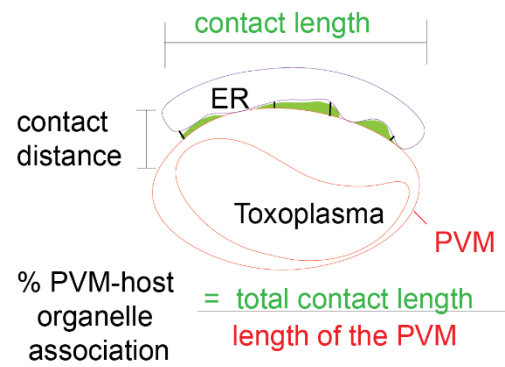


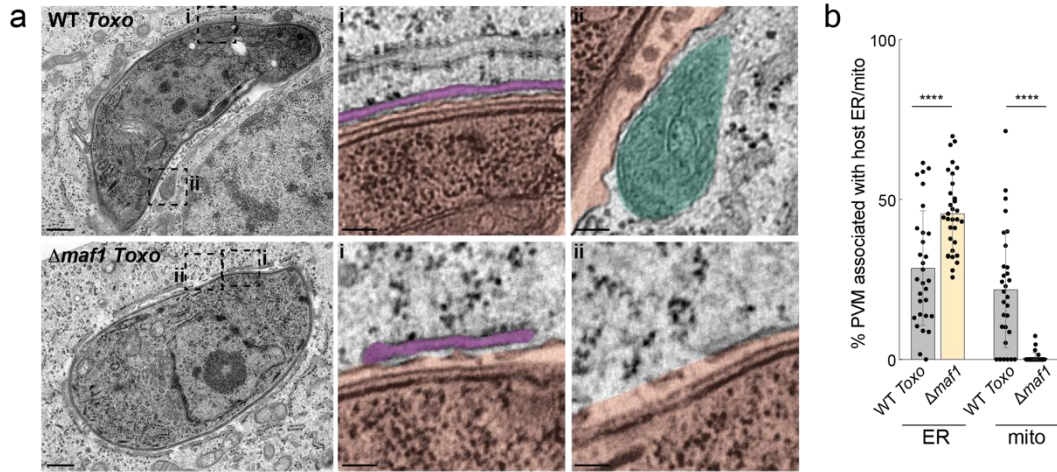
Toxoplasma effector TgROP1 establishes membrane contact sites with the endoplasmic reticulum during infection

In the format provided by the
authors and unedited



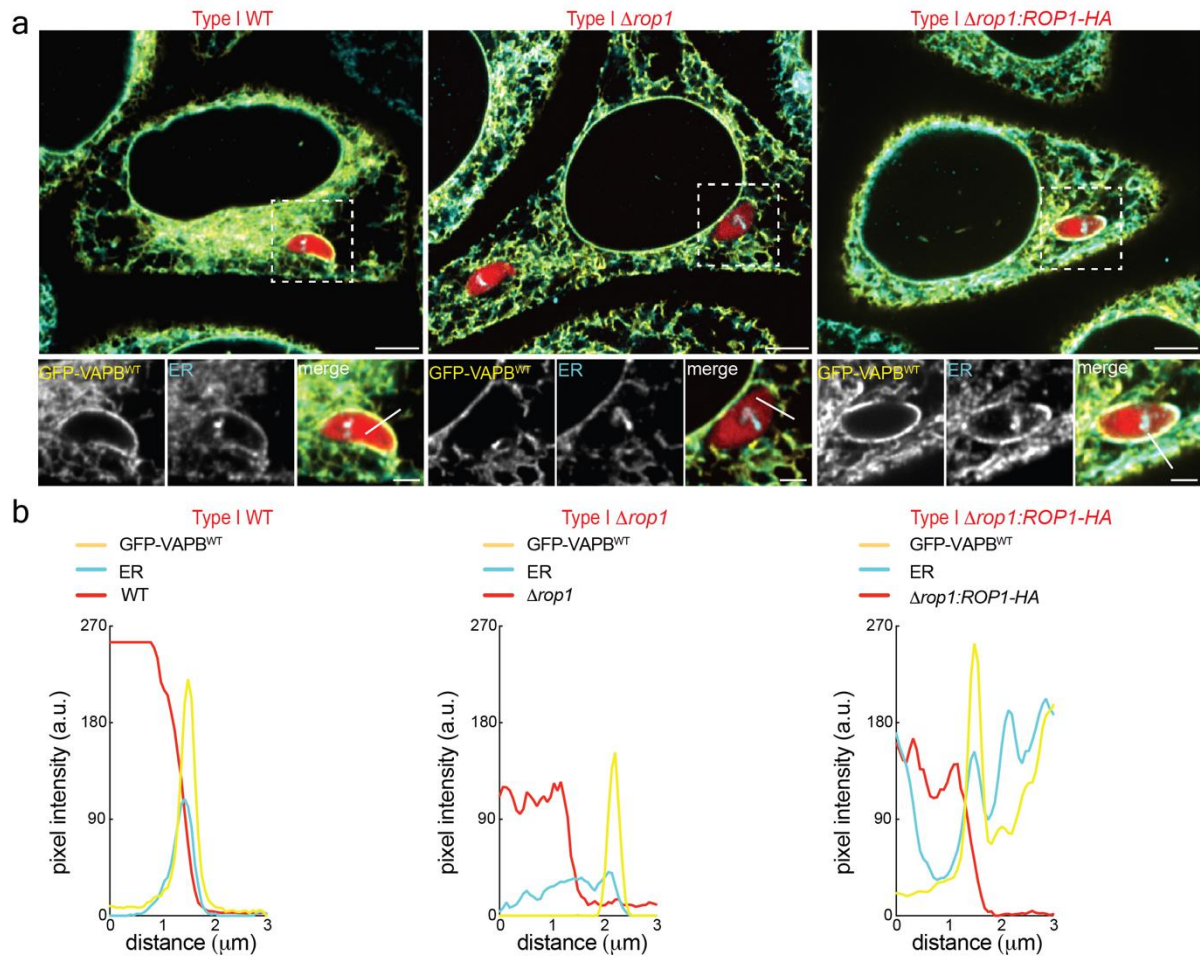
Supplementary Fig. 1: Schematic of host ER-*Toxoplasma* MCS analysis

Schematic of analyses of membrane contact sites (MCS) between host ER and *Toxoplasma* parasite vacuole membrane (PVM).



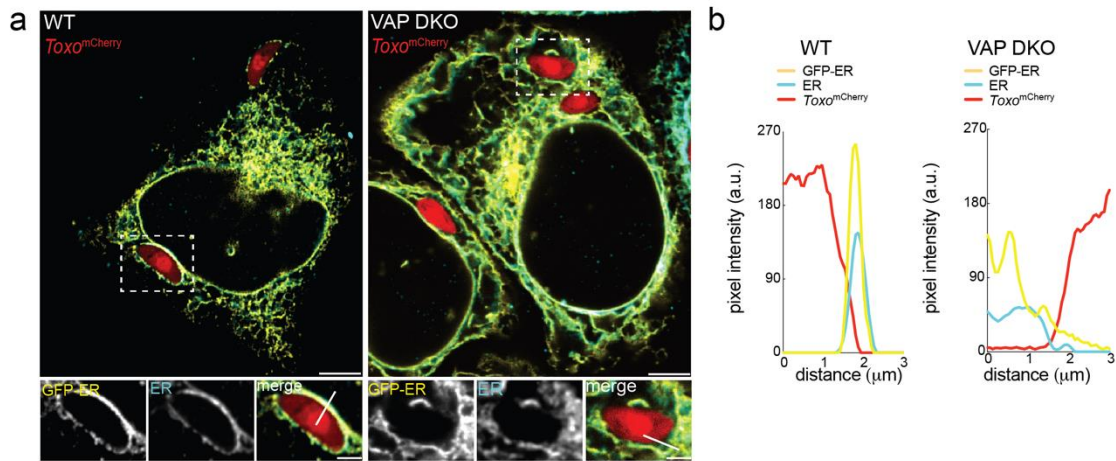
Supplementary Fig. 2: $\Delta maf1$ parasites have increased MCS with host ER

a, Representative electron micrograph images of ES-2 cells infected with *Toxo*^{PVM β 11} and $\Delta maf1$ *Toxoplasma* at 4 hours post infection. MCS sites between the *Toxoplasma* parasite vacuole membrane (PVM) and (i) host ER and (ii) host mito. Scale bars: 500 nm; inset, 100 nm. Red, PV; purple, ER; turquoise, mito. **b**, Percentage of *Toxoplasma* PVM associated with host ER and mitochondria in images as in (a). EM data are mean $\pm$ SD from n=1 biological replicate (WT: 30; $\Delta maf1$: 32 *Toxoplasma* vacuoles).. ****p < 0.0001 by means of unpaired two-tailed t-test.



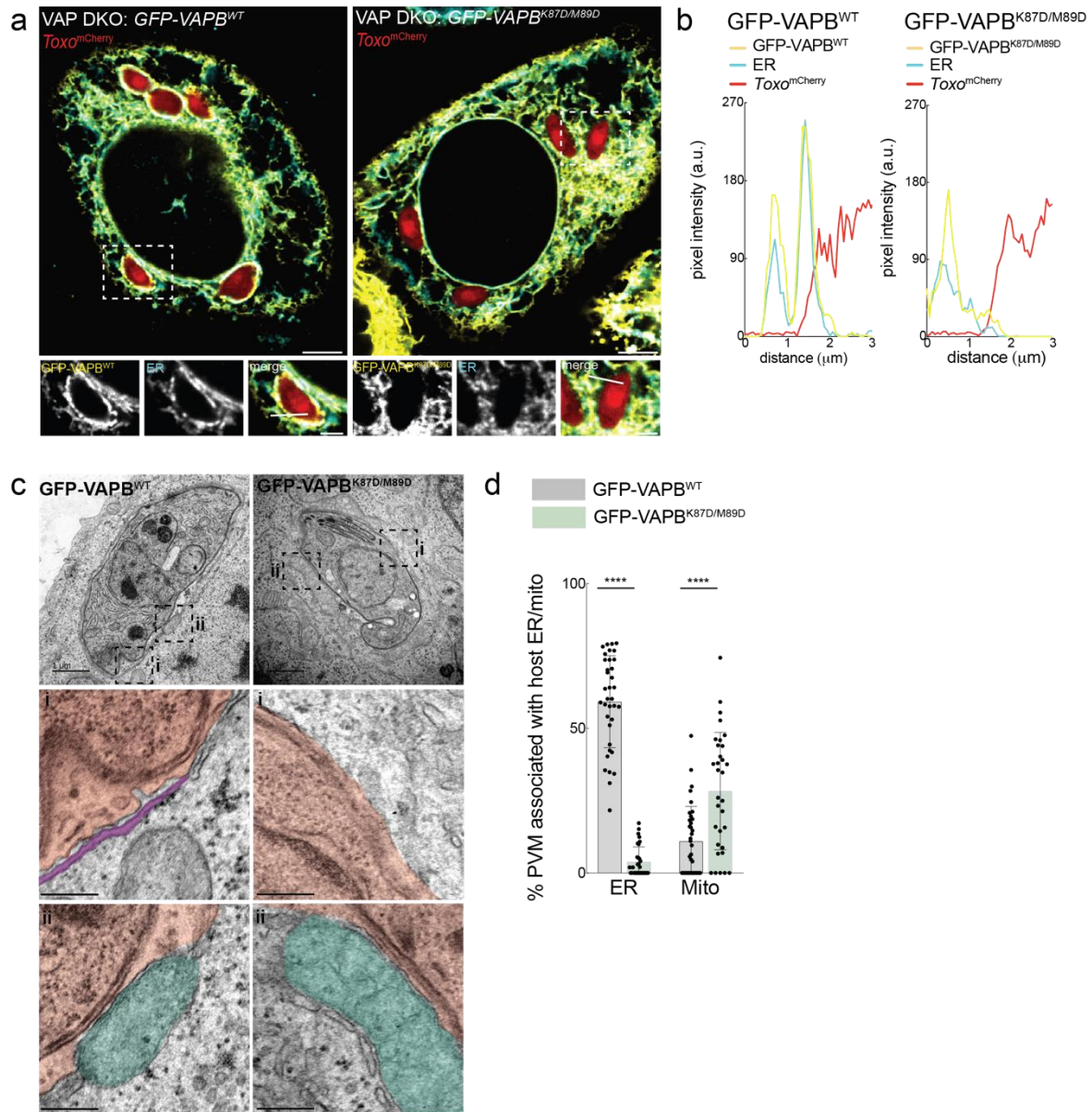
Supplementary Fig. 3: VAPB enrichment around Type I parasite vacuoles requires TgROP1

a, Representative immunofluorescence images of VAP DKO HeLa cells expressing GFP-VAPB^{WT} infected with *Toxoplasma* Type I WT (*Toxo*^{mCherry}), $\Delta rop1$, $\Delta rop1:ROP1-HA$ parasites at 3 hours after infection. ER (calnexin). Scale bars, 5 μm ; inset, 2 μm . **b**, Corresponding pixel intensity plots for white line in the (a) inset. Data is representative of n=1 biological replicate.



Supplementary Fig 4: Host ER enrichment around *Toxoplasma* is VAP-dependent

a, Immunofluorescence images of WT and VAP DKO HeLa cells expressing GFP on their ER membranes and infected with *Toxo*^{mCherry} at 3 hours post infection. ER (calnexin). Scale bars: 5 μm; inset, 2 μm. **b**, Corresponding pixel intensity plots for white line in the (a) inset. Data is representative of n=1 biological replicate.



Supplementary Fig. 5: The VAPB MSP domain is critical for host ER-*Toxoplasma* MCS
a, Representative immunofluorescence images of GFP-VAPB^{WT} and GFP-VAPB^{K87D/M89D} cells infected with *Toxo*^{mCherry} at 3 hours post infection (hpi). Data is representative of n=2 biological replicates. ER (calnexin). Scale bars: 5 μ m; inset, 2 μ m. **b**, Corresponding pixel intensity plots for white line in (a) inset. **c**, Representative electron microscopy images of GFP-VAPB^{WT} and GFP-VAPB^{K87D/M89D} cells infected with *Toxo*^{mCherry} at 3 hpi. Scale bars: 1 μ m; inset, 250 nm. Membrane contact sites between the *Toxoplasma* parasite vacuole membrane (PVM) and (i) host ER and (ii) host mito. Red, PV; purple, ER; turquoise, mito. **d**, Percentage of *Toxoplasma* PVM associated with host ER and mitochondria in images as in (c) from n=1 biological replicate (VAPB^{WT}: 36; GFP-VAPB^{K87D/M89D}: 32 *Toxoplasma* vacuoles). ****p<0.0001 by means of two-tailed unpaired t-test.

Additional Supplementary files included:

Supplementary Tables 1

List of gene targets and protospacer sequences used in the CRISPR screens.

Supplementary Table 2

List of *Toxoplasma* genes from the host mitochondria-*Toxoplasma* effector CRISPR screen showing the median log₂ fold change (log₂FC) in the sgRNA abundances between the GFP^{hi} and GFP^{neg} populations and robust ranking aggregation (RRA) scores.

Supplementary Table 3

List of *Toxoplasma* genes from the host ER-*Toxoplasma* effector CRISPR screen showing the median log₂ fold change (log₂FC) in the sgRNA abundance between the GFP^{hi} and GFP^{neg} populations and robust ranking aggregation (RRA) scores.

Supplementary Table 4

Analysis of transmembrane domain containing *Toxoplasma* rhoptry genes from the ER-*Toxoplasma* CRISPR screen for putative canonical or modified FFAT motifs, AlphaFold model scores and motif pLDDT values.

Supplementary Table 5

Proteomic analysis of GFP-IPs from uninfected and *Toxoplasma*-infected GFP-VAPA^{WT}-expressing VAP DKO HeLa cells.

Supplementary Table 6

Sequences of primers used in the study.

Supplementary Video 1

Time-lapse images of a live human foreskin fibroblast cell labelled with MitoTracker Deep Red and expressing OMM^{GFP-10} was infected with a parasite expressing PVM^{β11} (*Toxo*^{PVMβ11}). GFP is detected at the host mitochondria-*Toxoplasma* interface. Images were acquired every 3 minutes using a spinning-disk confocal microscope. PVM: parasite vacuole membrane; OMM: outer mitochondrial membrane. Scale bar, 5 μm

Supplementary Video 2

Time-lapse images of a live human foreskin fibroblast cell expressing GFP-VAPA^{WT} and infected with Type I *Toxoplasma* parasites. Images were acquired every 6 min using a spinning-disk confocal microscope. Scale bar, 5 μm.