Title: Cyr61 from adipose-derived stem cells promotes colorectal cancer metastasis and vasculogenic mimicry formation via integrin $\alpha_V\beta_5$

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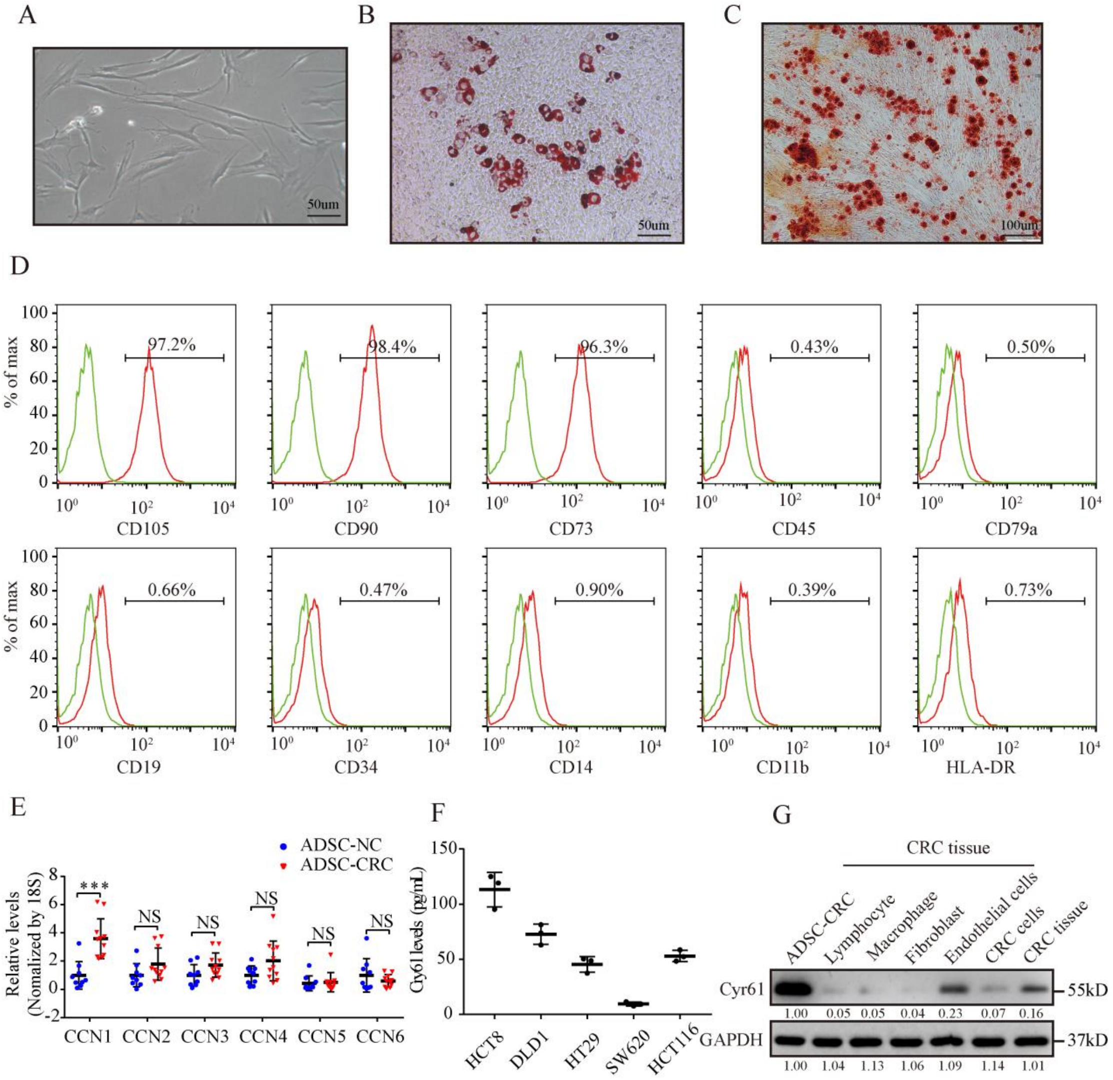
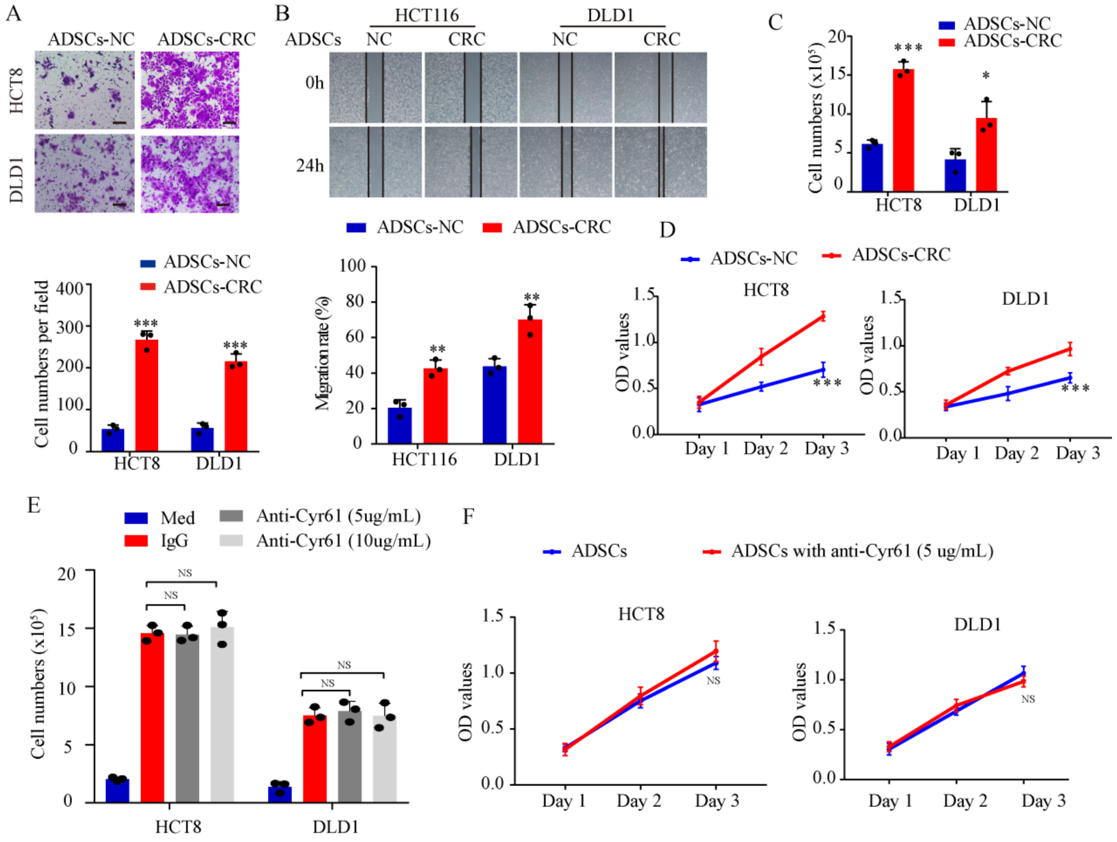
Supplemental Figures



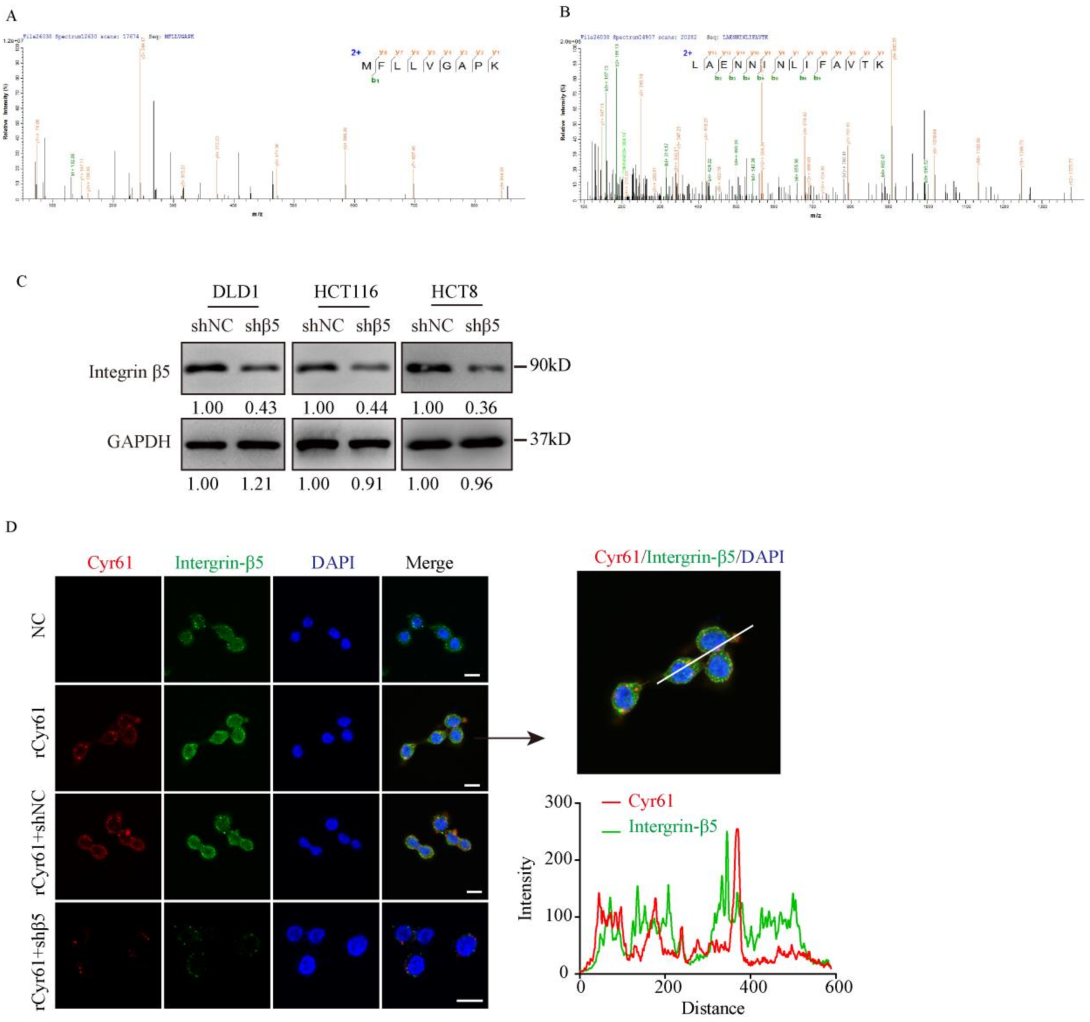
Supplementary Figure 1. Characterization of ADSCs

A Morphology of ADSCs. Scale bar = 50 μm. **B** ADSCs differentiated toward the adipogenic lineages, as evidenced by Oil Red O staining. Scale bar = 50 μm. **C** ADSCs differentiated toward the osteogenic lineages, as evidenced by Alizarin Red S staining. Scale bar = 100 μm. **D** Flow cytometric analysis of the ADSCs surface markers CD105, CD90, CD73, CD45, CD79a, CD19, CD34, CD14, CD11b and HLA-DR. IgG isotypes were used as negative controls. **E** qRT-PCR analysis of CCN protein family mRNA levels in ADSCs-NC (n=9) and ADSCs-CRC (n=11). **F** ELISA analysis of medium Cyr61 levels in CRC cell lines. **G** Western blot analysis of Cyr61 protein levels in ADSC-CRC, CRC tissues, and lymphocyte, macrophage, fibroblast endothelial cells and CRC cells isolated from CRC tissue. Values are represented as mean ± SD. NS, no significant, ***$p < 0.001$, by 2-tailed Student's t test (**E**).



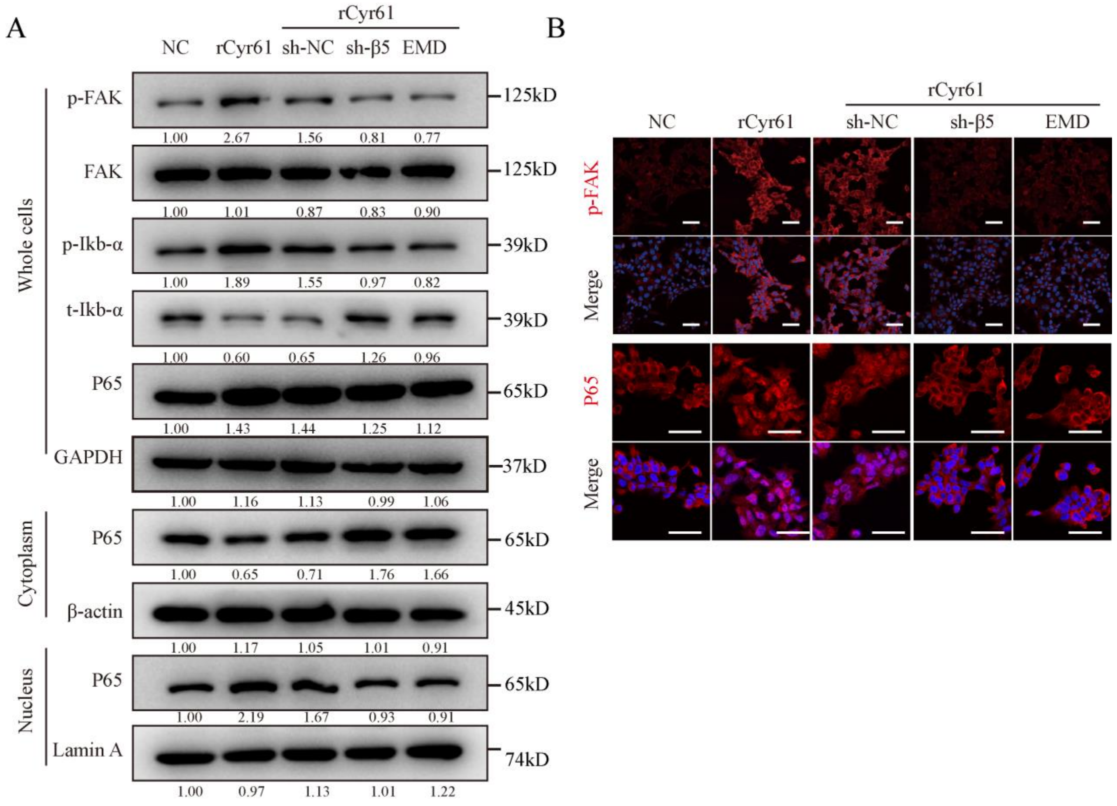
Supplementary Figure 2. ADSCs derived Cyr61 have no effect on CRC cell proliferation *in vitro*

A Representative images of transwell migration assays for HCT8 and DLD1 cells co-cultured with ADSCs-NC or ADSCs-CRC. Scale bar = 100 μm. **B** Representative images of wound-healing assays for HCT116 and DLD1 cells co-cultured with ADSCs-NC or ADSCs-CRC. **C** and **D** Cell counting and MTS assays for HCT8 and DLD1 cells co-cultured with ADSCs-NC or ADSCs-CRC. **E** and **F** Cell counting and MTS assays for HCT8 and DLD1 cells co-cultured with culture medium alone (Med) or ADSCs in the presence or absence of an anti-Cyr61 antibody at 5 or 10μg/mL, or an isotype-matched IgG control (IgG). Values are represented as mean ± SD. NS, no significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by 2-tailed Student's t test (**A**, **B**, **C**, **D** and **F**) and one-way ANOVA (**E**).



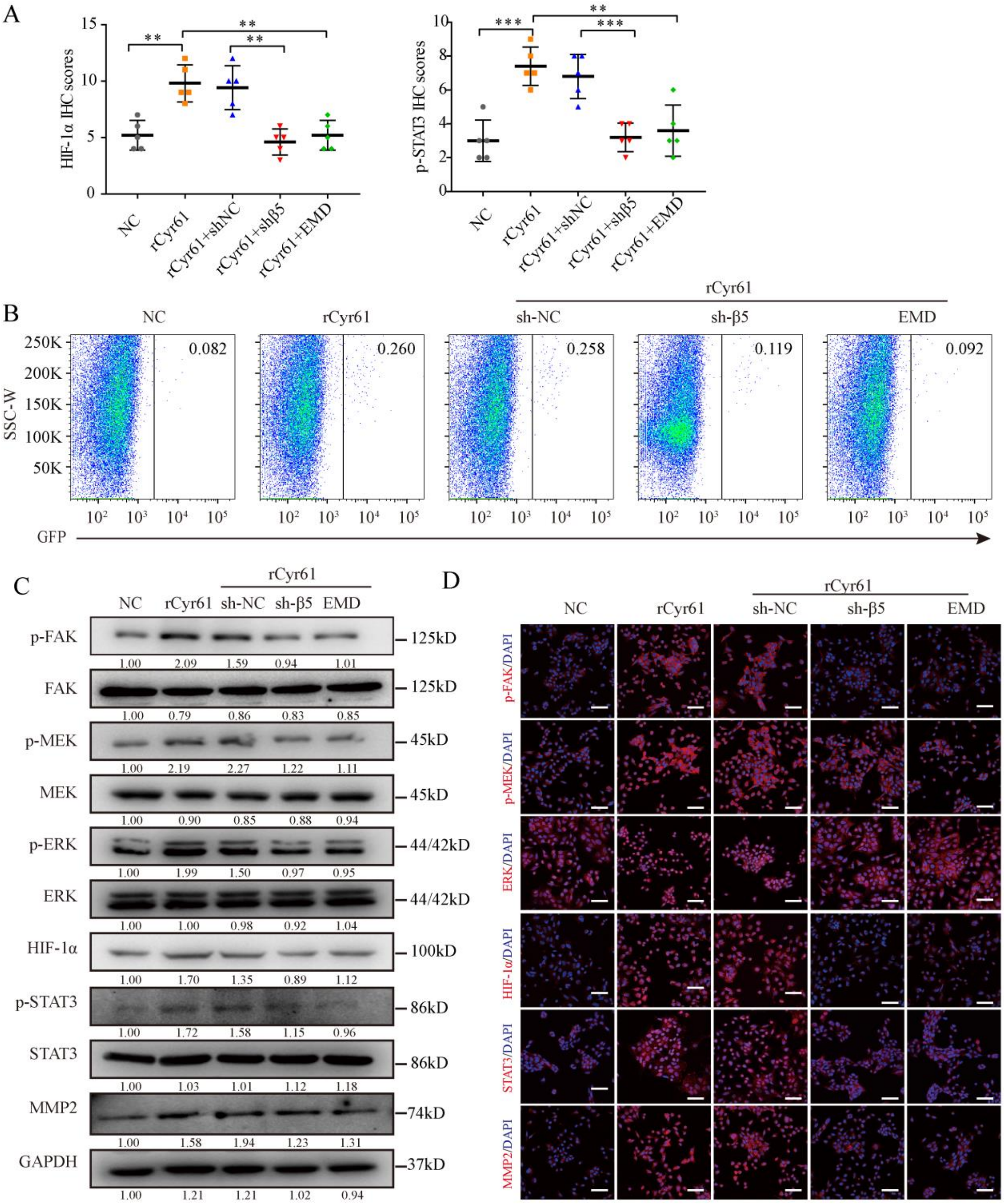
Supplementary Figure 3. Cyr61 receptor identification on CRC cells

A Mass spectra of a representative peptide fragment of integrin α_V from the bands. **B** Mass spectra of a representative peptide fragment of integrin β_5 from the bands. **C** Western blot analysis of the efficiency of shRNA for integrin β_5 in DLD1, HCT116 and HCT8 cells. **D** Confocal microscopy to confirm the colocalization between Cyr61 and integrin $\alpha_V\beta_5$ on HCT8 cells. Scale bar = 10 μm.



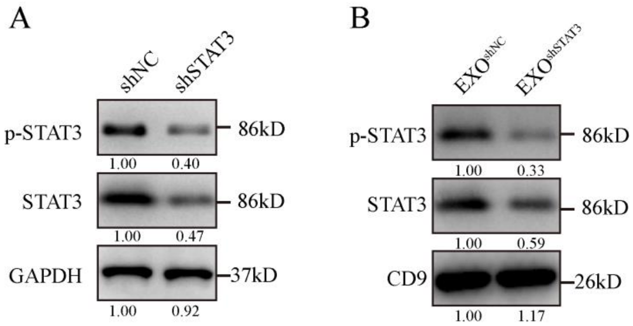
Supplementary Figure 4. The $\alpha_V\beta_5$/FAK/NF-κB signaling pathway changes in DLD1 cells

A Western blot analysis the expression of p-FAK, FAK, p-Ikb-α, t-Ikb-αP65 and P65 in DLD1 cells with integrinβ_5 knockdown or with integrin $\alpha_V\beta_5$ inhibitor EMD. GAPDH, β-actin and Lamin A were used as the controls. **B** Confocal microscopy of DLD1 cells with integrinβ_5 knockdown or treated with integrin $\alpha_V\beta_5$ inhibitor EMD for the expression of p-FAK and subcellular localization of P65. Scale bar = 50 μm.



Supplementary Figure 5. The $\alpha_V\beta_5$/FAK/HIF-1α/STAT3/MMP2 signaling cascade changes after Cyr61 treatment

A IHC scoring analyses of p-STAT3 and HIF-1α expression in the subcutaneous tumors. **B** Flow cytometric analysis of the GFP labeled CTCs from the nude mice whole-blood. **C** Western blot analysis of $\alpha_V\beta_5$/FAK/HIF-1α/STAT3/MMP2 signaling cascade in DLD1 cells pretreated with rCyr61, EMD or knockdown integrinβ5 expression. **D** Confocal microscopy analysis of $\alpha_V\beta_5$/FAK/HIF-1α/STAT3/MMP2 signaling cascade in HCT8 cells pretreated with rCyr61, EMD or knockdown integrinβ_5 expression. Scale bar = 50 μm.



Supplementary Figure 6. A Western blot analysis of the efficiency of shRNA for STAT3 in HCT8 cells. **B** Western blot analysis of p-STAT3 and STAT3 levels in exosomes.

Supplemental Tables

Table S1 Correlation between Cyr61 levels and clinicopathologic characteristics of CRC patients

Characteristics	Frequency	Cyr61 expression level		
		Low	High	*p*-value[a]
Gender				0.984
Female	118	10	108	
Male	246	21	225	
Age				0.237
≤ 59	166	11	155	
> 59	198	20	178	
T stage				< 0.001
T1/T2	96	26	70	
T3/T4	268	5	263	
N stage				< 0.001
N0	192	28	164	
N1/N2	172	3	169	
M stage				0.034
M0	271	28	243	
M1	93	3	90	
TNM Stage				< 0.001
I+II	179	28	151	
II+IV	185	3	182	

[a] Chi-square test

Table S2 The ROC curve assay of CEA, CA199, CA125and Cyr61

Variables	ACU	*p*-value[a]	95 % Confidence interval	
			Lower bound	Upper bound
CEA	0.667	< 0.001	0.611	0.723
CA199	0.522	0.516	0.460	0.584
CA125	0.600	0.003	0.540	0.661
Cyr61	0.933	< 0.001	0.906	0.960

[a] Null hypothesis: true area = 0.5

Table S3 List of surface membrane proteins of mass spectrometry results (score>35)

Num	Prot_acc	Prot_desc	Prot_score	Prot_mass (KD)	Coverage	Unique peptides	PSM
1	P06756	Integrin alpha-V	3401	117.048	39.1	41	162
2	P18084	Integrin beta-5	1811	91.303	36.5	26	82
3	Q02413	Desmoglein-1	98	114.702	3.7	3	3
4	Q9Y5I4	Protocadherin alpha-C2	57	110.010	4.7	6	12
5	O00522	Krev interaction trapped protein 1	49	84.979	2.9	2	8
6	Q9Y5X5	Neuropeptide FF receptor 2	46	60.858	4.4	4	7
7	Q8NGB9	Olfactory receptor 4F6	38	35.787	2.6	1	2
8	Q12934	Filensin	36	74.784	2.3	2	4
9	O95196	Chondroitin sulfate proteoglycan 5	36	60.720	2.3	1	5

Table S4 Oligonucleotide sequences

Name	Sequences(5’- 3’)
18S	F: CGGCTACCACATCCAAGGAA
	R: GCTGGAATTACCGCGGCT
Cyr61	F: CGCCTTGTGAAAGAAACCCG
	R: GGTTCGGGGGATTTCTTGGT
CCN2	F: GTTTGGCCCAGACCCAACTA
	R: GGCTCTGCTTCTCTAGCCTG
CCN3	F: TGATGGTCATTGGGACCTGC
	R: GGTGCTCTGTAGGTGTGCTT
CCN4	F: GCGTGGAATGTGTTTGCTCA
	R: GCCTGTACAAGAAAAGCCACC
CCN5	F: CTGTGCCTCTGTAAGCAGGA
	R: AGAAGCGGTTCTGGTTGGAC
CCN6	F: CTCACTGCGAAGGCAGGTTA
	R: GTACCCTGCAGCAGAACTGT
shβ5	F:CCGGAAAGATGATGTGCCCCACATCGCATTGCTCGAGCAATGCGATGTGGGGCACACATCTTTTTG
	R:AATTCAAAAAAAAGATGATGTGCCCCACATCGCATTGCTCGAGCAATGCGATGTGGGGCACATCATCT
shSTAT3	F:CCGGGGCCATCTTGAGCACTAAGCCCTCGAGGGCTTAGTGCTCAAGATGGCC TTTTTG
	R:AATTCAAAAAGGCCATCTTGAGCACTAAGCCCTCGAGGGCTTAGTGCTCAAGATGGCC