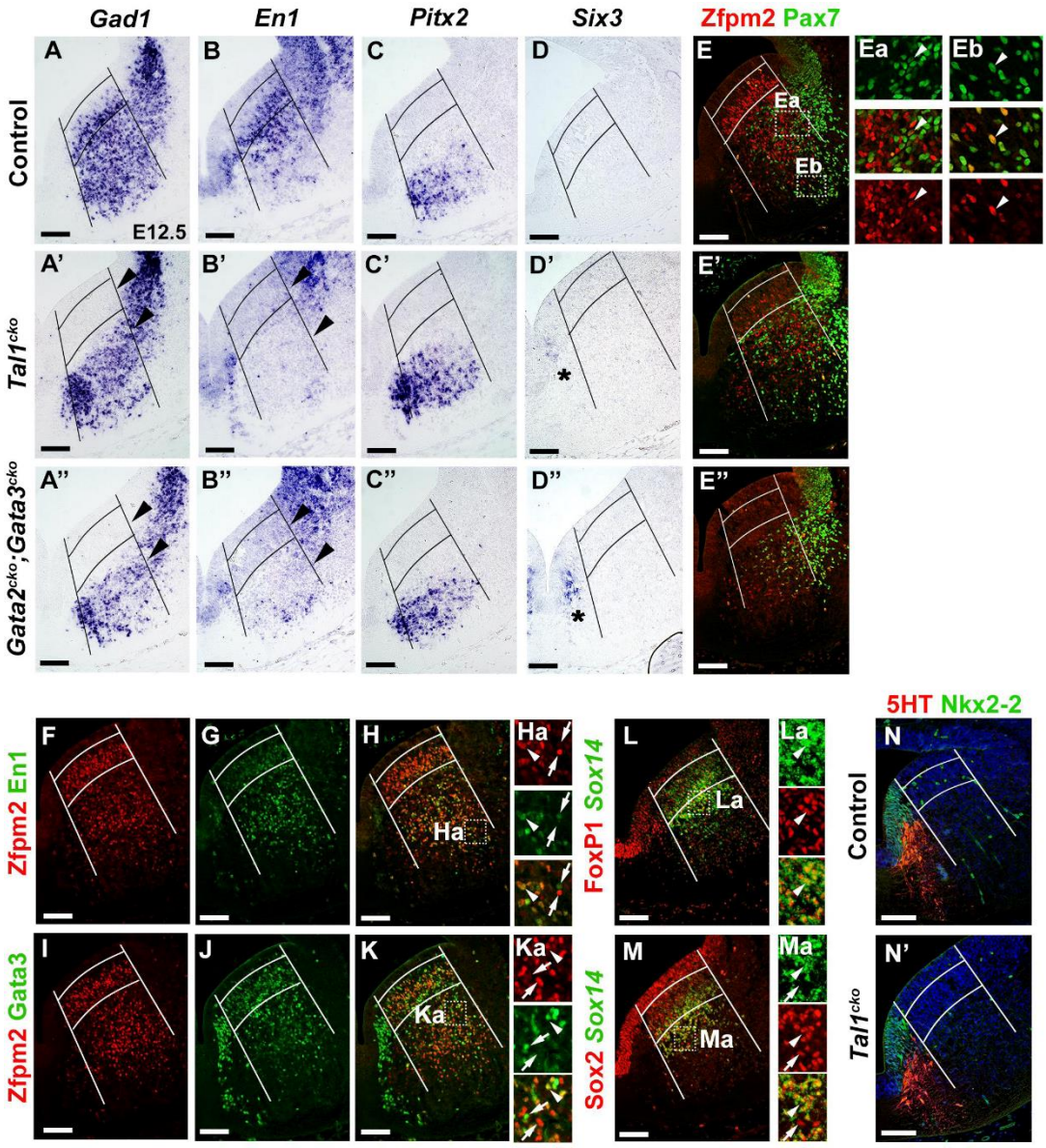
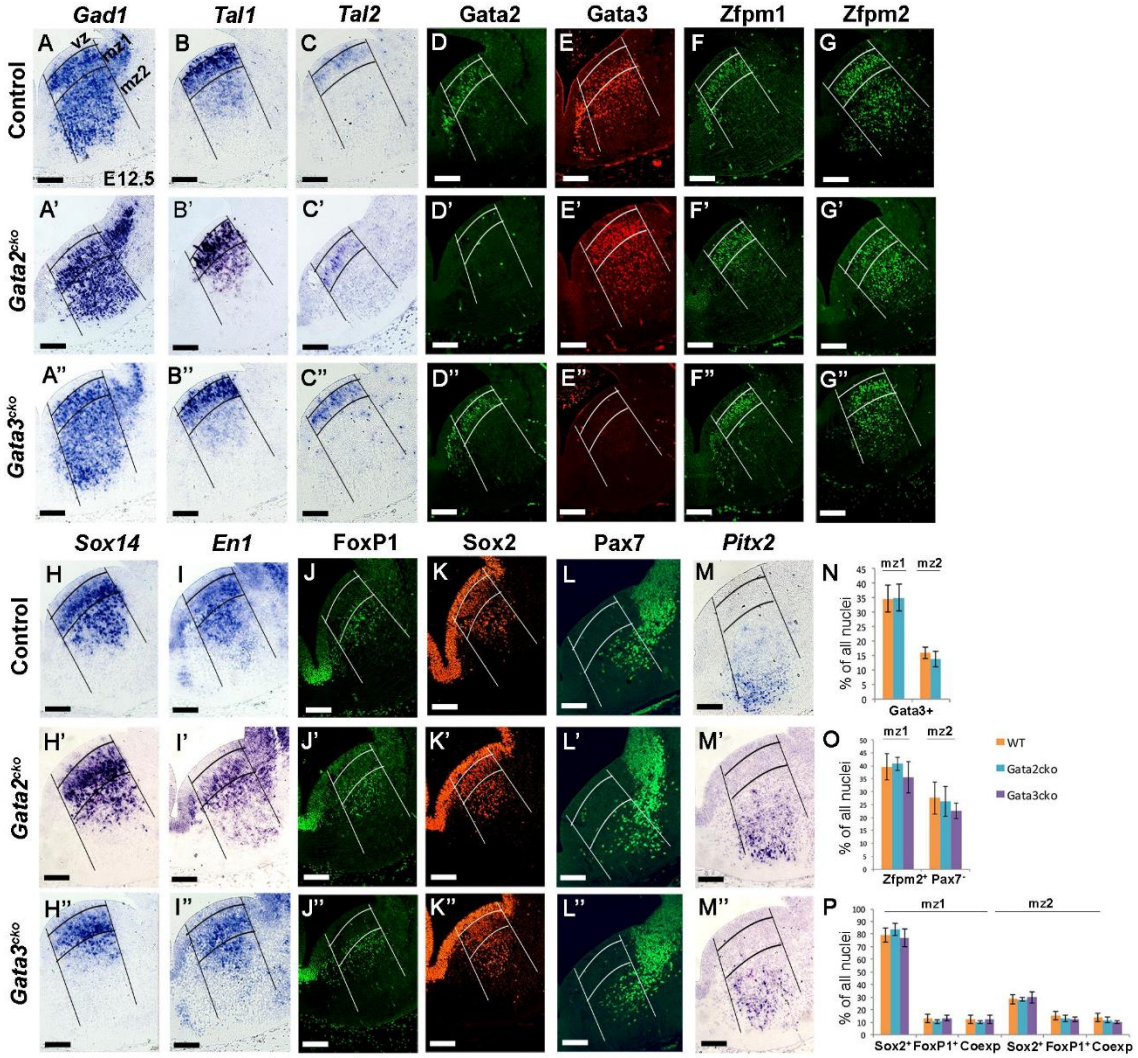


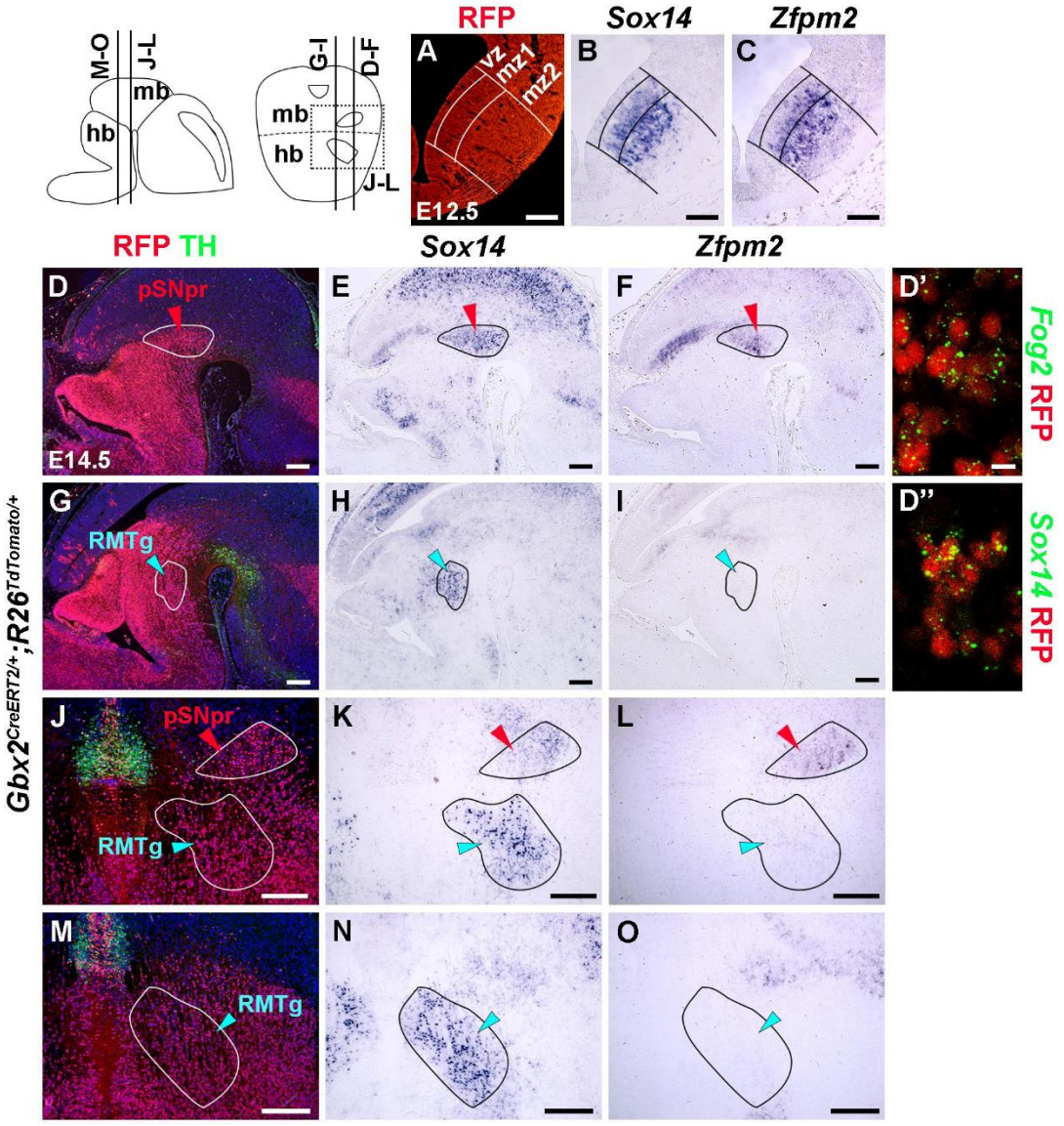
Supplementary Figure S1. Tal1 and Gata2/3 dependent and independent GABAergic neuron subtypes in r1.

(**A-N'**) ISH (A-D''), fluorescent ISH and IHC (L-M, close-ups in La,Ma) and IHC (E-E'',F-K,N,N', with higher magnification images in Ea,Eb,Ha-Ma) of transversal sections of r1. *Gad1* expression is shown here as a reference. The rV2 domain is indicated with lines, with boundaries of ventricular zone (vz), mantle zone 1 (mz1) and mantle zone 2 (mz2) defined similarly as in Figure 1. Black arrowheads (A'-B'') point to altered gene expression. White arrowheads indicate coexpression and white arrows lack of co-expression in (Ea-Ma). An asterisk (*) indicates ectopic Six3 expression, which is detected very weakly in *Tal1*^{cko} (D') and more strongly in *Gata2*^{cko};*Gata3*^{cko} (D'') mutants. This may be indicative of precursor fate transformation in the ventral Nkx2-2^{+} domain of the mutants (M.H., L.T., M.S., J.P., in preparation). Scale bars 200 μm.



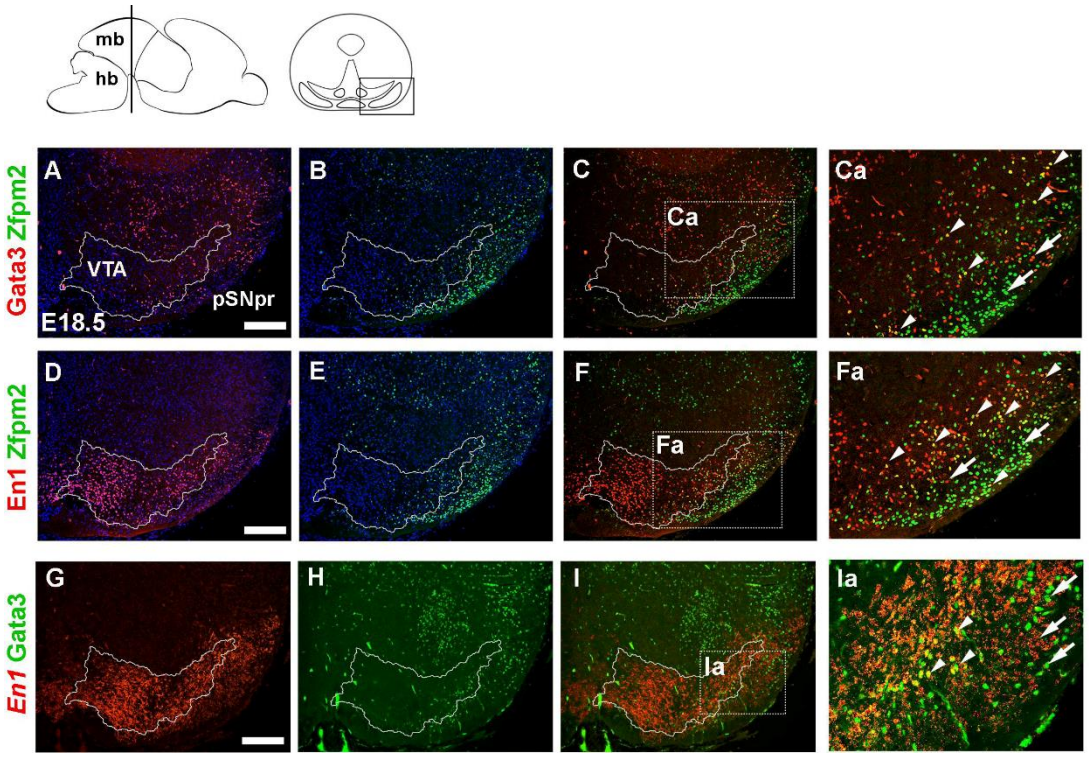
Supplementary Figure S2. GABAergic precursors in *Gata2*^{cko} and *Gata3*^{cko} r1.

(**A-M''**) ISH in (A-C'',H-I'',M-M'') and IHC (D-G'',J-L'') on transversal sections of E12.5 r1 showing no apparent alterations between the control and mutant embryos. The rV2 domain is indicated with lines, and the boundaries of ventricular zone (vz), mantle zone 1 (mz1) and mantle zone 2 (mz2) defined similarly as in Figure 1. (**N-P**) The percentage of Gata3^{+}, Zfpm2^{+}Pax7^{-}, and Sox2^{+}, FoxP1^{+}, Sox2^{+}FoxP1^{+} nuclei represented as averages with s.d. (n=3 in all genotypes). In all comparisons, p>0.05. Scale bars 200 μm.



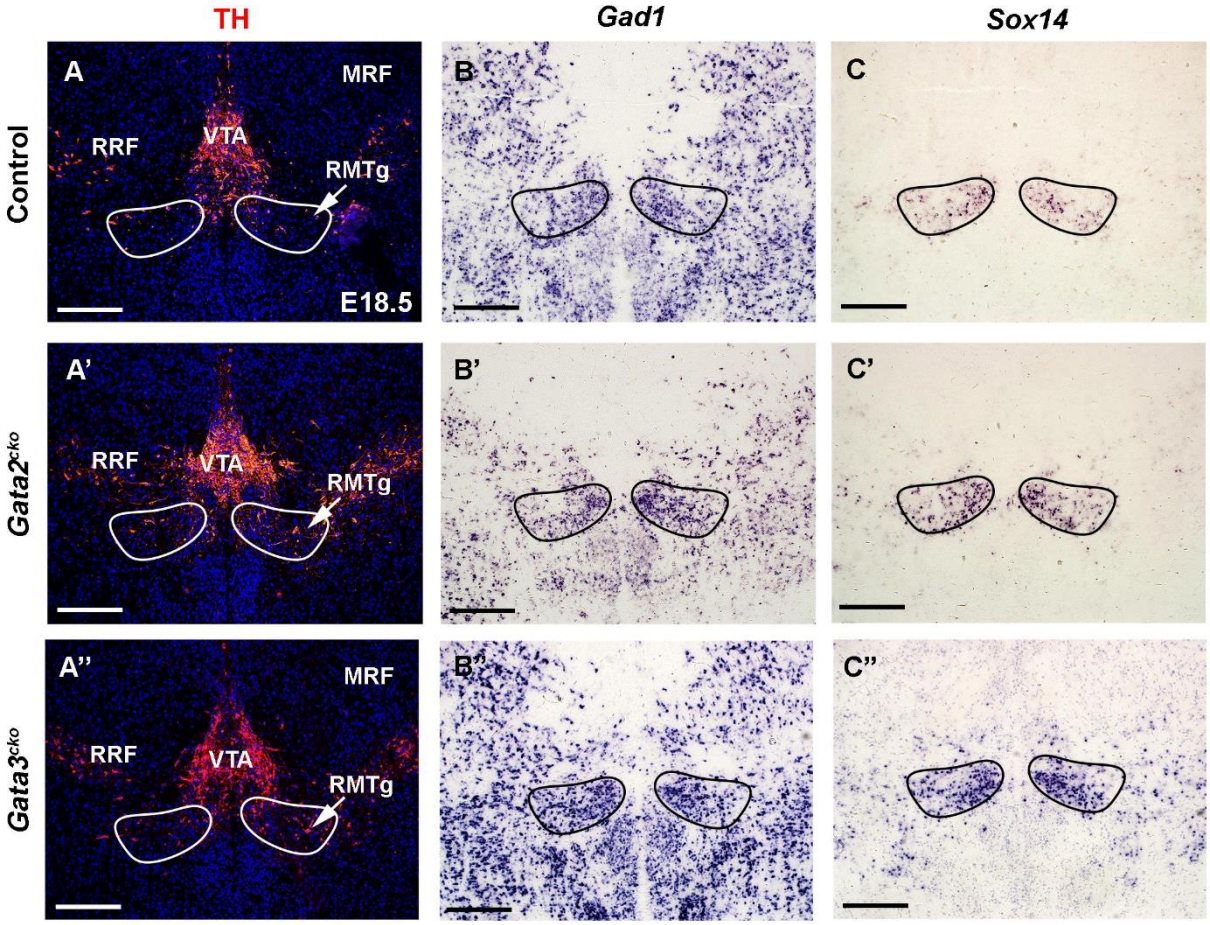
Supplementary Figure S3. Development of pSNpr and RMTg from *Sox14*$^{+}$ and *Zfpm2*$^{+}$ cells in r1.

(**A-C**) IHC (A) and ISH (B-C) on adjacent coronal sections of an E12.5 *Gbx2*^{CreERT2};*R26*^{TdTomato} embryo. The rV2 domain is indicated with lines together with ventricular zone (vz), mantle zone 1 (mz1) and mantle zone 2 (mz2). (**D-O**) IHC (D,G,J,M), ISH (E-F,H-I,K-L,N-O), and combined fluorescent ISH with IHC (D', D'') on sagittal (D-I, D´, D'') and coronal (J-O) sections of E14.5 *Gbx2*^{CreERT2};*R26*^{TdTomato} embryos. (D', D'') close-ups from the SNpr area (indicated in D). RMTg, rostromedial tegmental nucleus; pSNpr, posterior substantia nigra pars reticulata. Scale bars 100 μm in (A-C), 200 μm in (D-O) and 5μm in (D', D'').



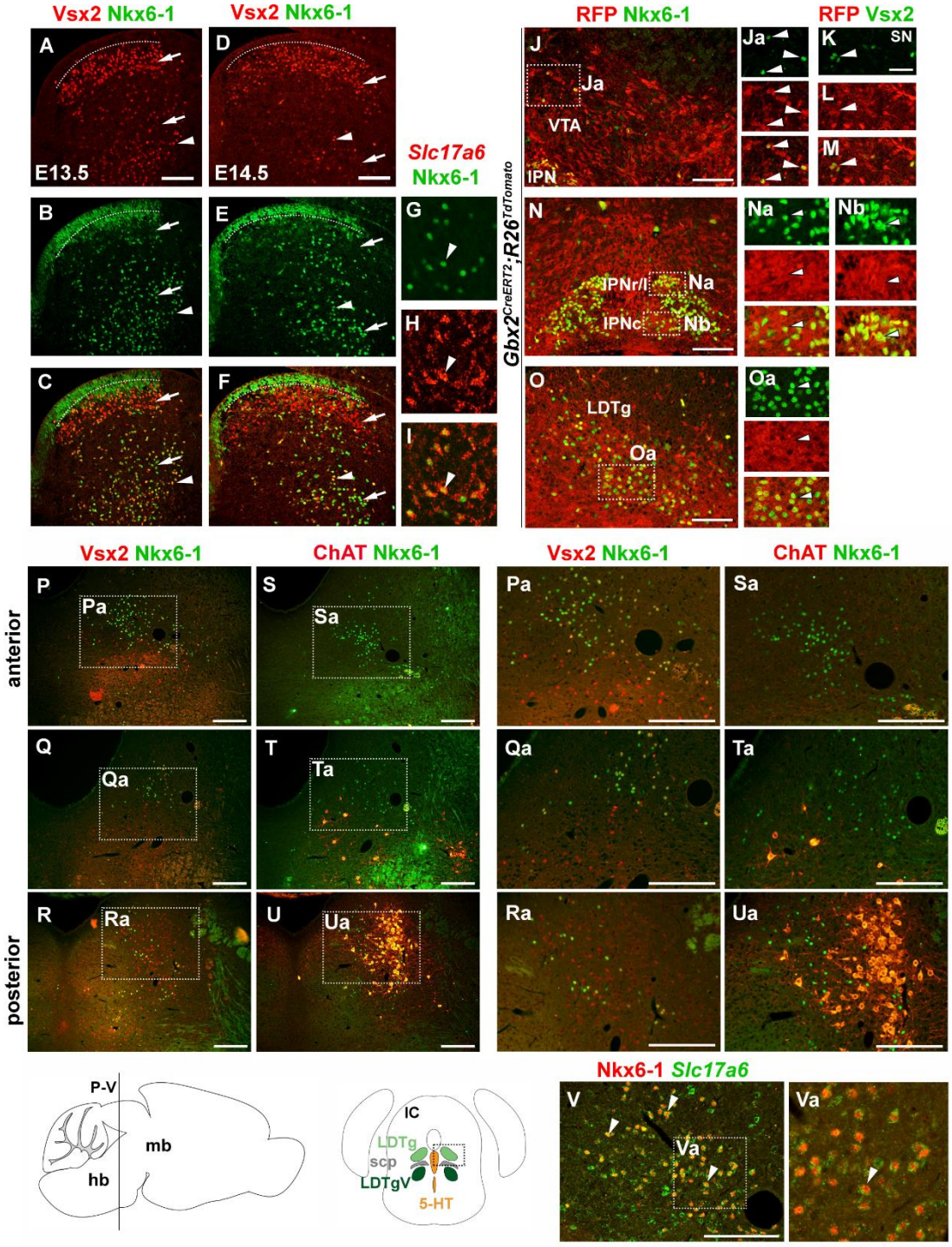
Supplementary Figure S4. GABAergic neuron subtypes in pSNpr.

(**A-Ia**) IHC (A-Fa) and ISH and IHC (G-Ia) on adjacent coronal sections of E18.5 VTA and pSNpr region, with higher magnification images of the boxed areas in (Ca,Fa,Ia). The boundaries of dopaminergic nuclei were based on TH-stainings on a parallel section (not shown). Arrowheads point to coexpressing and arrows to non-coexpressing cells. VTA, ventral tegmental area; pSNpr, posterior substantia nigra pars reticulata. Scale bars 200 μm.



Supplementary Figure S5. RMTg in *Gata2*^{cko} and *Gata3*^{cko} mutants.

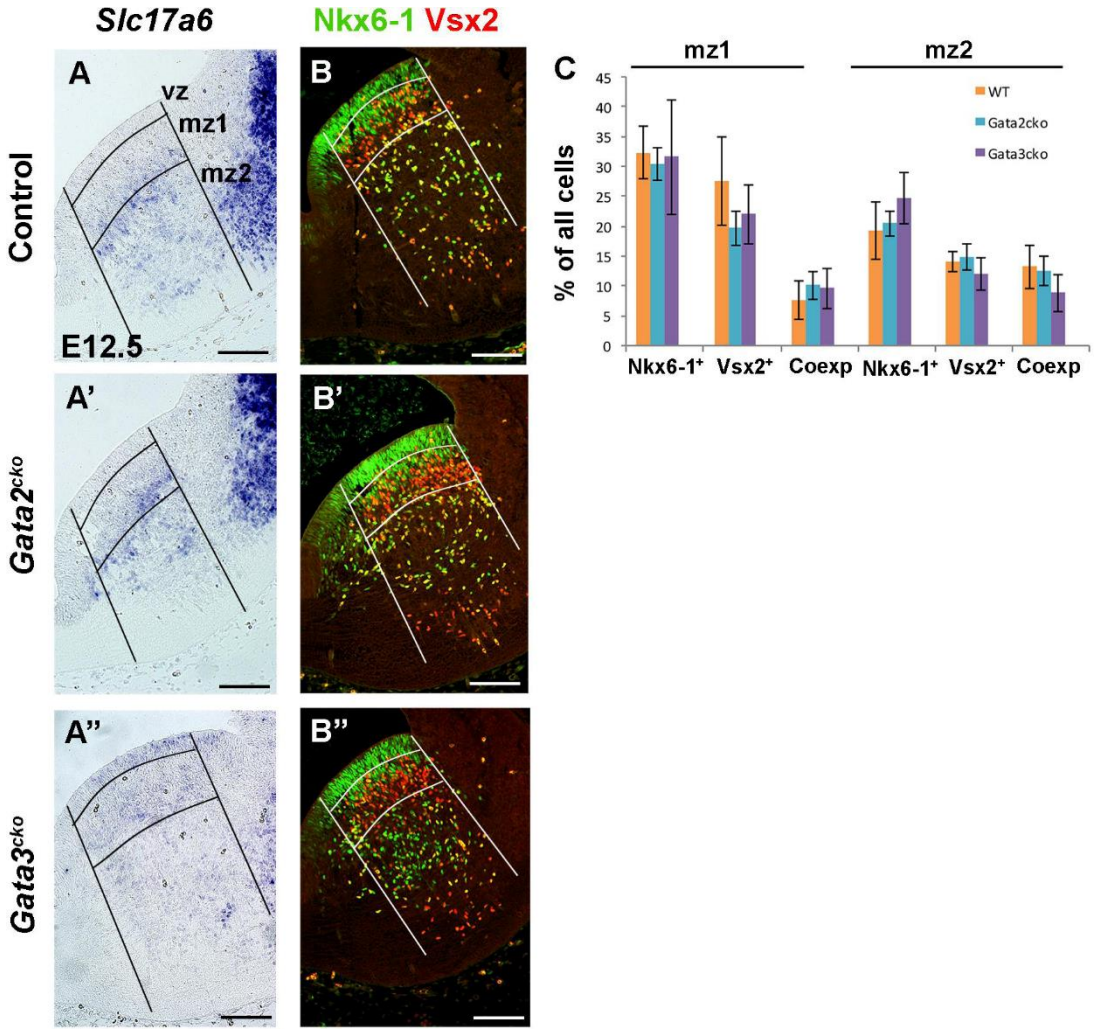
(**A-C''**) IHC (A-A'') and ISH (B-C'') on adjacent coronal sections. RMTg is circled based on *Sox14* expression. IPN, interpeduncular nucleus; MRF, midbrain reticular formation; RMTg, rostromedial tegmental nucleus; RRF, retrorubral field; VTA, ventral tegmental area. Scalebars 200 μm.



Supplementary Figure S6. Vsx2^{+} and Nkx6-1^{+} glutamatergic neurons in the embryonic and postnatal brain.

(**A-F**) IHC in E13.5 and E14.5 transversal sections of r1, depicting the separation of Vsx2^{+}, Nkx6-1^{+}, and double-positive neuronal populations. (**G-I**) ISH and IHC on E14.5 r1, showing coexpression of *Slc17a6* and Nkx6-1. (**J-O**) IHC on E18.5 *Gbx2*^{CreERT2};*R26*^{TdTomato} embryos from VTA (J,Ja), SN (K-

M), IPN (N-Nb) and LDTg (O-Oa). (**P-Ua**) IHC on adult brain LDTg region, the area indicated in the schematic figure below. Nkx6-1^{+} and Vsx2^{+} are localized near ChAT^{+} cholinergic neurons, but forming their own subnuclei. (**V**) *Slc17a6* ISH and Nkx6-1 IHC on an adjacent section to (P), showing glutamatergic identity of Nkx6-1^{+} neurons. Close-up view (Va). In all images, arrowheads indicate coexpression, and arrows lack of coexpression. 5-HT, serotonergic neurons; hb, hindbrain; IC, inferior colliculus; IPNc, central interpeduncular nucleus; IPNr/l, rostral/lateral interpeduncular nucleus; LDTg, laterodorsal tegmental nucleus; LDTgV, ventral part of laterodorsal tegmental nucleus; mb, midbrain; scp; superior cerebellar peduncle; SN, substantia nigra; VTA, ventral tegmental area. Scalebars 100 μm (A-F,J-O,V), 50 μm (K), 400 μm (P-Ua).



Supplementary Figure S7. Vsx2^{+} and Nkx6-1^{+} glutamatergic neuron precursors in *Gata2^{cko}* and *Gata3^{cko}* r1.

ISH (**A-A''**) and IHC (**B-B''**) on transversal sections of r1. The rV2 domain is indicated with lines, and the boundaries of ventricular zone (vz), mantle zone 1 (mz1) and mantle zone 2 (mz2) defined similarly as in Figure 1. (**C**) The percentages of Nkx6-1^{+}, Vsx2^{+}, and double-positive nuclei in mz1 and mz2, shown as averages with s.d. (n=3 in all genotypes). In all comparisons $p>0.05$. Scale bars 200 μm.

Supplementary Table S1. List of primary antibodies

Antigen	Species raised in	Supplier	Cat. No	Dilution
5-HT	Rabbit	Immunostar	20080	1:500
ChAT	Goat	Merck Millipore	AB144P	1:100
En1	Mouse	Developmental Studies Hybridoma Bank	4G11	1:200
FosB	Rabbit	Santa Cruz Biotechnology	sc-7203	1:400
FosB	Mouse	Abcam	ab11959	1:500
FoxP1	Rabbit	Abcam	ab16645	1:400
Gata2	Rabbit	Santa Cruz Biotechnology	sc-9008	1:200
Gata3	Mouse	Santa Cruz Biotechnology	HG3-3I sc-268	1:200
GFP	Rabbit	Abcam	ab290	1:800
GFP	Mouse	Merck Millipore	MAB3580	1:500
Nkx6-1	Mouse	Developmental Studies Hybridoma Bank	F55A10	1:1000
Olig2	Goat	Neuromics	GT15132	1:200
Pax7	Mouse	Developmental Studies Hybridoma Bank	Pax7	1:400
RFP	Rabbit	Rockland	600-401-379	1:400
Sox2	Rabbit	Merck Millipore	AB5603	1:400
Sox2	Mouse	Abcam	ab79351	1:800
TH	Rabbit	Merck Millipore	AB152	1:500
TH	Mouse	Merck Millipore	MAB318	1:500
Vsx2	Sheep	Abcam	ab16141	1:400
Zfpm1	Goat	Santa Cruz Biotechnology	M-20 sc-9361	1:400
Zfpm2	Rabbit	Santa Cruz Biotechnology	M-247 sc-10755	1:400
Zfpm2	Mouse	Santa Cruz Biotechnology	H-5 sc-398011	1:400