### **Supplemental Figures**

Shh <sup>Cre</sup> Cenpj <sup>/lox</sup>										
z = 1	z = 2	z = 4	z = 6	z = 8	z = 10	z = 12	z = 13	z = 14	z = 15	
z = 16	z = 17	z = 18	z = 19	z = 20	z = 21	z = 22	z = 23	z = 24	z = 25	
z = 26	z = 27	z = 28	z = 29	z = 30	z = 31	z = 32	z = 33	z = 34	z = 35	
z = 36	z = 37	z = 38	z = 39	z = 40	z = 41	z = 42	z = 43	z = 44	z = 45	
z = 46	z = 47	z = 48	z = 49	z = 50	z = 51	z = 52	z = 53	z = 54	z = 55	
z = 58	z = 59	z = 60	z = 61	z = 62	z = 63	z = 64	z = 65	z = 66	z = 67	
z = 68	z = 69	z = 70	z = 71	z = 72	z = 73	z = 74	z = 75	z = 76	z = 77	
z = 78	z = 79	z = 80	z = 81	z = 82	z = 83	z = 84	z = 85	z = 86	z = 87	
		(sec	Grid 1 ctions 1 to	44)	Grid 2 (sections 44 to 87)					
		17 WILLIAM MARKEN								

# Figure S1. Serial section transmission electron microscopy of *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>) lung epithelial cells, Related to Figure 1. Scale bar, 2 μm.

Shh<sup>Cre</sup> Cenpj<sup>/lox</sup>

$ \begin{array}{c} z = 17 \\ z = 19 \\ z = 21 \\ z = 23 \\ z = 23 \\ z = 23 \\ z = 24 \\ z = 25 \\ z = 27 \\ z = 27 \\ z = 29 \\ z = 29 \\ z = 29 \\ z = 31 \\ z = 3 \\ z = 31 \\ z = 31 \\ z = 3 \\ z = 31 \\ z = 41 \\ z = 43 \\ z = 45 \\ z = 45 \\ z = 47 \\ z = 47 \\ z = 66 \\ z = 68 \\ z = 70 \\ z = 70$	5	
$z = 35 \qquad z = 37 \qquad z = 39 \qquad z = 41 \qquad z = 43 \qquad z = 45 \qquad z = 47 \qquad z = 49 \qquad z = 50 \qquad z = 5$	3	
z = 55 $z = 57$ $z = 58$ $z = 60$ $z = 62$ $z = 64$ $z = 66$ $z = 68$ $z = 70$ $z = 7$	3	
	2	
z = 74  z = 75  z = 77  z = 78  z = 80  z = 81  z = 83  z = 84  z = 86  z = 8	8	
z = 90   z = 92   z = 94   z = 96   z = 98   z = 100   z = 102   z = 104   z = 106   z = 1   z = 10	08	
z = 110  z = 112  z = 114  z = 116  z = 117  z = 119  z = 121  z = 123  z = 125  z = 1	27	
z = 129 $z = 131$ $z = 133$ $z = 135$ $z = 137$ $z = 139$ $z = 141$		
Grid 1 Grid 2 Grid 3 Grid 4 (sections 1 to 7) (sections 8 to 66) (sections 68 to 116) (sections 117 to 141	Grid 4 ions 117 to 141)	



Figure S2. Serial section transmission electron microscopy of *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>) intestinal epithelial cells, Related to Figure 1. Scale bar, 2 μm.







F

E16.5



## Figure S3. Centrioles are not required for distal epithelial lung fate, but required for bronchiolar epithelial formation, Related to Figure 2.

(A) Immunostaining of Nkx2.1 (red), CDH1 (green) and nuclei (blue) in E12.5 control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>) lung sections. Scale bar, 50 μm.

(B) Immunostaining of Nkx2.1 (red), CDH1 (green) and nuclei (blue) in E16.5 control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>) lung sections. Scale bar, 25  $\mu$ m.

(C) Immunostaining of SFTPC (red), CDH1 (green) and nuclei (blue) in E16.5 control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>) lung sections. Scale bar, 25 μm.

(D) RT-qPCR analysis of DeltaN p63 and TAp63 isoform expression in E12.5 control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>) lung and trachea. n=4 and 3 for control and *Cenpj* loss of function respectively.

(E) Immunostaining of p73 (red), SOX2 (green), CDH1 (grey) and nuclei (blue) in E12.5 control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>) lung sections. Scale bar, 50 μm.

(F) Immunostaining of p73 (red), SOX2 (green), CDH1 (grey) and nuclei (blue) in E16.5 control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>-/lox</sup>) lung sections. Scale bar, 20 μm.



Shh<sup>Cre</sup> Cenpi<sup>+/lox</sup> p53<sup>+/lox</sup> Shh<sup>Cre</sup> Cenpi<sup>/lox</sup> p53<sup>-/lox</sup>



Shh<sup>Cre</sup> Ift88+/lox

Shh<sup>Cre</sup> Ift88<sup>/lox</sup>



### Figure S4. p53 deletion does not restore limb development in Shh<sup>Cre</sup> Cenpj<sup>/lox</sup> mice, Related to Figure 2 and Figure 3.

(A) Images of P7 control (Shh<sup>Cre</sup> Cenpj<sup>+/lox</sup> p53<sup>+/lox</sup>), Cenpj and p53 combined loss of function (Shh<sup>Cre</sup> Cenpj<sup>+/lox</sup> p53<sup>+/lox</sup>) mice. Higher magnification of right hind paw (boxed) was shown below. Arrows indicated the abnormal digits.

(B) Images of P7 control (Shh<sup>Cre</sup> Ift88<sup>+/lox</sup>), Ift88 loss of function (Shh<sup>Cre</sup> Ift88<sup>-/lox</sup>) mice. Higher magnification of right hind paw (boxed) was shown below. Arrows indicated the abnormal digits.

(C) Images of the 4 paws of 6 months old control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup> *p53*<sup>+/lox</sup>), *Cenpj* and *p53* combined loss of function (Shh<sup>Cre</sup> Cenpj<sup>+/lox</sup> p53<sup>+/lox</sup>) mice. Higher magnification of right hind paw (boxed) was shown below. Arrows indicated the abnormal digits.

(D) Images of the 4 paws of 6 months old control (Shh<sup>Cre</sup> Ift88<sup>+/lox</sup>), Ift88 loss of function (Shh<sup>Cre</sup> Ift88<sup>-/lox</sup>) mice. Higher magnification of right hind paw (boxed) was shown below. Arrows indicated the abnormal digits.



**50** μm

Apoptotic cells (%) p=0.60 SCH772984 Г ٦ 50 p=0.30 p=0.66 Τ

cKO

0

Control

- T T DKO

#### Figure S5. ERK inhibitor suppresses the growth of lung and intestinal organoids, Related to Figure 5.

(A) Control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup> *Rosa26*<sup>dTomato</sup> *Sox9*<sup>GFP</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup> *Rosa26*<sup>dTomato</sup> *Sox9*<sup>GFP</sup>) lung organoid cultures were treated with DMSO or 2.5 μM SCH772984 for 24 hours, then imaged for GFP and tdTomato fluorescence. Scale bar, 100 μm.

(B) Quantification of the diameter of control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup> *Rosa26*<sup>dTomato</sup> *Sox9*<sup>GFP</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>-/lox</sup> *Rosa26*<sup>dTomato</sup> *Sox9*<sup>GFP</sup>) (cKO) lung organoid cultures in (A). n=3.

(C) Quantification of the Sox9-GFP level of control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup> *Rosa26*<sup>dTomato</sup> *Sox9*<sup>GFP</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup> *Rosa26*<sup>dTomato</sup> *Sox9*<sup>GFP</sup>) (cKO) lung organoid cultures in (A). n=3

(D) Control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup> *Rosa26*<sup>tdTomato</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>-/lox</sup> *Rosa26*<sup>tdTomato</sup>) intestinal organoid cultures were treated with DMSO or 2.5 μM SCH772984 for 24 hours, then imaged for tdTomato fluorescence. Scale bar, 50 μm.

(E) Quantification of the diameter of control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup> *Rosa26*<sup>dTomato</sup>) and *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup> *Rosa26*<sup>dTomato</sup>) (cKO) intestinal organoid cultures in (D). n=3

(F) Control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>), *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>), and *Cenpj* and *p53* combined loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup> *p53*<sup>-/-</sup>) lung organoid culture were treated with DMSO or 2.5 μM SCH772984 for 24 hours, then subjected to frozen section and stained for Ki67 (green), CDH1 (red) and nuclei (blue). Scale bar, 50 μm.

(G) Quantification of the Ki67 level in Control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>), *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>), and *Cenpj* and *p53* combined loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>) lung organoid culture in (F). n=4, 3 for control; n=2,3 for *Cenpj* loss of function; n=2, 6 for *Cenpj* and *p53* combined loss of function.

(H) Control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>), *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>), and *Cenpj* and *p53* combined loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup> *p53*<sup>/-</sup>) lung organoid culture were treated with DMSO, 10 μM Nutlin-3a or 2.5 μM SCH772984for 24hours, then subjected to frozen section and stained for cleaved Caspase 3 (CC3, green), CDH1 (red) and nuclei (blue). Scale bar, 50 μm

(I) Quantification of the apoptotic cells in Control (*Shh*<sup>Cre</sup> *Cenpj*<sup>+/lox</sup>), *Cenpj* loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup>)(cKO), and *Cenpj* and *p53* combined loss of function (*Shh*<sup>Cre</sup> *Cenpj*<sup>/lox</sup> *p53*<sup>-/-</sup>)(DKO) lung organoid culture in (J). n=5, 4, 4 for control; n=4, 5, 5 for *Cenpj* loss of function; n=5, 4, 2 for *Cenpj* and *p53* combined loss of function.