**Supplementary Methods**

**IHC for mouse tumor xenografts**

Protocols for all animal studies were approved by the Norwegian Animal Research Authority (Project ID: 20124236). Tongue of each mouse were formalin fixed, paraffin embedded, cut into 5 micron sections and stained with HE, anti-S100A16 (11456-1-AP, Proteintech, 1:80 dilutions), anti- involucrin (NCL-INV, Novacastra, 1:500 dilutions), anti-Ki67 (M7240, clone MIB-1, DAKO, 1:1000 dilutions), anti-Bmi-1 (05-637, Millipore, 1:150 dilutions) antibodies. IHC for all of these antibodies were done essentially as described for human tissue specimens, except that citrate buffer pH 6 (S1699, DAKO) was used for the antigen retrieval of involucrin, Ki67 and Bmi-1.

**S100A16 IHC evaluation**

The P score (number of S100A16 positive cells) was determined as follows: 0, when there were 0 to 25% positive cells; 1, when there were 26 to 50 % positive cells; 2, when there were 51 to 75 % positive cells and 3 when there were >75 % positive cells. The L score (membranous or/and cytoplasmic localization of S100A16) was determined as follows: 2, if the proportion of cells with membrane to cytoplasmic staining was >1; 1, if the proportion was equal to 1; and 0.5, if the proportion was <0.5. The I score (intensity of S100A16 staining) was calculated as follows: 2, if the proportion of cells with strong to weak S100A16 intensity was >1; 1, if the proportion was equal to 1; and 0.5, if the proportion was <1. The final (PLI) score was calculated by multiplying the individual P, L and I scores and averaging PLI scores of the three evaluated fields. According to this scoring system, the NHOM is supposed to express the highest PLI score (12) with a maximum of P, L and I scores.

**Laser microdissection of FFPE specimens**

Briefly, 15 micron thick sections of FFPE specimens were placed on the glass slides (MembraneSlide NF 1.0 PEN, Zeiss, Germany) activated with UV light. Slides were then incubated at 56 °C for 2 hours, de-paraffinized in xylene, rehydrated in graded ethanol, stained with methylene green (S1962, DAKO), and dehydrated in reverse graded ethanol and xylene. Fifty to hundred µm2 tissue specimens of NHOM, ODL and paratumor epithelium, tumor center and the corresponding invading front/islands of each OSCC specimen were laser microdissected using a Zeiss Axiovert 200 inverted microscope equipped with a microlaser system (P.A.L.M Microlaser Technologies). Microdissected tissues were collected in nuclease free tubes (AdhesiveCap 500 clear, Zeiss) and subjected to RNA extraction.

**Construction of S100A16 expression and shRNA vectors and transfection**

Human cDNA encoding *S100A16* was amplified using primers pairs (F: 5' –ATCCCGCGGCAGGGAGATGTCAGACTGCTA-3' and R: 5'-TGAGGATCCCTAGCTGCTGCTCTGCTG-3') and subcloned into the pRetroX-IRES-ZsGreen1 retroviral expression vector (Clontech Laboratories, Inc., CA, USA). shRNA targeting *S100A1*6 mRNA was constructed using the following oligonucleotides: (F: 5’- GATCCCCGAACAAGATCAGCAAGAGCAGCTTCAAGAGAGCTGCTCTTGCTGATCTTGTTCTTTTTGGAAA -3’; R: 5’-AATTTTTCCAAAAAGAACAAGATCAGCAAGAGCAGCTCTCTTGAAGCTGCTCTTGCTGATCTTGTTCGGG -3’). Oligonucleotides were annealed and inserted in the RNAi-Ready pSIREN-RetroQ-DsRed-Express expression vector (cat. no: 632487, Clonetech). shRNA targeting *LacZ* gene was used as a control for the S100A16-shRNAs. Cancer cell-lines were infected with the retroviruses derived from packaging (Phoenix A) cells, sorted (DsRed as a marker), propagated, verified for knockdown of S100A16.

**RNA extraction, cDNA synthesis and qRT-PCR**

Frozen specimens of NHOM and OSCC were stored at -80 °C until mRNA extraction (Dynabeads mRNA Direct kit, Invitrogen) and cDNA synthesis (Transcriptor cDNA kit, Roche). RNeasy FFPE Kit (#73504, Qiagen) was used to extract RNA from laser microdissected tissues of NHOM, ODL and OSCCs. qRT-PCR amplification of *S100A16* mRNA was performed in duplicates in the LightCycler 480 qPCR system (Roche) using LightCycler® 480 Probes Master (#04707494001, Roche).*GAPDH* and *ACTB* were used as endogenous controls.

Total RNA was extracted from the RAC, LAC and MAC cells and p75NTRhigh and p75NTRlow CaLH3 cells using RNeasy fibrous tissue mini kit protocol (cat no: 74704, Qiagen Inc.). Following manufacturers’ instructions, 200-300 nanograms of total RNA was converted to cDNA using High-Capacity cDNA Archive Kit system (cat no: 4368814, Applied Biosystems). qRT-PCR amplifications was performed on ABI Prism Sequence Detector 7900 HT (Applied Biosystems) in triplicates as described previously [[1](#_ENREF_1)]. For details of the TaqMan assays used, see supplementary Table S1. *GAPDH* was used as endogenous control. Comparative 2-ΔΔ Ct method was used to quantify the relative mRNA expression.

**Fluorescent activated cell sorting (FACS) analyses for P75NTR and cytokeratin 13**

For P75NTR cell sorting, cells were trypsinized, washed and resuspended in PBS containing 1% FBS and 1% HEPES buffer and incubated with mouse monoclonal anti-P75NTR antibody (Sigma Aldrich, 1:250 dilutions) for 10 minutes in ice. Mouse IgG1 (DAKO) was used as an isotype control. Alexa Fluor® 488 F(ab1)2 fragment of goat anti-mouse H+L (Invitrogen) was used as the secondary antibody. FACS sorting was done in BD FACSAria TM IIu (BD biosciences) using 550/50 BP Filter. The 4-5% of cells with the highest and the lowest expression of P75NTR were designated respectively as the p75NTRhigh and p75NTRlow cell subsets. Post-sort was performed to ensure the quality of sorting.

For cytokeratin 13 staining, cells were trypsinized, washed and fixed with cold (-20 °C) methanol for 10 minutes, incubated with anti-cytokeratin 13 (Novacastra, 1:350 dilutions) antibody for 30 minutes at room temperature. Mouse IgG1 (DAKO) was used as an isotype control. Alexa Fluor® 647 goat anti-mouse H+L antibody (Invitrogen) was used as secondary antibody. FACS analysis of the stained cells was done in *Accuri6* cytometer (BD Biosciences). All FACS analyses were repeated three times and at least 10000 events were analyzed for each sample.

**Reference for supplementary methods:**

1. Sapkota D, Bruland O, Costea DE, Haugen H, Vasstrand EN, Ibrahim SO: **S100A14 regulates the invasive potential of oral squamous cell carcinoma derived cell-lines in vitro by modulating expression of matrix metalloproteinases, MMP1 and MMP9**. *Eur J Cancer* 2011, 47:600-610.

**Supplementary Tables**

Table S1. S100A16 expression and clinicopathological variables of the OSCC patiensts.

 PLI score at tumor center\*

Variables Low, n (%) High, n (%) *P*

Age\*\* (years)

 ≤64 16 (53.3) 14 (46.7) 0.399

 >64 15 (42.9) 20 (57.1)

Gender

 Female 9 (42.9) 12 (57.1) 0.590

 Male 22 (50.0) 22 (50.0)

Location

 Tongue 13 (41.9) 18 (58.1) 0.669

 Gingiva, buccal mucosa & oral lip 12 (52.2) 11 (47.8)

 Floor of mouth & oro-pharynx 6 (54.5) 5(45.5)

Differentiation

 Poor and moderate 17 (48.6) 18 (51.4) 0.878

 Well 14 (46.7) 16 (53.3)

Lymph node involvement

 Negative (N0) 18 (47.4) 20 (52.6) 0.951

 Positive (N1 & N2) 13 (48.1) 14 (51.9)

Tumor size

 T1 & T2 20 (55.6) 16 (44.4) 0.157

T3 & T4 11 (37.9) 18 (62.1)

Recurrence

 No 20 (43.5) 26 (56.5) 0.290

 Yes 11 (57.9) 8 (42.1)

Tumor stage

 Early (1 & 2) 13 (61.9) 8 (38.1) 0.113

Late (3 & 4) 18 (40.9) 26 (59.1)

\*OSCCs were stratified in to high and low S100A16 expression groups by using median S100A16 PLI score as a cut-off, \*\* patients were categorized into low- and high-age groups based on the median age.

Table S2. Details of the TaqMan assays used for qRT-PCR

Target Gene Protein encoded TaqMan assay ID

*S100A16* S100A16 Hs00293488\_m1

*IVL* Involucrin Hs00902520\_m1

*KRT10* Cytokeratin 10 Hs00166289\_m1

*MMP1* MMP1 Hs00233958\_m1

*MMP9* MMP9 Hs00957562\_m1

*GAPDH* GAPDH Hs99999905\_m1

*ACTB* Beta-actin Hs01060665\_g1

Table S3. Details of the antibodies used for immunoblotting

Target Species Catalog / Soruce Dilution

S100A16 P (rabbit) 11456-1-AP / Proteintech 1/500

InvolucrinM NCL-INV / Novacastra 1/200

Cytokeratin 13MNCL-CK13 / Novacastra 1/50

Cytokeratin 13Msc-58721 / Santa Cruz 1/200

Cytokeratin 10Msc-53253 / Santa Cruz 1/200

FilaggrinP (rabbit)sc-30229 / Santa Cruz 1/200

Transglutaminase 1P (rabbit)CVL-PAB0061 / Covalab 1/100

Bmi-1 M 05-637 / Millipore 1/1000

Oct 4 P (rabbit) sc-9081 / Santa Cruz 1/200

p38 P (rabbit) sc-7149 / Santa Cruz 1/200

p-p38 P (rabbit) sc-7149 / Santa Cruz 1/200

GAPDH P (rabbit) sc-25778 / Santa Cruz 1/5000

M monoclonal; P polyclonal