

Supplemental Figure 1: Alignment of CHMP1 protein sequences. The accession numbers and gene identifiers for the sequences used in this analysis are provided in the Methods section.
CLUSTALW - Protein Alignment, 215 amino acids

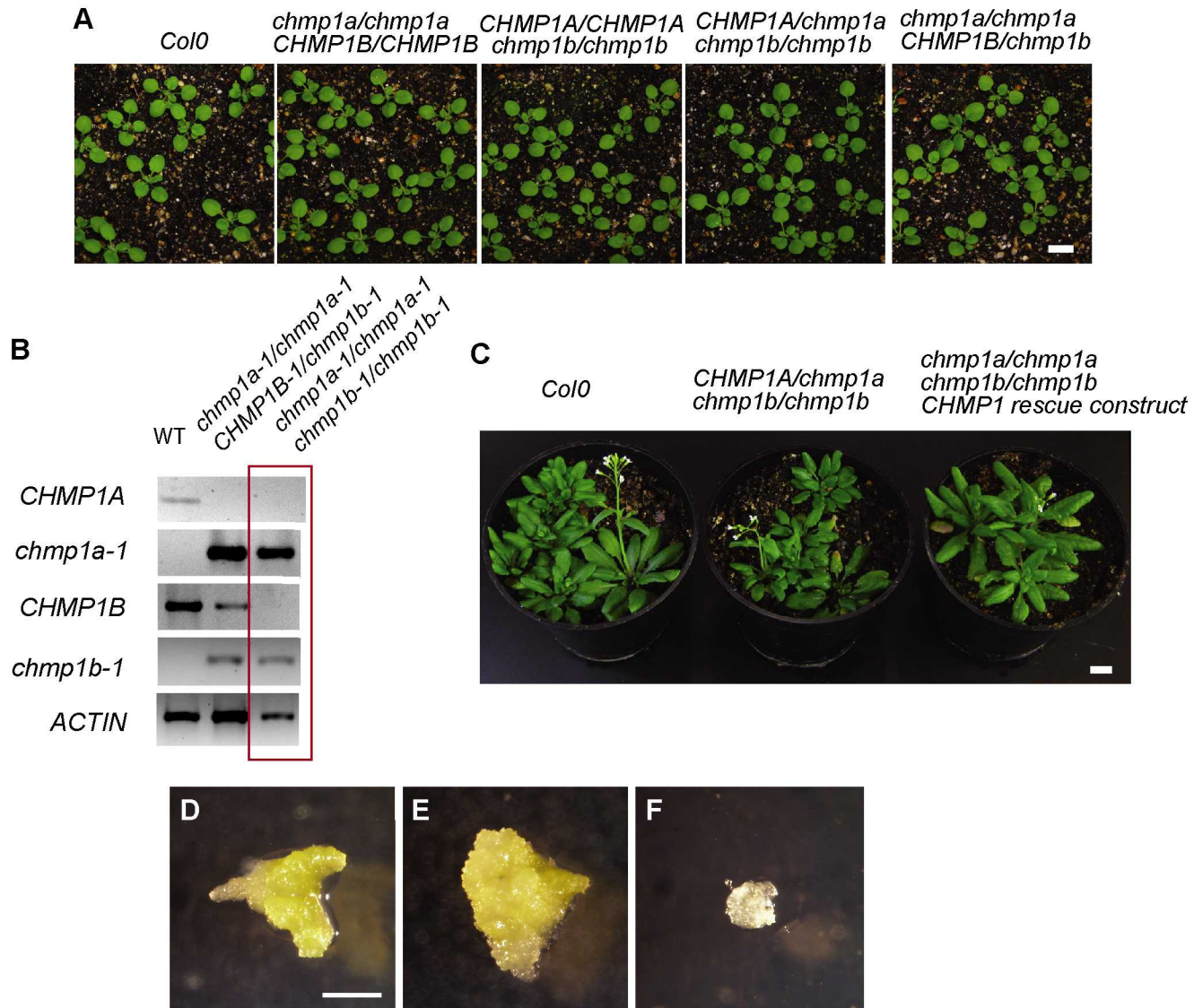
```

Gallus CHMP1A      -----M DDTLFLQKFT AKQLEKLAKK AEKDSKAEQA KVKKALQQKNVECARVYAEN AIRKKNEGLN WLRMASRVDA VASKVQTAVT MKGVTKNMAQ
Homo CHMP1A       -----M DDTLFLQKFT AKQLEKLAKK AEKDSKAEQA KVKKALLQKNVECARVYAEN AIRKKNEGVN WLRMASRVDA VASKVQTAVT MKGVTKNMAQ
Xenopus CHMP1A    -----M DDTLFLQKFT AKQLEKLAKK AEKDSNTEQA KVKKALQQKNVEVARVYAEN AIRKKNEGLN WLRMASRVDA VASKVQTAVT MKGVTKNMAQ
Danio CHMP1A      -----M DDTLFLQKFT SKQLERLAKK AEKDSKSEQA KVKKALQQKNVECARVYAEN AIRKKNEGLN WLRMASRVDA VASKVQTALT MKGVAKNMTQ
Strongyloc. CHMP1A ----- ---MFQLKFS SKQLERYAKK AEKEQKVQSG KVKKALEQKNPEGARIYAEN CIRKKNESLN FLRMSARIDA VSSRIKSAMV MKQVSKNMGQ
Gallus CHMP1B     -----MSNM EKHLFNLKFA AKELNRNSKR CDKEEKAEKA KIKKAIQKGNMEVARIHAEN AIRQKNQAVN FLRMSARVDA VAARVQTAVT MGKVTKSMAG
Homo CHMP1B       -----M EKHLFNLKFA AKELSRSAKK CDKEEKAEKA KIKKAIQKGNMEVARIHAEN AIRQKNQAVN FLRMSARVDA VAARVQTAVT MGKVTKSMAG
Xenopus CHMP1B    -----MSSM EKHLFNLKFA AKELNRNAKK CEKEEKTEKA KIKKAIQKGNTEIARIHAEN AIRQKNQGIN FLRMSARVDA VAARVQTAVT MGKVTKSMAG
Danio CHMP1B      -----MSSM EKHLFNLKFA AKELQRNSKK CDKEEKAEKV KVKKAIQKGNMEVARIHAEN AIRQKNQSVN FLRMSARVDA VAARVQTAVT MNQVTKSMAG
Strongyloc. CHMP1A -----MSSSL EKHLFNLKFA AKQMNRESKK CEKEEKAECT KLKKAIQKGNVEGARIHAEN AIRNKSQALN FLRMSARVDG VASRVQSAVA MKKVTSSMSG
Anopheles CHMP1  -----MSAM EKHLFNLKFA VKELERNAKK CEKEEKAAIL KTKKAIQKGNTEVARIHAEN AIRQKSQSLN YLRMSARVDA VASRVQTALT TRNVTNSMAG
Drosophila CHMP1 -----MSTSSM EKHLFNLKFA VKELERNNAKK CEKEEKLEKA KAKKAIQKGNMDVARIHAEN AIRQKNQAVN YLRMSARVDA VASRVQSALT TRKVTGSMAG
Caenorhabd. CHMP1 MGAGESSMAL EKHLFDLKFA AKQLEKNAQR CEKDEKVEKD KLTAAIKKGNKEVAQVHAEN AIRKKNEAVN YIKMAARIDA VAARVQTAAAT QKRVTASMSG
Ustilago DID2     -----MSGL EKSLFQKFT AKSLQRQARK ATKDETAEKA KLKALAQQGNTGARIYASN AIRKKNESLN LLRLGSRIDA VASRVETAVT MRQVSGSMAS
Laccaria DID2     -----MSNL EKTFLQKFT AKSLNRQAKK AOKDENSEKT RLKKALQQGNNDGARIYASN AIRKKSEALN LLRLSSRIDA VASRVETAVT MRQVTGNMTS
Candida DID2      -MSRNPAAAGL ENTFLQKFT SKQLQKQAAK ASKEEKQETN KLKALNE-NEEIARIYASN AIRKKNERLQ LLKLASRVDS VASRVQTAVT MRQVSSMAQ
Saccharomyces DID2 -MSRNSAAGL ENTFLQKFT SKQLQKQANK ASKEEKQETN KLKRALNE-NEDISRIYASN AIRKKNERLQ LLKLASRVDS VASRVQTAVT MRQVSASMGQ
Pichia DID2       -----MAGL EQSLFQKFT AKQLNRQASK AAKEELQEKA KIKKALQGNNDIAQLYAQN AIRKSNERVN LLRLASRIDA VASRVQTAVT MKSVTGNMTQ
Schizosach. DID2  -----MS-- ----LSMNFF TAHLS----- -IAIAITKGNSEIARIYASN AIRKQESLN LLKLSSRIDA VSSRLQTAVT MRAVSGNMAG
Physcom. CHMP1C   MWGRSGQDKL MDQIFQKFT SKSLVRAAKK CEKDEKSEKL KVKKAIEKGNMDGARIYAQN AIRKHNEQLN YLRLSSRLDA VVAQLGTQSK LQTVAKSMAG
Physcom. CHMP1D   MWGRSSQDKL MDQIFQKFT SKSLVRAAKK CEKEEKGEKL KVKKAIEKGNMDGARIYAQN AIRKHNEQLN YLRLSSRLDA VVARLGTQSK MQTISKSMAG
Physcom. CHMP1B   -MGGGAQEKL LDQIFQKFT SKSLVREAKK CEKDEKAEKL KVKKAMEKNNMDGARIYAQN AIRKHNEHLN YLRLSSRLDA VVAQLNTQSK MQTISKSMGS
Physcom. CHMP1A   -MGGG-QEKL LNQIFQKFT AKTLVRQAKK CESEEKAEKL KVKKAIEKGNMDGARIYAQN AIRKHNEQLN YLRLASRLDA VVSRLGTQSK MQTITKSMGS
Oryza CHMP1       -MGN--PEKL MTQIFDLKFT SKSLQRQARK CEKEEKEQKL KVKKAIEKGNMDGARIYAEN AIRKRTEHMN YLRLASRLDA VVARLDTQAK MQVIGKSMAN
Zea SAL1          -MGN--PEKL MNQIFDLKFT SKSLQRQARK CEKEEKEQKL KVKKAIEKGNMDGARIYAEN AIRKRTEHMN YLRLASRLDA VVARLDTQAK MQVIGKSMQS
Arabidopsis CHMP1B -MGN--TDKL MNQIFELKFT SKSLQRQARK CEKEERSEKL KVKKAIEKGNMDGARIYAEN AIRKRSEQMN YLRLSSRLDA VVARLDTQAK MATITKSMTN
Arabidopsis CHMP1A -MGN--TDKL MNQIFDLKFT SKSLQRQSRK CEKEEKAEKL KVKKAIEKGNMDGARIYAEN AIRKRSEQMN YLRLASRLDA VVARLDTQAK MTTITKSMTN
Nicotiana CHMP1   -MGN--TEKL MNQIMELKFT SKSLQRQARK CEKDEKSEKL KVKKAIEKGNMDGARIYAEN AIRKRSEQMN YLRLSSRLDA VVARLDTQAK MTTISKSMGN
Chlamyd. CHMP1    -MGG---EKL LDQIFNLKFT AKQLARSAVK CEKEEKAEKL KVKKAIEKGNMEGAKIYAQN AIRKKNEQLN YMKLASRLDA TVSRLETQAK MQMVQKNMAG
Trypanosoma CHMP1 -MS---VNQL QDLHFQKLYV VKQFNKNSTR CEKEQKAELG KCKKAMAKGNMDIARIFAEN SIRKRNESLS HLRLASRMDA VVSRLDTAIAK MNKVTRGMSQ
Leishmania CHMP1  -MS---VEKL YDIQFQKFT AKQFLKNASR CEKEQKQEMN KCKQAMEKNNMEGARIYAQN SIRKKNEALN HLRLSARMDA VVARLDTAIAK MKMVTKNMGQ

```

Gallus CHMP1A	VTKALDKALS	SMDLQKVS	AV	MDKFEQQVQ	N	LDVHTSV	MED	SMSSATTLTTPQE	QVDSLIV	QIAEENGL	EI	MDQLNQL	P	EG	AS----	AVG-	-ESSMRSQE-	
Homo CHMP1A	VTKALDKALS	TMDLQKVSS	V	MDRFEQQVQ	N	LDVHTSV	MED	SMSSATTLTTPQE	QVDSLIM	QIAEENGL	EV	LDQLSQL	P	EG	AS----	AVG-	-ESSVRSQE-	
Xenopus CHMP1A	VTKALDKALS	SMDLQKVS	AV	MDKFDQQVQ	N	LDVHTSV	MED	SMSSAMTLTTPQE	QVDNLIV	QIAEENGL	EV	MDQLNQL	P	QG	AS----	SVG-	-ESSTRTQE-	
Danio CHMP1A	VTKALDKALS	SMDLQKVS	AV	MDKFETQVQ	N	LDVHTSV	MED	SMSSATTLSTPQQ	QVDDLLI	QIAEESGL	EV	EDQLSQL	P	AG	AS----	ALG-	-ETSARAQEK	
Strongyloc. CHMP1A	VVKGLDKALQ	SMDLQKIS	GI	MEKFESQF	ED	LDVHTQV	LEG	SMGAATTLSTPQ	DQVDQLI	QVAEENGL	EM	ISDLAAAP	P	MA	GTGTLAS	ST-	-ASSSRTMVE	
Gallus CHMP1B	VVKSMDATLK	SMNLEKIS	SAL	MDKFEHQF	FET	LDVQTQQ	MEN	TMSNTTTLTTPQ	NQVDMLLQ	EMADEAGL	DL	NMELPQ	G	---	---	QTGSV	---	TS-VASAE
Homo CHMP1B	VVKSMDATLK	TMNLEKIS	SAL	MDKFEHQF	FET	LDVQTQQ	MED	TMSSTTTLTTPQ	NQVDMLLQ	EMADEAGL	DL	NMELPQ	G	---	---	QTGSV	---	TS-VASAE
Xenopus CHMP1B	VVKSMDATLK	SMNLEKIS	SAL	MDKFEHQF	FET	LDVQTQQ	MED	TMSNTTTLTTPQ	NQVDNLLH	EMADEAGL	DL	SMELPQ	G	---	---	QTGSV	---	TS-VASTE
Danio CHMP1B	VVKGMDATLK	SMNLEKIS	GL	MEKFERQF	FET	LDVQTAQ	MED	SMSSTTTLTTPQ	GQVDTLMM	EMADEAGL	DL	NMELPQ	G	---	---	QTGSV	---	TS-VASAE
Strongyloc. CHMP1A	VVKAMDSAMK	SMNLEKIS	SAL	MEKFEKQF	FED	MDVQTQC	MED	TMSASTTLTTPQ	SQVDSLMS	QVADEAGL	EL	NMELPSS	---	---	---	QTGSL	---	QS-TASAE
Anopheles CHMP1	VVKAMDAAMK	GMNLEKIS	GL	MDKFESQF	FED	LDVQSSY	MEN	TMSQTTTTAVP	QNDVESLMQ	RVADEAGL	EL	NMELPSG	P	---	---	SSIAI	---	ASTQASTE
Drosophila CHMP1	VVKAMDAAMK	GMNLEKIS	SSL	MEKFESQF	FED	LDVQSSV	MEG	TMSDVTVTSVP	QGDVDNLLQ	QVADEAGL	EL	NMELPSG	V	---	---	QSQSV	---	ASTAVSQE
Caenorhabd. CHMP1	VVKAMESAMK	SMNLEKIV	QQL	MDRFERDF	FED	LDVTTKT	MEK	TMDGTTVLNAP	KSQVDALIA	EAADKAGI	EL	NQELPSN	---	---	---	VPTAL	---	TGTQAVSE
Ustilago DID2	VVKGMDKAME	SMNLERMS	VM	MDKFETQF	FED	MDVQTSY	MEG	TIGATTAQSM	PQDQVDLLM	QVADENGI	EI	NHKLGEGL	L	LL	EGKVADL	APK	VPDAKVKDKE	
Laccaria DID2	VVKGMDKAME	SMNLERIS	MV	MDKFENQF	TD	LDVQTSI	MED	TMSATTAISTP	QDQIDQLLR	QTAEENIE	L	QHDLASK	DLN	A--	VADLN--	-SPNKVRE-E		
Candida DID2	VCKGMDKALQ	NMNLQQIT	IMI	MEKFEQQF	FED	LDTSVNV	YED	MGVASDAVLV	DNDKVDEL	MG	KVADENGM	EL	KQSARLE	N--	---	IPDIKQ-	-KET-VDDEK	
Saccharomyces DID2	VCKGMDKALQ	NMNLQQIT	IMI	MDKFEQQF	FED	LDTSVNV	YED	MGVNSDAMLV	DNDKVDEL	MS	KVADENGM	EL	KQSAKLD	N--	---	VPEIKA-	-KEVNVVDEK	
Pichia DID2	VIRGMDKALQ	TMNLERIS	LV	MDKFENQF	FED	LDASTNY	YET	ATNNVNALTTP	QEQVDELMS	QVADEAGI	EM	KQGLNET	K	---	---	VDIATP-	-PVSNMTEEK	
Schizosach. DID2	VVRGMDRAMK	TMNLEMIS	QV	MDKFEAQF	DD	VNVQTYG	MNK	AMGSVTAVDTP	QEVDVLLM	TVADEAGL	EF	NQNMNNNL	S-	---	---	VPAAS--	-VPTPAAPVE	
Physcom. CHMP1C	IVKSILDSALA	VGNMEKIS	QST	MDQFEKTF	FMN	MEVQSEFI	ET	AMAGSTSLSTP	EDDVNSLLH	QVADDYGL	EV	SVNLP	-----	---	---	QAGATAIP-	-VKETPTVNV	
Physcom. CHMP1D	IVKSILDSALA	VGNMEKIS	QST	MDQFEKTF	VN	MEVQSEFI	ET	AMAGSTSLSTP	EDVNSLLH	QVADDYGL	EV	SVNLP	-----	---	---	QAGATAMP-	-VKETATVNA	
Physcom. CHMP1B	IVKALDSSLA	VGNMEKIS	QST	MDQFEKTF	VN	MEVQAEFV	EN	AMAGSTSLSTP	EDVNSLIN	QVADDYGL	EV	SLGLP	-----	---	---	QAGATSVP-	-AKESSTESA	
Physcom. CHMP1A	IVKALDSALA	VGNMEKIS	QST	MDQFEKTF	VN	MEVQSEFV	ES	AMAGSTSLSTP	EDVNSLMH	QVADDYGL	EV	SVGLP	-----	---	---	QA-ASHMP-	-LGDAESTVS	
Oryza CHMP1	IVKSILDSALA	TGNLQKMS	SET	MDNFERQF	VN	MEVQAEFM	EG	AMAGSTSLSTP	ETEVNSLMQ	QVADDYGL	EV	SVGLP	-----	---	---	QAAAHAI	PA -AKEKEKAVD	
Zea SAL1	IVKSILDSSLA	TGNLQKMS	SET	MDNFERQF	VN	MEVQAEFM	EG	AMAGSTSLSTP	ETEVNSLMQ	QVADDYGL	EV	SVGLP	-----	---	---	QAAAHAI	PA -AKDKEK-VD	
Arabidopsis CHMP1B	IVKSLESSLT	TGNLQKMS	SET	MDSFEKQF	VN	MEVQAEFM	DN	AMAGSTSLSTP	EGEVNSLMQ	QVADDYGL	EV	SVGLP	-----	---	---	QPAGHAI	P -TKTEEK-VE	
Arabidopsis CHMP1A	IVKSLESSLA	TGNLQKMS	SET	MDSFEKQF	VN	MEVQAEFM	EN	AMAGSTSLSTP	EGEVNSLMQ	QVADDYGL	EV	SVGLP	-----	---	---	QPAGHAI	P -TKTEEK-VD	
Nicotiana CHMP1	IVKSLESSLA	TGNLQKMS	SET	MDKFEQQF	VN	MEVQAEFM	ES	SMAGSTSLSTP	EDQVNSLMH	QVADDYGL	EV	SVGLP	-----	---	---	QAAGHAI	P -TKDSEK-VD	
Chlamyd. CHMP1	IVKSLEKAMA	SNNLETIAN	T	MTQFEKQF	FEN	LDLQTVV	DD	VMGAQASLSTP	EDVVSALVA	QVAEEHGL	EL	AVGMP	-----	---	---	HAAAGTAR-	----PAAAKA	
Trypanosoma CHMP1	MVHGMDKVQ	SMNPEKISE	L	MEKFEKQF	FET	MDVASEY	MET	AIGQTTSTSM	PEDEVSLLLL	QVAEEEGL	AV	KEELFNK	KAK-	---	---	LPQQQP	VAP -EATKLAEPD	
Leishmania CHMP1	MVKGMDKVLQ	SMDPATIS	R	MDTFEQF	FET	MDVTSAY	MEG	AIGQSTAVTTP	EDVNNLMS	QVADEHGL	DI	REQLNDNL	Q-	---	---	IKNTNLTAQ	-KAAE-KQVE	

Gallus CHMP1A	-DQLSRRLAA	LRN--
Homo CHMP1A	-DQLSRRLAA	LRN--
Xenopus CHMP1A	-DQLSRRLAS	LRN--
Danio CHMP1A	EDQLSRRLAA	LRN--
Strongyloc. CHMP1A	EDRLTQRLQA	LRN--
Gallus CHMP1B	QDELSQRLAR	LRDQV
Homo CHMP1B	QDELSQRLAR	LRDQV
Xenopus CHMP1B	QDELSQRLAR	LRDQV
Danio CHMP1B	QDELSQRLAK	LRDQV
Strongyloc. CHMP1A	QDELSARLAE	LRKM-
Anopheles CHMP1	QDELTARLAR	LRQAE
Drosophila CHMP1	QDELTQRLAR	LRQAE
Caenorhabd. CHMP1	DKDLTERLAA	LRNM-
Ustilago DID2	DDALAERLRA	LRPAT
Laccaria DID2	DDKLAERLRA	LRPAT
Candida DID2	EDKLAERLRA	LRG--
Saccharomyces DID2	EDKLAQRLRA	LRG--
Pichia DID2	EDKLAERLRA	LRS--
Schizosach. DID2	DDNLQERLRA	LRS--
Physcom. CHMP1C	EDDLSRRLAE	LKSRG
Physcom. CHMP1D	EDDLSRRLAE	LKNRN
Physcom. CHMP1B	EDDLSRRLAE	LRNRG
Physcom. CHMP1A	EDDLSRRLAE	LKNRS
Oryza CHMP1	EDDLSRRLAE	LKARG
Zea SAL1	EDDLSRRLAE	LKARG
Arabidopsis CHMP1B	EDDLTRRLAE	LKARG
Arabidopsis CHMP1A	EDDLSRRLAE	LKARG
Nicotiana CHMP1	EDDLTRRLAE	LKARG
Chlamyd. CHMP1	EDDLTARLAE	LRGR-
Trypanosoma CHMP1	LDELSARLDM	LRGK-
Leishmania CHMP1	EDDLEAKFAQ	LRGR-



Supplemental Figure 2: Phenotype of wild type, *chmp1* mutant, and rescued *chmp1a chmp1b* mutant seedlings. PCR-genotyping of embryos and proliferating calli induced from WT and mutant embryos.

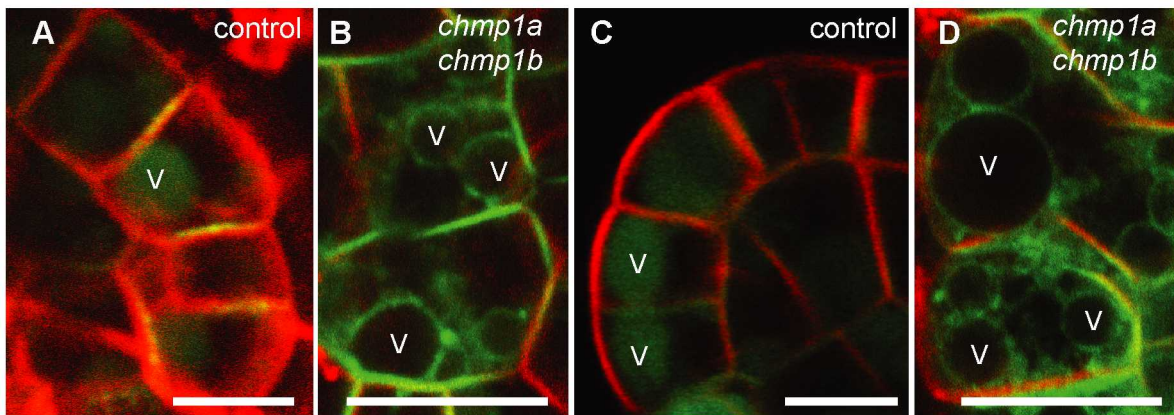
(A) Seedlings from WT (*Col-0*), single *chmp1a/chmp1a*, single *chmp1b/chmp1b*, *CHMP1A/chmp1a chmp1b/chmp1b*, and *chmp1a/chmp1a CHMP1B/chmp1b* mutants. Note that all mutant seedlings are indistinguishable from WT ones.

(B) PCR-genotyping of embryos from plants segregating the *chmp1a* and *chmp1b* mutant alleles.

(C) WT (*Col-0*), *CHMP1A/chmp1a chmp1b/chmp1b*, and double *chmp1a chmp1b* mutant plants rescued with a WT *CHMP1B* gene construct.

(D)-(F) Calli grown from excised WT **(D)**, WT-looking (containing two or more *chmp1* mutant alleles) **(E)** and *chmp1a chmp1b* mutant embryo **(F)**.

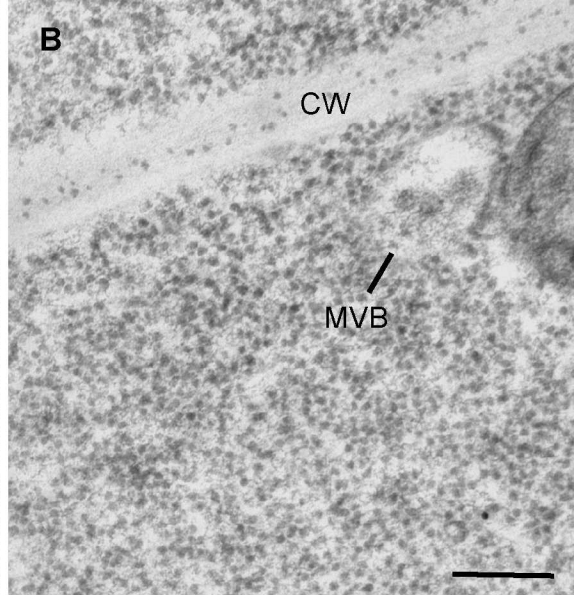
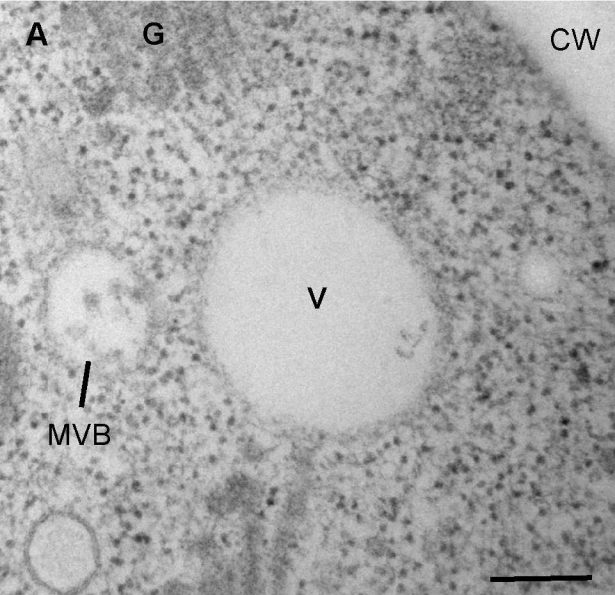
Bars in **(A)** and **(B)** = 10 mm; **(D)-(F)** = 1 mm



24h dark

48h dark

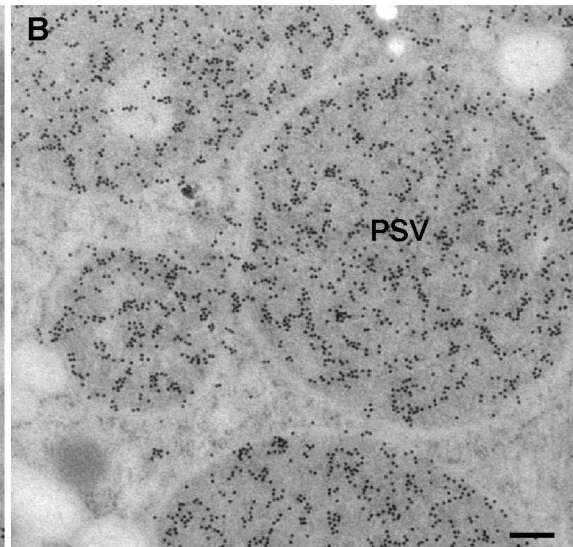
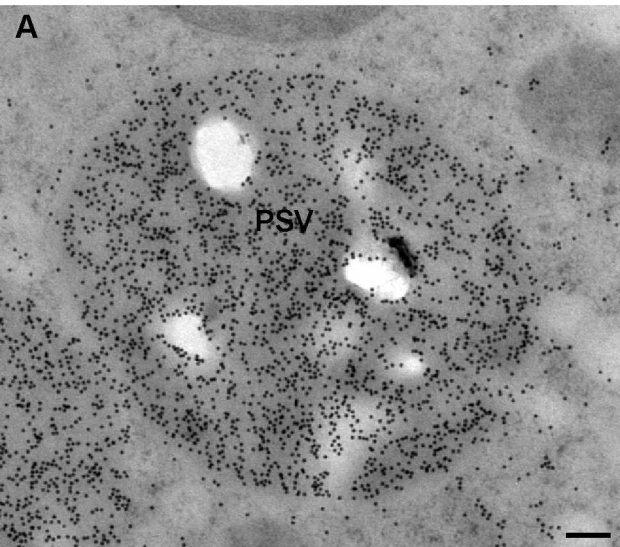
Supplemental Figure 3: Localization of PIN1-GFP in control and mutant embryos after dark treatment. Embryos were stained with FM4-64 to visualize cell outlines. Note the accumulation of PIN1-GFP in the vacuolar lumen in control embryo kept in the dark for 24h (A) and 48h (C). In *chmp1a chmp1b* mutant embryos dissected from the same plants (B) and (D), PIN1-GFP is only detected in the vacuolar membrane but not in the vacuolar lumen. V, vacuole. Bars = 10 μ m.



Supplemental Figure 4: Control labeling using anti-GFP antibodies on WT heart stage embryos. CW, cell wall; G, Golgi; V, vacuole. Bars = 200 nm

WT

chmp1a chmp1b



Supplemental Figure 5: Immunolabeling of 2S albumins in PSVs of WT (**A**) and *chmp1a chmp1b* mutant (**B**) embryos. Bars = 100 nm.