Supporting Information

Identification of an Atg8-Atg3 protein-protein interaction inhibitor from the Medicines for Malaria Venture Malaria Box active in blood and liver stage *Plasmodium falciparum* parasites.

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Supplemental Figure S1
Immunoblot of **1** treated *Plasmodium* cultures using cytocidal concentrations S2

Supplemental Figure S2
Coomassie stained protein loading control S3

Supplemental Figure S3
Intensity analysis of lipidated and unlipidated PfAtg8 S4

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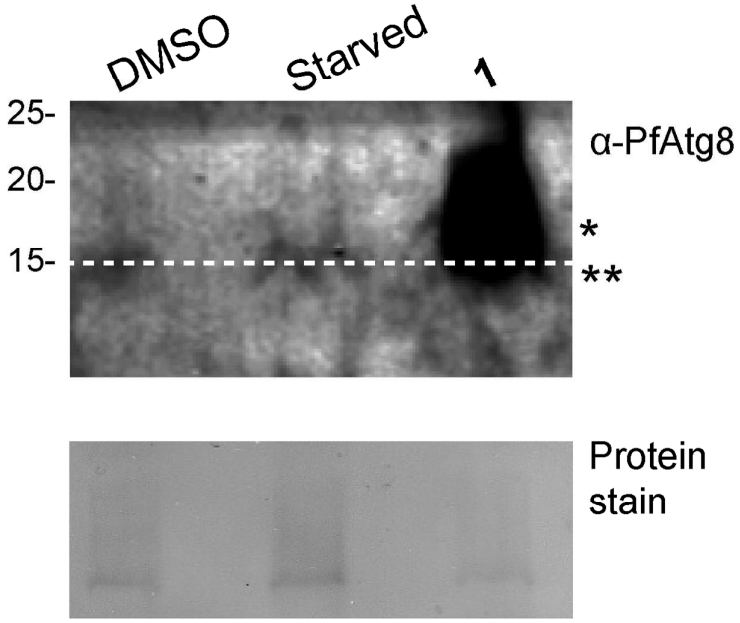
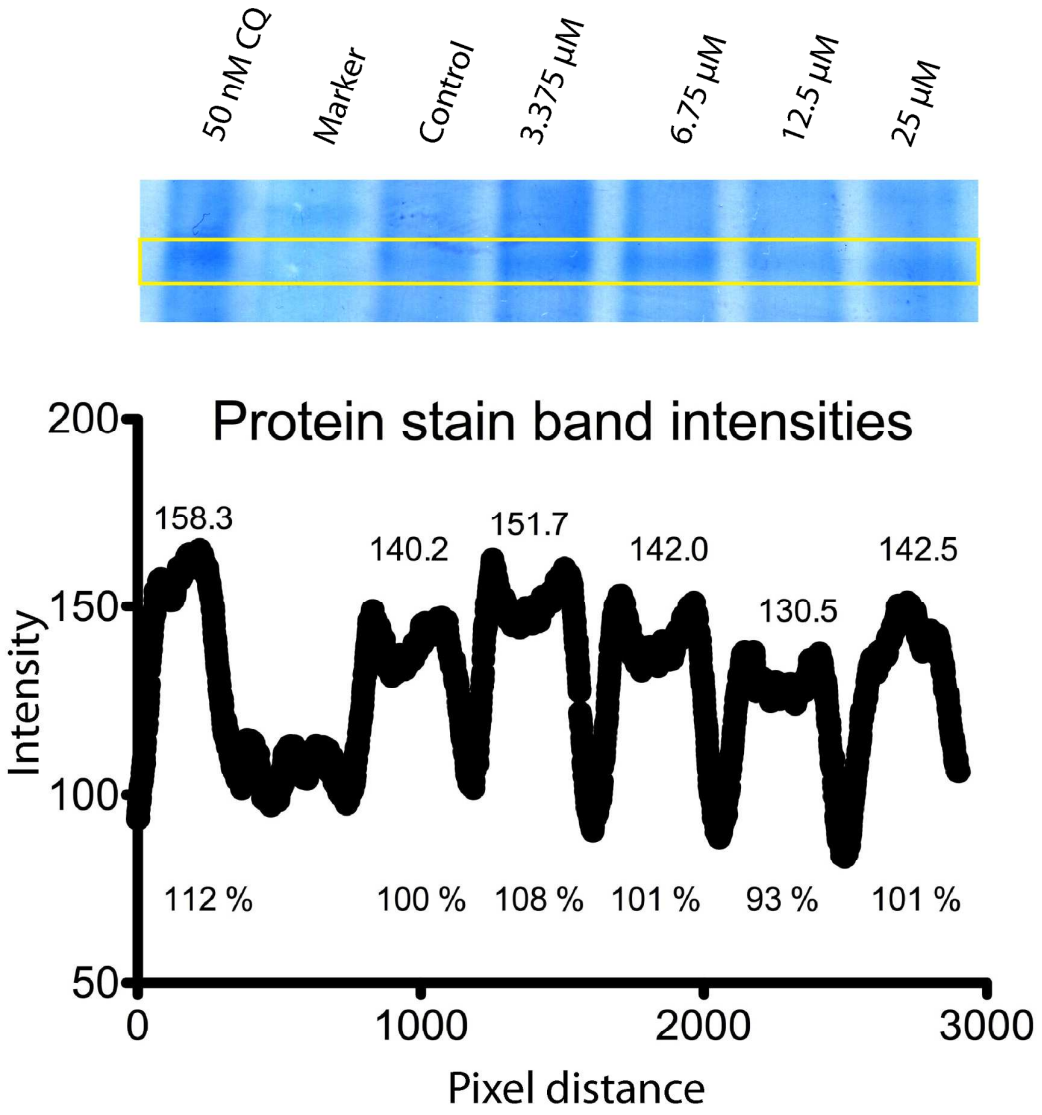
Complete Western blot used for analysis, indicating antibody specificity S5

Supplemental Figure S1



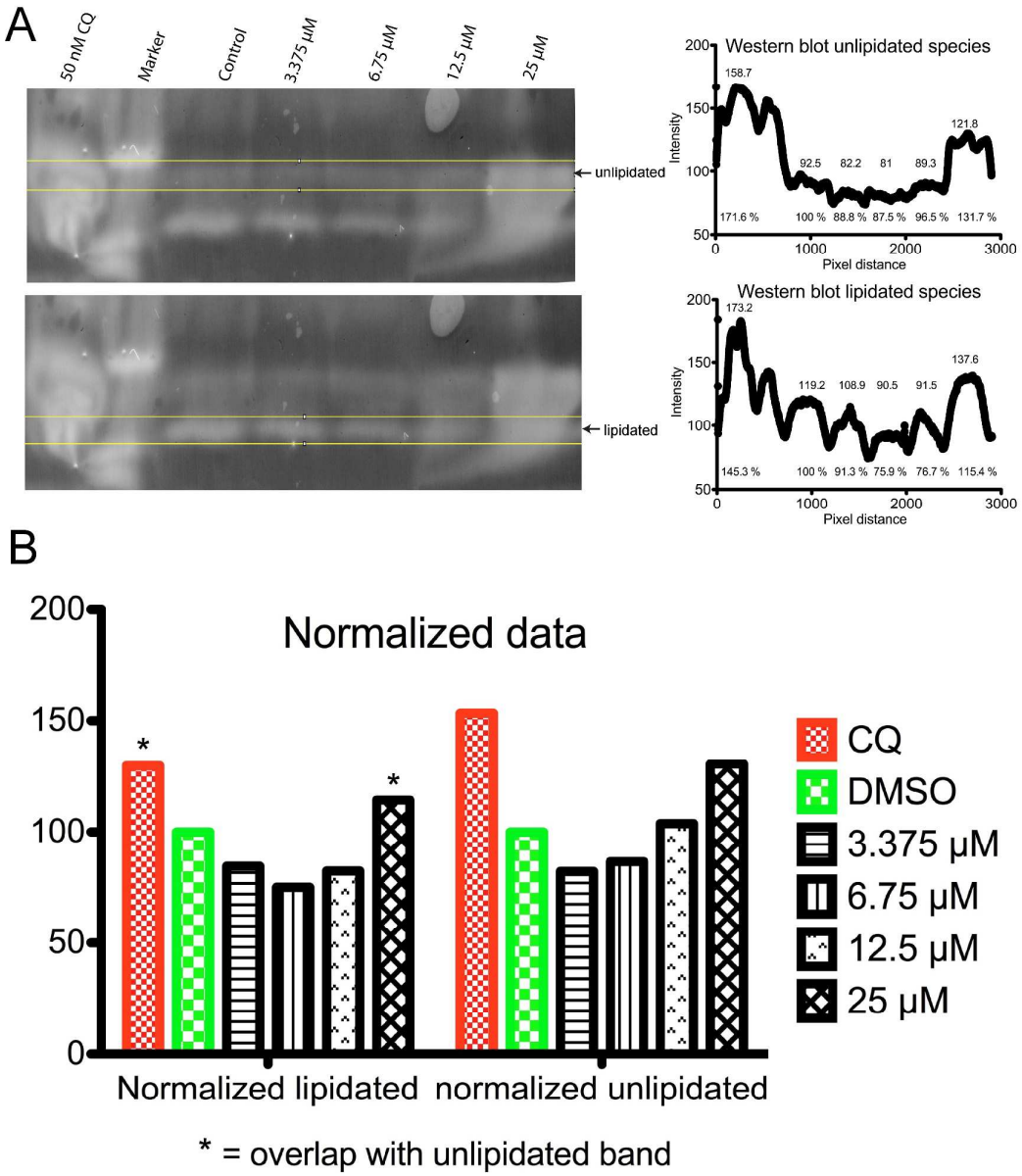
Immunoblot analysis of *P. falciparum* FCR3 cells treated with DMSO, DMSO and culture media lacking human serum, or 50 μM of compound **1** for five hours. Upper blot was probed with antibody against *Tg*Atg8, demonstrated to be cross reactive against *Pf*Atg8.[1] Asterisk indicates migration of *Pf*Atg8 in **1**-treated cells versus double asterisk indicating migration of *Pf*Atg8 in control and starved cells. Atg8-PE has a faster migration than unlipidated Atg8 with SDS-PAGE. Band intensity of *Pf*Atg8 was normalized to total protein on stained membrane with ImageJ.[2] *Pf*Atg8 was 23 times greater in the **1**-treated sample than in the control.

Supplemental Figure S2



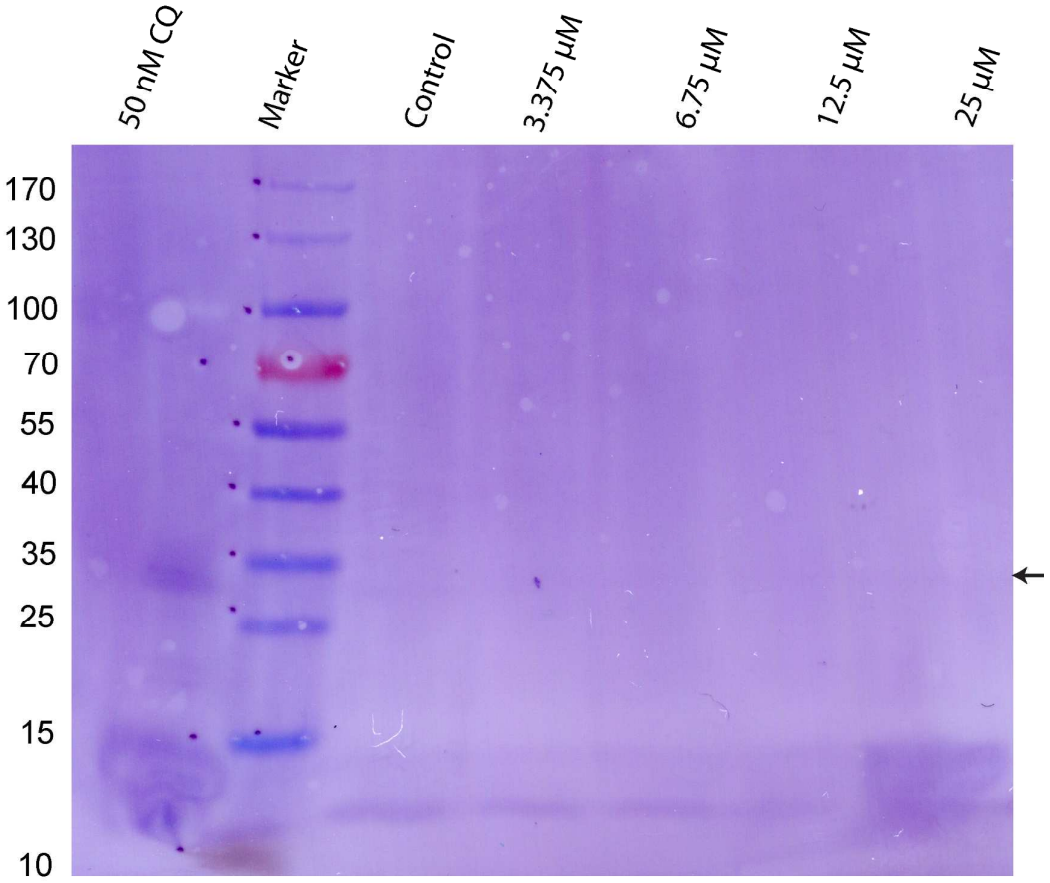
Analysis of the Comassie stained gel to correct for differences in protein loading levels.
The yellow box indicates the area analyzed in the profile plot below. Numbers reflect the average per peak. The marker lane was omitted from this analysis. All intensities were compared to the DMSO control, which was set to 100%. The 1-treated samples are within +/- 8 % of the total protein loading levels compared to the DMSO treated control lane.

Supplemental Figure S3



Intensity analysis of the two Atg8 species detected via Western blot. A) The Western blot was converted to a grey scale image and color inverted for ease of analysis in ImageJ.[2] Profile plots for the lipidated and unlipidated form of Atg8 are represented and the intensity values are normalized to the DMSO treated control. B) Intensity values normalized for total protein level, showing the corrected differences of lipidated and unlipidated Atg8 in the presence of **1** at four different concentrations. Analysis of either the Chloroquine (CQ, positive control) and the highest concentration of **1** for the lipidated and unlipidated form is difficult due to the bands not being completely resolved on the Western blot. The resulting graph is likely an underestimation of the true amount of unlipidated Atg8 at the highest concentration of **1**. The bar graph clearly indicates an increase of unlipidated Atg8 in the presence of **1**.

Supplemental Figure S4



Complete Western blot used for the analysis in figures 6A, S2 and S3 showing clear specificity of the mAB Tg-Atg8 antibody. Note that dimer formation is clearly visible in the CQ treated sample lane and less visible but still present in the other lanes between the 35 and 25 kDa marker lanes, estimated to migrate at approximately 30 kDa (indicated by an arrow).

References

(1) Gaviria, D.; Paguio, M. F.; Turnbull, L. B.; Tan, A.; Siriwardana, A.; Ghosh, D.; Ferdig, M. T.; Sinai, A. P.; Roepe, P. D. A Process Similar to Autophagy Is Associated with Cytocidal Chloroquine Resistance in *Plasmodium falciparum*. *PLoS ONE* **2013,** *8*, e79059.

(2) Abramoff, M. D.; Magalhaes, P. J.; Ram, S. J. Image Processing with ImageJ. *Biophotonics International* **2004,** *11*, 36-42.