

## **Supplemental Materials**

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## 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.

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MmPk4 -----MSVQNSDWSL----- 10
MmPk3 MFARGSRRRSRGRAPPEAEDFARGQPCNSCREQCPCGFLHGWRKICQKCKPREEHAVRT 60
MmPk1 ----- 0
MmPk2 -----MVTV 4

MmPk4 -----QQDNPIFREPDPPVYTDSDSGRRPVEDYEDTSAQAATCSSLGPCLD----- 57
MmPk3 VPDLEIRIMCLISDF-QRHSISDDSGC-ASEEY-----AHVPPGLRPEQVYQ 107
MmPk1 MPEMEPKMSKLVFGC-QRSSTDDSGC-ALAEY-----AHVPPGLRPEQIQ 47
MmPk2 MPEMEKTIKLMDFD-QRSSTDDSGC-ALAEY-----AHVPPGLRPEQVHQ 51
      . : . : : * * * * * * * *
      . : . : : * * * * * * * *

MmPk4 -----INQVSNWPGFRTLLQLPQSDERYCLAEGEELAQLRFLCAQRKQR 105
MmPk3 FFCLPPEKVPYVNSPGEKYRIKQLLHQLPHDSEAQYCTALEEEKELRAFSAQRKRE 167
MmPk1 YFACLPEEKVPYVNSPGEKRIKQLLHQLPHDNEVRICQSLSEEEKELQVFSQRKRE 107
MmPk2 YFACLPEEKVPYVNSAGEKLRKQLLHQLPHDNEVRICNSLDEEEKELALFSQRKRE 111
      : * . : : : * * * * * * * * : * * * * * * * *

MmPk4 SLGQGVARLLPPKLEGYTCKKCKLLDPGEYGVFAARAGEQSCWHRPCFACQACGGGLIN 165
MmPk3 NLGRATVRIFFVTITGAIACECGKQIGGGDIIVFASRAGLACWHPCFVCTCQELLVD 227
MmPk1 ALGRGTIKLLSRVHVAVCEQCGLQMGGEVAVFASRAGVGVWHPFCVCTCNEELLVD 167
MmPk2 NLGRGNVRFPPVTMTGAIACECGGQIKGGDIIVFASRAGHGICWHPCFVCTCNEELLVD 171
      * * . : : : : * * * * * * * * * * * * * * * * * * * * * *

MmPk4 LIYFYHEGLYGRHHAELLRPRCPACDQLIPSORCTAEGRWHENHFCCQDCAGPLDG 225
MmPk3 LIYFYHAGKVCYGRHHAELRPRCAQCEIIFSPPECTEAGRHWHMGGFCCEASLGG 287
MmPk1 LIYFYQDGKIHCGRHHALLKPRCAACDEIFADECTEAGRHWHMGGFCCECETVLGG 227
MmPk2 LIYFYQDGKIYGRHHAELKPRCAACDEIFADECTEAGRHWHMGGFCCECETVLGG 231
      * * * * * * * * * * * * * * * * * * * * * * * * * * * *

MmPk4 GRYPALPGGSPCCPSCFNRYSR--GSSVGVAGQASFEPEGDPDPSVGNRASSDDKI 282
MmPk3 QRVMNRQSRPHCCACYERHAEYCDGCGEIHGDQGMAYEGQH----WHASDRCCFC 341
MmPk1 QRVIKMDGRPPCCGCFESLYAEYCECTCGEIHGDVHAQMTYDGGH----WHATEACFSC 281
MmPk2 QRVIKMDGRPYCCHCFESLYAEYCDTCAQHIGDQGMAYEGQH----WHATECFCC 285
      * * : . * * * * * : . . . * : . * * : : : * : : .

MmPk4 TSRAALLSAVPTLTLETLN---GASKRQDRDGPQTPGSPIEDS----- 322
MmPk3 SRCRPLILGRPFLPRGLIFCSHACSLGSETTAPGPRRSWSAGVTPLTSTASFSAT 401
MmPk1 AQCKASLLGCPFLPKQGIYCSHTCSLGEDIHASDSDSAFQARSRSR--VRMGRS 339
MmPk2 AHCKKSLILGRPFLPKQGIIFCSHACSAAGEDPNGSDSDSAFQARAKESR--AKIGKN 343
      : * . * * . * * * * * . * * : : .

MmPk4 -----P-----CP-----TCSSSSE---SEPEGFFF 340
MmPk3 EGTSETASKGTCTKAEPANGPEEPSHFLRG--APHRHMPGLGRSAPEPT----- 451
MmPk1 SRSAQCQRLSLL--SPALNYK--FPGLSGNADTLRKLDDVSLASRQAGAFANEEFWK 395
MmPk2 KGKTEEAMLNQ---HSQLQVS--SNRLSA-DVDPLSVQMDLLELS--SQTPLNRPDIWR 395

MmPk4 G-----QRLPEPWKTPENLQADRD-I--SR---KHCTIC----- 369
MmPk3 -----ESPGHAPHPDDNAFGRQSTPRVSRDPLVSEGGPRRTLSAPPAQRPRSPPP- 505
MmPk1 ARVEQEASEDPEWAEHEDY-----MTQLLKFQDKHLFQ-QQSEVDPRASEHW 444
MmPk2 SRBEFPFYGNKMEQNSQ-S-----PLQLLSQCNIRTSYS-PGG-QGAGAQPDMW 442
      :

MmPk4 -----RT---PSCHH----- 369
MmPk3 IPDNMVTNKP--EVKPNHQLGASKKYQSDMY-----AQSDGLGDSAYGSHPGPASSRR 497
MmPk1 AKHFSNPKRSMALRKHGGSPFQECREYYPGRMSQESYSDMSQSFPNETRGSIPVK 502
MmPk2

MmPk4 ----- 369
MmPk3 LQEL-DLDHGAAGYTHDQSQWYEDSLECLSDLK-PEQSIKSDMSLALSNITGASVDGES 513
MmPk1 YEEEEEEEGGISTQCRRPRLSLSLKYTEDMTPEQTFRGSMESLALSNATGLSAEGGA 562
MmPk2

MmPk4 ----- 369
MmPk3 KPRPRLYS--LQNF-EEIEAEDCEKMSNMGLTNSMHLRSESLQSLNSGLCPEKILPEE 612
MmPk1 KRQELSRFSMPDLKSDGMNVSEKLSNMGLTNSMQLRSESVRSLLSAQYQEMEGNL 622
MmPk2

MmPk4 ----- 369
MmPk3 KPAHLVLR--SKSQR--PQVKFSDVDINDG--SYDIEIRQPPMERTRRRHYFERGS 669
MmPk1 HQLSNFLGYRDLQSHGRMHQSFDFDGIASSKLPQGEVHIQPMERTRRRTSRD-DNR 681
MmPk2

MmPk4 ----- 369
MmPk3 HHHHHHPGRHGHRC----- 543
MmPk1 RPHHRRRRSRKSRSDNALNLVTERKYS--KDRLRLVTPDNYEKFIQNSARELQAYMQ 727
MmPk2 RFRPHRRRRSRKSRSDNALHLASEREVIARLKERPPLRAREDYDQPMRQSFQESLGGGS 741

MmPk4 ----- 369
MmPk3 -----D-----LGSQSDSGSCSSPSSPSSSEDDGFLGERI 577
MmPk1 NANLYSQYAHATSDYALQNPQMRFLGLGEDDSDWCS--SSTSSDSEEGYFLGQPI 784
MmPk2 RRDLYSQCPRTVSDALQNAFGERWGPYF--TEYDWC--TCSSSSESDNEGYFLGPEI 796

MmPk4 ----- 369
MmPk3 PLPPLCRPRTTQDSTET-FNSPAQPLVQE-SHPVMPQTRDKNCIVA 624
MmPk1 PQPRPQRFTYYDLDLSSPA-SALPTQFTQRTKSKKKKGGKNCIIS 832
MmPk2 PQPARLYVTSDELLHYSSYGVPKSSTLGGGQLHRRKQKSKNCIIS 845

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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.

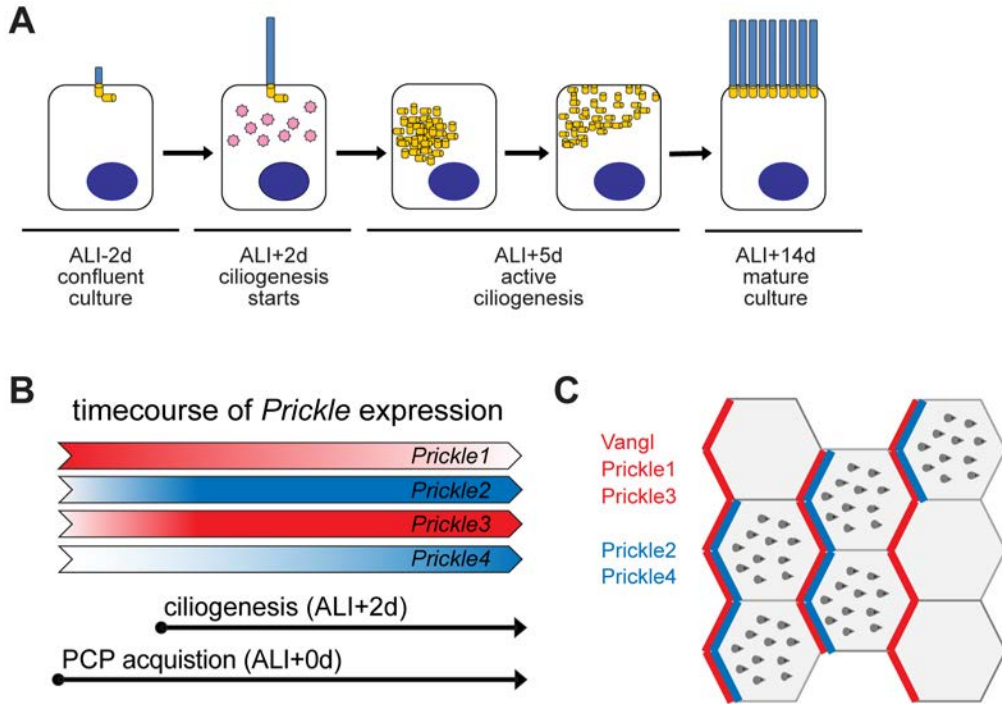
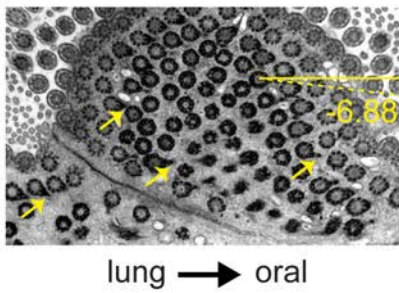


Figure S3. **Basal body misorientation in Pk mutants.** **A.** Example TEM image of basal feet on cilia and determination of ciliary orientation. Arrows point to basal feet. **B-C.** Table summary of the number of basal feet quantitated in adult (**B**) and perinatal (**C**) mice.

**A**



**B**

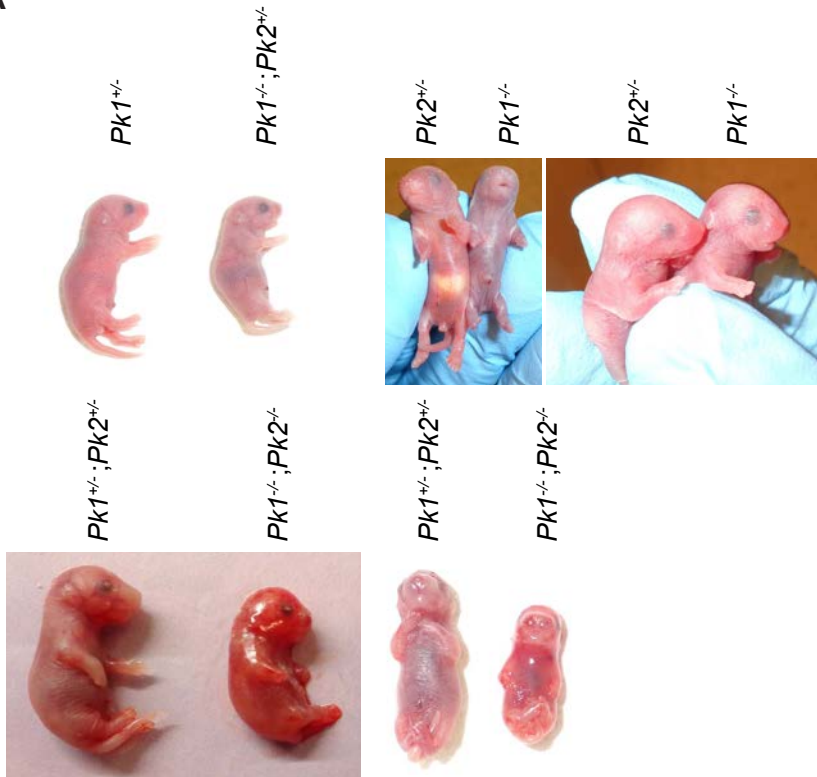
	genotypes						
	wildtype	<i>Pk1</i> <sup>+/-</sup>	<i>Pk2</i> <sup>-/-</sup>	<i>Pk1</i> <sup>+/-</sup> ; <i>Pk2</i> <sup>+/-</sup>	<i>Pk1</i> <sup>+/-</sup> ; <i>Pk2</i> <sup>-/-</sup>	<i>Vangl1</i> CKO <sup>Δ/Δ</sup>	<i>Pk2</i> <sup>-/-</sup> ; <i>Vangl1</i> CKO <sup>Δ/Δ</sup>
cells analyzed (n)	28	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**

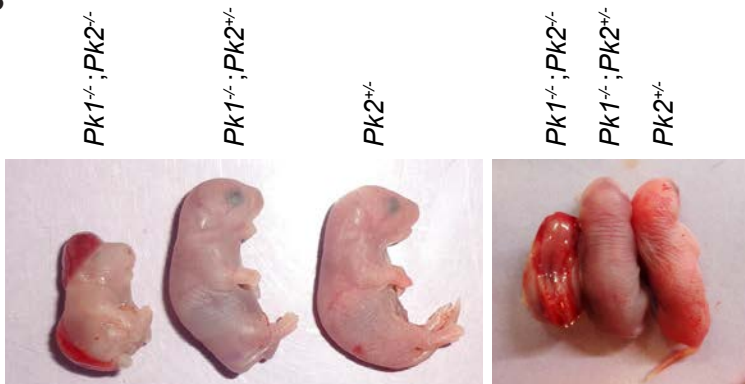
	genotypes					
	wildtype	<i>Pk1</i> <sup>-/-</sup>	<i>Pk2</i> <sup>-/-</sup>	<i>Pk1</i> <sup>+/-</sup> ; <i>Pk2</i> <sup>-/-</sup>	<i>Pk1</i> <sup>-/-</sup> ; <i>Pk2</i> <sup>+/-</sup>	<i>Pk1</i> <sup>-/-</sup> ; <i>Pk2</i> <sup>-/-</sup>
mouse age	E18.5	P0	P0	P16	P0	E18.5
mice (n)	3	2	2	1	2	3
basal 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.

**A**



**B**



## Supplemental Tables

Table S1. **Antibodies.**

<b>Protein</b>	<b>Antibody</b>	<b>Source</b>
<b>Vangl1</b>	HPA025235	Sigma Aldrich
<b>Prickle1</b>	-	Gibbs <i>et al.</i> , 2016
<b>Prickle2</b>	-	Deans <i>et al.</i> , 2007
<b>Frizzled6</b>	AF1526	R&D Systems
<b>ac. <math>\alpha</math>-Tubulin</b>	ab24610	Abcam
<b>GFP</b>	4745-1051	AbD Serotech

Table S2. **Primer sequences.**

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

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

wildtype				<i>Pk1</i> <sup>-/-</sup>				<i>Pk2</i> <sup>-/-</sup>				<i>Pk1</i> <sup>+/-</sup> ; <i>Pk2</i> <sup>-/-</sup>				<i>Pk1</i> <sup>-/-</sup> ; <i>Pk2</i> <sup>+/-</sup>				<i>Pk1</i> <sup>+/-</sup> ; <i>Pk2</i> <sup>+/-</sup>			
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)