Supporting Information

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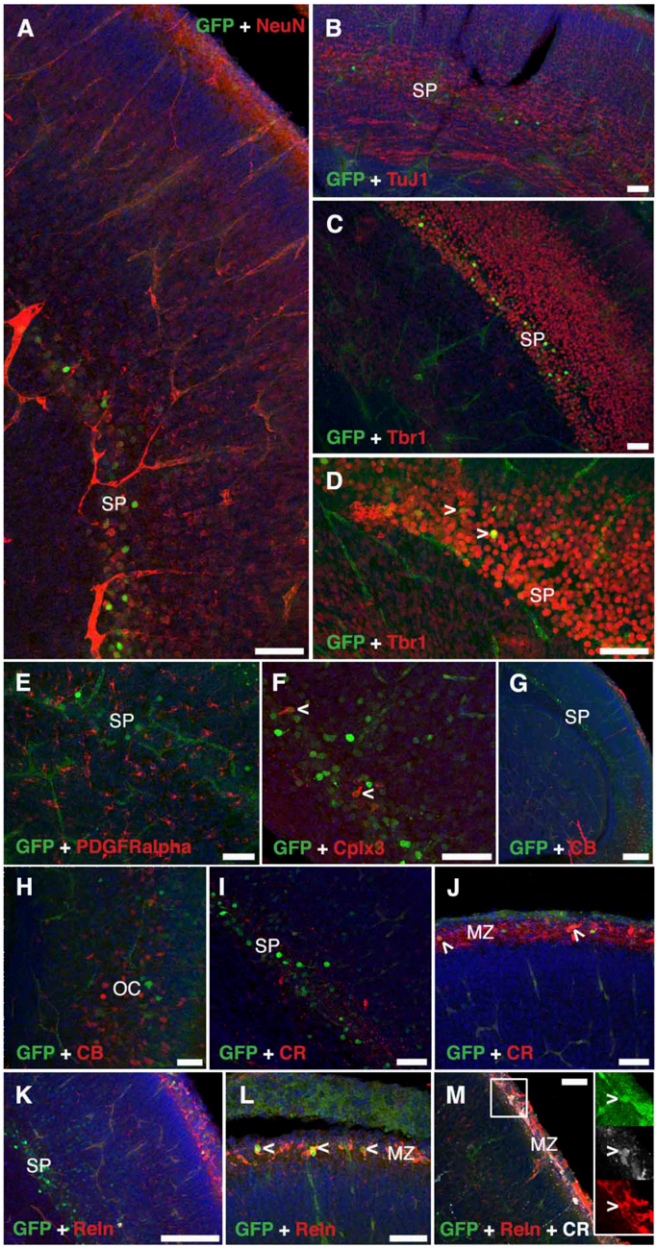


Fig. S1. Characterization of the rostromedial telencephalic wall (RMTW)-derived cells at embryonic day (E) 18. Injections were made into the ventricle, with subsequent electroporation of a GFP plasmid (green) into the RMTW of E11 embryos and immunohistochemical characterization at E18. (*A–E*) RMTW-derived cells in the subplate express neuronal, but not glial, markers at E18. GFP^+ cells express NeuN (*A*), TuJ1 (*B*), and Tbr1 (*C* and open arrowheads in *D*) in the subplate. At E18, PDGF receptor alpha (PDGFRα) is expressed in the neuroepithelium, but none of the carboxyfluorescein diacetate (CFDA)-labeled cells express this glial marker (*E*). (*F*) RMTW-derived cells in the subplate express subplate molecular markers at E18. Complexin 3 (Cplx3) is expressed in some of the RMTW-derived cells (*F*, open arrowheads). Some RMTW-derived, GFP-labeled cells in the cortical plate can be seen as well. (*G* and *H*) RMTW-derived cells do not express Calbindin (CB) at E18. CB is not expressed in the cortical neuroepithelium (*G*), but it is expressed in the olfactory cortex (*H*), where it does not colocalize with RMTW-derived GFP-labeled cells. (*I*, *J*, and *M*) RMTW-derived cells express Calretinin (CR) only in the marginal zone, and not in the subplate. At E18, few cell bodies and some fibers in the SP express CR (*I*), and none of these cells are labeled by GFP from the RMTW electroporation. In the marginal zone, colocalization between GFP and CR is seen (open arrowheads in *J*; *Inset* in *M*), as was observed at E13. (*K–M*) RMTW-derived cells express Reelin (Reln) only in the marginal zone. Reln expression is restricted to the marginal zone of the developing cortex (*K*), where some GFP cells coexpress this marker (*L* and *M*, open arrowheads). Blue cells have been counterstained with bisbenzimide. Some RMTW-derived cells are likely Cajal-Retzius cells, because they coexpress Reln and CR (*M, Inset*). Images are of 40-μm-thick coronal sections; midline is left, and dorsal is up. (Scale bars: 50 μm in *A–F*, *H–J*, *L*, and *M*: 50 μm in *G*; 200 μm in *K*.)

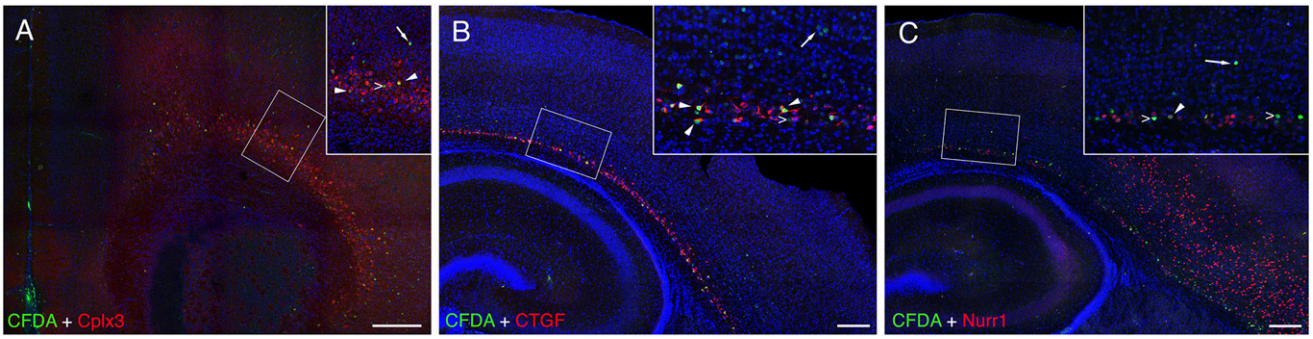
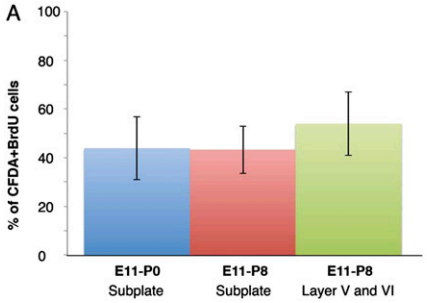


Fig. S2. Colocalization of RMTW-derived suplate cells and subplate neuronal markers. In utero ultrasound-guided injections of the green cell tracker CFDA into the RMTW of E11 embryos, analyzed at P8. (*A*) Cplx3 labels subplate neurons and is coexpressed in some CFDA-labeled RMTW-derived cells (solid arrowheads). Not all RMTW-derived cells coexpress Cplx3 (open arrowheads), and no nonsubplate CFDA-labeled cells were found to coexpress Cplx3 (arrows). (*B*) CTGF labels the majority of subplate neurons and is coexpressed in almost all CFDA-labeled RMTW-derived cells (solid arrowheads), although a few CFDA-labeled cells do not express CTGF (open arrowheads). Non-subplate, CFDA-labeled cells do not express CTGF (arrows). (*C*) Nurr1 labels subplate neurons in dorsal cortex and deep-layer neurons laterally. CFDA-labeled RMTW-derived cells rarely coexpress Nurr1 (solid arrowhead). The majority of CFDA-labeled cells in the subplate and RMTW-derived cells in lateral cortex layers V and VI do not coexpress Nurr1. Shown are 50-μm-thick coronal sections; midline is left, and dorsal is up. Blue cells have been counterstained with DAPI. (Scale bar: 200 μm.)



B

Injected at	% of CFDA+BrdU cells observed at: Subplate P0	Subplate P8	Neurons LV/VI P8
E10	N/D	N/D	N/D
E11	44±13	43±10	54±13
E12	0	0	0
E13	0	0	0

Fig. S3. Birthdating of RMTW-derived cells. (*A* and *B*) Quantification of the colocalization between RMTW-derived cells and BrdU labeling at either P0 or P8, after BrdU injections at E11 or E12. The cells seen in both the subplate and cortical layers V and VI share generation times. (*B*) At least one-half of the RMTW-derived cells are generated at E11 or at previous stages, but not later.

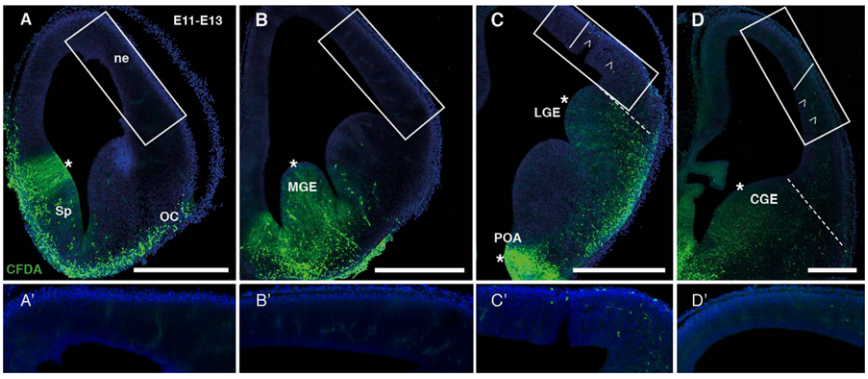


Fig. S4. Migratory paths of cells derived from the RMTW-adjacent zones. In utero ultrasound-guided injections of the green cell tracker CFDA in E11 embryos analyzed at E13. The injections were made into the following areas (denoted by asterisks): septum (Sp in *A*), medial ganglionic eminence (MGE; *B*), lateral ganglionic eminence (LGE; *C*), preoptic area (POA; *C*), and caudal ganglionic eminence (CGE; *D*). (*A*) Septum-derived cells migrate radially toward the outermost part of this area and subsequently change to migrate tangentially in the ventral direction to settle in the olfactory cortex (OC). They do not reach the cortical neuroepithelium by E13 (*A′*). (*B*) MGE-derived cells migrate toward its outermost part and do not ascend to the cortical neuroepithelium (ne; *B′*). (*C*) LGE- and POA-derived cells transgress the corticostriatal border (dashed line) and reach the cortical neuroepithelium (open arrowheads), although at E13 these cells do not cover its entire extension (solid line and enlargement in *C′*). (*D*) CGE-derived cells behave similarly to their companion cells shown in *C*. Some CGE-derived cells reach the lateral cortex by E13 (*D′*). Shown are 40-μm-thick coronal sections; midline is left, and dorsal is up. Blue cells have been counterstained with bisbenzimide. (Scale bars: 50 μm in *A*–*C*; 100 μm in *D*.)

Table S1. Experimental paradigm: combinations of injection time, injection site, tracer used, and postinjection survival time

Injection site	Age at tracer injection	Type of tracer	Survival until	Number of embryos	Mouse strain
RMTW	E11	CFDA	E13	16	C57
RMTW	E11	CFDA (birthdating studies)	P0	4	C57
RMTW	E11	CFDA	P0	8	C57
RMTW	E11	CFDA (birthdating studies)	P8	8	C57
RMTW	E11	GFP retrovirus	E12	9	C57
RMTW	E11	GFP retrovirus	E13	8	C57
RMTW	E11	GFP plasmid	E18	15	C57
RMTW	E11	DiI	P8	11	Lpar1-eGFP
RMTW	E12	CFDA (birthdating studies)	P0	7	C57
RMTW	E12	CFDA (birthdating studies)	P8	5	C57
RMTW	E13	CFDA (birthdating studies)	P8	8	C57
Septum	E11	CFDA	E13	8	C57
Ganglionic eminences	E11	CFDA	E13	7	C57
POA	E11	CFDA	E13	6	C57

DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetra-methyl-indocarbocyanine perchlorate.