Supplemental Materials

Inventory:

1. 4 Supplemental Figures
2. 3 Supplemental Tables

Supplemental Figures

Figure S1. **Alignment of mouse Pk1-4 protein sequences.** PET (green) and LIM (red) domains are highlighted. The four C-terminal amino acids represent the CAAX motif.

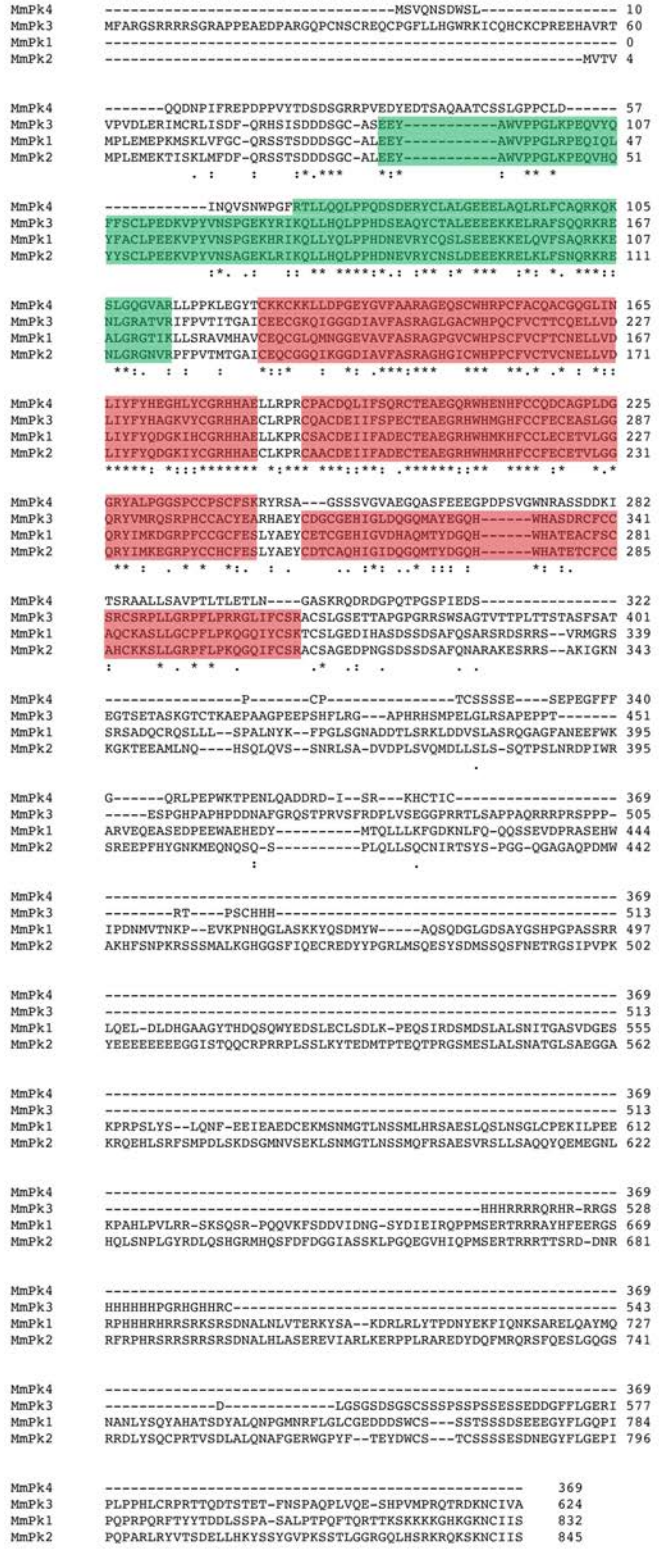


Figure S2. **Schematics of MTEC culture ciliogenesis and *Pk* expression. A.** Schematic of ciliogenesis during MTEC culture progression. Ciliated cell formation begins at ALI+2d. PCP acquisition indicated by crescent formation is evident slightly prior to the appearance of cilia. **B.** Schematic of *Pk1-4* expression during MTEC timecourse. **C.** Schematic of *Pk1-4* cell type specific enrichment in the airway epithelium.

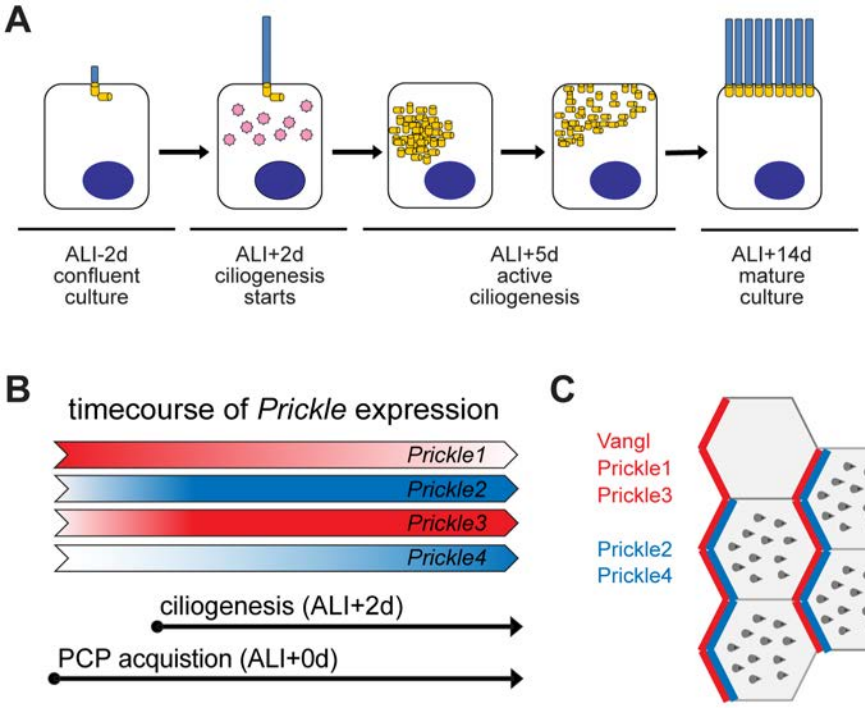
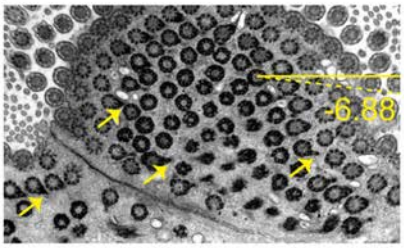


Figure S3. **Basal body misorientation in Pk mutants. A.** Example TEM image of basal feet on cilia and determination of ciliary orientation. Arrows point to basal feet. **B-C.** Table summary of the number of basal feet quantitated in adult (**B**) and perinatal (**C**) mice.

A



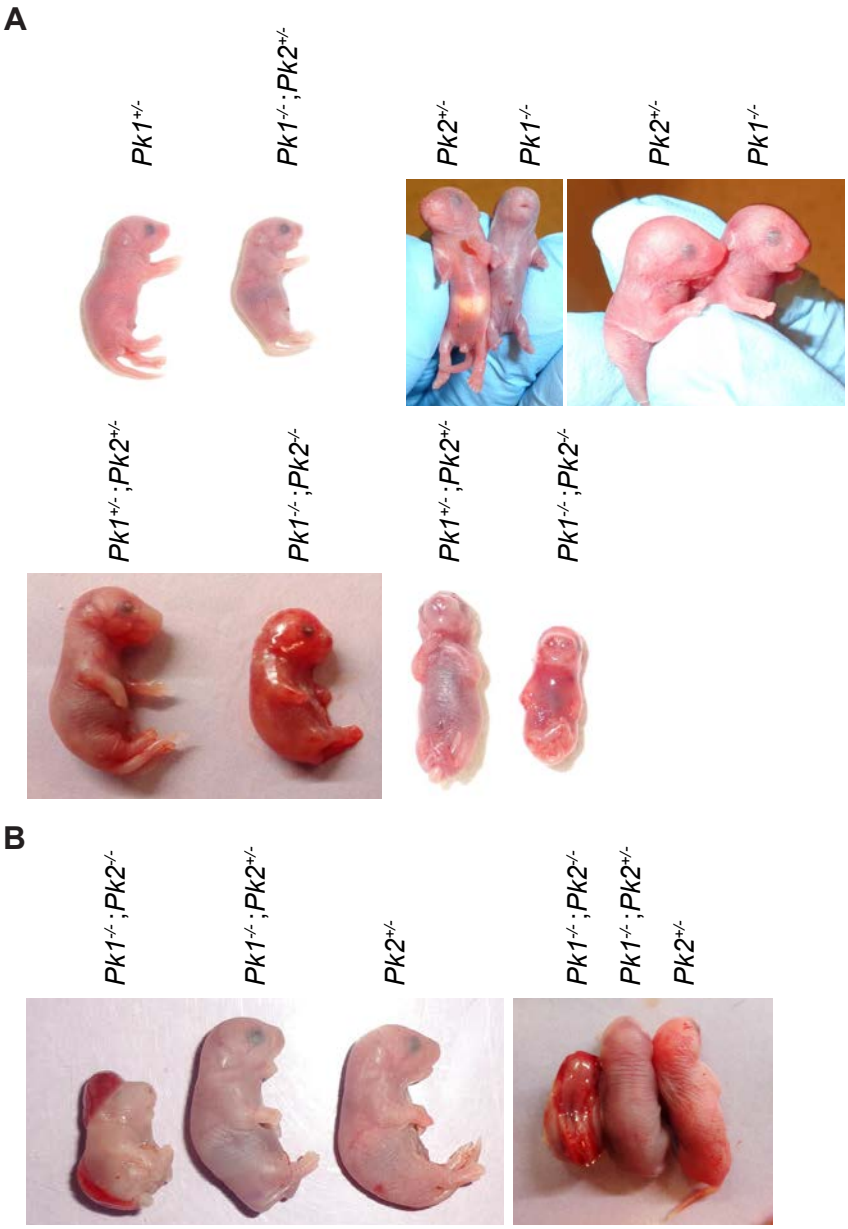
B

	genotypes						
	wildtype	*Pk1*$^{+/-}$	*Pk2*$^{-/-}$	*Pk1*$^{+/-}$; *Pk2*$^{+/-}$	*Pk1*$^{+/-}$; *Pk2*$^{-/-}$	*Vangl1CKO*$^{\Delta/\Delta}$	*Pk2*$^{-/-}$; *Vangl1CKO*$^{\Delta/\Delta}$
cells analyzed (n)	28	36	77	63	63	88	70
basal bodies analyzed (n)	277	444	1,039	743	595	1220	1060
ave. basal bodies per cell +/- SE	10.65 +/- 1.4	12.33 +/- 1.03	13.49 +/- 0.79	11.79 +/- 1.26	9.44 +/- 0.39	13.86 +/- 0.78	15.14 +/- 1.16

C

	genotypes					
	wildtype	*Pk1*$^{-/-}$	*Pk2*$^{-/-}$	*Pk1*$^{+/-}$; *Pk2*$^{-/-}$	*Pk1*$^{-/-}$; *Pk2*$^{+/-}$	*Pk1*$^{-/-}$; *Pk2*$^{-/-}$
mouse age	E18.5	P0	P0	P16	P0	E18.5
mice (n)	3	2	2	1	2	3
basal feet analyzed (n)	23	175	133	180	67	20
correctly oriented (n)	21	87	112	96	32	7
incorrectly oriented (n)	2	88	21	84	35	13

Figure S4. **Developmental phenotypes in Pk mutants. A.** Craniofacial and **B.** neural tube closure defects in Pk mutant litter mates from a *Pk1*$^{+/-}$*; Pk2*$^{+/-}$ x *Pk1*$^{+/-}$*; Pk2*$^{+/-}$ cross.



Supplemental Tables

Table S1. **Antibodies.**

Protein	Antibody	Source
Vangl1	HPA025235	Sigma Aldrich
Prickle1	-	Gibbs *et al*., 2016
Prickle2	-	Deans *et al*., 2007
Frizzled6	AF1526	R&D Systems
ac. α-Tubulin	ab24610	Abcam
GFP	4745-1051	AbD Serotech

Table S2. **Primer sequences.**

Target	Forward primer sequence	Reverse primer sequence
MmPrickle1	GATGGAGAAAGCAAGCCAAG	TGTGCAGCATGGAAGAGTTC
MmPrickle2	ACATGGGCACTCTCAACTCC	TGTATCCTAGGGGGTTGCTG
MmPrickle3	TGCTGTTTCGAGTGTGAAGC	CATCACAGTATTCCGCATGG
MmPrickle4	CCACAGGACAGTGATGAACG	CCTTCAAGCTTAGGAGGCAG
MmCentrin2	ACAGGGCAGAACAAGAGCAC	CCACTGCTTATGGTGACATGG
MmGapdh	GACTTCAACAGCAACTCCCAC	TCCACCACCCTGTTGCTGTA

Table S3. **Basal feet quantitation of Pk mutant mice from Fig. 4.**

wildtype				*Pk1*$^{-/-}$				*Pk2*$^{-/-}$				*Pk1*$^{+/-}$ *; Pk2*$^{-/-}$				*Pk1*$^{-/-}$ *; Pk2*$^{+/-}$				*Pk1*$^{-/-}$ *; Pk2*$^{-/-}$			
cell	BB - correct	BB - incorrect	**BB - total**	cell	BB - correct	BB - incorrect	**BB - total**	cell	BB - correct	BB - incorrect	**BB - total**	cell	BB - correct	BB - incorrect	**BB - total**	cell	BB - correct	BB - incorrect	**BB - total**	cell	BB - correct	BB - incorrect	**BB - total**
1	4	1	**5**	1	1	3	**4**	1	5	0	**5**	1	4	1	**5**	1	2	2	**4**	1	1	0	**1**
2	3	0	**3**	2	2	5	**7**	2	8	0	**8**	2	8	11	**19**	2	0	2	**2**	2	0	1	**1**
3	3	0	**3**	3	1	5	**6**	3	3	4	**7**	3	0	5	**5**	3	2	3	**5**	3	2	2	**4**
4	1	0	**1**	4	3	3	**6**	4	10	1	**11**	4	11	5	**16**	4	0	2	**2**	4	2	0	**2**
5	1	0	**1**	5	4	1	**5**	5	8	1	**9**	5	21	0	**21**	5	2	0	**2**	5	0	1	**1**
6	4	0	**4**	6	3	7	**10**	6	5	0	**5**	6	2	10	**12**	6	1	3	**4**	6	1	0	**1**
7	1	0	**1**	7	2	1	**3**	7	7	0	**7**	7	18	18	**36**	7	2	1	**3**	7	0	1	**1**
8	1	1	**2**	8	5	0	**5**	8	4	4	**8**	8	5	7	**12**	8	2	3	**5**	8	0	1	**1**
9	1	0	**1**	9	3	0	**3**	9	13	1	**14**	9	5	1	**6**	9	1	1	**2**	9	0	1	**1**
10	2	0	**2**	10	4	4	**8**	10	4	3	**7**	10	4	3	**7**	10	3	3	**6**	10	0	3	**3**
				11	0	3	**3**	11	8	0	**8**	11	3	1	**4**	11	4	1	**5**	11	0	1	**1**
				12	0	2	**2**	12	8	0	**8**	12	2	2	**4**	12	1	4	**5**	12	0	1	**1**
				13	2	0	**2**	13	8	2	**10**	13	1	1	**2**	13	4	2	**6**	13	0	2	**2**
				14	3	10	**13**	14	4	0	**4**	14	3	2	**5**	14	1	5	**6**	14	1	0	**1**
				15	1	0	**1**	15	5	1	**6**	15	2	2	**4**	15	3	0	**3**				
				16	3	6	**9**	16	4	1	**5**	16	0	1	**1**	17	4	3	**7**				
				17	2	1	**3**	17	5	2	**7**	17	1	2	**3**								
				18	0	8	**8**	18	3	1	**4**	18	5	3	**8**								
				19	5	0	**5**					19	0	6	**6**								
				20	12	0	**12**					20	1	3	**4**								
				21	2	3	**5**																
				22	4	0	**4**																
				23	3	3	**6**																
				24	3	2	**5**																
				25	2	2	**4**																
				26	3	3	**6**																
				27	4	2	**6**																
				28	0	3	**3**																
				29	4	3	**7**																
				30	0	3	**3**																
				31	0	3	**3**																
				32	4	0	**4**																
				33	2	2	**4**																
	21	**2**	**23**		**87**	**88**	**175**		**112**	**21**	**133**		**96**	**84**	**180**		**32**	**35**	**67**		**7**	**14**	**21**	**TOTAL (n)**