Supporting information

The genetic Ca^{2+} sensor GCaMP3 reveals multiple Ca^{2+} stores differentially coupled to Ca^{2+} entry in the human malaria parasite *Plasmodium falciparum*

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Running title: GCaMP3 studies of Ca^{2+} pools in *P. falciparum*

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List of the material included:

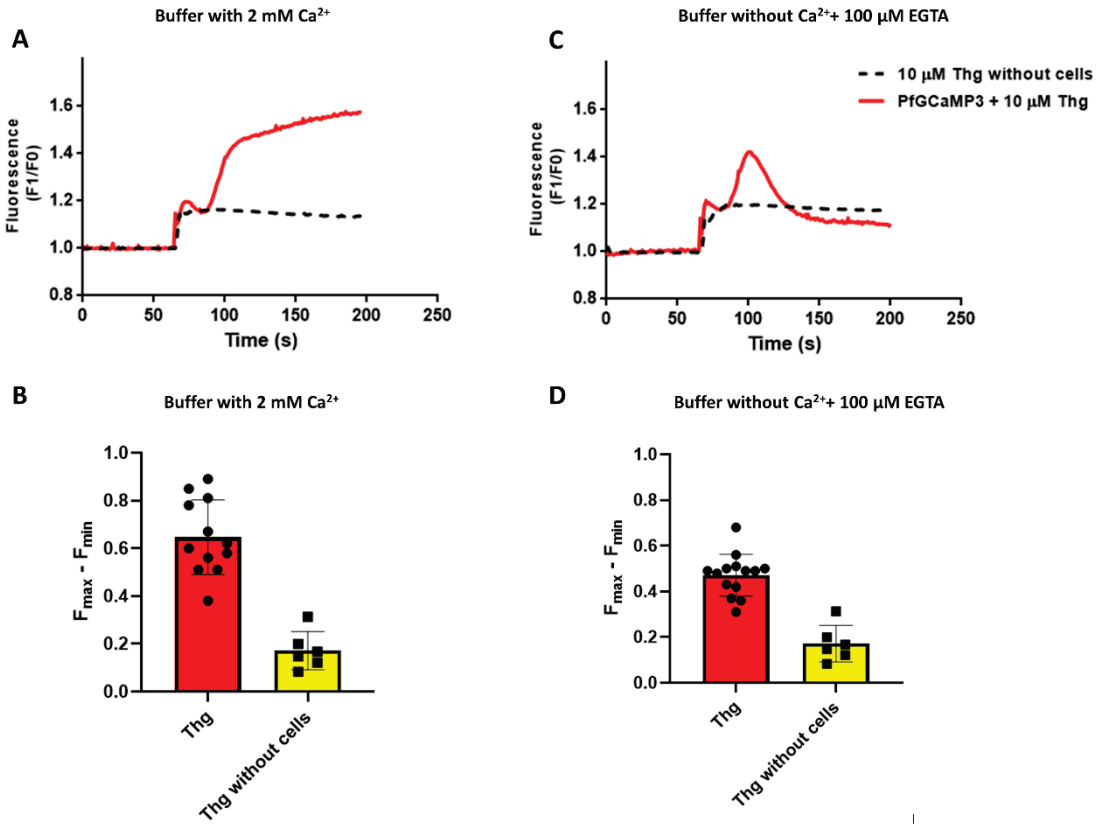

Fig. S1 Thg causes an addition artefact when added in the spectrofluorometer cuvette. Ten µM Thg was added in the spectrofluorometer cuvette in the absence of PfGCaMP3 parasites (buffer only). Thg *per se* showed a fluorescence artefact (dotted lines). This artefact was compared with the Ca^{2+} response elicited by Thg in PfGCaMP3 parasites (red lines) in the presence (A-B) and absence (C-D) of extracellular Ca^{2+}.

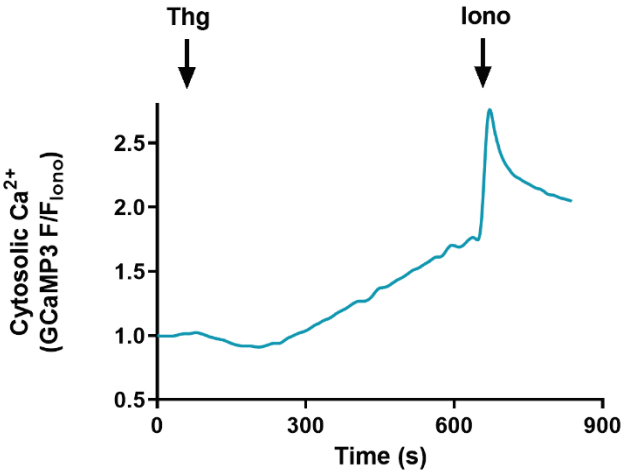

Fig. S2 Cell population average of single cell $[Ca^{2+}]_c$ responses to Thg.
Averaged $[Ca^{2+}]_c$ trace from 43 single trophozoite-stage *P. falciparum* parasites expressing GCaMP3 (PfGCaMP3). Cells were challenged with 5 µM Thg in the presence of extracellular Ca^{2+} (2 mM), followed by 10 µM Ionomycin (Iono). Responses in individual cells comprise complex oscillatory changes in $[Ca^{2+}]_c$ (see Fig. 4 C and F), whereas the population average is similar to fluorimeter cell population experiments (Figs. 1-3), albeit with a longer delay before the response, presumably because there is no mixing of drugs in the imaging experiments.

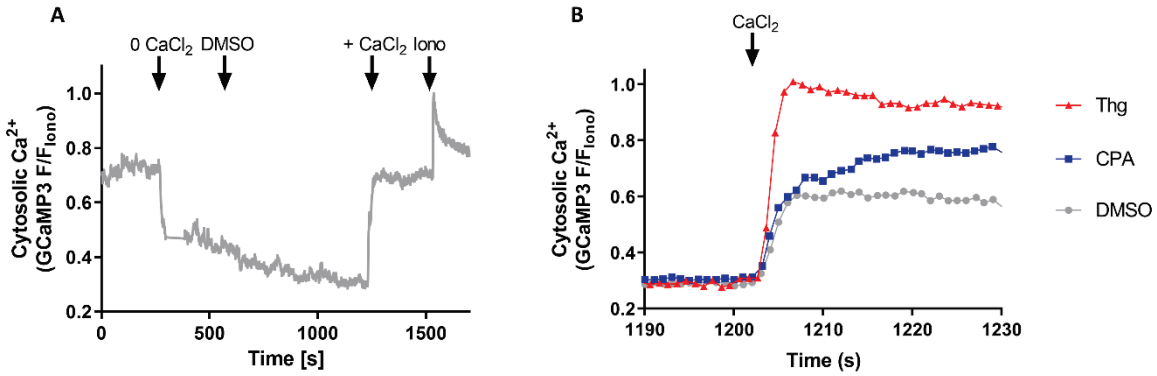

Fig. S3 Removal of extracellular Ca^{2+} reduces $[Ca^{2+}]_c$ to a new steady state in isolated *P. falciparum*. (A) Representative DMSO vehicle control trace to show the change in $[Ca^{2+}]_c$ upon removal and add back of extracellular Ca^{2+} in trophozoite-stage PfGCaMP3 parasites. Ionomycin (10 µM, Iono) was added at the end of each experiment. (B) Representative traces of the rise in $[Ca^{2+}]_c$ following addback of 2 mM Ca^{2+} after removal of extracellular Ca^{2+} and 10 min treatment with DMSO vehicle, 10 µM CPA or 5 µM Thg). The extracellular Ca^{2+} washout period shown in the first 400 s of panel A was omitted for the traces in Figure 4. Following addback of 2 mM $CaCl_2$, $[Ca^{2+}]_c$ returned to basal levels in DMSO- and CPA-treated parasites. By contrast, Thg-treated parasites showed a larger amplitude and faster rate of $[Ca^{2+}]_c$ rise, consistent with SOCE. All data are representative of 3 independent experiments and responses were normalized to the peak ionomycin (10 µM, Iono) response (F/F_{Iono}).