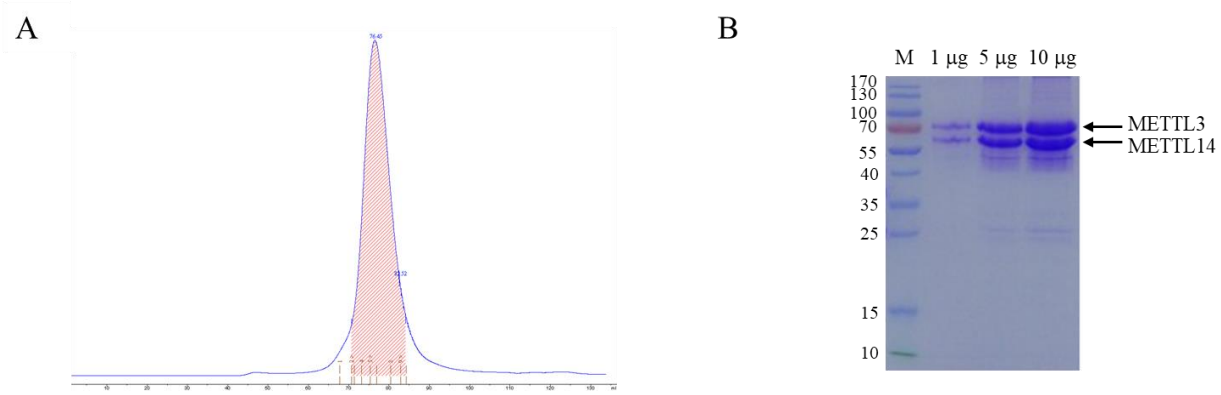
Supplemental Material

A Mass Spectrometric Assay of METTL3/METTL14 Methyltransferase Activity

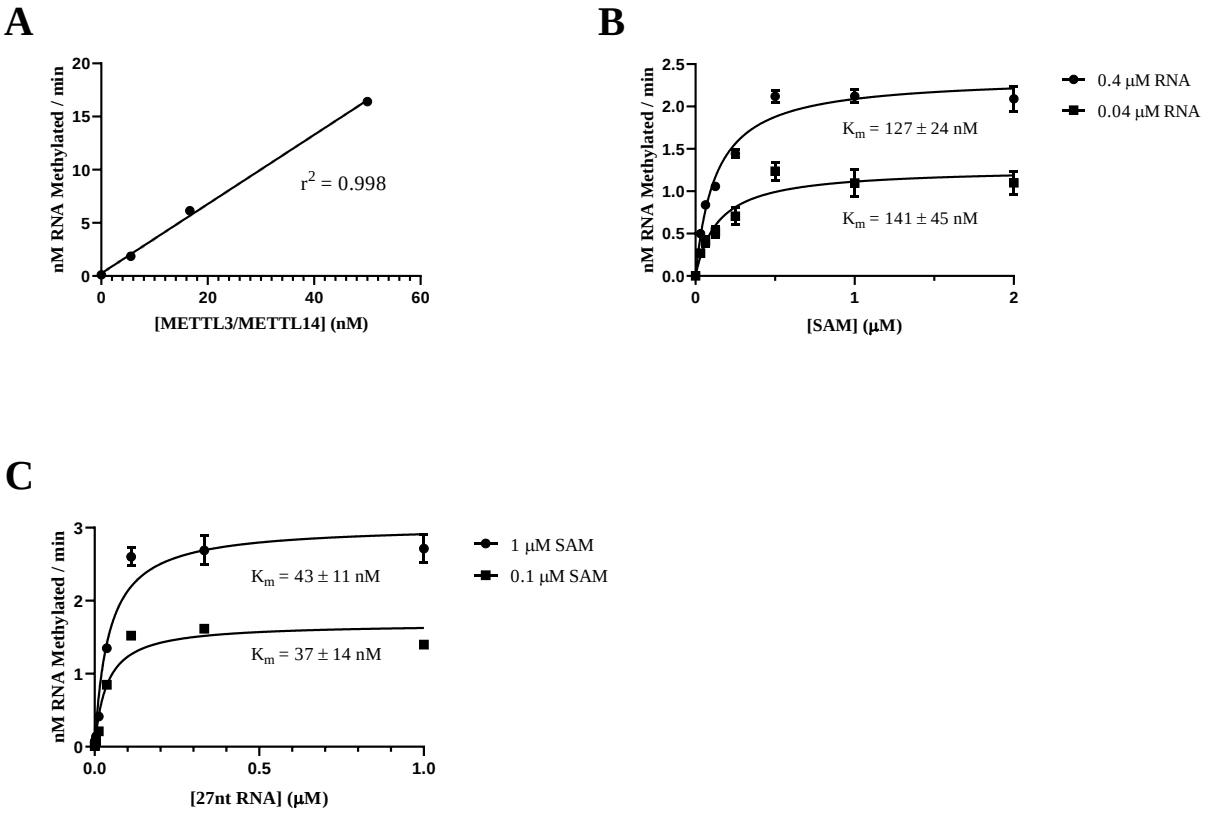
Shane M. Buker[1], Zachary A. Gurard-Levin[2], Benjamin D. Wheeler[3,4], Michael D. Scholle[2], April W. Case[1], Jeffrey L. Hirsch[3], Scott Ribich[1], Robert A. Copeland[1], P. Ann Boriack-Sjodin[1*]

[1]Accent Therapeutics, Cambridge, MA
[2]SAMDI Tech, Inc, Chicago, IL
[3]Confluence Discovery Technologies, St. Louis, MO
[4]Current address: University of California, San Francisco, Biomedical Science Program

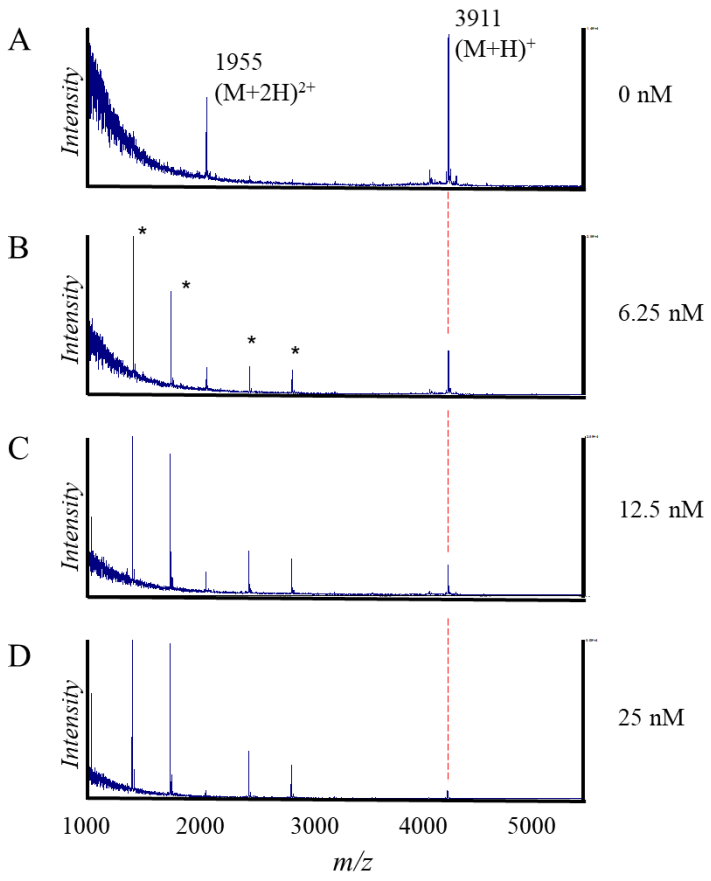
[*]Correspondence: P. Ann Boriack-Sjodin, Accent Therapeutics, 65 Hayden Ave, Lexington, MA 02421 E-mail aboriacksjodin@accenttx.com



Supplementary Figure S1: Characterization of the METTL3/METTL14 protein complex used in assay development. **(A)** METTL3/METTL14 complex elutes from a gel filtration column as a single peak. The final protein solution was generated by combining fractions indicated in red. **(B)** SDS-page gel of the final METTL3/METTL14 complex. Three protein amounts (1 μg, 5 μg and 10 μg) are shown. PageRuler Prestained Protein Ladder molecular weights (Thermo Scientific (#00677289); kD) and bands corresponding to METTL3 and METTL14 are labeled.



Supplementary Figure S2: Characterization of the METTL3/METTL14 radiometric assay using the 27-nucleotide substrate. Velocity was linear over a range of enzyme concentrations **(A)**. K_M values were determined for SAM **(B)** and the 27-nucleotide RNA **(C)** using conditions described in the Materials and Methods section; representative plots are shown. Data was generated in triplicate.



Supplementary Figure S3: RNA degradation is seen with increasing concentrations of METTL3/METTL14 protein in the absence of RNaseOUT. **(A)** Peaks from intact substrate (0 nM METTL3/METTL14) are shown as labeled. **(B)** Upon addition of METTL3/METTL14 (6.25 nM), degradation peaks (indicated with *) are seen. Degradation peaks increase in size with increasing protein concentrations as seen with 12.5 nM METTL3/14 **(C)** and 25 nM METTL3/14 **(D)**. Due to different ionization efficiencies of the degradation products compared to the 11-mer RNA, peak heights and AUC may not directly correlate to the extent of degradation.