Supplementary figure 1: Relationship between LAMP3 gene copy number and LAMP3 gene expression. Scatter plot of LAMP3/CEP7 ratios and LAMP3 protein (A) or mRNA (B) expression in human cervix tumors. A straight line was fitted through the data. (Spearman correlation test) C) LAMP3/CEP7 ratios in normal human cervix tissue, either squamous (cases 1-4) or glandular (cases 5-8).

Supplementary figure 2: In vitro validation ME180 host cell lines. EGFP fluorescence in ME180 cells expressing a doxycycline-inducible eGFP reporter gene. Shown are fluorescence microscopy images following 0, 24 and 48 hours of doxycycline incubation (1 μg/ml).

Supplementary figure 3: In vivo validation ME180 host cell lines. The in vivo growth and metastatic potential of two ME180 host cell lines was tested: clone 10.6 and clone 10.8. Both cell lines stably express the Tetracycline repressor and contain a single copy of the Flp Recombination Target (FRT) site. These two host line-features allow for rapid generation of ME180 sublines with isogenic, doxycycline-inducible expression of a transgene. The cervix tumors established from the host clones were grown orthotopically for a total 21 days and were daily exposed to the hypoxia treatment from day 9. A) Size orthotopic xenografts after 21 days of growth. Each symbol represents a tumor from one animal. As for the parental ME180 cells, the tumors derived from both host lines were considerably smaller following the hypoxia treatment. B) Total number of metastatic lymph nodes and C) metastatic score. Both ME180 clone 10.6 and 10.8 tumors had maintained the potential to spontaneously metastasize to the regional lymph node chain in an oxygen dependent manner. D) Clone 10.8 was used to construct a ME180 subline with doxycycline-inducible expression of the eGFP reporter gene. The
orthotopic tumors were grown for 21 days and were daily exposed to the hypoxia treatment from day 9. E and F) EGFP expression and the doxycycline treatment did not affect the growth of the cervix tumors or nodal metastasis. Horizontal lines represent the mean values for each group.

Supplementary figure 4: Validation ME180-GADD34-C cells. A) Induction kinetics of doxycycline regulated expression of the hamster GADD34 C-terminus region in ME180-GADD34-C cells. Levels of protein expression were measured by immunoblotting in cells grown in the presence or absence of doxycycline for the indicated periods of time. EIF4E was used as a loading control. B) ME180-GADD34-C and EV control (EV) cells were preincubated with doxycycline for 72 hours (+dox) or left untreated (-dox), and subsequently exposed to 0, 6 or 16 hours of anoxia. CHOP mRNA expression was measured by qRT-PCR. Mean and SEM of three independent experiments is shown. (two-way ANOVA, p < 0.05, **p < 0.005, (-)dox EV vs rest)

Supplementary figure 5: Inhibition of UPR signaling suppresses hypoxia-induced lymph node metastasis. A) Total number of colonized lymph nodes in each animal. Raw data referencing to figure 3C in the main text. B) The metastatic score. Raw data referencing to figure 3D in the main text.

Supplementary figure 6: LAMP3 expression measured in stable LAMP3 knockdown cells. A) LAMP3 deficient and empty vector control cells were exposed to anoxia for 24 hours and LAMP3 expression was quantified by qRT-PCR. Mean and SEM of two independent experiments is shown. (two-way ANOVA, **p < 0.01) B) LAMP3 protein levels in stable
LAMP3 knockdown cells (shLAMP3) and empty vector control cells (EV control) exposed to 24 hours of anoxia. Asterisks indicate non-specific protein bands.

**Supplementary figure 7: LAMP3 expression in LAMP3 knockdown and empty vector control tumors.** LAMP3 protein expression was assessed by immunofluorescence staining.

**Supplementary figure 8: Knockdown of LAMP3 inhibits hypoxia-induced lymph node metastasis.**

A) Total number of colonized lymph nodes in each animal. Raw data referencing to figure 4C in the main text. B) The metastatic score. Raw data referencing to figure 4D in the main text.

**Supplementary figure 9: Human VEGF mRNA expression in ME180-GADD34-C orthotopic xenografts.** Relative VEGF mRNA levels were measured by qRT-PCR using human-specific primers. Bars represent the mean levels of ten GADD34-C cervix tumors per group. Error bars depict SD.

**Supplementary figure 10: Microvessel density and hVEGF expression in shLAMP3 orthotopic xenografts.**

A) The microvessel density of the xenografts was evaluated by immunofluorescence staining (CD31). Bars represent the mean levels of ten tumors per group. Error bars depict SD. B) VEGF mRNA levels were measured by qRT-PCR using human-specific primers. Bars represent the mean levels of ten cervix tumors per group. Error bars depict SD.
Supplementary figure 11: Hypoxic fraction shLAMP3 orthotopic xenografts. A) Fraction of EF5-labeled tumor area. Horizontal lines represent the mean values for each group. B) Correlation between hypoxic fraction and tumor size. A straight line was fitted through the data. (Pearson correlation test)

Supplementary figure 12: LAMP3 promotes colony formation in soft agar. A) HeLa empty vector (pLKO1) and stable LAMP3 knockdown (shLAMP3 #388 and #842) cells were seeded into soft agar and allowed to form colonies. Graph shows the number of formed colonies after 4 weeks of incubation. Bars represent the mean of 3 24-wells from 1 experiment. Error bars depict SEM. Unpaired Student t-test, two-tailed, *p < 0.05). B) Depicted is the relative LAMP3 mRNA abundance in HeLa cells used in the soft agar assay in (A).

Supplementary figure 13: Transwell migration LAMP3 deficient HeLa cells (see supplementary figure 12). Assay was performed as described in figure 6B in the main text. Bars represent the mean of 3 24-wells from 1 experiment. Error bars depict SEM. Unpaired Student t-test, one-tailed, *p < 0.05).

Supplementary figure 14: Scratch wound healing assay. A) Closure of the scratch wound by CaSki cervical carcinoma cells following transient transfection with two different short hairpins against LAMP3 (#388 and #842) or an empty vector (pLKO1). The mean of 12 96-wells from 1 experiment is shown for each time point. Error bars depict SEM. B) Relative LAMP3 mRNA abundance CaSki cells in (A). C) Closure of the scratch wound after transient overexpression of the full LAMP3 cDNA or the empty vector (pcDNA3-EV) in ME180 cells. Mean and SEM of
two independent experiments is shown. D) Relative LAMP3 mRNA abundance in ME180 cells transiently overexpressing the full LAMP3 cDNA or the empty vector.

**Supplementary materials and methods**

*Western blot analysis*

Blots were probed with anti-GADD34 (sc-794, Santa Cruz), anti-eIF-4E (610270, BD Transduction Laboratories), anti-CHOP (MA1-250, Thermo Scientific), anti-LAMP3 (AF4087, R&D Systems), anti-phospho-eIF2α (Ser51) (3597, Cell Signaling) and anti-eIF2α (AHO1182, Invitrogen) detecting total eIF2α. Bound antibodies were visualized using HRP-linked secondary antibodies (anti-goat (Invitrogen), anti-mouse and anti-rabbit (GE healthcare)) and ECL luminescence (Pierce).

*Clonogenic assay*

Single cells were plated for clonogenic survival at a range of 100-400 cells per 10-cm dish in triplicate and were allowed to adhere for 6 hours before being exposed to anoxia for the indicated time periods. Colony formation was continued for 11-14 days at 21% O₂. Colonies were stained with methylene blue (Sigma). 50 cells or more were considered colonies.

*Immunohistochemical staining*

The expression of LAMP3 in orthotopic xenografts and cervix cancer patient biopsies was investigated as follows: Frozen sections (5 μm) were fixed for 20 min with 2% paraformaldehyde, rinsed 3 x 5 min each with PBS and then incubated with anti-LAMP3 antibody (AF4087, R&D Systems) at a 50-fold dilution overnight at room temperature in a
humidity chamber. Slides were rinsed 3 x 5 min each with PBS and incubated with donkey anti
Goat Cy3 at a 100-fold dilution for 1 h at room temperature in a humidity chamber. Slides were
then rinsed 3 x 5 min each with PBS and counterstained with DAPI at a concentration of 1 lg/ml
for 5 min. Samples were then rinsed 3 x 5 min each with PBS. Images were captured on the
TISSUEscope 4000 slide scanner (Biomedical Photometrics) at 0.5 μm resolution. Tumor-
infiltrating, LAMP3-positive, mature dendritic cells which show an intense, dot-like staining
pattern were excluded from the analysis by applying an upper LAMP3 signal threshold.

Spheroid formation and migration
Multicellular tumor spheroids were acquired as follows: V-shaped 96-wells plates (Greiner Bio
One) were coated with 0.5% (w/v) poly-HEMA (Sigma Aldrich) in 95% ethanol. The ethanol
was allowed to evaporate for 3 days. Per well 10000 cells were added, after which the plate was
centrifuged for 10 min at 1000 x g. Cells were allowed to form spheroids for 24 hours. Next,
spheroids were transferred to a flat-bottomed 96-wells plate (Greiner Bio One) and allowed to
migrate. Images were obtained using a Leica DM 6000 fluorescence microscope in combination
with IP-lab imaging software (Scanalytics Inc, Fairfax, VA, USA).

Transwell migration assays
Transwell migration assays were performed using non-coated inserts with 8 μm pores in a 24-
wells format (Greiner Bio One). Before the start of the assay, cells were deprived of serum
overnight. Per insert 40000 cells were seeded in the upper compartment. Serum containing
medium was added to the lower compartment as a chemoattractant. Cells were allowed to
migrate for 24 hours. For hypoxic assays this migration was performed under hypoxic
conditions. Afterwards, membranes were fixed in 70% ethanol and stained with 0.5% (w/v) crystal violet (Sigma Aldrich). Cells that had not migrated to the lower side of the membrane were removed with a cotton swab. Staining was solubilized with 1% (w/v) Sodium Dodecyl Sulphate (SDS, Sigma Aldrich) and absorbance was measured at 595 nm.

**FISH analysis**

RP11-1012H23 clone was selected from the Human UCSC Genome Browser assembly (Feb.2009 CRch37/hg19). The clone starts at 182,724,231 and ends at 182,902,423 bps on chromosome 3 thus covering the region of the LAMP3 gene (starts at 182,840,004 ends at 182,880,667). The BAC clone was directly labeled with SpO fluorochrome using a commercially available nick translation kit according to the manufacturer’s instructions (Abbott Laboratories).

TMA slides were deparaffinized in xylenes and incubated in citric buffer (pH=6.0) for 55 minutes at 80°C, followed by pepsin (Sigma) digestion (75,000 U in 0.01N HCl) for 22-25 minutes at 37°C. Probes and target tissues were codenatured for 7 minutes at 75°C and hybridized for two days at 37°C. After washings the slides were counterstained with 4',6-Diamidino-2-phenylindole, dihydrochloride (DAPI). The FISH analysis was performed on a Zeiss Imager.M1 microscope (Carl Zeiss Canada Ltd., Canada) equipped with a camera (JAI Inc., San Jose, CA, USA) and the MetaSystems Isis FISH Imaging System (MetaSystems GmbH, Altlussheim, Germany).

**Soft agar assay**

100 cells were suspended in 0.5 ml of 0.35% agarose (low gelling temperature; Sigma Aldrich, A9045) dissolved in MEM Alpha supplemented with 10% fetal bovine serum and overlaid onto a
0.5% agar solution in 24-well plates. Plates were incubated at 37°C in a humidified incubator for 4 weeks, after which images were taken using a Leica MZ FLIII fluorescent stereomicroscope. ImageJ was used to quantify the colonies.

*Scratch wound healing assay*

Cells were grown to confluence in a 96-well Essen ImageLock plate. A scratch wound was created using the 96-pin WoundMaker (Essen BioScience). Closure of the wound was monitored in real-time using the IncuCyte ZOOM (Essen BioScience).