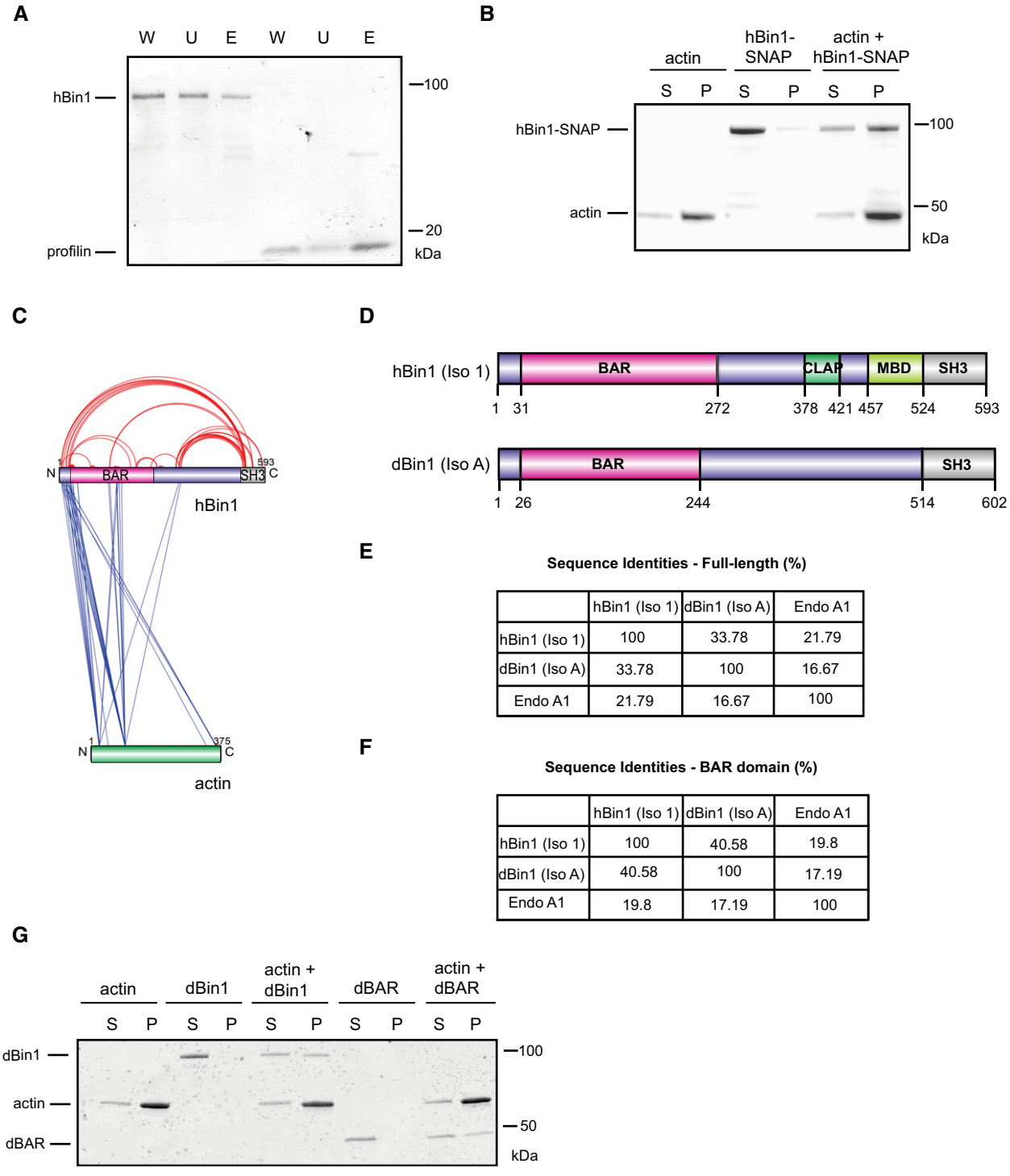
Expanded View Figures



	hBin1 (Iso 1)	dBin1 (Iso A)	Endo A1
hBin1 (Iso 1)	100	33.78	21.79
dBin1 (Iso A)	33.78	100	16.67
Endo A1	21.79	16.67	100

	hBin1 (Iso 1)	dBin1 (Iso A)	Endo A1
hBin1 (Iso 1)	100	40.58	19.8
dBin1 (Iso A)	40.58	100	17.19
Endo A1	19.8	17.19	100

Figure EV1.

Figure EV1. Conserved binding between BAR domain and F-actin within the Bin1 family.

A hBin1 isoform 1 binds slightly to G-actin. G-actin-coupled Sepharose beads were incubated with hBin1 and profilin (both 1.25 μM) and incubated for 1 h at RT before separating them from unbound protein via centrifugation at 6,000 *g* for 4 min. Fractions were resolved by SDS–PAGE followed by Coomassie Blue staining. W, whole input fraction; U, unbound fraction; E, eluted fraction.

B hBin1-SNAP (isoform 1; 1 μM) binds directly to 4 μM F-actin. Co-sedimentation assay resolved by SDS–PAGE followed by Coomassie Blue staining.

C Xvis-visualization of crosslink sites identified for the complex of hBin1 (isoform 1) with F-actin. Only the crosslinks with a score of 80 and higher are displayed. Intra-molecular crosslinks are indicated in red, whereas inter-molecular crosslinks are indicated in blue.

D Domain overviews of hBin1 (isoform 1) and the *Drosophila* orthologue of Bin1 isoform A [dBin1 (isoform A)]. Both isoforms share the N-terminal BAR domain and the C-terminal SH3 domain, but differ in the middle domains.

E Sequence identities of the full-length proteins hBin1 (isoform 1), dBin1 (isoform A), and the N-BAR family member endophilin A1 (Endo A1) analyzed with Clustal omega. Gaps in sequences were not considered.

F Sequence identities of the BAR domains including the amphipathic helix of hBin1 (isoform 1), dBin1 (isoform A), and Endo A1 analyzed with Clustal omega. Gaps in sequences were not considered.

G dBin1 (isoform A) and its BAR domain (dBAR; both 1 μM) bind directly to 4 μM F-actin. Co-sedimentation assay resolved by SDS–PAGE followed by Coomassie Blue staining.

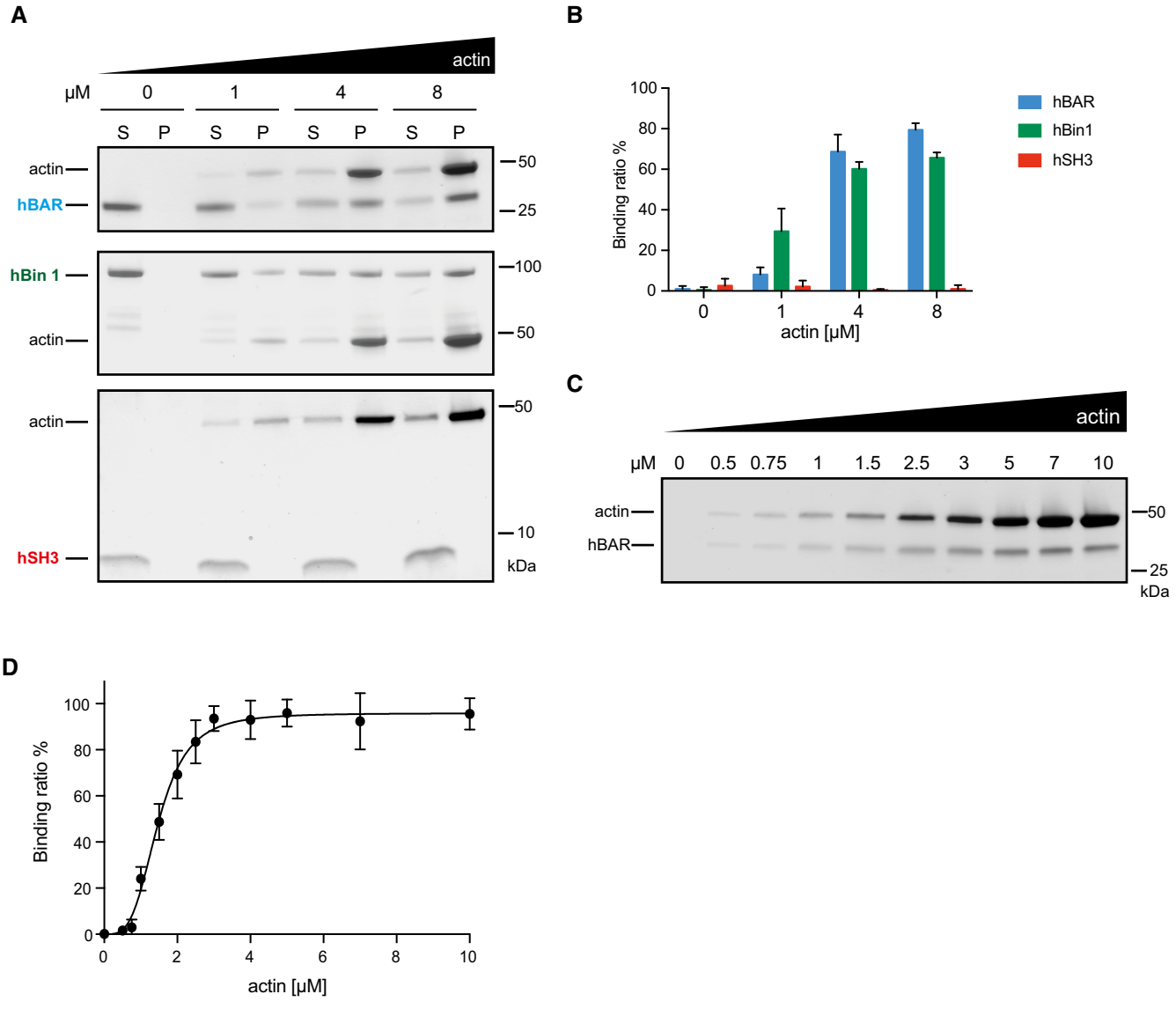


Figure EV2.

Figure EV2. Comparison of binding abilities of hBin1, hBAR, and hSH3.

A Effect of increasing amounts of F-actin on co-sedimentation of hBAR, hBin1, and hSH3 (all 2 μM). Different Bin1 proteins were mixed with F-actin at indicated concentrations before subjecting the samples to high-speed sedimentation. SDS–PAGE of supernatant and pellet fraction visualized by Coomassie Blue staining is shown.

B Densitometric quantitation of hBAR, hBin1, and hSH3 found in the pellet fractions from (A). The error bars represent mean ± SD (n = 3).

C Effects of increasing amounts of F-actin on co-sedimentation of hBAR. F-actin at indicated concentrations was incubated with 1 μM hBAR and then subjected to a co-sedimentation assay. SDS–PAGE followed by a Coomassie Blue staining of the proteins found in the pellet fraction is shown.

D Binding of hBAR to F-actin. Data represent mean values of three independent experiments similar to (C). Calculated K_d of binding: 1.47 ± 0.05 μM. Error bars represent ± SD.

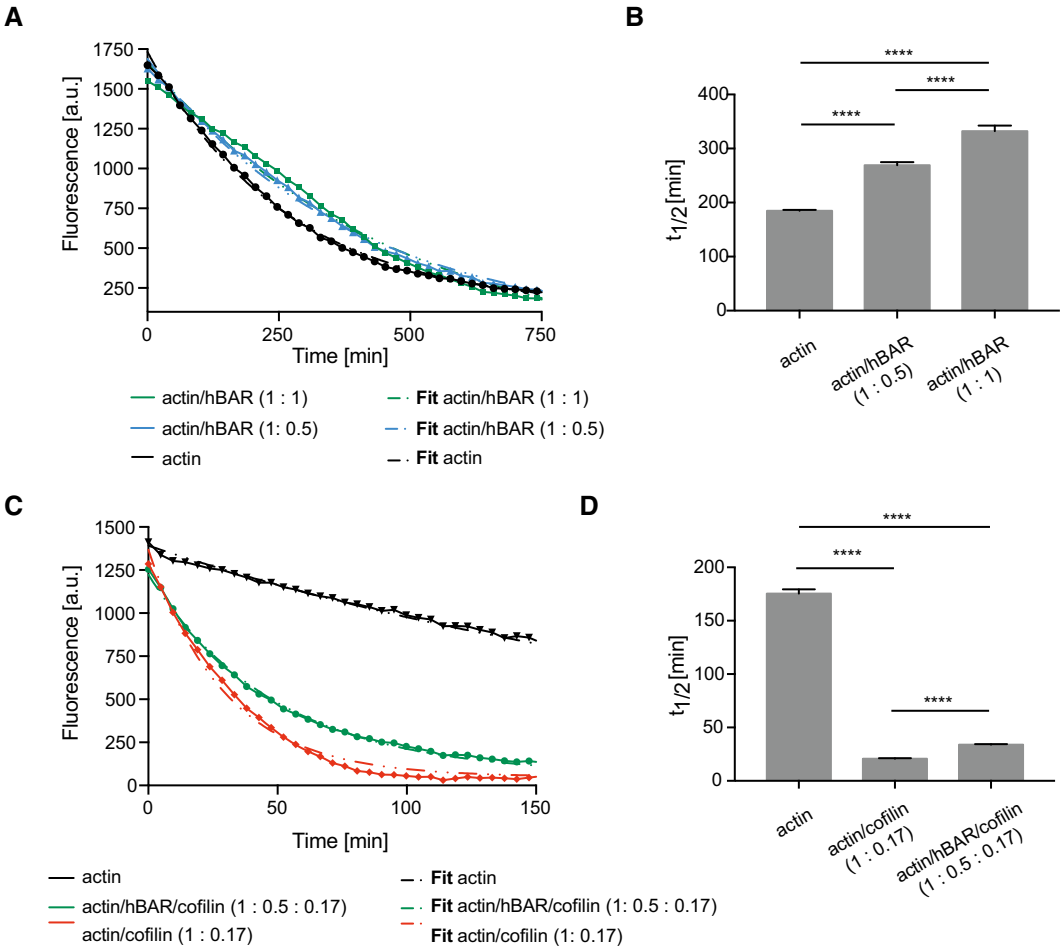


Figure EV3. hBAR stabilizes F-actin.

A Effect of hBAR on the rate of actin depolymerization. Dilution-induced depolymerization of pyrene-labeled actin in the presence or absence of hBAR at indicated molar ratios was measured following pyrene fluorescence intensity over time. Dotted lines indicate the one-phase exponential decay fitting.

B Calculated half-time of actin depolymerization from (A) after one-phase exponential decay fitting. Statistically significant differences are indicated (one-way ANOVA, ****P < 0.0001, n = 3). The error bars represent mean ± SD.

C Effects of cofilin and hBAR on dilution-induced depolymerization of F-actin. Spontaneous disassembly of pyrene-labeled F-actin in the presence or absence of hBin1 (isoform 1) and cofilin was followed, as measured by pyrene fluorescence. Dotted lines indicate the one-phase exponential decay fitting.

D Calculated half-time of actin depolymerization from (C) after one-phase exponential decay fitting. Statistically significant differences are indicated (one-way ANOVA, ****P < 0.0001, n = 3). The error bars represent mean ± SD.

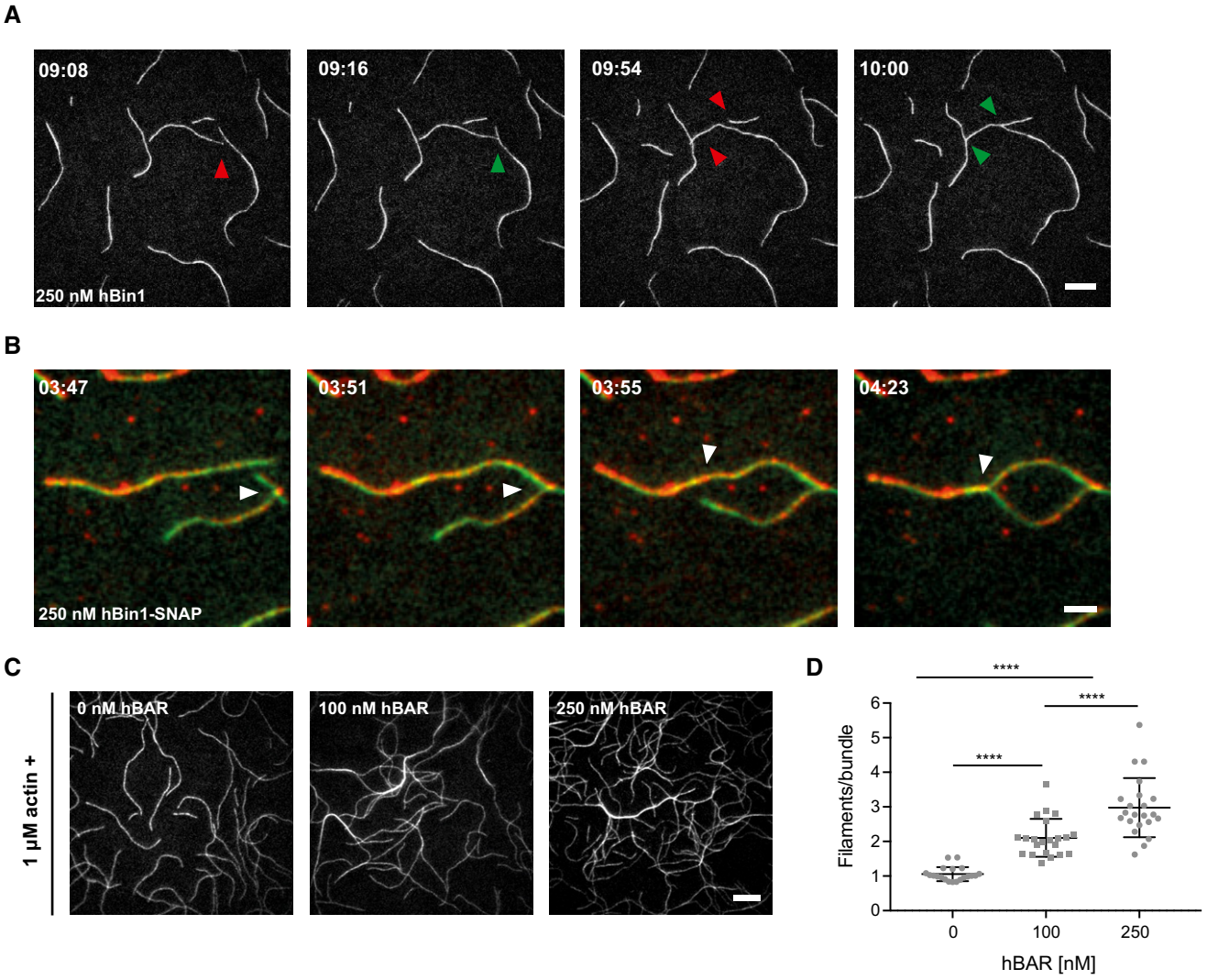


Figure EV4. hBin1, hBin1-SNAP, and hBAR induce actin bundling.

A Time course analysis visualizes hBin1 (isoform 1) induced actin bundling events visualized by TIRFM. Time is in minutes and seconds. Conditions are the same as in Fig 4A, hBin1 (isoform 1) = 250 nM. Red arrowheads indicate two filaments just before fusion and green arrowheads the filaments after the bundling event. Scale bar = 10 µm.

B Time course analysis visualizes hBin1-SNAP594 (red) associating with actin filaments (green) and inducing actin bundling events visualized by dual-color TIRFM. Time is in minutes and seconds. Conditions are the same as in Fig 4A, hBin1 (isoform 1) = 250 nM. White arrowheads indicate two filaments just before and after fusion. Scale bar = 4 µm.

C Concentration-dependent actin bundling induced by hBAR. 1 µM actin (10% ATTO-488 labeled) was polymerized for 20 min in the presence or absence of different concentrations of hBAR. Scale bar = 10 µm.

D Number of filaments/bundle formed in the presence of different hBAR concentrations. For each condition, 25 bundles were analyzed using fluorescence intensity measurements. Statistically significant differences are indicated (one-way ANOVA, ****$P < 0.0001$). The error bars represent mean ± SD.

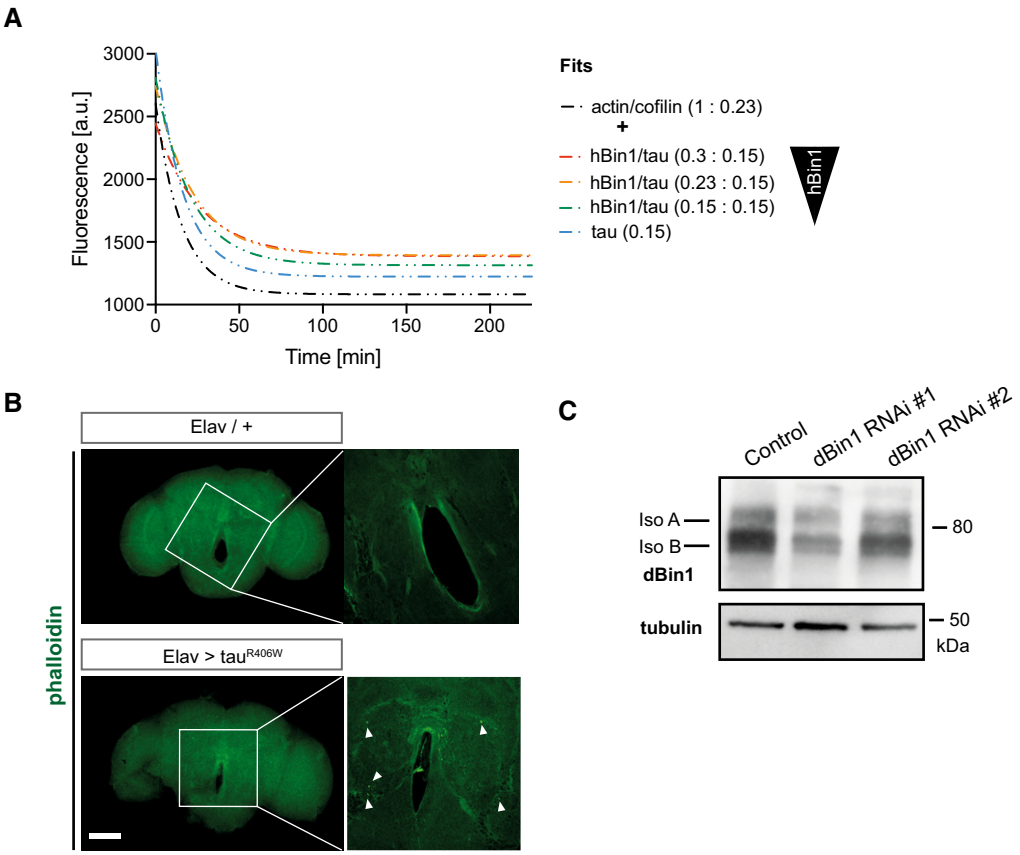


Figure EV5. Biochemical validation of dBin1 downregulation in fly brains.

A Exponential fitting of depolymerization of tau-induced actin bundles. Actin depolymerization data from Fig 5G were fit to a single exponential decay. The displayed fits are the basis for the half-time calculation from Fig 5H.

B Phalloidin staining of whole-mount brain (22 days old) dissected from tau^{R406W} transgenic flies and control flies. The zoom-in images represent one single section in the middle of the central body of the fly brain, and the white arrows highlight actin rods. Scale bar of brain overview: 100 μm and zoom-in: 25 μm.

C Knockdown efficiency of different dBin1 RNAi lines. The dBin1 lines were expressed pan-neuronally under the Elav promoter, and fly heads were subjected to protein extraction 10 days post-eclosion. Samples were resolved by SDS–PAGE and immunoblotted.