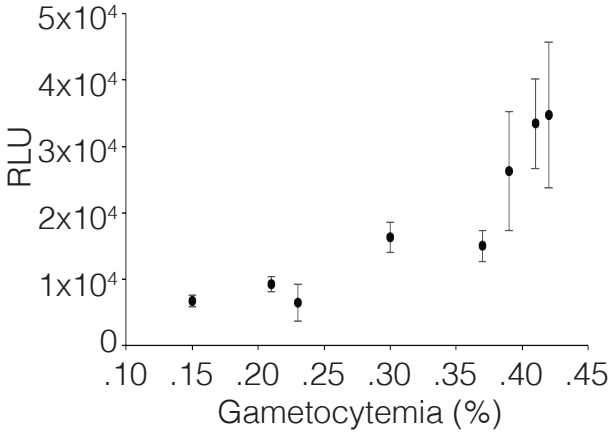
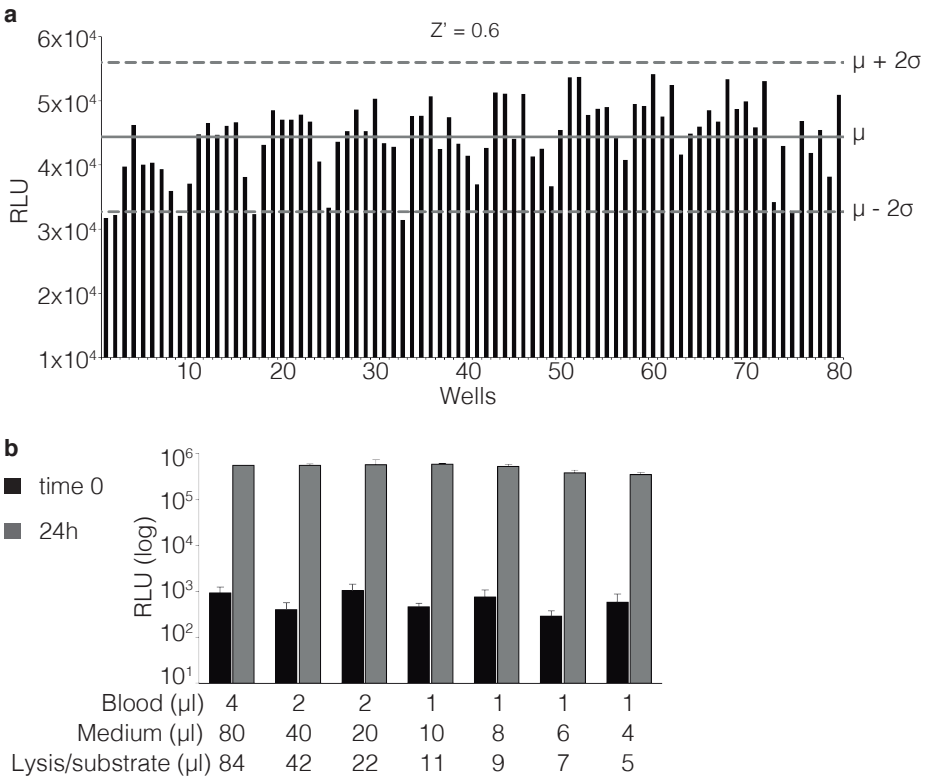
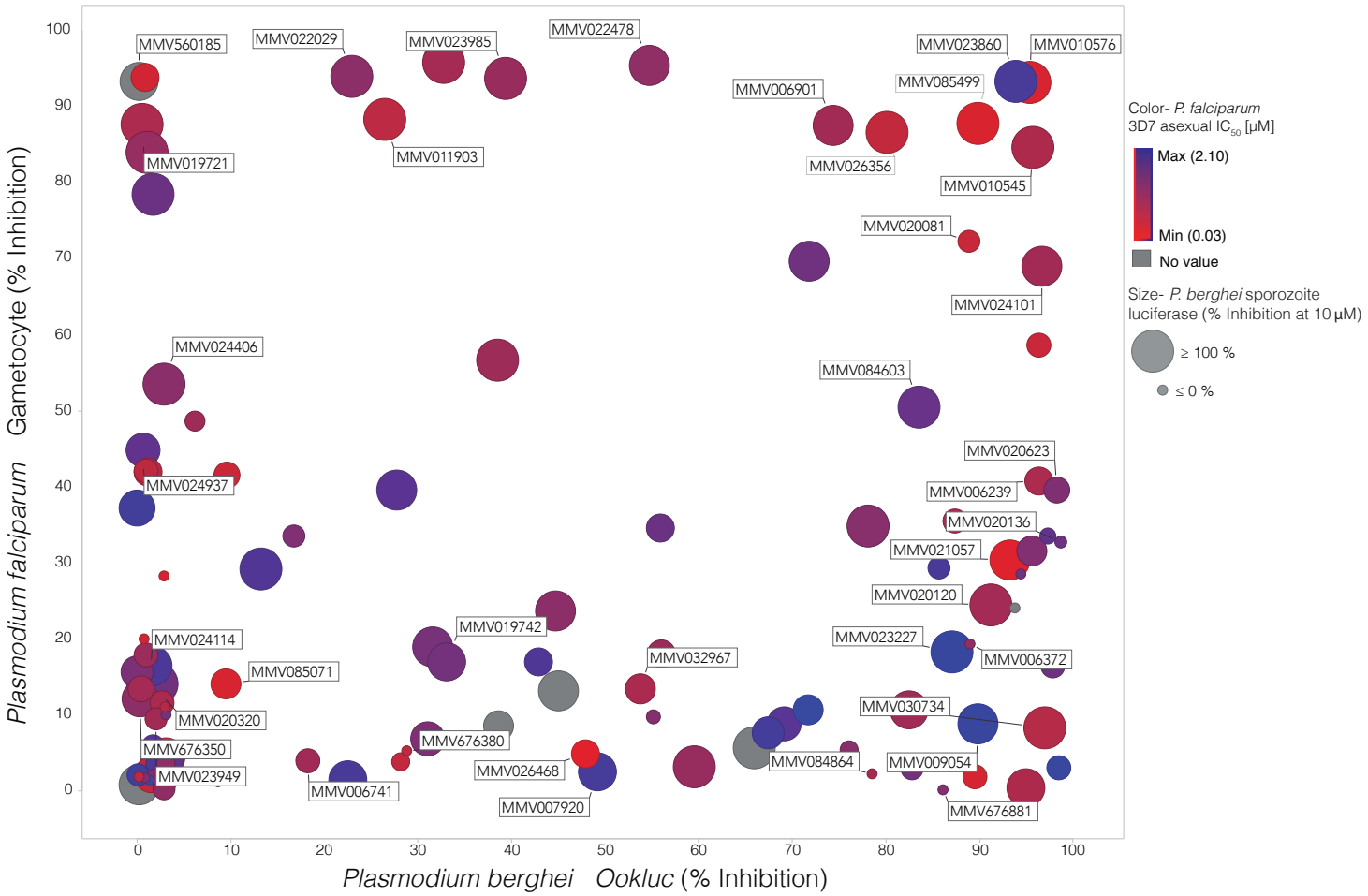
Supplementary Figure 1: Impact of the gametocytemia in *Ookluc* conversion.



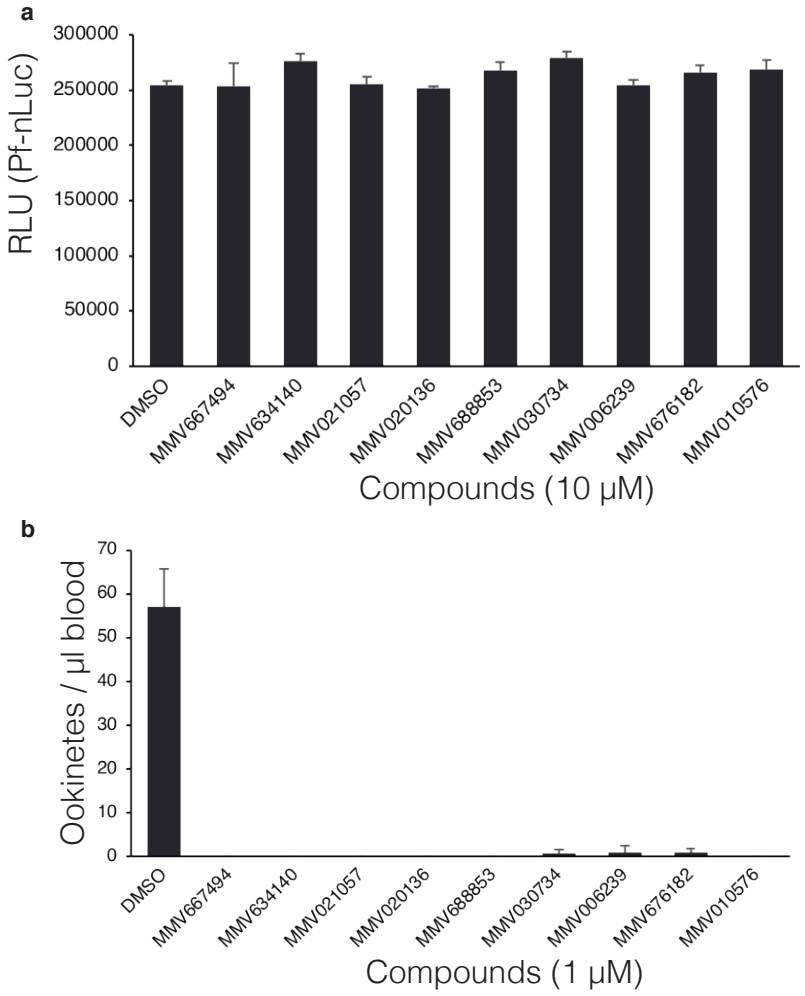
Supplementary Figure 2: *Ookluc* is compatible with HTS.



Supplementary Figure 3: Multidimensional scatterplot of *P. berghei Ookluc* activity compared to other datasets



Supplementary Figure 4: The most potent compounds do not inhibit nLuc activity in asexual stages of *P. falciparum* and specifically block ookinte formation in *P. berghei* conversion



Supplementary figure legends

Supplementary Figure 1. Impact of the gametocytemia in *Ookluc* conversion. nLuc activity, expressed as mean ± SD of Relative Light Units (RLU), after 24 hr of conversion assay with *Ookluc* from mouse donors with different gametocytemia. Results are representative of 3 independent experiments.

Supplementary Figure 2. *Ookluc* is compatible with HTS. a. Distribution of nLuc activity in a semi-HT conversion assay using a 96-well plate. Each bar represents the nLuc activity, expressed as Relative Light Units (RLU), in one well after 24 hours' conversion assay. The horizontal line represents the mean (μ) and the dashed lines represent ± two standard deviations (2σ). The calculated Z-factor is shown. **b.** Conversion assays were set using different volumes of blood, medium and lysis/substrate, and nLuc activities in time zero (black bars) or after 24 hours (grey bars) are represented as Relative Light Units (RLU) for each condition.

Supplementary Figure 3. Multidimensional scatterplot of *Plasmodium berghei Ookluc* activity compared to other datasets. Comparative activity of the MMV Pathogen Box malarial specific compounds screened previously within the MMV Malaria Box. Shown is the percent inhibition of the select compounds utilizing the *Ookluc* assay screened at a single 10 *μ*M concentration. Also shown is the percent inhibitory activity, again at a single 10 *μ*M concentration, against *P. falciparum* NF54 stage V gametocytes. Color indication reflects the IC50 against *P. falciparum* 3D7 asexual stage parasites with red representing higher potency (0.03 *μ*M) and blue representing lower potency compound activity (2.10 *μ*M). Size denotes activity in a *P. berghei* sporozoite liver stage assay (% inhibition at 10 *μ*M single concentration) with the largest size representing 100% inhibition and the smallest representing no detectable inhibition against the liver stage.

Supplementary Figure 4. The most potent compounds do not inhibit nLuc activity in asexual stages of *P. falciparum* and specifically block ookinete formation in *P. berghei* conversion. a. Samples of a culture of *Pf*-nLuc with 1%

parasitemia were incubated with the indicated compounds at a single 10 μM concentration, in triplicates, for 90 min prior to determination of nLuc activity, expressed as mean + SD of Relative Light Units (RLU). **b.** Ookinete counts (mean + SD) after 24 hr conversion assays of *P. berghei* in the presence of 1 μM of the indicated compounds. Results are representative of 2 independent experiments.