Construction of a Clickable Probe-Based Protein Chip Platform for Discovering Covalent mIDH1 Inhibitors from Natural Medicinal Extracts

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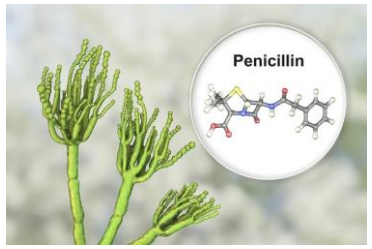
Zhao Cui, Jiameng Li, Caifeng Li, Mo Sun, Wei Liu, Xuxia Cao, Shiwen Deng, et al. 2026. Construction of a Clickable Probe-Based Protein Chip Platform for Discovering Covalent mIDH1 Inhibitors from Natural Medicinal Extracts. ***iMeta*** 5: e70107.
https://doi.org/10.1002/imt2.70107

Introduction

1. Advantages of Natural Products for Covalent Drugs

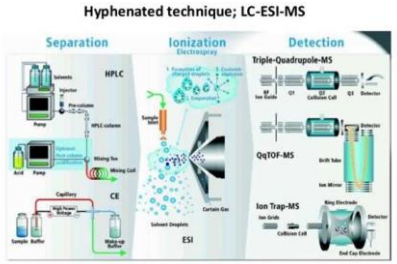


Penicillin (from *Penicillium*)

Aspirin (from willow bark)

- Natural products are key sources of covalent drugs, e.g., penicillin (from *Penicillium*), aspirin (from willow bark), and orlistat (from *Streptomyces toxitricini*).
- These molecules feature high safety, excellent drug-like properties, and lower clinical development failure risk.

2. Limitations of Existing Covalent Molecule Screening Technologies



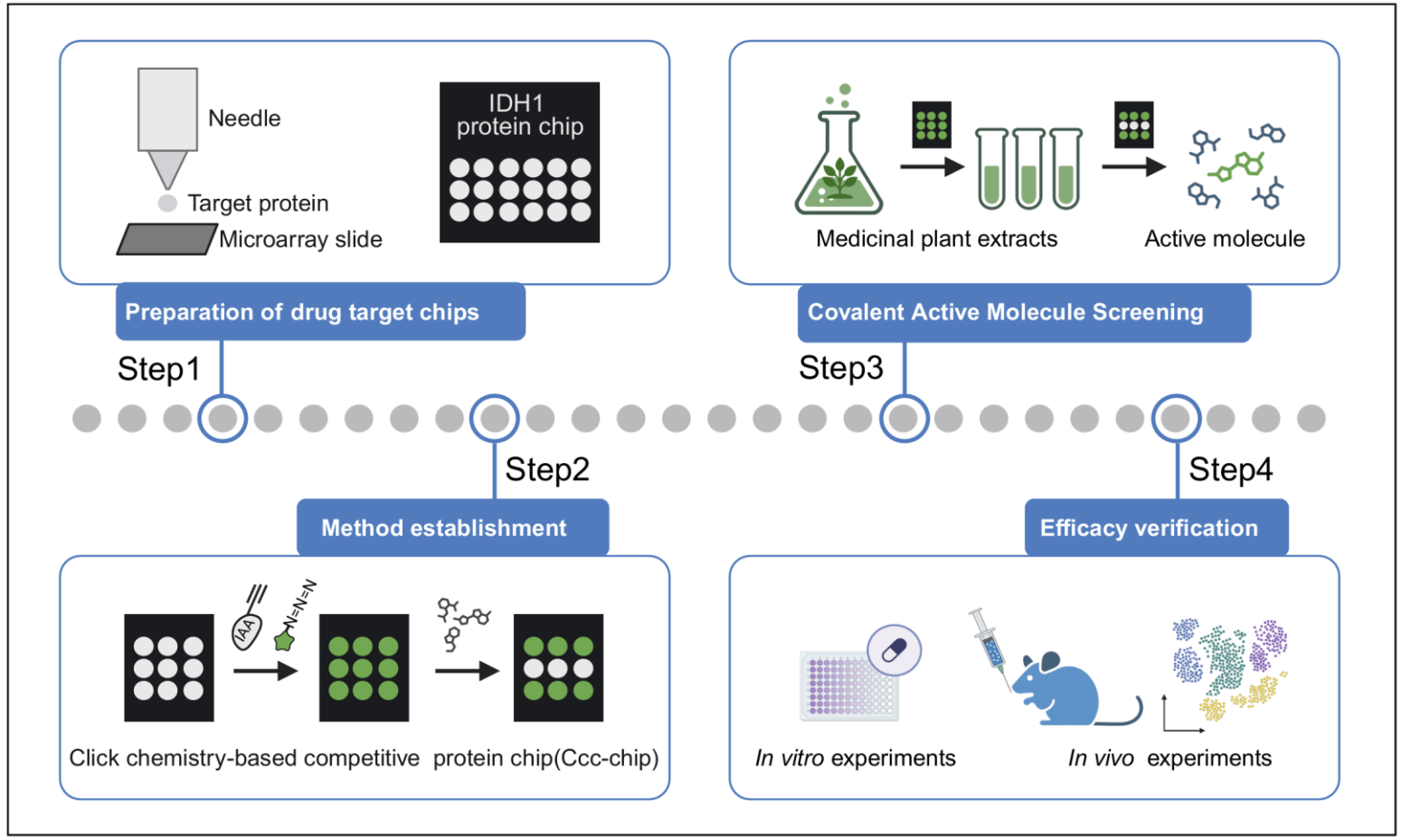
LC-ESI-MS

Library-Dependent

- Traditional LC-ESI-MS requires chromatographic separation and has low throughput.
- Bioorthogonal labeling needs pre-modification of ligands; competitive binding methods (gel/cell fluorescence imaging) are inefficient.
- Current methods rely on small-molecule compound libraries as screening sources, which are incompatible with the complex systems of medicinal plant extracts.

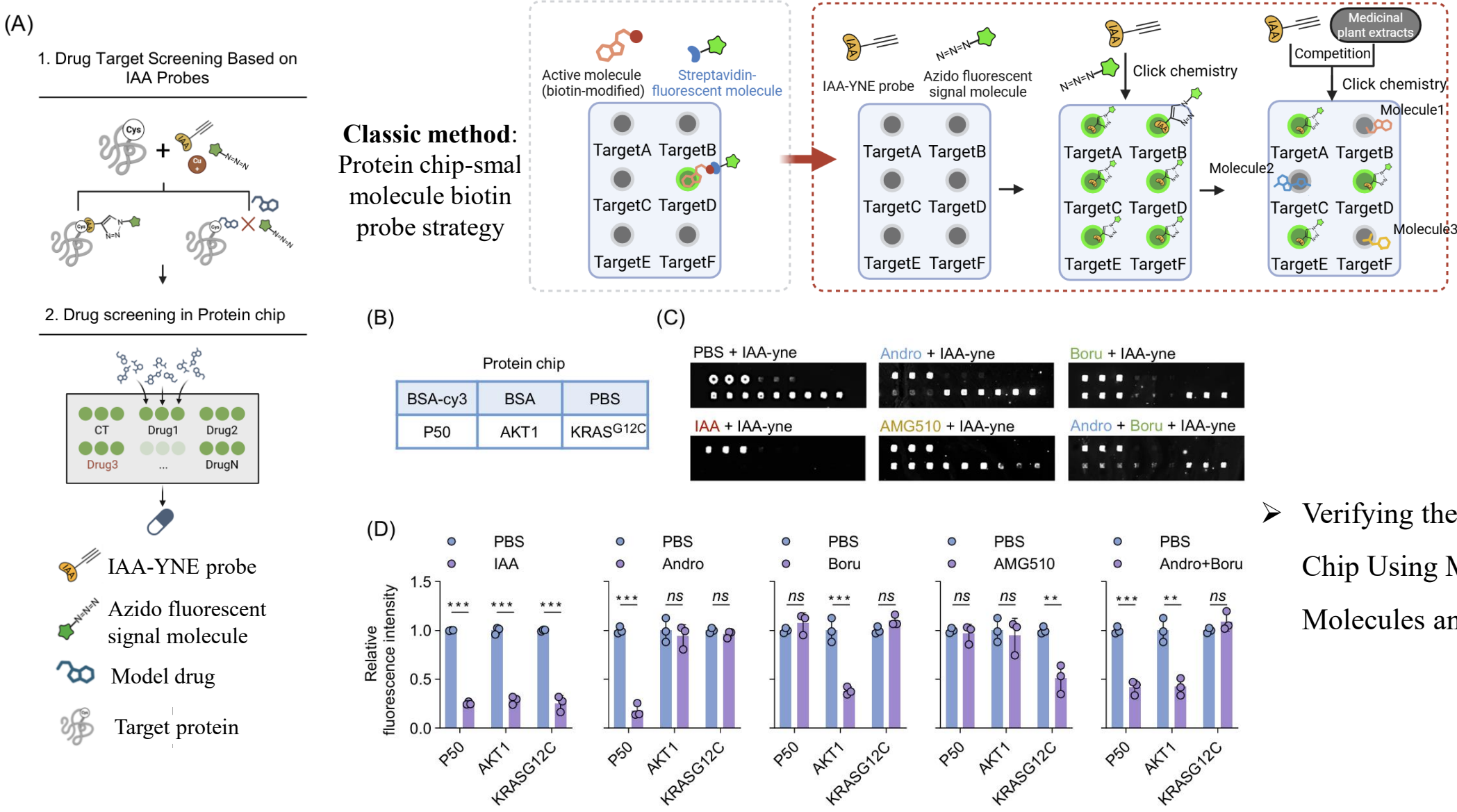
How to screen covalent active molecules from complex medicinal plant extracts?

Highlights



- Integrate and develop a bioorthogonal click chemistry-protein chip platform, which enables label-free, high-throughput screening of covalent inhibitors directly from the complex system of plant extracts.
- Identify Flavokawain C (Flc) as a novel covalent inhibitor of mIDH1 from 110 medicinal plant extracts; it can significantly reduce the level of the oncogenic metabolite 2-HG, and its combination with PD-1 antibodies synergistically enhances anti-tumor immunity.
- The platform is scalable, with flexibility for target proteins and specific probes, providing a simplified solution (high sensitivity and specificity) for mining covalent active molecules against refractory targets from natural sources.

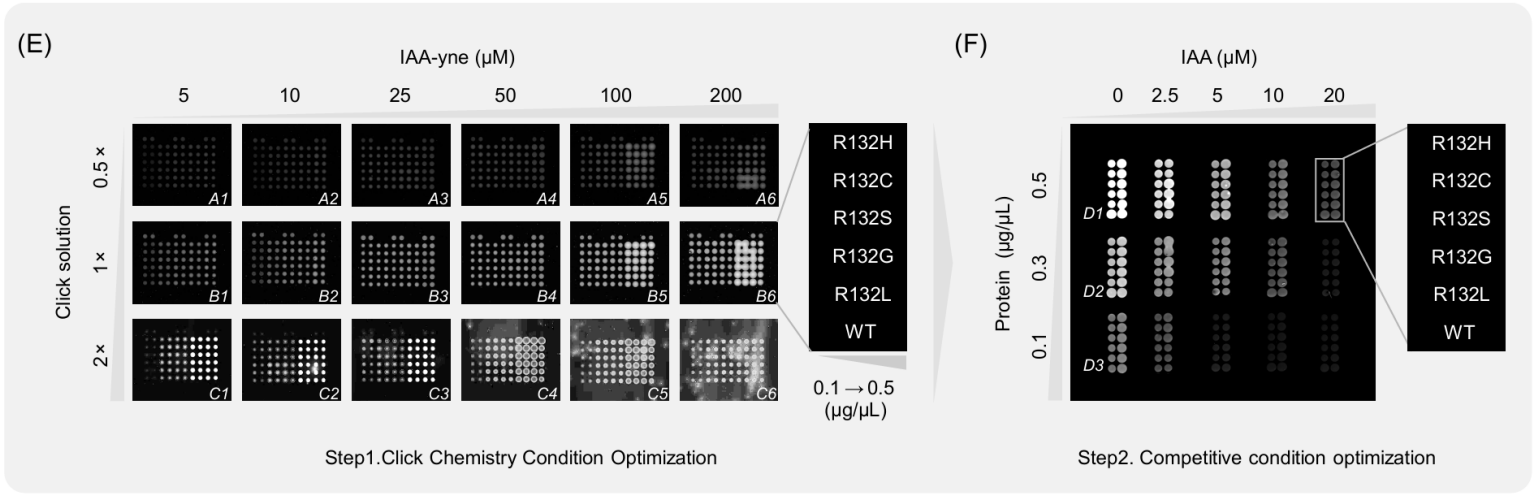
Establishment of the Ccc-Chip Method on the mIDH1 Protein Chip

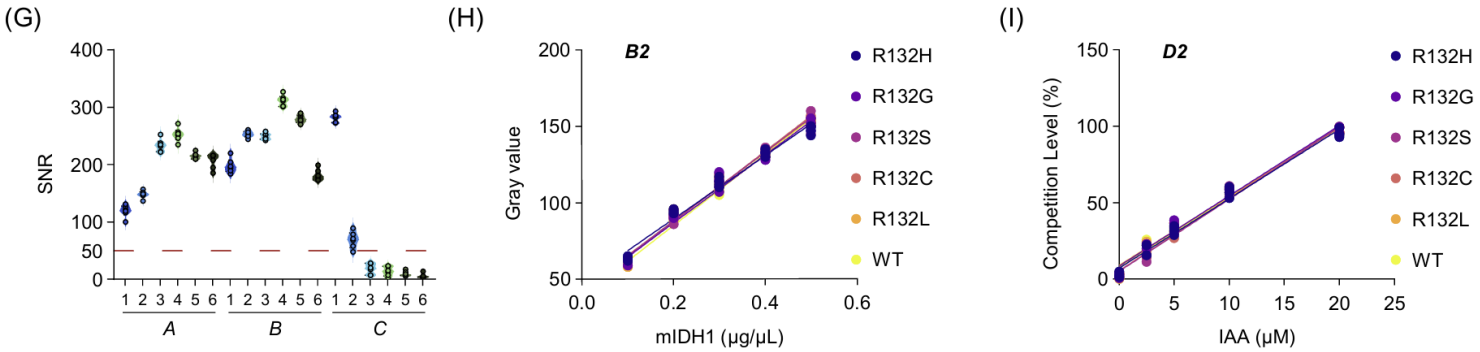


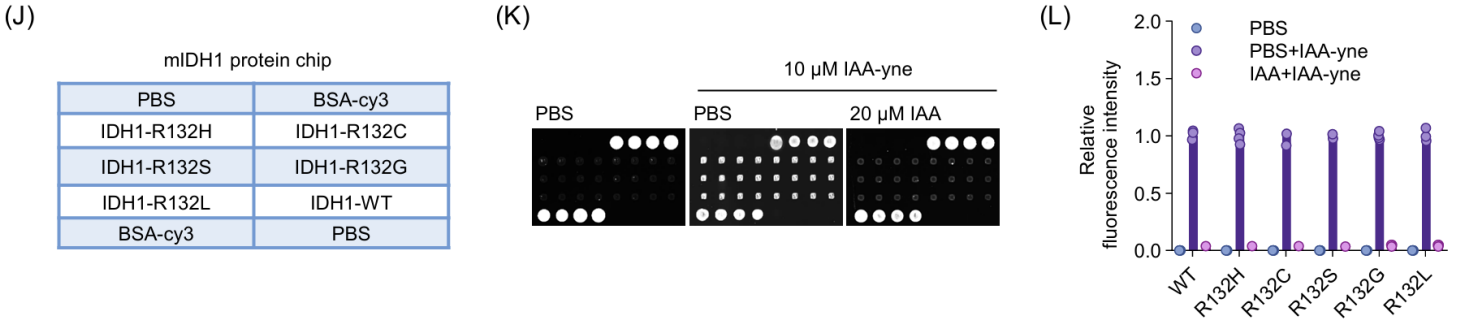
➢ Verifying the Feasibility of Ccc-Chip Using Model Covalent Molecules and Target Proteins.

Figure 1. Construction and Optimization of the mIDH1 Protein Chip for Ccc-Chip Screening

Establishment of the Ccc-Chip Method on the mIDH1 Protein Chip



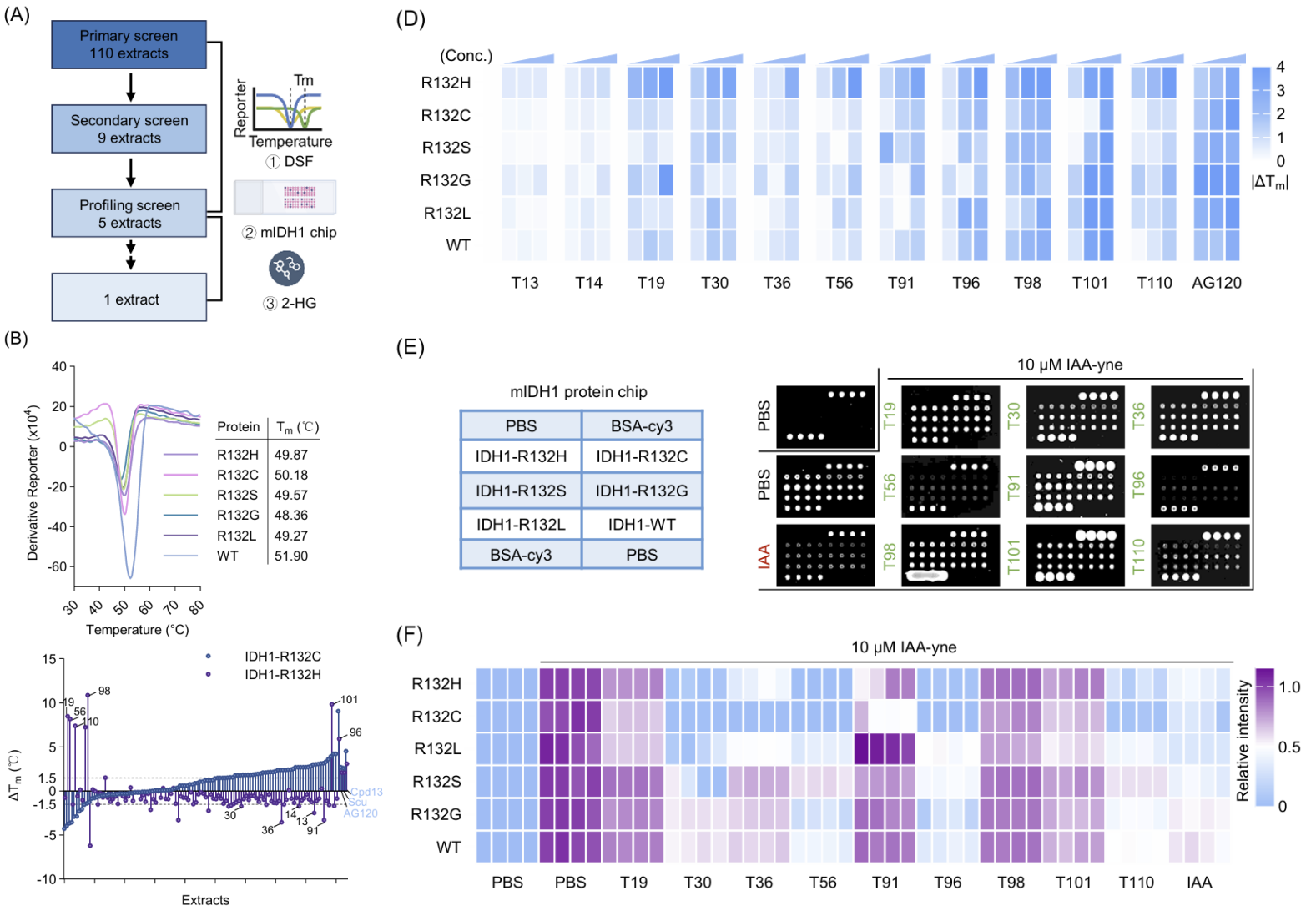




- Screening of optimal concentration conditions for IAA, IAA-yne, and click reaction solution in the mIDH1 chip.
- Construction and validation of the mIDH1 target chip.

Figure 1. Construction and Optimization of the mIDH1 Protein Chip for Ccc-Chip Screening

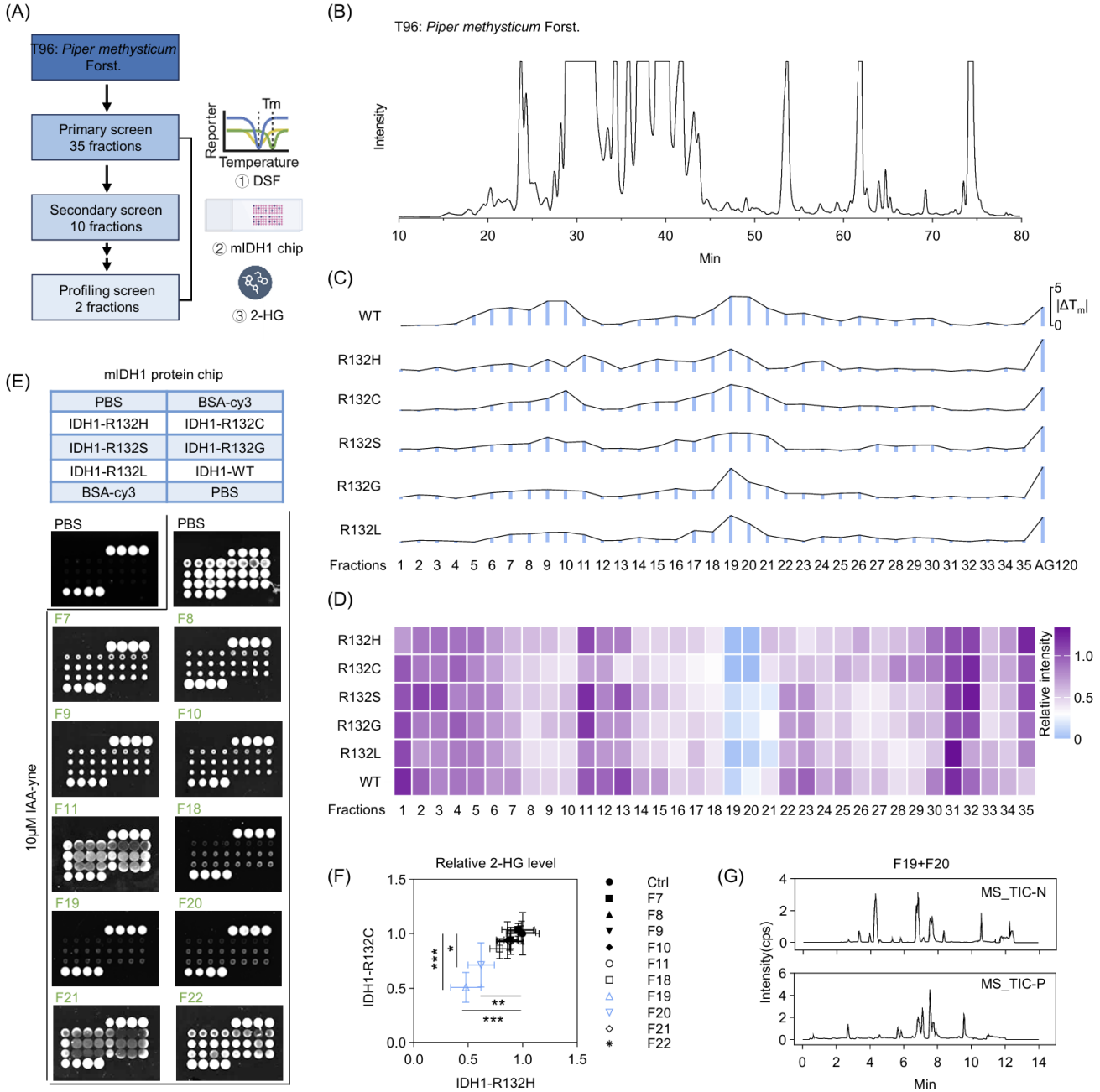
Screening of mIDH1-Targeting Components from Plant Extracts via the Ccc-Chip Method



- DSF assay for primary screening of medicinal plant extracts.
- Ccc-Chip assay for secondary screening of candidate medicinal plant.
- Cell-based assays to confirm medicinal plant extracts that inhibit 2-HG production.

Figure 2. Screening of mIDH1-Targeting Medicinal Plant Extracts Based on Ccc-Chip

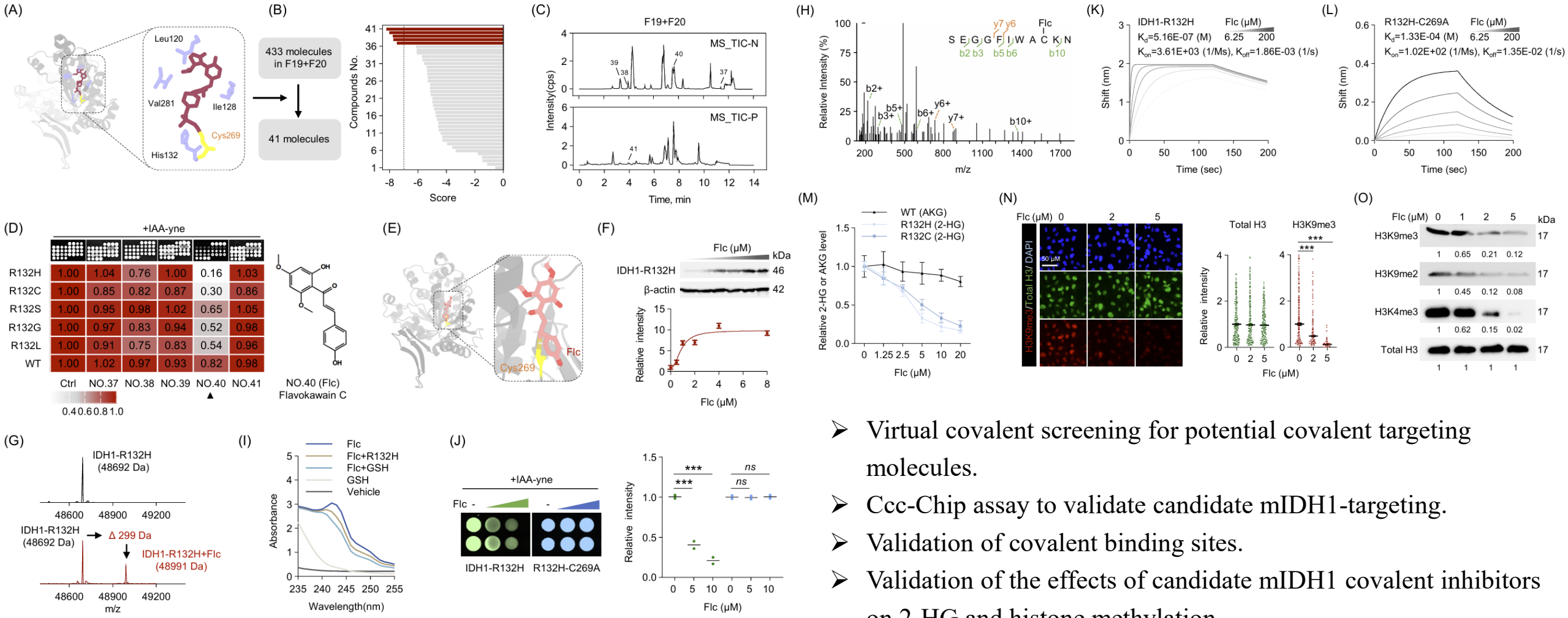
Screening of mIDH1-Targeting Components from Plant Extracts via the Ccc-Chip Method



- Preparative liquid chromatography to obtain sequential preparative fractions of candidate medicinal plant extracts.
- DSF combined with Ccc-Chip assay to screen effective preparative active fractions.
- Cell-based assays to validate effective preparative fractions that inhibit 2-HG production.
- LC-MS/MS to analyze the molecular composition of active fractions.

Figure 3. Identification of IDH1-Targeting Active Components from *Piper methysticum* Extract Using Ccc-Chip

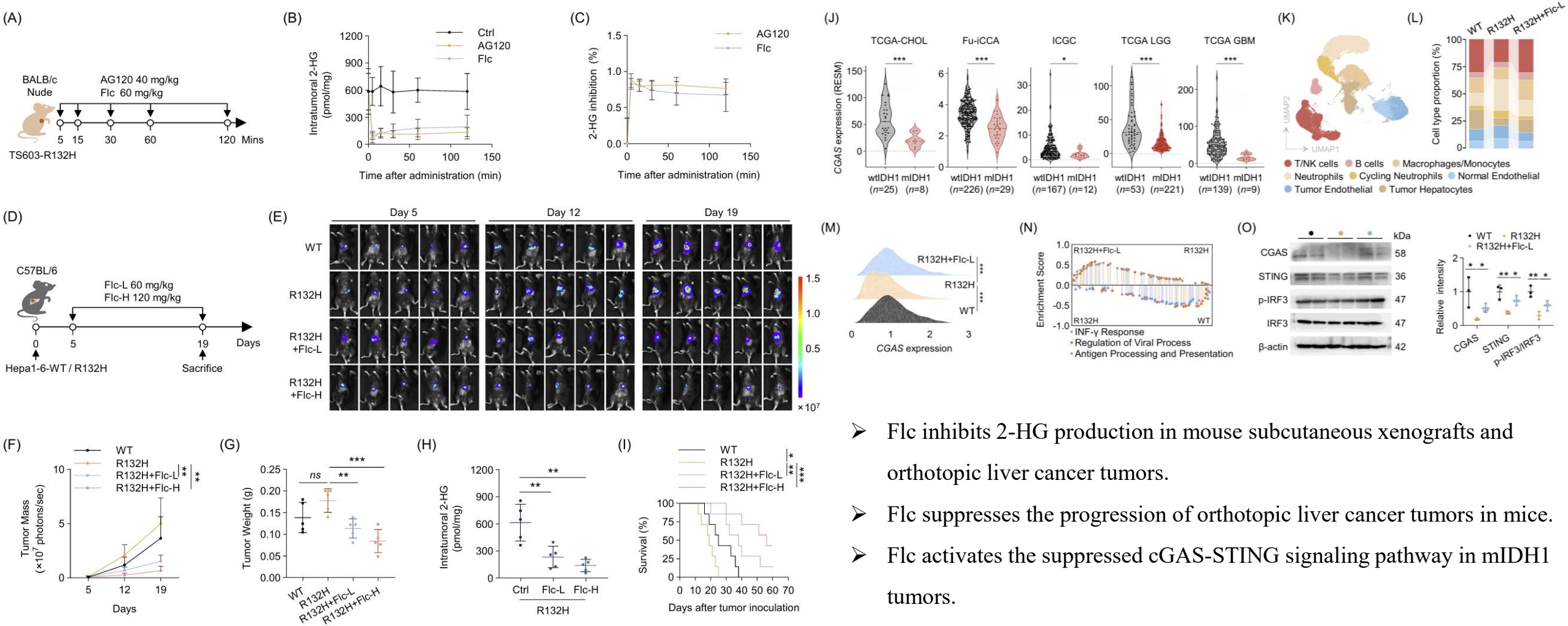
Flc is a Covalent Active Molecule Targeting mIDH1 in *Piper methysticum*



- Virtual covalent screening for potential covalent targeting molecules.
- Ccc-Chip assay to validate candidate mIDH1-targeting.
- Validation of covalent binding sites.
- Validation of the effects of candidate mIDH1 covalent inhibitors on 2-HG and histone methylation.

Figure 4. Flavokawain C (Flc) is a Covalent Inhibitor Targeting IDH1-R132H in *Piper methysticum* Extract

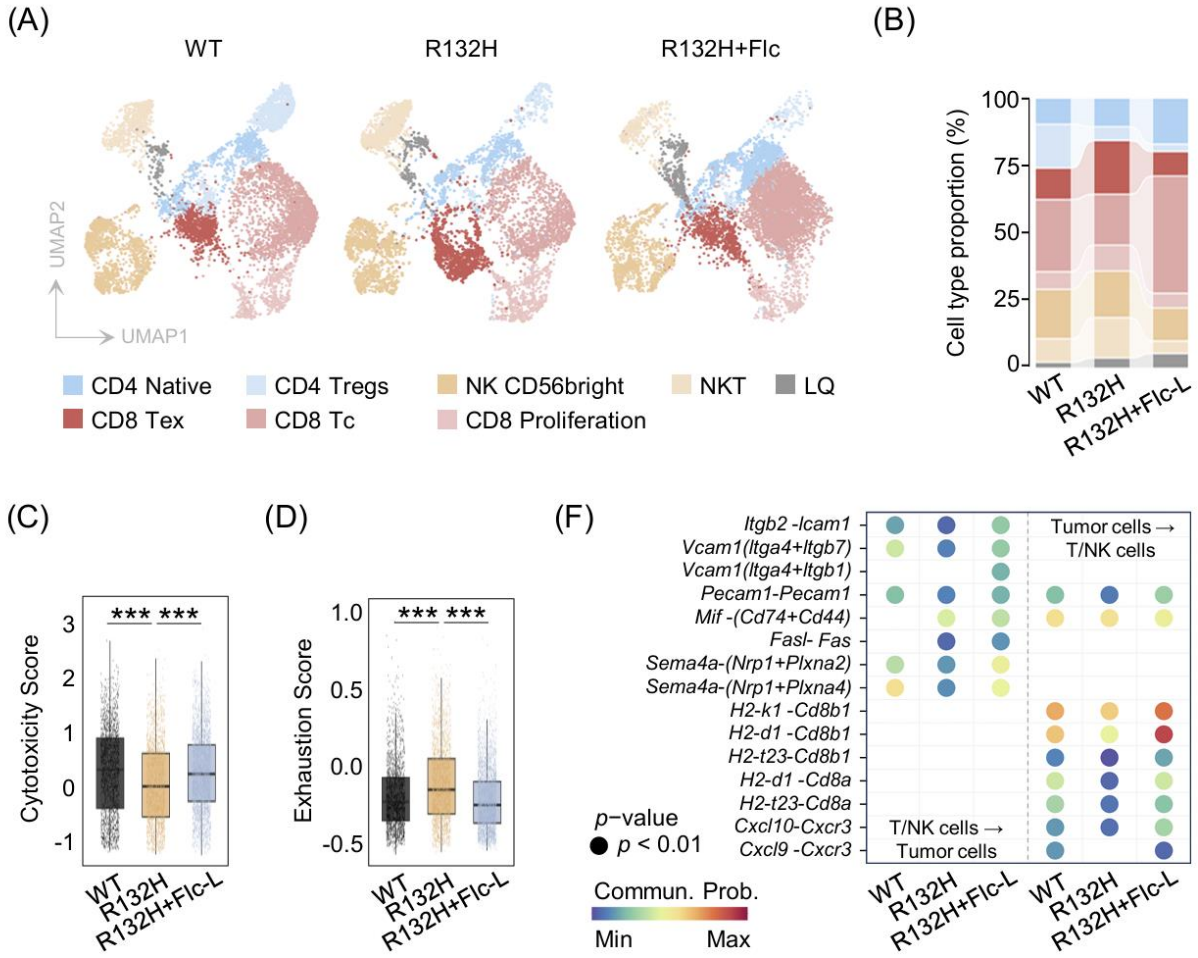
Flc Inhibits 2-HG in mIDH1 Tumors and Activates the cGAS-STING-T Cell Immune Axis



- Flc inhibits 2-HG production in mouse subcutaneous xenografts and orthotopic liver cancer tumors.
- Flc suppresses the progression of orthotopic liver cancer tumors in mice.
- Flc activates the suppressed cGAS-STING signaling pathway in mIDH1 tumors.

Figure 5. Flc Inhibits mIDH1 Liver Cancer and Activates the cGAS Signaling Pathway

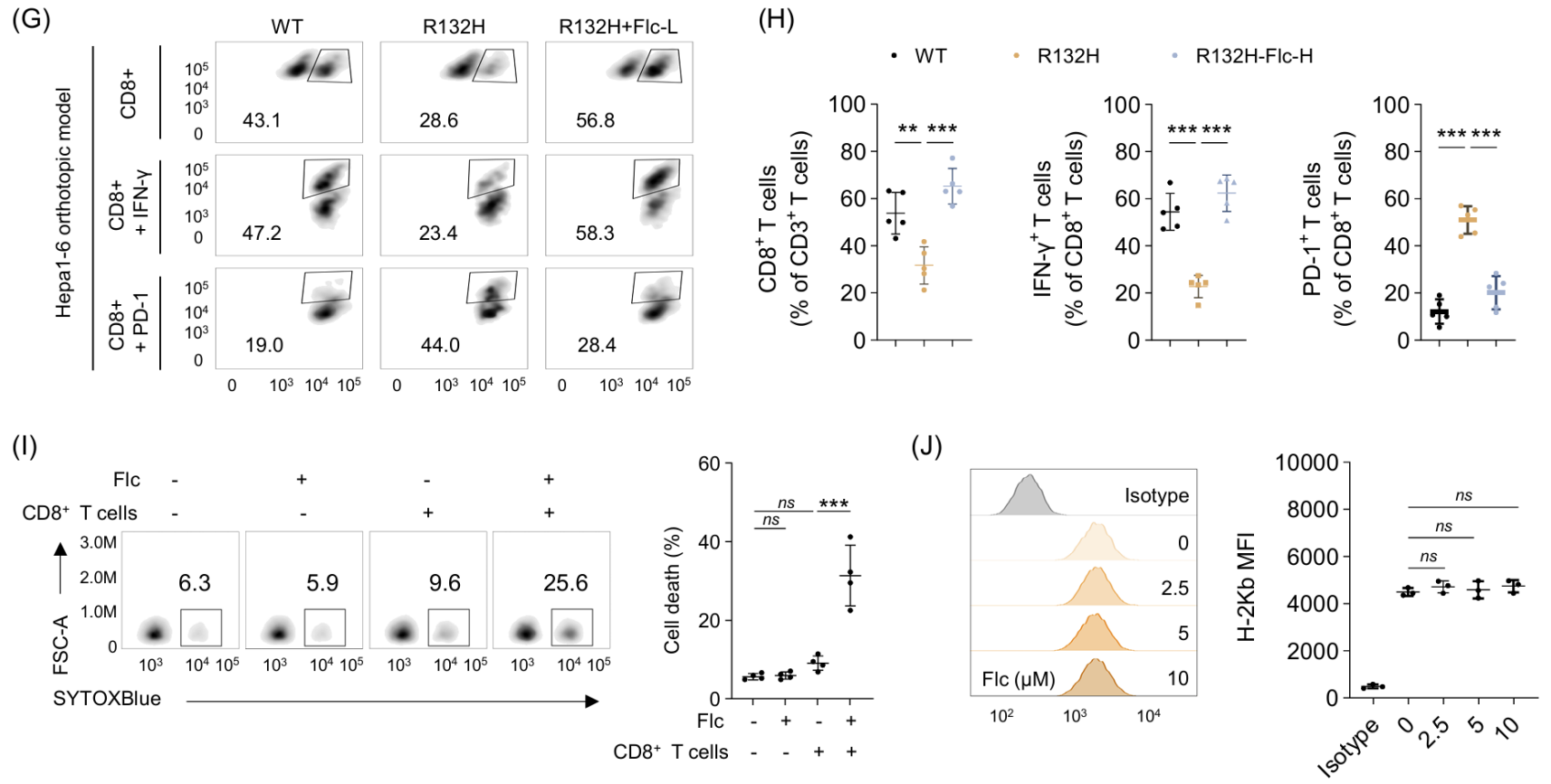
Flc Inhibits mIDH1 Orthotopic Tumor Growth by Activating CD8⁺T Cells



- scRNA-seq shows that Flc enhances the tumor-killing effect of CD8⁺ cells in mIDH1 tumors and reduces the proportion of exhausted CD8⁺ T cells.
- Flc increases the interaction between CD8⁺ cells and tumor cells, enhancing the anti-tumor immune response in mIDH1 tumors.

Figure 6. Flc Enhances CD8⁺T Cell-Mediated Anti-Tumor Immunity in mIDH1 Liver Cancer

Flc Inhibits mIDH1 Orthotopic Tumor Growth by Activating CD8⁺T Cells



- Flow cytometry assay validates the effect of Flc on the proportions of CD8⁺ IFNγ⁺ and CD8⁺ PD1⁺ T cells in mIDH1 tumors.
- Combined use of Flc and PD-1 antibody can synergistically inhibit tumor growth.

Figure 6. Flc Enhances CD8⁺T Cell-Mediated Anti-Tumor Immunity in mIDH1 Liver Cancer

Summary

- This study developed the Ccc-Chip integrated screening platform, enabling efficient screening of covalent inhibitors in complex natural extracts, and successfully identified Flc from *Piper methysticum*.
- The Ccc-Chip platform requires no pre-modification of ligands, and features high sensitivity and specificity, providing a solution for mining covalent molecules against refractory targets in natural product libraries.
- Flc inhibits 2-HG production via covalent binding to Cys269 of mIDH1, activates the cGAS-STING pathway and $CD8^{+}$T cell immunity, and can synergistically inhibit tumor growth when combined with PD-1 antibodies.
- This study not only verifies the practical value of the Ccc-Chip platform, but also establishes a technical framework linking natural product chemistry and functional proteomics for the development of covalent drugs from natural sources.

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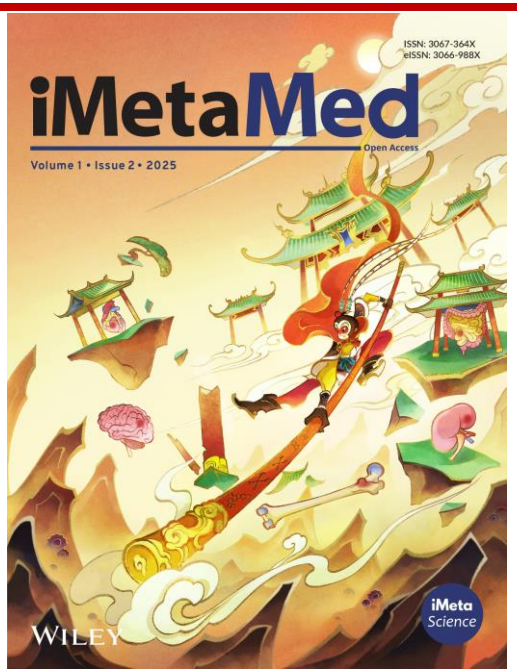

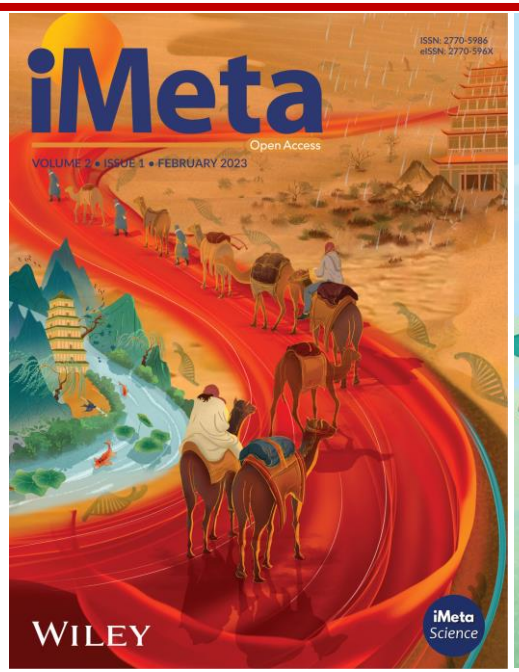

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