Supplementary Figure 5. Filopodia elicitation by KCl depolarization depends on protein synthesis and β-actin translocation. (a-c) Time-lapse DIC sequences showing the changes in filopodial number induced by KCl bath application without (a) and with protein synthesis inhibitors (b) or antisense oligonucleotides (c). Times indicate minutes after application of 40 mM KCl. (d) The bar graph shows the relative changes in filopodial number ($\Delta N/N_0$) at
various times with and without 40 mM KCl. Different drugs (PSI: protein synthesis inhibitors; AS: antisense; RS: control oligonucleotides) were applied to bath 20 min before the KCl treatment. Results were averaged from 10 growth cones for each group. Error bars represent S.E.M.

**Methods:** Overnight *Xenopus* neuronal cultures were used in KCl treatment experiments for their greater responsiveness to KCl depolarization. Each growth cone was monitored by differential interference contrast (DIC) enhanced microscopy on a Nikon Diaphot 300 with a 40X Plan Fluor objective. Video images were digitized and contrast-enhanced using an Argus-20 image processor and recorded to a personal computer before and every minute after KCl application. To depolarize the cells, we simply changed the bath solution to MR containing 40 mM KCl and protein synthesis inhibitors and oligonucleotides were applied to the bath 30 min before KCl application and imaging. We counted the number of filopodia per growth cone in each image and calculated the difference in filopodia number between image frames (ΔN), followed by dividing ΔN over the filopodia number before KCl (N₀) to obtain the relative increase in filopodia (ΔN/N₀, in percentage).