Supplementary Methods

Cell culture
Human fibrosarcoma cell line HT1080 was grown in high glucose Dulbecco's Modified Eagles Medium (DMEM, Mediatech, Manassas, VA, USA) consisting of 10% Fetal bovine serum (FBS, Hyclone Laboratories, South Logan, UT, USA) and 0.005% gentamicin (w/v). Human breast cancer cell line MDA-MB-231 was grown in DMEM supplemented with 10% FBS and 100 μU penicillin/ 100 μg streptomycin per mL of medium. Human ovarian cancer cell line HEY (CELLutions Biosystems, Ontario, CA) was grown in DMEM consisting of 10% FBS, 2mM L-Glutamine and 100 U penicillin/ 100 μg streptomycin per mL of medium. Human prostate cancer cell line DU-145 (ATCC, Manassas, VA, USA) was grown in Eagle’s Minimum Essential Medium (MEM, Mediatech, Manassas, VA, USA) consisting of 10% FBS and 100 U penicillin/ 100 μg streptomycin per mL of medium. Human T lymphocyte cell line Jurkat was grown in RPMI 1640 (Mediatech, Manassas, VA, USA) medium consisting of 10% FBS and 100 U penicillin/100 μg streptomycin per mL of medium. All cells were grown in presence of 5% CO₂ and 37°C.

2D and 3D Cell motility assay
For experiments on flat 2D substrates, 12 well plastic bottom plates were coated with 50-μg/ml collagen I or 20-μg/ml fibronectin (Jurkat cells) for one hour at room temperature. The plates were washed three times with 1X PBS and 2000 cells were seeded.

Cells were embedded inside collagen I (Corning Inc., Tewksbury, MA, USA) matrices as described previously¹,². Briefly, 18,000 cells were mixed with calculated amount of Collagen I in acetic acid, sodium hydroxide, cell culture media, and reconstitution buffer and added to 24-well plastic bottom plates. The well plates were incubated at 5% CO₂ and 37°C. For both 2D and 3D experiments, cells were incubated overnight, after which time-lapse movies were acquired.

Cell movements over time can be observed using a confocal or a wide-field light microscope. In our case, cells cultured on 2D plates or in 3D collagen I matrices were imaged using a 10X objective mounted on a Nikon TE-2000 Nikon microscope fitted with
a climate controlled incubator (37°C and 5% CO₂). Images were talked every two minutes for eight hours for 2D experiments and for 16.5 h for 3D experiments.

Cells in the time-lapse movies were tracked using MetaMorph software (Molecular Devices, Sunnyvale, CA). The results were output to an excel file which contained the x- and y- coordinates of the tracked cells. Alternative software, CellTracker, can be downloaded from the URL (http://group.szbk.u-szeged.hu/sysbiol/horvath-peter-lab-overview.html). In our experience, the tracking results from MetaMorph and CellTracker are in close agreement with each other.