Supplemental Information:

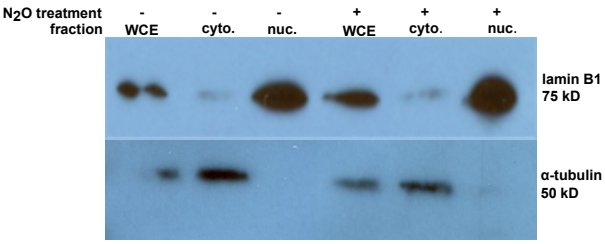

Figure S1. Western blot of whole cell, cytosolic, and nuclear fractions for nuclear (lamin B1) and cytosolic (α-tubulin) marker proteins from HeLa cells harvested in tandem with those cells used for quantifying nuclear one-carbon folate forms (refer to Fig. 2C). The absence of α-tubulin in the lanes corresponding to nuclear fractions confirms the lack of cytosolic contamination in nuclei. Lane designations: WCE, whole cell extract; cyto, cytosolic; nuc, nuclear. N_2O, nitrous oxide.

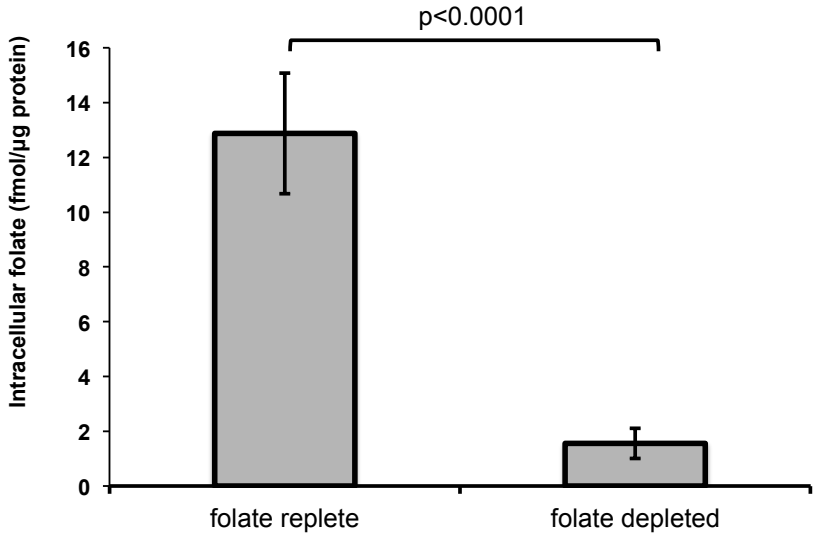

Figure S2. Intracellular folate concentrations in HeLa cells assayed for γH2AX at the time of staining and quantification (refer to Fig. 3). The intracellular folate concentrations were 8-fold higher in HeLa cells maintained in folate-replete (25 nM) media compared to those cultured in folate-depleted (5 nM) conditions ($p<0.0001$). Data are shown as the mean ± S.D. of n=4 groups for each folate replete or folate depleted condition. Significance was determined by using a Student's two-tailed t-test.

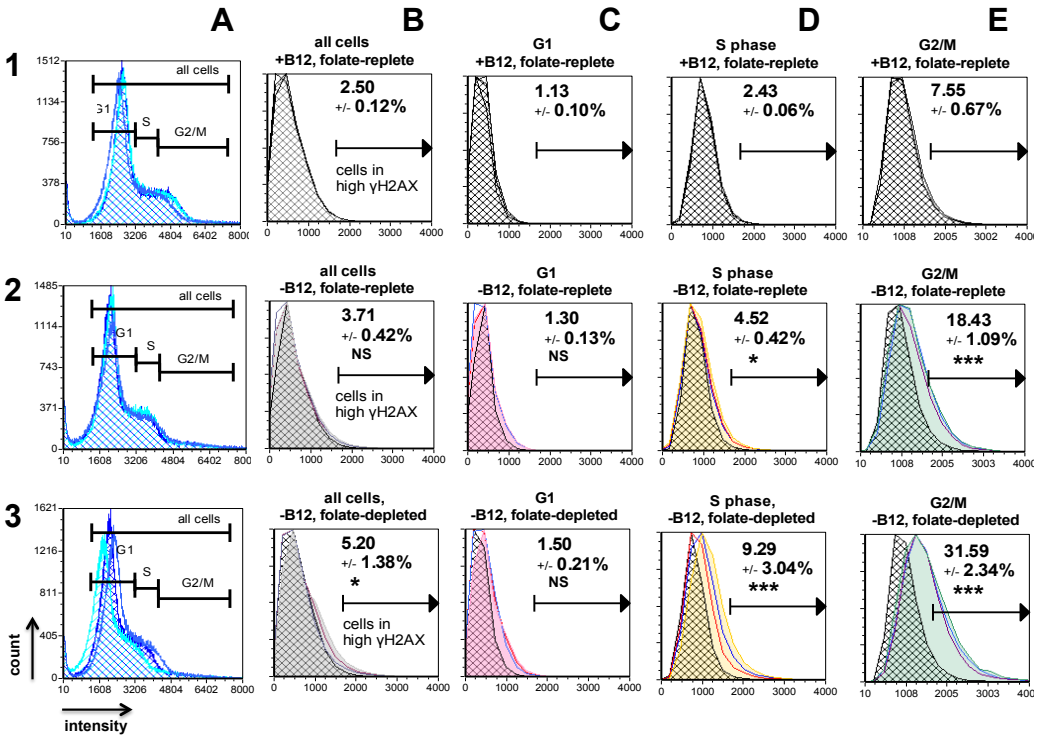

Figure S3. Vitamin B12 depletion induces changes in γH2AX, a marker of DNA damage, in the S and G2/M phases of the cell cycle in HeLa cells. Cells were stained for DNA content (Vybrant Violet; 1-3A) and γH2AX (FITC; 1-3 B-E); individual plots depict the cell count (Y-axis) versus fluorescence intensity (X-axis). The high γH2AX parameter is a threshold defined by the mean top 2.5% of cells in the G1, S, and G2/M ('all cells') stained for γH2AX in the vitamin B12- and folate-replete condition (1B), and this gate was uniformly applied to all conditions and cell cycle phases. Each plot shows the mean percent high γH2AX ± S.D. for triplicates for each experimental condition and cell cycle phase. Individual triplicates stained for γH2AX in each condition are plotted relative to the corresponding mean γH2AX values in the vitamin B12- and folate-replete condition (hatched histograms, 1B-E). Asterisks designate statistical significance in percent high γH2AX values between treatment conditions and cell cycle phase compared to the corresponding phases in the vitamin B12- and folate-replete condition (1B-E). The greatest percentage of cells in high γH2AX within conditions was observed in G2/M under vitamin B12- and folate-depleted conditions ($p<0.001$; 3E). A combined vitamin B12 and folate depletion exacerbated the percent high γH2AX observed in HeLa cells compared to cells maintained in vitamin B12-depleted and folate-replete culture conditions in all cells, S, and G2/M (compare 2A,D-E to 3A,D-E), and this difference in high γH2AX between conditions was significant for S phase ($p=0.01$) and G2/M ($p=0.0003$). Statistical significance was determined using a one-way ANOVA. The dependent variable was log-transformed percent high γH2AX, and the

independent variable was vitamin B12 and folate level. Folate-replete, 25 nM (6S) 5-formylTHF in culture media; Folate-depleted, 5 nM (6S) 5-formylTHF in culture media. The statistical significance is represented as follows: NS = Not significant ($p > 0.05$); * = $0.01 < p < 0.05$; ** = $0.01 < p < 0.001$; *** = $p < 0.001$

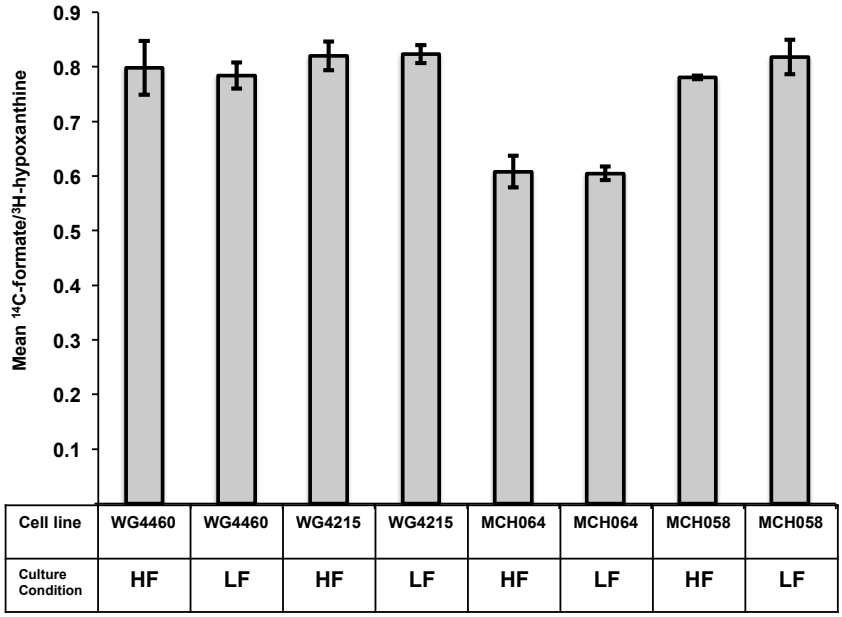

Figure S4. Mean ratio of ^{14}C-formate/^{3}H-hypoxanthine incorporation into nuclear DNA in cblG (WG4215 and WG4460) and control (MCH064 and MCH058) fibroblasts. Data are shown as mean ± S.D. for fibroblast line and treatment. A two-way ANOVA revealed a non-significant effect of folate exposure ($p>0.05$) and a significant effect of fibroblast genotype ($p=0.02$) on the rank-transformed mean ratios of ^{14}C/^{3}H incorporated into nuclear DNA. HF, 25 nM (6S) 5-formylTHF; LF, 5 nM (6S) 5-formylTHF.

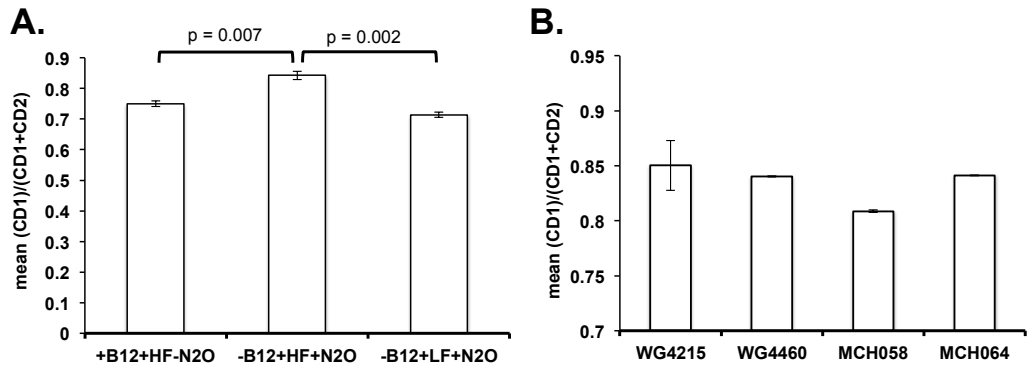

Figure S5. Mean ratio of isotopically labeled one-carbon units from MTHFD1 (CD1) to the total number of labeled one-carbons containing 1 or 2 deuterium atoms generated from MTHFD1 (CD1) or SHMT (CD2), respectively, into thymidine in nuclear DNA in (A) HeLa cells and (B) human fibroblasts. *(A)* Vitamin B12-depleted and folate-replete conditions increased the contribution of labeled one-carbon units from MTHFD1 relative to SHMT by 12% compared to vitamin B12 and folate-replete conditions in HeLa cells (p=0.007). The ratios between vitamin B12-depleted conditions were significant (p=0.002). *(B)* In human cblG (WG4215 and WG4460) and control fibroblasts (MCH058 and MCH064) grown under high folate conditions, there was no difference in the contributions of one-carbon units from MTHFD1 (p>0.05). For (A) and (B), cells were plated in duplicate, and the data are shown as mean ± S.D. Statistical significance between conditions was assessed using a one-way ANOVA. The dependent variable was the log-transformed ratio of mean CD1/(CD1+CD2) and the independent variable was folate and vitamin B12 exposure. HF, 25 nM (6S) 5-formylTHF; LF, 5 nM (6S) 5-formylTHF.

TABLE S1. Fold differences in percent high γH2AX[1] between control and experimental conditions[2] within cell cycle phase (to accompany Fig. 3).

	All cells	**G1**	**S**	**G2/M**
	Least Square (LS) Mean 95% CI (Lower, Upper)	LS Mean 95% CI (L,U)	LS Mean 95% CI (L,U)	LS Mean 95% CI (L,U)
Treatment	**B**	**C**	**D**	**E**
(1) B12- and folate-replete (control)	**2.50**	**0.76**	**2.46**	**8.99**
(2) B12-replete, folate-depleted	2.10*** (1.65, 2.66)	1.12^{NS} (0.89, 1.40)	1.55^{NS} (1.11, 2.16)	1.84** (1.39, 2.43)
(3) B12-depleted, folate-replete	3.16*** (2.42, 4.13)	2.25*** (1.74, 2.90)	4.50*** (3.10, 6.52)	2.80*** (2.05, 3.84)
(4) B12- and folate-depleted	5.38*** (4.23, 6.83)	4.83*** (3.85, 6.06)	9.02*** (6.47, 12.59)	4.85*** (3.66, 6.42)
(5) B12-depleted: folate-replete vs. depleted (row 3 vs. 4)	1.70** (1.30, 2.23)	2.15*** (1.67, 2.77)	2.01* (1.38, 2.91)	1.73* (1.26, 2.37)

[1]Percent (%) high γH2AX refers to the percentage of HeLa cells stained for γH2AX above the mean top 2.5% total γH2AX intensity in 'all cells' in the vitamin B12- and folate-replete (control) condition (Figure 3, 1B). Columns show the back-transformed least-squares (LS) means and 95% confidence intervals (CI) for Log_e transformed mean % high γH2AX for specific contrasts, indicating fold differences in % high γH2AX. Values for LS means in row 1B-E for control conditions are those mean % high γH2AX predicted by the model. LS means and 95% CI were back-transformed = exp^(LS Mean) = geometric mean % high γH2AX.

[2]*Post-hoc* comparisons (n=3) were made within cell cycle phase ('all cells', G1, S, G2/M) and compared the % high γH2AX in the control to that in row: 2) B12-replete, folate-depleted, 3) B12-depleted, folate-replete, and 4) B12- and folate-depleted conditions. A final comparison considered the difference in % high γH2AX between B12 depleted conditions (row 3 vs. 4). A Bonferroni correction was applied to all p-values to account for multiple comparisons (n=4).

Legend: NS = Not significant ($p \geq 0.05$); * = $0.01 < p < 0.05$; ** = $0.01 < p < 0.001$; *** = $p < 0.001$