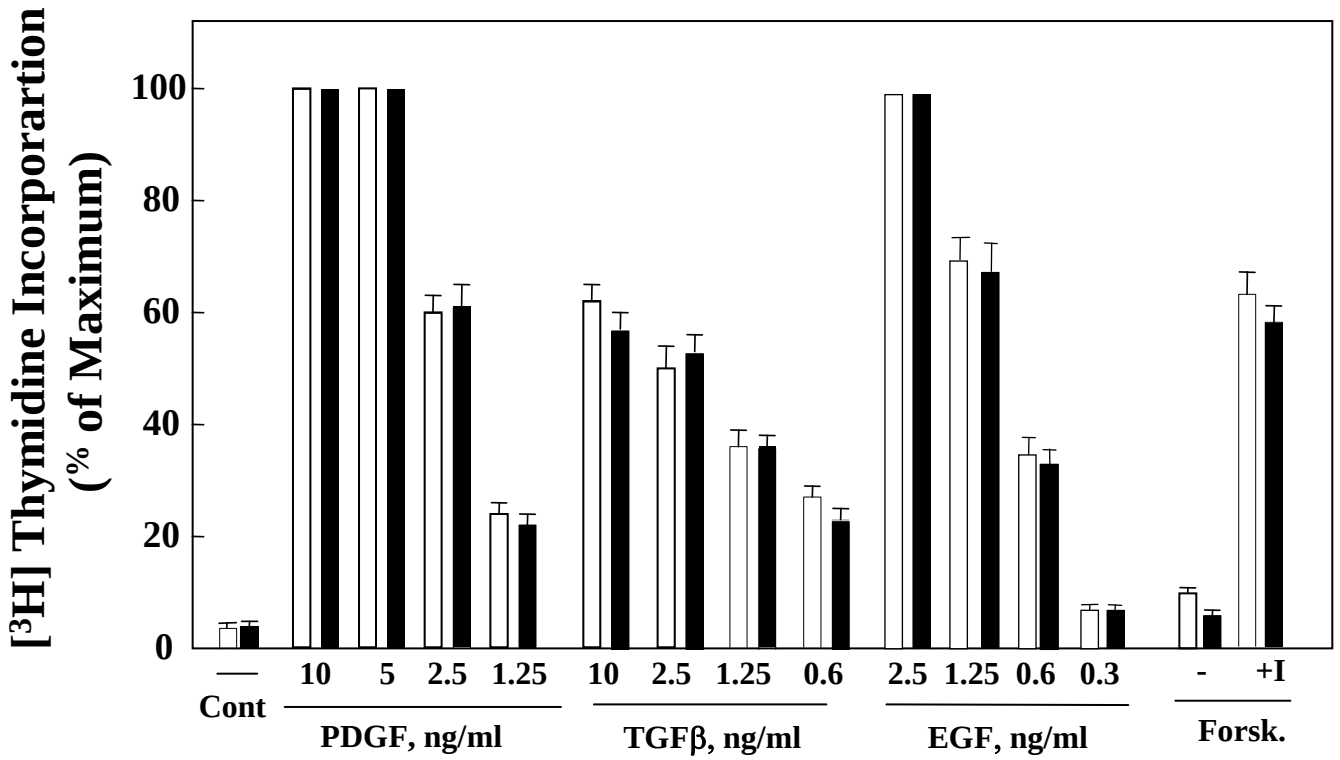
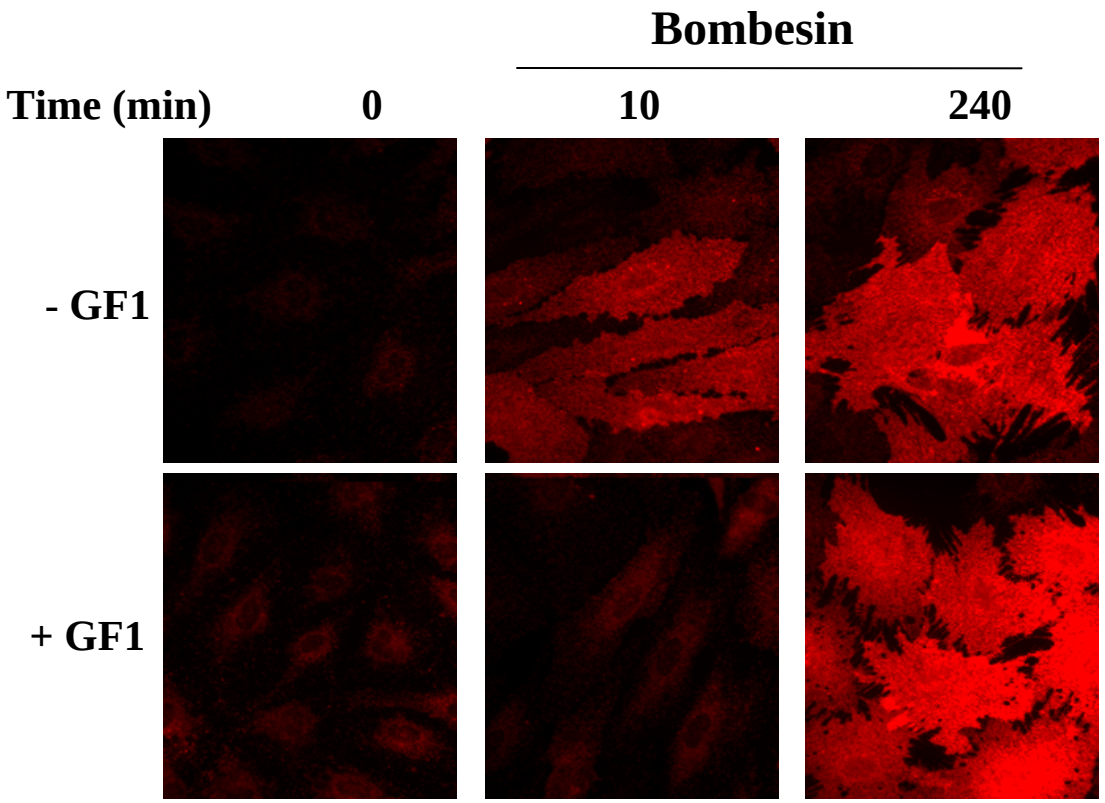
Supplemental Fig. S1



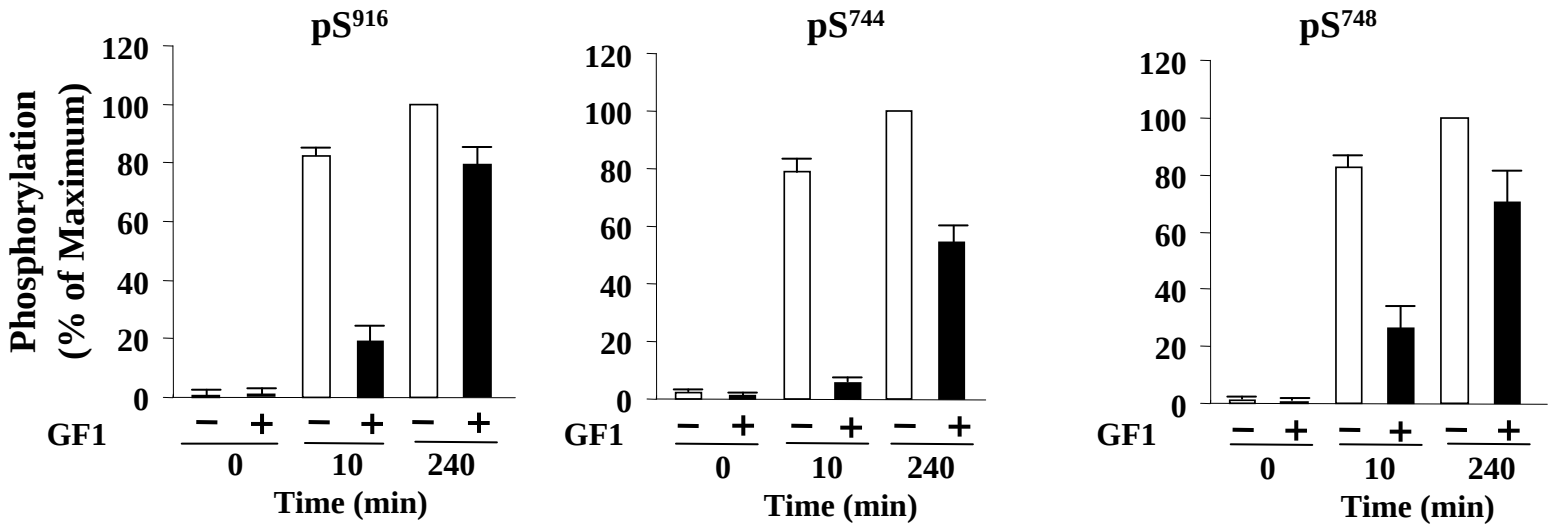
Supplemental Figure1**.** ***PKD overexpression does not increase DNA synthesis induced by PDGF, TGFb, EGF or forskolin in Swiss 3T3 cells.*** Confluent and quiescent cultures of Swiss 3T3 PKD.GFP cells (solid bars) and Swiss 3T3 GFP cells (open bars) were washed and incubated at 37°C in 2 ml of DMEM/Waymouth's medium containing [3H]-thymidine and the following growth-promoting factors: PDGF, TGFb and EGF, at the indicated concentrations. Parallel cultures were also stimulated with 10 mM forskolin either in the absence or prescence of 100ng/ml insulin (I). Insulin was added in this experiment to show the mitogenic activity of forskolin under the experimental conditions used. After 40 h, DNA synthesis was assessed by measuring the [3H]-thymidine incorporated into acid-precipitable material. Results are expressed as a percentage mean $\pm$ S.E. (n=3) of the maximal stimulation obtained with 10% fetal bovine serum (110 x 10-3 cpm/culture).

Supplemental Fig. S2



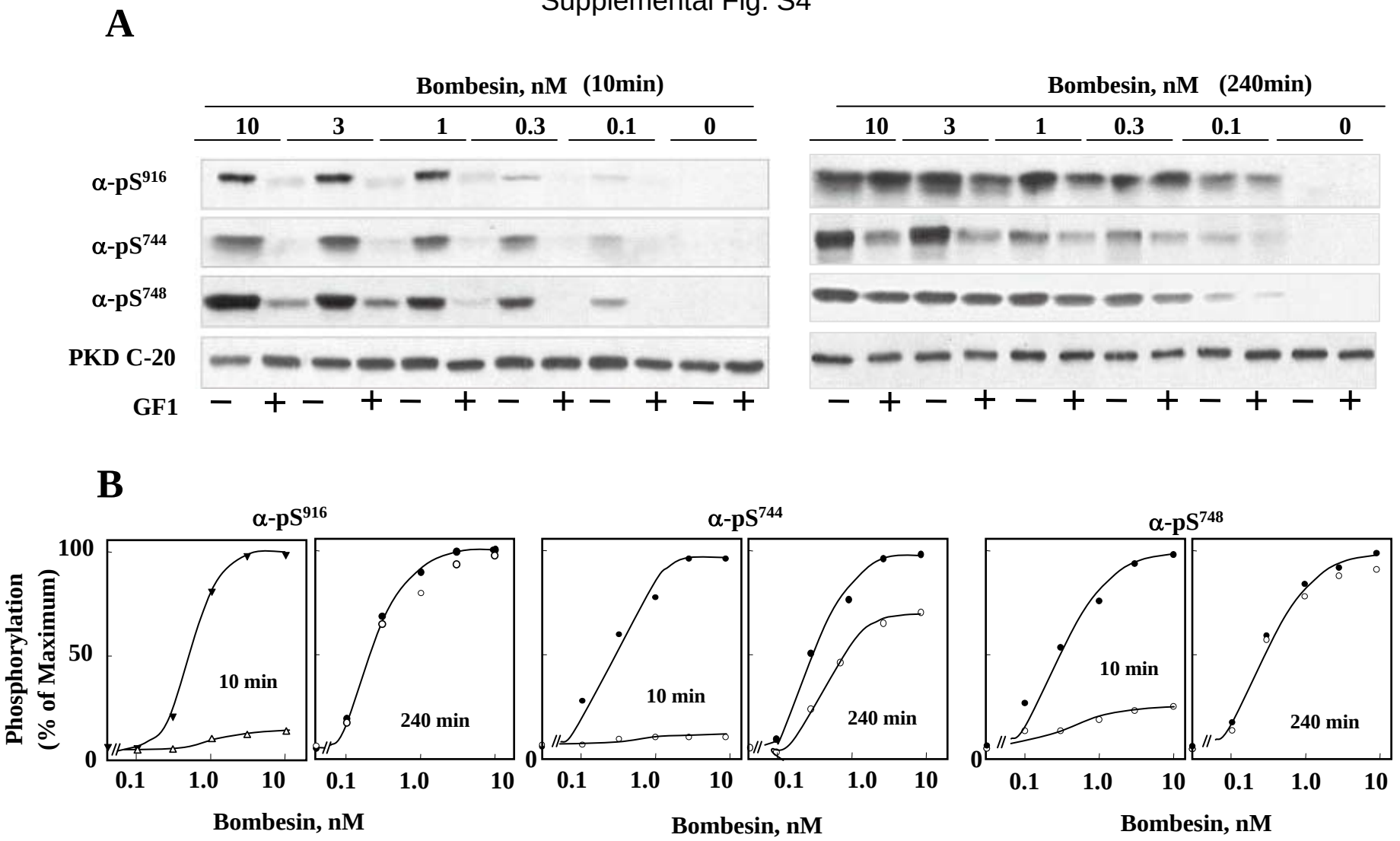
Supplemental Figure 2***. Bombesin induces early PKC-dependent and late PKC-independent PKD activation in individual Swiss 3T3 cells.*** Swiss 3T3 PKD.GFP cells were incubated in the presence (+) or in the absence (-) of 3.5 μM GF 109203X (GF1) for 1 h prior to stimulation of the cells with 10 nM bombesin for 10 min or 240 min as indicated. The cultures were fixed in 4% PFA for 20 min, rinsed with PBS, permeablized with 0.4% Triton-X, blocked with 5% Goat Serum + 2 % BSA, and then incubated overnight at 22 oC with pS916 antibody (anti-rabbit 1:1000). Cells were rinsed, and labeled with quantum dot (Qdots655) conjugated to goat anti-rabbit secondary antibody (1:100, 1.5 hrs.). Images were obtained with a cooled CCD Camera (Spot, Digital Diagnostics) connected to a Zeiss Axioskop epi-fluorescence microscope.

Supplemental Fig. S3



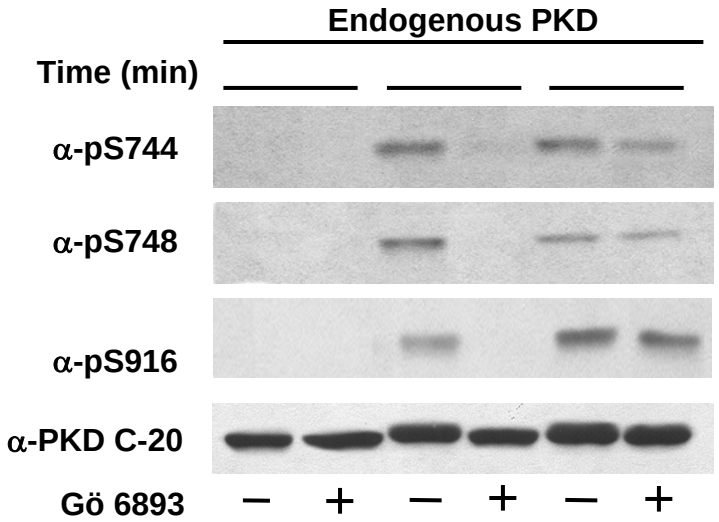
Supplemental Figure 3**.** ***Bombesin induces early PKC-dependent and late PKC-independent PKD activation in Swiss 3T3 cells.*** Swiss 3T3 PKD.GFP cells were incubated in the presence (+) or in the absence (-) of 3.5 μM GF 109203X (GF1) for 1 h prior to stimulation of the cells with 10 nM bombesin for 10 min or 240 min as indicated and then lysed with 2×SDS–PAGE sample buffer. The samples were analyzed by SDS-PAGE and immunoblotting with the following antibodies: phospho PKD pS916, pS744, pS748. Autoluminograms were quantified by densitometric scanning. The results shown are the mean ± S.E.M. n=8 and are expressed as percentage of the maximum increase induced by treatment with bombesin in cells preincubated in the presence (closed bars) or the absence (open bars) of GF1.

Supplemental Fig. S4



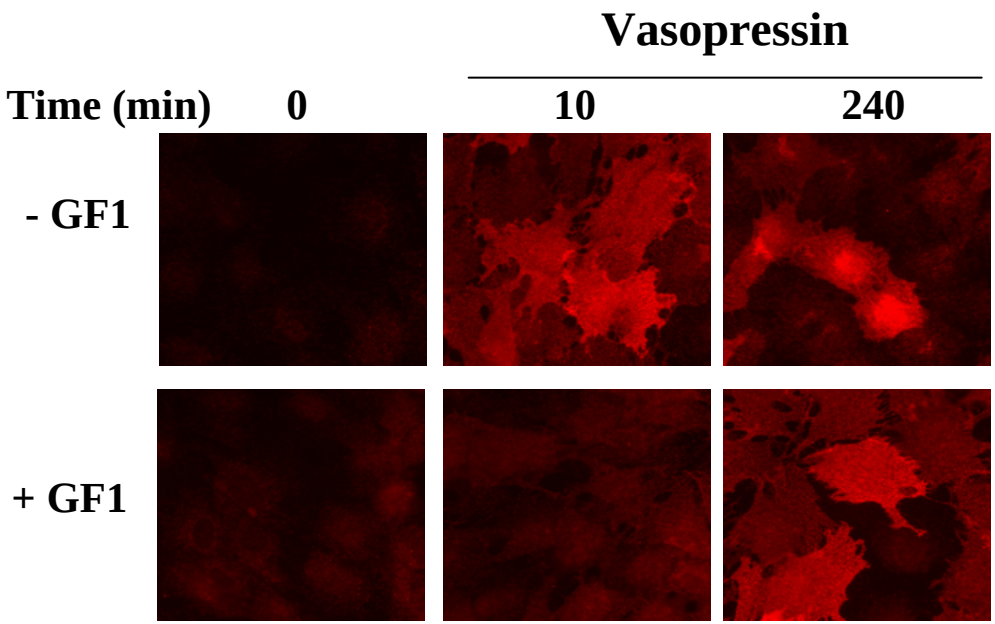
Supplemental Figure 4. ***PKC-dependent and PKC-independent PKD activation in response to various concentrations of bombesin. A.*** *Dose response of bombesin.* Swiss 3T3 PKD.GFP cells were incubated in the absence (-) or in the presence (+) of 3.5 μM GF1 for 1 h prior to stimulation of the cells with varying concentrations bombesin for 10 min and 240 min times. The cultures were then lysed with 2×SDS–PAGE sample buffer. All samples were analyzed by SDS-PAGE and immunoblotting with the site-specific antibodies phospho PKD pS916, pS744, pS748 as well as PKD-C20 to verify equal loading. The results shown here are representative autoluminograms; similar results were obtained in three independent experiments**. B,** Autoluminograms were quantified by densitometric scanning. The results shown are the mean ± S.E.M. n=3 and are expressed as percentage of the maximum increase induced by treatment with bombesin, in cells preincubated in the absence (closed symbols) or the presence (open symbols) of GF1.

Supplemental Fig. S5



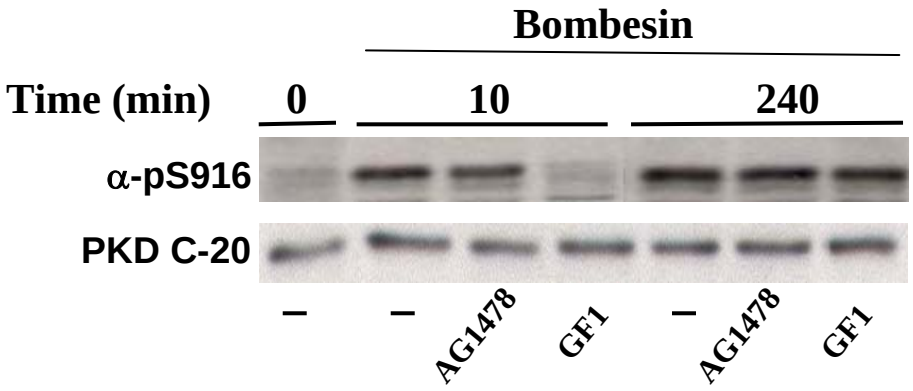
Supplemental Figure 5. ***PKC-dependent and PKC-independent phases of endogenous PKD activation in Swiss 3T3 cells stimulated with bombesin.*** Confluent Swiss 3T3 cells were incubated in the absence (-) or in the presence (+) of 3.5 μM GF1 for 1 h prior to stimulation of the cells with 10 nM bombesin for the indicated times. The cultures were then lysed with 2×SDS–PAGE sample buffer. All samples were analyzed by SDS-PAGE and immunoblotting with the following antibodies phospho PKD pS916, pS744, pS748 and PKD-C20 to verify equal loading. The results shown here are representative autoluminograms; similar results were obtained in three independent experiments.

Supplemental Fig. S6



Supplemental Figure 6**.** ***PKC-dependent and PKC-independent PKD activation in cells stimulated with vasopressin*****.** Swiss 3T3 PKD.GFP cells were incubated in the presence (+) or in the absence (-) of 3.5 μM GF1 for 1 h prior to stimulation of the cells with either 50 nM vasopressin for 10 min or 240 min, as indicated. The cells were fixed in 4% PFA for 20 min, rinsed with PBS, permeablized with 0.4% Triton-X, blocked with 5% Goat Serum + 2 % BSA, and then incubated overnight at 22oC with pS916 antibody (anti-rabbit 1:1000). Cells were rinsed, and labeled with quantum dot (Qdots655) conjugated to goat anti-rabbit secondary antibody (1:100, 1.5 h.). Images were obtained with a cooled CCD Camera (Spot, Digital Diagnostics) connected to a Zeiss Axioskop epi-fluorescence microscope.

Supplemental Fig. S7



Supplemental Figure 7**.** ***EGFR inhibitor AG1478 does not inhibit PKD kinase activity.*** Swiss 3T3 PKD.GFP cells were incubated in the presence (+) or in the absence (-) of either 500 nM AG1478 or 3.5 μM GF1 for 1 h prior to stimulation of the cells with 10 nM bombesin for 10 min or 240 min.