# Analysis of ESCO2 Degradation Using the FUCCI System

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## Introduction

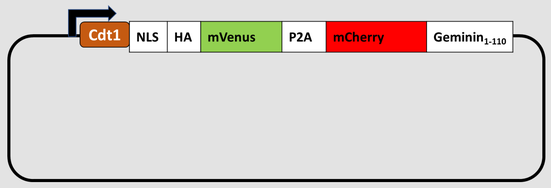
As cells prepare to divide, the genome is duplicated and the identical copies of each chromosome, called sister chromatids, are tethered together by a protein complex called cohesin. Sister chromatid cohesion ensures that chromosomes are accurately segregated to daughter cells during cell division. ESCO2 is an enzyme that plays an important part in stabilizing cohesin during S phase, ensuring cohesion of sister chromatids and thus their proper segregation.[1] ESCO2 is not present throughout the whole cell cycle, and several lines of evidence suggest that the protein is degraded during DNA replication or S phase. We speculate that ESCO2 may contain a "PIP degron." PIP degrons are short linear motifs that target proteins for destruction as they interact with the DNA replication machinery. Our goal in this project was to test four conserved motifs in ESCO2's N-terminus (called PIP A-D) to determine whether any of them act as PIP degrons.[2]


## Approach

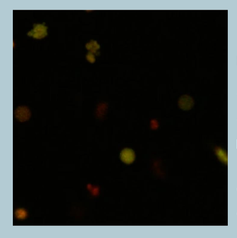
To determine the PIP degron, we modified an existing PIP-degron readout, the PCNA Interacting Protein Fluorescent Ubiquitylation Cell Cycle Indicator (PIP-FUCCI) system, replacing a known PIP degron with PIP A-D.[3] This system provides a fluorescence-based readout of protein stability using fluorescent microscopy or flow cytometry. The expected pattern of fluorescence was green to red to green if a true PIP degron was present in the plasmid and green to red-green double positive to green if the motif inserted was not a PIP degron.

## Methodology

- Recombinant plasmids were constructed, replacing the Cdt1 PIP box with motifs from ESCO2 (PIP A-D)
- The Cdt1 PIP-degron plasmid was regenerated, to serve as a positive control.
- Following sequence confirmation, each plasmid was transfected into HeLa cells.
- Cells were analyzed by fluorescence microscopy live cell imaging for 24 hours.
- In parallel, flow cytometry was also performed to characterize fluorescent cells in transfected populations.



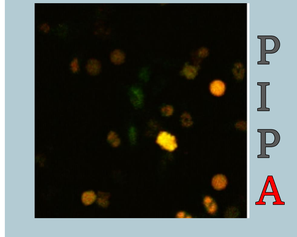

## Results – Live Cell Imaging



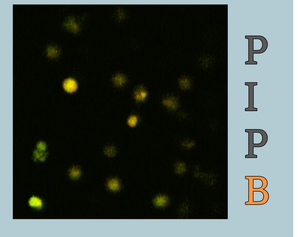
CDT1

**Positive Control** – The pattern of green single-positive (G1) to red single-positive (S) to green-red double-positive (G2/M) is displayed in the control, implying the motif is degraded as the green protein degrades.

**PIP Boxes**– The expected fluorescing pattern of the PIP degron is not displayed due to the lack of green degrading to reveal only single red fluorescing cells. Although PIP C and D are not displayed, they also showed the same lack of single red fluorescence in flow cytometry and fixed cell imaging.

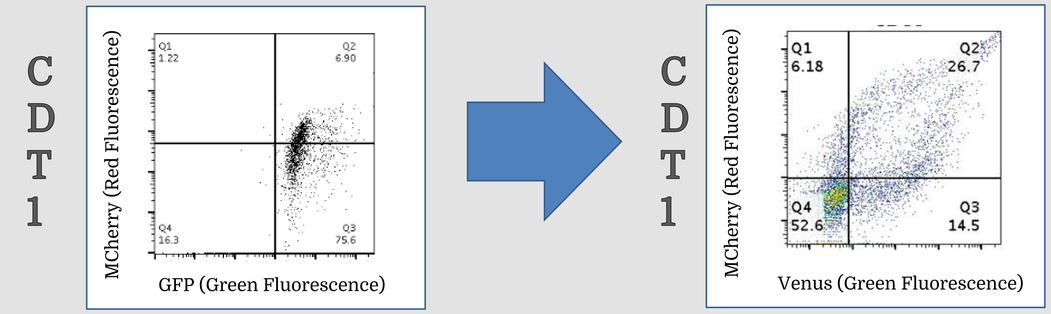


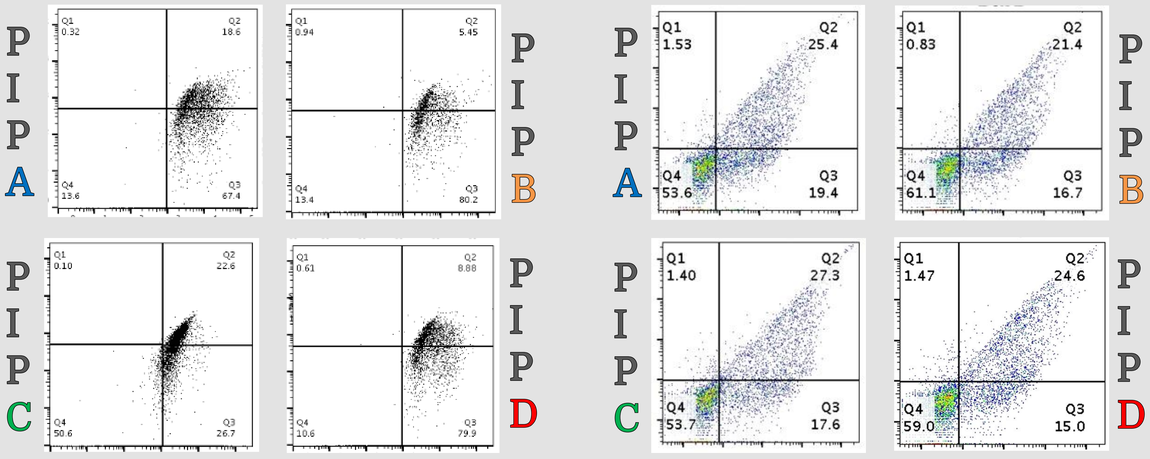
PIPA



PIPB

## Results – Flow Cytometry




## Conclusion

- Cells carrying the PIP boxes did not show similar fluorescence progression to the Cdt1 PIP degron, suggesting that none of the PIP boxes are PIP degrons
- Cells carrying the PIP box plasmids displayed green to green-red fluorescence, indicating that the mVenus (green) protein with the motifs were not being degraded in S phase.
- None of the motifs are likely to be PIP boxes – by creating a cell line, we can further confirm this

## Materials

1. Lelij, P. V. D.; Godthelp, B. C.; Zon, W. V.; Gosliga, D. V.; Oostra, A. B.; Steltenpool, J.; Groot, J. D.; Scheper, R. J.; Wolthuis, R. M.; Waisfisz, Q.; Darroudi, F.; Joenje, H.; Winter, J. P. D. The Cellular Phenotype of Roberts Syndrome Fibroblasts as Revealed by Ectopic Expression of ESCO2. PLoS ONE 2009, 4 (9).
2. Bender, D., Silva, E. M., Chen, J., Poss, A., Gawey, L., Rulon, Z., & Rankin, S. (2019). Multivalent interaction of ESCO2 with the replication machinery is required for cohesion. BioRXiv. doi:10.1101/666115
3. Grant, G. D., Kedziora, K. M., Limas, J. C., Cook, J. G., & Purvis, J. E. (2018). Accurate delineation of cell cycle phase transitions in living cells with PIP-FUCCI. Cell Cycle, 17(21-22), 2496-2516. doi:10.1080/15384101.2018.1547001