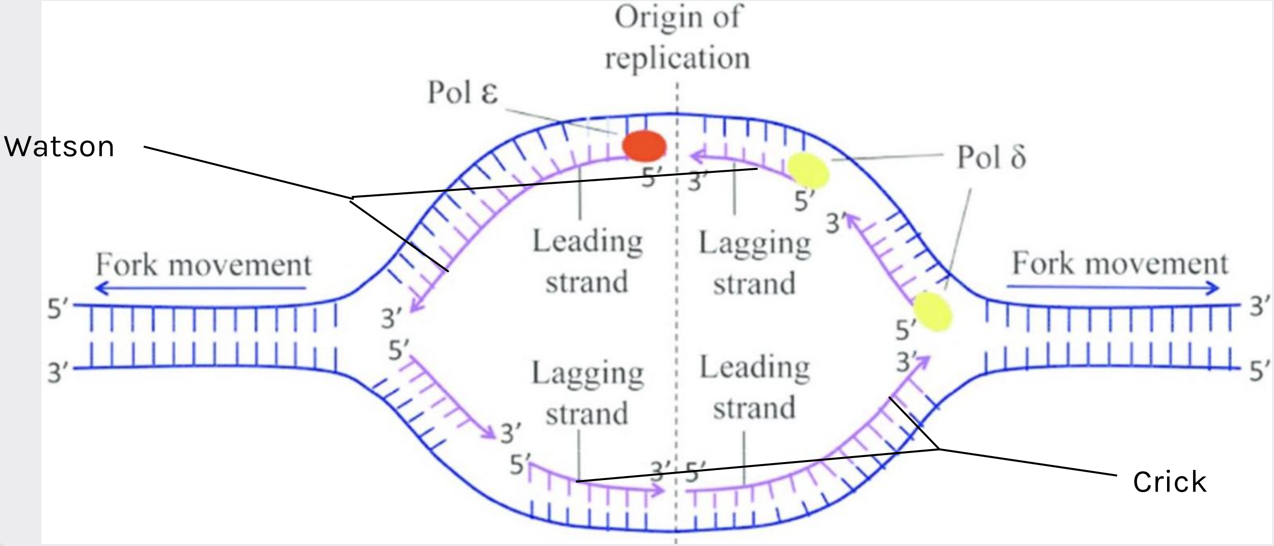
DNA Pol 32 governs parental histone transfer to DNA replication lagging strand

Kimin Nguyen, Jennifer Amador-Gonzalez, Meghana Nittala, Natalie Wang, James Mohn

Intro

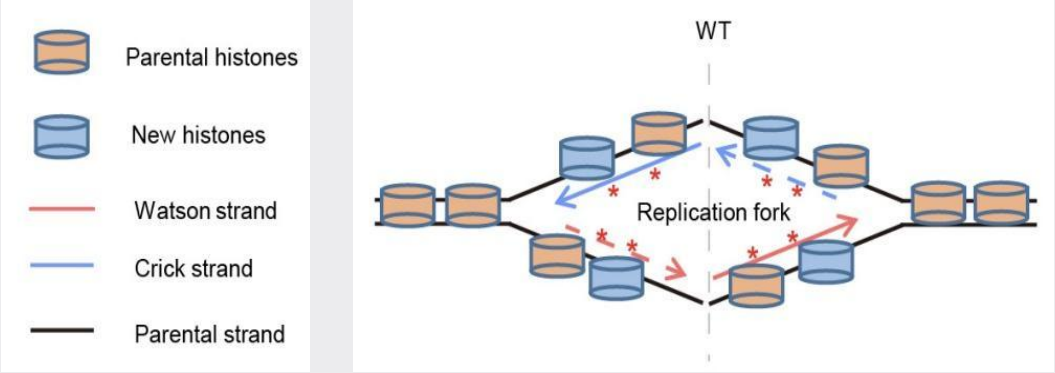
- Chromatin inheritance
  - Conservation of **cellular identity** during cell division
  - **chromatin** replication is linked with **DNA** replication



Chen, W. (n.d.). The schematic diagram of origin of replication of human. https://www.researchgate.net/figure/The-schematic-diagram-of-origin-of-replication-of-human-The-process-of-

System

- Nucleosome reassembly during replication
  - **Recycling** of **parental histones** + recruitment of **new histones** → restoration of chromatin in daughter strands
  - = **faithful** epigenetic inheritance



→ Daughter strands have **equal** amounts of **parental** and **new histones**

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Study

Known

- In **yeast**, recycling of **parental** histones is mediated by…
    - DNA Pol ε subunits **Dpb3/Dpb4**
        - Onto **leading** strand
    - MCM helicase subunit **Mcm2**
        - Onto **lagging** strand

Pathway?

Findings

- The researchers identified **Pol32…**
    - Non-essential subunit of DNA Pol δ
- Overall findings
    - Pol32 **binds H3/H4** *in vitro* and *in vivo*
    - Is a critical **histone chaperone** acting **downstream** of Mcm2

eSPAN

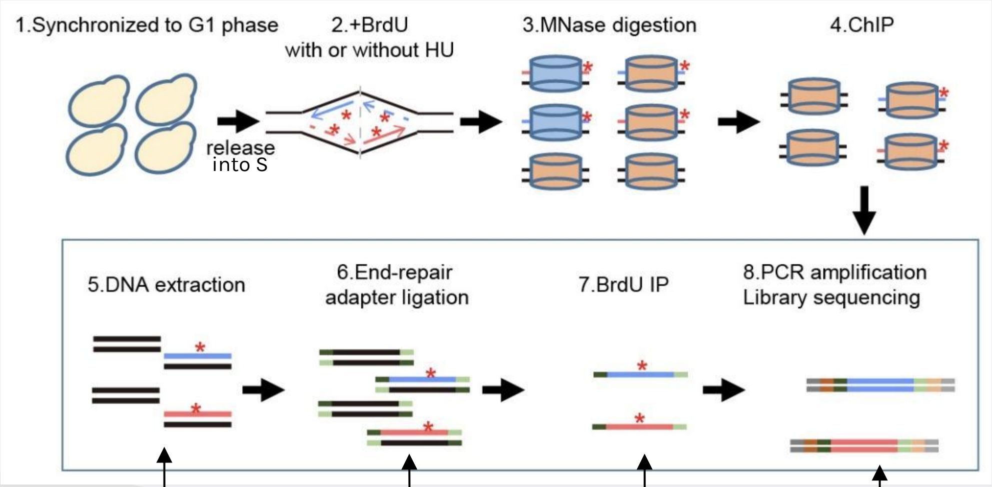
- **E**nrichment and **s**equencing of **p**rotein-**a**ssociated **n**ascent DNA
- Detects protein enrichment at replication forks w/ **strand specificity**
  - **Leading** vs. **lagging** strand

Yeast cells treated with alpha factor

- BrdU - thymidine analog
- HU - stops replication

Cleaves around nucleosomes

Against POI (histones)

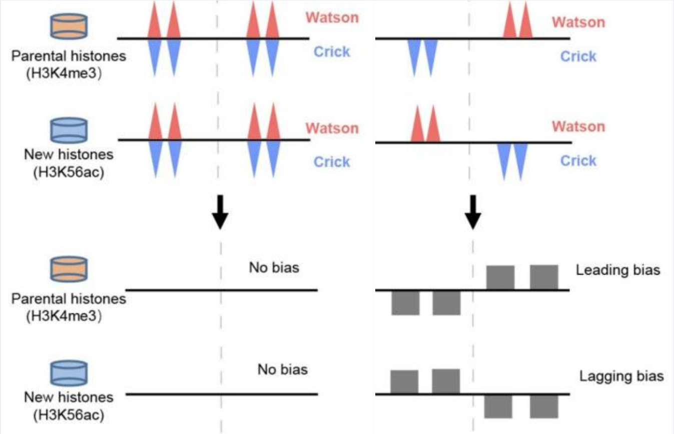


DNA that POI interacts with

Preserves strand specificity

ssDNA isolated

Map to reference genome



Bias calculation $\log_2$ (watson/crick)

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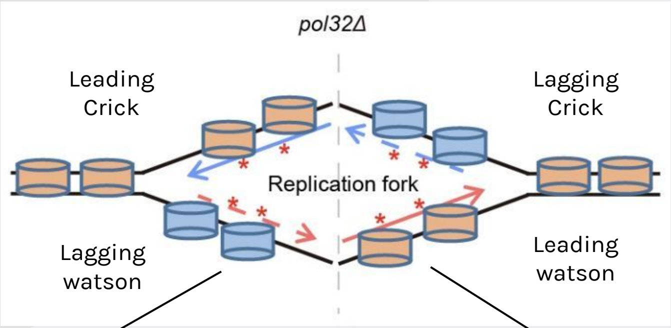
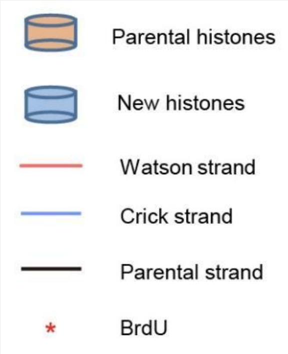
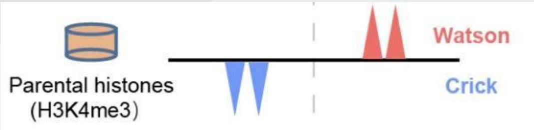
FIGURE 1

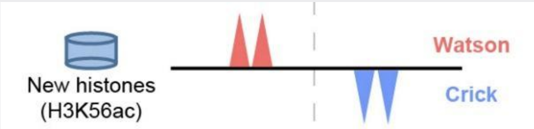
Pol32 Deposits Parental Histones - Histone Modifications

- **H3K4me3** - marks **parental** histones
- **H3K56ac** - marks **new** histones



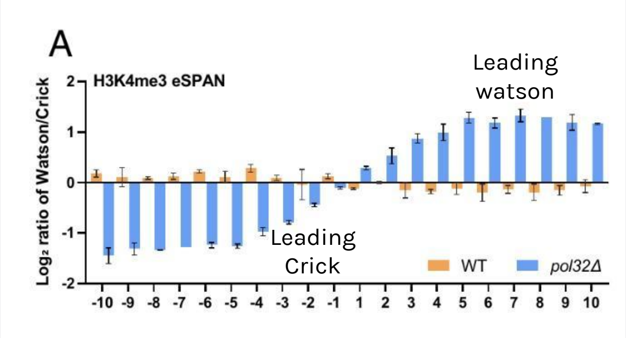


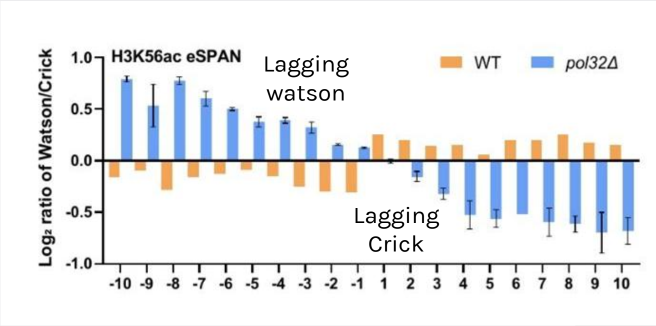




WT only shows slight bias

Parental histones show strong **leading strand bias** in Pol32Δ

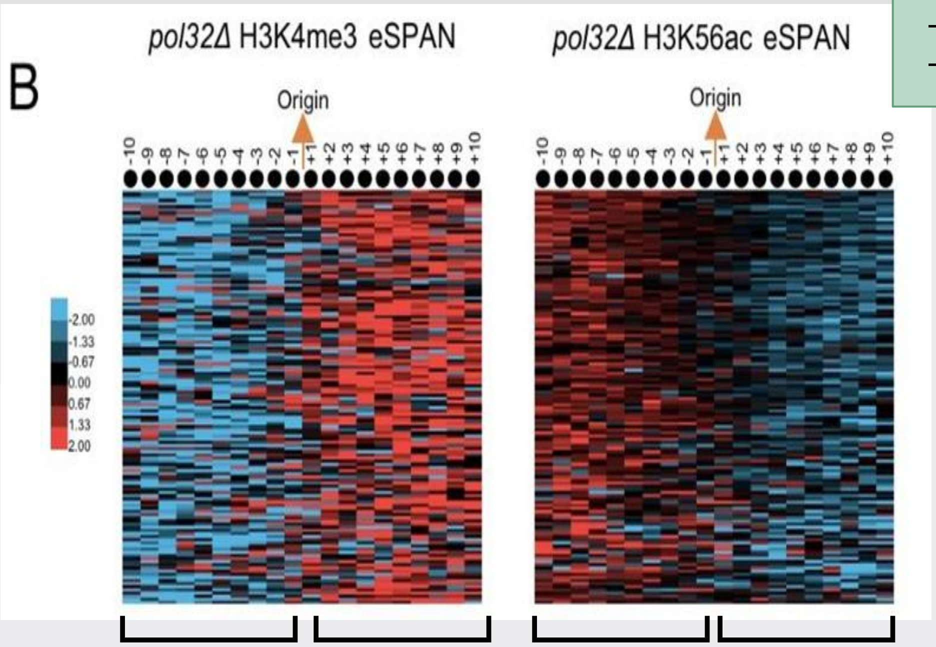




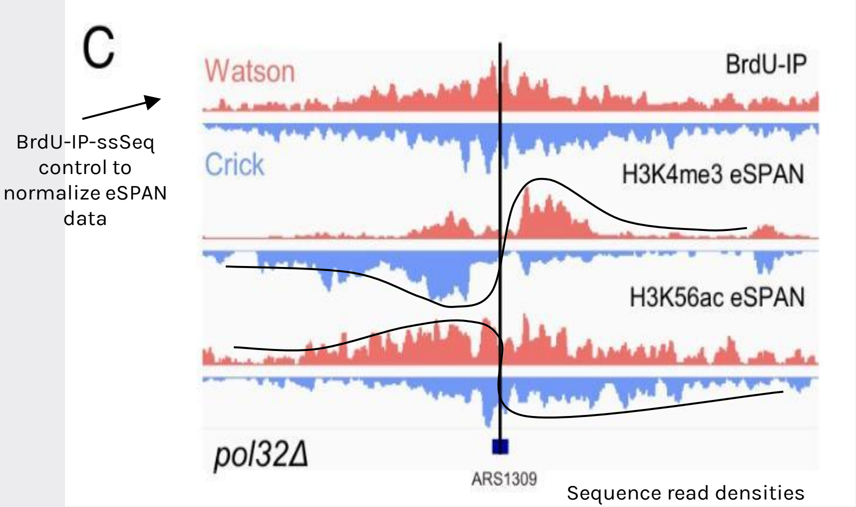
New histones show strong **lagging strand bias** in Pol32Δ

C. Tian, Q. Zhang, J. Jia, J. Zhou, Z. Zhang, S. Karri, J. Jiang, Q. Dickinson, Y. Yao, X. Tang, Y. Huang, T. Guo, Z. He, Z. Liu, Y. Gao, X. Yang, Y. Wu, K.M. Chan, D. Zhang, J. Han, C. Yu, H. Gan, DNA polymerase delta governs parental histone transfer to DNA replication lagging strand, Proc. Natl. Acad. Sci. U.S.A., 121 (20) e2400610121,, https://doi.org/10.1073/pnas.2400610121 (2024).

Pol32 Deposits Parental Histones - Histone Modifications



- Bias ratios of 10 flanking nucleosomes
- 134 early DNA replication origins



Clear color differences show Pol32Δ have strong histone deposition biases

Pol32 participates in **parental** histone H3-H4 deposition onto the **lagging** strand

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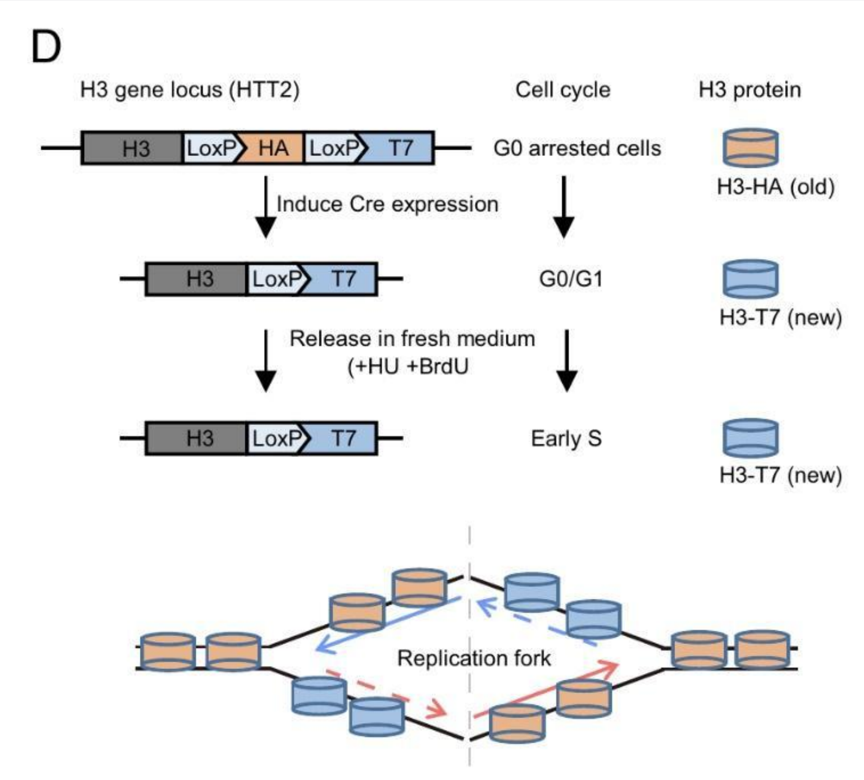
Pol32 Deposits Parental Histones - Histone Subunits



- Recombination-induced Tag Exchange System

1. Integrate cassette w/ floxed HA
2. Temporally control Cre expression
3. Cre excises HA tag
4. T7 tag now expressed

- H3-HA → **parental** histones
- H3-T7 → **new** histones

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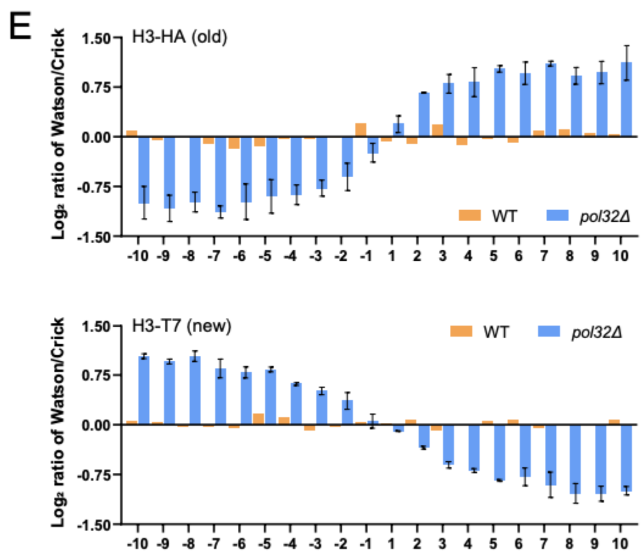
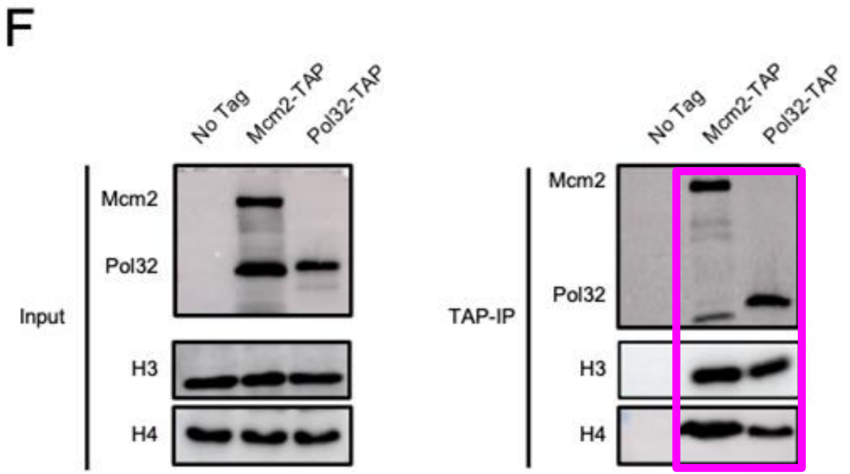
Pol32's Role in Histone Transfer





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Direct Interaction of Pol32 with Histones H3–H4 Confirmed by GST Pull-Down Assay

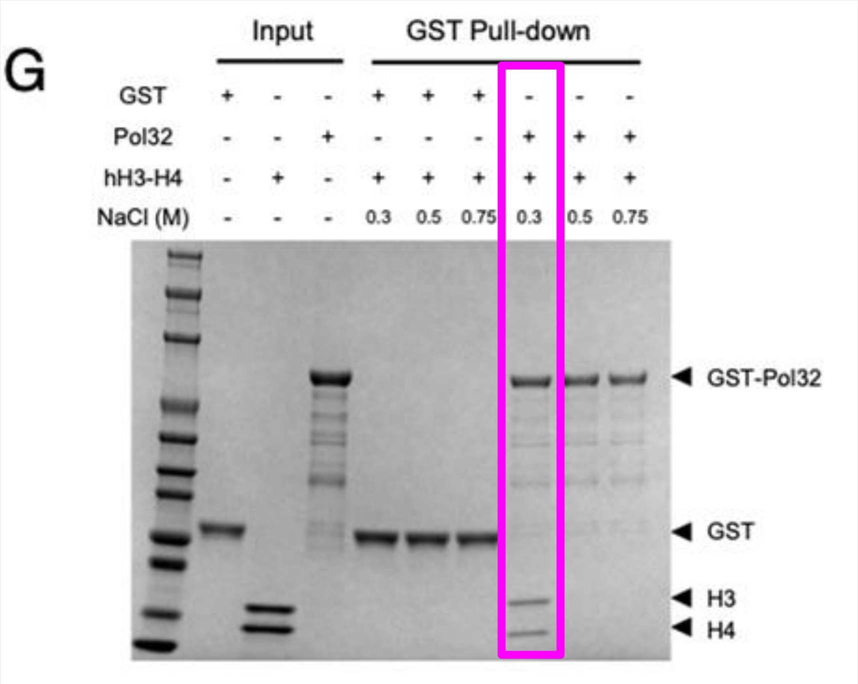

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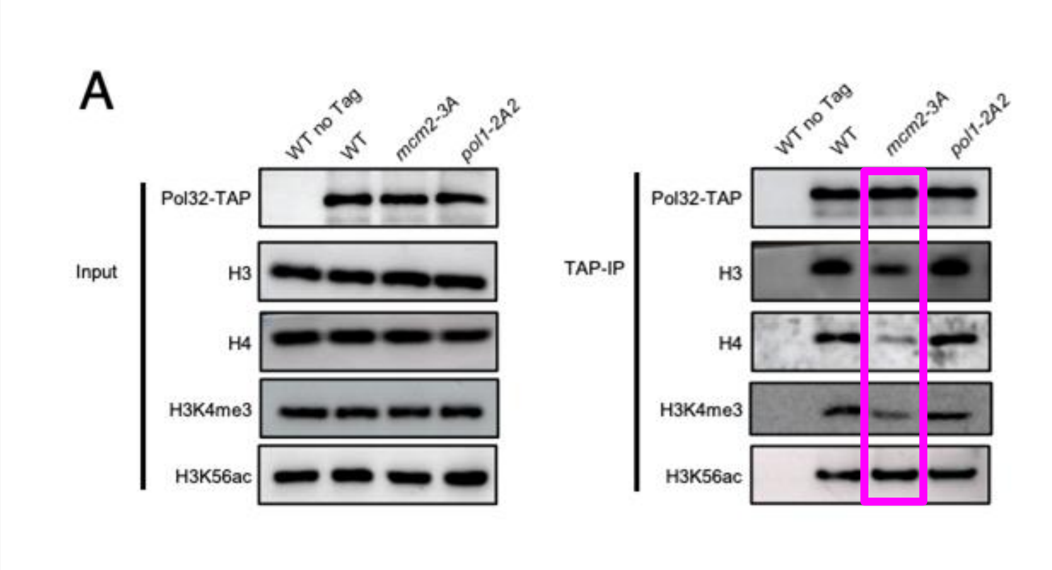
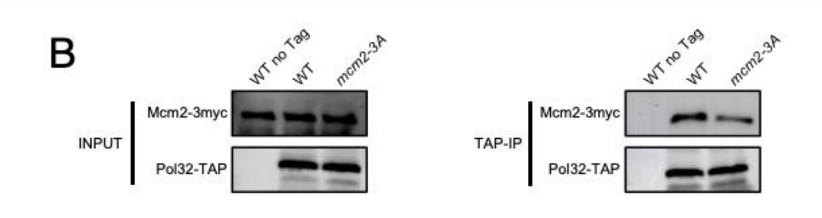
FIGURE 2

Mcm2 Transfers Parental Histone to Pol32





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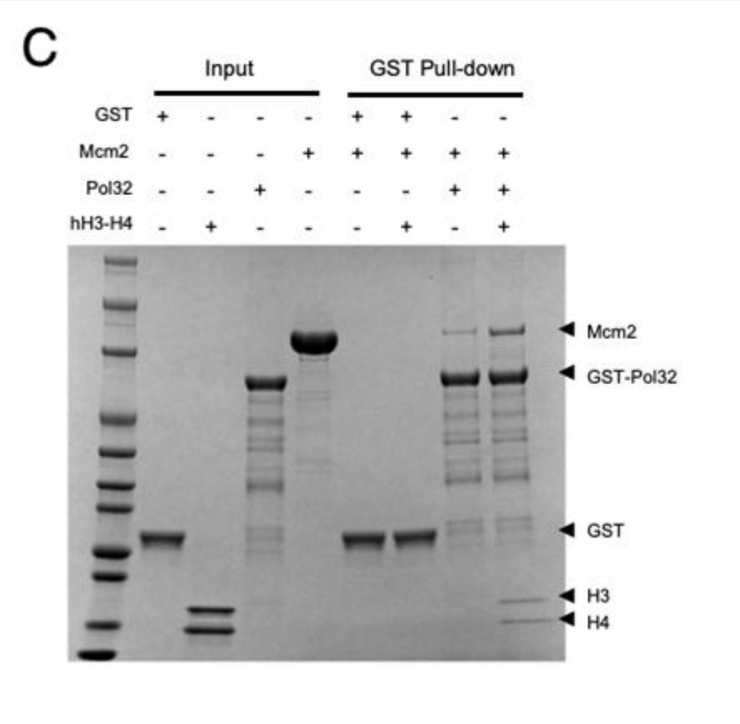
Histone H3–H4 Enhances the Pol32–Mcm2 Interaction



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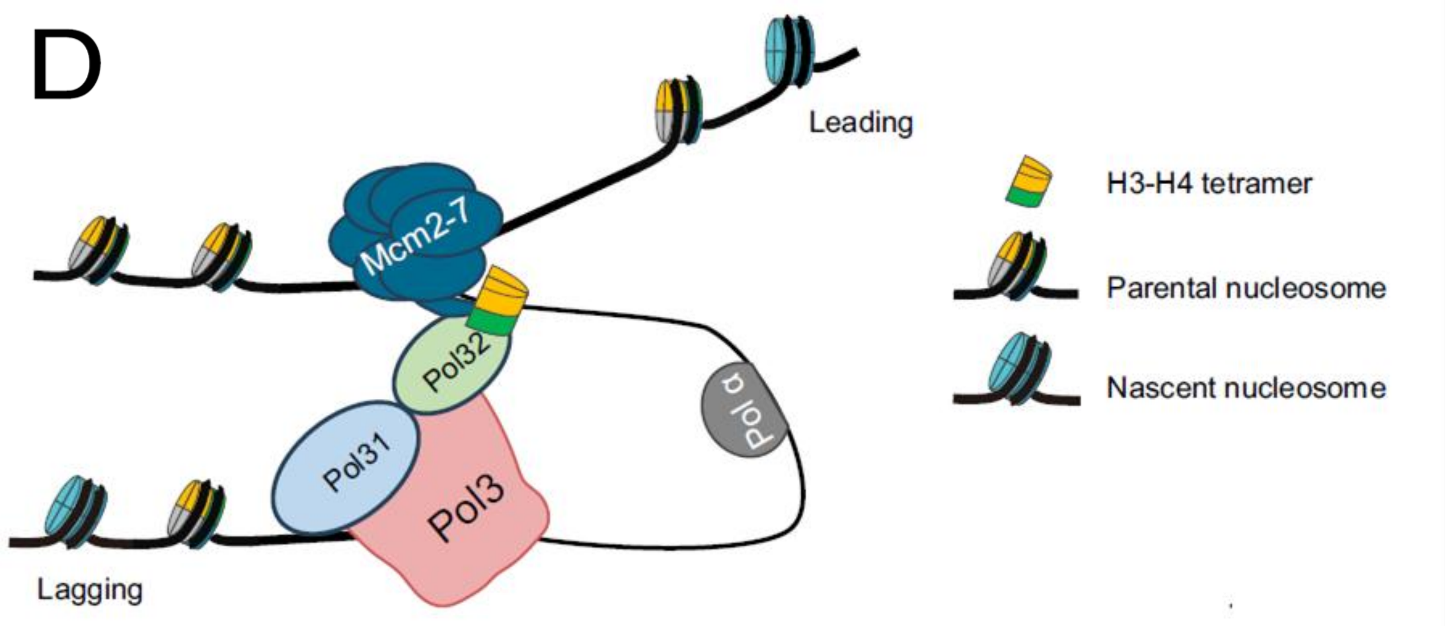
A Model of MCM2-7 and Pol32 Interaction



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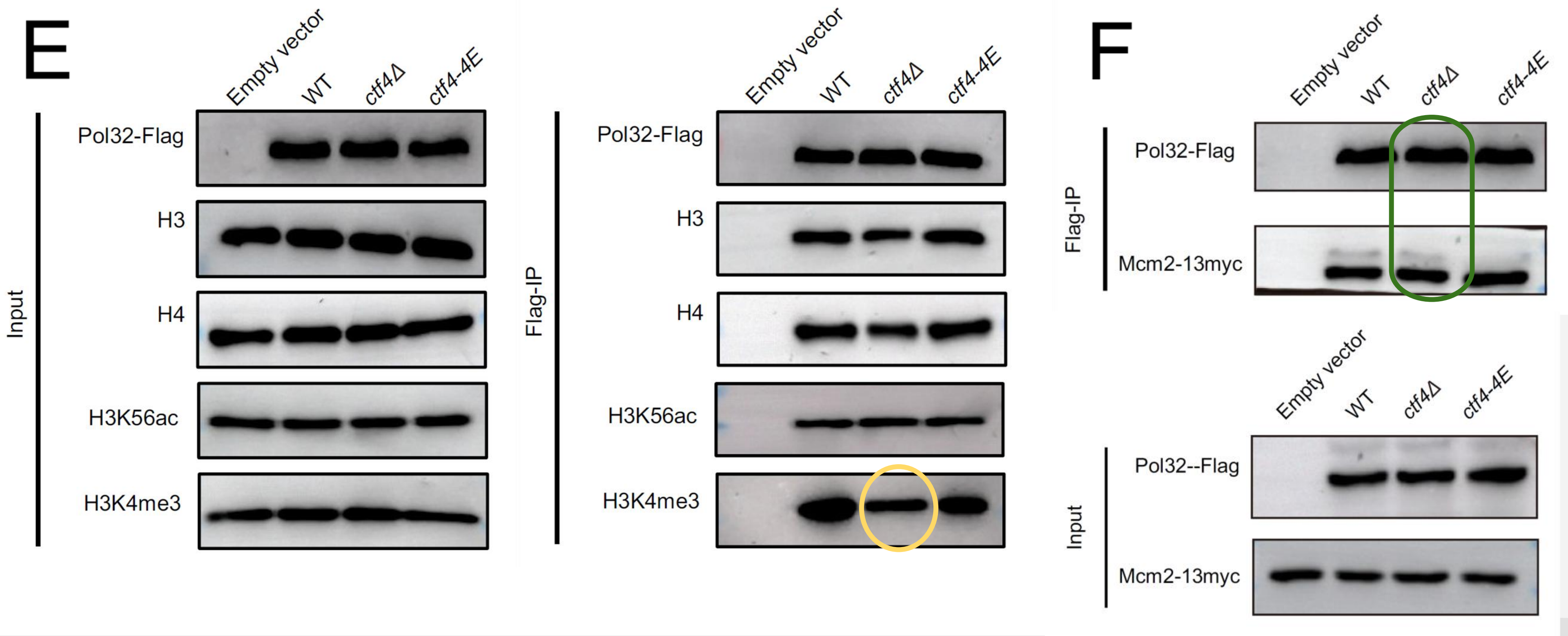
Ctf4 is not critical to the Pol32-Mcm2 Interaction



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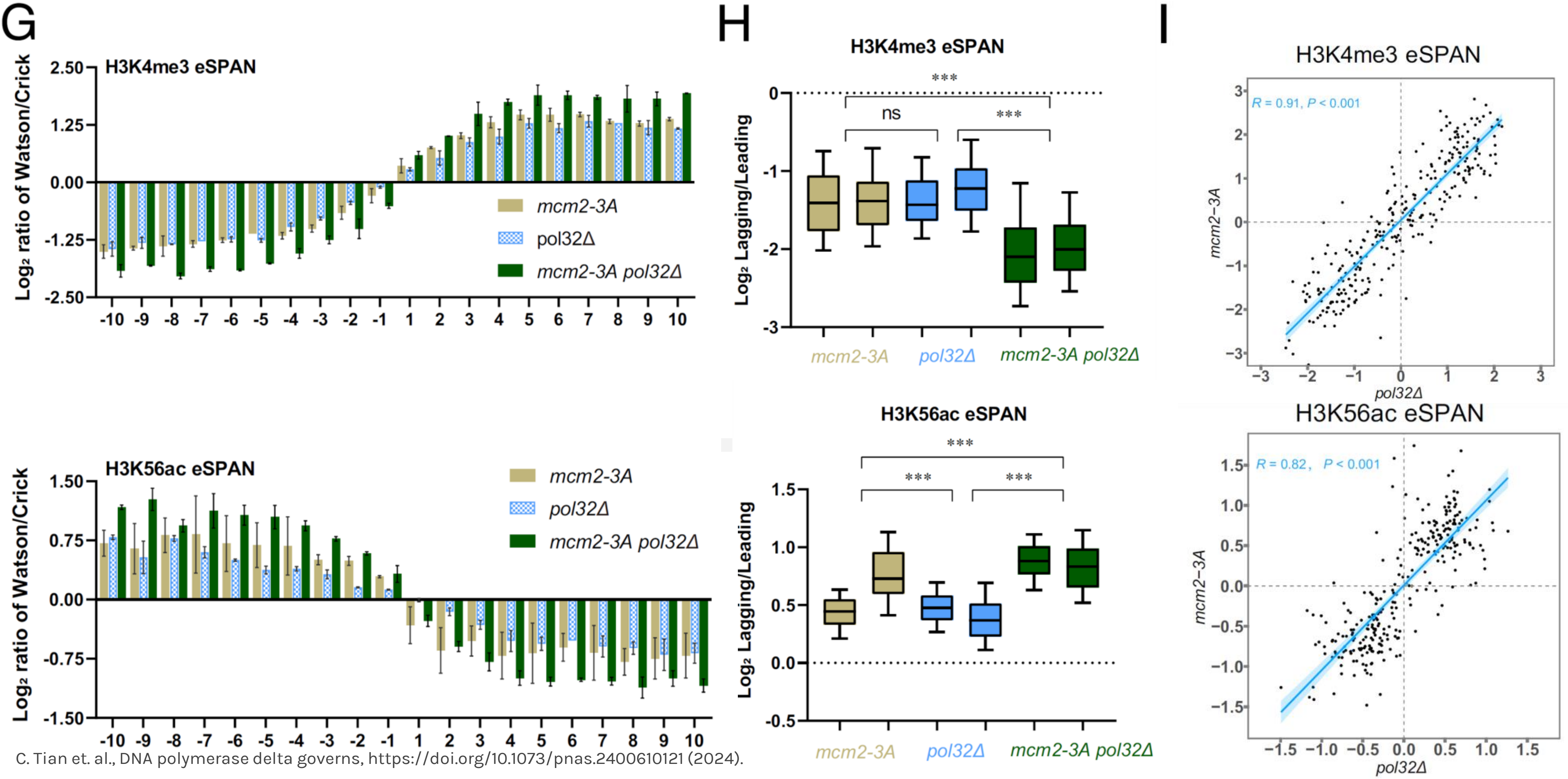
eSPANs of Double Mutants Show Synergistic Effects

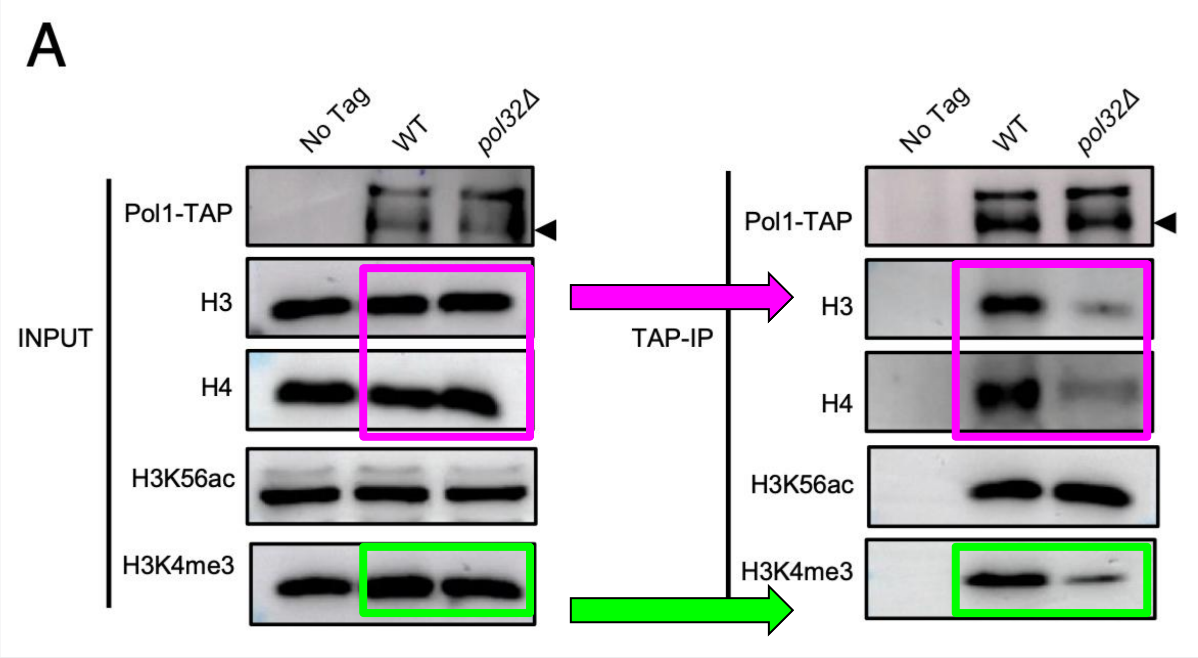


C. Tian et. al., DNA polymerase delta governs, https://doi.org/10.1073/pnas.2400610121 (2024).

FIGURE 3

Pol1-TAP pull-down in WT vs. pol32Δ cells

Observed **association** of histone **H3, H4, H3K56ac,** and **H3K4me3** with **Pol1** in both WT and pol32Δ cells.



Indicating **Pol32's** essential role in facilitating Pol1's interaction with modified parental **histones**

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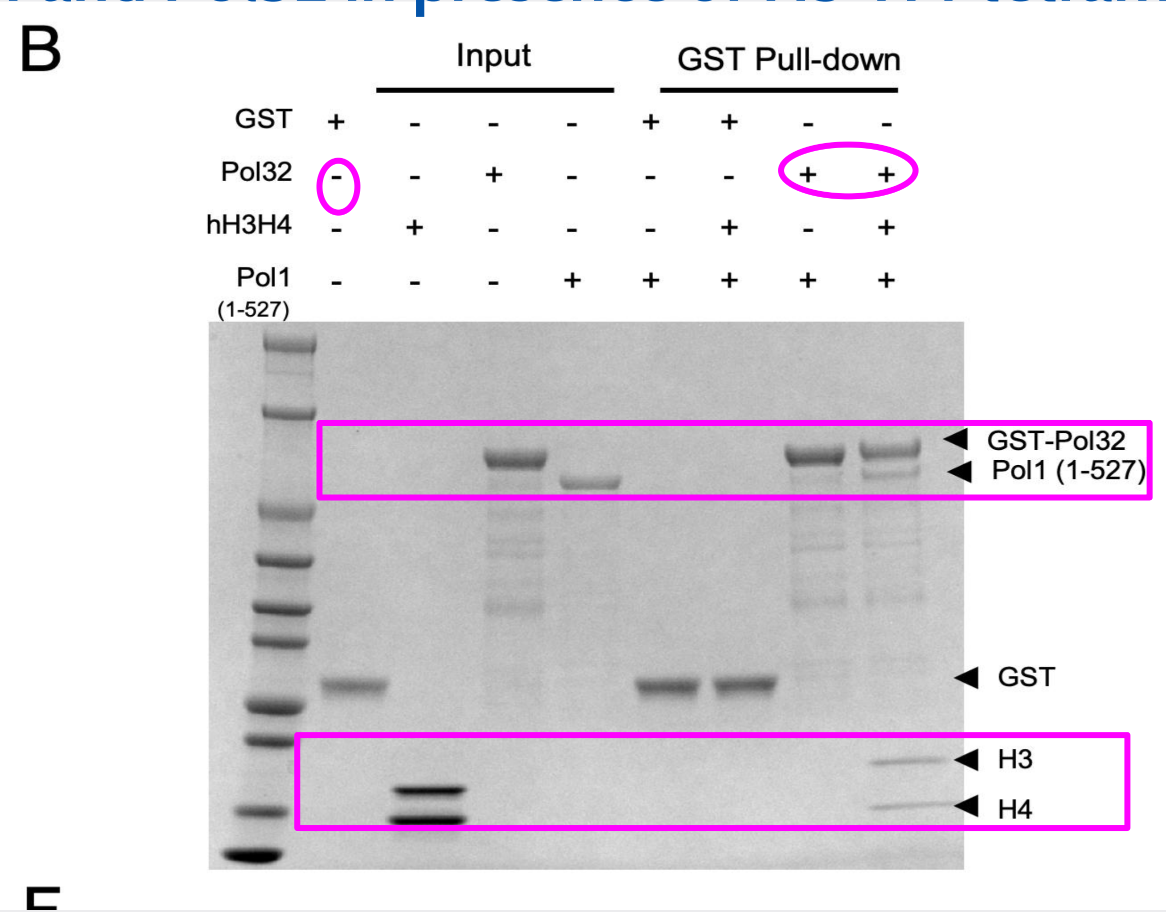
In vitro binding of Pol1 and Pol32 in presence of H3-H4 tetramers

Increased Pol1-Pol32 **interaction** when H3-H4 tetramers are **present**.

This supports the idea that Pol1 functions downstream of Pol32 in **histone** transfer.



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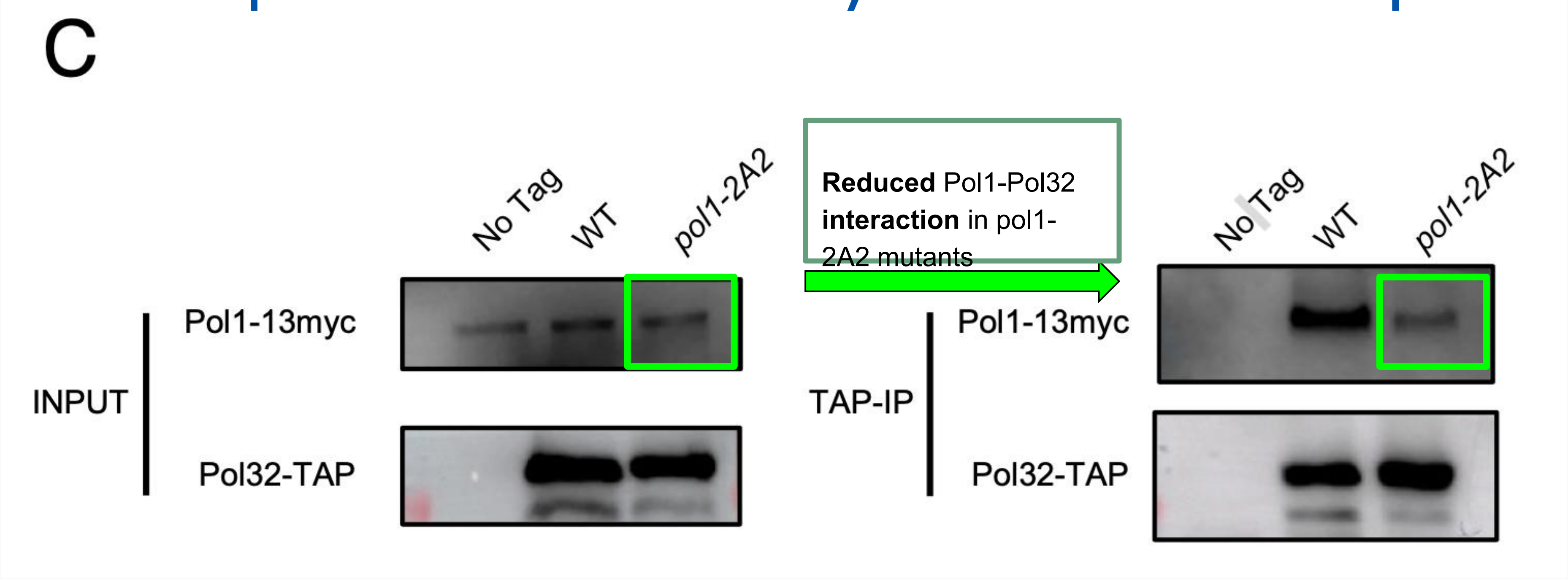
Pol32-TAP purification and Pol1-13myc detection in WT and pol1-2A2



Suggesting that **specific amino acid** changes in Pol1 (altered in the pol1-2A2 mutant) **impact** its **binding** with Pol32.

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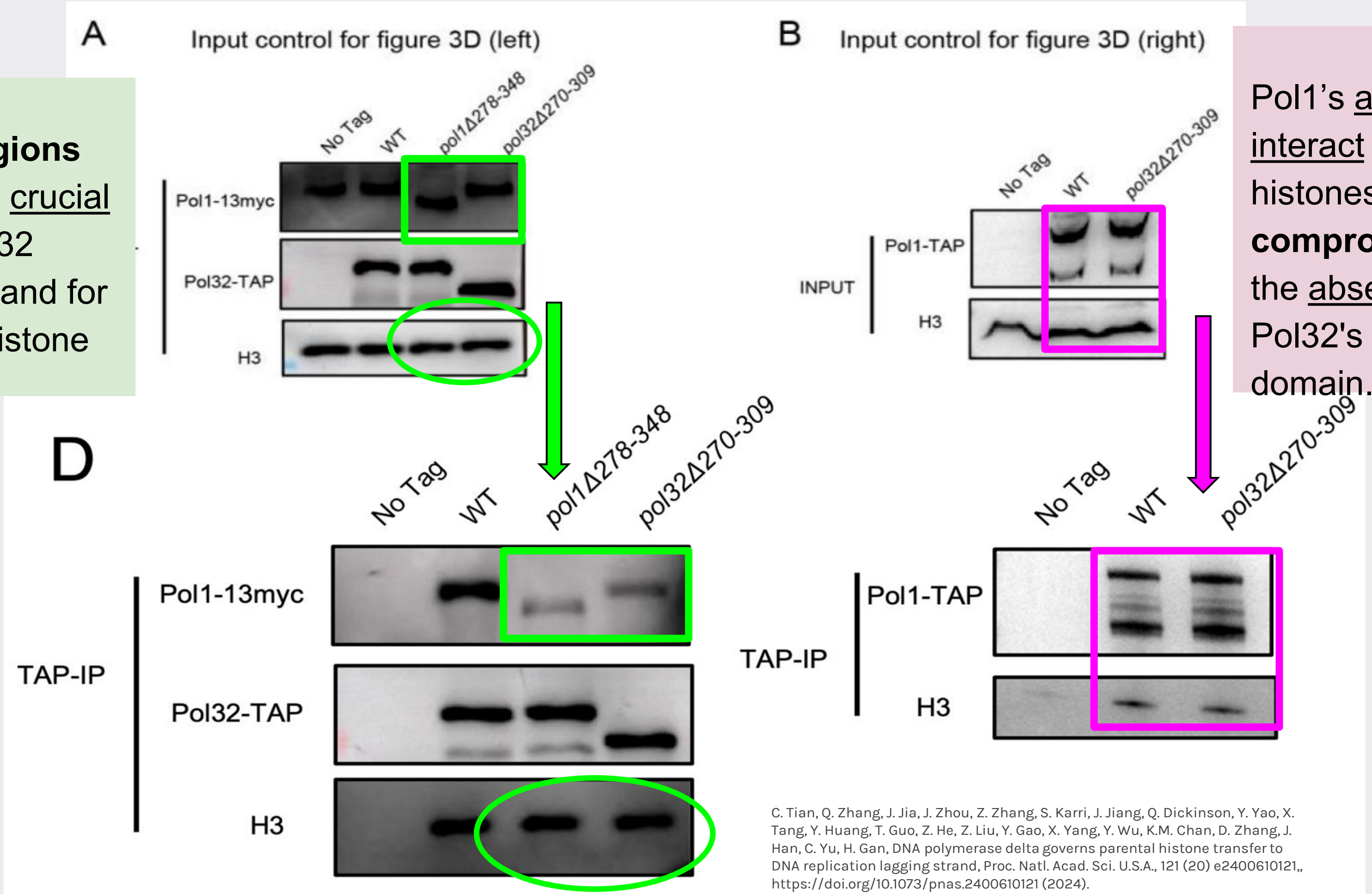
Effect of Pol1 and Pol32 deletion mutants on interactions

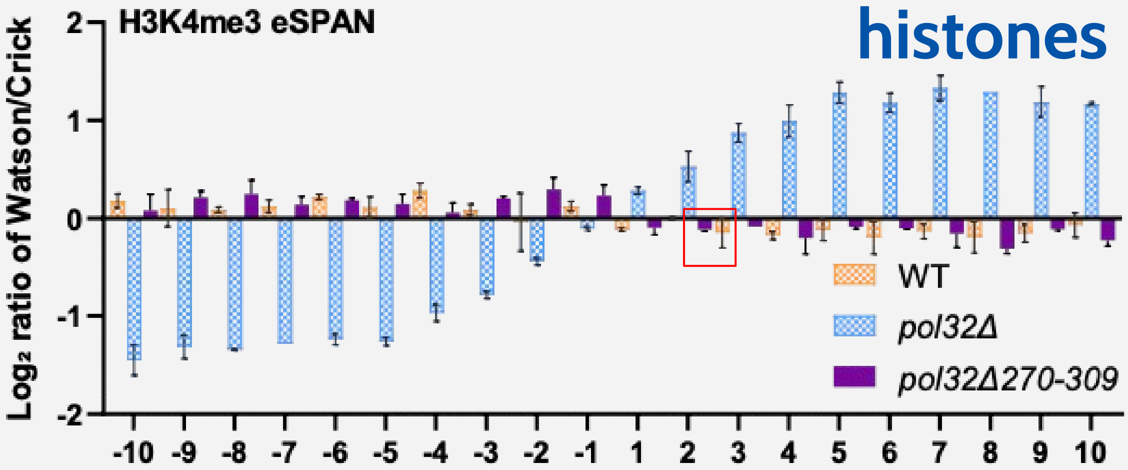
Specific regions of Pol32 are crucial for Pol1-Pol32 **interaction** and for facilitating histone H3 transfer



Pol1's ability to interact with histones is **compromised** in the absence of Pol32's C-terminal domain.

C. Tian, Q. Zhang, J. Jia, J. Zhou, Z. Zhang, S. Karri, J. Jiang, Q. Dickinson, Y. Yao, X. Tang, Y. Huang, T. Guo, Z. He, Z. Liu, Y. Gao, X. Yang, Y. Wu, K.M. Chan, D. Zhang, J. Han, C. Yu, H. Gan, DNA polymerase delta governs parental histone transfer to DNA replication lagging strand, Proc. Natl. Acad. Sci. U.S.A., 121 (20) e2400610121,, https://doi.org/10.1073/pnas.2400610121 (2024).

Pol1 and Pol32 collaborate in the transfer of parental histones



Disrupt interaction site of Pol32 and Pol1

=> **Pol32's interaction with the H3-H4 tetramer** is more important for parental histone transfer than its direct interaction with Pol1.

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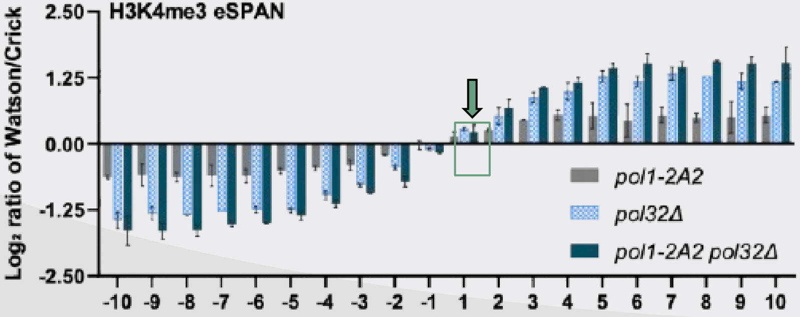
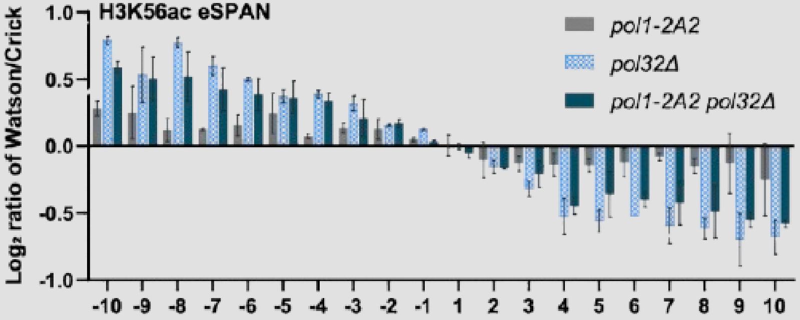
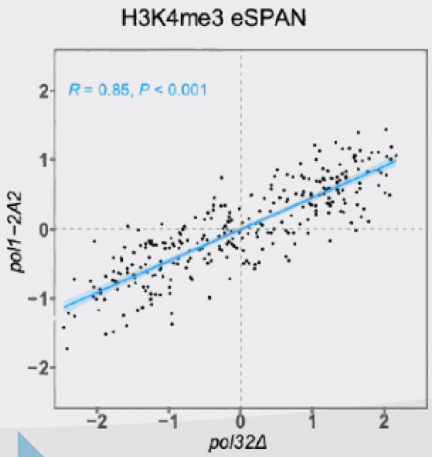
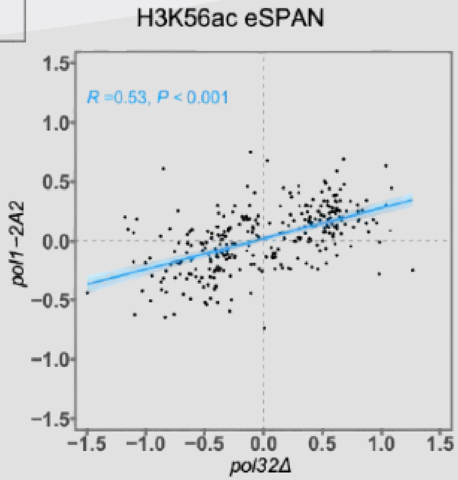
Strand Bias in Pol1 and Pol32 Mutants & Its correlation





Plotting the ratio of Watson/Crick between PolΔ32 and Pol1-2A2





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Limitation & Future change

- Model Organism Limitations
- Lack of Longitudinal Studies

Key Findings

- The DNA polymerase δ subunit Pol32 is identified as a crucial regulator of parental histone H3-H4 transfer to the lagging strand during DNA replication.

- Pol32 directly binds to histone H3-H4 and functions downstream of Mcm2 and upstream of DNA Pol1 in the histone transfer pathway

=> opens new avenues for exploring the mechanisms of epigenetic inheritance.

THANK YOU

IF YOU HAVE ANY QUESTIONS
PLEASE ASK!