

Supplementary Information

**Netrin-1 Derived from the Ventricular Zone, but not the Floor Plate,
Directs Hindbrain Commissural Axons to the Ventral Midline**

*Kenta Yamauchi^{1,2}, Maya Yamazaki³, Manabu Abe³, Kenji Sakimura³, Heiko Lickert⁴,
Takahiko Kawasaki¹, Fujio Murakami², and Tatsumi Hirata¹

¹ Division of Brain Function, National Institute of Genetics, Mishima, Shizuoka
411-8540, Japan

² Laboratory of Neuroscience, Graduate School of Frontier Biosciences, Osaka
University, Suita, Osaka 565-0871, Japan

³ Department of Cellular Neurobiology, Brain Research Institute, Niigata University,
Niigata 951-8585, Japan

⁴ Institute of Stem Cell Research and Institute of Diabetes and Regeneration Research,
Helmholtz Zentrum München, D-85764 Neuherberg, Germany

Correspondence to:

Kenta Yamauchi

Division of Brain Function, National Institute of Genetics

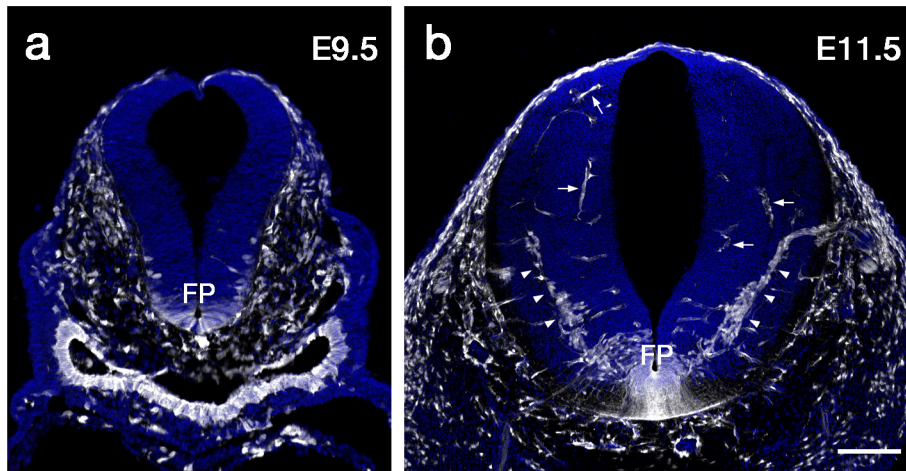
E-mail: kntymuci@gmail.com

Phone: +81-55-981-6721, Fax: +81-55-981-6722

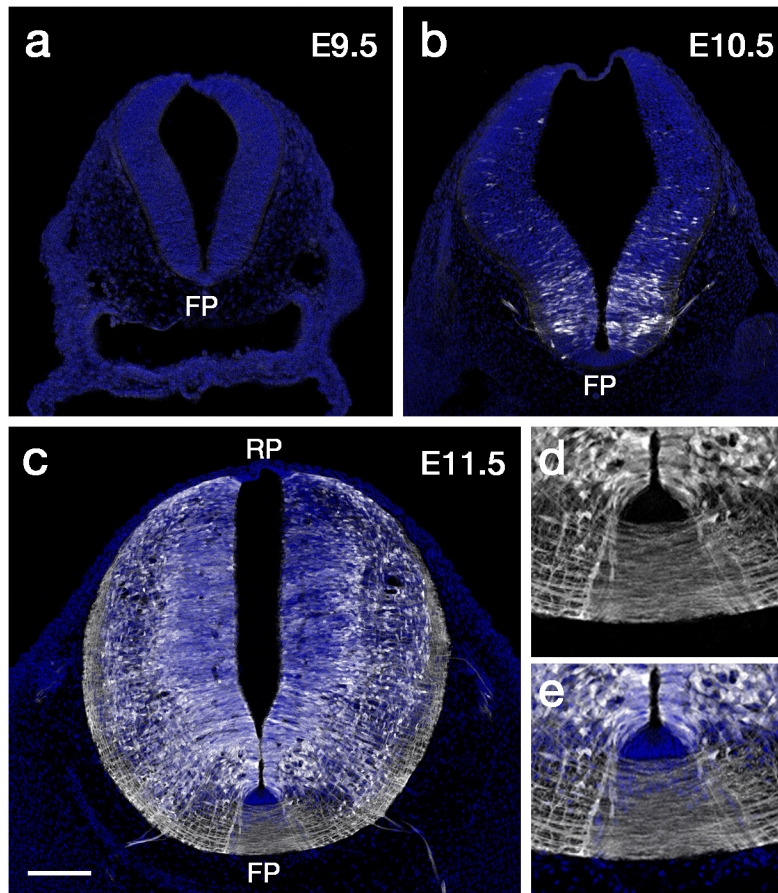
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Supplementary Figure S1-S6

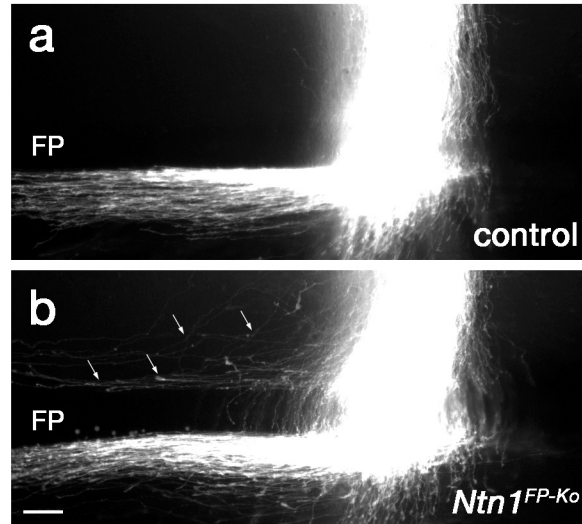
Supplementary Table S1



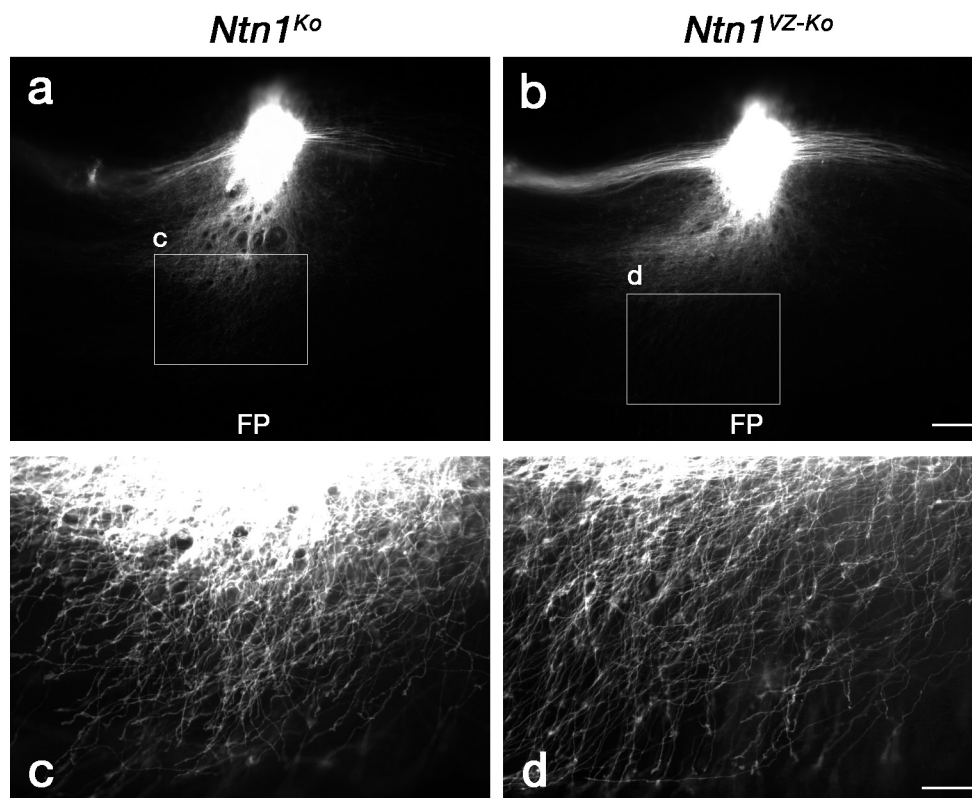
Supplementary Figure S1. EGFP reporter expression in the *Foxa2*^{+/*iCre*};*ZEG* mouse hindbrain. (a, b) GFP immunostaining in E9.5 (a) and E11.5 (b) *Foxa2*^{+/*iCre*};*ZEG* mouse hindbrain transverse sections (E9.5, n = 3; E11.5, n = 3). Sections are counter stained with PI (blue). EGFP reporter expression (white) is found in the FP at both E9.5 (a) and E11.5 (b). At E11.5, EGFP reporter is expressed in dorsally migrating vagal motor neurons (arrowheads), suggesting that the Cre-mediated recombination occurs in the P3 progenitor domain beyond the FP. Arrows point to blood vessels, which also express EGFP. Scale bar: (a) 150 μ m; (b) 100 μ m.



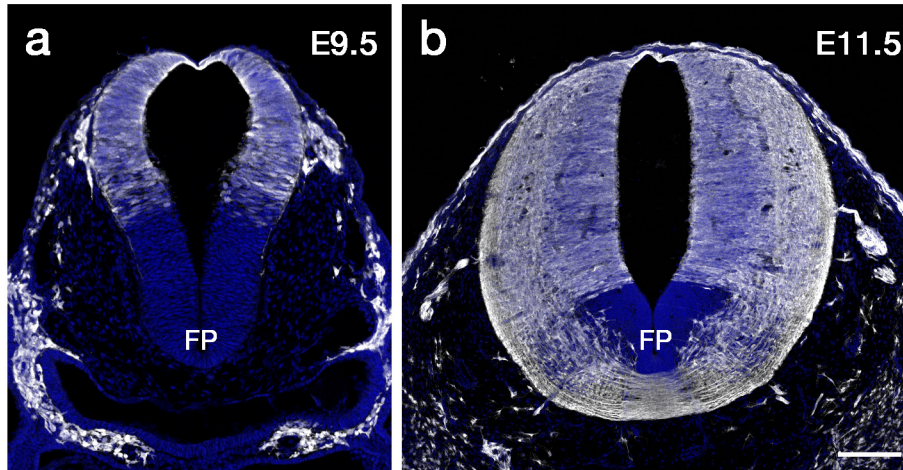
Supplementary Figure S2. EGFP reporter expression in the *NestinCre;ZEG* mouse hindbrain. (a-c) GFP immunostaining in E9.5 (a), E10.5 (b) and E11.5 (c) *NestinCre;ZEG* mouse hindbrain transverse sections (E9.5, n = 4; E10.5, n = 5; E11.5, n = 3). Nuclei are stained with PI (blue). EGFP reporter expression (white) is not detected at E9.5 (a) and appears by E10.5 (b). At E10.5, EGFP⁺ cells are mainly located in the ventral hindbrain and scattered in the dorsal region (b). By E11.5, EGFP reporter expression expands throughout the neural tube except the roof plate (RP) and FP (c). (d, e) Enlarged views of the FP region in (c). (d) GFP immunostaining without PI staining. (e) A merged image of GFP immunostaining and PI staining. Note the absence of EGFP reporter expression in cell bodies of the FP. EGFP⁺ axons crossing the midline are found at the basal region of the FP. Scale bar: (a) 150 μm ; (b, c) 100 μm ; (d, e) 200 μm .



Supplementary Figure S3. Misguidance of DiI-labeled axons near the FP in *Ntn1*^{FP-Ko} mice. (a, b) DiI-labeled axon trajectories near the FPs of control (*Foxa2*^{+iCre}; *Ntn1*^{+floxed}) (a) and *Ntn1*^{FP-Ko} mice (b) (control, n = 7; *Ntn1*^{FP-Ko}, n = 9). In control mice, DiI-labeled axons cross the FP and turn rostrally, forming a tightly fasciculated axon bundle (a). A limited number of axons are deflected from the bundle near the FP in *Ntn1*^{FP-Ko} mice (b). Arrows point to axons turning rostrally on the ipsilateral side. Note that the vast majority of DiI-labeled axons in the *Ntn1*^{FP-Ko} mice project normally. Rostral is towards the left. Scale bar: 100 μ m.



Supplementary Figure S4. Rostral deflection of DiI-labeled axons in *Ntn1^{Ko}* and *Ntn1^{VZ-Ko}* mice. (a, b) DiI-labeled axon trajectories in *Ntn1^{Ko}* (a) and *Ntn1^{VZ-Ko}* mice (b). (c) and (d) are higher magnification images of the areas indicated by rectangles in (a) and (b), respectively. In both genotypes, DiI-labeled axons growing in the ventral hindbrain tend to be rostrally deflected. Note that (a) and (b) are higher magnification images of Fig. 3f and h, respectively. Dorsal is upwards and rostral is towards the left. Hindbrains at the rhombomere 6-8 level are represented. Scale bars: (a, b) 100 μm ; (c, d) 50 μm . The bar in (b) and (d) apply to (a) and (c), respectively.



Supplementary Figure S5. EGFP reporter expression in the *Pax3^{+Cre};ZEG* mouse

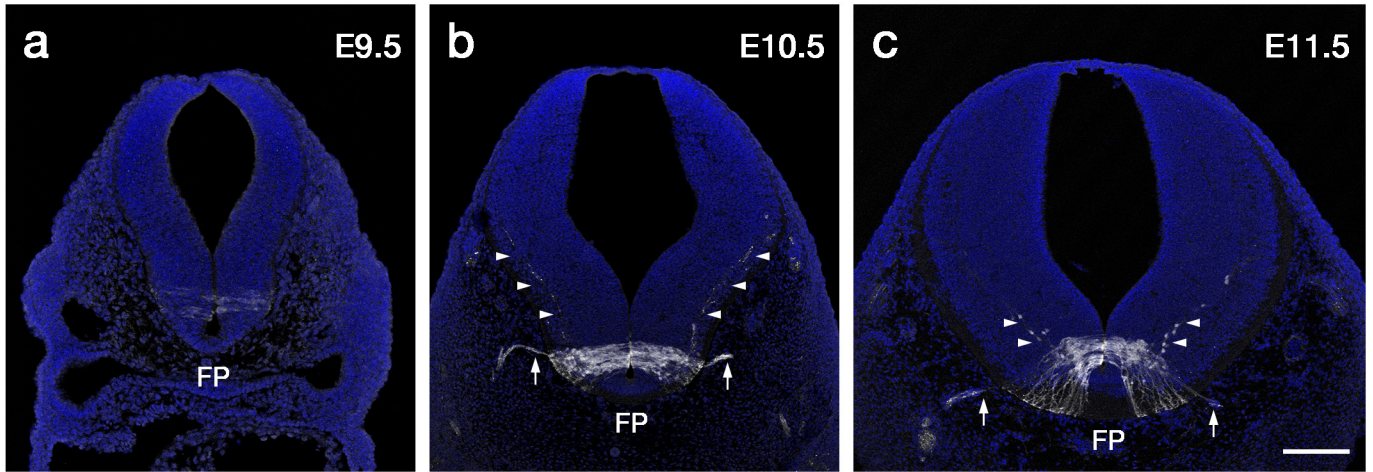
hindbrain. (a, b) GFP immunostaining in E9.5 (a) and E11.5 (b) *Pax3^{+Cre};ZEG* mouse

hindbrain transverse sections (E9.5, n = 4; E11.5, n = 4). Sections are counter stained with

PI (blue). EGFP reporter (white) is expressed in the dorsal VZ of the hindbrain at both E9.5

(a) and E11.5 (b). At E11.5, EGFP⁺ axons and cell bodies of apparent dorsal hindbrain origin

invade the ventral hindbrain (b). Scale bar: (a) 150 μ m; (b) 100 μ m.



Supplementary Figure S6. β -gal reporter expression in the *Olig2*^{+/*Cre*};*Rosa26*^{+/*LSLlacZ*} mouse hindbrain.

(a-c) β -gal immunostaining in E9.5 (a), E10.5 (b) and E11.5 (c) *Olig2*^{+/*Cre*};*Rosa26*^{+/*LSLlacZ*} mouse hindbrain transverse sections (E9.5, n = 8; E10.5, n = 3; E11.5; n = 5). Sections are counter stained with DAPI (blue). At E9.5, weak β -gal reporter expression (white) is detected in the pMN domain adjacent to the FP in some embryos (n = 5/8, a), but its expression is undetectable in the other embryos. β -gal reporter expression in the pMN domain becomes more prominent by E10.5 (b). Peripherally growing hypoglossal motor axons (arrows) and dorsally extending vagal motor axons (arrowheads) are also labeled with an anti- β -gal antibody (b). At E11.5, β -gal reporter expression is found in the pMN domain, hypoglossal axons (arrows) and some vagal motor neurons (arrowheads) (c). Scale bar: (a) 150 μ m; (b, c) 100 μ m.

Supplemental Table S1. Primer sequences for genotyping.

<i>Allele</i>	<i>Primer name</i>	<i>Primer sequence (5'-3')</i>	<i>Product size (bp)</i>
<i>Cre</i>	<i>Cre F3</i>	TAAAGATATCTCACGTACTGACGGTG	301
	<i>Cre R</i>	TCTCTGACCAGAGTCATCCTTAGC	
<i>Flpe</i>	<i>Flpe Fw</i>	CCTAAGGTCCTGGTTCGTCA	229
	<i>Flpe Rv</i>	TTGTTGCTTTTTGCGTCTTG	
<i>iCre</i>	<i>iCre 250 s</i>	GAGGGACTACCTCCTGTACC	630
	<i>iCre 880 as</i>	TGCCCAGAGTCATCCTTGGC	
<i>LacZ</i>	<i>JL32</i>	GCCGTCTGAATTTGACTGAGCGC	495
	<i>JL33</i>	GAAACCGACATCGCAGGCTTCTGC	
<i>Ntn1+</i>	<i>Ntn1 Frt Fw5</i>	GACATCCGCGTGGCTTTCAG	591
	<i>Ntn1 Frt Rv3</i>	CACTCCCTCCACCCCCTGAC	
<i>Ntn1FRT-neo</i>	<i>Pgk pr2</i>	GCTGCTAAAGCGCATGCTCC	369
	<i>Ntn1 Frt Rv3</i>	CACTCCCTCCACCCCCTGAC	
<i>Ntn1flox</i>	<i>Ntn1 Frt Fw5</i>	GACATCCGCGTGGCTTTCAG	770
	<i>Ntn1 Frt Rv3</i>	CACTCCCTCCACCCCCTGAC	
<i>Ntn1Δ</i>	<i>Ntn1 Δ Fw2</i>	GGCGGTTCAAGTTTGGAAGC	355
	<i>Ntn1 Frt Rv3</i>	CACTCCCTCCACCCCCTGAC	
<i>NestinCre</i>	<i>RNE1050</i>	GGCCATGAATGGAAACAGAC	211
	<i>RNE1260</i>	AACGCCAGTGGTTACATTCC	
<i>Olig2TvalresCre</i>	<i>IRES-F</i>	TGGCTCTCCTCAAGCGTATT	~600
	<i>oIMR1085</i>	GTGAAACAGCATTGCTGTCACTT	
<i>Pax3Cre</i>	<i>oIMR6977</i>	CTGCACTCAAGGGACTCCTC	190
	<i>oIMR9074</i>	AGGCAAATTTGGTGTACGG	
<i>Rosa26</i>	<i>oIMR8545</i>	AAAGTCGCTCTGAGTTGTTAT	Wild type: 603
	<i>oIMR8546</i>	GGAGCGGGAGAAATGGATATG	Mutant: 340
	<i>oIMR8052</i>	GCGAAGAGTTTGTCTCAACC	