FGF21 Induced by the ASK1-p38 Pathway Promotes Mechanical Cell Competition by Attracting Cells

Graphical Abstract

Highlights
- Depletion of Scribble induces FGF21
- FGF21 induces cell competition through FGFR1
- FGF21 promotes mechanical cell competition by attracting surrounding WT cells
- ASK1-p38 pathway induces FGF21 to cause cell competition

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In Brief
Using mammalian cell cultures, Ogawa et al. demonstrate that a secreted ligand, FGF21, induced from loser cells causes cell competition through FGFR1 of winner cells, which is a new type of cell-cell recognition mechanism in cell competition. FGF21 induced by the ASK1-p38 pathway promotes the compression of loser cells by attracting winner cells.

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FGF21 Induced by the ASK1-p38 Pathway Promotes Mechanical Cell Competition by Attracting Cells

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SUMMARY

Cell competition is a social cellular phenomenon in which unfit cells are selectively eliminated to maintain tissue homeostasis.1–3 Recent studies have revealed that mechanical forces induce competitive cell-cell interactions in Drosophila.4–6 This mechanical cell competition has also been reported to play an important role in mammalian cells, using Madin-Darby canine kidney (MDCK) cells depleted of a polarity regulator Scribble in a tetracycline-inducible manner (scribKD cells).7 scribKD cells are hypersensitive to crowding due to the lower homeostatic density than wild-type (WT) cells,7,8 and in the context of cell competition, scribKD cells are compacted and eliminated by WT cells.7–10 Although p38 and p53 are involved in this process,7,10 the molecular mechanism by which WT cells recognize and mechanically eliminate scribKD cells remains unclear. Here, we report that scribKD cells secrete fibroblast growth factor 21 (FGF21) to drive cell competition. Knockdown of FGF21 in scribKD cells or loss of FGFR1 in WT cells suppresses cell competition, suggesting that WT cells recognize scribKD cells through FGF21. FGF21-containing culture medium of scribKD cells activates cell motility. Moreover, FGF21 promotes the compression and elimination of scribKD cells by attracting surrounding WT cells. We also demonstrate that activation of the apoptosis signal-regulating kinase 1 (ASK1)-p38 pathway in scribKD cells induces FGF21 to drive cell competition. Our findings reveal a mechanism whereby WT cells mechanically eliminate scribKD cells and propose a new function for FGF21 in cell-cell communication.

RESULTS

As reported before, we confirmed that, when cocultured with wild-type (WT) cells, GFP-expressing scribKD cells were outcompeted by cell competition in a tetracycline (Tet)-dependent manner (Figure S1A).7–10 We also observed that the density of scribKD cells was remarkably increased after tetracycline addition compared to that of WT cells in cell competition, suggesting that scribKD cells were compacted (Figure S1B).7–9 A previous report showed that, when WT and scribKD colonies come into contact, both colonies move synchronously, with scribKD colony always followed by WT colony, suggesting that this migratory behavior promotes the compression and elimination of scribKD cells in cell competition.7 However, the precise mechanism by which the migratory behavior of WT and scribKD colonies is induced remains elucidated. Using live-cell imaging, we also observed that WT colonies migrated toward scribKD colonies after Scribble depletion, and upon contact, scribKD colonies appeared to be pushed by WT colonies (Figure 1A; Video S1). Moreover, it seemed that even WT cells not in direct contact with scribKD cells acquired enhanced motility in a Tet-dependent manner (Video S1). Thus, we speculated that certain soluble factor(s) derived from scribKD cells might activate the motility of WT cells, which may promote the compression of scribKD colonies. To precisely observe the movement of WT cells, we established a WT cell line stably expressing a red fluorescent reporter in the cell nucleus and treated the cells with conditioned medium derived from scribKD cell cultures. Strikingly, after treating with conditioned medium of scribKD cells cultured with Tet, WT cells acquired enhanced motility and showed a flattened morphology, while cell-cell contacts were mostly maintained (Figure 1B; small interfering RNA [siRNA] [−] Tet [−] in Video S2). In contrast, the addition of conditioned medium of scribKD cells cultured without Tet did not affect the movement of WT cells (Figure 1B; siRNA [−] Tet [−] in Video S2). These data suggest that scribKD cell-derived soluble factor(s) may activate the movement of WT cells. To search for the soluble factors that were upregulated by Scribble knockdown, we carried out a DNA microarray transcriptome analysis of scribKD cells with or without Tet treatment. Given that the protein expression of Scribble in scribKD cells was decreased at 30 h after Tet addition (Figure 1C) and that scribKD cells were markedly eliminated in cell competition from 47 to 57 h after Tet addition (Figure S1A), we performed the DNA microarray analysis using mRNAs of
Figure 1. *scrib*<sup>KO</sup> Cells Induce FGF21 that Activates the Motility of WT Cells

(A) Representative images from time-lapse movies of cocultures of tdTomato-expressing wild-type (WT) and GFP-expressing *scrib*<sup>KO</sup> cells with or without tetracycline (Tet). Live-cell imaging was performed from 53 to 68 h after Tet treatment. Scale bar, 50 μm.

(B and F) Representative images from time-lapse movies of WT cells stably expressing NLS-tdTomato after treatment with conditioned medium of *scrib*<sup>KO</sup> cells cultured with or without Tet for 64 h. Scale bar, 50 μm.

(F) *scrib*<sup>KO</sup> cells were treated with the indicated siRNA.

(C) Western blotting analysis of WT or *scrib*<sup>KO</sup> cells at the indicated times cultured with or without Tet.

(D) Quantitative PCR (qPCR) analysis of FGF21 in *scrib*<sup>KO</sup> cells at the indicated times cultured with or without Tet. Sample size: n = 4. *p < 0.05; **p < 0.01 (unpaired two-tailed Student’s t test). Data are represented as the mean ± SEM.
secreted from ture supernatants as well as lysates, indicating that FGF21 was dependent manner (Figure 1C). FGF21 was detected in cell cul-
protein was also increased following Tet treatment in a time-
does not exist in with that without Tet. N.D. means that two independent primers did not show any amplification of the FGF transcripts in either Tet (Figure 1D), and FGF21 protein was also increased following Tet treatment in a time-
dependent manner (Figure 1C). FGF21 was detected in cell culture supernatants as well as lysates, indicating that FGF21 was secreted from scribKD cells (Figure 1C). Interestingly, among FGF family members, only FGF21 was remarkably upregulated by Scribble knockdown, whereas most of the other members were not detected (Figure 1E). We then examined whether FGF21 is required to activate the motility of WT cells. Fluorescence-labeled WT Madin–Darby canine kidney (MDCK) cells were treated with conditioned medium derived from scribKD cells with or without siRNA knockdown of FGF21 (Figure S1E). We found that the active cell movement was hardly induced by the FGF21-depleted conditioned medium compared with the control (Figure 1F; Video S2). To quantify cell motility, we traced the centroid of the cell nucleus using a fluorescent reporter and found that FGF21-depleted conditioned medium of scribKD cells scarcely induced the movement of WT cells compared with the control (Figure 1G). The cumulative migration distance during 5 h after treatment with FGF21-depleted conditioned medium was shorter than that with FGF21-undepleted control medium (Figure 1H). Overall, these results suggest that FGF21 secreted from scribKD cells activates the motility of WT cells.

FGF21 Secreted by scribKD Cells Activates Cell Motility

The mRNA amount of fibroblast growth factor 21 (FGF21) was drastically upregulated following Tet treatment in a time-dependent manner (Figure S1C). FGF21 was one of the highly ranked soluble factors among the upregulated genes after Tet treatment (Figure S1D). FGF21 is a well-known metabolic activator in liver and adipose tissue. Although recent papers have demonstrated that FGF21 exists in skin and promotes cell migration, no reports have shown the involvement of FGF21 in cell competition. Upregulation of FGF21 mRNA in scribKD cells was confirmed using quantitative PCR (Figure 1D), and FGF21 protein was also increased following Tet treatment in a time-dependent manner (Figure 1C). FGF21 was detected in cell culture supernatants as well as lysates, indicating that FGF21 was secreted from scribKD cells (Figure 1C). Interestingly, among FGF family members, only FGF21 was remarkably upregulated by Scribble knockdown, whereas most of the other members were not detected (Figure 1E). We then examined whether FGF21 is required to activate the motility of WT cells. Fluorescein-labeled WT Madin–Darby canine kidney (MDCK) cells were treated with conditioned medium derived from scribKD cells with or without siRNA knockdown of FGF21 (Figure S1E). We found that the active cell movement was hardly induced by the FGF21-depleted conditioned medium compared with the control (Figure 1F; Video S2). To quantify cell motility, we traced the centroid of the cell nucleus using a fluorescent reporter and found that FGF21-depleted conditioned medium of scribKD cells scarcely induced the movement of WT cells compared with the control (Figure 1G). The cumulative migration distance during 5 h after treatment with FGF21-depleted conditioned medium was shorter than that with FGF21-undepleted control medium (Figure 1H). Overall, these results suggest that FGF21 secreted from scribKD cells activates the motility of WT cells.

FGF21-FGFR1 Axis Is Required for Cell Competition

We then examined the requirement for FGF21 in cell competition. scribKD cells were mixed with WT cells at a ratio of 1:3, and after 60 h cultured with Tet, the survival rate of scribKD cells was calculated (Figures S1F and S1G). Knockdown of FGF21 only in scribKD cells increased the survival rate of scribKD cells in cell competition (Figures 2A and 2B). We next examined whether FGF21 derived from scribKD cells directly regulates WT cells to cause cell competition. As one of the receptors of FGF21 is FGFR1 (FGFR1), we generated heterogeneous FGF21 knockout (KO) MDCK cell lines by a lentiviral-mediated CRISPR-Cas9 system (Figure S1H). Although the protein expression of FGFR1 in these cell lines was not completely abolished (possibly because some cells incorporated only the puromycin resistance gene), the survival rate of scribKD cells was increased when they were mixed with the FGFR1 KO cells (Figures 2C and 2D). Moreover, treatment with the FGFR kinase inhibitor AZD4547 in the cell competition system slightly but significantly increased the survival rate of scribKD cells (Figures 2E and 2F). Taken together, these data suggest that FGF21 secreted by scribKD cells is recognized, at least in part, via FGFR1 of WT cells to induce cell competition.

FGF21 Promotes Compression of scribKD Cells by Attracting WT Cells

Next, we asked whether FGF21 promoted WT colonies to compress scribKD colonies. As shown above, when WT and scribKD colonies came into contact, scribKD colonies were pushed by WT colonies (Figure 1A; Video S1). Given this behavior, we hypothesized that FGF21 activated the motility of WT colonies to mechanically push scribKD colonies. Knockdown of FGF21 in scribKD cells delayed the compression speed of scribKD colonies compared with that of scribKD control colonies under Tet treatment (Figure 3A; Video S3). Setting the direction of the centroid of scribKD colonies to the y axis, we traced the centroid of WT colonies and plotted the cumulative displacement measured every 2 h for 10 h after WT colonies came into contact with scribKD colonies (Figure 3B). The cumulative displacement of WT colonies showed the directionally persistent cell migration of WT colonies toward scribKD colonies under Tet treatment (Figure 3B). The cumulative migration distance of WT colonies toward scribKD colonies was considerably increased under Tet treatment (Figure 3C). However, when scribKD colonies depleted of FGF21 collided with WT colonies, the cumulative migration distance did not show a marked increase, even in the presence of Tet (Figure 3B), and was significantly shorter than that of WT colonies collided with control scribKD colonies cultured with Tet (Figure 3C). Using particle image velocimetry (PIV), we found that WT cells directly migrated toward scribKD cells and compressed them (Figure S2A). Considering the directional movement of WT colonies toward scribKD colonies, we then asked whether FGF21 attracts WT cells. We generated FGF21-overexpressing WT cells using FGF21-IRES-GFP (FGF21-overexpressing (OE) cells; Figure S2B). We observed that FGF21-OE cells acquired enhanced motility and showed a flattened morphology (Video S4). In coculture with FGF21-OE cells, WT colonies directionally migrated toward FGF21-OE colonies prior to contact and upon collision compressed them (Figure 3D; Video S4). The cumulative displacement of WT colonies showed the directional migration of WT colonies toward...
FGF21-OE colonies (Figure 3E). The migration distance of WT colonies toward FGF21-OE colonies was longer than that of WT colonies toward GFP-overexpressing WT colonies (Figure 3F). These data suggest that overexpression of FGF21 in WT cells is sufficient to promote directional cell migration. Overall, our results indicate that FGF21 may facilitate WT colonies to push "scribKD" colonies, presumably through attracting WT colonies.

Given these findings, we speculated that FGF21 promotes WT cells to compress and eliminate "scribKD" cells in cell competition.

To precisely quantify the potency of compression and elimination of "scribKD" cells in cell competition, we used a cell confrontation assay where we could observe the boundary between the two cell populations in a confluent culture. Upon collision with WT cells, "scribKD" cells treated with Tet appeared to be compressed, and marginal "scribKD" cells were eliminated, although WT cells continued to migrate forward and took over the space occupied by "scribKD" cells (control Tet [+] in Figure 3G; Video S5). However, in the absence of Tet, "scribKD" cells were scarcely eliminated by WT cells, as represented by the almost unchanged boundary, and they formed a stable monolayer (control Tet [−] in Figure 3G; Video S5). We measured the area occupied by GFP-expressing "scribKD" cells after collision with WT cells (Figure S2C). The GFP-positive area was significantly decreased after "scribKD" cells came into contact with WT cells following Tet treatment (control in Figures 3H and 3I). In contrast, when the same cell
Figure 3. FGF21 Attracts WT Cells to Compress and Eliminate scribKO Cells

(A) Representative images from time-lapse movies of cocultures of WT and GFP-expressing scribKO colonies. scribKO cells were treated with the indicated siRNA. In the movies, the colonies came into contact within 30–50 h after Tet treatment. Scale bar, 50 μm.

(B) Plot of the cumulative displacement of the centroid of WT colonies measured every 2 h for 10 h after collision with scribKO colonies. The direction of the centroid of scribKO colonies from the centroid of WT colonies was set to y axis. Each line corresponds to a different colony. Sample size: control Tet (−/C0, +; n = 10, 21); FGF21 #1 Tet (−/C0, +; n = 7, 22); and FGF21 #2 Tet (−/C0, +; n = 8, 24) from three independent experiments. **p < 0.01; ***p < 0.001 (one-way ANOVA followed by Tukey-Kramer’s multiple comparisons test).

(C) Cumulative migration distance of the centroid of WT colonies toward scribKO colonies (y axis) measured every 2 h for 10 h after collision. Data are represented by a boxplot. Individual values are presented as black points, and the line in the box shows the median. The data sample is the same as in (B). **p < 0.01; ***p < 0.001 (one-way ANOVA followed by Tukey-Kramer’s multiple comparisons test).

(D) Representative images from time-lapse movies of cocultures of WT cells expressing NLS-tdTomato and WT cells expressing GFP or FGF21-IRES-GFP (FGF21-OE cells). Scale bar, 50 μm.
populations collided, such as WT cells colliding with WT cells or scribKD cells colliding with scribKD cells, the boundary between the two cell populations did not move, even under Tet treatment (Figures S2D–S2F). Thus, Scribble-depleted cells were strongly compressed only when colliding with WT cells. We then examined the requirement for FGF21 in this phenomenon and found that knockdown of FGF21 in scribKD cells suppressed the compression and elimination of scribKD cells (Figures 3G–3I; Video S5). These data suggest that FGF21 promotes the compression and elimination of scribKD cells.

Enhanced motility without directionality of WT cells may be sufficient to drive the compression and elimination of scribKD cells. To test this possibility, we used the confront assay of scribKD cells depleted of FGF21 and added FGF21-containing culture medium of scribKD cells as exogenous FGF21 source. The compression of scribKD cells depleted of FGF21 was not increased, even after treating with FGF21-containing culture medium of scribKD cells, suggesting that the directionality of the movements of WT cells might be important to compress scribKD cells (Figures S2G–S2I).

**ASK1-p38 Axis Promotes Cell Competition via Induction of FGF21**

Finally, we investigated the molecular mechanism by which FGF21 was induced in scribKD cells. It has been shown that FGF21 is upregulated via activation of p38 in adipocytes. In addition, p38 activation is reported to be involved in cell death of scribKD cells in the context of cell competition. We therefore examined whether p38 activation induced FGF21 in scribKD cells. We found that the activating phosphorylation of p38 was spontaneously increased in monocultures of scribKD cells after Tet addition, and knockdown of p38 abolished FGF21 expression in scribKD cells (Figures 4A and 4B). Furthermore, three chemical inhibitors of p38 (Ph-797804, SB202190, and SB203580) also suppressed FGF21 upregulation, although SB202474, an inactive analog of SB202190 and SB203580, did not (Figures 4C and 4D). These data suggest that p38 activation is required for the induction of FGF21 under Scribble depletion. Apoptosis signal-regulating kinase 1 (ASK1) is one of the well-established upstream kinases that activate p38. In scribKD cells, ASK1 protein expression was slightly decreased following Tet treatment, but its activating phosphorylation was elevated (Figure 4E). Knockdown of ASK1 in scribKD cells simultaneously suppressed FGF21 upregulation and p38 activation induced by Tet treatment (Figures 4B, 4E, and 4F). Consistent with these results, the upregulation of FGF21 mRNA was significantly reduced by knockdown of p38 or ASK1 (Figure 4G). These findings suggest that the ASK1-p38 axis activated by Scribble knockdown induces FGF21 gene expression. As mentioned before, p38 plays a critical role in the elimination of scribKD cells in cell competition, but the requirement of ASK1 has not been reported. We found that depletion of ASK1 as well as p38 in scribKD cells increased the survival rate of scribKD cells (Figures 4H and 4I). These lines of evidence suggest that the ASK1-p38 axis activated by Scribble knockdown induces cell competition through FGF21 upregulation.

**DISCUSSION**

In this study, we suggest that FGF21 secreted from scribKD cells drives cell competition by promoting WT cells to compress scribKD cells. Loss of FGFR1 in WT cells increases the survival rate of scribKD cells, suggesting that WT cells may recognize scribKD cells through FGF21 to induce cell competition. These results reveal a mechanism by which WT cells mechanically eliminate scribKD cells and provide insights into the function of FGF21 in terms of cell-cell communication.

We show for the first time that FGF21 is induced by loss of Scribble, which is one of the cell polarity regulators. It would be of interest to examine whether loss of cell polarity, which is often observed in carcinogenesis, generally causes the induction of FGF21. As for the survival assay in cell competition, however, there is still a diminution of scribKD cells even after depletion of FGF21, ASK1, or p38, suggesting that other modes of elimination mechanisms may also exist in this cell competition. FGF21 KO mice have been reported to develop hepatocellular carcinoma (HCC) with consumption of an obesogenic diet. It is possible that loss of cell competition driven by FGF21 may facilitate the development of HCC at the initial stage of hepatocarcinogenesis.

A recent study suggested that the proliferation of WT cells is activated in areas mostly populated by scribKD cells. There is a possibility that FGF21 accelerates the proliferation of surrounding WT cells to compact scribKD cells. However, we did not observe the immediate induction of cell division in WT cells after treatment with conditioned medium of scribKD cells (Figure 1F; Video S2), and the effect of FGF21 on cell proliferation remains controversial.

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(E) Plot of the cumulative displacement of the centroid of WT colonies measured every 2 h for 10 h after collision with GFP-expressing WT colonies or FGF21-OE colonies. The direction of the centroid of GFP-expressing WT colonies or FGF21-OE colonies from WT colonies was set to y axis. Each line corresponds to a different colony. Sample size: GFP (n = 40); FGF21-IRES-GFP (n = 33) from three independent experiments.

(F) Cumulative migration distance of the centroid of WT colonies toward GFP-expressing WT colonies or FGF21-OE colonies (y axis) measured every 2 h for 10 h after collision with these results reveal a mechanism by which WT cells mechanically eliminate scribKD cells and provide insights into the function of FGF21 in terms of cell-cell communication.

(G) Representative images from time-lapse movies of the cell confront assay showing the collision of WT and GFP-expressing scribKD cells with or without Tet. scribKD cells were treated with the indicated siRNA. Scale bar, 200 μm.

(H) Plot of the area variation occupied by GFP-expressing scribKD cells measured every 20 min for 30 h after collision with WT cells. The lines show the mean of the area variation, and the shaded areas indicate SEM. Sample size: control Tet (–, +; n = 18, 25); FGF21 #1 Tet (–, +; n = 25, 32); and FGF21 #2 Tet (–, +; n = 35, 29) from four independent experiments.

(I) Violin plots of the area variation occupied by GFP-expressing scribKD cells at 30 h after collision with WT cells. The black dots show the median. The data sample is the same as in (H). *p < 0.001 (one-way ANOVA followed by Tukey-Kramer’s multiple comparisons test).

See also Figure S2 and Videos S3, S4, and S5.
Figure 4. The ASK1-p38 Pathway Activated by Scribble Depletion Induces FGF21 to Drive Cell Competition

(A) Effects of p38 depletion on the protein expression of FGF21 induced by Scribble knockdown, as determined by western blotting analysis.

(B and F) Quantification of the band intensities of FGF21 or p-p38 in lysates of scribKD cells treated with the indicated siRNA. Sample size: control #1 and #2 and ASK1 #1 and #2 (n = 6); p38 #1 and #2 (n = 5). *p < 0.05; **p < 0.01; ***p < 0.001 (one-way ANOVA followed by Tukey-Kramer’s multiple comparisons test).

(C) Effects of p38 inhibitors on the protein expression of FGF21 induced by Scribble knockdown, as determined by western blotting analysis. Cells were treated with the following chemical compounds at 5 μM for 12 h before lysis. PH-797804, SB202190, and SB203580 are p38 inhibitors, and SB202474 is an inactive analog of SB202190 and SB203580. Asterisk indicates non-specific bands.

(D) Quantification of the band intensities of FGF21 in lysates of scribKD cells treated with the indicated chemical compounds. Sample size: n = 8. ***p < 0.001 (one-way ANOVA followed by Dunnett’s multiple comparisons test).

(legend continued on next page)
Previous reports showed that the FGF/FGFR signaling regulates several collective migration events in vivo, such as trachea branch migration in Drosophila, and lateral line development in zebrafish, indicating that the cues of collective migration may be co-opted for mammalian cell competition in MDCK cells. It is possible that FGF21 might activate the motility of scribKD cells as well as WT cells in cell competition. However, because scribKD cells are spontaneously hypersensitive to compaction, we speculate that WT cells outcompete scribKD cells in cell competition.

Compaction-mediated p38 activation through Rho-associated protein kinase (ROCK) is reported to be important for cell death of scribKD cells in cell competition. Inhibition of p38 activity reduces p53 upregulation, which is necessary and sufficient for the vulnerability to compaction in scribKD cells. Here, we clearly show that p38 is spontaneously activated in monocultures of scribKD cells and induces FGF21 expression, suggesting that p38 also plays an alternative role in cell competition through FGF21. Our data suggest that FGF21 is gradually elevated after Scribble knockdown (Figures 1C and 1D) and may promote the compaction of scribKD cells by WT cells. Given these data, FGF21 might induce the compaction of scribKD cells, which further enhances p38 activation and accelerates upregulation of p53. As the significance of p53 in cell competition has also attracted attention in other cell competition models, clarifying the relationship between p53 and FGF21 is an important question for future studies. It would also be interesting to determine whether mechanical compaction further increases the expression of FGF21 through p38 activation in scribKD cells.

ASK1, which is a stress-responsive kinase, is activated by a wide range of stresses, such as tumor necrosis factor alpha (TNF-α), reactive oxygen species, and cold stress. In Drosophila, loss of Scribble promotes the transcriptional upregulation of Eiger, a Drosophila ortholog of TNF, which is required for the elimination of Scribble-deficient clones in cell competition. However, based on the DNA microarray data of scribKD cells, the expression of TNF-α was not increased following Tet treatment. We anticipate that mechanisms other than TNF-α induction may be responsible for ASK1 activation under mammalian Scribble depletion.

In summary, our findings have revealed a new function of FGF21 in the promotion of mechanical cell competition through FGFR1 of surrounding WT cells, which is a new type of cell-cell recognition mechanism in cell competition. Because loss of cell polarity is suggested to facilitate tumorigenesis, FGF21 might exert a tumor-suppressive role by promoting competitive interactions between cells.
REFERENCES

1. Morata, G., and Ripoll, P. (1975). Minutes: mutants of drosophila autonomously affecting cell division rate. Dev. Biol. 42, 211-221.

2. Clavería, C., and Torres, M. (2016). Cell competition: mechanisms and physiological roles. Annu. Rev. Cell Dev. Biol. 32, 411-439.

3. Maruyama, T., and Fujita, Y. (2017). Cell competition in mammals – novel homeostatic machinery for embryonic development and cancer prevention. Curr. Opin. Cell Biol. 48, 106–112.

4. Levayer, R., Duport, C., and Moreno, E. (2016). Tissue crowding induces caspase-dependent competition for space. Curr. Biol. 26, 670–677.

5. Brás-Pereira, C., and Moreno, E. (2018). Mechanical cell competition. Curr. Opin. Cell Biol. 51, 15–21.

6. Moreno, E., Valon, L., Levillayer, F., and Levayer, R. (2019). Competition for space induces cell elimination through compaction-driven ERK downregulation. Curr. Biol. 29, 23–34.e8.

7. Wagtstafl, D., Goscierska, M., Kozynka, K., Duclos, G., Kucinski, I., Chessel, A., Hampton-O’Neil, L., Bradshaw, C.R., Allen, E.G., Rawlins, E.L., et al. (2016). Mechanical cell competition kills cells via induction of lethal p53 levels. Nat. Commun. 7, 11373.

8. Gradeci, D., Bove, A., Vallardi, G., Lowe, A.R., Banerjee, S., and Charras, G. (2020). Cell-scale biophysical determinants of cell competition in epithelia. bioRxiv. https://doi.org/10.1101/729731.

9. Bove, A., Gradeci, D., Fujita, Y., Banerjee, S., Charras, G., and Lowe, A.R. (2017). Local cellular neighborhood controls proliferation in cell competition. Mol. Biol. Cell. 28, 3215–3228.

10. Norman, M., Wisniewska, K.A., Lawrenson, K., Garcia-Miranda, P., Tada, M., Kajita, M., Mano, H., Ishikawa, S., Ikegawa, M., Shimada, T., and Fujita, Y. (2012). Loss of Scribble causes cell competition in mammalian cells. J. Cell Sci. 125, 59–66.

11. Nies, V.J.M., Sancar, G., Liu, W., van Zutphen, T., Struik, D., Yu, R.T., Nasser, I.A., Flier, J.S., and Maratos-Flier, E. (2018). Deficiency of fibroblast growth factor 21 (FGF21) promotes hepatocellular carcinoma (HCC) in mice on a long term obesogenic diet. Mol. Metab. 13, 56–66.

12. Atkins, A.R., Evans, R.M., Jonker, J.W., and Downes, M.R. (2016). p53, cell competition and ribosomopathy in mammals and in Drosophila. Dev. Cell 446, 17–18.

13. de la Cova, C., Senoo-Matsuda, N., Ziosi, M., Wu, D.C., Bellosta, P., Quinzi, C.M., and Johnston, L.A. (2014). Supercompetitor status of Drosophila Myc cells requires p53 as a fitness sensor to reprogram metabolism and promote viability. Cell Metab. 19, 470–483.

14. Zhang, G., Xie, Y., Zhou, Y., Xiang, C., Chen, L., Zhang, C., Hou, X., Chen, J., Zong, H., and Liu, G. (2017). p53 pathway is involved in cell competition during mouse embryogenesis. Proc. Natl. Acad. Sci. USA 114, 498–503.

15. Poembacher, I., and Vincent, J.-P. (2018). Epithelial cells release adenosine to promote local TNF production in response to polarity disruption. Nat. Commun. 9, 4675.

16. Igaki, T., Kanda, H., Yamamoto-Goto, Y., Kanuka, H., Kurana, E., Aigaki, T., and Miura, M. (2002). Eiger, a TNF superfamily ligand that triggers the Drosophila JNK pathway. EMBO J. 21, 3009–3018.

17. Igaki, T., Pastor-Pareja, J.C., Aonuma, H., Miura, M., and Xu, T. (2009). Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Dev. Cell 16, 458–465.

18. Ohsawa, S., Sugimura, K., Takino, K., Xu, T., Miyawaki, A., and Igaki, T. (2011). Elimination of oncogenic neighbors by JNK-mediated engulfment in Drosophila. Dev. Cell 20, 315–326.
38. Yamamoto, M., Ohsawa, S., Kunimasa, K., and Igaki, T. (2017). The ligand Sas and its receptor PTP10D drive tumour-suppressive cell competition. Nature 542, 246–250.
39. Ellenbroek, S.I.J., Iden, S., and Collard, J.G. (2012). Cell polarity proteins and cancer. Semin. Cancer Biol. 22, 208–215.
40. Parnas, O., Jovanovic, M., Eisenhaure, T.M., Herbst, R.H., Dixit, A., Ye, C.J., Przybylski, D., Platt, R.J., Tiross, I., Sanjana, N.E., et al. (2015). A genome-wide CRISPR screen in primary immune cells to dissect regulatory networks. Cell 162, 675–686.
41. Campeau, E., Ruhl, V.E., Rodier, F., Smith, C.L., Rahmberg, B.L., Fuss, J.O., Campisi, J., Yaswen, P., Cooper, P.K., and Kaufman, P.D. (2009). A versatile viral system for expression and depletion of proteins in mammalian cells. PLoS ONE 4, e6529.
42. Stewart, S.A., Dykxhoorn, D.M., Palliser, D., Mizuno, H., Yu, E.Y., An, D.S., Sabatini, D.M., Chen, I.S.Y., Hahn, W.C., Sharp, P.A., et al. (2003). Lentivirus-delivered stable gene silencing by RNAi in primary cells. RNA 9, 493–501.
43. Sanjana, N.E., Shalem, O., and Zhang, F. (2014). Improved vectors and genome-wide libraries for CRISPR screening. Nat. Methods 11, 783–784.
44. Hsu, P.D., Scott, D.A., Weinstein, J.A., Ran, F.A., Konermann, S., Agarwala, V., Li, Y., Fine, E.J., Wu, X., Shalem, O., et al. (2013). DNA targeting specificity of RNA-guided Cas9 nucleases. Nat. Biotechnol. 31, 827–832.
45. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682.
46. Thielicke, W., and Stamhuis, E.J. (2014). PIVlab – towards user-friendly, affordable and accurate digital particle image velocimetry in MATLAB. J. Open Res. Softw. 2, e30.
## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Goat polyclonal anti-Scrib (Scribble) | Santa Cruz Biotechnology | Cat#sc-11049; RRID: AB_2254275 |
| Goat polyclonal anti-FGF-21 (FGF21) | R&D systems | Cat#AF3057; RRID: AB_2104611 |
| Rat monoclonal anti-α Tubulin (α-tubulin; clone YL1/2) | Santa Cruz Biotechnology | Cat#sc-53029; RRID: AB_793541 |
| Rabbit monoclonal anti-phospho-p38 MAPK (p-p38; clone D3F9, Thr180/Tyr182 in human p38) | Cell Signaling Technology | Cat#4511; RRID: AB_2139682 |
| Mouse monoclonal anti-p38α MAPK (p38; clone L53F8) | Cell Signaling Technology | Cat#9228; RRID: AB_490886 |
| Mouse monoclonal anti-Actin (Actin; clone AC-40) | Sigma-Aldrich | Cat#A3853; RRID: AB_262137 |
| Rabbit monoclonal anti-phospho-ASK1 (p-ASK1; Thr838 in human ASK1) | 25 | N/A |
| Rabbit monoclonal anti-ASK1 (ASK1; clone EP535Y) | Abcam | Cat#ab45178; RRID: AB_722915 |
| Rabbit polyclonal anti-FGFR1 (FGFR1) | Sigma-Aldrich | Cat#SAB4300488; RRID: AB_10621892 |
| Mouse anti-goat IgG-HRP | Santa Cruz Biotechnology | Cat#sc-2354; RRID: AB_628490 |
| Goat anti-rabbit IgG, HRP-linked | Cell Signaling Technology | Cat#7074; RRID: AB_2099233 |
| Horse anti-mouse IgG, HRP-linked | Cell Signaling Technology | Cat#7076; RRID: AB_330924 |
| Goat anti-rat IgG, HRP-linked | Cell Signaling Technology | Cat#7077; RRID: AB_10694715 |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| AZD4547 | ChemScene | CatCS-0971; CAS: 1035270-39-3 |
| Blasticidin S HCl | Invitrogen | Cat#A1113903; CAS: 3513-03-9 |
| CellTracker Red CMTPX Dye | Invitrogen | Cat#C34552 |
| G418 | Invitrogen | Cat#10131-035; CAS: 108321-42-2 |
| Hexadimethrine bromide (Polybrene) | Nacalai Tesque | Cat#17736-44; CAS: 28728-55-4 |
| Hoechst 33258 | Dojindo | Cat#C343-07961; CAS: 23491-45-4 |
| Lipofectamine RNAiMAX | Invitrogen | Cat#13778-150 |
| PH-797804 | Selleck Chemicals | Cat#S2726; CAS: 586379-66-0 |
| Phosphatase inhibitor cocktail | This paper | N/A |
| Polyethyleneimine “MAX” | Polysciences | Cat#24765; CAS: 49553-93-7 |
| Puromycin | GIBCO | Cat#A11138-03; CAS: 53-79-2 |
| SB202190 | Cayman Chemicals | Cat#10010399; CAS: 152121-30-7 |
| SB202474 | Cayman Chemicals | Cat#18749; CAS: 172747-50-1 |
| SB203580 | Calbiochem | Cat#559389; CAS: 152121-47-6 |
| Tetracycline | Sigma-Aldrich | Cat#T7660; CAS: 64-75-5 |
| **Deposited Data** |        |            |
| DNA microarray data | This paper | GEO: GSE153186 |

### Experimental Models: Cell Lines

| Canis: wild-type MDCK (WT) cells | 10 | N/A |
| Canis: MDCK-pTTR scribble shRNA (scribKO) cells | 10 | N/A |
| Canis: FGFR1 KO #1 MDCK cells | This paper | N/A |
| Canis: FGFR1 KO #2 MDCK cells | This paper | N/A |
| Canis: Non-targeting sgRNA-expressing MDCK cells | This paper | N/A |
| Canis: FGFR1:ires-GFP-expressing MDCK cells | This paper | N/A |
| Canis: GFP-expressing MDCK cells | This paper | N/A |
| Canis: tdTomato-expressing MDCK cells | This paper | N/A |

(Continued on next page)
### REAGENT or RESOURCE SOURCE IDENTIFIER

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Canis: 3 x NLS-tagged tdTomato-expressing MDCK cells | This paper | N/A |
| Human: HEK293T cells | ATCC | N/A |

### Oligonucleotides

| Control siRNA #1 (siGENOME Non-Targeting Control siRNA #1) | Dharmacon | Cat#D-001210-01 |
| Control siRNA #2 (siGENOME Non-Targeting Control siRNA #2) | Dharmacon | Cat#D-001210-02 |
| FGF21 siRNA #1 (siGENOME siRNA designed using siDESIGN Center from Dharmacon): 5’-GGUU UCGACCCUCCUGACUU-3’ | Dharmacon | N/A |
| FGF21 siRNA #2 (siGENOME siRNA designed using siDESIGN Center from Dharmacon): 5’-CGACGAUGCCAGGAGACAUU-3’ | Dharmacon | N/A |
| p38 siRNA #1 (siGENOME siRNA): 5’-CAAGGUCUCUGAGGACUUU-3’ | Dharmacon | Cat#D-003512-15 |
| p38 siRNA #2 (siGENOME siRNA): 5’-GUCAAGAAGCUUACAGAUGA-3’ | Dharmacon | Cat#D-003512-16 |
| ASK1 siRNA #1 (siGENOME siRNA designed using siDESIGN Center from Dharmacon): 5’-GCAAAUACUGAAGGGAUAAU-3’ | Dharmacon | N/A |
| ASK1 siRNA #2 (siGENOME siRNA designed using siDESIGN Center from Dharmacon): 5’-GCAAGUGCCACAGGGAUAAU-3’ | Dharmacon | N/A |
| Non-Targeting sgRNA forward: 5’-CACCGGGGGTAAGCTATGCTACGGA-3’ | 40 N/A |
| Non-Targeting sgRNA reverse: 5’-AAACTCCTCGTAATAGGCTACCGCCG-3’ | 40 N/A |
| FGFRI sgRNA #1 forward (designed using CRISPR Design Tool): 5’-CACCGTGTTCAGGCAAGGTCGGGGC-3’ | This paper | N/A |
| FGFRI sgRNA #1 reverse (designed using CRISPR Design Tool): 5’-AAACGCCCCGACCTTGCCTGAAC-3’ | This paper | N/A |
| FGFRI sgRNA #2 forward (designed using CRISPR Design Tool): 5’-CACCGCAACCGTACCCGCATCACGG-3’ | This paper | N/A |
| FGFRI sgRNA #2 reverse (designed using CRISPR Design Tool): 5’-AAACGCCGTAGCCGGGTACGGTTGC-3’ | This paper | N/A |

See Table S1 for primers of qPCR analysis.

### Recombinant DNA

| pLenti CMV/TO Puro DEST (670-1) | Addgene Plasmid | Cat#17293 |
| pCMV-VSV-G | Addgene Plasmid | Cat#8454 |
| psPAX2 produced by Dr. Didier Trono | Addgene Plasmid | Cat#12260 |
| lentiCRISPR v2 | Addgene Plasmid | Cat#52961 |

### Software and Algorithms

| CRISPR Design Tool | 44 | https://zlab.bio/guide-design-resources |
| Fiji (ver. 2.0.0) | 45 | https://fiji.sc/ |
| HCS Studio (ver. 6.4.3) | Thermo Fisher Scientific | N/A |
| Leica Application Suite Advanced Fluorescence (ver. 2.6.3.8173) | Leica Microsystems | N/A |
| Matlab2020a | Mathworks | https://fr.mathworks.com/ |
| PIVlab | 46 | https://pivlab.blogspot.com/ |
| R (ver. 3.6.1) | R Foundation | https://www.r-project.org/ |
| RStudio (ver. 1.2.1335) | RStudio | https://rstudio.com/ |
| siDESIGN Center | Dharmacon | http://dharmacon.horizondiscovery.com |
| Universal Probe Library Assay Design Center | Roche | https://lifescience.roche.com/global_en.html |

### Other

| Cell Imaging Plate, 24-well | Eppendorf | Cat#0030741021 |
| Culture-Insert 2well | ibidi GmbH | Cat#ib80209 |
RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Hidenori Ichijo (ichijo@mol.f.u-tokyo.ac.jp).

Materials Availability
Reagents generated in this study will be made available on request.

Data and Code Availability
Microarray data have been deposited in the NCBI Gene Expression Omnibus under accession number GSE153186.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines and cell culture
Wild-type MDCK (WT) cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM)-high glucose (Sigma-Aldrich, Cat#D5796) supplemented with 10% fetal bovine serum (FBS: BioWest, Cat#S1560-500 or GIBCO, Cat#10270-106) and 100 units/ml penicillin G (Meiji Seika, Cat#01028-85) in a 5% CO₂ atmosphere at 37°C supplemented with 10% fetal bovine serum (FBS: BioWest, Cat#S1560-500 or GIBCO, Cat#10270-106) and 100 units/ml penicillin G. Wild-type MDCK (WT) cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM)-high glucose (Sigma-Aldrich, Cat#D5796) supplemented with 10% fetal bovine serum (FBS: BioWest, Cat#S1560-500 or GIBCO, Cat#10270-106) and 100 units/ml penicillin G.

Cell lines and cell culture
Wild-type MDCK (WT) cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM)-high glucose (Sigma-Aldrich, Cat#D5796) supplemented with 10% fetal bovine serum (FBS: BioWest, Cat#S1560-500 or GIBCO, Cat#10270-106) and 100 units/ml penicillin G (Meiji Seika, Cat#01028-85) in a 5% CO₂ atmosphere at 37°C. MDCK-pTR scribble shRNA (scribbleKD) cells were constitutively expressing GFP with tetracycline-inducible scribble shRNA [10]. scribbleKD cells were cultured at the same conditions with WT cells, except that blasticidin (Invitrogen, Cat#A1113903) and G418 (Invitrogen, Cat#10131035) were added to the culture medium at a final concentration of 5 μg/ml and 800 μg/ml, respectively. To establish MDCK cell lines that stably express a red fluorescent protein (tdTomato), a nuclear red fluorescent protein (NLS-tdTomato), a green fluorescent protein (GFP), or FGF21-ires-GFP, tdTomato, GFP, or FGF21-IRES-GFP was cloned into a pLenti CMV/TO Puro DEST (670-1) (Addgene, Cat#17293) [41], and lentivirus infection was performed. Briefly, to produce lentivirus, HEK293T cells were transfected with the constructed plasmid, pCMV-VSV-G (Addgene, Cat#8454) [42], and psPAX2 (Addgene, Cat#12260) using PEI-MAX (Polysciences, Cat#24765). After cells were incubated for 24 hours, the culture medium was replaced with fresh medium supplemented with 1% BSA (Iwai Chemicals, Cat#A001). Lentivirus-containing culture supernatants were collected after 24 and 48 hours and filtered through a 0.45 μm-pore size filter (Millipore, Cat#SLHV033RS). WT cells were infected with the lentivirus in the presence of 8 μg/ml Polybrene (Hexadimethrine Bromide: Nacalai tesque, Cat#17736-44) and cultured for 18 hours. After cells were incubated for 24 hours with fresh medium, the selection was carried out with 1.0 μg/ml puromycin (Invitrogen, Cat#A11138-03) for 2–3 days. Cells were used for experiments immediately. FgfR1 knockout (KO) MDCK cells were generated using the CRISPR/Cas9 system. The sgRNA sequences were inserted into lentCRISPR v2 (Addgene, Cat#52961) [39]. These sequences were designed using the CRISPR Design Tool (https://zlab.bio/guide-design-resources) [44] or derived from the previous studies [45]. Pools of FgfR1 KO cells were established using the same protocol of lentivirus infection as mentioned above, and cells were used for experiments immediately. Knockout efficiency of FgfR1 was verified by western blotting analysis. The protein expression of FgfR1 in these cell lines was not completely abolished following puromycin selection, possibly because some cells incorporated only the puromycin resistance gene. All cell lines were verified to be negative for mycoplasma.

METHOD DETAILS

Treatment
Reagents were used at the following conditions. For Scribble knockdown, culture medium with tetracycline (Sigma-Aldrich, Cat#T7660) was added to cells at a final concentration of 5 μg/ml 6 hours after plating. For nuclear staining in Figures 2A, 2C, 2E, 4H, S1E, and S1F, cells were stained with Hoechst 33258 (Dojindo, Cat#343-07961) at 5 μg/ml at 5°C. For the cell lysis in Figure 2E, AZD4547 (ChemScene, Cat#CS-0971) dissolved in DMSO (>99.5% purity: Sigma-Aldrich, Cat#D5879) was added to cells at a final concentration of 5 μM (0.1% v/v DMSO) 36 hours before cells were fixed. For the cell lysis in Figure 4C, cells were treated with the following chemical compounds including PH-797804 (Selleck Chemicals, Cat#S2726), SB202190 (Cayman Chemicals, Cat#10010399), SB203580 (Calbiochem, Cat#559389), and SB202474 (Cayman Chemicals, Cat#18749) dissolved in DMSO at a final concentration of 5 μM (0.1% v/v DMSO) 12 or 24 hours before lysis.

Cell lysis and western blotting
Cells were plated in a 12-well plate or a 24-well plate at a density of 1.54 × 10⁵ or 7.7 × 10⁴ cells/well, respectively. Cells were treated with tetracycline 6 hours after plating and lysed 60–69 hours after tetracycline addition, unless otherwise stated. Before lysis, the supernatants were collected and mixed with an equal amount of 2 x SDS sample buffer (80 mM Tris-HCl pH 8.8, 80 μg/mL bromophenol blue, 28.8% glycerol, 4% SDS, and 10 mM dithiothreitol) to detect secreted FGF21 in the culture medium. Cells were lysed in a lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5% deoxycholate, 0.1% sodium deoxycholate, and 1% NP-40) supplemented with 1 mM phenylmethylsulfonfluryl fluoride (PMSF), 5 μg/mL leupeptin, and phosphatase inhibitor cocktail (8 mM NaF, 12 mM β-glycerophosphate, 1 mM Na₂VO₄, 1.2 mM Na₃MoO₄, 5 μM cantharidin, and 2 mM imidazole). The lysates...
were clarified by centrifugation for 10 min, and the supernatants were sampled by adding an equal amount of 2 × SDS sample buffer.

The protein concentration of the lysates was determined using DC Protein Assay (Bio-Rad, Cat#5000116JA) or Protein Assay BCA kit (Wako, Cat#294-7311), and the absorbance was measured at 562 nm (DC Protein Assay) or 592 nm (Protein Assay BCA kit) by a Varioskan Flash (Thermo Fisher Scientific). After boiling at 98 °C for 3 min, each sample was normalized to equivalent protein concentration, resolved by SDS-PAGE, and electroblotted onto a FluoroTrans W membrane (Pall, Cat#BSP0161) or an Immobilon-P membrane (Millipore, Cat#P8112). The membranes were blocked with 5% skim milk (Megmilk Snow Brand) in TBS-T (20 mM Tris-HCl pH 8.0, 137 mM NaCl, and 0.1% Tween 20) and then probed with the appropriate primary antibodies diluted by 1:100 to 1:1000 in 2% skim milk. Immunoreactive proteins were detected on X-ray films (FUJIFILM, Cat#47410-22167 or Cat#47410-26615) using an ECL system (GE Healthcare). Quantification was performed via densitometry using Fiji (ver. 2.0.0) software, using α-tubulin or Actin to normalize the samples. Quantified individual values and the mean ± SEM are presented as gray points and black bars, respectively. Kinase activity was defined as the ratio of phospho-protein to total protein. Representative images were adjusted linearly to the appropriate brightness using Fiji software. Representative data are shown in all western blotting and more than two additional experimental replicates showed similar results.

siRNA-mediated knockdown of FGF21, p38, and ASK1 was performed using Lipofectamine RNAiMAX Reagent (Invitrogen, Cat#133778-150) and Opti-MEM (Invitrogen, Cat#31985). Cells were reverse transfected at a final concentration of 10 or 30 nM.

**Cell competition assays**

WT and scribKO cells were mixed at a ratio of 3:1 and seeded in a 96-well plate at a density of 1.54 × 10^5 cells/well. Cells were treated with tetracycline 6 hours after plating and fixed with 3.7% paraformaldehyde (Wako, Cat#064-00406) in PBS 60–62 hours after tetracycline addition. The cells were permeabilized with PBS containing 1% Triton X-100 (Sigma-Aldrich, Cat#T9284) and stained with Hoechst 33258. The plate was measured and analyzed using CellInsight NXT (Thermo Fisher Scientific) with the optimized Cell Health Profiling BioApplication (Figures S1F and S1G). Representative images are shown and more than two additional experimental replicates showed similar results. Representative images were adjusted linearly to the appropriate brightness using Fiji software. Regarding the quantification of cell density in Figure S1B, we used Hoechst staining as a mask to segment the nuclei and used GFP intensity to determine the area of scribKO cells. We calculated the area of WT cells as the total area subtracted by the GFP-positive area of scribKO cells. Cell density was calculated as the number of the nuclei divided by the area.

**Quantitative PCR analysis**

Cells were seeded in a 12-well plate or a 24-well plate at a density of 1.54 × 10^5 or 7.7 × 10^5 cells/well, respectively. Cells were treated with tetracycline 6 hours after plating. Sixty to sixty-five hours after tetracycline addition (unless otherwise indicated, total RNA was isolated from cells using Isogen (Wako, Cat#319-90211) and reverse transcribed with ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Cat#F82-301), according to manufacturer’s instruction. Primers were designed using the Universal Probe Library Assay Design Center (Roche). Quantitative PCR was carried out using a LightCycler 96 (Roche) using FastStart Essential DNA Green Master (Roche, Cat#06924204001) or Kapa SYBR Fast qPCR Master Mix (Kapa Biosystems, Cat#KK4602). Data were normalized to GAPDH, and primer sequences are listed in Table S1.

**Live-cell imaging of cultured cells**

Cells were observed by a TCS SP5 confocal microscope (Leica Microsystems) using a 20 × 0.70 NA dry at 512 × 512 resolution. To perform time-lapse microscopy, cells were cultured in a humified stage top incubator (Tokai Hit, Cat#STXF-WSKMX-SET) at 37 °C with 5% CO₂. Imaging frequency was every 20 min as indicated in the movie time stamps. Representative images were adjusted linearly to the appropriate brightness using Fiji software.

For the cell competition assay in Figure S1A, WT and scribKO cells were mixed at a ratio of 3:1 and plated at a density of 7.7 × 10^4 cells/well in a 24-well glass bottom plate (Eppendorf, Cat#0030741021). Cells were treated with tetracycline 6 hours after plating, and live-cell imaging was performed from 37 to 57 hours after tetracycline addition. More than two additional experimental replicates showed similar results.

For the colony assay in Figure 1A and Video S1, tdTomato-expressing WT and scribKO cells were mixed at a ratio of 1:1 and plated at a density of 2.5 × 10^4 cells/well in a 24-well glass bottom plate. Cells were treated with tetracycline 6 hours after plating, and live-cell imaging was performed from 53 to 68 hours after tetracycline addition.

For the conditioned medium assay in Figures 1B and 1F and Video S2, scribKO cells were seeded in a 6-well plate at a density of 5.0 × 10^4 cells/well. The culture medium of scribKO cells was replaced with 1 mL fresh medium 46 hours after tetracycline addition. The conditioned medium of scribKO cells was collected 18 hours after replacing the fresh medium with tetracycline and filtered through a 0.45 μm-pore size filter. On the other hand, WT cells stably expressing with NLS-tdTomato were seeded in a 24-well glass bottom plate at a density of 2.5 × 10^4 cells/well, and live-cell imaging was performed 2 days after plating. The supernatant of recipient WT cells was replaced with conditioned medium of scribKO cells after the start of imaging. Displacement of cell movement was acquired every 20 min by tracking the position of the centroid of each cell nucleus using a red fluorescent marker. We discarded the data of the divided cells during the imaging for further analysis for accurate analysis.
For the colony assay in Figure 3A and Video S3, WT and scrib<sup>KD</sup> cells were mixed at a ratio of 1:1 and plated at a density of 1.0 x 10<sup>4</sup> cells/well in a 24-well glass bottom plate. Culture medium with or without tetracycline was added 20–26 hours after seeding, and live-cell imaging was performed 27–35 hours after tetracycline addition. Displacement of colony was acquired every 2 hours for 10 hours by tracking the position of the centroid of WT colonies. We analyzed the movies where WT colonies encountered with scrib<sup>KD</sup> colonies within 30–50 hours after tetracycline treatment. In Figure S2A, we carried out particle image velocimetry (PIV) analysis using the PIVlab package for MATLAB<sup>46</sup>.

For the colony assay in Figure 3D and Video S4, WT cells expressing NLS-tdTomato and WT cells expressing GFP or FGF21-IRES-GFP were mixed at a ratio of 1:1 and plated at a density of 5.0 x 10<sup>3</sup> or 1.0 x 10<sup>4</sup> cells/well in a 24-well glass bottom plate. Live-cell imaging was performed 48 hours after plating. Displacement of colony was acquired every 2 hours for 10 hours by tracking the position of the centroid of WT colonies expressing NLS-tdTomato.

For the cell confrontation assay in Figures 3G and S2D and Video S5, we referred to the previous report to set up the assay using cell culture inserts (ibidi GmbH, Cat#ib80209) with a modified protocol<sup>16</sup>. Briefly, WT and scrib<sup>KD</sup> cells were plated into one of the two compartments of the cell culture insert at a density of 3.0 or 4.0 x 10<sup>4</sup> cells/insert in a 24-well glass bottom plate. Cells were cultured for 5–7.5 hours before the inserts were removed. To suppress dome formation after cells are confluent, the culture medium was replaced with DMEM supplemented with 1% FBS 23–26 hours after removing the inserts. Live-cell imaging was performed 14–24 hours after switching the culture medium. In Figure S2D, one of the two populations (the right one) was stained with CellTracker Red CMTPX Dye (Invitrogen, Cat#C34552) at 20 µM before plating. The area of GFP-positive or red CMTPX-labeled cells was acquired every 20 min and measured using the analyze particle plugin in Fiji software (Figure S2C). We analyzed the movies where WT and scrib<sup>KD</sup> cells collided within 25–45 hours after replacing the culture medium. In Figures S2G–S2I, to collect conditioned medium of scrib<sup>KD</sup> cells, scrib<sup>KD</sup> cells were seeded in a 6-well plate at a density of 4.0 or 5.0 x 10<sup>5</sup> cells/well. The culture medium of scrib<sup>KD</sup> cells was replaced with fresh medium 46 hours after tetracycline addition. The conditioned medium of scrib<sup>KD</sup> cells was collected 18–24 hours after replacing the fresh medium and filtered through a 0.45 µm-pore size filter. Tetracycline was added to the collected medium cultured without tetracycline. On the other hand, WT and scrib<sup>KD</sup> cells were plated into the cell culture inserts as shown above. The conditioned medium of scrib<sup>KD</sup> cells was added to recipient cells before the start of imaging.

**DNA microarray analysis**

scrib<sup>KD</sup> cells were plated in a 12-well plate at a density of 1.54 x 10<sup>5</sup> cells/well. Cells were treated with tetracycline 6 hours after plating. Using Isogen, total RNA was extracted at 30, 45, and 60 hours after tetracycline addition from three independent wells of the same condition and mixed. RNA integrity was verified with an Agilent 2100 BioAnalyzer. Sense-strand DNA was generated from total RNA, fragmented, and labeled following the Agilent One-Color Microarray-Based Gene Expression Analysis protocol. Hybridization and scanning were performed by Canine (V2) Gene Expression Microarray (Agilent Technology, Cat#G2519F-021193). The data have been deposited in the NCBI Gene Expression Omnibus under accession number GSE153186.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All data are represented as the mean ± SEM, unless otherwise specified. The investigators were not blinded to the group allocation during the experiment. Statistical tests were performed using R (ver. 3.6.1) with RStudio (ver. 1.2.1335). For all statistical analyses, *p < 0.05, **p < 0.01, and ***p < 0.001. Unpaired two-tailed Student’s t test, one-way ANOVA followed by Dunnett’s multiple comparisons test, or one-way ANOVA followed by Tukey-Kramer’s multiple comparisons test was used in this study.