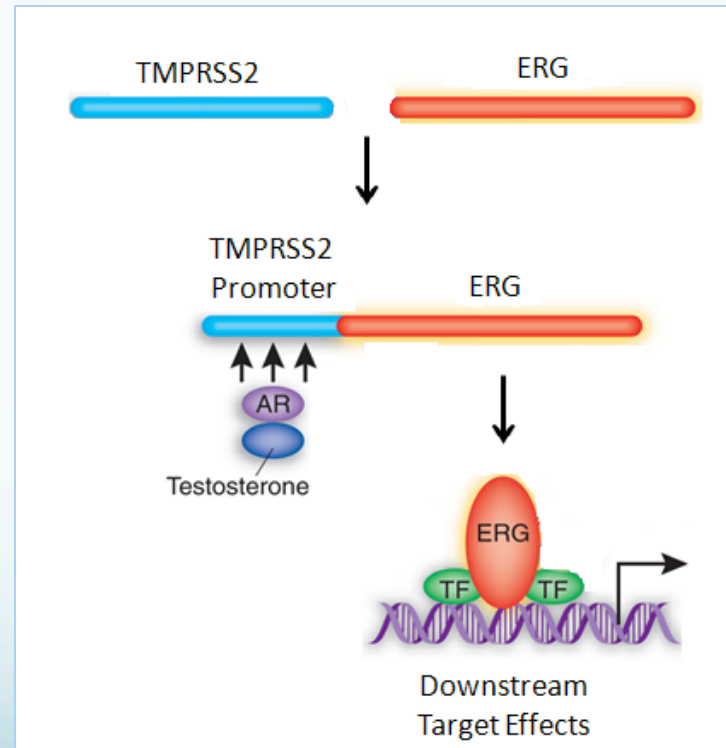


# Targeting Kinases Critical for TMPRSS2-ERG Function in Prostate Cancer

Kyle Nakatsuka  
David Takeda, MD, PhD  
Lab of William Hahn, M.D., Ph.D.  
Broad Institute  
Summer Research Program in Genomics

# TMPRSS2-ERG in Prostate Cancer

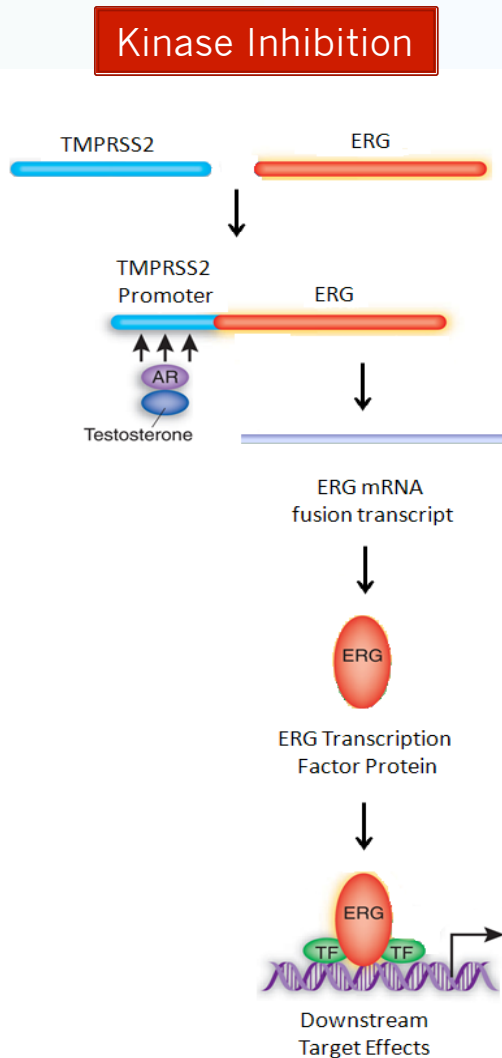
- TMPRSS2-ERG translocation causes ERG overexpression
  - Expressed in 50% of prostate cancers
  - Invasion, proliferation, survival
- Targets for prostate cancer therapy



# Kinase Screen Identifies Potential ERG Modulators

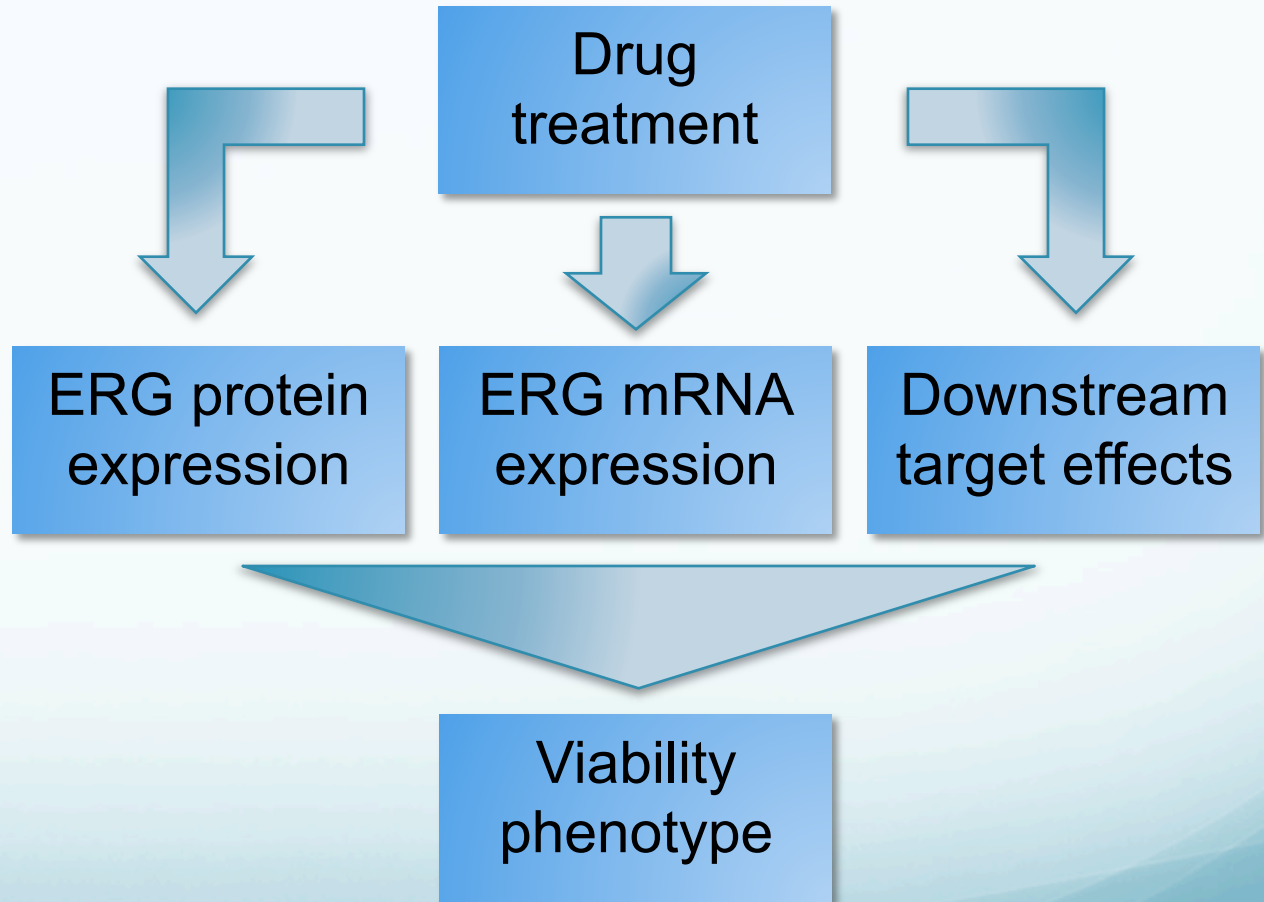
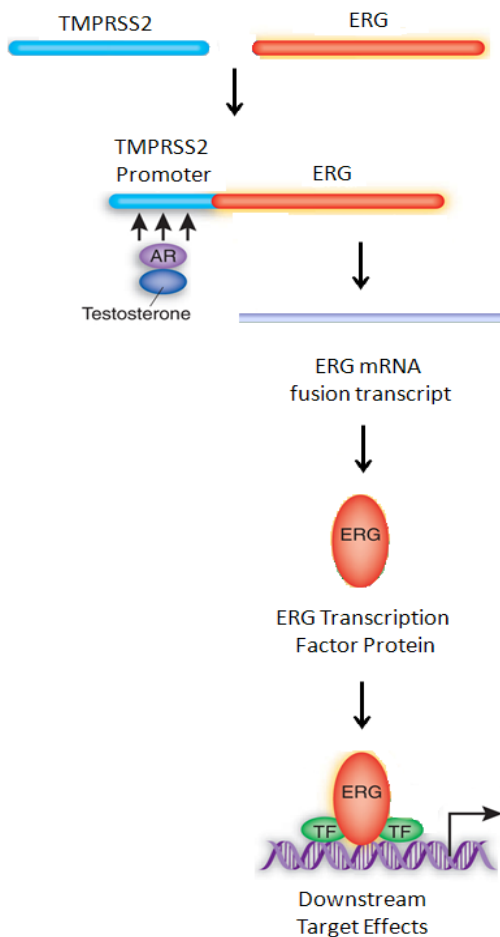
## Kinase Inhibition

- High-throughput screen identifies 40 kinases likely to affect ERG
  - Potential upstream modulators of ERG
  - Mechanisms unknown
- Several are targets of existing kinase-inhibiting drugs



# Methods

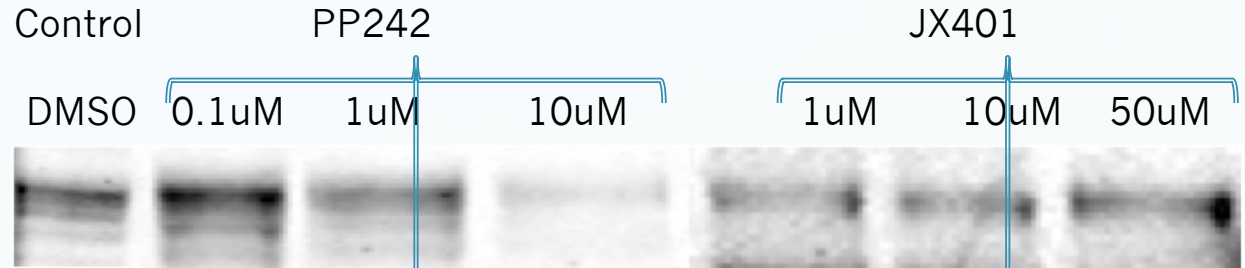
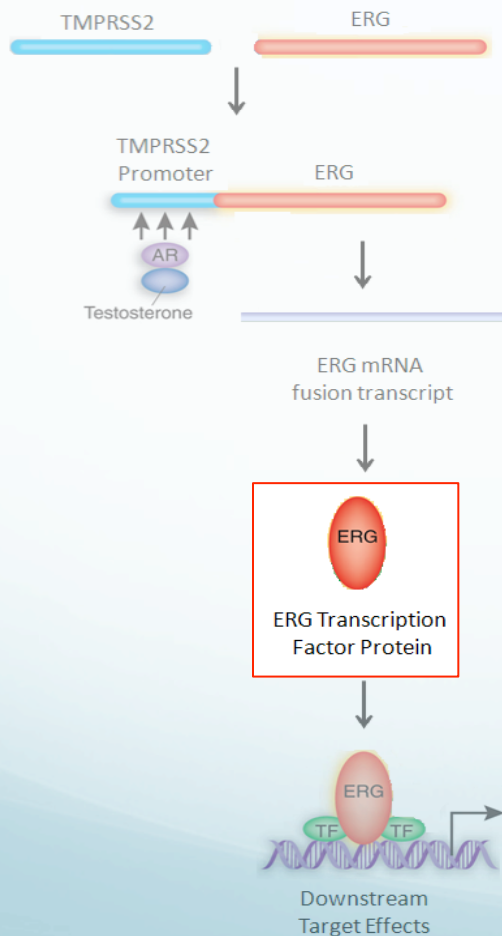
## Kinase Inhibition





# PP242 mTOR Inhibition Reduces ERG Protein Expression

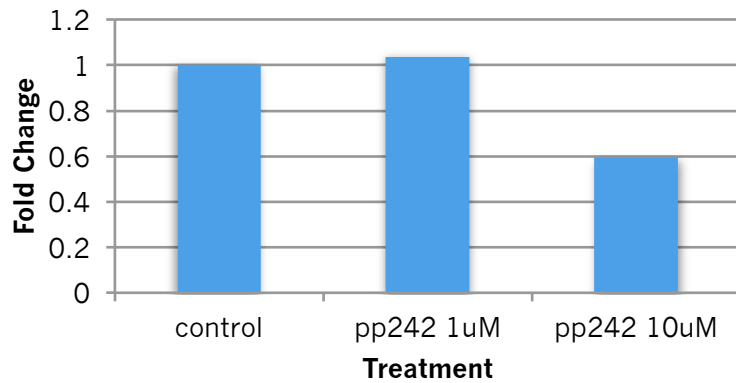
## Kinase Inhibition



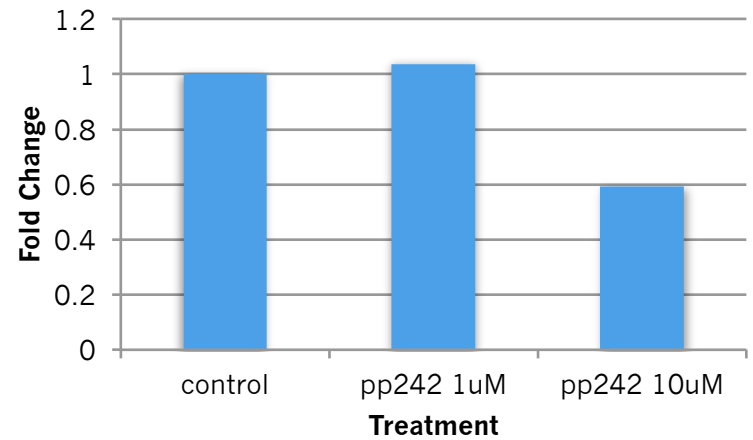
- Reduced protein expression with mTOR inhibition by PP242
- No significant change in protein expression with other drugs

## Downstream ERG Targets

### ARHGDIB mRNA Expression

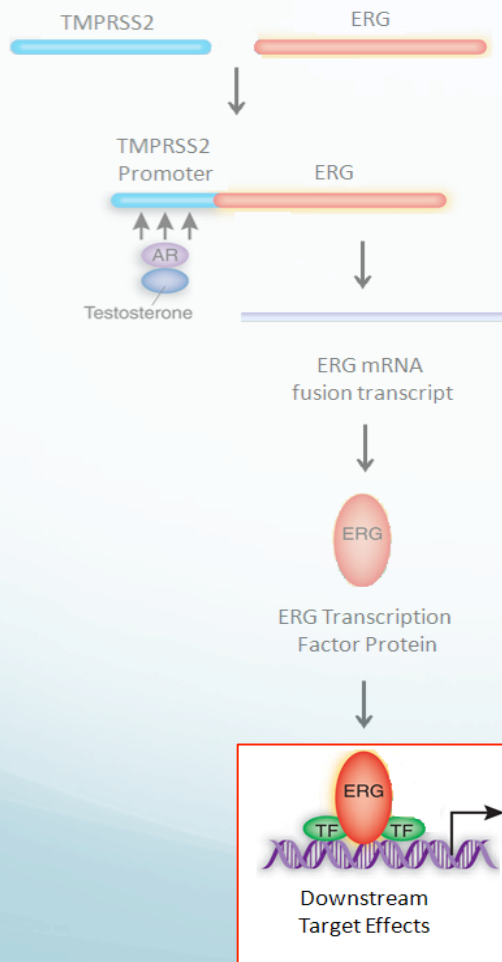


### PLA1A mRNA Expression

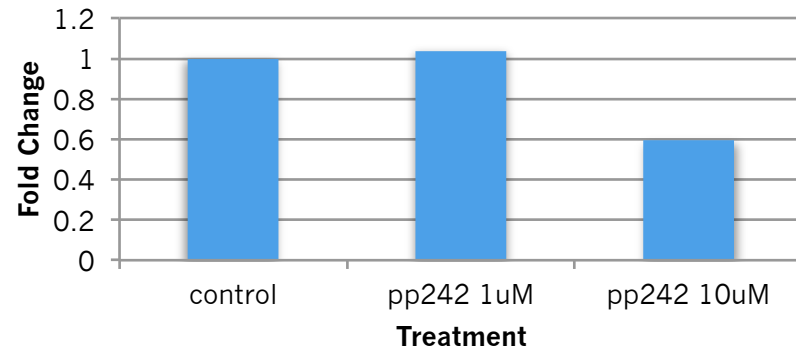


# PP242 mTOR inhibition downregulates ERG downstream target transcription

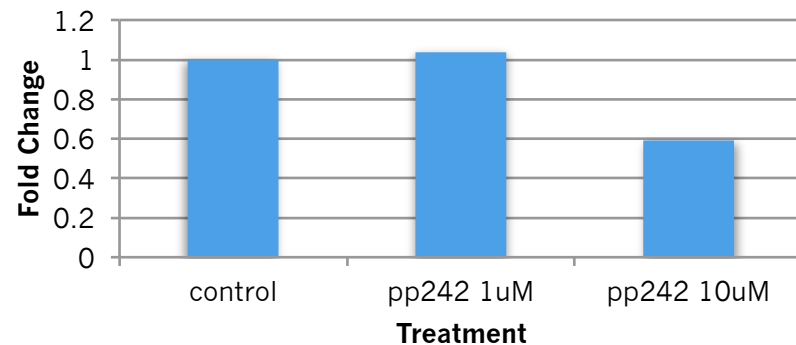
## Kinase Inhibition



## ARHGDIB mRNA Expression

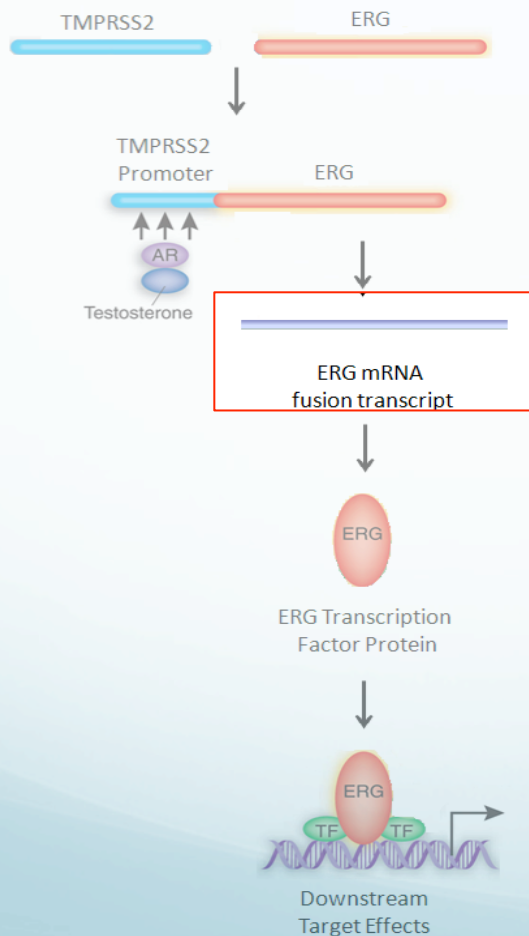


## PLA1A mRNA Expression

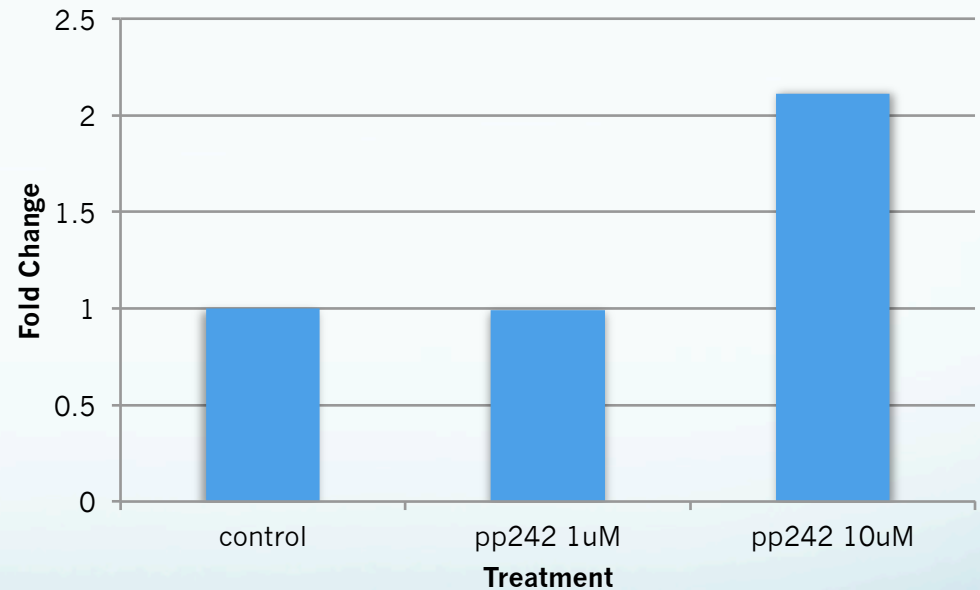


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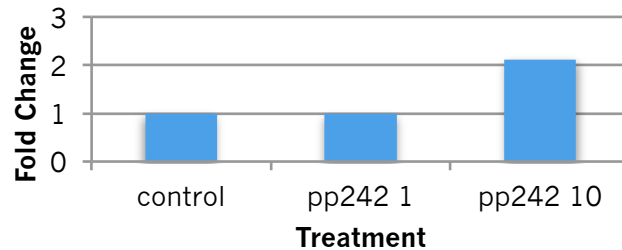


## mTOR Inhibition and ERG mRNA Expression

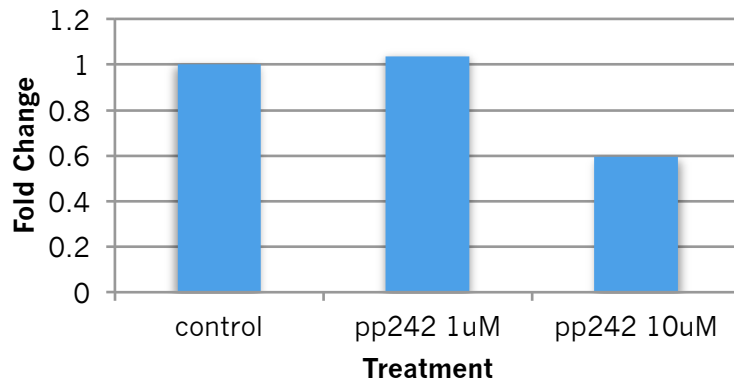


# mTOR Inhibition Reduces Downstream ERG Target Expression

## mTOR Inhibition and ERG mRNA Expression

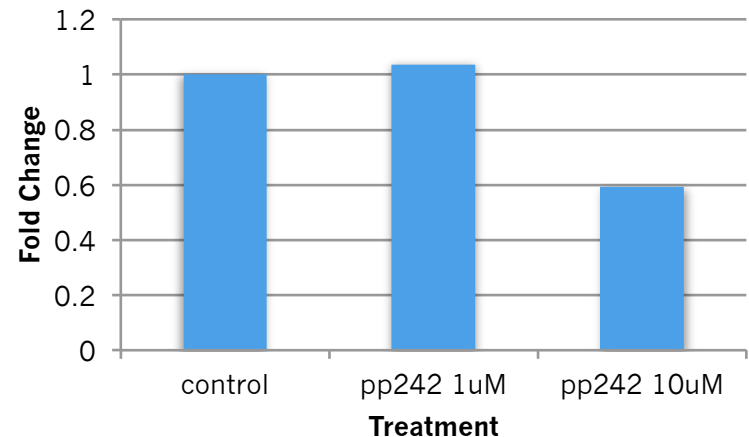


## ARHGDIB mRNA Expression



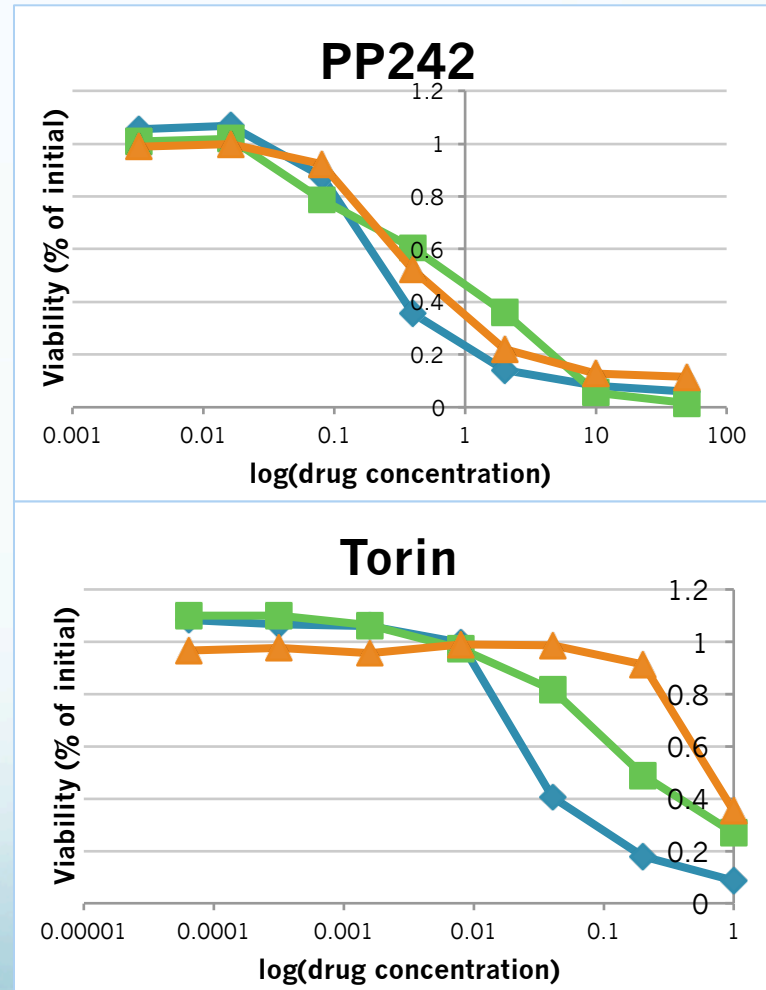
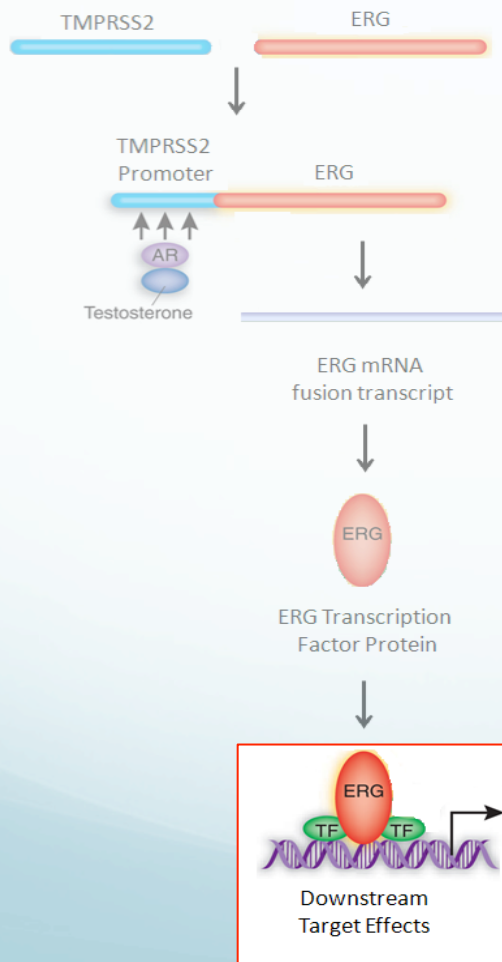
## Downstream ERG Targets

## PLA1A mRNA Expression



# Viability Phenotype

## Kinase Inhibition



# Conclusions

- mTOR inhibition reduces ERG protein levels, increases ERG transcription
  - Post-transcriptional ERG modification
- mTOR is an upstream regulator of ERG
  - Potential therapeutic target for ERG overexpressing tumors

# Future Directions

- Determine other phenotypic effects
  - Invasion, serum deprivation
- Confirm result in other mTOR inhibitor drugs
- Mechanism
  - Apply to other transcription factors



# Acknowledgements

## Hahn Lab

David Takeda

William Kim

Xiaoxing Wang

Diane Shao

Elizabeth Dwinell

Elsa Beyer

Mik Rinne

Leo Yao Luo

Yaara Zwang

William Hahn

## SRPG Program

Bruce Birren

Eboney Smith

Francie Latour

SRPG Students

# Questions?

# Results

## Cell treatment by various kinase inhibitor drugs



Toxicity by kinase inhibition is not specific to ERG

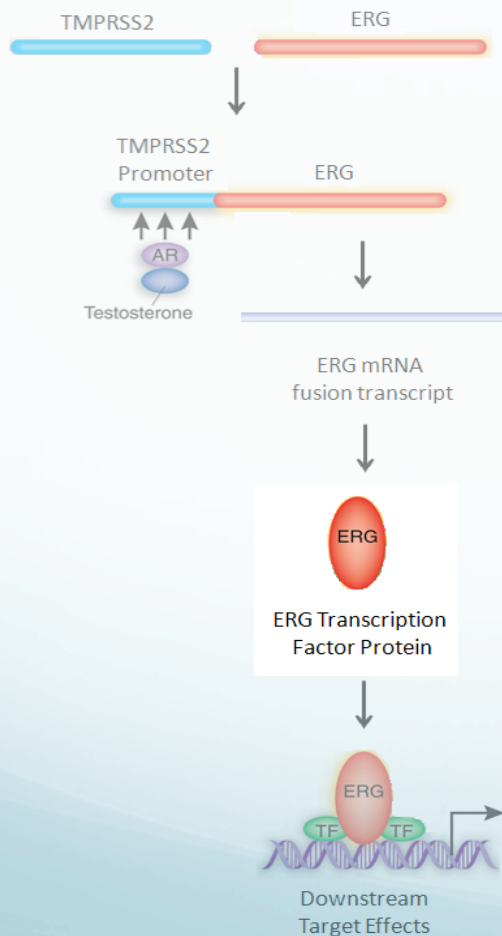
PP242 mTOR inhibition **downregulates** ERG protein expression

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# Methods

## Kinase Inhibition



Drug  
treatment

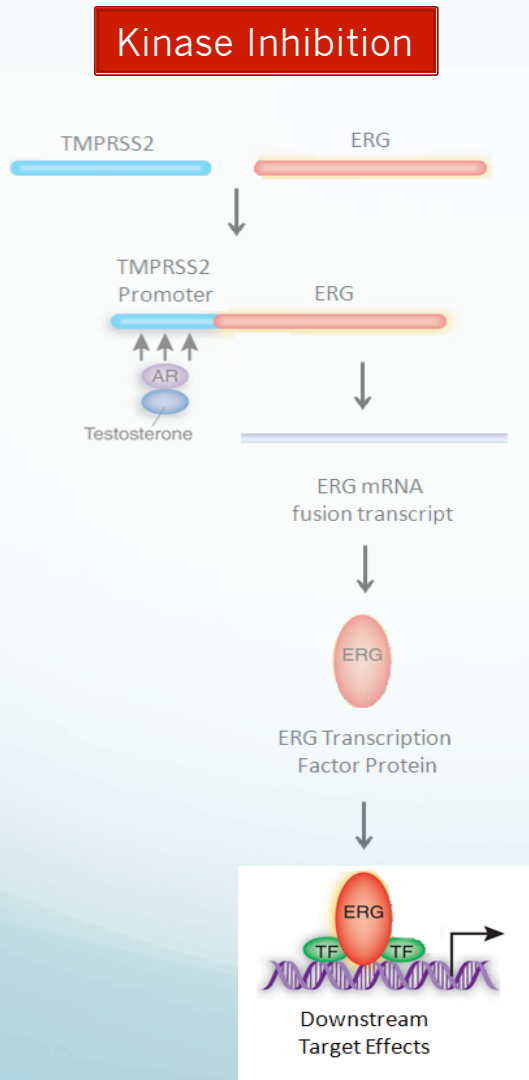
ERG protein  
expression

ERG mRNA  
expression

Downstream  
target effects

Viability  
phenotype

# Methods



Drug  
treatment

ERG protein  
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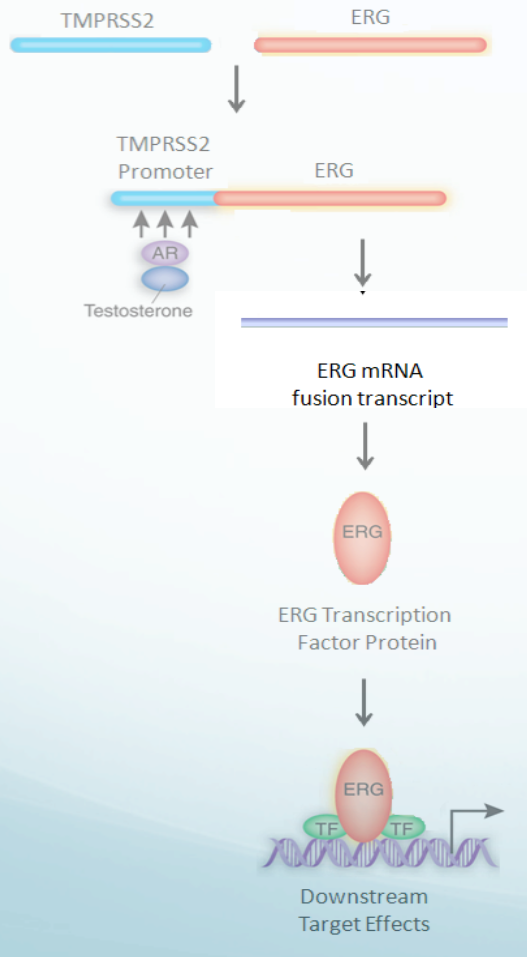
ERG mRNA  
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# Methods

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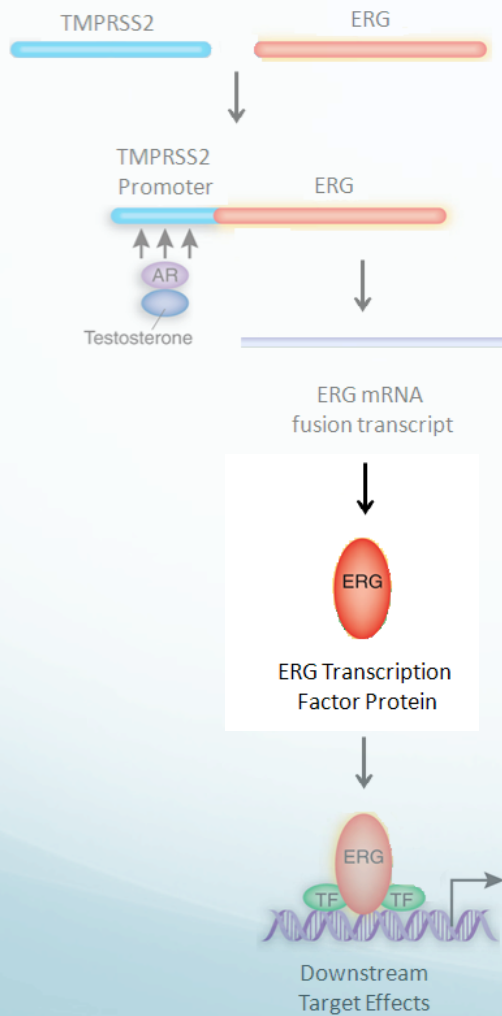
Drug  
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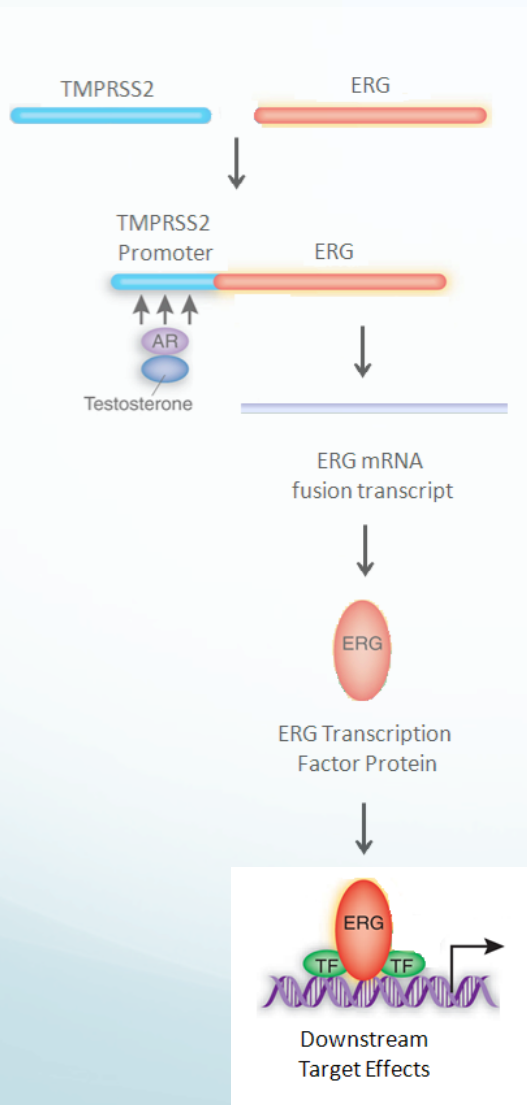
ERG protein  
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ERG mRNA  
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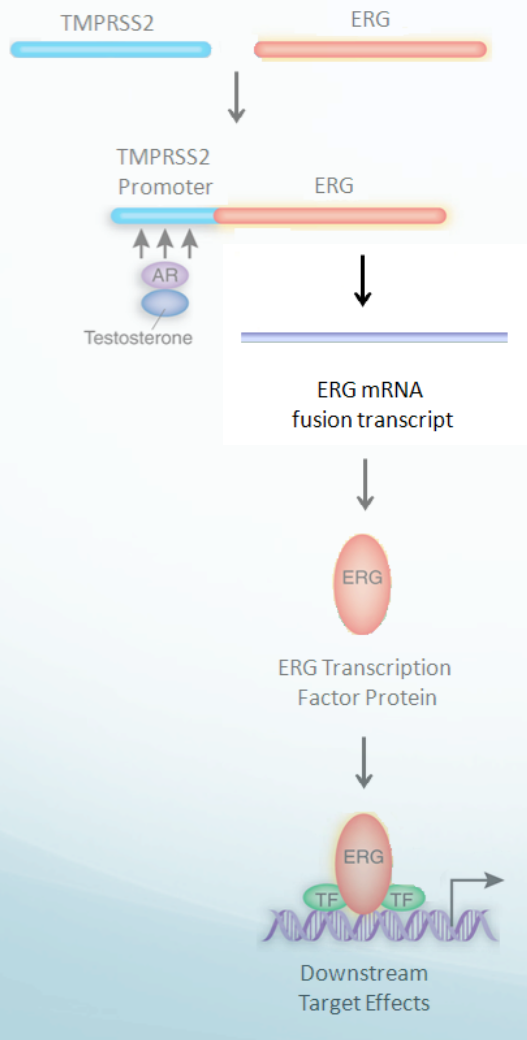
Downstream  
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# Methods

HT kinase screen identifies 40 ERG modulators



Cell treatment by various kinase inhibitor drugs



ERG protein  
expression by  
Western Blot



ERG mRNA  
expression  
by qRT-PCR



Downstream  
target mRNA  
Expression

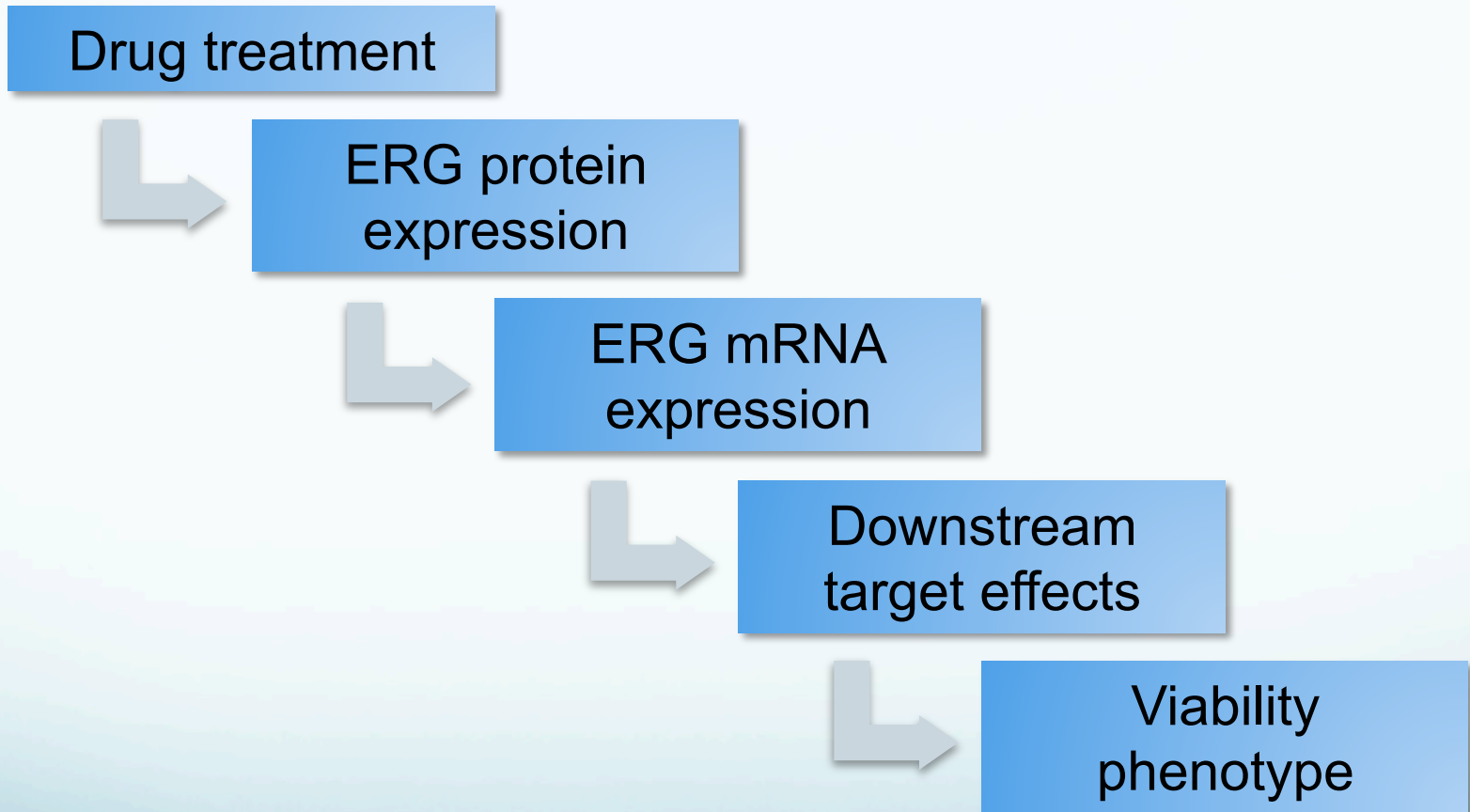


Mechanism,  
other mTOR  
inhibitors, etc.



Viability by  
cell titer-glo  
ATP assay

# Methods



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HT kinase screen identifies 40 ERG modulators

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expression  
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Downstream  
target mRNA  
Expression

# Methods

- Hypothesis:
  - If the kinases identified by the shRNA screen are involved in the ERG pathway, then their inhibition by drugs will reduce downstream signaling and phenotype

# Kinase Inhibition and Viability

IC50 Across Cell types

	LNCaP	VCaP (ERG)	PC3
<b>BIX</b>	12	24	36
<b>Olaparib</b>	9.0	-	-
<b>JX401</b>	32	34	46
<b>SB</b>	38	-	-
<b>PP242</b>	0.30	1.0	0.50
<b>Torin</b>	0.040	0.20	0.80
<b>nilotinib</b>	6.0	25	7.5
<b>BX912</b>	10	-	2.0

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# Results

Cell treatment by various kinase inhibitor drugs



Viability by  
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ATP assay



ERG protein  
expression by  
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ERG mRNA  
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Downstream  
target mRNA  
Expression

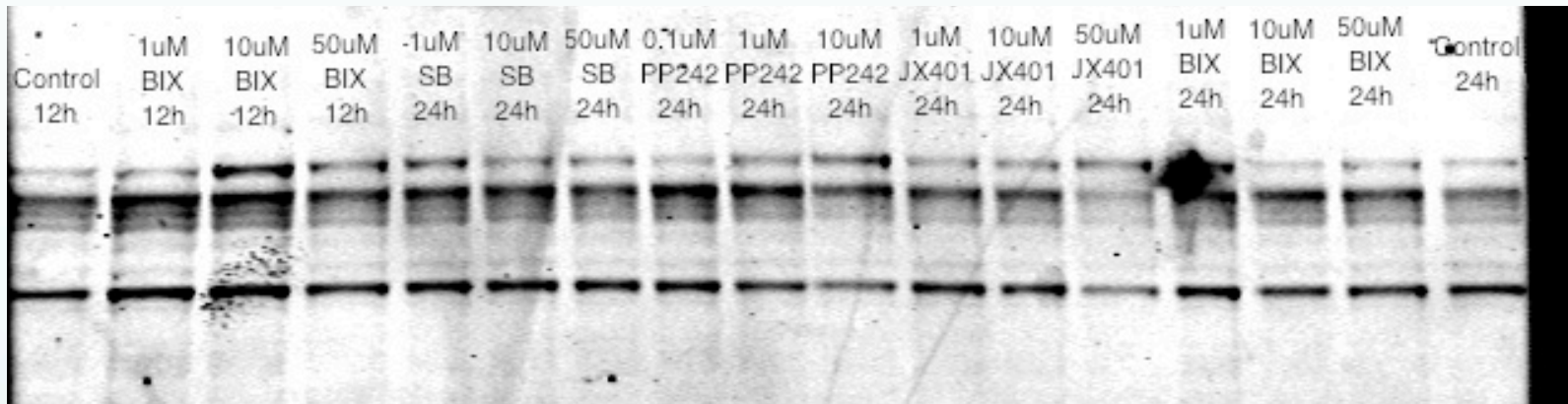


Mechanism,  
other mTOR  
inhibitors, etc.

Toxicity by kinase  
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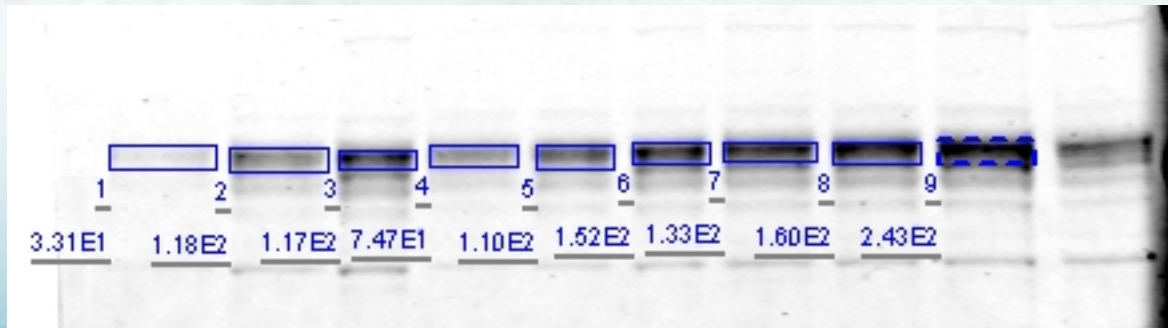
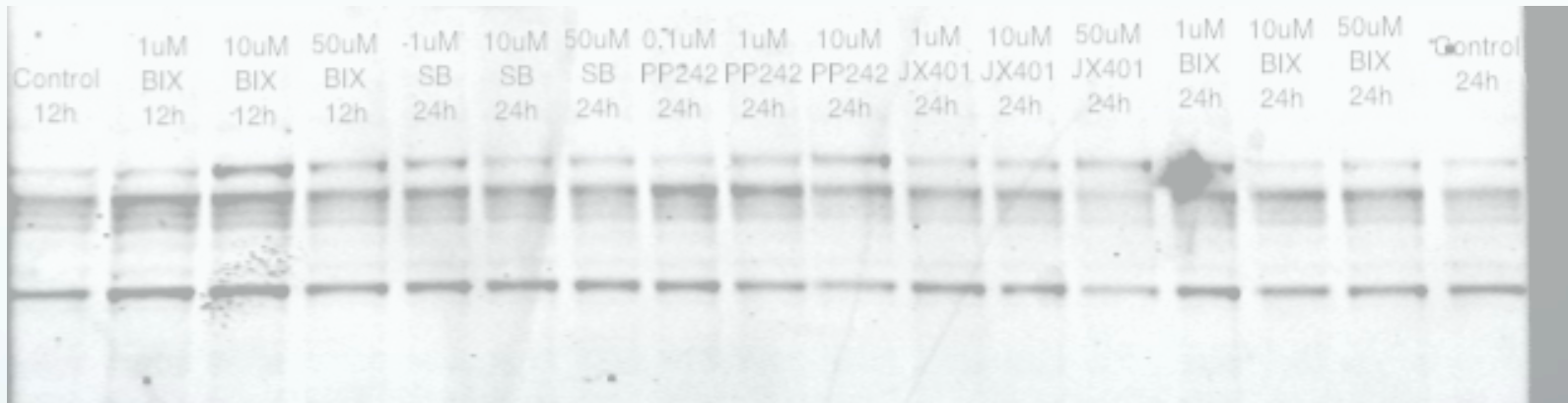
# Results

- Reduced protein expression at 12, 24 hours



# Results

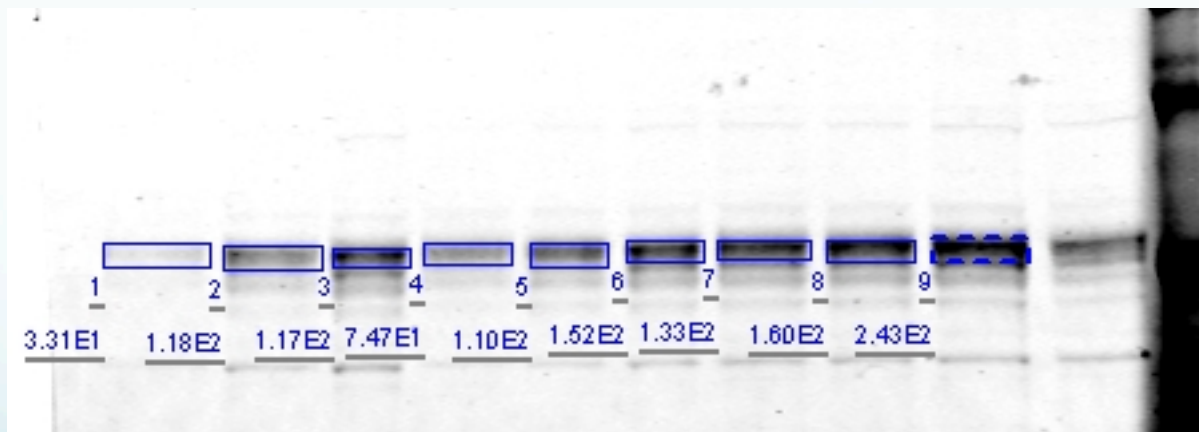
- Reduced protein expression at 12, 24 hours





# Results

- Reduced protein expression at 12, 24 hours
- Quantification? Incluir todo con actin?



# Results

Cell treatment by various kinase inhibitor drugs



Viability by  
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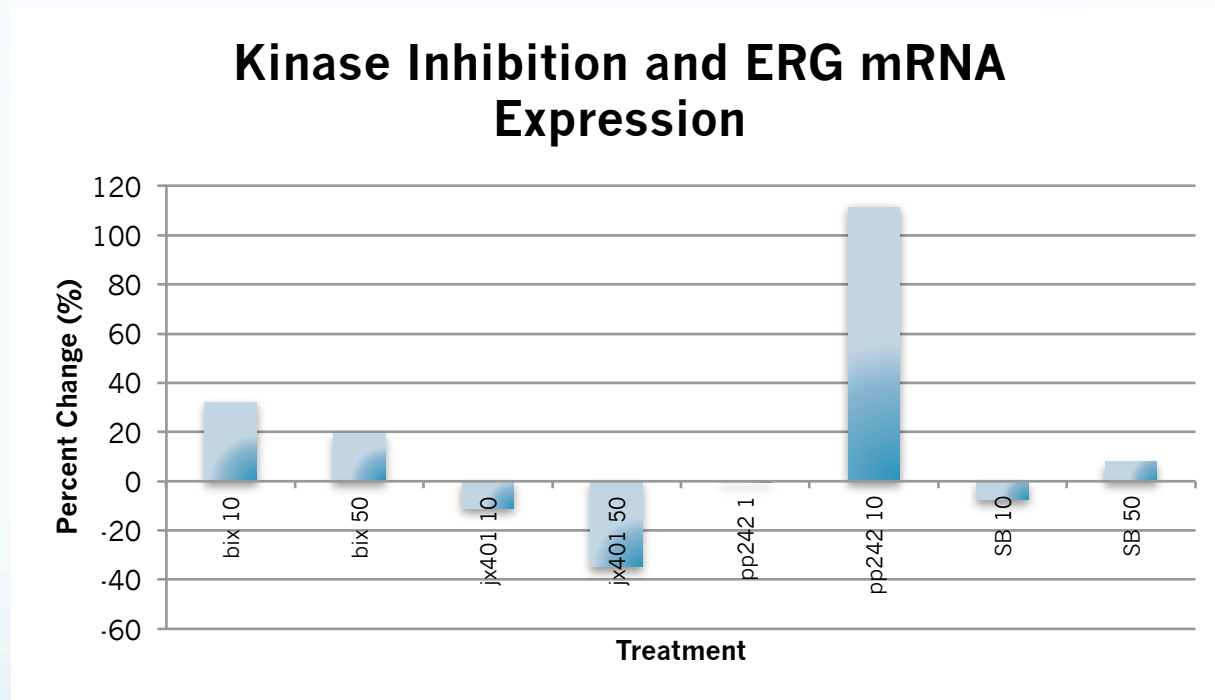


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PP242 mTOR  
inhibition  
downregulates  
ERG protein  
expression

# PP242 Increases ERG Transcription



Drug	Inhibits	Measured Effect
PP242	mTOR	Increased ERG mRNA levels

# Results

## Cell treatment by various kinase inhibitor drugs



Toxicity by kinase inhibition is not specific to ERG

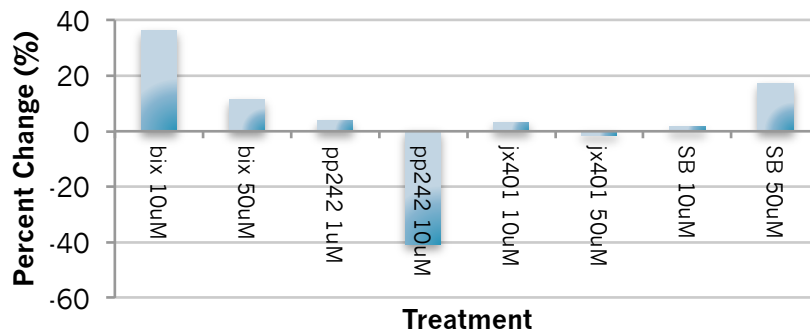
PP242 mTOR inhibition **downregulates ERG protein expression**

PP242 mTOR inhibition **upregulates ERG mRNA expression**

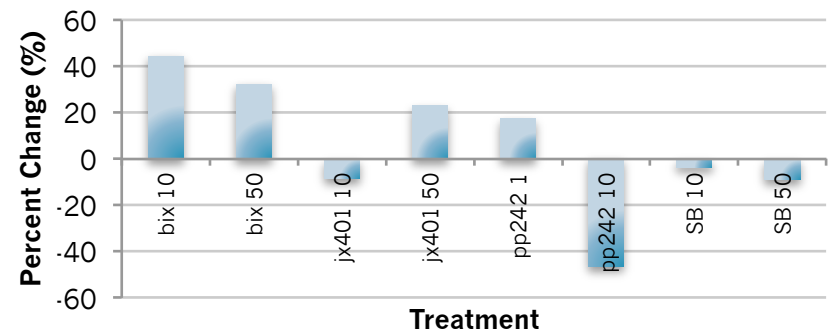
# Kinase Inhibition and ERG Target Transcription

- PLA1A and ARGHD1B are downstream targets of ERG

**Kinase Inhibition and ARGHDIB Transcription**



**Kinase Inhibition and PLA1A Transcription**



Drug	Inhibits	Measured Effect
PP242	mTOR	Reduced ERG target mRNA
BIX	MEK	Increased ERG target mRNA