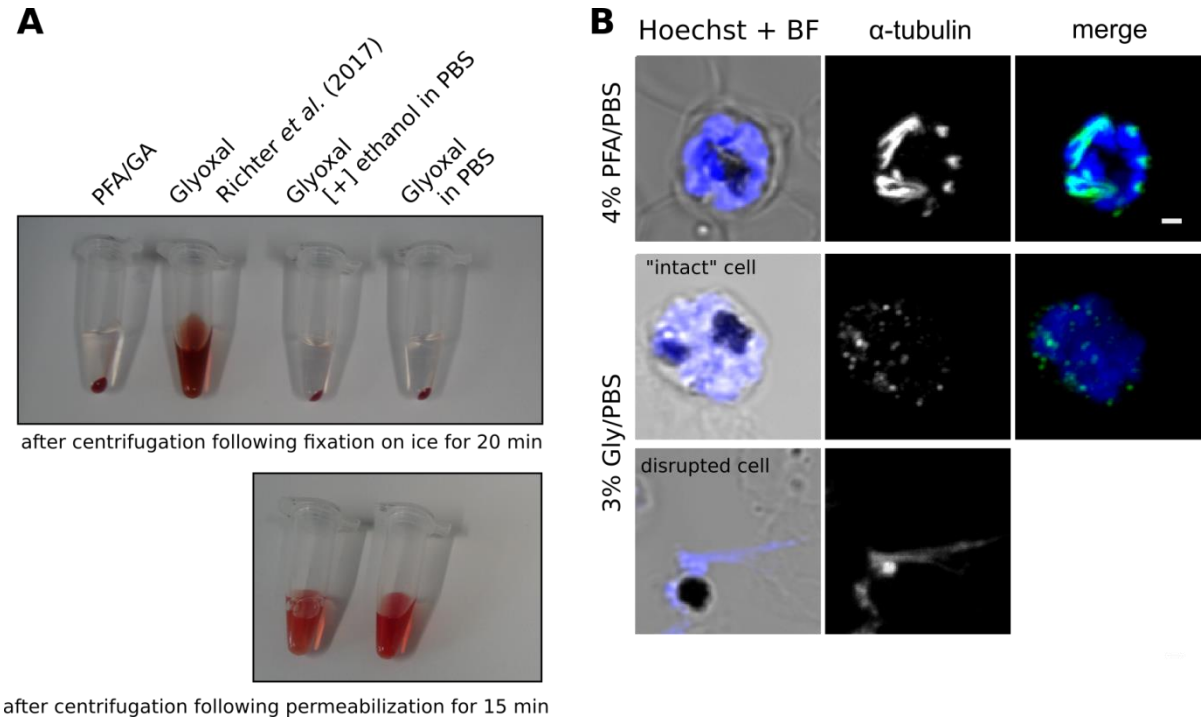
Supplemental Figures and Tables

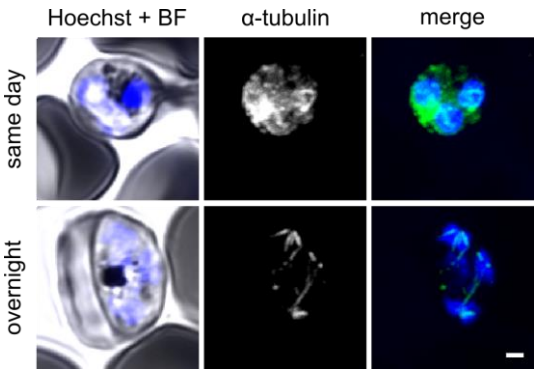


Supplemental Figure 1. Fixation by glyoxal in solution and on Lab-Teks.
Different glyoxal fixation solutions were tested on iRBCs in solution (A) or on Lab-Teks (B–C). If not indicated otherwise, fixation was performed on ice. (A) Exemplarily, the results of fixation by the following solutions are shown: 4% PFA/0.0075% GA in PBS, 3% glyoxal fixation solution according to Richter et al., 3% glyoxal/20% ethanol in PBS, and 3% glyoxal in PBS. Photos were taken after centrifugation following fixation on ice for 20 min (upper panel). Non-lysed suspensions were subjected to subsequent permeabilization and centrifugation (lower panel). (B) Confocal images of iRBCs fixed either by 3% glyoxal in PBS on ice or by 4% PFA in PBS on Lab-Teks at 37 °C. Cells were labeled with anti-α-tubulin antibody and DNA was stained with Hoechst. All images except brightfield (BF) are maximum intensity projections. Scale bar, 1 µm.

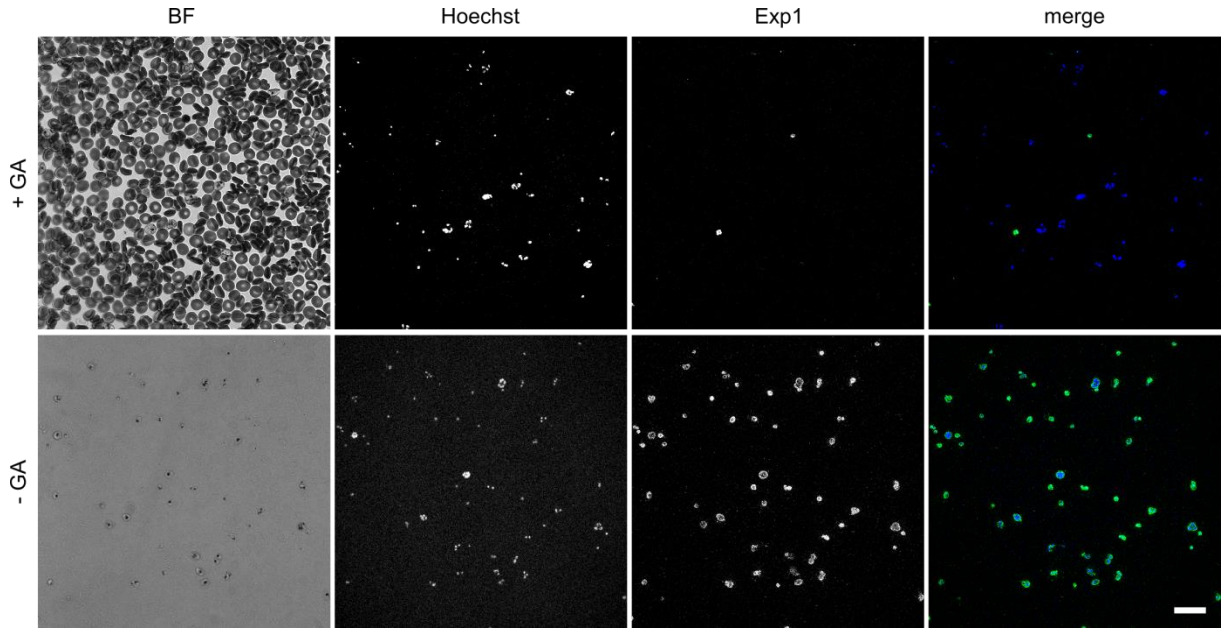
Supplemental Table 1: Tested glyoxal fixation solutions and conditions.

Fixation solution	pH	Temperature	Observation
3% glyoxal [+] 20% ethanol [+] 0.75% acetic acid in H_2O	4–5	on ice room temperature 37 °C	immediate RBC lysis immediate RBC lysis immediate RBC lysis
3% glyoxal [+] 20% ethanol [+] 0.75% acetic acid [+] 0.0075% glutaraldehyde in H_2O	4–5	37 °C	RBC lysis during fixation
3% glyoxal [+] 0.75% acetic acid in H_2O	n/a	37 °C	RBC lysis during fixation
3% glyoxal [+] 0.75% acetic acid [+] 0.0075% glutaraldehyde in H_2O	n/a	37 °C	RBC lysis during fixation
3% glyoxal [+] 20% ethanol [+] 0.75% acetic acid [+] 0.9% NaCl in H_2O	4–5	on ice 37 °C	immediate RBC lysis immediate RBC lysis
3% glyoxal [+] 20% ethanol in PBS	6	on ice 37 °C	RBC lysis during PBS wash after fixation RBC lysis during fixation
3% glyoxal in PBS	6.5	on ice 37 °C	RBC lysis upon addition of 0.1% Triton X-100/PBS RBC lysis during PBS wash after fixation

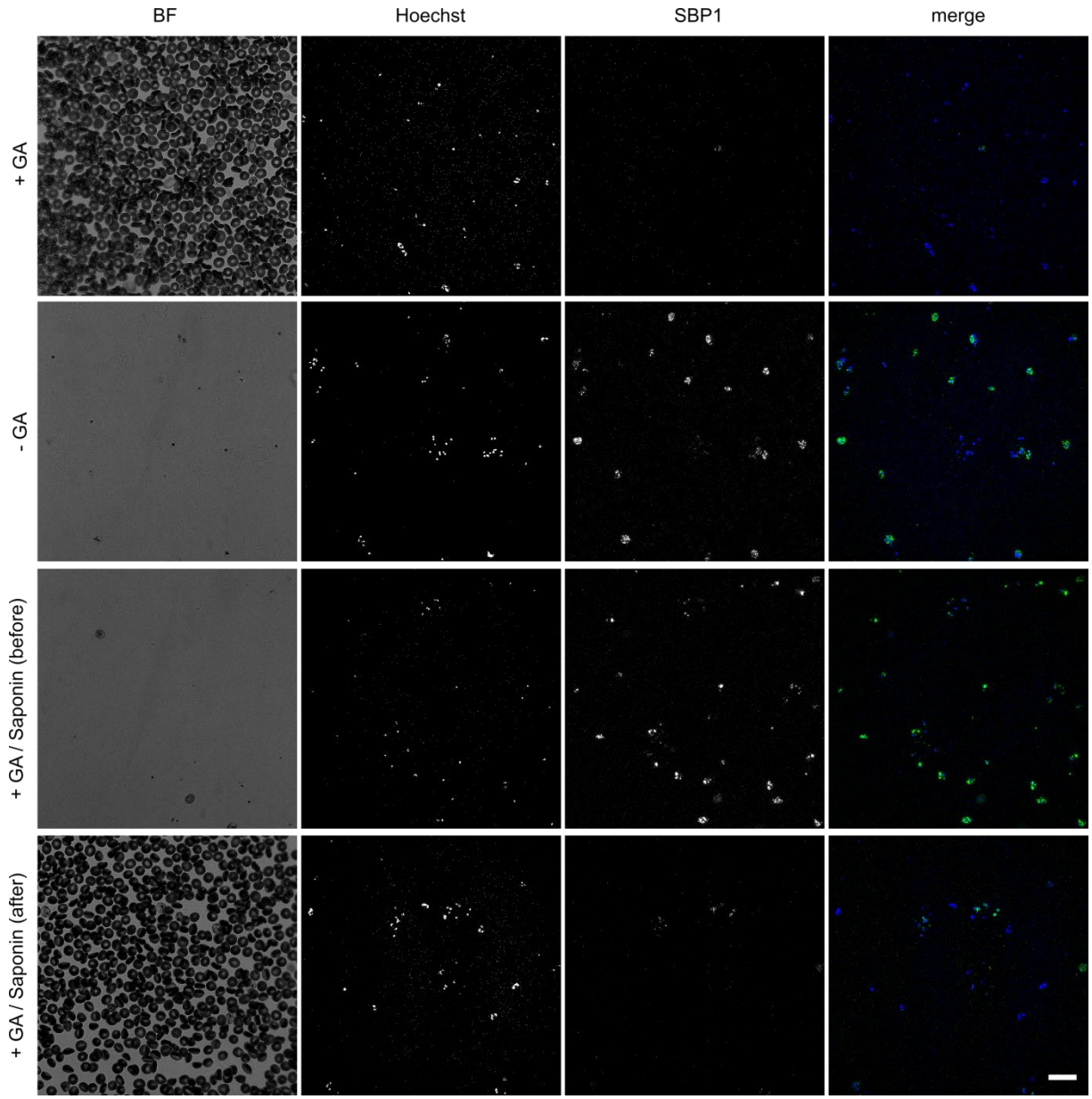
Resume of the different conditions that have been tested on iRBCs, based on the glyoxal fixative formulation introduced by Richter et al. 2018 None of them yielded satisfactory cellular preservation.



Supplemental Figure 2. Overnight seeding is required to detect spindle microtubules.
Infected RBCs were either seeded shortly before fixation (upper panel) or the day before (lower panel). Parasites were labeled with anti-α-tubulin antibody to visualize mitotic spindles after fixation with GA. Image acquisition settings, i.e. laser power, were adjusted accordingly to allow signal visibility despite fixation with GA. All images except brightfield (BF) are maximum intensity projections. Scale bar, 1 µm.

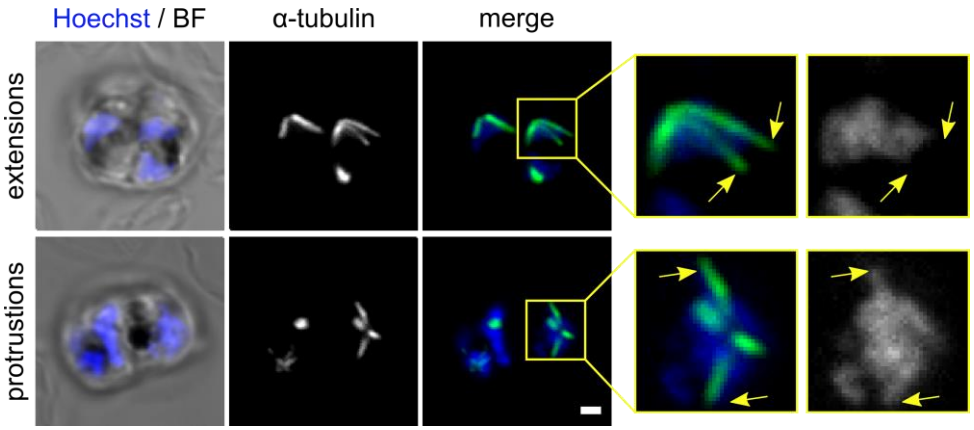


Supplemental Figure 3. Labeling density of iRBCs with and without GA.
Overview image of iRBCs stained with Hoechst and labeled with anti-Exp1 antibody after either fixation with (upper panels) or without GA addition (lower panels). All images are maximum intensity projections and contrast adjustment is identical for both conditions. Scale bar, 20 µm.



Supplemental Figure 4. Labeling density of iRBCs with Saponin permeabilization.
Overview image of iRBCs stained with Hoechst and labeled with anti-SBP1 antibody after either fixation with GA, without GA, with GA pretreated with 0.05% Saponin 30 sec, and with GA posttreated with 0.05% saponin for 5min. All images are maximum intensity projections and contrast adjustment is identical for all conditions. Scale bar, 20 µm. Table below shows quantification of labeling density for all conditions.

Fixation\Antibody	anti-SBP1
-GA	99.5% (189/190)
+GA	2.6% (3/117)
+GA Saponin pretreatement	89.8% (158/176)
+GA Saponin posttreatement	7.8% (19/241)



Supplemental Figure 5. Nuclear protrusions and tubulin extension across the nuclear border. Hemispindle microtubules stained by anti-α-tubulin antibody were occasionally found to extend across the nuclear border, approximated by Hoechst staining (upper panel). This could additionally be accompanied by protrusions in the Hoechst staining (lower panel). Extensions and protrusions are exemplarily marked by yellow arrows. Images are single slices acquired by confocal microscopy. Scale bar, 1 µm.

Supplemental Table 2: List of used antibodies and dilutions

Antibody target	Species	Source	Dilution
Exp1	Rabbit	Jude Przyborski	1:500
α-tubulin	Mouse	Sigma-Aldrich (T5168)	1:500
*Pf*Nup116	Rabbit	Julien Guizetti	1:50
SBP1	Rabbit	Jude Przyborski	1:500
*Pb*HSP70	Mouse	Ann-Kristin Mueller	1:100
Cdc48	Rabbit	Jude Przyborski	1:500
Centrin	Mouse	clone 20H5, Merck (04-1624)	1:1000
Anti-mouse ATTO 594	Goat	Sigma (76085-1ML-F)	1:200–1:500
Anti-rabbit ATTO 594	Goat	Sigma (77671-1ML-F)	1:200–1:500
Anti-mouse ATTO 647N	Goat	Sigma (50185-1ML-F)	1:200–1:500
Anti-rabbit ATTO 647N	Goat	Sigma (40839-1ML-F)	1:200–1:500