SUPPLEMENTAL FIGURES LEGENDS

Figure S1. Lamellipodial and filopodial markers in neuroblastoma cells. **(A)** Expression of GFP-VASP in control (top) or p34-Arc siRNA-treated (bottom) differentiated B35 cells shows VASP localization to the tips of filopodia and the leading edge of lamellipodia, which is less prominent in knockdown cells. **(B, C)** Localization of cortactin by immunostaining of NG-108 cells (B) or by expression of DsRed-cortactin in B35 cells (C). Cortactin localizes throughout the growth cone with some enrichment at the leading edge. Time lapse sequence (C) shows that during filopodia initiation cortactin concentrates in protruding regions (0:00 and 0:06 time points) and then redistributes to surrounding lamellipodia, while the growing filopodial bundles are depleted of cortactin (arrowheads). Time in min:sec. **(D)** Immunostaining of fascin in control (top) and p34-Arc siRNA-treated (bottom) B35 cells. **(E)** Immunostaining of myosin II in differentiated B35 cells double transfected with Arp3 siRNA and human Arp3. Arp3 expression along with siRNA (left cell) results in decreased amount of myosin II-containing bundles in neurites as compared to expression of siRNA only (right cell). Bars, 5 μm (A, B, D), 2 μm (C) and 10 μm (E).

Figure S2. Branched filaments in protrusions of neuronal cells. **(A)** Anaglyph stereo EM image of the growth cone of a differentiated B35 cell. The entire growth cone is shown in B. Selected regions from A are enlarged in small 3D panels. Stereo pair images are taken at ±20 degrees to emphasize the 3D organization of actin network. Red/cyan anaglyph glasses (left eye red) should be used to view the image. **(C, D)** Dendritic organization of actin network in lateral

protrusion in Xenopus neurons (C) and B35 neuroblastoma cells (D).Branched filaments are highlighted in green. Bars, 0.2 μm (A, C, D) and 1 μm (B).

Figure S3. (A-D) Nascent lamellipodium contains branched actin network. **(A)** Time lapse sequence of a growth cone shows initiation of a lamellipodium at 0:12 (arrow). **(B)** EM image of the field shown in A. The lamellipodium (arrow) protruded further during a few-second interval between the acquisition of the last live image and extraction. **(C, D)** Enlargement of boxed regions from B and C, respectively. Numerous branched filaments (red dots in D) are present throughout this nascent lamellipodium. Boxed regions in D are enlarged in insets to show branched filaments. **(E)** Immunogold (18 nm) staining with p16-Arc antibody of a growth cone of differentiated B35 cells. Gold particles are highlighted in yellow. **(F)** Rates of lamellipodia protrusion (left) and retrograde flow (right) in non-differentiated B35 cells transfected as indicated. Asterisks indicate statistical significance compared to control ($p<0.001$, N=30-35 events for each). Top and bottom of a box indicate 75th and 25th quartiles; whiskers indicate 10th and 90th percentiles; dot is the mean; line is the median. Time in min/sec. Bars, 5 μm (A), 2 μm (B) or 0.2 μm (D, E).

SUPPLEMENTAL VIDEOS LEGENDS

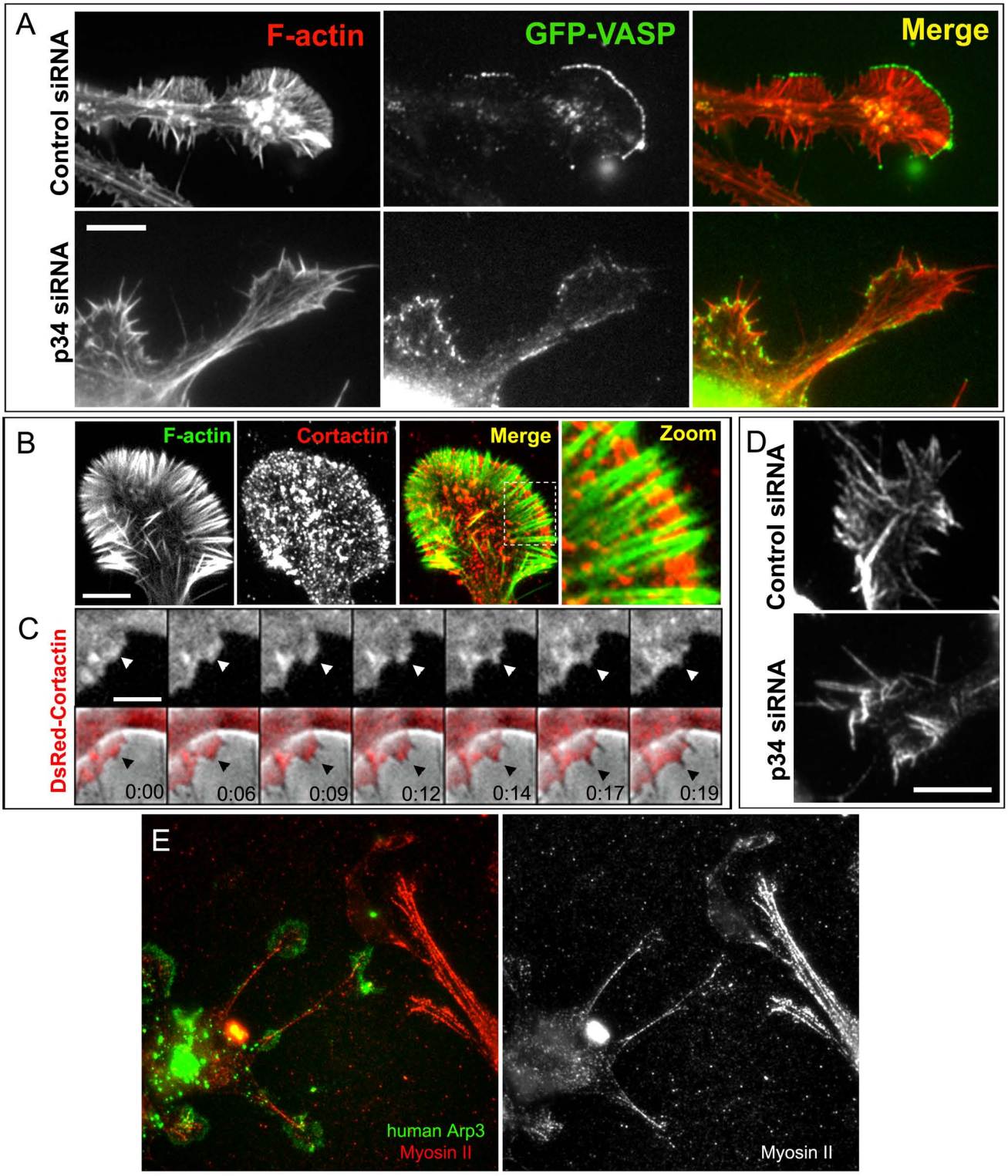
Video 1. Filopodia initiation in the growth cone of a differentiated B35 cell (left) and a hippocampal neuron (right). Arrows and arrowheads point to individual events of filopodia initiation. Filopodia arise from lamellipodia with a small bulge as an intermediate. Time in min:sec.

Video 2. Filopodia initiation from a neuronal shaft. Arrows mark sites on a secondary neurite of the hippocampal neuron where filopodia emerge from small lamellipodia. Time in min:sec.

Video 3. Growth cone motility in differentiated B35 cells treated with control siRNA (left), p34-Arc siRNA (middle) or co-transfected with Arp3 siRNA and human YFP-Arp3 (right). Note fast dynamics of lamellipodia and numerous filopodia in control (left) and rescued (right) cells, but slow dynamics of lamellipodia and infrequent filopodia in the inhibited cell (middle). Time in min:sec.

Video 4. Growth cone motility in hippocampal neurons treated with control (left) or p34-Arc siRNA (right). Arp2/3 depleted cell has slow lamellipodial dynamics and infrequent formation of filopodia, as compared to the control cell. Time in min:sec.

Video 5. Neuritogenesis in mixed populations of B35 neuroblastoma cells transfected with control or p34-Arc siRNA labeled by different colors. Differentiation is induced at the beginning of the movie. (A) This field contains mostly cells transfected with control siRNA, except one marked by an arrow, which is transfected by p34-Arc siRNA, but dies during the movie. Control cells persistently protrude 2-3 dominant long neurites. (B) All cells in this field are transfected with p34-Arc siRNA. Cells form short and broad processes which undergo erratic extension and retraction. Time in hrs:min.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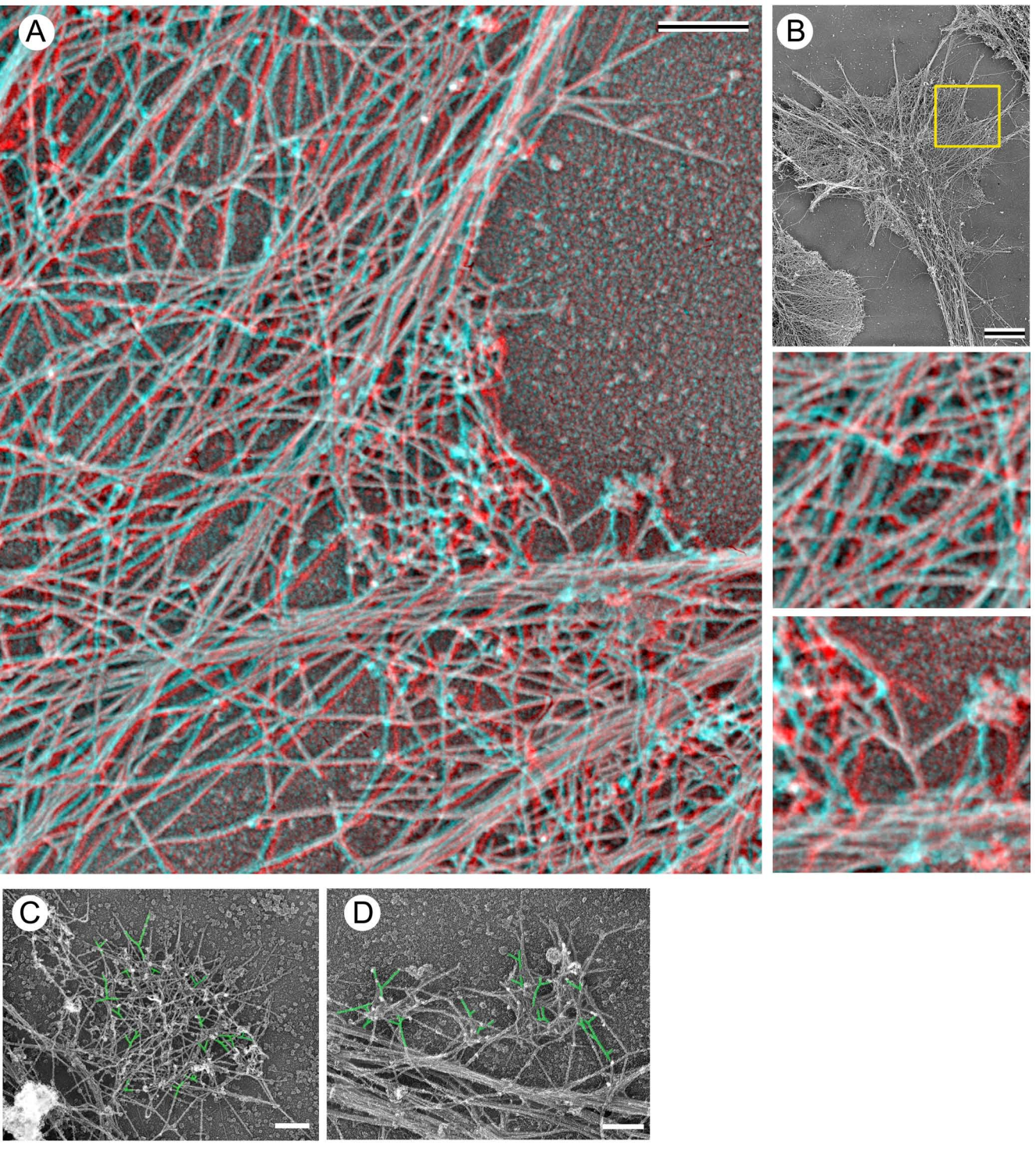
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