A

```
Tg      ----MVLFKDLNKSCADLLTKGYPH    21
Sc      --MSPPVYSDISRNINDLLNKDFYH    23
Hs      -MAVPPTYADLGKSARDVFTKGYGF    24
At      MVKGPGLYTEIGKKARDLLYRDYQG    25
              : ::.:.  *::  :.:
```

B

Species	Gene	Length (aa)	Homolgy to *T. gondii* VDAC (% identity)
T. gondii	TGME49_263300	290	n/a
H. hammondi	HHA_263300	290	99
N. caninum	NCLIV_024630	290	96
E. tenella	ETH_00023750	292	47
V. brassicaformis	Vbra_784	281	39
P. falciparum	PF3D7_1432100	289	29
T. brucei brucei	Tb927.2.2510	270	37
C. cayetanensis	cyc_02478	348	50
H. sapiens	VDAC2	294	33
A. thaliana	VDAC5	226	25
S. cervasiase	POR1	283	21
H. sapiens	VDAC3	283	26
H. sapiens	VDAC1	283	24

Fig. S1. Phylogeneti.alysis of VDAC from *T. gondii*

A) VDAC from *T. gondii* (Tg) contains glycine and lysine residues at positions 16 and 20 respectively which are conserved between human (Hs), yeast (Sc) and plants (At, D21 only). **B)** Table showing the gene ID, length (in amino acids) and percentage identity to *T. gondii* of VDACs from selected species.

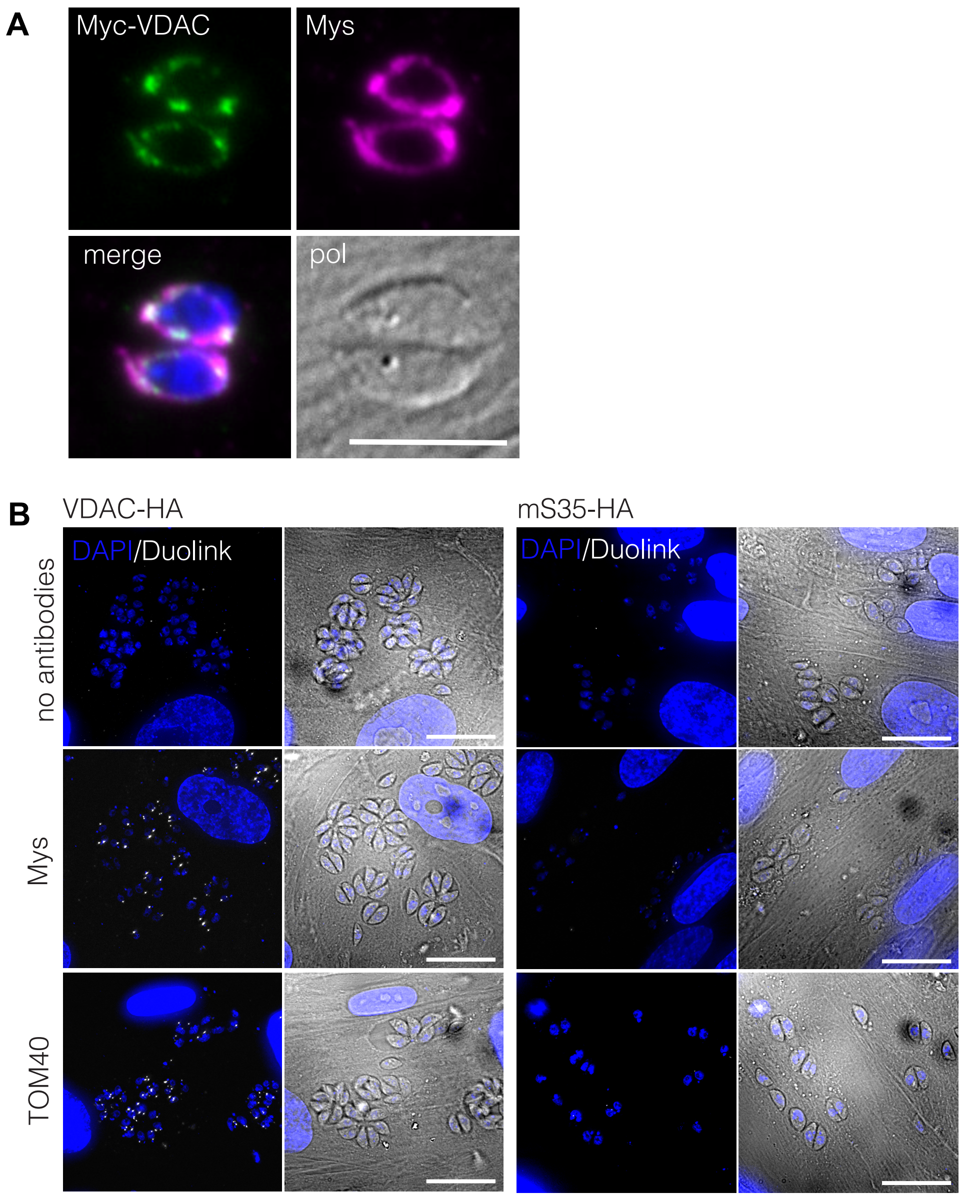

Figure S2. VDAC localises to the outer membrane of the mitochondrion
A) Overexpression of Myc-VDAC (green) co-localized with anti-Mys, a mitochondrial membrane marker. Scale bar 5 µm. **B)** Larger scale representative images of the DuoLink PLA, showing many vacuoles per field, demonstrating that when seen, PLA signal is seen in most/all cells. Scale bar 20 µm

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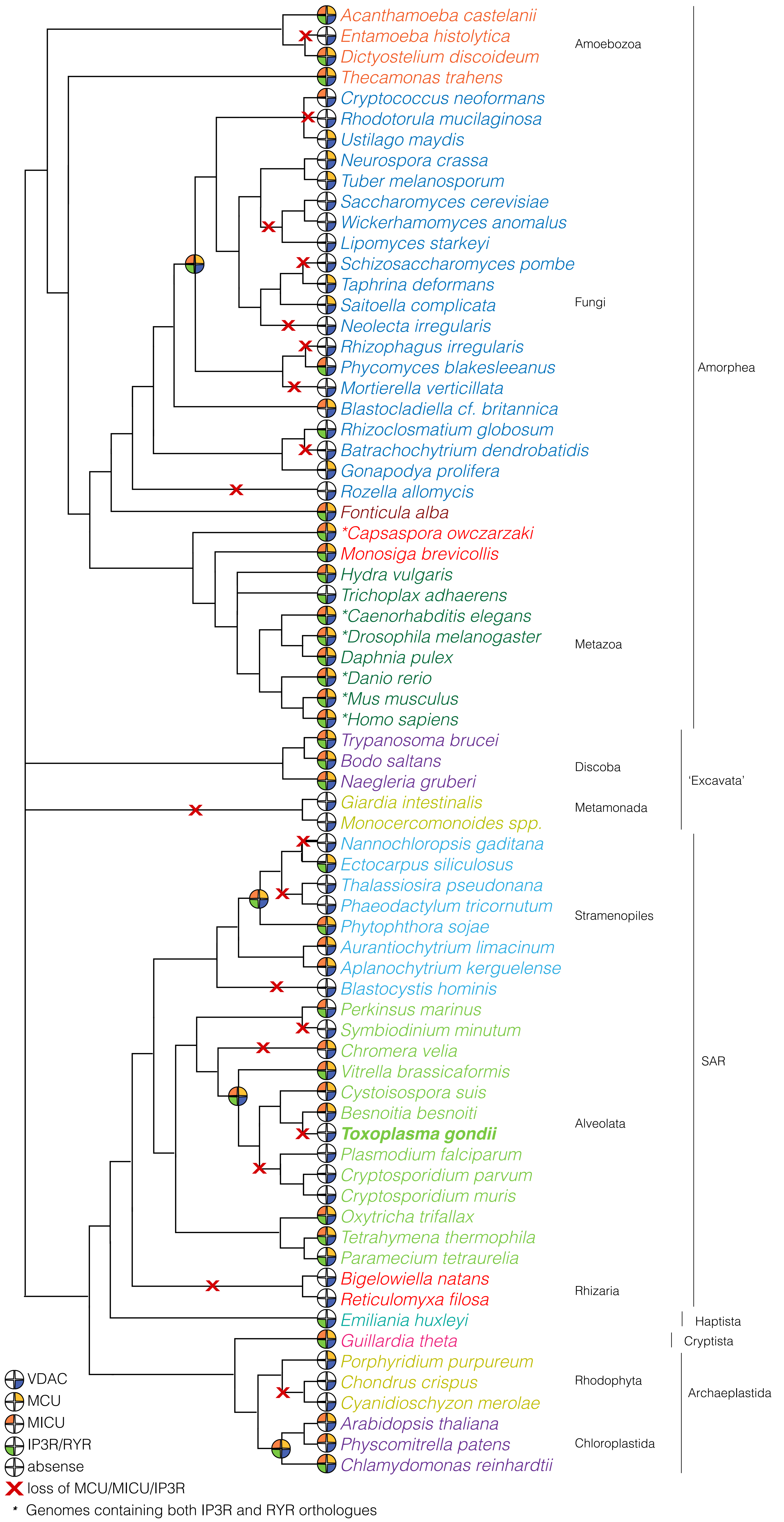
Acanthamoeba castelanii
Entamoeba histolytica
Dictyostelium discoideum
Thecamonas trahens
Cryptococcus neoformans
Rhodotorula mucilaginosa
Ustilago maydis
Neurospora crassa
Tuber melanosporum
Saccharomyces cerevisiae
Wickerhamomyces anomalus
Lipomyces starkeyi
Schizosaccharomyces pombe
Taphrina deformans
Saitoella complicata
Neolecta irregularis
Rhizophagus irregularis
Phycomyces blakesleeanus
Mortierella verticillata
Blastocladiella cf. britannica
Rhizoclosmatium globosum
Batrachochytrium dendrobatidis
Gonapodya prolifera
Rozella allomycis
Fonticula alba
*Capsaspora owczarzaki
Monosiga brevicollis
Hydra vulgaris
Trichoplax adhaerens
*Caenorhabditis elegans
*Drosophila melanogaster
Daphnia pulex
*Danio rerio
*Mus musculus
*Homo sapiens
Trypanosoma brucei
Bodo saltans
Naegleria gruberi
Giardia intestinalis
Monocercomonoides spp.
Nannochloropsis gaditana
Ectocarpus siliculosus
Thalassiosira pseudonana
Phaeodactylum tricornutum
Phytophthora sojae
Aurantiochytrium limacinum
Aplanochytrium kerguelense
Blastocystis hominis
Perkinsus marinus
Symbiodinium minutum
Chromera velia
Vitrella brassicaformis
Cystoisospora suis
Besnoitia besnoiti
Toxoplasma gondii
Plasmodium falciparum
Cryptosporidium parvum
Cryptosporidium muris
Oxytricha trifallax
Tetrahymena thermophila
Paramecium tetraurelia
Bigelowiella natans
Reticulomyxa filosa
Emiliania huxleyi
Guillardia theta
Porphyridium purpureum
Chondrus crispus
Cyanidioschyzon merolae
Arabidopsis thaliana
Physcomitrella patens
Chlamydomonas reinhardtii
Amoebozoa
Fungi
Metazoa
Amorphea
Discoba
Metamonada
'Excavata'
Stramenopiles
Alveolata
SAR
Rhizaria
Haptista
Cryptista
Rhodophyta
Chloroplastida
Archaeplastida
VDAC
MCU
MICU
IP3R/RYR
absense
loss of MCU/MICU/IP3R
* Genomes containing both IP3R and RYR orthologues

Fig. S3. MCU, MICU, and IP3R have been lost 19 times in the evolution of eukaryotes
Orthologues of MCU, MICU, and IP3R were identified using BLAST and phylogenetic reconstructions. Organisms with identified orthologues are marked as indicated in the figure legend. Most eukaryotes contain homologues that could not be unambiguously identified as ITPR1 or RYR3. Aster-isks indicate organisms containing both ITPR1 and RYR3 orthologues.

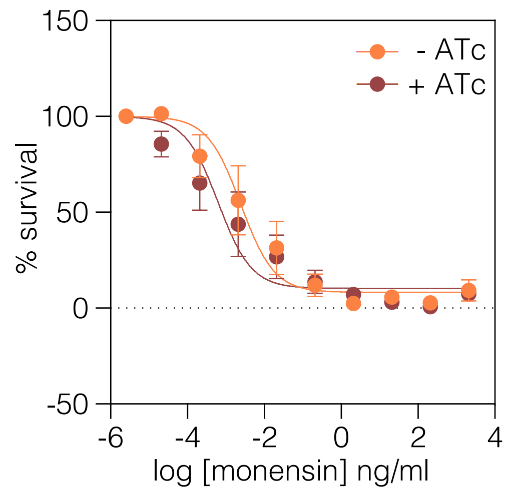

Fig. S4. Monensin IC50 is not affected by VDAC depletion
Growth curve generated using fluorescent iVDAC parasites grown with or without ATc in the prescenceof different concentrations of monensin. Results mean ± SEM, n = 4.

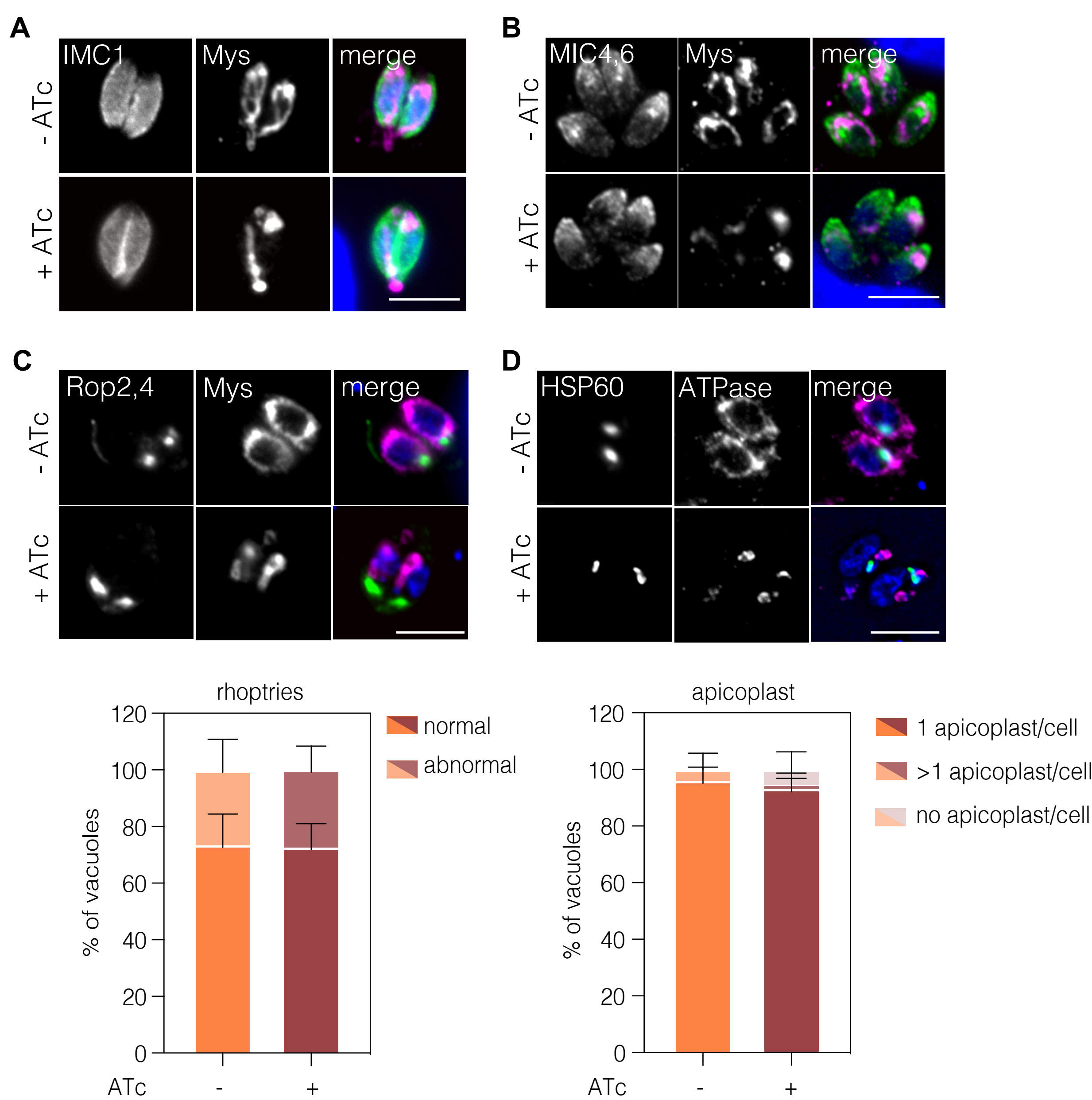

Fig. S5. Depletion of VDAC does not affect other organelles besides mitochondrion and ER
iVDAC parasites ATc treated for 48 h were fixed and stained using markers for various organelles. No difference in parasite structure (from at least 260 parasites), as visualized by anti-IMC1 **(A)** or micronemes (from at least 174 parasites), visualized using anti-MIC4, 5 **(B)** could be seen upon VDAC depletion. Imaging and quantification of the rhoptries **(C)** using anti-ROP2, 4 and the number of apicoplasts **(D)** using anti-HSP60. No significant differences could be seen in either organelle. Results mean of three independent experiments, + SD. Scale bar 5 µm.

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Table S1. List of primers used in this study

Primer number	Description	
P1	NsiI_vdac_R	cccatgcataggcacagatctcgagcttc
P2	PacI_vdac_R	cccttaattaattaggcacagatctcgagcttc
P3	VDAC_gR_F	aagttTGTACTCAACTCCGACAAAAg
P4	VDAC_gR_R	aaaacTTTTGTCGGAGTTGAGTACAa
P5	VDAC_PR_F	TCCCTCTCTCCGGGAATCTTAGCCGTTTCTGTGTACTCAACTCCGACAAA aagcttcgccaggctgtaaatcc
P6	VDAC_PR_R	CAATTTTCCGTACCCGCACAGCTTTTGTTCAAGTCCTTGAAGAGCACCAT ggttgaagacagacgaaagcagttg
P7	VDAC_test_ F	CCCTTTTTTTCCGTTTGCGGTGTTAC
P8	DHFR_R	CACGGTTATCAAACCCGAG
P9	VDAC_test_R	CACAGAAACAAGTTTTGGGCTTTAAACACG
P10	T7S4_F	CGGTTCGCTTGAAGAAGG
P11	qVDAC_F	TGAGAAGGCATGGGACTTG
P12	qVDAC_R	CGCATATTTGACGTCGATG

Table S2. List of antibodies used in this study

Antibody	**Species**	**Dilution (IFA unless indicated)**	**Source**	**Reference**
CPN60 (HSP60)	Rabbit	1:1000	Boris Striepen (University of Pennsylvania)	(Agrawal et al., 2009)
IMC1	Rabbit	1:2000	Gary Ward (University of Vermont)	(Wichroski et al., 2002)
Mys (TgME49_215430)	Rabbit	1:1000		(MacRae et al., 2012)
Myc	Mouse	1:1000	Cell Signaling	
Myc	Rabbit	1:1000	ThermoFisher	
GAP45	Rabbit	1:10000	Dominique Soldati (University of Geneva)	(Plattner et al., 2008)
Sag1	Mouse	1:1000	Sebastian Lourido (Whitehead Institute)	(Burg et al., 1988)
ROP2,4	Mouse	1:500		(Sadak et al., 1988)
F1-ATPase	Mouse	1:3000	Peter Bradley (University of California, Los Angeles)	(Beck et al., 2014)
MIC4	Rabbit	1:5000		(Reiss et al., 2001)
Actin	Mouse	1:100	Markus Meissner (Ludwig-Maximilians University of Munich)	(Angrisano et al., 2012)

Table S3. Accession numbers of identified proteins.

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