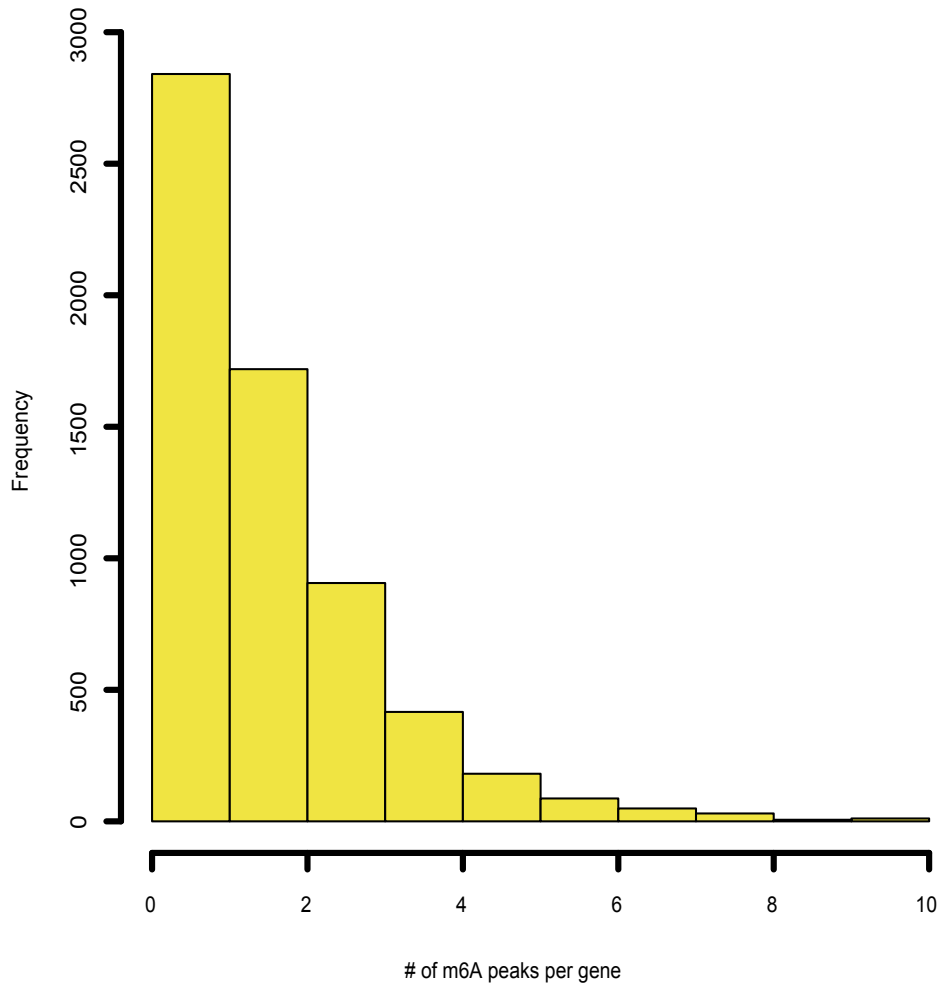
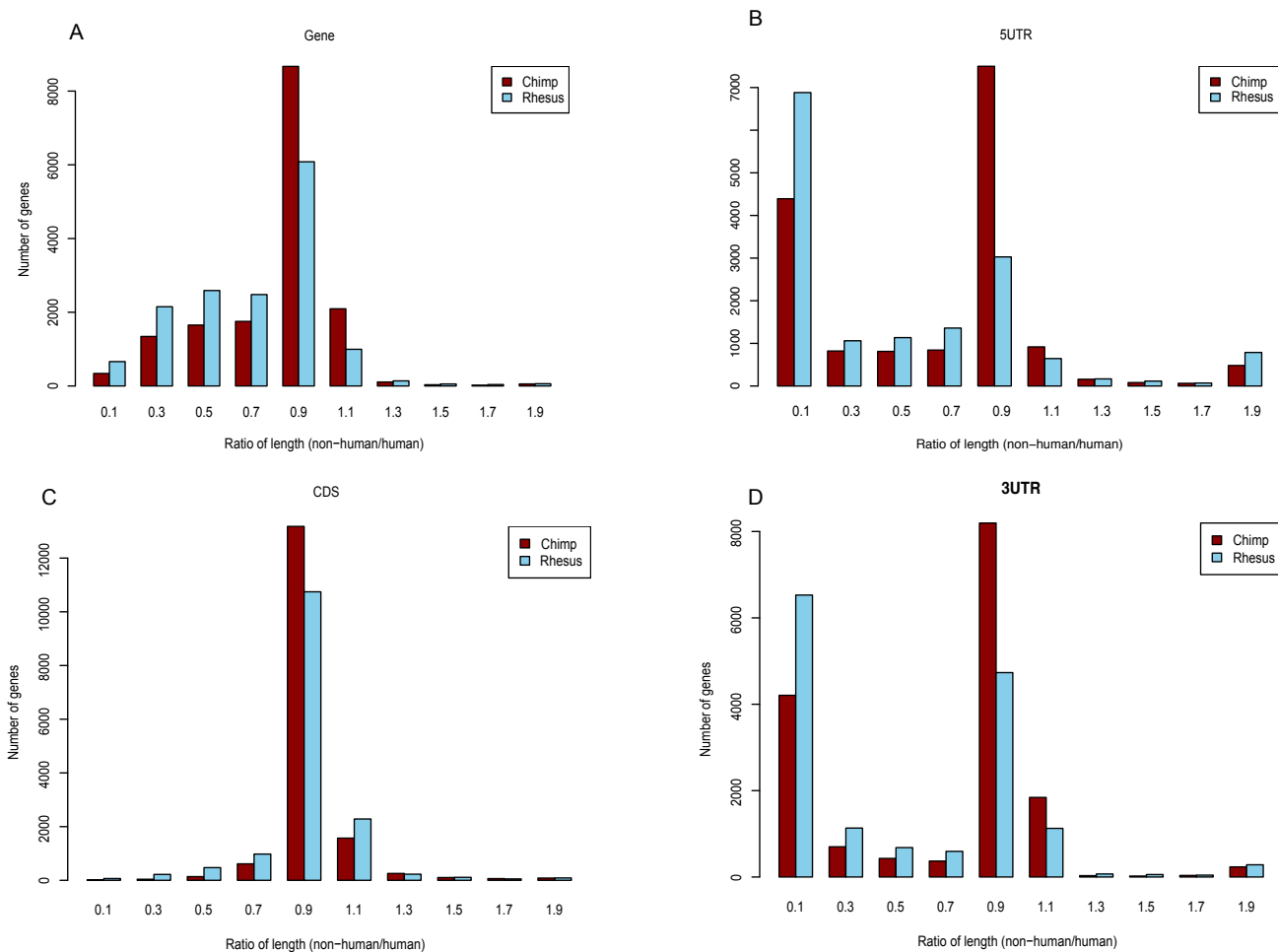


Supplementary Figure 1 Experimental design and analysis summary. Here we show the process by which we organize our data for analysis. At the top of flowchart we represent the individuals by large boxes, and the IP replicates and input are shown as solid boxes within the individuals. We represent the m⁶A peaks in hexagons. At the bottom, we show our process of intra- and inter- species comparison and the combination of them were used to define m⁶A evolutionary patterns (cyan box).



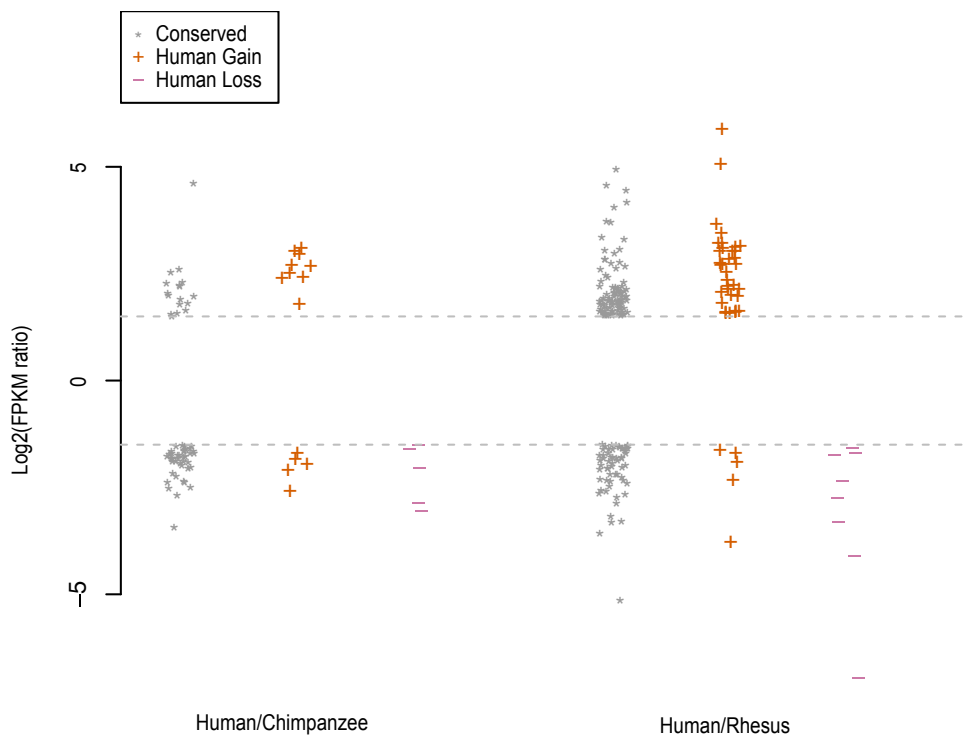
Supplementary Figure 2 Distribution of the number of m⁶A peaks per gene. We calculated the numbers of m⁶A peaks per m⁶A-modified gene, and the histogram shows their distribution.



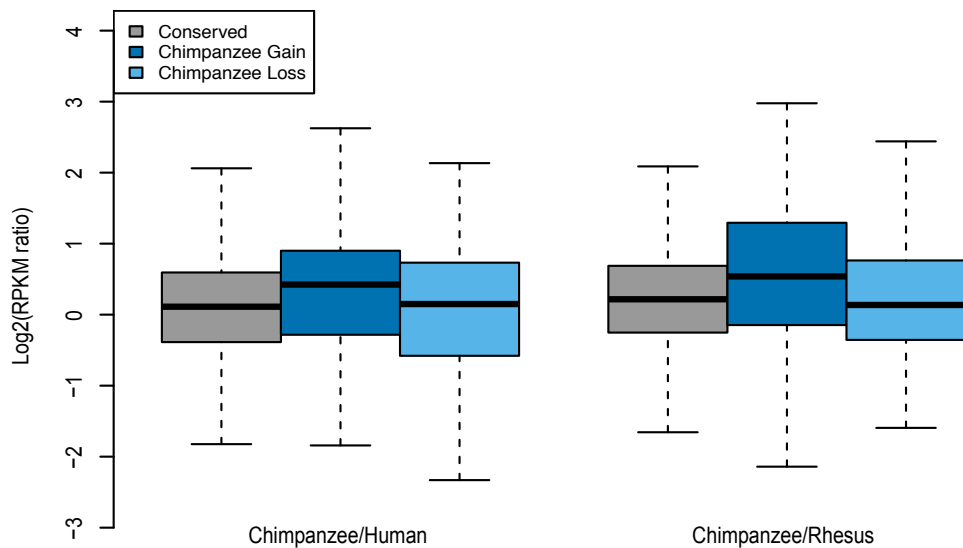
Supplementary Figure 3 The annotation quality of gene structures in human, chimpanzee and rhesus. We plotted the ratio of the length of orthologous genes between non-human species and human. **(a)** ratio of the entire gene length. **(b)** ratio of 5'UTR length. **(c)** ratio of CDS length. **(d)** ratio of 3'UTR length. The distribution indicates that the length of CDS and UTRs are shorter in chimpanzee and rhesus than most of the human genes, especially UTRs. Since m⁶A modification is enriched around the stop codon, the incomplete 3'UTR annotation will cause underestimation of the m⁶A modification in both chimpanzee and rhesus, especially in rhesus. Therefore, in the interspecies comparison, we considered and filtered for the sequence similarity along with the orthologous relationship.

		P-value	% of Targets	% of Back-ground	Motif
Human	Motif1	1e-303	48.45%	33.48%	★ GGACUUU
	Motif2	1e-46	22.82%	18.06%	AAGAGA
	Motif3	1e-40	12.21%	8.87%	CCUACC
Chimpanzee	Motif1	1e-289	65.56%	47.73%	★ GGACUGU
	Motif2	1e-69	42.31%	33.91%	AGAACU
	Motif3	1e-33	32.07%	26.63%	AAGAAAG
Rhesus	Motif1	1e-177	60.48%	44.87%	★ GGACUUU
	Motif2	1e-28	30.05%	25.06%	AASAAA
	Motif3	1e-25	27.09%	22.20%	CCUACCC

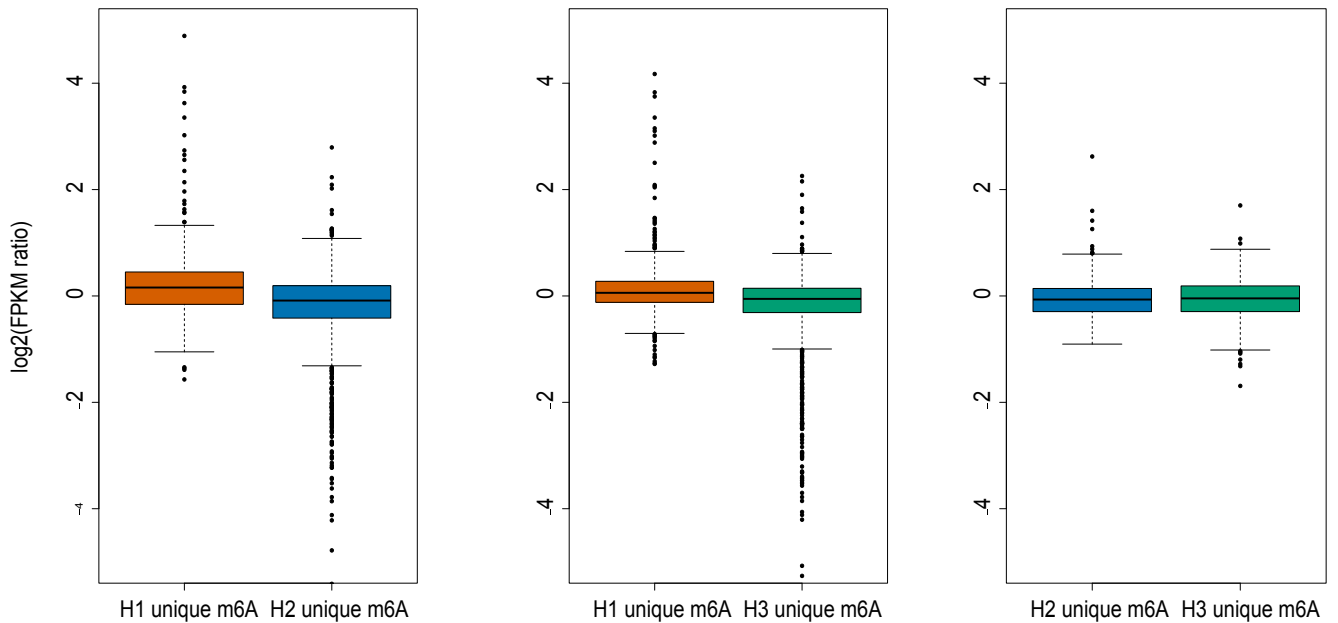
Supplementary Figure 4 Consensus sequence in m⁶A peaks. The well-known GGACU motif is consistently presented as the best motif in all three species. Here we present the three most significant motifs as determined by p-value from *de novo* motif search. We chose one individual from each species and show the results here.



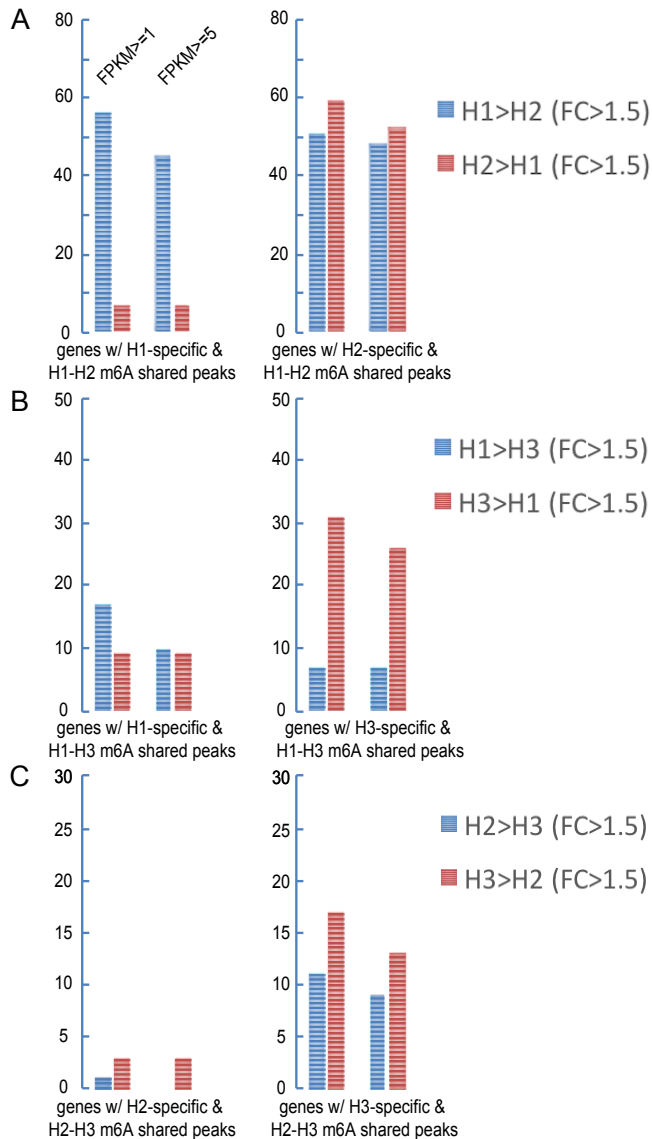
Supplementary Figure 5 m⁶A evolution and gene expression change (between human and chimpanzee, and human and rhesus). The expression change of orthologous genes was plotted against m⁶A conservation groups. We only included significant changed genes, and orthologous genes where expression fold change (log₂) less than 1.8 were excluded. Grey represents genes modified by conserved m⁶A, in which comparable number of up- and down-regulated genes were observed. Orange represents genes modified by human specific m⁶A, and most genes are up-regulated. Pink represents genes where the m⁶A modification was lost in human, and all genes are down-regulated.



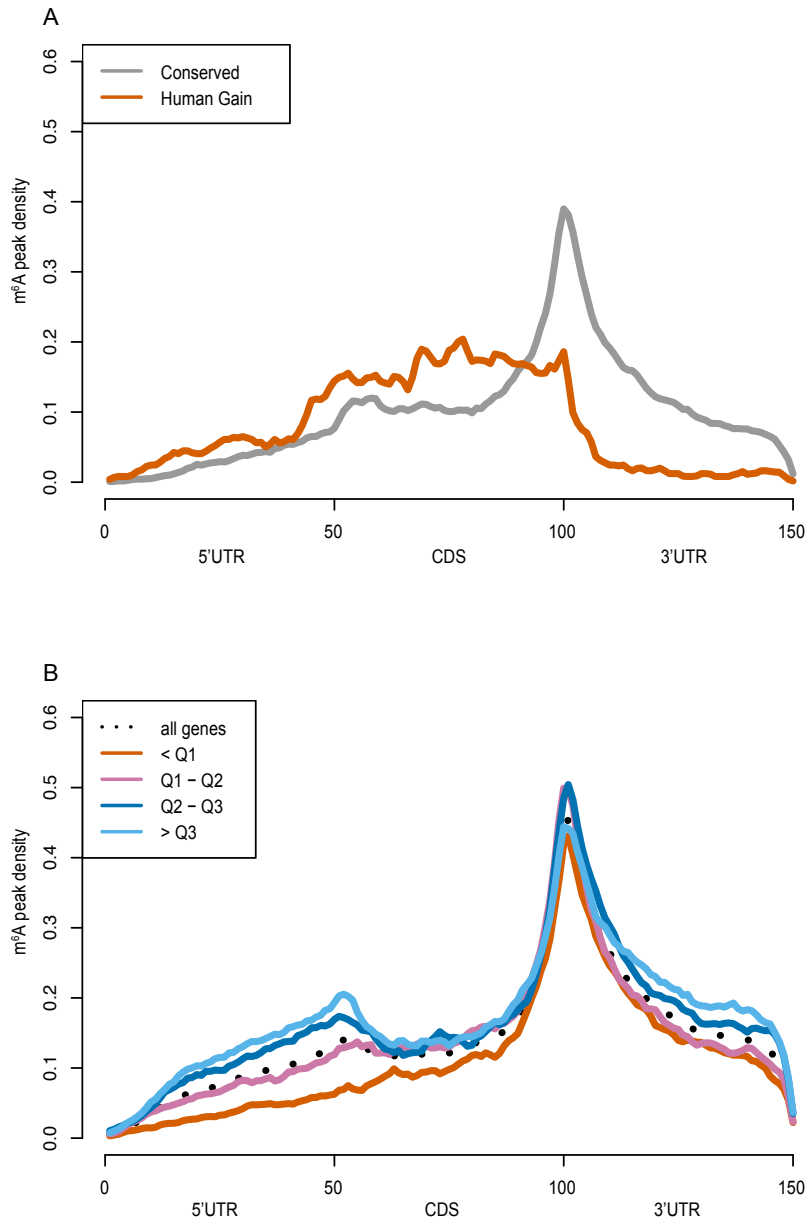
Supplementary Figure 6 m⁶A evolution and gene expression change (between chimpanzee and human/rhesus). The expression change of orthologous genes were plotted against groups of m⁶A-modified genes. The “conserved” group has comparable number of up- and down- regulated genes. Chimpanzee gain m⁶A-modified genes demonstrated more genes up-regulated compared to human and rhesus orthologs. Left panel: chimpanzee compare to human; Right panel: chimpanzee compare to rhesus (***: p<10⁻⁸, **: p<10⁻⁵, *: p<10⁻²; Wilcoxon test). (Conserved: N=2118; Chimpanzee gain: N=193; Chimpanzee loss: N=75.)



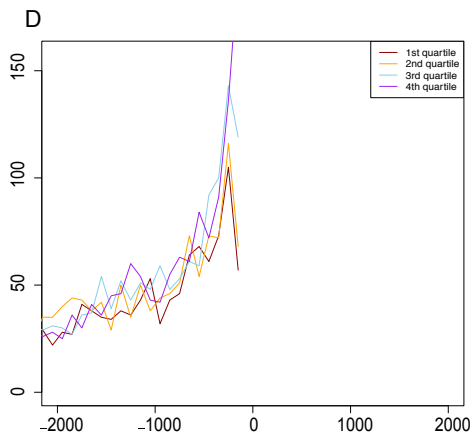
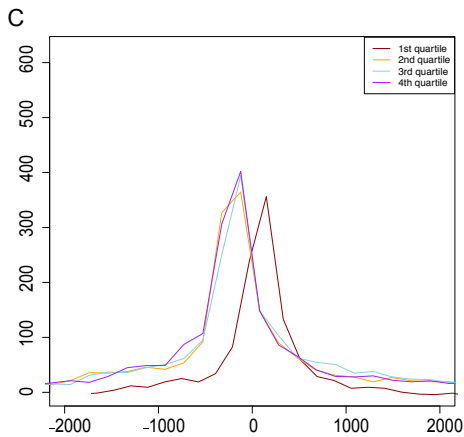
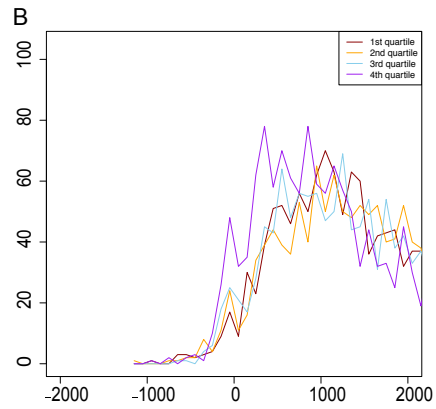
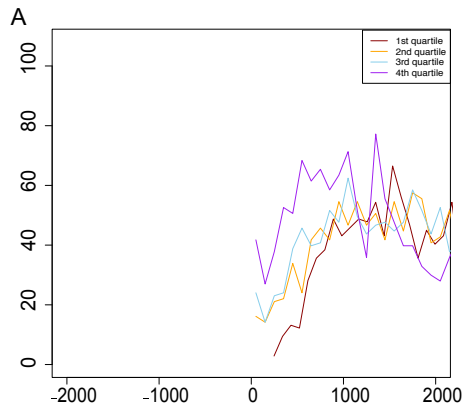
Supplementary Figure 7 Individual specific m⁶A peak associated with higher expression level. We plotted the expression divergence between human individuals based on groups of genes that are specifically m⁶A-modified in one individual (groups were labeled in x-axis: H1 unique, H2 unique and H3 unique). Pair-wise comparisons were performed between H1 and H2, H1 and H3 and H2 and H3. For example, between H1 and H2 (the left panel), genes that are uniquely m⁶A-modified in H1 are more likely to be up-regulated in H1 (outside of the upper whisker in the box plot). Similarly, genes that are uniquely m⁶A-modified in H2 are more likely to be up-regulated in H2 (outside of the lower whisker in the boxplot).



Supplementary Figure 8 One possible explanation of the positive correlation between the change of m⁶A level and the change of mRNA abundance is that m⁶A modifications in higher expressed genes are easier to detect. To demonstrate that such correlation is not just artifacts when detecting m⁶A modifications from genes with different expression levels, we devised a control analysis on a set of genes that carrying both shared and individual-specific m⁶A modifications between human individuals. We reasoned that the shared modifications between individuals provided a control for statistical power to detect m⁶A modification under given expression levels; such that the loss-of-modification in one of the two individuals is due to no detectable m⁶A signal rather than low detection power on lower expressed genes. We did pair-wise comparison on H1, H2 and H3, and demonstrated that the trend of positive correlation is still valid. From the top to the bottom: comparisons were performed between **(a)** H1 and H2, **(b)** H1 and H3, and **(c)** H2 and H3. In each comparison, the two panels (left bar-plot and right bar-plot) display differentially expressed genes carrying both shared and individual-specific m⁶A modifications. **For example**, the left panel in (a) represents a group of genes carrying both H1-specific and H1-H2 shared m⁶A modifications, and the right panel represents a groups of genes carrying both H2-specific and H1-H2 shared m⁶A modifications. The bars represent the number of genes differentially expressed between the two individuals (Fold change > 1.5). In most cases, the change of expression level and the change of m⁶A modification are in the same direction. We also applied two FPKM filters (FPKM \geq 1 and FPKM \geq 5) to further eliminate biases from the lower expressed genes.



Supplementary Figure 9 Distribution of m⁶A peaks density on transcript. We binned each human m⁶A-modified transcript into 150 windows divided equally between 5'UTR, CDS and 3'UTR, and calculated the m⁶A peak density. Here we plot the mean of density along the transcript. **(a)** The grey line is the group of genes with conserved m⁶A modification across species. The orange line is the group of genes identified with human-lineage specific m⁶A modification. **(b)** m⁶A-modified human genes were ranked according to their expression level, from low to high, and split into four groups using the lower quartile (Q1), median (Q2) and upper quartile (Q3). Shown here, the orange line represents the first 25% of genes with lowest expression levels. Cyan line represents the last 25% of genes with the highest expression level. Pink and dark blue lines are in between. Dashed line represents the m⁶A peak density on all modified genes.



Supplementary Figure 10 m⁶A distribution on human genes with different expression levels. Genes were divided into four groups as the same way of Fig. 3c. The number of m⁶A peaks were plotted according to their distance to TSS, SC, EC and TES. The Signal from H3 was used as example (TSS: Transcription start site; SC: Start of CDS; EC: End of CDS; TES: Transcription end site).