

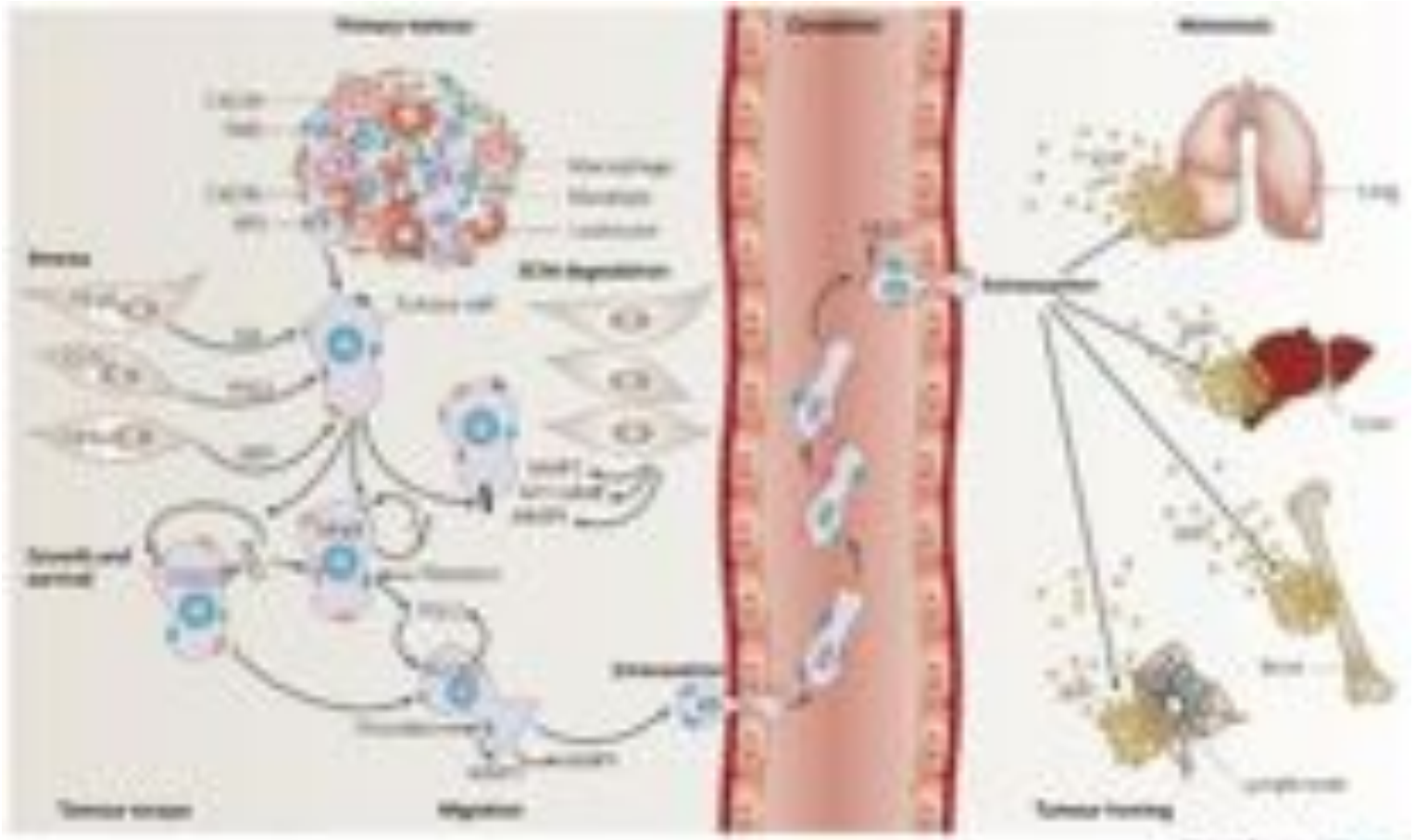
# A Translational Investigation of Metastasis

Ning Zhang  
Tianjin Medical University



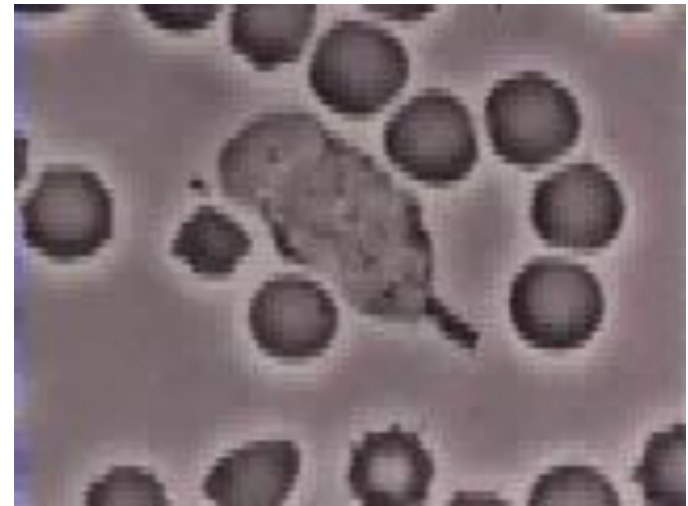
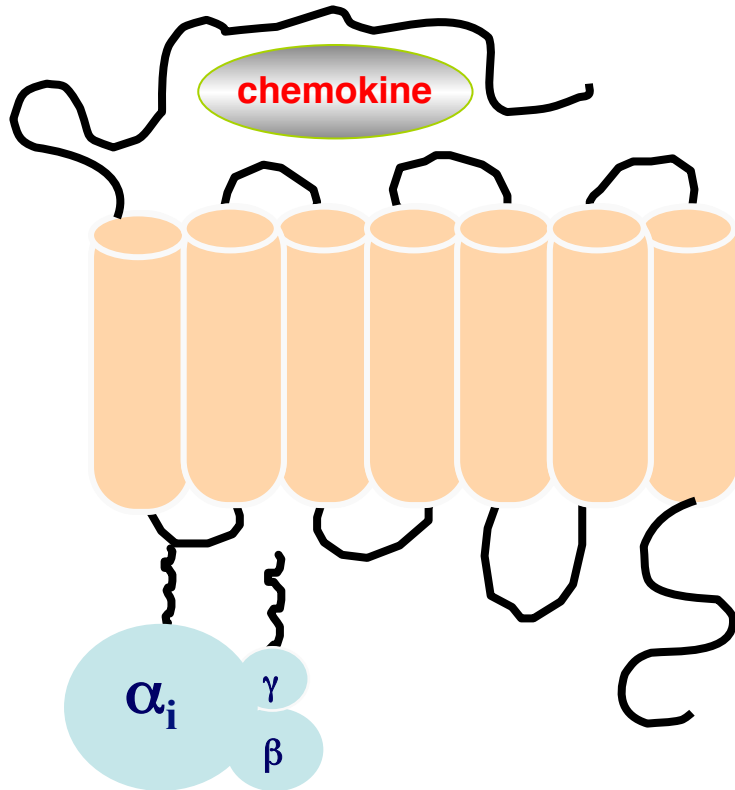


# Metastasis of Cancer Cells





# What is Chemotaxis?

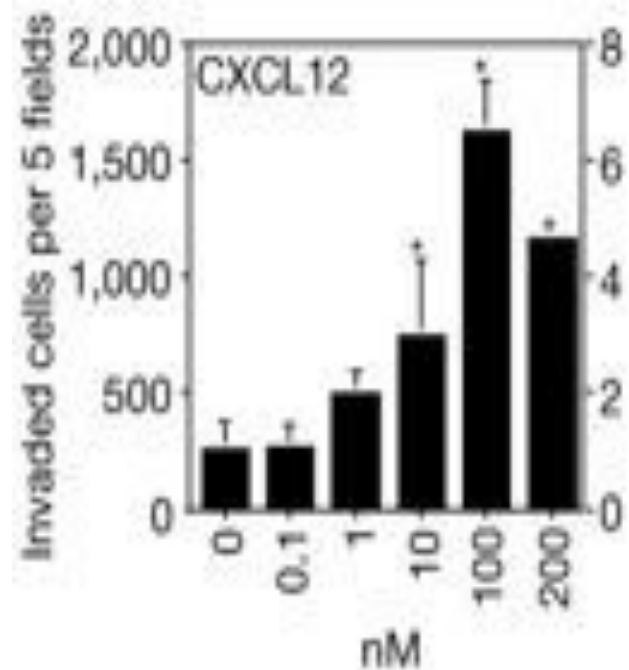
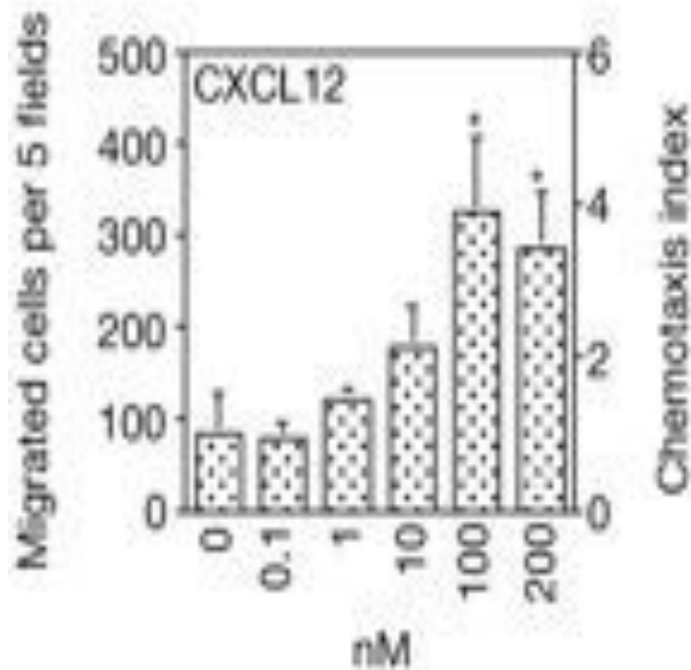


- Leukocyte trafficking
- Angiogenesis
- Wound healing
- Brain Development
- HIV infection



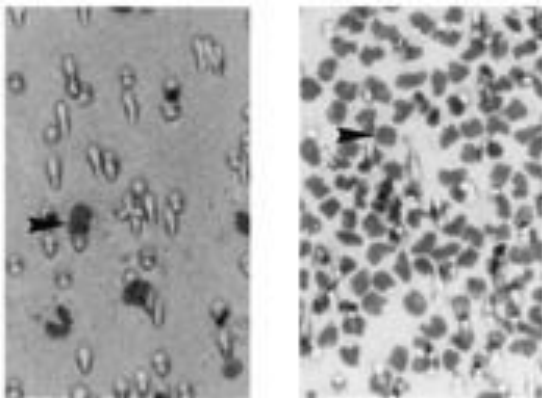
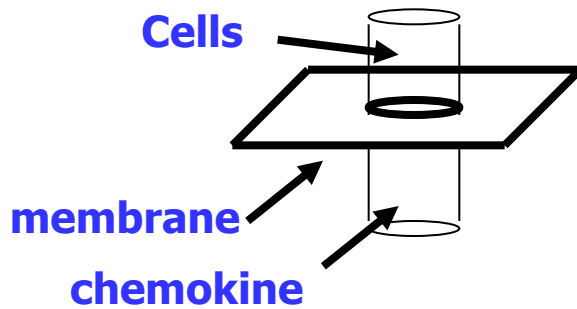
# Correlation between Chemotaxis and Cancer Metastasis

CXCR4 mediates chemotaxis of human breast cancer cells

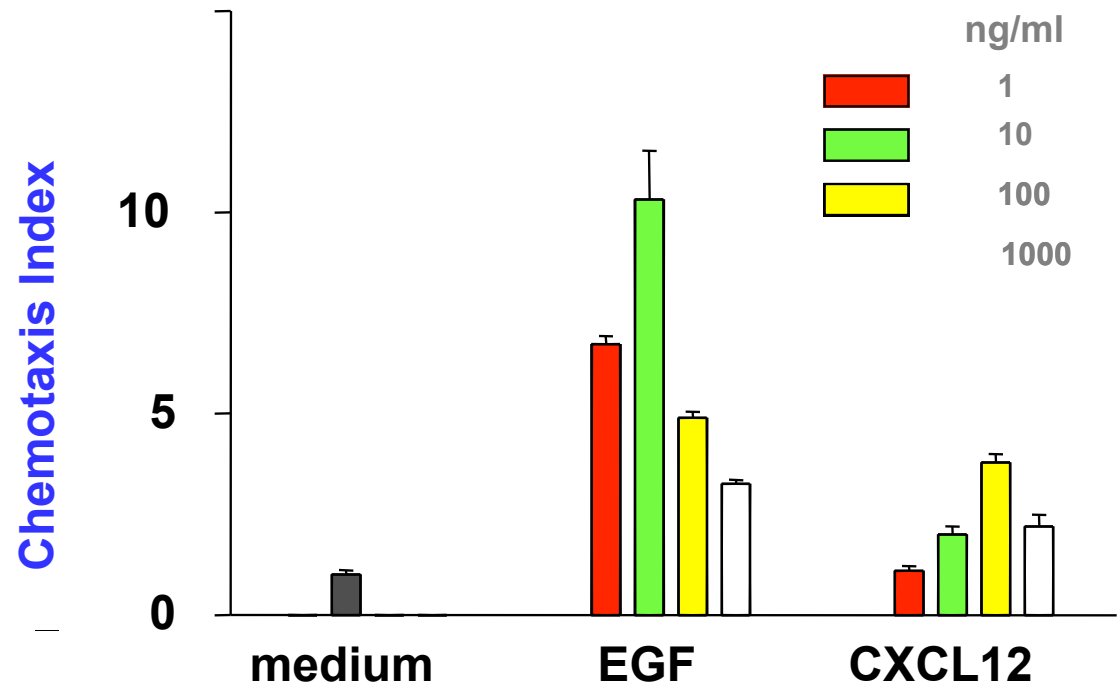


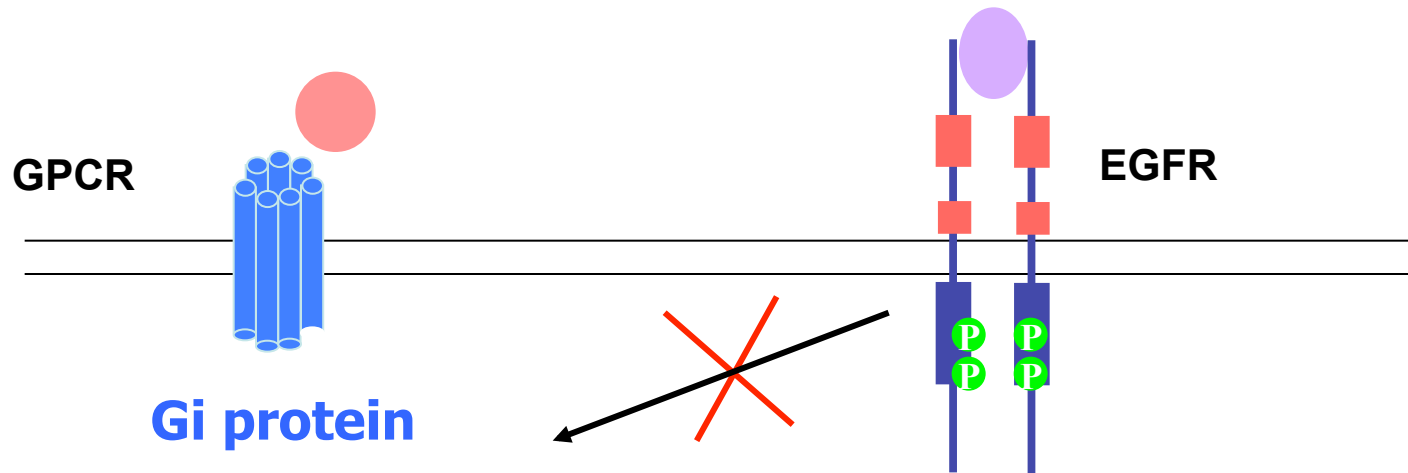


# EGF is a more potent chemoattractant than CXCL12



MDA-MB-231 cells





**PLC $\beta$ II**

**PLC $\gamma$**

**?**

**Chemotaxis**



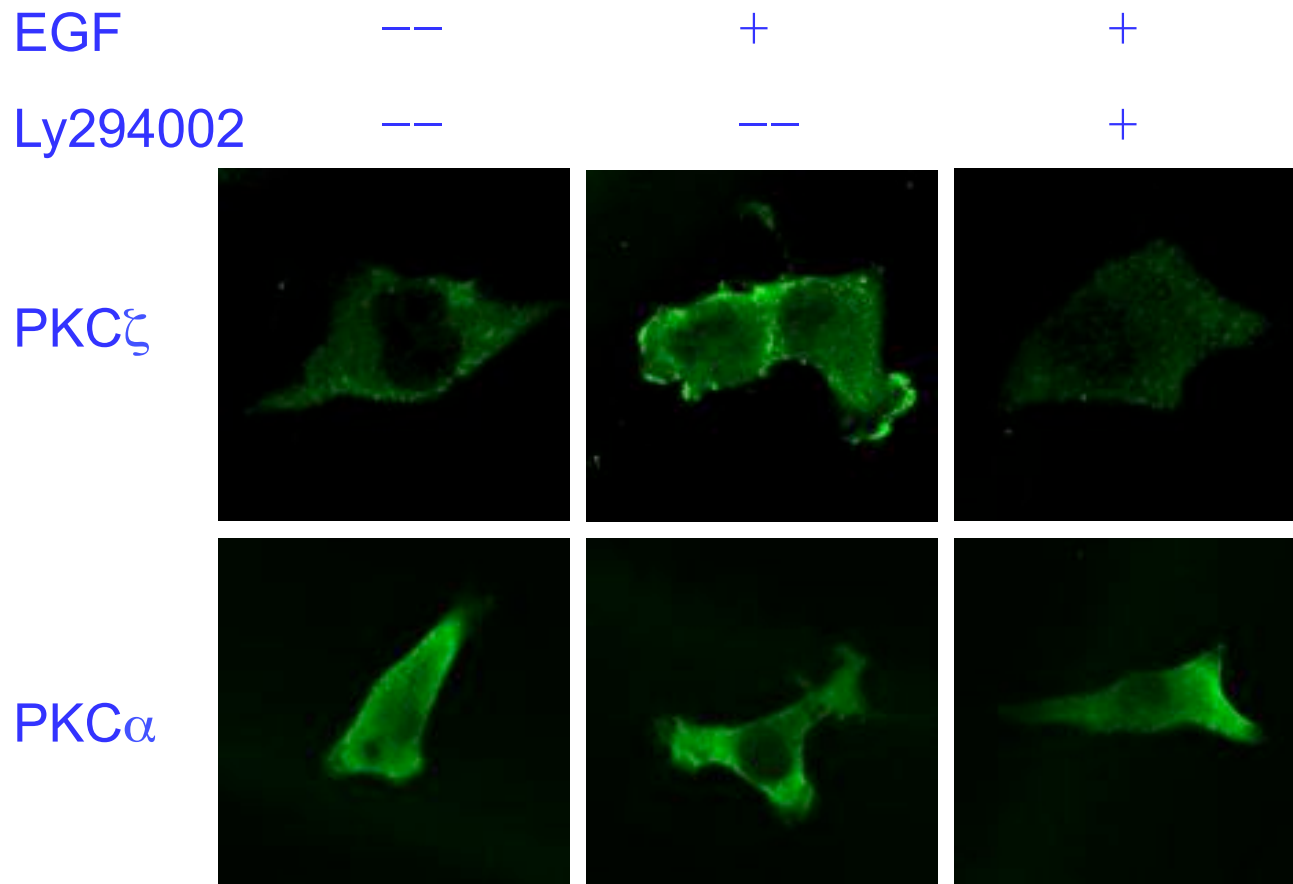
# PKC Family

$\beta I$ ,  $\beta II$ ,  $\gamma$

- Novel, DAG-dependent, such as  $\delta$ ,  $\epsilon$ ,  $\theta$ ,  $\eta$ .
- Atypical, doesn't require either  $Ca^{2+}$  or DAG, such as  $\zeta$  and  $\lambda$ .



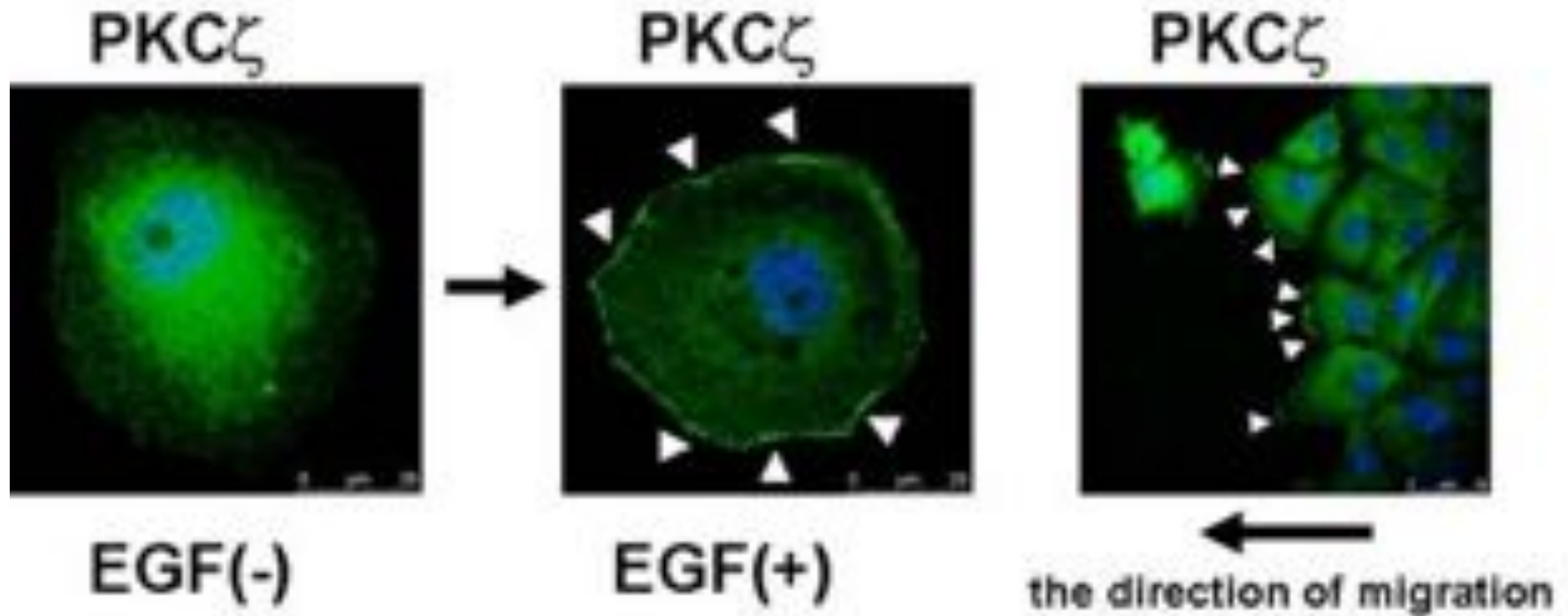
# EGF induces PKC $\zeta$ translocation





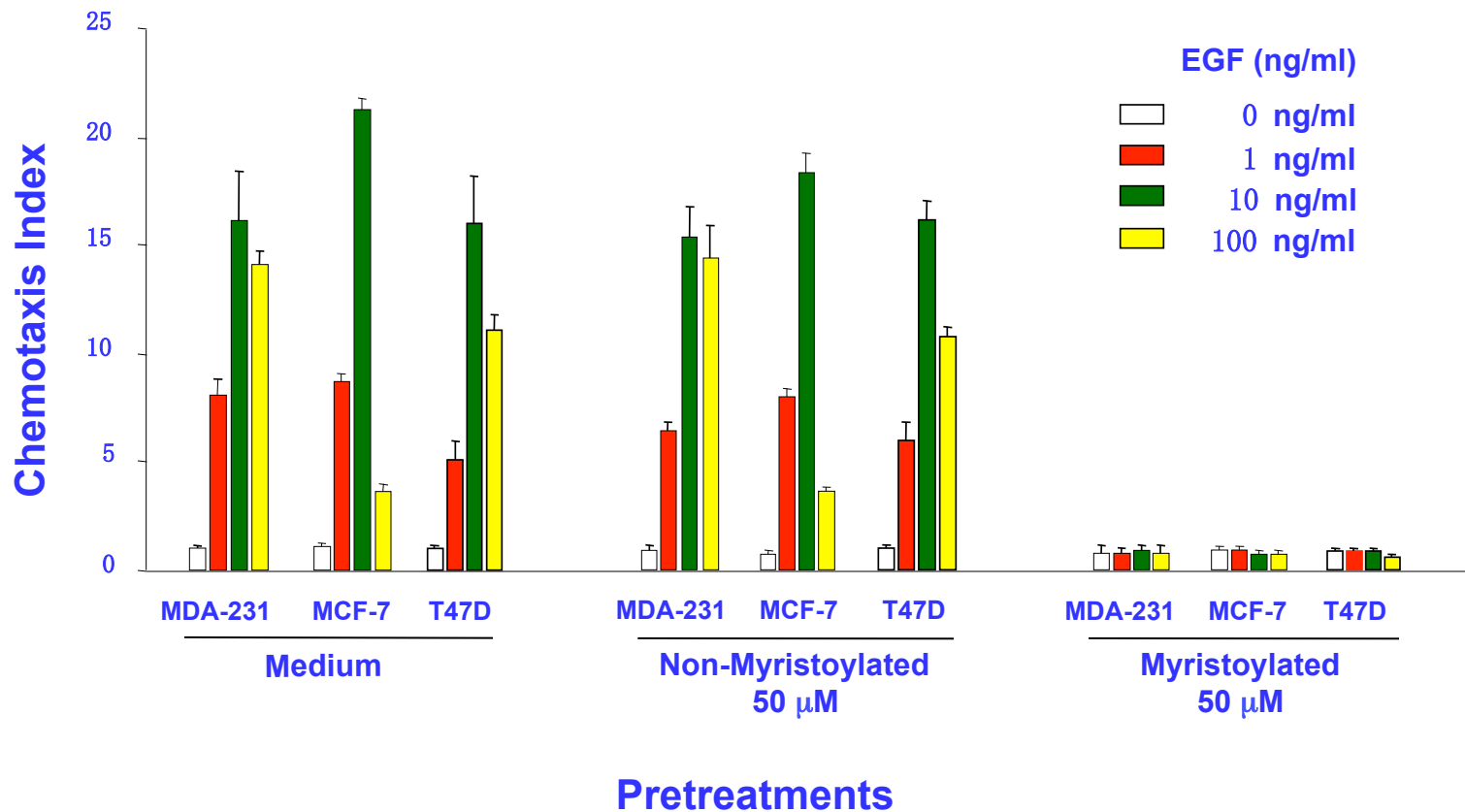


# Activated PKC $\zeta$ regulates directional cell migration and polarity



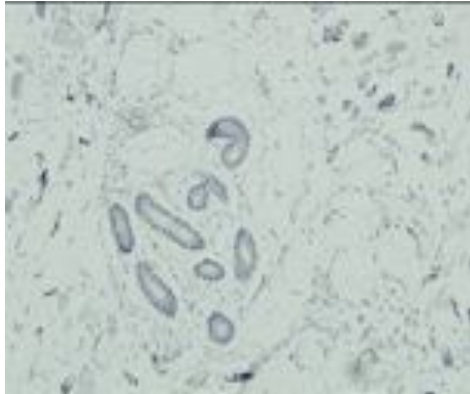


# PKC $\zeta$ Pseudosubstrate inhibits EGF-induced chemotaxis

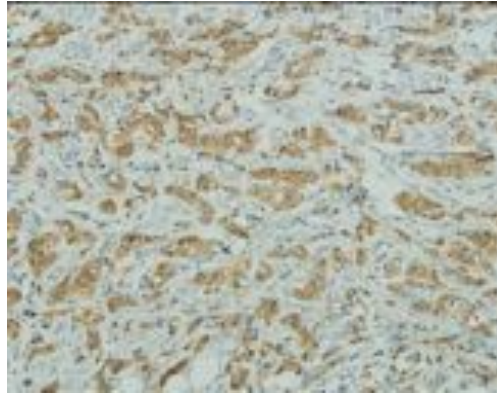




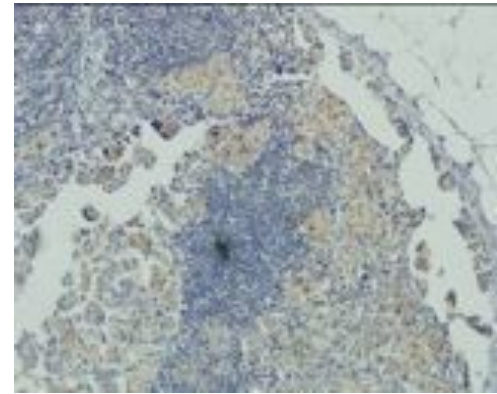
# Expression of PKC $\zeta$ Correlates with Lymph Node Metastasis



**Normal**



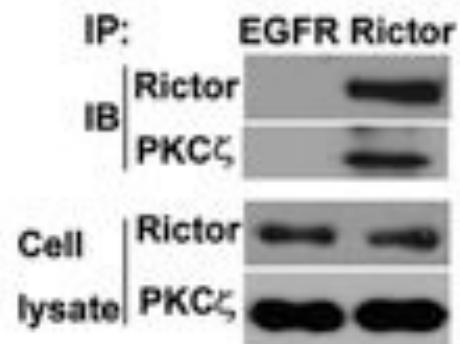
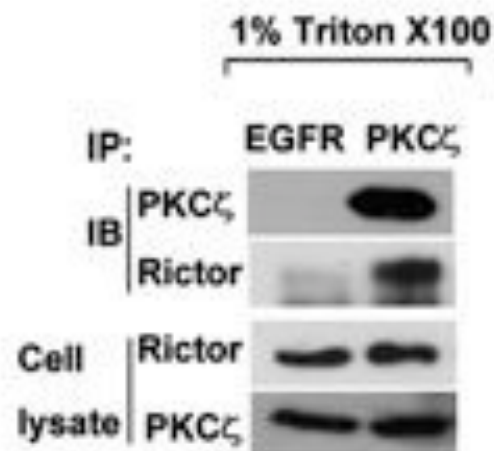
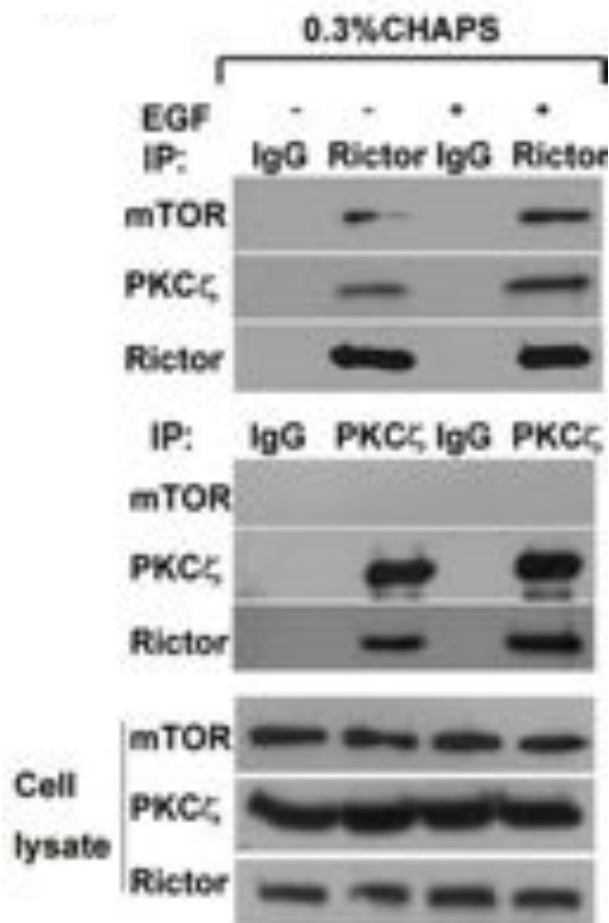
**Carcinoma**



**Lymph Node**

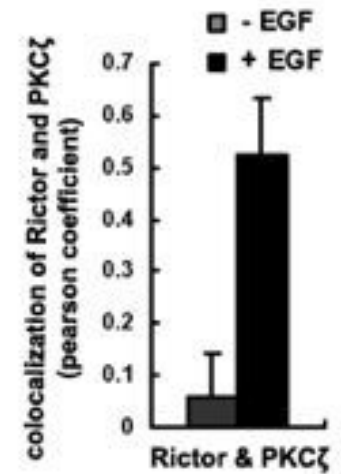
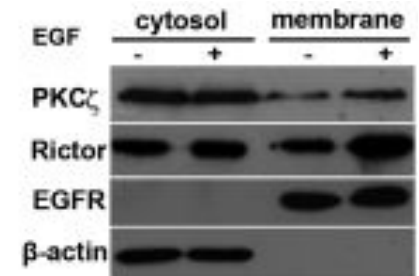
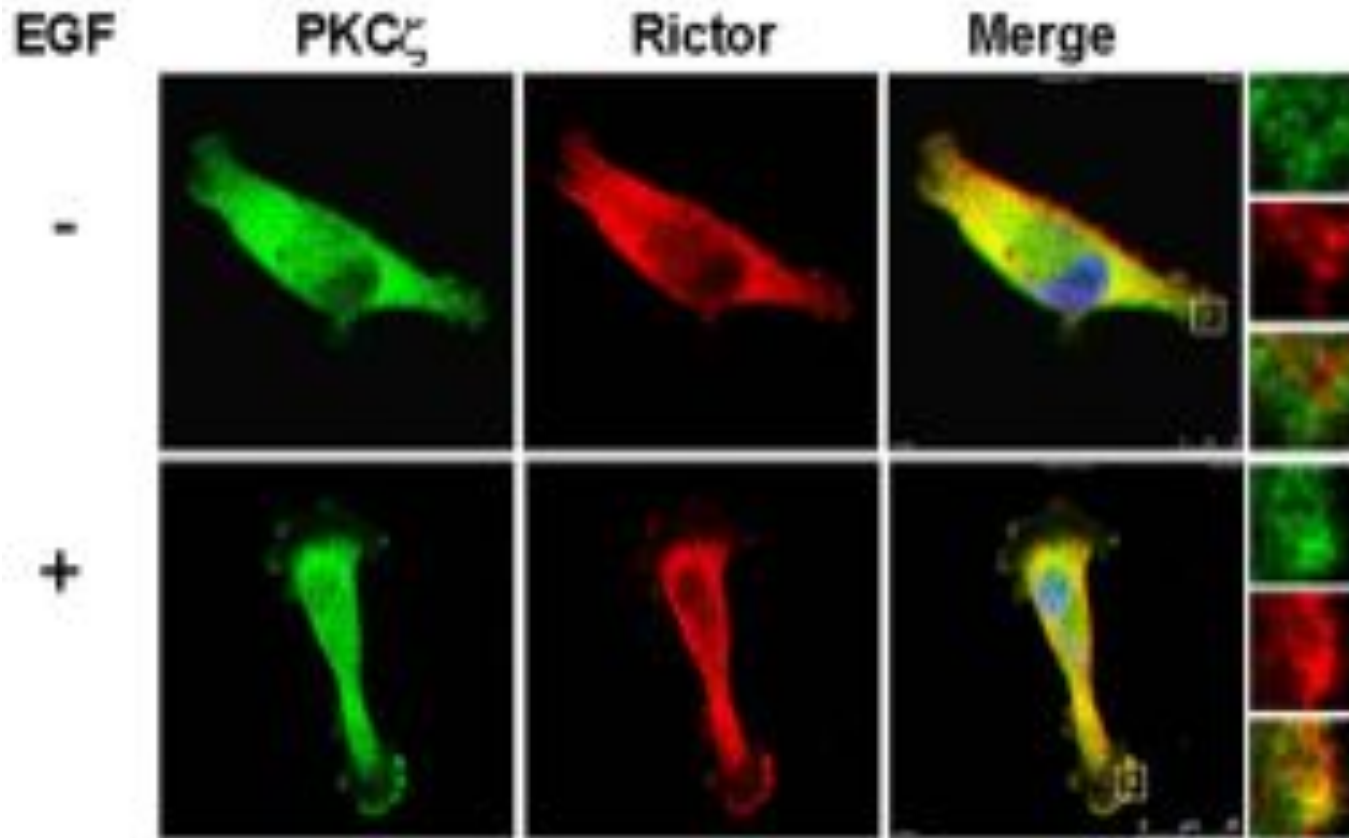


# Rictor Interacts with PKC $\zeta$



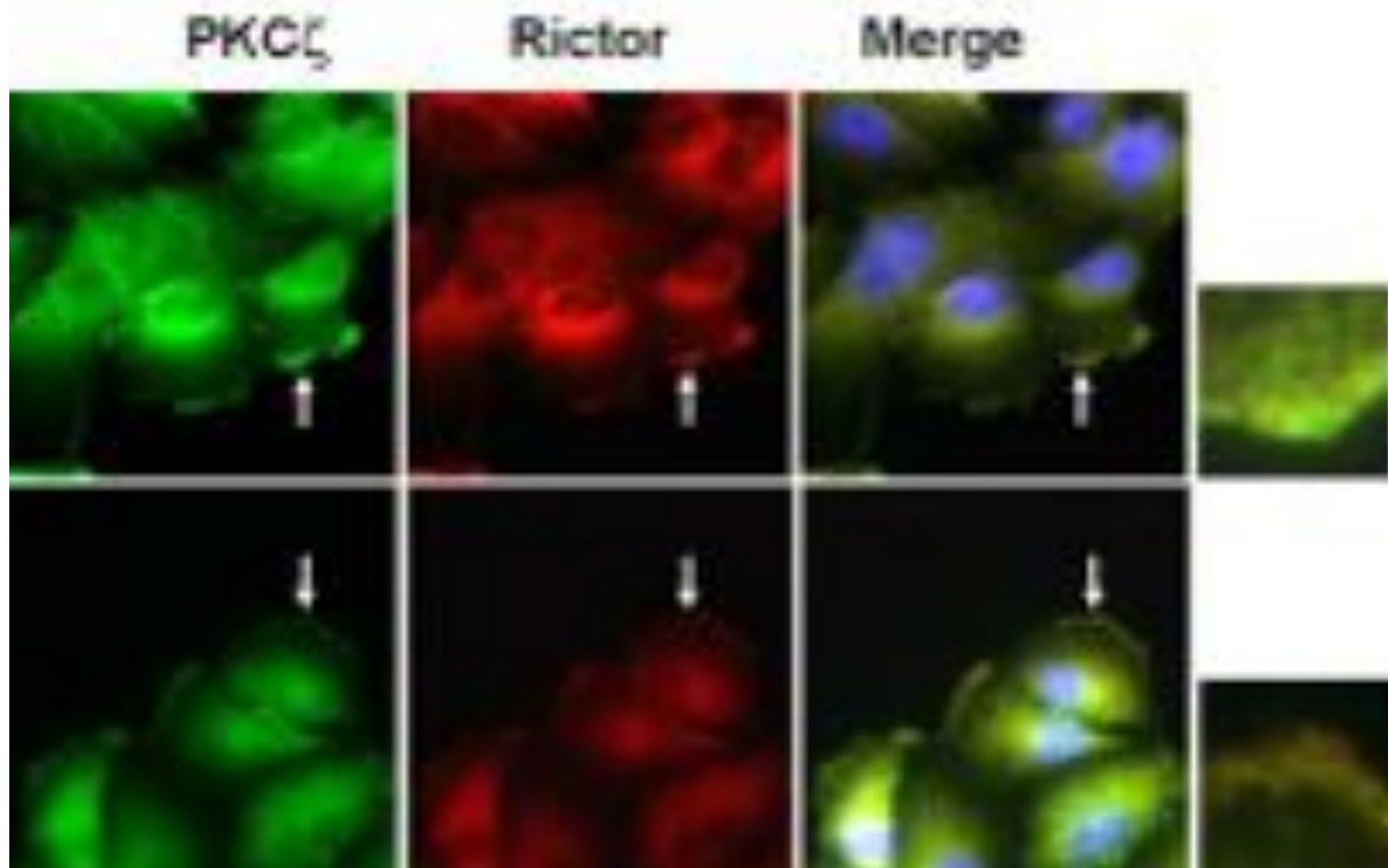


# PKC $\zeta$ colocalizes with Rictor along plasma membrane under EGF stimulation



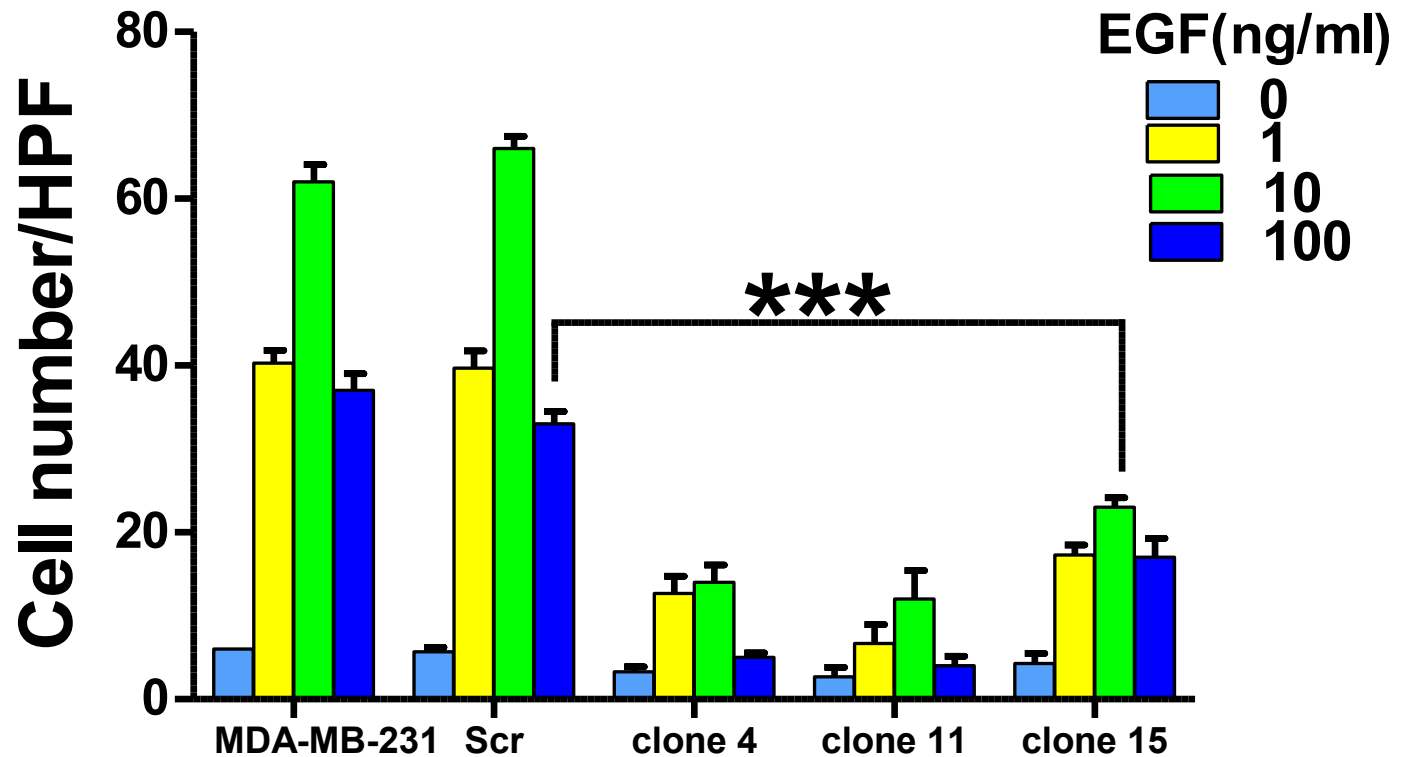


# PKC $\zeta$ colocalizes with Rictor at the leading edge of migratory cells





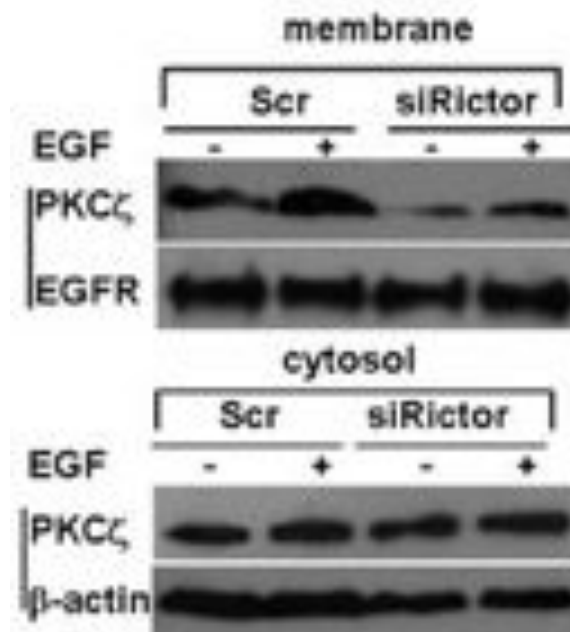
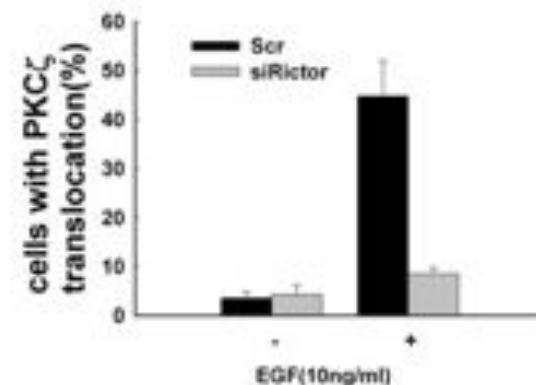
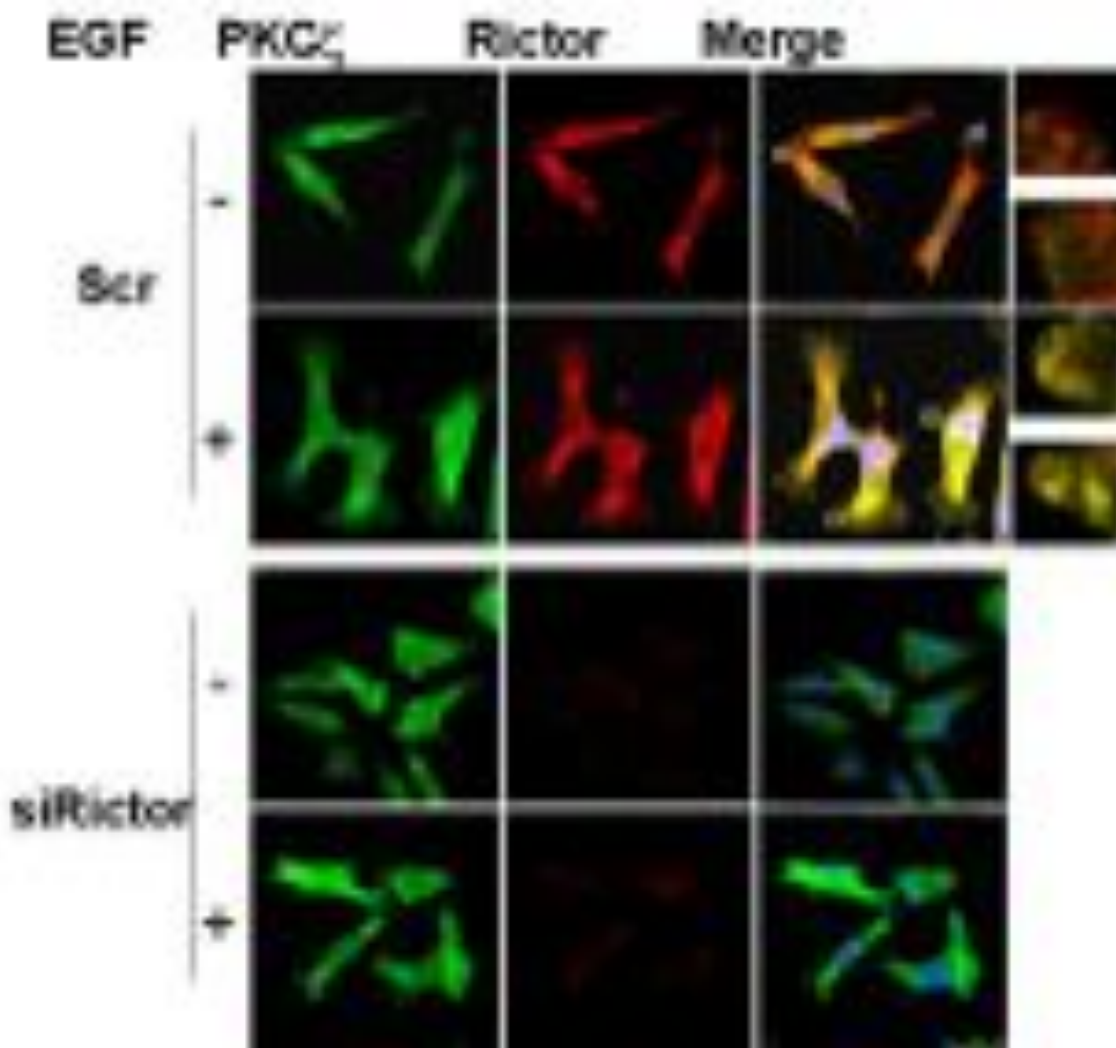
# Knockdown of Rictor by siRNA Decreases EGF Induced Chemotaxis







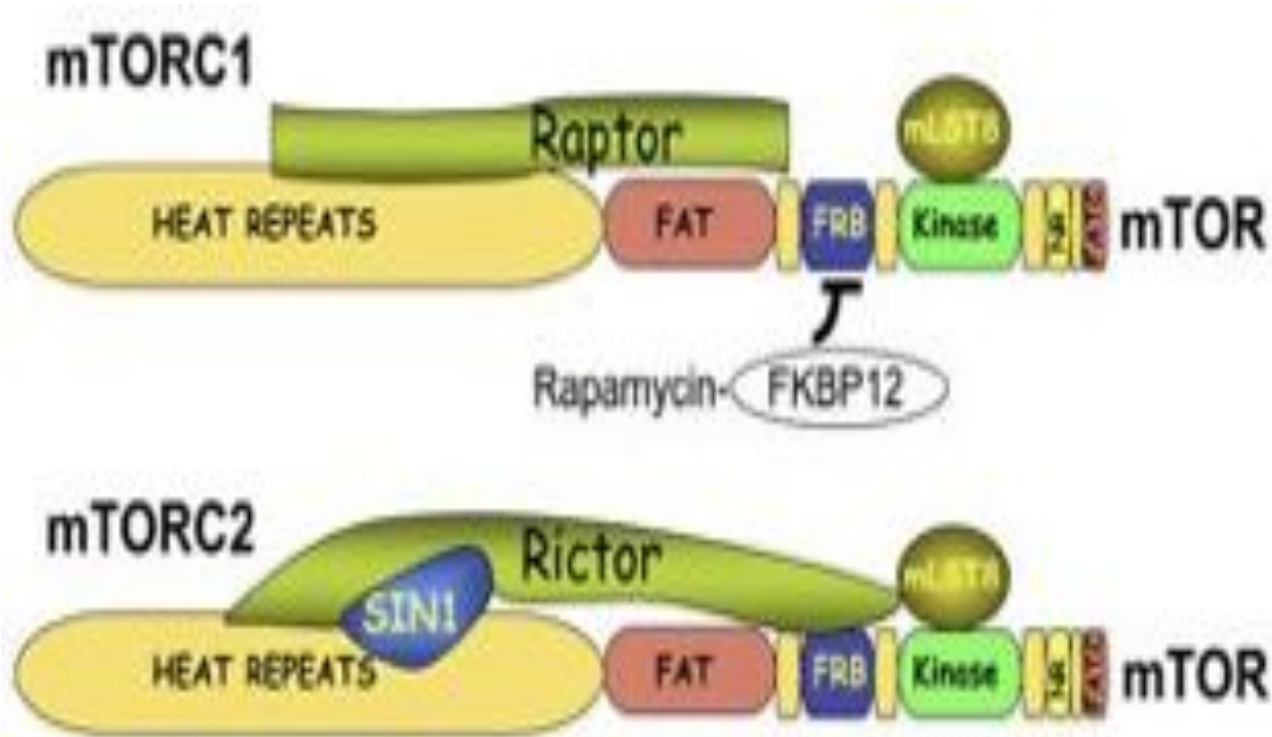
# Knockdown of Rictor Impairs EGF Induced PKC $\zeta$ Membrane Translocation







# Rictor and Raptor Define Two Distinct mTOR Containing Complexes



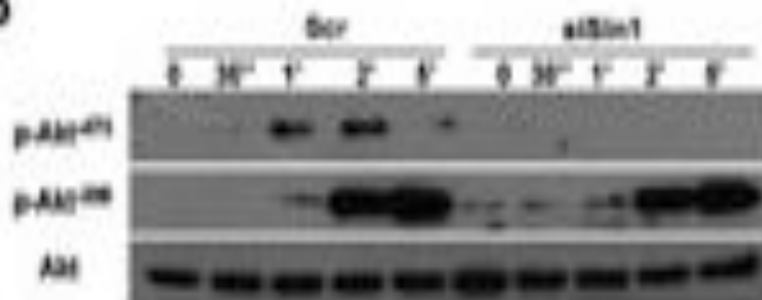


# Rictor still co-immunoprecipitated with PKC $\zeta$ in siSIN1 cells

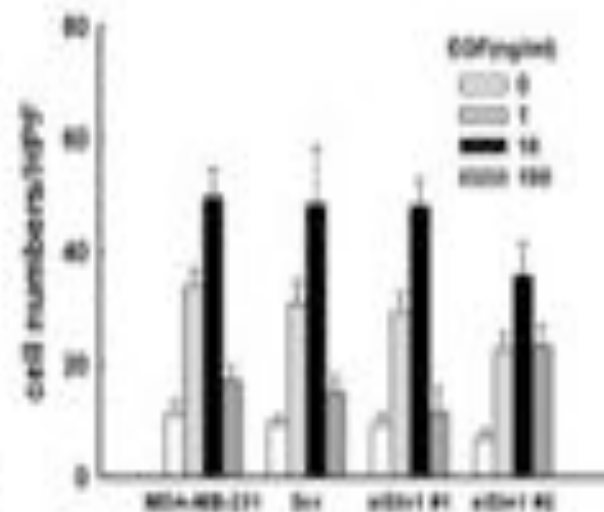
A



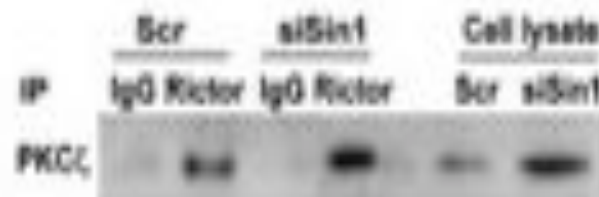
B



C

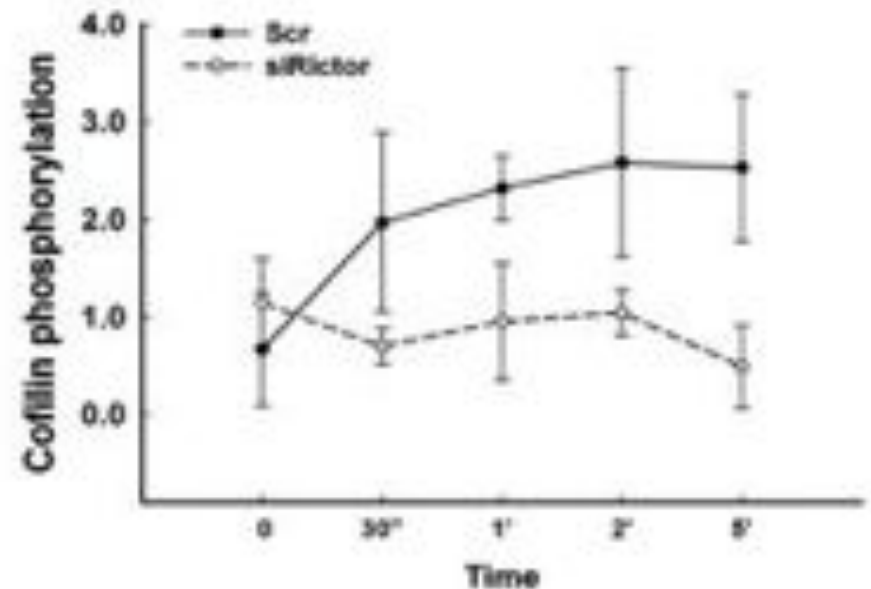
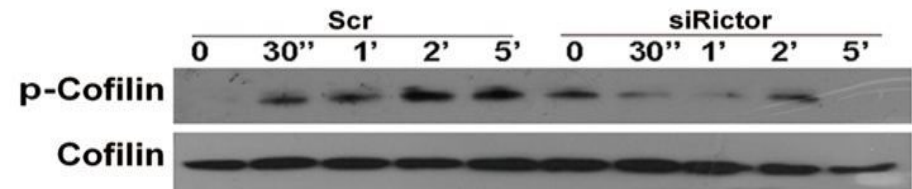
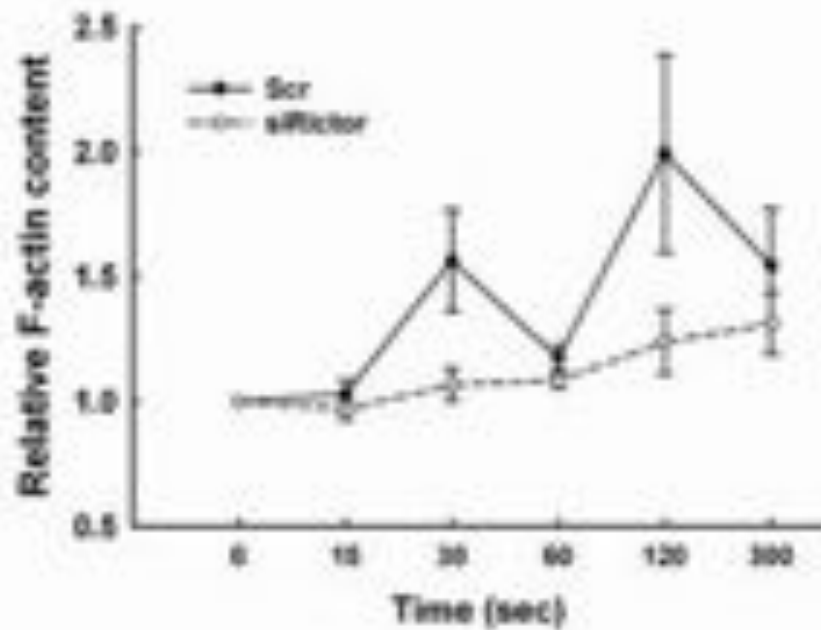


D



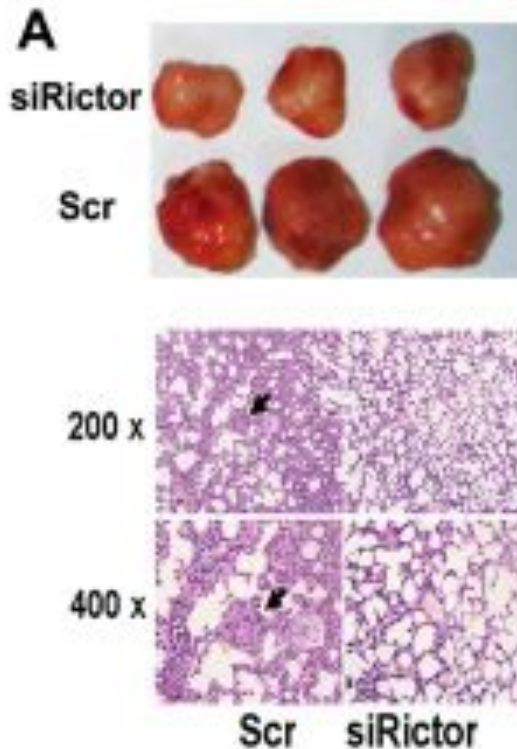


# EGF-induced actin polymerization was impaired in siRictor cells

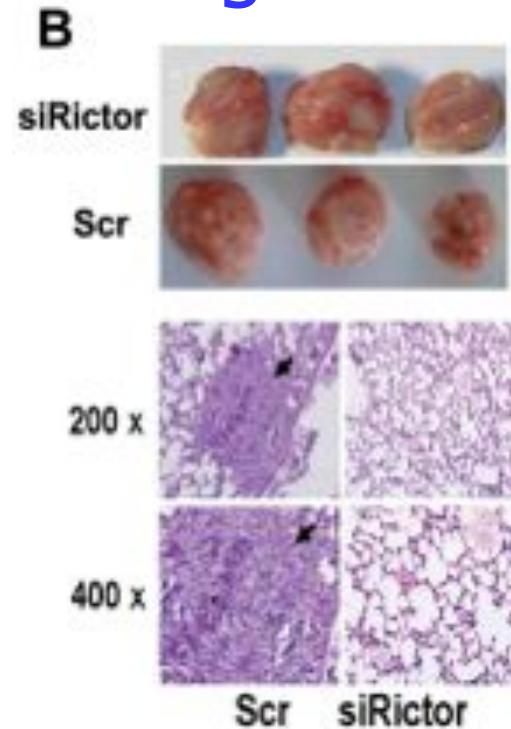




# Knockdown of Rictor Inhibited Spontaneous Metastasis of MDA-MB-231 Cells to SCID Mouse Lung



6 weeks after implantation

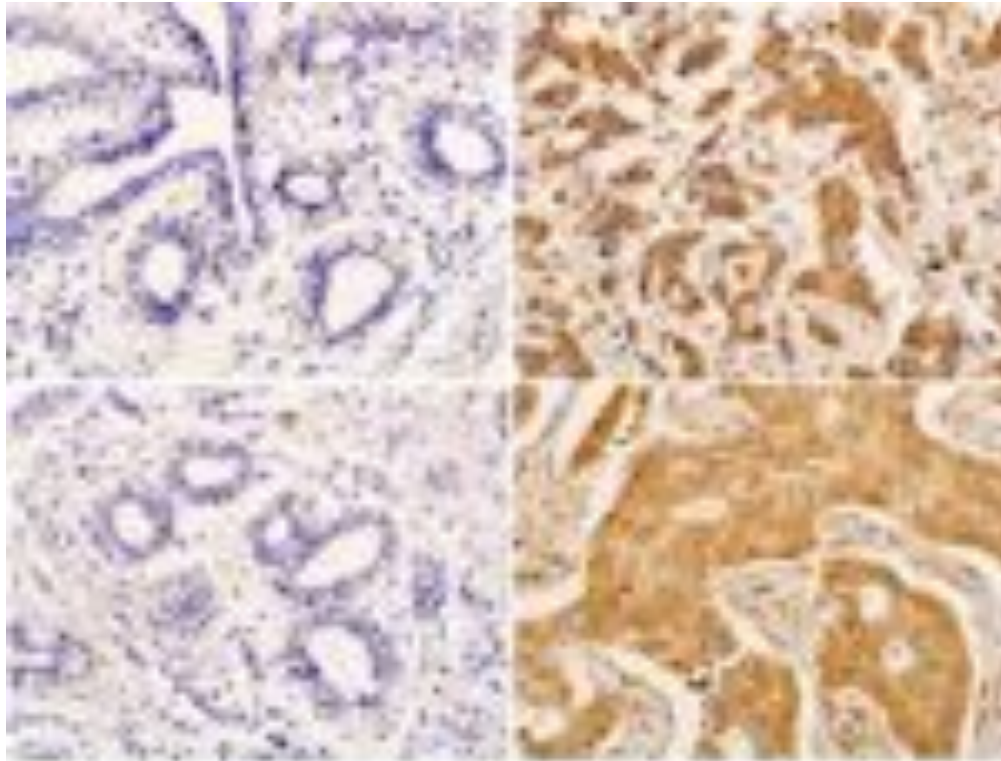


Scr: 6 weeks

siRictor: 9 weeks



# Expression of Rictor is Linked with Lymph Node Metastasis of Breast Cancer tissues



Normal breast tissue

invasive ductal carcinoma

	positive	negative	total
normal	3	36	39
*cancer	25	14	39
total	28	50	78

\*p=0.000

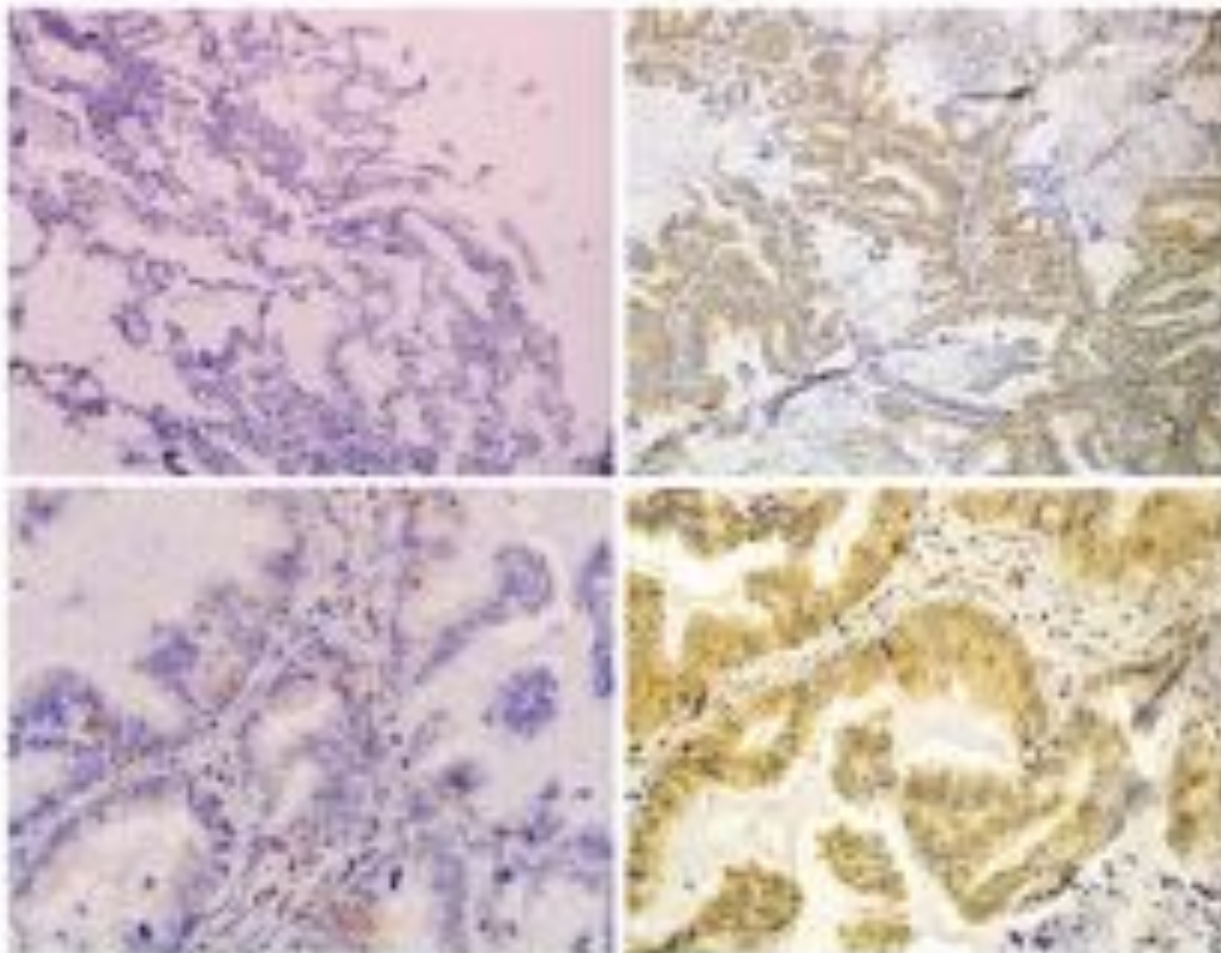


Parameters/Markers	Total	Positive	Factor	p
Menopausal				
Pre-menopausal	21	12	57.1	0.261
Post-menopausal	18	13	72.2	
Tumor Size				
<2 cm	7	5	62.5	.507
	32	20		
Lymph Node Status				
Negative	13	1	0.07	0.000
Positive	26	24	92.3	
Histological Grade				
G1	13	9	69.2	0.780
G2	22	14	63.6	
G3	4	2	50.0	
ER Status				
Negative	16	7	43.8	0.031
Positive	23	23	100	
PR Status				
Negative	18	10	55.6	0.243
Positive	21	15	71.4	
HER2/neu Protein				
Negative	29	16	55.2	0.049
Positive	10	9	90.0	





# Rictor is Expressed in Lung Cancer Tissues



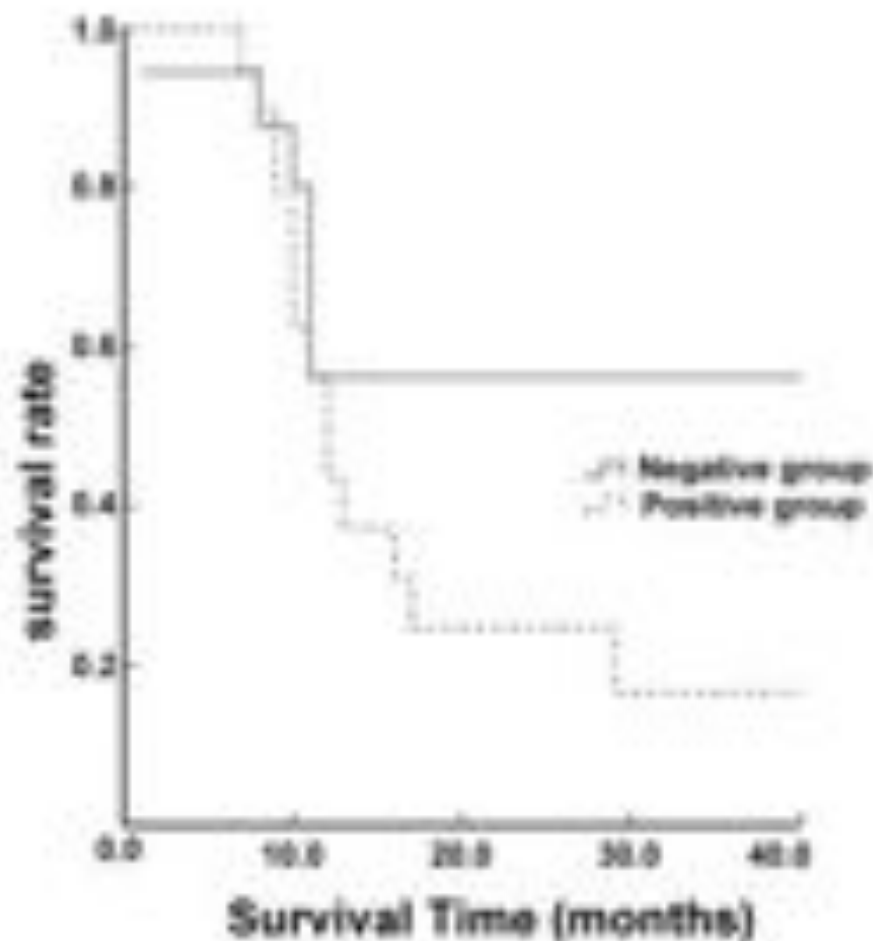
**Normal lung tissue**

**lung cancer**



# Expression of Rictor correlates with NSCLC lymph node metastasis and poor prognosis

Project	Rictor expression		P value
	positive	negative	
Age (years)			0.069
<60	16	8	
≥60	6	10	
Sex			0.154
male	11	13	
female	11	5	
Smoking			0.385
yes	8	9	
no	14	9	
TNM stages			0.257
I, II	6	8	
III, IV	16	10	
lymph node metastasis			0.038
yes	10	14	
no	12	4	







# Rictor was Expressed in Renal Cancer

**A**



Normal renal tissue(-)

**B**



Papillary renal cell carcinoma

**C**



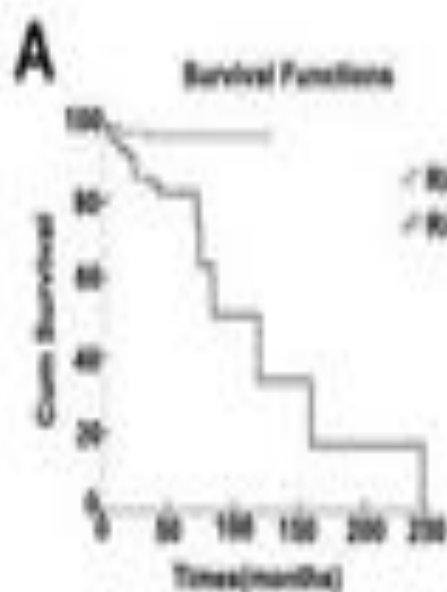
Clear cell renal cell carcinoma

Correlation of Plector expression with clinicopathologic parameters

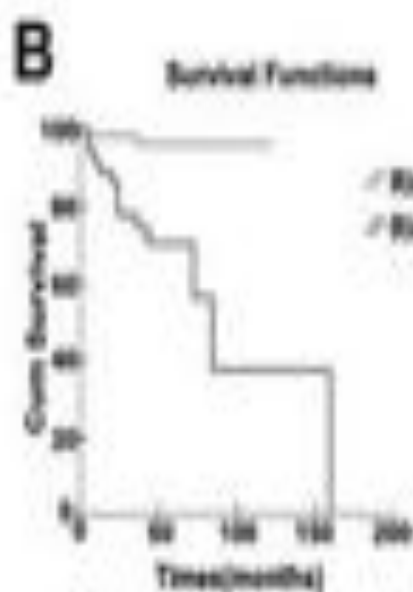
	Numbers	Plector expression		X <sup>2</sup>	P
		Negative	Positive		
Gender					
Male	144	55	89	0.258	0.613
Female	75	38	37		
Age(Y)					
≤ 55	88	35	53	0.484	0.486
>55	147	55	92		
Symptom presentation					
Negative	121	50	71	71.899	0.000 <sup>*</sup>
Positive	88	38	50		
Stages					
I-II	75	35	40	0.090	0.779
III-IV	108	58	50		
V-VI	45	24	21		
T stages					
T <sub>1</sub>	150	70	80	21.689	0.000 <sup>*</sup>
T <sub>2</sub>	28	15	13		
T <sub>3</sub>	35	9	26		
T <sub>4</sub>	12	2	10		
Grade					
I	67	39	28	1.288	0.258 <sup>*</sup>
II	108	58	50		
III-IV	65	35	30		
Lymph node metastasis					
No	264	158	106	99.923	0.000 <sup>*</sup>
Yes	14	4	10		
Living metastasis					
No	188	104	84	49.339	0.000 <sup>*</sup>
Yes	28	7	21		
Survival					
Living	188	104	84	23.290	0.000 <sup>*</sup>
Dead	28	9	19		



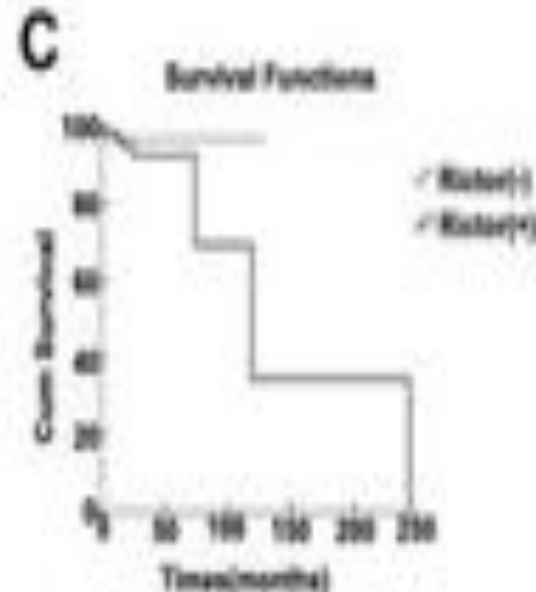
# Expression of Rictor in Renal Cancer Correlates with Poor Prognosis



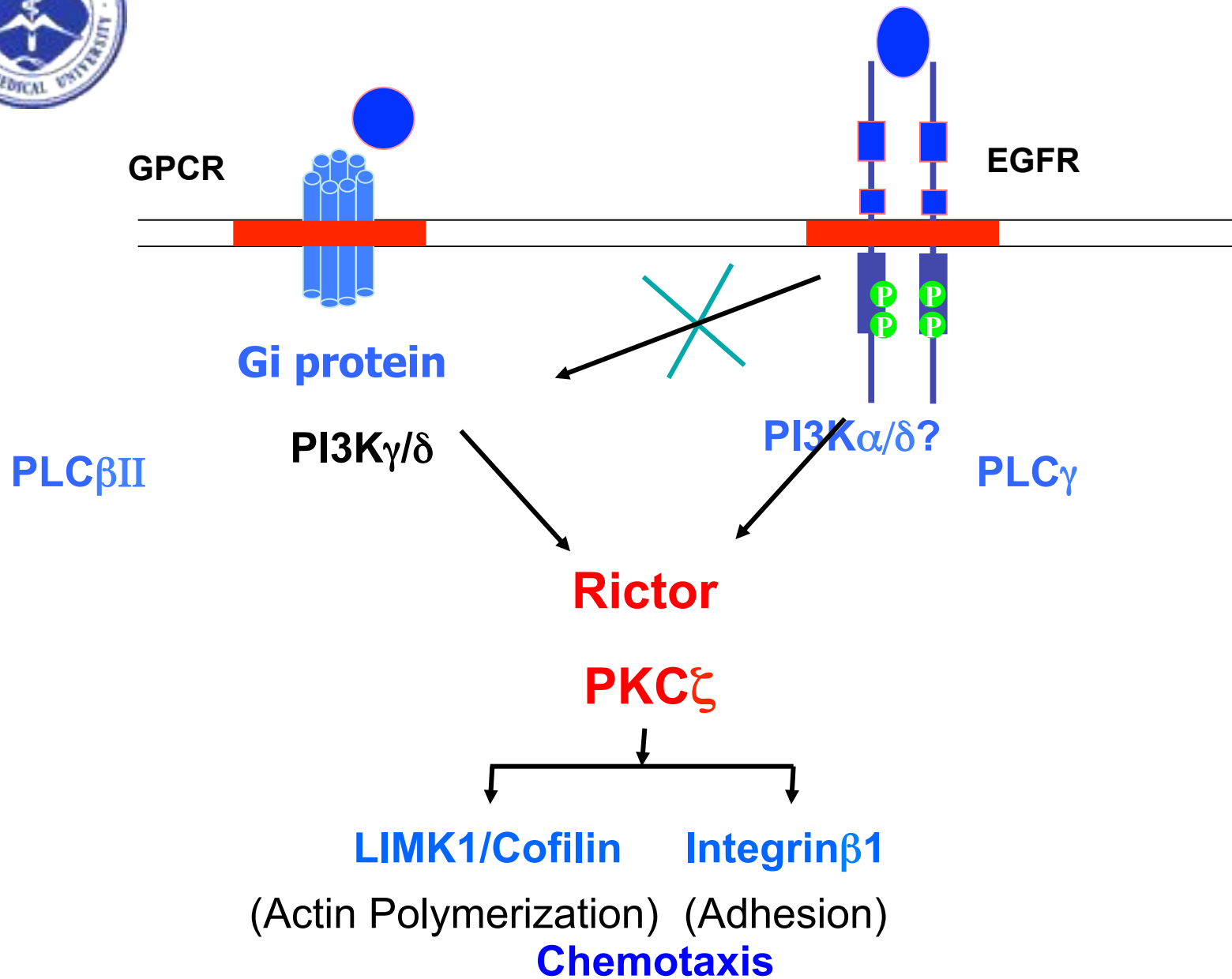
Total RCC survival function



PRCC survival function

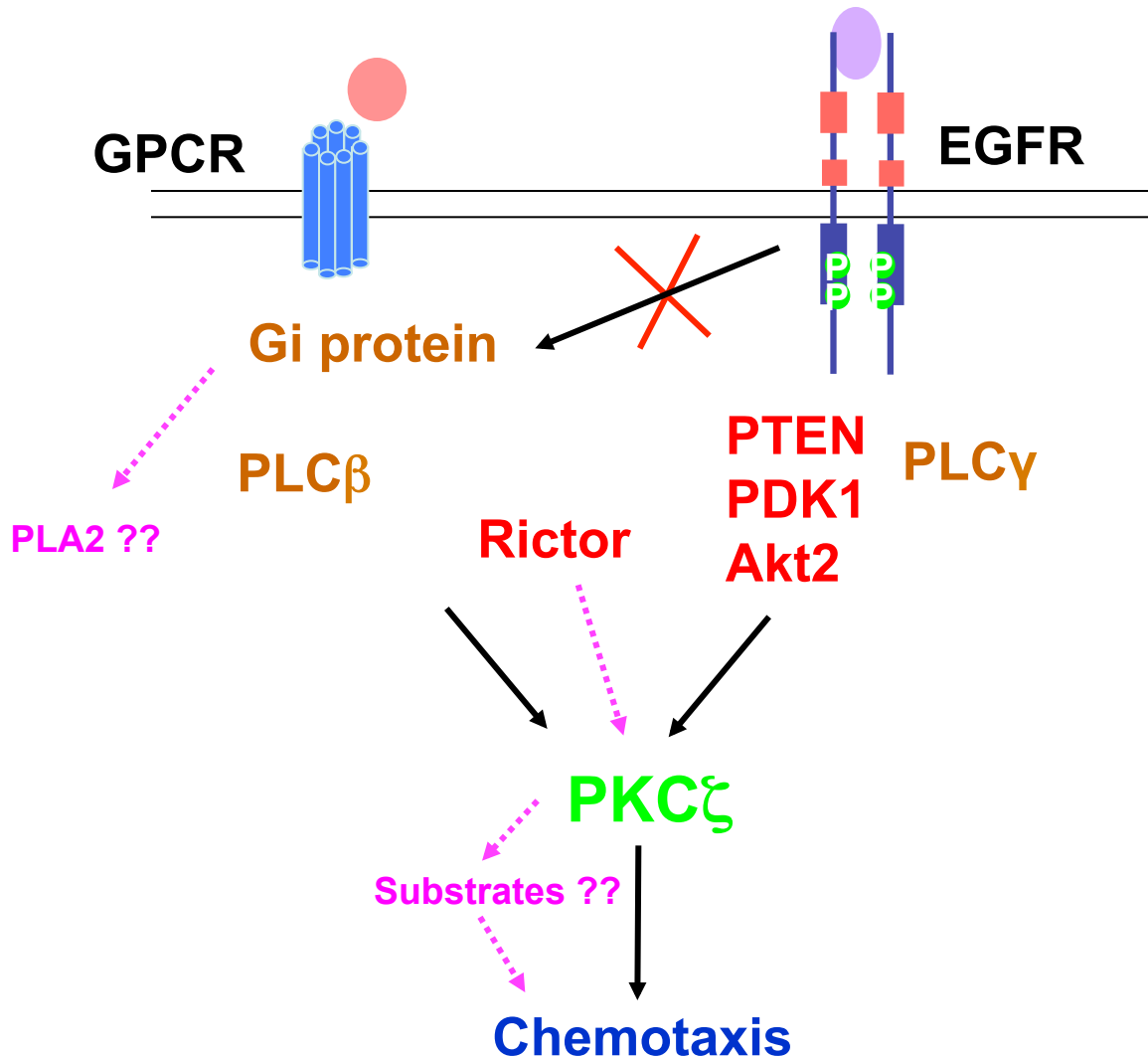


CCIRC survival function





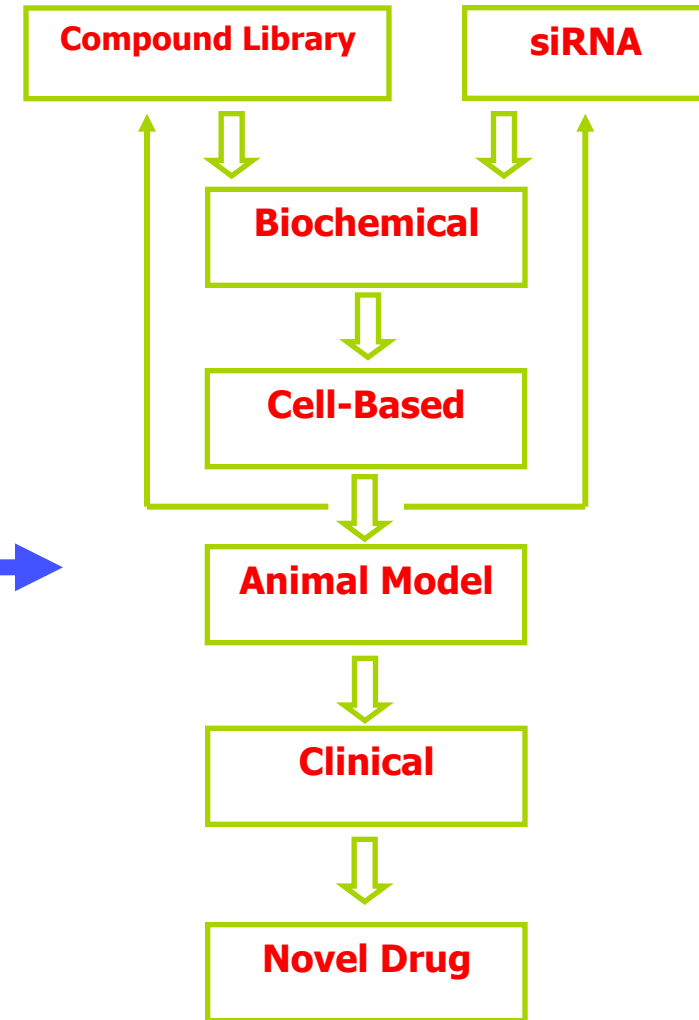
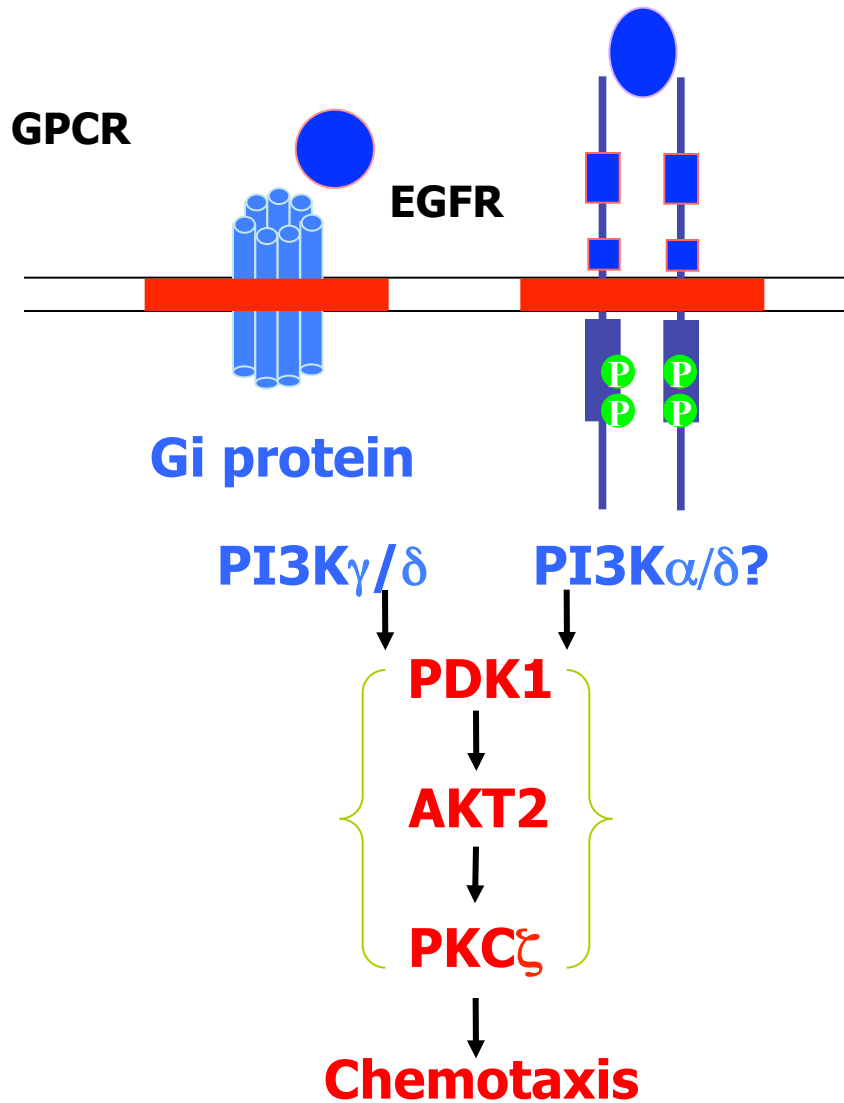
# Current Working Model



Cancer Research 2005  
Mol Membrane Bio 2007  
Cell Signal. 2007  
Cell Signal. 2008  
Lung Cancer 2008  
International J Cancer 2009  
Mol Cancer Research 2009  
J Proteome research 2009  
Cancer Research 2010  
J Clinical Invest. 2010

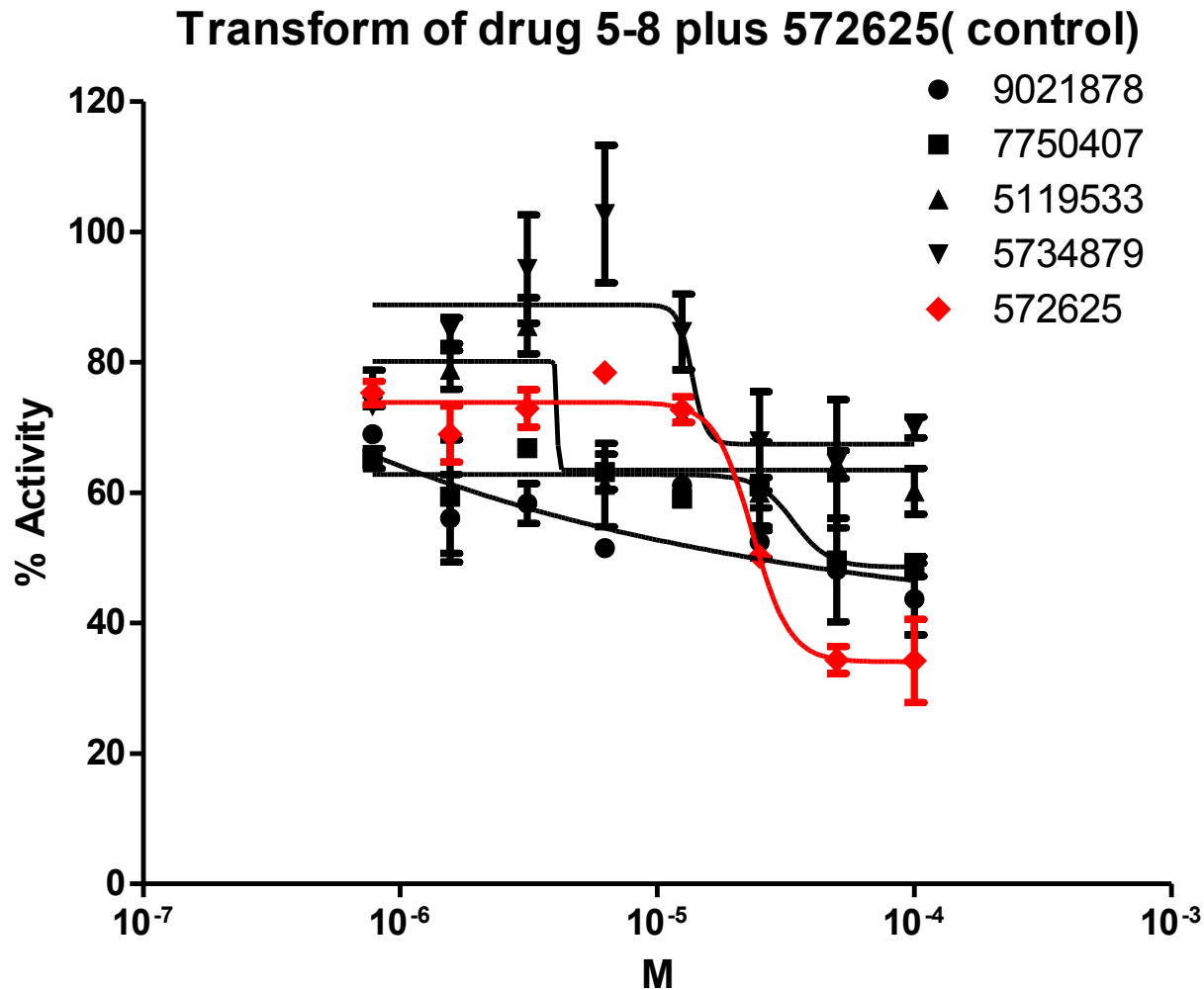


# So What?





# Screening for PKC $\xi$ inhibitors





# A Cell Based Screening for the Inhibitors of PKC $\zeta$

**EGF**

--

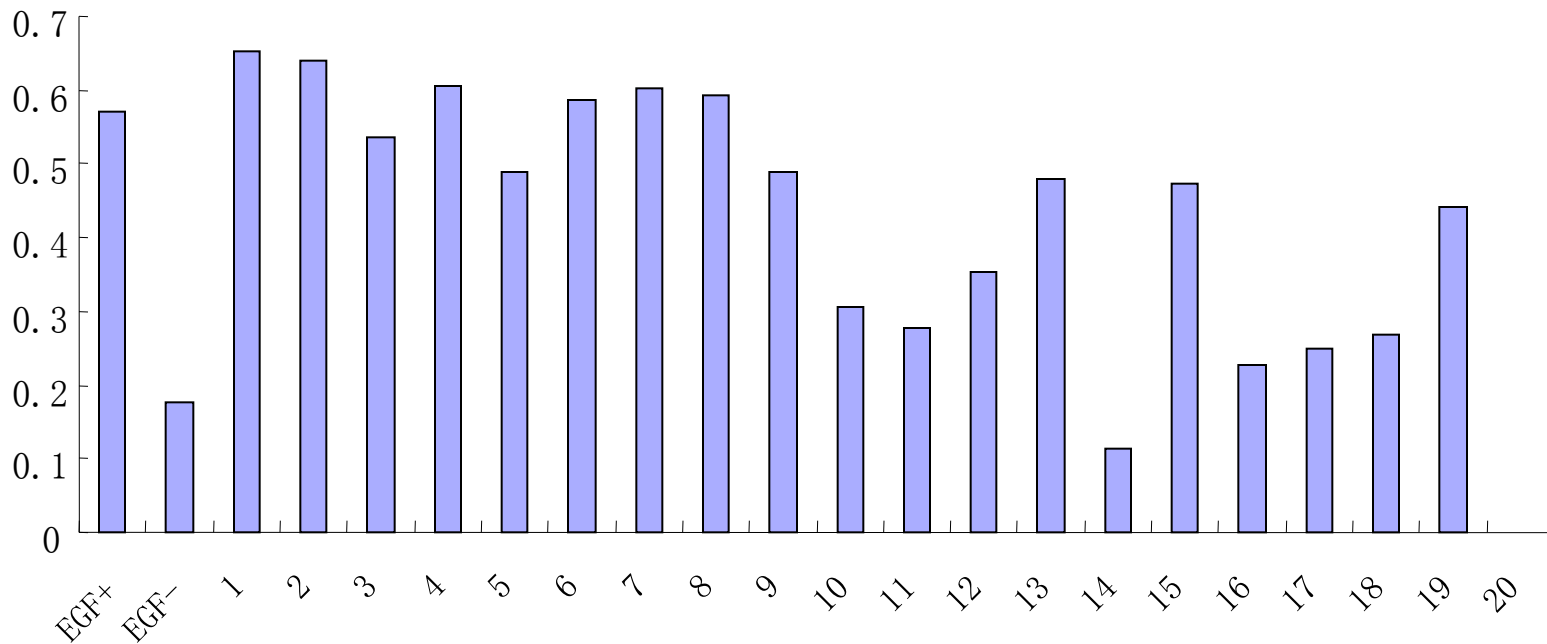
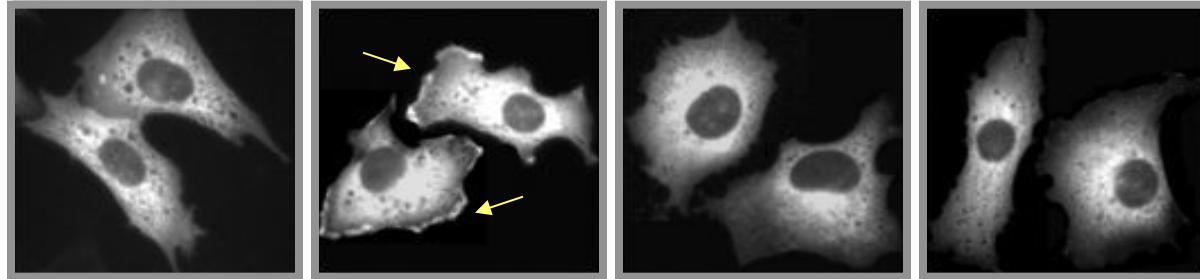
+

+

+

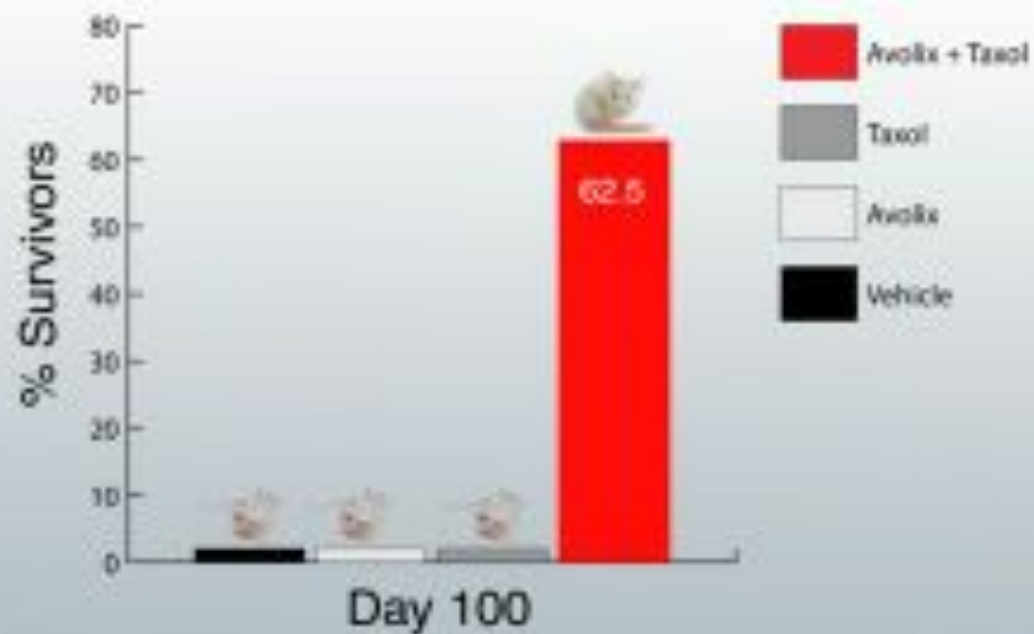
**Akt inhibitor**

**LY294002**





## Survivors



*Invest New Drugs 2009  
Cancer Letters 2010*



# Acknowledgements

Zhang Fei  
Guo Hua  
Zhang Baogang  
Zhang Xiaofang  
Tian Gang  
Liu Yan



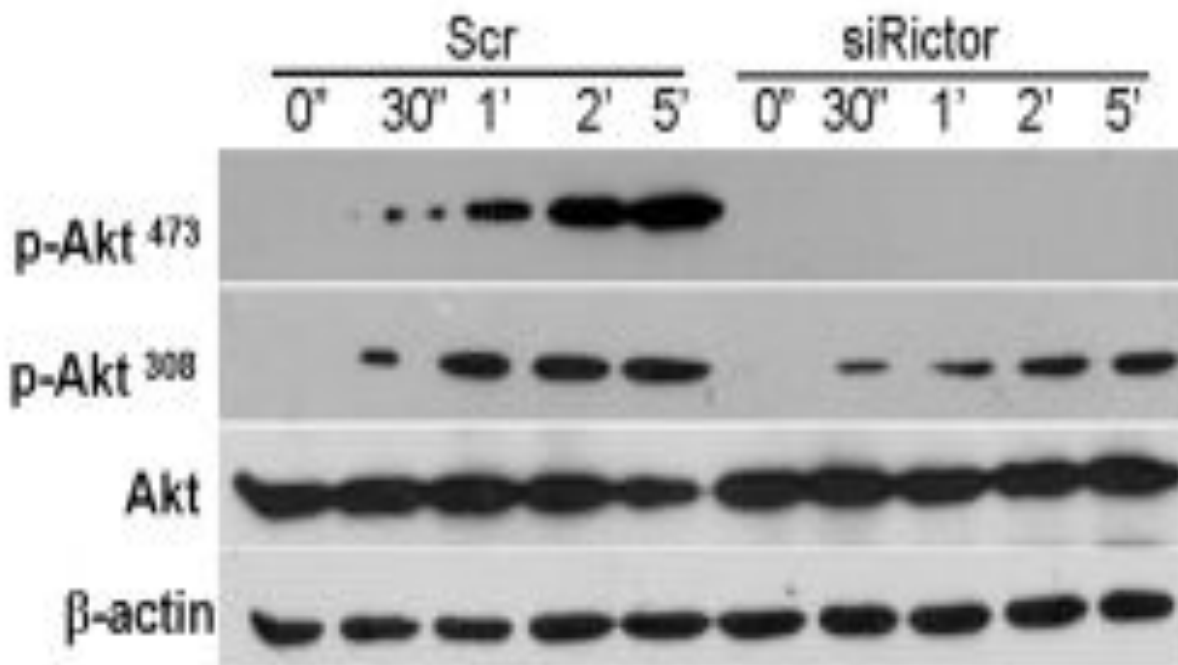
Sun Ronghua  
Liu Ying  
Wan Wuzhou  
Wang Jingna





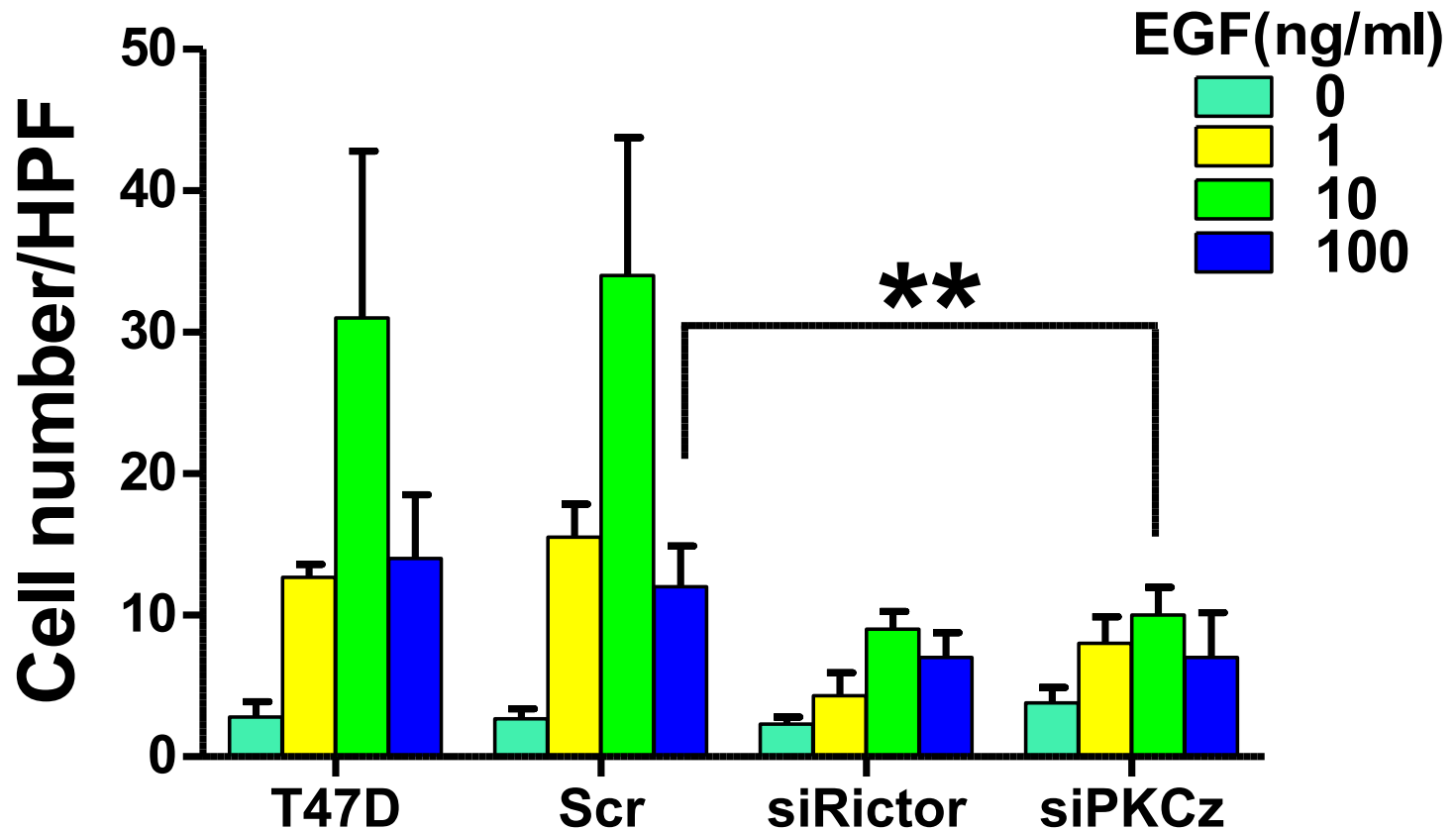
# Metastasis is the Major Cause of Morbidity





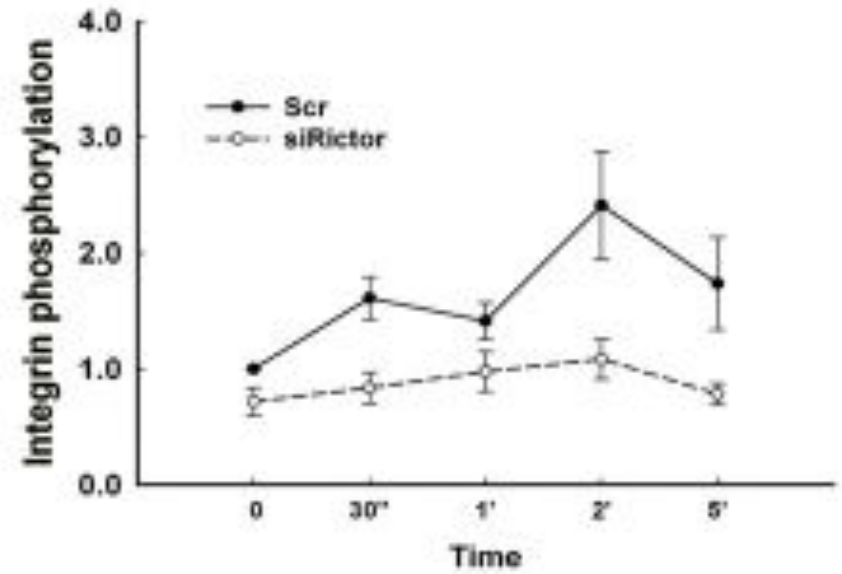
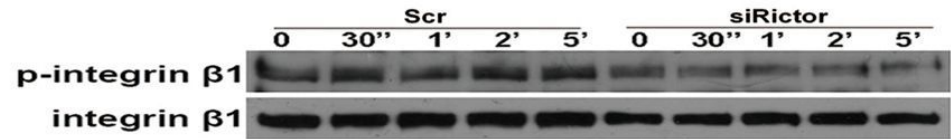
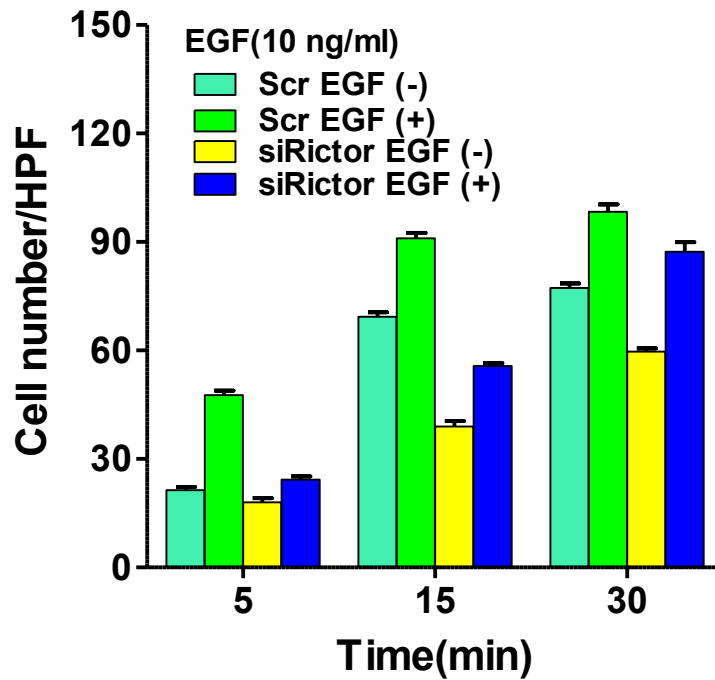


# Knockdown of Rictor by siRNA Decreases EGF Induced Chemotaxis



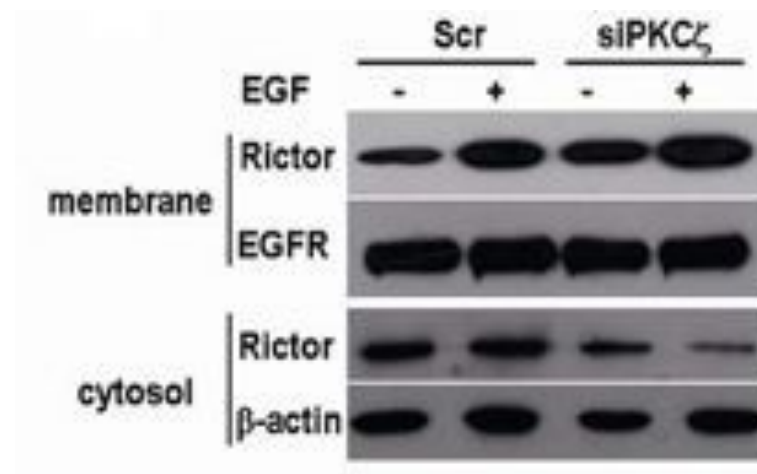
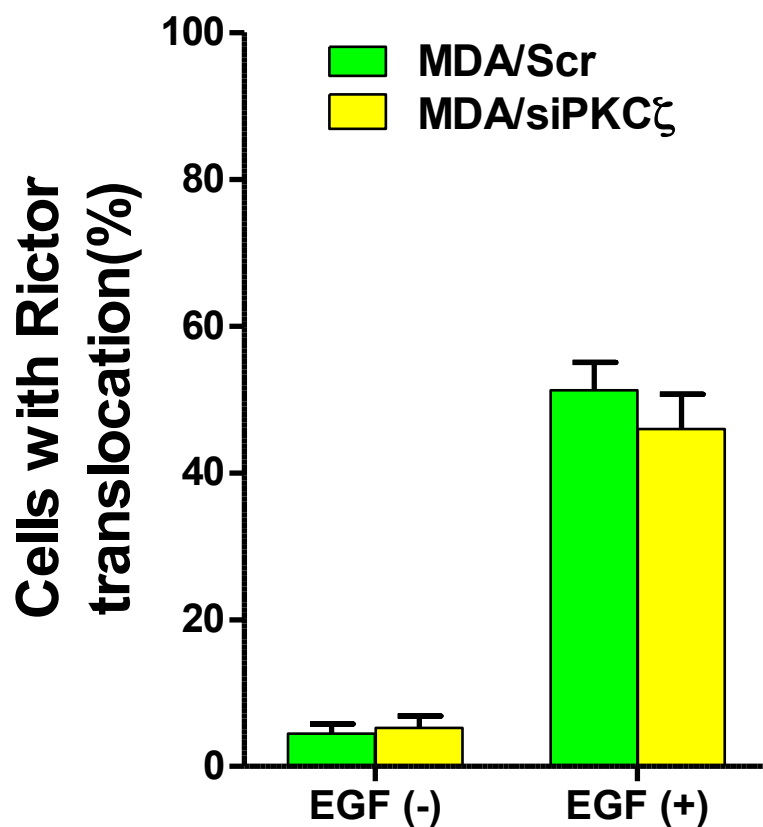


# Cell adhesion was Impaired in siRictor cells



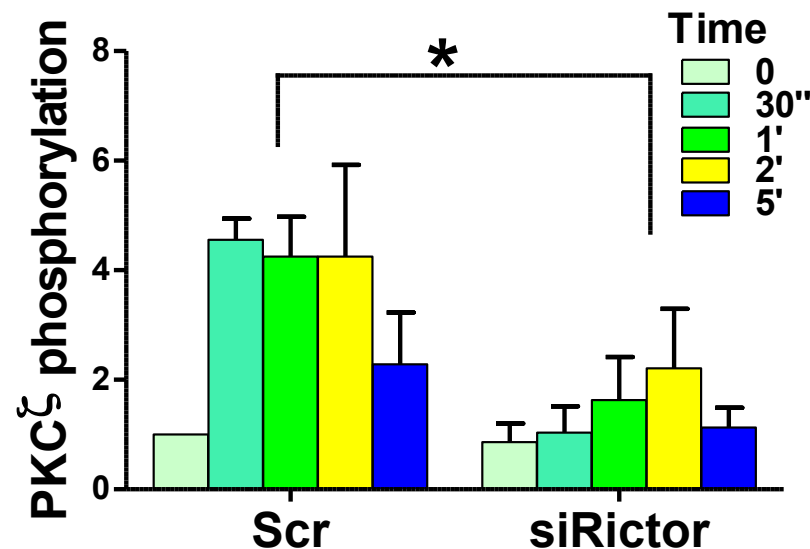
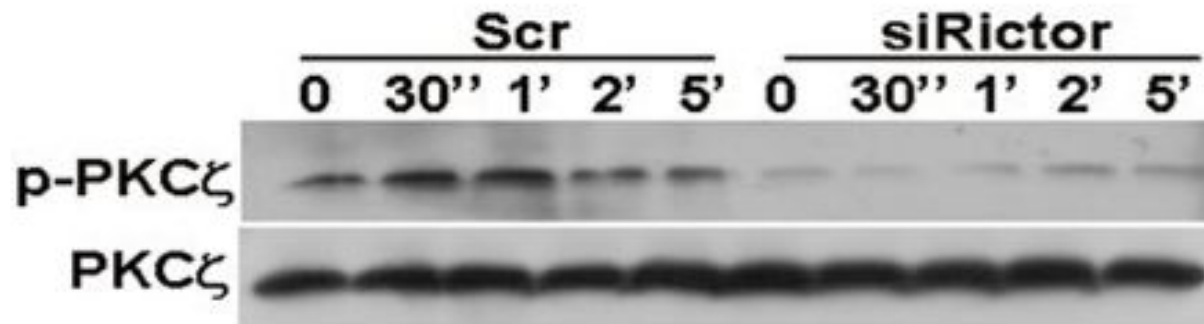


# EGF still induces Rictor membrane translocation in siPKC $\zeta$ cells





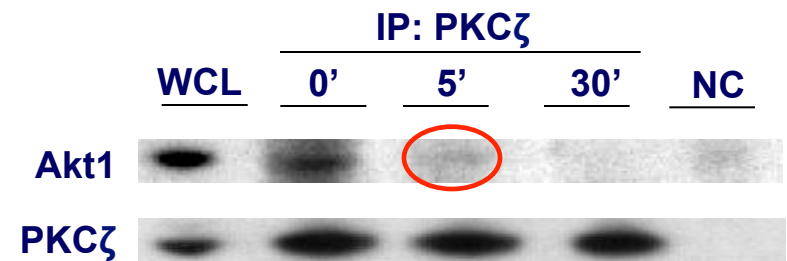
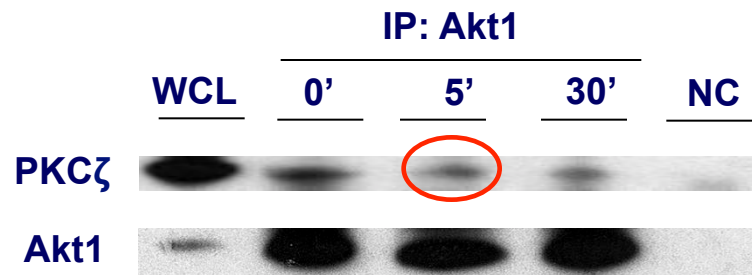
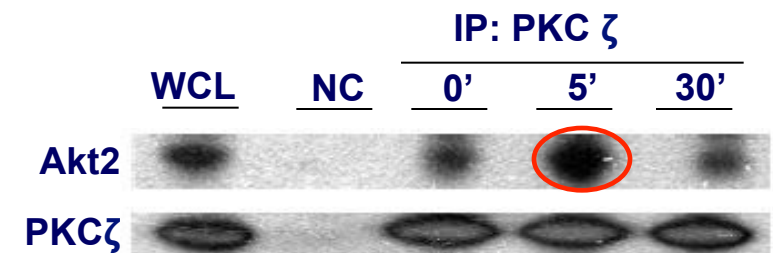
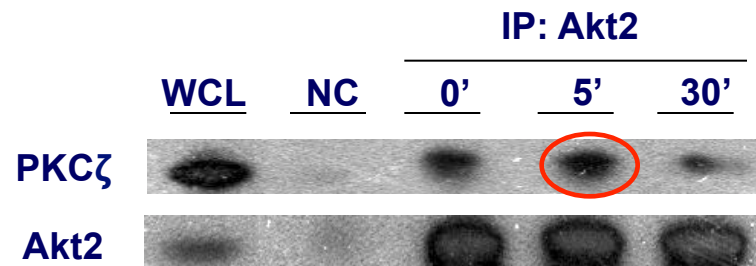
# EGF induced PKC $\zeta$ phosphorylation was impaired in siRictor cells





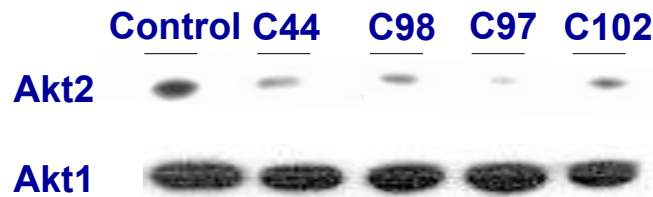
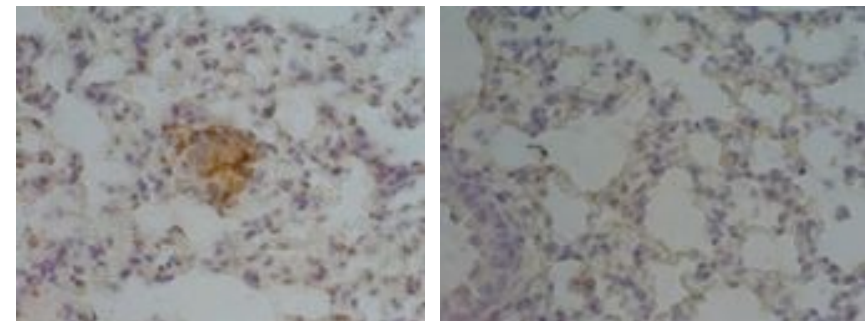
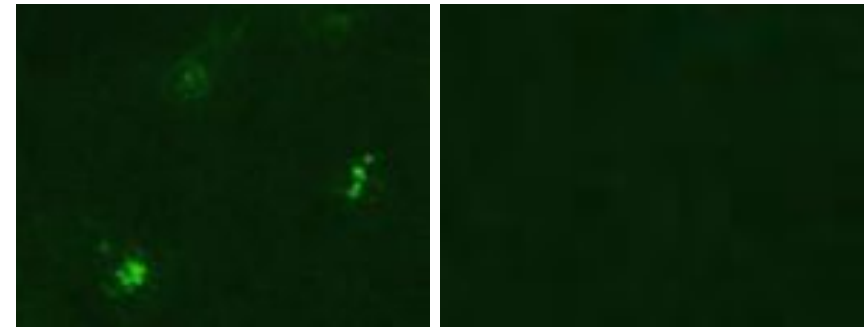
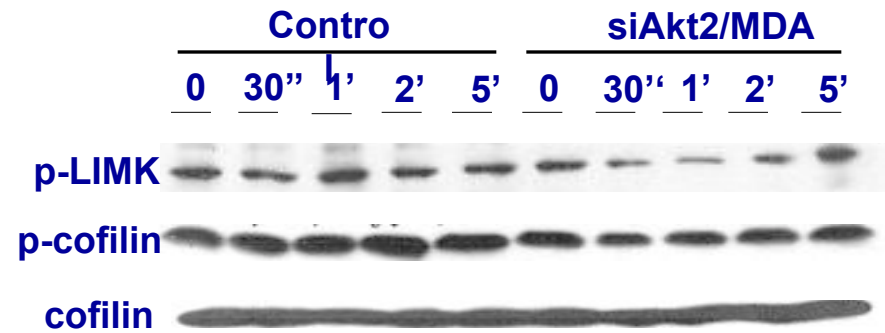
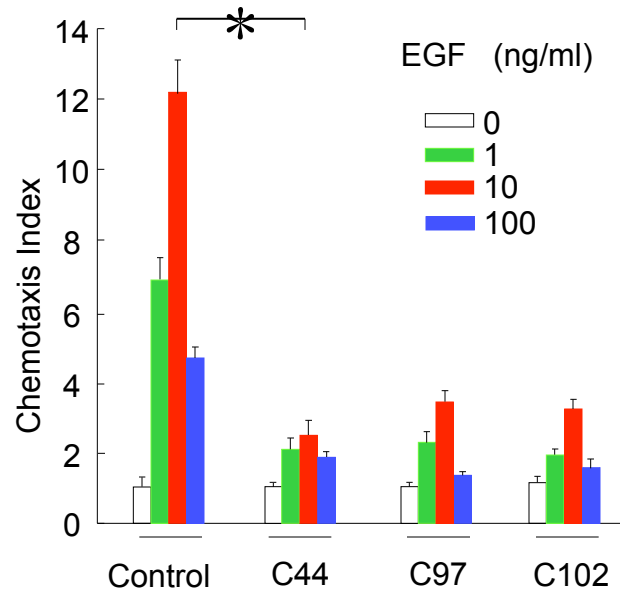


# EGF Induces Co-IP of Akt2 and PKC $\zeta$



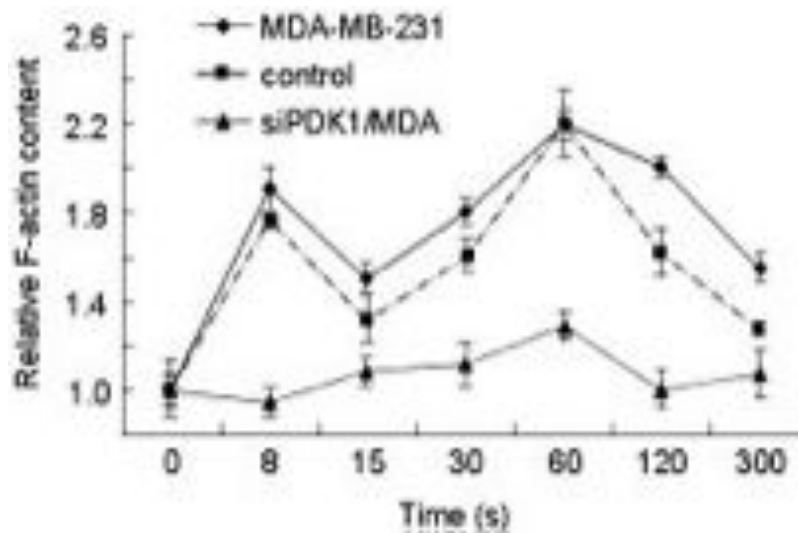
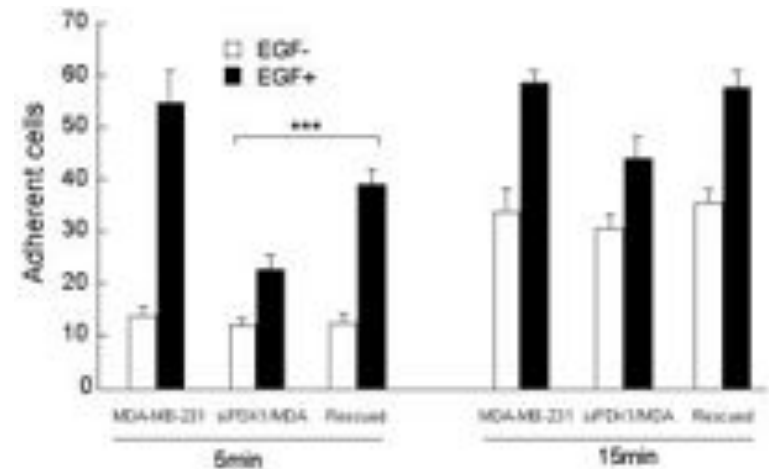
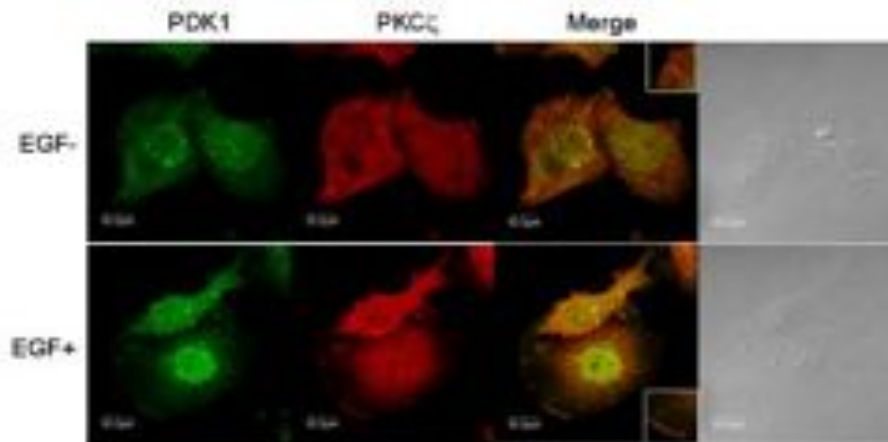


# Akt2 Plays a Critical Role in Metastasis



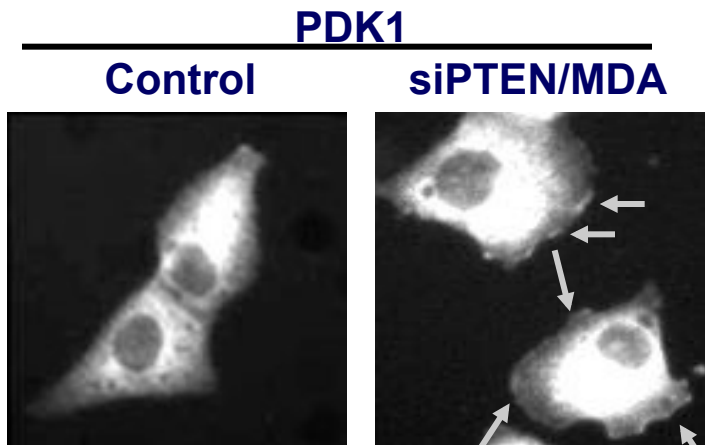
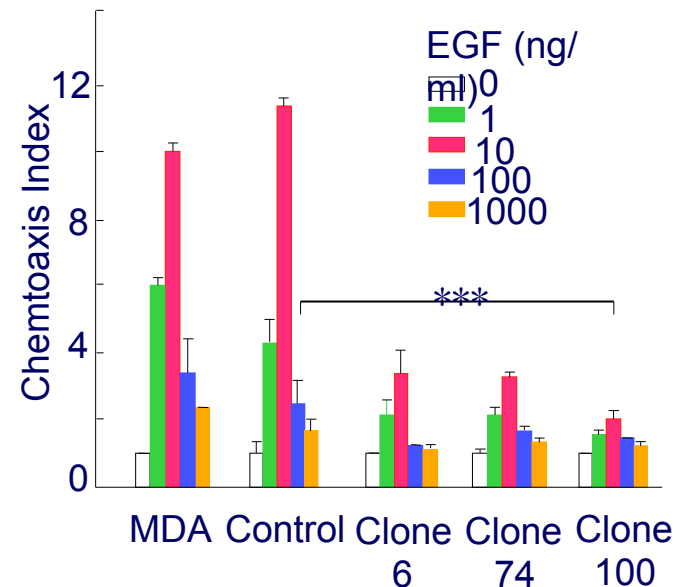
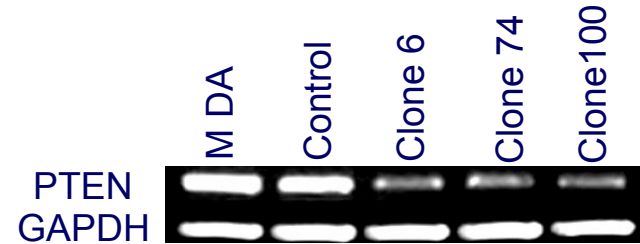
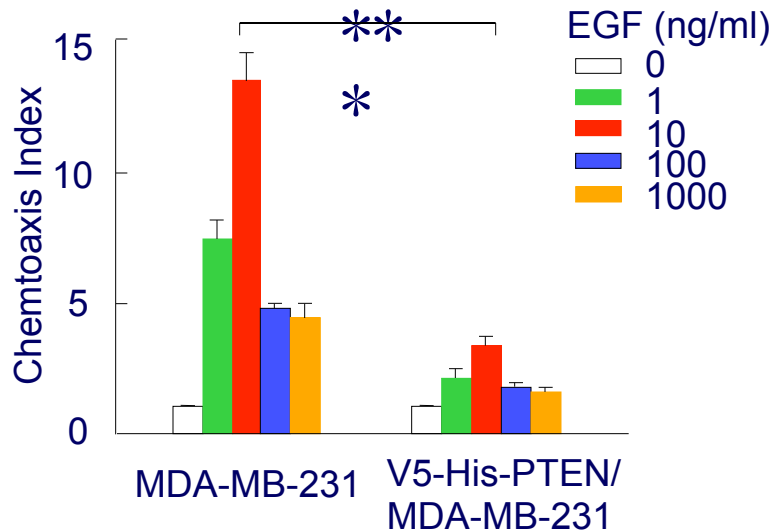
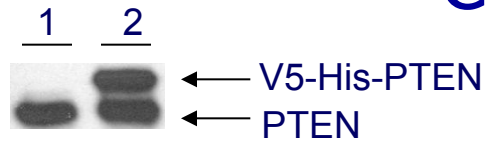


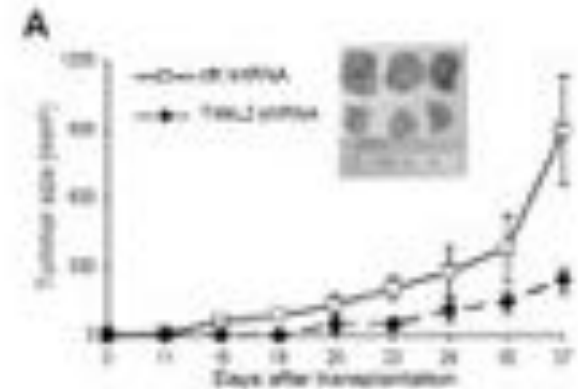
# PDK1 is Required for Metastasis





# An Optimal Level of PTEN is required for Chemotaxis

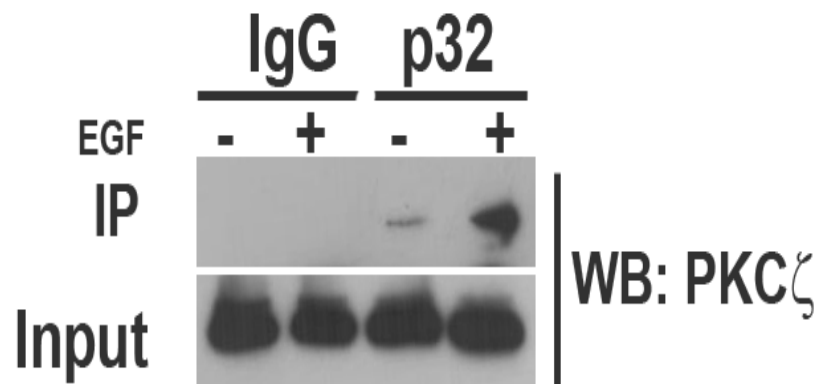




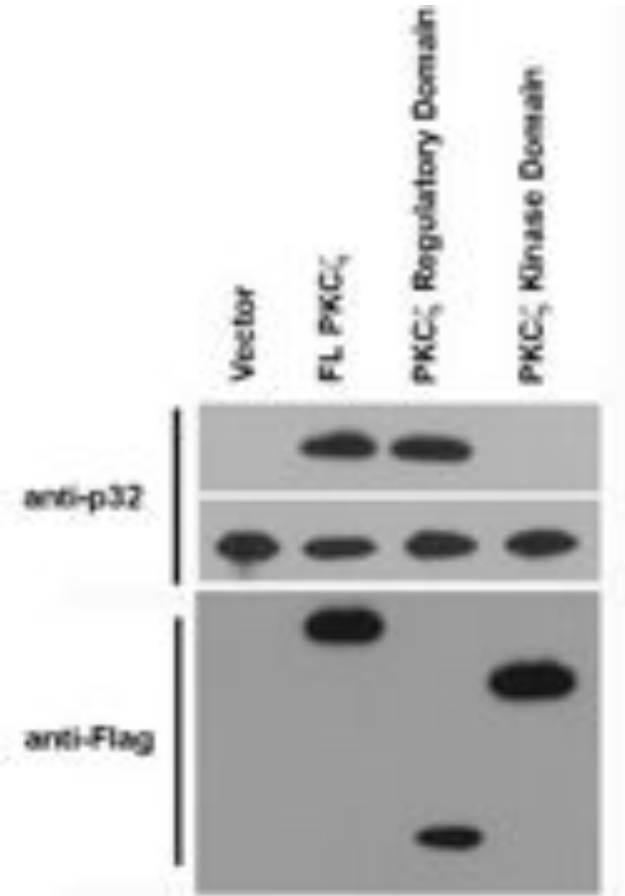


# What's Next?

## Co-immunoprecipitation



MDA-MB-231 cell

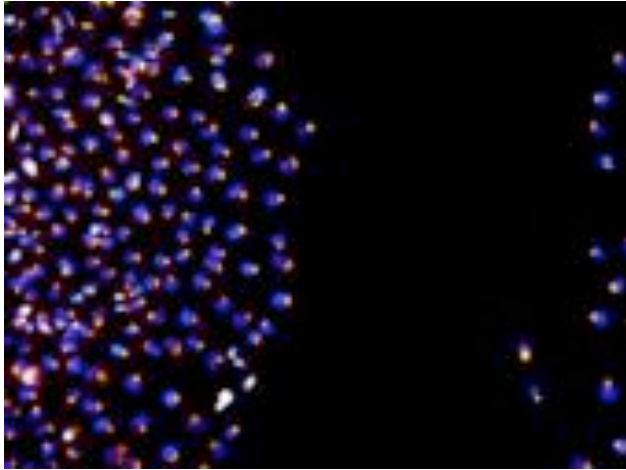


HEK293 cell

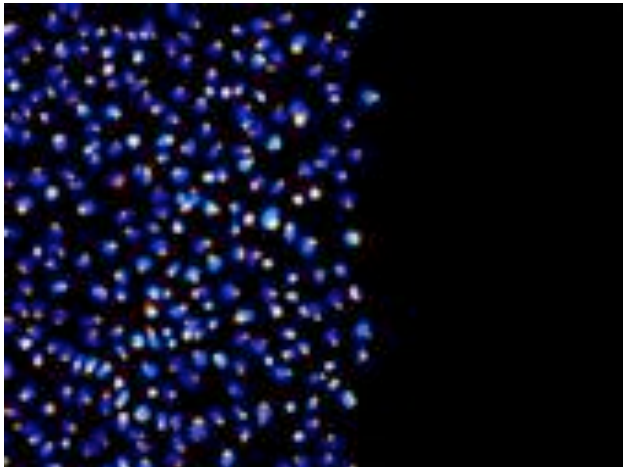


# P32

scr



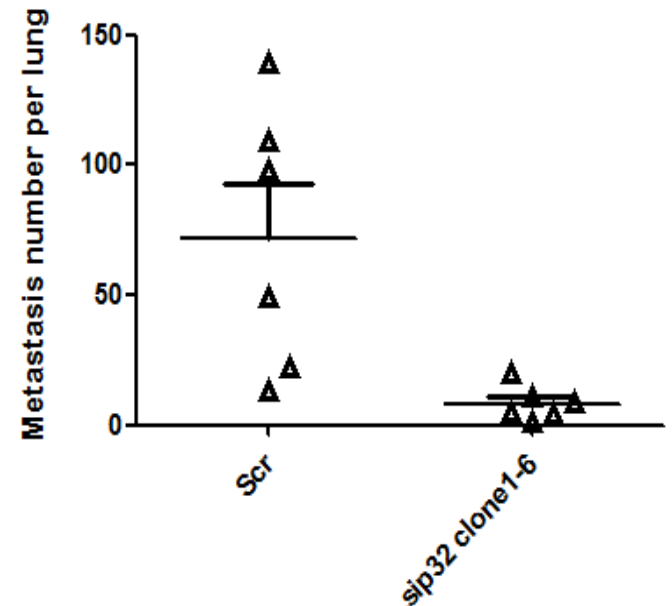
sip32



scr



sip32

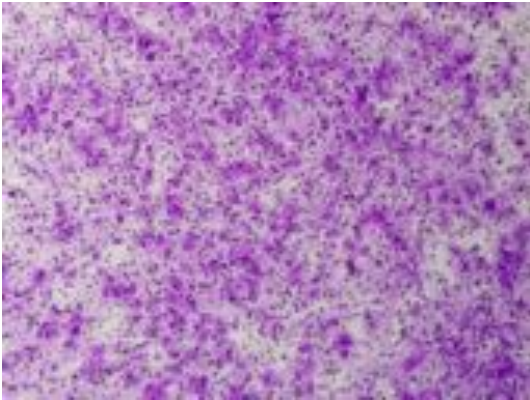




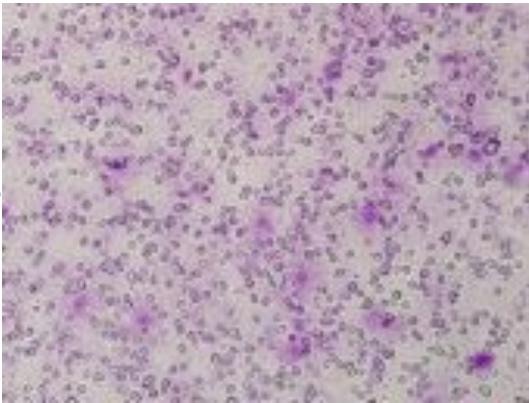


# B23

**SCR**

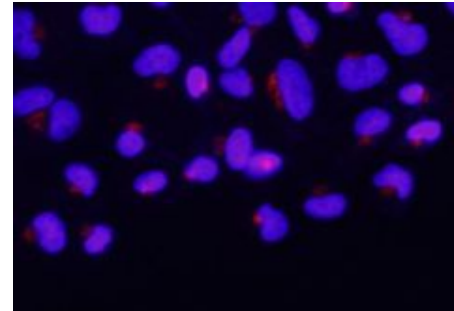


**siB23**

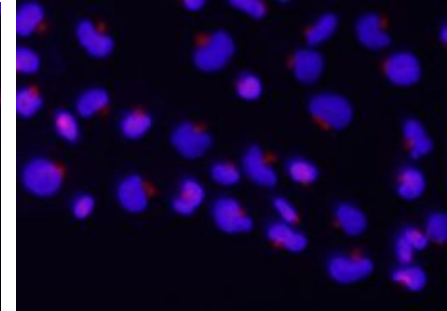


20min

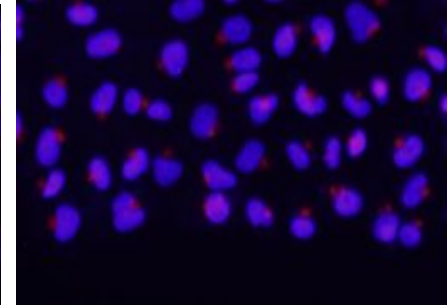
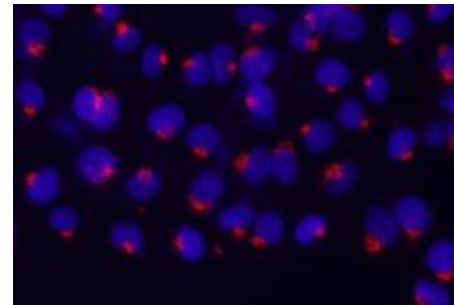
**SCR**



**SiB23**



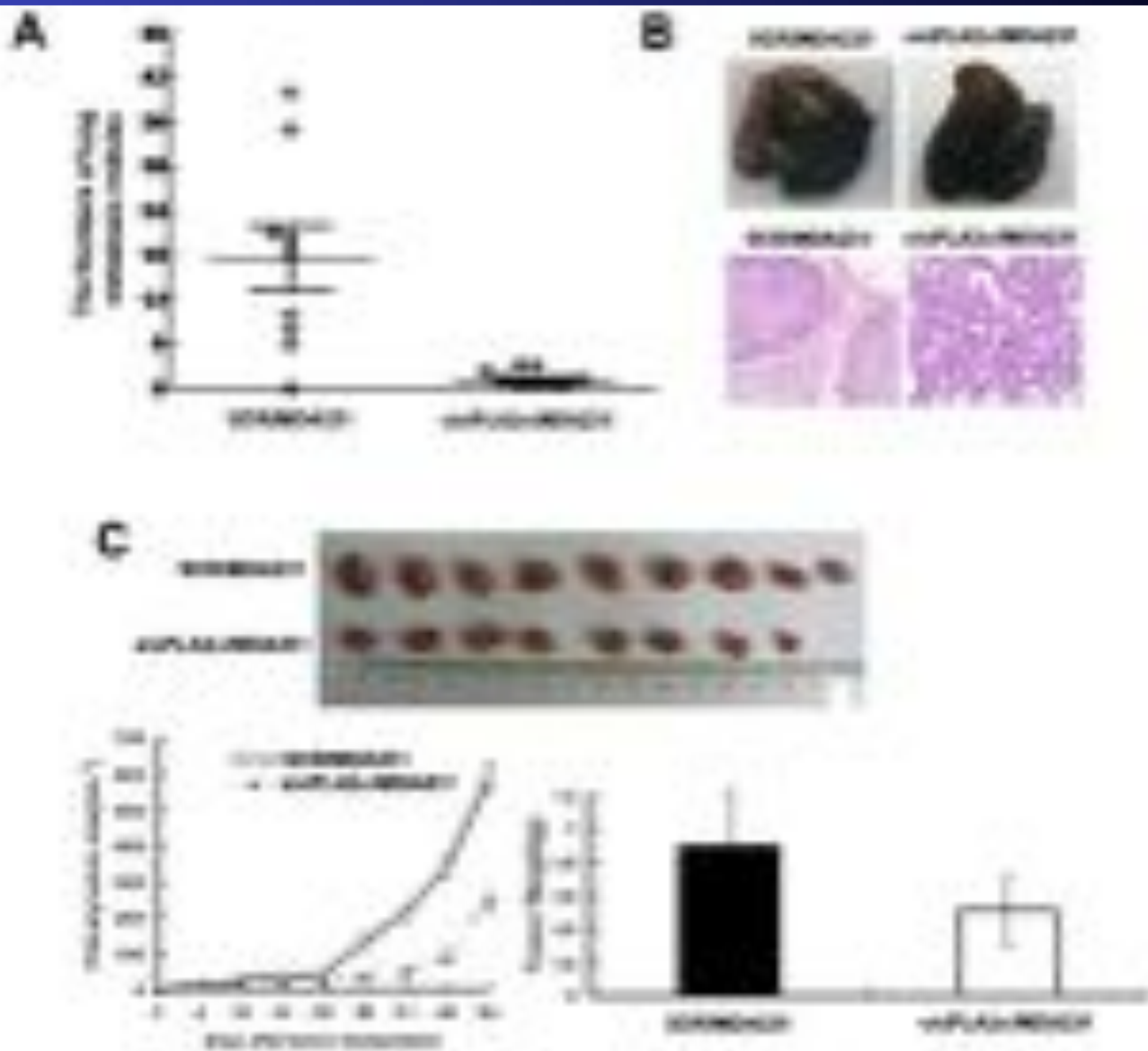
6h





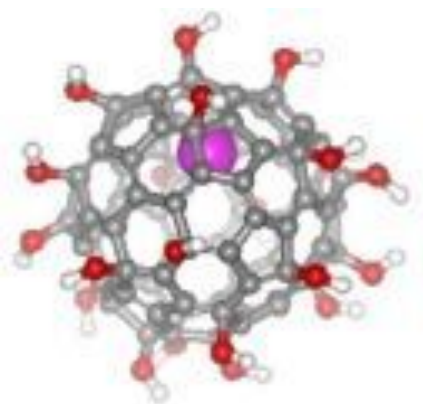


# cPLA2a

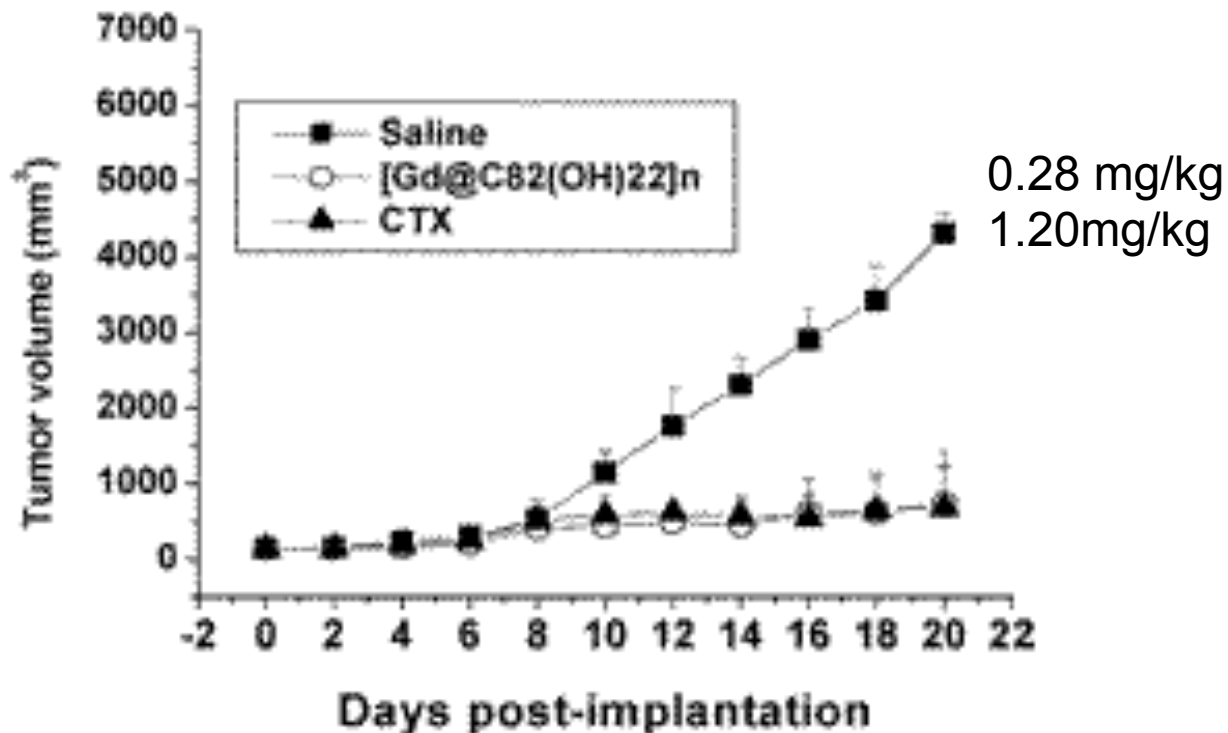




# Treatments with $\text{Gd}@C_{82}(\text{OH})_{22}$ Inhibit hepatoma growth in a mouse model



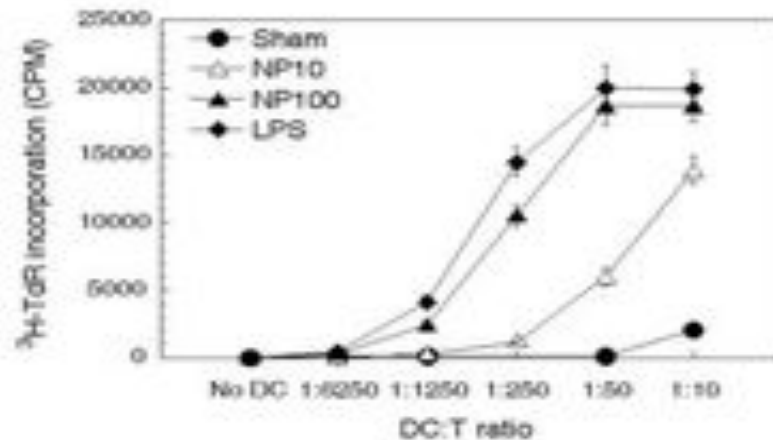
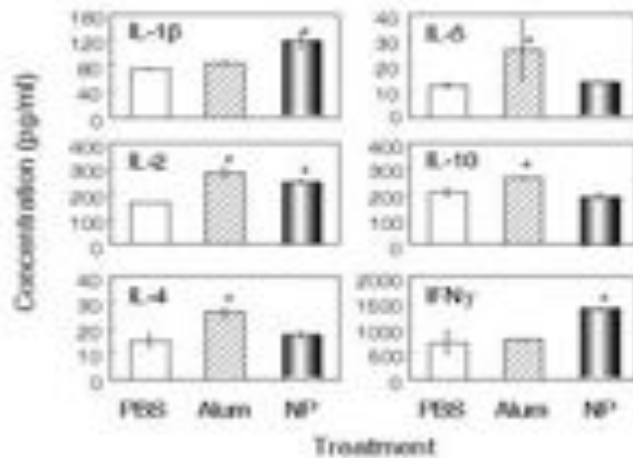
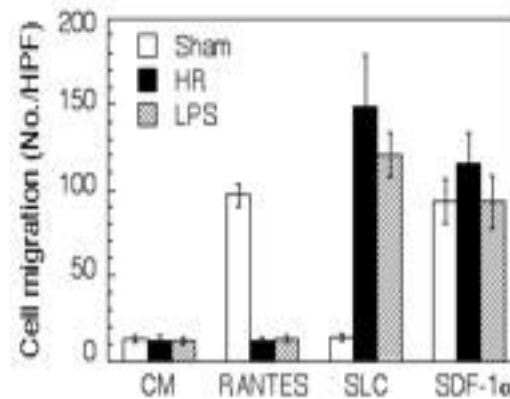
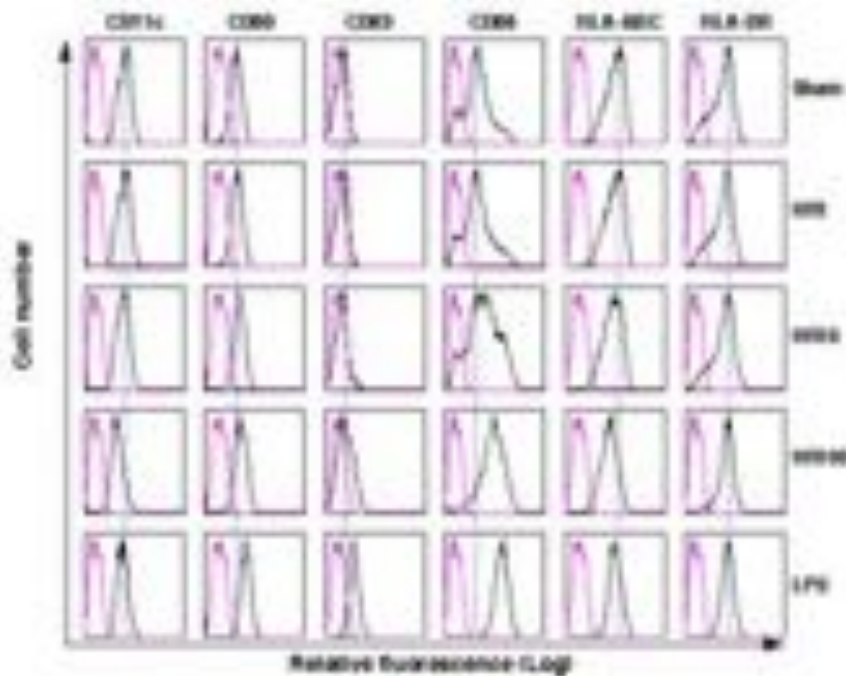
$\text{Gd}@C_{82}(\text{OH})_{22}$



$\text{Gd}@C_{82}(\text{OH})_{22}$  inhibits tumor growth in a breast tumor model.

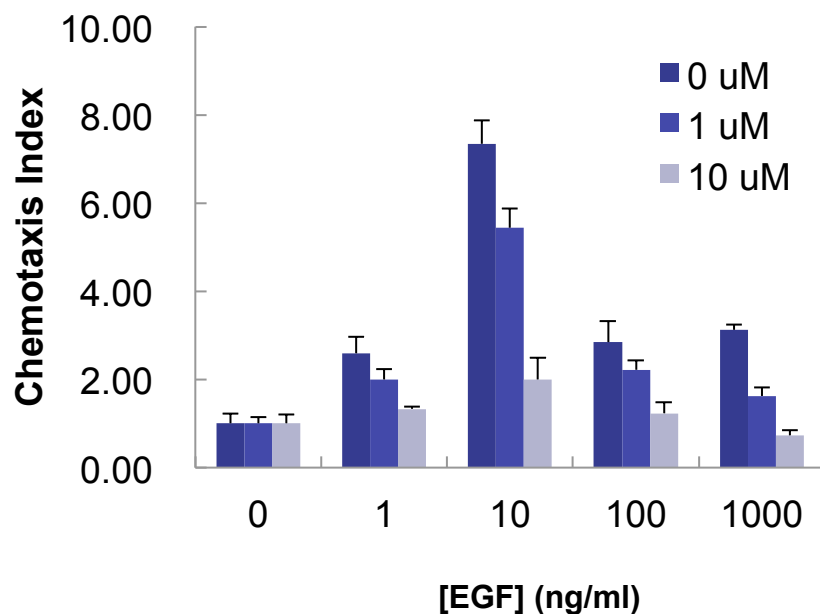
Nano Lett Vol 5, pg 2050

Gd@C82(OH)<sub>22</sub> induced iDC maturation and TH-1 response.





# Treatments with $\text{Gd}@\text{C}_{82}(\text{OH})_{22}$ cancer cell chemotaxis and metastasis



Treatments	Metastasis Rate
Saline q.d. ×20 day	66.7%
$\text{C}_{60}(\text{C}(\text{COOH})_2)_2$ (0.4 mg/kg, n = 10)	34.2%
$\text{C}_{60}(\text{OH})_{20}$ (0.4 mg/kg, n = 10)	38.0%
$\text{Gd}@\text{C}_{82}(\text{OH})_{22}$ , 0.35mg/kg q.d. ×20 day	4.3 %

## Summary

1. Treatment with  $\text{Gd}@C_{82}(\text{OH})_{22}$  inhibits tumor growth without detectable toxicity.
2.  $\text{Gd}@C_{82}(\text{OH})_{22}$  doesn't show cytotoxicity.
3.  $\text{Gd}@C_{82}(\text{OH})_{22}$  inhibits blood supply to tumor tissues.
4.  $\text{Gd}@C_{82}(\text{OH})_{22}$  induced tumor immunity.
5.  $\text{Gd}@C_{82}(\text{OH})_{22}$  inhibits cancer cell chemotaxis

