**Supplementary Information**

**Supplementary Movie Legends**

**Movie S1** 3D image of nuclear membrane blebbing and emerin mislocalization in BT-549 DIAPH3-depleted cells. DNA (Hoechst, blue), emerin (FITC, green) and membrane (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate, Dil, red) staining are shown.

**Movie S2** Nuclear membrane blebbing in amoeboid cells. Time-lapse analysis of DU145 DIAPH3-depleted cells treated with EGF and IL6 (exacerbates amoeboid features (13)) and stained with lipid fluorescent dye CellMask Orange.

**Movie S3** DU145 DIAPH3-depleted cells shed EV. Time-lapse analysis of DU145 DIAPH3-depleted cells treated with EGF and IL6 (exacerbates amoeboid features (13)) and stained with CellMask Orange. The cells were recorded over a period of 135s.

**Figure S1** Computer-assisted analysis of emerin-positive particles. The immunofluorescent stained slides were digitized by whole-slide scanning. Images of each core were individually extracted and analyzed. Images from the entire TMA were processed through an integrated image analysis workflow developed in KNIME (see methods)(**A**). Images are read into the workflow and integrated into a single data table where each core is annotated (**B**). Images are pre-processed for further analysis (**C**)and segmented to individual nuclei (HH3) and emerin particles(**D**).The emerin staining in individual segments (**E**) is subsequently used to classify emerin-positive particles(**F**). Emerin-positive particles are subsequently mapped back to the original image or the epithelial mask and the number of particles in each patient is quantified(**G**).

**Figure S2** Co-localization of DIAPH3 with emerin. Emerin mislocalization and increased cell deformability in DIAPH3-depleted cells. **A,** Representative tandem mass spectrum demonstrating the identification of a peptide (identification score 136.9) derived from emerin, a DIAPH3-interacting protein (data obtained from (14)). **B,** Confocal images of DU145 cells stably expressing GFP-DIAPH3 or GFP alone (scale bar 10 μm). Separate images of DIAPH3 (GFP, green) and emerin (Alexa Fluor 568, red) channels are shown. Inset in the bottom left shows enlarged areas of DIAPH3 co-localization with the nuclear envelope (yellow). **C,** Percentage retention of HMEC-HRASV12 control or DIAPH3-depleted cells subjected to PMF. **D,** Confocal 3D images of DU145 control or DIAPH3-depleted cells. DNA (Hoechst, blue), emerin (FITC, green) and membrane (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate, Dil, red) staining are shown (scale bar 10 μm). **E,** Graph represents the percentage of BT-549 control or DIAPH3-depleted cells with emerin mislocalization (n=50 cells). Graphs show mean + s.d. \*\*P<0.01, \*\*\*P<0.001. Unpaired t-test. **F,** Representative immunoblot of emerin, and β-actin levels in DU145 DIAPH3-depleted untreated or treated with 10m MG-132 for 6h.

**Figure S3** Nuclear shape instability promotes the shedding of EV containing DNA. **A,** EV shed from DU145 DIAPH3-depleted cells are enriched with DNA compared to EV shed from DU145 controls. Representative histograms of the EV sequentially stained with RNA (pyronin Y, PY) and DNA (Hoechst) dyes (see methods); 10,000 events were counted by flow cytometry. EV were normalized for cell number. Histograms show the peak separation for mean florescence intensity. **B,** Quantification of the percentage of permeable and impermeable membrane EV. EV were collected from EV-free complete medium in BT-549 control or DIAPH3-depleted cells after 24h. EV were normalized for cell number. Hoechst and propidium iodide (PI) positivity were quantified by flow cytometry; 10,000 events were analyzed. **C,** Quantification of the percentage of apoptotic (annexin positive) DU145 DIAPH3-depleted cells, untreated or treated with 200 ng/mL of TRAIL; 10,000 events were quantified by flow cytometry. **D,** Quantification of the percentage of permeable and impermeable membrane EV. EV were collected from serum free medium in DU145 DIAPH3-depleted cells, treated or untreated with 200 ng/mL of TRAIL after 24h. EV were normalized for cell number. Hoechst and PI positivity were quantified by flow cytometry; 10,000 events were analyzed. Note impermeable EV, which represent non-apoptotic EV. Graphs show mean + s.d. \*\*\*P<0.001. Unpaired t-test.

**Figure S4** Emerin-depleted cells display aggressive features *in vitro*. **A,** Sustained activation of ERK in emerin-silenced cells. Representative immunoblot of emerin, p-ERK, ERK, GAPDH and β-actin levels in DU145, BT-549, LNCaP and PC3, control or emerin-depleted cells. Enhanced phosphorylation of MLC2 in emerin-depleted cells. Representative immunoblot of p-MCL2 and MCL2 in DU145 control or emerin-depleted cells. **B,** Images of BT-549 and LNCaP control or emerin-depleted cells. DNA (Hoechst, blue) and lamin A (FITC, green) staining are shown (scale bar 10 μm). **C,** Images of DU145 emerin-depleted cells, related to Figure 2d. Lamin A (FITC, green) and membrane (1,1’-Dioctadecyl-3,3,3’,3’-Tetramethylindocarbocyanine Perchlorate, Dil, red) staining are shown (scale bar 10 μm). **D,** Quantification of the percentage of permeable and impermeable membrane EV. EV were collected from EV-free complete medium in DU145 control or emerin-depleted cells after 24h. EV were normalized for cell number. Hoechst and PI positivity were quantified by flow cytometry; 10,000 events were analyzed. **E,** DU145 emerin-depleted cells display enhanced migration quantified using a Boyden chamber assay, related to Figure 2j. **F,** PC3 emerin-depleted cells display increased invasion in comparison with the control cells, quantified using a Boyden chamber assay. Graph shows mean + s.d. \*\*P<0.01, \*\*\*P<0.001. Unpaired t-test.

**Figure S5** Emerin loss leads to wide spread metastasis in mice. Luciferase activity at weeks 3, 4 and 5 after intracardiac injection, **A,** n=8 mice for DU145 control and **B,** n=7 for DU145 emerin-depleted cells; related to Figure 3. Graphs on the right represent luciferase activity quantification in the distinct murine body parts (head, lungs, abdomen and bones). **C,** Representative image of the organs collected at necropsy. Organs from mice injected with DU145 control or emerin-depleted cells are shown (brain, lungs, liver and kidney). Arrows point to tumors. **D,** Representative images of H&E and pan-cytokeratin IHC staining of the brain (scale bar for control group = 300 μm, scale bar for emerin-depleted group = 50 μm), lungs (scale bar 300 μm), adrenal glands (scale bar 300 μm) and liver (scale bar 50 μm). Micro-CT images and H&E (scale bar 50 μm) of murine bones collected from the mice injected with emerin-depleted cells.

**Figure S6** Genomic alterations of the EMD gene in human prostate **A,** and breast **B,** cancers. 1Beltran H, et al.Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med. 2016;22:298–305. 2Baca SC, et al. Punctuated evolution of prostate cancer genomes. Cell. 2013;153:666–677. 3Abeshouse A, et al. The Molecular Taxonomy of Primary Prostate Cancer. Cell. 2015;163:1011–1025. 4Hieronymus H, et al. Copy number alteration burden predicts prostate cancer relapse. Proc Natl Acad Sci USA. 2014;111:11139–11144. 5Taylor BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18:11–22. 6Abida W, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. JCO Precis Oncol. 2017; doi:10.1200/PO.17.00029. 7Gao D, et al. Organoid cultures derive from patients with advanced prostate cancer. Cell. 2014;159:176–187.8Fraser M, et al. Genomic hallmarks of localized, non-indolent prostate cancer. Nature.2017;541:359–364. 9Barbieri CE, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet. 2012;44:685–689. 10Robinson D, et al. Integrative clinical genomics of advanced prostate cancer. Cell. 2015;161:1215–1228. 11Kumar A, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. Nat Med. 2016;22:369–378. 12Grasso CS, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012;487:239–243.13Eirew P, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518:422–426. 14Stephens PJ, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature. 2012;486:400–404.15Shah SP, et al. The clonal and mutational evolution spectrum of primary triple negative breast cancers. Nature.2012;486:395–399.16Lefebvre C, et al. Mutational Profile of Metastatic Breast Cancers: A Retrospective Analysis. PLoS Med. 2016;13: e1002201.17Pereira B, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat. Commun. 2016;7:11479. 18Ciriello G, et al. Comprehensive molecular portraits of invasive lobular breast cancer. Cell. 2015;163: 506–519. 19Banerji S, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature. 2012;486: 405–409.

**Figure S7** Lamin A/C-depletion leads to emerin mislocalization, nuclear shape instability and an increase in experimental metastatic sites. **A,** Lamin A/C-depleted DU145 and BT-549 cells using two different shRNA hairpins. Representative immunoblot for lamin A/C and β-actin levels. **B,** Images of BT-549 control or lamin A/C-depleted cells. DNA (Hoechst, blue), emerin (FITC, green) and membrane (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate, Dil, red) staining are shown (scale bar 10 μm). Left panels show enlarged images of the nuclei **C,** Luciferase activity quantification at weeks 3, 4, and 5 after intracardic injection (n=9 mice for DU145 lamin A/C-depleted cells). Graphs on the right represent luciferase activity of the distinct mouse body parts (head, lungs, abdomen and bones). Related with Figure 3A and Supplementary Figure 4A. **D,** Representative image of the organs collected at necropsy. Organs corresponding to mice injected with lamin A/C-depleted cells, brain, lungs, liver and kidney are shown. Arrows point to tumors. Related with Supplementary Figure 4C**. E,** Representative images of H&E and pan-cytokeratin IHC staining of the brain (scale bar 50 μm), lungs (scale bar 50 μm) and liver (scale bar 300 μm). Related with Supplementary Figure 4D. **F,** Representative images of emerin IHC staining of benign prostate and high-grade prostate cancer (images obtained from the The Human Protein Atlas database,https://www.proteinatlas.org). Arrows point to emerin-positive nuclear envelope blebs.

**Supplementary Table Legends**

**Supplementary Table 1** Distribution of metastasis in mice injected with control, emerin-depleted or lamin A/C-depleted cells.