THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

# Synthesis of Phosphatidylinositol 4,5-bisphosphate (PIP2) in Endosomal Compartments during Serum-Stimulated Cell Signaling

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# ABSTRACT

While epithelial cell migration facilitates normal processes such as wound repair and embryonic development, it can also underlie pathophysiological conditions like cancer metastasis. The ability of cells to become migratory relies on mechanisms involving turnover of focal adhesions and recycling of integrins through the cell's endosomal system. Prior research suggests that a phospholipid, Phosphatidylinositol 4,5-bisphosphate (PIP2), binds proteins known to facilitate integrin recycling. Therefore, this study aimed to elucidate potential locations of PIP2 synthesis and localization during serum-stimulated integrin recycling. Assays conducted in HeLa cells, a cervical cancer cell line, were designed to decrease PIP2 levels at specific endosomal populations in the cell (EHD1, Rab11, Rab7, Rab5, and Rab8). DNA constructs for fluorescent proteins were utilized to quantify colocalization between integrins and endosomes as a measure of the extent of integrin recycling. The results suggest that Rab5 and EHD1 endosomes may be key locations of PIP2 synthesis and localization during serum-stimulated integrin recycling.

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# Chapter 1

# Introduction

## Migration of Epithelial Cells

The epithelial cells that form barriers around tissues and compartments in the body are normally stationary. However, these cells can become migratory at specific times like embryonic development and wound repair (Ridley *et al.*, 2003). In pathophysiological scenarios, cell migration can also underlie cancer metastasis and cause epithelial tumors to spread in the body (Gao, *et al.*, 2019). The term "metastasis" is used to describe the spread of cancerous cells beyond their original locations or compartments. The onset of metastasis not only creates significant challenges to treatments, but is the major cause of death in cancer patients (Seyfried & Huysentruyt, 2013). This supports the urgency behind research efforts to uncover the mechanisms underlying epithelial cell migration.

Cell migration is facilitated by processes such as assembly and disassembly of transmembrane proteins at the cell surface, as well as recycling of those components through the cell's endosomal system. Integrins are a main class of proteins involved in this recycling process, and their trafficking to and from the cell membrane contributes to the mechanisms underlying cell migration (De Franceschi *et al.*, 2010).

## Integrins

Integrins are heterodimeric protein receptors consisting of non-covalently associated alpha (α) and beta (β) subunits. The two subunits each have cytosolic, extracellular, and transmembrane domains. Through combinations of 18 different alpha and 8 different beta subunits, over 24 different integrins can assemble. The type of integrin receptor formed depends on its binding function and the tissue type where it resides (Campbell & Humphries, 2011).

Being transmembrane protein receptors, integrins mediate a diverse set of cellular processes. Integrins are the principal cell receptors that help cells adhere to the extracellular matrix (ECM). ECM proteins that are known to bind integrins include collagen, laminin, and fibronectin (Alberts *et al.*, 2002). Extracellular matrix proteins interact with integrins' extracellular domains, whereas integrins' cytoplasmic domains interact with adaptor proteins inside the cell (Figure 1). Focal adhesions, a complex of various proteins, are formed inside the cell to link the integrin cytoplasmic domain to the actin cytoskeleton. Common adaptor proteins known to facilitate this include talin, filamin, and α-actinin (Calderwood & Ginsberg, 2003). These protein interactions are shown in Figure 1, although some additional proteins found in focal adhesion complexes are omitted for clarity. Since integrins effectively link the extracellular matrix to the intracellular actin cytoskeleton, integrins facilitate signal transduction through their transmembrane domains (Alberts *et al.*, 2002). Therefore, integrins have significant downstream effects since they activate intracellular signaling pathways and transduce signals across the plasma membrane. This functionality is what gives integrins the ability to mediate so many processes, such as those related to cell survival, growth, and proliferation (Alberts *et al.*, 2002).

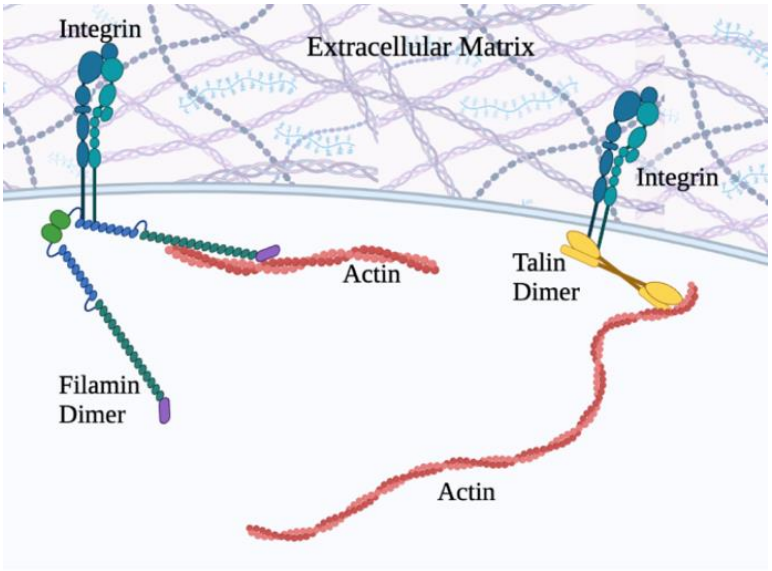


**Figure 1. Integrins Linking the Extracellular Matrix to the Actin Cytoskeleton**

Transmembrane integrins link the extracellular matrix to the actin cytoskeleton through interactions with adaptor proteins talin and actin. Image created using BioRender.com.

## Focal Adhesion Turnover

The focal adhesions connecting integrins to the actin cytoskeleton must be dissociated and reformed in order for cell migration to occur and for integrins to be recycled through the cell (Nagano *et al.*, 2012). Additional proteins facilitate this process and are recruited to the cell surface to help form or dissociate these adhesion complexes. Cytoskeletal proteins are recruited to the cytoplasmic domains of integrins to facilitate formation or disassembly of integrin-ECM interactions (Lawson & Schlaepfer, 2012). For instance, actin-binding proteins such as filamin and talin interact with the cytoplasmic domain of integrins to help them form adhesion complexes (Liu *et al.*, 2000).

## Integrin Recycling

Integrin recycling further facilitates the ability of epithelial cells to move during cell migration. Since integrins are transmembrane proteins, they are trafficked through the cell's endosomal system in vesicles (Moreno-Layseca, 2019). After being incorporated into vesicles, they are targeted to specific locations based on vesicular trafficking, then returned to the cell plasma membrane (Bridgewater *et al.*, 2012).

The process of integrin recycling is facilitated by integrins having lower binding affinity to their ligands compared to other types of receptors (such as those for hormones or signaling molecules). The advantage of this lower binding affinity is that integrins can form a large number of weaker adhesions that can still be broken when necessary. This transient binding is what contributes to the ability for integrins to be recycled through the cell's endosomal system (Alberts *et al*., 2002).

### Mechanism of Integrin Recycling

Through integrin recycling, cell movement occurs as integrins attach to and disassemble from the surrounding ECM in a cyclic manner. The process also relies on cell polarization, where different sides of the cell are regulated by distinct molecular processes and signaling cascades. At the leading edge of the cell, a protrusion must form so the cell can extend and adhere to the ECM. Integrins in the cell's plasma membrane facilitate these adhesions and provide traction while motor proteins exert the forces needed for movement. Adhesions must then be disassembled as the next adhesions begin to form at the leading edge to allow for

forward movement. The steps continue to cycle, allowing for cell movement that facilitates cell migration (Ridley *et al.*, 2003).

Cell migration is largely regulated by integrin expression and integrin recycling through the endosomal network. First, integrin expression plays an important role in the regulation of integrin recycling and cell migration, as studies have found correlations between increased integrin expression and disease progression in several types of cancer (Ganguly *et al.*, 2013) (Desgrosellier & Cheresh, 2010). Cell migration is also highly reliant on the endosomal compartments integrins are trafficked through after they are recycled from the plasma membrane (De Franceschi *et al.*, 2010).

## The Endosomal System

The cell's endosomal system consists of membrane-bound organelles and various membranous compartments. Different materials can be transported between these compartments, and materials can also be taken into or out of the cell through the endosomal system. Endocytosis is the process by which various materials, including transmembrane receptors such as integrins, are brought into the cell through an invagination of the plasma membrane (De Franceschi *et al.*, 2010). The membrane-bound vesicles formed inside the cell are called endosomes, and they can be classified based on the step of processing of the internalized materials. Endocytosis from the plasma membrane first results in formation of an early endosome, which acts as a compartment where materials are sorted based on their downstream fates (Jovic *et al.*, 2010). From the early endosome, materials are either trafficked to a late endosome or a recycling endosome. In the case of a late endosome, the material is next targeted to a lysosome for degradation. If the early

endosome is instead targeted to a recycling endosome, the material is moved through additional trafficking steps and ultimately returns to the plasma membrane (Naslavsky & Caplan, 2018).

## Endosomal Markers

Not only are endosomes classified based on the processing step they facilitate after endocytosis, different endosomes are “marked” based on their association with specific proteins. Examples of such proteins are those in the Rab-GTPase (Rab) and Eps15 Homology Domain (EHD) families of proteins, which are both known to have regulatory roles in intracellular trafficking as well (Shearer & Petersen, 2019).

## Rab Proteins

The Rab-GTPase (Rab) family of proteins is closely associated with the endosomal system given the regulatory role of Rabs in vesicular trafficking. Rab proteins are known to regulate the formation and targeting of vesicles in the cell by interacting with proteins required for vesicle trafficking. For instance, many Rab proteins interact with and regulate the SNARE proteins required for fusion of vesicles (Naslavsky & Caplan, 2011). Specific sets of Rab proteins have been found to localize to specific types of endosomal compartments, likely dependent on the regulatory role of the Rab protein at that location (Shearer & Petersen, 2019). Therefore, Rabs are often used as markers of specific endosomal compartments. Examples include Rab5 as a marker of early endosomes (Zerial & McBride, 2001), Rab7 as a marker of

late endosomes (Shearer & Petersen, 2019), and Rab8 (Henry & Sheff, 2008) and Rab11 (Martinez-Arroyo *et al.*, 2021) as markers of recycling endosomes.

**EHD1**

The Eps15 Homology Domain (EHD) family is another set of proteins that play regulatory roles in endosomal trafficking in the cell. EHD proteins have been shown to regulate effector proteins that play key roles in vesicle formation and targeting. Their role is further supported by the interaction of EHD proteins with different Rab proteins, such as the localization of EHD1 with Rab11 (Naslavsky & Caplan, 2011). With the localization and regulatory role of EHD1 at specific components, it is an endosomal marker that marks recycling endosomes. EHD1 has been found to regulate the trafficking of a variety of receptors and materials in the cell, and notably may play a role in the regulation of β1 integrin receptors in the cell (Naslavsky & Caplan, 2011).

**Integrin Recycling through the Endosomal System**

As mentioned previously, integrin recycling occurs through the internalization of integrins at the cell surface, followed by their return to the plasma membrane. Therefore, integrin recycling is closely related to the endosomal system. For a transmembrane integrin starting at the plasma membrane, the protein is first endocytosed into an early endosome. If the integrin is to be recycled, it is trafficked to a recycling endosome then returned to the cell surface. Once in the recycling endosome, the integrin could also be recycled in a longer recycling pathway before

returning to the plasma membrane (De Franceschi *et al*., 2010). Shown in Figure 2 is an example of these possible integrin recycling pathways, with Rab and EHD protein markers shown on the specific endosomal compartments.

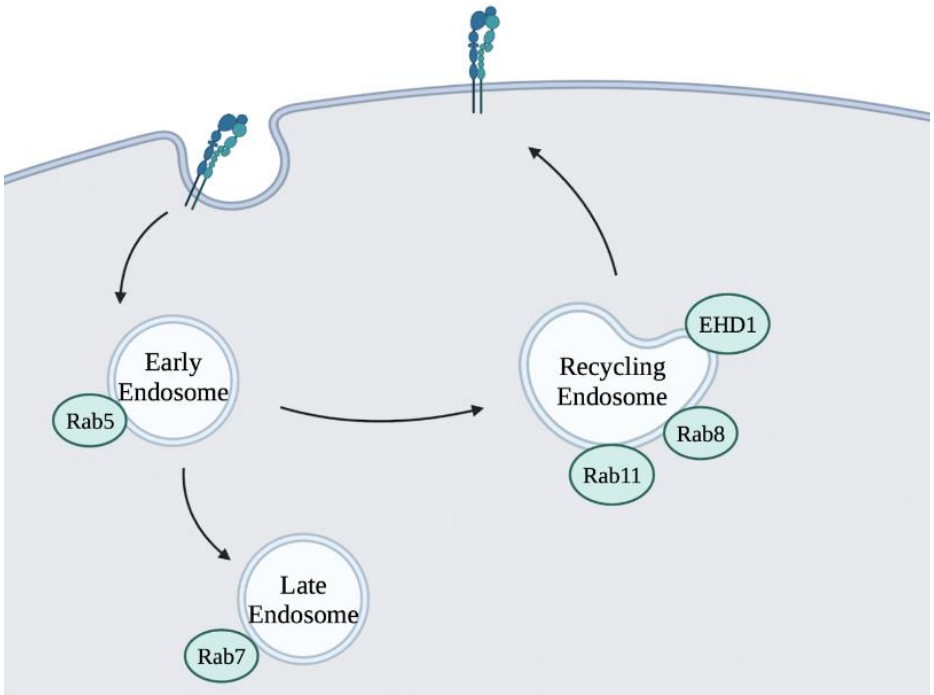


**Figure 2. Rab and EHD Protein Markers in The Endosomal System**

Heterodimeric integrin at the plasma membrane, with arrows showing potential directions of its endosomal trafficking following endocytosis. Markers of each endosome include Rab5 at the early endosome, Rab7 at the late endosome, and EHD1, Rab8, and Rab11 at the recycling endosome. Image created using BioRender.com.

**Phosphatidylinositol 4,5-bisphosphate (PIP2)**

Phosphatidylinositol 4,5-bisphosphate, often abbreviated as PI(4,5)P2 or “PIP2”, is a phospholipid with the structure shown in Figure 3. PIP2 is found in membranes and it localizes to both the plasma membrane and endosomes in the cell. PIP2 is part of a group of phospholipids called phosphoinositides, which are known for having a variety of secondary messenger and signaling functions, playing roles in vesicular trafficking and membrane dynamics. PIP2 is known to have diverse signaling roles in the cell, from those in cytoskeletal linkage to intracellular trafficking (Mandal, 2020).

PIP2 can be synthesized through the addition of phosphates to different forms of the phospholipid. Kinases PI4K and PIP5K add phosphates to the 4th and 5th carbon of the six-membered ring, respectively (Figure 3). PI4K converts PI to PI(4)P and PIP5K converts PI(4)P to PIP2 (Hansen *et al.*, 2022).

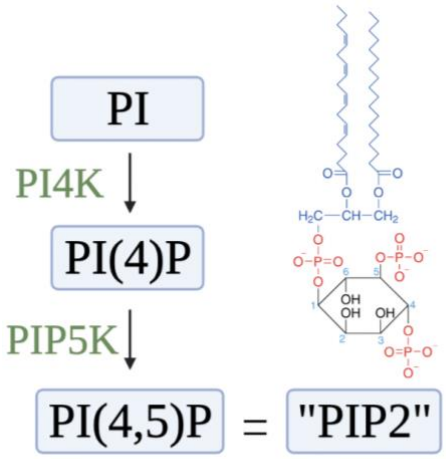


**Figure 3. Synthesis of Phosphatidylinositol 4,5-bisphosphate (PIP2)**

PI is converted from PI to PI(4)P by PI4K. PI(4)P is then converted to PI(4,5)P by PIP5K. Image created using BioRender.com.

**PIP2 in Integrin Recycling**

Previous studies have suggested a role for PIP2 in integrin recycling because it recruits proteins to endosomes where it is synthesized and localized (Oh & Santy, 2012). Therefore, PIP2 may impact the regulation of vesicle trafficking through the endosomal system. A specific protein called cytohesin-2, which is recruited and bound by PIP2, supports the potential role of PIP2 in the integrin recycling pathway. Cytohesin-2 is a protein known to promote β1 integrin recycling upon serum-stimulation (Oh, 2011). In addition, only cytohesins that can bind PIP2 are known to stimulate integrin recycling (Oh & Santy, 2012). Therefore, the endosomal location of PIP2 synthesis by PIP5 kinases may impact the β1 integrin recycling pathway.

## Experimental Objective & Research Question

Given the suggested role of PIP2 in integrin recycling, the goal of this experiment was to elucidate the potential locations of PIP2 synthesis within the endosomal system that impact integrin recycling. If PIP2 is produced at specific endosomes in the cell, uncovering the specific location(s) may give insight into which endosomal compartments integrins are recycled through.

To investigate this research question, the methodology involved investigating the impact of a *lack* of PIP2 at specific endosomal compartments. Five endosomal populations were tested: Rab5, Rab7, Rab8, Rab11, and EHD1. The trafficking of integrins through the endosomal system was visualized using fluorescent integrins and endosomal markers. It was hypothesized that at endosomes where PIP2 is synthesized, the integrin would become stuck and unable to move to the next endosomal compartment or be recycled back to the plasma membrane.

# Chapter 2

# Materials and Methods

## Cell Lines

HeLa cell cultures were used to conduct all assays. The cells were split regularly using a 1:10 dilution factor and incubated at 37°C and 5% $CO_2$. Cells were cultured in complete cell media consisting of 10% fetal bovine serum (FBS), 1% penicillin-streptomycin-fungizone (PSF), and 1% L-glutamine in Dulbecco's Modified Eagle Medium (DMEM).

## Collagen-Coating Procedure

Sterile glass coverslips were coated with 200 µL of solution containing collagen in filter sterilized 0.02M acetic acid, at a concentration of 50µg/ml. The plates were placed at 4°C overnight. The following day, they were moved to 37 °C for 1 hour then rinsed two times with phosphate buffered saline (PBS).

## Recruitment of PseudoJanin to Endosomal Compartments

PseudoJanin (PJ) is a synthetic enzyme with phosphatase activity, meaning it can hydrolyze phosphate groups from specific residues on a protein or lipid. PJ was utilized in the assays for its enzymatic activity of dephosphorylating PIP2. PJ contains two phosphatase domains: the inositol polyphosphate 5-phosphatase E (INPP5E) domain that removes the 5th carbon of the six-membered ring of PIP2 to convert it to PI(4)P, and the *S. cerevisiae* sac1

phosphatase (Sac) domain that subsequently removes the 4th carbon from PI(4)P to form PI (Hammond *et al.*, 2012).

To bring the PJ enzyme to specific endosomal populations, DNA constructs encoding specific domains were used. DNA constructs for the endosomal markers of interest each included an FKBP-rapamycin-binding (FRB) domain and the DNA construct for the PJ enzyme included an FKBP domain (Table 1). Using the heterodimerizer Rapamycin that links FRB and FKBP domains, the PJ enzyme was localized to specific endosomal markers in the cell (Figure 4). As endosomal markers are peripheral, membrane proteins, the PJ enzyme is targeted to the cytoplasmic side of the endosomes and hydrolyzes PIP2 found in the cytoplasmic leaflet of endosomal membranes.

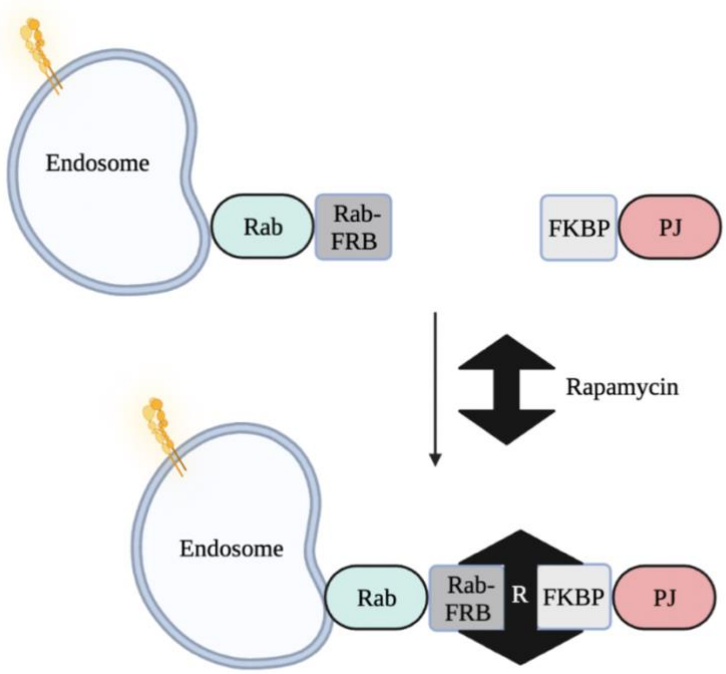


**Figure 4. Rapamycin to link the FKBP domain of the synthetic PJ enzyme to the FRB domain of Rab proteins.**

Image created using BioRender.com.

A “dead” form of the PJ enzyme was utilized as a control in this study. “Dead PJ” is catalytically inactive as both the INPP5E and Sac domains are inactivated. Experiments conducted by Hammond *et al*. (2012) validated the lack of hydrolytic activity in the dead PJ enzyme. The construct for the dead PJ enzyme still contains the FKBP domains which allow for its recruitment to specific endosomes during the assays.

**DNA Constructs for Protein Expression**

Depending on the endosomal marker being analyzed in the assay, two specific DNA constructs for the endosomal marker of interest were used. As seen in Table 1, for each endosome, there are two DNA constructs: one encoding the protein and cyan fluorescent protein (CFP), and another encoding the protein with an FRB domain but no fluorescence. For example, CFP-Rab8 was used to express and visualize Rab8 using cyan fluorescence for Rab8-specific assays. In addition, for a given endosome of interest, a Rab8 DNA construct with an FKBP-rapamycin-binding (FRB) domain was used.

For all assays, two additional DNA constructs were utilized to express and visualize specific proteins in the cell. mGold-Integrin5α was used to express and visualize integrins by encoding the mGold variant of yellow fluorescent protein (YFP) attached to Integrin5α. RFP-PJ or RFP-Dead PJ constructs were used to express and visualize PJ or dead PJ using red fluorescent protein (RFP).

**Table 1. DNA constructs used for Protein Expression and Visualization in PJ Assays**

| Name | Protein Expressed | Fluorescent Tag |
|---|---|---|
| mGold-Integrin5α | Integrin5α | Yellow |
| RFP-PJ + FKBP | PJ enzyme with FKBP domain | Red |
| RFP-Dead PJ + FKBP | Dead PJ enzyme with FKBP domain | Red |
| CFP-EHD1 | EHD1 | Cyan |
| FRB-EHD1 | EHD1 with FRB domain | none |
| CFP-Rab11 | Rab11 | Cyan |
| FRB-Rab11 | Rab11 with FRB domain | none |
| CFP-Rab8 | Rab8 | Cyan |
| FRB-Rab8 | Rab8 with FRB domain | none |
| CFP-Rab7 | Rab7 | Cyan |
| FRB-Rab7 | Rab7 with FRB domain | none |
| CFP-Rab5 | Rab5 | Cyan |
| FRB-Rab5 | Rab5 with FRB domain | none |

## 4-day PJ Assay Procedure

***Day 1:***

Cells were seeded onto 18mm collagen-coated coverslips in a 24-well plate, at a concentration of $10^5$ cells per coverslip. 0.5 mL of complete cell media was added to each well and cells were incubated at 37°C for approximately 24 hours.

***Day 2:***

Cells were transfected using a JetOPTIMUS transfection kit. A total of 0.5µg of DNA was used per well. The ratios of the DNA constructs used differed for the assays testing different endosomal populations), as shown in Table 2. The DNA was diluted in JetOPTIMUS buffer, vortexed 1 second, then spun down. JetOPTIMUS reagent was added to the mixture, vortexed 1 second, then spun down. The mixture was incubated at room temperature for 10 minutes then added to the wells, then cells were placed in incubation at 37°C. 4-6 hours after the initial transfection, the complete cell media in the wells was removed from the wells. The cells were rinsed with PBS, then 0.5 mL of serum-free media was put in each well. The cells were then returned to incubation at 37°C overnight.

**Table 2. Ratios of DNA constructs used in assays for each endosomal population.**

| DNA Construct | CFP-endosomal marker | FRB-endosomal marker | mGold-Integrin5α | PJ/Dead PJ |
|---|---|---|---|---|
| Rab5 | 2 | 6 | 10 | 1 |
| Rab7 | 2 | 4 | 8 | 1 |
| Rab8 | 2 | 3 | 2 | 1 |
| Rab11 | 4 | 8 | 6 | 1 |
| EHD1 | 2 | 3 | 2 | 1 |

***Day 3:***

500 mM of A/C heterodimerizer, a Rapamycin analog, was added to the cells to achieve a final concentration of 200 nM in each well, then cells were incubated for 10 minutes at 37°C. Undiluted and warmed (37°C) fetal bovine serum (FBS) was added to each well to achieve a solution consisting of 20% FBS. After addition of serum, the 24-well plate was placed back into 37°C incubation for 5 minutes. After 5 minutes, coverslips were rinsed with 0.5 mL cold PBS and fixed with 0.5 mL 4% paraformaldehyde (PFA) for 10 minutes. The coverslips were then rinsed with 0.5 mL PBS two times and 0.5 mL distilled water ($dH_2O$) one time. The coverslips were then removed from the wells, mounted onto slides using Hydromount, and cured overnight at room temperature and in the dark.

***Day 4:***

The cells were imaged at least one day after mounting the coverslips onto microscope slides or once the Hydromount had fully dried. Fluorescent markers for the proteins expressed using the DNA constructs were visualized using an Olympus IX83 microscope and Slidebook 6.0 software. Imaging of the slides was conducted using the 60x oil objective lens. The following fluorescent channels were used to visualize specific proteins: YFP to visualize integrin5α, CFP to visualize the endosome of interest, and RFP to visualize the PJ enzyme. 16-bit per pixel captures were taken using the 3 channels, then quantitative data analysis of the images was conducted using FIJI software (Schindelin *et al.*, 2012).

## Colocalization Analysis in FIJI

Images obtained from the assays were analyzed using FIJI software. Images for each of the three color channels (YFP, CFP, and RFP) were opened separately and background fluorescence was removed from each. A region of interest (ROI) was manually drawn around one cell of interest based on visualization of the plasma membrane from the YFP channel for integrin. Only cells that were adequately spread out and not showing signs of apoptosis (small, rounded cells) were chosen for further analysis.

Colocalization between the PJ/dead PJ enzyme (RFP channel) and endosomal marker (CFP channel) was first measured for the ROI using the “colocalization 2” setting in FIJI. Only cells with a Pearson correlation coefficient value above 0.70 for colocalization of these two channels were utilized in the final step of colocalization analysis.

Colocalization between the integrin (YFP channel) and endosomal marker (CFP channel) was then measured in the ROI, again using the “colocalization 2” setting in FIJI. Pearson correlation coefficient values were obtained then used in statistical tests to compare the extent of colocalization with active versus dead PJ enzyme.

## Statistical Analysis of Colocalization

To analyze the Pearson’s correlation coefficient values obtained in FIJI, a Difference in Means statistical test was performed for each of the five endosomes. The statistical test was used to determine if there was a difference in colocalization (of integrins to a given endosome) when active PJ versus dead PJ enzyme was localized to the endosome of interest. P-values were calculated and an $\alpha$ level of 0.05 was utilized to determine statistical significance.

# Chapter 3

## Results

In order to analyze which endosomal population(s) may be a key location of PIP2 synthesis or localization during serum-stimulated integrin recycling, assays tested the impact of decreasing PIP2 at specific endosomes in the cell. Endosomal populations with the following protein markers were tested: Rab5, Rab7, Rab8, Rab11, and EHD1.

For each assay, only one of the five endosomal populations was focused on. The objective was to analyze the impact of decreased PIP2 levels at each endosomal population. To achieve this, a synthetic enzyme called PseudoJanin (PJ) was used. PJ hydrolyzes both phosphates in PIP2 to convert it to PI. By using a form of the PJ enzyme with an FKBP domain attached, the PJ enzyme (or catalytically inactive “Dead PJ” as a control) was localized to specific endosomal locations in the cell. Therefore, the impact of decreasing PIP2 at those endosomes could be analyzed, to elucidate potential locations of PIP2 synthesis during serum-stimulated integrin recycling.

DNA constructs for fluorescent proteins were transfected into HeLa cells to visualize the endosomal markers, the PJ enzyme (or “dead” PJ enzyme as a control), and integrins. Pearson's Correlation Coefficient values (Pearson’s R values) were measured between different channels of fluorescence. The extent of colocalization between PJ and the endosome of interest was first analyzed to ensure they were properly linked by rapamycin (Pearson’s correlation coefficient values above 0.70 was considered sufficient colocalization). Next, colocalization between integrin and endosomes was analyzed. Pearson’s correlation coefficient values were compared between cells transfected with active PJ enzyme versus the “dead” PJ enzyme as a control.

Higher levels of colocalization between integrin and endosomes were considered indicative of integrins becoming “stuck” in the corresponding endosomal compartment, with decreased integrin recycling back to the cell surface.

It was hypothesized that assays targeting PJ to endosomal locations of PIP2 synthesis would result in increased colocalization of integrin to those endosomes. Data for each endosomal population was analyzed separately, given that integrin localization in different endosomal populations may differ between endosomes even prior to stimulation of integrin recycling. Therefore, Pearson’s correlation coefficient values were compared between cells with active PJ enzyme compared to the control “dead” PJ enzyme, and Difference in Means statistical tests were conducted for each of the five endosomal populations.

## EHD1 Results

**Table 3. EHD1 Statistical Analysis**

| | PJ | Dead PJ | **Difference in Means (PJ - Dead PJ)** |
|---|---|---|---|
| Average Pearson's Correlation Coefficient | 0.838 | 0.669 | **0.169** |
| Standard Deviation | 0.131 | 0.114 | **P-value < 0.0001** |
| n (number of cells) | 53 | 52 | |

Average Pearson's Correlation Coefficient values for integrin and EHD1 in cells transfected with active versus dead PJ enzyme. Sample size (n) consists only of cells with Pearson's Correlation Coefficient values above 0.70 for colocalization of PJ/Dead PJ to EHD1.

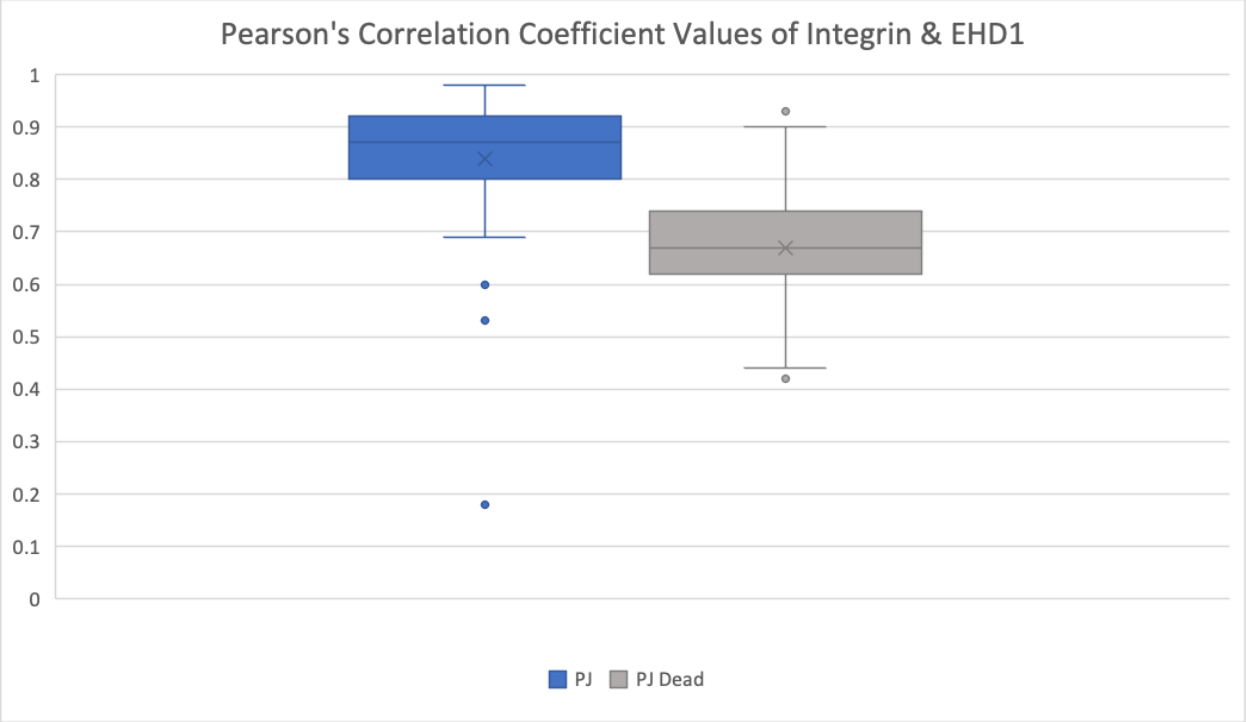


**Figure 5. Comparison of Pearson's Correlation Coefficient Values in cells transfected with PJ versus dead PJ enzyme targeted to EHD1 endosomes.**

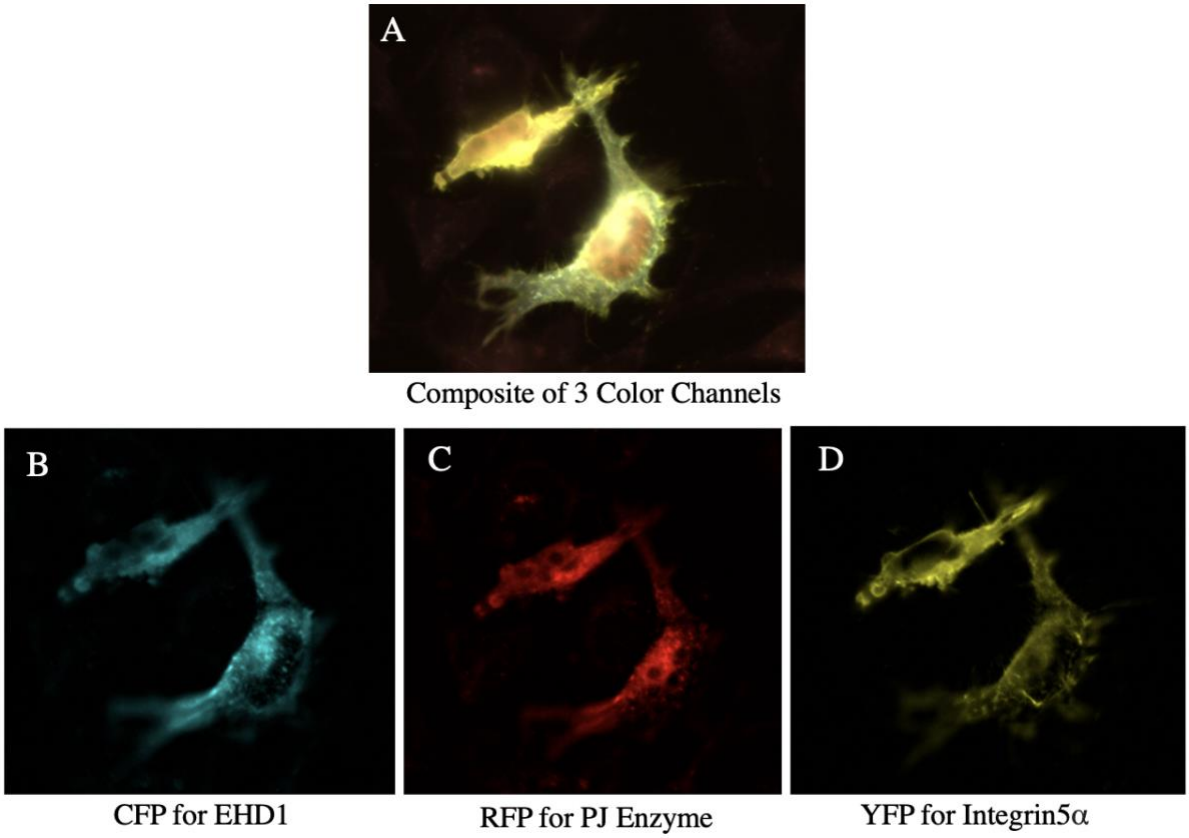


**Figure 6. PJ enzyme targeted to EHD1 compartments in HeLa cells**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize EHD1. C: RFP to visualize PJ enzyme. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.93 for CFP and RFP channels. Pearson's R value (no threshold) was 0.85 for CFP and YFP channels.
Cell 2: Pearson's R value (no threshold) was 0.90 for CFP and RFP channels. Pearson's R value (no threshold) was 0.78 for CFP and YFP channels.

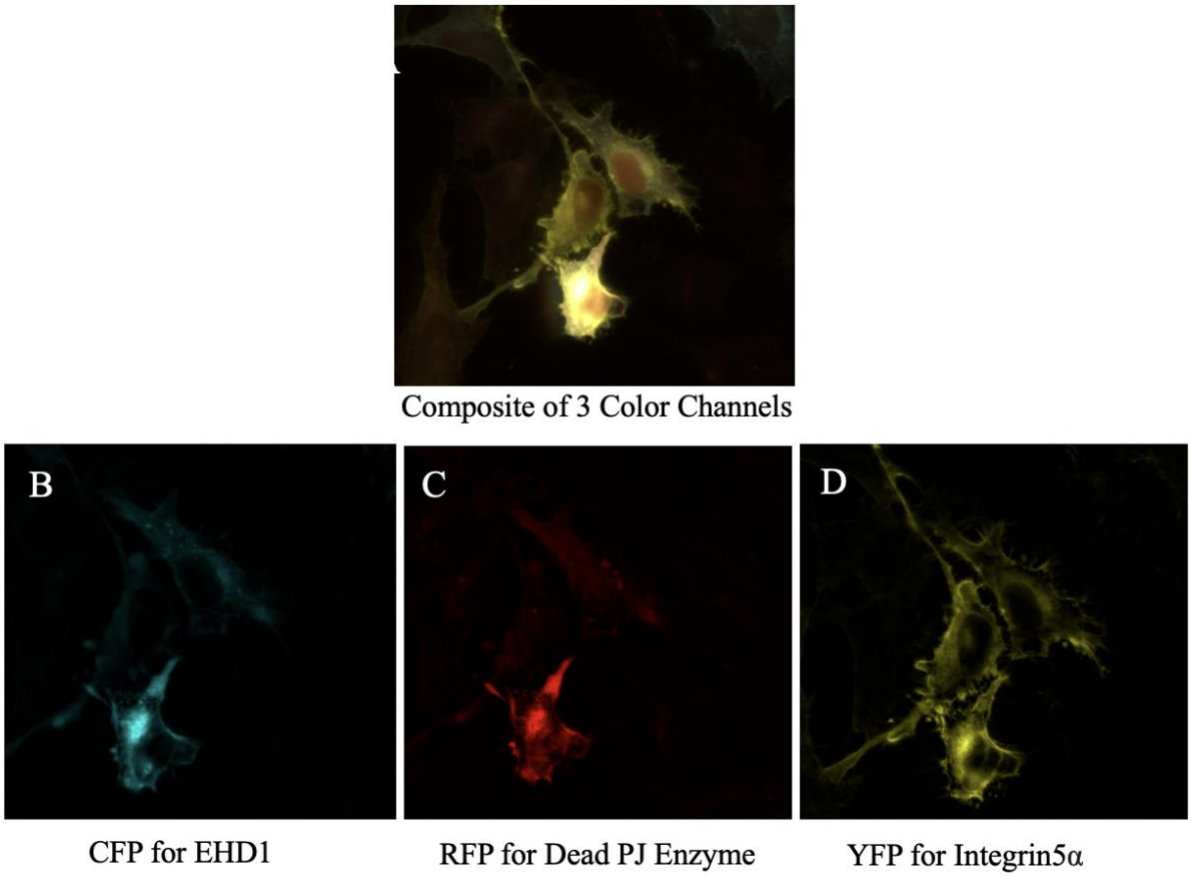


**Figure 7. Dead PJ enzyme targeted to EHD1 compartments in HeLa cells**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize EHD1. C: RFP to visualize Dead PJ enzyme. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.77 for CFP and RFP channels. Pearson's R value (no threshold) was 0.74 for CFP and YFP channels.
Cell 2: Pearson's R value (no threshold) was 0.78 for CFP and RFP channels. Pearson's R value (no threshold) was 0.67 for CFP and YFP channels.

Quantitatively, there was higher colocalization of integrin to EHD1 in cells transfected with active PJ enzyme compared to the control condition with dead PJ enzyme (Figure 5). As seen in Table 3, average Pearson's Correlation Coefficient value for cells with active PJ was 0.84, compared to 0.67 in cells containing dead PJ enzyme. Results of the difference in means test (PJ- Dead PJ) concluded a difference of 0.169 at a p-value of <0.0001 (Table 3). At an alpha

level of 0.05, the results were statistically significant. Therefore, there appears to be a difference in the extent of colocalization of integrin and EHD1 when active versus dead PJ enzyme is recruited to EHD1 compartments. This suggests EHD1 may be an important location of PIP2 synthesis or localization after the stimulation of integrin recycling.

Figures 6 and 7 show representative images of the quantitative results. The three separate color channels and a composite image are shown, with fluorescence indicating EHD1 endosomal markers (cyan), PJ/dead PJ (red), and integrin (yellow). In Figure 6, where active PJ was localized to EHD1 endosomes, it is qualitatively seen that yellow fluorescence in the same locations as the cyan fluorescence. This is indicative of integrins being largely colocalized with EHD1 compartments. Comparatively, in Figure 7, the integrin appears to have moved farther towards the cell plasma membrane. There appears to be less colocalization of yellow fluorescence with the cyan fluorescence for EHD1 within the cell. This suggests that integrin recycling may be lower in cells containing PJ enzyme targeted to EHD1 compartments in the cell (Figure 6) compared to normal integrin recycling (Figure 7).

## Rab11 Results

**Table 4. Rab11 Statistical Analysis**

| | PJ | Dead PJ | **Difference in Means (PJ - Dead PJ)** |
|---|---|---|---|
| Average Pearson's Correlation Coefficient | 0.670 | 0.644 | **0.026** |
| Standard Deviation | 0.137 | 0.158 | **P-value = 0.3472** |
| n (number of cells) | 66 | 50 | |

Average Pearson's Correlation Coefficient values for integrin and Rab11 in cells transfected with active versus dead PJ enzyme. Sample size (n) consists only of cells with Pearson's Correlation Coefficient values above 0.70 for colocalization of PJ/Dead PJ to Rab11.

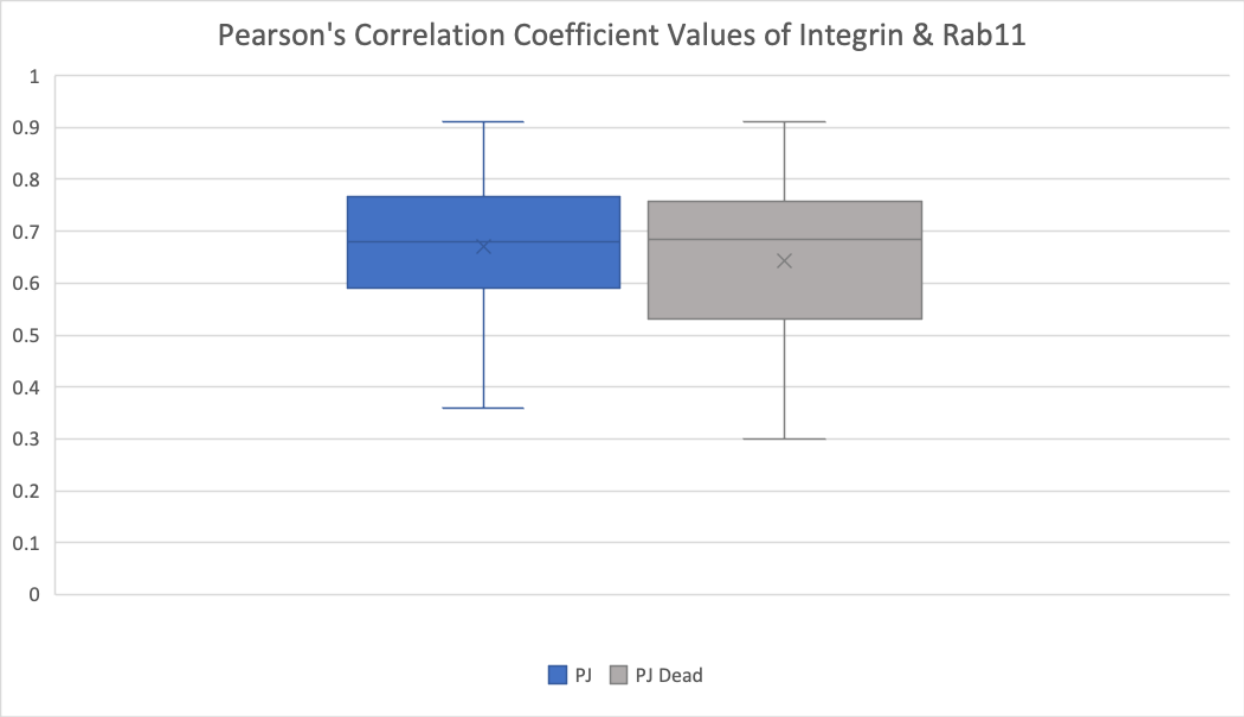


**Figure 8. Comparison of Pearson's Correlation Coefficient Values in cells transfected with PJ versus dead PJ enzyme targeted to Rab11 endosomes.**

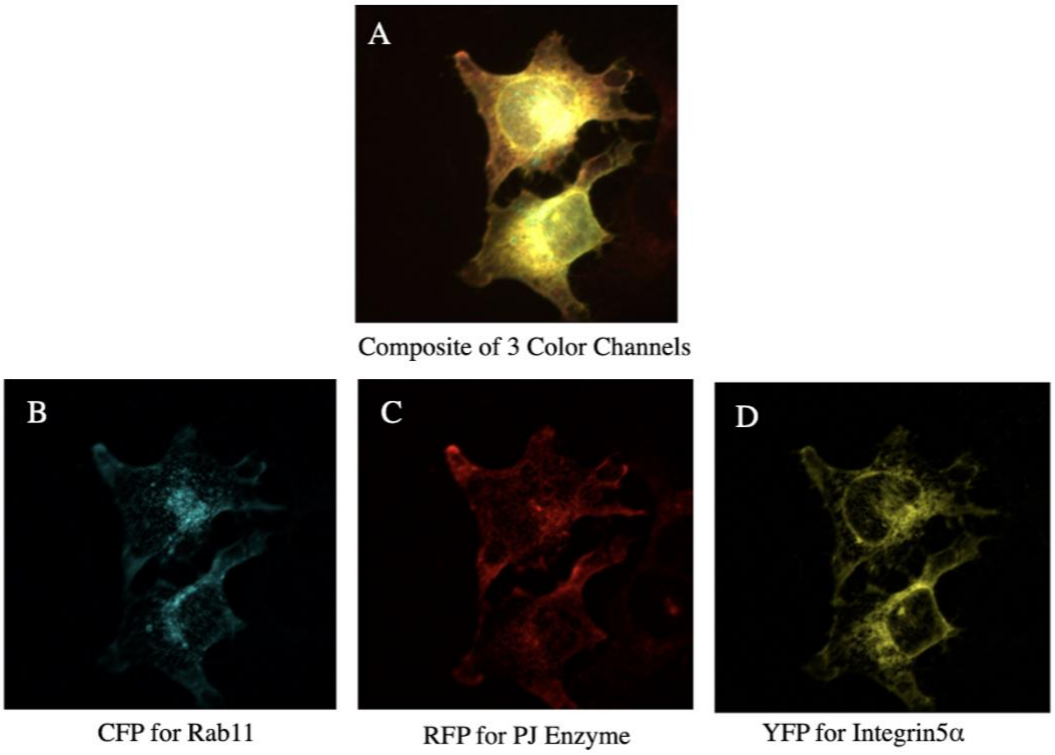


**Figure 9. PJ Enzyme targeted to Rab11 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab11. C: RFP to visualize PJ enzyme. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.72 for CFP and RFP channels. Pearson's R value (no threshold) was 0.53 for CFP and YFP channels.
Cell 2: Pearson's R value (no threshold) was 0.71 for CFP and RFP channels. Pearson's R value (no threshold) was 0.77 for CFP and YFP channels.

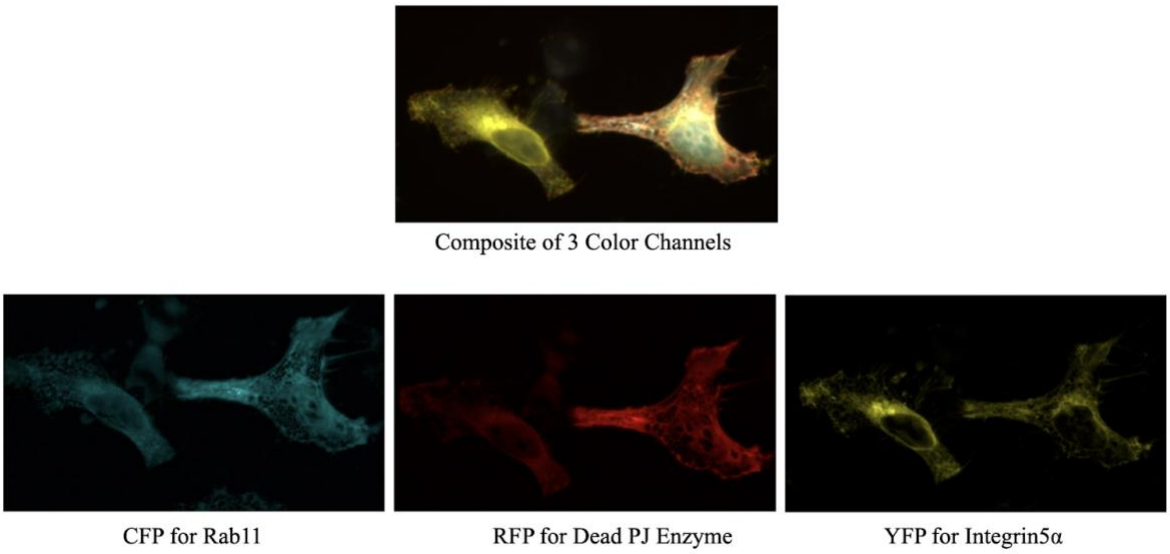


**Figure 10. Dead PJ Enzyme targeted Rab11 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab11. C: RFP to visualize Dead PJ enzyme. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.84 for CFP and RFP channels. Pearson's R value (no threshold) was 0.76 for CFP and YFP channels.
Cell 2: Pearson's R value (no threshold) was 0.87 for CFP and RFP channels. Pearson's R value (no threshold) was 0.67 for CFP and YFP channels.

For Rab11 assays, the difference in means test (PJ- Dead PJ) resulted in a p-value of 0.3472 based on the Pearson's Correlation Coefficient difference of 0.026 (Table 4). Therefore, there does not appear to be a significant difference in colocalization of integrin and Rab11 when active versus dead PJ enzyme is localized to Rab11 compartments (Figure 8). Representative sample images of colocalization shown in Figures 9 and 10 may support these results, as there do not appear to be distinct qualitative differences in integrin recycling when active versus dead PJ enzyme is localized to Rab11 endosomes.

## Rab7 Results

**Table 5. Rab7 Statistical Analysis**

| | PJ | Dead PJ | **Difference in Means (PJ - Dead PJ)** |
|---|---|---|---|
| Average Pearson's Correlation Coefficient | 0.676 | 0.641 | **0.035** |
| Standard Deviation | 0.160 | 0.177 | **P-value = 0.2780** |
| n (number of cells | 54 | 54 | |

Average Pearson's Correlation Coefficient values for integrin and Rab7 in cells transfected with active versus dead PJ enzyme. Sample size (n) consists only of cells with Pearson's Correlation Coefficient values above 0.70 for colocalization of PJ/Dead PJ to Rab7.

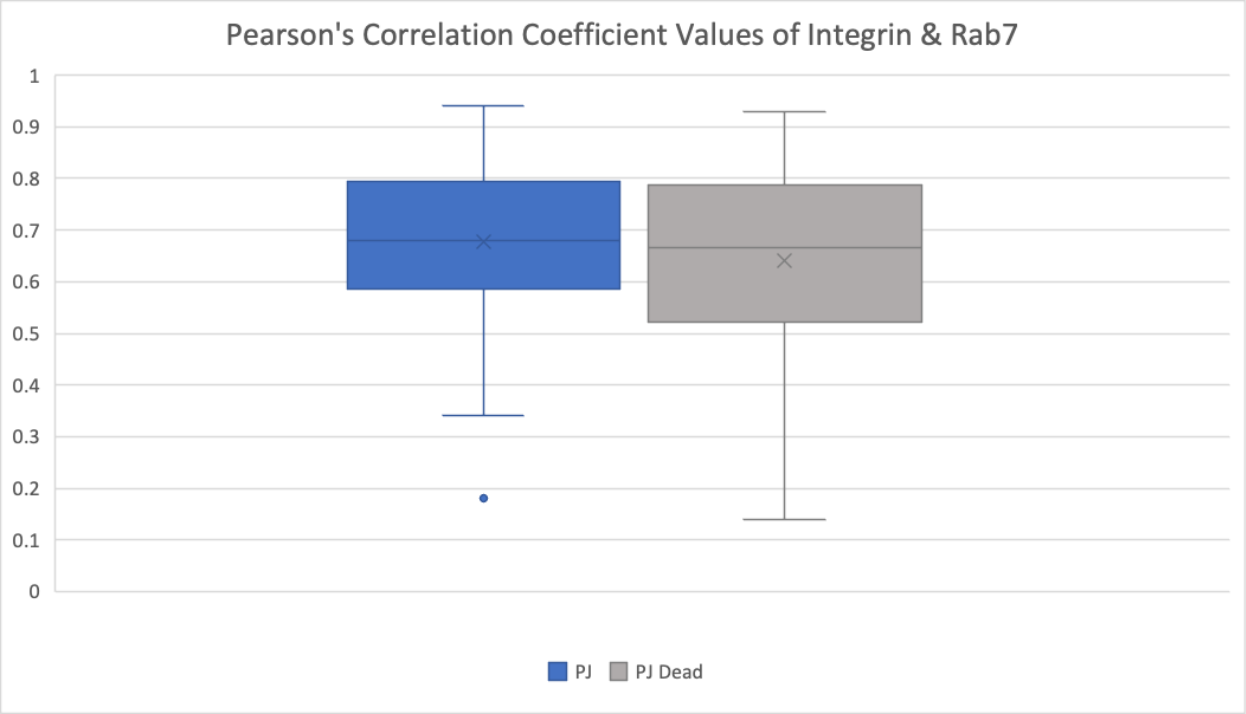


**Figure 11. Comparison of Pearson's Correlation Coefficient Values in cells transfected with PJ versus dead PJ enzyme targeted to Rab7 endosomes.**

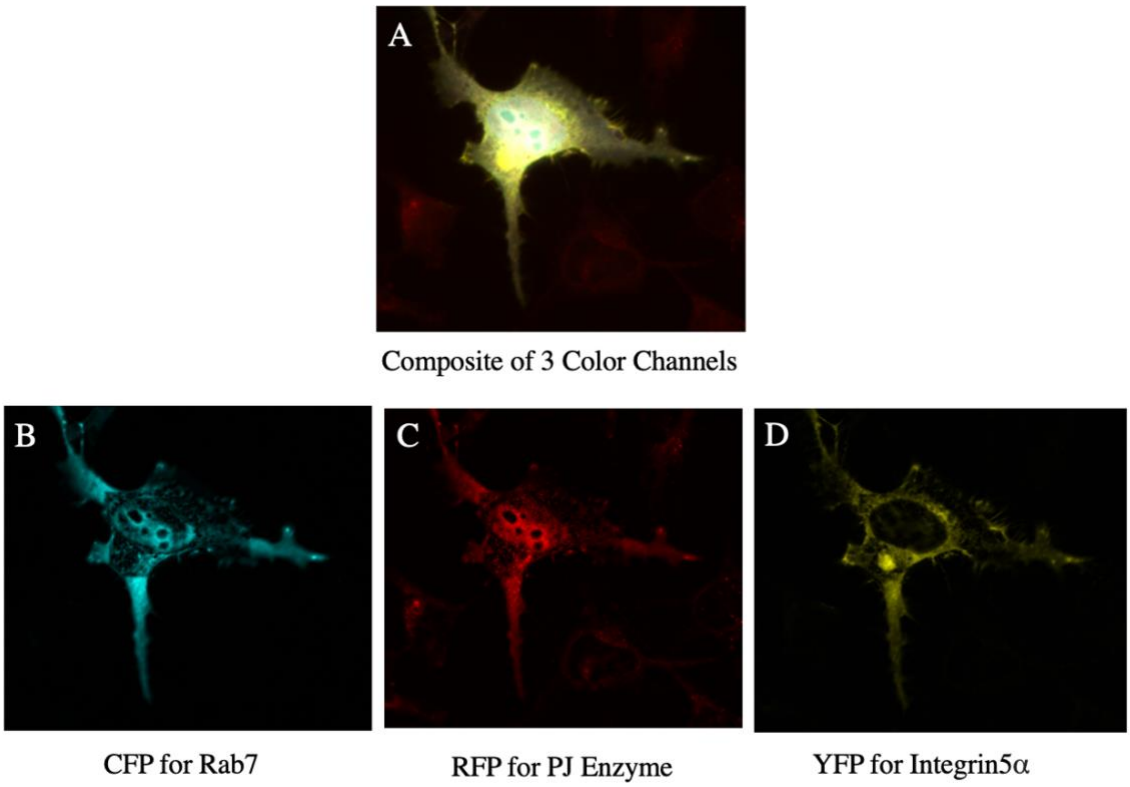


**Figure 12. PJ Enzyme targeted Rab7 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab7. C: RFP to visualize PJ. D: YFP to visualize Integrin5α.
Pearson's R value (no threshold) was 0.82 for CFP and RFP channels. Pearson's R value (no threshold) was 0.45 for CFP and YFP channels.

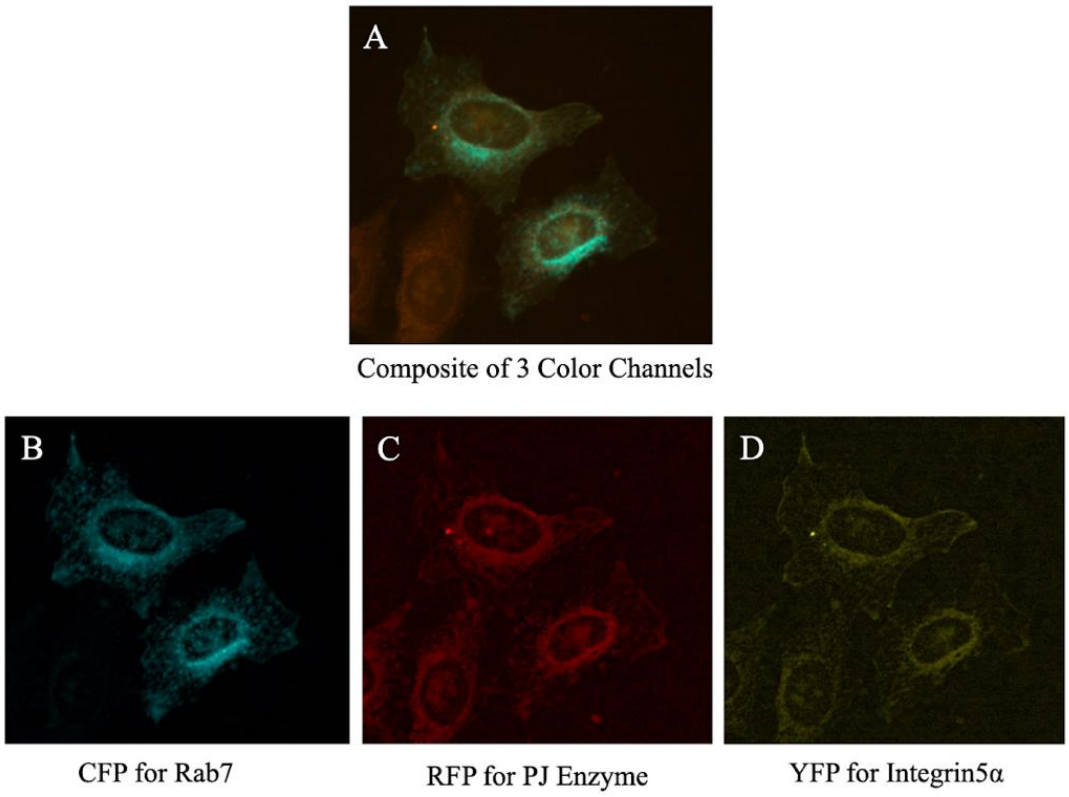


**Figure 13. Dead PJ Enzyme targeted to Rab7 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab7. C: RFP to visualize dead PJ enzyme. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.72 for CFP and RFP channels. Pearson's R value (no threshold) was 0.53 for CFP and YFP channels.
Cell 2: Pearson's R value (no threshold) was 0.56 for CFP and RFP channels. Pearson's R value (no threshold) was 0.43 for CFP and YFP channels.

The difference in means test conducted for Rab7 assays resulted in a p-value of 0.2780 with the difference in average Pearson's Correlation Coefficient values (PJ- Dead PJ) being 0.035 (Table 5). Therefore, there does not appear to be a significant difference in colocalization of integrin and Rab7 when active versus dead PJ enzyme is localized to Rab7 endosomes (Figure 11). Therefore, Rab7 endosomes may not be a key location of PIP2 synthesis or localization

during integrin recycling, as there did not appear to be a difference when PIP2 levels were intentionally decreased as those endosomes.

In comparing representative images chosen for Rab7 assays, qualitative analysis suggests similar results. As seen in Figures 12 and 13, there do not appear to be large differences in the extent of integrin recycling. Integrins appear to move towards the cell periphery to similar extents when both active (Figure 12) and dead PJ (Figure 13) are localized to Rab7 endosomes in the cell.

## Rab5 Results

**Table 6. Rab5 Statistical Analysis**

| | PJ | Dead PJ | **Difference in Means (PJ - Dead PJ)** |
|---|---|---|---|
| Average Pearson's Correlation Coefficient | 0.726 | 0.652 | **0.074** |
| Standard Deviation | 0.151 | 0.138 | **P-value = 0.0091** |
| n (number of cells) | 56 | 53 | |

Average Pearson's Correlation Coefficient values for integrin and Rab5 in cells transfected with active versus dead PJ enzyme. Sample size (n) consists only of cells with Pearson's Correlation Coefficient values above 0.70 for colocalization of PJ/Dead PJ to Rab5.

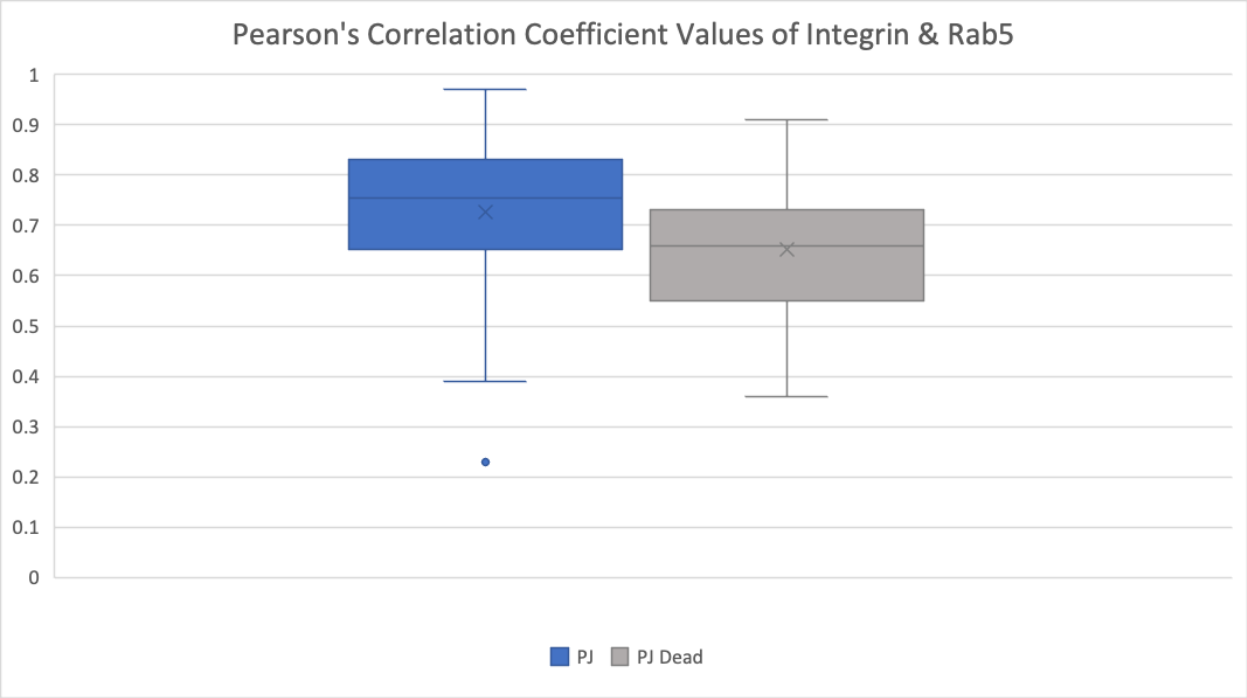


**Figure 14. Comparison of Pearson's Correlation Coefficient Values in cells transfected with PJ versus dead PJ enzyme targeted to Rab5 endosomes.**

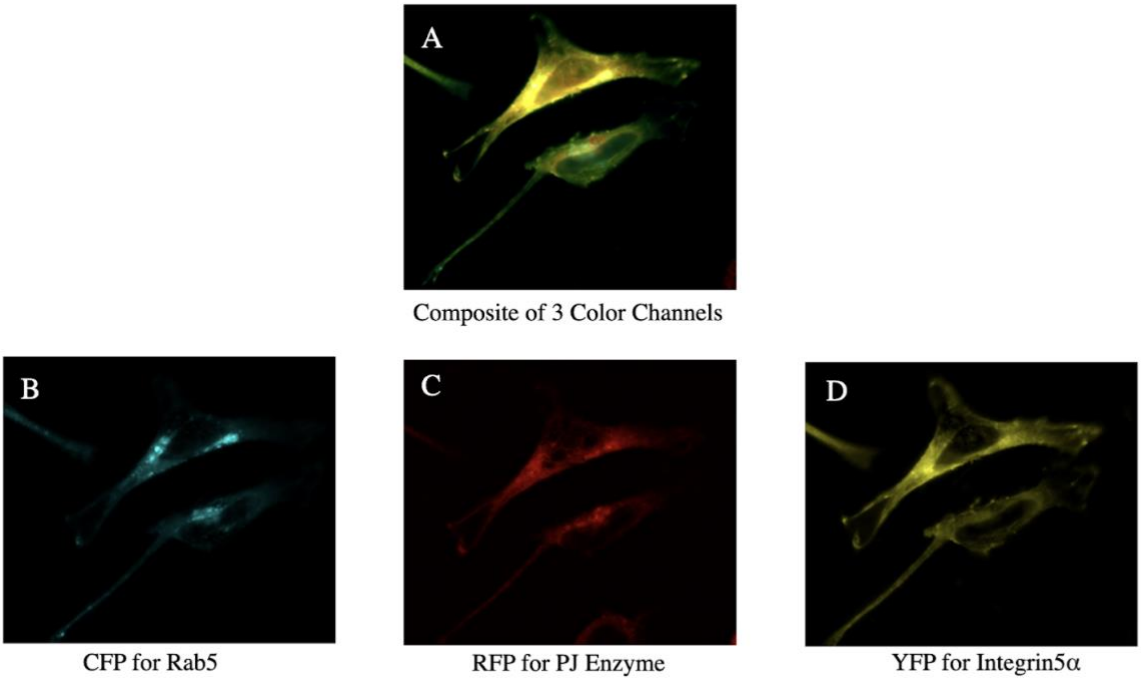


**Figure 15. PJ Enzyme targeted Rab5 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab5. C: RFP to visualize PJ. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.84 for CFP and RFP channels. Pearson's R value (no threshold) was 0.78 for CFP and YFP channels.
Cell 2: Pearson's R value (no threshold) was 0.82 for CFP and RFP channels. Pearson's R value (no threshold) was 0.71 for CFP and YFP channels.

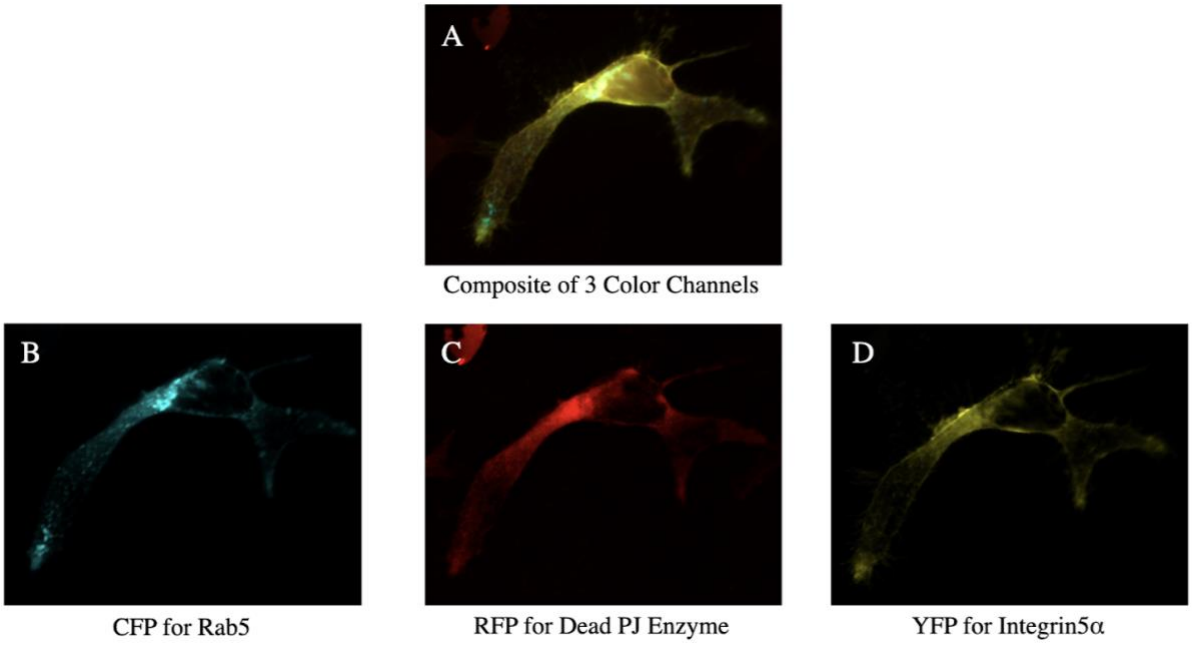


**Figure 16. Dead PJ Enzyme targeted to Rab5 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab5. C: RFP to visualize dead PJ enzyme. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.85 for CFP and RFP channels. Pearson's R value (no threshold) was 0.70 for CFP and YFP channels.

There appeared to be a greater level of colocalization when active PJ was recruited to Rab5 endosomes, compared to the control condition with dead PJ enzyme (Figure 14). The difference in means test done on the Pearson's Correlation Coefficient values (PJ- Dead PJ) of 0.074 resulted in a p-value of 0.0091 (Table 6). These results are statistically significant at an alpha level of 0.05. As there was more colocalization in samples containing active PJ versus dead PJ enzyme localized to Rab5 compartments, this suggests Rab5 endosomes may be important locations of PIP2 synthesis or localization during integrin recycling.

As seen in sample images for Rab5 assays, the extent of integrin recycling may differ in the two conditions. There appeared to be more integrin recycling, seen as yellow fluorescence towards the exterior of the cell, when dead PJ was localized to Rab5 endosomes (Figure 16). In comparison, integrin was more colocalized with Rab5 endosomes when active PJ was targeted to Rab5 endosomes (Figure 15). This suggests that the recycling of integrins back to the cell surface may be lower in cells when PJ is recruited to decrease PIP2 at Rab5 endosomes in the cell.

## Rab8 Results

**Table 7. Rab8 Statistical Analysis**

| | PJ | Dead PJ | **Difference in Means (PJ - Dead PJ)** |
|---|---|---|---|
| Average Pearson's Correlation Coefficient | 0.588 | 0.649 | **-0.062** |
| Standard Deviation | 0.158 | 0.164 | **P-value = 0.0656** |
| n (number of cells) | 45 | 50 | |

Average Pearson's Correlation Coefficient values for integrin and Rab8 in cells transfected with active versus dead PJ enzyme. Sample size (n) consists only of cells with Pearson's Correlation Coefficient values above 0.70 for colocalization of PJ/Dead PJ to Rab8.

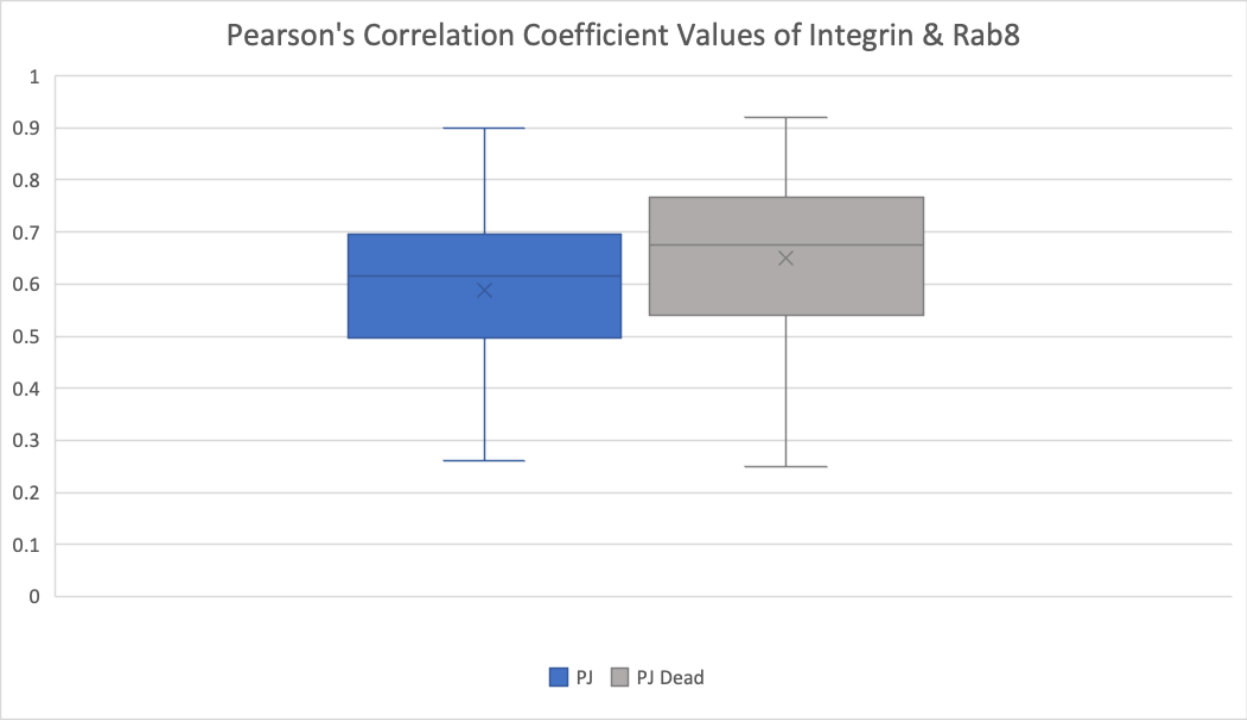


**Figure 17. Comparison of Pearson's Correlation Coefficient Values in cells transfected with PJ versus dead PJ enzyme targeted to Rab8 endosomes.**

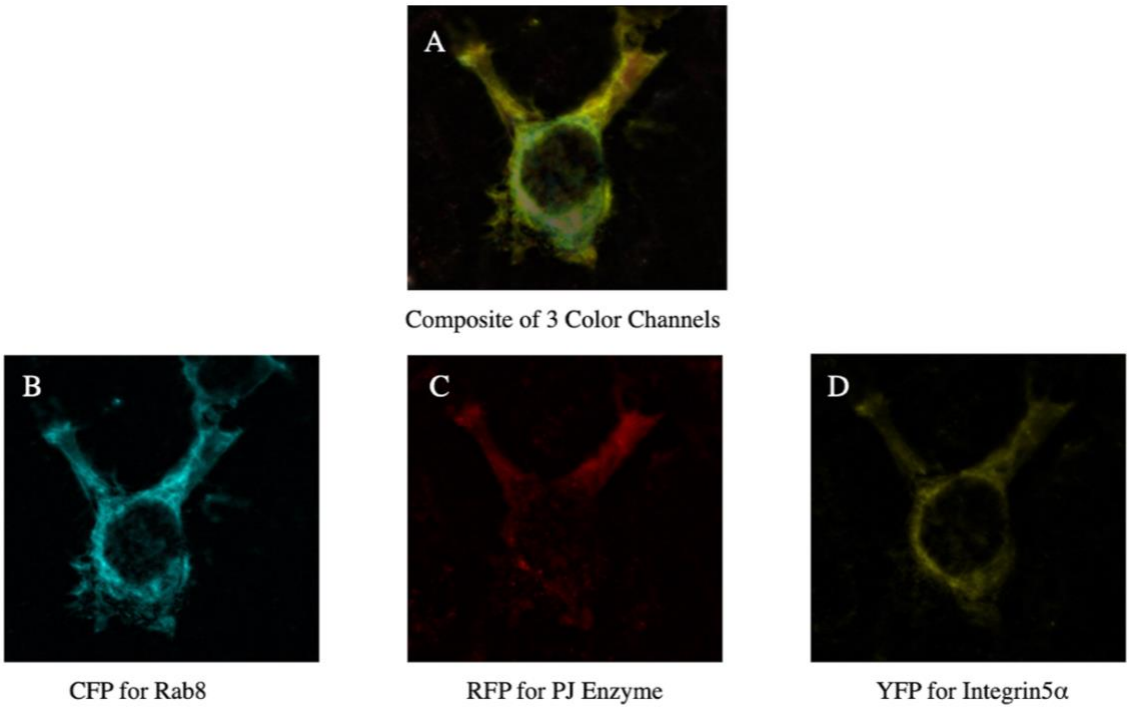


**Figure 18. PJ Enzyme targeted Rab8 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab8. C: RFP to visualize PJ. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.97 for CFP and RFP channels. Pearson's R value (no threshold) was 0.71 for CFP and YFP channels.

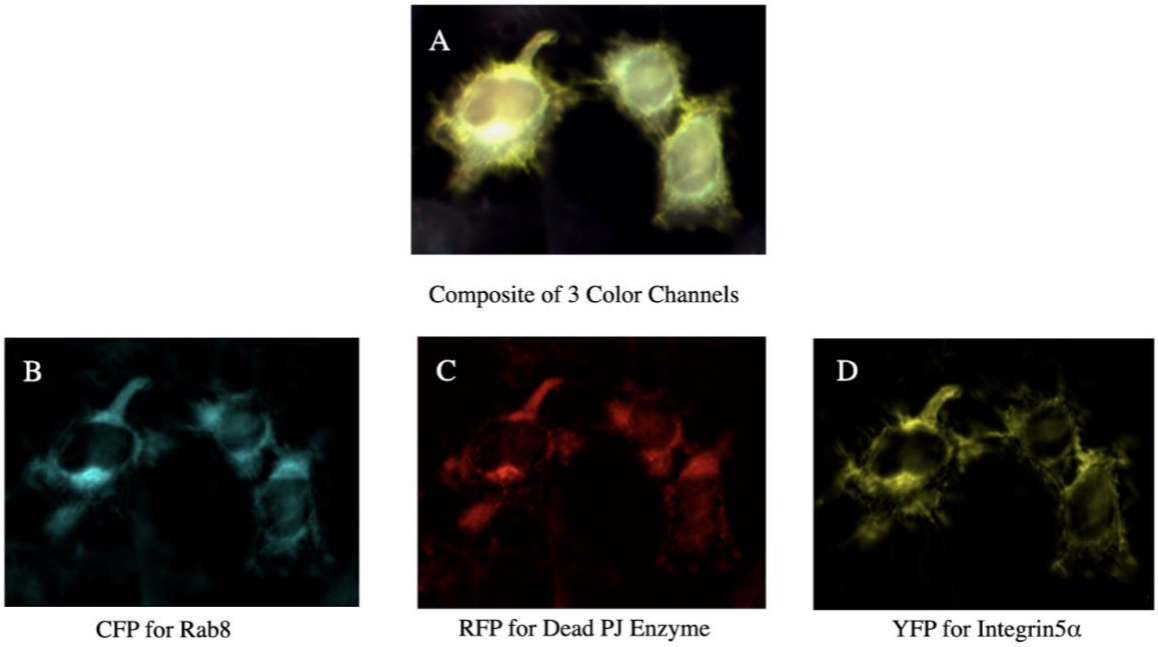


**Figure 19. Dead PJ Enzyme targeted to Rab8 compartments in HeLa cells.**

A: Composite image with CFP, RFP, and YFP channels. B: CFP channel to visualize Rab8. C: RFP to visualize dead PJ enzyme. D: YFP to visualize Integrin5α.
Cell 1: Pearson's R value (no threshold) was 0.86 for CFP and RFP channels. Pearson's R value (no threshold) was 0.83 for CFP and YFP channels.
Cell 2: Pearson's R value (no threshold) was 0.96 for CFP and RFP channels. Pearson's R value (no threshold) was 0.64 for CFP and YFP channels.
Cell 3: Pearson's R value (no threshold) was 0.95 for CFP and RFP channels. Pearson's R value (no threshold) was 0.68 for CFP and YFP channels.

Of the five endosomal locations tested, Rab8 was the only endosome that showed a lower level of colocalization with integrin when PJ was localized to Rab8 endosomes. This would suggest that integrin recycling actually increased when PIP2 levels were decreased at Rab8 endosomes, since less integrin remained colocalized with Rab8 endosomes. However, the difference in means test resulted in a p-value of 0.0656 based on the Pearson's Correlation Coefficient difference (PJ- Dead PJ) of -0.062. At an alpha level of 0.05, the results do not

appear to be statistically significant. Therefore, there is not enough evidence to support whether integrin recycling is impacted when PJ is targeted to Rab8 endosomes specifically. Figures 17 and 18 show sample images of the three fluorescent channels used to analyze colocalization in the Rab8 assays. There is yellow fluorescence for integrin shown towards the cell periphery both when active PJ (Figure 17) and dead PJ enzyme (Figure 18) are targeted to Rab8 endosomes, and differences in the extent of integrin recycling are not visually clear.

# Chapter 4

## Discussion

The aim of the assays conducted was to elucidate which endosomal populations may be key locations of PIP2 synthesis or localization during serum-stimulated integrin recycling. PIP2 levels were intentionally decreased at endosomes marked by EHD1, Rab11, Rab7, Rab5, and Rab8 proteins. Pearson's correlation coefficient values were utilized to analyze colocalization of integrins and endosomes, and difference in means tested indicated whether there were statistically significant differences between experimental (active PJ) and control (dead PJ) assays. The extent of colocalization of integrin with endosomes was used as an indicator of integrin recycling, as increased colocalization in an endosomal compartment could indicate the integrin becomes stuck in that endosome due to interference with the integrin recycling process. When PIP2 levels were decreased at EHD1 and Rab5 endosomes, colocalization increased and integrin recycling appeared to decrease. On the other hand, there did not appear to be a significant difference in integrin recycling when the PJ enzyme was targeted to Rab11, Rab8, and Rab7 endosomal locations in the cell.

The five endosomal locations tested in these assays are classified as different types of endosomes in the cell. Rab5 is a marker of early endosomes (Zerial & McBride, 2001). Rab7 is a marker of late endosomes (Shearer & Petersen, 2019). EHD1 (Naslavsky & Caplan, 2011), Rab8 (Henry & Sheff, 2008), and Rab11 (Martinez-Arroyo *et al*., 2021) are markers of recycling endosomes. The endosome type and its typical functions in the endosomal system may provide insight to explain the results of the assays, particularly in studying the impact of PIP2 at those locations during integrin recycling.

Rab5 is a marker of early endosomes in the cell, meaning internalized integrins may first reach Rab5-marked endosomes. Rab5 is known to be an activator of cell migration with its role in the internalization of β1 integrins through endocytosis (Paul *et al.*, 2015), but the *mechanism* behind this is largely unknown (Mendoza *et al.*, 2013). Since results of the assays suggest Rab5 endosomes are an important location of PIP2 during integrin recycling, this could be further probed to study how proteins recruited by PIP2 to Rab5-marked early endosomes could function in integrin recycling.

Given that Rab7 is a marker of late endosomes, the results of the associated assays align with the expected functions of those endosomal compartments. Rab7 is known to assist in the maturation of early endosomes into late endosomes (Girard *et al.*, 2014), as well as the downstream trafficking of late endosomes to lysosomes (Guerra & Bucci, 2016). Integrins moved into Rab7-marked late endosomes might not be intended for recycling back to the cell surface, as late endosomes typically do not connect with recycling pathways in the cell. Therefore, it is not surprising that integrins do not become stuck in Rab7 endosomes when decreasing PIP2 at those locations. While results of the assays do not conclude that integrins are not recycled through Rab7 endosomes, they may suggest that PIP2 synthesis and localization may not be the factor regulating this process at Rab7 endosomes.

EHD1 is a protein marker of recycling endosomes, which integrins are known to be recycled through. Studies have shown that PIP2 effectors commonly localize to EHD1 endosomes to regulate endosome sorting (Tan, *et al.*, 2015). Since PIP2 effector proteins are found at EHD1 endosomes, it suggests PIP2 may also be localized to those endosomes. This aligns with the results of the assays indicating the importance of PIP2 at EHD1 during integrin

recycling and suggests that PIP2 may be important for recruiting additional proteins needed for regulation of integrin trafficking.

Rab8, another protein marker of recycling endosomes, did not appear to be a significant location of PIP2 localization during integrin recycling. Research suggests Rab8 endosomes are critical to trafficking of newly synthesized proteins to the plasma membrane, rather than transport of endocytosed materials (Henry & Sheff, 2008). This is a potential explanation for the results seen in the assays, as it did not appear that PIP2 needs to be synthesized or localized to Rab8 endosomes to facilitate integrin recycling.

Rab11 is also a marker of recycling endosomes but is instead known to be involved in the “long” recycling pathway of the endosomal system. While results of the assays did not show that Rab11 endosomes may be key locations of PIP2 synthesis during integrin recycling, integrins could still be recycled through Rab11 endosomes. A potential explanation is that PIP2 instead plays a larger role in the “short” recycling pathway for trafficking integrins, and therefore may not localize to recycling endosomes like Rab11 involved in the “long” pathway of recycling.

Although EHD1, Rab8, and Rab11 are all markers of recycling endosomes, results of this study showed only EHD1 as a key location of PIP2 synthesis and localization during integrin recycling. Since PIP5 kinases must first be recruited to EHD1 endosomes to synthesize PIP2, it could be hypothesized that this is facilitated by proteins binding to the EH domain of EHD1. Studies have shown that the EH domain binds protein domains such as the asparagine-proline-phenylalanine (NPF) motif and the aspartate-proline-phenylalanine (DPF) motif (Kieken *et al.*, 2007). Therefore, this EH domain could be a reason why PIP2 is synthesized at EHD1

endosomes during integrin recycling, but not at other recycling endosomes like Rab8 and Rab11. Furthermore, the importance of PIP2 at EHD1 endosomes during integrin recycling may be due to its role in recruiting proteins that are needed in the recycling process. Such proteins may contain PIP2-binding domains, and the proteins recruited to EHD1 endosomes may further regulate the recycling of integrins through the endosomal system. For instance, cytohesin-2, a protein known to promote β1 integrin recycling, is known to bind PIP2 (Oh & Santy, 2012). Therefore, further investigating the downstream proteins that bind PIP2 may provide additional insight into the mechanisms and importance behind PIP2 synthesis and localization at EHD1 compartments, specifically.

Although these assays did not show Rab11, Rab7, and Rab8 endosomal locations as key locations of PIP2 localization during integrin recycling, they should not be disregarded completely as limitations in the assays could have impacted the final data collection and analysis. Increasing sample size could improve the ability to test the hypothesis and ensure variability in the data set was not the reason the statistical tests resulted as they did. It is still possible those endosomal locations are key locations for PIP2 synthesis or localization during integrin recycling, but these experimental assays did not show that statistically.

Overall, implications of this study could allow for more targeted approaches in future experiments by knowing that EHD1 and Rab5 endosomes may be key locations of PIP2 synthesis or localization during integrin recycling (Figure 20). Given this result, it could be hypothesized that these are two locations where PIP5 kinases are recruited to synthesize PIP2. Downstream of PIP2 synthesis, additional proteins necessary for integrin recycling could be recruited through binding PIP2. Further investigation could be useful to distinguish between the

roles of PIP2 at different types of endosomes, and whether PIP2 recruits different effector proteins to those locations.

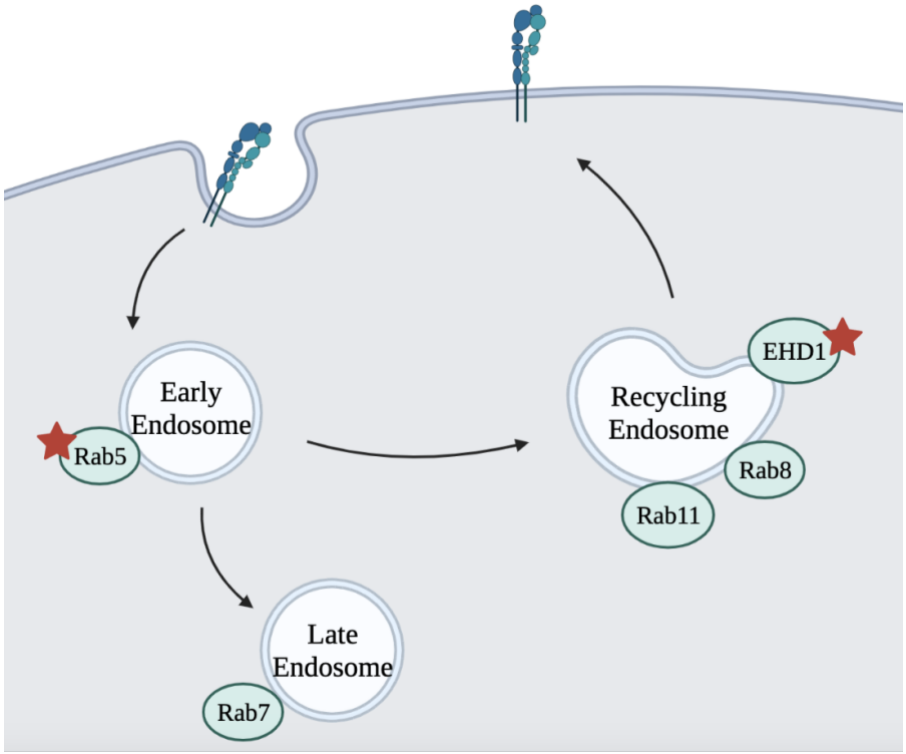


**Figure 20. Potential locations of PIP2 synthesis or localization during integrin recycling, on Rab5 and EHD1 compartments in the endosomal system.**

Image created using BioRender.com.

A limitation in this study was the ability to fully localize the PJ enzyme to the endosomes of interest. Although utilizing rapamycin to link PJ to specific endosomes was successful overall, the efficiency of this process could be further optimized. Pearson's Correlation Coefficient values of 0.70 or greater were utilized as the threshold for “sufficient” localization of PJ to the endosomes of interest, based on approximately half of the successfully transfected cells having achieved this level of colocalization. Therefore, a significant portion of the images collected for

the data set were omitted from final colocalization analysis due to insufficient colocalization of the PJ (or dead PJ) enzyme to the endosome of interest. Utilizing another method to ensure proper localization of PJ to endosomes could improve statistical measures since a larger data set could be utilized in data analysis. In addition, using an even more effective method for targeting PJ to endosomes could improve the accuracy of the results, since there was potential that the PJ enzyme decreased PIP2 levels at locations other than the intended endosome being tested.

Future research could build on the results of this study to probe EHD1 and Rab5 endosomes as potential locations where PIP5 kinases synthesize PIP2 when integrin recycling is initiated. Therefore, it may be insightful to investigate the locations of different PIP5 kinase isoforms, to study which isoforms synthesize PIP2 during integrin recycling. Used in conjunction with these assays probing PIP2 localization, this could provide a more complete understanding of the phospholipid and protein players involved in the integrin recycling process.

Overall, PIP2 plays significant roles in intracellular trafficking and recruitment of various effector proteins, such as the protein cytohesin-2 that regulates β1 integrin recycling. Elucidating the locations where PIP2 is synthesized and localized during integrin recycling can provide insight on how it recruits cytohesin-2 to regulate β1 integrin recycling at specific endosomes (Oh, 2011). Given the significance of integrin recycling as a mechanism behind epithelial cell migration, findings can potentially translate into better understandings of cancer metastasis.

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**ACADEMIC VITA**

# Ellen T. Lee

## EDUCATION

**The Pennsylvania State University** University Park, PA
Eberly College of Science, Schreyer Honors College
Bachelor of Science in Biochemistry & Molecular Biology

## RESEARCH EXPERIENCE

**Penn State Eberly College of Science** University Park, PA
*Undergraduate Researcher* in The Santy Lab *Aug 2020 – May 2023*

- Probe the co-localization of Phosphatidylinositol 4,5-bisphosphate (PIP2) and endosomes to study PIP2 synthesis and localization during serum-stimulated integrin recycling
- Perform assays involving HeLa and MCF-7 cell cultures, immunofluorescence staining, fluorescence microscopy, DNA transfection, and other molecular biology lab techniques

**The Cleveland Clinic** Cleveland, Ohio
*Research Intern* in The Lang Lab, Lerner Research Institute *Jun 2021 - Aug 2021*

- Performed literature reviews on lymphedema for a clinical trial proposal
- Compiled references and contributed to drafts for the proposed study protocol

*Clinical Research Student* in The Lou Ruvo Center for Brain Health

- Transferred documentation and information from patient files for clinical studies
- Attended appointments for Alzheimer's Disease clinical trials involving MRI scans and neuropsychological testing

**Penn State College of Health and Human Development** University Park, PA
*Research Fellow* in the Sleep Physiology Research Lab *Oct 2018 - June 2019*

- Organized files for entering patient data in Excel and logged collected specimens
- Cleaned and configured testing devices, organized biological specimens, and prepared materials for future studies

## ADDITIONAL EXPERIENCES/ACTIVITIES

**The Village at Penn State** State College, PA
*Student Volunteer* *Jun 2018 – Present*

- Lead activities such as arts and crafts, outdoor games, and interactive presentations
- Develop relationships with residents to provide them company and emotional support
- During the current COVID-19 pandemic, helped coordinate a "Pen-Pal" program for residents to interact with Penn State students through virtual modes of communication

**Student Anti-Hunger Program** University Park, PA
*Founding Secretary* *August 2021 – Present*

- Work with executive board members to implement the club's new constitution and goals
- Email club members about upcoming volunteer opportunities and help track participation
- Help spread awareness of the club, its initiatives, and food insecurity in the community

**Phi Epsilon Kappa Pre-Health Fraternity** University Park, PA
*Member | Service Chair 2021 & 2022* *Jan 2020 – Present*

- Participate in various activities with fellow pre-health students to enhance our preparations for entering the healthcare field, such as guest speaker talks and resume workshop sessions
- Attended weekly meetings to become inducted into the first pre-health fraternity at PSU
- As the Service Chair on the executive board, created community service events for the members, coordinated and organized the logistics of each event, and tracked attendance

**Alpha Epsilon Delta Pre-Health Honors Society** University Park, PA
*Member | THON 2020 Sub-Chair* *Sept 2019 - May 2022*

- Participated in informational meetings, speaker series, and career exploration events
- Received mentorship from a medical school student through the Penn State College of Medicine BRIDGE program
- Planned engagement activities and fundraising events as the THON 2020 Sub-Chair

## WORK EXPERIENCE

**UpGrade Tutoring** University Park, PA
*Staff Tutor* *Jan 2022- Present*

- Meet with students for tutoring in general chemistry, chemistry lab, and statistics
- Emphasize understanding of foundational concepts to improve problem-solving
- Foster the development of constructive learning habits for student success in future coursework

**University of Pittsburgh Medical Center (UPMC) Altoona** Altoona, PA
*Patient Care Technician I* in the Medical Progressive Care Unit *Aug 2022 – Jan 2023*

- Assisted patients with activities of daily living: toileting, ambulating, bathing, feeding
- Measured vital signs and blood glucose levels, set up cardiac telemetry and 12-lead EKGs
- Communicated with other healthcare providers to manage and distribute patient care

*Care Attendant* in the Resource Unit *Jan 2023 – Present*

- Ensure patient safety by providing continuous observation of assigned patients
- Utilize techniques for de-escalation such as verbal redirection of patients and alerting additional staff as needed

## HONORS & AWARDS

John Van Valzah Foster, Jr. M.D. and Carolyn Manifold Foster Memorial Scholarship Aug 2022
Jacqueline Hemming Whitfield Student Research Endowment May 2022
Evan Pugh Scholar Senior Award Feb 2022
Evan Pugh Scholar Junior Award Feb 2021
The President's Freshman Award Mar 2020