Figure S1. DNA fragmentation in EGF- or LL-37-treated OTSCC HSC-3 cells. DNA fragmentation in control, EGF- or LL-37-treated HSC-3 cells was determined after 48 h using the *In situ* Cell Death Detection Kit. The nuclei were stained with DAPI. Scale bar, 50 µm. EGF, epidermal growth factor; OTSCC, oral tongue squamous cell carcinoma; LL-37, antimicrobial peptide leucine-leucine-37.
Figure S2. Immunofluorescence staining of EGFR in EGF- or LL-37-treated OTSCC HSC-3 cells. Cells were starved for 24 h and stimulated with the indicated doses of EGF or LL-37 for 1 h. The nuclei were stained with DAPI. Scale bar, 50 µm. EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; OTSCC, oral tongue squamous cell carcinoma; LL-37, antimicrobial peptide leucine-leucine-37.
Figure S3. Immunoblot analysis of EMT markers in EGF- or LL-37-treated OTSCC cell lines and IHGK cells. Cells were treated with the indicated doses of EGF or LL-37 for 48 h. Cell homogenates (30 µg of soluble protein) were separated on SDS-PAGE gels. The amount of the epithelial marker E-cadherin (E-cad) and the mesenchymal marker vimentin was analyzed with immunoblots in (A) HSC-3, (C) SCC-25, (E) SAS and (G) IHGK cells. In A, C, E and G, representative immunoblots are shown. β-actin was used as a control to normalize the quantities of proteins in (B) HSC-3, (D) SCC-25, (F) SAS and (H) IHGK cells using Fiji software. The results represent average ± SD of two independent experiments, separated three times on SDS-PAGE gels. P-values were calculated with the independent-samples Kruskal-Wallis test and significance was adjusted by the Bonferroni correction for multiple tests. *P<0.05, compared with the control. EMT, epithelial-mesenchymal transition; EGF, epidermal growth factor; OTSCC, oral tongue squamous cell carcinoma; LL-37, antimicrobial peptide leucine-leucine-37.
Figure S4. Zymography of EGF- or LL-37-treated OTSCC cell lines and IHGK cells. Cells were treated with the indicated doses of EGF or LL-37 for 24 h. In zymography, conditioned concentrated media of (A) HSC-3, (C) SCC-25, (E) SAS and (G) IHGK cells were separated on SDS-PAGE casted in the presence of fluorescently labelled gelatin. (A) Purified gelatinase standards are shown on the left: pro-MMP2 (72 kDa), active MMP2 (62 kDa), pro-MMP9 (92 kDa) and active MMP9 (82 kDa). In A, C, E and G, a representative zymogram gel is shown. The amount of pro-MMP2 and MMP9, and active MMP2 in (B) HSC-3, (D) SCC-25, (F) SAS and (H) IHGK zymogram gels was quantified with Fiji software, and intensities were normalized to the cellular soluble protein concentration. The results represent the average ± SD of two separate sample sets each analyzed three times. P-values were calculated with the independent-samples Kruskal-Wallis test and significance was adjusted by the Bonferroni correction for multiple tests. *P<0.05, compared with the control. MMP, matrix metalloproteinase; EGF, epidermal growth factor; OTSCC, oral tongue squamous cell carcinoma; LL-37, antimicrobial peptide leucine-leucine-37.
Figure S5. Kaplan-Meier survival curves. Kaplan-Meier cumulative curves for (A) overall, (B) disease-specific and (C) disease-free survival of patients with OTSCC according to expression of hCAP18/LL-37. No significant difference was present in survival times between the high and low hCAP18/LL-37 expression groups (P>0.05). hCAP18/LL-37, human host defense cationic antimicrobial peptide-18/anti-microbial peptide leucine-leucine-37; OTSCC, oral tongue squamous cell carcinoma.