

FIGURE 1

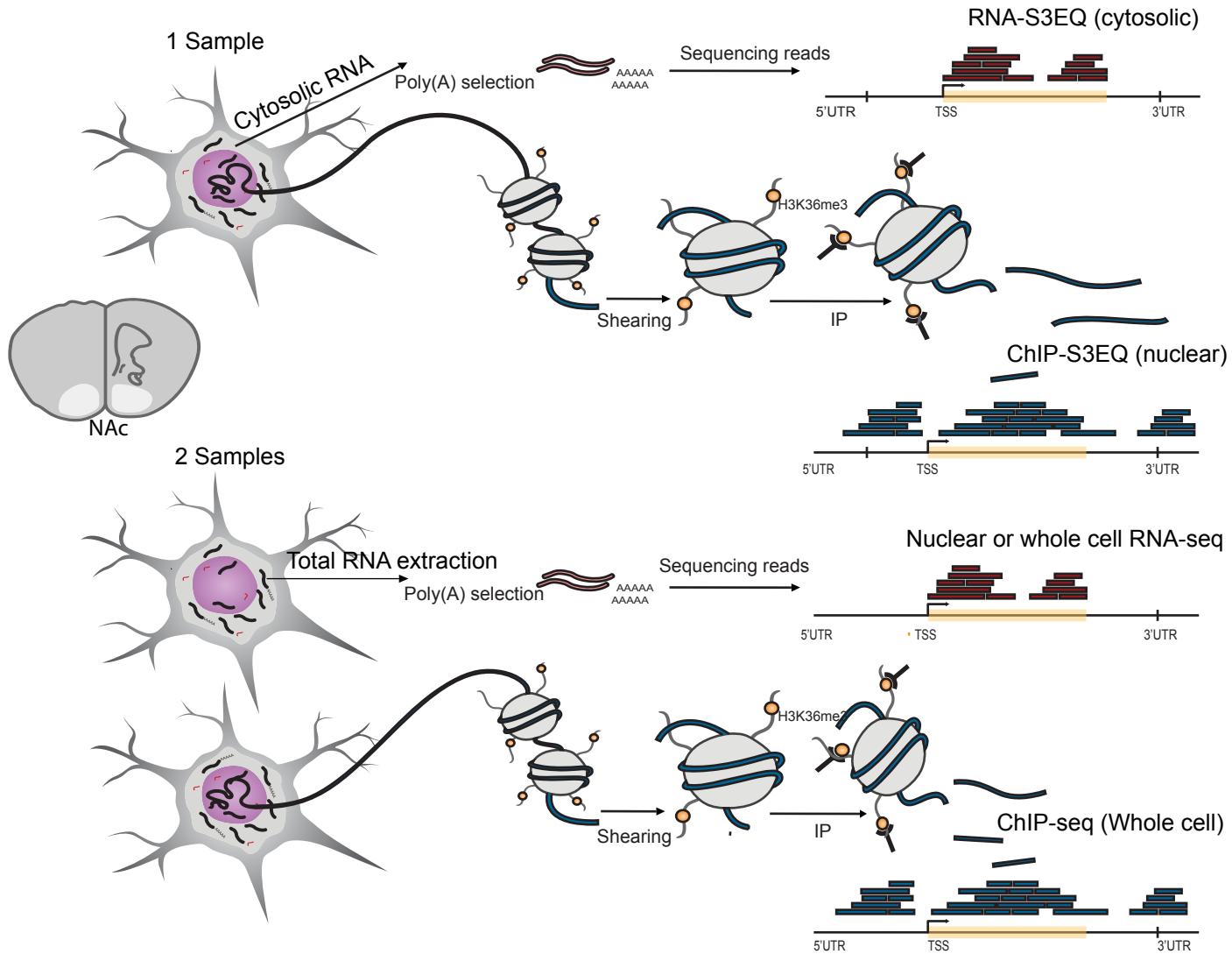


Figure 1. Development of single sample sequencing (S3EQ) for within-sample epigenomic and transcriptomic profiling.

Schematic representation of difference between S3EQ method and whole cell ChIP- and RNA-seq approaches. S3EQ method is used to generate cytosolic RNA-seq and nuclear ChIP-seq from same NAc sample, while classic methods use two sets of samples to generate whole cell ChIP- and whole cell RNA-seq respectively.

FIGURE 2

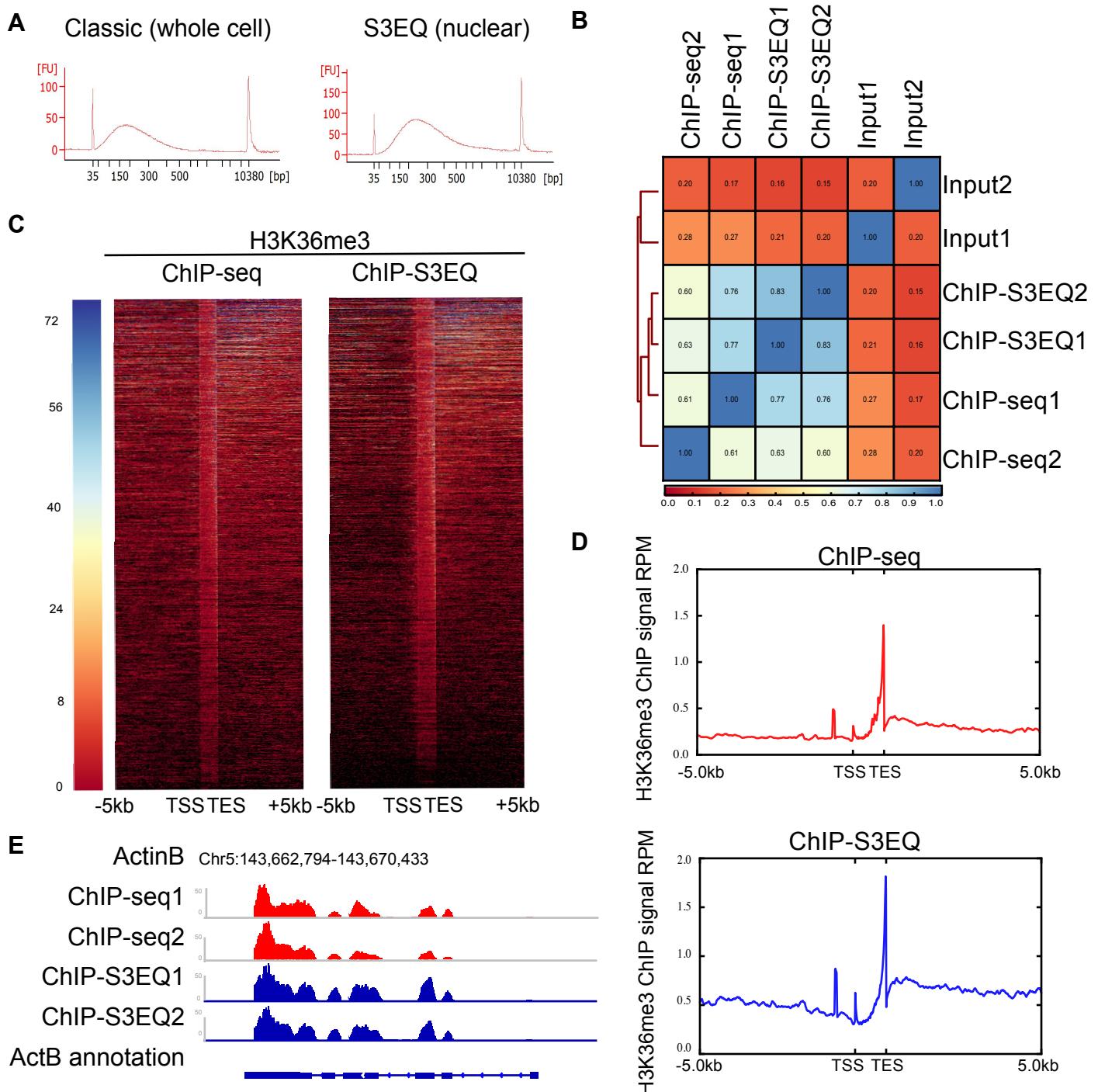


Figure 2. ChIP-S3EQ performs in similar accuracy comparing to whole-cell ChIP-Seq

(A)DNA fragment distribution from ChIP-S3EQ and ChIP approaches measured by Agilent Bioanalyzer. DNA fragments distribution showed no difference between the two methods. (B) Heatmap shows H3K36me3 signal of nuclear S3EQ and whole-cell ChIP-seq was highly correlated using Spearman's rank-order method based on the number of mapped reads using a bin size of 50kb. (C) Heatmap of H3K36me3 enrichment on annotated gene body regions, 5kb up- and down-stream from the transcription start and end sites (TSS/TES). (D) Density plot of H3K36me3 reads per million using ChIP-seq (whole cell) and ChIP-S3EQ (nuclear).

FIGURE 3

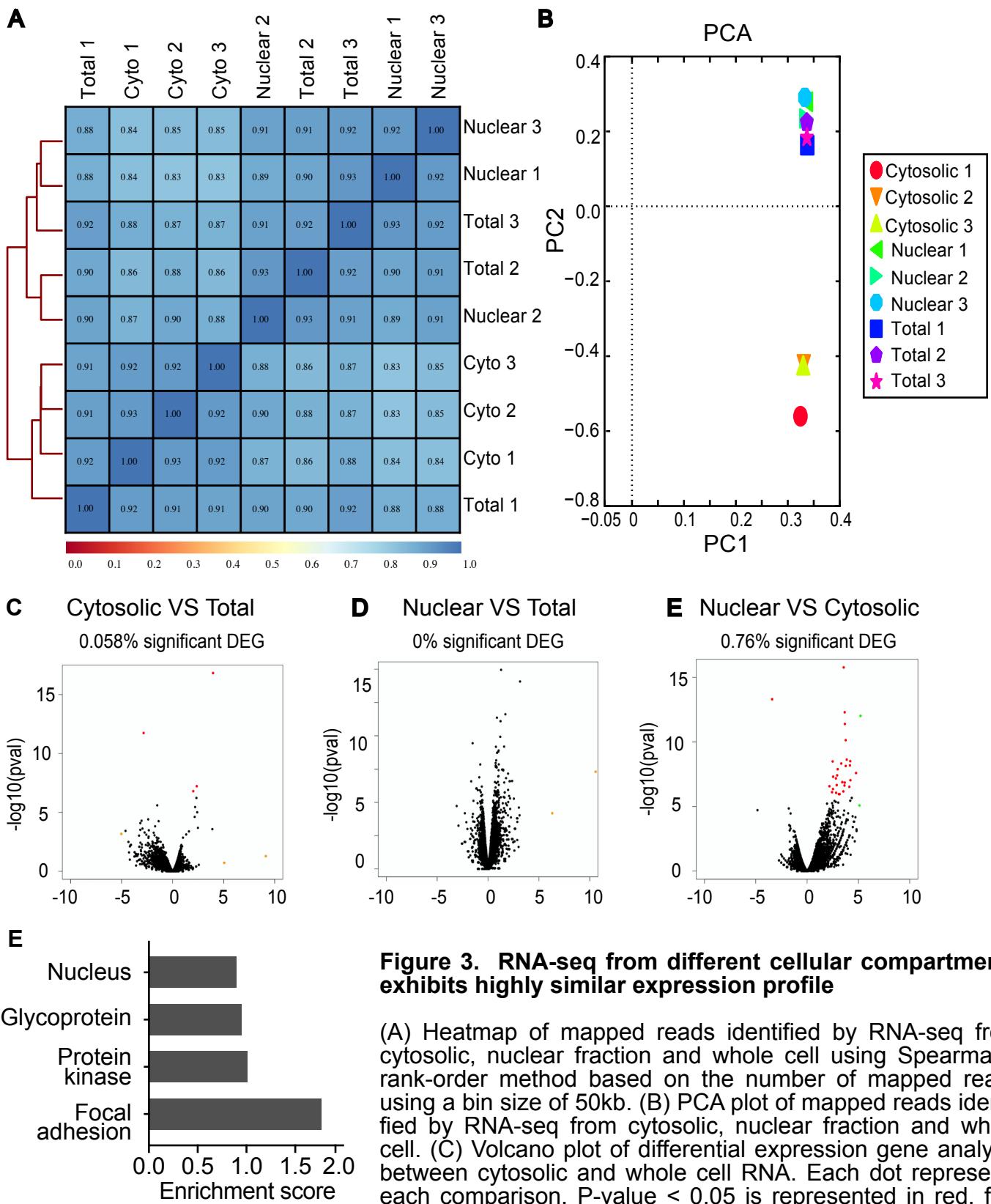
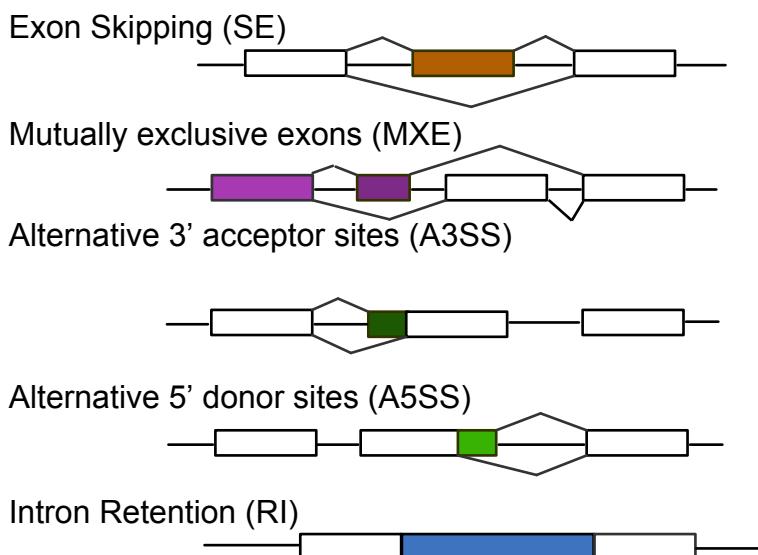


Figure 3. RNA-seq from different cellular compartments exhibits highly similar expression profile

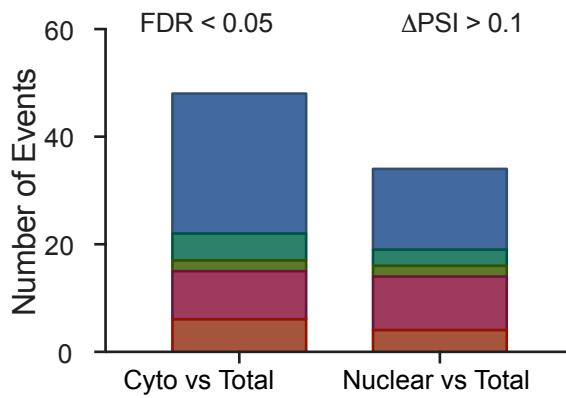
(A) Heatmap of mapped reads identified by RNA-seq from cytosolic, nuclear fraction and whole cell using Spearman's rank-order method based on the number of mapped reads using a bin size of 50kb. (B) PCA plot of mapped reads identified by RNA-seq from cytosolic, nuclear fraction and whole cell. (C) Volcano plot of differential expression gene analysis between cytosolic and whole cell RNA. Each dot represents each comparison. P-value < 0.05 is represented in red, fold change > 5 is represented in orange. (D) Volcano plot of differential expression gene analysis between nuclear and whole cell RNA. Each dot represents each comparison. Fold change > 5 is represented in orange. None of the difference is significant ($p < 0.05$). (E) Volcano plot of differential expression gene analysis between nuclear and cytosolic RNA. Each dot represents each comparison. P-value < 0.05 is represented in red, P-value < 0.05 and fold change > 5 is represented in green. (F) Go-term analysis on differential expression genes ($p < 0.05$) between cytosolic and nuclear RNA.

FIGURE 4

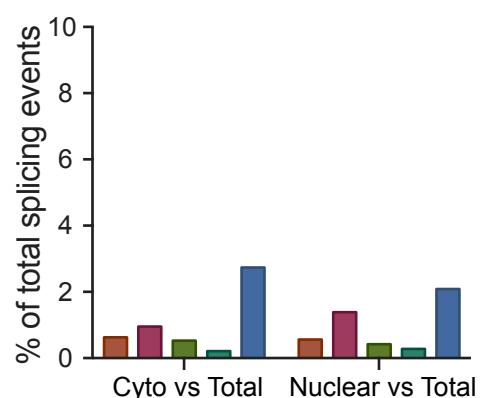
A



B

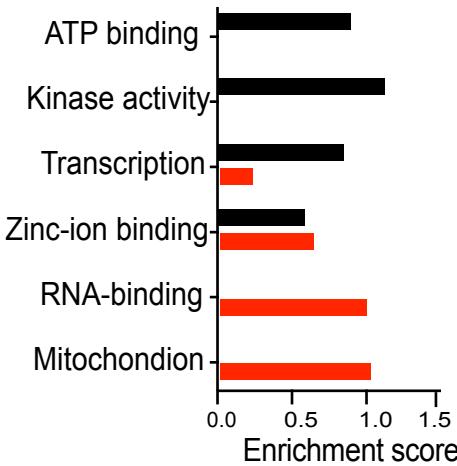


C

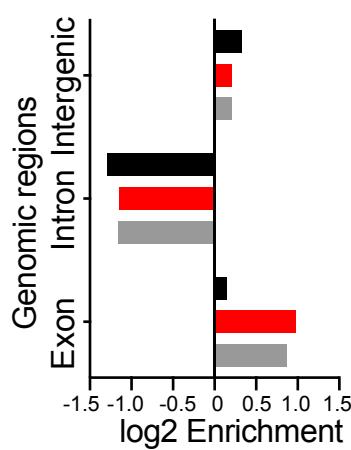


D

Cyto vs Total
Nuclear vs Total



E



F

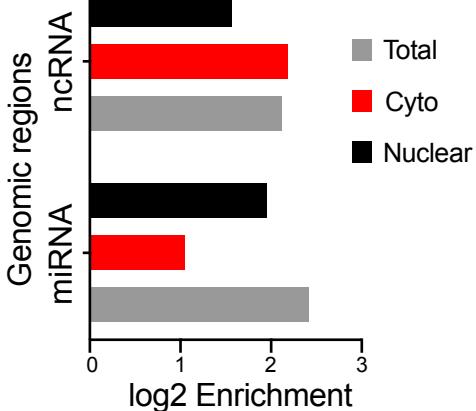
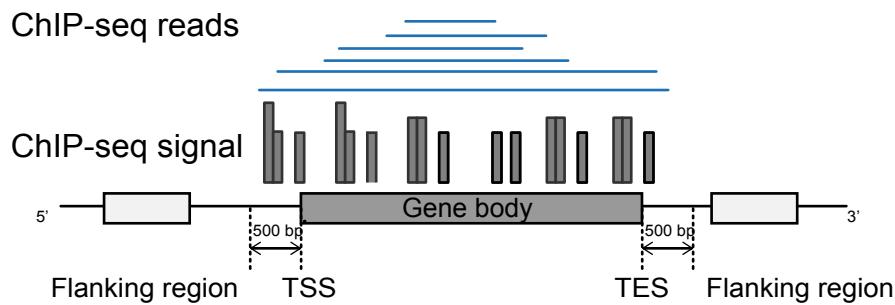


Figure 4. Cytosolic RNA resembles total RNA splicing profiles

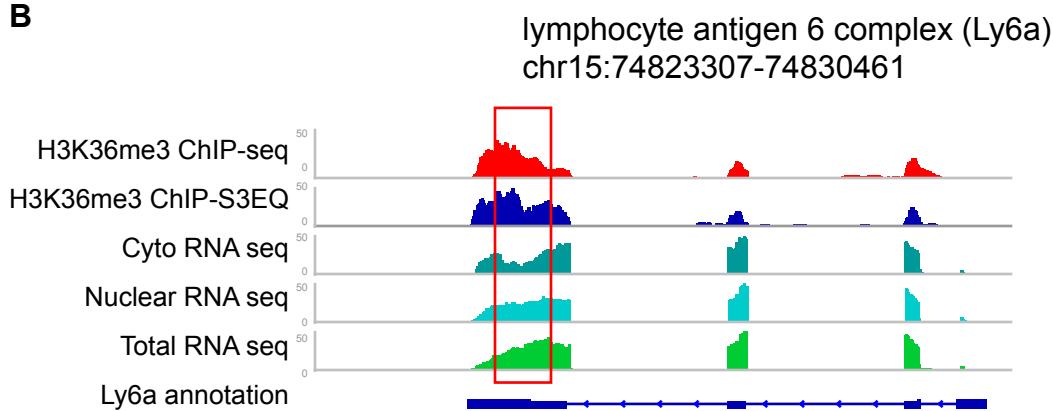
(A) Schematic representation on different splicing events. Colored block indicates spliced exon. (B) Number of alternative splicing events identified in cytosolic RNA comparing to whole cell RNA and nuclear RNA comparing to whole cell RNA ($FDR < 0.05$, $\Delta PSI > 0.1$). (C) Percentage of alternative splicing events ($FDR < 0.05$, $\Delta PSI > 0.1$) in total identified splicing events in comparisons between cytosolic RNA to total RNA and nuclear RNA to total RNA. (D) Function analysis on alternative splicing events of cytosolic and nuclear RNA comparing to total RNA. Enrichment score calculated by DAVID function cluster analysis. (E) Genomic annotation of intron, exon and intergenic regions by Homer on all mapped reads from RNA-seq in whole cell, cytosolic and nuclear RNA ($FDR < 0.05$). (F) Genomic annotation of ncRNA and non-coding RNA regions by Homer on all mapped reads from RNA-seq in whole cell, cytosolic and nuclear RNA ($FDR < 0.05$).

FIGURE 5

A



B



C

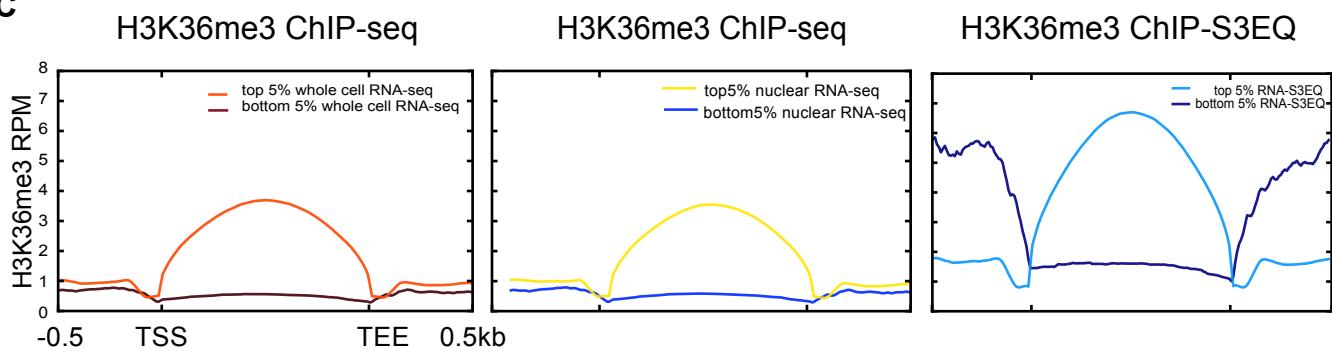
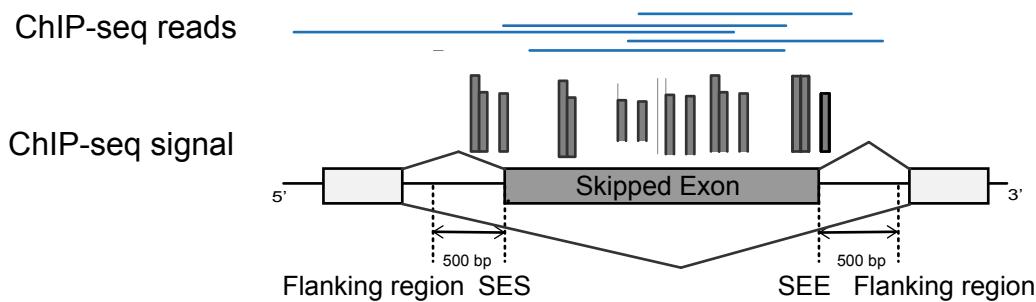


Figure 5. S3EQ H3K36me3 signal shows a stronger association in low-expressed RNA-S3EQ than whole cell ChIP- to RNA-seq

(A) Schematic representation of ChIP-seq reads in differential expression regions with 500bp flanking regions identified by RNA-seq. (B) H3K36me3 ChIP-seq enrichment levels of classic whole cell (red), S3EQ nuclear (blue) on highest differential expression gene (Ly6a) between cytosolic (dark green), nuclear (light blue) and total RNA (green) in RNA-seq. (C) Density plot of H3K36me3 ChIP-seq reads on whole cell RNA mapped reads of top 5% (red) and bottom 5% (dark red) in expression levels (left). Density plot of H3K36me3 ChIP-seq reads on nuclear RNA mapped reads of top 5% (green) and bottom 5% (dark green) in expression levels (middle). Density plot of H3K36me3 ChIP-S3EQ reads on RNA-S3EQ mapped reads of top 5% (blue) and bottom 5% (dark blue) in expression levels (right).

FIGURE 6

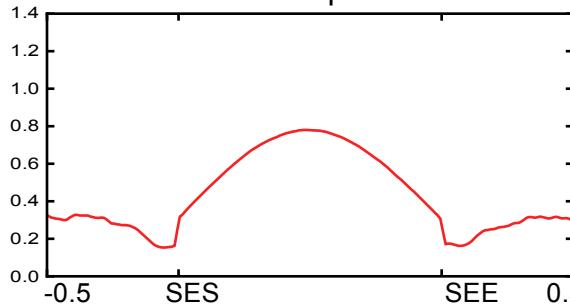
A



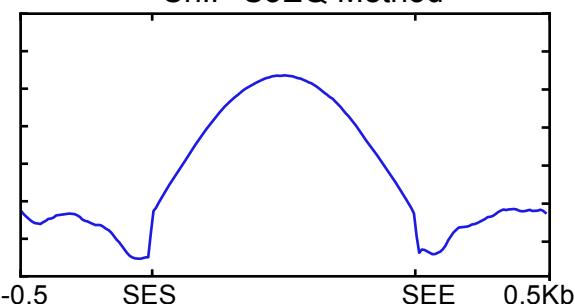
B

H3K36me3 signal on all annotated skipped exons

ChIP-seq Method



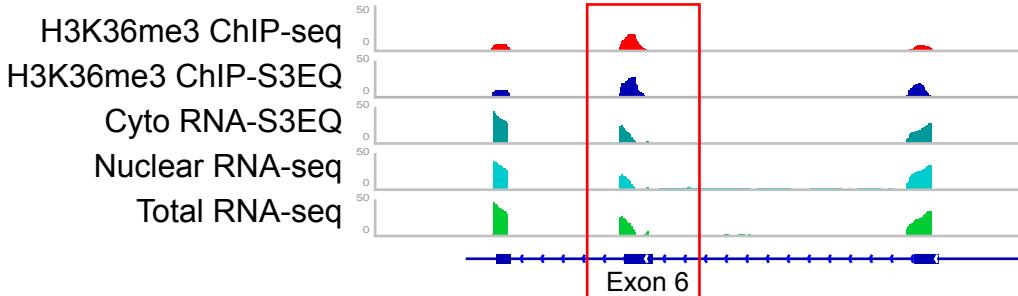
ChIP-S3EQ Method



C

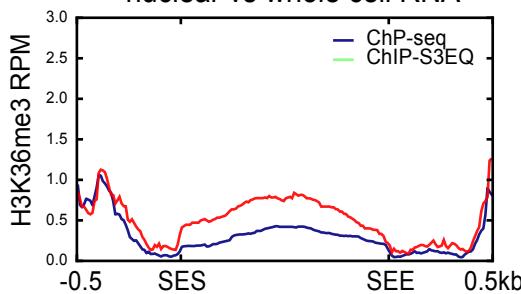
Transformer-2 protein homolog alpha (Tra_2a)

chr6:49191920-49216051



D

Alternatively skipped exon
nuclear vs whole cell RNA



Alternatively skipped exon
cytosolic vs whole cell RNA

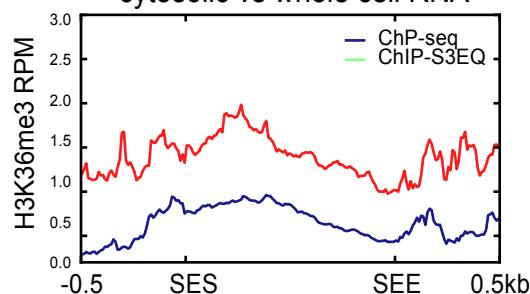


Figure 6. S3EQ accurately maps H3K36me3 to alternatively and differentially spliced transcripts

(A) Schematic representation of ChIP-seq reads in alternative splicing regions with 500bp flanking regions of skipped exons identified by rMATS(v3.4.2). (B) Density plot of H3K36me3 classic whole cell (red) and S3EQ nuclear (blue) ChIP-seq reads on all annotated skipped exons with 500bp up- and down-stream splicing junctions. (C) H3K36me3 ChIP-seq signals of whole cell (red), S3EQ nuclear (blue) on highest alternative splicing gene (Tra_2a) between nuclear (light blue) and total RNA (green) in RNA-seq. cytosolic (dark green) RNA-seq was also showing as control. (D) H3K36me3 ChIP-seq (blue) –S3EQ (red) signals of on alternative spliced skipped exons (as in Fig 4B, left bar) between nuclear and whole cell RNA. (E) H3K36me3 ChIP-seq (blue) –S3EQ (red) signals of on alternative spliced skipped exons (as in Fig 4B, right bar) between cytosolic and whole cell RNA.