

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 μ m; in C, 50 μ m.

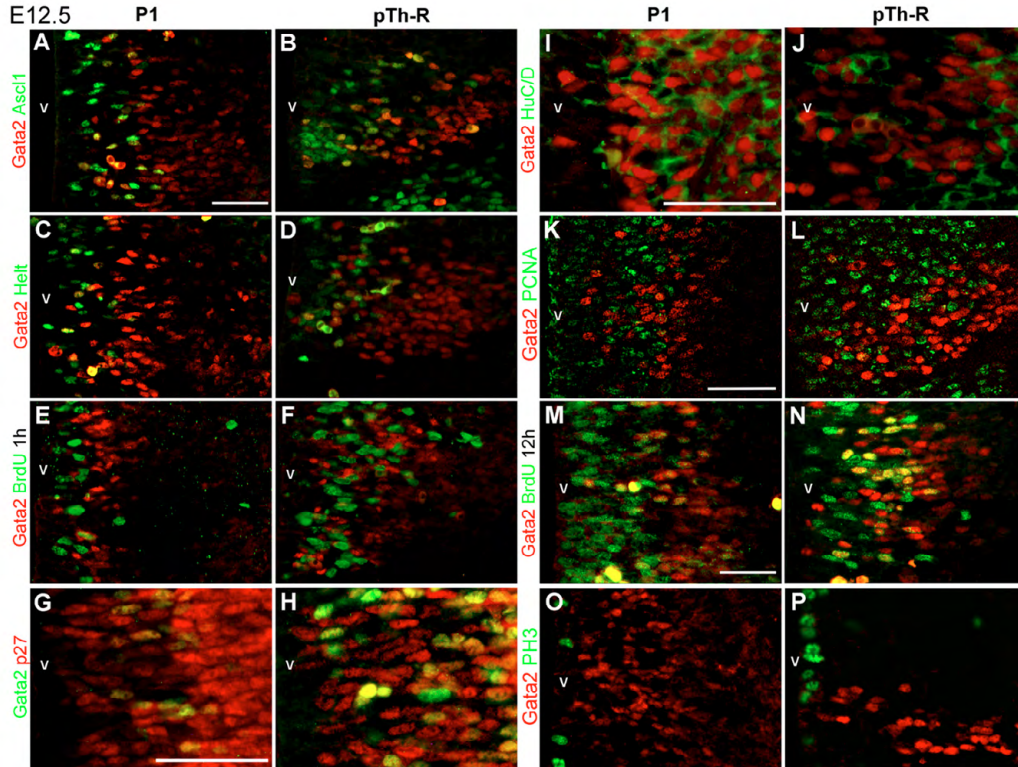


Fig. S2. Gata2 colocalizes with *Ascl1* and *Helt* and is expressed in the postmitotic GABAergic precursors in P1 and pTh-R. (A-P) IHC on was used to analyse the colocalization of *Gata2* and *Ascl1* (A,B), *Gata2* and *Helt* (C,D), *Gata2* and p27 (G,H), *Gata2* and HuC/D (I,J), *Gata2* and PCNA (K,L) 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 μ m.

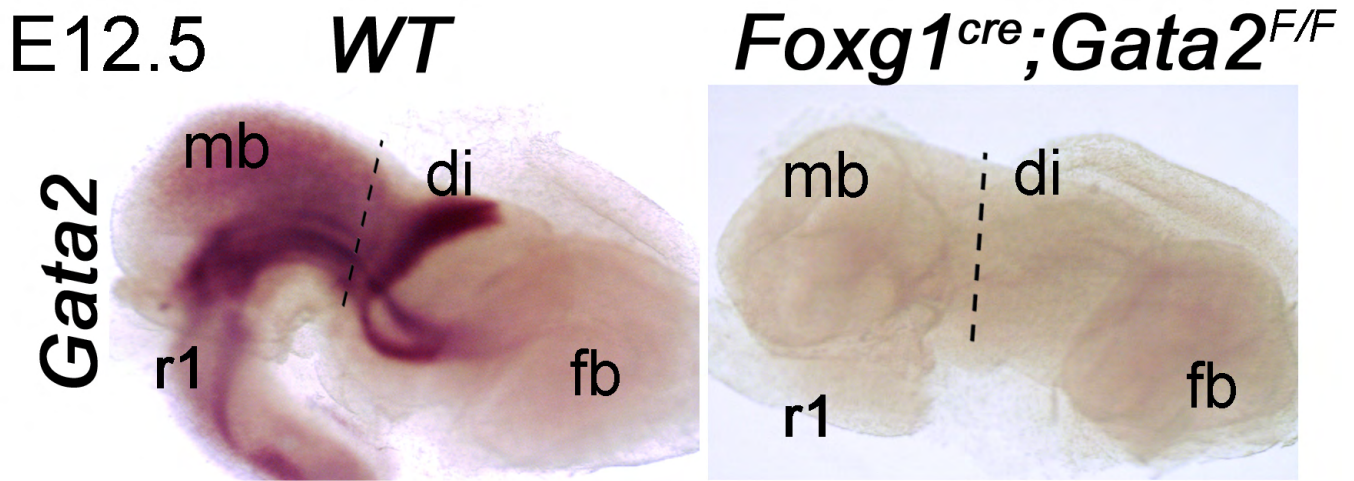


Fig. S3. Complete loss of *Gata2* expression in the *Foxg1^{cre};Gata2^{F/F}* brain. *Gata2* mRNA expression was analysed by whole mount in situ hybridization on E12.5 WT and *Foxg1^{cre};Gata2^{F/F}* embryonic brains. di, diencephalon; fb, forebrain; mb, midbrain.

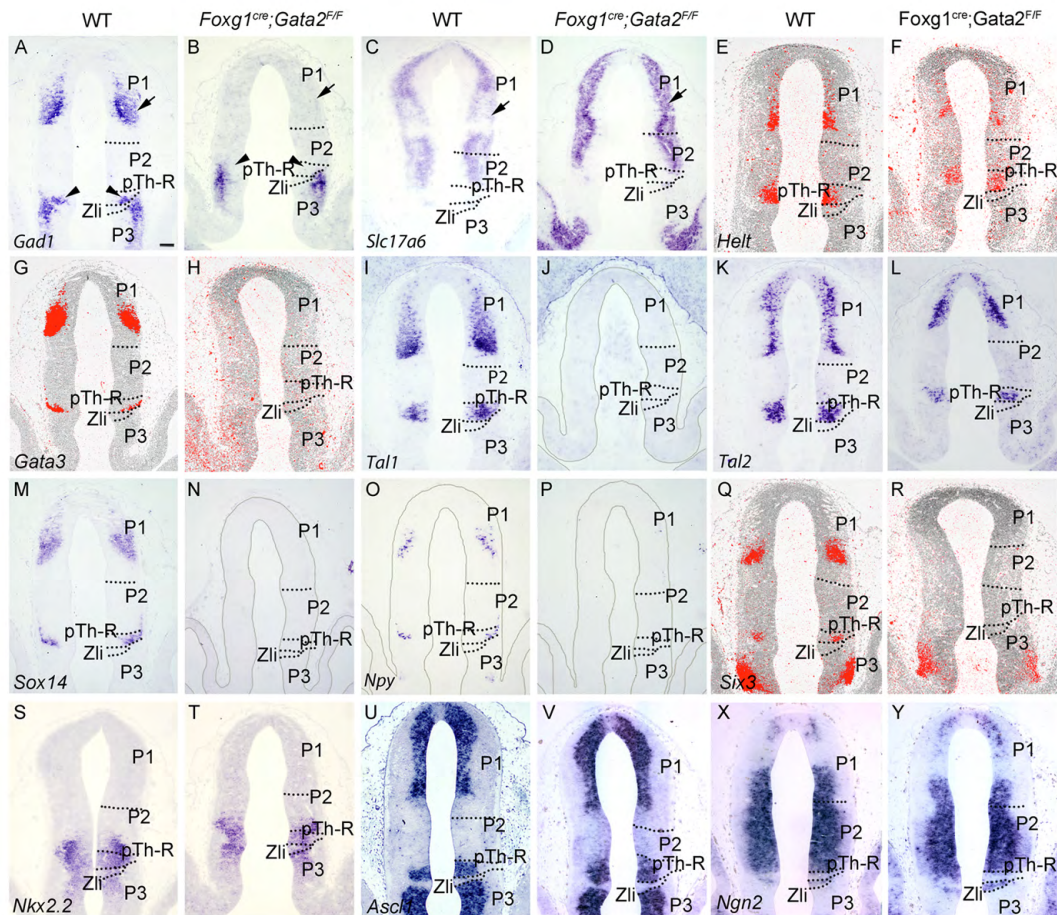


Fig. S4. GABAergic-to-glutamatergic fate transformation in the P1 and loss of GABAergic marker expression in the pTh-R in *Foxg1^{cre};Gata2^{F/F}* embryos at E12.5. (A-Y) ISH analysis of *Gad1*, *Slc17a6*, *Helt*, *Gata3*, *Tal1*, *Tal2*, *Sox14*, *Npy*, *Six3*, *Nkx2-2*, *Asc1* and *Ngn2* expression in WT and *Foxg1^{cre};Gata2^{F/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 μ m.

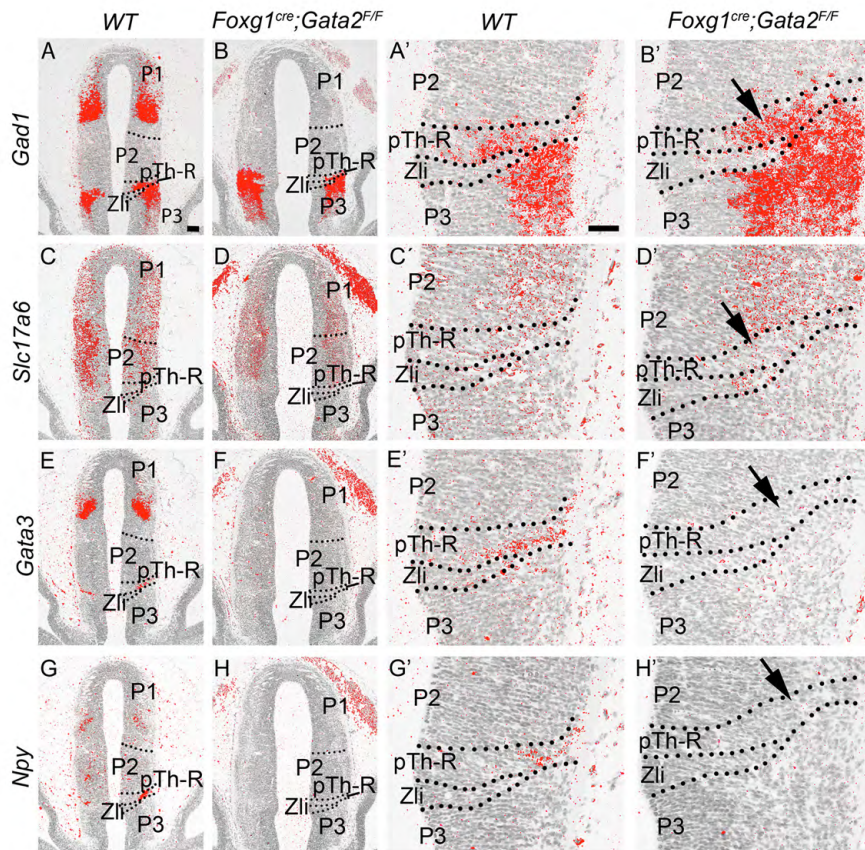


Fig. S5. pTh-R GABAergic precursors do not acquire glutamatergic phenotype in the absence of Gata2. (A-H') ISH analysis of *Gad1*, *Slc17a6*, *Gata3* and *Npy* in WT and *Foxg1^{cre}; Gata2^{F/F}* embryos at E13.5. Coronal view. (A'-H') Higher magnification views from the pTh-R. Arrows in B', D', F' and H' indicate the pTh-R GABAergic progenitors that lose *Gata3* and *Npy* expression but do not upregulate *Slc17a6*. Scale bars: 100 μ m.

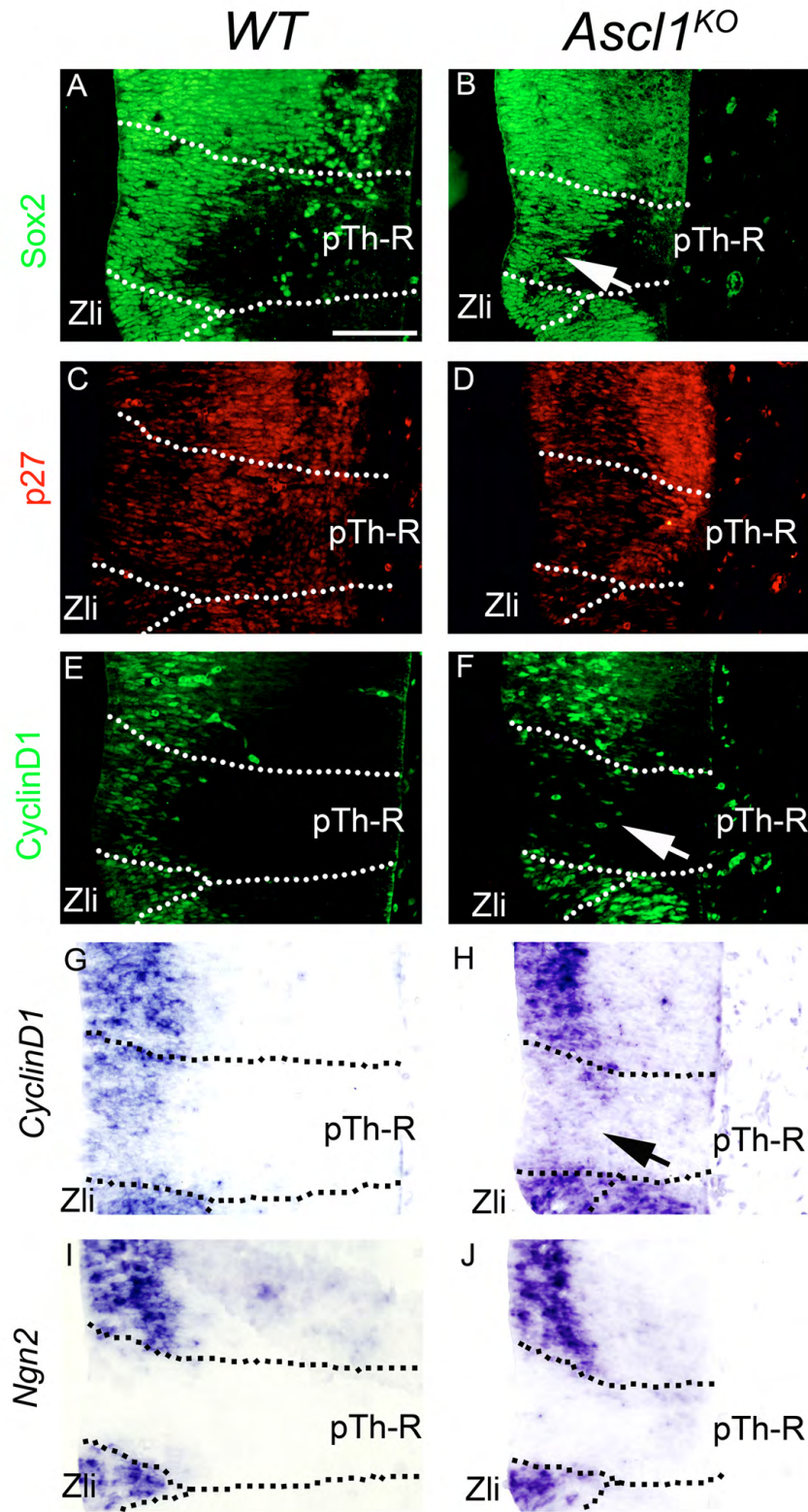


Fig. S6. Premature cell-cycle exit and reduction of progenitor cycling in the *Ascl1*^{KO} pTh-R. (A-J) IHC analysis of Sox2 and p27 (A-D), cyclin D1 (E,F) and ISH analysis of cyclin D1 and *Ngn2* (G-J) in the WT and *Ascl1*^{KO} pTh-R region at E12.5. Arrows indicate downregulation of Sox2 (B), cyclin D1 protein (F) and cyclin D1 mRNA (H) in the pTh-R. Scale bar: 100 μ m.

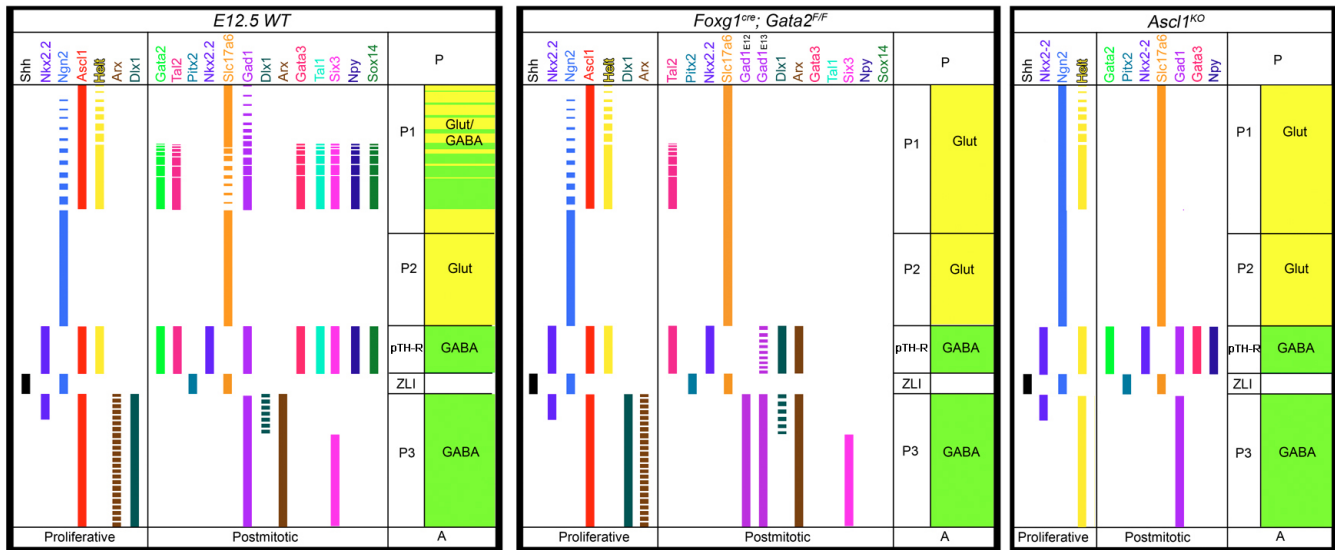


Fig. S7. Summary charts of gene expression in WT and changes in gene expression in *Foxg1^{cre}; Gata2^{F/F}* and *Ascl1^{KO}* diencephalon. P1-P3, diencephalic prosomeres; pTh-R, rostral part of P2; ZLI, zona limitans interthalamica; A, anterior; P, posterior.