

### **Supplemental Figure 1. Groove differentiation occurs before refinement is complete**

Early stage 12 embryo at the onset of groove formation, heat fixed and stained for aPKC, viewed en face (left) and in Z-section (right). aPKC is enriched in a stripe that is several cells wide. 3D visualization reveals that most cells with elevated aPKC have not yet initiated groove morphogenesis (examples marked with blue dots), although several cells are beginning to invaginate (yellow dots).

### **Supplemental Figure 2. En cell markers adjacent to groove cells**

The cell cortex of En cells was marked by expressing APC2::GFP, a fusion of the cytoskeletal protein APC2 to GFP, in the En cells using the Gal4, UAS system. GFP is visible in white. Embryos were also stained for En (green), Odd (red) and Cadherin (blue). Projections from the cell bodies of En cells were frequently observed that extend near to, but do not surround the Odd nuclei. APC2::GFP marking these projections was observed anterior to the Odd nuclei, but not posterior.

### **Supplemental Figure 3. Time lapse imaging of En-Gal4; UAS-APC2::GFP**

Time lapse imaging of En-Gal4; UAS-APC2::GFP embryos. Numbers indicate elapsed minutes during the imaging. Infrequent expression from the En-GAL4 driver in cells anterior to the En cells (arrowheads) demonstrates occasional ectopic expression from the En regulatory sequences used. If former En cells cease En expression, become Odd expressing cells and populate the groove, one would expect them to inherit APC2::GFP expression, but expression would fade as the En enhancer sequences are no longer

expressed. However, expression within the occasional labeled groove cells persists throughout the imaging period, and cannot therefore represent inherited expression of APC2::GFP from former En cells.

**Supplemental Figure 4. High magnification view of ectopic expression from time lapse imaging of En-Gal4; UAS-APC2::GFP**

Ectopic expression from En-Gal4; UAS-APC2::GFP sometimes labels not only groove cells, but posterior cells producing fine hairs, identified by accumulation of APC2::GFP, which labels the cytoskeleton.

**Supplemental Figure 5. Function of the En enhancer**

Adapted from Figure 2, Florence B., Guichet A., Ephrussi A. and Laughon A.

Development 124, 839-847 (1997): Ftz-F1 is a cofactor in Ftz activation of the *Drosophila* Engrailed gene.

The En enhancer has been molecularly characterized (Florence et al. 1997). The expression of reporter constructs driven by various elements was analyzed at the stage of early En expression (“Ftz stage”) or later at the two cell wide, Wg dependent stage (“Wg stage”). The expression patterns at these stages are indicated. Ftz and Ftz-F1 binding sites (red boxes) are required to initiate En transcription and are sufficient to drive expression when oligomerized. Interestingly this expression is maintained in a broad domain during the Wg dependent stage, implying the absence of Wg requirement for late expression of the Ftz/Ftz-F1 driven construct. The refinement of later expression depends

on the presence of the blue box, showing that switching off Ftz activation is an active process. Florence et al. proposed that Odd may bind this element, as Odd antagonizes En (Coulter and Wieschaus 1988). In our model, the blue box mediates the progression of cell identity. The requirement for the TCF binding sites (green box) was not tested specifically, but suggests a hypothesis to explain how Wg signaling prevents repression by the blue box. We propose that within the range of Wg signaling, the green box senses the Wg signal and prevents the blue box from triggering the progression from En to Odd expression. Therefore, the identity specified early by Ftz at the level of the red box is maintained.

Figure S1

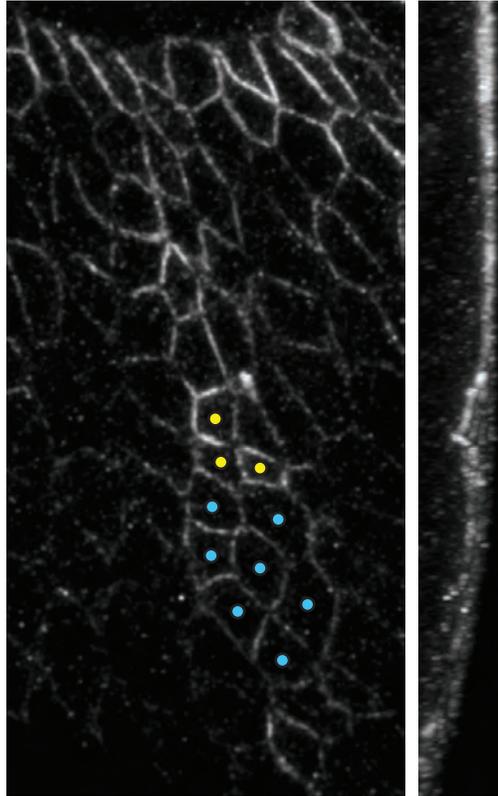


Figure S2

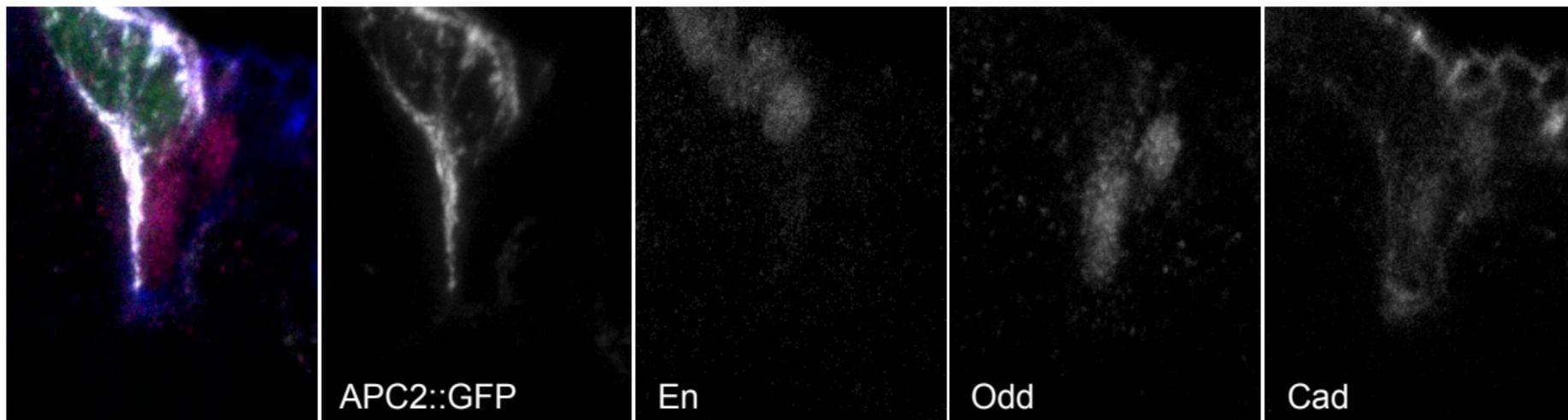


Figure S3

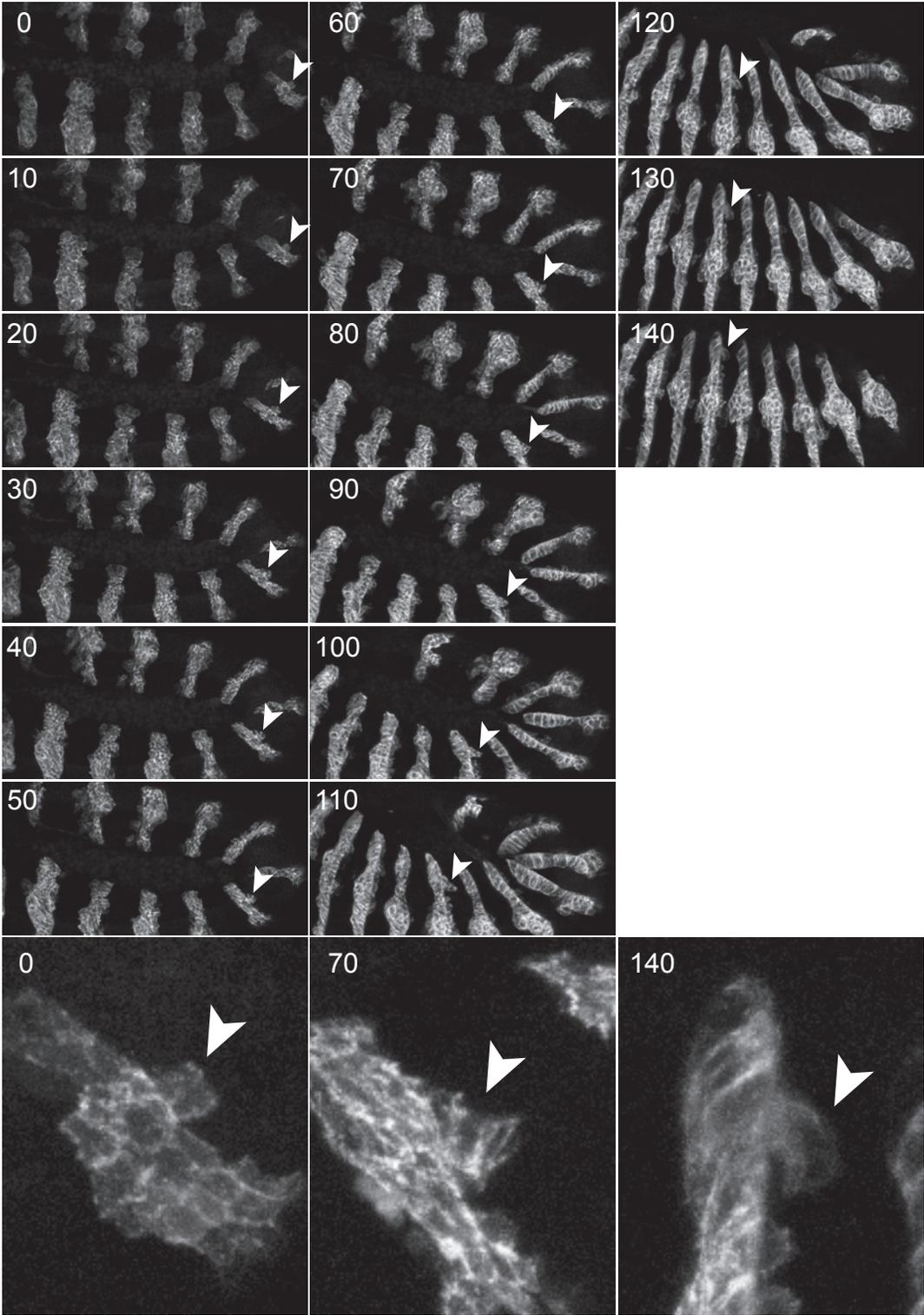
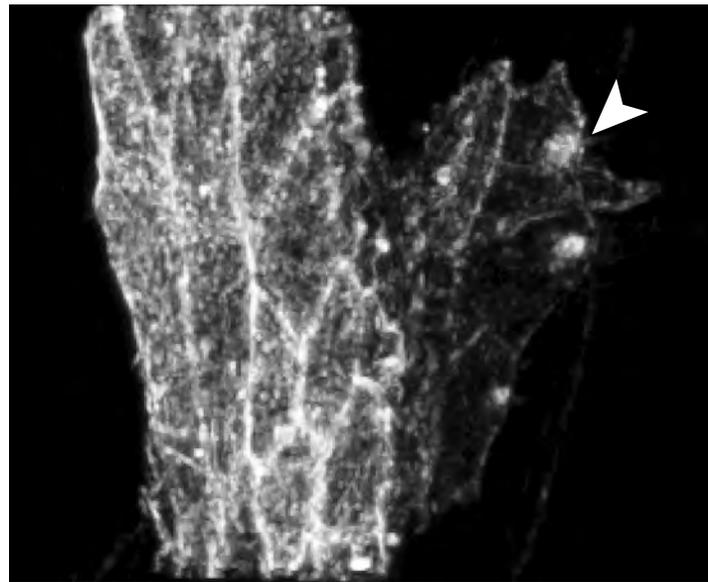
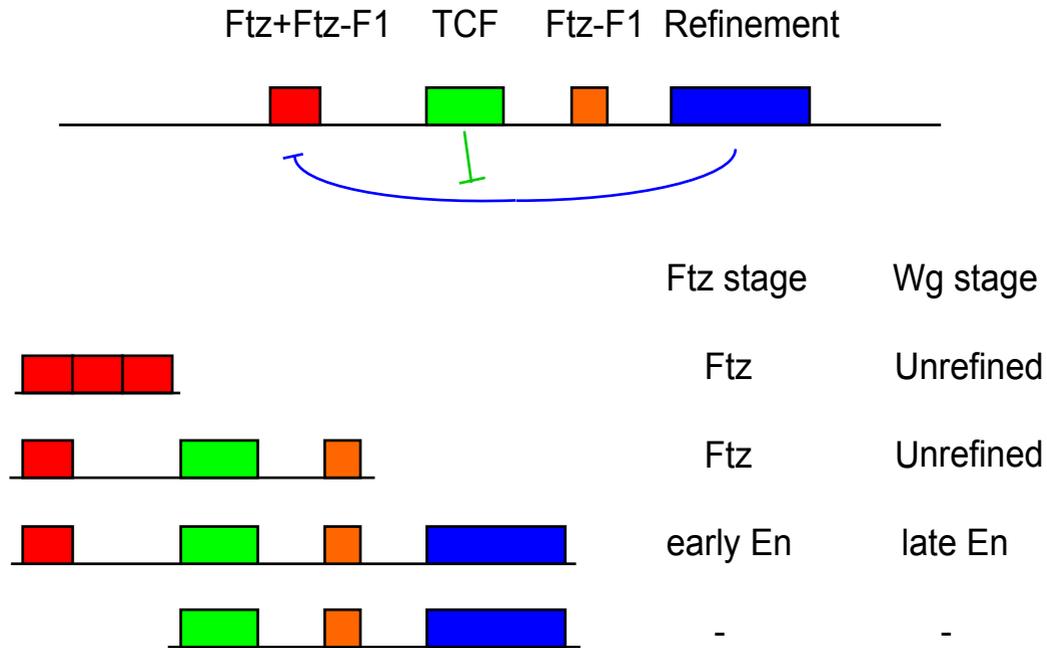


Figure S4



Supplemental Figure 4



Adapted from Fig. 2 in Florence B. Guichet A. Ephrussi. A & Laughon A. Development 124, 839-847 (1997)  
 "Ftz-F1 is a cofactor in Ftz activation of the *Drosophila engrailed* gene"