Supplemental information

Cell-type identity of the avian utricle

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Figure S1. Anatomy of the chicken utricle and identification of non-sensory single cells. Related to Figure 1 and Table S1.

(A) Whole mount immunostaining for Tenascin c (TNC, white) shows association with striolar type I hair cells, which are MYO7A-positive and SOX2-negative in a high magnification confocal plane image of the striola (right panel). Scale bars = 200µm and 10µm, as indicated.

(B) The tSNE plot shows single cells colored by affiliation with sensory epithelium (S1-3 and S5-8; green) and non-sensory cells (S4; magenta).
(C) Volcano plot showing the differential expression between sensory epithelium cells (S1-3 and S5-8; green) and non-sensory cells (S4; magenta). Dots represent genes. Dots above a log2 fold change > 2 and FDR < 0.01 are highlighted and some gene names are shown.

(D) tSNE plots of all cells showing USH1C expression in sensory epithelium cells and expression of COCH, DCN and OLFML3, which are found in non-sensory cells. Log2 expression scales for each gene is shown (Log2Ex).
Figure S2. Asymmetric mRNA location in supporting cells and hair cell markers. Related to Figure 2 and Table S1.

(A) *In situ* hybridization and HCR for *MATN4*, *ZBTB20*, *OTOGL* and *ADGRG6* in supporting cells. *MATN4* mRNA was restricted to the apical supporting cell region, *ZBTB20* mRNA in basal regions, and *OTOGL* and *ADGRG6* mRNAs were located in the whole cytoplasm of supporting cells.

(B) *In situ* hybridization for *GPX2*, *C4H4ORF50*, *B3GNTL*, and *SYT14* show robust expression in hair cells. Scale bar = 100µm for (A and B).
Figure S3. Striolar and extrastriolar hair cell marker gene expression. Related to Figure 3.

(A) HCR for SYT12 mRNA (magenta, white) and CABP1 mRNA (green, white). SYT12 mRNA was detected in striolar type I hair cells and CABP1 mRNA is expressed by extrastriolar type II hair cells. Scale bars = 100µm and 50µm, as indicated.

(B) HCR for KCNQ5 mRNA (magenta, white) reveals distinct expression in striolar type I hair cells. Scale bars = 100µm and 50µm, as indicated.
Figure S4. Striolar and extrastriolar hair cell marker gene expression. Related to Figure 3.

HCR for TMC2 mRNA (magenta, white) and RASD2 mRNA (green, white). TMC2 mRNA was detected in all striolar hair cells and RASD2 mRNA was restricted to extrastriolar hair cells. Scale bars = 100µm and 50µm, as indicated.
**Figure S5. Supporting cell marker gene expression. Related to Figure 4.**

HCR for *SMOC2* mRNA (magenta, white) reveals distinct expression in striolar supporting hair cells, particularly in the central region where the line of polarity reversal is located. *CXCL14* mRNA (green, white) is detected in supporting cells surrounding the SMOC2-expressing cells and in extrastriolar supporting cells. Scale bars = 100µm and 50µm, as indicated.
Figure S6. Reversal zone supporting cells and striolar type II hair cells. 
Related to Figure 4 and Table S1.

(A) Vibratome section of a P7 chicken utricle immunolabeled with antibodies to MYO7A (magenta) and GATA3 (white) and in situ hybridization for GATA3 to identify reversal zone striolar supporting cells and hair cells. Magnified panels on the right show GATA3-labeled nuclei exclusively in striolar supporting cells. Nuclei were stained with DAPI and F-ACTIN was labeled with phalloidin. Scale bars = 100µm and 20µm, as indicated.

(B) Whole mount immunostaining and in situ hybridization for TECTB (green, white as indicated) reveals striolar supporting cells associated with the reversal zone (arrow). MYO7A (magenta) and SOX2 (white) antibodies were used to label hair cells and supporting cells. Scale bars = 100µm and 20µm, as indicated.

(C) Expression levels of GATA3 and TECTB is projected into the CellTrails map and show association with striolar supporting cell group S1, transitional supporting cells S3, nascent hair cells S6, and striolar type II hair cell group S7.

(D) Shown are supporting cells that co-express GATA3 and TECTB. The volcano plot shows the result of a differential expression analysis of GATA3/TECTB double-positive supporting cells with all other supporting cells. Forty-two genes are expressed at distinct high levels in the GATA3/TECTB double-positive supporting cell group.

(E) Shown are cluster S7 striolar hair cells that co-express GATA3 and TECTB. The volcano plot shows the result of a differential expression analysis of GATA3/TECTB double-positive hair cells with all other hair cells. Thirty-four genes are expressed at distinct high levels in the GATA3/TECTB double-positive hair cell group.
Figure S7. Pathway enrichment analysis.
Related to Figures 3, 4, 5, 6, and 7, and Table S1.

(A) Enrichment map of GO processes and Reactome pathways enriched in supporting cells (S1-S2), dedifferentiating supporting cells (S3), nascent hair cells (S6), extrastriolar type II hair cells (S5), striolar type I (S8) and type II (S7) hair cells. Nodes (circles) in the enrichment map show distinct pathways, and edges (grey lines) represent overlapping genes between pathways. Nodes are colored by state affiliation.
(B) Heatmap of 565 expressed cell cycle process genes (GO:0022402) over all states shows four gene groups (clusters). Rows: genes; Columns: Mean Log₂ read counts in states as indicated; Color scale indicates expression values, pink: lowest; yellow: highest.

(C) S3-associated gene group cluster 1 (pink, 17 genes, log2mean > 2) and S6-associated gene group cluster 3 (blue, 158 genes, log2mean > 2) underwent pathway enrichment analysis (G:Profiler) to reveal GO processes and Reactome pathways. Pathway enrichment analysis results were visualized (EnrichmentMap) and interpreted (AutoAnnotate) in Cytoscape.