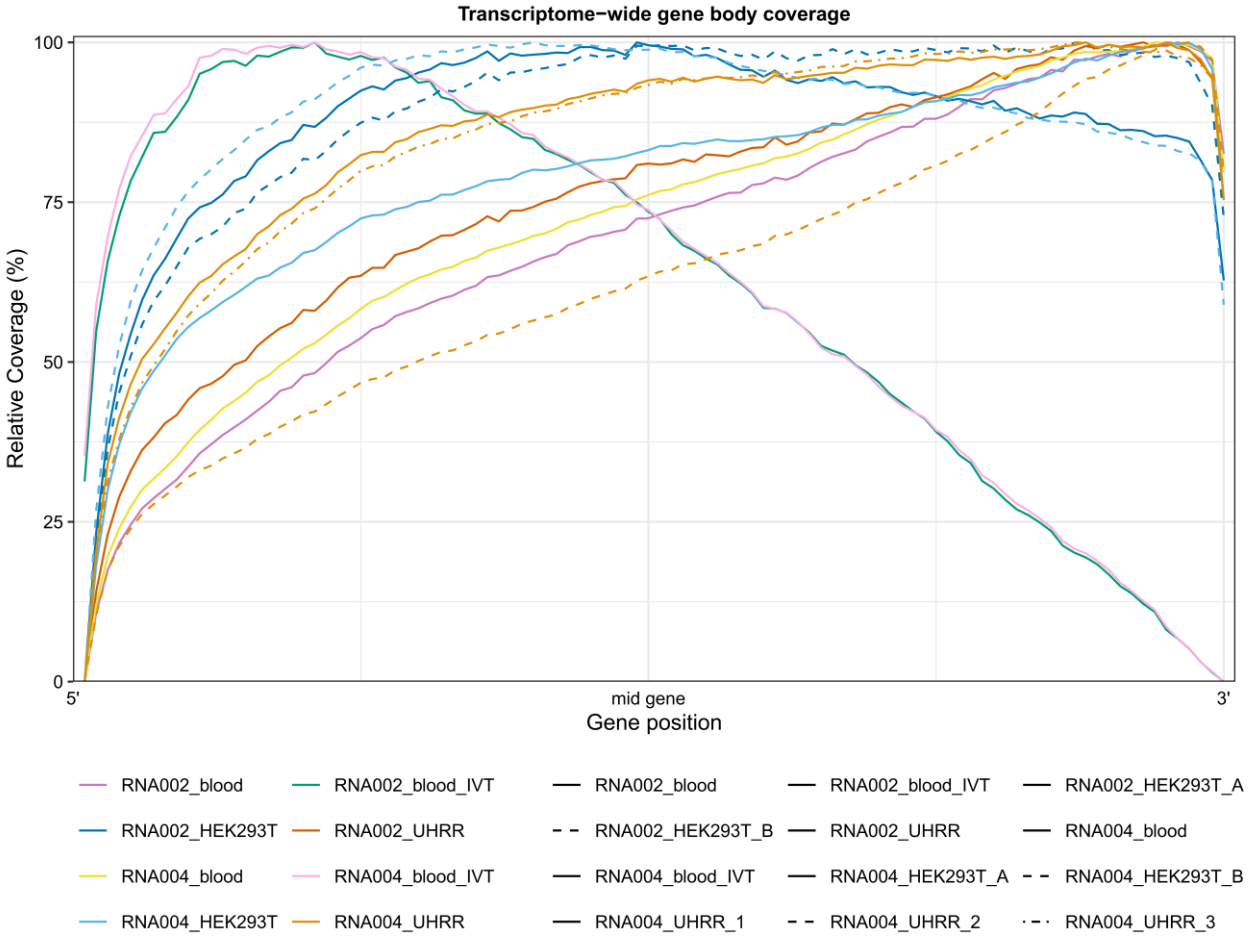
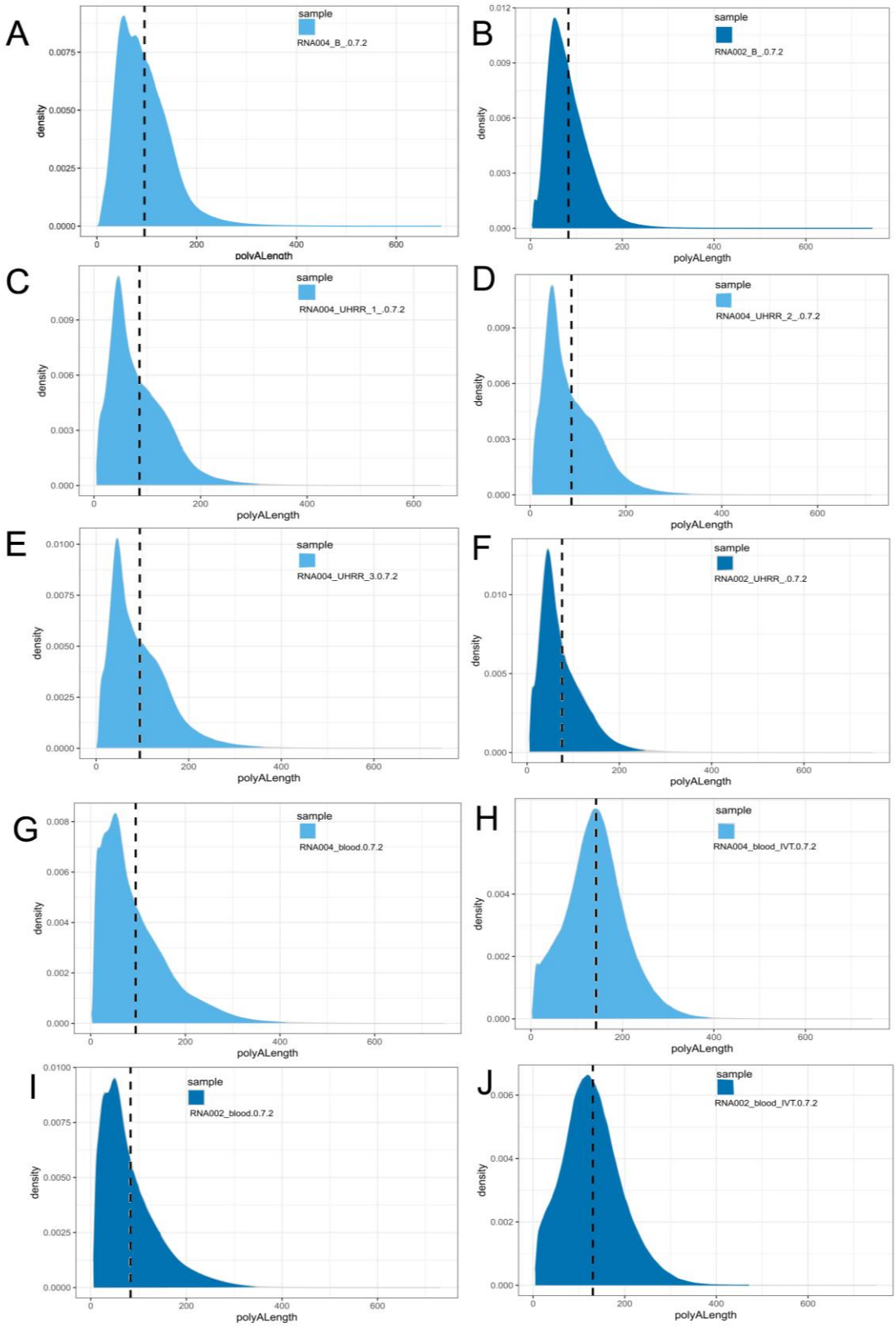
Supplementary Information

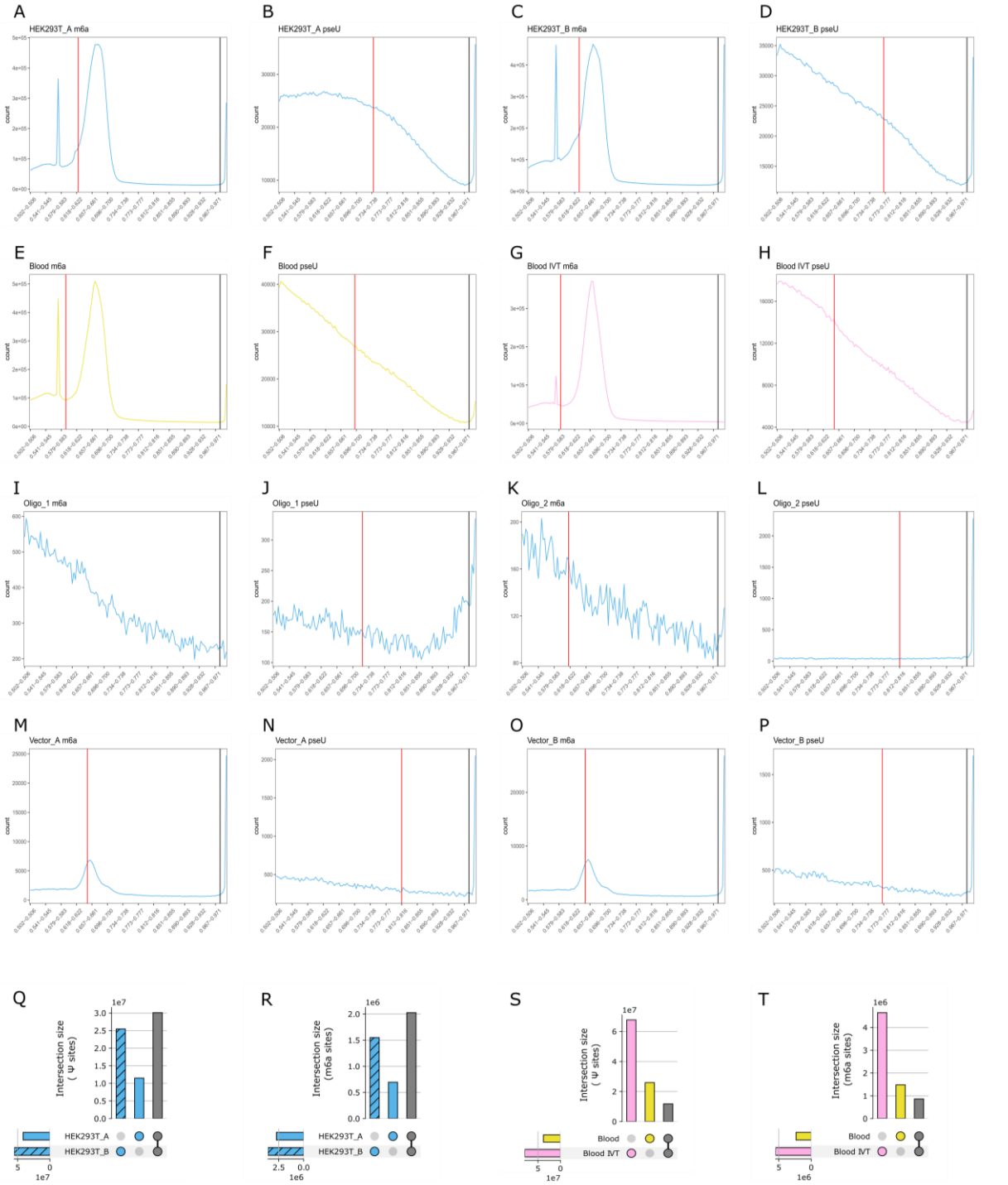
Direct RNA sequencing enables improved transcriptome assessment and tracking of RNA modifications for medical applications



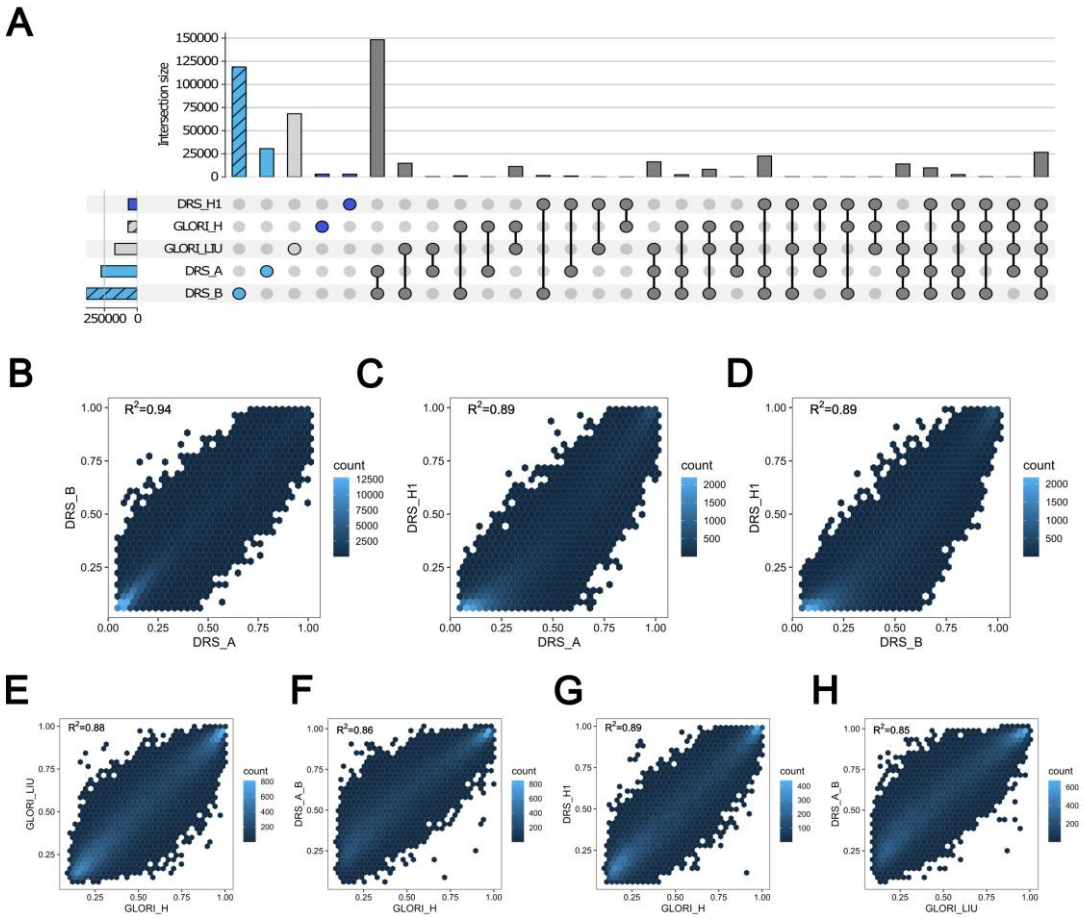
Supplementary Figure 1. Transcriptome wide gene Body coverage for all samples (RSeQC, GRCh38)



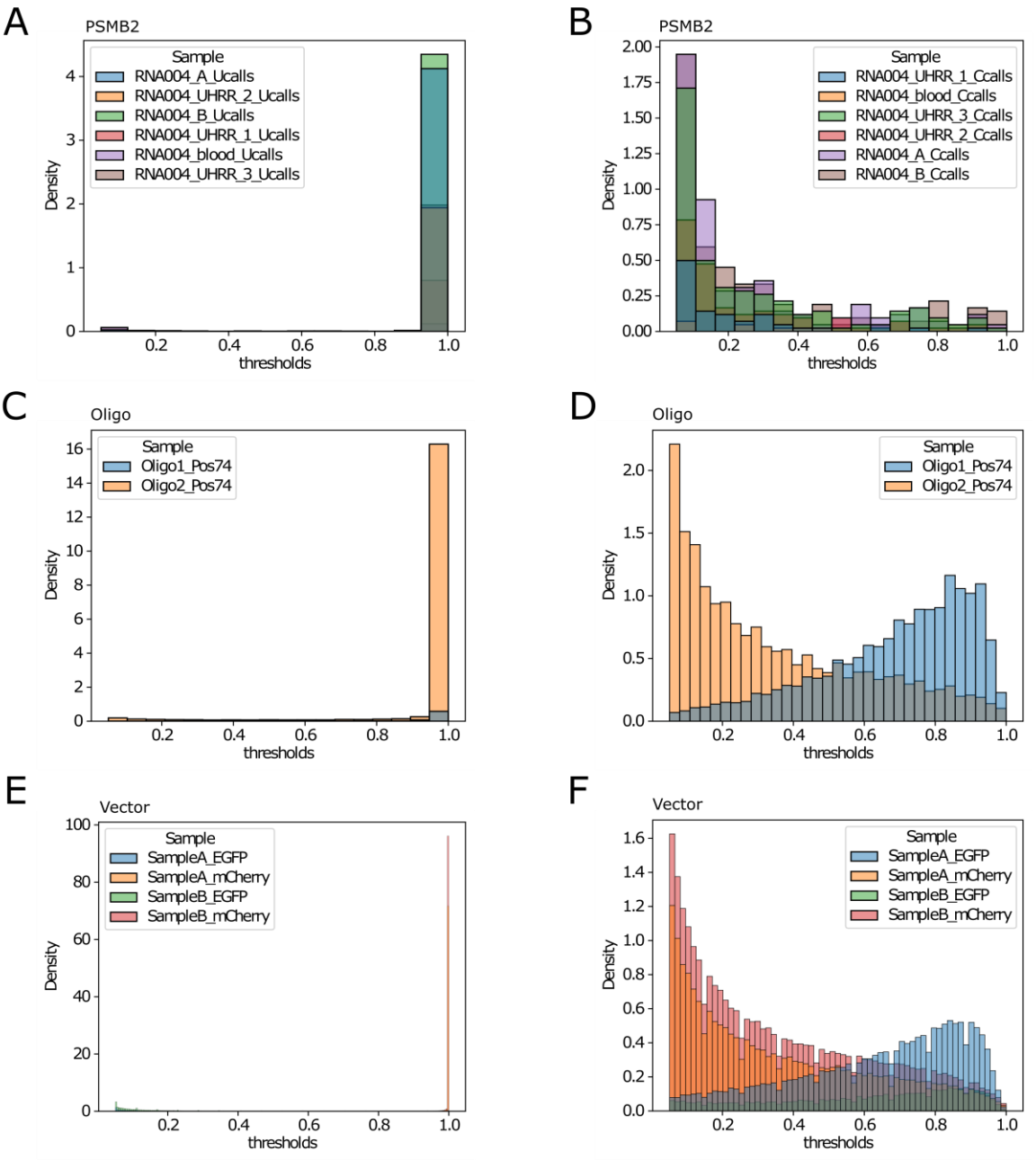
Supplementary Figure 2. Transcriptome-wide distribution of estimated poly(A) lengths. *Transcriptome-wide poly(A) length density plots for (A,B) HEK293T sample B, (C–F) Universal Human Reference RNA (UHRR), (G–J) peripheral blood and peripheral blood IVT. Light blue = RNA004 chemistry, dark blue = RNA002 chemistry. The dotted black line designates the mean length.*



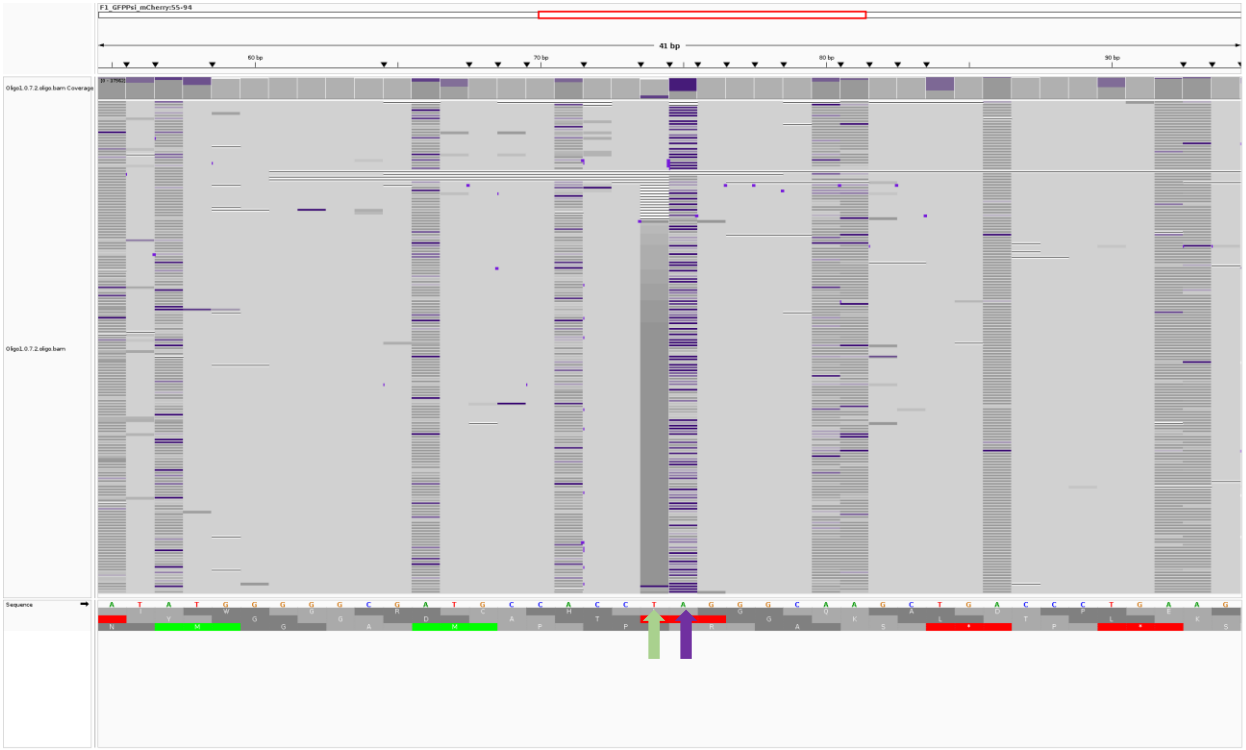
Supplementary Figure 3. Modification probability frequencies and unfiltered modification Upset plots. *Frequency plots of the modification probabilities of Ψ and m6A (as assigned by Dorado) for HEK293T cells (A–D), blood and blood IVT (E-H), custom Oligo1 and Oligo2 (I-L) and for the combined vector sequences of mCherry and EGFP for HEK293T sample A and B (M-P). The red lines indicate the filter thresholds that were selected by modkit as a default threshold (10th percentile). The black line indicates the modification threshold filter of 0.98 that was manually applied across all samples. (Q) Upset plot of transcriptome-wide Ψ sites in HEK293T samples A and B before the filter settings of n valid coverage of 20 reads and 5% site-level modification were applied. (R) Upset plot before filtering for HEK293T and m6A. (S) Upset plot for transcriptome-wide unfiltered Ψ sites between the peripheral blood and the peripheral blood IVT samples. (T) Upset plot for m6A between the peripheral blood and the peripheral blood IVT samples. All sequencing was done with a RNA004 kit.*



Supplementary Figure 4. M^{6}A modified sites throughout the HEK293T transcriptome. *A) UpSet plot of Nanopore DRS and GLORI control data sets. B) Correlation of m6A sites between PromethION sample HEK293T A vs HEK293T B. C) the PromethION sample A vs the MinION HEK293T sample, D) The PromethION sample B vs the MinION HEK293T Sample, E) the in-house GLORI data set (GLORI_H) vs the GLORI data set from Liu (GLORI_LIU), F) the in-house GLORI set vs the intersection of PromethION sample A and B, G) the in-house GLORI data vs the MionION Sample, H) the Glori sample from Liu et al vs the intersection of PromethION HEK293T sample A and B.*



Supplementary Figure 5. Single-site Ψ modification probabilities. *Plots shows Ψ modification probability for either U sites (Ucalls, left) assigned with the standard modification model or mis-base-called C sites (Ccalls, right) assigned with the modified Dorado model. (A,B) PSMB2 position chr1:35603333 for the cell line samples. (C,D) Motif-specific Ψ for the control oligos at motif1. (E,F) Ψ for the vectors EGFP and mCherry HEK293T samples A and B.*



Supplementary Figure 6 Integrated genomics viewer 's screenshot for false-positive m6A modification of the custom Oligo1. *The light green arrow designates the motif position with 100% pseudouridine. The purple arrow desginates the +1 site, where false-positive m6A calls seem to cluster. Unmodified reads are shown in gray and modified reads are purple.*