SUPPLEMENTARY METHODS

Plasmid Constructs
To generate the 6.4 kb pCG6 plasmid, a 0.6 kb cg6 insert was PCR amplified from *Plasmodium falciparum* Dd2 genomic DNA using the primers p11 and p12 (Supplementary Table 1) and subcloned as a BamHI-NotI fragment into the *P. falciparum* transfection plasmid pHD22Y1. This plasmid expresses the human dihydrofolate reductase (DHFR) gene under the control of the hrp3-5' and hrp2-3' untranslated regions (UTR). A 40 bp *Mycobacterium smegmatis* attB adaptor, generated by annealing the oligonucleotides p13 and p14, was ligated into the NotI site of pCG6 to form the plasmid pCG6-attB.

The 8.2 kb pBSD-GFP-INT-attP plasmid was assembled following several modifications to the *P. falciparum* transgene expression plasmid pDC/CRT-FLAG2. First, the GFP Mut2 coding sequence was amplified from pHD-GFP5 using the primers p15 and p16. This was ligated as a BglII-XhoI fragment into the pDC/CRT-FLAG plasmid digested to completion with BglII and partially with XhoI, yielding the plasmid pDC/CRT-FLAG-GFP. Second, the *bsd* selection marker4 was PCR amplified from pcam-BSD5 with primers p9 and p10, removing the stop codon in the process. This was ligated as a XhoI-SpeI fragment into pDC/CRT-FLAG-GFP in the place of the *pfenr* coding sequence, creating a *bsd*-FLAG-GFP fusion under the control of a 0.6 kb *calmodulin* 5' UTR and a 0.9 kb *hsp86* 3' UTR. Third, the 1507 bp Bxb1 integrase gene was PCR amplified from the pAIK5 plasmid6 using the primers p17 and p18, with a c-myc tag fused to the 3' end. This integrase-myc fragment was subcloned as a NcoI-KpnI fragment in the place of the human DHFR selectable marker1. Finally, we generated a 50 bp *attP* adaptor, flanked by EcoRI sites, by annealing the oligomers p19 and p20. The cohesive end double-stranded adaptor was ligated into the EcoRI site to give the final plasmid pBSD-GFP-INT-attP. Several versions of this plasmid were obtained that differed in the number of *attP* adaptors (one to three) ligated in tandem.

The 8.4 kb pLN-ENR-GFP plasmid was derived from pDC/CRT-FLAG-GFP in several steps. First, the 1.3 kb *pfenr* gene was PCR amplified from *P. falciparum* Dd2 genomic DNA using the primers p7 and p8. This fragment was ligated into the XhoI sites of pDC/CRT-FLAG-GFP in the place of the *pfenr*-FLAG-GFP fusion. This was necessary to engineer unique sites into the plasmid with *pfenr* and to ablate the downstream XhoI site. To create a *pfenr*-GGF fusion, the GFP Mut2 gene was amplified from pHH2-ACP-GFP7 using the primers p21 and p22. This was ligated downstream and in-frame of *pfenr* at the BsiWI-AflII site. Next, a 1.0 kb *calmodulin* 5' UTR was amplified from 3D7 genomic DNA with primers p23 and p24 and ligated into the SphI and AflII sites to replace the 0.6 kb *calmodulin* 5' UTR. This step removed the *XhoI* and BamHI sites leaving only the unique AflII site downstream of the *pfenr* and to ablate the downstream XhoI site. To create a *pfenr*-GGF fusion, the GFP Mut2 gene was amplified from pHH2-ACP-GFP7 using the primers p21 and p22. This was ligated downstream and in-frame of *pfenr* at the BsiWI-AflII site. Next, a 1.0 kb *calmodulin* 5' UTR was amplified from 3D7 genomic DNA with primers p23 and p24 and ligated into the SphI and AflII sites to replace the 0.6 kb *calmodulin* 5' UTR. This step removed the *XhoI* and BamHI sites leaving only the unique AflII site downstream of the *pfenr* and also introduced unique *Apal* and *BspEI* sites to replace the HindIII site upstream of this element. To introduce the *bsd* marker (flanked by *PcDT* 5' UTR and *hrp2* 3' UTR regulatory elements), its coding sequence was PCR amplified from pcam-BSD with primers p25 and p26 and ligated into the *NcoI* and *KpnI* sites in place of the human DHFR cassette. This gave rise to the intermediate plasmid pDC2-ENR-GFP. Last, the 50 bp *attP* adaptor was inserted at the EcoRI site to produce the final plasmid pLN-ENR-GFP with two tandem copies of the 50 bp *attP* adaptor positioned downstream of the *hrp2* 3' UTR.

pLN-GFP was made by first removing the AflII-AflII cloned *pfenr*-GGF insert from pLN-ENR-GFP and replacing it with an AflII-AflII full-length GFP Mut2 insert that was amplified from pHH2-ACP-GFP7 using the primers p27 and p22. We then replaced the 1.0 kb *calmodulin* 5' UTR with a 1.8 kb *hrp3* 5' UTR, using *Apal* – AflII sites.

The 8.0 kb integrase-expressing plasmid pINT was constructed as follows: an integrase-myc fusion was isolated following *NcoI*/*KpnI* digestion of pBSD-GFP-INT-attP and cloned into *NcoI*/*KpnI*-digested pDC2-ENR-GFP in the place of the *bsd* gene. Next, the neomycin phosphotransferase II gene was amplified from pPKDSneoII plasmid8 with primers p28 and p29, reconstituting the start codon of the gene. This neomycin gene was isolated following AflII and AflII digestion and used to replace the *pfenr*-GGF fusion. Last, a 0.9 kb *hsp86* 5' UTR element was amplified from pHH2-ACP-GFP7 with the primers p30 and p31 and cloned into...
the Apal and AvrII sites to replace the 1.0 kb calmodulin 5’ UTR, generating the final G418-resistant plasmid pINT that expresses the Bxb1 integrase gene under the control of PcDT 5’ UTR and hrp2 3’ UTR elements.

The pCBD-P plasmid was created from pMiniBSD (a gift of C. Ben Mamoun, U. Conn. Health Center) by replacing the hrp2 3’ UTR with the 3’ region of P. berghei dihydrofolate reductase. A new multicloning site (containing ClaI, BglII, SalI, KpnI, and Xhol sites) was inserted into the KpnI site by annealing and ligating two complementary 41-mer oligonucleotides such that the original KpnI site was ablated, to create the pCBD plasmid. An attP element was PCR amplified and subcloned into pCR2.1 (Invitrogen). After digestion with XhoI and KpnI, this element was subcloned into XhoI- and KpnI-digested pCBD, to yield pCBD-P. pCBD-P-Luc was created by the insertion of a KpnI- and BamHI-digested firefly luciferase cassette from the pALHi plasmid (derived from pHLH-1; Rebecca Muhle, in preparation) into KpnI- and BglII-digested pCBD-P.

All plasmid inserts were cloned into either pGEMT-Easy (Promega) or pCR2.1 (Invitrogen) and sequenced on both strands to identify error-free clones, prior to subcloning into the transfection plasmids.

Nucleic acid analyses
PCR primers p1 + p2 were used to detect integration of pCG6-attB and pCG6 into the cg6 locus on chromosome 7 (also known as PfGlp310; Fig. 1a). PCR was also used to detect integration into the 5’ UTR of the sub-telomeric hrp3 locus on chromosome 13 (Supplementary Fig. S2). Primers p2 + p3 and p2 + p4 were used respectively to detect pBSD-GFP-INT-attP and pLN-ENR-GFP integration into the attB+ clones (Fig. 2a, 3a). cg6 and pfnr probes were PCR amplified from Dd2 genomic DNA using the primers p5 + p6 and p7 + p8 respectively. The bsd probe was amplified from pcam-BSD using the primers p9 + p10. The pBluescript (pBS) probe was generated from ScaI-digested pBS plasmid.

REFERENCES