

Supplementary Materials

Non-cell autonomous control of precerebellar neuron migration by Slits and Robos.

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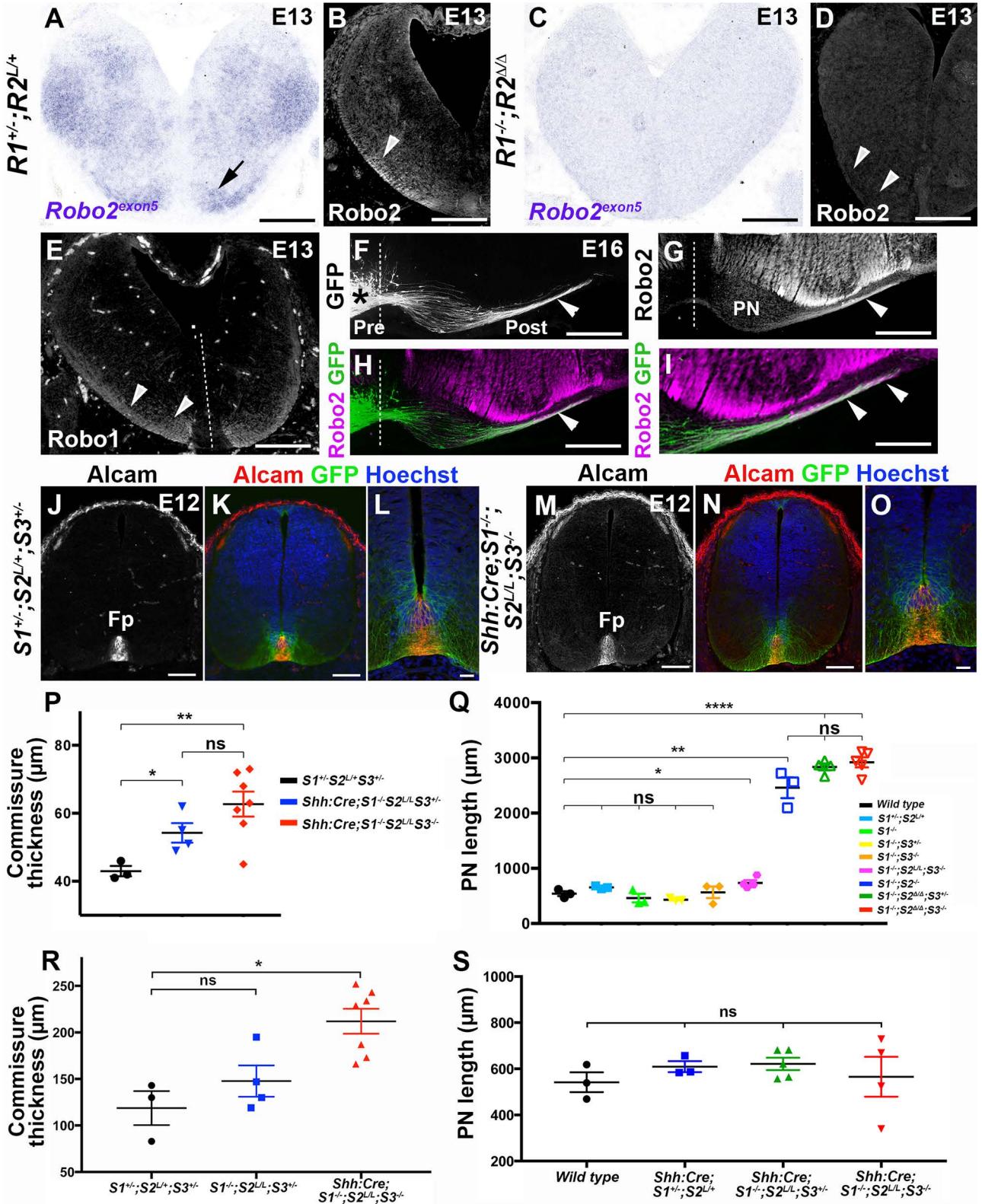


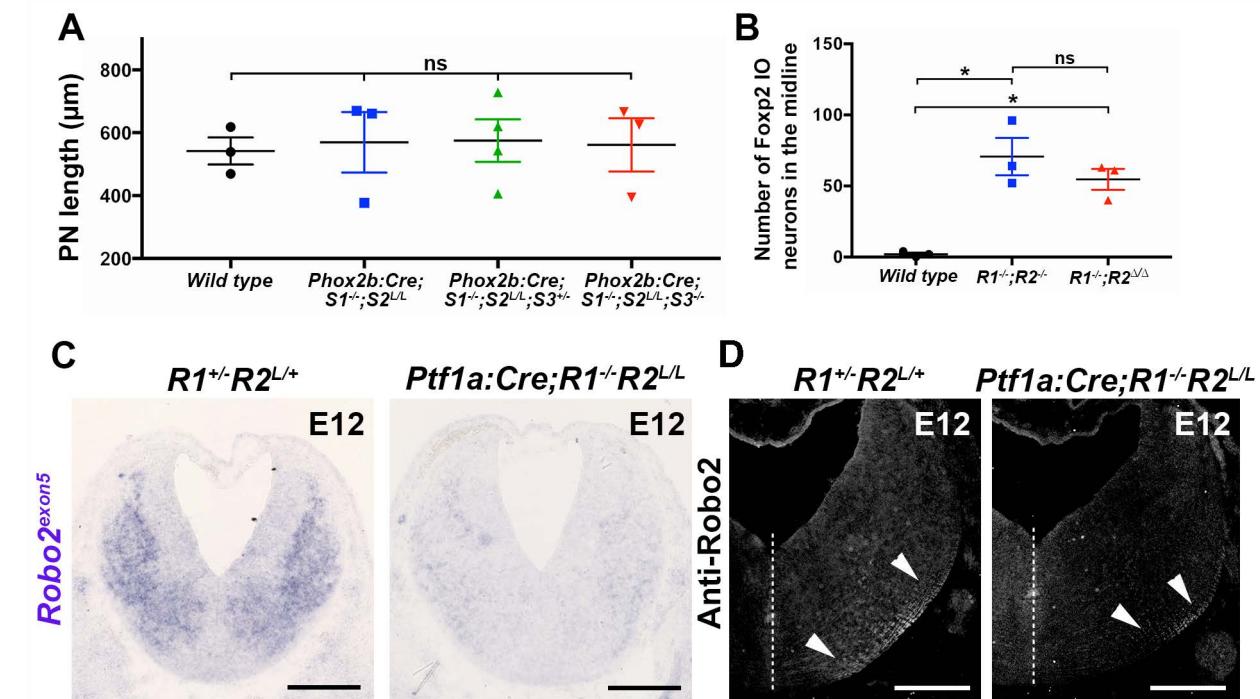
Figure S1

Figure S1**Validation of precerebellar neuron defects in Slit and Robo mutants.**

(A-D) Sections of E13 hindbrains, at the level of the inferior olive (arrow in A), hybridized with a *Robo2* exon5 specific probe (A, C) or immunolabeled with an anti-Robo2 antibody (B, D). Robo2 mRNA is not detected in *Robo1^{-/-};Robo2^{Δ/Δ}* embryo compared to *Robo1^{+/+};Robo2^{L/+}* embryo. Likewise, Robo2-immunoreactive processes seen in *Robo1^{+/+};Robo2^{L/+}* embryos are absent in *Robo1^{-/-};Robo2^{Δ/Δ}* mutant (arrowheads). (E) E13 hindbrain sections immunolabeled for Robo1. Robo1 is detected laterally in longitudinal axons (arrowheads) but not at the level of the floor plate (dashed lines). (F-I) Coronal sections at the level of the pontine nuclei (PN) of an E16 wild type embryo unilaterally electroporated at E13.5 with pCX-GFP and immunolabeled with Robo2 antibodies. GFP+ PN neurons (arrowhead in F) accumulate at the midline (dashed line) but their axons cross it and extend on the opposite side (arrowheads). Robo2 is highly expressed in hindbrain axons but not detected in PN neuron cell bodies. However, Robo2 is present on post-crossing PN axons (arrowhead in G-I). (J-O) Spinal cord sections of E12 *S1^{+/+};S2^{+/+};S3^{+/+}* (J-L) and *Shh:Cre;S1^{-/-};S2^{L/L};S3^{-/-}* (M-O) embryos immunolabeled with antibodies against Alcam and GFP. The floor plate (Fp) is labeled and similar in controls and mutants. (P) Quantification of spinal cord commissure thickness in *Shh:Cre;Slit* mutants (relates to Fig. 4). ns, not significant, P=0.1035. (Q) Quantification of PN nuclei length in *Slit* double and triple mutants (relates to Figs. 5-7). Comparison between wild type and each genotype. ns: *S1^{+/+};S2^{L/+}*, P=0.1080 ; *S1^{-/-}*, P=0.4221 ; *S1^{-/-};S3^{+/+}*, P=0.1130; *S1^{-/-};S3^{-/-}*, P=0.8440). (R) Quantification of hindbrain commissure thickness in E12 *Slit* mutants (relates to Fig. 6). ns, *S1^{-/-};S2^{L/L};S3^{+/+}*, P=0.2973. (S) Quantification of PN nuclei length in *Shh:Cre;Slit* mutants (relates to Fig. 6). Comparison between wild type and other genotypes. ns, *Shh:Cre;S1^{+/+};S2^{L/+}*, P=0.2613 ; *Shh:Cre;S1^{-/-};S2^{L/L};S3^{+/+}*, P=0.2008; *Shh:Cre;S1^{-/-};S2^{L/L};S3^{-/-}*, P=0.8173. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Welch's t-test. Error bar indicate standard error of the mean (s.e.m.).

Abbreviations: Pre, pre-crossing; Post, post-crossing.

Scale bars: 275 µm (A), 250 µm (B, C), 200 µm (D, E), 150 µm (F-G), 50 µm (I), 80 µm (J,K,M,N), 20 µm (L, O).

**Figure S2****Validation of Robo2 inactivation in *Ptf1a:Cre;Robo1/2* mutants.**

(A) Quantification of pontine neuron (PN) length in *Phox2b:Cre;Slit1/2/3* mutants. (B) Quantification of the number of *Foxp2*+ IO neurons invading the midline in Robo1/2 double mutants. Comparison between wild type and other genotypes.

*P<0,05. ns, *Phox2b:Cre;S1^{-/-};S2^{L/L}*, P=0.8134; *Phox2b:Cre;S1^{-/-};S2^{L/L};S3^{+/-}*, P=0.6994 ; *Phox2b:Cre;S1^{-/-};S2^{L/L};S3^{-/-}*, P=0.8493 (Welch's t-test). Error bar indicate standard error of the mean (s.e.m.). (C, D) Sections of E12 hindbrains, at the level of the inferior olive, hybridized with a *Robo2* exon5 specific probe (C) or immunolabeled with an anti-Robo2 antibody (D). Robo2 mRNA expression is strongly downregulated in *Ptf1a:Cre;Robo1^{-/-};Robo2^{L/L}* embryos (left panels) compared to *Robo1^{+/-};Robo2^{L/+}* embryos (right panels). Likewise, the density of Robo2+ immunoreactive axons is also decreased (arrowheads). Dashed lines indicate the midline. Scale bars: 300μm (C), 250μm (D).

Movies



Movie 1

3D movie of the migrating stream of pontine neurons in a wild type E16 embryo. Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 2

3D movie of the migrating stream of pontine neurons in a *Robo1^{-/-};Robo2^{-/-}* E16 embryo. Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 3

3D movie of the migrating stream of pontine neurons in a *Robo1^{-/-};Robo2^{Δ/Δ}* E16 embryo.

Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 4

3D movie of the migrating stream of pontine neurons in a *Slit1^{-/-};Slit2^{-/-}* E16 embryo.
Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 5

3D movie of the migrating stream of pontine neurons in a $Slit1^{-/-};Slit2^{-/-};Slit3^{-/-}$ E16 embryo.

Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 6

3D movie of the migrating stream of pontine neurons in a $Slit1^{-/-};Slit2^{\Delta/\Delta};Slit3^{-/-}$ E16 embryo. Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 7

3D movie of the migrating stream of pontine neurons in a *Shh:Cre;S1^{-/-};S2^{L/L};S3^{-/-}* E16 embryo. Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 8

3D movie of the migrating stream of pontine neurons in a *Phox2b:Cre;S1^{-/-};S2^{L/L};S3^{-/-}* E16 embryo. Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 9

3D movie of the migrating stream of pontine neurons in a *Shh:Cre;Phox2b:Cre;S1^{-/-};S2^{L/L};S3^{-/-}* E16 embryo. Whole-mount immunolabeling for Barhl1 (Green) and Robo3 (Red) and 3DISCO clearing.



Movie 10

3D stack of optical sections through the inferior olive of a wild type E13 embryo. Whole-mount immunolabeling for Foxp2.



Movie 11

3D stack of optical sections through the inferior olive of a *Robo1*^{-/-}; *Robo2*^{-/-} E13 embryo.

Whole-mount immunolabeling for Foxp2. Note that some Foxp2 neurons are found within the midline.



Movie 12

3D stack of optical sections through the inferior olive of a *Slit1*^{-/-}; *Slit2*^{Δ/Δ}; *Slit3*^{-/-} E13 embryo. Whole-mount immunolabeling for Foxp2. Note that some Foxp2 neurons are found within the midline.



Movie 13

3D stack of optical sections through the inferior olive of a *Shh:Cre;S1^{-/-};S2^{L/L};S3^{-/-}* E13 embryo. Whole-mount immunolabeling for Foxp2. Note that a few Foxp2 neurons are found within the midline.