SUPPLEMENTARY DATA:

Fig. S1. Analytical gelfiltration analysis of the interaction of Spir-2-KIND and the eFSI peptide and interaction analysis of Spir-KIND domains with formin-1-FH2 constructs lacking the FSI. Also the KIND domain of Spir-2 binds to the very C-terminus of formin-2 (eFSI), indicating a conserved binding site in the KIND domains of Spir-1 and Spir-2 (A). A formin-1-FH2 protein that lacks the FSI (formin-1-FH2) does neither bind to Spir-1-KIND (B) nor Spir-2-KIND (C), showing the necessity of intact FSI of Fmn-subfamily members. Black elution curve: Spir; red elution curve: formin; green elution curve: Spir and formin. Colors of the peak-numbering and elution-profiles correspond. Masses of molecular weight standards: $Mw_{Cytochrome\ C}$*: 12,4 kDa;* $Mw_{Carbonic\ Anhydrase}$*: 29 kDa;* Mw_{BSA}*: 66 kDa;* $Mw_{Alcohol\ Dehydrogenase}$*: 150 kDa;* $Mw_{b\text{-}Amylase}$*: 200kDa.* Observed molecular masses for the Spir- / formin proteins and complexes can be found in the table 1 (Tab. 1); mAU: milli absorption units, Ve/Vo: elution volume/void volume.

Fig. S2. Fluorescence anisotropy/polarization measurements probing the interaction of the KIND domain of Spir-2 with the extreme C-terminus of formin-2. (A) Spir-2-KIND binds with high affinity to the BodipyFl-labelled extended FSI-peptide of formin-2 (Fmn-2-eFSI). The affinity drops by a factor of ~ 2 when shortening the peptide to the core FSI-sequence (Fmn-2-FSI) (B). The affinity of Spir-2-KIND to the FSI-peptides is comparable to that of Spir-1-KIND (Tab. 2). ΔP: change in polarization; red boxes mark the BodipyFl-labelled proteins.

Fig. S3. Laemmli basic native PAGE analysis of the Spir-2-KIND/Fmn-2-eFSI (A) and Spir-2-KIND/Fmn-2-FSI interaction (B). 20 µM Spir-2-KIND was mixed with the eFSI peptide (0 and 60 µM) (A) or increasing amounts of FSI-peptide (0, 10, 20, …, 60 µM) (B) and incubated 20 minutes prior to native PAGE. 2.4 µg of Spir-2-KIND / lane were applied to the gels. The band pattern of Spir-2-KIND is not as pronounced as for Spir-1-KIND in absence of the formin-2-peptides. However with increasing Fmn peptide concentrations a novel prominent band appears, apparently presenting the complex (*). The multiple bands at higher molecular masses do not correspond to oligomers or impurities as judged by analytical gelfiltration and SDS-PAGE (C, C inset).

Fig. S4. SDS-PAGE showing the purity of formin and Spir constructs used in the study.

Fig. S5. Competition fluorescence polarization/anisotropy measurements probing the specificity of the Spir-KIND/Fmn2-eFSI interaction. Binding of the BodipyFl labelled Fmn-2-eFSI peptide to the Spir-1-KIND domain could efficiently be competed by addition of excess unlabeled eFSI peptide. Approximately 100 nM BodipyFL labelled eFSI peptide and 500 nM Spir-1-KIND were titrated with the indicated amounts of unlabelled eFSI peptide. The titration curve was fitted according to Vinson *et al*. (1998). As the equation is only an approximation one should consider the obtained K_d-value also as an approximation.

Fig. S6. Colocalization of Spir and eGFP proteins. Hela cells that transiently coexpress myc-tagged Spir-1 (Myc-Spir-1) or Spir-2 (Myc-Spir-2) with enhanced green fluorescent protein (eGFP) have been immunostained with a myc-epitope specific antibody and a TRITC-conjugated secondary antibody (red). The localization of proteins has been analyzed by fluorescence microscopy. The red immuno-fluorescence of the Myc-tagged proteins and the green autofluorescence of the eGFP proteins are shown. In addition an overlay (merge) of the two fluorescence channels is shown. Images are deconvoluted (blind) and further processed by Adobe Photoshop CS.

Table S1. Overview of Spir- and formin-constructs and their purpose in this study.

Construct	**Description**	**Fragment boundaries (Restriction sites)**	**Purification**	**Purpose**
pGex4T1-NTEV-Spir-1-KIND short	GST-Spir-1-KIND short	aa 35-236 (BamHI/XhoI)	GSH-Sepharose	GST-Pull-Down
pGex4T1-NTEV-Spir-2-KIND short	GST-Spir2-KIND short	aa 18-207 (BamHI/XhoI)	GSH-Sepharose	GST-Pull-Down
pGEX4T1-NTEV-Fmn-2-eFSI	GST-Fmn-2-eFSI / Fmn-2-eFSI	aa 1523-1578 (EcoRI/SalI)	GSH-Seph. FF, TEV, SP-Sepharose XL	Anisotropy / analyt. GF / native PAGE
pGEX4T1-NTEV-Fmn-2-FSI	GST-Fmn-2-FSI / Fmn-2-FSI	aa 1549-1578 (BamHI/SalI)	GSH-Seph. FF, TEV, SP-Sephaose XL	Anisotropy / analyt. GF / native PAGE
pProExHTb-Spir-1-KIND short	His-Spir-1-KIND short	aa 35-236 (BamHI/XhoI)	Ni-NTA HP, Sephadex G200	Anisotropy / analyt. GF / native PAGE
pProExHTb-Spir-2-KIND short	His-Spir-2-KIND short / Spir-2-KIND short	aa 18-207 (BamHI/XhoI)	Ni-NTA HP, TEV, Ni-NTA, Sephadex G200	Anisotropy / analyt. GF / native PAGE
pProExHTb-Fmn-1-FH2	His-Fmn-1-FH2	aa 757-1162 (BamHI/XhoI)	Ni-NTA HP, Sephadex G200	Anisotropy / analyt. GF
pProExHTb-Fmn-2-FH2	His-Fmn-2-FH2	aa 1134-1547 (BamHI/XhoI)	Ni-NTA HP, Sephadex G200	Anisotropy / analyt. GF
pQE80L-Fmn-2-FH2-FSI	His-formin-2-FH2-FSI	aa 1134-1578 (BamHI/KpnI)	Ni-NTA HP, Sephadex G200	Anisotropy / analyt. GF
pAcGFP-Fmn-2-eFSI	mGFP-formin-2-eFSI	aa 1523-1578 (BamHI (BglII) / SalI)	-	Fluorescence microscopy
pEGFP-C1-Fmn-1-FH2-FSI	eGFP-formin-1-FH2-FSI	aa 747-1204 (EcoRI / ApaI)	-	GST-Pull-Down / Fluorescence microscopy
pEGFP-C1-Fmn-2-FH2-FSI	eGFP-formin-2-FH2-FSI	aa 1134-1578 (KpnI / XbaI)	-	GST-Pull-Down / Fluorescence microscopy
pcDNA3-myc-Spir-1	myc-Spir-1 f.l.	aa 2-742 (BamHI / XhoI)	-	GST-Pull-Down / Fluorescence microscopy
pcDNA3-myc-Spir-2	myc-Spir2 f.l.	aa 2-714 (BamHI / NotI)	-	GST-Pull-Down / Fluorescence microscopy

Figure S1

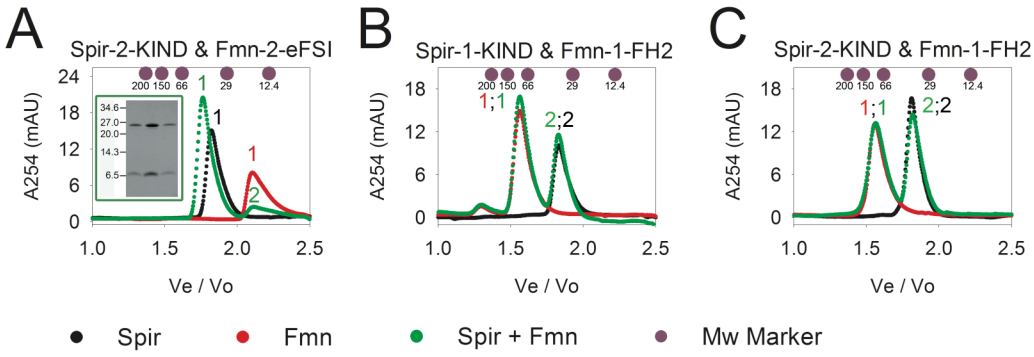

Figure S2

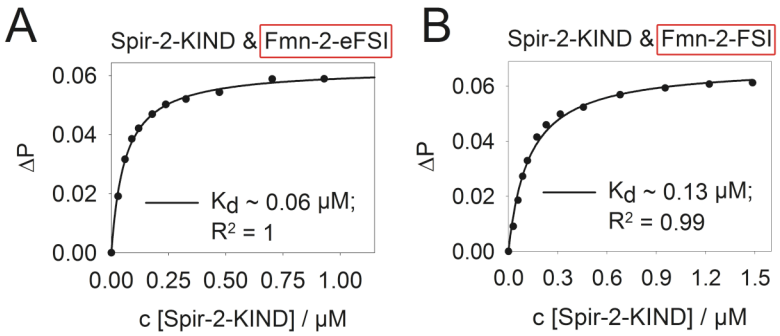

Figure S3

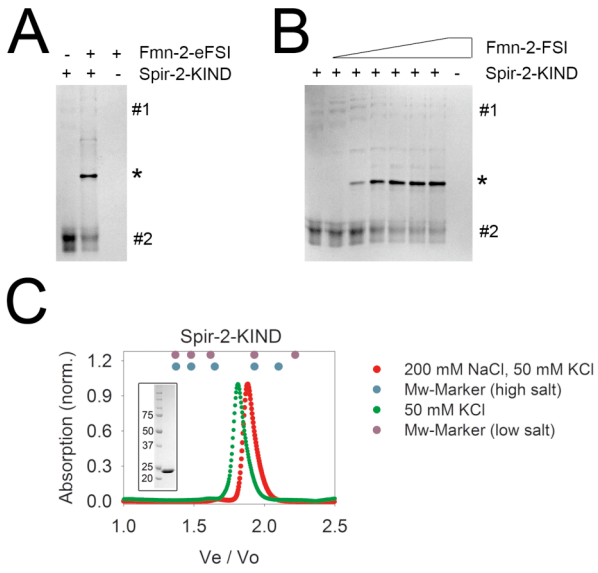

Figure S4

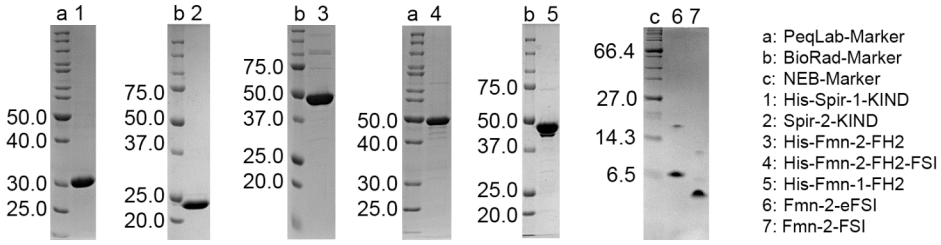

Figure S5

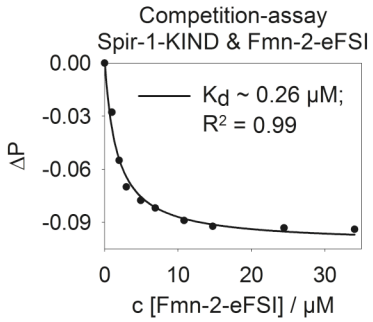

Figure S6