Fig. S1. Otx2 expression in developing dorsal mesencephalon. (**A-O'**) Immunohistochemistry experiments in E11, E13.5 and P5 wild type embryos and mice with Otx2 and Zic1 (A,A',L,L'), Pax7 (B,B',M,M'), Dbx1 (C,C'), Mash1 (D,D'), Helt (E,E'), Ngn1 (F,F'), Gata2 (G,G'), Lhx1 (H,H',N,N'), Pou4f1 (I,I',O,O'), Gad65 (J,J') and vGlut2 (K,K') show that Otx2 is expressed virtually in all progenitors while in post-mitotic neurons is prevalently restricted to the $Gata2^{+}$, $Lhx1^{+}$ and $Gata65^{+}$ GABAergic lineage. Note also that Zic1 expression defines two m1 sub-domains: the m1a and m1b. Abbreviations: m1, mesencephalic domain 1; m1a and m1b, mesencephalic sub-domain 1a and 1b.

Fig. S2. Expression in developing cerebellum of m1 mesencephalic and cerebellar markers. (**A-R**) Immunohistochemistry experiments performed at E12.5 and E13.5 on wild type cerebella with Otx2 (A,B), Pax6 and Pax7 (C,D), Lhx1 and Zic1 (E,F) Mash1 and Ngn1 (G,H), Lhx1 and Pax2 (I,J), Mash1 and Helt (K,L), Lhx1 and Gata2 (M,N) Pou4f1 and Pax7 (O,P) and Dbx1 and Pax7 (Q,R) show that Otx2 expression is restricted at these stages to the developing choroid plexus and in a portion of the adjacent rhombic lip (A,B), Pax6 is expressed in the rhombic lip, developing EGL and in the VZ/SVZ close to the rhombic lip (C,D), Pax7 is restricted to the VZ/SVZ and gradually down-regulated during development (C,D,O-R), Pax2 and Lhx1 are expressed respectively in post-mitotic precursors for GABAergic interneurons and Purkinje cells (I,J) and Helt, Gata2, Pou4f1 and Dbx1 are not expressed (K-R). Abbreviations: EGL, external granule cell layer; RL, rhombic lip; VZ/SVZ, ventricular/subventricular zone; chp, choroid plexus.

Fig. S3. Analysis of cerebellar markers in the mesencephalon of E18.5 Otx2 mutants. (**A-L**) Immunohistochemistry experiments performed at E18.5 on the mesencephalon and cerebellum of $Dbx1^{ICre/+}$ and on the mesencephalon of $Dbx1^{ICre/+}$; $Otx2^{flox/flox}$ embryos with Math1 and Pax6 (A-

C), Pax6 and Zic1 (D-F), Lhx1 and Pax2 (G-I) and Lhx1 and Calb (J-L) show a very similar expression pattern between the mutant m1a sub-domain and the control cerebellum. Abbreviations as in previous Figures.

Fig. S4. Cerebellar-like morphology in Otx2 mutants at P20. (A-H) External view (A,B) and sagittal sections (C-H) of *Dbx1*$^{ICre/+}$ and *Dbx1*$^{ICre/+}$; *Otx2*$^{flox/flox}$ brains at P20 are immunostained with Pax6 to assess the anterior-posterior extent of the cerebellar-like phenotype in Otx2 mutants (C-H); note that the posteriormost dorsal mesencephalon of Otx2 mutant brains does not exhibit Pax6 expression nor cerebellar-like morphology (arrow in E,H). Abbreviations as in previous Figures plus SuC, superior colliculus; InC, inferior colliculus; cu, culmen.

Fig. S5. Lhx1 and Pax2 are expressed in post-mitotic precursors exiting the cell-cycle prevalently between E11.5 and E12.5. (**A-D**) BrdU pulse experiments performed on the mesencephalon of *Dbx1*$^{ICre/+}$ and *Dbx1*$^{ICre/+}$; *Otx2*$^{flox/flox}$ embryos administered at E12.5 with BrdU for 20 min before to be sacrificed and immunostained with Lhx1 and BrdU (A,B) and Pax2 and BrdU (C,D) show that Lhx1 and Pax2 are expressed in BrdU^{-} post-mitotic precursors. (**E-J**) Immunohistochemistry experiments with Lhx1 and BrdU (E-G) and Pax2 and BrdU (H-J) are performed at E15.5 on the mesencephalon and cerebellum of *Dbx1*$^{ICre/+}$ and the mesencephalon of *Dbx1*$^{ICre/+}$; *Otx2*$^{flox/flox}$ embryos injected with BrdU at E11.7, E11.85 and E12. These experiments confirm that the majority of Lhx1 and Pax2 progenitors exit the cell-cycle between E11.5 and E12.5. Abbreviations as in previous Figures.

Fig. S6. Schematic representation summarizing the Otx2 requirement in the m1a and m1b sub-domains. (A) Otx2 is cell-autonomously required in the VZ/SVZ of the m1a (Pax7^{+}-Zic1^{+}) sub-domain to suppress cerebellar-like neurogenesis of granule cells and cerebellar GABAergic

neurons and instruct m1a neurogenesis; our data also suggest that suppression of granule cells fate requires Otx2 until approximately E11.5 while suppression of cerebellar-like GABAergic differentiation requires Otx2 until E14.5. The rectangular boxes correspond to the VZ/SVZ (yellow), the early post-mitotic mantle layer (orange) and the late post-mitotic mantle layer (red); for granule cells neurogenesis the RL (yellow), the EGL (orange) and the IGL (red) are indicated. Based on previous studies (Nakatani et al., 2007; Kala et al., 2009; Agoston and Schulte, 2009; Guimera et al., 2006; Liu and Joyner, 2001a; Hatten et al., 1997; Zhao et al., 2007; Morales and Hatten, 2006; Maricich and Herrup, 1999), we have indicated the presumptive genetic cascade of transcription factors operating along the entire m1 domain and in the cerebellum for granule cells and GABAergic neurogenesis. **B)** In the m1b ($Pax7^{+}$-$Zic1^{-}$) sub-domain Otx2 is not required to prevent cerebellar-like fate but it controls selectively the neurogenesis of GABAergic neurons by promoting the transition of $Helt^{+}$ progenitors into $Gata2^{+}$ early post-mitotic neurons. Our data do not reveal major Otx2-dependent abnormalities in the differentiation of glutamatergic neurons. Abbreviations: VZ/SVZ, ventricular zone/sub-ventricular zone; ML, mantle layer; RL, rhombic lip; EGL, external granule cell layer; IGL, internal granule cell layer.

Fig. S7. Expression analysis of the Otx2 interacting factors Grg4 and Meis2. Immunohistochemistry assays performed in $Dbx1^{ICre/+}$ and $Dbx1^{ICre/+}$; $Otx2^{flox/flox}$ embryos at E11.5, E12.5 and E13.5 with Grg4 and Meis2 show that lack of Otx2 does not remarkably affect the expression of both Meis2 and Grg4. Abbreviations as in previous Figures.

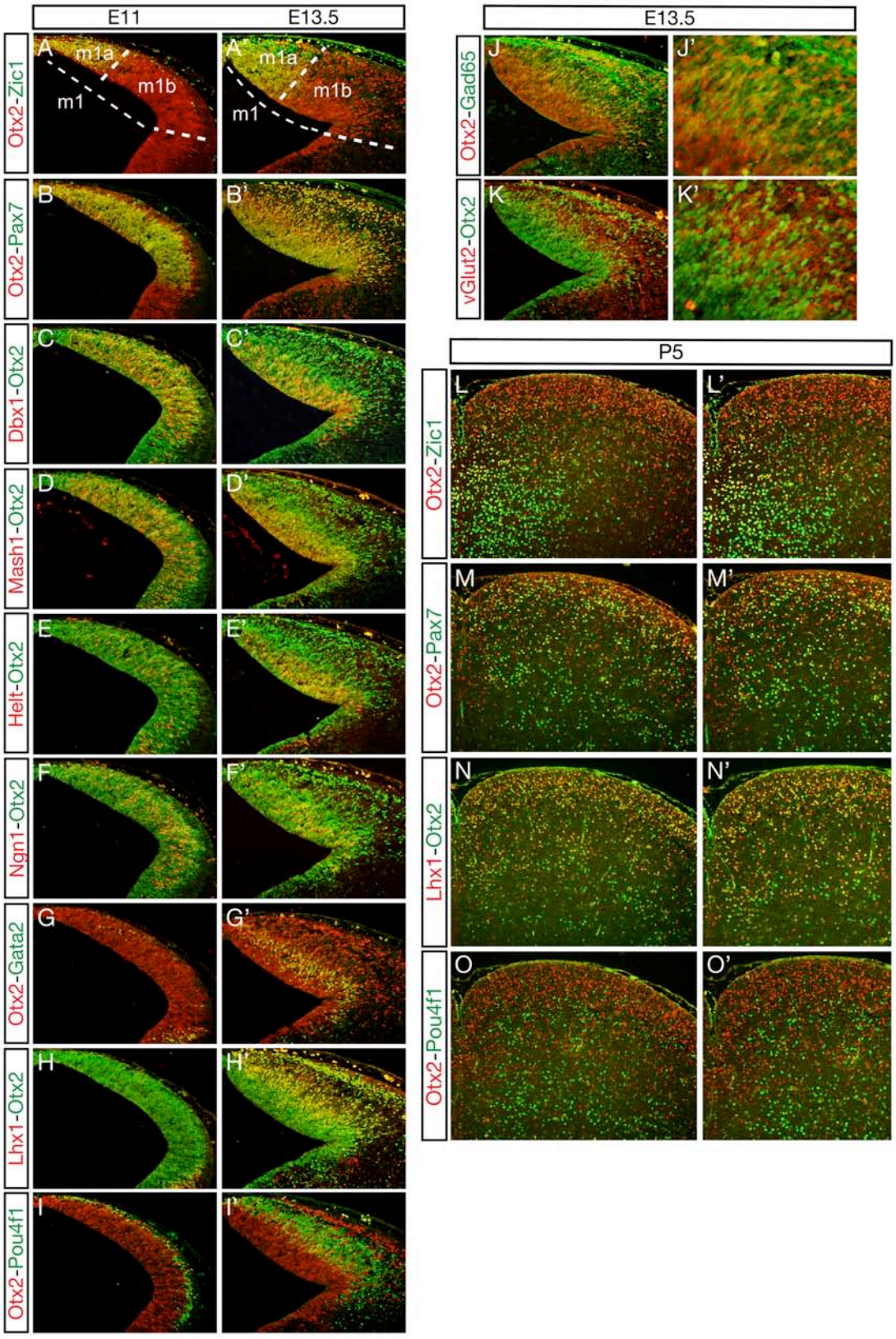
E11
E13.5
E13.5
P5
Otx2-Zic1
Otx2-Pax7
Dbx1-Otx2
Mash1-Otx2
Helt-Otx2
Ngn1-Otx2
Otx2-Gata2
Lhx1-Otx2
Otx2-Pou4f1
Otx2-Gad65
vGlut2-Otx2
m1a
m1b
m1
A
A'
B
B'
C
C'
D
D'
E
E'
F
F'
G
G'
H
H'
I
I'
J
J'
K
K'
L
L'
M
M'
N
N'
O
O'

Fig. S1

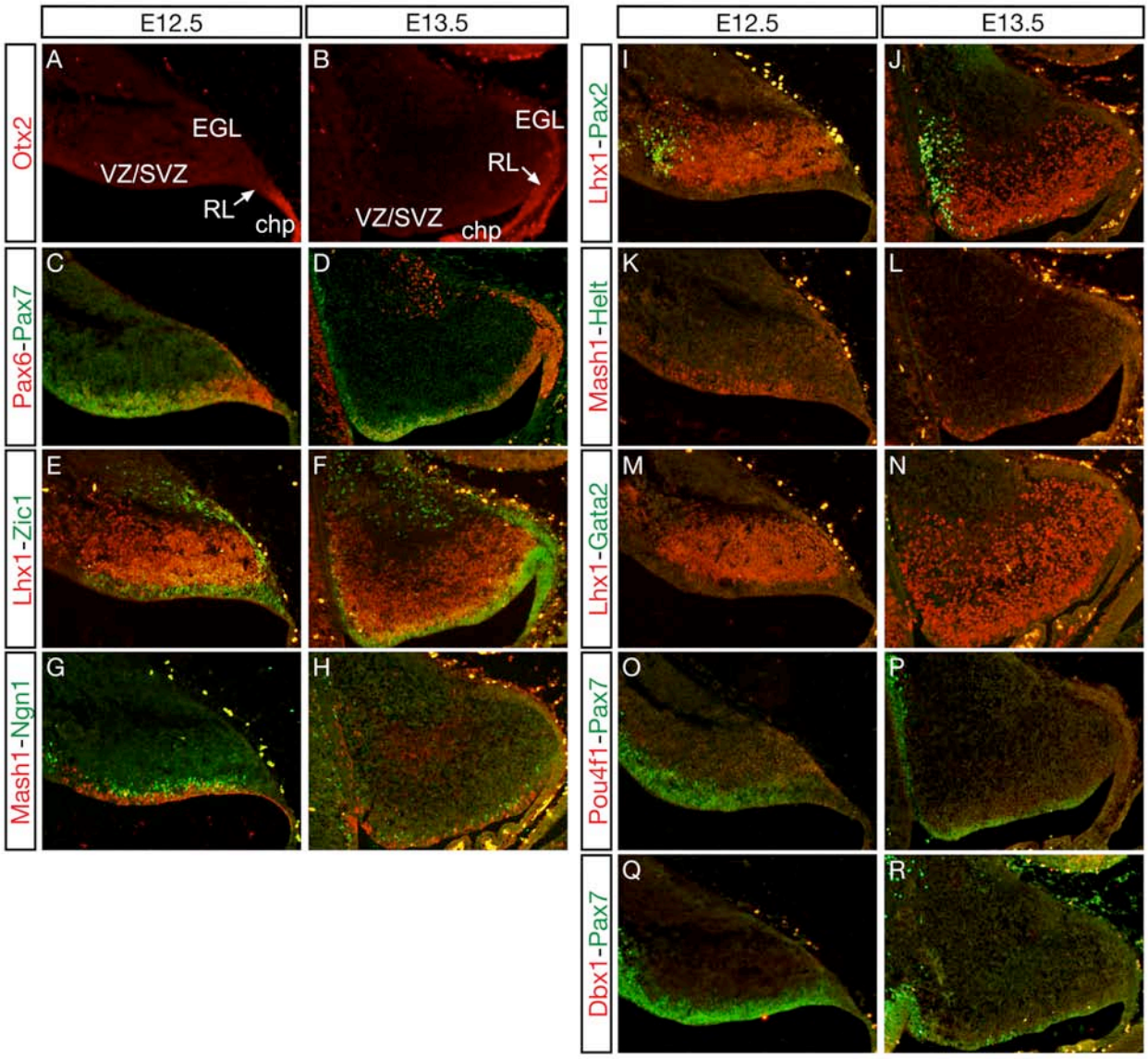
E12.5
E13.5
E12.5
E13.5
Otx2
Pax6-Pax7
Lhx1-Zic1
Mash1-Ngn1
Lhx1-Pax2
Mash1-Helt
Lhx1-Gata2
Pou4f1-Pax7
Dbx1-Pax7
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
EGL
VZ/SVZ
RL
chp
EGL
RL
VZ/SVZ
chp

Fig. S2

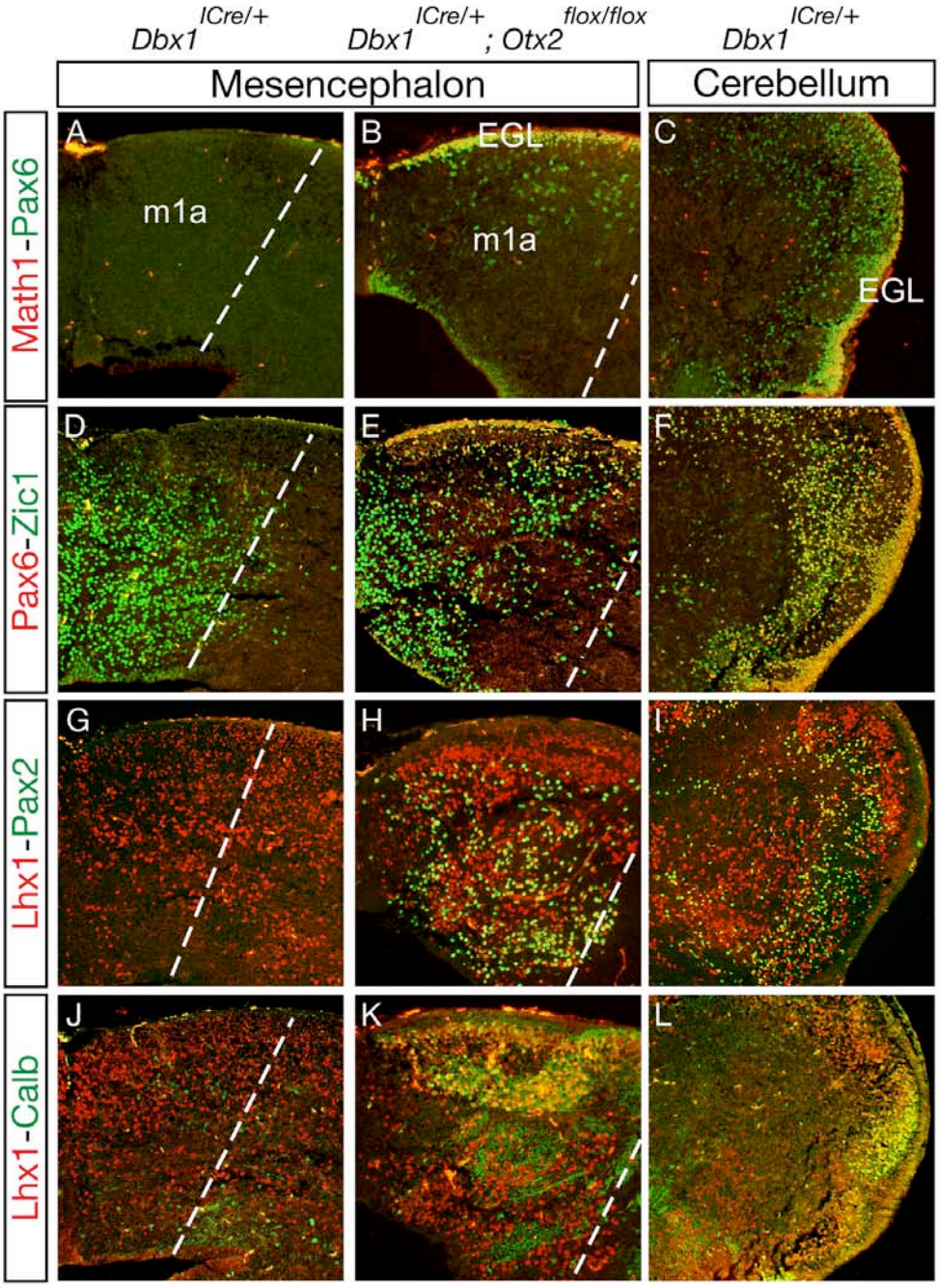
Dbx1 lCre/+
Dbx1 lCre/+ ; Otx2 flox/flox
Dbx1 lCre/+
Mesencephalon
Cerebellum
Math1-Pax6
Pax6-Zic1
Lhx1-Pax2
Lhx1-Calb
A
B
C
D
E
F
G
H
I
J
K
L
m1a
m1a
EGL
EGL

Fig. S3

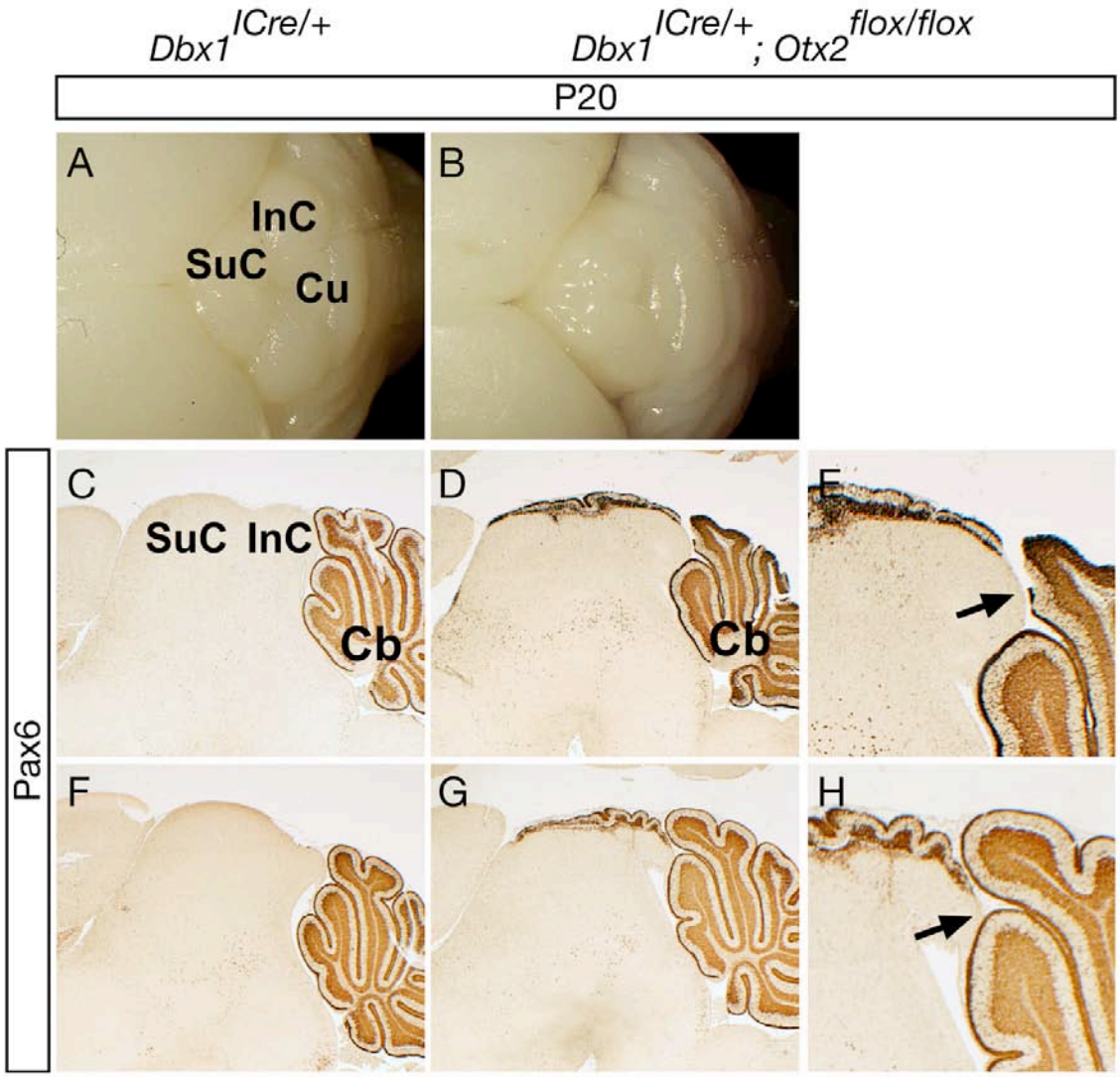
Dbx1ICre/+
Dbx1ICre/+; Otx2flox/flox
P20
A
InC
SuC
Cu
B
Pax6
C
SuC InC
Cb
D
Cb
E
F
G
H

Fig. S4

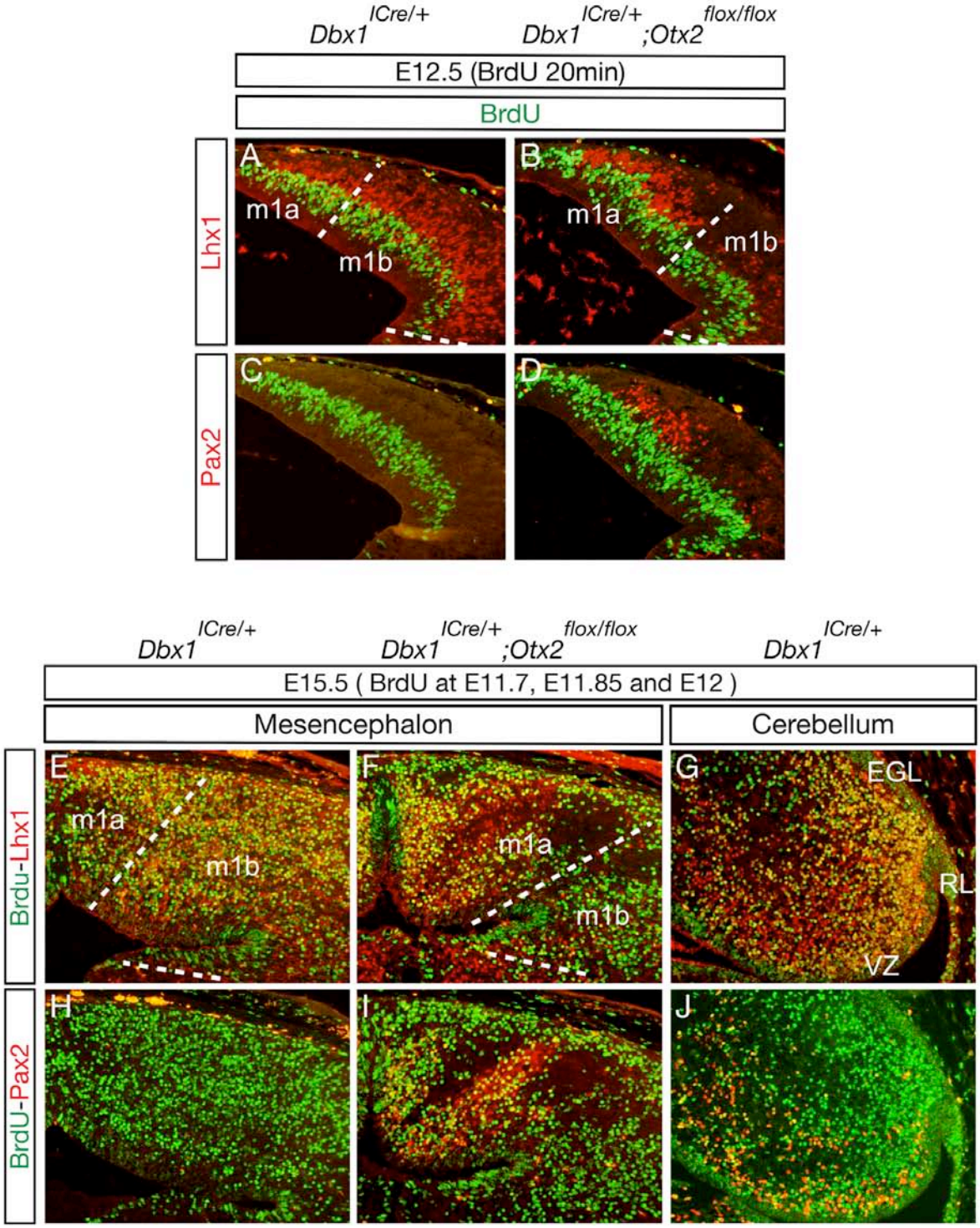
Dbx1 ICre/+
Dbx1 ICre/+ ;Otx2 flox/flox
E12.5 (BrdU 20min)
BrdU
Lhx1
Pax2
A
B
C
D
m1a
m1b
Dbx1 ICre/+
Dbx1 ICre/+ ;Otx2 flox/flox
Dbx1 ICre/+
E15.5 (BrdU at E11.7, E11.85 and E12)
Mesencephalon
Cerebellum
Brdu-Lhx1
BrdU-Pax2
E
F
G
H
I
J
m1a
m1b
EGL
RL
VZ

Fig. S5

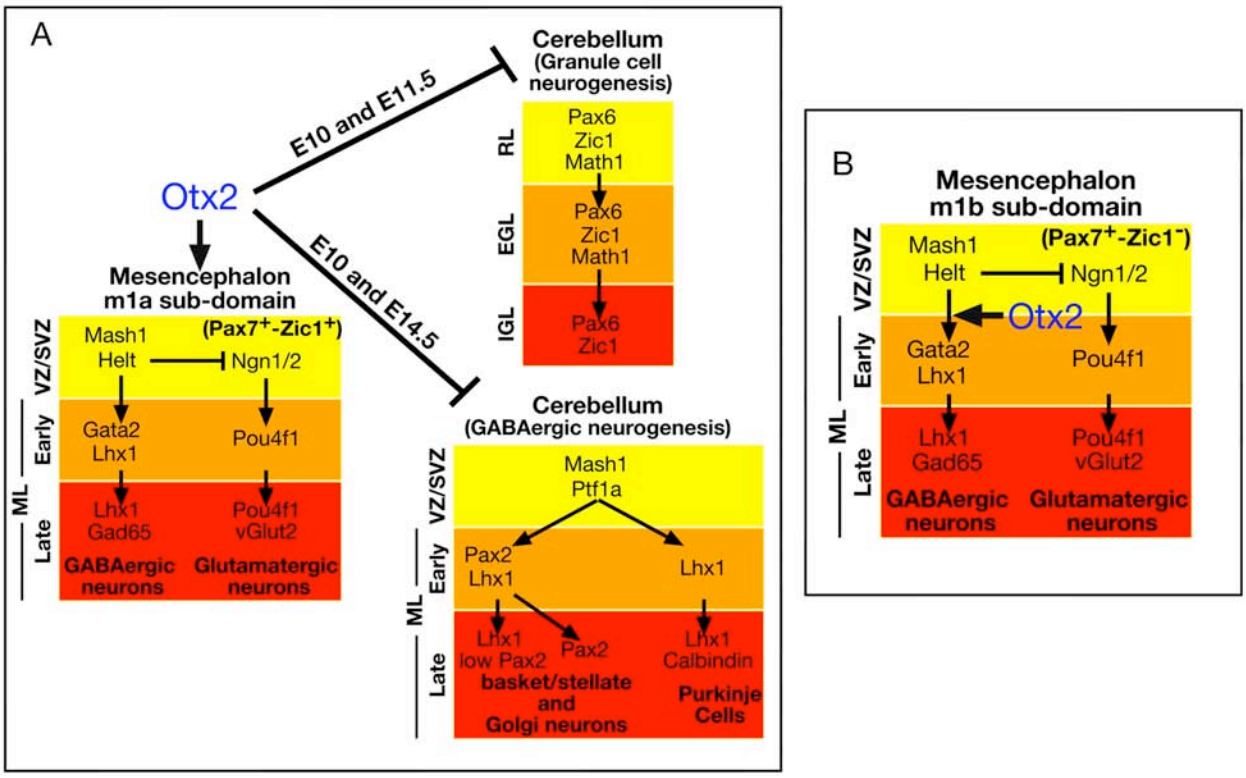
A
Otx2
E10 and E11.5
E10 and E14.5
Cerebellum
(Granule cell
neurogenesis)
RL
Pax6
Zic1
Math1
EGL
Pax6
Zic1
Math1
IGL
Pax6
Zic1
Mesencephalon
m1a sub-domain
VZ/SVZ
Mash1
Helt
(Pax7+-Zic1+)
Ngn1/2
ML
Early
Gata2
Lhx1
Pou4f1
Late
Lhx1
Gad65
Pou4f1
vGlut2
GABAergic
neurons
Glutamatergic
neurons
Cerebellum
(GABAergic neurogenesis)
VZ/SVZ
Mash1
Ptf1a
ML
Early
Pax2
Lhx1
Lhx1
Late
Lhx1
low Pax2
Pax2
Lhx1
Calbindin
basket/stellate
and
Golgi neurons
Purkinje
Cells
B
Mesencephalon
m1b sub-domain
VZ/SVZ
Mash1
Helt
(Pax7+-Zic1-)
Ngn1/2
Otx2
ML
Early
Gata2
Lhx1
Pou4f1
Late
Lhx1
Gad65
Pou4f1
vGlut2
GABAergic
neurons
Glutamatergic
neurons

Fig. S6

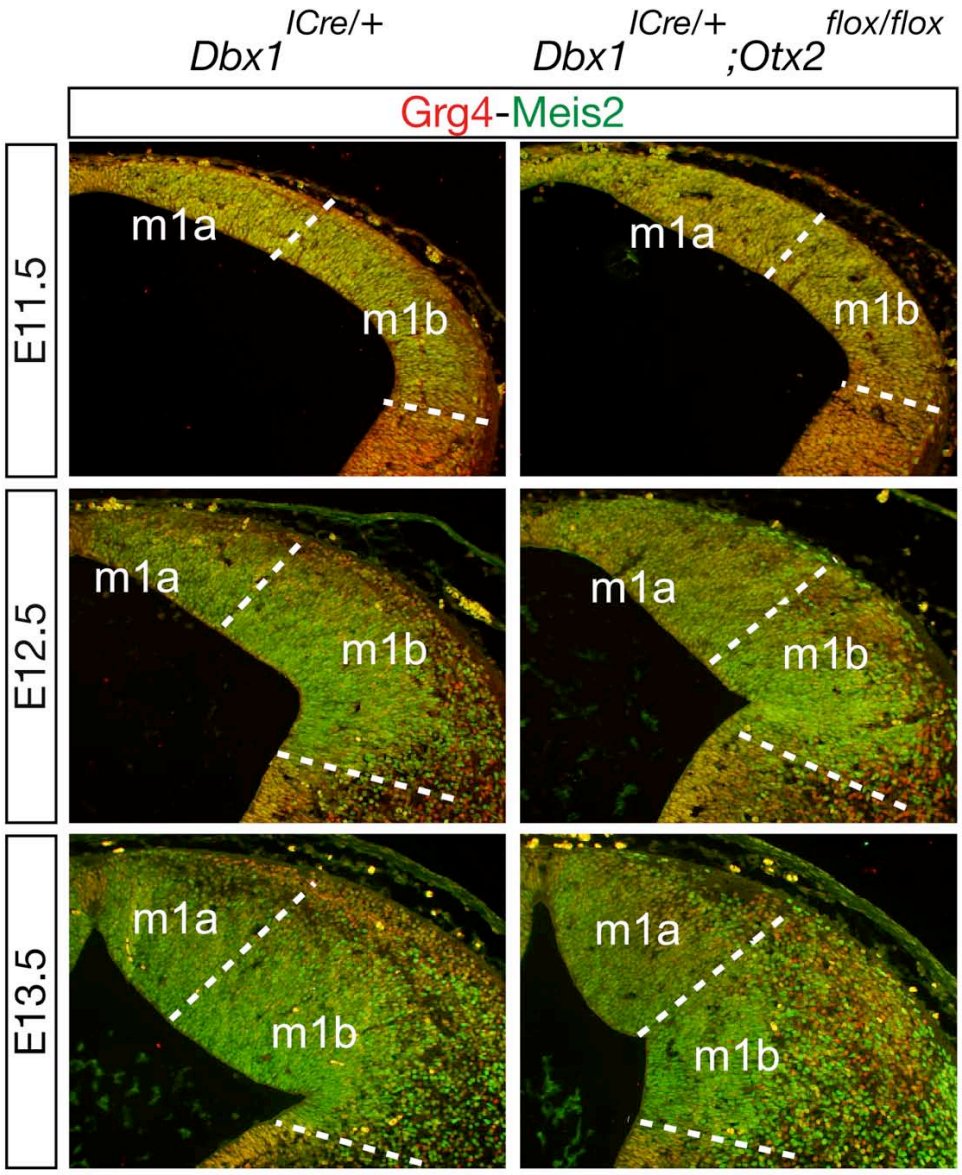
Dbx1 ICre/+
Dbx1 ICre/+ ;Otx2 flox/flox
Grg4-Meis2
E11.5
E12.5
E13.5
m1a
m1b

Fig. S7