A

5’ GGG AGG CGA GAA CAC ACC ACA ACG AAA ACG AGC AAA ACC CGG ΨAC GCA ACA CAA AAG CGA ACA ACG CGA AAA AGG ACA CCG AAG CGG AAG CAA AGA CAA C

B

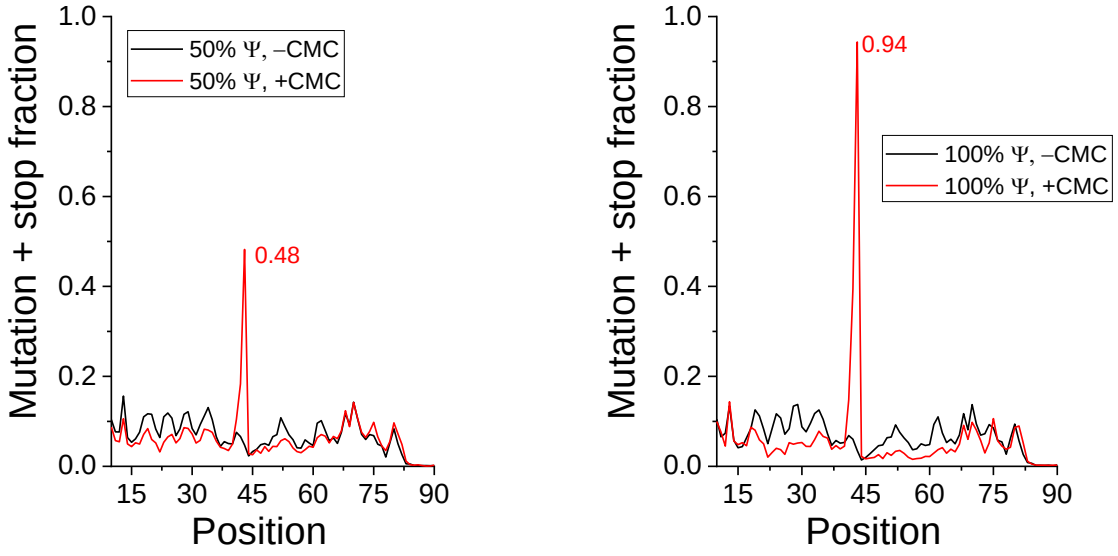

Fig. S1: RNA standard used for calibration. (A) The 100mer RNA contains a single U or Ψ at the underlined position. (B) Sequencing results using this RNA spiked-in total HEK293T RNA (data from GSE110247, NCBI GEO) showing a near complete conversion of Ψ to Ψ-CMC under our condition.

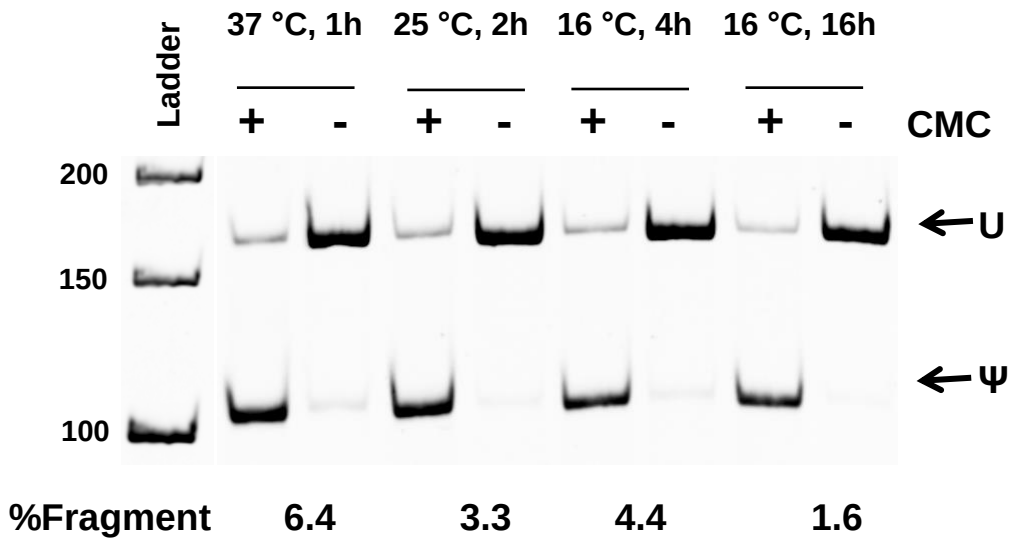

Fig. S2: Optimization of RNA-5 ligation conditions to reduce background. MALAT1 Ψ5590 site was tested here upon varying the incubation condition of the blocking 5mer RNA (RNA-5) ligation. The 16 °C, 16h condition was used for subsequent experiments.

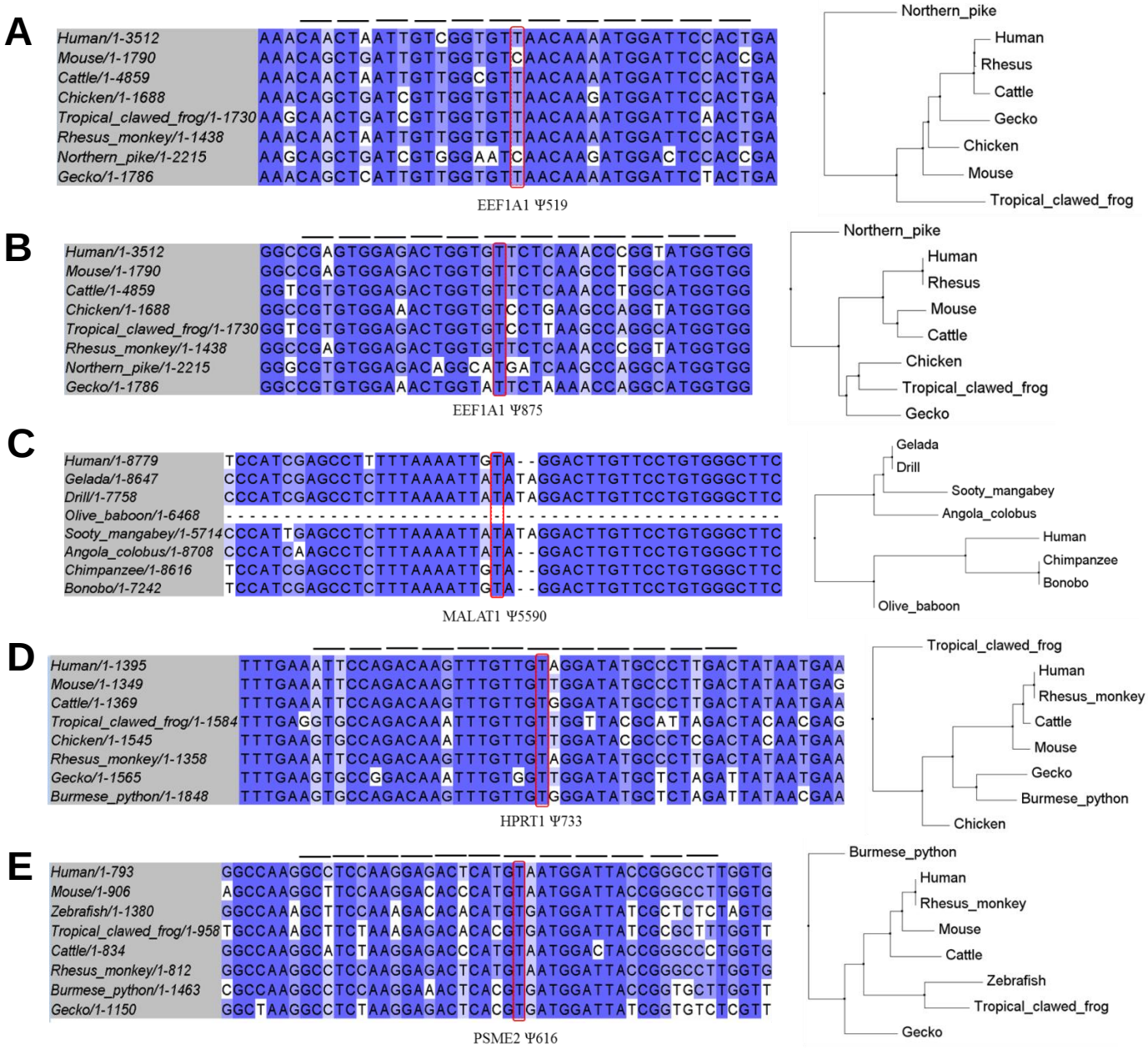

Figure S3: Conservation in vertebrates of the mRNA/lncRNA Ψ sites in this study. Sequence alignment around each target Ψ sites (boxed) are shown. The phylogenetic relationship of the organisms is shown on the right. For the 4 mRNA sites the codon positions are marked by lines on top. (A) eEF1A1 Ψ519; (B) eEF1A1 Ψ875; (C) MALAT1 Ψ5590; (D) HPRT1 Ψ733; (E) PSME2 Ψ616.