

Figure S1: Typical dorsoventral electrochemical gradients, as observed in the wt FE beginning with S10B, are absent in grk. Additional examples corresponding to Fig. 2, showing the variability between follicles of the same stage. a-I: Pseudocolor images of DiBAC ( $\mathrm{V}_{\mathrm{mem}}$ ) stained wt S9 (a-c) and wt S10B (d-f) as well as grk S9 (g-i) and grk S10B (j-I), respectively. $\mathbf{m - x}$ : Pseudocolor images of CFDA ( $\mathrm{pH}_{\mathrm{i}}$ ) stained wt 9 ( $\mathbf{m}-\mathbf{o}$ ) and wt S10B ( $\mathbf{p - r}$ ) as well as grk S9 (s-u) and grk S10B (v-x), respectively (scale bars represent $100 \mu \mathrm{~m}$; triangles indicate gradient's polarity; positions of the ON are marked with asterisks)

Table S1: Quantification of fluorescence intensities of transversal electrochemical gradients in the FE of wt and grk (S10B).

| Mean fluorescence intensity <br> in the FE of S10B follicles | FE $_{\mathbf{1}}$ | wt <br> $\mathrm{FE}_{\mathbf{2}}$ | ratio | $\mathrm{FE}_{\mathbf{1}}$ | grk <br> $\mathrm{FE}_{\mathbf{2}}$ | ratio |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | follicle 1 | 47.549 | 96.665 | 2.03 | 53.940 | 81.147 | 1.50 |
|  | follicle 2 | 87.134 | 93.925 | 1.08 | 54.312 | 64.269 | 1.18 |
|  | follicle 3 | 50.875 | 77.874 | 1.53 | 116.148 | 141.018 | 1.21 |
| $\mathbf{V}_{\text {mem }}$ | follicle 4 | 54.227 | 86.196 | 1.59 | 91.955 | 119.399 | 1.30 |
|  | follicle 5 | 66.310 | 93.636 | 1.41 | 58.158 | 59.392 | 1.02 |
|  | follicle 6 | 82.120 | 140.956 | 1.72 | 56.750 | 72.321 | 1.27 |
|  | follicle 7 | 44.366 | 97.796 | 2.20 | 28.814 | 33.256 | 1.15 |
|  | follicle 1 | 27.376 | 39.256 | 1.43 | 15.877 | 11.915 | 1.33 |
|  | follicle 2 | 7.963 | 13.977 | 1.76 | 1.091 | 1.354 | 1.24 |
|  | follicle 3 | 5.782 | 9.033 | 1.56 | 18.275 | 21.024 | 1.15 |
|  | follicle 4 | 9.352 | 10.494 | 1.12 | 26.991 | 25.883 | 1.04 |
| $\mathbf{p H}_{\mathbf{i}}$ | follicle 5 | 16.600 | 21.542 | 1.30 | 15.032 | 19.093 | 1.27 |
|  | follicle 6 | 8.997 | 13.623 | 1.51 | 11.018 | 10.802 | 1.02 |
|  | follicle 7 | 12.231 | 22.268 | 1.82 | 6.437 | 7.085 | 1.10 |

§Data corresponding to Table 2 and Fig. 3b. Fluorescence intensities ("mean grey value") of both sides of the $\mathrm{FE}\left(\mathrm{FE}_{1}\right.$ and $\mathrm{FE}_{2}$ ) were measured using ImageJ (see Fig. 1e). The ratio is $F E_{2} / \mathrm{FE}_{1}$, larger value vs. smaller value. In some wt follicles which were in late S10B (numbers 3 and 7), d-v polarity of the transversal Vmem-gradient was reversed (dorsal side depolarised), compared to earlier S10B follicles as shown in Figs. 2 and S1 (cf. [7]).

Table S2: Quantification of fluorescence intensities of anteroposterior electrochemical gradients in the FE of wt and grk (S10B).

| Mean fluorescence intensity <br> in the FE of S10B follicles | aFE | wt <br> pFE | ratio | aFE | grk <br> pFE | ratio |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | follicle 1 | 54.687 | 82.225 | 1.50 | 44.455 | 88.138 | 1.98 |
|  | follicle 2 | 87.093 | 92.574 | 1.06 | 93.915 | 121.706 | 1.29 |
| V $_{\text {mem }}$ | follicle 3 | 55.051 | 74.046 | 1.35 | 40.100 | 75.469 | 1.88 |
|  | follicle 4 | 65.824 | 68.068 | 1.03 | 51.718 | 67.134 | 1.30 |
|  | follicle 5 | 64.369 | 97.711 | 1.52 | 127.958 | 126.426 | 0.99 |
|  | follicle 6 | 97.739 | 114.622 | 1.17 | 53.689 | 80.274 | 1.74 |
|  | follicle 7 | 71.888 | 56.524 | 0.79 | 20.235 | 45.114 | 2.29 |
|  | follicle 1 | 21.080 | 45.383 | 1.43 | 7.476 | 14.197 | 1.90 |
|  | follicle 2 | 8.238 | 12.342 | 1.76 | 10.512 | 21.395 | 2.04 |
|  | follicle 3 | 6.710 | 8.905 | 1.56 | 3.685 | 2.927 | 0.79 |
|  | follicle 4 | 9.213 | 11.206 | 1.12 | 17.344 | 32.323 | 1.86 |
| $\mathbf{p H}_{\mathbf{i}}$ | follicle 5 | 11.663 | 26.749 | 1.30 | 11.908 | 23.356 | 1.96 |
|  | follicle 6 | 9.389 | 12.291 | 1.51 | 4.407 | 7.998 | 1.81 |
|  | follicle 7 | 12.306 | 21.521 | 1.82 | 4.103 | 9.043 | 2.20 |

§Data corresponding to Table 2 and Fig. 3c. Fluorescence intensities ("mean grey value") of the anterior and posterior half of the columnar FE (aFE and pFE) were measured using ImageJ (see Fig. 1e). The ratio is pFE/aFE.


Figure S2: The grk FE exhibits striking cytoskeletal differences compared to wt (S9 and S10B).
Additional examples corresponding to Fig. 4, showing the variability between follicles of the same stage. a-I: Fluorescent phalloidin (bMF) stained wt S9 (a-c) and wt S10B (d-f) as well as grk S9 ( $\mathbf{g - i}$ ) and grk S10B ( $\mathbf{j - l}$ ), respectively. m-x: Antitubulin (MT) stained wt $\mathrm{S9}$ ( $\mathbf{m}-\mathbf{o}$ ) and wt S10B (p-r) as well as grk S9 (s-u) and grk S10B (v-x), respectively (scale bars respresent 20 $\mu \mathrm{m})$.

Table S3: Numbers ( $n$ ) of follicles analysed for each condition and developmental stage.

| wt |  |  |  | grk |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $V_{\text {mem }}$ | n | pHi | n | $V_{\text {mem }}$ | n | pHi | n |
| S8 | 27 | S8 | 18 | S8 | 9 | S8 | 13 |
| S9 | 15 | S9 | 19 | S9 | 24 | S9 | 24 |
| S10A | 19 | S10A | 13 | S10A | 13 | S10A | 5 |
| S10B | 41 | S10B | 23 | S10B | 9 | S10B | 15 |
| S11 | 12 | S11 | 19 | S11 | 6 | S11 | 6 |
| S12 | 10 | S12 | 12 | S12 | 5 | S12 | 8 |
| bMF | n | MT | n | bMF | n | MT | n |
| S8 | 5 | S8 | 18 | S8 | 11 | S8 | 9 |
| S9 | 7 | S9 | 15 | S9 | 26 | S9 | 17 |
| S10A | 5 | S10A | 13 | S10A | 12 | S10A | 6 |
| S10B | 16 | S10B | 22 | S10B | 13 | S10B | 8 |
| S11 | 7 | S11 | 9 | S11 | 8 | S11 | 4 |
| S12 | 9 | S12 | 16 | S12 | 11 | S12 | 7 |

