Supporting Information Legends

Figure S1. Quantification of immunostaining. Box plots showing quantification of immunostaining under different conditions. (A) Quantification of data presented in Figures 1F and 1G. Su(H) accumulation in control socket cells at 23h (n = 17 control socket cells and 22 Ttk69-mutant socket cells) and at 28 h APF (n = 38 control socket cells and 33 Ttk69-mutant socket cells). Note that at 23h Su(H) accumulation was similar in both control and Ttk69-mutant socket cells. At 28h APF Su(H) accumulation was 3 times higher in control cells indicating that autoamplification occurred only in the control socket cells. (B) Quantification of data presented in Figures 4D and 4E. β-Gal accumulation in control pIIa cells in the 4.6WT-lacZ fly line (n = 21 control pIIa cells and 30 Ttk69-mutant pIIa cells) and in the 4.6m-lacZ fly line (n = 10 control pIIa cells and 16 Ttk69-mutant pIIa cells). Note that in both cases, ratios are similar and less than 1 indicating that Ttk69 continued to downregulate *CycE* expression when all canonical Ttk69 binding sites were mutated. (C) Quantification of data presented in Figures 5B and 5C. β-Gal accumulation in control pIIa cells in pupae expressing the *ΔD-lacZ* construct (n = 29 control pIIa cells and 30 Ttk69-mutant pIIa cells) and in pupae expressing the *D-lacZ* construct (n = 16 control pIIa cells and 17 Ttk69-mutant pIIa cells). Note that the *ΔD-lacZ* construct was not downregulated by Ttk69 and behaves similar as to when Ttk69 was absent. Furthermore, the *D-lacZ* construct behaves as control and has a ratio of about 0.3, similar to that observed in *4,6WT-lacZ* and *4,6mlacZ*. (D) Quantification of data presented in Figure S3. Box plot showing quantification of Ttk69 immunostaining in socket cells in control (n = 10) and after Ham (n= 12) or Seq (n= 13) overexpression. Note that ratios were unchanged between these three conditions showing that the overexpression of Seq or Ham did not affect Ttk69 expression. (E, F) Quantification of data presented in Figure 6B, C. Box plots showing quantification of Seq immunostaining (E, n = 5) and Ham immunostaining (F, n = 11). Note that Ham but not Seq accumulation was reduced after Ttk69 overexpression reflecting a specific downregulation of *ham* expression by Ttk69. ***: $p < 0{,}001$, ns: not significant (Mann-Whitney test).

Figure S2. Expression pattern of *CycE* transcriptional reporters in SOs. (A) Diagram of *CycE* transcriptional reporters aligned to the *CycE* promoter at the top . Transcriptional starts accordingly to GBrowse and referenced restriction sites are indicated (S: Sal1; H: HindIII; K: Kpn1; Xb: Xba1; B: BssHII; A: Ale1; N : Nco1; Xh : Xho1). The localization of four specific

regions (A to D) is depicted. Black dots indicate localization of canonical AGGAC Ttk-binding sites. 4.6WT *CycE* transcriptional reporter bearing A to D regions; 4.6m, bearing A to D regions in which the eight AGGAC binding sites are mutated; ΔAC, bearing the B and D regions; ΔB, bearing the A, C, and D regions; ΔD, bearing the A to C regions and D bearing only the D region. (B-G') Expression pattern of the six transcriptional reporters described in (A). Pupae at 28 h APF. Sensory cells were identified by Cut immunoreactivity (green) and expression of *CycE* transcriptional reporters was revealed by β Gal immunoreactivity (B' to G' and in red in B to G). Note that the *ΔD-lacZ* construct is expressed in all sensory cells, whereas the expression of the other constructs is higher in the inner cells than in the outer cells. Scale bars: 10 μm.

Figure S3. Hamlet and Sequoia do not regulate *ttk69* expression. (A-C) Ttk69 expression under *ham* and *seq* over-expression conditions. Pupae were maintained at 18°C from 0 to 19h APF, shifted to 30°, and analyzed 7 h later. Sensory cells were visualized by YFP expression. Socket cells were identified by Su(H) (blue) and Ttk69 (red) immunoreactivity. Individual, Su(H) and Ttk69 channels are shown in inverted fluorescence (Middle and right panels). Note that, under these conditions, no cell fate transformation occurred, assessed by the normal expression of Su(H) in socket cells. (D, E). Ttk69 expression in clones null for *ham* and *seq* at two developmental times. Clones null for *ham* (D) and *seq* (E) were detected by the absence of GFP (blue, white dashed lines indicating the border of the clones) in pupae at 20, 22, and 24 h APF. Sensory cells were identified by Cut (green) and Ttk69 (red) immunoreactivity (shown also in separate panels). Note that no modification of Ttk expression is observed in mutant SOs (SOs inside the clone) relative to control SOs (SOs outside the clone) at earlier developmental times. Modifications observed at later timepoints are related to *ham* or *seq*-mediated changes in cell identity, rather than the direct action of *ham* or *seq* on *ttk* expression. (F, G) Quantification of experiments shown in D and E. Histograms showing the percentage of SOs harboring two to five Ttk-positive cells in SOs in control (SOs outside clones) and mutant (inside clones) conditions for *ham* (F) and *seq* (G) clones at 20, 22, and 24 h APF. The percentage of clusters with two to five (grey bars) Ttk69 cells is indicated. Note that no significant modifications are observed at earlier developmental times. Scale bars: 10 μm.

Figure S4. Known partners of Ttk69 do not affect bristle-cell lineage. Identity of SO cells under conditions of RNAi-mediated loss of function for *Mep1* (A), *trl* (GAGA factor) (B),

and for *Mi-2* (C) in wild-type (left) and Ttk69 heterozygous mutant backgrounds (right). Sensory cells are shown in green (GFP staining). Socket and shaft cells were detected by their specific accumulation of Pdm1 (red), and neurons or sheath cells by ELAV or Pros immunoreactivity (blue). Note that, in all contexts, SOs contained four terminal cells with two outer cells and two inner cells as in control organs. Scale bars: 10 μm.

Supplementary Movies:

Movie S1. *In vivo* imaging of a control SO during 19 h period beginning at 16 h after pupal formation (APF). Sensory cells identified by the expression of H2B-YFP. Anterior is to the left and the view is dorsal. Each frame was obtained by combining a z-stack (composed of optical sections separated by 1μm) acquired every 3 minutes.

Movie S2. *In vivo* imaging of a SO inside a Ttk69-mutant clone during 10.5h period beginning at 19h after pupal formation (APF). The clone was identified by the lack of GFP expression in epithelial cells. Sensory cells identified by the expression of H2B-YFP. Anterior is to the left and the view is dorsal. Each frame was obtained by combining a z-stack (composed of optical sections separated by 1μm) acquired every 3 minutes. Note supplementary divisions of the posterior pIIa daughter cell corresponding to the presumptive shaft cell (Arrow).

Movie S3. *In vivo* imaging of a SO inside a Ttk69-mutant clone during 9h period beginning at 21h after pupal formation (APF). The clone was identified by the lack of GFP expression in epithelial cells. Sensory cells identified by the expression of H2B-YFP. Anterior is to the left and the perspective is dorsal. Each frame was obtained by combining a z-stack (composed of optical sections separated by 1μm) acquired every 3 minutes. Note supplementary divisions of the anterior pIIa daughter cell corresponding to the presumptive shaft cell (Arrows).