Supplementary Information

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

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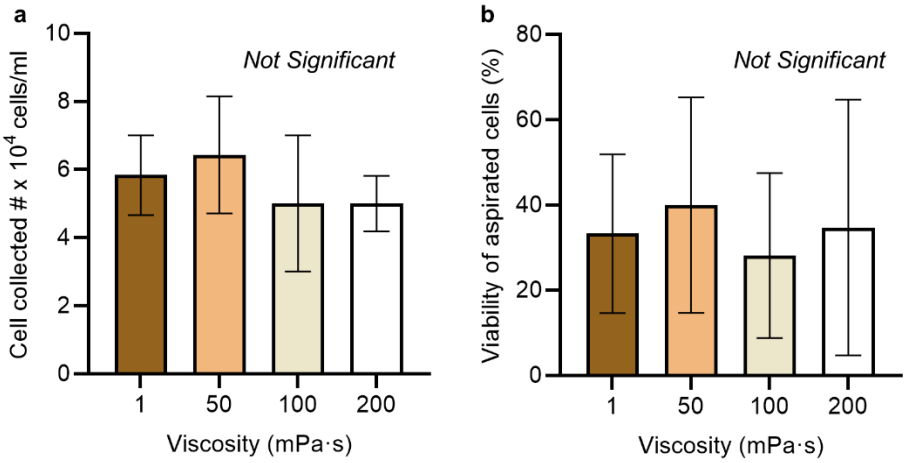
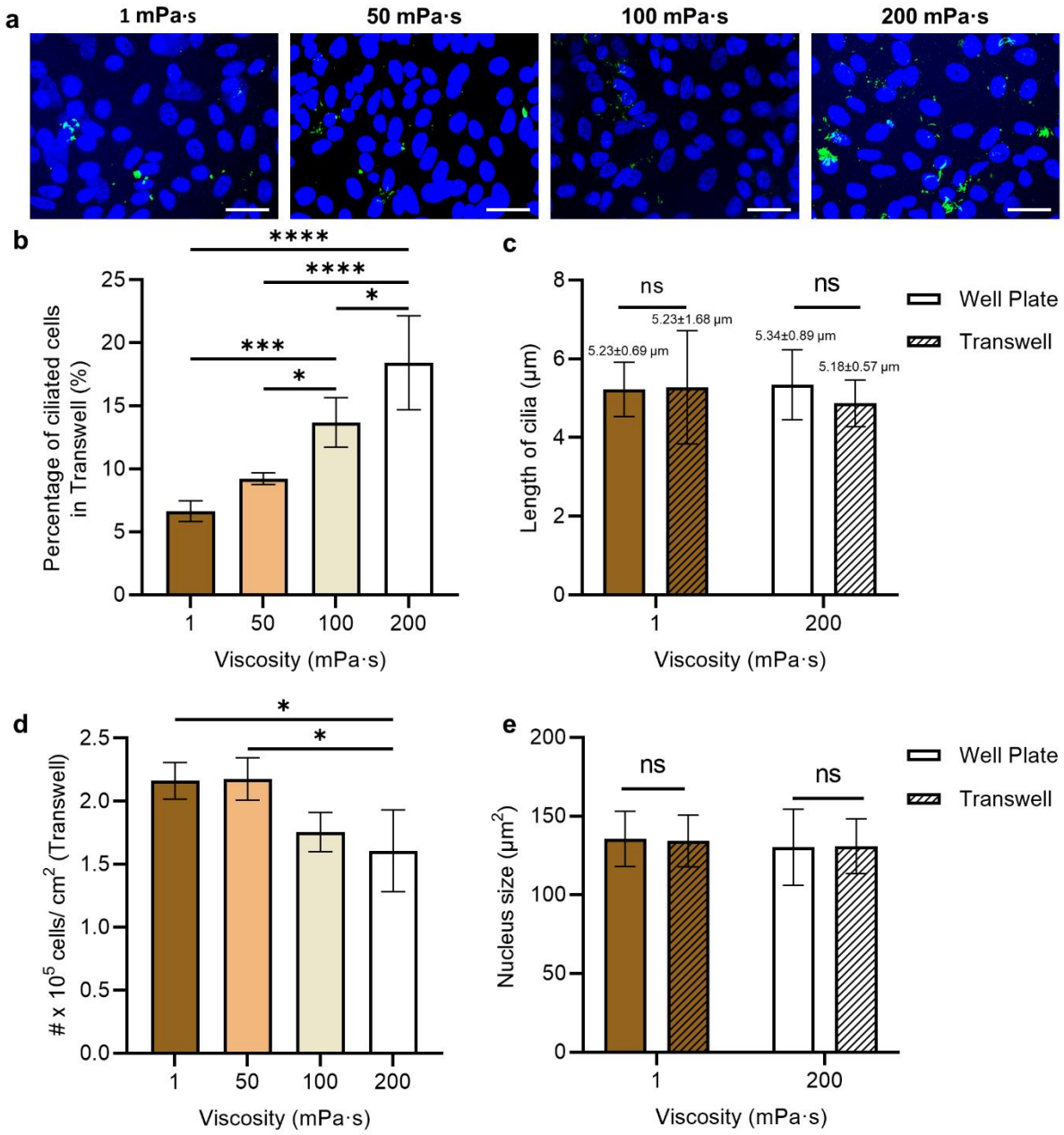
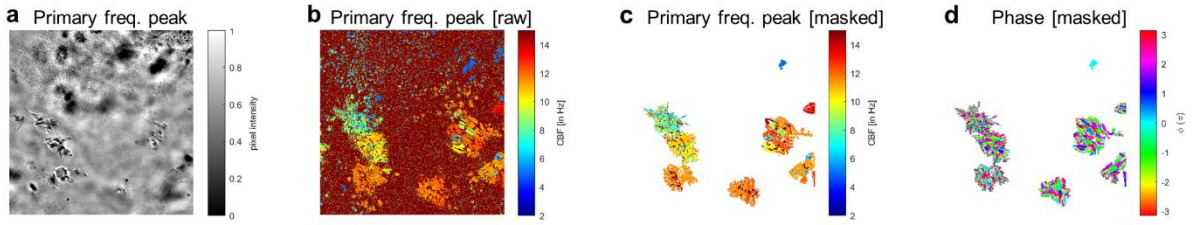
Supplementary Figures



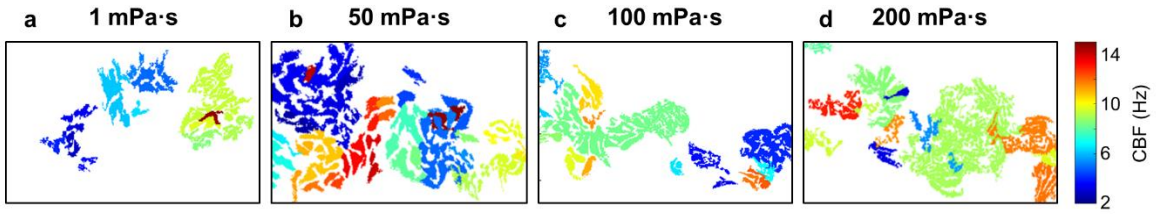
Supplementary Figure 1 | (**a**) Total number of cells aspirated when replacing the culture media, and (b) percentage of viability of the aspirated cells at the 72-hour timepoint. All data are represented as mean ± s.d. and analyzed using one-way ANOVA with Tukey post hoc testing (n = 9 images from three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



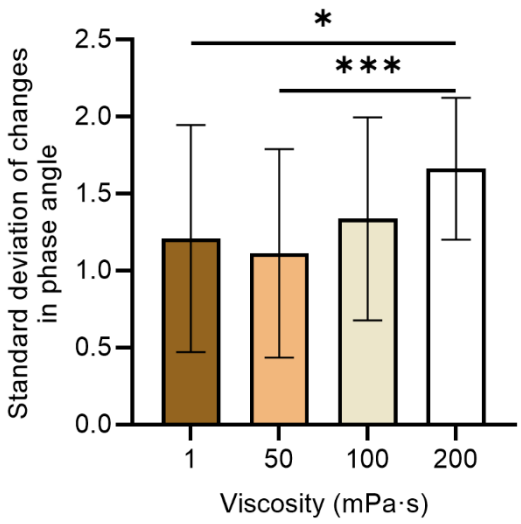
Supplementary Figure 2 | Comparison of the effects of culture media viscosity between well plate and transwell cultures. (**a**) Representative immunofluorescent images of FTEC 72 hours post-seeding in transwell, where the green stain indicates the cilia and the blue stain indicates nuclei. Scale bars, 30 µm. (**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^2) 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 ± 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 $****P \leq 0.0001$. $n \geq 200$ 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.

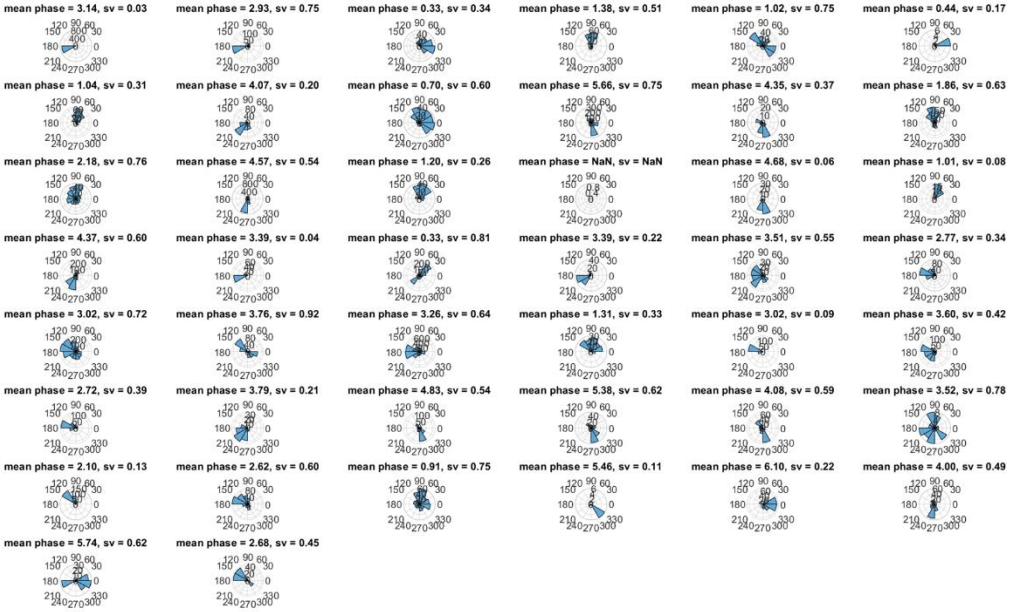


Supplementary Figure 4 | Representative images of frequency patches obtained by segmentation at (**a**) 1 mPa·s, (**b**) 50 mPa·s, (**c**) 100 mPa·s, and (**d**) 200 mPa·s.

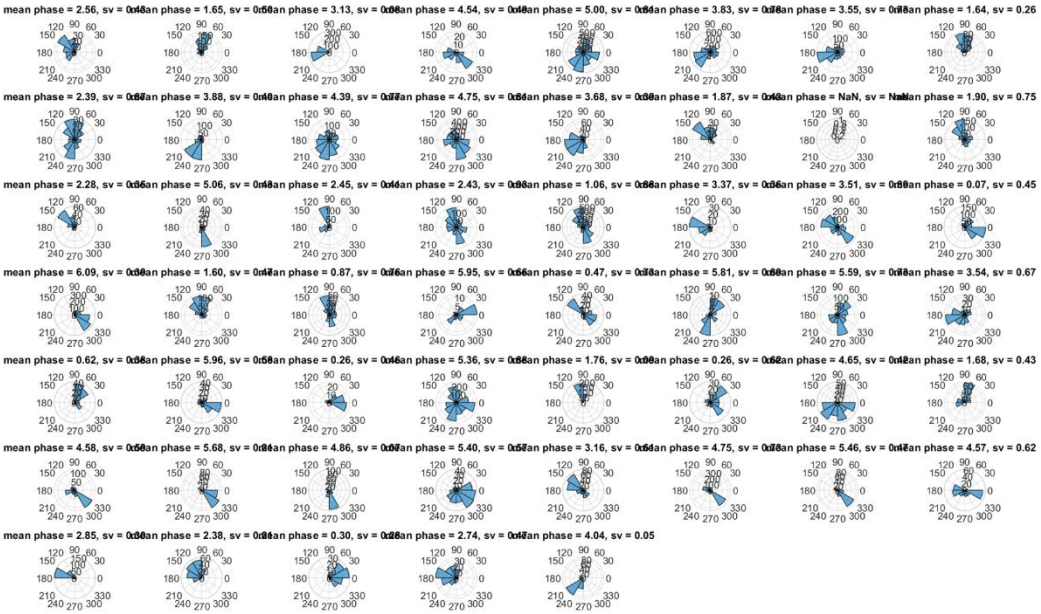


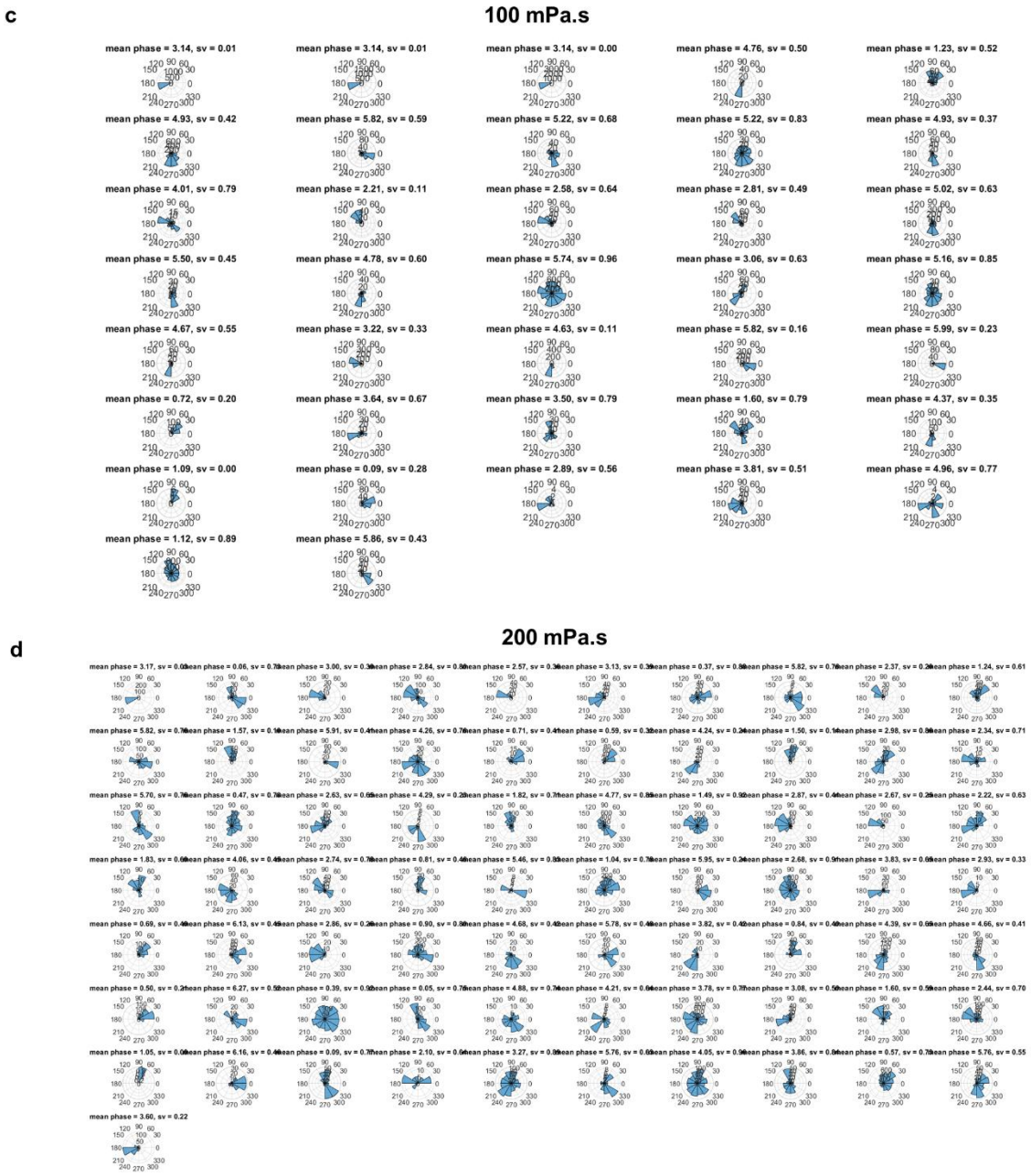
Supplementary Figure 5 | Standard deviation for changes in phase angle as a function of viscosity. Data are represented as mean ± s.d. and analyzed using one-way ANOVA with Tukey post hoc testing, $*P \leq 0.05$ and $***P \leq 0.001$. $n \geq 35$ images were analyzed from three biological replicates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

a **1 mPa.s**



b **50 mPa.s**

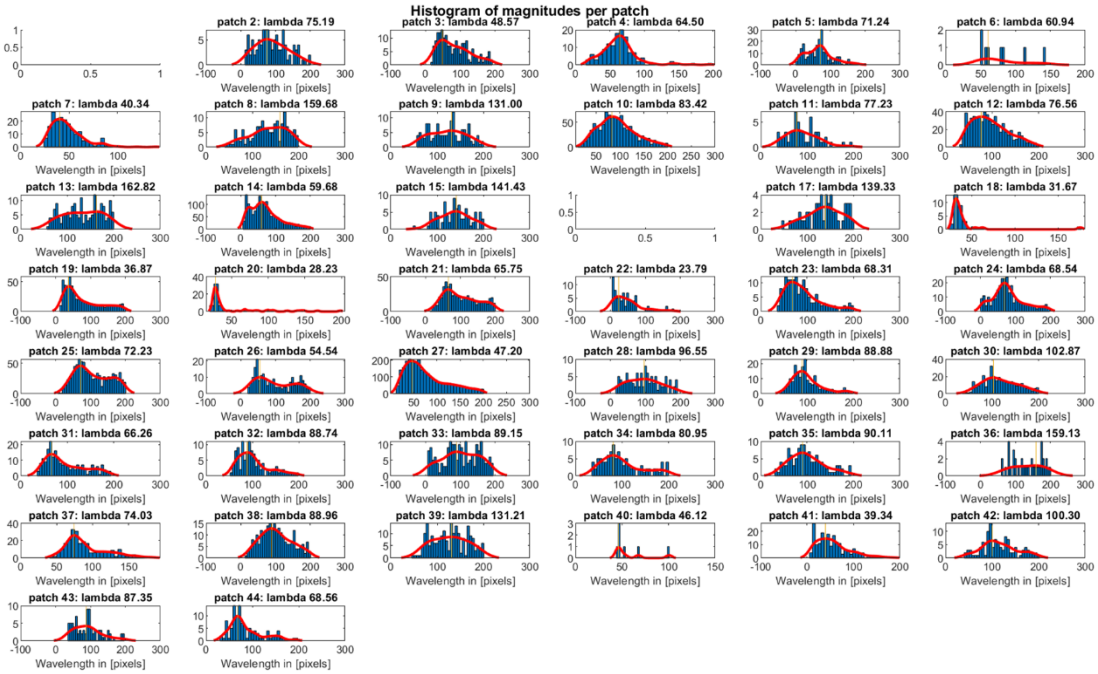




Supplementary Figure 6 | Representative images of wave direction computed as mean angle per patch at (**a**) 1 mPa·s, (**b**) 50 mPa·s, (**c**) 100 mPa·s, and (**d**) 200 mPa·s.

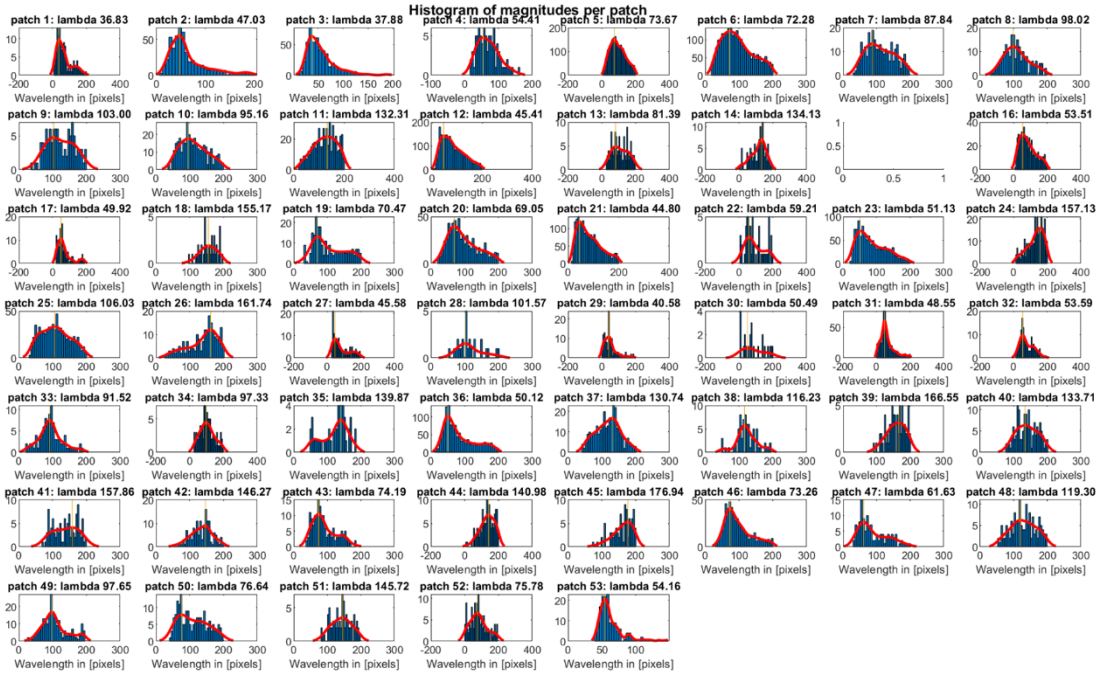
a

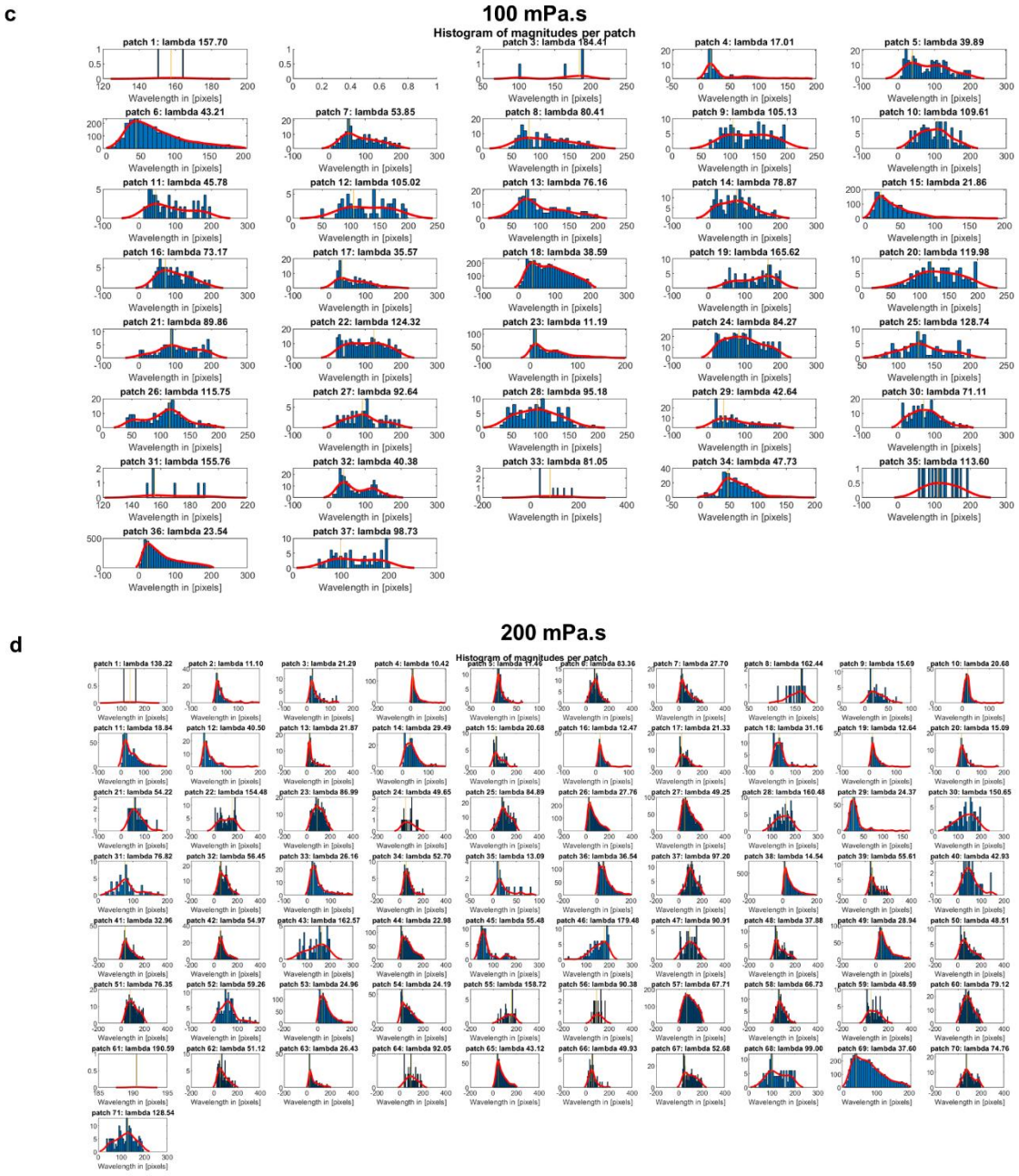
1 mPa.s



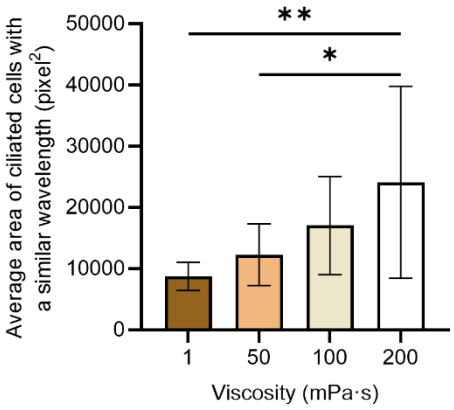
b

50 mPa.s

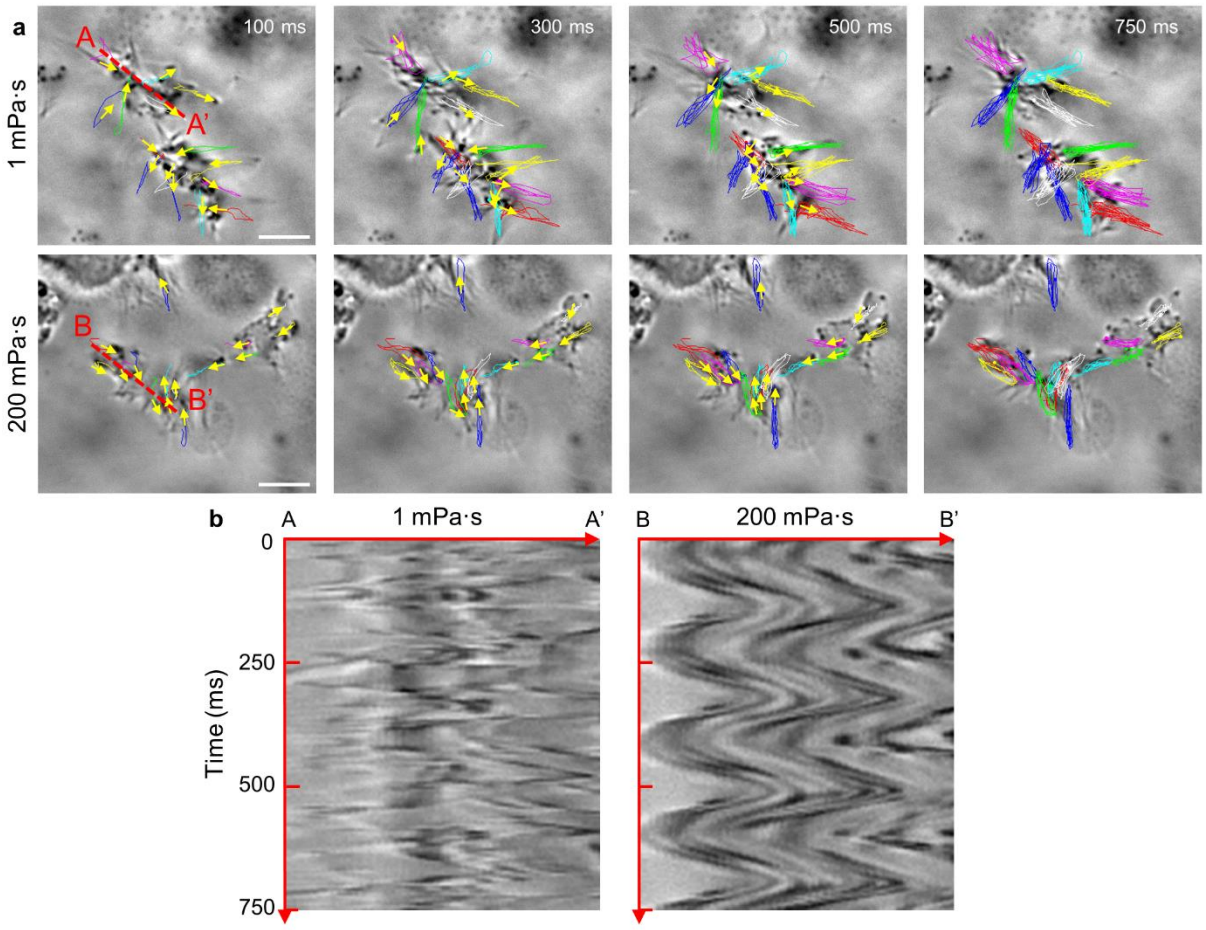




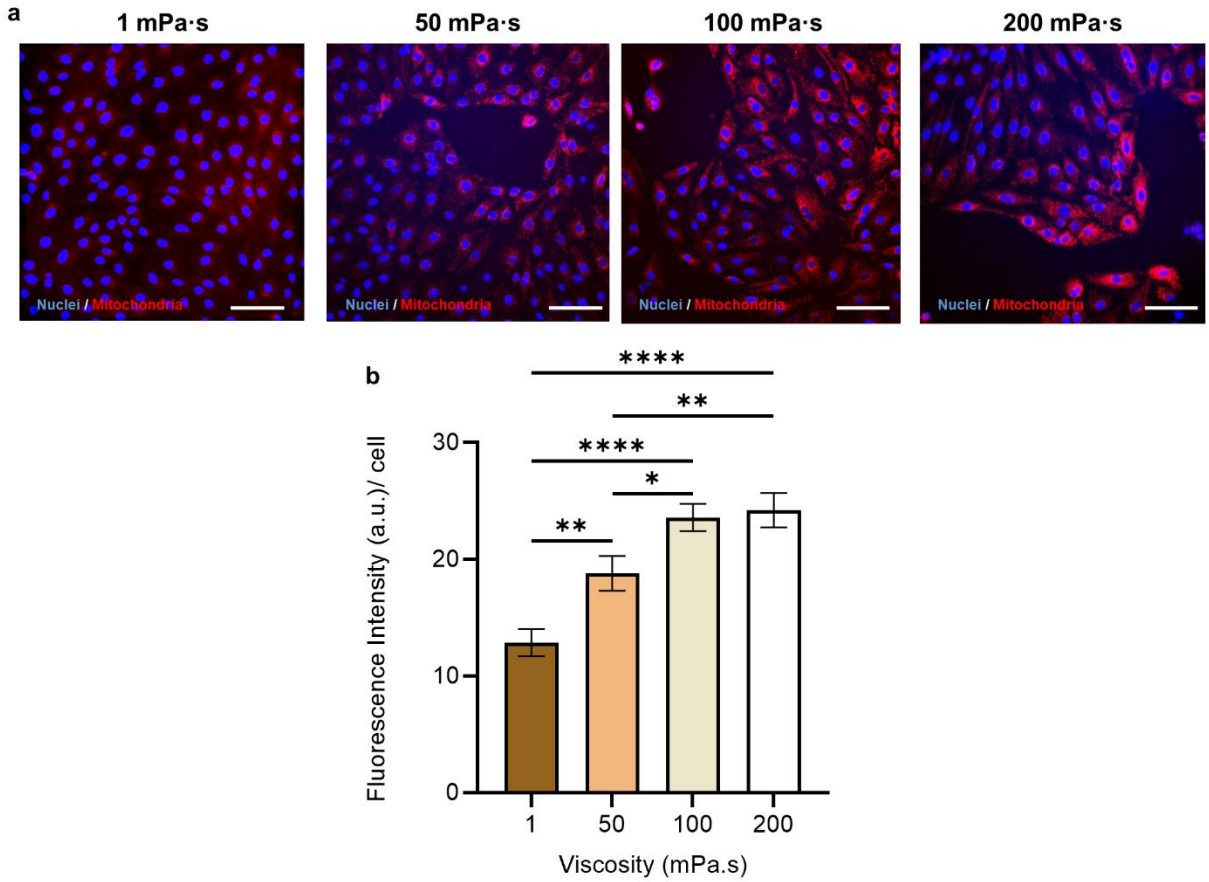
Supplementary Figure 7 | Representative images of wavelength computed as the mode magnitude per patch at (**a**) 1 mPa·s, (**b**) 50 mPa·s, (**c**) 100 mPa·s, and (**d**) 200 mPa·s.



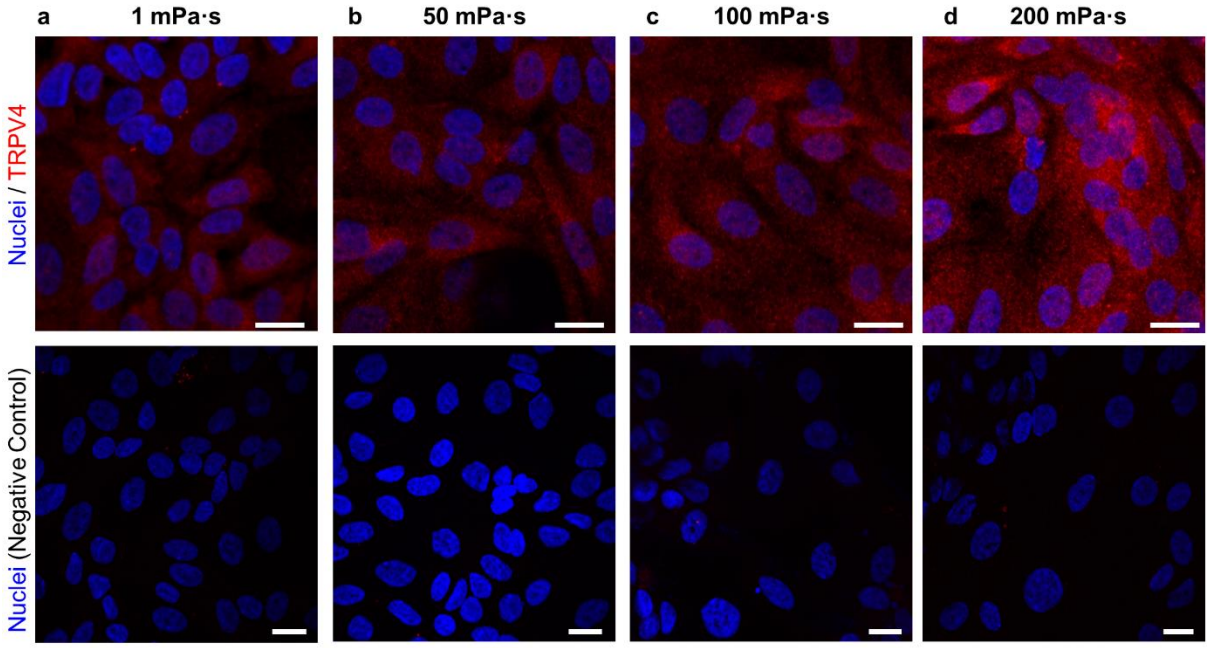
Supplementary Figure 8 | Average area of ciliated cells with a similar wavelength as a function of viscosity. Data are represented as mean ± s.d. and analyzed using one-way ANOVA with Tukey post hoc testing, $*P \leq 0.05$ and $**P \leq 0.01$. $n = 9$ images were analyzed from three biological replicates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



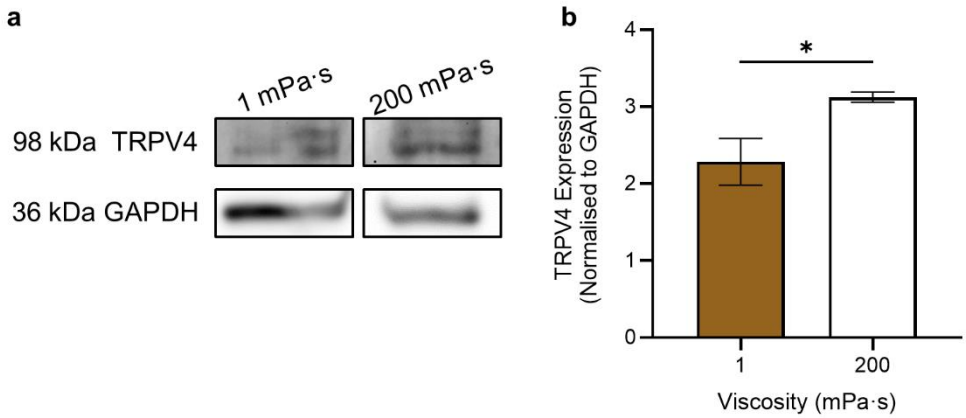
Supplementary Figure 9 | Cilia beating direction. (**a**) Cilia beat in a random manner in all directions at 1 mPa·s, while cilia beat in a coordinated and unidirectional manner at 200 mPa·s. Scale bars, 5 µm. (**b**) Kymographs of cilia beating along A-A' at 1 mPa·s and along B-B' at 200 mPa·s.



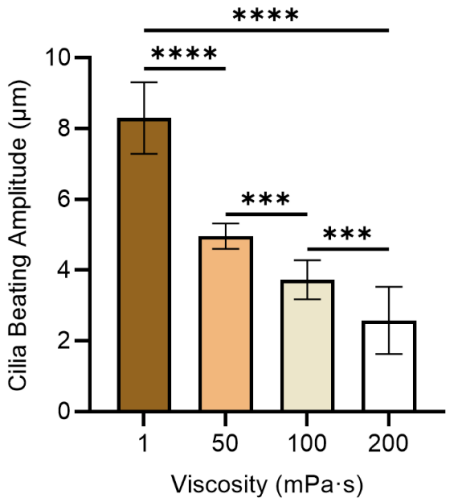
Supplementary Figure 10 | (**a**) Representative images of the epithelial cells stained with Mitotracker Deep Red and the corresponding (**b**) average fluorescent intensity per cell as a function of culture media viscosity. Cell nuclei were stained using blue-fluorescent Hoechst 33342. Scale bar, 80 µm. $n = 3$ from three biological replicates and ≥ 200 cells for each condition were analyzed. Data are represented as mean ± 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 μm.



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 ± s.d. and analyzed using Student's t-test, $*P \leq 0.05$ ($n = 3$ 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. $n \geq 15$ cells from three biological replicates per condition were traced manually. Data are represented as mean ± s.d. and analyzed using one-way ANOVA with Tukey post hoc testing, ***$P \leq 0.001$, ****$P \leq 0.0001$. 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 ± 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 ± s.d.

Viscosity (mPa·s)	Ki-67 positive cells for Passage 1 (%)	Ki-67 positive cells for Passage 3 (%)
1	75.3 ± 4.2	73.4 ± 3.1
50	72.7 ± 2.8	69.7 ± 1.7
100	69.3 ± 1.9	65.3 ± 2.7
200	73.3 ± 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*≤0.05, ***P*≤0.01, ****P*≤0.001, *****P*≤0.0001 and NS denotes not significant.

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

Fig.	Parameters / compared groups	a,b	a,c	a,d	b,c	b,d	c,d	d,f	e,f
1c	No of cells per cm^2	0.8867 *NS*	<0.0001 ****	<0.0001 ****	<0.0001 ****	<0.0001 ****	0.0218 *	n/a	n/a
1d	Area per cell (μm^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 *NS*	0.0290 *	0.0007 ***	>0.9999 *NS*	0.0171 *	0.5296 *NS*	n/a	n/a

Uncropped blot for Supplementary Figure 12