

A Vacuolar-H⁺-Pyrophosphatase (TgVP1) is Required for Microneme Secretion, Host Cell Invasion, and Extracellular Survival of *Toxoplasma gondii*

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Supporting Information

FIGURE S1: Gliding motility. (A) Eight-well glass chamber slides were coated overnight at 4°C with 50% fetal bovine serum in PBS (pH 7.4) and slides washed with PBS. Freshly lysed parasites, washed and suspended in HHE (Hank's Balanced Salt, 10mM HEPES, 1 mM EGTA) were allowed to glide on FBS coated slides, at 37°C for 15 min. Fixation was with 4% paraformaldehyde and staining was performed with rabbit anti-SAG1 antibody 1:1,000 directly conjugated to Alexa 488 fluorochrome 1:1,000. A trail was considered circular if the diameter was 11 μm or less; trails that were larger in diameter or straight were counted as non-circular. Approximately 100 trails were enumerated per strain in each experiment. Although $\Delta vp1$ show a small decrease in circular trails when compared to $\Delta ku80$ and $\Delta vp1\text{-cm}$, we did not find a significant difference among the three strains. Values are mean \pm SD. Data are the combined results of three independent experiments and analyzed by GraphPad Prism 5.

FIGURE S2. TgVP1 deficient parasites show normal maturation of MIC3, MIC5 and M2AP (A) Phosphorimages from pulse-chase metabolic labeling and immunoprecipitation of M2AP, MIC3 and MIC5 in $\Delta ku80$, $\Delta vp1$ and $\Delta vp1\text{-cm}$ parasites. Protocol is described in Experimental Procedures. Quantification of M2AP (B), MIC3 (C), and MIC5 (D) maturation by phosphorimage analyses of pulse-chase immunoprecipitates. One (MIC3 and MIC5) or two (M2AP) experiments were performed to avoid unnecessary use of radioactive materials since no apparent differences were observed between $\Delta ku80$ and $\Delta vp1$. Values for M2AP are means \pm SEM.

FIGURE S3. (A) $\Delta ku80$, $\Delta vp1$ and $\Delta vp1\text{-cm}$ parasites were treated with the solvent vehicle DMSO, or 50 and 100 nM bafilomycin A₁ inhibitor to reduce the V-ATPase activity. Maturation of M2AP was monitored by pulse-chase immunoprecipitation. (B) Maturation of M2AP was quantified and plotted by measuring the intensities of pro- and mature M2AP and calculating the percentage of mature M2AP in newly synthesized M2AP species (bars represent standard error of three independent experiments). Treatment with bafilomycin A₁

slowed down the processing of M2AP, however, there was no significant differences among *Δku80*, *Δvp1* and *Δvp1-cm* parasites, suggesting that TgVP1 does not contribute to M2AP processing.

FIGURE S4. Weight of infected mice: groups of 5 mice (Swiss Webster) were infected (i.v.) with 500 (A) or 50 (B) tachyzoites of the *Δvp1*, *Δku80* or *Δvp1-cm* strains. Each mouse was weighted every other day. Infected mice lost weight at 10 to 20 days p.i. Living mice recovered after 25-27 p.i and kept gaining weight even after being challenged at day 40 with 1,000 parasites of the RH strain (i.p. inoculation). Mice gain weight but they do not reach control values. Weight of living mice was monitored up to 80 d.p.i. Values are mean \pm SD.

Table S1

Intracellular Ca²⁺ measurements

<i>Δku80</i>	<i>Δvp1</i>	<i>Δvp1-cm</i>
60.58 ± 7.1 nM	170.52 ± 25.32 nM	51.57 ± 10.02 nM
SE: 4.10	SE: 14.62	SE: 10.98

Parasites were loaded with Fura-2AM as described in EP and calculations of cytosolic Ca²⁺ were done following published protocols (Moreno and Zhong, 1996). P = 0.002 when comparing *Δku80* with *Δvp1* and 0.003 comparing *Δvp1* and *Δvp1-cm*.

Figure S1

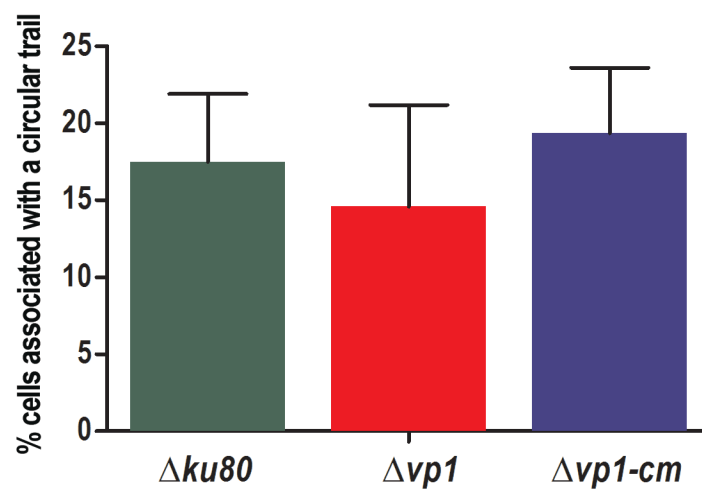


Figure S2

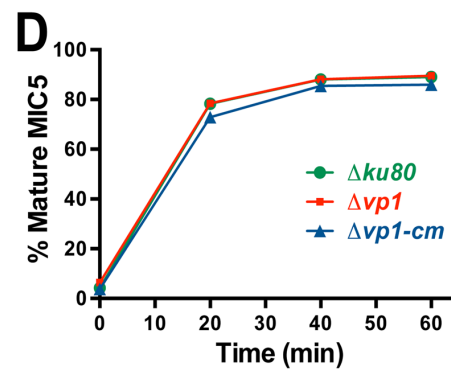
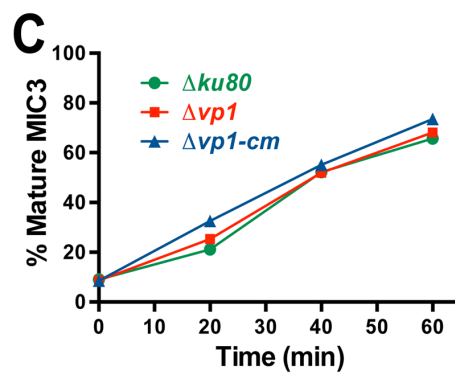
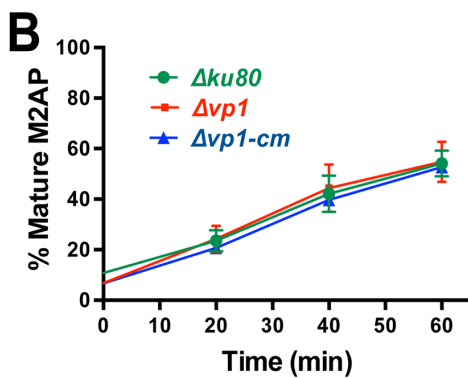
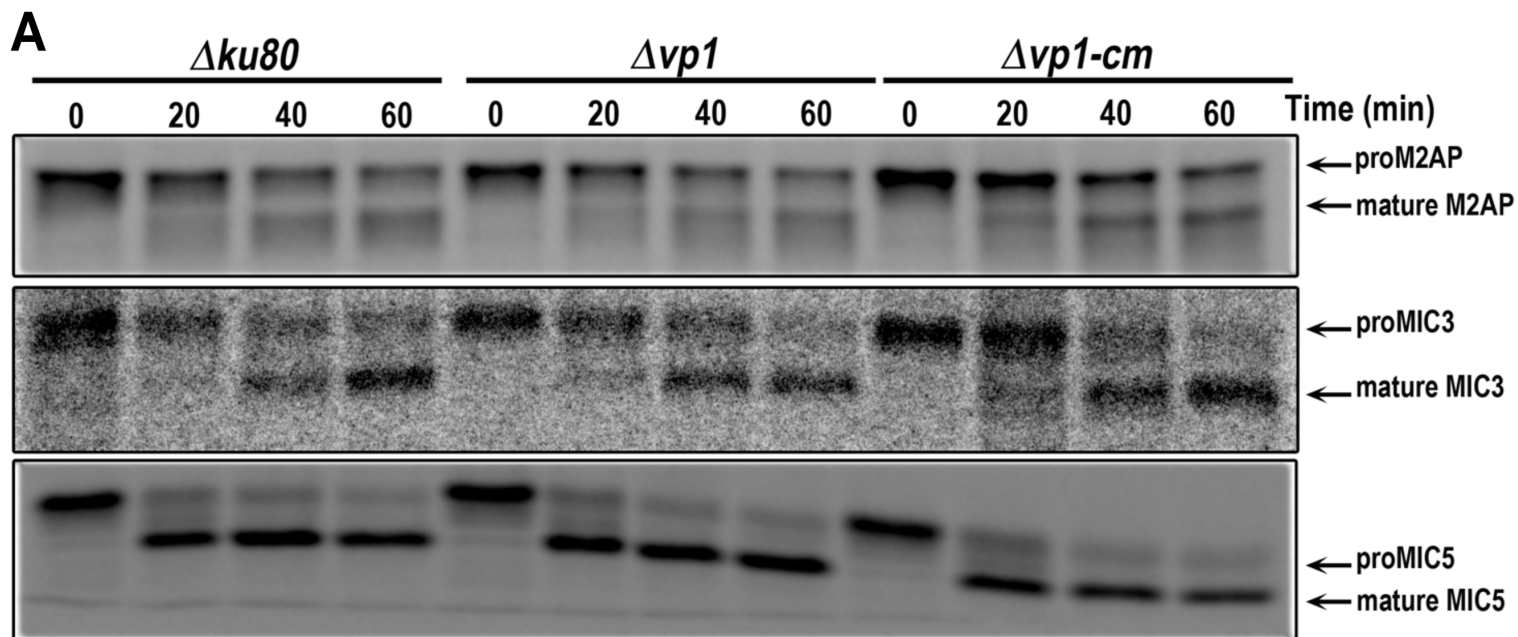


Figure S3

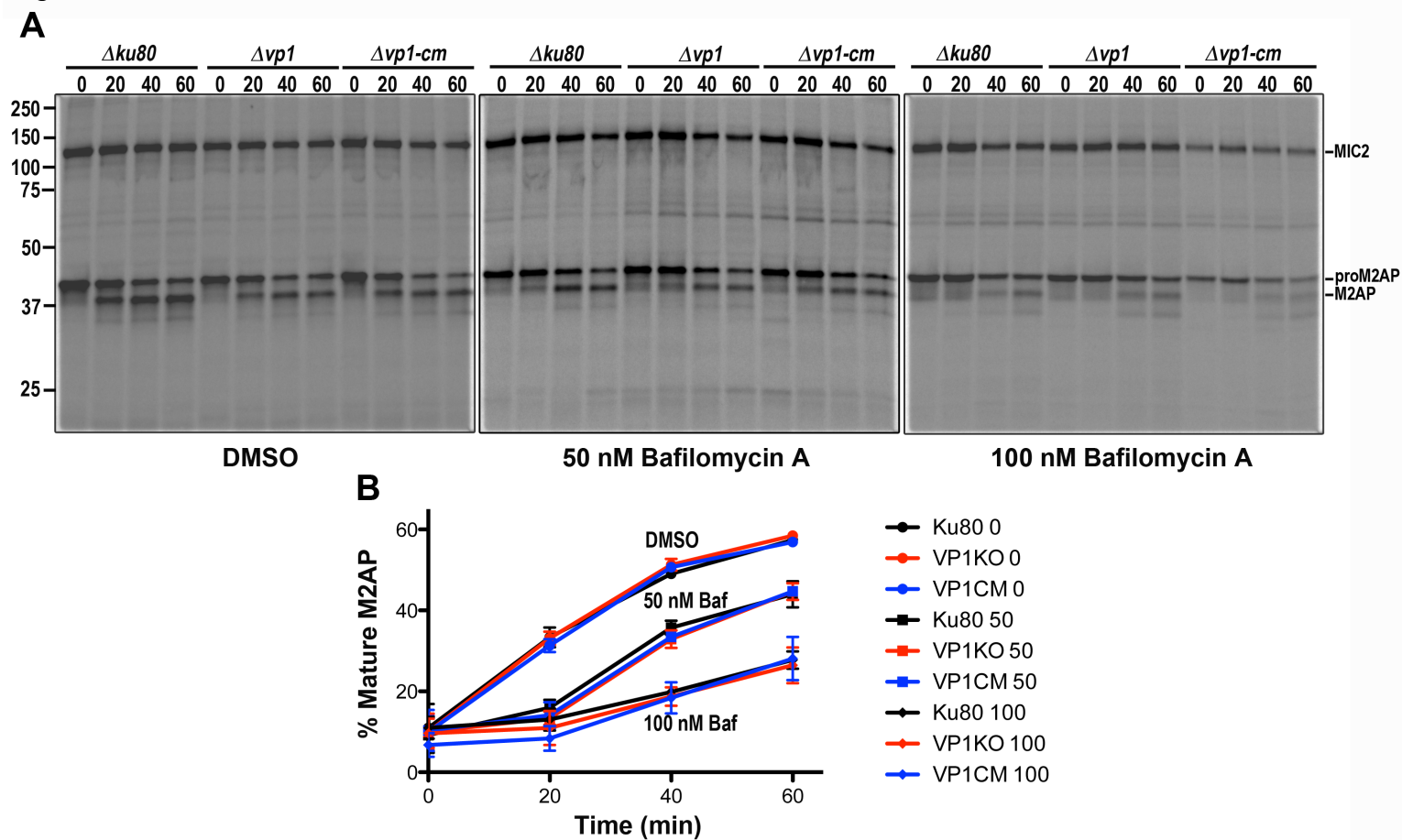


Figure S4

