Supplemental Information.

SHMT1 and SHMT2 anchor the nuclear *de novo* dTMP synthesis pathway to the nuclear lamina for DNA replication and repair.

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Inventory of Supplemental Information

Relationship of the supplemental figures to the main figures:

Figure S1, related to Figure 2, *Shows DHFR nuclear localization is cell cycle dependent.*

Figure S2, related to Figure 2, *Shows TYMS nuclear localization is cell cycle dependent.*

Figure S3, related to Figure 2, *Shows tandem affinity purification, which was used for determining SHMT1 interactors.*

Table S1, related to Figure 2 and Figure S3, *Shows list of proteins identified involved in DNA replication and repair.*

Table S2, related to Figure 2 and Figure S3, *Shows list of proteins identified involved in Purine metabolism.*

Table S3, related to Figure 2 and Figure S3, *Shows list of proteins identified involved in Cell cycle and checkpoints.*

Table S4, related to Figure 2 and Figure S3, *Shows list of proteins identified involved in chromatin modification.*

Table S5, related to Figure 2 and Figure S3, *Shows list of proteins identified involved in lamin binding.*

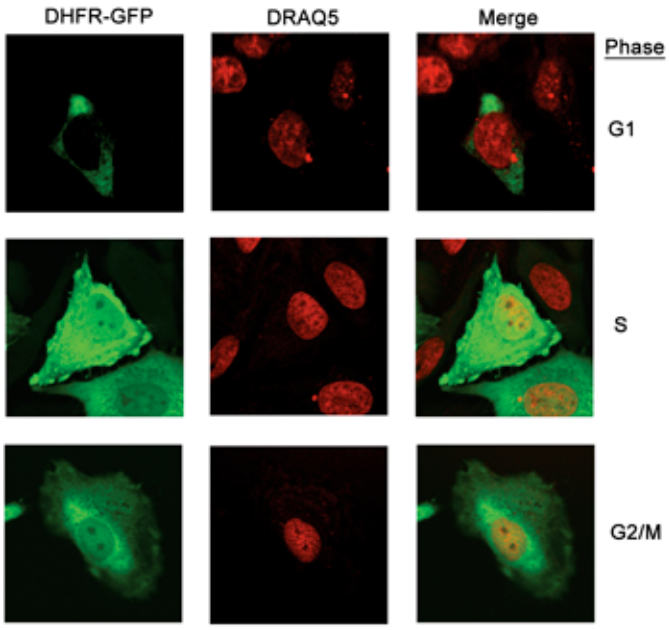
Figure S4, related to Figure 2 and S3, *Shows several validations identified from peptide sequencing.*

Figure S5, related to Figures 2 and 3, *Shows important control for LaminB1 over-expression.*

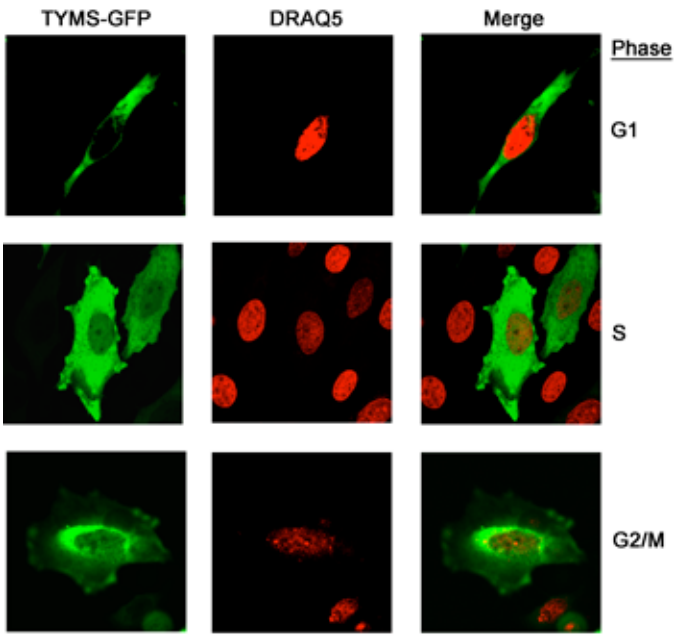
Figure S6, related to Figure 4, *Shows SHMT1 and SHMT2 are required for DHFR and LaminB1 co-localization.*

Figure S7, related to Figure 4, *Shows TYMS, DHFR, and SHMT2 are not required for SHMT1 and LaminB1 co-localization.*

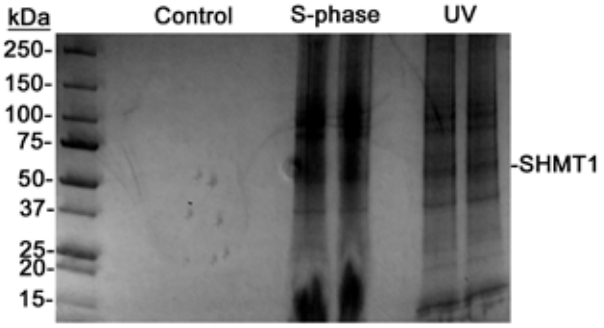
Figure S8, related to Figure 4, *Shows TYMS, DHFR, and SHMT1 are not required for SHMT2α and LaminB1 co-localization.*



Supplemental Figure 1. DHFR nuclear localization is cell cycle dependent. cDNAs encoding GFP-DHFR were transfected into cells and blocked in G1 (30 uM lovastatin), S phase (1 mM hydroxyurea), and G2/M (100 ng/mL nocodazole) phases of the cell cycle. DRAQ5 was used as the nuclear stain and cells were visualized with confocal microscopy. DHFR is present in nuclei during S and G2/M phases of the cell cycle.



Supplemental Figure 2. TYMS nuclear localization is cell cycle dependent. cDNAs encoding GFP-TYMS were transfected into cells and blocked in G1 (30 uM lovastatin), S phase (1 mM hydroxyurea), and G2/M (100 ng/mL nocodazole) phases of the cell cycle. DRAQ5 was used as the nuclear stain and cells were visualized with confocal microscopy. TYMS is present in nuclei during S and G2/M phases of the cell cycle.



Supplemental Figure 3. Tandem affinity purification of SHMT1. pCMV-FLAG-SHMT1-MAT-Tag-1 and pCMV-FLAG-MAT-Tag-1 empty vector (control) were transfected into HeLa cells. Cells were treated with hydroxyurea (1 mM) to block at S-phase or exposed to 10 mJ/cm^2 UV. Following treatment, cells were incubated for 24 hours. Nuclei were isolated, treated with benzonase and tandem affinity purified using FLAG and Nickel resins. The lanes were excised and peptide sequences were determined by MS/MS.

Table S1. Proteins identified which are involved in DNA replication and repair. Lamin binding proteins are highlighted in yellow.

Protein	S	UV	Both
APEX1			✓
FANCD2		✓	
FANCL		✓	
H2AFX			✓
MPG			✓
RAD50			✓
XAB2			✓
ALKBH2	✓		
CCNH	✓		
ERCC3			✓
GTF2H3			✓
LIG3			✓
LIG4		✓	
MDC1			✓
MNAT1	✓		
NTHL1			✓
POLE		✓	
POLD3		✓	
POLR2A	✓		
POLR2C	✓		
POLR2H	✓		
PCNA	✓		
RFC1			✓
ATM		✓	
TP53BP1			✓
PRIM1			✓
PRIM2			✓
H2AFZ			✓
DKC1			✓

Table S2. Proteins identified which are involved in purine metabolism. Lamin binding proteins are highlighted in yellow.

Protein	S	UV	Both
IMPDH2			✓
POLE		✓	

POLD3		✓	
POLR1A			✓
POLR1C	✓		
POLR1E			✓
POLR2A	✓		
POLR2C	✓		
POLR2H	✓		
POLR3A		✓	
PRIM1			✓
PRIM2		✓	
RRM1		✓	
MTHFD1			✓

Table S3. Proteins identified which are involved in cell cycle and checkpoints. Lamin binding proteins are highlighted in yellow.

Protein	S	UV	Both
RAD21	✓		
SKP1	✓		
ANAPC5			✓
ATR			✓
CDC27		✓	
CUL1			✓
CCNB1	✓		
CCNH	✓		
CDK4	✓		
PCNA	✓		
YWHAE			✓
ATM		✓	
STAG1			✓
YWHAB			✓
YWHAH	✓		
YWHAZ			✓
PSMD12		✓	
PSMB4	✓		

Table S4. Proteins identified which are involved in chromatin modification. Lamin binding proteins are highlighted in yellow.

Protein	S	UV	Both
HUWE1		✓	

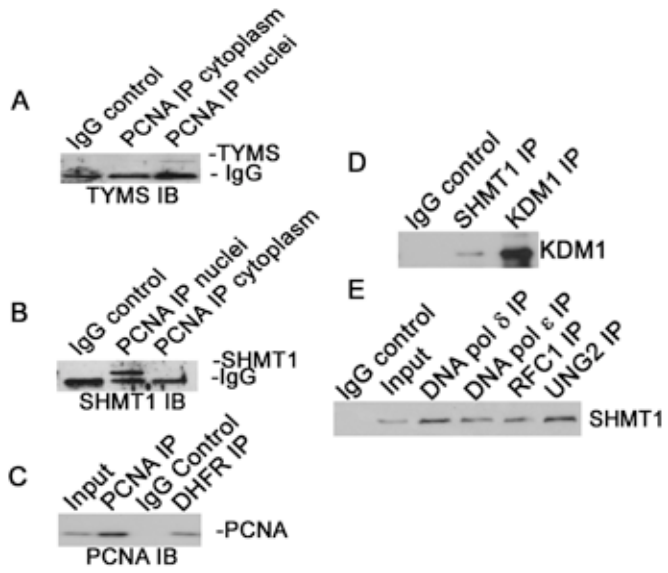
INO80	✓		
PHF21A		✓	
RBM14	✓		
SETD1A	✓		
SETD2	✓		
SMARCA2			✓
SRCAP	✓		
TAF5	✓		
BPTF			✓
BRD8	✓		
CENPV			✓
CHD7	✓		
EHMT2	✓		
HELLS			✓
HMG20B			✓
ING3	✓		
JMJD1C	✓		
KDM1	✓		
KDM3B		✓	
MBD3			✓
MORF4L2	✓		
NCOR1			✓
PHB			✓
PRMT5		✓	
RSF1			✓
RNF2	✓		
SUPT5H	✓		
SUPT6H			✓
USP22			✓

Table S5. Proteins identified that bin lamin.

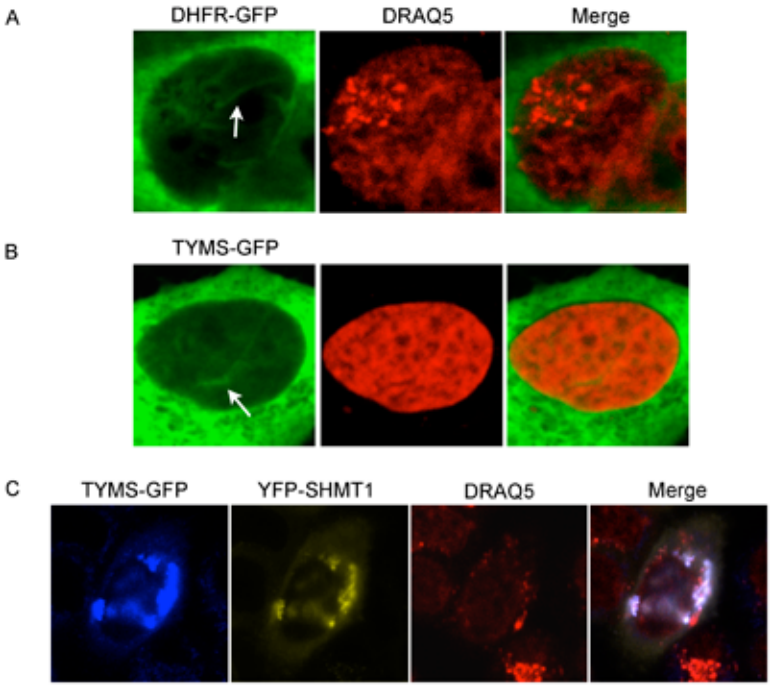
	Lamin Binding Proteins		
Protein	S	UV	both
DNAJA2	✓		
BAT1	✓		
IMO7			✓
LIMA1			✓
ANAPC5			✓
PDCD6			✓
CAPZB	✓		

CFL1		✓	
EEF2			✓
LUZP1		✓	
MED10	✓		
MED14			✓
MED19			✓
MED22	✓		
MED4	✓		
MED6			✓
MED7	✓		
MED8			✓
MED9	✓		
MYH9			✓
MYL12B			✓
POLR2A	✓		
POLR2C	✓		
POLR2H	✓		
PPP1CB	✓		
PPP1R12A			✓
RAI14			✓
RPL23A			✓
RPL26			✓
RPL30			✓
RPL7			✓
RPS11			✓
RPS28		✓	
RPS3A			✓
RPS5			✓
RPS9			✓
RPLP2			✓
SHMT2			✓
UBR5		✓	
RBM39			✓
ARPC3			✓
MED27	✓		
SNRPD1			✓
TMPO			✓
TMOD3		✓	
ARPC4			✓
EMD			✓
H2AFX			✓

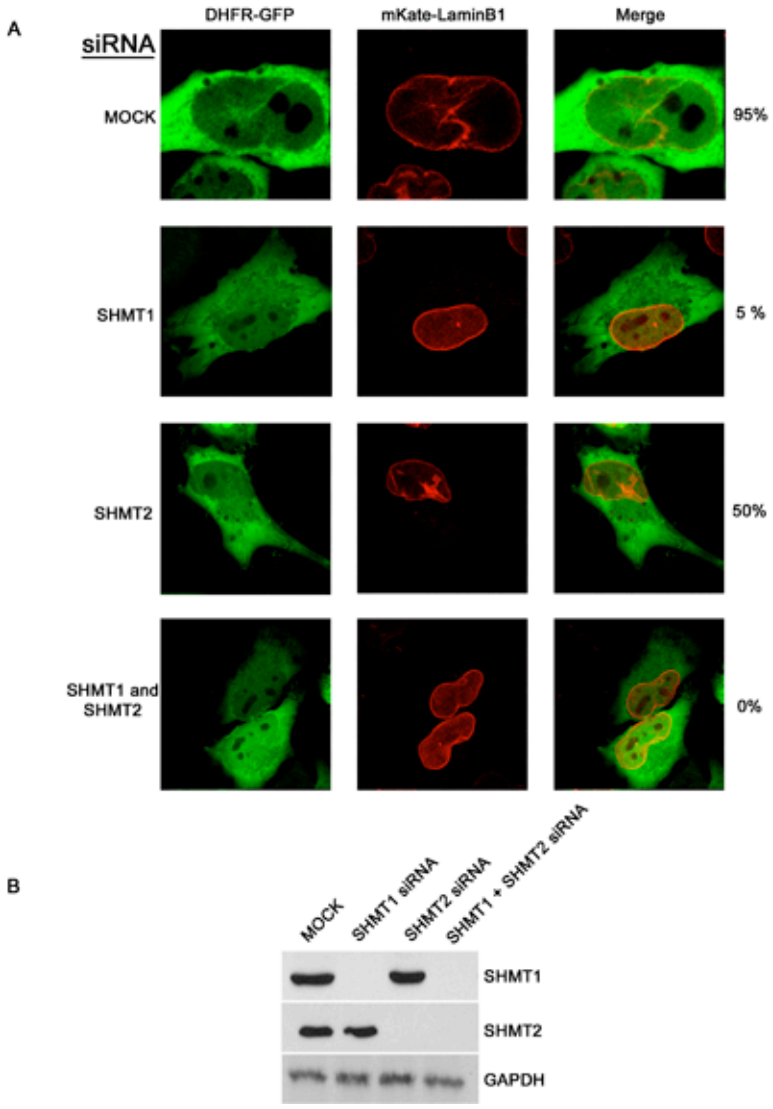
ING1			✓
LMNA			✓
LMNB1			✓
LMNB2	✓		
UBE2I	✓		
AHCTF1	✓		
PRMT5		✓	
AP2M1			✓
ATAD3A			✓
CCNC			✓
COPB1			✓
DDX6			✓
DNM2		✓	
TCEB1	✓		
EPPK1		✓	
IMPDH2			✓
MYH10			✓
MYO1C			✓
SLC9A3R2		✓	
NOLC1	✓		
PLEC1			✓
TMEM43		✓	
RPL10			✓
RPL35A		✓	
SMC4			✓
CTTN			✓
SRP9	✓		
TPM3			✓
THRAP3	✓		
XPO1		✓	
CSE1L		✓	
PCNA	✓		



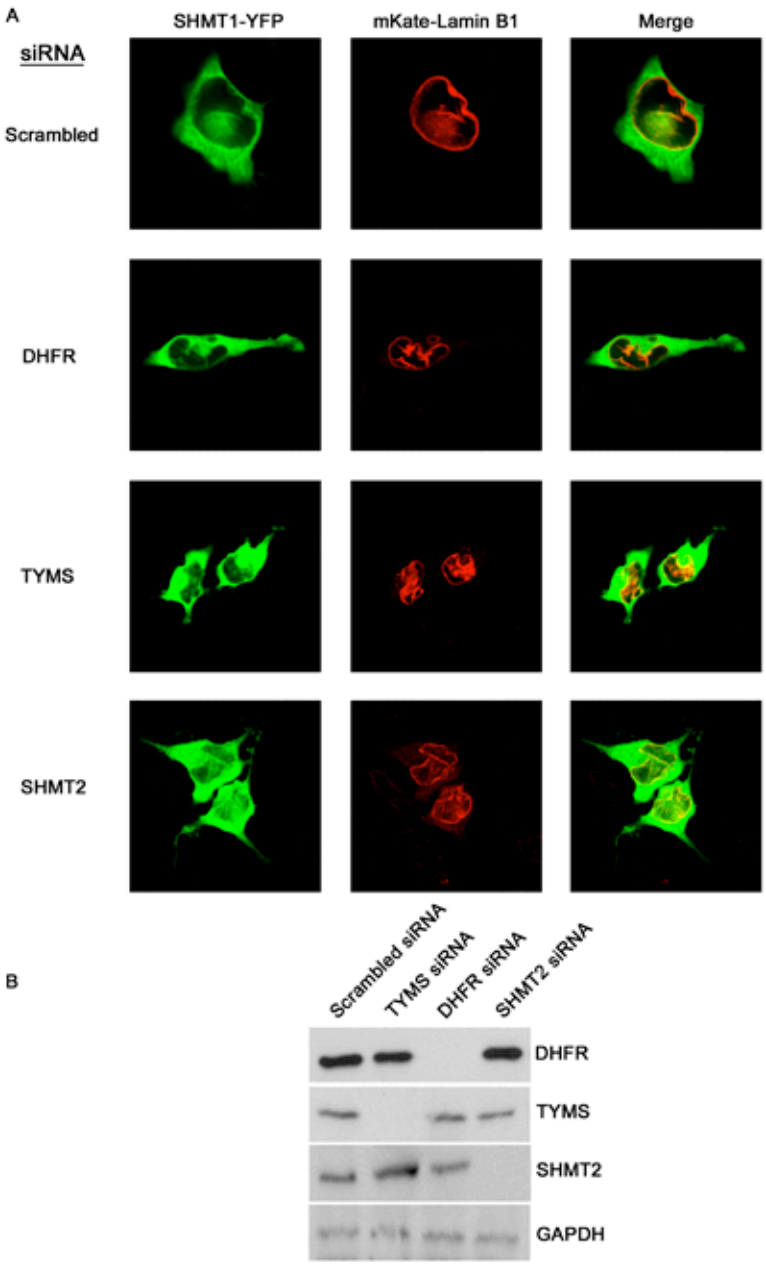
Supplemental Figure 4. Validation of SHMT1 interacting partners. Nuclear (A, B, C, and D) and cytosolic fractions (A and B) of HeLa cells were isolated for immunoprecipitation experiments. A) TYMS and B) SHMT1 co-precipitated with PCNA only in nuclear fractions. C) Nuclear fractions were subjected to PCNA, control, or DHFR immunoprecipitations. PCNA co-precipitates with DHFR, but not in control IgG. D) SHMT1 co-immunoprecipitates with KDM1 but not in IgG controls. E) SHMT1 co-immunoprecipitates with DNA polymerases δ and ε, RFC1, and UNG2, but not in IgG controls.



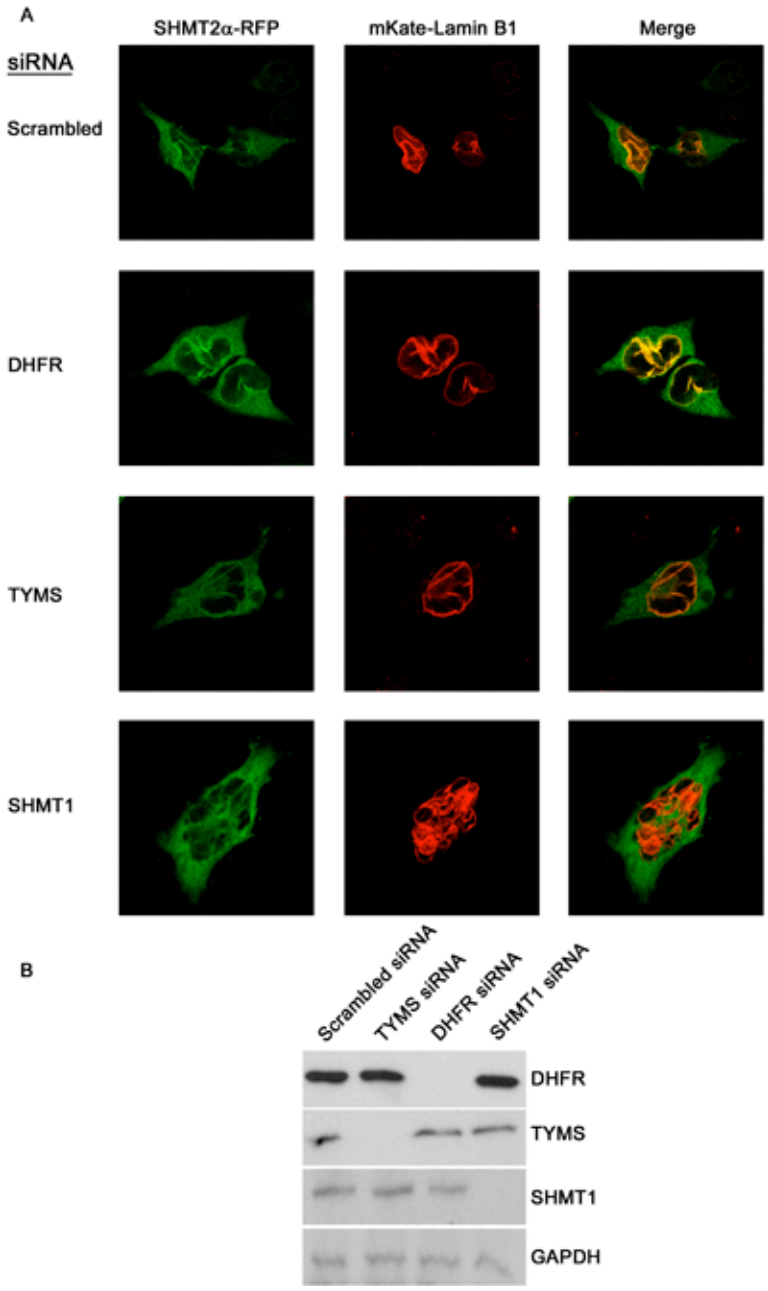
Supplemental Figure 5. TYMS, DHFR, and SHMT1 localize to linear structures within nuclei independent of LaminB1 over-expression. cDNAs encoding A) GFP-DHFR, B) and C) GFP-TYMS, and C) YFP-SHMT1 fusion proteins were transfected into HeLa cells. DRAQ5 was used as the nuclear stain to control for LaminB1 over-expression causing aggregation. Cells were visualized with confocal microscopy. Over-expression of LaminB1 in cells is not a requirement for visualization of linear structures within nuclei. DHFR, TYMS, and SHMT1 all form linear structures (white arrows) within nuclei.



Supplemental Figure 6. SHMT1 and SHMT2 are required for DHFR and Lamin B1 co-localization. A) cDNAs encoding GFP-DHFR and mKate-Lamin B1 fusion proteins, and siRNAs including scrambled (mock), SHMT1, SHMT2, or both SHMT1 and SHMT2 were transfected into HeLa cells. Cells were blocked in S-phase using hydroxyurea (2 mM) and visualized with confocal microscopy. For each siRNA treatment, 100 cells were counted. The presence of DHFR and Lamin B1 co-localizing structures occurred in 95% ± 4.7%, 5% ± 4.6 %, 50% ± 12%, and 0% ± 0.6% of cells transfected with scrambled, SHMT1, SHMT2, and both SHMT1 and SHMT2 siRNAs respectively. Error is expressed as standard deviation (n=3). B) Immunoblotting was performed on siRNA treated samples to ensure knockdown of SHMT1 and SHMT2. GAPDH was used as a loading control.



Supplemental Figure 7. TYMS, DHFR, and SHMT2 are not required for SHMT1 and Lamin B1 co-localization. A) cDNAs encoding SHMT1-YFP and mKate-Lamin B1 fusion proteins, and siRNAs including scrambled, DHFR,TYMS, or SHMT2 were transfected into HeLa cells. The formation of Lamin B1 and SHMT1 co-localizing structures was not inhibited by knockdown of DHFR, TYMS, or SHMT2. B) Immunoblotting was performed on siRNA treated samples to ensure knockdown of DHFR, TYMS and SHMT2. GAPDH was used as a loading control.



Supplemental Figure 8. TYMS, DHFR, and SHMT1 are not required for SHMT2α and Lamin B1 co-localization. cDNAs encoding SHMT2α-RFP and mKate-Lamin B1 fusion proteins, and siRNAs including scrambled, DHFR,TYMS, or SHMT1 were transfected into HeLa cells. DHFR, TYMS, and SHMT1 were not required for SHMT2α and Lamin B1 co-localization. B) Immunoblotting was performed on siRNA treated samples to ensure knockdown of DHFR, TYMS and SHMT1. GAPDH was used as a loading control.