

## Supplemental materials (Markgraf *et al.*)

### Figure S1. Vps21 compartment formation depends on Vps8 expression levels.

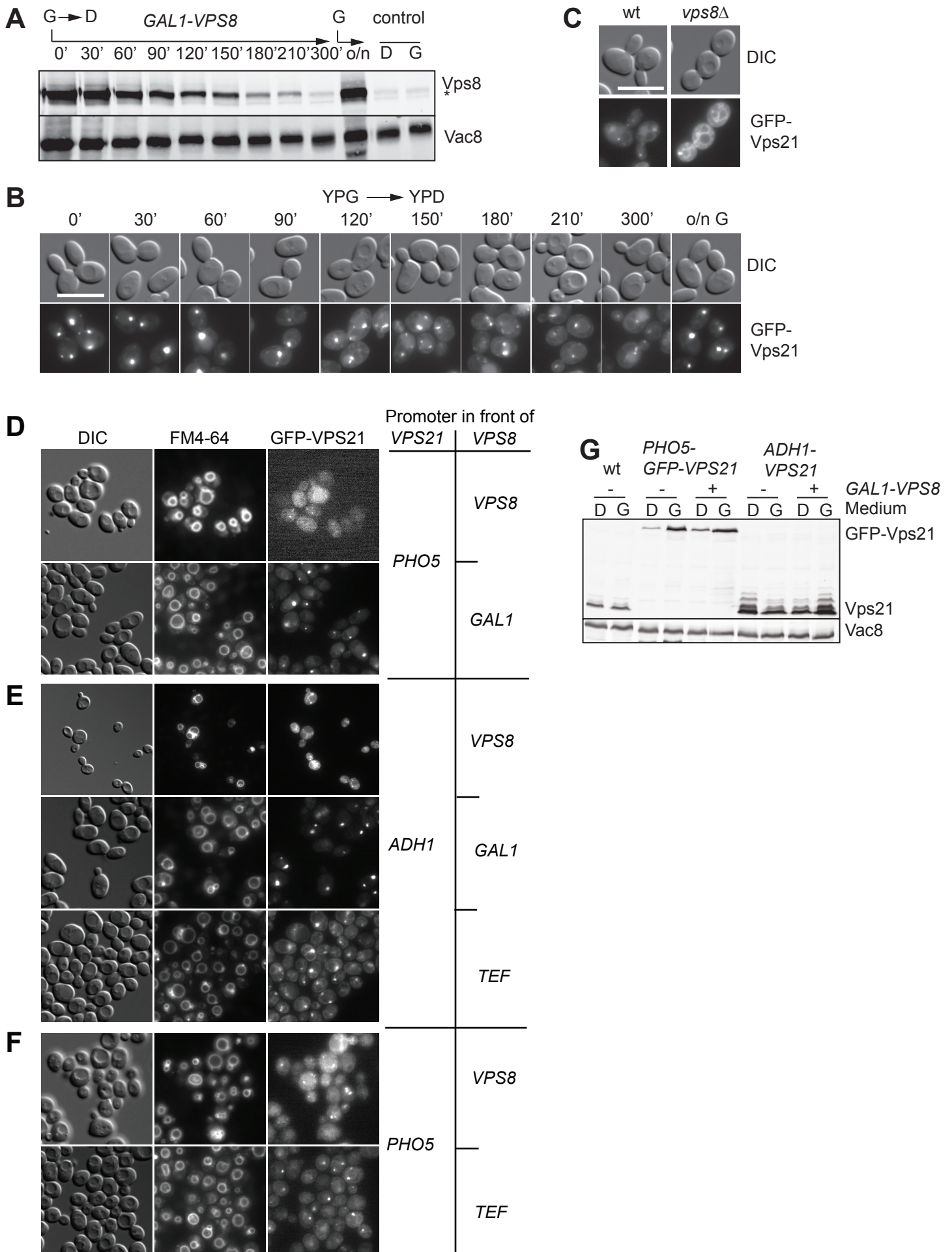
(A) Vps8 expression levels. Cells carrying GFP-Vps21 under the control of the *ADHI* promoter and overexpressing HA-Vps8 were grown in galactose and then shifted to the glucose containing medium. At the indicated time points, cell extracts were prepared and analyzed by SDS-PAGE followed by Western blotting using antibodies against the HA tag or Vac8 (loading control). Control cells contain HA-tagged Vps8 under the control of the authentic promoter.

(B) Localization of GFP-Vps21 upon Vps8 depletion. The cells were imaged at the same time points as in (A).

(C) Localization of GFP-Vps21 in wild type (wt) and *vps8* $\Delta$  cells. Fluorescence microscopy analysis was done as in Figure 1A. Size bar = 10 $\mu$ m.

(D-F) Comparison between strains expressing GFP-Vps21 either from the *PHO5* (D,F) or the *ADHI* promoter (E) in response to Vps8-overexpression. All strains were grown in YPG medium and analyzed as in (A). D,E are in a BY4741 background, F in a SEY6210 background strain.

(G) Vps21 expression levels in glucose (D)- and galactose (G)-containing media in presence or absence of Vps8. Cell extracts were analyzed by Western blotting using antibodies against the indicated proteins.



**Table S1**

Strain	Genotype	Reference
CUY100	BY4727 MATalpha <i>his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0</i>	EUROSCARF library
CUY105	BY4732 MATa <i>his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0</i>	EUROSCARF library
CUY106	BY4733 MATalpha <i>his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0</i>	EUROSCARF library
CUY473	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps8Δ::kanMX</i>	EUROSCARF library
CUY482	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps21Δ::kanMX</i>	EUROSCARF library
CUY520	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps33Δ::kanMX</i>	EUROSCARF library
CUY521	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps16Δ::kanMX</i>	EUROSCARF library
CUY523	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps18Δ::kanMX</i>	EUROSCARF library
CUY594	MATa <i>his3Δ leu2Δ met15Δ ura3Δ pep12Δ::kanMX</i>	EUROSCARF library
CUY827	CUY2008; <i>VPS21::natNT2-ADHpr</i>	This study
CUY902	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps45Δ::kanMX</i>	EUROSCARF library
CUY1014	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps3Δ::kanMX</i>	EUROSCARF library
CUY1030	MATa <i>his3Δ leu2Δ met15Δ ura3Δ VPS41::HIS5-PHO5pr-GFP-Myc</i>	Takeda et al., 2008
CUY1792	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VPS8::TAP-kanMX</i>	Peplowska et al., 2007
CUY1795	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 VPS3::TAP-URA3</i>	Peplowska et al., 2007
CUY1818	CUY100; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY1819	CUY473; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY1846	MATa <i>his3Δ leu2Δ met15Δ ura3Δ VPS3::TAP-URA3 VPS3::HIS3-GAL1pr</i>	Peplowska et al., 2007
CUY1874	CUY1792; <i>VPS8::HIS3-GAL1pr</i>	This study
CUY1896	CUY105; <i>VPS41::TRP1-GAL1pr</i>	This study
CUY2008	CUY106; <i>VPS8::TRP1-GAL1pr-3HA</i>	This study
CUY2123	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vps9Δ::kanMX</i>	EUROSCARF library
CUY2152	CUY1896; <i>VPS41::TAP-URA3</i>	This study
CUY2253	MATalpha <i>his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0 VPS8::TRP1-GAL1pr-3HA VPS21::URA3-PHO5pr-GFP</i>	Peplowska et al., 2007
CUY2260	CUY2008; <i>VPS3::TAP-URA3</i>	This study
CUY2267	CUY2123; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY2268	CUY902; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY2569	CUY100; <i>VPS18::TAP-URA3</i>	This study
CUY2270	CUY594; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY2278	MATa <i>his3Δ leu2Δ met15Δ ura3Δ vps3Δ::kanMX VPS21::URA3 PHO5pr-GFP VPS8::HIS3-GAL1pr-3HA</i>	Peplowska et al., 2007

CUY2590	CUY100; <i>URA3::pRS316-STE3-GFP</i>	This study
CUY2593	CUY100; <i>CPS1::URA3-PHO5pr-GFP</i>	This study
CUY2650	CUY1014; <i>CPS1::URA3-PHO5pr-GFP</i>	This study
CUY2653	CUY473; <i>CPS1::URA3-PHO5pr-GFP</i>	This study
CUY2682	CUY2268; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY2694	SEY6210 MATalpha <i>leu2-3,112 ura3-52 his3-Δ200 trp-Δ901 lys2-801 suc2-Δ9 GAL</i>	Robinson et al., 1988
CUY2695	CUY2694; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY2696	CUY106; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY2704	CUY1014; <i>URA3::pRS316-STE3-GFP</i>	This study
CUY2705	CUY473; <i>URA3::pRS316-STE3-GFP</i>	This study
CUY2715	CUY1818; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY2716	CUY2696; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY2719	CUY2008; <i>VPS21::natNT2-ADHpr-GFP</i>	This study
CUY2762	CUY2716; <i>pV2-PHO5pr-RFP-CPS1 (TRP1)</i>	This study
CUY2767	CUY2696; <i>pV2-PHO5pr-RFP-PEP12 (TRP1)</i>	This study
CUY2768	CUY2716; <i>pV2-PHO5pr-RFP-PEP12 (TRP1)</i>	This study
CUY2770	CUY827; <i>URA3::pRS316-STE3-GFP</i>	This study
CUY2771	CUY827; <i>VPS39::URA3-PHO5pr-GFP</i>	This study
CUY2772	CUY827; <i>VPS3::URA3-PHO5pr-GFP</i>	This study
CUY2773	CUY827; <i>VPS41::HIS3-PHO5pr-GFP</i>	This study
CUY2842	CUY106; <i>VPS21::natNT2-ADH1pr</i>	This study
CUY2843	CUY2842; <i>VPS8::HIS3-GAL1pr-GFP</i>	This study
CUY2844	CUY2267; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY2845	CUY2695; <i>VPS8::natNT2-TEFpr</i>	This study
CUY2871	CUY2270; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY2896	CUY106; <i>VPS8::HIS3-GAL1pr-GFP</i>	This study
CUY2900	CUY106; <i>VPS11::TAP-URA3</i>	This study
CUY2921	CUY1014; <i>VPS8::HIS3-GALpr-GFP</i>	This study
CUY2922	CUY482; <i>VPS8::HIS3-GAL1pr-GFP</i>	This study
CUY2935	CUY106; <i>VPS11::GFP-hphNT1</i>	This study
CUY2949	CUY2920; <i>VPS3::TAP-URA3</i>	This study
CUY2950	CUY827; <i>VPS3::TAP-URA3</i>	This study
CUY3025	CUY105; <i>VPS18::GFP-hpNT1</i>	This study
CUY3026	CUY105; <i>VPS16::GFP-hpNT1</i>	This study

CUY3217	CUY827; <i>VPS11::GFP-hphNT1</i>	This study
CUY3243	CUY106; <i>VAM3::URA3-PHO5pr-GFP</i>	This study
CUY3244	CUY106; <i>VPS3::URA3-PHO5pr-GFP</i>	This study
CUY3277	CUY827; <i>VPS18::GFP-hpNT1</i>	This study
CUY3279	CUY521; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY3289	CUY523; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY3404	CUY827; <i>VPS16::GFP-KanMX</i>	This study
CUY3311	CUY3283; <i>VPS21::natNT2-ADH1pr</i>	This study
CUY3312	CUY3279; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY3315	CUY3289; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY3324	CUY2922; <i>GAL1pr::pRS406-GAL1pr-VPS21 Q66L</i>	This study
CUY3337	CUY2694; <i>VPS21::KanMX-ADH1pr</i>	This study
CUY3338	CUY2694; <i>VPS8::natNT2-TEFpr-GFP</i>	This study
CUY3339	CUY520; <i>VPS8::HIS3-GAL1pr-3HA</i>	This study
CUY3358	CUY3339; <i>VPS21::URA3-PHO5pr-GFP</i>	This study
CUY3359	CUY3338; <i>VPS21::kanMX-ADH1pr</i>	This study
CUY3381	CUY106; <i>pV2-PHO5pr-RFP-VPS21 (TRP1)</i>	This study
CUY3382	CUY2896; <i>pV2-PHO5pr-RFP-VPS21 (TRP1)</i>	This study
CUY3391	CUY2253; <i>vps11Δ::HIS3</i>	This study
CUY3393	CUY2922; <i>GAL1pr::pRS406-GAL1pr-VPS21 S21N</i>	This study
CUY3399	CUY106; <i>VAC1::URA3-PHO5pr-GFP</i>	This study
CUY3402	CUY827; <i>VAC1::URA3-PHO5pr-GFP</i>	This study
CUY3403	CUY2253; <i>VPS3::kanMX-GAL1pr</i>	This study
CUY3422	CUY2696; <i>pV2-PHO5pr-RFP-VAM3 (TRP1)</i>	This study
CUY3423	CUY2716; <i>pV2-PHO5pr-RFP-VAM3 (TRP1)</i>	This study
CUY3564	CUY3359; <i>VPS4::URA3</i>	This study
CUY3593	CUY3337; <i>VPS8::natNT2-TEFpr</i>	This study
CUY3616	CUY3593; <i>CPS1::URA3-PHO5pr-GFP</i>	This study
CUY3625	MATalpha <i>leu2-3,112 ura3-52 his3-Δ200 trp-Δ901 lys2-801 suc2-Δ9 GAL vam3::TRP1</i>	This study
CUY3626	CUY2694; <i>CPS1::URA3-PHO5pr-GFP</i>	This study
CUY3632	CUY3625; <i>CPS1::URA3-PHO5pr-GFP</i>	This study
CUY3633	CUY2253; <i>MNN9::td-TOMATO-kanMX</i>	This study
CUY3667	CUY3391; <i>MNN9::td-TOMATO-kanMX</i>	This study
CUY3668	CUY827; <i>CPS1::URA3-PHO5pr-GFP</i>	This study

CUY3669	CUY3655; <i>CPS1::URA3-PHO5pr-GFP</i>	This study
CUY3677	CUY2715; <i>VPS8::TAP-TRP1</i>	This study
CUY3678	CUY3101; <i>VPS8::TAP-TRP1</i>	This study
CUY3722	CUY2921; <i>URA3::pRS406-GAL1pr-VPS21</i>	This study
CUY4007	CUY106; <i>VPS21::natNT2-GFP-ADHpr</i>	This study
CUY4043	CUY4007; <i>VPS8::kanMX-TEF</i>	This study
CUY4126	CUY2694; <i>VPS10::GFP-TRP1</i>	This study
CUY4128	CUY2694; <i>vps26Δ::HIS3</i>	This study
CUY4146	CUY3338; <i>vps4Δ::URA3</i>	This study
CUY4344	CUY3593; <i>VPS10::GFP-TRP1</i>	This study
CUY4345	CUY4128; <i>VPS10::GFP-TRP1</i>	This study