Supplemental Materials

Matrix Proteases Contribute to Progression of Pelvic Organ Prolapse in Mice and Humans

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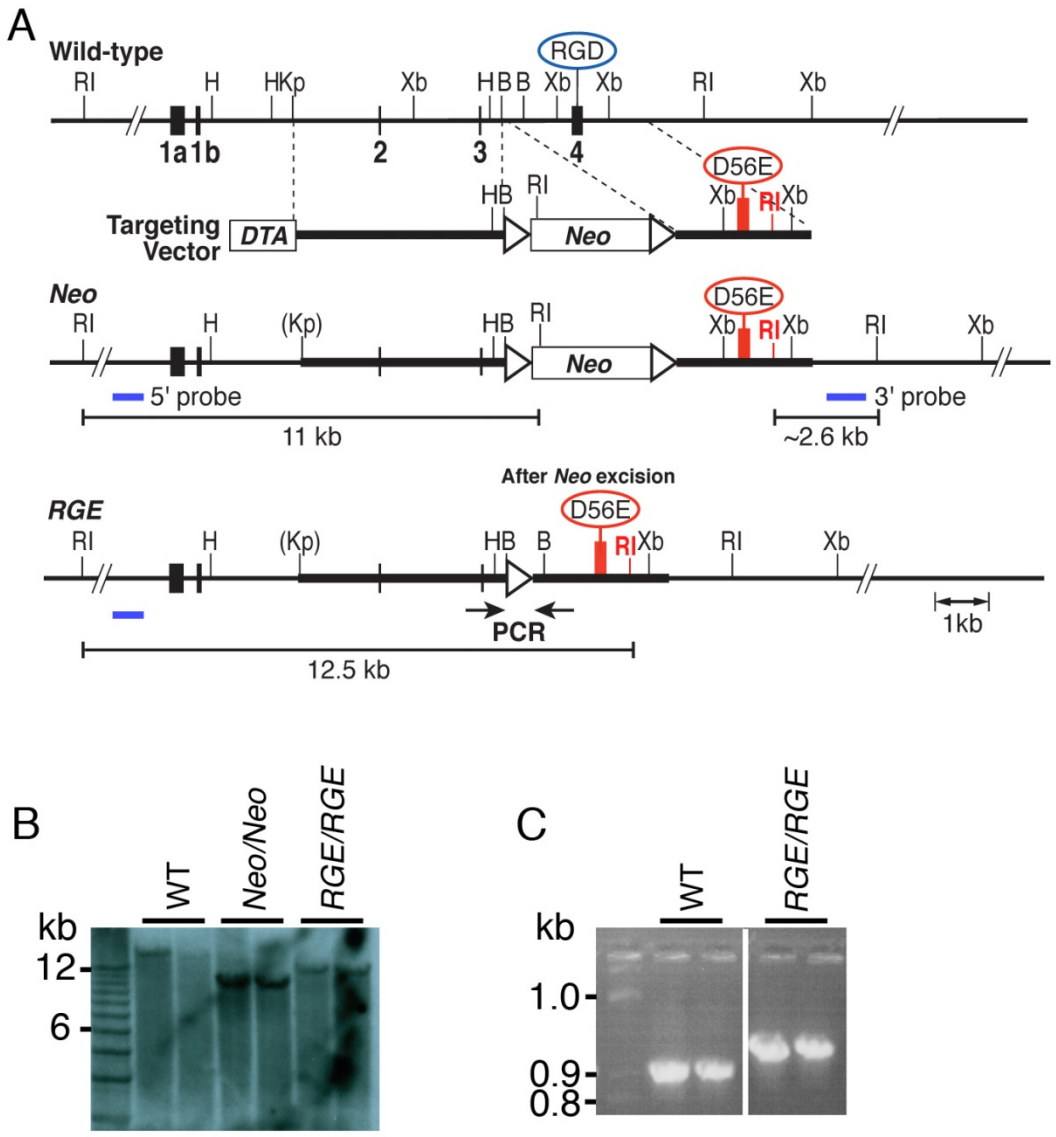

Figure S1. Generation of D56E knock-in mutant mice.

(**A**) Knock-in strategy. The RGD in exon 4 of the *Fbln5* gene is replaced with the short arm containing the RGE and a de novo EcoRI site. Exons 1 through 4 are numbered. WT allele, targeting vector and targeted alleles with or without a *Neomycin* cassette (*Neo*) are shown. *Cre*-mediated recombination removes *Neo* to generate a D56E allele (*RGE*). (**B**) Southern blot analysis with EcoRI-digested genomic DNA hybridizing with a 5' probe yields a 15-kb band for the WT, 11-kb for the *Neo* allele, and a 12-kb band for the *RGE* allele. (**C**) Genomic PCR detecting *RGE* alleles after *Neo* excision. WT and RGE alleles generate 900 bp and 930 bp bands, respectively.

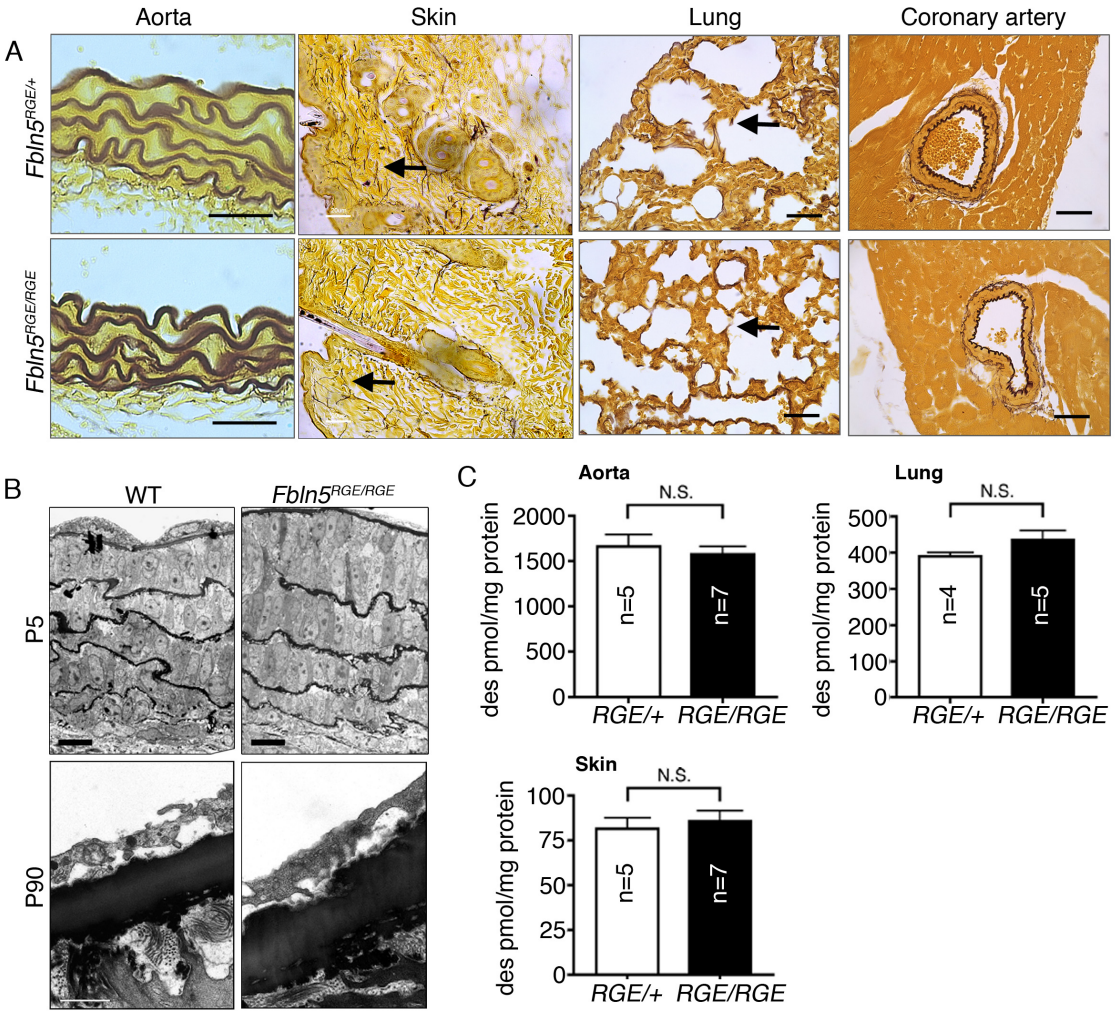

Figure S2. Evaluation of elastic fiber formation in *Fbln5*$^{RGE/RGE}$ mice.
(A) Hart's staining of elastic fibers in the aorta (Bars=40 µm), skin (Bars=20 µm), lungs (Bars=40 µm) and coronary arteries (Bars=40 µm) from *Fbln5*$^{RGE/+}$ and *Fbln5*$^{RGE/RGE}$ mice. Arrows indicate elastic fibers. (**B**) Electron microscopic observation of the aorta. Representative images of transverse sections of the aorta from WT and *Fbln5*$^{RGE/RGE}$ mice at P5 (n=3 per genotype) and P90 (n=4 per genotype) are shown. Bars=5 µm. Both WT and mutants show normal elastic fibers. (**C**) Desmosine levels were measured in the aorta, lungs and skin of 2 month old *Fbln5*$^{RGE/+}$ and *Fbln5*$^{RGE/RGE}$ mice. No statistical difference was observed between the genotypes. Means ± SEM.

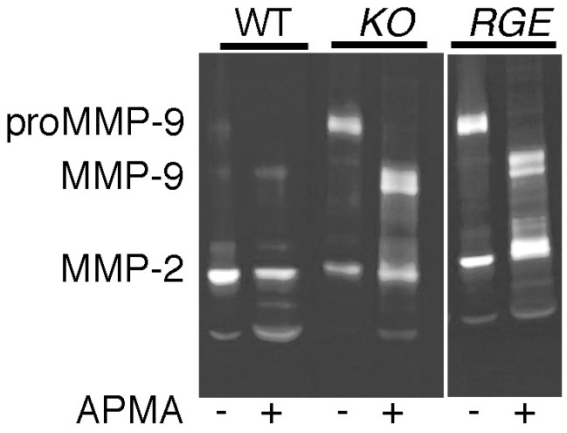

Figure S3. Predominant upregulation of proMMP-9 in vaginal tissues from *Fbln5* mutant mice.
Gelatin zymography with or without 2 mM 4-aminophenylmercuric acetate (APMA) distinguishes proMMP-9 and MMP-9 activity.

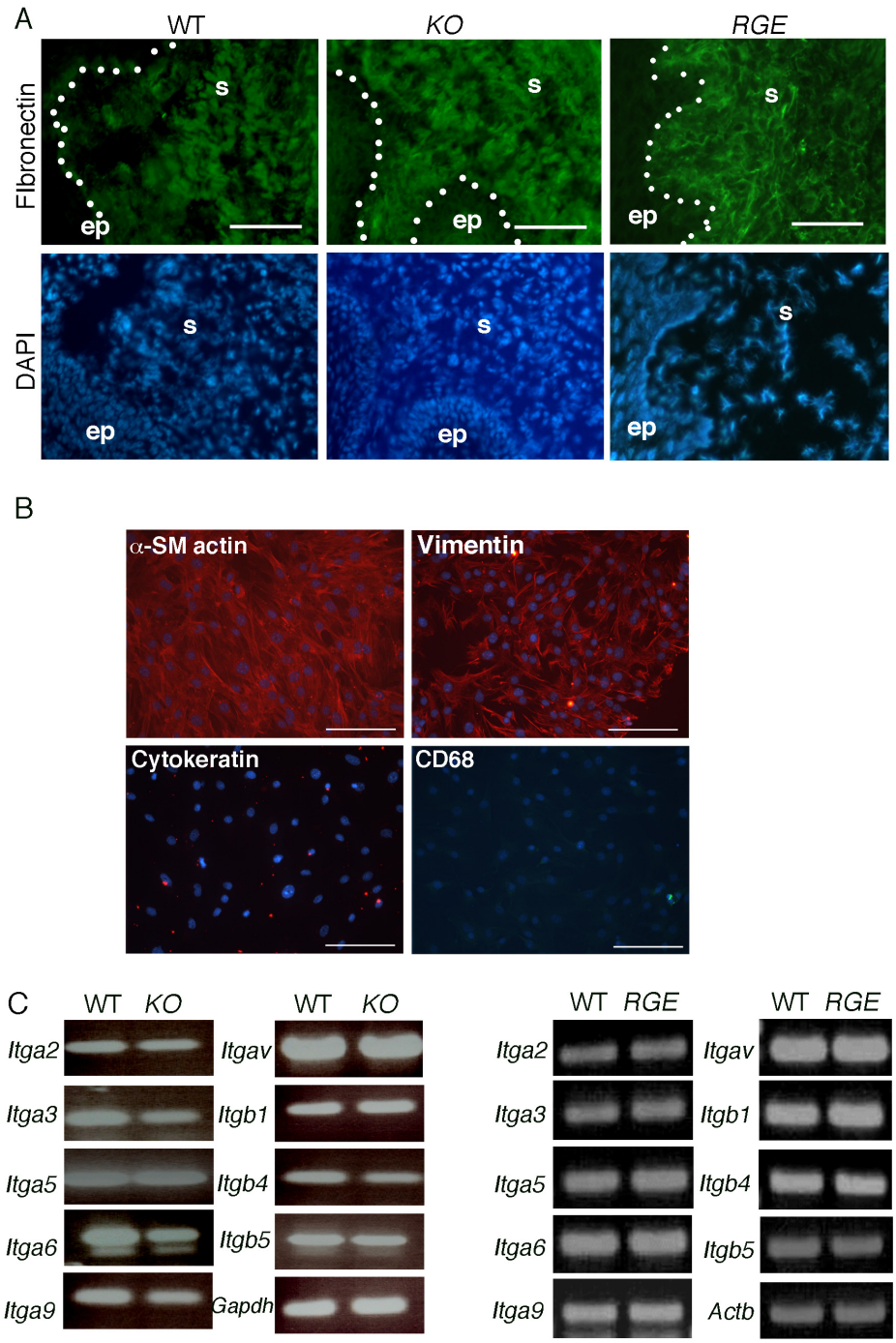

Figure S4. Characterization of primary vaginal stromal cells.
(A) Representative images of fibronectin immunostaining of the vagina from 4 week old WT, *Fbln5*$^{-/-}$ and *Fbln5*$^{RGE/RGE}$ mice. Corresponding DAPI staining is shown. Fibronectin is strongly expressed in the stroma (s). ep; epithelium. **(B)** Representative immunostaining of passage 1 WT vaginal stromal cells with α-SM actin, vimentin, cytokeratin and CD68. Note that stromal cells are positive (> 99%) for mesenchymal markers (upper panel) but negative (<1%) for epithelial or activated macrophage markers. **(C)** RT-PCR analysis of various integrin transcripts levels in vaginal stromal cells. No difference was observed between WT, *Fbln5*$^{-/-}$ and *Fbln5*$^{RGE/RGE}$ cells.

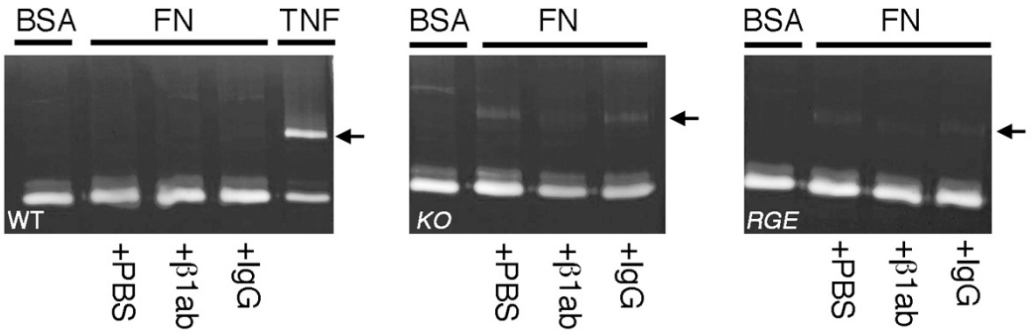

Figure S5. Effects of β1 integrin blocking antibodies or control IgG on fibronectin-mediated MMP-9 upregulation in primary vaginal stromal cells.
WT, *Fbln5*$^{-/-}$ and *Fbln5*$^{RGE/RGE}$ stromal cells were incubated with fibronectin (50 μg/ml) in the presence of ß1 blocking antibodies (10 μg/ml, ß1ab), IgG control (10 μg/ml) or PBS. Arrows indicate MMP-9. Note that fibronectin-mediated MMP-9 upregulation was inhibited by ß1ab but not by IgG.

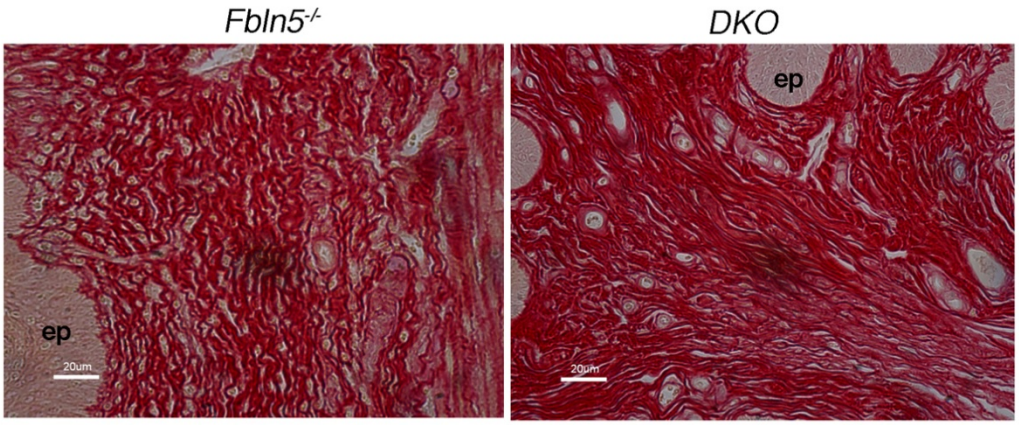

Figure S6. *DKO* vaginal tissues contain more collagen fibers compared to *Fbln5*$^{-/-}$ mice. Representative images of picrosirius red staining on transverse sections of vaginal tissues from *Fbln5*$^{-/-}$ (n=4) or *DKO* (n=6) mice. Bars=20 μm.

Table S1. Characterization of primary vaginal stromal cells.

Markers/Genotype	WT (%)	*KO* (%)	*RGE* (%)
α-SM actin	100	100	100
Vimentin	100	99.4 ± 0.9	98.8 ± 1.1
Cytokeratin	1.4 ± 2.2	1.0 ± 2.2	0
CD68	2.2 ± 0.6	1.2 ± 1.2	1.2 ± 0.9

Table shows % of positive cells per total nuclei in corresponding genotype. Value is the mean ± SD. At least 200 nuclei were counted from 5 fields taken under 20x objective lens. The experiments were repeated 3 times. No statistically significant difference was observed between genotypes.

Table S2. Absence of *Mmp9* alters the frequency of POP in nulliparous *Fbln5*$^{-/-}$ mice.

Age	*Fbln5*$^{+/+}$ */Mmp9*$^{+/+}$		*Fbln5*$^{-/-}$ */Mmp9*$^{+/+}$		*Fbln5*$^{-/-}$ */Mmp9*$^{+/-}$		*Fbln5*$^{-/-}$ */Mmp9*$^{-/-}$	
(weeks)	# ≥ Stage 2/n	%	# ≥ Stage 2/n	%	# ≥ Stage 2/n	%	# ≥ Stage 2/n	%
6-10	0/23	0	0/36	0	0/42	0	0/25	0
11-15	0/23	0	3/35	8.6	2/42	4.8	2/25	8.0
16-20	0/20	0	13/29	44.8	11/42	26.2	3/25	12.0*
21-25	0/18	0	19/26	73.1	20/41	48.8	6/23	26.1*
26-30	0/18	0	21/26	80.8	27/40	67.5	6/22	27.3*
31-35	0/17	0	24/25	96	29/40	72.5	7/20	35.0*
36-40	0/16	0	24/25	96	32/40	80	8/19	42.1*
41-45	0/16	0	25/25	100	35/40	87.5	8/19	42.1*
46-50	0/16	0	25/25	100	35/39	89.7	8/18	44.4*

The frequency of prolapse ≥ Stage 2 was determined as a function of age in all genotypes.*$p<0.05$ compared with *Fbln5*$^{-/-}$*;Mmp9*$^{-/-}$ or *Fbln5*$^{-/-}$*;Mmp9*$^{+/-}$.

Table S3. Demographics of human subjects from whom samples were used for qPCR.

	Premenopausal Controls (n=18)	Premenopausal Prolapse (n=19)	Menopausal Controls (n=5)	Menopausal Prolapse (n=34)
Age	41.8 ± 1.0	41.0 ± 1.1	53 ± 1.6*	66 ± 2.2*
Parity	2 [0,4.0]	3 [2,6]*	2 [2,3]	4 [2,8.3]*
Stage 2	-	11 (69%)	-	7 (21%)**
Stage 3	-	4 (25%)	-	10 (29%)
Stage 4	-	1 (6%)	-	17 (50%)**
Caucasian	1 (5%)	5 (26%)*	2 (40%)	14 (41%)*
Hispanic	6 (32%)	11 (58%)	0	13 (38%)
African Am	12 (63%)	2 (11%)	2 (40%)	5 (15%)
Asian	0	1 (5 %)	1 (20%)	2 (6 %)

Tissues were obtained from the apex of the anterior vaginal wall. Women with prolapse had descent of the anterior or central compartments as outlined in Materials and Methods. Age represents mean ± SEM. Parity represents median [range]. *$p \leq 0.01$ compared with other groups. **$p < 0.01$ compared with premenopausal prolapse.

Table S4. Demographics of human subjects from whom samples were used for gelatin zymography.

	Premenopausal Controls (n=6)	Premenopausal Prolapse (n=6)	Menopausal Controls (n=8)	Menopausal Prolapse (n=15)
Age	41.8 ± 1.0	41.0 ± 3.1	54 ± 2.6*	68 ± 4.2*
Parity	2 [0,4.0]	3 [2,6]*	2 [2,3]	4 [2,6.1]*
Stage 2	-	4	-	3 (20%)
Stage 3	-	2	-	4 (27%)
Stage 4	-	0	-	8 (53%)
Caucasian	0	2 (25%)	2 (25%)	6 (40%)
Hispanic	3 (50%)	5 (62%)	3 (37%)	6 (40%)
African Am	3 (50%)	1 (12%)	2 (25%)	3 (20%)
Asian	0	0	1 (12%)	0

Tissues were obtained from the apex of the anterior vaginal wall. Women with prolapse had descent of the anterior or central compartments as outlined in Materials and Methods. Age represents mean ± SEM. Parity represent median [range]. *$P \leq 0.01$ compared with premenopausal controls, premenopausal prolapse, and postmenopausal controls.

Table S5. PCR primer sequences

Mouse

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
Itga2	gcatggcattggtgactatccac	gcagcaaagggtggtgttggaa
Itga3	tgagaaccagcatccctaccatc	tgcctcttagcttcatacagggc
Itga5	accagccatttagccttcagtgtg	tgaagaagccgagcttgtagagga
Itga6	tcaccgctgctgctcagaatatca	aatgctgtcatcgtacctagagcg
Itga9	actgcaaccttagtgctcttccga	caccagcaaactgatggcgatgat
Itgav	agccagacccgttgtcactgtaaa	atggagaaacagtgctcgtcggat
Itgb1	tgtggacagtgtgtgtgtaggaag	gtctcacaagttggcccttgaaac
Itgb4	agaatctgcgagaatcccagccat	ttcaccaggtgctcagtgtcatca
Itgb5	actgtggagaatgcaaatgccacg	tttggaacttggcaaactctcggc
Mmp2	ggactatgaccgggataagaaatatg	gggcaccttctgaatttcca
Mmp9	agaccaagggtacagcctgttc	gcacgctggaatgatctaagc
Timp1	catggaaagcctctgtggatatg	aggcggcccgtgatg
Timp2	ggtggattccgggaatgac	tttgaacatctttatctgcttgatctc
Actb	acagtccgcctagaagcact	tccgatgccctgaggctctt
B2m	ccgagcccaagaccgtcta	aactggatttgtaattaagcaggttca
Gapdh	agtatgactccactcacggcaa	tctcgctcctggaagatggt

Human

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
MMP2	ttgatggcatcgctcagatc	tgtcacgtggcgtcacagt
MMP9	gaactttgacagcgacaagaagtg	gccgccacgaggaaca