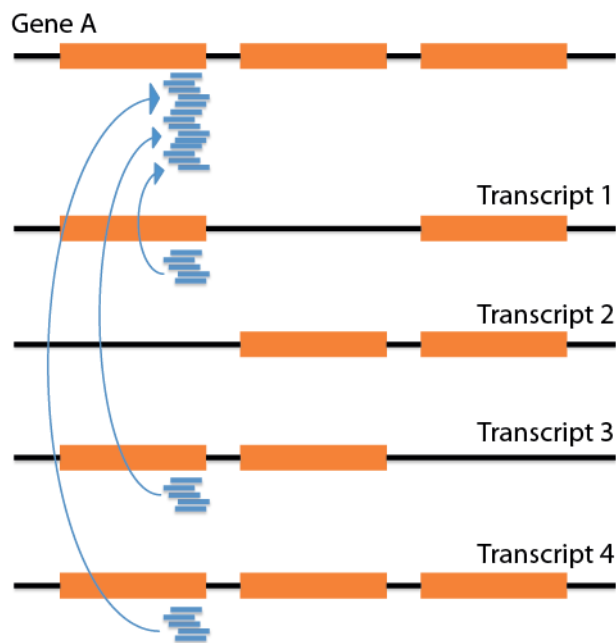
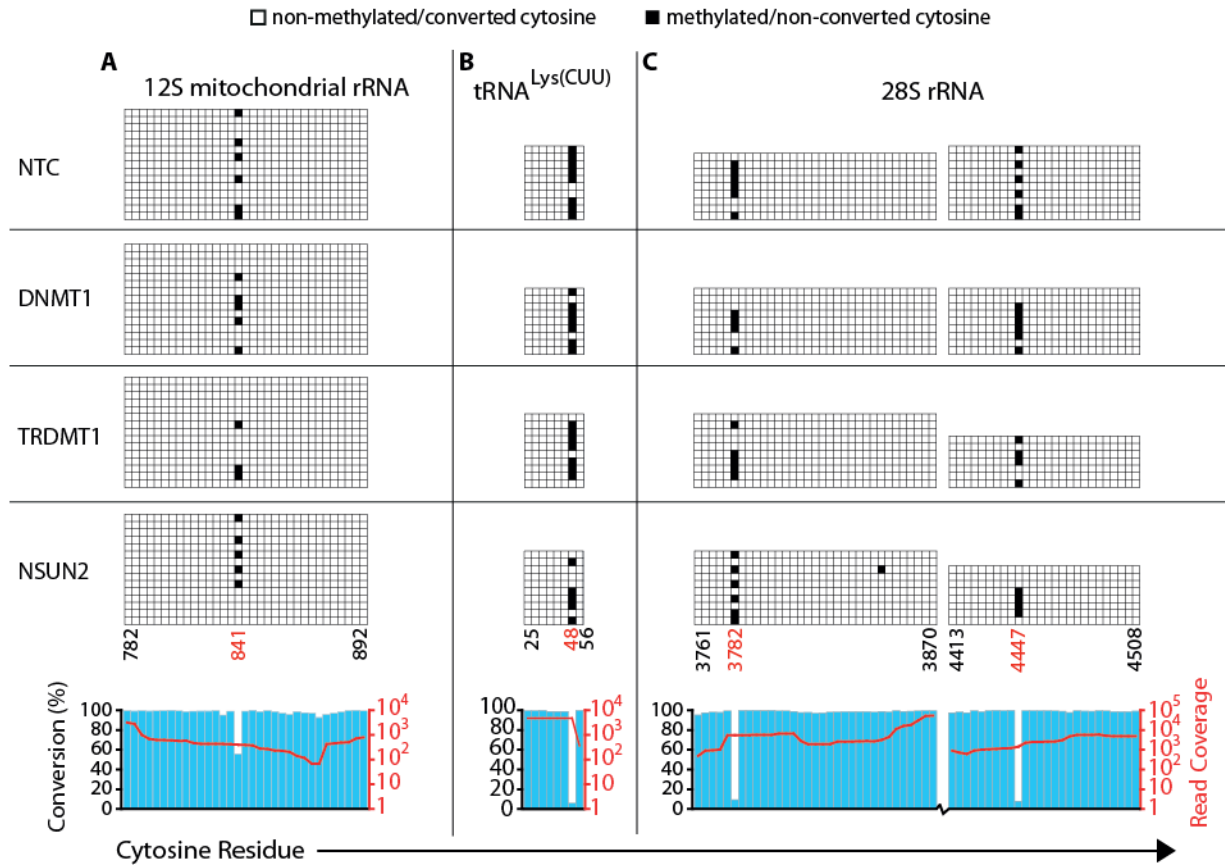


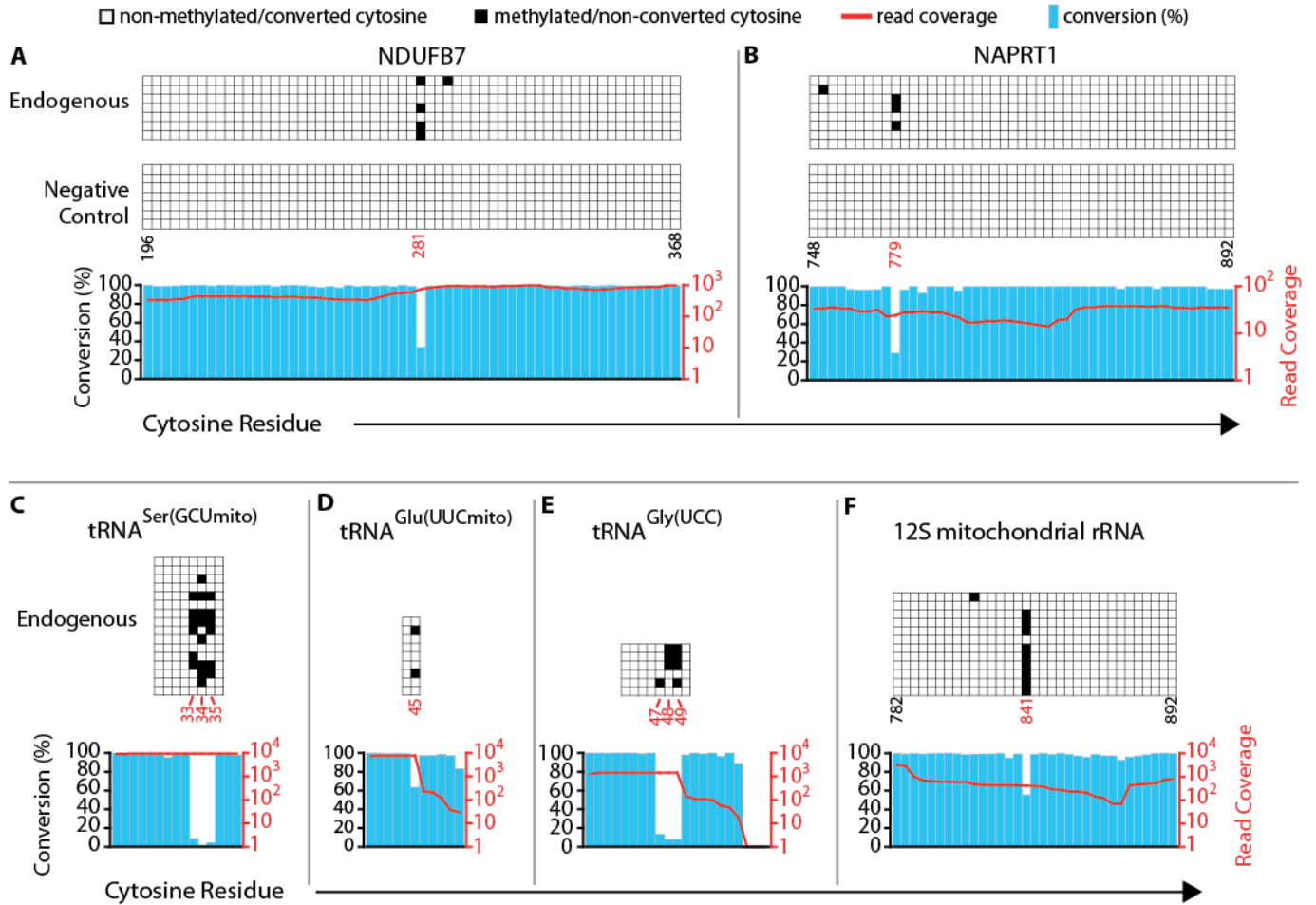
Supplementary Figures for Squires et al. ‘Widespread occurrence of 5-methylcytosine in human coding and noncoding RNA’



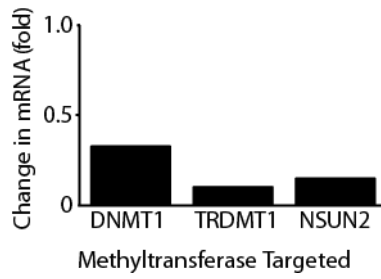
Supplementary Fig. 1. Schematic to illustrate transfer of mapped reads from transcript to genome coordinates.



Supplementary Fig. 2. Analysis of methyltransferase target sites. HeLa cells were transfected with siRNAs targeting *DNMT1*, *TRDMT1* and *NSUN2* or a non-targeting control siRNA (NTC) as indicated on the left. Conventional bisulfite sequencing data was obtained as described in Fig. 1 and is shown for novel sites in (A) 12S mitochondrial rRNA and (B) tRNA^{Lys(CUU)} as well as (C) two previously identified sites in 28S rRNA. Nucleotide positions highlighted in red designate m⁵C candidate sites identified by next generation sequencing. The corresponding next-generation sequencing results are also displayed (lower panels). See Fig. 4 for additional MTase target site data.



Supplementary Fig. 3. Validation of novel m⁵C candidate sites. Conventional bisulfite sequencing data is shown for novel sites in (A) NADH dehydrogenase 1 beta subcomplex 7 mRNA (*NDUF7*), (B) nicotinate phosphoribosyltransferase domain containing 1 (*NAPRT1*) mRNA, (C) mitochondrial tRNA^{Ser(GCU)}, (D) mitochondrial tRNA^{Glu(UUC)}, (E) tRNA^{Gly(UCC)}, and (F) 12S mitochondrial rRNA. Top panels display results for endogenous transcripts, while data for spiked-in *in vitro* transcribed negative controls harboring the same sequence flanked by unique priming sites are also shown in middle panels for (A&B). The corresponding next-generation sequencing results are also displayed (lower panels). Numbering of cytosine positions is as described in Fig. 1, positions highlighted in red designate m⁵C candidate sites identified by next generation sequencing. See Fig. 3 for additional validation data.



Supplementary Fig. 4. Quantitative PCR analysis to measure siRNA knockdown efficiency of targeted methyltransferases. The fold change in targeted MTase mRNA levels relative to non-targeting control MTase mRNA levels is shown. Data was normalized to *HPRT*. This data complements Fig. 4 and Supplementary Fig. 2.