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Supplemental Information

CCL22 induces pro-inflammatory changes in fibroblast-like synoviocytes

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Transparent Methods

Human participants

This study protocol was approved by the University of Calgary Human Research Ethics Board (REB15-0005 and REB15-0880). All individuals involved provided signed consent/assent. The study was carried out in accordance with the declaration of Helsinki. Matching SF and synovial membrane samples were obtained from every individual in the normal and OA cohorts.

Normal Group (n=10): Criteria for control cadaveric donations were an age of 18 years or older, no history of arthritis, joint injury or surgery (including visual inspection of the cartilage surfaces during recovery), no prescription anti-inflammatory medications, no co-morbidities (such as diabetes/cancer), and availability within 4 hours of death.

Knee Osteoarthritis (n=13): Inclusion criteria was based on a diagnosis of OA performed by an orthopedic surgeon at the University of Calgary based on clinical symptoms with radiographic evidence of changes associated with OA in accordance with American College of Rheumatology (ACR) criteria. Radiographic evidence of OA of any compartment of the knee with collapsed or near collapsed joint space of any compartment of the knee (Table 1).

FLS derivation

To obtain FLS for analysis, two biopsies (approximately 5mm in diameter) were obtained from each donor and placed in 1.5mL tubes with 1xDPBS (ThermoFisher) to keep the tissue hydrated. Each synovial membrane biopsy was digested for 1.5 hours at 37^0C in 1mg/mL filtered type IV collagenase (Sigma) in heat-inactivated FBS (ThermoFisher).

The resultant cell suspension was filtered at 70µm (ThermoFisher) and centrifuged at 5000rpm for 6 minutes. The resultant cell pellet was washed three times with 1ml of 1xDPBS. For *ex vivo* analysis, a sample of the cell suspension was collected at this point and processed for the reported outcome measures. For *in vitro* outcome measures and related analysis, an aliquot of the cell suspension was then expanded in T25 culture flasks (Primaria, Corning/ThermoFisher) in media containing DMEM F12, 10% FBS, 1%

Non-essential Amino Acids, and 1% Anti-anti (all ThermoFisher). Flasks were passaged when cells reached 80% confluence and all outcome measures were performed on FLS before passage 5.

CCL22, S100A12, CCR3, CCR4 and CCR5 analysis by flow cytometry

FLS were plated at 50,000 cells per well in 6-well plates, allowed to adhere overnight, and treated with the respective condition.

To determine expression of CCL22, S100A12 or CCR3, CCR4 and CCR5 on FLS, the cells were filtered and fixed in 500µl of 90% MeOH for 5-10 minutes at room temperature. The cells were then centrifuged, and washed with DPBS. The cells were centrifuged again, the liquid was removed, and 50µl of blocking buffer and 0.5µg of antibody CD68 (clone # Y1/82A: BD Biosciences); Cadherin-11/CDH-11 (clone # 16G5; BioLegend); CCR4 (clone # L291H4: BioLegend and clone # 1G1: BD Biosciences); CCR3 (clone # 5E8: BD Biosciences); CCR5 (clone # 2D7: BD Biosciences); CCL22 (clone # 57226, R&D systems); S100A12 (clone # 161205, R&D systems); fixable viability stain (FVS) 510 (BV510, BD Biosciences); and/or the appropriate isotype controls/unstained cells were added to each tube and incubated in the dark for 30-45 minutes at room temperature. The cells were washed three times with FACs buffer. The cells were then assayed with the BD LSR II Cytometer. The results were analyzed using FlowJo software. Briefly macrophages ($CD68^+$) as well as the dead cells ($FVS510^+$) were excluded. FLS ($CDH\text{-}11^+$) were gated upon and the remainder of the markers were examined in this cell population (**Figure S5**).

Cytokine expression analysis

FLS were plated (200,000 cells per well) in 12 well Primaria dishes 24 hours before cytokine treatment. Recombinant CCL22 (Peprotech) was added, so that the final concentrations were at 0.2ng/ml or 3ng/ml, which were the mean CCL22 concentrations in SF from normal and OA patients respectively based on our previous study(Ren *et al.*, 2018). FLS were incubated for 24 hours after cytokine treatment and culture media were collected for cytokine profiling analysis.

SF samples were collected without the use of lavage or any other diluting agent. The native SF samples

were aliquoted, centrifuged at 3000g for 15 minutes at 4^0C and stored in cryogenic vials at -80^0C. For standardization of the protocol, all SF samples were subjected to only one freeze-thaw event prior to the assessment.

Cytokine profiling analysis was performed by Eve Technologies (Calgary, AB Canada) using the Milliplex MAP Human Cytokine/Chemokine Panel (Millipore) according to the manufacturer's instructions. All samples were assayed in duplicate and prepared standards were included in all runs. The following cytokines were quantified in this study: GM-CSF, IFNy, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12(p70), IL-13, MCP-1, TNFα. CCL22 was assayed in single-plex for SF samples. The sensitivities of these makers range from 0.1 – 10.1pg/mL (average 2.359pg/ml) and the inter-array accuracies ranged from 3.5% – 18.9% coefficient of variation (average 10.7%).

RT-qPCR array analysis

RNA was extracted using Trizol Reagent (ThermoFisher). Total RNA was purified with RNeasy Plus Micro Kit (Qiagen) to remove genomic DNA. The RNA integrity number (RIN) was measured with Agilent RNA 6000 NanoChips on a 2100 Bioanalyzer (Agilent Technologies). The quantity was measured with a NanoDrop 1,000 (NanoDrop Technologies). Total mRNA from each well was extracted and purified using Trizol (ThermoFisher) and converted into cDNA according to the High Capacity cDNA Reverse Transcription Kits protocol (Applied Biosystems). cDNA along with SYBR™ Green PCR Master Mix (Applied Biosystems) was added to the Chemotaxis Tier 1-4 H384 predesigned qPCR array plate (Bio-Rad) and run on a QuantStudio 6 system (Applied Biosystems).

Relative quantification of gene expression

Total mRNA from each well was extracted and purified using Trizol (ThermoFisher) and converted into cDNA according to the High Capacity cDNA Reverse Transcription Kits protocol (Applied Biosystems). Two microliters of cDNA was added to a 96 well qPCR plate along with CCL22, S100A12, CCR3 and CCR5 TaqMan® validated probes and TaqMan® Universal PCR Master Mix. In addition, an 18S RNA

probe was used as an endogenous control. All samples were assayed in triplicate.

Histology, immunofluorescence (IF) and in situ hybridization

Both normal and OA synovium was fixed with formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin to identify synovitis (Krenn *et al.*, 2006). Three features of synovitis (enlargement of lining layer, cellular density of stroma, inflammatory infiltrate) were evaluated separately on a scale of from 0 to 3 (absent to strong) and then summed. The total score was interpreted as follows: no synovitis (0-1.9); low-grade synovitis (2–4.9); high grade synovitis (5-9).

Sections were also deparaffinized in CitraSolv (Fisher Scientific) and rehydrated through a series of graded ethanol to distilled water steps. Antigen retrieval (10mM sodium citrate, pH 6.0) and blocking (1:500 dilution; 100μL goat serum): 50mL TRIS-buffered saline, 0.1% Tween 20 (TBST) for 1hr), steps were performed prior to going through sequential wash (TBST) and primary antibody application steps. Primary antibodies (same as listed in the flow cytometry section) were directly conjugated to fluorescent probes (Abcam, Dylight system) and the nucleic acid stain DAPI (Sigma) were applied to sections. After antibody staining, sections were mounted using FluorSave reagent (Calbiochem) and coverslipped. A Zeiss Axio Scan.Z1 microscope was used to detect the signal localization and Zen software was used to quantify the signal intensity for each antibody. Fluorescent signal for each specific marker was quantified within the synovium (O'Brien *et al.*, 2017; Jablonski *et al.*, 2019). Briefly, n=3 tissue sections per biopsy (100mm2 fields of view) were assayed for each fluorescent filter (e.g. 488, 568). The mean fluorescent intensity (MFI) in these areas of interest was obtained from the Zen software.

For *in situ* hybridization, the dewaxed and rehydrated sections were boiled in sodium citrate for 10 min. The sections underwent protease digestion (0.2% pepsin/0.01 M HCl) at 37 °C for 10 min, and stringency washes in 0.1% NP-40/SSC buffer (150mM sodium chloride, 15mM sodium citrate, pH 7.0) at 37 °C for 30 min. The mixture of fluorescently labelled probes (IDT) was added to the sections in 50% formamide/SSC, heated to 85 °C (5 min) and incubated at 37 °C (overnight). The slides were washed with 50% formamide/2× SSC and then with 0.1% NP-40/SSC buffer at 45 °C, before they were counterstained

with DAPI. The slides were covered, imaged and MFI obtained as described above.

Western blot / dot blot analysis

Total protein was collected from FLS using a Tris-HCl/SDS based lysis/sample buffer and separated on a 10% poly-acrylamide gel. The gels were transferred to nitrocellulose membranes and probed with primary antibodies specific to the proteins CCL22 (clone # 57226, R&D systems) and Histone H3 (Cell Signaling). Histone H3 was utilized as a control, since it is constitutively expressed in most cell types. An appropriate infra-red secondary was utilized for detection of the signal with the Odyssey imaging system (LICOR). In the case of dot blot analysis, cell lysates were spotted directly onto nitrocellulose using a vacuum manifold in place of gel electrophoresis.

Transfection and shRNA knockdown

For gene knockdown of CCR3 and CCR5, we employed the MISSION® shRNA Plasmid system (Sigma). Plasmid DNAs including a non-sense shRNA control, were purified from bacterial cultures using the PureLink® HiPure Plasmid Midiprep Kit (Life Technologies). Cells were transfected using the TransIT-2020 (Mirus Bio LLC). After 24 h incubation at 37ºC, puromycin (Sigma) was added to the culture media to select for transfected cells for an additional 48h before processing for subsequent analysis.

Calcium flux assay

A modified method was employed based on a previous study(Nibbs *et al.*, 2000). FLS were washed in assay buffer (136mM NaCl, 4.8mM KCl, 5mM glucose, 1mM CaCl2, 0.025% BSA, and 25mM HEPES), and then incubated with 10mM fura-2-AM (Sigma) for 1 h at 37°C. FLS were washed in assay buffer and incubated at 37°C in Victor X3 (Perkin-Elmer) plate reader. Fluorescence emission was recorded every 1s for 10s, after which a specific chemokine ligand (or negative control – PBS) was added and fluorescence emission was recorded (500 nm) every 1s for an additional 40s. For all experiments, the highest point of the flux was calculated and all values were presented as a percentage of the maximal flux.

Data analysis

Graphpad was used for the statistical analysis. ANOVA with multiple comparison correction was used to compare cytokine profiling, gene expression or protein expression between treatments and controls. Linear regression was used to determine the R^2 value and significance for CCL22/S100A12 staining vs. CCL22 SF levels.

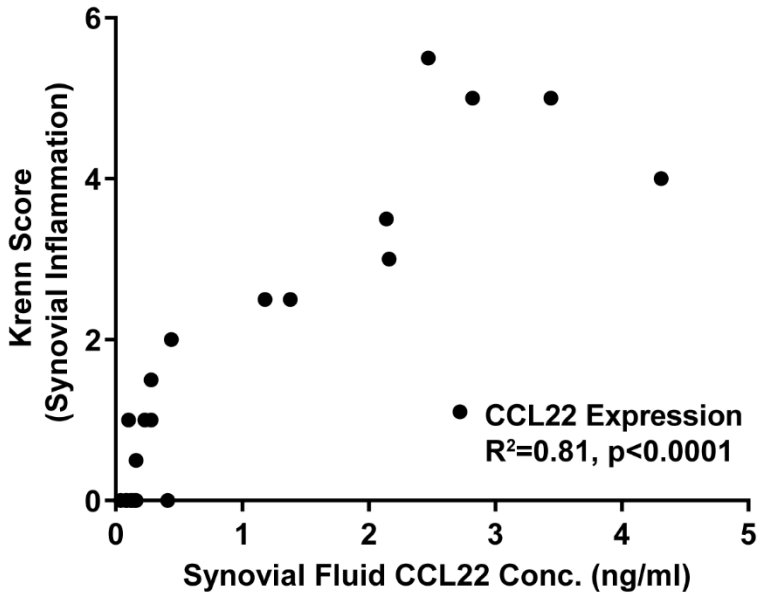

Figure S1. Relationship between Synovitis and SF CCL22 concentration, Related to Table 1. A positive and significant relationship was observed between synovitis score (Krenn) and SF concentration of CCL22. (R^2=0.81 p<0.0001).

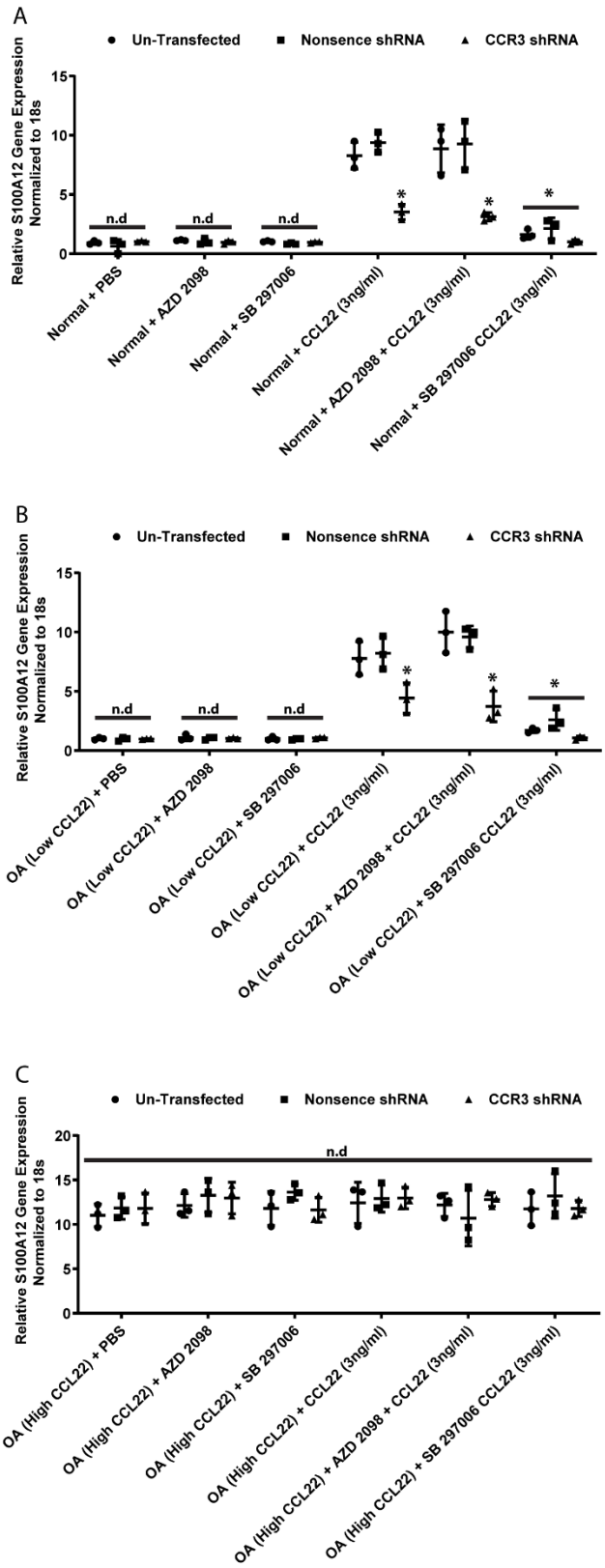

Figure S2. Inhibition of CCR4 has no effect on CCL22 induced *S100A12* expression, Related to Figure 7. FLS (normal n=3; OA low CCL22 n=3; OA high CCL22 n=3) transfected with a nonsense control shRNA or CCR3 shRNA were exposed to AZD 2098 (CCR4 inhibitor) or SB 297006 (CCR3 inhibitor) with/without CCL22 treatment. AZD 2098 has no effect on CCL22 induced *S100A12* expression, while SB 297006 inhibited *S100A12* expression in normal and OA low CCL22 FLS, but not in OA high CCL22 FLS. *$p<0.05$. n.d. = no difference. Data are represented as mean ± SD.

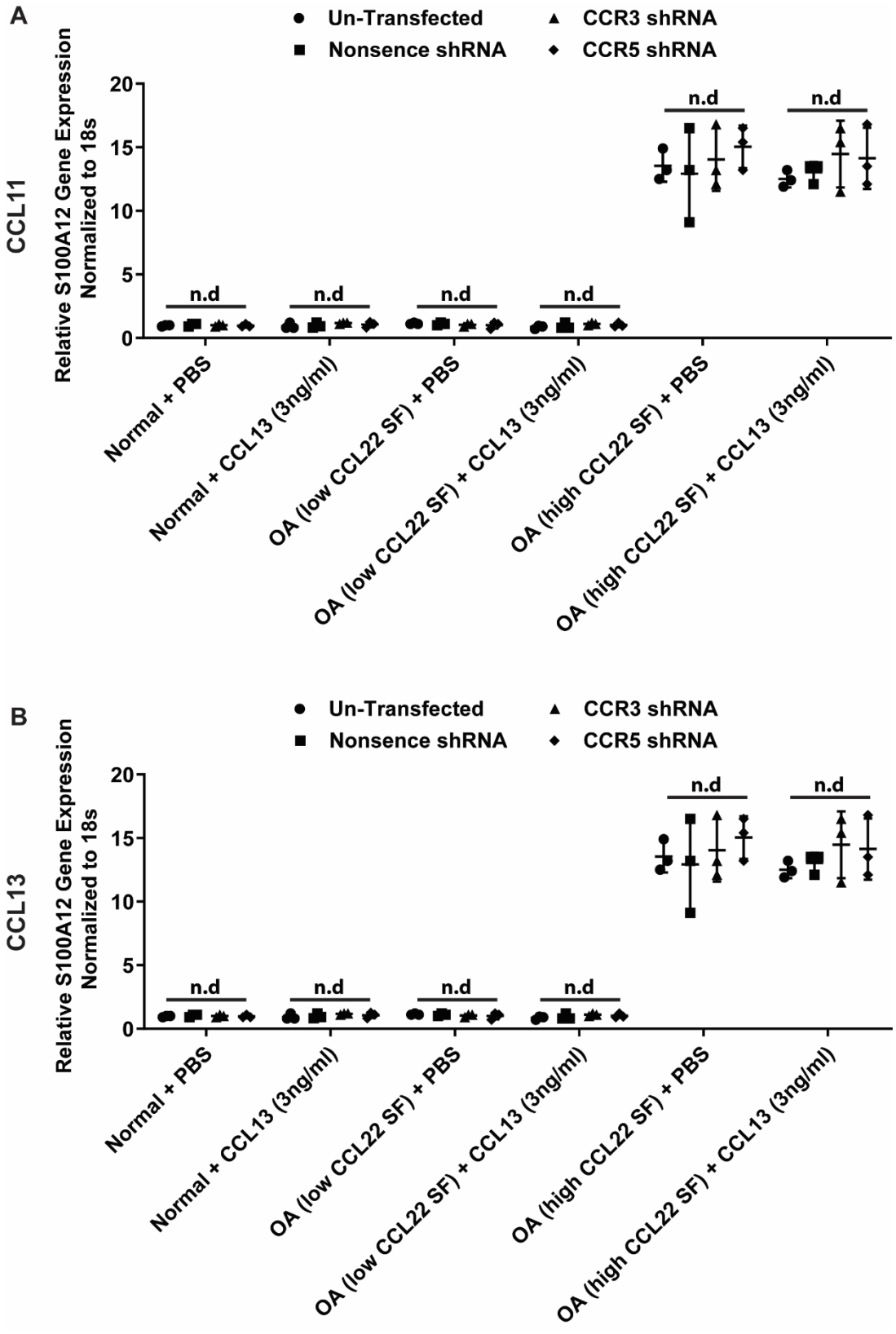

Figure S3. CCL11 and CCL13 do not induce expression of S100A12, Related to Figure 7. Control and transfected FLS (normal n=3; OA low CCL22 n=3; OA high CCL22 n=3) treated with CCL11 and CCL13 were assayed for S100A12 at the mRNA level with RT-qPCR. Neither CCL11 (A) nor CCL13 (B) induced S100A12 mRNA expression. n.d. = no difference. Data are represented as mean ± SD.

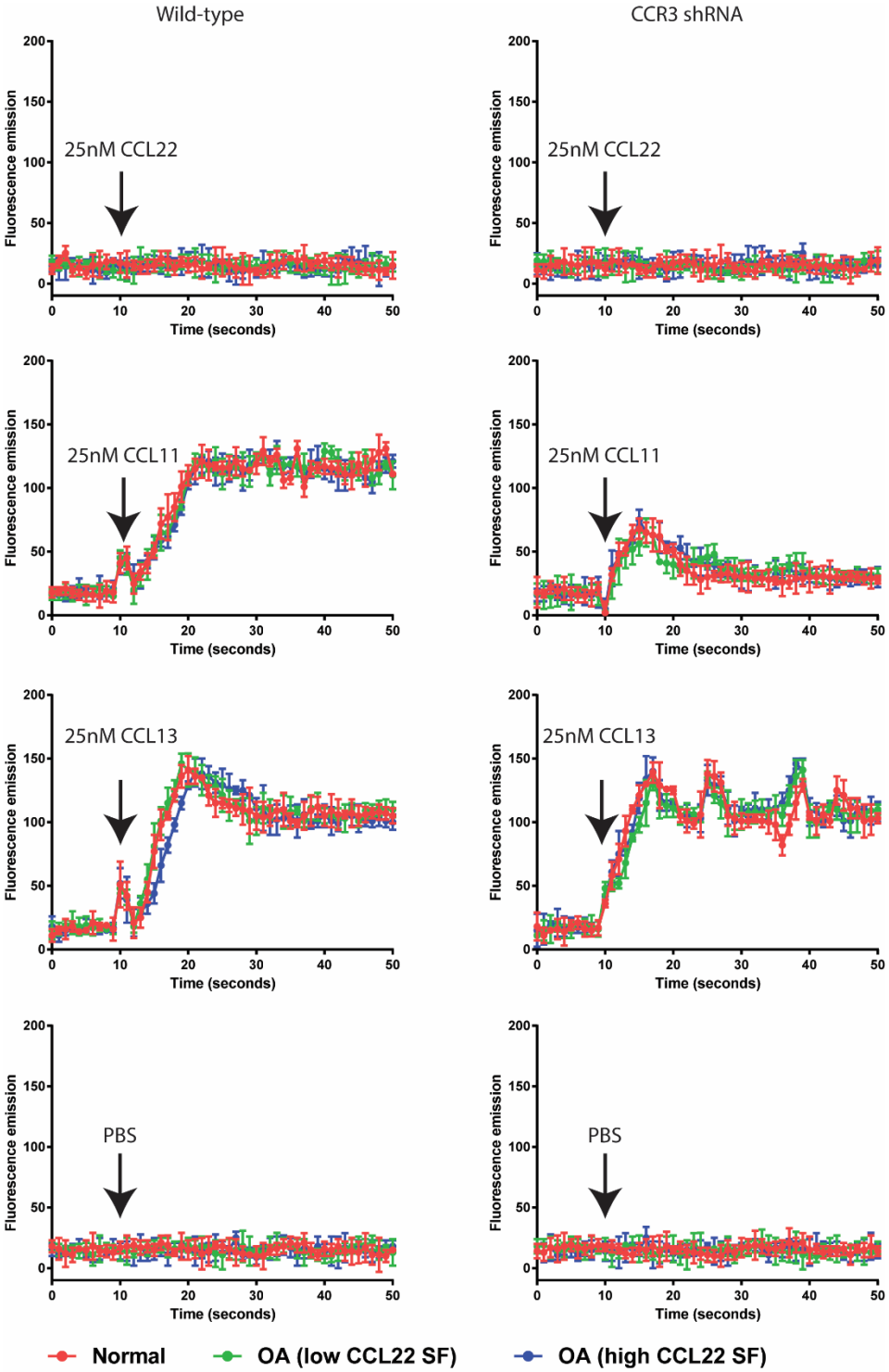

Figure S4. CCL11 and CCL13 induce calcium flux in FLS while CCL22 does not, Related to Figure 7. Control and transfected (CCR3 shRNA) FLS (normal n=3; OA low CCL22 n=3; OA high CCL22 n=3) treated with CCL11, CCL13 and CCL22 were assayed for calcium flux. CCL22 was not able to induce a calcium flux in control or CCR3 shRNA FLS (normal or OA); while CCL11 and CCL13 were both able to induce a calcium flux. CCR3 shRNA transfected FLS demonstrated a reduced calcium flux in the presence of CCL11.

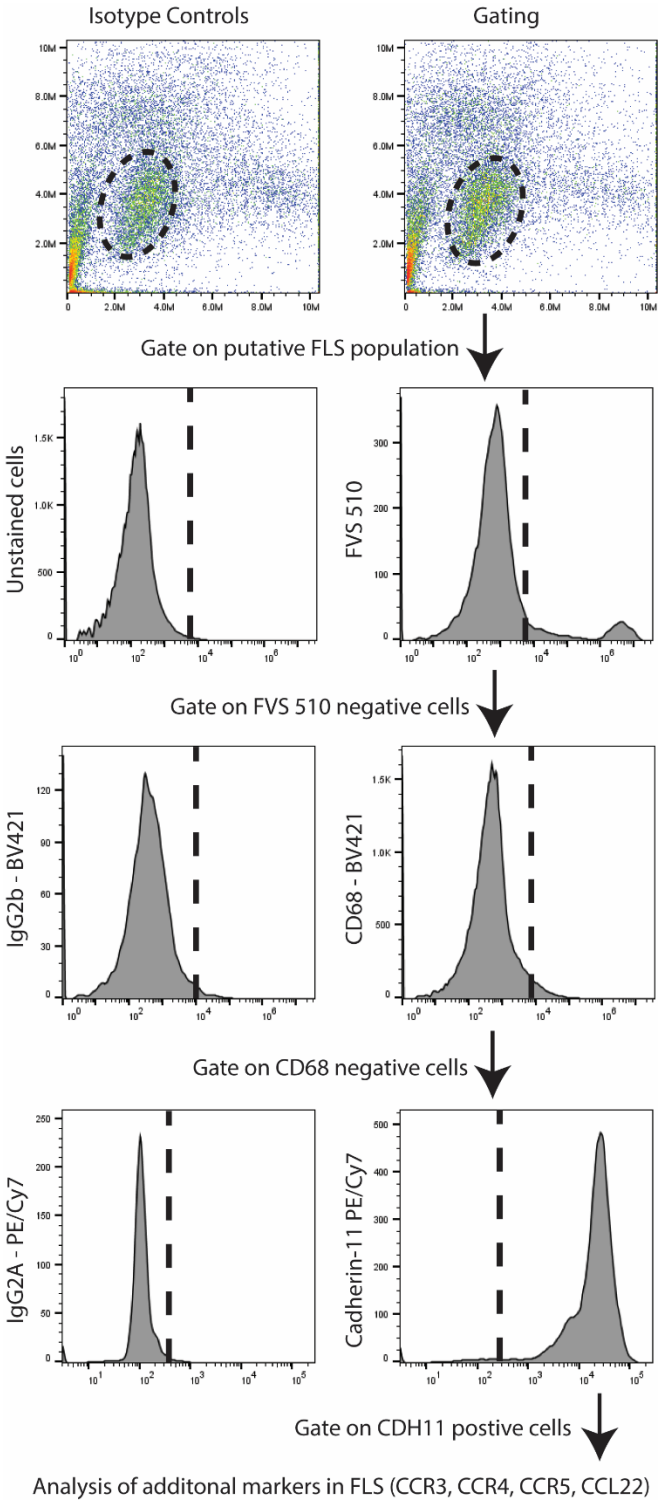

Figure S5. Flow cytometry gating strategy, Related to Figure 3, Figure 6 and Figure 7. Isotype controls and unstained cells were employed to determine background staining levels. The putative FLS population was identified based on forward and side scatter. Dead cells were excluded based on FVS 510 (viability dye) staining. Macrophage populations were excluded based on CD68 expression and the remaining population was gated on CDH-11 positive FLS.

Table S1. Complete list of gene expression differences between normal FLS with/without exposure to 3ng/ml CCL22 for 24hrs. Related to Figure 2.

Relative Fold Expression Difference	Gene Symbol	Gene Name	Notes
1.16	ACTA1	actin, alpha 1, skeletal muscle	
0.91	ACTA2	actin, alpha 2, smooth muscle, aorta	
1.06	**ACTB**	**actin, beta**	**Housekeeping gene**
0.99	ACTG1	actin, gamma 1	
1.10	ALCAM	activated leukocyte cell adhesion molecule	
1.07	ATF2	activating transcription factor 2	
0.81	ADRB2	adrenergic, beta-2-, receptor, surface	
0.91	AIMP1	aminoacyl tRNA synthetase complex-interacting multifunctional protein 1	
0.92	APP	amyloid beta (A4) precursor protein	
1.06	ANGPT1	angiopoietin 1	
0.93	AGTR1	angiotensin II receptor, type 1	
Not Detected	AGTR2	angiotensin II receptor, type 2	
0.94	ARRB2	arrestin, beta 2	
1.05	AZU1	azurocidin 1	
1.08	BMP4	bone morphogenetic protein 4	
1.15	BMP7	bone morphogenetic protein 7	
1.01	BMPR1B	bone morphogenetic protein receptor, type IB	
0.90	BDKRB1	bradykinin receptor B1	
0.95	BDKRB2	bradykinin receptor B2	
1.07	BDNF	brain-derived neurotrophic factor	
0.97	BCR	breakpoint cluster region	
0.96	BCAR1	breast cancer anti-estrogen resistance 1	
0.95	ABL1	c-abl oncogene 1, non-receptor tyrosine kinase	
0.91	CALCA	calcitonin-related polypeptide alpha	
0.94	CREB1	cAMP responsive element binding protein 1	
1.16	CREB3	cAMP responsive element binding protein 3	
0.88	CSNK2A1	casein kinase 2, alpha 1 polypeptide	
0.90	CTNNB1	catenin (cadherin-associated protein), beta 1	
1.13	CD14	CD14 molecule	
0.99	CD34	CD34 molecule	
0.96	CD4	CD4 molecule	
0.94	CD44	CD44 molecule	
0.95	CD8A	CD8a molecule	
1.16	CDC42	cell division cycle 42	
1.04	XCL1	chemokine (C motif) ligand 1	
0.93	CCL1	chemokine (C-C motif) ligand 1	
0.90	CCL11	chemokine (C-C motif) ligand 11	
0.93	CCL13	chemokine (C-C motif) ligand 13	
0.93	CCL16	chemokine (C-C motif) ligand 16	
2.75	**CCL17**	**chemokine (C-C motif) ligand 17**	**Reached significance**
0.89	CCL18	chemokine (C-C motif) ligand 18	
1.07	CCL19	chemokine (C-C motif) ligand 19	
0.92	CCL2	chemokine (C-C motif) ligand 2	
1.12	CCL20	chemokine (C-C motif) ligand 20	
0.93	CCL21	chemokine (C-C motif) ligand 21	
6.28	**CCL22**	**chemokine (C-C motif) ligand 22**	**Reached significance**
Not Detected	CCL23	chemokine (C-C motif) ligand 23	
0.94	CCL24	chemokine (C-C motif) ligand 24	
1.03	CCL25	chemokine (C-C motif) ligand 25	
0.95	CCL26	chemokine (C-C motif) ligand 26	
1.09	CCL27	chemokine (C-C motif) ligand 27	
1.18	CCL28	chemokine (C-C motif) ligand 28	
0.91	CCL3	chemokine (C-C motif) ligand 3	
1.06	CCL4	chemokine (C-C motif) ligand 4	
Not Detected	CCL4L1	chemokine (C-C motif) ligand 4-like 1	
1.03	CCL5	chemokine (C-C motif) ligand 5	
0.93	CCL7	chemokine (C-C motif) ligand 7	
Not Detected	CCL8	chemokine (C-C motif) ligand 8	
0.97	CCR1	chemokine (C-C motif) receptor 1	
1.04	CCR10	chemokine (C-C motif) receptor 10	
1.15	CCR2	chemokine (C-C motif) receptor 2	
1.14	CCR3	chemokine (C-C motif) receptor 3	
Not Detected	CCR4	chemokine (C-C motif) receptor 4	
0.85	CCR5	chemokine (C-C motif) receptor 5	
1.09	CCR6	chemokine (C-C motif) receptor 6	
1.03	CCR7	chemokine (C-C motif) receptor 7	
1.06	CCR8	chemokine (C-C motif) receptor 8	
1.09	CCR9	chemokine (C-C motif) receptor 9	
Not Detected	CCRL2	chemokine (C-C motif) receptor-like 2	
0.94	CX3CL1	chemokine (C-X3-C motif) ligand 1	
0.98	CX3CR1	chemokine (C-X3-C motif) receptor 1	
0.93	CXCL1	chemokine (C-X-C motif) ligand 1	
Not Detected	CXCL10	chemokine (C-X-C motif) ligand 10	
Not Detected	CXCL11	chemokine (C-X-C motif) ligand 11	
1.14	CXCL12	chemokine (C-X-C motif) ligand 12	
Not Detected	CXCL13	chemokine (C-X-C motif) ligand 13	
0.97	CXCL14	chemokine (C-X-C motif) ligand 14	
0.99	CXCL16	chemokine (C-X-C motif) ligand 16	
0.95	CXCL17	chemokine (C-X-C motif) ligand 17	
0.93	CXCL2	chemokine (C-X-C motif) ligand 2	
1.06	CXCL3	chemokine (C-X-C motif) ligand 3	

Relative Fold Expression Difference	Gene Symbol	Gene Name	Notes
1.01	CXCL5	chemokine (C-X-C motif) ligand 5	
1.11	CXCL6	chemokine (C-X-C motif) ligand 6	
0.95	CXCL9	chemokine (C-X-C motif) ligand 9	
Not Detected	CXCR1	chemokine (C-X-C motif) receptor 1	
Not Detected	CXCR2	chemokine (C-X-C motif) receptor 2	
1.06	CXCR3	chemokine (C-X-C motif) receptor 3	
0.94	CXCR4	chemokine (C-X-C motif) receptor 4	
1.01	CXCR5	chemokine (C-X-C motif) receptor 5	
1.01	CXCR6	chemokine (C-X-C motif) receptor 6	
Not Detected	CCBP2	chemokine binding protein 2	
Not Detected	CMKLR1	chemokine-like receptor 1	
1.08	CCKBR	cholecystokinin B receptor	
Not Detected	CHRM3	cholinergic receptor, muscarinic 3	
0.91	CLTC	clathrin, heavy chain (Hc)	
1.03	F2	coagulation factor II	
0.93	F2R	coagulation factor II receptor	
1.01	F2RL1	coagulation factor II receptor-like 1	
1.04	CFL1	cofilin 1	
0.86	COL1A2	collagen, type I, alpha 2	
1.06	COL4A1	collagen, type IV, alpha 1	
1.06	COL4A2	collagen, type IV, alpha 2	
1.05	COL4A3	collagen, type IV, alpha 3	
0.93	COL4A4	collagen, type IV, alpha 4	
1.11	CSF2	colony stimulating factor 2	
0.96	CSF3	colony stimulating factor 3	
1.03	CSF3R	colony stimulating factor 3 receptor	
1.08	C3	complement component 3	
0.96	C3AR1	complement component 3a receptor 1	
1.02	C5	complement component 5	
0.93	C5AR1	complement component 5a receptor 1	
1.09	CXADR	coxsackie virus and adenovirus receptor	
0.90	CDK5	cyclin-dependent kinase 5	
0.98	CYR61	cysteine-rich, angiogenic inducer, 61	
1.03	CYSLTR1	cysteinyl leukotriene receptor 1	
Not Detected	CYSLTR2	cysteinyl leukotriene receptor 2	
1.09	DOCK1	dedicator of cytokinesis 1	
1.06	DOCK2	dedicator of cytokinesis 2	
0.99	DEFA1	defensin, alpha 1	
Not Detected	DEFA3	defensin, alpha 3	
0.97	DEFB1	defensin, beta 1	
1.14	DEFB4A	defensin, beta 4A	
0.88	DPYSL2	dihydropyrimidinase-like 2	
0.91	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	
Not Detected	EMR2	egf-like module containing, mucin-like, hormone receptor-like 2	
0.97	ENG	endoglin	
Not Detected	ECSCR	endothelial cell-specific chemotaxis regulator	
0.93	EDN1	endothelin 1	
1.10	EDNRA	endothelin receptor type A	
1.06	EDNRB	endothelin receptor type B	
0.84	EPHA1	EPH receptor A1	
0.97	EPHA2	EPH receptor A2	
1.06	EPHA3	EPH receptor A3	
0.77	EPHA4	EPH receptor A4	
1.04	EPHB1	EPH receptor B1	
1.06	EPHB2	EPH receptor B2	
1.01	EPHB3	EPH receptor B3	
0.91	EPHB4	EPH receptor B4	
0.95	EPHB6	EPH receptor B6	
0.92	EFNA1	ephrin-A1	
1.01	EFNA3	ephrin-A3	
Not Detected	RP11-540D14.8	Ephrin-A3; cDNA FLJ57652, highly similar to Ephrin-A3	
1.06	EFNA5	ephrin-A5	
1.04	EFNB1	ephrin-B1	
0.87	EFNB2	ephrin-B2	
Not Detected	EGF	epidermal growth factor	
0.96	EGFR	epidermal growth factor receptor	
1.21	EZR	ezrin	
1.06	FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	
1.10	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor	
0.97	FES	feline sarcoma oncogene	
0.95	FGF2	fibroblast growth factor 2	
0.93	FGF7	fibroblast growth factor 7	
0.93	FGFR1	fibroblast growth factor receptor 1	
Not Detected	FLT1	fms-related tyrosine kinase 1	
1.04	FPR1	formyl peptide receptor 1	
1.08	FPR2	formyl peptide receptor 2	
0.90	FPR3	formyl peptide receptor 3	
0.91	FOSL1	FOS-like antigen 1	
0.95	FZD4	frizzled homolog 4	
0.89	FYN	FYN oncogene related to SRC, FGR, YES	
Not Detected	GPR44	G protein-coupled receptor 44	

Relative Fold Expression Difference	Gene Symbol	Gene Name	Notes
0.92	GATA3	GATA binding protein 3	
0.90	GDNF	glial cell derived neurotrophic factor	
1.10	**GAPDH**	**glyceraldehyde-3-phosphate dehydrogenase**	**Housekeeping gene**
1.09	GSK3A	glycogen synthase kinase 3 alpha	
0.85	GSK3B	glycogen synthase kinase 3 beta	
1.03	GAS6	growth arrest-specific 6	
3.68	**GRB2**	**growth factor receptor-bound protein 2**	**Reached significance**
0.97	HSPB1	heat shock 27kDa protein 1	
0.82	HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1	
0.97	HSP90AB1	heat shock protein 90kDa alpha (cytosolic), class B member 1	
0.88	HSP90B1	heat shock protein 90kDa beta (Grp94), member 1	
0.86	HGF	hepatocyte growth factor	
0.95	HMGB1	high mobility group box 1	
1.06	HRH1	histamine receptor H1	
0.97	HPRT1	hypoxanthine phosphoribosyltransferase 1	
1.19	IGF1	insulin-like growth factor 1	
1.06	ITGA1	integrin, alpha 1	
1.03	ITGA2	integrin, alpha 2	
1.13	ITGA2B	integrin, alpha 2b	
1.32	ITGA3	integrin, alpha 3	
1.04	ITGA4	integrin, alpha 4	
0.95	ITGA5	integrin, alpha 5	
0.90	ITGA6	integrin, alpha 6	
1.07	ITGA7	integrin, alpha 7	
1.04	ITGA9	integrin, alpha 9	
0.94	ITGAL	integrin, alpha L	
0.94	ITGAM	integrin, alpha M	
0.91	ITGAV	integrin, alpha V	
1.09	ITGAX	integrin, alpha X	
0.90	ITGB1	integrin, beta 1	
0.95	ITGB2	integrin, beta 2	
0.99	ITGB5	integrin, beta 5	
0.95	ITGB6	integrin, beta 6	
0.92	ITGB8	integrin, beta 8	
0.93	ICAM1	intercellular adhesion molecule 1	
0.97	IFNG	interferon, gamma	
1.04	IL1A	interleukin 1, alpha	
Not Detected	IL1B	interleukin 1, beta	
0.47	**IL10**	**interleukin 10**	**Reached significance**
0.88	IL13	interleukin 13	
0.82	IL16	interleukin 16	
1.01	IL17A	interleukin 17A	
0.95	IL17B	interleukin 17B	
Not Detected	IL18	interleukin 18	
Not Detected	IL2	interleukin 2	
0.62	**IL4**	**interleukin 4**	**Reached significance**
Not Detected	IL5	interleukin 5	
0.88	IL6	interleukin 6	
0.98	IL6R	interleukin 6 receptor	
0.96	IL6ST	interleukin 6 signal transducer	
1.06	IL8	interleukin 8	
0.96	JUND	jun D proto-oncogene	
1.13	JUN	jun proto-oncogene	
1.08	KDR	kinase insert domain receptor	
0.97	KISS1R	KISS1 receptor	
0.86	L1CAM	L1 cell adhesion molecule	
1.16	LAMA3	laminin, alpha 3	
1.06	LAMA5	laminin, alpha 5	
1.14	LTB4R2	leukotriene B4 receptor 2	
1.08	LIMK1	LIM domain kinase 1	
1.09	LBP	lipopolysaccharide binding protein	
1.01	LSP1	lymphocyte-specific protein 1	
0.88	LEF1	lymphoid enhancer-binding factor 1	
0.99	MIF	macrophage migration inhibitory factor	
1.12	MTOR	mechanistic target of rapamycin	
0.76	MET	met proto-oncogene	
1.05	MAPK1	mitogen-activated protein kinase 1	
1.08	MAPK11	mitogen-activated protein kinase 11	
0.90	MAPK14	mitogen-activated protein kinase 14	
0.94	MAPK3	mitogen-activated protein kinase 3	
0.97	MAPK8	mitogen-activated protein kinase 8	
0.84	MAP2K1	mitogen-activated protein kinase kinase 1	
0.95	MAP2K2	mitogen-activated protein kinase kinase 2	
1.24	MSN	moesin	
0.94	MPO	myeloperoxidase	
0.97	MYH9	myosin, heavy chain 9	
0.93	NCK1	NCK adaptor protein 1	
1.06	NCK2	NCK adaptor protein 2	
1.07	NGFR	nerve growth factor receptor	
0.93	NTN1	netrin 1	
1.07	NTN4	netrin 4	

Relative Fold Expression Difference	Gene Symbol	Gene Name	Notes
1.06	NCAM1	neural cell adhesion molecule 1	
Not Detected	NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	
0.91	NRP1	neuropilin 1	
1.18	NRP2	neuropilin 2	
0.84	NTRK1	neurotrophic tyrosine kinase, receptor, type 1	
0.92	NKX2-1	NK2 homeobox 1	
1.06	NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	
0.92	NR4A1	nuclear receptor subfamily 4, group A, member 1	
1.06	NR4A3	nuclear receptor subfamily 4, group A, member 3	
1.09	PAK1	p21 protein (Cdc42/Rac)-activated kinase 1	
1.08	PAK2	p21 protein (Cdc42/Rac)-activated kinase 2	
1.06	PARVA	parvin, alpha	
0.93	PTEN	phosphatase and tensin homolog	
0.91	PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	
1.11	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide	
1.08	PIK3CD	phosphoinositide-3-kinase, catalytic, delta polypeptide	
0.96	PIK3CG	phosphoinositide-3-kinase, catalytic, gamma polypeptide	
1.07	PIK3C2A	phosphoinositide-3-kinase, class 2, alpha polypeptide	
0.95	PIK3C2B	phosphoinositide-3-kinase, class 2, beta polypeptide	
0.97	PIK3C2G	phosphoinositide-3-kinase, class 2, gamma polypeptide	
0.91	PLA2G1B	phospholipase A2, group IB	
1.07	PLA2G2A	phospholipase A2, group IIA	
0.90	PLA2G4A	phospholipase A2, group IVA	
1.04	PLA2G7	phospholipase A2, group VII	
0.93	PLCG1	phospholipase C, gamma 1	
1.14	PLCG2	phospholipase C, gamma 2	
1.06	PLD1	phospholipase D1, phosphatidylcholine-specific	
0.90	PLAU	plasminogen activator, urokinase	
0.91	PLAUR	plasminogen activator, urokinase receptor	
1.04	PF4	platelet factor 4	
1.06	PTAFR	platelet-activating factor receptor	
1.16	PDGFA	platelet-derived growth factor alpha polypeptide	
0.93	PDGFB	platelet-derived growth factor beta polypeptide	
1.15	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	
0.90	PDGFRB	platelet-derived growth factor receptor, beta polypeptide	
1.03	PLXNB1	plexin B1	
1.04	PLXNC1	plexin C1	
1.04	PLXND1	plexin D1	
0.98	PROKR1	prokineticin receptor 1	
1.01	PPBP	pro-platelet basic protein	
1.12	PRKCA	protein kinase C, alpha	
0.97	PRKCB	protein kinase C, beta	
0.93	PRKCD	protein kinase C, delta	
0.88	PRKCE	protein kinase C, epsilon	
0.93	PRKCH	protein kinase C, eta	
0.95	PRKCG	protein kinase C, gamma	
1.06	PRKCI	protein kinase C, iota	
1.06	PRKCQ	protein kinase C, theta	
0.98	PRKCZ	protein kinase C, zeta	
0.92	PRKD1	protein kinase D1	
1.04	PRKD2	protein kinase D2	
0.92	PRKD3	protein kinase D3	
0.97	PTPN11	protein tyrosine phosphatase, non-receptor type 11	
Not Detected	PTPRC	protein tyrosine phosphatase, receptor type, C	
0.92	PTPRJ	protein tyrosine phosphatase, receptor type, J	
0.91	PTPRM	protein tyrosine phosphatase, receptor type, M	
0.86	PTK2	PTK2 protein tyrosine kinase 2	
0.90	RDX	radixin	
0.95	RHOA	ras homolog gene family, member A	
0.96	RHOB	ras homolog gene family, member B	
1.03	RHOC	ras homolog gene family, member C	
0.97	RHOG	ras homolog gene family, member G	
0.95	RAC1	ras-related C3 botulinum toxin substrate 1	
1.06	RAC2	ras-related C3 botulinum toxin substrate 2	
1.09	RRAS	related RAS viral (r-ras) oncogene homolog	
0.90	ARHGAP35	Rho GTPase activating protein 35	
1.04	ROCK1	Rho-associated, coiled-coil containing protein kinase 1	
1.09	ROCK2	Rho-associated, coiled-coil containing protein kinase 2	
Not Detected	RNASE2	ribonuclease, RNase A family, 2	
0.87	RPS6KA1	ribosomal protein S6 kinase, 90kDa, polypeptide 1	
1.05	RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	
0.91	RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5	
1.07	ROBO1	roundabout, axon guidance receptor, homolog 1	
12.47	**S100A12**	**S100 calcium binding protein A12**	**Reached significance**
1.08	S100A8	S100 calcium binding protein A8	
0.98	S100A9	S100 calcium binding protein A9	
1.01	SPP1	secreted phosphoprotein 1	
1.04	SCG2	secretogranin II	
1.03	SELL	selectin L	
1.03	SEMA3A	semaphorin 3A	
0.94	SEMA4D	semaphorin 4D	

Relative Fold Expression Difference	Gene Symbol	Gene Name	Notes
1.04	SEMA7A	semaphorin 7A, GPI membrane anchor	
Not Detected	SAA1	serum amyloid A1	
0.95	SPN	sialophorin	
1.11	SLIT2	slit homolog 2	
0.93	SMAD4	SMAD family member 4	
1.08	SOS1	son of sevenless homolog 1	
1.07	SHH	sonic hedgehog	
1.21	SYK	spleen tyrosine kinase	
0.87	SFN	stratifin	
1.06	SFTPD	surfactant protein D	
0.84	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin a4	
1.12	SDCBP	syndecan binding protein	
1.27	TACR1	tachykinin receptor 1	
1.05	TLN1	talin 1	
1.03	TBP	TATA box binding protein	
0.96	TYMP	thymidine phosphorylase	
1.11	TLR2	toll-like receptor 2	
0.92	TLR4	toll-like receptor 4	
1.09	TGFB1	transforming growth factor, beta 1	
0.95	TGFB2	transforming growth factor, beta 2	
0.95	TGFB3	transforming growth factor, beta 3	
1.07	TRPC6	transient receptor potential cation channel, subfamily C, member 6	
1.01	TREM2	triggering receptor expressed on myeloid cells 2	
1.12	TRIO	triple functional domain (PTPRF interacting)	
1.02	TSC2	tuberous sclerosis 2	
1.04	TNF	tumor necrosis factor	
1.03	TNFSF11	tumor necrosis factor (ligand) superfamily, member 11	
1.07	TNFRSF11A	tumor necrosis factor receptor superfamily, member 11a, NFKB activator	
0.88	TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	
1.04	TYROBP	TYRO protein tyrosine kinase binding protein	
1.08	YWHAB	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta	
0.99	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon	
0.75	YWHAH	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta	
0.91	YWHAQ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta	
1.10	YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta	
0.86	UNC5B	unc-5 homolog B	
0.91	ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2	
1.07	AKT1	v-akt murine thymoma viral oncogene homolog 1	
1.06	VEGFA	vascular endothelial growth factor A	
1.02	VEGFB	vascular endothelial growth factor B	
1.03	VEGFC	vascular endothelial growth factor C	
0.96	VASP	vasodilator-stimulated phosphoprotein	
1.18	VAV2	vav 2 guanine nucleotide exchange factor	
0.96	ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2	
0.98	HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	
0.93	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	
1.07	KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	
0.84	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	
1.15	RALA	v-ral simian leukemia viral oncogene homolog A	
1.18	SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog	
1.07	WNT2	wingless-type MMTV integration site family member 2	
1.05	WNT1	wingless-type MMTV integration site family, member 1	
1.04	WNT11	wingless-type MMTV integration site family, member 11	
1.05	WNT3A	wingless-type MMTV integration site family, member 3A	
0.90	WNT5A	wingless-type MMTV integration site family, member 5A	