

SUPPLEMENTARY METHODS

Generation of WAVE1- and WAVE3-specific antibodies. Polyclonal antisera were raised in rabbits against synthetic peptides derived from the murine sequences of WAVE1 and WAVE3 (for sequences see supplementary table 1). Anti peptide antisera were affinity-purified using the respective peptides immobilized on CNBr-Sepharose 4B (Amersham Biosciences). Specificity of the antisera was confirmed by Western Blot detection of the endogenous and ectopically expressed GFP-tagged antigens (see Fig. S2B, C).

cDNA cloning and expression constructs Murine WAVE1 and WAVE3 GFP-tagged constructs were obtained by PCR using IMAGE-clones (accession numbers BC016896 and BC027038) as templates, corrected by site directed mutagenesis according to published WAVE1 and-3 sequences (NM_031877, NM_145155), verified by sequencing and fused into pEGFP-C2 vectors. mRFP-Nap1 was obtained by exchanging EGFP in the EGFP-Nap1 construct for monomeric red fluorescent protein (mRFP; kindly provided by Roger Tsien, Howard Hughes Medical Institute, La Jolla, USA) (Campbell et al., 2002).

Two-colour fluorescence video microscopy and co-translocation analyses.

Separate recordings of differentially tagged proteins in the same cells was facilitated by a computer driven filter-wheel (Ludl electronic products Ltd., Hawthorne, USA). Excitation filters for mRFP and EGFP fluorescence in the filter wheel were used in combination with the corresponding dichroic beamsplitters and emission filters (Chroma Technology Corp., Rockingham, USA). Sequential images were recorded using IPLab 3.1 software and analysed using Scion Image 1.62. Signal intensity scans shown in Figure S3 and supplementary Movie 3 were undertaken from areas as indicated (20 x 110 pixels). Intensities averaged from the region width (20 pixels) were plotted (in percent of intensity maximum within the selected region) against region height (110 pixels). The top of the selected image region corresponded to the left and the bottom to the right of the plot abscissa.