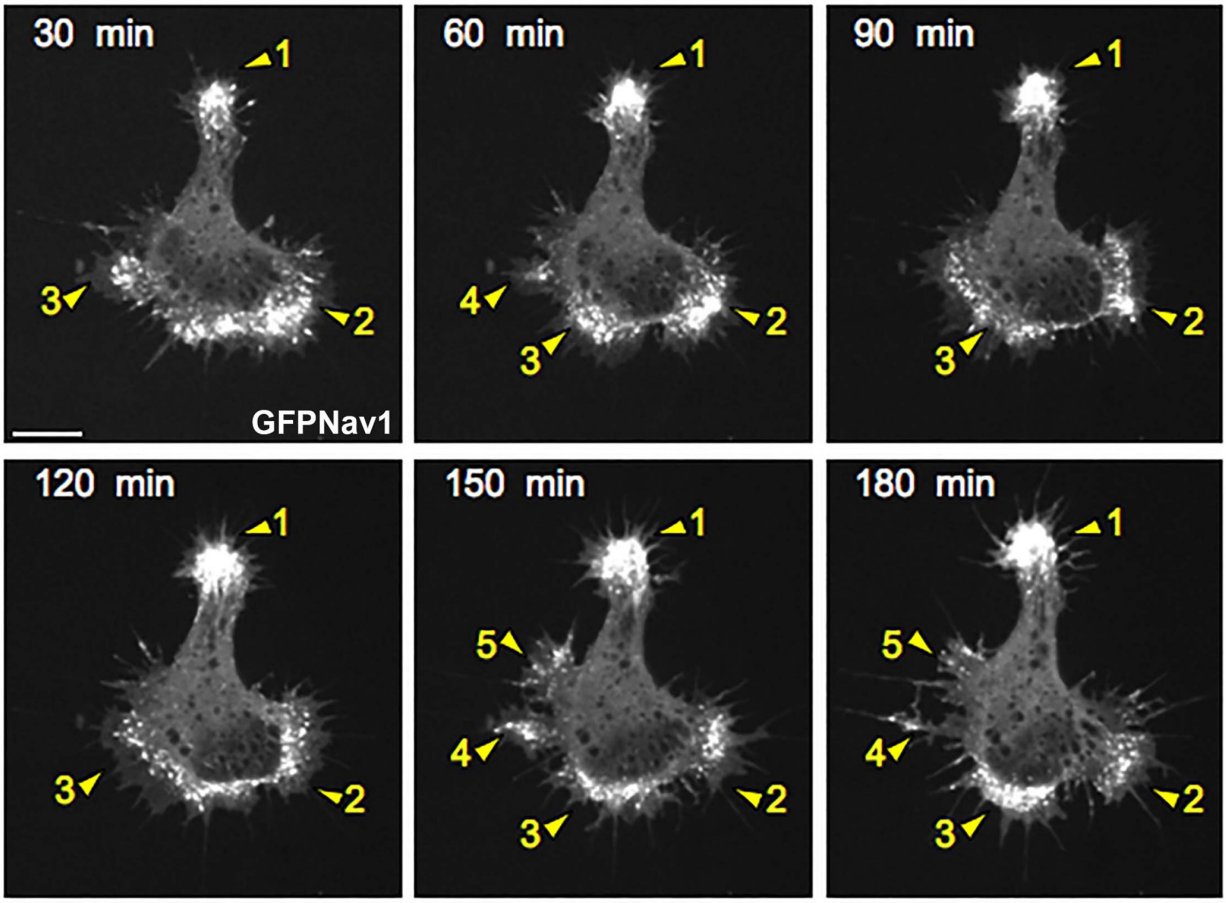
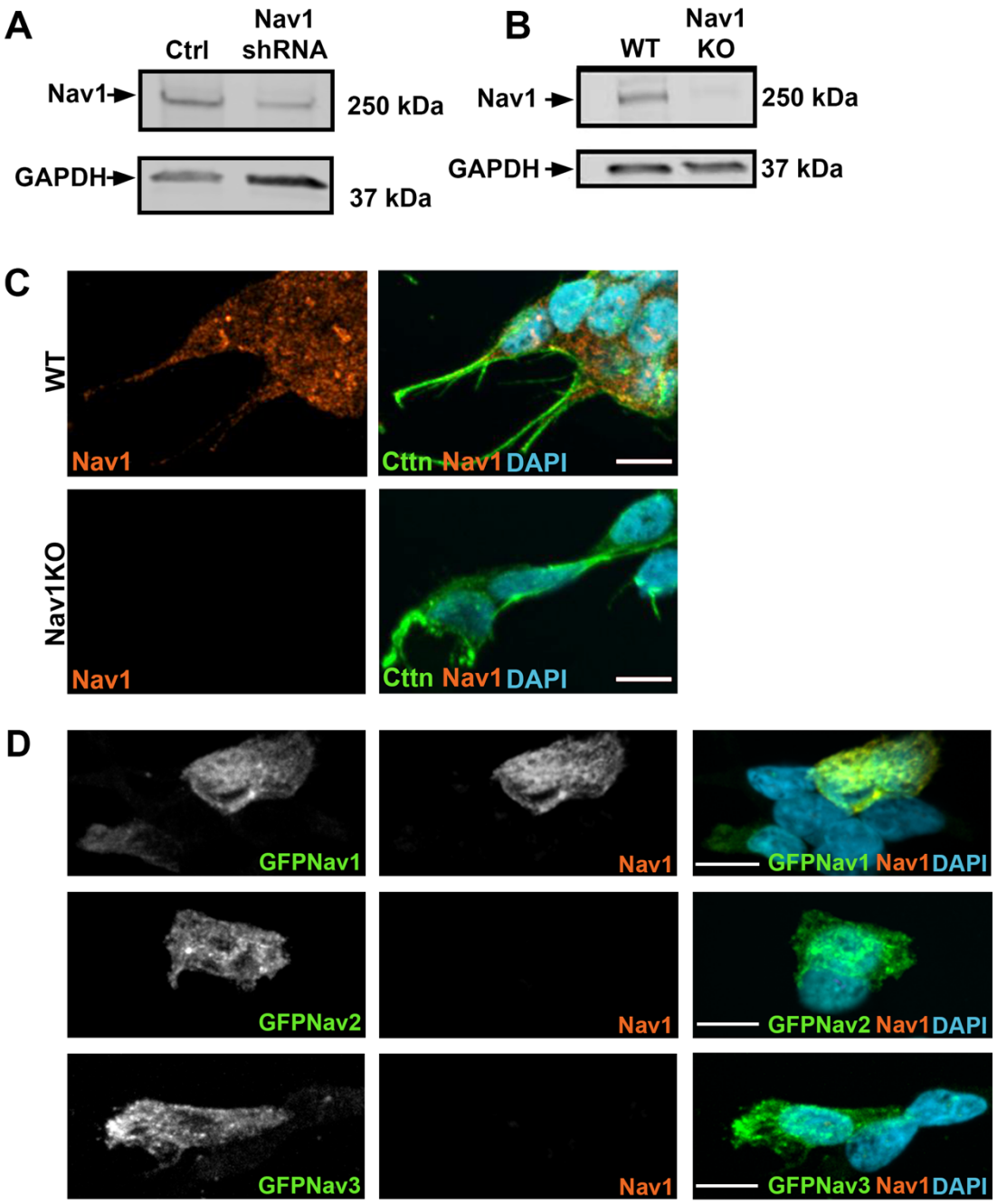
Supplementary Materials

Molecular Biology of the Cell

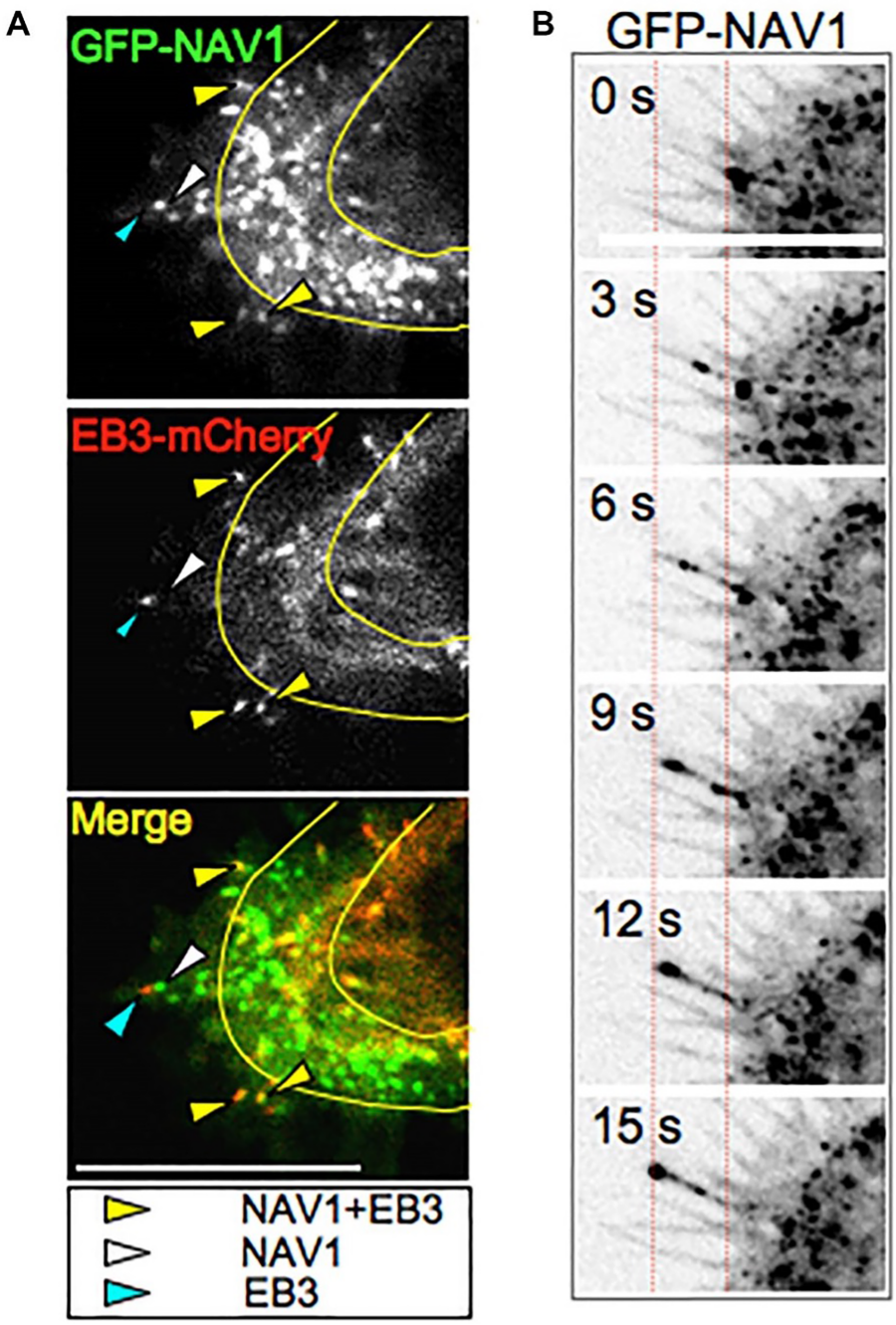
Powers *et al*.



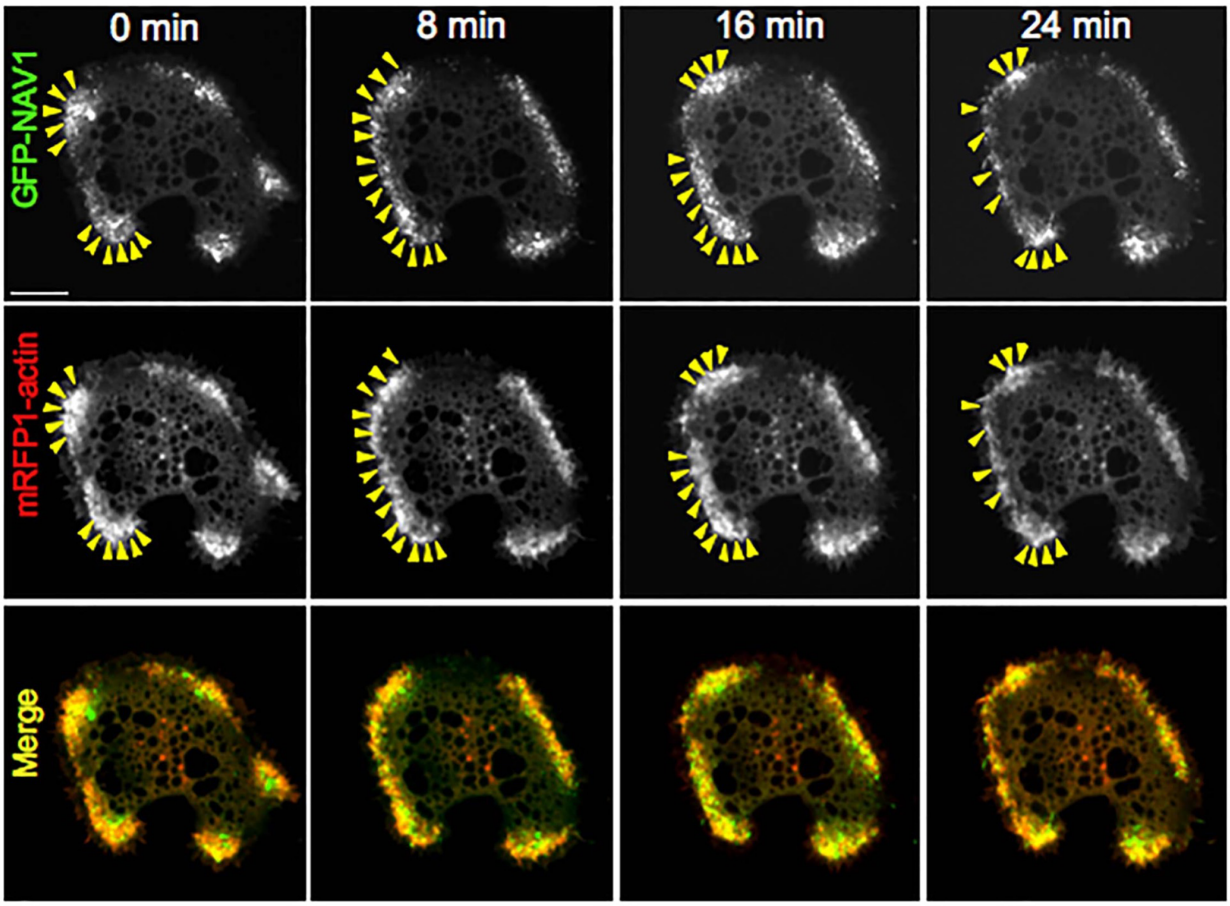
Supplementary Figure 1. GFP-Nav1 subcellular dynamics during neuritogenesis. Selected frames from a time-lapse sequence showing that Nav1 is enriched in subcellular regions (arrowheads) that extend from the cell body and might commit to becoming neurites. Arrowheads show the appearance of these nascent growth cones over neuronal morphogenesis. Cells presented the characteristic filopodial activity and segmentation of the initial lamellipodia surrounding the soma. Such membrane segmentation commonly precedes the selection of a neurite initiation site in cultured hippocampal neurons. Scale bar = 20μm. Images represent observations from 3 individual culture preparations.



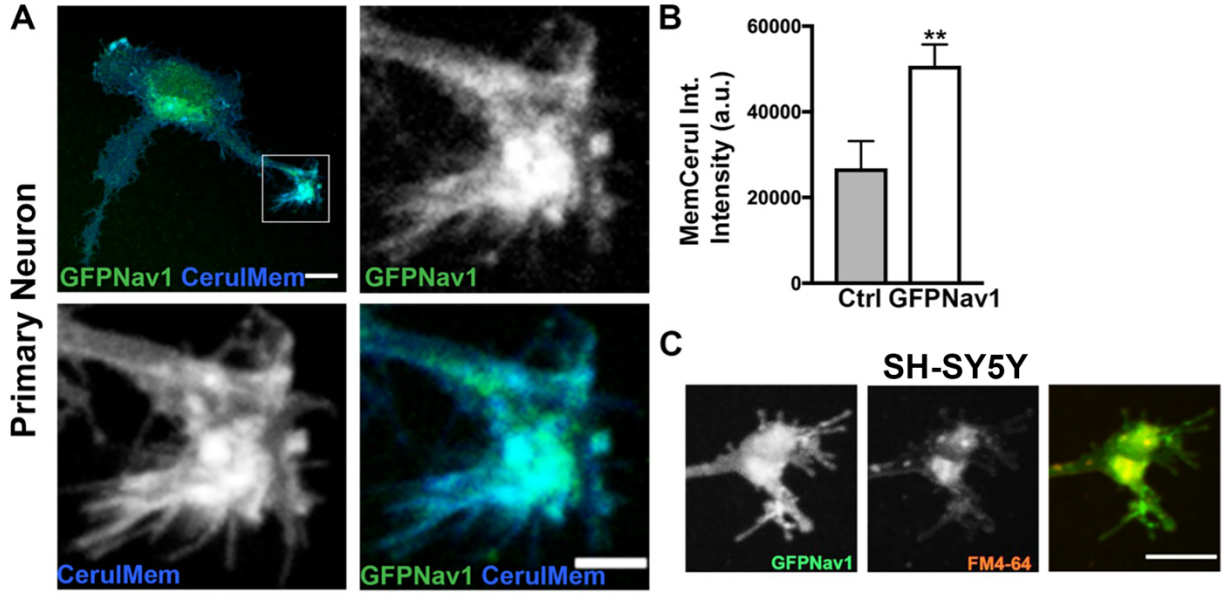
Supplementary Figure 2. Validation of Nav1 gene silencing and antibody specificity. (A) Immunoblot demonstrating the partial knockdown of Nav1 by shRNA in N1E cells. Arrows indicate band at indicated size. (B) Immunoblot of WT and Nav1KO SH-SY5Y cells demonstrating the lack of detectable expression of Nav1 expression in cells where the Nav1 gene was silenced via CRISPR/Cas9 gene editing. Arrows indicate band at indicated size. (C) Representative images of undifferentiated WT and Nav1KO SH-SY5Y demonstrating the lack of detectable Nav1 fluorescence signal in the Nav1KO cells using identical image collection and display settings. (D) Representative images demonstrating Nav1 antibody recognizes only GFPNav1, not GFPNav2 or GFPNav3 that were nucelofected into Nav1KO SH-SY5Y cells. Scale bar = 10μm. Images represent observations from 3 individual culture preparations.



Supplementary Figure 3. Nav1 associates with some EB3 puncta in the growth cone periphery. (A) Representative frame from a time-lapse sequence of a nascent growth cone cotransfected with GFP-Nav1 and EB3-mCherry. Yellow arrowheads indicate colocalized puncta of GFP-Nav1 and EB3-mCherry; white arrowhead indicates a GFP-Nav1 positive motile punctum lacking detectable EB3-mCherry; cyan arrowhead indicates an EB3-mCherry positive punctum lacking detectable GFP-Nav1. Transition zone (T-zone) is highlighted with a yellow line. Scale bar = 10μm (B) Time-lapse sequence of GFP-Nav1 in a nascent growth cone. Red dashed lines indicate the position in the first and last time points of a GFP-Nav1 motile punctum advancing from the T-zone and entering a filopodium in the peripheral domain. Scale bar = 10μm. Images represent observations from 3 individual culture preparations.



Supplementary Figure 4. Nav1 and actin colocalize in stage 1 neurons. Time-lapse sequence from a neuron cotransfected with GFP-Nav1and mRFP1-actin; images were acquired every 8 minutes. Note that mobile clusters (arrowheads) containing GFP-Nav1 and mRFP1-actin reorganize in coordinated fashion. Scale bar = 20μm. Images represent observations from 3 individual culture preparations.



Supplementary Figure 5. GFP-Nav1 promotes endocytosis and membrane accumulation. (A) Representative image of growth cone showing enriched pcs-membrane-cerulean-FP in the same growth cone areas where GFP-Nav1 is enriched. White box indicates growth cone. Scale bars = 10µm for large image, 5µm for zoomed image. (B) GFP-Nav1expressing growth cones have significantly higher intensity of pcs-CeruleanMembrane-FP than control growth cones. Statistics: Mann-Whitney, **p<0.01, n = 4 experiments; Control = 52 growth cones, GFP-Nav1 = 142 growth cones GFP-Nav1. (C) Representative image showing FM4-64 is taken up in GFP-Nav1-expressing WT SH-SY5Y growth cone. Scale bar = 10µm. Images represent observations from 3 individual culture preparations.

Supplementary Table 1. Target and primer sequences

Reagent	Sequence
Nav1 crRNA	GATATGGCCAAGGCGCCCAA
Forward primer for cut site amplification	CCAACCTGCGCAAGCAGAAGTCA
Reverse primer for cut site amplification	CCTGGAAGAGCGAGTGCTCCG
Nav1 shRNA target	GGATCAAGGTTCATGGACA