Temporal and spatial profiling of ALKBH5 activity through NAIL-MS and compartmentalized RNA Isolation

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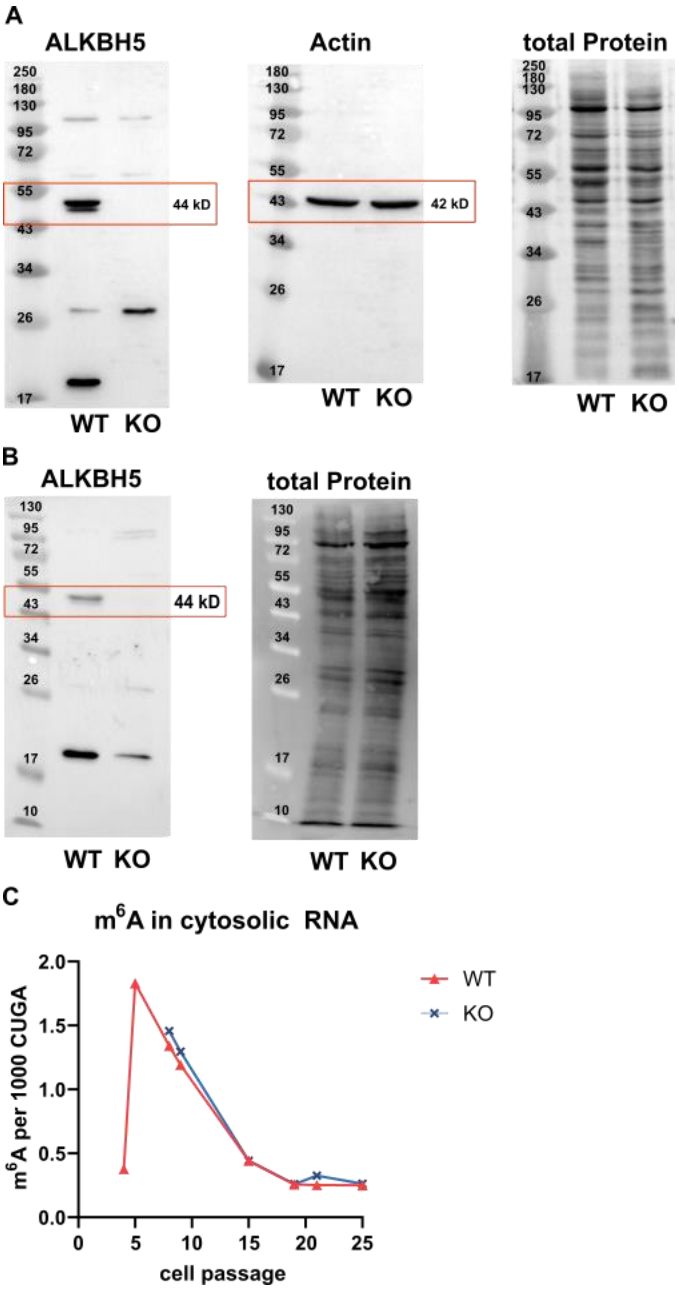
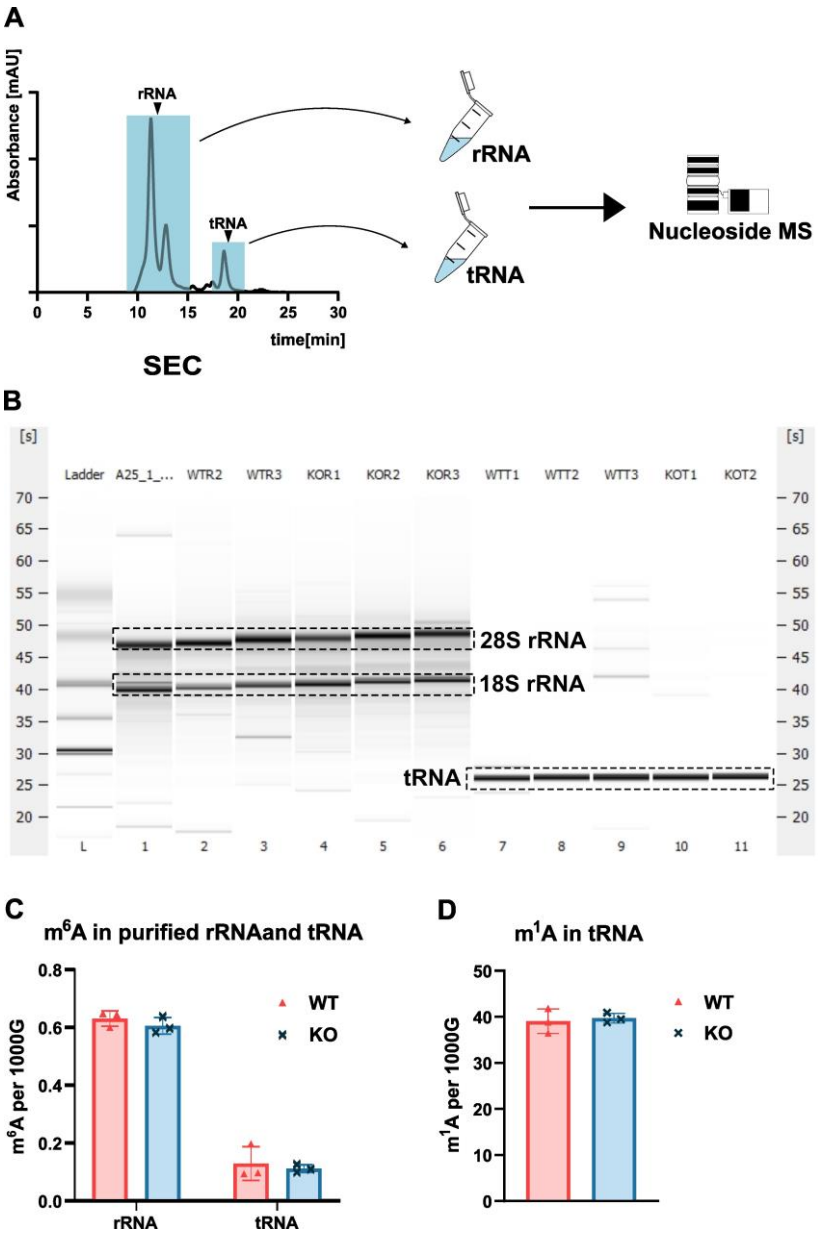
Supplemental Material

compound	labelling	mass [g/mol]	m/z
originals	/	281	282
hybrids	D_3	284	285
news	$^{15}N_5D_3$	289	290

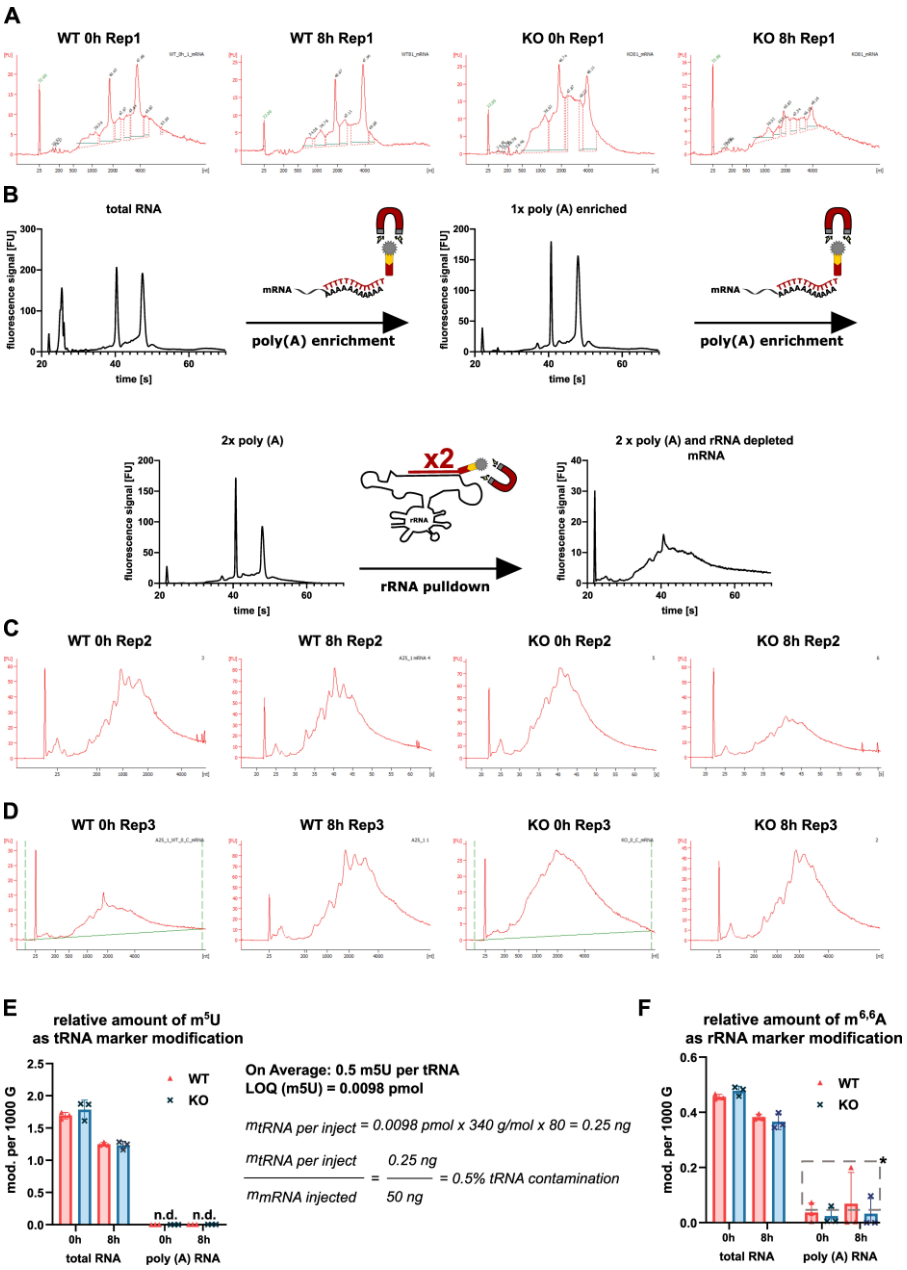
Supplemental Table_S1: list of m^6A isotopologues with mass und measured m/z



Supplemental Fig. S1: A: Western Blot with 20 µg total Protein of HEK 293T and ALKBH5 KO cells at passage 4. ALKBH5s absence was confirmed with an ALKBH5 primary Antibody, Actin was used as loading control. Afterwards total Protein was stained with No-Stain Protein Labelling Reagent. **B:** Western Blot at passage 23. ALKBH5s absence was confirmed with an ALKBH5 primary Antibody, afterwards total Protein was stained with No-Stain Protein Labelling Reagent. **C:** LC-MS results of m^6A quantification during passaging of HEK 293T and ALKBH5 deficient cells. m^6A concentrations in all datasets were normalized to 1000 Guanosines. Data was obtained from n=1 biological replicates.

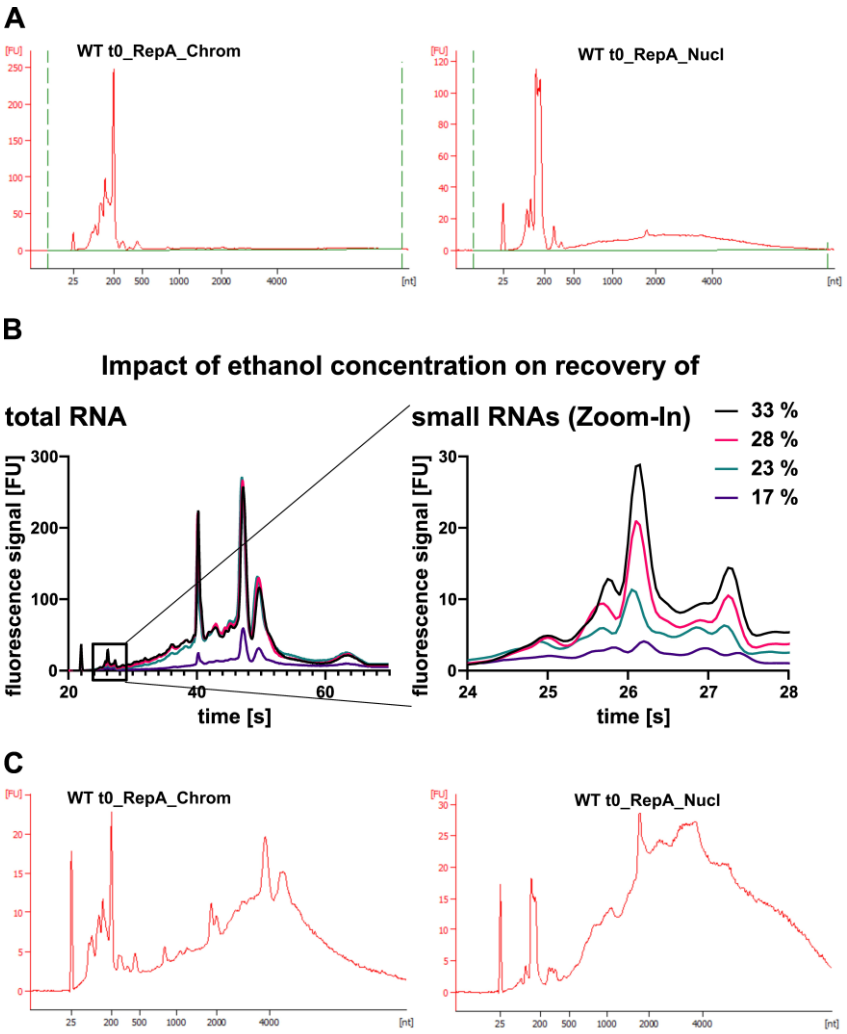


Supplemental Fig. S2: A: Workflow of rRNA and tRNA isolation from total RNA. Based on length (=elution time) RNAs were separated by size exclusion chromatography and afterwards digested and analysed by LC-MS. **B:** Bioanalyzer gel results showing purity of rRNA and tRNA samples. **C:** LC-MS results of m6A in rRNA and tRNA harvested and enriched from HEK 293T WT and ALKBH5 KO cells. **D:** LC-MS results of m^1A in tRNA from both cell types showing potential for dimroth re-arrangement which would explain m6A findings in tRNA fraction.



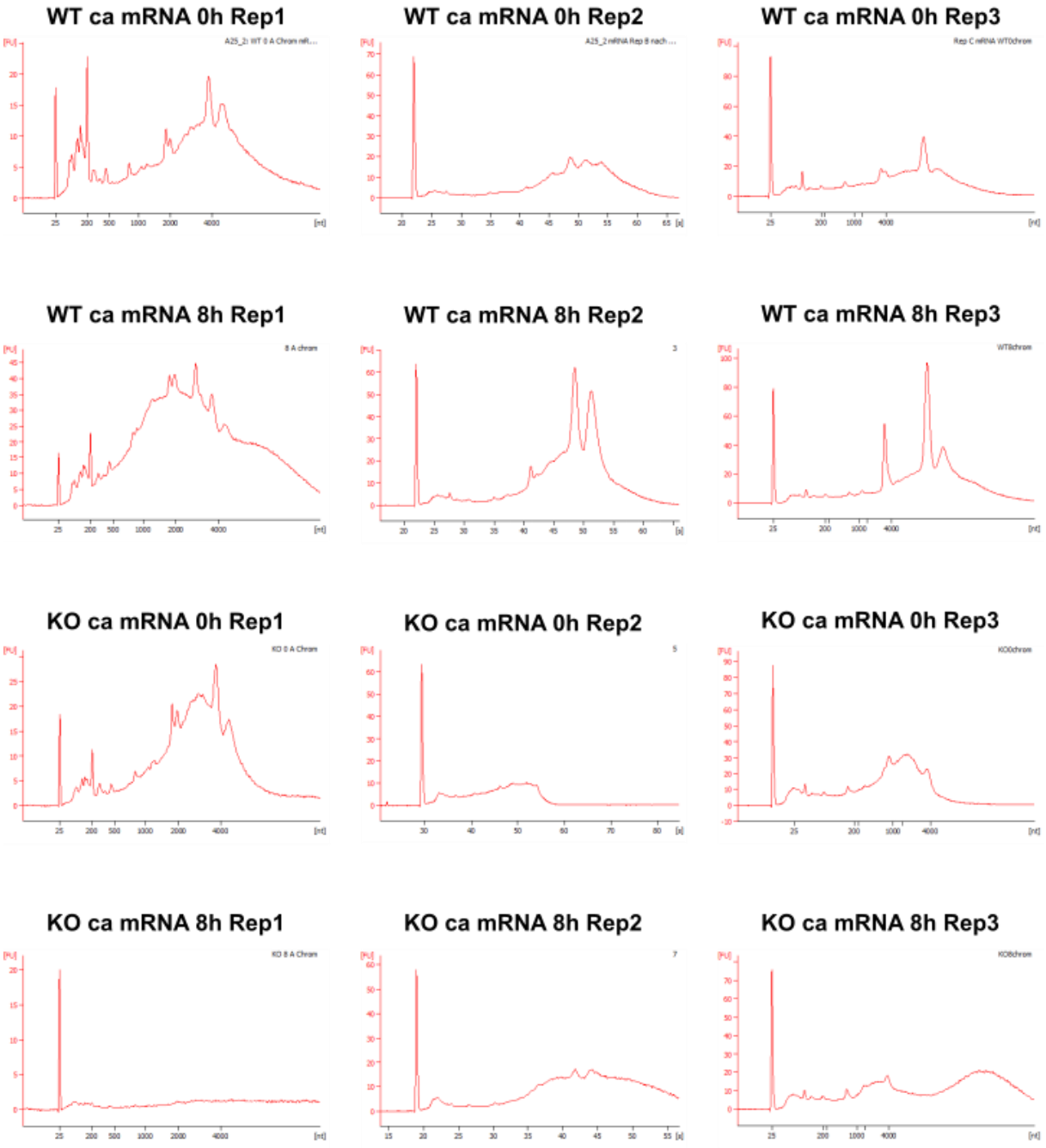
Supplemental Fig. S3: A: BioAnalyzer electropherograms indicating impurities with rRNA remnants after enriching mRNA by one step of poly (A) enrichment and rRNA depletion with standard DNA probe amount. **B:** experimental setup of improved mRNA enrichment protocol. **C and D:** electropherograms showing absence of contaminating RNA species for Replicates 2 and 3 of HEK 293T WT **(C)** and ALKBH5 KO **(D)** cells. **E:** Quantification by LC-MS of m^5U as tRNA marker modification from total RNA and poly (A) RNA obtained from WT and KO cells. n.d. = m^5U below 0.0098 pmol per inject, respectively 0.5% contaminating tRNA per inject **F:** Quantification by LC-MS of $m^{6,6}A$ as rRNA marker modification from total RNA and poly (A) RNA obtained from WT and KO cells. ***** marked

replicates were excluded from m6A analysis as rRNA contamination is still high in poly (A) enriched samples.

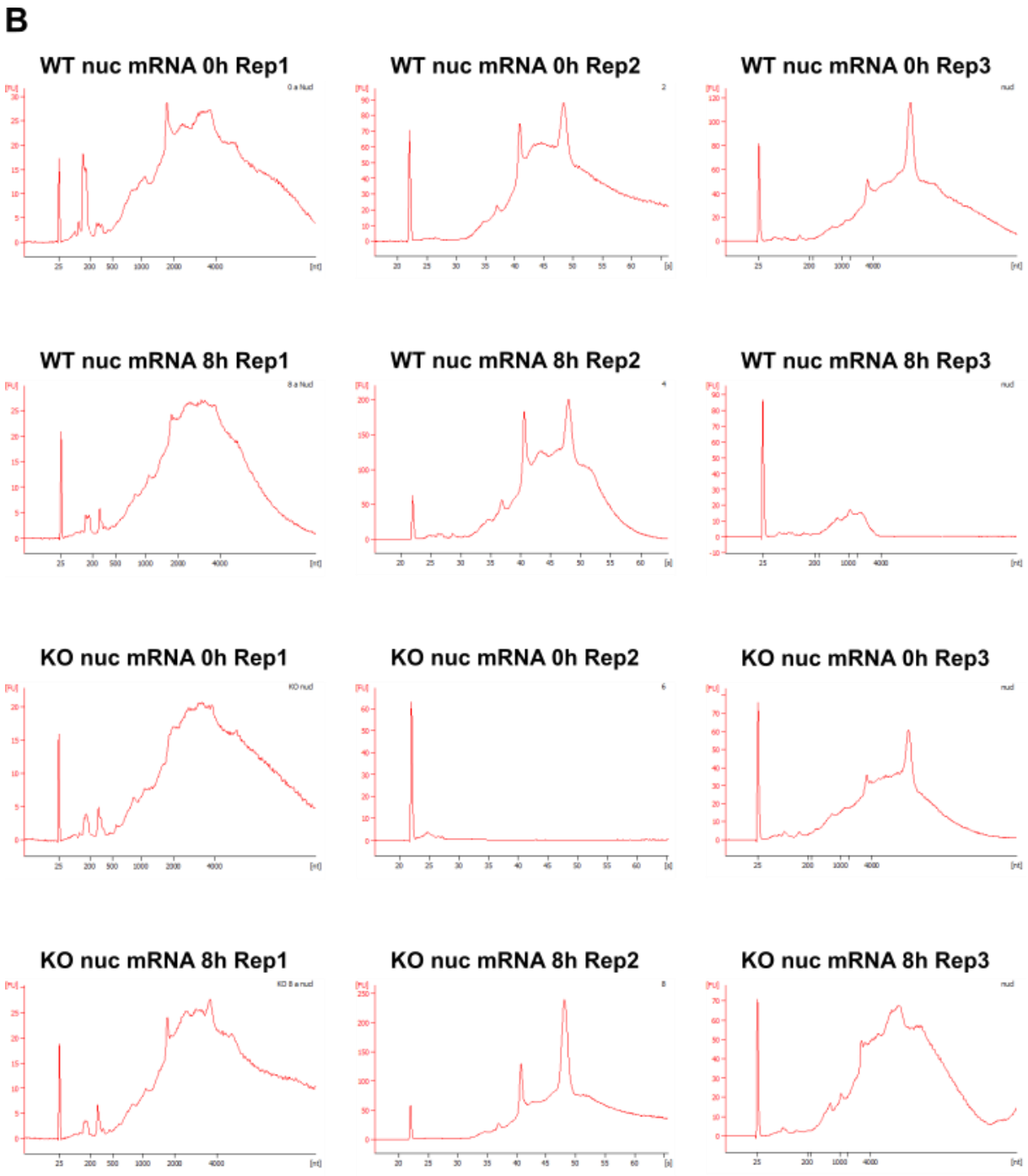


Supplemental Fig. S4: A: BioAnalyzer electropherogram showing mRNA purity after one step of small RNA and rRNA depletion. Chromatin-associated enriched "m"RNA is shown on the left, while on the right nucRNA is shown. Large amount of small RNA are still in the samples. **B:** Impact of ethanol concentration during small RNA enrichment. The lower the EtOH concentration, the lower the recoverd amount of total RNA. Small RNAs were acceptable removed at 23% Ethanol. **C:** Samples from Figure S4A after a second step of small RNA depletion. Typical mRNA peak from 1000-4000 nts is visible, small RNAs are much more depleted.

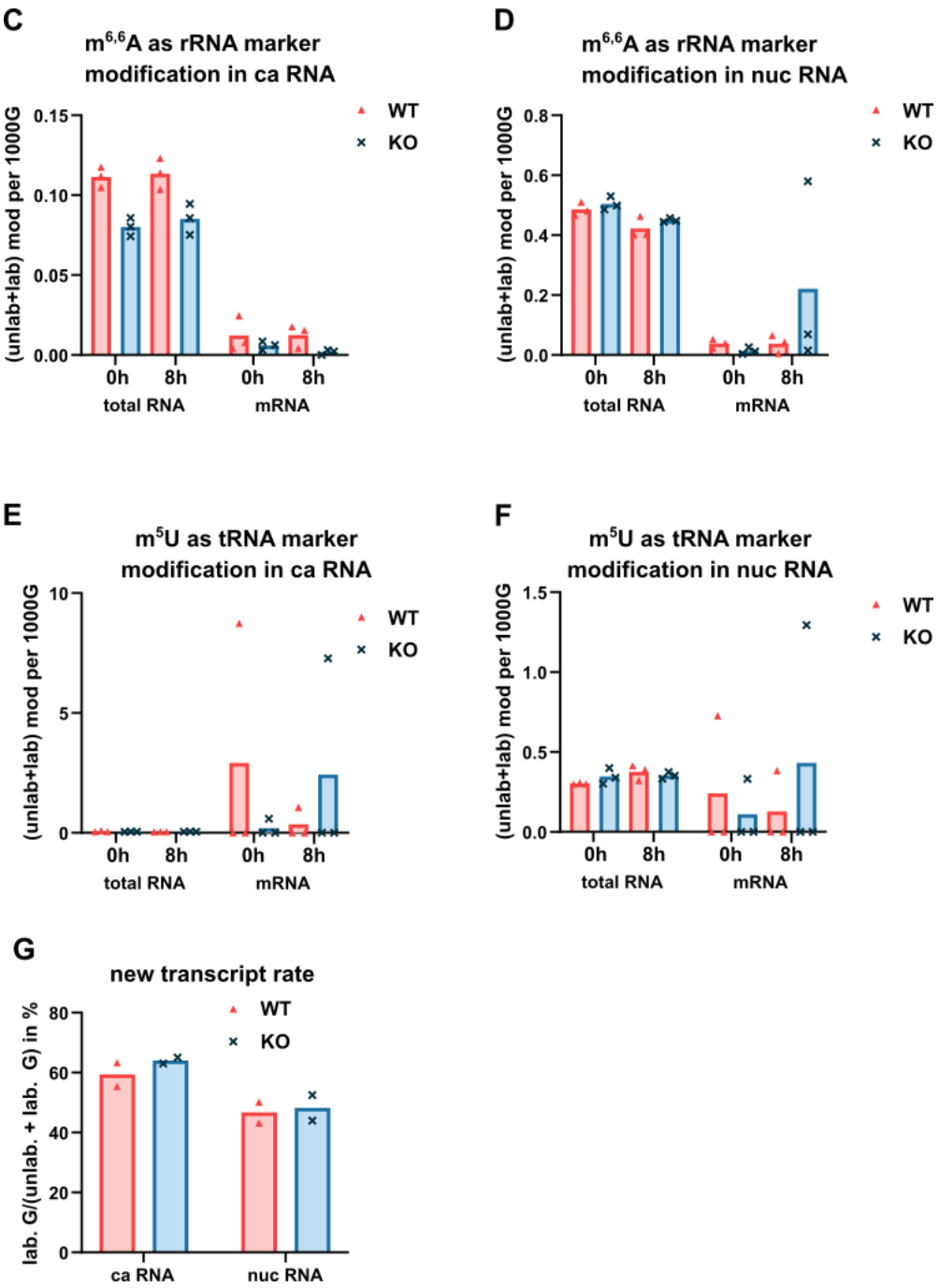
A



Supplemental Fig. S5A: electropherograms showing absence of contaminating tRNA and rRNA species for enriched mRNAs from chromatin-associated RNA of HEK 293T WT and ALKBH5 KO



Supplemental Fig. S5B: electropherograms showing absence of contaminating tRNA and rRNA species for enriched mRNAs from nucleoplasmic RNA of HEK 293T WT and ALKBH5 KO



Supplemental Fig. S5 C and D: Quantification by LC-MS of $m^{6,6}A$ as rRNA marker modification from ca **(C)** and nuc **(D)** RNA and enriched mRNA obtained from WT and KO cells. **E and F:** Quantification by LC-MS of m^5U as tRNA marker modification from ca **(E)** and nuc **(F)** RNA and enriched mRNA obtained from WT and KO cells. **G:** new transcript rate in chromatin-associated and nuclear mRNA. Calculated by dividing the amount of labelled G by the sum of labelled and unlabelled G. All datasets were obtained from HEK 293T cells (red) and ALKBH5 deficient mutants (blue). Except of the transcript rate dataset, all data was collected from n=3 biological replicates.