

Supplemental material

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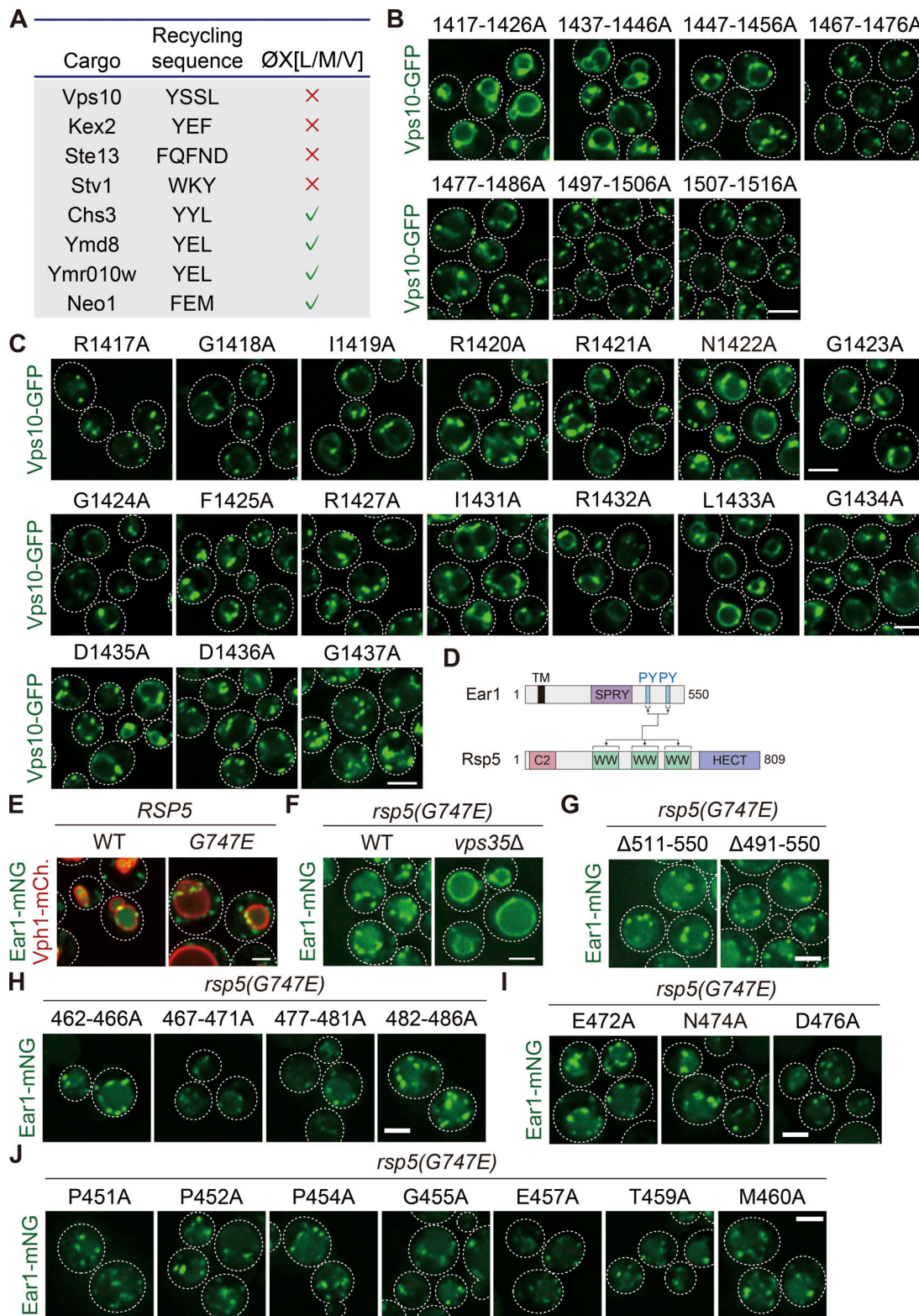


Figure S1. **The localization of Vps10-GFP and Ear1-mNeonGreen mutants. (A)** Schematic of the recycling sequence in yeast. **(B and C)** The localization of Vps10-GFP mutants. **(D)** Schematic of the Ear1-Rsp5 interaction. **(E)** Ear1-mNeonGreen localization in WT or *rsp5 (G747E)* cells. mCh, mCherry. **(F)** Ear1-mNeonGreen localization in *rsp5 (G747E)* or *rsp5 (G747E) vps35Δ* cells. **(G-J)** The localization of Ear1-mNeonGreen mutants in *rsp5 (G747E)* cells. Scale bars: 2 μm.

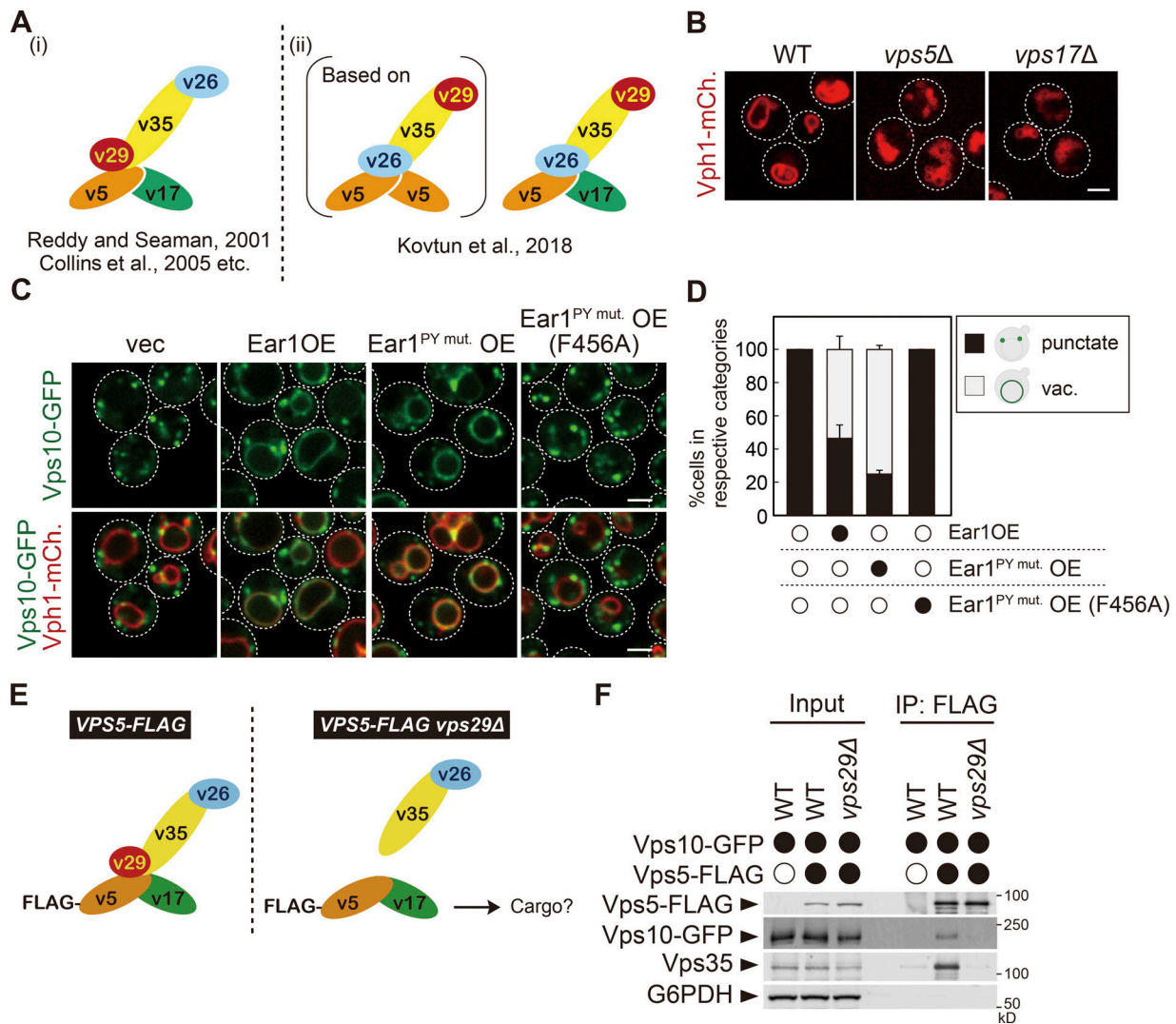


Figure S2. **Analysis of cargo recycling in retromer mutants. (A)** Two models for the retromer assembly. **(B)** The vacuole morphology in WT, *vps5*Δ, or *vps17*Δ cells. **(C)** Vps10-GFP localization in cells overexpressing Ear1 mutants. vec, vector. **(D)** The percentage of each category of Vps10-GFP mutant localization from C. vac, vacuole. **(E and F)** The Vps5-Vps10 interaction in *vps29*Δ cells. Vps5-FLAG was immunoprecipitated (IP) from cells lacking Vps29, and interacting Vps10-GFP was detected by immunoblotting using antibody against FLAG, GFP, Vps35, and G6PDH. For all quantification shown in this figure, at least 30 cells were classified and the data were obtained from three independent experiments. Error bars represent SD. Scale bars: 2 μm.

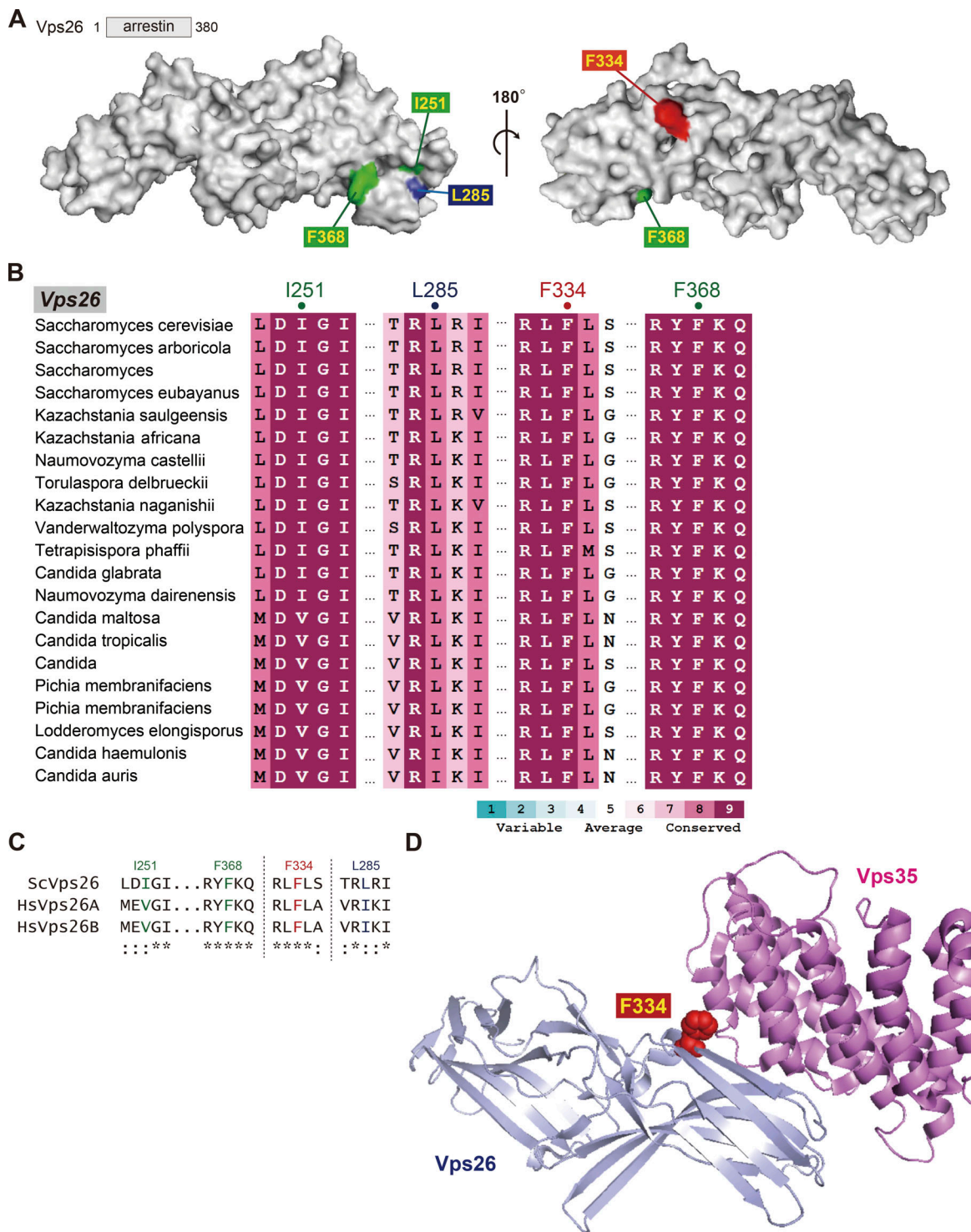


Figure S3. **Analysis of cargo recognition by Vps26.** (A) Mutation sites used in this study are shown on the crystal structure of *Mus musculus* Vps26 (Protein Databank ID code: 2R51). (B) Sequence comparison of Vps26 residues required for cargo retrieval among Vps26 homologues from related species. (C) Sequence comparison of Vps26 residues required for cargo retrieval in *S. cerevisiae* Vps26, *Homo sapiens* Vps26A, and *Homo sapiens* Vps26B. (D) F334 on Vps26 are shown on the crystal structure of *Homo sapiens* Vps26A-Vps35 complex (Protein Databank ID code: 5F0L).

Table S1. **Yeast strains used in this study**

Strain	Genotype	Source
SEY6210	<i>MATa ura3-52 his3-200 leu2-3,112 trp1-901 lys2-801 suc2-9</i>	Robinson et al., 1988
SEY6210.1	<i>MATa ura3-52 his3-200 leu2-3,112 trp1-901 lys2-801 suc2-9</i>	Robinson et al., 1988
YCY220	SEY6210, <i>VPH1-mCherry::TRP1</i>	This study
YCY950	SEY6210, <i>VPH1-mCherry::HIS3, vps10Δ::TRP1</i>	This study
YCY951	SEY6210, <i>VPH1-mCherry::HIS3, vps10Δ::TRP1, vps35Δ::KanMX6</i>	This study
YCY856	SEY6210, <i>rsp5Δ::HIS3, VPH1-mCherry::TRP1, pRS415-rsp5(G747E)</i>	This study
YCY755	SEY6210, <i>rsp5Δ::HIS3, EAR1-mNeonGreen::TRP1, pRS415-rsp5(G747E)</i>	This study
YCY722	SEY6210, <i>rsp5Δ::HIS3, EAR1-mNeonGreen::TRP1, vps35Δ::KanMX6, pRS415-rsp5(G747E)</i>	This study
YCY730	SEY6210, <i>rsp5Δ::HIS3, EAR1(Δ511-550)-mNeonGreen::TRP1, pRS415-rsp5(G747E)</i>	This study
YCY729	SEY6210, <i>rsp5Δ::HIS3, EAR1(Δ491-550)-mNeonGreen::TRP1, pRS415-rsp5(G747E)</i>	This study
YCY728	SEY6210, <i>rsp5Δ::HIS3, EAR1(Δ471-550)-mNeonGreen::TRP1, pRS415-rsp5(G747E)</i>	This study
YCY673	SEY6210, <i>rsp5Δ::HIS3, EAR1(Δ451-550)-mNeonGreen::TRP1, pRS415-rsp5(G747E)</i>	This study
YCY948	SEY6210.1, <i>VPS10-GFP::HIS3, VPH1-mCherry::TRP1, vps26Δ::KanMX6</i>	This study
YCY960	SEY6210, <i>rsp5Δ::HIS3, VPH1-mCherry::TRP1, pRS415-rsp5(G747E), vps26Δ::hphNT1</i>	This study
SSY896	SEY6210, <i>VPH1-mCherry::TRP1, vps5Δ::LEU2</i>	This study
SSY897	SEY6210, <i>VPH1-mCherry::TRP1, vps17Δ::KanMX6</i>	This study
SSY894	SEY6210, <i>VPH1-mCherry::TRP1, vps26Δ::LEU2</i>	This study
SSY895	SEY6210, <i>VPH1-mCherry::TRP1, vps29Δ::HIS3</i>	This study
SSY216	SEY6210, <i>VPH1-mCherry::TRP1, vps35Δ::KanMX6</i>	This study
SSY933	SEY6210, <i>VPS10-GFP::KanMX6, VPH1-mCherry::TRP1</i>	This study
SSY934	SEY6210, <i>VPS10-GFP::KanMX6, VPH1-mCherry::TRP1, vps5Δ::LEU2</i>	This study
SSY935	SEY6210, <i>VPS10-GFP::KanMX6, VPH1-mCherry::TRP1, vps17Δ::HIS3</i>	This study
SSY936	SEY6210, <i>VPS10-GFP::KanMX6, VPH1-mCherry::TRP1, vps35Δ::HIS3</i>	This study
SSY924	SEY6210, <i>VPS17-3xHA::TRP1</i>	This study
SSY926	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1</i>	This study
SSY923	SEY6210, <i>VPS5-3xFLAG::HIS3, vps17Δ::TRP1</i>	This study
SSY987	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1, vps26Δ::hphNT1</i>	This study
SSY1047	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1, vps29Δ::KanMX6</i>	This study
SSY1048	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1, vps35Δ::KanMX6</i>	This study
SSY970	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2</i>	This study
SSY981	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS5-3xFLAG::hphNT1</i>	This study
SSY1042	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS5-3xFLAG::hphNT1, vps29Δ::KanMX6</i>	This study
SSY982	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS26-3xFLAG::hphNT1</i>	This study
SSY1043	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS26-3xFLAG::hphNT1, vps5Δ::KanMX6</i>	This study
SSY1044	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS26-3xFLAG::hphNT1, vps17Δ::KanMX6</i>	This study
SSY1045	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS26-3xFLAG::hphNT1, vps29Δ::KanMX6</i>	This study
SSY1046	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS26-3xFLAG::hphNT1, vps35Δ::KanMX6</i>	This study
SSY983	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS35-3xFLAG::hphNT1</i>	This study
SSY1049	SEY6210, <i>pep4Δ::LEU2, prb1Δ::LEU2, VPS35-3xFLAG::hphNT1, vps26Δ::KanMX6</i>	This study
SSY1050	SEY6210, <i>VPS17-3xHA::TRP1, pep4Δ::KanMX6</i>	This study
SSY1051	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1, pep4Δ::KanMX6</i>	This study
SSY1052	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1, vps26Δ::hphNT1, pRS305-VPS26(I251E/F368E), pep4Δ::KanMX6</i>	This study
SSY1053	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1, vps26Δ::hphNT1, pRS305-VPS26(F334E), pep4Δ::KanMX6</i>	This study
SSY1054	SEY6210, <i>VPS5-3xFLAG::HIS3, VPS17-3xHA::TRP1, vps26Δ::hphNT1, pRS305-VPS26(L285E), pep4Δ::KanMX6</i>	This study

Table S2. **Plasmids used in this study**

Name	Genotype	Source
pRS416 (vector)	<i>CEN URA3</i>	Sikorski and Hieter, 1989
pRS416-VPS10-GFP	<i>pRS416-VPS10-GFP</i>	Lab stock
pRS416-VPS10(Δ C)-GFP	<i>pRS416-VPS10(2-1418)-GFP</i>	This study
pRS416-VPS10(Δ C)-EAR1(C-tail)-GFP	<i>pRS416-VPS10(2-1418)-EAR1(451-550)-GFP</i>	This study
pRS416-VPS10(Δ 1517-1579)-GFP	<i>pRS416-VPS10(2-1516)-GFP</i>	This study
pRS416-VPS10(1417-1426A)-GFP	<i>pRS416-VPS10(1417-1426A)-GFP</i>	This study
pRS416-VPS10(1427-1436A)-GFP	<i>pRS416-VPS10(1427-1436A)-GFP</i>	This study
pRS416-VPS10(1437-1446A)-GFP	<i>pRS416-VPS10(1437-1446A)-GFP</i>	This study
pRS416-VPS10(1447-1456A)-GFP	<i>pRS416-VPS10(1447-1456A)-GFP</i>	This study
pRS416-VPS10(1457-1466A)-GFP	<i>pRS416-VPS10(1457-1466A)-GFP</i>	This study
pRS416-VPS10(1467-1476A)-GFP	<i>pRS416-VPS10(1467-1476A)-GFP</i>	This study
pRS416-VPS10(1477-1486A)-GFP	<i>pRS416-VPS10(1477-1486A)-GFP</i>	This study
pRS416-VPS10(1487-1496A)-GFP	<i>pRS416-VPS10(1487-1496A)-GFP</i>	This study
pRS416-VPS10(1497-1506A)-GFP	<i>pRS416-VPS10(1497-1506A)-GFP</i>	This study
pRS416-VPS10(1507-1516A)-GFP	<i>pRS416-VPS10(1507-1516A)-GFP</i>	This study
pRS416-VPS10(R1417A)-GFP	<i>pRS416-VPS10(R1417A)-GFP</i>	This study
pRS416-VPS10(G1418A)-GFP	<i>pRS416-VPS10(G1418A)-GFP</i>	This study
pRS416-VPS10(I1419A)-GFP	<i>pRS416-VPS10(I1419A)-GFP</i>	This study
pRS416-VPS10(R1420A)-GFP	<i>pRS416-VPS10(R1420A)-GFP</i>	This study
pRS416-VPS10(R1421A)-GFP	<i>pRS416-VPS10(R1421A)-GFP</i>	This study
pRS416-VPS10(N1422A)-GFP	<i>pRS416-VPS10(N1422A)-GFP</i>	This study
pRS416-VPS10(G1423A)-GFP	<i>pRS416-VPS10(G1423A)-GFP</i>	This study
pRS416-VPS10(G1424A)-GFP	<i>pRS416-VPS10(G1424A)-GFP</i>	This study
pRS416-VPS10(F1425A)-GFP	<i>pRS416-VPS10(F1425A)-GFP</i>	This study
pRS416-VPS10(R1427A)-GFP	<i>pRS416-VPS10(R1427A)-GFP</i>	This study
pRS416-VPS10(F1428A)-GFP	<i>pRS416-VPS10(F1428A)-GFP</i>	This study
pRS416-VPS10(G1429A)-GFP	<i>pRS416-VPS10(G1429A)-GFP</i>	This study
pRS416-VPS10(E1430A)-GFP	<i>pRS416-VPS10(E1430A)-GFP</i>	This study
pRS416-VPS10(I1431A)-GFP	<i>pRS416-VPS10(I1431A)-GFP</i>	This study
pRS416-VPS10(R1432A)-GFP	<i>pRS416-VPS10(R1432A)-GFP</i>	This study
pRS416-VPS10(L1433A)-GFP	<i>pRS416-VPS10(L1433A)-GFP</i>	This study
pRS416-VPS10(G1434A)-GFP	<i>pRS416-VPS10(G1434A)-GFP</i>	This study
pRS416-VPS10(D1435A)-GFP	<i>pRS416-VPS10(D1435A)-GFP</i>	This study
pRS416-VPS10(D1436A)-GFP	<i>pRS416-VPS10(D1436A)-GFP</i>	This study
pRS416-VPS10(G1437A)-GFP	<i>pRS416-VPS10(G1437A)-GFP</i>	This study
pRS416-VPS10(L1438A)-GFP	<i>pRS416-VPS10(L1438A)-GFP</i>	This study
pRS416-VPS10(I1439A)-GFP	<i>pRS416-VPS10(I1439A)-GFP</i>	This study
pRS416-VPS10(E1440A)-GFP	<i>pRS416-VPS10(E1440A)-GFP</i>	This study
pRS416-VPS10(N1441A)-GFP	<i>pRS416-VPS10(N1441A)-GFP</i>	This study
pRS416-VPS10(N1442A)-GFP	<i>pRS416-VPS10(N1442A)-GFP</i>	This study
pRS416-VPS10(N1443A)-GFP	<i>pRS416-VPS10(N1443A)-GFP</i>	This study
pRS416-VPS10(T1444A)-GFP	<i>pRS416-VPS10(T1444A)-GFP</i>	This study
pRS416-VPS10(D1445A)-GFP	<i>pRS416-VPS10(D1445A)-GFP</i>	This study
pRS416-VPS10(R1446A)-GFP	<i>pRS416-VPS10(R1446A)-GFP</i>	This study

Table S2. **Plasmids used in this study (Continued)**

Name	Genotype	Source
pRS416-EAR1(Δ C)-GFP	pRS416-EAR1(2-67)-GFP	This study
pRS416-EAR1(Δ C)-VPS10(C-tail)-GFP	pRS416-EAR1(2-67)-VPS10(1416-1523)-GFP	This study
pRS416-VPS10(1492-1495A)-GFP	pRS416-VPS10(1492-1495A)-GFP	This study
pRS416-VPS10(1428-1433A)-GFP	pRS416-VPS10(1428-1433A)-GFP	This study
pRS416-VPS10(1428-1433A/1492-1495A)-GFP	pRS416-VPS10(1428-1433A+1492-1495A)-GFP	This study
pRS416-EAR1-mNeonGreen-3xHA	pRS416-EAR1-mNeonGreen-3xHA	This study
pRS416-EAR1(452-456A)-mNeonGreen-3xHA	pRS416-EAR1(452-456A)-mNeonGreen-3xHA	This study
pRS416-EAR1(457-461A)-mNeonGreen-3xHA	pRS416-EAR1(457-461A)-mNeonGreen-3xHA	This study
pRS416-EAR1(462-466A)-mNeonGreen-3xHA	pRS416-EAR1(462-466A)-mNeonGreen-3xHA	This study
pRS416-EAR1(467-471A)-mNeonGreen-3xHA	pRS416-EAR1(467-471A)-mNeonGreen-3xHA	This study
pRS416-EAR1(472-476A)-mNeonGreen-3xHA	pRS416-EAR1(472-476A)-mNeonGreen-3xHA	This study
pRS416-EAR1(477-481A)-mNeonGreen-3xHA	pRS416-EAR1(477-481A)-mNeonGreen-3xHA	This study
pRS416-EAR1(482-486A)-mNeonGreen-3xHA	pRS416-EAR1(482-486A)-mNeonGreen-3xHA	This study
pRS416-EAR1(P451A)-mNeonGreen-3xHA	pRS416-EAR1(P451A)-mNeonGreen-3xHA	This study
pRS416-EAR1(P452A)-mNeonGreen-3xHA	pRS416-EAR1(P452A)-mNeonGreen-3xHA	This study
pRS416-EAR1(P453A)-mNeonGreen-3xHA	pRS416-EAR1(P453A)-mNeonGreen-3xHA	This study
pRS416-EAR1(P454A)-mNeonGreen-3xHA	pRS416-EAR1(P454A)-mNeonGreen-3xHA	This study
pRS416-EAR1(G455A)-mNeonGreen-3xHA	pRS416-EAR1(G455A)-mNeonGreen-3xHA	This study
pRS416-EAR1(F456A)-mNeonGreen-3xHA	pRS416-EAR1(F456A)-mNeonGreen-3xHA	This study
pRS416-EAR1(E457A)-mNeonGreen-3xHA	pRS416-EAR1(E457A)-mNeonGreen-3xHA	This study
pRS416-EAR1(F458A)-mNeonGreen-3xHA	pRS416-EAR1(F458A)-mNeonGreen-3xHA	This study
pRS416-EAR1(T459A)-mNeonGreen-3xHA	pRS416-EAR1(T459A)-mNeonGreen-3xHA	This study
pRS416-EAR1(M460A)-mNeonGreen-3xHA	pRS416-EAR1(M460A)-mNeonGreen-3xHA	This study
pRS416-EAR1(E472A)-mNeonGreen-3xHA	pRS416-EAR1(E472A)-mNeonGreen-3xHA	This study
pRS416-EAR1(I473A)-mNeonGreen-3xHA	pRS416-EAR1(I473A)-mNeonGreen-3xHA	This study
pRS416-EAR1(N474A)-mNeonGreen-3xHA	pRS416-EAR1(N474A)-mNeonGreen-3xHA	This study
pRS416-EAR1(L475A)-mNeonGreen-3xHA	pRS416-EAR1(L475A)-mNeonGreen-3xHA	This study
pRS416-EAR1(D476A)-mNeonGreen-3xHA	pRS416-EAR1(D476A)-mNeonGreen-3xHA	This study
pRS416-EAR1(PY mut.)-mNeonGreen-3xHA	pRS416-EAR1(P398A/Y400A/P483A/Y485A)-mNeonGreen-3xHA	This study
pRS416-Ear1-pHluorin	pRS416-EAR1-pHluorin	This study
pRS416-Ear1(F456A)-pHluorin	pRS416-EAR1(F465A)-pHluorin	This study
pRS416-Ear1(L475A)-pHluorin	pRS416-EAR1(L475A)-pHluorin	This study
pCM189	pCM189-TETOFFpro-CYC1pro	This study
pCM189-EAR1 overexpressed	pCM189-TETOFFpro-CYC1pro-EAR1	This study
pCM189-EAR1(PY mut.) overexpressed	pCM189-TETOFFpro-CYC1pro-EAR1(P398A/Y400A/P483A/Y485A)	This study
pCM189-EAR1(PY mut./F456A) overexpressed	pCM189-TETOFFpro-CYC1pro-EAR1(P398A/Y400A/F456A/P483A/Y485A)	This study
pRS416-CPYpro-mCherry-PHO8	pRS416-CPYpro-mCherry-PHO8	This study
pRS305-VPS26	pRS305-VPS26	This study
pRS305-VPS26(I251E/F368E)	pRS305-VPS26(I251E/F368E)	This study
pRS305-VPS26(F334E)	pRS305-VPS26(F334E)	This study
pRS305-VPS26(L285E)	pRS305-VPS26(L285E)	This study
pRS416-VPS26	pRS416-VPS26	This study
pRS416-VPS26(I251E/F368E)	pRS416-VPS26(I251E/F368E)	This study
pRS416-VPS26(F334E)	pRS416-VPS26(F334E)	This study
pRS416-VPS26(L285E)	pRS416-VPS26(L285E)	This study

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