## **Supplementary Information**

# Fallopian tube rheology regulates epithelial cell differentiation and function to enhance cilia formation and coordination

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# **Supplementary Figures**



**Supplementary Figure 1** | (a) Total number of cells aspirated when replacing the culture media, and (b) percentage of viability of the aspirated cells at the 72-hour timepoint. All data are represented as mean  $\pm$  s.d. and analyzed using one-way ANOVA with Tukey post hoc testing (n = 9 images from three biological replicates per each condition). Source data are provided as a Source Data File.



Supplementary Figure 2 | Comparison of the effects of culture media viscosity between well plate and transwell cultures. (a) Representative immunofluorescent images of FTEC 72 hours post-seeding in transwell, where the green stain indicates the cilia and the blue stain indicates nuclei. Scale bars,  $30 \,\mu\text{m}$ . (b) The proportion of ciliated cells as a function of viscosity, and (c) cilia length at 1 mPa·s and 200 mPa·s for cells cultured in well plates compared to transwell systems, after 72 hours of culture. (d) Cell density (number of cells per cm<sup>2</sup>) for cells culture in transwell systems, and (e) nucleus size at 1 mPa·s and 200 mPa·s for cells cultured in well plates compared to transwell systems, after 72 hours of culture. Data are represented as mean  $\pm$  s.d. and analyzed using one-way ANOVA with Tukey post hoc testing in **b** and **d**, and using student t-test in **c** and **e**,  $*P \leq 0.05$ ,  $**P \leq 0.01$ ,  $***P \leq 0.001$  and  $****P \leq 0.001$ .  $n \geq 200$  cells analyzed in **b** and **d**, and  $n \geq 20$  cells analyzed in **c** and **e**, from three biological replicates per each condition. Source data are provided as a Source Data File.



**Supplementary Figure 3** | Fourier transforms analysis performed to obtain cilia beating frequency and its corresponding phase angle. (a) A bright field image of cilia beating and its corresponding pixel intensity is computed. (b) Cilia beating frequency is calculated by performing Fast Fourier transform on each pixel intensity. (c) Noise (area without cilia movement) was removed using 3×3 kernel to make a mask used in further analysis. (d) Phase angle was extracted from the complex Fourier output.



**Supplementary Figure 4** | Representative images of frequency patches obtained by segmentation at (a) 1 mPa·s, (b) 50 mPa·s, (c) 100 mPa·s, and (d) 200 mPa·s.



**Supplementary Figure 5** | Standard deviation for changes in phase angle as a function of viscosity. Data are represented as mean  $\pm$  s.d. and analyzed using one-way ANOVA with Tukey post hoc testing,  $*P \leq 0.05$  and  $***P \leq 0.001$ .  $n \geq 35$  images were analyzed from three biological replicates per each condition. Source data are provided as a Source Data File.

#### а

#### 1 mPa.s

mean phase = 3.14, sv = 0.03	mean phase = 2.93, sv = 0.75	mean phase = 0.33, sv = 0.34	mean phase = 1.38, sv = 0.51	mean phase = 1.02, sv = 0.75	mean phase = 0.44, sv = 0.17
150 800 30	150 90 60 150 100 30	150 90 60 40 30	150 90 60 30	150 90 60 30	150 90 6030
180 0	180	180 0	180 0	180 0	180 6 0
<sup>210</sup> / <sub>240270300</sub>	<sup>219</sup> 240 <sub>270</sub> 300	<sup>210</sup> 240270300	<sup>210</sup> 240270300	<sup>2</sup> 240 <sub>270</sub> 300	<sup>210</sup> 240270300
mean phase = 1.04, sv = 0.31	mean phase = 4.07, sv = 0.20	mean phase = 0.70, sv = 0.60	mean phase = 5.66, sv = 0.75	mean phase = 4.35, sv = 0.37	mean phase = 1.86, sv = 0.63
150 90 60 30	120 90 60 150 80 30	150 90 6930	150 396 30	150 20 690	120 90 60 150 160 30
180 🏶 0	180	180 0	180 100 0	180 🙀 0	180 🌱 0
<sup>210</sup> 2703880	<sup>2</sup> 240 <sub>270</sub> 3080	<sup>2</sup> 2402703030	<sup>2</sup> 1902703080	<sup>2</sup> 1902703030	<sup>2</sup> 240 <sub>270</sub> 3880
mean phase = 2.18, sv = 0.76	mean phase = 4.57, sv = 0.54	mean phase = 1.20, sv = 0.26	mean phase = NaN, sv = NaN	mean phase = 4.68, sv = 0.06	mean phase = 1.01, sv = 0.08
120 90 60 150 dm 30	120 800 30	120 90 60 30	150 0 8 30	120 90 60 30	120 90 60
180 🎒 0	180 0	180 0	180 64 0	180 😰 0	180 🧬 0
<sup>2</sup> 240270300	21240270300	22402703000	<sup>2</sup> 2402703030	<sup>2</sup> 240270300	<sup>2</sup> 2402703030
mean phase = 4.37, sv = 0.60	mean phase = 3.39, sv = 0.04	mean phase = 0.33, sv = 0.81	mean phase = 3.39, sv = 0.22	mean phase = 3.51, sv = 0.55	mean phase = 2.77, sv = 0.34
120 90 60	120 90 60 150 60 30	120 90 60 150 200 30	120 90 60 150 49 30	120 90 60	120 90 60 150 80 30
180 100 0	180 0	180 0	180 0	180 🗩 0	180 0
219 3330	<sup>219</sup> 220 <sub>270</sub> 300	<sup>210</sup> 240270300	<sup>210</sup> / <sub>240270300</sub>	<sup>2</sup> 190270300	<sup>210</sup> 240270300
mean phase = 3.02, sv = 0.72	mean phase = 3.76, sv = 0.92	mean phase = 3.26, sv = 0.64	mean phase = 1.31, sv = 0.33	mean phase = 3.02, sv = 0.09	mean phase = 3.60, sv = 0.42
120 90 60	120 90 60 150 80 30	120 90 60 150 600 30	150 20 30	120 90 60	120 90 60 150 100 30
180 0	180 0	180 💭 0	180 0	180 0	180 0
<sup>2</sup> 190270300	21902703030	<sup>2</sup> <sup>1</sup> 2 <sup>1</sup> 0270300	<sup>2</sup> 19 <sub>0270308</sub> 0	22402703030	<sup>219</sup> 240270300
mean phase = 2.72, sv = 0.39	mean phase = 3.79, sv = 0.21	mean phase = 4.83, sv = 0.54	mean phase = 5.38, sv = 0.62	mean phase = 4.08, sv = 0.59	mean phase = 3.52, sv = 0.78
120 90 60	120 90 60 150 20 30	120 90 60 150 100 30	120 90 60	120 90 60 60 30	120 90 60
180 0	180 🚽 0	180 0	180 👯 0	180 0	180 0
<sup>219</sup> / <sub>240270300</sub>	210 240270 300	<sup>2</sup> 190270300	<sup>210</sup> 240270300	219 3330 240270 308	210 330 240270300
mean phase = 2.10, sv = 0.13	mean phase = 2.62, sv = 0.60	mean phase = 0.91, sv = 0.75	mean phase = 5.46, sv = 0.11	mean phase = 6.10, sv = 0.22	mean phase = 4.00, sv = 0.49
120 90 60	120 90 60 150 80 30	120 90 6030	150 90 690	120 90 60 150 60 30	120 90 60 30
180 0	180 0	180 🙀 0	180 6 0	180 🕷 0	180 0
219 3330	2190270300 <sup>2</sup>	<sup>2</sup> 190270300	<sup>210</sup> / <sub>240270300</sub>	22402703030	210 330
mean phase = 5.74, sv = 0.62	mean phase = 2.68, sv = 0.45				
120 90 60	120.90.60				
150 - 30	150 40 30				
180 20 30	150 40 30				

b

50 mPa.s

mean phase = $2.56$ , sv = $006$	@an phase = 1.65, sv = 0rt	50an phase = 3.13, sv = 0d	06an phase = 4.54, sv = 00	48an phase = 5.00, sv = 0c	164an phase = 3.83, sv = 00	<b>16</b> an phase = 3.55, sv = 0d	Man phase = 1.64, sv = 0.26
	120 90 60 150 199 30	120 90 60 150 300 30	$120 \ 90 \ 60 \ 150 \ 20 \ 30 \ 10$	120 90 60 150 509 30	$120 \begin{array}{c} 90 \\ 500 \\ 150 \end{array}$	$120 90 60 \\ 150 100 30 \\ 150 100 30$	120 90 60 150 30
180 🔛 0	180 • 0	180 0	180 0	180 0	180	180	180 0
210 330 240 270 300	210 330 240 270 300	210 330 240 270 300	210 330 240 270 300	210 330 240 270 300	210 330 240 270 300	210 330 240 270 300	210 330 240 270 300
mean phase = 2.39, sv = 0d	dan phase = 3.88, sv = 0m	10an phase = 4.39, sv = 0d	l∉an phase = 4.75, sv = 0c	64an phase = 3.68, sv = 0c	1309an phase = 1.87, sv = 00	A&an phase = NaN, sv = Na	<b>M</b> an phase = 1.90, sv = 0.75
120 90 60 150 30 180 0 210 240 270 300	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} 120 & 90 & 60 \\ 150 & 60 & 30 \\ 180 & 0 \\ 210 & 330 \\ 240 & 270 & 300 \end{array}$	$\begin{array}{c} 120 & 90 & 60 \\ 150 & 30 & 30 \\ 180 & 0 \\ 210 & 330 \\ 240 & 270 & 330 \end{array}$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
mean phase = 2.28, sv = 0d	6an phase = 5.06. sv = 0m	18an phase = 2,45, sv = 0a	44an phase = 2.43. sv = 0c	98an phase = 1.06. sv = 0c	66an phase = 3.37. sv = 0a	66an phase = 3.51, sv = 0d	0an phase = 0.07, sv = 0.45
120 90 60 150 90 180 0 210 240 270 300	120 90 60 150 40 30 180 0 210 3330 240 270 300	120 90 60 150 100 30 180 90 0 210 330 240 270 300	120 90 60 150 00 30 180 0 210 330 240 270 300	120 90 60 150 0 180 0 210 240 270 300	120 90 60 150 70 30 180 70 0 210 240 270 300	120 90 60 150 200 30 180 200 0 210 333 240 270 300	120 90 60 150 158 30 180 0 210 240 270 300
mean phase = 6.09, sv = 0d	Man phase = 1.60, sv = 0m	Vēan phase = 0.87, sv = 0d	16an phase = 5.95, sv = 0c	66an phase = 0.47, sv = 0c	186an phase = 5.81, sv = 0a	60an phase = 5.59, sv = 0d	iðan phase = 3.54, sv = 0.67
120 90 60 30 150 90 0 30 180 0 210 240 270 300	120 90 60 150 00 180 0 210 330 240 270 300	120 90 60 150 90 30 180 0 210 233 240 270 300	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	120 90 60 150 49 30 180 0 210 240 270 300	120 90 60 150 16 30 180 0 210 240 270 300	120 90 60 150 100 30 180 0 210 240 270 300	120 90 60 150 30 30 180 30 210 330 240 270 300
mean phase = 0.62, sv = 0d	<b>Se</b> an phase = 5.96, sv = 0d	58an phase = 0.26, sv = 0a	46an phase = 5.36, sv = 0c	<b>66</b> an phase = 1.76, sv = 0a	100an phase = 0.26, sv = 0d	62an phase = 4.65, sv = 0n	42an phase = 1.68, sv = 0.43
120 90 60 150 90 60 30 180 0 210 210 330	120 90 60 150 90 630 180 0 210 240 270 300	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	120 90 60 150 200 30 180 210 330 240 270 300	150 90 60 180 0 210 240 270 300	120 90 60 150 0 180 0 210 240 270 300	120 90 60 150 90 30 180 0 210 3330 240 270 300	120 90 60 150 90 30 180 0 210 330 240 270 300
mean phase = 4.58, sv = 0d	60an phase = 5.68, sv = 0d	Mean phase = 4.86, sv = 0a	0∉an phase = 5.40, sv = 0c	5ēan phase = 3.16, sv = 0c	64an phase = 4.75, sv = 0d	18an phase = 5.46, sv = 0n	tēan phase = 4.57, sv = 0.62
120 90 60 150 100 30 180 0 210 330 240 270 300	120 90 60 150 90 630 180 0 210 240 270 300	120 99060 150 900630 180 0 210 3330 240 270 300	120 90 60 150 90 60 180 0 210 240 270 300	120 90 60 150 0 180 0 210 330 240 270 300	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	120 90 60 150 90 30 180 0 210 3330 240 270 300	120 90 60 150 90 30 180 230 0 210 240 270 300
mean phase = 2.85, sv = 0d	30an phase = 2.38, sv = 0m	Méan phase = 0.30, sv = 0d	26an phase = 2.74, sv = 0c	dean phase = 4.04, sv = 0.	05		
$\begin{array}{c} 120 & 90 \\ 150 & 150 \\ 180 & 80 \\ 210 & 330 \\ 240 & 370 & 300 \end{array}$	120 90 60 30 180 0 210 240 370 300	120 90 60 150 30 180 0 210 330 240 370 300	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$			
2/0 000	2/0 000	2/0 000	2/0 000	2/0 000			

#### 100 mPa.s

mean phase = 3. 100 100 200 200 200 100 200 200	4. sv = 0.01 90 0 0 0 0 0 0 0 0 0 0 0 0 0	mean phase = 3 150° 180° 230°270 mean phase = 5 150° 180° 230°270 mean phase = 3 180° 230°270 mean phase = 4 180° 230°270 mean phase = 3 180° 230°270 mean phase = 3 180°	14, sv = 0.01 (%) (%) (%) 82, sv = 0.59 (%) (%) (%) (%) (%) (%) (%) (%)	mean phase = 1300 230022 mean phase = 1300 1300 230022 mean phase = 1300 10	3.14, sv = 0.00 0 3.300 5.22, sv = 0.68 0 0 0 0 0 0 0 0 0 0 0 0 0	mean phase = 1800 1800 1800 1800 1800 1300 1300 1300 1300 1300 10000 1000 1000 1000 1000 1000 1000 1000 100	4.76, sv = 0.50 9.95 5.22, sv = 0.83 9.95 0.0300 5.22, sv = 0.49 9.95 0.0300 0.0300 2.21, sv = 0.49 9.95 0.03000 0.0300 0.03000 0.0300 0.0300 0.0300	mean phase = $\frac{1}{150^{\circ}}$ mean phase = $\frac{1}{150^{\circ}}$	-1.23, sv = 0.52
mean phase = 3.17, sv = 0.0	<b>S</b> ean phase = 0.06, sv = 0.	70eean phase = 3.00, sv = 0.3	<b>h</b> ean phase = 2.84, sv = 0.	<b>200 n</b> Minean phase = 2.57, sv = 0.	nPa.s Mean phase = 3.13, sv = 0.3	364ean phase = 0.37, sv = 0.8	Maean phase = 5.82, sv = 0.7	%nean phase = 2.37, sv = 0.2	Maean phase = 1.24, sv = 0.61
120 90 60 150 200 30 180 90 180 90 210 330 240 270 300	120 90 60 150 30 30 180 0 210 240 270 300	120 90 60 150 30 0 180 0 210 330 240 270 300	120 90 60 150 100 30 180 0 210 330 240 270 300	120 90 60 150 48 30 180 0 210 330 240 270 300	120 90 60 150 40 30 180 0 210 330 240 270 300	120 50 60 150 50 60 180 0 210 330 240 270 300	120 99 60 150 8 30 180 0 210 240 270 300	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	120 50 60 150 0 180 0 210 330 240 270 300
mean phase = 5.82, sv = 0.7 120 90 60 150 100 30 180 90 60 180 90 60 180 90 60 210 300 240 270 300 mean phase = 5.70, sv = 0.7	ean phase = 1.57, sv = 0. 120 90 60 150 30 180 0 210 330 240 270 330 ean phase = 0.47, sv = 0.	100 120 120 120 120 120 120 120 120 120	hean phase = 4.26, sv = 0. 120 90 60 150 30 30 180 0 210 200 240 270 300 Sean phase = 4.29, sv = 0.	76hean phase = 0.71, sv = 0. 120 90 60 150 155 30 180 0 210 330 240 270 300 26hean phase = 1.82, sv = 0.	Aftean phase = 0.59, sv = 0.3 120 90 60 150 0 180 0 210 210 210 770 300 2140 2770 300 2140 2770 300 2140 277 3 v = 0.4	abean phase = 4.24, sv = 0.2 120 90 60 150 49 30 180 0 210 330 240 270 300 abean phase = 1.49, sv = 0.9	Mean phase = 1.50, sv = 0.1 120 90 90 150 30 180 0 210 330 240 270 300 26ean phase = 2.87, sv = 0.4	Inhean phase = 2.98, sv = 0.8 120 90 60 150 30 180 330 210 20 70 300 2140 270 300 Inhean phase = 2.67, sv = 0.2	teen phase = 2.34, sv = 0.71 120 50 60 150 15 30 180 0 210 330 240 270 300 teen phase = 2.22, sv = 0.63
120 90 60 150 0 180 0 210 270 300 mean phase = 1.83, sy = 0.6	120 90 60 150 30 180 0 210 330 240 270 300	120 90 60 150 0 30 180 0 210 210 270 300 thean phase = 2.74, sy = 0.7	120 90 60 150 0 210 330 240 270 300	120 90 60 150 0 30 180 0 210 330 240 270 300	120 90 60 150 90 30 180 0 210 330 240 270 300	120 90 60 150 200 30 180 0 210 270 30 240 270 30 200 270 30 200 270 30 200 270 30 200 270 30 200 270 30 200 200 200 200 200 200 200 200 200 20	120 90 60 150 90 30 180 90 0 210 330 240 270 300	120 90 60 150 100 30 180 90 240 270 300 240 270 300	120 90 60 30 150 0 210 330 240 270 300
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	120 90 60 150 60 30 180 0 210 330 240 270 300	120 90 60 150 90 30 180 0 210 330 240 270 300	120 90 60 150 0 30 180 0 210 330 240 270 300	120 90 60 150 8 30 180 0 210 330 240 270 300	120 150 180 210 240 270 300	120 90 60 150 50 30 180 0 210 330 240 270 300	120 90 60 150 900 30 180 0 210 330 240 270 300	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	120 90 60 150 10 30 180 5 0 210 330 240 270 300
120 90 60 150 100 30 180 90 60 190 30 180 90 0 210 330 240 270 300	120 90 60 150 90 60 180 0 210 330 240 270 300	120 90 60 150 20 30 180 10 0 210 330 240 270 300	120 90 60 150 200 630 180 0 210 330 240 270 300	120 90 60 150 20 30 180 10 10 210 330 240 270 300	120 % 630 180 % 630 180 % 330 210 330 240 270 300	120 90 60 150 20 30 180 0 210 330 240 270 300	120 90 60 150 30 180 0 210 330 240 270 300	120 90 60 150 70 80 180 0 210 330 240 270 300	120 90 60 150 60 180 0 210 330 240 270 300
mean phase = 0.50, sv = 0.2 120 90, 60 150 10 10 180 0 210 270 300 210 270 300	fean phase = 6.27, sv = 0.1 120 90 60 150 20 30 180 0 210 330 240 270 300	300ean phase = 0.39, sv = 0.9 120 90 60 150 0 180 0 210 330 240 270 300	2000 210 210 210 210 210 210 210 210 210	766ean phase = 4.88, sv = 0. 120 90 60 150 10 30 180 0 210 330 240 270 300	Wean phase = 4.21, sv = 0.4   120 90 60   150 9 30   180 0 330   210 240 270 300	Been phase = 3.78, sv = 0.7 120 90 60 180 0 180 0 210 330 240 270 300	The phase = 3.08, sv = 0.5 120 90 60 150 99 30 180 0 210 330 240 270 300	Schean phase = 1.60, sv = 0.5 120 50 60 150 10 180 0 210 330 240 270 300	the an phase = 2.44, sv = 0.70 120 50 60 150 30 180 0 210 270 300
mean phase = 1.05, sv = 0.0 150 0 0 0 150 0 0 0 200 70 300 mean phase = 3.60, sv = 0.2 150 0 0 150 0 0 0 200 0 0 150 0 0	€ean phase = 6.16, sv = 0. <sup>120</sup> <sup>50</sup> <sup>50</sup> <sup>50</sup> <sup>50</sup> <sup>100</sup> <sup>100</sup> <sup>100</sup> <sup>200</sup> <sup>100</sup> <sup>200</sup>	<b>Stean</b> phase = 0.09, sv = 0.7 150 90 80 180 0 240 270 300	hean phase = 2.10, sv = 0.1 120 90 60 150 10 30 180 0 210 330 240 270 300	Senean phase = 3.27, sv = 0. 120 909 150 909 150 909 160 90 180 0 240 270 300	Stean phase = 5.76, sv = 0.4   150 9 60   150 9 0   180 0 0   240 270 300	689ean phase = 4.05, sv = 0.9 150 90 60 30 180 90 90 30 240 270 300	Rean phase = 3.86, sv = 0.8 120 00 60 150 00 60 180 0 210 330 240 270 300	Stean phase = 0.57, sv = 0.7   150 90, 80   150 90, 80   160 0   210 330   240 270	thean phase = 3.76, sr = 0.55 150 90 50 150 90 50 150 100 100 100 210 200 300 210 200 300

**Supplementary Figure 6** | Representative images of wave direction computed as mean angle per patch at (a) 1 mPa·s, (b) 50 mPa·s, (c) 100 mPa·s, and (d) 200 mPa·s.

d







#### 50 mPa.s





d



**Supplementary Figure 7** | Representative images of wavelength computed as the mode magnitude per patch at (a) 1 mPa $\cdot$ s, (b) 50 mPa $\cdot$ s, (c) 100 mPa $\cdot$ s, and (d) 200 mPa $\cdot$ s.



**Supplementary Figure 8** | Average area of ciliated cells with a similar wavelength as a function of viscosity. Data are represented as mean  $\pm$  s.d. and analyzed using one-way ANOVA with Tukey post hoc testing, \**P*  $\leq$  0.05 and \*\**P* $\leq$  0.01. *n* = 9 images were analyzed from three biological replicates per each condition. Source data are provided as Source Data File.



**Supplementary Figure 9** | Cilia beating direction. (a) Cilia beat in a random manner in all directions at 1 mPa·s, while cilia beat in a coordinated and unidirectional manner at 200 mPa·s. Scale bars, 5  $\mu$ m. (b) Kymographs of cilia beating along A-A' at 1 mPa·s and along B-B' at 200 mPa·s.



**Supplementary Figure 10** | (a) Representative images of the epithelial cells stained with Mitotracker Deep Red and the corresponding (b) average fluorescent intensity per cell as a function of culture media viscosity. Cell nuclei were stained using blue-fluorescent Hoechst 33342. Scale bar,  $80 \ \mu m$ . n = 3 from three biological replicates and  $\geq 200$  cells for each condition were analyzed. Data are represented as mean  $\pm$  s.d. and analyzed using one-way ANOVA with Tukey post hoc testing,  $*P \leq 0.05$ ,  $**P \leq 0.01$ ,  $***P \leq 0.001$ ,  $***P \leq 0.0001$ . Source data are provided as a Source Data File.



**Supplementary Figure 11** | Comparing TRPV4 expression as a function of viscosity using immunofluorescent sating. Representative images of FTEC stained with a TRPV4 antibody (indicating expression of TRPV4 activity in red fluorescence) and Hoechst 33342 (indicating nuclei with blue fluorescence) for cells cultured in (**a**) 1 mPa·s, (**b**) 50 mPa·s, (**c**) 100 mPa·s, and (**d**) 200 mPa·s. Staining images with both primary and secondary antibodies are shown at the top, and cells only stained with the secondary antibody (in the absence of the primary antibody) are shown as the negative control at the bottom. Scale bars, 15  $\mu$ m.



**Supplementary Figure 12** | Comparison of TRPV4 protein expression for FTEC as a function of viscosity. (a) Representative images of the Western blot analysis, and (b) quantification of TRPV4 protein expression in FTEC at 1 mPa·s and 200 mPa·s (normalized to GAPDH). Data are represented as mean  $\pm$  s.d. and analyzed using Student's t-test,  $*P \le 0.05$  (n = 3 from three biological replicates per condition). Source data are provided as a Source Data File.



Supplementary Figure 13 | Cilia beating amplitude as a function of culture media viscosity.  $n \ge 15$  cells from three biological replicates per condition were traced manually. Data are represented as mean  $\pm$  s.d. and analyzed using one-way ANOVA with Tukey post hoc testing, \*\*\* $P \le 0.001$ , \*\*\*\* $P \le 0.0001$ . Source data are provided as a Source Data File.

## **Supplementary Table**

Supplementary Table 1 | Osmolarity of culture media across viscosity range. Values are reported as mean  $\pm$  s.d. from 3 measurements.

Viscosity (mPa·s)	Methyl cellulose (%)	Osmolarity (mOsm/kg)
1	0	312 ± 1
50	0.7	271±2
100	0.9	265 ± 2
200	1	257±4

Supplementary Table 2 | Percentage of Ki-67 positive cells at 72-hour timepoint for passage 1 and passage 3. Values are reported as mean  $\pm$  s.d.

Viscosity (mPa·s)	Ki-67 positive cells for Passage 1 (%)	Ki-67 positive cells for Passage 3 (%)
1	$75.3 \pm 4.2$	73.4 ± 3.1
50	$72.7 \pm 2.8$	69.7 ± 1.7
100	69.3 ± 1.9	65.3 ± 2.7
200	$73.3 \pm 2.6$	72.1 ± 1.7

Supplementary Table 3 | Statistical analysis of presented data. *P* values were calculated using one-way ANOVA, with Tukey post hoc testing,  $*P \le 0.05$ ,  $**P \le 0.01$ ,  $***P \le 0.001$ ,  $****P \le 0.001$  and NS denotes not significant.

Group	Viscosity (mPa·s)
а	1
b	50
с	100
d	200
e	200 + Vehicle
f	200 + RN-1734

Fig.	Parameters /	a,b	a,c	a,d	b,c	b,d	c,d	d,f	e,f
	compared groups								
1c	No of cells per cm <sup>2</sup>	0.8867	<0.0001	<0.0001	<0.0001	<0.0001	0.0218	n/a	n/a
		143							
1d	Area per cell ( $\mu m^2$ )	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	n/a	n/a
1e	Percentage of ciliated cells (%)	0.0209 *	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	n/a	n/a
4e	Average CBF (Hz)	<0.0001 ****	<0.0001 ****	<0.0001 ****	0.0290 *	0.0045 **	0.9832 NS	0.0409 *	0.0125 *
4f	Coherence	0.5018 NS	0.0468 *	<0.0001 ****	0.6960 NS	<0.0001 ****	0.0031 **	0.0022 **	n/a
5d	Low MMP/ High MMP	0.8429 NS	0.0037 **	0.0261 *	0.0102 *	0.0824 NS	0.4693 NS	n/a	n/a
5e	TRPV4 Intensity	0.7044 <i>NS</i>	0.0290 *	0.0007 ***	>0.9999 NS	0.0171 *	0.5296 NS	n/a	n/a

# **Uncropped blot for Supplementary Figure 12**

