Yang et al., http://www.jcb.org/cgi/content/full/jcb.200811147/DC1

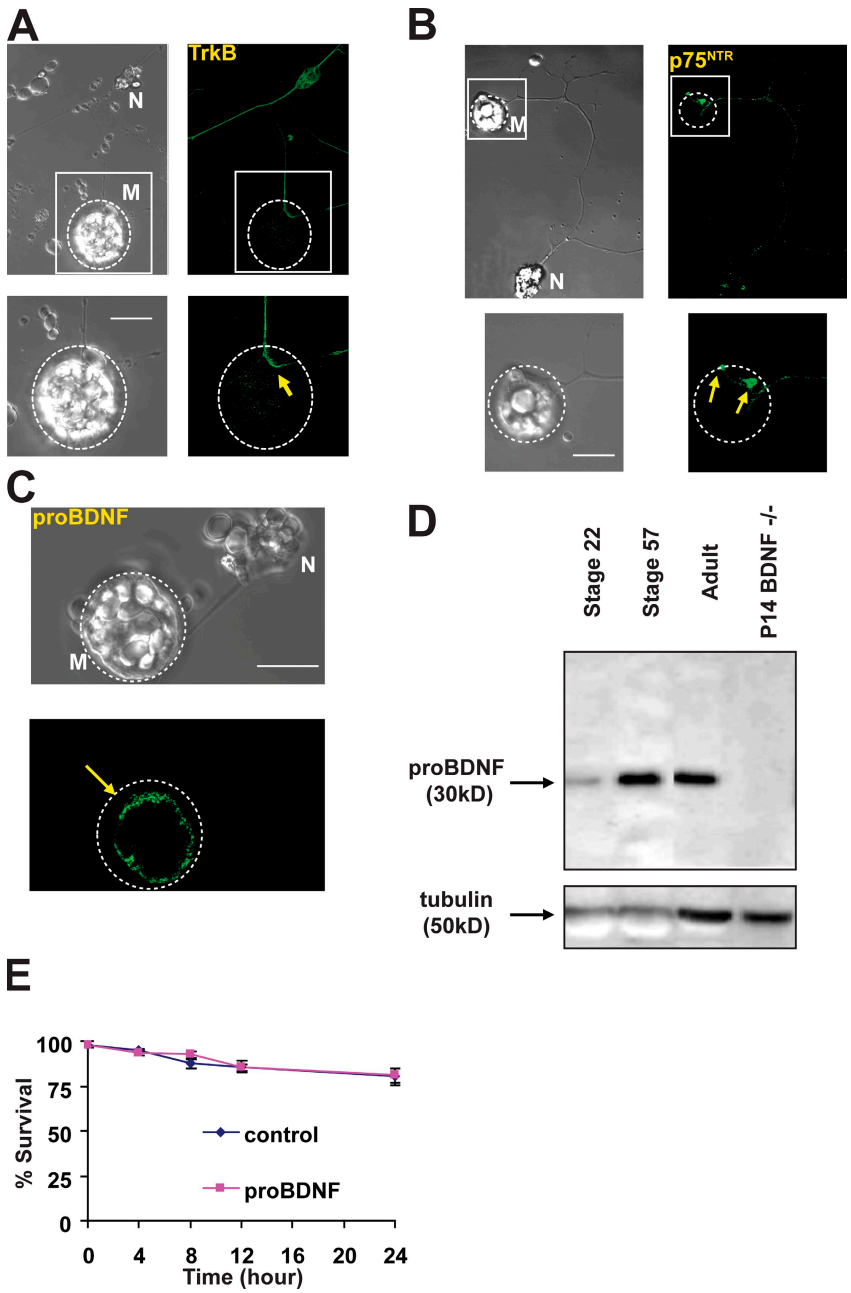

Figure S1. **Expression of pro-BDNF, p75^{NTR}, and TrkB in *Xenopus* nerve–muscle coculture system and survival of spinal neurons upon pro-BDNF treatment.** (A) Expression of TrkB. The nerve–muscle coculture was lightly fixed and processed for immunocytochemistry using an antibody specific for TrkB. (bottom) A higher magnification of the boxed area in the top panels is shown. Note that TrkB immunofluorescence is primarily found in the spinal neuron (N) and at the junction. (B) Expression of p75^{NTR}. Cultures were stained as in A using an antibody specific for p75^{NTR}. Higher magnifications of the boxed areas are shown below. Note the strong staining of p75^{NTR} at the junction. (C) Expression of pro-BDNF. Cultures were stained as in A using a polyclonal antibody specific for pro-BDNF. Blocking with recombinant pro-BDNF–HA fusion protein (mixing with the antibody and then pulled down by anti-HA affinity Matrix [Roche]) prevented the specific labeling. Note that pro-BDNF immunofluorescence is primarily found in the myocyte (M) but not in the spinal neuron innervating it. (A–C) The dotted circles indicate myocytes, and the arrows indicate fluorescence signals from axon terminals. (D) Expression of pro-BDNF in *Xenopus* muscles in vivo. Proteins were extracted from muscle of various developmental stages (as indicated from myotomal tissues at stage 22 and hindlimbs at stages 57 and 66) and processed for Western blotting using chicken anti–pro-BDNF antibody. Tubulin was used as loading control. (E) 2 ng/ml pro-BDNF was added to *Xenopus* spinal neuron cultures over a period of 24 h, and survival was assayed at 4, 8, 12, and 24 h after pro-BDNF treatment by using TUNEL assay. Error bars represent SEM. Bars, 10 µm.

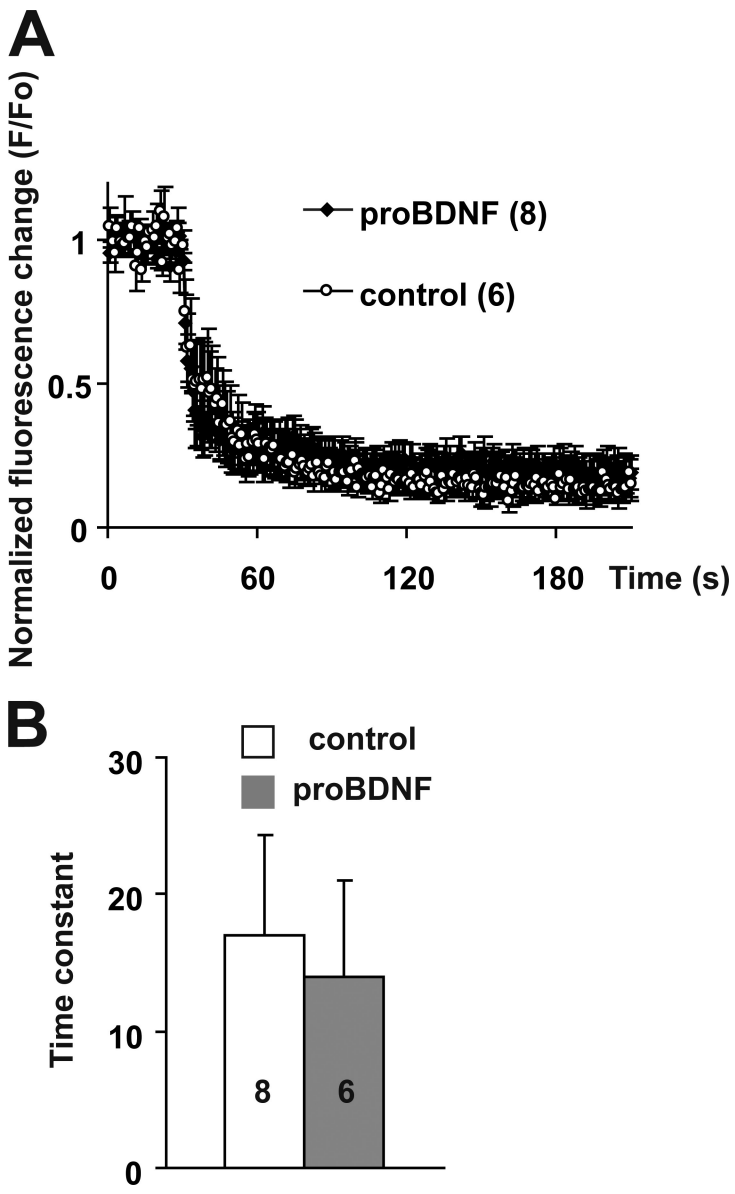

Figure S2. **Effect of pro-BDNF on FM 1-43 destaining.** Nerve–muscle cocultures were treated with vehicle or pro-BDNF for 20 min, and neurons were loaded with FM 1-43 by high K^+ solution. Subsequently, cells were rinsed extensively with wash solutions. After application of high K^+ destaining solution, FM dye–labeled varicosities lost most of their contents within 2 or 3 min. (A) Averaged destaining curves. (B) The time constant of destaining. The numbers in parentheses (A) and the bars (B) indicate the number of synapses recorded. Error bars represent SEM.

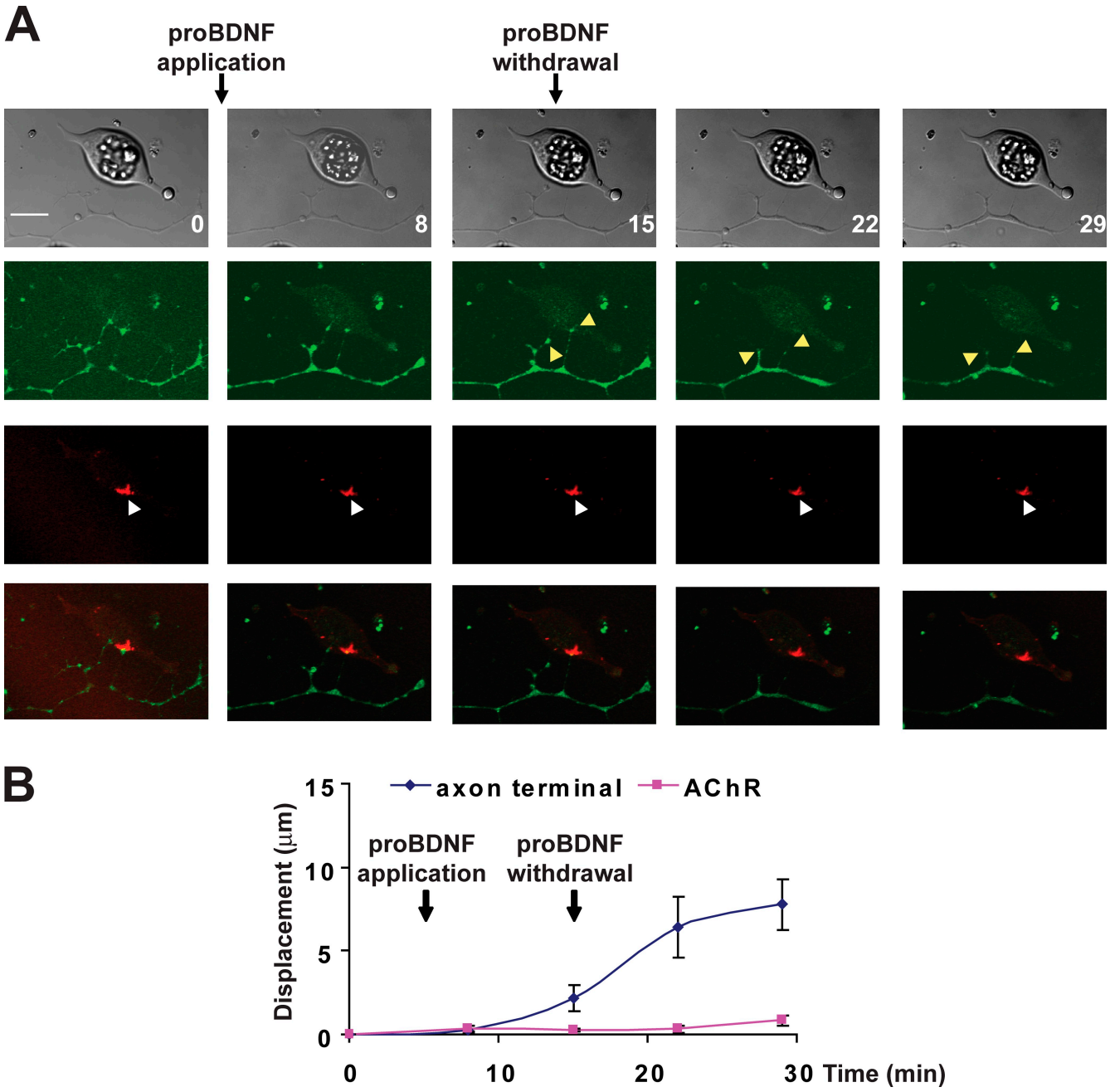

Figure S3. **Persistent synaptic retraction after pro-BDNF washout.** (A) Time-lapse images showing the retraction of a nerve terminal from its targeted myocyte after pro-BDNF treatment and subsequent removal. Axonal synaptic boutons were labeled with FM 1-43 (green) with high K^+ loading, and AChRs on the postsynaptic membrane were labeled with a low concentration of rhodamine-conjugated α-BTX (red). Pro-BDNF was applied at time 5 and removed (washout) at time 15. Synaptic boutons labeled with FM dye clearly occupied the postsynaptic site (colocalized with AChR) before treatment but progressively withdrew (yellow arrowheads) from the AChR clusters (white arrowheads) after pro-BDNF treatment for 10 min. (B) Quantification of pre- or postsynaptic displacement during the aforementioned time-lapse imaging. The displacement was calculated by using the formula

$$\text{displacement} = \sqrt{(X - Xo)^2 + (Y - Yo)^2},$$

where Xo and Yo are the initial centroid position of axonal bouton and AChR clustering, respectively. Note that although postsynaptic AChR clusters did not shift, axonal boutons displaced more than 10 μm even after pro-BDNF removal. Error bars represent SEM. Bar, 10 μm.

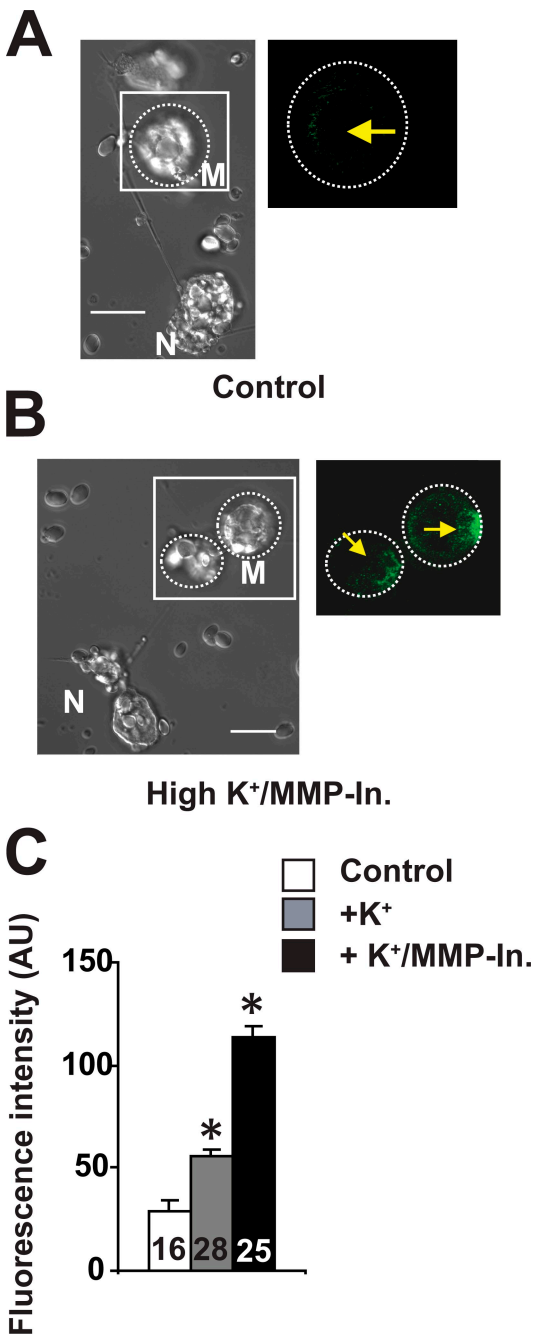

Figure S4. **Cell surface staining of secreted pro-BDNF.** Cultures were treated with drugs as indicated, lightly fixed under nonpermeable conditions, and processed for surface immunocytochemistry using a polyclonal antibody specific for pro-BDNF. Pro-BDNF immunoreactivities are shown as inset images on top of differential interference contrast images. (A) Pro-BDNF surface staining in control condition. (B) Pro-BDNF surface staining in high K^+ (50 mM) in the presence of 60 µM of MMP inhibitors. (A and B) Dotted circles indicate myocytes, and the arrows indicate fluorescence signals. Insets show a magnified view of myocytes. (C) Quantification of the immunofluorescence. The numbers in the bars indicate the number of muscle cells imaged. Error bars represent SEM. *, $P < 0.05$. AU, arbitrary unit; M, muscle cell; N, neuron. Bars, 10 µm.

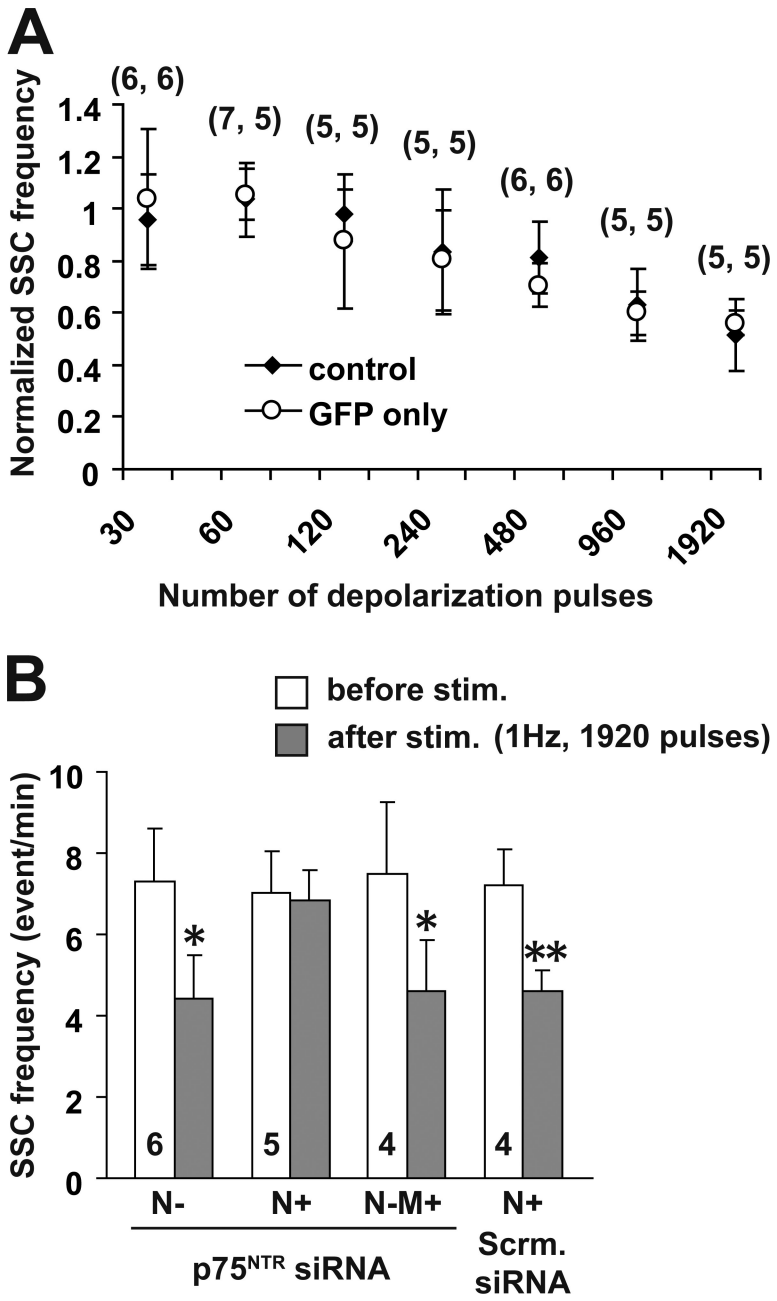

Figure S5. **Synaptic depression is triggered by different postsynaptic depolarization protocols, and p75^{NTR} knockdown in spinal neurons blocked synaptic depression induced by stronger postsynaptic stimulation (1 Hz and 1,920 pulses).** (A) Current pulses ranging from 30 to 1,920 were applied to singly innervated myocytes at a frequency of 1 Hz. Reduction in mean SSC frequency was normalized to the SSC frequency recorded from 10 min before postsynaptic stimulation. The numbers in parentheses associated with each stimulation pulse number are the numbers of cells in control and GFP-expressed myocytes examined, respectively. (B) p75^{NTR} or scrambled (Scrm.) siRNA was introduced into spinal neurons (N+) or myocytes (M+) as described in Fig. 2. The synaptic depression was induced when repetitive train stimulation (1 Hz and 1,920 pulses) was applied to muscle cell. The number of synapses recorded is indicated in each pair of columns. p75^{NTR} siRNA was effective in inhibiting synaptic depression when expressed in presynaptic neurons (N+) but not postsynaptic (M+) myocytes (*, $P < 0.05$; **, $P < 0.01$; *t* test). The neuronal expression of scrambled siRNA did not prevent muscle stimulation (stim.)–induced synaptic depression. Error bars represent SEM.