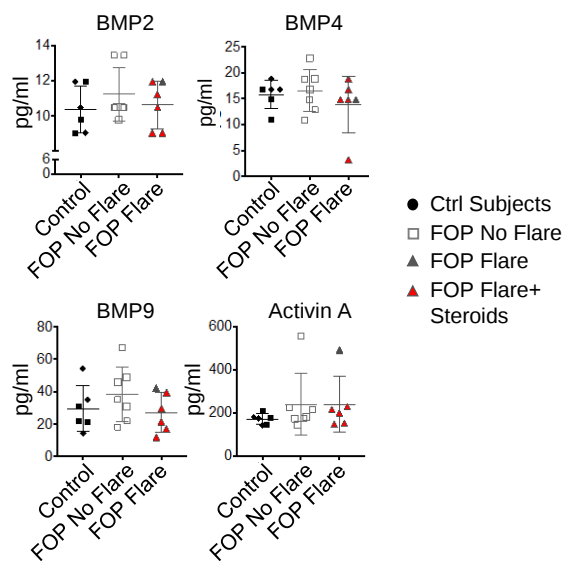
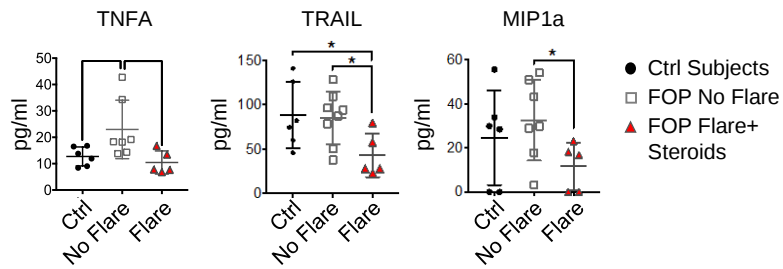
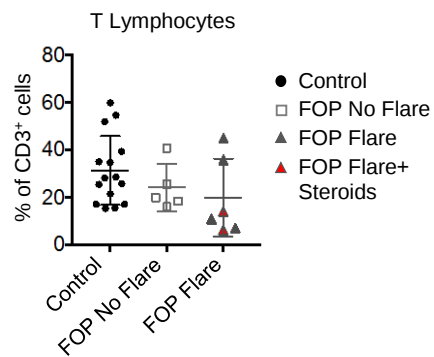




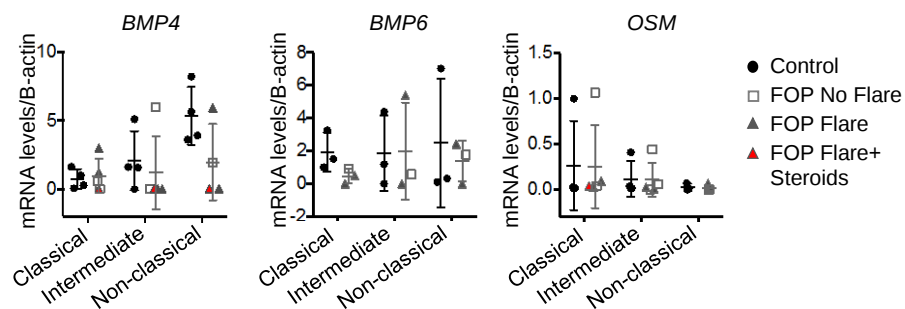
Supplemental Figure 1: No changes in BMPs and Activin A levels in the sera of FOP patients. Multiplex assay for 69 cytokines on serum of control (n=6) and FOP subjects with clinical documented flare (n=6) and FOP subjects without a flare (n=7). Levels of BMP2,4,9 and Activin A were not significantly different in FOP subjects compare to control. Error bars represent mean $\pm$ one SD.



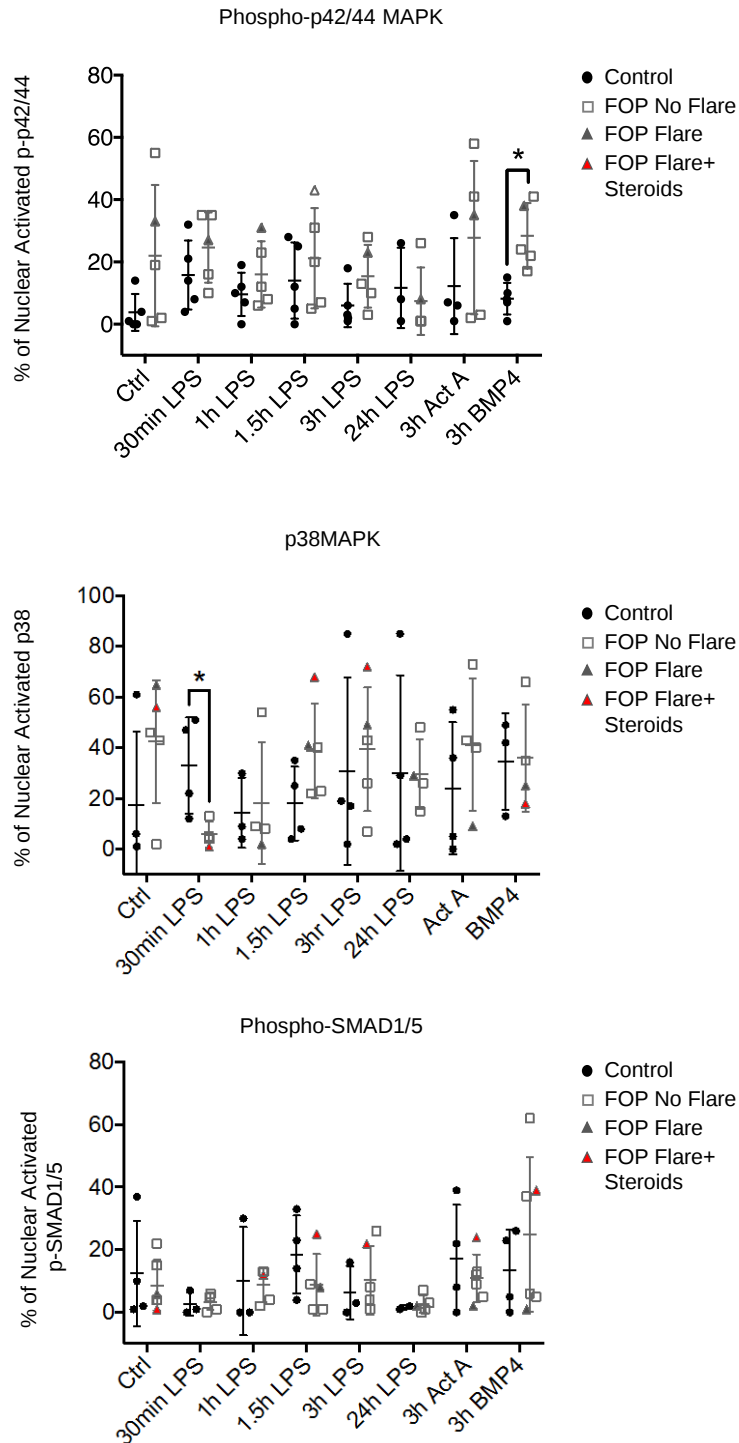
Supplemental Figure 2: Exclusion of the FOP subject having a flare and not taking steroids. Multiplex assay for 69 cytokines on serum of control (n=6) and FOP subjects with clinical documented flare (n=5) and Fop subjects without a flare (n=7). Levels of TNFA, TRAIL (TNF-Related Apoptosis-Inducing Ligand) and MIP1a of FOP No Flare and FOP Flare+Steroids become significantly different with the exclusion of FOP31, the FOP subject having a flare and not under steroid treatment. Error bars represent mean +/- one SD. *, p<0.05, by Student's t-test.



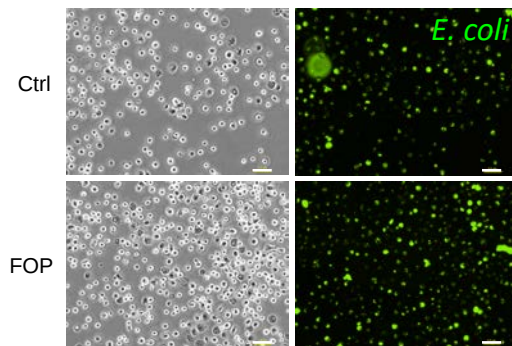
Supplemental Figure 3: No changes in T lymphocytes in FOP subjects. CD3⁺ cells from whole blood were analyzed by flow cytometry (control, n=15; FOP No Flare, n=5; FOP Flare, n=6). Red triangles in the FOP Flare group are FOP subjects taking steroids. Error bars represent mean $\pm$ one SD.



Supplemental Figure 4: Monocyte subsets expressed BMP4 and BMP6. Gene expression analysis of the three monocyte subsets for the BMPs. There were no significant differences between controls and FOP monocytes *OSM* expression. Error bars represent mean $\pm$ one SD of monocytes from control ($n \geq 3$) and FOP ($n \geq 3$) subjects.



Supplemental Figure 5: Time course of LPS-induced phospho-p42/44 MAPK (ERK1/2), p38MAPK, and phospho-SMAD1/5 nuclear translocation. Cells were stimulated with LPS, BMP4 and Activin A. Nuclear translocation percentages were statistically significant for *, $p < 0.05$ by student t test. Error bars represent means $\pm$ one SD of monocytes from control ($n \geq 3$) and FOP ($n \geq 3$) subjects.



Supplemental Figure 6: Control and FOP macrophages phagocytosis activity. FOP macrophages showed similar phagocytosis abilities to control macrophages in phagocytosis assay. Bar represents 0.2 μ m.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl9	54	M						
Ctrl10	45	M						
Ctrl12	44	F						
Ctrl13	36	F						
Ctrl14	36	M						
Ctrl15	50	F						
FOP12	34	M	No flare	n/a	no	Unknown	Unknown	None
FOP14	29	F	No flare	n/a	no	5 days	> 4 months	NSAID
FOP17	9	F	No flare	n/a	no	> 6 months	> 6 months	Albuterol
FOP18	12	F	No flare	n/a	no	1 months	51 days	NSAID
FOP19	38	M	No flare	n/a	no	2 months	2 months	Antihistamine, Oxycodone/APAP, Pseudoephedrine
FOP26	8	M	No flare	n/a	no	> 2 yrs	> 2 yrs	NSAID, Montelukast, Cromolyn
FOP32	9	M	No flare	n/a	no	> 1 yr	> 1 yr	None
FOP14	28	F	d5	L knee	yes	n/a	Unknown	NSAID
FOP17	10	F	d17 and d6	Concomitant neck/L knee	Unknown	n/a	2 months	NSAID
FOP20	10	F	d4	R hip/thigh	yes	n/a	> 4 months	Cetirizine, Prednisone
FOP28	7	F	d12	L shoulder	yes	n/a	57 days	NSAID, Imatinib, Ketoprofen gel
FOP30	9	M	d5	L lower back	yes	n/a	> 7 months	NSAID, Montelukast
FOP31	5	M	d9	L lower back	no	5 days	> 6 months	NSAID, Methylphenidate

Supplemental Table 1: Details of the clinical samples for the serum analysis.

Samples	Age	Gender	Flare	WBC Count/L	RBC Count/L	Hemoglobin (HGB) g/dL	Hematocrit %	MCV fL	MCH pg	MCHC g/dL	Platelet Count/L
FOP3	12	F	No	8.3	4.23	10.9	34	80	25.7	32	210
FOP7	26	F	No	9.1	3.79	12.3	37.1	98	32.5	33.2	270
FOP14	27	F	No	7.1	4.62	14.1	42.2	91.3	30.6	33.5	165
FOP35	18	F	No	10.1	4.54	13.7	39.6	87			211
FOP31	2	M	No	9.2	4.58	11.4	35.2	76.9	24.9	32.4	353
FOP47	12	F	No	7.2	4.52	12.9	38.5	85	28.5	33.5	224
Mean				8.5	4.4	12.6	37.8	86.4	28.4	32.9	238.8
Stdev				1.2	0.3	1.3	3.0	7.6	3.2	0.7	65.2
n				6	6	6	6	6	5	5	6
Ref Range				4.5 - 15.5 x10E9/L	4.0 - 5.0 x10E12/L	11.6 - 15.5 g/dL	35 - 45 %	77 - 95 fL	25 - 33 pg	31 - 36 g/dL	140 - 450 x10E9/L

Supplemental Table 2: Blood cell counts from FOP subjects.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl12	44	F						
Ctrl16	63	M						
Ctrl28	40	M						
Ctrl29	38	F						
Ctrl33	57	M						
Ctrl34	60	F						
Ctrl43	51	F						
Ctrl46	>30	M						
Ctrl47	34	F						
Ctrl50	68	F						
Ctrl51	23	M						
Ctrl52	43	M						
Ctrl55	15	M						
Ctrl56	34	F						
Ctrl8	51	F						
FOP24	13	M	No Flare	n/a	no	> 1 yr	> 6 months Minor flares	Cetirizine
FOP27	9	M	No Flare	n/a	no	2 months	> 5 yrs	Cromolyn, Montelukast, Ranitidine
FOP35	18	F	No Flare	n/a	no	unknown	2 months	NSAID
FOP36	19	F	No Flare	n/a	no	2 months	> 7 months	APAP, Aquaphor, Fexofenadine, Topical steroids for skin
FOP38	19	F	No Flare	n/a	no	no	1 yr	None
FOP13	33	F	d1	R thigh	no	2 days	6 months	Baclofen, Benadryl, Tramadol, Amlodipine, Atorvastatin, NSAID
FOP16	12	F	d1	L knee	yes	1 day	7 months	NSAID, Lorazepam, Prednisone
FOP30	10	M	d2	L hip	yes	1 day	> 9 months	NSAID, Montelukast
FOP34	11	F	d10	R shoulder	no	3 days	3 months	NSAID, Montelukast
FOP39	39	M	d1	L knee/calf	no	none	unknown	Unknown
FOP39	39	M	d1	L knee to calf	no	> 3 months	> 3 months	APAP, Lansoprazole, Hydrocodone

Supplemental Table 3: Details of the clinical samples for FACS analysis of whole blood.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl15	51	F						
Ctrl16	63	M						
Ctrl50	68	F						
Ctrl61	15	M						
FOP27	9	M	No Flare	n/a	no	2 months	>5 yrs	Cromolyn, Montelukast, Ranitidine
FOP47	13	F	No Flare	n/a	no	2 yrs	2 yrs	Albuterol
FOP16	12	F	d1	L knee	yes	1 day	7 months	NSAID, Lorazepam, Prednisone
FOP19	39	M	d1	R elbow	no	4 days	33 days	Benadryl, Loperamide, Oxy/APAP, Sudafed, ketoprofen 1% gel
FOP39	39	M	d1	L knee/calf	no	unknown	unknown	Unknown

Supplemental Table 4: Details of the clinical samples for gene expression analysis of the sorted monocyte subsets.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl21	50	M						
Ctrl22	50	F						
Ctrl24	46	F						
Ctrl26	48	F						
Ctrl29	39	F						
Ctrl34	60	M						
Ctrl38	54	M						
Ctrl40	42	M						
Ctrl41	37	F						
Ctrl44	49	M						
Ctrl45	45	F						
Ctrl56	34	F						
Ctrl55	15	M						
Ctrl57	49	F						
Ctrl61	15	M						
Ctrl64	38	F						
FOP3	17	M	No Flare	n/a	no	> 6 months asthma flare	> 1.5 yr	Pulmocort, NSAID, Montelukast
FOP28	8	F	No Flare	n/a	no	2.5 months	> 10 months	Aquaphor, NSAID
FOP29	10	M	No Flare	n/a	no	2 months	2 months	Montelukast, Cromolyn, Ranitidine
FOP31	6	M	No Flare	n/a	no	3 months	36 days	Imatinib, Unclear on other medication
FOP37	14	F	No Flare	n/a	no	Unknown	1 month	Iron, Montelukast, Cromolyn, Oxycodone
FOP47	13	F	No Flare	n/a	no	2 yrs	2 yrs	Albuterol
FOP13	33	F	d1	R thigh	no	10 days	7 months	Baclofen, Benadryl, Tramadol, Amlodipine, Atorvastatin, NSAID
FOP19	39	M	d1	R elbow	no	4 days	33 days	Benadryl, Loperamide, Oxy/APAP, Sudafed, ketoprofen 1% gel
FOP25	11	F	d1	R thigh, back	no	1 month	32 days	Cetirizine, Montelukast, Ranitidine
FOP29	10	M	d1	Back	no	no	4 months	Montelukast, Cromolyn, Ranitidine
FOP34	11	F	d1	R lower back	no	3 days	3 months	NSAID, Montelukast
FOP39	39	M	d1	L knee to calf	no	> 3 months	> 3 months	APAP, Lansoprazole, Hydrocodone

Supplemental Table 5: Details of the clinical samples for gene expression analysis of CD14+ monocytes stimulation.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl33	58	F						
Ctrl34	61	M						
Ctrl50	68	F						
Ctrl55	15	M						
Ctrl57	49	F						
FOP13	33	F	d1	R foot	no	4 days	6 months	Baclofen, Benadryl, Tramadol, Amlodipine, Atorvastatin, NSAID
FOP19	39	M	d1	R elbow	no	4 days	33 days	Benadryl, Loperamide, Oxy/APAP, Sudafed, ketoprofen 1% gel
FOP25	11	F	d1	R thigh, back	no	1 month	32 days	Cetirizine, Montelukast, Ranitidine
FOP39	39	M	d1	L knee to calf	no	> 3 months	> 3 months	APAP, Lansoprazole, Hydrocodone

Supplemental Table 6: Details of the clinical samples for the multiplex cytokine analysis of LPS stimulated monocytes.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl32	39	M						
Ctrl43	53	F						
Ctrl57	50	F						
Ctrl57	50	F						
Ctrl70	11	M						
Ctrl103	39	M						
Ctrl110	51	F						
Ctrl111	14	F						
Ctrl114	65	F						
Ctrl115	31	F						
FOP36	20	F	No Flare	n/a	no	> 9 months	> 9 months	NSAID
FOP37	15	F	No Flare	n/a	no	4 months	4 months	Montelukast, Cromolyn
FOP31	7	M	No Flare	n/a	no	10 months	> 2 years	Methylphenidate HCL
FOP39	40	M	No Flare	n/a	no	2 months	2 months	NSAID, Montelukast, Fentanyl, Pramipexole, Humalog
FOP43	9	M	No Flare	n/a	no	unknown	1 month	Celebrex, Cromolyn, Montelukast,
FOP65	20	F	No Flare	n/a	no	unknown	> 6 year	NSAID, Cromolyn, Ondansetron, Naltrexone, Montelukast
FOP68	14	F	No Flare	n/a	no	unknown	> 6 year	Antibiotics, NSAID
FOP25	12	M	d3	R Groin/ Hip	yes	3 days	> 9 months	None
FOP25	12	M	3 months	R Groin/ Hip	no	10 days	> 9 months	NSAID, Oxycodone, Baclofen, Ranitidine, Hydroxyzine, Mupirocin

Supplemental Table 7: Details of the clinical samples used for western blot analysis of primary monocytes.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl5	47	M						
Ctrl15	52	F						
Ctrl26	49	F						
Ctrl44	50	M						
Ctrl45	45	F						
Ctrl80	70	M						
Ctrl100	16	F						
Ctrl102	42	F						
FOP29	11	M	No Flare	n/a	no	1.5 month	1 month	NSAID, Montelukast, Ranitidine
FOP32	11	M	No Flare	n/a	no	1.5 year	6 months	None/Unknown
FOP37	15	F	No Flare	n/a	no	2 months	> 3 months	NSAID, Montelukast, Cromolyn, Ketoprofen gel, Oxycodone
FOP19	40	M	No Flare	n/a	no	7 months	7 months	Oxycodone, Benadryl, Sudafed, Imadium
FOP24	14	M	d14	R knee	yes	1 day	> 1 year	Meloxicam, Prednisone, 10% ketophen gel, prevacid, reactin, ventolin, NSAID
FOP24	15	M	12 weeks	R knee	no	4 months	> 1 year	Sterazin, 10% ketophen gel

Supplemental Table 8: Details of the clinical samples used for immunofluorescence staining and quantification.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl33	57	M						
Ctrl34	60	F						
Ctrl44	49	M						
Ctrl45	45	F						
Ctrl50	68	F						
Ctrl11	43	F						
Ctrl57	49	F						
Ctrl26	47	F						
FOP35	18	F	No Flare	n/a	no	unknown	> 2 months	Celecoxib
FOP25	12	M	3 months	R groin/ Hip	no	10 days	> 9 months	NSAID, Oxycodone, Baclofen, Ranitidine, Hydroxyzine, Mupirocin
FOP29	10	M	d1	Back	no	no	4 months	Montelukast, Cromolyn, Ranitidine
FOP19	39	M	d1	R elbow	no	4 days	33 days	Benadryl, Loperamide, Oxy/APAP, Sudafed, ketoprofen 1% gel
FOP13	33	F	d1	R thigh	no	10 days	7 months	Baclofen, Benadryl, Tramadol, Amlodipine, Atorvastatin, NSAID

Supplemental Table 9: Details of the clinical samples for the multiplex cytokine analysis of LPS stimulated M1/M2 macrophages.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl33	57	M						
Ctrl34	60	F						
Ctrl44	49	M						
Ctrl45	45	F						
Ctrl50	68	F						
Ctrl11	43	F						
Ctrl57	49	F						
Ctrl33	57	M						
FOP35	18	F	No Flare	n/a	no	unknown	> 2 months	Celecoxib
FOP25	12	M	3 months	R groin/ Hip	no	10 days	> 9 months	NSAID, Oxycodone, Baclofen, Ranitidine, Hydroxyzine, Mupirocin
FOP29	10	M	d1	Back	no	no	4 months	Montelukast, Cromolyn, Ranitidine
FOP13	33	F	d1	R foot	no	4 days	6 months	Baclofen, Benadryl, Tramadol, Amlodipine, Atorvastatin, NSAID
FOP13	33	F	d1	R thigh	no	10 days	7 months	Baclofen, Benadryl, Tramadol, Amlodipine, Atorvastatin, NSAID

Supplemental Table 10: Details of the clinical samples used for the TGFB analysis of LPS stimulated M2 macrophages.

Samples	Age	Gender	Day of flare ongoing	Flare site	Steroids	Last steroid dose before blood draw	Previous Flare	Other medications
Ctrl15	52	F						
Ctrl16	64	M						
Ctrl26	49	F						
Ctrl56	35	F						
Ctrl52	43	M						
Ctrl29	38	F						
Ctrl43	51	F						
Ctrl49	24	F						
FOP23	17	F	No Flare	n/a	no	>7 months	>7 months	Amoxicillin, Clindamycin phosphate 1% swab, Montelukast
FOP27	10	M	No Flare	n/a	no	>6 months	>6 months	NSAID, Montelukast, Cromolyn
FOP29	11	M	No Flare	n/a	no	>1 month	>1 month	NSAID, Montelukast, Ranitidine
FOP38	19	F	No Flare	n/a	no	no	1 yr	None
FOP24	14	M	No Flare	n/a	no	> 1 yr	> 6 months Minor flares	Cetirizine
FOP38	19	F	No Flare	n/a	no	no	1 yr	None
FOP30	10	M	d2	L hip	yes	d6	8 months	None

Supplemental Table 11: Details of the clinical samples used for protein quantification of "M1" and "M2" macrophages.

Name	Company	Catalog number	Conjugate	Clone	Dilution
CD3	Biolegend	300310	PE-Cyanine5	HIT3a	1:100
CD3	eBioscience	17-0037-42	APC	APC	1:100
CD11b	eBioscience	11-0118-42	FITC	ICRF44	1:100
CD14	eBioscience	25-0149-42	PE-Cyanine7	61D3	1:100
CD16	eBioscience	11-0168-42	FITC	eBioCB16	1;100
CD19	eBioscience	15-0199-42	PE-Cyanine5	H1B19	1:100
CD45	eBioscience	12-9459-42	PE	2D1	1:200
CD56	eBioscience	35-0567-41	PE-Cyanine5	CMSSB	1:100
CD66b	Biolegend	B215969	Biotin	G10F5	1:100
CD163	eBioscience	12-1639-42	PE	eBioGHI/61	1:100
CD206	eBioscience	17-2069-42	APC	19.2	1:100
HLA-DR	eBioscience	17-9956-42	APC	LN3	1:100
PE/CY5 Streptavidin	Biolegend	405205			1:1000
CD14 Microbeads	Miltenyi	130-059-201			20ul/10 ⁷ cells
b-actin	ThermoFisher	MA5-15739		BA3R	1:1000
p-Smad1/5/9	CST	13820		D5B10	1:1000
p-Smad1/5	CST	9516		41D10	1:1000
p-Smad2	CST	3108		138D4	1:1000
p-38MAPK	CST	9211			1:1000
p-NFkBp65	CST	3031			1:1000
NFkBp65	CST	6956		L8F6	1:1000
p-p44/42 MAPK (Erk1/2)	CST	4370		D13.14.4E	1:400
p-TAK1	CST	9339			1:1000
TAK1	NovusBio	NB100-56363			1:2000
Anti-Rabbit HRP	CST	7074P2			1:5000
Anti-Rabbit HRP	NOVEX	A24500			1:5000
Goat anti-rabbit 568	ThermoFisher	A11034			1:1000

Supplemental Table 12: List of antibodies used for FACS, western blot and immunofluorescence.

Gene	Assay ID
ACVR1	Hs00153836_m1
AP1	Hs00759776_s1
B-Actin	Hs01060665_g1
BMP2	Hs00154192_m1
BMP4	Hs00370078_m1
BMP6	Hs01099594_m1
BMP7	Hs00233476_m1
CCL5	Hs00982282_m1
CCR2	Hs00704702_s1
CCR7	Hs01013469_m1
CD14	Hs02621496_s
CD163	Hs00174705_m1
CREB1	Hs00231713_m1
CXCL10	Hs01124252_g1
CXCL11	Hs04187682_g1
CXCR4	Hs00607978_s1
INHBA	Hs01081598_m1
IRF3	Hs01547283_m1
TGFB	Hs00820148_g1
TLR2	Hs01872448_s1
TLR4	Hs00152939_s1
Oncostatin M	Hs00384276_m1

Supplemental Table 13: Taqman Primers-gene expression.