Supplement Results, Figures and Tables

Results

PET imaging

The SUV was significantly higher in tumours of the parental HSC-3 cell line than tumours of the EV and the MMP-8 overexpressing cell lines (Figure S4B upper panel), suggesting that the cloning procedure somehow affected the metabolism of the cells. Tongue tumours normally metastasize to lymph nodes in the neck, which overlap the sublingual and parotid salivary glands in mice. Salivary glands give background signal in ^{18}F-FDG PET-scans due to their metabolic activity. To identify lymph node metastases, the salivary gland area was delineated and the ^{18}F-FDG uptake in left and right salivary gland areas were compared **(**Figure S4A lower panel). In mice with tumours of the HSC-3 parental and control cell lines, there was more uptake in the left side compared to the right side, although the differences were not statistically significant (Figure S4B, lower panel). In mice injected with the MMP-8+ HSC-3 cells, the ^{18}F-FDG uptake was similar on the left and right side. However, the differences were not statistically significant. Figure S4C shows representative time-activity curves of ^{18}F-FDG uptake in liver, tumour and in the left and right salivary glands in an OSCC xenograft mouse based on a 60 min dynamic PET scan. This illustrates that the 20 min static scans, starting at about 40 min after ^{18}F-FDG injection, were within a stationary phase of the ^{18}F-FDG uptake.

Figures

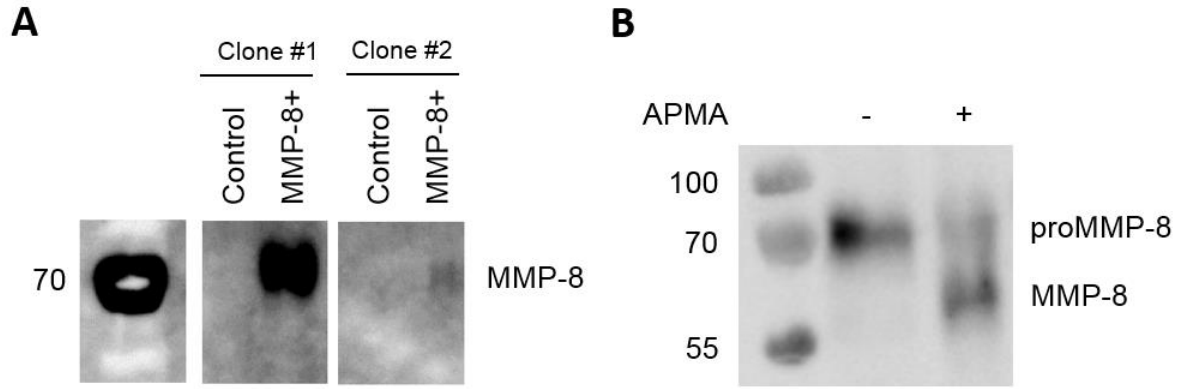


Figure S1. The overexpressed proMMP-8 level is higher in conditioned media of HSC-3 clone #1 compared to clone #2, and pro-MMP-8 was activated after treatment with APMA in MMP-8+ cells. Clone #1 of MMP-8+ HSC-3 cells showed higher expression of MMP-8 compared to the clone #2 cells in Western blot analysis (**A**). APMA activation led to reduction of proMMP-8 (~70kDA) and increase of mature MMP-8 (~58kDa) as shown by Western blot (**B**).

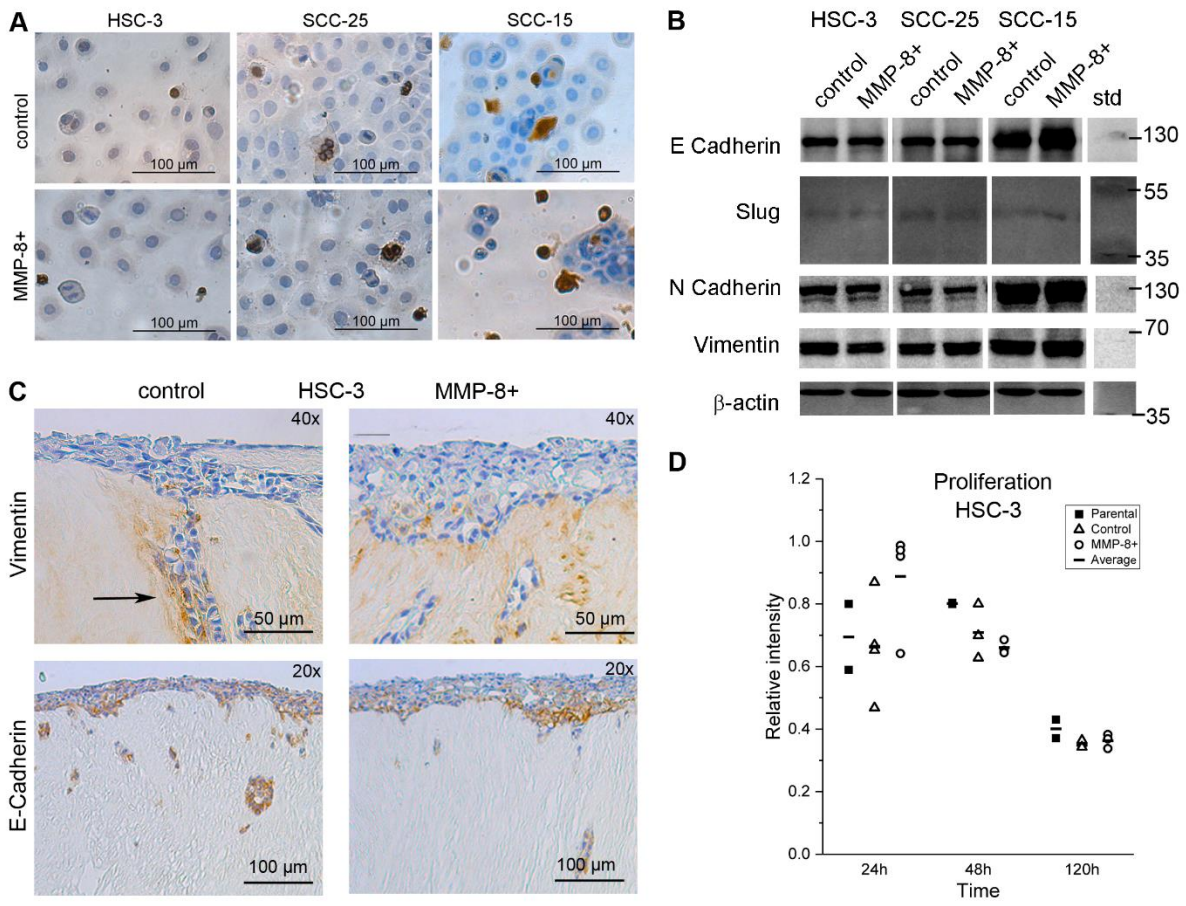


Figure S2. Apoptosis, EMT markers and proliferation in control and MMP-8+ OTSCC cells. Apoptosis was studied by TUNEL technology from cells seeded into wells of 96-well plates as described in the methods (**A**). The levels of E-cadherin, Slug, N-cadherin and vimentin were not changed in cell extracts (40 µg protein) studied by Western blot from control and MMP-8+ HSC-3, SCC-25 and SCC-15 cells cultured in monolayers. Representative results are shown (**B**). Levels of immunohistochemically detected vimentin was slightly reduced in MMP-8+ HSC-3 cells in organotypic 3D tissue cultures. In control cells, vimentin was stained in invasive cells (arrow), but the staining disappeared from invasive MMP-8+ HSC-3 cells; the amount of E-cadherin was not changed (**C**). Proliferation of HSC-3 cells was studied by BrdU kit. Two to four independent experiments (with different cell passages) were studied at each time point (**D**). Six samples of each cell group were analysed in the TUNEL assay. Western blots were performed using cell extracts from four different individual experiments. The myoma experiment was performed once with triplicate myoma disks per culture condition.

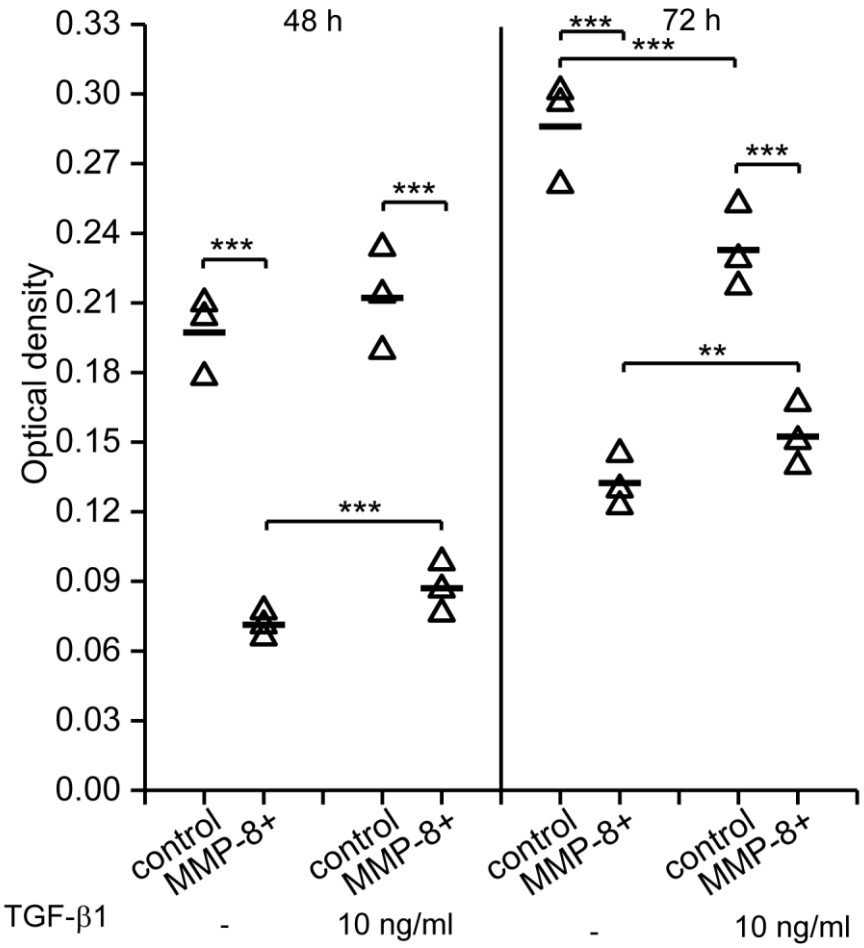


Figure S3. MMP-8 overexpression reduced the migration of HSC-3 cells and altered the response to TGF-β1 in clone #2. 10 ng/ml of recombinant human TGF-β1 was added to the serum-free medium in the upper Transwell® chamber and the amount of migrated MMP-8+ HSC-3 and control cells was measured as absorbance at 650 nm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

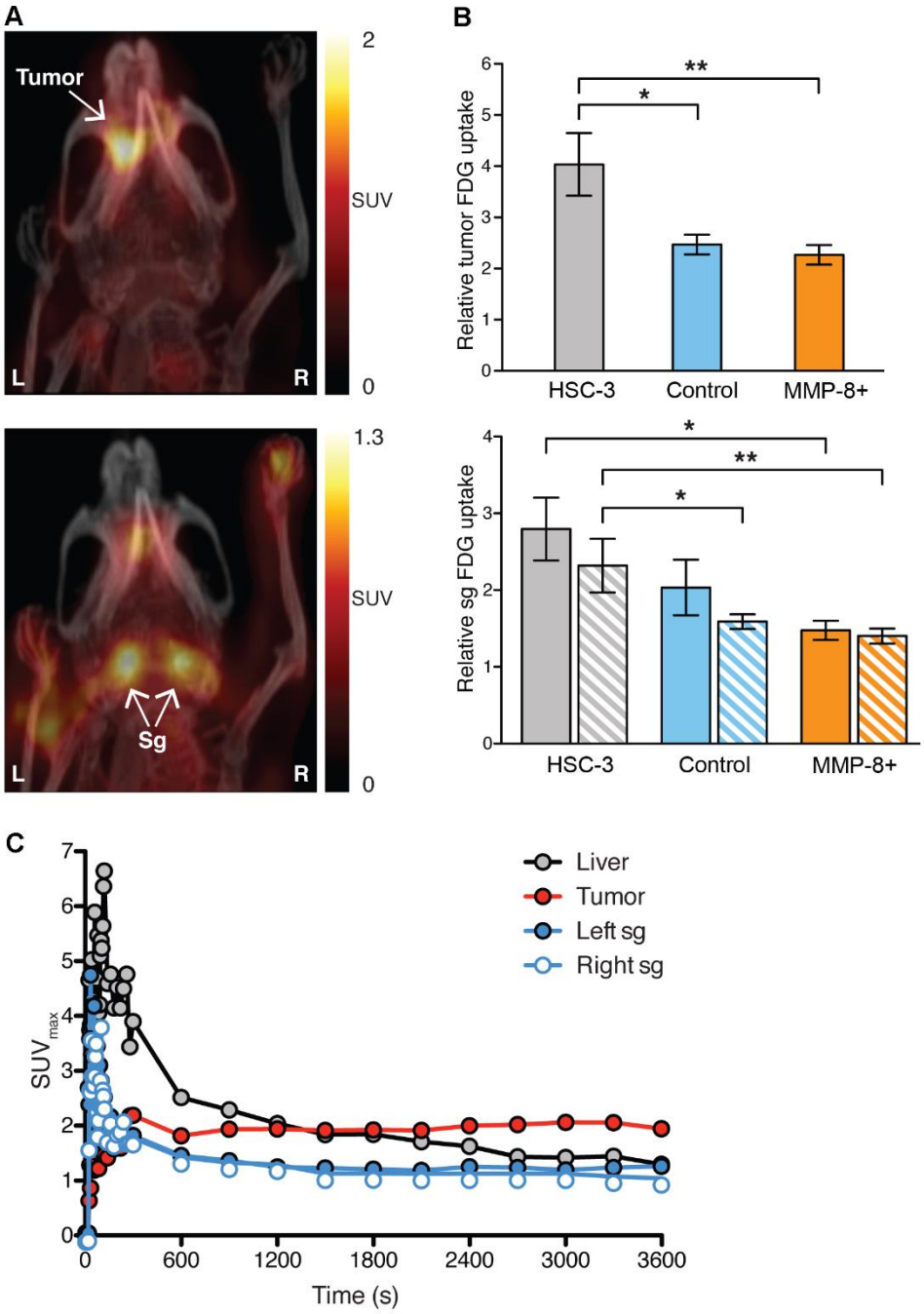


Figure S4. Evaluation of ^{18}F-FDG uptake and temporal pattern of ^{18}F-FDG uptake in OSCC xenograft mice (example graph). Coronal PET sections co-registered with a maximum intensity projection (MIP) CT image of an OSCC xenograft mouse showing average tumour and salivary gland (Sg) ^{18}F-FDG uptake 40-60 min post injection (**A**). Tumour and salivary gland ^{18}F-FDG uptake (SUV_{max}) relative to liver uptake (SUV_{mean}) in the different OTSCC cell groups. Lower panel, left salivary glands (filled bars) and right salivary glands (shaded bars). Results are mean ± SEM. *p<0.05, **p<0.01. Sg, salivary glands (**B**). An example graph shows representative time-activity curves of ^{18}F-FDG uptake in liver (grey symbols), tumour (red symbols), left (blue closed symbols) and right (blue open symbols) salivary glands (sg) in an OTSCC xenograft mouse (MMP-8+ HSC-3 group) during a 60 min PET acquisition (**C**).

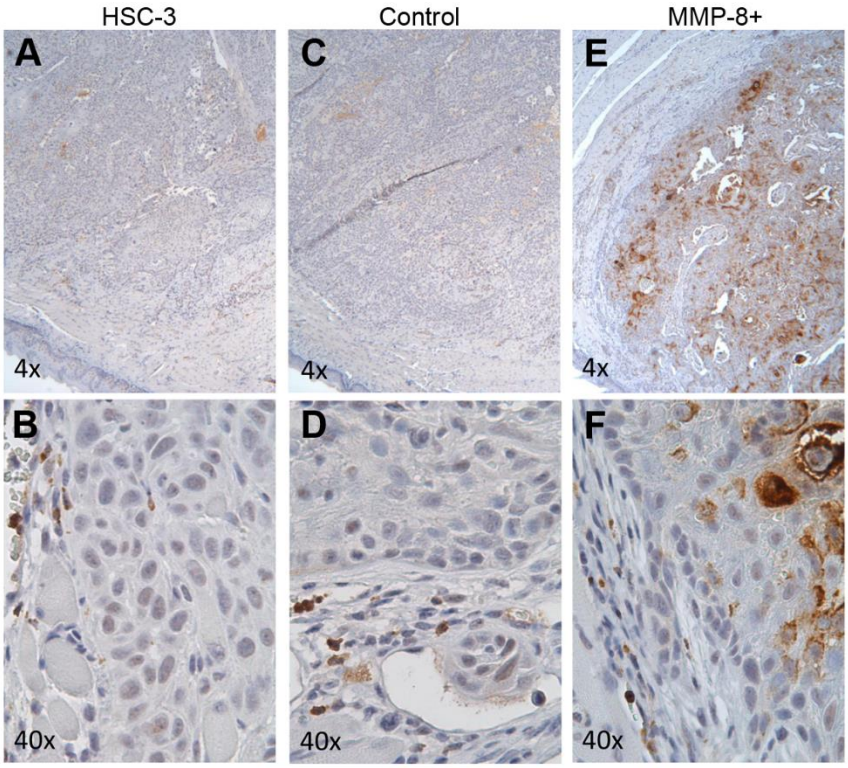
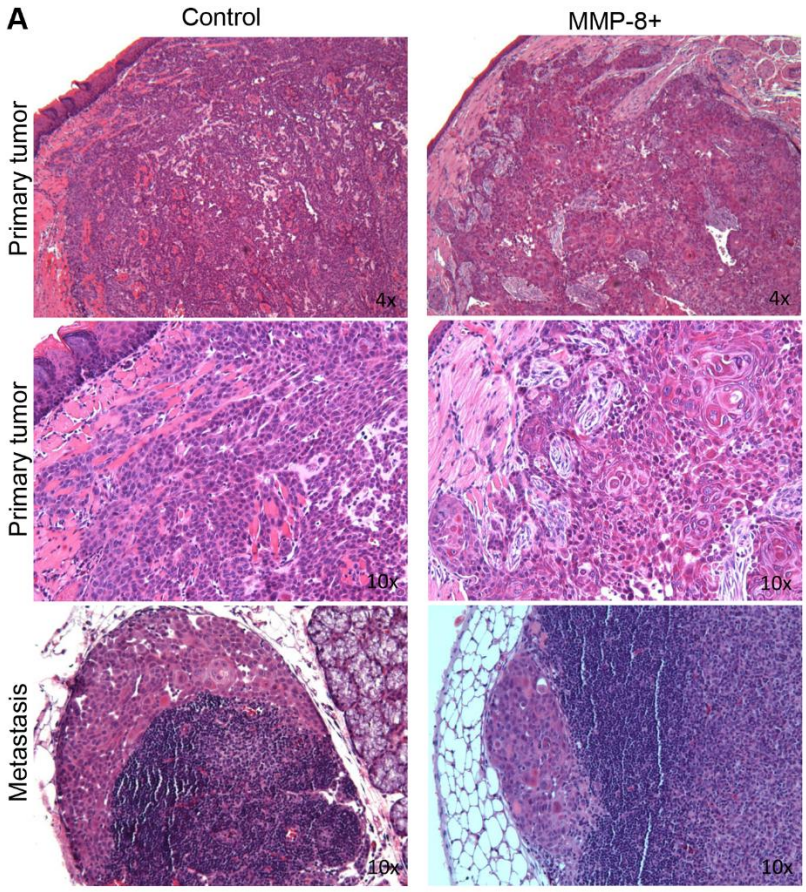


Figure S5. MMP-8 immunohistochemical staining of HSC-3 tumours in mouse tongues. Tumours of the HSC-3 parental cell line (**A-B**) and the control cell line (**C-D**) did not show cytoplasmic MMP-8 staining of the cancer cells. In the tumours of MMP-8 overexpressing cells, MMP-8 staining was mainly seen in the cells located in the centre of tumour islands. In the tumour-stroma interface, the cancer cells showed weak or absent cytoplasmic MMP-8 staining. Some of the mouse inflammatory cells were positive for MMP-8 (**E-F**). Some nuclear staining could be seen in the cancer cells of all clones.



B The % (n) of mice with lymph node metastasis (after 13 days)

	Positive % (n)	Unsure % (n)	Negative % (n)	Total (n)
Parental	44.4 (4)	11.1 (1)	44.4 (4)	100 (9)
Control	50.0 (5)	20.0 (2)	30.0 (3)	100 (10)
MMP-8+	55.6 (5)	11.1 (1)	33.3 (3)	100 (9)

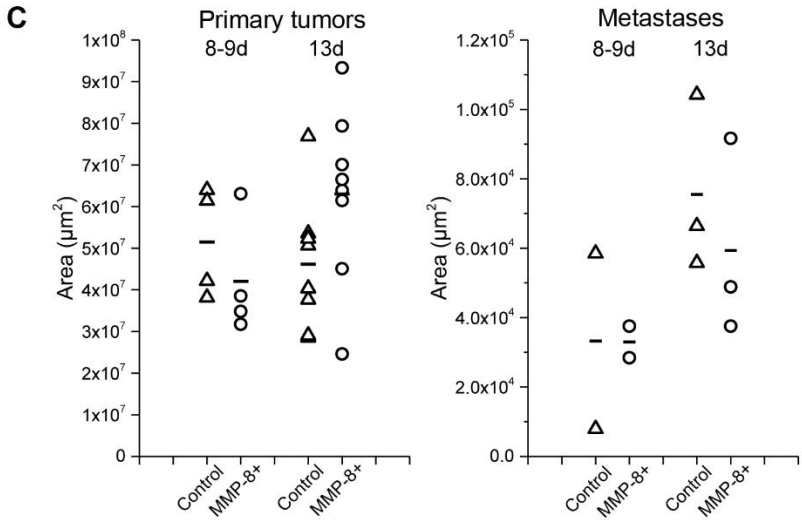


Figure S6. H/E staining of the primary tumours and lymph node metastasis in mice with MMP-8+ and control HSC-3 clones. Representative primary tumours and lymph node metastases in mice with MMP-8+ and control cells (**A**). The lymph nodes were analysed after 13 days to count the number of mice with and without metastases (**B**). The areas of primary tumours and metastases were measured (**C**).

Tables

Table S1. Patients' clinical data (OTSCC samples used in the VEGF-C and MMP-8 analyses).

Baseline patient characteristic

Patient clinical data		
	n	**%**
Total		
	57	**100.0**
Age at diagnosis		
<55 yrs	20	35.1
55-70 yrs	14	24.6
>70 yrs	23	40.4
Sex		
Male	25	43.9
Female	32	56.1
Tumour grade		
1	22	38.6
2	29	50.9
3	6	10.5
Tumour stage		
1-2	31	54.4
3-4	26	45.6
Neck lymph nodes		
Negative	43	75.4
Positive	14	24.6
Recurrence		
No	38	66.7
Yes	19	33.3
Adjuvant therapy		
No	38	66.7
Radiotherapy	16	28.1
Missing	3	5.3

Table S2. Differentially expressed genes in MMP-8 overexpressing stationary and migrating cells.

Top 20 genes upregulated in MMP-8 overexpressing cells compared to controls	
Stationary cells (43[a])	**Migrating cells (40[a])**
GJB6[b], CXCL14, FOS[b], KRT13, PHC1, GJB2, EGR1, MEST[b], KLK13[b], TUSC1[b], S100A8, TENM2[b], BLNK, ARMCX1[b], MAGED1[b], KLK10, ID1, GJA1[b], BCHE[b], MCAM	GJB6[b], TUSC1[b], TENM2[b], KLK10, KLK13[b], S100A8, MAGED1[b], EGR1, MEST[b], IL17D[b], GJB2[b], SCEL[b], BCHE[b], GAPDHS[b], NRG1, GAPDHS[b], NRG1, IL1RN, VGLL1, IL27RA[b], ARMCX1[b], FLRT3[b]
Top 20 genes downregulated in MMP-8 overexpressing cells compared to controls	
Stationary cells (40[a])	**Migrating cells (39[a])**
MMP1[b], RBM15B[b], MALAT1[b], LIMCH1[b], L1CAM[b], NNMT[b], CFB, LYN[b], C3[b], KLRC1/2, MAP2[b], SRPX[b], TNFAIP3, FAM20C[b], POLI[b], UGT1A1/3/4/5/6/7/8/9/10, BIRC3, C18orf54, NRG1, SNHG12	**MMP1**[b], L1CAM[b], NNMT[b], MAP2[b], AGPAT9, CFB, LYN[b], POLI[b], C3[b], KLRC1/2, PRTFDC1[b], ALS2CL[b], FLNC, UGT1A1/3/4/5/6/7/8/9/10, MUC1, GRIP2[b], FADS3, C18orf54[b], CROCC, POMZP3/ZP3
Changed genes only in migrating or stationary cells, top 15 ↑=upregulated, top 15 ↓= downregulated	
MMP-8 overexpressing cells vs. control cells	
Change only in stationary cell (42[a]): **↑**: CXCL14, FOS, KRT13, PHC1, BLNK, MCAM, LTBP1, NIN, TFCP2L1, HES1, FAM213A, CHCHD10, C6orf62, SSBP3, BCL11B **↓:** RBM15B, MALAT1, LIMCH1, SRPX, TNFAIP3, FAM20C, BIRC3, SNHG12, ABLIM3, SLC7A11,SLC39A8, PPAP2B, INSIG1,TWIST1, SLC47A2	
Change only in migrating cells (37[a]): **↑:** IL17D, SCEL, GAPDHS, TBX1, DOCK1, KREMEN1, MMP9, BAK1, ELAVL2, PTPLB, FAM89A, FAM101B, ASNS, MRPL43, TFPI2 **↓:** AGPAT9, PRTFDC1, FLNC, MUC1, ALS2CL, FADS3, POMZP3/ZP3, CROCC, SERPINA1, TMEM123, LOC284591, IL7R, CSRP2, NEXN, AKR1B10	

[a]Total number of changed genes (approx.) with fold change value 1.5.

Table S3. Differentially expressed genes in MMP-8 overexpressing cells involved in cancer-related events.

The changed genes (MMP-8+ vs. control cells) that are involved in cancer-related cell processes

Function	**Overexpressed**		**Underexpressed**	
	Stationary cells	Migrating cells	Stationary cells	Migrating cells
Actin cytoskeletal organisation		FLRT3, LAMA2	LIMCH1	
Cell adhesion	HES1, FLRT3	BCAM	L1CAM, OLR1	L1CAM
Cell motion	ID1	TBX1, GAPDHS, ID1	IL8, L1CAM, **VEGFC,** PPAP2B, TWIST1, NDE1	L1CAM
Transcriptional regulation	EGR1, HES1, VGLL1, MAGED1, FOS, ID1, BCL11B, TFCP2L1	ELAVL2, TBX1, EGR1, MAGED1, ID1, VGLL	SOD2, TWIST1, LRF1, YAP1, ABLIM3,	YAP1, SOD2, TRIM22
Regulation of cell growth		NRG1	NRG1, PAPPA2	
Inflammatory / immune resp.	S100A8 IL1RN	S100A8, IL1RN, IL27RA	CFB, LYN, IL8, PTX3, OLR1, C3	CFB, SERPINA1, C3, IL7R, LYN, TRIM22
Proteolysis	KLK10, KLK5	KLK10, KLK13, KLK5, **MMP9**	ECE1, ISG15, SENP7 OLR1, CFB, PAPPA2 TNFAIP3, **MMP1**, C3	CFB, **MMP1**, C3

Table S4. Clinical correlations with tumour MMP-8 and VEGF-C status.

	MMP-8 expression			**VEGF-C expression**		
	Weak, n (%)	Strong, n (%)	*p*-value	Weak, n (%)	Strong, n (%)	*p* value
Age at diagnosis						
<55 yrs	9 (45.0)	11 (55.0)		11 (55.0)	9 (45.0)	
55-70 yrs	11 (78.6)	3 (21.4)		7 (50.0)	7 (50.0)	
>70 yrs	14 (60.9)	9 (39.1)	0.144	10 (43.5)	13 (56.5)	0.751
Sex						
Male	13 (52.0)	12 (48.0)		15 (60.0)	10 (40.0)	
Female	21 (65.6)	11 (34.4)	0.298	13 (40.6)	19 (59.4)	0.147
Tumour grade						
1	12 (54.5)	10 (45.5)		12 (54.5)	10 (45.5)	
2	18 (62.1)	11 (37.9)		13 (44.8)	16 (55.2)	
3	4 (66.7)	2 (33.3)	0.806	3 (50.0)	3 (50.0)	0.789
Tumour stage						
1-2	19 (61.3)	12 (38.7)		18 (58.1)	13 (41.9)	
3-4	15 (57.7)	11 (42.3)	0.783	10 (38.5)	16 (61.5)	0.140
Neck lymph nodes						
Negative	23 (53.5)	20 (46.5)		25 (58.1)	18 (41.9)	
Positive	11 (78.6)	3 (21.4)	0.097	3 (21.4)	11 (78.6)	0.017
Recurrence						
No	21 (55.3)	17 (44.7)		22 (57.9)	16 (42.1)	
Yes	13 (68.4)	6 (31.6)	0.340	6 (31.6)	13 (68.4)	0.061
Adjuvant therapy						
No	21 (55.3)	17 (44.7)		21 (55.3)	17 (44.7)	
Radiotherapy	10 (62.5)	6 (37.5)	0.623	6 (37.5)	10 (62.5)	0.233