Wang et al., http://www.jcb.org/cgi/content/full/jcb.201510107/DC1

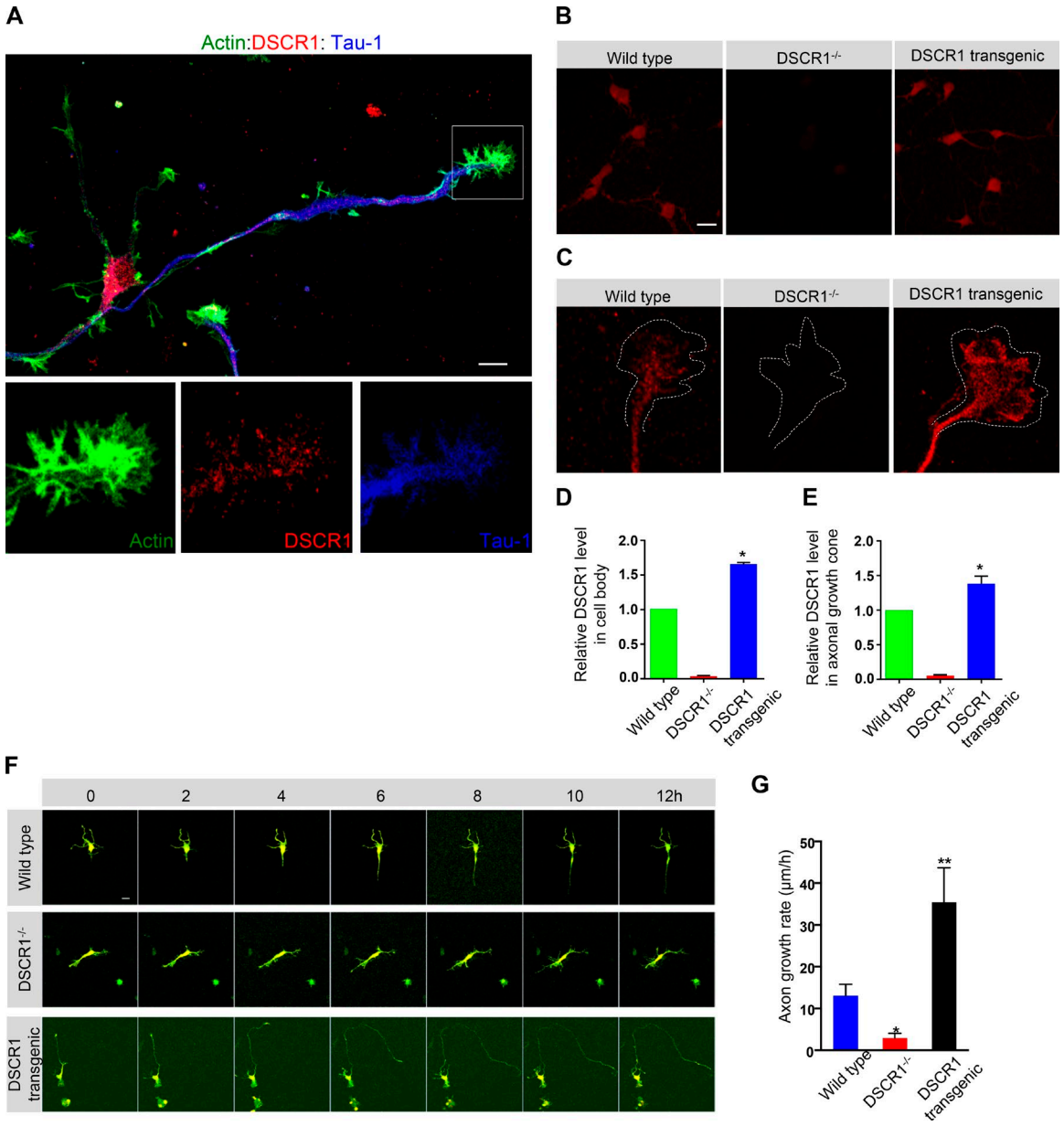

Figure S1. **DSCR1 is present in axonal growth cone.** (A) DIV 3 neuron was immunostained with antibodies against actin, DSCR1, and tau-1, as indicated. Note that DSCR1 is enriched in the axonal growth cone. Bar, 10 µm. (B) Wild-type, *DSCR1*$^{-/-}$, and *DSCR1* transgenic neurons at DIV 3 were immunostained with an antibody against DSCR1. Bar, 10 µm. (C) Axonal growth cones of wild-type, *DSCR1*$^{-/-}$, and *DSCR1* transgenic neurons at DIV 3 were immunostained with an antibody against DSCR1. Bar, 2 µm. (D) The level of DSCR1 expression is significantly increased in cell body of *DSCR1* transgenic neurons. *, $P < 0.0001$, $n = 45$ for wild type, $n = 26$ for *DSCR1*$^{-/-}$, and $n = 45$ for *DSCR1* transgenic neurons. (E) DSCR1 expression level is significantly increased in axonal growth cone of *DSCR1* transgenic neurons. *, $P < 0.001$, $n = 21$ for wild-type, $n = 10$ for *DSCR1*$^{-/-}$, and $n = 21$ for *DSCR1* transgenic neurons. Values shown are mean ± SEM and are tested for statistical significance by *t* test. (F and G) Development and growth of axons from wild-type and *DSCR1* mutant neurons were monitored by time-lapse microscopy by taking images every 2 h for 12 h. $n = 8$ neurons for each strain. *, $P < 0.02$; **, $P < 0.04$. Bar, 10 µm. Experiments were performed at DIV 2. Values shown are mean ± SEM and are tested for statistical significance by *t* test.

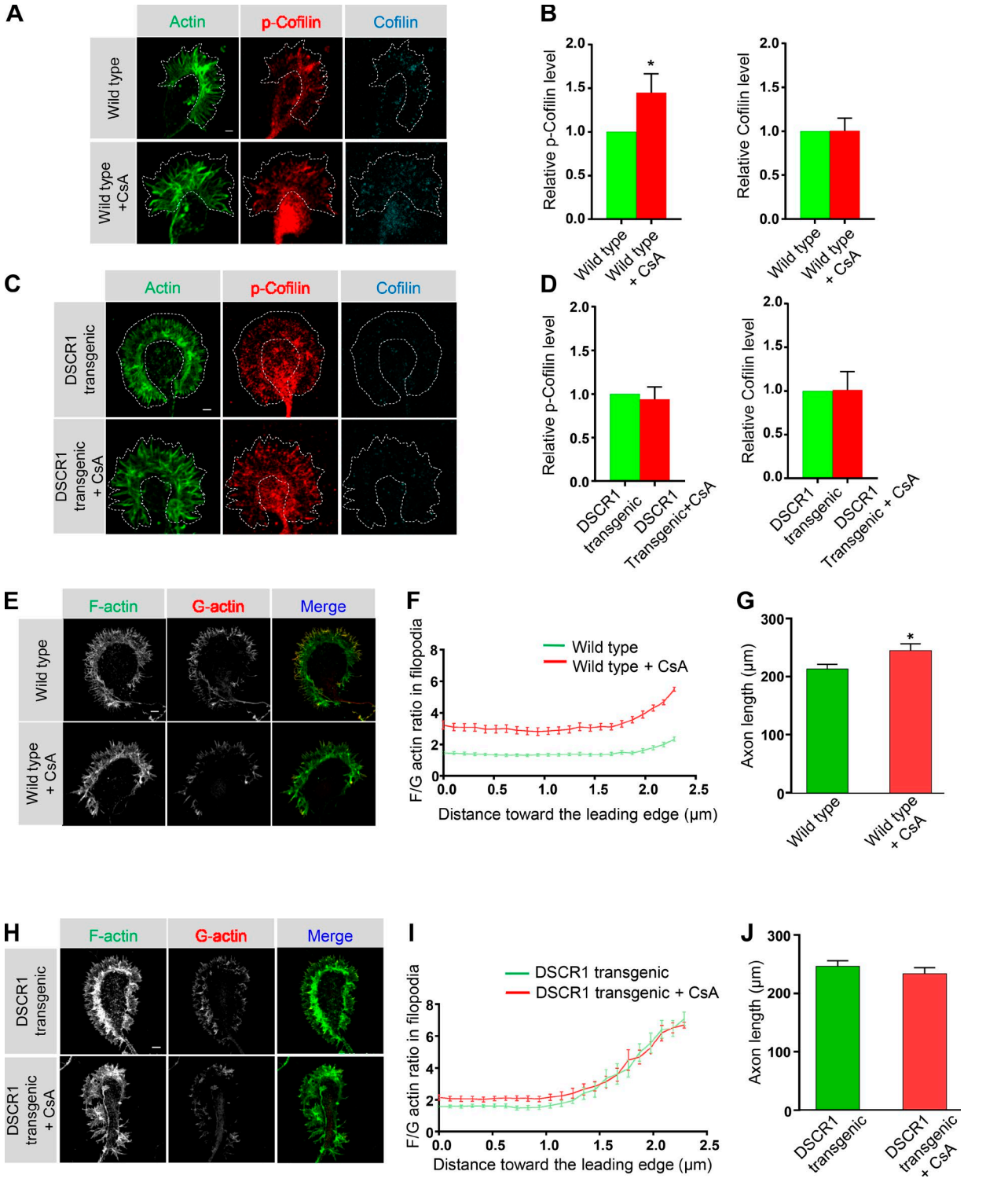

Figure S2. **Effect of CsA on the level of cofilin and phospho-cofilin in wild-type and *DSCR1* transgenic growth cones, as well as on F-actin/G-actin ratio of wild-type and *DSCR1* transgenic growth cone filopodia.** Axonal growth cones of wild type (A) and *DSCR1* (C) transgenic with and without treatment with CsA showed the expression of cofilin and phospho-cofilin. Bars, 2 µm. (B and D) Quantification of staining intensity of cofilin and phospho-cofilin level in axonal growth cones of the indicated genotypes. *, $P < 0.01$. $n = 24$ for wild type, $n = 29$ for wild type treated with CsA, $n = 23$ for *DSCR1* transgenic neurons, and $n = 28$ for *DSCR1* transgenic neurons treated with CsA. Values shown are mean ± SEM and are tested for statistical significance by *t* test. (E and H) Monomeric G-actin and actin filaments (F-actin) were labeled using antibodies against vitamin D–binding protein and fluorescent phalloidin, respectively. Bars, 2 µm. (F and I) The ratio of F-actin/G-actin in filopodia of wild type and wild type treated with CsA, and *DSCR1* transgenic neurons and *DSCR1* transgenic neurons treated with CsA. $n = 20$ (42) for wild-type growth cone, $n = 20$ (37) for wild-type growth cone treated with CsA, $n = 18$ (35) for *DSCR1* transgenic growth cone, and $n = 18$ (36) *DSCR1* transgenic growth cone treated with CsA. Parentheses indicate the number of filopodia. (G and J) CsA treatment increased axon length of wild-type neurons, but showed no effect on *DSCR1* transgenic neurons. *, $P < 0.02$. Values shown are mean ± SEM and are tested for statistical significance by *t* test.

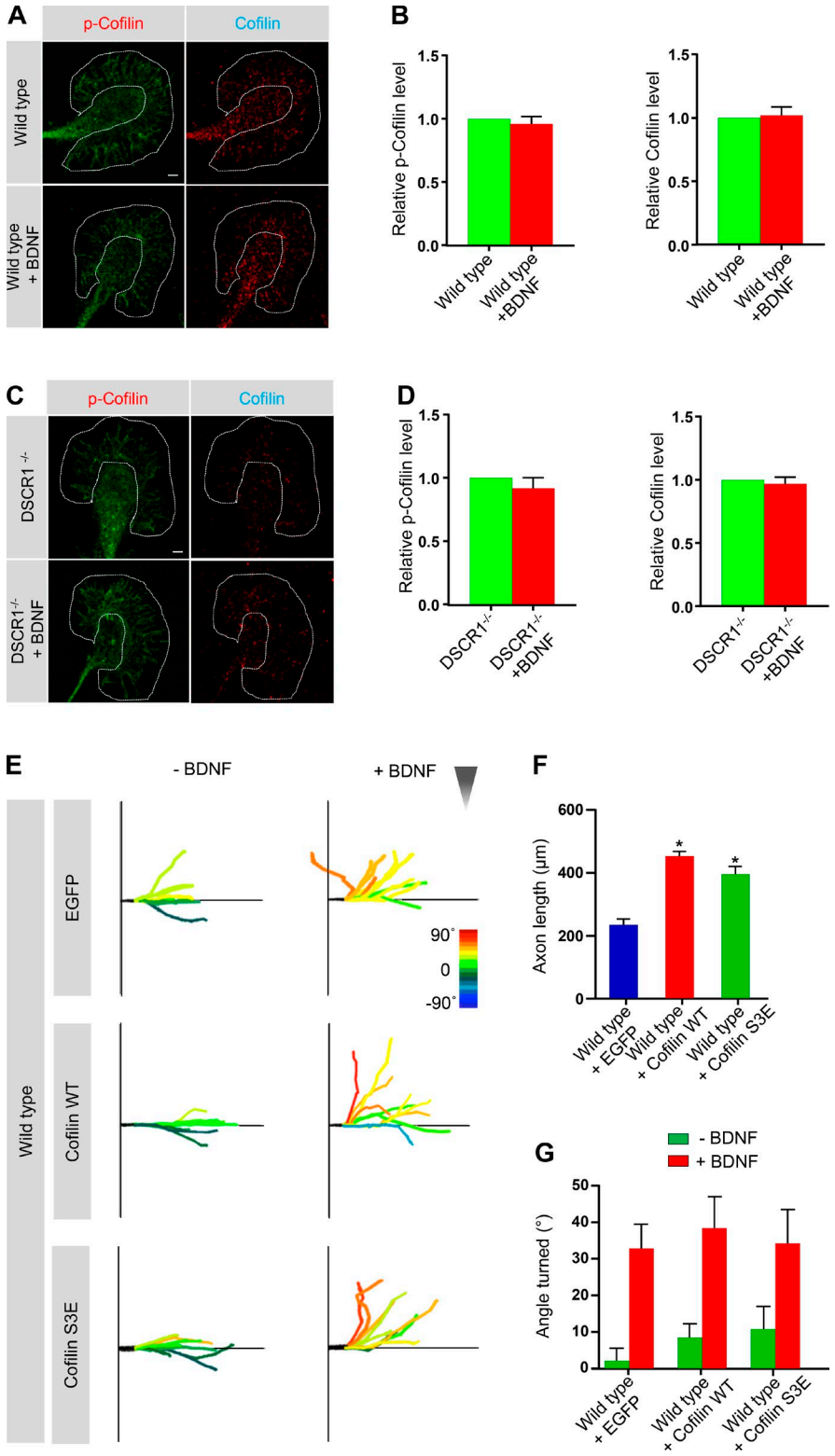

Figure S3. **Effect of BDNF on the level of phospho-cofilin and cofilin in axonal growth cones of wild-type and *DSCR1*$^{-/-}$ neurons, and effect of wild-type *cofilin* and *cofilin S3E* overexpression on the level of cofilin/phospho-cofilin in wild-type neurons.** Images and expression levels of phospho-cofilin and cofilin in axonal growth cones of wild-type (A and B) and *DSCR1*$^{-/-}$ (C and D) neurons with and without treatment with BDNF treatment. Bars, 2 μm. $n = 28$ for wild type and $n = 22$ for *DSCR1*$^{-/-}$ neurons in each condition. (E) Wild-type axonal growth cones were transfected with wild-type *cofilin* or *cofilin* S3E overexpression plasmid and then placed in Dunn chamber with or without BDNF. Trajectory plots show growth cone turning response of individual neurons of wild-type overexpressing *EGFP*, wild-type *cofilin*, or *cofilin* S3E. (F) Overexpression of wild-type *cofilin* or *cofilin* S3E shows no effect on axonal growth turning response to BDNF. $n > 10$ for each condition. (G) Axon length was significantly increased in wild-type neurons overexpressing wild-type *cofilin* or *cofilin* S3E. $n = 23$ for wild-type overexpressing *EGFP* and $n = 24$ for wild-type overexpressing wild-type *cofilin* or *cofilin* S3E. *, $P < 0.0001$. Values shown are mean ± SEM and are tested for statistical significance by *t* test.

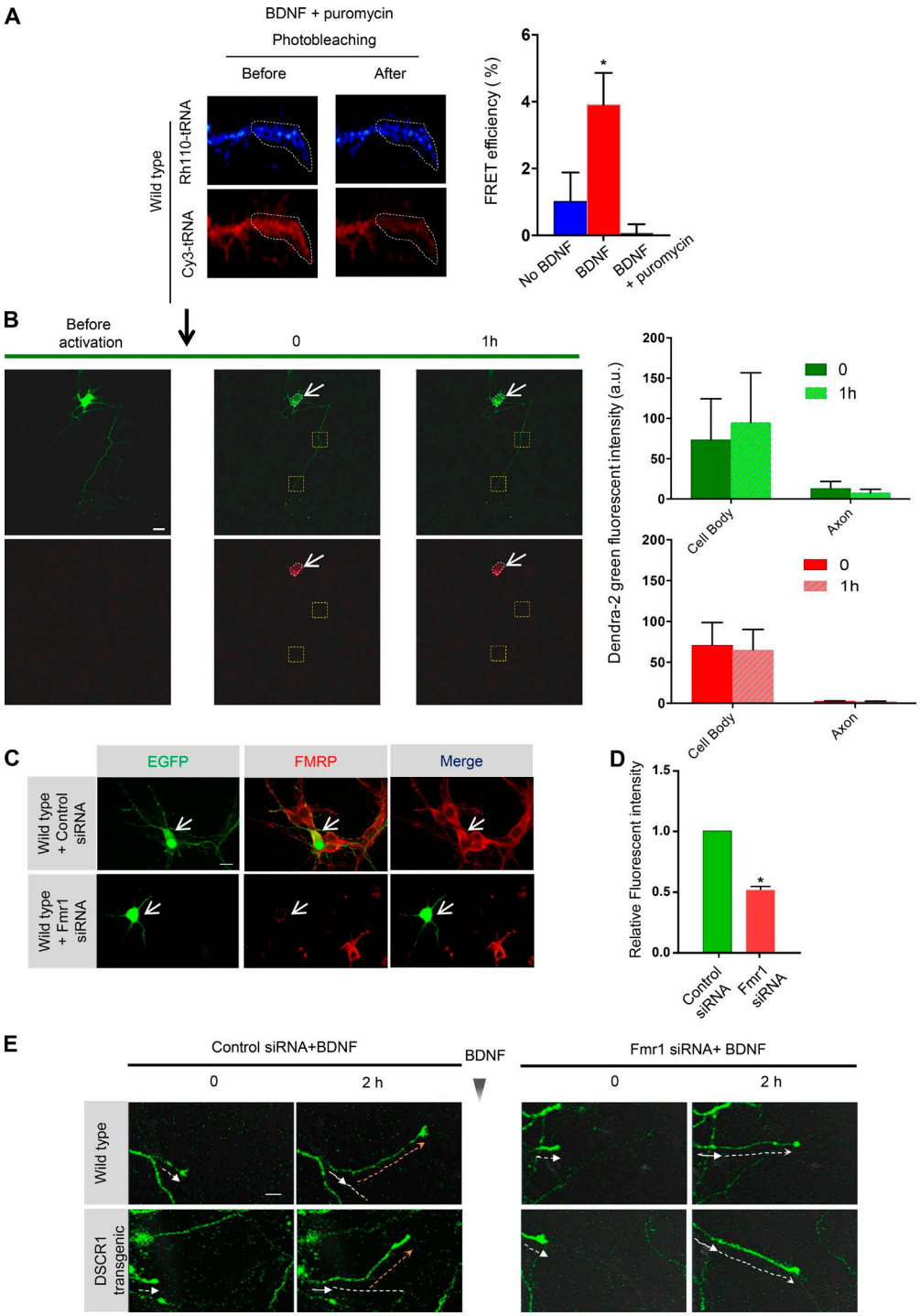

Figure S4. **Axonal growth cone turning in wild-type and *DSCR1* transgenic neurons transfected with *fmr1* siRNA.** Controls for local protein synthesis assay (A and B). (A) Puromycin treatment blocked local protein synthesis at the axonal growth cone. BDNF failed to increase FRET signals after photobleaching of Cy3-tRNA in wild-type axonal growth cones in the presence of a translation inhibitor, puromycin. The histogram is combined with the data shown in Fig. 6 B. *n* = 19 axons treated with BDNF and puromycin. *, P < 0.005. (B) Dendra-2 does not diffuse from the cell body or axon projections. Green dendra-2 protein was photoconverted from green to red fluorescence in the cell body (black arrow indicates UV illumination). Note that photoconversion is incomplete, and weak green dendra2 could be detected. To rule out the possibility that the increase in green signal at the growth cone is caused by diffusion of the remaining green dendra-2 protein from the cell body or the axon track, green fluorescence in the cell body and two different regions in the axon track were measured after photoconversion. There were no changes in the fluorescence intensities either in the cell body (white arrow) or axon track in both the green and the red channels, suggesting that the increased dendra-2 signals detected in the axonal growth cone is caused by local protein synthesis. Bar, 20 µm. (C) Wild-type neurons were transfected with *fmr1* siRNA or control siRNA, which were immunostained with antibody against FMRP. The white arrows indicate neurons transfected with siRNA. Bar, 10 µm. (D) FMRP expression level was significantly lower in neurons containing *fmr1* siRNA. *, P < 0.001. *n* = 31 for each condition. FMRP and phospho-FMRP antibodies show clear preference toward nonphosphorylated FMRP and phospho-FMRP, respectively. (E) No axonal growth cone turning toward BDNF was detected in wild-type or *DSCR1* transgenic neurons with *fmr1* siRNA. Lines drawn below the axons show the paths of growth cones. Bar, 10 µm.

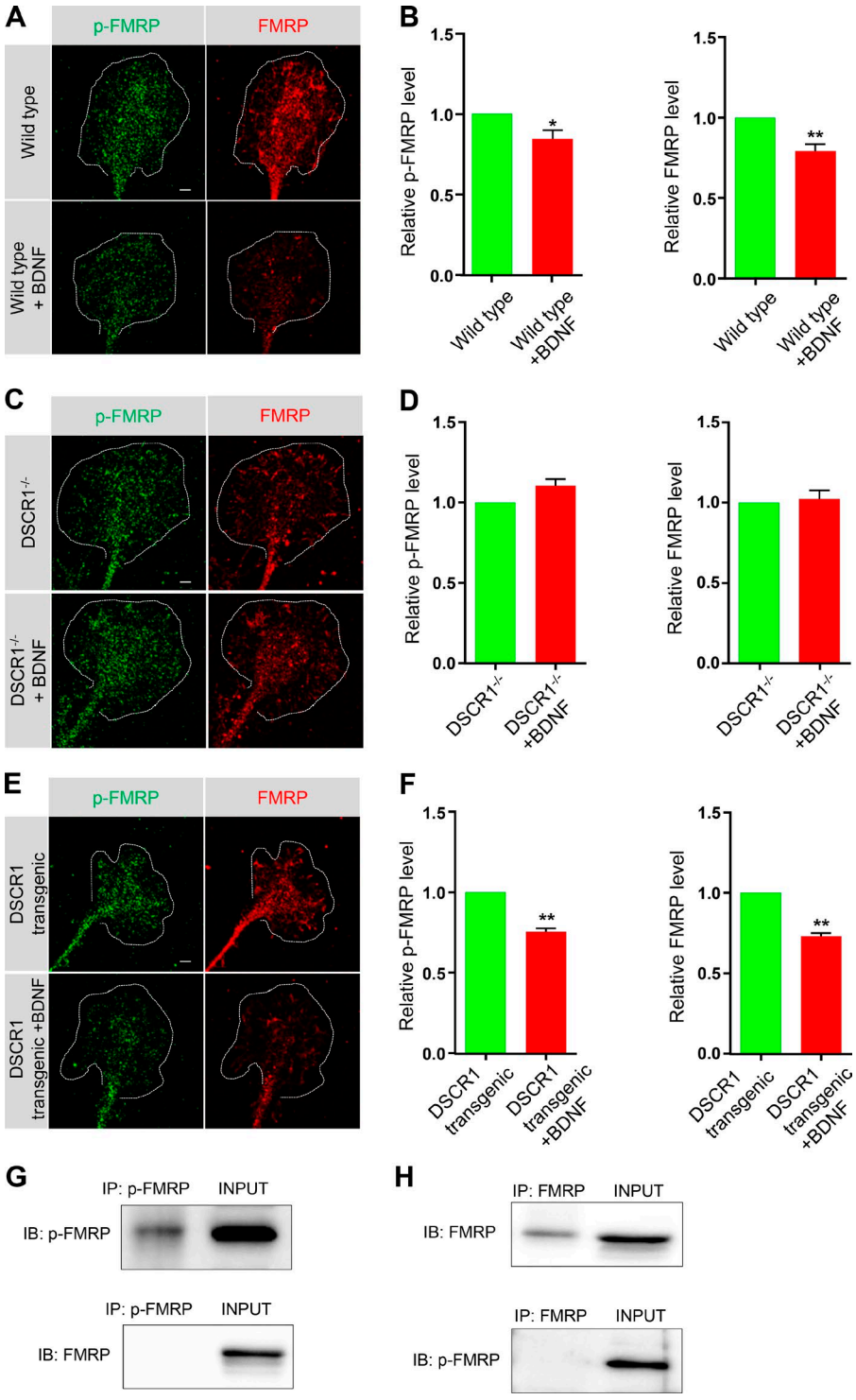

Figure S5. **Effect of BDNF on the level of phospho-FMRP and FMRP in axonal growth cones of wild-type, *DSCR1*$^{-/-}$, and *DSCR1* transgenic neurons.** Axonal growth cones of wild type (A), *DSCR1*$^{-/-}$ (C), and *DSCR1* transgenic (E) neurons with and without treatment with BDNF were immunostained with antibody against phospho-FMRP and FMRP. Bars, 2 µm. (B, D, and F) Relative levels of phospho-FMRP and FMRP in axonal growth cones of the indicated genotypes are shown. $n = 29$ for wild-type neurons, $n = 29$ for *DSCR1*$^{-/-}$ neurons, and $n = 24$ for *DSCR1* transgenic neurons. *, $P = 0.006$; **, $P < 0.001$. Values shown are mean ± SEM and are tested for statistical significance by *t* test. (G) Immunoprecipitation (IP) with phospho-FMRP antibody was followed by blotting (IB) with phospho-FMRP and FMRP antibodies. (H) Immunoprecipitation (IP) with FMRP antibody was followed by blotting (IB) with FMRP and phospho-FMRP antibodies.