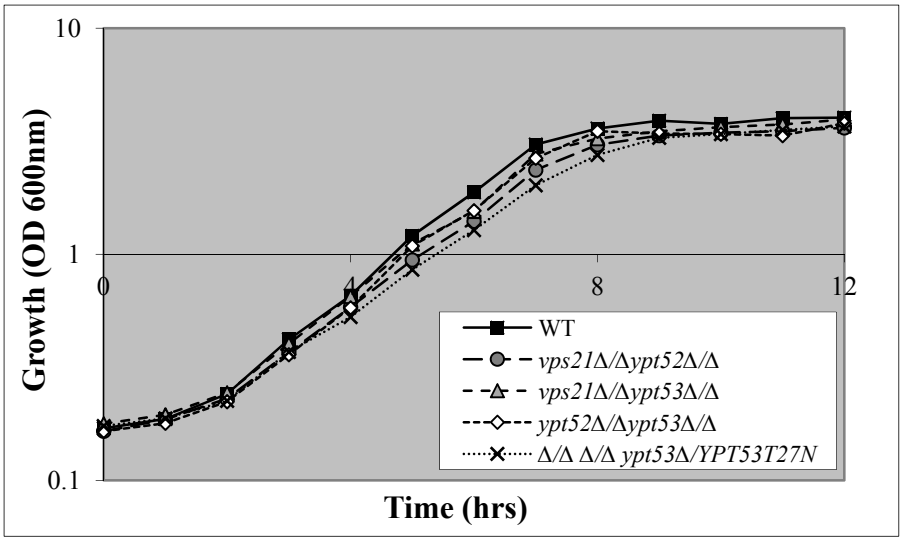
Figure S5. Ypt52p affects vacuole morphology in *C. albicans* yeast. Each strain was grown as yeast in YPD broth at 30ºC, and vacuoles stained with CMAC. Live cells were then observed by phase contrast and epifluorescence microscopy. Merged images are shown. Data is representative of 2 or more experimental replicates for each genotype.

Figure S6. Growth analysis of PVC GTPase mutants. Each strain was grown in YPD broth at 30ºC overnight, then sub-cultured to fresh YPD broth (pH 5.7 A, B, C; or pH 7 D, E, F) to an OD600 of 0.2, and incubated at 30ºC, 180 rpm. OD600 was determined at hourly intervals. WT = strain YJB6284. Data is representative of 2 experimental replicates.

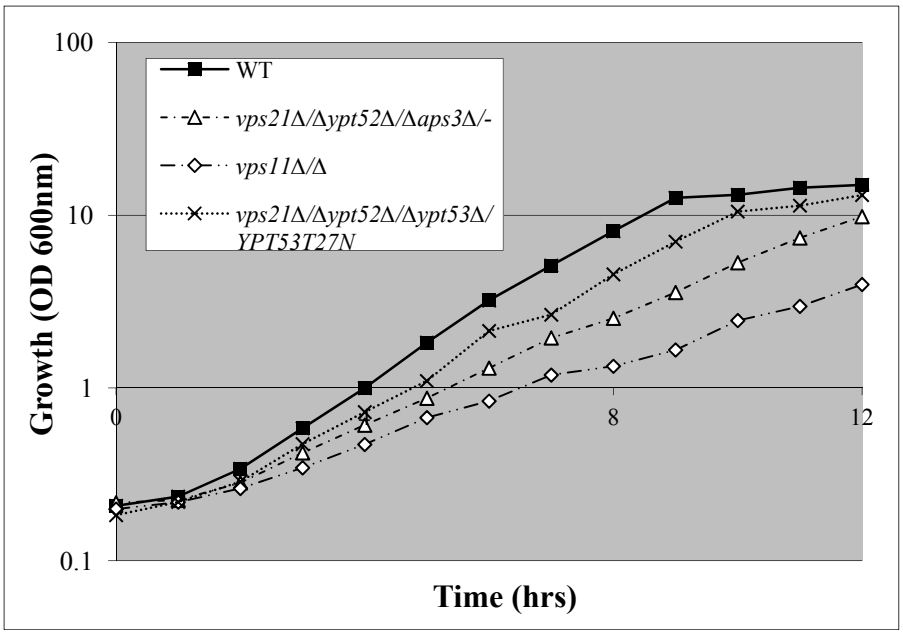
A.



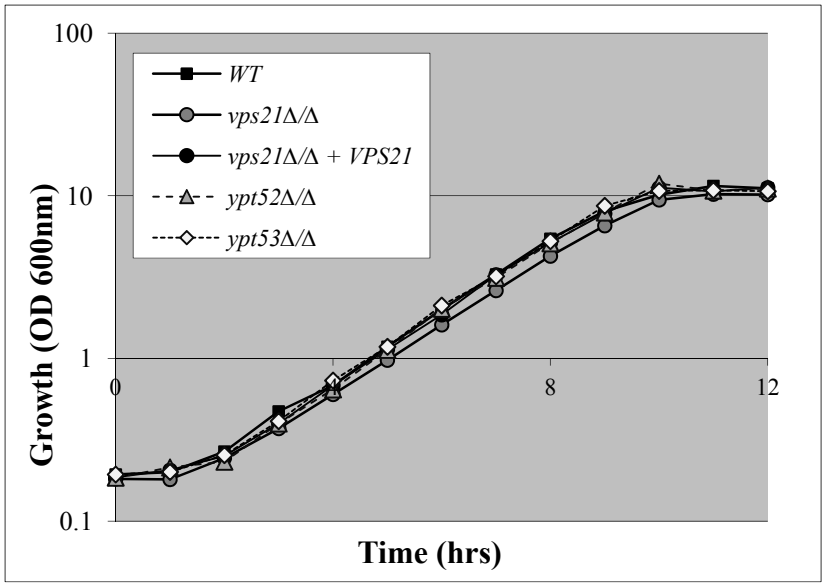
B.



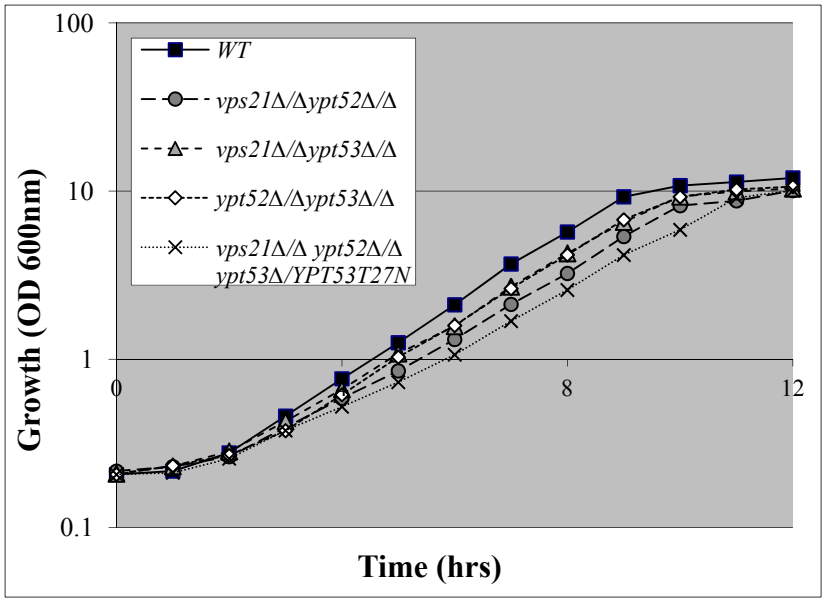
C.



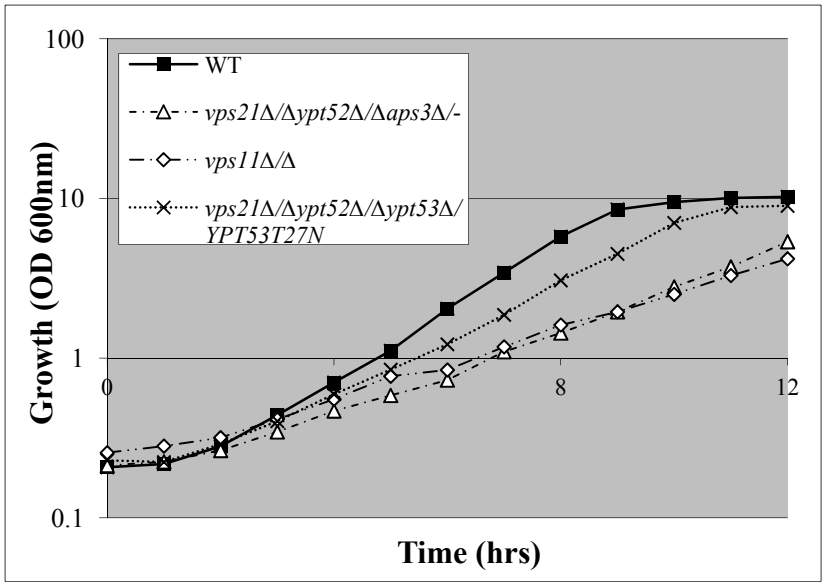
D.


100
10
1
0.1
0
8
12
Growth (OD 600nm)
Time (hrs)
WT
vps21Δ/Δ
vps21Δ/Δ + VPS21
ypt52Δ/Δ
ypt53Δ/Δ

E.


100
10
1
0.1
0
8
12
Growth (OD 600nm)
Time (hrs)
WT
vps21Δ/Δypt52Δ/Δ
vps21Δ/Δypt53Δ/Δ
ypt52Δ/Δypt53Δ/Δ
vps21Δ/Δ ypt52Δ/Δ ypt53Δ/YPT53T27N

F.


100
10
1
0.1
0
8
12
Growth (OD 600nm)
Time (hrs)
WT
vps21Δ/Δypt52Δ/Δaps3Δ/-
vps11Δ/Δ
vps21Δ/Δypt52Δ/Δypt53Δ/YPT53T27N

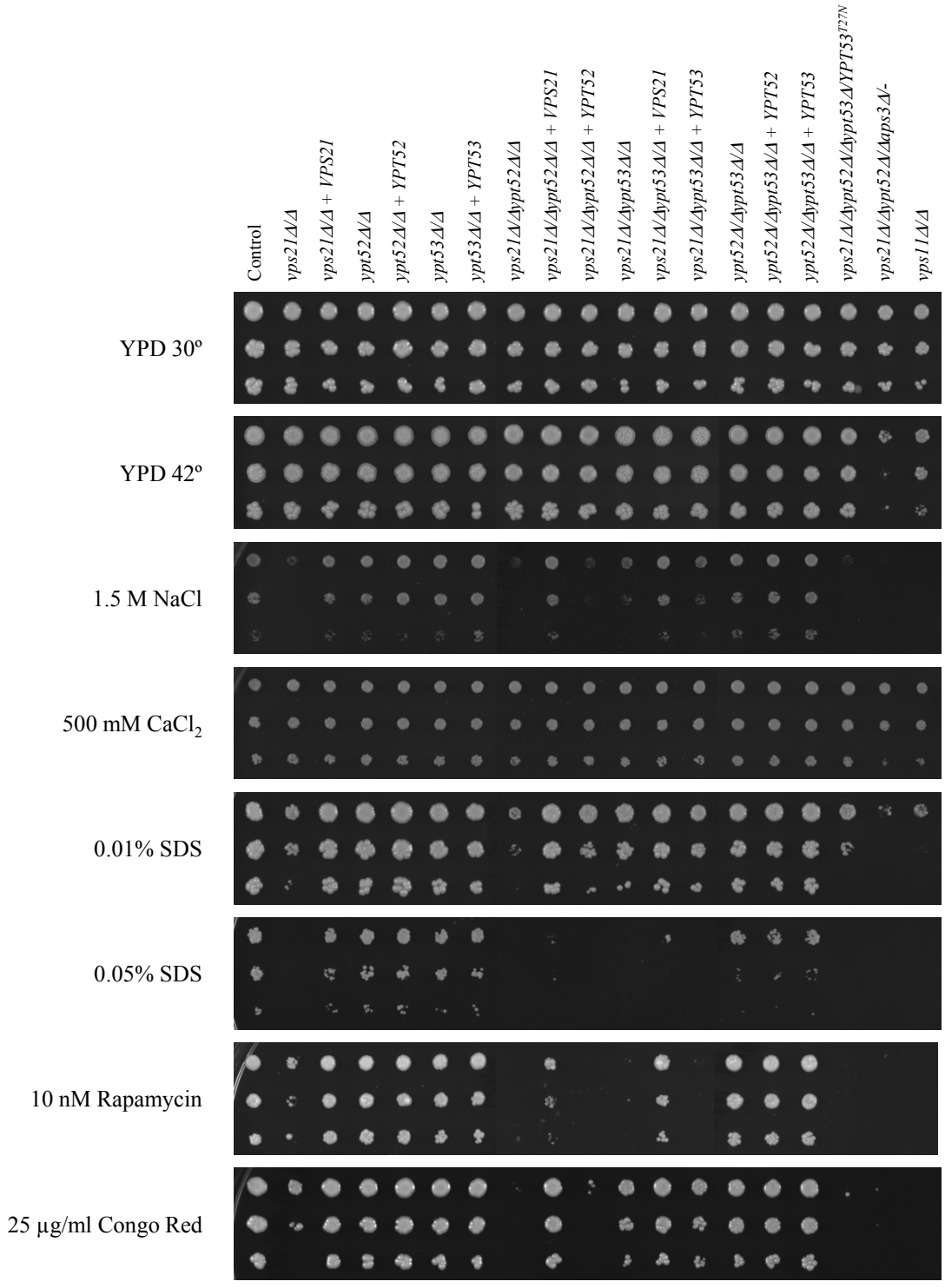


Figure S7. Loss of *VPS21* and *YPT52* causes synthetic stress phenotypes. Cell suspensions of each strain were prepared by serial dilution, applied to YPD agar or to YPD agar supplemented with each of the indicated stress inducing agents. Plates were incubated for 2 days at 30ºC, then imaged. Data from a representative experiment are shown, and the experiment repeated with similar results.

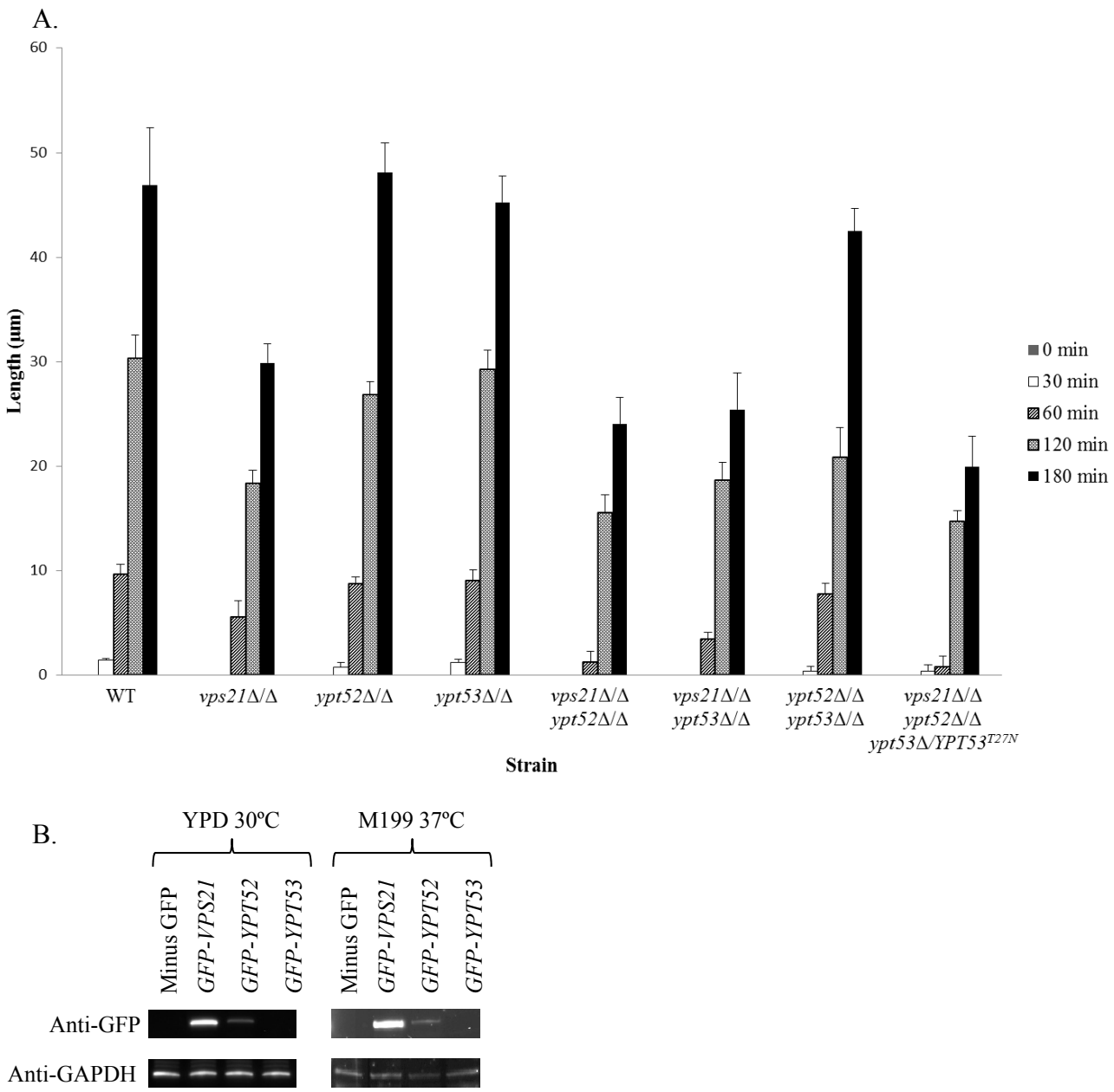


Figure S8. *vps21*Δ/Δ and *vps21*Δ/Δ*ypt52*Δ/Δ mutants are delayed in the initiation of hyphal growth and have reduced apical extension rates. (A) Each strain was induced to form hyphal in M199 liquid culture at 37ºC. Samples taken at the indicated time points were fixed and examined microscopically. The length of individual hyphae from the base of the germ-tube neck to the hyphal apex was measured from image analysis using CellSens Dimensions™ software (Olympus). Only cells in the true hyphal form were included in the analysis, yeast or pseudohyphal forms were excluded. The mean and standard deviation of at least 10 hyphae are shown for each strain at each time point, from a representative experiment. (B) *C. albicans* strains carrying *GFP-VPS21*, *GFP-YPT52* or *GFP-YPT53* fusions with the respective genes native 5' and 3' UTR sequences were grown as yeast (YPD 30ºC) or hyphae (M199 37ºC) for 4 hours, extracts prepared, and immunoblotted with anti-GFP or anti-GAPDH.

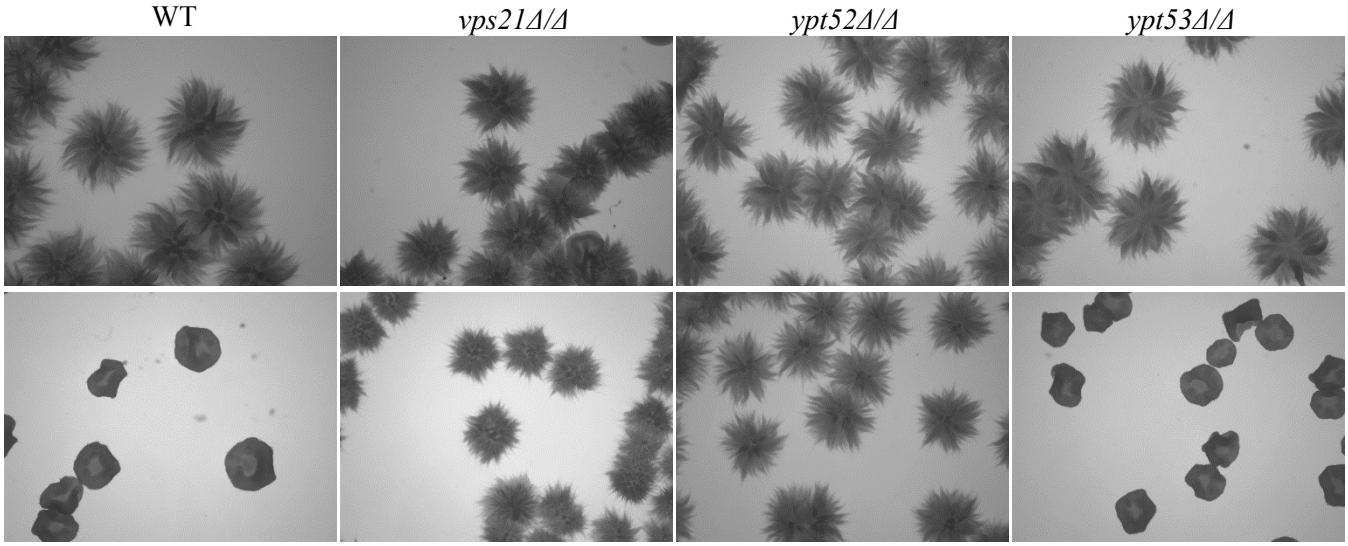


Figure S9. Ume6p overexpression does not suppress the hyphal growth defects of the endosomal GTPase mutants. An *ACT1-UME6* expression construct was introduced into each of the endosomal GTPase deletion mutants. Transformants of each genotype were then streaked to YPD agar and incubated at 30ºC for 2 days. At least four independent transformants were examined for each GTPase mutant genotype. Representative are shown for each genotype.

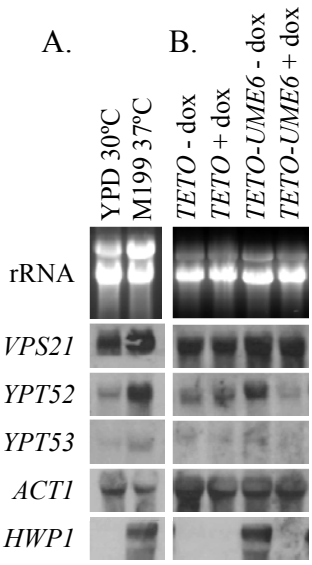


Figure S10. PVC Rab GTPase encoding genes are transcriptionally regulated during *C. albicans* hyphal growth. (A) *C. albicans* strain SC5314 was grown as yeast (YPD 30º) or hyphae (M199 37ºC), RNA extracted, and northern analysis performed with the indicated gene specific probes. (B) *C. albicans* carrying a doxycycline repressible *TETO-UME6* construct, or an isogenic control strain carrying vector alone (*TETO*), were grown in YPD 30ºC ± 1 µg/ml doxycycline for 4 hours, RNA extracted, and northern analysis performed as in part(A).

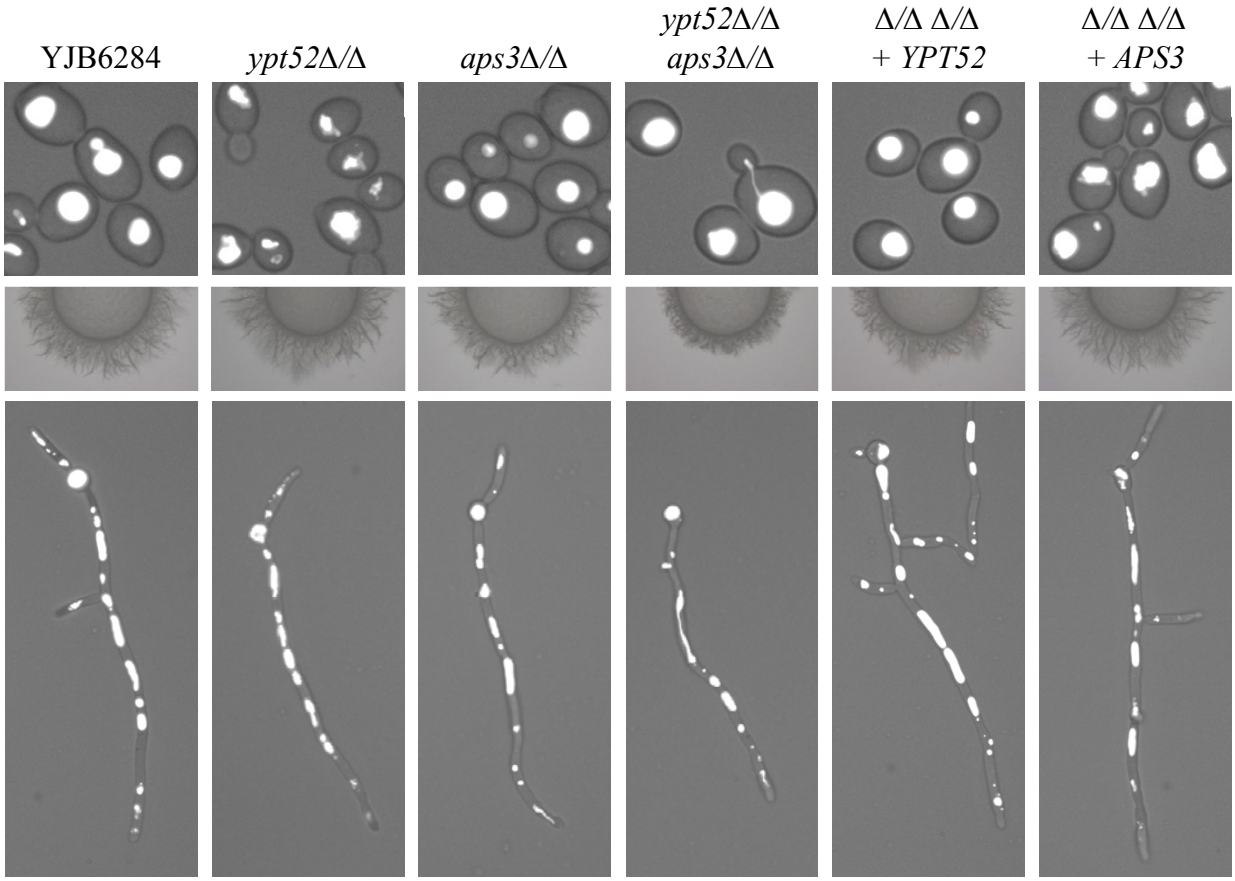


Figure S11. Loss of Ypt52p and AP-3 mediated trafficking causes hyphal growth defects. Vacuole morphology was determined using CMAC with yeast cells grown in YPD at 30ºC (top panel), or hyphal cells grown in M199 medium at 37ºC (bottom panel). Hyphal growth was also compared on 10% FBS agar (center panel).