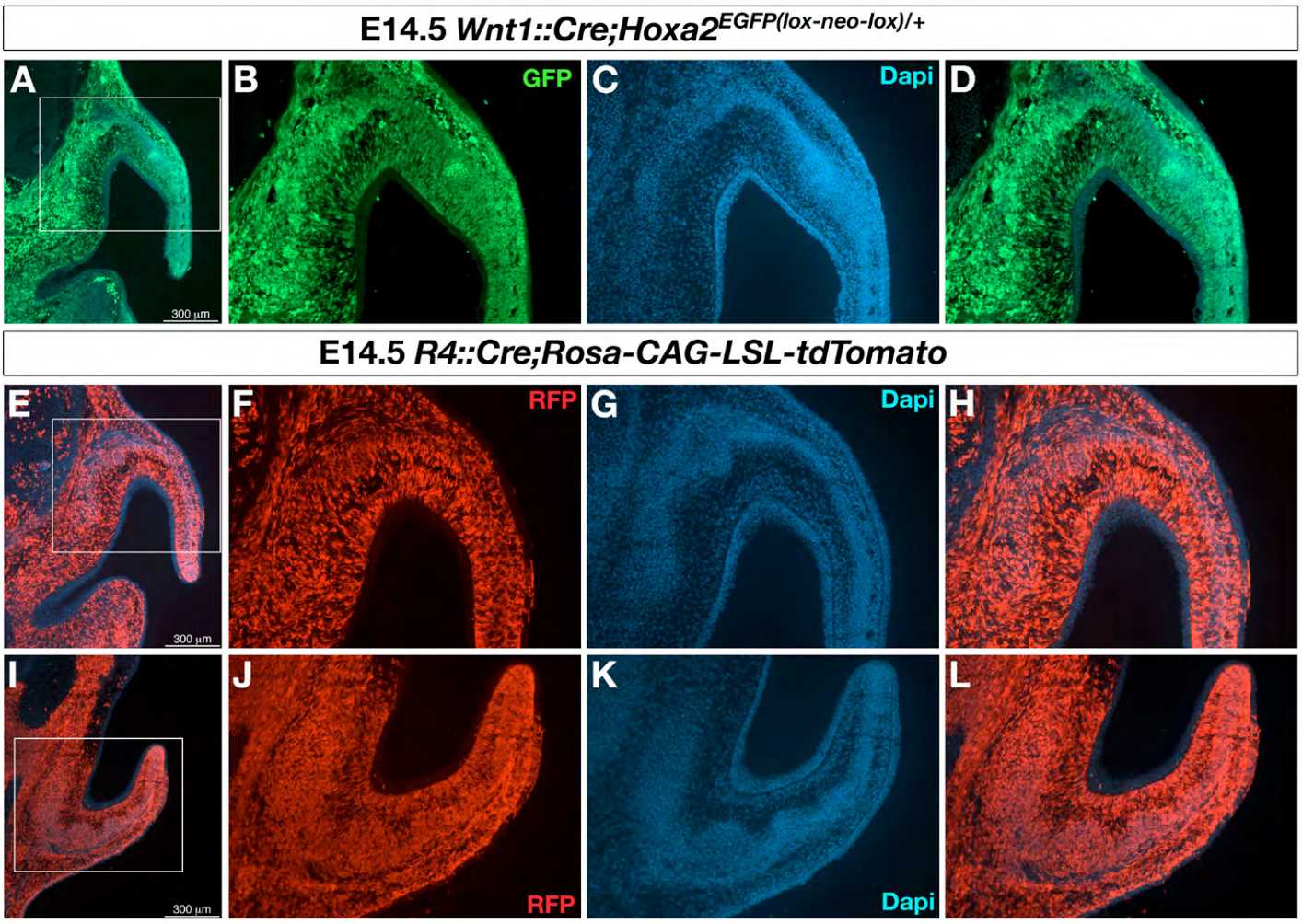

Fig. S1. Fate mapping of neural crest-derived pinna. (A-L) Anti-EGFP (A, B, D) and anti-RFP (E, F, H, I, J, L) immunostaining and DAPI staining (A, C, D, E, G, H, I, K, L) on frontal (A-D, E-H) and horizontal (I-L) sections through the external ear of E14.5 *Wnt1::Cre;Hoxa2*$^{EGFP(lox\text{-}neo\text{-}lox)/+}$ (A-D) and *R4::Cre;Rosa-CAG-LSL-tdTomato* (E-L) fetuses. In A and D, E and H, I and L, DAPI and anti-EGFP (A, D) or DAPI and anti-RFP (E, H, I, L) stainings are merged. B-D, F-H and J-L are higher magnifications of the domains outlined in A, E and I, respectively.

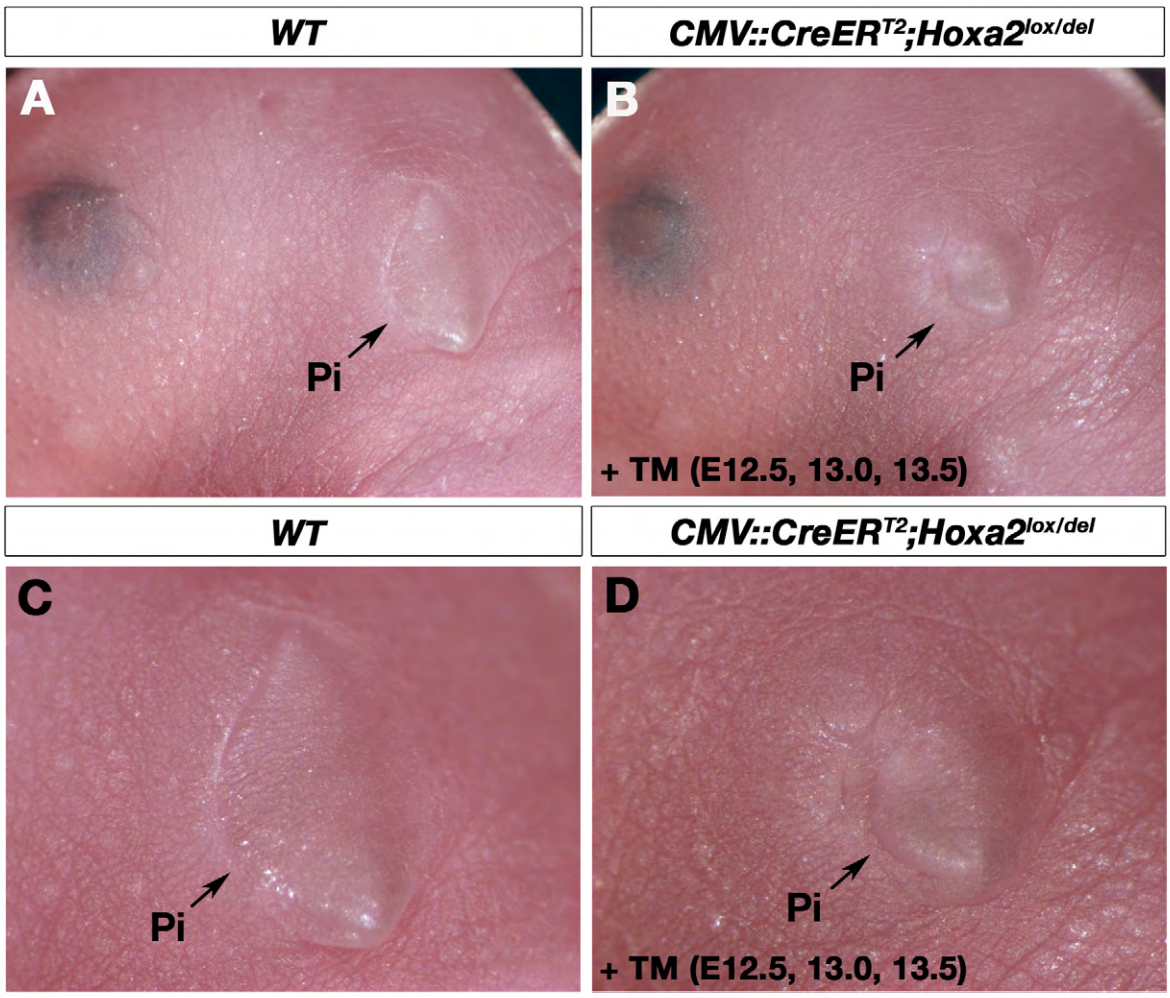

Fig. S2. Temporal requirement of Hoxa2 in pinna morphogenesis. (A-D) Lateral views of the head of E18.5 *wild-type* (*WT*) (A, C) and *CMV::CreER^{T2};Hoxa2$^{lox/del}$* mutant fetuses treated with tamoxifen (TM) at E12.5, E13.0 and E13.5 (B, D). C and D are enlarged views of pinnae (arrows, Pi) in A and B, respectively. Note the small pinna resulting from late Hoxa2 downregulation (B, D).

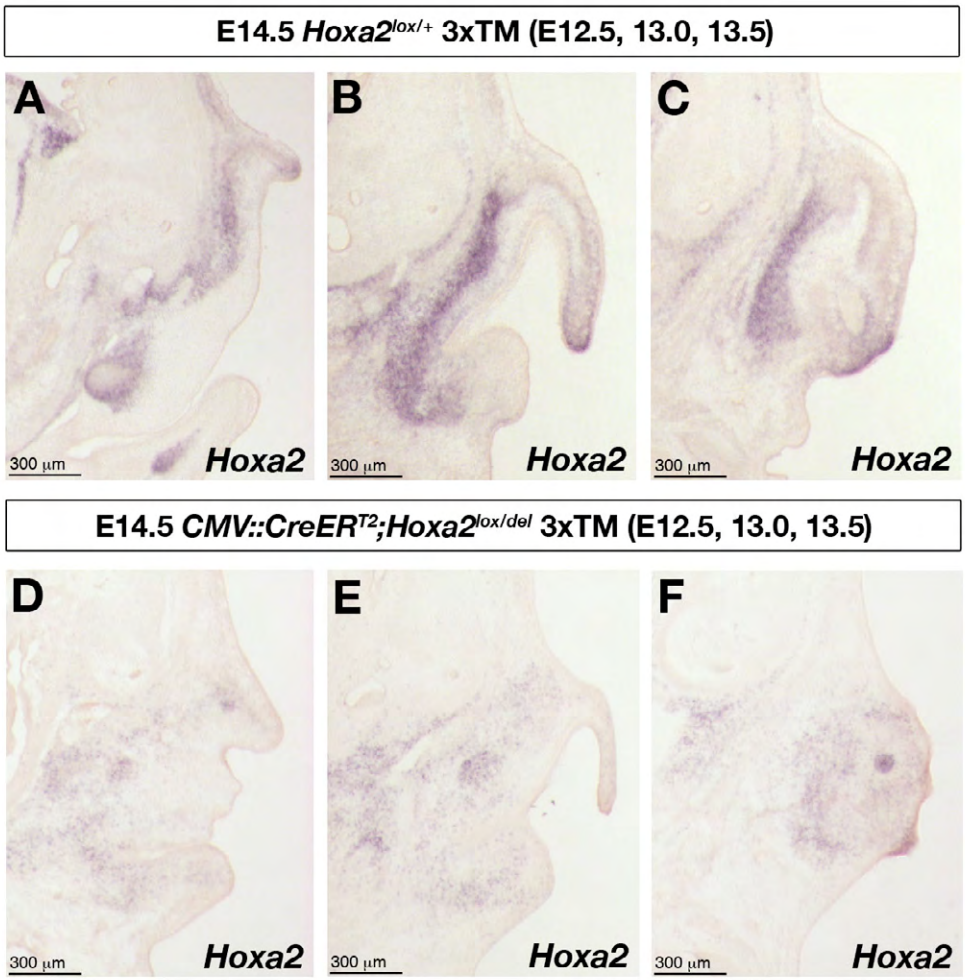

Fig. S3. Loss of *Hoxa2* expression in TM-treated mutants. (A-F) *Hoxa2 in situ* hybridization on frontal sections through the external ear of E14.5 *Hoxa2*$^{lox/+}$ control (A-C) and *CMV::CreER*^{T2}*;Hoxa2*$^{lox/del}$ mutant (D-F) fetuses treated with tamoxifen (TM) at E12.5, E13.0 and E13.5. Top is dorsal, bottom is ventral and sections are from anterior to posterior. Note the significant downregulation of *Hoxa2* expression in D-F.

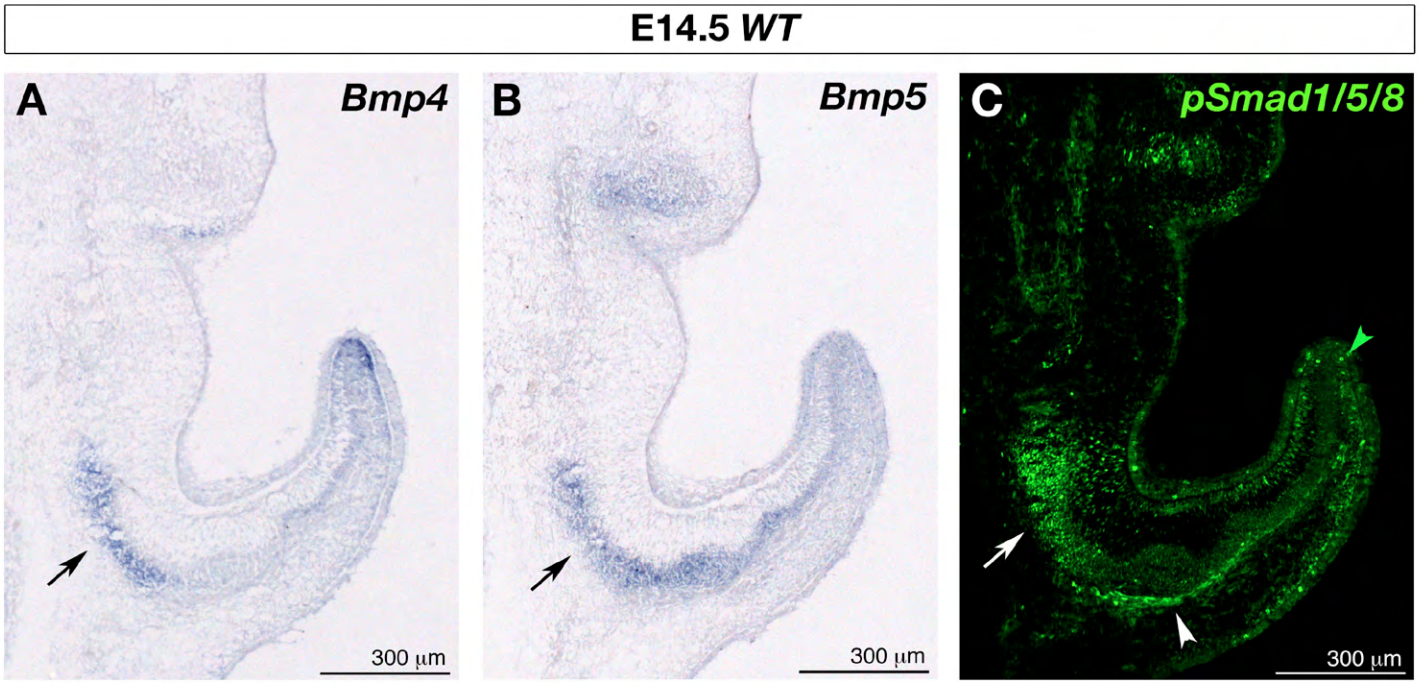

Fig. S4. Distribution of phosphoSmad1/5/8, *Bmp4* and *Bmp5* expression in the developing pinna. (A-C) *Bmp4* (A) and *Bmp5* (B) *in situ* hybridization and anti-phosphoSmad1/5/8 antibody immunostaining (C) on adjacent horizontal sections through the external ear of E14.5 *wild-type* (*WT*) fetuses. Black arrows in A and B, and the white arrow in C show *Bmp4*, *Bmp5* and phosphoSmad1/5/8 positive cells at the base the pinna. The white arrowhead in C shows the Eya1^{+} subpopulation of phosphoSmad1/5/8 positive cells next to the pinna mesenchymal core of *Bmp5* expression. The green arrowhead in C shows phosphoSmad1/5/8 activated cells at the tip of the pinna, just beneath the ectoderm. Top is anterior, bottom is posterior.

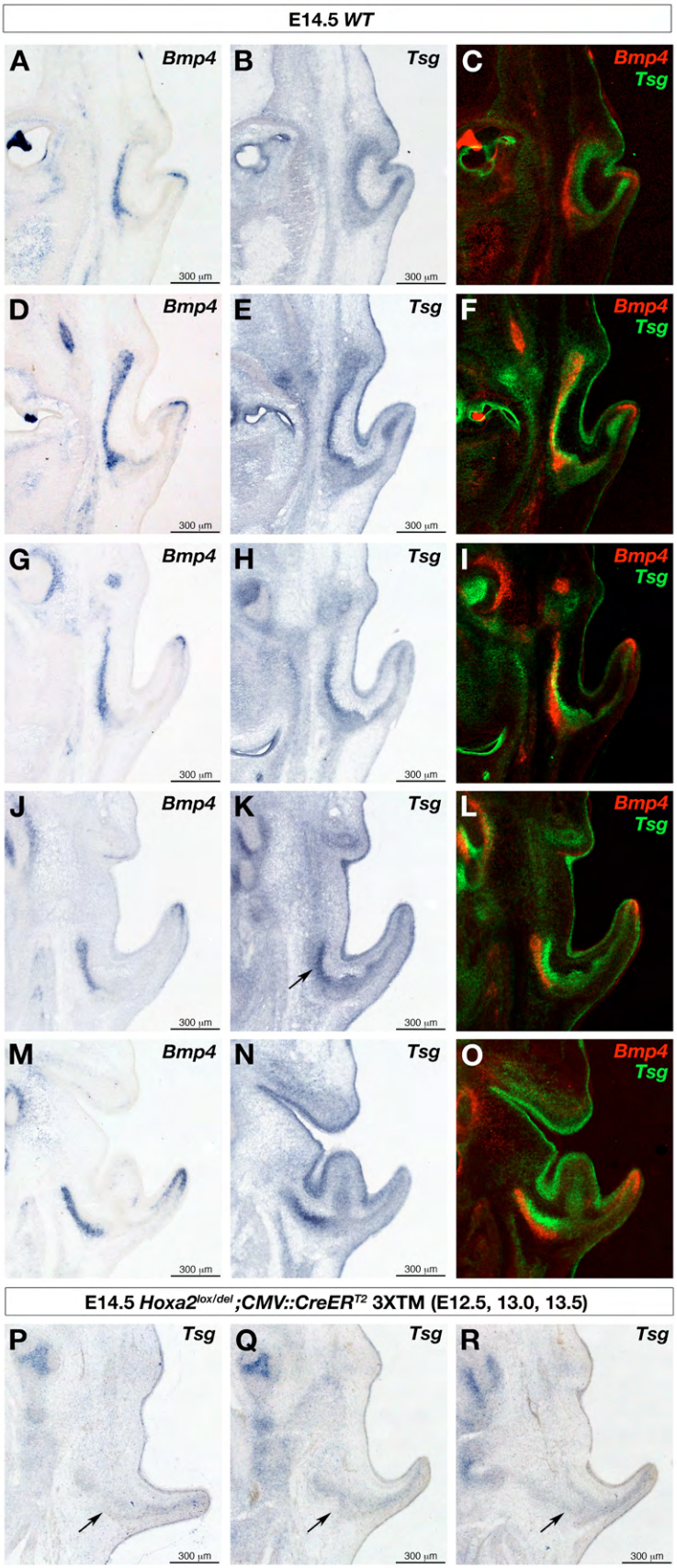

Fig. S5. Hoxa2 positively regulates *Tsg*. (A-O) *In situ* hybridization on adjacent horizontal sections through the external ear of E14.5 *wild-type* (*WT*) fetuses using *Bmp4* (A, D, G, J, M) and *Tsg* (B, E, H, K, N) probes. A merge between adjacent sections hybridised with *Bmp4* and *Tsg*, respectively (C, F, I, L, O) reveals that *Tsg* is highly expressed directly adjacent to the *Bmp4* positive domain. (P-R) *Tsg in situ* hybridization on horizontal sections through the external ear of E14.5 *CMV::CreER^{T2};Hoxa2$^{lox/del}$* mutant fetuses treated with tamoxifen (TM) at E12.5, E13.0 and E13.5. Top is anterior, bottom is posterior.

Fig. S6. Validation by qPCR of Hoxa2 binding at *Bmp4* and *Bmp5* non-coding regions. (A) Fold enrichment of Hoxa2 over IgG negative control antibody (Neg Ab) is shown for each Hoxa2-bound region. Values correspond to the average of duplicate samples and are representative of two independent experiments. Itih4 is a negative control gene (unbound region). The numbers in brackets correspond to the FDR (False Discovery Rate) of each bound region in Hoxa2 ChIP-seq (Donaldson et al., 2012). (B) Sequence alignment and conservation of Hoxa2-bound regions across Vertebrates, provided by the UCSC browser (Meyer et al., 2013).

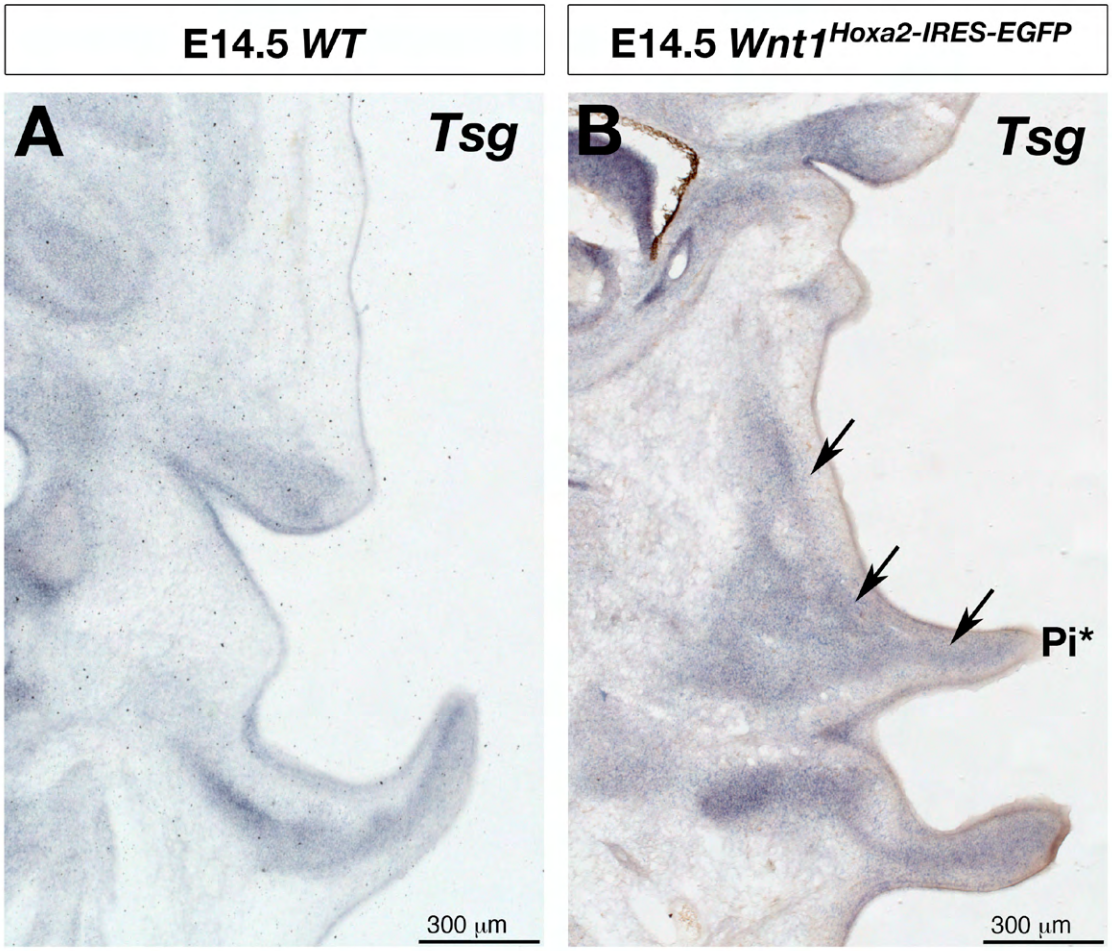

Fig. S7. *Tsg* is ectopically expressed in the Hoxa2-induced duplicated pinna. (A, B) *Tsg in situ* hybridization performed on horizontal sections through the external ear of E14.5 *Wild type* (*WT*) (A) and *Wnt1*$^{Hoxa2\text{-}IRES\text{-}EGFP}$ mutant (B) fetuses. Top is anterior, bottom is posterior. The black arrows indicate *Tsg* ectopic expression in the duplicated pinna (Pi*) and more anteriorly.