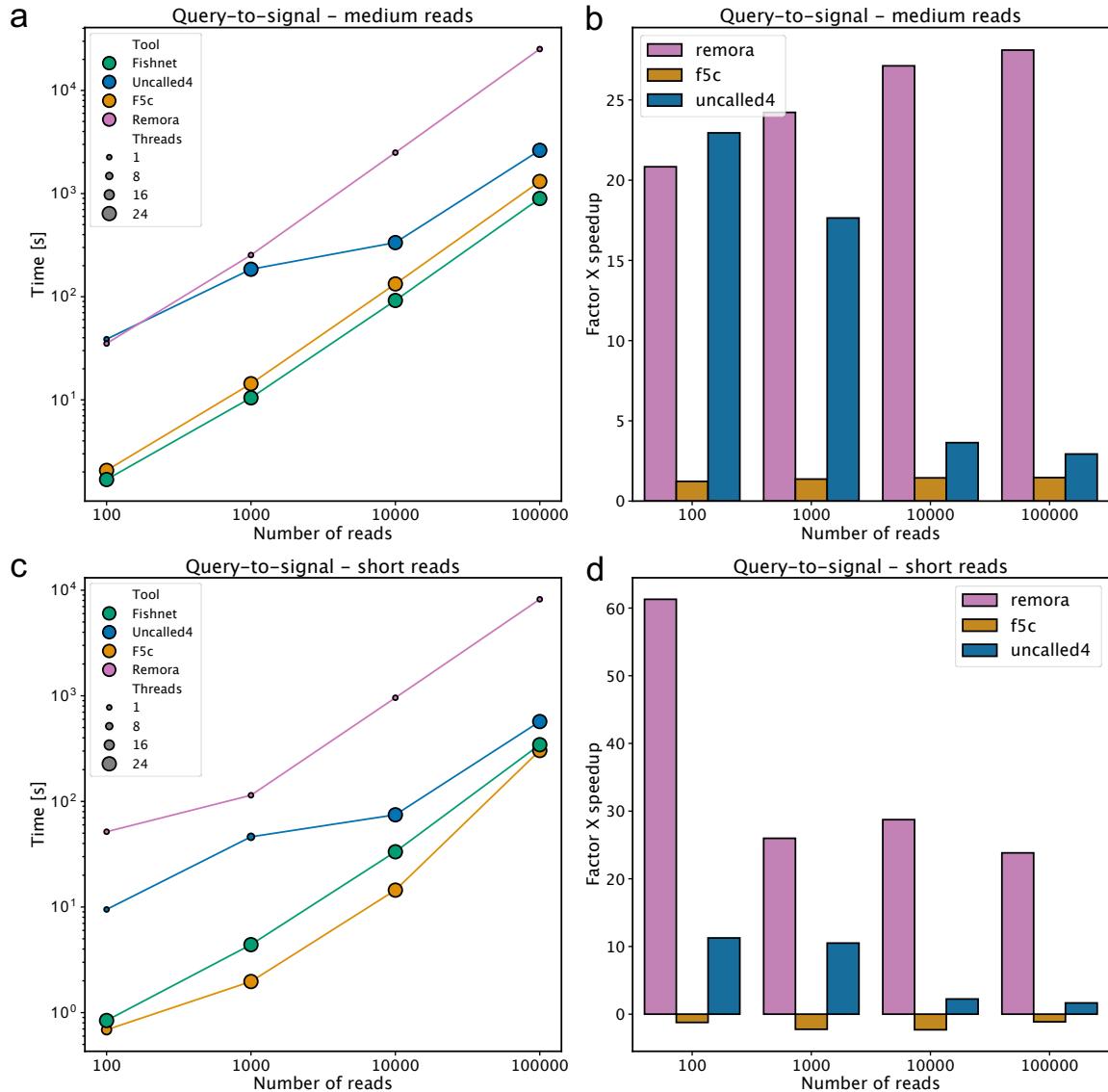
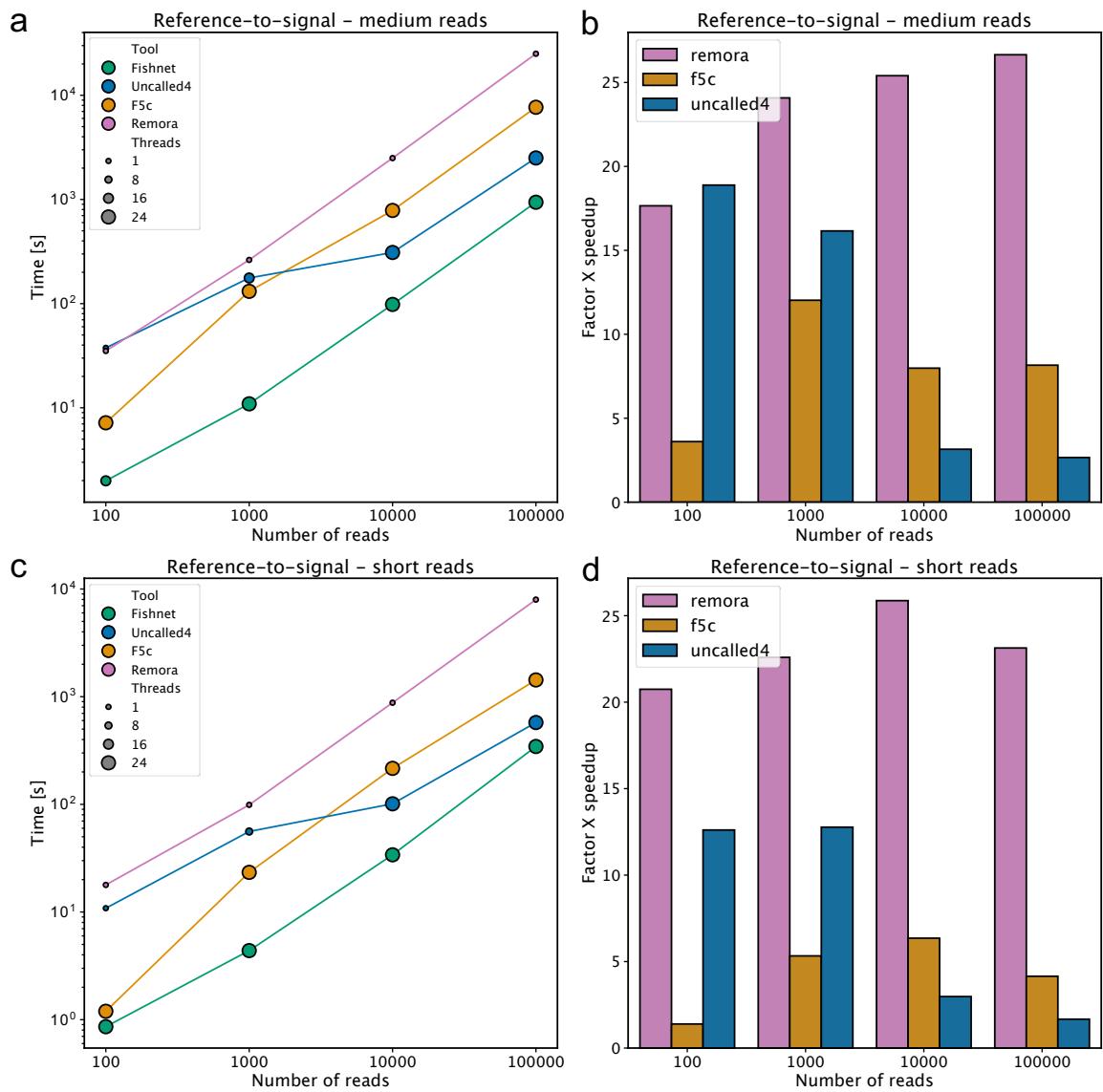


Supplementary figures

Comparing processing speed

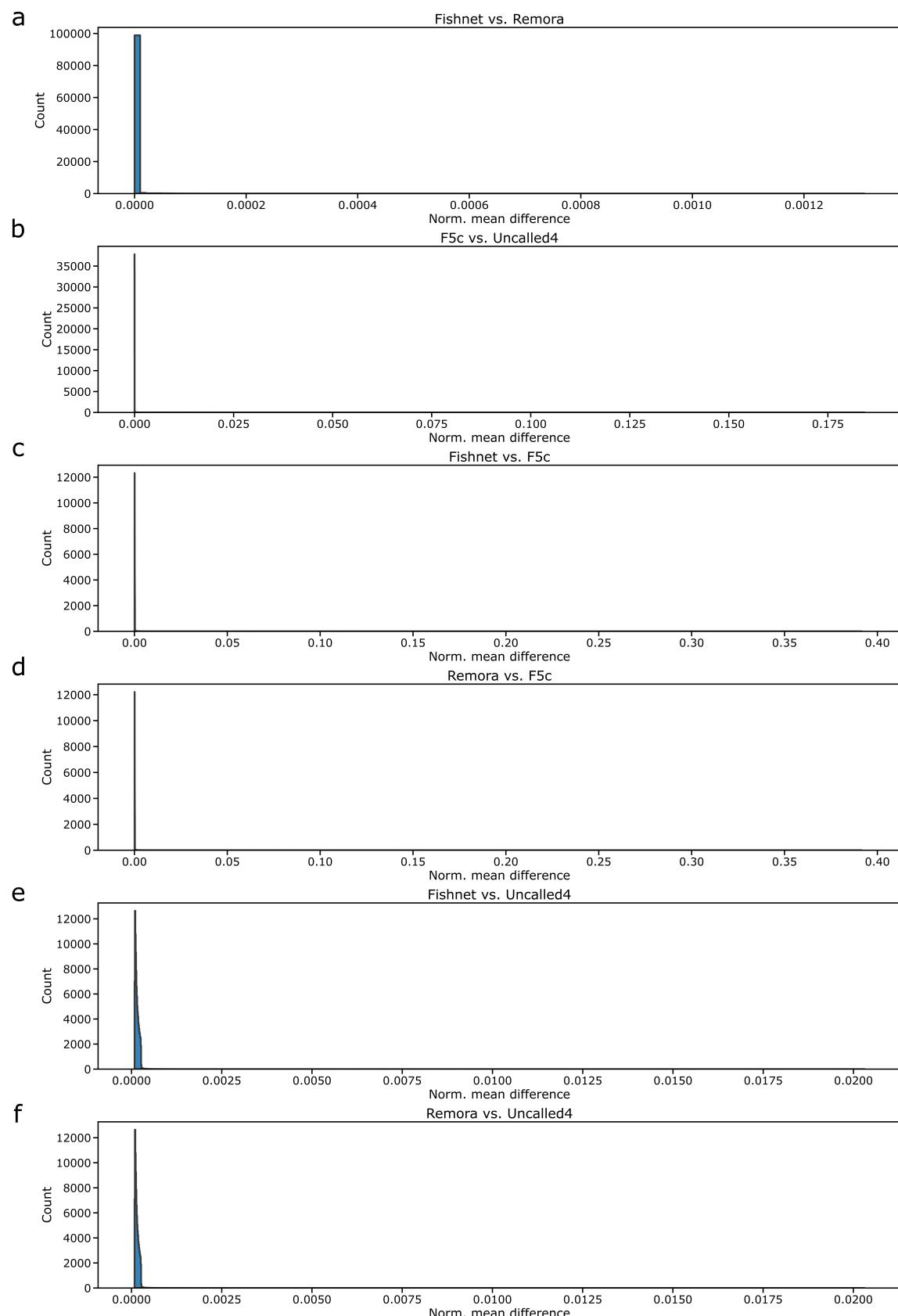


Supplementary Figure 1 Benchmarking results for query-to-signal alignments. Measured processing times for medium (a) and short reads (c). Factor X speedup of a given tool compared to the baseline time measured by Fishnet for medium (b) and short reads (d).

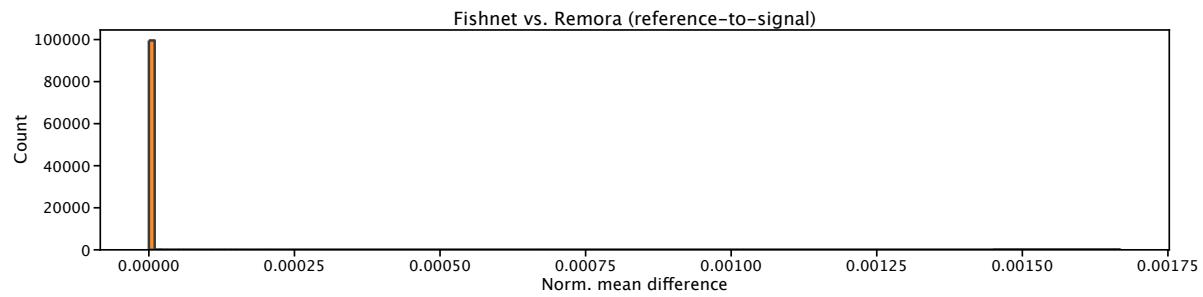


Supplementary Figure 2 Benchmarking results for reference-to-signal alignments. Measured processing times for medium (a) and short reads (c). Factor X speedup of a given tool compared to the baseline time measured by Fishnet for medium (b) and short reads (d).

Comparing produced alignments

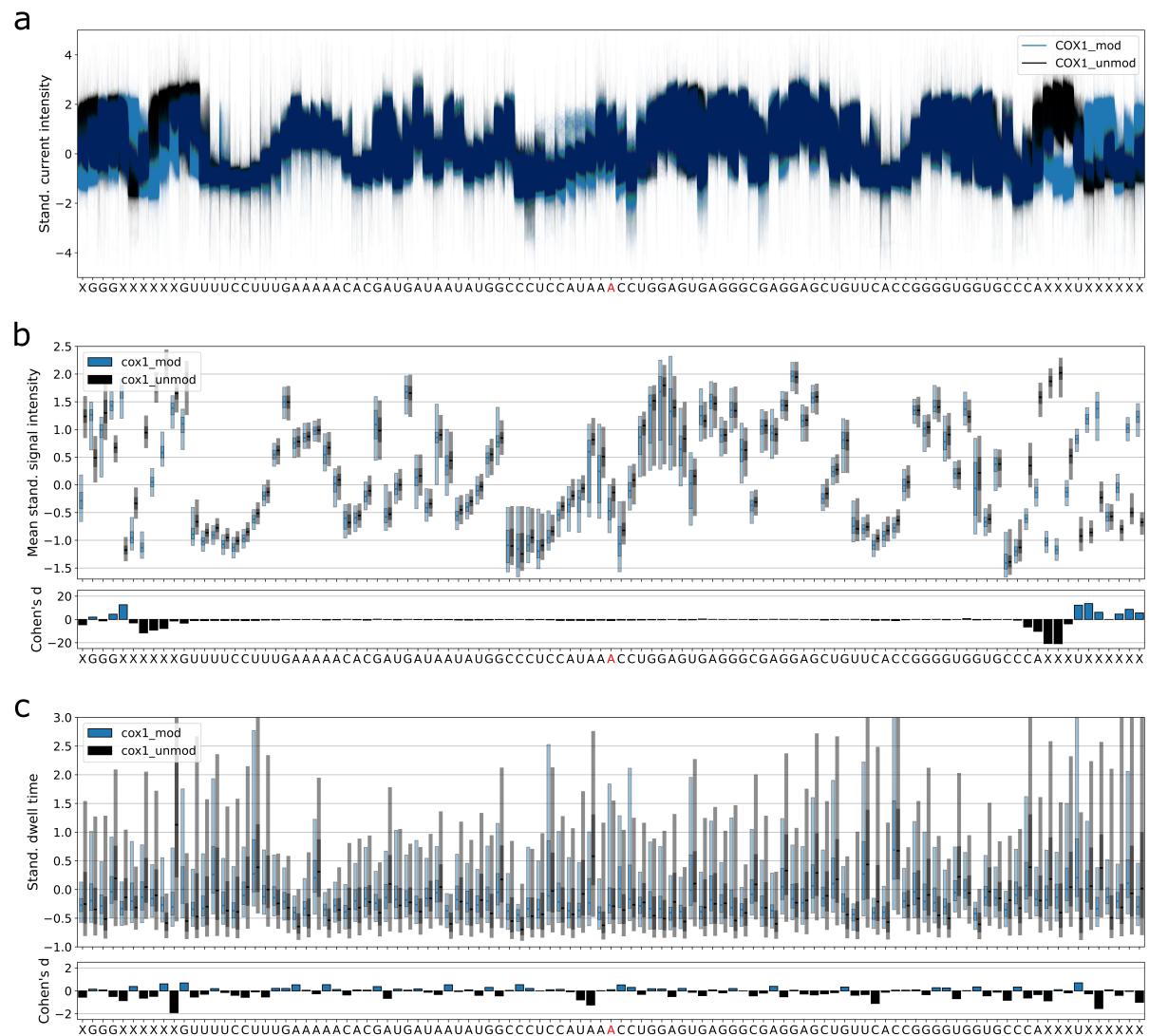


Supplementary Figure 3 Distributions of the normalized mean difference (NMD) in the alignments for all pairwise comparisons of the four tools for query-to-signal alignments with unsplit x-axis.

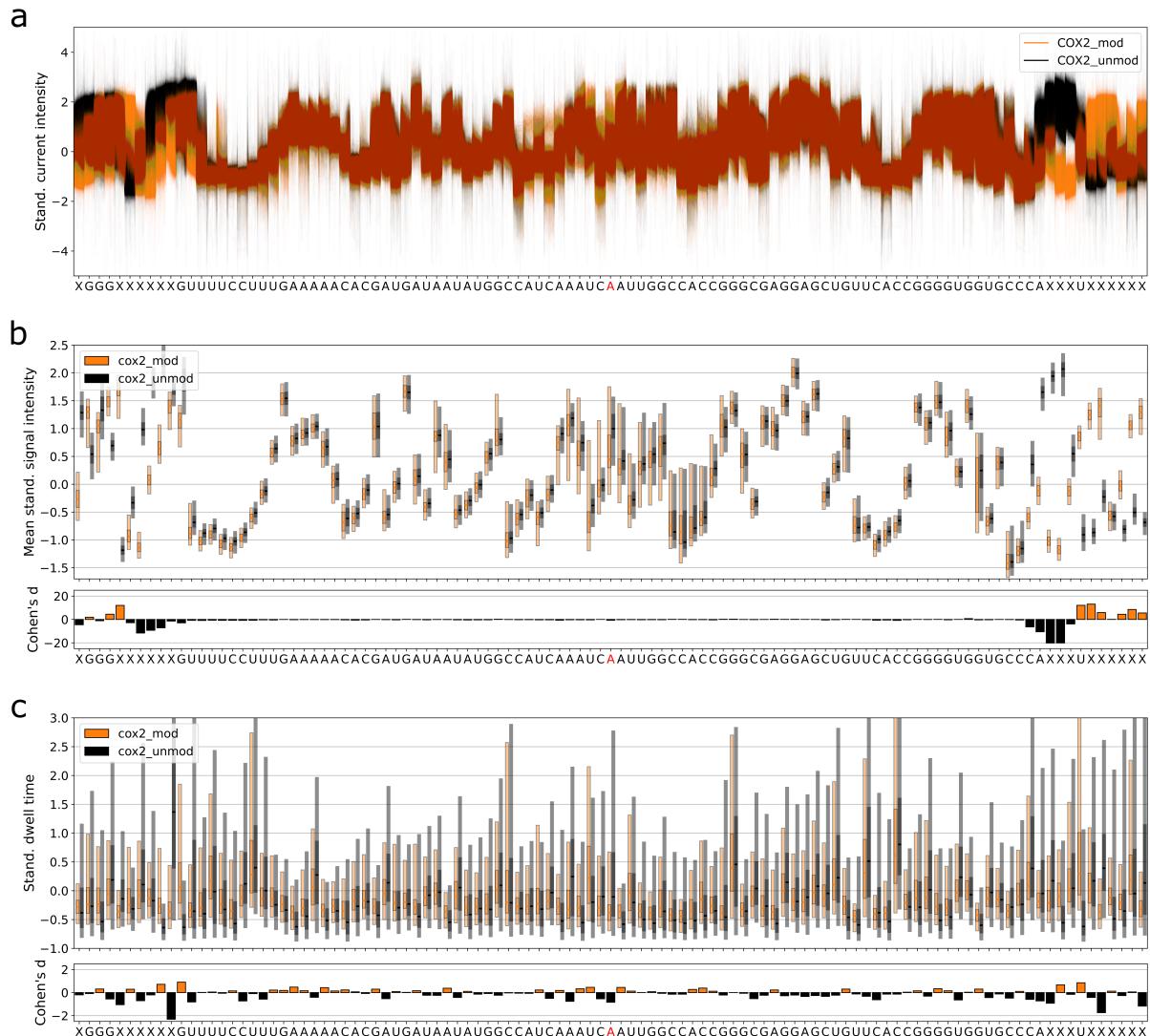


Supplementary Figure 4 Distribution of the NMD in the reference-to-signal alignment between Fishnet and Remora with unsplit x-axis.

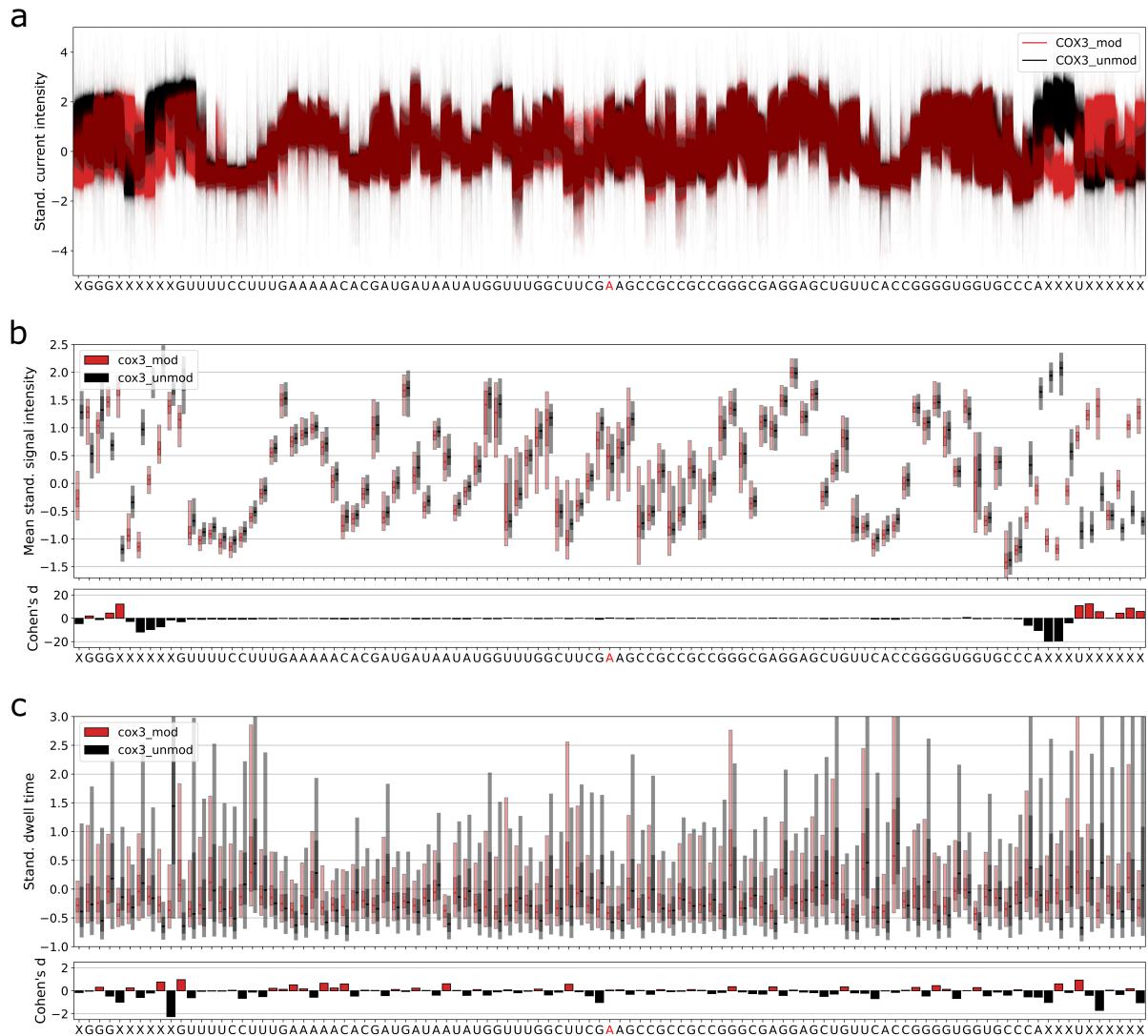
Analysis of an m¹A site in RNA constructs



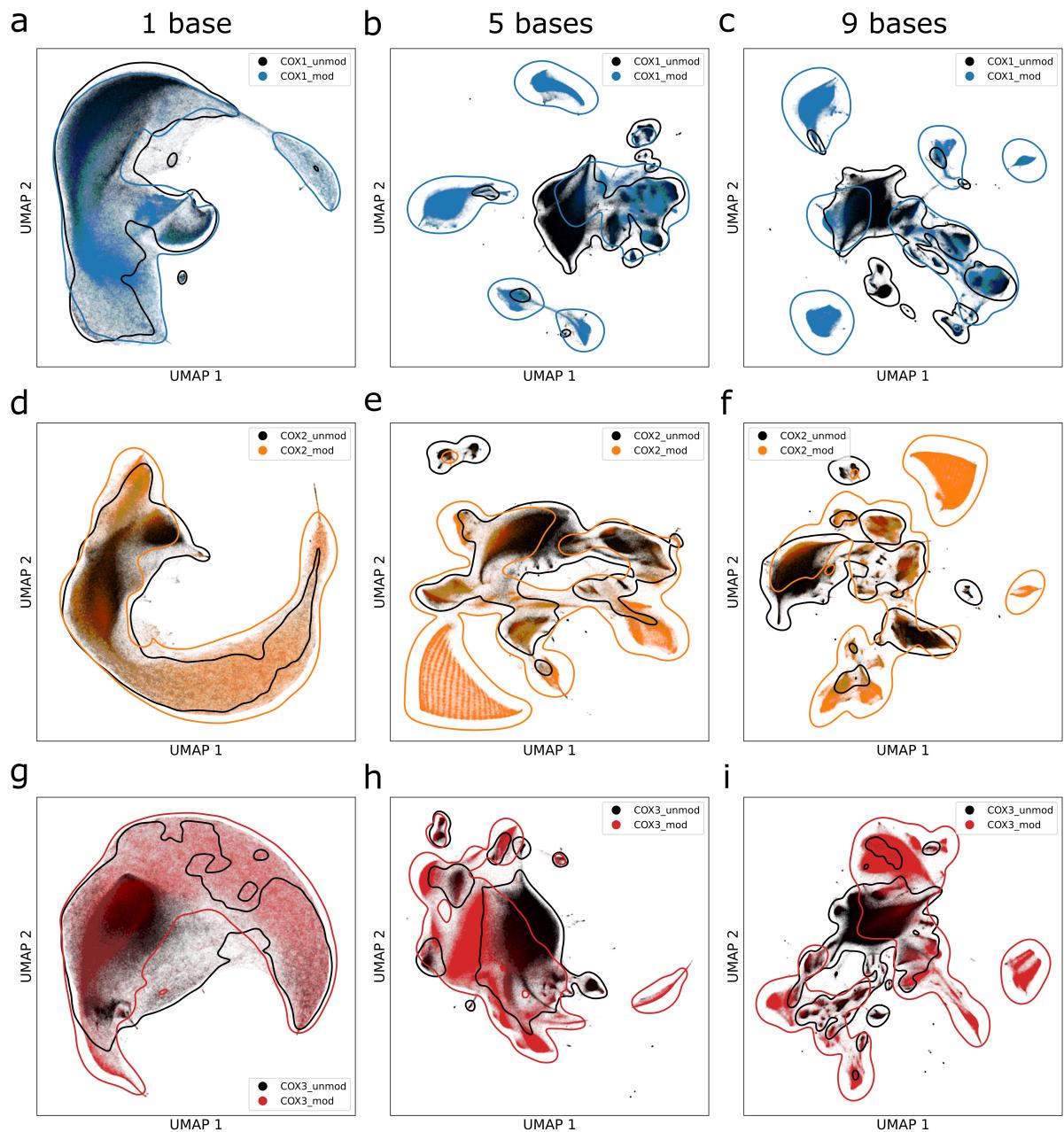
Supplementary Figure 5 Analysis of m1A COX1 construct. The unmodified sample is shown in black in all plots. **a:** Full sequence of the oligonucleotide surrounding the central m1A/A site (shown in red). **b:** Distribution of the mean standardized signal intensity. The boundaries of the bars correspond from bottom to top to the 5th percentile, 25th percentile, median, 75th percentile and 95th percentile of the distributions. Below is the effect size (Cohen's d) of the difference between the signal intensities in the modified and unmodified samples. **c:** Distribution of standardized dwell time and the corresponding effect sizes of the differences (same structure as second row).



Supplementary Figure 6 Analysis of m1A COX2 construct. The unmodified sample is shown in black in all plots. **a:** Full sequence of the oligonucleotide surrounding the central m1A/A site (shown in red). **b:** Distribution of the mean standardized signal intensity. The boundaries of the bars correspond from bottom to top to the 5th percentile, 25th percentile, median, 75th percentile and 95th percentile of the distributions. Below is the effect size (Cohen's d) of the difference between the signal intensities in the modified and unmodified samples. **c:** Distribution of standardized dwell time and the corresponding effect sizes of the differences (same structure as second row).



Supplementary Figure 7 Analysis of m1A COX3 construct. The unmodified sample is shown in black in all plots. **a:** Full sequence of the oligonucleotide surrounding the central m1A/A site (shown in red). **b:** Distribution of the mean standardized signal intensity. The boundaries of the bars correspond from bottom to top to the 5th percentile, 25th percentile, median, 75th percentile and 95th percentile of the distributions. Below is the effect size (Cohen's d) of the difference between the signal intensities in the modified and unmodified samples. **c:** Distribution of standardized dwell time and the corresponding effect sizes of the differences (same structure as second row).



Supplementary Figure 8 Scatterplots showing the UMAP axes on the interpolated signal data and dwell times from the central (un-)modified base (**a, d, g**). Increased amount of information by including two (**b, e, h**) and four surrounding bases (**c, f, i**).