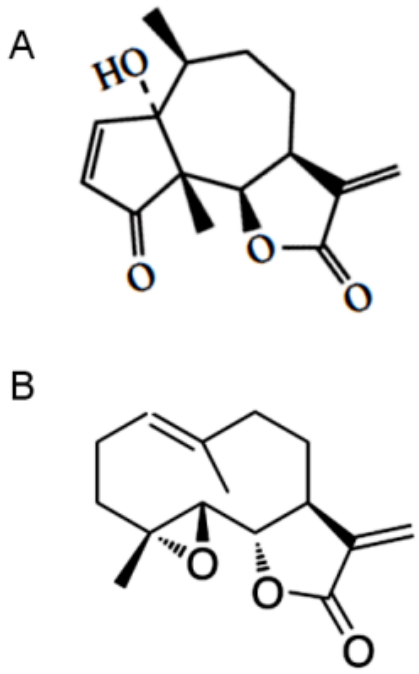
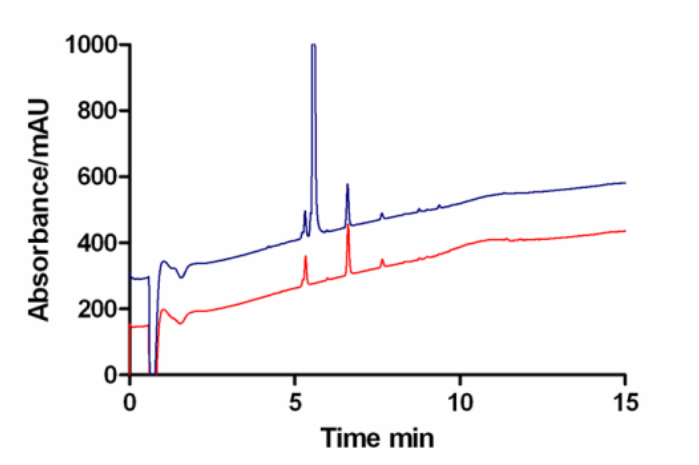
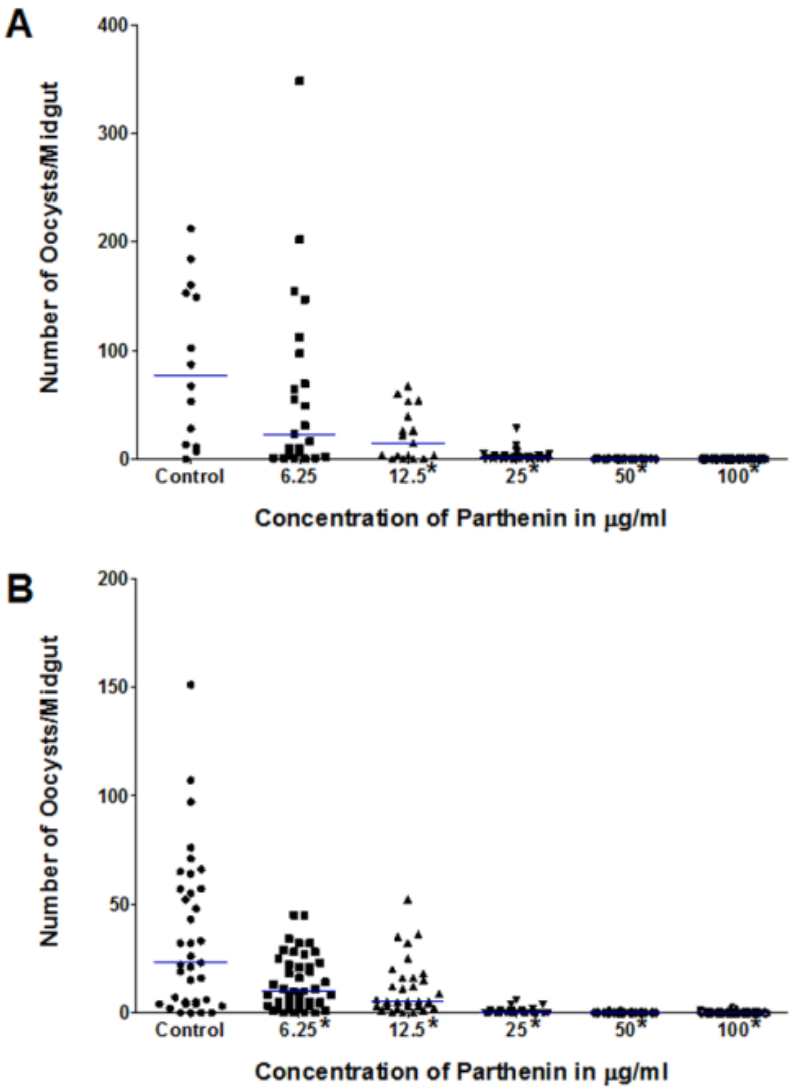
Supplemental Figures and Figure Legends



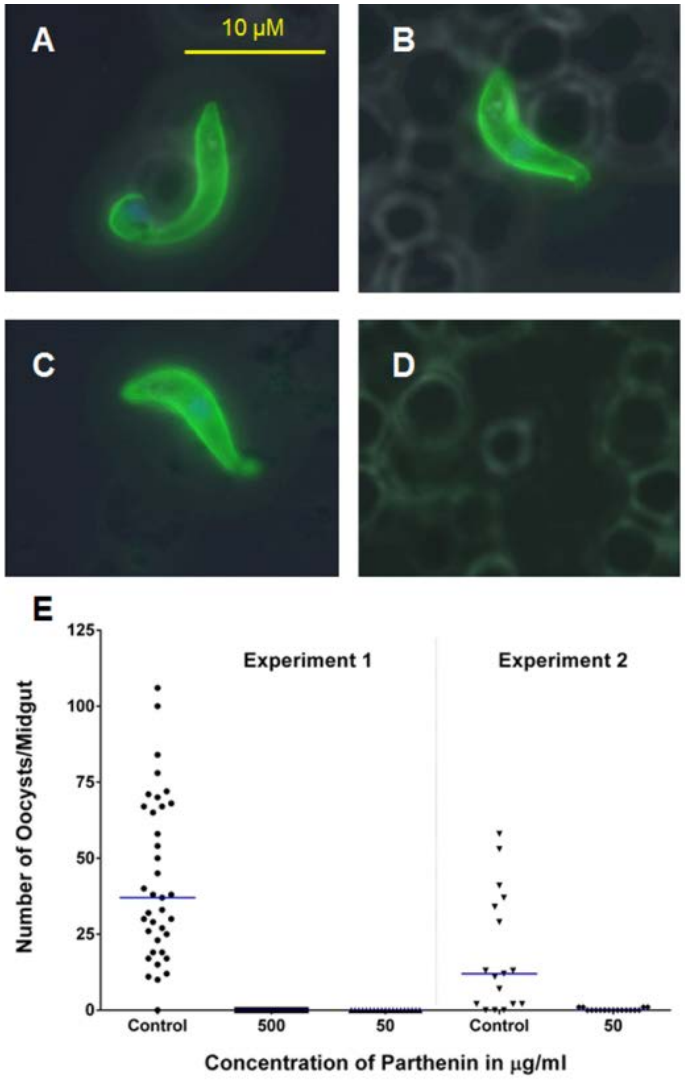
Supplemental Figure S1. Structures of parthenin (A) and parthenolide (B). The molar masses of parthenolide and parthenin are 248 g/mol and 262 g/mol, respectively.



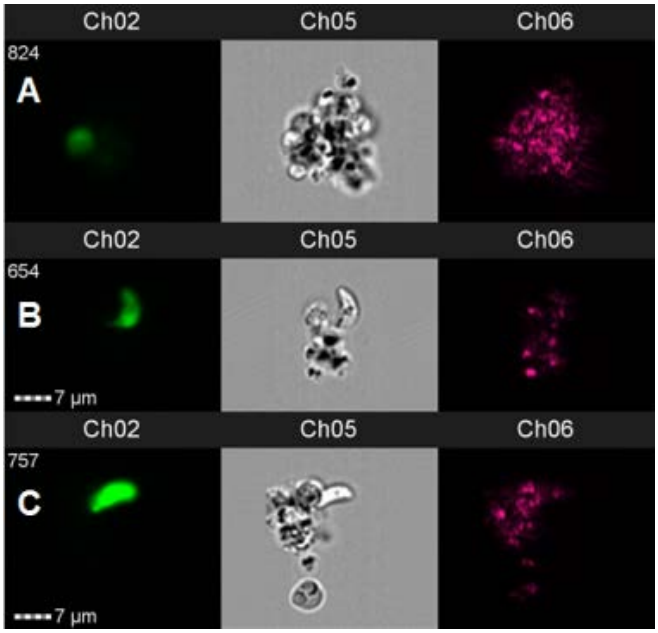
Supplemental Figure S2. Confirmation of parthenin purity by HPLC. The red line represents the water/acetone mixture used to dissolve the parthenin. The blue line is the same mixture with the addition of parthenin. The strong peak near 6 minutes and lack of additional peaks illustrates the purity of the parthenin.



Supplemental Figure S3. Replicates further illustrating the anti-parasite activity of parthenin. A. First replicate study from Figure 1 illustrating the effect of parthenin on oocyst development when added to the blood meal immediately before feeding. Asterisks indicate statistical significance as follows: 12.5 µg/mL (*P*= 0.0036), 25 µg/mL(*P*< 0.0001), 50 µg/mL (*P*< 0.0001), and 100 µg/mL (*P*< 0.0001). **B.** Replicate 3. Asterisks indicate statistical significance as follows: 6.25 µg/mL (*P* =0.0001) 12.5 µg/mL (*P* =0.0001), 25 µg/mL (*P*< 0.0001), 50 µg/mL (*P*< 0.0001), and 100 µg/mL (*P*< 0.0001).

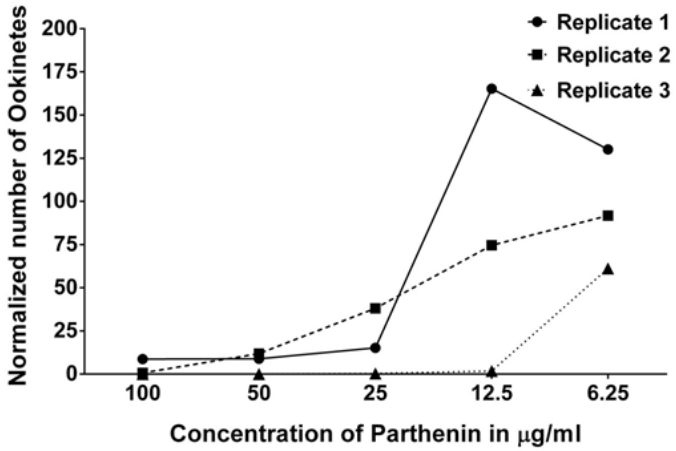


Supplemental Figure S4. Lack of ookinetes *in vivo* at 500 µg/mL parthenin. A-C. Example ookinete images from the control group (fed on the water-acetone mixture). **D.** An example image from the parthenin treated (500 µg/mL) mosquitoes showing that no ookinetes were present in any of the midguts, n=5 for both treatments. **E.** Standard Membrane Feeding Assay results showing complete lack of oocysts at high concentrations of parthenin that prompted the experiments in A-D.



Supplemental Figure S5. Sensitivity and precision of Amnis Imagestream^X Mark II instrument to decipher between GFP+ parasites and cellular debris. Ch02 is GFP, Ch05 is brightfield image and Ch06 is side scatter. **A.** Round zygote obscured by a clump of cells. **B-C.** Ookinetes obscured by cellular debris but still detectable by GFP. These images illustrate the ability of the Imagestream to capture parasites even when obscured by other cells or debris.

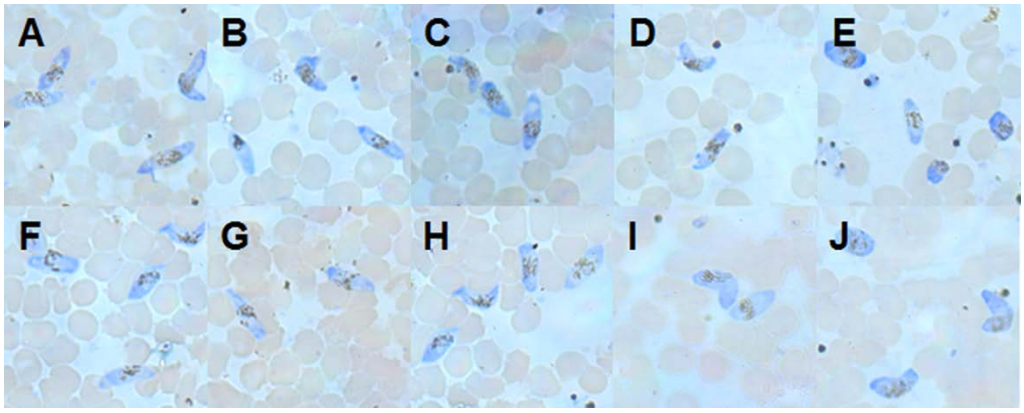
A



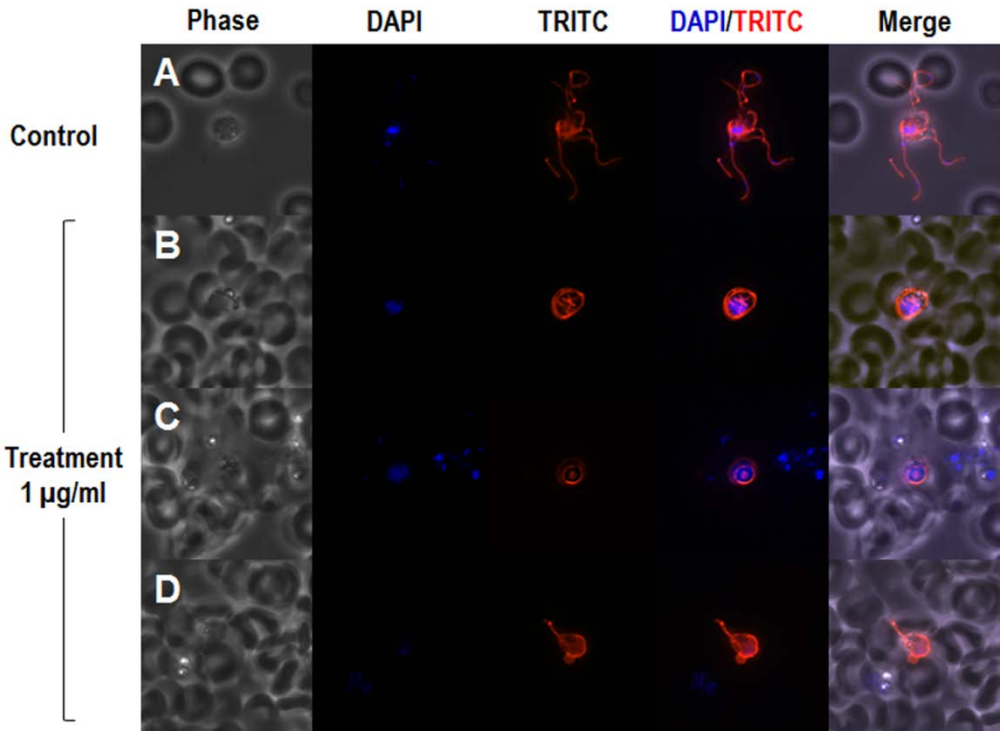
B

	Rep. 1	Rep. 2	Rep. 3	Totals
Total Gated Events	327	2266	648	3241
Total Verified Parasites	304	1033	544	1881
% Verified of Total	92.97	45.59	83.95	58.04
Estimated Total Parasites	1534	4270	5698	11502
Total Ookinetes	85	472	586	10.29
% Conversion	5.56	11.05	10.29	9.94
PBS	805	966	2708	4479
Acetone	170	1042	1830	3042
100	15	7	0	22
50	15	124	0	139
25	26	398	7	431
12.5	282	778	32	1092
6.25	222	956	1121	2299

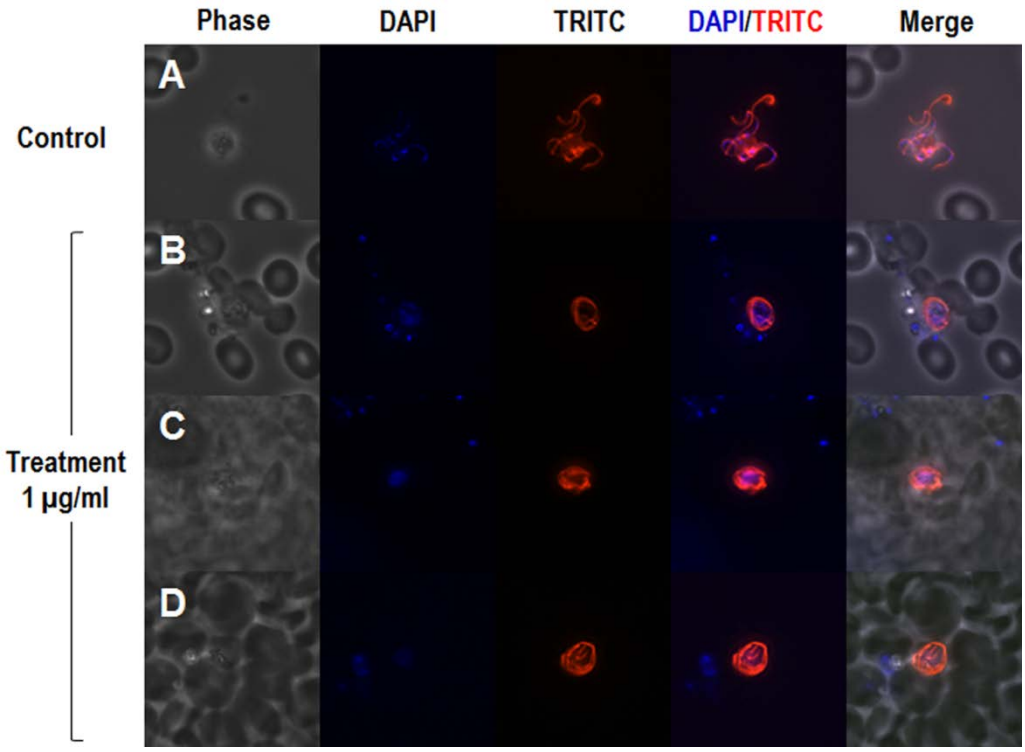
Supplemental Figure S6. Parthenin treatment post fertilization inhibits *P. berghei* ookinete maturation. A. Graph depicting three replicates of total fertilized parasite counts by the Amnis Imagestream from 100-6.25 µg/mL parthenin. **B.** Table summarizing total gated events, verified parasites from those events, percent of gated events that were verified to be parasites, estimated total parasites based on volume, total ookinetes, and percent conversion based on the total ookinetes divided by the total verified parasites. Also listed are total parasite numbers for each concentration and all three replicates.



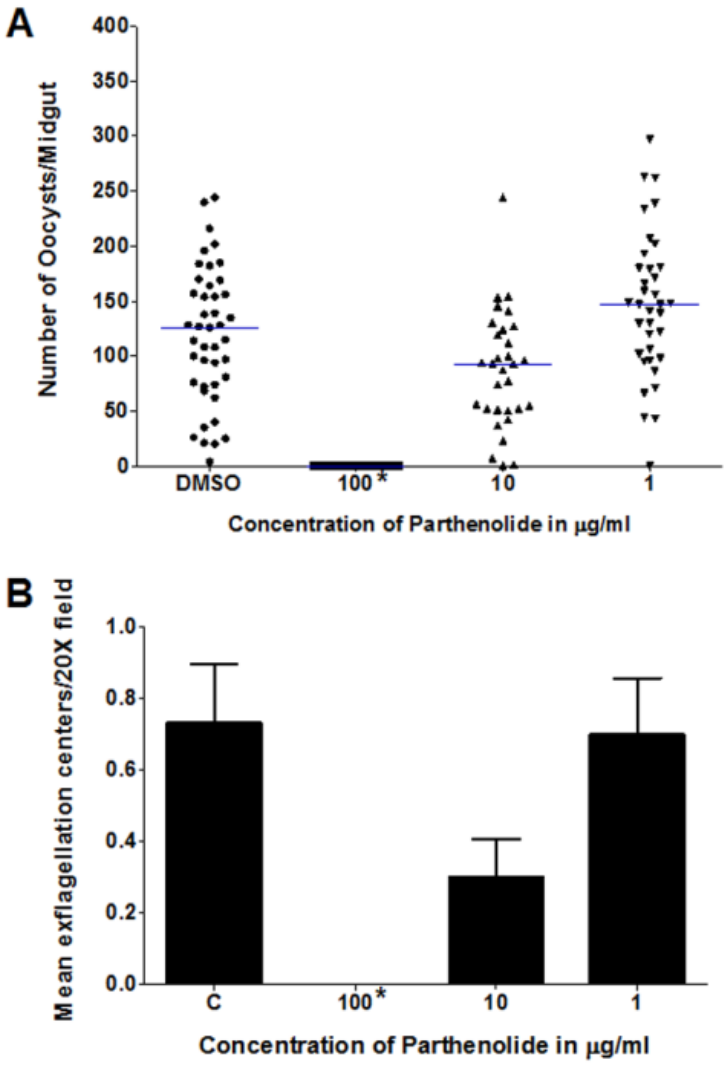
Supplemental Figure S7. Giemsa stain showing morphological similarity of treated and untreated stage V gametocytes. Gametocytes were exposed to the compound for 24 hours before the compound was removed and the gametocytes were stained. There appears to be no major observable differences between the treated and untreated control gametocytes. **A-E.** Representative images of the control gametocytes that were treated with the water-acetone mixture. **F-J.** Representative images of the treated gametocytes (1µg/mL parthenin). Scale bar = 10 µm.



Supplemental Figure S8. Tubulin staining illustrating the failure of microgametes to exflagellate. From left to right the channels are Phase Contrast, DAPI, TRITC, DAPI and TRITC overlay and a complete Merge (Phase, DAPI and TRITC). The parthenin treatment was 1 µg/mL for 24 hours on Day 15-16 gametocytes. The compound was then removed and fresh media added for 24 hours before induction. **A.** Representative control microgamete undergoing exflagellation. **B.** Treated microgametes with possible flagella beginning to form inside the cell. **C.** Possible flagella near the surface of the cell. **D.** Flagella beginning to exit the cell but failing to exflagellate properly. This type of cell was very rare and no properly exflagellating microgametes were seen in any of the treated samples. Scale bar = 4 µm.



Supplemental Figure S9. Tubulin staining illustrating the failure of microgametes to properly exflagellate when exposed to parthenolide. From left to right the channels are Phase Contrast, DAPI, TRITC, DAPI and TRITC overlay and a complete Merge (Phase, DAPI and TRITC). The parthenolide treatment was 1 µg/mL for 24 hours on Day 15-16 gametocytes. The compound was then removed and fresh media added for 24 hours before induction. **A.** Representative control microgamete undergoing exflagellation. **B-D.** Treated microgametes with possible flagella beginning to form and unable to exit the cell. Scale bar = 4 µm.



Supplemental Figure S10. Parthenolide shows similar activity to parthenin against *P. falciparum* when measuring exflagellation and oocyst development A. Parthenolide included in the blood meal causes a significant decrease at 100 µg/mL (*P*< 0.0001 using GLMM analysis) with loss of the effect at lower concentrations. **B.** 100 µg/mL parthenolide treatment results in a statistically significant decrease in exflagellation (*P*-value < 0.05, repeated measures ANOVA with a Dunnett's post-test).