



Figure S1: The effects of 4-TU incorporation and capture throughout *P. falciparum* 3D7^{cam} development within human erythrocytes. A) 4-TU has a concentration dependent effect on development. *P.f.* 3D7^{cam} growth in the presence of various concentrations of 4-TU (0, 20, 40, 80, 160 μ M) was monitored every 12hrs for 72hrs by Giemsa-stained thin blood films. Parasitemia percentages (infected/uninfected erythrocytes) are represented as an average of two independent experiments \pm s.d. across this timeframe, 40 μ M (red bars) or less had no effect on parasite growth. B) Transcript abundance profiles during the IDC were not altered by the addition of 40 μ M 4-TU. Total RNA isolated from 3D7^{cam} and 3D7^{pfs16} incubated for 12h with 40 μ M 4-TU was compared by DNA microarray. The Pearson correlation (r) of the $\text{Log}_2(\text{Cy}3/\text{Cy}5)$ ratio for each gene across the array at 0, 12, 24, and 36h was calculated. The median correlation of the log_2 ratio of each gene over time in either 3D7^{cam} (top panel) or 3D7^{pfs16} (middle panel) compared to a previously published 3D7 mRNA abundance timecourse (Kafsack et al. 2012) was 0.850 and 0.846, respectively. The median correlation between total RNA abundance of 3D7^{cam} and 3D7^{pfs16} was determined to be 0.815. C) To ensure that 4-TU is efficiently incorporated throughout the IDC, highly synchronized *P.f.* 3D7^{cam} was grown in the presence of 40 μ M 4-TU for various lengths of time (0, 1, 2, 4 hours) during three major developmental stages during the IDC (ring, trophozoite, and schizont). Following incubation, total RNA was extracted, biotinylated and separated on an agarose gel (2 μ g/lane) (top panel). Incorporation of 4-TU throughout the IDC was assessed by Northern blot and probed with streptavidin-HRP (bottom panel). D) Thiolated RNA can be separated from the parasite total RNA using streptavidin magnetic beads. Biotinylated RNA from both wild-type 3D7 and 3D7^{cam} eluted from streptavidin magnetic beads was run on an ethidium bromide stained gel as follows: (1) total RNA, (2) flow-through/non-biotinylated RNA, (3) last wash of 4, and (4) β -mercaptoethanol eluted/thiol-tagged RNA.