Supplementary Figure legends

Figure S1. Switching dynamics of the entire var gene family in the CSA-selected population following the down-regulation of var2csa by promoter titration. (A), Steady state mRNA levels of each individual var gene are presented before transfection (upper panel), under titration using selection on 15µg/ml blasticidin (second panel), two weeks (third panel), 2.5 months (fourth panel), four months (fifth panel) and 6 months (lower panel) after drug removal. Schematic of plasmid used for promoter competition is shown on the upper right. Each var gene is colored by its promoter type: Grey, UpsA; Orange, UpsA/B; Blue, UpsB; Green, UpsB/C; Red, UpsC; Black, UpsE (var2csa). Steady state mRNA levels of each individual var gene are presented as relative copy number. (B), pVBh copy numbers (left panel) and bsd mRNA levels (right panel) of the CSA-selected populations under selection using 15µg/ml blasticidin, as well as at 2 weeks and 1 month after drug removal. The number of pVBh copies was measured by qPCR using gDNA as template and transcript levels were measured by RT-qPCR using cDNA as template. All values are presented as relative copy number to the housekeeping genes arginine-tRNA ligase (PF3D7_1218600). (C), Dynamics in the relative transcription of each var gene subsets 2 weeks (upper pie), 2.5 months (second pie), four months (third pie) and 6 months (lower pie) after drug removal. The relative proportion of each var gene subsets, A, A/B, B, (telomeric) and B/C, C, (central) from the total transcripts is displayed for each population at each time point.

Figure S2. Forcing activation of the var2csa promoter by increasing concentrations of drug pressure does not elevate mRNA levels or change mRNA stability. Steady-state levels of bsd
transcripts expressed by either var (A & B) or var2csa (C & D) promoters by parasites growing in the presence of increasing blasticidin concentrations. Schematic of the plasmids used are presented under each graph (V stands for var promoter and V2 stands for var2csa promoter).

Transcript levels are presented as relative copy number to the housekeeping gene serine-tRNA ligase (PF3D7_0717700) (A & B) and to arginine-tRNA ligase (PF3D7_1218600) (C & D). (E - F), mRNA stability assay of bsd transcripts expressed by var or var2csa promoters. Parasites expressing bsd by either var or var2csa promoters were treated with actinomycin D (ActD) to stop mRNA synthesis and the rate of decay was measured by qRT–PCR over time as indicated (E).

Transcript levels of untreated parasites are shown in (F). Transcript levels are presented relative to arginine-tRNA ligase (PF3D7_1218600). A linear regression analysis for mRNA decay, presented as colored lines, was established by GraphPad Prism software.

Figure S3. Parasite resistance is due to bsd expression from the episomal var2csa-promoters. (A & C), Growth curves of the CSA and G6 populations before transfection. (E & G), Growth curves of the same populations stably transfected with pV2BbIDh and pV2Bh after drug pressure was removed for a month, allowing the parasites to shed all episomes. Each population was either grown on regular media (red curve) or re-selected on 2µg/ml blasticidin (blue curve) and the parasitemia was monitored for several generations. (B & D), Steady-state transcript levels of clag3.1 and clag3.2 in the unselected cultures CSA and G6 and in the same populations following var2csa-promoter selection on 10 µg/ml blasticidin (F & H). Transcript levels of clag3.1 and clag3.2 are presented as relative copy number to the housekeeping gene arginine-tRNA ligase (PF3D7_1218600). (I & J), Kinetics of osmotic lysis of transgenic parasites expressing bsd by either var (VB-myc) or var2csa (V2B-myc) promoters in isotonic sorbitol. (K & L), Similar
kinetic of osmotic lysis in isotonic PhTMA. In each panel, traces represent kinetics in isotonic sorbitol or PhTMA with 0, 1, 10, 50, 100, and 1000 nM ISG-21 PSAC inhibitor (top to bottom). (M & N) Dose responses showing permeability (P) as a function of ISG-21 concentration, normalized to 1.0 without inhibitor. Black and red symbols represent mean ± S.E.M. for VB-myc and V2B-myc, respectively (n = 5-6 each). There are no significant differences in either lysis kinetics or response to ISG-21 in either solute, excluding differences in uptake channels between these parasites.

Figure S4.

Western blot analysis of parasites expressing gfp-bsd with or without the fusion of the ER-retention KDEL motif expressed under the control of either a typical var promoter (V-GB and V-GB (KDEL), respectively) or a var2csa promoter (V2-GB and V2-GB (KDEL), respectively). BSD-GFP was detected using an anti-GFP antibody. Aldolase was used as a loading control.

Figure S5.

(A), Immuno-EM of parasites expressing the uORF-GFP fusion. uORF-GFP labeled with gold particles localizes mainly to membranous organelles surrounding the nucleus corresponding to the ER (Black arrows). Scale bar: 500nm. (B & C), High magnification of boxed areas in A. Scale bars: 200nm.
Figure S1

A

Before transfection

15µg/ml Blasticidin

Off Blasticidin (2 weeks)

Off Blasticidin (2.5 months)

Off Blasticidin (4 months)

Off Blasticidin (6 months)

B

Var 5’

Bsd

Off Blasticidin

C

Bsd expression

A/B

B

B/C

C

A/B

B

B/C

C

A/B

B

B/C

C

A/B

B

B/C

C
Figure S4

Anti-GFP

Anti-Aldolase

V-GB  V-GB (KDEL)  V2-GB  V2-GB (KDEL)

KDa

37
Figure S5

A

B

C
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**puORF-GFP**

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