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Supplemental Material to:

Bettina Maier, Michael Kirsch, Simon Anderhub, Hanswalter Zentgraf and Alwin Krämer

The novel actin/focal adhesion-associated protein FASP is involved in mitotic spindle positioning in human cells

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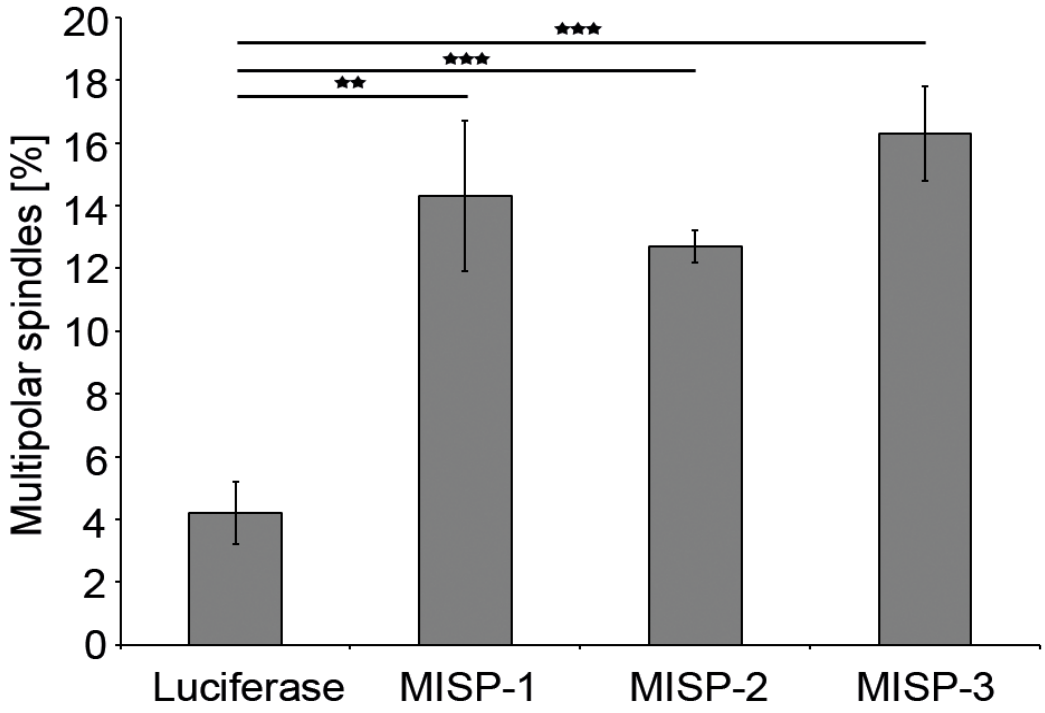

Figure S1. Depletion of MISP by different siRNAs induces multipolar mitotic spindles. RNAi-mediated depletion of MISP by three different MISP-siRNAs (MISP-1, MISP-1, MISP-3) significantly increases the amount of UPCI:SCC114 cells with multipolar mitotic spindles as compared to cells transfected with a siRNA against luciferase. Cells were treated with luciferase- or MISP-siRNAs for 48 h and immunostained with an antibody to α-tubulin. The graph shows the average of three independent experiments; mean ± SD.

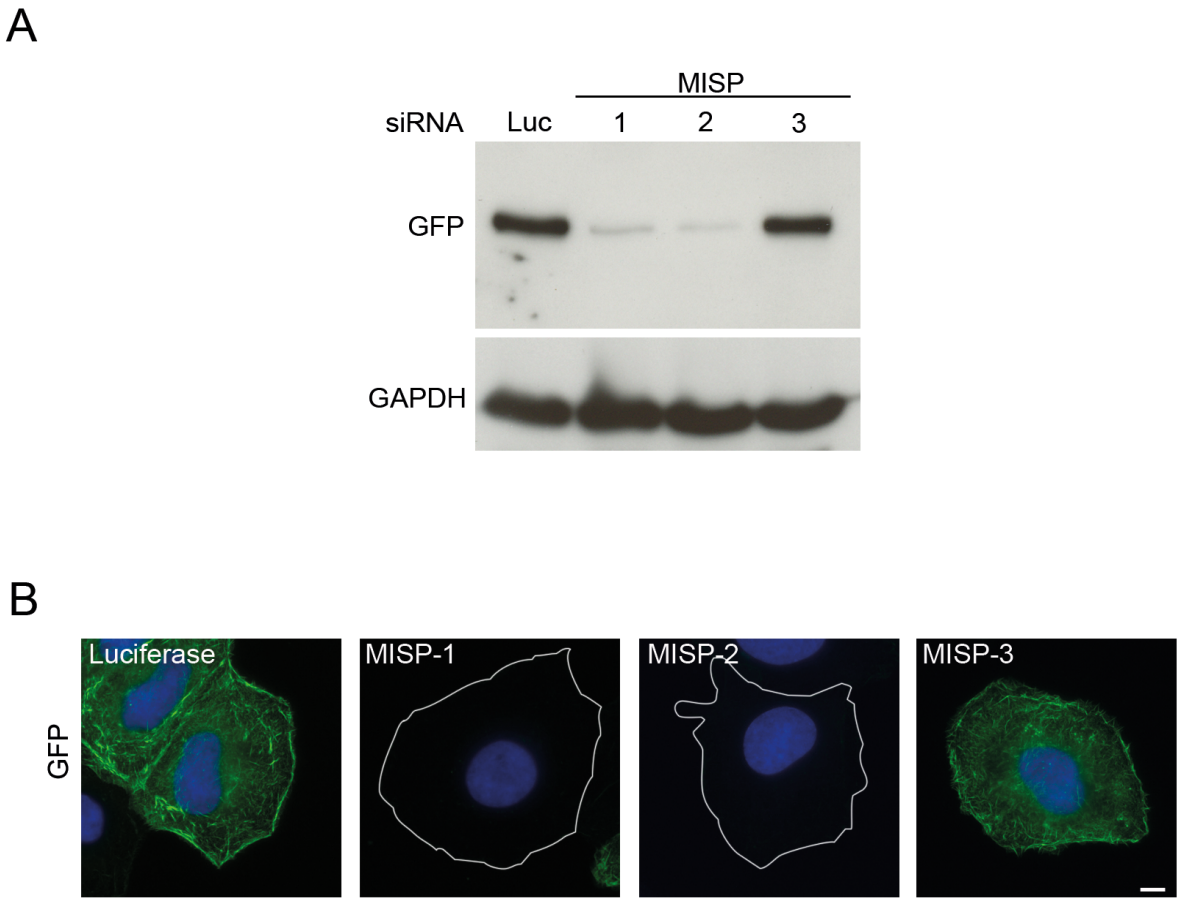

Figure S2. MISP-3 siRNA does not target exogenously overexpressed GFP-MISP. (A) Lysates of GFP-MISP-U2OS cells immunoblotted with an antibody to MISP (C118). GFP-MISP expression is clearly reduced by siRNAs targeting the coding region of MISP (MISP-1 and MISP-2) but not by a siRNA which is directed against the 3´UTR of MISP (MISP-3). Cells were transfected with indicated siRNAs for 48 h and expression of GFP-MISP was induced 24 h after siRNA-transfection for further 24 h. (B) Fluorescence microscopy of GFP-MISP-U2OS also reveals that GFP-MISP is depleted by siRNAs MISP-1 and MISP-2 but not by siRNA MISP-3. Cells were transfected with indicated siRNAs for 48 h and expression of GFP-MISP was induced 24 h after siRNA-transfection for further 24 h. Cell boundaries are depicted as white lines. Scale bar, 10 µm.

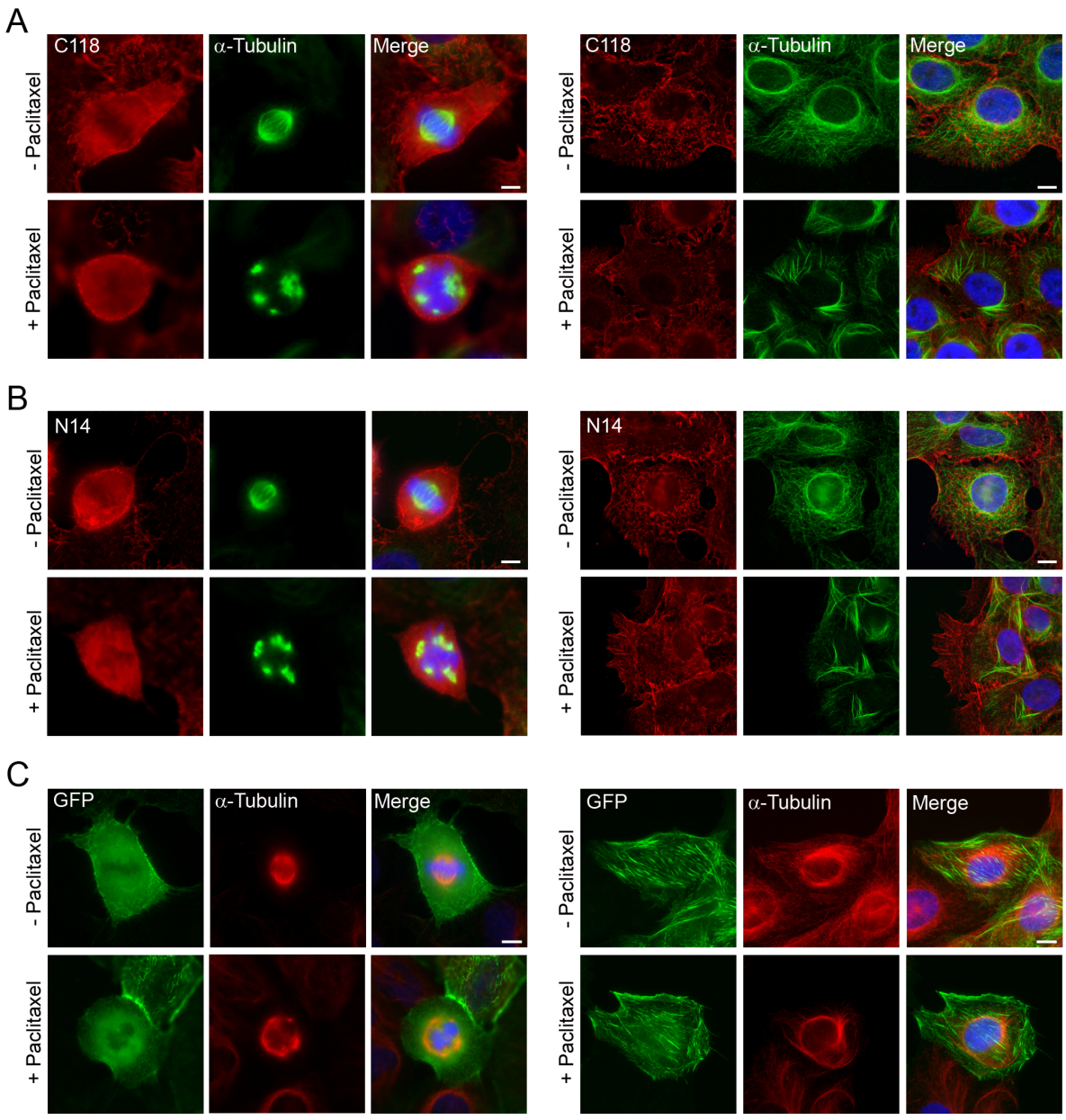

Figure S3. MISP does not localize to microtubules. (A) Co-immunostaining of MISP with antibodies directed against the C-terminus of the protein (C118) and α-tubulin does not reveal a colocalization to mitotic spindles (left panel) or interphase microtubules in UPCI:SC114 cells in the absence (-) or presence (+) of the microtubule-stabilizing agent paclitaxel. Scale bar, 10 μm. (B) Co-immunostaining of MISP with antibodies directed against the N-terminus of the protein (N14) and α-tubulin does not reveal a colocalization of MISP to neither mitotic spindles (left panel) nor interphase microtubules in UPCI:SC114 cells in the absence (-) or presence (+) of paclitaxel as well. Scale bar, 10 μm. (C) GFP-MISP does not colocalize to mitotic spindles (left panel) or interphase microtubules (right panel) in GFP-MISP-U2OS cells in the absence (-) or presence of (+) of paclitaxel. Scale bar, 10 μm.