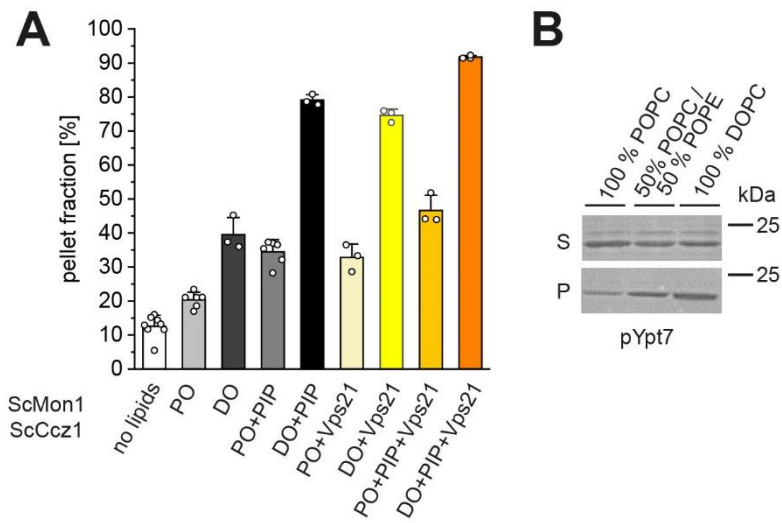
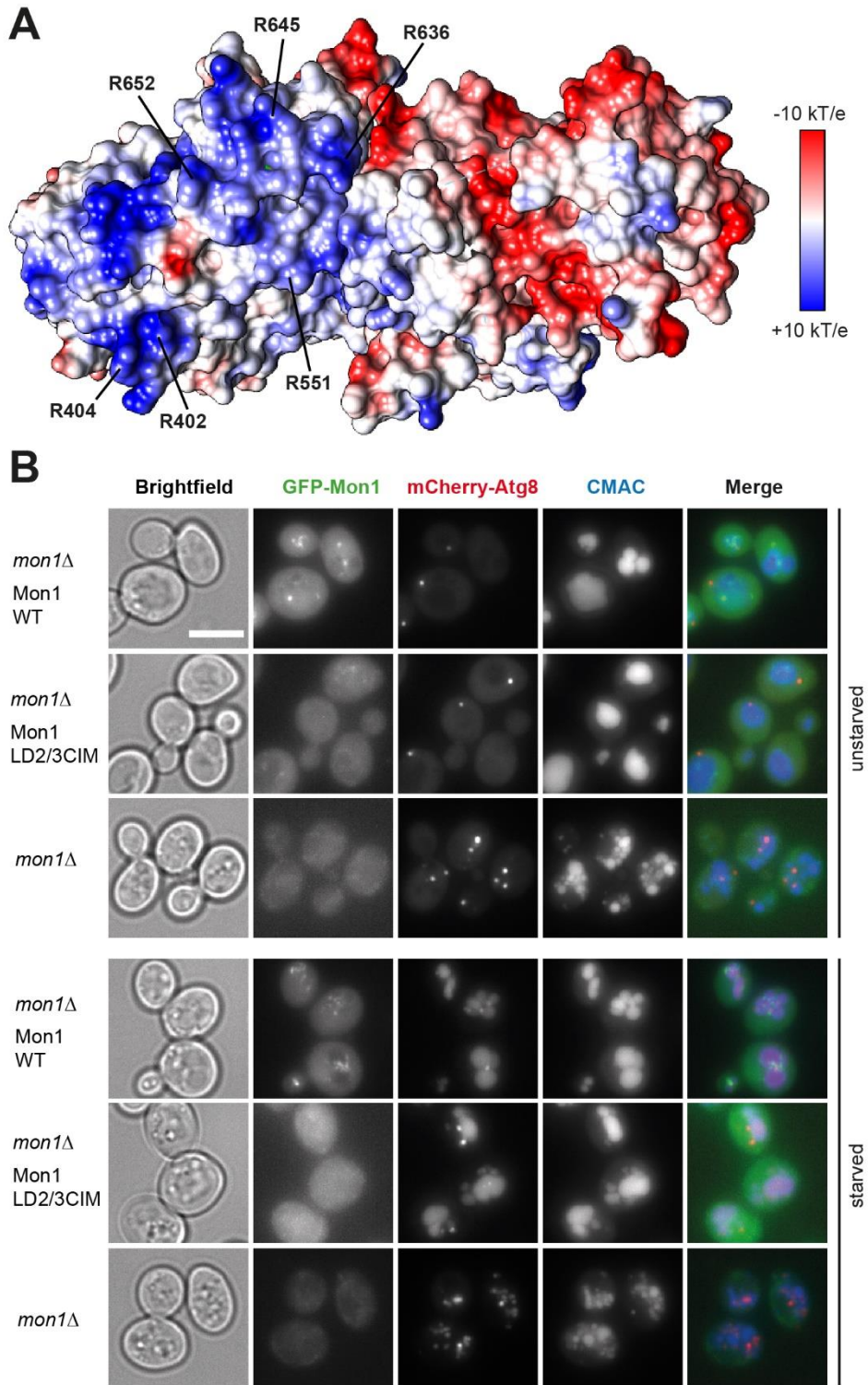


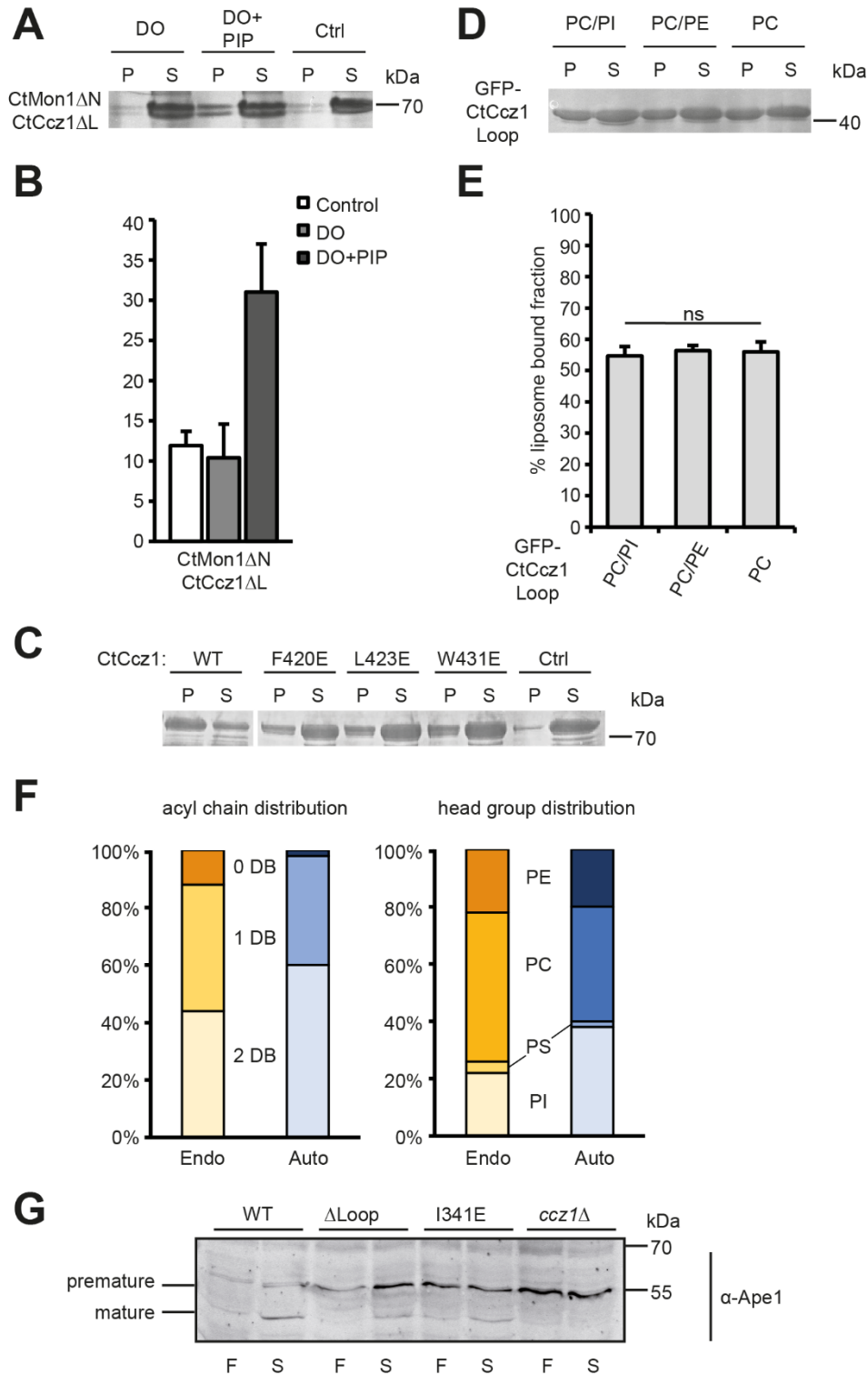
## Supporting information



**Figure S1:** (A) Quantification of binding of ScMC1 to liposomes without and with Vps21, high packing defects (DO), charged lipids (PIP), and different combinations (n=3-8). The graph summarizes data presented in Figures 2. (B) Liposome sedimentation assays with prenylated Ypt7 bound to REP (Rab escort protein) after incubation with GTP in the presence of liposomes of varying composition for 30 min.



**Figure S2:** Role of a charged patch on Mon1 in MC1 complex function. **(A)** Surface potential representation of the putative membrane binding interface of the CtMC1 complex. The locations of key basic residues of Mon1 involved in PIP binding are indicated. **(B)** Fluorescence microscopy images of *mon1* $\Delta$  yeast expressing mCherry-Atg8 and complemented with GFP-ScMon1<sup>WT</sup> or different GFP-ScMon1<sup>CIM</sup> variants. Vacuoles are stained with CMAC.



**Figure S3:** Characterization of the Ccz1 amphipathic helix. **(A)** Sedimentation assays of CtMC1 $\Delta$  (CtMon1 141-665/CtCcz1 1-360,461-796) with liposomes form DO (di-oleoyl) lipid mix with or without PIP **(B)** Quantification of A (n=4). **(C)** Reduced binding of CtCcz1 point mutants in the amphipathic helix to DO liposomes. **(D)** Binding of GFP-CtCcz1<sup>Loop</sup> to di-oleoyl phosphatidylcholine (PC) with or without 20% di-oleoyl phosphatidylinositol (PI) or di-oleoyl phosphatidylethanolamine (PE). **(E)** Quantification of D (n=3). Quantification data are

presented as mean  $\pm$ SD. **(F)** Phospholipid composition of yeast endosomes (endo) and autophagosomes (auto). DB: double bond; PI: phosphatidylinositol; PS: phosphatidylserine; PC: phosphatidylcholine; PE: phosphatidylethanolamine. **(G)** Ape1 processing assay of fed (F) and starved (S) *ccz1* $\Delta$  yeast complemented with GFP-ScCcz1<sup>WT</sup> or different GFP-ScCcz1 variants.

**Table S1:** Composition of lipid mixes used in this study

<b>Lipid mix</b>	<b>Composition</b>
PO	82 mol% 1-palmitoyl-2-oleoyl phosphatidylcholine 18 mol% 1-palmitoyl-2-oleoyl phosphatidylethanolamine
PO+PIP	79 mol% 1-palmitoyl-2-oleoyl phosphatidylcholine 18 mol% 1-palmitoyl-2-oleoyl phosphatidylethanolamine 2 mol% dipalmitoylphosphatidylinositol-3-phosphate 1 mol% dipalmitoylphosphatidylinositol-3,5-bisphosphate
DO	82 mol% dioleoyl phosphatidylcholine 18 mol% dioleoyl phosphatidylethanolamine
DO+PIP	79 mol% dioleoyl phosphatidylcholine 18 mol% dioleoyl phosphatidylethanolamine 2 mol% dipalmitoylphosphatidylinositol-3-phosphate 1 mol% dipalmitoylphosphatidylinositol-3,5-bisphosphate
PC	100 mol% 1-palmitoyl-2-oleoyl phosphatidylcholine
PC/PI	80 mol% 1-palmitoyl-2-oleoyl phosphatidylcholine 20 mol% 1-palmitoyl-2-oleoyl phosphatidylinositol
PC/PE	80 mol% 1-palmitoyl-2-oleoyl phosphatidylcholine 20 mol% 1-palmitoyl-2-oleoyl phosphatidylethanolamine
Endo	40 mol% dioleoyl phosphatidylcholine 12 mol% dipalmitoyl phosphatidylcholine 22 mol% 1-palmitoyl-2-oleoyl phosphatidylethanolamine 22 mol% 1-palmitoyl-2-oleoyl phosphatidylinositol 4 mol% dioleoyl phosphatidylserine
Auto	38 mol% dioleoyl phosphatidylcholine 2 mol% dipalmitoyl phosphatidylcholine 20 mol% dioleoyl phosphatidylethanolamine 38 mol% 1-palmitoyl-2-oleoyl phosphatidylinositol 2 mol% dioleoyl phosphatidylserine
Endo+PIP	40 mol% dioleoyl phosphatidylcholine 2 mol% 1-palmitoyl-2-oleoyl phosphatidylcholine 10 mol% dipalmitoyl phosphatidylcholine 22 mol% 1-palmitoyl-2-oleoyl phosphatidylethanolamine 20 mol% 1-palmitoyl-2-oleoyl phosphatidylinositol 4 mol% dioleoyl phosphatidylserine 2 mol% dipalmitoylphosphatidylinositol-3-phosphate
Auto+PIP	38 mol% dioleoyl phosphatidylcholine 2 mol% 1-palmitoyl-2-oleoyl phosphatidylcholine 20 mol% dioleoyl phosphatidylethanolamine 36 mol% 1-palmitoyl-2-oleoyl phosphatidylinositol 2 mol% dioleoyl phosphatidylserine 2 mol% dipalmitoylphosphatidylinositol-3-phosphate

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

<b>Plasmid</b>	<b>Construct</b>
DK65	pCDF6P-CtMon1 <sup>FL</sup> (GST-PreScission site CtMon1 1-665)
DK44	pCDF6P-CtMon1 <sup>ΔN</sup> (GST-PreScission site CtMon1 141-665)
DK123	pCDF6P-CtMon1 <sup>LD1</sup> (GST-PreScission site CtMon1 141-355)
DK1534	pCDF6P-CtMon1 <sup>LD2CIM</sup> (GST-PreScission site CtMon1 141-665; R402E, R404E, R551E)
DK1535	pCDF6P-CtMon1 <sup>LD3CIM</sup> (GST-PreScission site CtMon1 141-665; R636E, R645E, R652E)
CU3481	pRS406-GFP-ScMon1 <sup>WT</sup> (NOP1pr GFP-ScMon1 1-644)
DK1585	pRS406-GFP-ScMon1 <sup>LD2CIM</sup> (NOP1pr GFP-ScMon1 1-644; R374E, R376E)
DK1514	pRS406-GFP-ScMon1 <sup>LD3CIM</sup> (NOP1pr GFP-ScMon1 1-644; K620E, K624E, K631E)
DK1585	pRS406-GFP-ScMon1 <sup>LD2/3CIM</sup> (NOP1pr GFP-ScMon1 1-644; R374E, R376E, K620E, R624E, K631E)
DK46	pET28HS-CtCcz1 <sup>FL</sup> (6xHis-SUMO CtCcz1 1-796)
DK48	pET28HS-CtCcz1 <sup>LD1</sup> (6xHis-SUMO CtCcz1 1-249)
DK857	pET28HS CtCcz1 <sup>ΔLoop</sup> (6xHis-SUMO CtCcz1 1-360,461-796)
DK1052	pET28HS CtCcz1 <sup>ΔAH</sup> (6xHis-SUMO CtCcz1 1-407,441-796)
DK1149	pET28HS CtCcz1 <sup>L423E</sup> (6xHis-SUMO CtCcz1 1-796; L423E)
DK134	pET28GFP (6xHis-Thrombin site-GFP)
DK1287	pET28GFP-CtCcz1 <sup>Loop</sup> (6xHis-Thrombin site-GFP CtCcz1 360-460)
DK1677	pET28GFP-CtCcz1 <sup>LoopΔAH</sup> (6xHis-Thrombin site-GFP CtCcz1 360-407,441-460)
CU5525	pRS406-ScCcz1 <sup>WT</sup> -mNEON (CCZ1pr-ScCcz1 1-704 mNEON)
CU5528	pRS406-ScCcz1 <sup>ΔLoop</sup> -mNEON (CCZ1pr-ScCcz1 1-269,404-704 mNEON)
CU5527	pRS406-ScCcz1 <sup>I341E</sup> -mNEON (CCZ1pr-ScCcz1 1-704; I341E mNEON)
CU2452	pET24d-GST-TEV-Vps21
CU4527	pET24d GST-TEV-Ypt10
CU5326	pET-DUET-1-HIS-TEV-Atg8 (Gift from Sascha Martens)

**Table S3:** Recombinant proteins construct names and molecular weights.

<b>Short name</b>	<b>Construct</b>	<b>Molecular weight (kDa)</b>
CtMon1	GST-CtMon1 1-665	98.6
CtCcz1	HisSumo-CtCcz1 1-796	99.5
CtMon1-LD1	CtMon1 141-355	23.0
	GST- CtMon1 141-355	49.9
CtCcz1-LD1	CtCcz1 1-249	33.2
CtMon1 $\Delta$ N	CtMon1 141-665	57.5
	GST-CtMon1 141-665	74.4
CtMon1 $\Delta$ N LD2CIM	GST-CtMon1 141-665 R402E, R404E, R551E	74.4
CtMon1 $\Delta$ N LD3CIM	GST-CtMon1 141-665 R363E, R645E, R652E	74.4
Ccz1 $\Delta$ loop	HisSumo-CtCcz1 1-796 $\Delta$ 361-460	89.3
Ccz1 $\Delta$ AH	HisSumo-CtCcz1 1-796 $\Delta$ 408-440	96.0
CtCcz1 L423E	HisSumo-CtCcz1 1-796 L423E	99.5
GFP	eGFP	27.9
GFP-CtCcz1 Loop	eGFP-CtCcz1 360-460	38.7
GFP-CtCcz1 Loop $\Delta$ AH	eGFP-CtCcz1 360-460 $\Delta$ 408-440	35.2
ScMon1	ScMon1 1-644	73.5
ScCcz1	CaM-ScCcz1 1-704	97.7
Vps21	ScVps21 1-210	23.0
Ypt10	ScYpt10 1-199	21.8
Atg8	ScAtg8 1-117	13.6