Supplemental Figure 1. The mouse dorsolateral otocyst undergoes a rapid thinning and expansion similar to that occurring in the chick otocyst. (A-C) Transverse paraffin sections (stained with hematoxylin and eosin) at E9.5, 10.5, and 11.5. Asterisks mark the area of the otocyst undergoing rapid thinning and expansion. nt, neural tube; sg, statoacoustic ganglion; D, dorsal; M, medial. (A'-C') 3-D reconstructions of mouse otocysts from the sets of serial sections used to illustrate Figures A-C. Asterisks mark the area of the otocyst undergoing rapid thinning and expansion.

Supplemental Figure 2. Expression of *Bmp* transcripts in the chick otocyst and adjacent tissues suggests a role for BMP signaling in patterning the dorsolateral otocyst. Whole mount in situs (A-C, E-G) of *Bmp4* and *7*, and sectioned in situs (D, H) at the levels indicated by the lines in (B) and (F). D, dorsal; A, anterior; L, lateral. (A-D) At stages 16-18, *Bmp4* is expressed in the dorsolateral region of the otocyst (HH 16: n = 8; HH 18: n = 8), and at HH 20, it is expressed in two localized spots marking the anterior and posterior cristae (n = 8). ed, endolymphatic duct. (E-H) At stages 16-20, *Bmp7* is expressed predominantly in anterior and posterior regions of the otocyst. Fainter expression of *Bmp7* also extends into the dorsolateral otocyst (HH 16: n = 8; HH 18: n = 8; HH 20: n = 8). ed, endolymphatic duct.

Supplemental Figure 3. Expression of both type-I and type-II BMP receptors in the chick otocyst suggests a role for BMP signaling in patterning the dorsolateral otocyst. Both type-IA and type-II BMP receptors are expressed throughout the wall of the chick otocyst, but type-IB does not seem to be expressed (A-C, arrows; D-F, otocysts enlarged; type-IA: n = 12; type-IB: n

= 12; type-II: n = 12), as well as being broadly expressed in the cranial region of the embryo at stage 18; asterisks mark the dorsolateral otocyst. Sections (G-I) of the otocyst show expression, especially dorsolaterally (asterisks), in the otocyst of type-IA and type-II receptors, but the type-IB receptor is not expressed in the dorsolateral otocyst (asterisks). D, dorsal; A, anterior; L, lateral; nt, neural tube.

Supplemental Figure 4. Expression of both type-I and type-II BMP receptors in the mouse otocyst suggests a role for BMP signaling in patterning the dorsolateral otocyst. Both type-I and type-II BMP receptors are expressed throughout the wall of the mouse otocyst (A-C, arrows; D-F, otocysts enlarged; type-IA: n = 4; type-IB: n = 4; type-II: n = 4), as well as in being broadly expressed in the cranial region of the embryo at E10.0; asterisks mark the dorsolateral otocyst. Sections (G-I) of the otocyst show expression throughout the otocyst of type-IA and type-II receptors, but the type-IB receptor seems to be expressed only weakly in the dorsolateral otocyst (asterisks). D, dorsal; A, anterior; L, lateral; nt, neural tube.

Supplemental Figure 5. The mouse dorsolateral otocyst expresses pSMAD1/5/8, indicating that this portion of the otocyst receives and responds to BMP signaling as does the chick dorsolateral otocyst. (A-C) Transverse sections (labeled with pSMAD1/5/8 antibody and Hoechst stain) showing pSMAD1/5/8 expression in the mouse otocyst at E9.5, E10.5, and E11.5 (E9.5: n = 2; E10.5: n = 2; E11.5: n = 2). Asterisks mark the dorsolateral otocyst, which undergoes rapid thinning and expansion; the ventral otocyst, which in (B, C) is initiating outgrowth to form the cochlear duct (cd), also expresses pSMAD1/5/8; D, dorsal; L, lateral; nt, neural tube; ed, endolymphatic duct.

Supplemental Figure 6. Increased cell proliferation is not the cause of hyperexpansion of the dorsolateral otocyst in chick embryos in which *Bmp4* is over expressed. (A, B) Transverse sections pulsed with EdU (green) and counterstained with Hoechst stain (blue) and E-cadherin (red) from a control embryo and an experimental embryo at 48 hours after *Bmp4* over expression (HH 20; control: n = 3; Bmp4: n = 3). The embryos were labeled with EdU during the last 24 hours of incubation. (A', B') Enlargements of the boxes in A and B showing epithelial thinning in the dorsolateral otocyst wall. Only a few EdU-positive cells are present in the dorsolateral otocyst of control and experimental embryos, in contrast to the ventral otocyst. Asterisks mark the dorsolateral otocyst. D, dorsal; L, lateral; nt, neural tube. (C) Graph showing the quantification of EdU-labeled cells (means plus standard error of the mean from 3 control and 3 experimental embryos; EdU-labeled cells contained within 4-5 sections from individual embryos were counted) in the HH 20 dorsolateral otocyst. (D) Graph showing the percentage of EdU-positive cells present in the HH 20 dorsolateral otocyst (means plus standard error of the mean from 3 control and 3 experimental embryos; EdU-labeled cells within 4-5 sections from individual embryos were counted). *$P < 0.05$ (Student's T-test). *Bmp4* over expression did not increase the number of proliferating cells in the dorsolateral otocyst; rather, the statistically significant decrease in the percentage of dividing cells suggests that *Bmp4* over expression actually decreased the number of dividing cells in the dorsolateral otocyst.

Supplemental Figure 7. Programmed cell death is not the cause of failure of expansion of the dorsolateral otocyst in chick embryos in which *Noggin* is over expressed. (A, B) Transverse sections labeled with TUNEL and Hoechst stain from a control embryo and an

experimental embryo at 24 hours after *Noggin* over expression (HH 20; control: n = 4; Noggin: n = 4). In both the control and experimental embryo, no TUNEL-positive cells were detected in the dorsolateral otocyst (asterisks). Asterisks mark the dorsolateral otocyst, which fails to undergo normal expansion after treatment with *Noggin*. In contrast, scattered TUNEL-positive cells populate the head mesenchyme. D, dorsal; L, lateral; nt, neural tube; ed, endolymphatic duct.

Supplemental Figure 8. Programmed cell death is not the cause of attenuated expansion of the dorsolateral otocyst in chick embryos treated with Dorsomorphin. (A, B) Transverse sections labeled with TUNEL and Hoechst stain from a control embryo and an experimental embryo at 12 hours after Dorsomorphin treatment (HH 18; control: n = 3; Dorsomorphin: n = 3). In both the control and experimental embryo, very few TUNEL-positive cells are present in the otocyst, and none are present in its dorsolateral region. However, TUNEL-positive cells occupy the head mesenchyme and surface ectoderm overlying the otocyst of both the experimental and control embryos. Asterisks mark the dorsolateral otocyst. D, dorsal; L, lateral; nt, neural tube; ed, endolymphatic duct.

Supplemental Figure 9. Programmed cell death is not the cause of failure of expansion of the dorsolateral otocyst in chick embryos subjected to *Smad6* over expression. (A, B) Transverse sections stained with TUNEL and Hoechst stain from a control embryo and an experimental embryo at 24 hours after *Smad6* over expression (HH 20; control: n = 3; Smad6: n = 3). In both the control and experimental embryo, no TUNEL-positive cells are present in the dorsolateral (asterisks) otocyst. D, dorsal; L, lateral; nt, neural tube; ed, endolymphatic duct.