Supporting Information

Next generation chemiluminescent probes for antimalarial drug discovery

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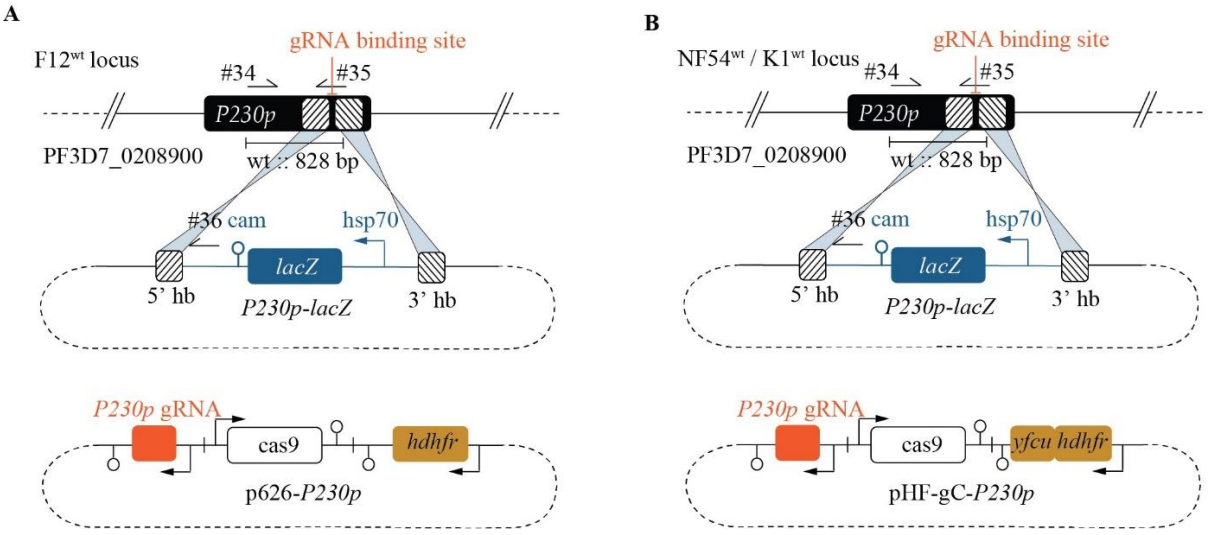


Figure S1. Overview of the two-plasmid CRISPR/Cas9-based gene editing strategy. (A) Schematic of the *P230p* locus in wild-type F12 parasites and the plasmids used for CRISPR/Cas9-mediated gene editing (*P230p-lacZ* and p626-*P230p*) to generate the F12^{lacZ} parasites. **(B)** Wild-type *P230p* loci in NF54 and K1 parasites and the plasmids used for CRISPR/Cas9-mediated gene editing (*P230p-lacZ* and pHF-gC-*P230p*) to generate the NF54^{lacZ} and K1^{lacZ} parasites. The heterologous expression cassette inserted into the *P230p* locus consists of an *hsp70* promoter, the *lacZ* gene and a *cam* terminator. Arrows indicate primers used for diagnostic PCRs. Striped boxes represent the homology regions used for CRISPR/Cas9-editing. The guideRNA binding site is indicated. *cas9*, recombinase; *yfcu*, negative selection marker[1] (yeast cytosine deaminase and uridyl phosphoribosyl transferase); hb, homology boxes; wt, wild-type.

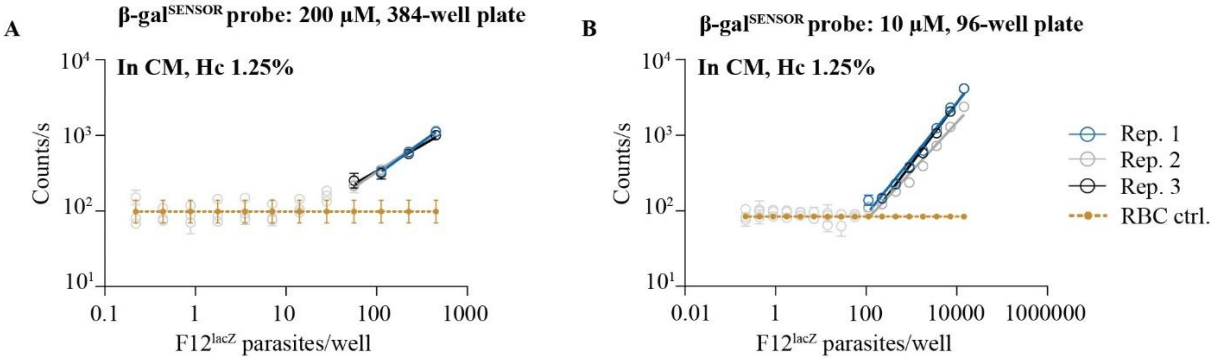


Figure S2. The β-gal^{SENSOR} probe allows detecting low numbers of F12^{lacZ} parasites under assay conditions. **(A)** Mixed-stage F12^{lacZ} parasites are detected at low numbers (~50 parasites/well) when incubated with β-gal^{SENSOR} probe at 200 µM in CM with a Hc of 1.25% and emitted chemiluminescence linearly correlates with parasite numbers. **(B)** Compared to (A), the LOD increases (~200 parasites/well) when reducing the β-gal^{SENSOR} probe to 10 µM and performing the experiments in 96-wel plates (assay conditions). n = 3; biological replicates (rep.) are shown individually with error bars indicating standard deviations of technical replicates; for the control samples (ctrl.), biological replicates are averaged and error bars indicate standard deviations of the mean.

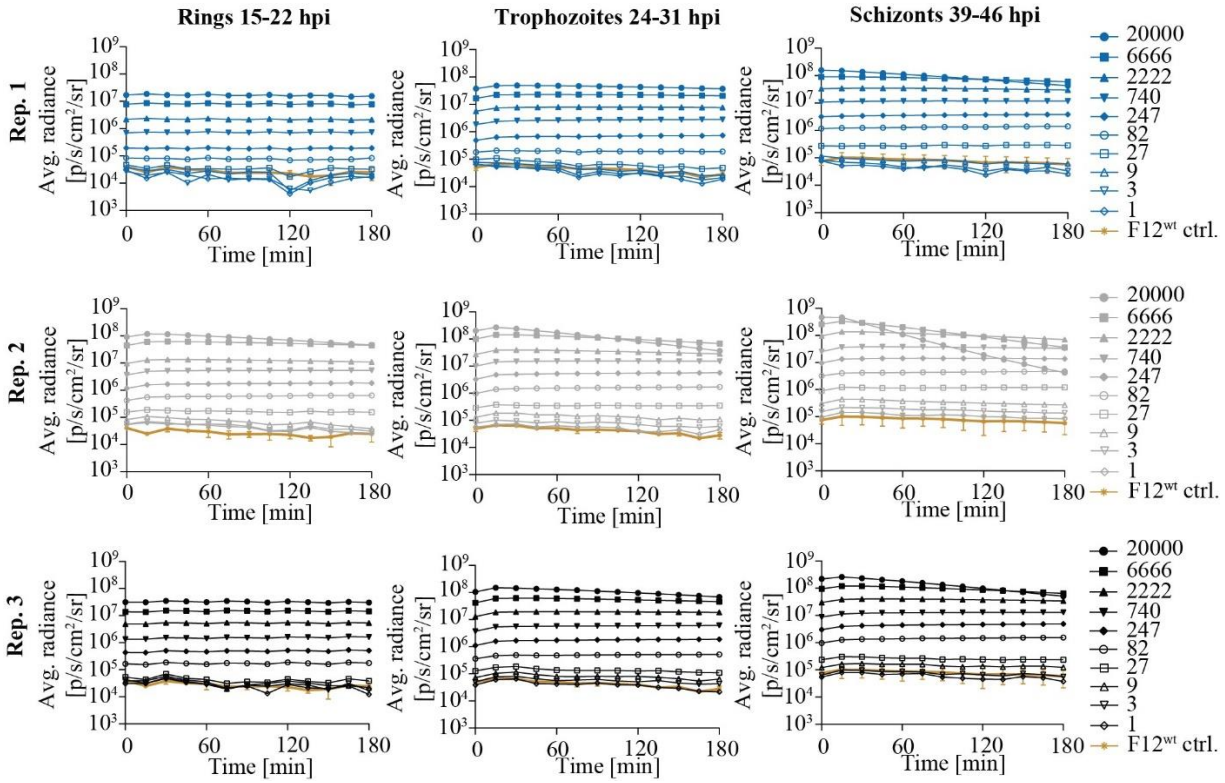
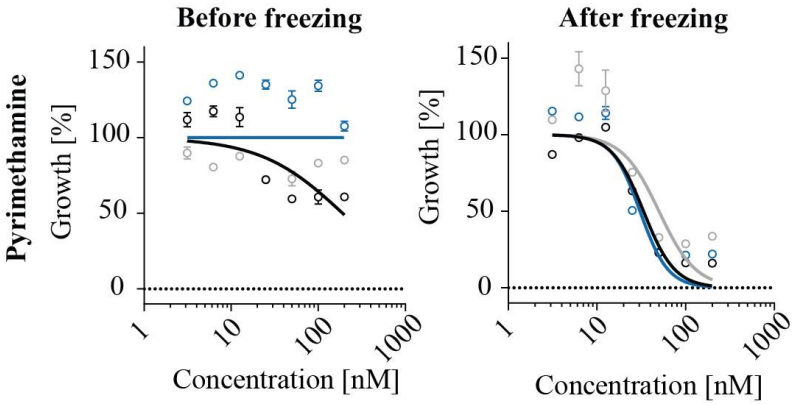
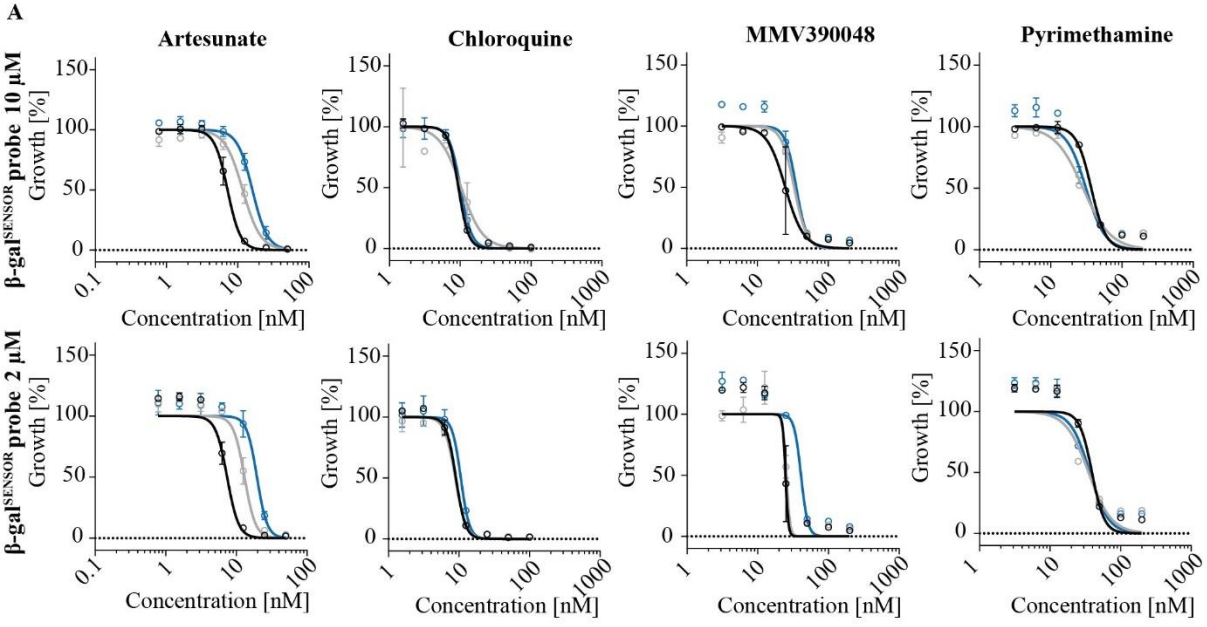


Figure S3. Luminescence emitted from F12^{lacZ} parasites remains stable. Following addition of the β-gal^{SENSOR} probe, luminescence flux is stable over a wide range of absolute parasite numbers for at least 180 min. Parasite numbers used per condition are indicated. 20`000 wild-type parasites (F12^{wt}) were used as a negative control sample. n = 3.



IC_{50} β-gal^{SENSOR} probe assay		
Pyrimethamine	**Before freezing**	**After freezing**
Rep. 1	-	30.51
Rep. 2	-	49.99
Rep. 3	-	33.44

Figure S4. Freezing of the β-gal^{SENSOR} probe assay plates prior to the readout is strongly recommended. For pyrimethamine, it was not possible to calculate the IC_{50} values when the assay plates were not frozen prior to the readout with 10 µM β-gal^{SENSOR} probe. Biological replicates (rep.) are shown individually. IC_{50} values [nM] are indicated.



	IC$_{50}$ [nM] F12^{lacZ}							
Drug	**Artesunate**		**Chloroquine**		**MMV390048**		**Pyrimethamine**	
β-gal^{SENSOR}	**10 µM**	**2 µM**	**10 µM**	**2 µM**	**10 µM**	**2 µM**	**10 µM**	**2 µM**
Rep. 1	7.19	7.39	9.44	9.03	24.72	24.68	36.99	39.22
Rep. 2	11.77	13.03	10.32	8.83	32.93	25.41	28.34	35.09
Rep. 3	16.06	19.55	10.06	10.58	35.30	40.89	31.17	36.44
Avg. IC$_{50}$	11.67	13.32	9.94	9.48	30.98	30.33	32.17	36.92
SD IC$_{50}$	3.62	4.97	0.37	0.78	4.53	7.48	3.60	1.72

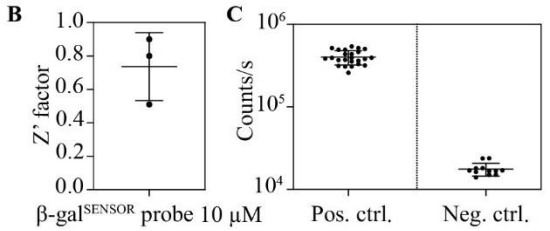


Figure S5. *lacZ*-expressing parasites allow monitoring dose-responses at different β-gal^{SENSOR} probe concentrations. (A) Comparison the F12^{lacZ}/β-gal^{SENSOR} probe assay formats using either 10 or 2 µM β-gal^{SENSOR} probe. Biological replicates (Rep.) are shown individually. IC$_{50}$ values [nM] are indicated. Avg., average; SD, standard deviation. **(B)** Assay robustness using β-gal^{SENSOR} probe at 10 µM determined by the Z'-factor. n = 3. **(C)** Luminescence derived from F12^{lacZ} parasite cultures lysed at the start of the experiment (negative control) and untreated F12^{lacZ} following the 72-hour incubation period (positive control).

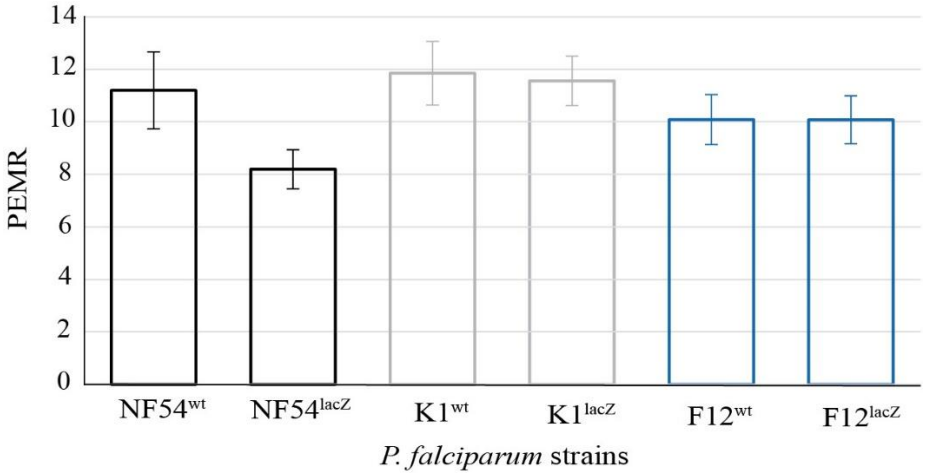


Figure S6: Parasitized Erythrocyte Multiplication Rate (PEMR) is comparable among parental *P. falciparum* strains and transgenic strains. n = 3. wt, wild type.

Table S1. The β-gal^{SENSOR} probe IC_{50} assay is cost effective. The price of one IC_{50} assay plate (96-well format) using the *lacZ*/β-gal^{SENSOR} probe 2 µM system can be reduced by one third compared to the [^{3}H]-hypoxanthine incorporation assay.

	IC_{50} assay costs per plate	
Currency: USD	**[^{3}H] incorporation**	**β-gal^{SENSOR} probe 2 µM**
Drug preparation	3.29	3.31
Plate preparation	9.15	12.85
Readout reagent	12.86	5.88
Readout consumables	7.82	-
Total per plate	33.12	22.04

References

1. Manzoni G, Briquet S, Risco-Castillo V, Gaultier C, Topçu S, Ivănescu ML, Franetich JF, Hoareau-Coudert B, Mazier D, Silvie O. A rapid and robust selection procedure for generating drug-selectable marker-free recombinant malaria parasites. Sci Rep. 2014;4:4760. doi:10.1038/srep04760.