SETD2 Maintains Nuclear Integrity Through Mitotic Lamin Phosphorylation

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Abstract

The modification of histone tails is responsible for vital cellular processes such as DNA repair, chromatin organization, and transcription regulation. However, mutations in modifier enzymes, such as SETD2, are known to be linked to cancer and tumorigenesis. Although SETD2's catalytic function of trimethylating H3K36 has been widely researched, we are focusing on its novel non-catalytic function with nuclear lamina, which is shown to be necessary for maintaining nuclear morphology and genome stability. The project provides evidence to the interaction between SETD2 and nuclear lamina by depleting SETD2 in synchronized cells and observing nuclear morphology post-mitosis. The results strengthen the causality of SETD2's novel non-catalytic role during mitosis and its effect on nuclear morphology in the subsequent interphase.

Background

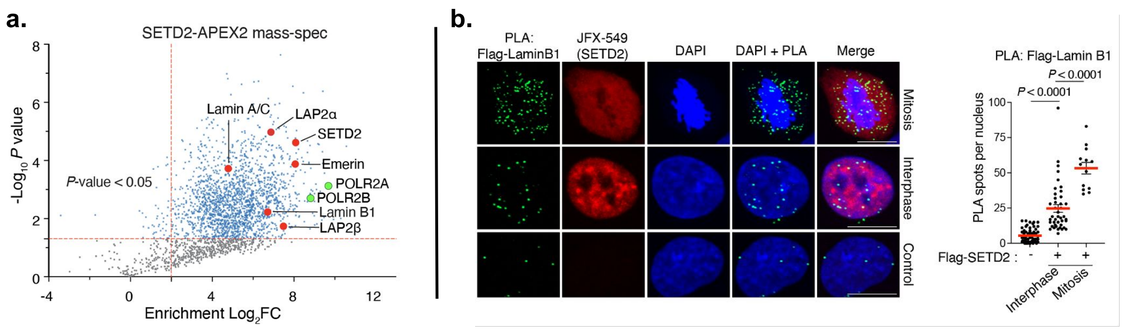
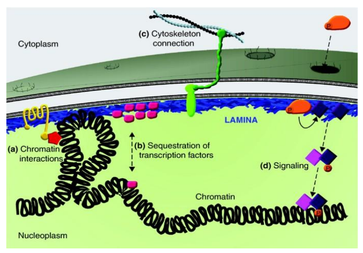


Figure 1a. Mass spectrometry shows the interaction between SETD2 and lamin proteins. **Figure 1b.** PLA result shows that the interaction occurs predominantly during mitosis.

Figure 2. Nuclear lamina is a filamentous meshwork of proteins, such as lamin A/C and lamin B1, that underline the nuclear envelope to uphold nuclear integrity.



Dittmer, T.A., Misteli, T. (2011)

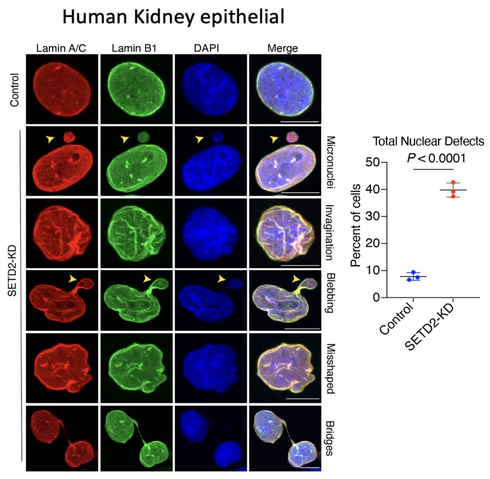
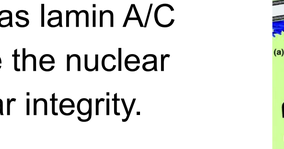


Figure 3. Depletion of SETD2 leads to significant increase in nuclear morphology defects including micronuclei, blebbing, misshaped, invagination, and bridges.

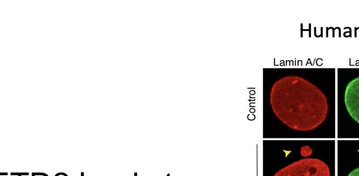
Question: Is mitotic phosphorylation defects after loss of SETD2 the cause of abnormal nuclear morphology in the subsequent interphase?

Method

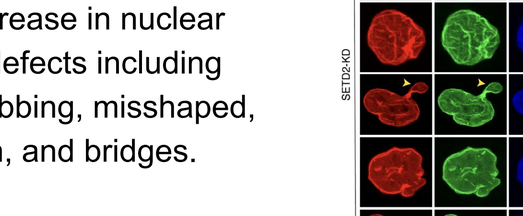
1. MEF SETD2 Flox



2. Cell Synchronization



3. SETD2 Deletion at Mitosis with Tamoxifen



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4. IF for Lamin Before and After Mitosis

Result

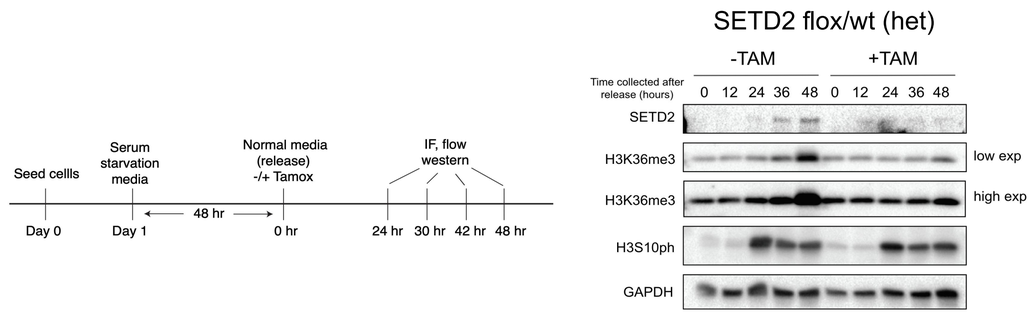


Figure 4. The result showed that cells begin to enter mitosis at 24 hours after release. Cells treated with tamoxifen showed a decreased level of SETD2 and H3K36me3.

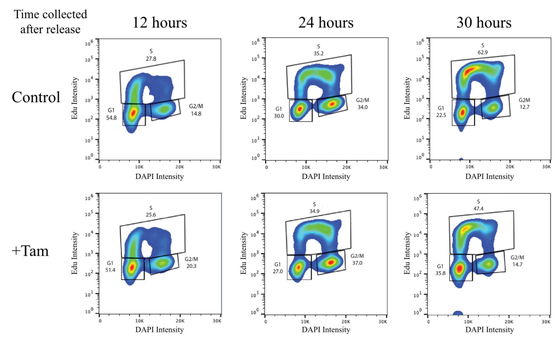


Figure 5. Flow cytometry data confirmed synchronization success in control and treated population. Cells treated with tamoxifen exhibited a degree of G1 arrest post-mitosis at 30 hours after release.

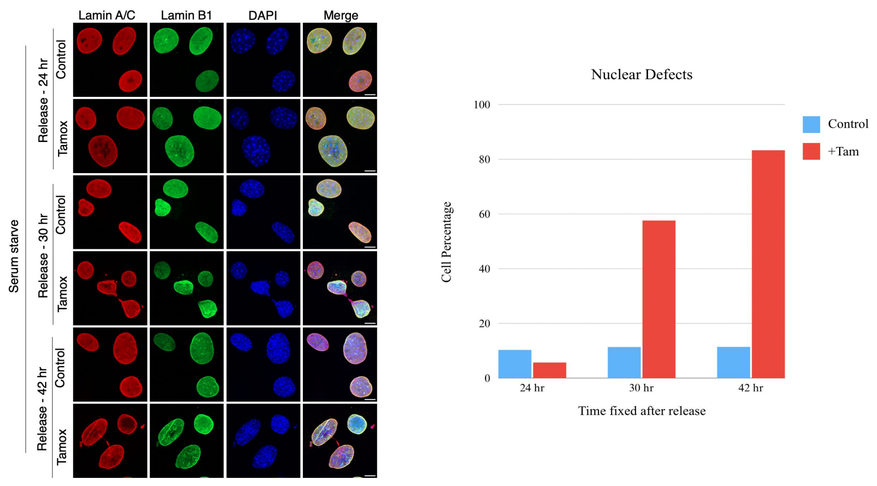


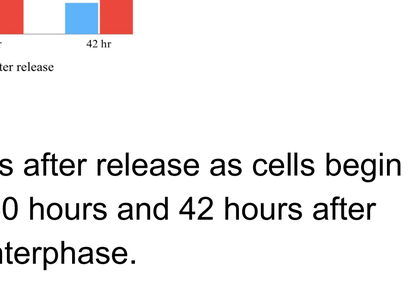
Figure 6. Minimal morphology defects detected at 24 hours after release as cells begin to enter mitosis. Gross morphology defects detected at 30 hours and 42 hours after release as cells exit mitosis and enter interphase.

Conclusions

- SETD2 knockout at mitosis showed significant increase in nuclear morphology defects from when cells are entering mitosis at 24 hours to when cells are entering interphase at 30 hours after release.
- Loss of SETD2 in asynchronous cell population showed gross nuclear morphology defects by 24 hours after treatment of tamoxifen.
- Loss of one SETD2 allele was sufficient for abnormal cellular phenotype.
- Mitotic phosphorylation defects after loss of one SETD2 copy causes abnormal nuclear morphology and genomic instability in the subsequent interphase.

Future Directions

- Optimization of synchronization in HKC SETD2-dtag cell line.
- Observe nuclear morphology in HKC before and after mitosis with dTAG treatment.
- Induce SETD2 deletion in post-mitotic cells for MEF and HKC.



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3. McDaniel, Stephen L, and Brian D Strahl. "Shaping the Cellular Landscape with SET2/SETD2 Methylation." *Cellular and Molecular Life Sciences : CMLS*, U.S. National Library of Medicine, Sept. 2017.