Supplementary Experimental Procedures and Data

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Spheroid assay. A stock Methocel solution was prepared by dissolving previously autoclaved 6 g of methylcellulose (4000 centipoises, Sigma-Aldrich) in 500 ml of DMEM. Transfected cells were trypsinized and 7.5×10^4 cells were resuspended in 15 ml of DMEM with 20% Methocel. 150 µl of the cell suspension was added per well of a U-bottom 96 well suspension plate (Greiner Bio-One, Stonehouse, UK) and incubated for 16h at 37ºC for spheroid formation. A solution of 1.5 mg/ml rat-tail collagen type-I (BD Biosciences) was prepared in DMEM and neutralised by dropwise addition of 1M NaOH. An initial layer of 100 µl of the collagen solution was added per well of a 12-well plate and allowed to set at 37ºC for 30 min. Spheroids were harvested and resuspended in a freshly prepared collagen solution, and 100 µl was added on top of the previously prepared layer, and allowed to set at 37ºC for 1.5h. After the collagen set, 1.5 ml of DMEM medium was added to each well and spheroids were left to invade for 40h before being fixed with 4% PFA in PBS. 10 spheroids were imaged from 2 different wells per condition and the total area of invasion for each spheroid was calculated using the Metamorph® imaging software (Molecular Devices, Sunnyvale, CA).

SUPPLEMENTARY FIGURE 1. MT1-MMP mono-ubiquitination does not regulate protein degradation. *A*. MCF-7 cells were transfected with pcDNA3.1, Ubiquitin-HA and MT1-MYC as indicated and cells were incubated for 3h with DMSO or 25, 50 or 75 µM MG-132. Cell extracts were immunoblotted with an anti-Myc, an anti-p21, an anti-HA and an anti-*β*-Actin antibody. *, non-specific band. *B*. MCF-7 cells were transfected with pcDNA3.1, MT1-WT, MT1-K581R or MT1-ΔICD and cell extracts were immunoblotted with an anti-MT1-MMP (LEM-2/15.8) or an anti-*β*-Actin antibody. The semi-quantitative analyses of band intensities of four different immunoblots are shown ± SEM.

SUPPLEMENTARY FIGURE 2. Ablation of MT1-MMP ubiquitination does not alter the localisation of MT1-MMP in respect to Rab7, Rab4, Rab6 or Rab11. MCF-7 cells were transfected with MT1-WT, MT1-Y573A, MT1-K581R or MT1-ΔICD and co-transfected with Rab7-, Rab4-, Rab6- or Rab11-EGFP. 16h after transfection the cells were fixed, permeabilised and stained with an anti-MT1-MMP (N175/6; Cy5 secondary, red) antibody. Inset panels show magnified portions of each merged image, as indicated (dashed squares). Bars represent a distance of 25 µm. The co-localisation of the different MT1-MMP constructs with the Rab-EGFP constructs was determined using the Volocity 3D imaging and analysis software of single confocal optical sections. Data represent the co-localisation co-efficients ±SEM of 6 optical sections.

SUPPLEMENTARY FIGURE 3. Ablation of MT1-MMP ubiquitination has no effect on invasion from 3-dimensional multicellular spheroids through type-I collagen. Wild type MCF-7 cells or MCF-7 cells expressing either pcDNA3.1, MT1-WT, MT1-Y573A, MT1-K581R or MT1-ΔICD, were assembled into multicellular spheroids and incubated within a 1.5 mg/ml rat-tail collagen type-I gel for 40 h. A. Representative images of spheroids containing different transfected cells after 40h incubation in collagen. White bars represent a distance of 100 µm. B. Total area of invasion was determined using the Metamorph® imaging software. Data represents total area of invasion per spheroid calculated for a total of 10 spheroids from 2 different wells per condition with $P<0.0001$ (***) and $P< 0.01$ (*). *ns*, not significant.