

# A Genome-Wide CRISPR/Cas9 Screen Reveals Epigenetic Immune Evasion Mechanisms in HPV+HNSCC

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Canchai Yang<sup>1</sup>, Hyun Hee Lee<sup>1</sup>, Mohamed Khalil<sup>1</sup>,  
Andrew Olive<sup>1</sup>, and Dohun Pyeon<sup>1</sup>**

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<sup>2</sup> College of Osteopathic Medicine, Michigan State University, East Lansing, MI



# Clinical Case

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**Pt:** 69 M

**CC:** R throat discomfort and enlarged lymph node for >3 months

**Hx/PE/Labs:** Enlarged R palatine lymph node with squamous cell carcinoma detected; p16+

**Dx:** Human papillomavirus (+)  
Head and Neck Squamous Cell Carcinoma  
(**HPV+HNSCC**)

**Tx:** Surgical dissection and Radiation

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**Remission for ~4 years**

**Cancer Relapses** with evidence of metastasis

Patient was admitted to palliative care

# Clinical Case

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**Poor prognosis** for ~20% patients (HPV+HNSCC)

→ Treatment non-responders, recurrent/metastatic disease

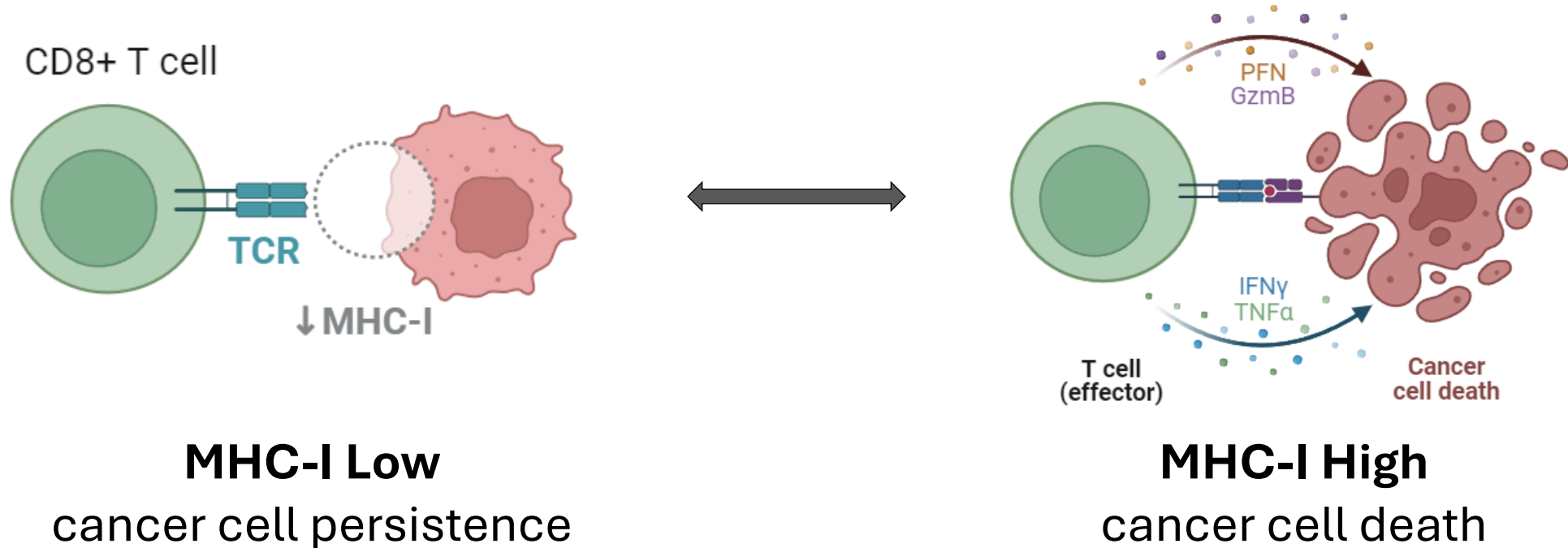
Lack of subsequent treatment options

# How do we help these patients?

Elucidate mechanisms underlying cancer immune evasion



Identification of novel targets for treatments



# Overall Findings

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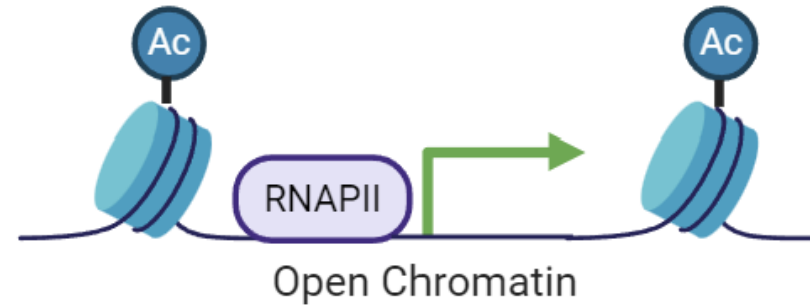
Genome-wide  
screens revealed  
**Epigenetic  
modifiers**  
negatively regulate  
MHC-I



# Overall Findings

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## Healthy Cells



Normal  
MHC-I expression

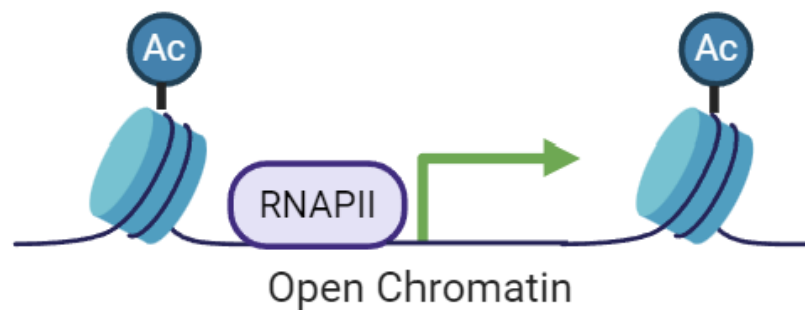
Genome-wide  
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# Overall Findings

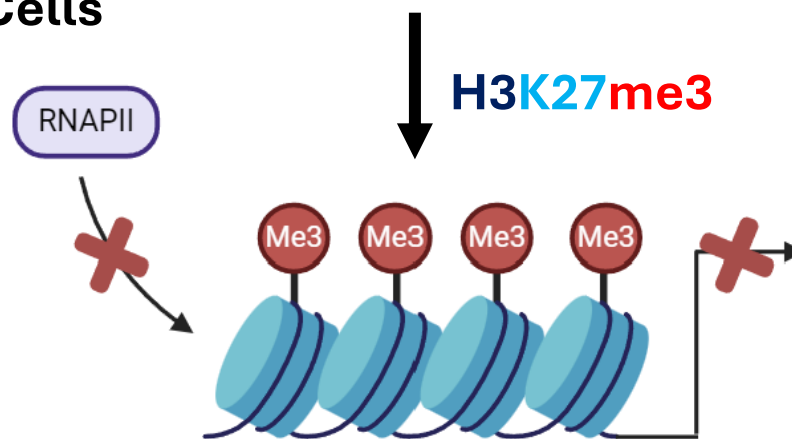
Genome-wide screens revealed **Epigenetic modifiers** negatively regulate MHC-I

## Healthy Cells



Normal MHC-I expression

## Cancer Cells



Decreased MHC-I expression



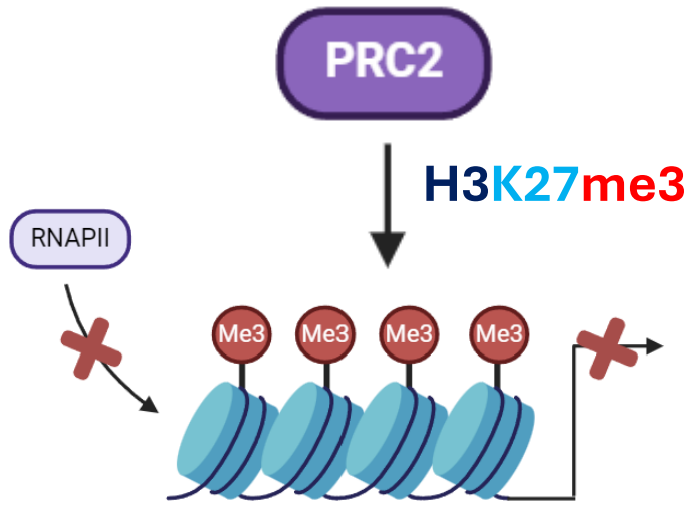


# Overall Findings

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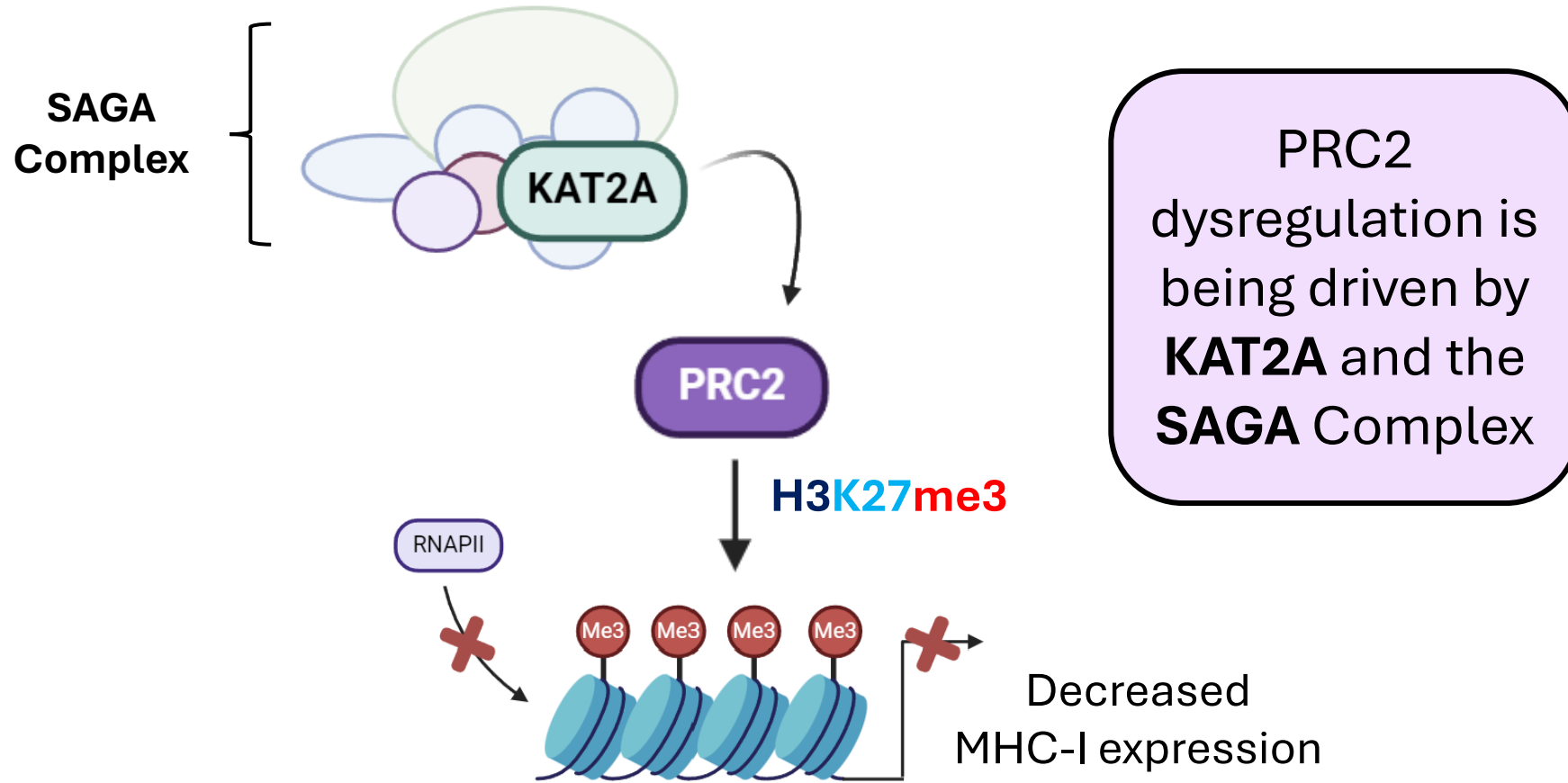


Epigenetic silencing is modulated by the polycomb repressor complex (**PRC2**)

Decreased  
MHC-I expression



# Overall Findings



# Overall Findings

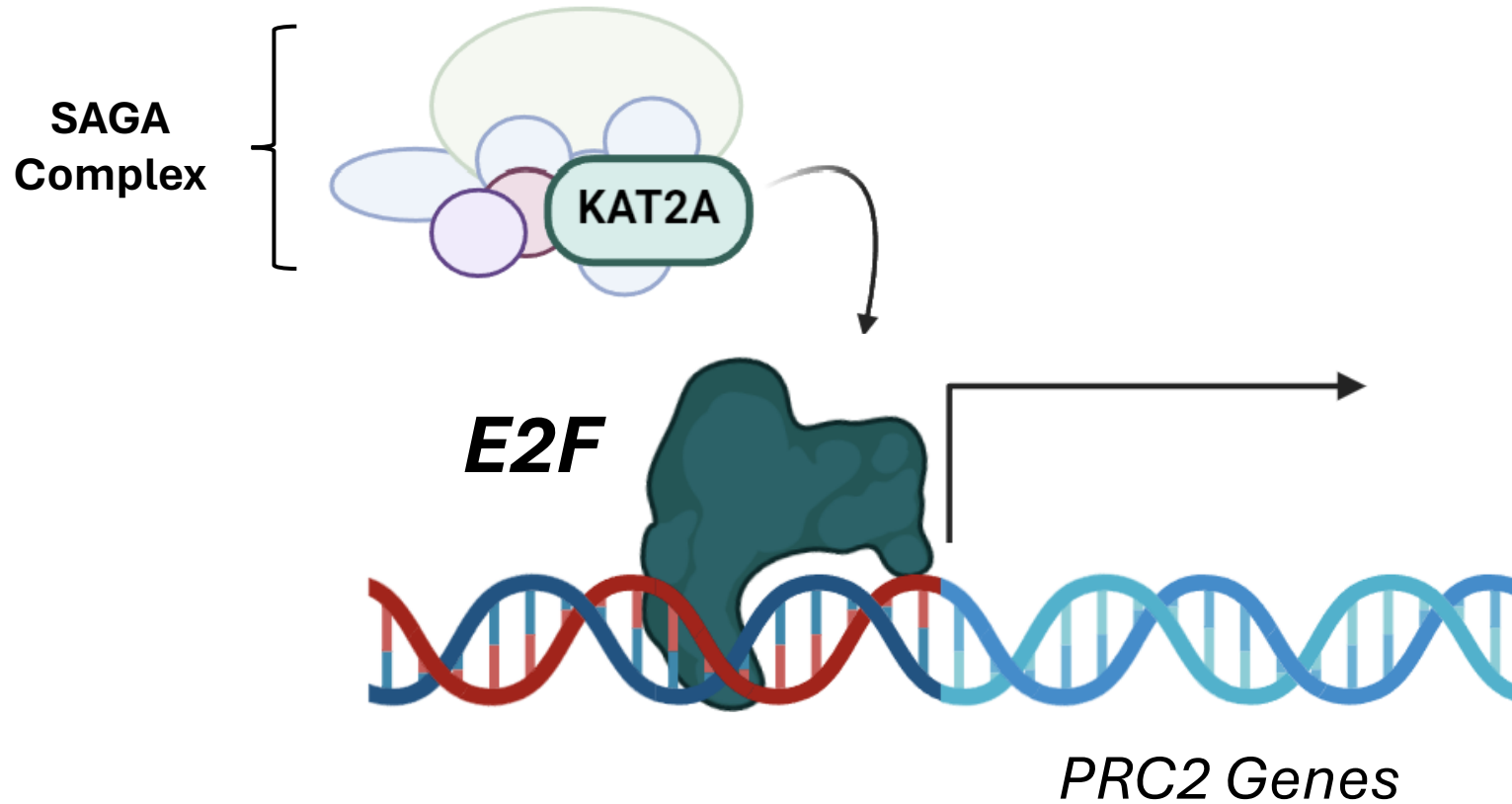
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KAT2A binds  
**E2Fs** to  
increase PRC2  
transcription



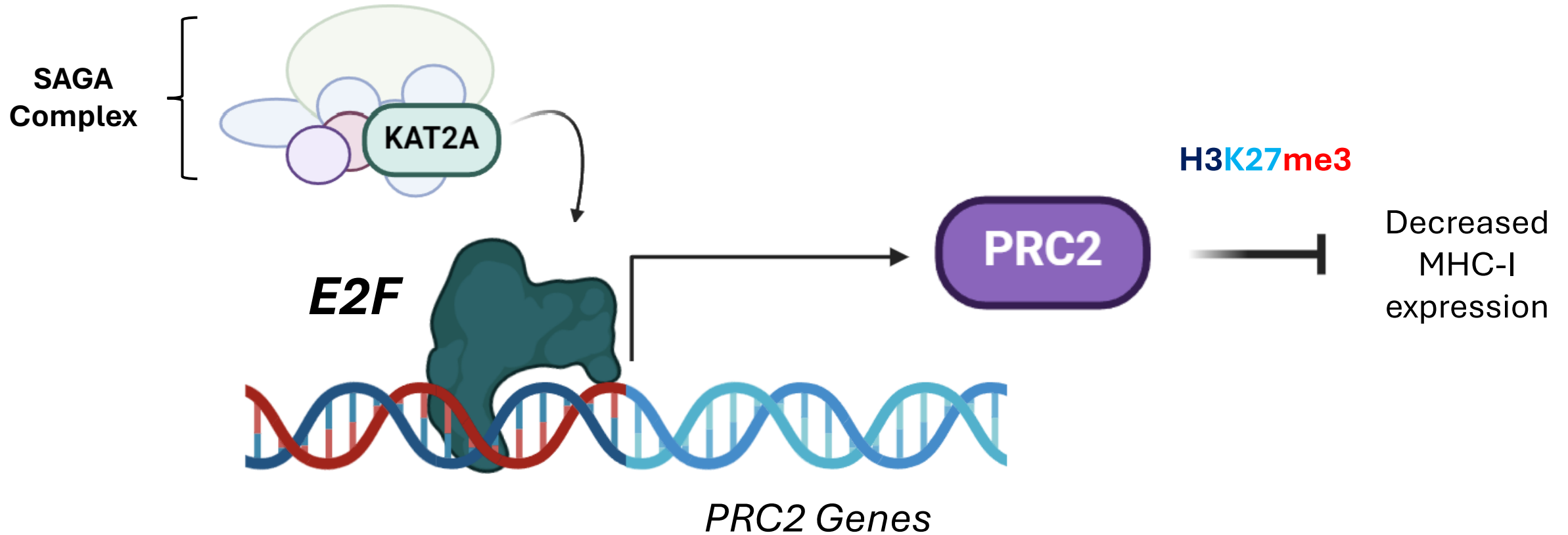
# Overall Findings

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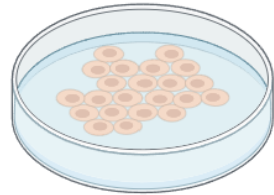
KAT2A binds **E2Fs** to increase PRC2 transcription

# Overall Findings



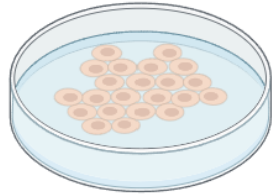
# Genome-wide CRISPR/Cas9 screen determines regulators of MHC-I expression

HPV+ Cancer Cells



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HPV+ Cancer Cells

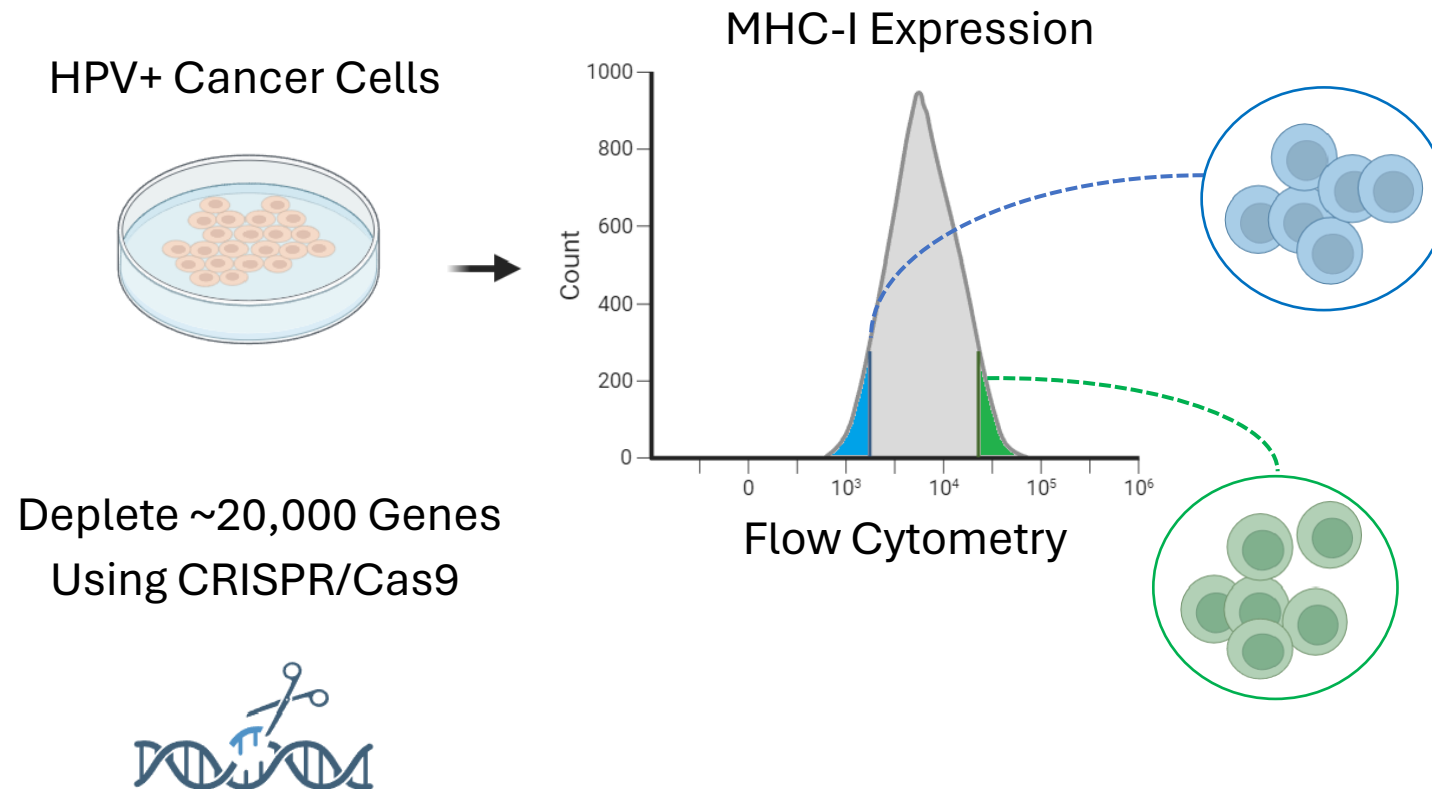


Deplete ~20,000 Genes  
Using CRISPR/Cas9



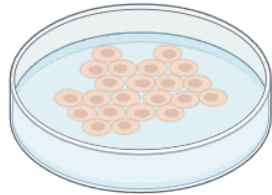


# Genome-wide CRISPR/Cas9 screen determines regulators of MHC-I expression



# Genome-wide CRISPR/Cas9 screen determines regulators of MHC-I expression

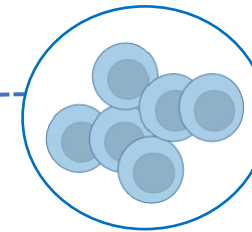
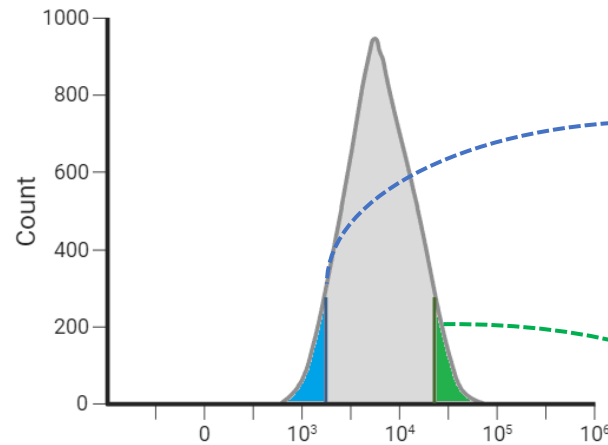
HPV+ Cancer Cells



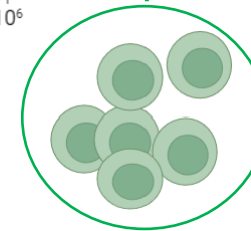
Deplete ~20,000 Genes  
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MHC-I Expression

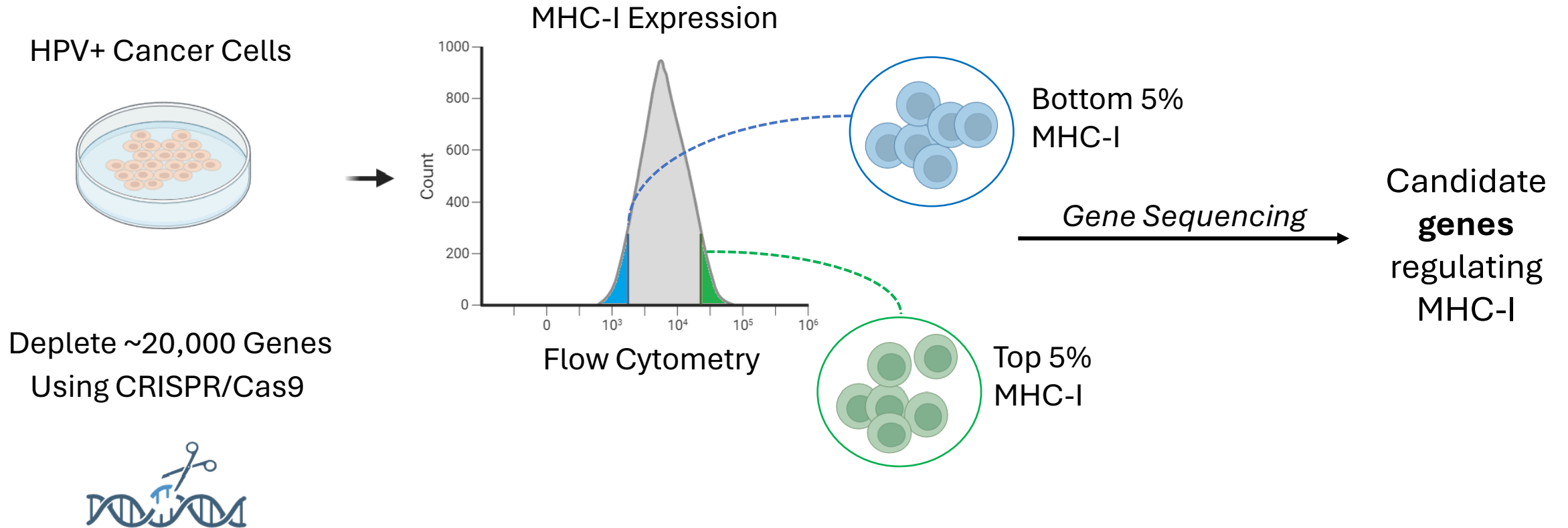


Bottom 5%  
MHC-I

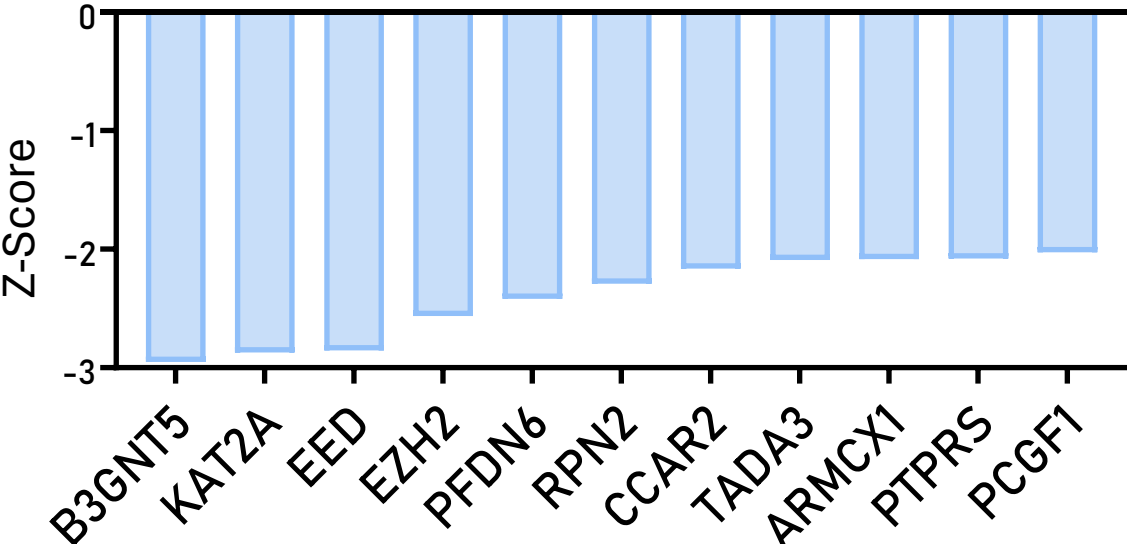
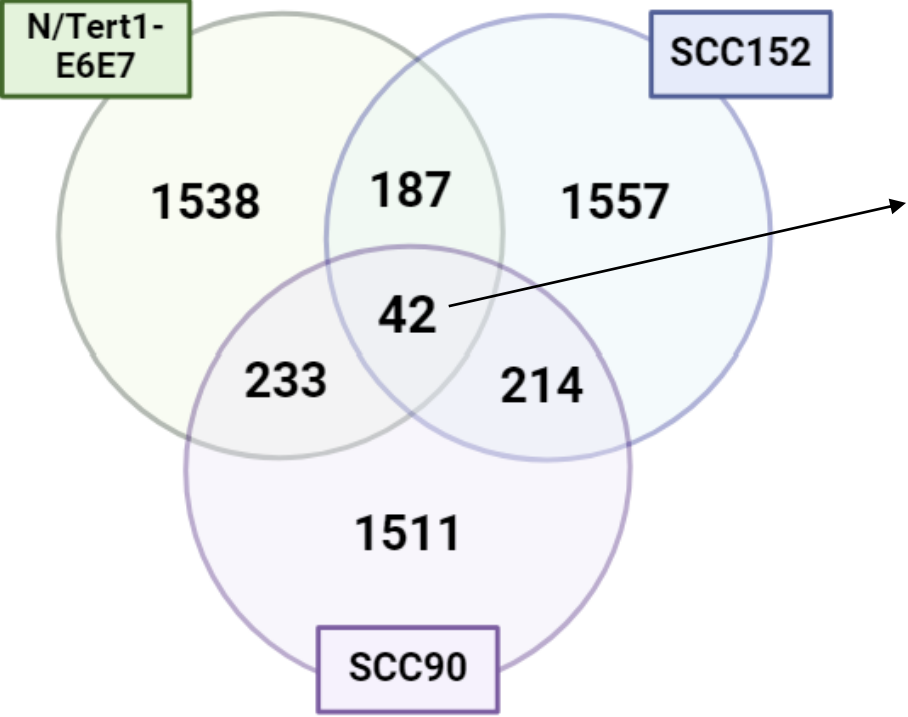


Top 5%  
MHC-I

# Genome-wide CRISPR/Cas9 screen determines regulators of MHC-I expression

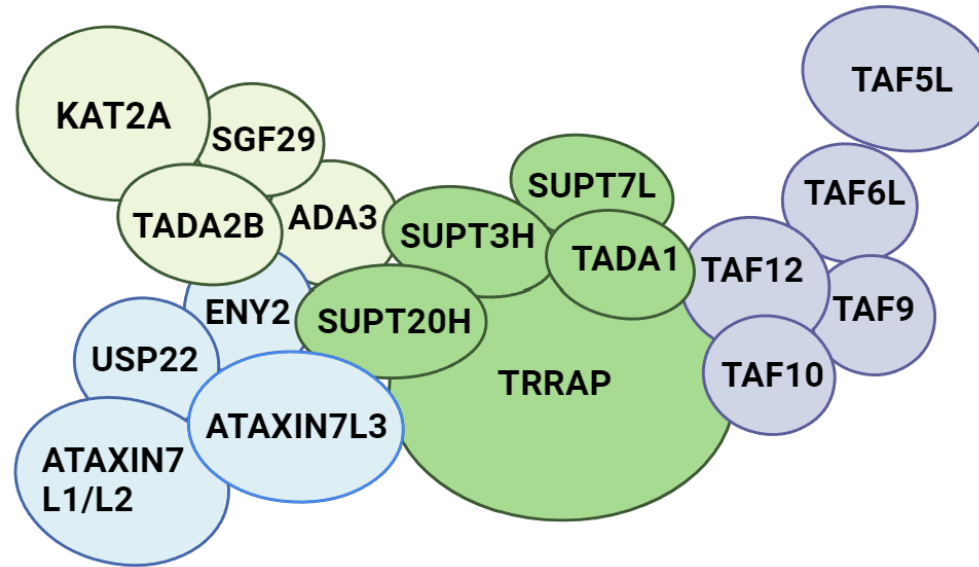


# Top Common Negative Regulators Among Screens

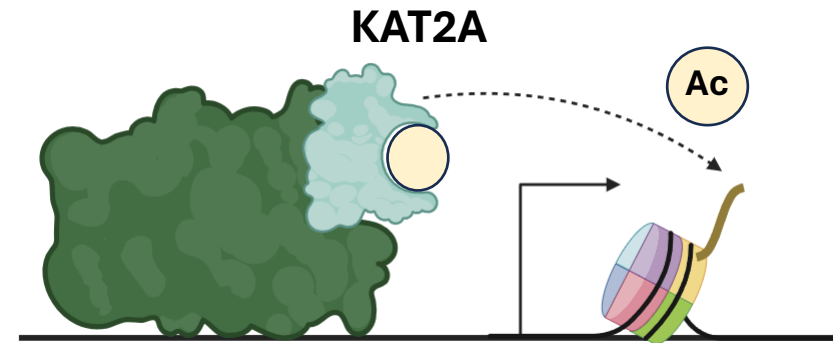
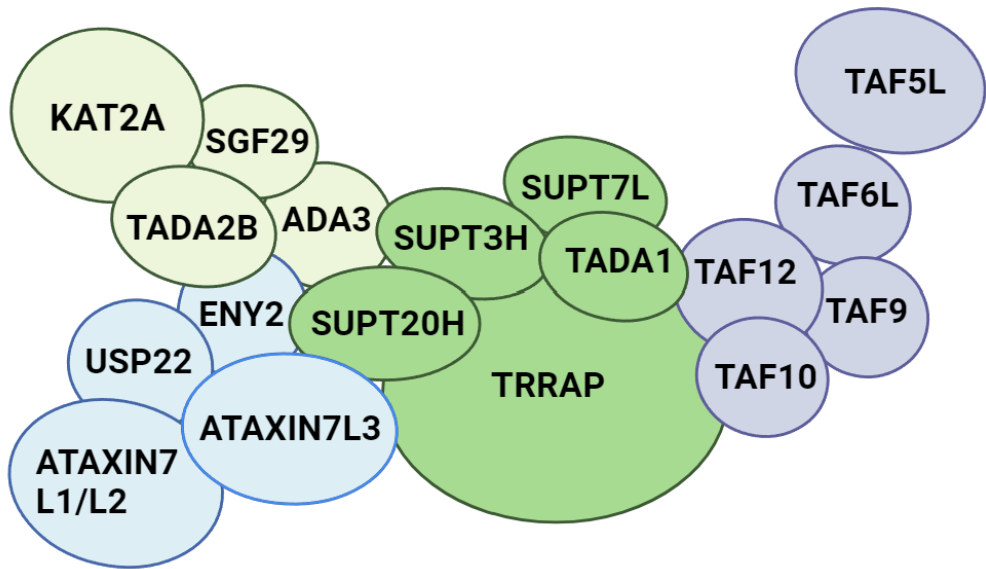


Known epigenetic regulators of gene transcription  
(PRC2 or SAGA complexes)

# The SAGA Complex



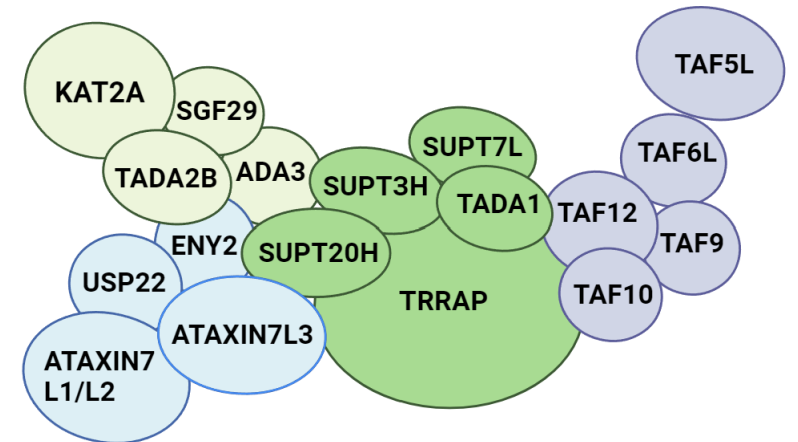
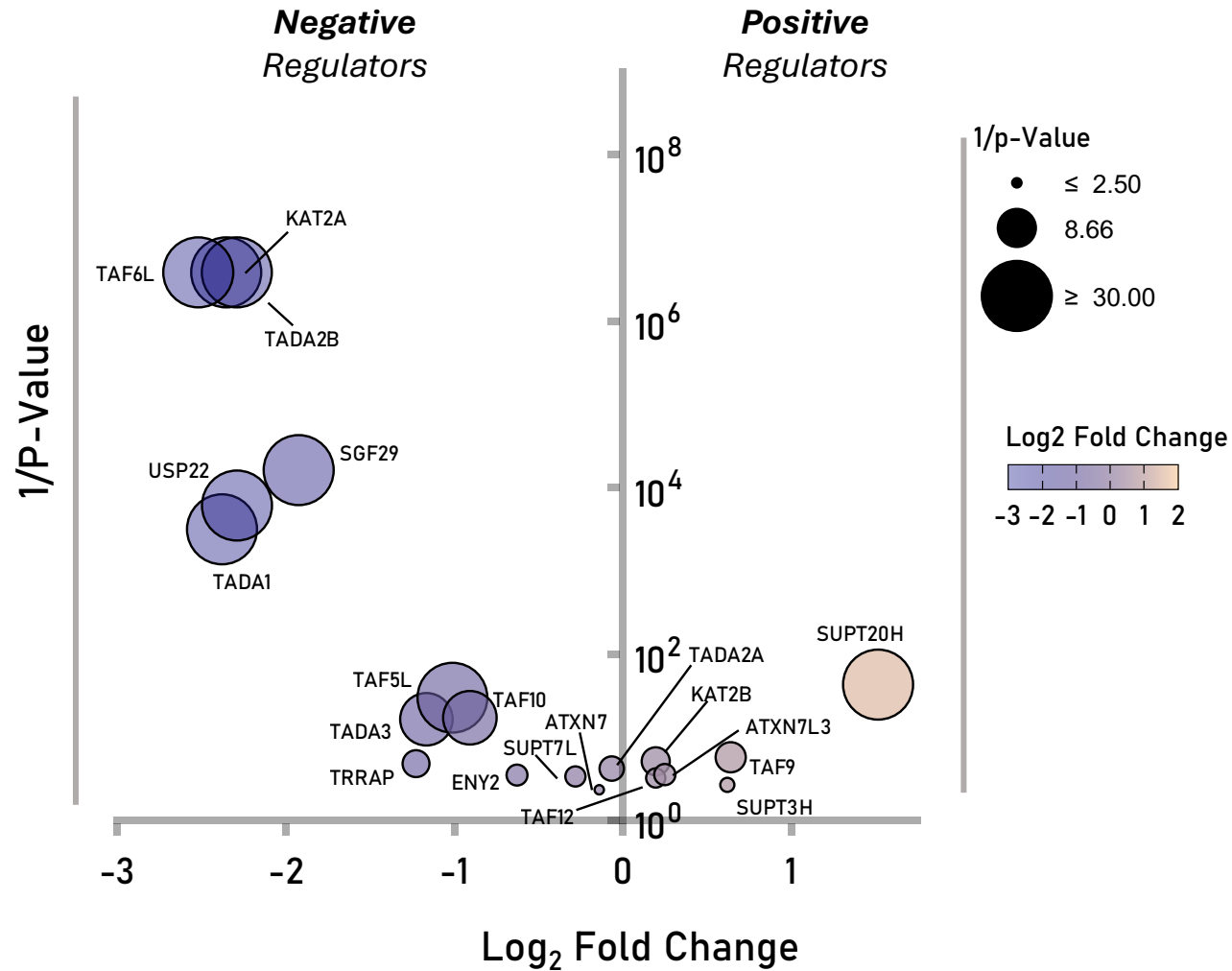
# The SAGA Complex



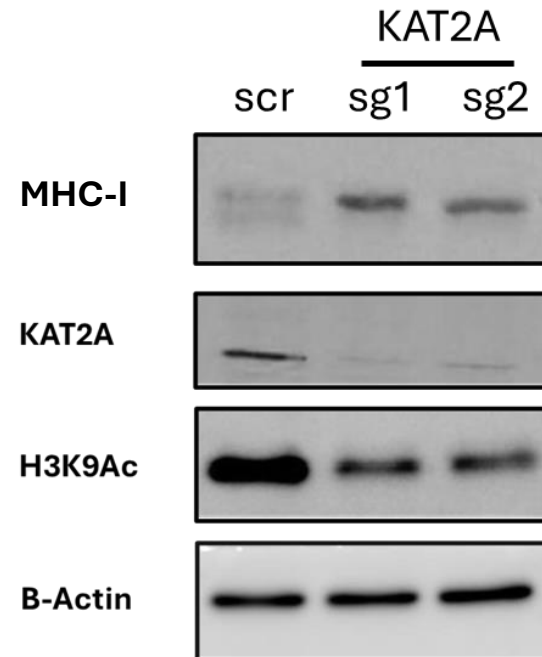
Histone Acetyltransferase

KAT2A is a regulator of transcription

# The SAGA Complex is a negative regulator of MHC-I

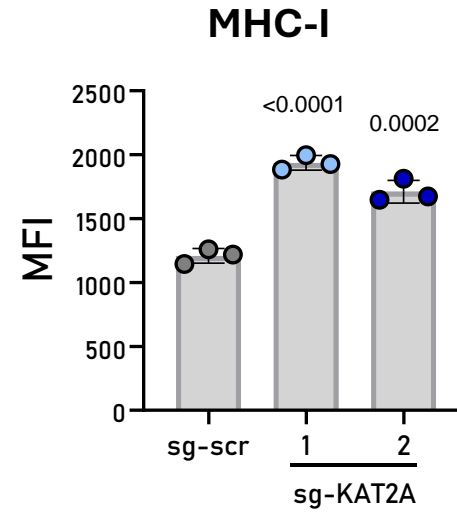
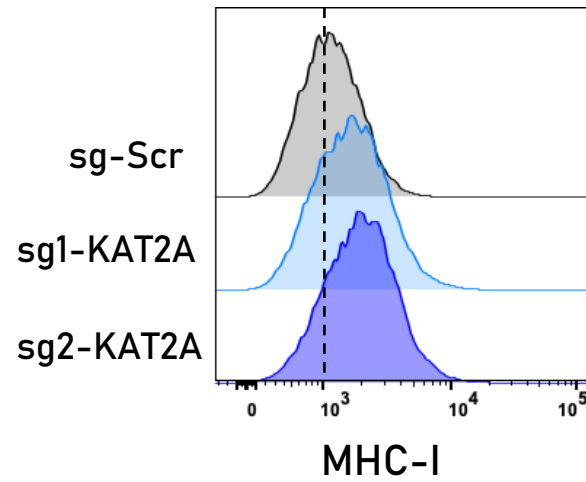
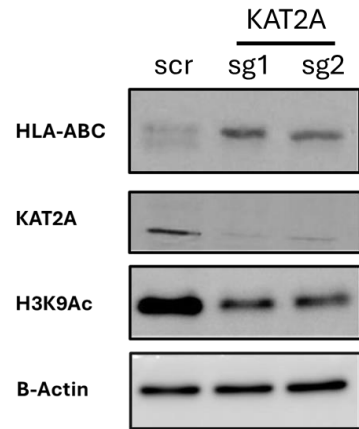


# Inhibition of KAT2A upregulates MHC-I Expression

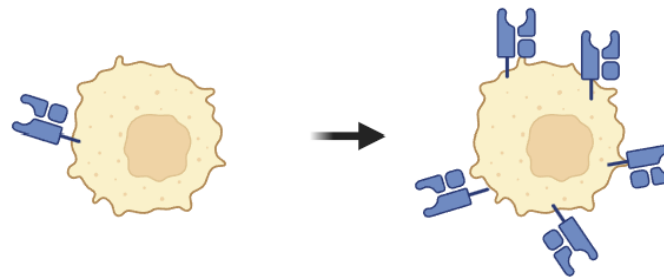
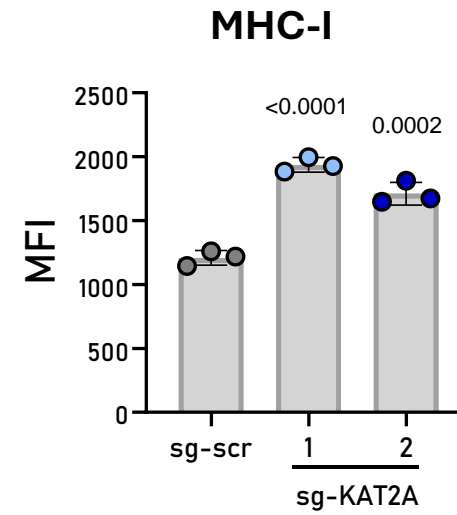
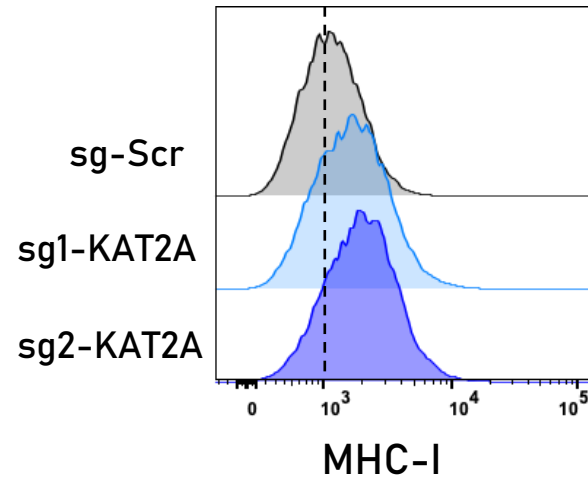
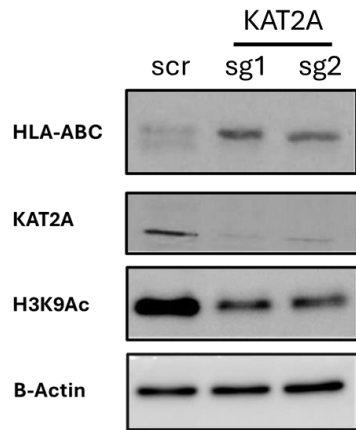




# Inhibition of KAT2A upregulates MHC-I Expression

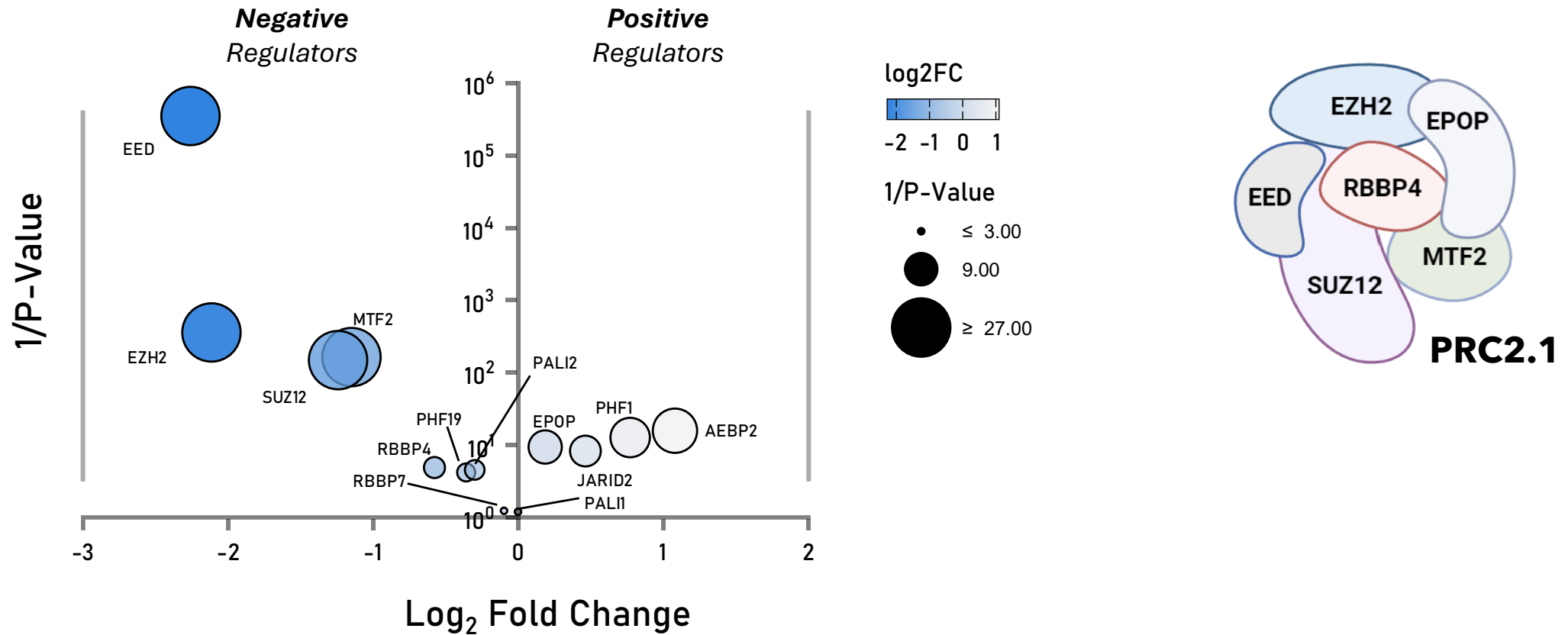


# Inhibition of KAT2A upregulates MHC-I Expression



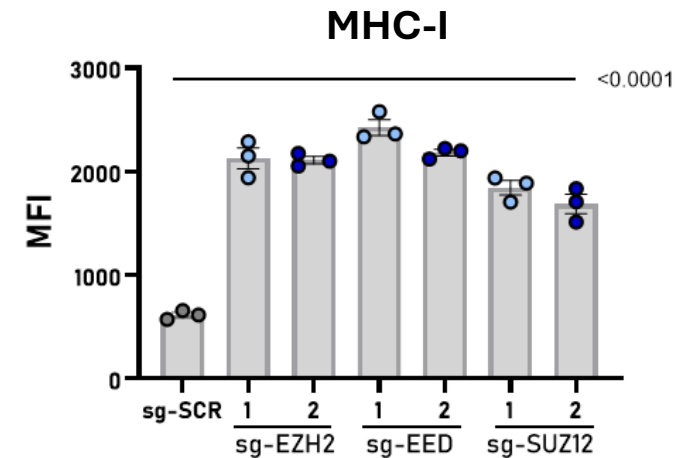
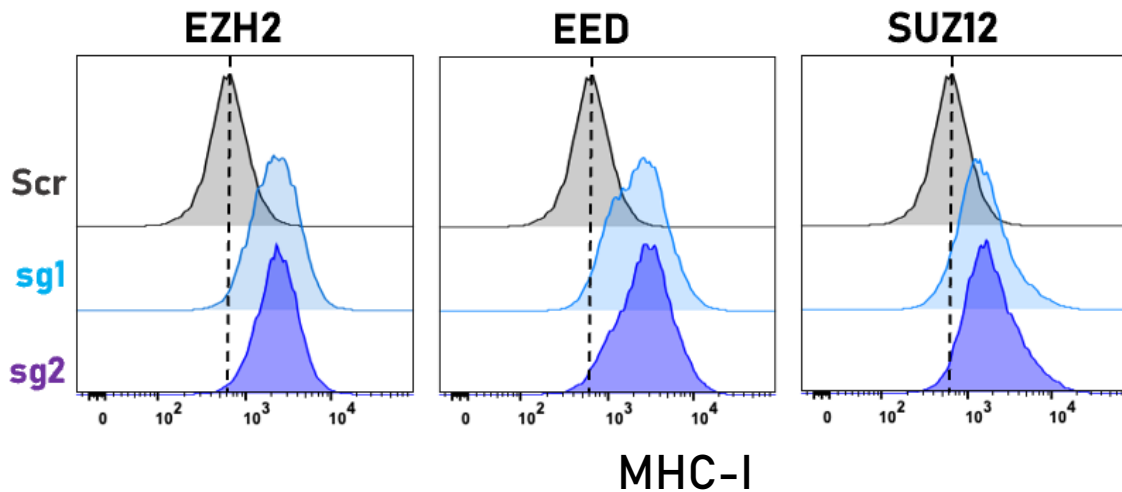
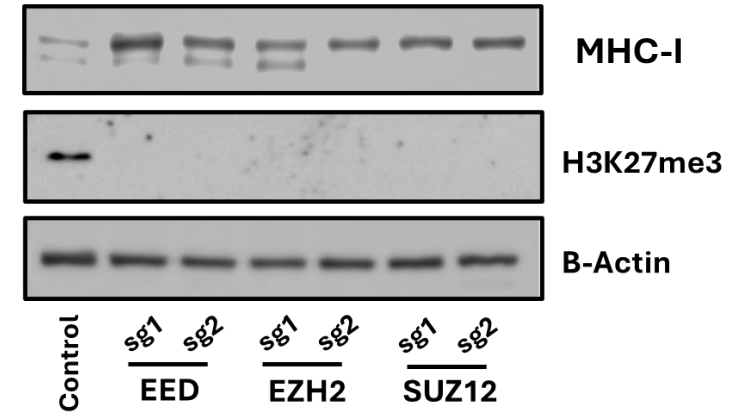
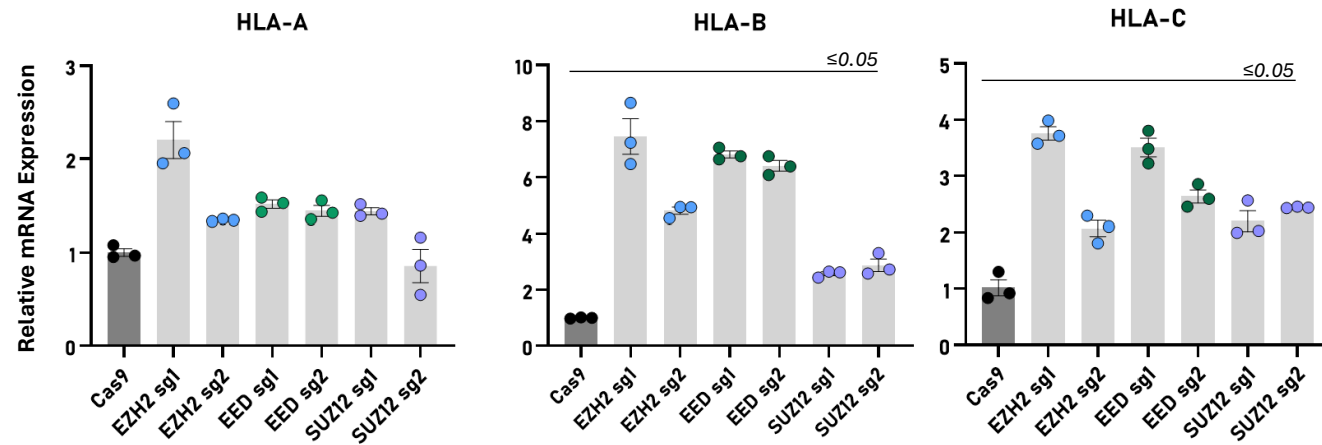
Increased MHC-I

# PRC2.1 is a negative regulator of MHC-I

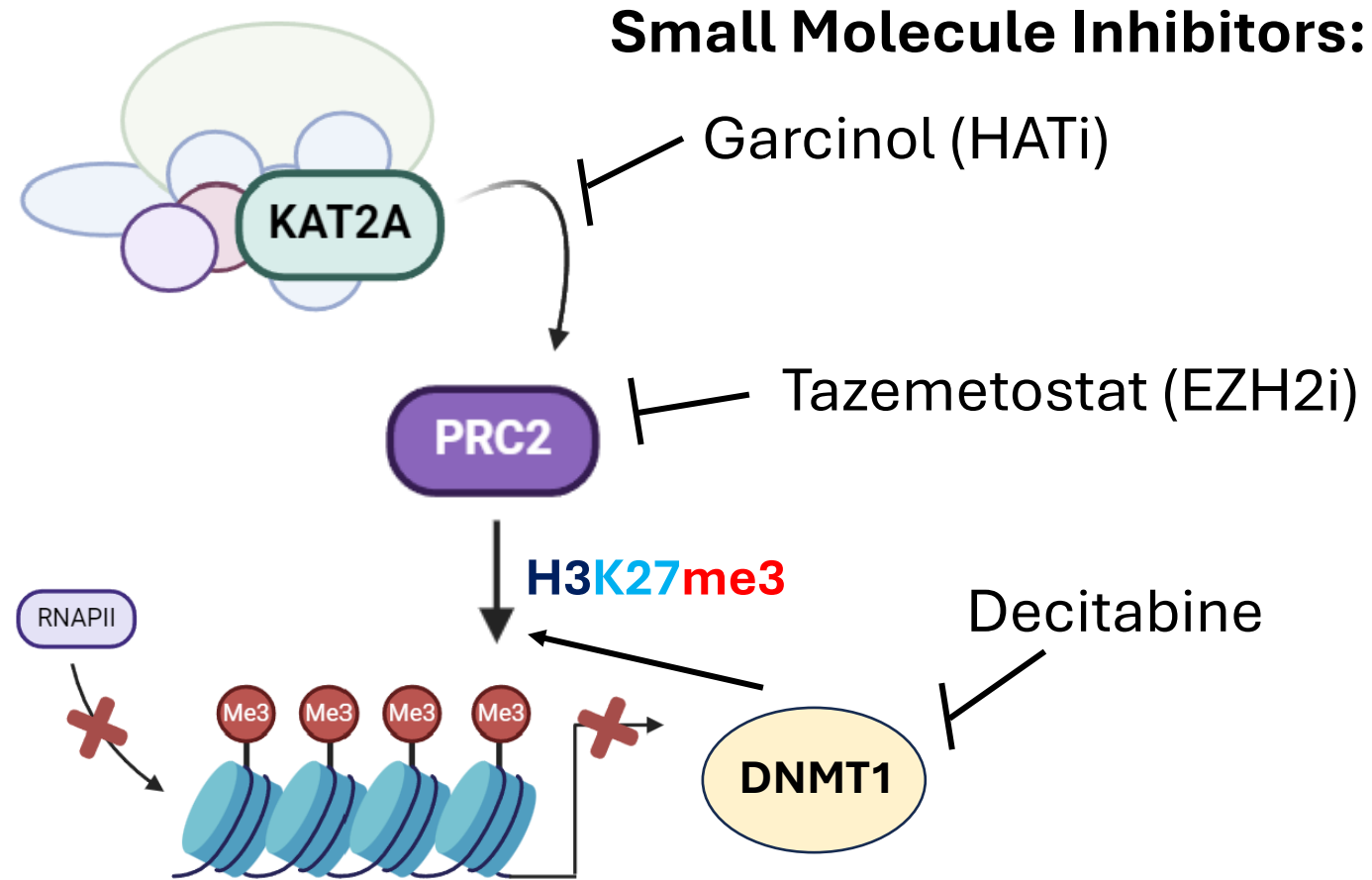


# Inhibition of PRC2 upregulates MHC-I Expression

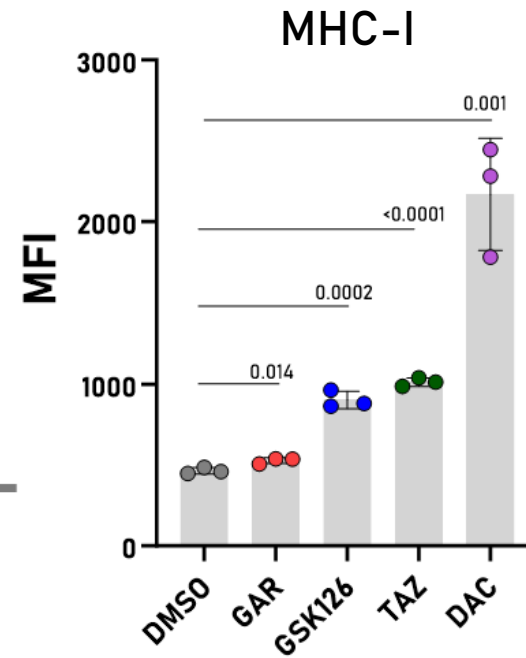
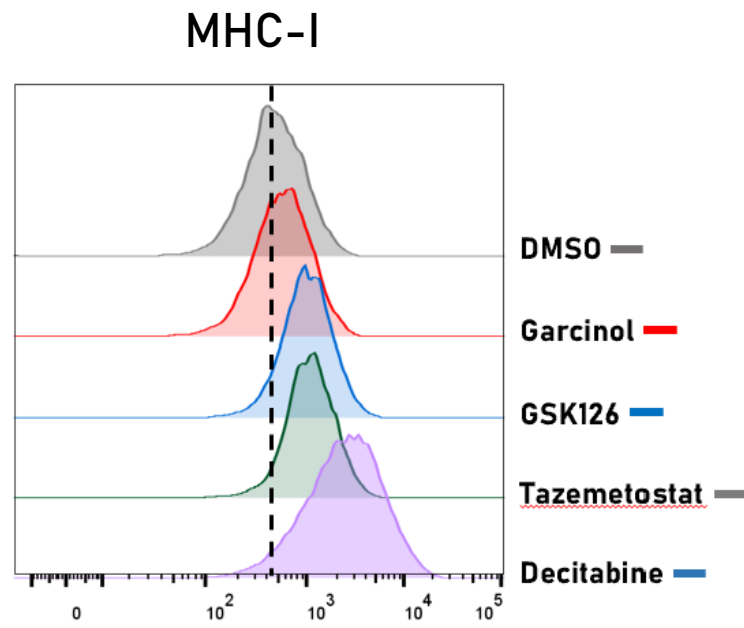
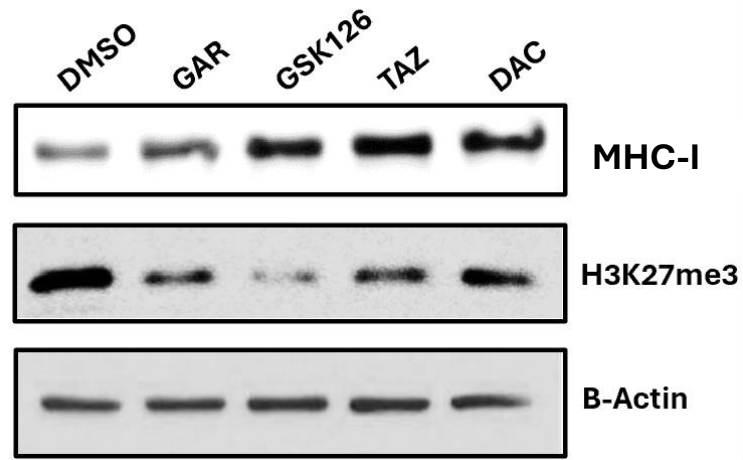
## MHC-I Gene Expression



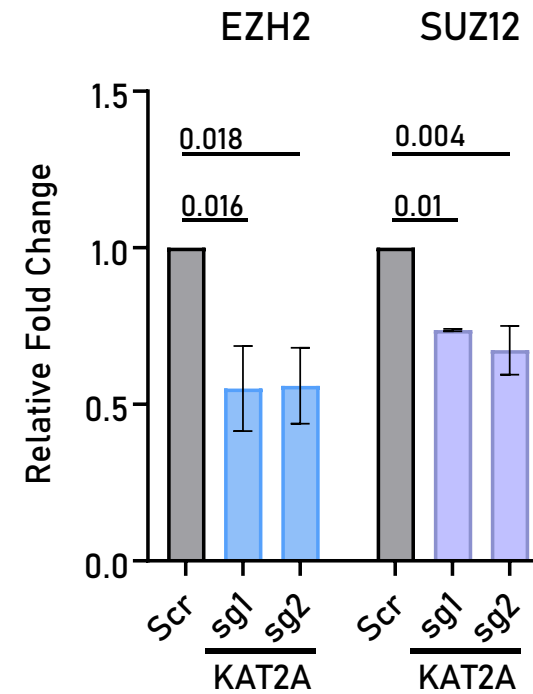
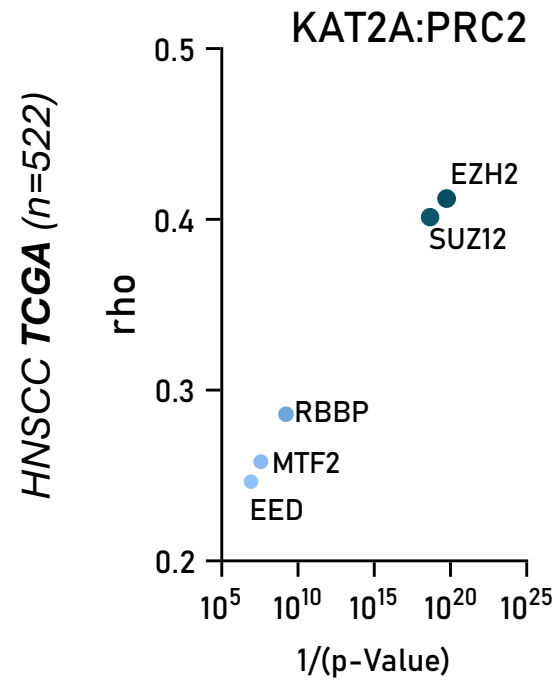
# Pharmacologic Validation and Drug Intervention



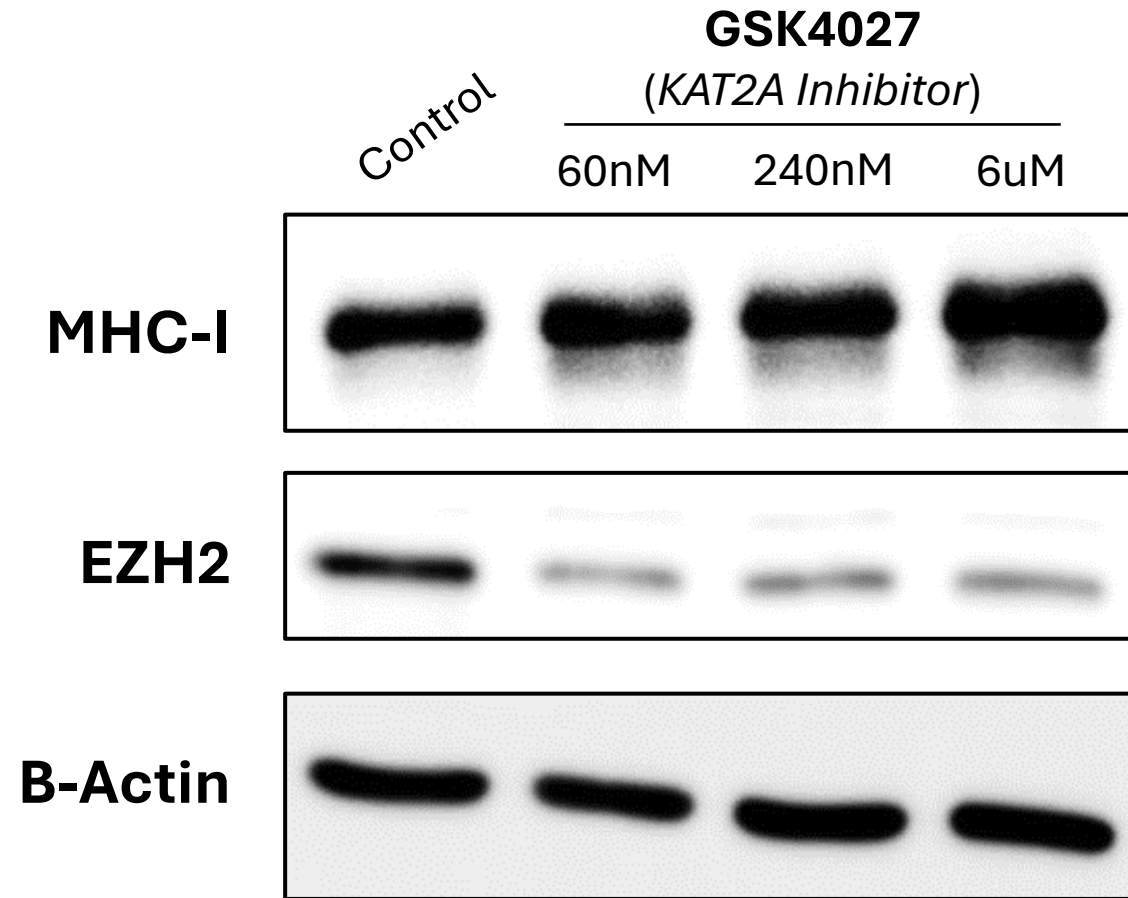
# Epigenetic inhibitors upregulate MHC-I Expression



# KAT2A controls PRC2 transcription

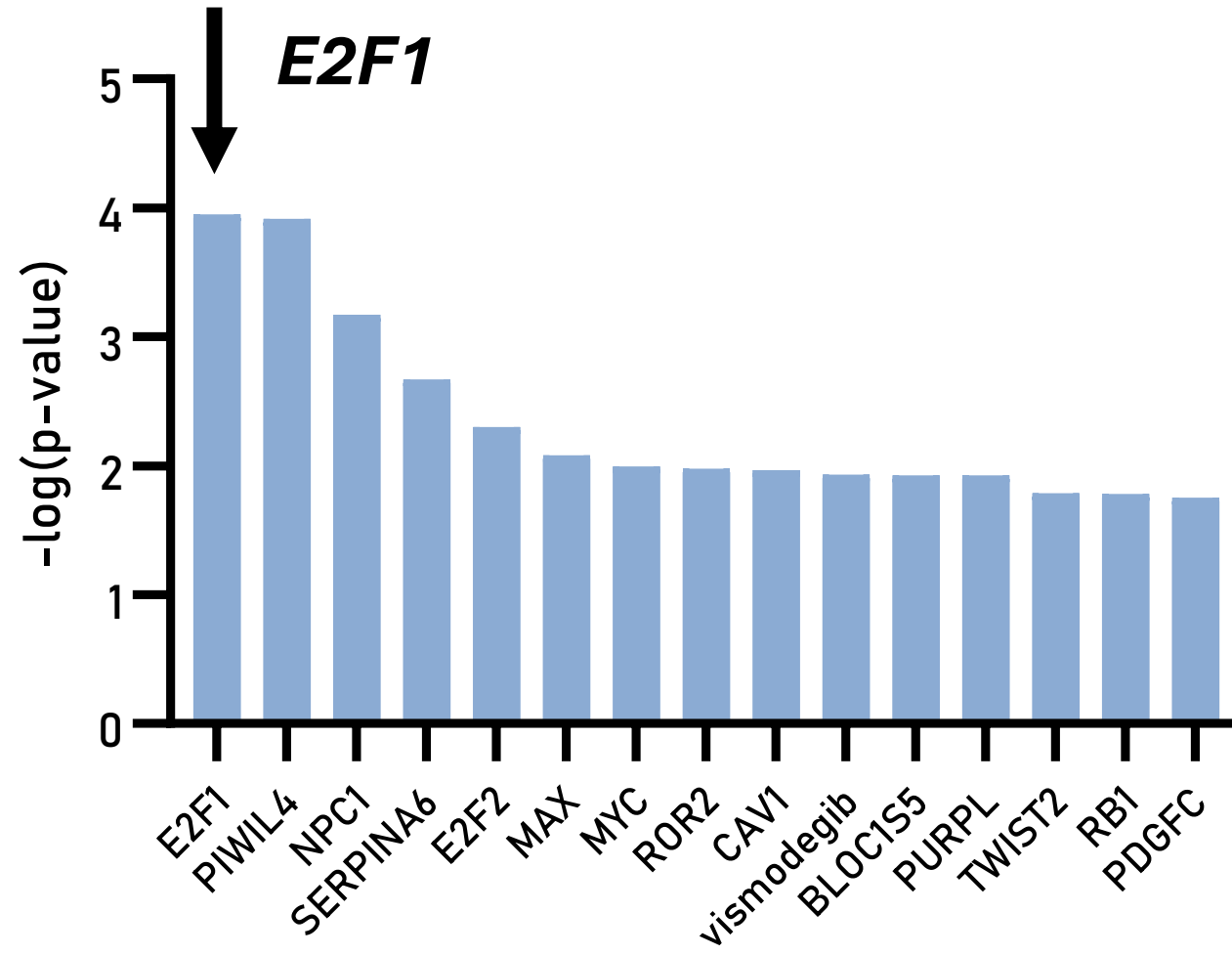


# Inhibition of KAT2A increases MHC-I and decreases PRC2 expression

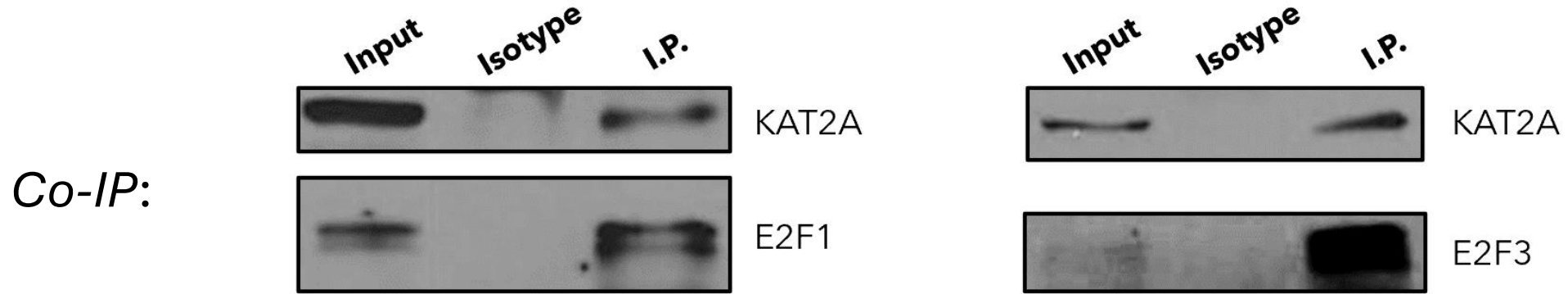




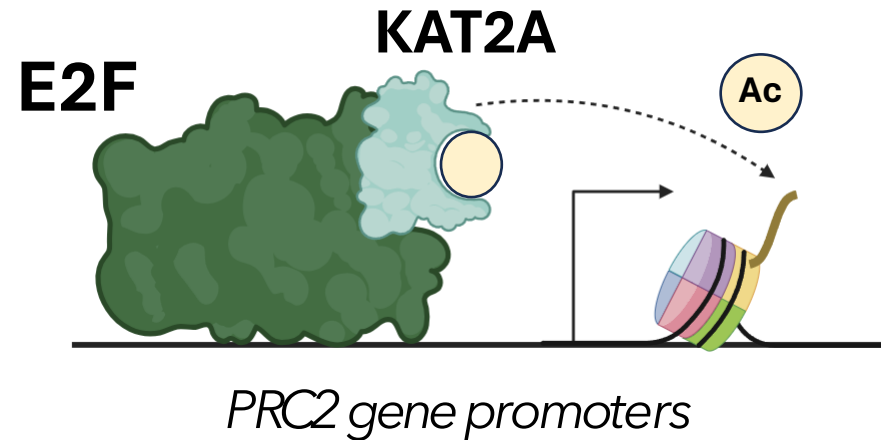
## Ingenuity Pathway Analysis: *Predicted Gene Regulators*



# KAT2A binds to E2Fs



*KAT2A fine-tunes E2F mediated transcription of PRC2 genes*

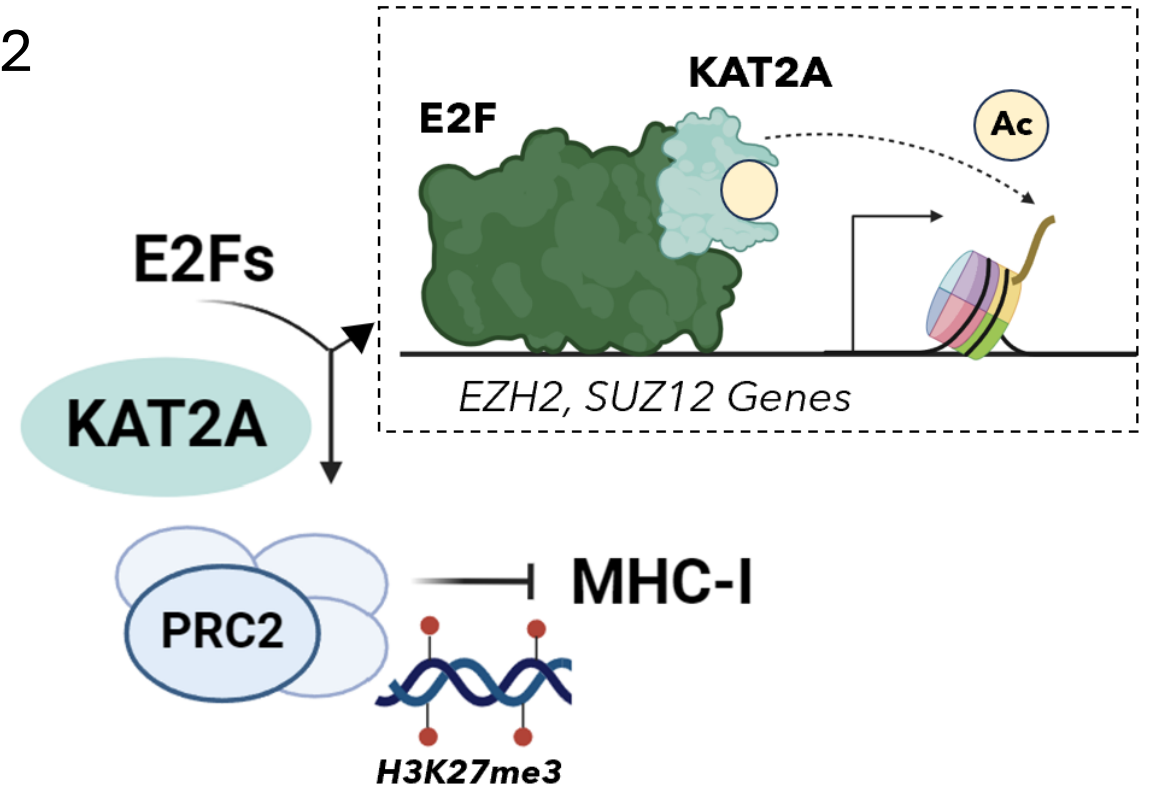


# Summary Messages

Epigenetic regulators KAT2A (SAGA) and PRC2 work in concert to repress MHC-I

Genetic and pharmacologic inhibition of KAT2A or PRC2 restored MHC-I

KAT2A and PRC2 may be **novel targets** for **treatment** of HPV+HNSCC



# Acknowledgements



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Saloni Shah

Anthony Schulte

Alex Wahl

Jessica de Assis



THE AITCH FOUNDATION

Hope. Heart. Health

Lauren Aitch

## **Committee**

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Dr. Eran Andrechek

Dr. Hua Xiao

Dr. Karen Liby

Dr. Yasser Aldhamen

**Software:**

*Illustrations made with Biorender*

*IPA Analysis: Qiagen*

## **Collaborators**

Dr. Andrew Olive

John Vusich

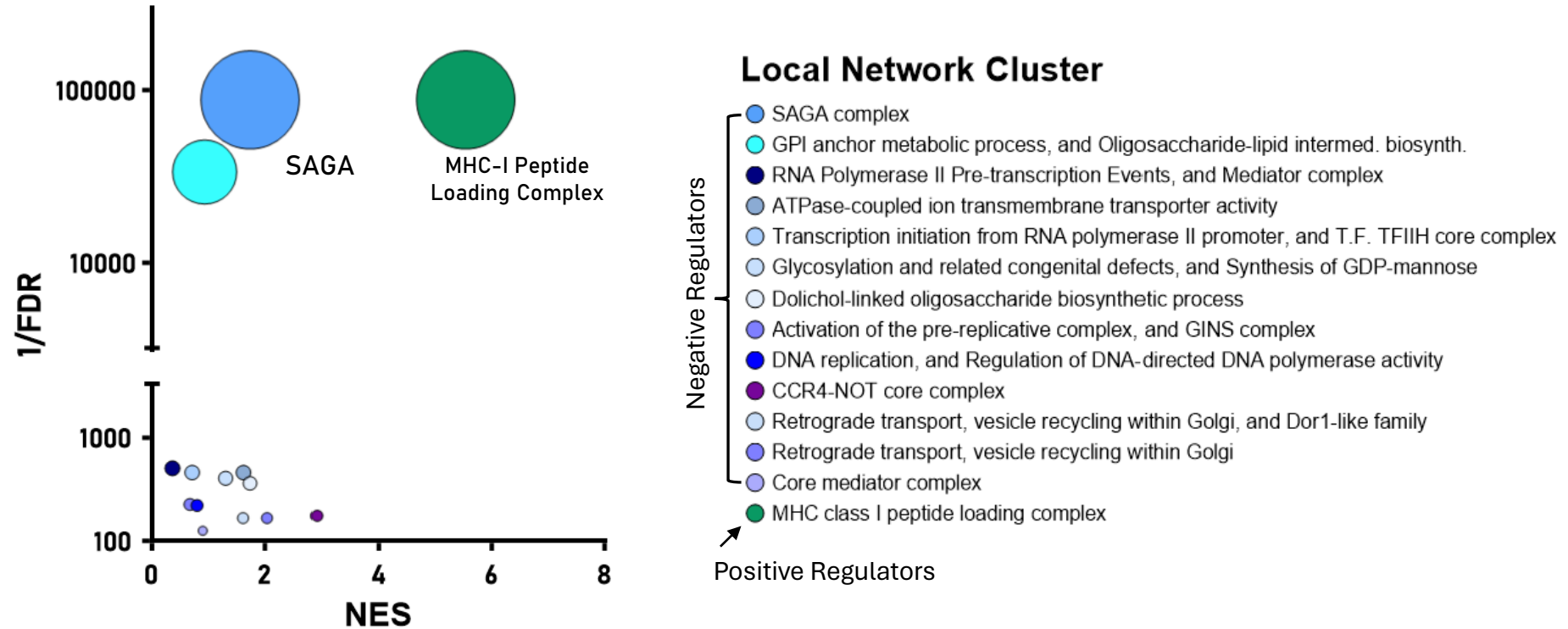
**Title Slide:**

*Henry Siddons Mowbray,*

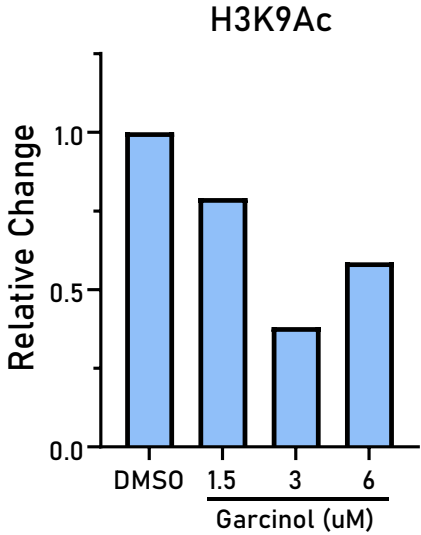
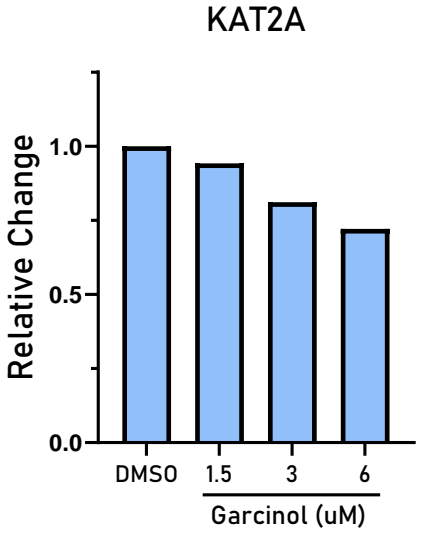
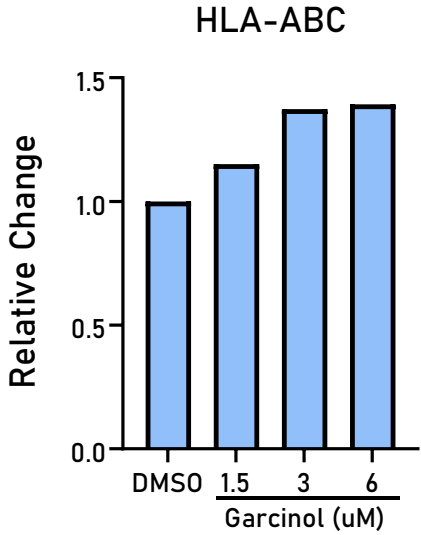
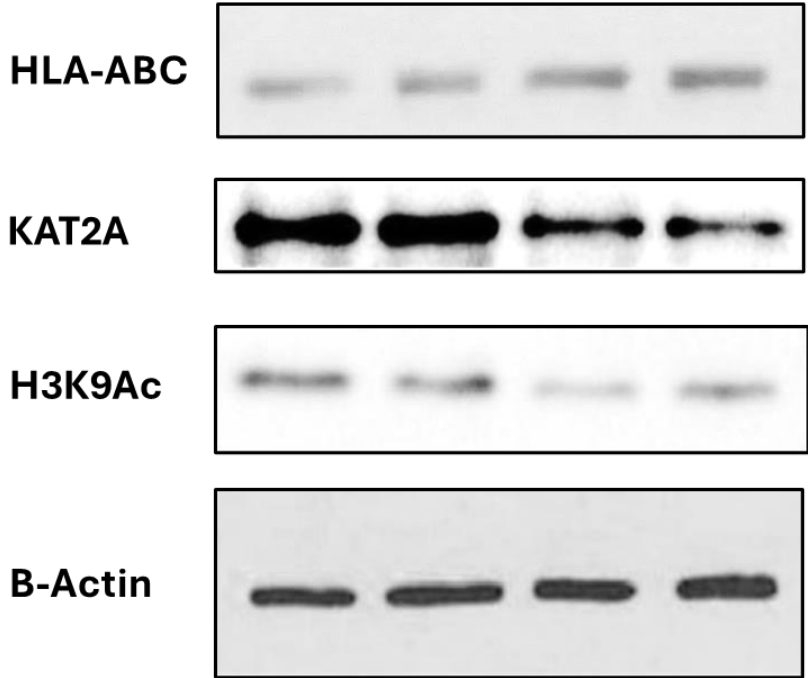
**Destiny, 1896**

*Museum of Fine Arts, Boston MA*

# SAGA complex is predominant network of MHC-I downregulation



# Garcinol upregulates MHC-I in HPV+HNSCC

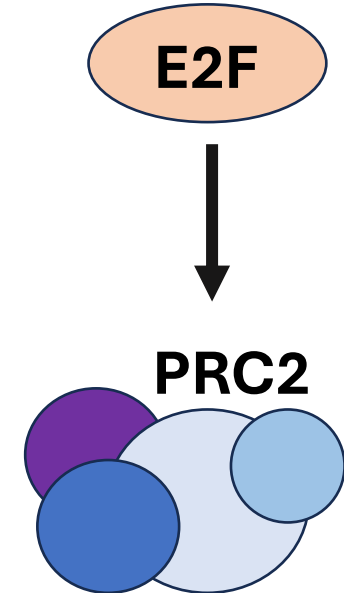


# How is E2F downregulating MHC-I?

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EZH2 is downstream of the pRB-E2F pathway,  
essential for proliferation and amplified in cancer

Adrian P Bracken<sup>1</sup>, Diego Pasini, Maria Capra, Elena Prosperini, Elena Colli, Kristian Helin  
EMBO J. **2003** Oct 15;22(20):5323-35.



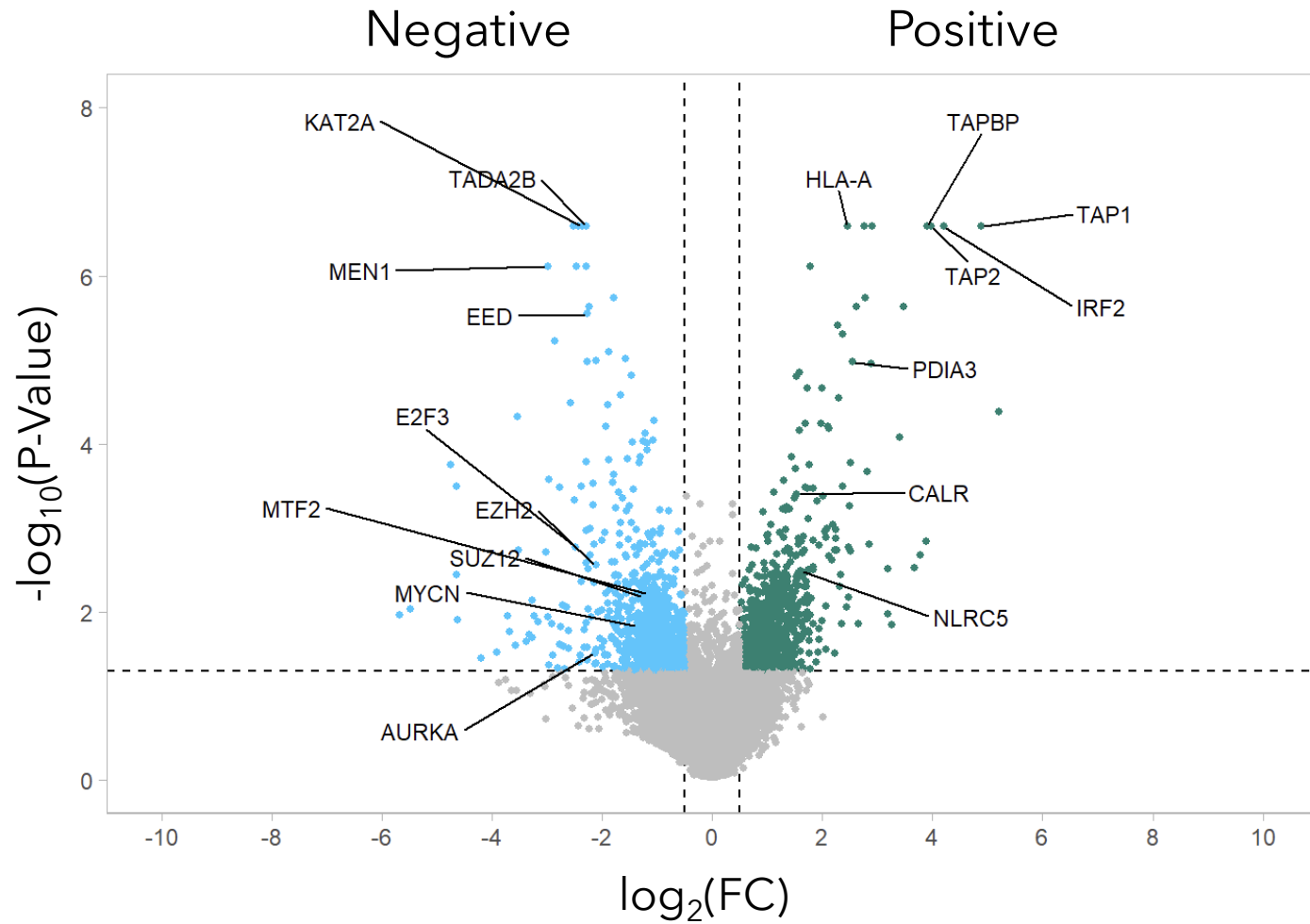
Identification of the polycomb group protein SU(Z)12  
as a potential molecular target for human cancer  
therapy

Antonis Kirmizis<sup>1</sup>, Stephanie M Bartley, Peggy J Farnham

Mol Cancer Ther. **2003** Jan;2(1):113-21.



# Regulators of MHC-I Expression





# KAT2A expression negatively correlates with immune cell infiltration

