

Additional File 1

Plasmids and transfections

The shMMP-1 and shMMP-13 sequences were the following:

	Oligonucleotide sequences
shpLKO.1-MMP-1(1)	5'-CCGG-TGAAGATGAAAGGTGGACCAACTCGAGTT GGTCCACCTTTCATCTTCA-TTTTT-3'
shpLKO.1-MMP-1(2)	5'-CCGG-AGCACATGACTTTCCTGGAATCTCGAGATTC CAGGAAAGTCATGTGCT-TTTTT-3'
shpLKO.1-MMP-13(1)	5'-CCGG-CCAACCGTATTGATGCTGCATCTCGAGATGCA GCATCAATACGGTTGG-TTTTTG-3'
shpLKO.1-MMP-13(2)	5'-CCGG-GCTCCGAGAAATGCAGTCTTCTCGAGAAAGA CTGCATTTCTCGGAGC-TTTTTG-3'

The MISSION pLKO.1-puro Non-Target shRNA Control Transduction Particles (Sigma-Aldrich) containing a shRNA insert that does not target any known genes from any species was used as control.

The proximal promoter regions of the human MMP-1 gene (pGL2-B-MMP-1._{1633/-1}, pGL2-B-MMP-1._{1040/-1}, pGL2-B-MMP-1._{738/-1} and pGL2-B-MMP-1._{216/-1}) were synthesized by PCR using the following oligonucleotides:

	Oligonucleotide sequences
pGL2-B-MMP-1. _{1633/-1} (Fw)	5'-CCGCTCGAGGAAATTGTAGTTAAATAATT-3'
pGL2-B-MMP-1. _{1040/-1} (Fw)	5'-CCGCTCGAGAGCCTTTCATCATCCGGTA-3'
pGL2-B-MMP-1. _{738/-1} (Fw)	5'-CCGCTCGAGCATGACTCCATGCTTGGCTA-3'
pGL2-B-MMP-1. _{216/-1} (Fw)	5'-CCGCTCGAGGAGTGTGTCTCCTTCGCACA-3'
Reverse primer (-20/-1)	5'-CCCAAGCTTATCCCAGCTAGGAAGCTCCC-3'

The proximal promoter regions of the human MMP-13 gene (pGL2-B-MMP-13._{1548/+2}, pGL2-B-MMP-13._{1329/+2}, pGL2-B-MMP-13._{1148/+2}, pGL2-B-MMP-13._{858/+2}, pGL2-B-MMP-13._{393/+2}, pGL2-B-MMP-1._{145/-1}) were synthesized by PCR using the following oligonucleotides:

	Oligonucleotide sequences
pGL2-B-MMP-13. _{1548/+2} (Fw)	5'- CCGCTCGAGCATCAAATGGGCATCTCCT-3'
pGL2-B-MMP-13. _{1329/+2} (Fw)	5'-CCGCTCGAGGGAACCAAGAATTAGAATAT-3'
pGL2-B-MMP-13. _{1148/+2} (Fw)	5'-CCGCTCGAGTCAGGGGTGTAATTCACAATTC-3'
pGL2-B-MMP-13. _{858/+2} (Fw)	5'-CCGCTCGAGTGCTAAAATTTAGATTACATTGA-3'
pGL2-B-MMP-13. _{393/+2} (Fw)	5'-CCGCTCGAGTGACATCTAAGGTGTTATCGTCAA-3'

pGL2-B-MMP-1 _{-145/+2} (Fw)	5'-CCGCTCGAGTGCAAAGTGGAGGTCTTCCT-3'
Reverse primer (-18/+2)	5'-CCCAAGCTTATCAACAGTCCCCAGGCATC-3'

Pit-1 siRNA

For silencing Pit-1, MCF-7 cells were transfected with two different Pit-1 siRNA (Pit-1 siRNA-1, Pit-1 siRNA-2). A scrambled siRNA was used as control. Primer sequences were the following:

	Oligonucleotide sequences
Pit-1 siRNA-1	site 1, 5'-AACCCCTTGTCTTTACAAGTTCCTGTCTC-3' (antisense) site-2, 5'-AATTAAGTTAGGATACACCCACCTGTCTC-3' (antisense) site-3, 5'-AATTGAATCTCGAGAAAGAAGCCTGTCTC-3' (antisense)
Pit-1 siRNA-2	site-1, 5'-AATGTTGCTGTAGACATCACACCTGTCTC-3' (antisense) site-2, 5'-AAGCTTTCAGTTTGCATGCATCCTGTCTC-3' (antisense) site-3, 5'-AACTTCTCCAGATTCAGTTCCTGTCTC-3' (antisense)
Scrambled siRNA	5'-AAGCTTCATAAGGCGCATAGC-3'

CHIP assay

MMP-1 and MMP-13 primer sequences were as follows:

	Oligonucleotide sequences
MMP-1	(A) Sense: 5'-ACAGTGGGAAAGGTGGGAGAC-3' Antisense 5'-GTCTATCTTCGCTCCTTGGTGCT-3' (B) Sense 5'-AGCACCAAGGAGCGAAGATAGAC-3' Antisense 5'-GAGGCTGCATTGAGCTGTGA-3' (C) Sense 5'-TCACAGCTCAATGCAGCCTC-3' Antisense 5'-CAGGGCAGAGGGTGGAAATTAC-3' (D) Sense 5'-GTAATTCCACCTCTGCCCTG-3' Antisense 5'-GTGATGGCTTCCCAGCCTCT-3'
MMP-13	(A) Sense: 5'-CATCATCAAAATGGGCATCT-3' Antisense 5'-ATGATGTCAGCAATGCCATC-3' (B) Sense 5'-GCACAGGTGTTTGGTAAATTAGTG-3' Antisense 5'-TGGA ACTCTTCATCTTGAGCA-3' (C) Sense 5'-TGCTAAAAGTTTAGATTACATTGA-3' Antisense 5'-GCATCTTGGCTTTTTCATGA-3' (D) Sense 5'-TCTCGGCAACCATATTAAGCT-3' Antisense 5'-ATGTACCAACCGCATCATCA-3' (E) Sense 5'-GGAGCCTCTCAGTCATGGAG-3' Antisense 5'-GATGCCTGGGGACTGTTGAT-3'.

The length of MMP-1 PCR products were 378 pb (A), 327 pb (B), 447 pb (C), and 425 pb (D). The length of MMP-13 PCR products were 315 pb (A), 635 pb (B), 470 pb (C), 444 pb (D), and 327 pb (D).

Real-Time PCR

The oligonucleotide sequences were as follows:

	Oligonucleotide sequences
Pit-1	Sense: 5'-GTGTCTACCAGTCTCCAACC-3' Antisense: 5'-ACTTTTCCGCCTGAGTTCCT-3
MMP-1	Sense: 5'-ATGCTGAAACCCTGAAGGTG -3' Antisense: 5'-CTGCTTGACCC- TCAGAGACC -3'
MMP-13	Sense: 5'-TAAGGAGCATGGCGACTTCT-3' Antisense: 5'-GGTCCTTGGAG-TGGTCAAGA-3'
18S	Sense: 5'-GTAACCCGTTGAACCCCAT-3' Antisense: 5'-CCATCCAATCGCTA GTAGCG-3'

Antibodies

Primary antibodies	Antibody dilution	Origin	Secondary antibody
MMP-1 (polyclonal)	1:1000	Millipore	Anti-rabbit
MMP-13 (polyclonal)	1:1000	Santa Cruz Biotechnology	Anti-rabbit
Pit-1 (polyclonal)	1:500	Santa Cruz Biotechnology	Anti-rabbit
β -Actin (monoclonal)	1:5000	Millipore	Anti-mouse
Ki-67 (MIB-1 clon)	1:200	Dako	Anti-mouse
CK7 (clon OV- TL12/30)	1:100	Dako	Anti-mouse
CK19 (clon RKC108)	1:100	Dako	Anti-mouse