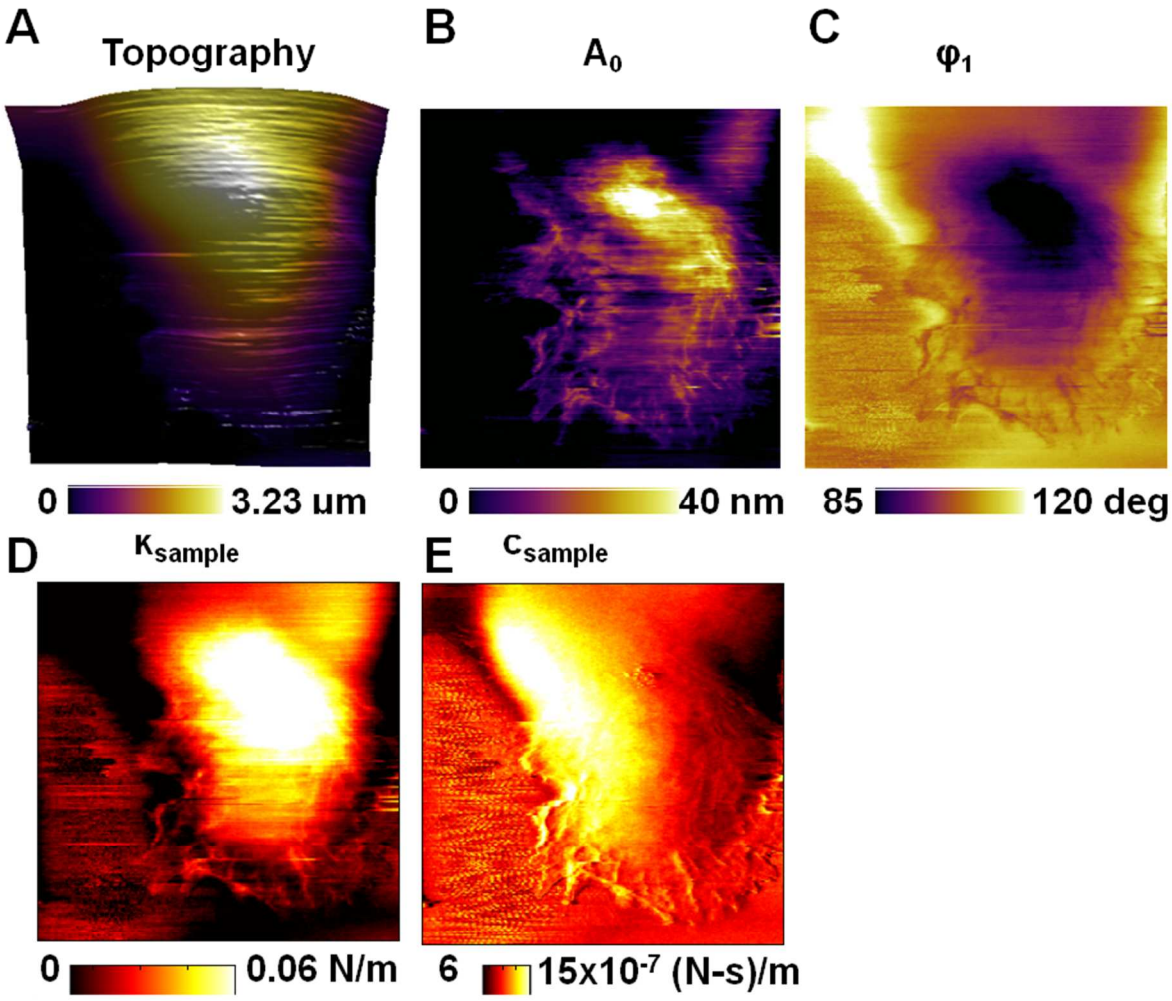
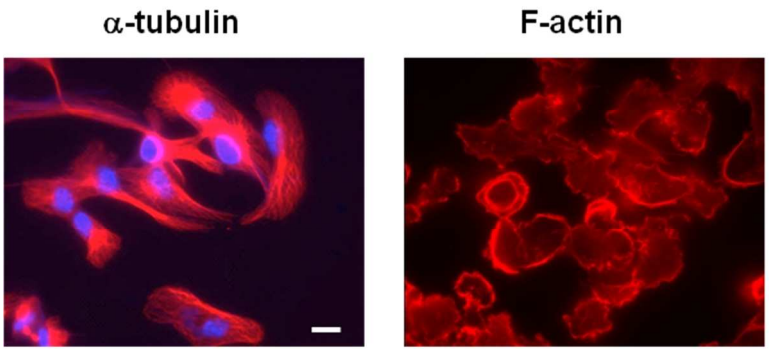
Supporting Information

Nanomechanical property maps of breast cancer cells by multi-harmonic atomic force microscopy reveal Syk-dependent changes in microtubule stability mediated by MAP1B

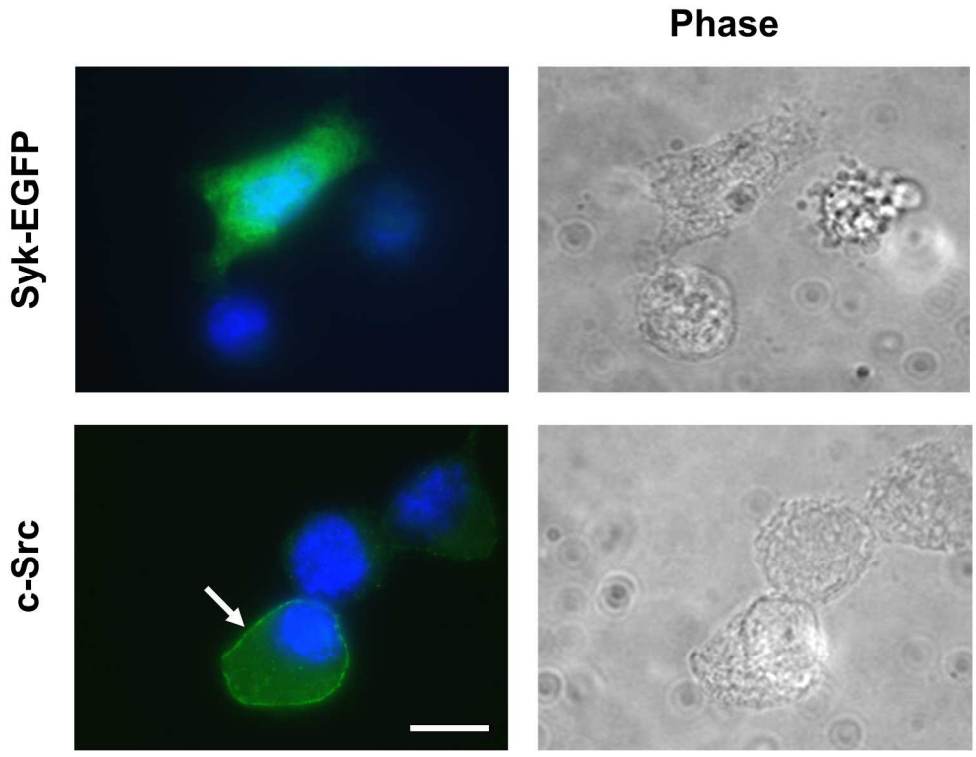
Mariya O. Krisenko, Alexander Cartagena, Arvind Raman, and Robert L. Geahlen



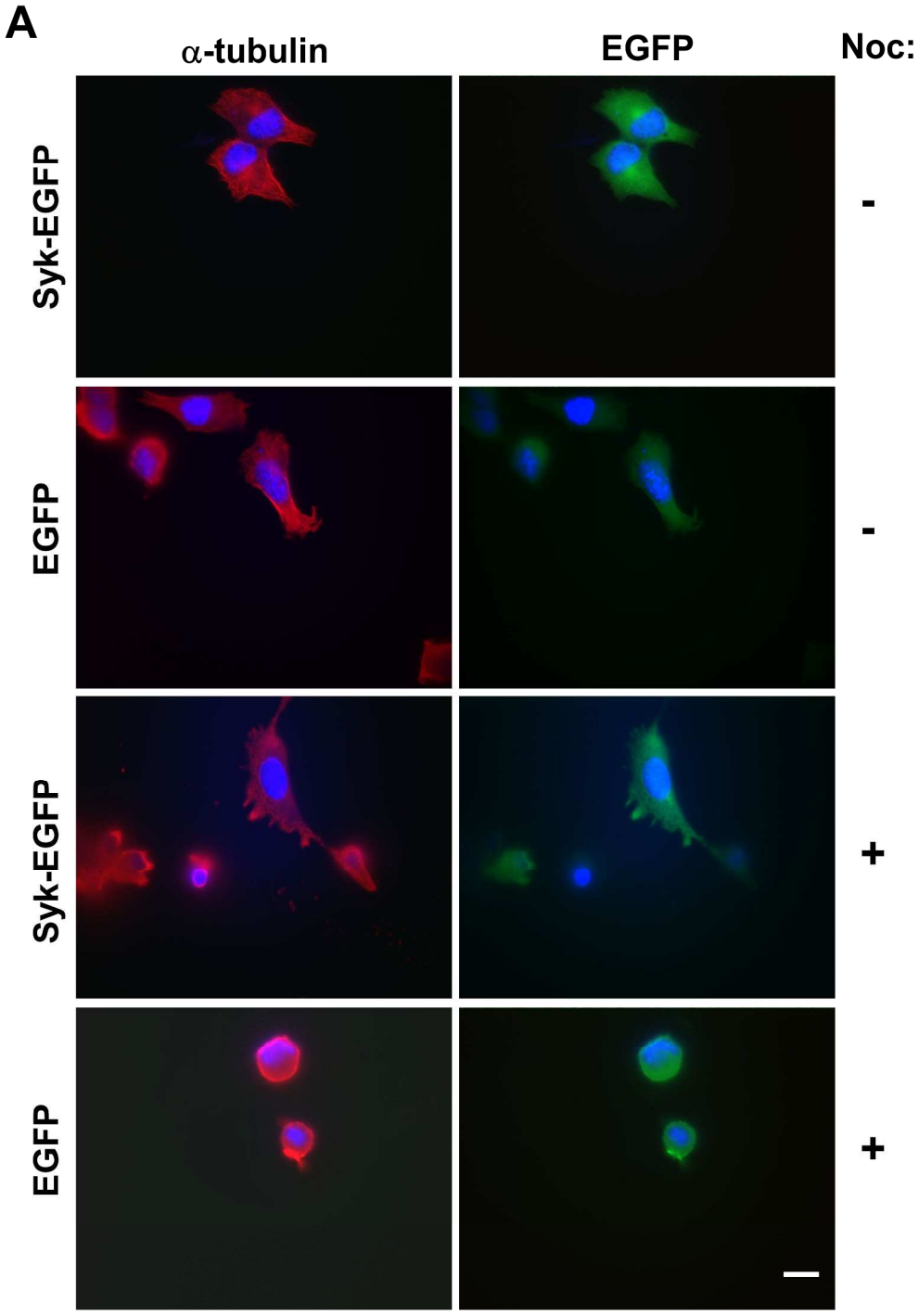
Supplemental Figure S1. High-resolution (256×256 pixels) AM-AFM images showing material compositional contrast in a cell expressing Syk-EGFP. Shown is a topography image (A), compositional contrast images of mean cantilever deflection A_0 (B) and phase lag (ϕ_1) (C), and maps of extracted local nanomechanical properties of dynamic stiffness k_{sample} (D) and damping c_{sample} (E).

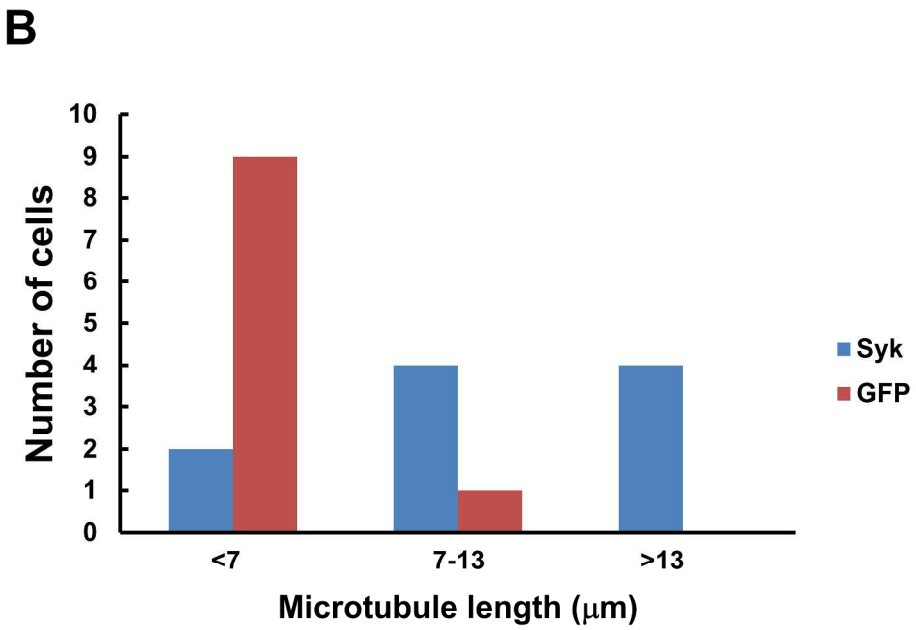


Supplemental Figure S2. Flourescence microscopy of cytoskeletal networks. MDA-MB-231 cells induced to express Syk-EGFP were fixed and stained with antibodies against α-tubulin or with rhodamine-phalloidin to stain F-actin. Bar = 10 μm.

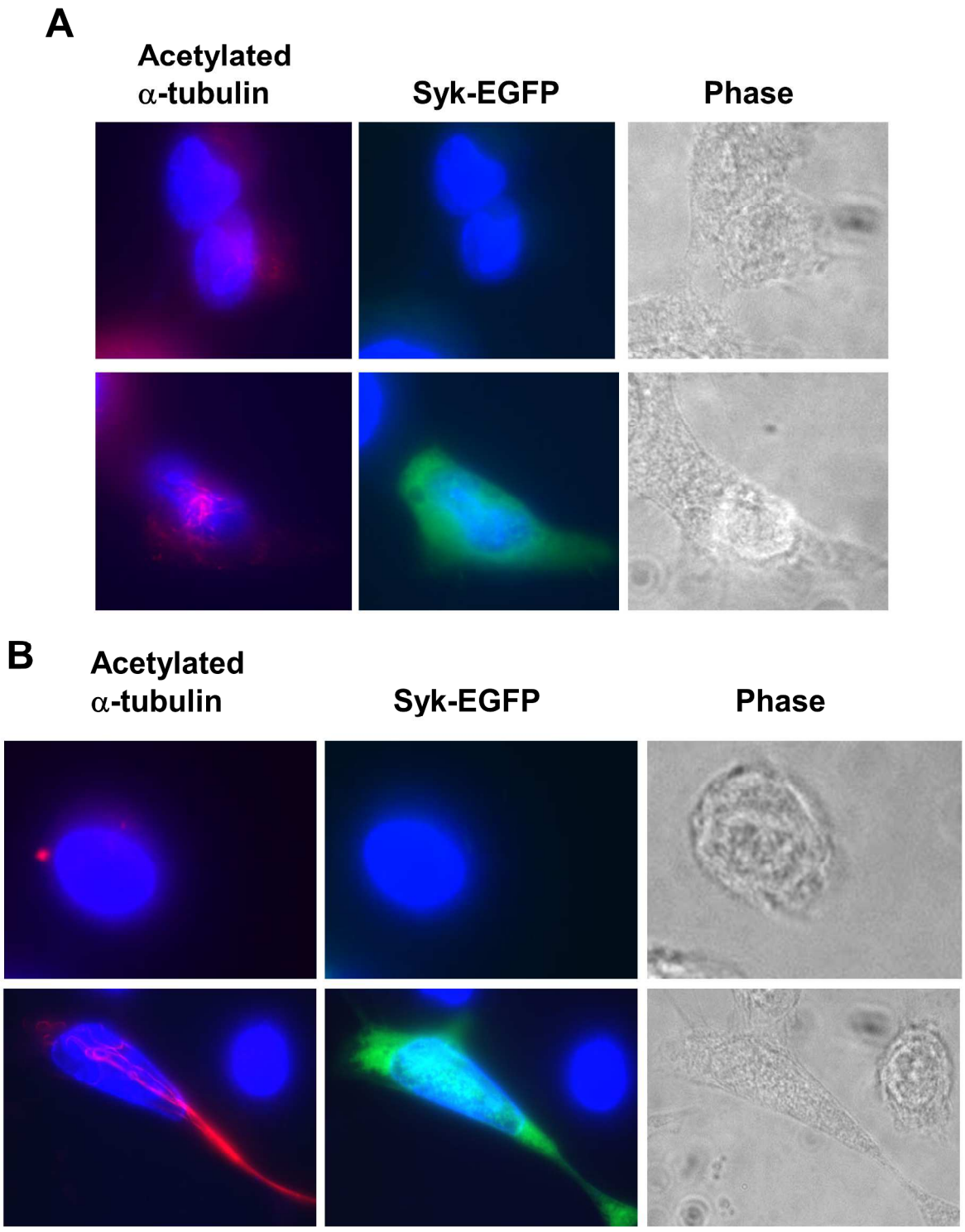


Supplemental Figure S3. Syk, but not c-Src, protects cells from nocodazole-induced rounding. MDA-MB-231 cells were transiently transfected to express Syk-EGFP or c-Src and treated with nocodazole. Syk-EGFP was detected by fluorescence and c-Src by immunofluorescence (green). Nuclei were detected by staining with DAPI (blue). An example of a cell over-expressing c-Src is indicated by the arrow. Bar = 10 μm.


A
α-tubulin
EGFP
Noc:
Syk-EGFP
-
EGFP
-
Syk-EGFP
+
EGFP
+

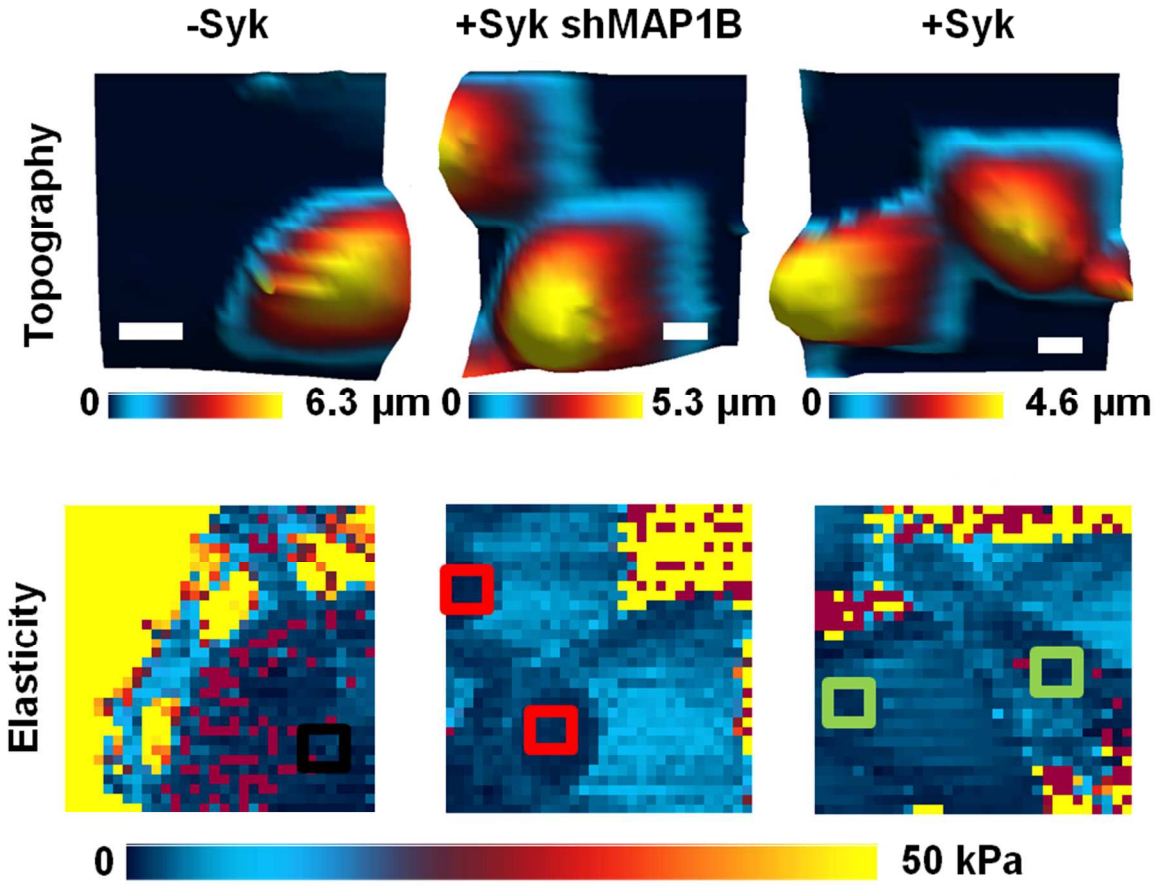


Supplemental Figure S4. Stabilization of microtubules through the expression of Syk in BT549 breast cancer cells. (A) BT549 cells were transiently transfected with expression plasmids for Syk-EGFP or enhanced green fluorescent protein (EGFP) and treated without (-) or with (+) nocodazole. Cells were fixed and stained with an antibody against α-tubulin and a fluorescently tagged secondary antibody, and with DAPI to mark the nucleus. Cells were examined by fluorescence microscopy to detect α-tubulin (red), nuclei (blue) and Syk-EGFP or EGFP (green). Bar = 10 μm. (B) The distance (μm) from the cell nucleus to the cell boundary as marked by α-tubulin fluorescence was measured for cells expressing Syk-EGFP (Syk) or EGFP (GFP). Cells were grouped into three categories as indicated.



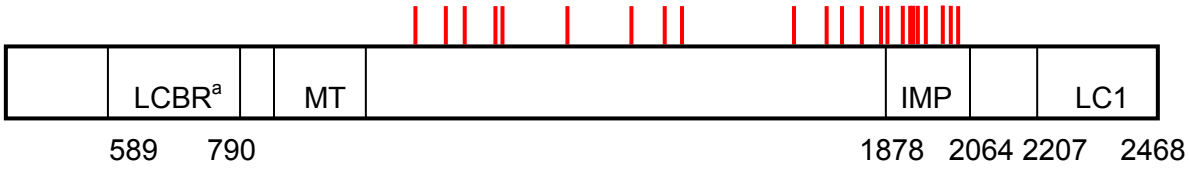
Supplemental Figure S5. Transient transfection of Syk enhances the stability of acetylated microtubules. MDA-MB-231 cells lacking (upper panels) or expressing Syk-EGFP (lower panels) and treated without (A) or with (B) nocodazole were fixed and stained with an antibody against acetylated α-tubulin and a fluorescently tagged secondary antibody, and with DAPI to

mark the nucleus. Cells were examined by phase contrast (phase) and fluorescence microscopy to detect α-tubulin (red) , nuclei (blue) and Syk-EGFP (green).



Supplemental Figure S6. Knockdown of MAP1B attenuates the Syk-dependent changes in cellular phenotype. Topographic images (upper panels) and elasticity maps (lower panels) of MDA-MB-231 cells lacking Syk (-Syk) or expressing Syk (+Syk) without or with shRNA directed against MAP1B (shMAP1B) were acquired by AFM using force-volume mode. On the elasticity maps are insets (squares) marking the locations of 3×3 pixels extracted to analyze the resulting cell height and elasticity. Images were taken at a trigger force of 2nN, 32×32 pixels. Bar = 10 µm.

Supplemental Table SI – Sites of tyrosine phosphorylation identified on MAP1B



Site sequence	Cell line[b]	YXXL/I
LKETEPVEAyVIQK	231, DG75	No
FEDEGAGFEESSETGDyEEK	DG75	No
HSPTEDEESAKAEADAyIR	DG75	No
MEAEDyVMAVVDK	DG75	No
AAEAGGAEEQyGFLTTPTK	DG75	Yes
TDATDGKDyNASASTISPPSSMEEDKFSR	231	No
TLEVVSPSQSVTGSAGHTPYyQSPTDEK	231	No
SPPLIGSESAyESFLSADDK	231, DG75	No
QGSPDQVSPVSEMTSTSLyQDKQEGK	231	No
QSPDHPTVGAGVLHITENGPTEVDySPSDMQDSSLSHK	231	No
DMSLyASLTSEK	DG75	Yes
ESSPLySPTFSDSTSAVK	231, DG75	No
TATCHSSSSPPIDAASAEPyGFR	231, DG75	No
TPGDFSYAyQKPEETTR	231, DG75	No
SPDEEDYDyESYEK	231	No
TPQASTYSyETSDLCYTAEK	231, DG75	No
TPEDGDySYEIIEK	DG75	No
TPEDGDYSyEIIEK	231, DG75	Yes
TPEEGGySYDISEK	231, DG75	No
TTSPPEVSGySYEK	231, DG75	No
LLDDISNGYDDSEDGGHTLGDPSySYETTEK	231	No
ITSFPESEGYSyETSTK	231, DG75	No
TPDTSTYCyETAEK	231	No

[a]Regions of MAP1B are: light chain binding region (LCBR), microtubule binding region (MT), imperfect repeat region (IMP) and light chain-1 (LC1).

[b]Cell lines reported are DG75 human B cell lymphoma (DG75) or MDA-MB-231 human breast carcinoma (231).[44,45]