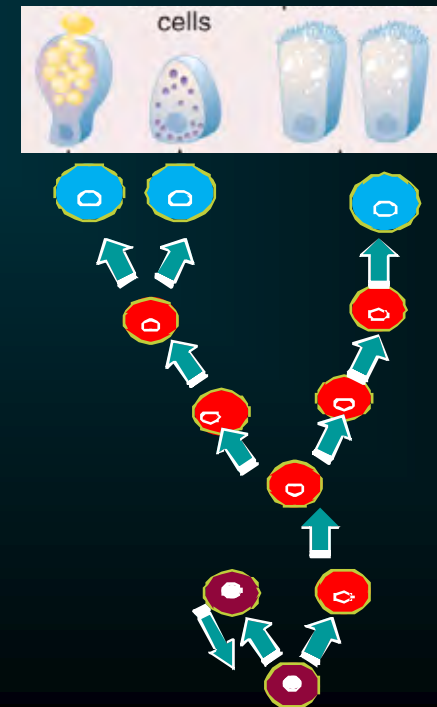
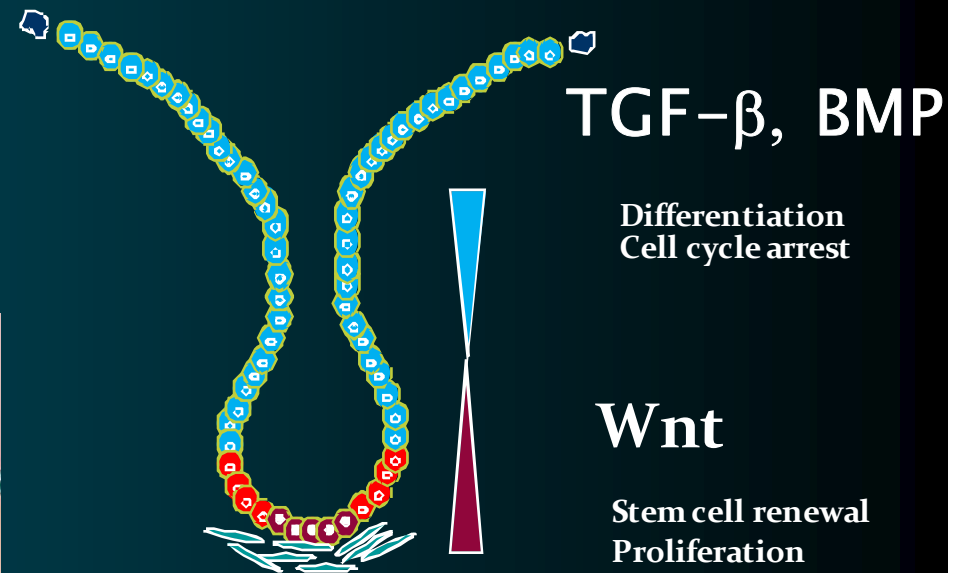
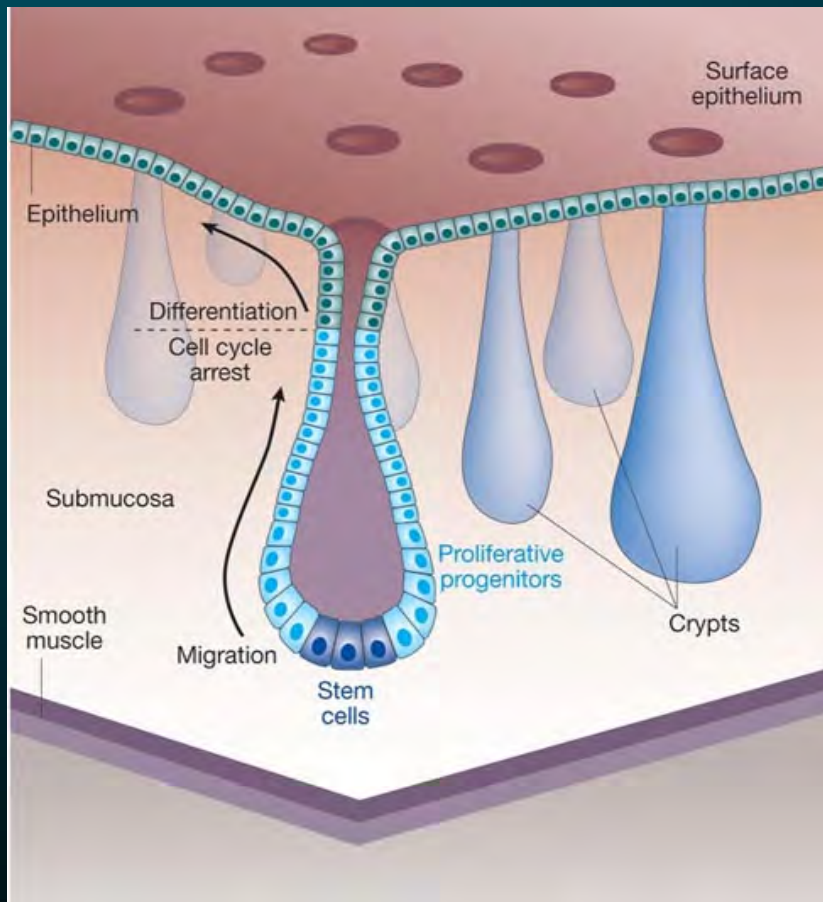




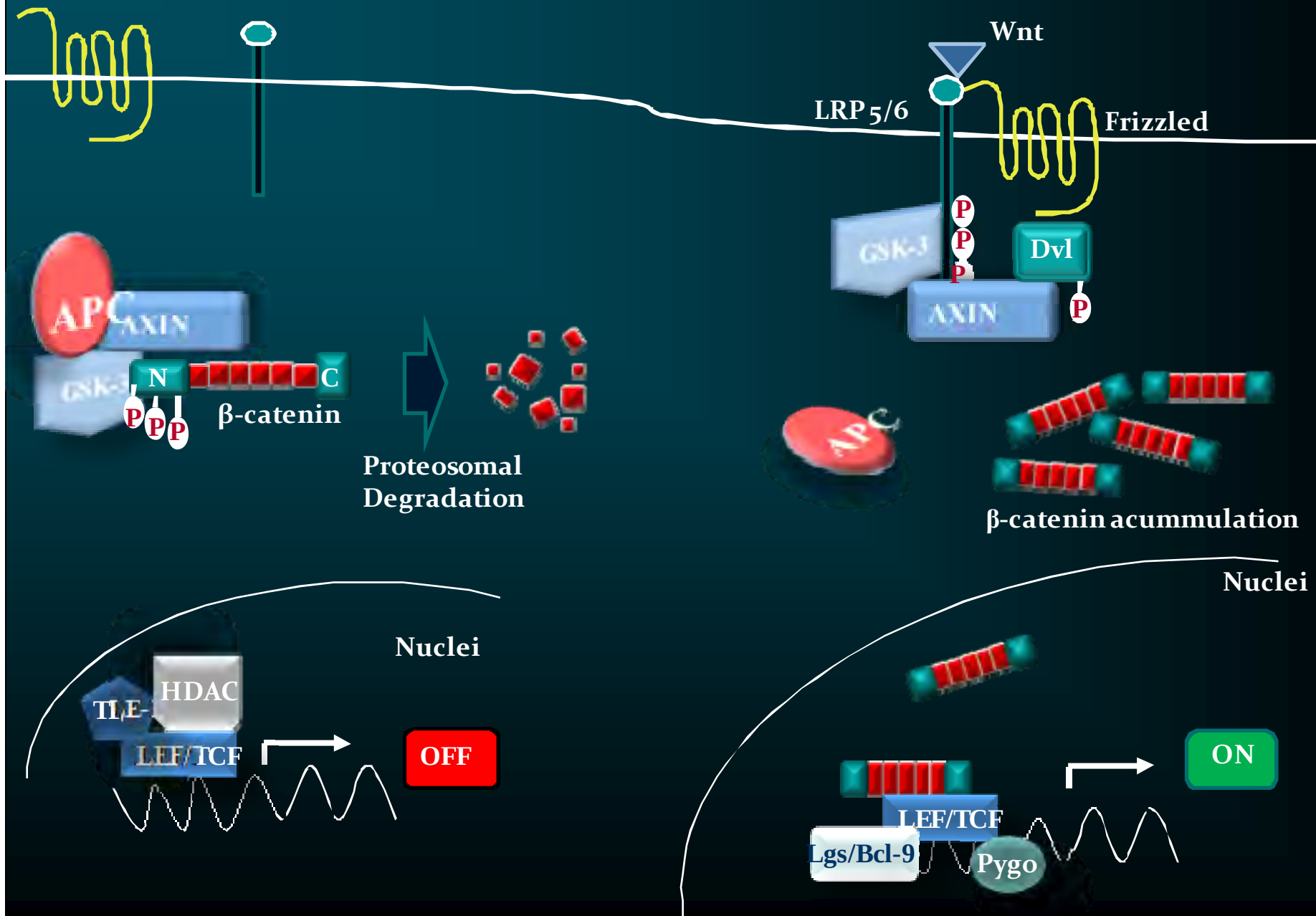
Protein Kinase C Modulates Wnt Signaling In Colon Tumoral Cell Lines

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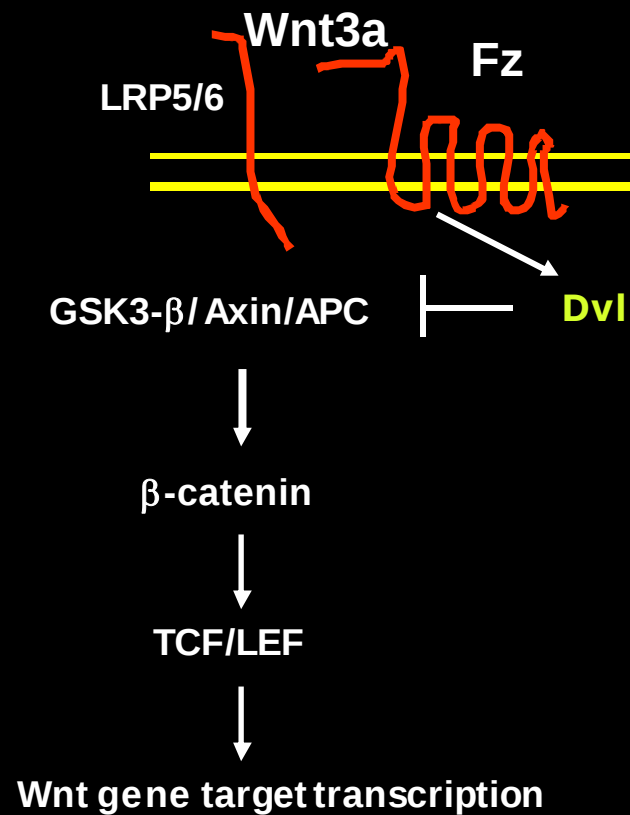
Tissue anatomy of the colonic epithelium



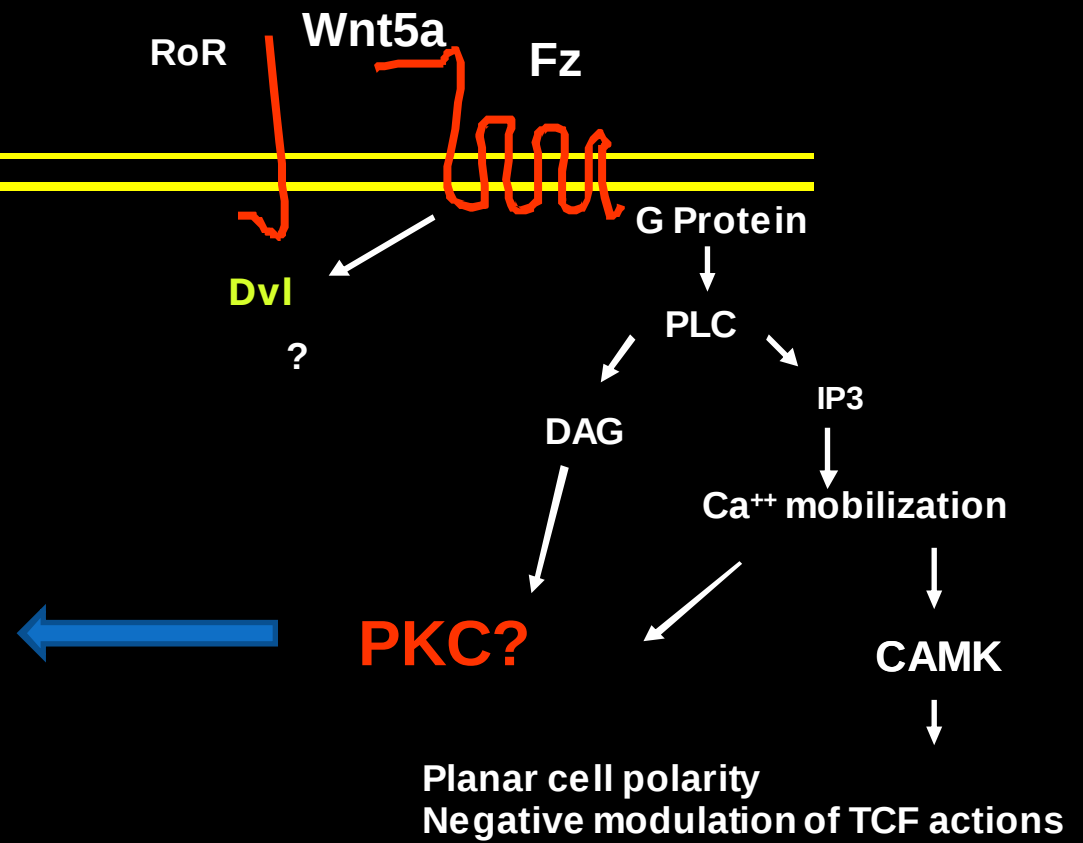
Canonical Wnt Signaling



Canonical Wnt Signaling (Wnt/ β -catenin)

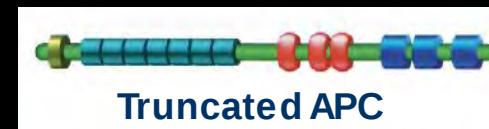


Non-canonical Wnt Signaling (Wnt/ Ca^{++})



PKC Isoform	APC ^{MIN} mice model	Colon cell lines	References
PKC α	Protein levels ↓	Cellular differentiation (IEC-18)	Frey 1997, Hizli, 2006, Black 2000.
PKC β I	Protein levels ↓	Cellular differentiation (SW-480)	Goldstein, 1995, Assert, 1999.
PKC δ	Protein and mRNA levels ↓	Apoptosis (CaCo-2)	Black, 2001, Cerda et al, 2006.
PKC β II	Protein and mRNA levels ↑	Cancer cell lines proliferation and migration	Yu, 2003, Murray, et al, 1999
PKC ϵ	Protein levels ↑	Cellular proliferation, migration (HT-29)	Heider et al, 2004; Perletti, 1998

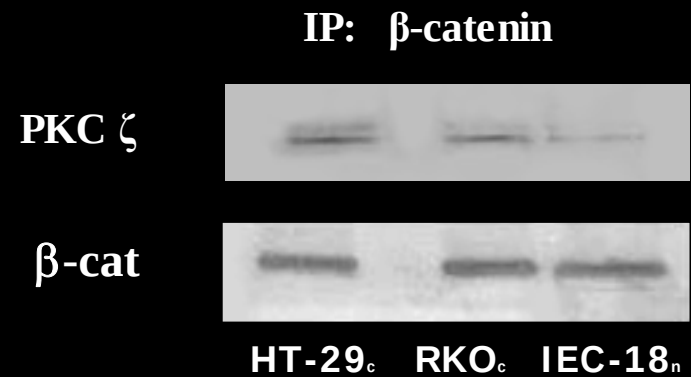
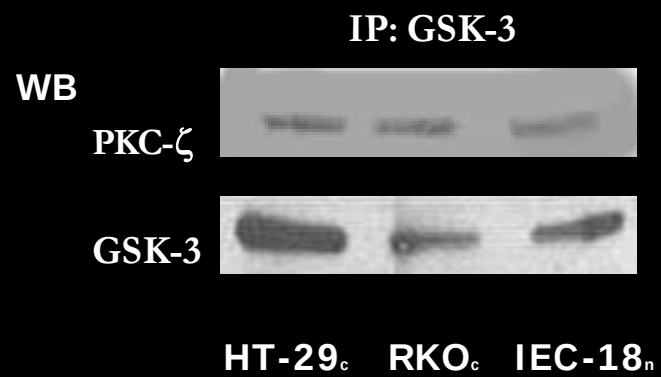
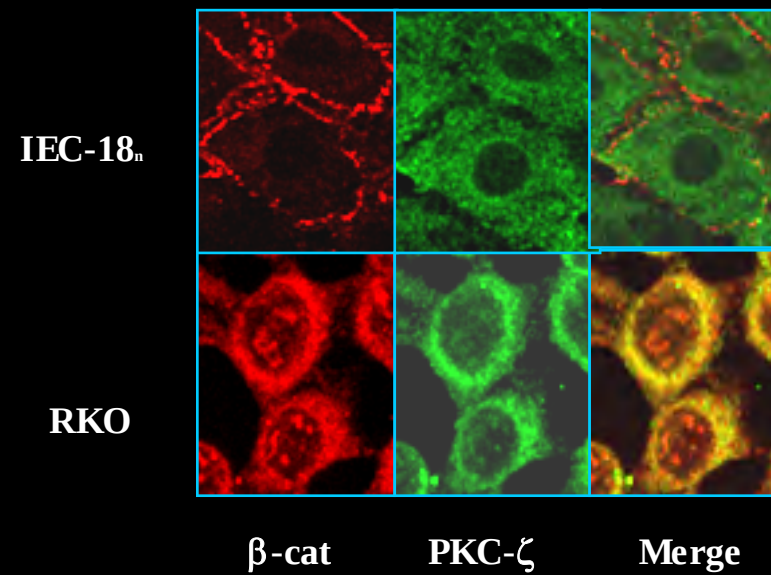
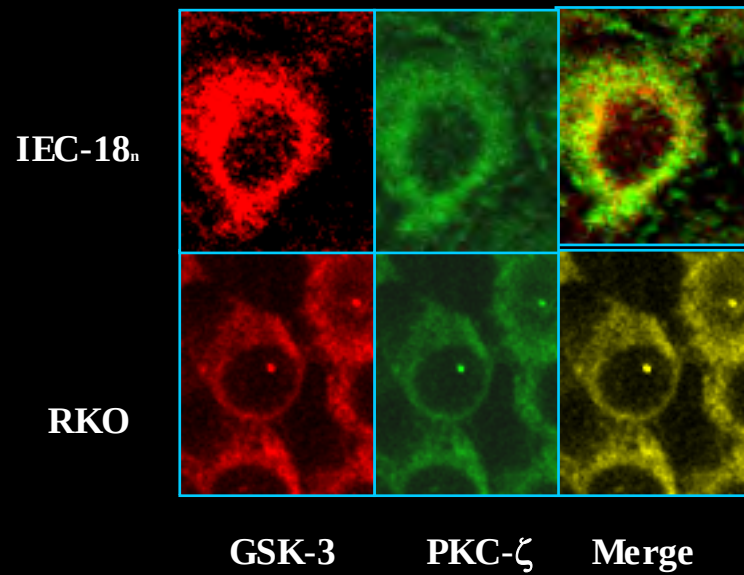
NON MALIGNANT CELL LINES		COLON CARCINOMA CELL LINES	
IEC-18 (rat)	112-CoN (human)	RKO (human)	SW-480 (human)
COMPLETE APC	COMPLETE APC	COMPLETE APC	TRUNCATED APC
Normal Wnt signaling	Normal Wnt signaling	Normal Wnt signaling	Altered Wnt signaling (constitutively active)



Comparative expression profile of PKC isoforms in colon cultured cell lines

PKC	Normal	Malignant	
	112 CoN	HT-29	RKO
α	1	0.2 ± 0.05	0
βI	1	0.05 ± 0.04	0
δ	1	0.1 ± 0.06	0.1 ± 0.02
βII	1	3 ± 0.07	4 ± 0.05
ϵ	1	2 ± 0.03	5 ± 0.09
η	0	4 ± 1	1.0 ± 0.02
ζ	1	4 ± 0.07	2 ± 0.01
μ	1	4 ± 0.1	6 ± 0.2

 DOWN-REGULATED
  UP-REGULATED



IP: APC

112-CoN IEC-18 HT-29

WB:

250

150

75

50

← APC

← PKC α

IP: APC

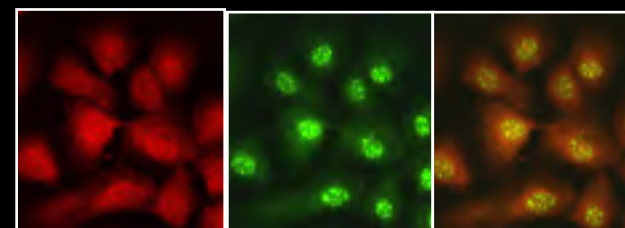
250

75

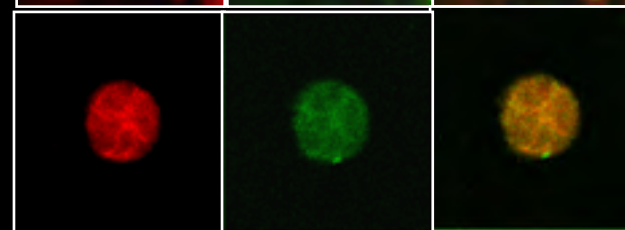
50

← APC

← PKC δ



IEC-18 (N)

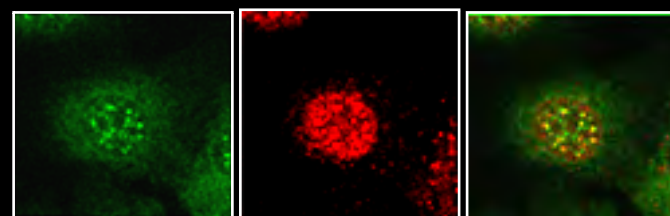


HT-29 (C)

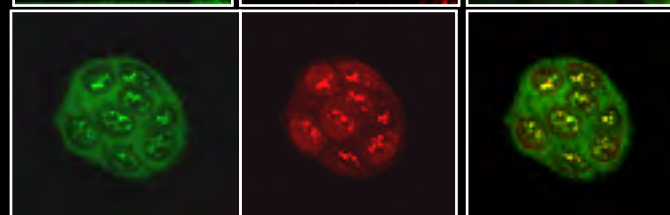
APC

PKC α

Merge



IEC-18 (N)



HT-29 (C)

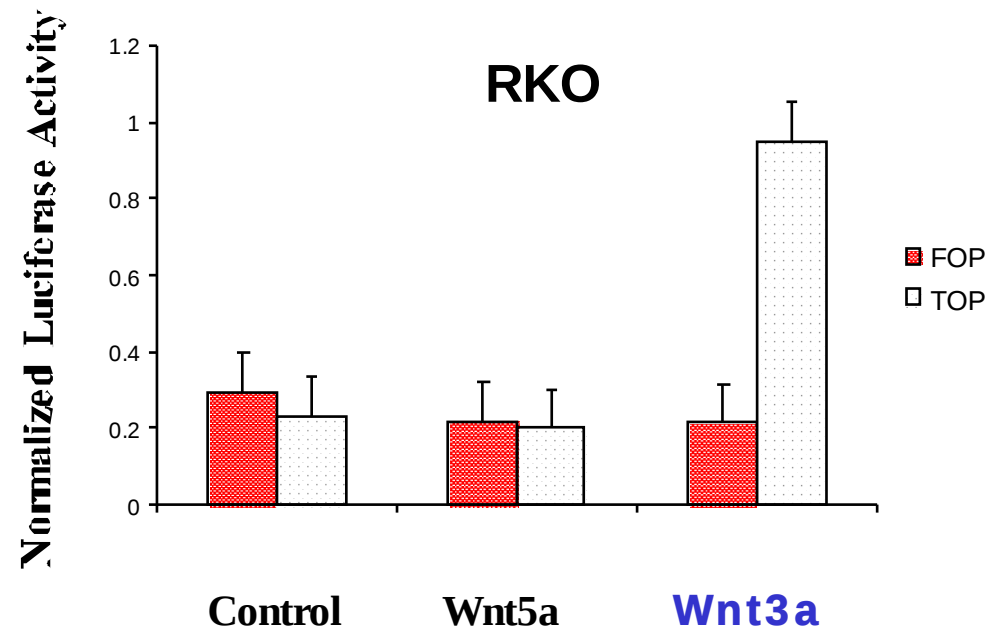
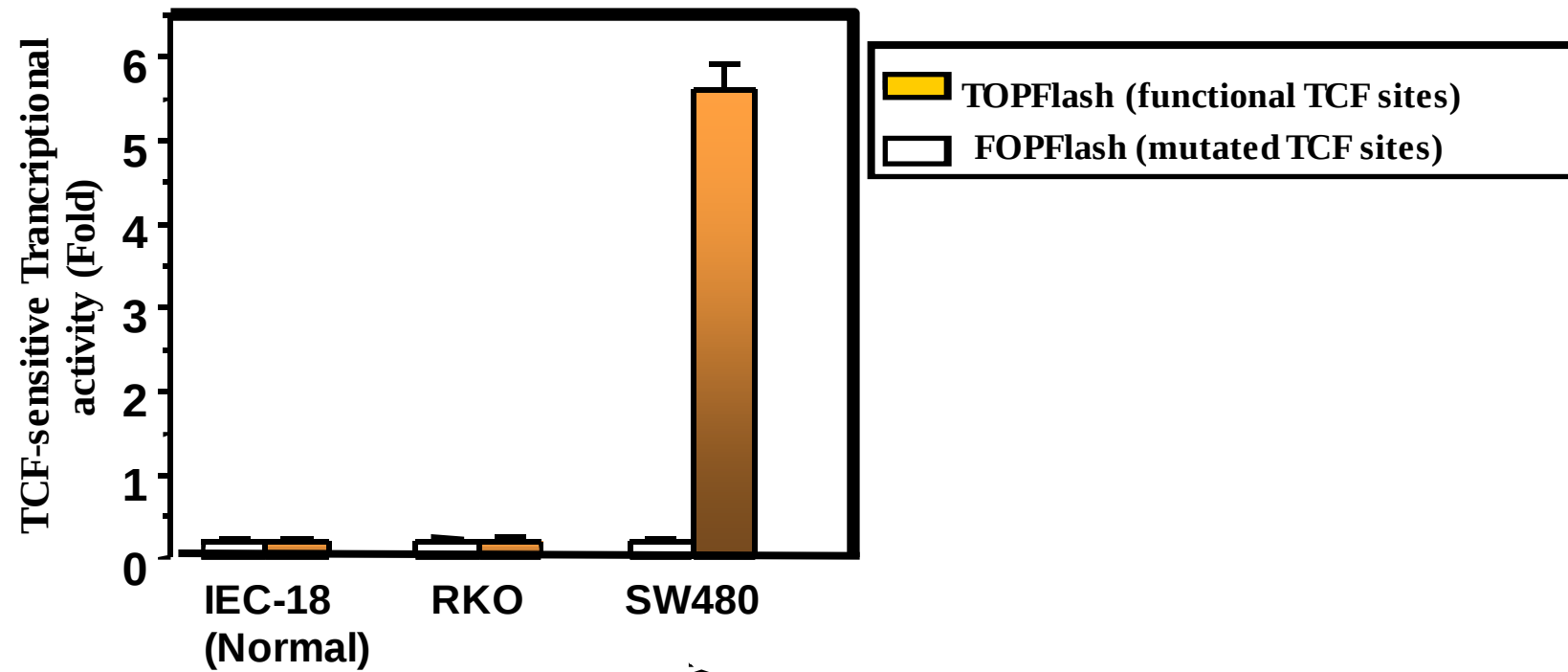
APC

PKC δ

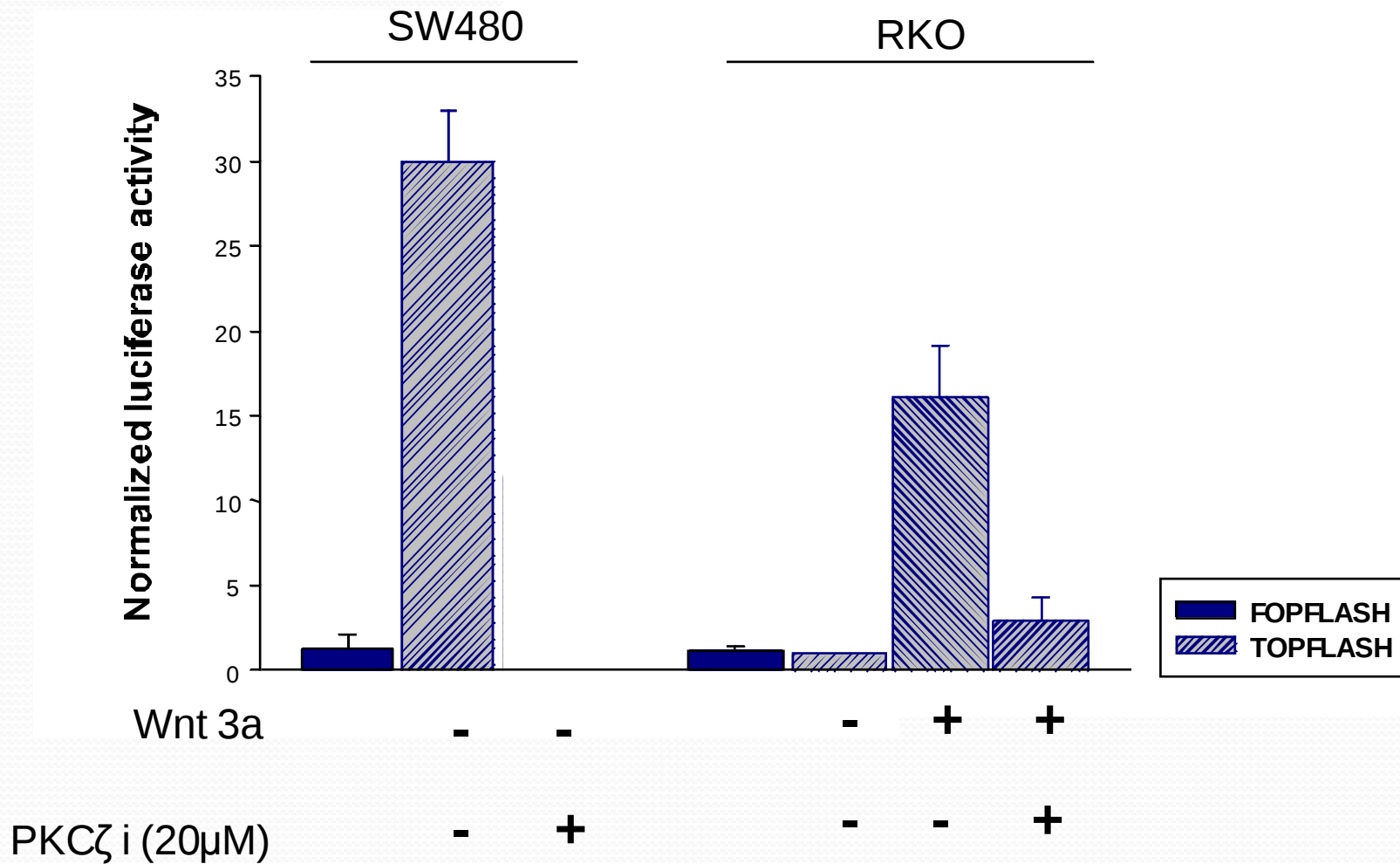
Merge

GOALS

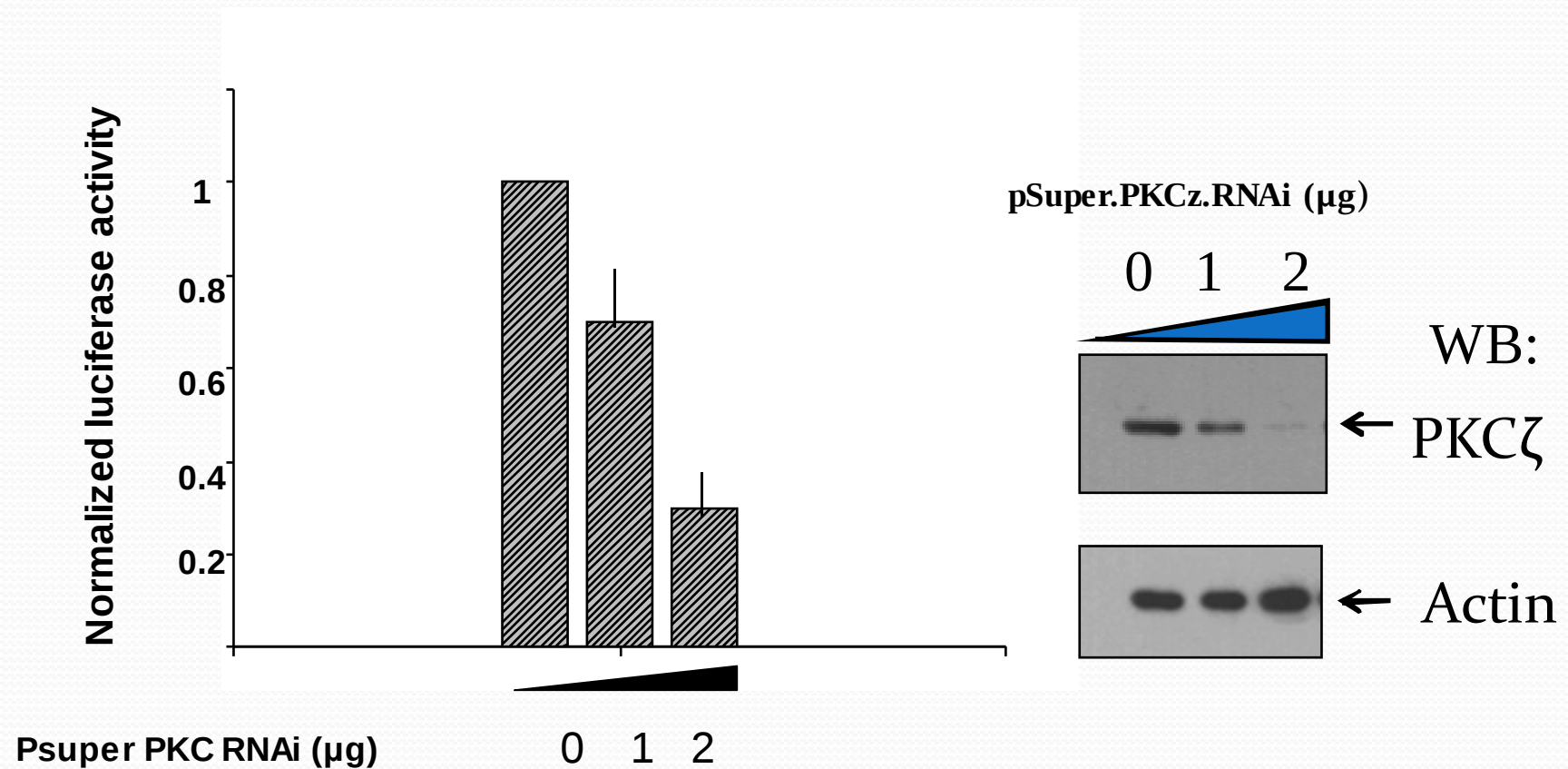
- ¿What is the biological meaning of PKC α/δ interaction with APC?
- ¿What is the biological meaning of β -catenin interaction with PKC ζ ?
- ¿Can PKC modulate canonical Wnt signaling?



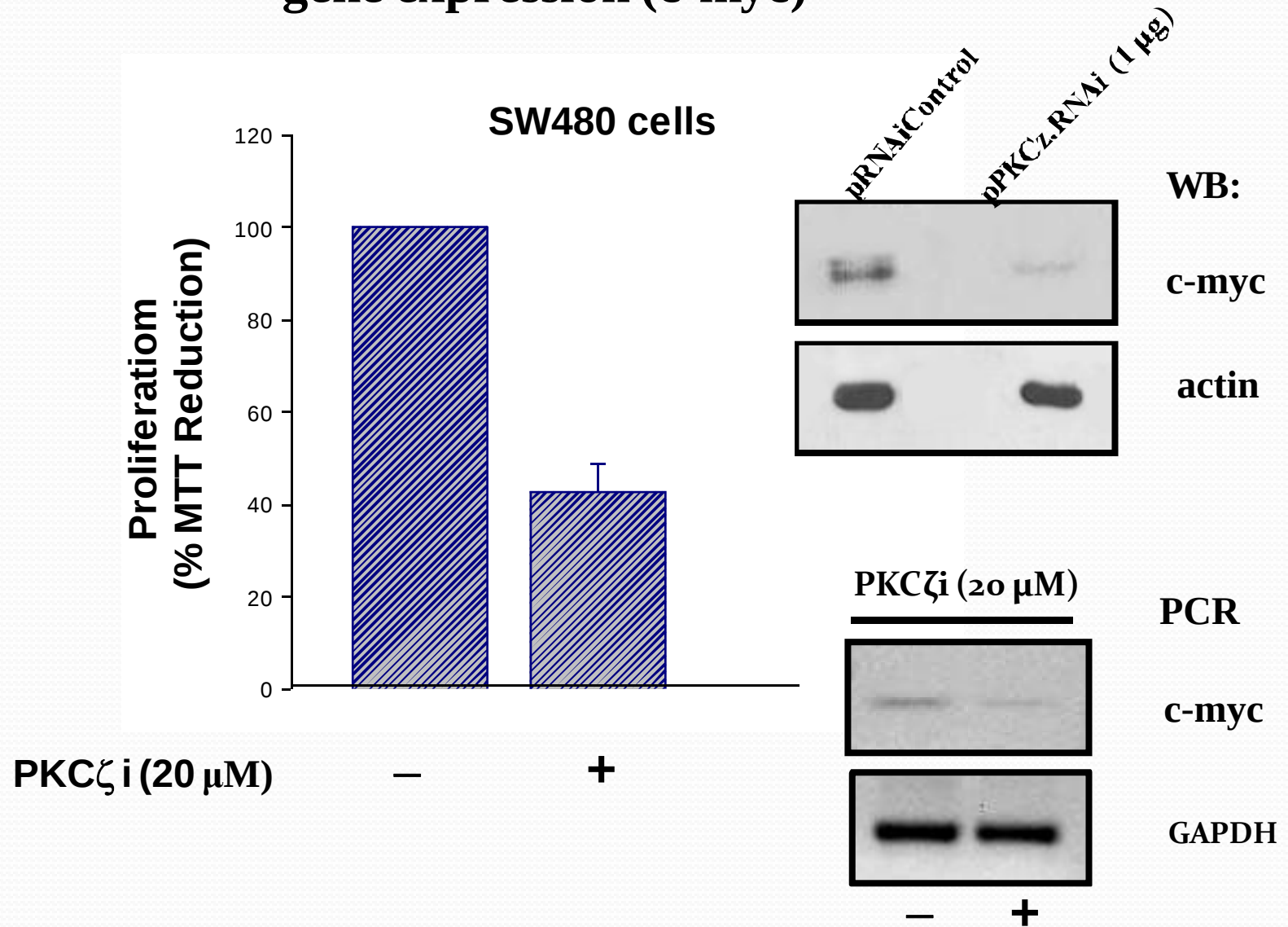
PKC ζ selective inhibitor blocks β -catenin-mediated transcriptional activity in both RKO and SW480 colon carcinoma cell lines



PKC ζ knockdown blocks in a dose-dependent manner the β -catenin-mediated transcriptional activity

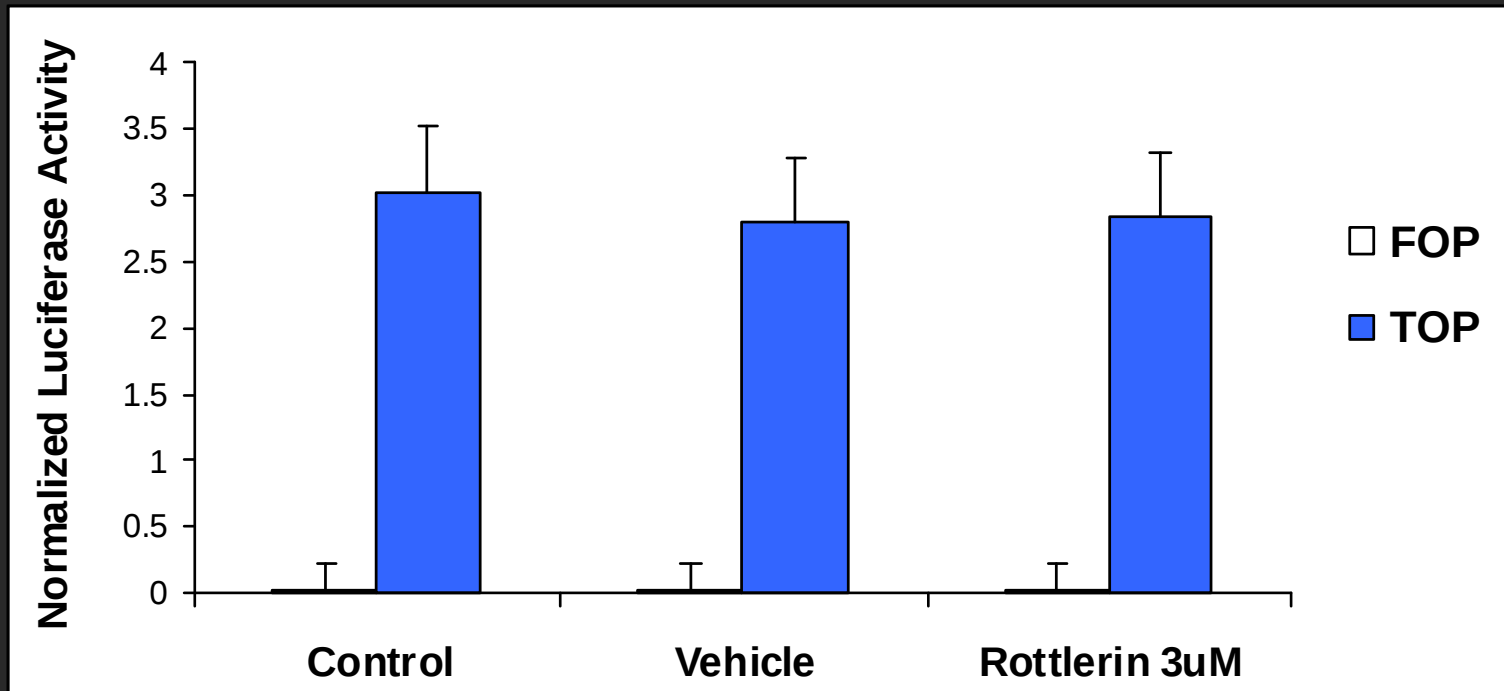


Effect of PKC ζ knockdown on cell proliferation and Wnt target gene expression (c-myc)

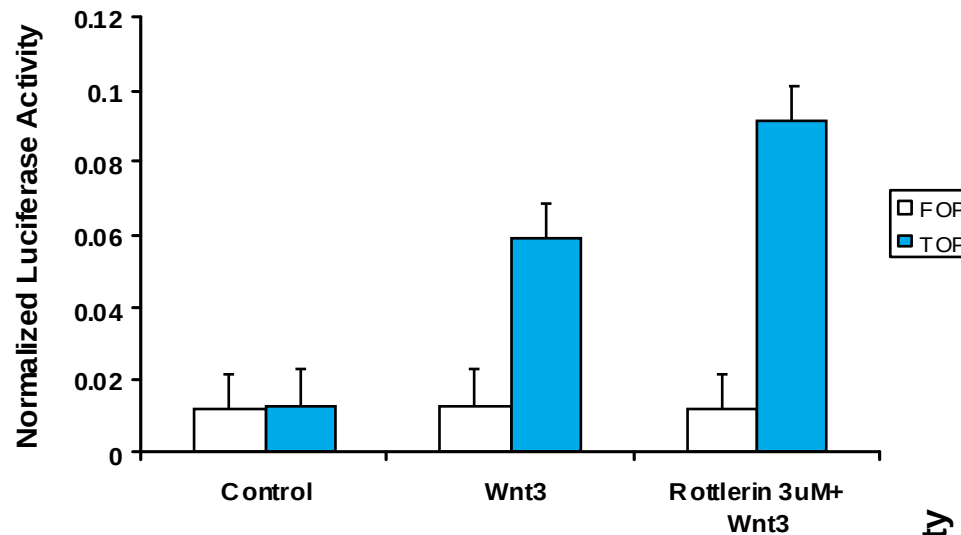


Effect of PKC δ inhibition on β -catenin-mediated transcriptional activity

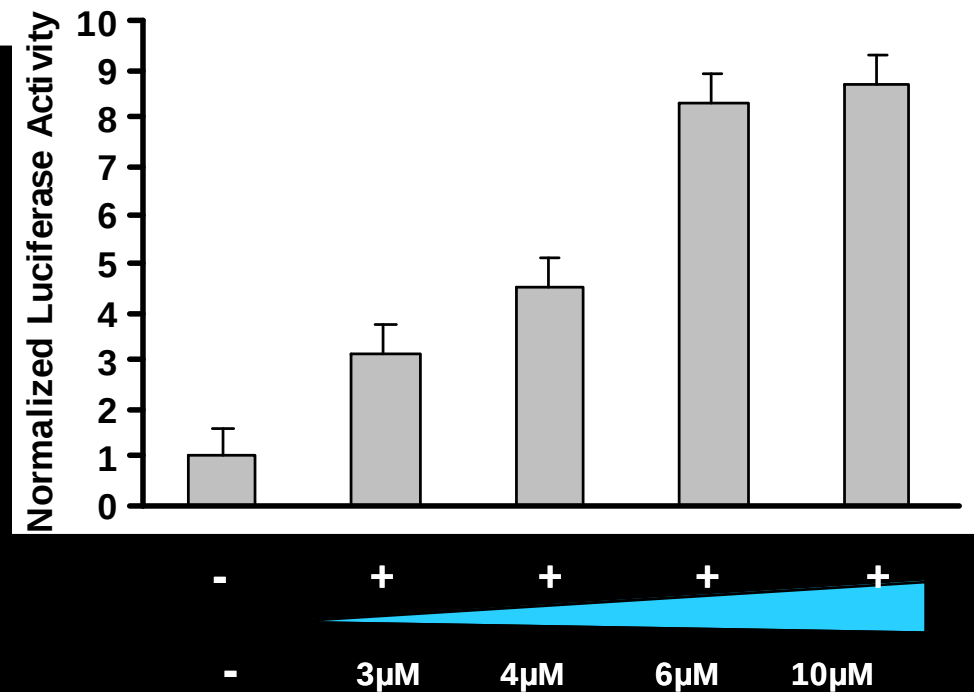
SW-480



Effect of PKC δ inhibition on β -catenin-mediated transcriptional activity



RKO



SUMMARY

- PKC isoforms associate in vivo with key Wnt canonical proteins: PKC ζ , upregulated in malignant cells, interacts with GSK3 β and with β -catenin mainly in cancerous cells. PKC δ , downregulated in malignant cells, interacts with APC in both normal and malignant cells.
- Pharmacological inhibition of PKC ζ , or its decreased expression blocked in a dose-dependent manner canonical Wnt activation in cancer cell lines, suggesting that PKC ζ modulates in a positive way canonical Wnt activation.
- Pharmacological inhibition of PKC δ or its decreased expression, improved in a dose-dependent way canonical Wnt activation in RKO cancer cells, suggesting that PKC δ modulates in a negative way canonical Wnt activation.

Altogether, our results indicate that PKC isoforms play an essential role in the regulation of canonical Wnt pathway.

