# Beta-Arrestin mediated chemotaxis

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Dr. Jin Zhang

# Outline

- Introduction
- Chemotactic system
  - Molecules involved in the beta-arrestin-mediated chemotaxis
  - Amplification of external gradient
  - Beta-arrestins as scaffold
  - Artifical chemotactic systems?
  - Vesicle trafficking is crucial
- Mathematical modeling
- Conclusions

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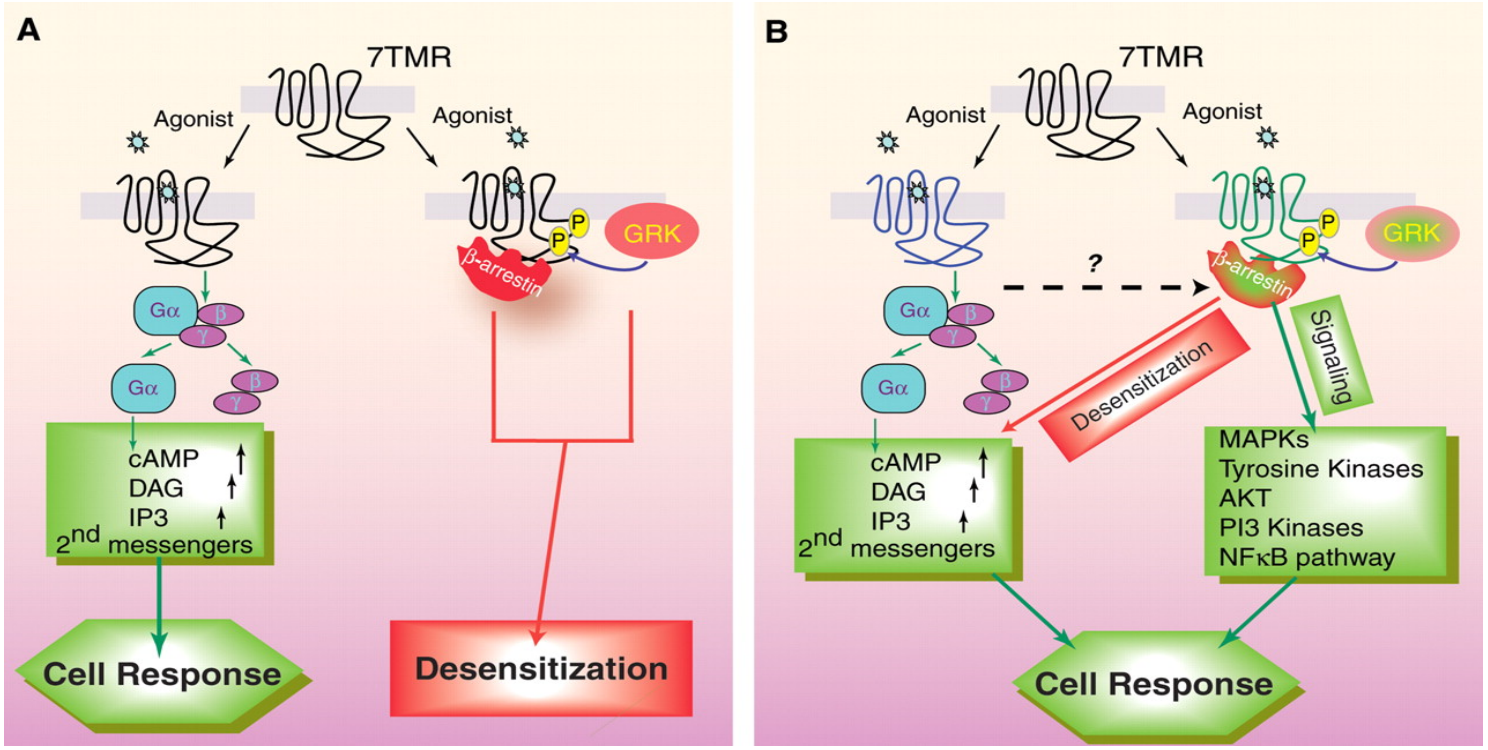
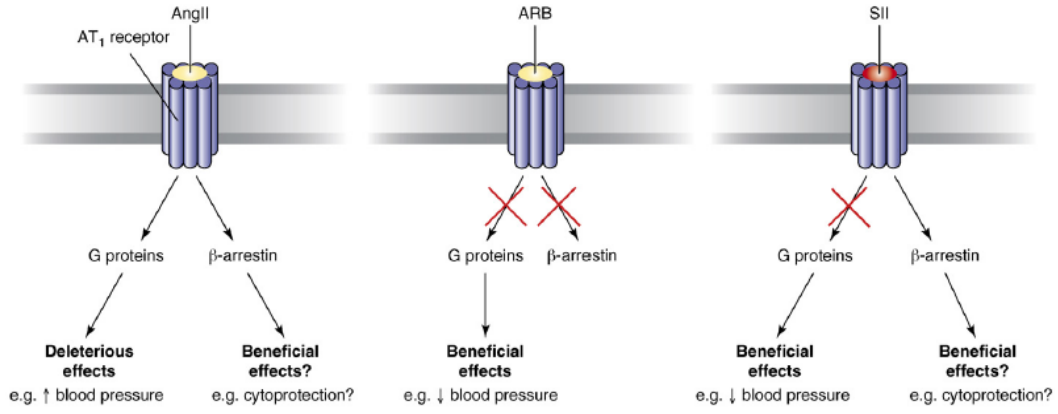
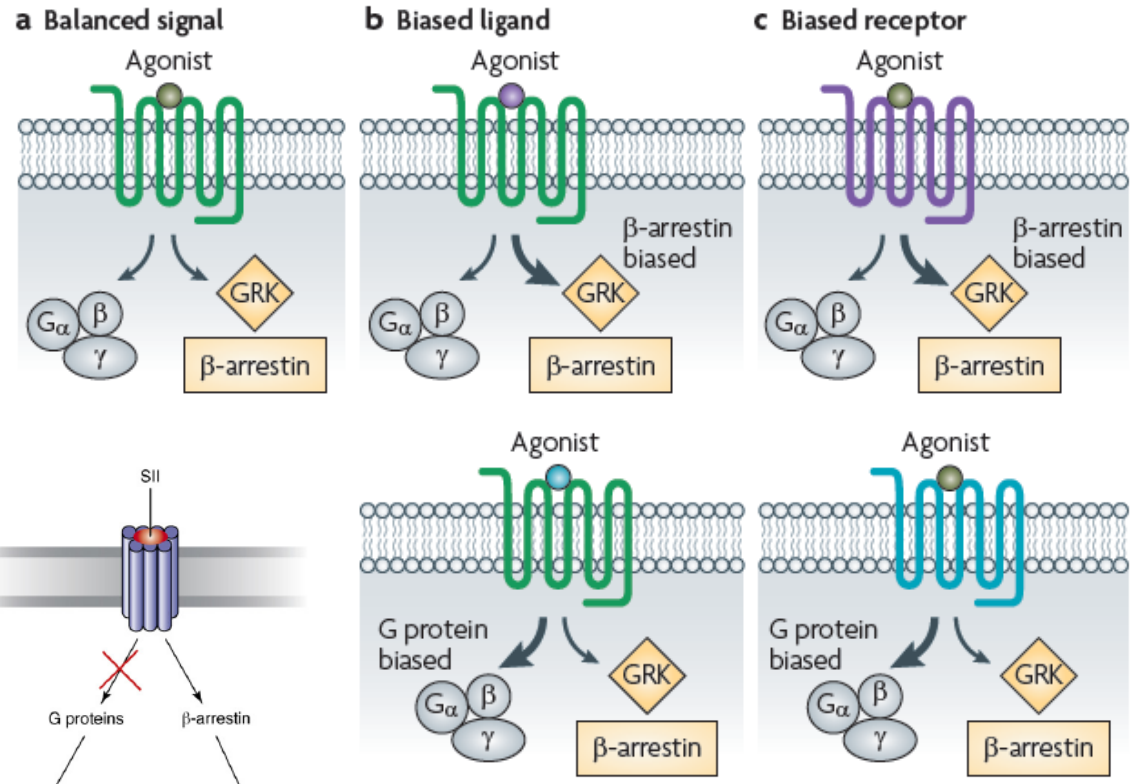
# Assembling different pathways




Lefkowitz *Science* 2005

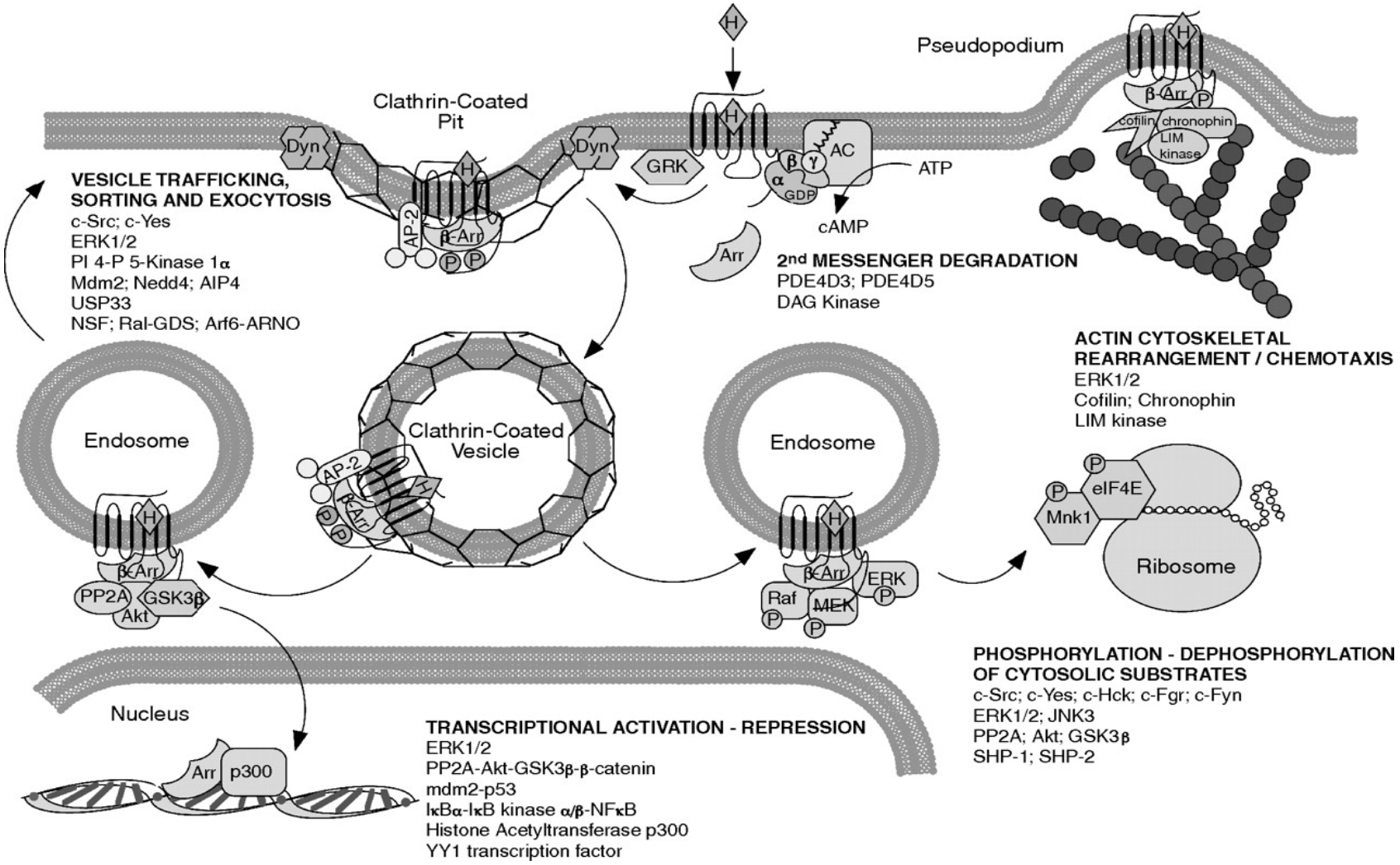
# Bypassing G-protein signaling



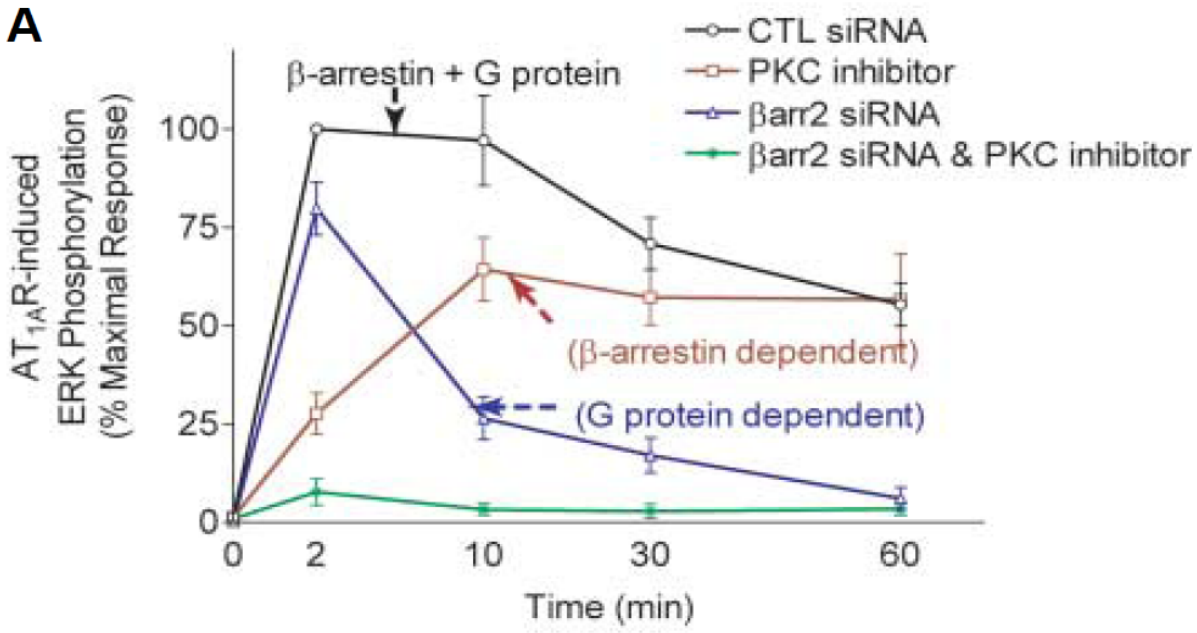

Lefkowtiz 2007, 2010

# Localization and scaffolding




Luttrell 2010, Pharmacol Rev

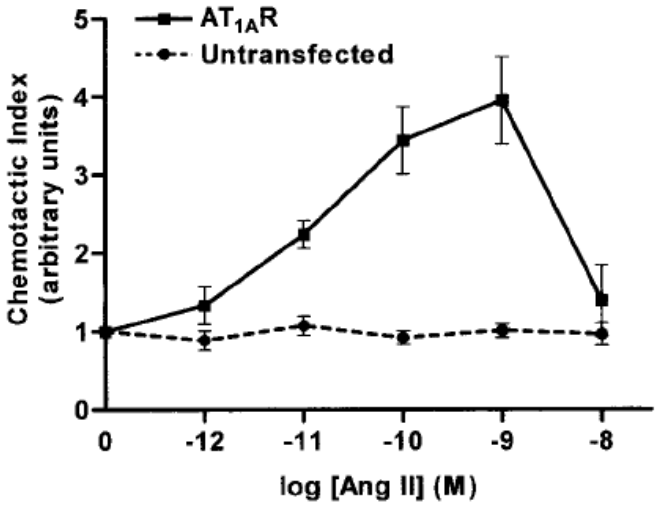
# Long-term effects

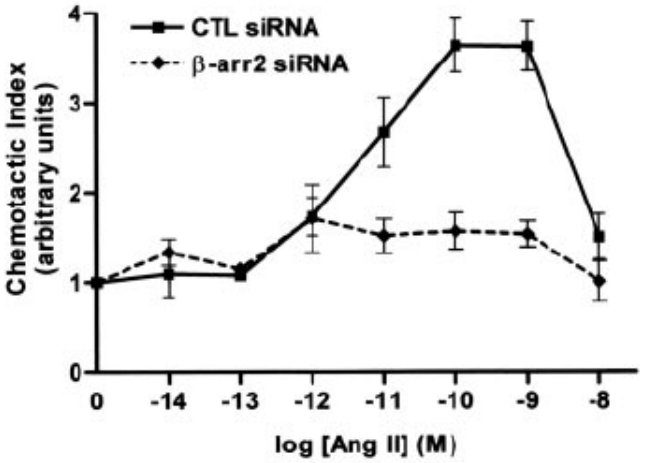



Lefkowitz *Science* 2005

# Beta-Arrestin2 (Arrb2) in Chemotaixs

- Trans-membrane



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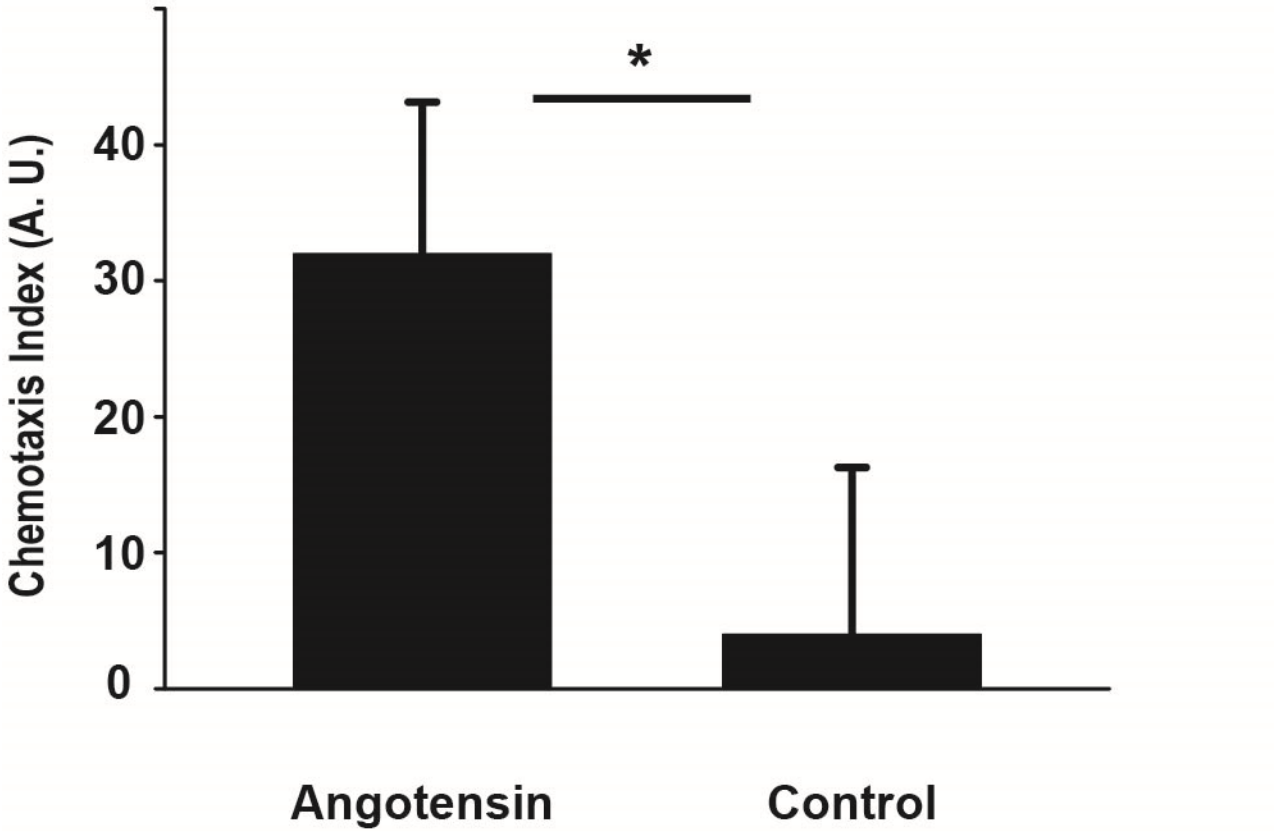
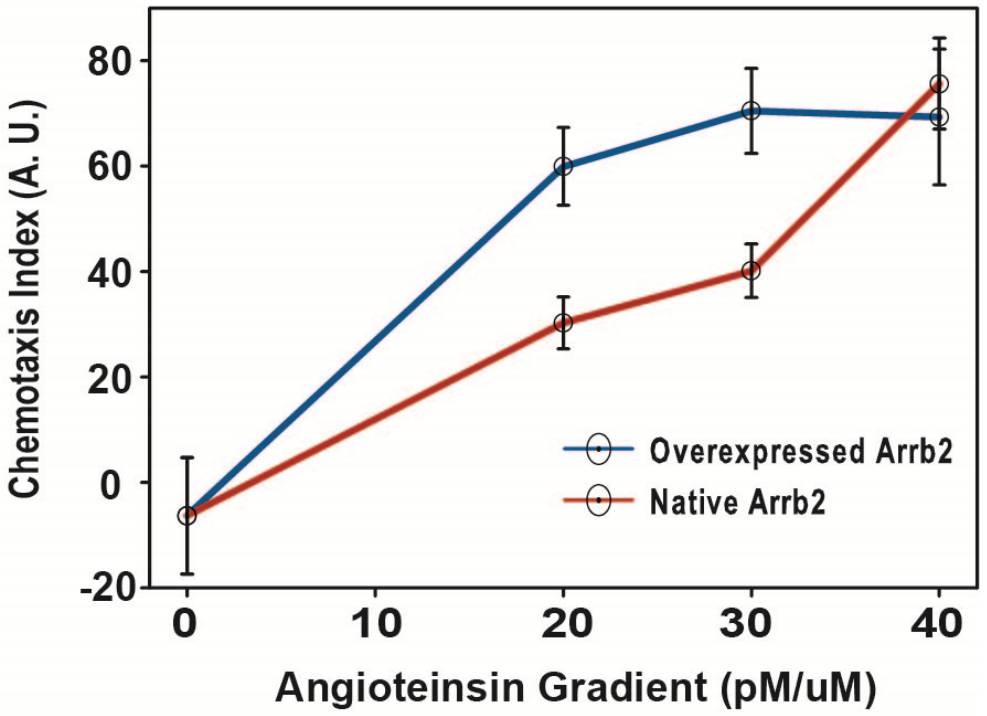
# Rat vascular smooth muscle cell

A7r5 cell line



# Our model cell system

- HEK (293)
- Rat AT1aR-CFP (or mCherry)
- Rat Arrb2-YFP

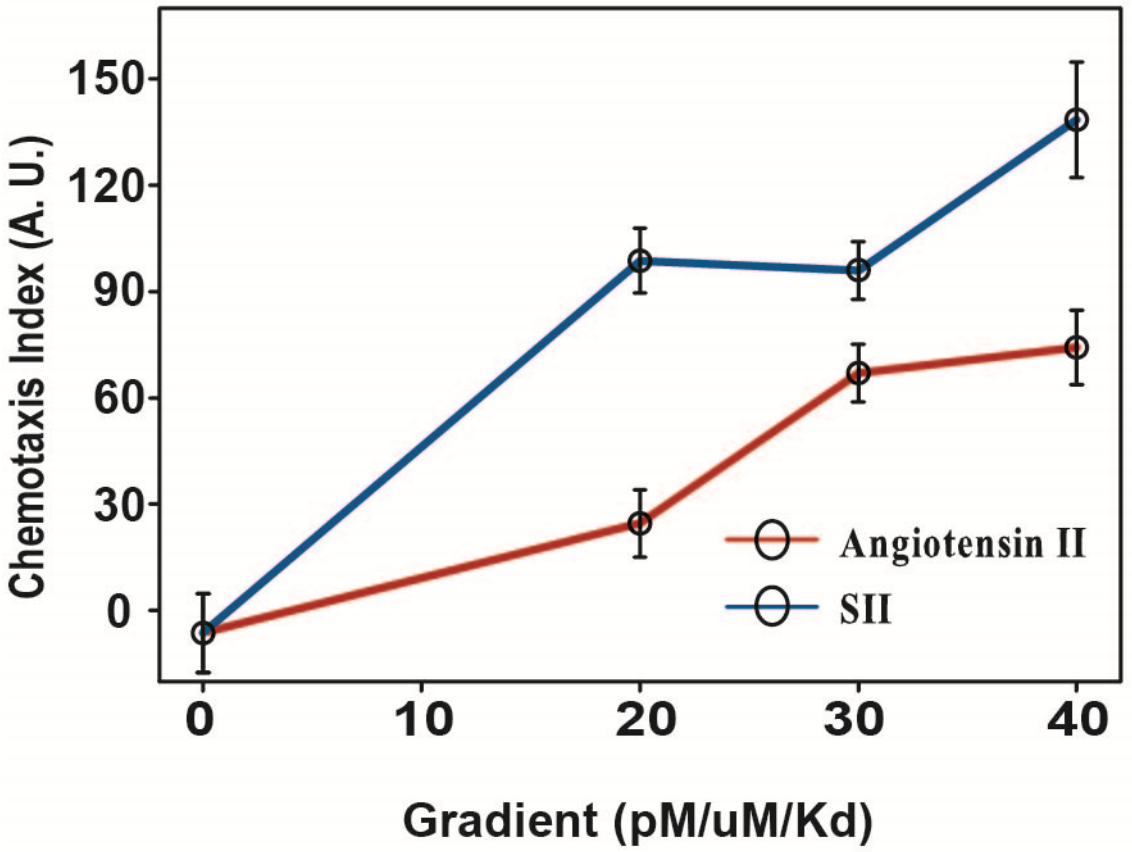



- EKAR FRET probe
- mCherry-Actin
- LifeAct-mCherry
- mCherry Rab11

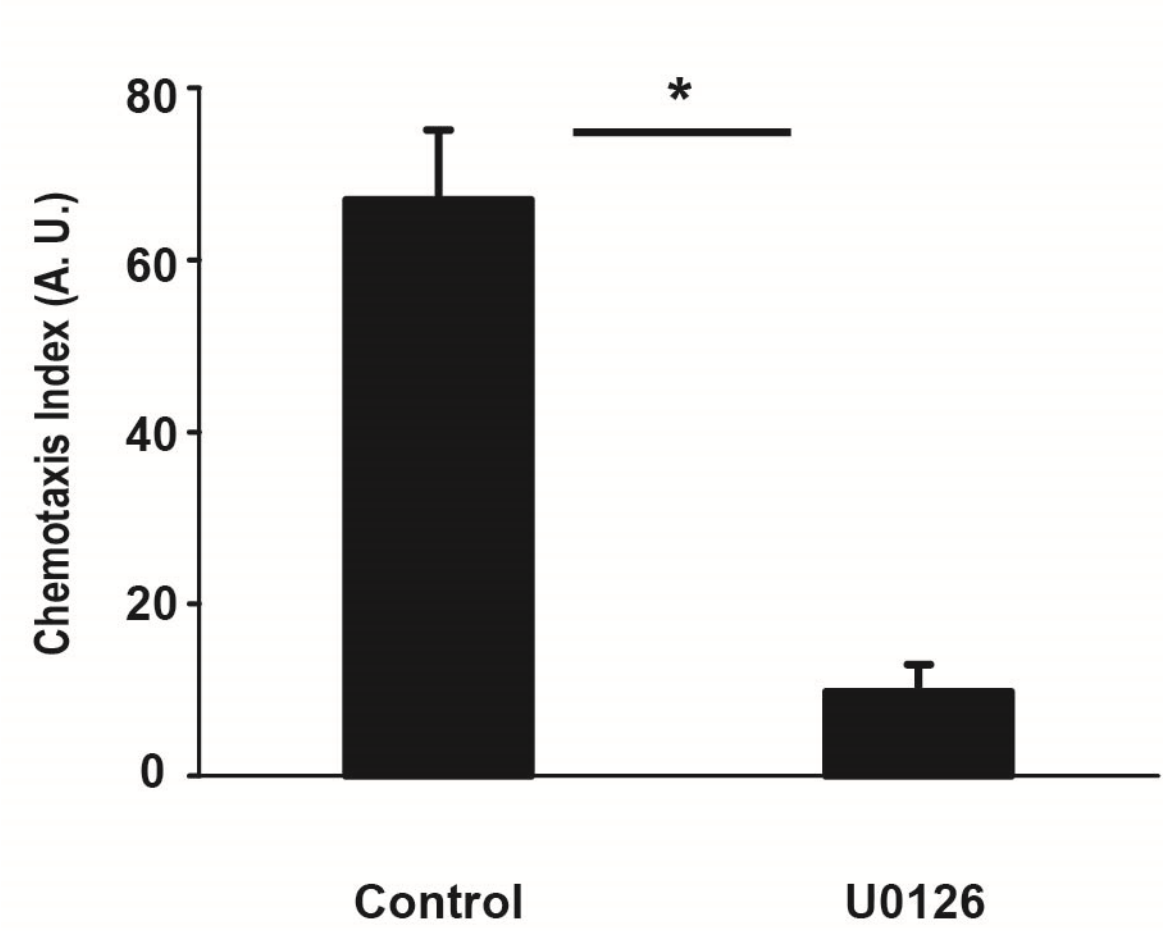
# To reduce uncertainty

- Clonal Cell lines express certain amount of:
  - AT1a Receptor
  - Arrb2-YFP overexpression (3 different levels)
  - Arrb2 knockdown (shRNA)
  - Actin-mCherry overexpression
  - LifeAct-mCherry overexpression
  - EKAR expression

# SII is Ang analog and only actives arrestin pathway



# ERK is necessary



# Base on above observations, we looked at:

- Molecular kinetics under uniform Angiotensin stimulation (at different dose)
- Molecule distribution under gradient

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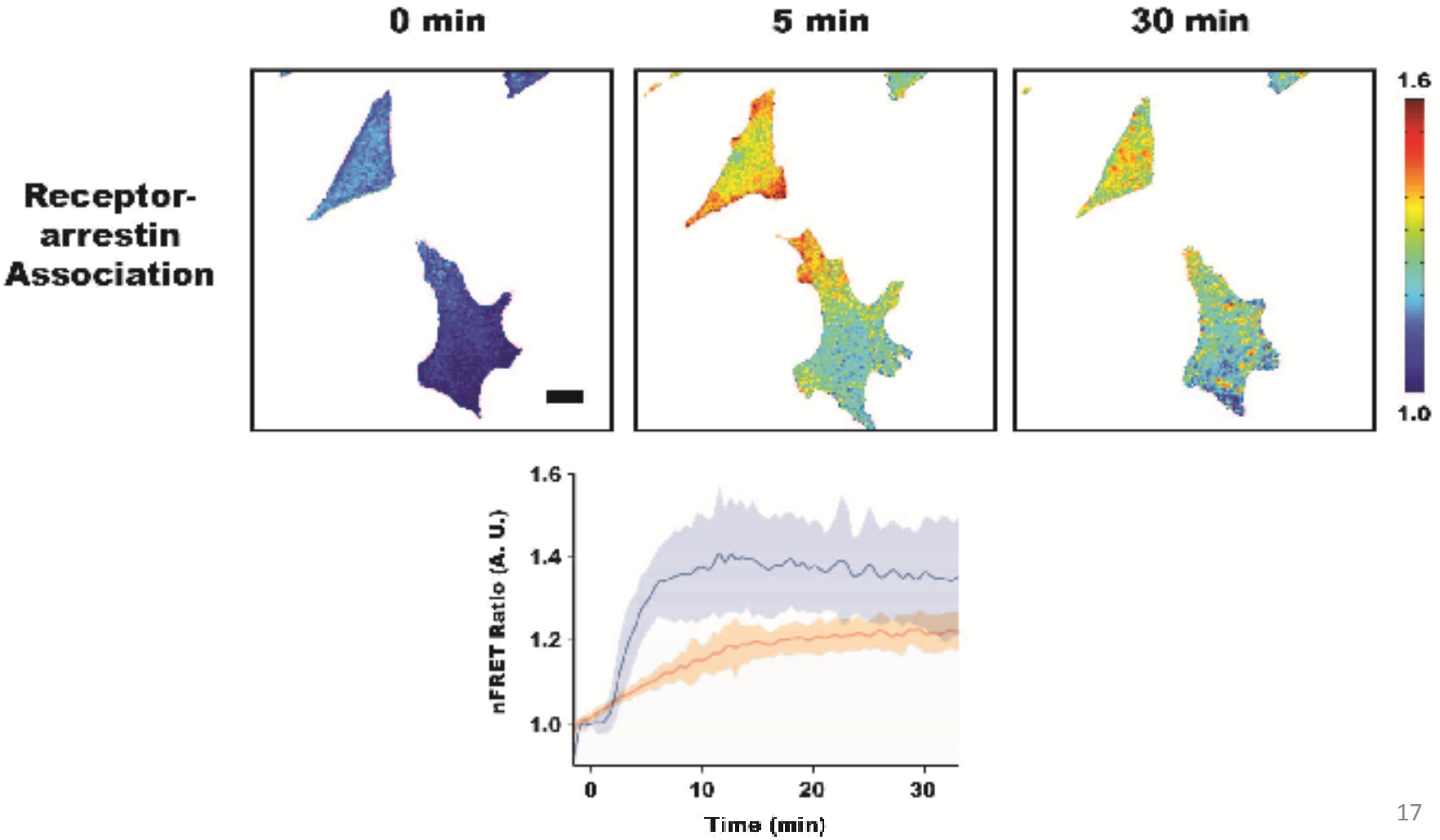
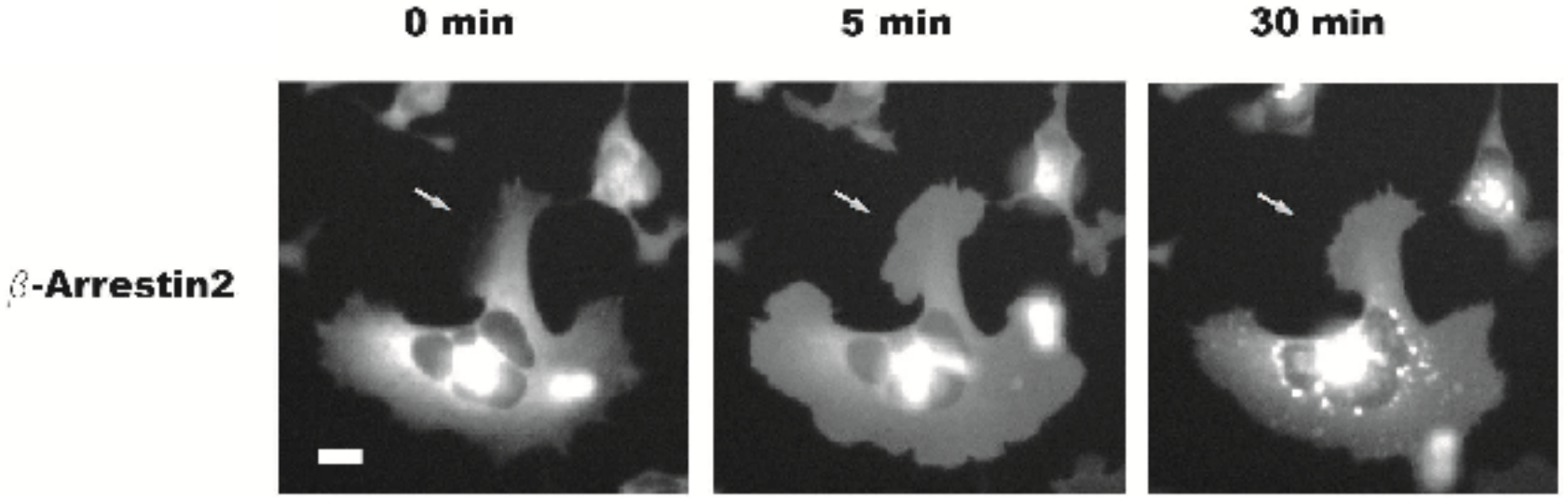
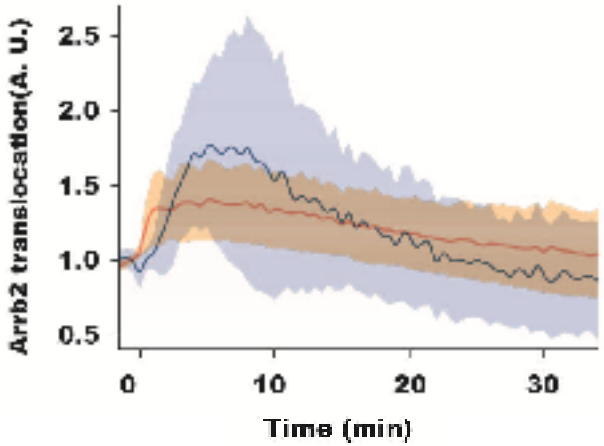
# Arrestin is recruited by receptor

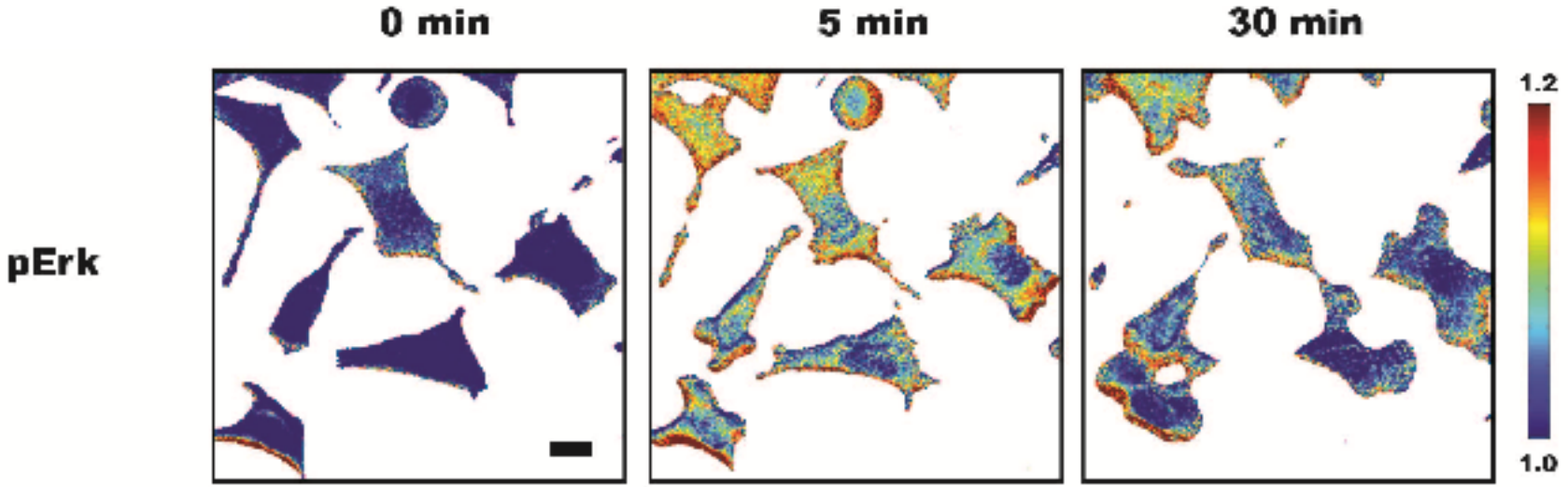


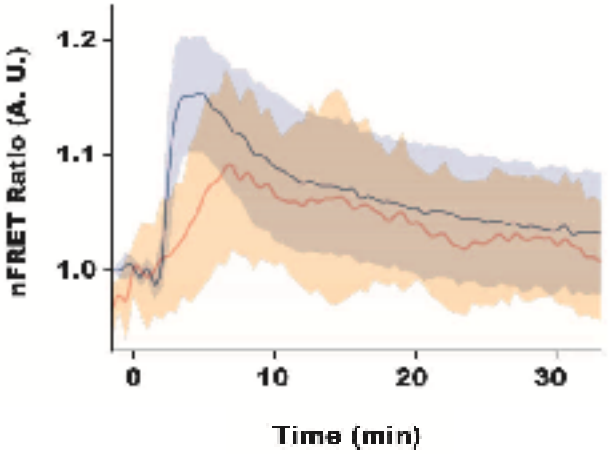
# Arrb2 translocation



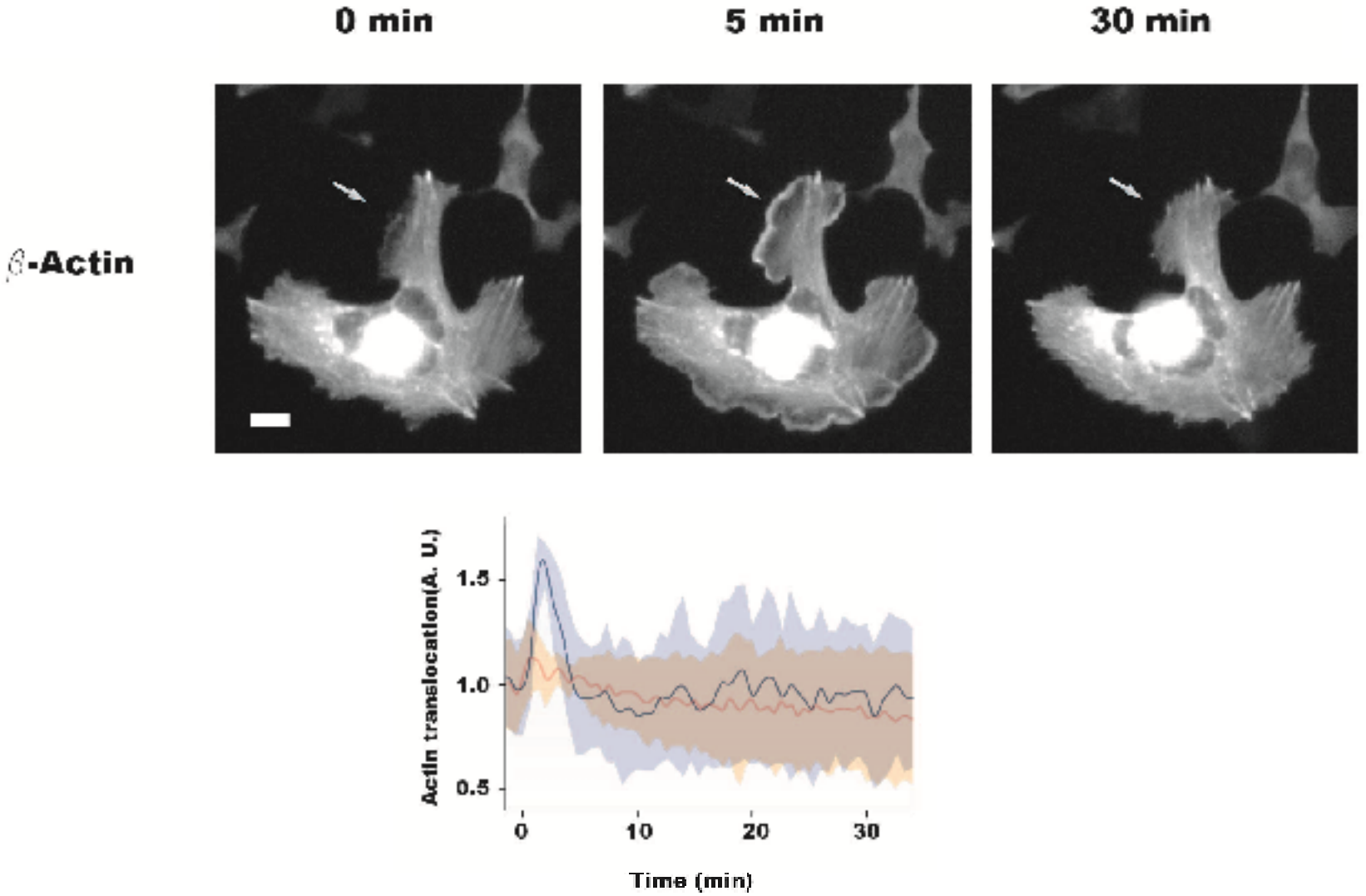



# ERK activation

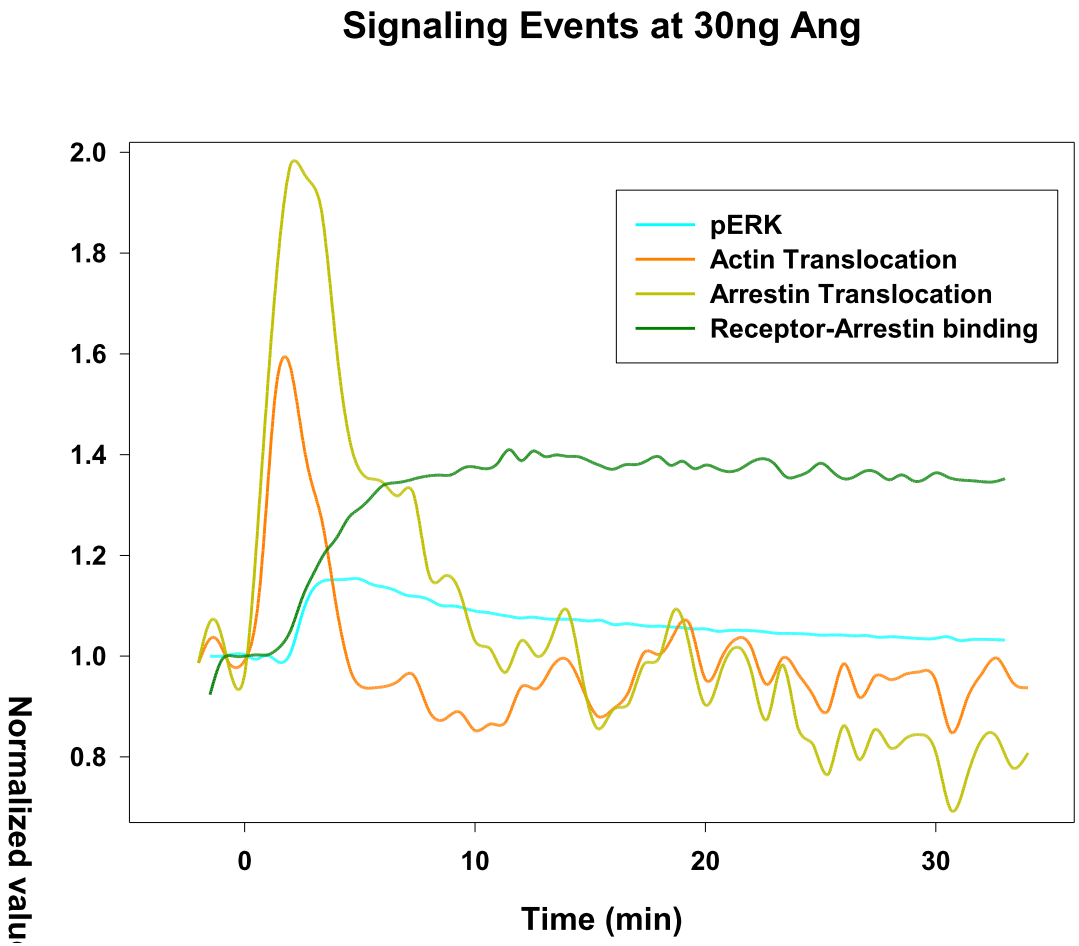


# Actin translocation

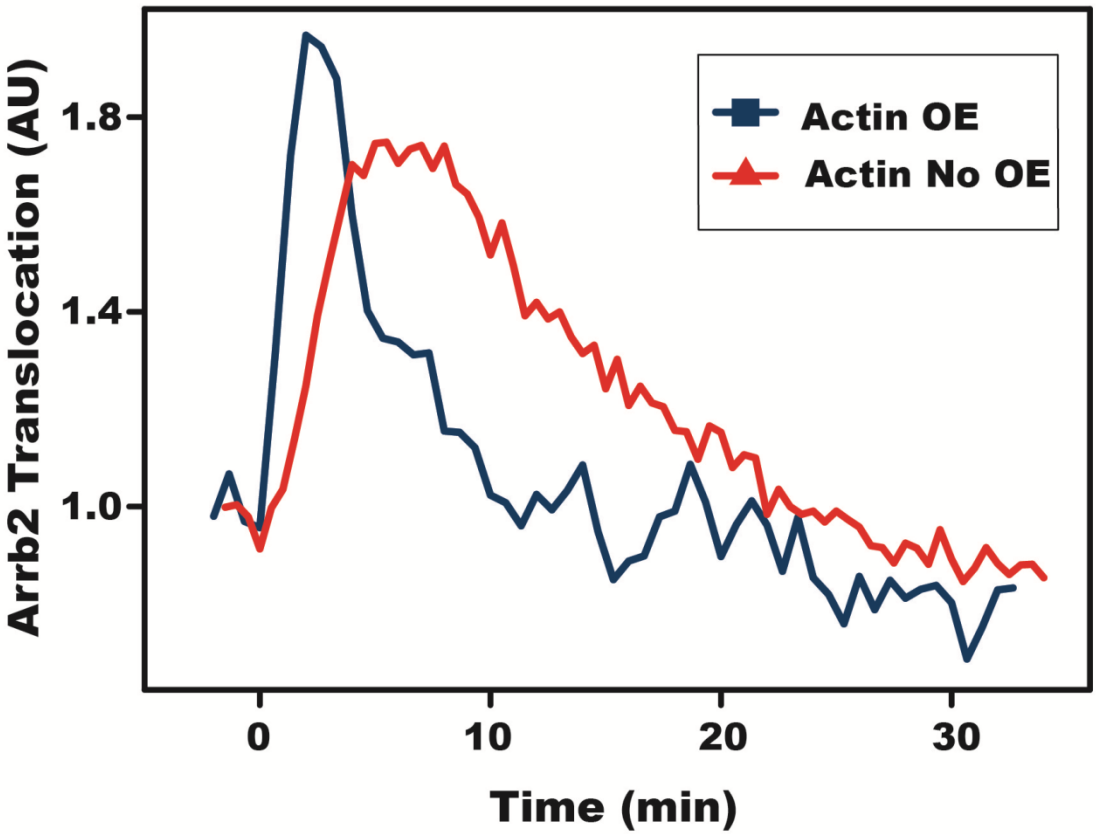


# Function together

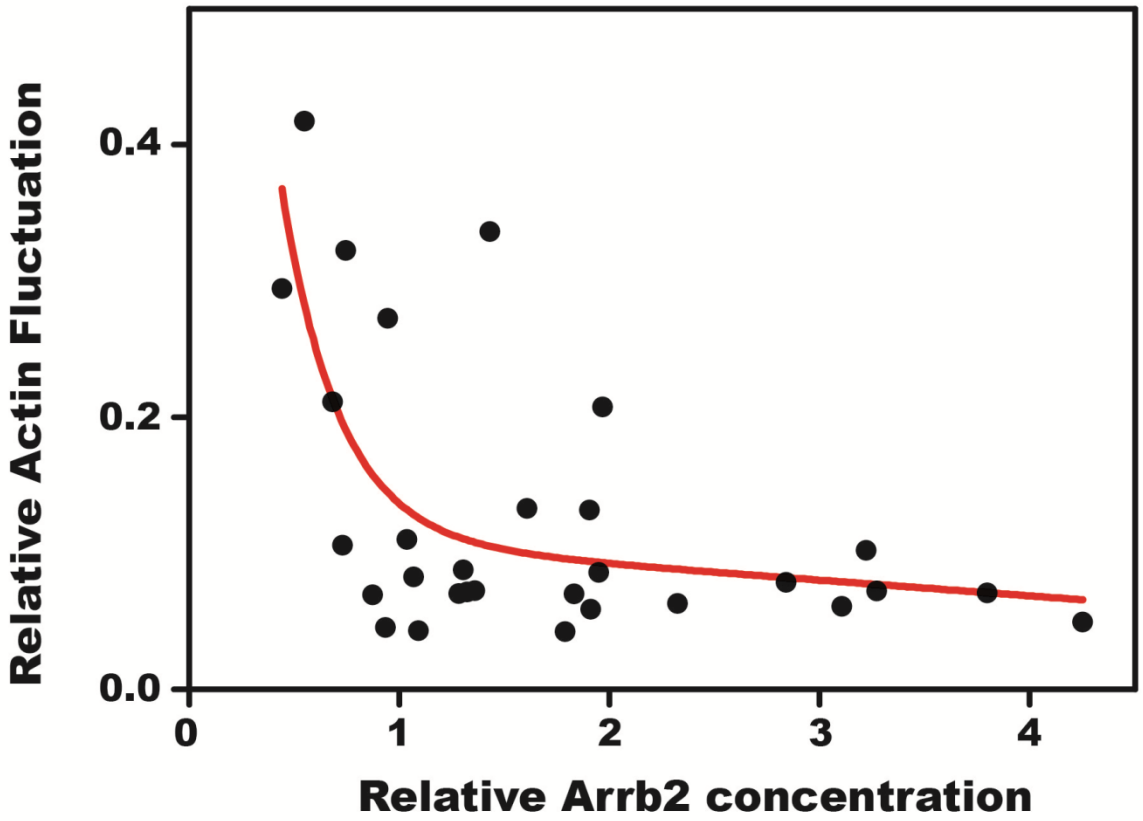


# Interplay between Actin & Arrb2

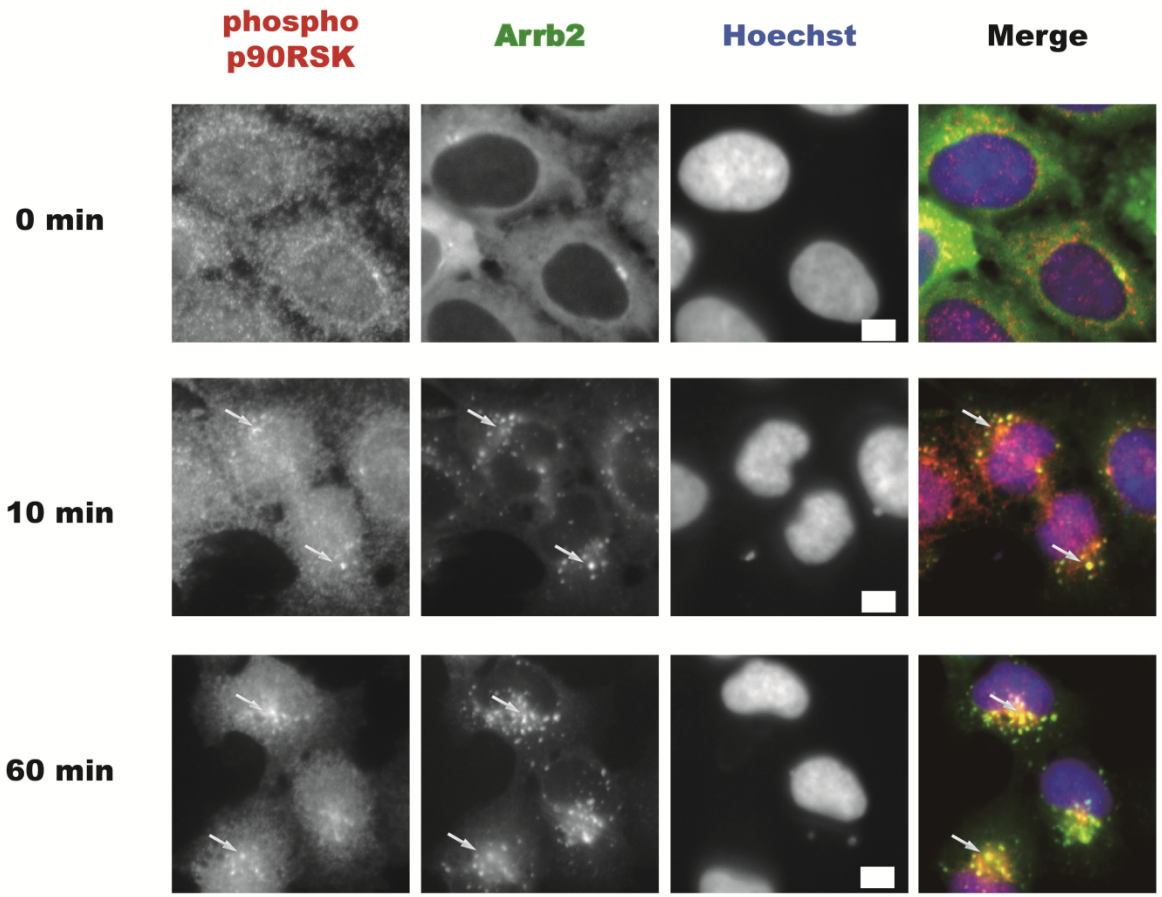
# Actin helps Arrb2 translocation



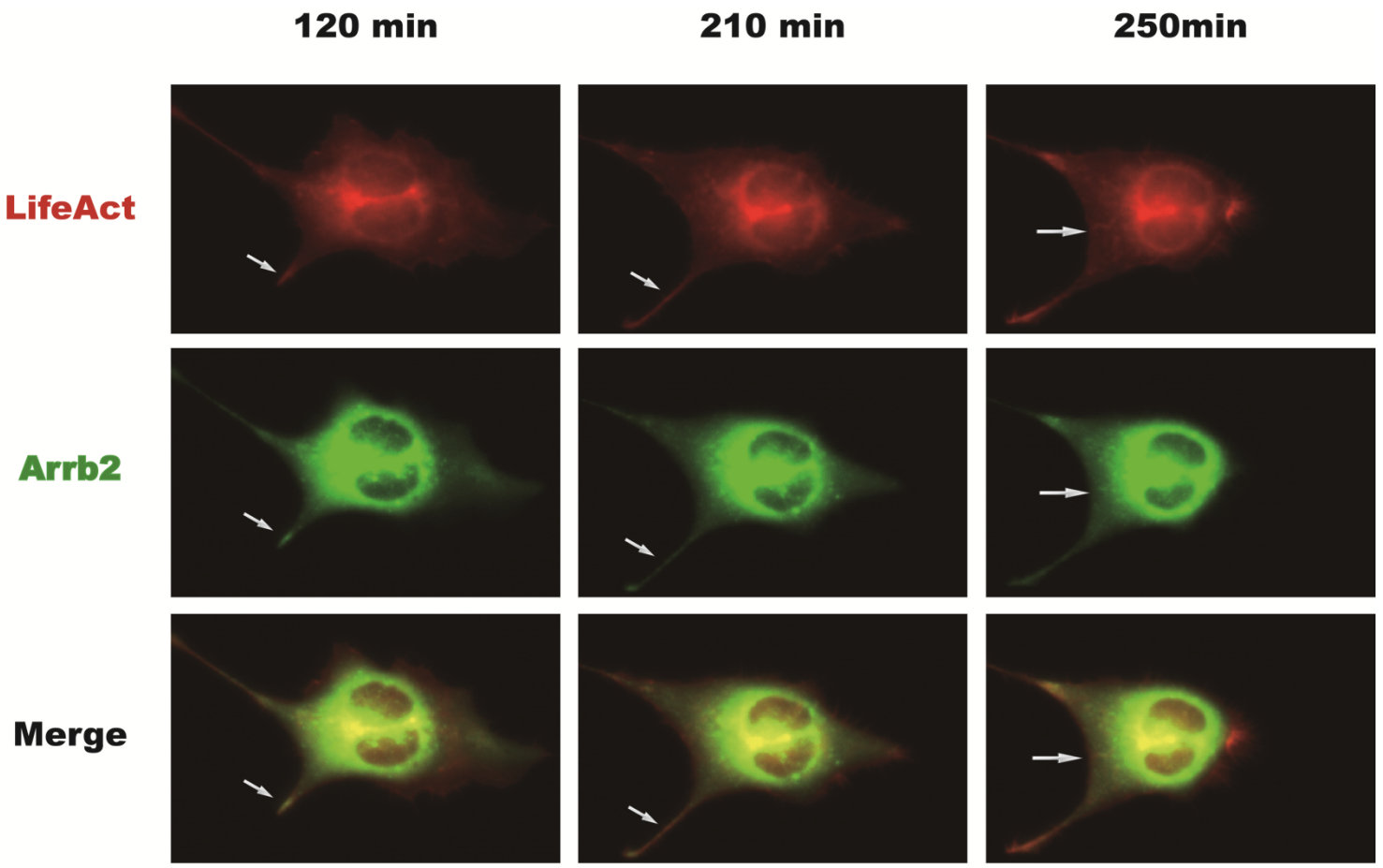
# Arrb2 stabilizes Actin



# Arrb2->p90RSK->FLNA->Actin



# Actin at filopodia



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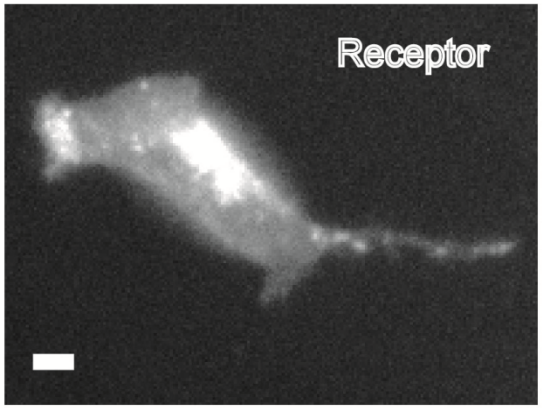
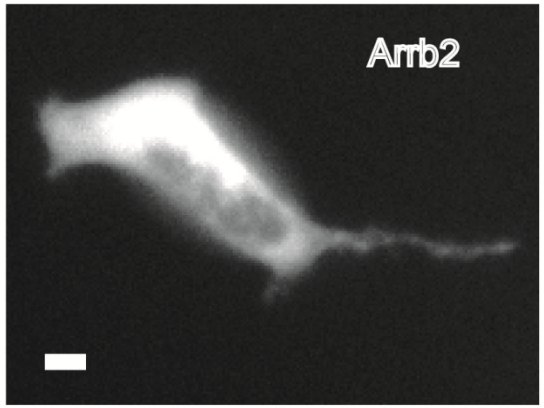
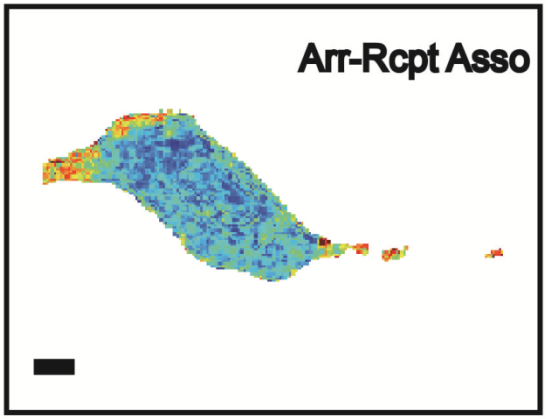
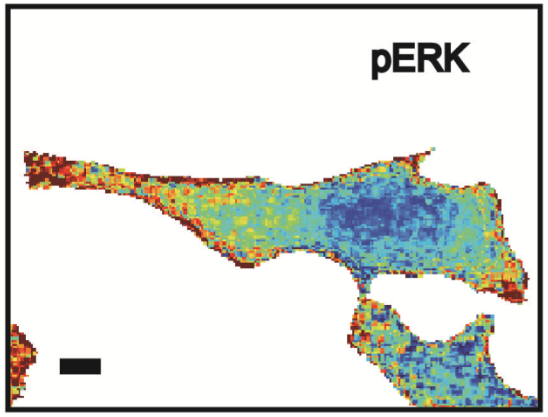
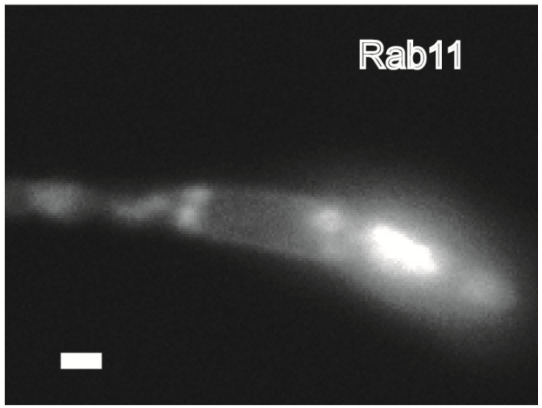
# With Angiotensin Gradient



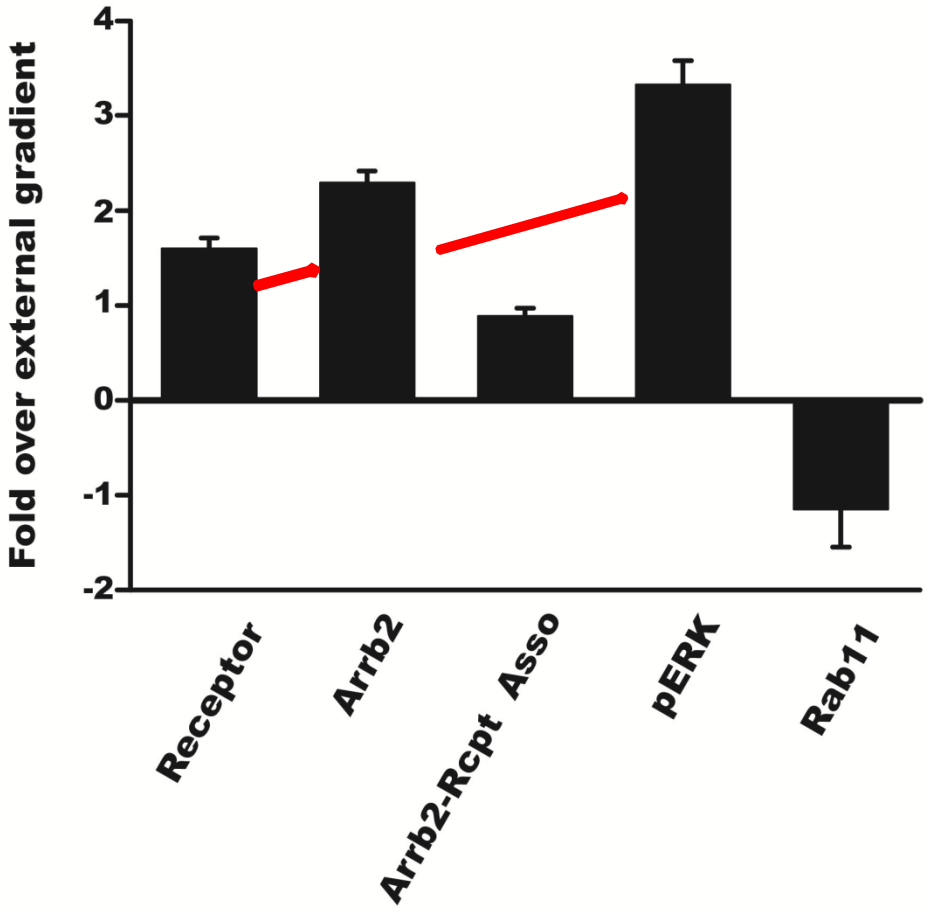



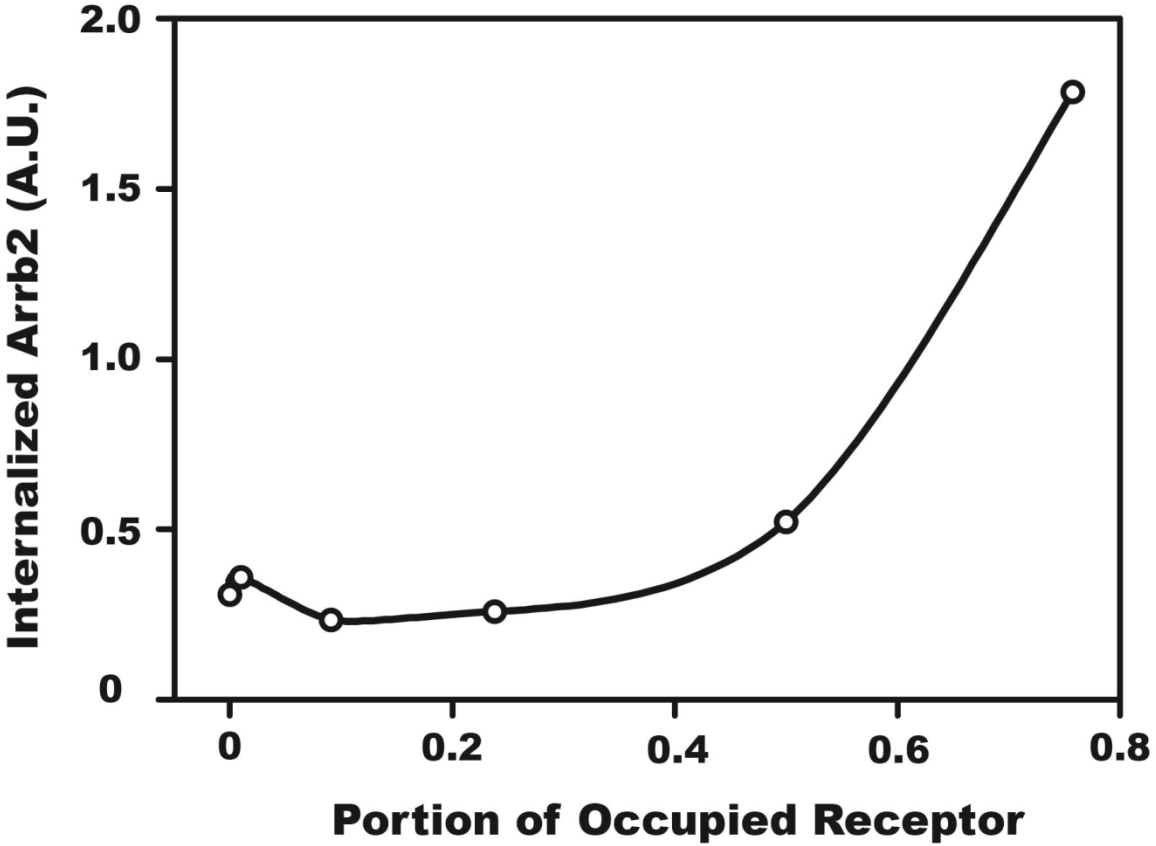




Dark Matter

# Amplification and more



# Nonlinear (Cooperative?)



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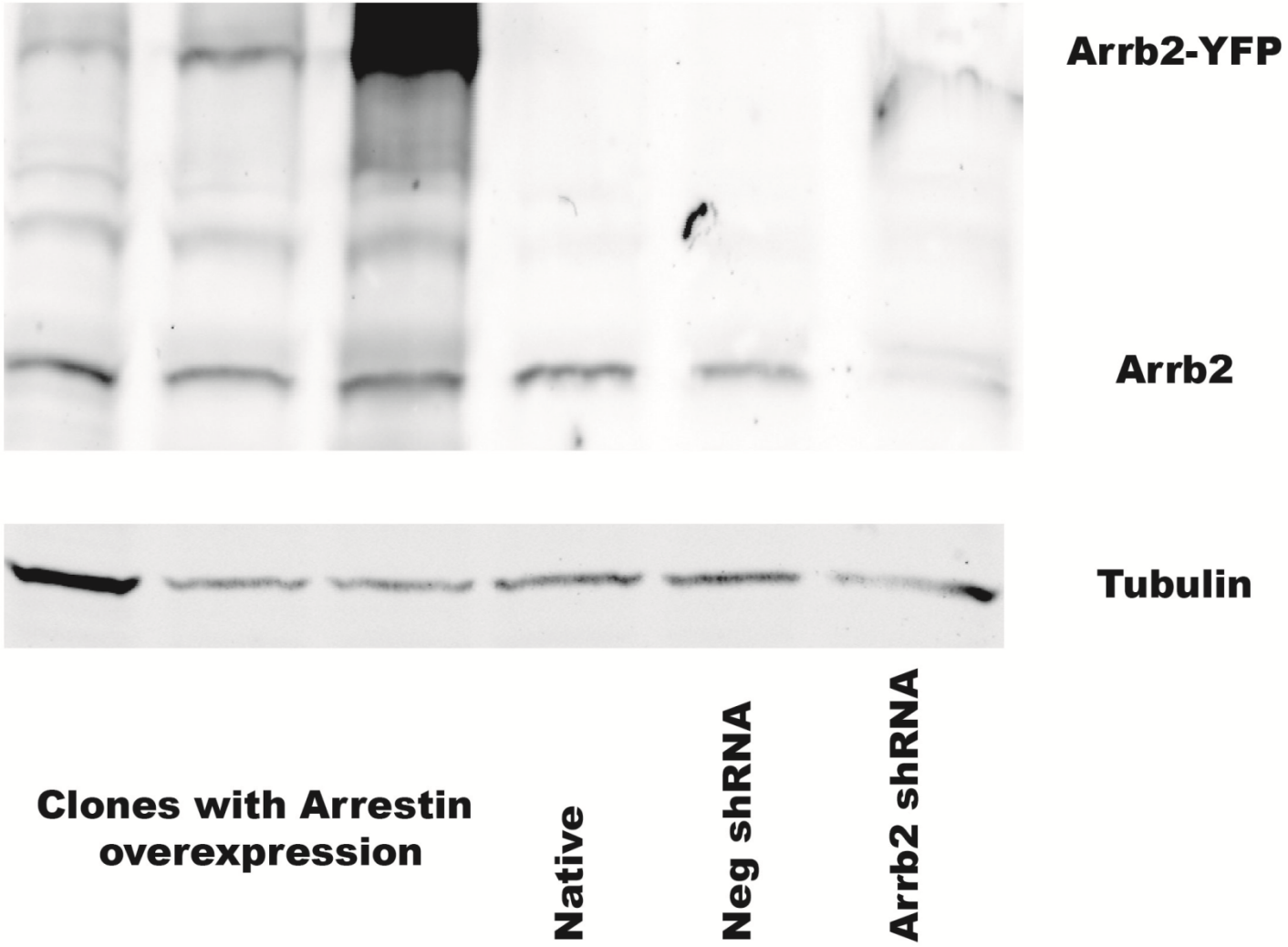
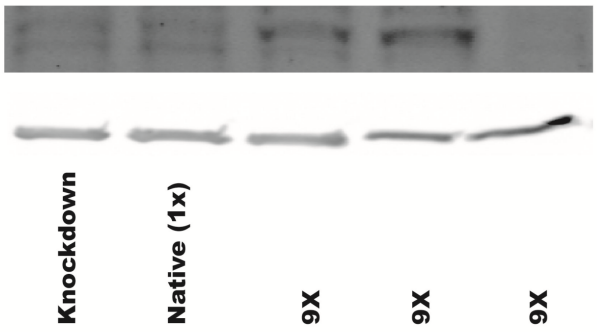
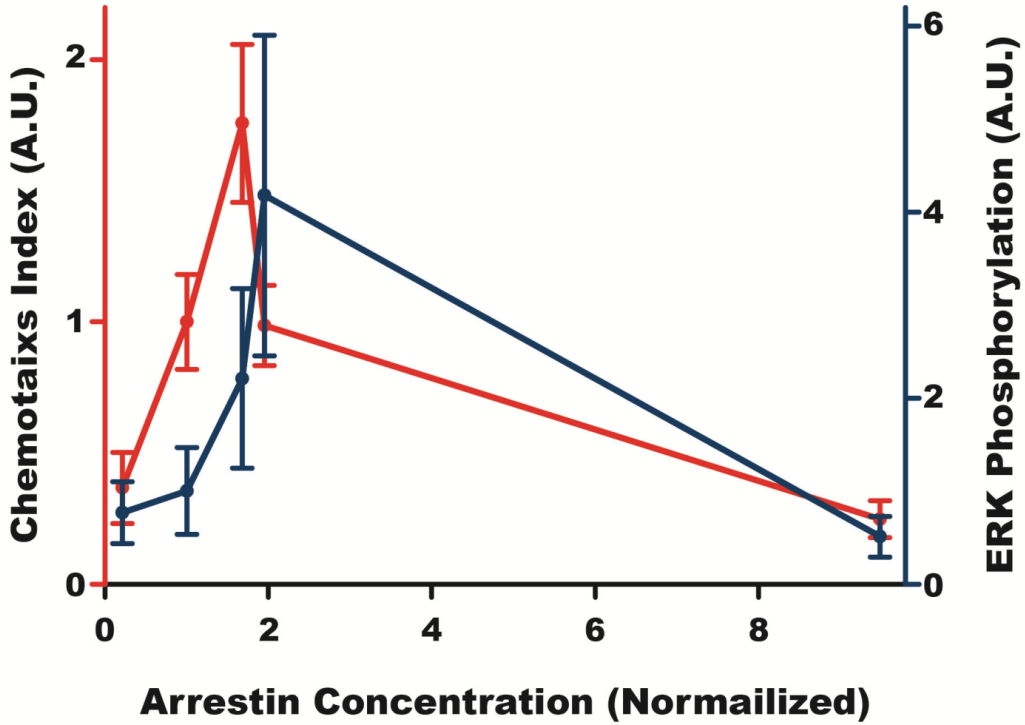
# Arrb2 as a scaffold:

# Cell lines with different Arrb2 expression levels

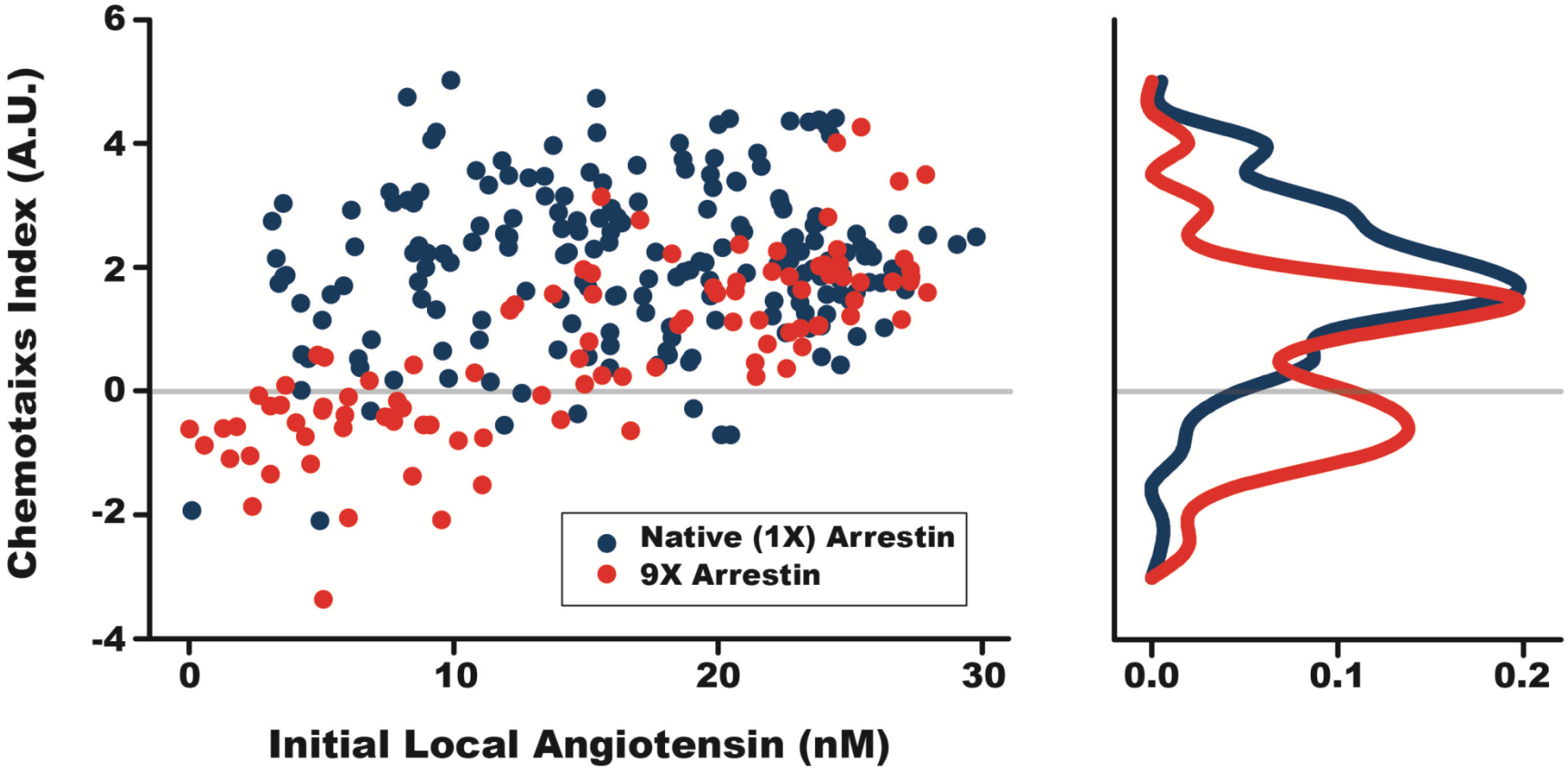


# Biphasic response of Arrb2

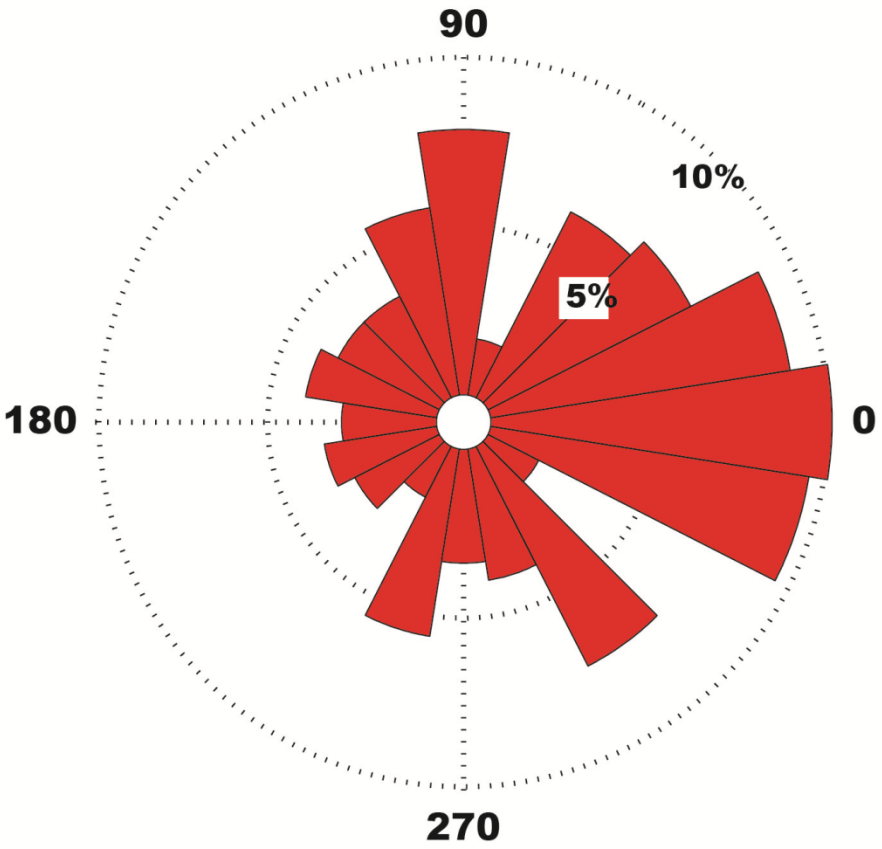


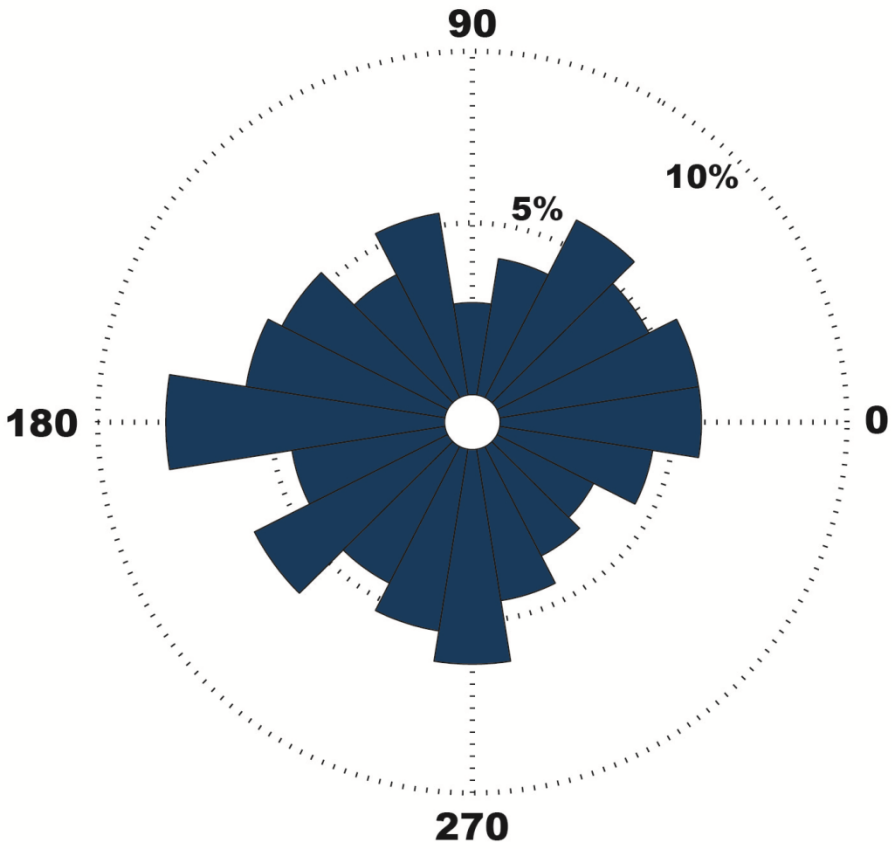
# Too much Arrb2: Chemorepulsion



# Too much Arrb2:
# Reversed polarization

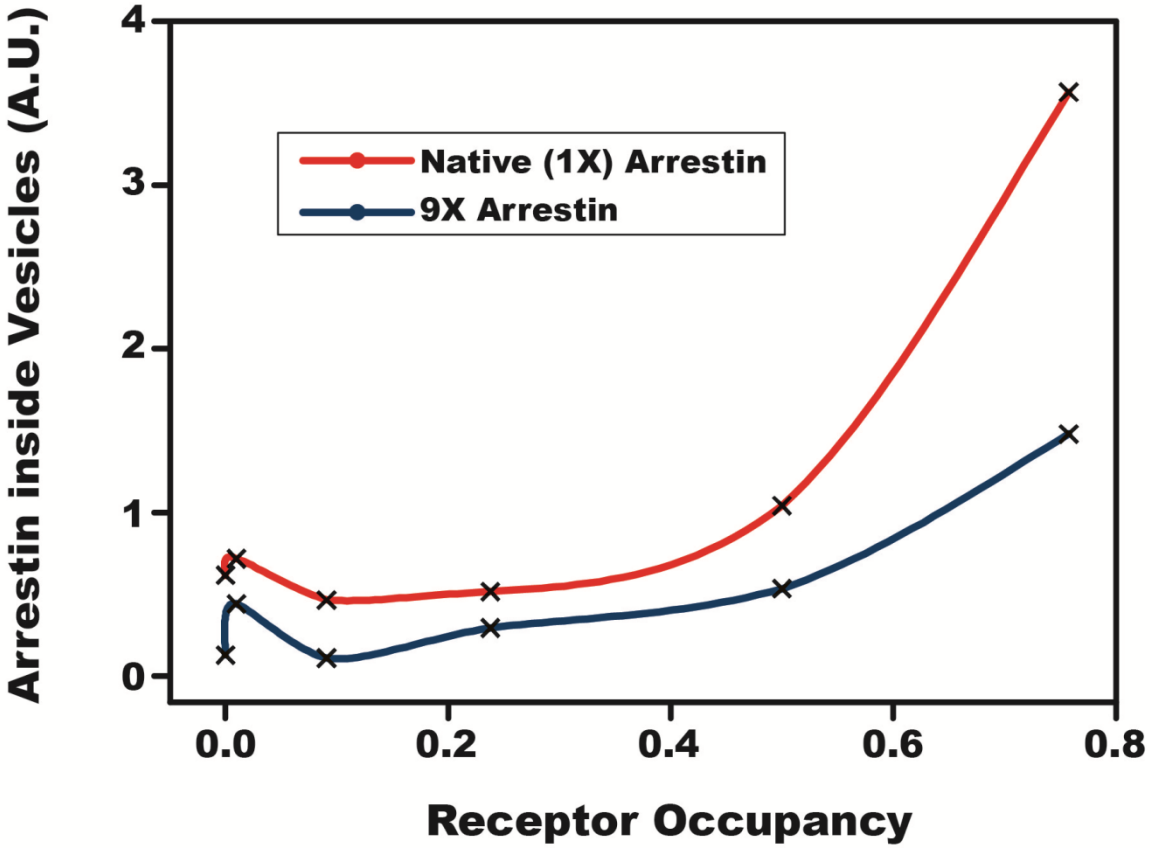



Optimal (2X) arrextin

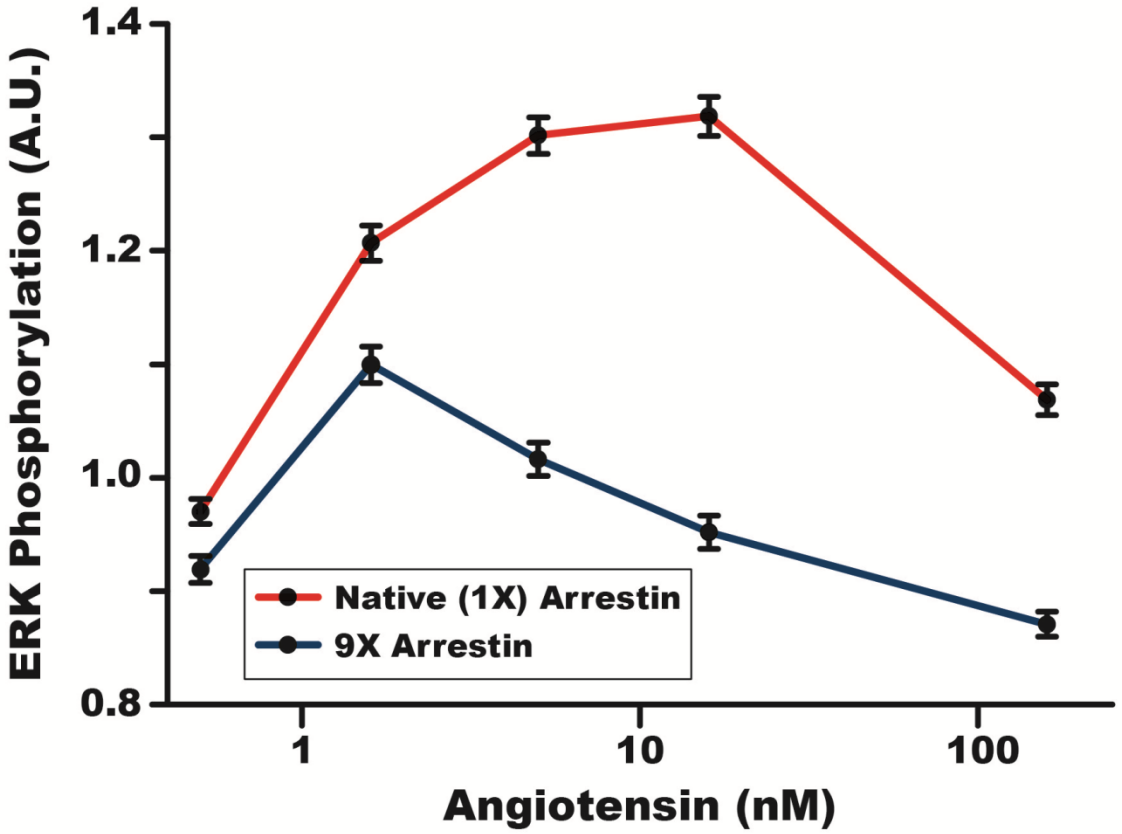



9x Arrestin

# Too much Arrb2: Weaker internalization



# Too much Arrb2: Reduced pERK



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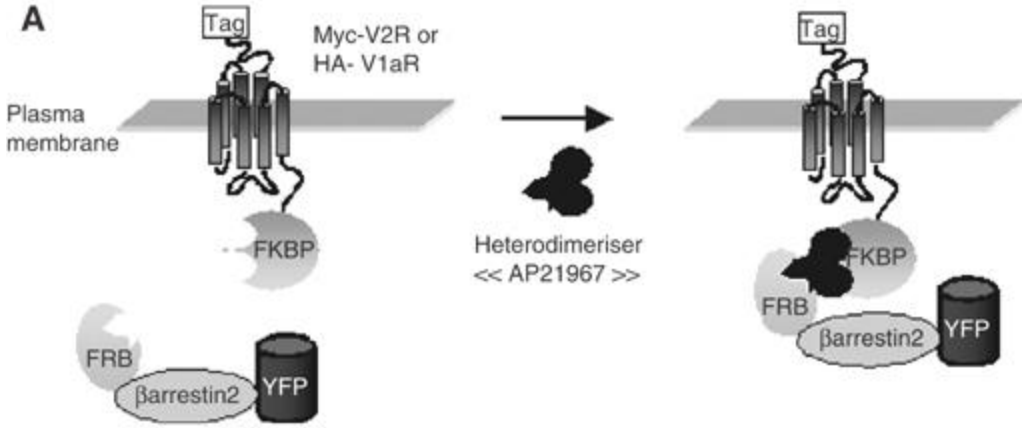
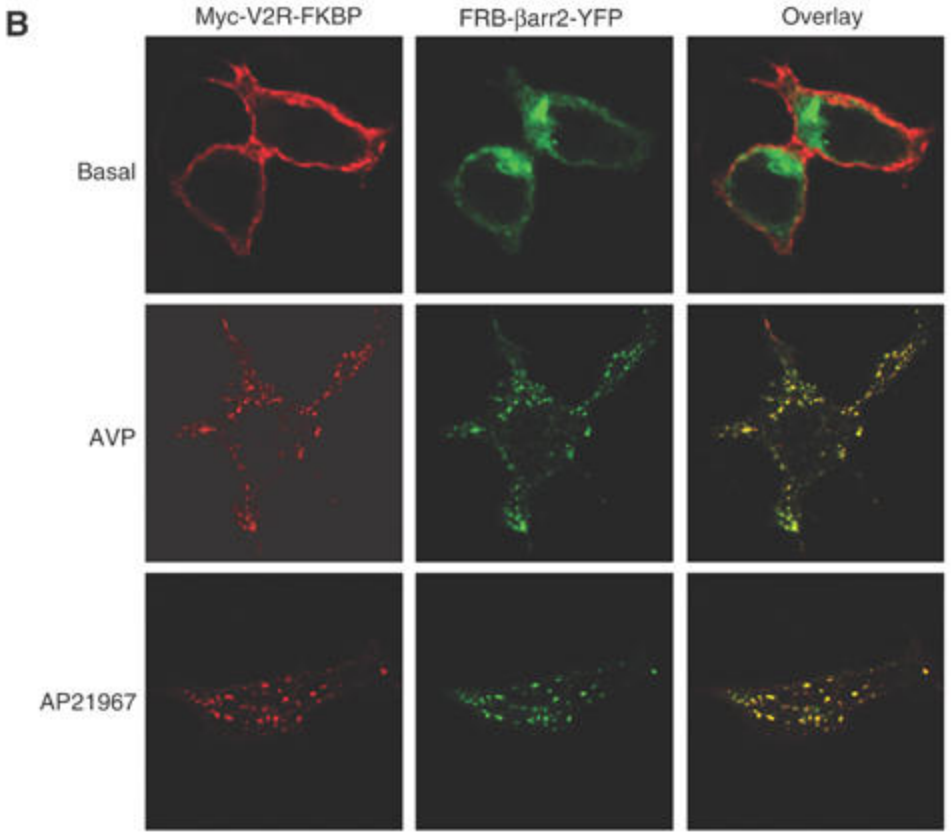
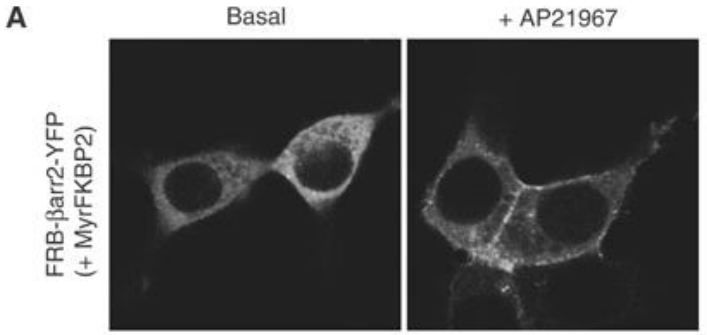
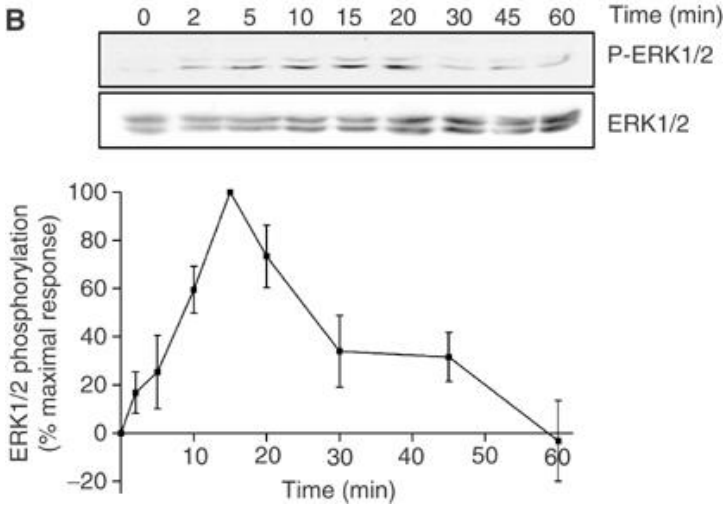
# So can we create an artificial chemotaxis?



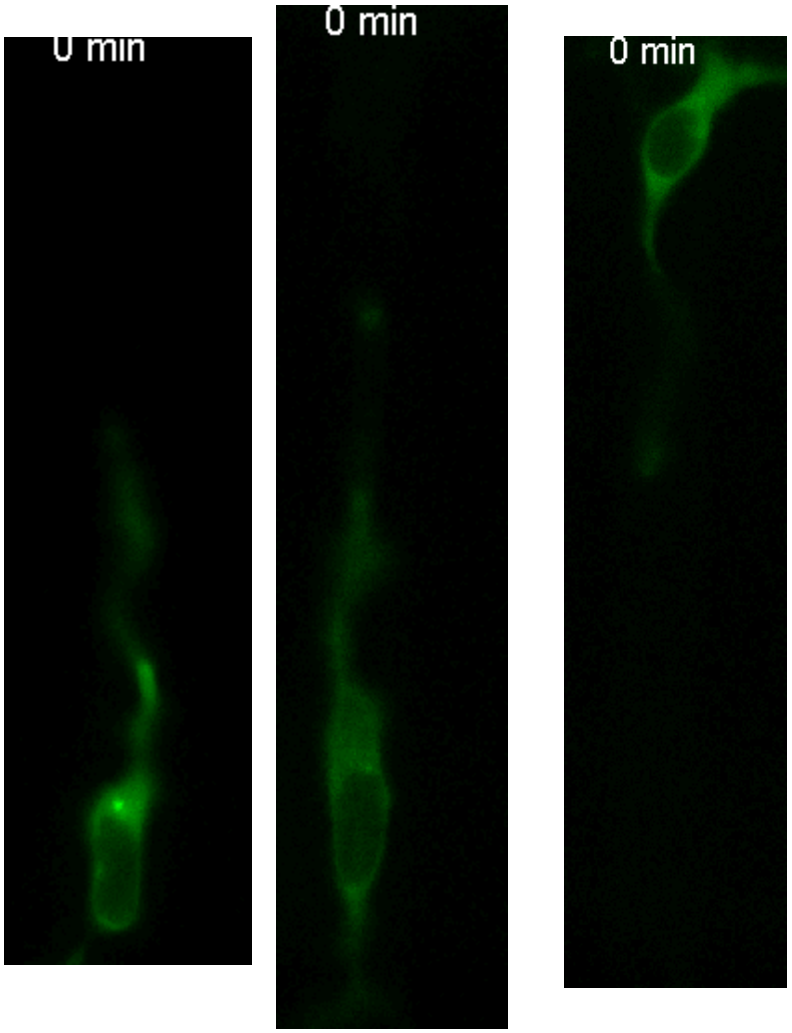




Terrillon 2004, EMBO J

# Graded translocation of B arrestin to the plasma membrane

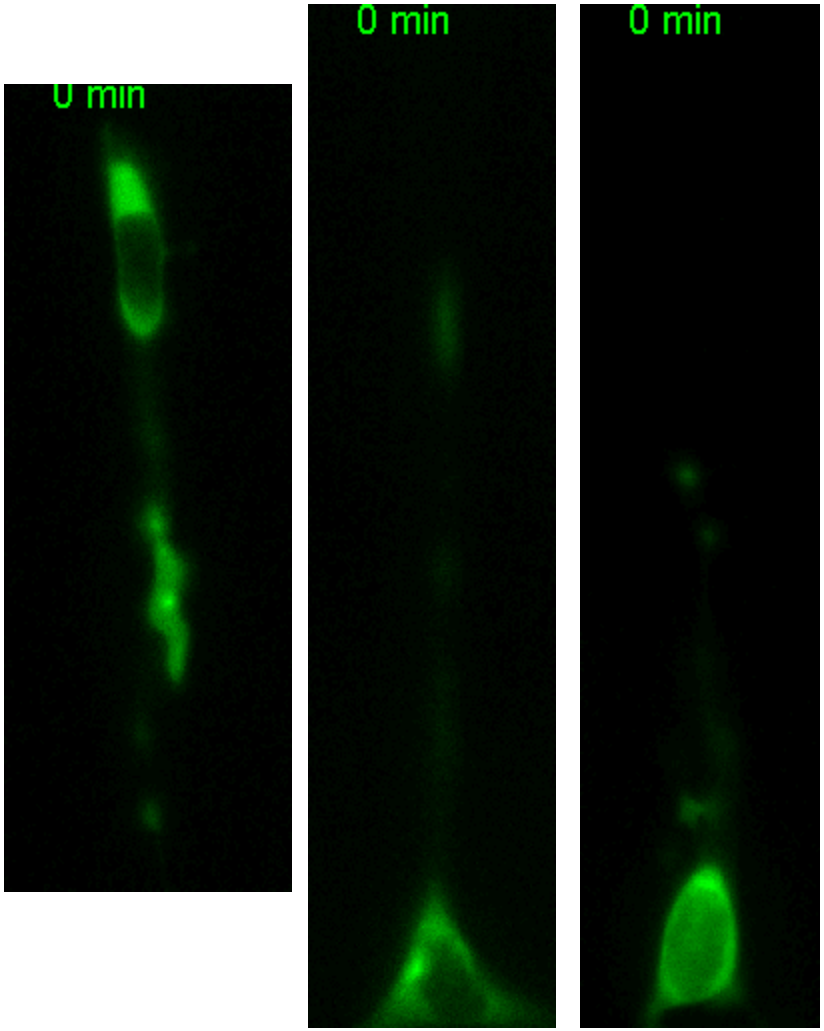



Blue- rapamycin, green- B arrestin

- Translocation of B arrestin induces some vesicle formation but there are no obvious physical changes towards the gradient
- Cells are surviving the experimental time period
- Based on these results we tried translocating B arrestin to the angiotensin receptor instead of the membrane

**From Ben**

# Graded translocation of B arrestin to the angiotensin receptor

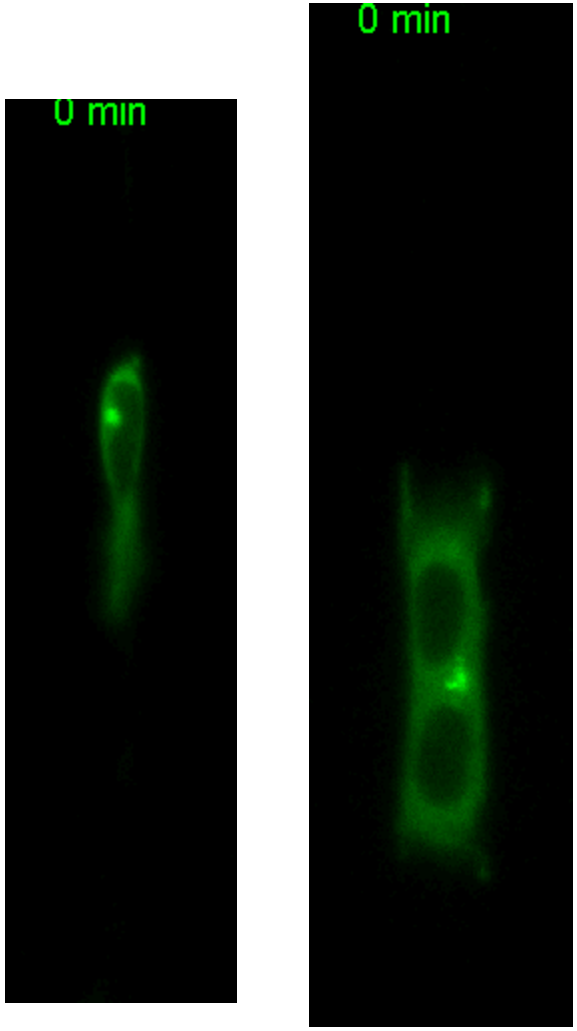



Green- B arrestin

- Gradient formation was confirmed by visualizing with Alexa 594 before and after experiment (Can't visualize at the same time with cfp, yfp)
- There seem to be increased vesicle formation but no real morphological changes (Gradient is applied from the top)
- Overall efficiency with the ATR construct is decreased, due to poorer transfection efficiency
- Based on these results, we reasoned that basal activity may need to be activated in order for a gradient of rapamycin to induce physical changes

**From Ben**

# Uniform application of Angiotensin + gradient of rapamycin




Green- B arrestin

- Translocation and vesicle formation as we have seen with angiotensin beforehand
- Unfortunately, there doesn't seem to be much physical changes
- Gradient is applied from the top

**From Ben**

# Conclusions as of now

- Graded translocation of B arrestin is sufficient to produce vesicles when translocated to either the plasma membrane or ATR
  - There doesn't seem to be any drastic physical changes
- Uniform application of angiotensin is also sufficient to produce vesicles in transfected cells
  - The addition of a rapamycin gradient also seems insufficient to produce additional responses

**From Ben**

# Good news or bad news?

# Other difficulties

- Co-existence of chemorepulsion and chemoattraction
- Amplification

# A possible solution: Vesicle trafficking

- Internalization -> recycled back
- The Dark Matter: Microtubule-Rab systems

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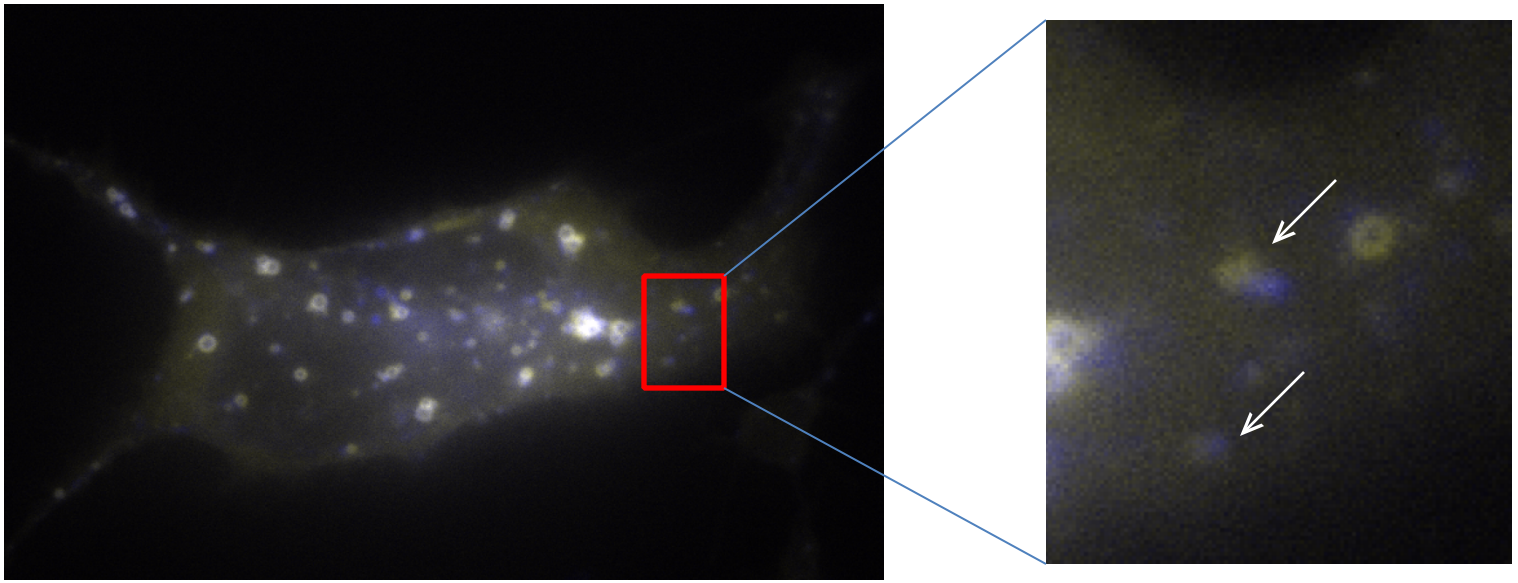
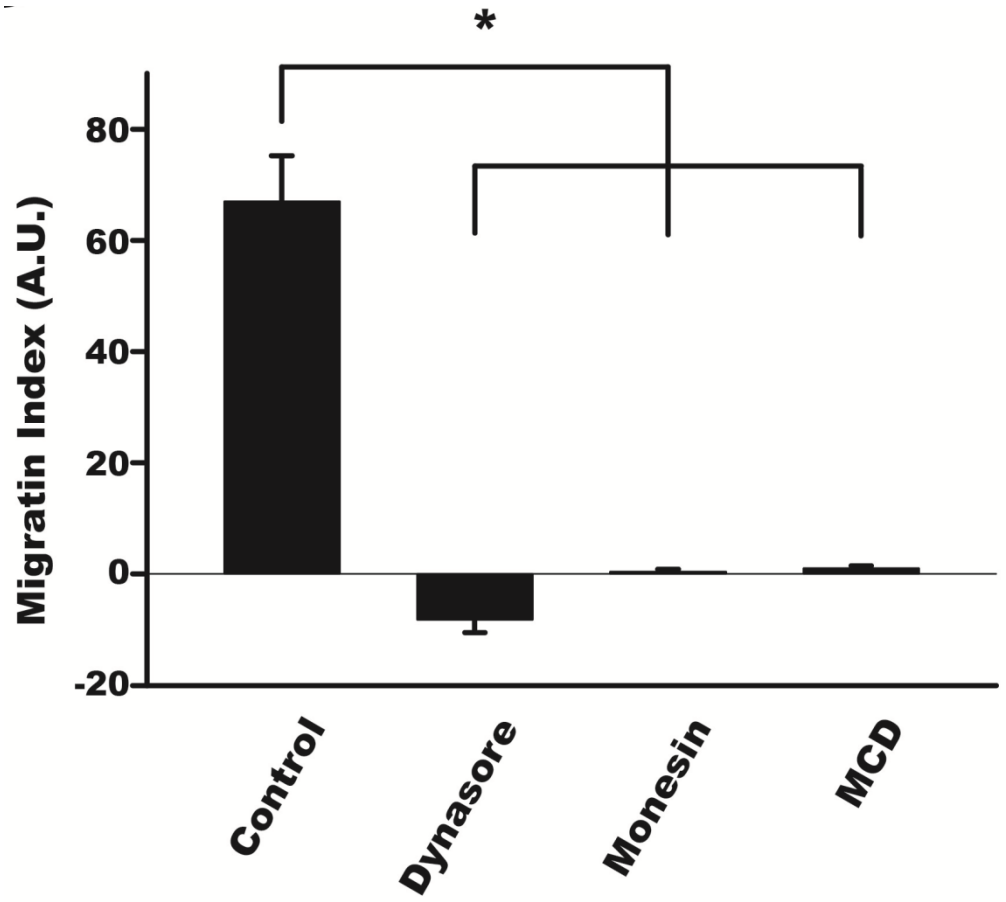
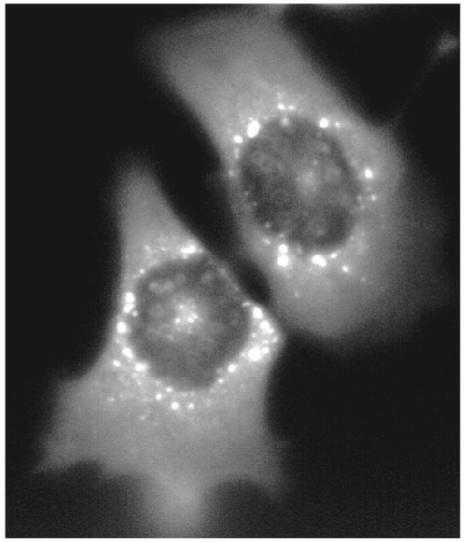
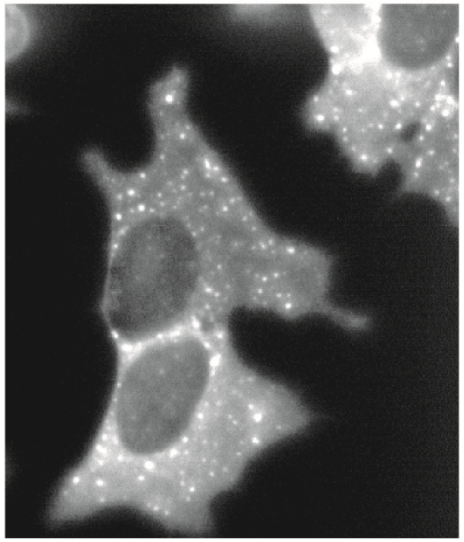



*
80
60
40
20
0
-20
Migratin Index (A.U.)
Control
Dynasore
Monesin
MCD

# Nocodazole effect on endosome trafficking

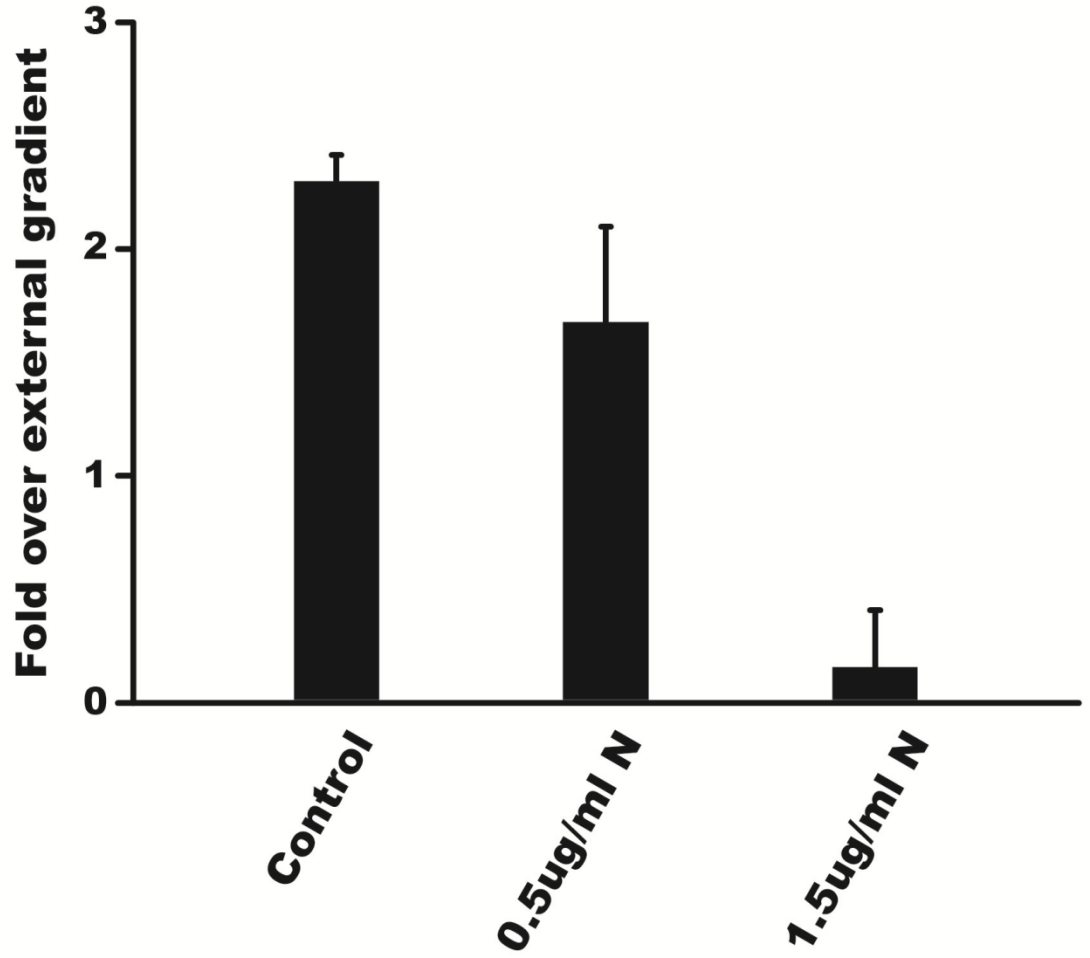


Control

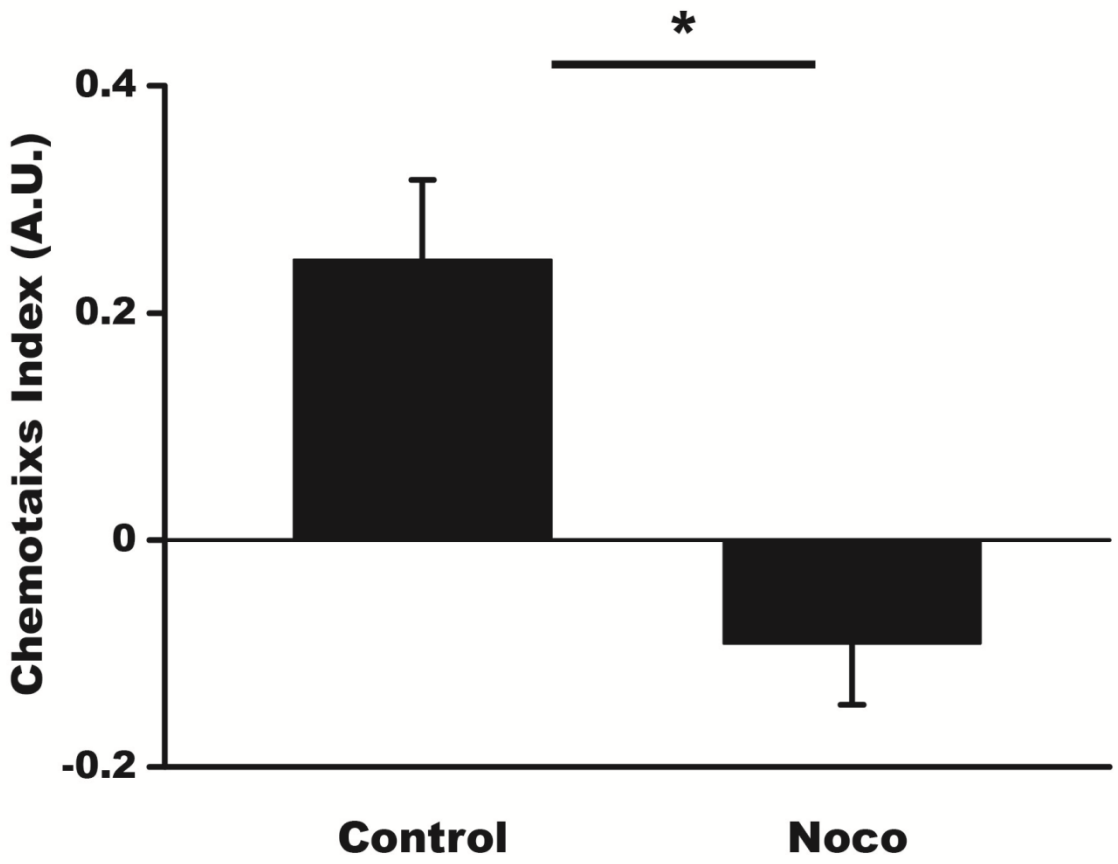


Nocodazole

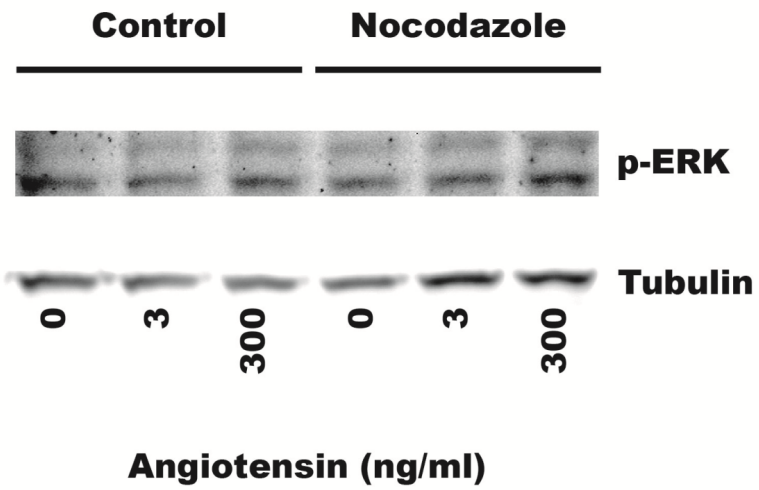
# Nocodazole inhibits Arrb2 polarization

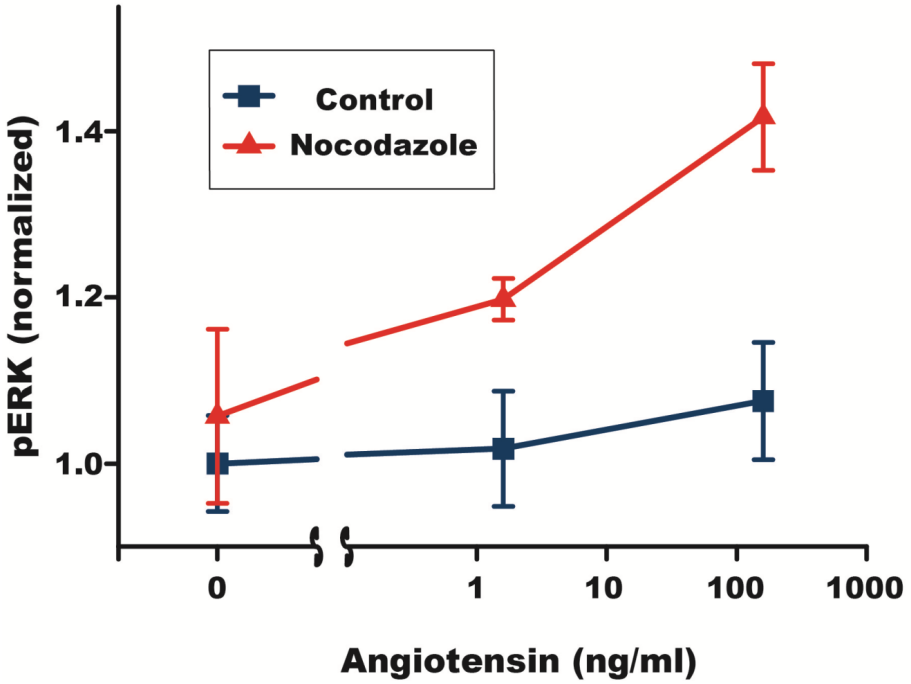


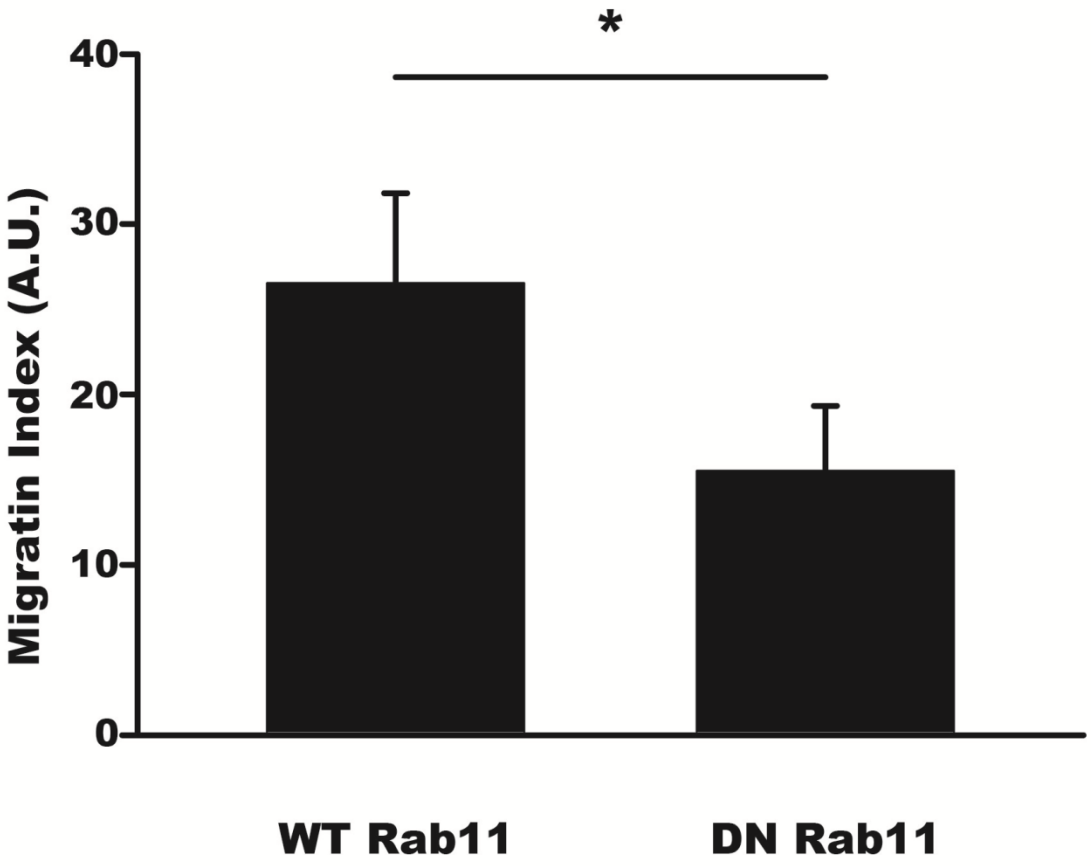
# Combine Nocodazole and lots of Arrb2



# Reduce trafficking increasing pERK




*
40
30
20
10
0
Migratin Index (A.U.)
WT Rab11
DN Rab11

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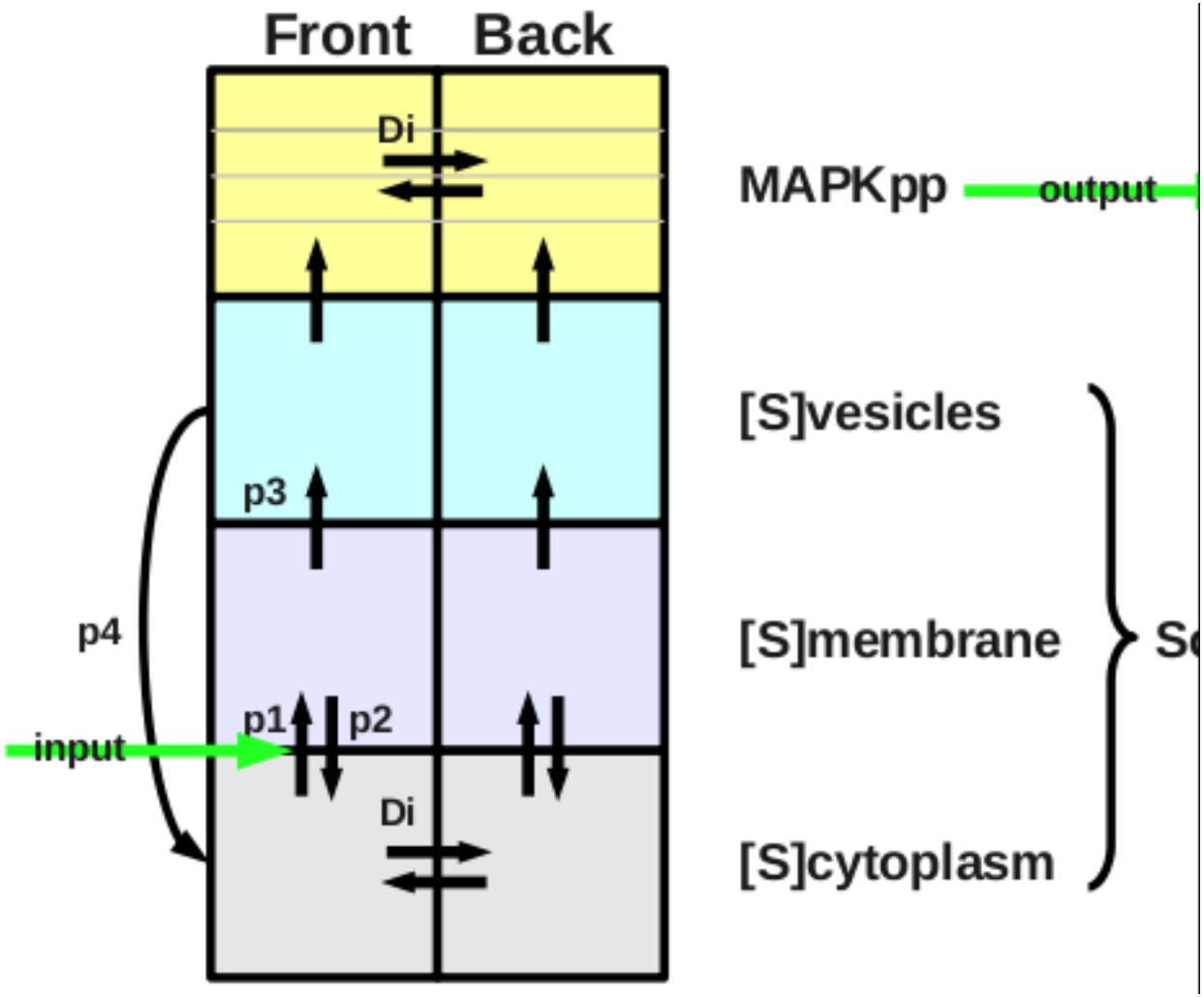
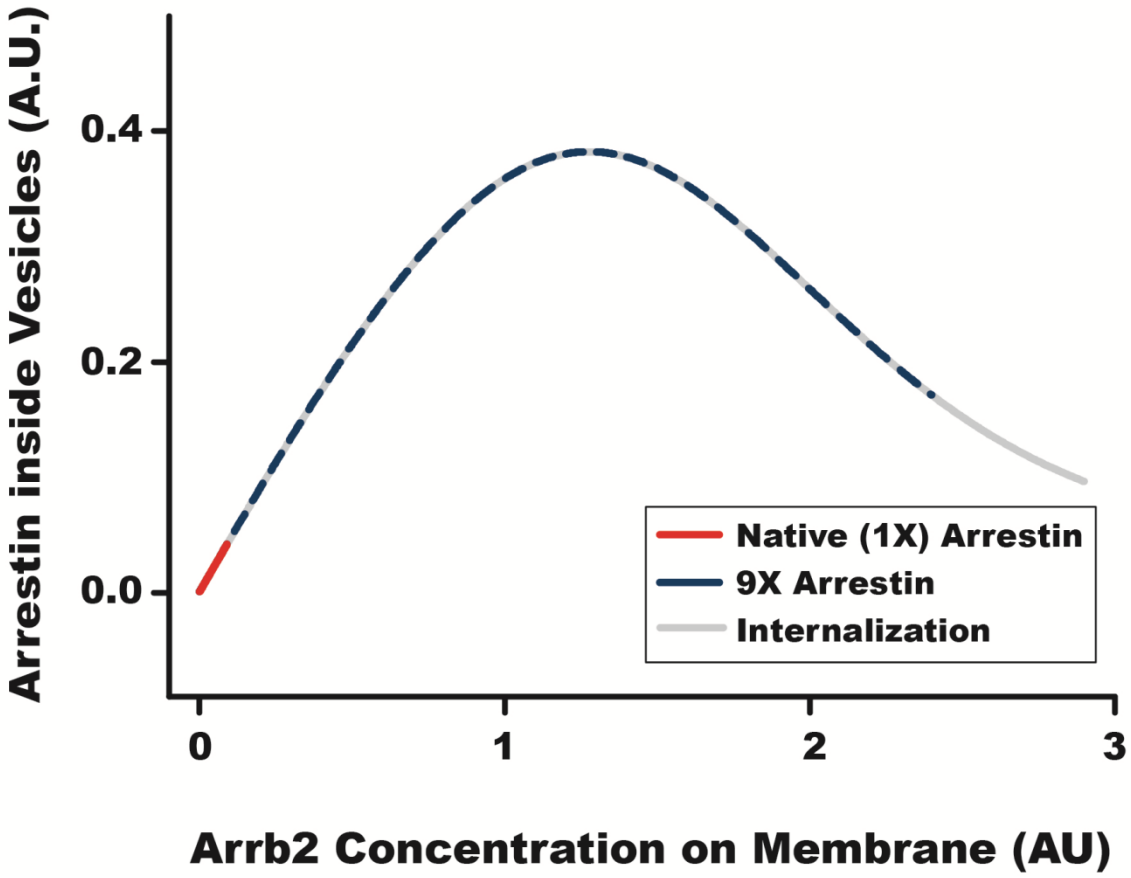
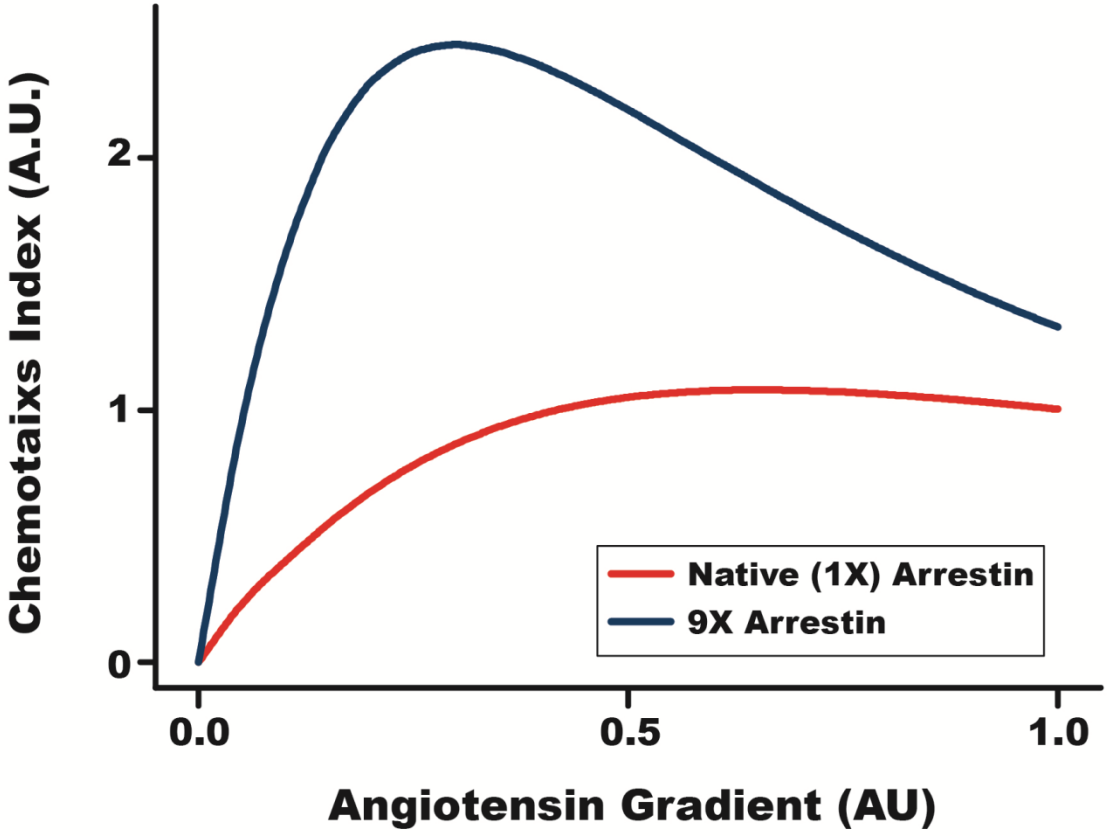
# Modeling by Adriel

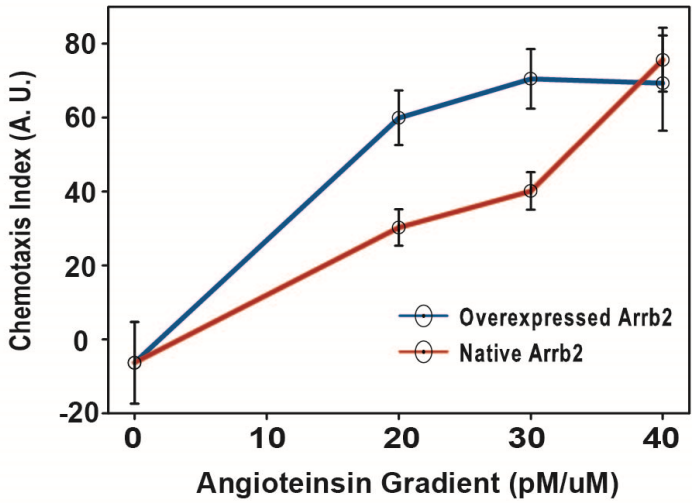



Arrestin inside Vesicles (A.U.)
0.4
0.2
0.0
0
1
2
3
Native (1X) Arrestin
9X Arrestin
Internalization
Arrb2 Concentration on Membrane (AU)

Modeling

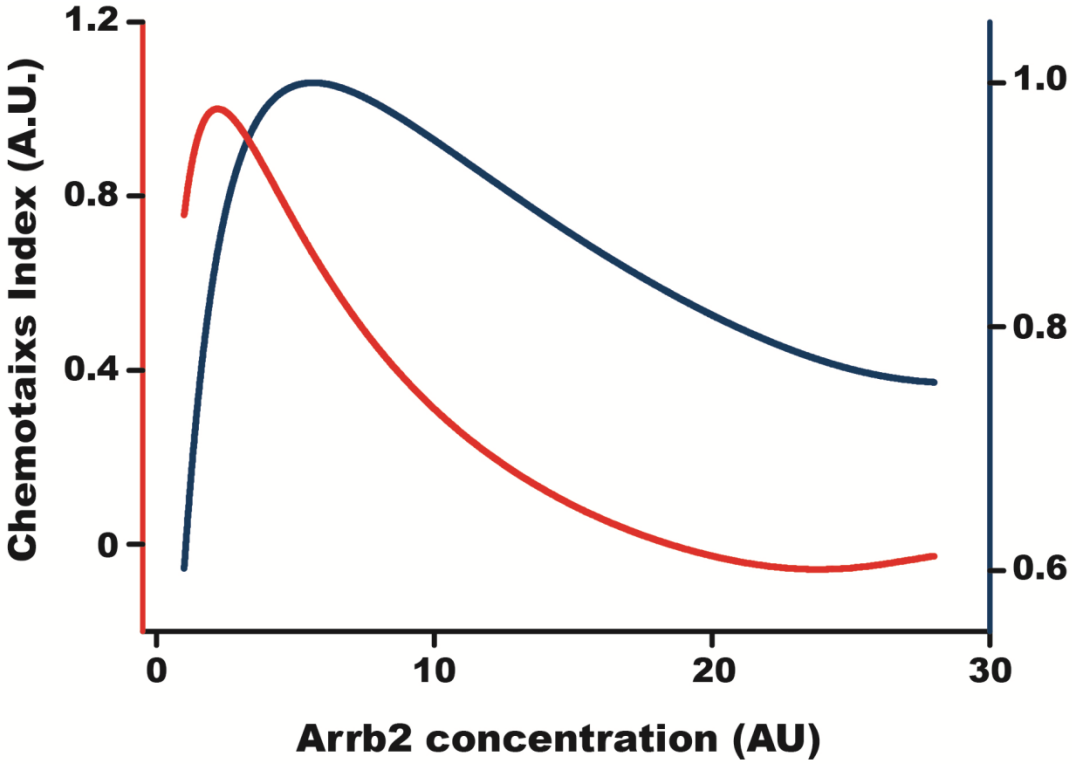
Experimental

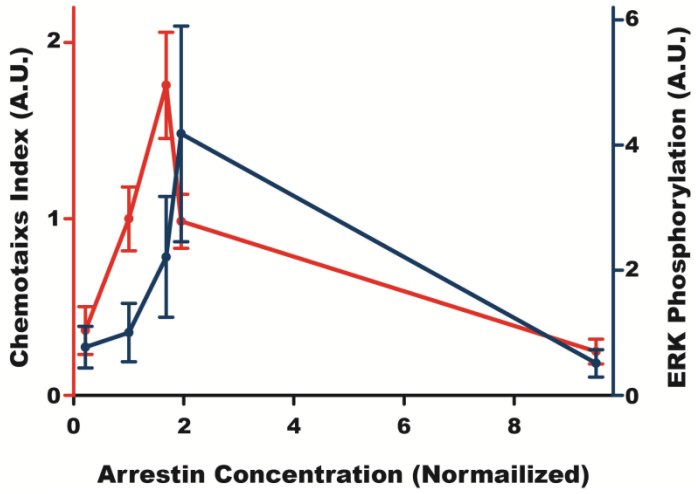


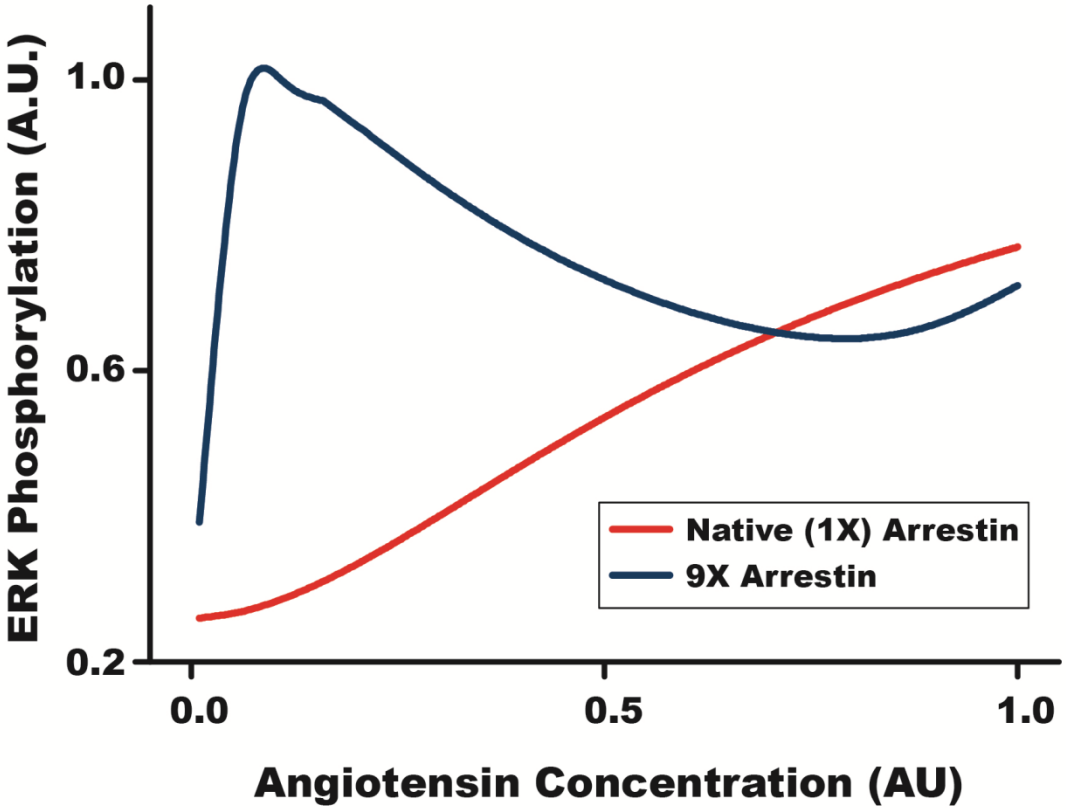
Modeling

Experimental

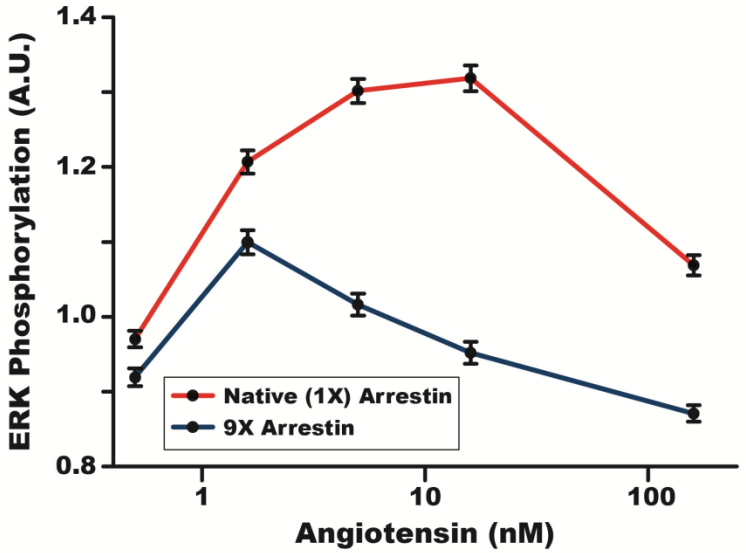


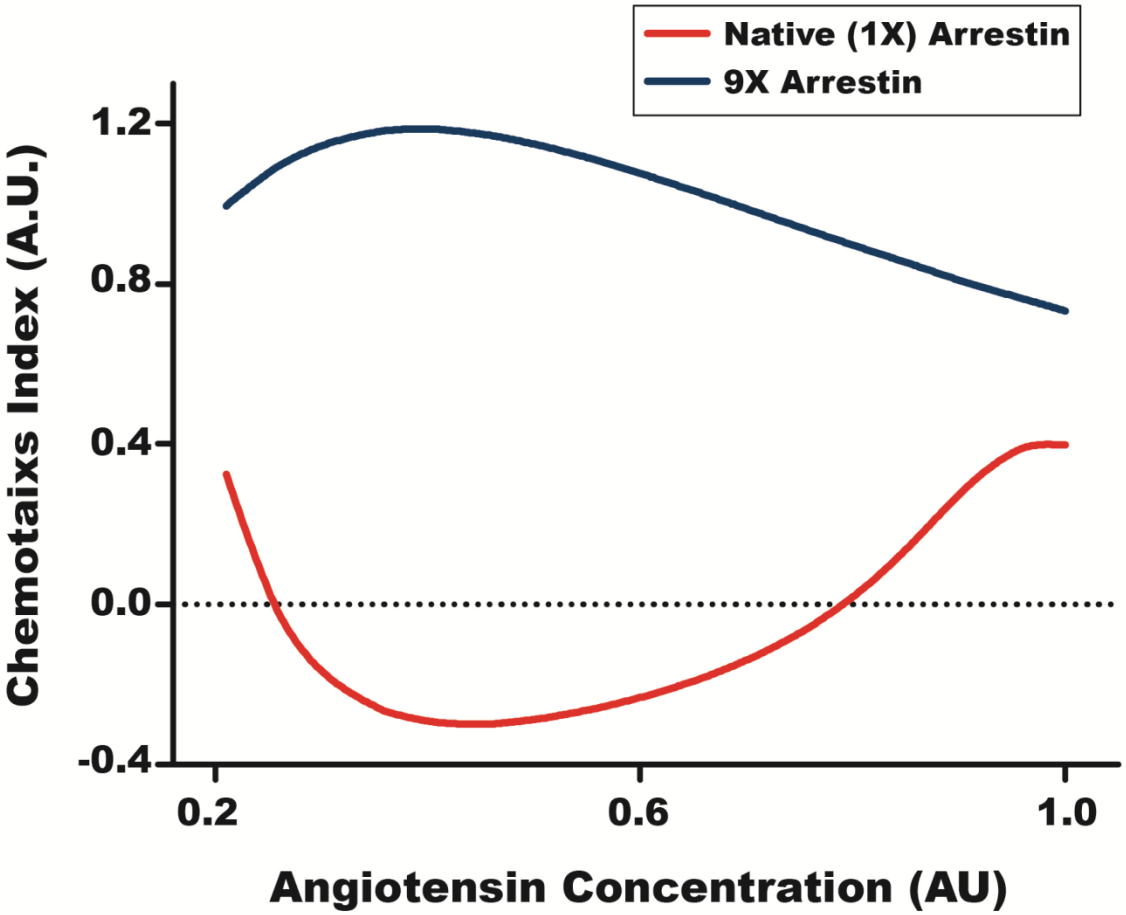
Modeling



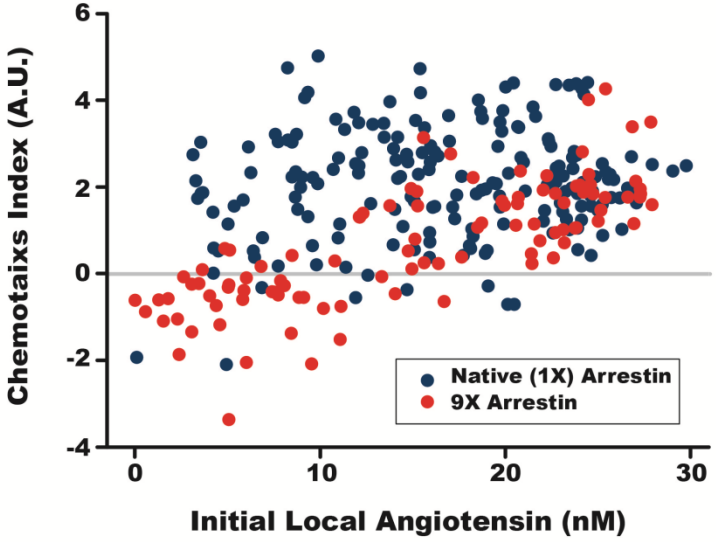

Experimental



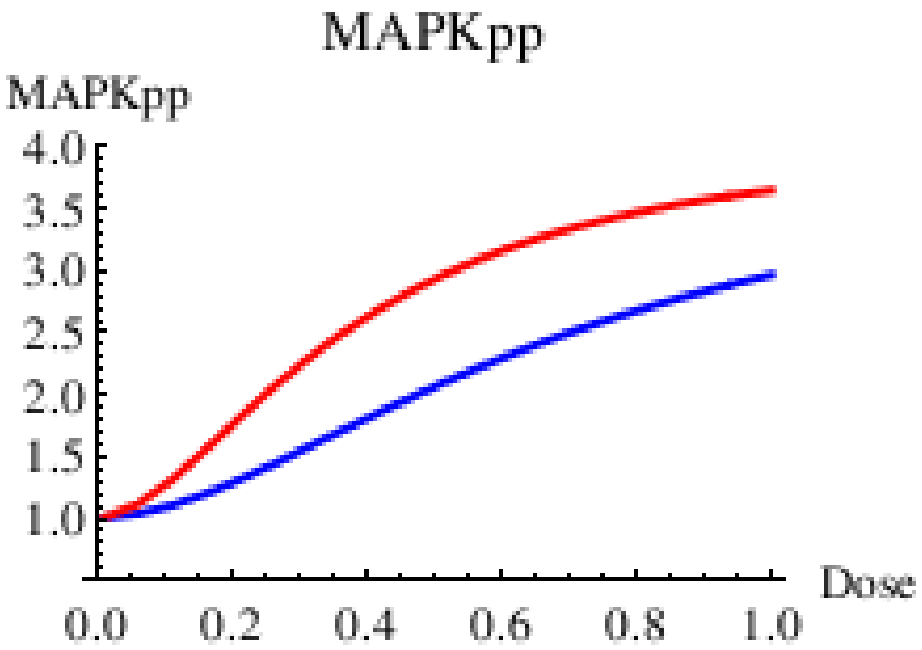
Modeling



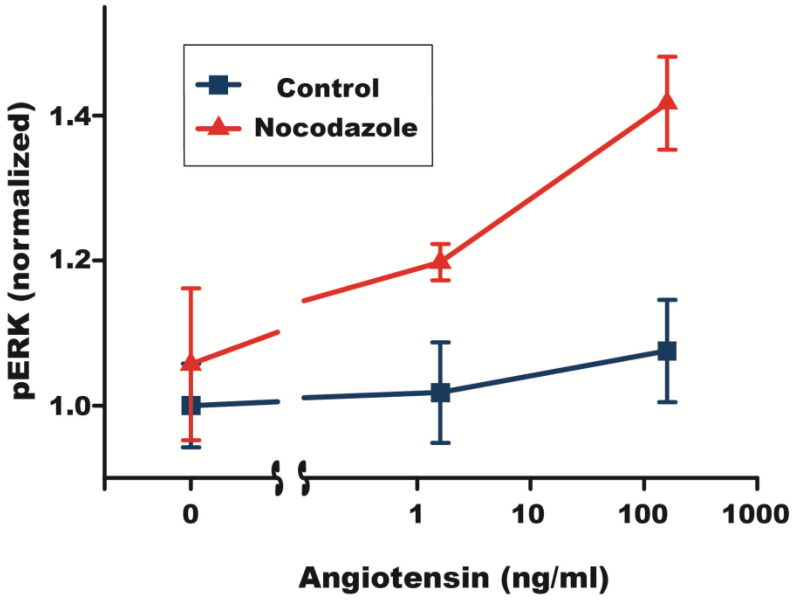

Experimental



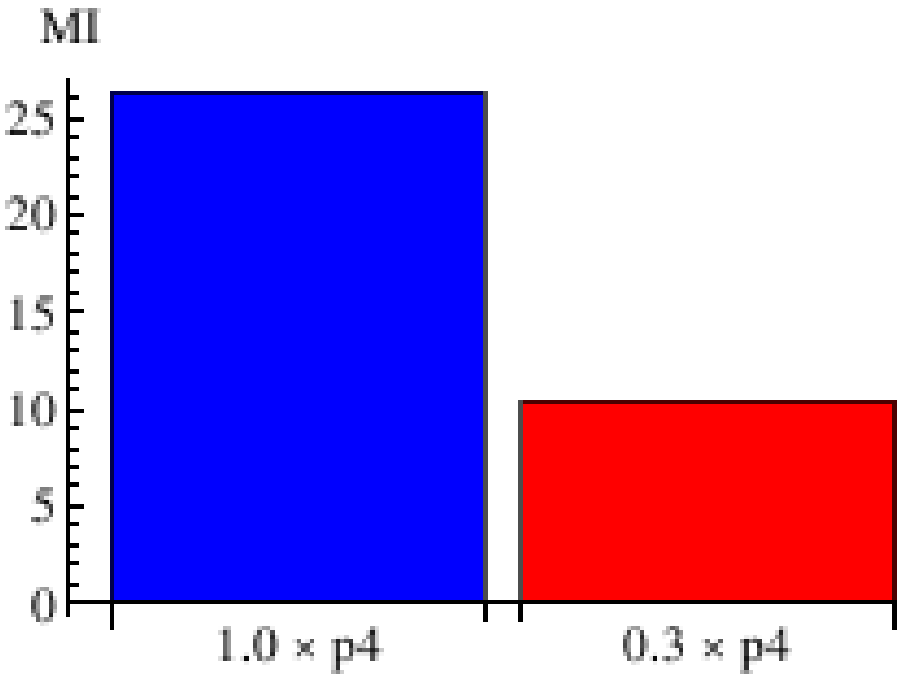
Modeling



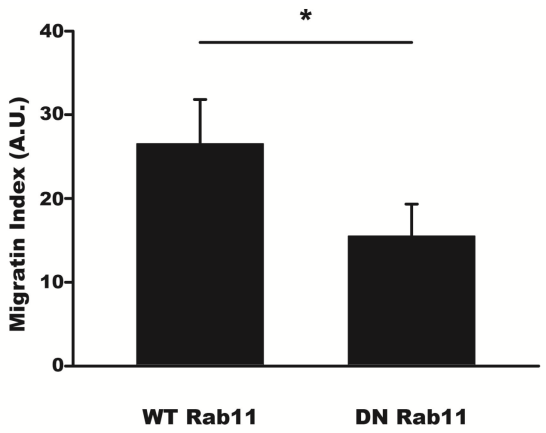

Experimental



Modeling




Experimental



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# Conclusions

- Arrb2 as: signal transducer, scaffold and amplifier
- Endosome recycling is important and controlled by Microtubule-Rab systems
- Simple model with simple rules coincide well with experiments

# Acknowledgement

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