FIGURE S1. Folate transporters fingerprint alignment. The protein sequences of PfFT1, PfFT2 and PF10_0215 from *P. falciparum* 3D7 (PlasmoDB5.3) are aligned to other characterised folate transporters. The predicted membrane-spanning domains (I - XII) for PfFT1 and PfFT2 are indicated by the solid lines at the top. Ruler follows PfFT1 coordinates. Limits between PfFT1 residues 45 and 455. Protein multiple alignment performed with ClustalX (1) and presented as fingerprint at 55% identity threshold with residues represented by vertical bars (TeXtshade) (2). GenBank accession numbers: *Synechocystis* (*Synechocystis sp.* PCC 6803, BAA10362), *Athaliana* (*A. thaliana* At2g32040, NP_565734), *Ldonovani-FT1* (*L. donovani*, AAD52046), *Lmexicana-BT1* (*L. mexicana*, AAC28092), *hPCFT* (*H. sapiens*, NP_542400), and *hRFC1* (*H. sapiens*, P41440). Segment ($D(X)_5GXRR$) part of a MFS motif, and the arginine residue R325/R311 (PfFT1/PfFT2) - equivalent to substrate-binding R497 of the *Leishmania* FT1 (3) - as described in main text are marked at the bottom of the alignment.

FIGURE S2. PfFT1 and PfFT2 predicted topology. An intracellular NH_2 terminus, 12 membrane-spanning domains (I – XII), and hydrophilic loops between membrane-spanning domains IV-V and VI-VII are predicted. Together with the highly conserved cytoplasmic loop motif $D[X]_nGXRR$ (black boxes) between II-III. This motif is shared with other transporters of the MFS (4). Conserved residues among the BT1 family of transporters are in gray scale. Residues R325 and R311 are the PfFT1 and PfFT2 equivalent of the *Leishmania* FT1 R497. Topology was calculated with HMMTOP (5) and drawn with TeXtopo (6).

FIGURE S3. Folate transporter global protein alignment. The protein sequences of PfFT1, PfFT2 and PF10_0215 from *P. falciparum* 3D7 (PlasmoDB5.3) are aligned to other members of the BT1 family and non-BT1 folate transporters such as the human folate transporters hPCFT and hRFC1. The predicted membrane-spanning domains (H1-H12) for PfFT1 and PfFT2 are indicated by the solid lines. Ruler follows PfFT1 coordinates. Protein multiple alignment was performed with ClustalX (1) and prepared at 45% identity threshold with TeXshade (2). GenBank accession numbers: *Synechocystis* (*Synechocystis sp.* PCC 6803, Slr0642, BAA10362), *Athaliana* (*A. thaliana* At2g32040, NP_565734), *Ldonovani-FT1* (*L. donovani*, AAD52046), *Lmexicana-BT1* (*L. mexicana*, AAC28092), *hPCFT* (*H. sapiens*, NP_542400), *hRFC1* (*H. sapiens*, P41440), and *P. falciparum* PF10_0215. Segment ($D(X)_5GXRR$) is part of an MFS motif. The Arginine residue R325/R311 (PfFT1/PfFT2) is equivalent to the substrate-binding R497 of the *Leishmania* FT1 (3). Other residues with PfFT1 coordinates labelled below the sequences have been found to be crucial for the folate transport activity of the *Synechocystis* Slr0642 BT1 transporter (7). Among them are residues N164 and N357 (between parentheses) that correspond to Aspartates (D145 and D341) found in the equivalent positions of Slr0642.

FIGURE S4. PfFT2 immunoprecipitation (IP) in *E. coli ΔpabA/ΔabgT*. A. Coomassie blue stained SDS-PAGE run with the protein elution from the Pierce Classic IP Kit columns (Thermo Scientific). Lane 1: *E. coli ΔpabA/ΔabgT* expressing pLOI-PfFT2. Lane 2: *E. coli ΔpabA/ΔabgT* expressing the plasmid only pLOI. The very prominent 52kDa bands correspond to the Heavy chains of anti-PfFT2 (IgGs) whose Light chains are also visible at 26kDa. Molecular size markers in kDa are represented by the lines drawn beside the picture of the SDS-PAGE. **B.** Western-blot film shows a band under the 52 kDa marker (the predicted size of PfFT2 is 51.35 kDa) in lane 1 which is absent in the sample from plasmid only transfectant shown in lane 2. Primary antibody anti-PfFT2 used at 1:500. The band observed for cells expressing pLOI-PfFT2 is absent in the plasmid only control. Membrane-enriched samples from *E. coli ΔpabA/ΔabgT* were prepared as in (8). IP with anti-PfFT2 was carried out following manufacturer's instructions.

FIGURE S5. Mass Spectrometry detection of 5-MTHF in bacterial and parasite culture media. Supernatant of bacterial and parasite cultures were probed for the presence of 5-MTHF at time point zero and 24h for the *E. coli* growth assays, as well as 48h and 72h for the *P. falciparum* inhibitory growth experiments. Spectra represent total ion current of the parent ion of 5-MTHF, m/z 460 for an equivalent injection volume of supernatant. 5-MTHF measured as area under the curve at different time points was compared to time zero. At 24h in bacterial culture supernatant 5-MTHF was

equivalent to 90% of the initial concentration. At 48h and 72h it was 88% and 80% of the initial concentration in the parasite culture supernatant, respectively.

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Figure S1

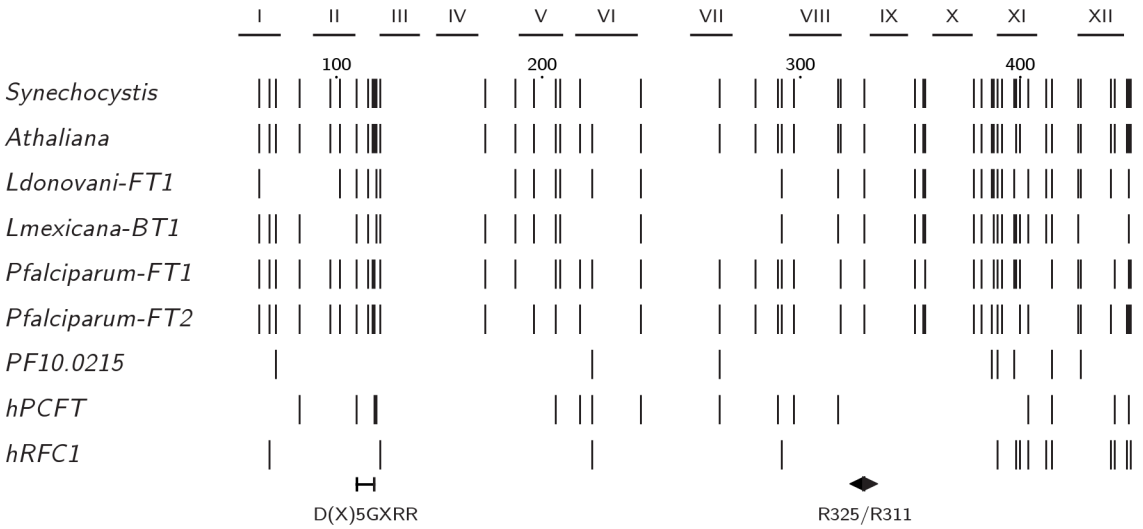
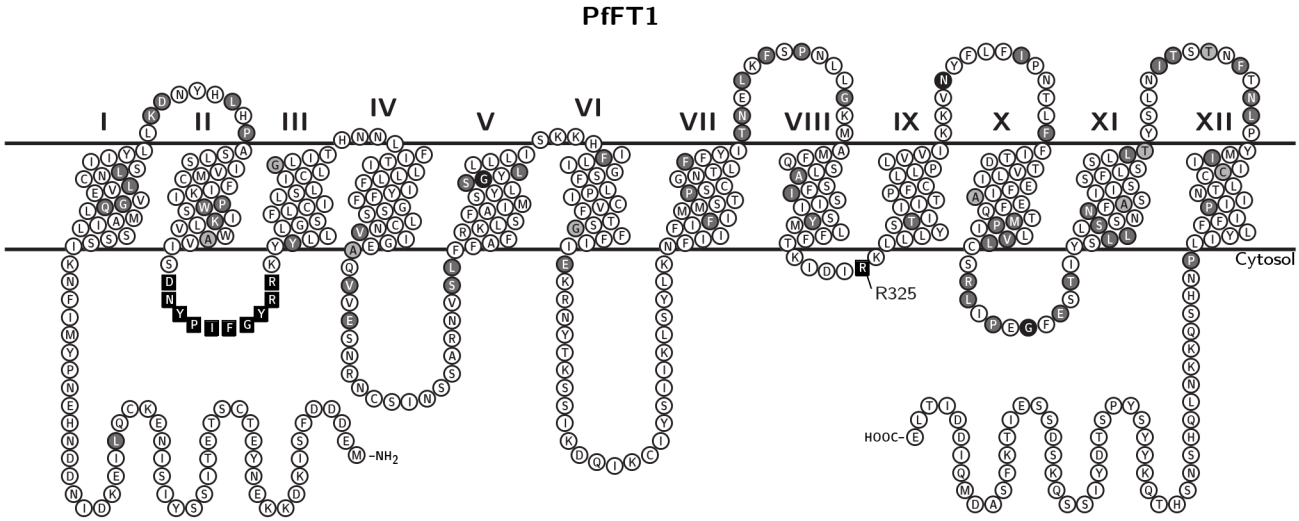

I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
100
200
300
400
Synechocystis
Athaliana
Ldonovani-FT1
Lmexicana-BT1
Pfalciparum-FT1
Pfalciparum-FT2
PF10.0215
hPCFT
hRFC1
D(X)5GXRR
R325/R311

Figure S2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V
VI
VII
VIII
IX
X
XI
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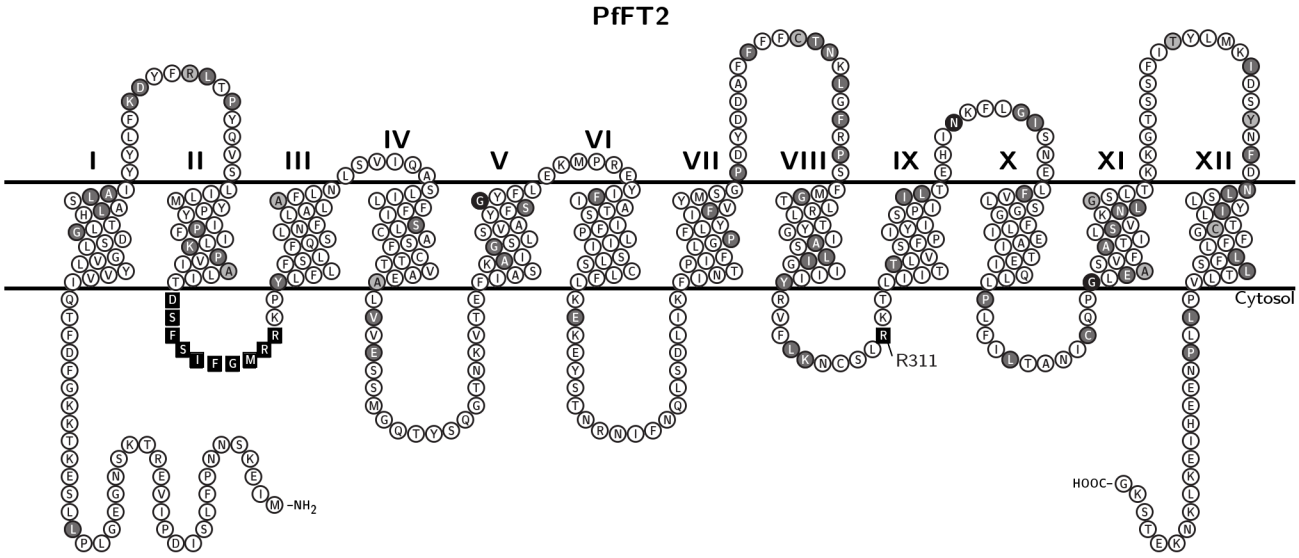
R325
HOOC-
-NH2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VII
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XI
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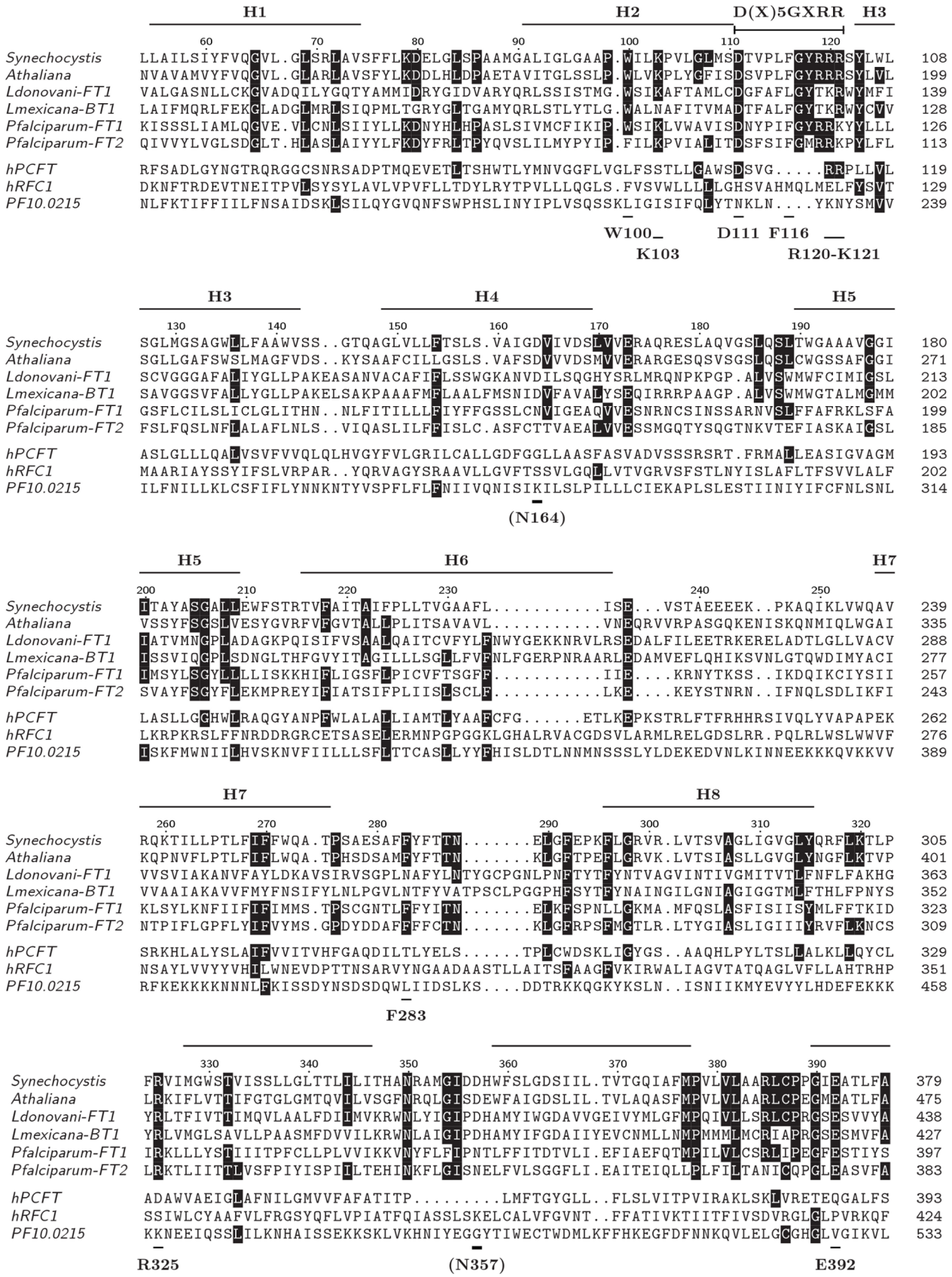
-NH2

Figure S3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H2
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H3
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Lmexicana-BT1
Pfalciparum-FT1
Pfalciparum-FT2
hPCFT
hRFC1
PF10.0215
W100
K103
D111
F116
R120-K121
H4
H5
H6
H7
H8
(N164)
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R325
(N357)
E392
H11
H12

Figure S4

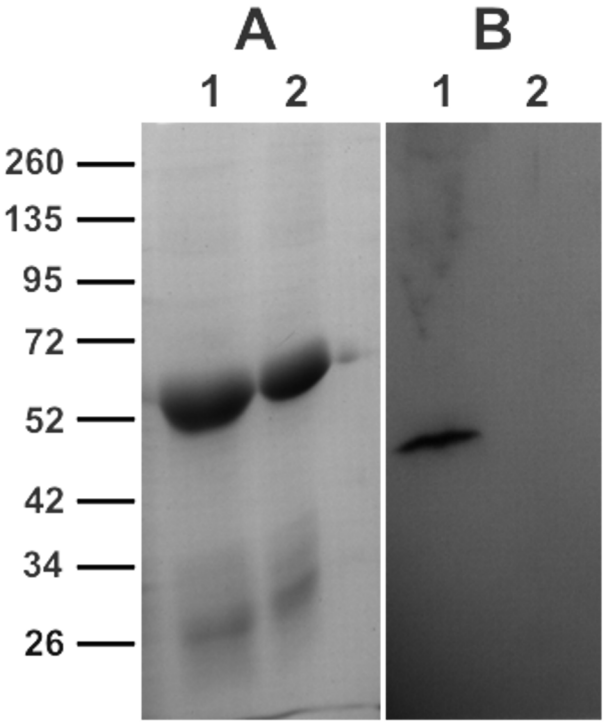
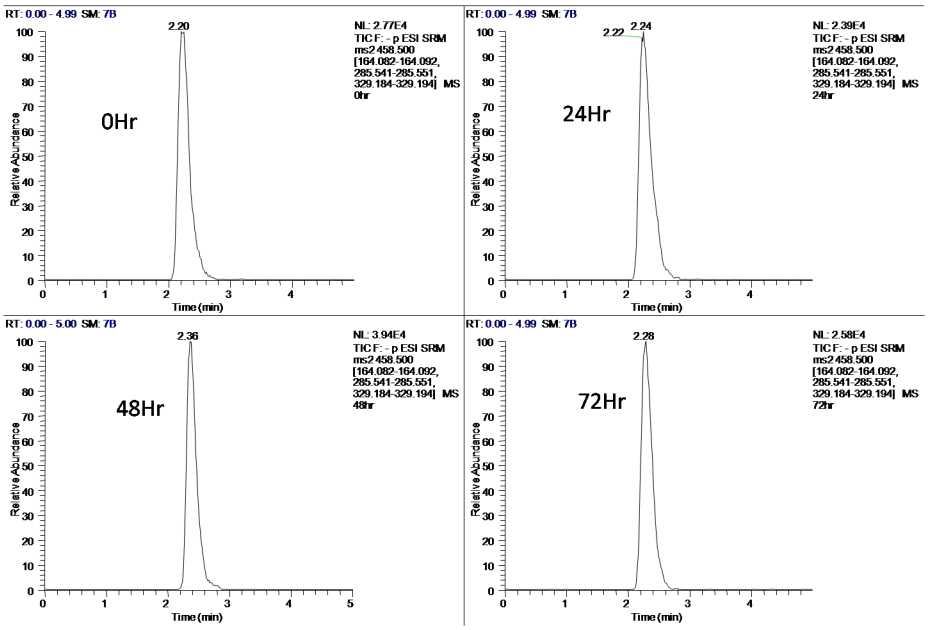


Figure S5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329.184-329.194] MS
0hr
0Hr
Relative Abundance
Time (min)
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2.24
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24Hr
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48Hr
Relative Abundance
Time (min)
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285.541-285.551,
329.184-329.194] MS
72hr
72Hr
Relative Abundance
Time (min)

TABLE S1

Effect of exogenous folate supplementation on the *in vitro* inhibitory activity of antifolates against *P. falciparum* strains.

Values shown media containing folate-free RPMI-1640 together with dialysed serum. Supplements were present at 7.3μM for *p*ABA and 2.3μM for the folate products 5-MTHF, FA and FoA. Values represent mean ± standard deviation with n = 4 - 7 for all experimental groups. Statistical analysis was performed using two-way ANOVA (antifolates vs supplements) with Bonferroni post-tests. **represents significant pABA dependent inhibition compared to control ($p < 0.001$). *represents significant folate dependent inhibition compared to control ($p < 0.01$).

	IC_{50} values (μM)			
	HB3			
	SDX	**PYR**	**DDS**	**CCG (nM)**
Control	175 ± 71.6	1.06 ± 0.65	26.53 ± 2.46	107 ± 13.90
***p*ABA**	1476 ± 36 **	5.6 ± 0.7 **	544 ± 188 **	277 ± 241
5-MTHF	451 ± 13 *	3.04 ± 1.90	40.15 ± 9.05	119 ± 33
FA	1272 ± 246 *	3.65 ± 0.35	213 ± 65 *	215 ± 149
FoA	1221 ± 106 *	6.5 ± 0.7 *	272 ± 29 *	310 ± 7 *
	3D7			
	SDX	**PYR**	**DDS**	**CCG (nM)**
Control	426 ± 85	0.05 ± 0.01	38 ± 0.63	4.36 ± 0.24
***p*ABA**	1952 ± 189 **	0.09 ± 0.01	524 ± 309 **	5.75 ± 2.35
5-MTHF	472 ± 42	0.07 ± 0.01	50.7 ± 4.18	4.64 ± 0.47
FA	1572 ± 413 *	0.1 ± 0.03	333 ± 275	6.52 ± 1.85
FoA	1336 ± 220 *	0.11 ± 0.02	283 ± 17 *	4.95 ± 1.12

	K1		
	SDX	**PYR**	**DDS**
Control	494 ± 47	2.23 ± 0.33	51.22 ± 2.62
***p*ABA**	2675 ± 533 **	2.94 ± 0.38	740 ± 144 **
5-MTHF	1116 ± 100 *	2.7 ± 0.71	64.42 ± 36.42
FA	2231 ± 67 *	3.53 ± 1.67	685 ± 239 *
FoA	2256 ± 90 *	2.99 ± 0.39	336 ± 5 *
	Dd2		
	SDX	**PYR**	**DDS**
Control	517 ± 206	2.29 ± 0.47	65.25 ± 6.07
***p*ABA**	1786 ± 438 **	4.02 ± 1.87	495 ± 195 **
5-MTHF	927 ± 145 *	2.35 ± 0.56	185 ± 17 *
FA	1491 ± 240 *	3.21 ± 1.52	498 ± 318 *
FoA	1498 ± 100 *	4.19 ± 2.54	416 ± 251 *