Electronic Supplementary Information

Nguyen et al. "Isolated *Saccharomyces Cerevisiae* Vacuoles contain Low-Molecular-Mass Transition-Metal Polyphosphate Complexes"

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Table S1: Batches of *S. cerevisiae* vacuoles isolation used in this study. "Sta" and "Exp" refer to stationary phase and exponential phase, respectively. Final supplemented metal concentrations are given in μM. Default supplemented Fe, Cu, Zn, and Mn concentrations were 40, 10, 0, and 0 μM, respectively.

Batch	Strain	Medium	Harvest	Non-default [M]
01	WT	MM	Sta	
02	WT	MM	Sta	
03	WT	MM	Sta	
04	WT	MM	Sta	
05	WT	MM	Exp	
06	WT	MM	Exp	
07	WT	MM	Exp	
08	WT	MM	Exp	
09	WT	MM	Exp	
10	WT	CSM	Sta	40 Cu; 200 Zn; 40 Mn
11	WT	CSM	Sta	40 Cu; 200 Zn; 40 Mn
12	WT	CSM	Sta	20 Cu; 100 Zn; 20 Mn
13	WT	CSM	Sta	
14	WT	CSM	Sta	
15	WT	CSM	Sta	
16	WT	CSM	Sta	
17	WT	CSM	Sta	
18	WT	CSM	Sta	
19	WT	CSM	Sta	1 Fe
20	WT	CSM	Sta	1 Fe
21	WT	CSM	Sta	20 Cu; 100 Zn; 20 Mn
22	*cox17*Δ	CSM	Sta	20 Cu; 100 Zn; 20 Mn
23	*cox17*Δ	CSM	Sta	20 Cu; 100 Zn; 20 Mn
24	*cup1*Δ	CSM	Sta	
25	*cup1*Δ	CSM	Sta	
26	*cup1*Δ	CSM	Sta	
27	*cup1*Δ	CSM	Sta	
28	WT	CSM	Sta	
29	WT	CSM	Sta	

Figure S1: Calibration Curve of Size Exclusion Column. Best-fit line was *Log (Molecular Mass, Da)* = -1.0529×(V_e/V_o) + 5.4287 where V_e and V_o are elution volume and void volume, respectively. The void volume was determined to be 14.94 mL using Blue Dextran (Fisher Scientific). The correlation coefficient R^2 was 0.9607. The molecular masses of standards used for calibration curve were from a previous study (66): cytochrome c *Saccharomyces cerevisiae* (12,384 Da), insulin (5,777 Da), cyanocobalamin (1,355 Da), ADP (427 Da), and AMP (327 Da).

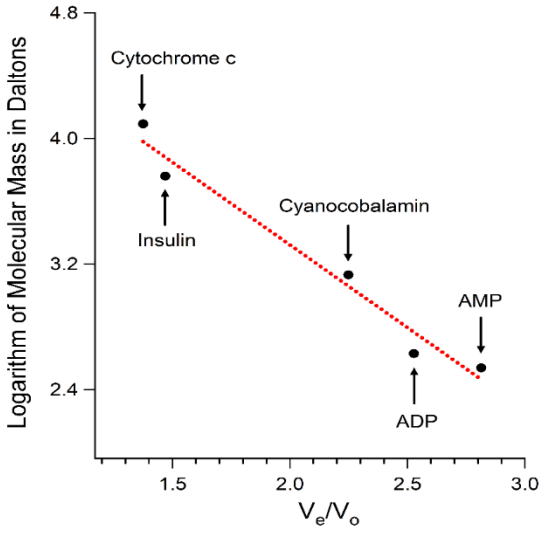

Figure S2: Iron-detected Chromatograms of Vacuolar FTSs. A, Vacuolar FTSs from cells grown in minimal media containing 40 μM Fe^{III} citrate and harvested during stationary (B1 – B4) and exponential (B5 – B9) phases. The mobile phase pH was 8.5. Some trace intensities were multiplied by the indicated factor. Numbers above vertical dashed lines indicate approximate mass in Da for species at that elution volume. B, Vacuole FTSs from cells grown in synthetic complete medium supplemented with 40 μM Fe^{III} citrate and harvested in stationary phase (B10 – B20). Vacuolar FTS of B19 and B20 were prepared in the same way but the media was supplemented with 1 μM Fe^{III} citrate. The mobile phase pH was 8.5. C, Vacuole FTSs prepared as in B with 40 (B10 – B18) or 1 (B19 – B20) μM Fe^{III} citrate. The mobile phase pH was 6.5.

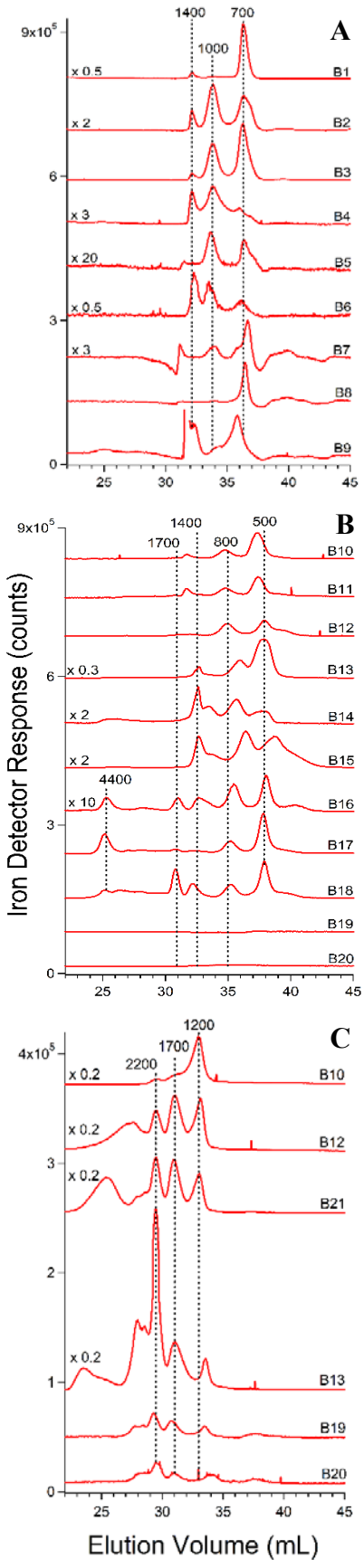

Figure S3: Phosphorous-detected chromatograms of FTS from isolated vacuoles. See the Figure S2 legend for other details.

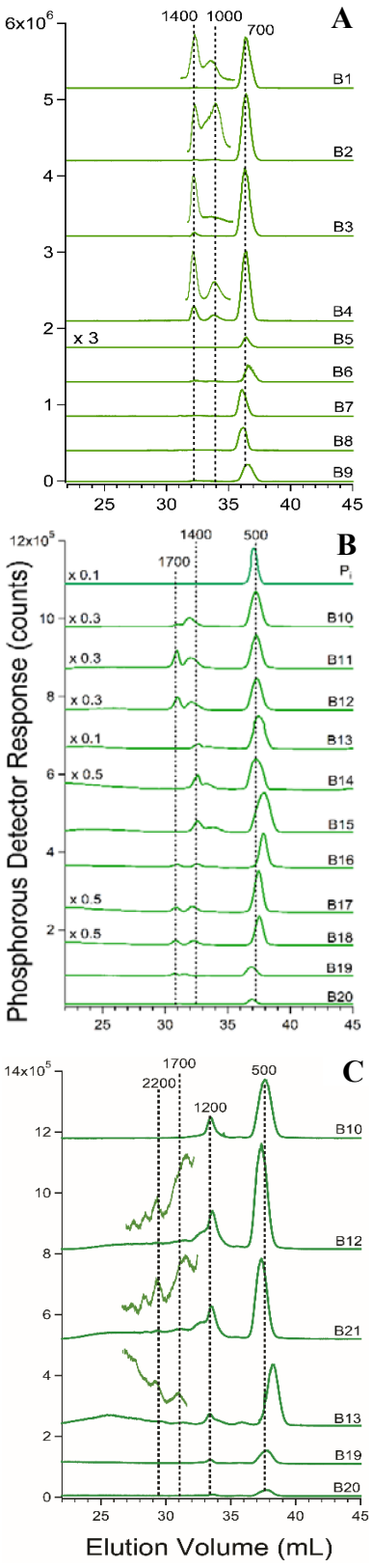

Figure S4: Zinc-detected chromatograms of FTSs from isolated vacuoles. See the Figure S2 legend for other details.

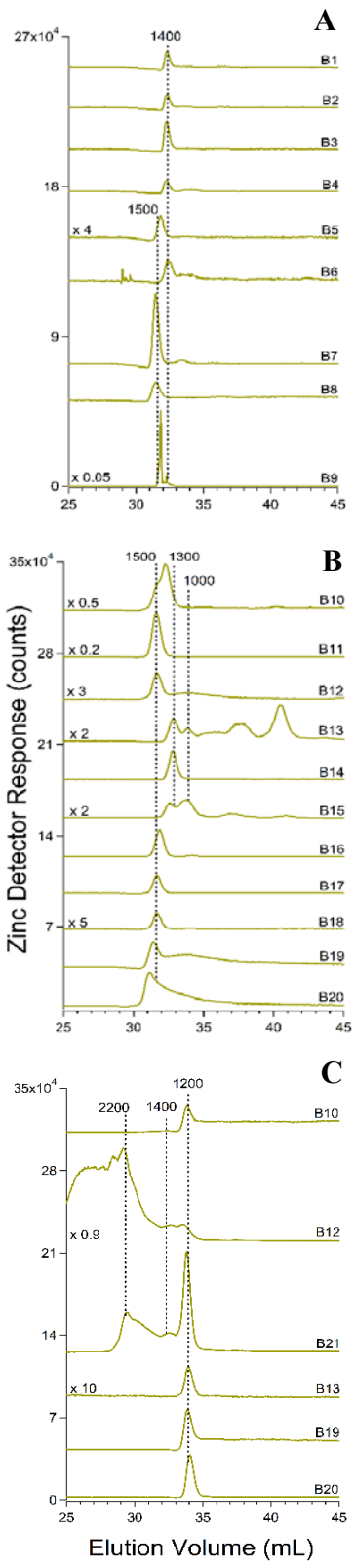

Figure S5: Manganese-detected chromatograms of FTSs from isolated vacuoles. See the Figure S2 legend for other details.

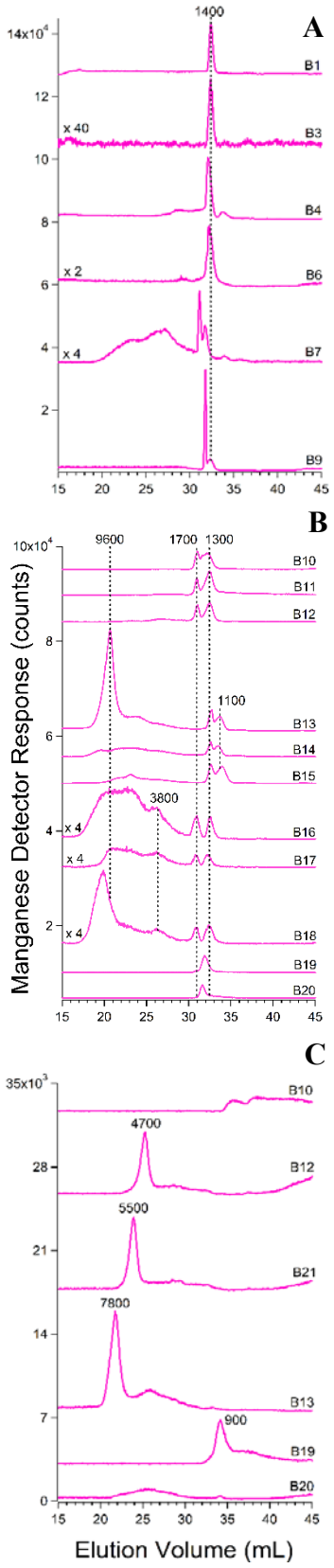

Figure S6: Overlaying of Mn and P for Batch 13, 16, 17, and 18 at pH 8.5 mobile phase.

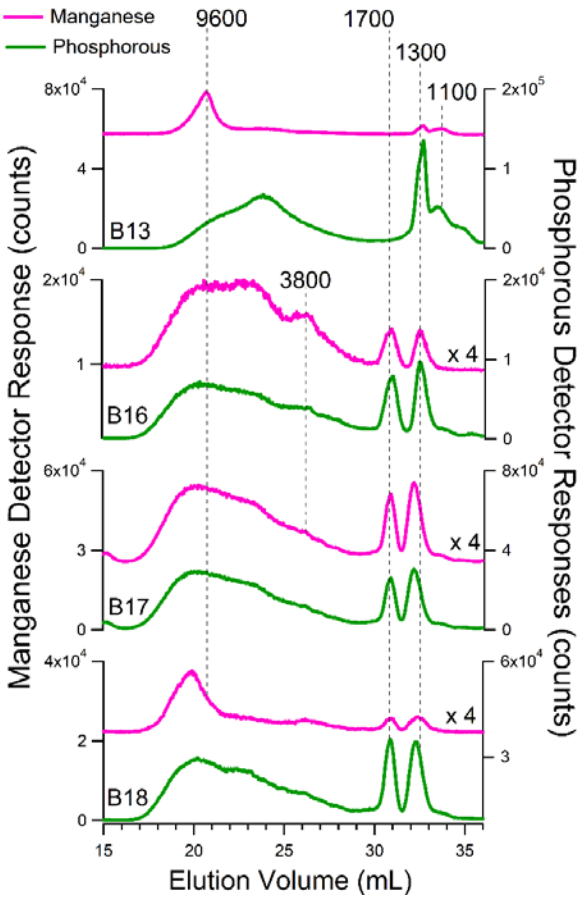

Figure S7: Phosphorous-detected Chromatograms of FTS from isolated vacuoles from WT strain in the presence of Phosphatase Inhibitors Cocktail. 10 mM stock of Phosphatase Inhibitors Cocktail (abcam, ab201113) containing imidazole, sodium fluoride, sodium molybdate, sodium orthovanadate, and sodium tartrate dihydrate was added to all buffers of vacuoles isolation procedure at a final concentration of 1 mM starting from the spheroplasting step and during the collection of vacuolar FTSs (n = 2).

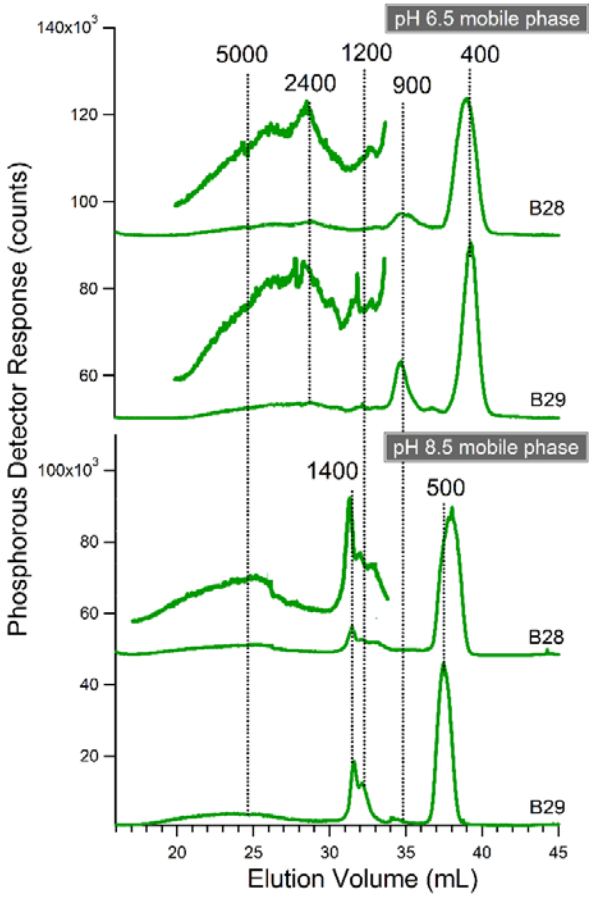

Figure S8: Copper-detected Chromatograms of FTS from isolated vacuoles from WT strain. See the Figure S3 legend for other details.

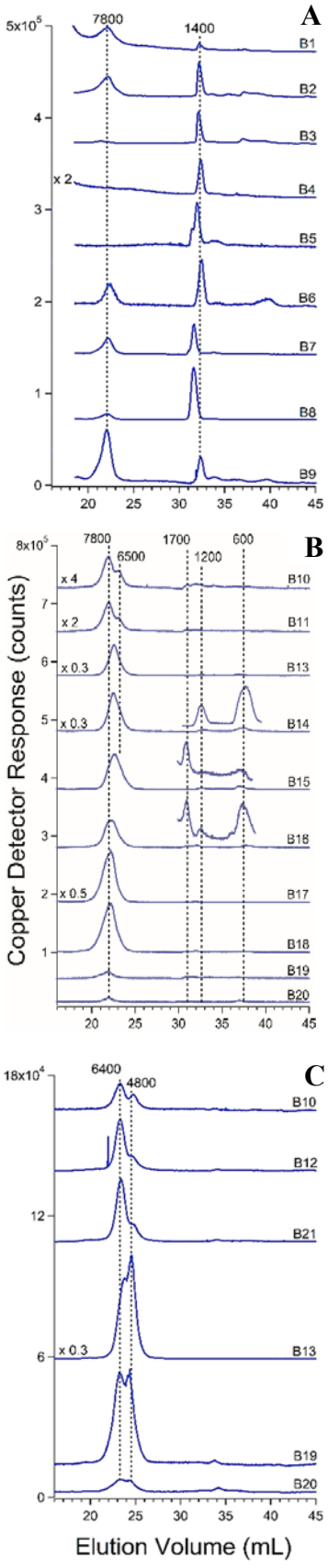

Figure S9: Copper-detected Chromatograms of FTS from isolated vacuoles from Cox17Δ (Panel A) and Cup1Δ (Panel B) cells. See the Figure S3 legend for other details.