

## Legends for Supplementary Material Figures:

### Supplementary Figure 1: NOS activity in *P. falciparum* gametocytes.

About  $2.4 \times 10^7$  3D7 stage II to IV gametocytes or unparasitized erythrocytes were homogenized and incubated for 30 min at 37 °C in a reaction buffer mixture containing [ $^3\text{H}$ ] L-arginine, NADPH and calcium in Tris-HCl buffer at pH 7.4. Results are expressed as a ration between eluted [ $^3\text{H}$ ] L-citrulline and unreacted [ $^3\text{H}$ ] L- arginine.

### Supplementary Figure 2: Genes possibly related for NO synthesis in *P. falciparum*.

Relative and absolute expression levels of PFL1555w and PF13\_0535 in intraerythrocytic 3D7 cultures (source: Plasmo DB: <http://plasmodb.org/plasmo/home.jsp>). Relative expression level (percentiled) of the genes intensity is compared relative to all other genes for a given experiment; absolute expression is the affimetrix MOID expression value normalized by experiment level. Green bars represent stage synchronization using sorbitol, purple bars represent stage synchronization by temperature and gray bars represent expression level that is close to the background. Abbreviations: ER, early rings; LR, late rings; ET, early trophozoites; LT, late trophozoites; ES, early schizonts; LS, late schizonts; M, merozoites; S, sporozoites; G, gametocytes.

### Supplementary Information 3:

The  $1359 \text{ cm}^{-1}$  shoulder in this spectrum is characteristic of ferrous high spin pentacoordinate. It is likely the result of reversible  $\{\text{FeNO}\}^7$  photolysis in the laser beam, a common property of heme carbonyl and heme nitrosyl complexes. It should be noted that, even though heme carbonyls and heme nitrosyls are formally considered to be Fe(II) complexes, the frequencies of their  $\nu_4$  bands fall within the range typical of Fe(III) complexes. This high frequency is attributed to  $\pi$  acid CO and NO ligands withdrawing  $\pi^*$  electron density from the

porphyrin ring. So, except for the small aforementioned buildup of the ferrous photolysis product, the spectra in traces **D-F** of **Fig 4** are clearly indicative of pentacoordinate heme nitrosyl. As hemoglobin is internalized by the FVs, the possibility of trace hemoglobin inside the FV cannot be ruled out. If present in sufficiently high concentration, hemoglobin would interfere with the rR spectrum of hemozoin and/or heme-NO. However, the rR spectra of the forms of hemoglobin would be distinct from the hemozoin or the heme-NO complexes discussed herein. None of these forms would contribute to the pentacoordinate HS ferric signature that dominates the spectra in **Figs 4A** and **4B**; oxy-hemoglobin has a low spin (LS) ferric signature, deoxy-hemoglobin has a pentacoordinate high spin (HS) ferrous signature, and methemoglobin gives a hexacoordinate HS ferric signature (Jin, et al., 2004). Thus, even if it is present, the dominance of 5c HS features in the spectrum of the untreated vacuoles suggests that hemoglobin is not contributing detectably to the spectrum. It is hence concluded that the heme responsible for the rR spectral features in **Fig 4** is from hemozoin.