Organelle Size Scaling of the Budding Yeast Vacuole Is Tuned by Membrane Trafficking Rates

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Supporting Material

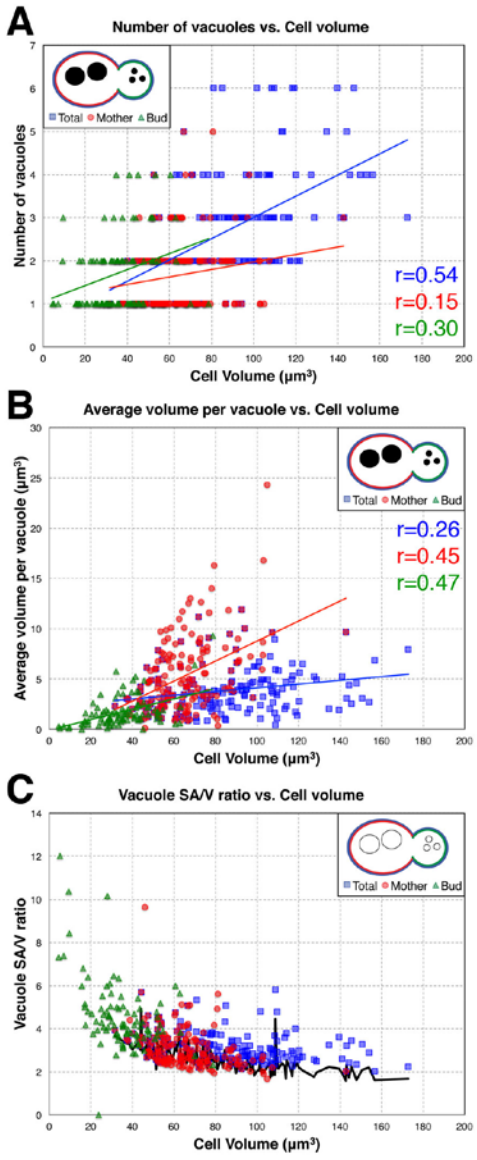

Figure S1 – Vacuole number, average volume, and surface area-to-volume ratios in W303A wild-type cells (related to Figure 2)
(A) Number of vacuoles, (B) Average volume per vacuole, and (C) Vacuole surface area-to-volume ratio plotted against cell volume. Plots contain data for total (mother+bud) cells (blue), individual mothers (red), and individual buds (green). Lines indicate least-squares linear regressions to the data with listed Pearson correlation r-values, except in (C), where the line shows the minimum surface area-to-volume ratio for total vacuoles. This was derived by taking the measured total vacuole volume in a cell, then calculating the surface area of a perfect sphere with that volume. Actual values above the theoretical minimum indicate vacuole morphology deviates from perfect sphericity.

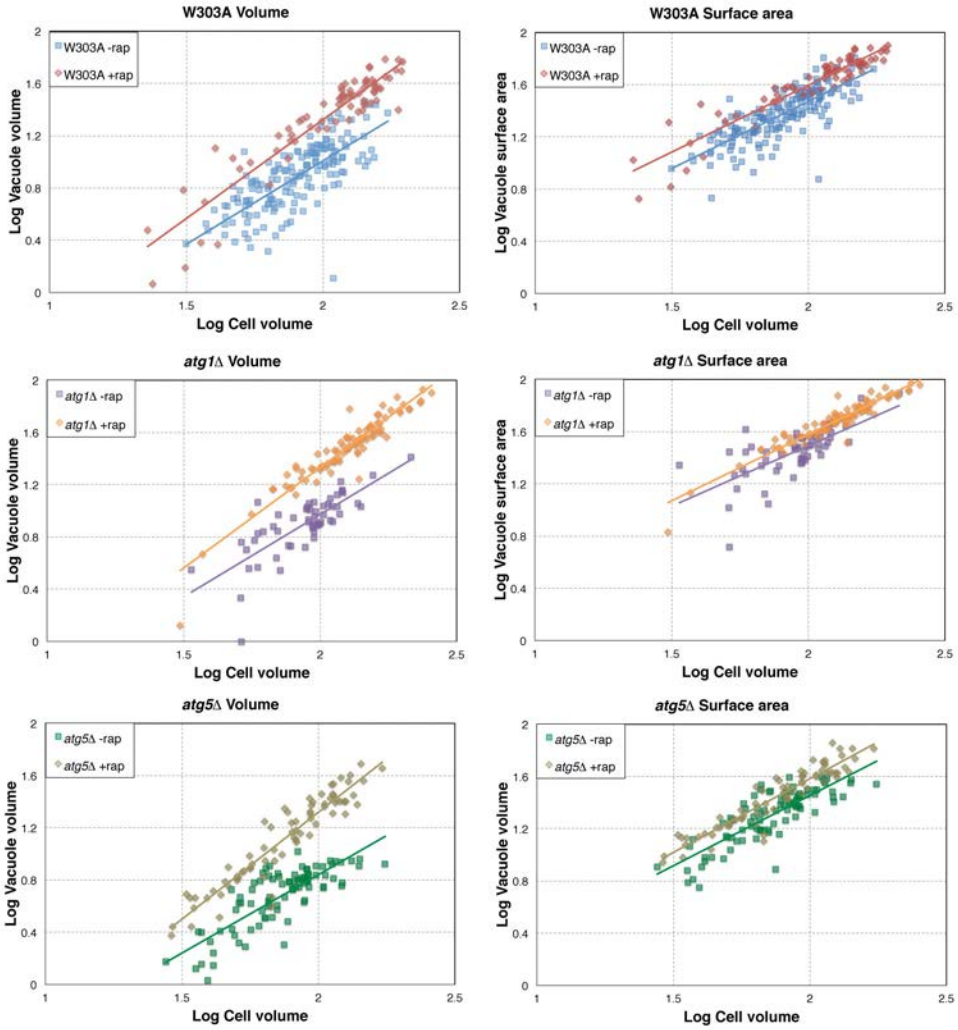

Figure S2 – Log-log plots of W303A, *atg1Δ*, and *atg5Δ* strains (related to Table 1)
For scaling analysis, raw data were converted and plotted on log-log axes. Linear regressions to these plots give slopes which are equal to the power-law exponents given in Table 1.

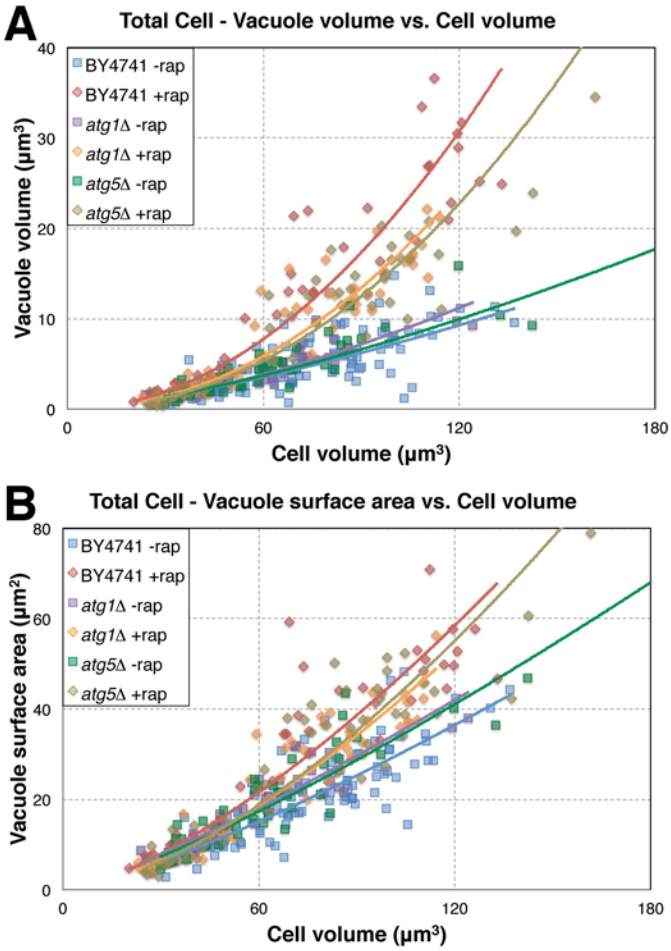

Figure S3 – Rapamycin increases vacuole size scaling in BY*atg1Δ* and BY*atg5Δ* (related to Figure 4)

Vacuole surface area-to-cell size scaling trends are similar in BY4741 and in BY*atg1Δ* and BY*atg5Δ* autophagy mutants transformed into the BY4741 background. Addition of rapamycin increases vacuole surface area similar to experiments using the W303A background. Lines indicate power-law fits to the data. Note that for all strains, both vacuole volume and surface area scaling trends are increased in rapamycin treated cells compared to untreated cells. Comparison of linear regressions shows a significant difference between the BY*atg1Δ* (p-value<0.001) and BY*atg5Δ* (p-value<0.001) vacuole-to-cells size scaling trends in the presence or absence of rapamycin, both for volume and surface area.

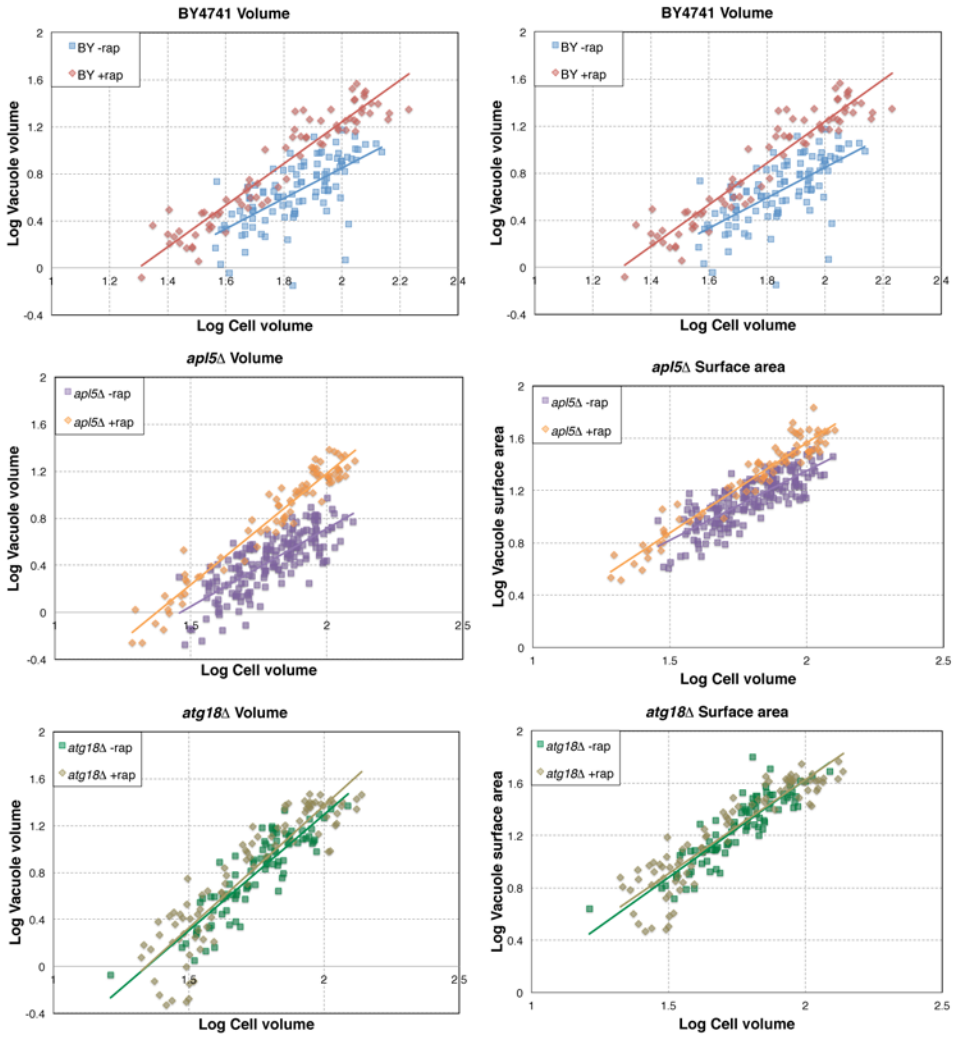

Figure S4 – Log-log plots of BY4741, *apl5Δ*, and *atg18Δ* strains (related to Table 2)
For scaling analysis, raw data were converted and plotted on log-log axes. Linear regressions to these plots give slopes which are equal to the power-law exponents given in Table 2.

Table S1 – List of strains used in this study (related to Experimental Procedures)

Strain	Parent	Genotype
BY4741 VPH1-GFP	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VPH1-GFP::HIS3*
W303A VPH1-GFP	W303A	*MATa leu2-3,112 trp1-1 can1-100 ura301 ade2-1 his3-11,15 VPH1-GFP::HIS3*
atg5Δ	W303A	*MATa leu2-3,112 trp1-1 can1-100 ura301 ade2-1 his3-11,15 atg5Δ::HIS3 VPH1-GFP::KANMX6*
atg1Δ	W303A	*MATa leu2-3,112 trp1-1 can1-100 ura301 ade2-1 his3-11,15 atg1Δ::KANMX6 VPH1-GFP::HIS*
fab1Δ	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 fab1Δ::KANMX6 VPH1-GFP::HIS3*
atg18Δ	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 atg18Δ::KANMX6 VPH1-GFP::HIS3*
apl5Δ	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 apl5Δ::KANMX6 VPH1-GFP::HIS3*
BY4741 *atg1Δ*	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 atg1Δ::KANMX6 VPH1-GFP::HIS3*
BY4741 *atg5Δ*	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 atg5Δ::KANMX6 VPH1-GFP::HIS3*

Table S2 – Comparison of cell and vacuole size measurements (related to Results and Discussion)

Values for vacuole size from this study show the average and standard deviation for the entire population. Average vacuole size ranges reported from Larabell et al (*) were estimated from Figures 3&4 in that paper which binned cells by cell cycle stage (1).

Strain	Cell Volume Range (μm^3)	Average vacuole volume (μm^3)	Average vacuole surface area (μm^2)
W303A (this study)	30-175	9.0±5.3	26.0±11.5
BY4741 (this study)	30-140	5.3±3.1	20.4±9.5
DDY904 (Larabell *et al.)*	10-100	1-6*	5-25*

Table S3- Summary of statistics for vacuole size scaling in different strains in the presence or absence of rapamycin. Pairs of vacuole size-to-cell size scatters were tested using an overall test for the coincidence of two regression lines.
Legend: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 (not significant)

Table S3a – Comparison of vacuole volume-to-cell volume regressions in W303A strains

Volume		W303A		*atg1Δ*		*atg5Δ*	
	rap	-	+	-	+	-	+
W303A	-		***	*		***	
	+				***		**
atg1Δ	-				***	***	
	+						NS
atg5Δ	-						***

Table S3b – Comparison of vacuole surface area-to-cell volume regressions in W303A strains

Surface Area		W303A		*atg1Δ*		*atg5Δ*	
	rap	-	+	-	+	-	+
W303A	-		***	NS		NS	
	+				NS		NS
atg1Δ	-				***	NS	
	+						NS
atg5Δ	-						***

Table S3c – Comparison of vacuole volume-to-cell volume regressions in BY4741 strains

Volume		BY4741		*atg18Δ*		*apl5Δ*	
	rap	-	+	-	+	-	+
BY4741	-		***	***	***	***	
	+			NS	***		***
atg18Δ	-				***	***	
	+						***
apl5Δ	-						***

Table S3d – Comparison of vacuole surface area-to-cell volume regressions in BY4741 strains

Surface Area		BY4741		*atg18Δ*		*apl5Δ*	
	rap	-	+	-	+	-	+
BY4741	-		***	***	***	***	
	+			NS	NS		**
atg18Δ	-				NS	***	
	+						*
apl5Δ	-						***