

Fig. S1. Dynamics of clathrin and AP2 (related to Fig. 2). (A) TIR-FM images of mRFP-clc and GFP-Ap2A1 showing examples of puncta localized in rings most likely around contractile vacuole bladders. Scale bar = $1\mu\text{m}$. (B-C) Box charts showing the durations of XFP-clc puncta (B) or GFP-Ap2A1 puncta (C) in the different cell lines. Crosses = maximum and minimum values; solid square = mean; error bars = s.d.; box = 25, 50 and 75 percentile. (D-E) Histograms of the duration on the surface for XFP-clc puncta (D) or GFP-Ap2A1 puncta (E) in the different cell lines. The mean duration \pm s.e.m and the number of puncta measured are given.

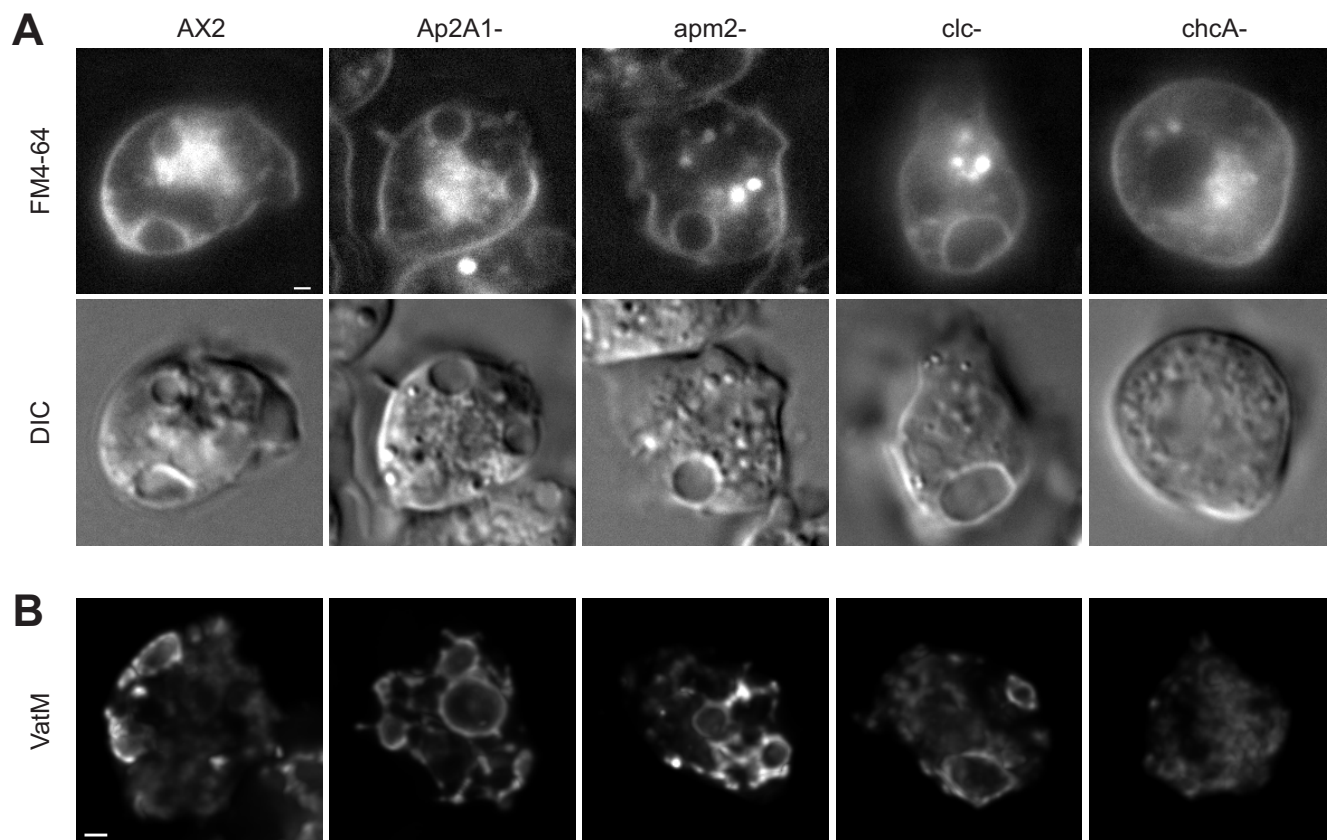


Fig. S2. Localization of contractile vacuole markers in AP2 and clathrin knockouts (related to Fig. 6). (A) Widefield microscopy images of FM4-64 labeling in AX2, *Ap2A1-*, *apm2-*, *clc-* and *chcA-* cells and corresponding DIC images. (B) Maximum intensity projection of VatM immunostaining in AX2, *Ap2A1-*, *apm2-*, *clc-* and *chcA-* cells. Scale bars = 1 μ m.

Table S1. AP genes in *Dictyostelium*

Name	Gene	Dictybase Gene ID	Percent identity with human (name and accession number)	Divergence	Experimental evidence
AP-1 γ subunit	ap1g1	DDB_G0281957	44.4 (AP-1 gamma-1 isoform b, NP_001119)	84.2	(Lefkir et al., 2003)
AP-1 β subunit	ap1b1	DDB_G0279141	52.2 (AP-1 beta-1 isoform b, NP_663782)	68.4	(Sosa et al., 2012)
AP-1 μ subunit	apm1	DDB_G0289247	67.4 (AP-1 mu-1 isoform 2, NP_115882)	38.4	(Lefkir et al., 2003)
AP-1 σ 1 subunit	ap1s1	DDB_G0279359	44.9 (AP-1 sigma-2, NP_003907)	89.1	
AP-1 σ 2 subunit	ap1s2	DDB_G0295711	57.8 (AP-1 sigma-2, NP_003907)	53.1	
AP-2 α subunit	ap2a1-1 ap2a1-2	DDB_G0273439 DDB_G0273501	38.3 (AP-2 alpha-2 isoform 2, NP_036437)	100.3	This study
AP-2 β subunit	ap1b1	DDB_G0279141	51.9 (AP-2 beta isoform b, NP_001273)	70.0	This study, (Sosa et al., 2012)
AP-2 μ subunit	apm2	DDB_G0277139	50.8 (AP-2 mu isoform a, NP_004059)	64.0	This study, (Sosa et al., 2012)
AP-2 σ subunit	ap2s1	DDB_G0289721	62.0 (AP-2 sigma isoform AP17, NP_004060)	52.6	This study
AP-3 δ subunit	ap3d1	DDB_G0279537	35.9 (AP-3 delta-1 isoform 2, NP_003929)	102.5	(Bennett et al., 2008)
AP-3 β subunit	ap3b-1 ap3b-2	DDB_G0272578 DDB_G0274003	31.6 (AP-3 beta-2, NP_004635)	113.5	(Bennett et al., 2008)
AP-3 μ subunit	apm3	DDB_G0277901	47.4 (AP-3 mu-1, NP_036227)	74.6	(Bennett et al., 2008)
AP-3 σ subunit	ap3s1	DDB_G0275405	64.9 (AP-3 sigma-1, NP_001275)	39.5	
AP-4 ϵ subunit	ap4e1	DDB_G0280427	21.6 (AP-4 epsilon-1, NP_031373)	170.5	
AP-4 β subunit	ap4b1	DDB_G0283319	24.0 (AP-4 beta-1, NP_006585)	157.9	
AP-4 μ subunit	apm4	DDB_G0276945	21.6 (AP-4 mu-1, NP_004713)	148.2	
AP-4	ap4s1	DDB_G0284905	55.4	59.3	

σ subunit		(AP-4 sigma-1 isoform 2, NP_001121598)		
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The genes annotated as belonging to AP complexes in *Dictyostelium* (www.dictybase.org) are shown. Homologous human proteins were identified by searching GenBank using BLASTP (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) and the *Dictyostelium* AP complex protein sequences. The top hit was used to calculate percent identity and phylogenetic divergence using the Clustal V method and MegAlign 9 software.

Table S2. Summary of LC-MS/MS Results (related to Fig. 1).

Protein	DDB ID	AX2 + GFP-Ap2A1			<i>apm2</i> - + GFP-Ap2A1		
		Number of unique peptides (total # peptides)	Percent coverage	Protein score	Number of unique peptides (total # peptides)	Percent coverage	Protein score
ap1b1 (β subunit)	G0279141	35 (103)	41%	3548	8 (11)	10%	116
apm2 (μ subunit)	G0277139	13 (71)	31%	2453	N/A	N/A	N/A
ap2s1 (σ subunit)	G0289721	7 (36)	44%	750	6 (15)	30%	136
ap1g1 (γ subunit)	G0281957	N/A	N/A	N/A	3 (5)	3%	76
eps15	G0287325	34 (118)	41%	4728	22 (62)	37%	1867

Summary of LC-MS/MS data for proteins identified from the gels shown in Fig. 1B,C.

Table S3. Kolmogorov-Smirnov (KS) test *P* values (related to Fig. 2 and Fig. S1).

KS test was performed to ask whether the distribution of the durations of cell surface puncta measured in different cell lines are drawn from the same distribution.

Data Set 1	Data Set 2	KS <i>P</i> value
GFP-clc (<i>clc-</i>)	GFP-Ap2A1 (AX2)	0.04594
GFP-clc minus 10s (<i>clc-</i>)	GFP-Ap2A1 (AX2)	0.06564
mRFP-clc (<i>clc-</i>)	GFP-Ap2A1 (AX2)	2.15915×10^{-8}
mRFP-clc minus 10s (<i>clc-</i>)	GFP-Ap2A1 (AX2)	0.0747
mRFP-clc (<i>clc-</i>)	mRFP-clc (<i>Ap2A1-</i>)	0.04744
mRFP-clc (<i>clc-</i>)	mRFP-clc (<i>apm2-</i>)	0.04795
GFP-Ap2A1 (AX2)	GFP-Ap2A1 (<i>Ap2A1-</i>)	0.00937
GFP-Ap2A1 (AX2)	GFP-Ap2A1 (<i>apm2-</i>)	2.22786×10^{-5}
GFP-Ap2A1 (AX2)	GFP-Ap2A1 (<i>clc-</i>)	0.01449
GFP-Ap2A1 (AX2)	GFP-Ap2A1 (<i>chcA-</i>)	3.80114×10^{-32}

Table S4. List of strains and expression plasmids used.

Strain Name	Expression Plasmid
AX2/[A15]:GFP:Ap2A1	339-3 (Bsn)
Ap2A1-/[A15]:GFP:Ap2A1	339-3 (Bsn)
apm2-/[A15]:GFP:Ap2A1	pDM304 (G418)
clc-/[A15]:GFP:Ap2A1	pDM304 (G418)
chcA-/[A15]:GFP:Ap2A1	pDM304 (G418)
AX2/[A15]:mRFP:clc	pDM304 (G418)
Ap2A1-/[A15]: mRFP:clc	pDM304 (G418)
apm2-/[A15]: mRFP:clc	pDM304 (G418)
clc-/[A15]: mRFP:clc	pDM304 (G418)
chcA-/[A15]: mRFP:clc	pDM304 (G418)
AX2/[A15]:GFP:Ap2A1	339-3 (Bsn)
[A15]:mRFP:clc	pDM304 (G418)
AX2/[A15]:GFP:Ap2A1	339-3 (Bsn)
[A15]:clc:mRFP	pDM304 (G418)
AX2/[A15]:dajumin:GFP	pDEXRH (G418)
[A15]:mRFP:clc	pDM326 (Bsn)
AX2/[A15]:dajumin:GFP	pDEXRH (G418)
[A15]:mRFP:Ap2A1	pDM358 (Hgr)
Ap2A1-/[A15]: dajumin:GFP	pDM326 (Bsn)
apm2-/[A15]: dajumin:GFP	pDEXRH (G418)
clc-/[A15]: dajumin:GFP	pDEXRH (G418)
chcA-/[A15]: dajumin:GFP	pDEXRH (G418)

Strain shows the genetic background and the promoter used to drive expression. Plasmids used for expression are listed along with the antibiotic resistance used for selection. Bsn = blasticidin, G418 = geneticin, Hgr = hygromycin.