MATERIAL AND METHODS

Cell culture
The human pancreatic cancer cell line L3.6pl was obtained by Christine J. Bruns (Department of Surgery, Clinic Grosshadern, LMU Munich, Germany) (1). L3.6pl cells were cultivated on Collagen G-coated culture flasks in RPMI 1640 (PAA, Coelbe, Germany) supplemented with 10 % FCS, non-essential amino acids (PAA, Coelbe, Germany) and 1 mM pyruvate (Sigma-Aldrich, Taufkirchen, Germany). MDA-MB-231 were received from CLS (Cell line services, Eppelheim, Germany) in the last 6 months and cultivated in DMEM (PAA, Coelbe, Germany) supplemented with 10 % FCS. The human mammary epithelial cell line MCF-10A was purchased from ATCC and was cultured in DMEM-F12 (PAA, Coelbe, Germany) supplemented with 5% horse serum, 100 mg/ml epidermal growth factor, 10 mg/ml insulin, 1 mg/ml hydrocortisone, 1 mg/ml cholera toxin and 0.1% penicillin/streptomycin.

Scratch Assay/Immunflourescence
SKBR3 and L3.6pl cells were seeded one day before stimulation (100,000 cells/well). Cells were stimulated at 70% confluence for 24 h. Cells were scratched and incubated at 37 °C for 10/16 h. Cells were fixed with 3% PFA and pictures were taken at the microscope (Axiovert, Zeiss, Oberkochen, Germany). The analysis was done by the WimScratch software (Wimasis, Munich, Germany).

For the localization of the V-ATPase subunit c during migration, a confluent cell layer of SKBR3 and MCF-10A was scratched. After 5 h of migration cells were fixed and permeabilized and stained for the V-ATPase subunit c (Ductin-antibody, ATP6L, Millipore, Eschborn, Germany) and f-actin (phalloidin-rhodamin, Sigma, Taufkirchen, Germany) and prepared for confocal microscopy (LSM 510 Meta; Zeiss, Oberkochen, Germany).

For the analysis of the role of the EGF-R in cell polarization, SKBR3 cells were treated with 10 nM Archazolid B for 30 h. Cells were collected, centrifuged and resuspended in fixation solution (2.5% glutaraldehyde in fixative buffer: 75 mM cadiclate, 75 mM NaCl, 2 mM MgCl_2, pH 7.0). After incubation of 1 h, cells were washed several times in fixative buffer and postfixed in 1% OsO_4 (diluted in fixative buffer). Cells were washed with aqua dest and dehydrated in a graded series of acetone. Finally, cells were embedded in Spurr low-viscosity epoxyresin and polymerized at 65°C. Several pictures were taken with a Zeiss EM 912 transmission electron microscope with integrated Ω-filter (in “zero-loss-mode”).

siRNA transfection
For the transfection of L3.6pl cells the Amaxa system was used, according to manufacturer's instructions. Briefly, 1.5 x 10^6 L3.6pl cells were transfected with control siRNA or siRNA targeting ATP6L (Thermo Scientific Dharmacoen, Surrey, UK). 24 h after transfection, cells were used for further assays and the downregulation of ATP6L was proved by real-time PCR.
REFERENCES

**Fig. S1: Archazolid inhibits the acidification of lysosomes. (A) Formula of archazolid A/B [adapted from Huss et al. (8)]. (B) Both compounds inhibit V-ATPase in tumor cells lines. Archazolid A as well as B inhibit the V-ATPase in lysosomes. Acidic lysosomes were stained with LysoTracker (red) and the nuclei with Hoechst (blue). After 2h of treatment with the V-ATPase inhibitor archazolid B or A (5 nM) a strong decrease of the red fluorescence was visible indicating an increase of the lysosomal pH. Representative images out of three independent experiments are shown.**
A.1

- Migration [% of +control] vs. concentration of arch (nM)

- Apoptotic cells [%] vs. concentration of arch (nM)

A.2

- Migration [% of +control] vs. concentration of arch (nM)
Fig. S2: Archazolid inhibits cancer cell migration. (A) L3.6pl (A.1) and MDA-MB-231 (A.2) cells were pretreated with archazolid B for 16/24 h followed by a Boyden Chamber assay. Cells were fixed and analyzed. Bars represent the mean ± S.E.M. of three independent experiments performed in duplicates. *p < 0.05 (One Way ANOVA, Dunnett) (B) SKBR3 (B.1) and L3.6pl (B.2) were treated with archazolid B for 24 h. The confluent cell layer was scratched with a pipette tip. Cells were allowed to migrate without additional treatment. Cells were fixed and analyzed. Bars represent the mean ± S.E.M. of three independent experiments performed in triplicates. *p < 0.05 (One Way ANOVA, Dunnett).
**Fig. S3: EGF-R and Her2 expression on the cell surface after archazolid treatment.** SKBR3 cells were treated for 24 h with archazolid. Cells were stained with specific antibodies against EGF-R and Her2 and analyzed via flow cytometry. Representative histograms out of three independent experiments are shown. Bars represent the mean ± S.E.M. of three independent experiments, *p < 0.05 (One Way ANOVA, t-test).
Fig. S4: EGF-R plays a role in cell polarization. SKBR3 cells were treated with Erlotinib-HCl (EGF-R inhibitor, Selleckchem, Boston, USA) for 24 h. The confluent cell layer was scratched with a pipette tip. Cells were allowed to migrate for 5 h. Cells were fixed, permeabilized and stained for F-actin and EGF-R. Formation of lamellipodia is abrogated upon EGF-R inhibition. As a control Western blots of downstream targets were performed for each experiment. Representative images and a Western Blot out of three independent experiments are shown.
Fig.S5: The downregulation of the V-ATPase subunit c affects the cell migration process also in L3.6pl cells. L3.6pl cells were transfected with siRNA for 24h, followed by a Boyden chamber assay. As control, a Real-time PCR was performed. Bars represent the mean ± S.E.M. of three independent experiments performed in duplicates, *p < 0.05 (One Way ANOVA, Dunnett/t-test).
Fig. S6: Rac1-GFP is less localized in Rab5-induced enlarged endosomes of archazolid treated cells. Rab5WT and Rac1-GFP were overexpressed in Hela cells followed by a treatment with 10 nM archazolid B for 24 h. Cells were fixed with 4 % PFA and a fluorescence staining using an antibody raised Rab5. Samples were analyzed via confocal microscopy. Representative images out of three independent experiments are shown. Arrows indicate enlarged endosomes.
Fig. S7: Dissemination of 4T1-Luc breast cancer cells is decreased in archazolid A (1mg/kg) pretreated mice. (A) On day eight, mice were sacrificed through cervical dislocation and lungs were harvested. Bioluminescence signals were recorded by imaging the lungs separately dorsoventral and ventrodorsal. Quantitative results of the measurement (flux/area) see Figure 6B. Color bar scales were equalized. (B) The harvested lungs were weighed. Bars represent the mean ± S.E.M. of ten lungs per group, *p < 0.05 (t-test).
Fig. S8: Scheme proposing the impact of V-ATPase inhibition on cell migration. Inhibition of endosomal Rac1 localization and Rab5-dependent Rac1 activation by archazolid leading to repression of actin assembly, which finally results in cell migration inhibition.
Fig. S9: Beside the localization in lysosomes the V-ATPase is also localized at the plasma membrane. The V-ATPase is localized at the leading edge in SKBR3, but not in non-tumorous MCF-10A cells. In migrating SKBR3 cells, V-ATPase subunit c was stained for confocal microscopy. Representative images out of three independent experiments are shown.
**Fig. S10: Archazolid affects cellular recycling.** (A) Archazolid B inhibits Dextran-uptake. SKBR3 cells were treated for 1h with archazolid B. Cells were exposed to Dextran-rhodamine for 5 min followed by analysis at the fluorescence microscope (LSM, Zeiss). Representative images out of three independent experiments are shown. (B) Archazolid B inhibits the uptake of transferrin-rhodamine after longtime treatment. SKBR3 (B.1) and L3.6pl (B.2) cells were treated with archazolid B for different timepoints. Transferrin-rhodamine was added after 2 h starvation and cells were fixed after 5 min uptake. Samples were analyzed by confocal microscopy (LSM, Zeiss). Representative images out of three independent experiments are shown. (C) Archazolid B leads to the amplification and size augmentation of multivesicular bodies (*). SKBR3 cells were treated with 10 nM archazolid B for 30 h. Cells were embedded in Spurr low-viscosity epoxyresin and sections were done. Cells were further prepared for electron microscopy and pictures were taken. (N-nuclei, M-mitochondria, V-vacuoles, *- multivesicular bodies)