**Supplemental File 1A.** **Yeast strains used in this study**

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| **Strain** | **Genotype** | **Source** |
| SEY6210SEY6210.1SSY208SSY209SSY342SSY413SSY414SSY778SSY667SSY252SSY703SSY659SSY33SSY970SSY37SSY615SSY212SSY490SSY98SSY1161SSY259SSY532SSY83SSY216SSY1134SSY479SSY1128SSY1129SSY1130SSY1131SSY491SSY474SSY493SSY477SSY1240SSY1271SSY1232SSY1178SSY1241SSY1180SSY1244SSY1068SSY1069SSY1272SSY1070SSY191SSY1268SSY1269SSY1274SSY1277SSY1258SSY1232SSY1248SSY727SSY728SSY439SSY441SSY445SSY1171SSY1169SSY1264SSY911SSY913SSY1032SSY1033SSY1034SSY1035SSY491SSY1229SSY792SSY1015SSY1217SSY12118SSY984SSY985SSY924SSY1084SSY1339SSY1329SSY1330 | *MAT* *ura3-52 his3-200 leu2-3,112 trp1-901 lys2-801 suc2-9**MATa ura3-52 his3-200 leu2-3,112 trp1-901 lys2-801 suc2-9*SEY6210, *vps35Δ::HIS3*SEY6210, *snx4Δ::TRP1*SEY6210, *mvp1Δ::TRP1*SEY6210, *snx41Δ::TRP1*SEY6210, *snx42Δ::TRP1*SEY6210, *ykr078wΔ::TRP1*SEY6210, *vps35Δ::KanMX6, snx4Δ::TRP1*SEY6210, *vps35Δ::KanMX6, mvp1Δ:: TRP1*SEY6210, *snx4Δ::KanMX6, mvp1Δ::TRP1*SEY6210, *vps35Δ::KanMX6, snx4Δ::TRP1, mvp1Δ::hphNT1*SEY6210, *pep4Δ::LEU2*SEY6210, *pep4Δ::LEU2, prb1Δ::LEU2*SEY6210, *vps4Δ::TRP1*SEY6210, *vps34Δ::HIS3*SEY6210, *pep12Δ::HIS3*SEY6210, *vps55Δ::KanMX6*SEY6210, *vps1Δ::KanMX6*SEY6210, *VPS1-GFP::KanMX6*SEY6210, *SEC7-mCherry::hphNT1*SEY6210, *NHX1-2xmCherry::hphNT1*SEY6210, *VPH1-mCherry::TRP1*SEY6210, *VPH1-mCherry::TRP1*, *vps35Δ::KanMX6*SEY6210, *VPH1-mCherry::TRP1*, *snx4Δ::KanMX6*SEY6210, *VPH1-mCherry::TRP1*, *mvp1Δ::KanMX6*SEY6210, *VPH1-mCherry::TRP1*, *vps35Δ::KanMX6, snx4Δ::TRP1*SEY6210, *VPH1-mCherry::TRP1*, *vps35Δ::KanMX6, mvp1Δ::TRP1*SEY6210, *VPH1-mCherry::TRP1*, *snx4Δ::TRP1, mvp1Δ::KanMX6*SEY6210, *VPH1-mCherry::TRP1*, *vps35Δ::KanMX6, snx4Δ::TRP1, mvp1Δ::hphNT1*SEY6210, *VPH1-mCherry::TRP1*, *vps55Δ::KanMX6*SEY6210, *VPS55-GFP::LEU2*, *SEC7-mCherry::TRP1*SEY6210, *VPS55-GFP::LEU2*, *mCherry-PEP12::URA3*SEY6210, *MVP1-GFP::LEU2*, *mCherry-PEP12::URA3*SEY6210, *VPS55-mNeonGreen-3xHA::KanMX6*, *mCherry-VPS21::LEU2*SEY6210, *VPS55-mNeonGreen-3xHA::KanMX6*, *VPS10-mCherry::TRP1*SEY6210, *MVP1-mNeonGreen-3xHA::KanMX6*, *mCherry-VPS21::LEU2*SEY6210, *VPS1-GFP::HIS3*, *SEC7-mCherry::hphNT1*SEY6210, *VPS1-GFP::KanMX6*, *mCherry-VPS21::LEU2*SEY6210, *VPS1-GFP::HIS3*, *NHX1-2xmCherry::hphNT1*SEY6210, *vps1Δ::hphNT1*, *MVP1-mRFP::URA3*SEY6210, *VPS55-GFP::KanMX6*, *VPH1-mCherry::TRP1*SEY6210, *VPS55-GFP::HIS3*, *VPH1-mCherry::TRP1*, *vps35Δ::KanMX6*SEY6210, *VPS55-GFP::HIS3*, *VPH1-mCherry::TRP1*, *snx4Δ::KanMX6*SEY6210, *VPS55-GFP::TRP1*, *VPH1-mCherry::TRP1*, *mvp1Δ::KanMX6*SEY6210, *VPS55-GFP::TRP1*SEY6210, *VPS55-GFP-Ub::HIS3*SEY6210, *VPS55-GFP-Ub::HIS3, vps4Δ::TRP1*SEY6210, *VPS55-mNeonGreen-3xHA::KanMX6*, *VPH1-mCherry::TRP1*SEY6210, *VPS55-mNeonGreen-3xHA::KanMX6*, *VPH1-mCherry::TRP1*, *vps1Δ::hphNT1*SEY6210, *VPS55-mNeonGreen-3xHA::KanMX6*, *mCherry-VPS21::LEU2*, *vps1Δ::hphNT1*SEY6210, *MVP1-mNeonGreen-3xHA::KanMX6*, *mCherry-VPS21::LEU2*SEY6210, *MVP1-mNeonGreen-3xHA::KanMX6*, *mCherry-VPS21::LEU2*, *vps1Δ::KanMX6*SEY6210, *GFP-PHO8::URA3*SEY6210, *GFP-PHO8::URA3*, *vps35Δ::KanMX6, snx4Δ::TRP1, mvp1Δ::hphNT1*SEY6210, *GFP-SNC1::URA3*SEY6210, *GFP-SNC1::URA3,* *snx4Δ::KanMX6*SEY6210, *GFP-SNC1::URA3*, *mvp1Δ::TRP1*SEY6210.1, *MUP1-pHluorin::KanMX6*SEY6210, *MUP1-pHluorin::natMX6*, *vps35Δ::KanMX6, snx4Δ::TRP1, mvp1Δ::hphNT1*SEY6210, *pep4Δ::LEU2*, *prb1Δ::LEU2*, *vps55Δ::KanMX6*, *MVP1-GFP::TRP1*SEY6210, *VPS5-3xFLAG::HIS3*SEY6210, *VPS17-3xFLAG::HIS3*SEY6210, *SNX4-3xFLAG::HIS3*SEY6210, *SNX41-3xFLAG::HIS3*SEY6210, *SNX42-3xFLAG::HIS3*, *SNX41-3xHA::TRP1*SEY6210, *YKR078w-3xFLAG::HIS3*SEY6210, *vps55Δ::KanMX6, VPH1-mCherry::TRP1*SEY6210, *pep4Δ::LEU2*, *prb1Δ::LEU2*, *vps1Δ::hphNT1, MVP1-3xFLAG::HIS3*SEY6210, *sec18ts, VPH1-mCherry::TRP1*SEY6210, *sec18ts, VPH1-mCherry::TRP1, VPS55-3xFLAG::HIS3*SEY6210, *sec18ts, VPH1-mCherry::TRP1, VPS55-GFP::LEU2*SEY6210, *sec18ts, VPH1-mCherry::TRP1, VPS10-3xFLAG::HIS3, VPS55-GFP::LEU2*SEY6210, *VPS17-3xHA::TRP1, VPS26-13xMyc::hphNT1*SEY6210, *VPS5-3xFLAG::HIS3*, *VPS17-3xHA::TRP1, VPS26-13xMyc::hphNT1*SEY6210, *VPS17-3xHA::TRP1*SEY6210, *VPS17-3xHA::TRP1,MVP1-3xFLAG::HIS3*SEY6210, *MVP1-GFP::HIS3,* *vam3Δ::LEU2*SEY6210, *vps35Δ::KanMX6, snx4Δ::TRP1, mvp1Δ::hphNT1, MVP1-3xFLAG::URA3*SEY6210, *vps35Δ::KanMX6, snx4Δ::TRP1, mvp1Δ::hphNT1, MVP1(I346E/Q468E/W496E)-3xFLAG::URA3* | (1)(1)This studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis study(2)This studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis study(2)This studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis study (2)This studyThis studyThis 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**Supplemental File 1B. Plasmids used in this study**

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| **Name** | **Genotype** | **Source** |
| pRS305 [vec]pRS416 [vec]pRS426 [vec]pRS416-VPS55-GFPpRS416-mNeonGreen-PEP12pRS416-MVP1-GFPpRS416-MVP1(R172E)-GFPpRS416-MVP1(I346E/Q468E/W496E)-GFPpRS306-MVP1-FLAGpRS306-MVP1(R172E)-FLAGpRS306-MVP1(I346E/Q468E/W496E)-FLAGpRS305-VPS55-GFPpRS305-VPS55(60-63A)-GFPpRS305-VPS55(64-67A)-GFPpRS305-VPS55(68-71A)-GFPpRS305-VPS55(72-75A)-GFPpRS305-VPS55(133-136A)-GFPpRS305-VPS55(137-140A)-GFPpRS305-VPS55(K60A)-GFPpRS305-VPS55(Y61A)-GFPpRS305-VPS55(H62A)-GFPpRS305-VPS55(T63A)-GFPpRS305-VPS55(S64A)-GFPpRS305-VPS55(D65A)-GFPpRS305-VPS55(F66A)-GFPpRS305-VPS55(M67A)-GFPpRS305-VPS55(Y61A/T63A)-GFPpRS305-VPS55(F66A/M67A)-GFPpRS305-VPS55(Y61A/T63A/F66A/M67A)-GFPpRS416-VPS55-FLAGpRS416-VPS55(Y61A/T63A/F66A/M67A)-FLAGpRS426-VPS55-GFPpRS416-VPS1-GFPpRS416-VPS1(K42A)-GFPpRS416-VPS1(G476D)-GFPpRS416-VPS1(K42A)-BFPpRS305-VPS1-GFPpRS416-GFP-VPS21pRS416-KEX2-GFPpRS416-GFP-NEO1pRS416-NHX1-GFPpRS426-NEO1pRS416-MUP1-GFPpRS416-GFP-CPSpRS416-mCherry-ALPpRS415-VPH1-mCherrypRS416-GFP-FYVEpRS425-VPS68pEGFP-C2pEGFP-SNX8pET28a-Mvp1pET28a-Vps1-GFP | *CEN URA3**2μ URA3**pRS416-VPS55pr-VPS55-GFP**pRS416-PEP12pr-mNeonGreen-PEP12**pRS416-MVP1pr-MVP1-GFP**pRS416-MVP1pr-MVP1(R172E)-GFP**pRS416-MVP1pr-MVP1(I346E/Q468E/W496E)-GFP**pRS306-MVP1pr-MVP1-3xFLAG**pRS306-MVP1pr-MVP1(R172E)-3xFLAG**pRS306-MVP1pr-MVP1(I346E/Q468E/W496E)-3xFLAG**pRS305-VPS55pr-VPS55-GFP**pRS305-VPS55pr-VPS55(60-63A)-GFP**pRS305-VPS55pr-VPS55(64-67A)-GFP**pRS305-VPS55pr-VPS55(68-71A)-GFP**pRS305-VPS55pr-VPS55(72-75A)-GFP**pRS305-VPS55pr-VPS55(133-136A)-GFP**pRS305-VPS55pr-VPS55(137-140A)-GFP**pRS305-VPS55pr-VPS55(K60A)-GFP**pRS305-VPS55pr-VPS55(Y61A)-GFP**pRS305-VPS55pr-VPS55(H62A)-GFP**pRS305-VPS55pr-VPS55(T63A)-GFP**pRS305-VPS55pr-VPS55(S64A)-GFP**pRS305-VPS55pr-VPS55(D65A)-GFP**pRS305-VPS55pr-VPS55(F66A)-GFP**pRS305-VPS55pr-VPS55(M67A)-GFP**pRS305-VPS55pr-VPS55(Y61A/T63A)-GFP**pRS305-VPS55pr-VPS55(F66A/M67A)-GFP**pRS305-VPS55pr-VPS55(Y61A/T63A/ F66A/M67A)-GFP**pRS416-VPS55pr-VPS55-3xFLAG**pRS416-VPS55pr-VPS55(Y61A/T63A/F66A/M67A)-3xFLAG**pRS426-VPS55pr-VPS55-GFP**pRS416-VPS1pr-VPS1-GFP**pRS416-VPS1pr-VPS1(K42A)-GFP**pRS416-VPS1pr-VPS1(G476D)-GFP**pRS416-VPS1pr-VPS1(K42A)-BFP**pRS305-VPS1pr-VPS1-GFP**pRS415-VPS21pr-GFP-VPS21**pRS416-KEX2pr-KEX2-GFP**pRS416-NEO1pr-GFP-NEO1**pRS416-NHX1pr-NHX1-GFP**pRS426-NEO1pr-NEO1**pRS416-MUP1pr-MUP1-GFP**pRS416-CPSpr-GFP-CPS**pRS416-CPYpr-mCherry-PHO8**pRS415-VPH1pr-VPH1-mCherry**pRS416-ADHpr-GFP-2xFYVEEEA1**pRS425-VPS68pr-VPS68**pCMVpr-EGFP**pCMVpr-EGFP-SNX8**pET28a-6xHis-SUMO-MVP1**pET28a-6xHis-SUMO-VPS1-GFP* | (3)(3)(3)This studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyThis studyClontech, #632481This studyThis studyThis study |

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