

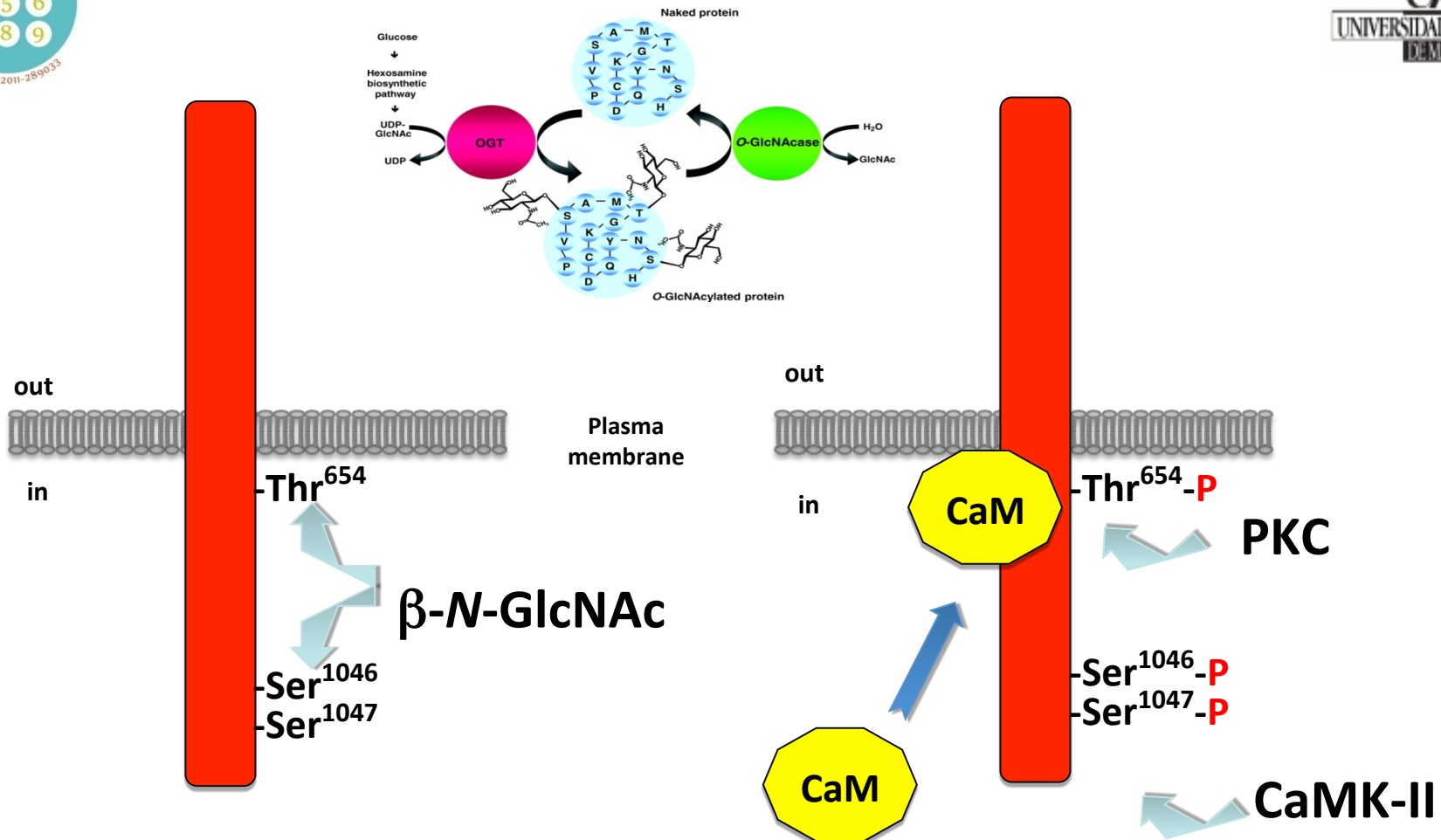


O-GlcNacylation of the EGFR and Systems Regulated by Calmodulin and Phospho-(Tyr)-Calmodulin

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Instituto de Investigaciones Biomedicas, CSIC & UAM



O-GlcNAcylation of Epidermal Growth Factor Receptor

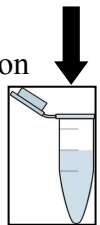


Kaleem *et al.*, 2008 Mol. Biol.

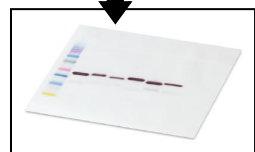
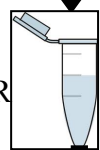
A431 cell line



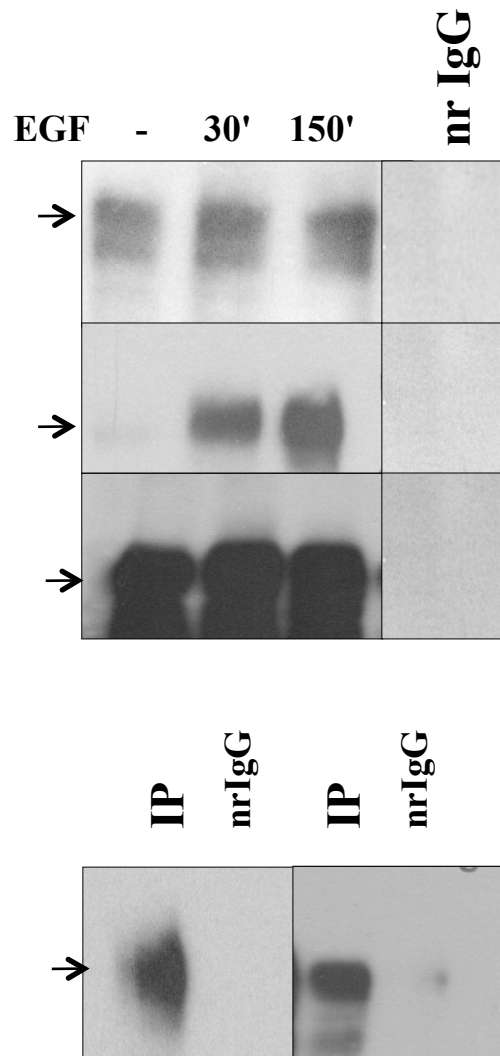
Protein extraction



IP EGFR



WB: O-GlcNAc
CD110.6



IP: EGFR
WB: O-GlcNAc
CTD 110

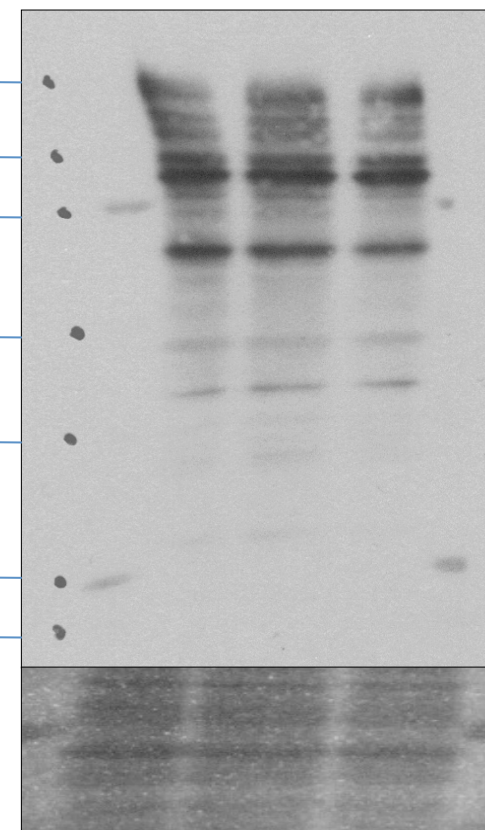
IP: EGFR
WB: 4G10

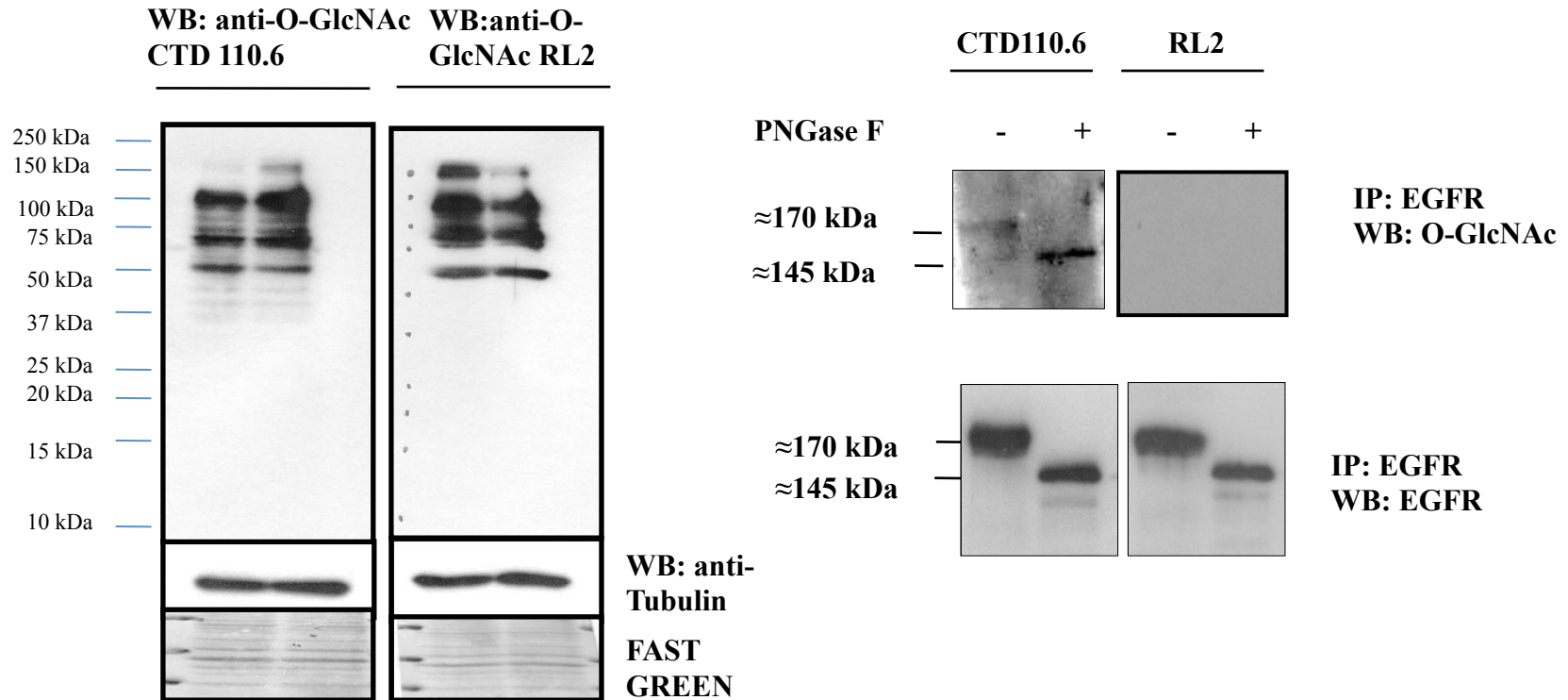
IP: EGFR
WB: EGFR

IP: O-GlcNAc
WB: EGFR

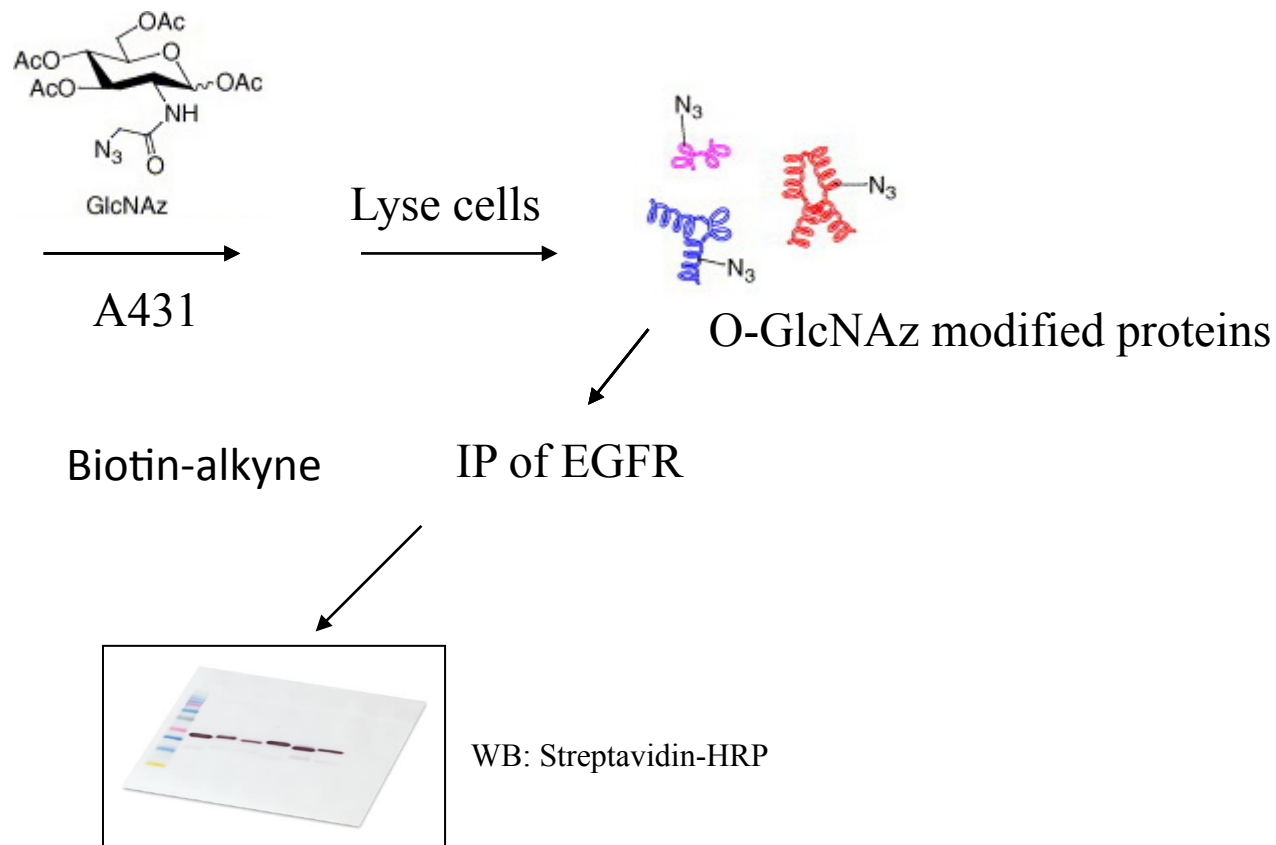
EGF - 30' 150'

150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa

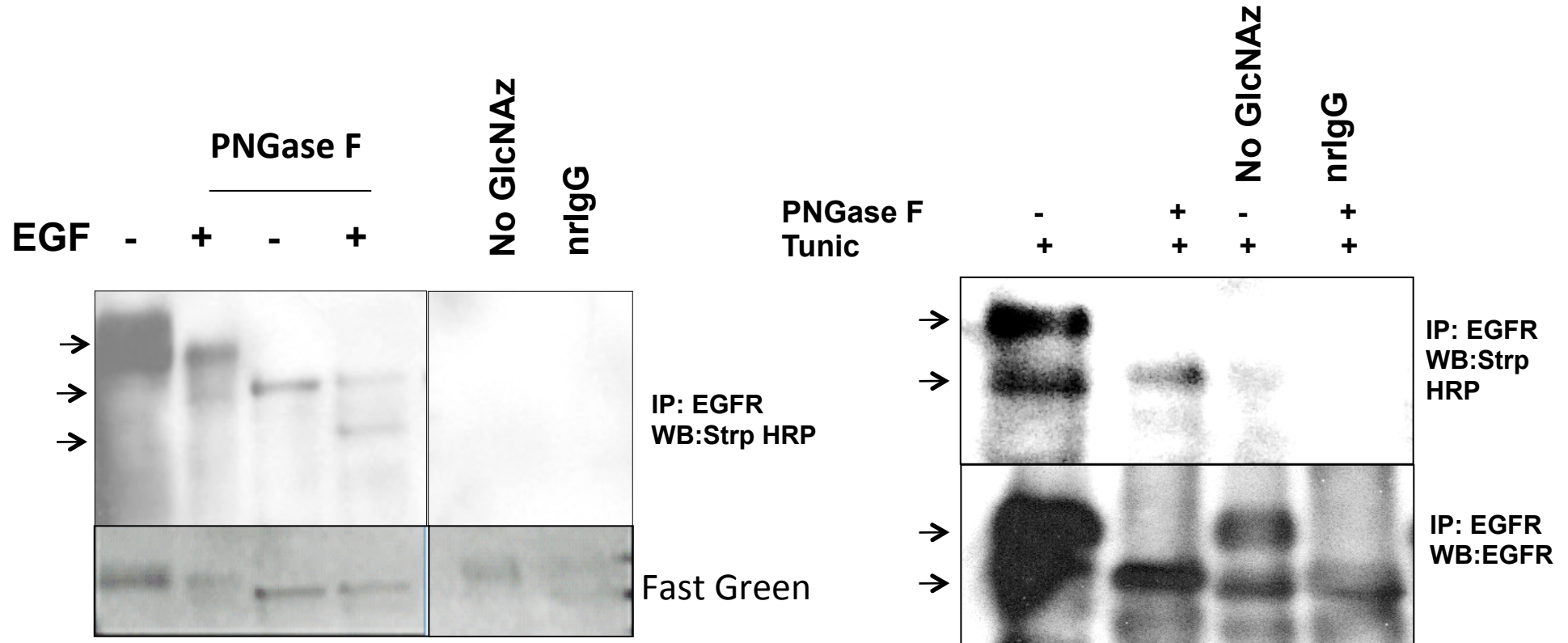




Click chemistry

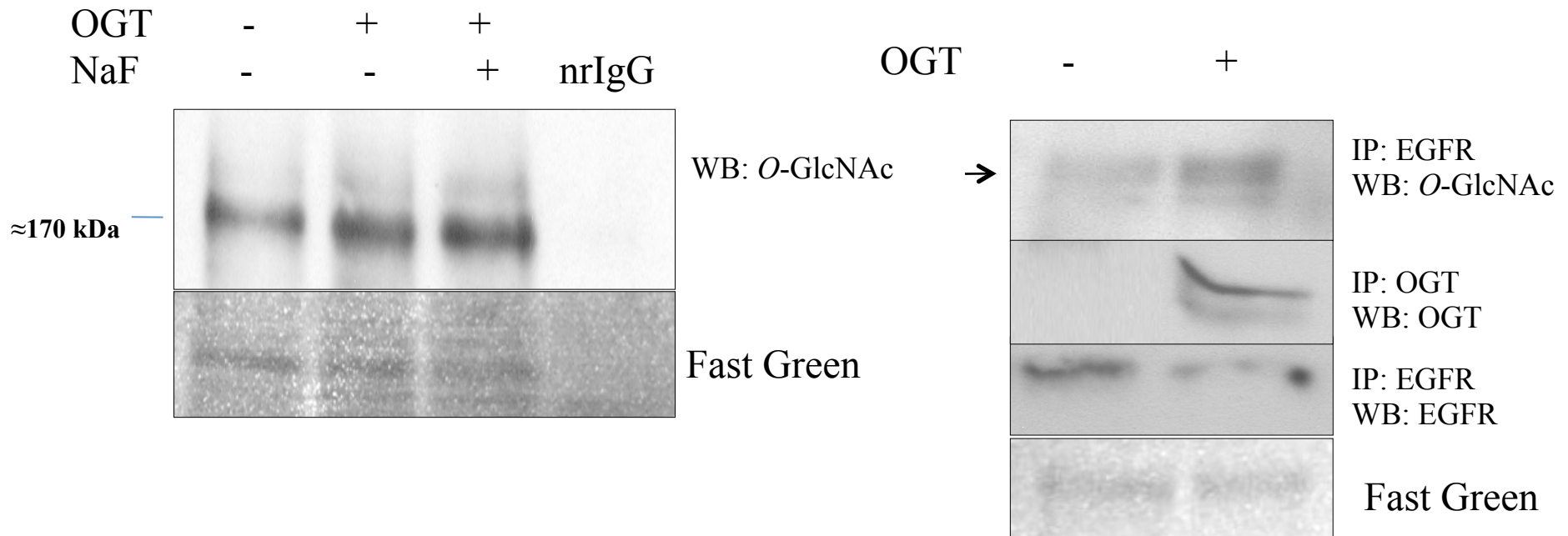


Click chemistry



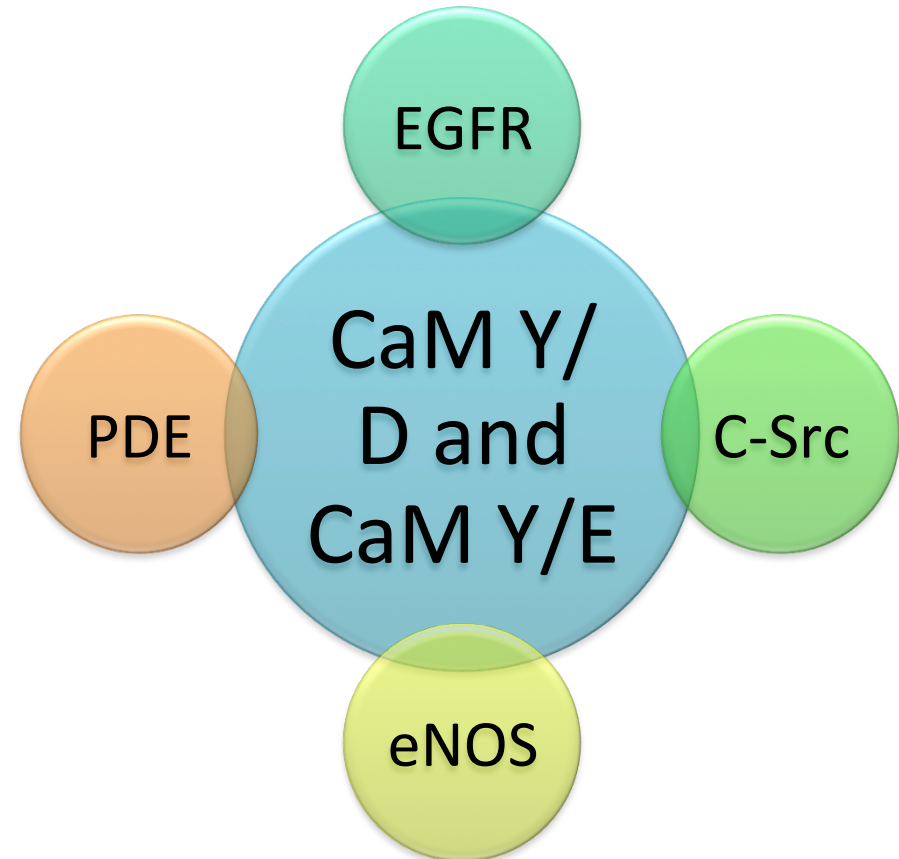
O-GlcNAcylation of Epidermal Growth Factor Receptor

In vitro O-GlcNAcylation

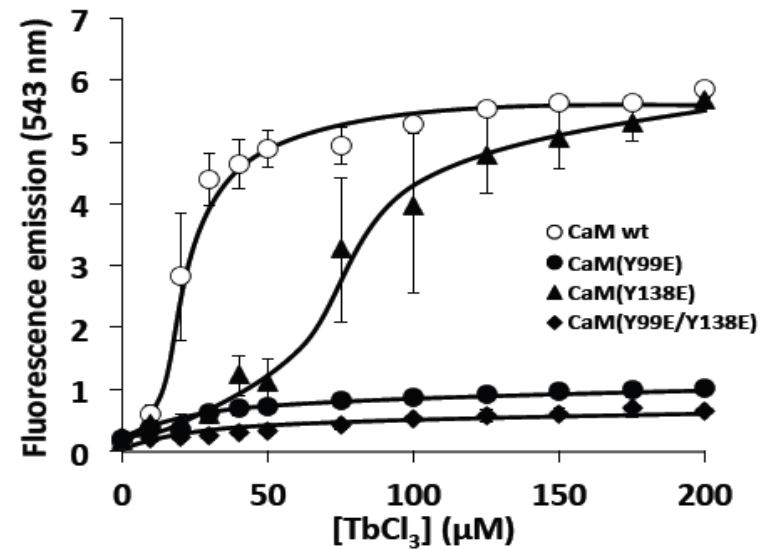
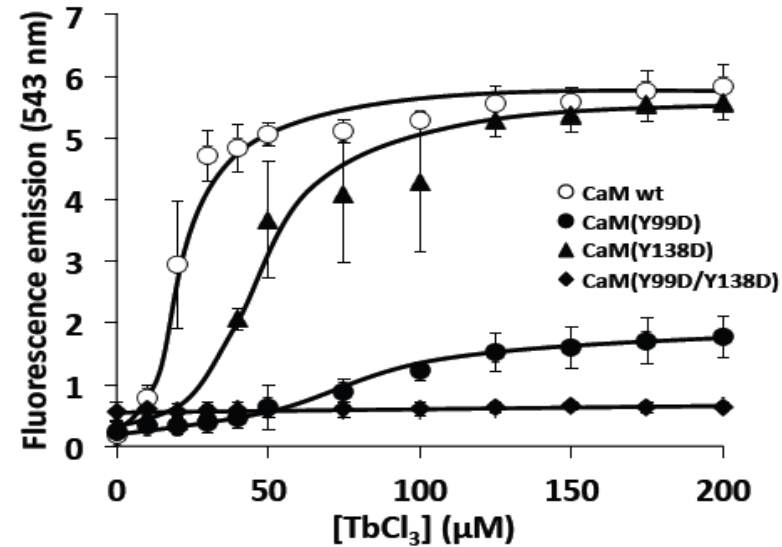
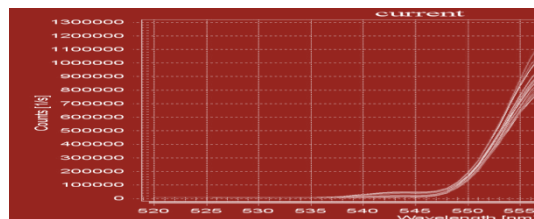
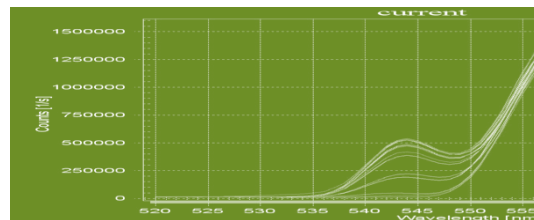
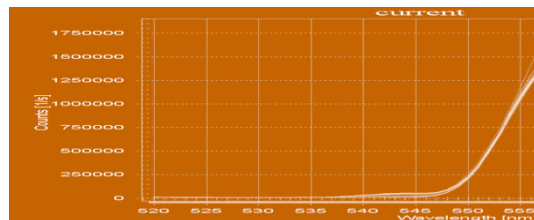
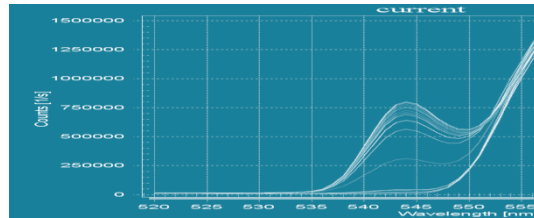


1. Generate and characterize phospho-mimetic CaM mutants
 - CaM Y/D (aspartic acid)
 - CaM Y/E (glutamic acid)

2. Test the effect of CaM (wt phospho-mimetic CaM mutants in the regulation of the **Epidermal Growth Factor Receptor (EGFR)**, **Proto-oncogene c-Src**, **Phosphodiesterase (PDE)** and **endothelial Nitric Oxide Synthetase (eNOS)** *in vitro*

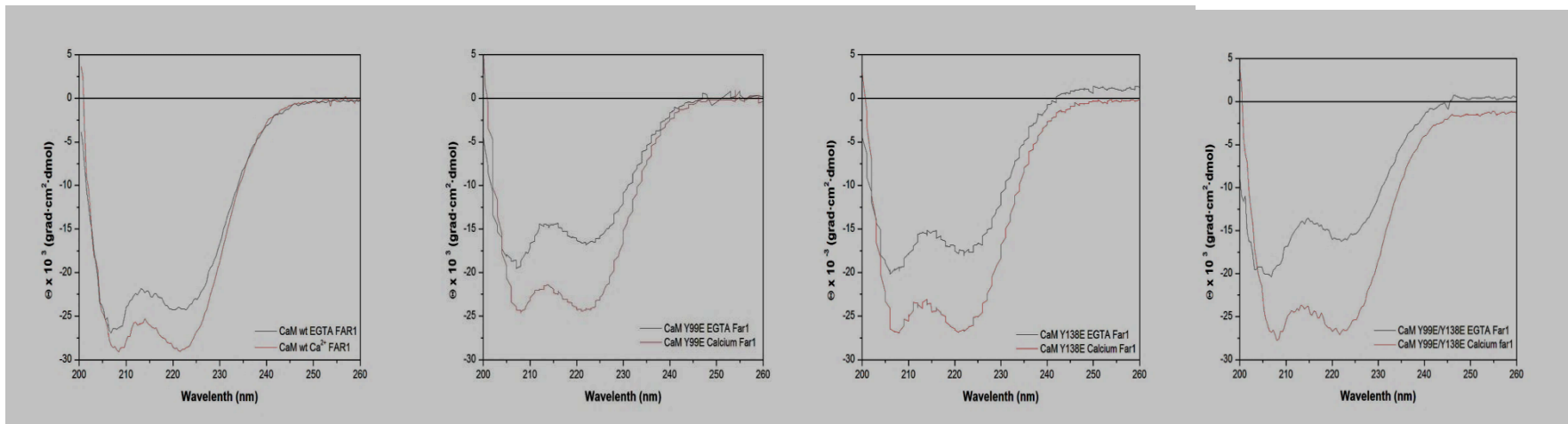
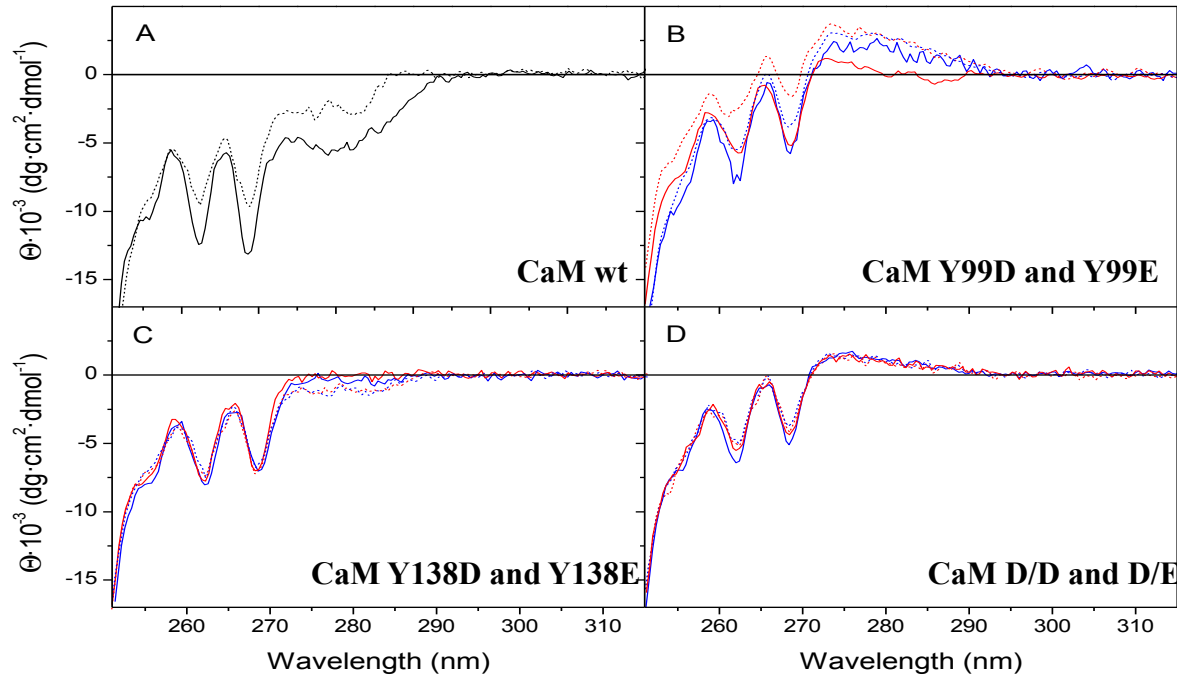


Generation and Characterization of Phospho-Tyr-CaM Mutants





Generation and Characterization of Phospho-Tyr-CaM Mutants



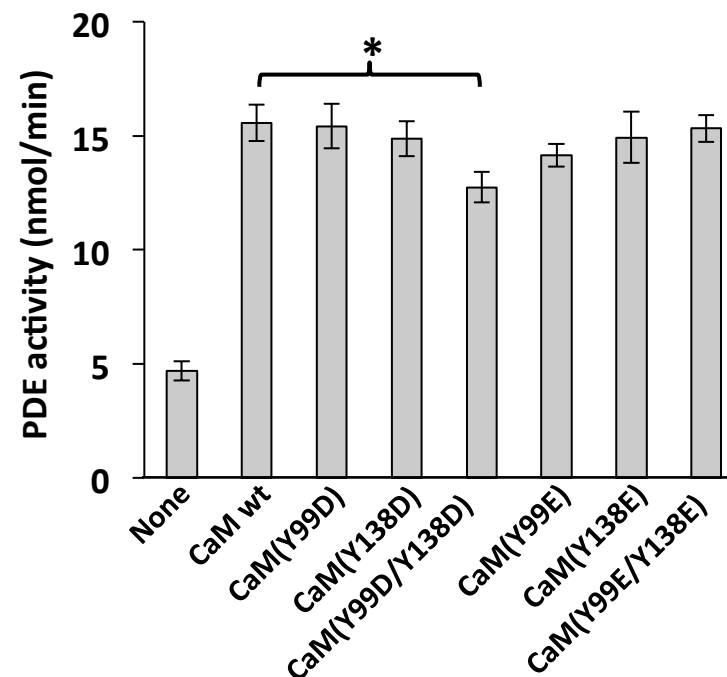
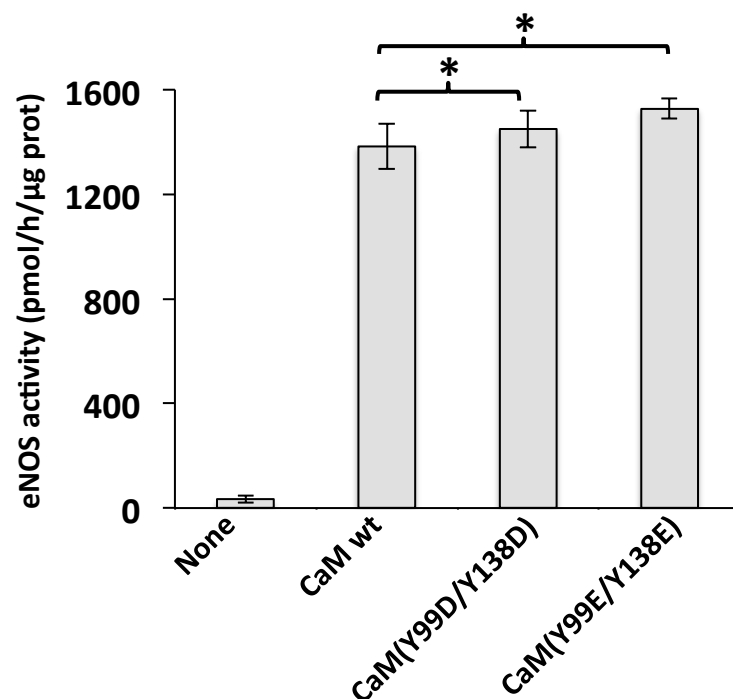
CaM wt

CaM Y99E

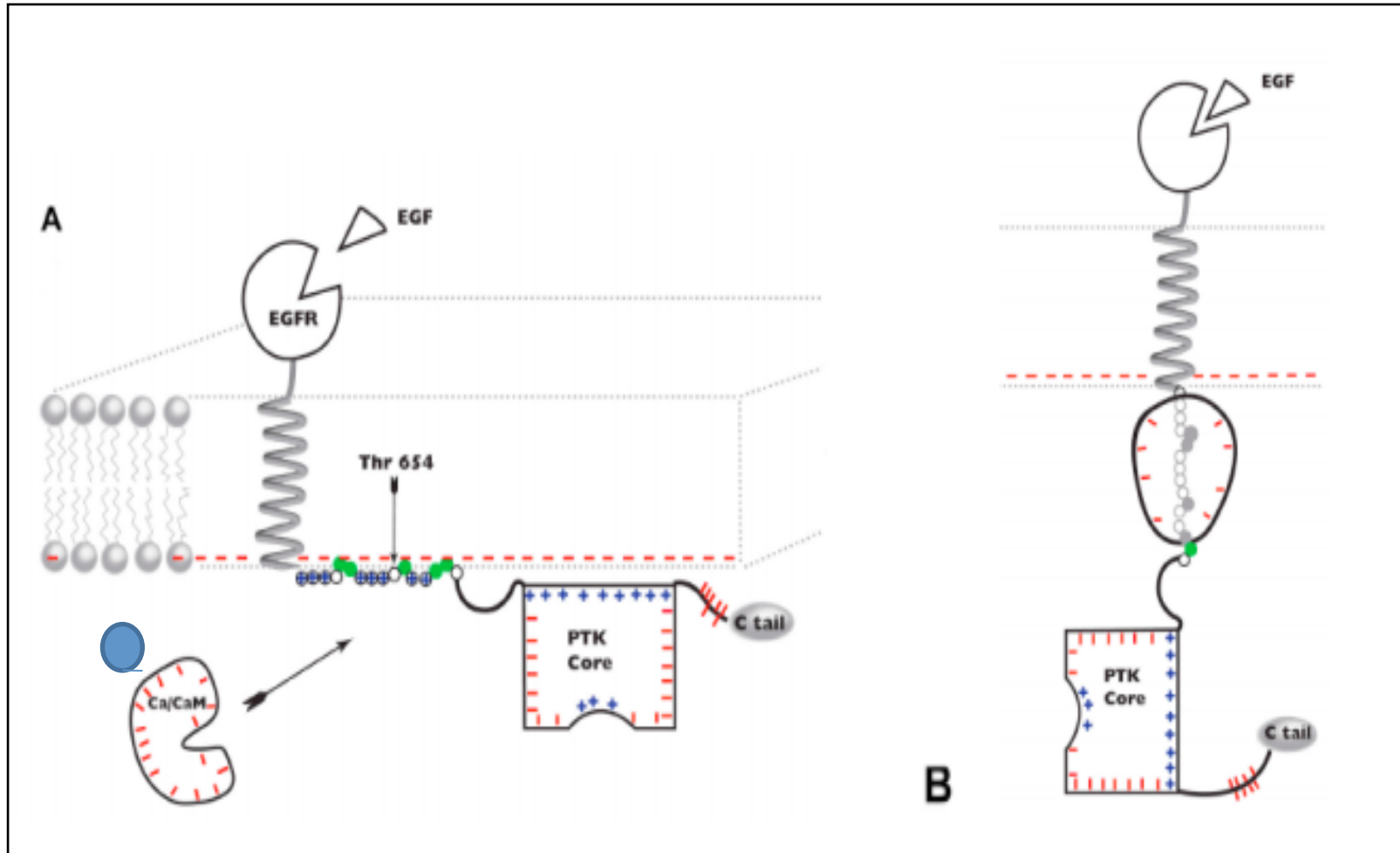
CaM Y138E

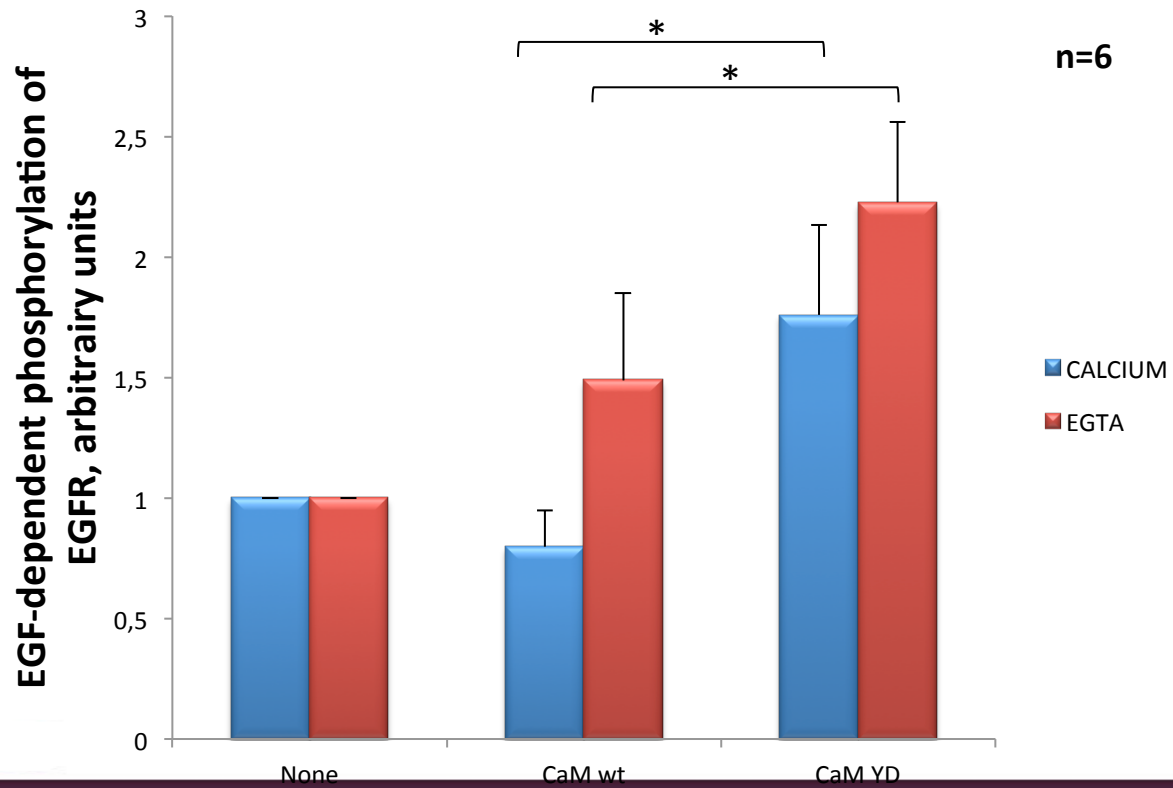
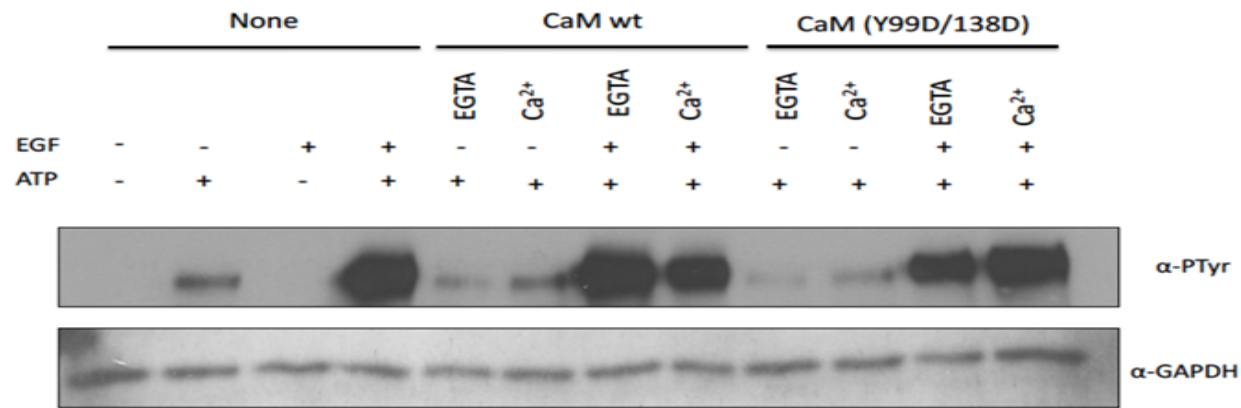
CaM Y99E/Y138E

Generation and Characterization of Phospho-Tyr-CaM Mutants

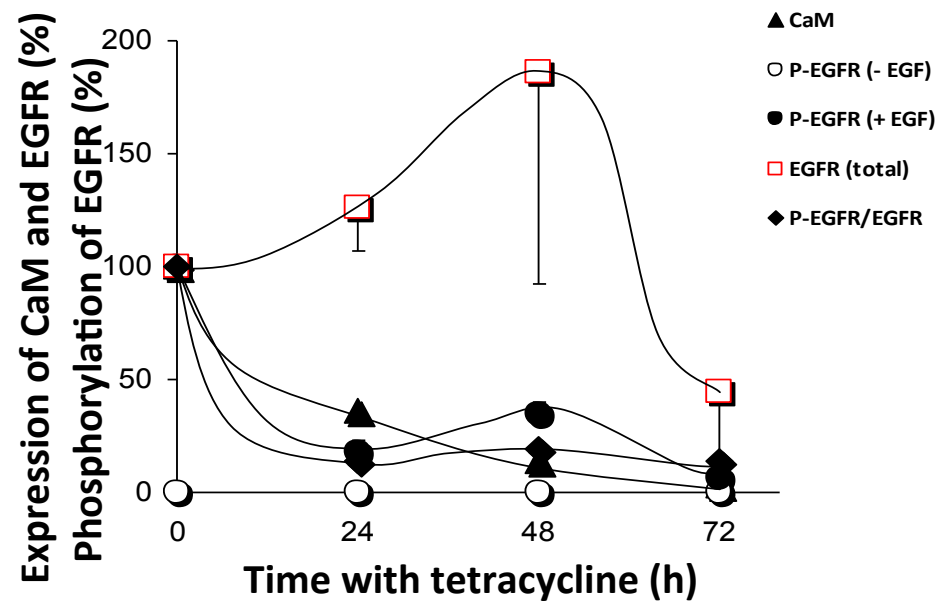
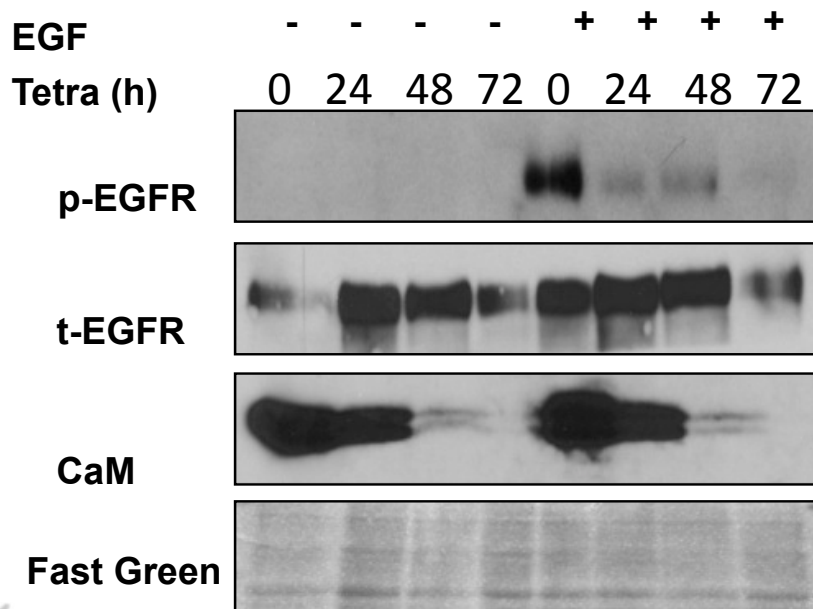
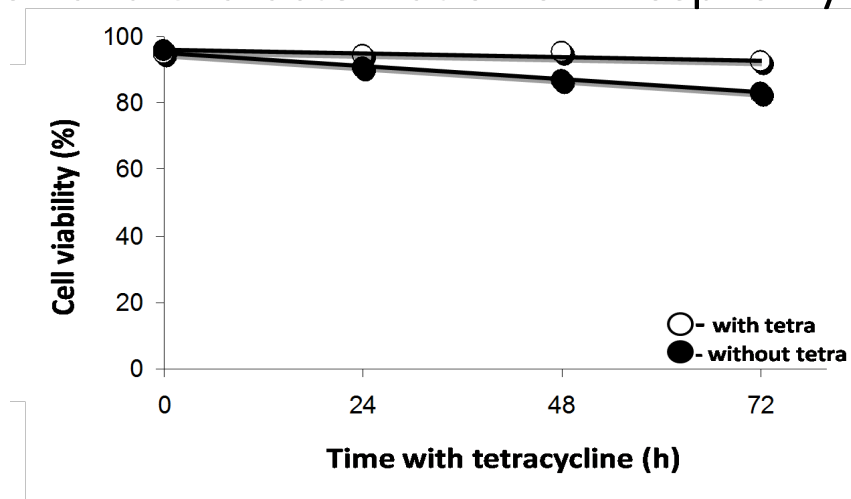


Generation and Characterization of Phospho-Tyr-CaM Mutants



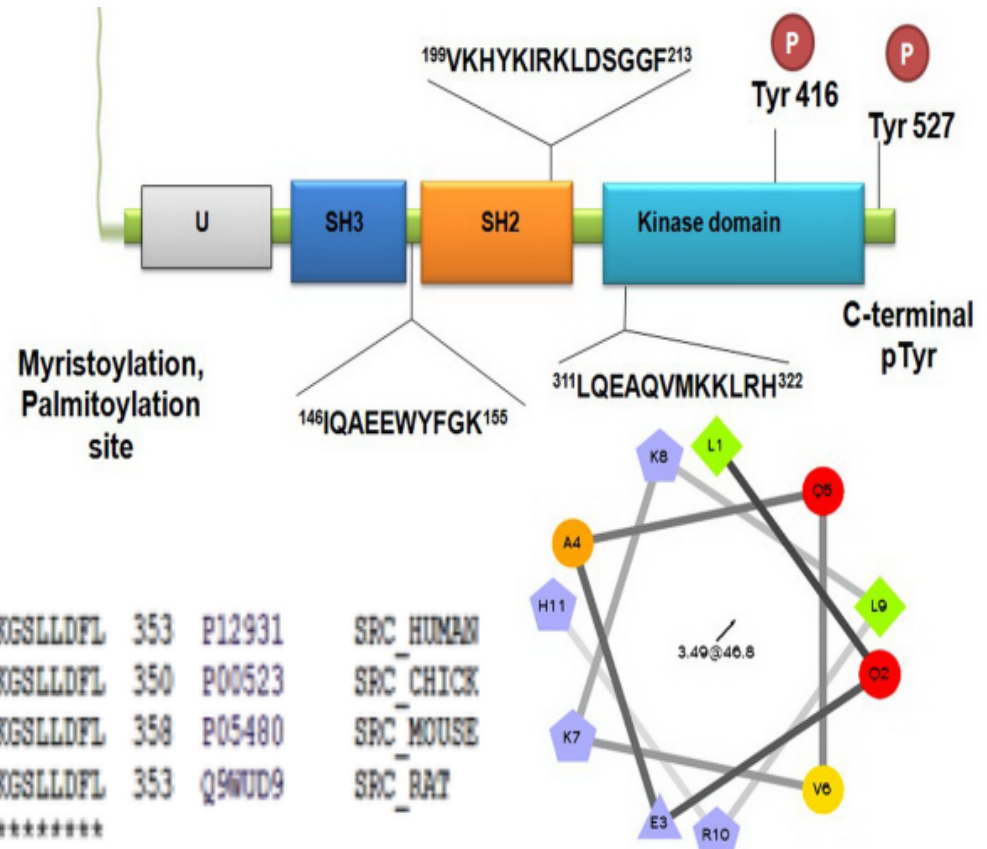


Generation and Characterization of Phospho-Tyr-CaM Mutants



CaM binds and regulate the activity of c-Src kinase

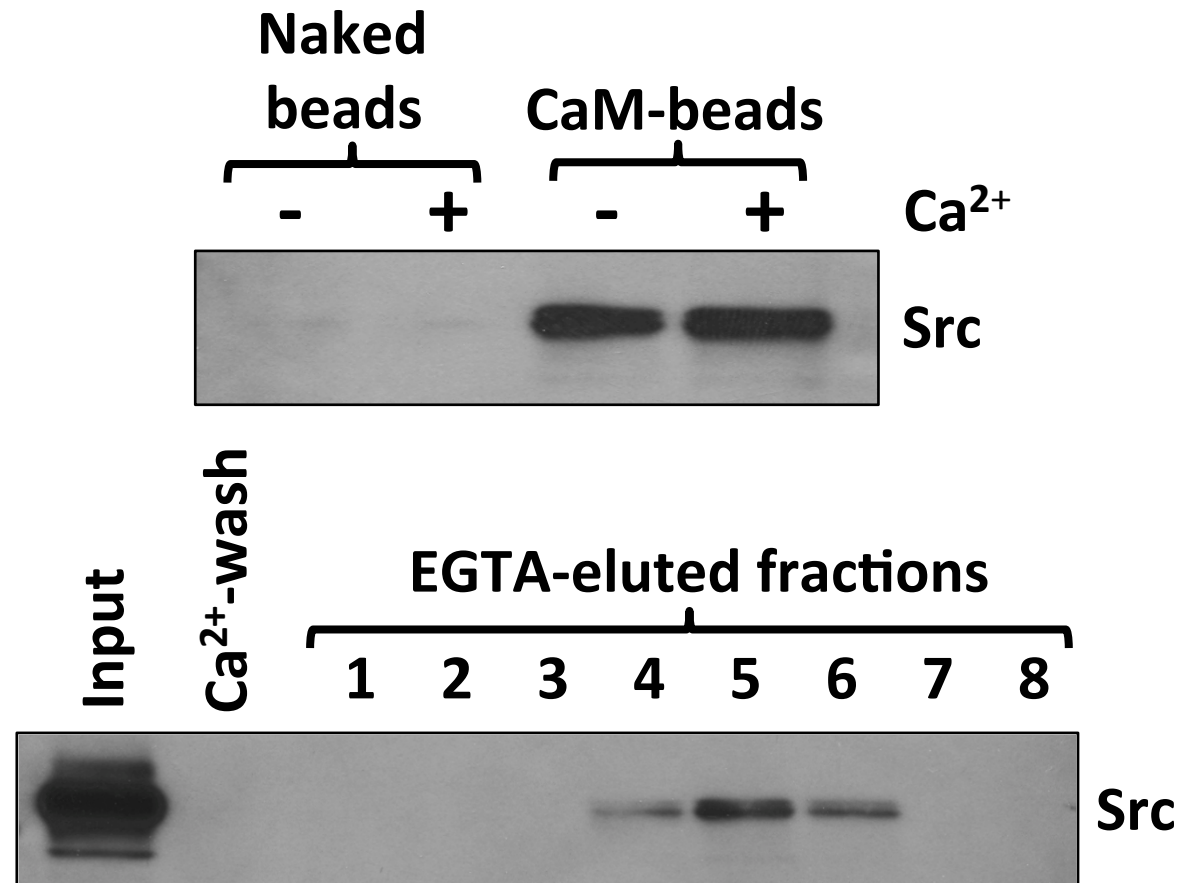
- Non-receptor tyrosine kinase (membrane attached)
- SH2, SH3, tyrosine kinase domain and short catalytic tail
- Activated by adhesion receptors, receptor tyrosine kinases, G-protein coupled receptors
- When activated it promotes survival, angiogenesis, proliferation and invasion pathways



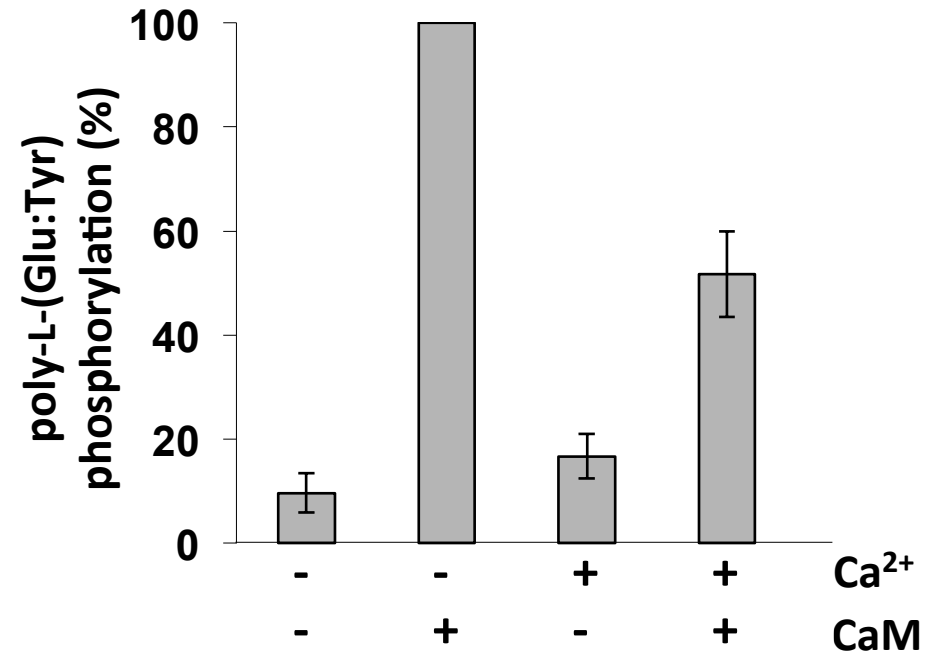
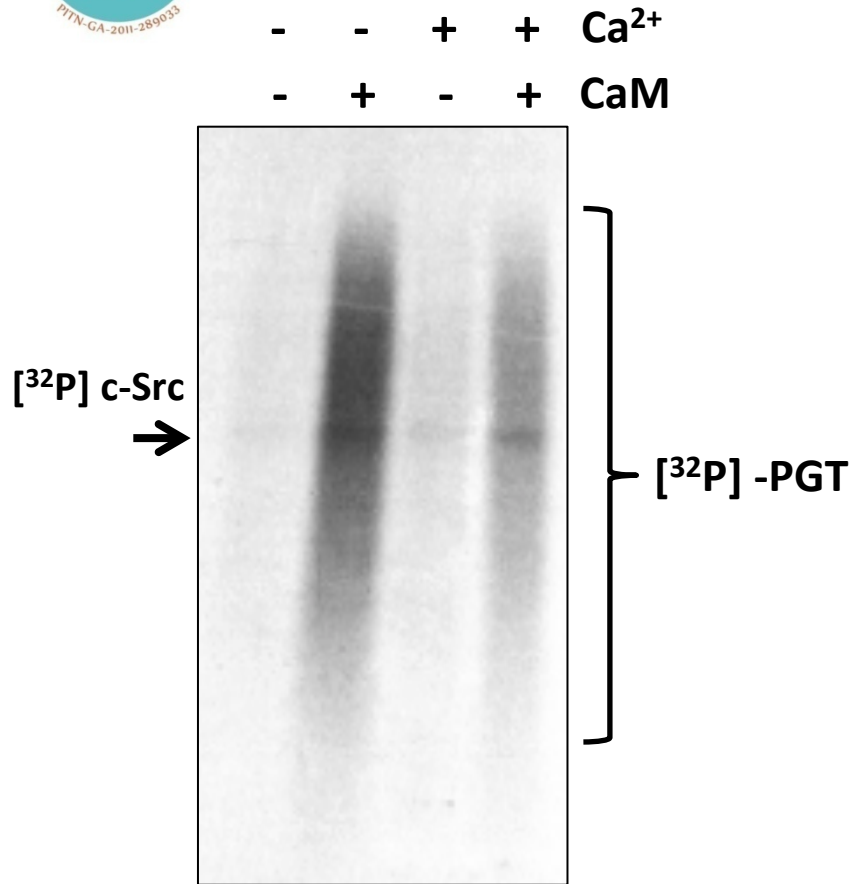
294	RVAIKTLKPGTMSPEAF	LQEAQVMKKLRHEK	LWQLYAVVSEEP	YIIVTEYMS	EGSLLD	DFL	353	P12931	SRC_HUMAN
291	RVAIKTLKPGTMSPEAF	LQEAQVMKKLRHEK	LWQLYAVVSEEP	YIIVTEYMS	EGSLLD	DFL	350	P00523	SRC_CHICK
299	RVAIKTLKPGTMSPEAF	LQEAQVMKKLRHEK	LWQLYAVVSEEP	YIIVTEYMS	EGSLLD	DFL	358	P05480	SRC_MOUSE
294	RVAIKTLKPGTMSPEAF	LQEAQVMKKLRHEK	LWQLYAVVSEEP	YIIVTEYMS	EGSLLD	DFL	353	Q9WUD9	SRC_RAT

***** , *****

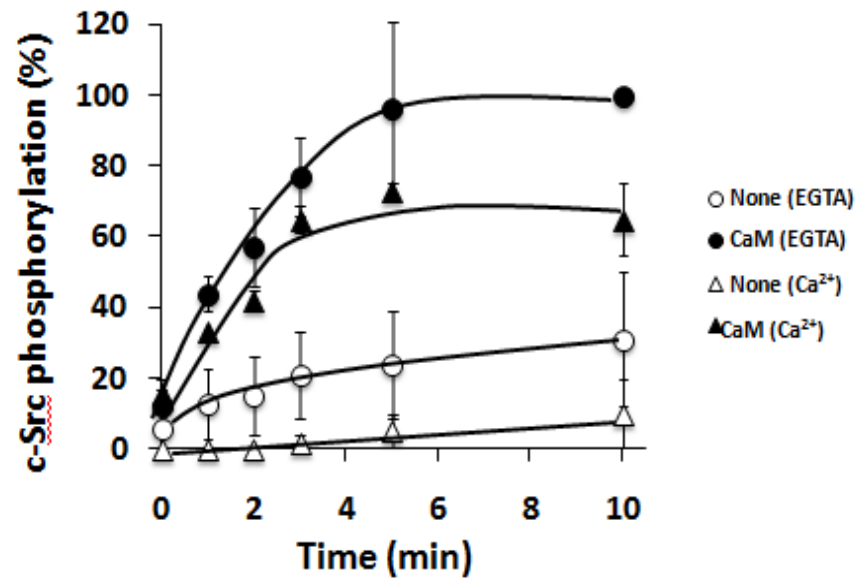
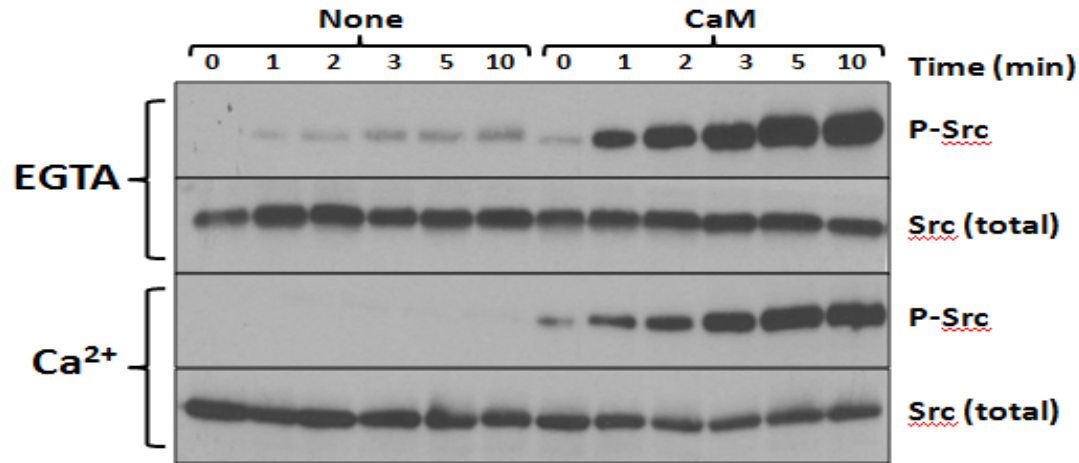
CaM binds and regulate the activity of c-Src kinase



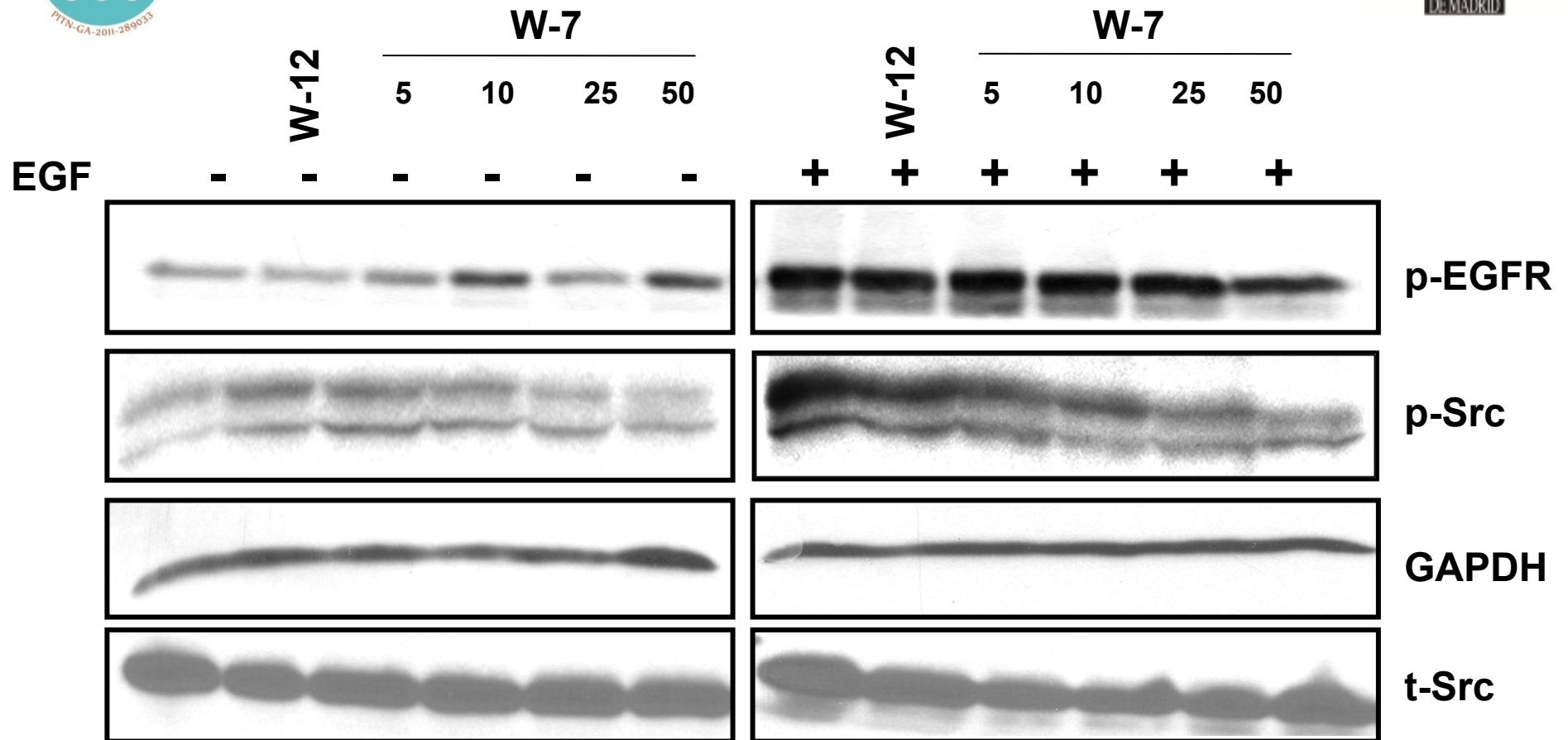
CaM binds and regulate the activity of c-Src kinase



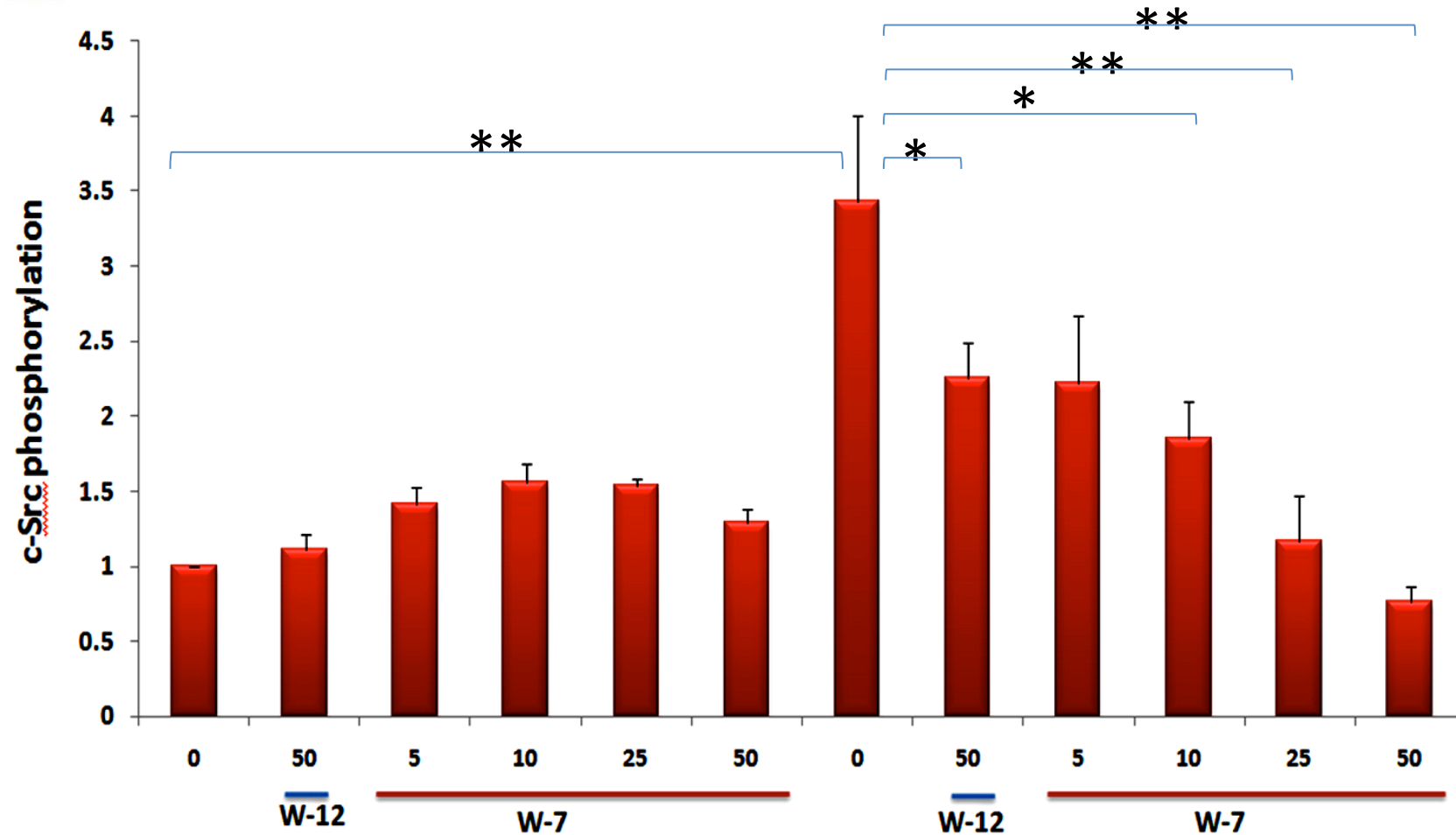
CaM binds and regulate the activity of c-Src kinase



CaM binds and regulate the activity of c-Src kinase In living cells

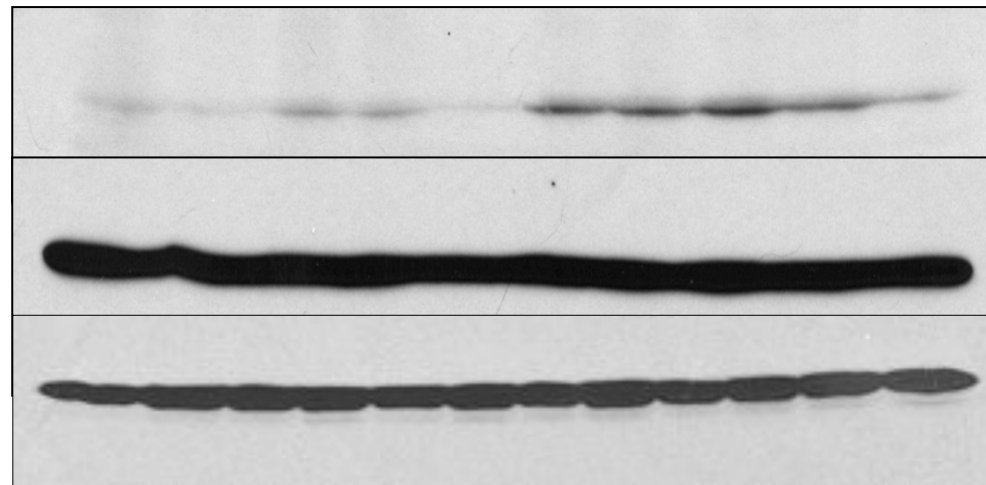


CaM binds and regulate the activity of c-Src kinase In living cells



CaM binds and regulate the activity of c-Src kinase In living cells

H2O2	-	-	-	-	-	+	+	+	+	+
W7- 20, 50 μ M	-	-	-	+	+	-	-	-	+	+
W12- - 20, 50 μ M	-	+	+	-	-	-	+	+	-	-



p-Src

GAPDH

t-Src

CaM binds and regulate the activity of c-Src kinase In living cells

