Moradi et al., https://doi.org/10.1083/jcb.201604117

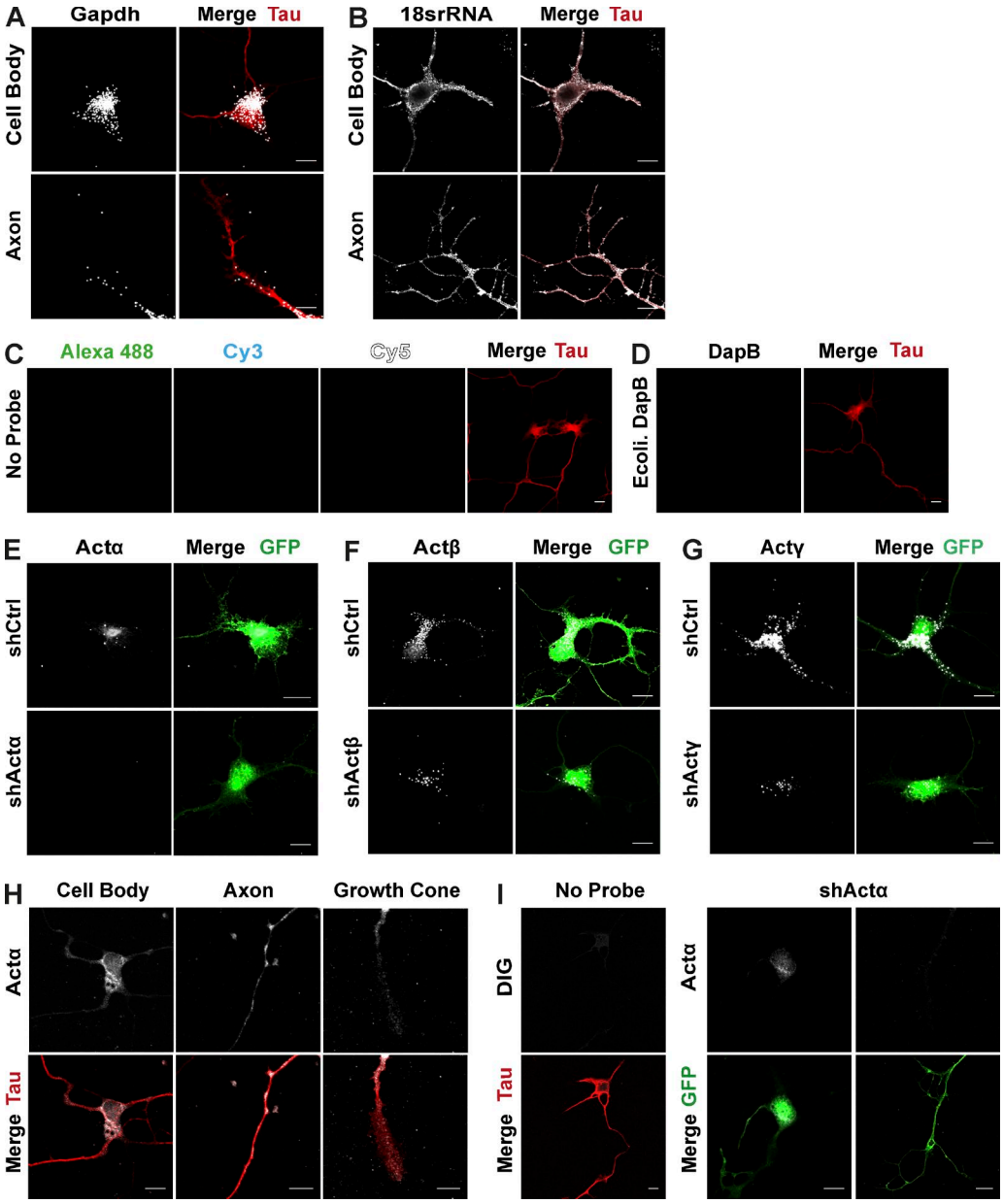


Figure S1. **High-resolution in situ hybridization.** (A and B) Probes for Gapdh mRNAs (A) and 18srRNA (B) were used as positive control for in situ hybridization experiments. Both transcripts are detected in soma and axons. (C) In "no-probe" conditions, only amplifier oligos coupled to different fluorescent dyes (i.e., Alexa Fluor 488, Cy3, and Cy5) were added. There is no distinct signal detectable in any of the three channels. (D) Scrambled probes targeting *E. coli*–DapB mRNAs were used to exclude unspecific binding of probes. (E–G) The specificity of FISH probes was examined by shRNA-mediated knockdown of individual actin isoforms. Fluorescent signals are decreased after down-regulation of Actα (E), Actβ (F), and Actγ (G; $n = 3$). (H) Actα transcripts are detected in soma, axons, and growth cones of motoneurons by in situ hybridization using an LNA probe. (I) In control experiments, the probe was omitted and cells were incubated only with the DIG antibody. No signal was detected. Specificity of the Actα LNA probe was further examined by knockdown of this isoform, which resulted in a marked reduction in signal intensity. Bars, 10 µm.

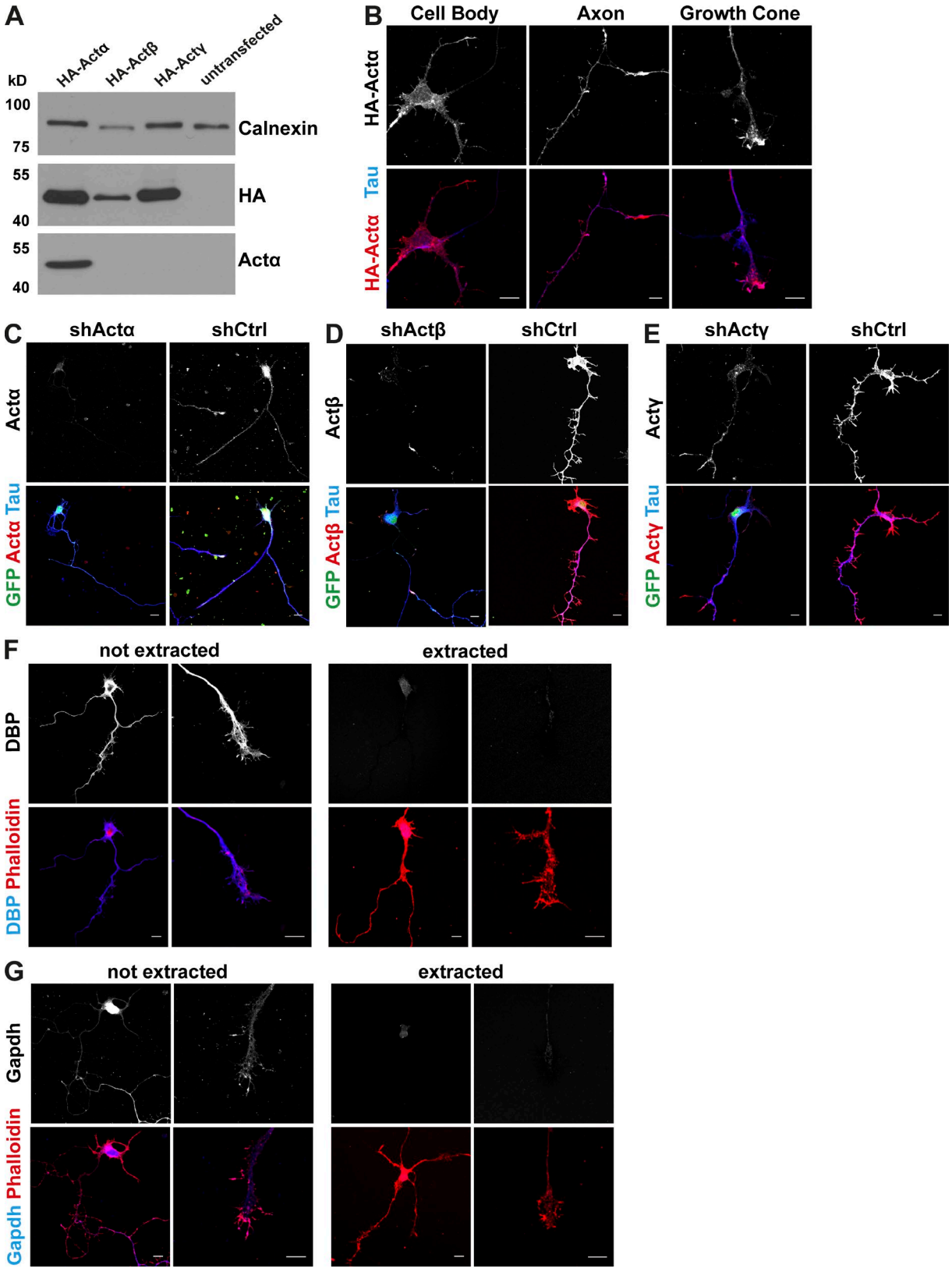


Figure S2. **Validation of Actα-, Actβ-, and Actγ-specific antibodies by Western blot and immunocytochemistry experiments.** (A) HEK^{293T} cells were transfected with plasmids expressing HA-tagged Actα, Actβ, or Actγ driven by the ubiquitin promoter. Actα antibody detects only HA-Actα and not HA-Actβ or HA-Actγ. (B) HA-tagged Actα lentiviral construct was expressed in motoneurons. Cells were fixed and stained with HA antibody. HA-Actα is detected in axonal branch points and in the growth cone. (C–E) Motoneurons were transduced with isoform-specific shRNAs and stained with respective antibodies. Corresponding signals for Actα, Actβ, and Actγ are reduced in somata and axons of motoneurons after shActα (C), shActβ (D), and shActγ (E) lentiviral transductions. (F and G) Motoneurons were stained with phalloidin and against DBP (F) and Gapdh (G). Treatment of cells with Triton X-100 before fixation extracted G-actin and other globular proteins, as DBP and Gapdh signals were reduced to undetectable levels. Bars, 10 µm.

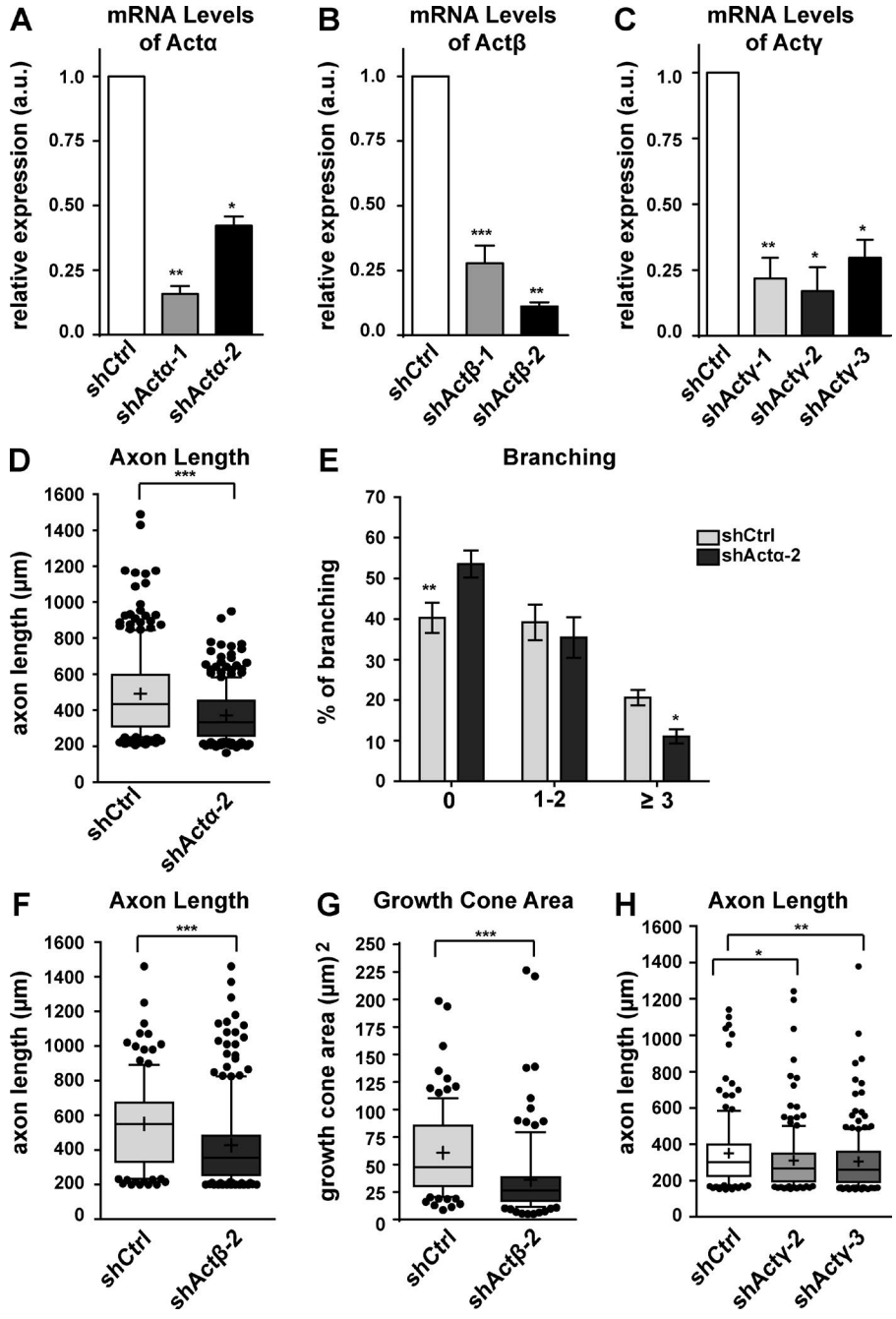


Figure S3. **shRNA knockdown of actin isoforms generates distinct phenotypes that are result of actin isoform–specific depletion and not off-target effects.** (A) Motoneurons were transduced with two different lentiviral shRNAs against Actα. Quantitative RT-PCR showed that shActα-1 leads to 85% reduction in Actα mRNA levels and shActα-2 leads to 60% reduction (*, $P < 0.01$; **, $P < 0.0037$, $n = 5$ for shActα-1 and $n = 4$ for shActα-2). (B) Actβ was knocked down using two different lentiviral shRNAs. ShActβ-1 leads to a 75% reduction in Actβ mRNA levels and shActβ-2 to a 90% reduction (**, $P < 0.008$; ***, $P < 0.0006$; $n = 6$ for shActβ-1 and $n = 3$ for shActβ-2). (C) Actγ was knocked down in motoneurons using three different shRNAs. Actγ mRNA levels are reduced by 70–85% after transduction with shActγ-1, shActγ-2, and shActγ-3 (*, $P < 0.014$; **, $P < 0.0013$; $n = 5$ for shActγ-1, $n = 4$ for shActγ-2, and $n = 3$ for shActγ-3). Statistical analysis was done using a one-tailed Mann–Whitney test. (D and E) shActα-2 lentiviral transduction causes a 20% reduction in axon length (***, $P < 0.0001$ for $n = 3$; sample size: shActα-2: 252, shCtrl: 242, by two-tailed Mann–Whitney test) and a significant increase in the number of axons without collateral branches and a significant decrease in the number of axons with three or more branches (*, $P < 0.05$; **, $P < 0.01$ for $n = 3$, by two-way ANOVA with Bonferroni post hoc test). (F and G) Knockdown of Actβ by shActβ-2 reduces axon length by 21% (***, $P < 0.0001$ for $n = 3$; sample size: shActβ-2: 220, shCtrl: 126) and growth cone size by 40% (***, $P < 0.0001$ for $n = 3$, sample size: shActβ-2: 108, shCtrl: 96, by two-tailed Mann–Whitney test). (H) Knockdown of Actγ by shActγ-2 causes a 12% reduction in axon length and knockdown by shActγ-3 a 15% reduction (*, $P < 0.012$; **, $P < 0.004$ for $n = 3$; sample size: shActγ-2: 150, shActγ-3: 184 and shCtrl: 144, by one-way ANOVA with Dunn's post-test). In A–C and E, data are shown as mean ± SEM.

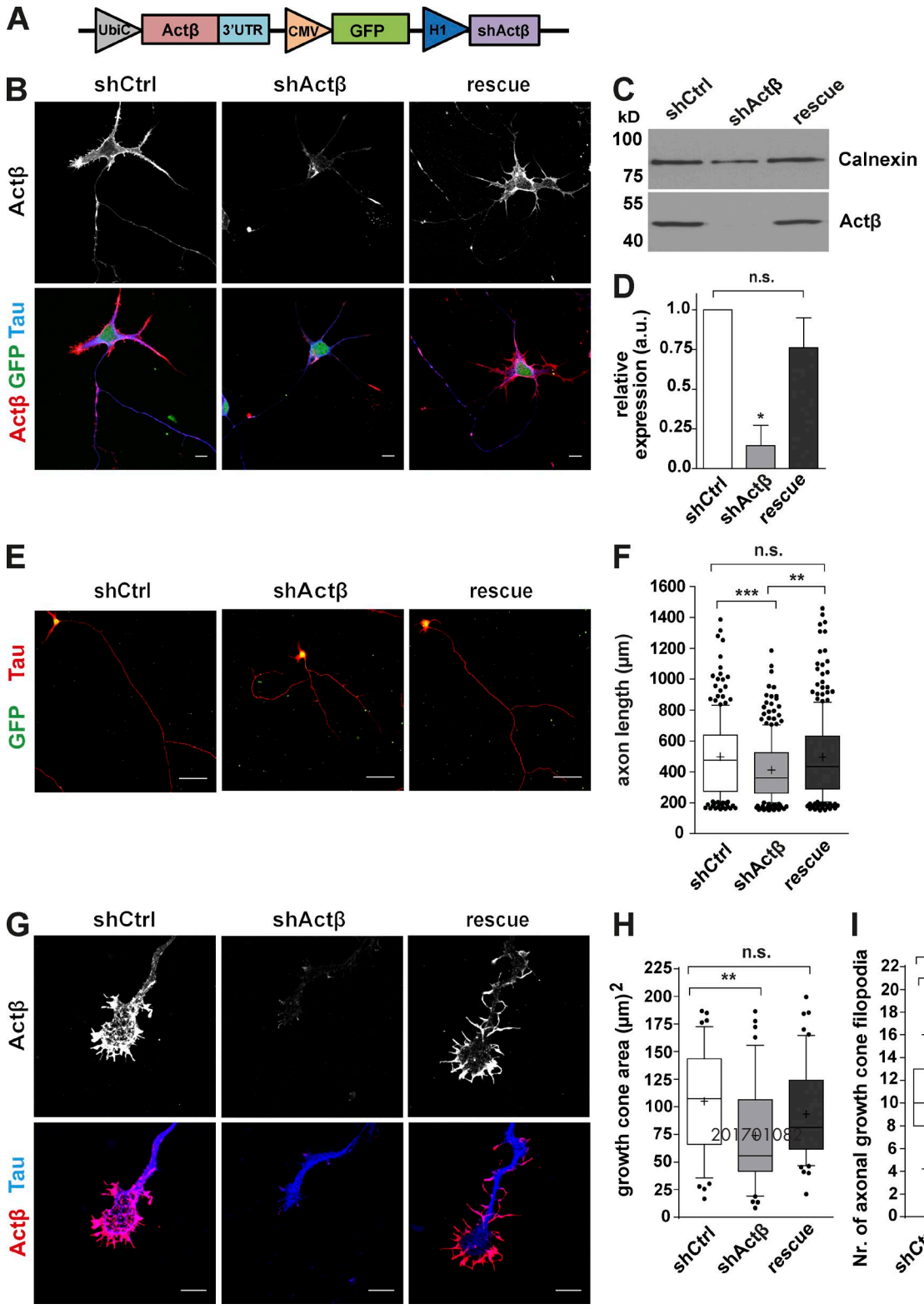


Figure S4. **Reexpression of Actβ rescues axon elongation and growth cone formation defects in Actβ-depleted motoneurons.** (A) Scheme of Actβ-rescue lentiviral construct. Expression of shRNA resistant Actβ is driven by the ubiquitin promotor and expression of shActβ by the H1 promotor. GFP was used to identify transduced neurons. (B) Motoneurons were transduced with shCtrl, shActβ, or Actβ rescue constructs and immunostained against Tau and Actβ. (C and D) Western blot analysis shows that Actβ protein levels are restored to 75% after application of the rescue construct (*, $P < 0.016$ for $n = 3$ by one-tailed Mann–Whitney test). Data are shown as mean ± SEM. (E and F) Reexpression of shRNA-resistant Actβ rescues axon growth defects in Actβ-depleted motoneurons (**, $P < 0.002$; ***, $P < 0.0002$ for $n = 3$; sample size: shActβ: 240, rescue: 266 and shCtrl: 217). (G–I) Expression of Actβ rescue construct attenuates defects in axonal growth cone formation (H: **, $P < 0.005$) and increases number of growth cone filopodia (I: ***, $P < 0.0003$ for $n = 3$; sample size: shActβ: 43, rescue: 56 and shCtrl: 48). Statistical analysis in F–I was done using a one-way ANOVA with Dunn's post-test. Bars: (B and G) 10 µm; (E) 50 µm. a.u., arbitrary units; n.s., not significant.

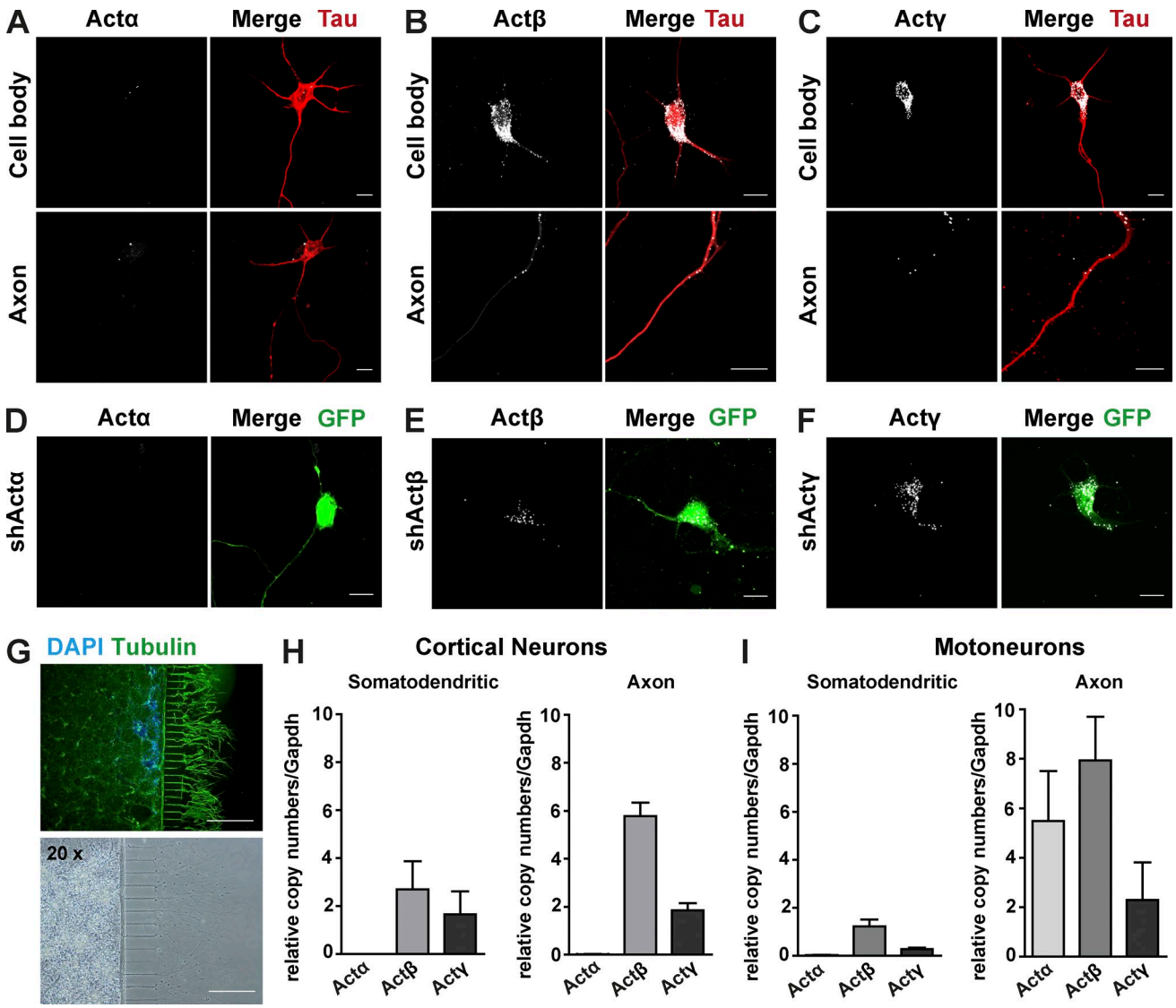
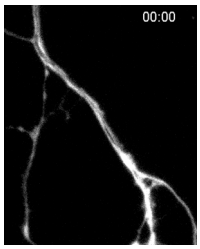
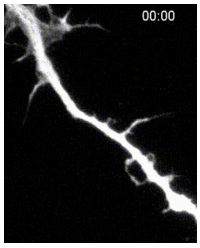


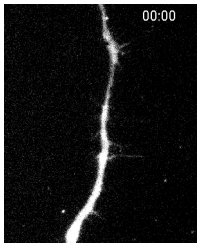
Figure S5. **Expression of actin isoforms in primary cortical neurons.** (A–C) In situ hybridization showing localization of Actα (A), Actβ (B), and Actγ mRNAs (C) in cortical neurons. In addition to Actβ, Actγ mRNAs are detected in the axon. (D–F) Control experiments with knockdown of Actα (D), Actβ (E), and Actγ (F) showing specificity of the signals in cortical neurons with applied probes. (G) Cortical neurons were grown in compartmentalized chambers for 5 d. (H) Actβ and Actγ mRNAs are detected in the axonal compartment by quantitative RT-PCR analysis ($n = 4$). (I) Compared with motoneurons, Actγ mRNAs are less abundant in the axon in cortical neurons. Bars: (A–F) 10 µm; (G) 200 µm.



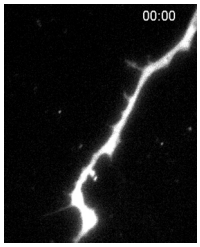
Video 1. **Axonal filopodia dynamics in cultured motoneurons.** Motoneurons transduced with lentiviral particles expressing GFP were imaged for 40 min every 20 s. Axonal filopodia form de novo along the axon shaft and show growth and retraction movements. Related to Fig. 6 (A–G).



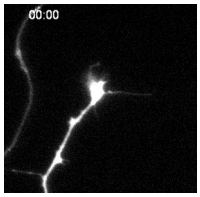
Video 2. **Depletion of Actα disturbs growth dynamics of axonal filopodia.** Motoneurons transduced with lentiviral shRNA targeting Actα and coexpressing GFP show decreased dynamics of axonal filopodia movements. Related to Fig. 6 (A–G).



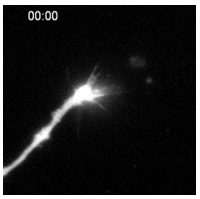
Video 3. **Depletion of Actβ does not affect growth dynamics of axonal filopodia.** Motoneurons expressing shRNA against Actβ exhibit unaltered axonal filopodia growth and retraction dynamics. Related to Fig. 6 (A–G).



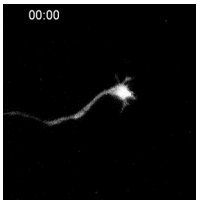
Video 4. **Depletion of Actγ reduces growth dynamics of axonal filopodia.** Axonal filopodia show reduced growth and retraction movements after shRNA-meditated knockdown of Actγ. Related to Fig. 6 (A–G).



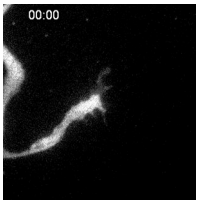
Video 5. **Axonal growth cones show highly dynamic filopodia activity.** Motoneurons were transduced with lentiviral particles that coexpress GFP and imaged for 40 min at 20-s intervals. Filopodia in growth cones are highly dynamic and show fast growth and retraction movements. Related to Fig. 7 (D–I).



Video 6. **Knockdown of Actα does not disturb axonal growth cone filopodia movements.** Dynamics of growth/retraction of filopodia in the axonal growth cone are not altered in Actα-depleted motoneurons. Related to Fig. 7 (D–I).



Video 7. **Dynamic movements of axonal growth cone filopodia are reduced in Actβ knockdown motoneurons.** ShRNA-mediated Actβ depletion decreases growth and retraction of filopodia in axonal growth cones. Related to Fig. 7 (D–I).



Video 8. **Actγ depletion does not impair axonal growth cone filopodia dynamics.** Actγ-depleted motoneurons show normal growth and retraction movements of axonal growth cone filopodia. Related to Fig. 7 (D–I).