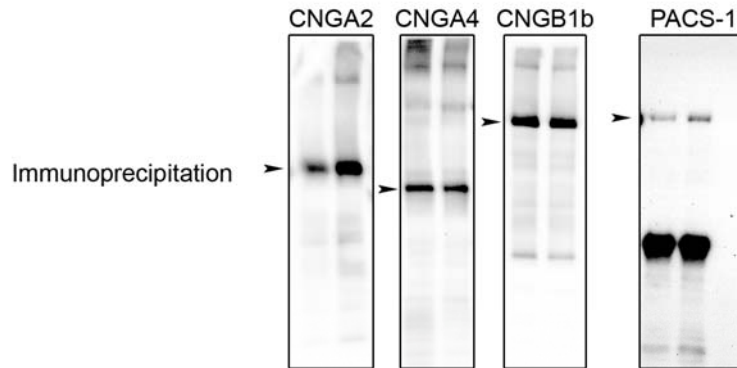
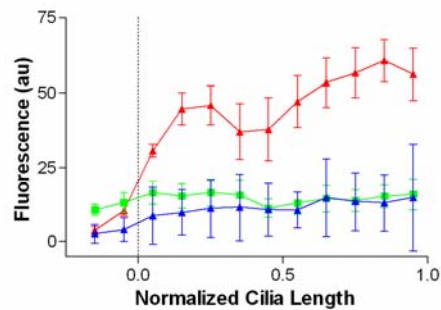
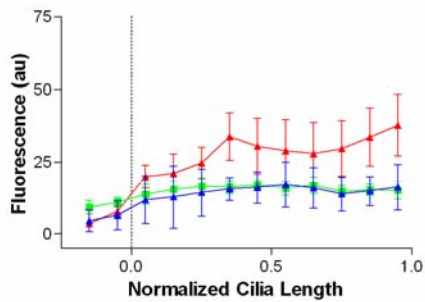
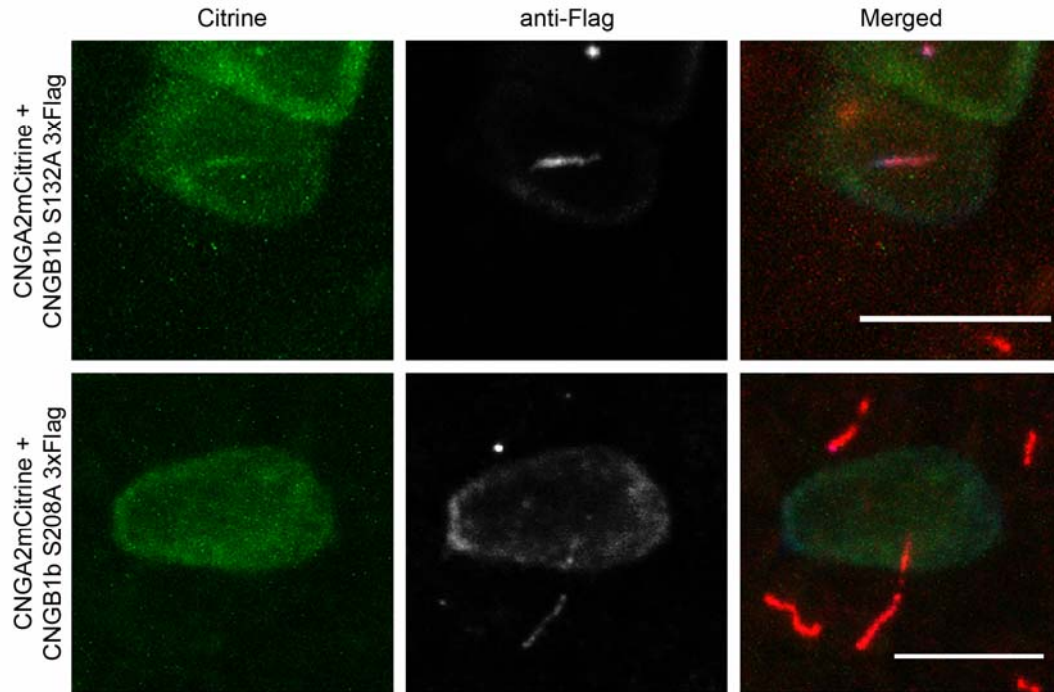


## Supplemental Material



### **Supplemental Figure 1 Immunoprecipitation of CNGA2, CNGA4, and CNGB1b prior to *in vitro* CK2 kinase reaction.**

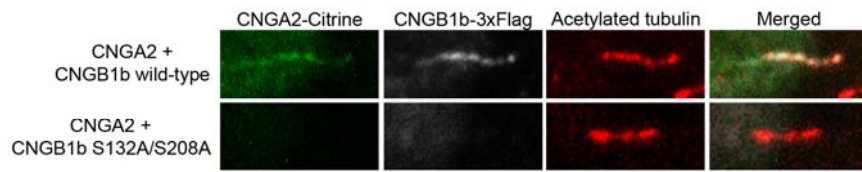
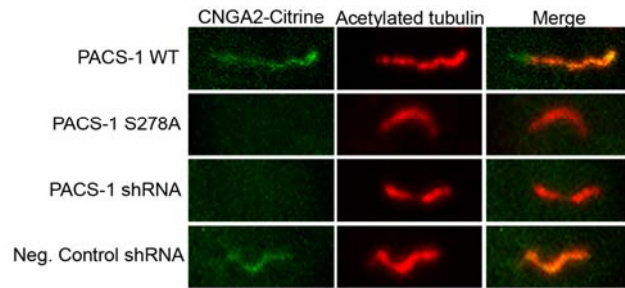
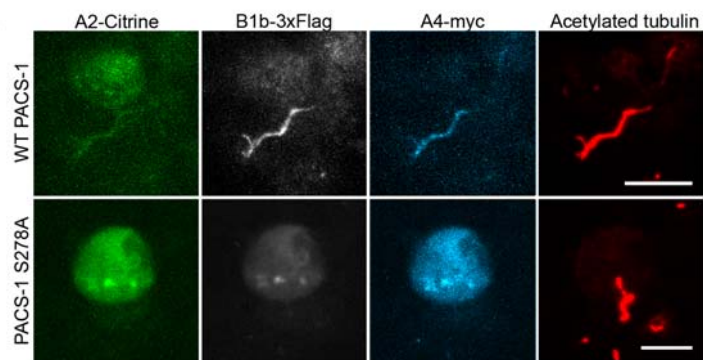
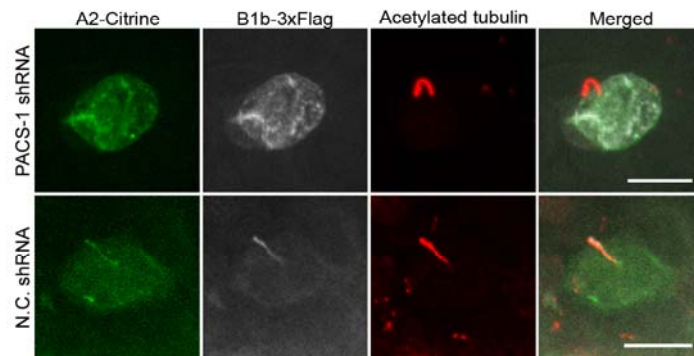
Western blot results from immunoprecipitation experiments from HEK293 cells transfected with CNGA2-3xFlag, CNGA4-3xFlag, CNGB1b3xFlag, or HA-PACS-1. Samples containing immunoprecipitates bound to beads were split, and half was used for the *in vitro* kinase reaction in Figure 2D. Arrowheads mark proteins of interest. Immunoprecipitations were performed with polyclonal anti-Flag (CNGA2, CNGA4, and CNGB1b) or polyclonal anti-HA (PACS-1) antibodies.



**Supplemental Figure 2 Mutation of Either S132 or S208 Leads to Diminished Ciliary Trafficking of the CNG Channel**

Representative confocal images of MDCK cells transfected with CNGA2-mCitrine and **(top images)** CNGB1b S132A-3xFlag or **(bottom images)** CNGB1b S208A-3xFlag. Citrine signal is on left (green). Flag immunostaining for CNGB1b is in middle (grayscale). Merged image with staining for acetylated  $\alpha$  tubulin (red) is

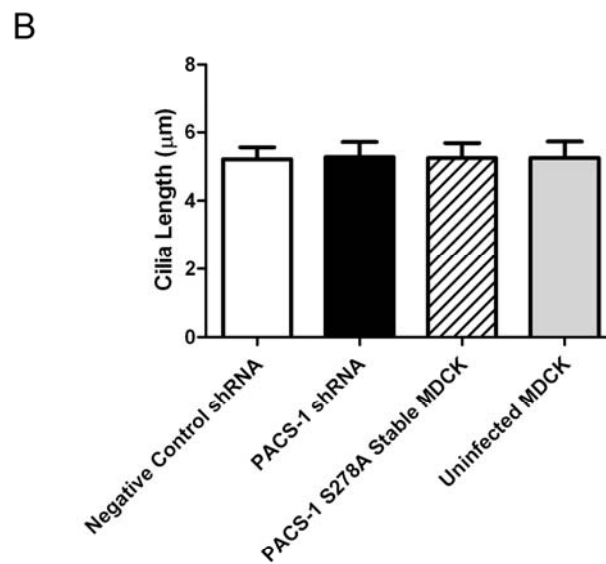
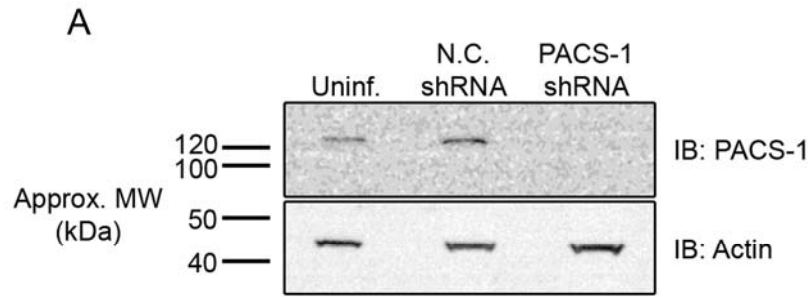
on right. Bar represents 10  $\mu\text{m}$ . Average data from multiple cells (n=6-7) shown for **(bottom left)** CNGB1b S132A or **(bottom right)** CNGB1b S208A. Data were apportioned into bins of  $1/10^{\text{th}}$  of normalized cilia length. Acetylated tubulin signal shown in red, CNGA2-mCitrine fluorescence shown in green, and anti-Flag immunostaining signal shown in blue (arbitrary fluorescent units).

**A****B****C****D**

### **Supplemental Figure 3 Alteration of PACS-1 Function Causes Impaired Ciliary Localization of the Complete CNG Channel Heterotetramer**

Representative collapsed confocal images from MDCK cells shown in Figure 3 **(A)** and Figure 4 **(B)**. These example images, used for line fluorescent intensity analysis, utilize only confocal planes containing cilia as determined by anti-acetylated tubulin immunostaining. **(A)** Representative confocal images of MDCK cells transfected with CNGA2-mCitrine and CNGB1b-3xFlag or CNGB1b S132A, S208A-3xFlag. Citrine signal is on left (green). Flag immunostaining for CNGB1b is in middle (grayscale). Merged image with staining for acetylated  $\alpha$  tubulin (red) is on right. **(B)** Representative confocal images of MDCK cells transfected with CNGA2-mCitrine and CNGB1b-3xFlag stably-expressing either PACS-1 WT, PACS-1 S278A, PACS-1 shRNA, or negative control shRNA. Signal from CNGA2-mCitrine is shown on the left (green) and acetylated tubulin signal marking the ciliary axoneme is in the middle (red), with the merged image on the right. **(C)** Representative confocal images of MDCK cells transfected with CNGA2-mCitrine, CNGA4-myc, and CNGB1b-3xFlag stably-expressing either PACS-1 WT or PACS-1 S278A. Signal from CNGA2-mCitrine is shown on the left (green), immunostaining for CNB1b-3x flag is shown 2<sup>nd</sup> from the left (grayscale), immunostaining for CNGA4-myc flag is shown 3<sup>rd</sup> from the left (cyan) and acetylated tubulin signal marking the ciliary axoneme is on the right (red). Bars represent 10  $\mu$ m. **(D)** Representative confocal images of MDCK cells transfected with CNGA2-mCitrine, and CNGB1b-3xFlag stably-expressing either PACS-1 shRNA (top) or negative control shRNA (N.C. shRNA, bottom). Signal

from CNGA2-mCitrine is shown on the left (green), immunostaining for CNB1b-3x flag is shown 2<sup>nd</sup> from the left (grayscale), acetylated tubulin signal marking the ciliary axoneme is 3<sup>rd</sup> from the left (red), and the merged image is on the right. Bars represent 10  $\mu\text{m}$ .



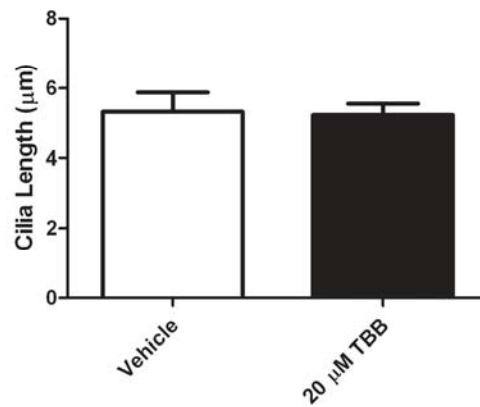
**Supplemental Figure 4 Retrovirally-Delivered shRNA Effectively Silences PACS-1 Expression but Does Not Affect Cilia Length**

**(A)** Western blot results from lysates prepared from uninfected MDCK cells (Uninf.), MDCK cells stably-expressing negative control shRNA (N.C. shRNA), or MDCK cells stably expressing shRNA directed against PACS-1 (PACS-1 shRNA). (Top) Blot probed for PACS-1 indicates loss of expression in PACS-1 shRNA stable MDCK cells. (Bottom) Loading control blot for actin demonstrates

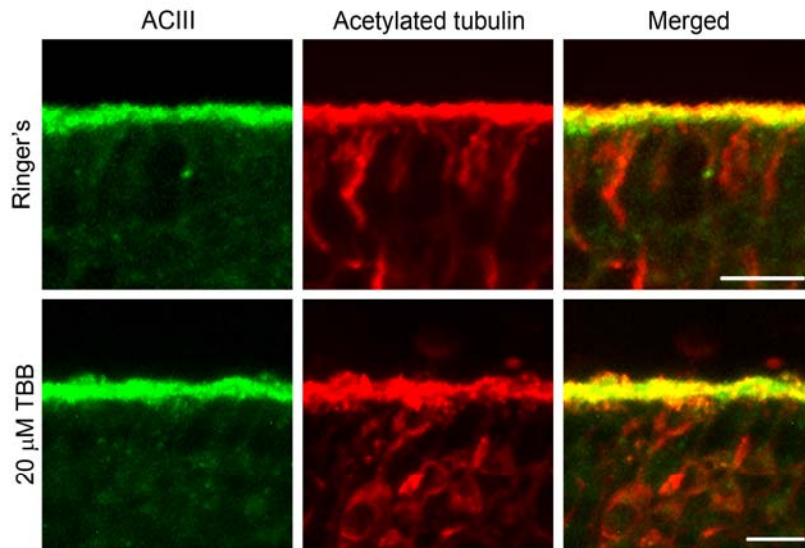
equal loading of protein. Markers for approximate molecular weight (kDa) are shown on left. **(B)** Average cilia length of filter-grown MDCK cells stably-expressing Negative Control shRNA, PACS-1 shRNA, PACS-1 S278A, versus uninfected MDCK cells. Data are represented as mean  $\pm$  SEM. No statistical significance was detected (n=15 for each condition; One-way ANOVA p=0.9998).



**A**



**B**

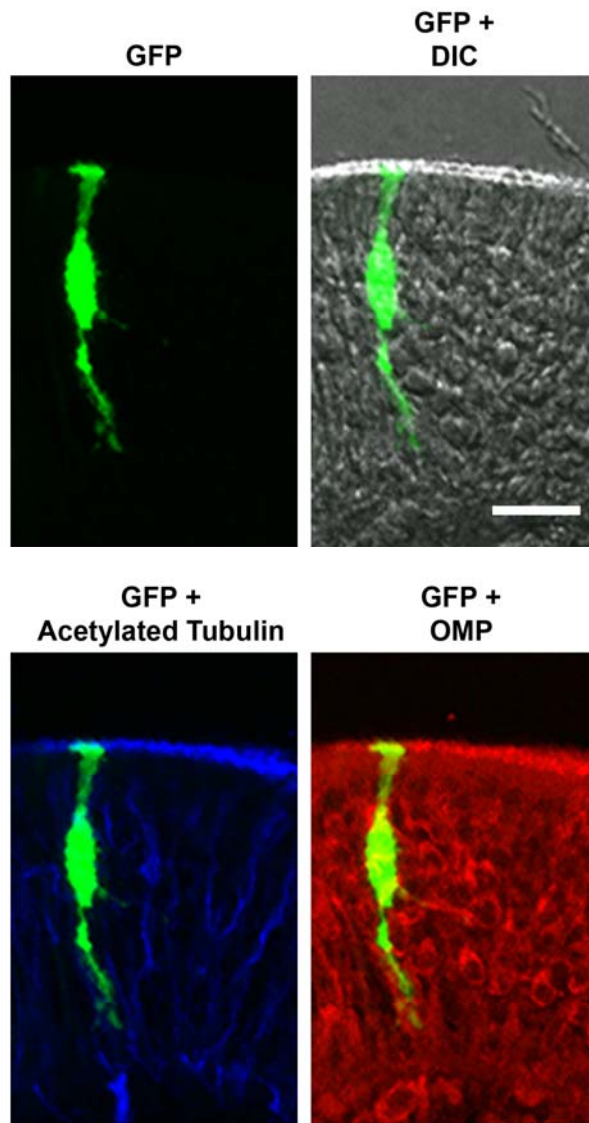


**Supplemental Figure 5 Cilia Length and ACIII Ciliary Localization are Unaffected by CK2 Inhibition.**

**(A)** Average cilia length of filter-grown MDCK cells treated with 0.01% DMSO vehicle (empty bar,  $5.323 \pm 0.552$   $n=33$ ) or 20 µM TBB (filled bar,  $5.230 \pm 0.329$   $n=33$ ).  $p=0.885$ , unpaired  $t$  test. Data are represented as mean  $\pm$  SEM. **(B)** Representative images taken from the medial surface of the olfactory turbinates

exposed to either Ringer's control (top) or 20  $\mu$ M TBB (bottom) for 25 minutes. Sections were coimmunostained with antibodies against ACIII (left, green) and acetylated  $\alpha$  tubulin (middle, red). Merged images shown on right. Bars represent 10  $\mu$ m.

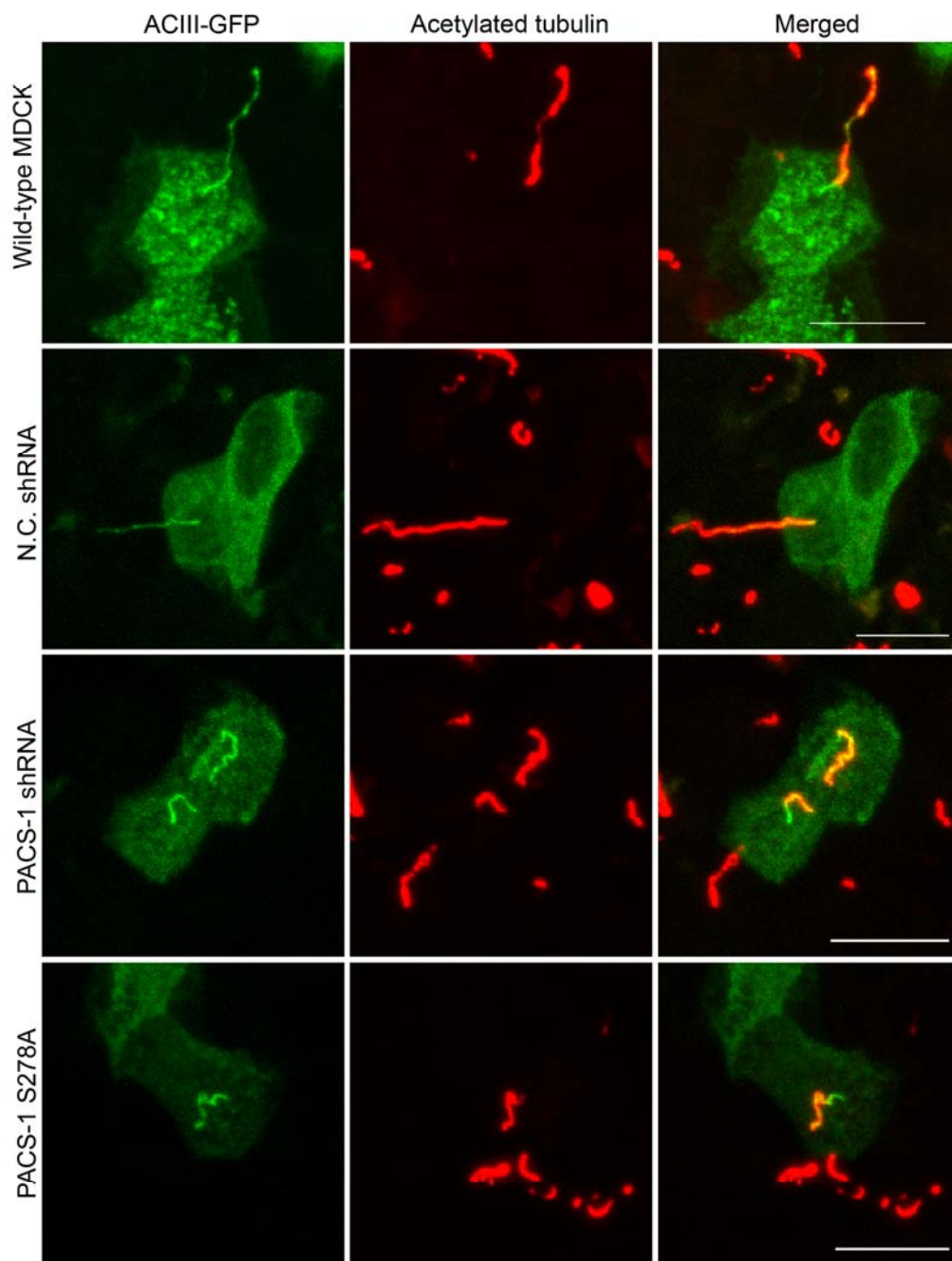
**PACS-1 S278A IRES GFP  
Adenovirus Infection**



**Supplemental Figure 6 Adenovirally-Infected Cells with Altered Morphology  
are Mature OSNs**

Representative collapsed confocal images of a coronal section of OE from adenovirally-infected mouse. A PACS-1 S278A IRES GFP-infected OSN (green,

top left) can be visualized deep in the layer of OSNs as determined by overlay with a differential interference contrast image (top right). This localization can be confirmed by immunostaining and colocalization with antibodies against the ciliary marker, acetylated  $\alpha$  tubulin (blue, bottom left) and the marker of mature OSNs, olfactory marker protein (OMP) (red, bottom right). Bar represents 20  $\mu$ m.



**Supplemental Figure 7 Ciliary Localization of ACIII-GFP is Unaffected by Alterations in PACS-1 Function**

Representative confocal images of wild-type MDCK cells (top) or MDCK cells stably-expressing either negative control shRNA, PACS-1 shRNA, or PACS-1 S278A transfected with ACIII-GFP. Signal from ACIII-GFP is shown on the left (green) and acetylated tubulin signal marking the ciliary axoneme is in the middle (red), with the merged image on the right. Bars represent 10  $\mu\text{m}$ .