Supplemental Figure 1

Localization of the exogenous Sema6B proteins in cultured hippocampal neurons



Hippocampal neurons were cotransfected with the expression plasmids for myc-tagged Sema6B and EGFP (*A*-*C*) or myc-tagged Sema6B alone (*D*-*F*). The exogenous Sema6B proteins were detected by anti-myc antibody A-14 (*B*, *E*). The neuron in *D*-*F* was also immunostained with anti-MAP2 antibody HM-2, a maker for dendrites. Note that axon-like cell processes (arrowheads in *A*, *D*) lack the Sema6B proteins. Scale bars: 50 μ m.

Supplemental Figure 2 Expression of PlxnA4 in the hippocampus of P10 mice



A, In situ hybridization (ISH) analysis. ISH signals for the PlxnA4 transcripts are detected in dentate granule cells (dgc), pyramidal cells (py) of CA and cells in the dentate hilus (DH). **B**, Immunohistochemistry with PlxnA4-specific monoclonal antibody Mab-A4F5. A dotted line demarcates the outer boundary of the molecular layer of the dentate gyrus. The antibody binds to mossy fibers in the suprapyramidal bundle (spb), the dentate hilus and axons of mossy cells in the inner molecular (in). The antibody also binds but weakly to fibers from the entorhinal cortex in the outer molecular layer (out). Scale bars: 200 μ m.

Supplemental Figure 3

Abnormal projection of mossy cell axons in Sema6A and Sema6B mutant mice



Calretinin immunohistochemistry of horizontal sections of the dentate gyrus of adult $Sema6A^{-/-}$ (*A*-*C*) and $Sema6B^{-/-}$ (*D*-*F*) mice. The supra- and infrapyramidal limbs of the dentate gyrus of *Sema6A* mutant mice (rectangular regions in *A*) are shown at a higher magnification in *B* and *C*, respectively, and *Sema6B* mutant mice (rectangular regions in *D*) in *E* and *F*, respectively. Calretinin-positive mossy cell axons ectopically spread into the outer molecular layer (out) in *Sema6A* mutants (asterisks in *B* and *C*) and *Sema6B* mutants (asterisks in *E* and *F*). DH: the dentate hilus; dgc: dentate granule cells; in: the inner molecular layer. Scale bars: *A*, *D*: 200 µm; *B*, *C*, *E*, *F*: 100 µm.

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