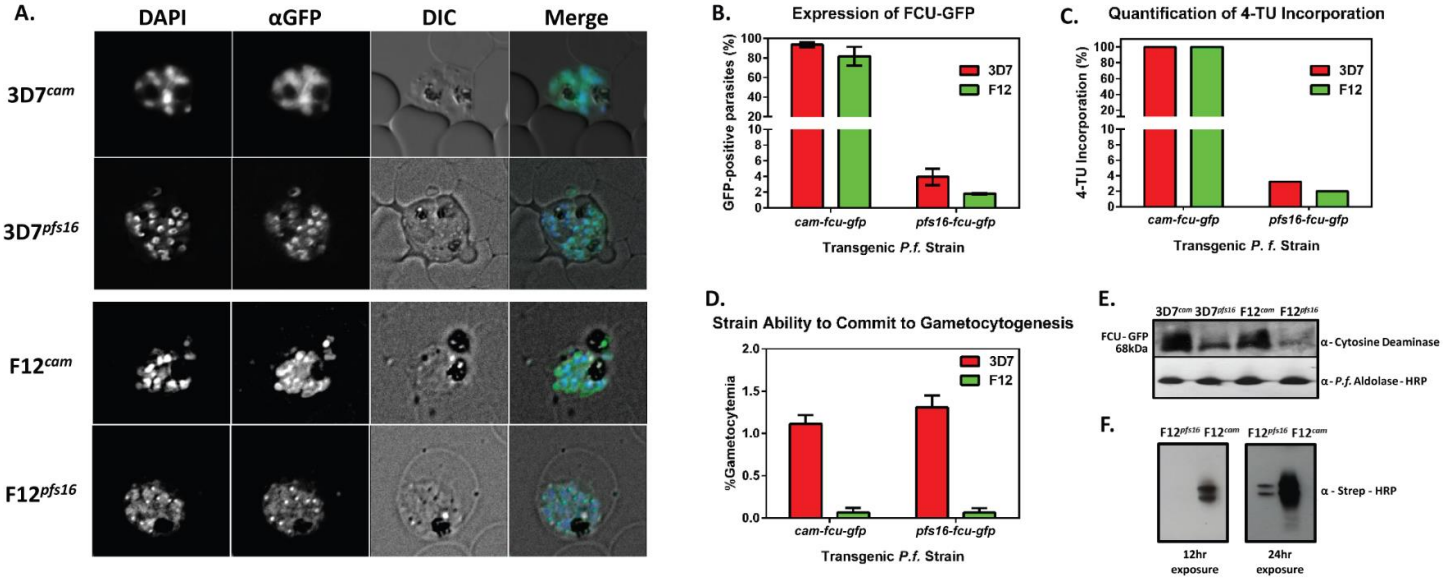

Figure S2: Confirmation of Various Transgenic Strains of *P. f.* Expressing FCU-GFP. A) Fluorescent microscopy images of each parasite line expressing *cam*- and *pfs16-fcu-gfp* (green) and DAPI dsDNA dye (blue) verified the presence of the protein fusion throughout the parasite. B) *P. falciparum* strains 3D7 (red) and F12 (green) were transgenically modified to express FCU-GFP under the control of the *calmodulin* or *pfs16* promoters. The proportion of mixed-stage asexual parasite populations expressing FCU-GFP was determined by calculating the percentage of FCU-GFP positive parasites (GFP positive/non-fluorescent) by fluorescence microscopy. Results represent the average of two independent experiments ± s.d. C) Thiol-incorporation and biotinylation are decreased in strains expressing *pfs16-fcu-gfp*. Percent incorporation of 4-TU into the total pool of RNA was quantified using ImageJ from Northern blots of 2µg of biotinylated RNA from 3D7 and F12 expressing FCU-GFP under the control of *cam* or *pfs16* probed with streptavidin-HRP. Blots utilized for this quantification are as follows 3D7 (Figure 3C) and F12 (Figure S2E). The level of thiolated-RNA in *pfs16-fcu-gfp* expressing parasites was calculated as a percentage of total labeling in *cam-fcu-gfp* expressing lines and normalized based on the length of time the film was exposed. D) Each transgenic strain maintained their parental gametocyte production phenotype. The percentage of stage III gametocytes was determined by Geimsa-stained thin-blood smears. Percentages represent the average of two biological replicates ± s.d. E) Western blot analysis of parasite protein extracts probed with α-cytosine deaminase confirmed a 68kDa band representing expression of either *cam*- or *pfs16-fcu-gfp* in *P.f.* strains 3D7 and F12. As a loading control, the membrane was probed with α-*P.f.* aldolase-HRP. F) Confirmation of promoter-driven thiol-tagged RNA in F12 *P.f.* transgenic lines (*pfs16-fcu-gfp* and *cam-fcu-gfp*). Total RNA from each parasite line grown in the presence of 40µM 4-TU for 12 hours was extracted and biotinylated. The presence of biotinylated, thiol-tagged RNA was assessed by Northern blot and probed with α-streptavidin-HRP (bottom panel). Film exposure times are noted and varied depending upon the time required to visualize biotinylation of RNA from F12^{pfs16}.