
Figures and figure supplements

Convergence of pontine and proprioceptive streams onto multimodal cerebellar granule cells

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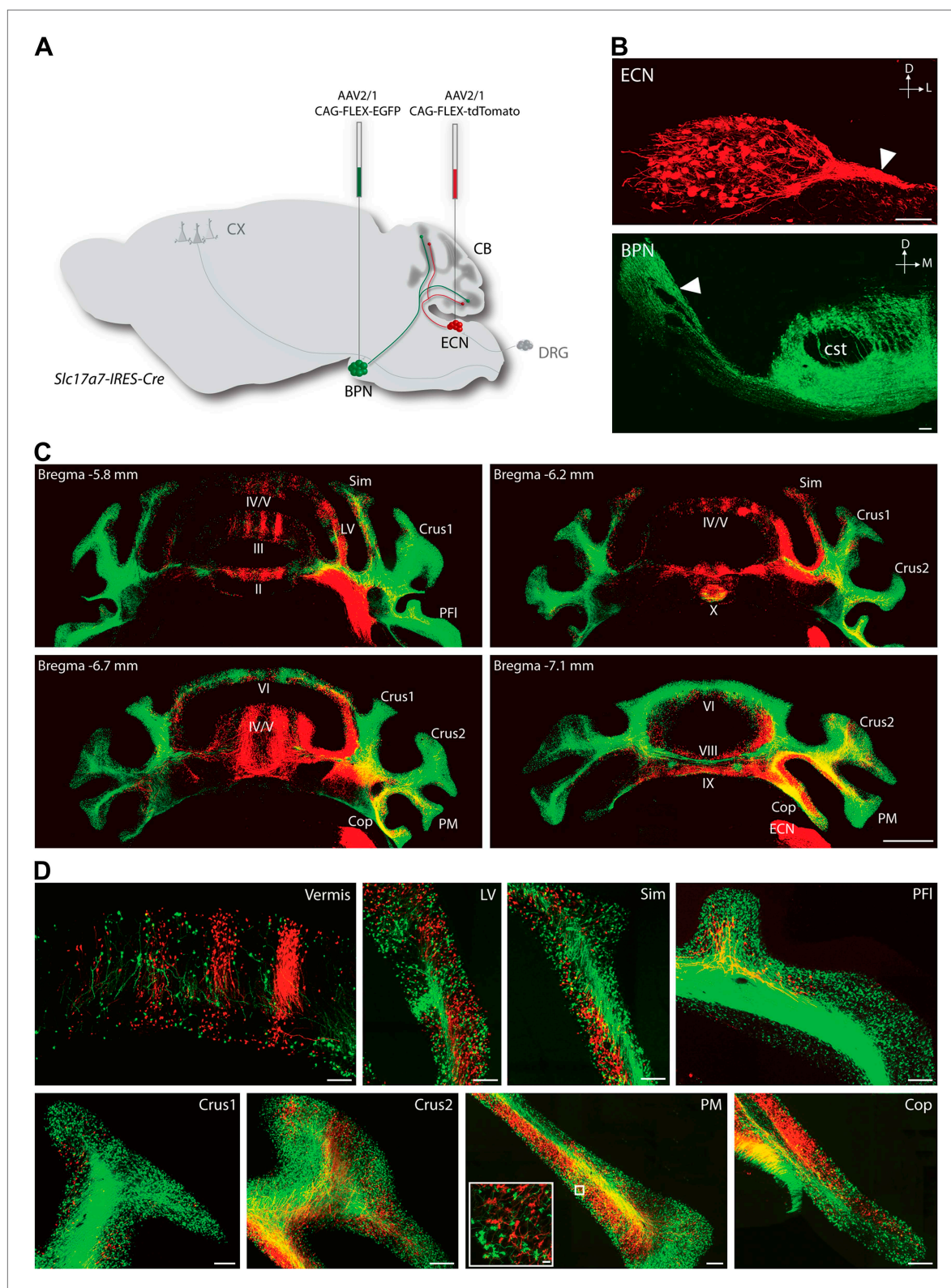


Figure 1. Termination patterns of ECN and BPN mossy fibers in the cerebellum. **(A)** Genetic and viral scheme to specifically label ECN and BPN mossy fibers. Cre-dependent AAVs expressing *tdTomato* and *EGFP* are stereotactically injected into ECN and BPN respectively in the *Slc17a7-IRES-Cre* mouse brain. CX, cortex; CB, cerebellum; DRG, dorsal root ganglia. **(B)** Confocal images of viral injection sites (ECN and BPN). White arrowheads, ECN and BPN axonal tracts; cst, corticospinal tract. D: dorsal; L: lateral; M: medial. Scale bars, 100 μ m. **(C)** Projection pattern of ECN (red) and BPN (green) mossy fibers

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in the cerebellum. Montage confocal images of the cerebellum from rostral to caudal (Bregma -5.8 to -7.1 mm) positions. Vermis (II, III, IV/V, VI VIII, IX, X); Copula of the pyramis (Cop); lateral vermis (LV); Paraflocculus (PFI); Paramedian lobule (PM); simple lobule (Sim). Scale bar, 1 mm. **(D)** Magnified co-termination fields of ECN (red) and BPN (green) mossy fibers in selected cerebellar lobules. Boxed area shows high density of ECN and BPN mossy fiber terminations in the paramedian lobule. Scale bars, 100 μm ; 10 μm in boxed area.

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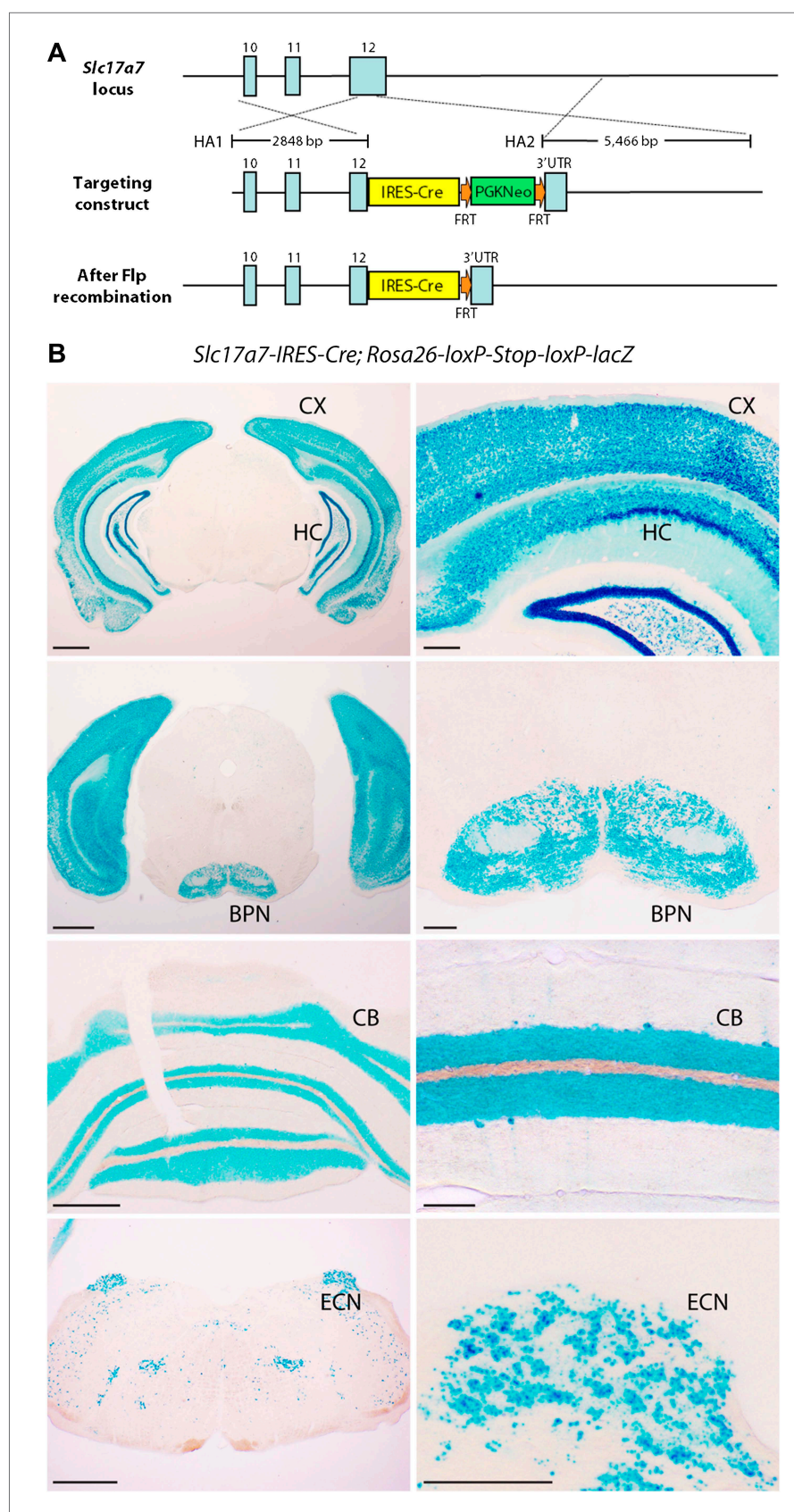


Figure 1—figure supplement 1. Expression pattern of *Slc17a7-IRES-Cre* mouse line. **(A)** Targeting strategy for generating *Slc17a7-IRES-Cre* mice. Exons: blue boxes. HA1 and HA2: homology arms; FRT: flippase recognition site. **(B)** In situ hybridization images showing lacZ expression in the brain of *Slc17a7-IRES-Cre; Rosa26-loxP-Stop-loxP-lacZ* mice. The images show lacZ expression in the cerebral cortex (CX), hippocampus (HC), basophilic nucleus (BPN), cerebellum (CB), and embryonic cerebellar nuclei (ECN). Continued on next page

Figure 1—figure supplement 1. Continued on next page

target; PGKneo: neomycin resistance cassette; UTR: untranslated region. **(B)** β -Galactosidase activity in four coronal levels (low and high magnification) of a *Slc17a7-IRES-Cre; Rosa26-loxP-Stop-loxP-lacZ* animal. Left scale bars, 1 mm; right scale bars, 250 μ m. CX: cortex; CB: cerebellum; HC: hippocampus.

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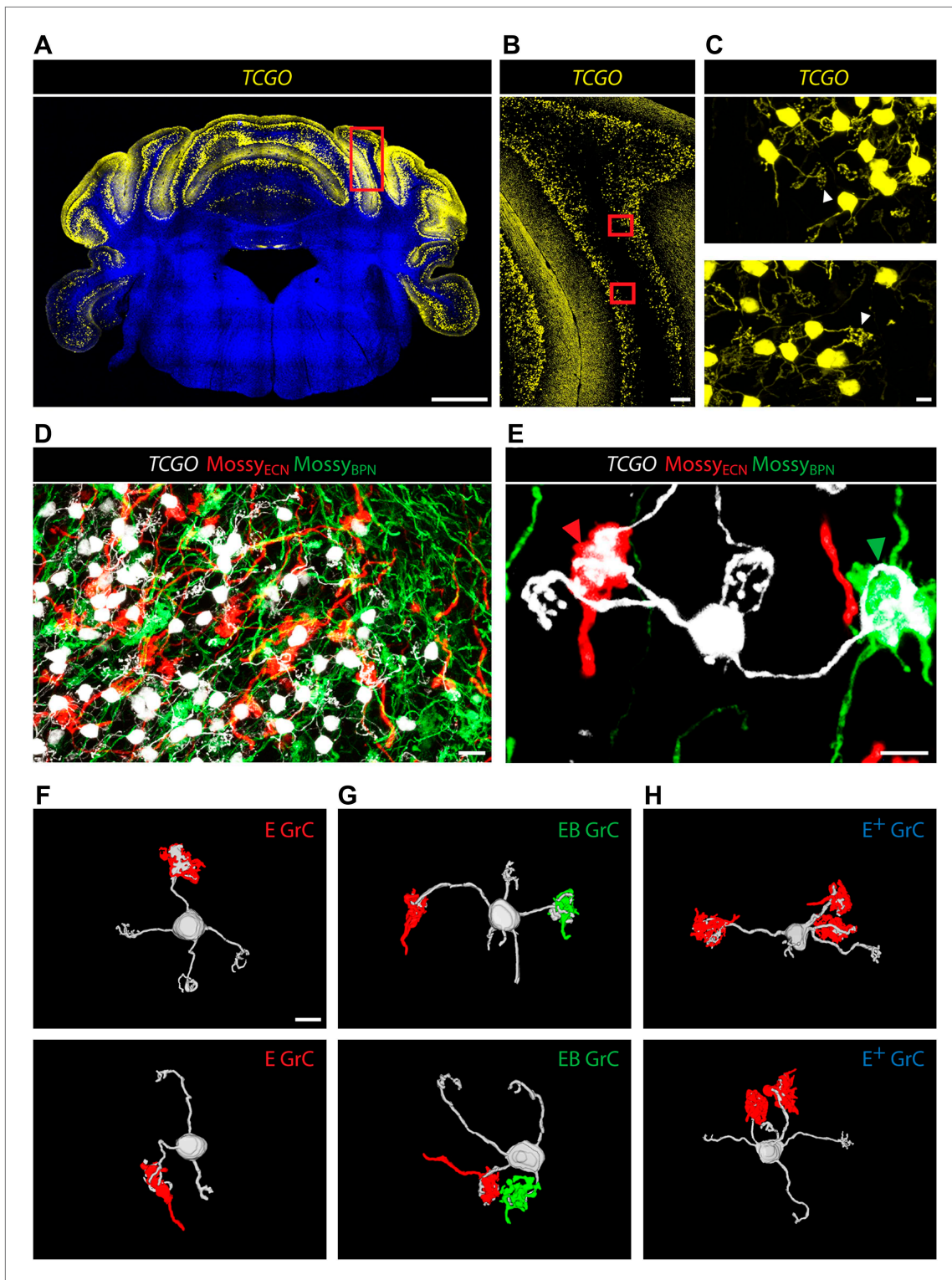


Figure 2. Convergence of ECN and BPN mossy fibers on cerebellar granule cells. **(A)** TCGO transgene expression in a representative section of cerebellum. Scale bar, 1 mm. **(B)** TCGO *mCitrine* expression in boxed area of **(A)**, simple lobule. Scale bar, 100 μ m. **(C)** Maximum projection of labeled granule cells in TCGO mice (white arrowhead: dendritic arborization) in boxed areas of **(B)**. Scale bar, 5 μ m. **(D)** Co-termination of ECN (red) and BPN (green) mossy fibers in paramedian lobule of a *Slc17a7-IRES-Cre*; TCGO mouse. Scale bar, 10 μ m. **(E)** Maximum projection of a labeled granule cell that receives mossy fiber inputs from ECN (red arrowhead) and BPN (green arrowhead) in a *Slc17a7-IRES-Cre*; TCGO mouse. Scale bar, 5 μ m. **(F)–(H)** 3D reconstruction of granule cells with associated mossy fiber terminations. E granule cell (GrC), granule cell with one ECN input and one other traceable dendrite; EB GrC, granule cell with ECN and BPN input(s); E⁺ GrC, granule cell with two or more ECN inputs but no BPN input. Scale bar, 5 μ m.
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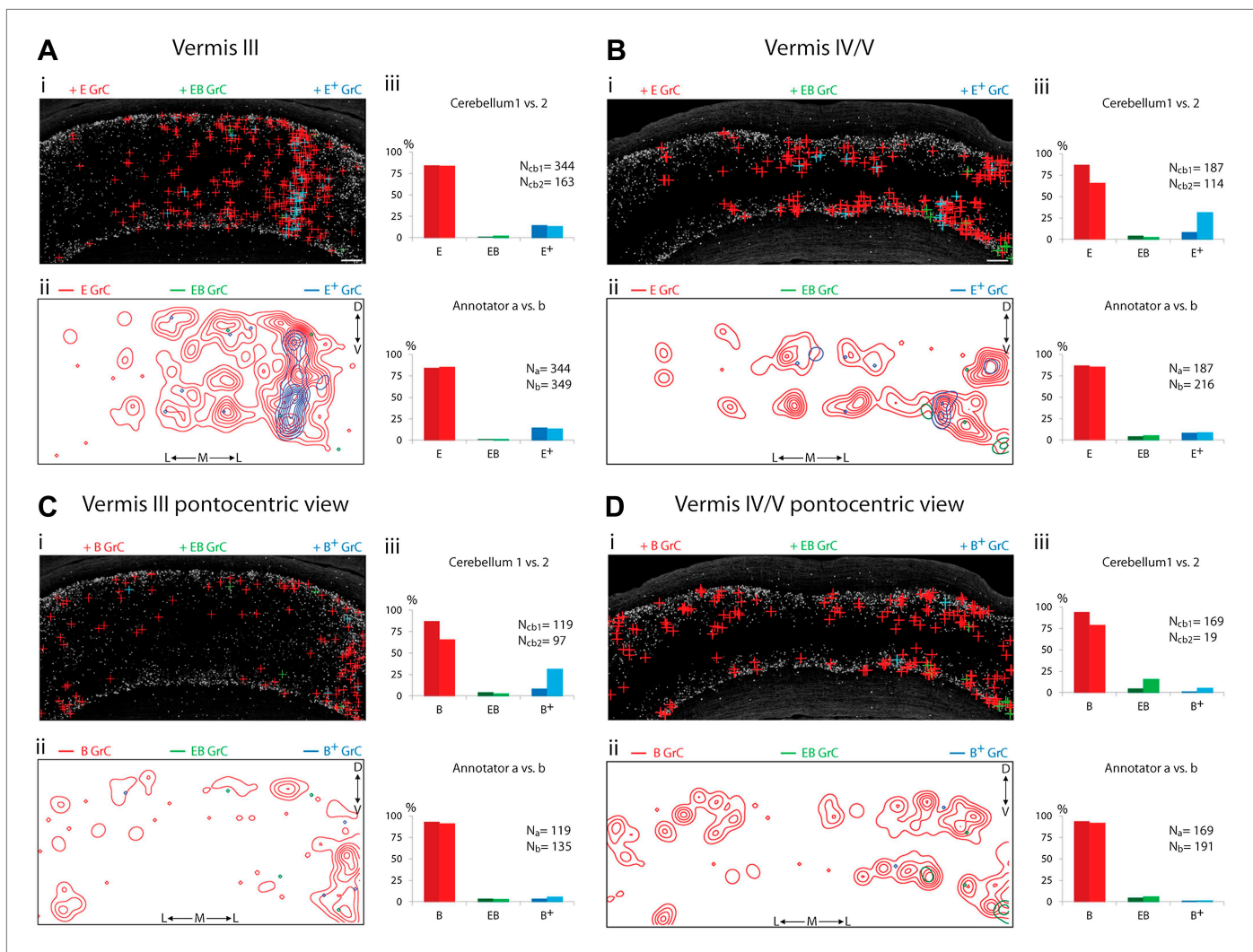


Figure 3. Cerebellar areas not exhibiting convergence of sensory and pontine inputs. Survey of ECN and BPN convergence in the anterior vermis. (A) Vermis III. (B) Vermis IV/V. (i) Granule cell (GrC) classification and distribution. Red cross, E GrC; green cross, EB GrC; blue cross, E⁺ GrC. Scale bars, 100 μ m. (ii) density contour map of E, EB and E⁺ granule cells. D: dorsal; V: ventral; M: medial; L: lateral. Red, green and blue lines in the contour map represent density of E, EB, and E⁺ granule cells respectively. (iii) upper, percentage of E, EB and E⁺ granule cells of two *Slc17a7-IRES-Cre*; *TCGO* cerebella. Lower, comparison between annotators in percentage of E, EB and E⁺ granule cells in a selected section. (C) Pontocentric view of vermis III. (D) Pontocentric view of vermis IV/V. (C and D) Same organization as in (A and B) but B replaces E and B⁺ replaces E⁺ granule cells. B GrC: granule cell with one BPN input and one other traceable dendrite; B⁺ GrC: granule cell with two or more BPN inputs but no ECN input. EB GrC is the same as in (A and B)

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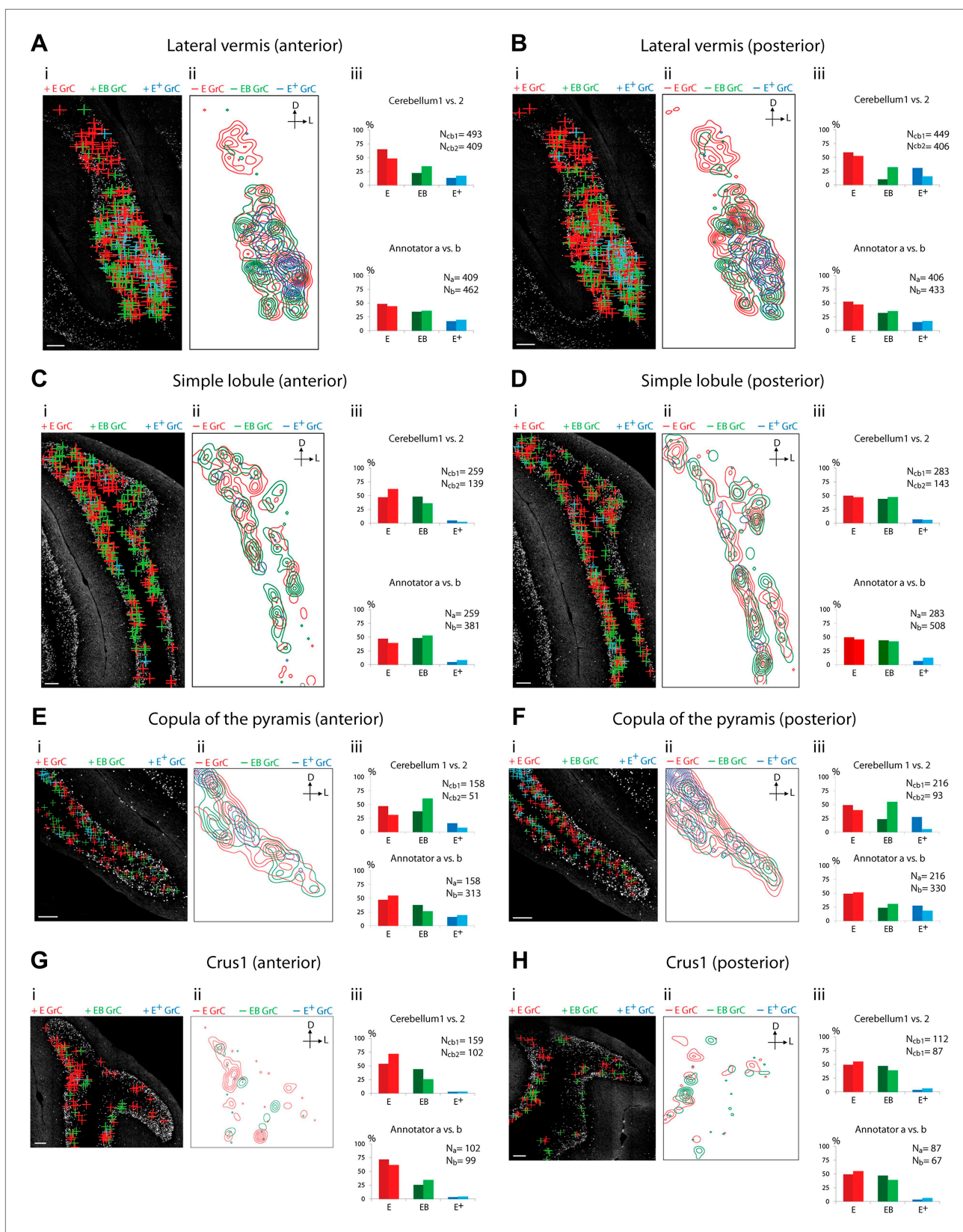


Figure 4. Cerebellar areas exhibiting mixtures of convergence and separation of sensory and pontine inputs. Survey of ECN and BPN convergence in the lateral vermis, simple lobule, copula of the pyramis and Crus1. (A) Lateral vermis, anterior section. (B) Lateral vermis, posterior section. (C) Simple lobule, anterior section. (D) Simple lobule, posterior section. (E) Copula of the pyramis, anterior section. (F) Copula of the pyramis, posterior section. (G) Crus1, anterior section. (H) Crus1, posterior section. Figure 4. Continued on next page

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lobule, anterior section. (D) Simple lobule, posterior section. (E) Copula of the pyramis, anterior section. (F) Copula of the pyramis, posterior section. (G) Crus1, anterior section. (H) Crus1, posterior section. (i) Granule cell (GrC) classification and distribution. Red cross: E GrC; green cross: EB GrC; blue cross: E⁺ GrC. Scale bars, 100 μ m. (ii) Density contour map of E, EB and E⁺ granule cells. D: dorsal; L: lateral. Red, green and blue lines in the contour map represent density of E, EB, and E⁺ granule cells, respectively. (iii) Upper, percentage of E, EB and E⁺ granule cells of two *Slc17a7-IRES-Cre*; TCGO cerebella. Lower, comparison between annotators in percentage of E, EB and E⁺ granule cells.

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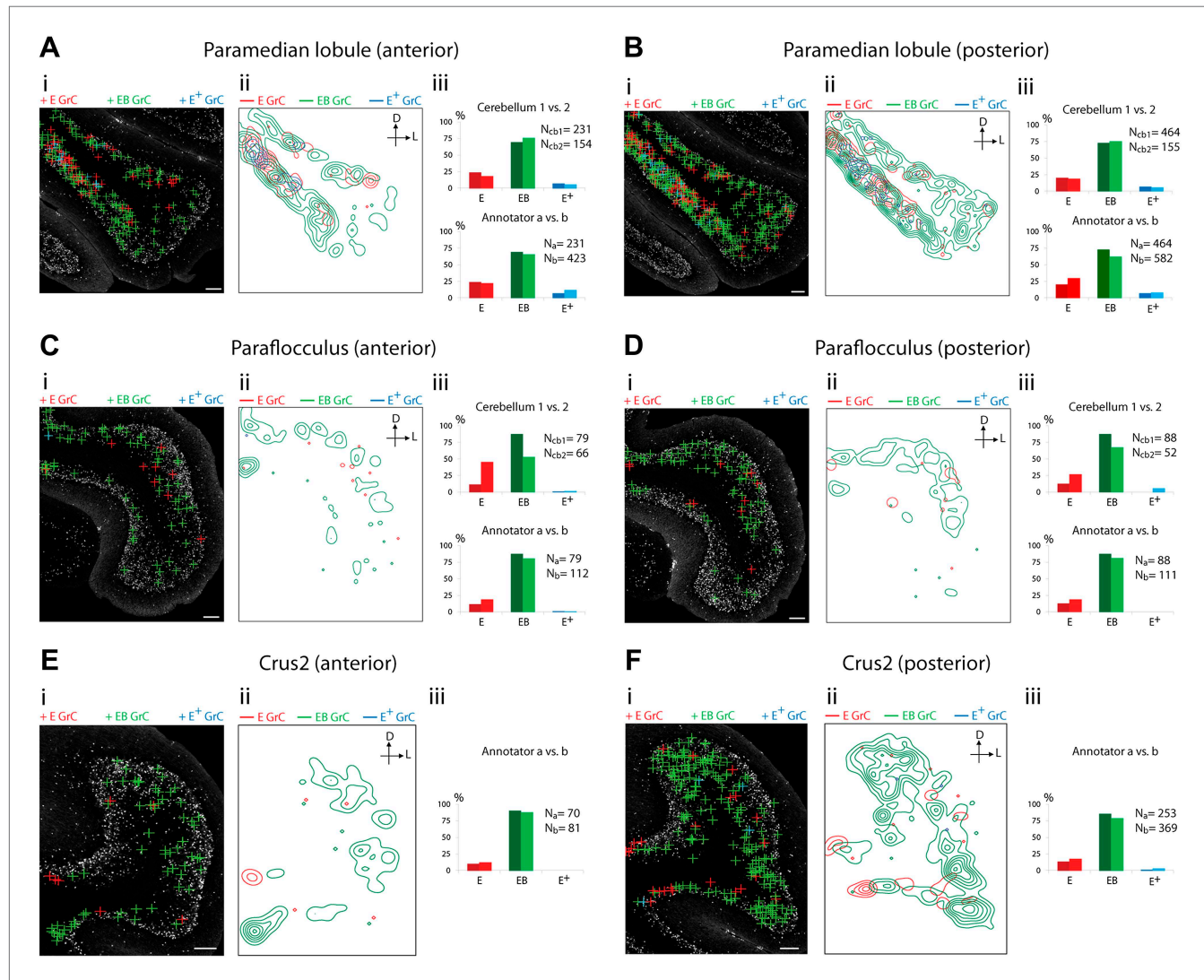


Figure 5. Cerebellar areas exhibiting abundant convergence of sensory and pontine inputs. Survey of ECN and BPN convergence in the hemispheric regions. (A) Paramedian lobule, anterior section. (B) Paramedian lobule, posterior section. (C) Paraflocculus, anterior section. (D) Paraflocculus, posterior section. (E) Crus2, anterior section. (F) Crus2, posterior section. (i) Granule cell (GrC) classification and distribution. Red cross: E GrC; green cross: EB GrC; blue cross: E⁺ GrC. Scale bars, 100 μ m. (ii) Density contour map of E, EB and E⁺ granule cells. D: dorsal; L: lateral. Red, green and blue lines in the contour map represent density of E, EB, and E⁺ granule cells respectively. (iii) Upper, percentage of E, EB and E⁺ granule cells from two *Slc17a7-IRES-Cre*; TCGO cerebella. Lower, comparison between annotators in percentage of E, EB and E⁺ granule cells in a selected section. (E and F) do not have comparisons across the two cerebella in (iii).

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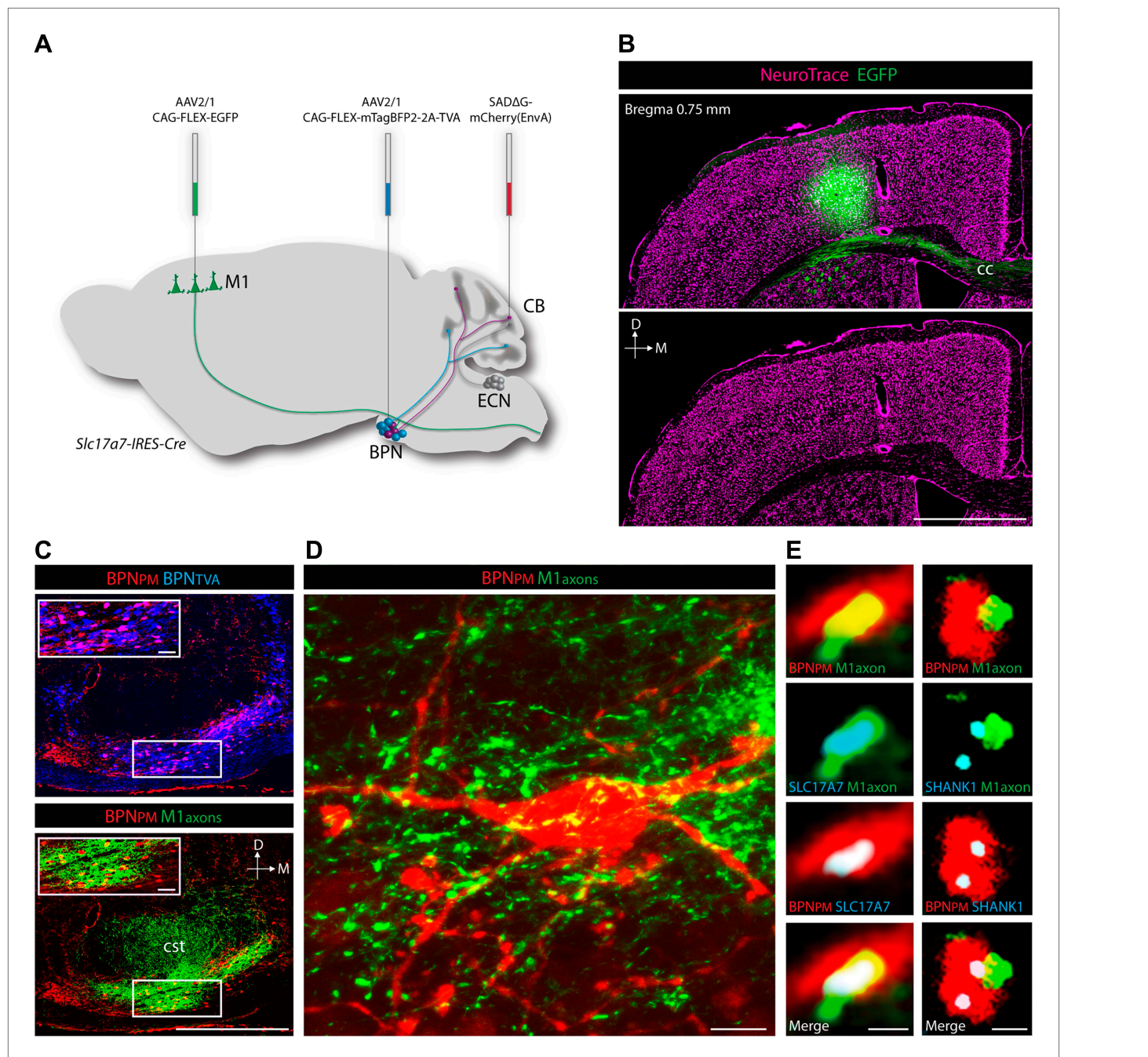


Figure 6. Cortical inputs to paramedian-projecting BPN neurons. Combining M1 anterograde tracing and paramedian lobule retrograde tracing. **(A)** Scheme to anterogradely label forelimb/upper body M1 cortical axons and retrogradely label paramedian-projecting BPN neurons (BPN_{PM}). Cre-dependent AAVs expressing *EGFP* and *mTagBFP2-2A-TVA* were stereotactically injected into M1 and BPN respectively in the *Slc17a7-IRES-Cre* mouse and SADΔG-mCherry(EnvA) rabies virus was injected into the paramedian lobule of the cerebellum. CB, cerebellum; M1, primary motor cortex. **(B)** Location of EGFP-expressing neurons with relation to cortical cytoarchitecture. cc: corpus callosum. D: dorsal; M: medial. Scale bar, 1 mm. **(C)** Upper, relationship of BPN_{PM} and TVA-expressing BPN neurons (BPN_{TVA}) sensitive to rabies infection. Lower, colocalization of M1 axons and BPN_{PM} neurons. D: dorsal; M: medial. Scale bars, 500 μm; 50 μm in magnified areas. **(D)** High-magnification image of BPN_{PM} neurons and the M1 axon termination field. Scale bar, 10 μm. **(E)** Synaptic arrangement between M1 axons and BPN_{PM}. Left, apposition of a M1 axon expressing the presynaptic marker SLC17A7 and a dendrite of a BPN_{PM} in a single confocal slice. Right, apposition of a M1 axon and a SHANK1-containing postsynaptic density of a BPN_{PM} in a single confocal slice. Scale bars, 1 μm.

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