Supplementary information

Enzymatic characterization of PCIF1, the mRNA cap adenosine-N6 methyltransferase, acting on uncapped RNAs

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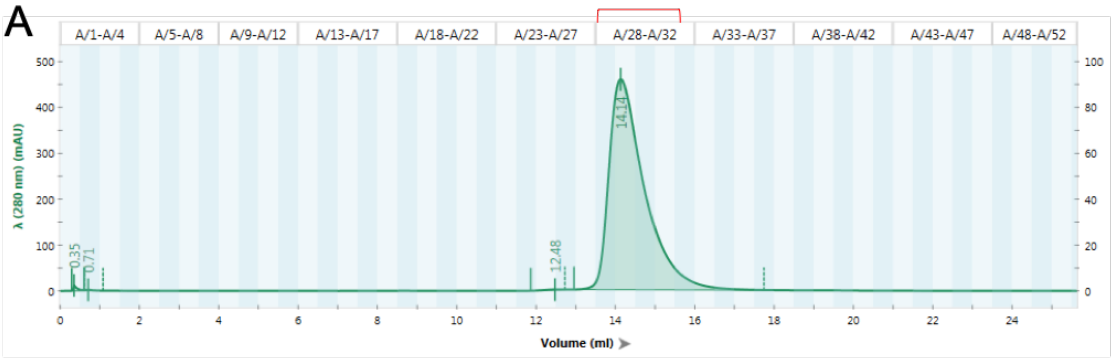
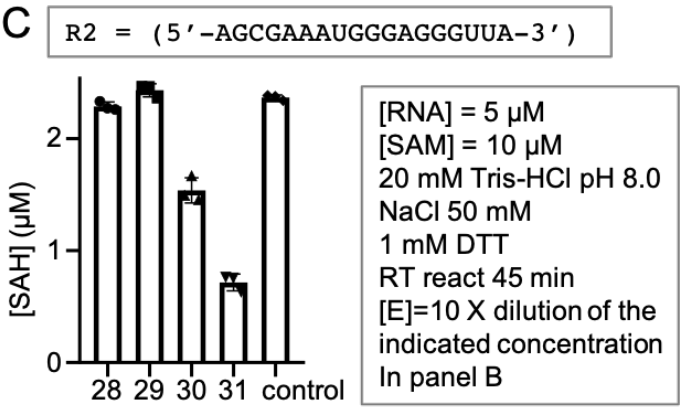


B

Fraction	[E]		[SAH]/[E] (h^{-1})
	mg/ml	(μM)	
28	0.46	5.7	5.6
29	0.51	6.3	5.3
30	0.28	3.4	5.7
31	0.12	1.5	6.3
control		5.0	6.2



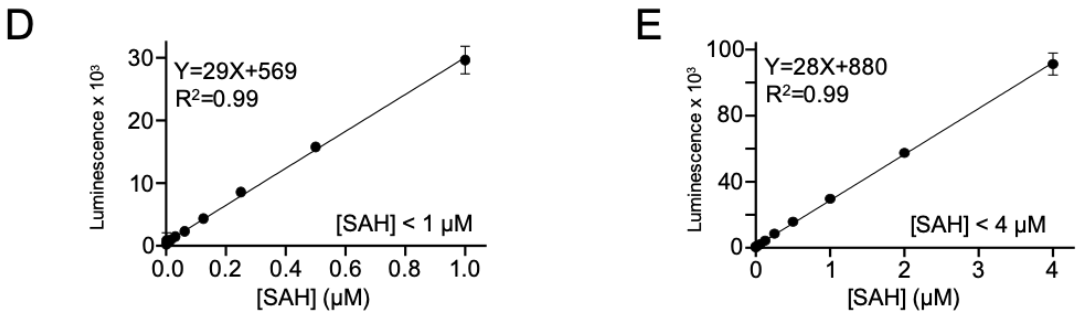
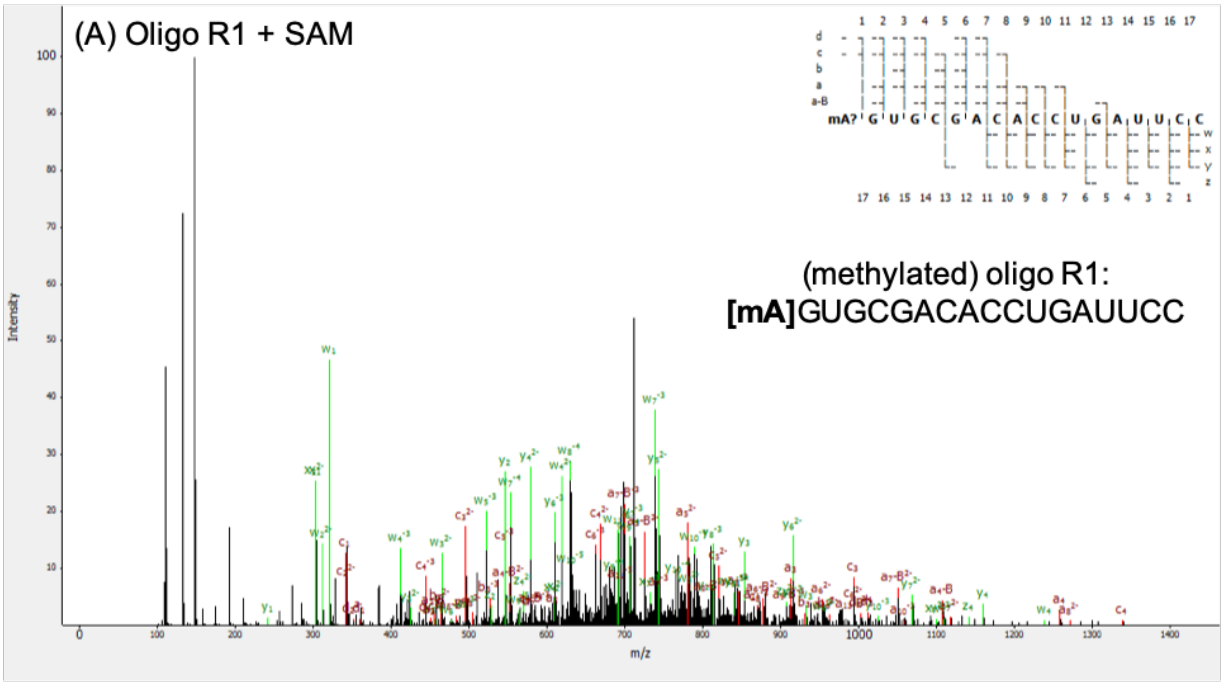


Figure S1. **PCIF1 activity assay**. (**A**) An example of analytic size exclusion chromatography (Superdex 200 Increase 10/300 GL) showing the peak fractions and retention volume. (**B**) Summary of four fractions and the corresponding enzyme concentrations and specific activity on RNA oligo R2, measured by the velocity of product SAH formation per enzyme molecule [SAH]/[E]. The control enzyme was taken from a previous purification. (**C**) The specific activity of each fraction. (D-E) Calibration curves for Promega bioluminescence assay as a function of SAH concentrations in the range of (**B**) below or (**E**) above 1 μM.



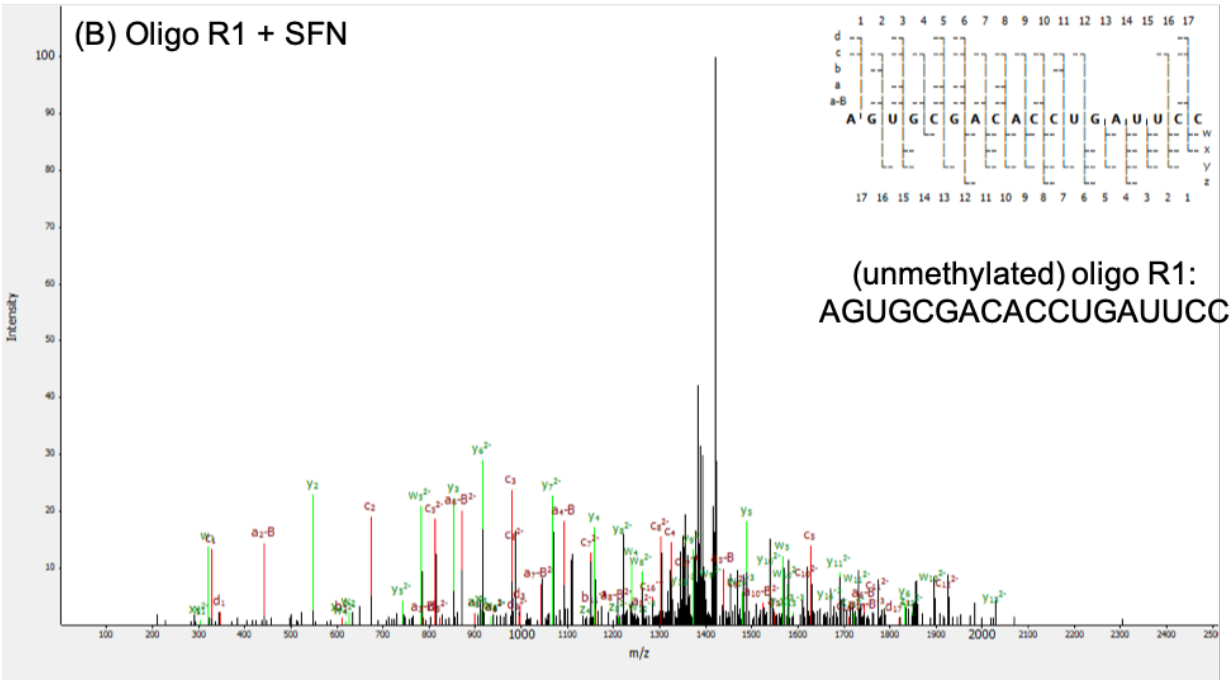


Figure S2. Representative tandem mass spectra of oligo R1 in the presence of (**A**) SAM or (**B**) sinfungin.

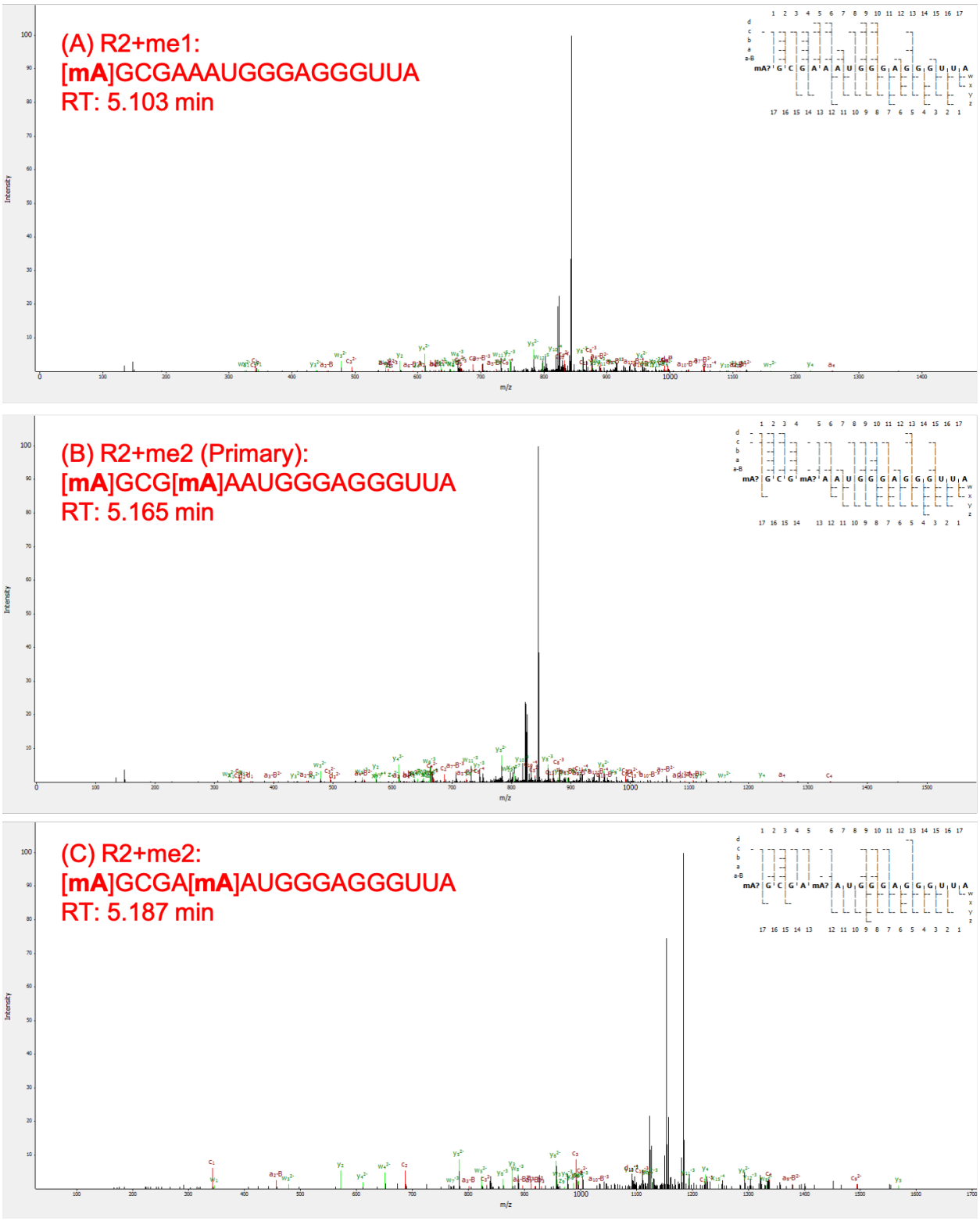


Figure S3. Representative tandem mass spectra of intact methylated R2 oligos (A-C).

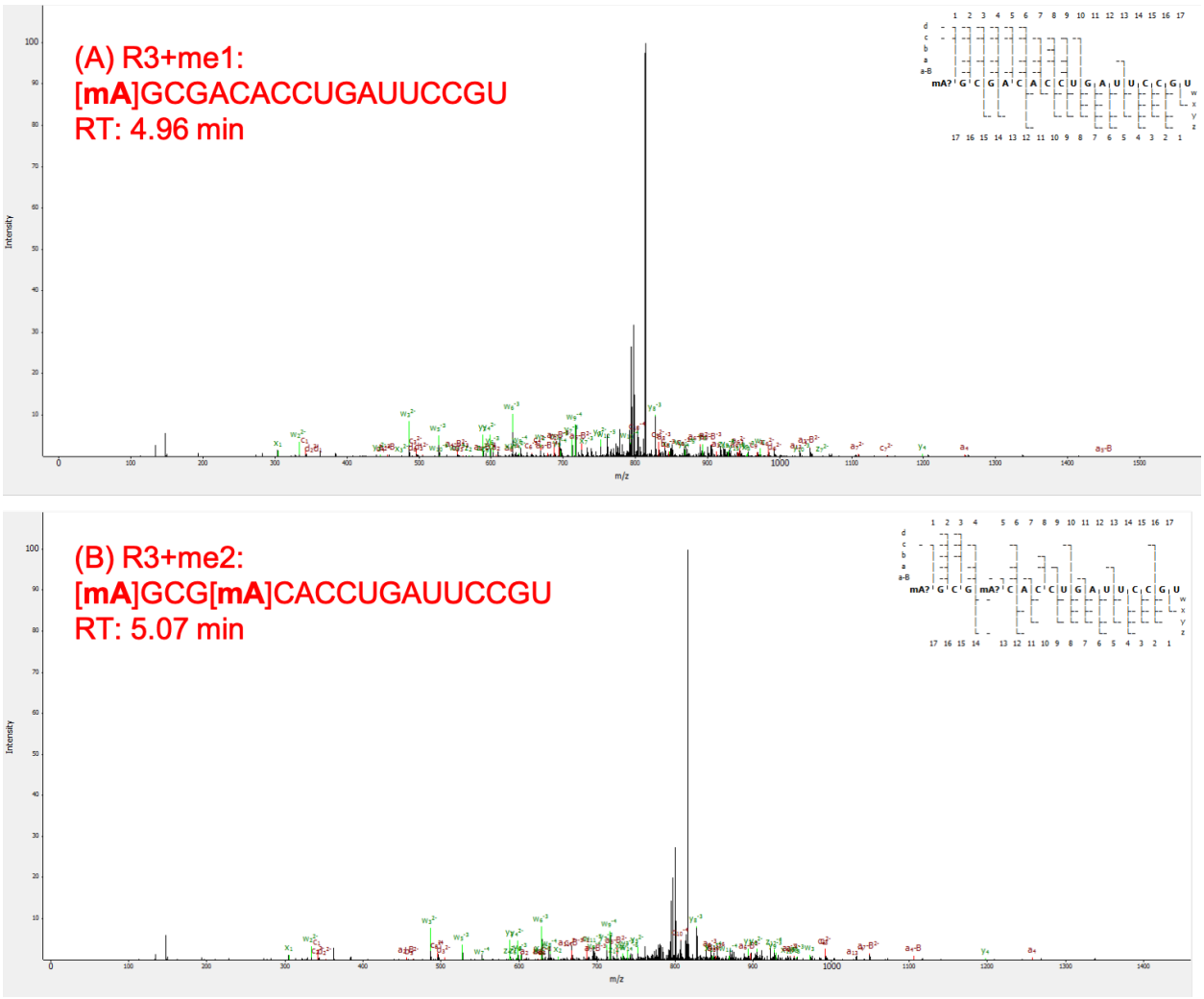


Figure S4. Representative tandem mass spectra of intact methylated R3 oligos (A-C).