Supplemental Information

Spatio-temporal and dynamic regulation of neurofascin alternative splicing in mouse cerebellar neurons

Satoko Suzuki, Noriko Ayukawa, Chisa Okada, Masami Tanaka, Susumu Takekoshi, Yoko Iijima, and Takatoshi Iijima

Supplemental Figure legends

Figure S1. Inclusion of *Nfasc* ex26-29 caused by high K^+ -induced depolarization

Cultured GCs were treated with addition of 25 mM K⁺ (final concentration: 30 mM) for 1 h, 3 h, 6 h, 1 day, 3 days or 5 days. All the cultures were harvested at DIV12. Brain-derived neurotrophic factor (*Bdnf*) expression was monitored to confirm immediate-early gene induction.

Figure S2. Representative gel images of the knockdown effect of *Rbfox1* and *Rbfox3* siRNAs on alternative splicing of *Nfasc* (related to Figs. 6d, 7b, and 7c). PCR products from the same samples used for qRT-PCR (Figs. 6d, 7b, and 7c) were loaded onto agarose gels to confirm the knockdown effects.

Figure S3. Western blot analysis of total cell lysates from untreated control and depolarized GC cultures with anti-Rbfox1 antibody.

Protein levels of Rbfox1 were normalized to that of ubiquitously expressed SAM68. (n = 3 cultures)

Figure S4. Areas of somata (left) and nuclei in control and depolarized cerebellar GCs (related to Fig. 7h).

(a) The mean area and cumulative distribution of MAP2⁺ somata. There was no significant difference in size of MAP2⁺ somata (n >20 neurons each group in two sister cultures)

Figure S5. Uncropped images of blots presented in Figures 1–7.

(a1)–(a3) Fig 1b. (b1)–(b5) Fig 2b. (c1)–(c4) Fig 3a. (d1)–(d3) Fig 4a. (e) Fig 4b. (f1)–(f2) Fig 4d. (g1)–(g5) Fig 5b. (h1)–(h2) Fig 5d. (i1)–(i3) Fig 5e. (j1)–(j2) Fig 6b. Images show agarose gels for splicing assays and the membranes used in western blotting with the indicated antibodies.

Figure S6. Uncropped images of blots presented in Figures S1-4.

(a1)-(a5) Fig S1. (b1)-(b9) Fig S2. (c1)-(c2) Fig S3. Images show agarose gels for

splicing assays and the membranes used in western blotting with the indicated antibodies.

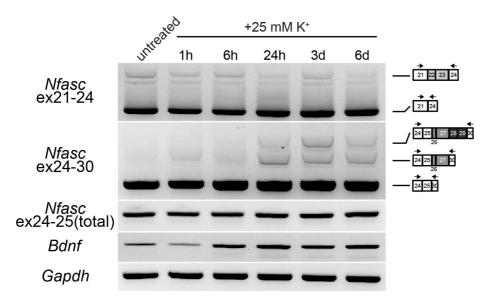


Figure S1

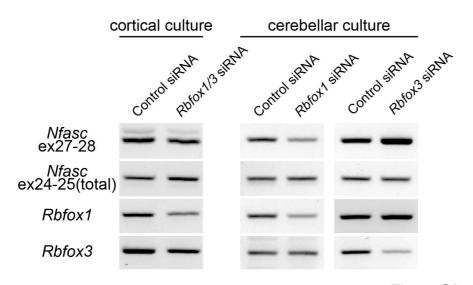
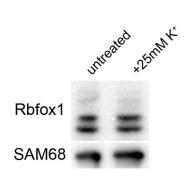


Figure S2



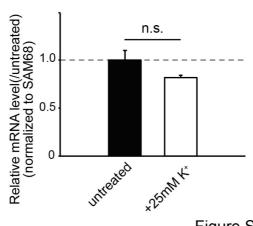


Figure S3

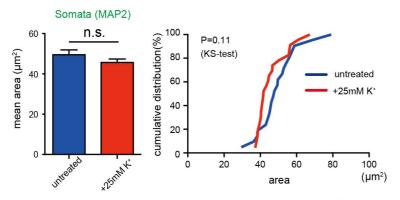


Figure S4

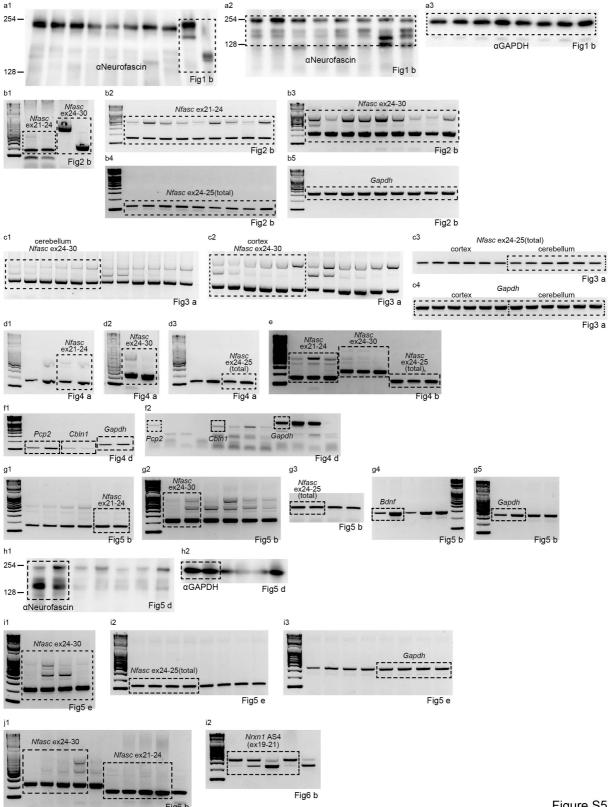


Figure S5

