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Original Paper

SUPPLEMENTARY DATA

Post-transcriptional regulation of the human reduced folate carrier as a novel adaptive mechanism in response to folate excess or deficiency

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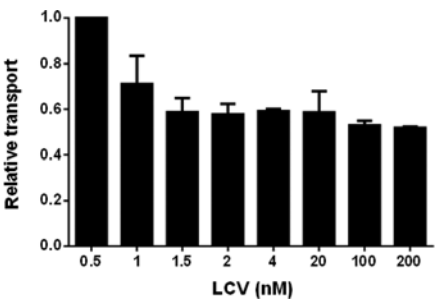

Figure S1 Characterization of Hela cell responses to exogenous folate availability

Hela cells were depleted of endogenous folates by growth in complete folate-free media, in the presence of thymidine (10 μM) and adenosine (100 μM) for 10 days. Cells were then cultured in complete folate-free media in the presence of a range of LCV concentrations (0.5–200 nM) for 96 h, and then assayed for transport with [^{3}H]Mtx (0.5 μM) for 2 min at 37°C. Transport results were normalized to transport measured at 0.5 nM LCV, which was assigned a value of 1. Results are reported as mean values $\pm$ standard errors (error bars) from three separate experiments. The transport differences measured at 0.5 and 200 nM LCV were statistically significant ($P<0.05$).

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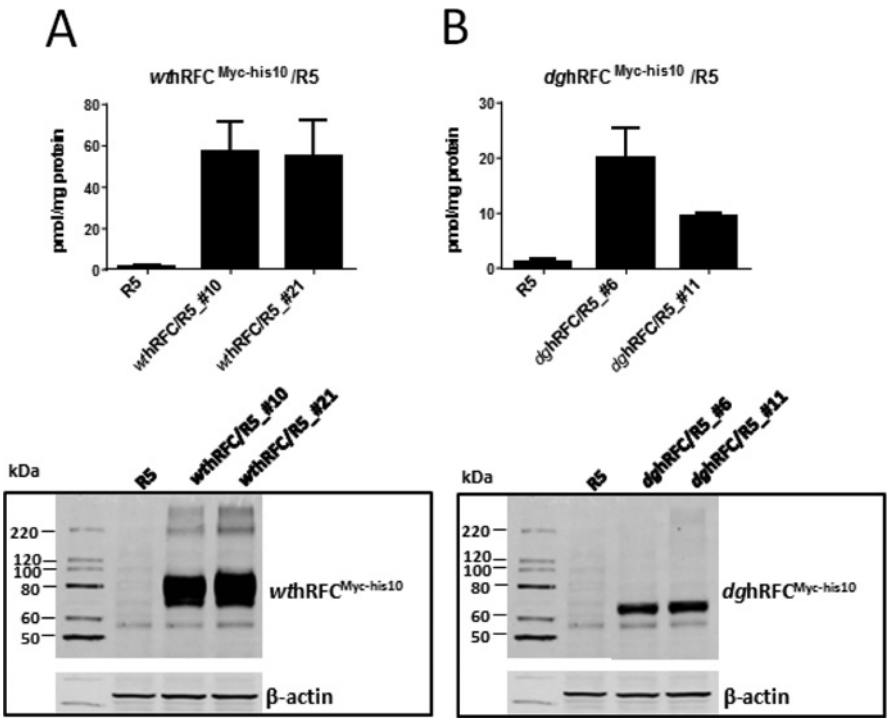

Figure S2 **Characterization of *wt* and *dg*hRFC$^{Myc-his10}$/R5 stable clones**

Both *wt* and *dg*hRFC$^{Myc-his10}$/R5 stable clones and RFC-null Hela derivative R5 cells were assayed for transport with [^{3}H]Mtx (0.5 μM) for 2 min at 37 °C. hRFC expression was analysed by Western blotting of crude membrane protein fractions. Detection of immunoreactive hRFC was with anti-Myc antibody and IRDye800-conjugated secondary antibody with an Odyssey® Infrared Imaging System. [^{3}H]Mtx transport results are reported as mean values±range (error bars) from two separate experiments for *wt*hRFC$^{Myc-his10}$/R5 cells (**A**, upper panel) and for *dg*hRFC$^{Myc-his10}$/R5 cells (**B**, upper panel). A representative Western blot is shown for *wt*hRFC$^{Myc-his10}$/R5 cells (**A**, lower panel) and for *dg*hRFC$^{Myc-his10}$/R5 cells (**B**, lower panel). β-actin was used as a loading control. The molecular mass markers for SDS–PAGE are noted.

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