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Protocol

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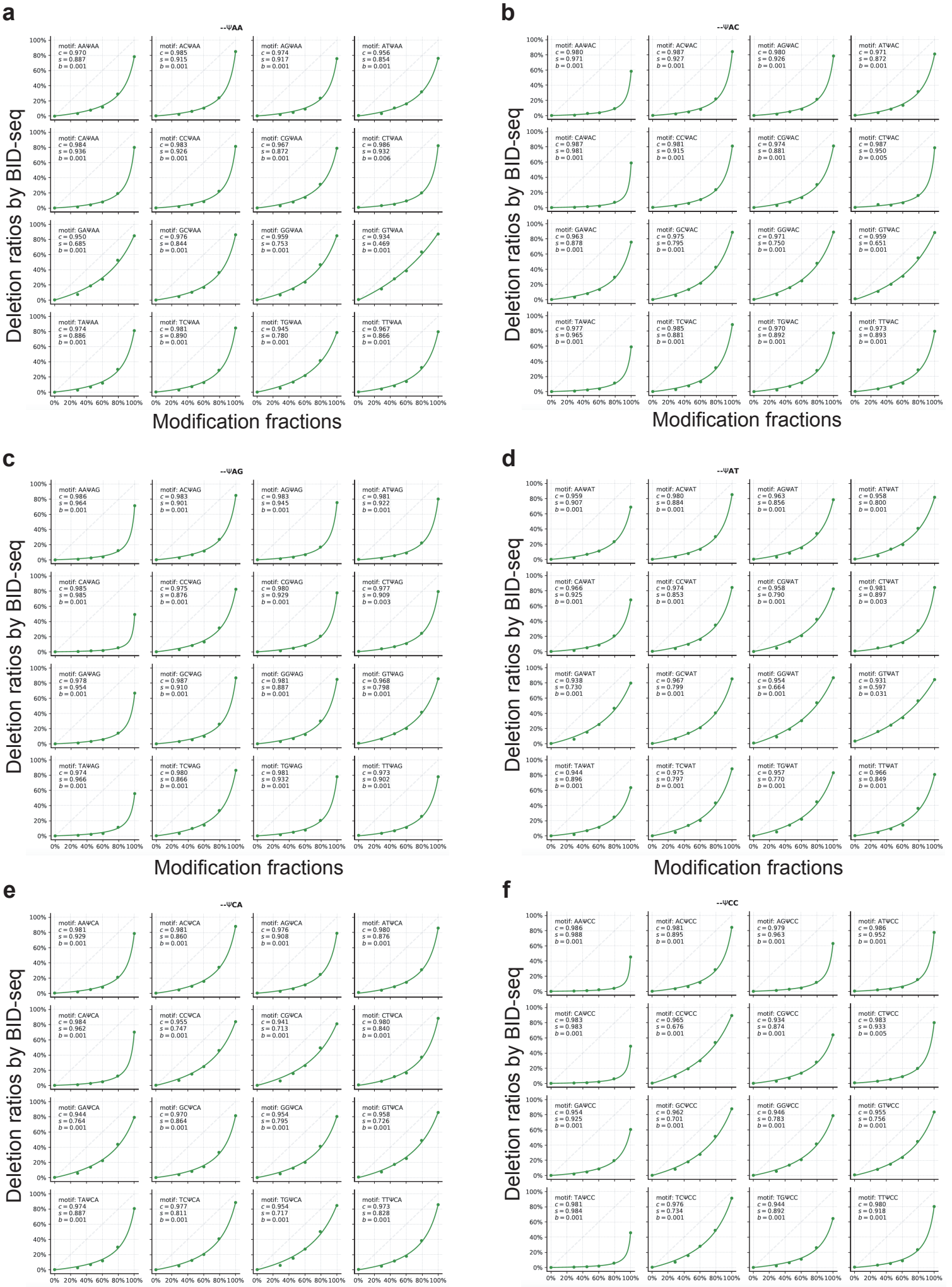
BID-seq for transcriptome-wide quantitative sequencing of mRNA pseudouridine at base resolution

In the format provided by the authors and unedited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Deletion ratios by BID-seq
Modification fractions
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Deletion ratios by BID-seq
Modification fractions
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Deletion ratios by BID-seq
Modification fractions
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Deletion ratios by BID-seq
Modification fractions
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Deletion ratios by BID-seq
Modification fractions
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motif: GGΨCC c = 0.946 s = 0.783 b = 0.001
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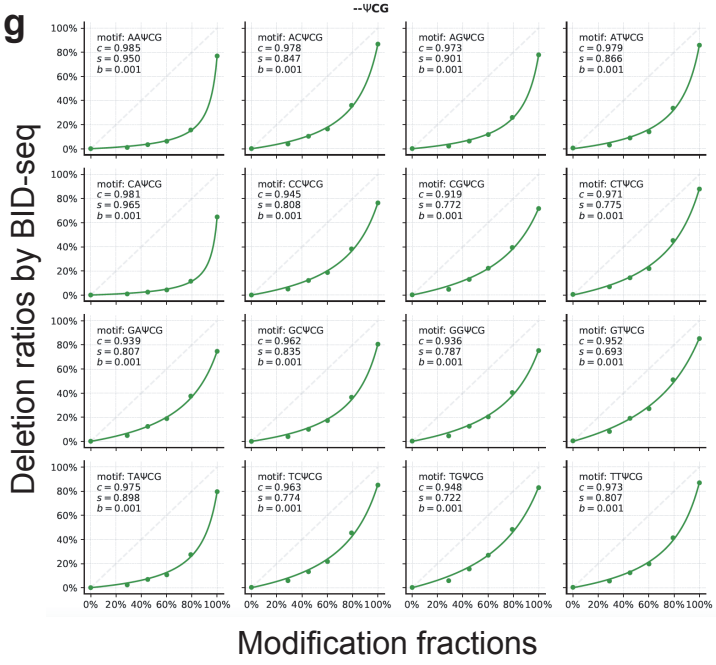
Deletion ratios by BID-seq
Modification fractions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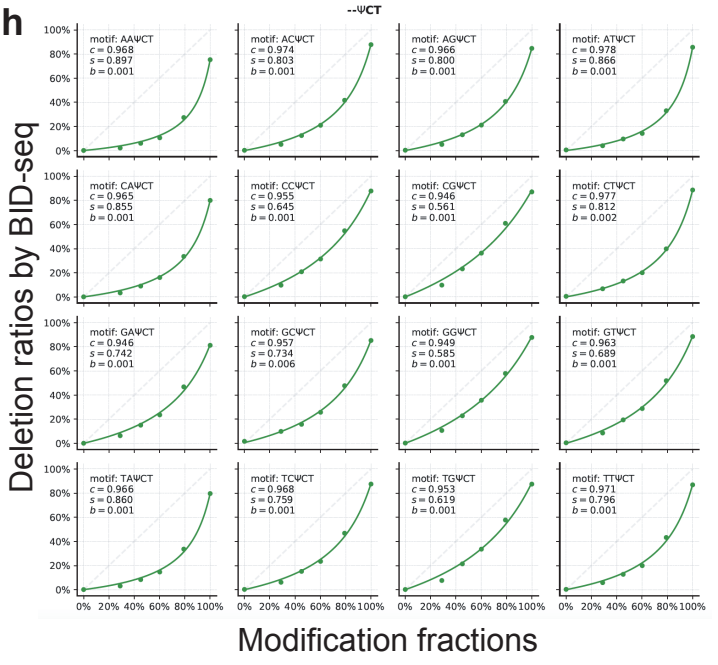
Deletion ratios by BID-seq
Modification fractions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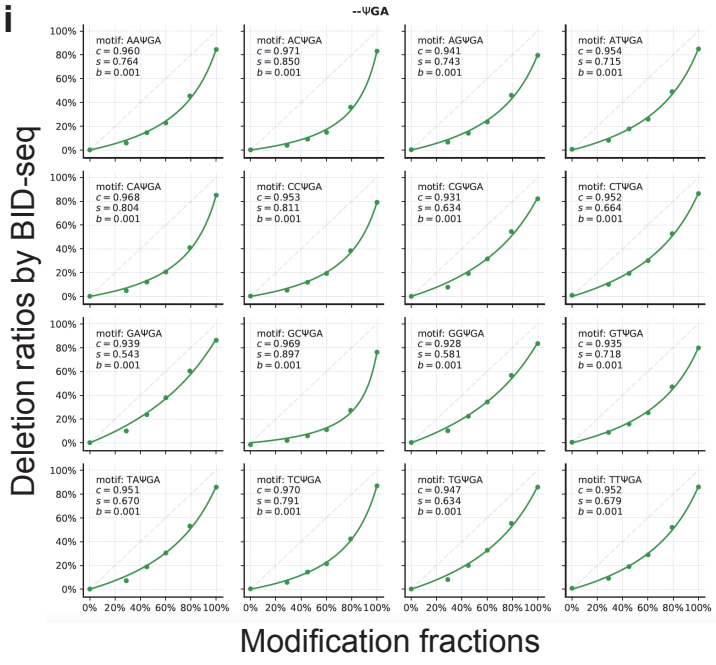
Deletion ratios by BID-seq
Modification fractions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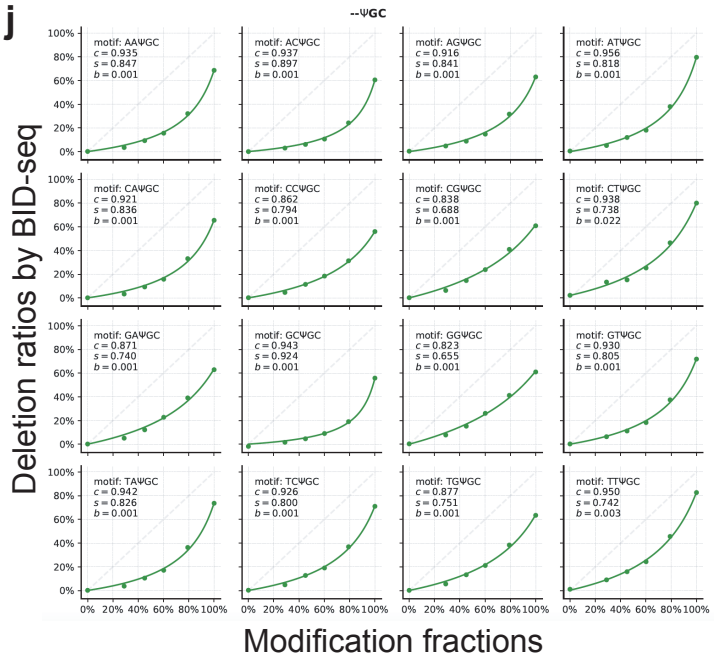
Deletion ratios by BID-seq
Modification fractions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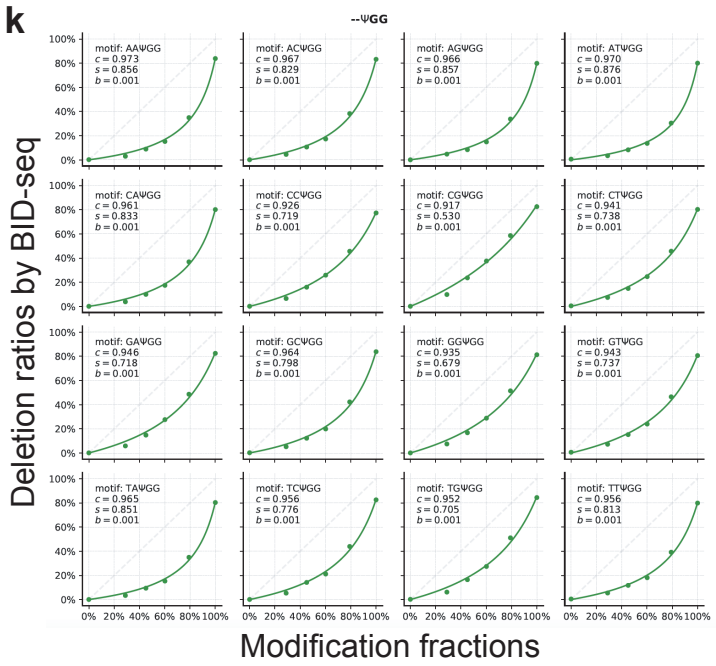
Deletion ratios by BID-seq
Modification fractions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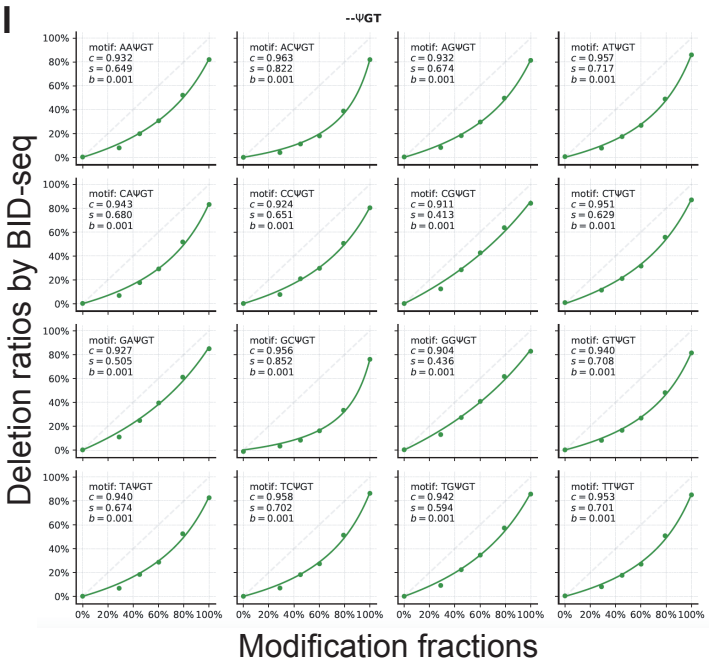
Deletion ratios by BID-seq
Modification fractions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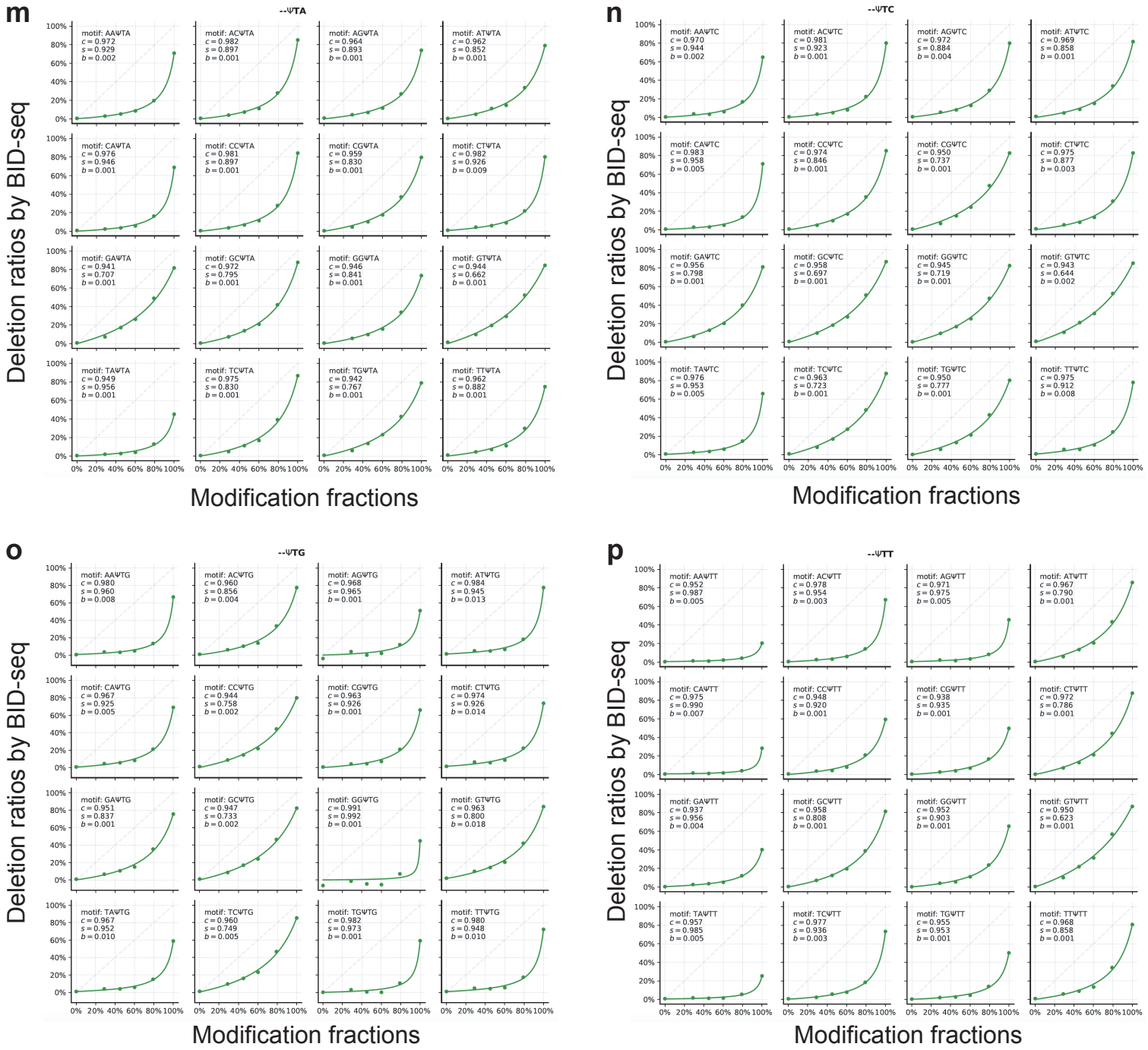
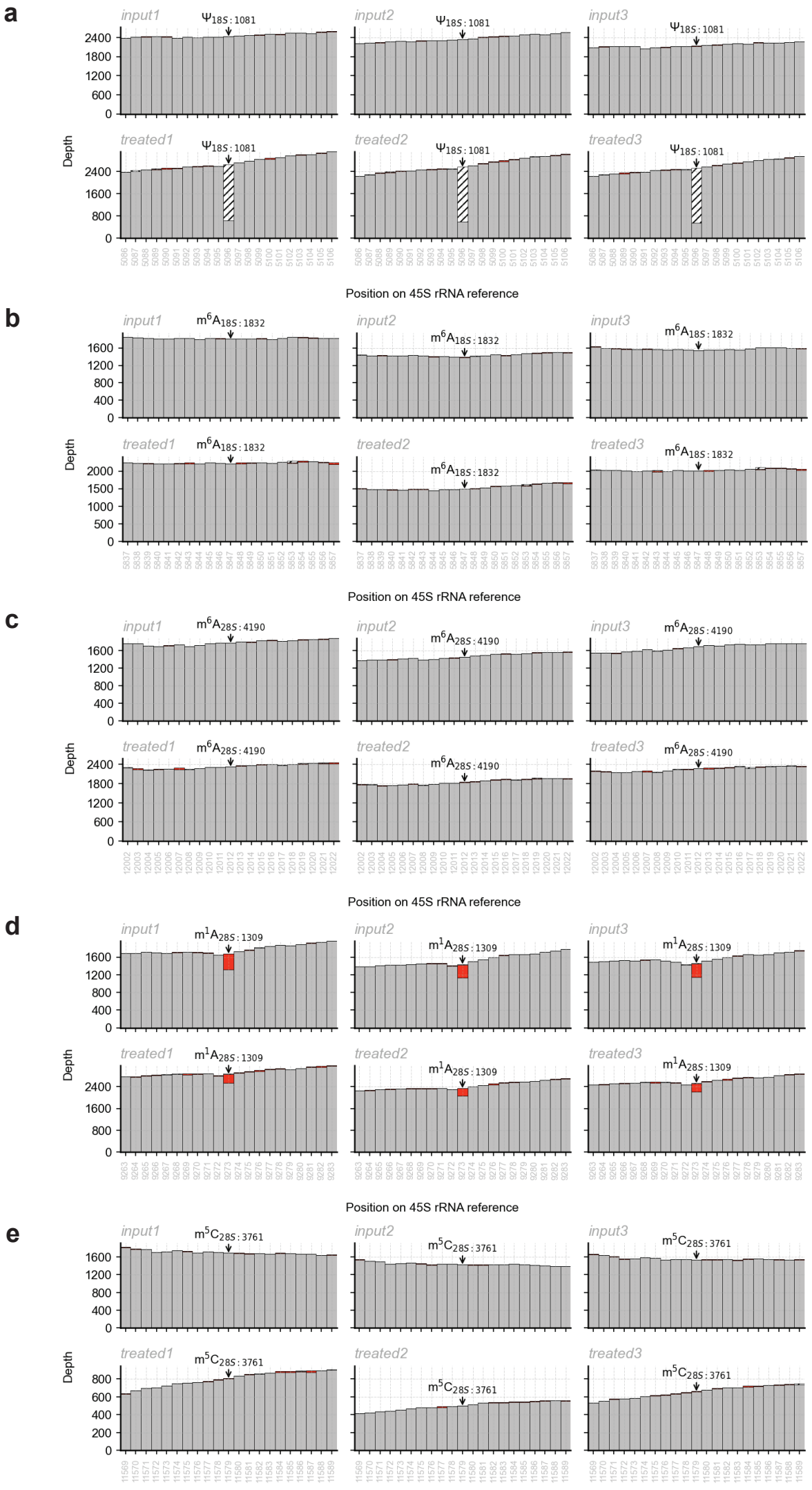
Deletion ratios by BID-seq
Modification fractions



Supplementary Fig. 1 | The calibration curves for BID-seq, based on BID-pipe analysis. a, The BID-seq calibration curves for motif contexts containing NN-ΨAA. **b**, The BID-seq calibration curves for motif contexts containing NN-ΨAC. **c**, The BID-seq calibration curves for motif contexts containing NN-ΨAG. **d**, The BID-seq calibration curves for motif contexts containing NN-ΨAU. **e**, The BID-seq calibration curves for motif contexts containing NN-ΨCA. **f**, The BID-seq calibration curves for motif contexts containing NN-ΨCC. **g**, The BID-seq calibration curves for motif contexts containing NN-ΨCG. **h**, The BID-seq calibration curves for motif contexts containing NN-ΨCU. **i**, The BID-seq calibration curves for motif contexts containing NN-ΨGA. **j**, The BID-seq calibration curves for motif contexts containing NN-ΨGC. **k**, The BID-seq calibration curves for motif contexts containing NN-ΨGG. **l**, The BID-seq calibration curves for motif contexts containing NN-ΨGU. **m**, The BID-seq calibration curves for motif contexts containing NN-ΨUA. **n**, The BID-seq calibration curves for motif contexts containing NN-ΨUC. **o**, The BID-seq calibration curves for motif contexts containing NN-ΨUG. **p**, The BID-seq calibration curves for motif contexts containing NN-ΨUU.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input2
input3
treated1
treated2
treated3
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Position on 45S rRNA reference
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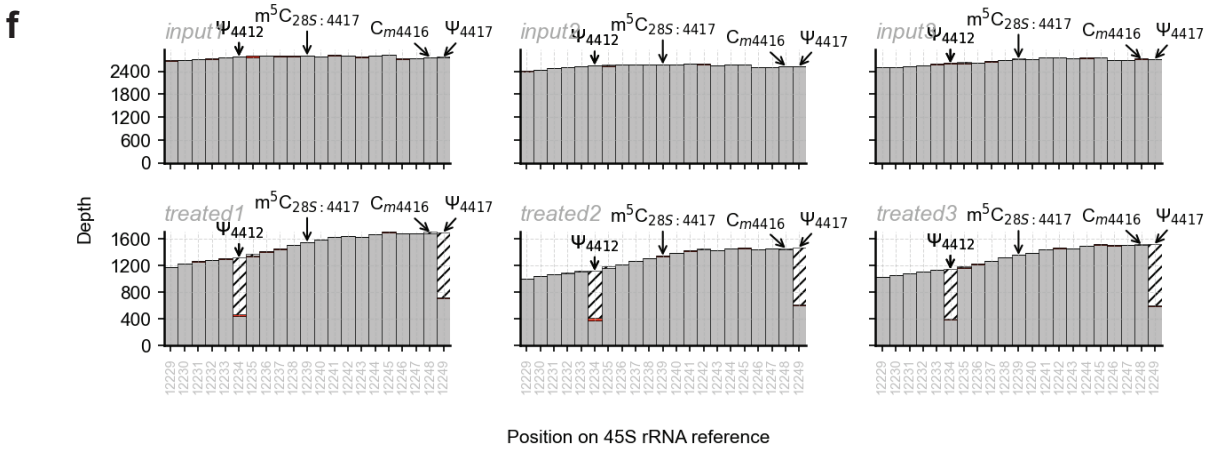
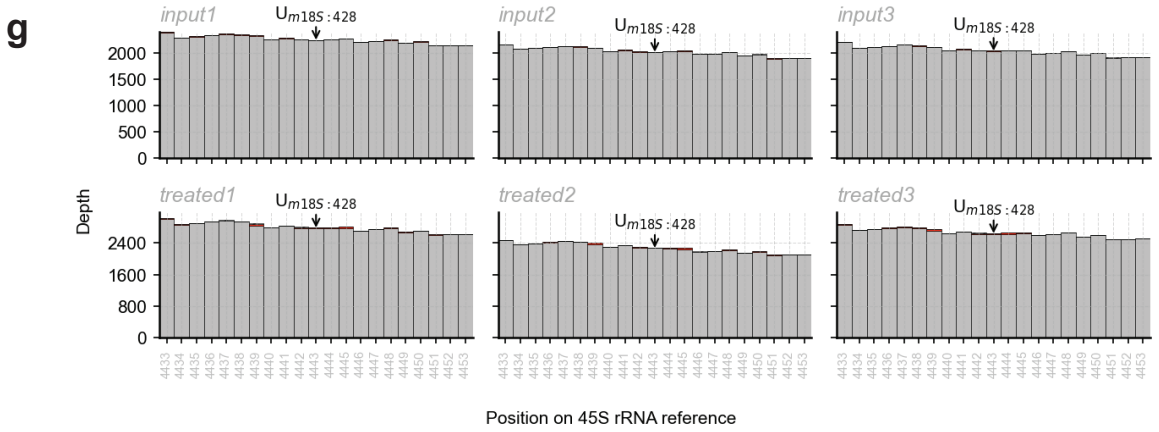
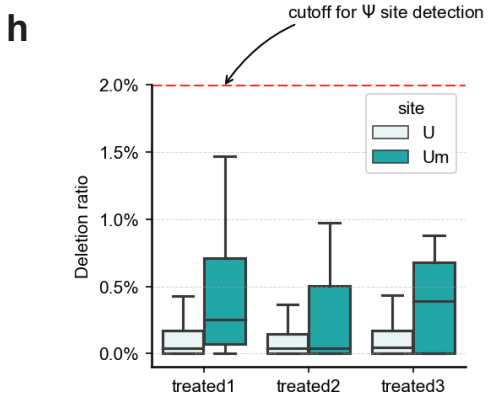
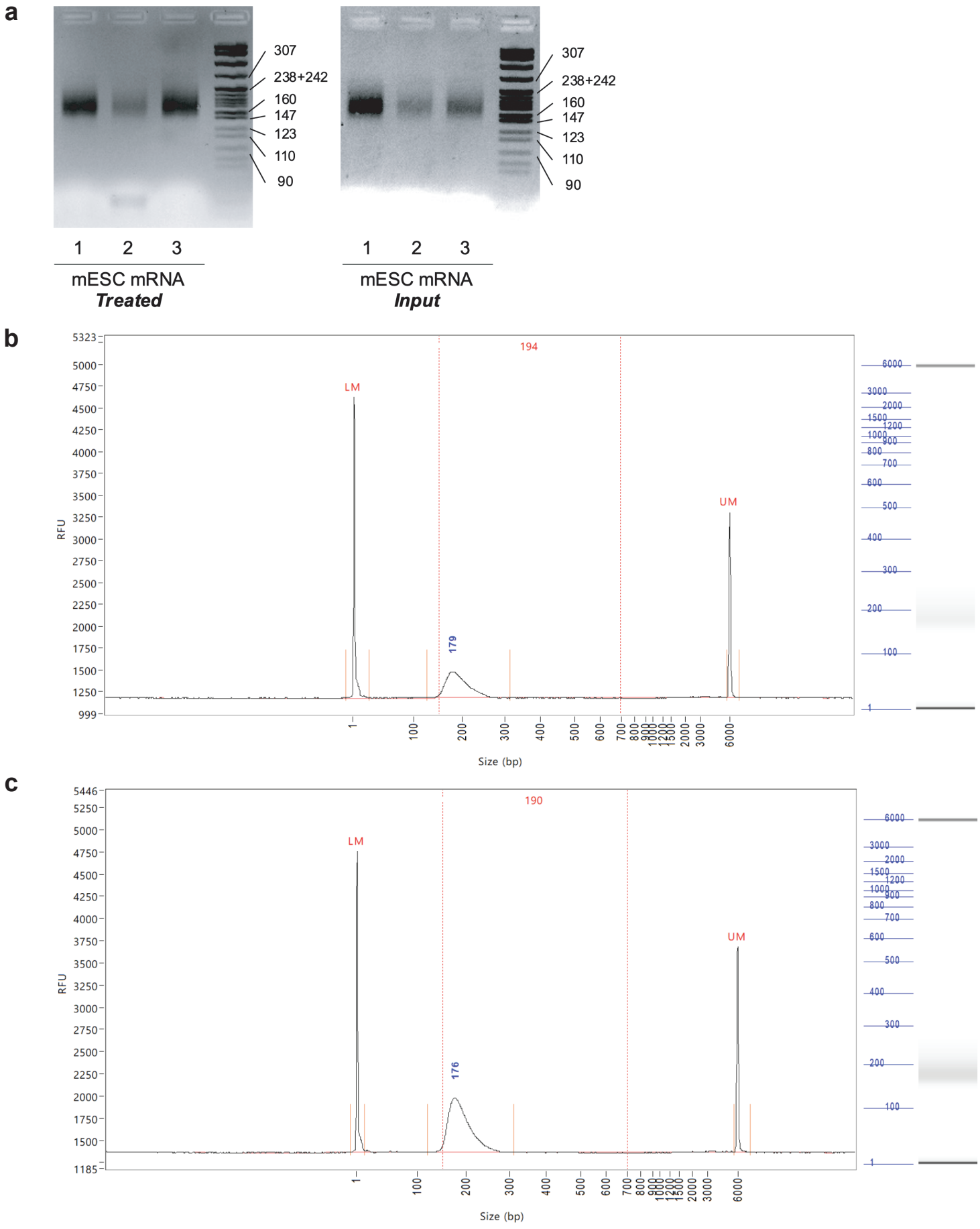






Supplementary Fig. 2 | The deletion profiles of SuperScript IV RT at different RNA modifications on mESC rRNA. **a**, BID-seq reveals dramatic deletion signatures at Ψ1081 of mESC 18S rRNA. **b**, BID-seq does not reveal any deletion signatures at m^6A1832 of mESC 18S rRNA. **c**, BID-seq does not reveal any deletion signatures at m^6A4190 of mESC 28S rRNA. **d**, BID-seq does not reveal any deletion signatures at m^1A1309 of mESC 28S rRNA. **e**, BID-seq does not reveal any deletion signatures at m^5C3761 of mESC 28S rRNA. **f**, BID-seq does not reveal any deletion signatures at m^5C4417 of mESC 28S rRNA. **g**, BID-seq does not reveal any deletion signatures at Um428 of mESC 18S rRNA. **h**, For all Um sites on mESC rRNA, the deletion ratios at Um sites are much lower than Ψ detection cutoff in BID-pipe.



Supplementary Fig. 3 | The Agarose gel and Bioanalyzer plot to show the size of BID-seq libraries. **a**, The agarose gel image to show the sizes of 'Input' and 'Treated' libraries built with mESC polyA$^+$ RNA, in BID-seq. **b**, The Bioanalyzer measurement to show the size of 'Input' library built with mESC polyA$^+$ RNA, in BID-seq. **c**, The Bioanalyzer measurement to show the size of 'Treated' library built with mESC polyA$^+$ RNA, in BID -seq.