Supplementary Materials and Methods

Fly strains

The following strains were used:

1) y w; FRT42D seq^{441}/CyO, hs-hid (this study) 2) y w; FRT42D seq^{U5}/CyO, hs-hid (this study) 3) y w; FRT42D seq^{N22}/CyO, hs-hid (this study) 4) y w; FRT42D seq^{R1}/CyO, hs-hid (this study) 5) y w; FRT42D seq^{Q8}/CyO, hs-hid (this study) 6) y w; FRT42D iso (this study) 7) y w; FRT42D y+ 8) y w; FRT42D seq^{22}/CyO (Brennan et al, 2001) 9) w; FRT82B pros^{17}/TM3, Sb (Manning & Doe, 1999) 10) y w, Ubx-FLP; ham E7-2-36 FRT40A/CyO (Moore et al, 2004) 11) UAS-D-Pax2 (Kavaler et al, 1999) 12) UAS-Pros (Manning & Doe, 1999; Reddy & Rodrigues, 1999b) 13) UAS-Ham (Moore et al, 2004) 14) w; FRT42D seq^{U5}/CyO; UAS-Dpax (this study) 15) w; FRT42D seq^{U5}/CyO; UAS-Pros (this study) 16) w; FRT42D seq^{U5}/CyO; UAS-Ham (this study) 17) w; FRT42D iso; UAS-D-Pax2 (this study) 18) w; FRT42D iso; UAS-Pros (this study) 19) w; FRT42D iso; UAS-Ham (this study) 20) y w Ubx-FLP; FRT42D Ubi-GFPnls (this study) 21) y w Ubx-FLP; Ubi-GFPnls FRT40A (this study) 22) y w, Ubx-FLP; FRT82B M(3) Ubi-GFPnls (this study) 23) y w, Ubx-FLP; FRT42D tub-GAL80 y^{+}; neur-GAL4 UAS-CD8::GFP/SM5-TM6B, Tb (this study) 24) y w, hs-FLP UAS-CD8::GFP; FRT42D tub-GAL80 y^{+}; tub-GAL4 25) UAS-numb-GFP.

Immunostainings

Dissections and stainings were performed as described previously (Berdnik et al, 2002). Primary antibodies utilized in this study are rat α-Elav 1:500 (7E8A10; DSHB),

**S2 cell transfection and luciferase assays**

Transfection and luciferase assays were performed according to (Giagtzoglou et al, 2005). All constructs were verified by sequencing.

**SEM**

Adult animals were collected and aged on fresh food for 2–3 days and then taken through an EtOH dehydration series. The flies were washed in 1 ml of 30, 50, 70, 90, 100, and 100% EtOH in water for 12–24 hr per step. Hexamethydisilizane (HMDS; Electron Microscopy Sciences, Fort Washington, PA) was then used to chemically dry the samples. The flies were washed for 30 min in 500 μl of the following solutions: 75% EtOH + 25% HMDS, 50% EtOH + 50% HMDS, 25% EtOH + 75% HMDS, 100% HMDS, 100% HMDS, and 100% HMDS. The final 100% HMDS wash was allowed to evaporate under vacuum in the presence of anhydrous calcium sulfate (Drierite; W. A. Hammond Drierite, Xenia, OH). The samples were then mounted on double-stick carbon
tabs (Ted Pella, Redding, CA), which had been previously mounted onto aluminum specimen mounts (Electron Microscopy Sciences). Next, the samples were coated under vacuum using a Balzer MED 010 evaporator (Technotrade International, Manchester, NH) with platinum alloy for a thickness of 25nm and then flash carbon coated. Images were captured with a JSM-5910 scanning electron microscope (JEOL, USA, Inc., Peabody, MA) and processed with Adobe Photoshop software.
Supplementary Figure Legends

Supplementary Figure 1: Gain of socket cells in seq mutant clusters

(A) A 100X SEM image of a fly which harbors Ubx-FLP induced sequoia clones. (B) A 500X SEM image of a fly which harbors Ubx-FLP induced sequoia clones. (C) A 2000X image of a wild-type bristle/socket. (D) A 2000X image of a sequoia mutant multiple socket structure.

Supplementary Figure 2: Specificity of anti-Seq antibody

(A) Sequoia is absent in ESO clusters within negatively marked (by the absence of GFP) clones of seq^{A41} mutant tissue. (B-C) Single channel representations of Cut (B), which marks all the cells of the ESO clusters, and Seq (C).

Supplementary Figure 3

The socket phenotype of sequoia is not due to a sensitized genetic background. Clones of seq^{A41} (A) and seq^{U5} (B) generated by a heat shock induced FLPase demonstrate the “multi-socket” phenotype (compare with Figure 1A-B). The arrowheads point to sockets and the asterisk in panel A points to abnormal bristles that are occasionally observed within seq mutant clones.

Supplementary Figure 4: Seq has a dynamic expression pattern in the ESO lineage.

Anterior is up and Sens or Cut (red) mark the ESO cells at different stages of development in panels A-D. In panel E, Elav (red) marks the neuron. Seq expression in wild-type precursor cells is shown in blue, which is represented in grayscale below all the panels. (A/A’) Seq is expressed in the dividing SOP I cell (div pI). (B/B’) Seq is initially
expressed equally in the pIIa and the pIIb, but is downregulated in the pIIa at the late pII stage. (C/C’) Seq is expressed strongly in the pIIIb and glia (g), but is weakly expressed, and eventually downregulated in, the hair (h) and socket (so) cells. (D/D’, E/E’) Seq is expressed in the internal cells at the 4 cell stage, but by ~28 APF is restricted to the neuron. (F) Cartoon representing the expression pattern of Seq in the ESO lineage. Striped circles represent cells where Seq is initially expressed and is later downregulated.

**Supplementary Figure 5: Senseless is unaffected in seq mutant clusters**

(A and A’) The level of Senseless in sequoia mutant clones is comparable to that of wild-type.

**Supplementary Figure 6: Seq can act as an activator of the UAS-tk-luc reporter in S2 cell transcription assays**

Schematics of the UAS-tk-luc reporter construct and S2 cell transcription assays using the UAS-tk-luc reporter. Full-length Seq is able induce strong expression from the reporter. Amino acids (a.a) within the C terminal domain of Seq carry the ability to activate transcription from the reporter. Each series of four columns represents increasing transfection concentrations from 10, 50, 100 to 200 ng of Seq.

**Supplementary Figure 7: The expression of D-Pax2 and Prospero is not affected in seq mutant embryos.**

Pax2, Prospero are not lost from seq mutant embryos. Hamlet although reduced is not completely abolished. These results suggest that different regulatory relationships exist between these genes at different developmental stages. Note however that the number and position of different neurons in the embryonic peripheral nervous system is affected.
in seq mutant embryos, suggesting that there are developmental abnormalities. The red asterisk signifies the position of the lateral chordotonal organs. The red arrow in E, F indicates non-specific staining of Hamlet in a tubular structure, presumably part of the trachea. In all images, the GFP channel has been removed for clarity of images. A-B) D-Pax2. C,D) Prospero, E, F) Hamlet immunostainings in control seq^{A41}/CyO, twist-GFP (A, C, E) and seq^{A41}/ seq^{A41} (B, D, F) homozygous mutant embryos. All images are projections of confocal slices from embryos of stage 15. All embryos are aligned along the anterior (left)-posterior (right) axis.
Supplementary Figure 2

A: GFP (wt) Cut Sequoia

B: Cut

C: Sequoia

10 μm
Supplemental Figure 5

A

WT Senseless

A'

Red dots indicate cells with a specific marker.