Extended data

A cryptic pocket in METTL3-METTL14 regulates m^{6}A conversion and sensing

Shan Qi[1,2], Yogesh K. Gupta[1, 2*]

[1] Greehey Children's Cancer Research Institute, University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA

[2] Department of Biochemistry and Structural Biology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA

[*]Corresponding author:

Y.K.G. email: guptay@uthscsa.edu

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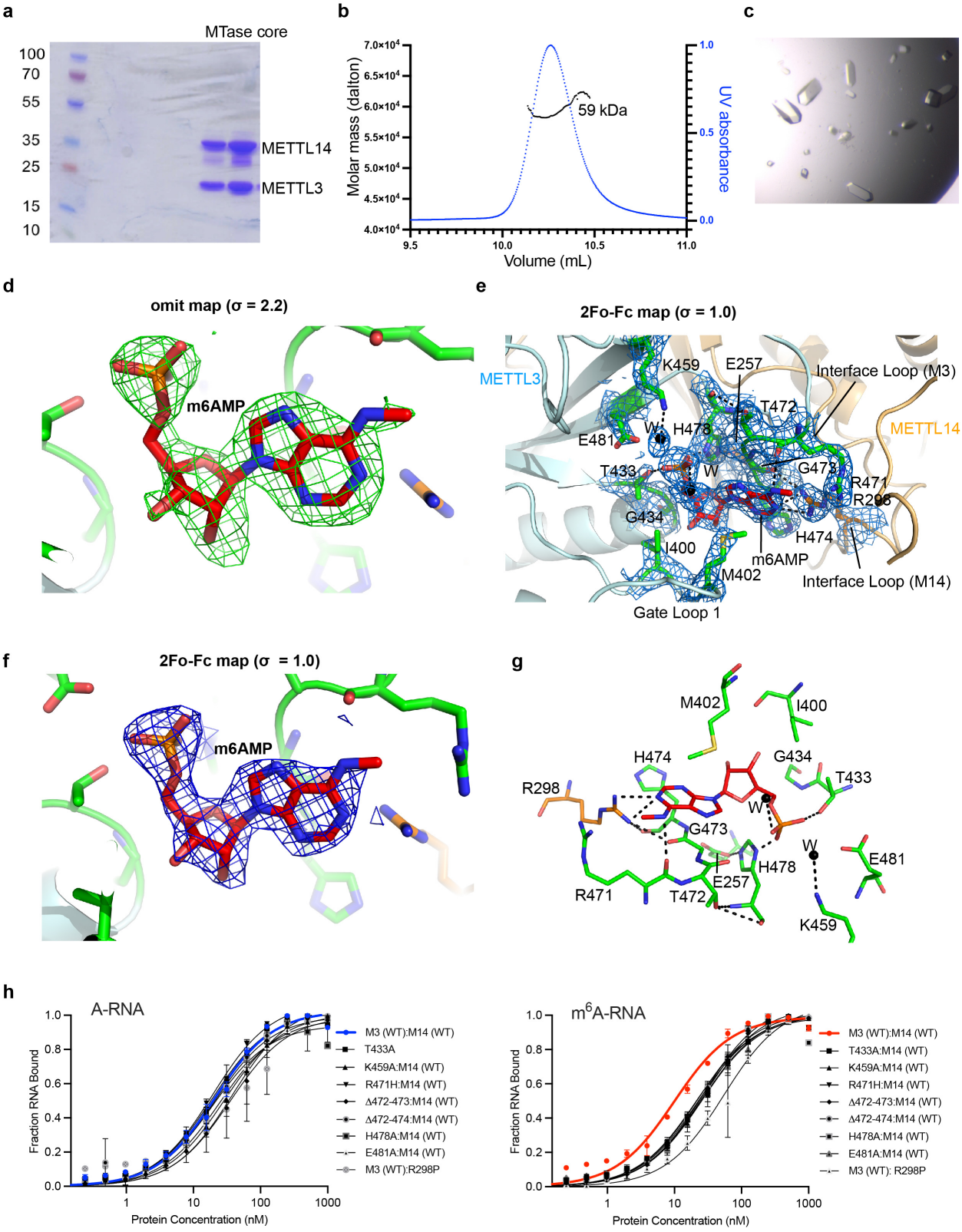
Table 1. Data collection and refinement statistics (molecular replacement)

	METTL3-METTL14-m6A (PDB: 8TDS)
Data collection	
Wavelength (Å)	1.033
Space Group	$P4_12_12$
Cell dimensions	
a=b, c (Å)	95.98, 121.61
α=β=γ (°)	90
Resolution (Å)	2.5
R-merge (%)	18.8 (156)*
I/σI	10.6 (1.76)
Completeness (%)	100 (100)
Redundancy	9.7 (10.3)
CC1/2	0.99 (0.50)*
Wilson B-factor	47
Refinement	
Resolution (Å)	37.34 – 2.5
Number of reflections	20306 (2238)
R_{work} (%) / R_{free} (%)	23 / 26.2
Nonhydrogen atoms	
Protein	3986
Ligands	32
Water	90
Average B-factors (Å^2)	
Protein	53.2
Ligands (m^6AMP)	70.9
Water	47.3
Root mean square deviations	
Bond lengths (Å)	0.016
Bond angles (°)	1.4
Ramachandran favored (%)	94.6
Ramachandran allowed (%)	4
Ramachandran outliers (%)	1.4

*Values for outermost shell are given in parentheses.

Extended data Figure 1



Extended data Figure 1 | a, Coomassie-stained SDS-PAGE showing high purity of METTL3-METTL14 MTase core. **b,** Size-exclusion chromatography coupled with multi-angle scattering estimated a molecular mass of this complex of ~59 kDa, in excellent agreement with theoretical mass (~ 60 kDa). **c,** crystals of METTL3-METTL14 MTase core used for soaking N^6-methyladenosine monophosphate (m^6AMP or m^6A). **d,** A green mesh showing an unbiased electron density omit map countered at 2.2σ, confirming the presence of m^6A. **e-f,** A blue mesh representing a 2Fo-Fc map (σ = 1.0) showing the final refinement of the m^6A-METTL3-METTL14 structure. The map confirms the excellent agreement between calculated and observed electron density for the region surrounding m^6A and for m^6A itself (**f**). **g,** Network of interaction between m^6A and residues from METTL3 (green) and METTL14 (orange). **g,** Quantitative measurement of 30-mer RNA (left panel, A-RNA; right panel, m^6A-RNA) binding (n = 3) to the WT and mutant enzymes shown as binding isotherms fitted with a one-site specific binding model, (Y=Bmax*X/(K_d + X). The equilibrium dissociation constant (K_d) was derived from three independent experiments, with error bars indicating the range of data points (n = 3). m^6A replaces the central adenine base within the GGACU motif in A-RNA in m^6A-RNA. See the methods section and source data for details.