



Supplementary information, Figure S1 Transcriptome-wide identification of m⁵C sites by RNA-BisSeq. (A) For ribonucleoside standards, 5 fmol of each of m⁵C and hm⁵C, 20 fmol of each of C, G, and A, 400 fmol of U were used. 40 ng of mRNA for each sample was used to

analyze m⁵C and hm⁵C, and 0.02 ng was used to analyze C, U, G and A. **(B)** m⁵C sites within *FURIN* (chr15:91425541) and *PLOD3* (chr7:100849671) (hg19) identified by RNA-BisSeq were validated. cDNA was amplified by PCR using normal primers for untreated mRNAs and specific primers for bisulfite treated mRNAs. **(C)** Venn diagram showing the overlap of m⁵C sites within RNAs between two HeLa replicates. **(D)** The Pearson correlation of common m⁵C levels between two HeLa replicates. **(E)** Percentage of m⁵C sites within various RNA categories. **(F)** Bar chart showing numbers of m⁵C sites within representative noncoding RNAs. **(G)** Bar chart showing the percentage of CG, CHG and CHH contexts within 5' UTR, CDS, intron and 3' UTR regions in transcriptomes. **(H)** Distribution of m⁵C sites and C sites along mRNA transcripts. The moving averages of percentages of mRNA m⁵C and C sites were shown. **(I)** Relationship between m⁵C levels and mRNA abundance in HeLa cells ($R = 0.002$). **(J)** Representative gene ontology (GO) terms enriched in m⁵C-containing mRNAs.