Supplementary Figure 1: **Characterization of bacterially expressed fusion proteins used in biochemical analysis of the N66 F-actin binding domain.** (A) Schematic depictions of fusion proteins. (B). Relative mobility of purified fusion proteins on a 6% SDS-PAGE gels. (C). An F-actin co-sendimentation binding assay was performed with the NusA-9-52 fusion protein. Input and pelleted fractions were separated by SDS-PAGE and stained with Coomassie blue, followed by densitometric analysis. We estimated that 43% of the N9-52 fusion protein is truncated does not appear in the pellet fraction, indicating that it is unable to bind F-actin to appear in the pellet when mixed and centrifuged at 430,000 x g with F-actin.

Supplementary Figure 2: Results obtained from live-cell extraction, under conditions that preserve the cytoskeleton. (A). Various parts of the IPTKA actin binding domain N66 were fused to GFP and expressed in C6 glioma cells (green), fixed, and co-labeled with phalloidin (red) to visualize F-actin (left panels). Detail of these cells is also depicted in Figure 4. When cells expressing the various truncation fusion proteins were extracted in 1% TX-100 in a cytoskeleton stabilization buffer, 1-66 and 9-66 fluorescence was retained on the F-actin (right panels). By contrast, residues 9-52 were completely extracted under this condition, suggesting a low affinity interaction with F-actin (9-52, right). Unexpectedly, residues 15-66 and 25-66, despite their cytosolic localization in non-extracted cells, were retained on the F-actin following extraction in TX-100, suggesting that the polybasic region (residues 52-66) can participate in an interaction with F-actin under some conditions (bottom, right; see also Supplementary Movies 1 and 2). However, full-length N66 containing the L34P mutation was not associated with F-actin following live-cell extraction, indicating that the mere presence of residues 52-66 is insufficient to interact with F-actin if the helical region is destroyed. (B). Semi-quantitative determination of resistance of fusion proteins to detergent extraction in living cells, as decribed in the Materials and Methods. In the top group, C6 glioma cells were transfected with EGFP-tagged fragments from the truncation series depicted in Figure 4. ITPKA was in general less resistant to extraction in neurons, perhaps owing to the greater amount of actin dynamics in these cells. (C) Summary of results from all constructs tested under 2 extraction conditions described in the methods. Residues 9-52 comprised a minimal stretch capable of targeting EGFP to the F-actin cytoskeleton in cells, but appeared to have a low affinity for F-actin because it was not resistant to live cell extraction in the BRB cytoskeletal stabilization buffer. A low concentration of crosslinking fixative in the PEM-GA buffer (0.05% glutaraldehyde) preserved some of this weak interaction during extraction, while reducing the degree of interaction of the 15-66 construct.

Supplementary Movie 1: **Time-lapse movie of a C6 cell expressing the partial F-actin binding domain N25-66-mEGFP.** The protein appears completely cytosolic, with no obvious tendency to associate with F-actin-rich structures. Images were collected at 6 frames/ min for 11.5 min (timestamp). MP4 file format.

Supplementary Movie 2: Time-lapse movie of a C6 cell expressing the partial F-actin binding domain N25-66 during extraction of live cells in 1% TX-100. Following extraction, N25-66-mEGFP rapidly translocates to F-actin between the time the detergent is added (seen as a brief distortion in the movie) and the next frame collected. Also note the tendency for some of the GFP fusion protein to escape its source cells and bind to F-actin in neighboring untransfected cells. Images were collected at 12 frames/min for 3.25 min (timestamp). MP4 file format.

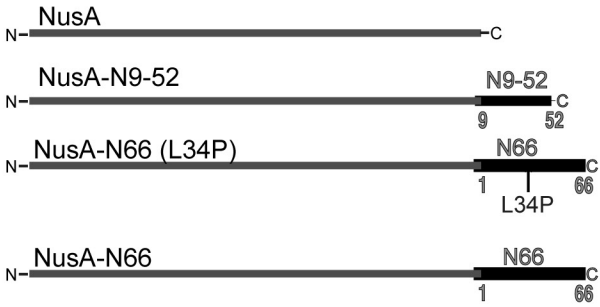
Supplementary Movie 3: Representative FRAP experiment. C6 glioma cells expressing N66-mEGFP were treated with the actin stabilizer jasplakinolide before confocal live cell imaging. A peripheral region of one cell is shown, in which a small rectangular region on a stress fiber was bleached and recovery in the region monitored. The movie depicts 49 seconds of recovery (timestamp). MP4 format.

Supplementary Movie 4: Time-lapse movie of a hippocampal neuron (18 DIV) expressing the ITPKA full actin binding/bundling domain fused to mEGFP. Images were collected at a rate of 3 frames/minute for 47 minutes (timestamp). MP4 format.

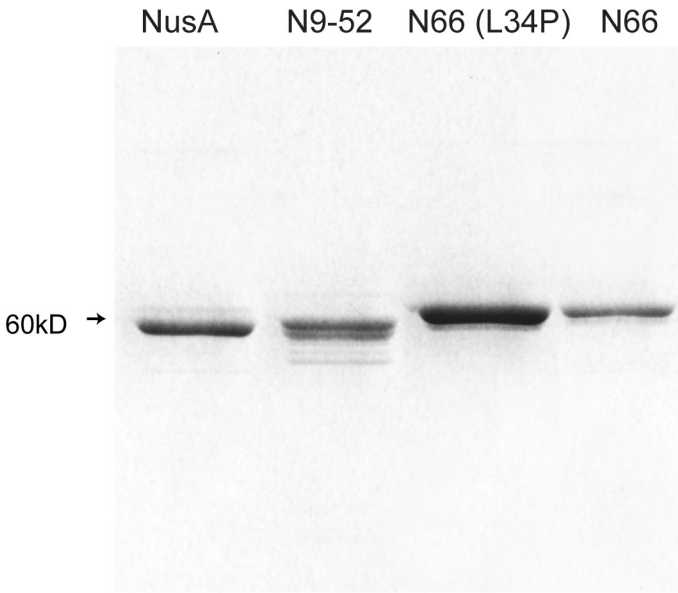
Supplementary Movie 5: Time-lapse movie of a hippocampal neuron (18 DIV) expressing the minimal ITPKA F-actin binding domain (N9-52) fused to mEGFP. Images were collected at a rate of 3 frames/minute for 47 minutes (timestamp). Arrows in the first frame of the movie demark areas on presumed axons (thin caliber processes), which displayed motile "flares" during time-lapse. MP4 format.

Johnson & Schell, Supplementary Figure 1.

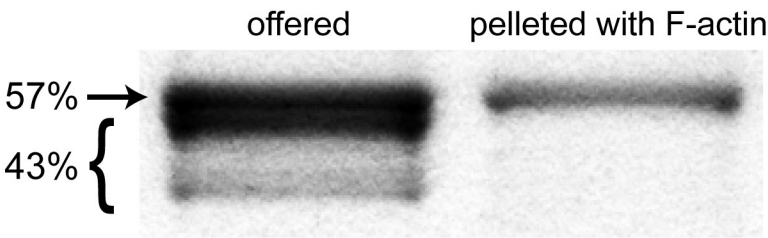
A. Diagrams of fusion proteins



B. Electrophoretic properties of purified fusion proteins

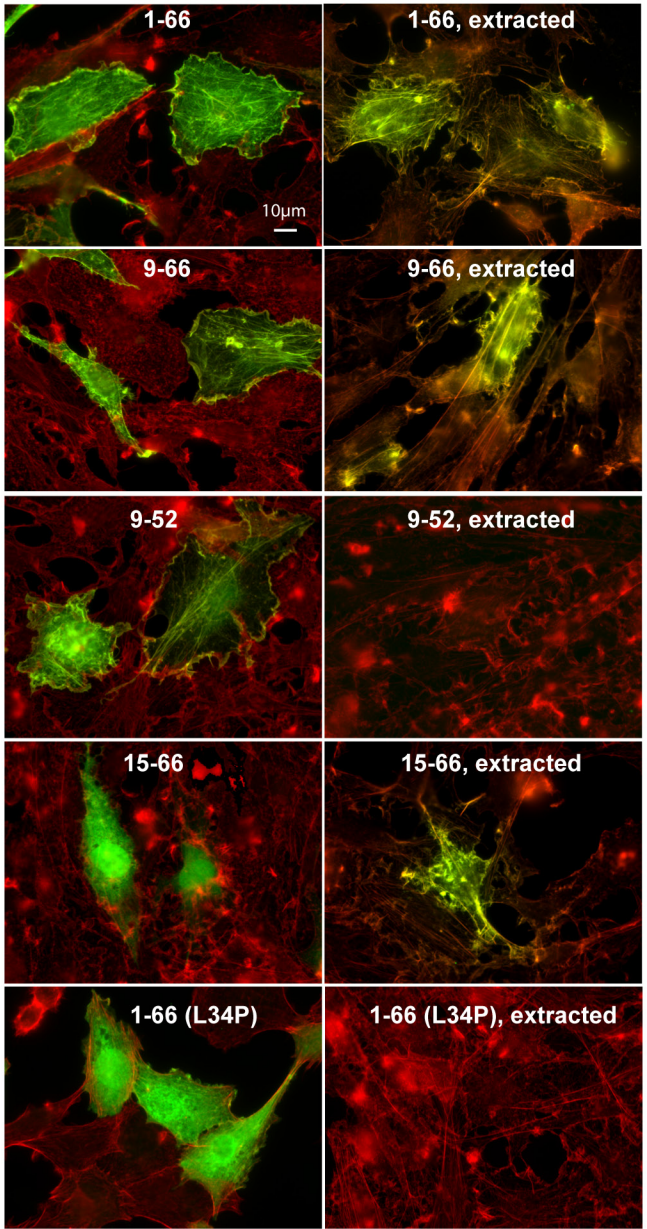


C. 43% of purified NusA-N9-52 is truncated, and unable to bind F-actin



Johnson & Schell, Supplementary Figure 2

A.



B.

Cells, construct	Extracted/non-extracted (# cells/coverslip)	% TX-100 resistant
C6, N66-mEGFP	4020/4770	84%
C6, N9-66-mEGFP	1589/3116	51
C6, N25-66, mEGFP	1415/3307	43
C6, N15-66, mEGFP	1208/3482	35
C6, N9-52, mEGFP	33/3180	1
C6, mEGFP	0/3519	0
Neurons, N66-mEGFP	156/278	56%
Neurons, ITPKA-EGFP	74/237	31
Neurons, ITPKA-mEGFP	21/73	29
Neurons, N66(L34P)	0/162	0

C.

Construct	*Localized w/ phalloidin?*	*Extracted BRB?*	*Extracted PEM-GA?*
mEGFP	*no*	*yes*	*yes*
1-66-mEGFP	*yes*	*no*	*no*
1-66 (L34P)-mEGFP	*no*	*yes*	*yes*
9-66-mEGFP	*yes*	*no*	*no*
15-66-mEGFP	*no*	*no*	*no*
25-66-mEGFP	*no*	*no*	*yes*
30-66-mEGFP	*no*	*yes*	*not tested*
33-66-mEGFP	*no*	*yes*	*not tested*
1-52-mEGFP	*yes*	*yes*	*yes*
9-52-mEGFP	*yes*	*yes*	*no*
1-33-mEGFP	*no*	*yes*	*not tested*
ITPKA-mEGFP	*yes*	*no*	*no*

Summary of resistance to TX-100 extraction:

N66 > N15-66 > N25-66 > N9-52 >> N66(L34P) = EGFP