UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM COMPREHENSIVE CANCER CENTER
NATHAN SCHNAPER INTERN PROGRAM

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

# A Novel Genetically Encoded Reporter System for Functional Single-Cell Tracking in Cultured Mammalian Cells

*Shylah Healy[1,2], Jinghui Wang[2], Miroslaw Janowski[2], Piotr Walczak[2], Yajie Liang[2]*

[1]Johns Hopkins University

[2]Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland, Baltimore

NSIP
Nathan Schnaper Intern Program
in Translational Cancer Research

## INTRODUCTION:

Tumor heterogeneity, characterized by distinct changes between cells within the same tumor, is the main cause of drug resistance and relapse of cancers. The development of the genetically encoded fluorescent ubiquitination-based cell cycle indicator (FUCCI) system (Fig. 1) [1] has expanded the toolset for investigating tumor heterogeneity, allowing for the visualization of the cell cycle phase in cancer cells. If a single cell's physical position, progression through the cell cycle, and activation of intracellular calcium signaling pathways can be tracked, then we can tell if there is any cellular heterogeneity present within a cancer cell population. However, the blue fluorescent protein (BFP), conventionally used in imaging, is not ideal for in vitro single-cell tracking because of the high background in culture medium. There are also no current sensors that can detect both calcium and the cell cycle, as calcium is an important second messenger for intracellular signaling pathways. Lastly, limited access to instruments capable of high throughput long-term imaging has prevented the exploration of this method.

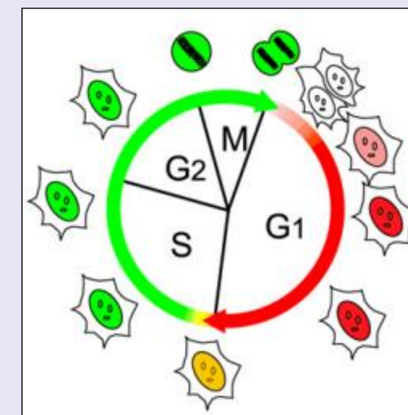
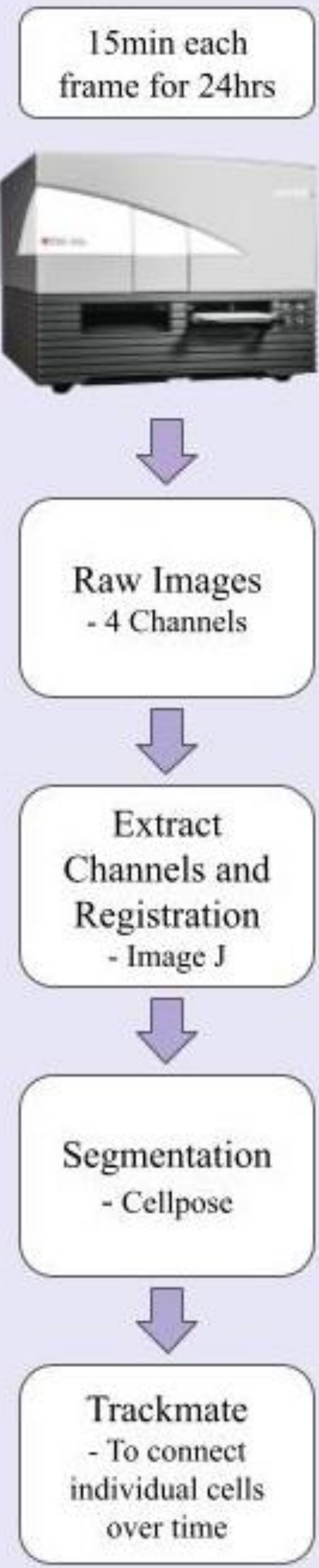
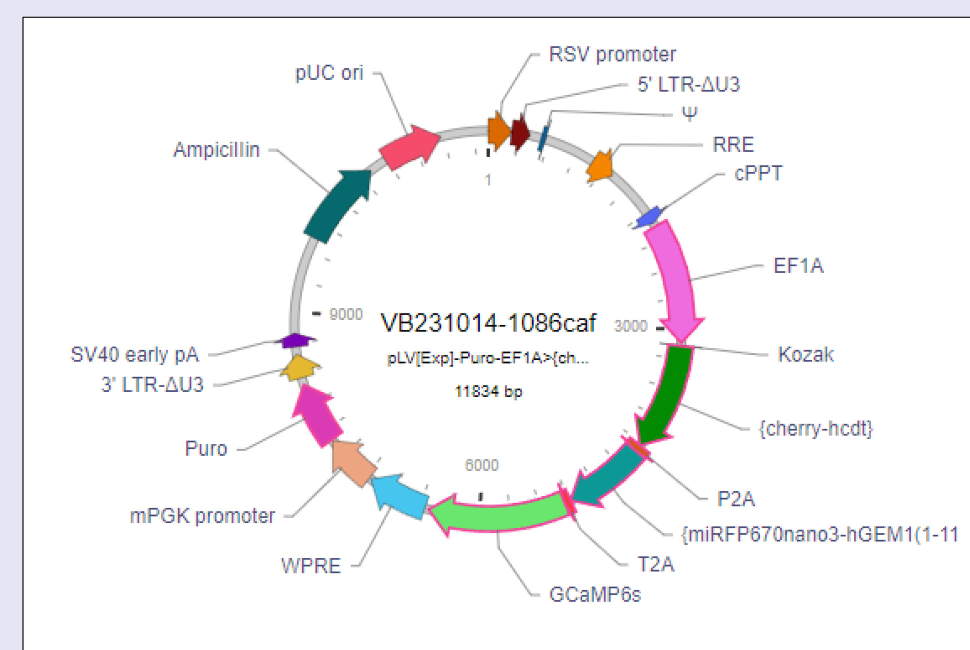



**Figure 1. Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI)** Red fluorescence expressed by mKO2-hCdt during G1 phase of the cell cycle and green fluorescence expressed by mAG-hGem during S/G2/M phases.

## METHODS:

We replaced the BFP in the FUCCI system with infrared fluorophore (miRFP670nano) and incorporated a calcium indicator (GCaMP6s [3]) to create the construct named functional FUCCI, illustrated in Fig. 2. We then packaged lentivectors and transduced HEK293 cells and performed long-term single-cell tracking through repetitive imaging of these cells at 15 min intervals for 24 hours using Tecan Spark Cyto. These images from four channels (red, green, infrared, and bright field) were stacked and registered in ImageJ. In Cellpose [4], a customized segmentation model for HEK293 cells was then trained through manual annotation and applied to a cropped stack of the recorded images. Finally, Trackmate [5] was utilized to link the segmented cells together over consecutive time frames. The cell track labeled 'Track 1' was chosen for analysis of fluorescent intensity regarding both cell cycle indicator and GCaMP6s [5] calcium indicator.



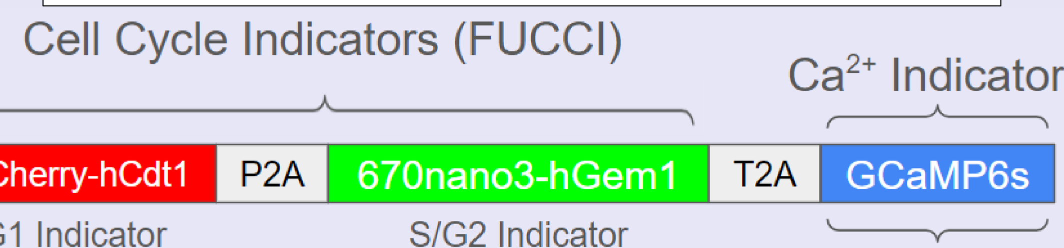
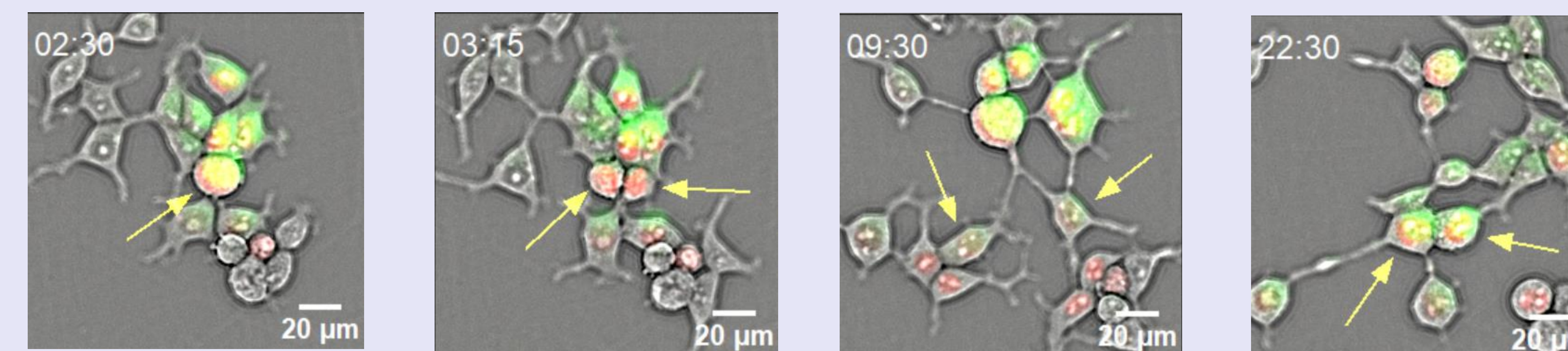




**Figure 2. Plasmid Vector of pLV-puro-EF1A-Cherry-hCdt-p2a-miRFP670nano3-hGem1(1-110):t2a-GCaMP6s , Composition of Functional FUCCI, and Schematic Flowchart of Methodology**

## OBJECTIVE:

We aim to explore whether a new genetically encoded reporter construct can reliably report migration, cell cycle progression, and calcium through long-term single-cell tracking.

## RESULTS:



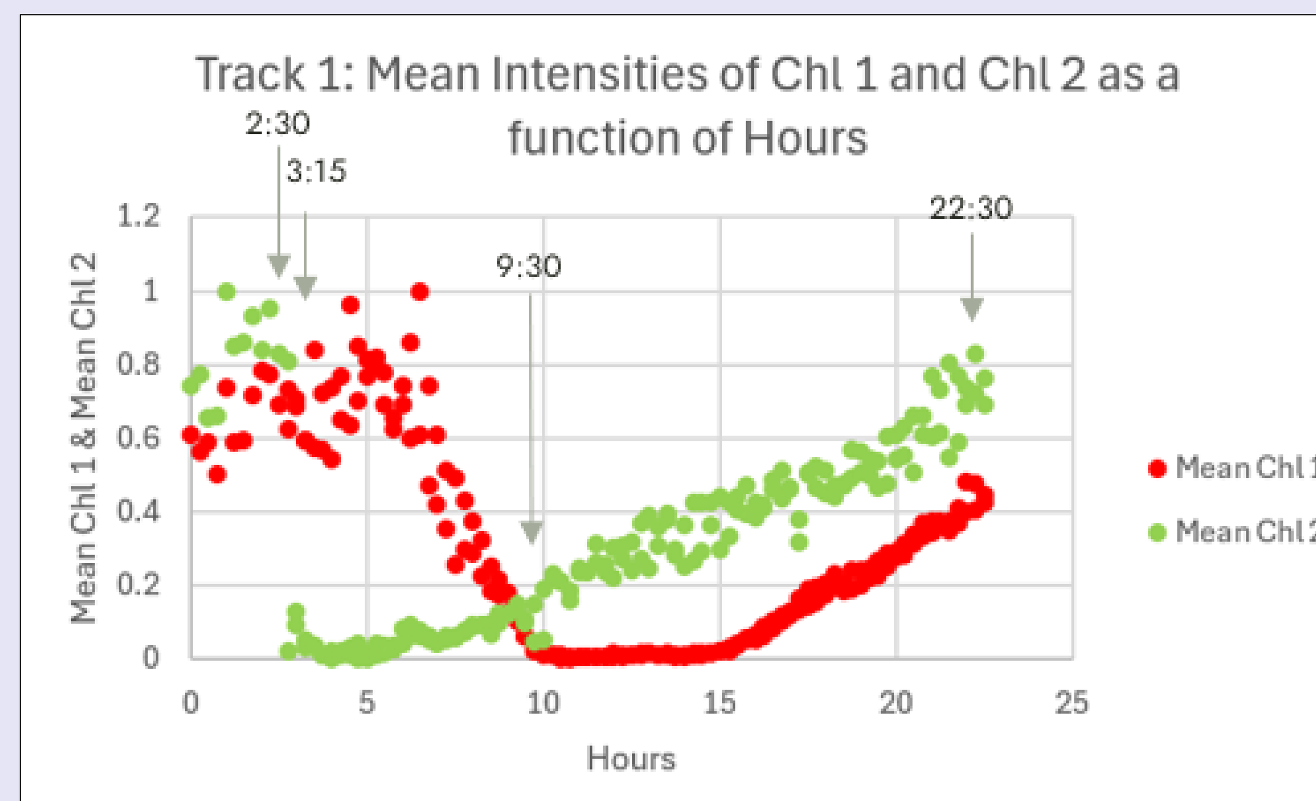




**Figure 3. Mean Intensities of Cell Cycle Indicators in HEK293 Cell Track 1** Cell tracking images depict cell's progression through the cell cycle with corresponding intensities of red (mCherry-hCdt1) and green (670nano3-hGem1) fluorescence.

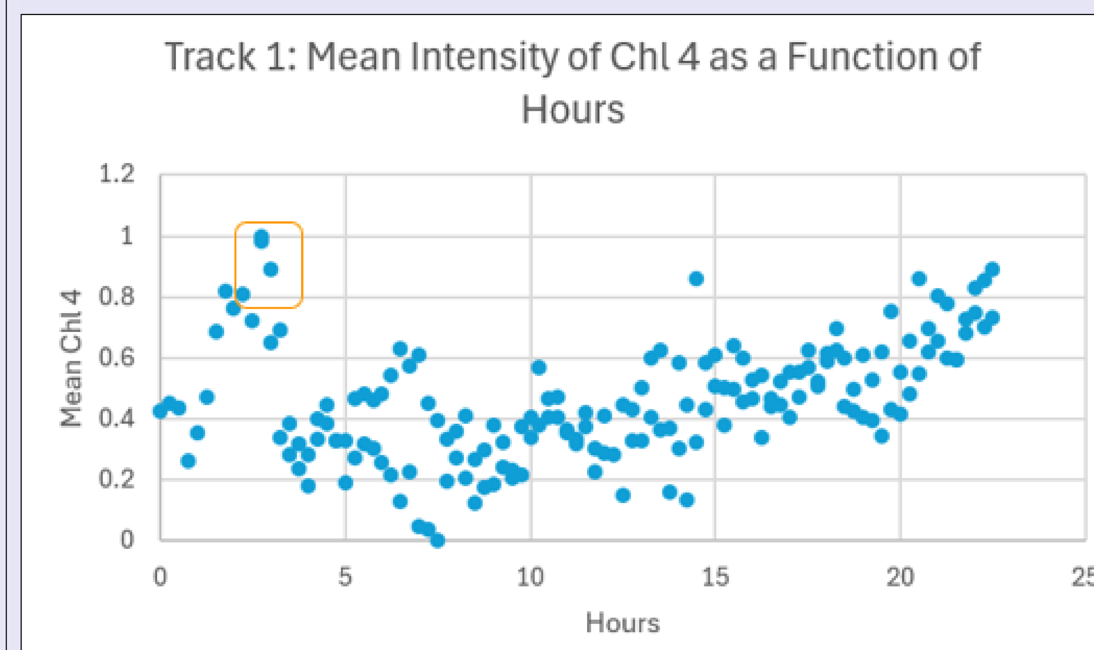
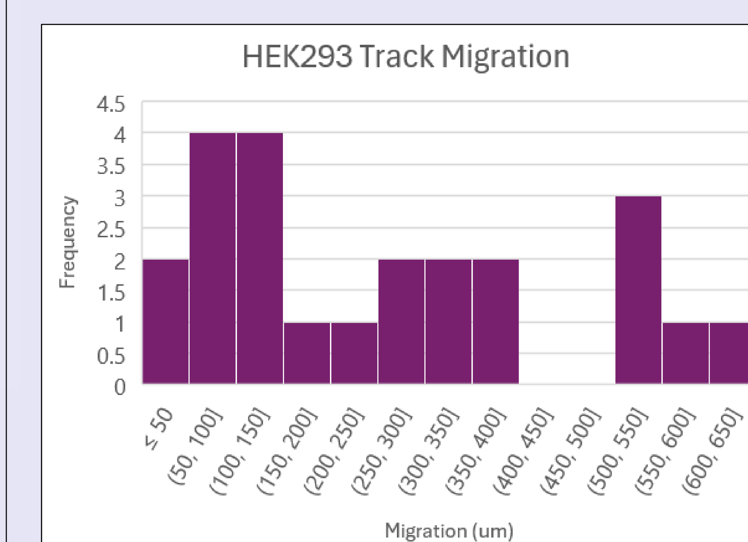



**Figure 4. Mean Intensity of Calcium Indicator in HEK293 Cell Track 1** Highlighted focus area and associated cell tracking images depict concentration of calcium via GCaMP6s.



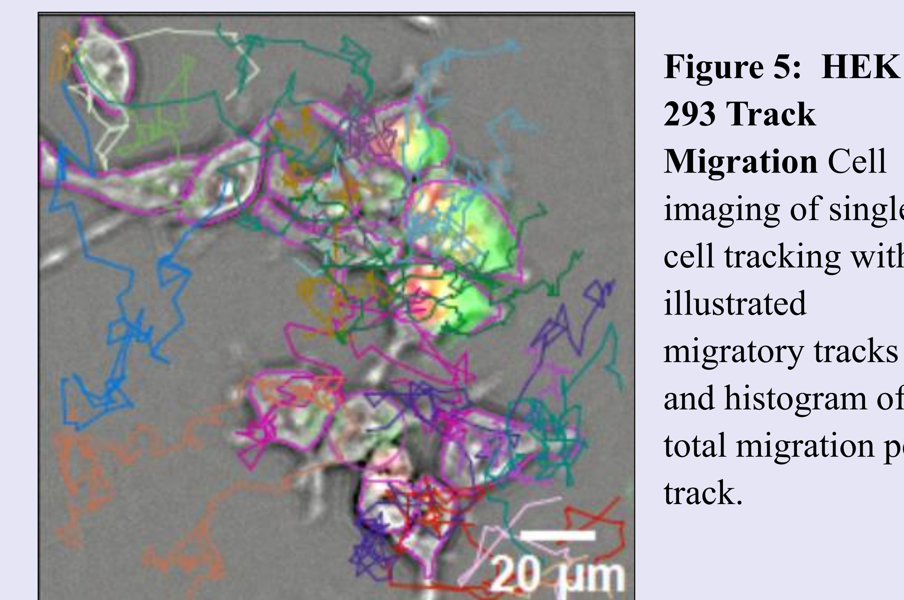
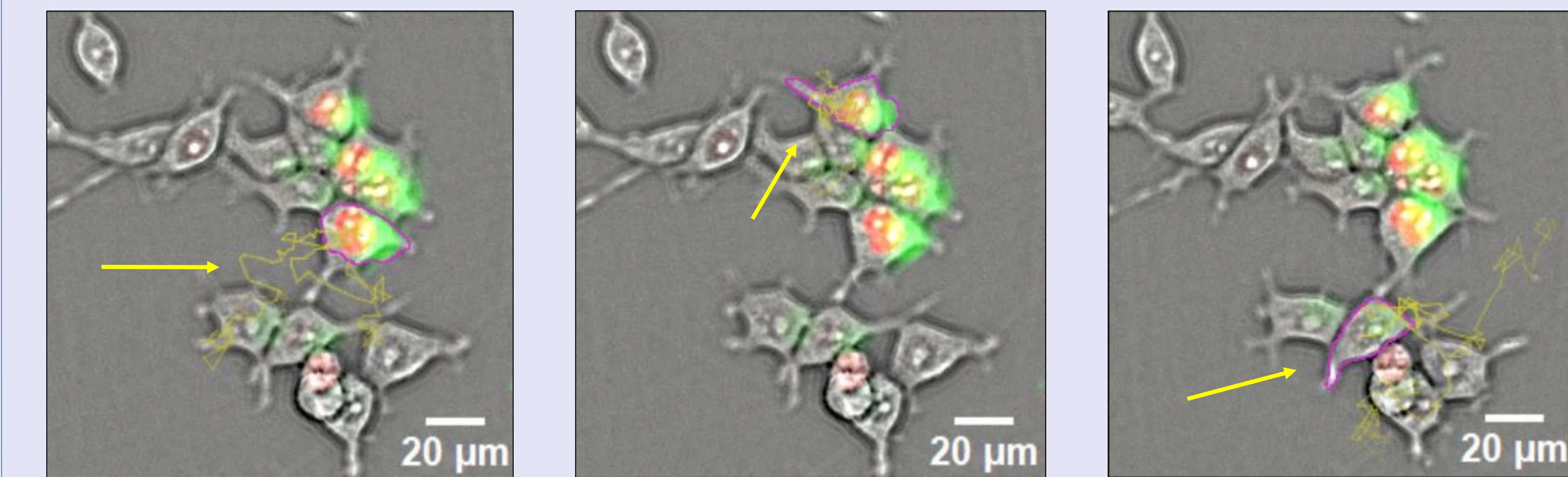




**Figure 5: HEK 293 Track Migration** Cell imaging of single-cell tracking with illustrated migratory tracks and histogram of total migration per track.



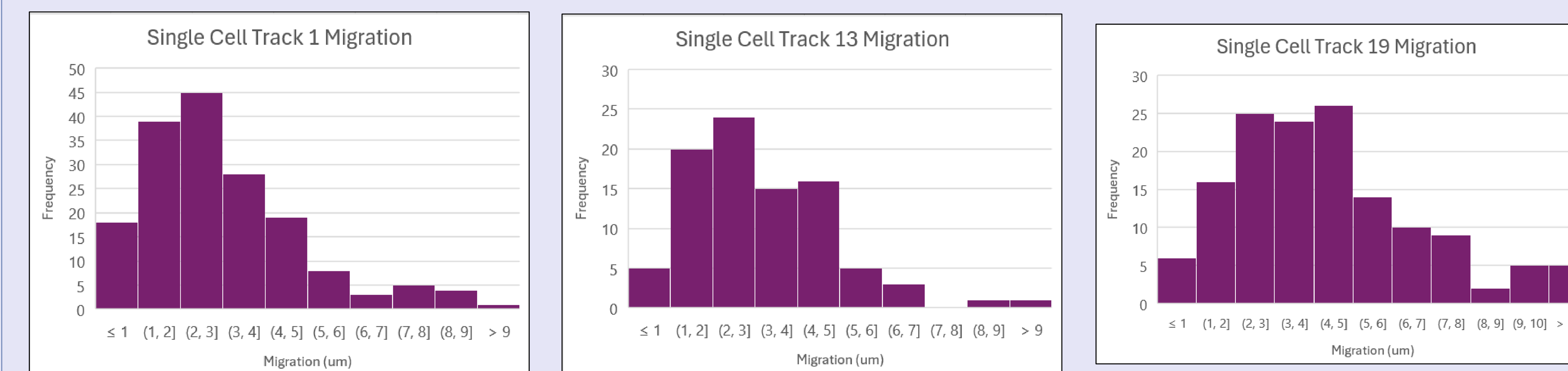




**Figure 6. Variation in Single-Cell Track Migration** Cell imaging of single-cell tracking with illustrated migratory tracks and histogram of select single tracks.

## CONCLUSION:

- We developed a genetically encoded reporter system that successfully tracks changes in fluorescence intensity of functional FUCCI, tracking cell cycle progression and calcium dynamics of single cells.
- We successfully tracked the dynamic change in the fluorescence intensity of functional FUCCI regarding cell cycle progression and calcium dynamics in HEK293 cells.

## FUTURE DIRECTIONS:

- Future studies should be performed to evaluate tumor heterogeneity and if similar behaviors can be observed in differing cell lines or in vivo. Moreover, the quantification of such behaviors may eventually allow for discovery of new drugs and potential treatment to combat cancer relapse.

## ACKNOWLEDGEMENTS:

Special thanks to Dr. Liang and the Liang lab for their continued guidance and support. Thank you to the organizing committee of the Alavi-Bradley Symposium for the opportunity to participate in this event. This research was supported in part by the Nathan Schnaper Internship Program (NSIP), University of Maryland School of Medicine Office of Student Research. Generous support provided by Dr. Bret Hassel and Dr. Heather Ezelle; Dr. Taofeek Owonikoko, Director, Greenebaum Comprehensive Cancer Center; Dr. Lauren Schnaper and Family, Dr. Greg Carey and Ms. Fiama Romero.

National Cancer Institute grants:

R25CA186872 to Bret A. Hassel

P30CA134274 to Taofeek Owonikoko

## REFERENCES:

1. Sakaue-Sawano A, Kurokawa H, Morimura T, Hanyu A, Hama H, Osawa H, Kashiwagi S, Fukami K, Miyata T, Miyoshi H, Imamura T, Ogawa M, Masai H, Miyawaki A. Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. Cell. 2008 Feb 8;132(3):487-98. doi: 10.1016/j.cell.2007.12.033. PMID: 18267078.
2. Liang Y, Walczak P. Long term intravital single cell tracking under multiphoton microscopy. J Neurosci Methods. 2021 Feb 1;349:109042. doi: 10.1016/j.jneumeth.2020.109042. Epub 2020 Dec 16. PMID: 33340557.
3. Chen, TW., Wardill, T., Sun, Y. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499, 295–300 (2013). https://doi.org/10.1038/nature12354
4. Stringer, C., Wang, T., Michaelos, M. et al. Cellpose: a generalist algorithm for cellular segmentation. Nat Methods 18, 100–106 (2021). https://doi.org/10.1038/s41592-020-01018-x
5. Ershov, D., Phan, MS., Pylvänäinen, J.W. et al. TrackMate 7: integrating state-of-the-art segmentation algorithms into tracking pipelines. Nat Methods 19, 829–832 (2022). https://doi.org/10.1038/s41592-022-01507-