

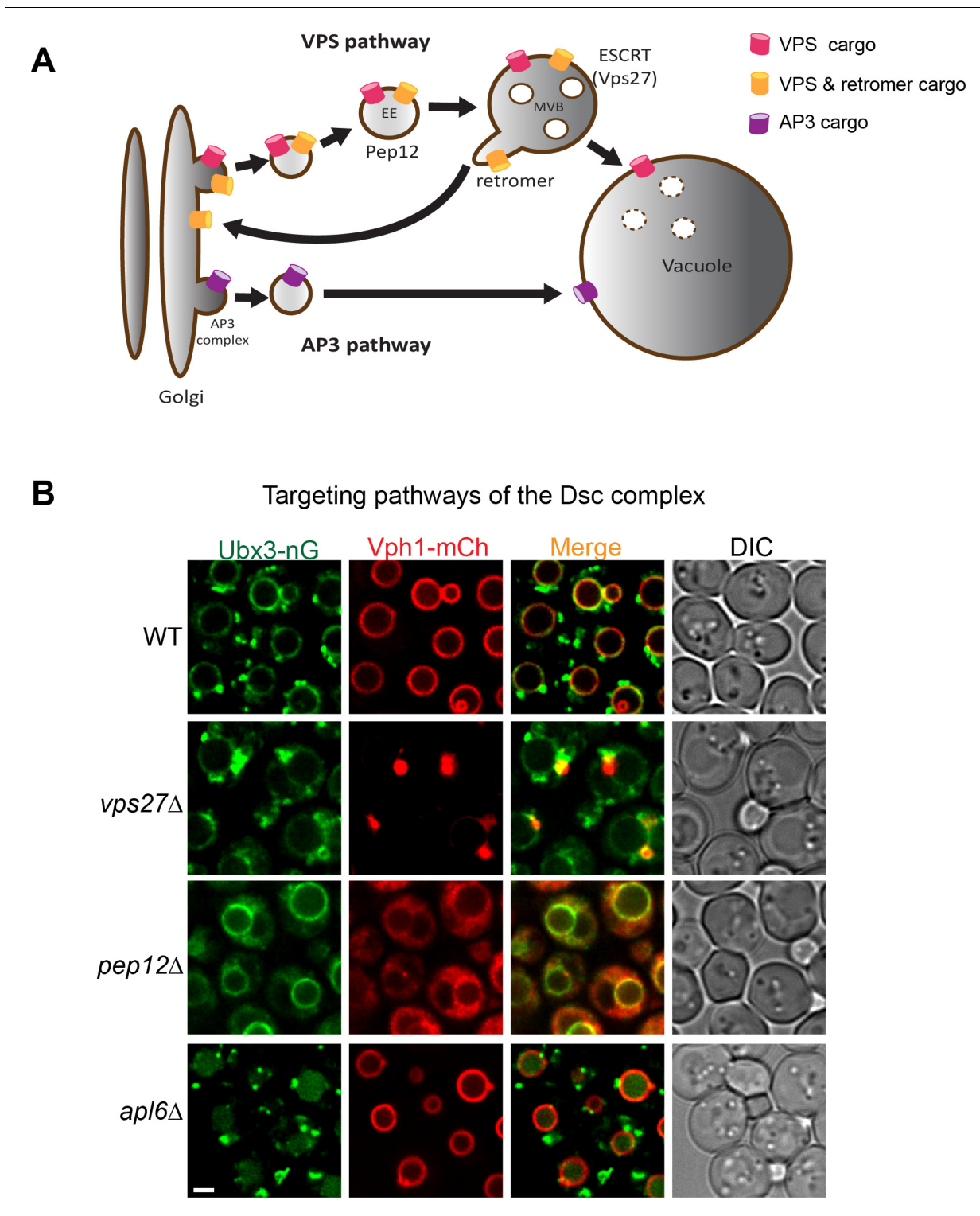


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## Figures and figure supplements

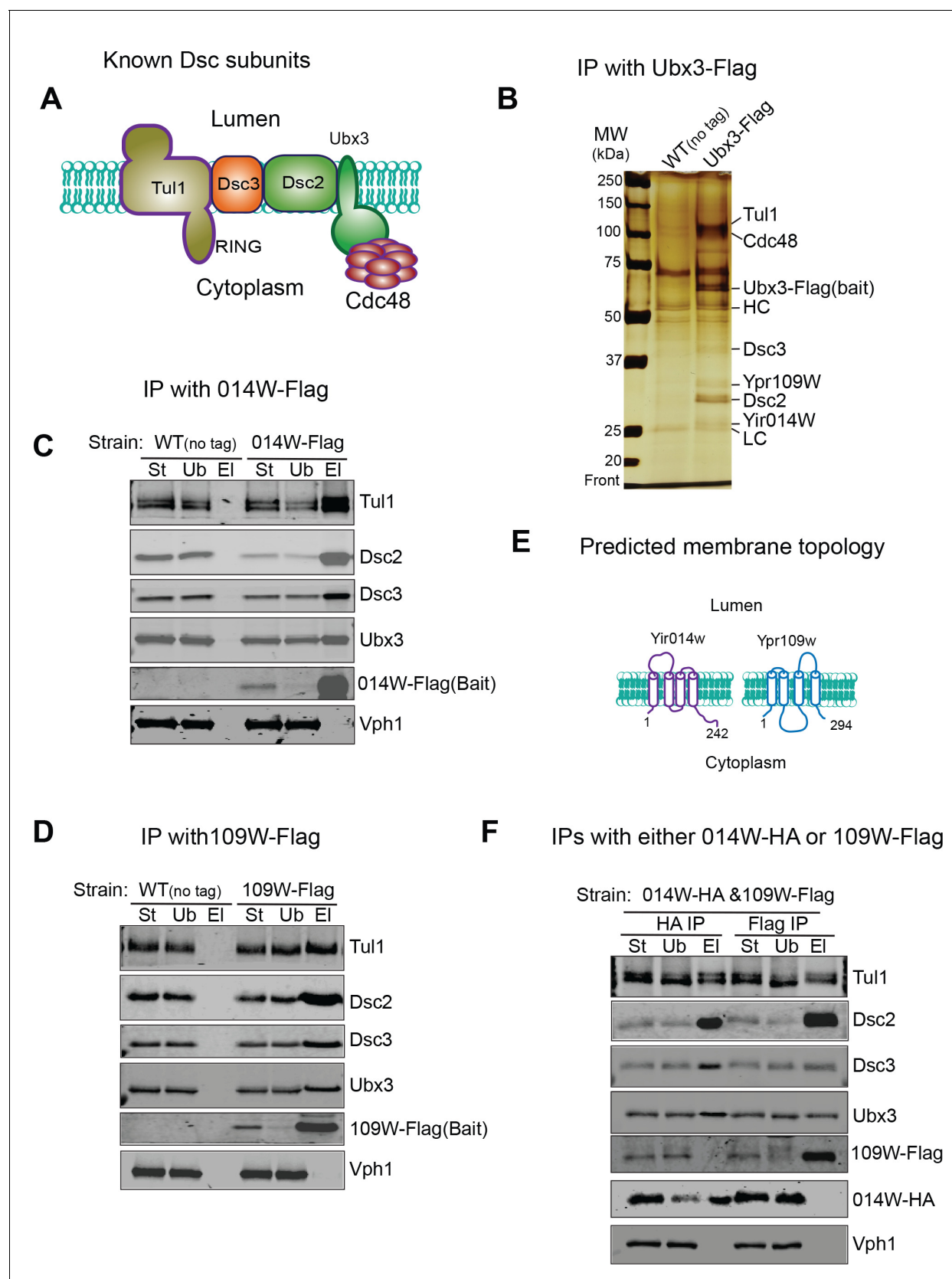
Sorting of a multi-subunit ubiquitin ligase complex in the endolysosome system

**Xi Yang et al**



**Figure 1.** Targeting pathways utilized by the Dsc complex. (A) A cartoon model showing two conserved pathways (VPS and AP3) for transporting proteins from Golgi to vacuole. Some VPS cargoes can be recycled from the endosome to Golgi by the retromer complex before reaching the vacuole. EE: early endosome, MVB: multivesicular body. (B) Localization of Ubx3-nG and Vph1-mCh in WT, *vps27Δ*, *pep12Δ*, and *apl6Δ* strain cells. Scale bar: 2 μm.

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**Figure 2.** Identification of two new Dsc components. (A) A cartoon model showing known Dsc components in budding yeast. (B) A silver staining gel showing that Ypr109W and Yir014W co-immunoprecipitate with Ubx3-Flag, together with other known Dsc components. HC, heavy chain. LC, light chain. Figure 2 continued on next page

*Figure 2 continued*

chain. (C) 014W-Flag can selectively pull down known Dsc components. (D) 014W-Flag can selectively pull down with known Dsc components. (E) Cartoon representations of 014W and 109W. Both proteins are predicted to be tetra-spanning membrane proteins. (F) 014W-HA and 109W-Flag do not pull down each other. St: Starting material. Ub: Unbound. El: Elution.

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**A**

Yir014W:

17 unique peptides, 113/242 amino acids (47% coverage)

**MLHLEDDNGRQ**RSVIANL**QKFVYCCLYLRF**IKDGSFLILLGWIISSLCDFIQELTLRYL  
**KKNYLEVGRDNDQEDDES**LAIRG**LETPIVRMI**INKAIRYY**QGLILLE**TAYCIVYHIRLDV  
 SRDICKSPYGFVIMLLIRE**FTCPVPTAFPSK**LLLVLDDILLFCQIVIINGSLSSSLQNV  
**KLIVKELNAEEEGALN**ILKLNTWHMDATGPELIVLKNHDK**SIPQQADGDDATEITPL**LN**IAE**

**B**

Yir109W:

28 unique peptides, 181/294 amino acids (62% coverage)

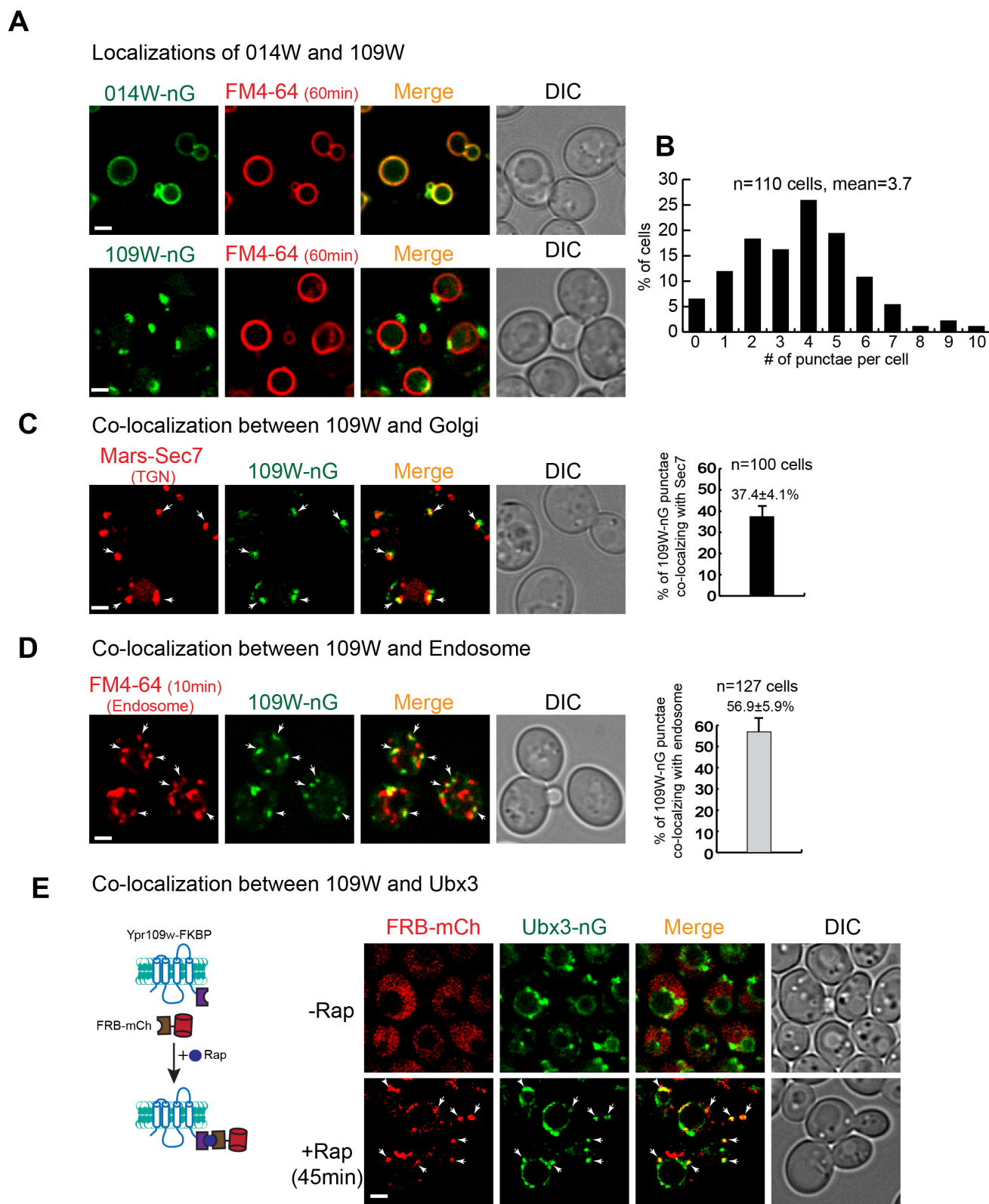
**MDNFESTAEGNLSIGNKRVYELRK**RNFQRLVNNLSFLGYVLISLEYIKYDR**TVWTLI-**  
**TRAI**VOSLISS**PFPSDAKLRL**LATLGADNNTTG**VATLP**GGRSIR**FPGMFGTE**MLYNS**SE**  
**AEQQDHDDTAIVSMKKQIRKFLFHGCLSLNMLFI**ILTILFPIDFF**EPLSGSE**PVDDG**PKN**  
**TPSPFSNSDGLLLGERRGGLFLQMIGERLPKSNFSGNLGLVMFEFSILIVQFTLFS**LCV  
**VLADLDFE**EPER**LEPVNSDGYDGS**VIVARI**PLNKT**MNAILNDGNINDNNENASNSV

**C**

Yir014W	-----MLHLEDDNGRQ	RSVIANLQKFVYCCLYLRF	IKDGSFL-ILL
Ypr109W	MDNFESTAEGNLSIGNKRVYELR-KRNFQRLVNNLSFLGYVLISLEYIKYDR	TVWTLIT	
		: .*. . . **.: : *. : * : *.:** . . : :	
Yir014W	GWIISSLC--DFIQELTLRYLKKNYLEVGRDN-----		
Ypr109W	RAIVQSLISSPFPSDAKLRR----LATLGADNNTTG	VATLP	GGRSIRFPGMFGTEMLYNS
	*.:** * .: .**	:* **	
Yir014W	---DQEDDES	LAIRGLETPIVRMIINKAIRYYQGLILLE	TAYCIVYHIRLDVSRDIC--
Ypr109W	SSEAEQQDHDDTAIVSMKKQIRKFLFHGCLSLNMLFI	ILTILFPIDFF	EPLSGSEPVDDG
	:*:*. . . ** . . . *	: : : : : :	:*: * : * . * . *
Yir014W	----SKPYGFVIMLLIRE-----	FTCPV-PTAFPSKLLLVLDDILLFCQIVIING	
Ypr109W	PKNTPSPFSNSDGLLLGERRGGLFLQMIGERLPKSNFSGNLGLVMFEFSILIVQFTLFS-		
	.*: . ** : *	: : : *	:* **: : : *: *: : .
Yir014W	SLSSSLQNVKLIVKELNAEEEGALN	ILKLNTWHMDATGPELIVLKNHDKSIPQQADGDDA	
Ypr109W	----LT--CVVLADLDFEEPE-----		RLEPVNSDGYDG
	*	: : : *: *	: * :*: *
Yir014W	TEITPLLNIAE-----		
Ypr109W	SVIVARIPLNKT	MNAILNDGNINDNNENASNSV	

**Figure 2—figure supplement 1.** Identification of two new Dsc subunits by mass spectrometry. (A) Mass spectrometry peptide coverage for Yir014W. The peptides identified by mass spectrometry are highlighted in red. The underlined sequences are the predicted transmembrane helices. (B) Mass spectrometry peptide coverage for Ypr109W. The peptides identified by mass spectrometry are highlighted in red. The underlined sequences are the predicted transmembrane helices. (C) Sequence similarity between Yir014W and Ypr109W. The alignment was performed with Clustal Omega (Sievers et al., 2011).

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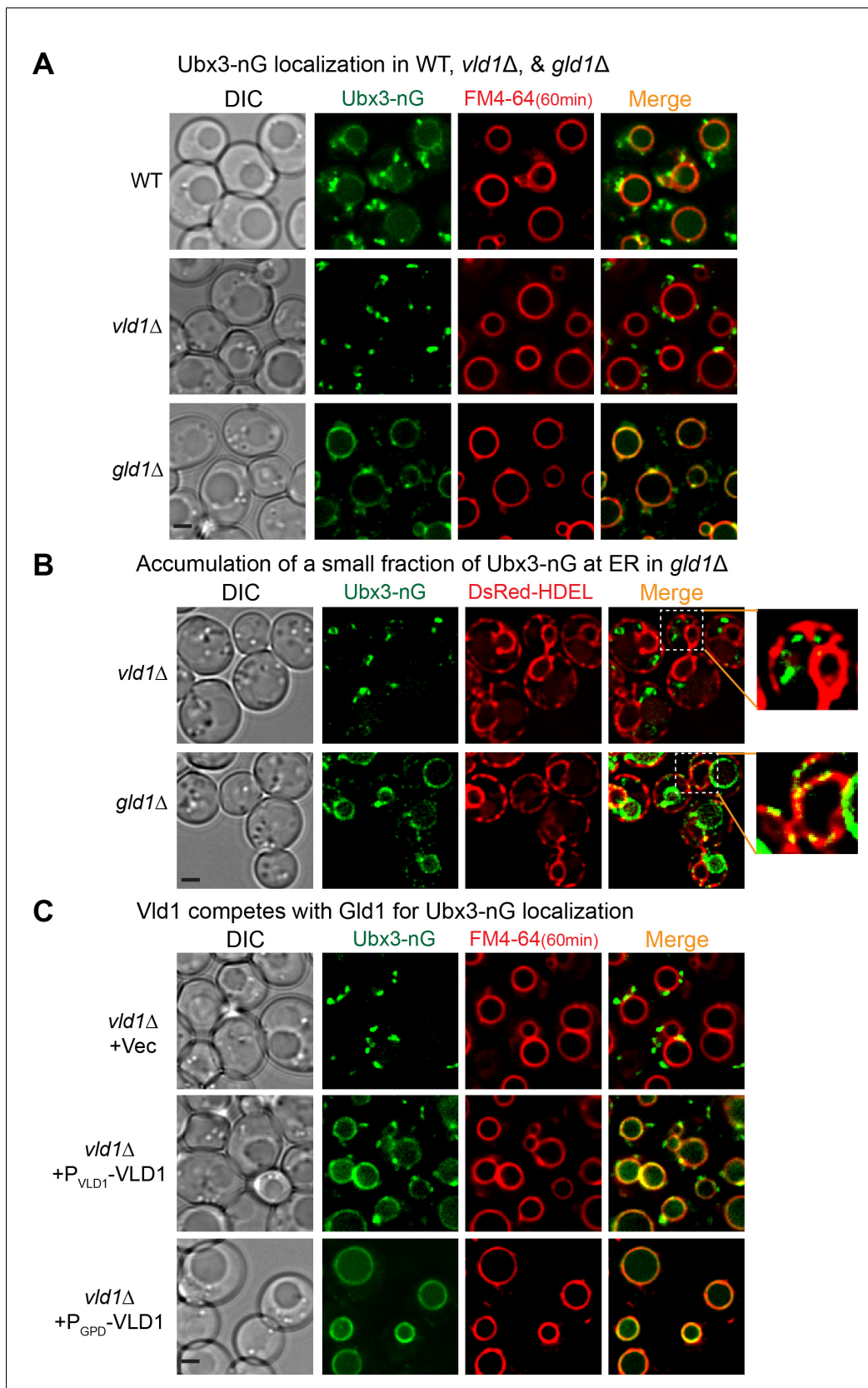
**Figure 3.** 109W and 014W have distinct subcellular localizations. (A) 014W-nG co-localizes with FM4-64 labelled vacuole membrane, whereas 109W-nG localizes to the intracellular punctae. (B) Quantification of the number of 109 W-nG punctae. (C) Left, 109W-nG partially co-localizes with Mars-Sec7

Figure 3 continued on next page

*Figure 3 continued*

labelled trans-Golgi (white arrows). Right, quantification of the co-localization. Error bar represents the Standard Error of the Mean (SEM). (D) Left, 109W-nG partially co-localizes with FM4-64 labelled endosomes (white arrows). Right, quantification of the co-localization. Error bar represents the SEM. (E) Co-localization between 109W and Ubx3 using rapamycin induced co-localization (RiCo) assay. Left: A cartoon diagram showing the principle of the assay. Right: FRB-mCherry localization before and after rapamycin treatment. White arrows indicate the co-localization. Scale bar: 2  $\mu$ m.

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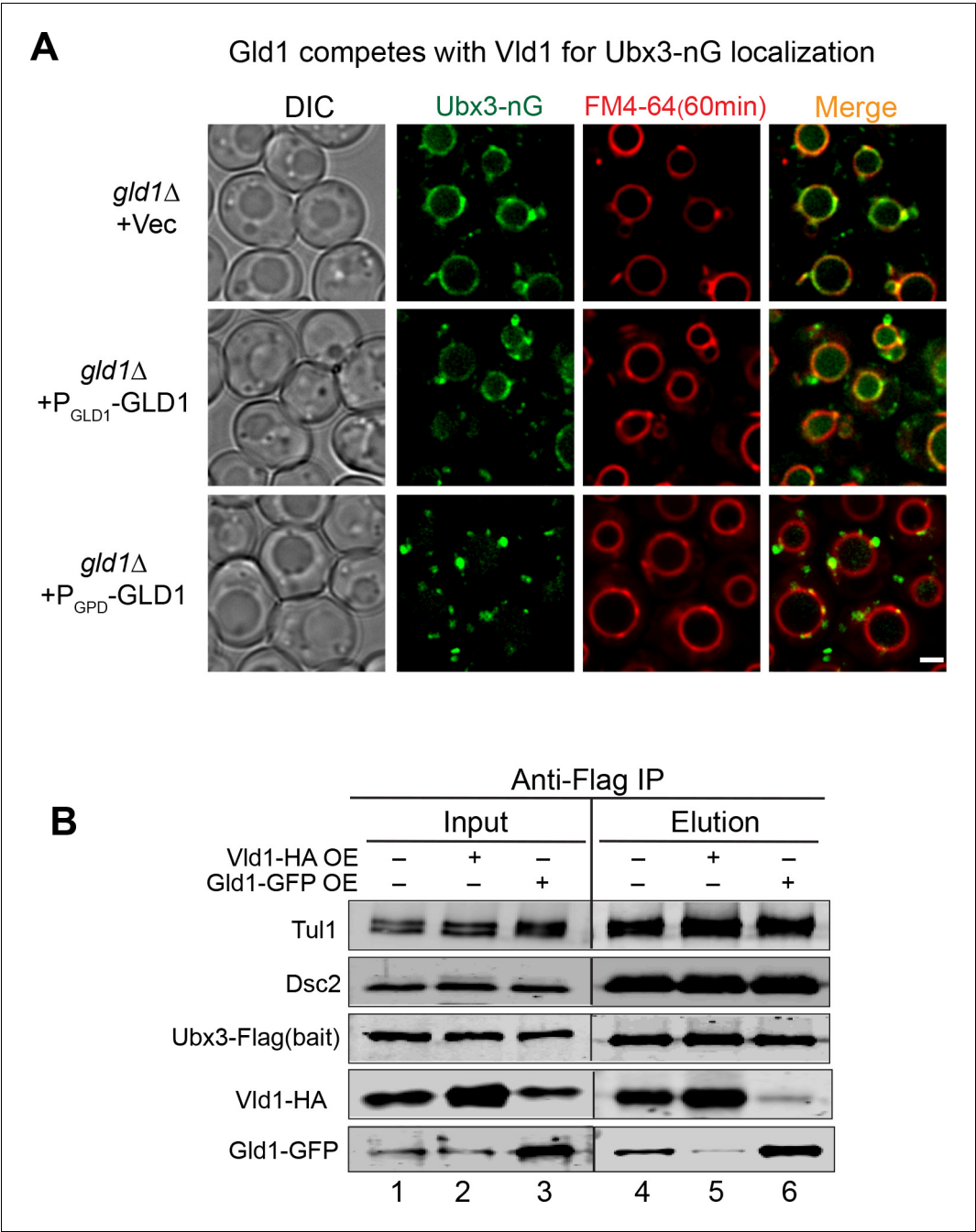


**Figure 4.** Vld1 competes with Gld1 to determine the Dsc subcellular localizations. (A) Localization of Ubx3-nG after either *VLD1* or *GLD1* deletion. Note a small amount of punctae are also observed outside the vacuole in *gld1Δ* cells. (B) The small Ubx3-nG punctae in the *gld1Δ* cells co-localize with Figure 4 continued on next page

*Figure 4 continued*

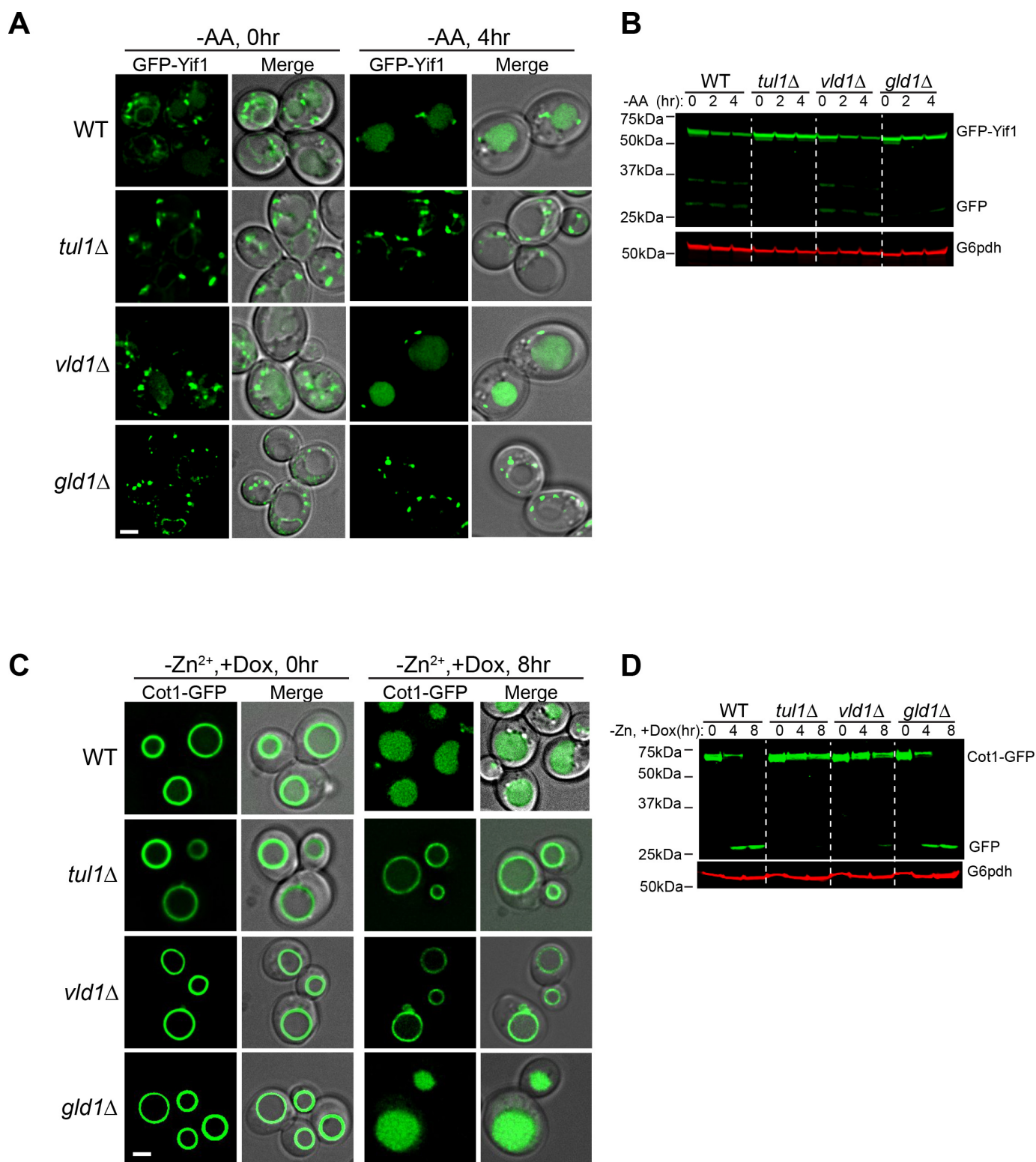
ER marker, DsRed-HDEL, whereas the punctae in *vld1Δ* cells do not co-localize with ER marker. (C) A competition assay to show elevating the Vld1 expression level in *vld1Δ* cells gradually diverts Ubx3-nG from punctae to vacuole membrane. Scale bar: 2  $\mu$ m.

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**Figure 4—figure supplement 1.** Gld1 competes with Vld1 to determine the subcellular localization of the Dsc complex. (A) A competition assay to show the localization of Ubx3-nG in strains with different *GLD1* expression levels. The vacuoles were labeled with FM4-64. Vec: empty vector. (B) Immunoprecipitation experiments to show the interactions between Ubx3-Flag and other Dsc components in WT, Vld1-HA overexpression, and Gld1-GFP overexpression strains. Note for the Input, 1OD<sub>600</sub> cells were loaded for each sample except Vld1-HA, lane 2 and Gld1-GFP OE, lane 3. Only 0.1 OD<sub>600</sub> cells were loaded for these two samples due to the overexpression. Scale bar: 2 μm.  
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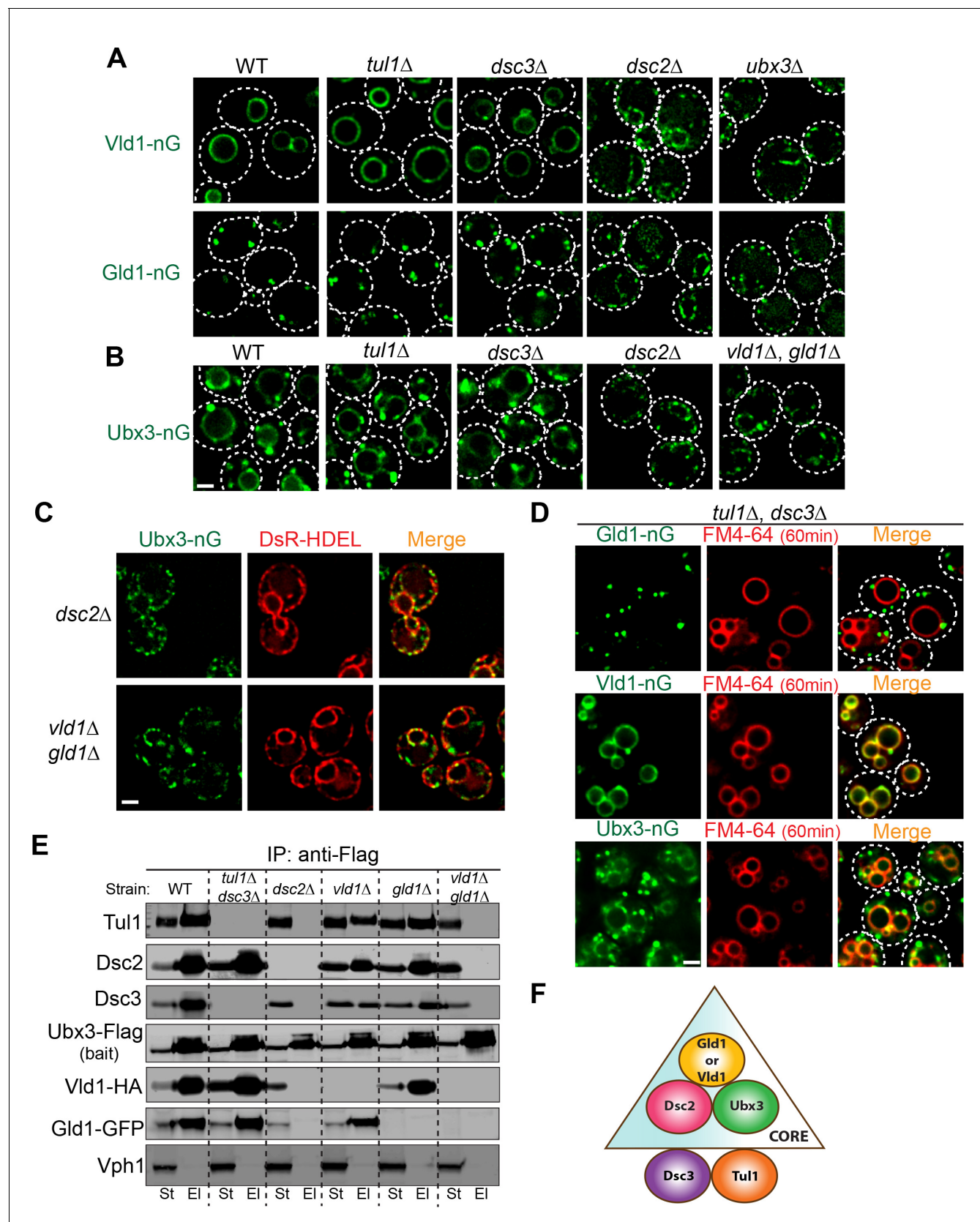
**Figure 5.** The Golgi/endosome and vacuole subcomplexes are functionally independent. (A) Subcellular localizations of GFP-Yif1 before (0 hr) and after (4 hr) amino acid starvation. (B) Western blot analysis of the GFP-Yif1 starvation assay. 1 OD<sub>600</sub> cells were loaded in each lane. (C) Subcellular localizations of Cot1-GFP before (0 hr) and after (8 hr) zinc starvation. (D) Western blot analysis of the Cot1-GFP starvation assay. 1 OD<sub>600</sub> cells were loaded in each lane. Figure 5 continued on next page

*Figure 5 continued*

localizations of Cot1-GFP before (0 hr) and after (8 hr)  $\text{Zn}^{2+}$  withdrawal. (D) Western blot analysis of the Cot1-GFP degradation assay. Same volume of cells was loaded, with 0.25 OD<sub>600</sub> cells loaded at 0 hr. Scale bar: 2  $\mu\text{m}$ .

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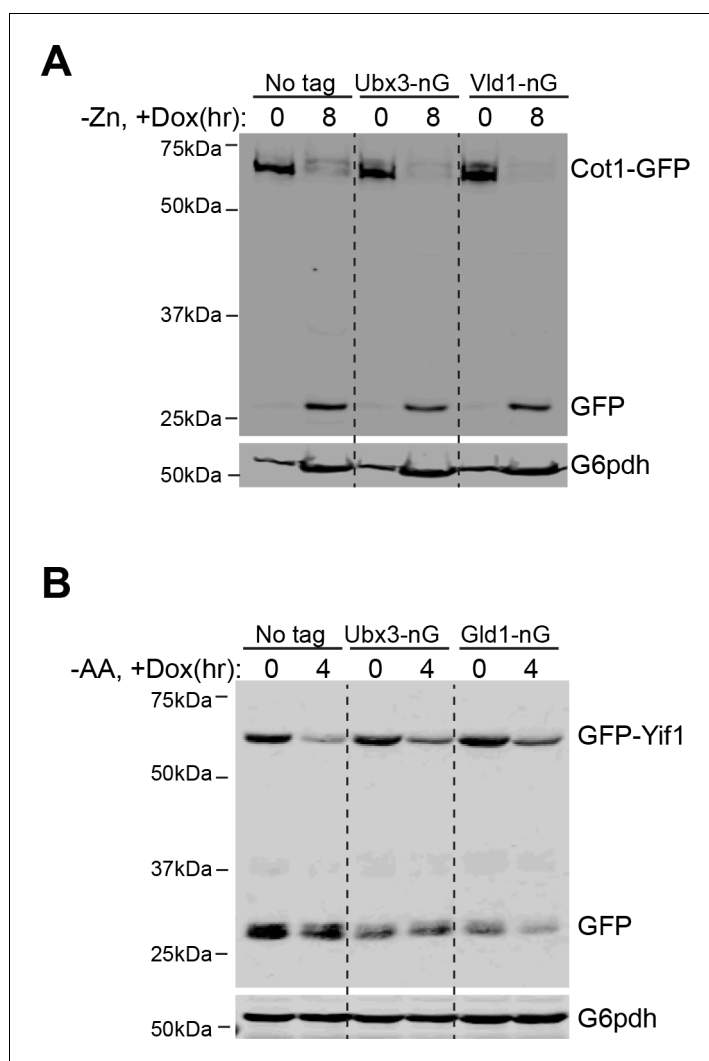


**Figure 6.** Two core complexes that determine the subcellular localizations. (A) Deletion analysis for Vld1-nG and Gld1-nG localizations. (B) Deletion analysis for Ubx3-nG localization. (C) Co-localization between the Ubx3-nG punctae and ER marker DsRed-HDEL in either *dsc2Δ* or *vld1Δ gld1Δ* strains. Figure 6 continued on next page

Figure 6 continued

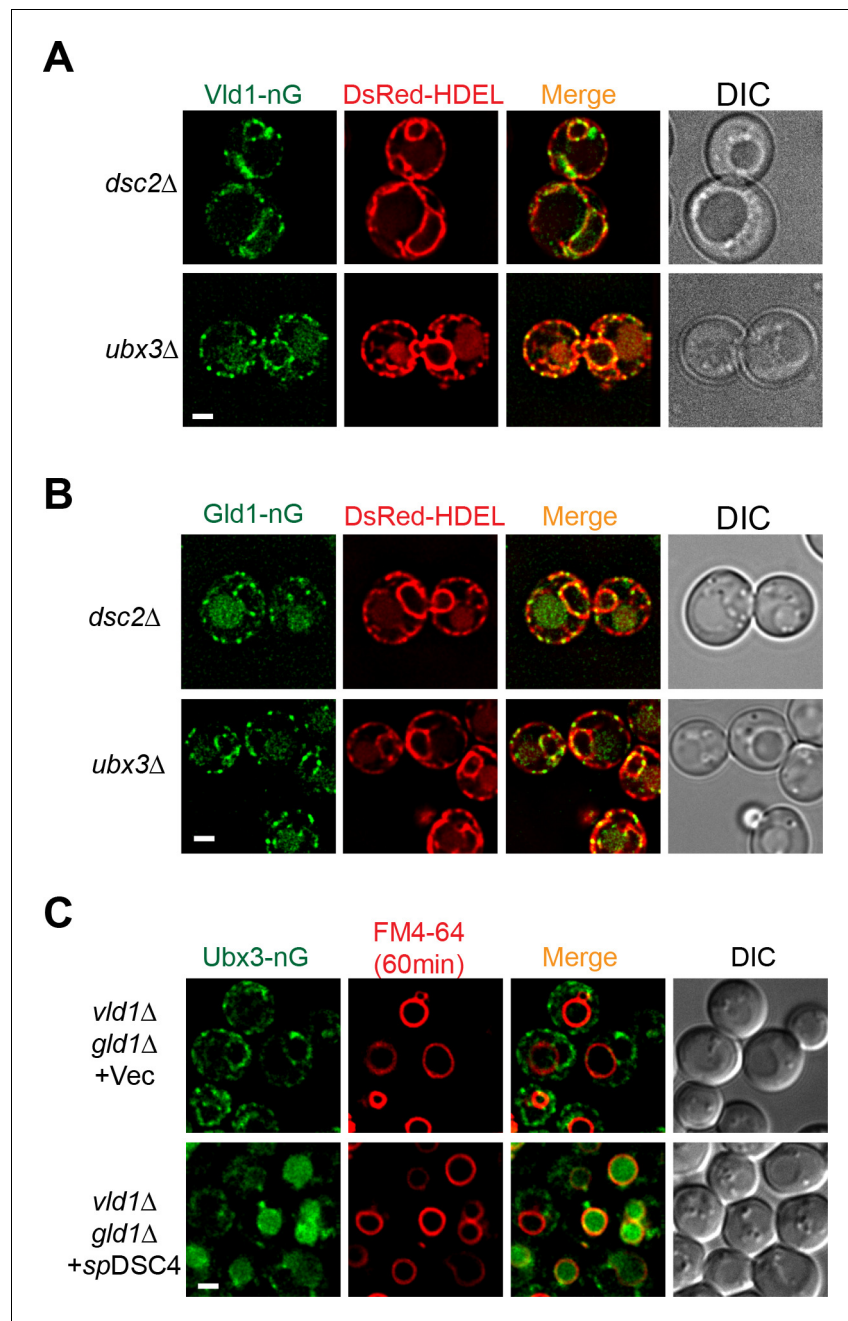
(D) Double deletion of *TUL1* and *DSC3* did not change the localization patterns of Vld1-nG, Gld1-nG, or Ubx3-nG. (E) Western blot analysis of the Dsc complex assembly after deleting indicated Dsc components. St: Starting material, El: Elution. (F) A model to summarize the core complex concept. White dashed lines indicate the periphery of yeast cells. Scale bar: 2  $\mu$ m.

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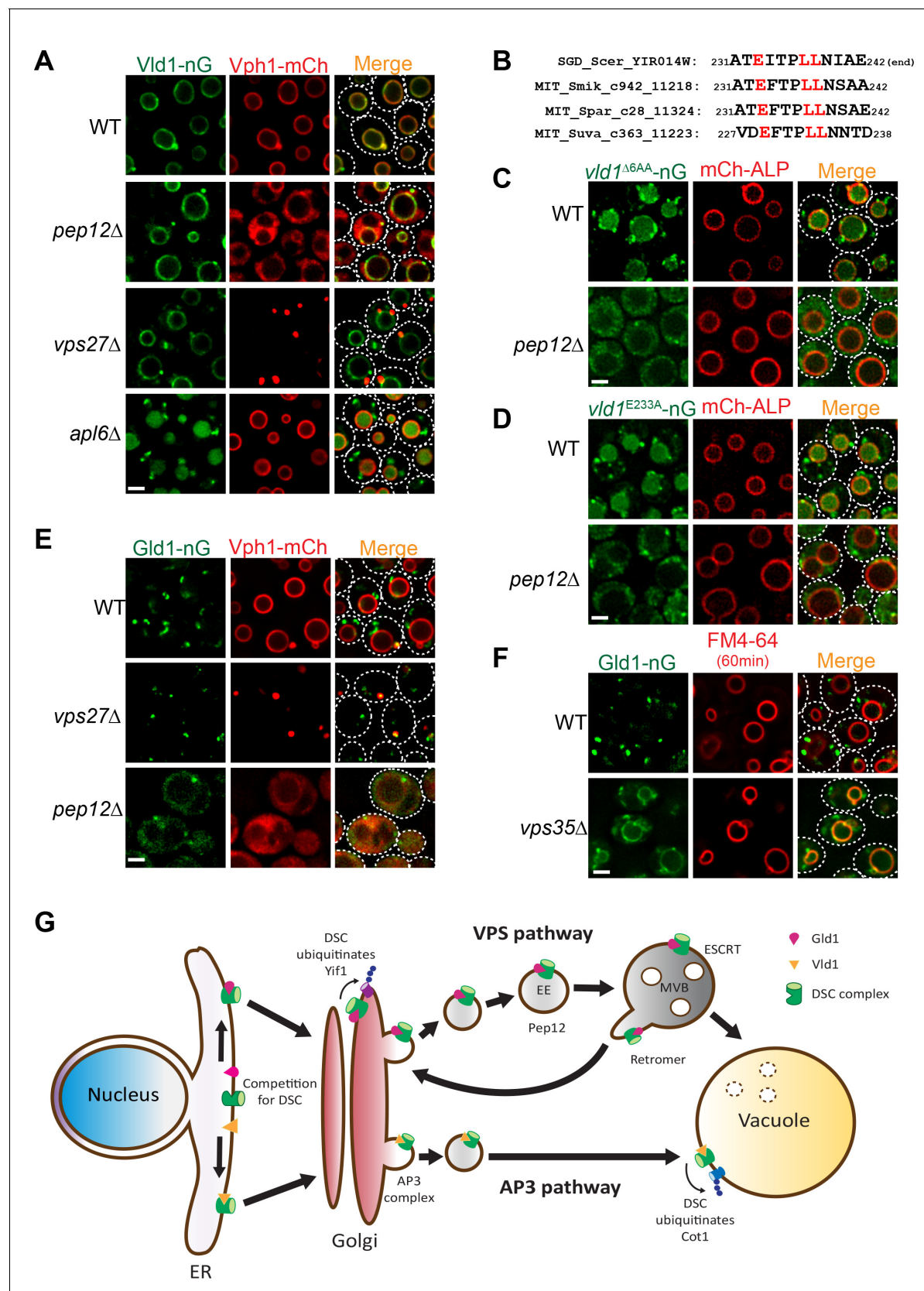
**Figure 6—figure supplement 1.** Ubx3, Vld1 and Gld1 still support the degradation of Cot1-GFP and GFP-Yif1 after neonGreen tagging. **(A)** Western blot analysis of the Cot1-GFP degradation after  $\text{Zn}^{2+}$  withdrawal in WT, Ubx3-nG, and Vld1-nG strains. Same volume of cells was loaded for each group, with 0.25 OD<sub>600</sub> cells loaded at 0 hr. **(B)** Western blot analysis of the GFP-Yif1 degradation after amino acid starvation in WT, Ubx3-nG, and Gld1-nG strains. Same volume of cells was loaded, with 0.5 OD<sub>600</sub> cells loaded at 0 hr.

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**Figure 6—figure supplement 2.** Both Vld1-nG and Gld1-nG are trapped in the ER after deleting either *DSC2* or *UBX3*. (A) Co-localization of Vld1-nG punctae with the ER marker DsRed-HDEL in either *dsc2Δ* or *ubx3Δ* strains. (B) Co-localization of Gld1-nG punctae with the ER marker DsRed-HDEL in either *dsc2Δ* or *ubx3Δ* strains. (C) Localization of Ubx3-nG in *vld1Δ gld1Δ* cells expressing either an empty vector or *spDsc4*. The vacuoles were labeled with fluorescent dye FM4-64. Scale bar: 2  $\mu$ m.

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**Figure 7.** Vld1 and Gld1 subcomplexes travel through the AP3 and VPS pathways, respectively. (A) Subcellular localizations of Vld1-nG and Vph1-mCh in WT, *pep12Δ*, *vps27Δ*, and *apl6Δ* cells. (B) Vld1 contains a conserved acidic di-leucine motif at its C-terminus. (C) Subcellular localizations of *vld1*<sup>Δ6AA</sup>. Figure 7 continued on next page

*Figure 7 continued*

nG (last 6 amino acids of Vld1 deleted) in WT cell and *pep12Δ* cells. (D) A single E233 to A mutation caused the trafficking defects of *vld1<sup>E233A</sup>*-nG in both WT and *pep12Δ* cells. (E) Subcellular localizations of Gld1-nG and Vph1-mCh in WT, *vps27Δ*, and *pep12Δ* cells. (F) Subcellular localizations of Gld1-nG in WT and *vps35Δ* cells. (G) A model summarizing the key findings of this study. White dashed lines indicate the periphery of yeast cells. Scale bar: 2 μm.

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