SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Nuclear positioning of an unrelated active gene (PFF1125c) to a *var* locus in different activation states. Nuclei are stained with DAPI (blue). Two different microscopic fields are shown for each experimental condition. A. Nuclear positioning of actively transcribed PFF1125c (red) and a silent *bsd* cassette under control of a chromosomal *var* promoter (green). B. Nuclear positioning when both PFF1125c (red) and the endogenous *bsd* cassette (green) are actively expressed. C. Quantification of co-localization shown in A and B. Error bars display standard deviations of multiple, independent counts.

Figure S2: Nuclear positioning of two simultaneously active *var* promoters by DNA-FISH. Nuclei are stained with DAPI (blue). Nuclear positioning of actively transcribed pVLH/IDH-FP episomes (red) and of an active *bsd* cassette under control of a chromosomal *var* promoter (green) is shown.

SUPPLEMENTAL METHODS:

Plasmid Constructs

The *bsd* cassette was recovered from pVBb/IDH(Dzikowski et al., 2006) and cloned into pVLH to create pVBH. The *hrp2* promoter was cloned from pHLH(Wu et al., 1995) into pVLH to create pVLH/*hrp.* The *hsp86* and *rRNA* promoters were amplified from *P. falciparum* NF54 DNA and cloned into pVLH to create pVLH/*hsp86* and pVLH/rRNA, respectively.

Fluorescent in situ hybridization (FISH)

DNA FISH technique was carried out on ring stage parasites as described by Freitas-Junior *et al*.(Freitas-Junior et al., 2000) with slight modifications. RNA FISH was performed on ring stage parasites as previously described by Thompson *et al.*(Thompson, 2002) with minor changes. DNA probes were labeled with biotin and fluorescein using Roche High-Prime Kits. Biotin was detected using streptavidin AlexaFluor 594 (Molecular Probes). RNA probes were labeled using a Promega Riboprobe in vitro Transcription Kit and fluorescein-12-UTP and DIG-11-UTP (Roche). DIG was detected using sheep anti-DIG antibody followed by donkey anti-sheep-antibody AlexaFluor 594 (Molecular Probes). After washing, the slides were mounted in antifade medium and visualized using an Olympus M081 fluorescent microscope. For DNA FISH, slides were blindly counted by three different individuals using over 100 nuclei for each experiment. Composite images were produced using Photoshop 6.0 and the images collated.

Nuclear positioning of two *var* genes: To label the endogenous transgenic *var* gene a *bsd* DNA probe (450 bp) was labeled with fluorescein. A biotin-conjugated DNA probe complementary to the Bluescript plasmid (3000 bp) was generated for detection with streptavidin conjugated with AlexaFluor 594 to detect the transfected episomes.

Nuclear positioning of PFF1125c and a *var* gene: to compare the rate of co-localization of two *var* genes to the rate of co-localization of a *var* gene and an unrelated gene we chose to probe for PFF1125c, an RNA-binding protein *mei2* homologue which is expressed throughout the cell cycle (http://www.plasmodb.org). We confirmed the reported micro-array expression profile by Q-RT-PCR. A biotin-conjugated DNA probe

complementary to the sequence of that gene (Chromosome 6, bp 950224-951507) was generated for detection with streptavidin conjugated with AlexaFluor 594.

For production of RNA probes, *bsd* (450 bp) and *luciferase* (1300 bp) gene sequences were sub-cloned into pGEM-T vector. The vector was then cut with SpeI and NcoI respectively. mRNA probes were made using Riboprobe System SP6 (Promega) according to manufacturer instructions. Both sense and anti-sense probes were labeled and used for hybridization. *bsd* probes were labeled with fluorescein and the *luciferase* probes were biotin-labeled for detection with streptavidin conjugated with AlexaFluor 594. Only probes that directly hybridized to the mRNAs gave visible signals. The hybridization patterns shown in Figure 4B were observed in all nuclei in several independent experiments.

REFERENCES

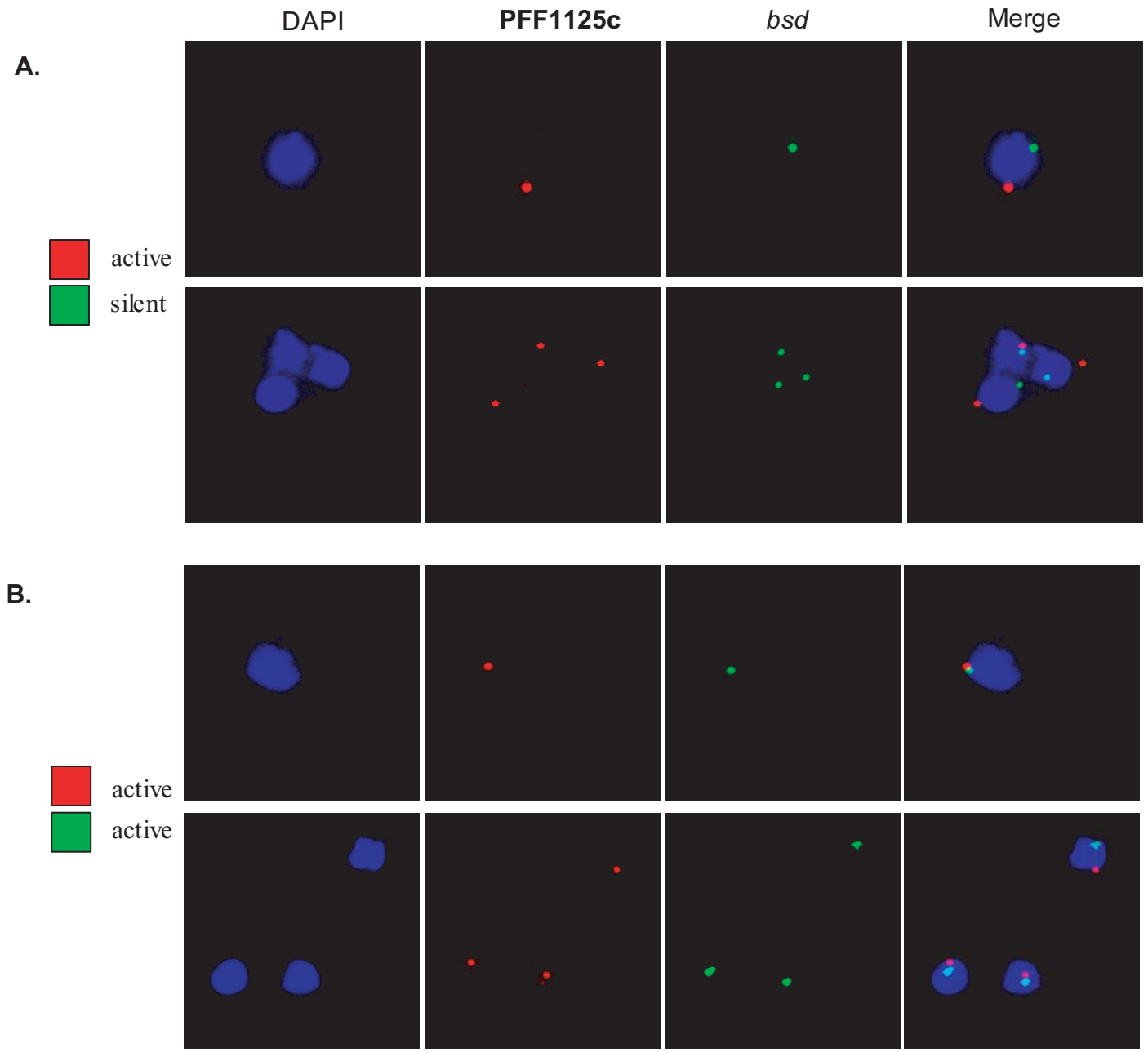
1. Dzikowski,R., Frank,M., and Deitsch,K. (2006). Mutually Exclusive Expression of Virulence Genes by Malaria Parasites Is Regulated Independently of Antigen Production. *PLoS. Pathog.,* **2,** e22.

2. Freitas-Junior,L.H., Bottius,E., Pirrit,L.A., Deitsch,K.W., Scheidig,C., Guinet,F., Nehrbass,U., Wellems,T.E., and Scherf,A. (2000). Frequent ectopic recombination of virulence factor genes in telomeric chromosome clusters of P. falciparum. *Nature,* **407,** 1018-1022.

3. Thompson,J. (2002). In situ detection of RNA in blood- and mosquito-stage malaria parasites. *Methods in Molecular Medicine,* **72,** 225-233.

4. Wu,Y., Sifri,C.D., Lei,H.-H., Su,X., and Wellems,T.E. (1995). Transfection of *Plasmodium falciparum* within human red blood cells. *Proceedings of the National Academy of Sciences USA,* **92,** 973-977.

Figure S1


DAPI
PFF1125c
bsd
Merge
A.
active
silent
B.
active
active

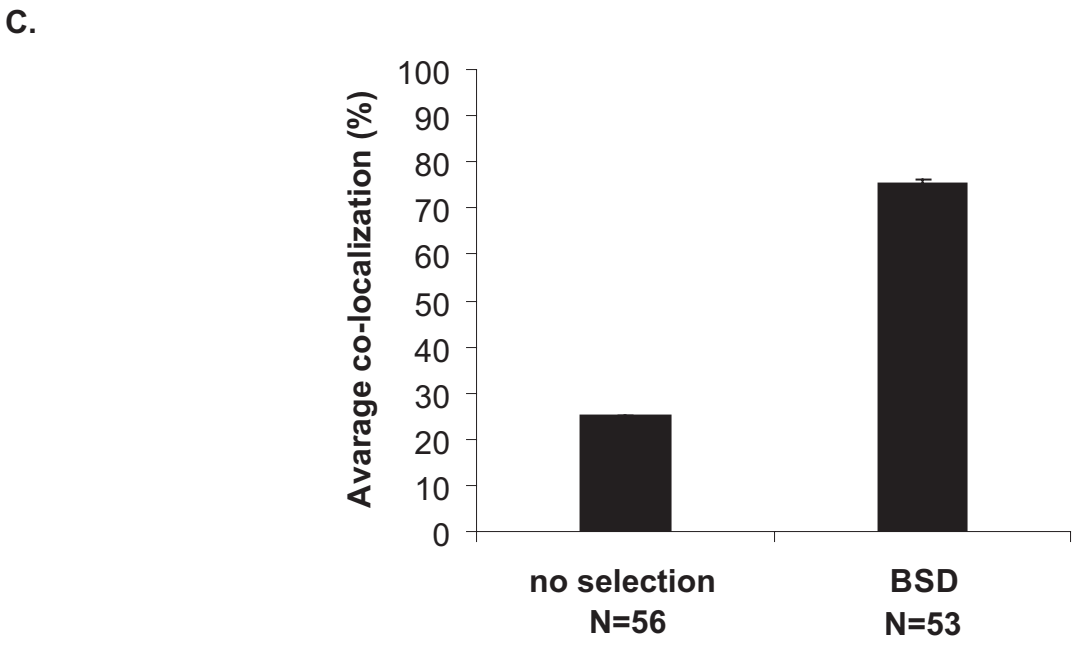

C.
Avarage co-localization (%)
100
90
80
70
60
50
40
30
20
10
0
no selection
N=56
BSD
N=53

Figure S2.