SUPPLEMENTARY METHODS

Cell lines and reagents

Human Embryonic Kidney 293 cells (HEK293) were transduced using lentivectors carrying SK3 channels cDNA to generate HEK293-$SK3^+$ and were grown as previously described (12, 13). Human melanoma 518A2 cell line was transduced using lentivectors carrying shSK3 to generate 518A2-$SK3^-$ cells or a non-targeting shRNA to generate 518A2-$SK3^+$ cells and cells were maintained in culture as previously described (12). Apamin (specific SKCa blocker), Ca^{2+} ionophore A23187 and Orai1 inhibitor 2APB were purchased from Sigma-Aldrich. Calpain inhibitor Z-LLY-FMK were purchased from Promokine (Heidelberg, Germany). 1-O-hexadecyl-2-O-methyl-sn-glycero-3-lactose named Ohmline was synthetized as previously described (13).

Cell proliferation and migration assays

Cell proliferation was determined using the tetrazolium salt reduction method (MTT), as described elsewhere (6). Cells were seeded on 24-well plates at a density of 20,000 cells per well and measurements were performed in triplicate daily for 5 days. Note that drugs and high external calcium concentration used in trans-well migration assays had no effect on cell proliferation/viability (48 h).

Cell migration was analysed in 24-well plates receiving 8-µm pore size polyethylene terephthalate membrane cell culture inserts (Becton Dickinson, France), as previously described (12). Briefly, $4x10^4$cells were seeded in the upper compartment with medium culture supplemented with 5% of FBS (± drugs/high external calcium concentration). The lower compartment was filled with medium culture supplemented with 5% FBS (± drugs/high external calcium concentration). Two-dimensional migration assays were performed without coating. After 24 h, stationary cells were removed from the topside of the membrane, whereas

migrated cells in the bottom side of the inserts were fixed, nuclei were stained and automatically counted (31). At least three independent experiments were performed and each of them in triplicate.

Immunofluorescence

Anti-SK3-ATTO-594 (Alomone Lab.) and a rabbit anti-Orai1 (H-46, Santa Cruz Biotech.) associated with Alexa Fluor 488-conjugated anti-rabbit (A11070, Invitrogen) were used for MDA-MB-435s cells and tumors samples. MDA-MB-435s cells were fixed and permeabilized by methanol. The immunolabelled samples were examined using an Olympus IX71 epifluorescence microscope (Olympus, Tokyo, Japan) and confocal images were acquired by means of Confocal Laser Scanning Microscopy (CLSM) using an Olympus confocal system (confocal FV-1000 station). For immunofluorescence on human samples, 3µm frozen sections from prostate cancer were incubated 60 mm at room temperature with the primary antibody, either SK3 or Orai1 (dilution 1:50). For Orai1, the secondary antibody (Alexa Fluor 488-conjugated anti-rabbit) was then applied for 60mn after rinsing, and slides were finally counterstained with DAPI (ZytoVision, Bremerhaven, Germany). Double staining was achieved by incubation first with Orai1, and then with SK3 antibody. Fluorescent images were captured with a JAI camera (model CV-M4+CL), with the use of an automated filter wheel coupled to a Leica DMRB fluorescence microscope (Leica Microsystems).

Electrophysiology

Experiments were performed in the whole-cell configurations of the patch-clamp technique. Whole-cell potassium currents were generated by stepwise 10-mV depolarizing pulses (500 ms duration; 5 sec intervals) from a constant holding potential of -70 mV up to +70 mV. In some experiments currents were generated by ramp protocol from -70 mV to +70 mV in 500

ms from a constant holding of -70 mV. The voltage-ramp protocol was checked by comparing the I-V relation obtained from the voltage ramp and the current measured at the end of the 500 ms voltage steps and the results were identical. Signals were filtered at 1 KHz and digitized at 10 kHz. Cell capacitance of the electrode and the junction potentials between the electrode and the bath were cancelled using respectively the pipette capacitance compensating and the voltage pipette offset control of the amplifier. During the experiments series resistances and cell capacitances were not compensated. Patch pipettes (3.0-4.5 MΩ) were pulled from borosilicate glass capillary tubes in a two-stage vertical puller (PP-830, Narishige, Tokyo, Japan) and were filled with a pipette solution contained (in mM): K-glutamate 125, KCl 20, $MgCl_2$ 1, Mg-ATP 1, HEPES 10, $CaCl_2$ 0.37, EGTA 10, adjusted to pH 7.2 with KOH, pCa7. Estimation of free Ca^{2+} concentrations was performed using WebMaxC (http://www.stanford.edu/~cpatton/webmaxc2.htm) and was controlled by a Ca^{2+} sensitive electrode (WPI, UK).

The physiological saline solution (PSS) contained (in mM): NaCl 140, $MgCl_2$ 1, KCl 4, $CaCl_2$ 2, D-glucose 11.1 and HEPES 10, adjusted to pH 7.4 with NaOH. The high Ca^{2+}-PSS was prepared by adding 3 mM of $CaCl_2$ and the results were compared to a PSS in which 3 mM $MgCl_2$ was added in order to keep the same concentration of divalent ions in both solutions

Western blot experiments

The antibodies used were the following : rabbit anti-Orai1 (H-46, Santa Cruz Biotech., dilution 1/500), rabbit anti-Stim1 (GOK**,** ProScience, dilution 1/500), mouse anti-Hsc70 (sc7298, Santa Cruz Biotech., dilution 1/3000), rabbit anti-SK3 (P0608, Sigma-Aldrich, dilution 1/250), mouse anti-luciferase (mAb21, Invitrogen, dilution 1/2000), mouse anti-talin (clone 8d4, Sigma-Aldrich, dilution 1/2000), rabbit anti-caveolin (D46G3, Cell Signaling Tech, dilution 1/200), goat anti-β-adaptin (sc 6425, Santa Cruz Biotech., dilution 1/1000) and

horseradish peroxidase conjugated anti-rabbit, anti-goat or anti-mouse (Jackson Immuno-Research Laboratories).

Luciferase assay *in vitro*

Luciferase activity in cell culture was measured by chemiluminescence using the Bright-Glo luciferase assay system (Promega). Briefly, cells were seed in 96-wells plate and lysed 24 h later with the reagent containing all the components required for starting the chemiluminescent reaction. Lysates were transferred in white 96-well plates and photo emission was quantitated using a luminometer (Berthold, Centro LB960). Three independent experiments were each performed in triplicate.

FACS analysis

Briefly, 5.10^5 cells were washed with cold PBS then incubated for 20 min with 100 µL of Cytofix/Cytoperm™Fixation/Permeabilization Kit (BD Biosciences). Cells were incubated with a mouse anti-luciferase antibody (1:100; Invitrogen) for 1 h at 4°C, washed, then incubated with an anti-mouse coupled to R-phycoerythin (BD Biosciences) for 45 min at 4°C. Fluorescence was analysed using an Epics XL-MCL flow cytometer (Beckman-Coulter). MDA-MB-435s wild-type were used as negative control.

RT-qPCR

Reverse transcription-qPCR experiments were done according to standard protocols. Briefly, total RNAs were extracted using the Nucleospin RNA II kit (Macherey-Nagel) and 2.5 µg of total RNA was used for cDNA synthesis using hexanucleotide primers and a Ready-to-Go You-Prime First-Strand Beads kit (GE Healthcare, UK). Quantitative PCR was performed using PlatiniumSyBR Green qPCRSuperMix-UDG (Invitrogen) with the LightCycler 480

instrument (Roche Diagnostics, Basel, Switzerland) and with the following parameters: 45 cycles of 10 sec at 95 °C, 30 sec at 60 °C and 20 sec at 72°C. The experiments were performed in triplicate, the amplifications were analysed with the LC480 software and the mRNA expression of target genes was normalized using the mRNA level of HRPT-1. Amplifications were performed using the sequences of primers used: STIM1: forward 5' GCGGGAGGGTACTGAG 3' and reverse 5' TCCATGTCATCCACGTCGTCA 3', for Orai1: forward 5' AGGTGATGAGCCTCAACGAG 3' and reverse 5' CTGATCATGAGCGCAAACAG 3', for HPRT-1 : Forward 5' CGAGCAAGACGTTCAGTCCT 3' and reverse 5' TGACCTGATTTATTTTGCATACC 3' (Sigma Aldrich).

Calpain activity assay.

A calpain activity assay kit from Promokine was used according to the manufacturer's recommendation. The fluorometric assay is based on the detection of cleavage of calpain substrate Ac-LLY-AFC. Fluorescence emission was measured at 505 nm using a microplatespectrofluorimeter (Molecular devices Spectra max 190, Ltd) with an excitation light at 400 nm.

Membrane fractionation. Membranes were fractionated on sucrose gradient (90%, 45%, 35% and 5%) as described (28). Upon centrifugation a total of 12 fractions were collected. Caveolin 1 was used as a marker for lipid-rafts fractions and β-adaptin was used as a marker for non-lipid-rafts fractions. Both proteins were detected by western blot following SDS–polyacrylamide gel electrophoresis. Equal volumes of each fraction were loaded onto the gel.

Short hairpin RNA interference and RNA interference assays

To establish stable pools of MDA-MB-435s with Orai1 knockdown, we used a short hairpin RNAs (shRNAs) targeting human Orai1 or control shRNA cloned onto the pRS vector .Cells were transfected with either pRS-shControl (TR30012, Origene Inc) or pRS-shOrai1 (TR301009, Origen Inc) using Lipofectamine 2000 (Invitrogen), and selected by 1 mg/mL puromycin during 3 weeks. Results indicated that shRNA targeting Orai1 exerted inhibition of protein expression without affecting the cell viability.

Cells were transfected using Lipofectamine RNAi max (Invitrogen) with 20 nM of siRNA for 48 h. The siRNA sequences directed against Orai1 were purchased from Santa Cruz Biotechnologies (sc-76589). For Control siRNA we used the following sequences: 5'CUGUAUCGAAUGUUAUGAGCC[dT][dT]3' and 5'GCUCAUAACAUUCGAUACAG [dT] [dT] 3' (Invitrogen).

Bioluminescence Imaging (BLI). All of mice were assessed weekly using whole-body bioluminescent imaging to quantify relative amounts of tumor burden (ΦimageurTM; BIOSPACE Lab). Each mouse was given 150mg/kg body-weight of D-luciferin potassium salt (Interchim) by intraperitoneal injection (ip) and anesthetized with Ketamin/xylasinip injection as above. Bioluminescence images were acquired 3-5 min after injection and were collected in real time until saturation plate was reached in the lateral, ventral and dorsal positions. Photons count emitted by the tumor was performed by a photon imager system. Regions of interest were drawn around the MFP tumor and metastases. The amount of tumor burden was quantified as the relative amount of photons produced from the luciferase activity in cells and expressed in cpm using the software Photovision+ (version 1.3; Biospace Lab). At necropsy, *ex-vivo* BLI measurement for each collected organ was performed within 15min after D-luciferin injection.

Incorporation of Omhline in tissues. Total lipids from primary tumors, bones and lungs were extracted as previously described (29). Lipids were treated with Vitride® (30) to remove ester-linked lipids. Ether-linked lipids including Ohmline were separated and quantified against a calibration curve (0.05 to 10 µg/spot) of Ohmline, by HPTLC densitometry (using Linomat5 sample applicator and TLC Visualizer with Wincats 1.4 software, CAMAG, Switzerland).

Microscanner and radiologic analyses. At necropsy, legs and rachis were dissected and fixed in formaldehyde. Microscanner experiments were done using the high-resolution X-ray micro-computed tomography (micro-CT) system for small animal imaging SkyScan-1072 (SkyScan, Belgium) with source voltage of 50 Kv, source current of 179 µA, rotation step of 0.675° (to 180°) and an image pixel size of 19.19 µm. Architectural analyses were performed using Nrecon and CT Vox (Skyscan Belgium). Radiographs were taken with a mammography PLANMED Sophie apparatus (SN RAH 40710).