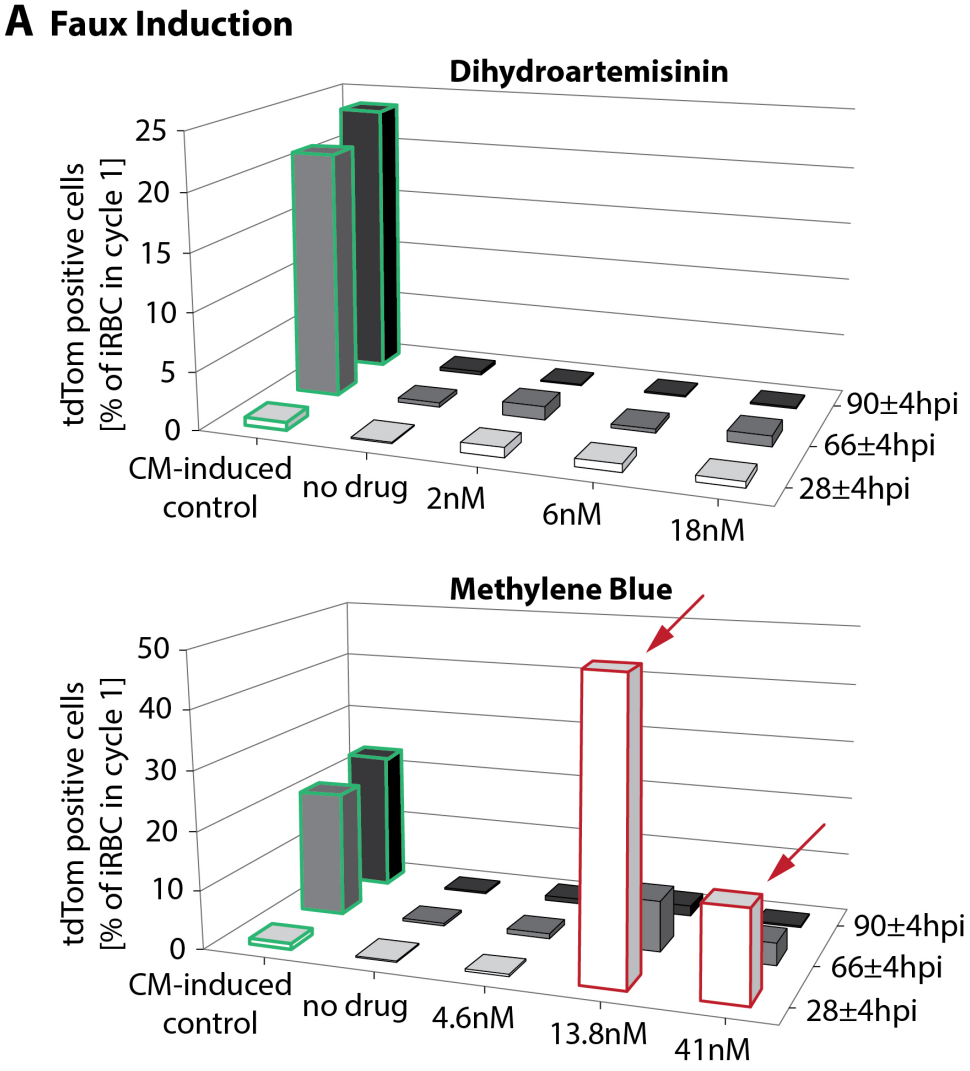
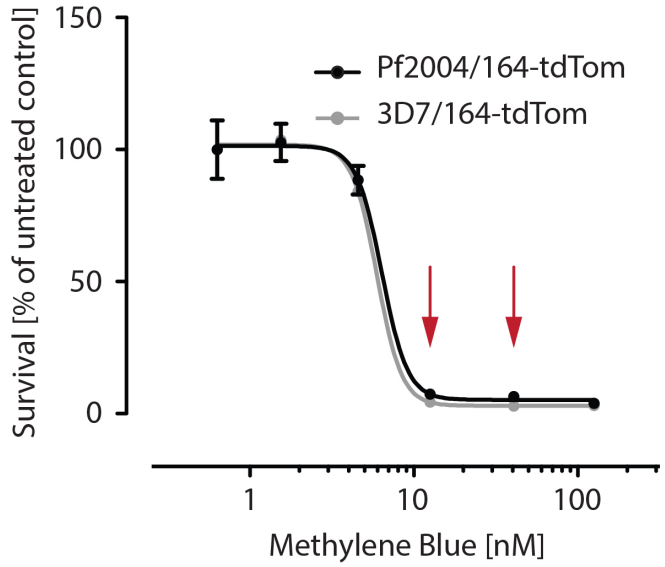
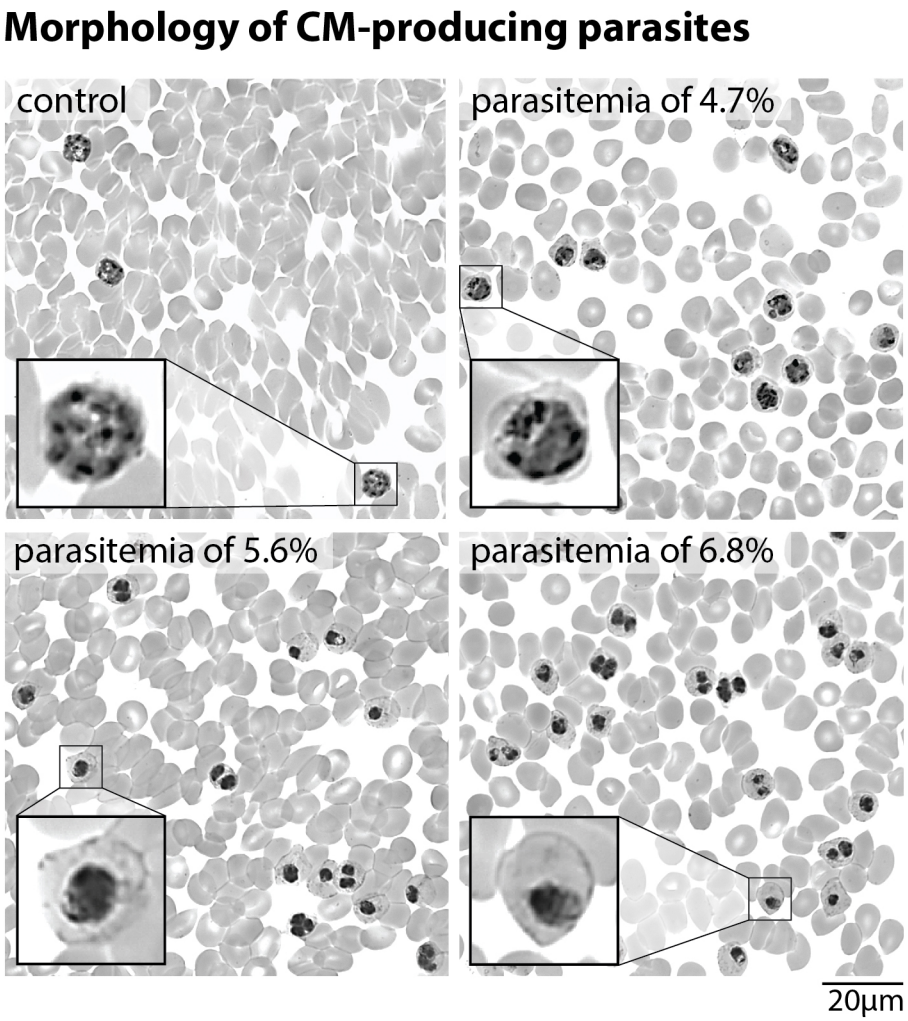

A Faux Induction
Dihydroartemisinin
tdTom positive cells [% of iRBC in cycle 1]
25
20
15
10
5
0
CM-induced control
no drug
2nM
6nM
18nM
90±4hpi
66±4hpi
28±4hpi
Methylene Blue
tdTom positive cells [% of iRBC in cycle 1]
50
40
30
20
10
0
CM-induced control
no drug
4.6nM
13.8nM
41nM
90±4hpi
66±4hpi
28±4hpi


B Dose response curve for Methylene Blue
Survival [% of untreated control]
150
100
50
0
Pf2004/164-tdTom
3D7/164-tdTom
1
10
100
Methylene Blue [nM]

Supplementary Figure 1

Cytostatic drugs cause a faux activation of the tdTomato reporter.

A. Bar graphs showing the proportion of red fluorescent parasites (Pf2004/164-tdTom) after treatment with different concentrations of dihydroartemisinin (upper panel) and methylene blue (lower panel). Except for the CM-induced control parasites, cells were cultured in absence of conditioned medium, i.e. under conditions that do not induce gametocyte production. High concentrations of methylene blue induce a short-lived activation of the tdTom reporter (highlighted in red). Note that this faux induction precedes proper reporter expression in early gametocytes of the control cells (highlighted in green). Drugs were added for 24 hours (from 28±4hpi of the preceding IDC to 4±4hpi). Bars show the mean of technical triplicates **B**. Effect of methylene blue on asexual parasite multiplication (mean ± SEM, n=3). Concentrations triggering a faux induction of the tdTom reporter are highlighted.



Supplementary Figure 2

Conditions during CM production hamper parasite development.

Diff-Quick stained blood smears show the morphology of cells used to produce CM. Smears were made at the time point of CM collection (44±4hpi). Parasitemia is indicated. Note that the development of CM-producing parasites is delayed compared to control cells kept at a low parasitemia. In the assay, highest rates of sexual conversion were achieved by using CM produced from cells at a parasitemia of 5.6% (data not shown).