# An Epigenetics Platform for Exploring Tumorigenesis Mechanism and Developing Potential Anti-Tumor Drug

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## Introduction

In the specific fields of genetics, epigenetics is the study of heritable changes in gene expression and cell phenotype caused by DNA methylation, histone modification and regulation of non-coding RNA without altering the DNA sequence. Abnormal methylation modification of mRNA and abnormal expression of miRNA and other non-coding RNA often promote the tumorigenesis. Therefore, tumor epigenetic therapy has become an urgent research direction for scholars.

ICE Bioscience has established an epigenetic screening platform for exploration on tumor epigenetic therapy. The platform mainly includes methylation screening platform, acetylation screening platform and mRNA screening platform. It includes about 100 types of epigenetic hot targets, which allows the platform being supportive for preclinical research and anticancer drug validation. Meanwhile, several biochemical and cell-based assays are demonstrated for promisingly conducting in-vitro experiments. Thus, ICE Bioscience epigenetic platform can support tumorigenesis research and new-generation anti-tumor drug development.

## Epigenetics Platform

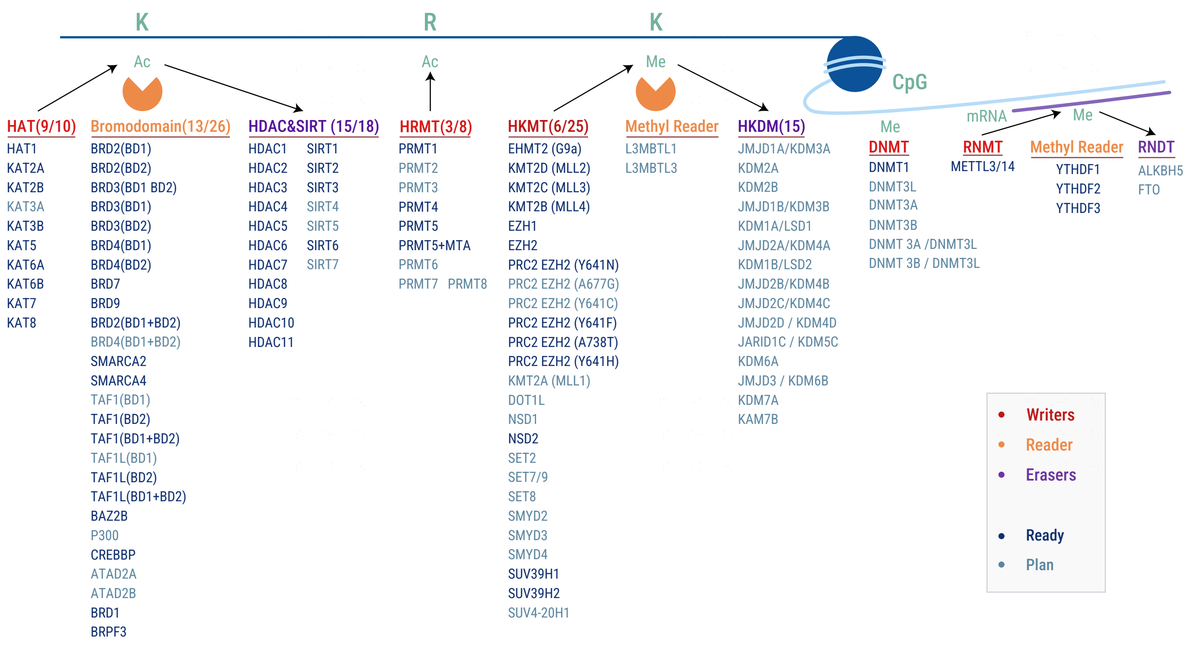



Figure 1: Target list overview.

## Results

### 1. Methylation Screening Platform

#### a. Biochemical Assay

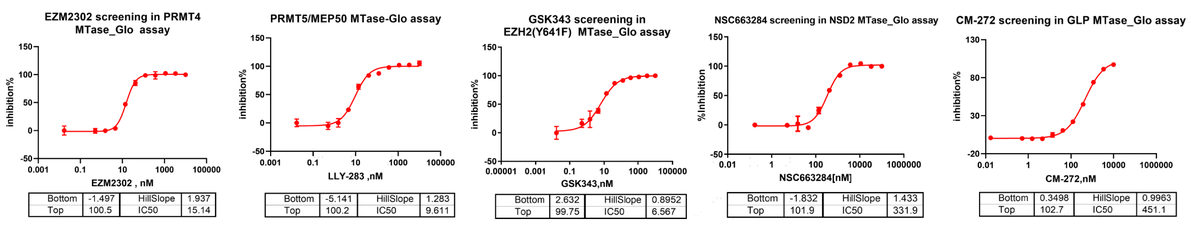



Figure 2: MTase-Glo assays (Promega) were established for compound screening. This method can efficiently and quickly screen methylation inhibitors of different targets at the biochemical level.

#### b. Cellular Assay

*KO Cell Line Generation and Proliferation Assay*

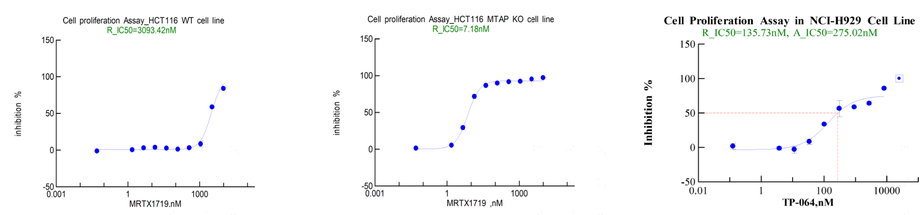



Figure 3: The MTAP KO cell line has been successfully constructed. The function of KO cell line was verified by proliferation assay. The results showed that the KO cell line was more sensitive to the test compound than the WT cell line. Cellular assay showed inhibition of the NCI-H929 cell line using a dose-range of TP-064.

*In Cell Western Assay and Western Blot Assay*

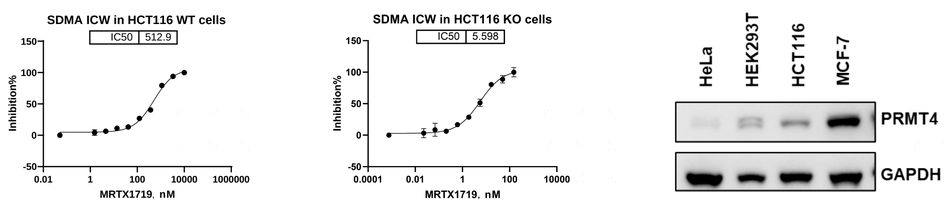
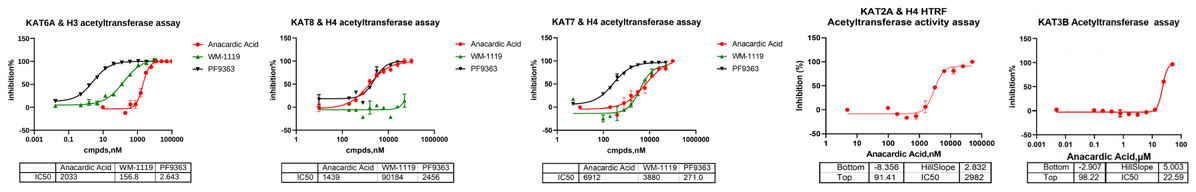



Figure 4: In Cell Western assay and Western Blot assay were used to evaluate protein expression and the effect of inhibitors. We can verify the expression levels of different targets in cells by WB assay and screen target inhibitors by In Cell Western assay.

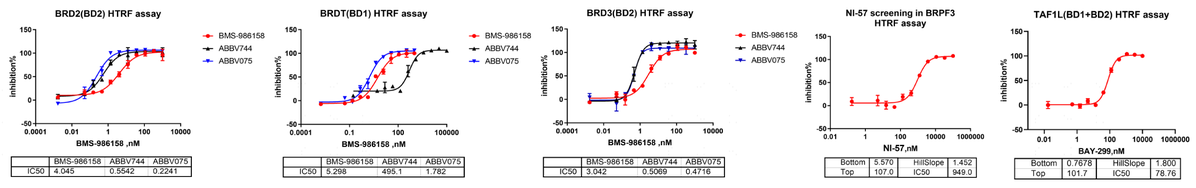
### 2. Acetylation Screening Platform

#### a. Biochemical Assay

*Acetyltransferase Assay*




*HTRF Assay*






*Fluorescent Intensity (FI) Assay*

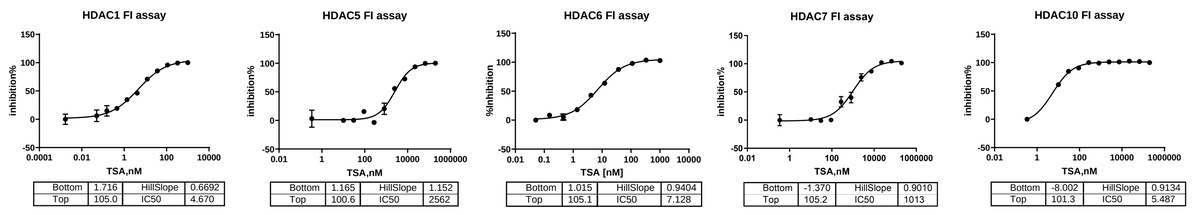
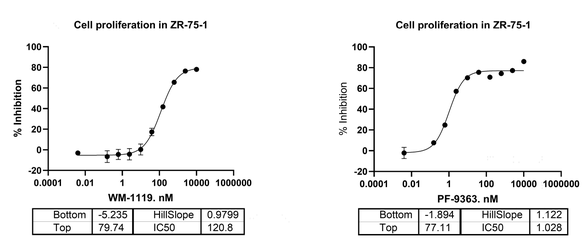



Figure 5: Acetyltransferase assays, HTRF assays and FI assays were utilized to evaluate the potency of different Acetyltransferase target inhibitors.

#### b. Cellular Assay

*Proliferation Assay*




*H3K23Ac/ H3K27Ac*

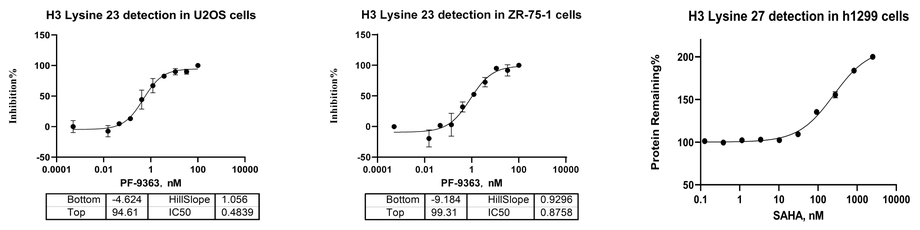



Figure 6: In Cell Western assay was used to evaluate the effect of compounds on the acetylation of H3K23 and H3K27.

*Western Blot Assay*

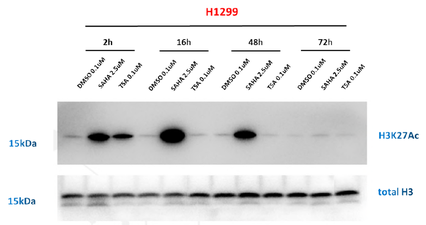



Figure 7: Western Blot assay was performed to determine the expression of H3K27Ac on H1299 cells for 2h,16h,48h and 72h to evaluate the effect of inhibitors on the acetylation of H1299.

### 3. mRNA Screening Platform

#### a. Biochemical Assay

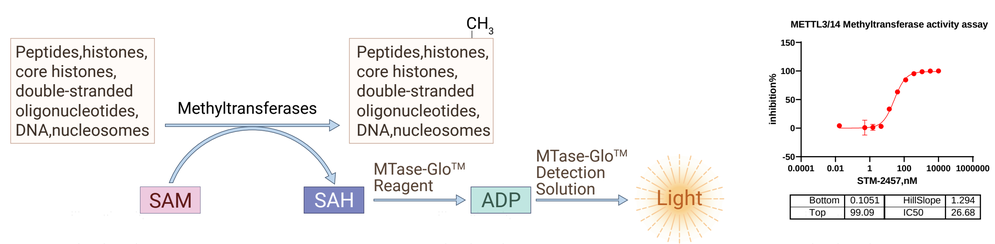



Figure 8: Biochemical activity assay showed inhibition of the METTL3/14 enzyme complex using a dose-range of STM2457[1].

#### b. Cellular Assay

*Proliferation assay*

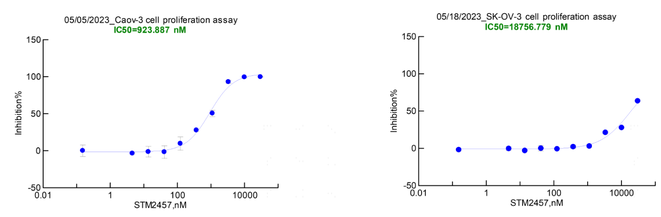



Figure 9: Cellular assay showed inhibition of the Caov-3 and SK-OV-3 cell line using a dose-range of STM2457.

*RNA modification*

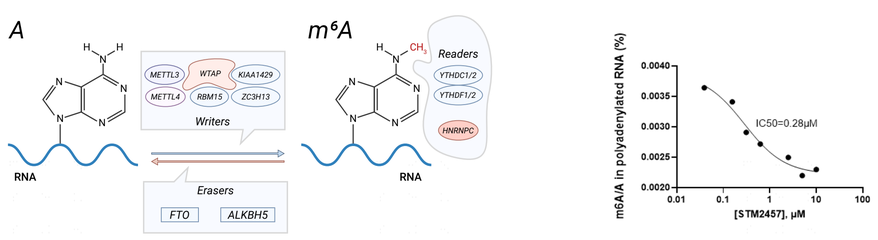



Figure 10: m6A RNA modification is involved in all phases of the RNA life cycle, including splicing, RNA translation regulation and RNA degradation[2]. The overall m6A/A ratio of polyadenylated RNA was measured using A kit and results showed that STM2457 mediated downregulation of the m6A/A ratio at the cellular level.

Methylation Screening Platform, Acetylation Screening Platform and mRNA Screening Platform constructed by ICE Bioscience can achieve high-throughput screening of inhibitors, which facilitates epigenetic anti-tumor drug screening.

## Summary

Compounds targeting epigenetic pathways do not induce the immediate death of cells, which differs from other anti-cancer therapy such as chemotherapy. Their role is to reactivate cellular pathways and promote cancer cell death. Epigenetic modification is a reversible process, so treating cancer from epigenetics would take a whole new promising direction. This study builds an epigenetic screening platform that can be used for drug combination, targeted therapy and immunotherapy. The platform provides a powerful tool for drug discovery and screening.

## References

1. Yankova E, Blackaby W, Albertella M, et al. Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia. Nature. 2021;593(7860):597-601. doi:10.1038/s41586-021-03536-w.
2. Zhuang H, Yu B, Tao D, Xu X, Xu Y, Wang J, Jiao Y, Wang L. The role of m6A methylation in therapy resistance in cancer. Mol Cancer. 2023 Jun 1;22(1):91. doi: 10.1186/s12943-023-01782-2.