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Supplemental Information

GFRα1 Regulates Purkinje Cell Migration by Counteracting NCAM Function

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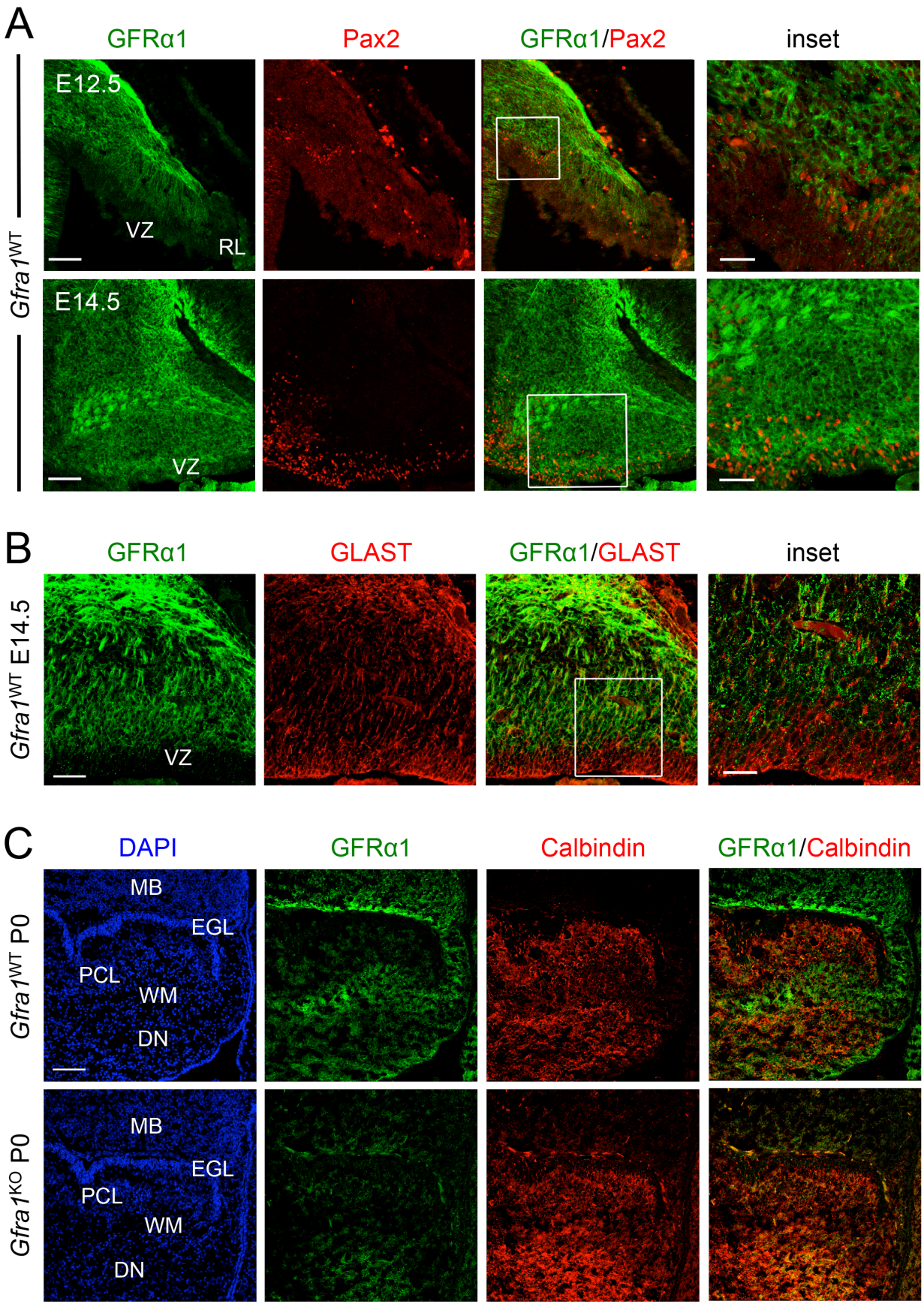

Figure S1. Lack of GFRα1 expression in PCs at birth. (Related to Figure 1).

(A) Immunostaining for GFRα1 and Pax2 of cerebellar sections from E12.5 (bottom row) and E14.5 (upper row) wild type mice. Insets show higher magnification of boxed areas. VZ, ventricular zone; RL, rhombic lip. Scale bar, 100μm (50μm for inset).

(B) Immunostaining for GFRα1 and GLAST of cerebellar sections from E14.5 wild type mice. Inset show higher magnification of boxed area. VZ, ventricular zone. Scale bar, 50μm (25μm for inset).

(C) Immunostaining for GFRα1 (green) and Calbindin (red) of cerebellar sections from wild type (*Gfra1*^{WT}) and *Gfra1*^{KO} newborn (P0) mice. DAPI counterstaining is shown in blue. In wild type animals, GFRα1 immunostaining can be seen in the midbrain (MB) and cerebellar white matter (WM) but not in the Purkinje cell layer (PCL) or external granule layer (EGL). DN, deep nuclei. Scale bar, 100 μm.

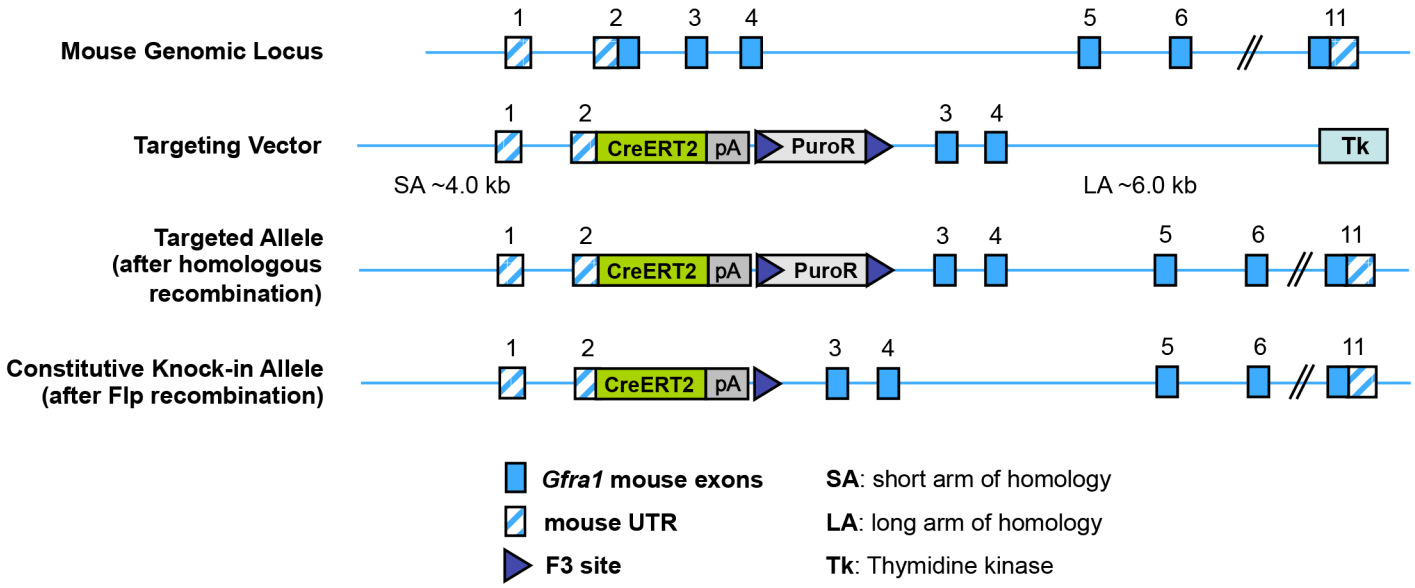

Figure S2. Generation of *Gfra1*^{CreERT2} knock-in mice. (Related to Figure 1).

Schematic of the *Gfra1* locus (not at scale) with strategy for generation of the *Gfra1*^{CreERT2} knock-in allele.

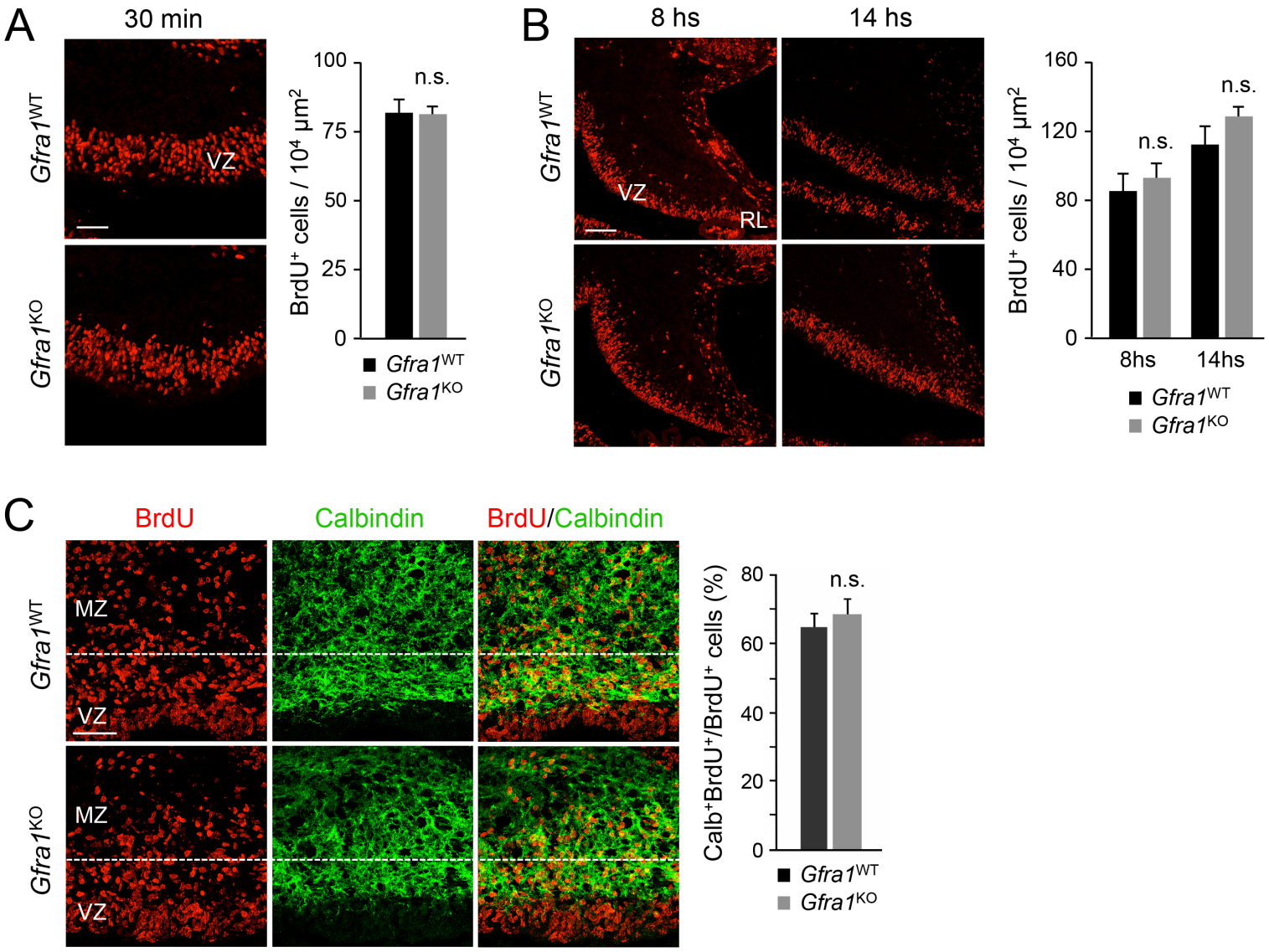

Figure S3. Normal PC proliferation and differentiation in GFRα1 knock-out mice. (Related to Figure 2).

(A) Representative sagittal sections of cerebella from *Gfra1*^{WT} and *Gfra1*^{KO} E12.5 embryos injected with BrdU 30min prior to collection to assess cell proliferation. Histogram to the right shows quantitative analysis of BrdU-labeled cells in ventricular zone (VZ) as average ± SEM (N=5 and 7 for WT and KO, respectively). n.s., not significant (p=0.11).

(B) Representative sagittal sections of cerebella from *Gfra1*^{WT} and *Gfra1*^{KO} E12.5 embryos injected with BrdU 8 and 14hs prior to collection. Histogram to the right shows quantitative analysis of BrdU-labeled cells in ventricular zone (VZ) as average ± SEM (N=3). n.s., not significant (p=0.82).

(C) Representative sections from E14.5 wild type (*Gfra1*^{WT}) and *Gfra1* knock-out (*Gfra1*^{KO}) embryos injected with BrdU at E12.5 stained for BrdU (red) and calbindin (green). VZ, ventricular zone; MZ, mantle zone. Scale bar, 50µm. Histogram to the right shows quantitative analysis of the proportion of PC progenitors that migrated from the VZ to the MZ in *Gfra1*^{WT} and *Gfra1*^{KO} embryos as average ± SEM (N=4, 3 for WT and KO, respectively). n.s., not significant (p=0.52).

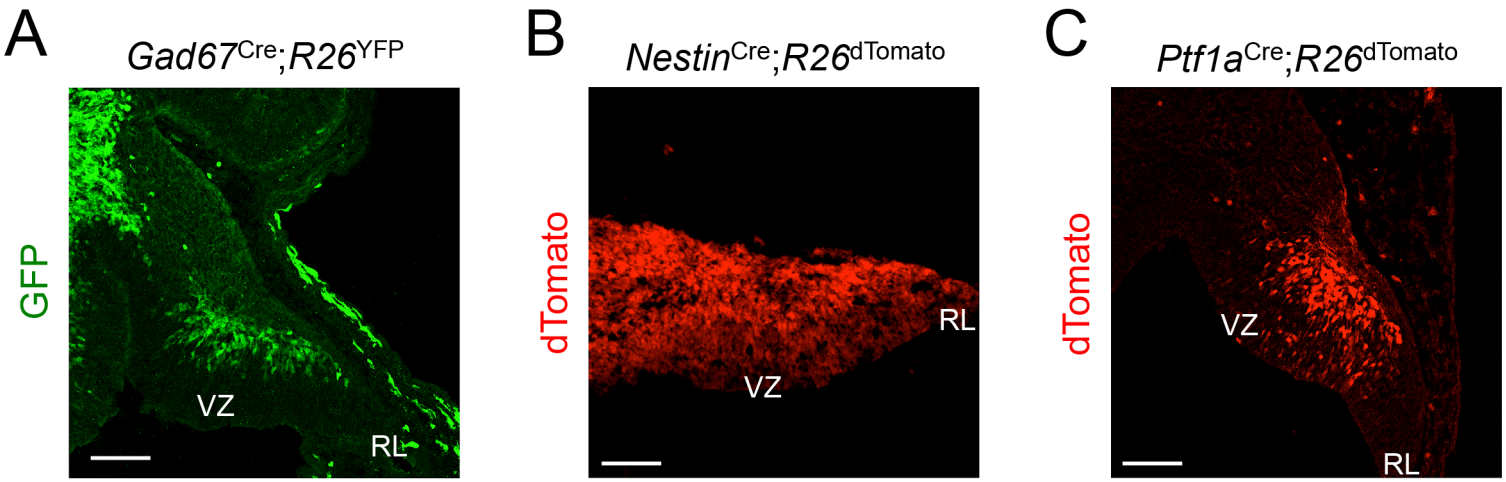

Figure S4. Expression of GAD67, Ptf1a and Nestin in the E12.5 cerebellar primordium. (Related to Figure 2).

(A) Representative section of cerebellum primordium from *Gad67*^{Cre};*R26*^{YFP} E12.5 embryos immunostained for GFP. VZ, ventricular zone; RL, rhombic lip.

(B) Representative section of cerebellum primordium from *Nestin*^{Cre};*R26*^{dTomato} E12.5 embryos showing dTomato fluorescence.

(C) Representative section of cerebellum primordium from *Ptf1a*^{Cre};*R26*^{dTomato} E12.5 embryos showing dTomato fluorescence.

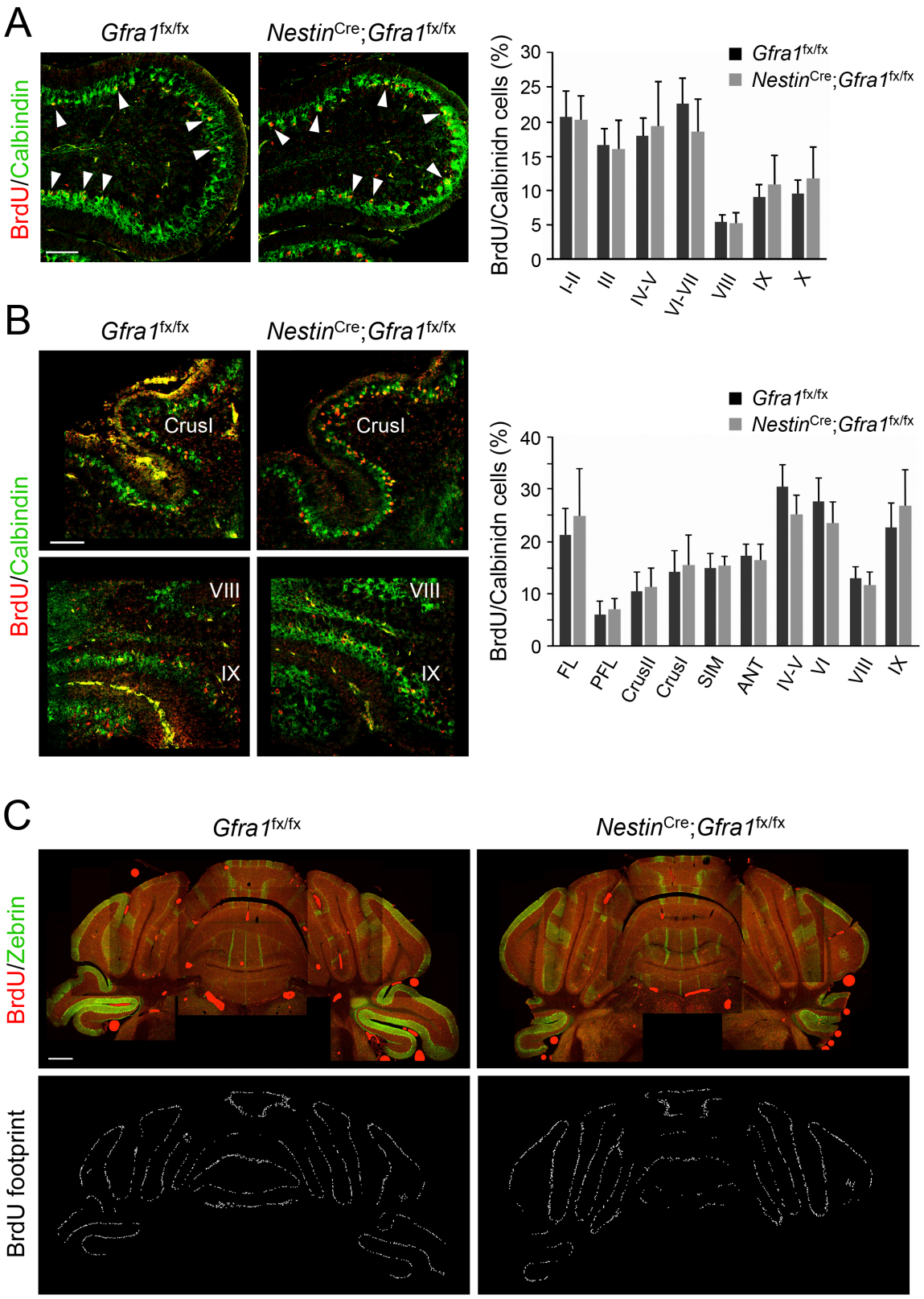

Figure S5. Normal PC distribution in postnatal cerebellum of *Gfra1* conditional mutant mice. (Related to Figure 2).

(A) Representative sagittal sections of cerebellum from postnatal day 5 (P5) *Gfra1*$^{fx/fx}$ and *Nestin*^{Cre};*Gfra1*$^{fx/fx}$ mice injected with BrdU at E12.5. Sections were immunostained for BrdU (red) and calbindin (green). Arrowheads point to double positive cells in the Purkinje cell layer (PCL). Histogram to the right shows quantitative analysis of BrdU/calbindin double-labeled cells in each cerebellar folia (I to X) relative to the total number of double-labeled cells found in each section expressed as average ± SEM (N=4). Scale bar, 100 μm.

(B) Representative coronal sections of cerebellum from P5 *Gfra1*$^{fx/fx}$ and *Nestin*^{Cre};*Gfra1*$^{fx/fx}$ mice injected with BrdU at E12.5. Sections were immunostained for BrdU (red) and calbindin (green). Histogram to the right shows quantitative analysis of BrdU/calbindin double-labeled cells in each cerebellar lobule (FL to IX) relative to the total number of double-labeled cells found in each section expressed as average ± SEM (N=5). FL: flocculus, PFL: paraflocculus, CrusI and II: lobulus ansiformis, SIM: lobulus simplex, ANT: anterior lobe. Scale bar, 100 μm.

(C) Representative coronal sections of cerebellum from P25 *Gfra1*$^{fx/fx}$ and *Nestin*^{Cre};*Gfra1*$^{fx/fx}$ mice injected with BrdU at E12.5. Top row shows sections immunostained for BrdU (red) and zebrin (green). Zebrin staining was performed to assess whether PCs labeled with BrdU at E12.5 ended up in different Zebrin^{+} or Zebrin^{-} zones the different genotypes. Bottom row shows BrdU footprint in the PCL in which each dot represents one BrdU-labeled cell. There is no difference in the distribution of BrdU^{+} cells along the mediolateral axis between mutant and control cerebella. Scale bar, 500 μm.