Science
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SWISS NETWORK FOR
INTERDISCIPLINARY EDUCATION
IN CHEMICAL BIOLOGY

Menin “reads” H3K79me2 mark in a nucleosomal context

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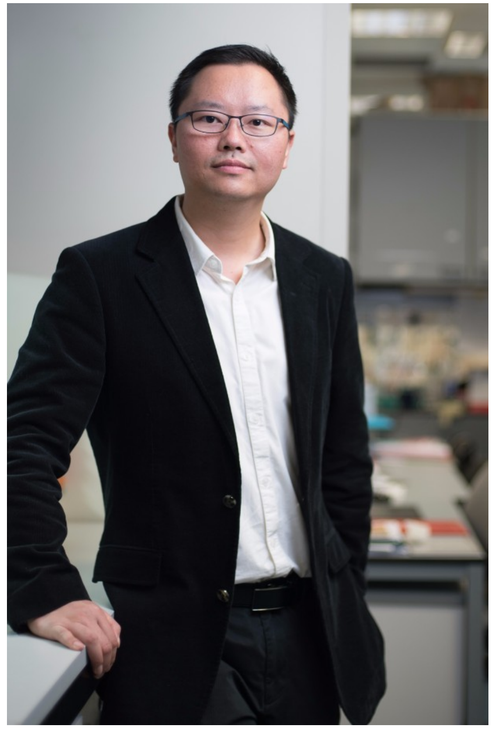


Xiang David Li

Hong kong University

- ✦Chemical proteomics and epigenetic
- ✦Chemical biology approach to identify epigenetic “Readers”, ”Erasers” and “Writer”

Yuan Liang Zhai

Hong kong University

- ✦Chromatin Dynamics
- ✦Cryo-EM in structure determination

Background

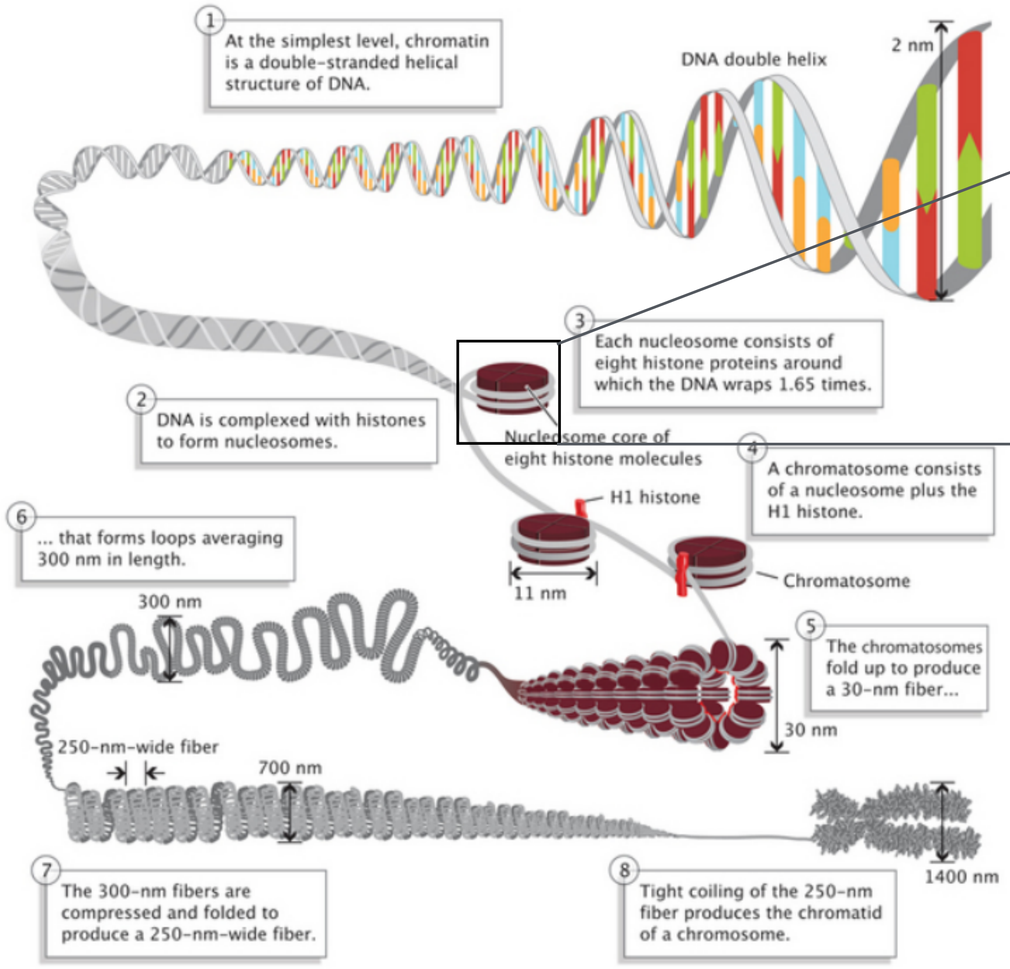
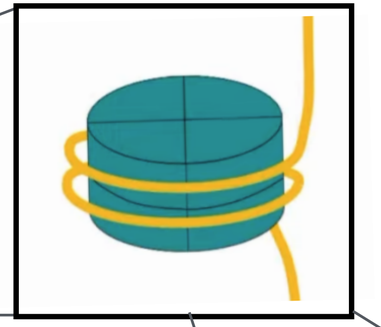
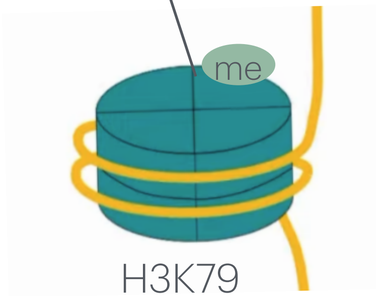


Fig 1. How do genes stored in our cells

Genes are stored in cells in a highly compressed form

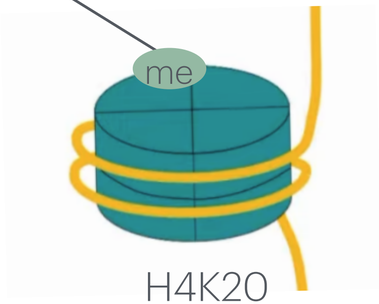


The gene expression will be differently influenced by the different methylated sites



H3K79

Activate transcription



H4K20

Suppress transcription

"Reader"

Pray, L. (2008) Eukaryotic genome complexity. Nature Education 1(1):96

Background

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How to find this “Reader”?

Pull-down Assay

- An in vitro method used to determine the interaction between proteins
- A “bait” protein will be used to capture the “prey” protein

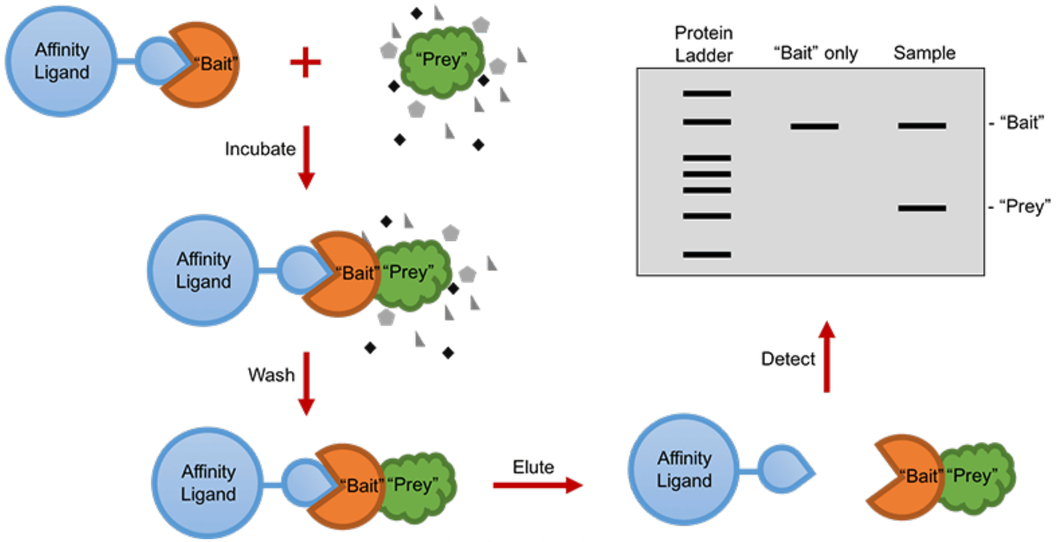

Fig 1. Protocol of Pull-down assay

✳ *“Identification of H3K79 methylation readers is challenging because the posttranslational modification (PTM)–mediated protein-protein interactions (PPIs) can be* ***weak and transient****”*

✳ *“Recognition of H3K79 and its methylation is* ***dependent on nucleosome context****”*

Solve this problem by Chemical biology approach

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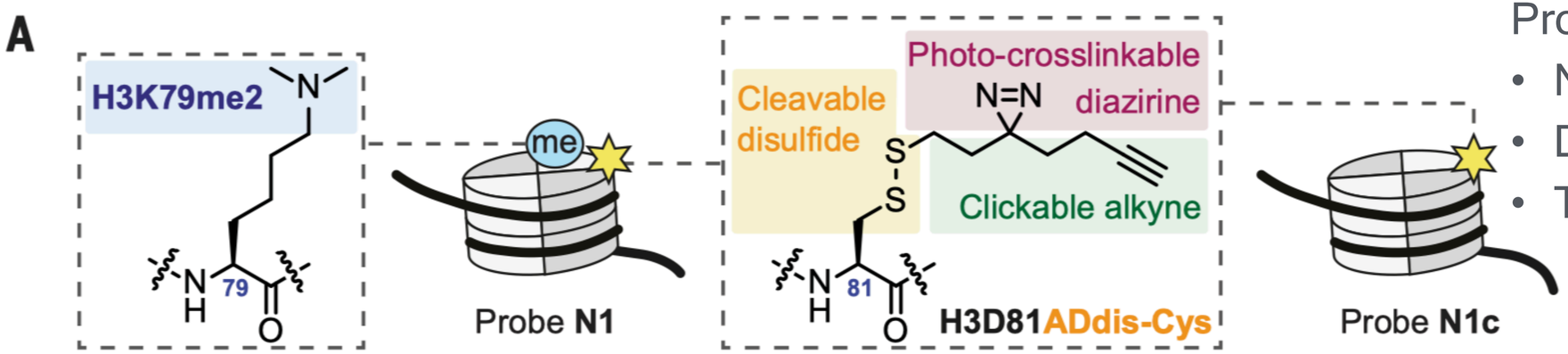

Fig 1. Illustration and partial structure of probes N1 and N1c

Probe N1:

- Nucleosome
- Dimethylation at H3K79 site
- Tri-functional group at H3K81 site
  - Photo-crosslinkable diazirine: Form covalent bonds upon UV irradiation
  - Clickable Alkyne: For the selective isolation of cross-linked proteins
  - Cleavable Disulfide: For releasing once the cross-linked peptides are isolated.

Fig 2. Workflow for H3K79me2 “reader” identification using probe

Identification of Probe

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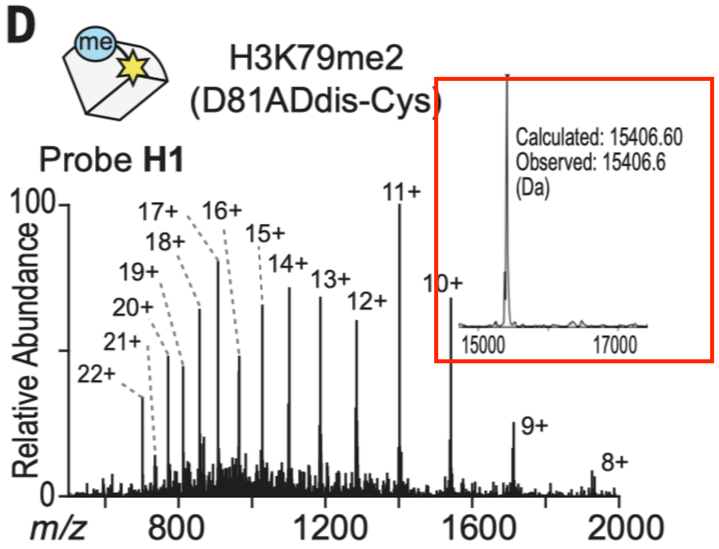

Fig 1. ESI-MS analysis of probe H1.

- Based on the charge state and the m/z ratio, calculated the mass of product, compare it with the theoretical value

- b ions include the N-terminal peptide, essential for amino acids sequence.
- y ions include the C-terminal peptide, essential for detection of modification.

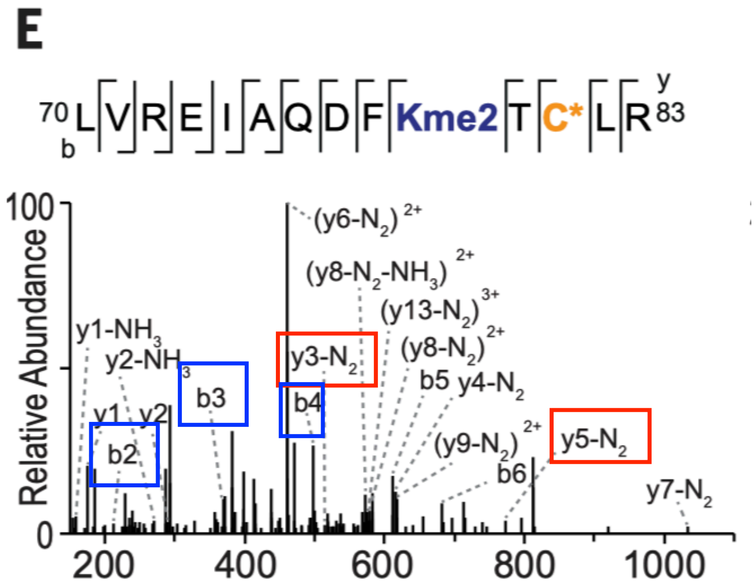

Fig 2. MS/MS analysis of probe H1.

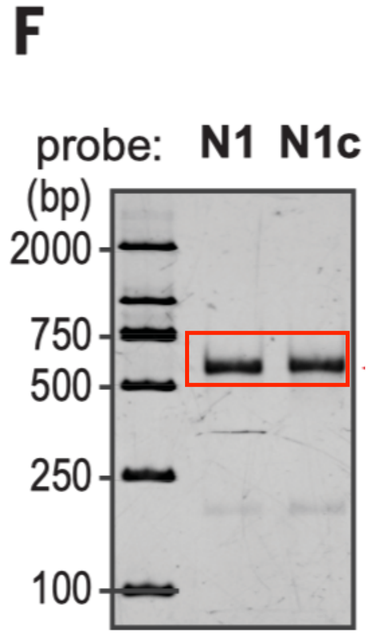

Fig 3. Western blot of Probe N1

- Display the successful synthesis of Probe N1 and N1c

Identification of the protein bind to H3K79me

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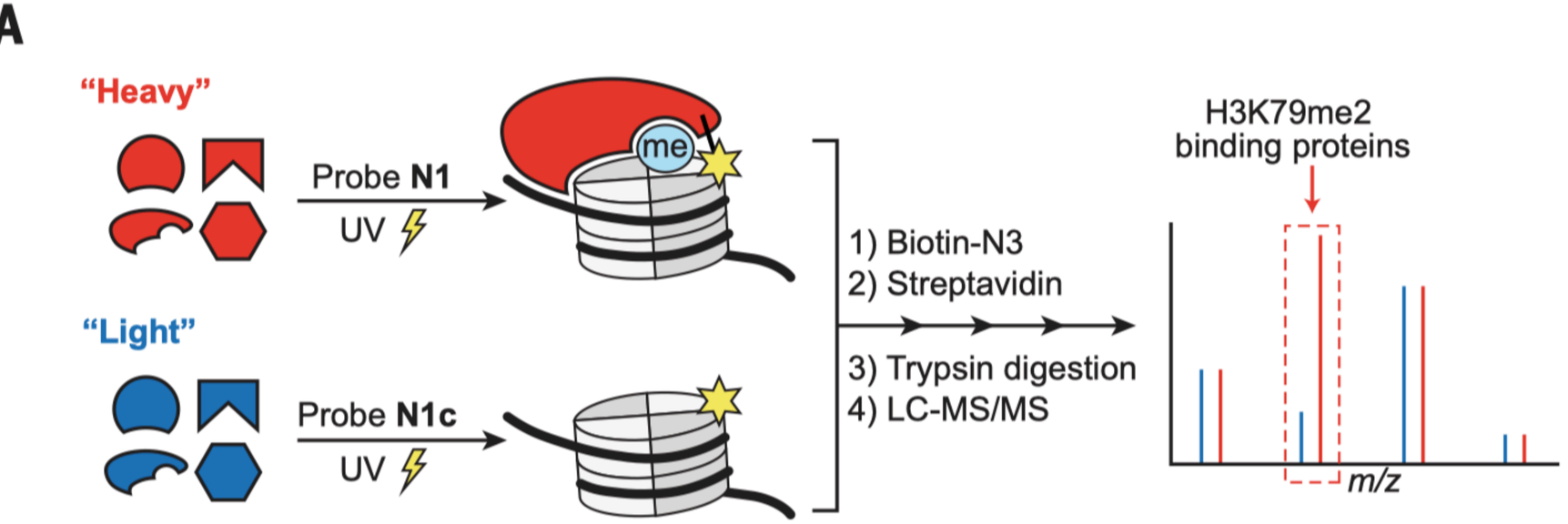

Fig 1. Workflow of the CLASPI approach to identify H3K79me2 binders.

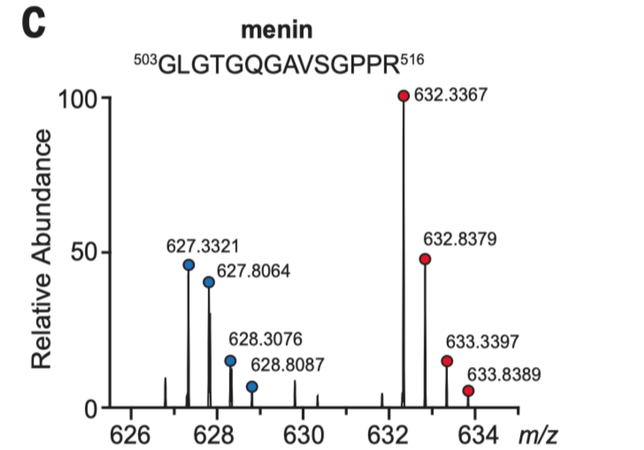

Fig 2. A representative MS1 spectrum from menin

	Forward	Reverse
Heavy label protein	Probe N1	Probe N1c
Light label protein	Probe N1c	Probe N1
H/L	Higher	Lower
L/H	Lower	Higher

Table1. Experiment design

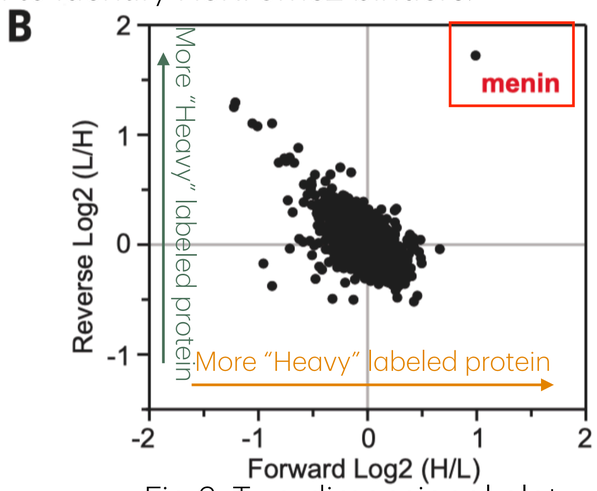

Fig 3. Two-dimensional plot

- Menin has a higher abundance in the "Heavy" label group
- Menin stands out from the forward and reverse experiments.

Menin selectively binds to H3K79me2 nucleosomes

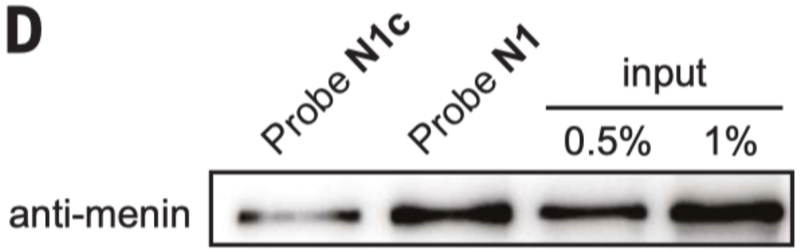

Fig 1. Pull-down of menin by probes N1 and N1c in nuclear extract.

- menin was enriched in the Probe N1 group

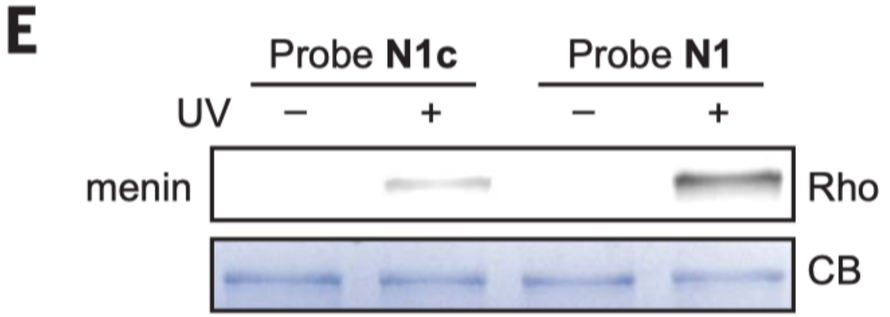

Fig 2. Photocrosslinking assay of recombinant menin by probes N1 and N1c

- Probe N1 captured menin more efficiently than probe N1c

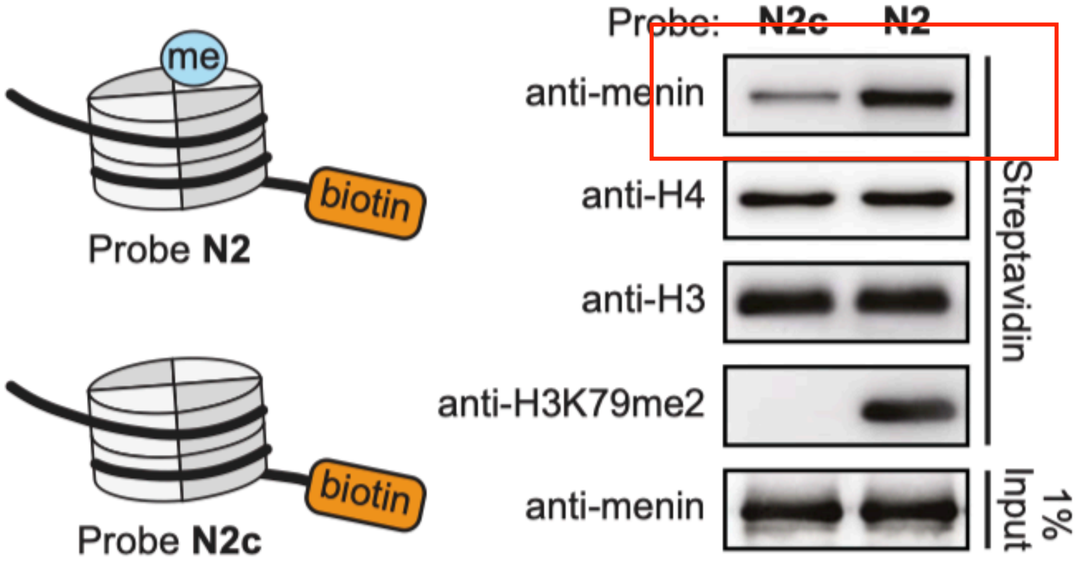

Fig 3. Non-crosslinking pull-down

- The observed interactions in photo-crosslinking experiments are not artifacts of the crosslinking process but reflect true biological interactions.

Menin forms a stable complex with nucleosome in higher concentration

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✳ *EMSA is designed to detect if a specific protein (or protein complex) binds to a specific DNA or RNA sequence by observing changes in the mobility of the DNA or RNA when it is run through a gel.*

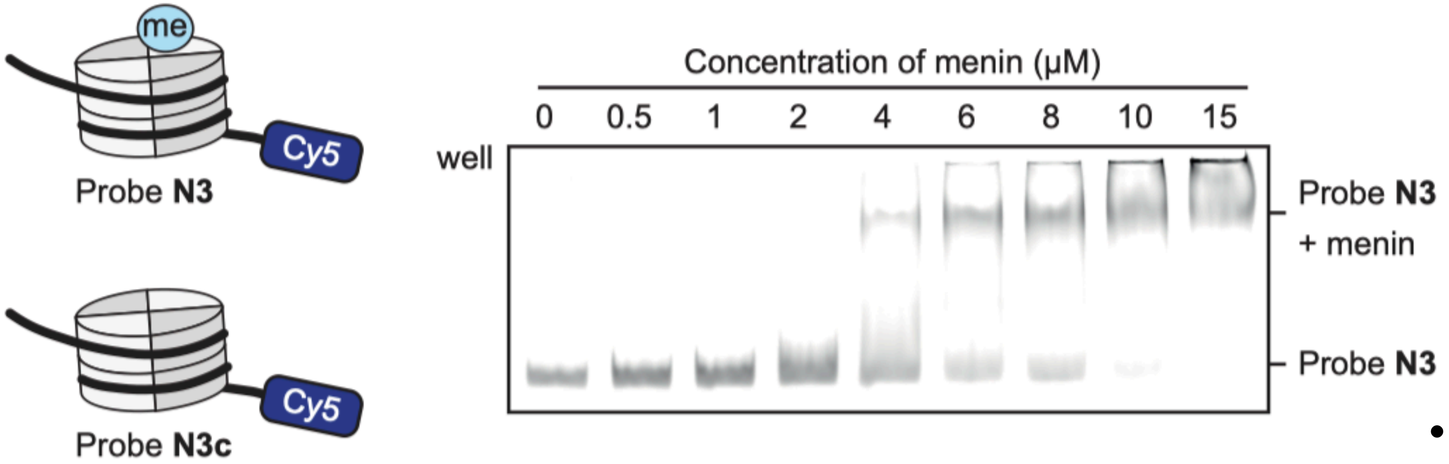

Fig 1.EMSA result of Cy5-labeled H3K79me2 nucleosome probe N3 titrated with menin

- The formation of a new band with lower mobility (a shifted band), which indicates the formation of a stable complex between menin and the H3K79me2 nucleosome.

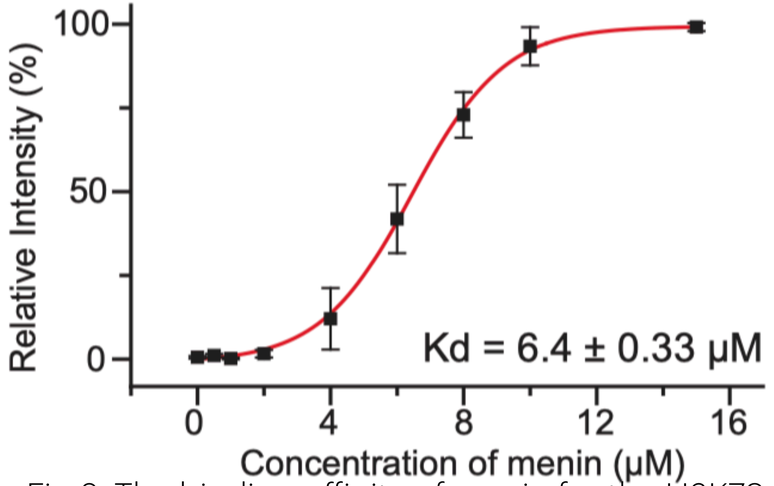

Fig 2. The binding affinity of menin for the H3K79me2 nucleosome

- The dissociation constant (Kd) is 6.4 μM, which represents a higher binding affinity of menin for the H3K79me2 nucleosome

Structural overview of menin bound to H3K79me2

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✳ *In the Menin-H3K79me2 structure, menin **binds to only one face** of the nucleosome disk , **covering almost half** of the nucleosome face*

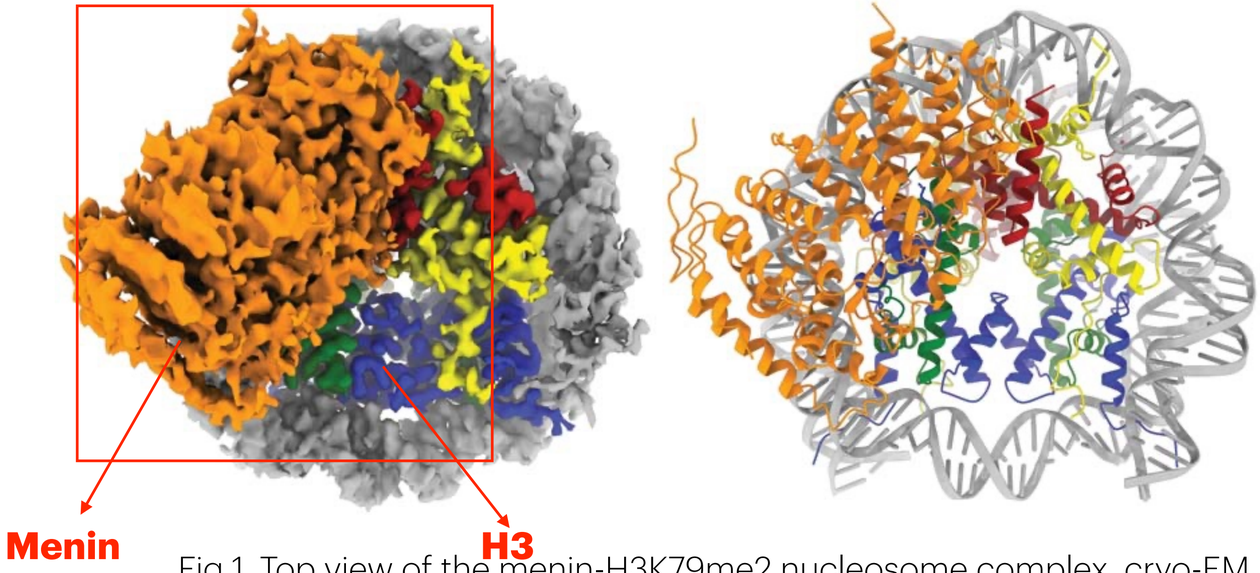

Fig 1. Top view of the menin-H3K79me2 nucleosome complex, cryo-EM map(Left), atomic model(Right)

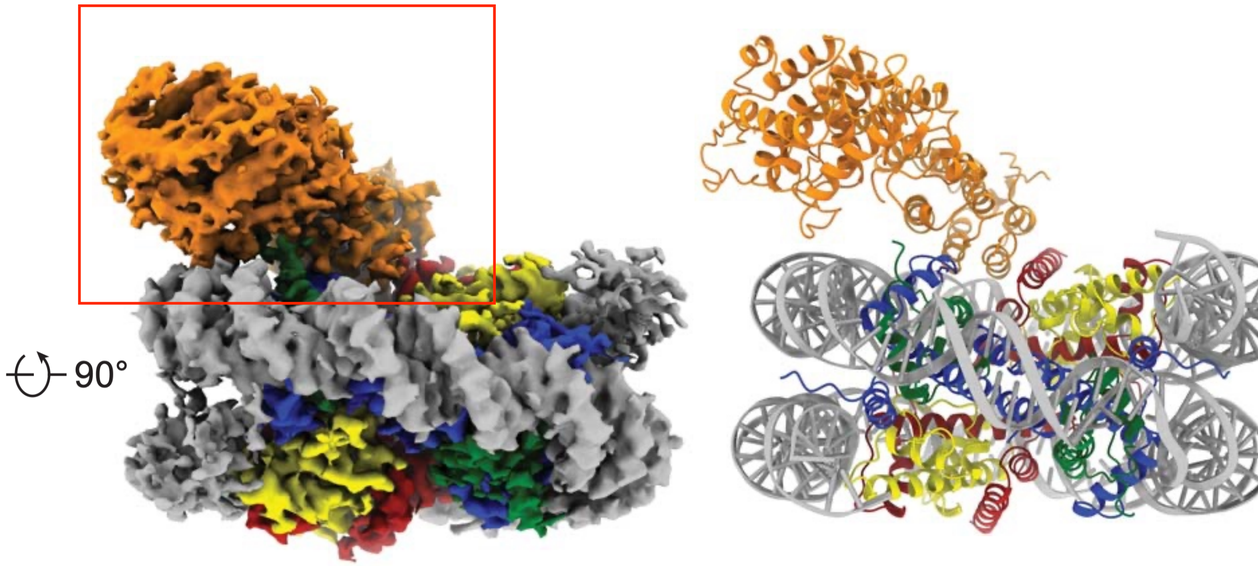

Fig 2. Side view of the menin-H3K79me2 nucleosome complex, cryo-EM map(Left), atomic model(Right)

Menin binds to H3K79me2 through its fingers and palm domains

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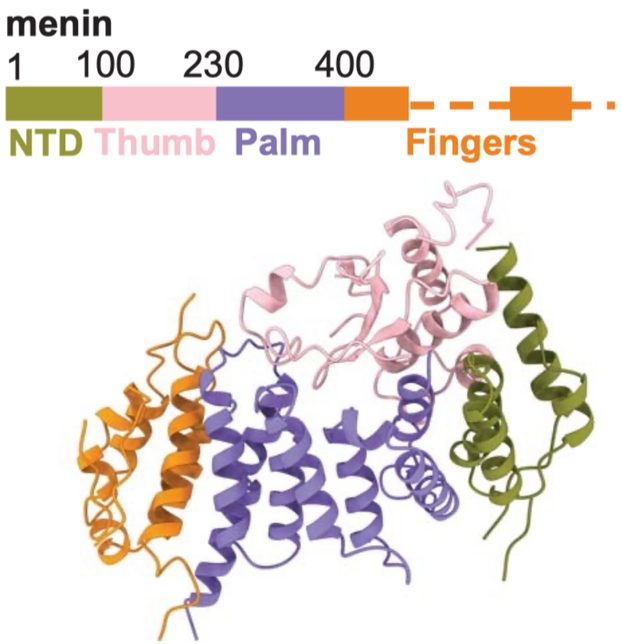
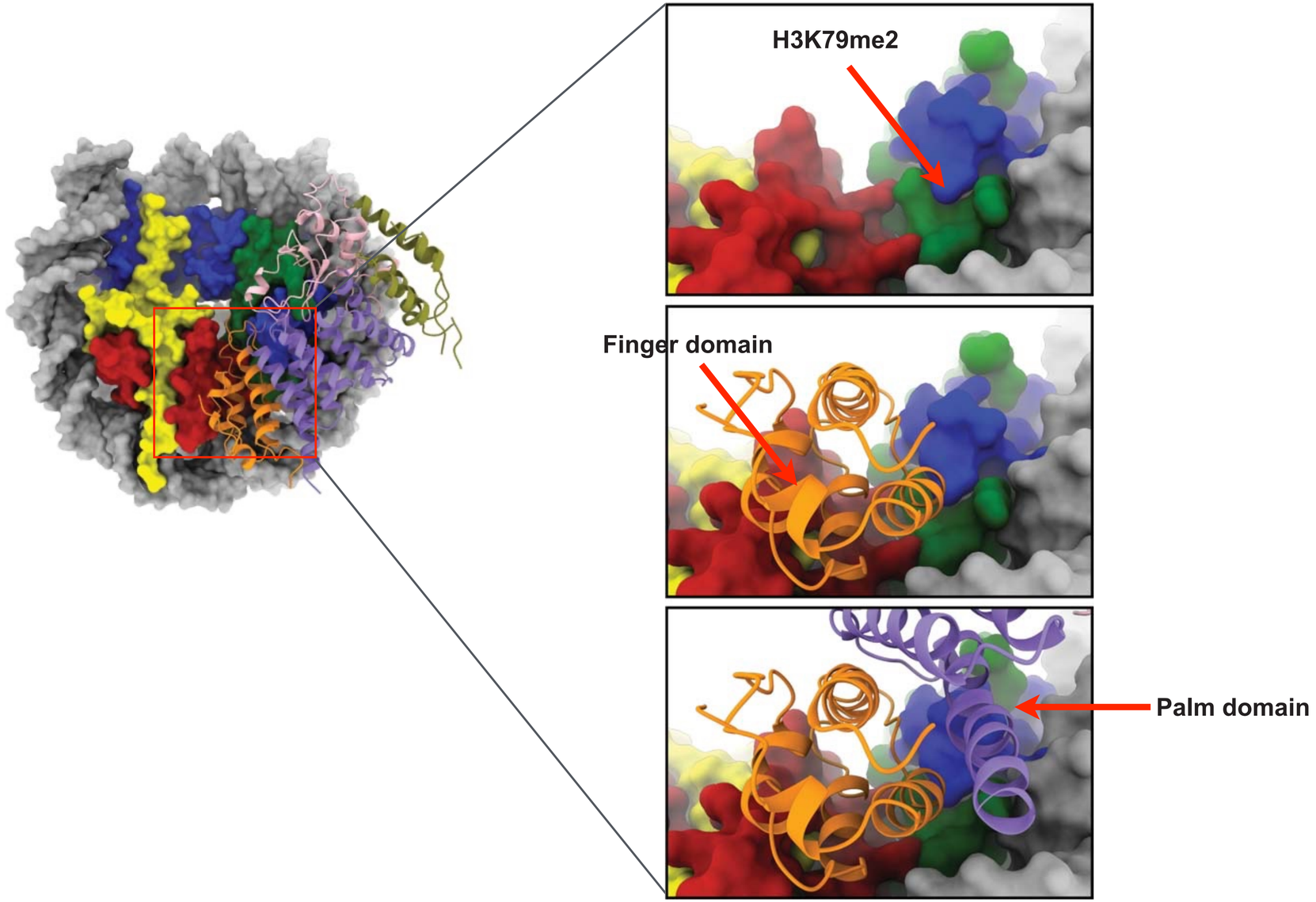


Fig 1. Schematic domain organization of menin.



Menin binds to H2B and H3 to secure the position for interaction

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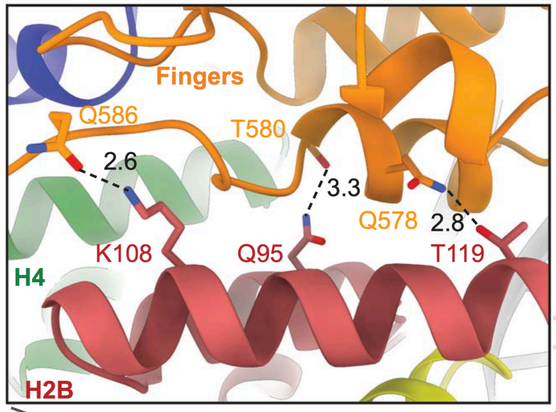
- The Finger domain engages with the C-terminal a-helix of H2B
- Palm domain forms four hydrogen bonds with the H3 L1 loop and a1 helix



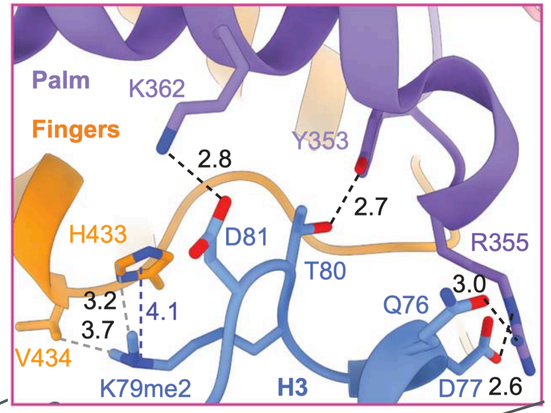


Fig 1. The Atomatic model from different view of Menin interacting with H3K79me2

H433 of menin is a key residue for the recognition of H3K79me2

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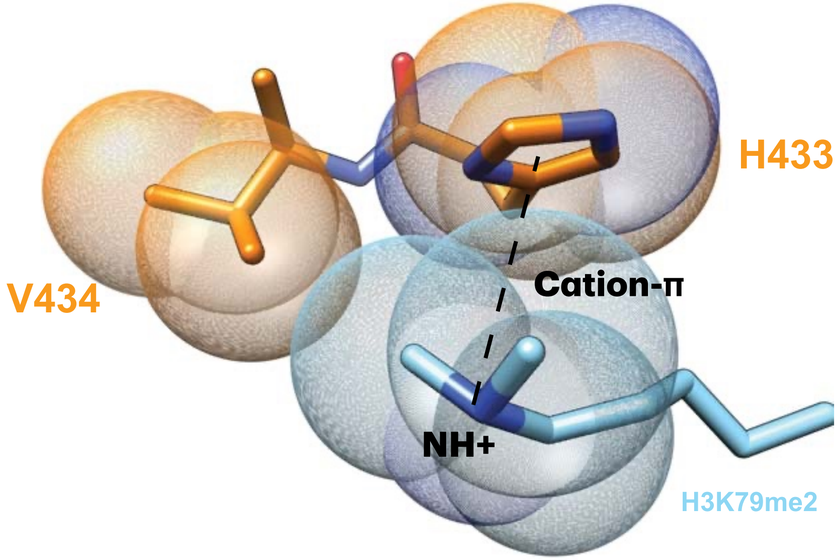

Fig 1. Magnified view of the p-cation interaction between menin H433 and H3K79me2

- The H433 and V434 residue of Finger domain stabilize the methylation site of the H3K79me2 through Cation-π and hydrophobic interaction.

- Hydrophobic interaction mediated by V434 is not essential for H3K79me2 recognition and the H433 region is crucial for recognition.

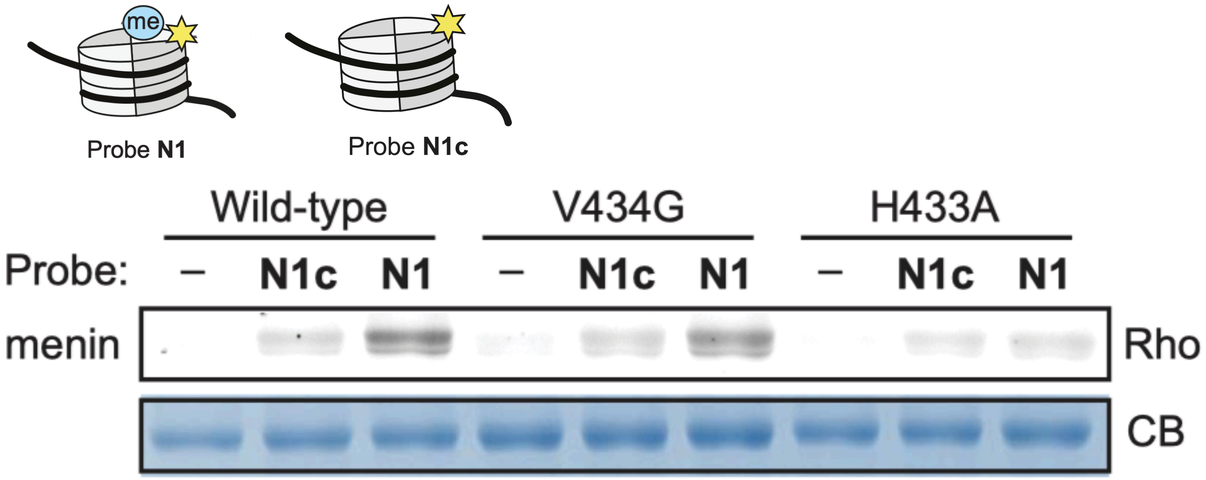

Fig 2. Photocrosslinking assay of recombinant wild-type, V434G, and H433A menin by probe N1 and N1c.

F365 of menin is a key residue for the recognition of Tri-functional handle

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The experimental methods used to map binding site:

*"The trifunctional handle of probe N1 enabled **crosslinking of the H3K79me2-binding region of menin** for enrichment and allowed the release of the cross-linked fragment for **MS analysis**"*

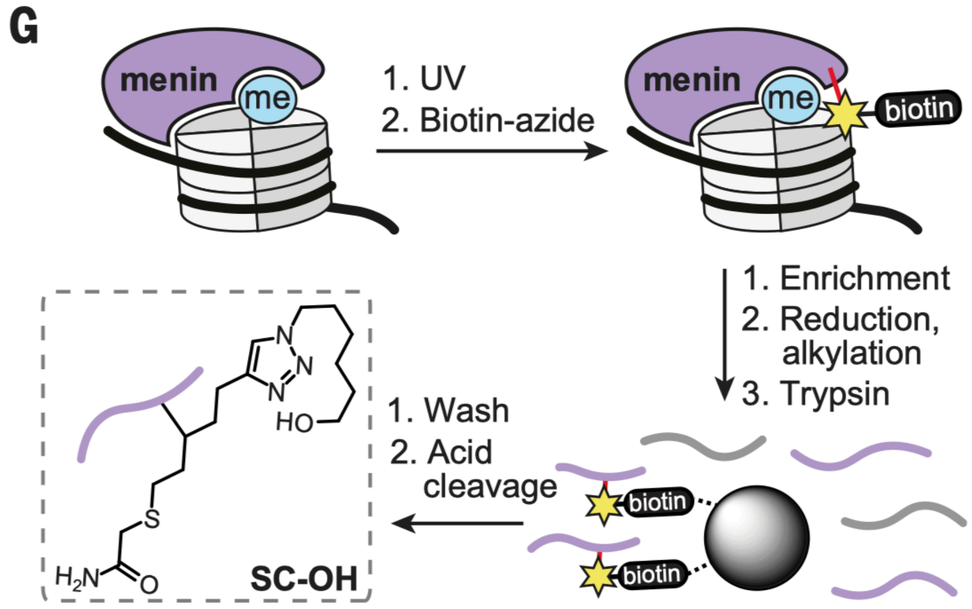

Fig 1. Workflow for mapping the specific sites on menin that interact with H3K79me2

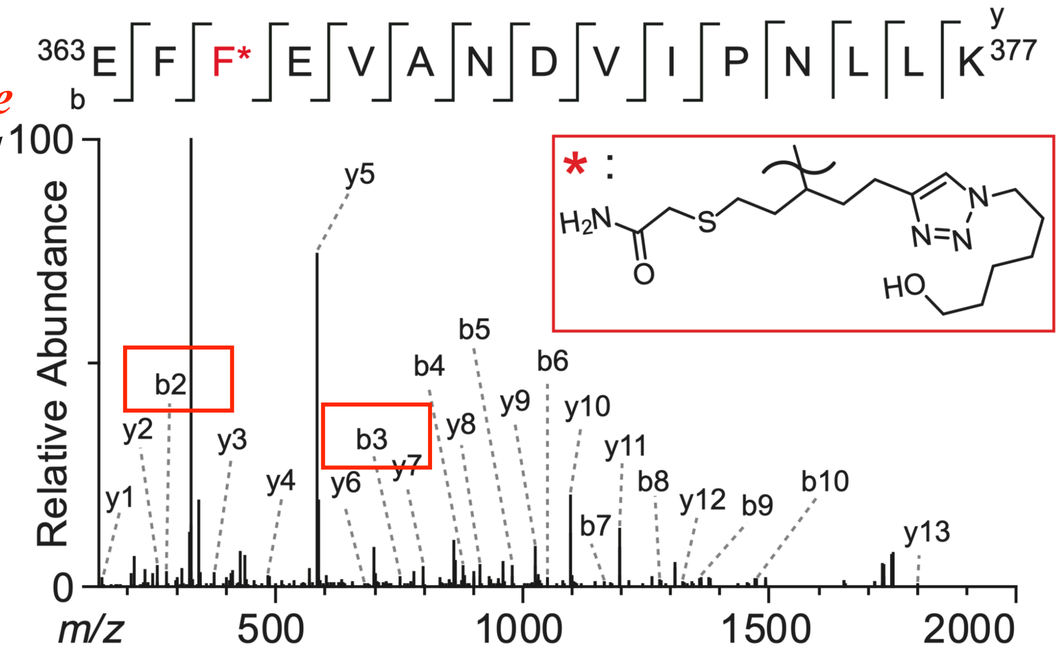

Fig 2. MS/MS spectrum for identified SC-OH–labeled menin peptide

- Based on the MS spectrum, the F365 is the residue which can binds with the tri-functional handle

The MLL1 and its inhibitor will not affect menin binding with H3K79me2

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✳ *" Menin also interacts with mixed lineage leukaemia protein 1 (MLL1), a histone H3 lysine 4 methyltransferase"*

——J. Huang et al., Nature 482

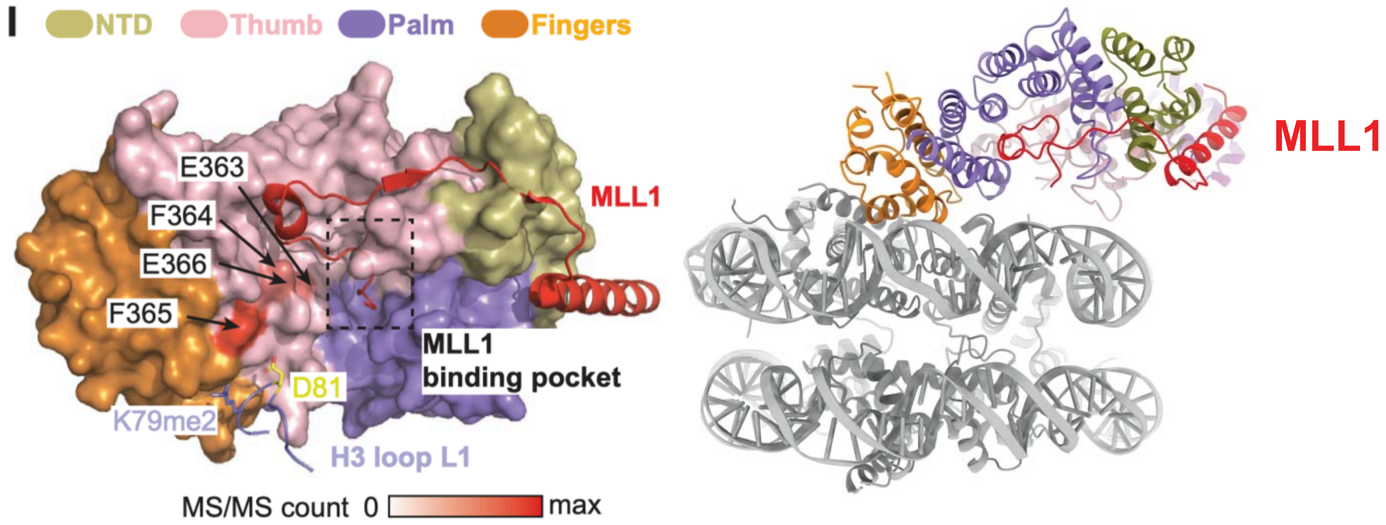

Fig 1. Mapping of the identified H3K79me2- binding sites

- The MLL1 binding site is distal to the F365 and H3K79me2 binding site

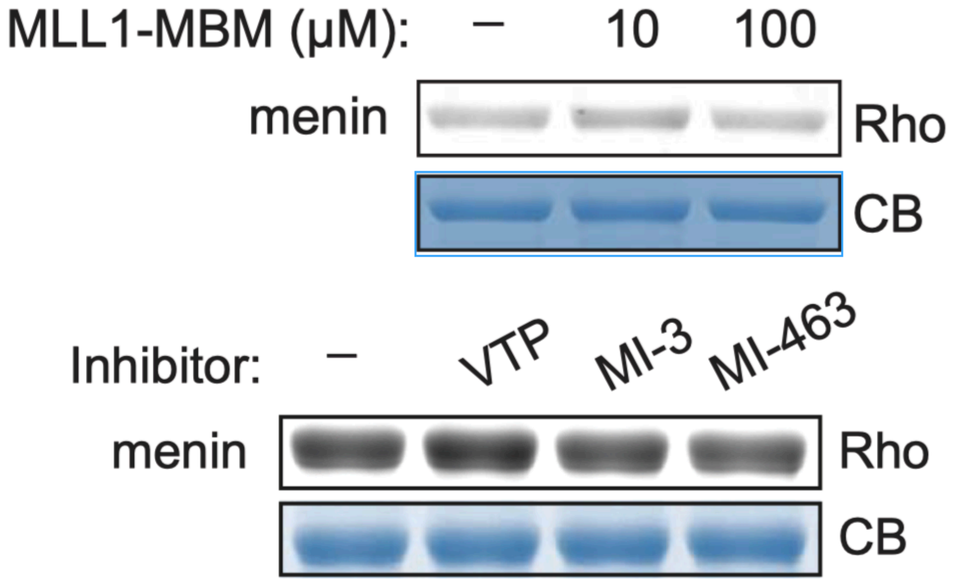

Fig 2. Photocrosslinking assay of menin by probe N1 in the presence of MLL1-MBM (up) or menin-MLL1 inhibitors(down)

- The MLL1 and its inhibitor will not affect the binding between menin and nucleosome

ChIP-seq illustrates a strong correlation between H3K79me2 and menin

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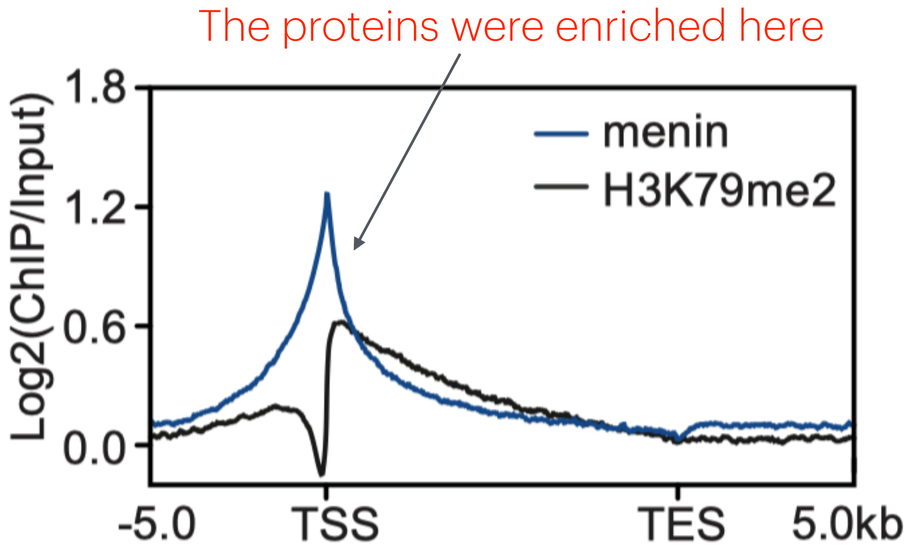

Fig 1. Menin and H3K79me2 log2(ChIP-seq/Input) signal across the gene body.

- The menin was enriched at TSS and H3K79me2 is also enriched here, which means these two protein might function simultaneously on gene bodies

- The menin signal was strongly predictable by H3K79me2

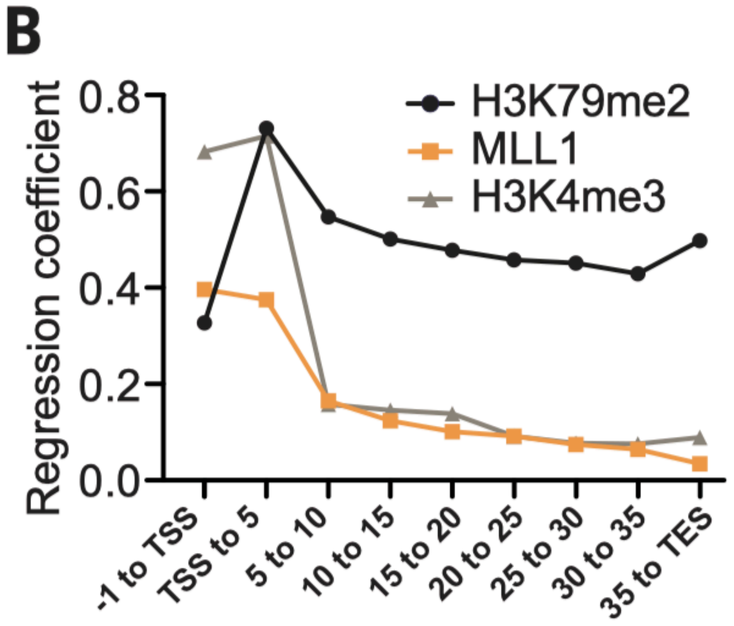

Fig 2. Linear regression coefficient of H3K79me2, H3K4me3, and MLL1 ChIP-seq signal/input against menin

The Loss of H3K79me2 will result in less interaction between menin and chromatin

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✳ *"We treated cells with EPZ5676, a DOT1L inhibitor, which resulted in a near-complete* ***global loss of H3K79me2****"*

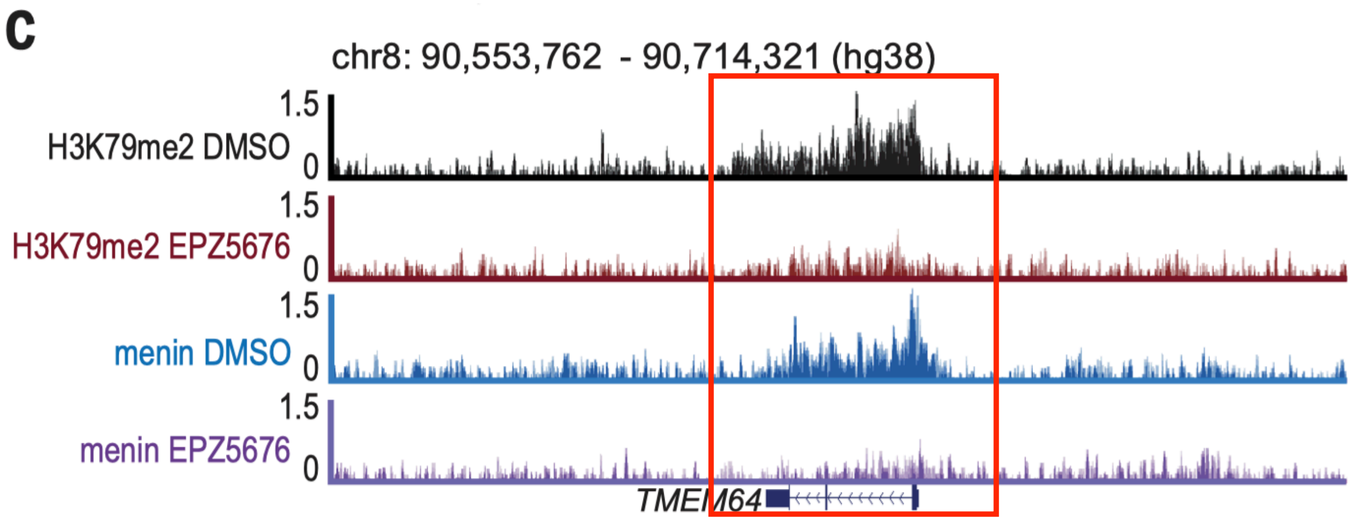

Fig 1. ChIP-seq signal of H3K79me2 and menin with DMSO or EPZ5676

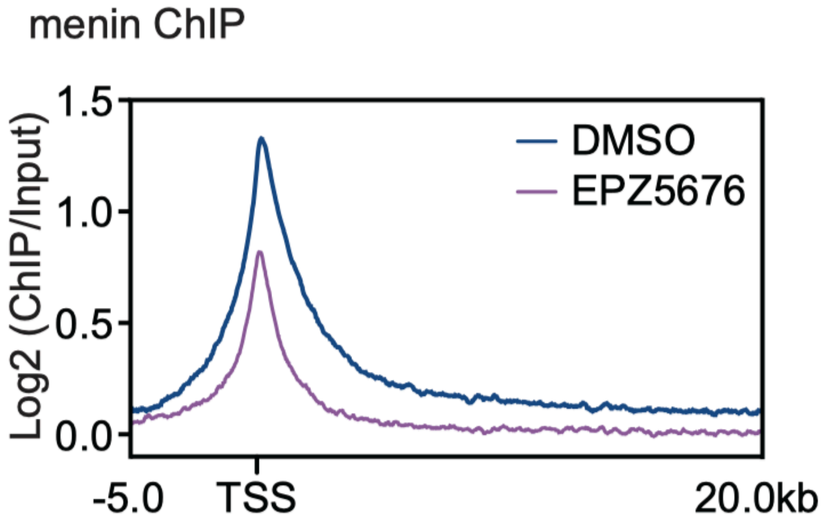

Fig 2. Menin enrichment with or without EPZ5676

✳ *"The loss of H3K79me2 attenuated the interaction between menin and chromatin"*

The menin associated with the chromatin in an H3K79me2 dependent manner

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- The loss of H3K79me2 strongly interfere the enrichment of menin.

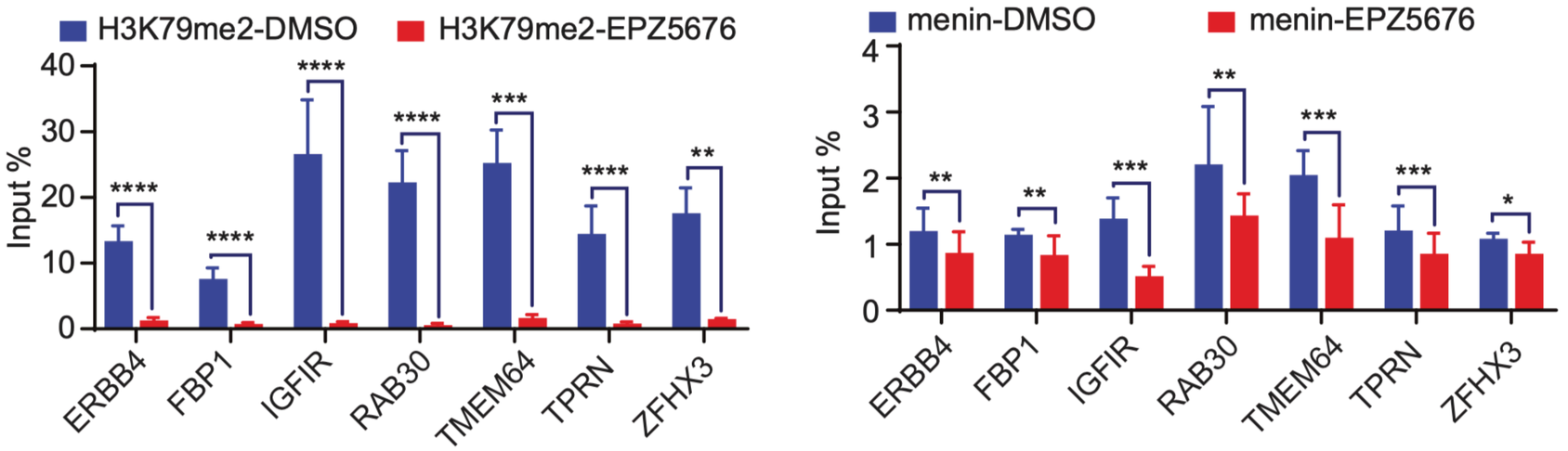

Fig 1. ChIP-qPCR analyses showing the changes in chromatin loci of H3K79me2(left) and menin(right)

- The loss of H433 also strongly interfere the menin's association with chromatin

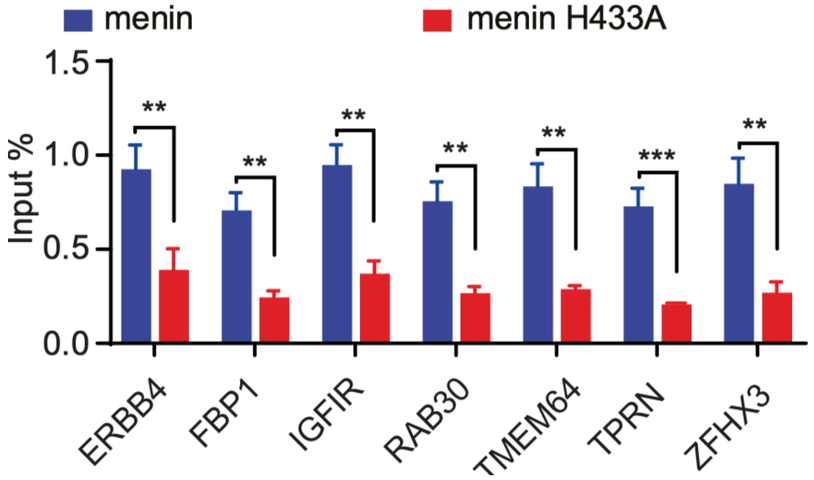

Fig 2. ChIP-qPCR analyses showing the changes in chromatin loci of H433 mutation

Menin might recognize the H3K79me2 mark at intragenic enhancer

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✳ *"H3K79me2 could activate the expression of target genes through the maintenance of enhancer–promoter interactions"*

——-*L. Godfrey et al.*

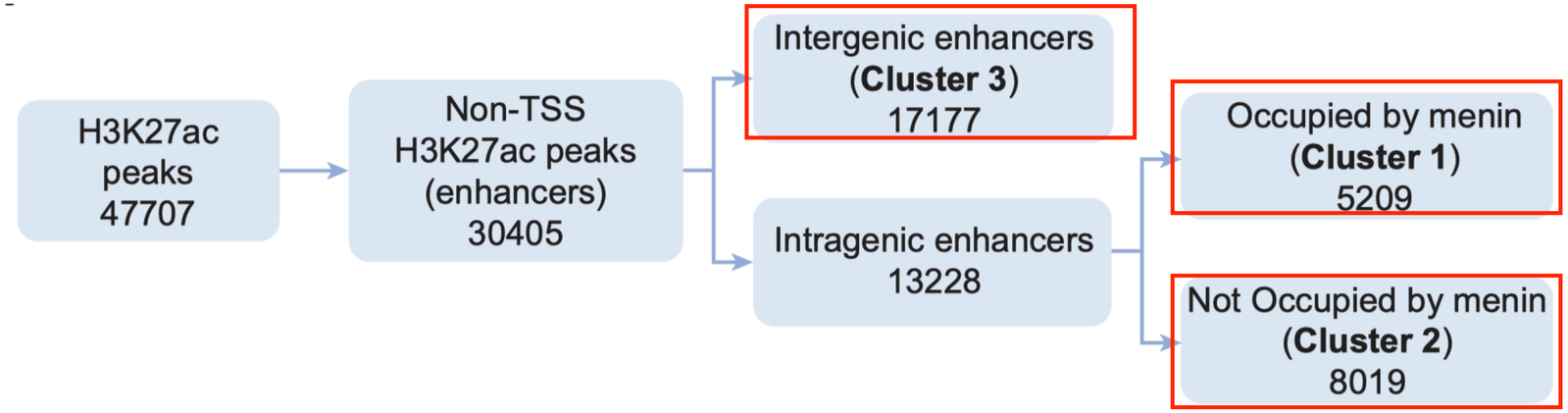

Fig 1. Schematic diagram illustrating how different categories of enhancers are defined.

- H3K27ac peaks help distinguish non-transcription start site (non-TSS) regions of the genome where enhancer activity might be present.
- After EPZ5676 treatment significantly down-regulated the the expression of Cluster1

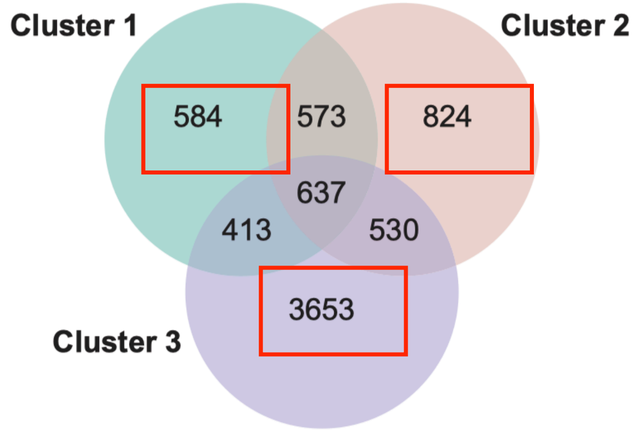

Fig 2. Venn diagram showing overlap of genes associated with enhancers from different clusters.

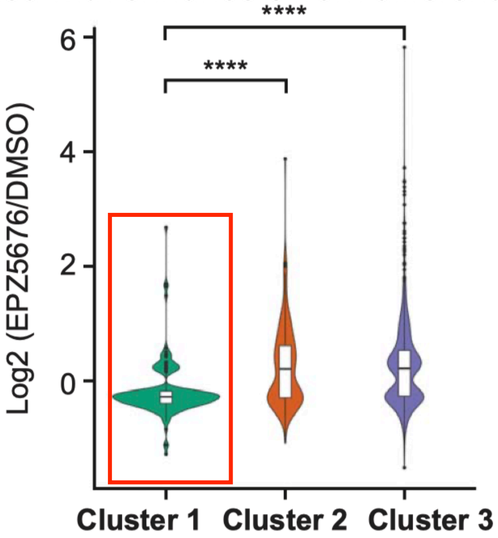

Fig 3. Violin plot of RNA-seq data representing differential gene expression

Conclusion and Limitation

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- **Conclusion**
  - How to identify the "Reader"
    - Synthesis and identification of the Probe N1
    - Identification of menin and its association with H3K29me2
  - What is the structure basis of such reader bind with H3K79me2?
    - Menin binds to H3K79me2 nucleosome through its fingers and palm domains
    - H433 of menin is a key residue for the recognition of H3K79me2
    - Menin binds H3K79me2 nucleosome and MLL using two different pockets
  - How this "Reader" involved in transcriptional regulation?
    - The menin associated with chromatin in an H3K79me2 dependent manner
    - Menin is involved in transcriptional regulation through binding to H3K79me2 at potential intragenic enhancers

Limitations

This identification is performed in vitro

Do not explain why the interaction is weak and transient

Questions

- What further research we can do based on these findings?

  There are some diseases caused by the H3K79me2, we can study the role of menin in the development of these diseases.