Supplemental Materials

Figure S1

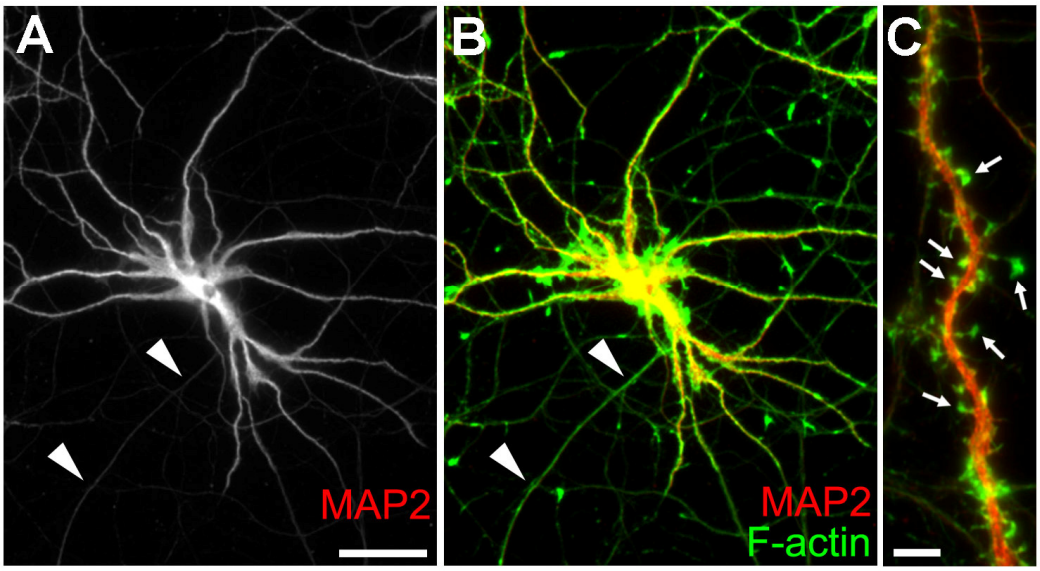

Figure S1. Immunofluorescence staining of the dendritic marker MAP2 (red) in 21 DIV hippocampal neurons co-stained with phalloidin (green). (A, B) Dendrites are enriched in MAP2 and F-actin, while axons are relatively depleted in both (arrowheads). (C) A segment of a dendrite with MAP2 staining in the shaft (red) and actin-rich (green) dendritic spines (arrows) protruding from the sides. Bar, 10 µm (A, B) or 5 µm (C).

Figure S2

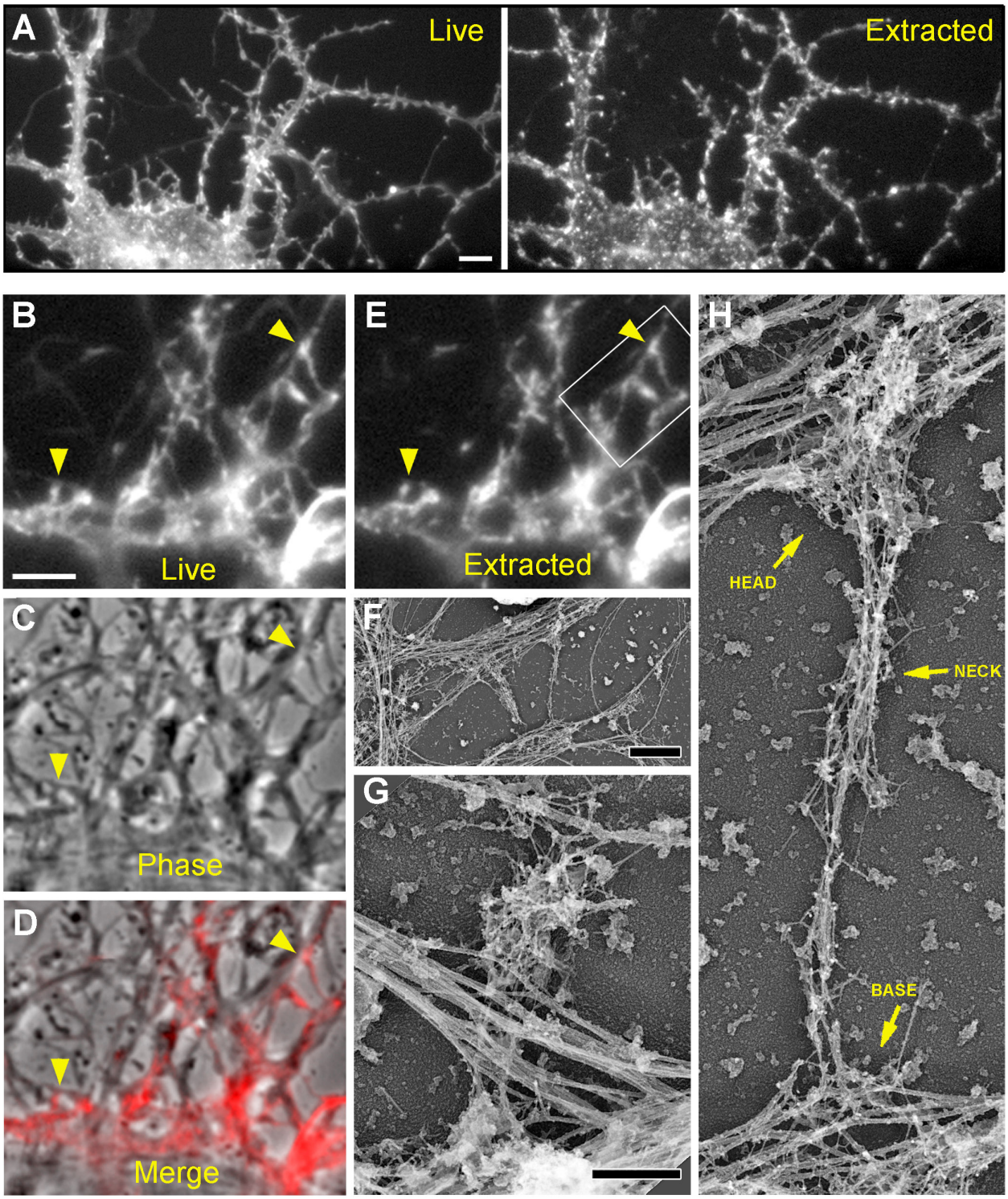
A
Live
Extracted
B
Live
E
Extracted
H
HEAD
NECK
BASE
C
Phase
F
G
D
Merge

Figure S2. Correlative microscopy of dendritic spines. (A) Effect of detergent extraction on actin distribution. A 14 DIV hippocampal neuron expressing mCherry-actin is shown before (left) and after (right) detergent extraction. No dramatic changes occur after extraction except for some loss of diffuse fluorescence apparently corresponding to the soluble actin pool. (B-H) Correlative light and EM of dendritic spines. The same region of a YFP-actin expressing 14 DIV hippocampal neuron is shown in fluorescence (B, E), phase contrast (C), and phase/fluorescence merged (D) images before (B-D) and after (E) detergent extraction. Low magnification EM of the boxed region in E is shown in F. Spines marked by arrowheads in B-E are shown in G and H. The head, neck and base regions of the spine in H are labeled. Dendritic spines are well preserved during extraction and after EM processing. Bars, 5 µm (A, B-E), 2 µm (F), or 0.5 µm (G, H).

Figure S3

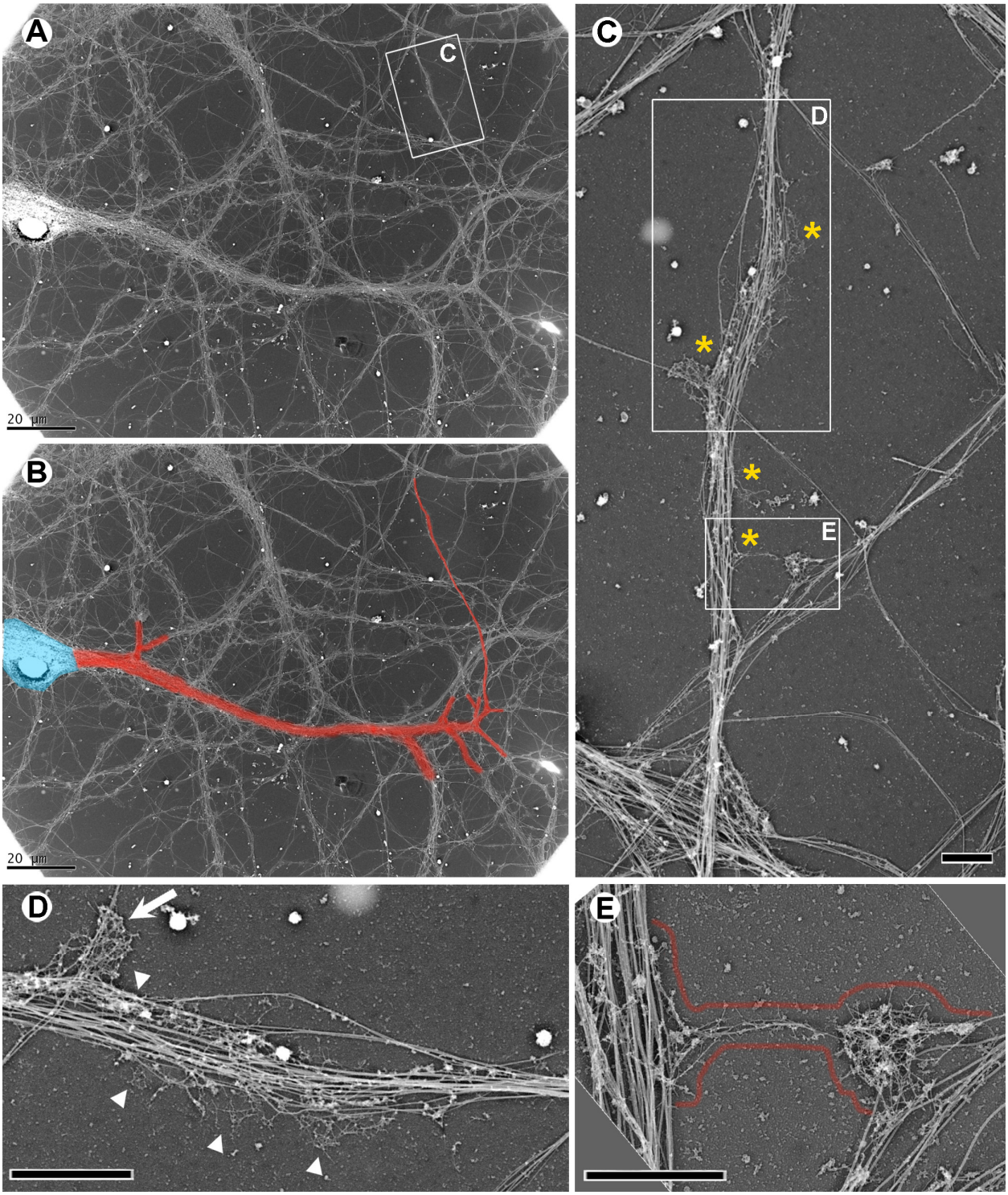

Figure S3. Identification of spine orientation in replica EM samples. (A,B) Low magnification EM image of a neuron with a thick neurite (red in B) extending from the soma (blue in B) and branching in several places. Based on the thickness, this neurite is identified as a dendrite. Only the beginning of a branch is colorized except for the branch of interest that is shown at higher magnifications in C-E. (C) Enlarged boxed region from A showing that this neurite contains several actin-rich protrusions (asterisks) consistent with its identification as a dendrite. (D) Enlarged box from C showing lamellipodia-like actin-rich protrusions (arrowheads) and a potential stubby spine (arrow) emerging from the dendrite. (E) Enlarged box from C showing a mushroom spine (red outline) emerging from the dendrite and contacting an axon. The axon is relatively thin and depleted of actin, as compared to the dendrite. The base of the spine has a delta-like shape, as compared to more bulbous head, and it is less rich in actin than the head. See Figure 2A for more detail of this spine structure. Bars, 20 µm (A, B) and 2 µm (C-E).

Figure S4

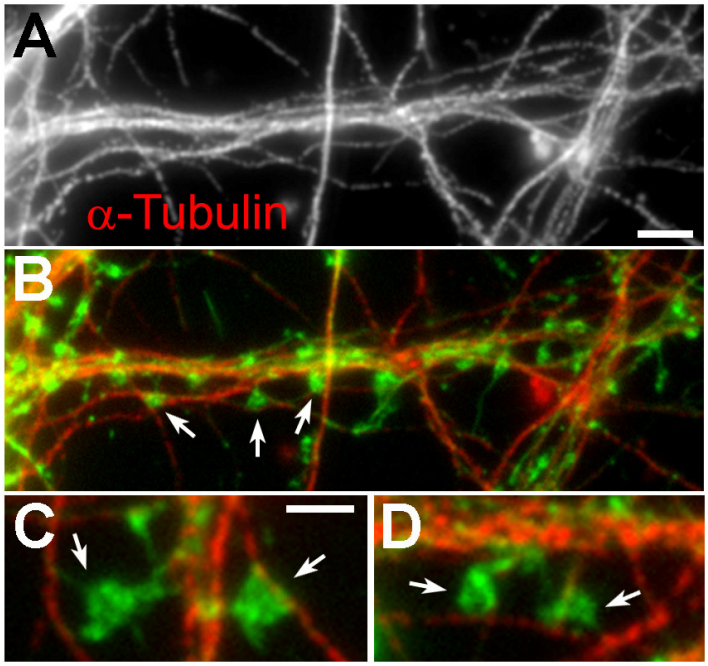

Figure S4. Relative distribution of F-actin and microtubules in hippocampal neurons. Non-extracted 14 DIV hippocampal neurons were stained with phalloidin (green) and α-tubulin antibody (red). Dendritic spines are marked by arrows. Bars, 5 µm (A, B) or 2 µm (C, D).

Figure S5

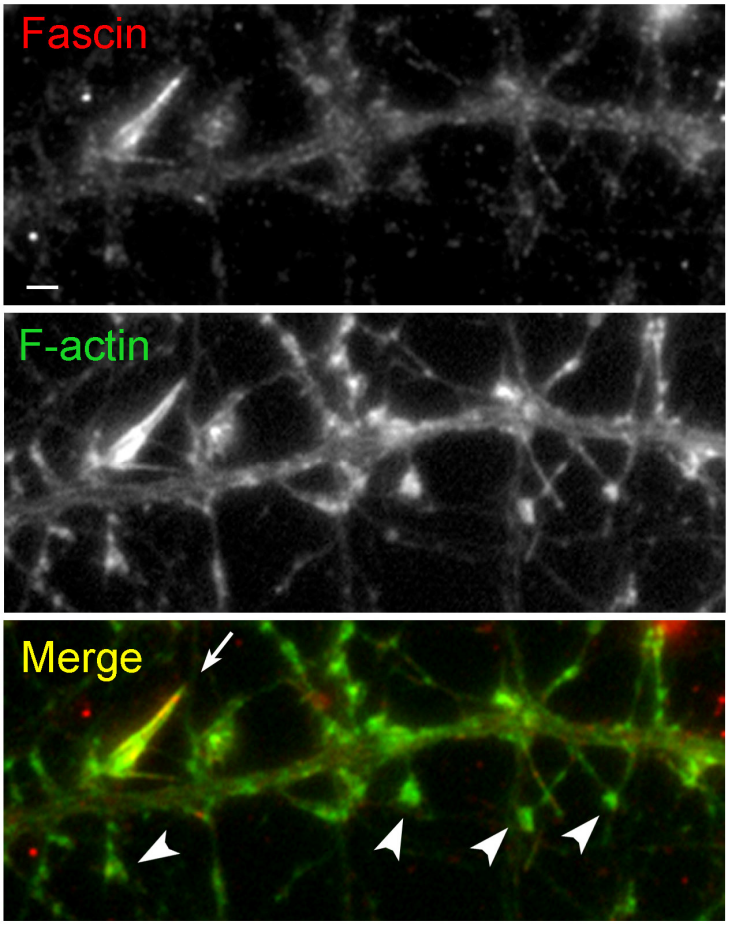

Figure S5. Immunofluorescence staining of fascin (red) in 21 DIV hippocampal neurons co-stained with phalloidin (green). Growth cone filopodia are enriched in fascin and F-actin (arrow), while dendritic spines are depleted in fascin, but contain F-actin (arrowheads). Bar, 2 µm.

Figure S6

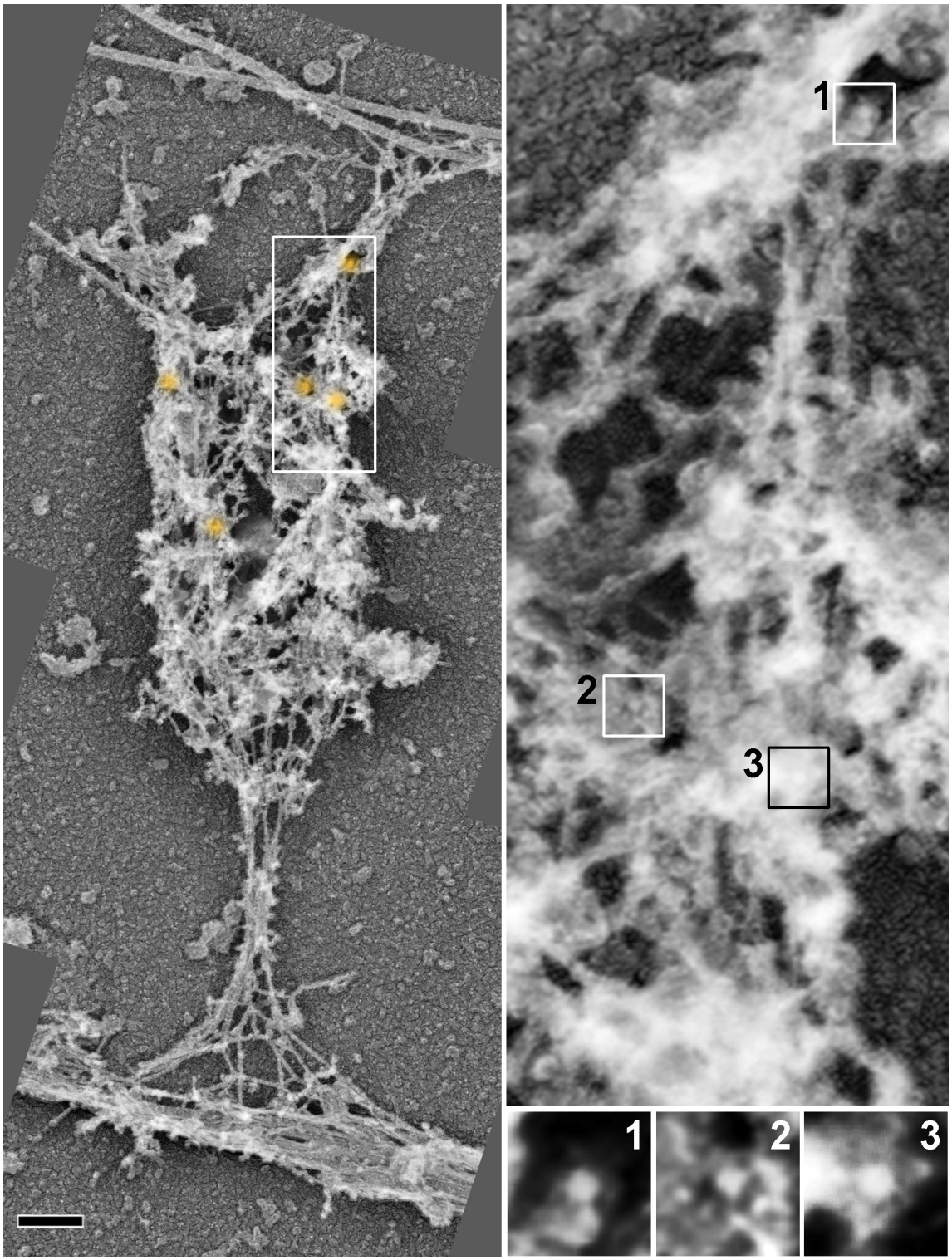

Figure S6. Immunogold EM of PSD-95 in dendritic spines. (A) Overview of a mushroom spine; gold particles showing PSD-95 localization are highlighted in orange. (B) Enlarged boxed region from A; numbered boxes outlining gold particles are enlarged and contrast-adjusted in bottom panels to optimally show gold particles. Bar, 0.2 µm.

Figure S7

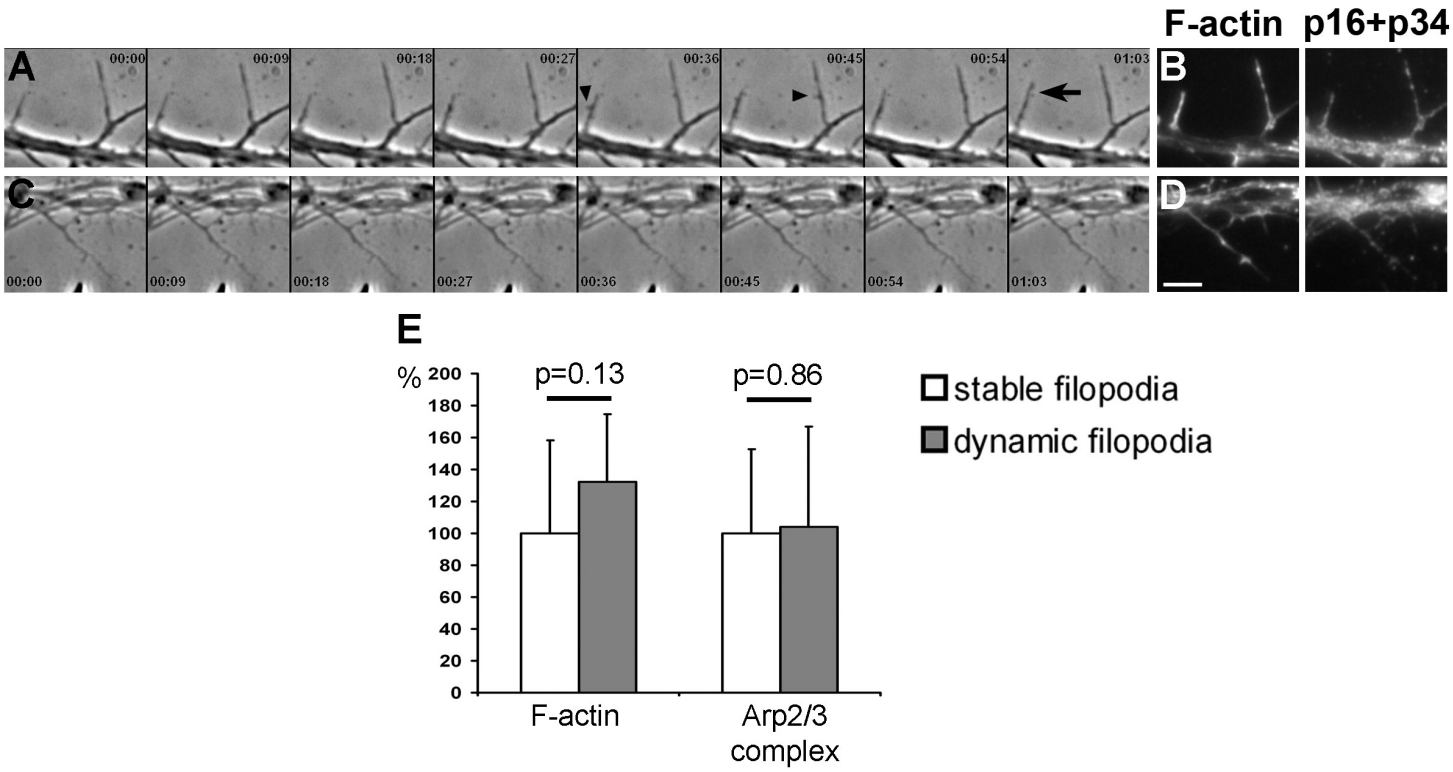

Figure S7. F-actin and Arp2/3 complex contents in dynamic and stable dendritic filopodia in 11 DIV hippocampal neurons. (A,C) Selected frames from time-lapse sequences showing dynamic (A) and stable (C) filopodia. Arrow in A points to a filopodium elongated during the sequence; arrowheads mark lateral activity in filopodia. (B, D) Cells from A and C, respectively, fixed immediately after sequence acquisition and stained with phalloidin and Arp2/3 complex antibodies as indicated. Fluorescence staining reveals little or no difference in the F- actin or Arp2/3 complex contents between stable and dynamic filopodia. Bar, 5 µm. (E) Average intensities of the Arp2/3 complex and F-actin fluorescence for dynamic and stable filopodia, expressed as a percentage of the respective mean values for stable filopodia. A filopodium was considered stable if it did not change its length or shape during the last minute

before fixation; filopodia that changed their length or expressed lateral activity were scored as dynamic. Data are pooled from two experiments. Error bars show standard deviations; p-values of Student's test are shown above the bars; N=15 for stable filopodia and 11 for dynamic filopodia.

Supplementary Videos

Video S1. Time-lapse phase-contrast sequence showing initiation of dendritic filopodia from phase-dense spots in the dendrite of a 10 DIV neuron. Right frame shows the same sequence in false colors to emphasize the phase-dense patches (purple). Time is shown in min:sec.

Video S2. Time-lapse phase-contrast sequence showing initiation of dendritic filopodia from lamellipodia in the dendrite of a 10 DIV neuron. Time is shown in min:sec.