



Title	The color pattern inducing gene wingless is expressed in specific cell types of campaniform sensilla of a polka-dotted fruit fly, <i>Drosophila guttifera</i>
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1 **The color pattern inducing gene *wingless* is expressed in specific cell types of**  
2 **campaniform sensilla of a polka-dotted fruit fly, *Drosophila guttifera***

3

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13

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16

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18

19 **Abstract**

20 A polka-dotted fruit fly, *Drosophila guttifera*, has a unique pigmentation pattern on its wings  
21 and is used as a model for evo-devo studies exploring the mechanism of evolutionary gain of  
22 novel traits. In this species, a morphogen-encoding gene, *wingless*, is expressed in species-  
23 specific positions and induces a unique pigmentation pattern. To produce some of the  
24 pigmentation spots on wing veins, *wingless* is thought to be expressed in developing  
25 campaniform sensilla cells, but it was unknown which of the four cell types there express(es)  
26 *wingless*. Here we show that two of the cell types, dome cells and socket cells, express  
27 *wingless*, as indicated by *in situ* hybridization together with immunohistochemistry. This is a  
28 unique case in which non-neuronal SOP (sensory organ precursor) progeny cells produce  
29 Wingless as an inducer of pigmentation pattern formation. Our finding opens a path to  
30 clarifying the mechanism of evolutionary gain of a unique *wingless* expression pattern by  
31 analyzing gene regulation in dome cells and socket cells.

## 32 Introduction

33 Animal color patterns are an example of the morphological diversity of organisms.  
34 Ecological roles of color patterns have been studied (Cott 1940), and another major issue  
35 regarding color patterns is their formation process. In particular, color pattern formation of  
36 insects has been investigated to gain insight into the relationship between regulation of gene  
37 expression and evolution of morphological novelty. As a result of studies to elucidate the  
38 process of pattern formation, patterning genes whose expression induces the color formation  
39 have been identified. For example, the pattern of *optix* expression determines the position of  
40 red pigmentation in adult wings of *Heliconius* butterflies (Reed et al. 2011; Martin et al.  
41 2014; Zhang et al. 2017), *WntA* expression determines the border of pigmentation in adult  
42 wings of Nymphalidae butterflies (Martin et al. 2012; Martin and Reed 2014; Mazo-Vargas et  
43 al. 2017), and *pannier* expression determines the aposematic color pattern in adult ladybird  
44 beetles (Ando et al. 2018; Gautier et al. 2018). Interestingly, these genes are also expressed  
45 during ontogenesis. This indicates an evolutionary process in which genes with roles in  
46 ontogenesis were co-opted for color pattern formation (Jiggins et al. 2017). In order to  
47 examine in detail the evolutionary process that produces novel color patterns, it is necessary  
48 to elucidate the mechanism of spatiotemporal regulation of patterning genes (Fukutomi and  
49 Koshikawa 2021).

50 Various pigmentation patterns are found in adult wings of *Drosophila* fruit flies  
51 (Insecta, Diptera, Drosophilidae) (Wittkopp et al. 2002; Massey and Wittkopp 2016; Dufour  
52 et al. 2020; Koshikawa 2020; Werner et al. 2020). These patterns have been studied in a  
53 context of mechanisms of phenotypic evolution. For example, the *cis*-regulatory evolution of  
54 the *yellow* pigmentation gene has been identified and analyzed in a novel wing spot of  
55 *Drosophila biarmipes* (Gompel et al. 2005; Le Poul et al. 2020). Wing pigmentation patterns  
56 of *Drosophila* provide a unique opportunity to explore general questions regarding the  
57 genetic backgrounds of morphological novelties. Adult *Drosophila guttifera* flies have a  
58 species-specific wing spot pattern (Fig. 1A). The spots occur at specific positions, such as  
59 around campaniform sensilla on wing veins (Werner et al. 2010; Koshikawa et al. 2015;  
60 Fukutomi et al. 2017; Fukutomi et al. 2021). The spot formation around campaniform sensilla  
61 is a suitable model for examining the spatiotemporal regulation of the patterning gene  
62 (Koshikawa et al. 2017). This formation is induced by the species-specific expression of the  
63 patterning gene *wingless* (*wg*) during the pupal stage (Fig. 1C; Werner et al. 2010;  
64 Koshikawa et al. 2015). *wg* is expressed at campaniform sensilla on wing veins of *D.*

65 *guttifera* (Fig. 1C), and this expression is not detected in other *Drosophila* species  
66 (Koshikawa et al. 2015). The expression begins at mid-pupa (late stage 6) and then induces  
67 the subsequent expression of pigmentation genes such as *yellow* (Werner et al. 2010). In *D.*  
68 *melanogaster*, each campaniform sensillum, which is a mechanoreceptor involved in flight  
69 control by sensing wing flexion (Tuthill and Wilson 2016), consists of four differentiated  
70 cells: a socket (tormogen) cell, a dome (trichogen) cell, a sheath (thecogen) cell, and a neuron  
71 (Fig. 1B; Van De Bor et al. 2000; Van De Bor et al. 2001). The formation of a campaniform  
72 sensillum resembles that of a bristle, a type of mechanoreceptor. The sensory organ precursor  
73 (SOP) selected from a proneural cluster (Furman and Bukharina 2008; Gómez-Skarmeta et  
74 al. 1995) divides into the two secondary precursors, PIIa and PIIb (Van De Bor et al. 2000;  
75 Van De Bor and Giangrande 2001). The socket and dome cells are generated by the division  
76 of the PIIa, and the sheath cell and neuron are generated by the division of the tertiary  
77 precursor PIIIb, which is a progeny of the PIIb (Van De Bor et al. 2000; Van De Bor and  
78 Giangrande 2001). Subsequently, the differentiation process of each of these cells follows  
79 (Furman and Bukharina 2008), but the detailed gene expression profiles involved in the  
80 differentiation have not been well investigated. The expression of the patterning gene *wg*  
81 begins during the differentiation stage of the four cells of the campaniform sensilla in *D.*  
82 *guttifera* (Werner et al. 2010), indicating that the cell differentiation and *wg* expression are  
83 synchronized. Understanding the relationships between campaniform sensilla differentiation  
84 and *wg* expression is a key for unravelling the process of evolutionary gain of species-  
85 specific *wg* expression.

86 Proteins of the Wnt family, including Wingless (Wg) protein, are involved in the  
87 formation of structures characteristic of the neuronal network, such as synapses and axons  
88 (Packard et al. 2002; He et al. 2018). Considering that *wg* may be expressed in neurons in  
89 general, although *wg* is not expressed at campaniform sensilla of *D. melanogaster*, it is  
90 possible that co-option of *wg* expression occurs in neurons of campaniform sensilla of *D.*  
91 *guttifera*. Here, as the first step to elucidate the relationships between the differentiation of  
92 cells composing campaniform sensilla and the pigmented spot formation, we investigated  
93 whether neurons express the patterning gene *wg*, and if not, which cells express *wg*. The  
94 identification was performed by the dual detection of *wg* transcripts by *in situ* hybridization  
95 and of specific marker protein or cell membrane of campaniform sensilla by  
96 immunohistological staining in pupal wings.

97

## 98 **Materials and methods**

### 99 Flies and genomic DNA

100 We used *D. melanogaster* Oregon-R (wild-type) and *D. guttifera* (stock no. 15130-  
101 1971.10, obtained from the *Drosophila* Species Stock Center at the University of California,  
102 San Diego), for genomic DNA preparation and gene expression analysis. Both fly lines were  
103 reared on standard food containing cornmeal, sugar, yeast, and agar at room temperature  
104 (Fukutomi et al. 2018).

105

### 106 Dissection and Fixation

107 Dissection of pupal wings was performed as described previously (Werner et al.  
108 2010). After the pupal membrane was removed, pupal wings were fixed in PBS (phosphate  
109 buffered saline, Takara Bio) with 4% paraformaldehyde (PFA) for 20 minutes (min) at room  
110 temperature. Fixed samples were washed three times in PBT (0.1% Triton X-100 in PBS) and  
111 stored in methanol at -20°C.

112

### 113 Immunohistochemistry

114 Stored wing samples were incubated with primary antibodies in PBT overnight at  
115 4°C. Following three washes with PBT, samples were incubated with fluorescent secondary  
116 antibodies in PBT for 2 h at room temperature and washed three times with PBT. After the  
117 last PBT wash, samples were mounted with Vectashield Mounting Medium with DAPI  
118 (Vector Laboratories). Each wash was 5 min long. The following primary antibodies were  
119 used at the indicated dilutions: mouse anti-Cut, 1:1000 [2B10; Developmental Studies  
120 Hybridoma Bank (DSHB)]; mouse anti-Elav (Embryonic lethal abnormal vision), 1:1000  
121 (9F8A9, DSHB); mouse anti-Futsch, 1:1000 (22C10; DSHB); mouse anti-Na<sup>+</sup>/K<sup>+</sup>-ATPase $\alpha$   
122 (Chicken homolog of *Drosophila* Atpa), 1:50 (a5; DSHB). For secondary antibodies, anti-  
123 mouse-Alexa555 conjugate (Abcam) or anti-mouse-Alexa488 conjugate (Abcam) was used at  
124 1:500.

125

### 126 *In situ* hybridization

127 Digoxigenin-labeled antisense RNA probes of *wg* and *Suppressor of Hairless*  
128 [*Su(H)*] were produced as described previously (Werner et al. 2010). Genomic DNA was  
129 extracted by using a DNeasy Blood & Tissue Kit (QIAGEN). The following forward and  
130 reverse primers were used to amplify a 377 bp DNA fragment of *wg* exon 2 in *D. guttifera*:

131 5'-CACGTTTCAGGCGGAGATGCG-3' and 5'-GGCGATGGCATATTGGGATGATG-3', a  
132 525 bp DNA fragment of *Su(H)* exon 2 in *D. guttifer*: 5'-CAGTGATCAGGATATGCAGC-  
133 3' and 5'-TGCGAAACAGGATCATCAGC-3', and a 402 bp DNA fragment of *Su(H)* exon 2  
134 in *D. melanogaster*: 5'-AGCTGGATCTCAATGGCAAG-3' and 5'-  
135 CATTATTACGGAGCCACAG-3'. DNA fragments were cloned into pGEM-T Easy  
136 Vector (Promega). DNA templates for *in vitro* transcription were amplified using M13F and  
137 M13R primers. RNA probes were transcribed *in vitro* with T7 or SP6 polymerase (Promega)  
138 and DIG (Digoxigenin) RNA Labeling Mix (Roche). Each probe was purified using a  
139 ProbeQuant G-50 Micro Column (Cytiva) and stored in RNase-free water at -20°C. Stored  
140 wing samples were treated with methanol containing 2% H<sub>2</sub>O<sub>2</sub> for 20 min at room  
141 temperature, as described previously (Lauter et al. 2011). Then they were washed twice with  
142 ethanol, incubated in a mixture of xylene and ethanol (1:1 v/v) for 60 min, washed three  
143 times with ethanol, and rehydrated by two washes with methanol and two washes with PBT.  
144 After treatment with a mixture of acetone and PBT (4:1 v/v) for 14 min at -20°C, samples  
145 were washed twice with PBT, post-fixed in PBS with 4% PFA for 20 min and washed three  
146 times with PBT. The fixation in acetone was performed with reference to Nagaso et al.  
147 (2001). The hybridization process and anti-DIG antibody incubation were performed as  
148 described previously (Sturtevant et al. 1993, Werner et al. 2010), except that the  
149 hybridization buffer contained 5% dextran sulfate and the hybridization temperature was  
150 57°C. Signals of transcripts were detected using Anti-DIG-AP (alkaline phosphatase) and Fab  
151 fragments from sheep (Roche) and developed in Fast Red TR and naphthol-AS-MX-  
152 phosphate in 0.1 M Tris-HCl pH 8.2 (tablet set; Sigma). After three washes with PBT,  
153 samples were mounted in PBT or 50% glycerol diluted with PBS.

154

## 155 Dual detection

156 The pretreatment and hybridization processes were performed as described above.  
157 After washes with PBT, hybridized wings were incubated with Anti-DIG-AP Fab fragments  
158 (Roche) diluted 1:6000 and primary antibodies in Pierce Immunostain Enhancer (PIE)  
159 (Thermo Scientific) overnight at 4°C. Then they were washed three times with PBT,  
160 incubated with fluorescent secondary antibodies in PIE for 2 h at room temperature and  
161 washed three times with PBT. Detection of transcripts, subsequent washes, and mounting  
162 were performed as described in the “*In situ* hybridization” section.

163

## 164 Microscopy and image analysis

165 Preparations were observed using a BX60 microscope (Olympus) equipped with a  
166 dry 20 x or an oil-immersion 60 x objective, or an LSM700 confocal microscope (Carl Zeiss)  
167 equipped with an oil-immersion 63 x objective. The filter sets on a BX60 microscope  
168 contained a BP 535/30 nm excitation filter and LP 580 nm emission filter for Alexa555 and  
169 Fast Red, and a BP 480/20nm excitation filter and BP 530/40 nm emission filter for  
170 Alexa488. On a LSM700 confocal microscope, Fast Red was excited at 555 nm and detected  
171 with a LP 560 emission filter, and Alexa488 was excited at 488 nm and detected with a SP  
172 555 emission filter. Confocal images were taken at z-intervals of 0.4  $\mu$ m. The brightness,  
173 contrast, and color of images were adjusted with Fiji (Schindelin et al. 2012).

174

## 175 **Results and discussion**

176 Comparison of gene expression patterns in pupal wings of *D. melanogaster* and *D. guttifer*

177 We first investigated whether *cut*, *futsch*, *embryonic lethal abnormal vision (elav)*,  
178 and *Suppressor of Hairless [Su(H)]* are expressed in the campaniform sensilla on pupal wings  
179 of *D. guttifer* (Fig. 1). These are marker genes expressed in the cells of sensilla in *D.*  
180 *melanogaster*. Cut protein (Blochlinger et al. 1990; 1993) is localized in the nuclei of all four  
181 types of cells composing campaniform sensilla during the developmental stages in *D.*  
182 *melanogaster* (Van De Vor et al. 2000). Futsch, also known as the antigen of 22C10  
183 antibodies, and Elav proteins are localized in the cytoplasm and nucleus of neurons,  
184 respectively (Fig. 1F, H; Hummel et al. 2000; Aigouy et al. 2004; Robinow and White 1988;  
185 Van De Bor et al. 2000). *Su(H)* is a gene specifically expressed in the socket cells composing  
186 bristles of *D. melanogaster* (Barolo et al. 2000).

187 In *D. melanogaster*, we found that Cut expression was observed in the sensilla of the  
188 wing margin, the third longitudinal vein, and the anterior cross vein of pupal wings (Fig. 1D).  
189 The magnified image of L3-1 (Fig. 1D') shows four cells with a high level of accumulation  
190 of Cut, as in other campaniform sensilla of the third longitudinal vein (L3-2 and L3-3). In *D.*  
191 *guttifer*, Cut was localized in the campaniform sensillum on the fifth longitudinal vein (L5)  
192 in addition to the third longitudinal vein (Fig. 1E). This is in accord with the previous report  
193 that there is a campaniform sensillum on the fifth longitudinal vein in the wing of *D. guttifer*  
194 (Sturtevant 1921), but not in *D. melanogaster*. In a magnified view of the *D. guttifer* wing  
195 (L3-1, Fig 1E'), four cells containing a high level of Cut protein are seen, as in *D.*  
196 *melanogaster*, indicating that a campaniform sensillum of *D. guttifer* also consists of four  
197 types of cells.

198 Futsch protein, also known as 22C10 antigen, was detected throughout the  
199 cytoplasm of neurons in *D. melanogaster* (Fig. 1F; Hummel et al. 2000; Aigouy et al. 2004).  
200 A similar pattern of Futsch localization in pupal wings was observed in *D. guttifer* (Fig.  
201 1G). Elav protein is localized in nuclei of neurons of *D. melanogaster* (Fig. 1H; Robinow and  
202 White 1988; Van De Bor et al. 2000). A similar pattern of Elav localization in pupal wings  
203 was observed in *D. guttifer* (Fig. 1I), suggesting that Elav is localized in nuclei of neurons  
204 of *D. guttifer*. In summary, there was no substantial difference in the morphology or  
205 position of neurons in pupal wings between the two species.

206 The expression of *Su(H)* was detected at the estimated position of the campaniform  
207 sensilla in *D. melanogaster* (Fig. 1J). The known expression of *Su(H)* in the socket cells  
208 composing bristles of *D. melanogaster* (Barolo et al. 2000) and the structural homology  
209 between bristles and campaniform sensilla suggest that the socket cells composing the  
210 campaniform sensilla of *D. melanogaster* express *Su(H)*. Similarly, the expression of *Su(H)*  
211 was observed in the campaniform sensilla of *D. guttifer* (Fig. 1K). This indicates that the  
212 position of socket cells in pupal wings of *D. guttifer* can be visualized by detecting the  
213 expression of *Su(H)*. However, it is unknown whether the detection of *Su(H)* could visualize  
214 the entire area of the socket cells.

215

216 Neurons and sheath cells do not express the patterning gene *wg*

217 Next, we analyzed whether *wg* is expressed in the neurons composing campaniform  
218 sensilla in the pupal wings of *D. guttifer*. We simultaneously detected *wg* transcripts and the  
219 (putative) neuronal marker gene expression in the four sensilla, L3-1, L3-2, L3-3, and L5 (Fig  
220 2), where *wg* is expressed at a high level. We found that the signals of Elav protein localized  
221 in neuronal nuclei (Fig. 2A-D) and *wg* transcripts (Fig. 2A'-D') were not colocalized within  
222 the four campaniform sensilla (Fig. 2A''-D''). In addition, we did not observe colocalization  
223 of Futsch protein, which was localized throughout the neuronal cytoplasm (Fig. 2E-H), and  
224 *wg* transcripts (Fig. 2E'-H') in the four campaniform sensilla (Fig. 2E''-H''). These results  
225 indicate that the patterning gene *wg* is not expressed in the neurons in the campaniform  
226 sensilla.

227 The signal of *wg* transcripts was observed in contact with the tip of the dendrite  
228 labeled with anti-Futsch antibody at the level of detection by light microscopy (Fig. 2E''-  
229 H''). With reference to the structure of a campaniform sensillum (Fig. 1B; Chevalier 1969;  
230 Gullan and Cranston 2014), the relatively large socket and dome cells surround the dendrite



231 within the layer of epidermal cells. In contrast, the sheath cell covers the neuron from the  
232 inner dendrite to the cell body and appears not to have a large volume around the tip of the  
233 dendrite. In addition, *wg* transcripts appeared to be localized in two spots in some images  
234 (Fig. 2G', H'), indicating the possibility that two cells express *wg*. The *wg* expression pattern  
235 suggests that *wg* is expressed not in sheath cells but in either socket cells or dome cells, or  
236 both.

237

238 Both socket and dome cells express the patterning gene *wg*

239 In order to determine whether socket and/or dome cells composing campaniform  
240 sensilla express *wg*, we furthermore analyzed the expression pattern of *wg*. As cytoplasmic  
241 markers of sheath, socket and dome cells have not been identified in *D. guttifer*, we labeled  
242 the campaniform sensilla with anti- $\text{Na}^+/\text{K}^+$ -ATPase  $\alpha$  subunit (*Atp $\alpha$* ) antibody (Lebovitz et al.  
243 1989), a marker of the cell membranes. The signals visualized three concentric layers within  
244 the sensilla (Fig 3A). To identify the cell type of each layer, we simultaneously detected  
245 *Su(H)* transcripts, which are expressed in the socket cells in the mechanoreceptor bristles. We  
246 found that *Su(H)* signals (Fig. 3B', C') were limited to the outermost layer (Fig 3B'', C''),  
247 indicating that the outermost layer is composed of the socket cell, as shown in Fig 1B.

248 Although we have not identified the cell types of all of the three layers with specific markers,  
249 based on the cell arrangement shown in Fig 1B, we conclude that the innermost layer is the  
250 neuronal dendrite, the second most is the dome cell, and the outermost is the socket cell.

251 Among these three concentric layers, we found that *wg* is expressed in the two outer layers,  
252 both socket and dome cells (3D'', D''', E'', E''').

253 This study revealed that *wg* expression which induces pigmentation spots around  
254 campaniform sensilla of *D. guttifer* occurs in the socket and dome cells, but not neurons.  
255 Considering that Wg protein is known to act as a morphogen, it is expected that Wg produced  
256 by these two cells is secreted to the surrounding epidermal cells and induces expression of the  
257 pigmentation genes in the recipient cells. This process of color pattern formation, in which  
258 co-option of the patterning gene occurs in specific cells composing nerve tissues, has not  
259 been reported before.

260 Although it is still unclear what gene regulatory network is responsible for the co-  
261 option of *wg* in these two cell types, a clue was obtained from an abnormal individual of *D.*  
262 *guttifer* (Werner et al. 2010). In the abnormal individual, the dome structure of a  
263 campaniform sensillum in the adult wing was converted to the bristle structure, and the

264 pigmentation spot was not formed around the structure. Considering our findings,  
265 suppression of the *wg* expression by the fate change of socket and/or dome cells can be  
266 assumed to have been the reason for the abnormality of pigmentation in that individual. This  
267 suggests that we can approach the regulatory process of the spatiotemporal expression of *wg*  
268 by clarifying the state of gene expression underlying the fate change of these two cells.  
269 Furthermore, in *D. melanogaster*, overexpression of the *hindsight* gene in sensory organ  
270 precursors (SOPs) on the future wing vein of the wing disc transformed the dome structure of  
271 some campaniform sensilla to the bristle-like structure (Szablewski and Reed 2019). The  
272 expression of *hindsight* was confirmed in SOPs (Buffin and Gho 2010), and *hindsight*  
273 encodes a transcription factor that is necessary to positively regulate EGFR signaling (Kim et  
274 al. 2020). If the transformation mechanism applies to the reason why the described  
275 abnormality of *D. guttifera* occurred, the expression of *wg* could be under the control of  
276 EGFR signaling, and overactivation of the signaling could downregulate the *wg* expression.  
277 In order to confirm whether EGFR signaling influences the spatiotemporal expression of *wg*  
278 in socket and dome cells, it will be necessary to investigate whether the spot formation of *D.*  
279 *guttifera* is influenced by manipulating the expression of genes involved in EGFR signaling,  
280 such as *hindsight*.

281 Our study revealed the expression of the color pattern inducing gene *wingless* in  
282 specific types of cells. Elucidation of the detailed evolutionary mechanisms of the gain of this  
283 gene expression and the resultant novel pigmentation pattern, and comparison between  
284 different lineages of *Drosophila* which evolved pigmentation spots independently will give a  
285 unique insight into the general mechanism of morphological evolution.

286

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290

### 291 **Data availability**

292 The datasets generated during and/or analysed during the current study are available from the  
293 corresponding author on reasonable request.

294

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297

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439

440 **Figure legends**

441

442 Figure 1. Expression patterns of *wingless* (*wg*) and developmental marker genes in pupal  
 443 wings at late stage 6. Transcripts were visualized by *in situ* hybridization using Fast Red, and  
 444 proteins were visualized by immunostaining with Alexa555. **A** An adult wing of *D. guttifer*;  
 445 arrowheads indicate the pigmentation around campaniform sensilla. **B** Transverse section  
 446 diagram of campaniform sensillum (after Gullan and Cranston, 2014). **C** Expression pattern  
 447 of the patterning gene *wg* in a pupal wing of *D. guttifer*; arrowheads indicate the expression  
 448 at campaniform sensilla. **D and D'** Localization of Cut protein in *D. melanogaster*. **E and E'**  
 449 Localization of Cut protein in *D. guttifer*. The rectangles in D and E indicate the area of L3-  
 450 1 (but different samples are shown in D, D', E and E') and the arrow indicates the position of  
 451 L5 (E). In D' and E', the magnified area of L3-1 is shown and arrowheads indicate the nuclei  
 452 at L3-1. Cut was observed in nuclei of all cells composing campaniform sensilla in both  
 453 species. **F** Localization of Futsch protein in *D. melanogaster*. **G** Localization of Futsch  
 454 protein in *D. guttifer*. Futsch was observed in cytoplasm of neurons in both species. The low  
 455 accumulation of Futsch in L5 sensillum was probably caused by the existence of only one  
 456 axon. **H** Localization of Elav (Embryonic lethal abnormal vision) protein in *D. melanogaster*.  
 457 **I** Localization of Elav protein in *D. guttifer*. Elav protein was observed in nuclei of neurons  
 458 in both species. **J** Localization of *Suppressor of Hairless* [*Su(H)*] transcripts in *D.*  
 459 *melanogaster*. **K** Localization of *Su(H)* transcripts in *D. guttifer*. *Su(H)* transcripts were  
 460 observed in socket cells in both species. Scale bar indicates 100  $\mu\text{m}$  (A, B, D-K) or 5  $\mu\text{m}$  (D',  
 461 E'). Distal is to the right.

462

463 Figure 2. The patterning gene *wingless* (*wg*) is not expressed in neurons of campaniform  
 464 sensilla. Signals of Elav (Embryonic lethal abnormal vision) protein (**A, B, C, D**) localized in  
 465 neuronal nuclei were compared with those of *wg* transcripts (**A', B', C', D'**). Signals of Elav  
 466 did not overlap with those of *wg* (**A'', B'', C'', D''**). Signals of Futsch protein (**E, F, G, H**)  
 467 localized in neuronal cytoplasm compared with those of *wg* transcripts (**E', F', G', H'**).  
 468 Arrows indicate the cell bodies and arrowheads indicate the dendrites. Signals of Futsch did  
 469 not overlap with signals of *wg* (**E'', F'', G'', H''**). Scale bar indicates 5  $\mu\text{m}$ . Distal is to the  
 470 right. Elav and Futsch proteins were visualized by immunostaining with Alexa488. *wg*  
 471 transcripts were visualized by *in situ* hybridization using Fast Red.

472



473 Figure 3. *wg* transcripts were observed in the socket and dome cells. The signal of Na<sup>+</sup>/K<sup>+</sup>-  
474 ATPase $\alpha$  (Atp $\alpha$ , green in the figures) visualizes the cell membrane at campaniform sensilla  
475 (**A**, **B**, **C**, **D**). *Suppressor of Hairless* [*Su(H)*, magenta in the second left and third left figures]  
476 transcripts were detected at campaniform sensilla (**B'**, **C'**). Cell types can be identified  
477 according to their morphology and relative position. The relatively large cell surrounding the  
478 other structures of the campaniform sensillum and expressing *Su(H)* transcripts was identified  
479 as a socket cell (**A'**, **B''**, **B'''**, **C''**, **C'''**). The cell surrounded by a socket cell was identified  
480 as a dome cell. The sheath cell was not obvious in this plane, while the dendrite surrounded  
481 by a sheath cell was visible. *wg* transcripts (**C'**, **D'**) were localized in the socket cell and the  
482 inner dome cell (**C''**, **C'''**, **D''**, **D'''**). All panels show L3-1 sensillum. Scale bar indicates 1  
483  $\mu$ m. Atp $\alpha$  protein was visualized by immunostaining with Alexa488. *wg* and *Su(H)*  
484 transcripts were visualized by *in situ* hybridization using Fast Red.

**Fig.1**

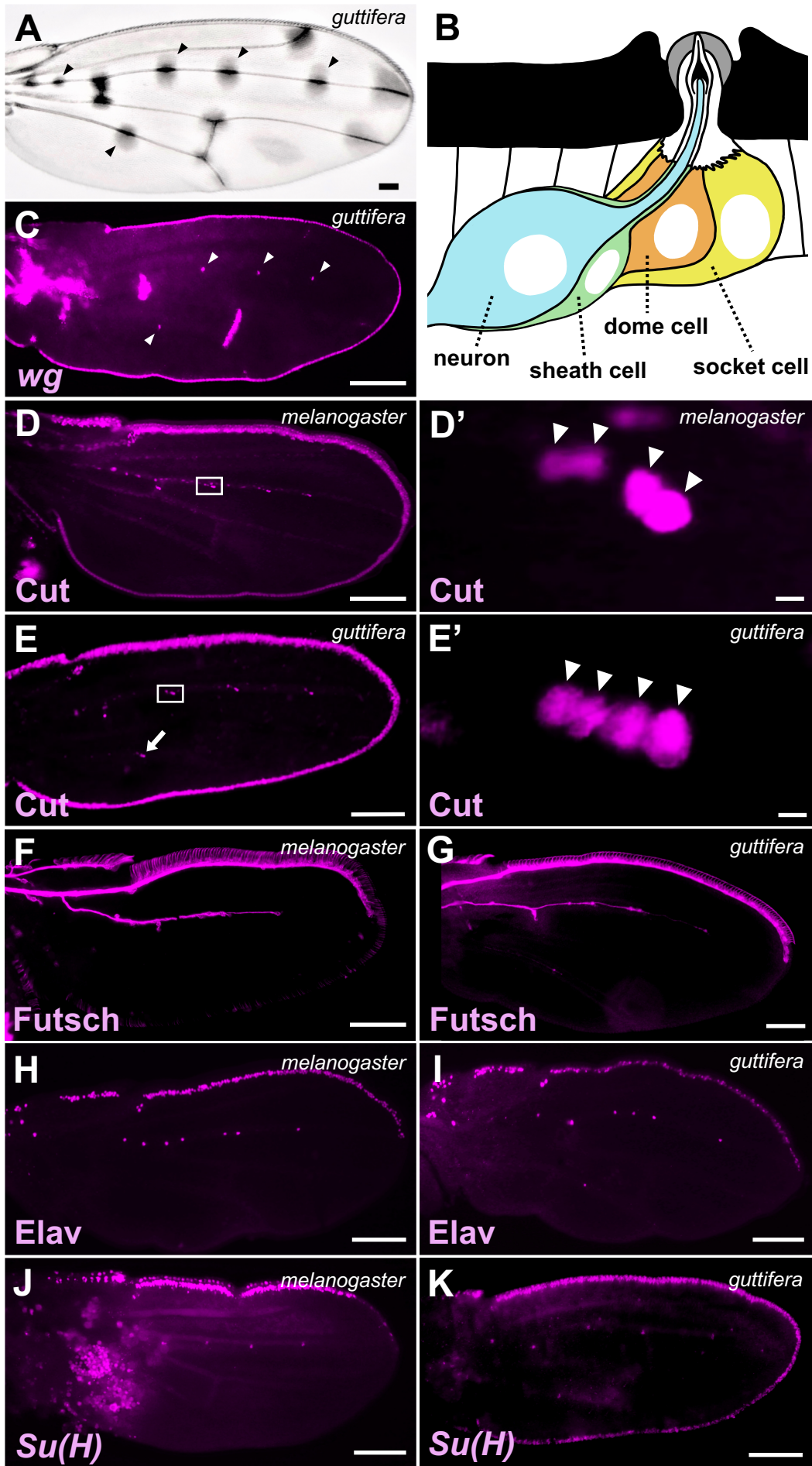


Fig.2

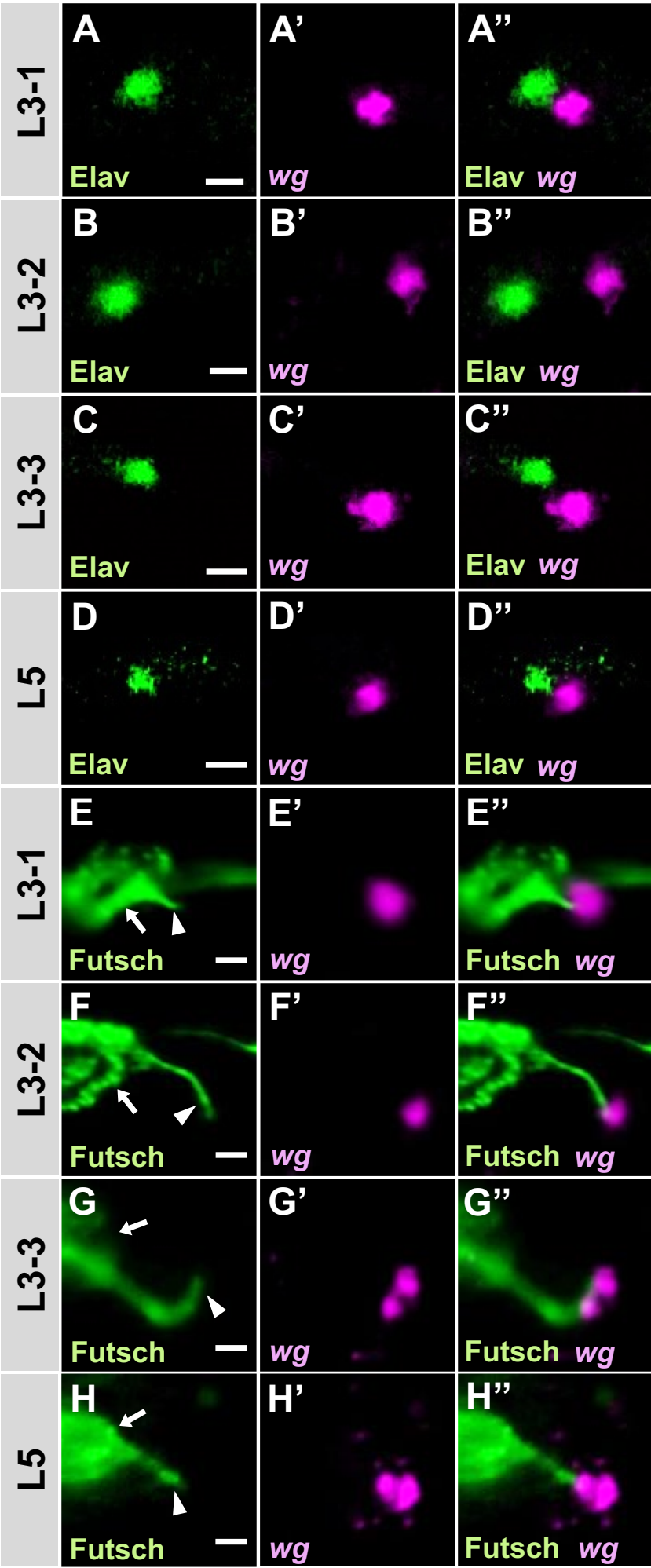


Fig.3

