



Title	Expression of Indian hedgehog signaling in murine oviductal infundibulum and its relationship with epithelial homeostasis
Author(s)	Hosotani, Marina; Ichii, Osamu; Namba, Takashi; Masum, Md Abdul; Nakamura, Teppei; Hasegawa, Yasuhiro; Watanabe, Takafumi; Kon, Yasuhiro
Citation	Cell and tissue research, 391, 595-609 https://doi.org/10.1007/s00441-022-03722-w
Issue Date	2022-12-29
Doc URL	http://hdl.handle.net/2115/91042
Rights	This version of the article has been accepted for publication, after peer review (when applicable) and is subject to Springer Nature 's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at: http://dx.doi.org/10.1007/s00441-022-03722-w
Type	article (author version)
File Information	R2_Main_manuscript_kizou.pdf



[Instructions for use](#)

1 **Expression of indian hedgehog signaling in murine oviductal infundibulum and its**
2 **relationship with epithelial homeostasis**

3 **Authors:** Marina Hosotani^{1*}, Osamu Ichii^{2,3}, Takashi Namba², Md. Abdul Masum², Teppei
4 Nakamura^{2,4}, Yasuhiro Hasegawa⁵, Takafumi Watanabe¹, Yasuhiro Kon²

5 **Addresses:** ¹Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen
6 University, Ebetsu, Hokkaido 069-8501, Japan

7 ²Laboratory of Anatomy, Department of Basic Veterinary Science, Faculty of Veterinary
8 Medicine, Hokkaido University, Sapporo, Hokkaido 060-0818, Japan

9 ³Laboratory of Agrobiomedical Science, Faculty of Agriculture, Hokkaido University, Sapporo,
10 Hokkaido 060-0818, Japan

11 ⁴Department of Biological Safety Research, Chitose Laboratory, Japan Food Research
12 Laboratories, Chitose, Hokkaido 066-0052, Japan

13 ⁵Department of Food Science and Human Wellness, College of Agriculture, Food and
14 Environment Science, Rakuno Gakuen University, Ebetsu, Japan

15

16 ***Corresponding author: Marina Hosotani, DVM,**

17 Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University,
18 Midorimachi 582, Bunkyo-dai, Ebetsu 069-8501, Japan

19 Tel & Fax: +81-11-388-4763

20 Email: m-hosotani@rakuno.ac.jp

21

22 **Acknowledgements and Funding Information**

23 This work was supported in part by JSPS KAKENHI Grant (No. 19K23708 and 21K05976),

24 Rakuno Gakuen University Research Fund (No. 2020-01), Suhara Memorial Foundation Research

25 Grants, and Uehara Memorial Foundation Research Incentives Grants (M. H.). The research

26 described in this paper was presented in part and awarded at the 163rd Japanese Association of

27 Veterinary Anatomists, 14-30 September 2020, online.

28 **Abstract**

29 Homeostasis of the oviductal infundibulum epithelium is continuously regulated by signaling
30 pathways under physiological and pathological conditions. Herein, we investigated the expression
31 of hedgehog (Hh) signaling-related components in the murine oviductal infundibulum, which is
32 known to maintain homeostasis in the adult epithelium. Additionally, using autoimmune disease-
33 prone MRL/MpJ-Fas^{lpr/lpr} (MRL/lpr) mice showing abnormal morphofunction of the ciliated
34 epithelium of the infundibulum related to the oviductal inflammation, we examined the
35 relationship between Hh signaling and pathology of the infundibulum. The expression and
36 localization of Pax8, a marker for progenitor cells in the oviductal epithelium, and Foxj1, a marker
37 for ciliogenesis, were examined in the infundibulum. The results showed that Pax8 was
38 downregulated and Foxj1 was upregulated with aging, suggesting that homeostasis of the
39 infundibulum epithelium of MRL/lpr mice was disturbed at 6 months of age. In all mice, the motile
40 cilia of ciliated epithelial cells in the infundibulum harbored Hh signaling pathway-related
41 molecules: patched (Ptch), smoothened (Smo), and epithelial cells harbor Gli. In contrast, *Ptch*,
42 *Smo*, and *Gli2* were significantly downregulated in the infundibulum of MRL/lpr mice at 6 months
43 of age. The expression levels of *Pax8* and *Foxj1* were significantly positively correlated with those
44 of *Ptch1*, *Smo*, and *Gli2*. Hh signaling is thought to be involved in homeostasis of the ciliated
45 epithelium in the infundibulum. In MRL/lpr mice, which show exacerbated severe systemic

46 autoimmune abnormalities, molecular alterations in Hh signaling-related components are
47 considered to interact with local inflammation in the infundibulum, leading to disturbances in
48 epithelial homeostasis and reproductive function.

49

50 **Keywords:** autoimmune abnormality, ciliated epithelium, hedgehog signaling pathway,
51 homeostasis, oviduct

52 **Introduction**

53 Mammalian oviducts are divided into three parts, the infundibulum, ampulla, and isthmus, in
54 order from the distal to proximal parts. The different composition ratios of ciliated epithelial cells
55 and secretory cells in the epithelium of each part reflect the unique reproductive function in each
56 section (Li et al. 2017; Koyama et al. 2019). Particularly, the infundibulum epithelium is composed
57 mostly of ciliated epithelial cells, so that ciliary beating effectively moves oocytes produced in the
58 ovaries into the oviductal lumen; this process is known as oocyte pick-up. Oocyte pick-up and
59 transportation are disturbed by abnormal ciliary morphofunction in the infundibulum caused by
60 pathological conditions such as smoking (Talbot and Riveles 2005), hormonal dysregulation (Raidt
61 et al. 2015), and inflammation (Hosotani et al. 2020). Physiologically, the proportion of ciliated
62 epithelial cells and secretory cells in the infundibulum epithelium changes under hormonal
63 dynamics through the estrous cycle (Ito et al. 2016). In addition, the infundibulum is constantly
64 exposed to follicular fluid containing inflammatory molecules during each ovulation, which
65 damages the ciliary epithelium (Palma-Vera et al. 2017). Therefore, the infundibulum epithelium
66 continuously undergoes epithelial turnover to maintain its healthy histology and reproductive
67 function under both physiological and pathological conditions.

68 Adult epithelial homeostasis is maintained by the proliferation and differentiation of epithelial
69 stem cells, which are regulated by activation of signaling pathways such as the Wnt/ β -catenin,

70 Notch, and Hedgehog (Hh) signaling pathways (Sancho et al. 2004; Carlier et al. 2020). In
71 oviductal epithelial homeostasis, secretory cells act as progenitors by self-renewing and/or
72 differentiating into ciliated epithelial cells (Ghosh et al. 2017). The molecular mechanism
73 underlying these effects is not fully understood; however, several studies have focused on the
74 involvement of Wnt/ β -catenin signaling (Ghosh et al. 2017) and Notch signaling (Zhu et al. 2019)
75 in homeostasis of the oviductal epithelium. In contrast, although homeostasis of the adult tracheal
76 ciliated epithelium is maintained by activation of the Hh signaling pathway (Peng et al. 2015), the
77 involvement of this pathway in maintaining the oviductal epithelium has not been explored.

78 In a murine model of systemic autoimmune disease, MRL/MpJ-Fas^{lpr/lpr} (MRL/lpr) mice
79 develop severe inflammation of the lamina propria in the oviductal infundibulum. Chronic
80 abnormal immune conditions result in abnormal morphofunction in the ciliated epithelium, such
81 as decreased numbers of ciliated epithelial cells, elongation of the cilia, and disorientation of ciliary
82 beating (Hosotani et al. 2018, 2020). These pathological conditions are closely related to
83 disturbances in epithelial homeostasis but the underlying molecular mechanism is unclear.

84 In this study, we investigated expression of the Hh signaling pathway in the murine
85 infundibulum epithelium and its relationship with homeostasis of the infundibulum epithelium. In
86 addition, we examined the expression of Hh signaling pathway-related molecules in MRL/lpr mice
87 as a destruction model of the infundibulum epithelium and compared the results with those

- 88 obtained the healthy infundibulum of C57BL/6N mice (B6) as a general strain and MRL/MpJ mice
89 (MRL/+) as wild-type MRL/lpr mice.

90 **Material and methods**

91 *Animals*

92 Animal experiments were approved by the School of Veterinary Medicine, Rakuno Gakuen
93 University (approval no. VH19A6). The animals were handled in accordance with the Guide for
94 the Care and Use of Laboratory Animals, Rakuno Gakuen University, Japan. Female B6, MRL/+,
95 and MRL/lpr mice at 3 and 6 months of age were obtained from Japan SLC, Inc. (Hamamatsu,
96 Shizuoka, Japan). Previous studies reported that autoimmune disease is severely exacerbated in
97 female MRL/lpr mice at 6 months of age compared to at 3 months of age (Hosotani et al. 2018,
98 2020). The mice were housed in groups within plastic cages at 18–26°C under a 12-h light/dark
99 cycle and had free access to a commercial diet and water. The estrous cycle of each mouse under
100 the natural estrous cycle was confirmed by monitoring vaginal smears (Byers et al. 2012). All mice
101 were euthanized by either severing the carotid artery or cervical dislocation under deep anesthesia
102 using a mixture of medetomidine (0.3 mg/kg), midazolam (4 mg/kg), and butorphanol (5 mg/kg).
103 The spleen was collected from the mice and weighed as a marker of autoimmune disease.

104

105 *Immunostaining*

106 Mouse female reproductive organs were collected and fixed with 4% paraformaldehyde at 4°C
107 overnight, embedded in paraffin, and cut into 3- μ m-thick sections, which were then used for

108 immunohistochemistry (IHC) and immunofluorescence (IF). Detailed information on the
109 antibodies, antigen retrieval, and serum blocking is listed in Table 1. The sections were incubated
110 in 20 mM Tris-HCl (pH 9.0) for 15 min at 110°C, 10 mM citrate buffer (pH 6.0) for 15 min at
111 110°C, or 0.1% pepsin for 5 min at 37°C. The sections for IHC were soaked in methanol containing
112 0.3% hydrogen peroxide. Sections incubated with blocking serum for 60 min at room temperature
113 were incubated overnight at 4°C with primary antibodies. Negative controls were performed with
114 normal mouse IgG (sc-2025, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), normal rat
115 IgG (sc-2026, Santa Cruz Biotechnology) and normal rabbit IgG (sc-2027, Santa Cruz
116 Biotechnology). After three washes in 0.01 M PBS, the sections were incubated with secondary
117 antibodies for 30 min and washed. The sections for IHC were incubated for 30 min at room
118 temperature, using a streptavidin-biotin complex (SABPRO Kit, Nichirei, Tokyo, Japan), and then
119 incubated with 3,3'-diaminobenzidine tetrahydrochloride-hydrogen peroxide solution, and lightly
120 stained with hematoxylin. The stained sections of the outer infundibulum were examined using a
121 BZ-X710 microscope (Keyence, Osaka, Japan).

122 Three semi-serial sections stained with IHC for detection of Foxj1 with 50- μ m intervals were
123 used, and the percentage of expelled non-ciliated epithelial cells and the percentage of Foxj1
124 positive expelled non-ciliated epithelial cells were calculated as follows, respectively: percentage
125 of expelled non-ciliated epithelial cells (%) = $100 \times$ number of expelled non-ciliated epithelial

126 cells / number of total 80-100 epithelial cells in the field. Percentage of Foxj1 positive expelled
127 non-ciliated epithelial cells (%) = $100 \times$ number of Foxj1 positive expelled non-ciliated epithelial
128 cells / number of total 10-20 expelled non-ciliated epithelial cells in the field.

129

130 ***Reverse Transcription and Quantitative Real-time Polymerase Chain Reaction***

131 The oviducts were manually separated into the proximal (including the isthmus and ampulla)
132 and distal (including infundibulum) parts and homogenized using a BioMasher (Nippi Inc., Tokyo,
133 Japan). Total RNA was purified using the NucleoSpin® RNA Plus kit (Macherey-Nagel, Düren,
134 Germany) according to the manufacturer's instructions. The purified total RNA was used as a
135 template to synthesize cDNA using ReverTra Ace qPCR RT Master Mix (Toyobo Co., Ltd., Osaka,
136 Japan). Quantitative real-time polymerase chain reaction (qPCR) analysis of the cDNA was
137 performed using THUNDERBIRD SYBR qPCR Mix (Toyobo Co., Ltd.) and gene-specific
138 primers (Table 2, Sigma-Aldrich, St. Louis, MO, USA). The qPCR cycling conditions were 95°C
139 for 1 min, followed by 40 cycles of 95°C for 15 s and 52°C or 60°C for 45 s. Data were normalized
140 against the expression level of actin, beta (*Actb*) and analyzed using the Δ Ct method to compare
141 the expression of genes encoding hedgehog ligands; the $\Delta\Delta$ Ct method was used to compare the
142 expression of other genes.

143

144 ***In Situ Hybridization***

145 Paraformaldehyde-fixed paraffin-embedded specimens of female reproductive organs were
146 cut into 5 μ m-thick sections, air-dried overnight, and baked in an oven for 60 min at 60°C. RNA *in*
147 *situ* hybridization was performed using an RNAscope® 2.5 HD Detection Regent-Brown kit
148 (Advanced Cell Diagnostics, Newark, CA, USA) according to the manufacturer's instructions. The
149 RNAscope® positive control probe-Mm-Polr2a (Cat. No. 312471, Advanced Cell Diagnostics),
150 RNAscope® negative control probe-DapB (Cat. No. 310043, Advanced Cell Diagnostics),
151 RNAscope® probe-Mm-Shh (Cat. No. 314361, Advanced Cell Diagnostics), RNAscope® probe-
152 Mm-Dhh (Cat. No. 415031, Advanced Cell Diagnostics), and RNAscope® probe-Mm-Ihh-noXHs
153 (Cat. No. 413091, Advanced Cell Diagnostics) were used.

154

155 ***Statistical Analysis***

156 The results are expressed as the mean \pm standard error (s.e.). Data among three or more groups
157 were compared using Tukey's test ($P < 0.05$). Data between two groups were compared using
158 Student's *t*-test ($P < 0.05$). Correlations between two parameters were analyzed using Spearman's
159 correlation test ($P < 0.05$). Statistical analysis was conducted using JMP 14.2.0 (SAS Institute, Inc.,
160 Cary, NC, USA).

161 **Result**

162 ***Expression and Localization of Epithelial Homeostasis-Related Molecules in the Infundibulum***

163 In the oviductal epithelium, Pax8 is a marker of secretory cells (i.e., progenitor cells), whereas
164 Foxj1 is a marker of ciliogenesis. Pax8 was localized in the nucleus of secretory cells in the
165 infundibulum of mice, except for in MRL/lpr mice at 6 months of age (Figure 1a-c and a'-c').
166 Foxj1 was localized in the nucleus of ciliated epithelial cells in the infundibulum of mice, except
167 for MRL/lpr mice at 6 months of age, in which Foxj1 expression was observed not only in the
168 nucleus of ciliated epithelial cells as well as that of non-ciliated epithelial cells expelled from the
169 epithelium (Figure 1d-f and d'-f'). There was no significant differences in the percentage of
170 expelled non-ciliated epithelial cells composing the infundibulum epithelium among the strains
171 and ages (Figure 1h), while the percentage of Foxj1 positive expelled non-ciliated epithelial cells
172 was significantly higher in MRL/lpr mice at 6 months of age than other strains and MRL/lpr mice
173 at 3 months of age (Figure 1i).

174 We compared the transcriptional expression levels of *Pax8* and *Foxj1* in the infundibulum as
175 the distal part of the oviduct with those in the ampulla and isthmus as the proximal part (Figure 2a
176 and b). *Pax8* expression was lower in the distal part than in the proximal part of all strains at 3
177 months of age. In the entire oviduct of MRL/lpr mice at 6 months of age, *Pax8* expression showed
178 an age-related decrease and was significantly lower than that in the other strains. In contrast, *Foxj1*

179 expression was higher in the distal part than in the proximal part of all mice. In the entire oviduct
180 of MRL/lpr, *Foxj1* expression was higher than in other strains, particularly at the distal part which
181 showed a significant age-related increase.

182

183 ***Expression and Localization of Hedgehog Signaling-Related Molecules in Infundibulum***

184 The canonical Hh signaling pathway in primary cilia is activated by binding of Hh ligands such
185 as sonic, indian, and desert Hh (Shh, Ihh, and Dhh, respectively) to the transmembrane protein
186 patched (Ptch), followed by an interaction with smoothened (Smo) and the activation of GLI
187 family zinc finger 1–3 (Gli1–3) transcription factors (Briscoe and Thérond 2013). There were no
188 significant differences in the expression of Hh signaling pathway-related genes during the estrous
189 cycle (Figure 4a-e); therefore, we focused on the infundibulum of mice at estrus. In all mice, Ptch1
190 and Smo were localized in the cilia of ciliated epithelial cells in the infundibulum (Figure 3a-a''''',
191 b-b''''', c-c''''', d-d''''', e-e''''', and f-f'''''). The fluorescence intensity of Ptch1 and Smo in the
192 infundibulum did not differ between mice of different strains and ages. In B6 and MRL/+ mice,
193 Gli2 was highly expressed in the nucleus of secretory cells and faintly in the nucleus of ciliated
194 epithelial cells in the infundibulum (Figure 3g-g''''', h-h'''''). In MRL/lpr mice at both 3 and 6
195 months of age, Gli2 was ubiquitously observed in the nuclei of cells comprising the infundibulum
196 epithelium (Figure 3i-i''''').

197 In transcriptional analysis, *Ptch1* expression did not significantly differ among the oviductal
198 parts of all mice (Figure 5a). *Ptch1* expression showed an age-related decrease in the proximal part
199 of B6 and at both the proximal and distal parts in MRL/lpr mice. In the entire oviduct of MRL/lpr
200 mice, *Ptch1* expression was significantly lower than that in B6 or/and MRL/+ mice. *Smo*
201 expression was significantly higher in the distal part than in the proximal part of B6 and MRL/+
202 mice at 3 months of age (Figure 5b). *Smo* expression showed an age-related decrease at the distal
203 part in all mice. In the entire oviduct of MRL/lpr, *Smo* expression was significantly lower than that
204 in the other strains. *Gli1* expression was significantly lower at the distal part than at the proximal
205 part: *Gli2* and *Gli3* expression showed no significant differences among the oviductal parts (Figure
206 5c-e). In the entire oviduct of MRL/lpr, *Gli1* expression showed an age-related decrease and was
207 significantly lower than that in the other strains (Figure 5c). The mean C_t value of *Gli1* expression
208 in the distal parts was high above around 32 in all mice (data not shown), so that the localization
209 level of *Gli1* protein seems to be very low in the distal part. In the entire oviduct of MRL/lpr, *Gli2*
210 expression was significantly higher than in other strains, particularly at the distal part at 6 months
211 of age (Figure 5d). *Gli3* expression tended to be higher in the entire oviduct of MRL/lpr mice at 3
212 months of age than in other strains, whereas that of MRL/lpr at 6 months of age showed an age-
213 related decrease and was significantly lower than that in other strains (Figure 5e).

214 Among the transcription factors of the hedgehog ligands (*Shh*, *Dhh*, and *Ihh*), *Shh* and *Dhh*

215 were not expressed in the infundibulum epithelium or ovarian granulosa cells (Figure 6a-f and a'-
216 f'), whereas *Ihh* was highly expressed in ovarian granulosa cells and slightly in infundibulum
217 epithelial cells (Figure 6a''-f''). *Ihh* expression in ovarian granulosa cells was lower in MRL/lpr
218 mice than in B6 mice (Figure 6a'' and e''). The *Shh* and *Dhh* expression levels were not
219 significantly different among the oviductal parts, whereas *Ihh* expression was lower in the distal
220 part than in the proximal part in all mice (Figure 7a-f).

221

222 ***Relation between Hh Signaling Pathway and Oviductal Epithelial Homeostasis***

223 Correlation analysis of transcriptional expression in the oviduct of mice at 3 months of age
224 was performed (Table 3). In the proximal part, *Ptch1* and *Pax8* expression was significantly
225 positively correlated with the expression of all Hh signaling pathway-related genes. *Foxj1*
226 expression showed the same results, except for *Gli1*. In contrast, at the distal part, *Ptch1* expression
227 was significantly positively correlated with the expression of *Smo* and *Gli2* and significantly
228 negatively correlated with *Gli3* expression. *Pax8* and *Foxj1* expression was significantly positively
229 correlated with the expression of *Ptch1*, *Smo*, and *Gli2*.

230 **Discussion**

231 The molecular mechanism that maintains homeostasis of the infundibulum epithelium has been
232 greatly altered in autoimmune disease model mice. This molecular alteration causes an abnormal
233 morphology of the ciliated epithelium of the infundibulum of MRL/lpr mice at 6 months of age,
234 such as a decreased proportion of ciliated epithelial cells, elongated cilia, and disorientation of
235 ciliary alignment (Hosotani et al. 2020). The transcription factor Pax8 governs the expression of a
236 series of genes pivotal to tissue development in thyroid follicular cells and Müllerian ducts
237 (Plachov et al. 1990; Grote et al. 2006). Pax8 in the oviductal secretory cells is a marker of the
238 self-renewal and differentiation of these cells to ciliated epithelial cells (Ghosh et al. 2017);
239 therefore, the significant downregulation of Pax8 transcripts and proteins suggests that Pax8
240 attenuates epithelial homeostasis in the infundibulum of MRL/lpr at 6 months of age. The
241 transcription factor Foxj1 is required for the late steps of ciliogenesis, including docking of
242 centrioles at the apical membrane to form basal bodies and axoneme elongation (You et al. 2004).
243 Foxj1 upregulation promotes abnormal ciliogenesis, resulting in disorganized, dense, and
244 lengthened cilia in the airway ciliated cells of patients with chronic mucosal inflammation (Li et
245 al. 2014). Considering that the direction of coordinated ciliary beating requires basal body
246 polarization (Kunimoto et al. 2012), in the infundibulum of MRL/lpr mice at 6 months of age,
247 significant upregulation of Foxj1 may promote abnormal ciliogenesis, resulting in cilia elongation

248 and disoriented ciliary alignment. Foxj1 localization in the expelled non-ciliated epithelial cells
249 was significantly observed in the infundibulum of MRL/lpr mice at 6 months of age, which implies
250 the increase of inadequate ciliogenesis and the promoted elimination of epithelial cells failed to
251 differentiate to ciliated epithelial cells. Otherwise, significantly high expression of Foxj1 may have
252 been a reaction to the decreased number of ciliated epithelial cells following disturbed ciliogenesis
253 and loss of Pax8⁺ epithelial progenitor cells. The abnormal morphofunction of the ciliated
254 epithelium of the infundibulum caused by alterations in homeostasis-related molecules causes
255 dysfunction in oocyte pick-up (Hosotani et al. 2018, 2020).

256 This study revealed that the motile cilia of ciliated epithelial cells in the infundibulum harbor
257 Hh signaling pathway-related molecules: the transmembrane receptors, Ptch and transmembrane
258 protein adjacent to Ptch, and Smo (Carpenter et al. 1998). In addition, Hh signaling effectors in
259 the Gli family are expressed in the nucleus of infundibulum epithelial cells. Considering the
260 significant positive correlations between Ptch1 and Smo/Gli2 in the infundibulum, motile cilia in
261 the infundibulum epithelium contain the Hh signaling pathway, although whether this pathway is
262 canonical or non-canonical matter has not been determined. Although primary cilia (i.e., immotile
263 cilia) are generally considered as sensor cilia in which canonical/non-canonical Hh signaling is
264 transduced (Abou Alaiwi et al. 2009; Bangs and Anderson 2017), recent studies reported that
265 motile cilia also act as sensors of the pericellular environment. The motile cilia of the tracheal

266 epithelium express sensing receptors such as the bitter taste receptor and *Ptch1/Smo* (Shah et al.
267 2009; Nordgren et al. 2014), which sense injury and chronic inflammation in the airway. The
268 motile cilia in oviducts harbor progesterone receptors, which regulate the ciliary beat frequency
269 (Teilmann et al. 2006; Bylander et al. 2010). Thus, motile cilia in the infundibulum epithelium
270 may play a sensing role via Hh signaling-related components. Hh ligands were not significantly
271 expressed in the infundibulum, and the present and previous studies (Russell et al. 2007) revealed
272 *Ihh* expression in ovarian granulosa cells, where Hh signaling may induce granulosa cell
273 proliferation. Therefore, cilia in the infundibulum may receive *Ihh* produced by ovarian granulosa
274 cells at the time of ovulation, rather than through the paracrine effect of Hh produced by
275 infundibulum epithelial cells.

276 In the infundibulum, *Pax8* and *Foxj1* expression showed a significant positive correlation with
277 the gene expression of Hh signaling pathway-related *Ptch1*, *Smo*, and *Gli2*. This suggests that the
278 Hh signaling pathway is closely related to the regulation of epithelial homeostasis in the
279 infundibulum. To the best of our knowledge, direct molecular interactions between *Pax8* and/or
280 *Foxj1* and the Hh signaling pathway during development and homeostasis maintenance have not
281 been observed previously. However, *Pax8* has been predicted as a target gene activated via the *Shh*
282 signaling pathway in oncogenesis (Harter et al. 2015). During the development of central nervous
283 system, *Foxj1* expression is regulated by the *Shh* signaling pathway and alters the response of cells

284 to Shh signaling (Cruz et al. 2010). Although further studies are needed to determine the molecular
285 relationship between Pax8, Foxj1, and Hh signaling-related components, the significant
286 downregulation of the Ptch1 and Smo transcripts in the infundibulum of MRL/lpr mice at 6 months
287 of age may be related to the abnormal pathology of the ciliated epithelium. The alternation of Gli2
288 localization in the infundibulum of MRL/lpr mice compared to other strains would relate to the
289 downregulation of Ptch1 and Smo transcripts and the disruption of Hh signaling

290 Aged MRL/lpr mice show autoimmune disease-prone phenotypes in systemic organs, including
291 the oviductal infundibulum with infiltration of autoreactive immune cells because of a mutation in
292 the Fas cell surface death receptor (*Fas*) gene (Andrews et al. 1978; Watanabe-Fukunaga et al.
293 1992; Hosotani et al. 2020). MRL/lpr at 3 months of age showed expression of *Ptch1*, *Smo*, and
294 *Gli1-3* in the infundibulum, and thus specific genetic alterations in the MRL/lpr strain do not cause
295 significant downregulation of Hh signaling-related components. In contrast, alterations in Hh
296 signaling transduction were reported to trigger immunological modulation. In the skin of atopic
297 dermatitis mouse models and the central nervous system with autoimmune neuroinflammation,
298 Shh signaling suppresses immune reactions via T-regulatory cell signaling and reduces tissue
299 pathology and disease severity (Papaioannou et al. 2019; Benallegue et al. 2021). In adult intestinal
300 mesenchyme, Shh and Ihh act as anti-inflammatory epithelial modulators and modulate
301 tolerogenic versus proinflammatory signaling (Zacharias et al. 2010). Therefore, significant

302 downregulation of *Ptch1* and *Smo* transcripts may interact with severe autoimmune inflammation
303 in the oviductal lamina propria of MRL/lpr mice at 6 months of age (Hosotani et al. 2020).
304 Furthermore, the deteriorated supply of fresh Hh signaling-related components in the
305 infundibulum of MRL/lpr mice at 6 months of age may be related to abnormal homeostasis of the
306 ciliated epithelium. The damages on ciliary morphofunction caused by variations in levels of
307 inflammatory cytokines in the infundibulum of MRL/lpr mice (Hosotani et al. 2020) are thought
308 to reflect the physiological damage of ciliated epithelium of infundibulum caused by inflammatory
309 follicular fluid (Palma-Vera et al. 2017), in terms of inflammatory cytokines altering the
310 morphofunction of the ciliated epithelium of infundibulum. Therefore, the downregulation of Hh
311 signaling-related components caused by the inflammatory molecules would also relate to the
312 turnover of the infundibulum epithelium under physiological conditions.

313 We observed a difference in the molecular expression between the infundibulum and
314 ampulla/isthmus. Epithelial cells in the distal and proximal parts of the oviducts are from
315 intrinsically different lineages and are maintained separately (Ford et al. 2020). The properties and
316 populations of Pax8⁺ epithelial cells differ between the distal and proximal parts; the infundibulum
317 shows a low population of Pax8⁺ secretory cells (Ford et al. 2020). Foxj1 upregulation in the
318 infundibulum compared to in the proximal part reflects greater activation of ciliogenesis and a
319 larger population of ciliated epithelial cells in the infundibulum. Hh signaling-related components

320 were transcribed in the proximal part, despite the sparse presence of cilia. In contrast, correlation
321 analysis indicated that Gli1 is involved in Hh signaling in the proximal part and that Gli1 and Gli3
322 are involved in epithelial homeostasis. Further investigations are required to reveal the effects of
323 the difference in molecular expression patterns among oviductal parts.

324 In summary, we propose the different mechanism of Hh signaling transduction between in
325 healthy and autoimmune disease conditions (Figure 8a and b). We propose that homeostasis of
326 ciliated epithelium in the infundibulum is regulated not only by Wnt/ β -catenin and Notch signaling,
327 but also possibly by Hh signaling. In addition, alterations in Hh signal transduction related to the
328 abnormal immune condition in the oviduct of MRL/lpr disrupts the epithelial morphology in the
329 infundibulum, resulting in oocyte pick-up dysfunction. Further functional study on the change of
330 the epithelial morphology in infundibulum under the experimental manipulation of Hh signaling
331 would strengthen the role of Hh signaling in the epithelial homeostasis in oviducts. Our results
332 improve the understanding of the physiology and pathology of mammalian female reproductive
333 function.

334 **Author contributions**

335 Conceptualization: M.H. and O.I.; Methodology: M.H., O.I., Ta.N., M.A.M, Te.N. and Y. H.;
336 Validation: M.H., O.I., and T.N.; formal analysis: M.H. and O.I.; Investigation: M.H., O.I., Ta.N.
337 and M.A.M.; Resources: M.H., O.I., and M.A.M.; Data curation: M.H.; Writing - original draft:
338 M.H. and O.I.; Writing - review & editing: M.H., O.I., T.W., and Y.K.; Visualization: M.H. and
339 O.I.; Supervision: M.H. and Y.K.; Project administration: O.I. and YK; Funding acquisition: M.H.

340

341 **Conflicts of interest**

342 The authors declare no conflicts of interest.

343

344 **Ethical approval**

345 Animal experimentation was approved by the School of Veterinary Medicine, Rakuno Gakuen
346 University (approval no. VH19A6). Animals were handled in accordance with the Guide for the
347 Care and Use of Laboratory Animals, Rakuno Gakuen University, Japan.

348

349 **Data, material, and/or code availability**

350 The data that support the findings of this study are available from the corresponding author
351 upon reasonable request.

352 **References**

- 353 Abou Alaiwi WAA, Lo ST, Nauli SM (2009) Primary cilia: Highly sophisticated biological
354 sensors. *Sensors (Basel)* 9:7003–7020
- 355 Andrews BS, Eisenberg RA, Theofilopoulos AN, et al (1978) Spontaneous murine lupus-like
356 syndromes. Clinical and immunopathological manifestations in several strains. *J Exp Med*
357 148:1198–1215
- 358 Bangs F, Anderson KV (2017) Primary cilia and Mammalian Hedgehog signaling. *Cold Spring*
359 *Harb Perspect Biol* 9. <https://doi.org/10.1101/cshperspect.a028175>
- 360 Benallegue N, Kebir H, Kapoor R, et al (2021) The hedgehog pathway suppresses
361 neuropathogenesis in CD4 T cell-driven inflammation. *Brain* 144:1670–1683.
362 <https://doi.org/10.1093/brain/awab083>
- 363 Briscoe J, Théron PP (2013) The mechanisms of Hedgehog signalling and its roles in
364 development and disease. *Nat Rev Mol Cell Biol* 14:416–429
- 365 Byers SL, Wiles M V., Dunn SL, Taft RA (2012) Mouse estrous cycle identification tool and
366 images. *PLoS One* 7:e35538. <https://doi.org/10.1371/JOURNAL.PONE.0035538>
- 367 Bylander A, Nutu M, Wellander R, et al (2010) Rapid effects of progesterone on ciliary beat
368 frequency in the mouse Fallopian tube. *Reprod Biol Endocrinol* 8:48.
369 <https://doi.org/10.1186/1477-7827-8-48>

370 Carlier FM, Dupasquier S, Ambroise J, et al (2020) Canonical WNT pathway is activated in the
371 airway epithelium in chronic obstructive pulmonary disease. *Ebiomedicine* 61.
372 <https://doi.org/10.1016/j.ebiom.2020.103034:103034>

373 Carpenter D, Stone DM, Brush J, et al (1998) Characterization of two patched receptors for the
374 vertebrate hedgehog protein family. *Proc Natl Acad Sci U S A* 95:13630–13634.
375 <https://doi.org/10.1073/pnas.95.23.13630>

376 Cruz C, Ribes V, Kutejova E, et al (2010) *Foxj1* regulates floor plate cilia architecture and
377 modifies the response of cells to sonic hedgehog signalling. *Development* 137:4271–4282.
378 <https://doi.org/10.1242/dev.051714>

379 Ford MJ, Harwalkar K, Pacis AS, et al (2021) Oviduct epithelial cells constitute two
380 developmentally distinct lineages that are spatially separated along the distal-proximal axis.
381 *Cell Rep* 36:109677. <https://doi.org/10.1101/2020.08.21.261016>

382 Ghosh A, Syed SM, Tanwar PS (2017) In vivo genetic cell lineage tracing reveals that oviductal
383 secretory cells self-renew and give rise to ciliated cells. *Development* 144:3031–3041.
384 <https://doi.org/10.1242/dev.149989>

385 Grote D, Souabni A, Busslinger M, Bouchard M (2006) Pax2/8-regulated Gata3 expression is
386 necessary for morphogenesis and guidance of the nephric duct in the developing kidney.
387 *Development* 133:53–61. <https://doi.org/10.1242/dev.02184>

388 Harter PN, Baumgarten P, Zinke J, et al (2015) Paired box gene 8 (PAX8) expression is
389 associated with sonic hedgehog (SHH)/wingless int (WNT) subtypes, desmoplastic
390 histology and patient survival in human medulloblastomas. *Neuropathol Appl Neurobiol*
391 41:165–179. <https://doi.org/10.1111/nan.12186>

392 Hosotani M, Ichii O, Nakamura T, et al (2018) Autoimmune abnormality affects ovulation and
393 oocyte-pick-up in MRL/MpJ-Fas lpr/lpr mice. *Lupus* 27:82–94.
394 <https://doi.org/10.1177/0961203317711772>

395 Hosotani M, Ichii O, Nakamura T, et al (2020) Altered ciliary morphofunction in the oviductal
396 infundibulum of systemic autoimmune disease-prone MRL/MpJ-Fas lpr/lpr mice. *Cell*
397 *Tissue Res* 380:627–641. <https://doi.org/10.1007/s00441-020-03175-z>

398 Ito S, Kobayashi Y, Yamamoto Y, et al (2016) Remodeling of bovine oviductal epithelium by
399 mitosis of secretory cells. *Cell Tissue Res* 366:403–410. [https://doi.org/10.1007/s00441-](https://doi.org/10.1007/s00441-016-2432-8)
400 [016-2432-8](https://doi.org/10.1007/s00441-016-2432-8)

401 Koyama H, Shi D, Fujimori T (2019) Biophysics in oviduct: Planar cell polarity, cilia, epithelial
402 fold and tube morphogenesis, egg dynamics. *Biophys Physicobiol* 16:89–107.
403 https://doi.org/10.2142/biophysico.16.0_89

404 Kunimoto K, Yamazaki Y, Nishida T, et al (2012) Coordinated ciliary beating requires Odf2-
405 mediated polarization of basal bodies via basal feet. *Cell* 148:189–200.

406 <https://doi.org/10.1016/j.cell.2011.10.052>

407 Li S, O'Neill SRS, Zhang Y, et al (2017) Estrogen receptor α is required for oviductal transport
408 of embryos. *FASEB J* 31:1595–1607. <https://doi.org/10.1096/fj.201601128R>

409 Li YY, Li CW, Chao SS, et al (2014) Impairment of cilia architecture and ciliogenesis in
410 hyperplastic nasal epithelium from nasal polyps. *J Allergy Clin Immunol* 134:1282–1292.
411 <https://doi.org/10.1016/j.jaci.2014.07.038>

412 Nordgren TM, Wyatt TA, Sweeter J, et al (2014) Motile cilia harbor serum response factor as a
413 mechanism of environment sensing and injury response in the airway. *Am J Physiol Lung
414 Cell Mol Physiol* 306:L829–L839

415 Palma-Vera SE, Schoen J, Chen S (2017) Periovulatory follicular fluid levels of estradiol trigger
416 inflammatory and DNA damage responses in oviduct epithelial cells. *PLOS ONE*
417 12:e0172192. <https://doi.org/10.1371/journal.pone.0172192>

418 Papaioannou E, Yáñez DC, Ross S, et al (2019) Sonic Hedgehog signaling limits atopic
419 dermatitis via Gli2-driven immune regulation. *J Clin Invest* 129:3153–3170.
420 <https://doi.org/10.1172/JCI125170>

421 Peng T, Frank DB, Kadzik RS, et al (2015) Hedgehog actively maintains adult lung quiescence
422 and regulates repair and regeneration. *Nature* 526:578–582.
423 <https://doi.org/10.1038/nature14984>

424 Plachov D, Chowdhury K, Walther C, et al (1990) Pax8, a murine paired box gene expressed in
425 the developing excretory system and thyroid gland. *Development* 110:643–651

426 Raidt J, Werner C, Menchen T, et al (2015) Ciliary function and motor protein composition of
427 human fallopian tubes. *Hum Reprod* 30:2871–2880. <https://doi.org/10.1093/humrep/dev227>

428 Russell MC, Cowan RG, Harman RM, et al (2007) The hedgehog signaling pathway in the
429 mouse ovary. *Biol Reprod* 77:226–236. <https://doi.org/10.1095/biolreprod.106.053629>

430 Sancho E, Batlle E, Clevers H (2004) Signaling pathways in intestinal development and cancer.
431 *Annu Rev Cell Dev Biol* 20:695–723

432 Shah AS, Ben-Shahar Y, Moninger TO, et al (2009) Motile cilia of human airway epithelia are
433 chemosensory. *Science* 325:1131–1134. <https://doi.org/10.1126/science.1173869>

434 Talbot P, Riveles K (2005) Smoking and reproduction: The oviduct as a target of cigarette
435 smoke. *Reprod Biol Endocrinol* 3:52. <https://doi.org/10.1186/1477-7827-3-52>

436 Teilmann SC, Clement CA, Thorup J, et al (2006) Expression and localization of the
437 progesterone receptor in mouse and human reproductive organs. *J Endocrinol* 191:525–535.
438 <https://doi.org/10.1677/joe.1.06565>

439 Watanabe-Fukunaga R, Brannan CI, Copeland NG, et al (1992) Lymphoproliferation disorder in
440 mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 356:314–317.
441 <https://doi.org/10.1038/356314a0>, [1372394](https://doi.org/10.1038/1372394)

442 You Y, Huang T, Richer EJ, et al (2004) Role of f-box factor foxj1 in differentiation of ciliated

443 airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 286:L650–L657.

444 <https://doi.org/10.1152/ajplung.00170.2003>

445 Zacharias WJ, Li X, Madison BB, et al (2010) Hedgehog is an anti-inflammatory epithelial

446 signal for the intestinal lamina propria. *Gastroenterology* 138:2368–77, 2377.e1.

447 <https://doi.org/10.1053/j.gastro.2010.02.057>

448 Zhu M, Iwano T, Takeda S (2019) Estrogen and EGFR pathways regulate Notch signaling in

449 opposing directions for multi-ciliogenesis in the Fallopian tube. *Cells* 8:933

450 <https://doi.org/10.3390/cells8080933>

451 **FIGURE LEGENDS**

452 **Fig. 1** Localization of Pax8 and Foxj1 in the oviductal infundibulum. IHC of Pax8 (a-c and a'-c'),
453 Foxj1 (d-f and d'-f'), and negative control (g) are shown. Bar = 25 μ m. Black arrows:
454 immunoreactive positive epithelial cells; red arrowheads: expelled non-ciliated epithelial cells
455 from the epithelium. (h) The percentage of expelled non-ciliated epithelial cells from the
456 epithelium in distal parts of oviducts. There were no significant differences between ages at the
457 same strains (Student's *t*-test, $P < 0.05$) and among strains at the same age (Tukey's test, $P < 0.05$).
458 (i) The percentage of Foxj1 positive expelled non-ciliated epithelial cells from the epithelium distal
459 parts of oviducts. *: Significant differences between 3 and 6 months of age at the same strains and
460 same part (Student's *t*-test, $P < 0.05$). B&M: Significant differences between B6 or MRL/+ mice
461 at the same age (Tukey's test, $P < 0.05$). B6 = C57BL/6N, MRL/+ = MRL/MpJ, MRL/lpr =
462 MRL/MpJ-*Fas*^{lpr/lpr}

463
464 **Fig. 2** Expression levels of *Pax8* (a) and *Foxj1* (b) in the oviduct. Data are the mean \pm s.e. *:
465 Significant differences between 3 and 6 months of age at the same strains and same part (Student's
466 *t*-test, $P < 0.05$). #: Significant differences between proximal and distal parts of oviducts from mice
467 of the same strains and age (Student's *t*-test, $P < 0.05$). B&M: Significant differences between B6
468 or MRL/+ mice at the same age (Tukey's test, $P < 0.05$). *Pax8*: Paired box 8; *Foxj1*: Forkhead box

469 protein J1. B6 = C57BL/6N, MRL/+ = MRL/MpJ, MRL/lpr = MRL/MpJ-*Fas*^{lpr/lpr}

470

471 **Fig. 3** Localization of hedgehog signaling-related proteins in the oviductal infundibulum revealed
472 by immunofluorescence. Localization of Ptch1 (a-a''''', b-b''''', and c-c'''''), Smo (d-d''''', e-e''''',
473 and f-f'''''), Gli2 (g-g''''', h-h''''', and i-i'''''), and negative control (j-j'') are shown. Bar = 25 μ m.
474 Arrows: immunoreactive positive cells in the epithelium. B6 = C57BL/6N, MRL/+ = MRL/MpJ,
475 MRL/lpr = MRL/MpJ-*Fas*^{lpr/lpr}

476

477 **Fig. 4** Expression levels during estrous cycle of hedgehog signaling-related genes in the oviduct
478 of C57BL/6N at 3 months of age. Expression levels of *Ptch1* (a), *Smo* (b), *Gli1* (c), *Gli2* (d), and
479 *Gli3* (e) are shown. Data are the mean \pm s.e. *: Significant differences between 3 and 6 months of
480 age at the same cycle and same part (Student's *t*-test, $P < 0.05$). #: Significant differences between
481 proximal and distal parts of oviducts from mice of the same cycle and age (Student's *t*-test, $P <$
482 0.05). There were no significant differences among estrous cycles at the same age and part
483 (Tukey's test, $P < 0.05$). P: proximal part; D: distal part 3m: 3 months of age, 6m: 6 months of age.
484 *Ptch1*: Patched-1, *Smo*: Smoothened, *Gli1-3*: GLI family zinc finger 1–3.

485

486 **Fig. 5** Expression levels of hedgehog signaling-related genes in the oviduct. Expression levels of

487 *Ptch1* (a), *Smo* (b), *Gli1* (c), *Gli2* (d), and *Gli3* (e) are shown. Data are the mean \pm s.e. *: Significant
488 differences between 3 and 6 months of age for the same strains and same part (Student's *t*-test, *P*
489 < 0.05). #: Significant differences between proximal and distal parts of oviducts from mice of the
490 same strains and age (Student's *t*-test, *P* < 0.05). B&M: Significant differences between B6 or
491 MRL/+ mice at the same age (Tukey's test, *P* < 0.05). *Ptch1*: Patched-1, *Smo*: Smoothened, *Gli1*-
492 3: GLI family zinc finger 1-3. B6 = C57BL/6N, MRL/+ = MRL/MpJ, MRL/lpr = MRL/MpJ-
493 *Fas*^{lpr/lpr}

494

495 **Fig. 6** Expression of transcriptions of hedgehog in ovarian granulosa cells (a-a'', c-c'', and e-e'')
496 and epithelial cells of the oviductal infundibulum (b-b'', d-d'', and f-f'') revealed by *in situ*
497 hybridization. Negative controls are shown in (g and g'). Brown dots indicate positive reactions to
498 *in situ* hybridization. Bar = 25 μ m. Arrows: reaction-positive cells in the oviductal infundibulum.
499 B6 = C57BL/6N; MRL/lpr = MRL/MpJ-*Fas*^{lpr/lpr}

500

501 **Fig. 7** Expression levels of hedgehog genes in the oviduct of B6 at 3 months of age (a and d),
502 MRL/+ at 3 months of age (b and e), and MRL/lpr at 3 months of age (c and f). Data are the mean
503 \pm s.e. *: Significant differences between proximal and distal parts of oviducts from mice of the
504 same strains and age (Student's *t*-test, *P* < 0.05). #: Significant differences between 3 and 6 months

505 of age in mice of the same strains and from the same part (Student's *t*-test, $P < 0.05$). B&M:
506 Significant differences between B6 or MRL/+ mice at the same age (Tukey's test, $P < 0.05$). B6 =
507 C57BL/6N, MRL/+ = MRL/MpJ, MRL/lpr = MRL/MpJ-*Fas*^{lpr/lpr}

508

509 **Fig. 8** Estimated modulation mechanisms of hedgehog signaling in the oviductal infundibulum
510 between healthy (a and a') and autoimmune disease conditions (b and b'). In healthy condition,
511 hedgehog signaling transduced by *Ihh* released by the ovarian granulosa cells, which is received
512 by *Ptch1* and *Smo* localized on the motile cilia of the oviductal infundibulum. The hedgehog
513 signaling mediated by *Ptch1*, *Smo*, and *Gli2* in the ciliated epithelial cells would lead the constant
514 transcription and protein production of *Ptch1* and *Smo* and maintain the proliferation of *Pax8*
515 positive secretory cells and differentiation of those into *Foxj1* positive healthy ciliated epithelial
516 cells in the oviductal infundibulum. In autoimmune disease condition, the declined levels of *Ihh*
517 production and transcription of *Ptch1* and *Smo* causes the modulation of hedgehog signaling
518 transduction, which resulted in the disturbances in the homeostasis of ciliated epithelium of the
519 oviductal infundibulum and the appearance of strongly *Foxj1* positive ciliated epithelial cells
520 which are morphologically abnormal. CEC: ciliated epithelial cell, SC: secretory cell, NCEC: non
521 ciliated epithelial cell, GCs: granulosa cells, *Foxj1*: forkhead box J1, *Pax8*: paired box 8, *Hh*:
522 Hedgehog, *Ihh*: indian hedgehog, *Ptch1*: Patched-1, *Smo*: Smoothened, *Gli2*: GLI family zinc

523 finger 2.

524 **Table 1. Primary antibody information in immunostaining.**

Anti gen	Cat. No	Source	Host	Dilution	Antigen retrieval reagent	Blocking serum	Secondary antibody for IF (1:300)	Biotinylated secondary antibody for IHC
Gli2	-	Novus Biologicals LLC., Littleton, CO, USA	Rabbit	1:200	20 mM tris-HCl (pH 9.0)	10% goat normal serum	Goat anti-rabbit IgG H&L CF568, 20103-1 (Biotium.Inc, Hayward, CA)	-
Ptch 1	MAB 41051	R&D Systems Inc., Minneapolis, MN, USA	Rabbit	1:200	0.1% pepsin	10% goat normal serum	Goat anti-rat IgG H&L CF568, 20096-1 (Biotium.Inc, Hayward, CA)	-
Smo	GTX 60154	Gene Tex Inc., Irvine, CA, USA	Rabbit	1:200	0.1% pepsin	10% goat normal serum	Goat anti-rabbit IgG H&L CF568, 20103-1 (Biotium.Inc, Hayward, CA)	-
Tubulin- α	MS-581-R7	Thermo Scientific, Waltham,	Mouse	Undiluted	No need	10% goat normal serum	Goat anti-mouse IgG H&L CF488A, 20018-1 (Biotium.Inc, Hayward, CA)	-

526 **Table 2. Primer list used for quantitative PCR analysis.**

Genes	Accession Number	Primer Sequence (5'-3') F: Forward, R: Reverse		Product size (bp)	Annealing temp. (°C)
<i>Actb</i>	NM_007393.5	F: TGTTACCAACTGGGACGACA	R: GGGGTGTTGAAGGTCTCAA	165	60
<i>Dhh</i>	NM_007857.5	F: TTGGCACTCTTGGCACTATCT	R: CTTTGCAACGCTCTGTCATC	277	60
<i>Foxj1</i>	NM_008240.3	F: ACTATGCCACCAACCCACA	R: GGATGGAATTCTGCCAGGT	171	60
<i>Gli1</i>	NM_010296.2	F: CGACCTGCAAACCGTAATC	R: CTTGCCAACCATCATATCCA	289	60
<i>Gli2</i>	NM_001081125.1	F: TGGAGAAGAAAGAAGCCAAGAG	R: TCATGTCAATCGGCAAAGG	159	60
<i>Gli3</i>	NM_008130.3	F: CCTGCTCCAACATTCCAAC	R: CTTGACTAGGGTTGTTCCCTTCC	240	60

		F: CCTCTTGCCTACAAGCAGTTC		
<i>Ihh</i>	NM_010544.3		214	60
		R: AGATGGCCAGTGAGTTCAGAC		
<hr/>				
		F: AAGCATCGACTCACAGAGCA		
<i>Pax8</i>	NM_011040.4		285	60
		R: GAATGAGGATCTGCCACCAC		
<hr/>				
		F: CCATGACAAAGCCGACTACA		
<i>Ptch1</i>	NM_001328514.1		293	60
		R: GGAAGACTGCGCACACTAGAA		
<hr/>				
		F: CAAGTACGGCATGCTGGCTC		
<i>Shh</i>	NM_009170.3		254	52
		R: AAGGTGAGGAAGTCGCTGTA		
<hr/>				
		F: GTCTCTGCACGCTCTTCACA		
<i>Smo</i>	NM_176996.4		269	60
		R: CCAGACTACTCCAGCCATCAA		
<hr/>				

527 *Dhh*: desert hedgehog, *Foxj1*: forkhead box J1, *Gli1-3*: GLI family zinc finger 1–3, *Ihh*: Indian
528 hedgehog, *Pax8*: paired box 8, *Ptch1*: Patched 1, *Shh*: sonic hedgehog, *Smo*: smoothed, frizzled
529 class receptor.

530 **Table 3. Spearman’s correlation coefficient (ρ) between hedgehog signaling-related genes**
 531 **expression and secretory/ciliated epithelial cells marker expression in the oviduct of mice at**
 532 **3 months of age.**

533

		Parameters					
		Hedgehog receptor gene expression			Secretory and ciliated epithelial cells marker expression		
		at proximal part			at distal part		
		<i>Ptch1</i>	<i>Pax8</i>	<i>Foxj1</i>	<i>Ptch1</i>	<i>Pax8</i>	<i>Foxj1</i>
<i>Ptch1</i>	ρ	1	0.55***	0.38**	1	0.63***	0.62***
	<i>P</i>	-	<0.0001	0.0071	-	<0.0001	<0.0001
<i>Smo</i>	ρ	0.60***	0.62***	0.42**	0.48***	0.48***	0.59***
	<i>P</i>	<0.0001	<0.0001	0.0026	0.0007	0.0007	<0.0001
<i>Gli1</i>	ρ	0.63***	0.49***	0.20	0.19	0.13	0.23
	<i>P</i>	<0.0001	0.0005	0.1690	0.1981	0.3862	0.1226
<i>Gli2</i>	ρ	0.56***	0.72***	0.69***	0.43**	0.61***	0.69***

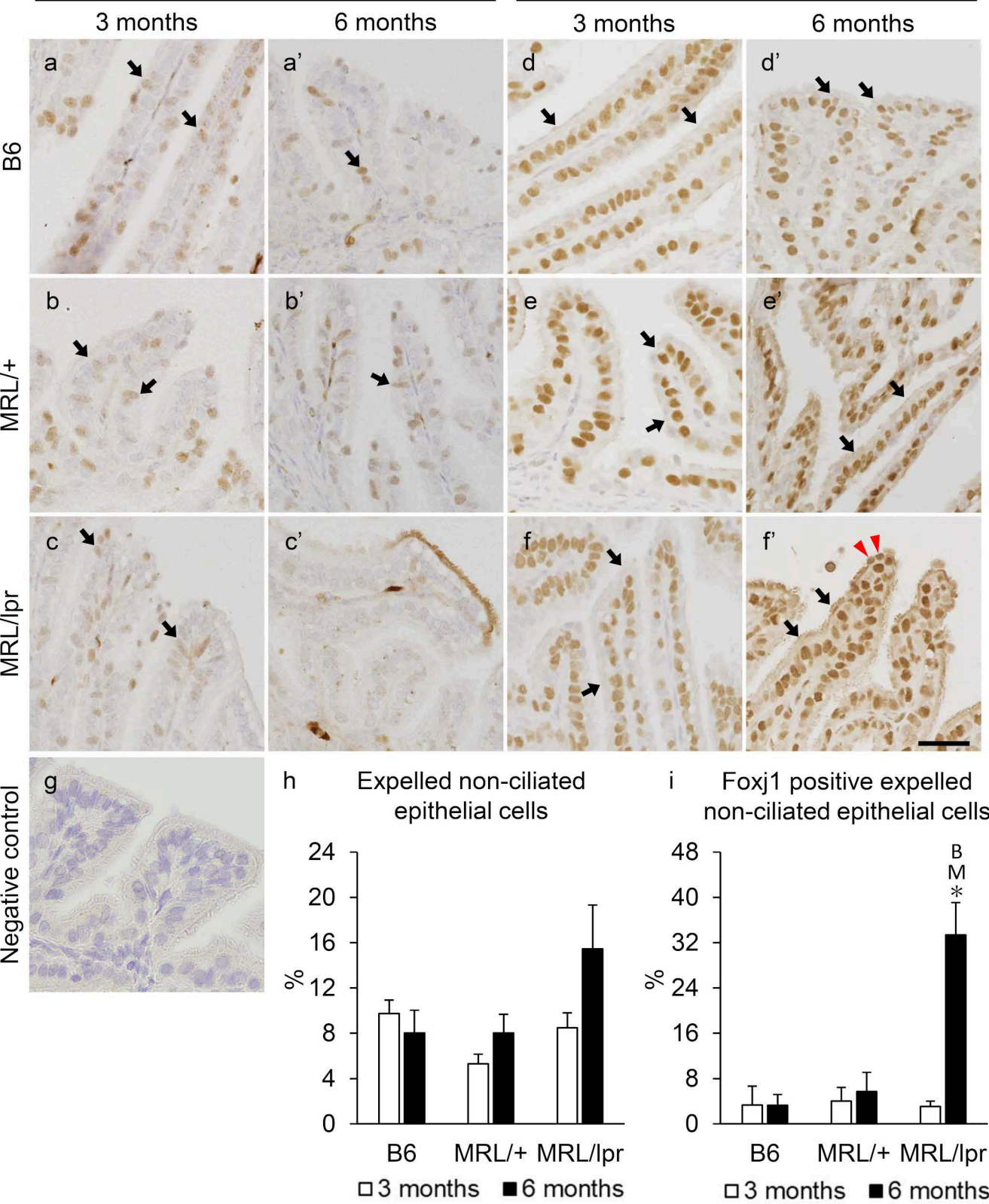
	<i>P</i>	<0.0001	<0.0001	<0.0001	0.0027	<0.0001	<0.0001
<i>Gli3</i>	ρ	0.41**	0.39**	0.35*	-0.30*	-0.09	0.07
	<i>P</i>	0.0038	0.0059	0.0141	0.0431	0.5554	0.6623

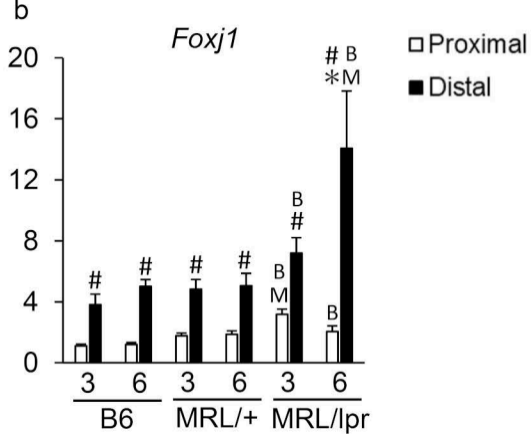
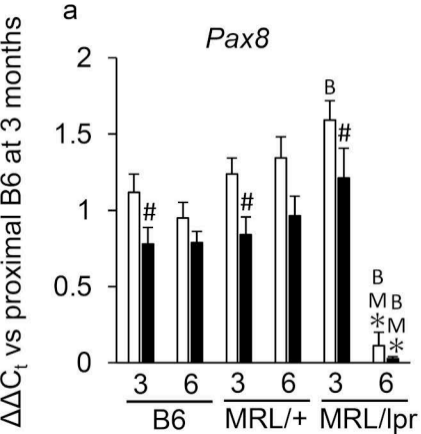
534 *Ptch1*, Patched-1; *Smo*, Smoothened; *Gli1-3*, GLI family zinc finger 1–3, *Pax8*, paired box 8;

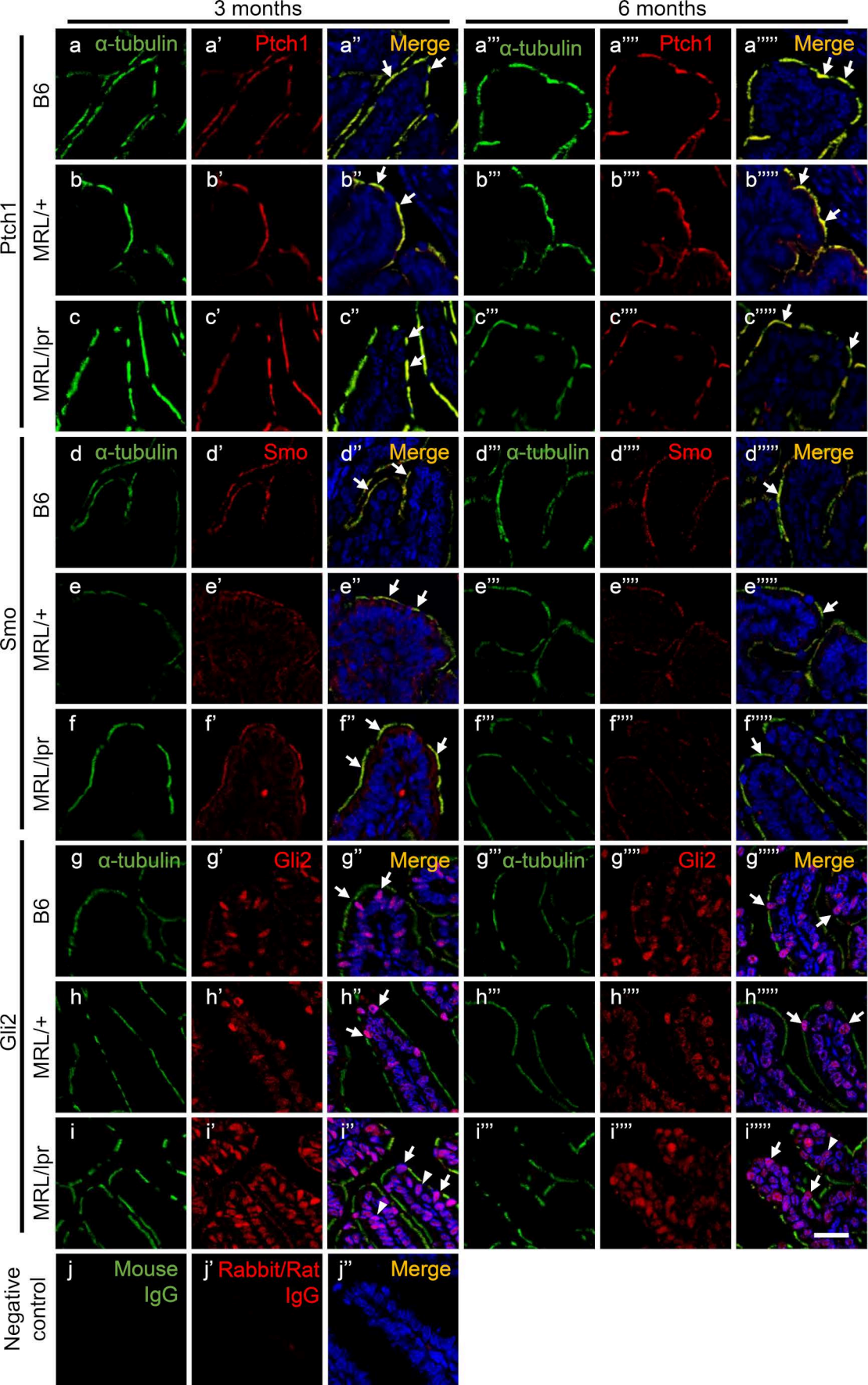
535 *Foxj1*, forkhead box J1.

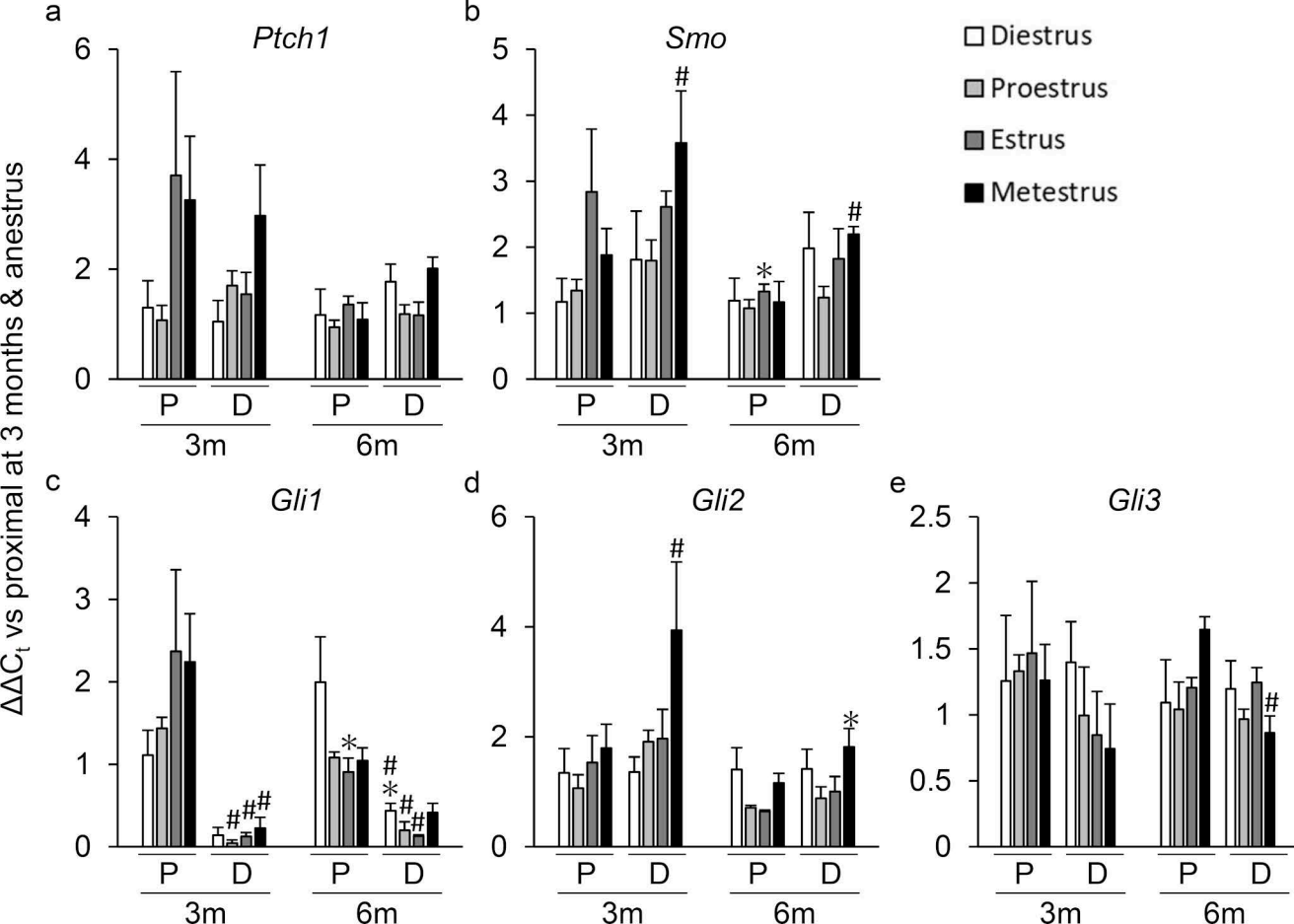
Pax8

Foxj1

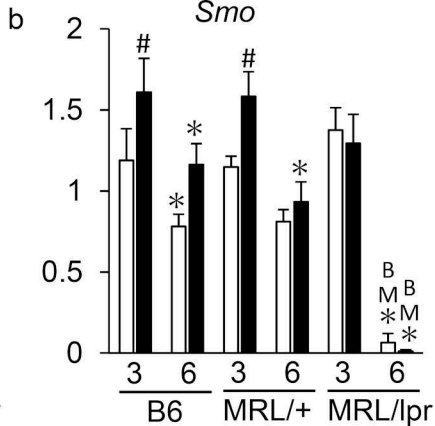
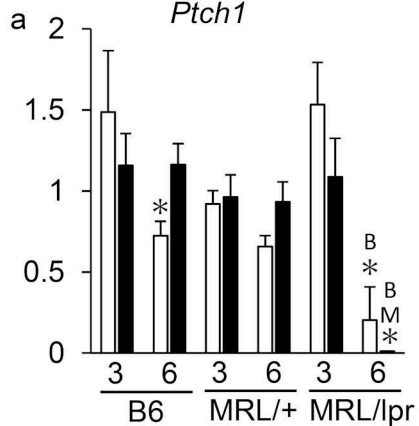








$\Delta\Delta C_t$ vs proximal B6 at 3 months



□ Proximal
■ Distal

