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Information about the manuscript

Selective YAP activation in Procr cells is essential for ovarian stem/progenitor expansion and epithelium repair

Tracking no: 10-11-2021-RA-eLife-75449

Competing interests: Yi Ariel Zeng: Reviewing editor, *eLife*

Author contributions:

Yi Ariel Zeng: Conceptualization; Supervision; Funding acquisition; Writing - original draft; Project administration
Jingqiang Wang: Conceptualization; Data curation; Formal analysis; Funding acquisition; Validation; Investigation; Writing - original draft; Project administration
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Junfen Fu: Supervision; Funding acquisition
Lei Zhang: Resources; Supervision; Funding acquisition; Writing - review and editing

Data Availability:

All data generated or analysed during this study are included in the manuscript and supporting file; Source Data files have been provided for Figure 3-figure supplement 1-source data 1

N/A

Ethics:

Human Subjects: No
Animal Subjects: Yes
Ethics Statement: All mice were housed in the SIBCB animal facility under IVC standard with a 12-hr light/dark cycle at room temperature. Experimental procedures were approved by the Animal Care and Use Committee of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, with a project license number of IBCB0065.

1 **Selective YAP activation in Procr cells is essential for ovarian**
2 **stem/progenitor expansion and epithelium repair**

3

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26 Running Title:

27 YAP senses OSE rupture and stimulates progenitor expansion

28

29 Keywords:

30 Ovarian surface epithelium, ovulatory rupture repair, adult stem cells, YAP,
31 Procr

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33

34 **Abstract**

35 Ovarian surface epithelium (OSE) undergoes recurring ovulatory rupture and
36 OSE stem cells rapidly generate new cells for the repair. How the stem cell
37 senses the rupture and promptly turns on proliferation is unclear. Our previous
38 study has identified that Protein C Receptor (*Procr*) marks OSE progenitors.
39 In this study, we observed decreased adherent junction and selective
40 activation of YAP signaling in *Procr* progenitors at OSE rupture site. OSE
41 repair is impeded upon deletion of *Yap* in these progenitors. Interestingly,
42 *Procr*⁺ progenitors show lower expression of *Vgll4*, an antagonist of YAP
43 signaling. Overexpression of *Vgll4* in *Procr*⁺ cells hampers OSE repair and
44 progenitor proliferation, indicating that selective low *Vgll4* expression in
45 *Procr*⁺ progenitors is critical for OSE repair. In addition, YAP activation
46 promotes transcription of the OSE stemness gene *Procr*. The combination of
47 increased cell division and *Procr* expression leads to expansion of *Procr*⁺
48 progenitors surrounding the rupture site. These results illustrate a YAP-
49 dependent mechanism by which the stem/progenitor cells recognize the
50 ovulatory rupture, and rapidly multiply their numbers, highlighting a YAP-
51 induced stem cell expansion strategy.

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61 **Introduction**

62 During the adult reproductive cycles, the OSE undergoes recurring
63 ovulatory rupture and repair^{1,2}. After ovulation, to maintain the physiological
64 function and morphology of the ovary, the wound is completely closed within
65 12 hours to 3 days following rupture³⁻⁵. Cells surrounding the damaged sites
66 are required to respond to the wound by turning on cell proliferation to supply
67 sufficient cells as building block for regeneration⁶. Our previous study has
68 identified that Procr+ OSE stem/progenitor cells are the major contributor for
69 ovulatory rupture repair. Targeted ablation of these cells hampers the repair⁷.
70 Interestingly, we observed that Procr+ cells expand instantly upon ovulation,
71 reminiscent of a result of symmetric division⁷. It remains unknown how the
72 stem cell senses the ovulation event, and what is the signal that triggers the
73 instant stem cell expansion at the rupture site.

74 The cue for this stem/progenitor cell amplification likely comes from a
75 particular extracellular signal occurring upon ovulation. One possibility is that
76 the follicular fluid expelled during ovulation consists of Wnts and other potential
77 niche signals⁸⁻¹¹, which may regulate Procr+ stem/progenitor cell expansion.
78 Another possibility is the involvement of mechanical force-induced signals
79 during ovulation, resulting in Procr+ stem/progenitor cell expansion.

80 YAP (Yes-associated protein, also known as YAP1) signaling is an
81 evolutionarily conserved pathway and a master regulator of organ size and
82 tissue growth during animal development¹². As a downstream effector, YAP is
83 critical for regeneration in different organs, through triggering cell proliferation,
84 cell survival or expansion of stem and progenitor cell compartments¹³⁻¹⁹. YAP
85 is a transcriptional coactivator protein that shuttles between the cytoplasm
86 and nucleus, and regulate expression of target genes, such as *Cyr61* and
87 *Ctgf*, through binding with TEAD transcription factors²⁰⁻²⁴. Vgll4, a member of
88 Vestigial-like proteins, serves as a transcriptional repressor of YAP through

89 direct interactions with TEADs²⁵. Previous studies from us and others have
90 demonstrated the important roles of Vgll4 plays during development and
91 regeneration in various tissues²⁶⁻²⁹. Cell-cell junctions links cells to each other
92 in epithelial tissues, and is an upstream negative regulator of YAP^{30,31}.
93 Mechanical forces regulate cell-cell adhesion stability, and cell-cell adhesion
94 junctions may be intrinsically weak at high forces³². It has been shown that
95 disruption of adherent junctions turns on YAP nuclear activities in lung
96 stem/progenitor cells³³. However, whether YAP signaling is implicated in
97 ovulatory rupture repair is unknown.

98 In this study, we investigated how OSE stem/progenitor cells sense the
99 rupture post ovulation and divide subsequently. We found that, in the
100 proximity of rupture site, decreased adherent junction is associated with Yap
101 nuclear localization in all cells, and conditional deletion of Yap in Procr+ cells
102 hampers OSE repair. Interestingly, only Procr+ OSE cells displayed a low
103 level of Vgll4, allowing YAP signaling activation. We generated a new *tetO-*
104 *Vgll4* mouse. Ectopic expression of Vgll4 in the stem/progenitor cells using
105 *Procr-rtTA;tetO-Vgll4* mice blocked OSE ovulatory repair. Moreover, we found
106 that YAP signaling activation resulted in Procr+ cells expansion at the rupture
107 site, through the combination of inducing cell division, and directly activating
108 Procr transcription. The activation of Procr is essential, as when Procr was
109 deleted, stemness property was lost and OSE repair was hindered.

110

111 **Results**

112 **Decreased E-Cadherin expression at the rupture site and selective** 113 **activation of YAP signaling in Procr+ cells**

114 To investigate what could be the potential extracellular stimuli at the
115 rupture site, we performed immunostaining of various adherent or tight
116 junction components on ovary sections. To increase rupture incidences,

117 superovulation was induced by injection of PMSG and HCG, and the ovaries
118 were harvested at 0.5 days after HCG injection, when ovulation just occurred
119 (Fig. S1a). Interestingly, we found that E-Cadherin staining is markedly
120 decreased at the proximal region of rupture (defined as within 20 cells on one
121 side of the rupture in section) compared to other regions, i.e., rupture distal
122 region (Fig. 1a) and non-rupture region (Fig. S1b). As adherent junction has
123 been implicated as a modulator of YAP signaling^{31,34-36}, we examined YAP
124 activities at the rupture area by immunostaining. We observed increased
125 incidence of nuclear YAP at the proximal region of rupture compared to other
126 regions (Fig. 1b-c, Fig. S1c). These results suggest that compromised
127 adherent junction resulted from ovulatory rupture could induce YAP nuclear
128 localization in OSE cells surrounding the wound.

129 Our previous study has established that Procr+ progenitor cells
130 surrounding the wound instantly proliferate upon rupture and are responsible
131 for OSE repair⁷. We therefore investigated whether Procr+ cells close to the
132 rupture site are associated with YAP signaling activities. We performed YAP
133 immunostaining using *Procr-rtTA;tetO-H2B-GFP* reporter, in which H2B-GFP
134 signal marks Procr-expressing cells. Superovulation was induced in these
135 animals by PMSG and HCG injections, and ovaries were harvested 0.5 days
136 after HCG injection. We found that Procr+ (H2B-GFP+) cells at the rupture
137 proximal region (referred to as rupture site from here on) have significantly
138 higher nuclear YAP staining ($75.9\pm 1.7\%$) compared to Procr- cells
139 ($39.6\pm 1.0\%$) (Fig. 1d, 1f), or compared to Procr+ cells at the non-rupture
140 region (Fig. 1e-f). This was further validated by RNA double *in situ*
141 hybridization with *Procr* and a YAP target gene *Cyr61*. We found that, at the
142 rupture site, *Cyr61* is preferentially activated in Procr+ OSE cells, with
143 $44.8\pm 2.9\%$ of Procr+ cells being Cyr61+, which is markedly higher than that of
144 Procr- cells ($8.9\pm 0.8\%$) (Fig. 1g, 1i). At the non-rupture site, both Procr+ and

145 Procr- cells had rather low expression of *Cyr61* expression (Fig. 1h-i).

146 Together, these results suggest that YAP signaling was specifically activated
147 in Procr+ cells at the rupture site. Considering the role of YAP signaling in
148 promoting cell proliferation, these results are in line with our previous
149 observations that only Procr+, but not Procr-, cells at the rupture site
150 displayed increased proliferation⁷.

151

152 **Deletion of Yap in Procr+ cells hinders rupture repair and progenitor** 153 **proliferation**

154 To investigate whether YAP signaling is important for OSE repair, we
155 deleted YAP specifically in Procr+ cells using *Procr-CreER; Yap^{fl/fl}* mice (Yap-
156 cKO). *Yap^{fl/fl}* mice were used as control (Ctrl). Tamoxifen (TAM) was
157 administered in 4-week-old mice, followed by superovulation at 2 days after
158 TAM injection (Fig. 2a). The impact on OSE repair by Yap deletion was
159 analyzed by ovary whole-mount imaging. At 4.5d pi (ovulation), the two
160 groups had similar ruptures (Fig. 2b, 2e). At 6d pi, Ctrl ovaries underwent
161 rapid repairing (Fig. 2c), and the OSE was completely recovered by 7.5d pi
162 (Fig. 2d). In contrast, the OSE repair in Yap-cKO ovaries was significantly
163 delayed at both 6d pi and 7.5d pi (Fig. 2c-e). The efficacy of Yap deletion and
164 the reduced expression of the target gene *Cyr61* in OSE cells were validated
165 by qPCR analyses (Fig. S1d).

166 To analyze the proliferative capacity of Procr+ OSE cells, mice were
167 subjected to 12 h of EdU incorporation before harvesting the ovaries (Fig. 2a).
168 When analyzed at 4.5d pi (ovulation), the number of proliferating OSE cells at
169 rupture site (defined as 20 cells on one side from the opening) were
170 significantly decreased from 5.5±0.3 EdU+ in Ctrl to 1.4±0.2 EdU+ in Yap-cKO
171 (Fig. 2f-h). The impact to cell proliferation was further analyzed *in vitro*. Our
172 previous study has established that Procr+, but not Procr-, OSE cells can

173 form colonies *in vitro*⁷. At 4.5d pi, total OSE cells were isolated from both Ctrl
174 and Yap-cKO mice (Fig. 2i), and placed in culture as previously described⁷.
175 Deletion of Yap in Procr+ cells drastically inhibited OSE colony formation (Fig.
176 2j-k).

177 To visualize the contribution of Procr+ progenitors toward the repair in the
178 presence or absence of Yap, we performed *in vivo* lineage tracing. TAM was
179 administered to 4-week-old mice to simultaneously delete Yap and initiate
180 lineage tracing in Procr+ cells (Fig. 2l). At 4.5d pi, control (*Procr-CreER;R26-*
181 *mTmG*) ovary displayed a zone of mGFP+ cells that are the progeny of
182 Procr+ progenitors surrounding the rupture sites (Fig. 2m). In contrast, Yap-
183 cKO (*Procr-CreER;Yap^{fl/fl};R26-mTmG*) ovaries have markedly fewer mGFP+
184 cells around the wound (Fig. 2m, 2o), supporting the notion that the activity of
185 Procr+ progenitors was hampered at the beginning of the repairing process.
186 At 7d pi, control ovaries had generated patches of mGFP+ cells covering the
187 newly formed corpus luteum (Fig. 2n). However, Yap-cKO ovaries still had
188 obvious openings with few mGFP+ cells (Fig. 2n-o). Together, these results
189 suggest that YAP signaling activation is crucial for the proliferation of Procr+
190 progenitor cells and the timely repair of OSE after rupture.

191

192 **An intrinsic lower level of *Vgll4* in Procr+ cells is essential for their** 193 **progenitor property and OSE rupture repair**

194 Next, we investigated what could be the reason that YAP signaling is
195 specifically activated in Procr+ cells. *Vgll4* is a negative regulator of YAP by
196 inhibiting the binding of YAP and TEAD4²⁶. We FACS-isolated Procr+ cells
197 and Procr- cells from the rupture sites (Fig. 3a). qPCR analysis indicated that
198 Procr+ cells have lower level of *Vgll4* compared to Procr- cells (Fig. 3b). This
199 was further validated by *Vgll4* immunostaining using *Procr-rtTA;tetO-H2B-*
200 *GFP* reporter, in which H2B-GFP signals mark Procr-expressing cells.

201 Consistent with the qPCR results, Procr+ cells also exhibited lower Vgll4
202 protein expression compared to Procr- cells (Fig. 3c-e).

203 To examine whether the reduced level of Vgll4 is significant for the
204 selective YAP signaling activation in Procr+ cells and rupture repair, we set to
205 overexpress Vgll4 specifically in Procr+ cells. A new *tetO-Vgll4* mouse line
206 was generated, by inserting a tetO-Vgll4-Flag-wpre-polyA cassette behind the
207 3'UTR of the *Col1a1* gene (Fig. 3f and Fig. S2a-c). Subsequently, *Procr-*
208 *rtTA;tetO-Vgll4* (Vgll4-OE) mice were generated by genetic crosses with *TetO-*
209 *Vgll4* as control (Ctrl). The efficacy of overexpression was validated by
210 western blotting and qPCR, showing increased expression of Vgll4 and
211 decreased expression of *Cyr61* in Vgll4-OE cells (Fig. S2d-e). Furthermore,
212 immunostaining confirmed the increased number of Vgll4-high cells in the
213 OSE layer of Vgll4-OE mice (Fig. 3g). For this experiment, superovulation was
214 performed to 4-week-old mice and DOX was fed throughout the process (Fig.
215 3h). The impact of Vgll4 overexpression was analyzed throughout the
216 repairing process, at 4.5d pi (ovulation), 6d pi (OSE repair ongoing) and 7.5d
217 pi (OSE repair completed) by ovary whole-mount imaging. We found that the
218 rupture in Ctrl and Vgll4-OE ovaries are comparable at 4.5d pi (Fig. 3i, 3l). At
219 6d pi, while Ctrl ovaries had sights of wound closure, Vgll4-OE ovaries still
220 showed larger areas of rupture (Fig. 3j, 3l). At 7.5d pi, Ctrl ovaries displayed
221 complete OSE, whereas the repair in Vgll4-OE ovaries was obviously delayed
222 (Fig. 3k-l).

223 Next, we examined whether overexpression of Vgll4 affects progenitor
224 proliferation. At 4.5d pi (ovulation), the number of proliferating OSE cells at
225 rupture site were significantly decreased from 3.7 ± 0.3 in Ctrl to 1.0 ± 0.2 in
226 Vgll4-OE (Fig. 3m-o). At 4.5d pi, total OSE cells were isolated and cultured *in*
227 *vitro* for 7 days (Fig. 3p). Consistently, overexpression of Vgll4 inhibits cell
228 proliferation and colony formation (Fig. 3q-r). Together, these results suggest

229 that overexpression of *Vgll4* in *Procr*⁺ cell impaired *Procr*⁺ cell proliferation
230 and ovulatory rupture repair.

231

232 **YAP signaling promotes *Procr*⁺ cells expansion at rupture site**

233 We have previously found that *Procr*⁺ progenitor cells expand instantly
234 at the periphery of the rupture site upon ovulation⁷. To investigate whether
235 YAP signaling activation is linked to the expansion of *Procr*⁺ progenitor cells,
236 TAM was administered to 4-week-old *Procr-CreER; Yap^{fl/fl}* (Yap-cKO) and
237 *Yap^{fl/fl}* (Ctrl) mice for 2 times, followed by superovulation. At 4.5d pi
238 (ovulation), FACS analysis showed a dramatic decrease of *Procr*⁺ progenitor
239 population when *Yap* was deleted (Fig. 4a-c).

240 To better visualize the change of *Procr*⁺ progenitor cells under the
241 influence of Yap signaling, we generated *Procr-rtTA; TetO-H2B-GFP^{+/-}; TetO-*
242 *Vgll4^{+/-}* mice (Vgll4-OE). Superovulation was performed to 4-week-old mice
243 and DOX was fed throughout the experiments to maintain the expression of
244 H2B-GFP in *Procr*⁺ cells (Fig. 4d). When analyzed at 4.5d pi (ovulation), at
245 the wound edge (defined as 20 cells on one side from the opening) of control
246 ovary (*Procr-rtTA; TetO-H2B-GFP^{+/-}*), there were about 7.4±0.3 H2B-GFP⁺
247 cells expressing the peak level of GFP (Fig. 4e, 4g). In contrast, in Vgll4-OE
248 ovary (*Procr-rtTA; TetO-Vgll4; TetO-H2B-GFP^{+/-}*), only 2.4±0.2 H2B-GFP⁺ cells
249 were observed at the wound edge (Fig. 4f-g). FACS analysis also showed that
250 the percentage of *Procr*⁺ progenitor population decreased significantly from
251 18.5±1.0% in Ctrl to 7.4±1.2% in Vgll4-OE at ovulation stage (Fig. 4h-i).

252 The proliferative activity of *Procr*⁺ cells was further evaluated *in vitro*. We
253 isolated OSE cells from control (*Procr-rtTA; TetO-H2B-GFP^{+/-}*) and Vgll4-OE
254 (*Procr-rtTA; TetO-Vgll4; TetO-H2B-GFP^{+/-}*) mice and placed in culture, followed
255 by live imaging to document the division of H2B-GFP⁺ (*Procr*⁺) cells (Fig. S3a-
256 b). In control cells, we observed frequent division of *Procr*⁺ cells, and in most

257 cases, it was one Procr⁺ cell dividing into two Procr⁺ cells (Fig. S3a). But in
258 Vgll4-OE, we could hardly observe cell division (Fig. S3b). Together, these
259 results suggest that inhibition of YAP signaling, by either Yap-deletion or Vgll4-
260 OE, impairs the expansion of Procr⁺ progenitors upon rupture.

261

262 **YAP signaling enhances Procr expression**

263 It is unclear how YAP maintains Procr expression during or after cell
264 division. Thus, we investigated the association of YAP activation and Procr
265 expression. OSE cells were isolated from *Procr-rtTA;TetO-H2B-GFP^{+/+}* mice,
266 and cultured on glass (YAP activation) or soft condition (0.48kPa, YAP
267 inactivation) (Fig. S4a-b). DOX was added 2 days before harvest. Consistent
268 with the notion, we found that, in soft condition, YAP was mostly cytoplasmic
269 and most OSE cells are H2B-GFP⁻ (Fig. S4a). In contrast, most OSE cells are
270 H2B-GFP⁺ in stiff condition and YAP was found in the nucleus (Fig. S4b).

271 These observations suggest that YAP activation might induce Procr
272 expression. We verified by qPCR that *Procr* expression is upregulated in stiff
273 conditions (Fig. S4c). Our results support the notion that YAP activation
274 induces Procr expression.

275 To further investigate whether YAP regulates Procr expression, we
276 knocked down YAP by shRNA in OSE culture and found that this inhibits Procr
277 expression (Fig. S4d-e). Furthermore, blocking YAP activation by Verteporfin
278 (VP) or Vgll4 overexpression also resulted in lower Procr expression (Fig. S4f-
279 g). These results suggest that inhibiting YAP signaling suppresses Procr
280 expression.

281 To investigate whether YAP/TEAD4 directly regulate Procr expression, we
282 analyzed the promoter of *Procr*. A Tead4 binding motif (5'-CATTCC-3') was
283 found at the proximal promoter of *Procr* (-1486 to -1481 bp) (Fig. 4j). ChIP-
284 qPCR showed that Tead4 could directly bind to the Procr promoter (Fig. 4k).

285 Therefore, we examined whether this Tead4 binding motif is responsible for
286 induction of Procr expression by Yap. While Yap induced the wild type
287 promoter-luciferase in a dose-dependent manner (Fig. 4l), it could not activate
288 the mutant reporter with the deletion of the Tead4 binding motif (Fig. 4m).
289 These results suggest that YAP directly promotes Procr expression. Together,
290 our data support a model that YAP signaling promotes expansion of Procr+
291 cells at rupture site through a combination of increased cell division and Procr
292 expression (Fig. 4n).

293 **Procr is essential for the progenitor property.**

294 The up-regulation of Procr expression coupled with YAP-induced cell
295 division implies that the expression of Procr may be important for keeping the
296 stem cell property in OSE. To assess the significance of Procr, we utilized a
297 *Procr-flox* allele (Liu and Zeng unpublished) and specifically deleted Procr in
298 the progenitor using *Procr*^{CreER/fl} (Procr-cKO) mice. TAM was administered in 4-
299 week-old mice for two times, followed by superovulation at 2 days after TAM
300 injection (Fig. 5a), and the phenotype was analyzed by ovary whole-mount
301 imaging. Ctrl and Procr-cKO ovaries formed comparable ruptures at 4.5d pi
302 (ovulation) (Fig. 5b, 5e). At 6d pi (OSE repair ongoing), Procr-cKO ovaries
303 showed bigger openings compared to control (Fig. 5c, 5e). At 7d pi (OSE repair
304 completed), Ctrl ovaries were covered by complete OSE, whereas Procr cKO
305 ovaries still had regions with unrepaired OSE (Fig. 5d-e). Furthermore, at 4.5d
306 pi (ovulation), the ovaries were harvested after 12 h of EdU incorporation. The
307 number of proliferated OSE cells at rupture site decreased from 5.5±0.3 cells
308 in control (*Procr*^{fl/+}) to 1.4±0.2 cells in Procr-cKO (Fig. 5f-h). After deletion of
309 Procr *in vivo*, total OSE cells were isolated and cultured *in vitro* for 7 days (Fig.
310 5i). We found that deletion of Procr inhibits the proliferation of progenitor cells,
311 resulting in reduced colony sizes (Fig. 5j-l). Overall, these data suggest that
312 Procr is essential for progenitor property upon rupture.

313

314 **Discussion**

315 In this study, we addressed the molecular mechanism in which OSE
316 stem/progenitor cells sense the ovulatory rupture and promptly turns on
317 proliferation and repair the wound. Our findings support the following model.
318 Procr⁺ OSE progenitors have intrinsically lower level of Vgll4. Upon ovulatory
319 rupture, decreased adherent junction at the proximity of rupture site promotes
320 Yap nuclear localization. These intrinsic and extrinsic factors together lead to
321 Yap signaling activation in Procr⁺ progenitors around the wound, which
322 sequentially stimulates proliferation of the progenitors. Importantly, YAP
323 activation directly upregulates Procr expression in the dividing cells, resulting
324 in the expansion of Procr⁺ progenitors around the wound (Fig. 5m). Blocking
325 Yap signaling in the progenitors by Yap-cKO or Vgll4-OE impairs the
326 progenitors' activities and hinders OSE repair. Furthermore, Procr function is
327 essential for these progenitors. When Procr was deleted, stem cell property
328 was lost hindering OSE repair.

329 YAP signaling promotes Procr⁺ cell expansion at rupture site through a
330 combination of increased cell division and Procr expression. In the current study,
331 YAP is particularly activated in Procr⁺ progenitor cells at rupture site. During
332 the late stage of follicle development, the pre-ovulatory follicle forms a
333 protrusion towards OSE. Subsequently, ovulation generates a rupture on OSE.
334 These contiguous events might induce the thinning of OSE surrounding the pre-
335 ovulatory follicles or at the proximity of the rupture site, leading to YAP activation.
336 Another possibility is that the pre-ovulatory follicle protrusion or the release of
337 oocytes induce special mechanical force on the OSE surrounding the wound,
338 subsequently activates YAP signaling. Interestingly, at the rupture sites, Vgll4 is
339 highly expressed in Procr⁻ cells, preventing YAP pathway activation in those
340 cells around the rupture sites. We showed here that reduced levels of Vgll4 in

341 Procr+ progenitors likely contribute to the selective activation of YAP signaling
342 in these cells. Further study should investigate what mechanism determines
343 the lower expression of Vgll4 in Procr+ progenitor cells.

344 In the current study, we generated a new *tetO-Vgll4* mouse model that
345 enables the overexpression of Vgll4 in specific cell type. The overexpression
346 of Vgll4 in the progenitor of OSE has been validated using *Procr-rtTA* (Fig. 3g
347 and Fig. S2d-e). The advantages brought by our *tetO-Vgll4* reporter will be of
348 broad value in studies of Hippo-Yap signaling across all tissues.

349 Procr expression is initially found on the surface of vascular cells exerting
350 an anti-coagulation role, by binding and activating protein C (PC) in the
351 extracellular compartment³⁷. More recently, studies from us and others have
352 identified Procr as a stem cell surface marker in multiple tissues^{7,38-40}, but less
353 is known regarding the function of Procr in stem/progenitor cells. In the
354 current study, we demonstrate that, Procr is essential for the proliferation of
355 Procr+ progenitor cells and OSE repair upon rupture. Our previous report
356 indicated that PROCR concomitantly activates multiple pathways including
357 ERK, PI3K-Akt-mTOR and RhoA-Rock-P38 signaling in breast cancer cells⁴¹.
358 We speculate that similar intracellular pathways might be involved in the
359 Procr+ OSE cells. Procr is regarded as a Wnt target gene from an *in vitro*
360 screen in mammary stem cell culture³⁹. In this study, we identify YAP as a
361 novel upstream regulator of Procr. ChIP-qPCR and promoter luciferase
362 experiments demonstrate that *Procr* transcription can be directly upregulated
363 by YAP activation.

364 The phenomena of YAP promoting stem/progenitor cell expansion has
365 been reported in various tissues^{30,33,34,42-44}. Yet, in this process, less is known
366 about how YAP maintains stem cell properties. To the best of our knowledge,
367 this is the first report illustrating a mechanism through which YAP promotes
368 cell proliferation, and simultaneously upregulates the expression of an

369 essential stemness gene to maintain cell fate, leading to a rapid expansion of
370 stem cell numbers around the wound. In summary, our study provides new
371 evidence and molecular insights into how the OSE stem cell senses ovulatory
372 rupture and promptly expands their numbers for repair. This may have a
373 broad implication to understand the action of tissue stem cells during wound
374 healing in other tissues.

375

376

377

378 **ACKNOWLEDGMENTS**

379 Thank to Dr. Chi Chung Hui of University of Toronto for helpful comments on
380 the manuscript. This research was supported by grants from National Natural
381 Science Foundation of China (31625020 to Y.A.Z. ; 32030025 and 31625017
382 to L. Z.); National Key Research and Development Program of China
383 (2019YFA0802000 and 2020YFA0509002 to Y.A.Z.); the Chinese Academy of
384 Sciences (XDA16020200 to Y.A.Z.); Fundamental Research Funds for the
385 Central Universities (2020XZZX002-22 to J.F.); China Postdoctoral Science
386 Foundation (2020TQ0260 to J.W.); Zhejiang Provincial Preferential
387 Postdoctoral Foundation (ZJ2020150 to J.W.); Postdoctoral Exchange
388 Fellowship Program 2021 (No.160 Document of OCPC, 2021) to J.W.;
389 Shanghai Leading Talents Program to L. Z..

390

391 **AUTHOR CONTRIBUTION**

392 J.W. and Y.A.Z. designed the project and wrote the manuscript. J.W. performed
393 most of the experiments including genetic crosses, FACS, RNA *in situ*, staining,
394 cell culture, luciferase assay, qPCR and ovary phenotype analysis. L.H. and
395 W.Y. performed ChIP-qPCR and provided YAP overexpression plasmids. Z.X.
396 cloned the Procr promoter luciferase plasmids. L.B. and C.L. performed genetic
397 crosses and superovulation. Y.L., Z.W. and Z.L. constructed the *TetO-Vgll4*
398 mice and provided *YAP^{fl/fl}* mice. L.H, Z.L. and J.F. helped project design.

399 **DECLARATION OF INTERESTS**

400 The authors declare no competing interests.

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408 **Methods**

409 **Key Resource Table**

410

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Biotin Rat anti mouse CD31 (clone MEC 13.3)	BD	Cat#553371; RRID: AB_394817
Biotin Rat anti mouse CD45 (clone 30-F11)	BD	Cat#553080; RRID: AB_394610
Biotin Rat anti mouse Ter119 (clone TER-119)	BD	Cat#553672; RRID: AB_394985
FITC Rat anti mouse Cd31 (clone MEC 13.3)	BD	Cat# 553372; RRID: AB_394818
FITC Rat anti mouse Cd45 (clone 30-F11)	BD	Cat# 553079; RRID: AB_394609
FITC Rat anti mouse Ter119 (clone TER-119)	BD	Cat# 557915; RRID: AB_396936
APC Rat anti-mouse EpCAM (clone G8.8)	Thermo Fisher	Cat#17-5791-82; RRID: AB_2716944
Streptavidin-apc-Cy7	BioLegend	Cat# 405208; RRID: N/A
Streptavidin-V450	BD	Cat# 560797; RRID: AB_2033992
Rat anti-mouse K8 (clone TDM1)	DSHB	Cat# TROMA-I; RRID: AB_531826
Biotin Rat anti mouse Procr (clone)	Thermo Fisher	Cat# 13-2012-82; RRID: AB_657694
PE Rat anti mouse Procr (clone 1560)	Thermo Fisher	Cat# 12-2012-82; RRID: AB_914317

Mouse anti-E-Cadherin (clone 36)	BD	Cat# 610181; RRID: AB_397581
Rabbit anti-YAP (D8H1X)	CST	Cat# 14074; RRID: AB_2650491
Rabbit anti-YAP (polyclonal)	ABclonal	Cat# A1002; RRID: AB_2757539
Chicken anti-GFP (polyclonal)	Thermo fisher	Cat#A10262; RRID: AB_2534023
Rabbit anti-Vgll4	Self-made	Cat# N/A; RRID: N/A
Mouse anti-Flag (clone M2)	Sigma	Cat#F1804; RRID: AB_262044
Rabbit anti-Vgll4 (polyclonal)	ABclonal	Cat# A18248; RRID: AB_2862024
Rabbit anti-Laminin (polyclonal)	Sigma	Cat# L9393; RRID: AB_477163
Normal mouse IgG	Santa cruz	Cat# sc-2025; RRID: AB_737182
Mouse anti-TEAD4 (5H3)	Abcam	Cat# ab58310; RRID: AB_945789
Bacterial and Virus Strains		
N/A		
Biological Samples		
N/A		
Chemicals, Peptides, and Recombinant Proteins		
RNAiso plus	Takara	Cat#9109
HEPES	Sigma-Aldrich	Cat#H4034
Type IV collagenase	Worthington	Cat#LS004189
PBS	Thermo Fisher	Cat#10010049
RPMI 1640	Thermo Fisher	Cat#12633-012
DMEM/F12	Thermo Fisher	Cat#11320
DMEM, high glucose	Thermo Fisher	Cat#11965092

Opti-MEM	Thermo Fisher	Cat#31985070
0.25% trypsin-EDTA	Thermo Fisher	Cat#25200056
Penicillin-Streptomycin	Thermo Fisher	Cat#15140-122
ITS	Thermo Fisher	Cat#41440
DNase I	Sigma-Aldrich	Cat#D4263
Matrigel	BD	Cat#354230
EGF	BD	Cat#354001
2-mercaptoethanol	Millipore	Cat#ES-007-E
Hydrocortisone	Sigma-Aldrich	Cat#614517
MEM Non-Essential Amino Acids Solution	Thermo Fisher	Cat#11140050
L-Glutamine	Thermo Fisher	Cat#25030081
Sodium pyruvate	Thermo Fisher	Cat#11360070
Dispase	BD	Cat#354235
Red Blood Cell Lysing Buffer	Sigma-Aldrich	Cat#R7757
DMSO	Sigma-Aldrich	Cat#D2650
Paraformaldehyde, PFA	Sigma-Aldrich	Cat#P6148
Triton-X 100	Sigma-Aldrich	Cat#T9284
Tamoxifen	Sigma-Aldrich	Cat#T5648
Doxycycline hyclate	Sigma-Aldrich	Cat#D9891
DAPI	Thermo Fisher	Cat#D1306
Hoechst33342	Thermo Fisher	Cat#H21492
OCT	Thermo Fisher	Cat#D6506
Fetal bovine serum, FBS	PAN	Cat#P30-3302
Critical Commercial Assays		
Click-iT™ EdU Cell Proliferation Kit for Imaging	Thermo Fisher	Cat#C10337
Dual-Luciferase® Reporter Assay System Technical Manual	Promega	Cat#E1910
Opal 4-Color Automation IHC Kit	PerkinElmer	Cat#NEL8200001KT
RNAscope® Multiplex Fluorescent Detection Kit v2	ACD	Cat#323110
SYBR green Mix	Roche	Cat#04913914001
PrimeScript RT master Mix	Takara	Cat#RR036A
Deposited Data		

N/A	N/A	N/A
Experimental Models: Cell Lines		
Human: HEK293T cells	ATCC	Cat# CRL-3126
Experimental Models: Organisms/Strains		
Mouse: <i>Procr-creER</i>	<i>Procr^{CreER 39}</i>	N/A
Mouse: <i>Procr-rtTA</i>	<i>Procr^{rtTA 7}</i>	N/A
Mouse: <i>R26-mTmG</i>	The Jackson Laboratory	Jax: 007576
Mouse: <i>TetO-H2B-GFP</i>	The Jackson Laboratory	Jax: 005104
Mouse: <i>YAP^{fl/+}</i>	<i>YAP^{fl/+ 26}</i>	N/A
Mouse: <i>Procr^{fl/+}</i>	(unpublished, Liu and Zeng)	N/A
Mouse: ICR	SLAC	N/A
Mouse: C57BL/6	SLAC	N/A
Mouse: <i>TetO-Vgll4-Flag</i>	this paper	N/A
Oligonucleotides		
Sequence for qPCR primers, see Table S1	This paper	N/A
Sequence for mouse genotyping, see Table S2	This paper	N/A
Recombinant DNA		
pLKO.1-EGFP-shYAP	This paper	N/A
pcDNA3.1-YAP	This paper	N/A
PGL3.1 <i>Procr</i> promoter	This paper	N/A
PGL3.1 <i>Procr</i> promoter TEAD4 binding motif mutation	This paper	N/A
Software and Algorithms		
GraphPad Prism 6	GraphPad software	https://www.graphpad.com

Adobe Photoshop CC2017	Adobe	https://www.adobe.com/product/photoshop.html
Adobe Illustrator CC2017	Adobe	https://www.adobe.com/product/illustrator.html
Adobe Premiere CC2017	Adobe	https://www.adobe.com/cn/products/premiere.html
Flow Jo vX	Flow Jo	https://www.flowjo.com
Other		
N/A		

411

412 LEAD CONTACT AND MATERIALS AVAILABILITY

413 Further information and requests for resources and reagents should be
 414 directed to and will be fulfilled by the Lead Contact, Yi Ariel Zeng
 415 (yzeng@sibcb.ac.cn). All unique/stable reagents generated in this study are
 416 available from the Lead Contact with a completed Materials Transfer
 417 Agreement.

418

419 EXPERIMENTAL MODEL AND SUBJECT DETAILS

420 Experiment animals

421 *TetO-H2B-GFP^{+/-}* (Stock: 005104), *R26-mTmG* (Stock: 007576) from Jackson
 422 Laboratories, *Procr^{CreER}*³⁹, *Procr^{rtTA}*⁷, *YAP^{fl/+}*²⁶, *Procr^{fl/+}* (*unpublished*, Liu and
 423 Zeng), *TetO-Vgll4* were used in this study. The *TetO-Vgll4* mouse line was
 424 generated by knocking in a cassette of TetO-Vgll4-Flag-wpre-polyA behind
 425 3'UTR of *Col1a1* gene (Fig. S2). All mice were housed in the SIBCB animal
 426 facility under IVC standard with a 12-hr light/dark cycle at room temperature.
 427 Both ovaries were used per mice and the number of mice per experiment was
 428 shown in figure legends. For targeted knockout *in vivo*, 4-5 weeks mice were

429 administered with TAM diluted in sunflower oil by intraperitoneal (IP) injection
430 at a concentration of 2mg per 25g body weight for two or three times (on every
431 second day). For superovulation experiments, 4-5 weeks old mice were
432 injected with 10 IU of pregnant mare serum gonadotropin (PMSG) by IP,
433 followed by IP injection of 10IU of human chorionic gonadotropin (HCG) about
434 48 h later. For DOX feeding, doxycycline hyclate (DOX) was dissolved in drink
435 water at a concentration of 1 mg/ml. Experimental procedures were approved
436 by the Animal Care and Use Committee of Shanghai Institute of Biochemistry
437 and Cell Biology, Chinese Academy of Sciences, with a project license number
438 of IBCB0065.

439 **OSE cells isolation and flow cytometry**

440 Ovaries from super-ovulated or 4-12 weeks old female mice were isolated, and
441 the oviduct and bursa were carefully cleared out under dissect microscope. The
442 ovaries were minced into pieces as small as possible, and then placed in 10 ml
443 digest buffer (RPMI 1640with 5% fetal bovine serum, 1% penicillin-
444 streptomycin, 25 mM HEPES and 300U/ml collagenase IV). After digestion at
445 37 °C, 100 rpm for about 1 hour, ovarian cells were obtained after centrifugation
446 at 1000 rpm for 5 min. The red blood cells were lysed with buffer at room
447 temperature for 5 min, and then single cells were obtained with 0.25% trypsin
448 treatment at 37 °C for 5 min, followed by 0.1 mg/ml DNaseI incubation at 37 °C
449 for 5 min with gently pipetting before filtering through 70 μ m cell strainers. The
450 single cells were incubated on the ice and in dark with the following antibodies
451 at a dilution of 1:200: FITC conjugated, PE conjugated or biotinylated CD31,
452 CD45, EpCAM-APC, Procr-PE, Procr-Biotin, Streptavidin-APC-Cy7,
453 Streptavidin-V450. All analysis and sorting were performed using a FACSJazz
454 (Becton Dickinson). The purity of sorted population was routinely checked and
455 ensured to be > 95%.

456 **OSE cells 3D culture assay**

457 FACS sorted OSE cells were resuspended with 60 μ l 100% growth factor-
458 reduced Matrigel and placed around the rim of a well of a 24-well plate, and

459 allowed to solidify for at least 15 min at 37 °C in a 5% CO₂ incubator before
460 adding 0.5-1 ml culture medium. Colonies were grown for 7–9 days and the
461 medium was changed every second days. The culture medium was prepared
462 by adding 5% FBS, 4 mM L-glutamine, 1 mM sodium pyruvate, 10 ng/ml
463 epidermal growth factor, 500 ng/ml hydrocortisone, 5 mg/ml insulin, 5 mg/ml
464 transferrin, 5 ng/ml sodium selenite, 0.1 mM MEM non-essential amino acids,
465 10⁻⁴ M 2-mercaptoethanol into DMEM/F12. The organoid images were
466 captured by Zeiss inverted microscope at day7-day9.

467 **Immunohistochemistry**

468 For section staining, ovarian tissues were fixed in 4% PFA at room temperature
469 for 15 min, following by washed with PBS for 3 times, dehydrated in 30%
470 sucrose at 4°C overnight and embedded with Optimum Cutting Temperature
471 (OCT). 16-18 µm tissue sections were incubated in 0.1% or 0.5% TritonX100
472 diluted with PBS (PBST) for 20 min and then 1 hour blocking using 10% FBS
473 in PBST. Then sections were incubated with primary antibodies diluted in
474 blocking buffer at 4°C overnight, followed by washes for 3 times (20 min per
475 time). After wash, the sections were further incubated with secondary
476 antibodies and DAPI diluted in blocking buffer for 2 hours at room temperature
477 in dark, followed by washes for 3 times (20 min per time) and mounted with
478 mounting medium.

479

480 For staining of cultured colonies, colonies were released from Matrigel by
481 incubating with dispase for 20-30 min. Then the colonies were fixed in 4% PFA
482 on ice for 10 min, followed by cytospin (Thermo Fisher) into slides and staining
483 protocol described above.

484 For whole mouse ovary immunohistochemistry, mouse ovaries that cleared
485 without bursa and oviduct were fixed with fresh 4% PFA at room temperature
486 for 15 min in 4 ml Eppendorf tubes, followed by washing with PBST for three
487 times (20 min per time). The staining of whole ovaries was then transferred into
488 the 2 ml Falcon tubes using a dropper carefully. Ovaries were blocked for 1
489 hour using 10% FBS in PBST. Then, the ovaries were incubated with primary
490 antibodies diluted in blocking buffer at 4 °C for 48 hours on a transference

491 shaker with 10 rpm, followed by washing for three times (20 min per time) at
492 room temperature. After washing, the ovaries were incubated with secondary
493 antibodies diluted in blocking buffer for 24 hours at 4 °C in dark, and
494 counterstained with DAPI. The ovaries could be stored in PBST at 4 °C for at
495 least 2 weeks.

496 For YAP staining, Tyramide signal amplification assay (TSA staining) was used.
497 Briefly, paraffin sections were rehydrated in histo-clear and gradual ethanol
498 (100%, 100%, 95%, 85%, 75%, 50%, 30%) and the TSA staining was performed
499 using the Opal 4-Color Automation IHC Kit (PerkinElmer) following the
500 manufacturer's instructions. After TSA staining for YAP, staining for GFP and
501 Krt8 was performed following protocol described above.

502 Tissue sections and organoids fluorescent images were captured using Leica
503 DM6000 TCS/SP8 laser confocal scanning microscope with a 20×/0.75 or
504 40×/0.75 or 63×/0.75 IMM objective with 1-3 μm z-step. Confocal images were
505 processed with maximum intensity projections.

506 Whole mouse ovarian fluorescent images were captured with inverted Leica
507 TCS SP8 WLL at a 10×/0.75 objective, z-stack was ~ 50–80 layers with 6-7 μm
508 per layer, and the area was about 1.5 mm x 1.5 mm, which was about 1/6-1/4
509 of the adult ovary surface.

510 Primary antibodies used were: rat anti-Krt8 (1:250), rabbit anti-YAP (1:200),
511 chicken anti-GFP (1:500), mouse anti-E-Cadherin (1:500), rabbit anti-Vgll4
512 (1:500), rabbit anti-Laminin (1:500). The secondary antibodies used were
513 donkey anti-rat Cy3, donkey anti-rat Alexa Fluor 488, donkey anti-mouse Alexa
514 Fluor 488, donkey anti-rabbit Alexa Fluor Cy3, donkey anti-mouse Alexa Fluor
515 Cy3, donkey anti-rat Alexa Fluor Cy3, donkey anti-rat Alexa Fluor 647, donkey
516 anti-mouse Alexa Fluor 647, donkey anti-rabbit Alexa Fluor 647, all secondary
517 antibodies were used as 1:1000. At least three times repeats were done per
518 tissue block. Only representative images were shown.

519 **Western Blotting**

520 Digested cells were lysed in SDS-PAGE loading buffer and boiled for 10min.
521 Proteins were separated by SDS-PAGE and transferred to nitrocellulose
522 membrane (GE company). Bolts were blocked with 3% BSA in TBST (50 mM
523 Tris-HCl, 150 mM NaCl, 0.05% Tween-20, pH 7.5) for 1 hour and incubated
524 with primary antibodies at 4 °C overnight, followed by incubated with secondary
525 IgG-HRP antibodies for 2 hours at room temperature. Protein bands were
526 visualized with chemiluminescent reagent and exposed to Mini
527 Chemiluminescent Imager.

528 **RNA *in situ***

529 In situ hybridization was performed using the RNA scope kit (Advanced Cell
530 Diagnostics) following the manufacturer's instructions. *Procr* probes
531 (REF#410321) and *Cyr61* probes (REF#429001) were ordered from Advanced
532 Cell Diagnostics. After in situ hybridization, TSA method was used for Krt8
533 staining following the using the Opal 4-Color Automation IHC Kit (PerkinElmer)
534 following the manufacturer's instructions. The images were captured using
535 Leica DM6000 TCS/SP8 laser confocal scanning microscope with a 63×/0.75
536 IMM objective.

537

538 **EdU labelling assays**

539 The proliferation of OSE cells *in vivo* or *in vitro* was measured by 5- ethynyl-29-
540 deoxyuridine (EdU) uptake. Briefly, mice were injected with 100 µl EdU (2.5
541 mg/ml in dimethyl sulfoxide) for 12 h. Then ovaries were harvested for section,
542 following by EdU color staining using Click-iT EdU Alexa Fluor Imaging Kit
543 (prepared according to the manufacturer's instructions). After washed with PBS
544 for 3 times (10 min per time), EdU color development was performed following
545 manufacturer's protocol. After EdU signal developing, sections were blocked in
546 blocking buffer for 1 h at room temperature followed by antibody staining and
547 mounted with mounting medium for imaging and quantification.

548

549 **Living image of cultured OSE cells**

550 OSE cells were isolated from the mice and cultured on glass for 3-4 days. DOX
551 was added into the medium 1 day and Hoechst33342 was added 30 mins

552 before image. Live-cell imaging was performed at 37 °C on a Zeiss
553 Celldiscoverer 7 with perfect focus system. Cells were imaged at 1 time per 5
554 mins for 24 hours with 70% laser power.

555 **Chromatin Immunoprecipitation-qPCR (ChIP-qPCR)**

556 Cultured primary OSE cells were crosslinked in a final concentration of 1%
557 formaldehyde (Sigma) PBS buffer for 15 min at 37 °C, then added glycine to
558 stop crosslinking. Chromatin from nuclei was sheared to 200–600 bp fragments
559 using ultrasonic apparatus, then immunoprecipitated with antibody of TEAD4
560 (ab58310, Abcam) or normal mouse IgG (sc-2025, Santa Cruz) overnight.
561 Antibody/antigen complexes were recovered with Protein A/G PLUS-Agarose
562 (sc-2003, Santa Cruz Biotechnology) for 2 hours at 4 °C. After washing, the
563 chromatin was eluted, de-crosslinked and digested. The immunoprecipitated
564 DNA was collected with QIAQUICK PCR Purification Kit (QIAGEN)). Purified
565 DNA was performed with ChIP-qPCR. Assessing the enrichment of the proteins
566 of interest on the targeting region by calculating the value of “fold over IgG”.
567 ChIP-qPCR primers used were as follows.

568 Ctf_g CHIP-F, TGTGCCAGCTTTTTCAGACG;
569 Ctf_g CHIP-R, GAACTGAATGGAGTCCTACACA;
570 Procr CHIP-F, ATATCCGAGCTACACACGGC;
571 Procr CHIP-R, GTGAATGCACACACACACC;
572 Negative Ctrl CHIP-F, GATCAACGCAGGGGAGAGAG;
573 Negative Ctrl CHIP-R, TATCCCCACTGCCCAAGA.

574

575 **Preparation of Procr promoter luciferase reporter and luciferase assay**

576 The DNA sequence of Procr promoter containing TEAD4 binding sites (about
577 2kb before the initiation codon) were amplified by PCR, separated by agarose
578 gel, purified by Gel Extraction Kit, and then cloned into pGL3-promoter vector.
579 Luciferase assays were performed in 293T cells with the pGL3-Procr
580 promoter luciferase reporter described above 0.2 mg reporter plasmid were
581 transfected together with CMV-Renilla (0.005 mg) to normalize for transfection
582 efficiency. For luciferase assays in overexpression plasmid-transfected cells,

583 cells were transfected with the indicated plasmids and reporter plasmid
584 together, and then the luciferase activity was measured 36 hours later using
585 Dual-Luciferase® Reporter Assay System Technical Manual kit following
586 manufacturer's protocol.

587 **Cell Culture, viral production and infection**

588 HEK293T, C3H10 were obtained from American Type Culture Collection
589 (ATCC) and cultured in Dulbecco modified essential medium (DMEM)
590 supplemented with 10% FBS plus 1% penicillin and streptomycin antibiotics at
591 37 °C in 5% CO₂ (v/v). For cells cultured on different modulus of elasticity,
592 hydrogel substrates with tunable mechanical properties were prepared
593 following the previous protocol ⁴⁵, and the glass was as solid control. HEK
594 293T cells were used to produce lentivirus. When cells were up to 80-90%,
595 indicated constructs and packaging plasmids transfection was performed in
596 Opti-MEM, and the media were replaced 12 hours later. Viral supernatants
597 were collected 48-72 hours after medium change and filtered through a 0.45
598 µm filter, followed by concentration. For primary OSE cells infection,
599 concentrated virus was diluted in the culture medium along with 1:100
600 polybrene.

601 **RNA isolation and quantitative real-time PCR**

602 Total RNA was isolated from fresh OSE cells or cultured cells lysed with Trizol
603 according to the manufacturer's instructions. The cDNA was generated from
604 equal amounts of RNA using the SuperScriptIII kit. qPCR was performed on a
605 StepOne Plus (Applied Biosystems) with Power SYBR Green PCR Master Mix.
606 RNA level was normalized to *Gapdh*. The cycling condition was as 10 min at
607 95°C for initial denaturing, 40 cycles of 15 s at 95°C for denaturing, 1 min at
608 60°C for annealing and extension, following by melt curve test. Primers used
609 were as follows.

610 *Procr-F*, CTCTCTGGGAAAACCTCCTGACA;

611 *Procr-R*, CAGGGAGCAGCTAACAGTGA;

612 *Vgll4-F*, ATGAACAACAATATCGGCGTTCT;

613 *Vgll4-R*, GGGCTCCATGCTGAATTTCC;

614 *YAP-F*, GCCATGCTTTCGCAACTGAA;

615 *YAP-R*, CAAAACGAGGGTCCAGCCTT;

616 *Cyr61-F*, TCGCAATTGGAAAAGGCAGC;

617 *Cyr61-R*, CCAAGACGTGGTCTGAACGA.

618

619 **Quantification and statistical analysis**

620 For quantification of YAP+, Vgll4+ and EdU+ cells, 40 OSE cells at the both
621 edges of ruptured sites (20 OSE cells at one side of rupture site) was identified
622 as rupture regions, while other regions as non-rupture regions. At least 30
623 rupture regions and 30 non-rupture regions were counted. For quantification of
624 the diameter of rupture, the longest diameter was counted, and at least 20
625 rupture sites were counted. For quantification of mG+ clone sizes, about 0.3
626 mm² circle centered on ruptured sites was identified as rupture regions. At least
627 30 rupture regions were counted. For quantification of colonies size, diameters
628 of the colonies were measured using Zeiss software.

629

630 Statistical analyses were calculated in GraphPad Prism (Student's t-test or
631 One-way ANOVA). For all experiments with error bars, the standard error of
632 measurement (s.e.m) was calculated to indicate the variation within each
633 experiment. All the p values were calculated using GraphPad PRISM 6 with the
634 following significance: n.s. p > 0.05; * p < 0.05; ** p < 0.01; *** p < 0.001.
635 Statistical details for each experiment can be found in the figures and the
636 legends.

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649 **Figure Legends**

650 **Fig 1. Rupture-induced YAP signaling activation is preferentially activated** 651 **in Procr+ progenitors at the rupture sites.**

652 a, Sections from wildtype ovaries at ovulation stage were stained with Krt8 (K8)
653 and E-cadherin (E-Cad). Confocal images showed less E-cad in the OSE of
654 proximal regions surrounding the rupture sites (view #1, #3 in a) compared with
655 distal regions (view #2, #4 in a). Scale bar, 100 μ m for zoom out and 10 μ m for
656 zoom in. n=3 mice and 15 images.

657 b-c, Sections from wildtype ovaries at ovulation stage were stained with K8 and
658 YAP. Confocal images showed YAP nuclear localization in the OSE was only
659 observed in the proximal regions surrounding the rupture sites (b), but not in
660 the distal regions (b) or the non-rupture sites (c). Scale bar, 100 μ m for zoom
661 out and 20 μ m for zoom in. n=3 mice and 15 images.

662 d-f, *Procr-rtTA;TetO-H2B-GFP^{+/-}* mice were fed with doxycycline for 3 days and
663 harvested at ovulation stage. Confocal images of ovarian sections with Krt8 (K8)
664 and YAP staining (d-e) and quantification (f) were showed. Nuclear YAP
665 staining is preferentially detected in Procr+ (histone 2B-GFP+) cells in rupture
666 proximal region (arrowheads in d), whereas at the non-rupture site, YAP
667 staining was cytoplasmic regardless in Procr+ (arrows in e) or Procr- cells (e).
668 Scale bar, 100 μ m for zoom out and 10 μ m for zoom in. n=3 mice and 15 images.
669 One-way ANOVA with Tukey test is used for comparison of multiple groups.
670 ***P<0.001.

671 g-i, Combination of *Procr* and *Cyr61* double fluorescent *in situ* with K8 antibody
672 immunohistochemistry staining (g-i). Confocal images showed co-localization
673 of *Procr* and *Cyr61* in the OSE at the rupture sites (arrowhead in g), while at
674 non-rupture regions, both Procr+ and Procr- cells had low incidence of *Cyr61*
675 expression (h). Quantification showed increased *Cyr61* expression in Procr+
676 cells at rupture sites compared with Procr- cells at rupture sites or Procr+ cells
677 at non-rupture regions (i). Scale bar, 100 μ m for zoom out and 1 μ m for zoom in.
678 n=3 mice and 15 images. One-way ANOVA with Tukey test is used for
679 comparison of multiple groups. ***P<0.001.

680

681 **Fig 2. Deletion of YAP in Procr+ cells hinders OSE rupture repair and**
682 **progenitor proliferation.**

683 a, Illustration of TAM induction and superovulation strategy.

684 b-e, Yap was deleted in Procr+ cells using *Procr-CreER;Yap^{fl/fl}* mice (Yap-cKO),
685 and *YAP^{fl/fl}* mice was used as control (Ctrl). Ovary whole-mount staining with
686 K8 and Laminin was performed (b-d) and the wound size in diameter was
687 quantified (e). At 4.5d (ovulation) (star) Ctrl and Yap-cKO ovaries had
688 comparable wound size (* in b, e). At 6d (OSE repair ongoing), the wounds in
689 Ctrl ovary were significantly smaller than those in Yap-cKO ovary (c, e). At 7.5d
690 (repair completed), the wound was completely repaired in Ctrl, while the Yap-
691 cKO ovary still showed obvious wounds (star) (* in d, e). Scale bar, 100 μ m. n=3
692 pairs of mice.

693 f-h, Ctrl and Yap-cKO mice were subjected to 12hrs EdU incorporation and
694 were harvested at 4.5 days (Ovulation stage). Representative images (f-g) and
695 quantification (h) were showed. Out of 20 cells next to the rupture on one side,
696 the numbers of EdU+ cells (arrowhead) in the OSE of rupture site decreased
697 from 5.52 ± 0.33 cells in Ctrl to 1.42 ± 0.16 cells in Yap-cKO. Scale bar, 100 μ m
698 for zoom out and 20 μ m for zoom in. n=3 pairs of mice. Unpaired two-tailed t
699 test is used for comparison. ***P<0.001.

700 i-k, Total OSE cells from *Procr-CreER;Yap^{fl/fl}* mice (Yap-cKO), and *Yap^{fl/fl}* mice
701 (Ctrl) were isolated by FACS at 4.5 days (Ovulation stage) (i), followed by
702 culture in 3D Matrigel for 7 days. Representative bright-field and confocal
703 images of K8 staining were shown (j). Colony sizes in diameter were measured
704 (k). Scale bar, 20 μ m. Data are pooled from three independent experiments and
705 displayed as mean \pm s.e.m. Unpaired two-tailed t test is used for comparison.
706 ***P < 0.001.

707 l, Illustration of lineage tracing, deletion of YAP and superovulation strategy.

708 m-o, *Procr-CreER;YAP^{fl/fl};R26-mTmG* (Yap-cKO) and *Procr-CreER;R26-*
709 *mTmG* (Ctrl) mice were used. At 4.5pi (ovulation), ovary whole-mount confocal
710 imaging showed zones of concentrated GFP+ cells surrounding the rupture site
711 in Ctrl, while fewer GFP+ cells were seen in Yap-cKO ovary (m). At 7pi (repair
712 completed), ovary whole-mount confocal imaging showed large GFP+ patches

713 located at corpus luteum (C.L.) in Ctrl, while rare GFP⁺ cells surrounding the
714 unrepaired wound in Yap-cKO ovary (n). Quantification showed significantly
715 fewer GFP⁺ cells in Yap-cKO compared with Ctrl in both ovulation stage and
716 repair completed stage. Quantification showed an expansion of GFP⁺ cell
717 numbers in Ctrl mice during the tracing and no expansion in Yap-cKO (o). Scale
718 bar, 100µm. n=3 pairs of mice. ***P < 0.001.

719

720 **Fig 3. An intrinsic lower level of Vgll4 in Procr⁺ cells is essential for**
721 **Procr⁺ cells' stemness and OSE rupture repair.**

722 a-b, At ovulation stage, Procr⁺ and Procr⁻ OSE cells (Lin⁻, EpCAM⁺) were
723 FACS-isolated (a). qPCR analysis showed the lower *Vgll4* level in Procr⁺ cells
724 (b). Data are pooled from 3 independent experiments and presented as
725 mean±s.e.m. ***P<0.001.

726 c-e, *Procr-rtTA;TetO-H2B-GFP* mice were administered with PMSG and HCG
727 to induce superovulation, and fed with doxycycline for 3 days before harvest.
728 Ovarian sections were stained with Vgll4 and K8. Representative images
729 showed that at both rupture proximal region (c) and non-rupture region (d),
730 H2B-GFP⁻ (Procr⁻) OSE cells have high Vgll4 expression (arrows in c, d), while
731 H2B-GFP⁺ (Procr⁺) OSE cells have no Vgll4 expression (arrowheads in c, d).
732 Scale bar, 20µm for zoom out and 5µm for zoom in. Quantification of the
733 staining was shown in (e). n=3 mice and 15 images. Unpaired two-tailed t test
734 is used for comparison. ***P<0.001.

735 f-g, Targeting strategy and validation of *TetO-Vgll4* knock-in mouse (f-g). A
736 cassette of TetO-Vgll4-Flag-wpre-polyA was knocked in behind 3'UTR of
737 *Col1a1* gene (f). Immunohistochemistry staining of Vgll4 in the ovaries
738 indicated more Vgll4⁺ OSE cells at the rupture sites (g). Scale bar, 10µm. n=3
739 pairs of mice.

740 h-l, Illustration of superovulation and overexpression of Vgll4 in Procr⁺ cells (h).
741 Ovary whole-mount confocal images of K8 and Laminin showed that at 4.5d
742 (ovulation), Ctrl (*TetO-Vgll4*) and Vgll4-OE (*Procr-rtTA;TetO-Vgll4*) ovaries have
743 similar wound sizes (* in i). At 6d (repair ongoing), the wound sizes in Ctrl mice
744 were smaller than those in Vgll4-OE (* in j). At 7.5d (repair completed), Ctrl
745 ovary had completely repaired, while Vgll4-OE mice had obvious opening (* in

746 k). Scale bar, 100 μ m. The sizes of the wound in diameter were quantified (l).

747 n=3 pairs of mice.

748 m-o, The mice were harvested at 4.5 days (ovulation) after 12hrs EdU
749 incorporation. Representative images (m, n), and quantification (o) showed the
750 number of EdU+ cells in the OSE surrounding the rupture site (arrowheads in
751 m) decreased from 3.73 \pm 0.26 in Ctrl to 1.04 \pm 0.15 in Vgll4-OE (arrowheads in
752 n). Scale bar, 100 μ m for zoom out and 20 μ m for zoom in. n=3 pairs of mice.
753 Unpaired two-tailed t test is used for comparison. ***P<0.001.

754 p-r, Total OSE cells were isolated by FACS from Ctrl and Vgll4-OE at 4.5 days
755 (ovulation) (p), and cultured in 3D Matrigel. At day 7 in culture, colony sizes
756 were measured in diameter (q), and representative images were shown (r) out
757 of 15 images in each group. Scale bar, 20 μ m. Data are pooled from three
758 independent experiments and displayed as mean \pm s.e.m. Unpaired two-tailed t
759 test is used for comparison. ***P < 0.001.

760

761 **Fig 4. YAP signaling promotes Procr+ cells expansion at rupture sites**
762 **through a combination of promoting cell division and enhancing Procr**
763 **expression.**

764 a-c, Illustration of superovulation and analysis strategy as indicated using
765 *YAP^{fl/fl}* (Ctrl) and *Procr-CreER;YAP^{fl/fl}* (Yap-cKO) mice (a). At ovulation stage,
766 the percentage of Procr+ OSE cells in Ctrl and Yap-cKO were FACS analyzed
767 (b) and quantified (c). n = at least 3 mice in each group and displayed as
768 mean \pm s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001.

769 d-i, Illustration of superovulation and analysis strategy as indicated using *Procr-*
770 *rtTA;TetO-H2B-GFP^{+/-}* (Ctrl) and *Procr-rtTA;TetO-H2B-GFP^{+/-};TetO-Vgll4^{+/-}*
771 (Vgll4-OE) mice (d). At ovulation stage, ovary section imaging showed that at
772 the rupture sites, the number of H2B-GFP+ (Procr+) cells in Ctrl (arrowheads
773 in e) are higher than those in Vgll4-OE (arrowheads in f). Scale bar, 100 μ m.
774 Quantification was shown in (g). n=3 pairs of mice and 15 images in each group.
775 Unpaired two-tailed t test is used for comparison. ***P<0.001.

776 The percentage of Procr+ OSE cells were analyzed by FACS at ovulation stage
777 (h). The percentage of Procr+ cells in Ctrl are higher than that in Vgll4-OE (h,
778 i). n = at least 3 mice and displayed as mean \pm s.e.m. Unpaired two-tailed t test

779 is used for comparison. ***P < 0.001.

780 j-k, Illustration of Tead4 motif in Procr promoter region (j). TEAD4 ChIP-qPCR
781 analysis using cultured primary OSE cells showed the enrichment of Procr
782 promoter, and Ctgf promoter was used as positive control (k). n=2 biological
783 repeats. Unpaired two-tailed t test is used for comparison. **P<0.01, *P<0.05,
784 n.s, not significant.

785 l-m, Analysis of luciferase reporter activity driven by WT (l) and Tead4 motif (-
786 1486 to-1481bp) deleted- Procr promoter (m) in HEK193T cells transfected with
787 increased amount of YAP overexpression plasmids. Data are pooled from three
788 independent experiments and displayed as mean±s.e.m. Unpaired two-tailed t
789 test is used for comparison. ***P < 0.001, **P < 0.01, n.s, not significant.

790 n, A proposed model of which YAP signaling promotes Procr+ cells expansion
791 at rupture site through a combination of promoting cell division and enhancing
792 Procr expression.

793

794 **Fig 5. Procr is essential for the progenitor property.**

795 a-e, Illustration of superovulation and deletion of *Procr* in Procr+ cells using
796 *Procr*^{CreER/fl} mice (Procr-cKO), and *Procr*^{fl/+} mice (Ctrl) (a). Ovary whole-mount
797 confocal imaging of K8 and Laminin showed that at 4.5d (ovulation), Ctrl and
798 Procr-cKO have similar wound sizes (* in b). At 6d (OSE repair ongoing), the
799 wound sizes in Ctrl mice were smaller than those in Procr-cKO (* in c). At 7.5d
800 (repair completed), Ctrl ovary had completely repaired, while Procr-cKO
801 remained obvious opening (* in d). Scale bar, 100µm. Quantification of the
802 wound size in diameter was shown in (e). n=3 pairs of mice. Unpaired two-tailed
803 t test is used for comparison. ***P<0.001. n.s, not significant.

804 f-h, Post 12hrs EdU incorporation, the mice were harvested at 4.5 days
805 (ovulation) (a). Representative images showed the number of EdU+ cells
806 (arrowhead) in the OSE surrounding the rupture site decreased from 4.73±0.40
807 in Ctrl (arrowheads in f) to 1.62±0.20 in Procr-cKO (arrowheads in g). Scale bar,
808 100µm for zoom out and 20µm for zoom in. Quantification of was shown in (h).
809 n=3 pairs of mice. Unpaired two-tailed t test is used for comparison. ***P<0.001.

810 i-l, Total OSE cells from Ctrl and Procr-cKO were isolated by FACS (i), followed
811 by culture in 3D Matrigel. At culture d7, representative bright-field and confocal

812 images with K8 staining showed that OSE cells with Procr-cKO form markedly
813 smaller colonies compared to Ctrl (j). Colony sizes were quantified in (k). qPCR
814 analysis validated the deletion efficiency of *Procr* in OSE cells of Procr-cKO (l).
815 Data are pooled from three independent experiments and displayed as
816 mean±s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001.
817 Scale bar, 20µm.

818 m, A proposed model of YAP activation in Procr+ cells promoting OSE
819 progenitor cell expansion. Procr+ OSE progenitors have intrinsically lower level
820 of Vgll4 compared to Procr- OSE cells. At ovulation, cell-cell junctions at rupture
821 site were disrupted, which induces the possibility of YAP activation in all OSE
822 cells surrounding the rupture. However, the lower expression of Vgll4 in Procr+
823 cells allowed YAP activation in the progenitor cells at this area. YAP activation
824 in Procr+ cells promoted cell division, and importantly, it directly upregulates
825 Procr expression in the dividing cells, resulting in expansion of Procr+
826 progenitors around the wound.

827

828 **Fig S1. Increased YAP signaling activity at OSE of rupture sites.**

829 a, Illustration of superovulation strategy. 4-week old mice were administrated
830 with PMSG, following by HCG 2 days later. The ovaries were harvested 0.5 day
831 after HCG injection (ovulation).

832 b, Confocal images showed abundant E-cad expression in the OSE of non-
833 rupture sites. Scale bar, 100µm for zoom out and 10µm for zoom in. n=3 mice
834 and more than 15 images.

835 c, Quantification of the percentage of OSE cells with YAP nuclear localization
836 at the rupture sites and non-rupture sites. n=3 mice. Unpaired two-tailed t test
837 is used for comparison. ***P < 0.001.

838 d, qPCR analysis validated the deletion efficiency of *Yap* and the
839 downregulation of the expression of YAP target *Cyr61* in total OSE cells of Yap-
840 cKO mice compared with Ctrl mice. Data are pooled from three independent
841 experiments and displayed as mean±s.e.m. Unpaired two-tailed t test is used
842 for comparison. ***P < 0.001, *P < 0.05.

843

844 **Fig S2. Construction of TetO-Vgll4 mouse model.**

845 a, Targeting strategy for the generation of *TetO-Vgll4* knock-in mouse. Designs
846 of ES clone genotyping primers (red) and mouse genotyping primers (green)
847 are as indicated.
848 b, ES clone genotyping PCR indicating three successful knock-in (KI) clones.
849 NC, negative control with no DNA input.
850 c, Genotyping PCR results indicate pup #1,2,3 is heterozygote, #4 are
851 wildtypes. A wild-type mouse was used as negative control (NC) and a positive
852 ES clone was used as positive control (PC).
853 d, Western blotting validated the overexpression of Flag and *Vgll4* in the cells
854 of *Vgll4*-OE mice compared with Ctrl mice. One of 3 independent experiments
855 is shown.
856 e, qPCR analysis validated the overexpression of *Vgll4* and downregulation of
857 *Cyr61* in total OSE cells of *Vgll4* OE mice compared with Ctrl mice (d). Data are
858 pooled from three independent experiments and displayed as mean \pm s.e.m.
859 Unpaired two-tailed t test is used for comparison. ***P < 0.001, **P < 0.01.

860

861 **Fig S3. YAP promotes Procr⁺ cells expansion.**

862 a-b, OSE cells were isolated from *Procr-rtTA;TetO-H2B-GFP^{+/+}* (Ctrl) or *Procr-*
863 *rtTA;TetO-Vgll4^{+/+};TetO-H2B-GFP^{+/+}* (*Vgll4*-OE) mice and cultured on the glass.
864 In control, almost all cells are H2B-GFP⁺ (Procr⁺) in such stiff culture condition
865 (a). Living images for 6 hours showed many cases of H2B-GFP⁺ (Procr⁺) cells
866 (a) (examples in *, arrow, arrowhead in a). In *Vgll4*-OE cells, there were
867 drastically less H2B-GFP⁺ (Procr⁺) cells, and living imaging of 24 hours
868 showed no incidence of cell division (b). Scale bar, 50 μ m. n=at least 3 views.

869

870 **Fig S4. YAP induces Procr expression.**

871 a-c, OSE cells were isolated from *Procr-rtTA;TetO-H2B-GFP^{+/+}* mice and
872 cultured upon the soft hydrogel (a) or glass (b). Confocal images showed more
873 H2B-GFP⁺ cells upon glass compared with soft hydrogel (a-b). Scale bar, 20 μ m,
874 n=15 images. qPCR indicated Procr expression was upregulated upon glass
875 culture (c).

876 d-e, OSE cells isolated from wildtype mice were infected with Scramble (Sc) or
877 Yap shRNA (shYap) virus. and then cultured on glass. OSE cells were

878 harvested on culture day 4 (d). qPCR showed knockdown of Yap repressed

879 *Procr* expression (e). Scale bar, 100 μ m, n=15 images.

880 f, OSE cells were isolated from wildtype mice and cultured on glass. Verteporfin

881 (VP) was added into the medium before harvest. qPCR showed that VP

882 treatment inhibits *Cyr61* and *Procr* expression.

883 g, OSE cells were isolated from *Procr-rtTA;TetO-H2B-GFP^{+/-}* (Ctrl) and *Procr-*

884 *rtTA;TetO-Vgll4^{+/-};TetO-H2B-GFP^{+/-}* (Vgll4-OE) mice and cultured on the glass.

885 Confocal images showed dimmer H2B-GFP expression in Vgll4-OE compared

886 to Ctrl. Scale bar, 10 μ m, n=15 images.

887 For all qPCR results, data are pooled from 3 independent experiments and

888 presented as mean \pm s.e.m. Unpaired two-tailed t test is used for comparison.

889 ***P<0.001, **P<0.01, *P<0.05.

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913 Reference

914 1 Gaytan, M. *et al.* Cyclic changes of the ovarian surface epithelium in the rat.
915 *Reproduction* **129**, 311-321, doi:10.1530/rep.1.00401 (2005).

916 2 Nelly Auersperg, A. S. T. W., Kyung-Chul Choi, Sung Keun Kang, and Peter
917 C. K. Leung. Ovarian Surface Epithelium: Biology, Endocrinology, and
918 Pathology. *Endocrine reviews* **22**, 255–288 (2001).

919 3 Ng, A. & Barker, N. Ovary and fimbrial stem cells: biology, niche and cancer
920 origins. *Nature reviews. Molecular cell biology* **16**, 625-638,
921 doi:10.1038/nrm4056 (2015).

922 4 Burdette, J. E., Kurley, S. J., Kilen, S. M., Mayo, K. E. & Woodruff, T. K.
923 Gonadotropin-induced superovulation drives ovarian surface epithelia
924 proliferation in CD1 mice. *Endocrinology* **147**, 2338-2345,
925 doi:10.1210/en.2005-1629 (2006).

926 5 Tan, O. L. & Fleming, J. S. Proliferating cell nuclear antigen
927 immunoreactivity in the ovarian surface epithelium of mice of varying ages
928 and total lifetime ovulation number following ovulation. *Biology of*
929 *reproduction* **71**, 1501-1507, doi:10.1095/biolreprod.104.030460 (2004).

930 6 Wang, Y., Yu, A. & Yu, F.-X. The Hippo pathway in tissue homeostasis and
931 regeneration. *Protein & Cell* **8**, 349-359, doi:10.1007/s13238-017-0371-0
932 (2017).

933 7 Wang, J., Wang, D., Chu, K., Li, W. & Zeng, Y. A. Procr-expressing
934 progenitor cells are responsible for murine ovulatory rupture repair of
935 ovarian surface epithelium. *Nature communications* **10**,
936 doi:10.1038/s41467-019-12935-7 (2019).

937 8 Ahmed, N. *et al.* Molecular pathways regulating EGF-induced epithelio-
938 mesenchymal transition in human ovarian surface epithelium. *Am J Physiol*
939 *Cell Physiol* **290**, C1532-1542, doi:10.1152/ajpcell.00478.2005 (2006).

940 9 Boyer, A., Goff, A. K. & Boerboom, D. WNT signaling in ovarian follicle
941 biology and tumorigenesis. *Trends in endocrinology and metabolism: TEM*
942 **21**, 25-32, doi:10.1016/j.tem.2009.08.005 (2010).

943 10 Jeff A. Parrott, R. M., Grace Kim, And Michael K. Skinner. Autocrine

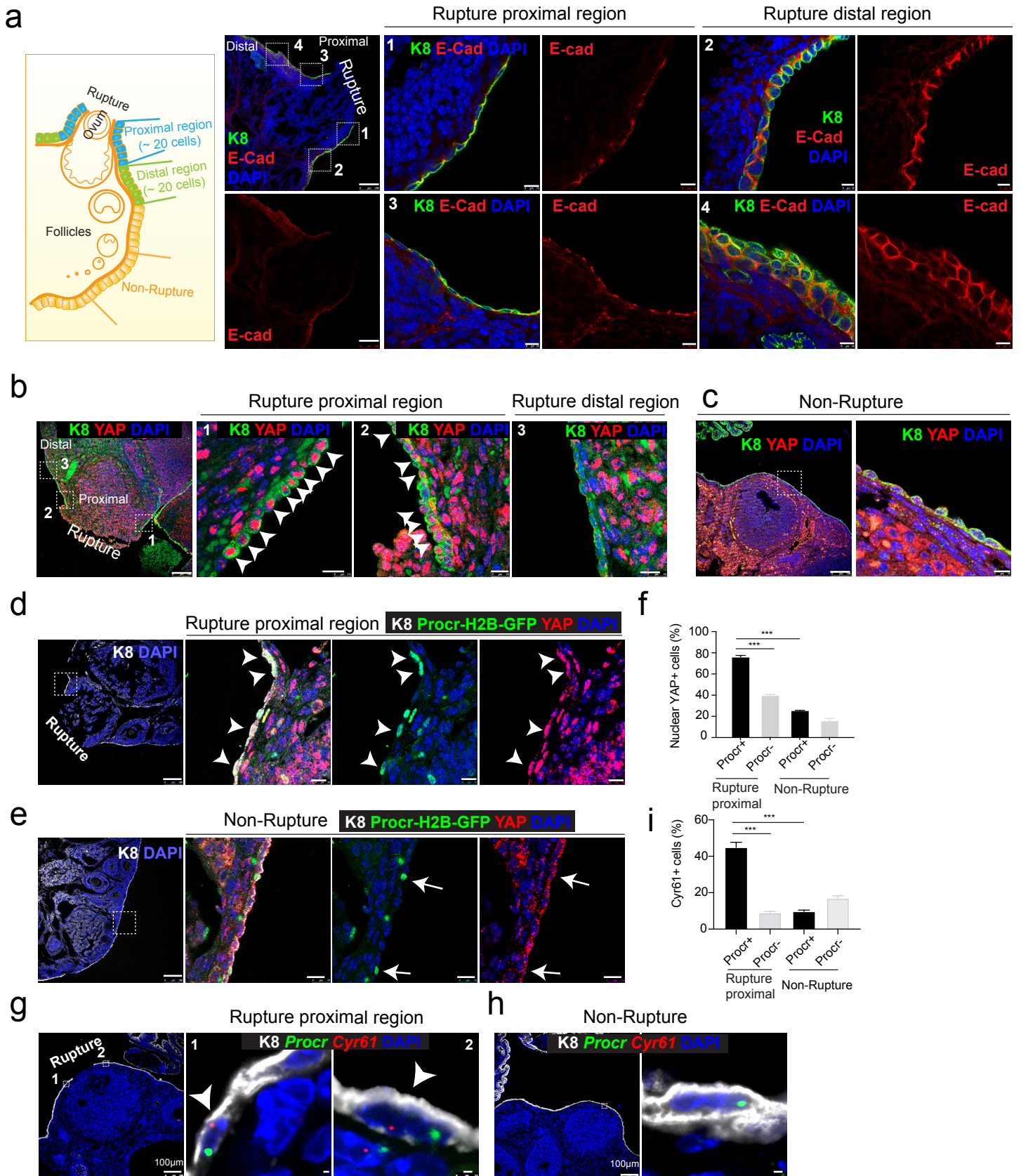
- 944 Interactions of Keratinocyte Growth Factor, Hepatocyte Growth Factor, and
945 Kit-Ligand in the Regulation of Normal Ovarian Surface Epithelial Cells.
946 *Endocrinology* **141**, 2532-2539 (2000).
- 947 11 Eric Nilsson, V. D., Jeff A. Parrott , Michael K. Skinner. Expression and action
948 of transforming growth factor beta (TGFb1, TGFb2, TGFb3) in normal
949 bovine ovarian surface epithelium and implications for human ovarian
950 cancer. *Molecular and cellular endocrinology* **281**, 145-155 (2001).
- 951 12 Moya, I. M. & Halder, G. Hippo–YAP/TAZ signalling in organ regeneration
952 and regenerative medicine. *Nature Reviews Molecular Cell Biology* **20**,
953 211-226, doi:10.1038/s41580-018-0086-y (2018).
- 954 13 Johnson, R. & Halder, G. The two faces of Hippo: targeting the Hippo
955 pathway for regenerative medicine and cancer treatment. *Nature Reviews*
956 *Drug Discovery* **13**, 63-79, doi:10.1038/nrd4161 (2013).
- 957 14 Moya, I. M. & Halder, G. The Hippo pathway in cellular reprogramming and
958 regeneration of different organs. *Current Opinion in Cell Biology* **43**, 62-
959 68, doi:10.1016/j.ceb.2016.08.004 (2016).
- 960 15 Patel, S. H., Camargo, F. D. & Yimlamai, D. Hippo Signaling in the Liver
961 Regulates Organ Size, Cell Fate, and Carcinogenesis. *Gastroenterology* **152**,
962 533-545, doi:10.1053/j.gastro.2016.10.047 (2017).
- 963 16 Xiao, Y., Leach, J., Wang, J. & Martin, J. F. Hippo/Yap Signaling in Cardiac
964 Development and Regeneration. *Current Treatment Options in*
965 *Cardiovascular Medicine* **18**, doi:10.1007/s11936-016-0461-y (2016).
- 966 17 Zhang, Y. & Del Re, D. P. A growing role for the Hippo signaling pathway
967 in the heart. *Journal of Molecular Medicine* **95**, 465-472,
968 doi:10.1007/s00109-017-1525-5 (2017).
- 969 18 Panciera, T. *et al.* Induction of Expandable Tissue-Specific Stem/Progenitor
970 Cells through Transient Expression of YAP/TAZ. *Cell stem cell* **19**, 725-737,
971 doi:10.1016/j.stem.2016.08.009 (2016).
- 972 19 Lin, C. *et al.* YAP is essential for mechanical force production and epithelial
973 cell proliferation during lung branching morphogenesis. *eLife* **6**,
974 doi:10.7554/eLife.21130 (2017).
- 975 20 Lin, K. C., Park, H. W. & Guan, K.-L. Regulation of the Hippo Pathway
976 Transcription Factor TEAD. *Trends in Biochemical Sciences* **42**, 862-872,

- 977 doi:10.1016/j.tibs.2017.09.003 (2017).
- 978 21 Piccolo, S., Dupont, S. & Cordenonsi, M. The Biology of YAP/TAZ: Hippo
979 Signaling and Beyond. *Physiological Reviews* **94**, 1287-1312,
980 doi:10.1152/physrev.00005.2014 (2014).
- 981 22 Zhao, B. *et al.* TEAD mediates YAP-dependent gene induction and growth
982 control. *Genes & development* **22**, 1962-1971, doi:10.1101/gad.1664408
983 (2008).
- 984 23 Chen, C.-C., Mo, F.-E. & Lau, L. F. The Angiogenic Factor Cyr61 Activates a
985 Genetic Program for Wound Healing in Human Skin Fibroblasts. *Journal of*
986 *Biological Chemistry* **276**, 47329-47337, doi:10.1074/jbc.M107666200
987 (2001).
- 988 24 Totaro, A., Panciera, T. & Piccolo, S. YAP/TAZ upstream signals and
989 downstream responses. *Nature cell biology* **20**, 888-899,
990 doi:10.1038/s41556-018-0142-z (2018).
- 991 25 Chen, H.-H., Mullett, S. J. & Stewart, A. F. R. Vgl-4, a Novel Member of the
992 Vestigial-like Family of Transcription Cofactors, Regulates α 1-Adrenergic
993 Activation of Gene Expression in Cardiac Myocytes. *Journal of Biological*
994 *Chemistry* **279**, 30800-30806, doi:10.1074/jbc.M400154200 (2004).
- 995 26 Feng, X. *et al.* Dual function of VGLL4 in muscle regeneration. *The EMBO*
996 *journal* **38**, doi:10.15252/emj.2018101051 (2019).
- 997 27 Jinlong Suo, X. F., Jiayi Li, Jinghui Wang, Zuoyun Wang, Lei Zhang, Weiguo
998 Zou. VGLL4 promotes osteoblast differentiation by antagonizing TEADs-
999 inhibited Runx2 transcription. *SCIENCE ADVANCES* **6**, eaba4147 (2020).
- 1000 28 Yu, W. *et al.* VGLL4 plays a critical role in heart valve development and
1001 homeostasis. *PLoS genetics* **15**, e1007977,
1002 doi:10.1371/journal.pgen.1007977 (2019).
- 1003 29 Lin, Z. *et al.* Acetylation of VGLL4 Regulates Hippo-YAP Signaling and
1004 Postnatal Cardiac Growth. *Developmental cell* **39**, 466-479,
1005 doi:10.1016/j.devcel.2016.09.005 (2016).
- 1006 30 Ramos, A. & Camargo, F. D. The Hippo signaling pathway and stem cell
1007 biology. *Trends in cell biology* **22**, 339-346, doi:10.1016/j.tcb.2012.04.006
1008 (2012).
- 1009 31 Yang, C.-C. *et al.* Differential regulation of the Hippo pathway by adherens

- 1010 junctions and apical–basal cell polarity modules. *Proceedings of the*
1011 *National Academy of Sciences* **112**, 1785–1790,
1012 doi:10.1073/pnas.1420850112 (2015).
- 1013 32 Pinheiro, D. & Bellaïche, Y. Mechanical Force-Driven Adherens Junction
1014 Remodeling and Epithelial Dynamics. *Developmental cell* **47**, 3–19,
1015 doi:10.1016/j.devcel.2018.09.014 (2018).
- 1016 33 Zhou, B. *et al.* Claudin-18–mediated YAP activity regulates lung stem and
1017 progenitor cell homeostasis and tumorigenesis. *Journal of Clinical*
1018 *Investigation* **128**, 970–984, doi:10.1172/jci90429 (2018).
- 1019 34 Schlegelmilch, K. *et al.* Yap1 Acts Downstream of α -Catenin to Control
1020 Epidermal Proliferation. *Cell* **144**, 782–795, doi:10.1016/j.cell.2011.02.031
1021 (2011).
- 1022 35 Varelas, X. *et al.* The Crumbs Complex Couples Cell Density Sensing to
1023 Hippo-Dependent Control of the TGF- β -SMAD Pathway. *Developmental*
1024 *cell* **19**, 831–844, doi:10.1016/j.devcel.2010.11.012 (2010).
- 1025 36 Nam-Gyun Kim, E. K., Xiao Chen, and Barry M. Gumbiner. E-cadherin
1026 mediates contact inhibition of proliferation through Hippo signaling-
1027 pathway components. *PNAS* **108**, 11930–11935 (2011).
- 1028 37 Fukudome, K. *et al.* Activation mechanism of anticoagulant protein C in
1029 large blood vessels involving the endothelial cell protein C receptor. *J Exp*
1030 *Med* **187**, 1029–1035, doi:10.1084/jem.187.7.1029 (1998).
- 1031 38 Wang, D. *et al.* Long-Term Expansion of Pancreatic Islet Organoids from
1032 Resident Procr(+) Progenitors. *Cell* **180**, 1198–1211 e1119,
1033 doi:10.1016/j.cell.2020.02.048 (2020).
- 1034 39 Wang, D. *et al.* Identification of multipotent mammary stem cells by protein
1035 C receptor expression. *Nature* **517**, 81–85, doi:10.1038/nature13851 (2015).
- 1036 40 Yu, Q. C., Song, W., Wang, D. & Zeng, Y. A. Identification of blood vascular
1037 endothelial stem cells by the expression of protein C receptor. *Cell research*
1038 **26**, 1079–1098, doi:10.1038/cr.2016.85 (2016).
- 1039 41 Wang, D. *et al.* Protein C receptor stimulates multiple signaling pathways
1040 in breast cancer cells. *The Journal of biological chemistry* **293**, 1413–1424,
1041 doi:10.1074/jbc.M117.814046 (2018).
- 1042 42 Camargo, F. D. *et al.* YAP1 Increases Organ Size and Expands

- 1043 Undifferentiated Progenitor Cells. *Current Biology* **17**, 2054-2060,
1044 doi:10.1016/j.cub.2007.10.039 (2007).
- 1045 43 Beverdam, A. *et al.* Yap Controls Stem/Progenitor Cell Proliferation in the
1046 Mouse Postnatal Epidermis. *Journal of Investigative Dermatology* **133**,
1047 1497-1505, doi:10.1038/jid.2012.430 (2013).
- 1048 44 Cao, X., Pfaff, S. L. & Gage, F. H. YAP regulates neural progenitor cell
1049 number via the TEA domain transcription factor. *Genes & development* **22**,
1050 3320-3334, doi:10.1101/gad.1726608 (2008).
- 1051 45 Tse, J. R. & Engler, A. J. Preparation of Hydrogel Substrates with Tunable
1052 Mechanical Properties. *Current Protocols in Cell Biology* **47**,
1053 doi:10.1002/0471143030.cb1016s47 (2010).
- 1054

Fig 1. Rupture-induced YAP signaling activation is preferentially activated in Procr+ progenitors at the rupture sites.



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Fig 1. Rupture-induced YAP signaling activation is preferentially activated in *Procr*⁺ progenitors at the rupture sites.

a, Sections from wildtype ovaries at ovulation stage were stained with Krt8 (K8) and E-cadherin (E-Cad). Confocal images showed less E-cad in the OSE of proximal regions surrounding the rupture sites (view #1, #3 in a) compared with distal regions (view #2, #4 in a). Scale bar, 100 μ m for zoom out and 10 μ m for zoom in. n=3 mice and 15 images.

b-c, Sections from wildtype ovaries at ovulation stage were stained with K8 and YAP. Confocal images showed YAP nuclear localization in the OSE was only observed in the proximal regions surrounding the rupture sites (b), but not in the distal regions (b) or the non-rupture sites (c). Scale bar, 100 μ m for zoom out and 20 μ m for zoom in. n=3 mice and 15 images.

d-f, *Procr-rtTA;TetO-H2B-GFP*^{+/-} mice were fed with doxycycline for 3 days and harvested at ovulation stage. Confocal images of ovarian sections with Krt8 (K8) and YAP staining (d-e) and quantification (f) were showed. Nuclear YAP staining is preferentially detected in *Procr*⁺ (histone 2B-GFP+) cells in rupture proximal region (arrowheads in d), whereas at the non-rupture site, YAP staining was cytoplasmic regardless in *Procr*⁺ (arrows in e) or *Procr*⁻ cells (e). Scale bar, 100 μ m for zoom out and 10 μ m for zoom in. n=3 mice and 15 images. One-way ANOVA with Tukey test is used for comparison of multiple groups. ***P<0.001.

g-i, Combination of *Procr* and *Cyr61* double fluorescent in situ with K8 antibody immunohistochemistry staining (g-i). Confocal images showed co-localization of *Procr* and *Cyr61* in the OSE at the rupture sites (arrowhead in g), while at non-rupture regions, both *Procr*⁺ and *Procr*⁻ cells had low incidence of *Cyr61* expression (h). Quantification showed increased *Cyr61* expression in *Procr*⁺ cells at rupture sites compared with *Procr*⁻ cells at rupture sites or *Procr*⁺ cells at non-rupture regions (i). Scale bar, 100 μ m for zoom out and 1 μ m for zoom in. n=3 mice and 15 images. One-way ANOVA with Tukey test is used for comparison of multiple groups. ***P<0.001.

Fig 2. Deletion of Yap in Procr+ cells hinders OSE repair and progenitor proliferation.

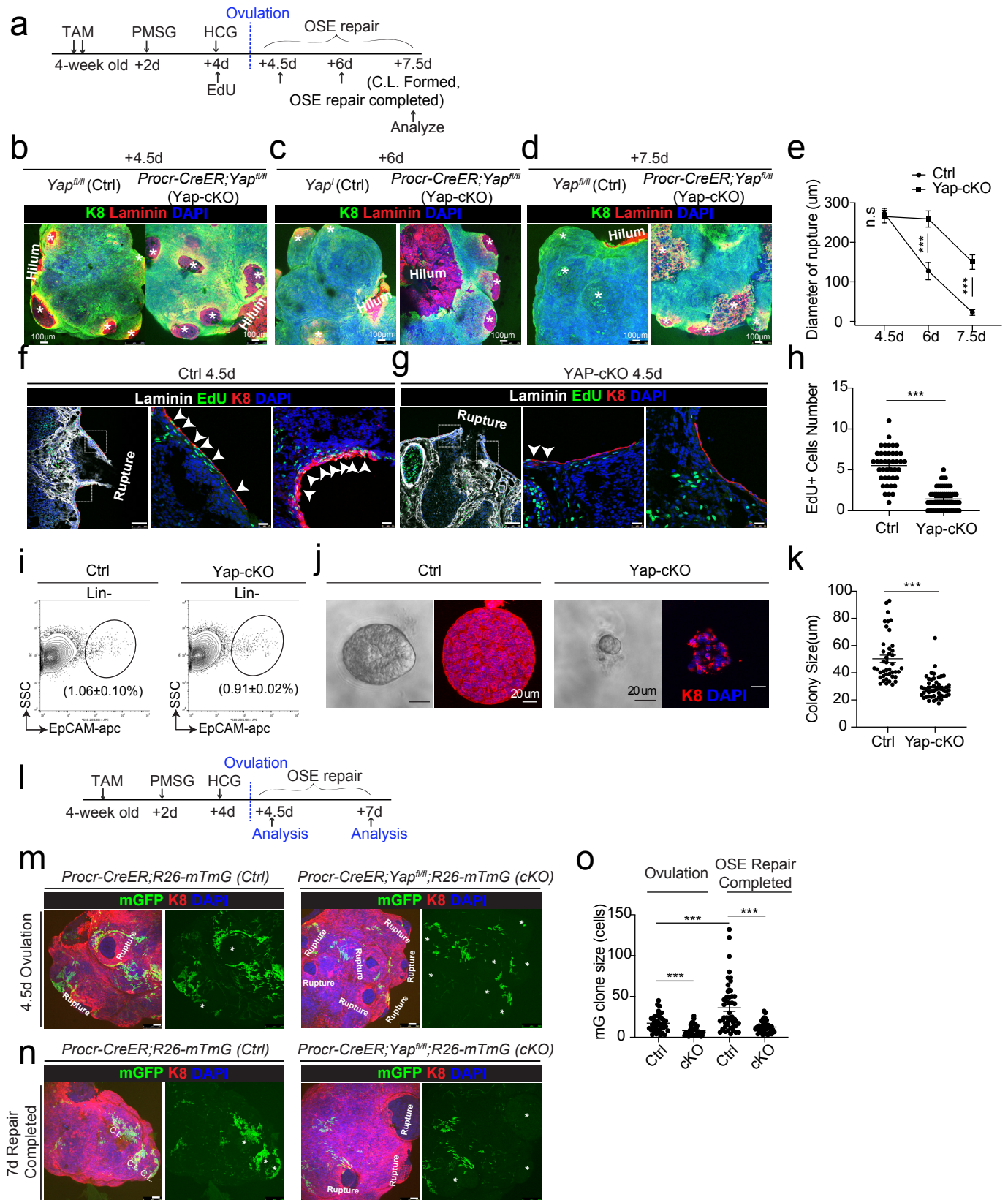


Fig 2. Deletion of Yap in Procr+ cells hinders OSE rupture repair and progenitor proliferation.

a, Illustration of TAM induction and superovulation strategy.
b-e, Yap was deleted in Procr+ cells using *Procr-CreER; Yap^{fl/fl}* mice (Yap-cKO), and *Yap^{fl/fl}* mice was used as control (Ctrl). Ovary whole-mount staining with K8 and Laminin was performed (b-d) and the wound size in diameter was quantified (e). At 4.5d (ovulation) (star) Ctrl and Yap-cKO ovaries had comparable wound size (* in b, e). At 6d (OSE repair ongoing), the wounds in Ctrl ovary were significantly smaller than those in Yap-cKO ovary (c, e). At 7.5d (repair completed), the wound was completely repaired in Ctrl, while the Yap-cKO ovary still showed obvious wounds (star) (* in d, e). Scale bar, 100 μ m. n=3 pairs of mice.
f-h, Ctrl and Yap-cKO mice were subjected to 12hrs EdU incorporation and were harvested at 4.5 days (Ovulation stage). Representative images (f-g) and quantification (h) were showed. Out of 20 cells next to the rupture on one side, the numbers of EdU+ cells (arrowhead) in the OSE of rupture site decreased from 5.52 ± 0.33 cells in Ctrl to 1.42 ± 0.16 cells in Yap-cKO. Scale bar, 100 μ m for zoom out and 20 μ m for zoom in. n=3 pairs of mice. Unpaired two-tailed t test is used for comparison. ***P<0.001.
i-k, Total OSE cells from *Procr-CreER; Yap^{fl/fl}* mice (Yap-cKO), and *Yap^{fl/fl}* mice (Ctrl) were isolated by FACS at 4.5 days (Ovulation stage) (i), followed by culture in 3D Matrigel for 7 days. Representative bright-field and confocal images of K8 staining were shown (j). Colony sizes in diameter were measured (k). Scale bar, 20 μ m. Data are pooled from three independent experiments and displayed as mean \pm s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001.
l, Illustration of lineage tracing, deletion of Yap and superovulation strategy.
m-o, *Procr-CreER; YAP^{fl/fl}; R26-mTmG* (Yap-cKO) and *Procr-CreER; R26-mTmG* (Ctrl) mice were used. At 4.5pi (ovulation), ovary whole-mount confocal imaging showed zones of concentrated GFP+ cells surrounding the rupture site in Ctrl, while fewer GFP+ cells were seen in Yap-cKO ovary (m). At 7pi (repair completed), ovary whole-mount confocal imaging showed large GFP+ patches located at corpus luteum (C.L.) in Ctrl, while rare GFP+ cells surrounding the unrepaired wound in Yap-cKO ovary (n). Quantification showed significantly fewer GFP+ cells in Yap-cKO compared with Ctrl in both ovulation stage and repair completed stage. Quantification showed an expansion of GFP+ cell numbers in Ctrl mice during the tracing and no expansion in Yap-cKO (o). Scale bar, 100 μ m. n=3 pairs of mice. ***P < 0.001.

Fig 3. An intrinsic lower level of Vgll4 in Procr+ cells is essential for Procr+ cells' stemness and OSE rupture repair.

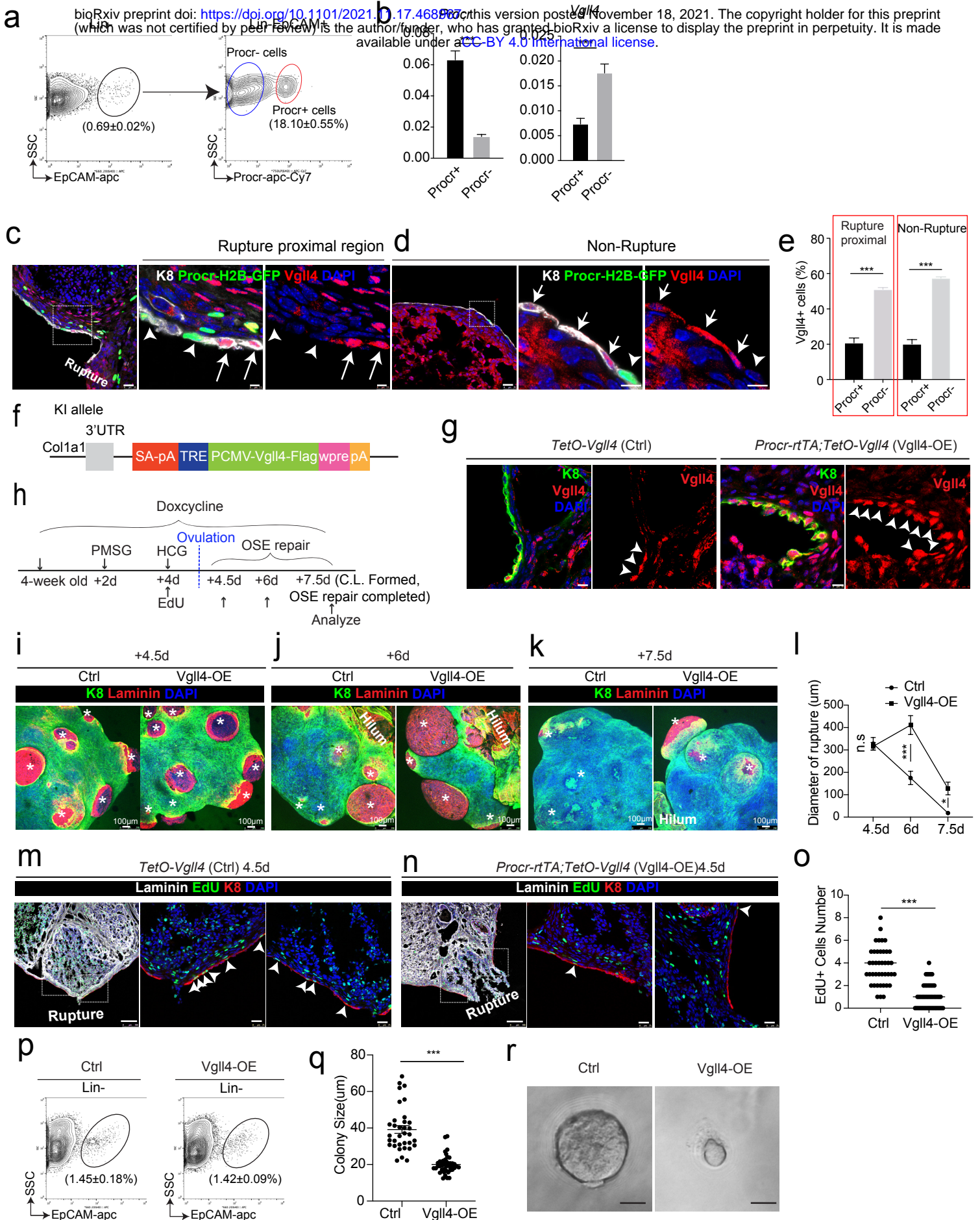


Fig 3 An intrinsic lower level of Vgll4 in Procr+ cells is essential for Procr+ cells' stemness and OSE rupture repair.

a-b, At ovulation stage, Procr+ and Procr- OSE cells (Lin-, EpCAM+) were FACS-isolated (a). qPCR analysis showed the lower *Vgll4* level in Procr+ cells (b). Data are pooled from 3 independent experiments and presented as mean±s.e.m. ***P<0.001.

c-e, *Procr-rtTA;TetO-H2B-GFP^{+/+}* mice were administered with PMSG and HCG to induce superovulation, and fed with doxycycline for 3 days before harvest. Ovarian sections were stained with Vgll4 and K8. Representative images showed that at both rupture proximal region (c) and non-rupture region (d), H2B-GFP- (Procr-) OSE cells have high Vgll4 expression (arrows in c, d), while H2B-GFP+ (Procr+) OSE cells have no Vgll4 expression (arrowheads in c, d). Scale bar, 20µm for zoom out and 5µm for zoom in. Quantification of the staining was shown in (e). n=3 mice and 15 images. Unpaired two-tailed t test is used for comparison. ***P<0.001.

f-g, Targeting strategy and validation of *TetO-Vgll4* knock-in mouse (f-g). A cassette of TetO-Vgll4-Flag-wpre-polyA was knocked in behind 3'UTR of *Col1a1* gene (f). Immunohistochemistry staining of Vgll4 in the ovaries indicated more Vgll4+ OSE cells at the rupture sites (g). Scale bar, 10µm. n=3 pairs of mice.

h-l, Illustration of superovulation and overexpression of Vgll4 in Procr+ cells (h). Ovary whole-mount confocal images of K8 and Laminin showed that at 4.5d (ovulation), Ctrl (*TetO-Vgll4*) and Vgll4-OE (*Procr-rtTA;TetO-Vgll4*) ovaries have similar wound sizes (* in i). At 6d (repair ongoing), the wound sizes in Ctrl mice were smaller than those in Vgll4-OE (* in j). At 7.5d (repair completed), Ctrl ovary had completely repaired, while Vgll4-OE mice had obvious opening (* in k). Scale bar, 100µm. The sizes of the wound in diameter were quantified (l). n=3 pairs of mice.

m-o, The mice were harvested at 4.5 days (ovulation) after 12hrs EdU incorporation. Representative images (m, n), and quantification (o) showed the number of EdU+ cells in the OSE surrounding the rupture site (arrowheads in m) decreased from 3.73±0.26 in Ctrl to 1.04±0.15 in Vgll4-OE (arrowheads in n). Scale bar, 100µm for zoom out and 20µm for zoom in. n=3 pairs of mice. Unpaired two-tailed t test is used for comparison. ***P<0.001.

p-r, Total OSE cells were isolated by FACS from Ctrl and Vgll4-OE at 4.5 days (ovulation) (p), and cultured in 3D Matrigel. At day 7 in culture, colony sizes were measured in diameter (q), and representative images were shown (r) out of 15 images in each group. Scale bar, 20µm. Data are pooled from three independent experiments and displayed as mean±s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001.

Fig 4. YAP signaling promotes Procr+ cells expansion at rupture sites through

a combination of promoting cell division and enhancing Procr expression

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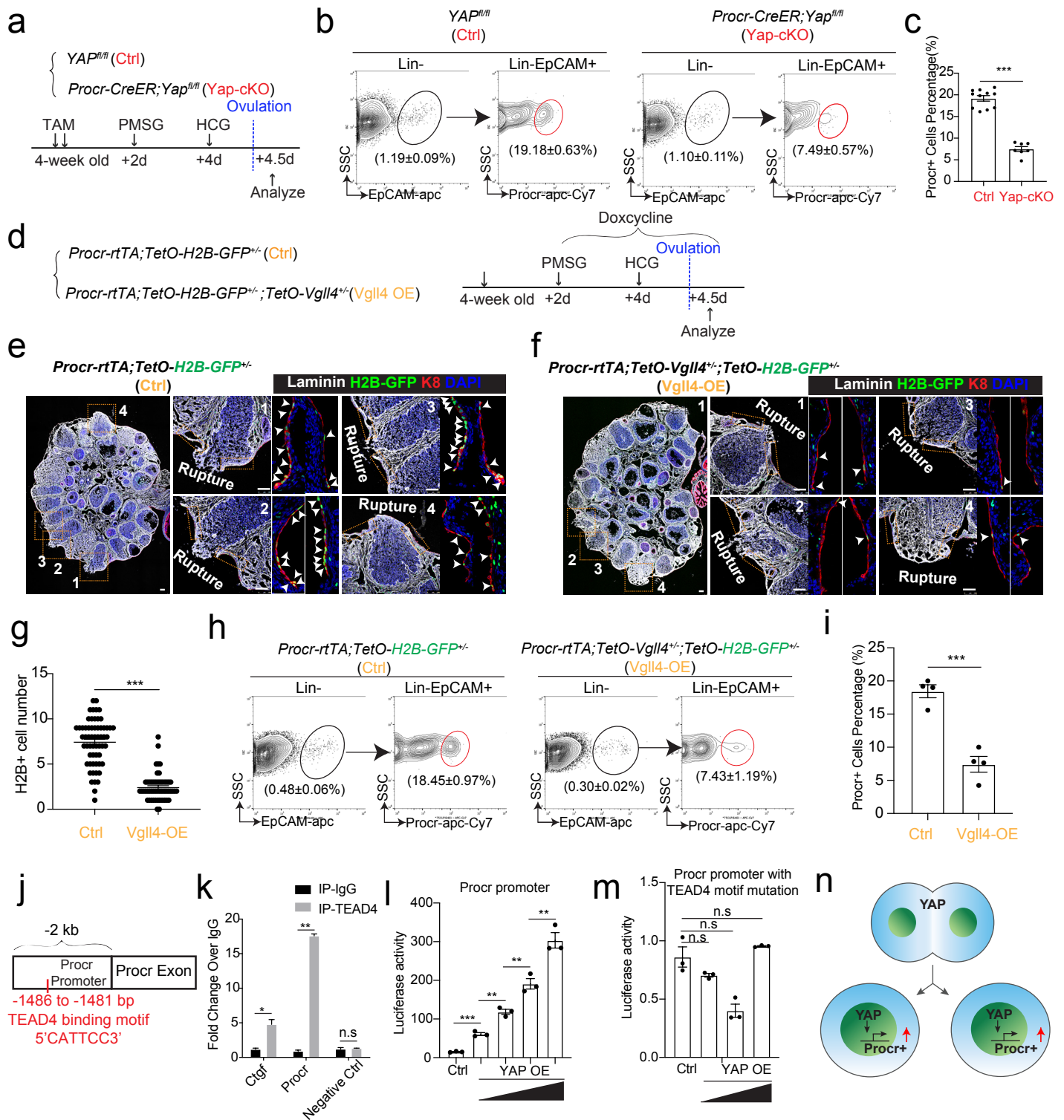


Fig 4. YAP signaling promotes Procr+ cells expansion at rupture sites through a combination of promoting cell division and enhancing Procr expression.

a-c, Illustration of superovulation and analysis strategy as indicated using *Yap^{fl/fl}* (Ctrl) and *Procr-CreER;Yap^{fl/fl}* (Yap-cKO) mice (a). At ovulation stage, the percentage of Procr+ OSE cells in Ctrl and Yap-cKO were FACS analyzed (b) and quantified (c). n = at least 3 mice in each group and displayed as mean±s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001.

d-i, Illustration of superovulation and analysis strategy as indicated using *Procr-rtTA;TetO-H2B-GFP^{+/+}* (Ctrl) and *Procr-rtTA;TetO-H2B-GFP^{+/+};TetO-Vgll4^{+/-}* (Vgll4-OE) mice (d). At ovulation stage, ovary section imaging showed that at the rupture sites, the number of H2B-GFP+ (Procr+) cells in Ctrl (arrowheads in e) are higher than those in Vgll4-OE (arrowheads in f). Scale bar, 100µm. Quantification was shown in (g). n=3 pairs of mice and 15 images in each group. Unpaired two-tailed t test is used for comparison. ***P<0.001.

The percentage of Procr+ OSE cells were analyzed by FACS at ovulation stage (h). The percentage of Procr+ cells in Ctrl are higher than that in Vgll4-OE (h, i). n = at least 3 mice and displayed as mean±s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001.

j-k, Illustration of Tead4 motif in Procr promoter region (j). TEAD4 ChIP-qPCR analysis using cultured primary OSE cells showed the enrichment of Procr promoter, and Ctgf promoter was used as positive control (k). n=2 biological repeats. Unpaired two-tailed t test is used for comparison. **P<0.01, *P<0.05, n.s, not significant.

l-m, Analysis of luciferase reporter activity driven by WT (l) and Tead4 motif (-1486 to-1481bp) deleted- Procr promoter (m) in HEK193T cells transfected with increased amount of YAP overexpression plasmids. Data are pooled from three independent experiments and displayed as mean±s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001, **P < 0.01, n.s, not significant.

n, A proposed model of which YAP signaling promotes Procr+ cells expansion at rupture site through a combination of promoting cell division and enhancing Procr expression.

Fig 5. Procr is essential for the progenitor property.

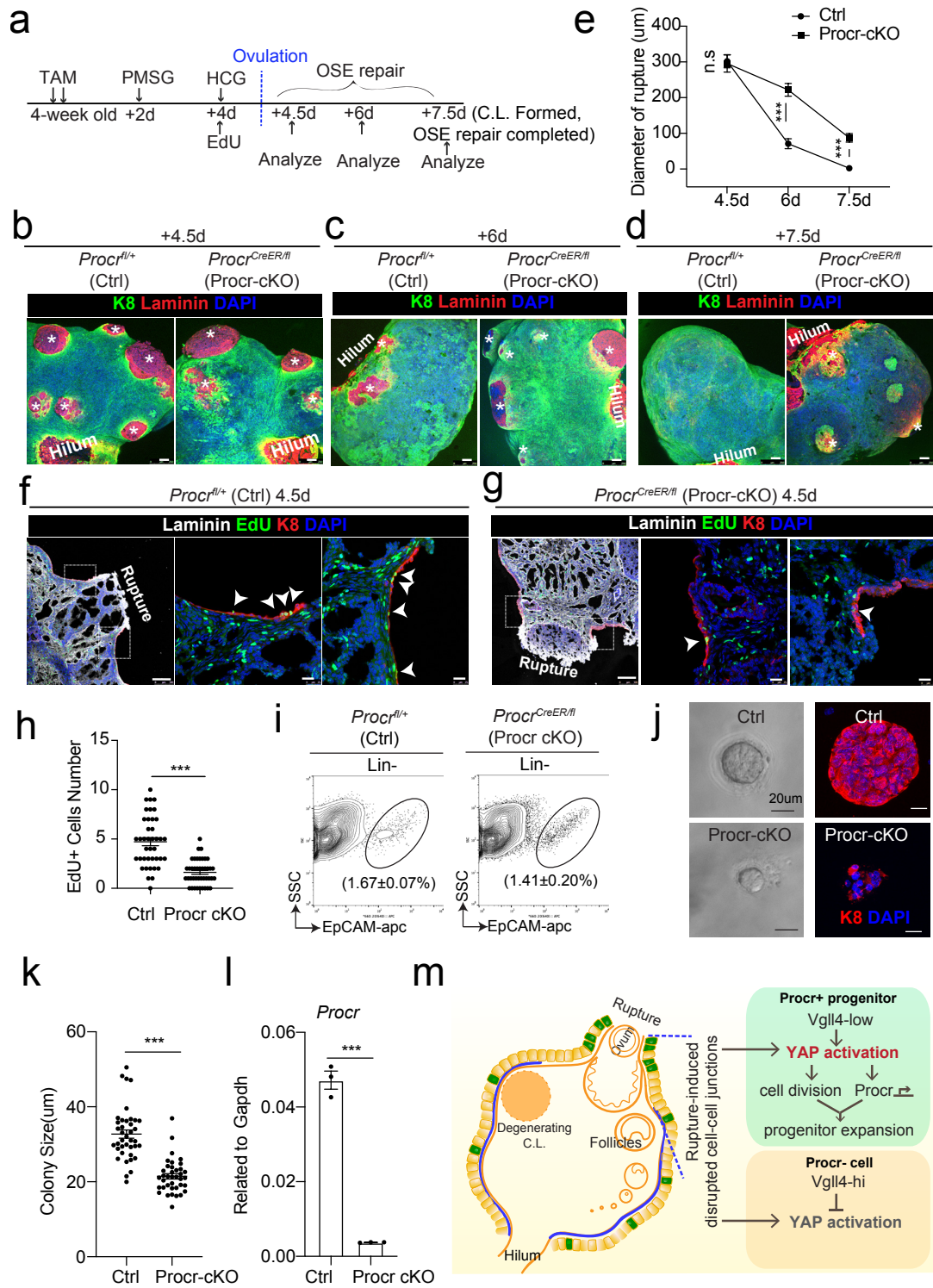


Fig 5. Procr is essential for the progenitor property

a-e, Illustration of superovulation and deletion of Procr in Procr⁺ cells using *Procr*^{CreER/fl} mice (Procr-cKO), and *Procr*^{fl/+} mice (Ctrl) (a). Ovary whole-mount confocal imaging of K8 and Laminin showed that at 4.5d (ovulation), Ctrl and Procr-cKO have similar wound sizes (* in b). At 6d (OSE repair ongoing), the wound sizes in Ctrl mice were smaller than those in Procr-cKO (* in c). At 7.5d (repair completed), Ctrl ovary had completely repaired, while Procr-cKO remained obvious opening (* in d). Scale bar, 100 μ m. Quantification of the wound size in diameter was shown in (e). n=3 pairs of mice. Unpaired two-tailed t test is used for comparison. ***P<0.001. n.s, not significant.

f-h, Post 12hrs EdU incorporation, the mice were harvested at 4.5 days (ovulation) (a). Representative images showed the number of EdU⁺ cells (arrowhead) in the OSE surrounding the rupture site decreased from 4.73 \pm 0.40 in Ctrl (arrowheads in f) to 1.62 \pm 0.20 in Procr-cKO (arrowheads in g). Scale bar, 100 μ m for zoom out and 20 μ m for zoom in. Quantification of was shown in (h). n=3 pairs of mice. Unpaired two-tailed t test is used for comparison. ***P<0.001.

i-l, Total OSE cells from Ctrl and Procr-cKO were isolated by FACS (i), followed by culture in 3D Matrigel. At culture d7, representative bright-field and confocal images with K8 staining showed that OSE cells with Procr-cKO form markedly smaller colonies compared to Ctrl (j). Colony sizes were quantified in (k). qPCR analysis validated the deletion efficiency of Procr in OSE cells of Procr-cKO (l). Data are pooled from three independent experiments and displayed as mean \pm s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001. Scale bar, 20 μ m.

m, A proposed model of YAP activation in Procr⁺ cells promoting OSE progenitor cell expansion. Procr⁺ OSE progenitors have intrinsically lower level of Vgll4 compared to Procr⁻ OSE cells. At ovulation, cell-cell junctions at rupture site were disrupted, which induces the possibility of YAP activation in all OSE cells surrounding the rupture. However, the lower expression of Vgll4 in Procr⁺ cells allowed YAP activation in the progenitor cells at this area. YAP activation in Procr⁺ cells promoted cell division, and importantly, it directly upregulates Procr expression in the dividing cells, resulting in expansion of Procr⁺ progenitors around the wound.

Fig S1. Increased YAP signaling activity at OSE of rupture sites.

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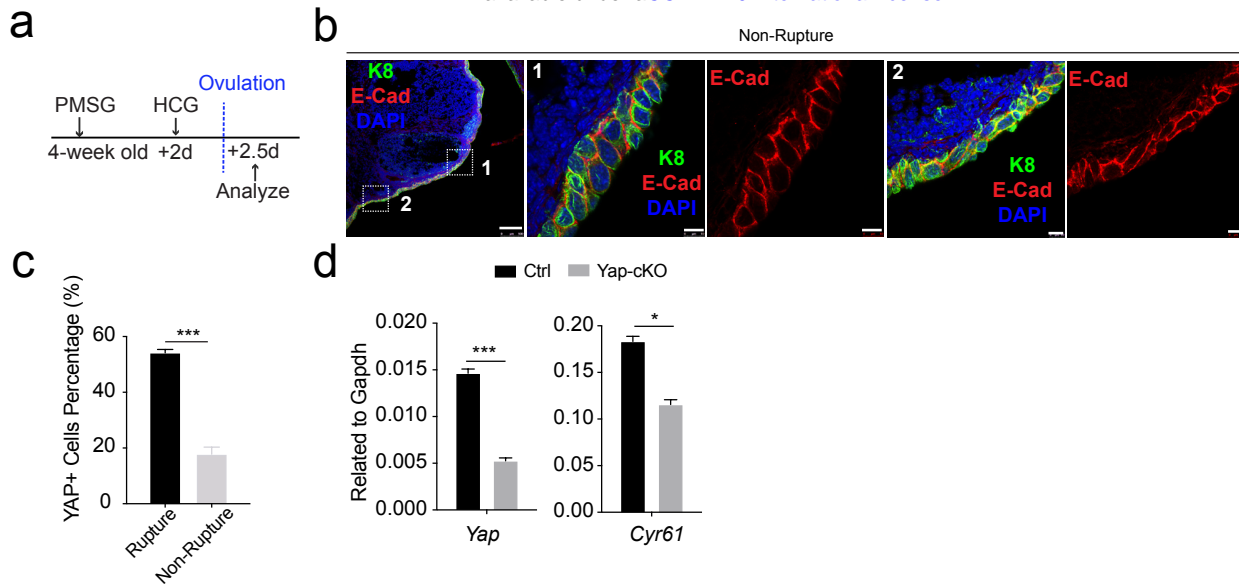


Fig S1. Increased YAP signaling activity at OSE of rupture sites.

a, Illustration of superovulation strategy. 4-week old mice were administrated with PMSG, following by HCG 2 days later. The ovaries were harvested 0.5 day after HCG injection (ovulation).

b, Confocal images showed abundant E-cad expression in the OSE of non-rupture sites. Scale bar, 100µm for zoom out and 10µm for zoom in. n=3 mice and more than 15 images.

c, Quantification of the percentage of OSE cells with YAP nuclear localization at the rupture sites and non-rupture sites. n=3 mice. Unpaired two-tailed t test is used for comparison. ***P < 0.001.

d, qPCR analysis validated the deletion efficiency of *Yap* and the downregulation of the expression of YAP target *Cyr61* in total OSE cells of Yap-cKO mice compared with Ctrl mice. Data are pooled from three independent experiments and displayed as mean±s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001, *P < 0.05.

Fig S2. Construction of *TetO-Vgll4* mouse model.

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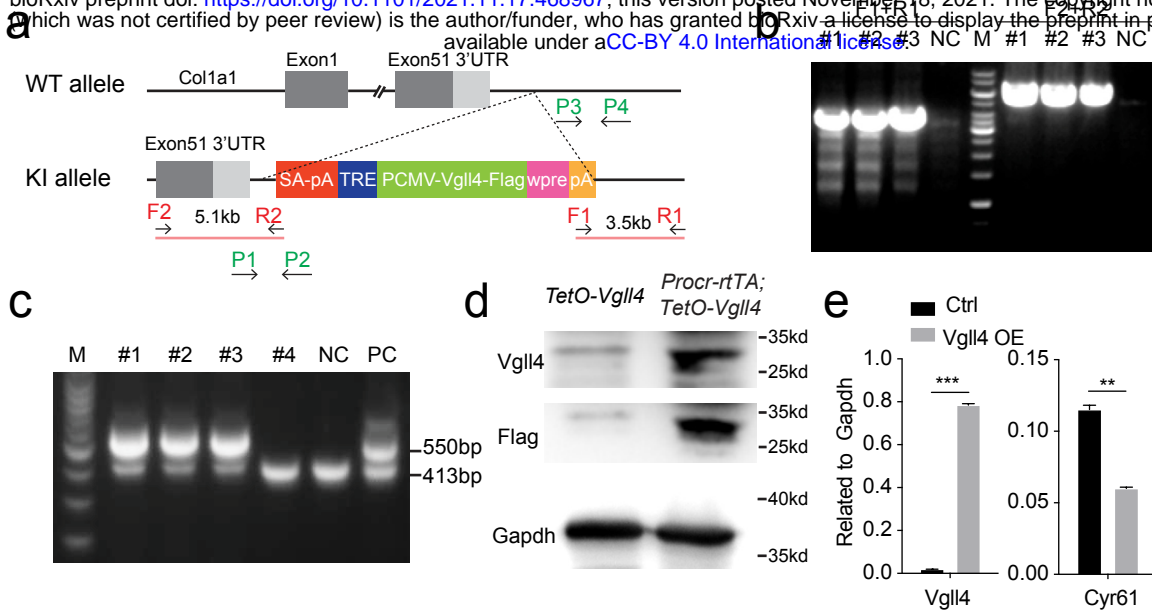


Fig S2. Construction of *TetO-Vgll4* mouse model.

a, Targeting strategy for the generation of *TetO-Vgll4* knock-in mouse. Designs of ES clone genotyping primers (red) and mouse genotyping primers (green) are as indicated.

b, ES clone genotyping PCR indicating three successful knock-in (KI) clones. NC, negative control with no DNA input.

c, Genotyping PCR results indicate pup #1,2,3 is heterozygote, #4 are wildtypes. A wild-type mouse was used as negative control (NC) and a positive ES clone was used as positive control (PC).

d, Western blotting validated the overexpression of Flag and Vgll4 in the cells of Vgll4-OE mice compared with Ctrl mice. One of 3 independent experiments is shown.

e, qPCR analysis validated the overexpression of *Vgll4* and downregulation of *Cyr61* in total OSE cells of Vgll4-OE mice compared with Ctrl mice (d). Data are pooled from three independent experiments and displayed as mean \pm s.e.m. Unpaired two-tailed t test is used for comparison. ***P < 0.001, **P < 0.01.

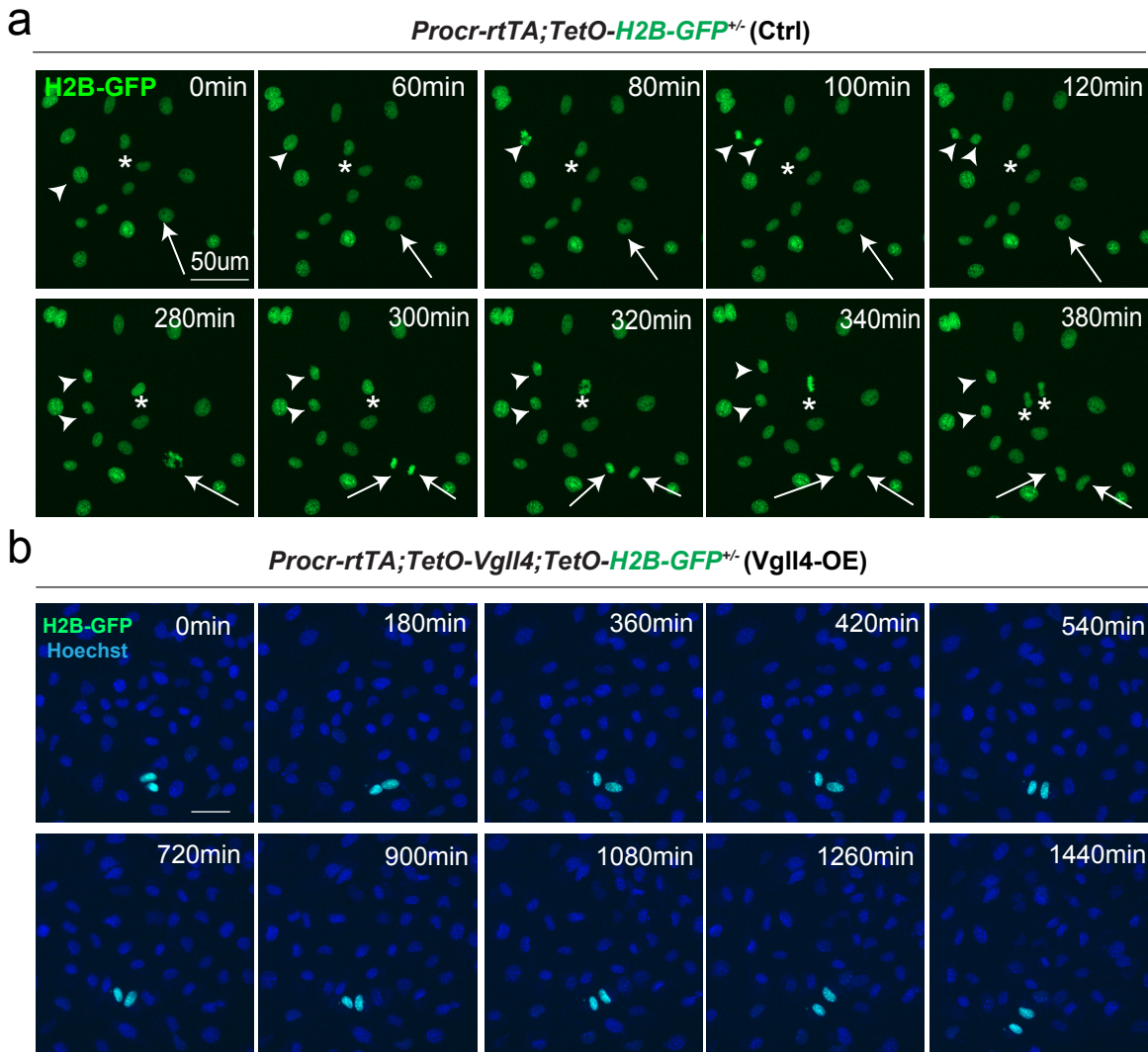


Fig S3. YAP promotes Procr⁺ cells expansion.

a-b, OSE cells were isolated from *Procr-rtTA;TetO-H2B-GFP^{+/+}* (Ctrl) or *Procr-rtTA;TetO-Vgll4^{+/+};TetO-H2B-GFP^{+/+}* (Vgll4-OE) mice and cultured on the glass. In control, almost all cells are H2B-GFP⁺ (Procr⁺) in such stiff culture condition (a). Living images for 6 hours showed many cases of H2B-GFP⁺ (Procr⁺) cells (a) (examples in *, arrow, arrowhead in a). In Vgll4-OE cells, there were drastically less H2B-GFP⁺ (Procr⁺) cells, and living imaging of 24 hours showed no incidence of cell division (b). Scale bar, 50µm. n=at least 3 views.

Fig S4. YAP induces Procr expression.

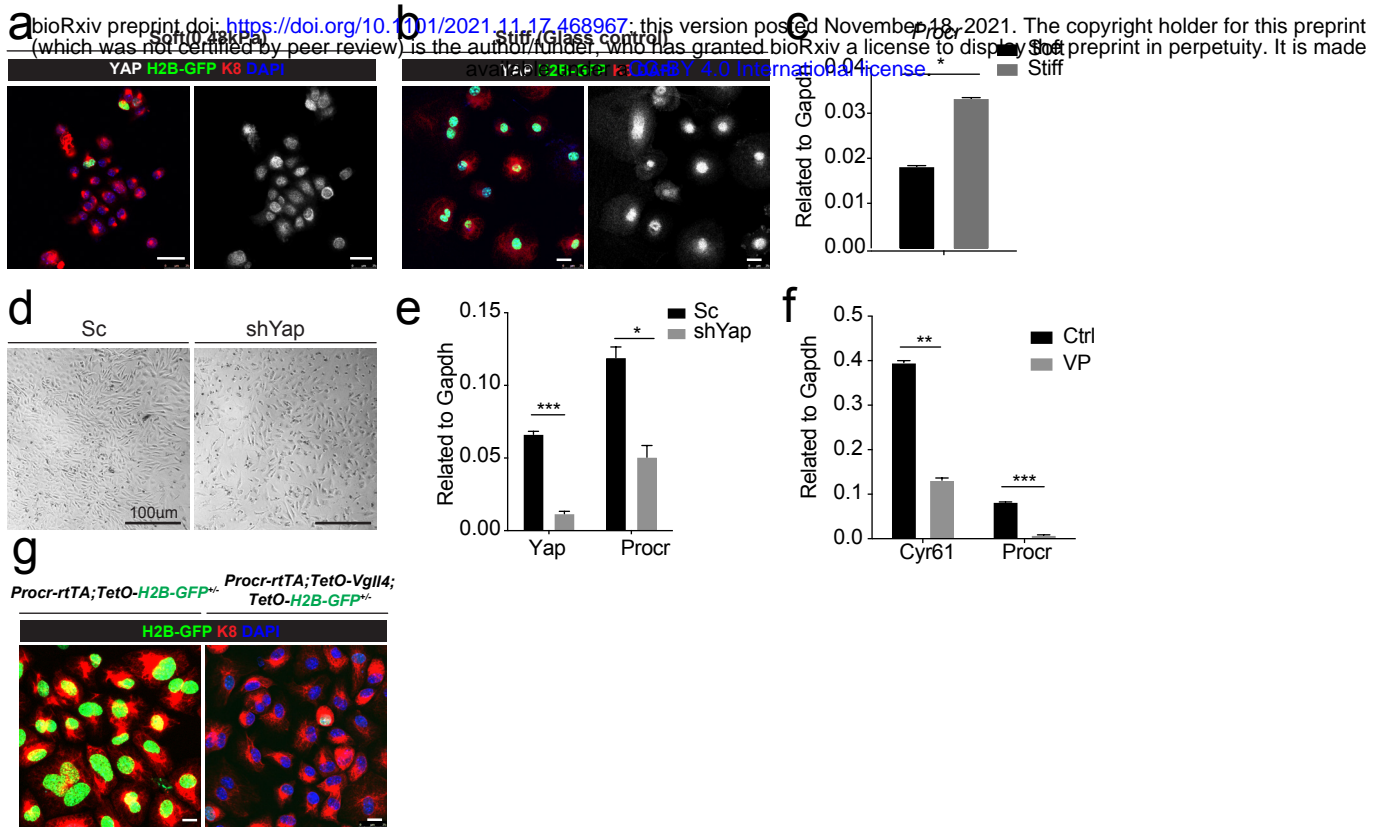


Fig S4. YAP induces Procr expression.

a-c, OSE cells were isolated from *Procr-rtTA;TetO-H2B-GFP^{+/+}* mice and cultured upon the soft hydrogel (a) or glass (b). Confocal images showed more H2B-GFP⁺ cells upon glass compared with soft hydrogel (a-b). Scale bar, 20 μ m, n=15 images. qPCR indicated *Procr* expression was upregulated upon glass culture (c).

d-e, OSE cells isolated from wildtype mice were infected with Scramble (Sc) or Yap shRNA (shYap) virus, and then cultured on glass. OSE cells were harvested on culture day 4 (d). qPCR showed knockdown of Yap repressed *Procr* expression (e). Scale bar, 100 μ m, n=15 images.

f, OSE cells were isolated from wildtype mice and cultured on glass. Verteporfin (VP) was added into the medium before harvest. qPCR showed that VP treatment inhibits *Cyr61* and *Procr* expression.

g, OSE cells were isolated from *Procr-rtTA;TetO-H2B-GFP^{+/+}* (Ctrl) and *Procr-rtTA;TetO-Vgll4^{+/+};TetO-H2B-GFP^{+/+}* (Vgll4-OE) mice and cultured on the glass. Confocal images showed dimmer H2B-GFP expression in Vgll4-OE compared to Ctrl. Scale bar, 10 μ m, n=15 images.

For all qPCR results, data are pooled from 3 independent experiments and presented as mean \pm s.e.m. Unpaired two-tailed t test is used for comparison. ***P<0.001, **P<0.01, *P<0.05.