Suppression of MTHFD2 in MCF-7 breast cancer cells increases glycolysis, dependency on exogenous glycine and sensitivity to folate depletion

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Supporting Information

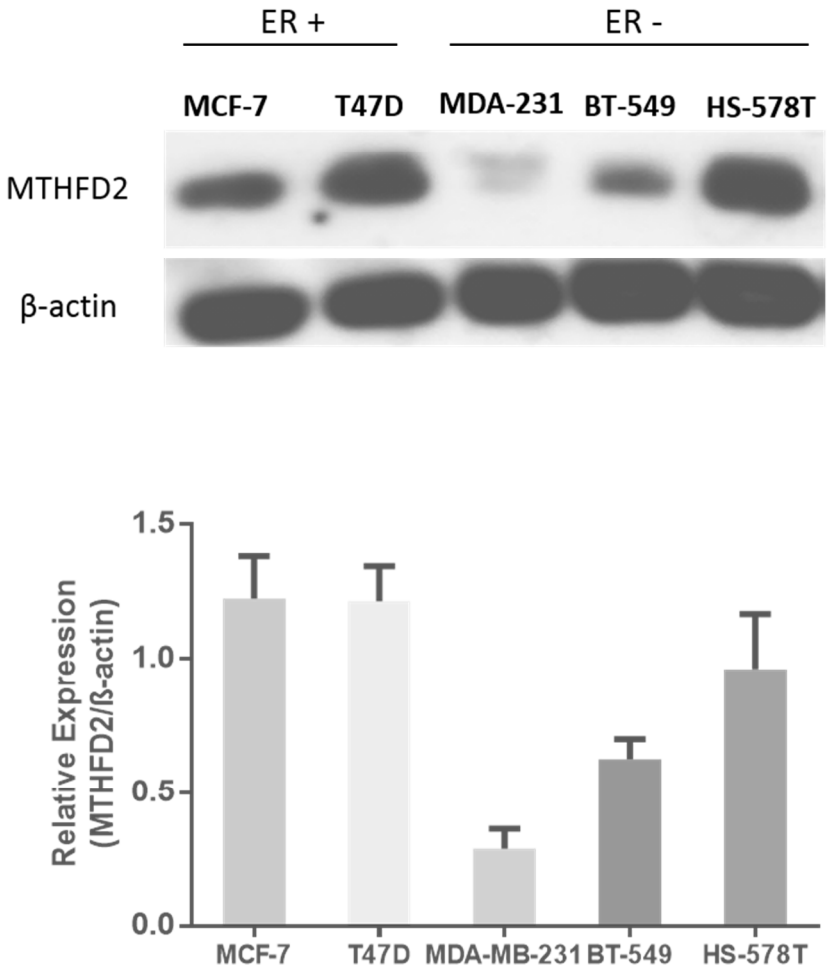


Figure S1. Expression of MTHFD2 in breast cancer cell lines from NCI60 cancer cell line panel (**A**) Characteristic immunoblot of MTHFD2 and β-actin in breast cancer cell lines ;(**B**) Quantification of MTHFD2 normalised to β-actin in NCI-60 breast cancer cell lines. Error bars indicate SD n=3.

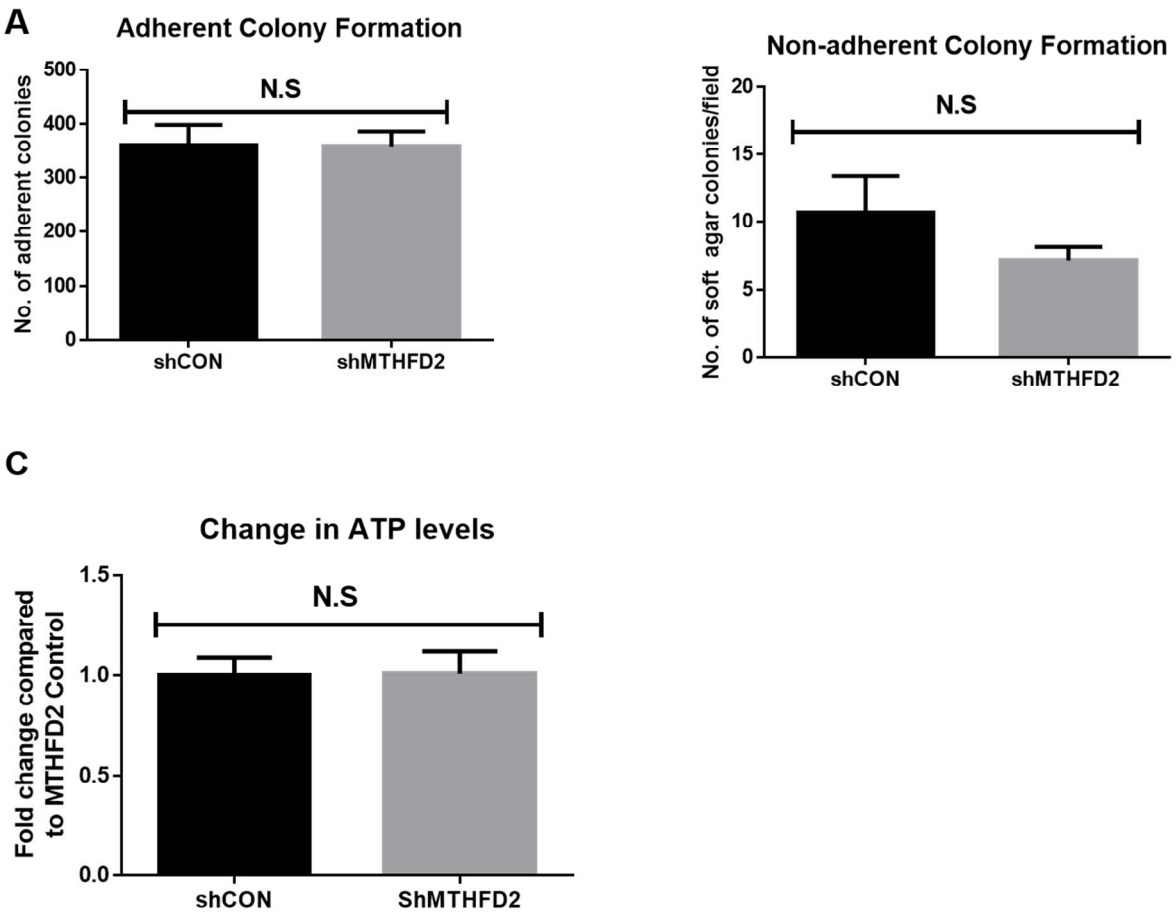


Fig.S2 Characterisation of effects of stable MTHFD2 suppression on MCF-7 breast cancer cell lines (**A**) Anchorage dependent colony formation assay for shControl and shMTHFD2 cell lines. The number of adherent colonies was counted on day 9 after fixing and staining ;(**B**) Anchorage-independent soft agar assay for shControl and shMTHFD2 cell lines. The cells were plated in semi-solid agar mixed with media and the number of soft agar colonies per 10 random fields were counted on day 13 ; (**C**) shControl and shMTHFD2 cells were seeded at a density of 1000000 in T25 flasks and ATP levels were determined using the ATP Bioluminescence Assay Kit HSII after two days. n.s. non-significant with $p<0.05$ as threshold with Student's t-test for statistical significance. Error bars indicate SD. For colony formation n=6, for ATP assay n=3.

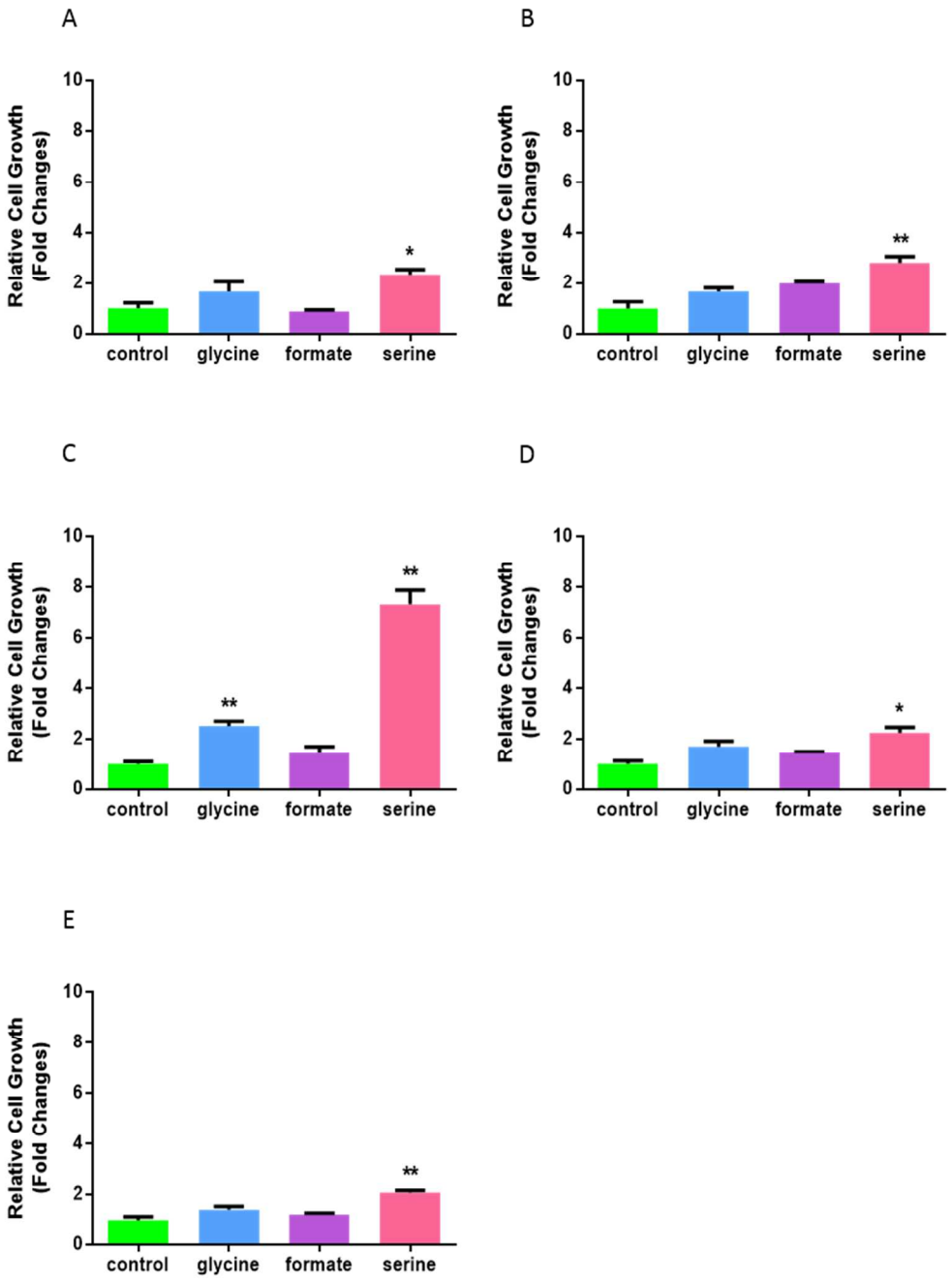
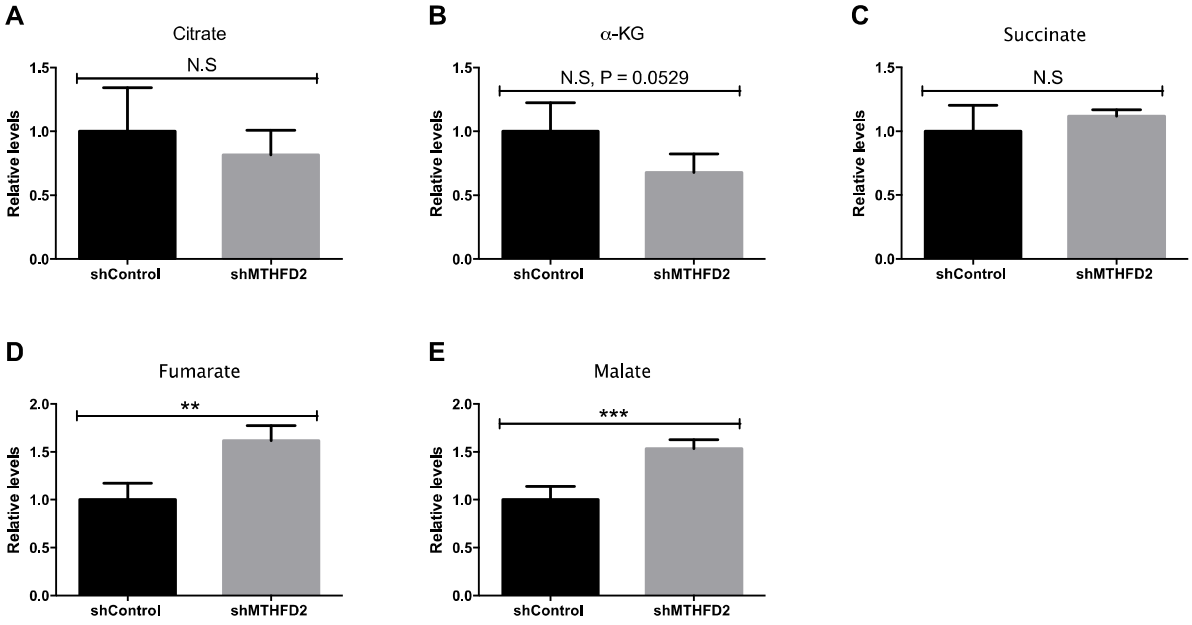


Fig.S3 Growth of cell lines after serine, glycine or formate supplementation. Growth of **(A)** MCF-7 (n=3) ; **(B)** T47D (n=3) ; **(C)** MDA-MB-231 (n=3); **(D)** BT-549 (n=3) ; **(E)** HS-578T (n=3) after supplementation of glycine (2mM), formate (2mM) and serine (2mM) for 72 hours, expressed as fold changes in cell number from control over 72 hours. The control group was cultured in MEM containing dFBS for the same duration for comparison. Cell numbers were determined using a haemocytometer and trypan blue exculsion. Error bars denote SEM (Mann-Whitney U-test; *p<0.05 vs control, **p<0.01 vs control).



Suppl.Fig.4 Changes in relative intracellular levels of TCA metabolites in shControl and shMTHFD2 cells. A: Citrate. B: α-ketoglutarate. C: Succinate. D: Fumarate. E: Malate. The MCF-7 MTHFD2 Control and KD cells were seeded at a specified density. The samples were run on GC-MS and an in-house GaVIn 1.06 Matlab script was used for metabolite validation and peak integration. Metabolite levels were normalised to the sum of all peak integrals (total peak area). The data are expressed as means ± SD of four replicates. Student's T-test was performed to compare the different treatments (**P < 0.01).