Supplementary Materials

TITLE PAGE

Title

Nerve modulation therapy in gouty arthritis: targeting increased sFRP2 expression in dorsal root ganglion regulates macrophage polarization and alleviates endothelial damage

Author

Jingtian Mei[1, *], Feng Zhou[1, *], Han Qiao[1], Hanjun Li[1], Tingting Tang[1],

* These authors contributed equally to this work
Correspond to Tingting Tang
E-mail: ttt@sjtu.edu.cn, Tel/fax: +8621 6313 7020, Shanghai Key Laboratory of Orthopaedic Implants, Department of Orthopaedic Surgery.

Abstract

Gouty arthritis (GA) is a form of arthritis caused by uric acid deposition in the joints that results in intense inflammation and pain. Accumulating evidence showed the importance of the sensory neurons signal upon immune cells by releasing neuropeptides and chemokines to regulate associated immune-inflammatory response. In this study, we investigated the significance of sensory neuron neuropeptides and chemokine signals on inflammation-induced macrophages polarization during GA.

Methods: We screened the mRNA expression profile during GA in dorsal root ganglion (DRG) neurons to identify the most likely candidate that mediates neuro-immune communication. Then, we silenced specific gene expression in the DRG by lentiviral vectors in the monosodium urate (MSU)-induced ankle GA mouse model and evaluated alterations in the inflammatory response. In vitro, primary macrophages were used to investigate the neural impact on M1/M2 subtype polarization, proinflammatory cytokine production, and downstream endothelial damage. The mechanism by which macrophage inflammation is induced in the DRG was evaluated by Western blot, immunofluorescence, and immunoprecipitation.

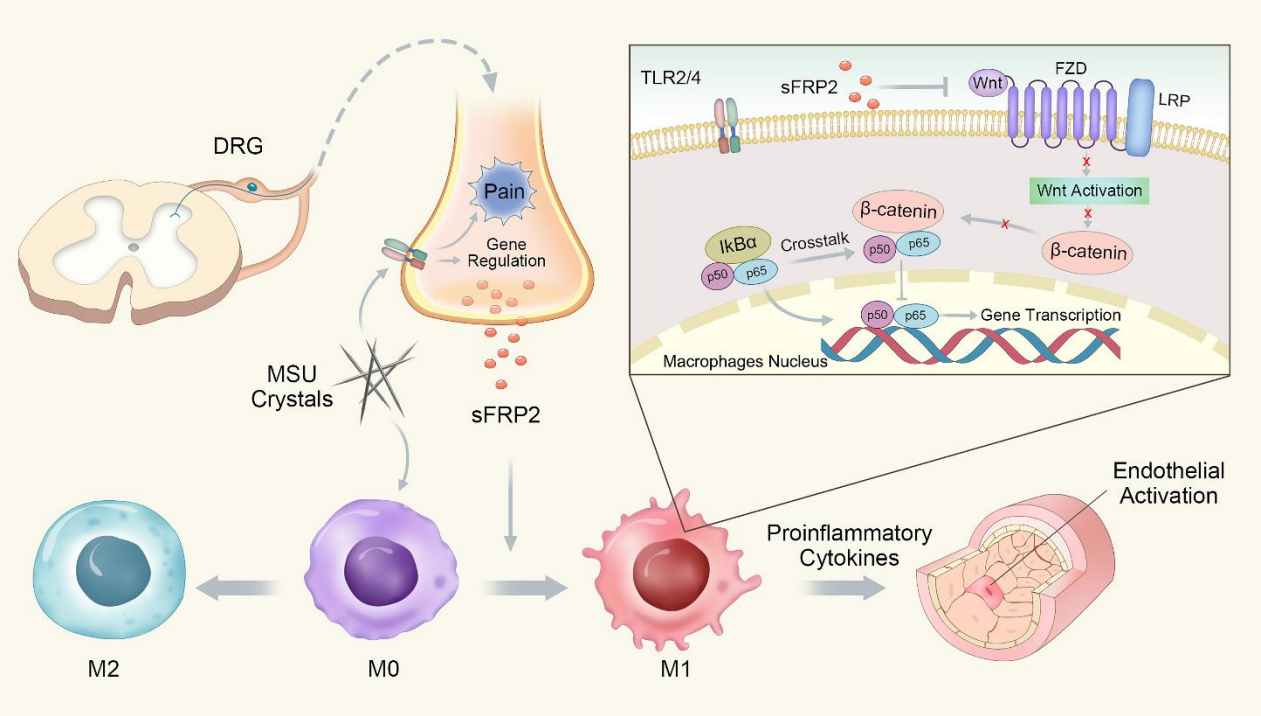
Results: We found that secreted frizzled-related protein 2 (sFRP2) was the most upregulated gene in dorsal root ganglion (DRG) neurons in response to monosodium urate (MSU) deposition. Injection of LV-sFRP2-shRNA into the L5 and S1 DRG significantly suppressed inflammatory cell infiltration and M1 polarization in the synovial membrane, attenuating hyperalgesia and ankle swelling in the GA mouse model. In vitro, DRG neurons-derived sFRP2 promoted M1 polarization and macrophage migration, thereby upregulating the production of proinflammatory cytokines and preventing endothelial apoptosis. Furthermore, DRG-derived sFRP2 activated the nuclear factor (NF)-κB pathway by destabilizing the β-catenin and p65 complex.

Conclusion: We demonstrated the involvement of a sensory neuron-macrophage axis in GA pathology that was regulated by sFRP2 expression in a paracrine manner. Targeting increased sFRP2 expression in DRG provide novel insights for future GA research in both pain alleviation and treatment of gout inflammation.

Key Words

Dorsal root ganglion, Gout, sFRP2, Macrophages, Wnt/β-catenin

Graphical abstract



sFRP2 from DRG neurons contributed to inflammation by promoting M1 polarization and migration via the regulation of Wnt/β-catenin and NF-*κ*B signaling. The proinflammatory cytokines subsequently induced endothelial damage.

Supplementary Materials

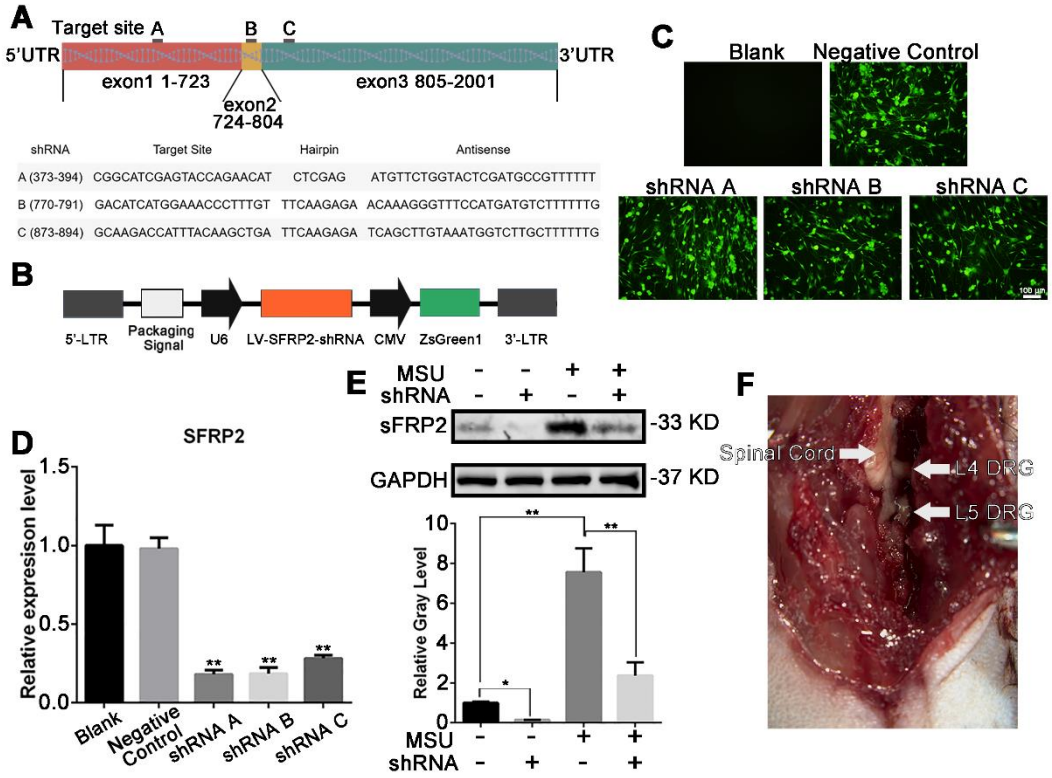


Figure S1. Construction and silencing efficacy of mouse SFRP2 shRNA lentiviral vectors

Herein, we first evaluated the capacity of shRNA to silence sFRP2 expression by infecting primary DRG neurons with lentiviral vectors expressing sFRP2 short hairpin RNA1-3 (LV-SFRP2-shRNA1-3) or negative control (Fig. S1A-B). Seventy-two hours after infection, similar ZsGreen1 fluorescence signals were detected in the shRNA and negative control group, demonstrating a similar infection efficiency of the three vectors (Fig. S1C). Subsequently, the Sfrp2 mRNA expression levels in infected DRG neurons were quantified. We found that the silencing vectors including shRNA1, shRNA2, and shRNA3 significantly inhibited sFRP2 expression by 82.1 ±2.8%, 81.6 ±3.9%, and 71.9 ±2.2%, respectively (Fig. S1D) compared with the negative control. The efficiency of shRNA1 was further verified by Western blot (Fig. S1E), showing that sFRP2 expression was inhibited by 86.0 ±6.8% and 68.3 ±9.4% with/without MSU crystals, respectively. Therefore, we used shRNA1 for further experiments in vivo.

(A), Three candidate shRNA sequences against sFRP2 and the schematic presentation of the target sites of the shRNAs. The black bars above the schematic of the mouse SFRP2 cDNA indicate the location of the target sites of shRNA A, B, and C. (B), Schematic presentation of LV-SFRP2-shRNA. LTR: long terminal repeat. (C), Fluorescence in mouse DRG neurons at 72 h after transduction with lentiviral vectors. Scale bar, 50 μm. (D), mRNA expression of sFRP2 in DRG neurons at 72 h after LV-SFRP2-shRNA transduction. (E), Western blotting for the sFRP2 protein level of transduced and control DRG neurons treated with MSU crystals. In vitro experiments were conducted in biological triplicate. (F), Dissection of mouse dorsal root ganglia. Every experiment was repeated at least three times with the same results. Values are the means ± SD (*P < 0.05 compared with control. **P<0.01 compared with control). All statistical significance was determined using one-way ANOVA and Tukey's post comparison test.

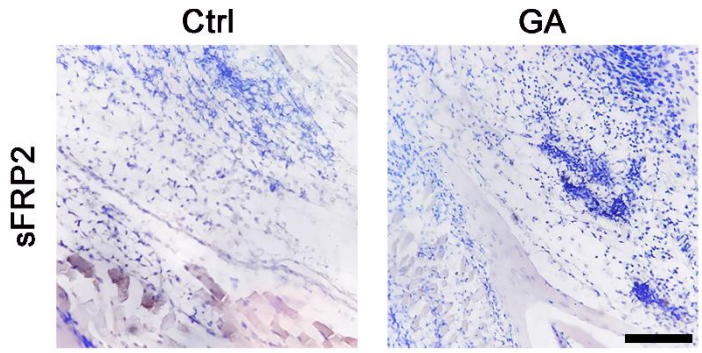


Figure S2 Immunohistochemistry of sFRP2 expression in the immune cells in the synovium. Scale bar, 200 μm.

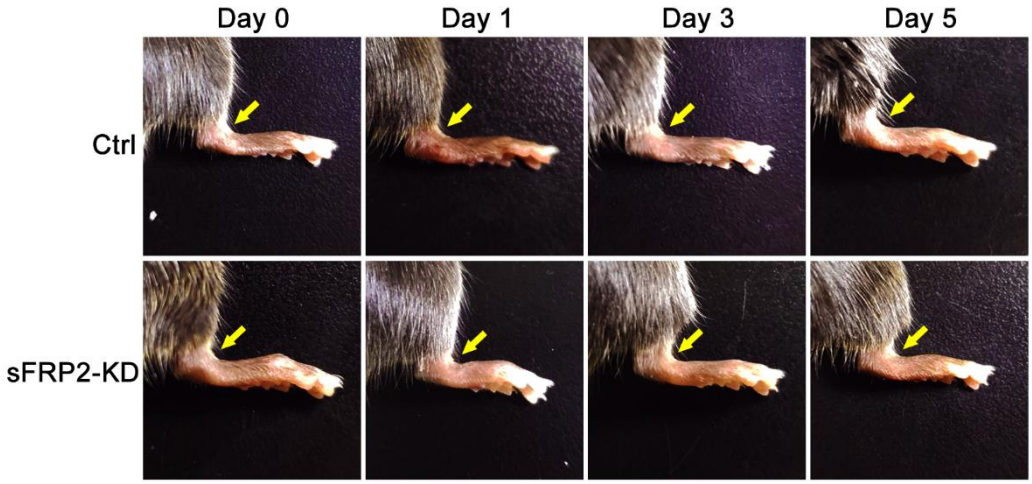


Figure S3. Representative images of the right ankle swelling. Arrows indicate the position of the measurement.

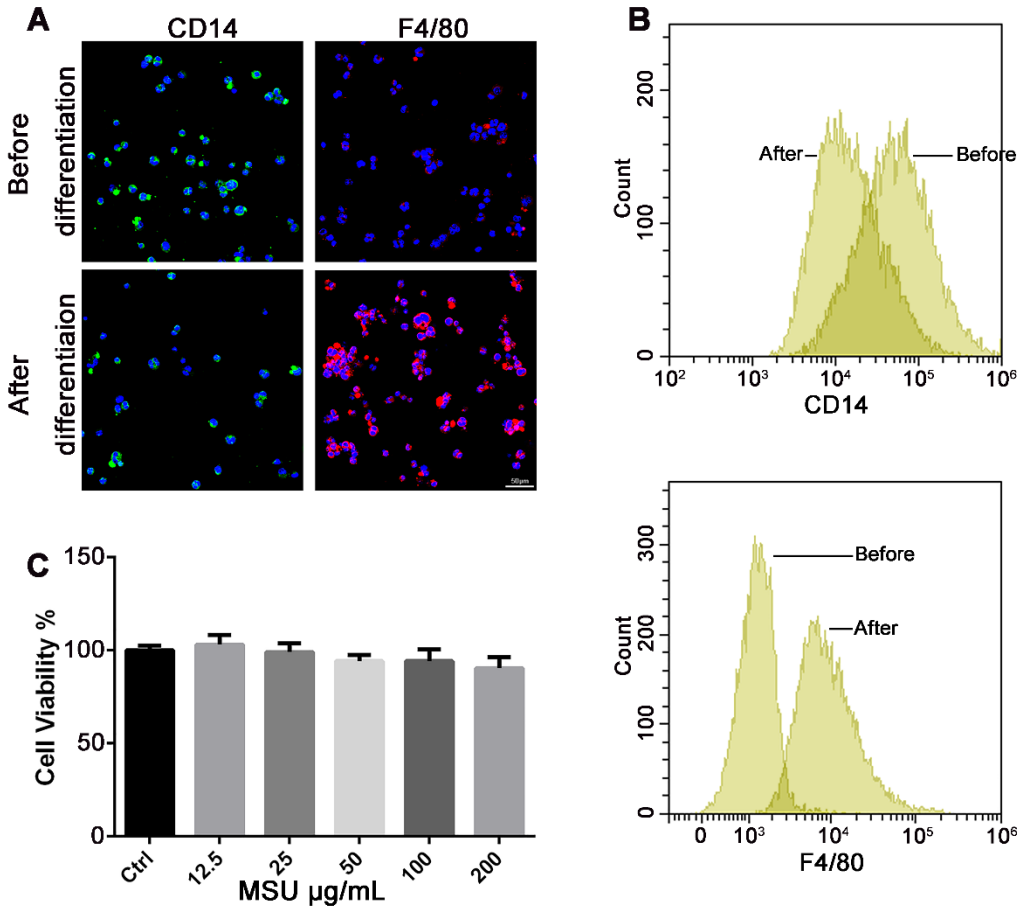


Figure S4. Validation of the homogenous population of macrophages

(A), Primary bone marrow monocyte/macrophage progenitors differentiated into a homogenous population of macrophages. Cells were fixed and immunolabeled for CD14 (green) and F4/80 (red) culture in the presence of M-CSF. Cell nuclei were detected with DAPI (blue). Scale bar, 50 μm. (B), Expression of CD14 and F4/80 of primary bone marrow monocytes/macrophage progenitors after M-CSF stimulation compared to that in the control group. (C), CCK-8 assay indicated that 200 μg/mL MSU did not compromise the viability of macrophages. In vitro experiments were conducted in biological triplicate. Every experiment was repeated at least three times with the same results. Values are the means ± SD (**P<0.01 compared with control). All statistical significance was determined using one-way ANOVA and Tukey's post comparison test.

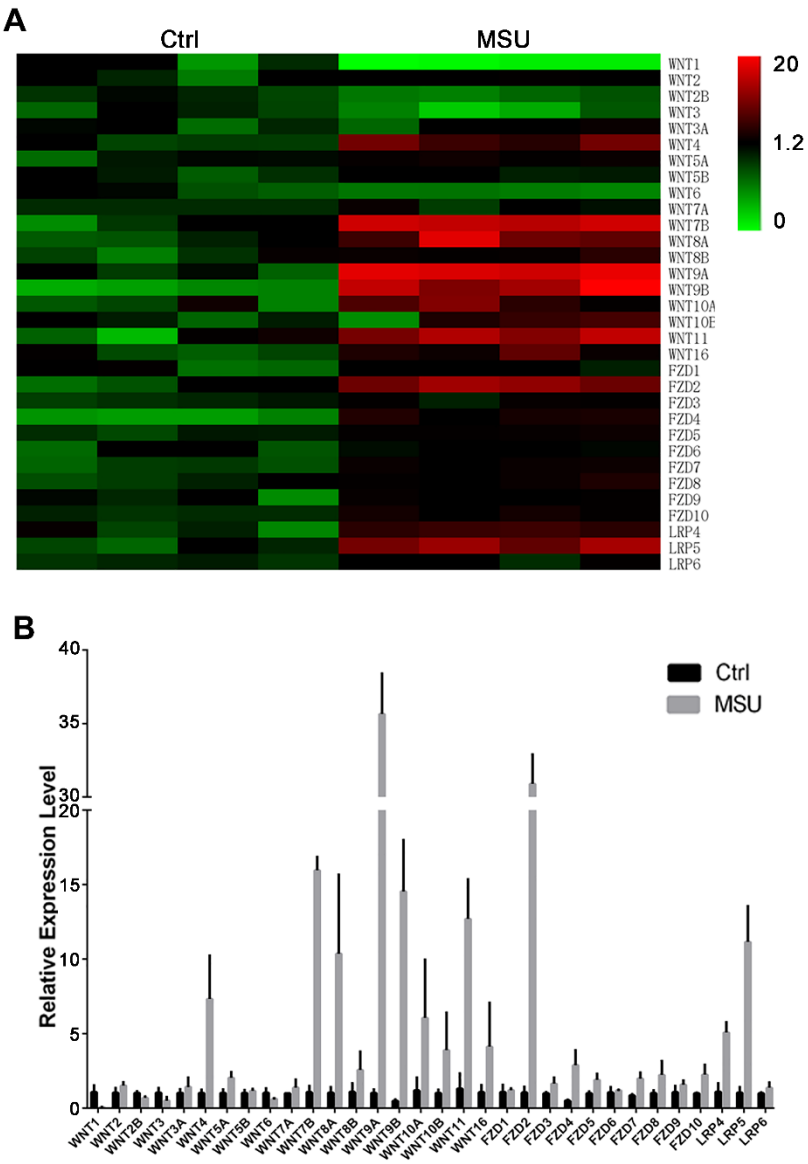


Figure S5. Heat map and bar chart diagrams of expression profiles of the Wnt family of genes in normal and MSU-stimulated macrophages detected by RT-PCR

(A), The left and right four rows represent four replicates of macrophages treated with control or 200 μg/ml MSU crystals for 24 h. Green indicates downregulation, and red indicates upregulation. (B), a Bar chart of Wnt family gene expression in normal and MSU-stimulated macrophages detected by RT-PCR.

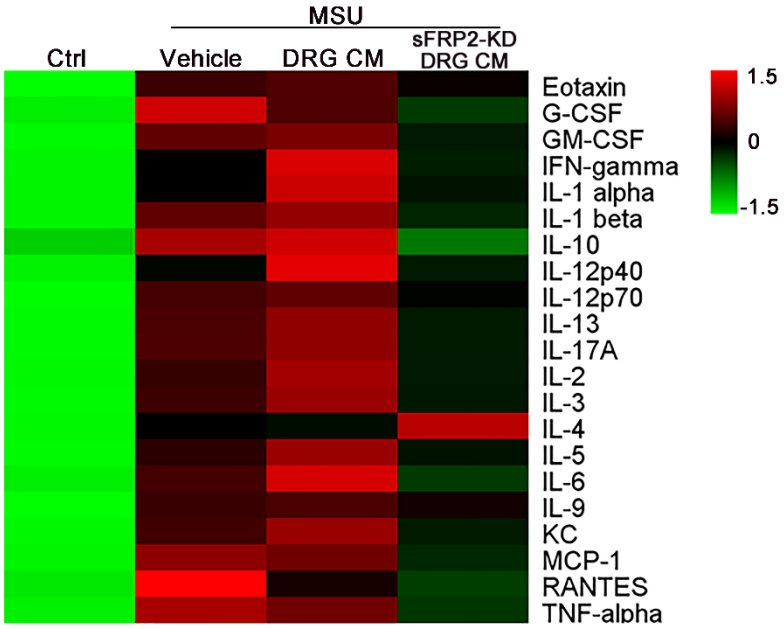


Figure S6. Measurement of cytokines by using a Luminex assay in conditioned medium from macrophages stimulated with either DRG CM, sFRP2-KD DRG CM, or control for 24 h.
The raw measurement was transformed into Z-score. Green indicates downregulation, and red indicates upregulation.

Table S1. The primers of genes used in the article

Genes	Forward	Reverse
GAPDH	5’-GGAGAGTGTTTCCTCGTCCC-3’	5’-ATGAAGGGGTCGTTGATGGC-3’
NGF	5’-GCGTTTTTGATCGGCGTACA-3’	5’-AGGGCTGTGTCAAGGGAATG-3’
CTGF	5’-AGAACTGTGTACGGAGCGTG-3’	5’-GTGCACCATCTTTGGCAGTG-3’
ENPP2	5’-GGTAGAGCCAAAGAACAAATTGGA-3’	5’-GCAGGTCGTCCATACAGGAG-3’
ANGPTL4	5’-AGATCCCCAAGGCGAGTTCT-3’	5’-AATTGGCTTCCTCGGTTCCC-3’
SERPINE1	5’-GCCACCGACTTCGGAGTAAA-3’	5’-TGAGCTGTGCCCTTCTCATT-3’
EREG	5’-TGCTTTGTCTAGGTTCCCACC-3’	5’-GGCGGTACAGTTATCCTCGG-3’
PTGS	5’-AGCCAGGCAGCAAATCCTT-3’	5’-GGGTGGGCTTCAGCAGTAAT-3’
MMP9	5’-GCCGACTTTTGTGGTCTTCC-3’	5’-TACAAGTATGCCTCTGCCAGC-3’
SFRP2	5’-GAAGCTCCCAAGGTGTGTGA-3’	5’-CACTTTGATTTTCAGTGCGAAGT-3’
CY61	5’-AACCACAACAGGACCAGAGC-3’	5’-AGGAAAAGGGCCACGTTGAA-3’
GREM1	5’-TGAATCGCACCGCATACACT-3’	5’-TGGCTCCTTGGGAACCTTTC-3’
LGALS1	5’-GCCAAGAGCTTTGTGCTGAA-3’	5’-TGGGCATTGAAGCGAGGATT-3’
IL-6 (IL6)	5’-ACAAAGCCAGAGTCCTTCAGAG-3’	5’-TCTGTGACTCCAGCTTATCTCTTG-3’
TNF-A (TNF)	5’-GATCGGTCCCCAAAGGGATG-3’	5’-CCACTTGGTGGTTTGTGAGTG-3’
INOS（NOS2）	5’-TCCTGGACATTACGACCCCT-3’	5’-CTCTGAGGGCTGACACAAGG-3’
IL-12B（IL12B）	5’-TGGGAGTACCCTGACTCCTG-3’	5’-AGGAACGCACCTTTCTGGTT-3’
TGF-β（TGF）	5’-AGCTGCGCTTGCAGAGATTA-3’	5’-AGCCCTGTATTCCGTCTCCT-3’
ARG-1	5’-ACAAGACAGGGCTCCTTTCAG-3’	5’-GGCTTATGGTTACCCTCCCG-3’
TGM2	5’-GCTGGACCAACAGGACAATGT-3’	5’-CTCTAGGCTGAGACGGTACAG-3’

IL-10	5’-GGCGCTGTCATCGATTTCTC-3’	5’-ATGGCCTTGTAGACACCTTGG-3’
GAPDH	5’-CCACTCCTCCACCTTTGACG-3’	5’-CCACCACCCTGTTGCTGTAG-3’
E-SELECTIN	5’-AGAGTGGAGCCTGGTCTTACA-3’	5’-CCTTTGCTGACAATAAGCACTGG-3’
ICAM-1	5’-ATGCCCAGACATCTGTGTCC-3’	5’-GGGGTCTCTATGCCCAACAA-3’
VCAM-1	5’-GGGAAGATGGTCGTGATCCTT-3’	5’-TCTGGGGTGGTCTCGATTTTA-3’
MCP-1（CCL2）	5’-CAGCCAGATGCAATCAATGCC-3’	5’-TGGAATCCTGAACCCACTTCT-3’
WNT1	5’-GATGGTGGGGCATCGTGAAC-3’	5’-ATTGCCATTTGCACTCTCGC-3’
WNT2	5’-CTCGGTGGAATCTGGCTCTG-3’	5’-CACATTGTCACACATCACCCT-3’
WNT2B	5’-AAGAGGCTTAAGGATGCCCG-3’	5’-GGAATCTCCGAACAGCCGTG-3’
WNT3	5’-TACCCAATTTGGTGGTCCCTG-3’	5’-GCTGGGCATGATCTCGATGT-3’
WNT3A	5’-CTCCTCTCGGATACCTCTTAGTG-3’	5’-CCAAGGACCACCAGATCGG-3’
WNT4	5’-AAGAGGAGACGTGCGAGAAAC-3’	5’-GTCCCTTGTGTCACCACCTT-3’
WNT5A	5’-CAACTGGCAGGACTTTCTCAA-3’	5’-CCTTCTCCAATGTACTGCATGTG-3’
WNT5B	5’-TCCTGGTGGTCACTAGCTCTG-3’	5’-TGCTCCTGATACAACTGACACA-3’
WNT6	5’-CTCCTACAGTGTGGTTGTCAGG-3’	5’-GCGCATCCATAAAGAGTCTTGA-3’
WNT7A	5’-GGCTTCTCTTCGGTGGTAGC-3’	5’-TGAAACTGACACTCGTCCAGG-3’
WNT7B	5’-CTTCACCTATGCCATCACGG-3’	5’-TGGTTGTAGTAGCCTTGCTTCT-3’
WNT8A	5’-CCCGTGTGCGTTCTTCTAGTC-3’	5’-GTAGACCAGGTAAGCCTTTGGA-3’
WNT8B	5’-CCCGTGTGCGTTCTTCTAGTC-3’	5’-GTAGACCAGGTAAGCCTTTGGA-3’
WNT9A	5’-TCGTGGGTGTGAAGGTGATA-3’	5’-GTTTTAGGTGCTTGCCCACC-3’
WNT9B	5’-ACCTGAAGCAGTGTGACCTAC-3’	5’-GCTCCTGCCTGAACTGGAA-3’
WNT10A	5’-CAGATCGCCATCCATGAGTG-3’	5’-ACCGCAAGCCTTCAGTTTACC-3’
WNT10B	5’-GCGGGTCTCCTGTTCTTGG-3’	5’-CCGGGAAGTTTAAGGCCCAG-3’
WNT11	5’-GCACTGAATCAGACGCAACAC-3’	5’-CGACAGGGCATACACGAAGG-3’
WNT16	5’-CAGGGCAACTGGATGTGGTT-3’	5’-CTCGTGTCGGAACTGGCTTC-3’
FZD1	5’-GAGTTCTGGACCAGTAATCCGC-3’	5’-ATGAGCCCGTAAACCTTGGTG-3’
FZD2	5’-GCCGTCCTATCTCAGCTATAAGT-3’	5’-TCTCCTCTTGCGAGAAGAACATA-3’
FZD3	5’-ATGGCTGTGAGCTGGATTGTC-3’	5’-GGCACATCCTCAAGGTTATAGGT-3’
FZD4	5’-AACCTCGGCTACAACGTGAC-3’	5’-GGCACATAAACCGAACAAAGGAA-3’
FZD5	5’-GGTGTGCCAGGAAATCACG-3’	5’-CACAAGCGGCCAGAATTGG-3’
FZD6	5’-TCTGCCCCTCGTAAGAGGAC-3’	5’-GGGAAGAACGTCATGTTGTAAGT-3’
FZD7	5’-GCCACACGAACCAAGAGGAC-3’	5’-CGGGTGCGTACATAGAGCATAA-3’
FZD8	5’-GGGTTACCTGTTGGAAGTGAC-3’	5’-GGCACCGTGATCTCTTGGC-3’
FZD9	5’-CGCACGCACTCTGTATGGAG-3’	5’-GCCGAGACCAGAACACCTC-3’
FZD10	5’-CATGCCCAACCTGATGGGTC-3’	5’-GCCACCTGAATTTGAACTGCTC-3’
LRP4	5’-GCACACGGAATAGCCAGCA-3’	5’-GGATACAGGTACATTCGCCAAG-3’
LRP5	5’-ACGTCCCGTAAGGTTCTCTTC-3’	5’-GCCAGTAAATGTCGGAGTCTAC-3’
LRP6	5’-TGCAAACAGACGGGACTTGAG-3’	5’-CGGGGACAATAATCCAGAAACAA-3’

Table S2. Measurement of cytokines by using a Luminex assay in conditioned medium from macrophages stimulated with either DRG CM, sFRP2-KD DRG CM, or control.

			MSU	
	CTRL	VEHICLE	DRG CM	SFRP2-KD DRG CM

EOTAXIN	1.71±0.14	7.45±0.48	7.59±0.29	7.09±0.25
G-CSF	32.05±15.7	70367.65±4954.99	60390.74±4249.82	40779.8±2049.64
GM-CSF	9.13±1.86	44.81±0.54	45.86±0.4	37.28±2.02
IFN-GAMMA	5.02±0.19	1716.24±99.2	2232.33±100.09	1502.19±35.72
IL-1 ALPHA	4.08±0.65	50.53±1.56	64.17±1.56	47.16±4.27
IL-1 BETA	0.48±0.4	21.67±0.66	22.99±0.4	16.3±0.91
IL-10	1200.86±0.71	1255.41±176.7	1258.21±9.67	1218.77±6.8
IL-12P40	2.92±0.18	921.26±36.02	1259.54±10.05	852.24±14.59
IL-12P70	38.68±0.55	148.36±7.07	152.15±2.14	137.12±16.65
IL-13	9.4±4.91	108.54±14.14	117.07±3	89.58±5.69
IL-17A	0.69±0.08	5.2±0.35	5.57±0.45	4.3±0.08
IL-2	0.2±0.21	8.34±0.35	9.48±0.97	6.97±0.2
IL-3	0.19±0.1	2.89±0.1	3.2±0.06	2.43±0.07
IL-4	1.3±0.88	2.08±0.08	2.04±0.02	2.28±300.54
IL-5	0.75±0.1	7.08±0.27	7.97±0.2	6.27±1.32
IL-6	0.56±0.06	11113.61±58.98	13210.04±1419.35	7600.95±106.64
IL-9	2.58±0.18	10.11±0.08	10.29±0.16	9.78±0.71
KC	1.9±0.14	16.04±0.51	17.56±0.87	13.41±0.69
MCP-1	252.52±59.24	13359.13±138.79	12912.18±1166.54	9361.71±155.88
RANTES	561.73±81.68	13702.52±2654.22	10548.89±958.03	7599.73±734.5
TNF-ALPHA	75.89±30.4	119745.56±2478.37	112339.38±1789.93	74982.83±339.5

(pg/mL)