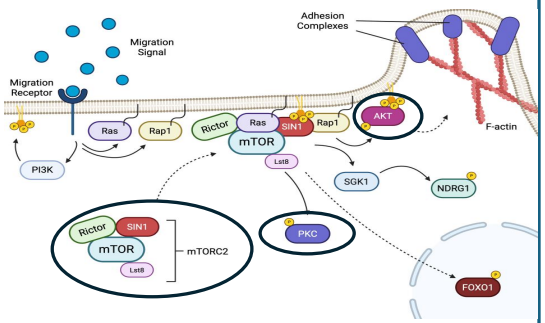
Investigating Chemoattractant-Specific Temporal Activation of mTORC2–PKC Signaling Pathways in Human Breast Epithelial Cells

Research Questions

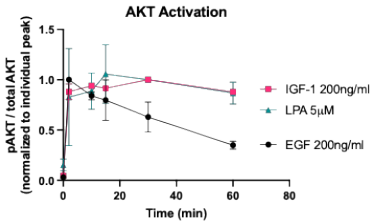
1. Do different chemoattractants activate mTORC2 with distinct timing patterns?
2. Does mTORC2 activate different downstream PKC proteins depending on the stimulus?



mTORC2–PKC Signaling Schematic

Key Findings / Results

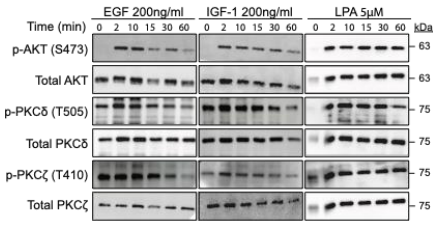
- All chemoattractants activated mTORC2 (via AKT S473 phosphorylation)
- EGF induced rapid, transient AKT phosphorylation
- IGF-1 and LPA induced sustained AKT phosphorylation
- IGF-1 produced early PKCδ phosphorylation
- EGF induced PKCζ phosphorylation
- LPA exhibited minimal PKCδ and PKCζ engagement



AKT (S473) phosphorylation time interval

Methodology

- MCF10A human breast epithelial cells
- Stimulated with EGF (200 ng/mL), IGF-1 (200 ng/mL), and LPA (5 μM)
- Data collection in the span of 0–60 minutes
- Western blot analysis
- Measured: p-AKT (S473) as a readout of mTORC2 activity, p-PKCδ (T505), p-PKCζ (T410)
- Band quantification normalized to total protein



Representative Western Blots

Interpretation & Conclusion

1. mTORC2 activation timing differs depending on the chemoattractant.
2. Different PKC proteins are activated depending on the stimulus.
3. The timing of activation, not just signal strength, varies by stimulus.
4. mTORC2 uses timing differences to help distinguish extracellular signals.

Figures by Isaiah Toth and the student researcher using BioRender (2025/26) based on results by the student researcher.

Introduction

Why Cell Migration Signaling Matters

- Cells migrate in response to chemical cues (chemoattractants).
- Migration (chemotaxis) is essential for survival processes such as finding nutrients, immune defense, development, and wound healing.
- **mTORC2** (mechanistic Target Of Rapamycin Complex 2) is a signaling complex that regulates cell movement by affecting the cytoskeleton [1].
- It helps cells organize their internal structure and move in a directed way by activating downstream key proteins needed for cell motility [4].
- It regulates migration-related signaling through AKT and PKC pathways.
- Errors in migration signaling can lead to disease, including chronic inflammation and tumor spread (metastasis).

It remains unclear whether mTORC2 encodes chemoattractant identity through stimulus-specific temporal signaling dynamics.

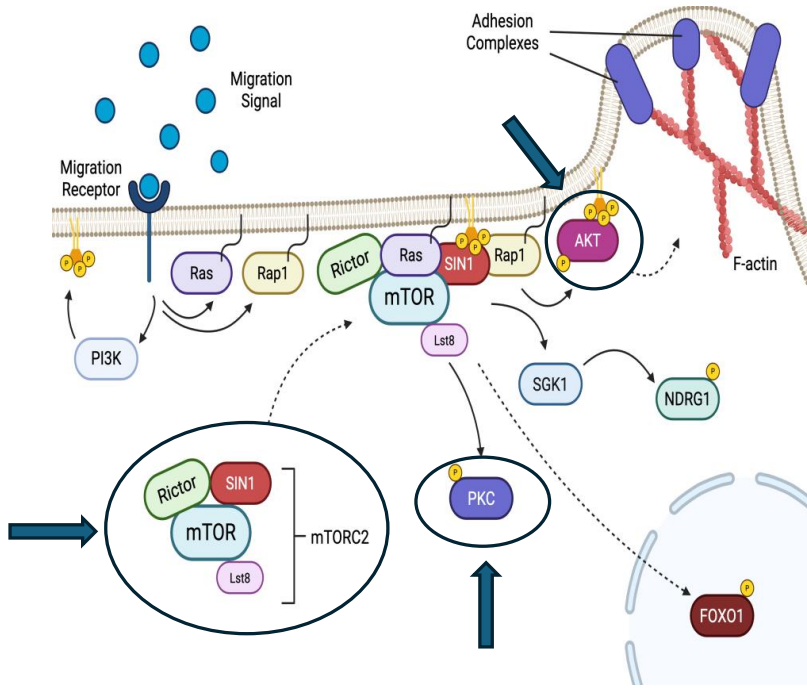


Figure 1. mTORC2 regulates cell migration through multiple downstream effectors, including AKT and PKC pathways.
Figure by Isaiah Toth and the student researcher using BioRender (2025/26).

Introduction

mTORC2 Signaling and the Knowledge Gap

- mTORC2 activity is commonly measured by phosphorylation (activation) of AKT at S473, a chemical modification that “turns on” proteins involved in cell migration.
- However, measuring AKT alone does not fully explain how mTORC2 signaling differs between stimuli.
- mTORC2 through AKT activates additional downstream proteins, including members of the PKC family, such as PKCδ (Delta) and PKCζ (Zeta), which help regulate cytoskeletal organization and cell movement.
- This project focuses specifically on PKCδ and PKCζ to examine whether different chemoattractants activate different members of this family over time.
- Different chemoattractants are known to activate distinct downstream targets, but only a limited number of these targets have been examined.
- It remains unclear whether different chemoattractants activate different downstream proteins in a time-dependent manner.

To address this gap, this study tests whether chemoattractant identity shapes the timing of mTORC2 activation and leads to activation of different downstream PKC proteins.

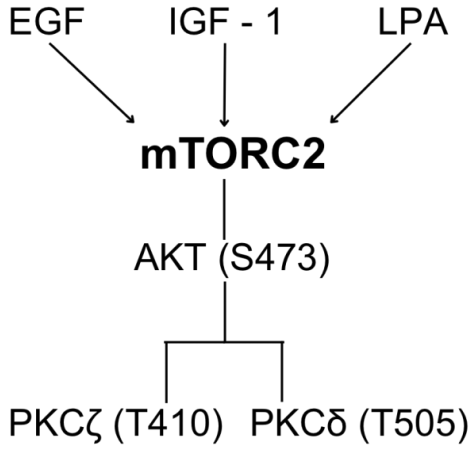


Figure 2. Simplified signaling framework tested in this study. Figure by the student researcher using Canva (2026).

Research Question and Prediction

Temporal Encoding of Chemoattractant Identity

Research Questions

1. Do different chemoattractants activate mTORC2 with distinct timing patterns?
2. Does mTORC2 activate different downstream PKC proteins depending on the stimulus?

Predictions

- Different chemoattractants will produce unique time-dependent patterns of AKT (S473) phosphorylation.
- Differences in activation timing (not just signal strength) will distinguish between downstream activation of different PKC proteins.
- If activation timing matters, changes over time may help distinguish how cells respond to different chemoattractants.

Investigative Methods

Chemoattractant Stimulation and Western Blot Analysis

Model System: MCF10A human breast epithelial cells

Time Interval: 0–60 minute stimulation

Independent Variable: Chemoattractant identity
(EGF (Epidermal Growth Factor) 200 ng/mL; IGF-1 (Insulin-like Growth Factor) 200 ng/mL; LPA (Lysophosphatidic Acid) 5 μM)

Dependent Variables
p-AKT (S473): indicator of mTORC2 activation;
p-PKCδ (T505) and p-PKCζ (T410): downstream signaling proteins involved in cell movement

Controls: Total AKT and total PKC (to ensure equal protein loading)

Procedure
- MCF10A cells stimulated with each chemoattractant
- Cells collected at multiple time points (0, 2, 10, 15, 30, 60 minutes)
- Proteins separated and transferred to membranes (Western blot)
- Activated (phosphorylated) proteins detected using antibodies
- Band intensity measured and normalized to total protein
- Biological replication: AKT (N=2); PKCδ and PKCζ (N=1)

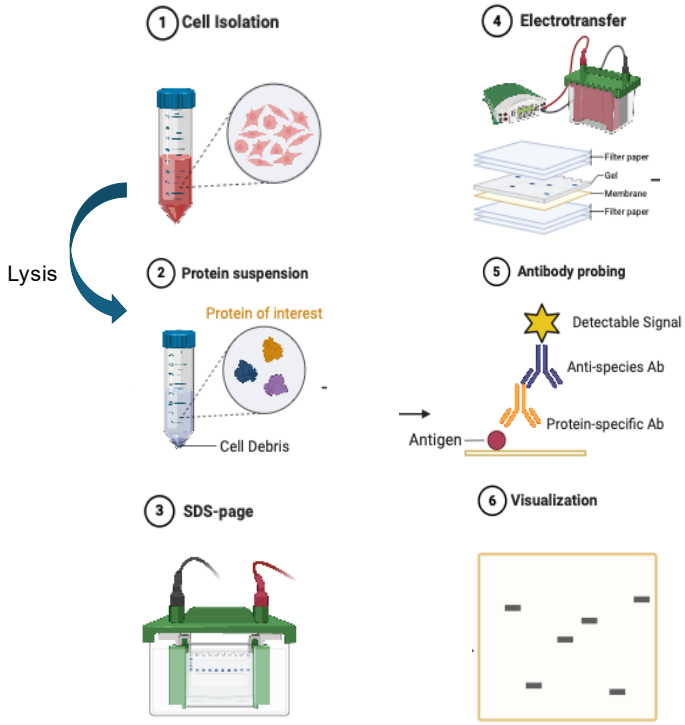


Figure 3. Experimental workflow used to assess mTORC2 pathway activation following chemoattractant stimulation.
Figure by Isaiah Toth using BioRender (2025) based on methods used by the student researcher.

Results

mTORC2 Activation Over Time (AKT S473 Phosphorylation)

- Image shows representative Western blots used for data analysis and imaged on a chemiluminescent detection system.
- Phosphorylation of AKT (S473) was used as a readout of mTORC2 activation.
- Phosphorylation of PKCδ (T505) and PKCζ (T410) was examined as downstream targets.
- EGF produced a rapid, transient increase in AKT phosphorylation (*Figure 4: top 2 rows, left*).
- IGF-1 and LPA produced more sustained activation patterns (*Figure 4: top 2 rows, middle and right*).

Key Result:

The blots give an overall visual picture of protein activation. To precisely compare differences between conditions, band intensity was measured and normalized to total protein.

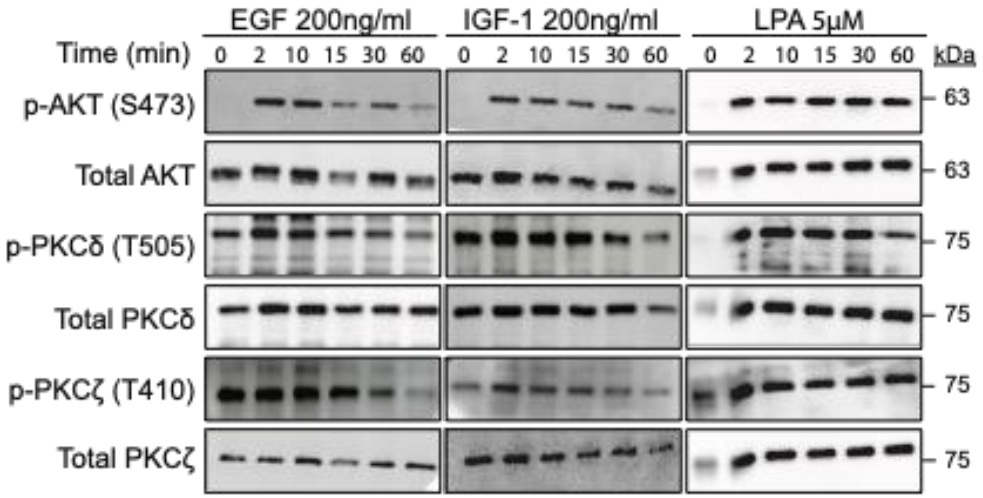


Figure 4. Western blot analysis of mTORC2 pathway activation over time following EGF, IGF-1, or LPA stimulation (pAKT S473).
Figure by Isaiah Toth using BioRender (2025) based on findings by the student researcher.

Results

mTORC2 Activation Over Time Reveals Stimulus-Specific AKT Dynamics

- p-AKT (S473) is used as the readout for mTORC2 activity
- EGF triggers a rapid, transient p-AKT response (early peak, then declines)
- IGF-1 and LPA produce more sustained pAKT activation, with IGF-1 showing the strongest persistence

Key Result:

Quantitative analysis confirms stimulus-specific temporal mTORC2 activation kinetics, with EGF exhibiting transient dynamics and IGF-1 demonstrating sustained signaling.

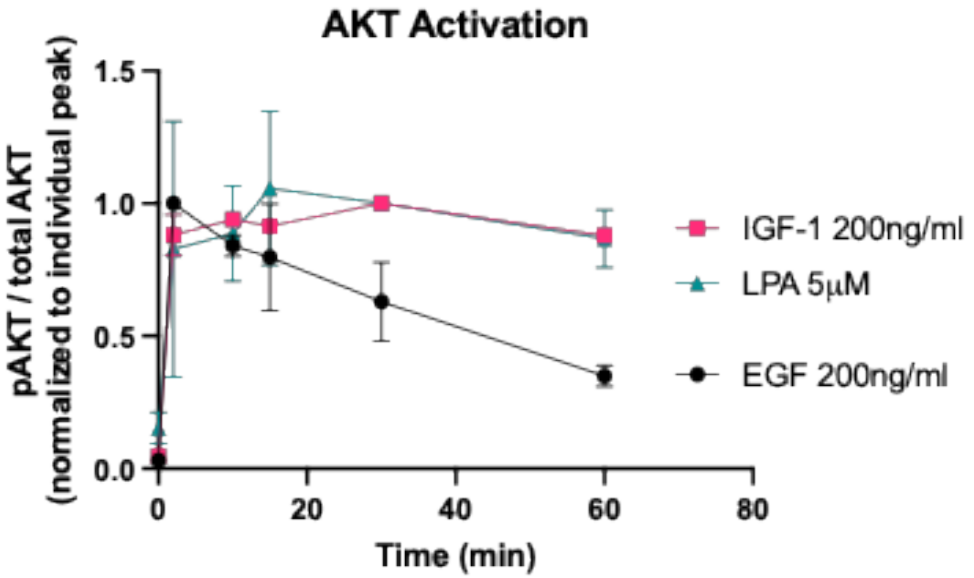


Figure 5. Quantification of AKT (S473) phosphorylation over time following EGF, IGF-1, or LPA stimulation. Figure by Isaiah Toth using BioRender (2025) based on findings by student researcher.

Results

PKCδ Phosphorylation Is Chemoattractant-Specific

- Downstream mTORC2 signaling was examined by measuring PKCδ phosphorylation (T505)
- IGF-1 stimulation produced a rapid, early peak in p-PKCδ (~2 minutes)
- EGF and LPA showed early and rapid PKCδ phosphorylation by 2 minutes but lacked a distinct activation peak as the decay was slow and remained elevated for the full 60 minutes

Key Result:

Although all stimuli activate mTORC2, only IGF-1 produces a rapid, short-lived PKCδ phosphorylation peak, indicating stimulus-specific downstream routing rather than uniform pathway activation.

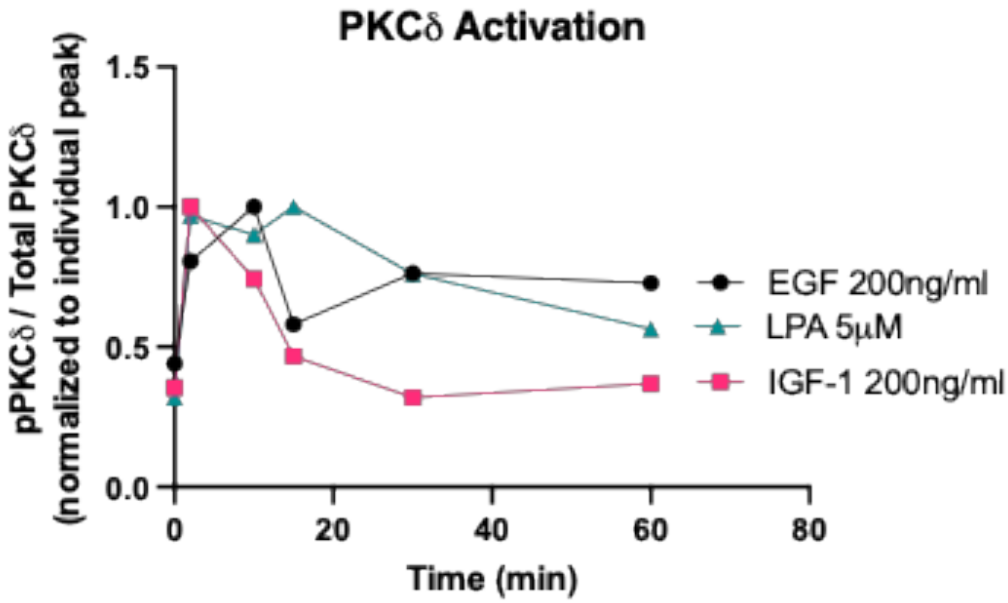


Figure 6. Quantification of PKCδ (T505) phosphorylation over time following EGF, IGF-1, or LPA stimulation. Figure by Isaiah Toth using BioRender (2025) based on findings by student researcher.

Results

PKCζ Displays Distinct Temporal Regulation

- PKCζ phosphorylation (T410) was measured as an additional downstream readout
- EGF stimulation caused a delayed response in p-PKCζ for the first 10 minutes, followed by a rapid rise to peak at 15 minutes and a decline afterwards
- p-PKCζ levels following EGF stimulation fell below the initial (0-minute) level at later time points
- IGF-1 produced a small peak in p-PKCζ at 2 minutes that declined almost as rapidly before returning to initial (0-minute) level by 30 minutes
- LPA generated maximum phosphorylation of PKCζ at the 0-minute. The level dropped rapidly to below half the maximum by 2 minutes, reached a minimum at 30 minutes before a slow rise to half of maximum by 60 minutes.
- PKCζ dephosphorylates in response to LPA activation of mTORC2.

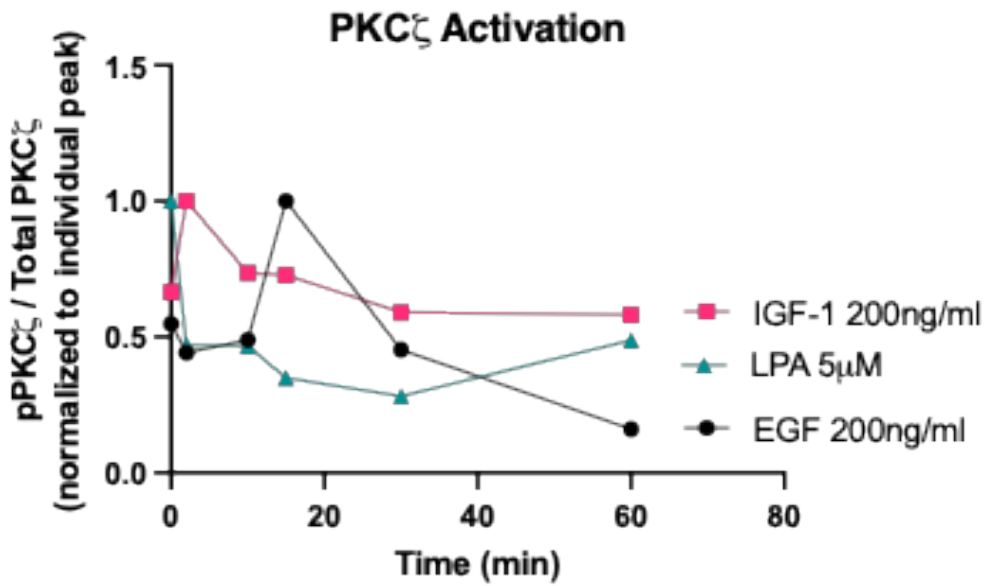


Figure 7. PKCζ (T410) phosphorylation dynamics following EGF, IGF-1, or LPA stimulation over time. Figure by Isaiah Toth using BioRender (2025) based on findings by student researcher.

Key Result: Together with PKCδ results, these data demonstrate that chemoattractants do not merely alter signaling magnitude, but selectively route mTORC2 output through distinct PKC pathways in a timing-dependent manner.

Discussion

Key Insight 1: Temporal Activation Patterns

Interpretation of Findings

- Time-sampled Western blot analysis with normalization to total protein revealed stimulus-specific activation profiles.
- All three chemoattractants activated mTORC2, but with distinct temporal dynamics.
- EGF induced rapid, transient AKT activation with PKCζ engagement.
- IGF-1 produced sustained AKT activation with early PKCδ phosphorylation.
- LPA generated sustained mTORC2 activation with minimal PKC engagement.

If signaling depended only on pathway identity

- All stimuli would activate mTORC2 in the same way. AKT timing and duration would be similar.
- PKCδ and PKCζ activation patterns would overlap.Graph curves would look nearly identical.
- In that model, mTORC2 would function as a simple ON/OFF switch.

Connection to Research Question 1

- Chemoattractants generate distinct temporal patterns of mTORC2 activation.
- mTORC2 activation kinetics vary depending on the extracellular stimulus.

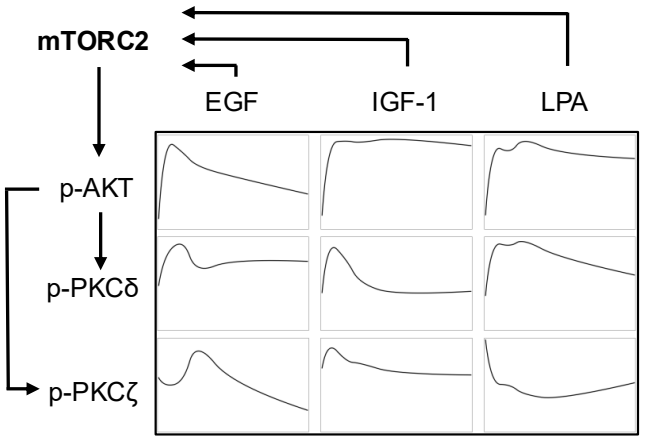


Figure 8. How timing allows cells to distinguish chemoattractants.
Different stimuli activate the same mTORC2 pathway, but with distinct timing patterns. These temporal differences influence which downstream PKC proteins are engaged, allowing cells to generate stimulus-specific responses.
Figure by the student researcher using Mathematica (2026).

Discussion

Key Insight 2: Stimulus-Dependent PKC Pathway Engagement

Interpretation of Findings

- Chemoattractant identity changes the response over time of mTORC2 activation
- Different response patterns bias signaling toward specific PKC pathways
- EGF favors PKCζ engagement
- IGF-1 favors PKCδ engagement
- LPA induces an elevated and sustained phosphorylation response in AKT and PKCδ. It dephosphorylates PKCζ

Connection to Research Question 2

- These results suggest that mTORC2 does not activate all PKC proteins equally.
- mTORC2 timing influences which downstream PKC pathway is engaged.

Together, the findings support a model in which cells distinguish chemoattractants not only by whether mTORC2 is activated, but by differences in activation timing and downstream protein engagement.

Conclusion

Temporal Dynamics and Selective PKC Engagement in mTORC2 Signaling

1. All tested chemoattractants (EGF, IGF-1, LPA) activate mTORC2, but with stimulus-specific temporal activation patterns.

2. Activation timing influences downstream PKC pathway engagement.

3. Identical signaling pathways can produce distinct cellular outcomes through timing-dependent pathway selection.

mTORC2 does not simply turn on or off; its activation is dynamic and time-dependent.

The duration and shape of the response may influence downstream signaling and migration-related behaviors relevant to wound healing, immune responses, and cancer progression.

Implications

Broader Significance of Temporal Signaling

What This Study Shows

- The same signaling pathway, when activated, produces different response patterns depending on the stimulus.
- Cells may use timing (not just signal strength) to decide how to behave and to interpret their environment
- These differences could influence how cells organize their cystoskeleton and move.

Cell movement is critical for:

- Immune cells reaching infection sites
- Wound healing
- Embryonic development
- Cancer metastasis

If signal timing changes how cells interpret external cues, then these differences could affect how persistently or aggressively cells migrate.

Limitations and Future Research

Evaluating Scope and Next Steps

Limitations

- Conclusions are based on phosphorylation dynamics rather than direct migration assays.
- mTORC2 activity was assessed using AKT (S473) as a proxy readout.
- Downstream analysis focused on PKCδ and PKCζ; other pathways were not examined.
- Experiments were performed in the MCF10A model under defined conditions.
- Biological replication was limited due to time and access constraints.

Future Research

- Perform functional migration assays to directly link signaling timing with cellular behavior.
- Use targeted perturbations (PKCδ or PKCζ inhibition or overexpression) to test causal pathway routing.
- Increase biological replication and refine temporal resolution.
- Test whether temporal encoding extends to additional chemoattractants or cell types.

References

1. Liu, P., Gan, W., & Chin-Sang, I. (2014). mTORC2 and cancer cell migration. *Journal of Molecular Signaling*, 9(1), 1-7
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4. Smith SF, Collins SE, Charest PG. Ras, PI3K and mTORC2 - three's a crowd? J Cell Sci. 2020 Oct 8;133(19):jcs234930. doi: 10.1242/jcs.234930. PMID: 33033115; PMCID: PMC7561475.