

Figure S1, related to Figure 1. Injection of hKir-mCherry DNA into the D1.1 blastomere at the 16-cell stage labels single neurons in stage 41 embryos and suppresses Ca^{2+} spike frequency in single neurons during one hour imaging period.

(A) Diagram illustrating the injection.

(B) Arrowheads identify some of the labeled spinal neurons in a stage 41 embryo.

(C) Distribution of neurons targeted on ventral and dorsal sides of the spinal cord.

(D) Each symbol is the spike frequency for a single neuron. Stages 23-25. Kir-mCh cells, hKir2.1-mCherry-expressing neurons; mCh cells, mCherry alone-expressing neurons. Control, neurons expressing neither construct. $n \geq 7$ embryos per group. ***, $p < 0.001$. Mann-Whitney U test.

Figure S2

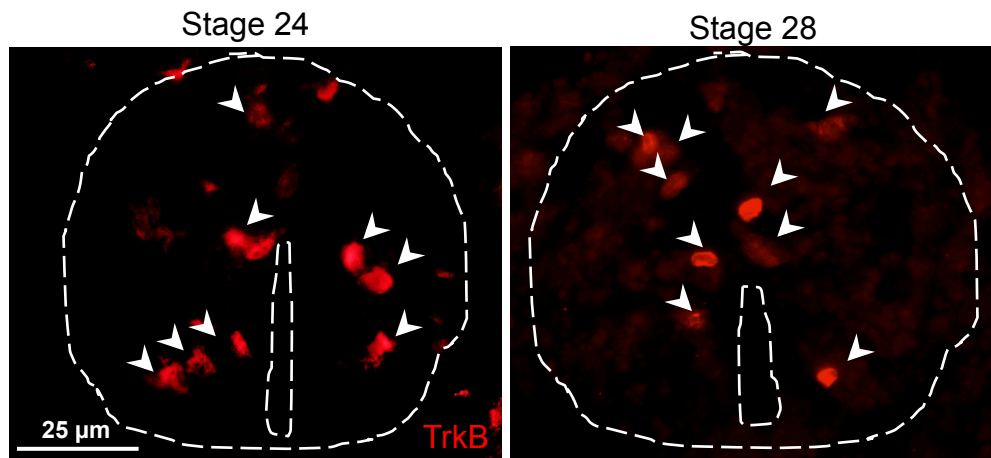


Figure S2, related to Figure 3. TrkB receptors are expressed during the critical period for neurotransmitter specification.

TrkB staining of the neural tube at stage 24 and 28 shows sparse labeling of neurons. $n \geq 3$ embryos.

Figure S3

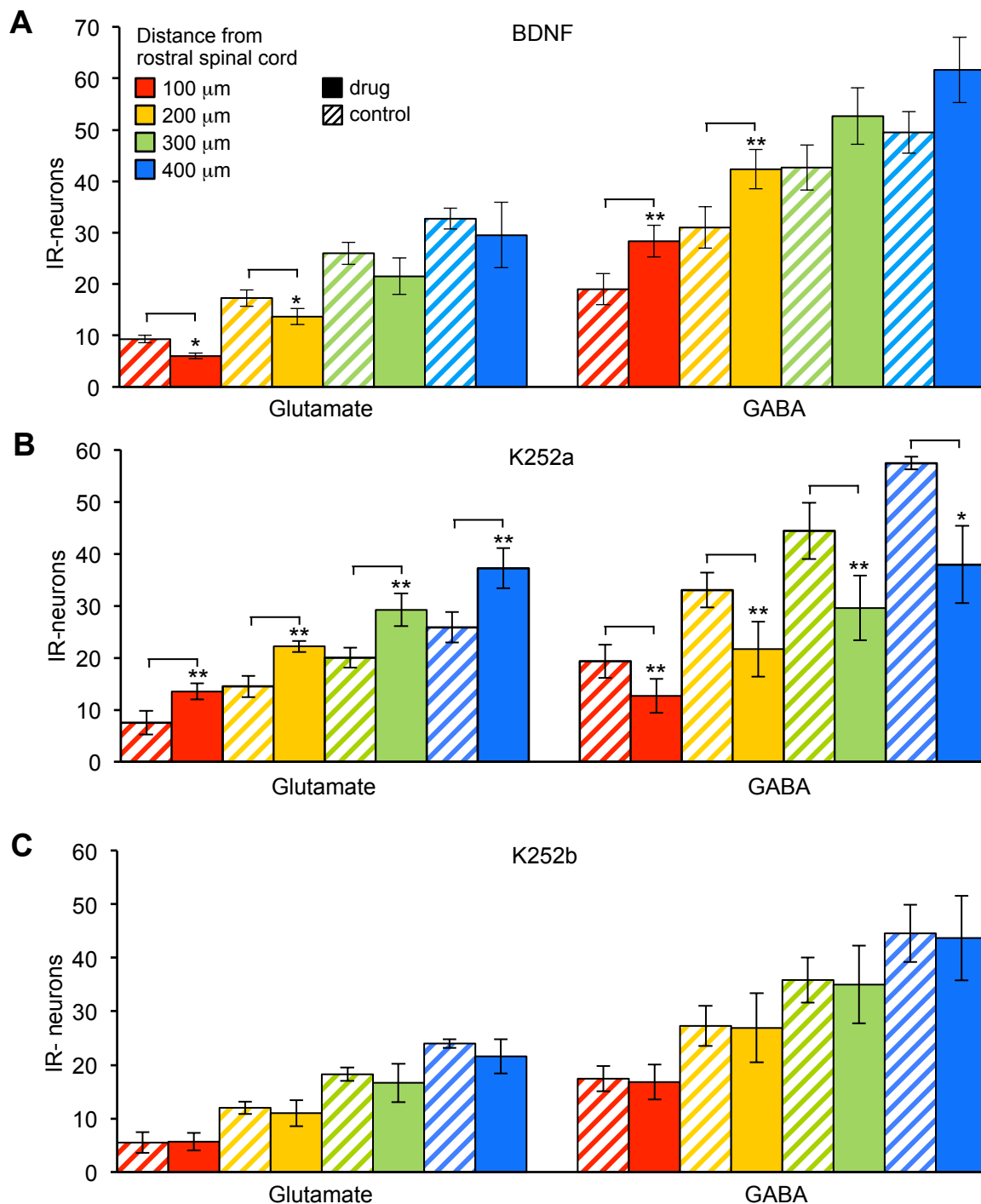


Figure S3, related to Figure 4. BDNF regulates neurotransmitter phenotype via Trk receptors.

(A-C) Glutamate and GABA staining following implantation of beads containing BDNF, K252a and K252b, a membrane impermeable analog of K252a. Striped columns, controls. Solid columns, beads with 100 ng/ml BDNF or 50 mM K252a/K252b. Colors indicate distance from the beginning of the spinal cord. Beads were located adjacent to the most rostral 100 μm of the spinal cord. $n \geq 5$ embryos per condition. Data are mean \pm SEM. *, $p < 0.05$; **, $p < 0.01$. Mann-Whitney U test.

Figure S4

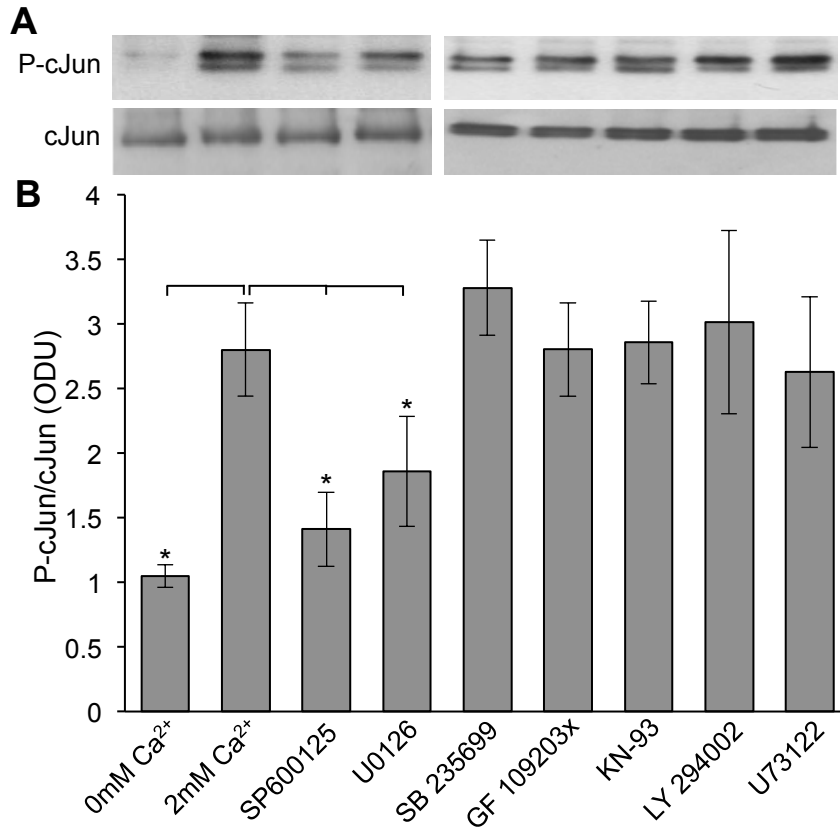


Figure S4, related to Figure 5. Signaling pathway regulating activity-dependent cJun phosphorylation. (A) Neuronal cultures were prepared from stage 15 embryos and plated in dishes containing 0 mM or 2 mM Ca²⁺ medium. Cells were maintained for 9 hr in the presence or absence of 10 mM of different kinase inhibitors as indicated and protein levels were determined by Western blot. (B) Bars represent the ratio between the phosphorylated proteins and total protein expressed in optical density units (O.D.U). n≥5 cultures per condition. Data are mean±SEM. *, p<0.05. Mann-Whitney U test. Full-length Western blot is shown in **Figure S5**.

Figure S5

Figure 5

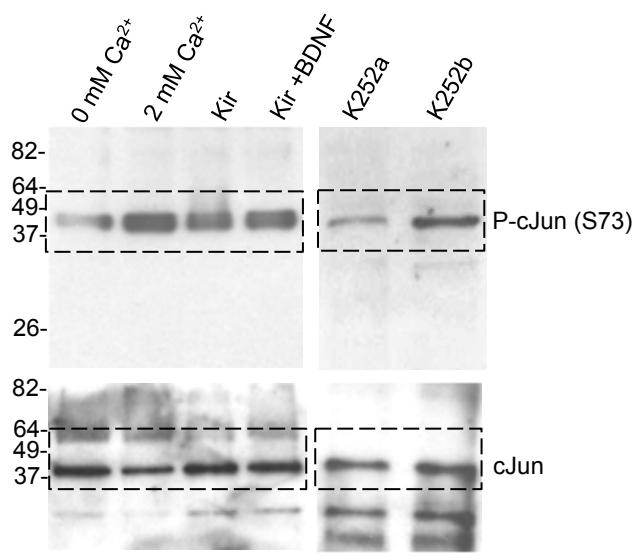


Figure 6

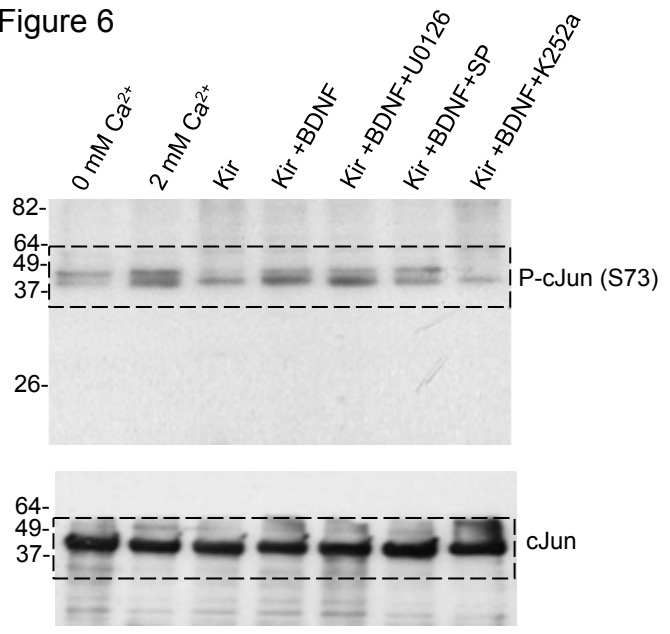


Figure S4

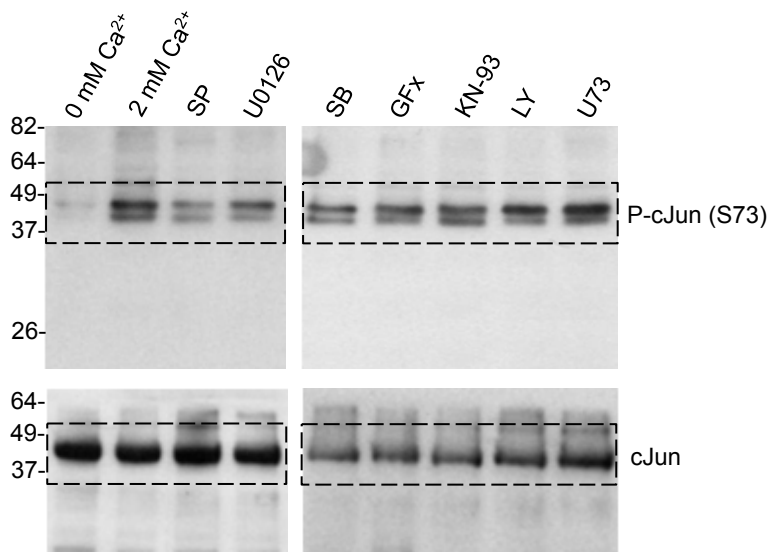


Figure S5. Full-length Western blots for Figures 5 and 6 and Figure S4
Samples were loaded on a 12% gel and immunoblotted with the indicated antibodies. Dashed lines indicate the bands presented in the other figures.