

Introduction :

N⁶-methyladenosine (m⁶A) is the most prevalent modification in messenger RNA (mRNA) of higher eukaryotes, and it plays an important role in the regulation of biological processes. Transcriptome-wide mapping has revealed that m⁶A is favorably enriched in 3'UTR(3' untranslated Region) and stop codon of mRNA in mammalian systems. The METTL3/METTL14/WTAP complex has been identified as the core component of human methyltransferase complex. But the whole methyltransferase complex has yet to be fully identified and the mechanism of the enrichment of m⁶A in 3'UTR of mammalian mRNA remains unknown. Here we report new components including VIRILIZER, ZC3H13, HAKAI, and HNRNPH as candidates of the methyltransferase complex and among them, VIRILIZER can regulate m⁶A modification specificity in Hela cells.

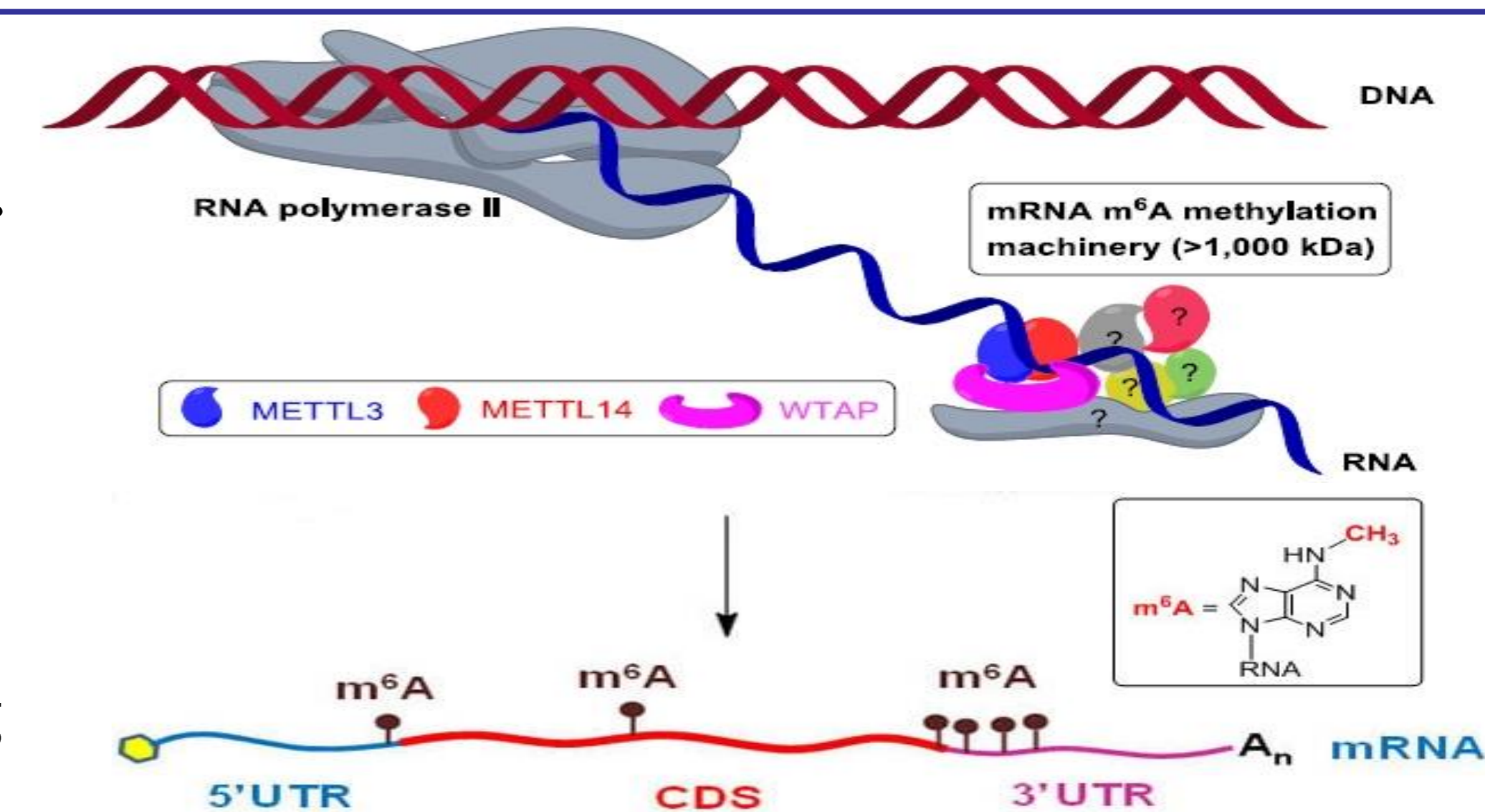
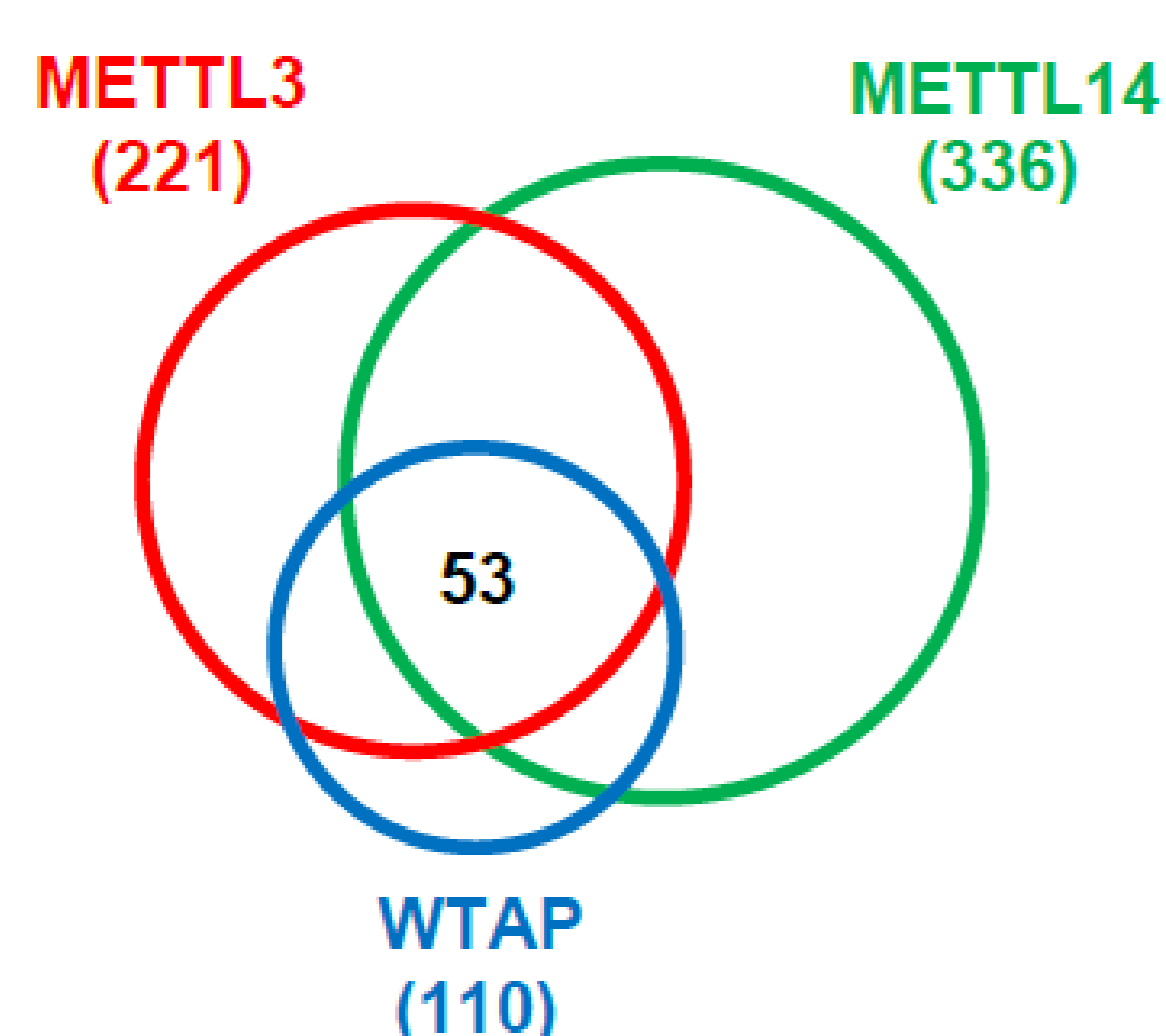


Fig. 1 The m⁶A methylation complex and its site specificity in mammalian systems

Part I : Identification of New Components of Methyltransferase in Mammalian Cells

Identified proteins from co-IPs



Common targets:

VIRILIZER (KIAA1429) HNRNPH
HAKAI (CBLL1) HNRNPK
ZC3H13 HNRNPU
TRIM28 (KAP-1) ...

Fig. 2 Identification of candidate proteins within the methyltransferase complex through proteomic profiling of the co-immunoprecipitated products of METTL3, METTL14 and WTAP

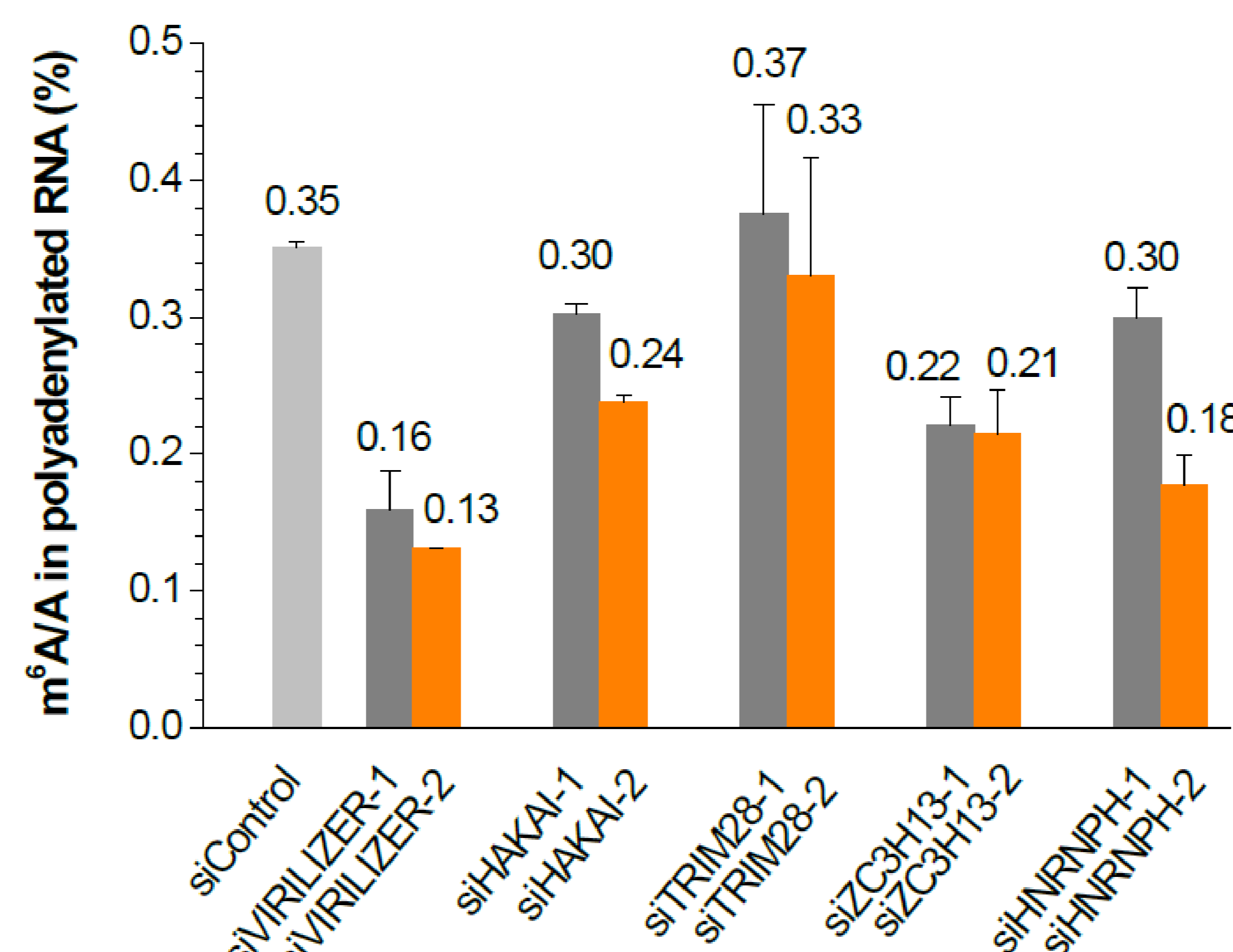


Fig. 3 m⁶A level (m⁶A/A) decreased after knocking down genes identified from the co-immunoprecipitated products of METTL3/METTL14/WTAP

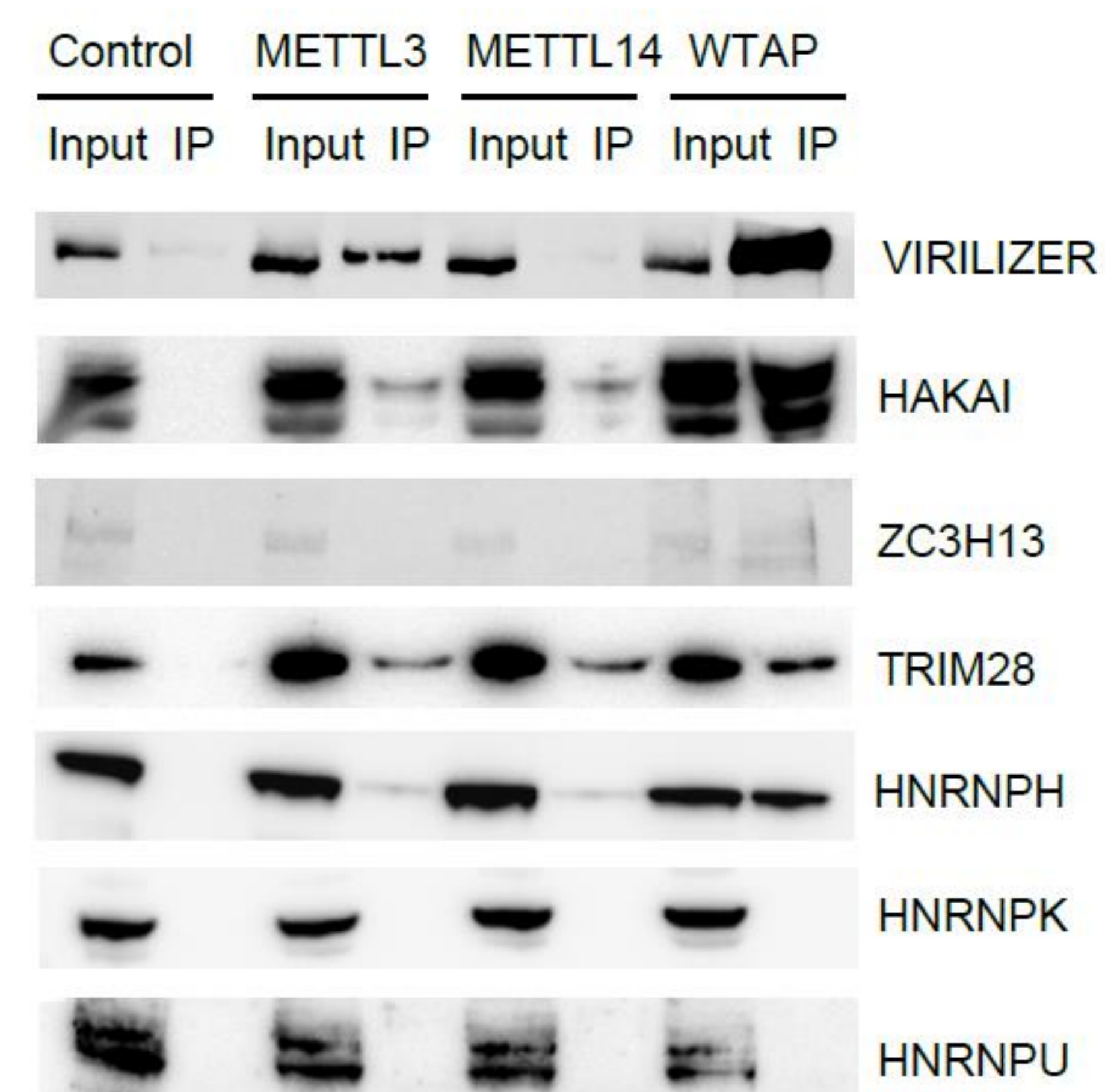


Fig. 4 Interaction between VIRILIZER/ZC3H13/HAKAI/HNRNPH and METTL3, METTL14 or WTAP was verified by western blotting with the co-immunoprecipitated products

Part II : Site Specificity of m⁶A Methylation in Mammalian Cells

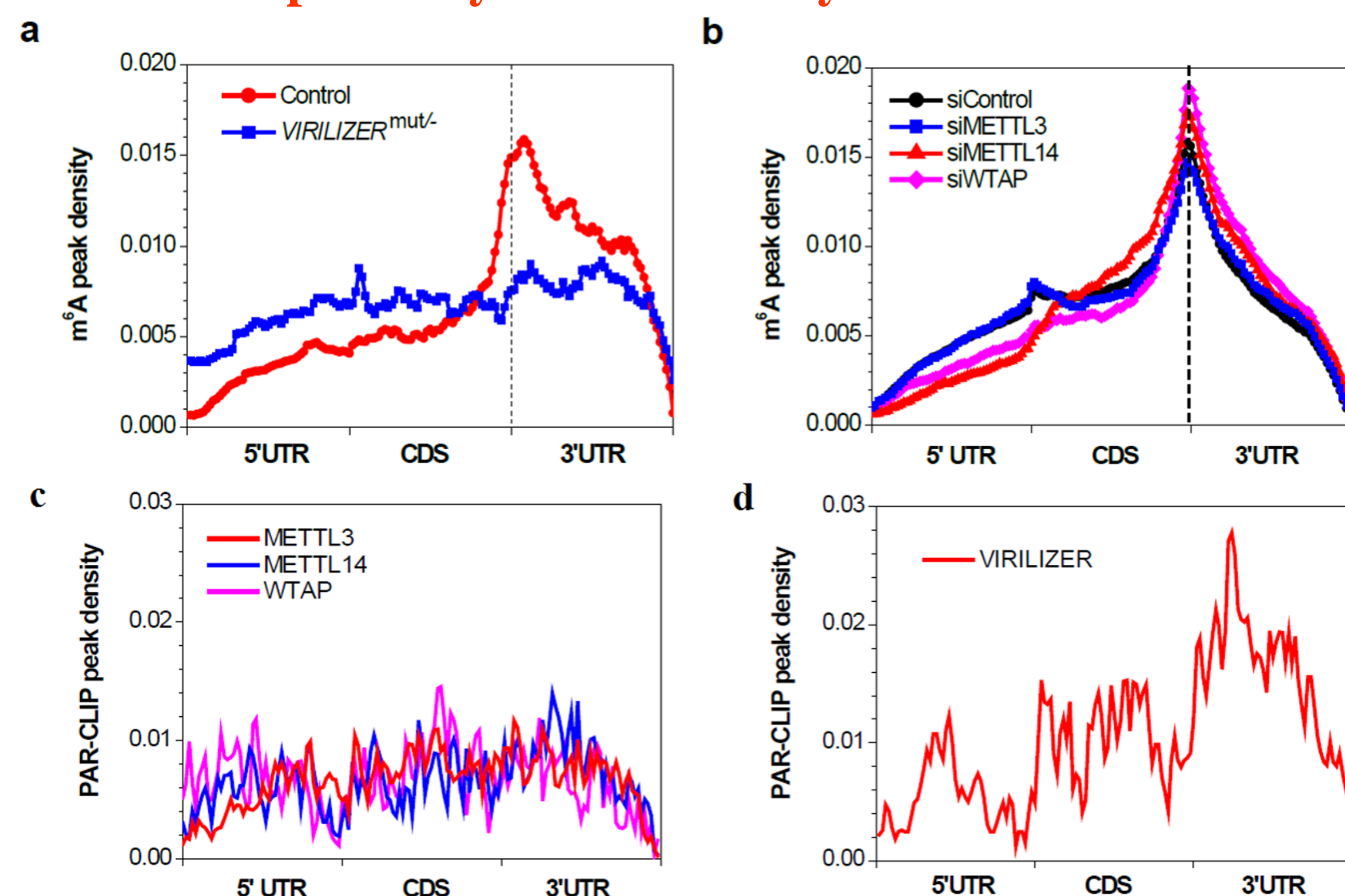


Fig. 5 The enrichment of m⁶A in 3'UTR disappeared in VIRILIZER stably knocked down cell line VIRILIZER^{mut/-}(a); while knock down of METTL3 or METTL14 didn't effect its site specificity(b) ; PAR-CLIP manifested that the binding site of METTL3/METTL14/WTAP distribute averagely in mRNA(c); while the major binding sites of VIRILIZER are located in 3'UTR(d)

Conclusion

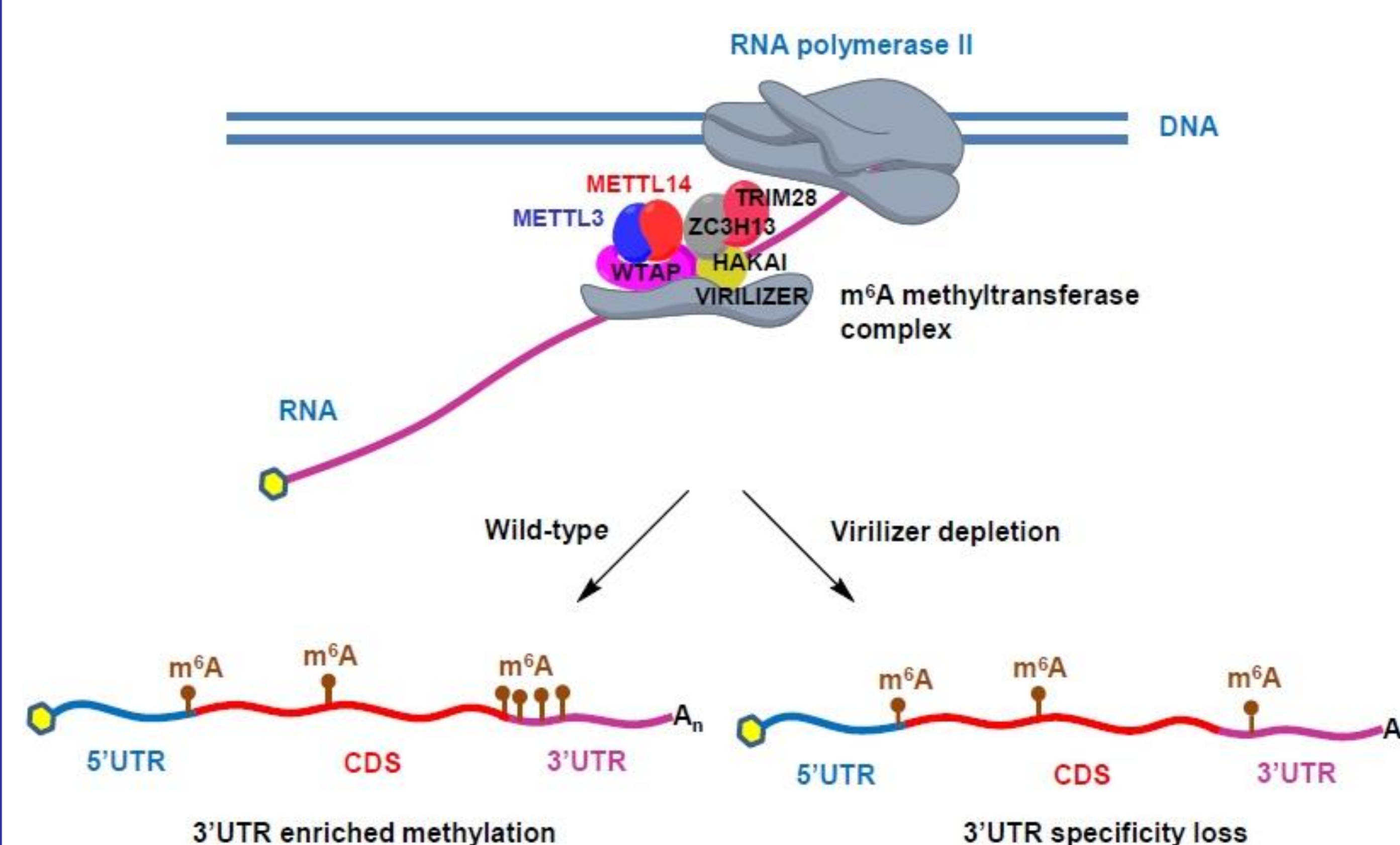


Fig. 6 VIRILIZER/HAKAI/ZC3H13 are identified as new components of m⁶A methyltransferase complex , among them VIRILIZER can regulate m⁶A methylation specificity

Summary :

Our study focused on characterization of the full methyltransferase complex besides previously identified components of METTL3, METTL14, and WTAP in order to decipher the mechanism for m⁶A modification specificity. Based on proteomic search and biochemical validation, we found out new components of m⁶A methyltransferase complex including VIRILIZER, HAKAI and ZC3H13. We chose VIRILIZER as our focus due to its huge effect on m⁶A modification. In the m⁶A methylome of VIRILIZER^{+/+} cell line, the signature pattern of m⁶A enrichment in 3'UTR and near stop codon disappeared, indicative of loss of m⁶A modification specificity. PAR-CLIP data further manifested that the major binding sites of VIRILIZER are located in 3'UTR. Together we propose a model that VIRILIZER may serve as a scaffold, target 3'UTR of specific set of mRNAs, and recruit METTL3/METTL14 mainly through WTAP.

Acknowledgements

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References

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