

## Electronic Supplementary Material

Cellular and Nuclear Alignment Analysis for Determining Epithelial Cell Chirality

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**Table S1.** Cell Alignment Data of Each Ring

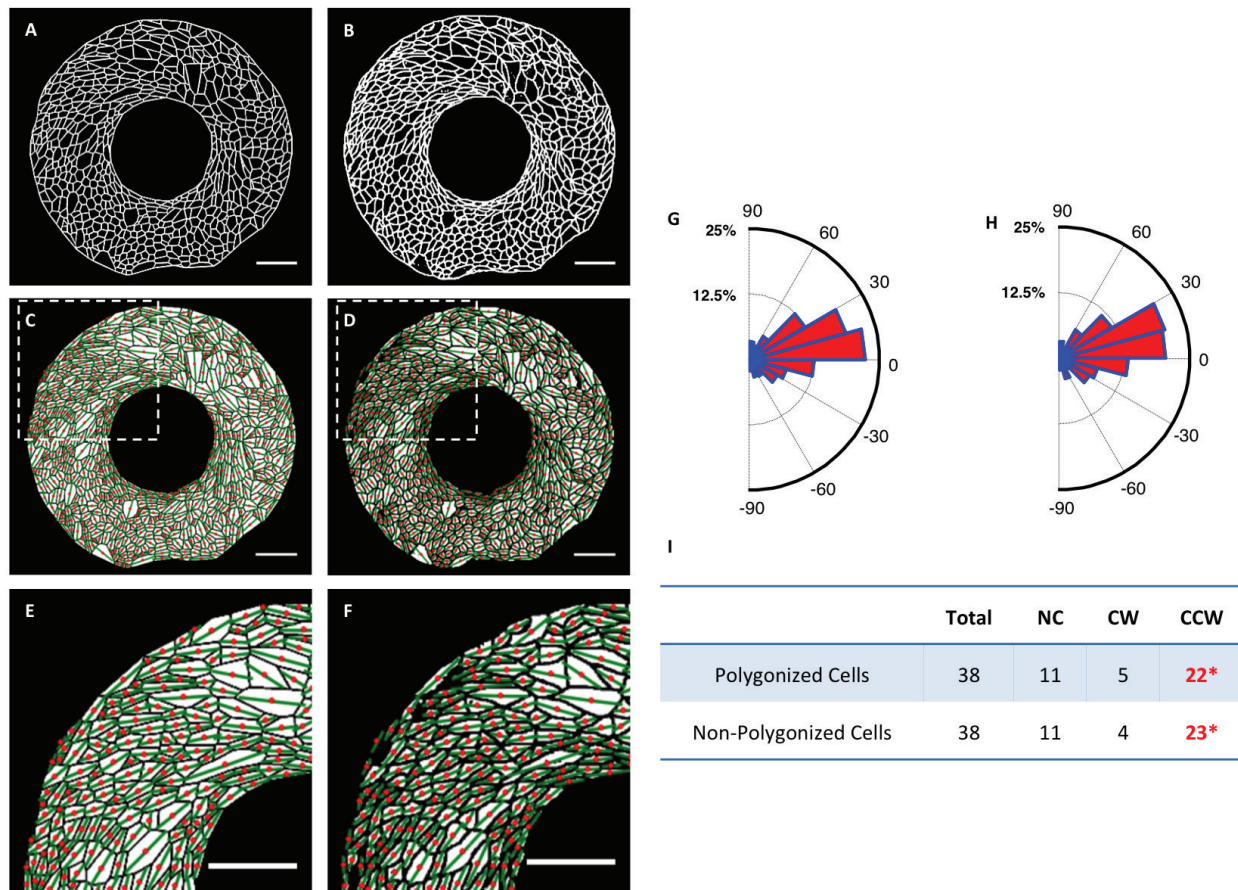
Ring ID	Alignment Angle (mean $\pm$ SEM)	CW	CCW	NC	p Value
1	1.07 $\pm$ 2.65	73	76	17	8.1E-01
2	5.02 $\pm$ 2.39	123	152	37	8.0E-02
3	13.58 $\pm$ 2.02	75	<b>199</b>	34	6.8E-14
4	5.22 $\pm$ 2.29	95	<b>140</b>	32	3.3E-03
5	12.97 $\pm$ 2.03	84	<b>204</b>	32	1.5E-12
6	15.42 $\pm$ 1.97	71	<b>205</b>	34	7.3E-16
7	13.26 $\pm$ 1.99	80	<b>215</b>	27	3.8E-15
8	4.42 $\pm$ 2.10	139	162	39	1.9E-01
9	1.31 $\pm$ 2.32	134	121	32	4.2E-01
10	19.47 $\pm$ 1.79	34	<b>185</b>	28	1.9E-24
11	18.65 $\pm$ 1.78	37	<b>186</b>	35	1.9E-23
12	1.50 $\pm$ 2.71	86	104	31	1.9E-01
13	-9.57 $\pm$ 1.47	<b>323</b>	168	68	2.7E-12
14	6.93 $\pm$ 1.97	107	<b>166</b>	47	3.6E-04
15	13.66 $\pm$ 2.48	81	<b>172</b>	24	1.1E-08
16	9.36 $\pm$ 2.06	85	<b>174</b>	33	3.2E-08
17	9.21 $\pm$ 1.81	129	<b>243</b>	38	3.4E-09
18	15.28 $\pm$ 2.61	70	<b>156</b>	22	1.1E-08
19	13.29 $\pm$ 2.49	52	<b>132</b>	27	3.7E-09
20	2.99 $\pm$ 2.01	158	176	52	3.3E-01
21	-2.87 $\pm$ 2.72	133	110	26	1.4E-01
22	-1.22 $\pm$ 2.36	99	111	25	4.1E-01
23	8.83 $\pm$ 2.35	87	<b>159</b>	44	4.4E-06
24	23.57 $\pm$ 2.18	35	<b>178</b>	12	1.2E-22
25	7.13 $\pm$ 2.25	118	<b>191</b>	34	3.3E-05
26	4.94 $\pm$ 1.50	173	<b>269</b>	78	4.0E-06
27	16.79 $\pm$ 1.63	136	<b>331</b>	51	1.8E-19
28	10.48 $\pm$ 1.74	151	<b>298</b>	64	4.0E-12
29	6.76 $\pm$ 1.39	229	<b>427</b>	78	1.1E-14
30	-4.04 $\pm$ 1.59	<b>303</b>	241	68	7.9E-03
31	-4.92 $\pm$ 1.47	<b>302</b>	221	50	4.0E-04
32	-5.97 $\pm$ 1.15	<b>424</b>	289	106	4.3E-07
33	-6.16 $\pm$ 1.55	<b>255</b>	201	57	1.1E-02
34	0.56 $\pm$ 2.21	173	168	52	7.9E-01
35	0.85 $\pm$ 1.79	260	279	48	4.1E-01
36	1.10 $\pm$ 2.01	178	195	50	3.8E-01
37	-0.91 $\pm$ 1.98	208	218	47	6.3E-01
38	4.82 $\pm$ 1.64	267	<b>341</b>	57	2.7E-03

CW: clockwise alignment, CCW: counter-clockwise alignment, and NC: not significantly biased to CW or CCW.

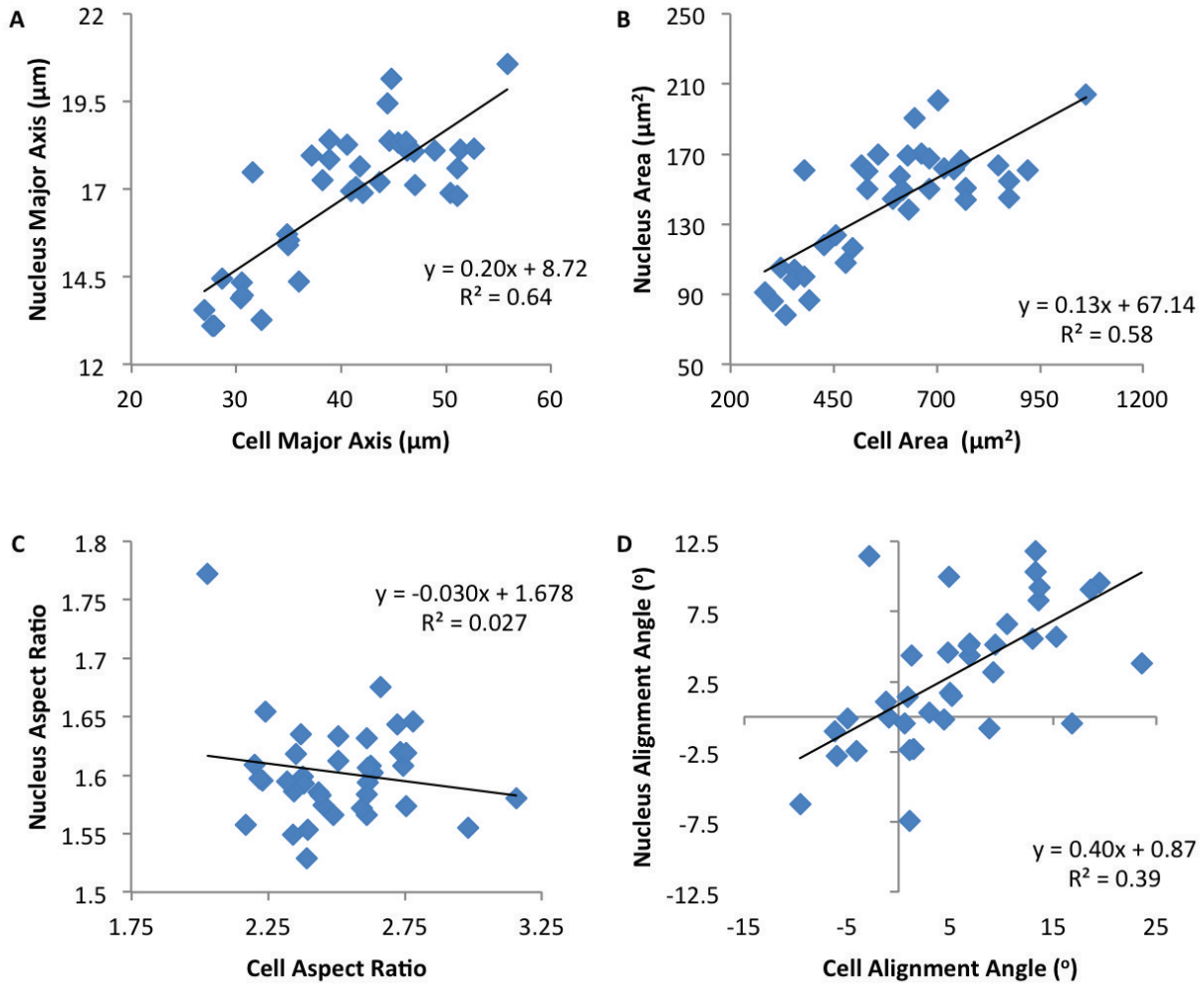
**Table S2.** Nuclear Alignment Data of Each Ring

Ring ID	Alignment Angle (mean $\pm$ SEM)	CW	CCW	NC	p Value
1	-7.41 $\pm$ 3.11	80	67	19	2.8E-01
2	1.71 $\pm$ 2.61	138	146	28	6.4E-01
3	8.32 $\pm$ 2.38	96	<b>181</b>	31	3.3E-07
4	1.50 $\pm$ 2.53	112	120	35	6.0E-01
5	5.59 $\pm$ 2.42	105	<b>176</b>	39	2.3E-05
6	13.18 $\pm$ 2.20	88	<b>195</b>	27	2.0E-10
7	11.82 $\pm$ 2.09	94	<b>198</b>	30	1.2E-09
8	-0.19 $\pm$ 2.45	151	160	29	6.1E-01
9	4.34 $\pm$ 2.47	127	134	26	6.7E-01
10	9.56 $\pm$ 2.36	66	<b>152</b>	29	5.7E-09
11	9.06 $\pm$ 2.32	73	<b>146</b>	39	8.1E-07
12	-2.28 $\pm$ 3.02	104	96	21	5.7E-01
13	-6.22 $\pm$ 1.84	<b>284</b>	229	46	1.5E-02
14	5.22 $\pm$ 2.19	122	<b>164</b>	34	1.3E-02
15	9.20 $\pm$ 2.72	95	<b>155</b>	27	1.5E-04
16	5.11 $\pm$ 2.46	118	<b>153</b>	21	3.4E-02
17	3.19 $\pm$ 2.05	169	203	38	7.8E-02
18	5.71 $\pm$ 2.87	95	<b>130</b>	23	2.0E-02
19	10.34 $\pm$ 2.65	66	<b>117</b>	28	1.6E-04
20	4.38 $\pm$ 2.31	151	<b>199</b>	36	1.0E-02
21	0.29 $\pm$ 2.89	119	132	18	4.1E-01
22	11.47 $\pm$ 2.61	67	<b>138</b>	30	7.1E-07
23	1.10 $\pm$ 2.73	120	141	29	1.9E-01
24	-0.84 $\pm$ 3.21	105	103	17	8.9E-01
25	3.83 $\pm$ 2.56	121	<b>156</b>	32	3.6E-02
26	10.00 $\pm$ 2.14	154	<b>247</b>	41	3.4E-06
27	-0.49 $\pm$ 1.93	204	214	49	6.3E-01
28	6.64 $\pm$ 1.92	159	<b>238</b>	52	7.3E-05
29	5.07 $\pm$ 1.65	244	<b>349</b>	63	1.6E-05
30	-2.41 $\pm$ 1.80	254	220	70	1.2E-01
31	-0.12 $\pm$ 1.90	241	240	42	9.6E-01
32	-2.78 $\pm$ 1.67	350	301	62	5.5E-02
33	-1.01 $\pm$ 1.99	216	191	49	2.2E-01
34	-0.44 $\pm$ 2.41	158	152	31	7.3E-01
35	1.42 $\pm$ 1.95	237	262	40	2.6E-01
36	-2.35 $\pm$ 2.27	176	165	32	5.5E-01
37	-0.08 $\pm$ 2.23	197	189	40	6.8E-01
38	4.57 $\pm$ 1.89	245	<b>315</b>	48	3.1E-03

CW: clockwise alignment, CCW: counter-clockwise alignment, and NC: not significantly biased to CW or CCW.



**Figure S1.** Chirality analyses based on polygonized and non-polygonized images. Scale bars = 100  $\mu\text{m}$ . **(A)** Polygonized cell image. **(B)** Non-polygonized cell image. **(C-D)** Alignment of the cells in polygonized and non-polygonized images indicated by green lines over cell centroid (red dots), respectively. **(E-F)** Region of interest of C-D. **(G-H)** Rose diagrams of alignment angle distributions. **(I)** Table for chirality analysis of polygonized and non-polygonized images. \* Significant bias towards CCW among chiral rings.



**Figure S2.** Correlations between cellular and nuclear morphological parameter: (A) Major axis length of cells vs nuclei, (B) Cellular vs nuclear area, (C) Aspect ratio, and (D) Alignment angles. Strong correlations ( $R^2 > 0.5$ ) in major axis length, area, and alignment angles between cells and their nuclei were observed.