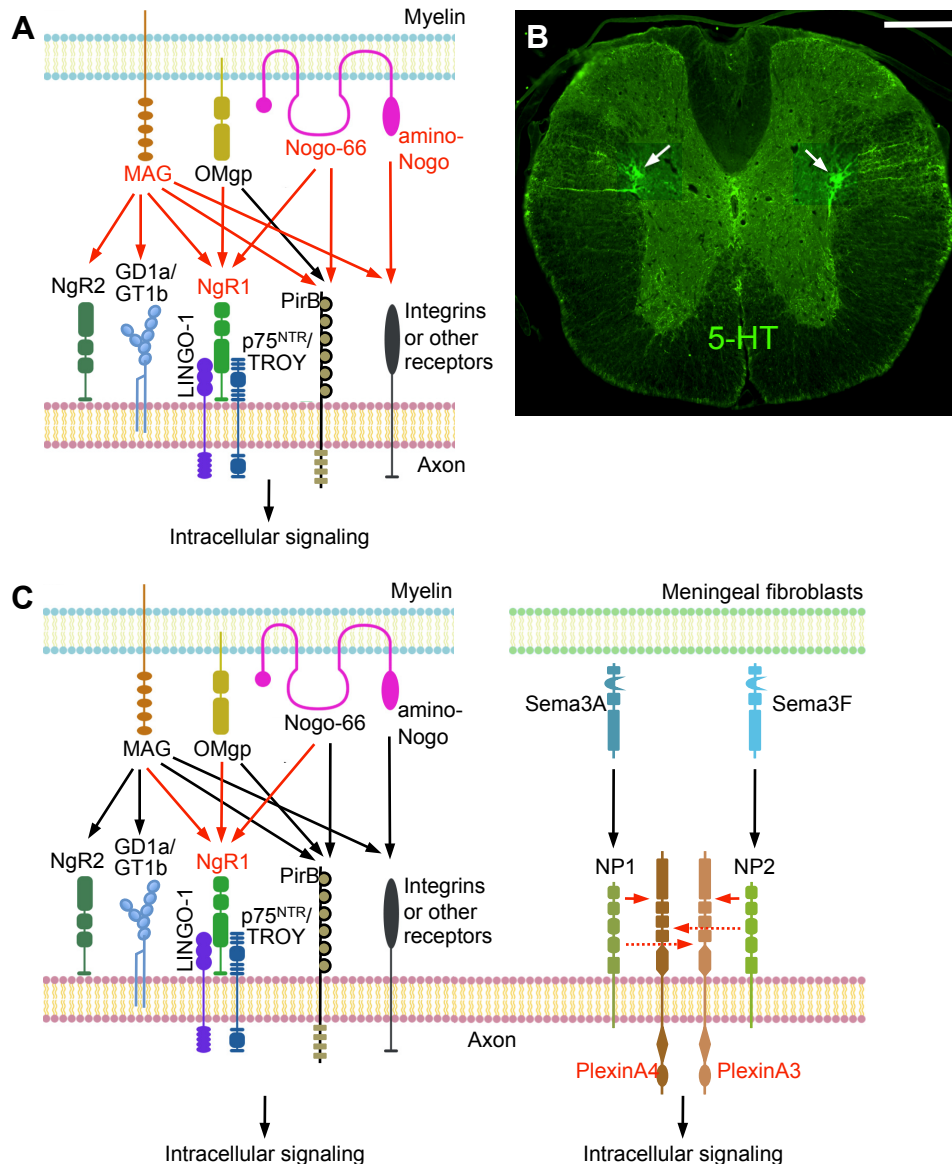


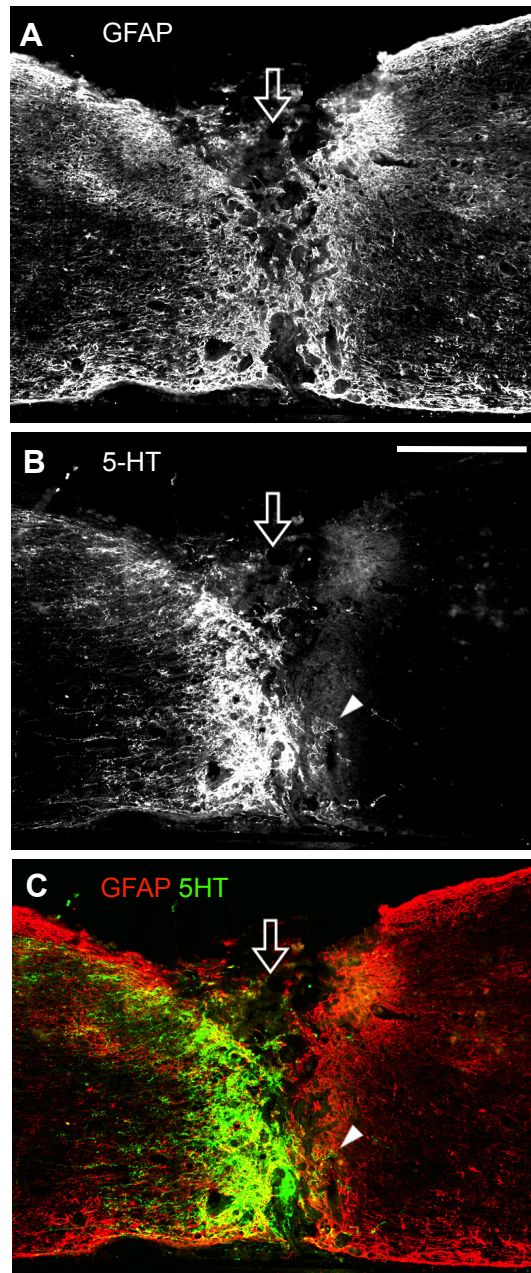
Supplemental Materials for

Combined genetic attenuation of myelin and Semaphorin-mediated growth inhibition is insufficient to promote serotonergic axon regeneration

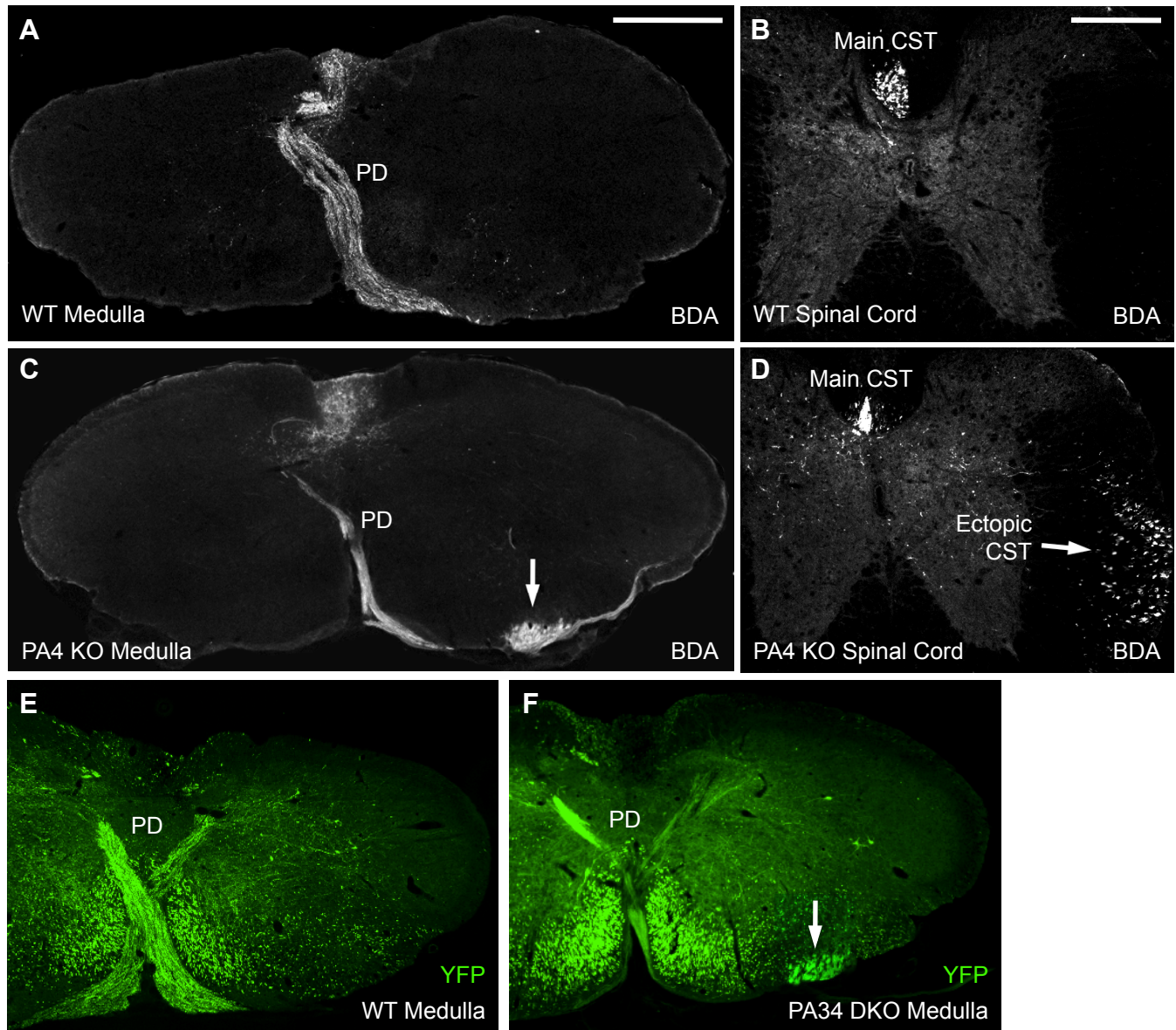
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Supplemental Figure 1. The inhibitory pathways and the raphespinal serotonergic axons in the mouse spinal cord. (A) The prototypical myelin inhibitory pathway showing the molecular components and associated inhibitory signals disrupted in the Nogo/MAG/NgR triple mutant mice in red. GD1a and GT1b, gangliosides that may serve as receptors for MAG. Note that the only known inhibitory signal left intact in the triple mutants is from OMgp to PirB (black arrow). (B) A representative image as an illustration for the distribution of 5-HT axons in a transverse section of adult mouse spinal cord at a thoracic level. Note prominent 5-HT staining in the gray matter and the intermediolateral columns (white arrows). Scale bar = 250 μ m. (C) Both the prototypical myelin inhibitory pathway and the Sema3A/3F signaling pathway showing the molecular components and associated inhibitory signals disrupted in the PlexinA3/PlexinA4/NgR triple mutant mice in red. NP1 and NP2, Neuropilins 1 and 2 respectively. Sema3A and Sema3F may signal through NP1/PlexinA4 and NP2/PlexinA3 receptor complexes respectively, but there is significant cross-talk (dotted lines with arrow) within the signaling pathway (Yaron et al., 2005).



Supplemental Figure 2. 5-HT axons occasionally traversed GFAP-positive bridges at the injury site (arrows) into the caudal spinal cord for short distances (arrowheads) regardless of the genotype in the Plexin mutant study. The example shown here is from a wild type mouse, but this phenomenon occurred in a small subset of mice from all genotypes. (A) GFAP immunoreactivity; (B) 5-HT immunoreactivity; (C) Merged image of GFAP and 5-HT immunoreactivity. Scale bar = 500 μ m.



Supplemental Figure 3. Corticospinal tract (CST) axon defect in Plexin mutants. In WT mice (A), the main CST bundle crosses the midline (or decussates) at the medullary pyramids (marked as PD, or pyramidal decussation) and descend down the spinal cord in the ventral portion of the dorsal funiculus (B). Note that CST is unilaterally labeled in (A-D) with the surgical axon tracer BDA. In PlexinA4 knockout mice (C), a significant portion of the CST bundle fails to cross the midline at the pyramids (arrow), and remains in the ipsilateral white matter in the spinal cord (arrow in D). This phenotype can be recapitulated with a genetic tracer with the *Thy1-YFP-H* transgenic line (comparing the WT mouse in E and, in this case, a PlexinA3/PlexinA4 double knockout mouse in F). Note the bilateral labeling of CST axons and the labeling of other neurons/axons with the genetic tracer in (E,F). Scale bar = 500 μ m (A,C,E,F), 200 μ m (B,D).