

Supplementary Figure 1

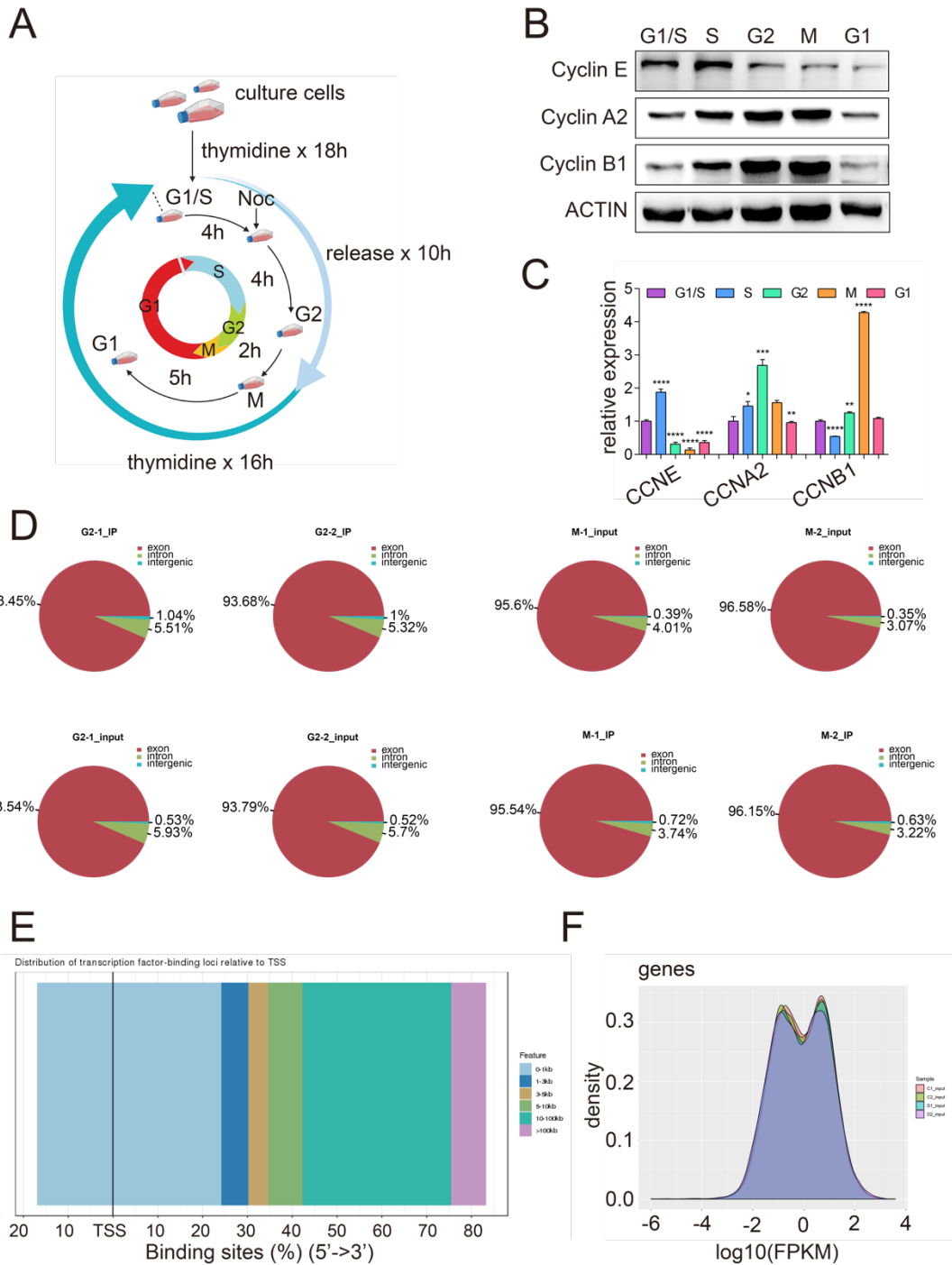


Figure S1. (A) Schematic of the double-block method with thymidine and nocodazole to synchronize the cell cycle. At the first block, HeLa cells were treated with 2.5 mM thymidine for 18 hours and released in thymidine-free medium for 10 hours. After that, an additional block with 2.5 mM thymidine for 16 hours, cells in S phase and G1 phase were harvested respectively at 4 and 15 hours later. G2 and M phase cells

were further incubated with 100 ng/ml nocodazole for 8h and 10 hours after being released, respectively. Next, cells arrested in distinct phases were processed for further detection.

(B) Cell cycle synchronization efficiency of HeLa cells was examined by Western blotting of specific cell cycle markers (Cyclin A2, Cyclin B1 and Cyclin E). ACTIN served as a loading control.

(C) qPCR of *CCNA2*, *CCNB1* and *CCNE* was performed to detect cell cycle synchronization efficiency across differential cell cycle stages of HeLa cell. Relative mRNA expression levels were normalized by 18S rRNA. Data are presented as the mean \pm SD of triplicate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared to the control group.

(D) Mapped region of each sample.

(E) Analysis of different m⁶A peaks and Transcription Start Sites (TSS).

(F) Density of Different Expression Genes.

Table S1

| Genes | Organism | Sequence (5'->3') |
|-------------|------------|--|
| si YTHDF1#1 | homosapien | GGACAGUCAAAUCAGAGUAdTdT UACUCUGAUUUUGACUGUCCdTdT |
| si YTHDF1#2 | homosapien | CCUACGGACAGCUCAGUAAAdTdT UUACUGAGCUGUCCGUAGGdTdT |
| siNC | | UUCUCCGAACGUGUCACGUdTdT ACGUGACACGUUCGAGAAAdTdT |
| shMETTL3#1 | homosapien | AATCCGTCAGTATCTTGGGCAAGTTCTCGAGAACTTGCCCAAGATACTGACGTTTTTT CTAGAAAAACGTCAGTATCTTGGGCAAGTTCTCGAGAACTTGCCCAAGATACTGACGG |
| shMETTL3#2 | homosapien | AATTCGCCAAGGAACAATCCATTGTTCTCGAGAACAATGGATTGTTCTTGCTTTTTT CTAGAAAAAGCCAAGGAACAATCCATTGTTCTCGAGAACAATGGATTGTTCTTGCTGCG |
| shNC | homosapien | CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG |

Table S2

| Genes | Primers | Organism | Sequence (5'->3') |
|---------|---------|------------|---------------------------|
| GAPDH | Forward | homosapien | ACAAC TTTGGTATCGTGAAGG |
| GAPDH | Reverse | homosapien | GCCATCACGCCACAGTTTC |
| CCNA2 | Forward | homosapien | GAGGATATTCACACATACCTTAGGG |
| CCNA2 | Reverse | homosapien | TCCACGAGGATAGCTCTCATAC |
| CCNB1 | Forward | homosapien | CTTTCGCCTGAGCCTATTTTG |
| CCNB1 | Reverse | homosapien | ACATCAGAGAAAGCCTGACAC |
| CCNE | Forward | homosapien | GCCAAAATCGACAGGACGG |
| CCNE | Reverse | homosapien | ATCTTCTTTGTCAGGTGTGGG |
| METTL3 | Forward | homosapien | AGAGCCTTCTGAACCAACAG |
| METTL3 | Reverse | homosapien | CAATGGATTGTTCTTGGCTG |
| METTL14 | Forward | homosapien | GAGCTGAGAGTGCGGATAGC |
| METTL14 | Reverse | homosapien | GCAGATGTATCATAGGAAGCCC |
| FTO | Forward | homosapien | ACTTGGCTCCCTTATCTGACC |
| FTO | Reverse | homosapien | TGTGCAGTGTGAGAAAGGCTT |
| ALKBH5 | Forward | homosapien | CATACGGCCTCAGGACATC |
| ALKBH5 | Reverse | homosapien | TCAGGGACTTTGTTTCCAACC |
| CDC25B | Forward | homosapien | CAGGTCTCTGCATGGATTCC |
| CDC25B | Reverse | homosapien | TGCTCGTTTCGAATGATCCG |
| YTHDF1 | Forward | homosapien | GACTTTGAGCCCTACCTTACTG |
| YTHDF1 | Reverse | homosapien | CAATGGACGGCGGGTAATAG |

Table S6

| Term | Count | % | PValue | Genes | List Total | Pop Hits | Pop Total | Fold Enrichment | FDR |
|---|-------|--------|-----------|--|---------------|-------------|--------------|--------------------|--------|
| GO:0000398~mRNA splicing, via spliceosome | 39 | 0.0187 | 5.65E-07 | RALY, POLR2F, POLR2L, POLR2J, TRA2B, SYNCRIP, SART3, BUD31, CACTIN, XAB2, SART1, HNRNPM, DHX38, METTL3, HNRNPF, SNRNP70, DHX35, DDX41, DDX39A, SNRPA1, PRPF31, CSTF3, SPEN, SF3A2, SF3A3, UBL5, PPIE, PCF11, EIF4A3, SRSF5, RBPMS, PAPOLA, AQR, GTF2F1, RSR1, SNRNP25, PDCD7, CPSF1, SNRPG POLL, ING5, ACHE, RECQL5, DTL, POLG, LIG1, TREX1, IGF1, NFIX, RMI2, RBBP7, MCM5, RPA3, CDT1, SET, ORC4, SUPT16H, CHTF18, POLG2, E4F1, RTEL1-TNFRSF6B, ATRIP KIF22, RAD51C, STUB1, RPA3, EPC2, UBE2D3, CHRNA4, POLG2, RTEL1-TNFRSF6B, INO80C, INO80B, POLL, RECQL5, NUDT1, LIG1, TRIM28, SMG1, TREX1, RAD51, DOT1L, PNKP, UHRF1, SUPT16H, RAD18, RUVBL2, OGG1, GADD45A, USP45 ANAPC2, PSMD14, PSMC5, PSMD12, PSME1, PSMB2, PSMD2, UBC, PSMD4, ANAPC11, CDC26, UBE2E1 | 1217 | 222 | 16792 | 2.423949011 | 0.001 |
| GO:0006260~DNA replication | 23 | 0.011 | 0.0018118 | RPA3, CDT1, SET, ORC4, SUPT16H, CHTF18, POLG2, E4F1, RTEL1-TNFRSF6B, ATRIP KIF22, RAD51C, STUB1, RPA3, EPC2, UBE2D3, CHRNA4, POLG2, RTEL1-TNFRSF6B, INO80C, INO80B, POLL, RECQL5, NUDT1, LIG1, TRIM28, SMG1, TREX1, RAD51, DOT1L, PNKP, UHRF1, SUPT16H, RAD18, RUVBL2, OGG1, GADD45A, USP45 ANAPC2, PSMD14, PSMC5, PSMD12, PSME1, PSMB2, PSMD2, UBC, PSMD4, ANAPC11, CDC26, UBE2E1 | 1217 | 155 | 16792 | 2.047424921 | 3.2978 |
| GO:0006281~DNA repair | 28 | 0.0134 | 0.0116046 | RTEL1-TNFRSF6B, ATRIP KIF22, RAD51C, STUB1, RPA3, EPC2, UBE2D3, CHRNA4, POLG2, RTEL1-TNFRSF6B, INO80C, INO80B, POLL, RECQL5, NUDT1, LIG1, TRIM28, SMG1, TREX1, RAD51, DOT1L, PNKP, UHRF1, SUPT16H, RAD18, RUVBL2, OGG1, GADD45A, USP45 ANAPC2, PSMD14, PSMC5, PSMD12, PSME1, PSMB2, PSMD2, UBC, PSMD4, ANAPC11, CDC26, UBE2E1 | 1217 | 235 | 16792 | 1.644000769 | 19.414 |
| GO:0051436~negative regulation of ubiquitin- protein ligase activity involved in mitotic cell cycle | 12 | 0.0057 | 0.0126872 | ANAPC2, PSMD14, PSMC5, PSMD12, PSME1, PSMB2, PSMD2, UBC, PSMD4, ANAPC11, CDC26, UBE2E1 | 1217 | 71 | 16792 | 2.332033284 | 21.031 |

Table S6 Continued

| | | | | | | | | | |
|--|----|--------|-----------|--|------|-----|-------|-------------|--------|
| GO:0045931~positive regulation of mitotic cell cycle | 6 | 0.0029 | 0.0481613 | SHB, EIF4EBP1, PKN2, PAFAH1B1, CDC25B, TERF1 | 1217 | 28 | 16792 | 2.956685057 | 59.859 |
| GO:0006977~DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest | 9 | 0.0043 | 0.0776215 | CNOT9, BAX, CNOT10, CNOT3, UBC, ARID3A, RBM38, PIDD1, GADD45A | 1217 | 62 | 16792 | 2.002915684 | 77.556 |
| GO:0008283~cell proliferation | 36 | 0.0172 | 0.0555665 | ACHE, HRAS, PPARD, GNAI2, ERBB2, BCAR1, SIPA1, BOP1, SCRIB, PRMT5, CKLF, ILK, E4F1, DDX41, BHLHE41, NUDC, MATK, EGFR, IRS2, CRIP1, PDXK, IGF1, RBBP7, TACC2, UHRF1, IGSF8, PRDM4, LAMA5, SIX1, H3F3A, TCF19, BIN1, EMP2, EMP1, IGFBP4, MAP3K11, MAD1L1, CLTA, LZTS2, HAUS5, GNAI3, GNAI2, BCAR1, NEDD9, CDC34, ANAPC11, CDC37, SPC25, FIGN, CCDC124, KLHL21, E4F1, NSUN2, KMT5A, NUDC, TERF1, CSNK1A1, PARD6A, ANAPC2, RECQL5, LIG1, PSRC1, PKN2, KIF18B, CDC26, PPP1CC, MARK4, MCM5, CDC25B, CCND1, CENPT | 1217 | 366 | 16792 | 1.357166911 | 65.257 |
| GO:0051301~cell division | 35 | 0.0167 | 0.0493404 | E4F1, NSUN2, KMT5A, NUDC, TERF1, CSNK1A1, PARD6A, ANAPC2, RECQL5, LIG1, PSRC1, PKN2, KIF18B, CDC26, PPP1CC, MARK4, MCM5, CDC25B, CCND1, CENPT | 1217 | 350 | 16792 | 1.37978636 | 60.769 |

Table S6 Continued

| | | | | | | | | | |
|--|----|--------|-----------|--|------|-----|-------|-------------|--------|
| GO:0006977~DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest | 9 | 0.0043 | 0.0776215 | CNOT9, BAX, CNOT10, CNOT3, UBC, ARID3A, RBM38, PIDD1, GADD45A | 1217 | 62 | 16792 | 2.002915684 | 77.556 |
| | | | | MRPL2, EGFR, MRPL4, SLC25A6, MRPS12, RPL26, RPS6KB2, RPL36, RPL37, RPL38, UCP3, RPL31, UCP2, RPS14, SLC25A10, MRPL17, SLC25A22, RPL26L1, SLC25A29, SLC25A37, MRPL55, SLC25A44, SLC25A39, EEFSEC, MRPL43, RMND1 | | | | | |
| GO:0006412~translation | 26 | 0.0124 | 0.0701683 | | 1217 | 253 | 16792 | 1.417962267 | 73.955 |

Table S7

| ANTIBODIES | SOURCE | CAT No |
|--|-----------------------------|------------|
| GAPDH Polyclonal antibody | Proteintech Group | 10494-1-AP |
| METTL3 Polyclonal antibody | Proteintech Group | 15073-1-AP |
| FTO Polyclonal antibody | Proteintech Group | 27226-1-AP |
| ALKBH5 Polyclonal antibody | Proteintech Group | 16837-1-AP |
| Anti-cyclin E Antibody (E-4) | Santa Cruz Biotechnology | sc-377100 |
| Cyclin A2 Polyclonal antibody | Proteintech Group | 18202-1-AP |
| Cyclin B1 Polyclonal antibody | Proteintech Group | 55004-1-AP |
| Beta Actin Polyclonal antibody | Proteintech Group | 20536-1-AP |
| CDC25B Polyclonal antibody | Proteintech Group | 10644-1-AP |
| YTHDF1 Polyclonal antibody | Proteintech Group | 17479-1-AP |
| Anti-METTL14 antibody produced in rabbit | MilliporeSigma | HPA038002 |
| WTAP Monoclonal antibody (4A10G9) | Proteintech Group | 60188-1-Ig |