

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 MFARGSRRRRSGRAPPEAEDPARGQPCNSCREQCPGFLHGWRKICQHKCKPREHVAVRT 60
MmPk1 ----- 0
MmPk2 -----MVTV 4

MmPk4 -----QQDNPIFREPDPVYVYDSDSGRRPVEDYEDTSAQAATCSSLGPCLD----- 57
MmPk3 VPDVLERIMCRLISDF-QRHSISDDSGC-ASEEY-----AWVPPGLKPEQVYQ 107
MmPk1 MPLEMEPKMSKLVFGC-QRSSTSDSGC-ALEEY-----AWVPPGLRPEQIQI 47
MmPk2 MPLEMEKTIKLMFDF-QRSSTSDSGC-ALEEY-----AWVPPGLKPEQVHQ 51
      . : . : : * . * * * * : * * *

MmPk4 -----INQVSNWPGFRLLQQLPPQSDERYCLALGEEELAQLRFLCAQRKOR 105
MmPk3 FFSCLPEKVPYVYVNSPGEKYRIKQLLHQLPHDSEAQYCTALEEEKELRAFSAQRKRE 167
MmPk1 YFACLPEEKVPYVYVNSPGEKRIKQLLYQLPHDNEVRYCQSLSEEEKELQVFAQRKRE 107
MmPk2 YFACLPEEKVPYVYVNSAGEKLRKQLLHQLPHDNEVRYCNSLDEEEKRELKLFNSQRKRE 111
      : * . : . : : * * * * * : * : * * * * : * : * * * * :

MmPk4 SLGQGVARLLPPKLEGYTCKCKKLLDPGEYGVFAARAGEQSCWHRPCFACQACGGLIN 165
MmPk3 NLGRATVRIFPVTITGAICEECKGQIGGGIVAFASRAGLACWHPQCFVCTTCQELLVD 227
MmPk1 ALGRGTIKLRSRAVHACVCEQGLQMGGEVAVFASRAGPGVWCWHPFCVCTCNEELLVD 167
MmPk2 NLGRGNVRFPFVMTGAICEQCGGQIKGGIVAFASRAGHGICWHPFCVCTVNEELLVD 171
      * * : . : : : * : * : * : * * * * * * * * * * * * * * : * :

MmPk4 LIYFYHGHLYCGRHBAELLRPRCPACDQLIFSQRCTAEGQRWHENHFCCQDCAGPLDG 225
MmPk3 LIYFYHAGKYCGRHBAELRPRCAQACDEIFSPPECTAEGRHHWHMGGHFCCEASLGG 287
MmPk1 LIYFYQDGKIHCGRHBALLKPRCSACDEIFADECTAEGRHHWHMGGHFCCECETVLGG 227
MmPk2 LIYFYQDGKIYGRHBAELKPRCAACDEIFADECTAEGRHHWHMGGHFCCECETVLGG 231
      * * * * * : * : * * * * * * * * * * * * * * * * * * * * * *

MmPk4 GRVALPGGSPCCPSCFSAIRYRSA--GSSSVGVAEQASFEPEGDPDSVGMNRASDDKI 282
MmPk3 QRVMNRQSRPHCCACYEARHAEYCDGCGEHLGDDQGMAYEGQH-----WHASDRCFCC 341
MmPk1 QRVMNRGRPFCCGCFESLYAEYCTCGEHLGDDHAQMTYDGH-----WHATEACFSC 281
MmPk2 QRVMNRGRPYCCHCFESLYAEYCDTCAOHIGIDQGMAYEGQH-----WHATECFCC 285
      * * : . * * * * . : . : . . * * * * * : * : * : * :

MmPk4 TSRAALLSAVPTLLETLN---GASKRQDRDGPQTGPSPIEDS----- 322
MmPk3 SRCRSRPLLRPFLPRRGLFCSACSLGSETTAPGPRRSWSACTVTTPLTSTASFSAT 401
MmPk1 AQCKASLLGCPFLPKQGIYCSHTCSLGEDIHASDSDSAFQARSRSRRS--VRMGRS 339
MmPk2 AHCKKSLLRPFLPKQGIYCSACSAAGEDPNGSDSDSAFQARAKESRRS--AKIGRN 343
      : * * * * . * * : . * * * *

MmPk4 -----P-----CP-----TCSSSE---SEPEGFFF 340
MmPk3 EGTSETASKGTCTKAEPAGPEEPSSHFRG---APRRHSMPELGLRSAFEPT----- 451
MmPk1 SRSADQCRQSLLL--SPALNYK--FPLSGNADDTLSRKLDDVSLASRQAGAFANEFWK 395
MmPk2 KGTTEAMLNQ---HSQLQVS--SNRLSA-DVDPLSVQMDLLSL-SQTPSLNRDPIWR 395

MmPk4 G-----QRLPEPWKTPENLQADDRD-I--SR---KHCTIC----- 369
MmPk3 -----ESPGHPAPHDDNAPGRQSTPRVSRFDPLVSEGGPRRTLSAPPAQRRRPPPP- 505
MmPk1 ARVEQEAASEDPEEWAHEHEDY-----MTQLLLKFGDKNLFQ-QSSEVDPRASEHW 444
MmPk2 SREEPFHYGNKMEQNQSQ-S-----PLQLLSQCNIRTSYS-PGG-QGAGAQPDMW 442
      :

MmPk4 ----- 369
MmPk3 -----RT---PSCHH----- 513
MmPk1 IPDNMVTNKP--EVKPNHQGLASKKYQSDMYN----AQSDGLGDSAYGSHPGPASSRR 497
MmPk2 AKHFSNPKRSSSMALHGGSFIQECREDIYGRMLMSQESYSDMSSQSFNETRGSIPVK 502

MmPk4 ----- 369
MmPk3 ----- 513
MmPk1 LQEL-DLDHGAAGYTHDQSQWYEDSLECLSDLK-PEQSIKSDMSLALSNITGASVDGES 555
MmPk2 YEEEEEEGGISTQCRRRPLSLKYTEDMTPTTEQTPRGSMSLALSNATGLSABGGA 562

MmPk4 ----- 369
MmPk3 ----- 513
MmPk1 KPRPRLYS--LQNF-EEIEAEDCEKMSNMGLTNSMMLHRSAESLQSLNSGLCPEKILPEE 612
MmPk2 KRQEHLSRFSMPDLKSDSGMNVSEKLSNMGLTNSMMLQFRSAESVRSLLSAQYQEMENL 622

MmPk4 ----- 369
MmPk3 -----HHRRRRRQRHR-RRGS 528
MmPk1 KPAHLPLVLR-SKSQSR-PQOVKFSDDVIDNG-SYDIEIRQPPMSERTRRRHYFEERGS 669
MmPk2 HQLSNPLGYRDLQSHGRMHQSFDDGFIASSKLPQGEVHVIQPMSETRRRRTSRD-DNR 681

MmPk4 ----- 369
MmPk3 ----- 543
MmPk1 RPHHHRRRRSRKSRSDNALNLVTERKYSY--KDRRLVTPDHYEKFIQNKSARELQAYMQ 727
MmPk2 RFRPHRRRRSRKSRSDNALHLASEREVIARLKERPLRAREDYDQFMRQSRFQESLGGGS 741

MmPk4 ----- 369
MmPk3 -----D-----LGSQSDSGSCSSSPSSPSSSEDDGFFLGERI 577
MmPk1 NANLYSQYAHATSDYALQNGMNRFLGCGEDDSDWCS---SSTSSSDSEEYGLQPI 784
MmPk2 RRDLYSQCPRTVSDLALQNAFGRNGPYF--TEYDWCSS---TCSSSESDEYGLGPEI 796

MmPk4 ----- 369
MmPk3 PLPPLCRPRTTQDTSTET-FNSPAQLVQE-SHPVMPQTRDKNCIVA 624
MmPk1 PQPRPQRFYTYDGLSSPA-SALPTPOFTQRTKSKKKKGGKNCIIS 832
MmPk2 PQPARLYVTSDELLHKYSSYGVPKSSTLGGRGQLHSRKRQKSKNCIIS 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.

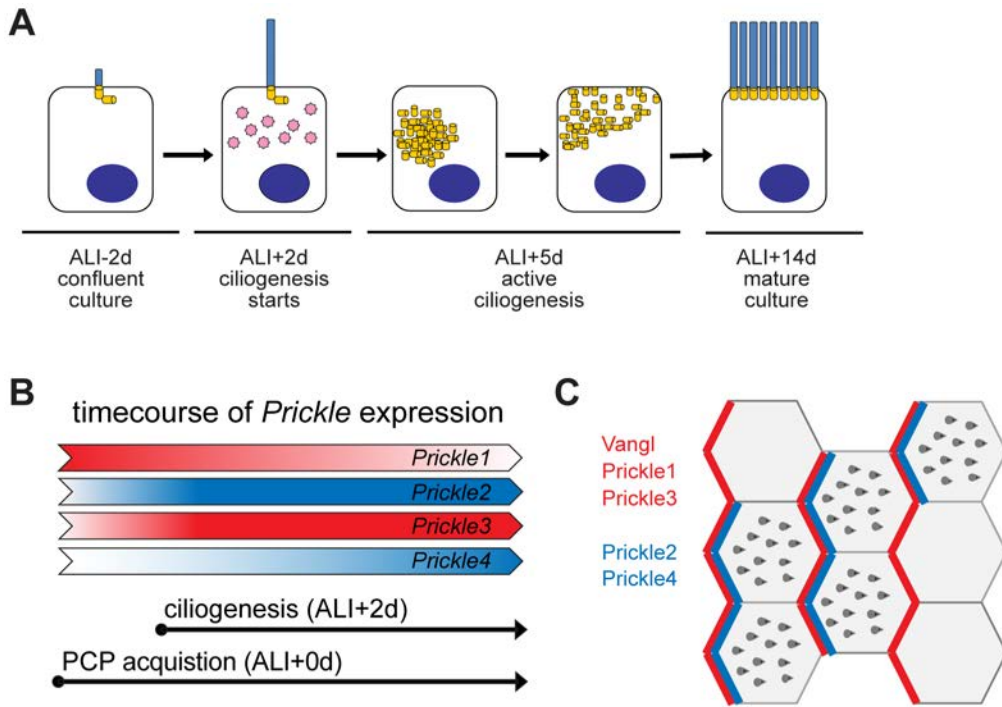
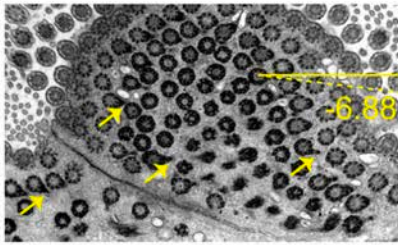


Figure S3. **Basal body misorientation in Pk mutants.** **A.** Example TEM image of basal feet on cilia and determination of ciliary orientation. Arrows point to basal feet. **B-C.** Table summary of the number of basal feet quantitated in adult (**B**) and perinatal (**C**) mice.

A



lung → oral

B

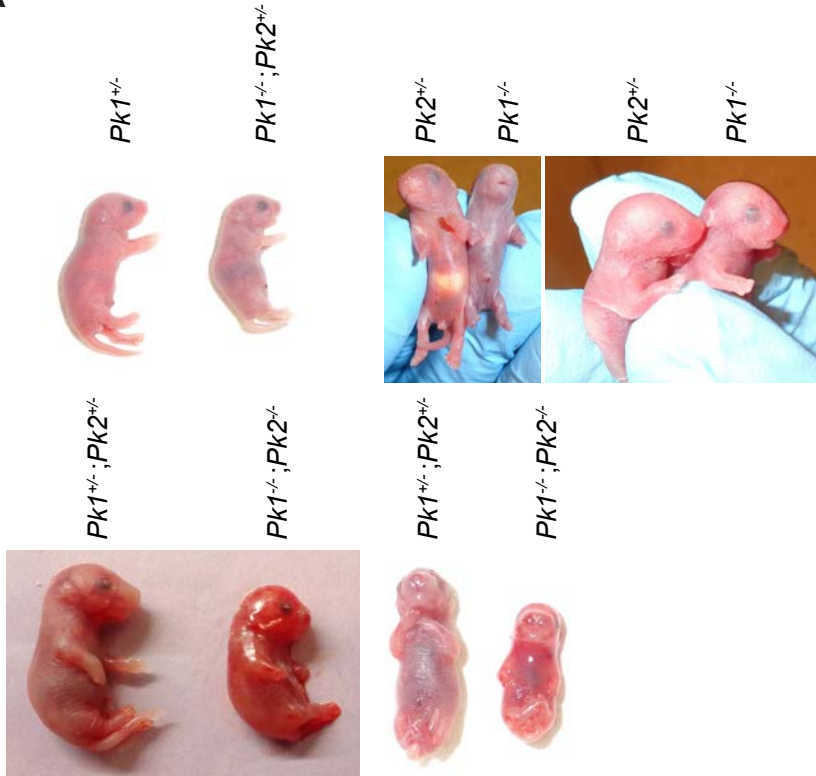
	genotypes						
	wildtype	<i>Pk1</i> ^{+/-}	<i>Pk2</i> ^{-/-}	<i>Pk1</i> ^{+/-} ; <i>Pk2</i> ^{+/-}	<i>Pk1</i> ^{+/-} ; <i>Pk2</i> ^{-/-}	<i>Vangl1CKO</i> ^{Δ/Δ}	<i>Pk2</i> ^{-/-} ; <i>Vangl1CKO</i> ^{Δ/Δ}
cells analyzed (n)	28	36	77	63	63	88	70
basal bodies analyzed (n)	277	444	1,039	743	595	1220	1060
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

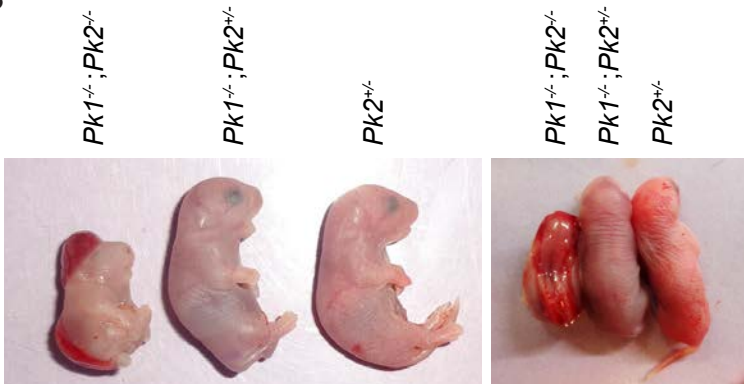
	genotypes					
	wildtype	<i>Pk1</i> ^{-/-}	<i>Pk2</i> ^{-/-}	<i>Pk1</i> ^{+/-} ; <i>Pk2</i> ^{-/-}	<i>Pk1</i> ^{+/-} ; <i>Pk2</i> ^{+/-}	<i>Pk1</i> ^{-/-} ; <i>Pk2</i> ^{-/-}
mouse age	E18.5	P0	P0	P16	P0	E18.5
mice (n)	3	2	2	1	2	3
basal feet analyzed (n)	23	175	133	180	67	20
correctly oriented (n)	21	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.**

Protein	Antibody	Source
Vangl1	HPA025235	Sigma Aldrich
Prickle1	-	Gibbs <i>et al.</i> , 2016
Prickle2	-	Deans <i>et al.</i> , 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
<i>MmPrickle1</i>	GATGGAGAAAGCAAGCCAAG	TGTGCAGCATGGAAGAGTTC
<i>MmPrickle2</i>	ACATGGGCACTCTCAACTCC	TGTATCCTAGGGGGTTGCTG
<i>MmPrickle3</i>	TGCTGTTTCGAGTGTGAAGC	CATCACAGTATTCCGCATGG
<i>MmPrickle4</i>	CCACAGGACAGTGATGAACG	CCTTCAAGCTTAGGAGGCAG
<i>MmCentrin2</i>	ACAGGGCAGAACAAGAGCAC	CCACTGCTTATGGTGACATGG
<i>MmGapdh</i>	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	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)				