Supplemental Materials

Molecular Biology of the Cell

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Figure S1: Validation of Src2 and cortactin expression approach.

(A) DIC and fluorescent images of growth cones stained for total Src2 and pSrc2 after control injections with Alexa 568 G-actin only or in combination with CASrc2 and MDSrc2 mRNAs, respectively. Src2 was detected throughout the growth cone; activated Src2 was enriched in the growth cone P domain (region demarked with yellow dashed line). Scale bar: 20 μm. (B) Quantification of total Src2 and activated Src2 levels in the P domain. Average
fluorescent intensity values normalized against control (CTL) condition are given ± SEM. Number of growth cones analyzed are indicated. One-way ANOVA with Dunnett’s post hoc test; *p<0.05, ***p<0.001. CASrc2, DNSrc2, and MDSrc2 expression increased total Src2 levels by 111%, 77%, and 54%, respectively, compared to control levels. Activated Src2 levels were increased by 94% in CASrc2-expressing growth cones, not significantly different in DNSrc2-expressing growth cones, and reduced by 34% in MDSrc2-expressing growth cones when compared to controls. Uninjected (Unj) cells had similar Src2 levels as control cells, whereas no signal was detected in growth cones that received no primary antibody (Unj-NoPA). (C) Cortactin immunolabeling in control and cortactin expressing growth cones. Scale bar: 20 μm. (D) Quantification of cortactin and activated Src2 signals in control growth cones, cortactin-expressing growth cones, and cortactin phosphorylation mutant (CortF)-expressing growth cones. Cortactin levels were increased by 52% and 48% in growth cones expressing full-length cortactin and CortF, respectively, whereas Src2 activation was not affected in either case. Average fluorescent intensity values normalized against control condition are given ± SEM. Number of growth cones analyzed are indicated. One-way ANOVA with Dunnett’s post hoc test; *p<0.05, ***p<0.001.
Figure S2: DNSrc2-expression results in similar but more moderate growth cone phenotypes compared to MDSrc2-expression.

(A) Lamellipodial length, (B) % area in lamellipodia occupied by actin filaments, (C) filopodial length, (D) filopodial density, (E) fraction of filopodia undergoing small scale lateral movements, (F) fraction of filopodia undergoing large scale lateral movements in control growth cones, growth cones expressing DNSrc2, or MDSrc2, respectively. Data are represented as means ± SEM; numbers in parentheses are number of growth cones selected from at least three experiments; p values are determined in one-way ANOVA with Dunnett’s post hoc test comparing to control condition: **p<0.01, ***p<0.001.
Figure S3: Src inhibition by PP2 promotes retraction of lamellipodia and lateral movements of filopodia.

(A) DIC image of a live *Aplysia* growth cone in medium containing 0.1% DMSO. (B) Same growth cone after a 15 min treatment with 25 µM PP2, which caused retraction of lamellipodia, a less smooth leading edge, as well as filopodia that were not perpendicular to the leading edge. (C) The growth cone completely recovered after PP2 washout. Scale bar as indicated. (D) DIC kymograph of a growth cone undergoing PP2 treatment. The line was drawn perpendicular to the leading edge. Slow protrusion was observed in DMSO control condition, significant leading edge retraction after PP2 application, and more persistent protrusion following drug washout. (E) Comparison of lamellipodial length before and after 15 min treatment with 25 µM PP2 for 5 individual growth cones. Data are represented as means ± SEM; p value was determined using paired t-test: *p<0.05. (F) Src inhibition by PP2 increased the fraction of filopodia undergoing large scale lateral movements as shown for 5 individual growth cones. The mean values are significantly different after Src inhibition according to a paired t-test: **p<0.01.
Figure S4: CortF abolishes CASrc2-induced effects on lamellipodial and filopodial motility.

Lamellipodial protrusion and retraction frequency (A), time (B), rates (C), filopodial protrusion and retraction frequency (D), time (E), rates (F) of Aplysia growth cones expressing CASrc2, CortF or both together compared to control (CTL) growth cones. Data are represented as means ± SEM; numbers in parentheses are number of growth cones selected from at least three experiments; p values are determined by one-way ANOVA with Dunnett’s post hoc test, comparing each experimental group against a pooled control group: *p<0.05, ***p<0.001.
Figure S5: Actin dynamics in lamellipodia.

(A) Plot of filopodial motility rates versus the difference between the experimentally determined actin assembly and retrograde flow rates in filopodia (left), actin assembly rates (center), or retrograde flow rates (right) and linear regression analysis, demonstrating that filopodial motility rate can be modeled as the difference between actin assembly and retrograde flow rates. Each circle represents a single measurement of the motility rate of filopodial tips, actin assembly and retrograde flow rate from all Src2 or cortactin expression conditions independent of protrusion state (1575 observations in total). (B-B”) Comparison of actin assembly rates in lamellipodial veils during protrusion (B), retraction (B’) or pausing (B”) phases. These assembly rates were calculated as the sum of the leading edge protrusion and actin retrograde flow rates. (C-C”) Comparison of experimentally determined actin retrograde flow rates in lamellipodial veils in protrusion (C), retraction (C’) or pausing (C”) phases. Mean values ± SEM; numbers in parentheses are number of growth cones selected from at least three experiments; one-way ANOVA with Dunnett’s post hoc test was separately performed for Src2 and cortactin conditions. No significant differences were found between the different conditions.