

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: **Collagenolytic invadopodia grow overtime and push collagen fibers away.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were plated on top of a thin type I collagen layer (magenta) and analyzed by confocal spinning-disk microscopy. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Asterisk: position of an unlabeled cell in the field. Scale bar, 10 μ m.

File Name: Supplementary Movie 2

Description: **Collagenolytic rupture of invadopodia/collagen fiber ensemble reveals typical visco-elastic movement.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were plated on top of a thin type I collagen layer (magenta) and analyzed by confocal spinning-disk microscopy. Boxed regions and corresponding insets document invadopodia/collagen fiber rupture events (pointed by red arrowheads) in separated channels. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Scale bar, 10 μ m.

File Name: Supplementary Movie 3

Description: **Inhibition of MMP proteolytic activity inhibits invadopodia expansion and elongation.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were treated with MMP-inhibitor GM6001 (40 μ M) and plated on top of a thin type I collagen layer (magenta) before analysis by confocal spinning-disk microscopy. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Asterisk: position of a cell with low Tks5^{GFP} expression in the field. Scale bar, 10 μ m.

File Name: Supplementary Movie 4

Description: **Laser-mediated rupture of invadopodia/collagen fiber ensemble.** MDA-MB-231 cells expressing Tks5^{GFP} (green) Mock- or GM6001-treated (40 μ M) were plated on top of a thin type I collagen layer (magenta) and analyzed by confocal spinning-disk microscopy. Photo-ablation was performed along the region shown in red. Images were taken every 15 s (time is in hr:min:s). Representative movie from three independent experiments. Scale bar: 5 μ m.

File Name: Supplementary Movie 5

Description: **Invadopodia force generation requires actin polymerization.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were plated on top of a thin type I collagen layer (magenta) in DMSO-treated medium and analyzed by confocal spinning-disk microscopy. Cytochalasin D (0.5 μ M) was added 15 min after starting the time-lapse. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Scale bar, 10 μ m.

File Name: Supplementary Movie 6

Description: **Arp2/3 complex function is required for invadopodia-based force generation.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were plated on top of a thin type I collagen layer (magenta) in DMSO-treated medium and analyzed by confocal spinning-disk microscopy. CK-666 (200 μ M) was added 15 min after starting the time-lapse. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Scale bar, 10 μ m.

File Name: Supplementary Movie 7

Description: **Invadopodia dynamics is not perturbed upon ROCK inhibition.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were plated on top of a thin type I collagen layer (magenta) in complete medium and analyzed by confocal spinning-disk microscopy. Y27632 (20 μ M) was added 15 min after starting the time-lapse. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Scale bar, 10 μ m.

File Name: Supplementary Movie 8

Description: **Myosin II inhibition by blebbistatin does not affect invadopodia dynamics.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were plated on top of a thin type I collagen layer (magenta) in DMSO-treated medium and analyzed by confocal spinning-disk microscopy. Paranitro-Blebbistatin (10 μ M) was added 15 min after starting the time-lapse. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Scale bar, 10 μ m.

File Name: Supplementary Movie 9

Description: **Tks5-positive invadopodia form as ring-like structures in 3D collagen.** Z-stack of MDA-MB-231 cells expressing Tks5^{GFP} (green) embedded in a 3D fibrillar collagen-I gel (magenta) and analyzed by confocal spinning-disk microscopy. Z-steps were taken every μ m as indicated and the full stack corresponds to a 15 μ m-thick region in the 3D collagen gel (total width of the collagen gel is 100-150 μ m). The maximal intensity z-projection corresponding to every z-steps is shown as indicated. Collagen fibers can be seen beneath and above the cell body, indicating that cells were completely embedded in the gel. Tks5-positive structures form as a ring surrounding the cell body along collagen fibers. Middle and right panels: separated channels for Tks5^{GFP} and collagen signal respectively. Arrowheads point at Tks5-positive ring-like structures along collagen fibers. This stack corresponds to the 1 hr:30 min time point of Movie 2. Scale bar, 10 μ m.

File Name: Supplementary Movie 10

Description: **Ring-like Tks5-positive invadopodia during 3D collagen invasion.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were embedded in 3D fibrillar collagen-I (magenta) and analyzed by confocal spinning-disk microscopy. Images were taken every 10 min during 15 hr (time is in hr:min). Representative movie from three independent experiments. Right panel, Tks5^{GFP} signal. Arrowheads point at Tks5-positive ring-like structures along collagen fibers. Asterisk, position of cell nucleus. Scale bar, 10 μ m.

File Name: Supplementary Movie 11

Description: **Abortive ring-like Tks5-positive invadopodia forming upon inhibition of MT1-MMP in 3D collagen.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were treated with GM6001 (40 μ M) and embedded in 3D fibrillar collagen-I (magenta) and analyzed by confocal spinning-disk microscopy. Images were taken every 10 min during 15 hr (time is in hr:min). Representative movie from two independent experiments. Right panel, Tks5^{GFP} signal. Arrowheads point at Tks5-positive ring-like structures along collagen fibers. Asterisk, position of cell nucleus. Scale bar, 10 μ m.

File Name: Supplementary Movie 12

Description: **Collagen cross-linking impairs invadopodia expansion.** MDA-MB-231 cells expressing Tks5^{GFP} (green) were plated on top of a chemically (4% PFA) cross-linked type I collagen layer (magenta) and analyzed by confocal spinning-disk microscopy. Images were taken every min during 1 hr (time is in hr:min). Representative movie from three independent experiments. Asterisk: position of a cell with low Tks5^{GFP} expression in the field. Scale bar, 10 μ m.