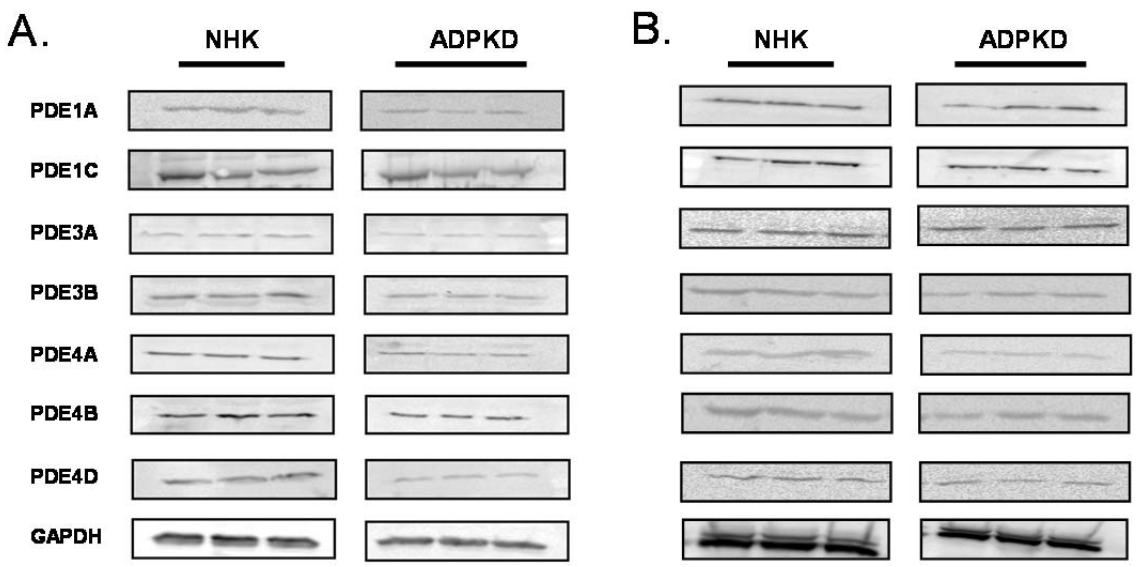
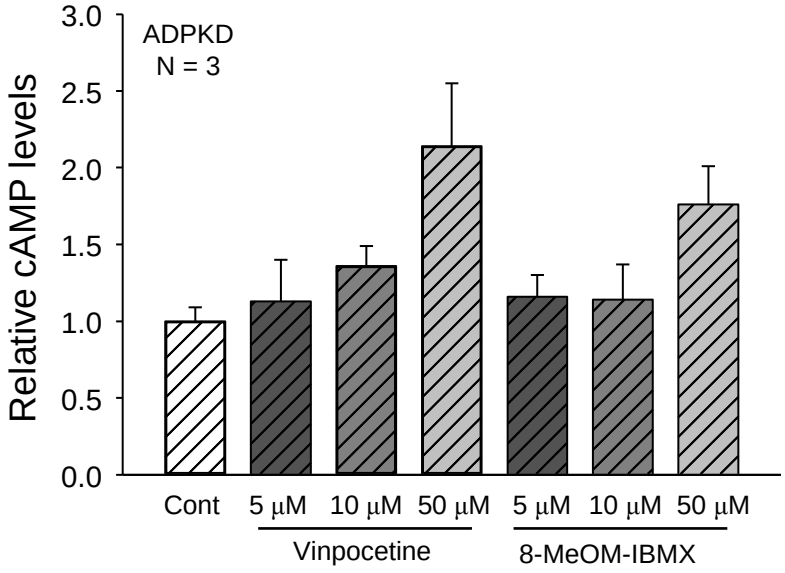
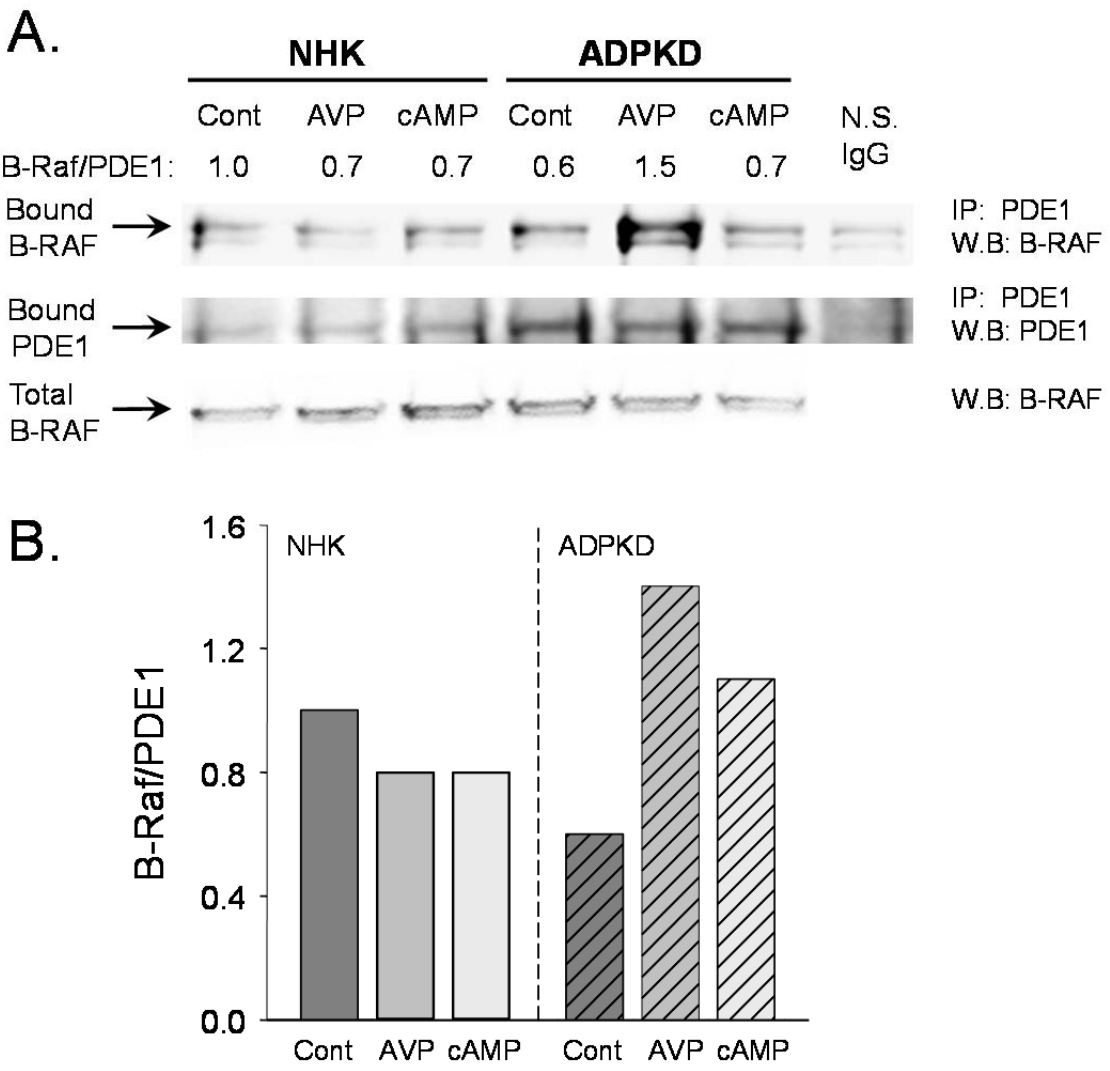


Supplemental Figure S1. Representative western blots for specific PDE isoforms in normal human kidney (NHK) and ADPKD. Lysates (40 μg/well) of NHK and ADPKD cells (A) and tissues (B) were loaded into 10% polyacrylamide gels for western blot analysis. Proteins were separated by electrophoresis and transferred to a nitrocellulose membrane. Membranes were blotted with 5% milk and probed with PDE isoform specific antibodies (1:200 dilution). Bands were visualized using enhanced chemiluminescence and the protein mass was estimated from the relative position of molecular weight markers using a Fluor-S MAX imager (BioRad, Hercules, CA). Antibodies detected bands of predicted molecular masses as reported in the literature[5] and in product data sheets (Santa Cruz, Dallas, TX and Abcam, Cambridge, MA). Each pair of images, comparing PDE and GAPDH bands in NHK and ADPKD tissues or cells (N = 3 each), were from the same blot and the images were handled identically in regard to contrast and brightness. Measurements of the band intensities for Figure 1 were from unaltered images of the blots.



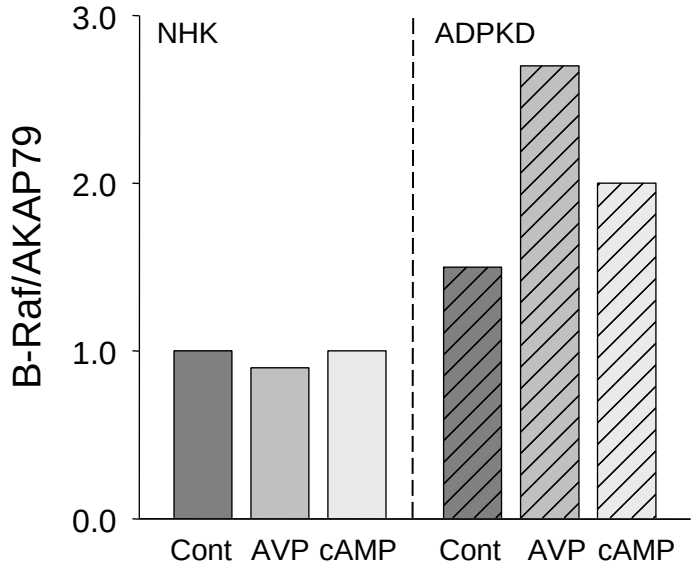
Supplemental Figure S2. Effect of PDE1 inhibitors vinpocentine and 8-MeOM-IBMX on intracellular cAMP levels in ADPKD cells. Cells were treated with vinpocentine or 8-MeOM-IBMX, a potent PDE1 inhibitor, at concentrations ranging from 5 to 50 μM for 15 min. cAMP was extracted and measured using a cAMP EIA kit (Cayman Chemical, Ann Arbor, MI). The effect of the two PDE1 inhibitors caused an increase in cAMP levels that were similar in magnitude. Bars ± SE are fold changes from control, set to 1.0 (N = 3).



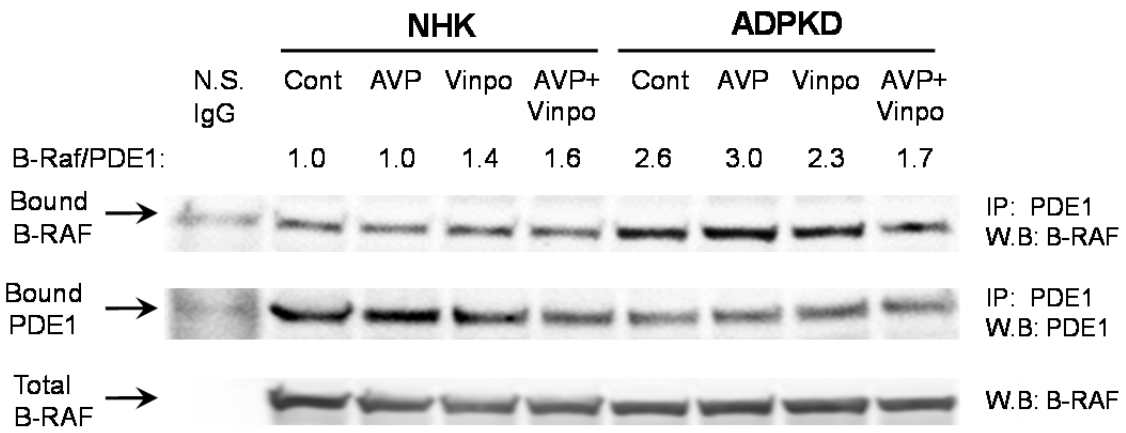
Supplemental Figure S3. Effect of AVP and cAMP on B-Raf/PDE1 interaction in ADPKD and NHK cells. The relative level of B-Raf that was pulled down by the PDE1 antibody/bead complex was normalized to the amount of PDE1 in the immunoprecipitate. A. NHK and ADPKD cells were treated with control, 10 nM AVP or 100 μM 8-Br-cAMP for 15 minutes, and then cell lysates were prepared**.** B-Raf was immunoprecipitated with a PDE1 antibody bound to magnetic beads (BioMag, Polyscience, Inc., Warrington, PA), and levels of B-Raf were detected by immunoblot analysis using a rabbit B-Raf polyclonal antibody (C-19, Santa Cruz). Total bound PDE1

in the immunoprecipitate was used to normalize the amount of bound B-Raf. Bands on the far right demonstrate the amount of B-Raf and PDE1 binding to the magnetic beads coated with a non-specific (N.S.) IgG. Total B-Raf levels were also detected in cell lysates by western blot. B. Summary of the effect of AVP and cAMP on the interaction between PDE1 and B-Raf. Bars ± SE are relative bound B-Raf/bound PDE1. B-Raf/PDE1 in NHK cells treated with control media were set to 1.0 (N = 2). AVP caused an increase in the interaction between PDE1 and B-Raf, consistent the results of Figure 7.

Supplemental Figures



Supplemental Figure S4. Effect of AVP and cAMP on B-Raf/AKAP-79 interactions in ADPKD and NHK cells. The relative level of B-Raf pulled down by the AKAP-79 antibody/bead complex was normalized to the amount of AKAP-79 in the immunoprecipitate. NHK and ADPKD cells were treated with control vehicle or 100 μM 8-Br-cAMP for 15 minutes and cell lysates were prepared. AKAP-79 was immunoprecipitated with a rabbit AKAP79 polyclonal antibody (H-105, Santa Cruz, Dallas, TX) and levels of B-Raf were detected by immunoblot using a rabbit B-Raf polyclonal antibody (C-19, Santa Cruz). Total bound AKAP-79 was used to normalize the amount of B-Raf pulled down by the immunoprecipitation. Bars ± SE are relative bound B-Raf/bound AKAP79. B-Raf/AKAP79 in NHK cells treated with control media were set to 1.0 (N = 2).



Supplemental Figure S5. Effect of vinpocentine on the PDE1/B-Raf interaction in ADPKD and NHK cells. NHK and ADPKD cells were treated with 50 μM vinpocentine for 15 min, and then 10 nM AVP was added for an additional 15 minutes. B-Raf was immunoprecipitated with a PDE1 antibody (ab14602, Abcam, Cambridge, MA) and detected with a mouse monoclonal primary antibody (clone 1H12, Thermo Fisher Scientific, Rockford, IL). Bound PDE1 in the immunoprecipitate was used to normalize the amount of B-Raf. Bands on the left demonstrate low levels of non-specific binding of B-Raf and PDE1 to the beads coated with a non-specific (N.S.) IgG. Bars ± SE are bound B-Raf/bound PDE1 levels, normalized to control-treated NHK cells. Similar results were observed in a different pair of NHK and ADPKD cells. These results suggest that PDE1 inhibition was not sufficient to cause an AVP-mediated increase in the interaction between B-Raf and PDE1 in NHK cells.