



Supplementary information, Figure S2 Conserved, tissue-specific and dynamic features of mRNA m^5C methylation across mammalian transcriptomes. **(A)** UHPLC-MRM-MS/MS analysis of m^5C and hm^5C in mRNAs from six mouse tissues (small intestine, heart, muscle, brain, kidney and liver). The same ribonucleoside standards and mRNA amount as cell lines

were used. **(B)** Bar charts showing numbers of m⁵C sites and m⁵C-containing mRNAs in six mouse tissues. **(C)** Bar charts showing the normalized (left panel) and unnormalized (right panel) fractions of mRNA m⁵C sites identified in each sequence context: CG, CHG and CHH, where H = A, C, or U in six mouse tissues. **(D)** Bar charts showing the percentage of m⁵C sites within distinct RNA regions: CDS, intron, 5' UTR and 3' UTR among six mouse tissues. **(E)** Sequence frequency logo for the sequences proximal to mRNA m⁵C sites in mouse tissues. **(F)** Distribution of m⁵C sites (pooled from mouse tissues) in CDS regions of mRNA transcripts. The distance of m⁵C sites in mRNA transcripts to translation initiation sites was determined and plotted. There was a peak of m⁵C number at around 79 nt after translation initiation sites. **(G)** Heatmap showing tissue-specific m⁵C sites and expression levels of mRNAs containing them. Blue lines represent presence of tissue-specific m⁵C sites. The representative GO term enrichment in mRNA with tissue-specific sites in each tissue was shown. **(H)** Bar charts showing numbers of tissue-specific m⁵C sites within mRNAs expressed in all six tissues. **(I)** Scatter plot showing the levels of common m⁵C sites between 3- and 4-week testis samples. The m⁵C sites with increased or decreased methylation levels in 4-week testis compared to 3-week testis were highlighted with orange or blue color, respectively. **(J)** Gene ontology analysis of mRNAs containing m⁵C sites with differential levels in 4-week stage testis.