Supplementary Material:

Analysis of the Matrix Metalloproteinase Family Reveals *MMP8* is often mutated in Melanoma

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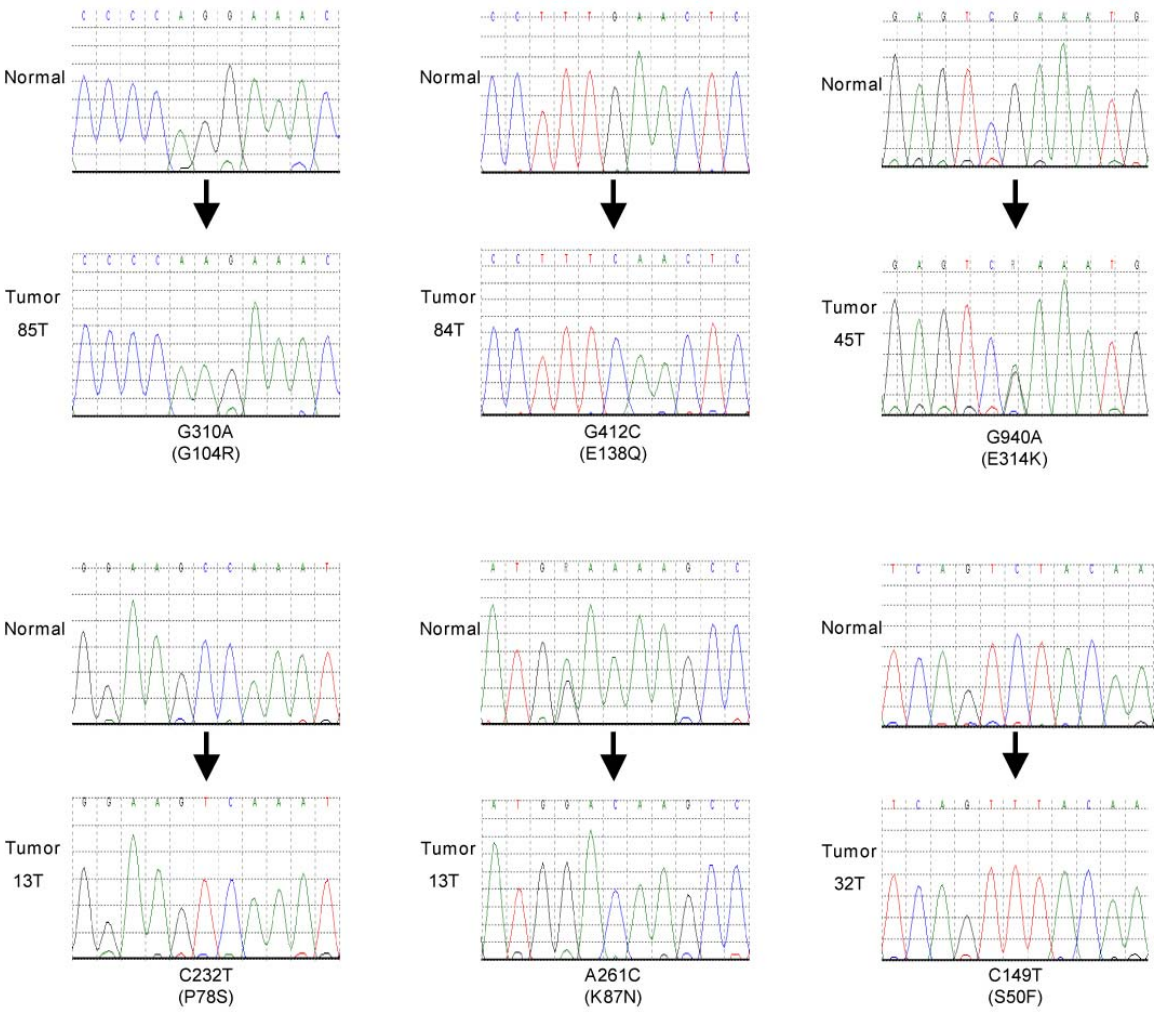
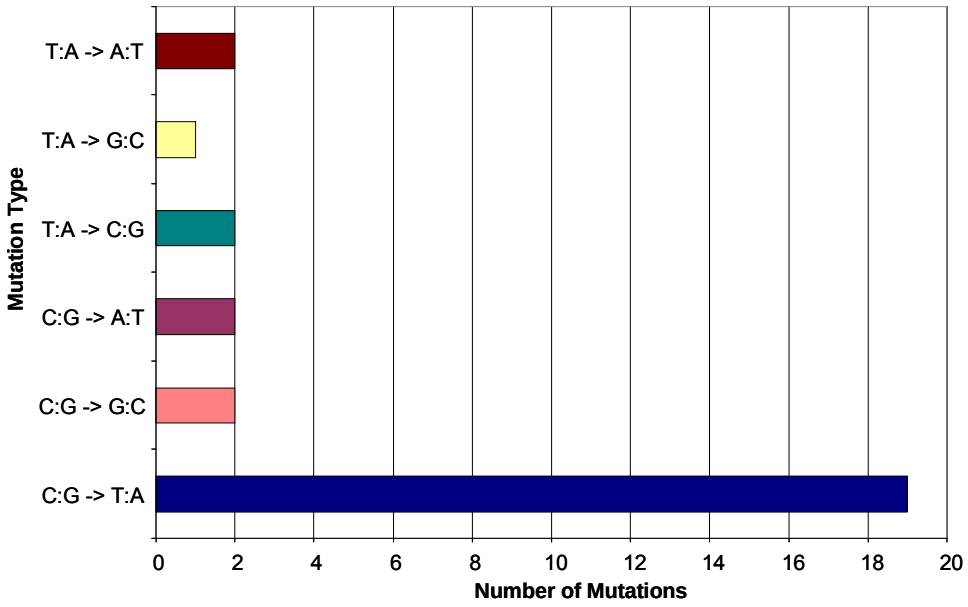
Supplementary Figures



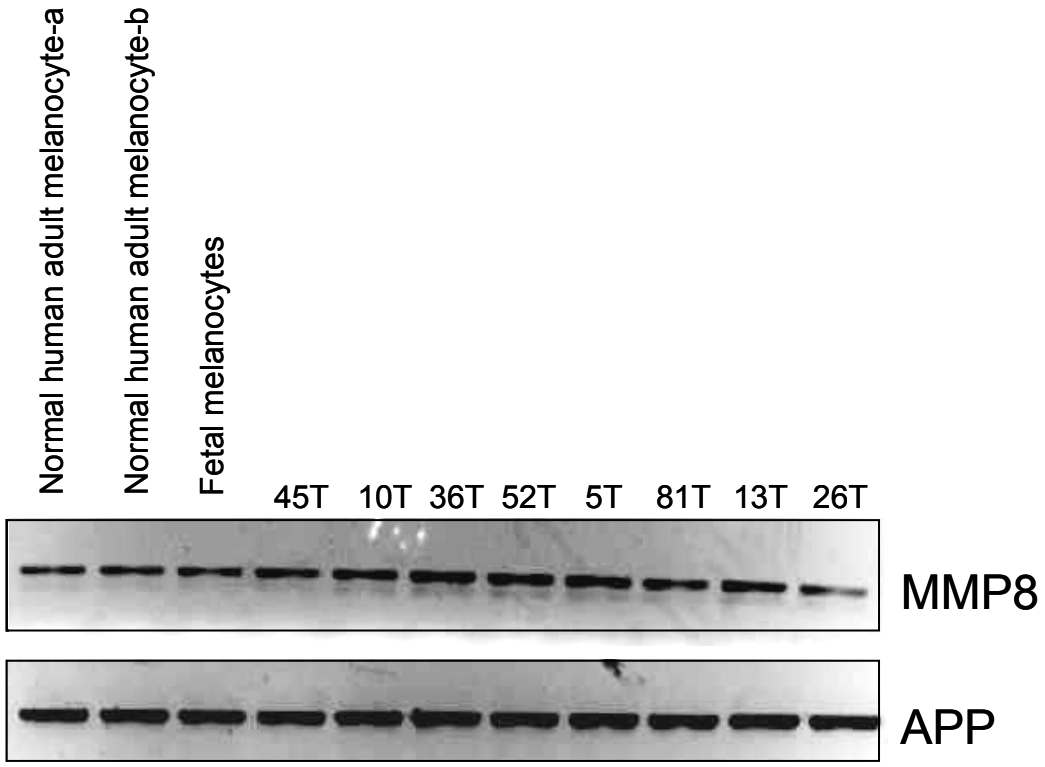
Supplementary Figure 1. Somatic mutations in the *MMP8* gene. In each case, the top sequence chromatogram was obtained from normal tissue and the lower sequence chromatogram from the indicated tumors. Arrows indicate the location of missense mutations. The nucleotide and amino acid alterations are indicated below the tumor chromatograms.



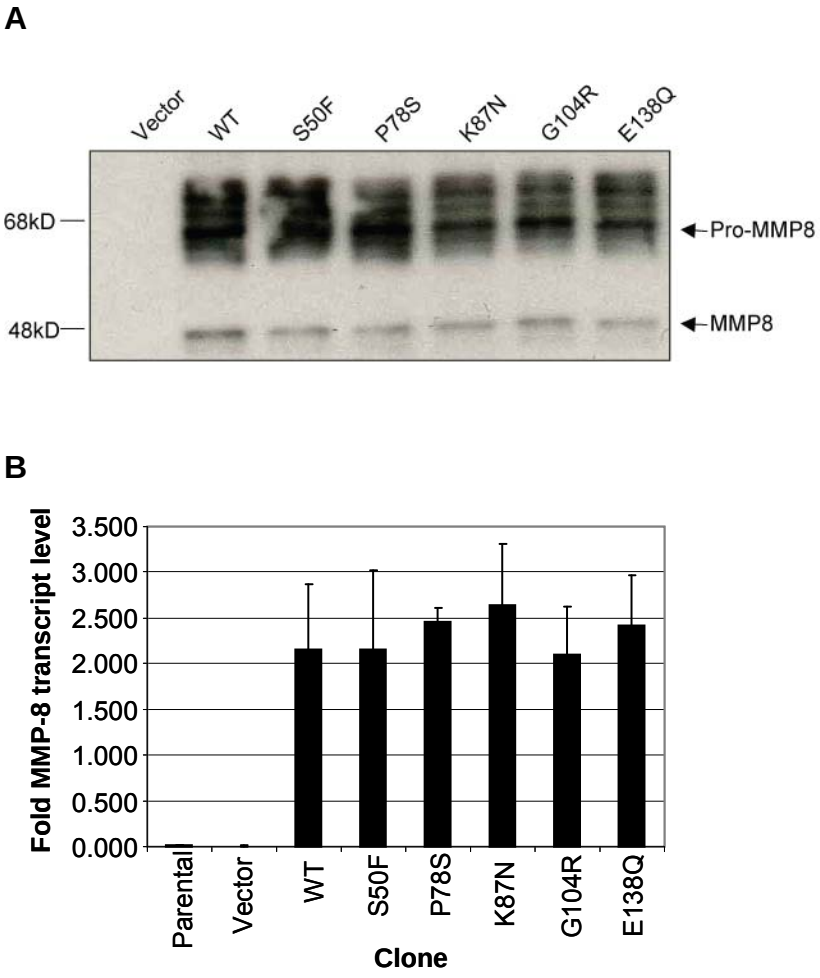
Supplementary Figure 2. Mutation spectra of single base pair substitutions. The number of each of the six classes of base substitutions resulting in non-synonymous changes is shown.

MMP	Fraction mutated	
MMP-27	7/79	(7.6%)
MMP-8	5/79	(6.3%)

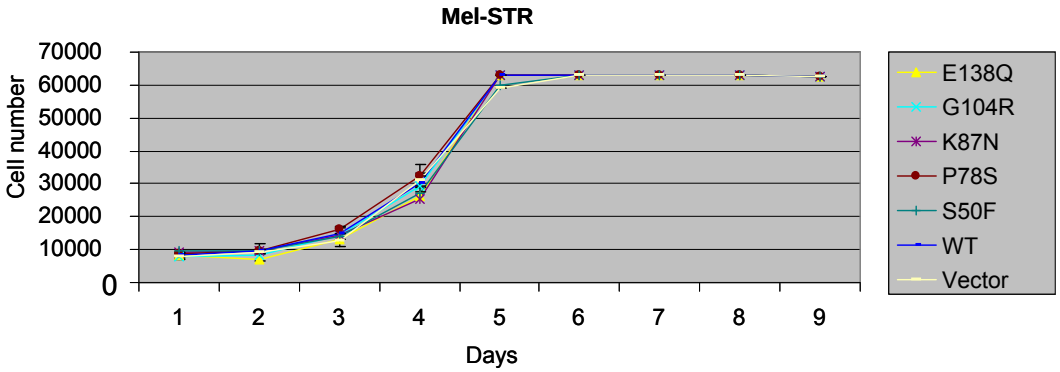
Supplementary Figure 3. Distribution of mutations in MMP-27 and MMP-8. Arrows indicate the location of somatic mutations, black stars indicate locations where both alleles of the gene were mutated, and boxes represent functional domains (P, leader sequence; PGBD, proteoglycan binding domain; catalytic; H, hinge domain; hemopexin). Red stars indicate MMP-8 mutants evaluated for metalloproteinase activity and effects on cell growth *in vitro* and *in vivo* (Fig. 1A, 1B, 1D and 1E). Fraction of tumors with mutations is noted above. Nucleotide positions of mutations are listed in Table 1.



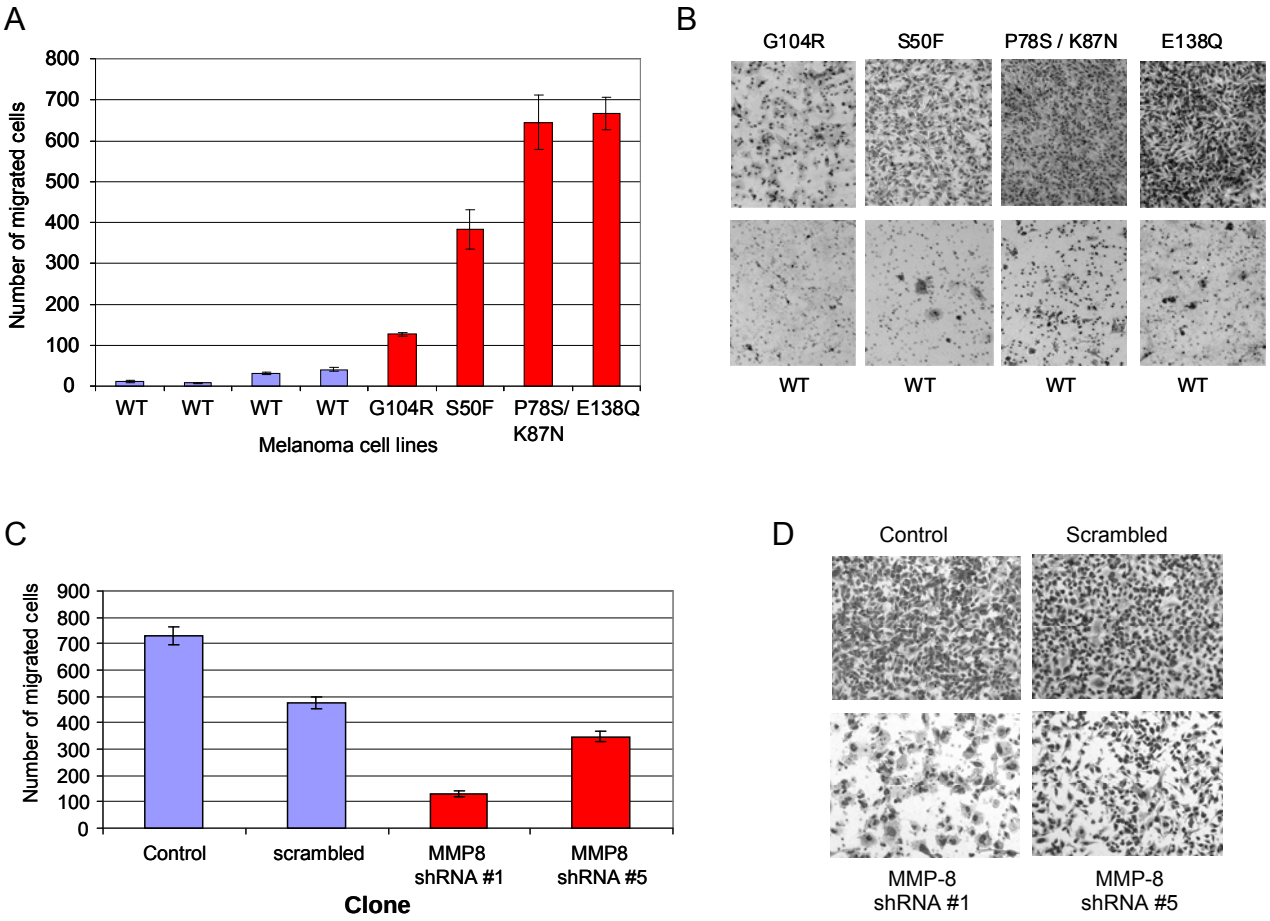
Supplementary Figure 4. MMP-8 mRNA expression in normal melanocytes and melanoma tumors. MMP-8 expression was evaluated by reverse transcription of total RNA followed by PCR amplification using a forward primer from exon 3 and a reverse primer from exon 4. Expression analysis of amyloid precursor protein gene (APP) was performed as a control. Melanoma tumors and normal melanocytes analyzed are indicated above.



Supplementary Figure 5. Generation of stable transfectants. Detection of MMP-8 protein expression in stable transfectants of Mel-STR melanoma cells by western blot analysis (A) and real-time PCR (B). A. Conditioned media from the different clones stably transfected with an empty vector, human MMP-8 or the indicated MMP-8 mutants were immunoprecipitated and immunoblotted with anti-FLAG antibody. Bands corresponding to the various MMP-8 forms are indicated. B. The
expression of MMP-8 in Mel-STR stable cells was evaluated using real-time PCR and compared with that of the amyloid precursor protein gene.



Supplementary Figure 6. Effect of *MMP8* mutations on cell growth. Cellular proliferation of Mel-STR pooled clones transfected with an empty vector, human *MMP8* or the indicated *MMP8* mutants was assessed in plastic culture plates. Average cell number at each time point was measured by determining DNA content in eight replicate wells using SYBR Green I.



Supplementary Figure 7. Effect of MMP-8 mutations on cell migration. (A) Melanoma cells (2.5x 10^5) of the indicated genotypes were grown in Boyden chambers and assessed for their ability to migrate through 8 μm porous membranes. The graph indicates the number of cells that migrated 24hrs after seeding. Medium containing 10% FBS was used as chemo attractant. Cells that had migrated were fixed in methanol and stained using crystal violet. Cells were counted and photographed in 4 random fields of view under a bright field microscope (magnification, 100x). (B) Representative pictures of migrated cells. (C) Cell migration of infected SK-Mel-2 cell clones expressing the indicated constructs was assessed as described in 1C. (D) Representative pictures of migrated cells.

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Supplementary Tables

Supplementary Table 1. MMP genes analyzed

CCDS accession and amplimer number	Ref Seq accession and amplimer number	Gene Name	Gene Description
CCDS8322.1	NM_002421	MMP-1	Fibroblast collagenase
CCDS5753.1	NM_032549	IMMP-2L	IMP2 inner mitochondrial membrane protease-like
CCDS10752.1	NM_004530	MMP-2	Gelatinase A
CCDS8323.1	NM_002422	MMP-3	Stromelysin 1
CCDS8317.1	NM_002423	MMP-7	Uterine matrilysin
CCDS8320.1	NM_002424	MMP-8	Neutrophil collagenase
CCDS13390.1	NM_004994	MMP-9	Gelatinase B
CCDS8321.1	NM_002425	MMP-10	Stromelysin 2
CCDS13816.1	NM_005940	MMP-11	Stromelysin 3
CCDS8324.1	NM_002427	MMP-13	collagenase 3
CCDS9577.1	NM_004995	MMP-14	matrix metallopeptidase 14 (membrane-inserted)
CCDS10792.1	NM_002428	MMP-15	matrix metallopeptidase 15 (membrane-inserted)
CCDS6246.1	NM_005941	MMP-16	matrix metallopeptidase 16 (membrane-inserted)
CCDS31927.1	NM_016155	MMP-17	matrix metallopeptidase 17 (membrane-inserted)
CCDS8895.1	NM_002429	MMP-19	Matrix metalloproteinase RASI
CCDS8318.1	NM_004771	MMP-20	Enamel metalloproteinase
CCDS7647.1	NM_147191	MMP-21	Matrix metallopeptidase 21
N/A	NM_006690	MMP-24	Matrix metalloproteinase 24 (membrane-inserted)
CCDS10492.1	NM_022468	MMP-25	Membrane-type matrix metalloproteinase 6
CCDS7752.1	NM_021801	MMP-26	Matrix metallopeptidase 26
CCDS8319.1	NM_022122	MMP-27	Matrix metalloproteinase 27
N/A	NM_024302	MMP-28	Matrix metallopeptidase 28

Coding exons of 22 out of the 23 MMP family members were genetically analyzed for somatic mutations. MMP-23B was omitted from this analysis.

Supplementary Table 2. Primers used for PCR amplification and sequencing

Gene and Exon Name	CCDS accession and amplimer number	Ref Seq accession and amplimer number	Forward Primer	Reverse Primer	Sequencing Primer
MMP1-1	CCDS8322.1-Amp 1	NM_00242-Amp 1	GTAAAACGACGGCCAGTCTGGGATATTGGAGCAGCAAG	GTGGAGGGTGCTGTTTCTAGC	GTAAAACGACGGCCAGT
MMP1-2	CCDS8322.1-Amp 2	NM_00242-Amp 2	GTAAAACGACGGCCAGTCTGCGAAATCTAAACACAAGGTT	ATCTGCTCTTGGCAAATCTGG	GTAAAACGACGGCCAGT
MMP1-3	CCDS8322.1-Amp 3	NM_00242-Amp 3	CTGAAGGTGATGAAGCAGCC	GTAAAACGACGGCCAGTTCAACTGTGATGCAGTTTCCC	GTAAAACGACGGCCAGT
MMP1-4	CCDS8322.1-Amp 4	NM_00242-Amp 4	GTAAAACGACGGCCAGTAAGCTAATGCTTGCCCTTCTG	GAGTGGCCTAGTTGTCACTGC	GTAAAACGACGGCCAGT
MMP1-5	CCDS8322.1-Amp 5	NM_00242-Amp 5	ATAAATGCATGCCTTCTGCC	GTAAAACGACGGCCAGTCAAGGATATCGCTAATGGCTG	GTAAAACGACGGCCAGT
MMP1-6	CCDS8322.1-Amp 6	NM_00242-Amp 6	GTAAAACGACGGCCAGTCAGCCATTAGCGATATCCTTG	GCATGAAATAAGTAACATGTGAAGC	GTAAAACGACGGCCAGT
MMP1-7	CCDS8322.1-Amp 7	NM_00242-Amp 7	GGAGCTGGAGCTGGATATCTG	GTAAAACGACGGCCAGTTCATTGATTTGGTTGGTGAGAC	GTAAAACGACGGCCAGT
MMP1-8	CCDS8322.1-Amp 8	NM_00242-Amp 8	GTAAAACGACGGCCAGTAATCTGCAGCCAGGGTACTG	TGAGACTCACTTTGAAATGGGC	GTAAAACGACGGCCAGT
MMP1-9	CCDS8322.1-Amp 9	NM_00242-Amp 9	GTAAAACGACGGCCAGTTCCACCTTATCCCATCCACTC	GAGAAACCAATTCATCTGTGAGC	GTAAAACGACGGCCAGT
MMP1-10	CCDS8322.1-Amp 10	NM_00242-Amp 10	GTAAAACGACGGCCAGTTGCTCACAGATGAATTGGTTTC	GAATCCATAAGCCACAAACTTGAC	GTAAAACGACGGCCAGT
IMMP2L-1	CCDS5753.1-Amp 1	NM_032549-Amp 1	ACAAGATTGCCCTTCGGTAAC	GTAAAACGACGGCCAGTTCCCAGAATAGGAATAATAATTAGCAC	GTAAAACGACGGCCAGT
IMMP2L-2	CCDS5753.1-Amp 2	NM_032549-Amp 2	GTAAAACGACGGCCAGTCGAGCTGTGAAGTGCAAGAAC	CACCAAATCCAAATATGTTAGTCAAC	GTAAAACGACGGCCAGT
IMMP2L-3	CCDS5753.1-Amp 3	NM_032549-Amp 3	GTAAAACGACGGCCAGTGGAACCGAAGTGAGACTGATG	TTTGGCCCTCATGGACTTC	GTAAAACGACGGCCAGT
IMMP2L-4	CCDS5753.1-Amp 4	NM_032549-Amp 4	AAGCTTCATGGTTAACCTGTCTG	GTAAAACGACGGCCAGTTGATCAGTCTTGGCTGAGTTACC	GTAAAACGACGGCCAGT
IMMP2L-5	CCDS5753.1-Amp 5	NM_032549-Amp 5	GTAAAACGACGGCCAGTCTCCCTGGGATGTTAAACCTG	CGCACAGCATCATATTGTCAG	GTAAAACGACGGCCAGT
MMP2-1	CCDS10752.1-Amp 1	NM_004530-Amp 1	GTAAAACGACGGCCAGTGGAGCAGGCTCCAACCAG	TCCAATAGCTTGCCAAAGACC	GTAAAACGACGGCCAGT
MMP2-2	CCDS10752.1-Amp 2	NM_004530-Amp 2	TGATTGCTACAGCCTGCTTTG	GTAAAACGACGGCCAGTCTGAGTGGAGAGAATGCATGG	GTAAAACGACGGCCAGT
MMP2-3	CCDS10752.1-Amp 3	NM_004530-Amp 3	CTGTGCACACACACATACTTGC	GTAAAACGACGGCCAGTACTGTCCTATCTAAGCCGCCC	GTAAAACGACGGCCAGT
MMP2-4	CCDS10752.1-Amp 4	NM_004530-Amp 4	GTAAAACGACGGCCAGTAGCTCCTGTTGACAGTCCTGG	CTCTCTGGTCCAAGCAAATGG	GTAAAACGACGGCCAGT
MMP2-5	CCDS10752.1-Amp 5	NM_004530-Amp 5	CATTTGCTTGGACCAGAGAGG	GTAAAACGACGGCCAGTAGTGTGGAGGGAAGAGCACAC	GTAAAACGACGGCCAGT
MMP2-6	CCDS10752.1-Amp 6	NM_004530-Amp 6	GTAAAACGACGGCCAGTTGCATCAACTTCACTGGTGTG	TGGGCACTCGGAGTCTTATG	GTAAAACGACGGCCAGT
MMP2-7	CCDS10752.1-Amp 7	NM_004530-Amp 7	GTAAAACGACGGCCAGTTGTGGTTCATTAAGGTCAGCG	ATGCTTGAAGTGGGTGAGGTC	GTAAAACGACGGCCAGT
MMP2-8	CCDS10752.1-Amp 8	NM_004530-Amp 8	CCAGGGTTCTCAGCCTTACTG	GTAAAACGACGGCCAGTATGAGATCTTGCTGGTCCCAC	GTAAAACGACGGCCAGT
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MMP2-10	CCDS10752.1-Amp 10	NM_004530-Amp 10	TCCAACCTTCCTTTGATCCTG	GTAAAACGACGGCCAGTAGGAACTGTGGTTTGAGAGGG	GTAAAACGACGGCCAGT
MMP2-11	CCDS10752.1-Amp 11	NM_004530-Amp 11	GTAAAACGACGGCCAGTTGGACAGACAGATGATGGGAG	CACCAACCTCTGAACTCAGCC	GTAAAACGACGGCCAGT
MMP2-12	CCDS10752.1-Amp 12	NM_004530-Amp 12	GAAGGGCTAGGTCCAGTTTCC	GTAAAACGACGGCCAGTGTGTATTTGCAAGGAGCTGGG	GTAAAACGACGGCCAGT
MMP2-13	CCDS10752.1-Amp 13	NM_004530-Amp 13	CCTCAGCCTTTCAAAGCTCTC	GTAAAACGACGGCCAGTCTGTAGAGCTGAAGGCACGG	GTAAAACGACGGCCAGT
MMP3-1	CCDS8323.1-Amp 1	NM_002422-Amp 1	GTAAAACGACGGCCAGTCGAGGTTGGACCTACAAGGAG	AAGCCCAAATGGTGTGATAATG	GTAAAACGACGGCCAGT
MMP3-2	CCDS8323.1-Amp 2	NM_002422-Amp 2	TTTGCCATTATTTCAGCAAGC	GTAAAACGACGGCCAGTTGTATAATTCACAATCCTGTAGGAGAA	GTAAAACGACGGCCAGT
MMP3-3	CCDS8323.1-Amp 3	NM_002422-Amp 3	GTAAAACGACGGCCAGTCACTTCAGAACCTTTCCTGGC	CAACAACACCAGATAGATGAATGG	GTAAAACGACGGCCAGT
MMP3-4	CCDS8323.1-Amp 4	NM_002422-Amp 4	CTCACATTCTCCAGGCTGTATG	GTAAAACGACGGCCAGTCCAGAACATCTCATTTCTTGAGC	GTAAAACGACGGCCAGT
MMP3-5	CCDS8323.1-Amp 5	NM_002422-Amp 5	CCAATACAATGCACAGCAGAC	GTAAAACGACGGCCAGTTGCTTTCCAAACATTCTCACG	GTAAAACGACGGCCAGT
MMP3-6	CCDS8323.1-Amp 6	NM_002422-Amp 6	AAAGCACCGTGAGAATGTTTG	GTAAAACGACGGCCAGTAAGGCAGCACCAGATCTAAATG	GTAAAACGACGGCCAGT
MMP3-7	CCDS8323.1-Amp 7	NM_002422-Amp 7	GAAGCTGGGTGAGGCAAGAC	GTAAAACGACGGCCAGTCCCTGGTTATTTCTTCATTACCG	GTAAAACGACGGCCAGT
MMP3-8	CCDS8323.1-Amp 8	NM_002422-Amp 8	GTAAAACGACGGCCAGTAAAGCCCACTGTAAGCTGGTG	AGAGAAGCAGGCCTAAGGTTG	GTAAAACGACGGCCAGT
MMP3-9	CCDS8323.1-Amp 9	NM_002422-Amp 9	GTAAAACGACGGCCAGTCTGTTCCGGATGGAGATATGG	TTTCAATGCTCCTCTTCCCTC	GTAAAACGACGGCCAGT
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MMP7-1	CCDS8317.1-Amp 1	NM_002423-Amp 1	ATTTCCACATTCGAGGCTGAG	GTAAAACGACGGCCAGTGCAATGCTAACATGGGAAGG	GTAAAACGACGGCCAGT
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MMP7-3	CCDS8317.1-Amp 3	NM_002423-Amp 3	GTAAAACGACGGCCAGTGACTTCCAAAGTGGTCACCTACAG	CATAGTTTGCCAACTGCTGC	GTAAAACGACGGCCAGT
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MMP7-6	CCDS8317.1-Amp 6	NM_002423-Amp 6	ATCCTGCCTTCTTAGCCTCTG	GTAAAACGACGGCCAGTTTGCTAAATGGAGTGGAGGAAC	GTAAAACGACGGCCAGT
MMP8-1	CCDS8320.1-Amp 1	NM_002424-Amp 1	GTAAAACGACGGCCAGTGCTGTGAGTGACACATGATGC	GTCCTTACCAGCTTGGAACCC	GTAAAACGACGGCCAGT
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MMP9-2	CCDS13390.1-Amp 2	NM_004994-Amp 2	GTGTCTGGAGGGAGTCCTTTG	GTAAAACGACGGCCAGTCAAGACAGGACATGTTCACCG	GTAAAACGACGGCCAGT
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MMP9-4	CCDS13390.1-Amp 4	NM_004994-Amp 4	GTAAAACGACGGCCAGTATTTCAGCCCGCACTTATTTC	GAACCACTGCAGGAGGAGG	GTAAAACGACGGCCAGT
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MMP9-6	CCDS13390.1-Amp 6	NM_004994-Amp 6	ACCATCCATGGGTCAAAGAAC	GTAAAACGACGGCCAGTCAAACTGATGGGAGGGAAGAG	GTAAAACGACGGCCAGT
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MMP9-8	CCDS13390.1-Amp 8	NM_004994-Amp 8	GTAAAACGACGGCCAGTTCGAACTTTGACAGCGACAAG	AGACAGACGTTGAGGTCCCAC	GTAAAACGACGGCCAGT
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MMP9-10	CCDS13390.1-Amp 10	NM_004994-Amp 10	TGGGAACCAGCTGTATTTGTTC	GTAAAACGACGGCCAGTTTTCGTGGGTTATCGATGGTC	GTAAAACGACGGCCAGT
MMP9-11	CCDS13390.1-Amp 11	NM_004994-Amp 11	GTAAAACGACGGCCAGTAATCTCCCTCCTCGCGTTC	CACCTCGGTACTGGAAGACG	GTAAAACGACGGCCAGT
MMP9-12	CCDS13390.1-Amp 12	NM_004994-Amp 12	GTAAAACGACGGCCAGTGTGTGGGTGTACACAGGCG	ATTTGTTTCCATTTCCAAGGG	GTAAAACGACGGCCAGT
MMP9-13	CCDS13390.1-Amp 13	NM_004994-Amp 13	GTAAAACGACGGCCAGTACAGCATCTCACAGGTTGGG	AGTTTGTATCCGGCAAACTGG	GTAAAACGACGGCCAGT
MMP10-1	CCDS8321.1-Amp 1	NM_002425-Amp 1	GTAAAACGACGGCCAGTCTGTTGGTGATCTCAGCATTG	CACCTCCAGCCTGTTAATTATTC	GTAAAACGACGGCCAGT
MMP10-2	CCDS8321.1-Amp 2	NM_002425-Amp 2	TGACTAGCAAACTAACATTGCAATAAA	GTAAAACGACGGCCAGTTTCACAATCCTGGAGGAGAAA	GTAAAACGACGGCCAGT
MMP10-3	CCDS8321.1-Amp 3	NM_002425-Amp 3	CATGCCGAAGTGGAGGAAA	GTAAAACGACGGCCAGTGGTACCATCTATGAGGAATAGGCTC	GTAAAACGACGGCCAGT
MMP10-4	CCDS8321.1-Amp 4	NM_002425-Amp 4	GTAAAACGACGGCCAGTTGCAGCAGACACCAACATTC	CGATTTATTGCTCGTTCTAGATAATCC	GTAAAACGACGGCCAGT
MMP10-5	CCDS8321.1-Amp 5	NM_002425-Amp 5	GTAAAACGACGGCCAGTACATTGTGGTTTCAAGGATGG	AAGTGCTTCAGGCCAAATCAG	GTAAAACGACGGCCAGT
MMP10-6	CCDS8321.1-Amp 6	NM_002425-Amp 6	GTAAAACGACGGCCAGTTGAAAGGCACACAAATTGACA	AACAAACCTGCACATGTACCC	GTAAAACGACGGCCAGT
MMP10-7	CCDS8321.1-Amp 7	NM_002425-Amp 7	TTACCCTGGAATCACATGCAG	GTAAAACGACGGCCAGTAAGTGTTAGCACAAGGTCTTTCAG	GTAAAACGACGGCCAGT
MMP10-8	CCDS8321.1-Amp 8	NM_002425-Amp 8	TAAGGACTGGCTGTGAACTGG	GTAAAACGACGGCCAGTAGAGGGTGGGTGTAGGGTAGG	GTAAAACGACGGCCAGT
MMP10-9	CCDS8321.1-Amp 9	NM_002425-Amp 9	GTAAAACGACGGCCAGTTTTGTCTAGGATTCCACACTGG	ACACAAATTTAAACCCACTTTGC	GTAAAACGACGGCCAGT
MMP10-10	CCDS8321.1-Amp 10	NM_002425-Amp 10	GTAAAACGACGGCCAGTCTTAGCTCAGTGCTTGGCATAG	TCTGCAAGGCTCATCTTCTTC	GTAAAACGACGGCCAGT
MMP11-1	CCDS13816.1-Amp 1	NM_005940-Amp 1	CGGCTGCTAGGAGAGTTCAG#	GTAAAACGACGGCCAGTACTGCAAGCTCGCTGTCTATC#	GTAAAACGACGGCCAGT
MMP11-2	CCDS13816.1-Amp 2	NM_005940-Amp 2	GTAAAACGACGGCCAGTCACGTGTGTTTGCTGACAGG	GGGTTCAGTGGACACACAGG	GTAAAACGACGGCCAGT
MMP11-3	CCDS13816.1-Amp 3	NM_005940-Amp 3	GTAAAACGACGGCCAGTCTGAGTGTTCACACACCCACC	CCCATGCCAGTACCTACAGATG	GTAAAACGACGGCCAGT
MMP11-4	CCDS13816.1-Amp 4	NM_005940-Amp 4	GTAAAACGACGGCCAGTTGACGCCACTCACCTTTACTG	CTCAGTGACCTTGGCGAGTC	GTAAAACGACGGCCAGT
MMP11-5	CCDS13816.1-Amp 5	NM_005940-Amp 5	GTAAAACGACGGCCAGTGGGCAACCGAAGATCATAAAG	AAAGCCCGCTTTGAAGAAA	GTAAAACGACGGCCAGT
MMP11-6	CCDS13816.1-Amp 6	NM_005940-Amp 6	ACACCAATGAGATTGCACCG	GTAAAACGACGGCCAGTTCTCCCTATCCACTGTGTCCC	GTAAAACGACGGCCAGT
MMP11-7	CCDS13816.1-Amp 7	NM_005940-Amp 7	GTAAAACGACGGCCAGTGGGTCAAGCAGGTCCACAG	GGACAAAGGACCTGTGATGAAG	GTAAAACGACGGCCAGT
MMP11-8	CCDS13816.1-Amp 8	NM_005940-Amp 8	GTAAAACGACGGCCAGTAGGACTCGAGGAACTCAGCAG	AGATGGCCATGGGTCTCTAGC	GTAAAACGACGGCCAGT
MMP13-1	CCDS8324.1-Amp 1	NM_002427-Amp 1	GTAAAACGACGGCCAGTTGATGACTCACCATTGCAGG	AAAGACTGCATTTCTCGGAGC	GTAAAACGACGGCCAGT
MMP13-2	CCDS8324.1-Amp 2	NM_002427-Amp 2	GTAAAACGACGGCCAGTTGATGATGCGGTTGGTACATC	TCGGCAACCATATTAAAGCTATTC	GTAAAACGACGGCCAGT
MMP13-3	CCDS8324.1-Amp 3	NM_002427-Amp 3	CCACATGGTCAGATATTTGGAG	GTAAAACGACGGCCAGTCTTAGCACAGGTGTTTGGTAAATTAG	GTAAAACGACGGCCAGT
MMP13-4	CCDS8324.1-Amp 4	NM_002427-Amp 4	GTAAAACGACGGCCAGTACACTAATTTACCAAACACCTGTGC	CCCTCAAGCCACAGTGACAG	GTAAAACGACGGCCAGT
MMP13-5	CCDS8324.1-Amp 5	NM_002427-Amp 5	GCCAAGAAAGAAACATACCAGATAG	GTAAAACGACGGCCAGTGCATGCGCTTCACTGTACC	GTAAAACGACGGCCAGT
MMP13-6	CCDS8324.1-Amp 6	NM_002427-Amp 6	CTGGAGAAACCCTCTCCCAC	GTAAAACGACGGCCAGTTTTGGCAATATGCAGAAGCAG	GTAAAACGACGGCCAGT
MMP13-7	CCDS8324.1-Amp 7	NM_002427-Amp 7	GTAAAACGACGGCCAGTACAGGTGGGAGGTATTGGTTG	GATTCATGGTAGAATGGTTTCAGG	GTAAAACGACGGCCAGT
MMP13-8	CCDS8324.1-Amp 8	NM_002427-Amp 8	AAAGTGGGCCTGTCTTTAAGC	GTAAAACGACGGCCAGTGCCTGAAAGAGCTGACATGG	GTAAAACGACGGCCAGT
MMP13-9	CCDS8324.1-Amp 9	NM_002427-Amp 9	GCTACCAGATGCTTTAAATGGAC	GTAAAACGACGGCCAGTTTTGGTTTGCTGTCTTTGTAGG	GTAAAACGACGGCCAGT
MMP13-10	CCDS8324.1-Amp 10	NM_002427-Amp 10	TCCTACAAATGGGATCTAAGAGATTG	GTAAAACGACGGCCAGTTCTTCAATGTGGTTCCAGCC	GTAAAACGACGGCCAGT

Gene and Exon Name	CCDS accession and amplimer number	Ref Seq accession and amplimer number	Forward Primer	Reverse Primer	Sequencing Primer
MMP14-1	CCDS9577.1-Amp 1	NM_004995-Amp 1	AGTGCCTACCGAAGACAAAGG	GTAAAACGACGGCCAGTCTCTCCGAATAGAGGCTGTCC	GTAAAACGACGGCCAGT
MMP14-2	CCDS9577.1-Amp 2	NM_004995-Amp 2	GGTCTAGCCTGGGAGTTCCTC	GTAAAACGACGGCCAGTACAAGCCAGGCATTCAGGTAG	GTAAAACGACGGCCAGT
MMP14-3	CCDS9577.1-Amp 3	NM_004995-Amp 3	ACCCTGACCTCATAACCTTGG	GTAAAACGACGGCCAGTAGTCCCTGCATAAGCACAATG	GTAAAACGACGGCCAGT
MMP14-4	CCDS9577.1-Amp 4	NM_004995-Amp 4	CAGGGAAGGAGAATGTTGCC	GTAAAACGACGGCCAGTTCTGTGGTCCCTTCTAGCCC	GTAAAACGACGGCCAGT
MMP14-5	CCDS9577.1-Amp 5	NM_004995-Amp 5	GTAAAACGACGGCCAGTTGGAGGTGAAACCAAAGCTG	GAGGCTGAAGCAACCAACTG	GTAAAACGACGGCCAGT
MMP14-6	CCDS9577.1-Amp 6	NM_004995-Amp 6	GTAAAACGACGGCCAGTCTGCCCATCTGTCTGTCCTTC	CTATGTTGGCCAGCCTGC	GTAAAACGACGGCCAGT
MMP14-7	CCDS9577.1-Amp 7	NM_004995-Amp 7	GTAAAACGACGGCCAGTTGGAAGTGACAACAGCTGGAC	AAACACCCAATGCTTGTCTCC	GTAAAACGACGGCCAGT
MMP14-8	CCDS9577.1-Amp 8	NM_004995-Amp 8	GTAAAACGACGGCCAGTTCAAAGGTAACCAGGCACTCC	TTTCTAGGCAACAGCAGAGGC	GTAAAACGACGGCCAGT
MMP14-9	CCDS9577.1-Amp 9	NM_004995-Amp 9	GTAAAACGACGGCCAGTCTGTCCACAGCTATCCTTTGC	ACCTTCGTGAGCATCAGCTTC	GTAAAACGACGGCCAGT
MMP14-10	CCDS9577.1-Amp 10	NM_004995-Amp 10	TTAACAGAGCTTCCCTCGCTC	GTAAAACGACGGCCAGTCACCACCTGCTGCCACTG	GTAAAACGACGGCCAGT
MMP15-1	CCDS10792.1-Amp 1	NM_002428-Amp 1	ATTTGCCGAGGCGACCTA	GTAAAACGACGGCCAGTCCAGAACAGGGCTGTAGGAGT	GTAAAACGACGGCCAGT
MMP15-2	CCDS10792.1-Amp 2	NM_002428-Amp 2	GTAAAACGACGGCCAGTCTCAGATAGAGCAGCGGTGG	AGTGACATGGAGGACCAAACC	GTAAAACGACGGCCAGT
MMP15-3	CCDS10792.1-Amp 3	NM_002428-Amp 3	TTGAGCGAACCCTCCATTACT	GTAAAACGACGGCCAGTCTGGTCTCATCAGCGTGAAAG	GTAAAACGACGGCCAGT
MMP15-4	CCDS10792.1-Amp 4	NM_002428-Amp 4	GTAAAACGACGGCCAGTCACTCTTGGGAACATGCCAG	ACTCAGCAGGGTGTTGTTGC	GTAAAACGACGGCCAGT
MMP15-5	CCDS10792.1-Amp 5	NM_002428-Amp 5	CTTCCAAGAGGGAAGCAAGG	GTAAAACGACGGCCAGTGATGGCAGCAATGCCTACTC	GTAAAACGACGGCCAGT
MMP15-6	CCDS10792.1-Amp 6	NM_002428-Amp 6	TTTACTTTCCGCGTGGGTG	GTAAAACGACGGCCAGTTAGAGCTGAGGGCTTAATGGC	GTAAAACGACGGCCAGT
MMP15-7	CCDS10792.1-Amp 7	NM_002428-Amp 7	GTAAAACGACGGCCAGTCCACTGCCTGCCTCAGTATC	CTTCTCTCAGATCCAGCAGGG	GTAAAACGACGGCCAGT
MMP15-8	CCDS10792.1-Amp 8	NM_002428-Amp 8	CGGGTGACCTCATTGTATGTTG	GTAAAACGACGGCCAGTCCACAGGCAGTGGATTCTTTC	GTAAAACGACGGCCAGT
MMP15-9	CCDS10792.1-Amp 9	NM_002428-Amp 9	GTAAAACGACGGCCAGTCCAGAAAGAATCCACTGCCTG	AAGCTAGGTAAGCTCCCAGCC	GTAAAACGACGGCCAGT
MMP15-10-1	CCDS10792.1-Amp 10	NM_002428-Amp 10	CAACCCAGCAGGCTATAGGAC	GTAAAACGACGGCCAGTCATCACCACGTTCACCGTC	GTAAAACGACGGCCAGT
MMP15-10-2	CCDS10792.1-Amp 11	NM_002428-Amp 11	TACAAGGGCACCAAATACTGG	GTAAAACGACGGCCAGTGACATAAATAATGAGGGCGGG	GTAAAACGACGGCCAGT
MMP16-1	CCDS6246.1-Amp 1	NM_005941-Amp 1	GTAAAACGACGGCCAGTTTGAAAGGAAACGAGGGAGG	GCTACAAGGATCCCAGGAGTG	GTAAAACGACGGCCAGT
MMP16-2	CCDS6246.1-Amp 2	NM_005941-Amp 2	GTAAAACGACGGCCAGTAAATACTGTATGCCCTTGTGGAG	CAAATTACTCCATTTCTGGTTGG	GTAAAACGACGGCCAGT
MMP16-3	CCDS6246.1-Amp 3	NM_005941-Amp 3	TCATCACTGTCTCAGATTAACCTCTT	GTAAAACGACGGCCAGTGAGCCCAAGTGTCTCAGAGTG	GTAAAACGACGGCCAGT
MMP16-4	CCDS6246.1-Amp 4	NM_005941-Amp 4	GAGACAAGAGGAATGAATACGTG	GTAAAACGACGGCCAGTAAGTTTGTAAATTGTTAAATCTCTTGG	GTAAAACGACGGCCAGT
MMP16-5	CCDS6246.1-Amp 5	NM_005941-Amp 5	AGGAAAGCCAGTGATGAAACC	GTAAAACGACGGCCAGTAAGATACCTGGGACCTTTGCC	GTAAAACGACGGCCAGT
MMP16-6	CCDS6246.1-Amp 6	NM_005941-Amp 6	GTAAAACGACGGCCAGTCTGTGCCTTCTGCTGATAAGTG	TCTAACTGTGAGAGGGCTGGG	GTAAAACGACGGCCAGT
MMP16-7	CCDS6246.1-Amp 7	NM_005941-Amp 7	TTTGTGATCATTGCGGTCAAG	GTAAAACGACGGCCAGTTTGTGTGCACCACAGGGTC	GTAAAACGACGGCCAGT
MMP16-8	CCDS6246.1-Amp 8	NM_005941-Amp 8	TTCCAGATGGGACAACTAATAACT	GTAAAACGACGGCCAGTTCTTCAACAGCTGATGCCTCT	GTAAAACGACGGCCAGT
MMP16-9	CCDS6246.1-Amp 9	NM_005941-Amp 9	GTCAAGCACACAGCAAAGGTC	GTAAAACGACGGCCAGTCTCTAGTATAGCACTTTCCAATGGC	GTAAAACGACGGCCAGT
MMP16-10	CCDS6246.1-Amp 10	NM_005941-Amp 10	AGCCACAGCCATCTTAACAGA	GTAAAACGACGGCCAGTCAGCATAACAGCTCTTGTCTTGA	GTAAAACGACGGCCAGT
MMP17-1	CCDS31927.1-Amp 1	NM_0161550-Amp 1	GTAAAACGACGGCCAGTGCATACATTTGCATGGCCC[#]	ACCCAGGAAATGGGTCCAG[#]	GTAAAACGACGGCCAGT
MMP17-2	CCDS31927.1-Amp 2	NM_0161550-Amp 2	GAGACAAGAGGTGCCTTGTGG	GTAAAACGACGGCCAGTCAAGCATAAGACGGGACTGTG	GTAAAACGACGGCCAGT
MMP17-3	CCDS31927.1-Amp 3	NM_0161550-Amp 3	GTAAAACGACGGCCAGTCAAAGATGACGTGGGAGGAG	AGACCCTGTTCCTCACCCTG	GTAAAACGACGGCCAGT
MMP17-4	CCDS31927.1-Amp 4	NM_0161550-Amp 4	GTAAAACGACGGCCAGTGGGCGTTCAGGGAACCTC	AGATCGGCCTAGTGGGAAATG	GTAAAACGACGGCCAGT
MMP17-5	CCDS31927.1-Amp 5	NM_0161550-Amp 5	GTAAAACGACGGCCAGTTAACCCTGCAGAATCCCTCTG	ATCCGTGGAGGGAACAGC	GTAAAACGACGGCCAGT
MMP17-6	CCDS31927.1-Amp 6	NM_0161550-Amp 6	ACAGAGCCTGTCTGCTGAGTG	GTAAAACGACGGCCAGTTCCCAGGACTCTGAGCCAC	GTAAAACGACGGCCAGT
MMP17-7	CCDS31927.1-Amp 7	NM_0161550-Amp 7	ACACAAGGATGCCTGTCCC	GTAAAACGACGGCCAGTAGAAGACGATCTTGTGGTCGC	GTAAAACGACGGCCAGT
MMP17-8	CCDS31927.1-Amp 8	NM_0161550-Amp 8	GTAAAACGACGGCCAGTTCTTCAAAGGTACCTCCAGGG	AGACGTAGGGCTTACACGCAG	GTAAAACGACGGCCAGT
MMP17-9	CCDS31927.1-Amp 9	NM_0161550-Amp 9	GTAAAACGACGGCCAGTTGACACAGGAGAAGGAAAGGC	ACCTCTTGGCTGCCCTGTAG	GTAAAACGACGGCCAGT
MMP17-10	CCDS31927.1-Amp 10	NM_0161550-Amp 10	GTAAAACGACGGCCAGTCCCACTGCAGTCTTGTGC	ACTCCGGCACAGTCCTTG	GTAAAACGACGGCCAGT
MMP19-1	CCDS8895.1-Amp 1	NM_002429-Amp 1	GTAAAACGACGGCCAGTCTGGTAGGGCAAGGGTTAATG	CAGGCTGAAGGTCAGGAGG	GTAAAACGACGGCCAGT
MMP19-2	CCDS8895.1-Amp 2	NM_002429-Amp 2	GTAAAACGACGGCCAGTTTCCATATCAGGTCCCTCCTC	AATCCCTGGCATGGTTTAGG	GTAAAACGACGGCCAGT
MMP19-3	CCDS8895.1-Amp 3	NM_002429-Amp 3	GTAAAACGACGGCCAGTCAGCTTCAGGGATTCTGTGC	GGTCCAGACTGTCCTAATGCC	GTAAAACGACGGCCAGT
MMP19-4	CCDS8895.1-Amp 4	NM_002429-Amp 4	ACCTGGTTGTCACCTGTCTTG	GTAAAACGACGGCCAGTCAGCCCAAACATCAAGGTTC	GTAAAACGACGGCCAGT
MMP19-5	CCDS8895.1-Amp 5	NM_002429-Amp 5	GTAAAACGACGGCCAGTGGGAGAAAGAAGCATCCAAGAG	GCTAGACCTGTCTCTTCAAGCC	GTAAAACGACGGCCAGT
MMP19-6	CCDS8895.1-Amp 6	NM_002429-Amp 6	AAACCTAGAACGTGTCTCCCG	GTAAAACGACGGCCAGTTAACCTCCATTGCTCATGCTG	GTAAAACGACGGCCAGT
MMP19-7	CCDS8895.1-Amp 7	NM_002429-Amp 7	AGGGCAAATTGTTAGTCAGCC	GTAAAACGACGGCCAGTCCTCCGGTCCACCTATTCAG	GTAAAACGACGGCCAGT
MMP19-8	CCDS8895.1-Amp 8	NM_002429-Amp 8	GTAAAACGACGGCCAGTCAGTGTCTTACTTGAGGAGGGC	GCAGAACATCTCCCTTCAAGC	GTAAAACGACGGCCAGT
MMP19-9	CCDS8895.1-Amp 9	NM_002429-Amp 9	GTAAAACGACGGCCAGTGCTTGAAGGGAGATGTTCTGC	ATTAGGCCTTAGGCTTCTGGG	GTAAAACGACGGCCAGT
MMP20-1	CCDS8318.1-Amp 1	NM_004771-Amp 1	GTAAAACGACGGCCAGTAAGGAGCTCAAGGTCGAAGTG	TGCACCAGACACCAATCTAGG	GTAAAACGACGGCCAGT
MMP20-2	CCDS8318.1-Amp 2	NM_004771-Amp 2	GTAAAACGACGGCCAGTAATACATCAGGAATGCTGTCCC	CATTGTCATTGCCTGACGG	GTAAAACGACGGCCAGT
MMP20-3	CCDS8318.1-Amp 3	NM_004771-Amp 3	CCGGATTATCCCAACTGTCTC	GTAAAACGACGGCCAGTGTGCGAAGGAGGAGTGTGTG	GTAAAACGACGGCCAGT
MMP20-4	CCDS8318.1-Amp 4	NM_004771-Amp 4	GTAAAACGACGGCCAGTTTTCTGTAATATGATGCGCCC	AGTTAAAGGGTGGCTTGGGAC	GTAAAACGACGGCCAGT
MMP20-5	CCDS8318.1-Amp 5	NM_004771-Amp 5	GTGCCAGAATAACCTACCAACC	GTAAAACGACGGCCAGTAAATGCACTTTCTTTAATTCGGAG	GTAAAACGACGGCCAGT
MMP20-6	CCDS8318.1-Amp 6	NM_004771-Amp 6	ATCACACAGCTCAGCAACCAG	GTAAAACGACGGCCAGTTCATACAAGGCAGCAACAAGG	GTAAAACGACGGCCAGT
MMP20-7	CCDS8318.1-Amp 7	NM_004771-Amp 7	CAAGAGCAAAGGGCATTTAGTG	GTAAAACGACGGCCAGTTGCTGGCAGGGCTAGATATG	GTAAAACGACGGCCAGT
MMP20-8	CCDS8318.1-Amp 8	NM_004771-Amp 8	GTAAAACGACGGCCAGTCCATGGCAAGTATCCCATAAAC	CTTGCTTTGCATTCTTTCGTG	GTAAAACGACGGCCAGT
MMP20-9	CCDS8318.1-Amp 9	NM_004771-Amp 9	CTGGGCAACAGTGAGACCC	GTAAAACGACGGCCAGTCCACTTGGAAATCCACTTTCTG	GTAAAACGACGGCCAGT
MMP20-10	CCDS8318.1-Amp 10	NM_004771-Amp 10	GTAAAACGACGGCCAGTAAGACAAGCTAACTGCCACCTG	CCTTTGGAAGAATCCCTCTCC	GTAAAACGACGGCCAGT
MMP21-1	CCDS7647.1-Amp 1	NM_147191-Amp 1	GTAAAACGACGGCCAGTGAGAAATGCTTGGAAACCTGG	GAGCCACTCCTCCCTCTCAG	GTAAAACGACGGCCAGT
MMP21-2-1	CCDS7647.1-Amp 2-1	NM_147191-Amp 2-1	ATTAGGGTGCCTTTCTCTCCC[#]	GTAAAACGACGGCCAGTTGCTCTTACCTCTCCCAAAGC[#]	GTAAAACGACGGCCAGT
MMP21-2-2	CCDS7647.1-Amp 2-2	NM_147191-Amp 2-2	CCAGGCCTTCTCCAAGAGG	GTAAAACGACGGCCAGTTGACCATGATTCCGACAAGAC	GTAAAACGACGGCCAGT
MMP21-3	CCDS7647.1-Amp 3	NM_147191-Amp 3	GGAGCTGCCACTCTGTCTACC	GTAAAACGACGGCCAGTCTCAGAGGTTGCGGACACTAC	GTAAAACGACGGCCAGT
MMP21-4	CCDS7647.1-Amp 4	NM_147191-Amp 4	ACAGGCCTGCGTCTGTTATG	GTAAAACGACGGCCAGTAATGGCATCAGGGAGCTAGAG	GTAAAACGACGGCCAGT
MMP21-5	CCDS7647.1-Amp 5	NM_147191-Amp 5	CCCAGCTTTGCAACTGTCTTC	GTAAAACGACGGCCAGTAGAGCATTACAACAGATTCCTTGG	GTAAAACGACGGCCAGT
MMP21-6	CCDS7647.1-Amp 6	NM_147191-Amp 6	GTAAAACGACGGCCAGTTGTACAGGATTGGCTGAGACC	TTTGTTGAGACCGAAACATGG	GTAAAACGACGGCCAGT
MMP24-1	N/A	NM_006690-Amp 1	GTAAAACGACGGCCAGTCCCTCCTGCTCAGGGCTAC[#]	ACCTCGGCAGTAGGTGCG[#]	GTAAAACGACGGCCAGT
MMP24-2	N/A	NM_006690-Amp 2	GTAAAACGACGGCCAGTCTCGTGTTCAAGGCACATCTC	AGGCCATGACAAATGTGAACC	GTAAAACGACGGCCAGT
MMP24-3	N/A	NM_006690-Amp 3	GATGCACATTGCTTTCCAGAC	GTAAAACGACGGCCAGTGTGAGCCTTTGAAACTCAGGC	GTAAAACGACGGCCAGT
MMP24-4	N/A	NM_006690-Amp 4	TGAGCCACATTGTCTCAGGTA	GTAAAACGACGGCCAGTCATCTAGGATGTCCTCCAAGTTCT	GTAAAACGACGGCCAGT
MMP24-5	N/A	NM_006690-Amp 5	GTAAAACGACGGCCAGTTCGGTGTTTGCTGAGGTAATG	GTGCTGATGATAGCAACGTCC	GTAAAACGACGGCCAGT
MMP24-6	N/A	NM_006690-Amp 6	GTGATGCTGAGACTGGCAATG	GTAAAACGACGGCCAGTAATCCCAAATCAGGAGCCTTC	GTAAAACGACGGCCAGT
MMP24-7	N/A	NM_006690-Amp 7	TAATGGACAGTCTCGGTGGAG	GTAAAACGACGGCCAGTCCGCCTCTTCCAGTTTAGATG	GTAAAACGACGGCCAGT
MMP24-8	N/A	NM_006690-Amp 8	GTAAAACGACGGCCAGTACCTGACACCCTGAAGATGG	CCATGGGCACTGAGTATGC	GTAAAACGACGGCCAGT
MMP24-9	N/A	NM_006690-Amp 9	GTAAAACGACGGCCAGTAGTGCCTGTGCCCTCCTT	CTGGCCCTGGTGAGCACT	GTAAAACGACGGCCAGT
MMP25-1	CCDS10492.1-Amp 1	NM_022468-Amp 1	GTAAAACGACGGCCAGTATCCCTCCCTACTCGGTGC	CCATCCTCAGTCACTCTTTGG	GTAAAACGACGGCCAGT
MMP25-2	CCDS10492.1-Amp 2	NM_022468-Amp 2	CAGACAAGACTGGCAAACCC	GTAAAACGACGGCCAGTTGAGGTCTAAAGGCTGTTGGG	GTAAAACGACGGCCAGT
MMP25-3	CCDS10492.1-Amp 3	NM_022468-Amp 3	AGCTGGCTTCCAGAGACAGAC	GTAAAACGACGGCCAGTGGGAGTGAGGTGTAAGGGTGG	GTAAAACGACGGCCAGT
MMP25-4	CCDS10492.1-Amp 4	NM_022468-Amp 4	GTAAAACGACGGCCAGTGAAGAAGCGAACCCTGACAT	CTGAGCAACAGAGCAAGACCT	GTAAAACGACGGCCAGT
MMP25-5	CCDS10492.1-Amp 5	NM_022468-Amp 5	GTAAAACGACGGCCAGTTGATGGAGAGTTCAGGAAGGG	CCCTGTGAGGAAAGAGTCAGG	GTAAAACGACGGCCAGT
MMP25-6	CCDS10492.1-Amp 6	NM_022468-Amp 6	GTAAAACGACGGCCAGTTGCAGCAACTCTATGGTAGGG	CTCACATCGATCAGGGATGG	GTAAAACGACGGCCAGT
MMP25-7	CCDS10492.1-Amp 7	NM_022468-Amp 7	CCTGACTCTTTCCTCACAGGG	GTAAAACGACGGCCAGTCTTCCAGGGTAAGCCCACAG	GTAAAACGACGGCCAGT
MMP25-8	CCDS10492.1-Amp 8	NM_022468-Amp 8	CCTTGATTTCCAGATGGGACC	GTAAAACGACGGCCAGTCAGGTAGGTCTTCCCGTTCTG	GTAAAACGACGGCCAGT
MMP25-9	CCDS10492.1-Amp 9	NM_022468-Amp 9	GTAAAACGACGGCCAGTCCTCCTCTTTAGCGGTGAGTG	AGGAGCAGAGCTTGAGTCCC	GTAAAACGACGGCCAGT
MMP25-10	CCDS10492.1-Amp 10	NM_022468-Amp 10	TGTAAAACGACGGCCAGTGATGTCACCGTCAGCAACG	CAGGAAACAGCTATGACCGTCCGTGGGAACAGACAAG	CAGGAAACAGCTATGACC
MMP26-1	CCDS7752.1-Amp 1	NM_021801-Amp 1	GTAAAACGACGGCCAGTGGCAGAGTGAGTCATTGGATG	GGCTGAATTAGGCACAGAAGG	GTAAAACGACGGCCAGT
MMP26-2	CCDS7752.1-Amp 2	NM_021801-Amp 2	GTAAAACGACGGCCAGTCTCAAAGAAGGCTATGCCCAG	GCTGCCAATGAAAGCAGAAG	GTAAAACGACGGCCAGT
MMP26-3	CCDS7752.1-Amp 3	NM_021801-Amp 3	GTAAAACGACGGCCAGTTTTGCATGTTTAATTCCTTAAGTTTC	AGGATTTGCTTTGCAGTAGGG	GTAAAACGACGGCCAGT
MMP26-4	CCDS7752.1-Amp 4	NM_021801-Amp 4	GTAAAACGACGGCCAGTCCCAGTGGTAGACTGTTGCAC	GGATCCCTAAATGGAAACCATC	GTAAAACGACGGCCAGT
MMP26-5	CCDS7752.1-Amp 5	NM_021801-Amp 5	CCTGTAACATTTGCCCTTTC	GTAAAACGACGGCCAGTCAGTAGCGTTTTGAGTGTCG	GTAAAACGACGGCCAGT
MMP26-6	CCDS7752.1-Amp 6	NM_021801-Amp 6	GCAGCACTCTGGGAATC	GTAAAACGACGGCCAGTAGGCTGCTCTAGTGCTTTCA	GTAAAACGACGGCCAGT
MMP27-1	CCDS8319.1-Amp 1	NM_022122-Amp 1	GTAAAACGACGGCCAGTTGATACAAGCCAATTCATGCC	AAACATCCCTTCATTTGTGACC	GTAAAACGACGGCCAGT
MMP27-2	CCDS8319.1-Amp 2	NM_022122-Amp 2	TTTCCTCCTTCACTCCTCACTTT	GTAAAACGACGGCCAGTGCACACAGGAAGTGCTCAGTC	GTAAAACGACGGCCAGT
MMP27-3	CCDS8319.1-Amp 3	NM_022122-Amp 3	TCAGCCTCCTTTGTTGAAGTG	GTAAAACGACGGCCAGTGACTGAGATACAATTTATGCGAGG	GTAAAACGACGGCCAGT
MMP27-4	CCDS8319.1-Amp 4	NM_022122-Amp 4	ATACTCCGGATATGGCACGAG	GTAAAACGACGGCCAGTTGAACACTTCCATCAAGGTCATC	GTAAAACGACGGCCAGT
MMP27-5	CCDS8319.1-Amp 5	NM_022122-Amp 5	GTAAAACGACGGCCAGTTAGCATTGCAATACCTCCCAG	TGCCTACACAAGGGTCTTACC	GTAAAACGACGGCCAGT
MMP27-6	CCDS8319.1-Amp 6	NM_022122-Amp 6	TCCAGTCCATCTATGGTGAGC	GTAAAACGACGGCCAGTCACATATGGTAAAGCATTCAAACAG	GTAAAACGACGGCCAGT
MMP27-7	CCDS8319.1-Amp 7	NM_022122-Amp 7	TGGAAGTCCCAAACTGAACTG	GTAAAACGACGGCCAGTACAGCCACAGAGAATCCTGG	GTAAAACGACGGCCAGT
MMP27-8	CCDS8319.1-Amp 8	NM_022122-Amp 8	GTAAAACGACGGCCAGTGAAAGTGGTAGGAATGAATGTGG	GGATCTCTTGAAAGCAGGAGG	GTAAAACGACGGCCAGT
MMP27-9	CCDS8319.1-Amp 9	NM_022122-Amp 9	GTAAAACGACGGCCAGTTCCCTGCTTGACTCTAAGTTGTG	GATTCAGTTTGTGATAATGTTTGTGG	GTAAAACGACGGCCAGT
MMP27-10	CCDS8319.1-Amp 10	NM_022122-Amp 10	TGTGATTCCACAAATTTCTTCG	GTAAAACGACGGCCAGTGGACCAGCAACTTGTTGTTAAAG	GTAAAACGACGGCCAGT
MMP28-1	N/A	NM_024302-Amp 1	GCAGAGCAGTCCCATCCC	GTAAAACGACGGCCAGTACGCAGATTGTCTGCTCACAG	GTAAAACGACGGCCAGT
MMP28-2	N/A	NM_024302-Amp 2	ACTGGGCAGGATGTATGTGC	GTAAAACGACGGCCAGTGCTCTGTCAGGAGGAAAGGAC	GTAAAACGACGGCCAGT
MMP28-3	N/A	NM_024302-Amp 3-1	GTAAAACGACGGCCAGTCAAGCTCTGACCATTTCCTCC	CTGACAAAGTCCAGTCCCAGG	GTAAAACGACGGCCAGT
MMP28-4	N/A	NM_024302-Amp 4	CCCTCGCCATCCTACTGG	GTAAAACGACGGCCAGTAGGAGACTGAGGGTTCTCTGC	GTAAAACGACGGCCAGT
MMP28-5	N/A	NM_024302-Amp 5	GTAAAACGACGGCCAGTGAGAACAGCCACACACACCC	GGGAAATCCTATTTGCAGAGC	GTAAAACGACGGCCAGT
MMP28-6	N/A	NM_024302-Amp 6	ACCAAGGGAAGGTCTTGAGTG	GTAAAACGACGGCCAGTCTTGTGGCTTGGGATTTCTG	GTAAAACGACGGCCAGT
MMP28-7	N/A	NM_024302-Amp 7	ATGCTTGTCCATTTGGGAAAC	GTAAAACGACGGCCAGTTCCCTAATCCATCCCTGAAAG	GTAAAACGACGGCCAGT
MMP28-8-1	N/A	NM_024302-Amp 8-1	GTAAAACGACGGCCAGTGGGCTCCAGCTTAAAGGAGAG	TGAGAGCACCACCAGTTTCTG	GTAAAACGACGGCCAGT
MMP28-8-2	N/A	NM_024302-Amp 8-2	GCAAGTGGAGCCCTACTACCC	GTAAAACGACGGCCAGTTCTGCAGAGGGACTCAGAGG	GTAAAACGACGGCCAGT

[#]The primer pair did not meet our quality criteria that ≥90% of bases in the target region have a Phred quality score of at least 20 in three quarters of the tumor samples analyzed.

Supplementary Table 3. Characteristics of melanoma patients with MMP mutations

Sample	Patient age (years) #	Gender	Tumor source	Matched normal source	NRAS/BRAF mutation
5T	47	M	External iliac soft tissue	Blood	BRAF
8T	61	M	Inguinal soft tissue	Blood	BRAF
10T	55	M	Axillary soft tissue	Blood	BRAF
12T	53	M	Upper arm, subcutaneous	Blood	NRAS
13T	49	M	Chest wall, subcutaneous	Blood	none
17T	33	M	Shoulder, subcutaneous	Blood	NRAS
32T	61	M	Omentum	Blood	BRAF
39T	56	M	Mesentery	Blood	none
44T	56	M	Lung	Blood	NRAS
45T	48	M	Mediastinum	Blood	BRAF
52T	39	F	Lung	Blood	BRAF
54T	26	M	Subcutaneous	Blood	BRAF
55T	60	M	Lung	Blood	none
63T	30	M	Jejunum	Blood	NRAS
64T	32	F	Ovary	Blood	BRAF
71T	67	M	Lung	Blood	BRAF
76T	40	M	Neck	Blood	none
81T	60	F	Upper arm, subcutaneous	Blood	BRAF
84T	60	F	Thigh, subcutaneous	Blood	BRAF
85T	44	M	Chest wall, subcutaneous	Blood	BRAF

Patient age refers to age at which patient MM sample was obtained. "None" refers no mutation observed.
Abbreviations: F, female; M, male.

Supplementary Table 4. Effects of MMP-8 mutations on growth *in vivo.*

Clone	# of mice with ulceration	# of mice with micrometastases
Vector	1	1
WT	0	0
S50F	5	2
P78S	3	2
K87N	5	3
G104R	3	0
E138Q	7	0

NOD/SCID mice were subcutaneously injected with $1x10^6$ Mel-STR cell clones expressing the indicated constructs and examined on a bi-weekly basis. After 20 days, mice were sacrificed and the number of mice with ulcerations and lung metastases was quantified.

Supplementary Methods

Tumor Tissues

A panel of pathology-confirmed metastatic melanoma tumor resections, paired with apheresis-collected peripheral blood mononuclear cells, was collected from 79 patients enrolled in IRB-approved clinical trials at the Surgery Branch of the National Cancer Institute. Pathology-confirmed melanoma cell lines were derived from mechanically or enzymatically dispersed tumor cells, which were then cultured in RPMI 1640 + 10% FBS at 37°C in 5% CO_2 for 5-15 passages. Genomic DNA was isolated using DNeasy Blood & Tissue kit (Qiagen, Valencia, CA). For all samples, matching between germline and tumor DNA was verified by direct sequencing of 26 single nucleotide polymorphisms (SNP) at 24 loci.

PCR, sequencing and mutational analysis

PCR and sequencing primers were designed using Primer 3 (http://www-genome.wi.mit.edu/cgibin/primer/primer3_www.cgi) and synthesized by Invitrogen (Carlsbad, CA) (Supplementary Table S2). PCR primers were designed to amplify the selected exons and the flanking intronic sequences, including splicing donor and acceptor regions. PCR products were ~500 bp in length, with multiple overlapping amplimers for larger exons. PCRs were done in both 384- and 96-well formats in 10-μl reaction volumes, containing 1mM deoxynucleotide triphosphates, 1 μM each of the forward and reverse primers, 6% DMSO, 1x PCR buffer, 6 ng/μl DNA, and 0.5 unit/μl Platinum Taq (Invitrogen, Carlsbad, CA). A touchdown PCR program was used for PCR amplification.

PCR conditions were as follows: 94°C for 2 min; three cycles of 94°C for 10 s, 67°C for 30 s, 68°C for 30 s; three cycles of 94°C for 10 s, 64°C for 30 s, 68°C for 30 s; three cycles of 94°C for 10 s, 61°C for 30 s, 68°C for 30 s; and 35 cycles of 94°C for 10 s, 58°C for 30 s, and 68°C for 30 s, followed by 68°C for 3 min and 10°C thereafter.

PCR products were purified using exonuclease (Epicentre Biotechnologies, Madison, WI) and shrimp alkaline phosphatase (USB Corporation, Cleveland, Ohio). Cycle sequencing was carried out using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) with an initial denaturation at 96°C for 2 min, for 25 cycles at 96°C for 30 s, 50°C for 15 s, and 60°C for 4 min. Sequencing products were purified with rehydrated Sephadex G-50 powder (GE Healthcare, Piscataway, NJ). Sequence data was collected on an ABI3730xl (Applied Biosystems, Foster City, CA). Sequence traces were assembled and analyzed to identify potential genomic alterations using the Mutation Surveyor software package (SoftGenetics, State College, PA).

Construction of wild-type and mutant MMP8 expression vector

The CDS MMP8 sequence was cloned by PCR using Phusion™ Hot Start High-Fidelity DNA Polymerase (New England Biolabs, Inc., Ipswich, MA) from human melanoma cDNA with primers TGACCAgaattcATGTTCTCCCTGAAGACGCTT

and TGGTCActcgagGCCATATCTACAGTTAAGCCATTTAT. The PCR product was cloned into the C-terminal FLAG of pCMV-Tag 4A Epitope Tagging Mammalian Expression Vector (Stratagene, La Jolla, CA) via the EcoR I & Xho I restriction sites. The S50F, P78S, K87N, G104R, E138Q, and E314K mutants were made using Fusion PCR.

Cell culture and transient expression

HEK 293T cells (ATCC, Manassas, VA) were grown in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 0.075% sodium bicarbonate (Invitrogen, Carlsbad, CA), 1X NEAA (Invitrogen, Carlsbad, CA), and 2mM L-glutamine (Mediatech, Inc, Herndon, VA) in a T-75 flask. SK-Mel-2 cells (National Cancer Institute, Division of Cancer Treatment, Developmental Therapeutics Program, Frederick, MD) were grown in RPMI-1640 (Lonza, Walkersville, MD) and supplemented with 10% fetal bovine serum (HyClone, Logan, UT). Confluent HEK 293T cells were transfected using Lipofectamine™ 2000 Transfection Reagent (Invitrogen, Carlsbad, CA) with 2.5 µg of empty vector or with vector containing wild-type *MMP8* or mutant *MMP8* DNA. Cells were harvested 24 hours after transfection.

Mel-STR cell culture and pooled stable expression

2.5×10^5 Mel-STR cells (generous gift from Robert Weinberg, Whitehead Institute) were grown in RPMI-1640 (Lonza, Walkersville, MD) and supplemented with 5% fetal bovine serum (HyClone, Logan, UT) in a 6-well plate. Twenty four hours later, the cells were transfected using Effectene Transfection Reagent (Qiagen, Valencia, CA) with 1 µg of empty vector or with vector containing wild-type *MMP8* or mutant *MMP8* DNA. Cells were dosed with 300 ng/ml of geneticin (Invitrogen, Carlsbad, CA) 48 hours after transfection. Media was changed every 4-5 days.

Lentiviral shRNA

Constructs for stable depletion of MMP-8 were obtained from Open Biosystems (Huntsville, AL). Two pre-made constructs were identified to be able to achieve efficient knockdown of MMP-8 at the protein level. Negative control constructs in the same vector system (vector alone (pLK0.1) and scrambled shRNA) were created by Drs. Robert Weinberg (Massachusetts Institute of Technology) and David Sabatini (Massachusetts Institute of Technology) and obtained from Addgene (Cambridge, MA). The lentiviral helper plasmids pHR'8.2ΔR and pCMV-VSV-G were obtained from Dr. Todd Waldman (Georgetown University). All plasmids were prepped, and their integrity was confirmed by restriction analysis.

To prepare viral stocks, 1.5×10^6 HEK 293T cells were plated in T-75 flasks. The following day cells were co-transfected with shRNA constructs (3 µg), together with pHR'8.2ΔR and pCMV-VSV-G helper constructs (3 µg and 0.3 µg, respectively), using Arrest-In (Open Biosystems). The viral-containing medium was harvested 48-50 hours post transfection. The viral stocks were centrifuged and filtered to remove any non-adherent HEK 293T cells.

Next, SK-Mel-2 cells were infected with shRNA lentiviruses for each condition (vector and scrambled controls and two independent *MMP8* specific shRNAs). To do this, cells were plated at sub-confluent densities in T-75 flasks. The next day, cells were infected with a cocktail of 4 ml viral-containing medium, 4 ml regular medium and 8 μg/ml polybrene. The medium was changed 1 day post-infection, and selective medium was added 2 days post-infection (2 μg/ml puromycin for all cells). After 3 days of puromycin selection, the mock-infected cells had all died. Stably infected pooled clones were tested in functional assays.

Immunoprecipitations

Transfected cells' growth media from a T-75 flask was removed and placed into Amicon Ultra-15 centrifugal filter unit with Ultracel-30 membrane (Millipore, Billerica, MA) and spun at 4150 rpm at 4°C for 25 minutes. The transfected cells remaining in the flask were washed with phosphate buffered saline, then lysed for 20 minutes on ice by using 810 μL EBC medium (50mM Tris pH 7.5, 100mM NaCl, 1/100 NaF, 1/400 $NaVO_4$, 5% protease inhibitor) and 90 μl of 1,10 phenanthroline (Sigma, St. Louis, MO) in 0.1N HCl per T-75 flask. Cells were removed from flasks and centrifuged at 14,000 rpm at at 4°C for 10 minutes. The supernatant from the lysed cells and the concentrated media were collected and incubated with 100 μl blocked EBC (EBC medium + 5% milk) plus 30 μl beads/sample of ANTI-FLAG® M2 Affinity Gel (Sigma-Aldrich, St. Louis, MO). The mixtures were incubated overnight at 4°C. The supernatant from each sample was aspirated off and the immunoprecipitates were washed three times with EBC buffer.

SK-Mel-2 pooled clones were seeded at $5x10^5$ cells per T-75 flask and incubated for 5 days. Media was harvested by centrifugation at 2,000 rpm for 10 minutes followed by concentration in an Amicon Ultra-15 centrifugal filter and spun at 4150 rpm at 4°C for 25 minutes. The remaining cells were washed with phosphate buffered saline and then lysed in EBC medium for further analysis via immunoblot. Cells were removed from flasks and centrifuged at 14,000 rpm at at 4°C for 10 minutes. The concentrated media was immunoprecipitated using 5μg / sample of anti-MMP-8 (R&D Systems) mixed with 20 μl of Protein-A sepharose beads (Amersham Pharmacia). The mixtures were incubated overnight at 4°C. The supernatant from each sample was aspirated off and the immunoprecipitates were washed three times with EBC buffer. Immunoprecipitates and lysates were analyzed by immunoblotting with anti-MMP-8 (Chemicon-Millipore) or anti-Tubulin (Millipore), respectively.

Zymographys

Collagen zymographys were made using 0.3% rat tail collagen type I (BD, Bedford, MA) in a 10% polyacrylamide gel. Immunoprecipitates were treated with 30 μl Laemmli solution (non-reducing) and incubated for 10 minutes at RT. Fifteen μl of each sample was loaded onto the zymographys. After electorphoresis, the gel was incubated in 100 ml 1X Zymogram Renaturing Buffer (Invitrogen, Carlsbad, CA) for 30 minutes at RT. The renaturing buffer was

decanted and 100 ml 1X Zymogram Developing Buffer (Invitrogen, Carlsbad, CA) was added to the gel, which was equilibrated for 30 minutes at RT. The developing buffer was decanted and 100 ml of 1X Zymogram Developing Buffer was added and then incubated O/N at 37°C. The gel was stained with 100 ml 0.5% Coomassie Blue R-250 (BioRad, Hercules, CA) for 30 minutes. The gel was destained using 100 ml destaining solution (Methanol:Acetic Acid:dH_2O (40:10:50)) until clear bands appeared against the blue background.

Western-blot analysis

After the zymography, the remaining agarose beads were treated with 30 µl denaturing Laemmli solution and incubated for 3 minutes at 95°C. Fifteen µl of each sample was loaded onto a Novex® 10% Tris-Glycine Gel (Invitrogen, Carlsbad, CA). After electrophoresis, the gel was blocked in 3% BSA & 0.05% Tween in PBS for 1 hour in RT, and then incubated with primary antibody, Monoclonal ANTI-FLAG® M2-Peroxidase (HRP) antibody produced in mouse (Sigma-Aldrich, St. Louis, MO) for 1 hour at RT. Finally, it was washed 4 x 5 minutes with 0.5X TBST and developed.

Colony formation assay in soft agar

Mel-STR or SK-Mel-2 pooled clones were plated in duplicate at 1000 cells/well in top plugs consisting of sterile 0.33% Bacto-Agar (BD, Sparks, MD) and 10% fetal bovine serum (HyClone, Logan, UT) in a 24-well plate. The lower plug contained sterile 0.5% Bacto-Agar and 10% fetal bovine serum. After two weeks, the colonies were photographed and counted.

Cell growth assay

Mel-STR pooled clones were plated in a 96-well plate in RPMI-1640 (Lonza, Walkersville, MD) and supplemented with 5% fetal bovine serum (HyClone, Logan, UT) and 300 ng/ml of geneticin (Invitrogen, Carlsbad, CA). Every 24 hours for a total of 10 days, the media was decanted from the 96 well plate with all the cell lines. The cells were lysed with 50 µl of 0.2% SDS. SYBR Green I (Invitrogen, Carlsbad, CA) solution was then added and the number of cells was counted using the Fluorostar Optima equipment and Optima software.

Mouse Studies

NOD/SCID (NOD.CB17-*Prkdc*<*scid*>/NCrCrl) mice were purchased from Charles River Labs. All mice were housed in a pathogen-free facility and were given autoclaved food and water. Mel-STR pooled clones with empty vector or with wild-type MMP8 or mutant MMP8 were grown up in T-75 flask to 90% confluency. $1x10^6$ cells were resuspended in 100 µl of sterile PBS and injected subcutaneously into 11 week old male NOD/SCID mice. Mice were monitored bi-weekly and tumor diameters were measured using precision calipers.

Supplementary Note

Tumor Tissue Collection

A panel of 79 pathology-confirmed metastatic melanoma tumor resections was assembled from patients referred to the Surgery Branch of the National Cancer Institute for enrollment in IRB-approved Surgery Branch clinical trials of immunotherapy. All of the patients had progressive metastatic melanoma that had failed available conventional treatments, were 16 years or older and passed eligibility testing for the relevant protocol. None had received therapy for 1 month prior to entering the protocol. Before tissue was acquired all patients signed written informed consents. The melanoma tissue panel was selected on the basis of an available pathology-confirmed, melanoma tissue culture line, paired with freshly frozen tissue from the resected melanoma metastasis and apheresis-collected, peripheral blood mononuclear cells.