Vitamin D receptor inhibits EMT *via* regulation of epithelial mitochondrial function in intestinal fibrosis

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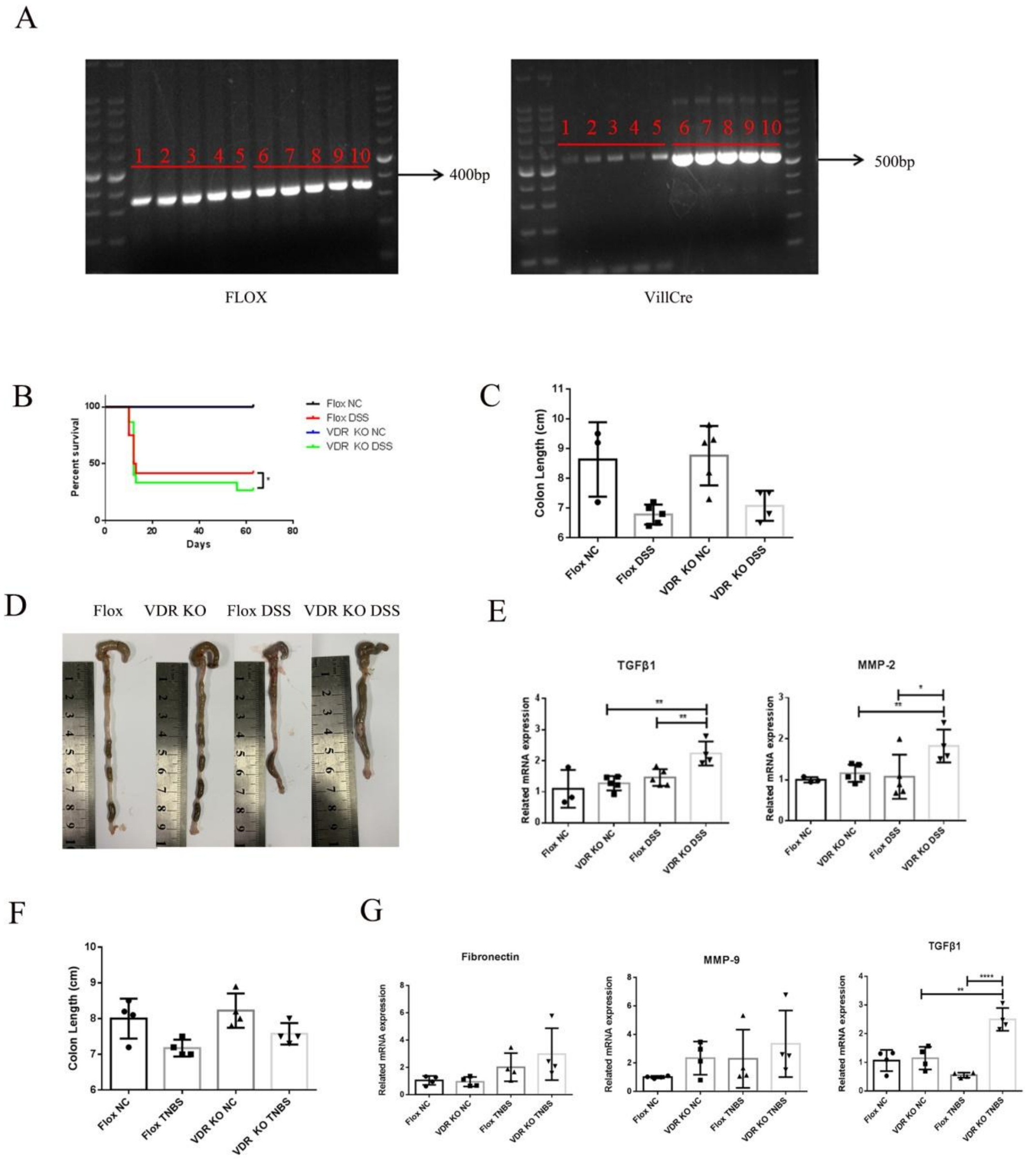
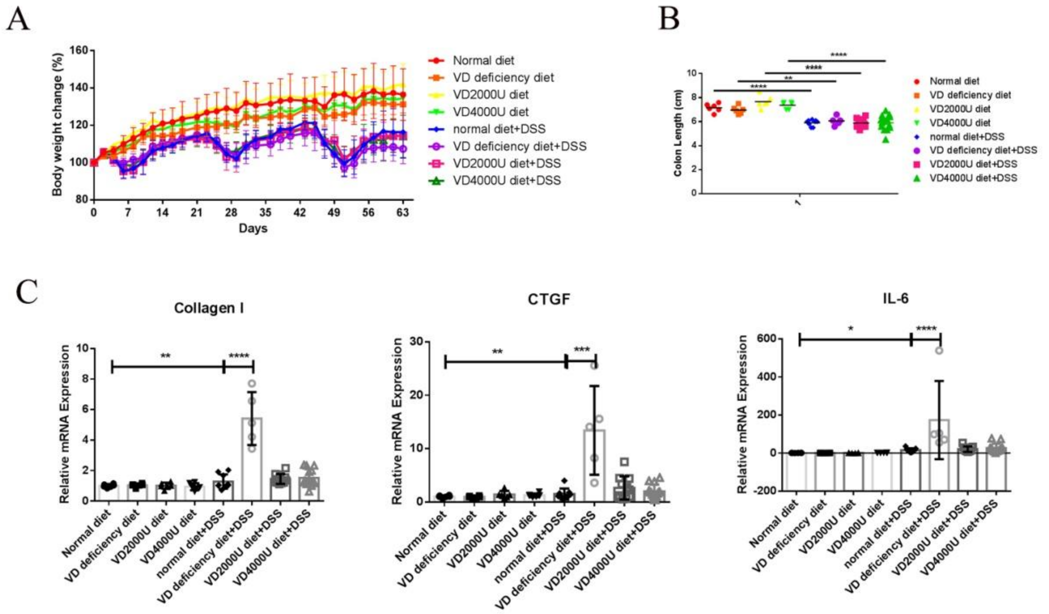
Supplementary materials

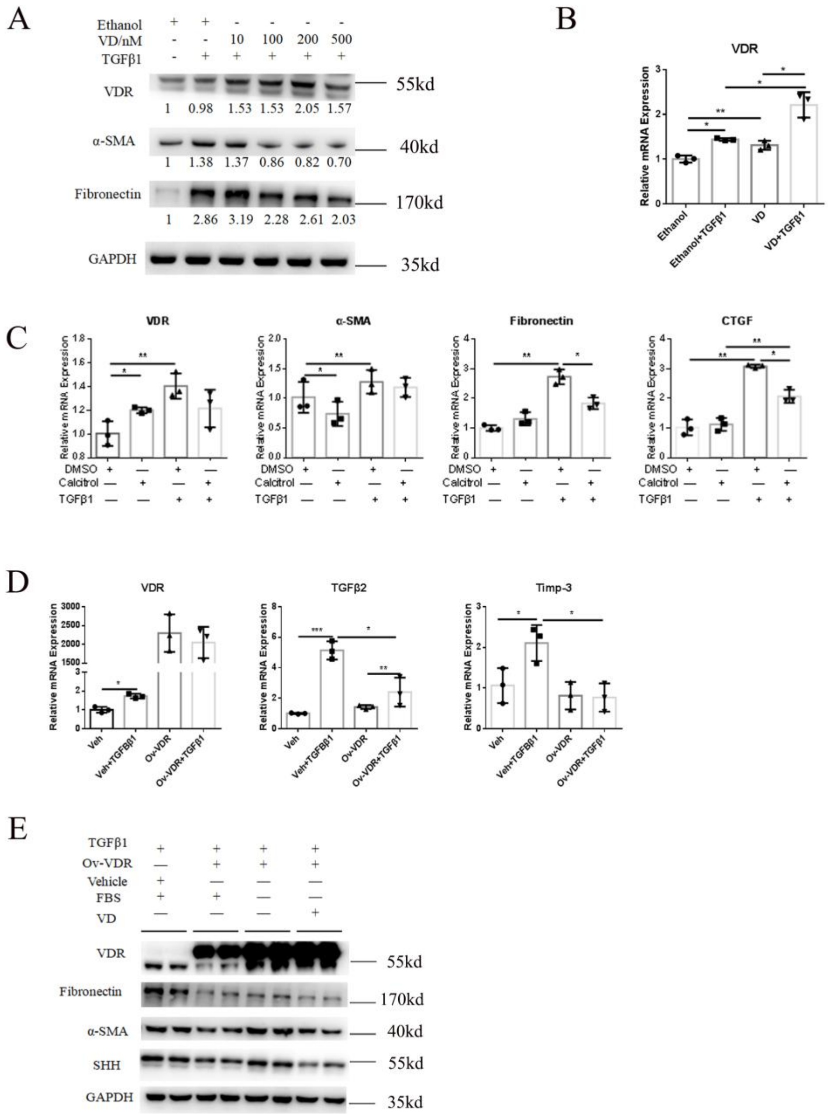
Supporting tables



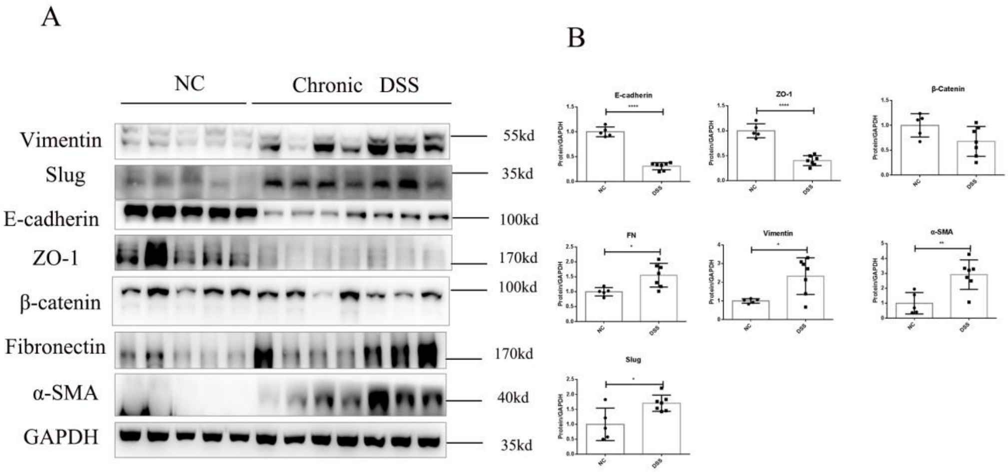
Supplementary Figure 1: VDR specific knockout in intestinal epithelium exacerbated mouse intestinal fibrosis. (A) PCR analysis of genomic DNA. VDR $^{IEC\text{-}KO}$ mice were generated by homologous recombined flox VDR allele, which removed by Cre-mediated recombination (VDR $^{IEC\text{-}KO}$, 6 to 10; VDR$^{fl/fl}$,1 to 5). (B) Survival rates of DSS-induced intestinal fibrosis model mice. (*P<0.05, two-tailed, Flox DSS vs VDR KO DSS). (C) Colon length of DSS-induced fibrosis. (D) Representative pictures of mice colons. (E) Quantitative PCR analysis of DSS administrated mice (*P<0.05, **P<0.01, two-tailed). (F) Colon length of TNBS experiment group. (G) Quantitative PCR analysis of TNBS administrated mice (**P<0.01, ****P<0.0001, two-tailed).



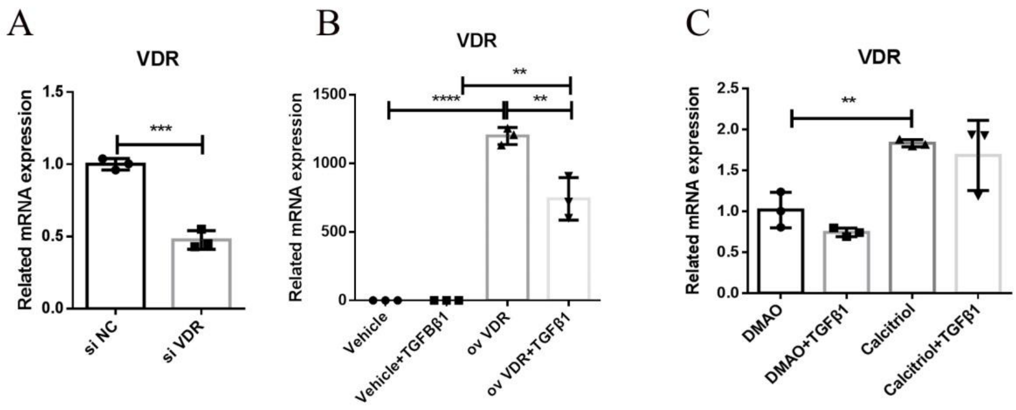
Supplementary Figure 2: VD dietary intervention influenced DSS-induced intestinal fibrosis. (A) Body weight change of VD dietary intervention experiment. (B) Colon length of each group. (C) Quantification of gene expression in RNA levels (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, two-tailed).



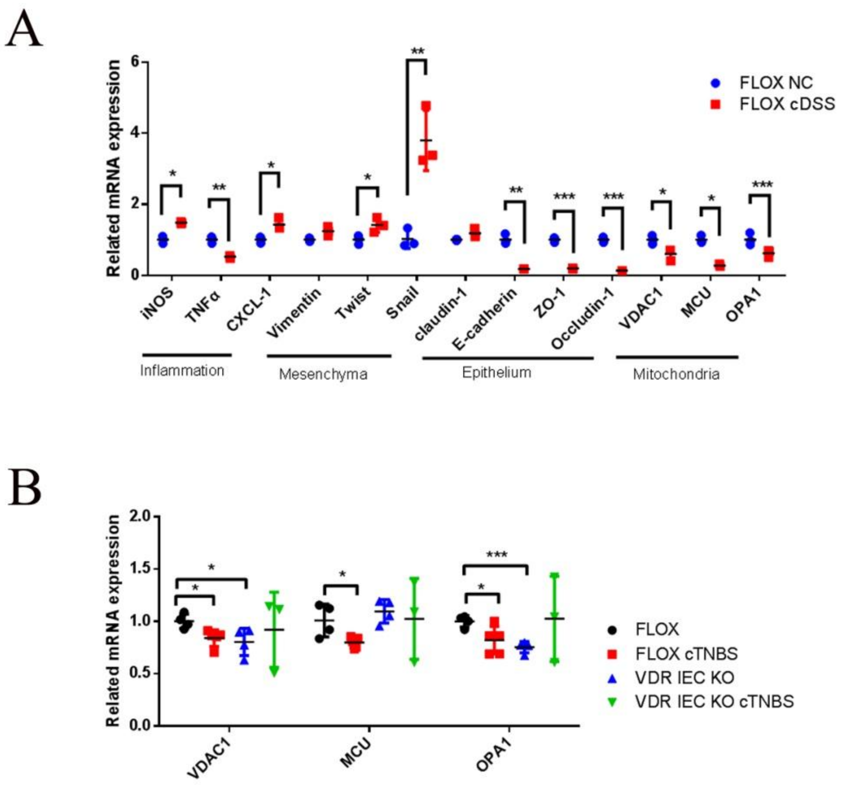
Supplementary Figure 3: VD dose-independent experiment in primary human intestinal fibroblasts and VDR partially modulated CCD-18Co cells activation *in vitro*. (A) Western blot analysis of VDR and fibrosis markers expression in primary human intestinal fibroblasts under different concentrations of VD. (B) VDR expression was measured by quantification PCR in VD-treated CCD-18Co cells (n=3, *P<0.05 **P<0.01, two-tailed). (C) Quantitative PCR analysis of genes in Calcitriol -treated CCD-18Co cells (n=3, *P<0.05 **P<0.01, two-tailed). (D) Quantitative PCR analysis of genes in VDR overexpression CCD-18Co cells (n=3, *P<0.05, ***P<0.001, ****P<0.0001, two-tailed). (E) Western blot analysis of VDR, SHH and fibrosis markers expression in primary human intestinal fibroblasts under the treatment of FBS or/and VD.



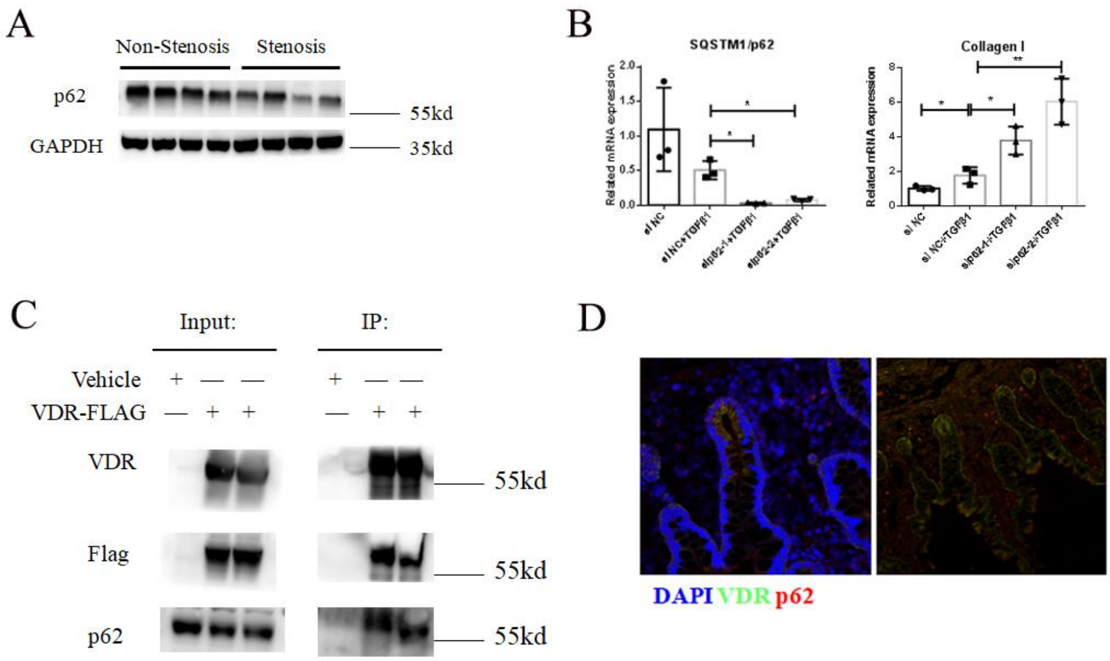
Supplementary Figure 4: EMT activated in mice chronic DSS-induced intestinal fibrosis. (A) Western blot analysis of mice colon tissues. (B) Quantification of western blot (NC=5, DSS=7, **P*<0.05 ***P*<0.01, *****P*<0.0001, two-tailed).



Supplementary Figure 5: VDR expression in different experiments of HT29 cells. (A) Quantitative PCR analysis of VDR gene after siRNA transfection (n=3, ***P<0.001, two-tailed). (B) Quantitative PCR analysis of VDR gene after VDR plasmid transfection (n=3, *P<0.05 **P<0.01, ****P<0.0001, two-tailed). (C) Quantitative PCR analysis of VDR gene after Calcitriol administration (n=3, **P<0.01, two-tailed).



Supplementary Figure 6: The expression of mitochondrial functional genes in mice. (A) Quantitative PCR analysis of isolated mice epithelial tissues (n=3, *P<0.05 **P<0.01, ***P<0.001, two-tailed). (B) Genes expression were measured by quantitative PCR (Flox NC n=4; Flox TNBS n=4; VDR KO NC n=4; VDR KO TNBS n=4, *P<0.05,***P<0.001, two-tailed).



Supplementary Figure 7: p62 regulated VDR to modulated intestinal fibrosis. (A) Representative Western blot images of p62 expression. (B) Quantitative PCR analysis of primary human intestinal fibroblasts (n=3, **P*<0.05 ***P*<0.01). (C) HEK293 cells were infected with a FLAG-tagged VDR overexpression plasmid or the empty vector (NC) for 48h were collected, and total FLAG-tagged VDR was immunoprecipitated using FLAG beads. (B) Representative images of immunofluorescence staining of human colon sections. Magnification: 600×.

Supporting Table S1

The characteristics of CD patients

Number of patients		19
Age	[0-30]	1
	[30-45]	12
	[45-60]	3
	[>60]	3
Male		14
Female		5
Localization	Terminal ileum	14
	Colon	2
	Terminal ileum and colon	3
Complications	Obstruction	18
	Perforation	2
	Sinus formation	5
	Bleeding	1
Medcinine	Azathioprine	16
	Hormonotherapy	12
	Anti-TNFα	2

Supporting Table S2

Primers for mice identification

Primer name	Sequence 5'-3'
EGE-LZX-019-5'loxP-F3	CAGTGACATTGTACTCACATACATGAC
EGE-LZX-019-5'loxP-R3	TGCATGCGTCTGTGGGGAGG
Vil1-ProF1	GTGTTTGGTTTGGTTTCCTCTGCATAAGA
Cre5R1	GCAGGCAAATTTTGGTGTACGGTCA

Primers for Quantitative PCR dectection in mice

Primer name	Sequence 5'-3'
m-VDR-F	GAATGTGCCTCGGATCTGTGG
m-VDR-R	ATGCGGCAATCTCCATTGAAG
m-GAPDH-F	TGGCCTTCCGTGTTCCTAC
m-GAPDH-R	GAGTTGCTGTTGAAGTCGCA
m-αSMA -F	CCCAACTGGGACCACATGG
m-αSMA -R	TACATGCGGGGGACATTGAAG
m-Fibronectin-F	ATGTGGACCCCTCCTGATAGT
m-Fibronectin-R	GCCCAGTGATTTCAGCAAAGG
m-mmp-9-F	CTGGACAGCCAGACACTAAAG
m-mmp-9-R	CTCGCGGCAAGTCTTCAGAG
m-TIPM-1-F	GCAACTCGGACCTGGTCATAA
m-TIPM-1-R	CGGCCCGTGATGAGAAACT
m-Collagen I-F	TGGGATTCCCTGGACCTAA
m-Collagen I-R	GCTCCAGCTTCTCCATCTTT

m-CTGF-F	GGGCCTCTTCTGCGATTTC
m-CTGF-R	ATCCAGGCAAGTGCATTGGTA
m-TGFβ1-F	CCACCTGCAAGACCATCGAC
m-TGFβ1-R	CTGGCGAGCCTTAGTTTGGAC
m-VDAC1-F	CCCACATACGCCGATCTTGG
m-VDAC1-R	GTGGTTTCCGTGTTGGCAGA
m-MCU-F	GAGCCGCATATTGCAGTACG
m-MCU-R	CGAGAGGGTAGCCTCACAGAT
m-OPA1-F	TGGAAAATGGTTCGAGAGTCAG
m-OPA1-R	CATTCCGTCTCTAGGTTAAAGCG
m-Vimentin-F	CGTCCACACGCACCTACAG
m-Vimentin-R	GGGGGATGAGGAATAGAGGCT
m-Twist-F	GGACAAGCTGAGCAAGATTCA
m-Twist-R	CGGAGAAGGCGTAGCTGAG
m-Snail-F	CACACGCTGCCTTGTGTCT
m-Snail-R	GGTCAGCAAAAGCACGGTT
m-Claudin1-F	GGGGACAACATCGTGACCG
m-Claudin1-R	AGGAGTCGAAGACTTTGCACT
m- E-Cadherin-F	CAGGTCTCCTCATGGCTTTGC
m- E-Cadherin-R	CTTCCGAAAAGAAGGCTGTCC
m-ZO-1-F	GCCGCTAAGAGCACAGCAA
m-ZO-1-R	TCCCCACTCTGAAAATGAGGA
m-Occludin-F	TTGAAAGTCCACCTCCTTACAGA
m-Occludin-R	CCGGATAAAAAGAGTACGCTGG
m-TNF-α-F	GCCACCACGCTCTTCTGTCT
m-TNF-α-R	GTCTGGGCCATAGAACTGAT
m-CXCL1-F	CTGGGATTCACCTCAAGAACATC
m-CXCL1-R	CAGGGTCAAGGCAAGCCTC
m-iNOS- F	GTTCTCAGCCCAACAATACAAGA
m- iNOS-R	GTGGACGGGTCGATGTCAC

Primers for Quantitative PCR dectection in human

Primer name	Sequence 5’-3’
h-VDR-F	GTGGACATCGGCATGATGAAG
h-VDR-R	GGTCGTAGGTCTTATGGTGGG
h-αSMA -F	AAAAGACAGCTACGTGGGTGA
h-αSMA -R	GCCATGTTCTATCGGGTACTTC
h-Fibronectin-F	CGGTGGCTGTCAGTCAAAG
h-Fibronectin-R	AAACCTCGGCTTCCTCCATAA
h-mmp-9-F	TGTACCGCTATGGTTACACTCG
h-mmp-9-R	GGCAGGGACAGTTGCTTCT
h-mmp-2-F	CCCACTGCGGTTTTCTCGAAT
h-mmp-2-R	CAAAGGGGTATCCATCGCCAT
h-Collagen Iα1-F	GAGGGCCAAGACGAAGACATC
h-Collagen Iα1-R	CAGATCACGTCATCGCACAAC

h-CTGF-F	CAGCATGGACGTTCGTCTG
h-CTGF-R	AACCACGGTTTGGTCCTTGG
h-Zeb1-F	GATGATGAATGCGAGTCAGATGC
h-Zeb1-R	ACAGCAGTGTCTTGTTGTTGT
h-Vimentin-F	ATGAAGGAGGAAATGGCTCGTC
h-Vimentin-R	GGGTATCAACCAGAGGGAGTGAA
h-Claudin-1-F	CCTCCTGGGAGTGATAGCAAT
h-Claudin-1-R	GGCAACTAAAATAGCCAGACCT
h- E-Cadherin-F	TGGAGGAATTGTTGCTTGC
h- E-Cadherin-R	CGCTCTCCTCCGAAGAAC
h-ZO-1-F	CAACATACAGTGACGCTTCACA
h-ZO-1-R	CACTATTGACGTTTCCCCACTC

Abbreviations: m-, mouse; h-, human