Characteristics of Colorectal Cancer Cell (CRC) Lines

The panel of 18 human CRC cell lines used in this study reflects the range of genetic changes associated with colon cancer development[1] (Supplementary Table 1): most were mutant for both APC and K-Ras, with a minority harboring mutations in β-catenin rather than APC (i.e., HCT116 and LS180), or in B-Raf rather than K-Ras (i.e., Colo205).[2-6] Notably, while RKO cells have mutant B-Raf, they are wild-type for both APC and β-catenin, and show no aberrant activation of β-catenin signaling.[4, 7] The study also included an isogenic series of cell lines comprised of parental FET cells and FET cells transfected with TGFα (FET-6α), wild-type TGFβ receptor II (FET-RII), or a dominant negative form of TGFβRII (FET/DNR). These cell lines differ in tumorigenic potential, with FET/DNR and FET-6α being more tumorigenic than FET cells, and FET-RII exhibiting the lowest tumorigenicity.[8, 9] HCT116 and HCT116b cells were derived from the same primary tumor; HCT-116 cells have a more progressed tumor phenotype than HCT-116b cells, as characterized by more rapid proliferation and markedly higher tumorigenicity.[7] The HCT116T cell line is a clone isolated after transfection of HCT116 cells with a TGFα antisense vector, and is unaggressive.[10]

Supplementary Table 1

CRC Cell Line	APC Mutation	β-catenin Mutation	K-Ras Mutation	Other mutations associated with intestinal tumorigenesis	Differentiation status	Aggressiveness	References
FET[#]	+	-	+	p53	Well	Unaggressive	[6, 9, 11-14]
FET-6α[#]	+	-	+	p53	Moderate	Intermediate	[9]
FET-RII[#]	+	-	+	p53	Well	Unaggressive	[9]
FET/DNR[#]	+	-	+	p53	Poor	Aggressive	[9]
HCT-15	+	-	+	p53, TGFβ-RII, PI3KCA	Moderate	Intermediate	[3, 6, 14-18]
CBS4	+	NA	+	NA	Well	Unaggressive	[11-13, 15]
RKO	-	-	-	B-Raf, p53, PI3KCA	Poor	Aggressive	[12-16]
TENN	+	NA	-	PI3KCA	Poor	Aggressive	[12, 13]
RCA	+	+	NA	NA	Moderate	Intermediate	[11, 13]
Moser	+	NA	NA	NA	Moderate	Intermediate	[11]
GEO	+	NA	+	NA	Well	Unaggressive	[2, 11-13, 16]
DLD-1	+	-	+	p53, PI3KCA	Poor	Intermediate	[3, 14, 15, 18]
Colo205	+	+	-	p53, B-Raf, SMAD4	Poor	Aggressive	[2, 3, 14, 15, 17]
SW620	+	-	+	p53	Poor	Aggressive	[2, 3, 14, 15, 17]
HCT116T[*]	-	+	+	TGFβ-RII	Poor	Unaggressive	[10, 19]
HCT116b[*]	-	+	+	TGFβ-RII	Poor	Unaggressive	[7, 12]
HCT116[*]	-	+	+	TGFβ-RII, E2F-4, PI3KCA	Poor	Aggressive	[2, 6, 7, 12-18]
LS180	-	+	+	TGFβ-RII, TCF-4, PIK3CA	Well	Aggressive	[2, 3, 6, 11, 14, 15, 17]

***** HCT116 and HCT116b cell lines were established from the same primary tumor. HCT116T denotes HCT116 cells transfected with a TGF-α antisense vector and stably selected.

FET cell line derivatives include FET-6α cells, which overexpress TGF-α; FET-RII cells, which overexpress TGF-β RII; and FET/DNR cells, which express dominant negative TGF-β RII.

NA = Not available.

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SUPPLEMENTARY INFORMATION

Supplementary Figure Legends

Supplementary Figure 1. CRC cells infected with LacZ, PKCα, or PKCδ adenovirus were stained with propidium iodide, and percentage of cells in G1, S, and G2/M phases was determined by flow cytometry. Data represent the average of 2 independent experiments ± s.e.

Supplementary Figure 2A. PKCα expression does not affect cyclin D1 protein stability. *Left Panel*: LacZ- and PKCα-transduced DLD-1 cells were treated with 30 μg/mL CHX for various times and subjected to anti-cyclin D1 immunoblotting. Fast green-stained membranes are shown as loading controls. A longer exposure is shown for PKCα-transduced cells to facilitate comparison between samples. The graph shows densitometric quantification of cyclin D1 levels normalized to 0 h control. *Right panel*: Lighter exposure at time 0 confirms down-regulation of cyclin D1 steady-state levels in PKCα-transduced cells. Data are representative of 2 independent experiments.

Supplementary Figure 2B. Northern blot (NB) analysis of cyclin D1 mRNA in DLD-1 cells treated with 2 μg/mL ActD for the indicated times. The graph shows relative levels of cyclin D1 mRNA normalized to 28S RNA. Data are representative of ≥2 independent experiments.

Supplementary Figure 3. CRC cells were infected with LacZ, PKCα, or PKCδ adenovirus as indicated and plated in soft agarose. Colonies were imaged after 1-2 weeks.

Supplementary Figure 4A. Immunoblot analysis of cyclin D1, phospho-EGFR (Tyr^{1173}) (indicative of EGFR activation), and total EGFR in FET and GEO cells transduced with LacZ or PKCα adenovirus. Actin: loading control. Data are representative of ≥2 independent experiments.

Supplementary Figure 4B. SW620 cells, which do not express EGFR, were infected with 20 moi LacZ, PKCα, or PKCδ adenovirus. After 48 h expression of the indicated proteins was detected by immunoblot analysis. Fast Green staining shows even protein loading. Data are representative of ≥2 independent experiments.

Supplementary Figure 1

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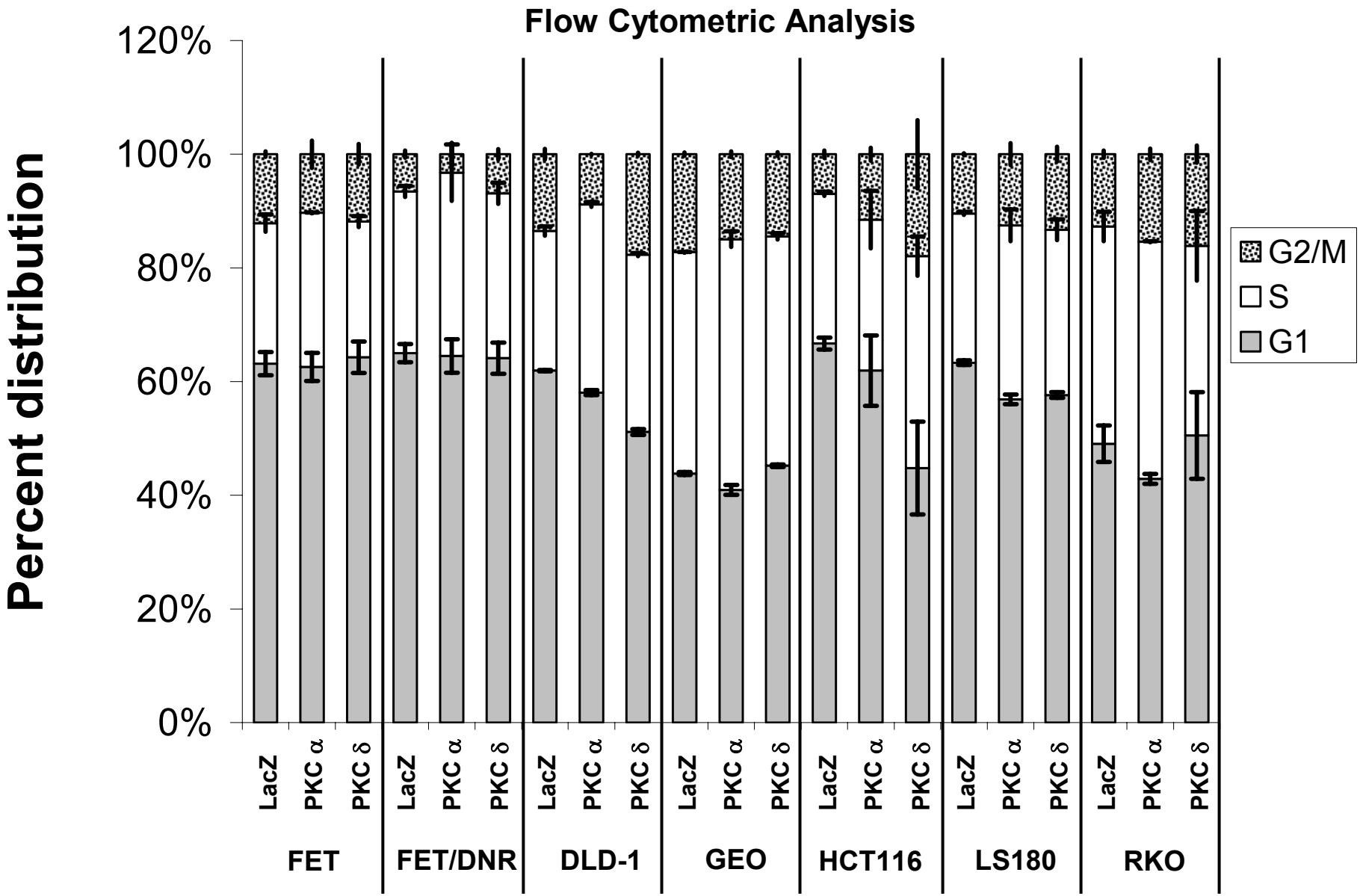

Supplementary Figure 2A

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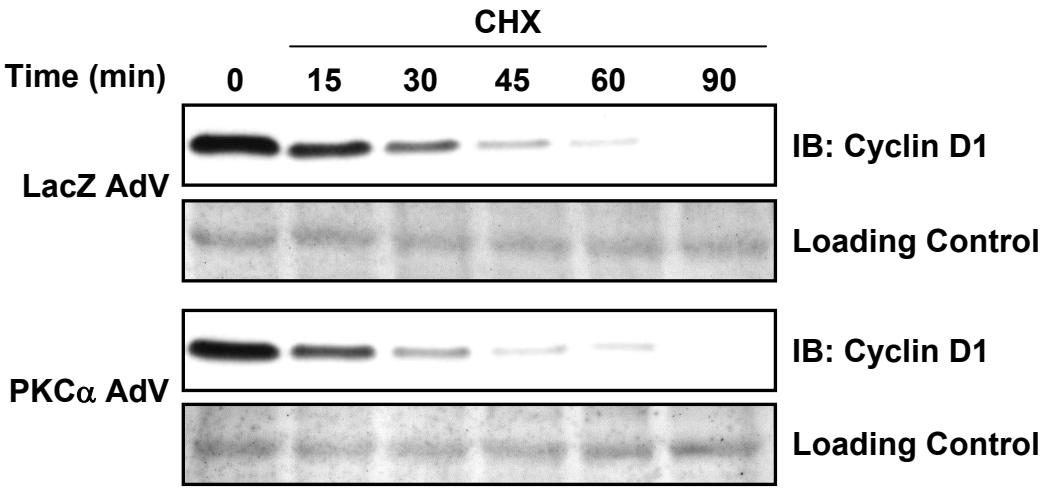
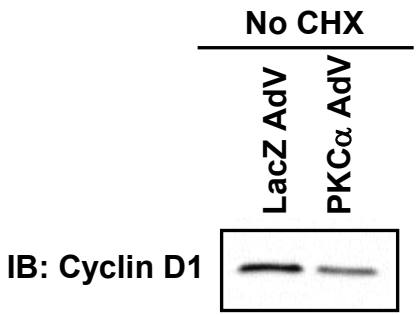
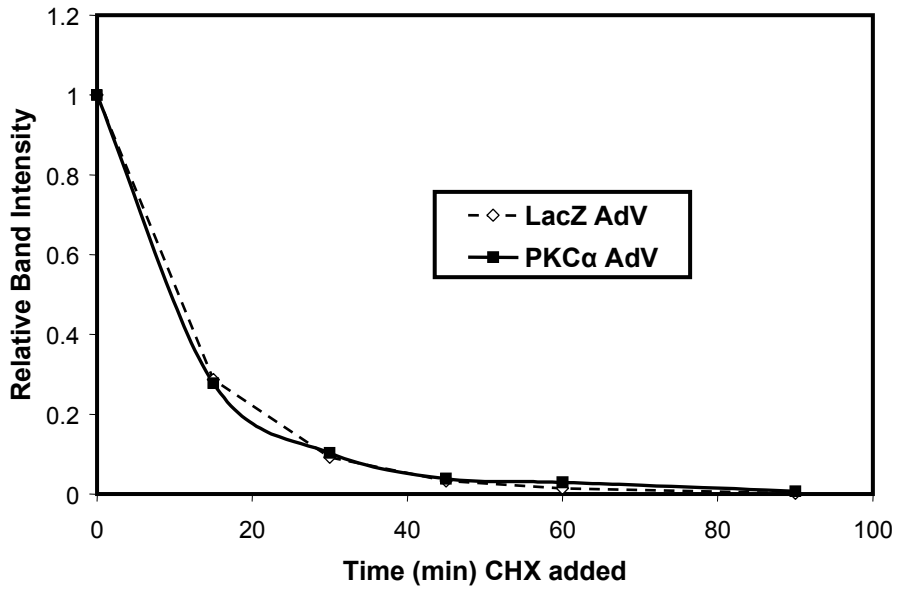




Quantification of IB:Cyclin D1



Supplementary Figure 2B

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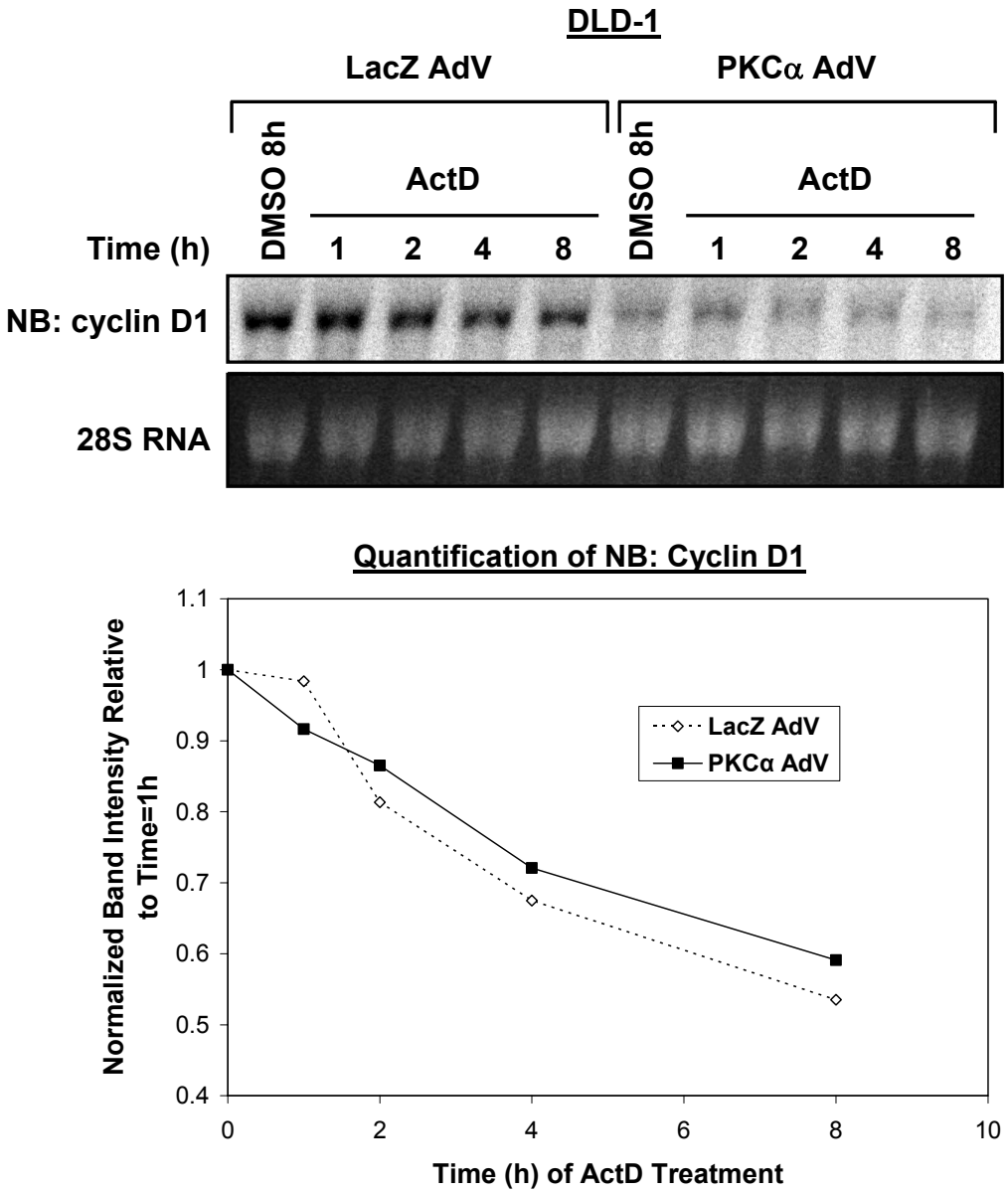

Supplementary Figure 3

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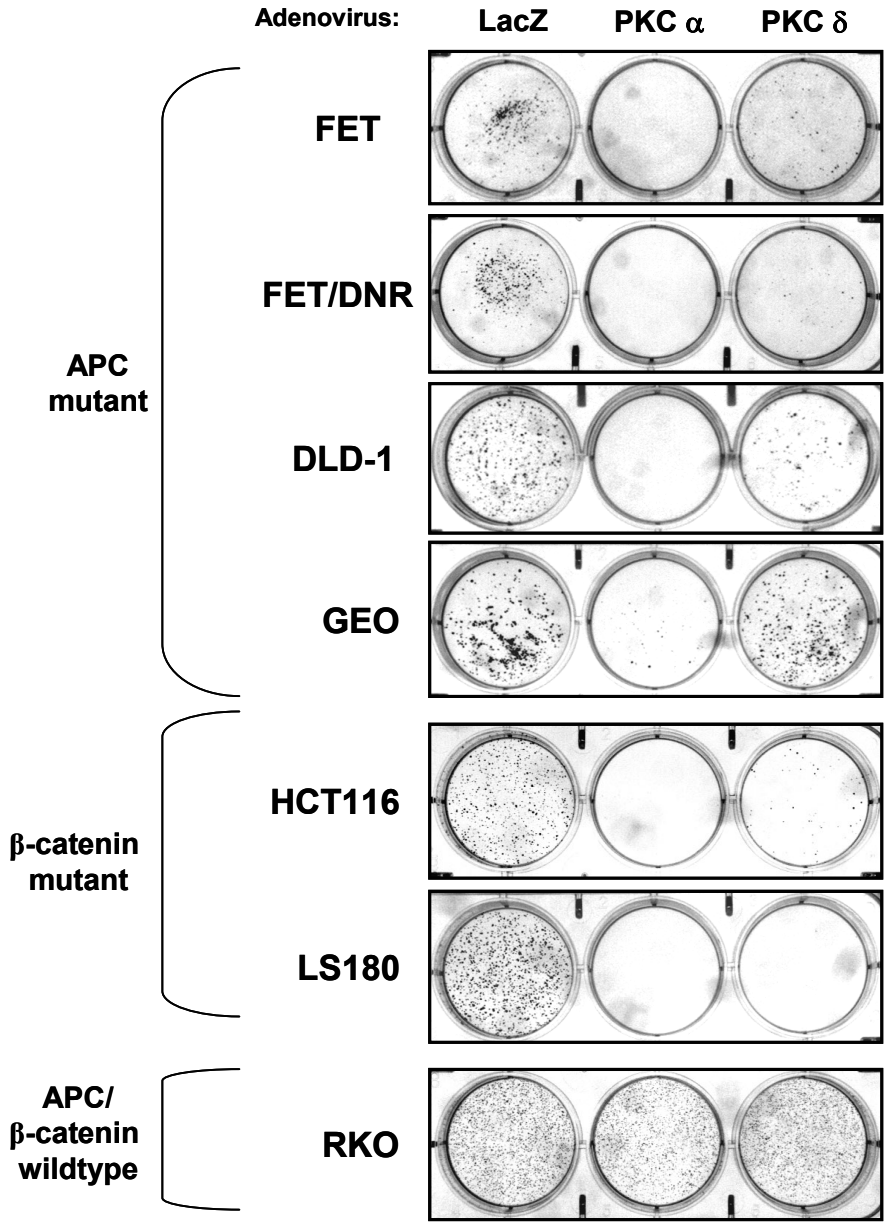

Supplementary Figure 4

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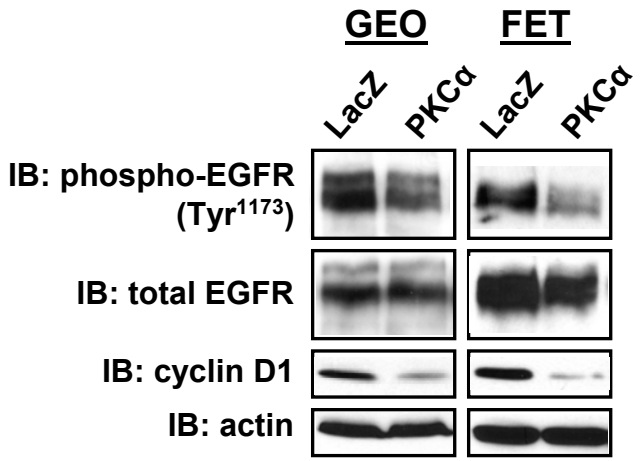
A.



B.