Supporting Information

Fig. S1. MDCK cystogenesis in Matrigel. (**A**) MDCK cells embedded in Matrigel form cysts. Phase images were taken at multiple time points as indicated. (**B**) Day 6 MDCK cysts were fixed to coverslips and stained or immunostained for apical markers as indicated. The images are representative of at least 3 separate experiments. Scale bar represents 50μM.

Fig. S2. The effect of PKCι knockdown on polarized morphogenesis of C-Raf and p110α MDCK spheroids. MDCK oncogenic variant cell lines were treated with two separate siRNA-PKCι (siPKCι1 & siPKCι2) and two scrambled controls (siSCRAM1 & siSCRAM2) for 24h prior to culture in Matrigel for 6 days. (**A**) Representative single confocal images with phalloidin (Red) stained actin and Hoechst (Blue) identified nuclei. Scale bar represent 50μm. (**B**) Quantification of number of Predominant Single Apical Lumens (PSALs). At least 100 spheroids were counted per condition and the mean and SEM of at least 3 separate experiments are presented. There were no statistical differences in PSALs upon PKCι depletion for Raf-CAAX or p110-CAAX MDCK spheroids. (**C**) PKCι protein depletion was confirmed in adherent cells 2 days after re-seed (72h after transfection) by Western Blot.

Fig. S3. Exogenous protein expression in MDCK cells. Western blot showing the level of exogenous protein in MDCK cells transfected with PKCι/PKCι mutants.