

1 **Dermal appendage-dependent patterning of zebrafish *atoh1a*+ Merkel cells**

2

3 Tanya L. Brown^{1,†}, Emma C. Horton^{1,†}, Evan W. Craig¹, Madeleine N. Hewitt^{2,3,4}, Nathaniel G.
4 Yee¹, Camille E.A. Goo¹, Everett T. Fan¹, Erik C. Black^{1,2}, David W. Raible^{3,4,5}, Jeffrey P.
5 Rasmussen^{1,5}

6

7 ¹ Department of Biology, University of Washington, Seattle, WA, 98195, USA

8 ² Molecular and Cellular Biology Program, University of Washington, Seattle, WA,
9 98195, USA

10 ³ Department of Biological Structure, University of Washington, Seattle, WA, 98195, USA

11 ⁴ Department of Otolaryngology - Head and Neck Surgery, University of Washington,
12 Seattle, WA 98195, USA

13 ⁵ Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle,
14 WA 98109, USA

15 † Equal contribution

16

17 **Corresponding author:** JPR (rasmuss@uw.edu)

18

19 **Keywords:** somatosensory neuron, epidermis, skin, ectodysplasin, Fgf8

20 **ABSTRACT**

21

22 Touch system function requires precise interactions between specialized skin cells and
23 somatosensory axons, as exemplified by the vertebrate mechanosensory Merkel cell-neurite
24 complex. Development and patterning of Merkel cells and associated neurites during skin
25 organogenesis remains poorly understood, partly due to the *in utero* development of mammalian
26 embryos. Here, we discover Merkel cells in the zebrafish epidermis and identify Atonal homolog
27 1a (Atoh1a) as a marker of zebrafish Merkel cells. We show that zebrafish Merkel cells derive
28 from basal keratinocytes, express neurosecretory and mechanosensory machinery, extend actin-
29 rich microvilli, and complex with somatosensory axons, all hallmarks of mammalian Merkel cells.
30 Merkel cells populate all major adult skin compartments, with region-specific densities and
31 distribution patterns. *In vivo* photoconversion reveals that Merkel cells undergo steady loss and
32 replenishment during skin homeostasis. Merkel cells develop concomitant with dermal
33 appendages along the trunk, and preventing dermal appendage formation reduces Merkel cell
34 density by affecting both cell differentiation and maintenance. By contrast, altering dermal
35 appendage morphology changes the distribution, but not density, of Merkel cells. Overall, our
36 studies provide insights into touch system maturation during skin organogenesis and establish
37 zebrafish as an experimentally accessible *in vivo* model for the study of Merkel cell biology.

38

39

40 **INTRODUCTION**

41

42 Skin functions as our primary interface with the physical environment and can distinguish a range
43 of tactile inputs with exquisite acuity. As the skin undergoes organogenesis, the epidermis
44 transforms from a simple, uniform epithelium into a complex, diverse tissue. During these
45 dramatic changes, the skin develops regionally specialized sensory structures and becomes
46 innervated by specific types of somatosensory neurites (reviewed by Jenkins and Lumpkin, 2017).
47 Interactions between somatosensory neurites and cutaneous cell types regulate diverse tactile
48 responses (reviewed by Handler and Ginty, 2021). Altered tactile sensitivity during early
49 mammalian development has been associated with neurodevelopmental disorders (reviewed by
50 Orefice, 2020), underscoring the importance of understanding the cellular and molecular basis of
51 touch system development and function.

52

53 Merkel cells (MCs), a specialized mechanosensory cell type found in the vertebrate epidermis
54 (reviewed by Hartschuh et al., 1986), densely populate many highly sensitive regions of skin
55 (Lacour et al., 1991). MCs have several defining cellular characteristics that distinguish them from
56 other epidermal cell types: they are relatively small, extend actin-rich microvilli, contain
57 cytoplasmic granules reminiscent of synaptic vesicles, and form contacts with somatosensory
58 axons (Hartschuh and Weihe, 1980; Mihara et al., 1979; Smith, Jr, 1977; Toyoshima et al., 1998).
59 In mammals, a subset of cutaneous somatosensory axons known as A β slowly adapting type I
60 low-threshold mechanoreceptors (SAI-LTMRs) innervate MCs, forming the MC-neurite complex.
61 MCs detect mechanical inputs via the cation channel Piezo2 (Ikeda et al., 2014; Maksimovic et
62 al., 2014; Woo et al., 2014) and play an active role in touch sensation by releasing
63 neurotransmitters to activate neighboring neurites (Chang et al., 2016; Chang and Gu, 2020;
64 Hoffman et al., 2018). Genetic ablation of rodent MCs indicates they are required for specific
65 aspects of touch system function, including promoting the static phase of the slowly adapting
66 response of A β SAI-LTMRs and sensory tasks such as texture discrimination (Maricich et al.,
67 2012, 2009).

68

69 Molecular control of MC development has primarily been studied in rodent hairy skin (reviewed
70 by Oss-Ronen and Cohen, 2021). While this system has been useful for understanding many
71 aspects of MC development and function, it also has several significant limitations. First,
72 vertebrates have diverse types of skin and MCs are found in both hairy and glabrous (non-hairy)
73 skin, as well as mucocutaneous regions such as the gingiva and palate (Lacour et al., 1991;
74 Moayed et al., 2021). Importantly, MC populations within different skin compartments share
75 similar transcriptional profiles (Nguyen et al., 2019). Thus, the establishment of complementary
76 genetic systems in different types of skin could help reveal both shared and divergent principles
77 of MC development. Second, because *in utero* development of mammalian skin limits access to
78 the developing touch system—combined with technical limitations of imaging intact mammalian
79 skin—the dynamics of MC development and innervation remain essentially unknown. Third,
80 unbiased screens for regulators of MC development would be difficult or impractical in rodents
81 due to the prohibitive cost of animal housing and difficulty of visualizing MCs *in situ*.

82

83 Anamniote model systems, such as the genetically tractable zebrafish, provide the potential to
84 overcome these limitations. Interestingly, despite the different tactile environments encountered
85 by terrestrial and aquatic vertebrates, MCs have been described by transmission electron
86 microscopy (TEM) in a wide variety of anamniotes, including teleost (ray-finned) fish, lungfish,

87 and lamprey (Fox et al., 1980; Lane and Whitear, 1977; Whitear and Lane, 1981). Here, we
88 identify and characterize a population of zebrafish epidermal cells that we propose are bona fide
89 MCs. Our studies establish the zebrafish as a promising new model to investigate the
90 developmental and cell biology of MCs.

91

92

93 **RESULTS**

94

95 ***Ultrastructural identification of presumptive MCs in the adult epidermis***

96

97 Given the presence of cells with the ultrastructural characteristics of MCs in several teleosts (Lane
98 and Whitear, 1977), we reasoned that the zebrafish epidermis may contain similar cells. Whitear
99 (1989) defined five ultrastructural criteria for the identification of vertebrate MCs: 1) a relatively
100 small volume of cytoplasm; 2) an association with a nerve fiber; 3) the presence of cytoplasmic
101 granules; 4) desmosomal attachments to neighboring cells; and 5) peripheral microvilli.

102

103 We previously demonstrated that somatosensory axons densely innervate the epidermis above
104 scales (Rasmussen et al., 2018), dermal appendages that cover the adult zebrafish trunk (Figure
105 1A). By TEM, we found that many of the axon endings in the scale epidermis arborize between
106 keratinocyte membranes (Rasmussen et al., 2018). Interestingly, however, we identified
107 additional axon-associated epidermal cells that were distinct from the large, cuboidal
108 keratinocytes that comprise most of the epidermis based on several characteristics. The cells
109 appeared relatively small and spherical with a low cytoplasmic-to-nuclear ratio (Figure 1B,C),
110 contained cytoplasmic vesicles that in some instances localized adjacent to axon contacts (Figure
111 1B') and formed desmosomal-like attachments with neighboring keratinocytes (Figure 1B'',B''').
112 Furthermore, the cells extended spike-like microvillar processes that contacted adjacent cells
113 (Figure 1C,C'). Thus, based on established TEM criteria, we identified presumptive MCs in the
114 adult scale epidermis.

115

116 ***atoh1a reporters label MCs in the adult epidermis***

117

118 To date, a lack of genetically encoded reagents has hindered in-depth study of anamniote MCs.
119 Since the TEM studies of Whitear and colleagues decades ago, molecular markers have been
120 identified that distinguish mammalian MCs from other epidermal cells. For example, expression

121 of Atoh1 uniquely identifies MCs in rodent skin and is necessary and sufficient for MC
122 development (Morrison et al., 2009; Ostrowski et al., 2015; Van Keymeulen et al., 2009). The
123 zebrafish genome contains three genes (*atoh1a*, *atoh1b*, and *atoh1c*) encoding Atoh1 homologs
124 (Chaplin et al., 2010; Kani et al., 2010). To determine if the adult epidermis contained cells
125 expressing an Atoh1 homolog, we focused on characterizing the expression pattern of *atoh1a*
126 due to the availability of an enhancer trap line that expresses a nuclear localized version of the
127 photoconvertible fluorescent protein Eos (nls-Eos) from the endogenous *atoh1a* locus
128 (*Tg(atoh1a:nls-Eos)*) (Pickett et al., 2018). Confocal imaging of the adult trunk revealed that
129 *Tg(atoh1a:nls-Eos)* labeled hair cells of the posterior lateral line, which formed tight clusters within
130 neuromasts in interscale regions (Figure 1E,E'). In addition to *atoh1a*⁺ cells of the lateral line, we
131 identified a second, spatially distinct population of *atoh1a*⁺ cells dispersed across the scale
132 surface (Figure 1E,E'',F). Reconstructed cross-sections showed that this population of *atoh1a*⁺
133 cells resided within the epidermis above scales (Figure 1F), in a similar axial position to the cells
134 we identified by TEM.

135
136 The numerous, actin-rich microvilli that emanate from the MC surface distinguish them from other
137 epidermal cells morphologically (Lane and Whitear, 1977; Toyoshima et al., 1998; Yamashita et
138 al., 1993). To determine whether the dispersed epidermal *atoh1a*⁺ cells extended microvilli, we
139 created an *atoh1a* enhancer trap line that expresses Lifeact-EGFP, a reporter for filamentous
140 actin (Riedl et al., 2008). Similar to *Tg(atoh1a:nls-Eos)*, *Tg(atoh1a:Lifeact-EGFP)* labeled hair
141 cells of the lateral line and inner ear in larvae (Figure 1—figure supplement 1). *atoh1a*⁺ cells were
142 notably absent from regions above the larval eye, yolk sac, or caudal fin (Figure 1—figure
143 supplement 1), where neuroepithelial cells (NECs), a morphologically distinct population of
144 sensory cells, have been described in larval skin (Coccimiglio and Jonz, 2012). Confocal
145 microscopy of the scale epidermis in *Tg(atoh1a:Lifeact-EGFP)* adults revealed actin-rich microvilli
146 densely decorating *atoh1a*⁺ cells in close proximity to neighboring keratinocytes (Figure 1G),
147 further suggesting that the epidermal *atoh1a*⁺ cell population shared key characteristics with the
148 candidate MCs identified by TEM. Immunostaining for Sox2, a transcription factor required for
149 murine MC maturation (Bardot et al., 2013; Perdigoto et al., 2014), demonstrated that the
150 epidermal *atoh1a*⁺ cells expressed Sox2 (Figure 1—figure supplement 2). Together, these results
151 define molecular and cellular properties of a previously uncharacterized epidermal cell population
152 in zebrafish and identify genetic reagents for the study of this cell type. Anticipating the conclusion
153 of our analysis below, we shall hereafter refer to the epidermal *atoh1a*⁺ cells as MCs.

154

155 ***Somatosensory axons innervate zebrafish MCs, which display neurosecretory and***
156 ***mechanosensory characteristics***

157

158 We next sought to determine whether zebrafish MCs displayed other key characteristics of MCs
159 defined in mammals, including innervation by somatosensory axons and expression of
160 neurosecretory and mechanosensory machinery.

161

162 Our ultrastructural observations suggested that cutaneous axons innervate MCs (Figure 1B).
163 Staining scales with zn-12, a monoclonal antibody that labels several types of peripheral axons
164 (Metcalf et al., 1990), revealed that >90% of MCs tightly associated with axons (Figure 2A,C).
165 To determine the type of axon(s) innervating MCs, we examined expression of genetically
166 encoded somatosensory axon reporters that we previously characterized in adult scales
167 (Rasmussen et al., 2018). Analysis of reporters for three somatosensory neuron-expressed genes
168 (*p2rx3a*, *p2rx3b*, and *trpa1b*) (Kucenas et al., 2006; Palanca et al., 2013; Pan et al., 2012)
169 demonstrated that somatosensory axons innervated up to 99% of MCs (Figure 2B,C). Consistent
170 with ultrastructural analyses of MCs in the skin of other teleosts (Whitear, 1989), some axons
171 formed ring-like structures that wrapped around MCs and MC-axon contacts frequently contained
172 varicosities or swellings (Figure 2B, inset and 2D-F; Supplemental Video 1). Additionally, we
173 observed examples of axons forming both bouton- and en passant-like contacts with MCs (Figure
174 2G,H).

175

176 Based on our observation that MCs contained cytoplasmic granules (Figure 1B), we postulated
177 that they would display neurosecretory characteristics. We began by staining scales with an
178 antibody against synaptic vesicle glycoprotein 2 (SV2), a component of secretory vesicle
179 membranes (Buckley and Kelly, 1985). Essentially all MCs contained SV2-positive structures
180 (Figure 3A), suggesting they express neurosecretory machinery that may contain
181 neurotransmitter(s). Indeed, immunostaining revealed that MCs expressed serotonin (5-
182 hydroxytryptamine; 5-HT) (Figure 3B), similar to mammalian MCs (Chang et al., 2016; English et
183 al., 1992; García-Caballero et al., 1989). Both 5-HT and SV2 appeared in a speckled pattern
184 within MCs (Figure 3A,B), consistent with a vesicular localization.

185

186 Do zebrafish MCs exhibit properties consistent with mechanosensory function? To address this
187 question, we began by staining scales with AM1-43, an activity-dependent fluorescent styryl dye
188 that labels a variety of sensory cells, including mammalian MCs (Meyers et al., 2003). Following

189 a short preincubation, AM1-43 robustly stained MC membranes and punctate structures
190 reminiscent of vesicular compartments (Figure 3C), suggestive of ion channel expression in MCs
191 (Meyers et al., 2003). Mammalian MCs express the mechanically activated cation channel Piezo2,
192 which is required for MC mechanosensory responses (Ikeda et al., 2014; Maksimovic et al., 2014;
193 Woo et al., 2014). Fluorescent in situ hybridization with an antisense probe against *piezo2*
194 strongly labeled MCs in adult scales (Figure 3D). Together, these data suggest that
195 somatosensory peripheral axons innervate adult MCs, which possess neurosecretory and
196 mechanosensory properties.

197

198 ***MCs arise from basal keratinocyte precursors***

199

200 What are the precursors of MCs in zebrafish? Analysis of MC progenitors have come to conflicting
201 results in avians and rodents: quail/chick chimeras suggest a neural crest origin for avian MC
202 (Grim and Halata, 2000), whereas Cre-based lineage tracing studies in mouse demonstrate an
203 epidermal origin (Morrison et al., 2009; Van Keymeulen et al., 2009).

204

205 To investigate a possible neural crest origin, we crossed a Cre driver expressed in neural crest
206 progenitors (*Tg(sox10:Cre)*; (Kague et al., 2012)) to a reporter transgene that stably expresses
207 DsRed upon Cre-mediated recombination from a quasi-ubiquitous promoter (*Tg(actb2:LOXP-*
208 *BFP-LOXP-DsRed)*; (Kobayashi et al., 2014)) (Figure 4A). DsRed⁺ neural crest-derived cell
209 types, such as Schwann cells, appeared along scales, indicative of successful recombination
210 (Figure 4B). However, we observed <0.5% colocalization between the neural crest lineage trace
211 and a MC reporter (Figure 4B',E). Based on these results, we concluded that zebrafish MCs likely
212 derive from a non-neural crest lineage.

213

214 To investigate a possible epidermal origin, we considered basal keratinocytes, an epidermal-
215 resident stem cell population, the most likely candidate progenitors. To follow this lineage, we
216 engineered a transgene to express a tamoxifen-inducible Cre recombinase from regulatory
217 sequences of *ΔNp63* (*TgBAC(ΔNp63:Cre-ERT2)*), a basal keratinocyte marker (Bakkers et al.,
218 2002; Lee and Kimelman, 2002). We crossed this transgene to the Cre reporter transgene and
219 treated embryos with 4-OHT at 1 day post-fertilization (dpf) to induce Cre-ERT2 activity, which
220 resulted in permanent DsRed expression in basal keratinocytes and their derivatives (Figure
221 4C,D; Figure 4—figure supplement 1). 4-OHT-treated animals showed extensive co-labeling

222 between the basal keratinocyte lineage trace and a MC reporter (Figure 4D',E). These
223 observations strongly support a basal keratinocyte origin of zebrafish MCs.

224

225 ***MCs continuously turn over in adult skin***

226

227 The longevity and turnover of murine MCs is controversial. Several studies concluded that MC
228 numbers fluctuate with hair cycle stages (Marshall et al., 2016; Moll et al., 1996; Nakafusa et al.,
229 2006), while another found no correlation (Wright et al., 2017). To determine the turnover rate of
230 zebrafish MCs, we photoconverted small regions of the scale epidermis in *Tg(atoh1a:nls-Eos)*
231 adults and tracked individual cells over time. Exposure to UV light irreversibly photoconverts nls-
232 Eos, allowing us to distinguish pre-existing cells (containing photoconverted nls-Eos) from newly
233 added cells (without photoconverted nls-Eos) (Figure 5A-C). By longitudinally tracking individual
234 fish over the course of 28 days, we found a decrease of ~15% of the photoconverted MCs every
235 7 days (Figure 5D). In addition to the gradual loss of MCs over time, we noted a steady addition
236 of new MCs, resulting in a nearly constant total cell number (Figure 5E). Thus, MCs undergo
237 constant cell loss and renewal in adult skin, albeit at a slower rate than *atoh1a*-expressing hair
238 cells of the lateral line (Cruz et al., 2015).

239

240 ***MCs are widely distributed across the body, in compartment-specific patterns***

241

242 MCs localize to specific regions of mammalian skin, such as in crescent-shaped touch domes
243 adjacent to hair follicles in hairy skin and at the bottom of rete ridges in glabrous skin (Boot et al.,
244 1992; Fradette et al., 1995; Iggo and Muir, 1969; Lacour et al., 1991). To determine the distribution
245 pattern of zebrafish MCs, we used confocal microscopy to survey multiple regions of the adult
246 skin. In addition to the MCs found on the trunk, MCs appeared in the epidermis above the eyes,
247 gill covers (opercula), and fins (Figure 6A-E). While MC morphology was similar across the skin
248 compartments (Figure 6A-E, insets), MC densities and spatial distributions varied across skin
249 compartments (Figure 6F). For example, MCs were distributed uniformly across the eye (Figure
250 6B). By contrast, in the caudal fin, MCs localized specifically to the epidermis above bony rays
251 and in medial regions of the interray epidermis between bony rays (Figure 6E). Along the trunk,
252 MCs appeared in patches, similar to the pattern of dermal scales beneath the epidermis (Figure
253 6D). Altogether, our results demonstrate that MCs are widely distributed across the adult zebrafish
254 skin and localize in specific patterns in each skin compartment.

255

256 ***Trunk MCs develop concomitant with dermal appendage morphogenesis***

257

258 To examine the mechanisms that generate a compartment-specific MC pattern, we focused on
259 the trunk skin because of its molecular and cellular similarities to murine hairy skin (Aman et al.,
260 2018; Harris et al., 2008). Both during ontogeny and at post-embryonic stages, murine MCs
261 associate with primary (guard) hairs, a subclass of dermal appendages (Jenkins et al., 2019;
262 Nguyen et al., 2018). Based on these studies in mice, and our previous work showing that
263 epidermal diversification and somatosensory remodeling coincides with scale development in
264 zebrafish (Rasmussen et al., 2018), we postulated that MCs would appear during squamation
265 (scale formation).

266

267 Zebrafish post-larval development is staged by standard length (SL) in millimeters (mm) (Parichy
268 et al., 2009). Squamation begins at ~9 mm SL (Figure 7A) (Aman et al., 2018; Harris et al., 2008;
269 Sire et al., 1997a). Using reporters that label MCs and scale-forming osteoblasts, we observed
270 only rare MCs in the epidermis prior to the onset of squamation (Figure 7B). By contrast, MC
271 density rapidly increased between 10-15 mm SL, a period of active scale growth (Figure 7C-F).
272 The density and number of MCs positively correlated with scale area (Figure 7G,H). These data
273 indicate that MC development coincides with dermal appendage growth along the trunk.

274

275 ***Ectodysplasin signaling promotes trunk MC development***

276

277 Since appearance of MCs in the trunk epidermis tightly correlated with scale growth, we examined
278 the consequences of blocking dermal appendage morphogenesis on MC development.
279 Ectodysplasin (Eda) signaling regulates the formation of many types of skin appendages,
280 including mammalian hair follicles and zebrafish scales (Biggs and Mikkola, 2014; Harris et al.,
281 2008). To determine whether MC development requires Eda-dependent signals, we measured
282 MC density in animals homozygous for a presumptive null allele of *eda* that do not develop scales
283 (*eda*^{dt1261/dt1261}; hereafter *eda*^{-/-}) (Harris et al., 2008). Immediately prior to squamation, we found
284 that there was no difference in MC density between *eda* mutants and sibling controls (Figure
285 8A,B,G). However, after the onset of squamation, *eda* mutants had significantly fewer MCs, a
286 difference that persisted into adulthood (Figure 8A-G). In addition to the decrease in cell density,
287 we observed a dramatic change in the spatial distribution of MCs across the epidermis in *eda*
288 mutants compared to controls (Figure 8H). Specifically, in siblings, MCs appeared in patches
289 corresponding to the location of the underlying scales (Figure 8C,H). By contrast, the few MCs

290 that developed in *eda* mutants were distributed uniformly across the trunk (Figure 8D,H). Although
291 we found a decrease in MC density in the trunk skin of the mutants, we observed no change in
292 MC density above the eye or operculum (Figure 8I), suggesting that the reduced density was
293 specific to the trunk skin.

294
295 The decreased MC density in *eda* mutant trunk skin could be due to decreased cell addition,
296 increased cell turnover rate, or a combination of the two. Using *in vivo* photoconversion, we found
297 that the rate of MC addition was significantly reduced in *eda* mutants compared to siblings (Figure
298 8—figure supplement 1A-D). Additionally, the rate of cell loss was higher in mutants compared to
299 siblings (Figure 8—figure supplement 1E). Thus, our observations indicate that the decrease in
300 MC cell density in *eda* mutants is likely due to both reduced MC production and increased MC
301 turnover. Together, these data suggest that *Eda* signaling is required for MC development,
302 maintenance, and distribution along the trunk.

303

304 ***Altering dermal appendage shape and size redistributes MCs***

305

306 Since blocking dermal appendage formation inhibited MC development, we next examined the
307 consequences of altering dermal appendage size and shape on MC patterning. Zebrafish scale
308 morphogenesis is regulated by Fibroblast growth factor (FGF) signaling (Aman et al., 2018;
309 Daane et al., 2016; De Simone et al., 2021; Rohner et al., 2009). To determine whether alterations
310 to scale patterning impacted MC development, we examined animals heterozygous for an allele
311 of *hagoromo* (*hag; fgf8a^{dhiD1Tg/+}*), which results in *fgf8a* overexpression in the post-embryonic skin
312 due to a viral insertion near the *fgf8a* locus (Amsterdam et al., 2009). An independent allele of
313 *hag* (*fgf8a^{dhi4000Tg/+}*) was previously shown to result in large, disorganized sheets of scale-forming
314 osteoblasts during squamation (Aman et al., 2018). *fgf8a^{dhiD1Tg/+}* juveniles showed dramatic
315 variability in scale size and shape, with both smaller and larger scales compared to the remarkably
316 uniformly patterned scales observed in sibling controls (Figure 9A-D; Figure 9—figure supplement
317 1A-C). We found no significant differences in MC density between the genotypes (Figure 9—
318 figure supplement 1D,E). Nevertheless, the distribution of MCs tracked with the altered scale size
319 and shape in the mutants, suggesting the MC pattern is not predetermined (Figure 9). Based on
320 these data, we concluded that altering dermal appendage morphogenesis is sufficient to
321 redistribute MCs within the trunk skin compartment.

322

323

324 **DISCUSSION**

325

326 Here, we discover a zebrafish epidermal cell type that we classify as a MC based on ultrastructural
327 criteria (Whitear, 1989). We further present several lines of evidence that suggest zebrafish MCs
328 share molecular, cellular, and lineage properties with mammalian MCs. First, we show that
329 zebrafish MCs express the transcription factors *Atoh1a* and *Sox2*, the orthologs of which uniquely
330 mark MCs in mammalian skin (Maricich et al., 2009; Nguyen et al., 2018; Ostrowski et al., 2015;
331 Van Keymeulen et al., 2009). Second, zebrafish MCs extend numerous short, actin-rich microvilli
332 and complex with somatosensory axons, classic morphological hallmarks of MCs (Mihara et al.,
333 1979; Smith, Jr, 1977; Toyoshima et al., 1998). Third, Cre-based lineage tracing revealed that
334 basal keratinocytes give rise to zebrafish MCs, akin to studies in mouse (Morrison et al., 2009;
335 Van Keymeulen et al., 2009). Fourth, we demonstrate that zebrafish MCs contain neurosecretory
336 machinery and express the neurotransmitter serotonin, release of which has been proposed to
337 regulate somatosensory responses to touch (Chang et al., 2016; Chang and Gu, 2020; English
338 et al., 1992). Finally, we show that zebrafish MCs express the cation channel *Piezo2*, which is
339 cell-autonomously required for MC mechanosensory function (Ikeda et al., 2014; Maksimovic et
340 al., 2014; Woo et al., 2014). Importantly, our results extend previous histological studies of MCs
341 in various teleost fish (Hartschuh and Weihe, 1980; Lane and Whitear, 1977; Whitear, 1989;
342 Whitear and Lane, 1981; Zachar and Jonz, 2012) by identifying the first genetically encoded
343 reagents for the study of this cell type in zebrafish.

344

345 ***Teleost Merkel cells and somatosensory physiology***

346

347 In mammalian skin, the MC-neurite complex regulates slowly adapting type I responses to light
348 touch (Iggo and Muir, 1969; Ikeda et al., 2014; Maksimovic et al., 2014; Maricich et al., 2009; Woo
349 et al., 2014). Although physiological studies of somatosensory responses in adult zebrafish have
350 not been reported, extracellular recordings in adult rainbow trout demonstrated that a subset of
351 somatosensory neurons exhibited slowly adapting responses to mechanical skin stimulation
352 (Ashley et al., 2007, 2006; Sneddon, 2003). We postulate that the slowly adapting responses to
353 mechanical skin stimulation in adults requires MCs. Nevertheless, the exact physiological roles
354 of teleost MCs in regulating somatosensory responses and resulting behaviors remain unknown
355 and will require the development of tools to selectively ablate and activate MCs. Interestingly,
356 recordings from zebrafish Rohon-Beard neurons, a transient larval somatosensory population,
357 suggest they have rapidly, but not slowly, adapting mechanosensory responses (Katz et al.,

358 2021). Together these studies correlate with our finding that MCs develop at post-larval stages
359 and suggest that the teleost somatosensory system undergoes significant functional maturation
360 during the juvenile period.

361

362 What are the subtypes of somatosensory neurons in fish and how do they correspond to MC
363 innervation? Several studies have identified molecularly distinct subsets of somatosensory
364 neurons through mRNA, protein, and transgene expression analysis in larvae (Faucherre et al.,
365 2013; Gau et al., 2017, 2013; Kucenas et al., 2006; Palanca et al., 2013; Pan et al., 2012; Patten
366 et al., 2007; Slatter et al., 2005). Adult trout somatosensory neurons have been classified based
367 on their responses to mechanical, chemical, and thermal stimuli (Ashley et al., 2007, 2006;
368 Sneddon, 2003). However, to date, a detailed molecular characterization of the diversity of
369 somatosensory subtypes present in adult fish has not been performed. Our data suggest that
370 somatosensory neurons expressing reporters for *p2rx3a*, *p2rx3b*, or *trpa1b* innervate MCs.
371 Whether these neurons represent a dedicated class of MC-innervating neurons remains
372 unknown. The development of Cre drivers for specific somatosensory subtypes (Bai et al., 2015;
373 Li et al., 2011; Luo et al., 2009; Rutlin et al., 2014; Zylka et al., 2005) and single-cell transcriptional
374 profiling (Sharma et al., 2020; Usoskin et al., 2015; Zeisel et al., 2018) have been fruitful in
375 characterizing the diversity of somatosensory neurons in mammals. The application of these
376 technologies to the teleost somatosensory system is an interesting avenue for further
377 investigation.

378

379 ***Merkel cell lineage and homeostasis***

380

381 The developmental lineage of MCs has been a long-standing question with both epidermal and
382 neural crest origins posited (Hartschuh et al., 1986). Our Cre-based lineage tracing identified
383 basal keratinocytes as MC progenitors. These results extend previous studies in the zebrafish
384 epidermis showing that basal keratinocytes serve as precursors for diverse post-larval cell types,
385 including periderm (superficial epidermis) and immune cells (Lee et al., 2014; Lin et al., 2019).
386 Although previous work in mouse unambiguously identified *keratin 14*-expressing basal
387 keratinocytes as MC precursors (Morrison et al., 2009; Van Keymeulen et al., 2009), the precise
388 nature of murine MC progenitors varies across skin compartments (Nguyen et al., 2019). Future
389 studies characterizing the molecular properties and cellular behaviors of zebrafish MC precursors
390 will be informative for identifying conserved properties of skin stem cells.

391

392 The turnover of MCs in mammalian skin has been a source of controversy. Several studies
393 reported that MC numbers fluctuate with the natural hair cycle in mouse (Marshall et al., 2016;
394 Moll et al., 1996; Nakafusa et al., 2006). By contrast, Wright et al. (2017) found no evidence for
395 changes in MC density based on stages of the hair cycle and demonstrated that MCs could live
396 for months. These types of analyses in murine skin have relied either on histology, which limits
397 tissue sampling, or required use of advanced (2-photon) microscopy in combination with hair
398 shaving, a mild form of skin injury. Using photoconversion and confocal imaging, we non-
399 invasively tracked individual MCs during normal skin homeostasis *in vivo* for weeks. We found
400 that trunk MCs have a steady turnover in adult animals, with a half-life of approximately 1 month.
401 Additionally, this further distinguishes MCs from hair cells in the adult lateral line, which have a
402 shorter half-life (Cruz et al., 2015). Whether MC turnover varies at different stages of
403 development, across skin compartments, or following skin insults will require further study.

404

405 ***Merkel cell distribution and patterning***

406

407 Regionally specific sensory structures allow our skin to distinguish tactile inputs with remarkable
408 acuity (Corniani and Saal, 2020). For example, MC densities vary greatly across human skin
409 compartments, with the highest numbers found in particularly sensitive regions such as fingertips
410 and lips (Boot et al., 1992; Lacour et al., 1991). We observed that MCs populate several major
411 skin compartments and have regional-specific densities in adult zebrafish, with the highest
412 densities found in the face (above the eye and operculum). We speculate this may bestow the
413 juvenile and adult skin with the ability to detect innocuous tactile inputs across almost the entire
414 body surface, with perhaps the greatest sensitivity along facial structures.

415

416 Although most studies of MC development have centered on the formation of MC aggregates in
417 the touch domes of murine hairy skin, MCs are found in a range of distribution patterns in other
418 types of skin. For example, MCs are found as dispersed, single cells arrayed across the skin of
419 human toe pads (Boot et al., 1992). Similarly, we found that MCs have a dispersed, rather than
420 clustered, pattern in all skin compartments examined. Few studies have addressed how MCs
421 adopt specific distributions, and zebrafish present a promising model to understand mechanisms
422 of MC pattern formation *in vivo*.

423

424 ***Dermal appendages and Merkel cell development***

425

426 Our developmental analysis showed that trunk MC density rapidly increases during dermal
427 appendage morphogenesis. Previous genetic analysis in mouse hairy skin revealed that MC
428 development requires *Eda* signaling (Vielkind et al., 1995; Xiao et al., 2016). We show that
429 zebrafish *eda* mutants have decreased MC density in trunk, but not facial, skin. These
430 observations suggest that MC development in mouse and zebrafish likely share similar genetic
431 pathways, akin to the shared molecular and cellular mechanisms that regulate dermal appendage
432 formation (Aman et al., 2018; Biggs and Mikkola, 2014; Daane et al., 2016; Harris et al., 2008;
433 Rohner et al., 2009). They further support a model whereby MC development requires
434 compartment-specific signals, akin to recent observations on MC development in mouse hairy
435 and glabrous skin (Nguyen et al., 2019). By taking advantage of the ability to image large skin
436 areas in intact zebrafish, we show that *eda* mutants have altered MC distribution compared to
437 controls. Furthermore, we use *in vivo* photoconversion to demonstrate the reduction in MC density
438 is largely due to decreased production, but also reflects increased turnover. One explanation for
439 these results is that *Eda* signaling regulates the differentiation of MC progenitors. Alternatively,
440 since *eda* mutants have decreased epidermal innervation (Rasmussen et al., 2018), MC
441 development may require somatosensory neuron-derived signals.

442

443 We found that a gain-of-function allele of *fgf8a* leads to a change in the overall size and shape of
444 scales. Intriguingly, the MC distribution modifies to accommodate the altered scale size and shape
445 in the *fgf8a* mutants but retains the same MC density as sibling controls. This result suggests that
446 the number of MCs per scale is not predetermined, but rather is titrated relative to appendage
447 size. How are MCs able to populate the much larger scales in *fgf8a* mutants? Does the size of
448 the MC progenitor domain expand with increases in scale size? Are MCs, or their progenitors,
449 able to migrate to their final destination? Distinguishing between these possibilities will require
450 tracking the behaviors of MCs and their progenitors *in vivo*.

451

452 **Summary**

453

454 Our results establish a promising new system to investigate MC biology. This model will allow for
455 the identification of deeply conserved mechanisms used to regulate vertebrate MC biology.
456 Furthermore, the advantages of zebrafish—such as non-invasive *in vivo* imaging, genetic and
457 chemical screens, and high regenerative capacity—will complement the strengths of rodent
458 models. Specifically, the ability to track individual cells over time has the potential to answer key
459 and long-standing questions surrounding MC biology, including how the MC-neurite relationship

460 is established, how MCs interact with neighboring cell types, and their progenitor dynamics.
461 Addressing these questions, as well as potential novel insights provided by the zebrafish system,
462 represent exciting directions for future research.

463 **MATERIALS AND METHODS**

464

465 **Key Resource Table**

466

Reagent type	Designation	Source or reference	Identifiers	Additional information
Antibody	Rabbit anti-Serotonin	MilliporeSigma	Cat #: S5545	Used at 1:1000
Antibody	Mouse anti-Sv2	DSHB; (Buckley and Kelly, 1985)	Cat #: SV2	Used at 1:50
Antibody	Rabbit anti-Sox2	GeneTex	Cat #: GTX124477	Used at 1:500
Antibody	Sheep Anti-Fluorescein Polyclonal Antibody, POD Conjugated	Roche	Cat# 11426346910, RRID:AB_840257	Used at 1:2000
Antibody	GFP Polyclonal Antibody	Thermo Fisher Scientific	Cat #: A11122, RRID:AB_221569	Used at 1:1000
Antibody	Mouse monoclonal zn-12	Zebrafish International Resource Center	Cat# zn-12, RRID:AB_10013761	Used at 1:200
Antibody	Goat anti-Rabbit Alexa Fluor 647	Thermo Fisher Scientific	Cat #: A32733, RRID:AB_2633282	Used at 1:500
Antibody	Goat anti-Mouse Alexa Fluor 647	Thermo Fisher Scientific	Cat #: A32728, RRID:AB_2633277	Used at 1:500
Antibody	Goat anti-Rabbit Alexa Fluor 568	Thermo Fisher Scientific	Cat #: A-11036, RRID:AB_10563566	Used at 1:500
Antibody	Goat anti-Mouse Alexa Fluor 568	Thermo Fisher Scientific	Cat #: A-11031, RRID:AB_144696	Used at 1:500
Antibody	Goat anti-Rabbit Alexa Fluor 488	Thermo Fisher Scientific	Cat #: A32731, RRID:AB_2633280	Used at 1:500
Commercial assay or kit	TSA Plus Cyanine 5	Akoya Biosciences	NEL705A001KT	Used at 1:50

Other	10x PBS	Thermo Fisher Scientific	Cat #: AM9624	
Other	Triton X-100	Thermo Fisher Scientific	Cat #: BP337-500	
Other	Tween-20	Promega	Cat #: H5152	
Other	20x SSC	Thermo Fisher Scientific	Cat #: AM9763	
Other	Formaldehyde	Thermo Fisher Scientific	Cat #: 29806	
Other	Fetal bovine serum	Gibco	Cat #: 10082-139	
Other	Normal goat serum	Abcam	Cat #: ab7481, RRID:AB_2716553	
Other	ProLong gold antifade mountant	Thermo Fisher Scientific	Cat #: P36930	
Sequence-based reagent	<i>piezo2</i> in situ probe (originally referred to as <i>piezo2b</i>)	(Faucherre et al., 2013)	N/A	
Sequence-based reagent	<i>atoh1a</i> gRNA, 5'-GGA GAC TGA ATA AAG TTA TG-3'	(Pickett et al., 2018)	N/A	
Sequence-based reagent	Mbait gRNA, 5'-GGC TGC TGC GGT TCC AGA GGT GG-3'	(Kimura et al., 2014)	N/A	
Chemical compound	DAPI	MilliporeSigma	Cat #: 508741	Used at 5 ng/μl
Chemical compound	AM1-43	Biotinium	Cat #: 70024	Used at 15 μm
Chemical compound	MS-222	MilliporeSigma	Cat #: E10521	
Chemical compound	Alizarin Red S	ACROS Organics	Cat #: 400480250	Used at 0.01%

Chemical compound	Methanol	VWR	Cat #: K977-4L	
Chemical compound	Proteinase K	Thermo Fisher Scientific	Cat #: 100005393	Used at 0.1 mg/ml
Chemical compound	Hoechst 3342	Thermo Fisher Scientific	Cat #: H3570	Used at 5 ng/μl
Chemical compound	(Z)-4-Hydroxytamoxifen (4-OHT)	MilliporeSigma	Cat #: H7904	Used at 10 μM
Strain (<i>Danio rerio</i>)	AB (Wild-Type)	Zebrafish International Resource Center	ZIRC Cat# ZL1, RRID:ZIRC_ZL1	
Strain (<i>Danio rerio</i>)	<i>Tg(actb2:LOXP-BFP-LOXP-DsRed)</i>	(Kobayashi et al., 2014)	<i>Tg(actb2:LOXP-BFP-LOXP-DsRed)^{sd27Tg}</i>	ZFIN: ZDB-TGCONSTRCT-141111-5
Strain (<i>Danio rerio</i>)	<i>Tg(atoh1a:nls-Eos)</i>	(Pickett et al., 2018)	<i>Tg(atoh1a:nls-Eos)^{w214Tg}</i>	ZFIN: ZDB-TGCONSTRCT-190701-2
Strain (<i>Danio rerio</i>)	<i>Tg(atoh1a:lifect-EGFP)</i>	This study	<i>Tg(atoh1a:lifect-EGFP)^{w259Tg}</i>	
Strain (<i>Danio rerio</i>)	<i>TgBAC(ΔNp63:Cre-ERT2)</i>	This study	<i>TgBAC(ΔNp63:Cre-ERT2)^{w267Tg}</i>	
Strain (<i>Danio rerio</i>)	<i>Tg(sox10:Cre)</i>	(Kague et al., 2012)	<i>Tg(Mmu.Sox10-Mmu.Fos:Cre)^{zf384}</i>	ZFIN: ZDB-TGCONSTRCT-130614-2
Strain (<i>Danio rerio</i>)	<i>Gt(ctnna-citrine)</i>	(Trinh et al., 2011)	<i>Gt(ctnna-citrine)^{ct3aGt}</i>	ZFIN: ZDB-ALT-111010-23
Strain (<i>Danio rerio</i>)	<i>Tg(sp7:mCherry)</i>	(Singh et al., 2012)	<i>Tg(Ola.Sp7:mCherry-Eco.NfsB)^{pd46Tg}</i>	ZFIN: ZDB-TGCONSTRCT-120503-4
Strain (<i>Danio rerio</i>)	<i>Tg(p2rx3a>mCherry)</i>	(Palanca et al., 2013)	<i>Tg(Tru.P2rx3a:LEXA-VP16,4xLEXOP-mCherry)^{la207Tg}</i>	ZFIN: ZDB-TGCONSTRCT-130307-1
Strain (<i>Danio rerio</i>)	<i>Tg(trpa1b:EGFP)</i>	(Pan et al., 2012)	<i>TgBAC(trpa1b:EGFP)^{a129Tg}</i>	ZFIN: ZDB-TGCONSTRCT-120208-2

Strain (<i>Danio rerio</i>)	<i>Tg(p2rx3b:EGFP)</i>	(Kucenas et al., 2006)	<i>Tg(p2rx3b:EGFP)_{sl1Tg}</i>	ZFIN: ZDB-TGCONSTRUCT-070117-110
Strain (<i>Danio rerio</i>)	<i>Tg(krt4:DsRed)</i>	(Rieger and Sagasti, 2011)	<i>Tg(krt4:DsRed)^{la2}_{03Tg}</i>	ZFIN: ZDB-TGCONSTRUCT-120127-5
Strain (<i>Danio rerio</i>)	<i>eda^{dt1261}</i>	(Harris et al., 2008)	<i>eda^{dt1261}</i>	ZFIN: ZDB-ALT-090324-1
Strain (<i>Danio rerio</i>)	<i>fgf8a^{dhiD1Tg/+}</i>	(Amsterdam et al., 2009)	<i>fgf8a^{dhiD1Tg/+}</i>	ZFIN: ZDB-ALT-010427-4

467

468 **Animals**

469

470 *Zebrafish*

471 Zebrafish were housed at 26-27°C on a 14/10 h light cycle. See Key Resource Table for strains
 472 used in this study. Animals of either gender were used. Zebrafish were staged according to
 473 standard length (SL) (Parichy et al., 2009). SL of fish was measured using the IC Measure
 474 software (The Imaging Source) on images captured on a Stemi 508 stereoscope (Zeiss) equipped
 475 with a DFK 33UX264 camera (The Imaging Source). All zebrafish experiments were approved by
 476 the Institutional Animal Care and Use Committee at the University of Washington (Protocol:
 477 #4439-01).

478

479 *Creation of Tg(atoh1a:Lifeact-EGFP)*

480 *Tg(atoh1a:Lifeact-EGFP)^{w259Tg}* was generated by CRISPR-mediated knock-in as previously
 481 described (Kimura et al., 2014). A donor plasmid containing the Mbait, minimal *hsp70l* promoter,
 482 *Lifeact-EGFP*, and *bgh poly(A)* sequences was created using Gibson assembly. The insertion
 483 was targeted 372 bp upstream of the endogenous *atoh1a* coding sequence using a previously
 484 published guide RNA (gRNA) (Pickett et al., 2018). The *Mbait-hsp70l-Lifeact-EGFP* plasmid,
 485 Mbait and *atoh1a* gRNAs, and Cas9 protein were prepared and injected into single cell embryos
 486 of the AB strain as previously described (Thomas and Raible, 2019). Larvae were screened for
 487 Lifeact-EGFP expression at 3 dpf and raised to adulthood. A founder adult was identified and
 488 outcrossed to generate a stable transgenic line.

489

490 *Creation of TgBAC(Δ Np63:Cre-ERT2) and induction with 4-OHT*

491 The Δ Np63:EGFP-2xFYVE bacterial artificial chromosome (BAC) was created by modifying the
492 previously generated BAC DKEY-263P13-iToI2-amp (Rasmussen et al., 2015). The predicted
493 Δ Np63 start codon was replaced by a Cre-ERT2-pA-KanR cassette that contained a zebrafish
494 codon-optimized Cre-ERT2 (Kesavan et al., 2018) using a previously described protocol (Suster
495 et al., 2011). TgBAC(Δ Np63:Cre-ERT2)^{w267Tg} was created by injecting *tol2* mRNA, which was
496 transcribed from pCS2-zT2TP (Suster et al., 2011), and BAC DNA into one-cell stage embryos
497 and screening adults for germline transmission. To activate Cre-ERT2, 1 dpf embryos were
498 treated with 10 μ M 4-OHT for 24 h. 4-OHT was prepared as described (Felker et al., 2016).

499

500 *Mutant identification and analysis*

501 *eda* mutants and siblings were sorted by visible phenotype starting at 7 mm SL. Mutants were
502 grown separately from siblings. *fgf8a*^{dhiD1Tg/+} fish were identified based on altered scale patterning
503 and/or pigmentation (Kawakami et al., 2000).

504

505 **Imaging and photoconversion**

506

507 *Electron microscopy*

508 Isolated scales were prepared for TEM as described (Sire et al., 1997b), with the following
509 modifications: after dehydration, scales were treated with propylene oxide (PO), infiltrated with
510 PO:Eponate 12, and embedded in Eponate 12. Semithin sections (0.2 μ m) stained with toluidine
511 blue were used for orientation. Thin sections (50 nm) were placed on Formvar coated copper slot
512 grids, stained with saturated uranyl acetate and Reynolds' lead citrate, and examined on a JEOL
513 100CX at 60 kV or a Philips CM100 at 80 kV.

514

515 *Confocal image acquisition*

516 Confocal z-stacks were collected using a A1R MP+ confocal scanhead mounted on an Ni-E
517 upright microscope (Nikon) using a 16 \times water dipping objective (NA 0.8) for live imaging or 40 \times
518 oil immersion objective (NA 1.3) for fixed image acquisition. Images acquired in resonant scanning
519 mode were post-processed using the denoise.ai function in NIS-Elements (Nikon). For live
520 imaging, zebrafish were anesthetized in a solution of 0.006-0.012% MS-222 in system water for
521 5 min. Anesthetized fish were mounted in a custom imaging chamber, partially embedded in 1%
522 agarose and covered with tricaine solution. For Supplemental Video 1, a FLUOVIEW FV3000

523 scanning confocal microscope (Olympus) equipped with a 100× objective (NA 1.49) was used to
524 collect a z-stack and 3D rendered with Imaris (Bitplane).

525

526 *Whole animal photoconversion*

527 Prior to imaging, *Tg(atoh1a:nls-Eos)* zebrafish were exposed to light from a UV LED flashlight
528 (McDoer) for 15 min in a reflective chamber constructed from a styrofoam box lined with aluminum
529 foil. A similar lateral region of the trunk was imaged over subsequent days identified by
530 approximate body position below the dorsal fin and relative to underlying pigment stripes.

531

532 *Regional photoconversion*

533 After anesthetization and mounting as described above, the *Tg(atoh1a:nls-Eos)* reporter was
534 photoconverted using the stimulation program of NIS-Elements with the 405 nm laser at 14-18%
535 power for 30-45 s within a 500X500 pixel ROI with an area of 67055 μm^2 . The same lateral region
536 of the trunk was imaged over subsequent days identified by body position under the dorsal fin,
537 position relative to underlying pigment stripes, and presence of photoconverted cells.

538

539 **Staining**

540

541 *Alizarin Red S Staining*

542 To visualize mineralized bone, live animals were stained for 15 min in a solution of 0.01% (wt/vol)
543 Alizarin Red S dissolved in system water, and subsequently rinsed 3×5 min in system water prior
544 to imaging as described (Bensimon-Brito et al., 2016).

545

546 *Antibody staining*

547 Zebrafish were anesthetized in a solution of 0.012% MS-222 in system water for 5 min. Using
548 metal forceps, up to 10 scales were removed from the lateral side of the trunk in the region below
549 the dorsal fin. Scales were fixed in 4% PFA/PBS at 4°C overnight. Scales were washed 4×5 min
550 in 1x PBS + 0.3% triton-X (PBST) at room temperature and then blocked for 1.5 h with PBST
551 containing 5% normal goat serum. Incubation with primary antibodies occurred at 4°C overnight,
552 followed by 4×15 min washes in PBST. Scales were incubated in appropriate secondary
553 antibodies for 2 h at room temperature and washed 4×15 min in PBST. To label nuclei, scales
554 were incubated with DAPI for 5 min at 4°C and washed in PBST 4×5 min at room temperature.
555 Scales were mounted between a microscope slide and coverslip in Prolong gold. All steps were
556 performed on a rotating platform.

557

558 *AM1-43 staining*

559 Scales were removed from adult *Tg(atoh1a:nls-Eos)* zebrafish as described above, and placed
560 into the center of a petri dish. 1 mL of L-15 media was added to the dish containing newly plucked
561 scales no longer than 2 min after the scales had been removed. 1.5 μ l of 10 mM AM1-43 was
562 added to the dish for a final concentration of 15 μ M AM1-43. Scales were incubated for 5 min in
563 this solution to allow for incorporation. Prior to confocal imaging, regional photoconversion of nls-
564 Eos was carried out as described above.

565

566 *Fluorescent in situ hybridization (FISH)*

567 *piezo2* antisense RNA was transcribed *in vitro* from a previously generated plasmid (Faucherre
568 et al., 2013) using SP6 and fluorescein-dUTP. The FISH protocol for adult zebrafish scales was
569 previously described (Lin et al., 2019). Briefly, scales from *Tg(atoh1a:Lifeact-EGFP)* adults were
570 plucked and fixed in 4% PFA overnight at 4°C then washed three times with 1x PBS + 0.1%
571 Tween-20 (PBSTw). Scales were dehydrated in sequential washes of 75% PBSTw:25% methanol
572 (MeOH), 50% PBSTw:50% MeOH, 25%PBSTw:75%MeOH, then placed in 100% MeOH at -20°C
573 overnight. Scales were rehydrated in sequential washes of 25% PBSTw:75% MeOH, 50%
574 PBSTw:50% MeOH, 75%PBSTw:25% MeOH, then washed 3x in PBSTw. Scales were treated
575 with 0.1mg/ml proteinase K for 5 min, then re-fixed in 4% PFA for 20 min. Scales were washed
576 once in PBSTw, washed once in 50% PBSTw:50% hybridization buffer, then incubated in
577 hybridization buffer for 2 h at 65°C. Scales were incubated in hybridization buffer with probe (~1
578 ng/ μ l) overnight at 65°C. Scales were sequentially washed at 65°C in 75% hybridization
579 buffer:25% 2xSSC + 0.1%Tween20 (SSCT), 50% hybridization buffer:50% 2x SSCT, 25%
580 hybridization buffer:75% 2xSSCT, followed by 3 washes at room temperature in 2x SSCT,
581 followed by 3 washes in 0.2x SSCT. Scales were then washed 3x in 1x PBS + 0.2% Triton X-100
582 (PBSTr), then blocked for 2 h in PBSTr + 5% FBS. Scales were incubated in blocking buffer with
583 anti-fluorescein POD fragments (1:2000) overnight at 4°C. Scales were washed 6x in PBSTr,
584 followed by staining with TSA Plus Cyanine 5 (1:50 dilution) for 10 min.

585 Following FISH, scales were incubated in PBSTr + 10%NGS for 2 h at room temperature. Scales
586 were stained with an anti-GFP antibody (1:1000) in PBSTr + 10% NGS overnight at 4°C. Scales
587 were washed in PBSTr, then incubated in secondary antibodies (1:200) for 2 h at room
588 temperature. Scales were washed in PBSTr, stained with Hoechst (3.24 nM) for 10 min at room
589 temperature, washed in PBSTr, mounted under coverslips in ProLong Gold, and imaged.

590

591 **Image analysis**

592

593 *Axon contact quantification*

594 Innervation of *Tg(atoh1a:nls-Eos)*-expressing cells was scored using a custom ImageJ macro. A
595 cell was scored as innervated if an axon passed within a sphere (created using the “3D project”
596 function) centered around the nuclear center of mass that was 10% larger than the maximum
597 nuclear diameter. In some cases, the *Tg(atoh1a:nls-Eos)* reporter was photoconverted prior to
598 image acquisition as described above.

599

600 *Cell density analysis*

601 Maximum intensity projections of confocal z-stacks were converted to 8-bit images and
602 thresholded in ImageJ. Cell density was quantified using the “Analyze particles” function of
603 ImageJ. For low magnification quantification of MC cell density across the trunk of *fgf8a^{dhiD1Tg/+}*
604 and siblings, tiled images were collected that included multiple scales per region. Cell density was
605 quantified as described above using ImageJ. For high magnification cell density quantification in
606 the epidermis directly above scales, a small region centered in the epidermis of each full scale in
607 view and positioned based on scale lobe was quantified. For scales with multiple lobes, a density
608 measurement was collected from the center of each lobe and averaged.

609

610 *Statistical analysis*

611 Statistical tests used are listed in individual figure legends. Plots were created using R or Python.

612

613 **ACKNOWLEDGEMENTS**

614

615 We thank the LSB Aquatics staff for animal care; Wai Pang Chan and Marianne Cilluffo for TEM
616 support; the labs of Ajay Dhaka, Jacqueline Lees, and Alvaro Sagasti for sharing zebrafish stocks;
617 the lab of Chris Joplin for sharing the *piezo2* plasmid. The authors are grateful to all members of
618 the Rasmussen lab for discussion, technical assistance, and support.

619

620

621 **CONFLICT OF INTERESTS**

622 The authors declare that they have no conflict of interest.

623

624

625 **FUNDING**

626

627 This work was funded in part by a Postdoctoral Fellowship (#2011008) from the National Science
628 Foundation to TLB, a Graduate Research Fellowship (DGE-2140004) from the National Science
629 Foundation to EWC, R01HD107108 from the Eunice Kennedy Shriver National Institute of Child
630 Health and Human Development to JPR, A153025 from the University of Washington Research
631 Royalty Fund to JPR, and a New Investigator Award from the University of Washington/Fred
632 Hutchinson Cancer Research Center Cancer Consortium, which is supported by the NIH/NCI
633 Cancer Center Support Grant P30 CA015704, to JPR. JPR is a Washington Research Foundation
634 Distinguished Investigator.

635 **REFERENCES**

- 636
- 637 Aman AJ, Fulbright AN, Parichy DM. 2018. Wnt/ β -catenin regulates an ancient signaling network
638 during zebrafish scale development. *eLife* **7**. doi:10.7554/eLife.37001
- 639 Amsterdam A, Lai K, Komisarczuk AZ, Becker TS, Bronson RT, Hopkins N, Lees JA. 2009.
640 Zebrafish Hagoromo mutants up-regulate fgf8 postembryonically and develop
641 neuroblastoma. *Mol Cancer Res MCR* **7**:841–850. doi:10.1158/1541-7786.MCR-08-0555
- 642 Ashley PJ, Sneddon LU, McCrohan CR. 2007. Nociception in fish: stimulus-response properties
643 of receptors on the head of trout *Oncorhynchus mykiss*. *Brain Res* **1166**:47–54.
644 doi:10.1016/j.brainres.2007.07.011
- 645 Ashley PJ, Sneddon LU, McCrohan CR. 2006. Properties of corneal receptors in a teleost fish.
646 *Neurosci Lett* **410**:165–168. doi:10.1016/j.neulet.2006.08.047
- 647 Bai L, Lehnert BP, Liu J, Neubarth NL, Dickendeshler TL, Nwe PH, Cassidy C, Woodbury CJ,
648 Ginty DD. 2015. Genetic Identification of an Expansive Mechanoreceptor Sensitive to Skin
649 Stroking. *Cell* **163**:1783–1795. doi:10.1016/j.cell.2015.11.060
- 650 Bakkers J, Hild M, Kramer C, Furutani-Seiki M, Hammerschmidt M. 2002. Zebrafish DeltaNp63
651 is a direct target of Bmp signaling and encodes a transcriptional repressor blocking neural
652 specification in the ventral ectoderm. *Dev Cell* **2**:617–627. doi:10.1016/s1534-
653 5807(02)00163-6
- 654 Bardot ES, Valdes VJ, Zhang J, Perdigoto CN, Nicolis S, Hearn SA, Silva JM, Ezhkova E. 2013.
655 Polycomb subunits Ezh1 and Ezh2 regulate the Merkel cell differentiation program in skin
656 stem cells. *EMBO J* **32**:1990–2000. doi:10.1038/emboj.2013.110
- 657 Bensimon-Brito A, Cardeira J, Dionísio G, Huysseune A, Cancela ML, Witten PE. 2016. Revisiting
658 in vivo staining with alizarin red S—a valuable approach to analyse zebrafish skeletal
659 mineralization during development and regeneration. *BMC Dev Biol* **16**:2.
660 doi:10.1186/s12861-016-0102-4
- 661 Biggs LC, Mikkola ML. 2014. Early inductive events in ectodermal appendage morphogenesis.
662 *Semin Cell Dev Biol* **25–26**:11–21. doi:10.1016/j.semcdb.2014.01.007
- 663 Boot PM, Rowden G, Walsh N. 1992. The distribution of Merkel cells in human fetal and adult
664 skin. *Am J Dermatopathol* **14**:391–396. doi:10.1097/00000372-199210000-00003
- 665 Buckley K, Kelly RB. 1985. Identification of a transmembrane glycoprotein specific for secretory
666 vesicles of neural and endocrine cells. *J Cell Biol* **100**:1284–1294.
667 doi:10.1083/jcb.100.4.1284
- 668 Chang W, Gu JG. 2020. Effects on tactile transmission by serotonin transporter inhibitors at
669 Merkel discs of mouse whisker hair follicles. *Mol Pain* **16**:1744806920938237.
670 doi:10.1177/1744806920938237
- 671 Chang W, Kanda H, Ikeda R, Ling J, DeBerry JJ, Gu JG. 2016. Merkel disc is a serotonergic
672 synapse in the epidermis for transmitting tactile signals in mammals. *Proc Natl Acad Sci*
673 *U S A* **113**:E5491-5500. doi:10.1073/pnas.1610176113
- 674 Chaplin N, Tendeng C, Wingate RJT. 2010. Absence of an external germinal layer in zebrafish
675 and shark reveals a distinct, anamniote ground plan of cerebellum development. *J*
676 *Neurosci Off J Soc Neurosci* **30**:3048–3057. doi:10.1523/JNEUROSCI.6201-09.2010
- 677 Coccimiglio ML, Jonz MG. 2012. Serotonergic neuroepithelial cells of the skin in developing
678 zebrafish: morphology, innervation and oxygen-sensitive properties. *J Exp Biol* **215**:3881–
679 3894. doi:10.1242/jeb.074575
- 680 Corniani G, Saal HP. 2020. Tactile innervation densities across the whole body. *J Neurophysiol*
681 **124**:1229–1240. doi:10.1152/jn.00313.2020
- 682 Cruz IA, Kappedal R, Mackenzie SM, Hailey DW, Hoffman TL, Schilling TF, Raible DW. 2015.
683 Robust regeneration of adult zebrafish lateral line hair cells reflects continued precursor
684 pool maintenance. *Dev Biol* **402**:229–38. doi:10.1016/j.ydbio.2015.03.019
- 685 Daane JM, Rohner N, Konstantinidis P, Djuranovic S, Harris MP. 2016. Parallelism and Epistasis

- 686 in Skeletal Evolution Identified through Use of Phylogenomic Mapping Strategies. *Mol Biol*
687 *Evol* **33**:162–173. doi:10.1093/molbev/msv208
- 688 De Simone A, Evanitsky MN, Hayden L, Cox BD, Wang J, Tornini VA, Ou J, Chao A, Poss KD,
689 Di Talia S. 2021. Control of osteoblast regeneration by a train of Erk activity waves. *Nature*
690 **590**:129–133. doi:10.1038/s41586-020-03085-8
- 691 English KB, Wang Z-Z, Stayner N, Stensaas LJ, Martin H, Tuckett RP. 1992. Serotonin-like
692 immunoreactivity in Merkel cells and their afferent neurons in touch domes from the hairy
693 skin of rats. *Anat Rec* **232**:112–120. doi:10.1002/ar.1092320112
- 694 Faucherre A, Nargeot J, Mangoni ME, Jopling C. 2013. piezo2b regulates vertebrate light touch
695 response. *J Neurosci Off J Soc Neurosci* **33**:17089–17094.
696 doi:10.1523/JNEUROSCI.0522-13.2013
- 697 Felker A, Nieuwenhuize S, Dolbois A, Blazkova K, Hess C, Low LWL, Burger S, Samson N,
698 Carney TJ, Bartunek P, Nevado C, Mosimann C. 2016. In vivo performance and properties
699 of tamoxifen metabolites for CreERT2 control. *PloS One* **11**:e0152989.
700 doi:10.1371/journal.pone.0152989
- 701 Fox H, Lane E, Whitear M. 1980. Sensory nerve endings and receptors in fish and amphibians
702 In: Spearman R, Riley P, editors. The Skin of Vertebrates, Linnean Society Symposium
703 Series. Academic Press. pp. 271–281.
- 704 Fradette J, Godbout MJ, Michel M, Germain L. 1995. Localization of Merkel cells at hairless and
705 hairy human skin sites using keratin 18. *Biochem Cell Biol Biochim Biol Cell* **73**:635–639.
706 doi:10.1139/o95-070
- 707 García-Caballero T, Gallego R, Rosón E, Basanta D, Morel G, Beiras A. 1989. Localization of
708 serotonin-like immunoreactivity in the Merkel cells of pig snout skin. *Anat Rec* **225**:267–
709 271. doi:10.1002/ar.1092250402
- 710 Gau P, Curtright A, Condon L, Raible DW, Dhaka A. 2017. An ancient neurotrophin receptor code;
711 a single Runx/Cbfb complex determines somatosensory neuron fate specification in
712 zebrafish. *PLoS Genet* **13**:e1006884. doi:10.1371/journal.pgen.1006884
- 713 Gau P, Poon J, Ufret-Vincenty C, Snelson CD, Gordon SE, Raible DW, Dhaka A. 2013. The
714 zebrafish ortholog of TRPV1 is required for heat-induced locomotion. *J Neurosci* **33**:5249–
715 5260. doi:10.1523/JNEUROSCI.5403-12.2013
- 716 Grim M, Halata Z. 2000. Developmental origin of avian Merkel cells. *Anat Embryol (Berl)* **202**:401–
717 10. doi:10.1007/s004290000121
- 718 Handler A, Ginty DD. 2021. The mechanosensory neurons of touch and their mechanisms of
719 activation. *Nat Rev Neurosci* **22**:521–537. doi:10.1038/s41583-021-00489-x
- 720 Harris MP, Rohner N, Schwarz H, Perathoner S, Konstantinidis P, Nüsslein-Volhard C. 2008.
721 Zebrafish eda and edar mutants reveal conserved and ancestral roles of ectodysplasin
722 signaling in vertebrates. *PLoS Genet* **4**:e1000206. doi:10.1371/journal.pgen.1000206
- 723 Hartschuh W, Weihe E. 1980. Fine structural analysis of the synaptic junction of Merkel cell-axon-
724 complexes. *J Invest Dermatol* **75**:159–65. doi:10.1111/1523-1747.ep12522555
- 725 Hartschuh W, Weihe E, Reinecke M. 1986. The Merkel Cell In: Bereiter-Hahn J, Matoltsy AG,
726 Richards KS, editors. Biology of the Integument: 2 Vertebrates. Berlin, Heidelberg:
727 Springer Berlin Heidelberg. pp. 605–620. doi:10.1007/978-3-662-00989-5_30
- 728 Hoffman BU, Baba Y, Griffith TN, Mosharov EV, Woo S-H, Roybal DD, Karsenty G, Patapoutian
729 A, Sulzer D, Lumpkin EA. 2018. Merkel Cells Activate Sensory Neural Pathways through
730 Adrenergic Synapses. *Neuron* **100**:1401-1413.e6. doi:10.1016/j.neuron.2018.10.034
- 731 Iggo A, Muir AR. 1969. The structure and function of a slowly adapting touch corpuscle in hairy
732 skin. *J Physiol* **200**:763–796. doi:10.1113/jphysiol.1969.sp008721
- 733 Ikeda R, Cha M, Ling J, Jia Z, Coyle D, Gu JG. 2014. Merkel cells transduce and encode tactile
734 stimuli to drive Aβ-afferent impulses. *Cell* **157**:664–675. doi:10.1016/j.cell.2014.02.026
- 735 Jenkins BA, Fontecilla NM, Lu CP, Fuchs E, Lumpkin EA. 2019. The cellular basis of
736 mechanosensory Merkel-cell innervation during development. *eLife* **8**.

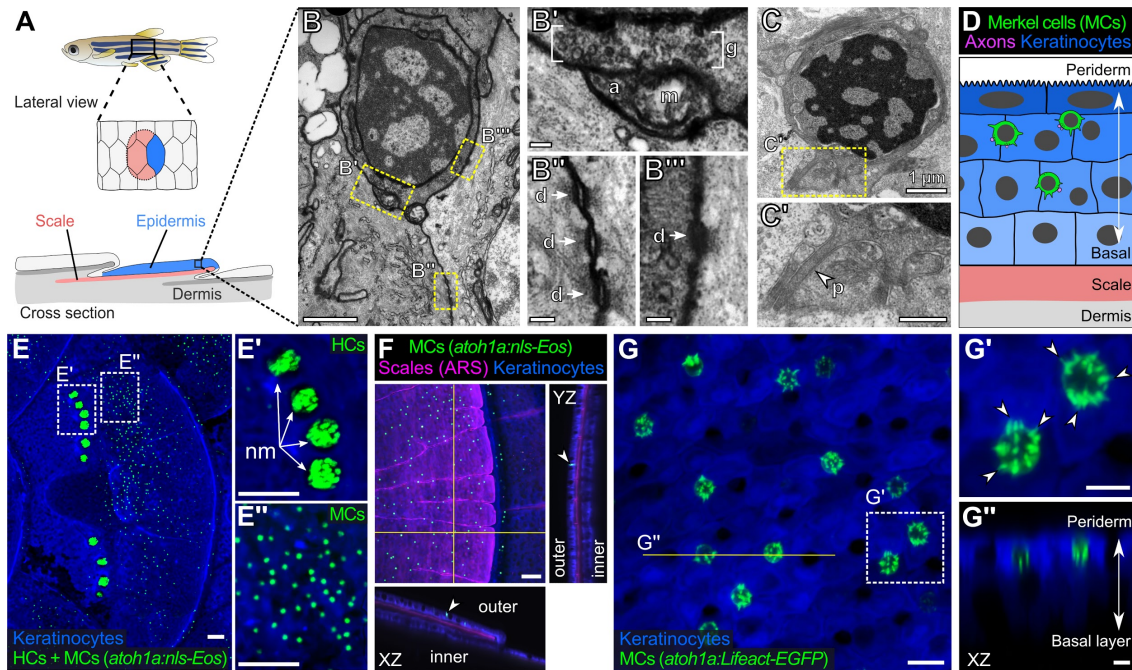
- 737 doi:10.7554/eLife.42633
738 Jenkins BA, Lumpkin EA. 2017. Developing a sense of touch. *Dev Camb Engl* **144**:4078–4090.
739 doi:10.1242/dev.120402
740 Kague E, Gallagher M, Burke S, Parsons M, Franz-Odenaal T, Fisher S. 2012. Skeletogenic
741 fate of zebrafish cranial and trunk neural crest. *PLoS One* **7**:e47394.
742 doi:10.1371/journal.pone.0047394
743 Kani S, Bae Y-K, Shimizu T, Tanabe K, Satou C, Parsons MJ, Scott E, Higashijima S, Hibi M.
744 2010. Proneural gene-linked neurogenesis in zebrafish cerebellum. *Dev Biol* **343**:1–17.
745 doi:10.1016/j.ydbio.2010.03.024
746 Katz HR, Menelaou E, Hale ME. 2021. Morphological and physiological properties of Rohon-
747 Beard neurons along the zebrafish spinal cord. *J Comp Neurol* **529**:1499–1515.
748 doi:10.1002/cne.25033
749 Kawakami K, Amsterdam A, Shimoda N, Becker T, Mugg J, Shima A, Hopkins N. 2000. Proviral
750 insertions in the zebrafish hagaromo gene, encoding an F-box/WD40-repeat protein,
751 cause stripe pattern anomalies. *Curr Biol CB* **10**:463–466. doi:10.1016/s0960-
752 9822(00)00444-9
753 Kesavan G, Hammer J, Hans S, Brand M. 2018. Targeted knock-in of CreER T2 in zebrafish
754 using CRISPR/Cas9. *Cell Tissue Res* **372**:41–50. doi:10.1007/s00441-018-2798-x
755 Kimura Y, Hisano Y, Kawahara A, Higashijima S. 2014. Efficient generation of knock-in transgenic
756 zebrafish carrying reporter/driver genes by CRISPR/Cas9-mediated genome engineering.
757 *Sci Rep* **4**:6545. doi:10.1038/srep06545
758 Kobayashi I, Kobayashi-Sun J, Kim AD, Pouget C, Fujita N, Suda T, Traver D. 2014. Jam1a-
759 Jam2a interactions regulate haematopoietic stem cell fate through Notch signalling.
760 *Nature* **512**:319–23. doi:10.1038/nature13623
761 Kucenas S, Soto F, Cox JA, Voigt MM. 2006. Selective labeling of central and peripheral sensory
762 neurons in the developing zebrafish using P2X(3) receptor subunit transgenes.
763 *Neuroscience* **138**:641–652. doi:10.1016/j.neuroscience.2005.11.058
764 Lacour JP, Dubois D, Pisani A, Ortonne JP. 1991. Anatomical mapping of Merkel cells in normal
765 human adult epidermis. *Br J Dermatol* **125**:535–42. doi:10.1111/j.1365-
766 2133.1991.tb14790.x
767 Lane EB, Whitear M. 1977. On the occurrence of Merkel cells in the epidermis of teleost fishes.
768 *Cell Tissue Res* **182**:235–46. doi:10.1007/BF00220592
769 Lee H, Kimelman D. 2002. A dominant-negative form of p63 is required for epidermal proliferation
770 in zebrafish. *Dev Cell* **2**:607–616. doi:10.1016/s1534-5807(02)00166-1
771 Lee RTH, Asharani PV, Carney TJ. 2014. Basal keratinocytes contribute to all strata of the adult
772 zebrafish epidermis. *PLoS One* **9**:e84858. doi:10.1371/journal.pone.0084858
773 Li L, Rutlin M, Abaira VE, Cassidy C, Kus L, Gong S, Jankowski MP, Luo W, Heintz N, Koerber
774 HR, Woodbury CJ, Ginty DD. 2011. The functional organization of cutaneous low-
775 threshold mechanosensory neurons. *Cell* **147**:1615–1627. doi:10.1016/j.cell.2011.11.027
776 Lin X, Zhou Q, Zhao C, Lin G, Xu J, Wen Z. 2019. An Ectoderm-Derived Myeloid-like Cell
777 Population Functions as Antigen Transporters for Langerhans Cells in Zebrafish
778 Epidermis. *Dev Cell* **49**:605-617.e5. doi:10.1016/j.devcel.2019.03.028
779 Luo W, Enomoto H, Rice FL, Milbrandt J, Ginty DD. 2009. Molecular identification of rapidly
780 adapting mechanoreceptors and their developmental dependence on ret signaling.
781 *Neuron* **64**:841–856. doi:10.1016/j.neuron.2009.11.003
782 Maksimovic S, Nakatani M, Baba Y, Nelson AM, Marshall KL, Wellnitz SA, Firozi P, Woo S-H,
783 Ranade S, Patapoutian A, Lumpkin EA. 2014. Epidermal Merkel cells are
784 mechanosensory cells that tune mammalian touch receptors. *Nature* **509**:617–621.
785 doi:10.1038/nature13250
786 Maricich SM, Morrison KM, Mathes EL, Brewer BM. 2012. Rodents rely on Merkel cells for texture
787 discrimination tasks. *J Neurosci Off J Soc Neurosci* **32**:3296–300.

- 788 doi:10.1523/JNEUROSCI.5307-11.2012
- 789 Maricich SM, Wellnitz SA, Nelson AM, Lesniak DR, Gerling GJ, Lumpkin EA, Zoghbi HY. 2009.
- 790 Merkel cells are essential for light-touch responses. *Science* **324**:1580–2.
- 791 doi:10.1126/science.1172890
- 792 Marshall KL, Clary RC, Baba Y, Orlowsky RL, Gerling GJ, Lumpkin EA. 2016. Touch receptors
- 793 undergo rapid remodeling in healthy skin. *Cell Rep* **17**:1719–1727.
- 794 doi:10.1016/j.celrep.2016.10.034
- 795 Metcalfe WK, Myers PZ, Trevarrow B, Bass MB, Kimmel CB. 1990. Primary neurons that express
- 796 the L2/HNK-1 carbohydrate during early development in the zebrafish. *Dev Camb Engl*
- 797 **110**:491–504.
- 798 Meyers JR, MacDonald RB, Duggan A, Lenzi D, Standaert DG, Corwin JT, Corey DP. 2003.
- 799 Lighting up the senses: FM1-43 loading of sensory cells through nonselective ion
- 800 channels. *J Neurosci Off J Soc Neurosci* **23**:4054–4065.
- 801 Mihara M, Hashimoto K, Ueda K, Kumakiri M. 1979. The specialized junctions between Merkel
- 802 cell and neurite: an electron microscopic study. *J Invest Dermatol* **73**:325–34.
- 803 doi:10.1111/1523-1747.ep12550322
- 804 Moayed Y, Michlig S, Park M, Koch A, Lumpkin EA. 2021. Somatosensory innervation of healthy
- 805 human oral tissues. *J Comp Neurol* **529**:3046–3061. doi:10.1002/cne.25148
- 806 Moll I, Paus R, Moll R. 1996. Merkel cells in mouse skin: intermediate filament pattern,
- 807 localization, and hair cycle-dependent density. *J Invest Dermatol* **106**:281–286.
- 808 doi:10.1111/1523-1747.ep12340714
- 809 Morrison KM, Miesegaes GR, Lumpkin EA, Maricich SM. 2009. Mammalian Merkel cells are
- 810 descended from the epidermal lineage. *Dev Biol* **336**:76–83.
- 811 doi:10.1016/j.ydbio.2009.09.032
- 812 Nakafusa J, Narisawa Y, Shinogi T, Taira K, Tanaka T, Inoue T, Misago N. 2006. Changes in the
- 813 number of Merkel cells with the hair cycle in hair discs on rat back skin. *Br J Dermatol*
- 814 **155**:883–889. doi:10.1111/j.1365-2133.2006.07441.x
- 815 Nguyen MB, Cohen I, Kumar V, Xu Z, Bar C, Dauber-Decker KL, Tsai P-C, Marangoni P, Klein
- 816 OD, Hsu Y-C, Chen T, Mikkola ML, Ezhkova E. 2018. FGF signalling controls the
- 817 specification of hair placode-derived SOX9 positive progenitors to Merkel cells. *Nat*
- 818 *Commun* **9**:2333. doi:10.1038/s41467-018-04399-y
- 819 Nguyen MB, Valdes VJ, Cohen I, Pothula V, Zhao D, Zheng D, Ezhkova E. 2019. Dissection of
- 820 Merkel cell formation in hairy and glabrous skin reveals a common requirement for
- 821 FGFR2-mediated signalling. *Exp Dermatol* **28**:374–382. doi:10.1111/exd.13901
- 822 Orefice LL. 2020. Peripheral Somatosensory Neuron Dysfunction: Emerging Roles in Autism
- 823 Spectrum Disorders. *Neuroscience* **445**:120–129.
- 824 doi:10.1016/j.neuroscience.2020.01.039
- 825 Oss-Ronen L, Cohen I. 2021. Epigenetic regulation and signalling pathways in Merkel cell
- 826 development. *Exp Dermatol* **30**:1051–1064. doi:10.1111/exd.14415
- 827 Ostrowski SM, Wright MC, Bolock AM, Geng X, Maricich SM. 2015. Ectopic Atoh1 expression
- 828 drives Merkel cell production in embryonic, postnatal and adult mouse epidermis. *Dev*
- 829 *Camb Engl* **142**:2533–2544. doi:10.1242/dev.123141
- 830 Palanca AMS, Lee S-L, Yee LE, Joe-Wong C, Trinh LA, Hiroyasu E, Husain M, Fraser SE,
- 831 Pellegrini M, Sagasti A. 2013. New transgenic reporters identify somatosensory neuron
- 832 subtypes in larval zebrafish. *Dev Neurobiol* **73**:152–167. doi:10.1002/dneu.22049
- 833 Pan YA, Choy M, Prober DA, Schier AF. 2012. Robo2 determines subtype-specific axonal
- 834 projections of trigeminal sensory neurons. *Development* **139**:591–600.
- 835 doi:10.1242/dev.076588
- 836 Parichy DM, Elizondo MR, Mills MG, Gordon TN, Engeszer RE. 2009. Normal table of
- 837 postembryonic zebrafish development: staging by externally visible anatomy of the living
- 838 fish. *Dev Dyn Off Publ Am Assoc Anat* **238**:2975–3015. doi:10.1002/dvdy.22113

- 839 Patten SA, Sihra RK, Dhama KS, Coutts CA, Ali DW. 2007. Differential expression of PKC isoforms
840 in developing zebrafish. *Int J Dev Neurosci Off J Int Soc Dev Neurosci* **25**:155–64.
841 doi:10.1016/j.ijdevneu.2007.02.003
- 842 Perdigoto CN, Bardot ES, Valdes VJ, Santoriello FJ, Ezhkova E. 2014. Embryonic maturation of
843 epidermal Merkel cells is controlled by a redundant transcription factor network. *Dev Camb*
844 *Engl* **141**:4690–6. doi:10.1242/dev.112169
- 845 Pickett SB, Thomas ED, Sebe JY, Linbo T, Esterberg R, Hailey DW, Raible DW. 2018. Cumulative
846 mitochondrial activity correlates with ototoxin susceptibility in zebrafish mechanosensory
847 hair cells. *eLife* **7**. doi:10.7554/eLife.38062
- 848 Rasmussen JP, Sack GS, Martin SM, Sagasti A. 2015. Vertebrate epidermal cells are broad-
849 specificity phagocytes that clear sensory axon debris. *J Neurosci Off J Soc Neurosci*
850 **35**:559–70. doi:10.1523/JNEUROSCI.3613-14.2015
- 851 Rasmussen JP, Vo N-T, Sagasti A. 2018. Fish scales dictate the pattern of adult skin innervation
852 and vascularization. *Dev Cell* **46**:344–359.e4. doi:10.1016/j.devcel.2018.06.019
- 853 Riedl J, Crevenna AH, Kessenbrock K, Yu JH, Neukirchen D, Bista M, Bradke F, Jenne D, Holak
854 TA, Werb Z, Sixt M, Wedlich-Soldner R. 2008. Lifeact: a versatile marker to visualize F-
855 actin. *Nat Methods* **5**:605–607. doi:10.1038/nmeth.1220
- 856 Rieger S, Sagasti A. 2011. Hydrogen peroxide promotes injury-induced peripheral sensory axon
857 regeneration in the zebrafish skin. *PLoS Biol* **9**:e1000621.
858 doi:10.1371/journal.pbio.1000621
- 859 Rohner N, Bercsényi M, Orbán L, Kolanczyk ME, Linke D, Brand M, Nüsslein-Volhard C, Harris
860 MP. 2009. Duplication of *fgfr1* permits Fgf signaling to serve as a target for selection during
861 domestication. *Curr Biol CB* **19**:1642–1647. doi:10.1016/j.cub.2009.07.065
- 862 Rutlin M, Ho C-Y, Abraira VE, Cassidy C, Bai L, Woodbury CJ, Ginty DD. 2014. The cellular and
863 molecular basis of direction selectivity of A δ -LTMRs. *Cell* **159**:1640–1651.
864 doi:10.1016/j.cell.2014.11.038
- 865 Sharma N, Flaherty K, Lezgiyeva K, Wagner DE, Klein AM, Ginty DD. 2020. The emergence of
866 transcriptional identity in somatosensory neurons. *Nature* **577**:392–398.
867 doi:10.1038/s41586-019-1900-1
- 868 Singh SP, Holdway JE, Poss KD. 2012. Regeneration of amputated zebrafish fin rays from de
869 novo osteoblasts. *Dev Cell* **22**:879–86. doi:10.1016/j.devcel.2012.03.006
- 870 Sire J-Y, Allizard F, Babiari O, Bourguignon J, Quilhac A. 1997a. Scale development in zebrafish
871 (*Danio rerio*). *J Anat* **190** (Pt 4):545–561. doi:10.1046/j.1469-7580.1997.19040545.x
- 872 Sire J-Y, Quilhac A, Bourguignon J, Allizard F. 1997b. Evidence for participation of the epidermis
873 in the deposition of superficial layer of scales in zebrafish (*Danio rerio*): A SEM and TEM
874 study. *J Morphol* **231**:161–174. doi:10.1002/(SICI)1097-4687(199702)231:2<161::AID-
875 JMOR5>3.0.CO;2-H
- 876 Slatter CAB, Kanji H, Coutts CA, Ali DW. 2005. Expression of PKC in the developing zebrafish,
877 *Danio rerio*. *J Neurobiol* **62**:425–38. doi:10.1002/neu.20110
- 878 Smith, Jr KR. 1977. The haarscheibe. *J Invest Dermatol* **69**:68–74. doi:10.1111/1523-
879 1747.ep12497883
- 880 Sneddon LU. 2003. Trigeminal somatosensory innervation of the head of a teleost fish with
881 particular reference to nociception. *Brain Res* **972**:44–52. doi:10.1016/s0006-
882 8993(03)02483-1
- 883 Suster ML, Abe G, Schouw A, Kawakami K. 2011. Transposon-mediated BAC transgenesis in
884 zebrafish. *Nat Protoc* **6**:1998–2021. doi:10.1038/nprot.2011.416
- 885 Thomas ED, Raible DW. 2019. Distinct progenitor populations mediate regeneration in the
886 zebrafish lateral line. *eLife* **8**:e43736. doi:10.7554/eLife.43736
- 887 Toyoshima K, Seta Y, Takeda S, Harada H. 1998. Identification of Merkel cells by an antibody to
888 villin. *J Histochem Cytochem Off J Histochem Soc* **46**:1329–34.
889 doi:10.1177/002215549804601113

- 890 Trinh LA, Hochgreb T, Graham M, Wu D, Ruf-Zamojski F, Jayasena CS, Saxena A, Hawk R,
891 Gonzalez-Serricchio A, Dixson A, Chow E, Gonzales C, Leung H-Y, Solomon I, Bronner-
892 Fraser M, Megason SG, Fraser SE. 2011. A versatile gene trap to visualize and interrogate
893 the function of the vertebrate proteome. *Genes Dev* **25**:2306–2320.
894 doi:10.1101/gad.174037.111
- 895 Usoskin D, Furlan A, Islam S, Abdo H, Lönnerberg P, Lou D, Hjerling-Leffler J, Haeggström J,
896 Kharchenko O, Kharchenko PV, Linnarsson S, Ernfors P. 2015. Unbiased classification of
897 sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* **18**:145–
898 153. doi:10.1038/nn.3881
- 899 Van Keymeulen A, Mascré G, Youseff KK, Harel I, Michaux C, De Geest N, Szpalski C, Achouri
900 Y, Bloch W, Hassan BA, Blanpain C. 2009. Epidermal progenitors give rise to Merkel cells
901 during embryonic development and adult homeostasis. *J Cell Biol* **187**:91–100.
902 doi:10.1083/jcb.200907080
- 903 Vielkind U, Sebzda MK, Gibson IR, Hardy MH. 1995. Dynamics of Merkel cell patterns in
904 developing hair follicles in the dorsal skin of mice, demonstrated by a monoclonal antibody
905 to mouse keratin 8. *Acta Anat (Basel)* **152**:93–109. doi:10.1159/000147688
- 906 Whitear M. 1989. Merkel cells in lower vertebrates. *Arch Histol Cytol* **52 Suppl**:415–22.
- 907 Whitear M, Lane EB. 1981. Fine structure of Merkel cells in lampreys. *Cell Tissue Res* **220**:139–
908 51. doi:10.1007/BF00209973
- 909 Woo S-H, Ranade S, Weyer AD, Dubin AE, Baba Y, Qiu Z, Petrus M, Miyamoto T, Reddy K,
910 Lumpkin EA, Stucky CL, Patapoutian A. 2014. Piezo2 is required for Merkel-cell
911 mechanotransduction. *Nature* **509**:622–626. doi:10.1038/nature13251
- 912 Wright MC, Logan GJ, Bolock AM, Kubicki AC, Hemphill JA, Sanders TA, Maricich SM. 2017.
913 Merkel cells are long-lived cells whose production is stimulated by skin injury. *Dev Biol*
914 **422**:4–13. doi:10.1016/j.ydbio.2016.12.020
- 915 Xiao Y, Thoresen DT, Miao L, Williams JS, Wang C, Atit RP, Wong SY, Brownell I. 2016. A
916 cascade of wnt, eda, and shh signaling is essential for touch dome merkel cell
917 development. *PLoS Genet* **12**:e1006150. doi:10.1371/journal.pgen.1006150
- 918 Yamashita Y, Toida K, Ogawa H. 1993. Observation of Merkel cells with scanning electron
919 microscopy. *Neurosci Lett* **159**:155–158. doi:10.1016/0304-3940(93)90822-3
- 920 Zachar PC, Jonz MG. 2012. Confocal imaging of Merkel-like basal cells in the taste buds of
921 zebrafish. *Acta Histochem* **114**:101–115. doi:10.1016/j.acthis.2011.03.006
- 922 Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J, Häring M, Braun
923 E, Borm LE, La Manno G, Codeluppi S, Furlan A, Lee K, Skene N, Harris KD, Hjerling-
924 Leffler J, Arenas E, Ernfors P, Marklund U, Linnarsson S. 2018. Molecular Architecture of
925 the Mouse Nervous System. *Cell* **174**:999-1014.e22. doi:10.1016/j.cell.2018.06.021
- 926 Zylka MJ, Rice FL, Anderson DJ. 2005. Topographically distinct epidermal nociceptive circuits
927 revealed by axonal tracers targeted to Mrgprd. *Neuron* **45**:17–25.
928 doi:10.1016/j.neuron.2004.12.015

929 FIGURES AND FIGURE LEGENDS



930

931

932

Figure 1. The adult scale epidermis contains *atoh1a*+ Merkel cells.

933

(A) Illustration of the adult zebrafish trunk anatomy showing the organization of epidermis, scales,

934

and dermis. Scales are flat bony discs arranged in an overlapping, imbricated pattern and coated

935

on their external surface by epidermis. (B) TEM of a presumptive MC from the scale epidermis.

936

Dotted boxes indicate regions of magnification in B'-B'''. (B') Magnification of B showing

937

cytoplasmic granules (g, brackets) juxtaposed to a putative axon (a) contact containing a

938

mitochondrion (m). (B'', B''') Magnifications of B showing desmosomal-like (d, arrows)

939

attachments between keratinocytes (B'') and between a presumptive MC and keratinocyte (B''').

940

(C, C') TEM of a presumptive MC from the scale epidermis showing a microvillar process (p,

941

arrowhead). (D) Illustration of a cross section of the scale epidermis based on TEM observations.

942

Superficial periderm cells (dark blue) are in the uppermost epidermal stratum and basal

943

keratinocytes (light blue) are in the lowermost epidermal stratum. MC containing cytoplasmic

944

granules, extending microvillar processes, and contacting axons localize between keratinocytes.

945

(E) Lateral confocal micrograph of the trunk epidermis in an adult expressing reporters for

946

keratinocytes (*Tg(actb2:LOXP-BFP-LOXP-DsRed)*) and *atoh1a*-expressing cells (*Tg(atoh1a:nls-*

947

Eos)). Dotted boxes indicate areas of magnification in E' and E''. (E') Magnification of E showing

948

atoh1a+ hair cells (HCs) and progenitors within neuromasts (nm) of the posterior lateral line. (E'')

949

Magnification of E showing *atoh1a*+ MCs scattered throughout the scale epidermis. (F) Lateral

950

and reconstructed cross sectional confocal micrographs of the trunk in an adult expressing

951

reporters for keratinocytes (*Tg(actb2:LOXP-BFP-LOXP-DsRed)*) and *atoh1a*-expressing cells

952

(*Tg(atoh1a:nls-Eos)*) and stained with Alizarin Red S (ARS) to label the mineralized scale matrix.

953

Note that *atoh1a*+ MCs localize to the epidermis above scales (arrowhead). (G) Lateral confocal

954

micrograph of the scale epidermis in an adult expressing reporters for keratinocytes

955

(*Tg(krt4:DsRed)*) and F-actin within *atoh1a*+ MCs (*Tg(atoh1a:Lifeact-EGFP)*). Note that all

956

atoh1a+ MCs extend multiple microvilli. (G') Magnification of G with arrowheads indicating

957

individual microvillar processes on the surface of MCs. (G'') Reconstructed cross section

958

along the yellow line in G. MCs localize to the upper epidermal strata as diagrammed in D. Note that

959

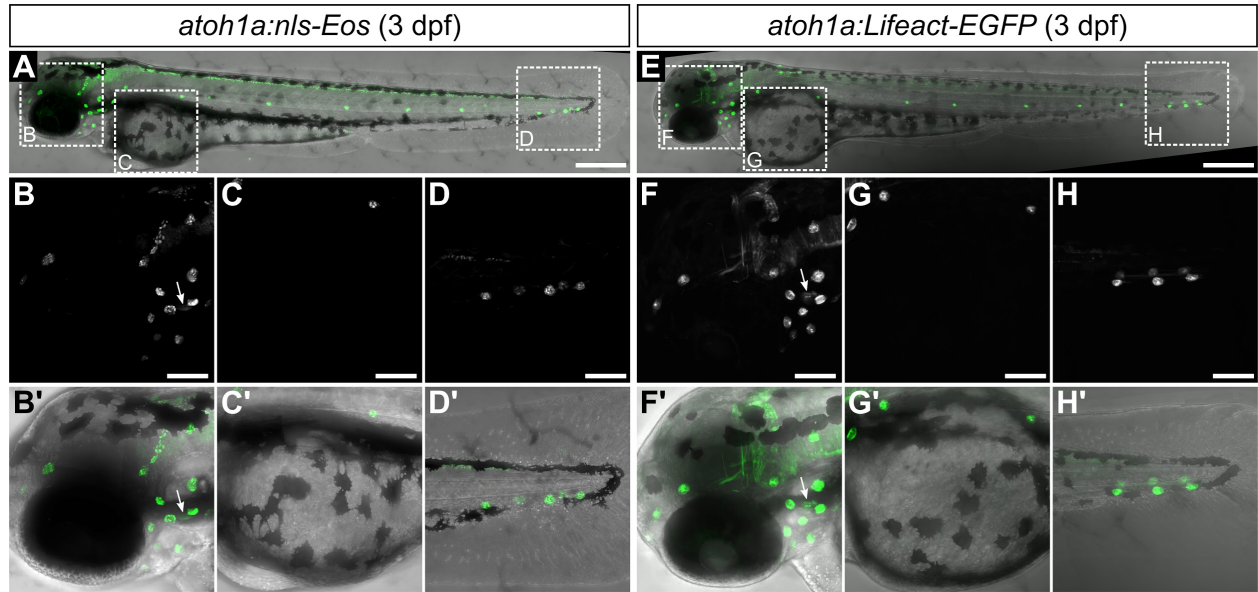
Tg(krt4:DsRed) (blue) preferentially labels keratinocytes in the upper epidermal strata, but not in

960

the basal cell layer. Scale bars: 1 μ m (B,C), 0.1 μ m (B'-B'''), 0.5 μ m (C'), 50 μ m (E-E'',F), 10 μ m

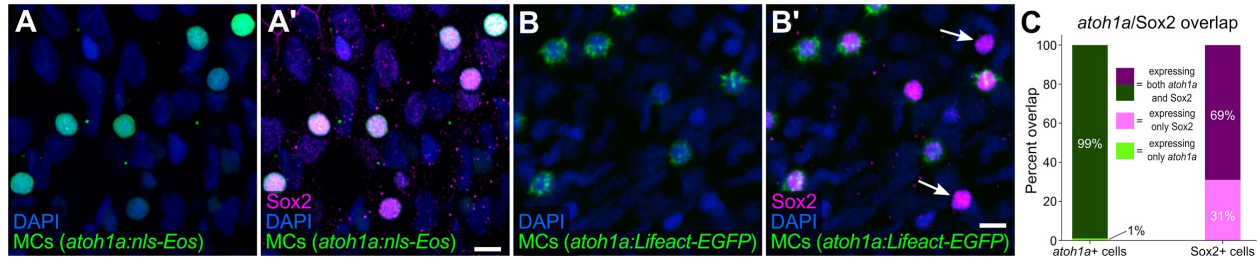
961

(G) and 5 μ m (G',G'').



962
963
964
965
966
967

Figure 1—figure supplement 1. Characterization of *atoh1a* reporter transgenes in larvae. Confocal micrographs of 3 dpf larvae expressing the indicated transgenes. Dotted boxes in A and E indicate areas of magnification for panels below. Arrows indicate expression by hair cells of the inner ear. Scale bars: 300 μ m (A,E) and 100 μ m (B-D,F-H).



968
969

970

971 **Figure 1—figure supplement 2. MCs in the adult epidermis express Sox2.**

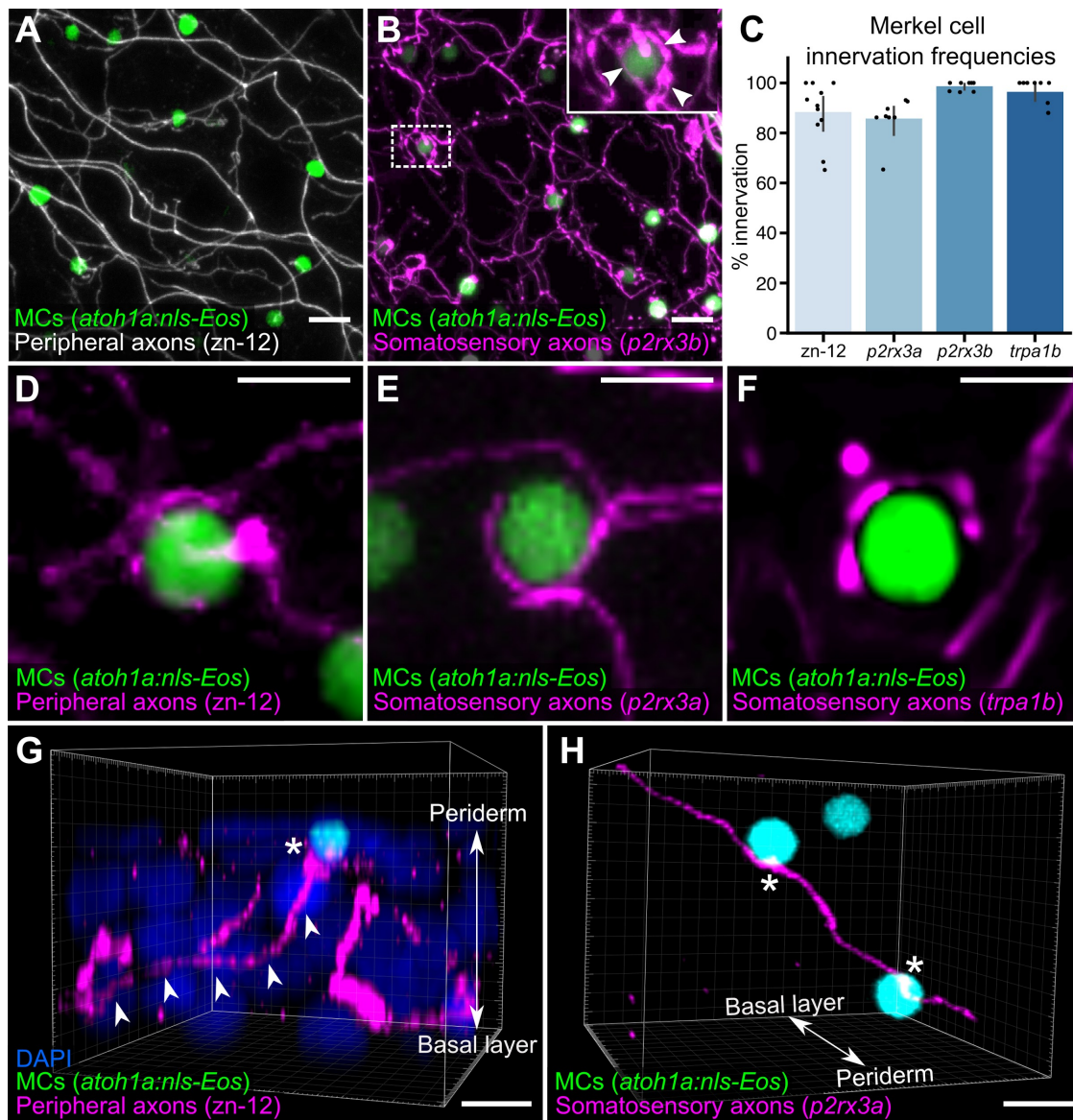
972 (A-B') Lateral confocal micrographs of the scale epidermis showing anti-Sox2 immunostaining of

973 MCs labeled by either *Tg(atoh1a:nls-Eos)* (A,A') or *Tg(atoh1a:Lifeact-EGFP)* (B,B'). Arrows

974 indicate examples of Sox2+/*atoh1a*- cells. DAPI labels epidermal nuclei. (C) Quantification of the

975 overlap between *atoh1a*+ MCs and Sox2 immunostaining. 99% of *atoh1a:nls-Eos*+ MCs

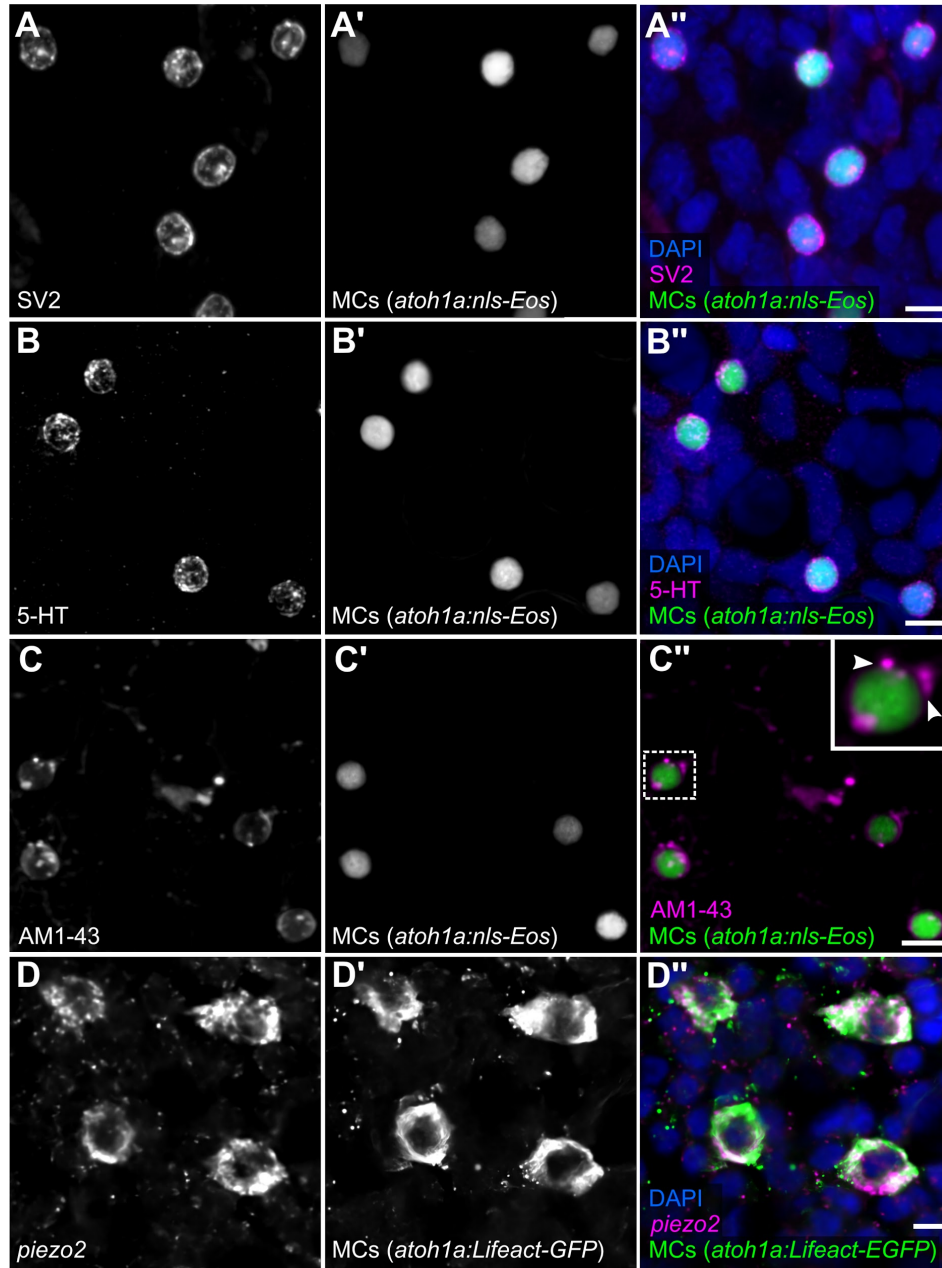
expressed Sox2 (769/774 cells from N=5 fish). Scale bars: 5 μ m.



976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992

Figure 2. Somatosensory axons innervate MCs in the adult epidermis.

(A) Lateral confocal micrograph of the scale epidermis from an adult expressing a MC reporter immunostained for peripheral axons (*zn-12*). (B) Lateral confocal micrograph of the scale epidermis showing that somatosensory peripheral axons (*Tg(p2rx3b:EGFP)*) innervate MCs. Inset of dotted region shows axonal varicosities adjacent to a MC (arrowheads). (C) Quantification of MC innervation with various axon markers. Each dot represents measurements from an individual scale. Innervation frequencies: *zn-12*, 91% (284/311 cells; *N*=3 adults); *Tg(p2rx3a>mCherry)*, 86% (196/228 cells; *N*=4 adults); *Tg(p2rx3b:EGFP)*, 99% (225/228 cells; *N*=4 adults); *Tg(trpa1b:EGFP)*, 96% (217/225 cells; *N*=9 adults). (D-F) High-magnification confocal micrographs showing examples of somatosensory axons forming extended, ring-like contacts with MCs within the scale epidermis. (G) Three-dimensional (3D) reconstruction of an axon (*zn-12* immunostaining, arrowheads) forming a bouton-like ending (asterisk) that terminates near a MC. DAPI staining labels epidermal nuclei. (H) 3D reconstruction of a single somatosensory axon (*Tg(p2rx3a>mCherry)*) that forms en passant-like contacts (asterisks) with multiple MCs. Scale bars: 10 μ m (A, B), 5 μ m (D-H).



993

994

995

996

997

998

999

1000

1001

1002

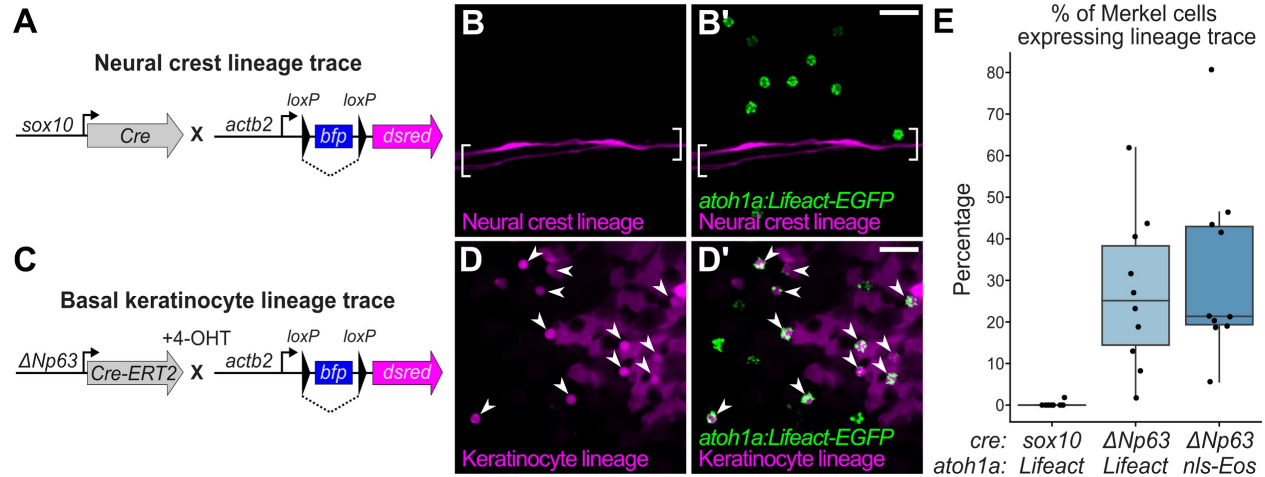
1003

1004

1005

Figure 3. MCs in the adult epidermis express neurosecretory and mechanosensory machinery.

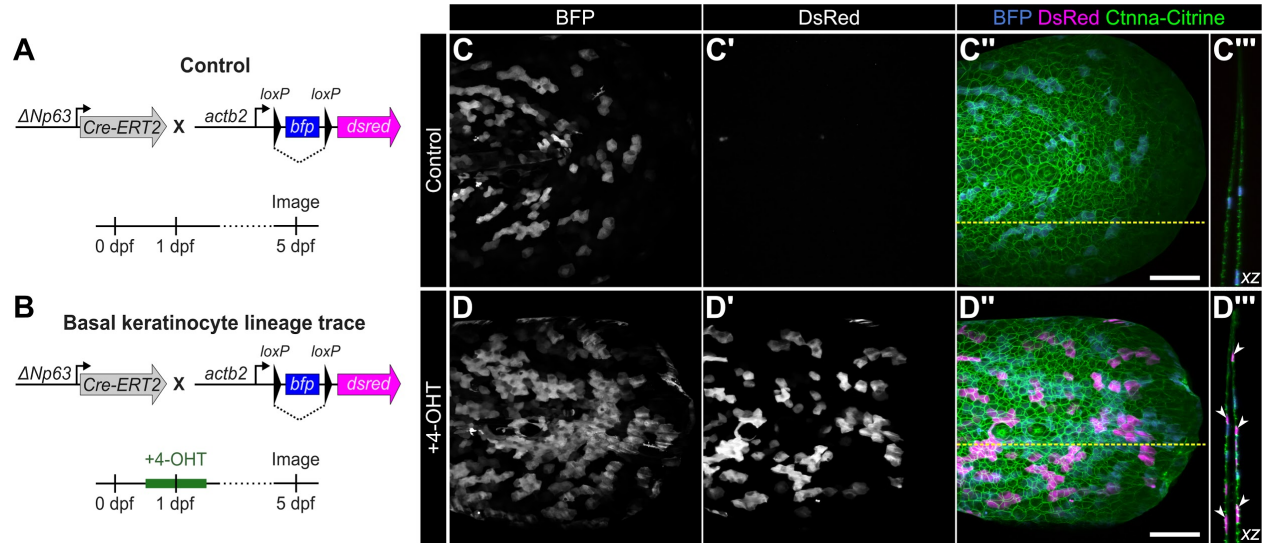
(A,B) Anti-SV2 (A-A'') or anti-5-HT (B-B'') immunostaining of the scale epidermis from an adult expressing a MC reporter. Note the punctate localization of SV2 and 5-HT staining in MCs, consistent with a vesicular localization. 96% of MCs (178/185) were SV2+. 98% of MCs (326/332) were 5-HT+. Cells analyzed from $n=3$ scales from $N=2$ adults (25-27 mm SL). DAPI labels epidermal nuclei. (C) Scale epidermis from an adult expressing a MC reporter stained with AM1-43. 98% of MCs (90/92) were AM1-43+. Cells analyzed from $n=6$ scales from $N=2$ adults. Inset of dotted region shows puncta within a MC labeled by AM1-43 (arrowheads). (D) Fluorescent in situ hybridization with an antisense probe against *piezo2*. 99% of MCs (246/248) were *piezo2*+. Cells analyzed from $n=3$ individual scales from $N=2$ adults (23-27 mm SL). Scale bars: 5 μ m.



1006
 1007
 1008
 1009
 1010
 1011
 1012
 1013
 1014
 1015
 1016
 1017
 1018
 1019
 1020
 1021

Figure 4. Merkel cells derive from the basal keratinocyte lineage.

(A) Schematic of Cre-based neural crest lineage tracing strategy. (B) Confocal micrograph of the scale epidermis in an adult expressing neural crest lineage (*Tg(sox10:Cre)*; *Tg(actb2:LOXP-BFP-LOXP-DsRed)*) and MC (*Tg(atoh1a:Lifeact-EGFP)*) reporters. Brackets denote Schwann cells associated with a nerve along a scale radius. (C) Schematic of Cre-based basal keratinocyte lineage tracing strategy. (D) Confocal micrograph of the scale epidermis in an adult expressing basal keratinocyte lineage (*TgBAC($\Delta Np63$:Cre-ERT2)*; *Tg(actb2:LOXP-BFP-LOXP-DsRed)*) and MC (*Tg(atoh1a:Lifeact-EGFP)*) reporters, which was treated with 4-OHT at 1 dpf. Arrowheads indicate MCs labeled by the basal keratinocyte lineage reporter. Note that recombination is not complete, therefore not all MCs express the lineage reporter. (E) Boxplots of the percentage of MCs expressing the lineage tracing reporters diagrammed in panels A and C. Each dot represents an individual scale. Overall percentage of MCs expressing lineage trace reporters: *sox10/Lifeact*, 0.3% (1/323 cells; N=6 adults); $\Delta Np63/Lifeact$, 29.7% (299/1005 cells; N=6 adults); $\Delta Np63/nls-Eos$, 32.3% (386/1195 cells; N=4 adults). Scale bars: 20 μ m.



1022
1023

1024

1025 **Figure 4—figure supplement 1. Validation of basal keratinocyte lineage tracing strategy.**

1026 (A,B) Schematics of the experimental design. (C-C'',D-D'') Lateral confocal micrographs of the

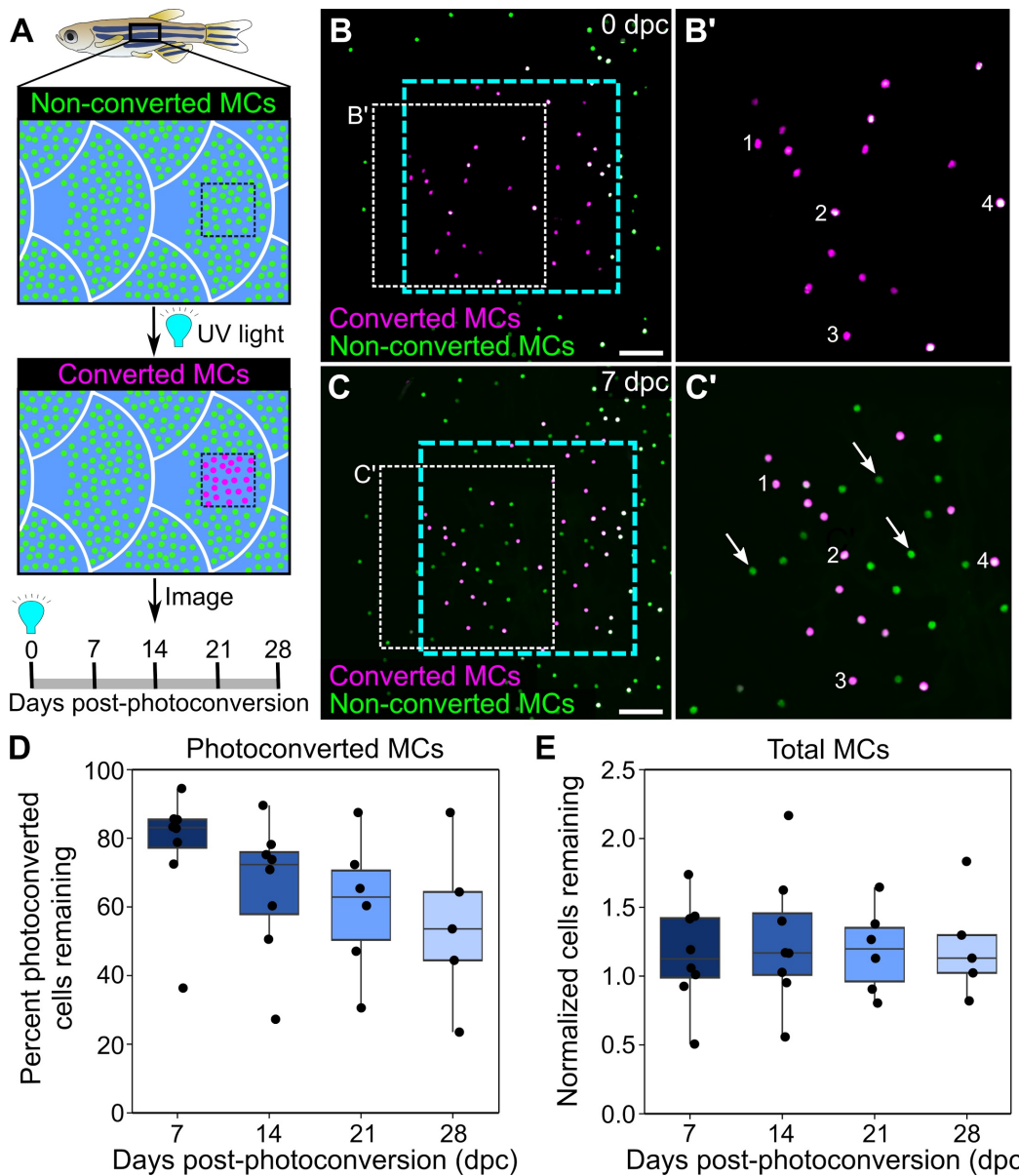
1027 caudal fin of *TgBAC(ΔNp63:Cre-ERT2); Tg(actb2:LOXP-BFP-LOXP-DsRed)* larvae treated as

1028 indicated. *Gt(Ctnna-Citrine)* labels keratinocyte membranes. (C''',D''') Reconstructed cross

1029 sections along the dashed yellow line in C'' or D''. Arrowheads indicate examples of basal

1030 keratinocytes that have undergone Cre recombination as evidenced by DsRed expression. Scale

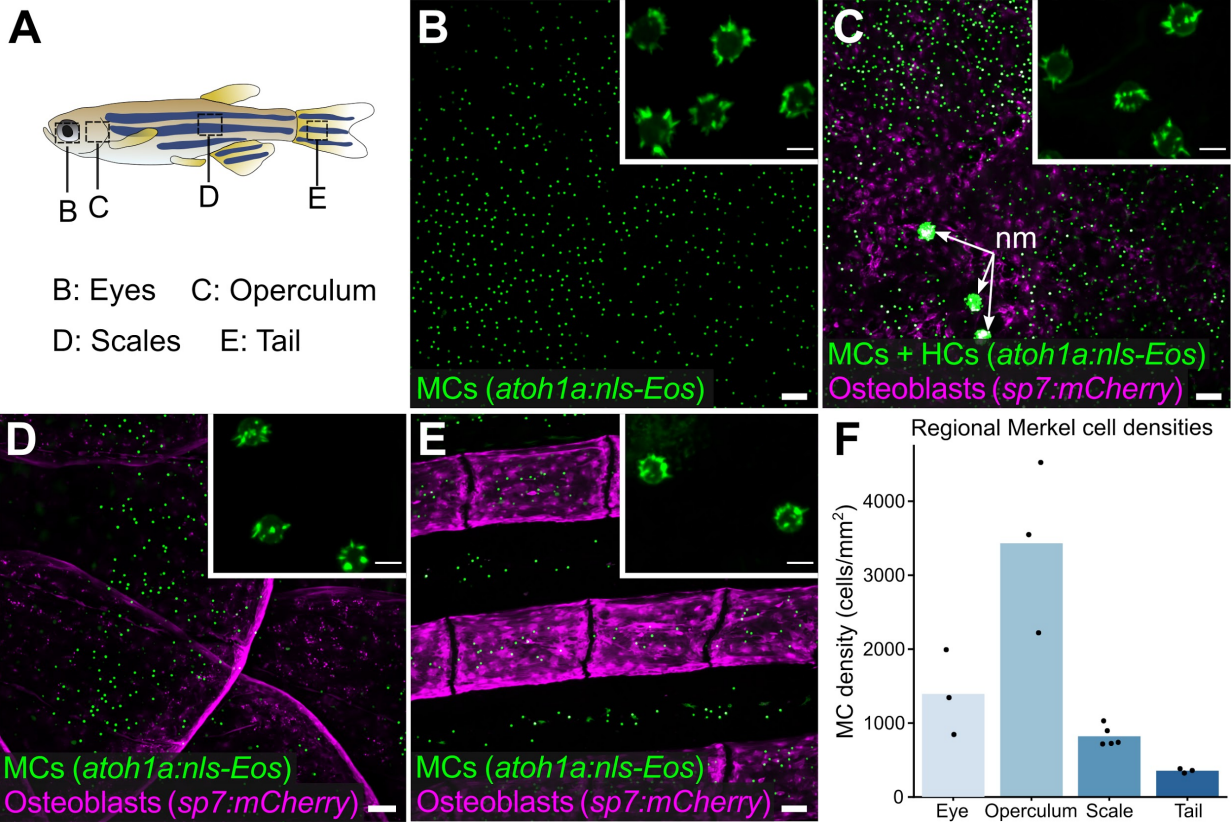
bar: 100 μm.



1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044

Figure 5. Homeostatic replacement of MCs in the adult epidermis.

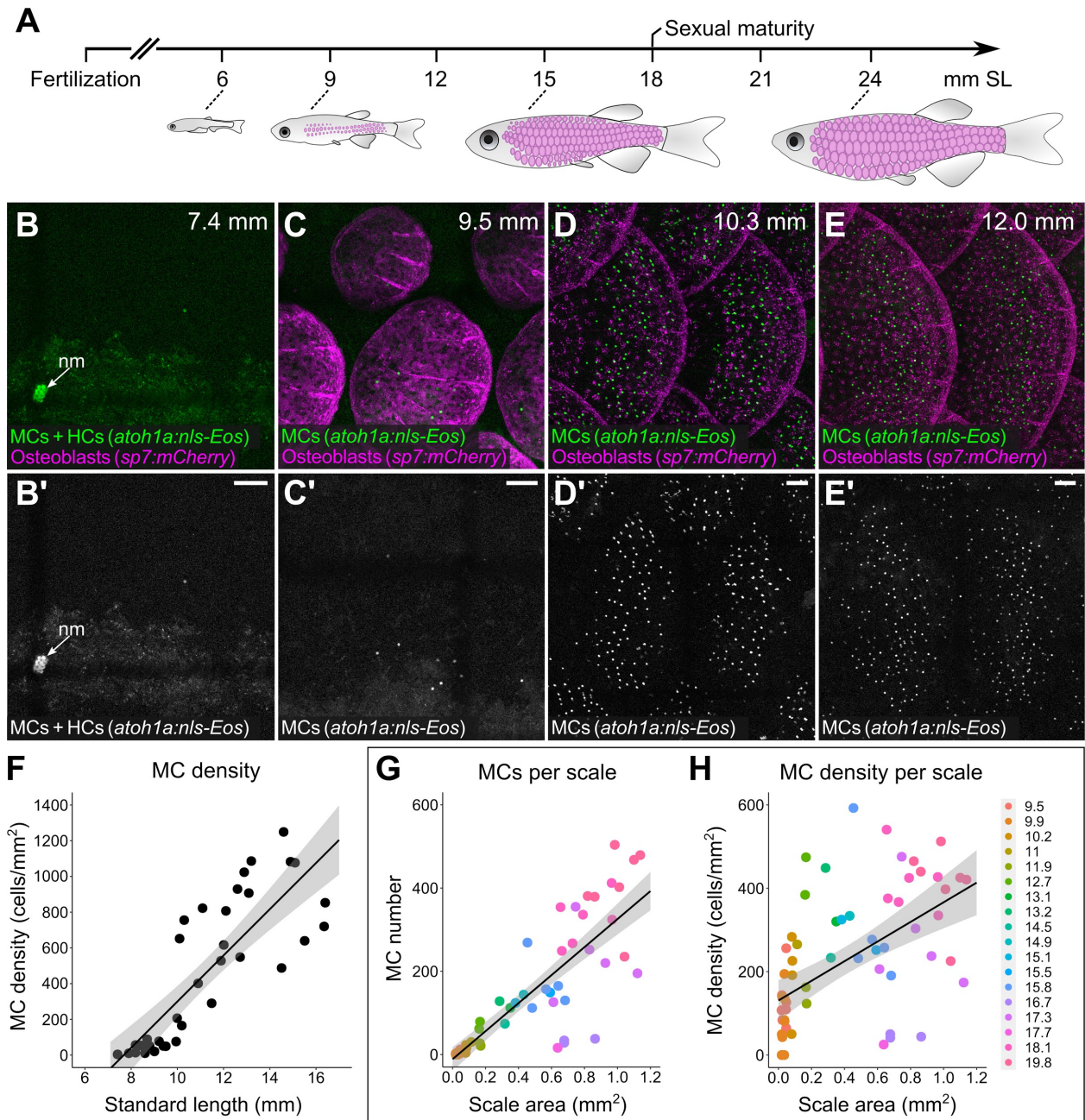
(A) Illustration of the photoconversion experiment showing the epidermis (blue), non-converted MCs (green), and converted MCs (magenta) after exposure of a region of the scale epidermis to UV light. **(B,C)** Representative images of MCs labeled by *Tg(atoh1a:nls-Eos)* at 0 (B) or 7 (C) days post-conversion (dpc) from a single adult. Cyan dotted box indicates the photoconverted region. White dotted box indicates the area magnified in B', C'. **(B',C')** Numbers label examples of individual cells present at 0 and 7 dpc. Arrows indicate examples of newly added cells, which appear green due to the presence of non-converted nls-Eos (green) and absence of converted nls-Eos (magenta). **(D)** Boxplots of the percent of photoconverted MCs remaining compared to 0 dpc. Each dot represents an individual fish. **(E)** Boxplots of the total number of MCs (converted + non-converted) present at each day compared to 0 dpc. Each dot represents an individual fish. Scale bars: 50 μ m.



1045
1046
1047
1048
1049
1050
1051
1052
1053

Figure 6. MCs are widely distributed across the skin, in compartment-specific patterns.

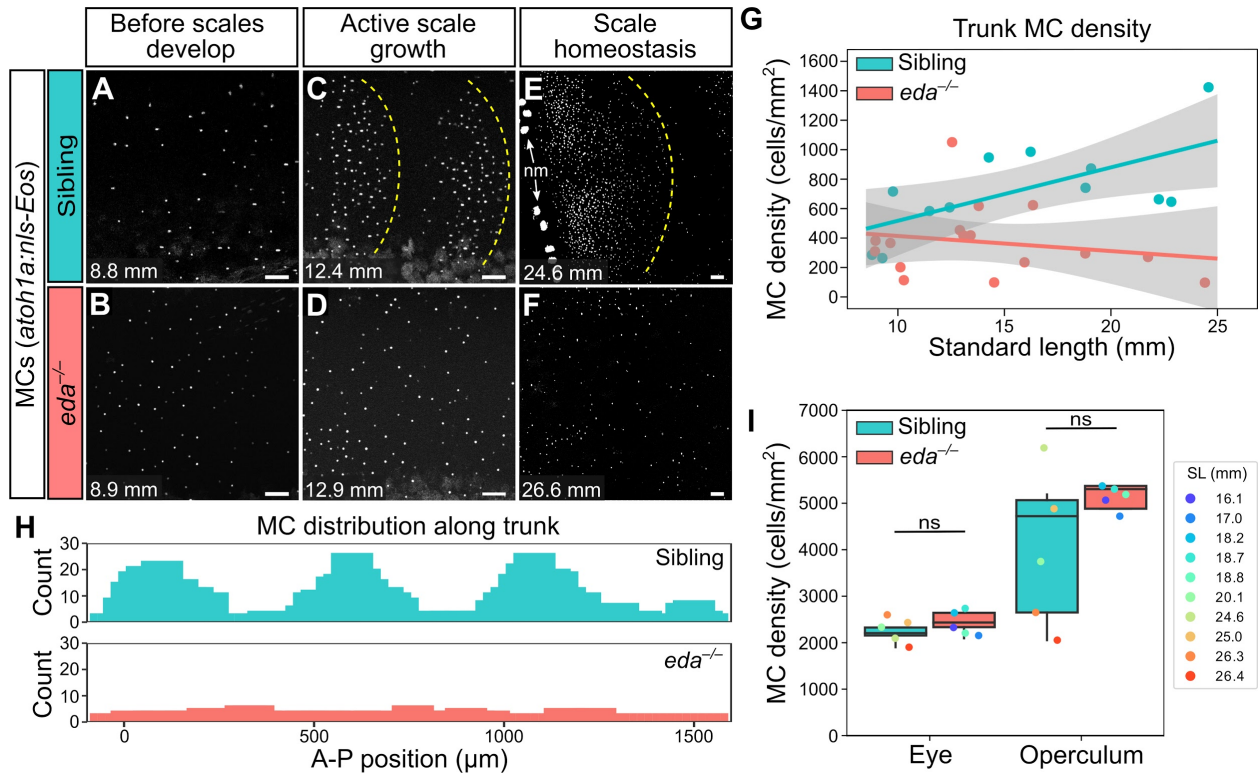
(A) Illustration indicating the regions imaged in adult zebrafish. (B-E) Lateral confocal micrographs of MCs in the regions from animals expressing the indicated reporters. The regions imaged are indicated in A. Insets show MCs expressing *Tg(atoh1a:Lifeact-EGFP)* have a similar morphology across skin compartments. nm, neuromasts of the posterior lateral line. (F) Quantification of MC densities in the specified regions. Each dot represents an individual fish (27-29 mm SL). Scale bars: 50 μ m (B-E), 5 μ m (B-E, insets).



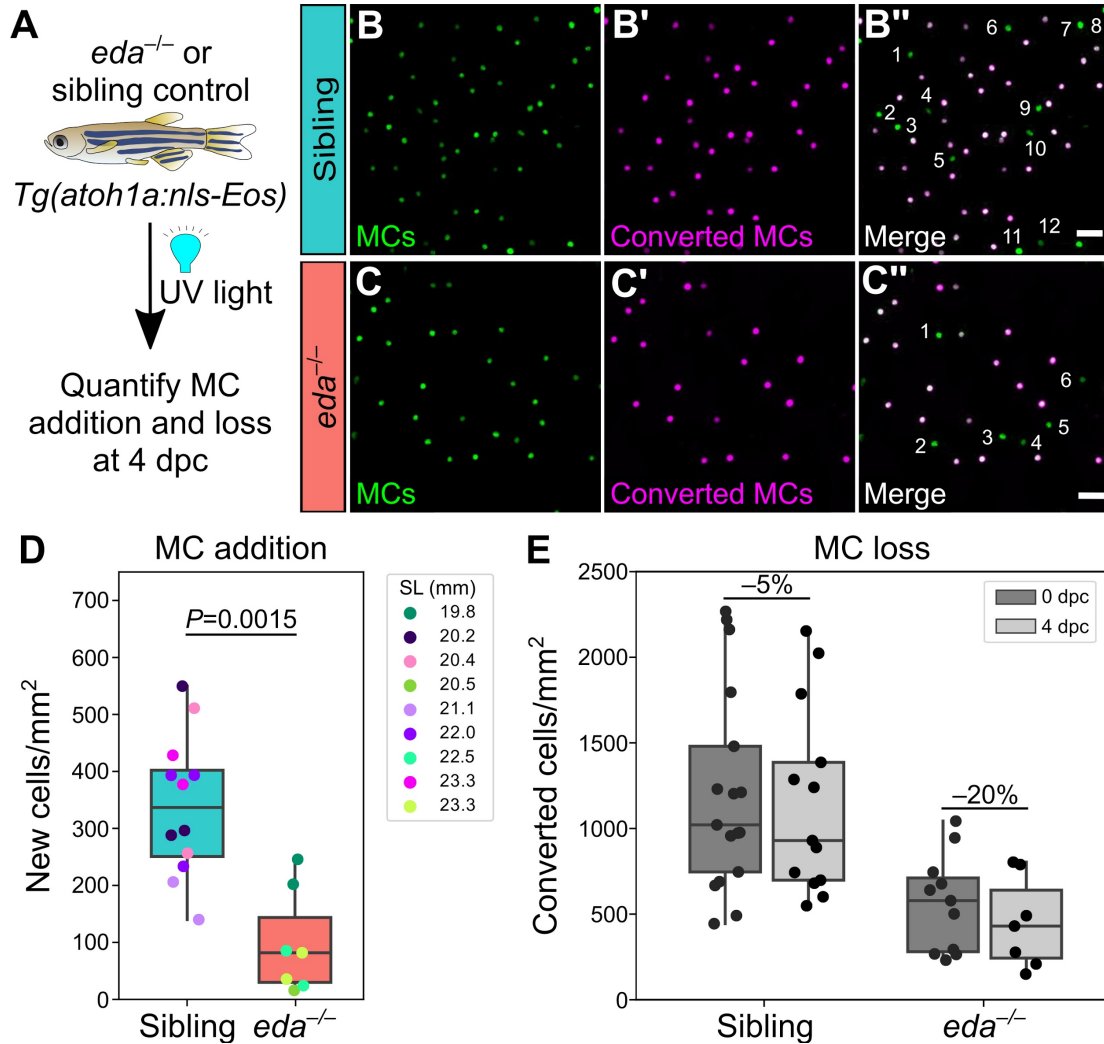
1054
 1055
 1056
 1057
 1058
 1059
 1060
 1061
 1062
 1063
 1064
 1065
 1066

Figure 7. MCs develop concomitant with dermal appendage morphogenesis.

(A) Abbreviated zebrafish developmental timeline showing standard length (SL) in millimeters. Developing scales are drawn in magenta below the approximate corresponding stage. (B-E) Representative lateral confocal micrographs of MCs and osteoblasts along the trunk at the indicated stages. Note that MCs increase in number and density as scale-forming osteoblasts develop below the epidermis. nm, neuromast of the posterior lateral line. (F) Quantification of MC density according to SL. Each dot represents an individual fish. (G,H) Quantification of the number (G) or density (H) of MCs relative to scale area. Each dot represents an individual scale. Dot colors represent animal SL as indicated in the legend. Shading indicates a 95% confidence interval around the linear regression lines in F-H. Correlation coefficients (R^2): 0.33 (F), 0.73 (G), 0.31 (H). Scale bars: 50 μ m.



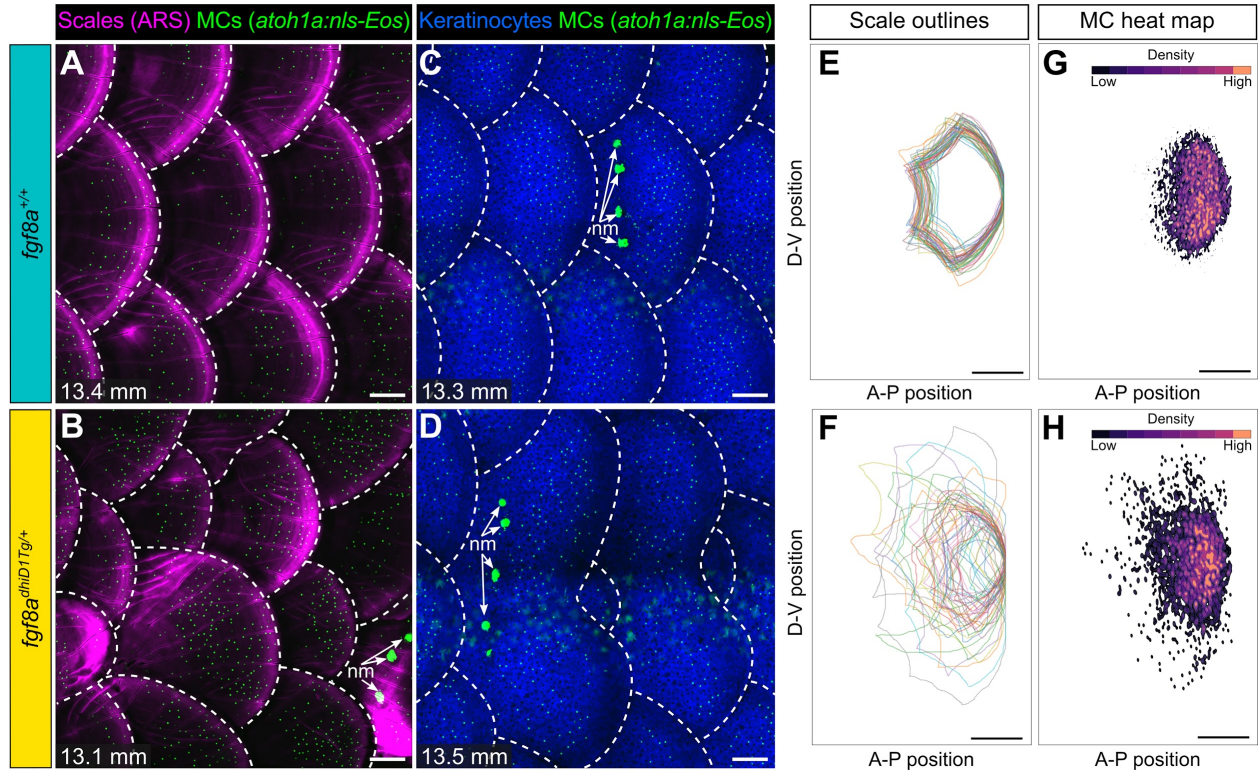
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080



1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093

Figure 8—figure supplement 1. *eda* mutants exhibit decreased MC addition and increased MC loss.

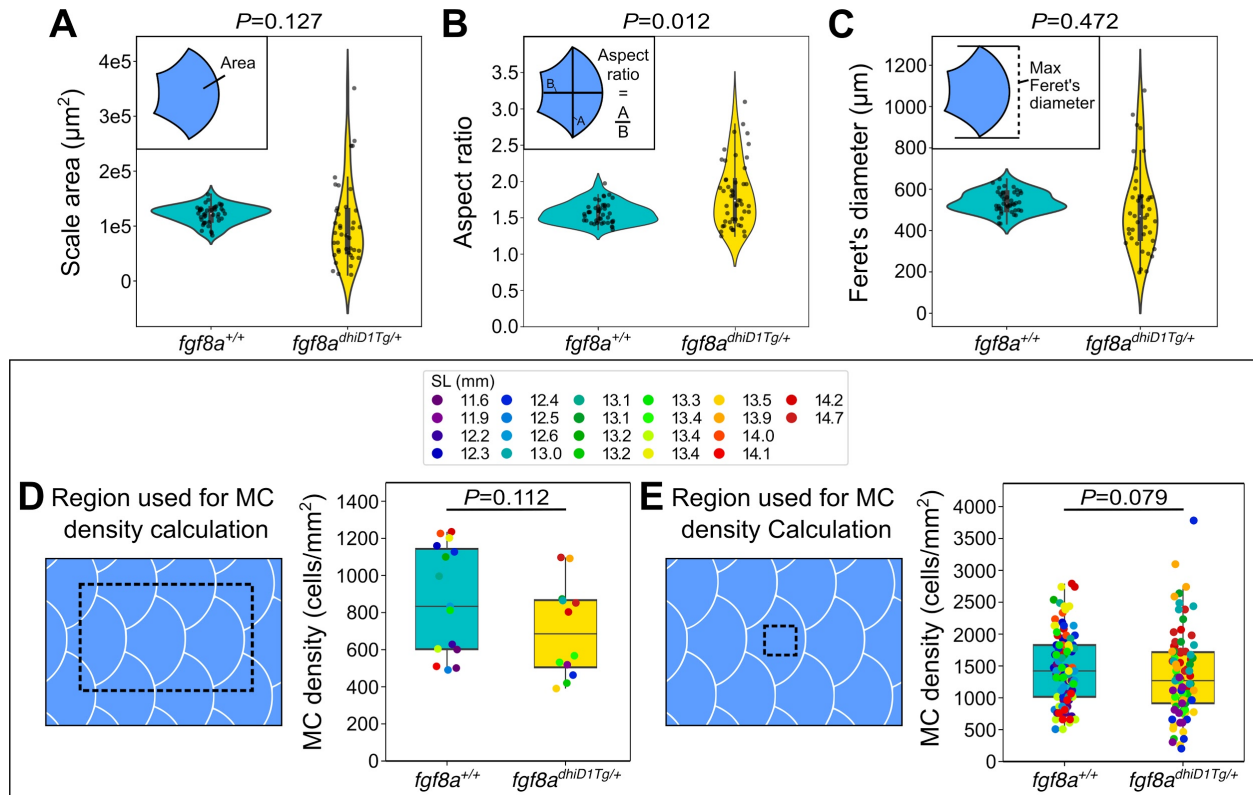
(A) Schematic of experimental approach. Following whole animal photoconversion, densities of converted and non-converted MCs were quantified at 4 dpc. (B-C'') Representative lateral confocal micrographs of MCs at 4 dpc in adults of the indicated genotypes. Numbers label newly added cells, distinguishable by the absence of photoconverted nls-Eos (magenta). (D) Boxplots of MC addition at 4 dpc in the indicated genotypes. 1-3 independent regions were analyzed per animal. *eda* mutants show a significantly lower rate of MC cell addition (Mann-Whitney test). (E) Boxplots of photoconverted MC density in animals of the indicated genotypes. Average percentage cell density loss between 0 and 4 dpc is listed above the boxplots for each genotype. Scale bars: 20 μ m (B,C).



1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104

Figure 9. Altering dermal appendage patterning redistributes MCs.

(A-D) Representative images of juvenile of the indicated genotypes expressing a MC reporter and stained with ARS to visualize scales (A,B) or co-expressing MC and keratinocyte (*Tg(krt4:DsRed)*) reporters (C,D). Dotted lines indicate scale boundaries. nm, neuromasts of the posterior lateral line. (E-H) Tracings of scale outlines (E,F) and density plots of MC position (G,H) from juvenile animals (11.6-14.7 mm SL) of the indicated genotypes. Scales tracings were aligned at the dorsal-ventral midpoint of the posterior scale margin. Note the variability in scale shape and size and corresponding increased spread of MC position in *fgf8a*^{dhiD1Tg/+} juveniles compared to sibling controls. Scale bars: 100 μm (A-D), 200 μm (E-H).



1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117

Figure 9—figure supplement 1. $fgf8a^{dhiD1Tg/+}$ juveniles show altered dermal appendage size and shape, but not MC density.

(A-C) Violin plots of scale area, aspect ratio, and Feret's diameter from juveniles (11.6-14.7 mm SL) of the indicated genotypes. P -values (Mann-Whitney test), listed above each plot, indicate a significant difference between the genotypes for the scale aspect ratio, but not scale area or Feret's diameter. Data represent $n=42$ scales from $N=13$ fish ($fgf8a^{+/+}$) and $n=32$ scales from $N=9$ fish ($fgf8a^{dhiD1Tg/+}$). Insets illustrate the various measurements. (D,E) Boxplots of MC density across the trunk epidermis (D) or the epidermis directly above individual scales (E) as indicated by the dotted boxes in juveniles expressing a MC reporter ($Tg(atoh1a:nls-Eos)$). Dot colors represent animal SL as indicated in the legend. Total fish analyzed: $fgf8a^{+/+}$ ($N=13$); $fgf8a^{dhiD1Tg/+}$ ($N=9$). P -values (Mann-Whitney test) are listed above each plot.

1118 **SUPPLEMENTAL VIDEO LEGENDS**

1119

1120 **Supplemental Video 1.**

1121 3D rotation of somatosensory axons (green) and photoconverted MCs (green and magenta) in

1122 the adult scale epidermis. Arrows indicate axonal varicosities in close proximity to MCs.