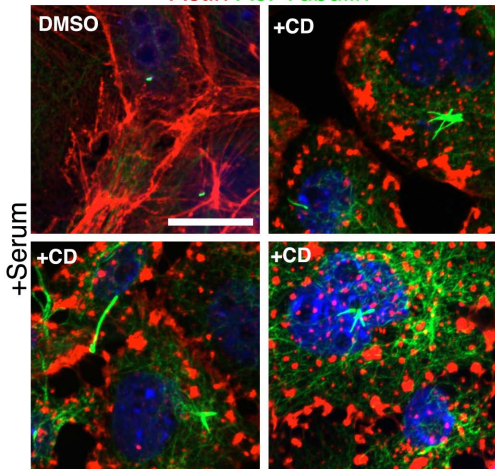
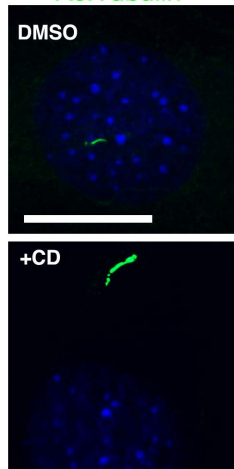


**A**

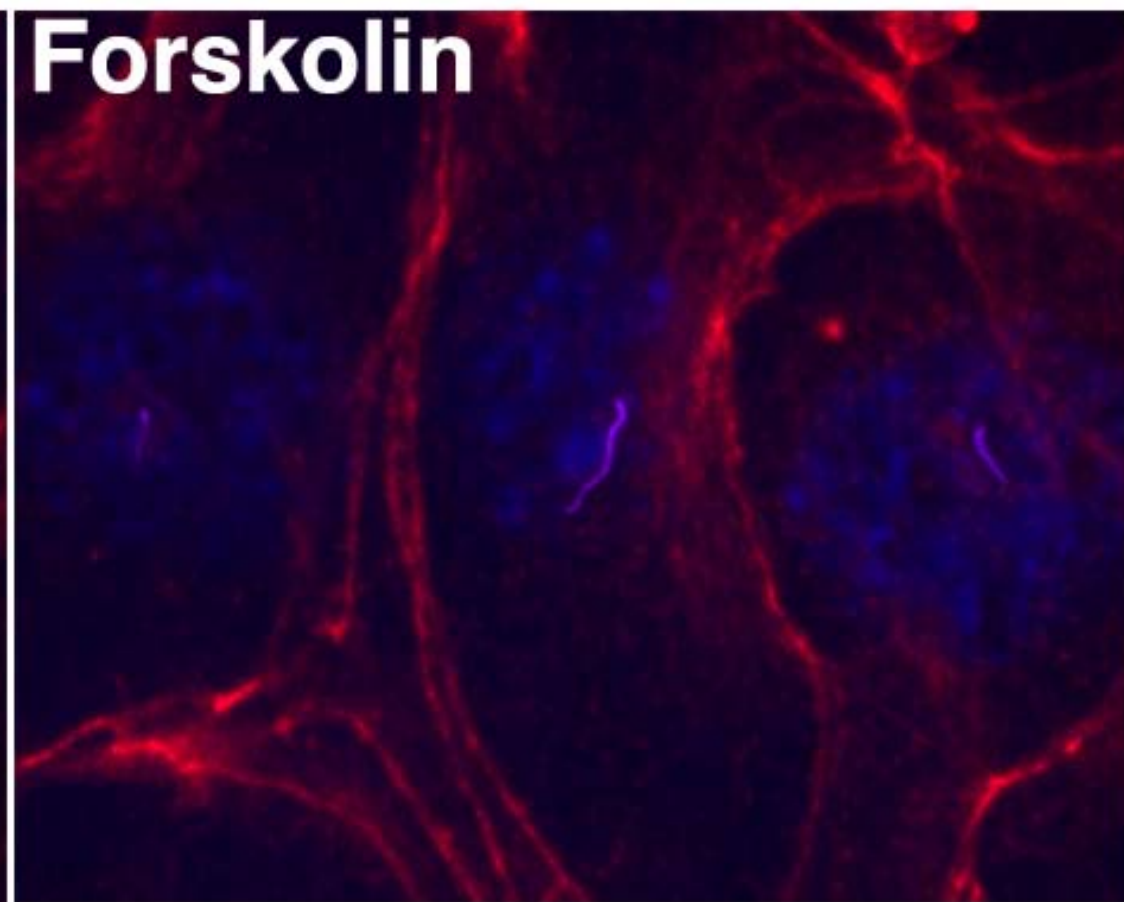
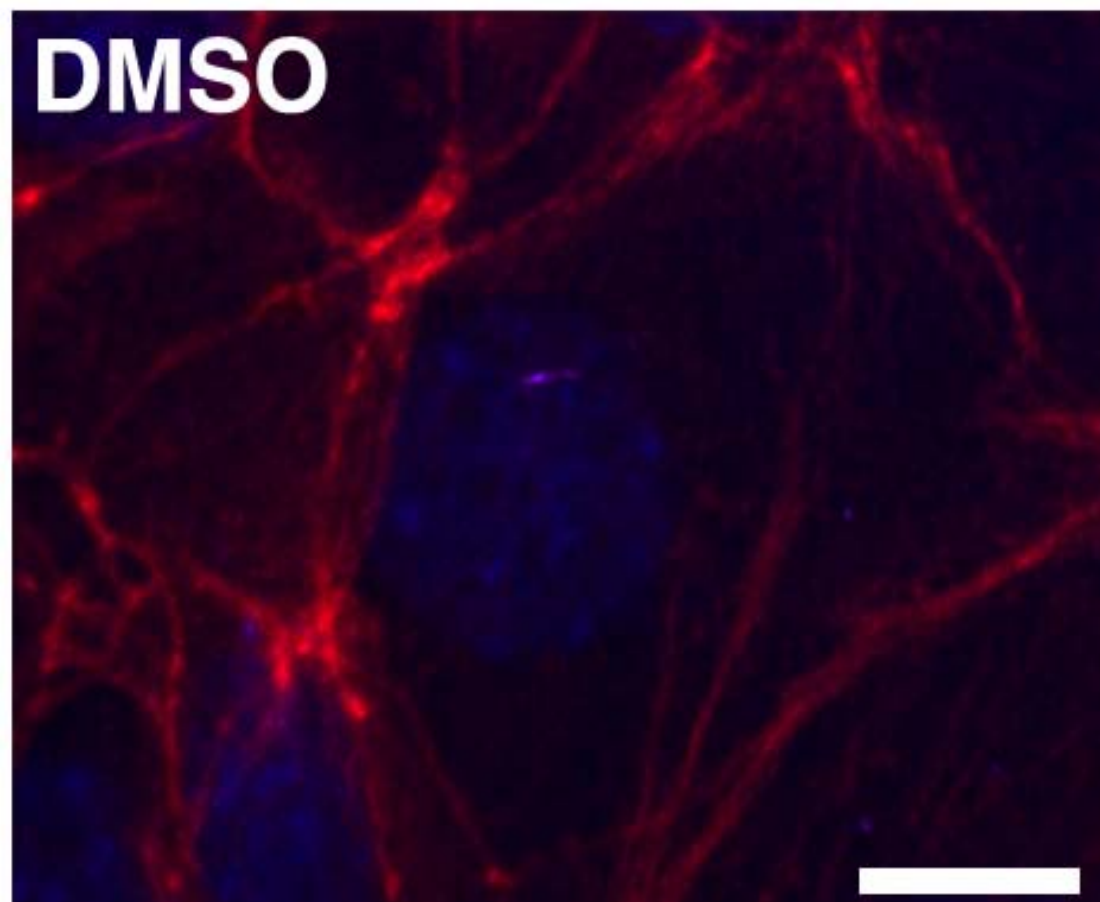
Actin Ac. Tubulin

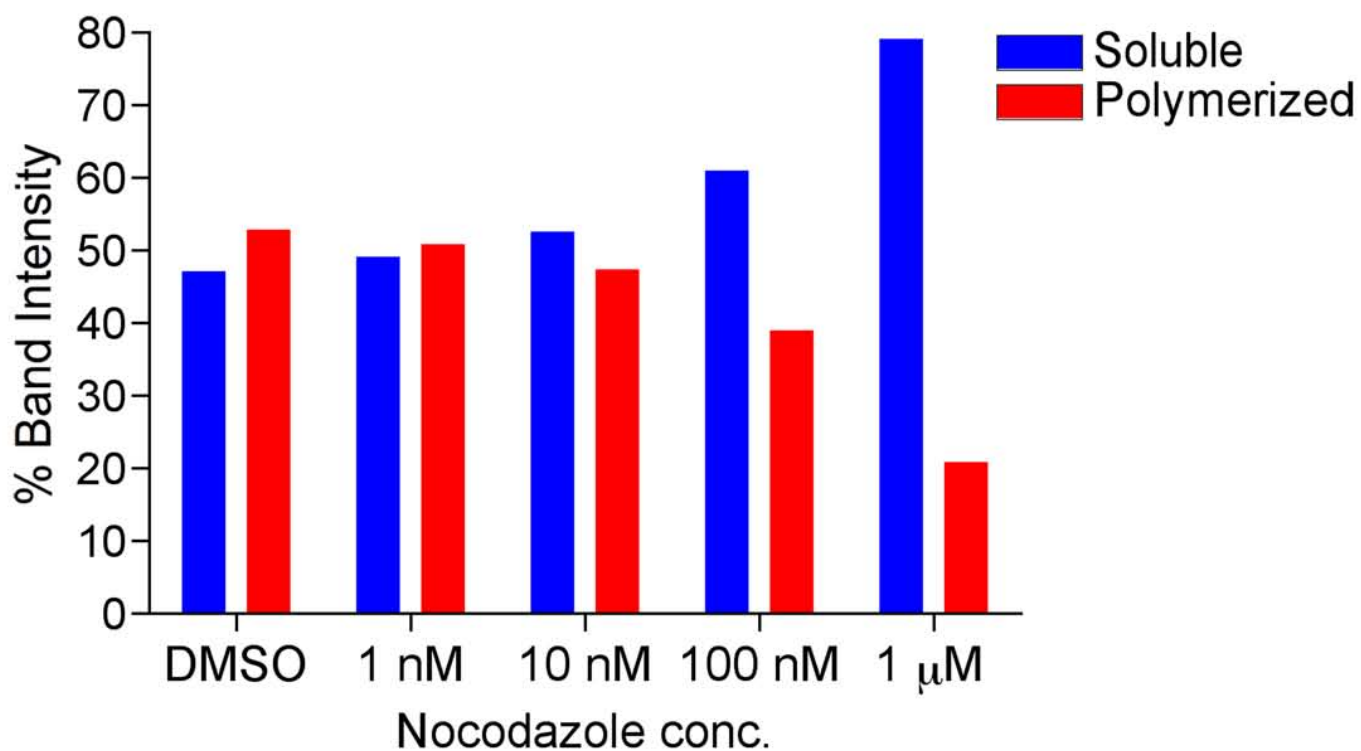
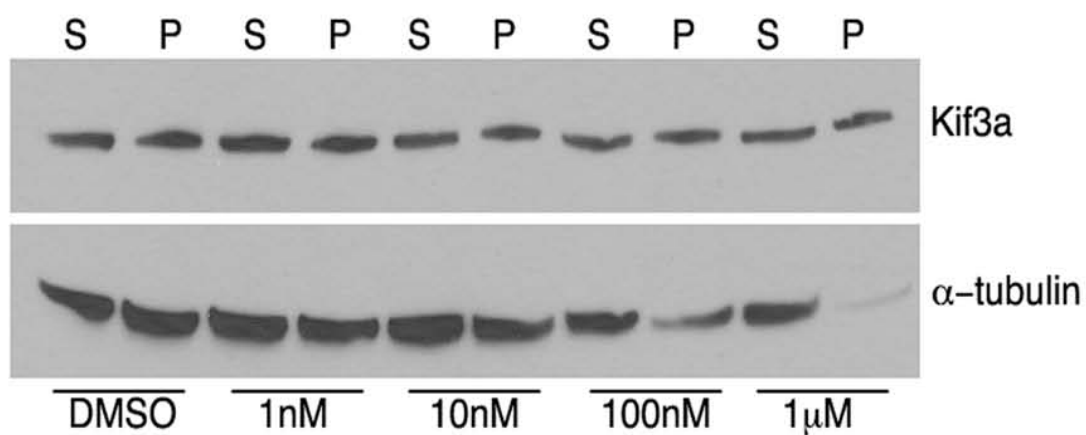
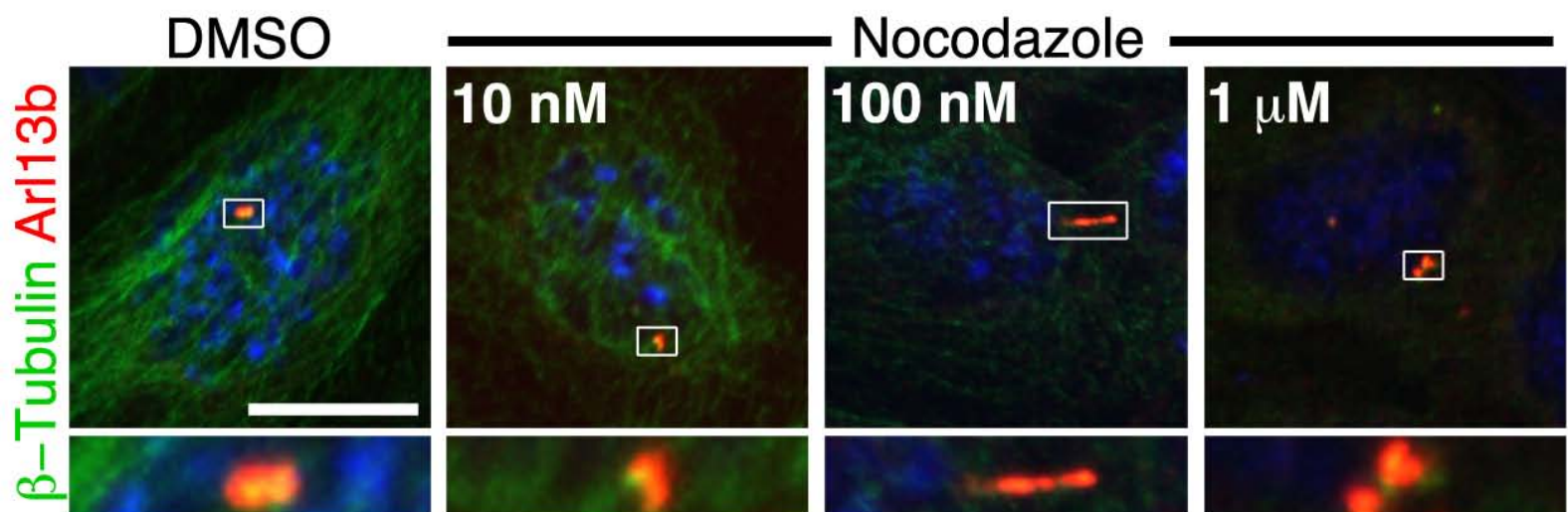
**B**

Ac.Tubulin



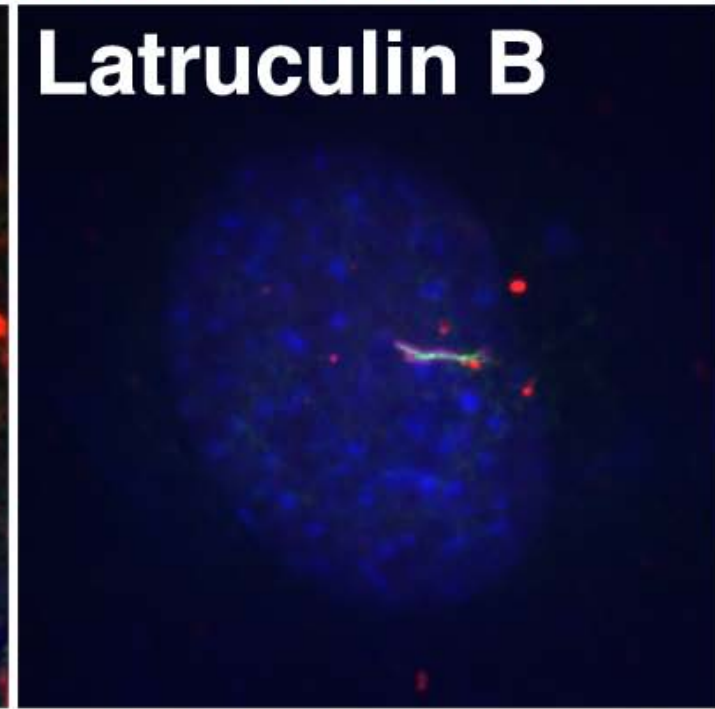
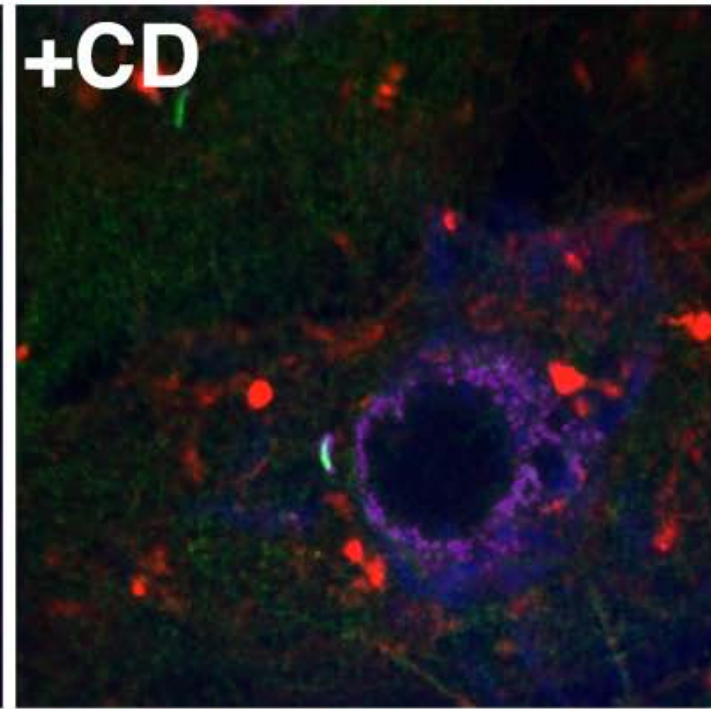
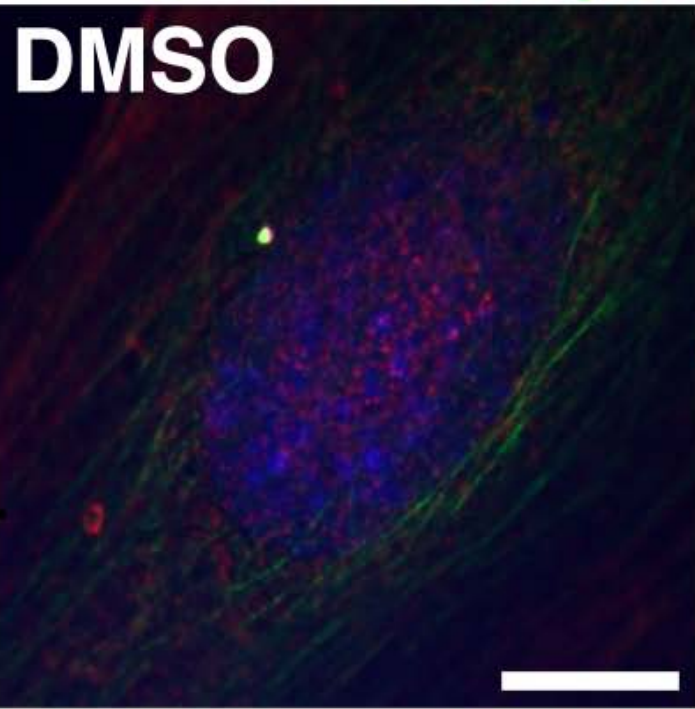
# Ac. Tubulin Actin



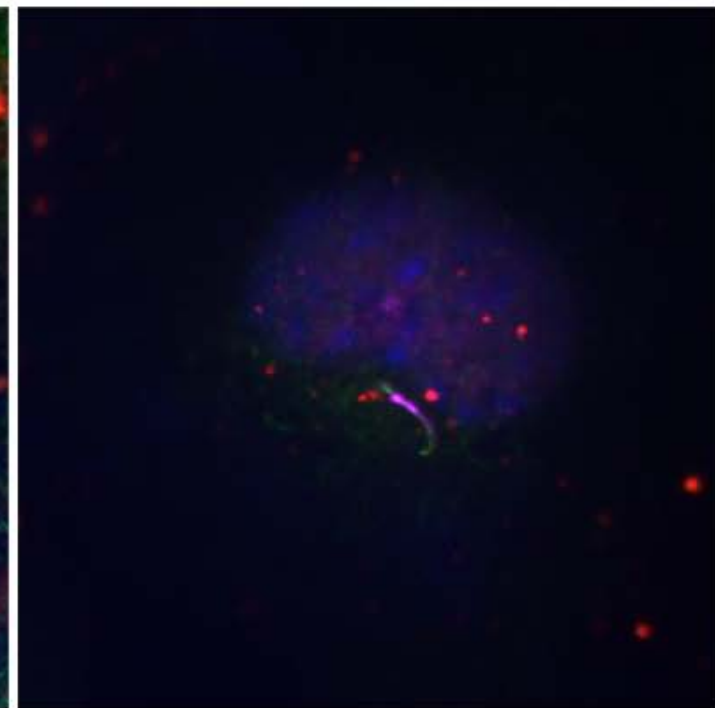
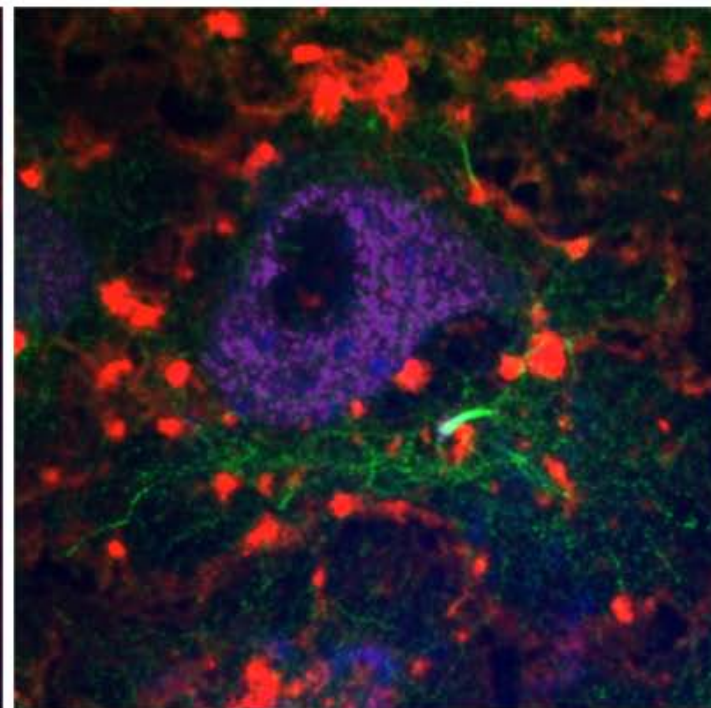
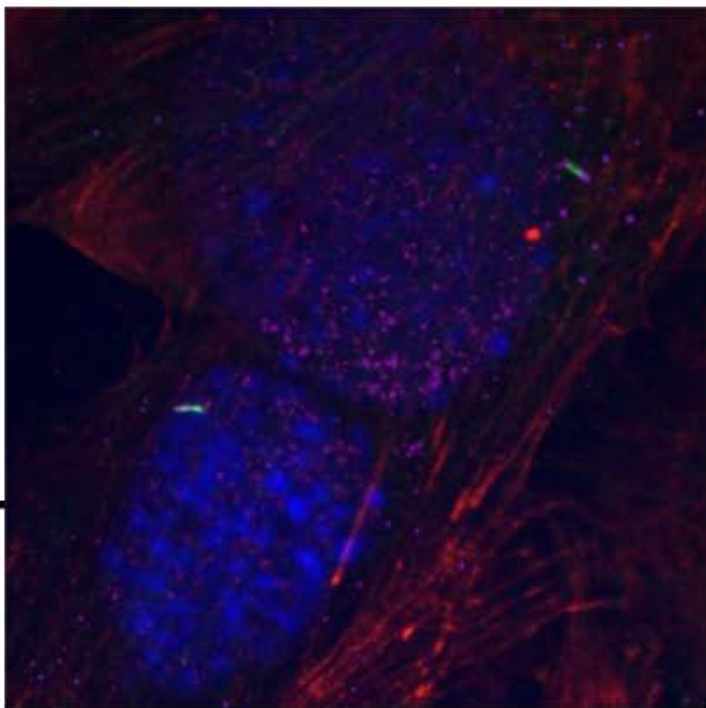


IFT88/Acetylated tubulin/Rhodamine Phalloidin

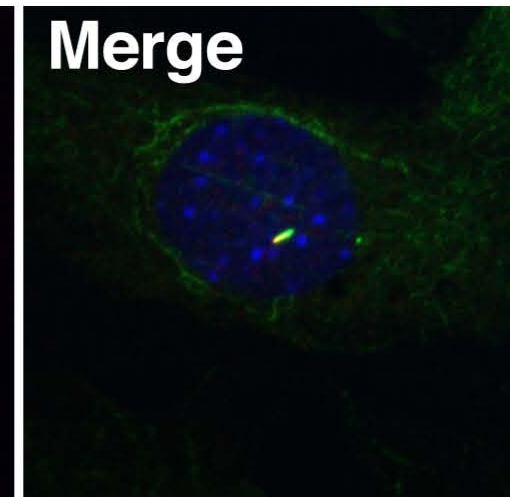
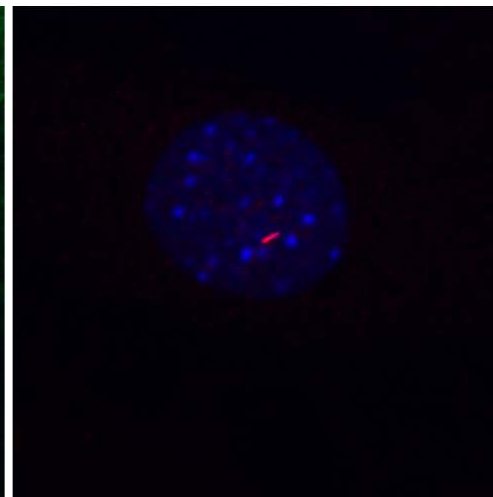
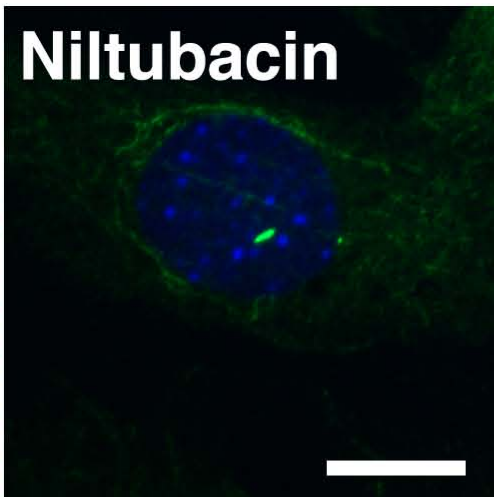
Orpk mutant



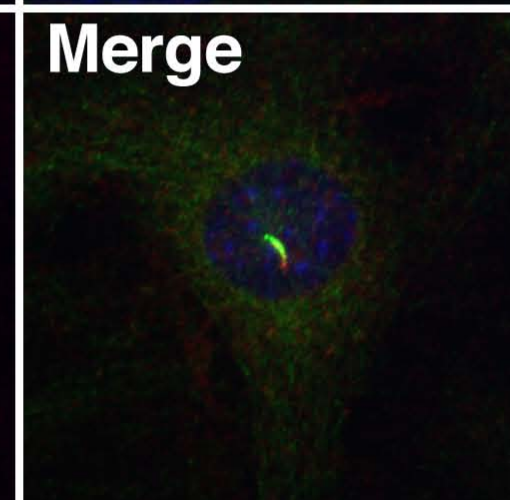
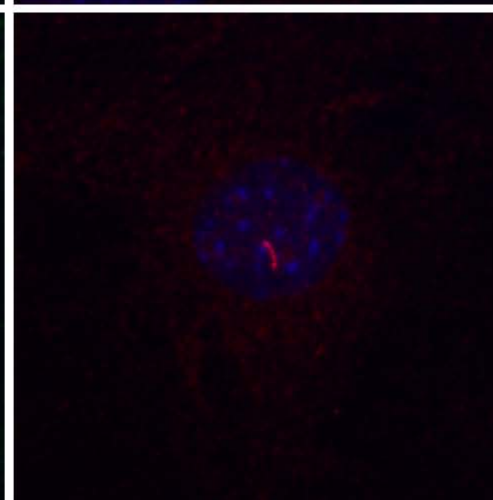
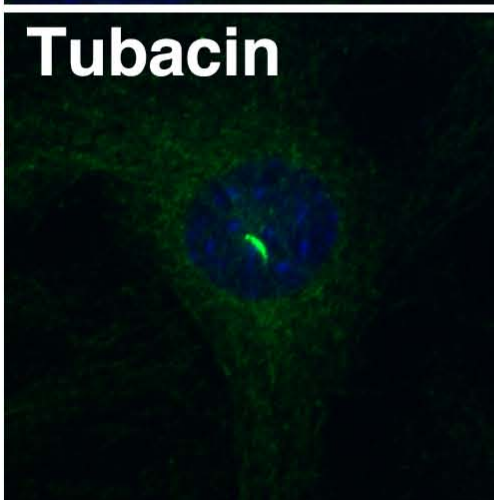
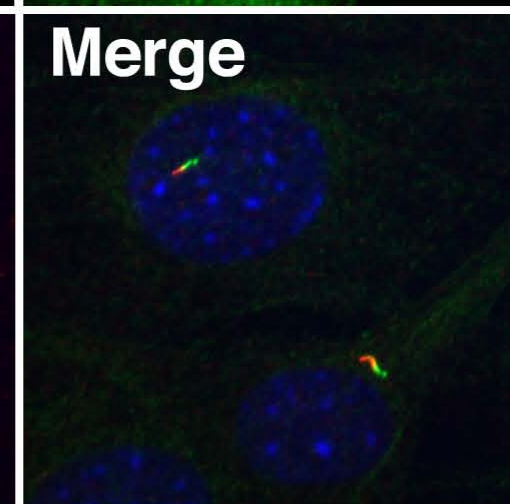
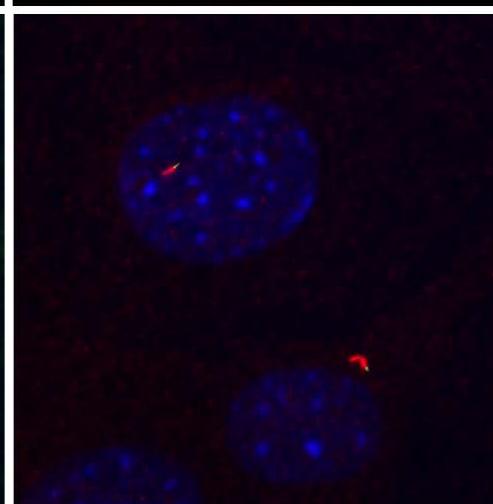
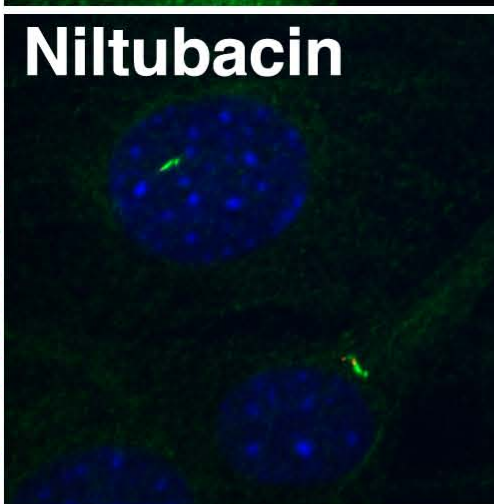
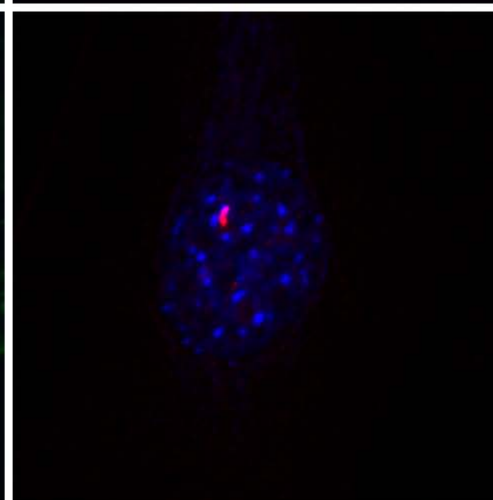
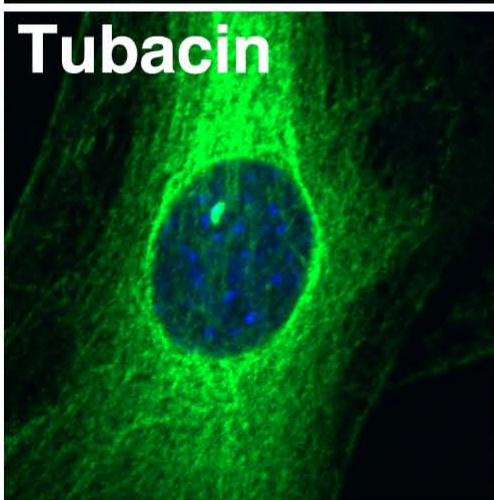
Orpk rescue

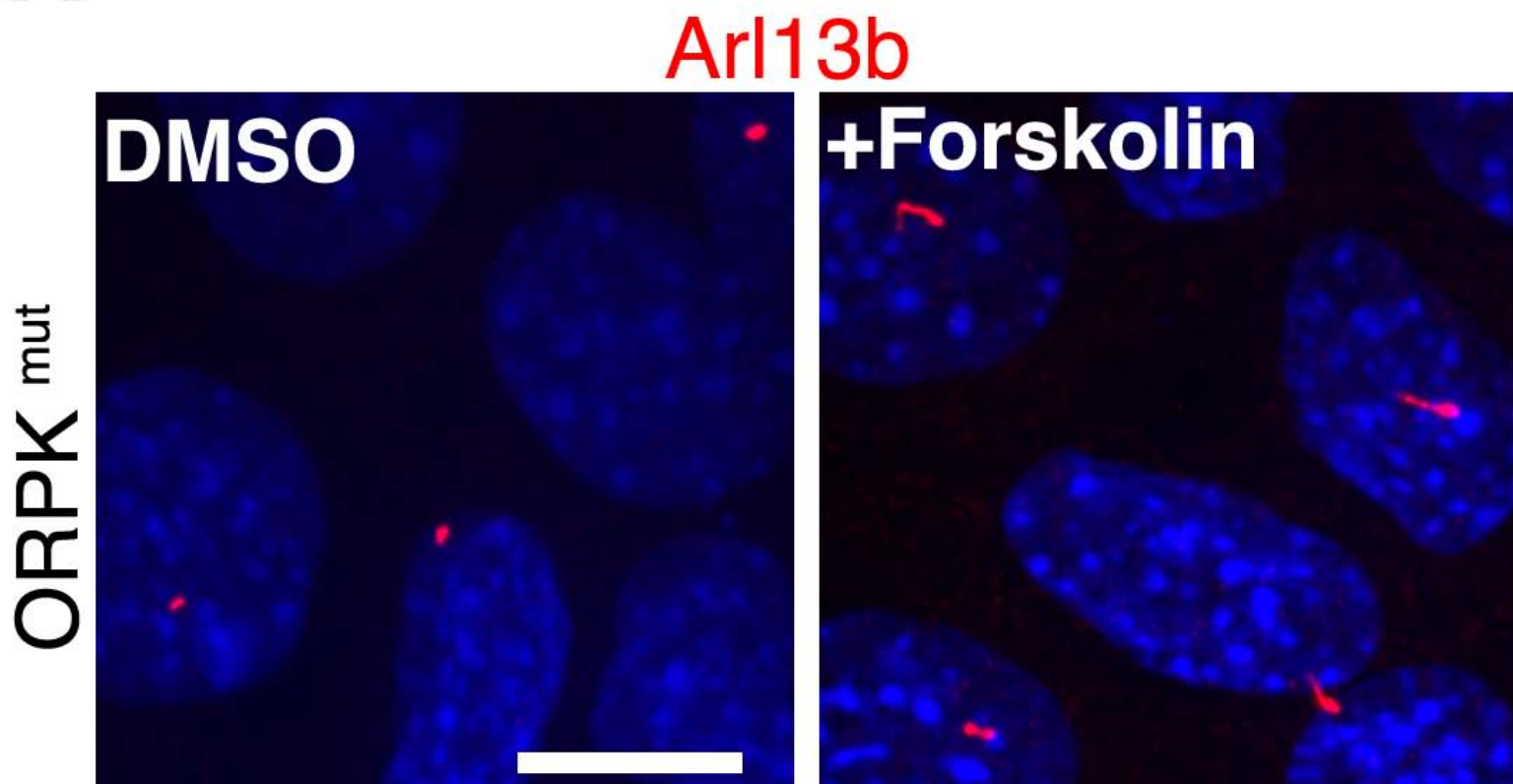
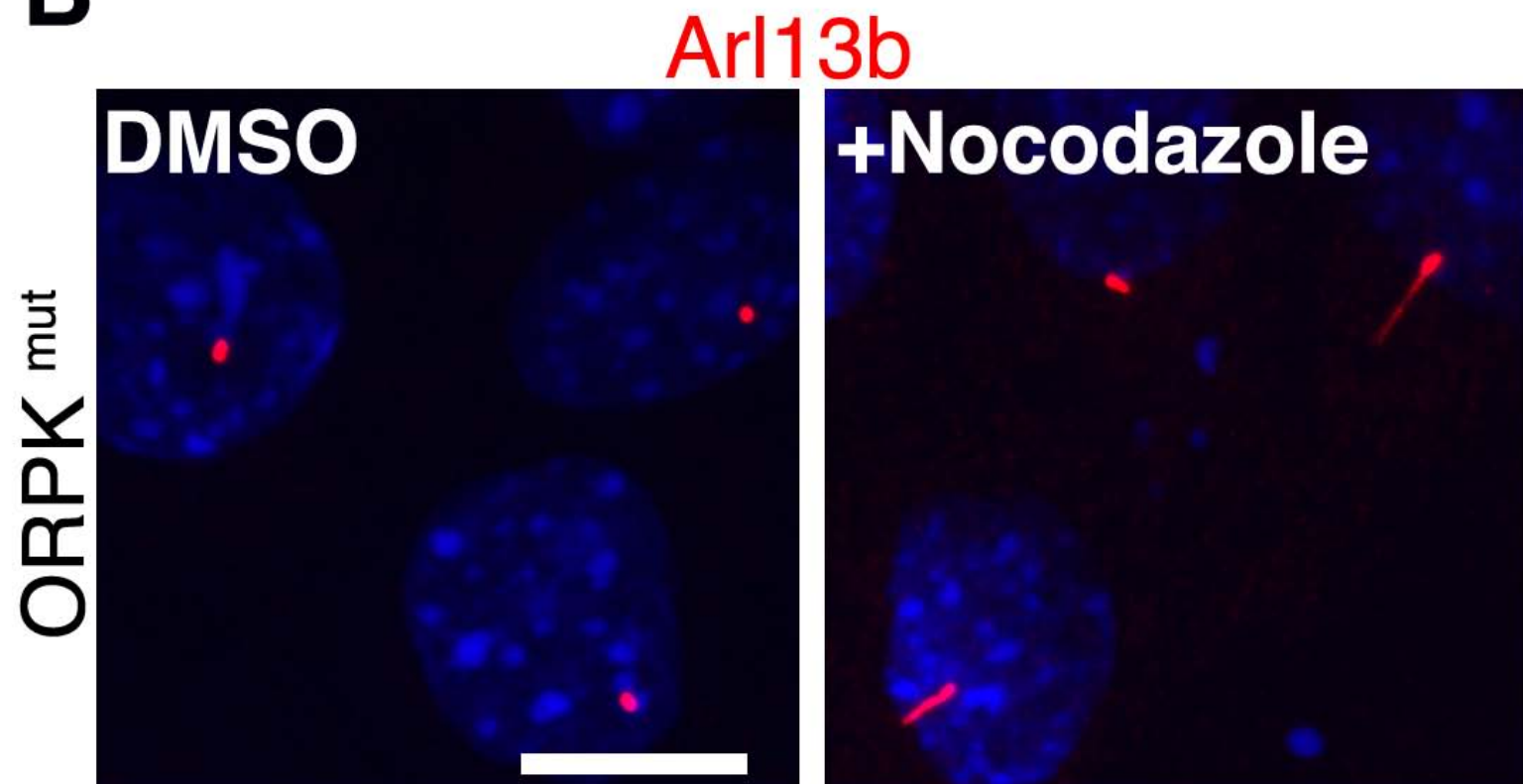


Ac.Tubulin Arl13b



GT335 Arl13b



**A****B**

## **Supplementary Information**

### **Supplementary figure legends**

#### **Figure S1: Actin depolymerization results in multiciliated cells and elongation of cilia**

**A.** Representative confocal images are shown for multiciliated and multinucleated IMCD cells when treated with 1  $\mu$ M CD for 24 hrs. Cells were fixed and stained with Rhodamine-phalloidine (red) and anti-acetylated tubulin antibody (green). The distribution of multiciliated cells was non-uniform. Only multinucleated cells exhibit multiple cilia suggesting there are defects in cytokinesis in dividing cells. Serum starved non-CD treated cells did not show multiple cilia defects (data now shown). Scale bar 15  $\mu$ m and nuclei stained blue with Hoechst. **B.** Wild type mouse embryonic fibroblasts (MEFs) were treated with DMSO and 1  $\mu$ M CD for 3 hrs and immunolabeled with anti-acetylated tubulin antibody (green). CD treatment resulted in longer cilia. Scale bar 15  $\mu$ m and nuclei stained blue with Hoechst.

#### **Figure S2: Forskolin does not affect actin cytoskeleton**

IMCD cells were treated with forskolin (0.05 mM) for 2 hrs and stained with anti-actin (red) and anti-acetylated tubulin (Cy5; purple) antibodies. Forskolin results in increase in cilia length. Actin filaments appear to be unaffected in forskolin treated cells. Scale bar 10  $\mu$ m and nuclei stained blue with Hoechst.

#### **Figure S3: Nocodazole treatment result in elongation of primary cilia.**

Kif3a<sup>fl/fl</sup> renal collecting duct or IMCD (data not shown) were treated with different concentrations of nocodazole for 2 hrs at 37 °C. Cells were fixed with -20 °C methanol and stained with anti- $\beta$ -tubulin (green) and anti-Arl13b (red). 100 nM nocodazole treatment causes increase in cilia length. DMSO and 10 nM nocodazole treatments do not affect cilia length. 1  $\mu$ M nocodazole treatment causes loss of cilia along with complete depolymerization of cell body

microtubules. Scale bar 15  $\mu\text{m}$  and nuclei stained blue with Hoechst. Western blot and quantification of  $\alpha$ -tubulin band intensities from cells treated with different doses of nocodazole for 3 hrs. Soluble and polymerized fractions were probed with anti- $\alpha$ -tubulin antibodies. Blot was reprobed with anti-Kif3a antibodies (as a loading control) to quantify  $\alpha$ -tubulin signal intensity in all respective fractions. Relative amount of tubulin present in soluble and polymerized fractions in each treatment is represented by bar graph.

**Figure S4: Actin-depolymerization rescues short stunted cilia phenotype in the hypomorphic *ift88<sup>orpk</sup>* mutants**

Hypomorphic *ift88<sup>orpk</sup>* mutant and rescued collecting cell lines were treated with CD (1  $\mu\text{m}$ ) or Latruculin B (0.5  $\mu\text{m}$ ) and processed for immunofluorescence using anti-IFT88 (Cy5; purple), anti-acetylated tubulin (green), and Rhodamine-phalloidine (red). Scale bar 10  $\mu\text{m}$  and nuclei stained blue with Hoechst. IFT88 localization in the nucleus is nonspecific as it is also present in IFT88 null mutant cell lines (Haycraft et al. 2005).

**Figure S5: Increase in tubulin acetylation does not affect cilia length or ciliogenesis**

MEFs were treated with Niltubacin (1  $\mu\text{M}$ ) and Tubacin (1  $\mu\text{M}$ ) in DMSO for 4 hrs at 37  $^{\circ}\text{C}$ . Cells were fixed and stained with anti-acetylated  $\alpha$ -tubulin (611B1 in green) and anti-Arl13b (red) antibodies or with anti-polyglutamylated tubulin (GT335 in green) and anti-Arl13b (red) antibodies. Note increase in acetylation signal (green) in Tubacin treated cells, which can be detected by 611B1 antibody and presence of cilia (in green and red). No significant changes in GT335 signal were detected on cell body and ciliary microtubules when cells were treated with Tubacin. Scale bar 15  $\mu\text{m}$  and nuclei stained blue with Hoechst.



**Figure S6: Forskolin and nocodazole treatment rescues short stunted cilia phenotype in the hypomorphic *ift88<sup>orpk</sup>* mutants**

**A.** Hypomorphic *ift88<sup>orpk</sup>* collecting cell lines were treated with forskolin (0.05 mM) for 2 hrs and processed for immunostaining with anti-Arl13b (red) antibody. **B.** Hypomorphic *ift88<sup>orpk</sup>* collecting cell lines were treated with 100 nM nocodazole for 2 hrs and processed for immunostaining with anti-Arl13b (red) antibody. Note increase in cilia length when treated with forskolin or nocodazole. Scale bar 10  $\mu$ m and nuclei stained blue with Hoechst.

## **Supplementary Tables**

### **Supplementary Table 1**

Taxol/CD comparison	$P_{calc}$	Significant for cilia length Critical $P$ value for Significance
CD::Taxol	1.02E-26	0.0083
CD:: (Taxol+CD)	1.38E-22	0.0100
CD:: DMSO	1.45E-16	0.0125
DMSO:: (Taxol)	2.27E-09	0.0167
DMSO:: (Taxol+CD)	2.07E-06	0.0250
(Taxol+CD):: Taxol		No significant difference

### **Supplementary Table 2**

Taxol/CD comparison	$P_{calc}$	Significance for ciliated cells count Critical $P$ value for significance
CD:: (Taxol+CD)	1.36E-10	0.0083
CD:: Taxol	3.38E-08	0.0100
DMSO:: (Taxol+CD)	5.17E-08	0.0125
DMSO:: Taxol	4.28E-06	0.0167
(Taxol+CD):: Taxol		No significant difference
CD:: DMSO		No significant difference

### **Supplementary Table 3**

Taxol/Forskolin comparison	$P_{calc}$	Significance for cilia length Critical $P$ value for significance
Forskolin:: Taxol	1.58E-34	0.0083
Forskolin:: DMSO	3.58E-27	0.0100
Forskolin:: (Taxol+Forskolin)	6.48E-14	0.0125
DMSO:: Taxol	4.76E-07	0.0167
(Taxol+Forskolin):: Taxol	2.33E-05	0.0250
(Taxol+Forskolin):: DMSO		No significant difference

### **Supplementary Table 4**

Taxol/Forskolin comparison	$P_{calc}$	Significance for ciliated cells count Critical $P$ value for significance
Forskolin:: Taxol	1.25E-10	0.0100
Forskolin:: (Taxol+Forskolin)	6.48E-14	0.0125
DMSO:: Taxol	4.76E-07	0.0167
(Taxol+Forskolin):: DMSO	2.33E-05	0.0250
(Taxol+Forskolin):: Taxol		No significant difference
DMSO:: Forskolin		No significant difference

### **Supplementary Table 5**

KT5720/Forskolin comparison	$P_{\text{calc}}$	Significance for cilia length Critical $P$ value for significance
Forskolin::DMSO	5.22E-020	0.0083
Forskolin::KT5720	4.99E-015	0.0100
(Forskolin+KT5720)::KT5720	4.99E-015	0.0125
Forskolin:(Forskolin+KT5720)	2.09E-007	0.0167
(Forskolin+KT5720)::DMSO	4.43E-006	0.0250
KT5720::DMSO		No significant difference

### **Supplementary Table 6**

KT5720/CD comparison	$P_{\text{calc}}$	Significance for cilia length Critical $p$ value for significance
CD::DMSO	3.08E-040	0.0083
(CD+KT5720)::DMSO	1.51E-038	0.0100
CD::KT5720	1.44E-033	0.0125
(CD+KT5720)::KT5720	2.40E-033	0.0167
(CD+KT5720)::CD		No significant difference
KT5720::DMSO		No significant difference

### **Supplementary Table 7**

KT5720/CD comparison	$P_{\text{calc}}$	Significance for ciliated cells count Critical $p$ value for significance
KT5720::DMSO	3.0E-04	0.0083
(CD+KT5720)::DMSO	1.4E-04	0.0100
		No other differences are significant