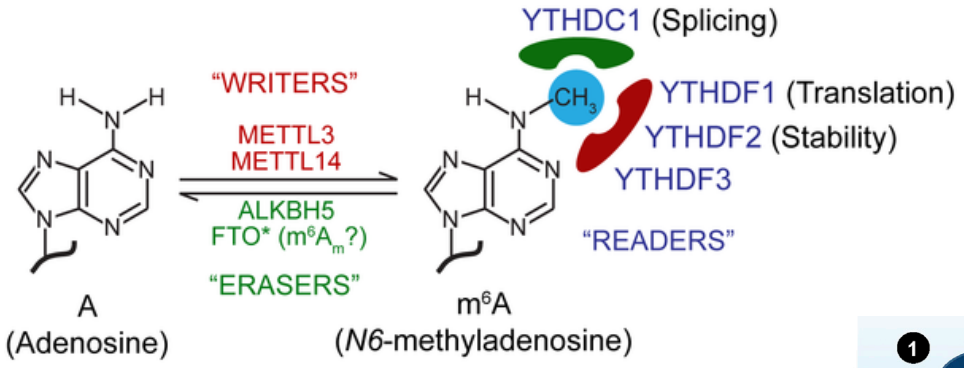
RNA modifications: methodology and examples in disease

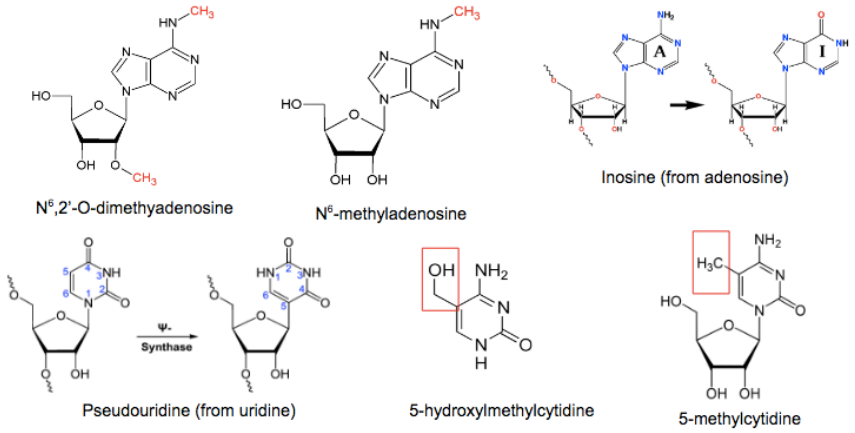
Evgenia Ntini
evgenia.ntini@imbb.forth.gr
RNA & Gene Regulation
IMBB-FoRTH

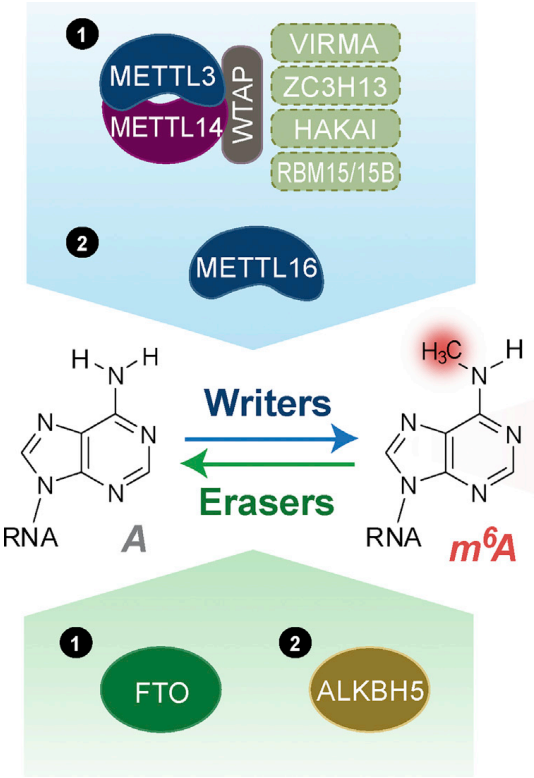
RNA modifications: dynamic and reversible

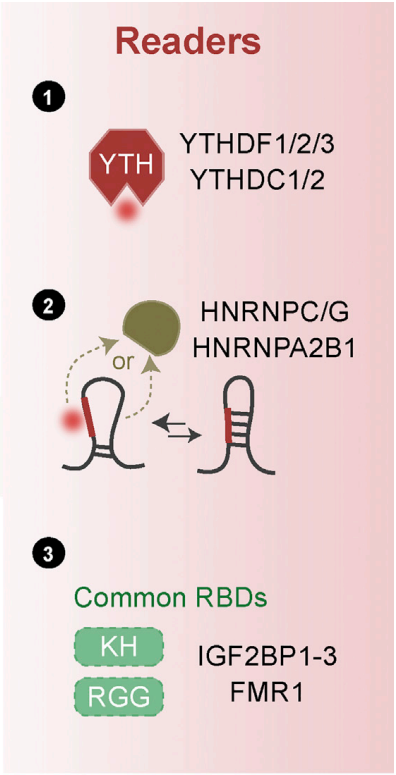


Gokhale and Horner SM, PLOS Pathogens 2017

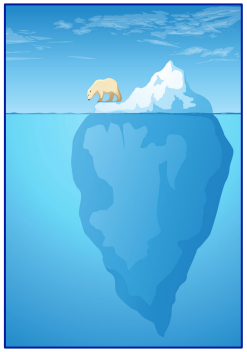
Shi, Wei and Chuan He, Mol Cell 2019

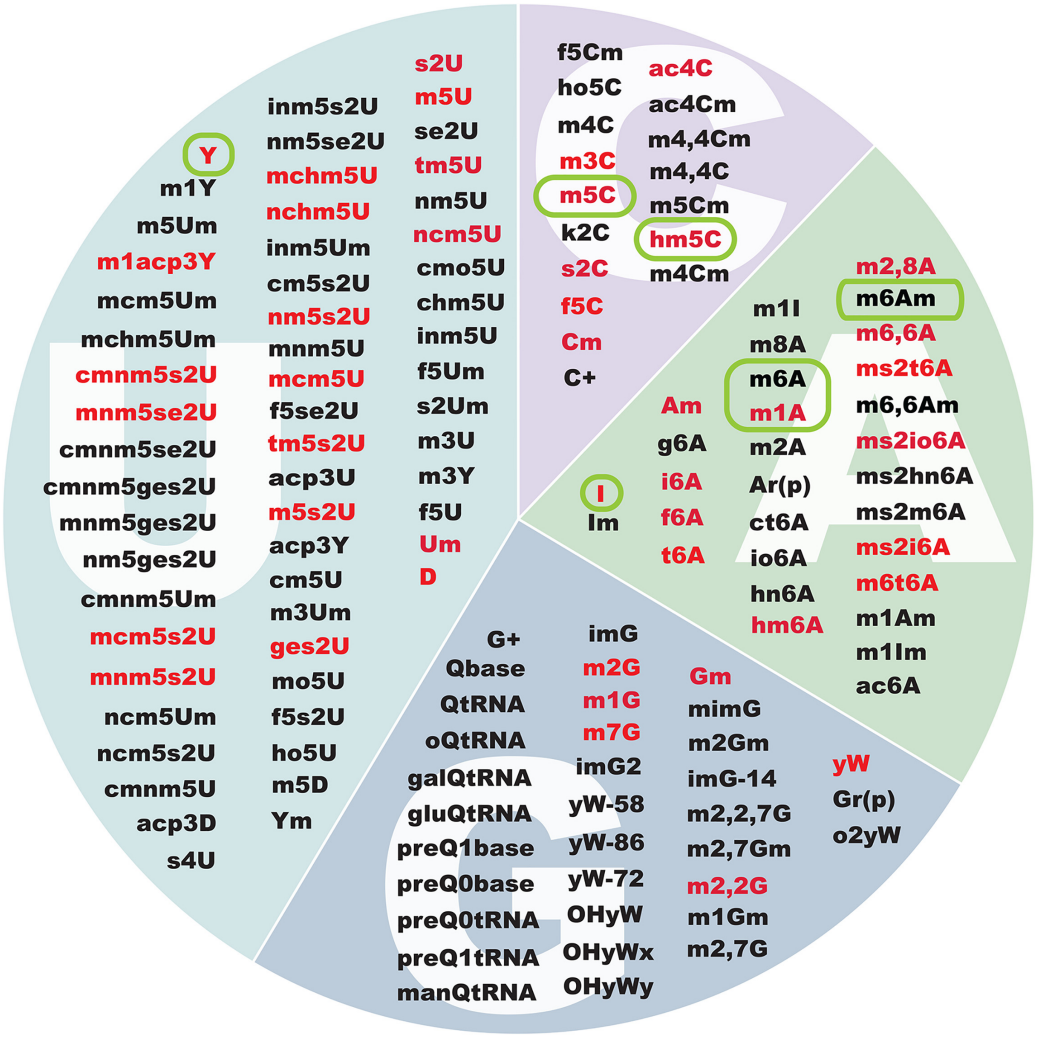






RNA modifications: dynamic and reversible





Disease related

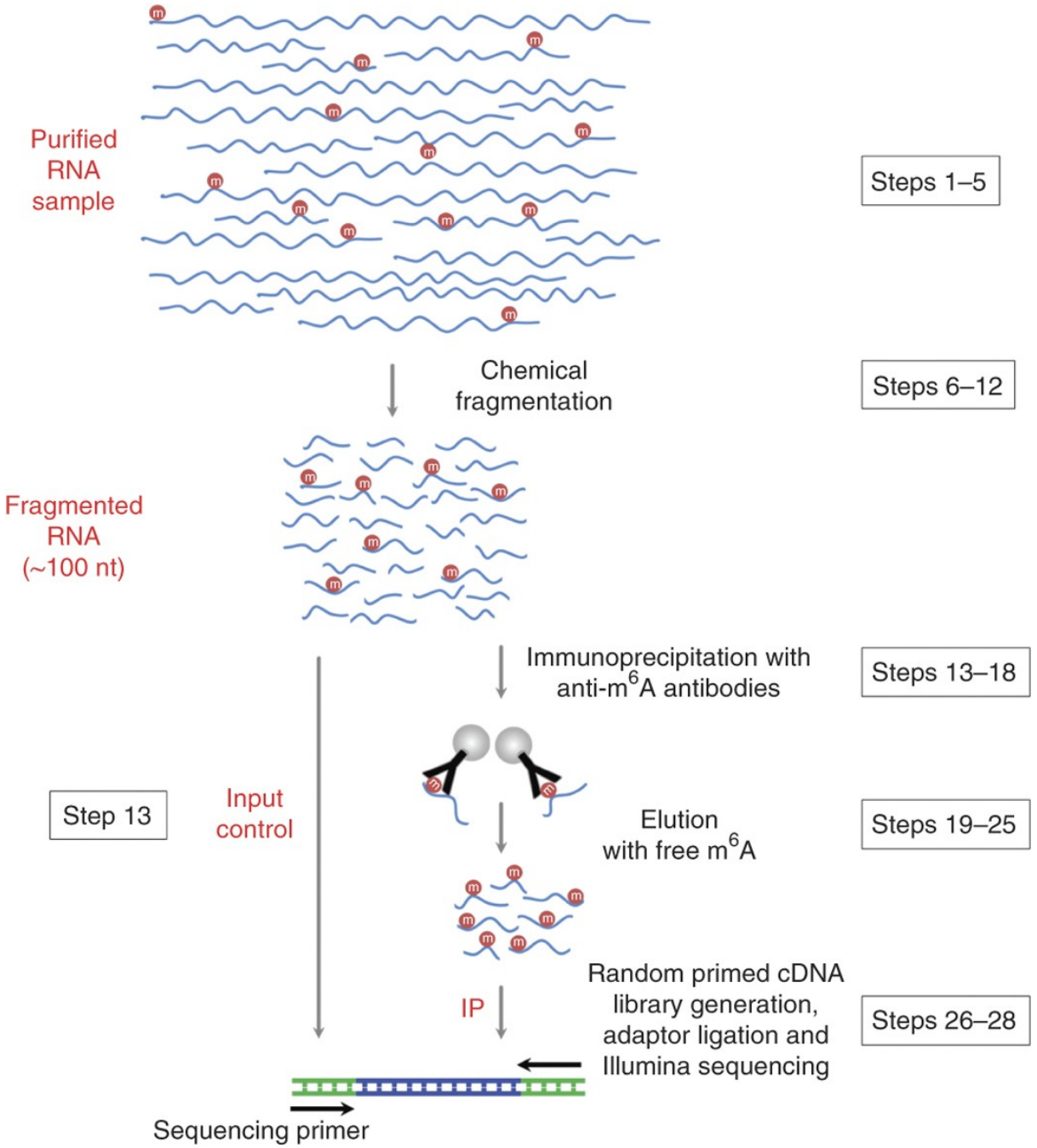
Detection technique available

Johnkhout et al., RNA 2017
The RNA modification landscape in human disease
PMID: 28855326

Me-RIP/m6A-seq

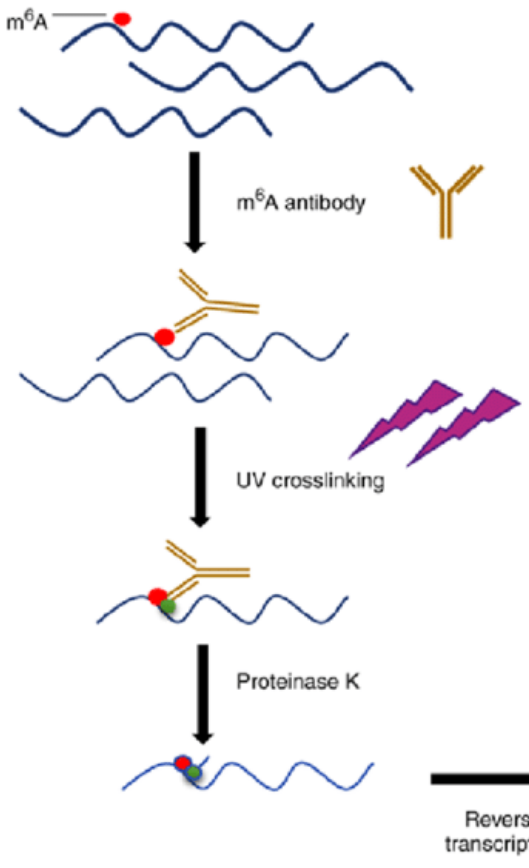
- MeRIP-Seq: Meyer K. D., Saletore Y., Zumbo P., Elemento O., Mason C. E., et al. (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 149: 1635-1646
- m6A-Seq:Dominissini D. et al. (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485: 201-206
- Dominissini D. et al. (2013) Transcriptome-wide mapping of N6-methyladenosine by m6A-seq based on immunocapturing and massively parallel sequencing

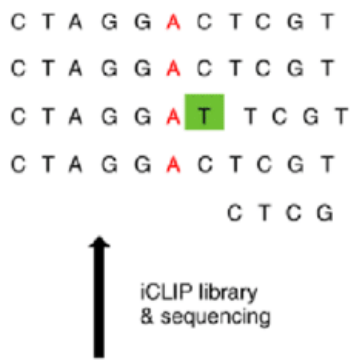
Me-RIP/m6A-seq

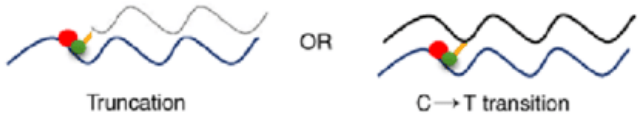


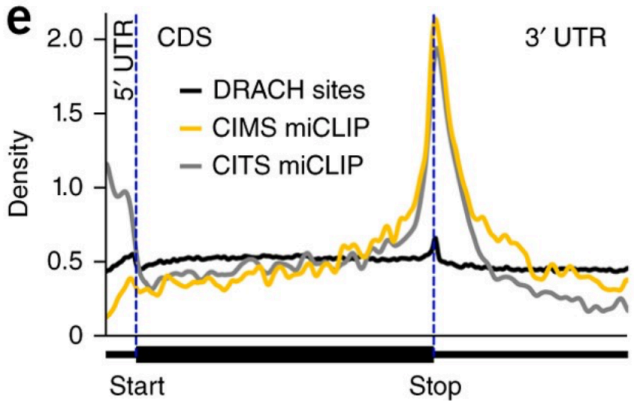
miCLIP −m6A

- miCLIP-m6A: Linder, et al. (2015) Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat Methods 12, 767–772 (2015)

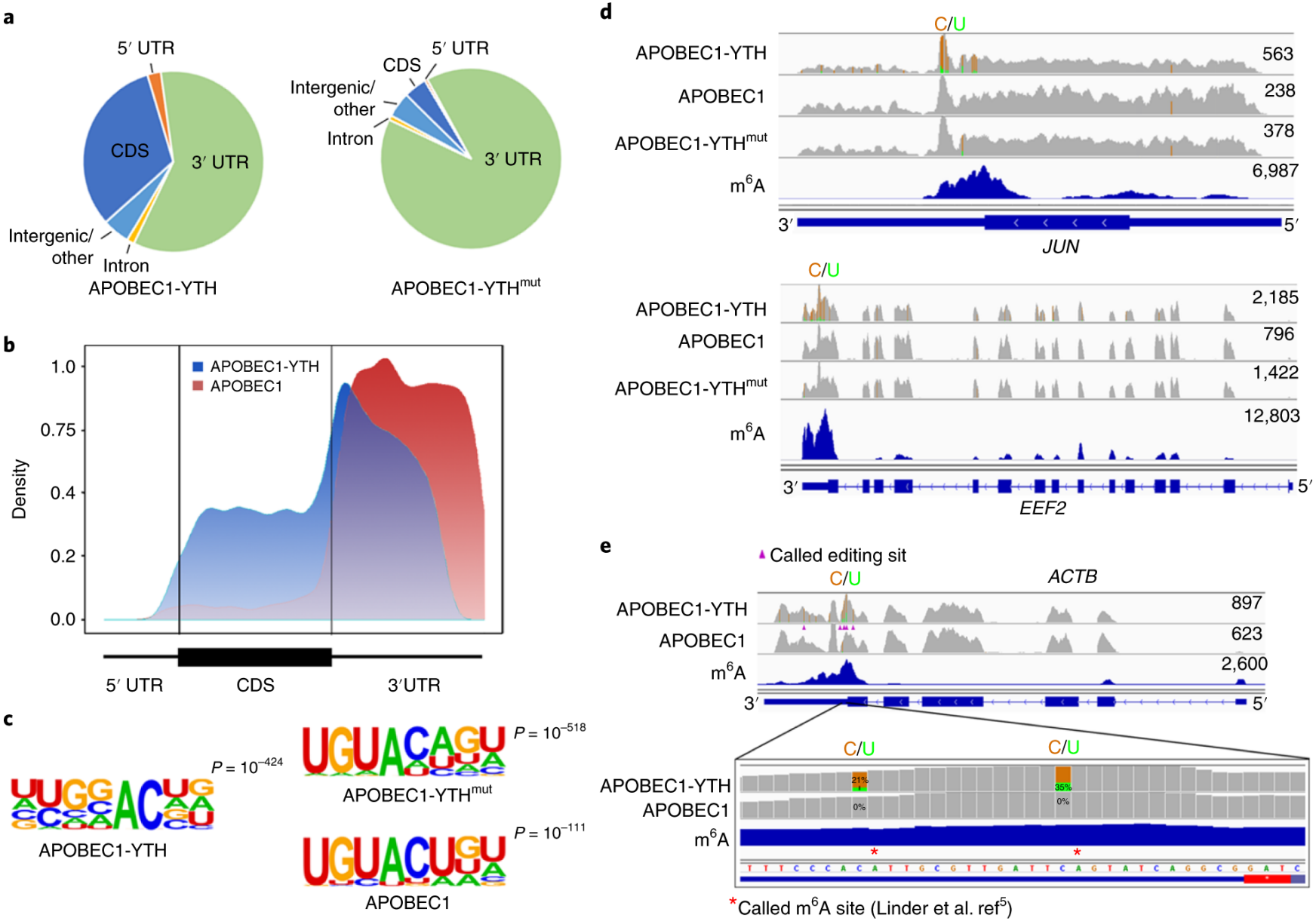








Antibody-free methods:
Meyer KD. DART-seq: an antibody-free method for global m6A detection
Nature Methods v 16, p 1275–1280 (2019)



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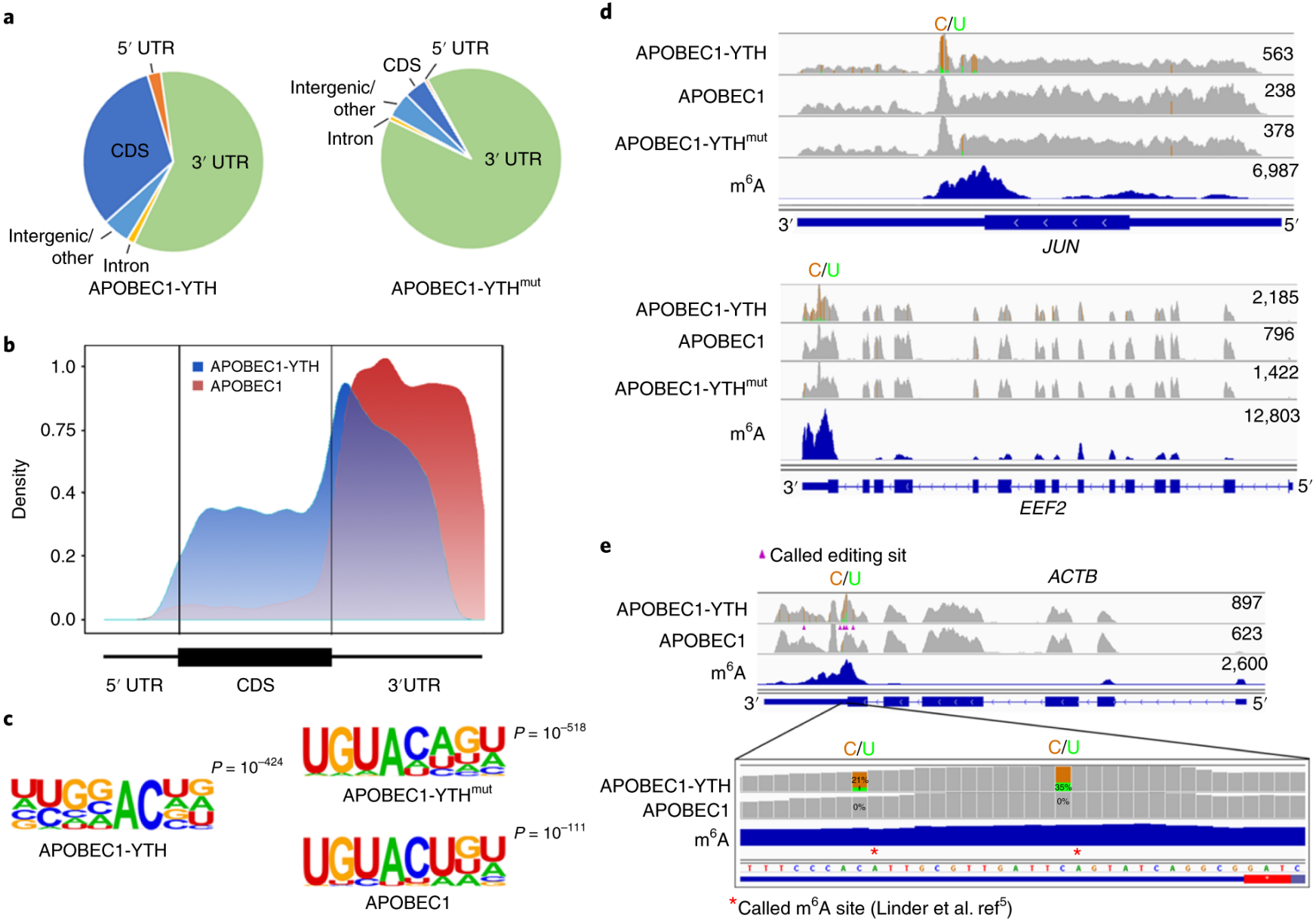
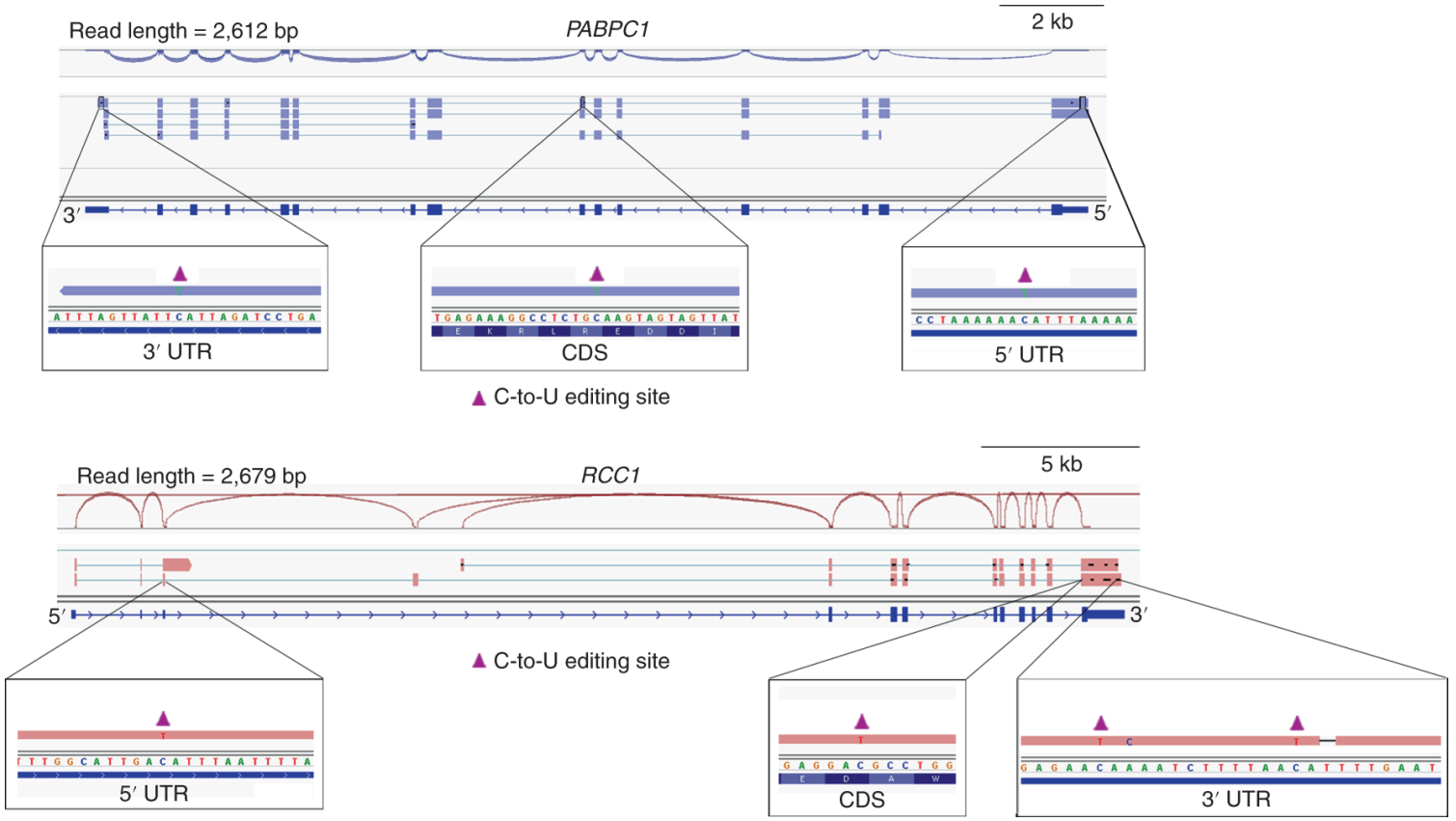
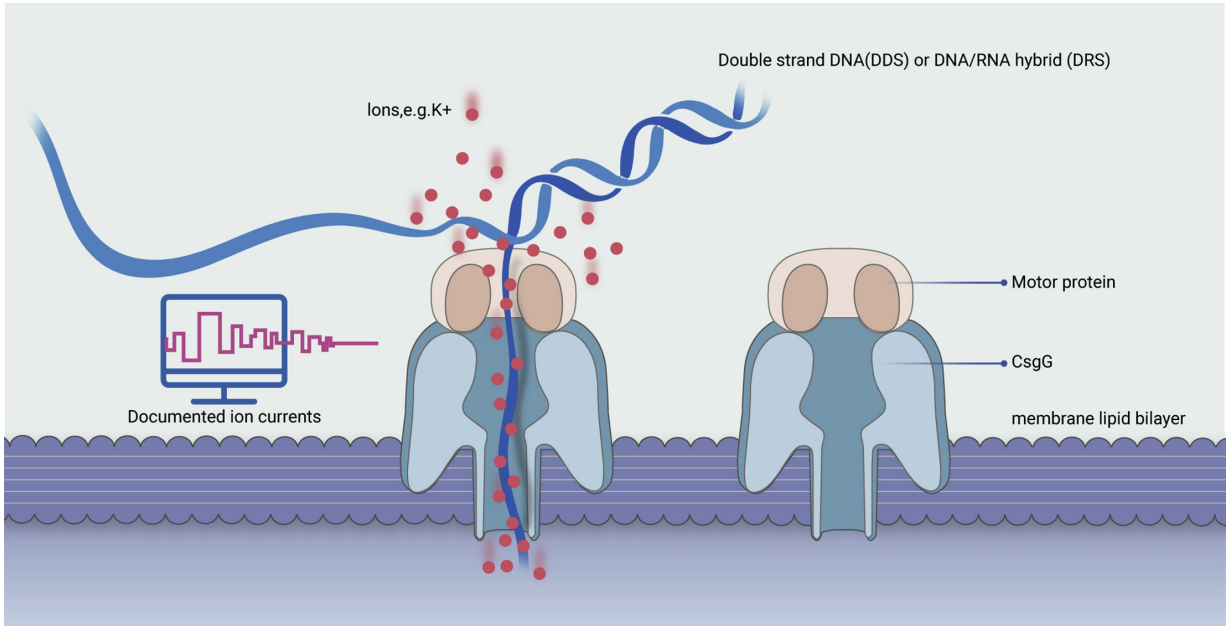


Meyer KD. DART-seq: an antibody-free method for global m6A detection
Nature Methods v 16, p 1275–1280 (2019)

Long-read DART-seq (with PacBio) reveals m6A distribution within individual RNA molecules.

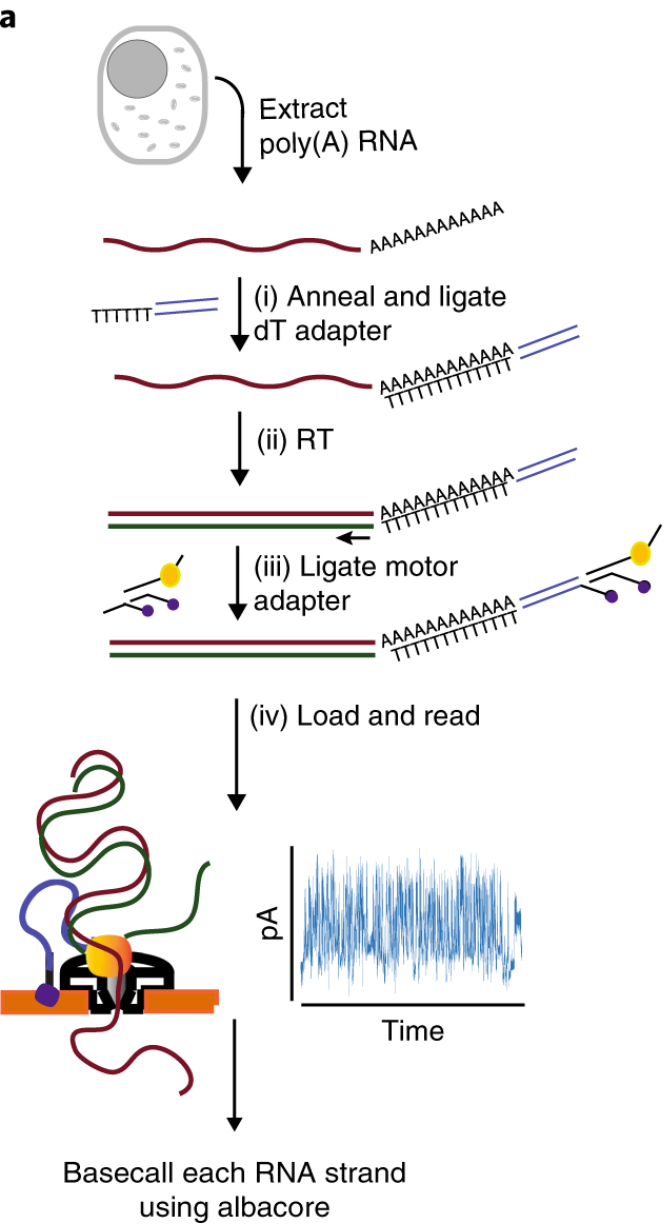


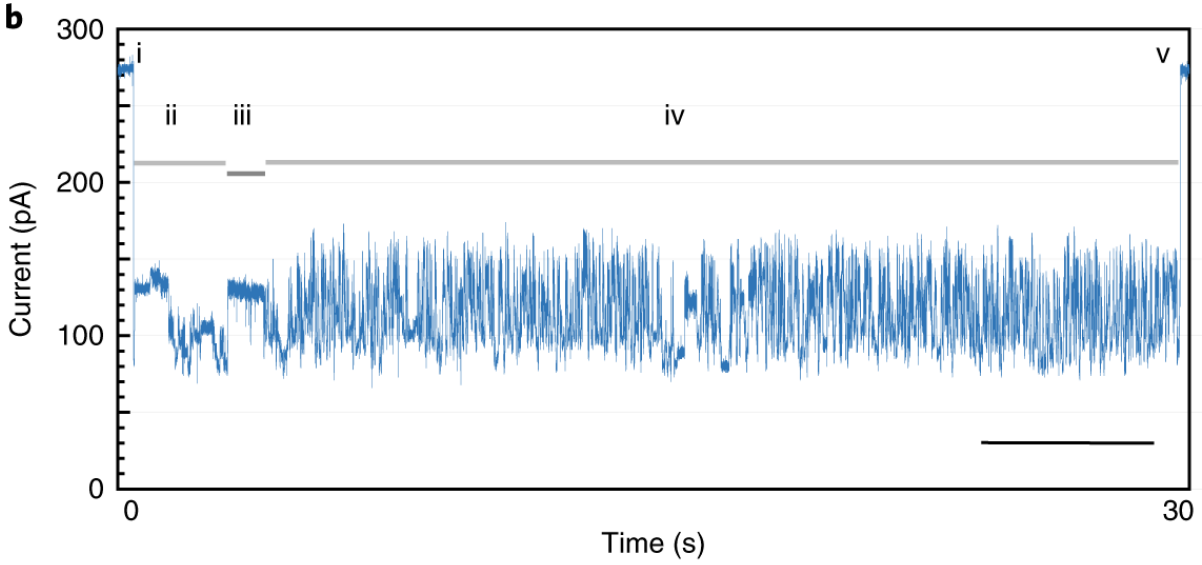
Direct RNA sequencing
with Oxford Nanopore Technology

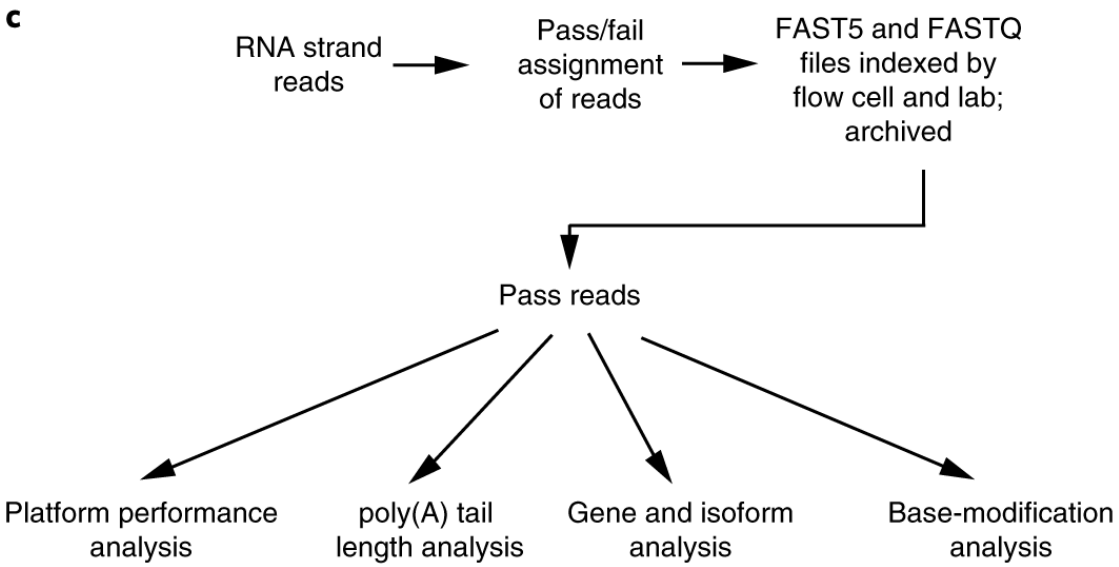


https://doi.org/10.1016/j.xinn.2021.100153

Oxford Nanopore Direct RNA sequencing

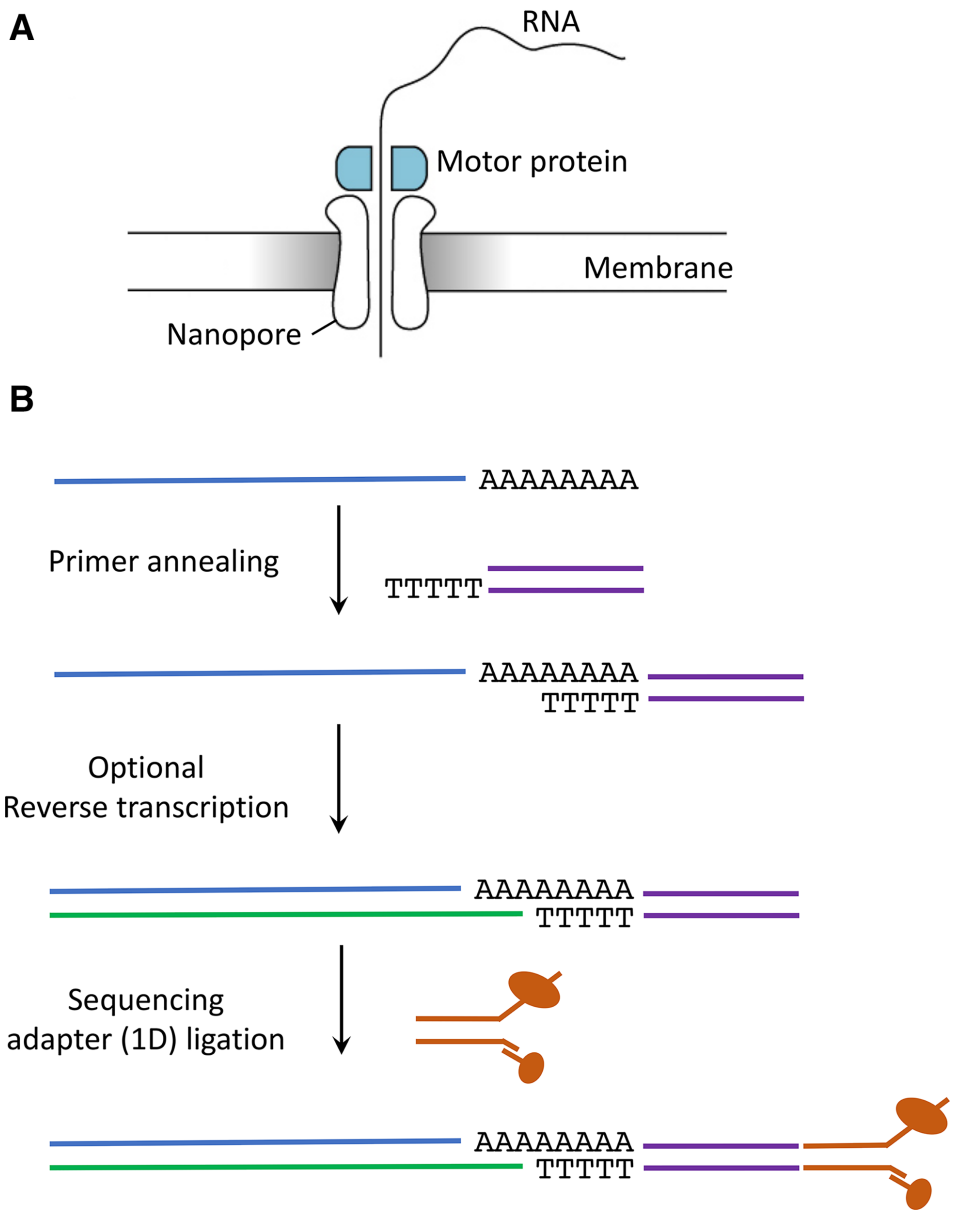






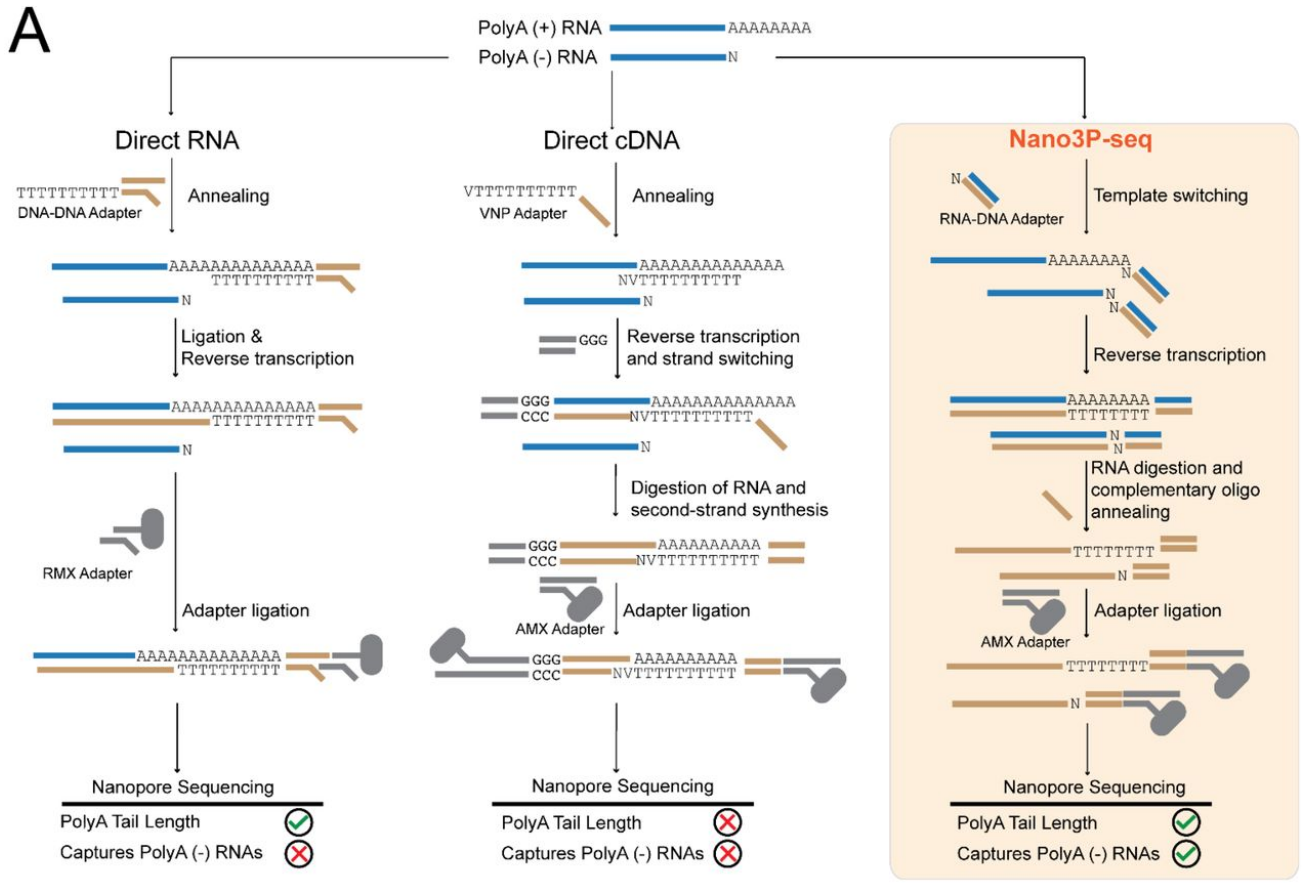
Nature Methods 16, p. 1297–1305 (2019)
doi: 10.1038/s41592-019-0617-2

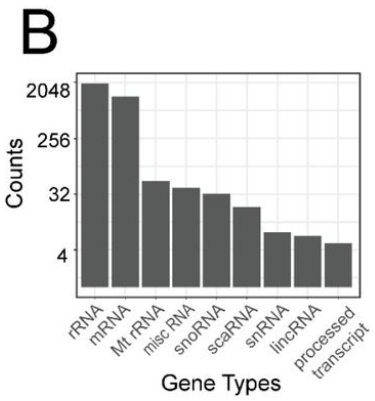
Oxford Nanopore Direct RNA sequencing

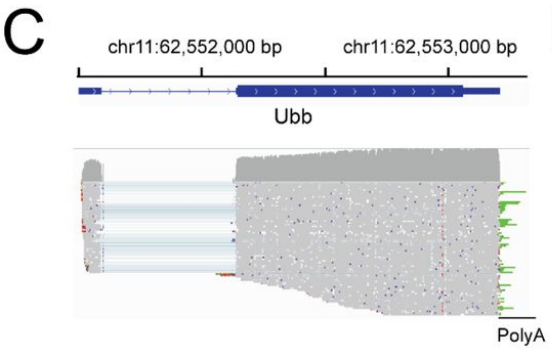


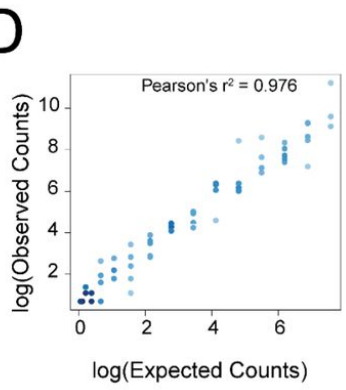
Johnkhout et al., RNA 2017
DOI:10.1261/rna.063503.117

With Nanopore DRS study RNA dynamics



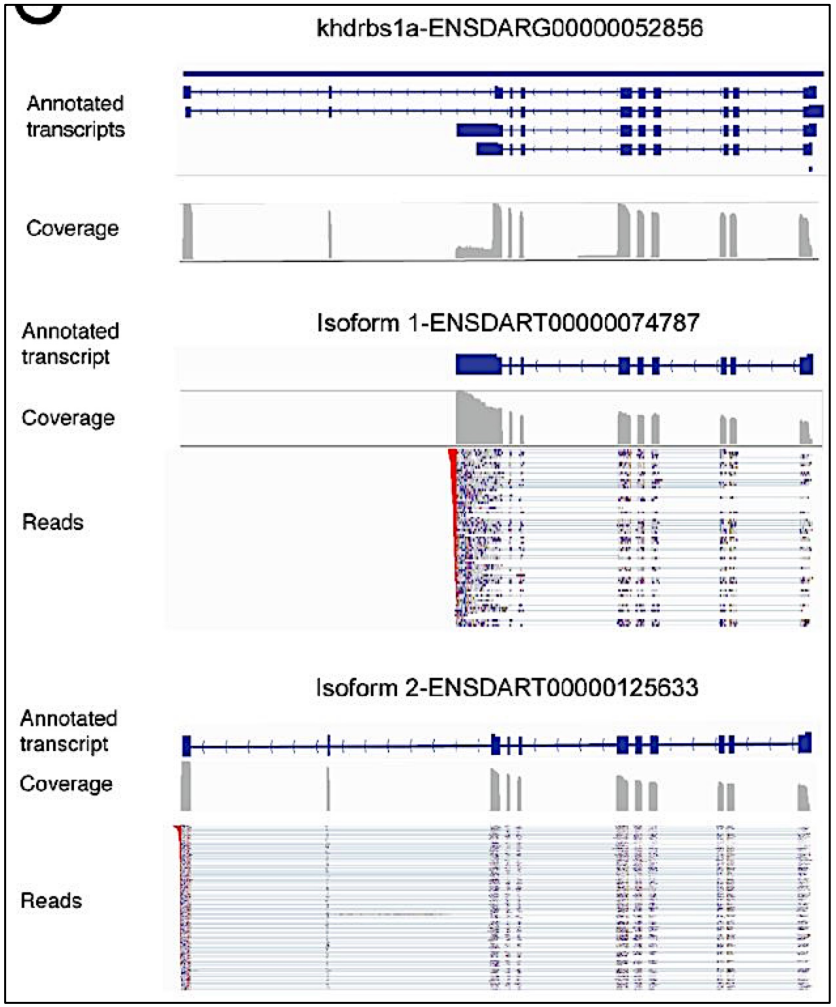






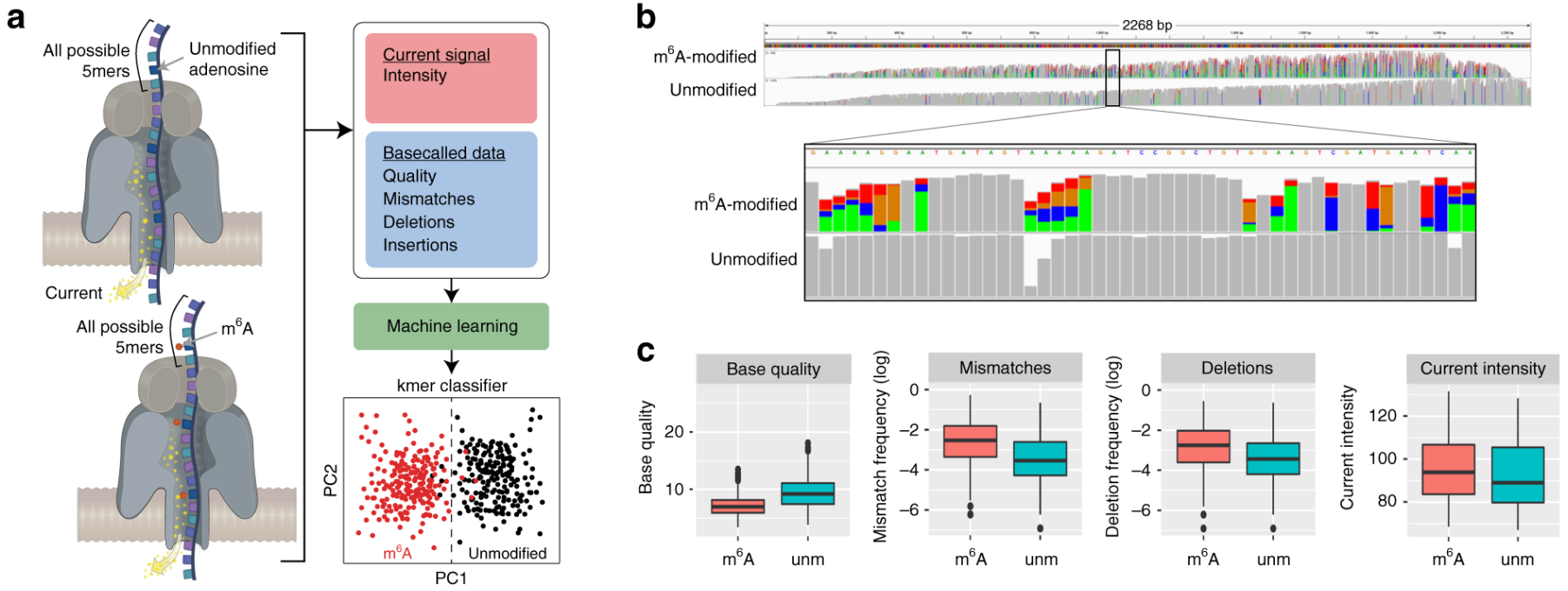
doi: https://doi.org/10.1101/2021.09.22.461331

✓ Study isoform-specific polyA-tail dynamics



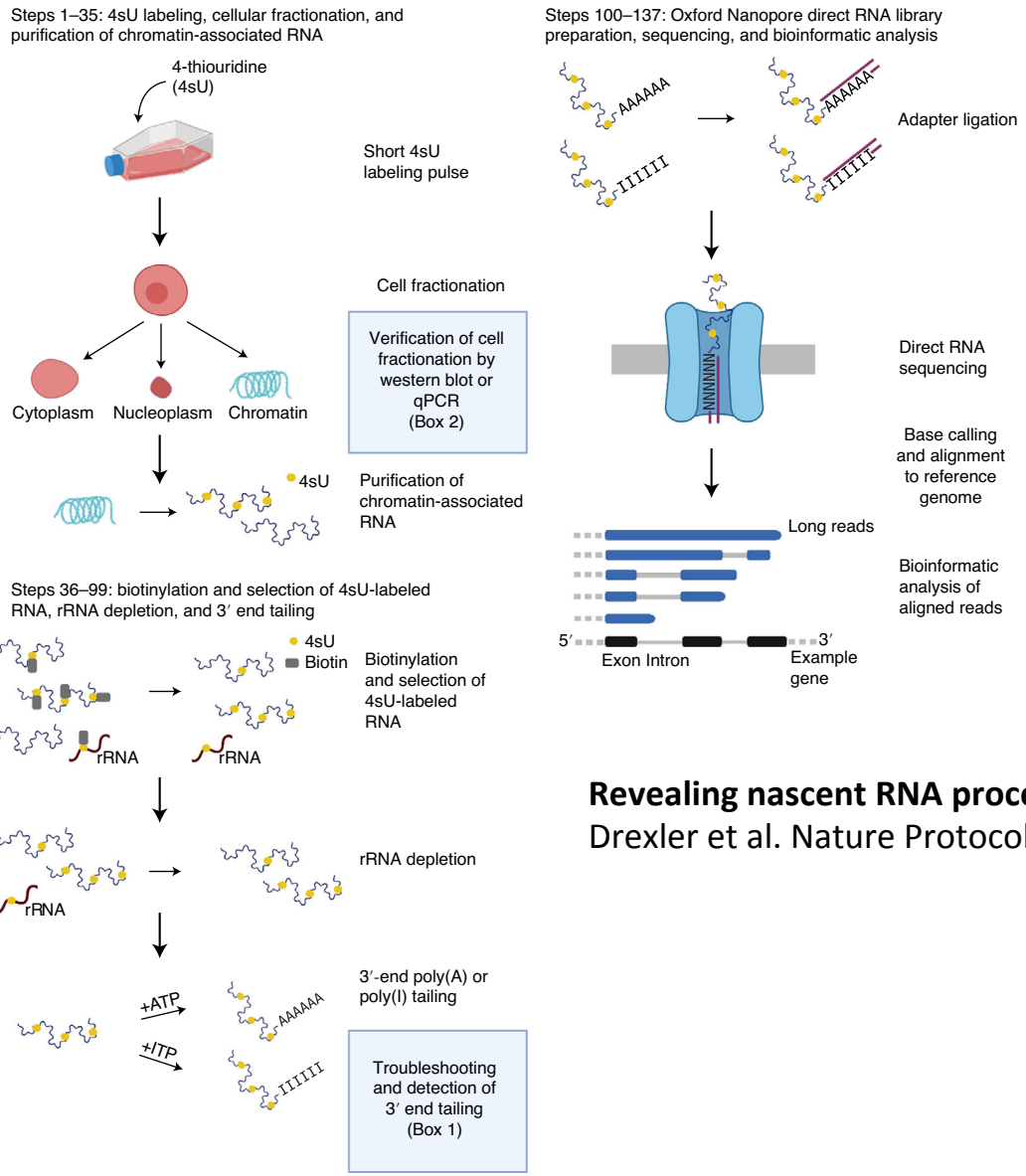
doi: https://doi.org/10.1101/2021.09.22.461331

✓ Predict RNA modifications



Accurate detection of m6A RNA modifications in native RNA sequences
Liu et al. Nature Communications 10: 4079 (2019)

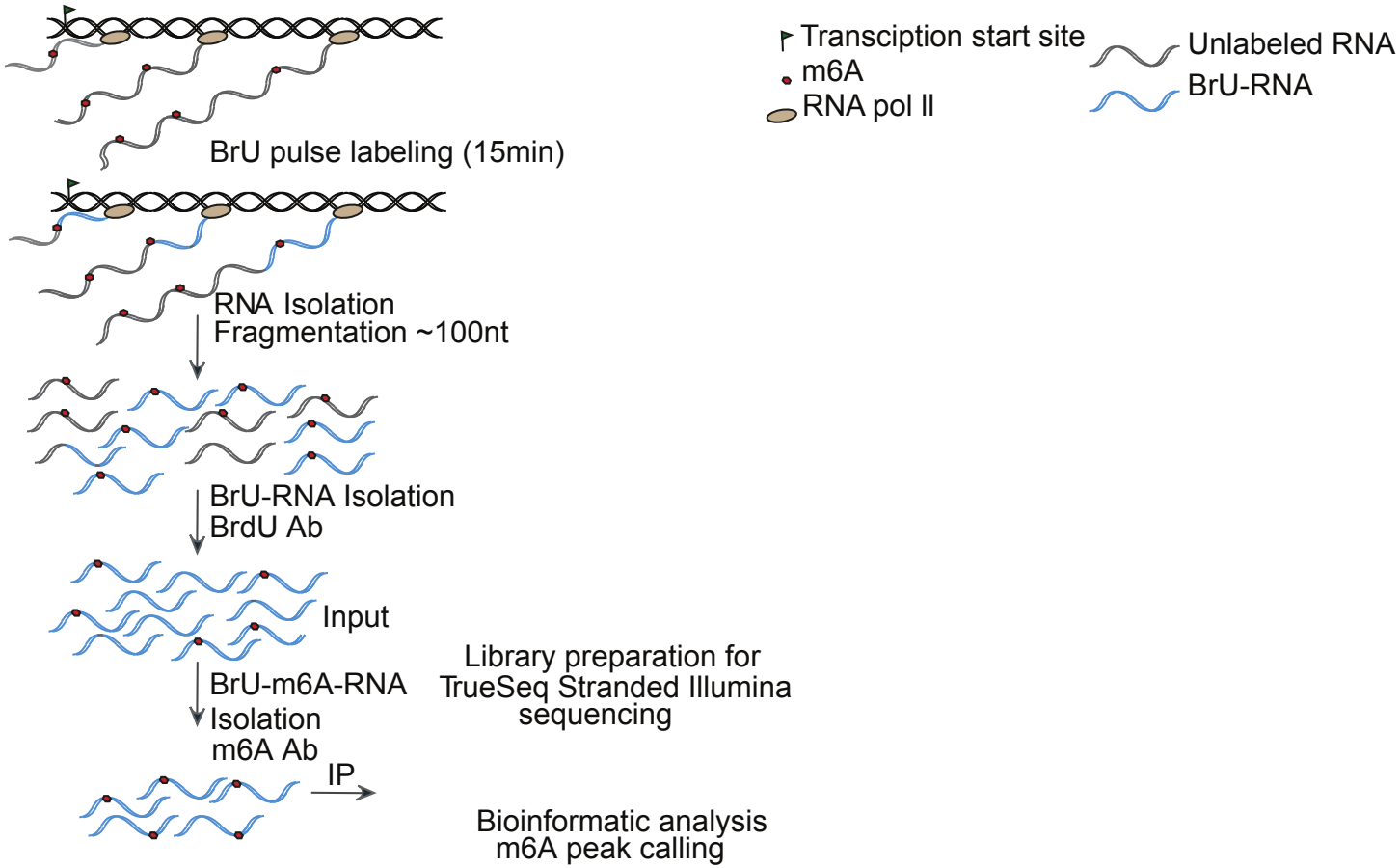
Long-read sequencing of nascent RNA (combine long read-sequencing with ~chromatin fractionation and ~metabolic labeling)



Revealing nascent RNA processing dynamics with nano-COP
Drexler et al. Nature Protocols 16, 1343–1375 (2021)

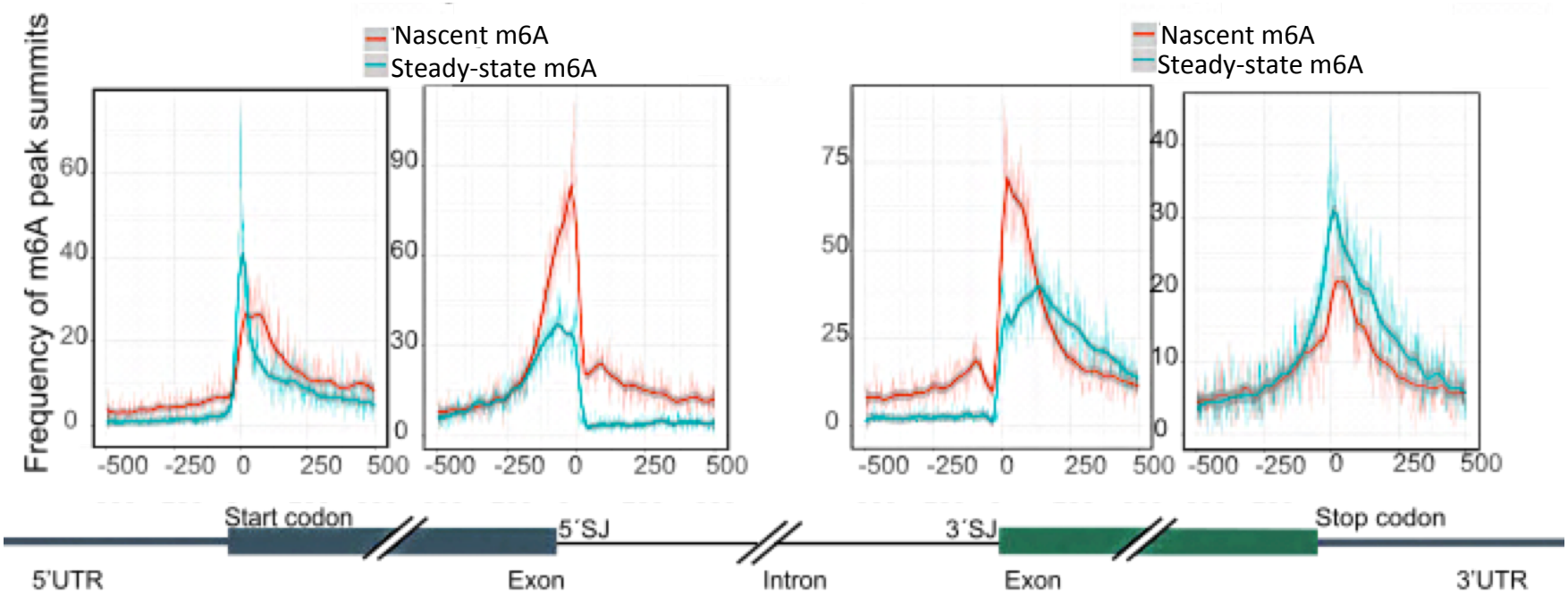
Functions of co-transcriptional RNA modifications

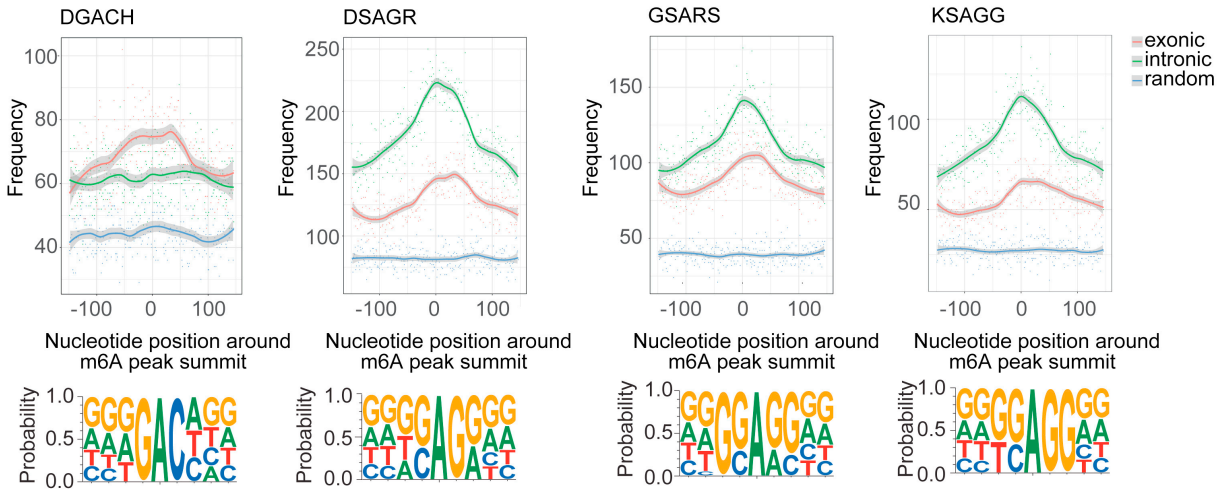
Capturing **nascent** N6-methyladenosine (m6A) RNA modifications:



Louloupi*, Ntini* et al., Cell Rep 2018

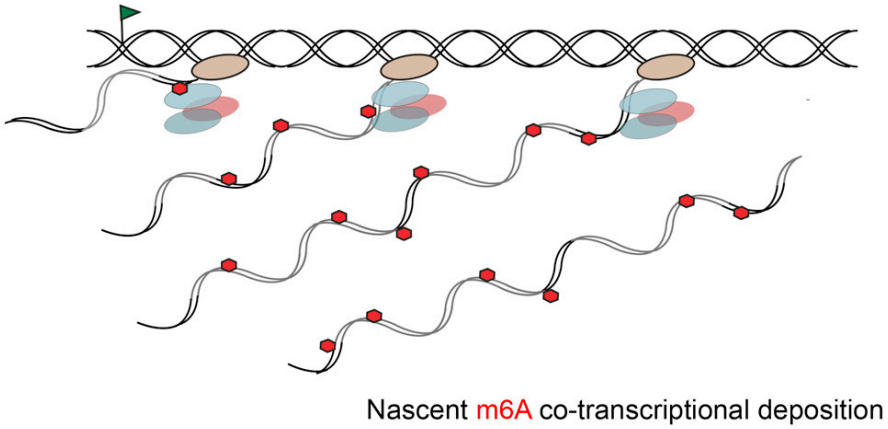
Nascent m6A affects co-transcriptional RNA processing



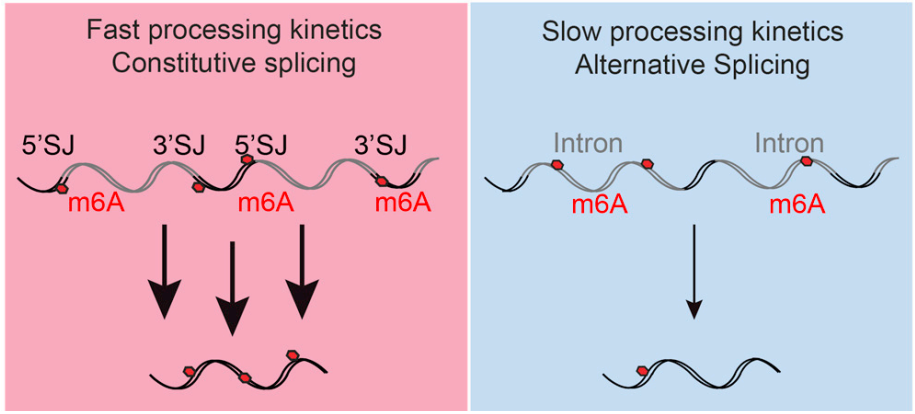


Louloupi*, Ntini* et al., Cell Rep 2018

Nascent RNA m6A modification affects co-transcriptional RNA processing

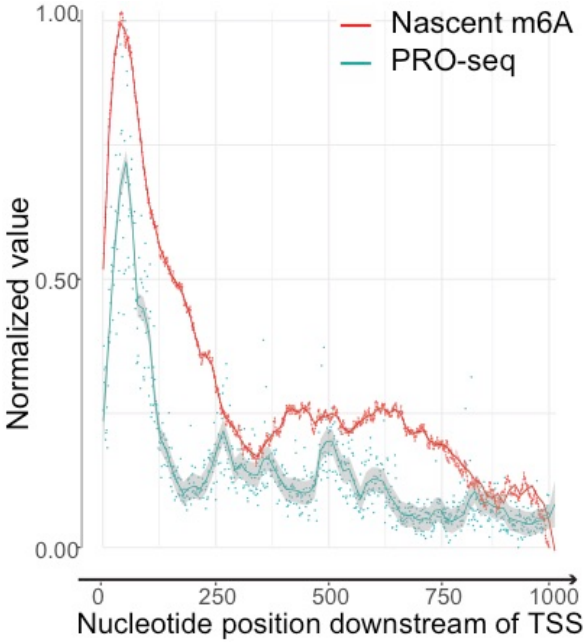


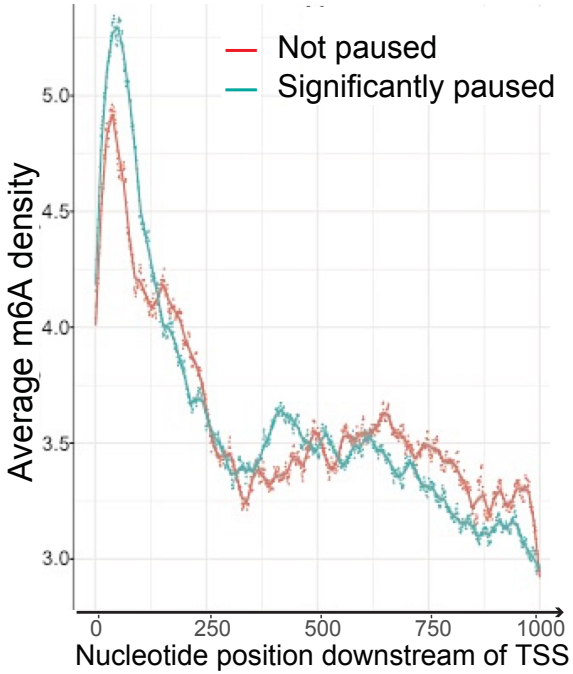
Model:

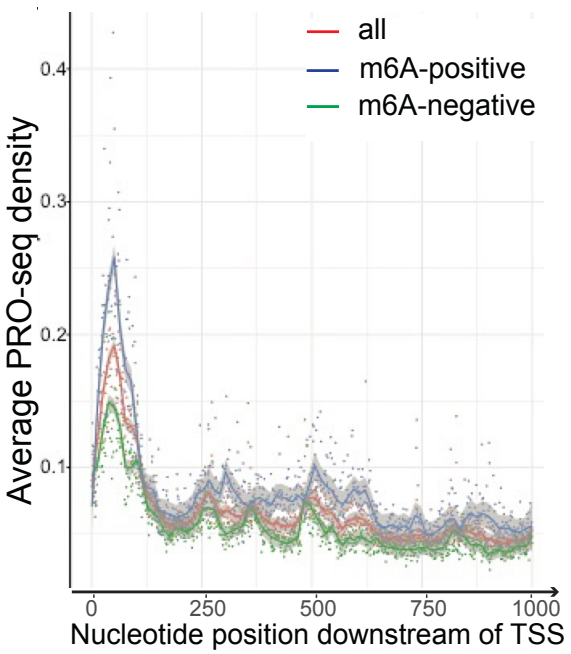


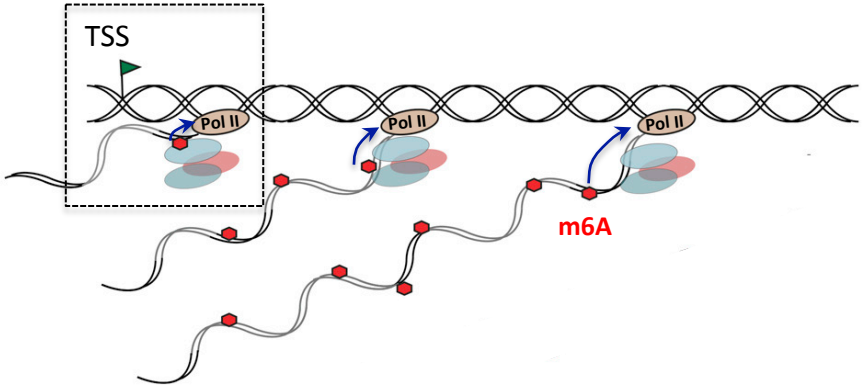
Louloupi*, Ntini* et al., Cell Rep 2018

Nascent RNA modifications in crosstalk with transcription



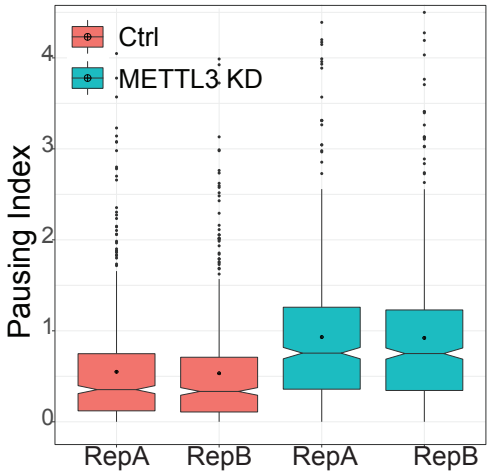


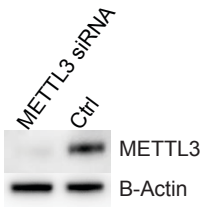


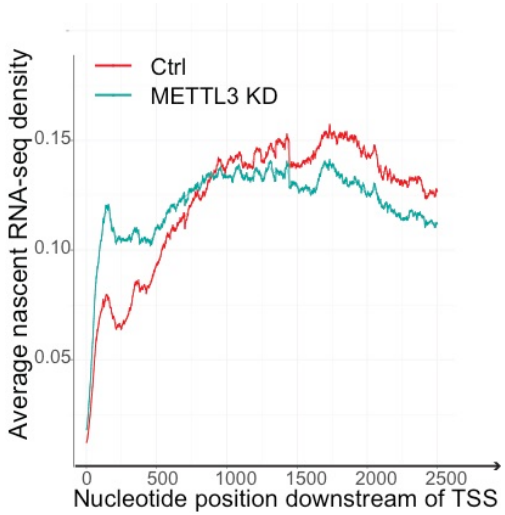


Nascent m6A in crosstalk with transcription

Pausing Index: Read density [0_+300]/[+300_+3000]







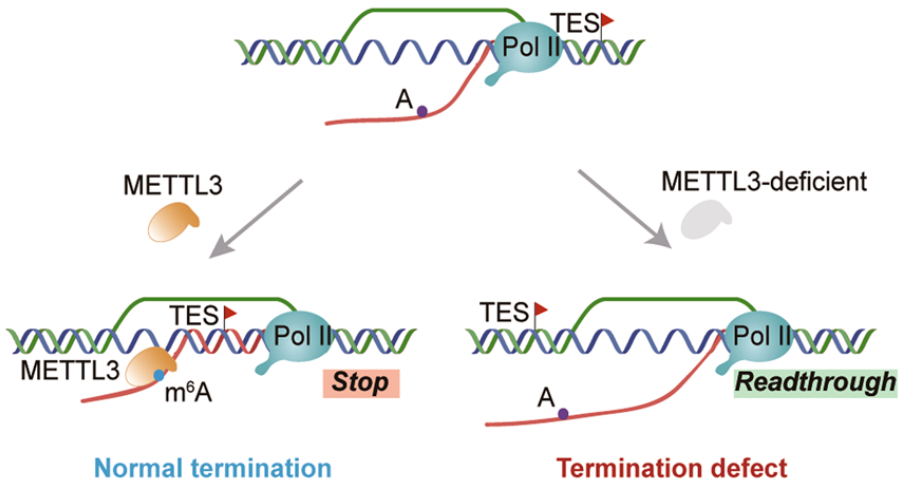
Nascent m6A in crosstalk with transcription

LETTER TO THE EDITOR

m^6A promotes R-loop formation to facilitate transcription termination

Cell Research (2019) 0:1–4; https://doi.org/10.1038/s41422-019-0235-7

Yang et al. Cell Res 2019



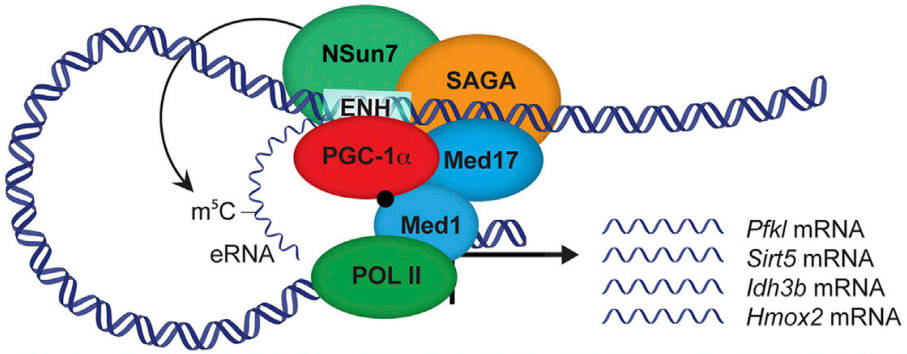
Nascent RNA modifications on non-coding RNA transcripts

Article

Cell Reports

Deposition of 5-Methylcytosine on Enhancer RNAs Enables the Coactivator Function of PGC-1α

Aguilo et al., Cell Rep 2016

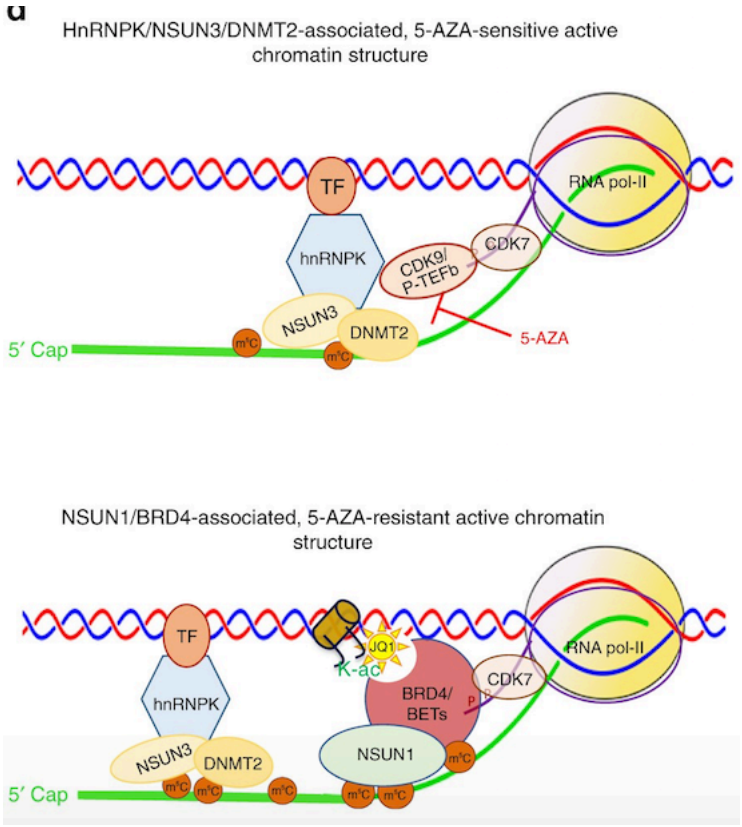


- METTL3-dependent RNA m6A dysregulation contributes to neurodegeneration in Alzheimer's disease through aberrant cell cycle events
  Zhao et al., Molecular Neurodegeneration 16: 70 (2021)

- m6A mRNA methylation-directed myeloid cell activation controls progression of NAFLD and obesity (in mice)
  Kin et al., Cell Rep 2021, 37:6 doi.org/10.1016/j.celrep.2021.109968

Review: Epitranscriptomics in liver disease: Basic concepts and therapeutic potential
Zhao et al., Journal of Hepatology 2020

Cheng, J. X. et al. RNA cytosine methylation and methyltransferases mediate chromatin organization and 5-azacytidine response and resistance in leukaemia. Nat. Commun. 9, 1163 (2018).



Yankova et al., Nature 2021 Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia, Nature 2021

https://www.stormtherapeutics.com