Fig. S1. Gata2 expression is initiated at ~E10.5. (A-H) ISH analysis of Gad1, Gata2, Tal2, Ascl1 and Gata3 expression on coronal sections of WT diencephalon at E10.5 (A,B) and E11.5 (D-H). Gata2 and HuC/D co-IHC in P1 at E10.5 (C). Scale bars: in A,D, 100 μm; in C, 50 μm.

Fig. S2. Gata2 colocalizes with Ascl1 and Helt and is expressed in the postmitotic GABAergic precursors in P1 and pTh-R. (A-P) IHC on was used to analyse the colocalization of Gata2 and Ascl1 (A,B), Gata2 and Helt (C,D), Gata2 and p27 (G,H), Gata2 and HuC/D (I,J), Gata2 and PCNA (K,L) and Gata2 and PH3 (O,P) on coronal sections of E12.5 mouse diencephalon. BrdU incorporation in Gata2+ cells was analysed after a 1-hour BrdU pulse (E,F) and a 12-hour BrdU pulse (M,N). Images from the P1 and pTh-R regions are shown as indicated. v, ventricle. Scale bars: 50 μm.
Fig. S3. Complete loss of Gata2 expression in the Foxg1cre;Gata2F/F brain. Gata2 mRNA expression was analysed by whole mount in situ hybridization on E12.5 WT and Foxg1cre, Gata2F/F embryonic brains. di, diencephalon; fb, forebrain; mb, midbrain.

Fig. S4. GABAergic-to-glutamatergic fate transformation in the P1 and loss of GABAergic marker expression in the pTh-R in Foxg1cre; Gata2F/F embryos at E12.5. (A-Y) ISH analysis of Gad1, Slc17a6, Helt, Gata3, Tal1, Tal2, Sox14, Npy, Six3, Nkx2-2, Ascl1 and Ngn2 expression in WT and Foxg1cre; Gata2F/F embryos at E12.5. Arrows in A-D indicate the regions where the fate transformation is most apparent. Arrowheads in A and B indicate the loss of Gad1 expression in pTh-R. The border between P1 and P2 was defined by the expression of Gbx2 on adjacent sections. Scale bar: 100 μm.
Fig. S5. pTh-R GABAergic precursors do not acquire glutamatergic phenotype in the absence of Gata2. (A–H’) ISH analysis of Gad1, Slc17a6, Gata3 and Npy in WT and Foxg1<sup>cre</sup>; Gata2<sup>F/F</sup> embryos at E13.5. Coronal view. (A’–H’) Higher magnification views from the pTh-R. Arrows in B’, D’, F’ and H’ indicate the pTh-R GABAergic progenitors that lose Gata3 and Npy expression but do not upregulate Slc17a6. Scale bars: 100 μm.
Fig. S6. Premature cell-cycle exit and reduction of progenitor cycling in the $Ascl1^{KO}$ pTh-R. (A-J) IHC analysis of Sox2 and p27 (A-D), cyclin D1 (E,F) and ISH analysis of cyclin D1 and $Ngn2$ (G-J) in the WT and $Ascl1^{KO}$ pTh-R region at E12.5. Arrows indicate downregulation of Sox2 (B), cyclin D1 protein (F) and cyclin D1 mRNA (H) in the pTh-R. Scale bar: 100 μm.
Fig. S7. Summary charts of gene expression in WT and changes in gene expression in Foxg1<sup>−/−</sup>; Gata2<sup>+/−</sup> and Ascl1<sup>−/−</sup> diencephalon. P1-P3, diencephalic prosomeres; pTh-R, rostral part of P2; ZLI, zona limitans interthalamica; A, anterior; P, posterior.