

MMSET

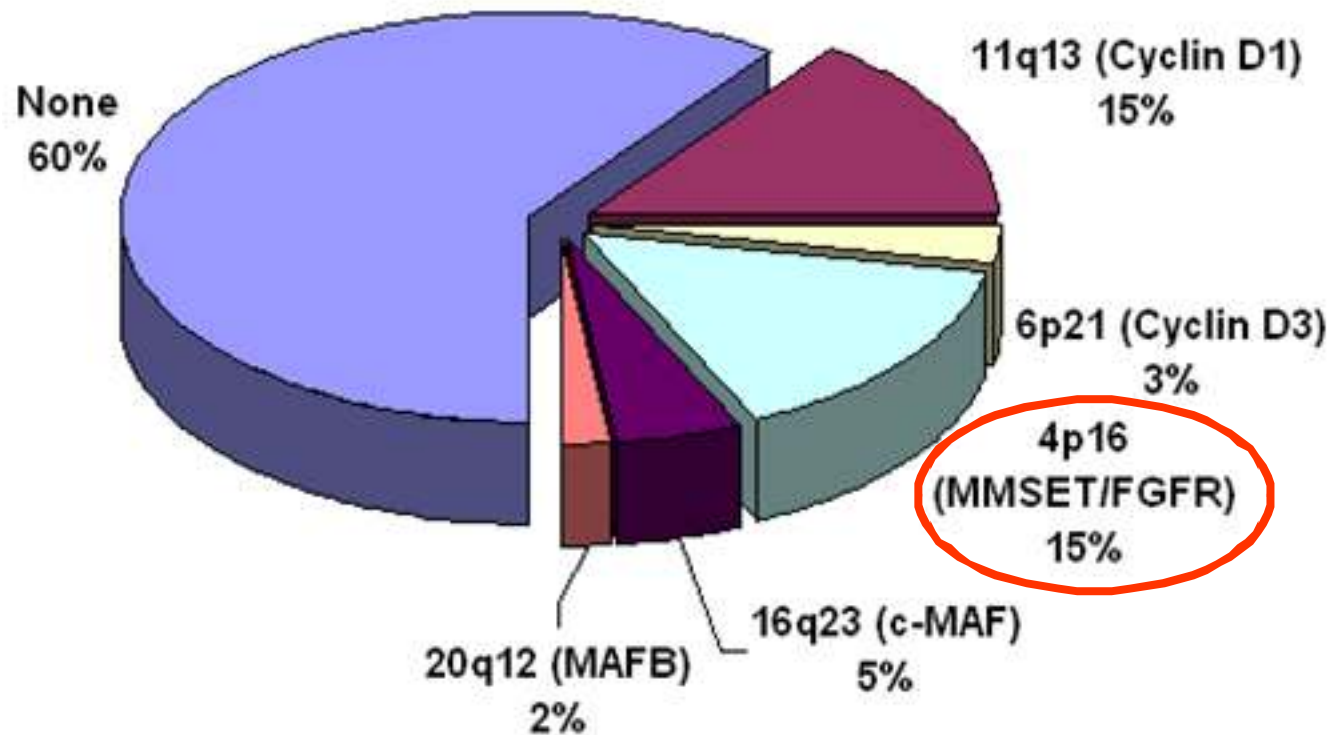
The Histone Methyl Transferase Affected In (t4;14) Multiple Myeloma

Jonathan D. Licht

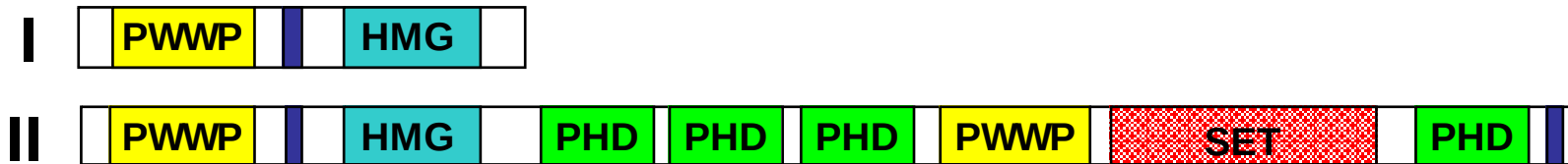
Hematology/Oncology

Robert H. Lurie Comprehensive Cancer Center

Chromosomal Aberrations In Multiple Myeloma Drive Oncogene Expression



The MMSET Protein Contains Domains Involved In Chromatin Function



 **SET** = **S**uvar, **E**nhancer-of-Zeste and **T**rithorax domain; catalyzes lysine methylation in histones and transcription factors.

 **HMG** = **H**igh **M**obility **G**roup; Possible DNA binding domain.

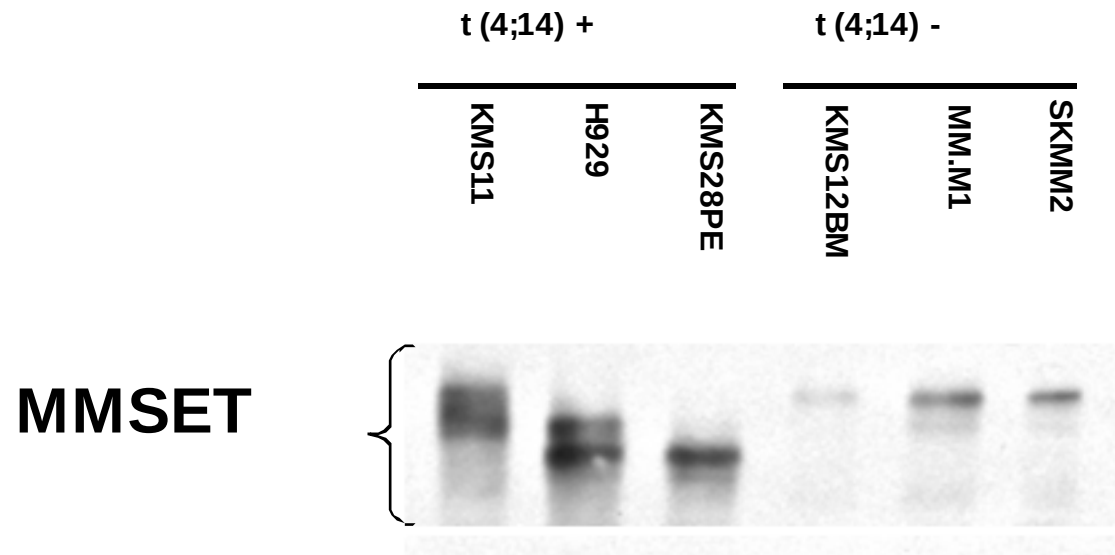
 **PWWP** = Pro-Trp-Trp-Pro; found in nuclear proteins.

 **PHD** = **P**lant **H**omeo**D**omain; methyl-lysine binding module.

 **NLS** = **N**uclear **L**ocalization **S**equence

MMSET Overexpressed in t(4;14)

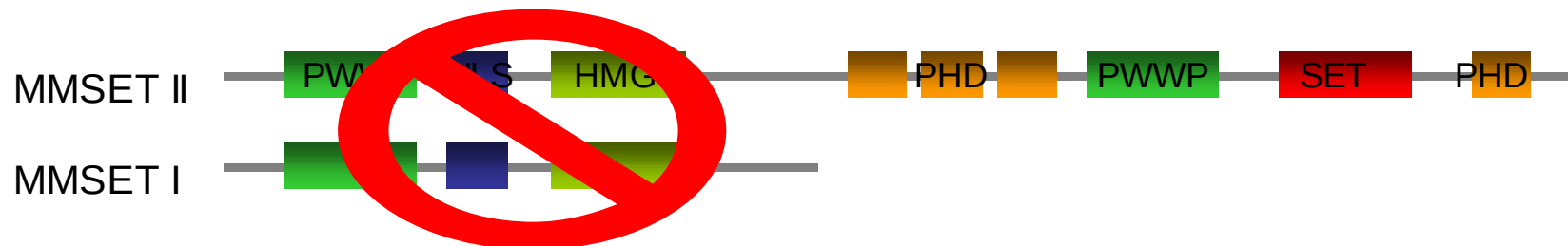
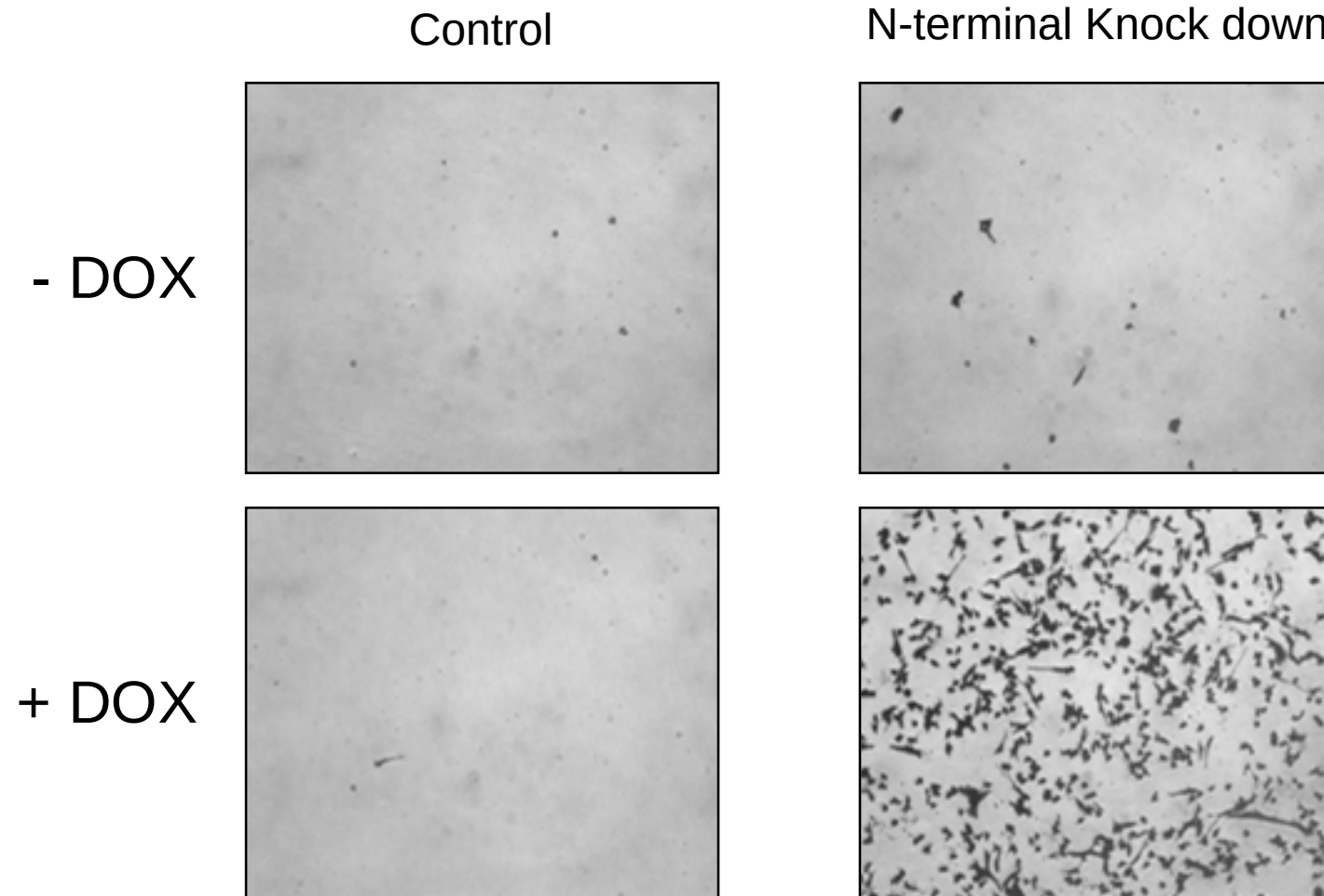
What Are the Consequences?



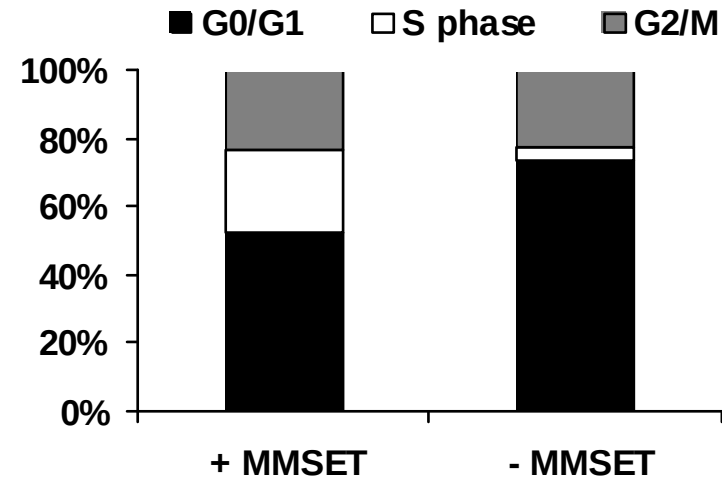
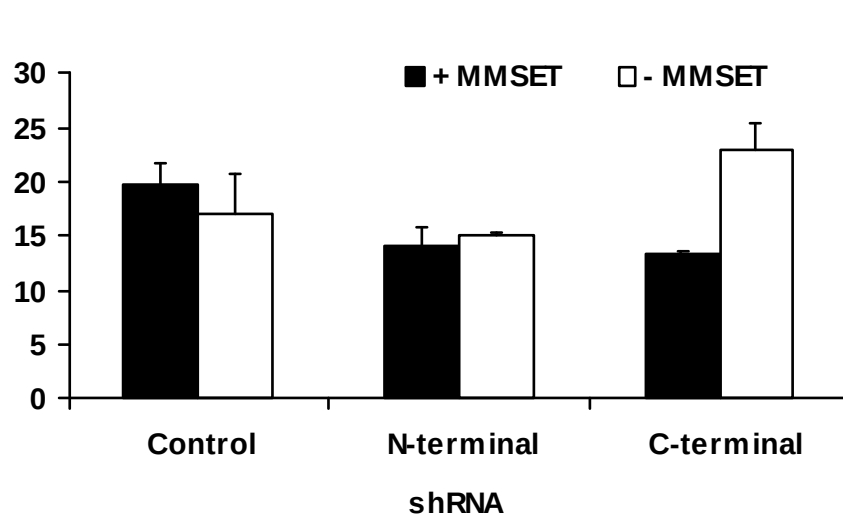
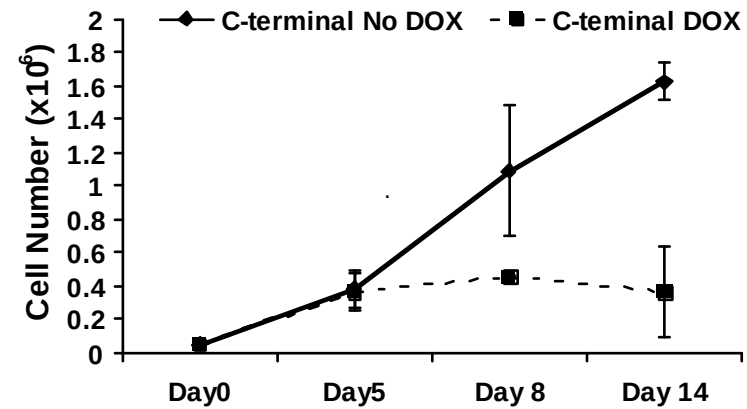
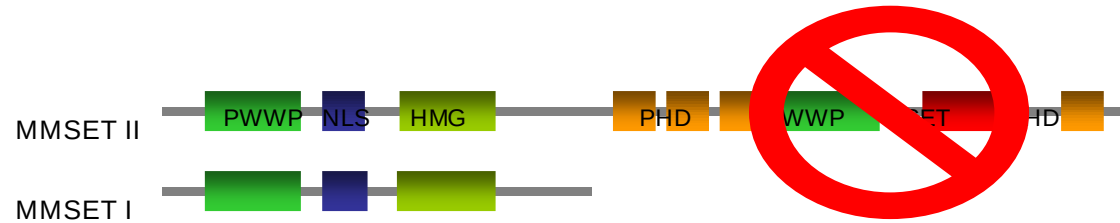
MMSET in Myeloma

- Biological Function - Is MMSET Overexpression Important?
- Can Biochemical Analysis Lead to Insights into Disease Pathogenesis and Therapy?

MMSET Effects on **Adhesion** Properties



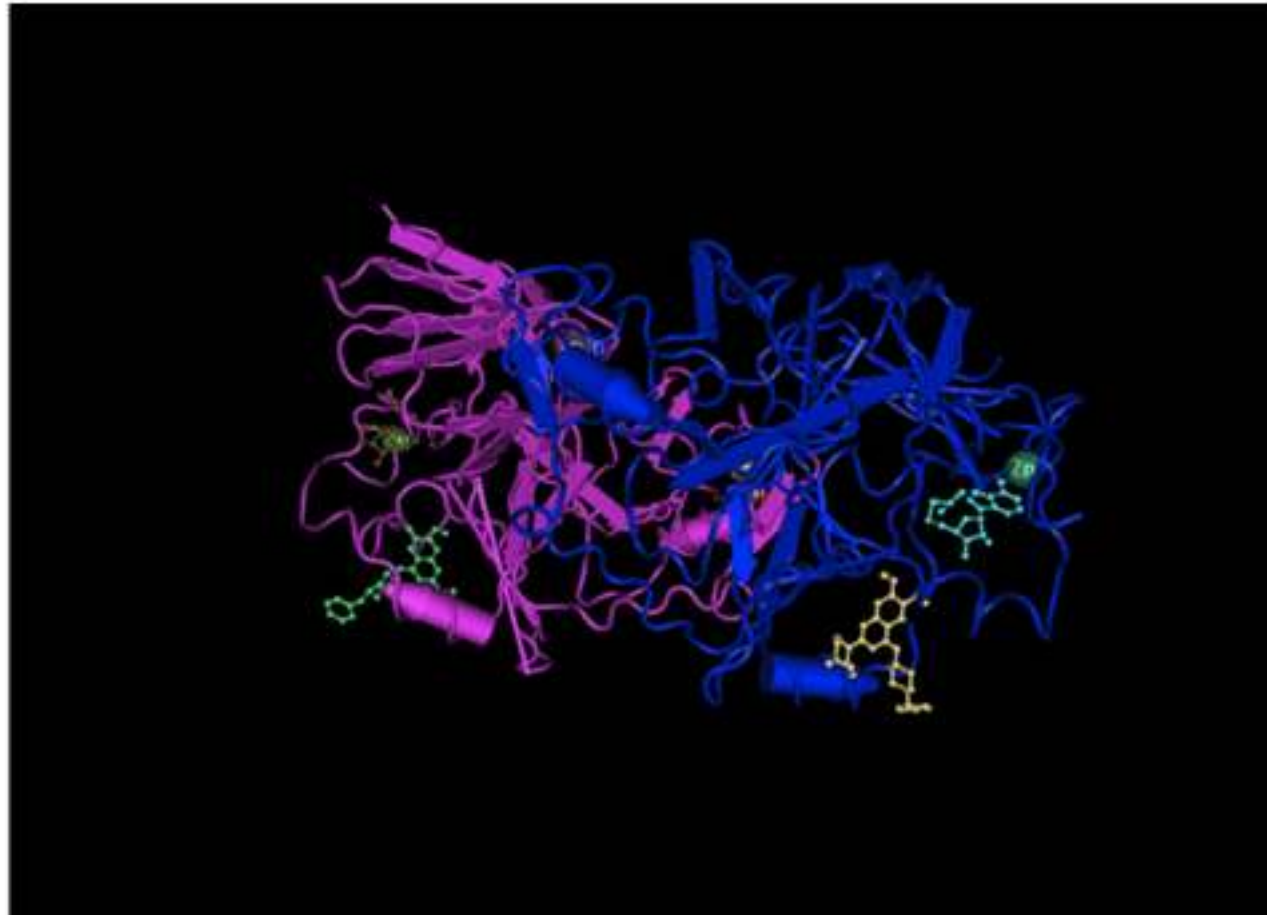
MMSET Effects on Growth Properties



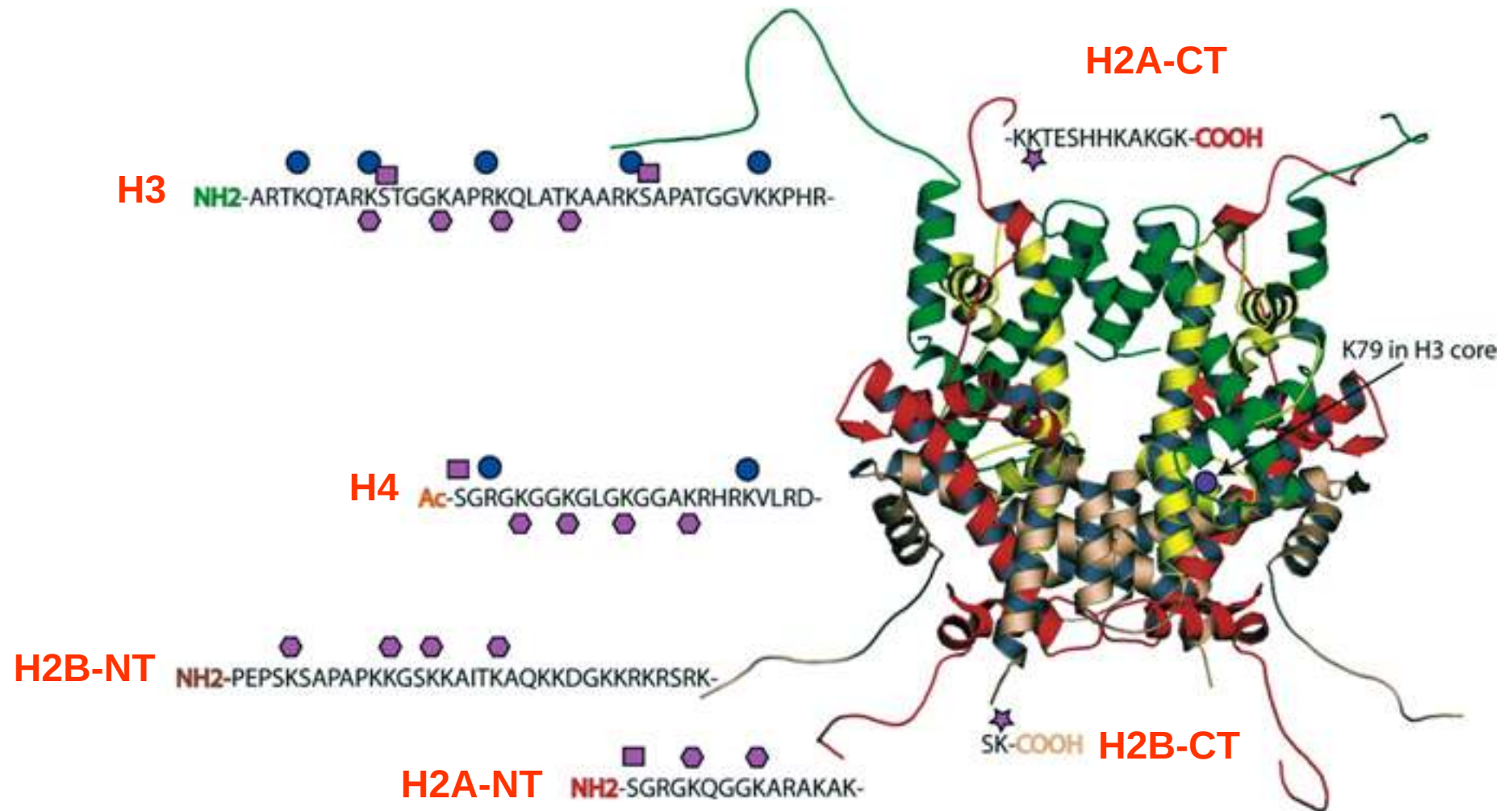
MMSET in Myeloma

- Biological Function - Is MMSET Overexpression Important?
- Can Biochemical Analysis Lead to Insights into Disease Pathogenesis and Therapy?

SET Domain

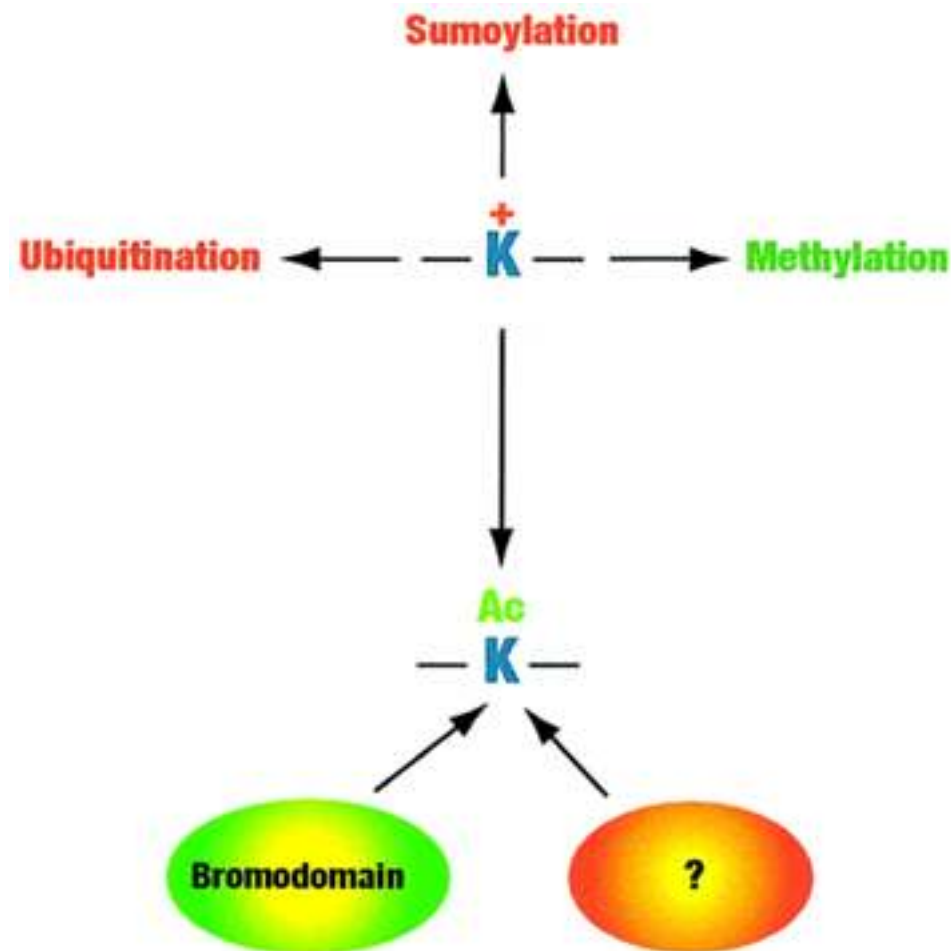


Histone Post-translational Modifications

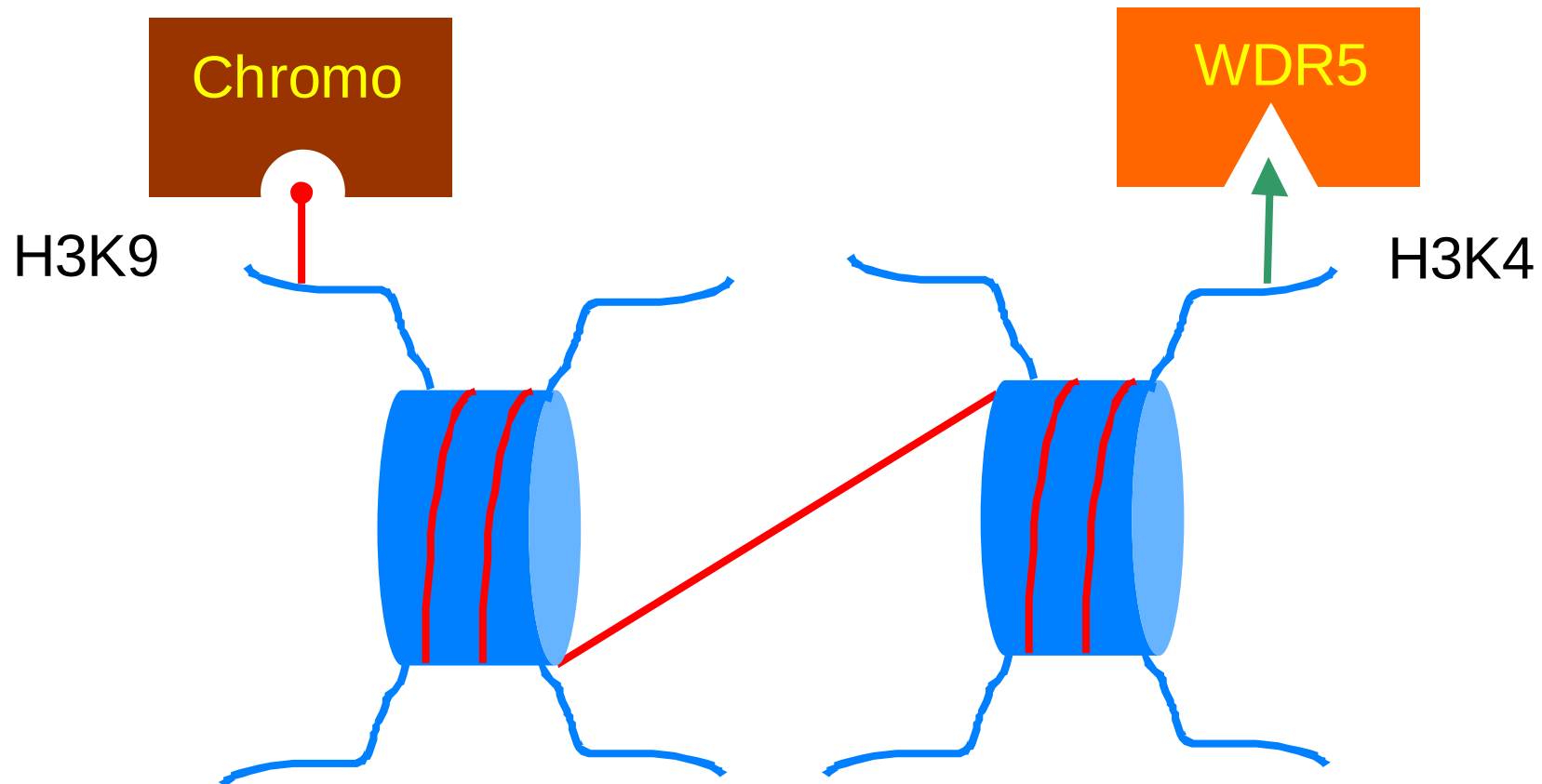


Histone Lysine Modifications

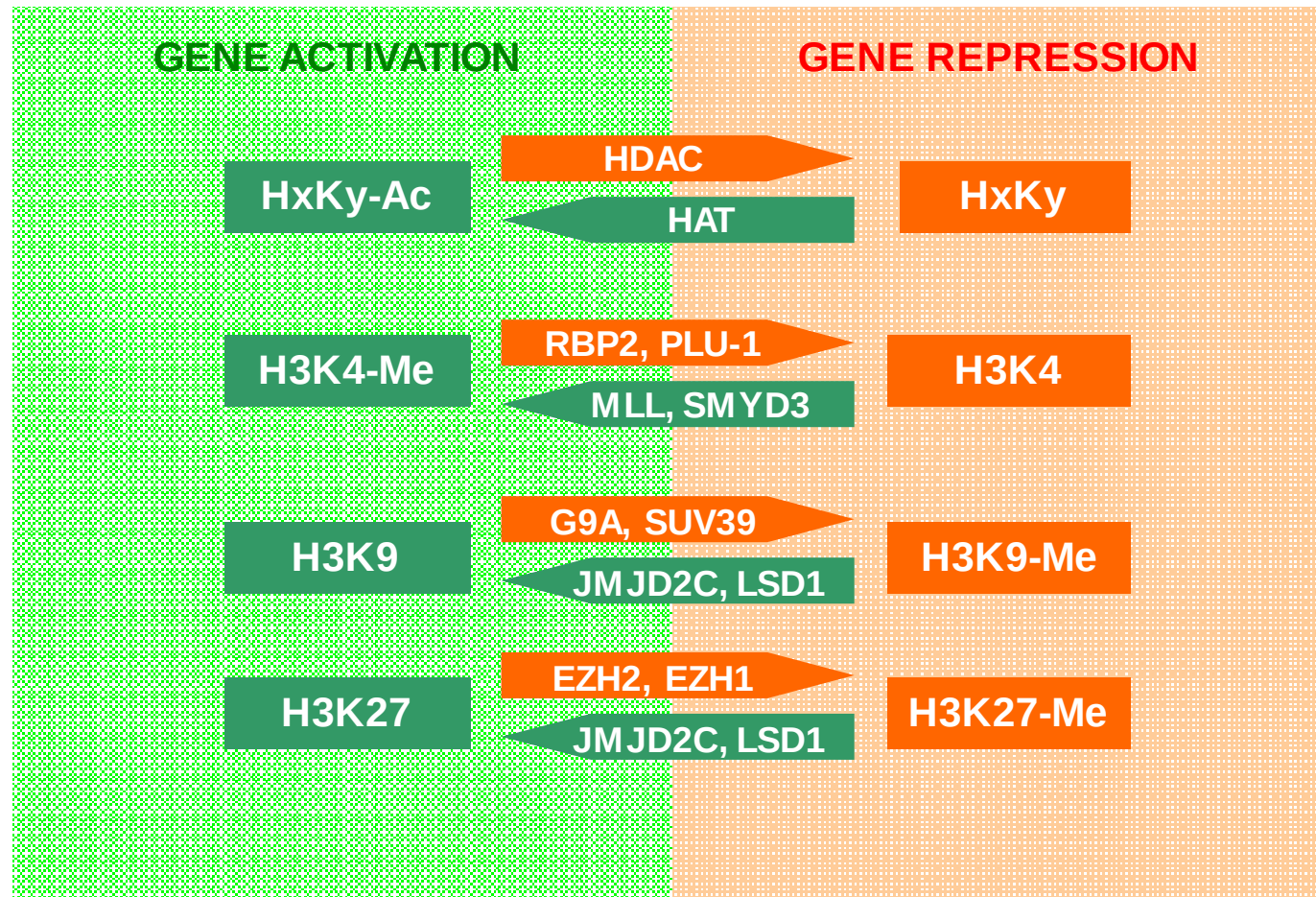
Lysines are the most diversely modified residues in histones.



Histone Modifications are “Read” By Specific Modules



Different Chromatin Modifications and Enzymes Are Associated With Alternative Transcriptional Outcomes



Hypothesis: MMSET is a Reader and a Writer of the Histone Code

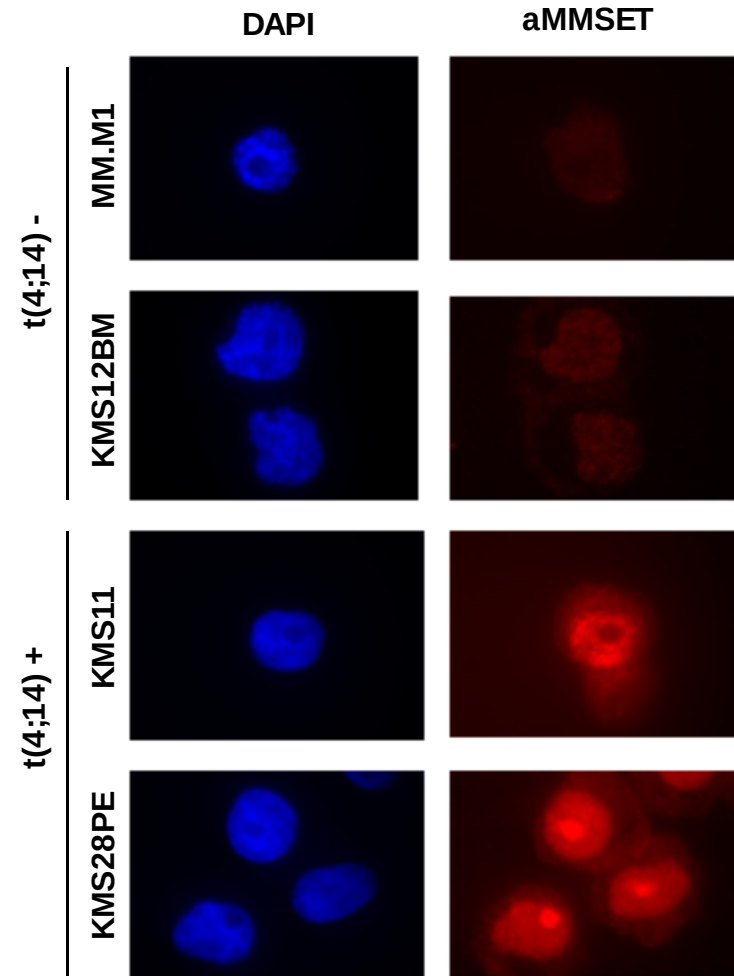
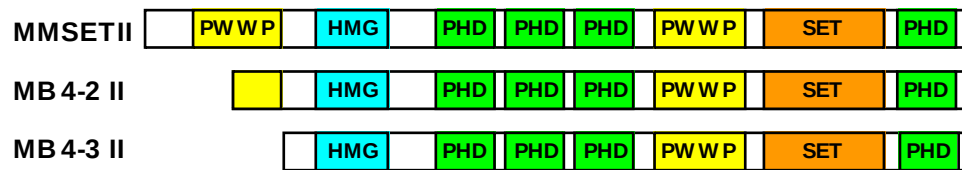
What Mark(s) Does MMSET Read?

What Mark(s) Does MMSET Make?

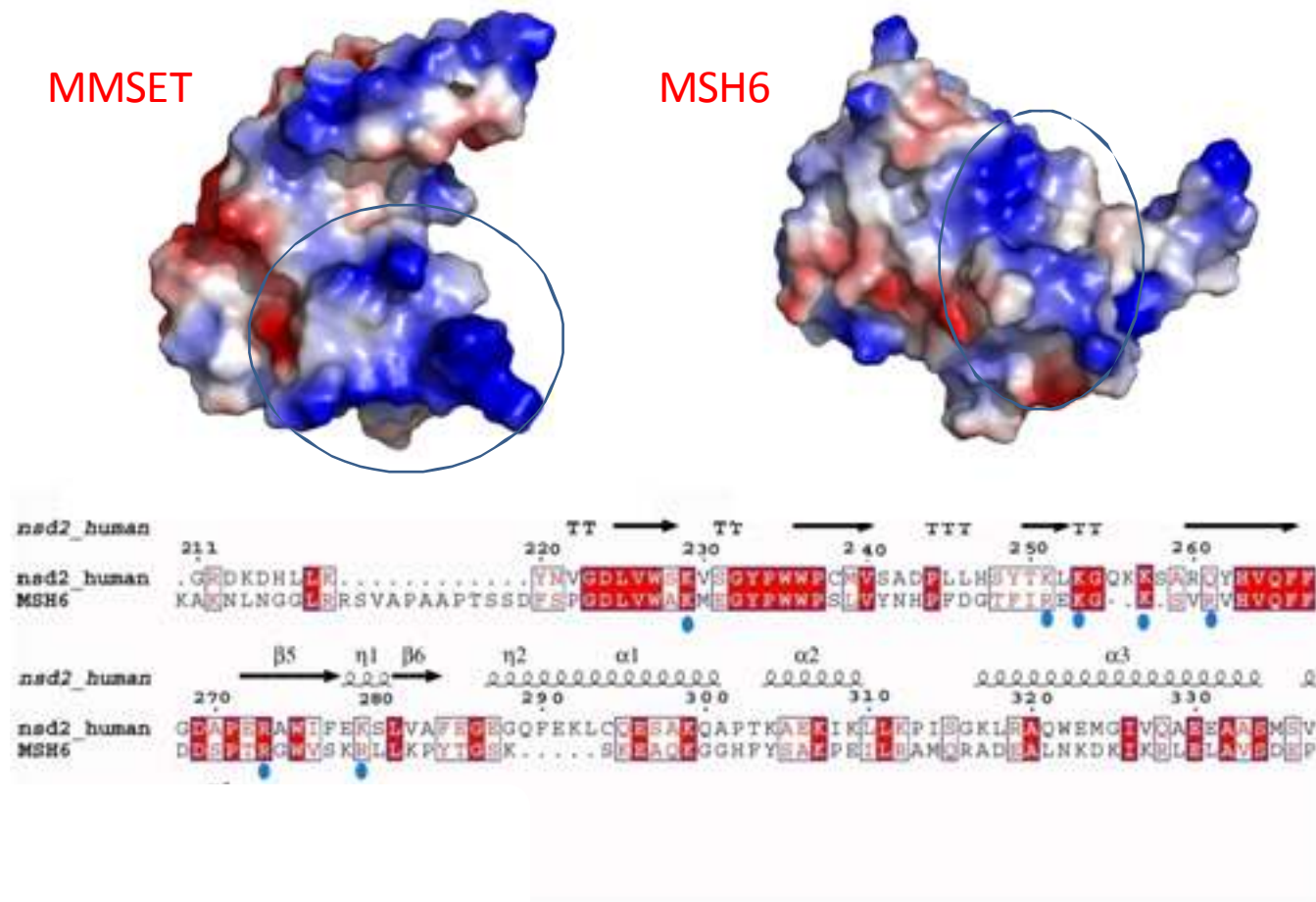
How Does This Mark Affect Gene Expression?

Can MMSET Be Inhibited?

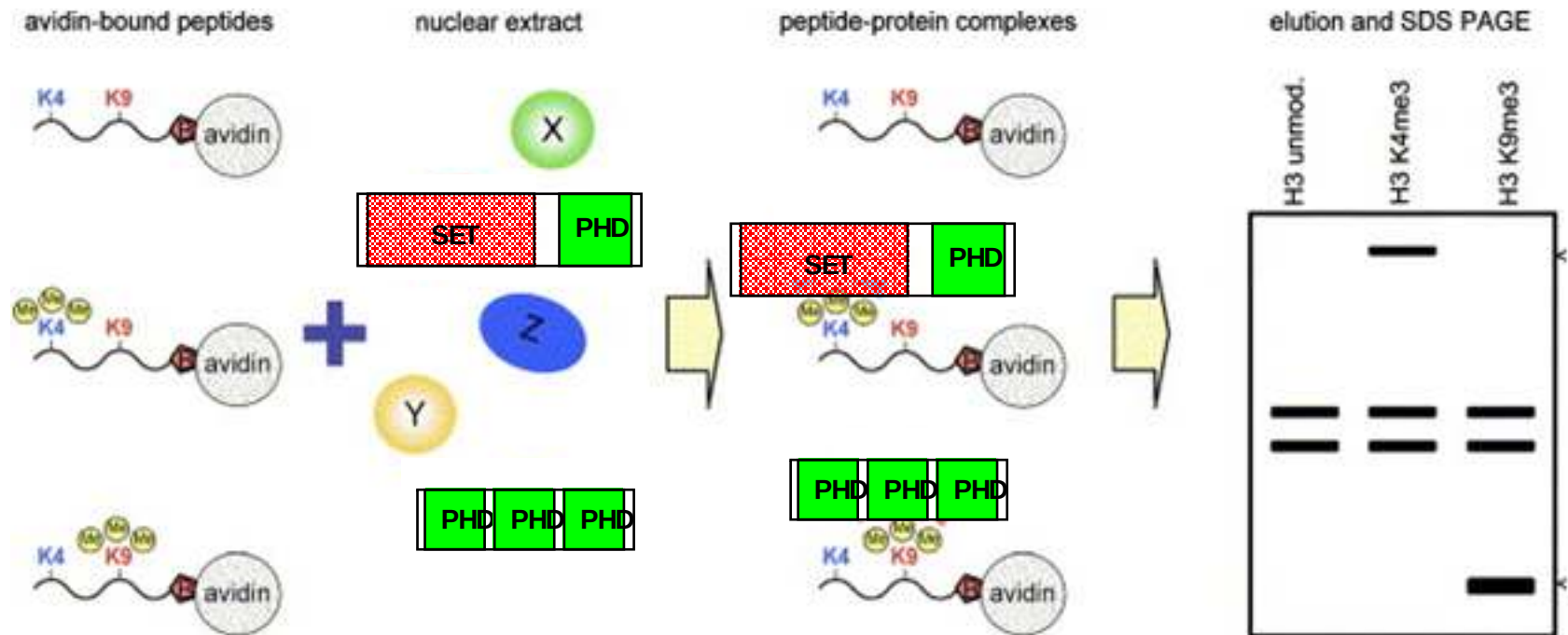
The N-terminal PWWP Motif Is Necessary for Tight Chromatin Binding



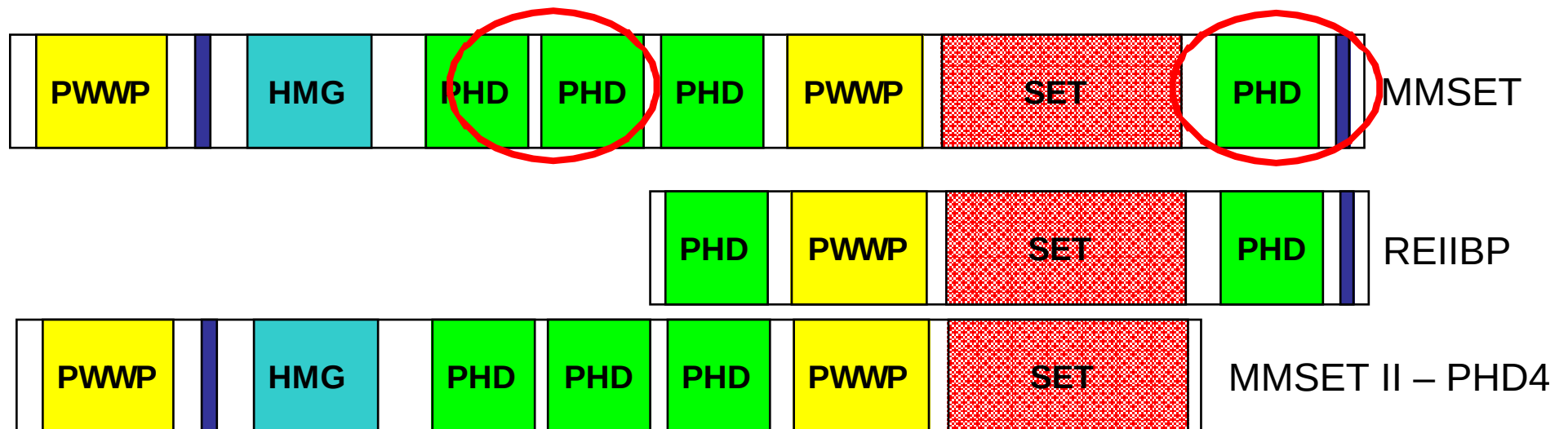
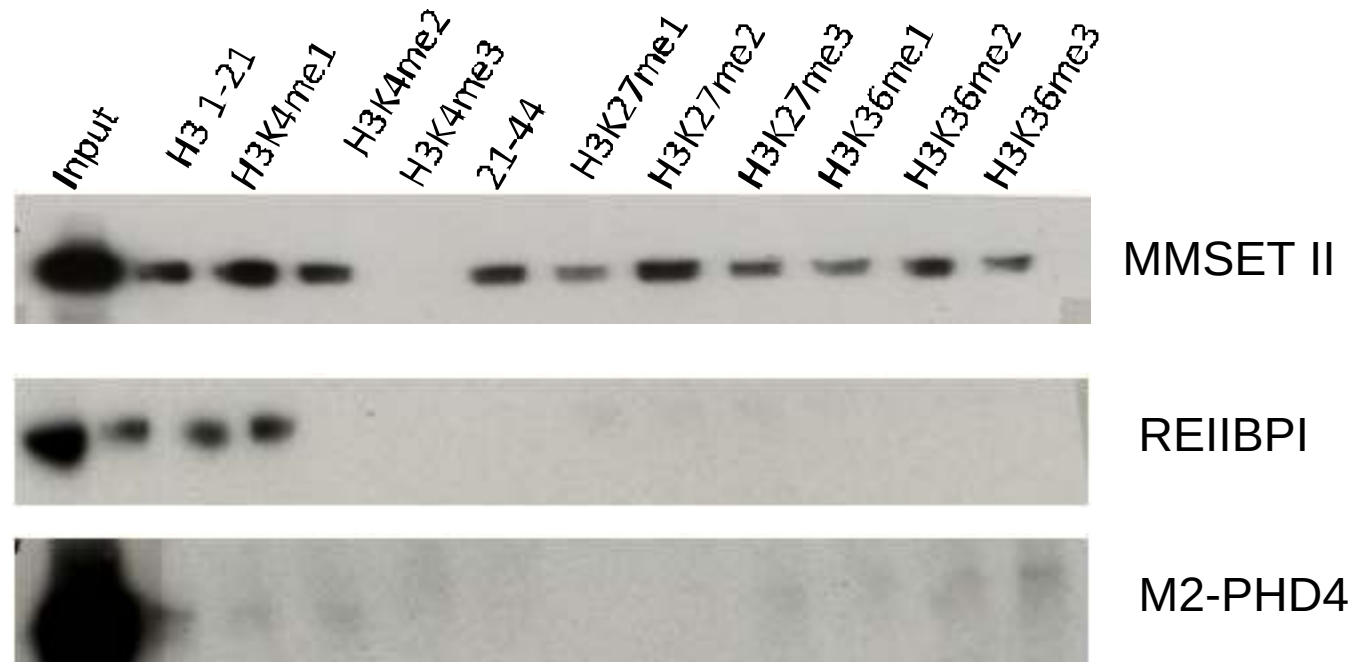
The first PWWP domain in MMSET is also a potential DNA binding module



How does MMSET Read the Histone Code?



How does MMSET Read the Histone Code?



MMSET- Reads DNA/Chromatin

- DNA Binding - Not specific
- H3K36, H3K27 Mark Binding
- PHD Domains- Possible Targets

Hypothesis: MMSET is a Reader and a Writer of the Histone Code

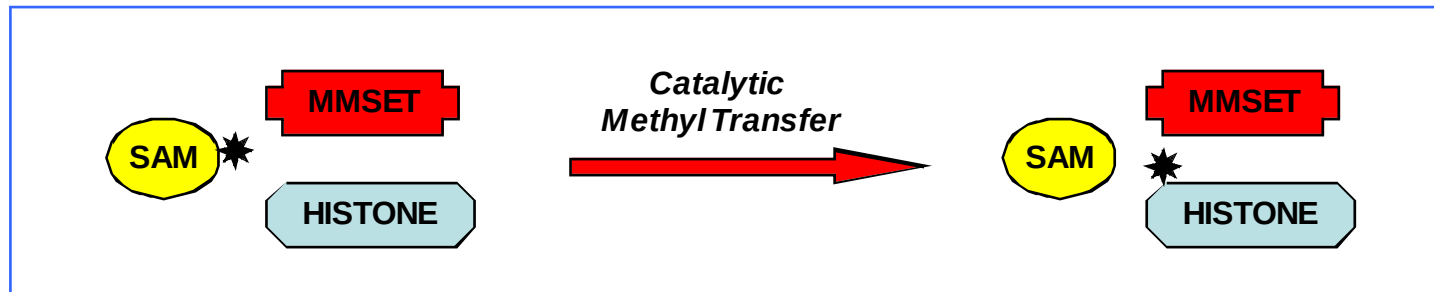
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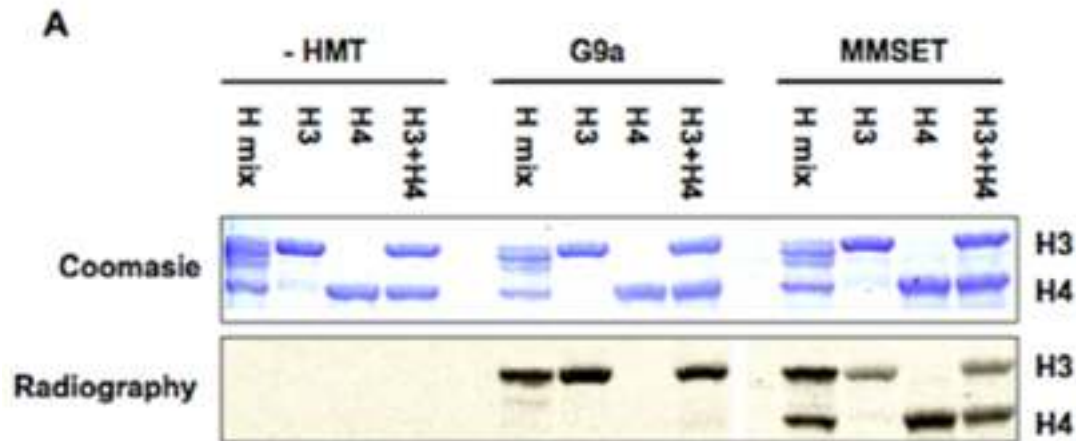
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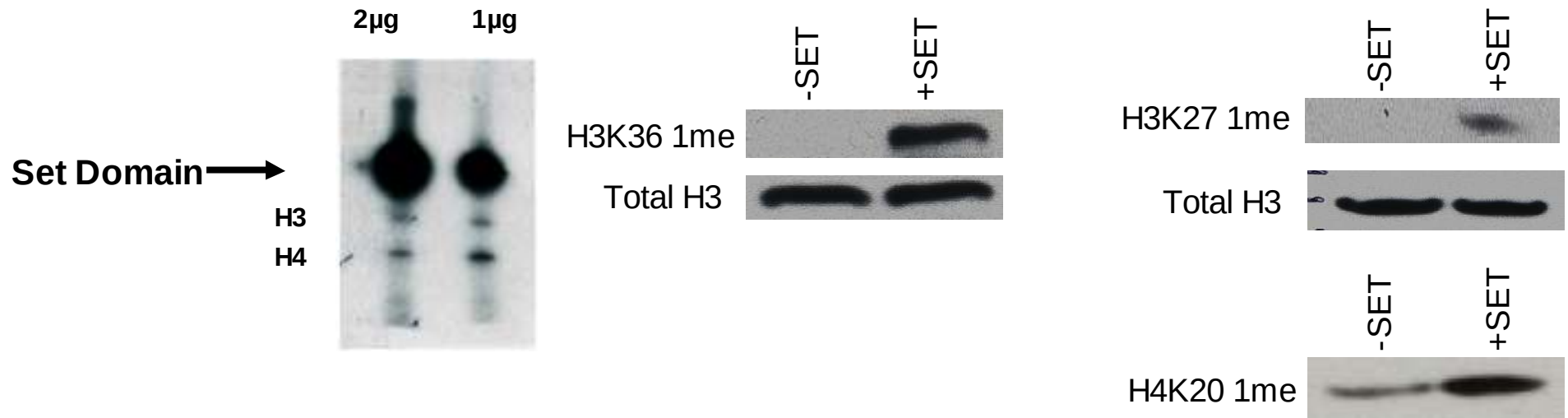
MMSET: *in vitro* Methyltransferase Activity For Histone H3 and Histone H4



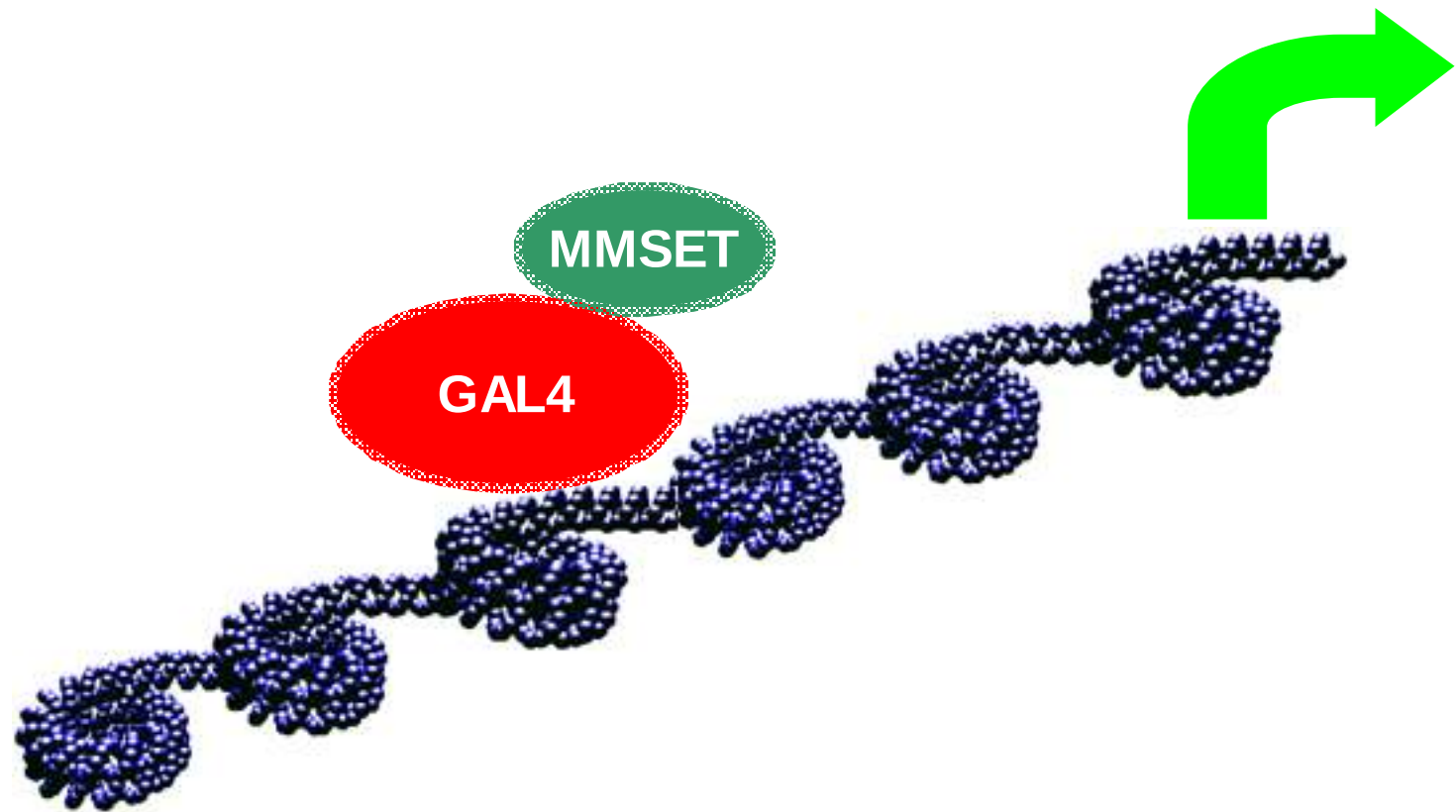
MMSET+ GST PRE SET SET POST SET PHD



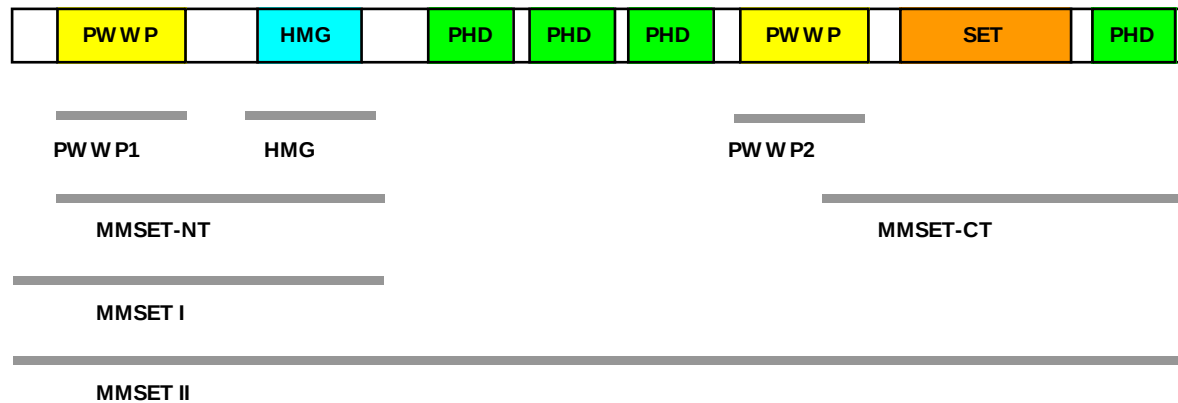
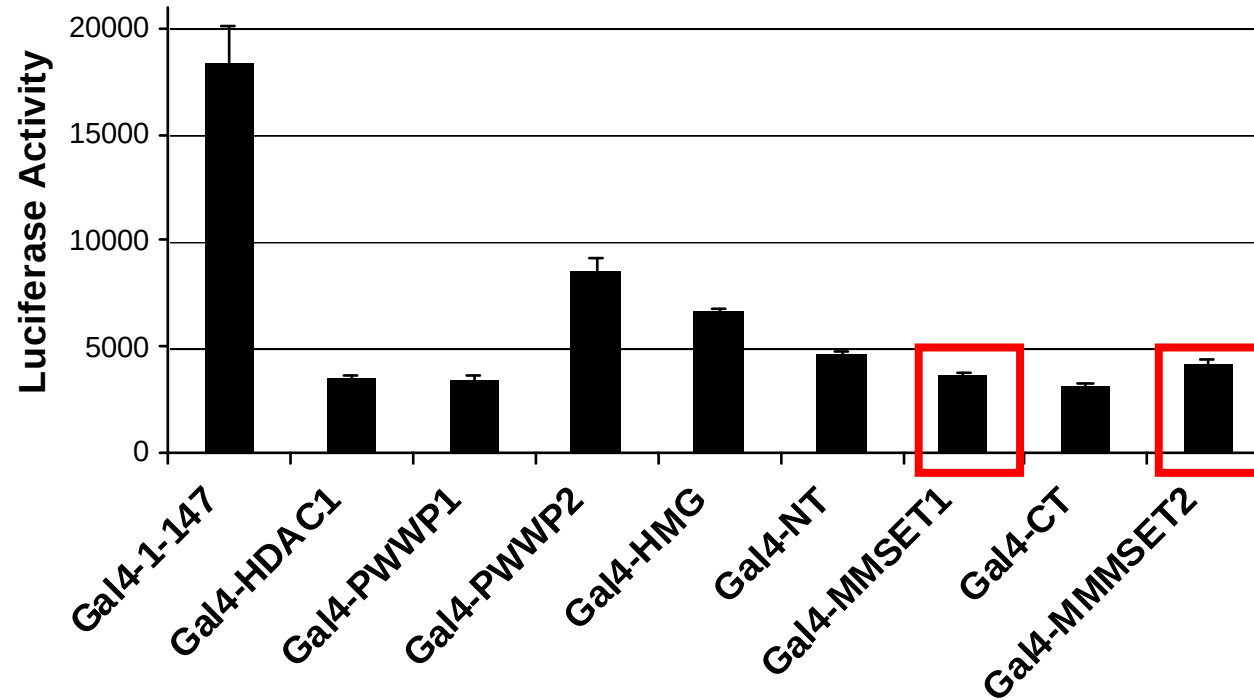
In Vitro- MMSET Can methylated Many Different Histone Sites



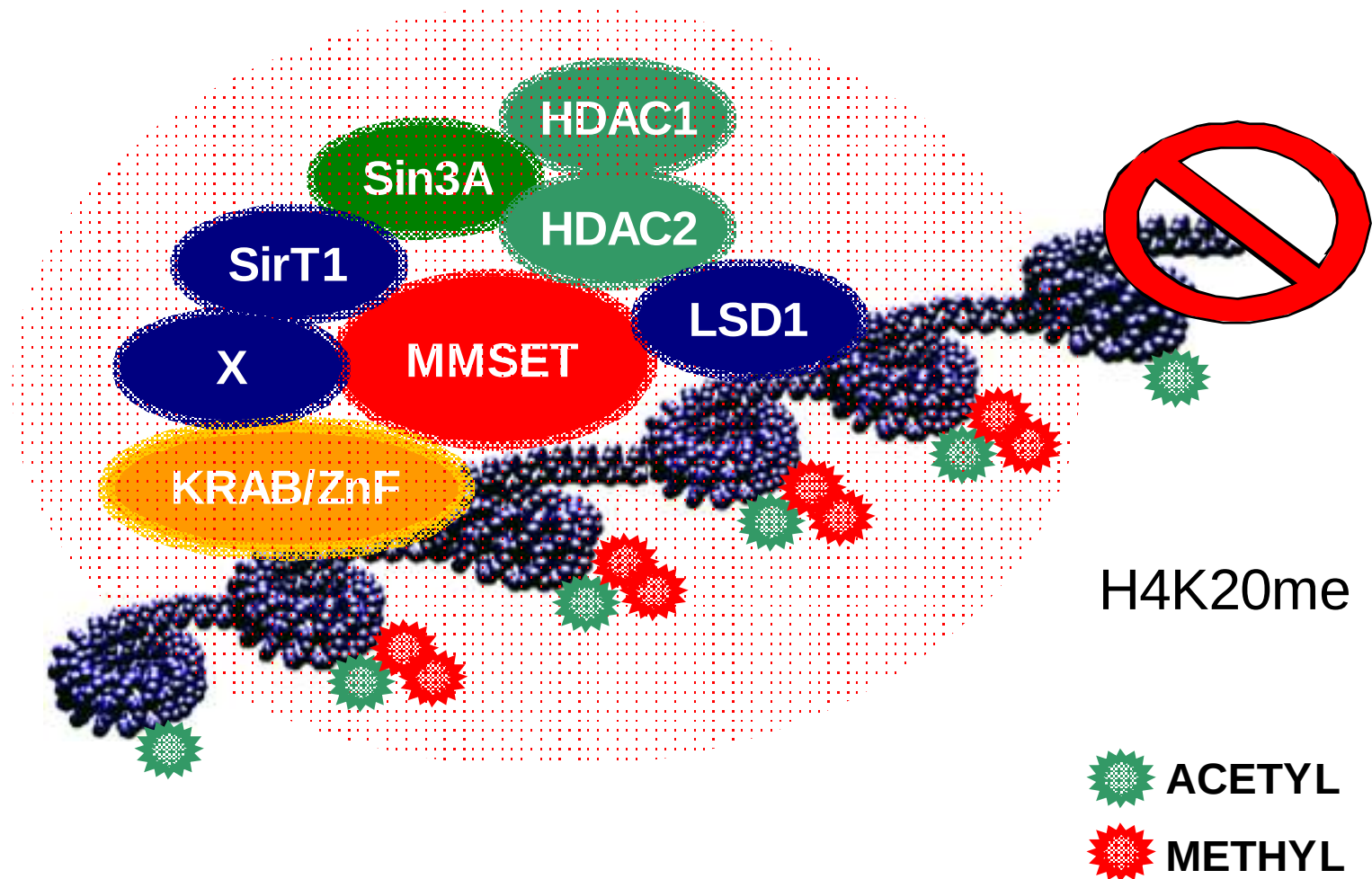
GAL4 Tethering to Test MMSET Function



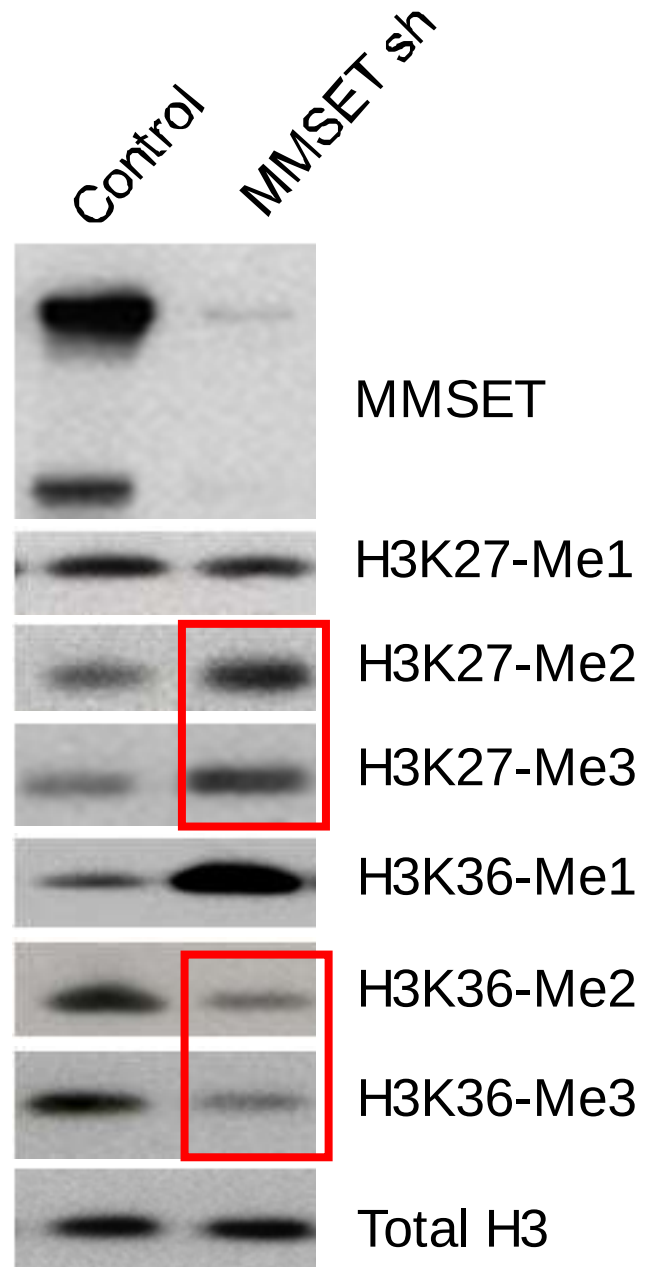
Multiple Repression Domains within MMSET



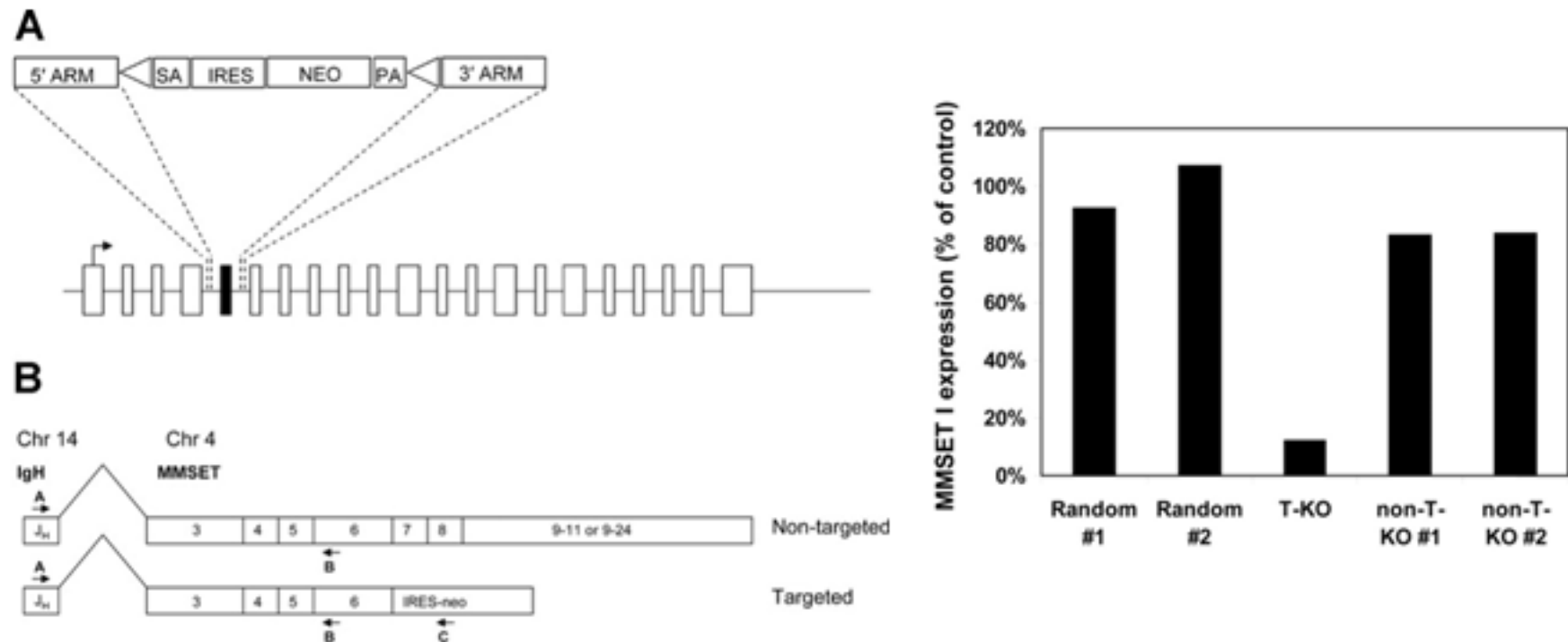
MMSET-Potential Transcriptional Repressor



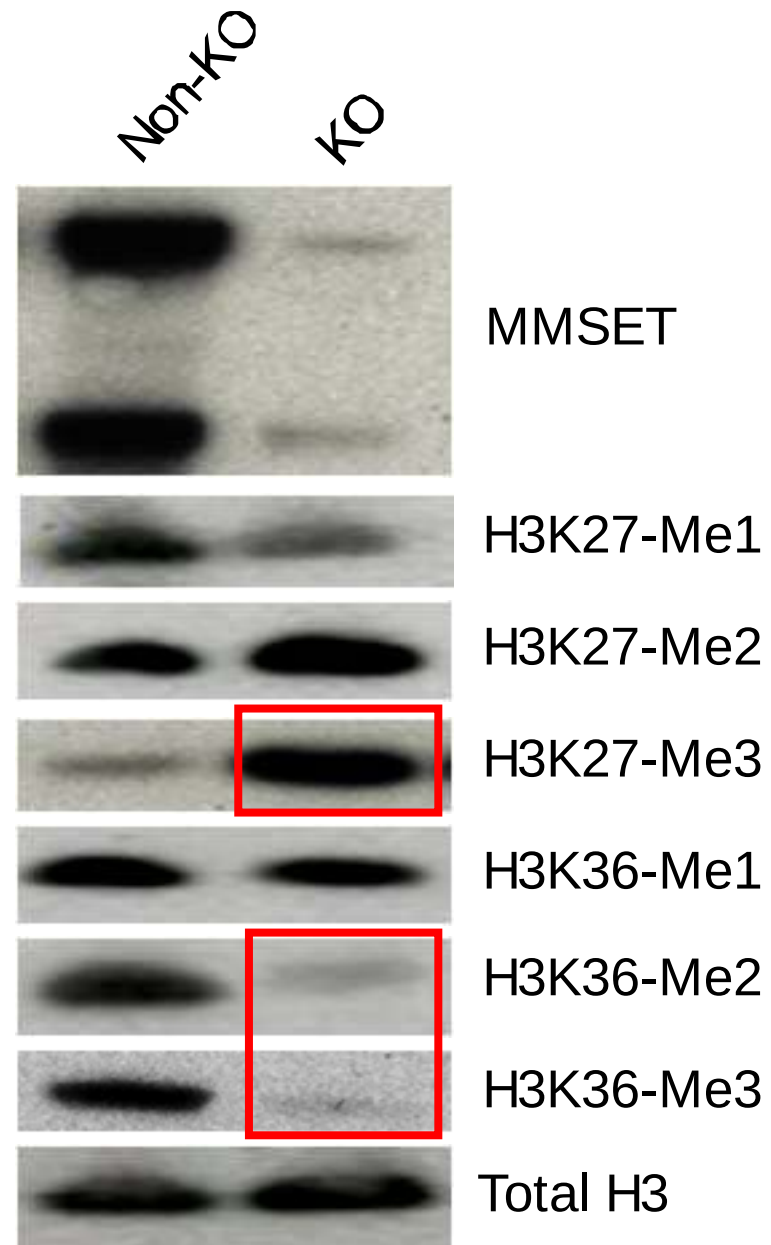
Global Chromatin Changes in Response to MMSET



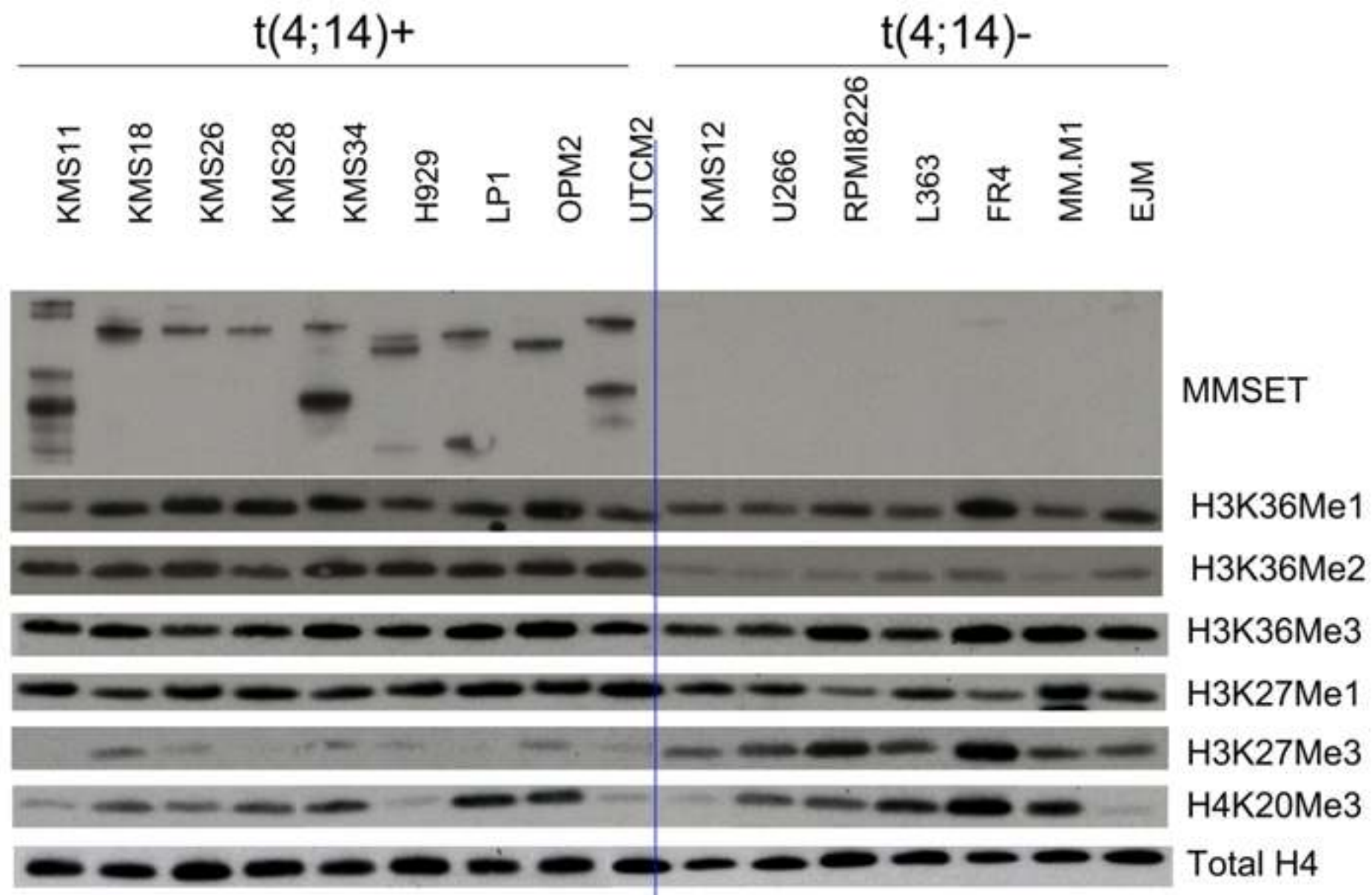
KNOCK OUT SYSTEM FOR MMSET



MMSET Knock out (KO)-Global Effects on Chromatin



MMSET Global Effects on Chromatin



MMSET-H36me2/H3K27me3 Switch

t(4;14)+

t(4;14)-

KMS11

KMS18

KMS26

KMS28

KMS34

H929

LP1

OPM2

UTCM2

KMS12

U266

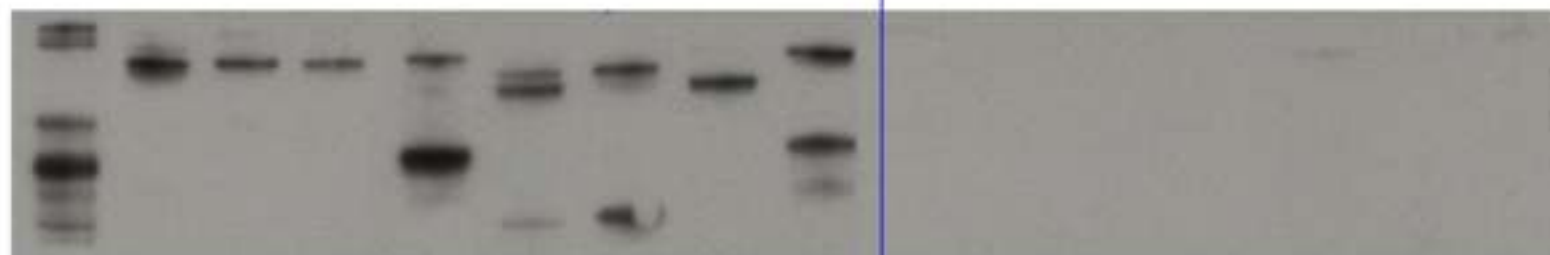
RPMI8226

L363

FR4

MM.M1

EJM



MMSET



H3K36Me2

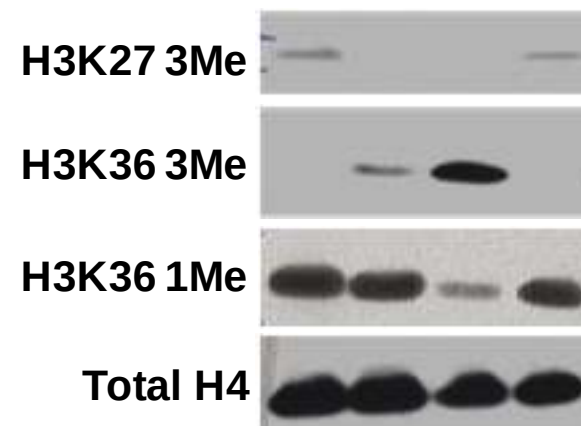
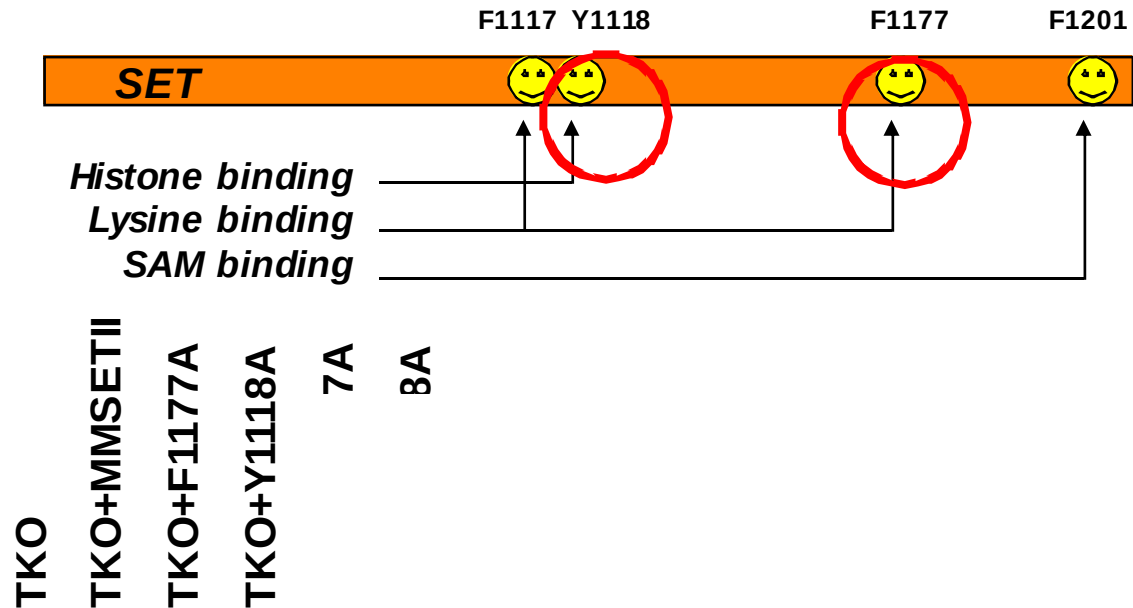
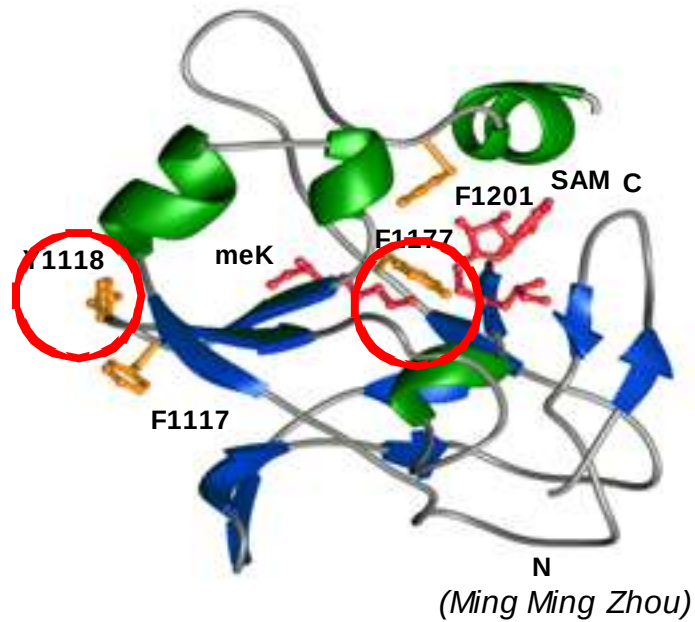


H3K27Me3

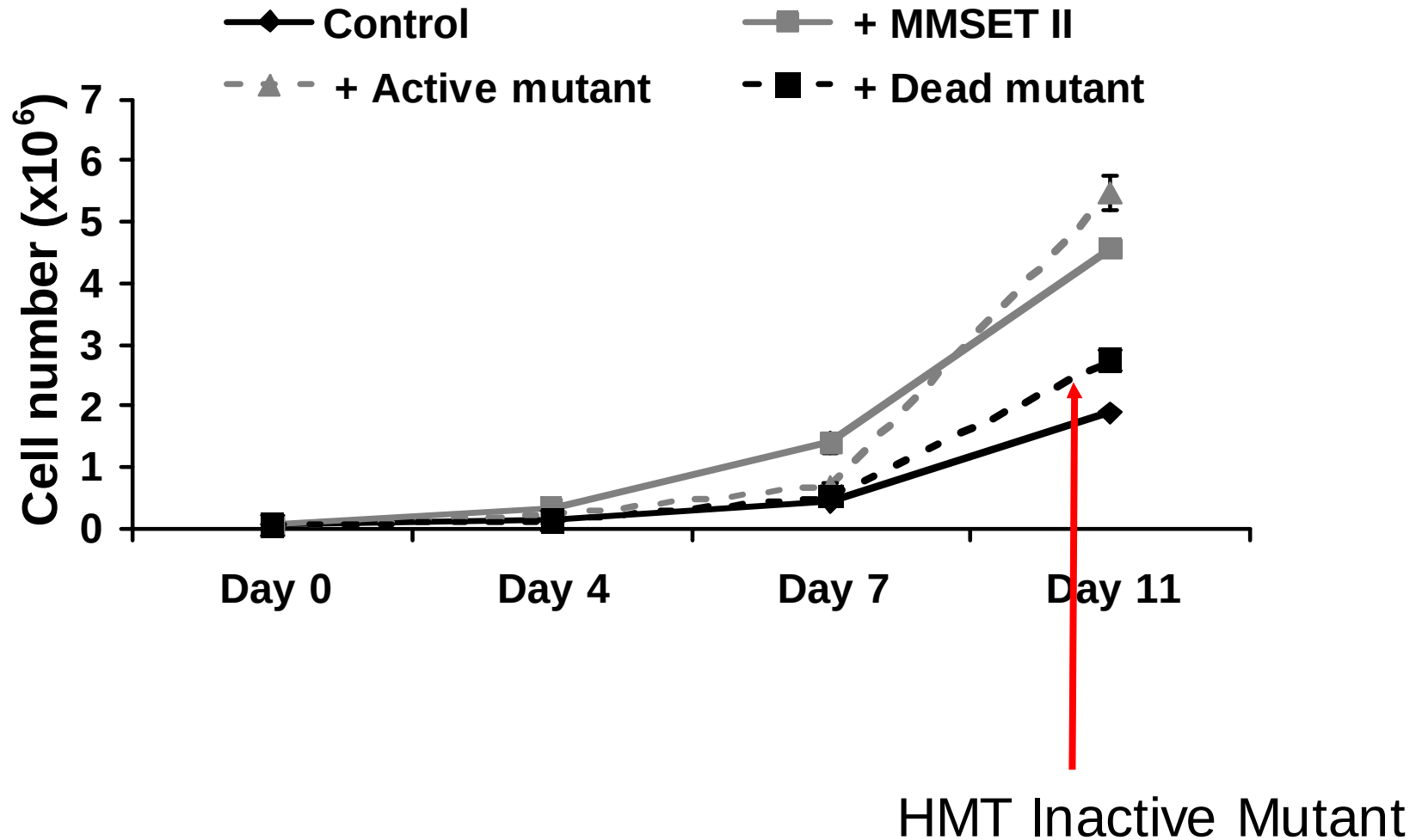


Total H4

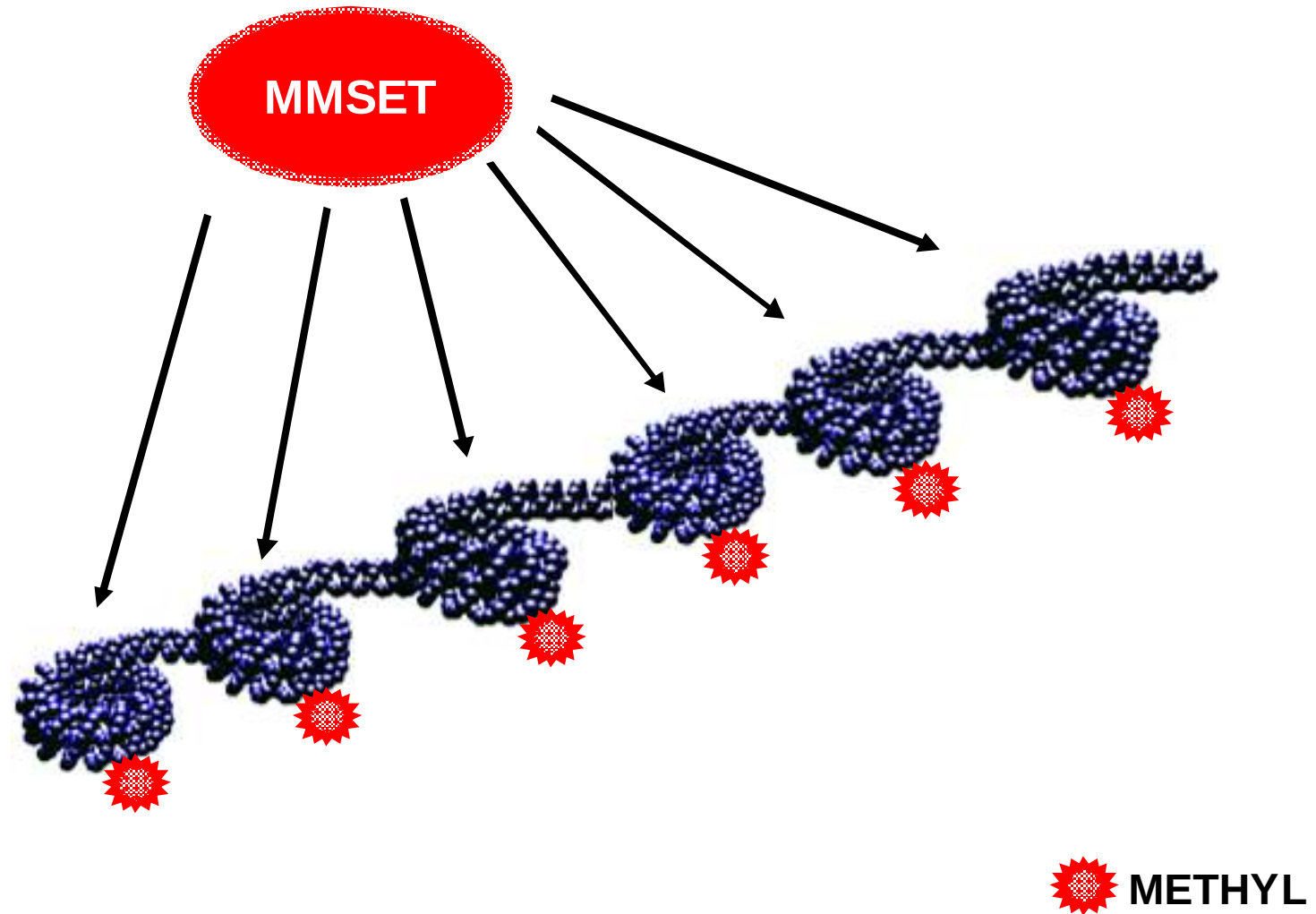
MMSET Re-Addition Reverses the Switch



MMSET Methylation Activity Critical to Myeloma Growth



HMTs Gone Wild!--K36/K27 Switch



Hypothesis: MMSET is a Reader and a Writer of the Histone Code

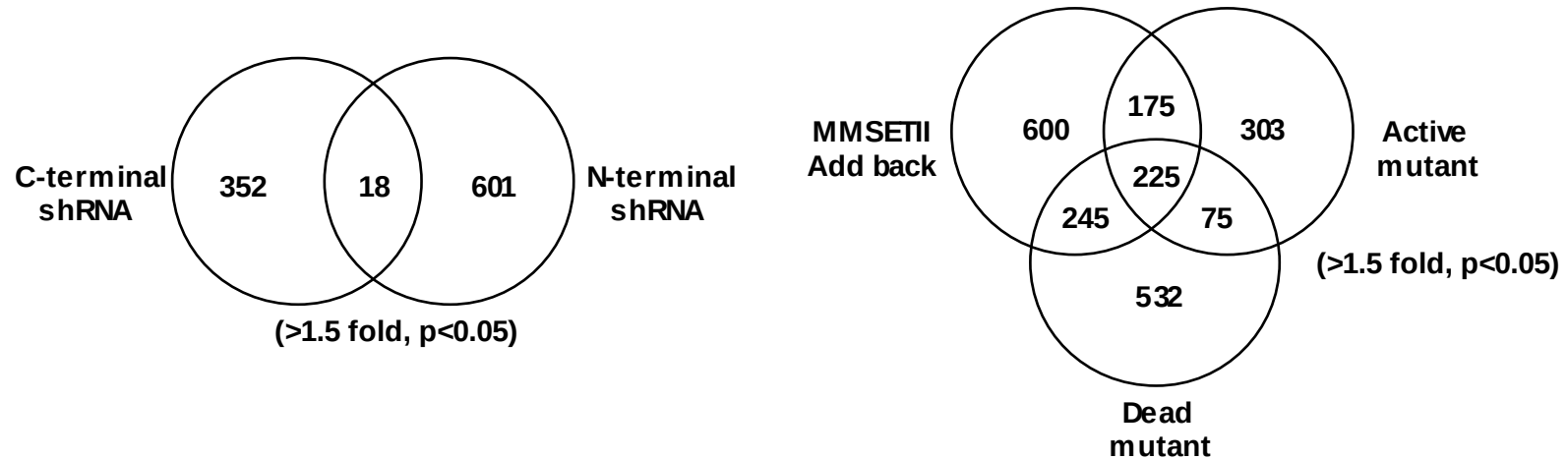
What Mark(s) Does MMSET Read?

What Mark(s) Does MMSET Make?

How Does This Mark Affect Gene Expression?

Can MMSET Be Inhibited?

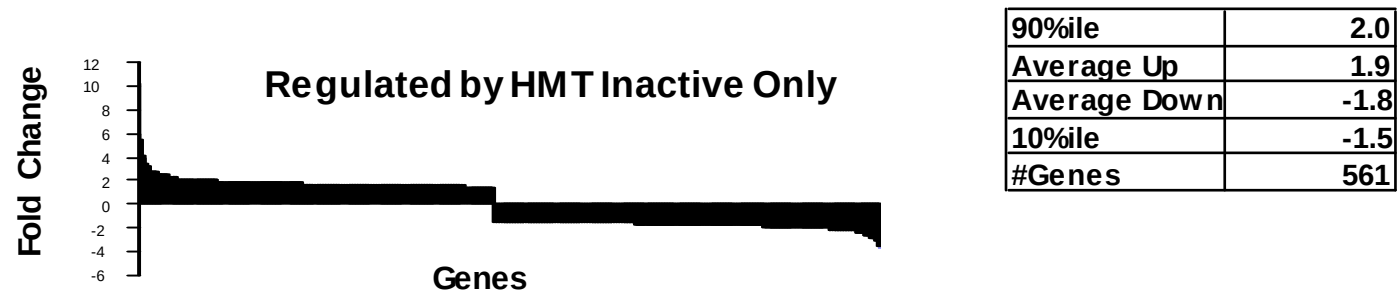
Which Genes are Affected by MMSET Expression?



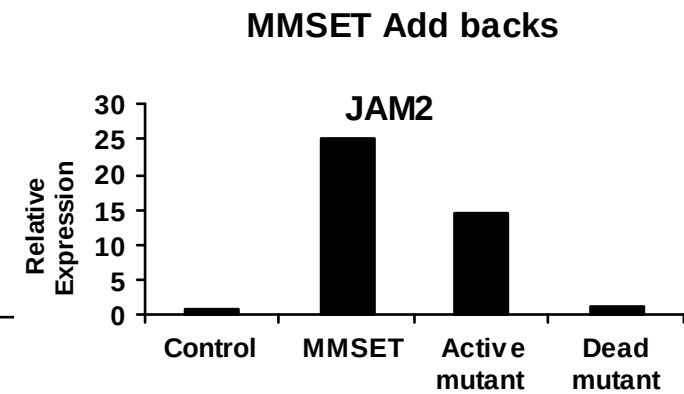
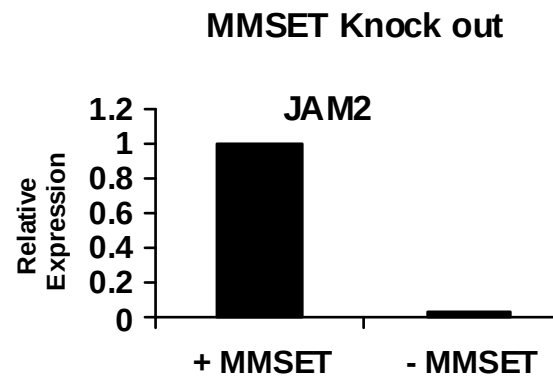
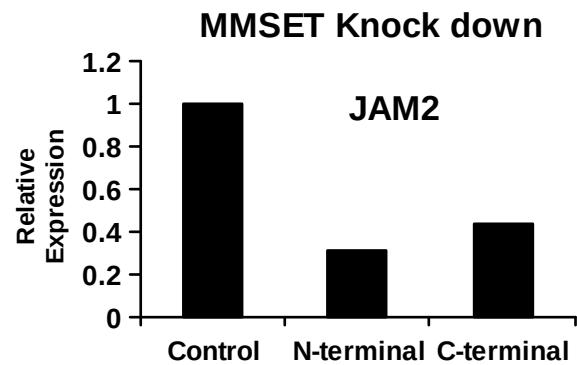
d

Ontology	p-value	Examples
Knock down		
Apoptosis	5.20E-02	BCL2, HDAC4, PDCD10, STAT3
DNA repair	3.70E-04	RAB23, RRAS, ERCC1, IGF1
Chromatin packaging and remodeling	8.10E-03	MLL3, IRF7, HAT1, SETDB2
Cell cycle control	8.30E-02	BTG2, CCND2, CCNE2, GADD45A
+ MMSET II		
Apoptosis	1.40E-03	BAX, ITGB1, PRKCA
Cell cycle	1.40E-03	BTG2, E2F2, CDC25A
Cell motility	2.30E-02	CXCR4, ITGB7, TUBB4, ROCK2

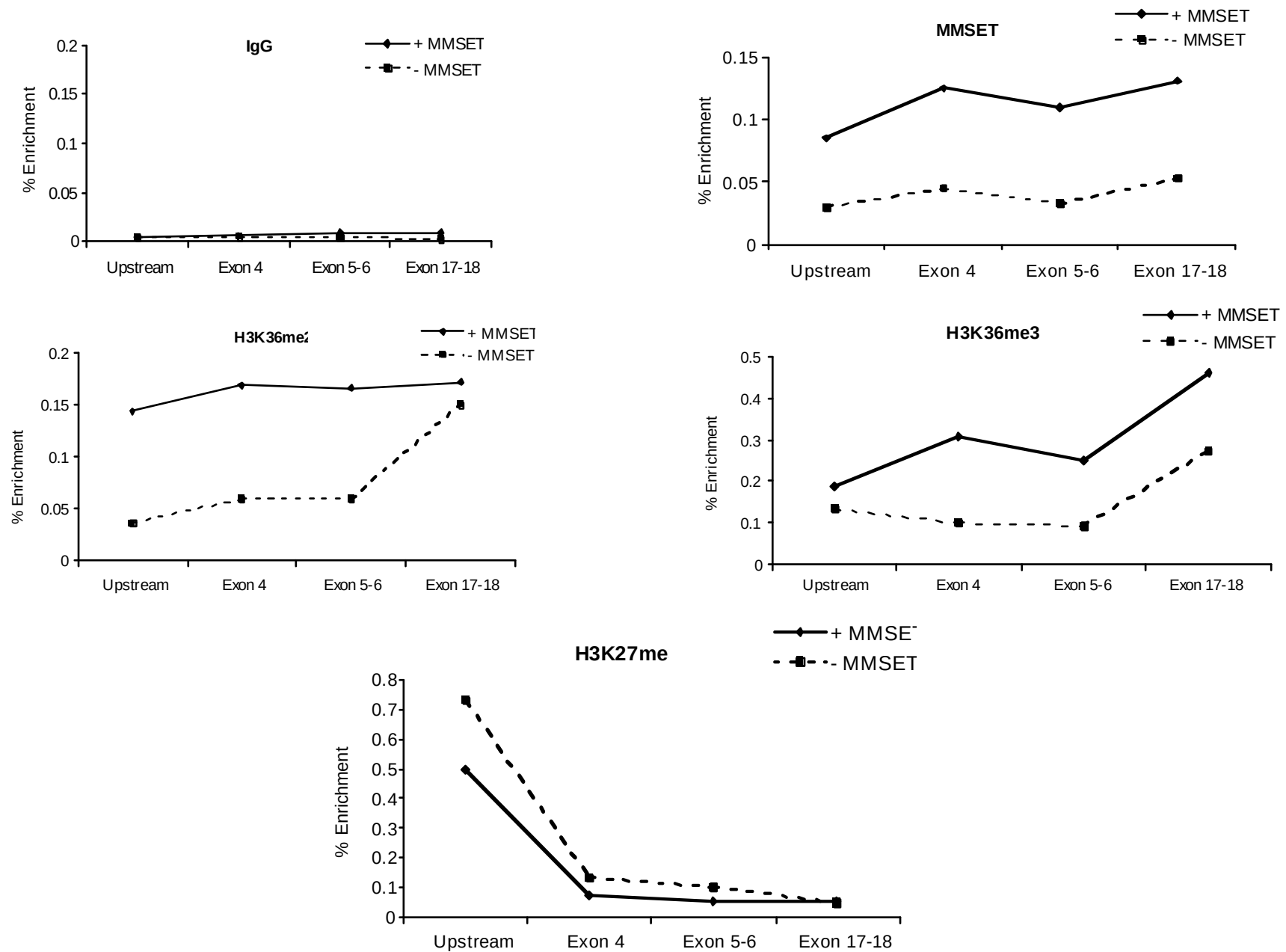
The HMT Activity of MMSET Yields Gene Activation



Which Genes are Affected by MMSET Expression?



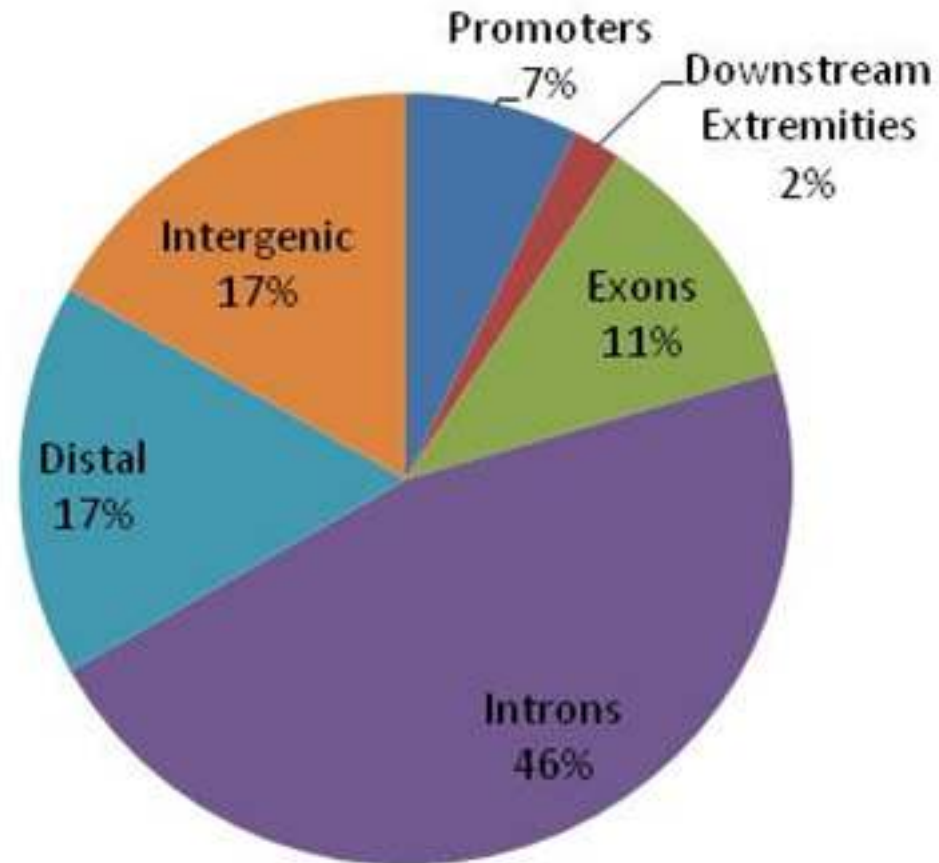
ChIP in MMSET knockout cells- GLS2



MMSET- Genome Wide Profiling

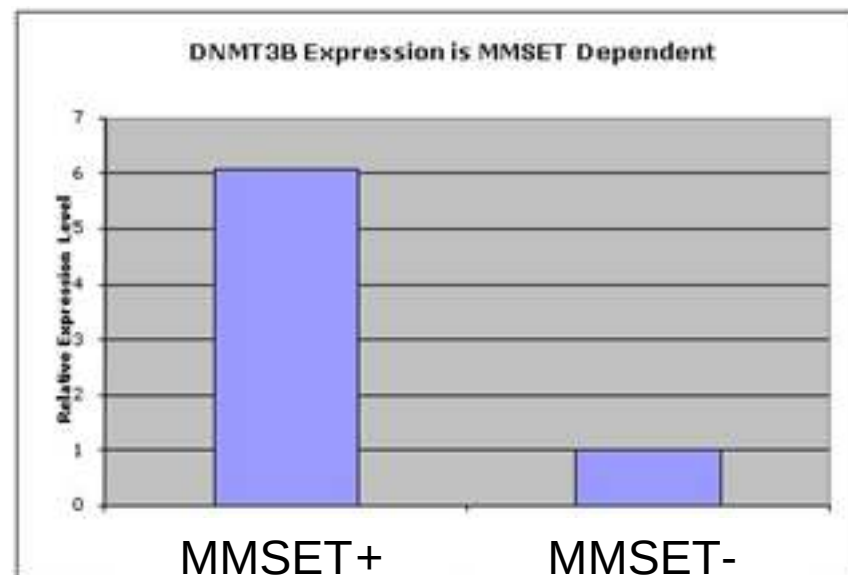
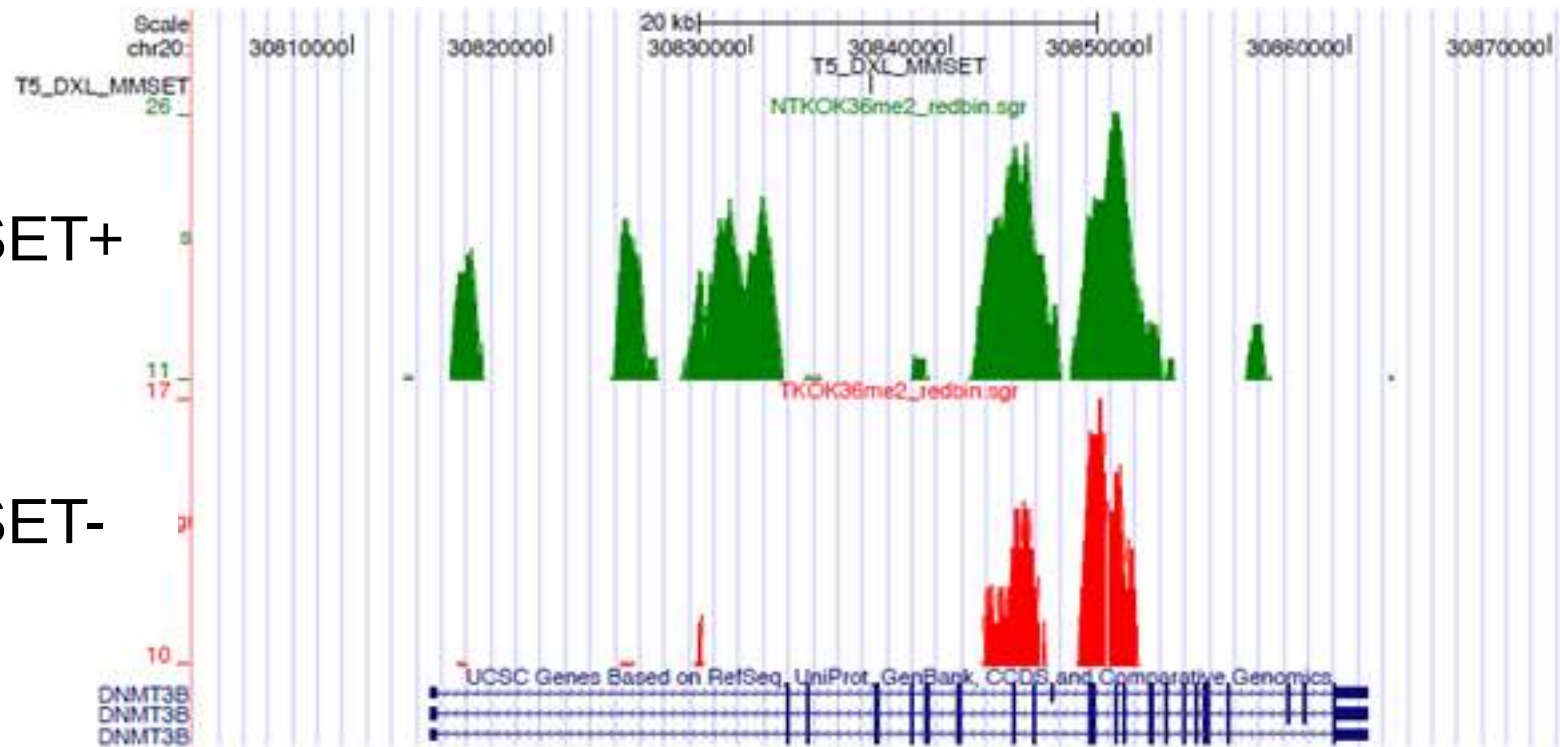
KMS11 cells- double crosslinking; ChIPseeqer software;

4736 Peaks Identified



MMSET+

MMSET-

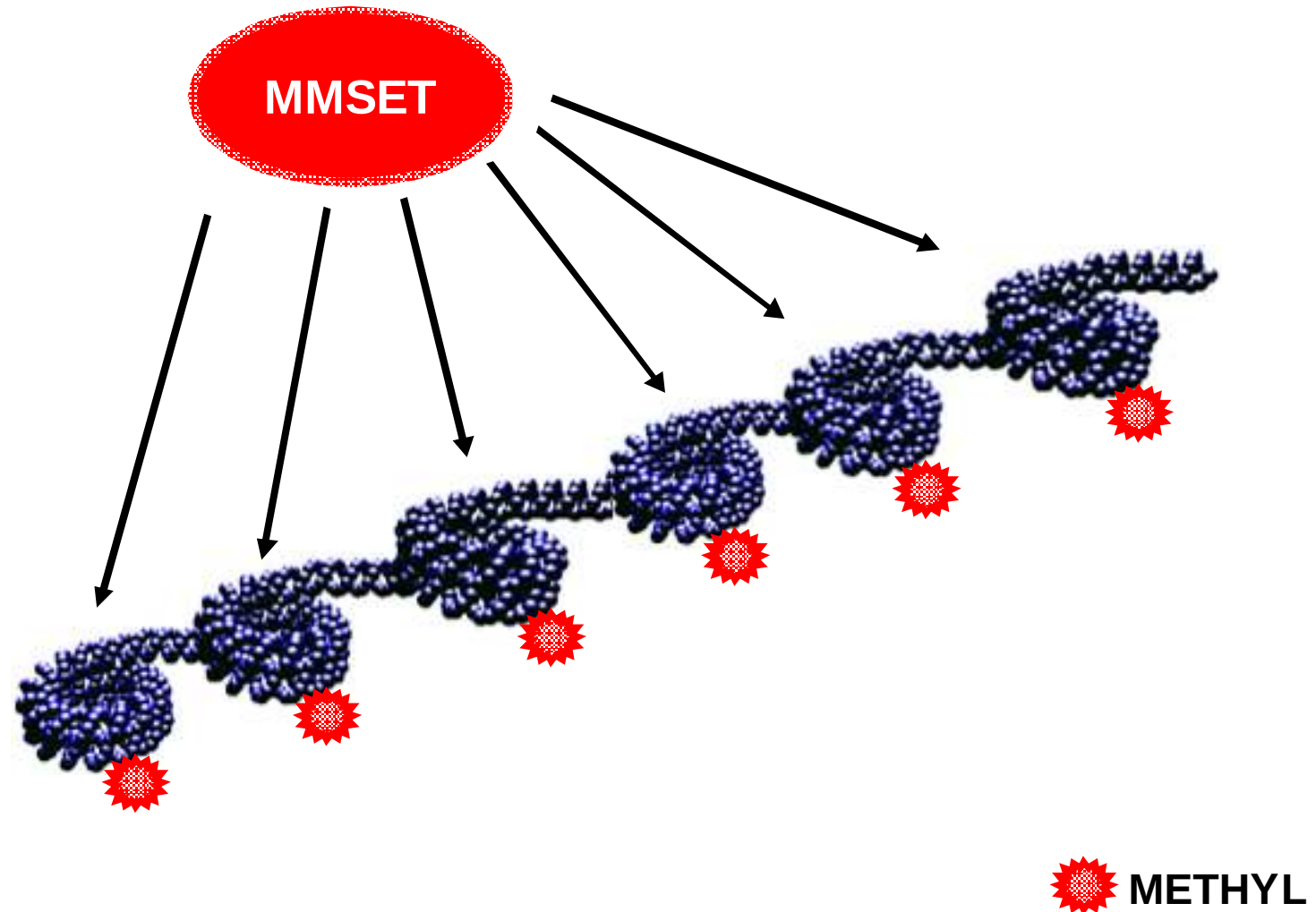


MMSET Targets

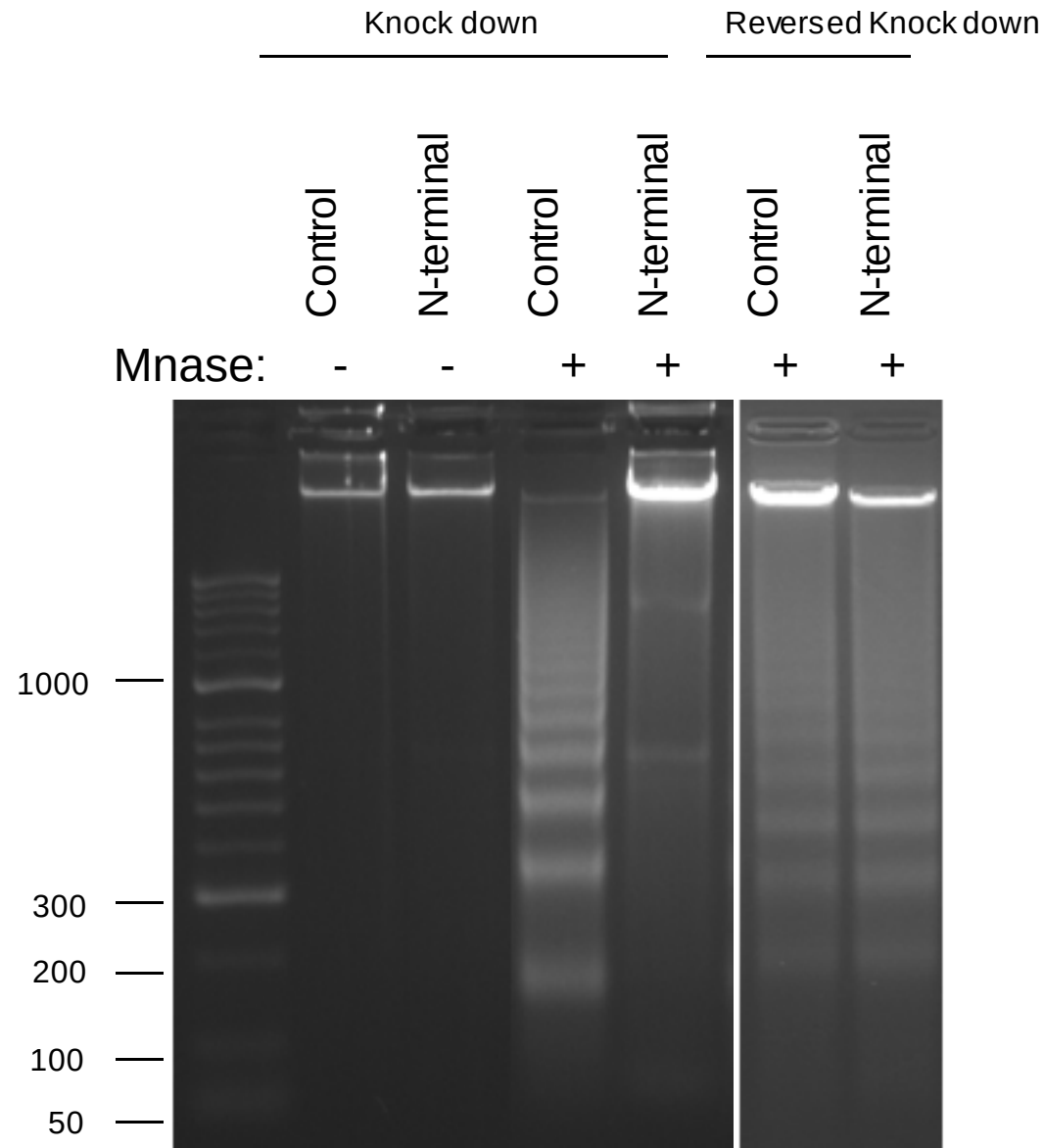
- SET Dependent
 - Cell Cycle, Adhesion, Apoptosis Genes
Chromatin Regulators Among Targets
 - This Activity Critical for Growth

Which Targets are involved in Pathogenesis?

HMTs Gone Wild- Non Transcriptional Effects?



MMSET effects on chromatin accessibility



Control

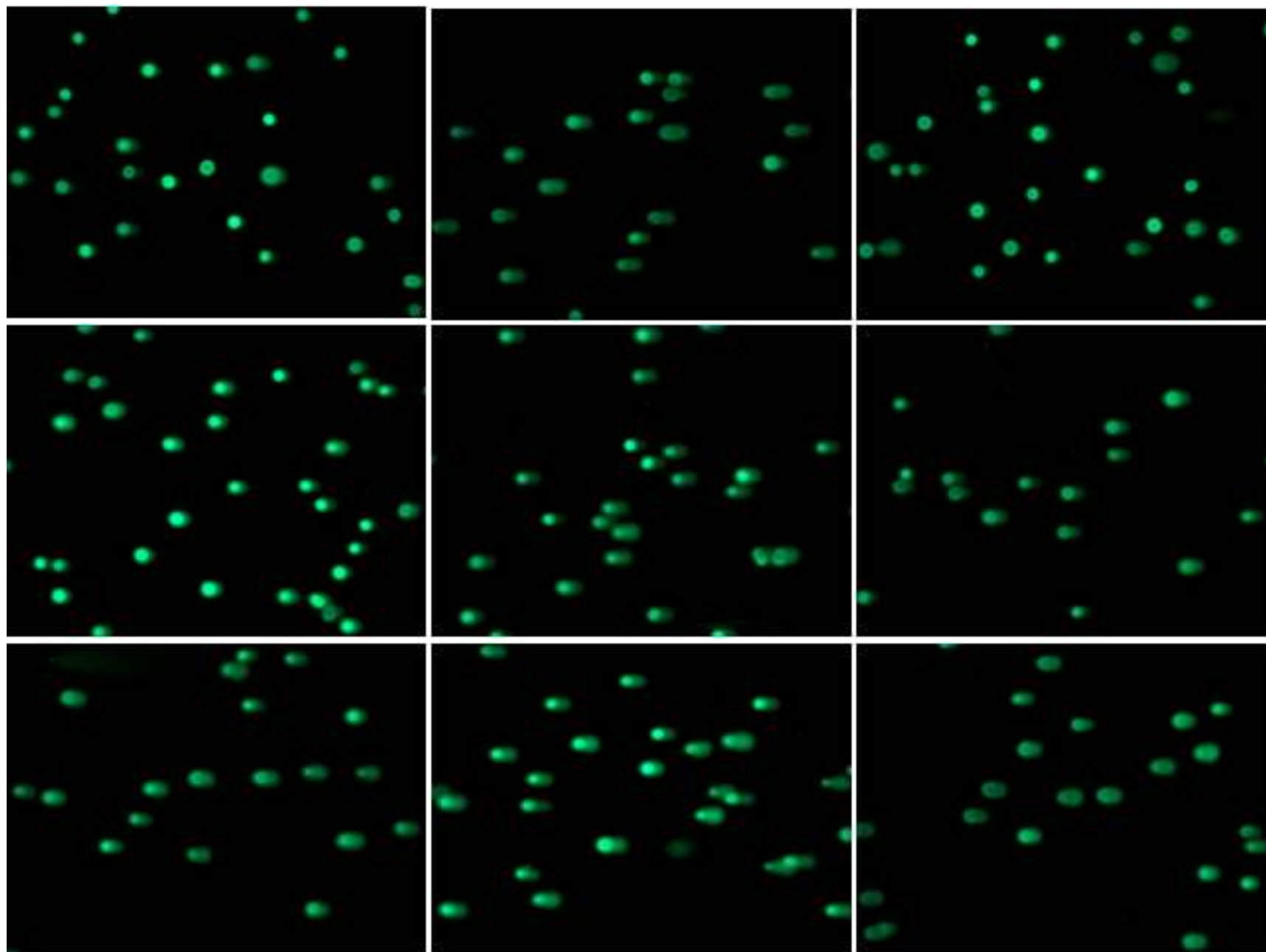
+MMSET II

+Inactive Mutant

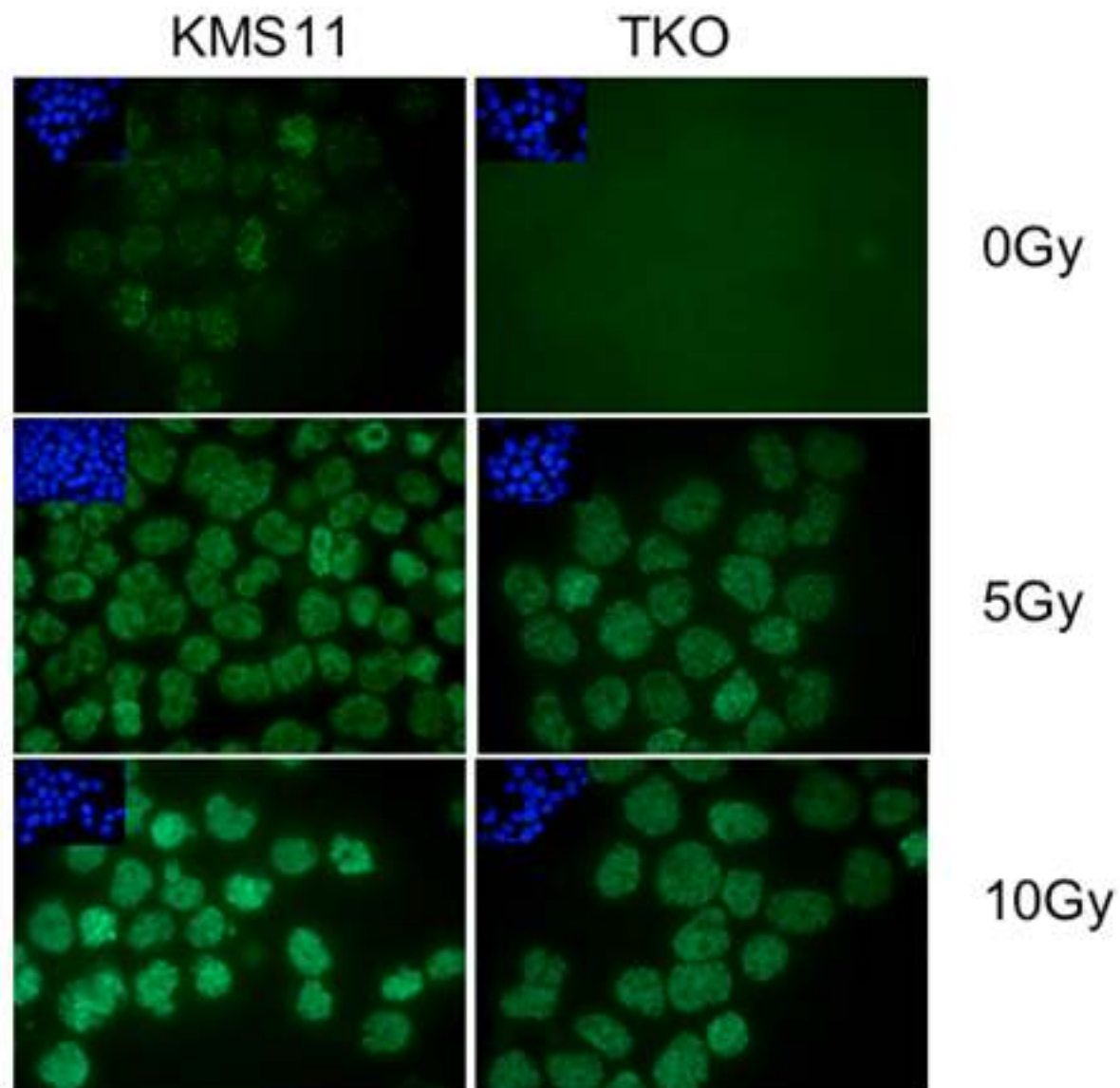
0 Gy

5 Gy

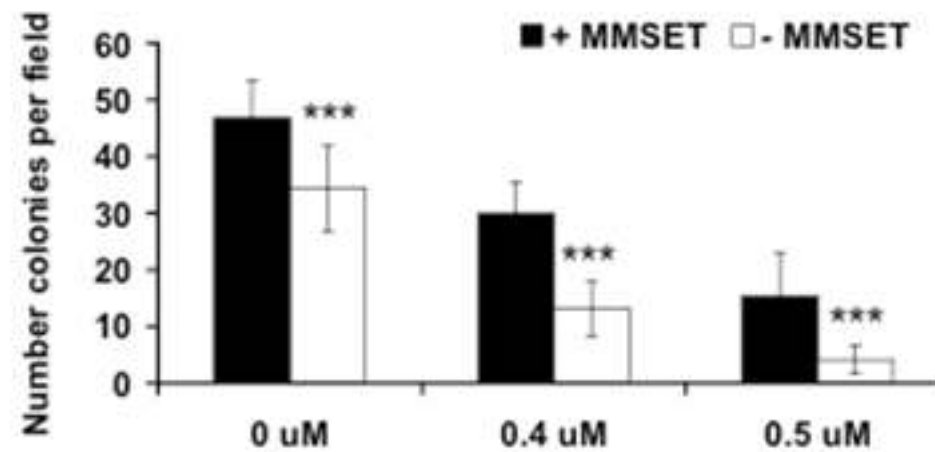
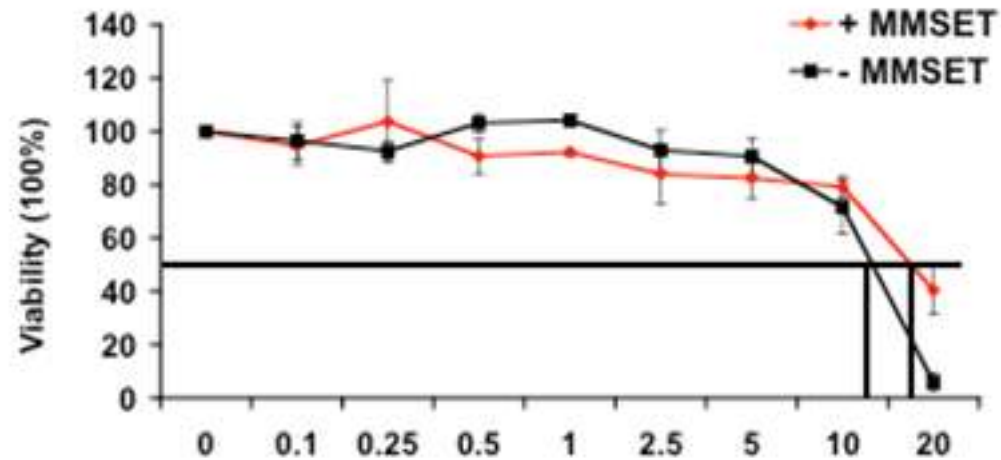
10Gy



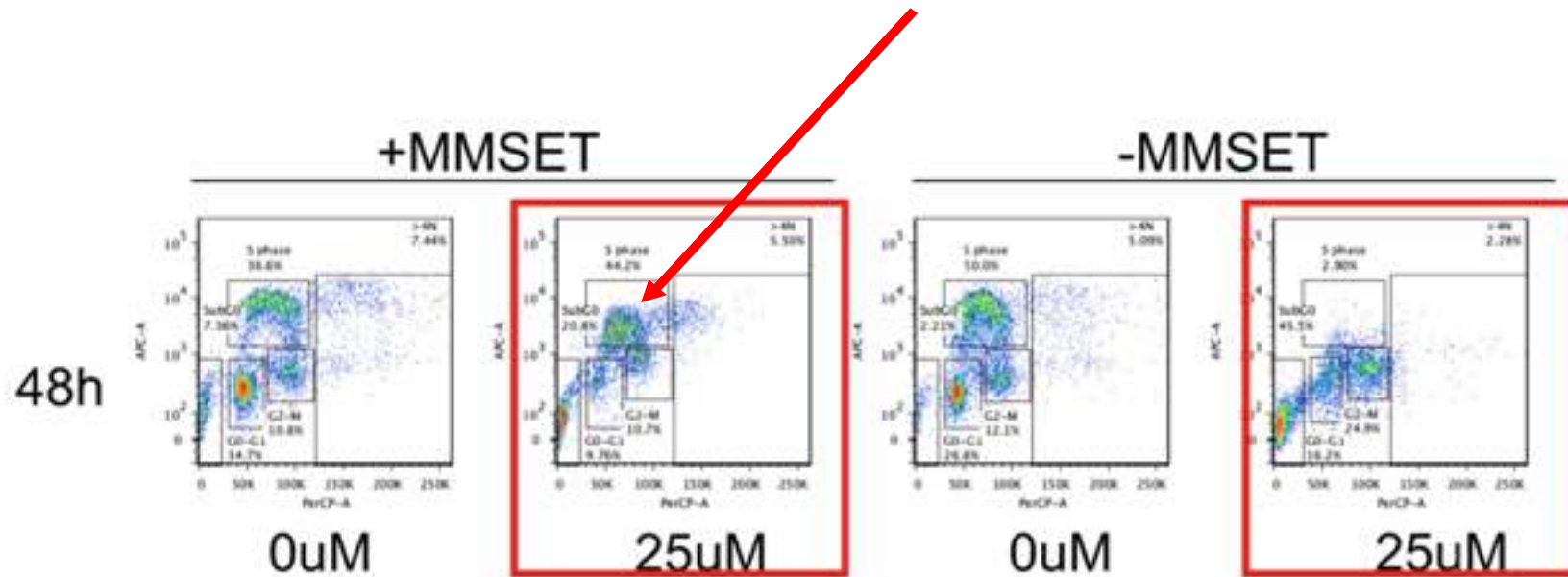
γ H2AX in Knock out system 30min after radiation



MMSET- An Effect on DNA Damage Response



MMSET+ Cells Fail to Undergo Cell Cycle Arrest of DNA Damage



Hypothesis: MMSET is a Reader and a Writer of the Histone Code

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How Does This Mark Affect Gene Expression?

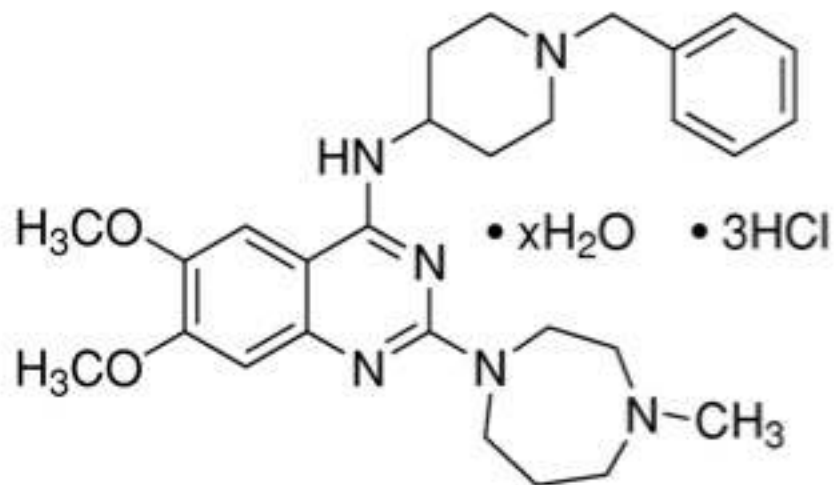
Can MMSET Be Inhibited?

BIX 01294

G9a/GLP Inhibitor

Inhibits H3K9me3

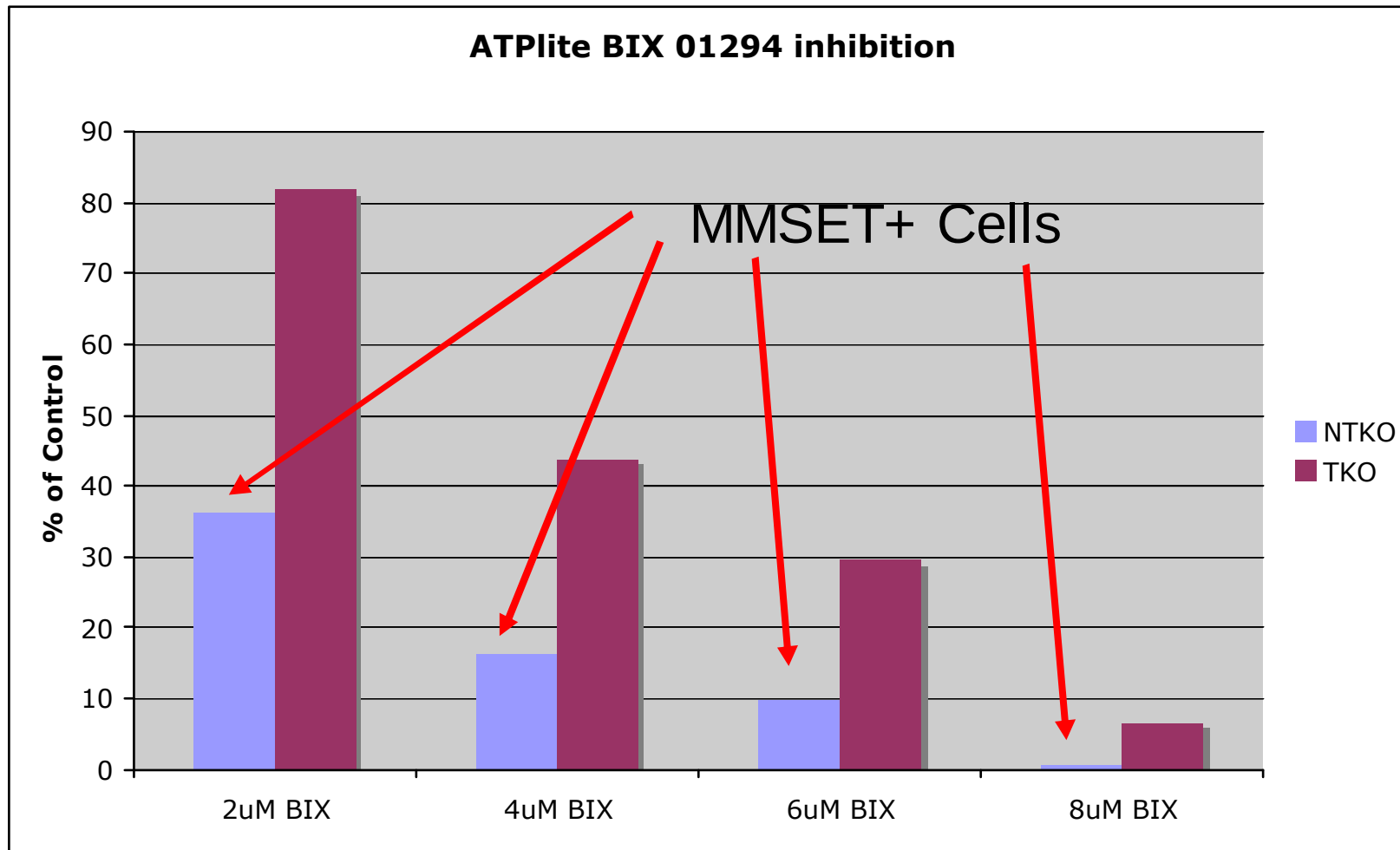
Reprograms Somatic to IPS cells



H3K36 Me3 Ab



HMT Inhibitor Killed MMSET+ Cell More Readily

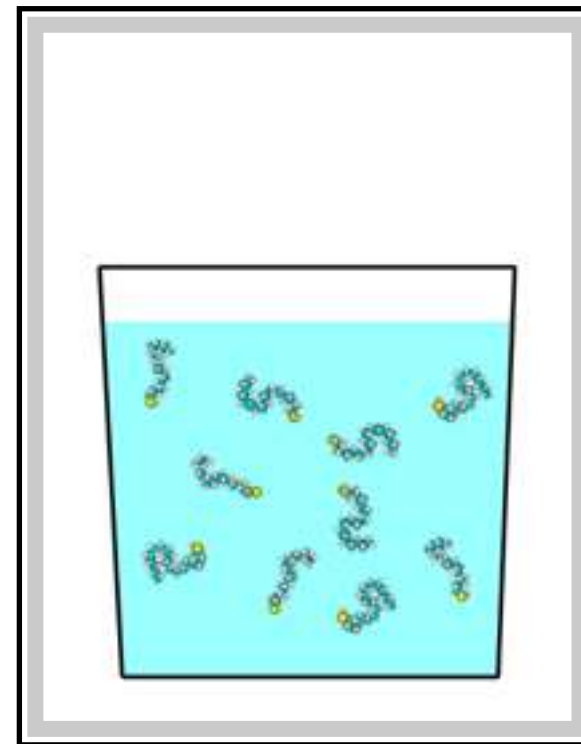
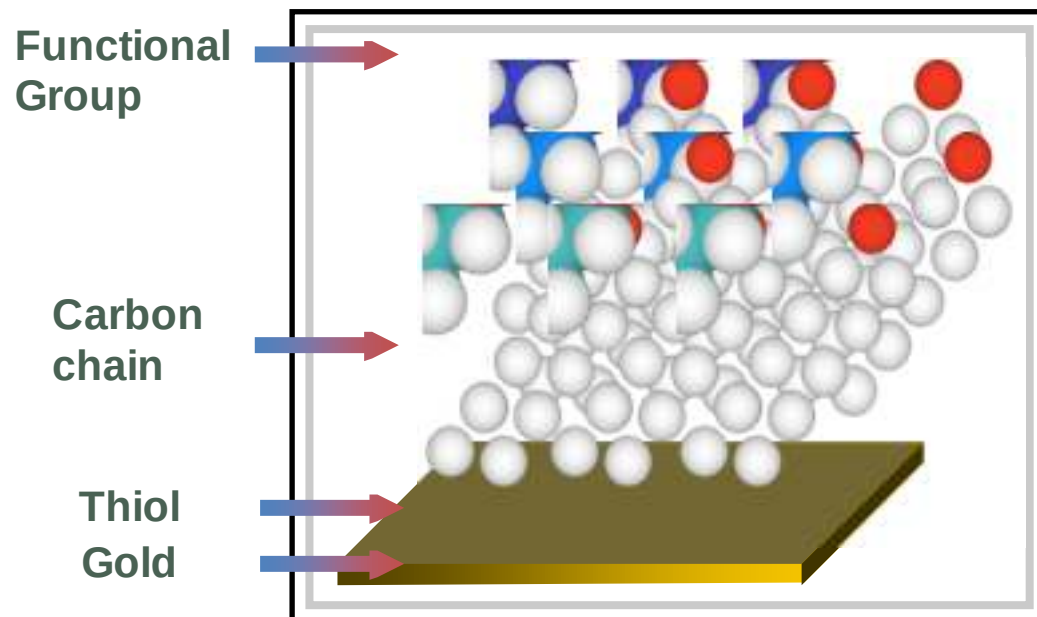


SET Domain model for CADD Prediction of Inhibitors



Mass Spec Based Screen for MMSET Inhibitors

Self-Assembled Monolayers (SAMs) on Gold

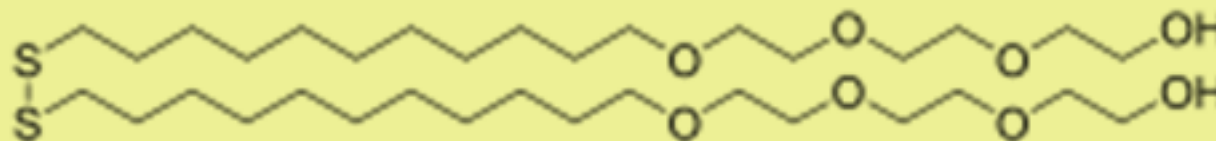


Ⓢ Well-defined structure

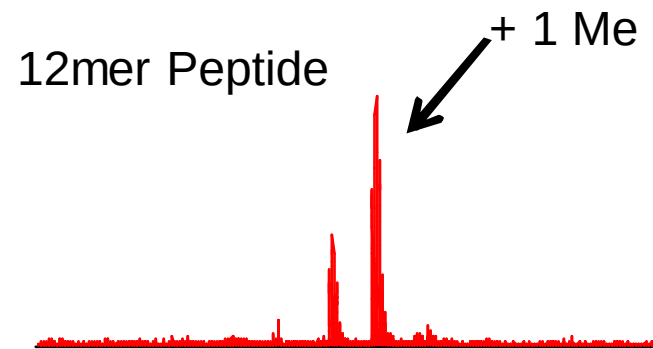
Ⓢ Inert background environment

Ⓢ Controllable ligand density

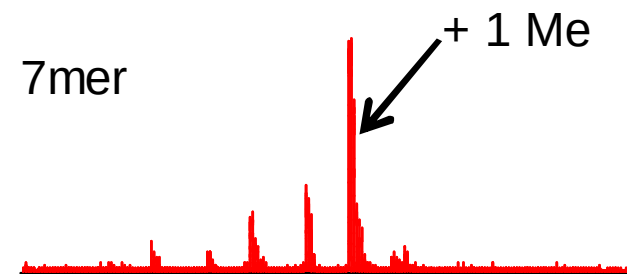
Ⓢ Compatible with MALDI-TOF MS



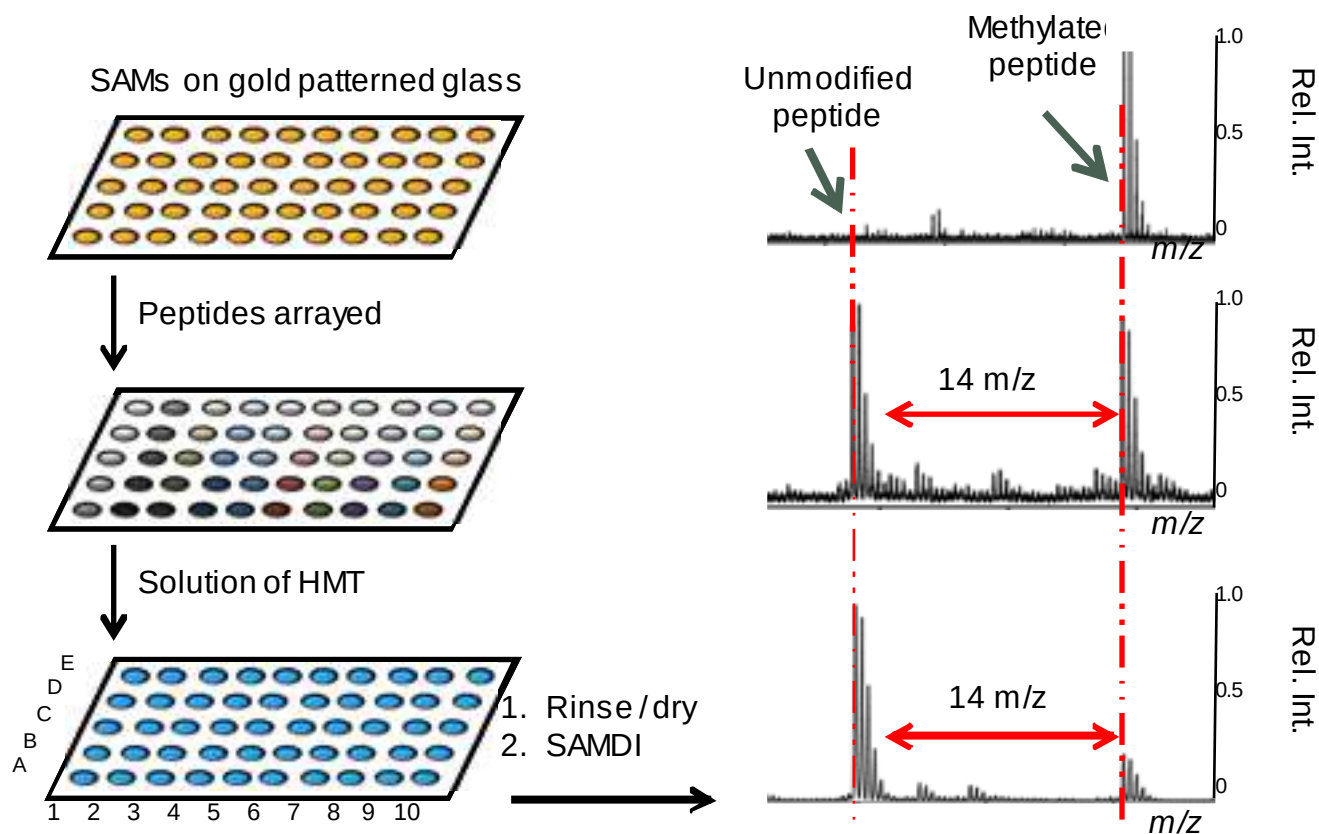
Ac-GGVKKPHRYC-NH₂



Ac- GVKKPHR C-NH₂



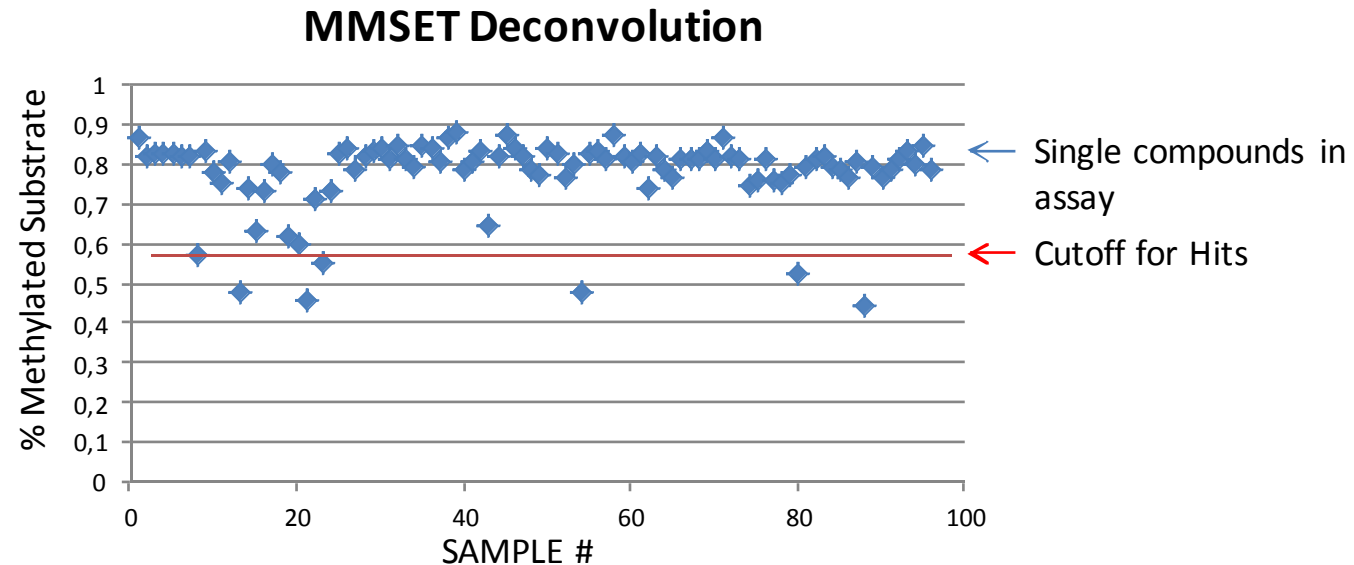
High-Throughput Mass Spec



Applied Biosystems 4800
MALDI TOF/TOF

MMSET Inhibitor Screen Summary

- 10,000 compounds screened in pools of 8
 - 5 μ M MMSET, 2 μ M peptide, 1 mM SAM, 12.5 μ M each compound
- Total of 11 pools with significant inhibition (20-30%)
- Deconvoluted 11 pooled hits to singletons (Data below)
 - 6 compounds with >40% inhibition at 10 μ M



Conclusions

- MMSET Appears to be a Therapeutic Target in Myeloma
- MMSET Inhibitors exist and more will be developed
 - The HMT Activity of MMSET is Important for Biological Effects
- MMSET Can Activate and Repress Genes
 - Exact Critical Targets being determined
- MMSET May Have Effects on other Chromatin-Dependent Processes
 - DNA Repair
 - DNA replication?
- MMSET Myeloma May Have Specific “Achilles Heels”



Northwestern University

- Dong-Joon Min
- Relja Popovic
- Eva Martinez
- Christine Will
- Teresa Ezponda
- Marianne Kim

Mayo Clinic- Scottsdale

- Marta Chesi
- Leif Bergsagel

National Cancer Institute

- Lou Staudt

MSKCC

- Dinshaw Patel

U Chicago

- Milan Mrksch
 - Zack Guard- Levin

U Maryland

- Alex MacKarrell

DFCI

- Jay Bradner



SCOR Consortium

- Jay Hess- U Mich
- Dave Allis-Rockefeller
- Robert Roeder- Rockefeller
- Dinshaw Patel-MSKCC
- Ari Melnick-Weil-Cornell

