Supporting Information

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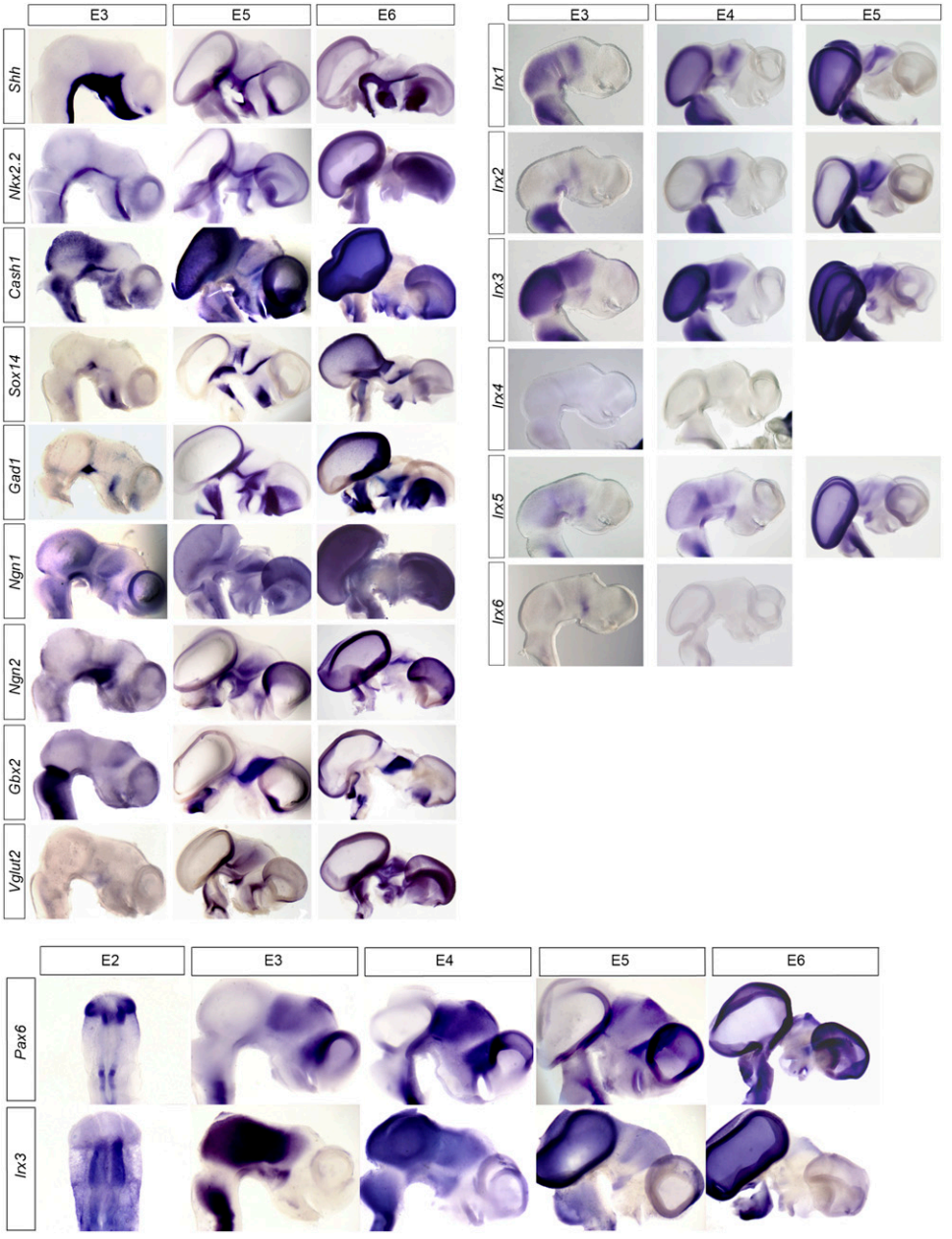

Fig. S1. Spatiotemporal expression patterns of genes discussed in this study. Lateral views of embryonic day (E)2–6 chick brains stained by in situ hybridization for the expression of the genes indicated on the left of each row of panels. Anterior points to the right. Note persistent expression of *Irx1*, *Irx2*, *Irx3*, and *Irx5* in the thalamic primordium and progressive down-regulation of *Pax6* immediately posterior to the ZLI.

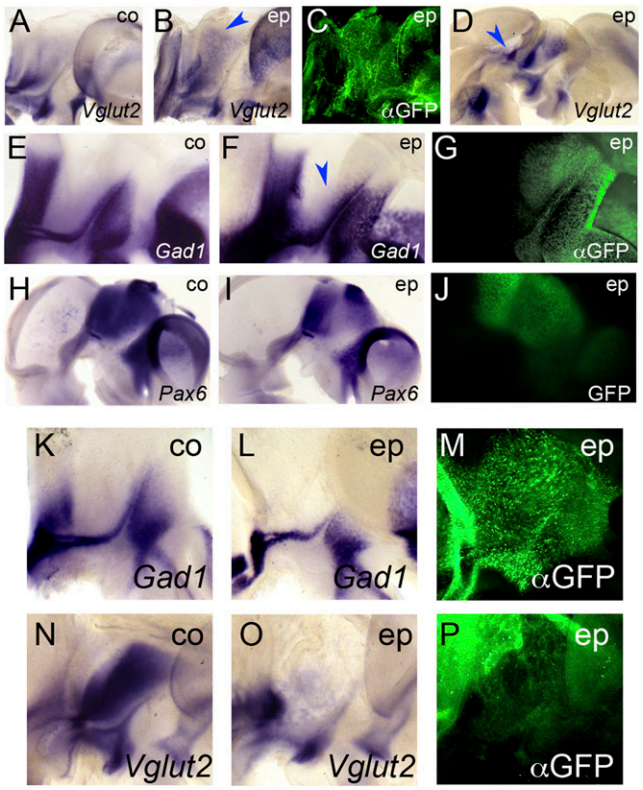

Fig. S2. Ectopic activation of Shh signaling results in ectopic induction of *Vglut2* and *Gad1* and in down-regulation of *Pax6* between the forebrain–midbrain boundary (FMB) and zona limitans intrathalamica (ZLI); Irx3 repressor function is required for thalamic *Gad1* and *Vglut2* expression. Lateral views of hemisected chick brains electroporated (ep) with SmoM2 (*A*–*J*) or Irx3-VP16 (*K*–*P*) between E2.5 and E3 and fixed 3 dpe (*A*–*C*, *E*–*G*, and *K*–*P*), 4 dpe (*D*), or 2 dpe (*H*–*J*). Anterior points to the right. (*A*, *E*, *H*, *K*, and *N*) Unelectroporated control halves (co). In situ hybridization for *Vglut2* (*A*, *B*, *D*, *N*, and *O*), *Gad1* (*E*, *F*, *K*, and *L*) and *Pax6* (*H* and *I*). (*C*, *G*, *M*, and *P*) Anti-GFP immunofluorescence (αGFP); *J* shows pre-in situ GFP fluorescence. Blue arrowheads point to areas of ectopic gene induction. Note formation of ectopic *Vglut2*+ cluster of cells in the pretectum in *D* (compare Fig. S1).

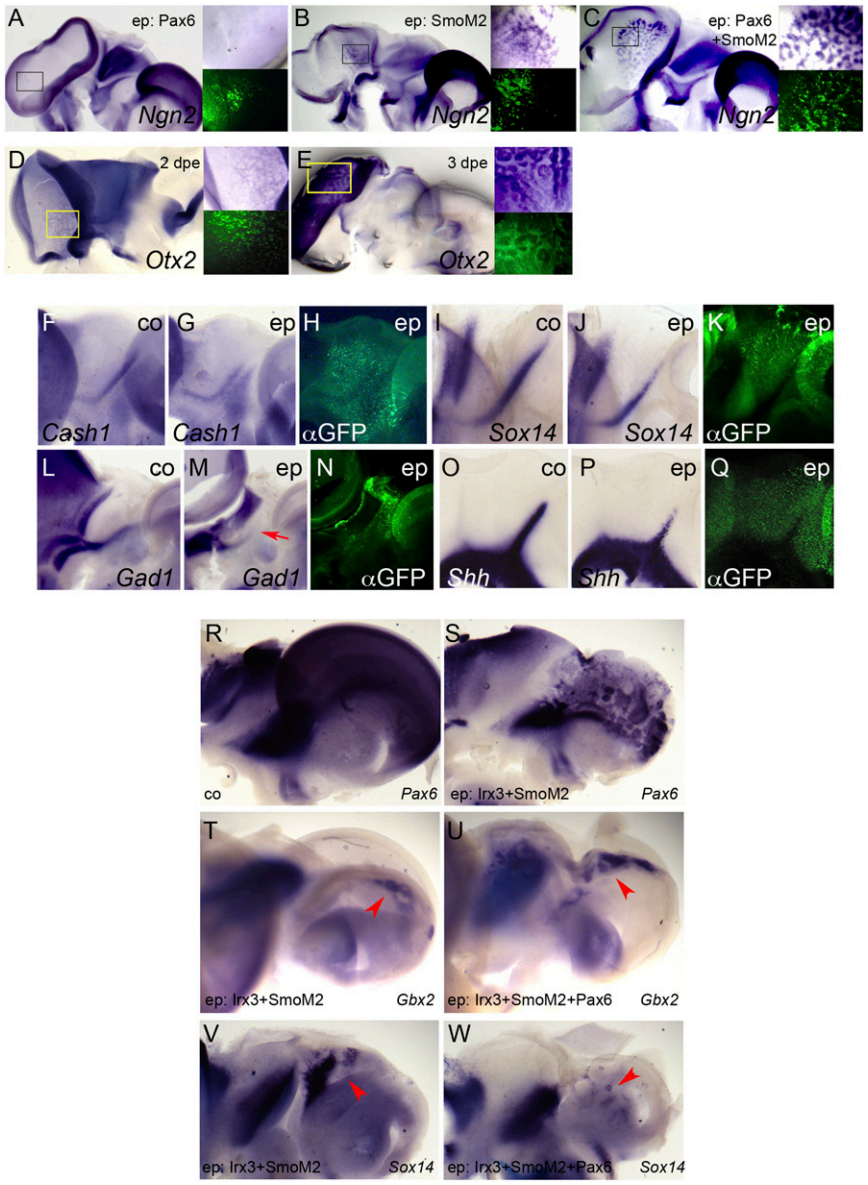

Fig. S3. Pax6 mediates competence for cTh induction by Shh and antagonizes rTh identity and *Shh* expression. Lateral views of chick brains electroporated between E2.5 and E3 with Pax6 (*A*), SmoM2 (*B*), or Pax6+SmoM2 (*C*–*E*) into the midbrain, with Pax6 into the diencephalon (*F*–*Q*) or with Irx3+SmoM2 (*R*, *S*, *T*, and *V*) or Irx3+SmoM3+Pax6 (*U* and *W*) into the telencephalon and fixed at 1 dpe (*O*–*Q*), 2 dpe (*D* and *F*–*H*) or 3 dpe (*A*–*C*, *E*, *I*–*N*, and *R*–*W*). Anterior points to the right. In situ hybridization for *Ngn2* (*A*–*C*), *Otx2* (*D* and *E*), *Cash1* (*F*–*H*), *Sox14* (*I*–*K*, *V*, and *W*), *Gad1* (*L*–*N*), *Shh* (*O*–*Q*), *Pax6* (*R* and *S*) or *Gbx2* (*T* and *U*). (*Insets* in *A*–*E*) Magnification of boxed area in main panel (*Upper Right*) and corresponding anti-GFP immunofluorescence (*Lower Right*). Note weak induction of *Ngn2* by SmoM2 alone (*B*) compared with strong induction by Pax6+SmoM2 (*C*). Note down-regulation of *Otx2* in the thalamus by E3 (*E*). Red arrow in *M* points to rudimentary ZLI. Red arrowheads point at domains of ectopic gene expression in *T*–*W*.