

Figure S1. Semi-quantification and statistical analysis of western blotting results. (A) Semi-quantification of western blotting results of ZNF384 expression in human CRC and adjacent normal tissues. (B) Semi-quantification of western blotting results of ZNF384 expression in various CRC cell lines. *P<0.05, **P<0.01 vs. FHC. (C) Semi-quantification of western blotting results of ZNF384 expression in SW480 and Caco-2 cells transfected with LV-Control and LV-ZNF384. **P<0.01 vs. LV-Control. (D) Semi-quantification of western blotting results of ZNF384 expression in SW620 and LoVo cells transfected with LV-shControl, LV-shZNF384-1 and LV-shZNF384-2. **P<0.01 vs. LV-shControl. (E) Semi-quantification of western blotting results of MMP2 expression in SW480 and Caco-2 cells transfected with LV-Control and LV-ZNF384. *P<0.05, **P<0.01 vs. LV-Control. (F) Semi-quantification of western blotting results of MMP2 expression in SW620 and LoVo cells transfected with LV-shControl, LV-shZNF384-1 and LV-shZNF384-2. **P<0.01 vs. LV-shControl. (G) Semi-quantification of western blotting results of ZNF384 expression in SW480-ZNF384 cells transfected with LV-shControl, LV-MMP2-1 and LV-MMP2-2. (H) Semi-quantification of western blotting results of MMP2 expression in SW480-ZNF384 cells transfected with LV-shControl, LV-shMMP2-1 and LV-shMMP2-2. **P<0.01 vs. LV-shControl. (I) Semi-quantification of western blotting results of ZNF384 expression in SW620-shZNF384 cells transfected with LV-Control and LV-MMP2. (J) Semi-quantification of western blotting results of MMP2 expression in SW620-shZNF384 cells transfected with LV-Control and LV-MMP2. *P<0.05 vs. LV-Control. (K) Semi-quantification of western blotting results of ZNF384 expression in SW480 and Caco-2 cells cultured in a hypoxic atmosphere (0.5% O₂) for the indicated time intervals. *P<0.05, **P<0.01 vs. 0 h. (L) Semi-quantification of western blotting results of HIF-1 α expression in SW480 and Caco-2 cells cultured in a hypoxic atmosphere (0.5% O₂) for the indicated time intervals. *P<0.05 vs. 0 h. (M) Semi-quantification of western blotting results of HIF-1 α expression in SW480 cells transfected with LV-Control and LV-HIF-1 α , and SW620 cells transfected with shControl and LV-shHIF-1 α . *P<0.05 vs. LV-Control; #P<0.05 vs. LV-shControl. (N) Semi-quantification of western blotting results of ZNF384 expression in SW480 cells transfected with LV-Control and LV-HIF-1 α , and SW620 cells transfected with shControl and LV-shHIF-1 α . *P<0.01 vs. LV-Control; #P<0.05 vs. shControl. (O) Semi-quantification of western blotting results of HIF-1 α expression in various CRC cell lines. *P<0.01 vs. FHC. (P) Semi-quantification of western blotting results of MMP2 expression in various CRC cell lines. *P<0.05, **P<0.01 vs. FHC. (Q) Semi-quantification of western blotting results of MMP2 expression in SW480 cells transfected with LV-shControl, LV-shMMP2-1 and LV-shMMP2-2. **P<0.01 vs. LV-shControl. (R) Semi-quantification of western blotting results of MMP2 expression in SW620 cells transfected with LV-Control and LV-MMP2. *P<0.05 vs. LV-Control. (S) Semi-quantification of western blotting results of ZNF384 expression in SW480 cells transfected with pCMV-Control and pCMV-ZNF384. *P<0.05 vs. pCMV-Control. (T) Semi-quantification of western blotting results of HIF-1 α expression in SW480 cells transfected with pCMV-Control and pCMV-HIF-1 α . *P<0.05 vs. pCMV-Control. ZNF384, zinc finger protein 384; sh, short hairpin RNA; HIF-1 α , hypoxia-inducible factor 1 α ; ns, not significant.

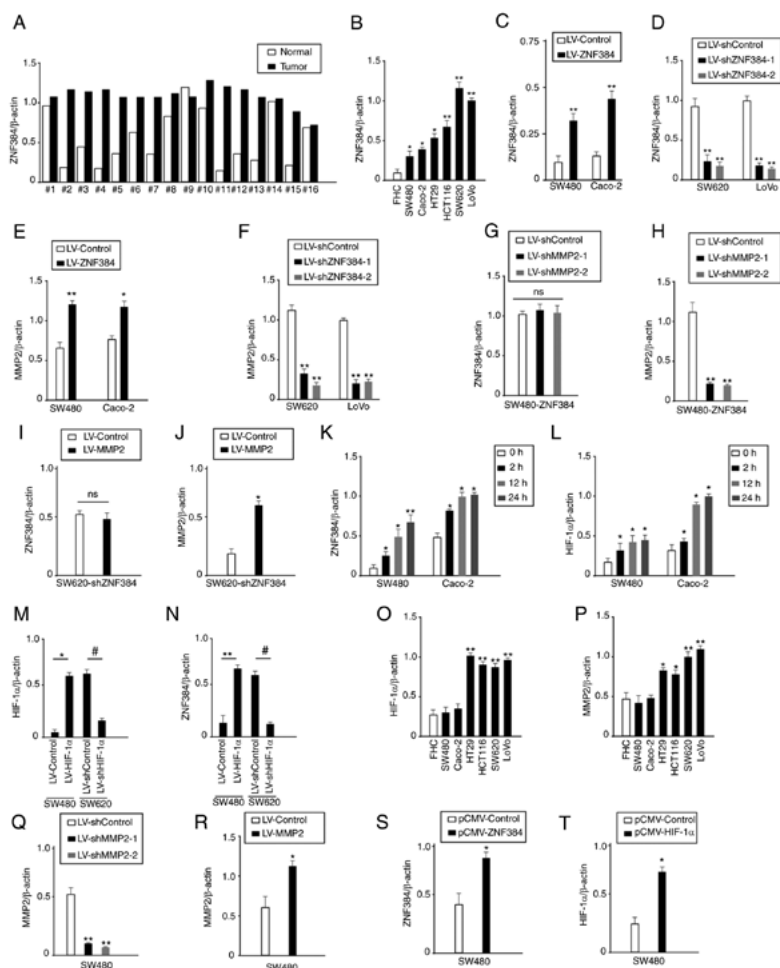


Figure S2. Kaplan-Meier survival curves demonstrating the association between (A) HIF-1 α and (B) MMP2 expression, and overall survival outcomes in patients with CRC. HIF-1 α , hypoxia-inducible factor 1 α ; CRC, colorectal cancer.

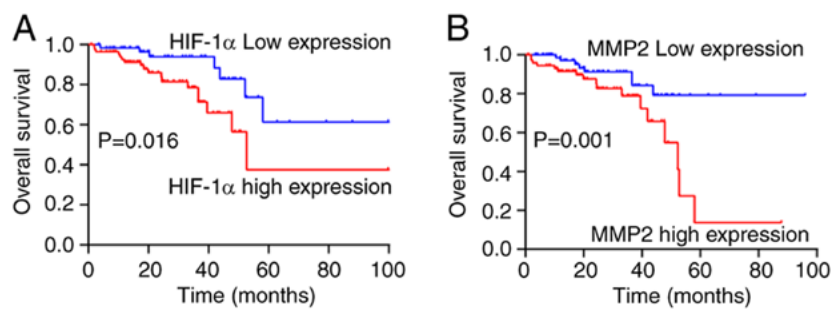


Figure S3. Proliferation of (A) SW480 cells overexpressing ZNF384 and (B) SW620 cells with ZNF384 knockdown, as detected using MTT assays. ZNF384, zinc finger protein 384; sh, short hairpin RNA; ns, not significant.

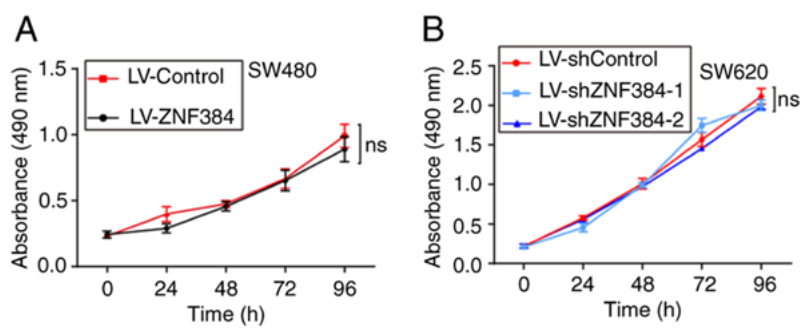


Figure S4. Western blot analysis was carried out to determine the protein expression levels of (A) HIF-1 α and (B) MMP2 in different colorectal cancer cell lines. HIF-1 α , hypoxia-inducible factor 1 α .

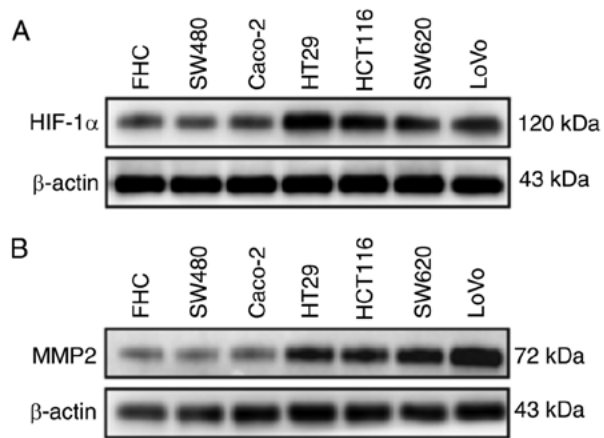


Figure S5. Migration of cells overexpressing ZNF384 (SW480 and Caco-2) and cells with ZNF384 knockdown (SW620 and LoVo), as detected using wound healing assays (magnification, x20). ZNF384, zinc finger protein 384; sh, short hairpin RNA. **P<0.01 vs. LV-Control, #P<0.05, ##P<0.01 vs. LV-shControl.

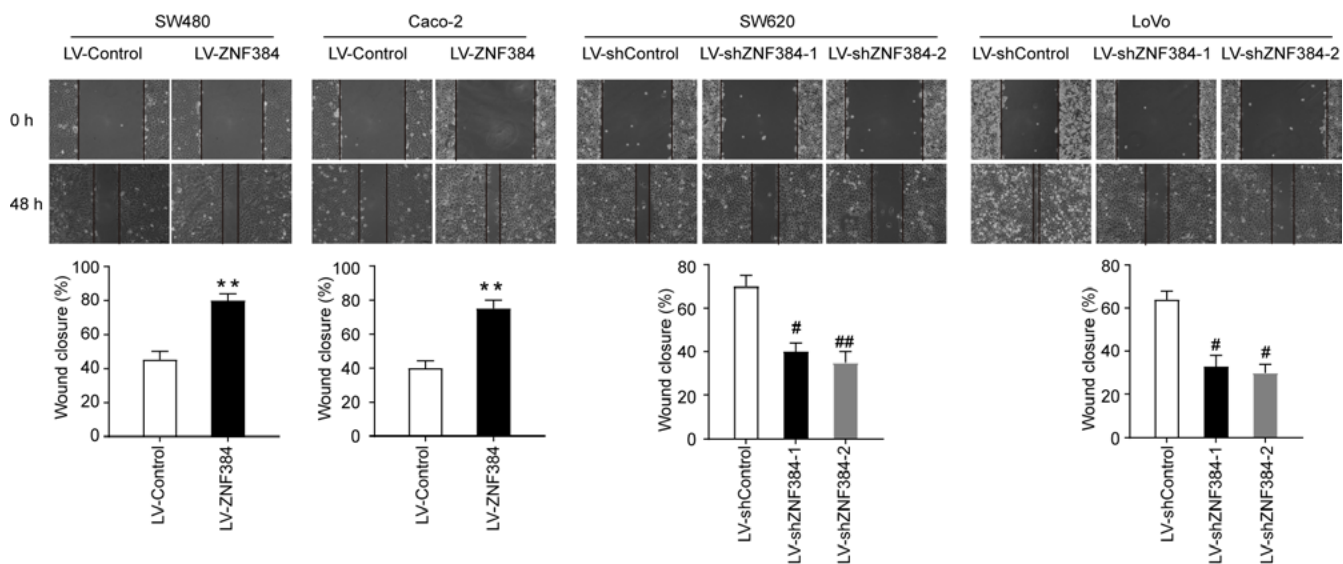


Figure S6. Western blot analysis was carried out to determine the expression levels of (A) MMP2, (B) ZNF384 and (C) HIF-1 α in specific colorectal cancer cell lines. HIF-1 α , hypoxia-inducible factor 1 α ; ZNF384, zinc finger protein 384; sh, short hairpin RNA.

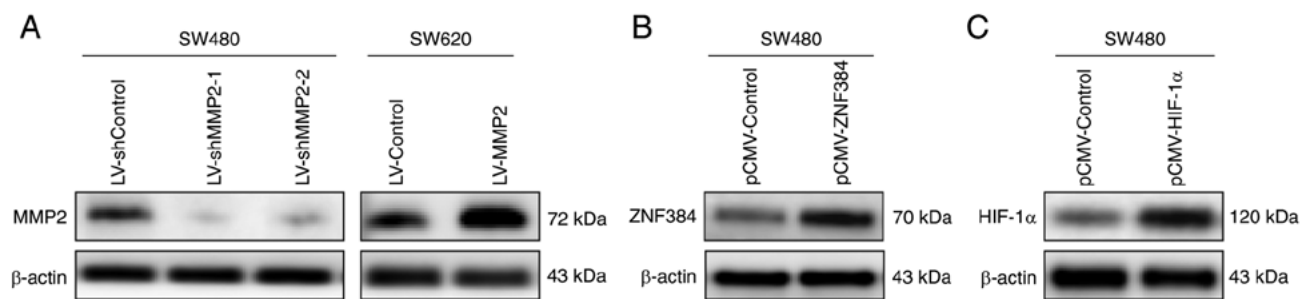


Figure S7. Migration of cells overexpressing MMP2 (SW620-shZNF384) and cells with MMP2 knockdown (SW480-ZNF384), as detected using wound healing assays (magnification, x20). ZNF384, zinc finger protein 384; sh, short hairpin RNA. *P<0.05 vs. LV-shControl; **P<0.01 vs. LV-Control.

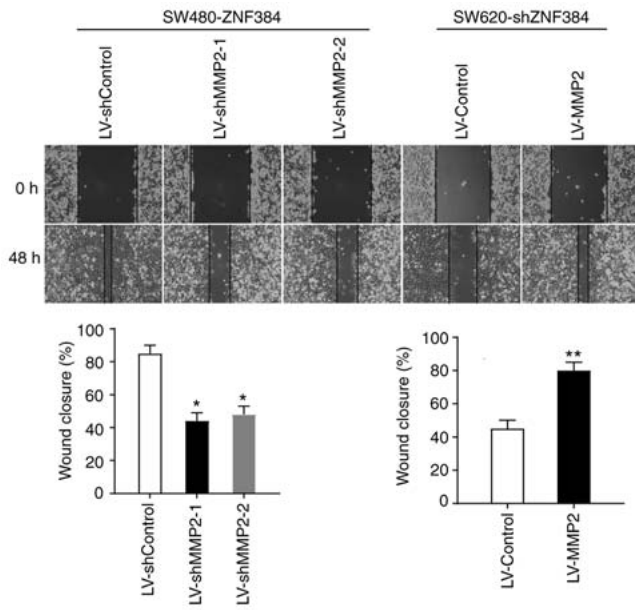


Table SI. Clinical characteristics of patients with colorectal cancer.

Clinical characteristic	Case, n (%)
Age, years	
<60	55 (33.5)
≥60	109 (66.5)
Sex	
Male	83 (50.6)
Female	81 (49.4)
T stage	
T1 and T2	57 (34.8)
T3 and T4	107 (65.2)
Lymphatic invasion	
Absent	114 (69.5)
Present	50 (30.5)
Differentiation	
Well	35 (21.3)
Moderate	73 (44.5)
Poor	56 (34.2)
AJCC stage	
I	47 (28.7)
II	67 (40.9)
III	50 (30.4)
Tumor location	
Colon	76 (46.3)
Rectum	88 (53.7)

AJCC, American Joint Committee on Cancer.

Table SII. Primer and shRNA sequences used in the present study.

Primer/shRNA name	Primer sequence	Enzyme
Primers for reverse transcription-quantitative PCR		
ZNF384 sense	5'-GTCTCAGGTCAGATCGAGAACA-3'	
ZNF384 antisense	5'-ACTCTGTGTCCATACTGATGCC-3'	
MMP2 sense	5'-CTCATCGCAGATGCCTGGAA-3'	
MMP2 antisense	5'-TTCAGGTAATAGGCACCCTTGAAGA-3'	
GAPDH sense	5'-GCACCGTCAAGGCTGAGAAC-3'	
GAPDH antisense	5'-TGGTGAAGACGCCAGTGGA-3'	
Primers for MMP2 promoter construct		
(-1,919/+99) MMP2 sense	5'-TATAGGTACCCCAAGTTAAGGCTTACACAT-3'	<i>KpnI</i>
(-1,332/+99) MMP2 sense	5'-TATAGGTACCGCATCCAGACTTCCTCAG-3'	<i>KpnI</i>
(-679/+99) MMP2 sense	5'-TATAGGTACCCTTCAGCATAACAGCAGTG-3'	<i>KpnI</i>
(-278/+99) MMP2 sense	5'-TATA GGTACCCATTTGGGAAGTGATTGGG-3'	<i>KpnI</i>
Antisense	5'-ATATAAGCTTTCTCTGCTAGGCTATCC-3'	<i>HindIII</i>
Primers for MMP2 promoter site-directed mutagenesis		
Binding site 1 mutation sense	5'-GAAAAGAGGTGGAGAAActacgcgctAGGAGAAAAGTGG-3'	
Binding site 1 mutation antisense	5'-CCACTTTTCTCCTacgcgctagTTCTCCACCTCTTTTC-3'	
Binding site 2 mutation sense	5'-GGGGGATCTTTTGTActacgctcgcACCTTTGATAAACA-3'	
Binding site 2 mutation antisense	5'-TGTTTATCAAAGGTgcgagcctagTACAAAAGATCCCCC-3'	
Binding site 3 mutation sense	5'-CGGAGAGGGACCTcgcgctcctAGCTCGAGGGGGCCATT-3'	
Binding site 3 mutation antisense	5'-AAATGGCCCCCTCGAGCTagcagcgcgAGGTCCCTCTCCG-3'	
Primers used for ChIP in the MMP2 promoter		
Distant region sense	5'-GCCATTGCCATCTCTGAA-3'	
Distant region antisense	5'-GTATTGCCTTGTATGTGACTC-3'	
Binding site 1 sense	5'-GCCAGGAGTAGCAGGCGGCCGG-3'	
Binding site 1 antisense	5'-CACTCGGGCCCCCTCTCTGC-3'	
Binding site 2 sense	5'-CTTTGGCAAGCTATTGGAGTGAT-3'	
Binding site 2 antisense	5'-TCGCCCCACTGCTGTATGCTGAAG-3'	
Binding site 3 sense	5'-CCTTCGGCACAGATCCGGAGAG-3'	
Binding site 3 antisense	5'-CAGCCCTTGACCAATTTGTCCAT-3'	
Primers used for ChIP in the ZNF384 promoter		
Distant region sense	5'-GAGAAGCATTGGCACAGA-3'	
Distant region antisense	5'-GTGGTCTAATCAGGCAGTAA-3'	
Binding site 1 sense	5'-GCTGACGGAGACACGGAGAC-3'	
Binding site 1 antisense	5'-AGGTGGGAACAGGAAATAGTAAT-3'	
Binding site 2 sense	5'-AGTTTCTAACTGGACCTAAGTA-3'	
Binding site 2 antisense	5'-ACCCCCAAGAATGTCTCAATGCTT-3'	
Primers for ZNF384 promoter construct		
(-1,996/+115)ZNF384 sense	5'-TATAGGTACCTAAGGAGTTCAAAGCACTTG-3'	<i>KpnI</i>
(-1,752/+115)ZNF384 sense	5'-TATAGGTACCAAGAGCAGGAGGAGAACC-3'	<i>KpnI</i>
(-317/+115)ZNF384 sense	5'-TATAGGTACCGCTTATTCAACATCCAGAGG-3'	<i>KpnI</i>
Antisense	5'-ATATAAGCTTAAGCACTTGTTCATCACAC-3'	<i>HindIII</i>

Table SII. Continued.

Primer/shRNA name	Primer sequence	Enzyme
Primers for ZNF384 promoter site-directed mutagenesis		
Binding site 1 mutation sense	5'-ACACGGAGACCCAGTgcagtatCAGTTAACATAAAAT-3'	
Binding site 1 mutation antisense	5'-ATTTATGTTAACTGatactgcaCTGGGTCTCCGTGT-3'	
Binding site 2 mutation sense	5'-CCGAAAAACAAGGAgctagatgAGTCCTTTCCCGCA-3'	
Binding site 2 mutation antisense	5'-TGCGGGAAAGGACTcatctagcTCCTTGTTTTTCGG-3'	
shRNA sequences		
LV-shZNF384-1 sense	5'-ccgg CCCGAGATGAATGACCCTTATCTCGAGATAAGGGTCATTCATCTCGGTTTTT g-3'	
LV-shZNF384-1 antisense	5'-aattcAAAAACCCGAGATGAATGACCCTTATCTCGAGATAAGGGTCATTCATCTCGGG-3'	
LV-shZNF384-2 sense	5'-ccgg CGGCAACACAACAAGATAAACTCGAGTTTATCTTTGTTGTGTTGCCGTTTTT g-3'	
LV-shZNF384-2 antisense	5'-aattcAAAAACGGCAACACAACAAGATAAACTCGAGTTTATCTTTGTTGTGTTGCCG-3'	
LV-sh HIF-1 α -1 sense	5'-ccgg CCGCTGGAGACACAATCATATCTCGAGATATGATTGTGTCTCCAGCGTTTTT g-3'	
LV-sh HIF-1 α -1 antisense	5'-aattcAAAAACCGCTGGAGACACAATCATATCTCGAGATATGATTGTGTCTCCAGCGG-3'	
LV-sh HIF-1 α -2 sense	5'-ccgg CCAGTTATGATTGTGAAGTTACTCGAGTAACTTCACAATCATAACTGTTTTT g-3'	
LV-sh HIF-1 α -2 antisense	5'-aattcAAAAACCAGTTATGATTGTGAAGTTACTCGAGTAACTTCACAATCATAACTGG-3'	
LV-shMMP2-1 sense	5'-ccgg GCTGAAGGACACACTAAAGAACTCGAGTTCTTTAGTGTGTCCTTCAGTTTTT g-3'	
LV-shMMP2-1 antisense	5'-aattcAAAAAGCTGAAGGACACACTAAAGAACTCGAGTTCTTTAGTGTGTCCTTCAGC-3'	
LV-shMMP2-2 sense	5'-ccgg GCCGCCTTTAACTGGAGCAAACCTCGAGTTTGCTCCAGTTAAAGGCGGTTTTT g-3'	
LV-shMMP2-2 antisense	5'-aattcAAAAAGCCGCCTTTAACTGGAGCAAACCTCGAGTTTGCTCCAGTTAAAGGCGGC-3'	
LV-shControl sense	5'-ccgg TTCTCCGAACGTGTCACGTCTCGAGACGTGACACGTTCCGAGAATTTTT g-3'	
LV-shControl antisense	5'-aattcAAAAATTCTCCGAACGTGTCACGTCTCGAGACGTGACACGTTCCGGAGAA-3'	

Text in bold indicates the core sequence. ChIP, chromatin immunoprecipitation; HIF-1 α , hypoxia inducible factor 1 α ; sh/shRNA, short hairpin RNA; ZNF384, zinc finger protein 384.

Table SIII. List of genes differentially expressed in SW480-ZNF384 vs. SW480-Control cells using a human tumor Metastasis RT² Profiler PCR Array.

Symbol	SW480-ZNF384/ SW480-Control	Description
MMP2	6.803169	Matrix metalloproteinase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)
PLAUR	5.729974	Plasminogen activator, urokinase receptor
CXCR2	4.875143	Chemokine (C-X-C motif) receptor 2
RPLP0	4.527235	Ribosomal protein, large, P0
CST7	4.258437	Cystatin F (leukocystatin)
APC	4.210097	Adenomatous polyposis coli
EPHB2	4.117362	EPH receptor B2
FLT4	4.102342	Fms-related tyrosine kinase 4
RORB	4.040211	RAR-related orphan receptor B
IL18	4.038544	Interleukin 18 (interferon-gamma-inducing factor)
TIMP4	4.038133	TIMP metalloproteinase inhibitor 4
CDH11	3.952675	Cadherin 11, type 2, OB-cadherin (osteoblast)
TP53	3.603238	Tumor protein p53
SMAD4	3.122566	SMAD family member 4
NR4A3	3.12027	Nuclear receptor subfamily 4, group A, member 3
MYC	3.000382	V-myc myelocytomatosis viral oncogene homolog (avian)
MMP11	2.89206	Matrix metalloproteinase 11 (stromelysin 3)
CTSL	2.888349	Cathepsin L1
ETV4	2.823738	Ets variant 4
KRAS	2.736422	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
HRAS	2.516215	V-Ha-ras Harvey rat sarcoma viral oncogene homolog
B2M	2.516204	Beta-2-microglobulin
TSHR	2.213144	Thyroid stimulating hormone receptor
SYK	1.630847	Spleen tyrosine kinase
IGF1	1.967176	Insulin-like growth factor 1 (somatomedin C)
MMP13	1.944491	Matrix metalloproteinase 13 (collagenase 3)
SET	1.938329	SET nuclear oncogene
SRC	1.842958	V-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)
MMP9	1.784612	Matrix metalloproteinase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)
ITGB3	1.712388	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
RPSA	1.651114	Ribosomal protein SA
MET	1.633829	Met proto-oncogene (hepatocyte growth factor receptor)
VEGFA	1.620416	Vascular endothelial growth factor A
COL4A2	1.541384	Collagen, type IV, alpha 2
CXCR4	1.491561	Chemokine (C-X-C motif) receptor 4
CHD4	1.486695	Chromodomain helicase DNA binding protein 4
MMP7	1.455853	Matrix metalloproteinase 7 (matrilysin, uterine)
MTA1	1.40839	Metastasis associated 1
FN1	1.389357	Fibronectin 1
(fibronectin)		
CXCL12	1.36986	Chemokine (C-X-C motif) ligand 12
MMP10	1.32662	Matrix metalloproteinase 10 (stromelysin 2)
MCAM	1.299941	Melanoma cell adhesion molecule
TGFB1	1.272172	Transforming growth factor, beta 1
FGFR4	1.254272	Fibroblast growth factor receptor 4
CCL7	1.19974	Chemokine (C-C motif) ligand 7
ITGA7	1.076854	Integrin, alpha 7
HGF	-1.0191	Hepatocyte growth factor (hepapoietin A; scatter factor)
SMAD2	-1.03338	SMAD family member 2
CTSK	-1.03616	Cathepsin K
NME4	-1.17847	Non-metastatic cells 4, protein expressed in
TCF20	-1.23492	Transcription factor 20 (AR1)
CDKN2A	-1.23649	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
(p16)		
TRPM1	-1.24047	Transient receptor potential cation channel, subfamily M, member 1

Table III. Continued.

Symbol	SW480-ZNF384/ SW480-Control	Description
METAP2	-1.25391	Methionyl aminopeptidase 2
FXVD5	-1.32096	FXVD domain containing ion transport regulator 5
CTNNA1	-1.47336	Catenin (cadherin-associated protein), alpha 1, 102 kDa
TIMP3	-1.5016	TIMP metalloproteinase inhibitor 3
PNN	-1.60503	Pinin, desmosome associated protein
FAT1	-1.63334	FAT tumor suppressor homolog 1 (Drosophila)
KISS1	-1.66165	KiSS-1 metastasis-suppressor
CDH1 (E-cadherin)	-1.67911	Cadherin 1, type 1, E-cadherin (epithelial)
KISS1R	-1.72975	KISS1 receptor
IL1B	-1.76472	Interleukin 1, beta
MTSS1	-1.76564	Metastasis suppressor 1
BRMS1	-1.81771	Breast cancer metastasis suppressor 1
TIMP2	-1.83205	TIMP metalloproteinase inhibitor 2
NF2	-1.86952	Neurofibromin 2 (merlin)
TNFSF10	-1.90731	Tumor necrosis factor (ligand) superfamily, member 10
CTBP1	-1.97557	C-terminal binding protein 1
GNRH1	-1.97803	Gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)
NME1	-1.97907	Non-metastatic cells 1, protein (NM23A) expressed in
MDM2	-1.9799	Mdm2 p53 binding protein homolog (mouse)
CDH6	-2.37518	Cadherin 6, type 2, K-cadherin (fetal kidney)
CD82	-2.3845	CD82 molecule
GAPDH	-2.41314	Glyceraldehyde-3-phosphate dehydrogenase
MMP3	-2.69297	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)
RB1	-2.82507	Retinoblastoma 1
MYCL	-2.85002	V-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)
EWSR1	-2.92875	Ewing sarcoma breakpoint region 1
DENR	-2.95771	Density-regulated protein
SSTR2	-2.9907	Somatostatin receptor 2
PTEN	-2.99449	Phosphatase and tensin homolog
MGAT5	-3.15121	Mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase
SERPINE1 member 1	-3.18434	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1),
HPSE	-3.23465	Heparanase
ACTB	-3.32727	Actin, beta
HPRT1	-3.3346	Hypoxanthine phosphoribosyltransferase 1
CD44	-3.40678	CD44 molecule (Indian blood group)
HTATIP2	-3.45619	HIV-1 Tat interactive protein 2, 30 kDa