Supplementary Figure 1

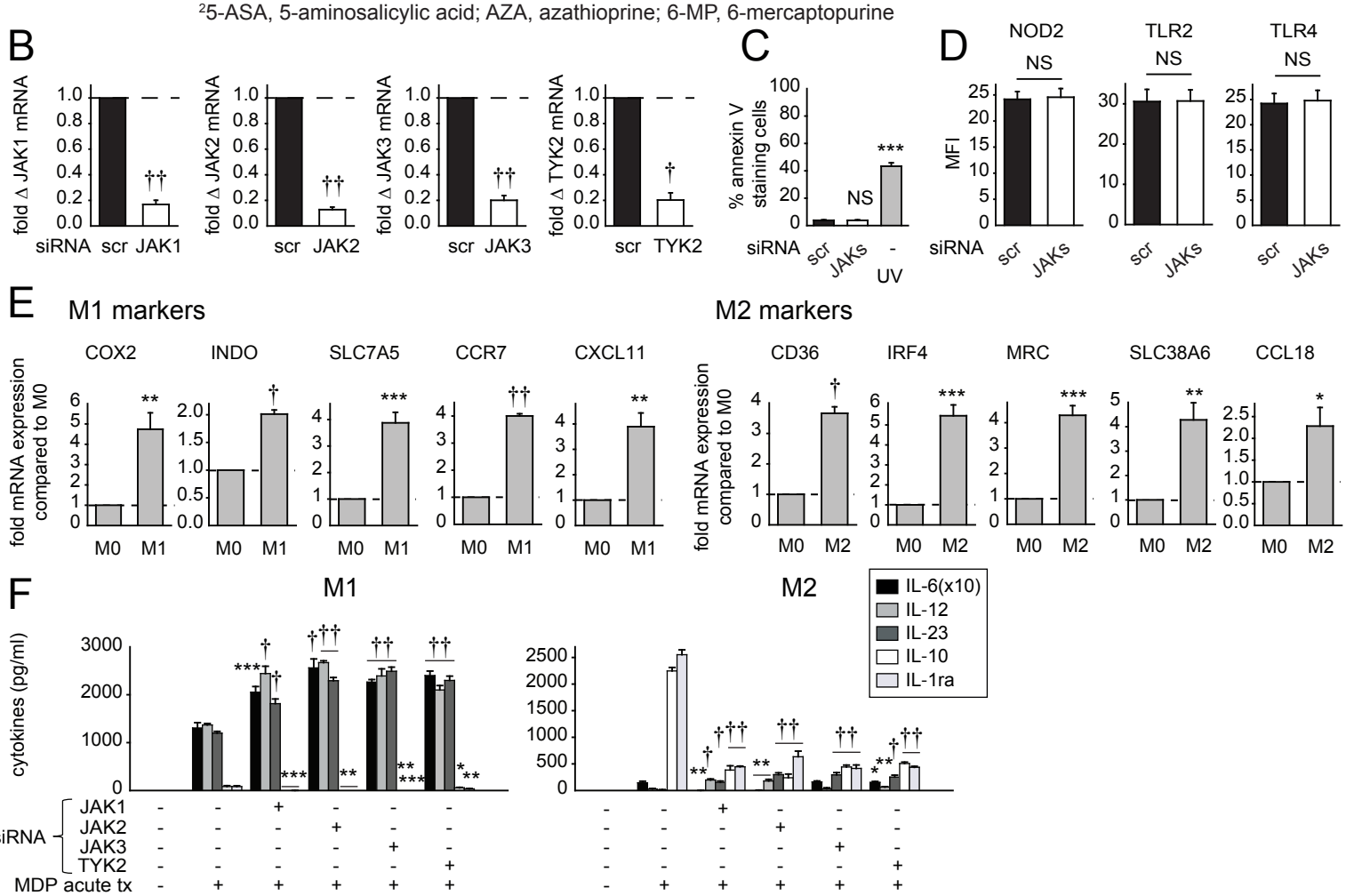
A

Description of Ulcerative Colitis Patients

Patient Characteristics	Genotype			p value[1]
	CC (n=8)	CA (n=8)	AA (n=8)	
Age (years±SEM)	38.25±3.62	41.50±6.29	44.12±6.68	NS
Gender(Male, %)	62.5%	50.0%	75.0%	NS
Disease duration (years±SEM)	6.88±2.15	7.88±1.98	10.25±4.60	NS
Medications (% patients)[2]				
5-ASA	75.0%	62.5%	62.5%	NS
AZA/6-MP	37.5%	37.5%	25.0%	NS
Steroids	0%	12.5%	12.5%	NS
anti-TNFα	12.5%	12.5%	12.5%	NS
Antibiotics	12.5%	0%	0%	NS

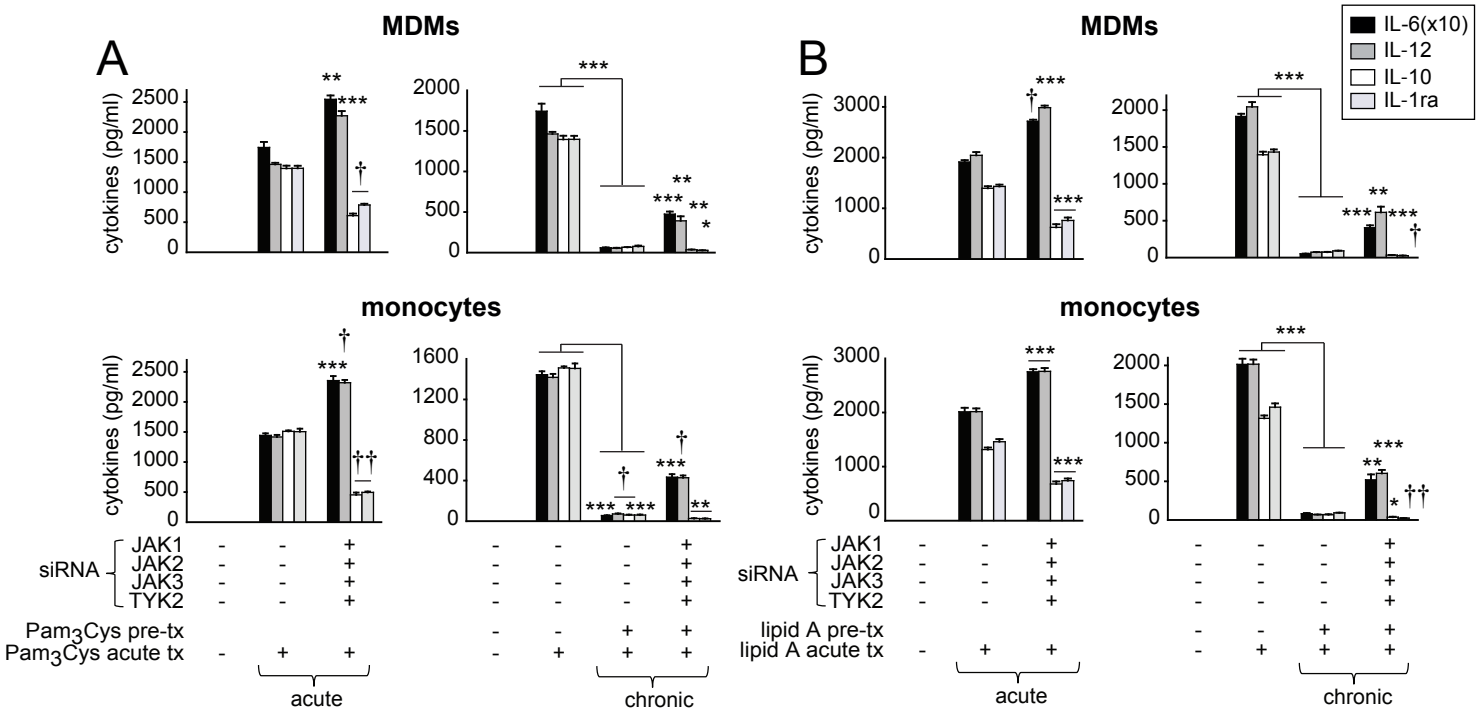
[1]NS, not significant.

[2]5-ASA, 5-aminosalicylic acid; AZA, azathioprine; 6-MP, 6-mercaptopurine



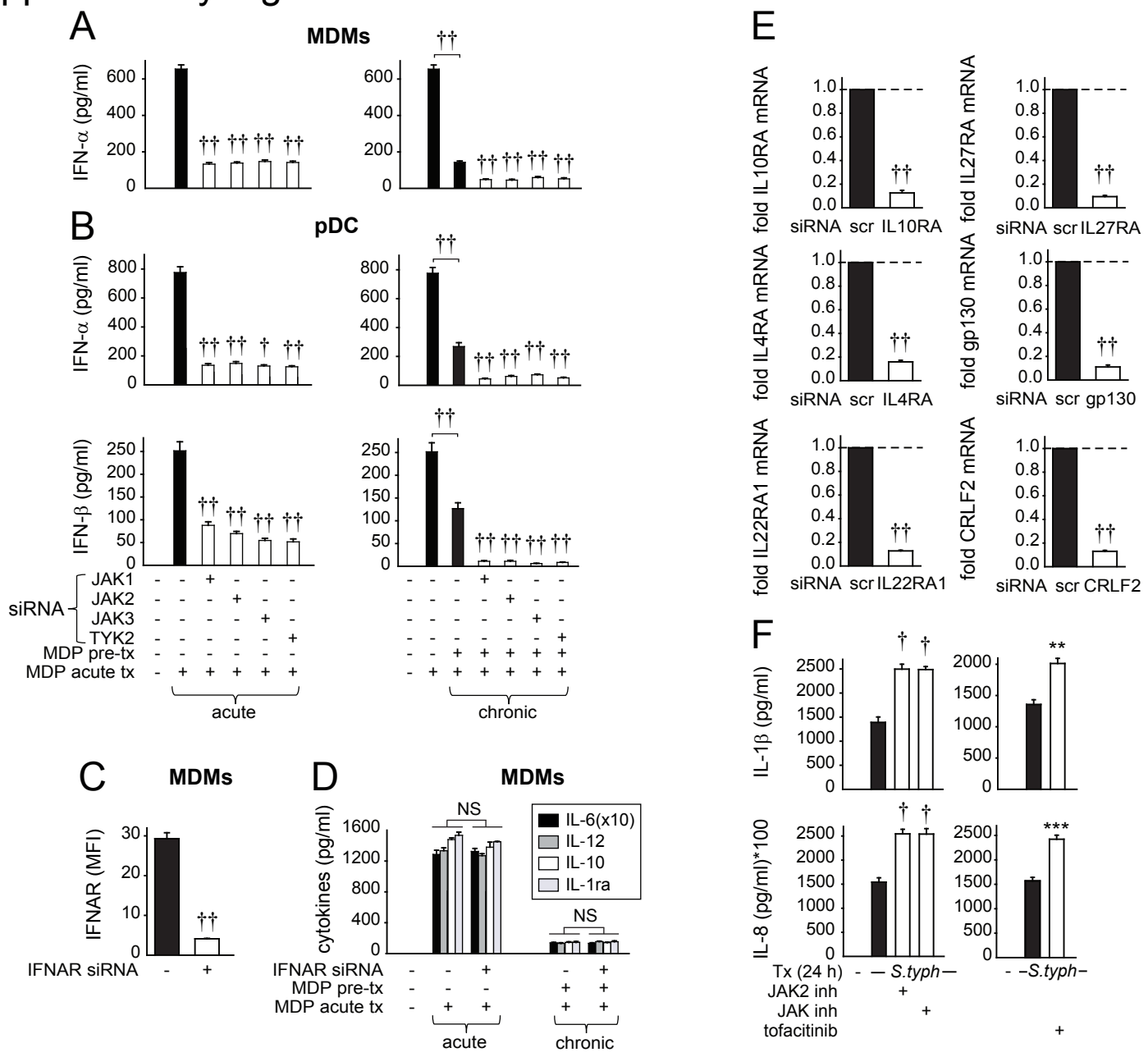
Supplementary Figure 1. JAK signaling regulates cytokine secretion following NOD2 stimulation in M1 and M2 macrophages. **(A)** Shown is a table describing the demographics and disease characteristics of the ulcerative colitis patients used in this study. **(B)** MDMs (n=4 donors) were transfected with the indicated siRNA. Fold mRNA expression compared to scrambled siRNA-transfected cells (represented by the dotted line at 1). **(C)** MDMs (n=4 donors) were transfected with JAK1, JAK2, JAK3 and TYK2 siRNA in combination, then stained with annexin V (eBiosciences). Shown is percent annexin V^+ cells as a measure of cell death. UV stimulation at 50-100 J/m^2 is shown as a positive control. **(D)** MDMs (n=8 donors) were transfected with JAK1, JAK2, JAK3 and TYK2 siRNA in combination. Summarized flow cytometry data for NOD2 (Cayman Biochemicals, Ann Arbor, MI), TLR2 or TLR4 (BD Biosciences) protein expression. **(E)** MDMs (n=8 donors) were left non-polarized (M0) or polarized for 24h with 100ng/ml LPS (Sigma-Aldrich, St. Louis, MO) and 20ng/ml IFN-γ (R&D Systems, Minneapolis, MI) (M1 polarization), or 20ng/ml IL-4 (R&D Systems) (M2 polarization). Summarized data are represented as fold mRNA induction of M1 or M2 markers normalized to non-polarized (M0) MDMs for each individual (represented by the dotted line at 1). **(F)** MDMs were polarized for 24h as in 'E', then transfected with the indicated siRNAs for 24h. Cells were then treated with 100μg/ml MDP for 24h. Cytokine secretion + SEM for n=8 donors. Significance is compared to scrambled siRNA-transfected, acute MDP-treated cells. Tx, treatment; scr, scrambled; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$. **(C,F)** Bonferroni-Holm correction was used for multiple comparisons.

Supplementary Figure 2



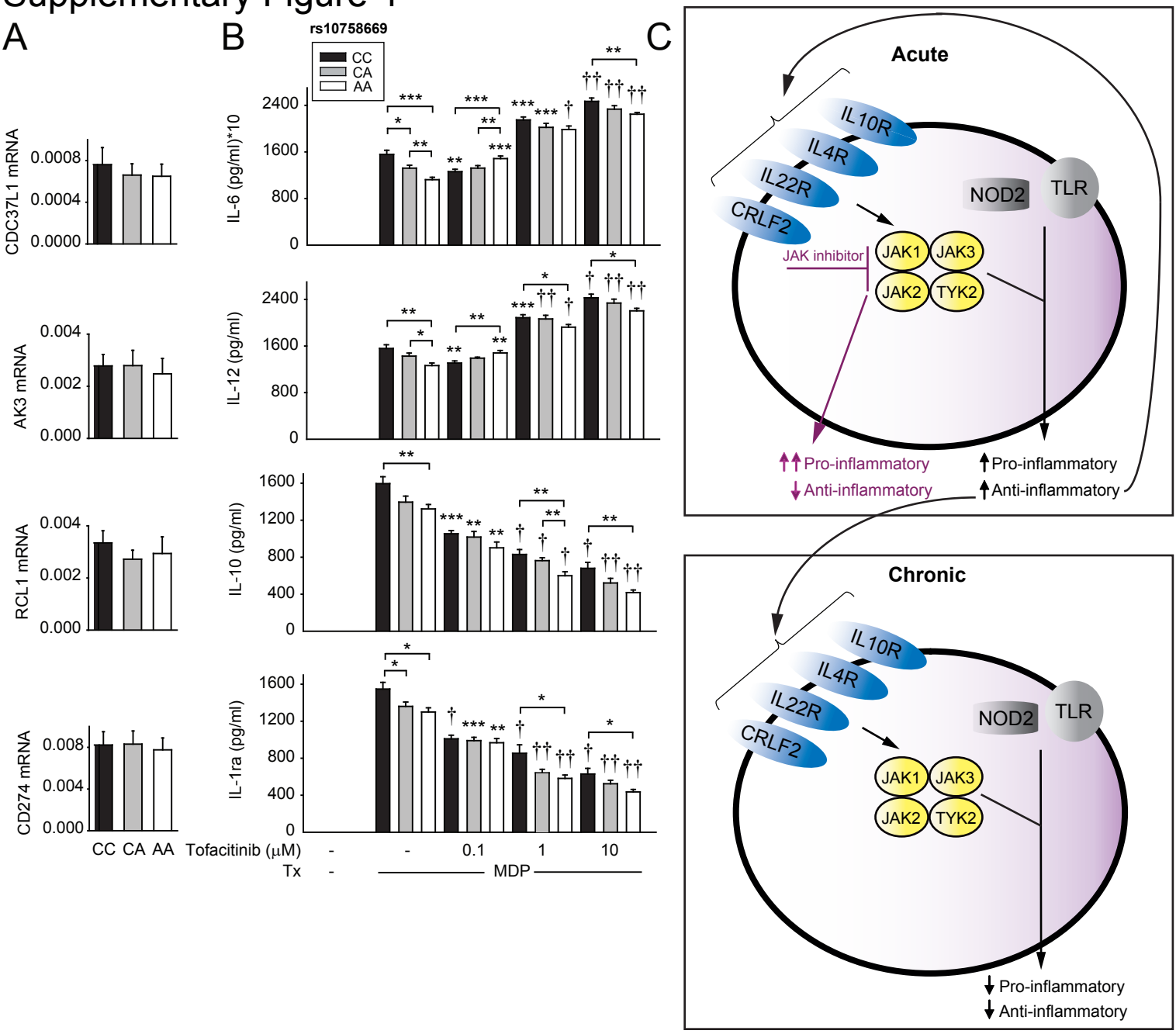
Supplementary Figure 2. JAK pathway signaling differentially regulates pro-inflammatory and anti-inflammatory cytokine secretion following TLR2 and TLR4 stimulation. MDMs (n=4 donors, *top*) or monocytes (n=4 donors, *bottom*) were transfected with the indicated siRNAs for 24h and left untreated (acute) or pre-treated with **(A)** 10μg/ml Pam3Cys (TLR2 ligand) or **(B)** 0.1μg/ml lipid A (TLR4 ligand) for 48h (chronic). Cells were then re-treated with the same respective TLR ligand for 24h. Cytokine secretion + SEM. Significance is shown compared to scrambled siRNA-transfected, acute TLR2 or TLR4-treated cells (*left*, acute), or to scrambled siRNA-transfected, TLR2 or TLR4 pre-treated and TLR2 or TLR4 retreated cells (*right*, chronic), or as indicated. Tx, treatment. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$. Bonferroni-Holm correction was used for multiple comparisons.

Supplementary Figure 3



Supplementary Figure 3. Autocrine type I IFN secretion is not sufficient to regulate acute or chronic NOD2-induced secretion of additional cytokines in human MDMs. MDMs were **(A)** left unpolarized or **(B)** differentiated for 24h with 100ng/ml FLT-3 ligand (Peprotech, Rocky Hill, NJ) (pDC differentiation) and then transfected with the indicated siRNAs for 24h. Cells were then left untreated (for acute) or pre-treated with 100μg/ml MDP for 48h (for chronic), and then treated with 100μg/ml MDP for 24h. Type I IFN secretion from MDMs (n=12 donors, similar results were seen in an additional n=8) and pDCs (n=8 donors, similar results were seen in an additional n=4) in supernatants. Significance is shown compared to scrambled siRNA-transfected, acute MDP-treated cells (*left*, acute), or scrambled siRNA-transfected, MDP-pre-treated and MDP-retreated cells (*right*, chronic), or as indicated. **(C)** MDMs (n=4) were transfected with scrambled or IFNAR siRNA. Summarized flow cytometry data for IFNAR MFI (BD Bioscience). **(D)** MDMs (n=8) were transfected with IFNAR siRNA for 24h and left untreated (acute) or pre-treated with 100μg/ml MDP for 48h (chronic). Cells were then treated with 100μg/ml MDP for 24h. Cytokine secretion + SEM. **(E)** MDMs (n=4 donors) were transfected with scrambled, IL-10RA, IL-4RA, IL-22RA1, IL-27RA, gp130 (required for EBI3 signaling) or CRLF2 (TSLP receptor) siRNA. Fold mRNA expression compared to scrambled siRNA-transfected cells (represented by the dotted line at 1). **(F)** Peripheral MDMs were preincubated with 10μM SD-1029 (JAK2 inhibitor) (n=6 donors), JAK inhibitor I (JAK1, JAK2 and JAK3 inhibitor) (n=6 donors), or tofacitinib (n=5 donors) and then co-cultured with *S. typhimurium* (*S. typh*) at MOI 10:1 for 24h. Cytokine secretion+SEM. Tx, treatment; NS, not significant. **, p<0.01; ***, p<0.001; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$. **(A,B,F)** Bonferroni-Holm correction was used for multiple comparisons.

Supplementary Figure 4



Supplementary Figure 4. Expression of additional genes in the *JAK2* region is not modulated by rs10758669 genotype in MDMs and PRR-induced reciprocal regulation of pro- and anti-inflammatory cytokines depends on the tofacitinib dose. (A) MDMs from rs10758669 CC, CA or AA healthy control carriers were assessed for expression of the indicated genes (change in CT values normalized to GAPDH and represented as a linear scale) (n=10 donors/genotype). **(B)** MDMs from rs10758669 CC, CA or AA healthy control carriers (n=8 donors/ genotype) were pre-incubated with tofacitinib at the indicated doses and then treated with 100μg/ml MDP for 24h. Cytokine secretion + SEM. Significance is compared to MDP-treated cells of the same genotype or as indicated. Tx, treatment. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; †, $p<1x10^{-4}$; ††, $p<1x10^{-5}$. **(C) Model for JAK-dependent regulation of PRR-induced cytokines**. (*Acute*) Acute PRR stimulation in MDMs induces secretion of both pro- and anti-inflammatory cytokines. Secretion of the inhibitory mediators IL-10, IL-4, IL-22 and TSLP feedback in an autocrine/paracrine loop to activate JAK1, JAK2, JAK3 and TYK2, which then amplifies anti-inflammatory cytokine secretion in a feedforward manner. The threshold of JAK expression and signaling determines how pro-inflammatory cytokines are regulated. JAK signaling below this threshold, as can be achieved when using JAK inhibitors, results in decreased anti-inflammatory and increased pro-inflammatory cytokine secretion. Consequently, MDMs carrying the rs10758669 CC IBD-risk variant in the *JAK2* region, which show increased JAK2 expression and increased PRR-induced JAK2 activation and cytokine secretion, also show less sensitivity to JAK inhibitor doses than the lower JAK2 expressing non-risk *JAK2* genotype carriers with respect to the 'switch' to increase pro-inflammatory cytokines. (*Chronic*) With chronic PRR stimulation, as is observed with prolonged residence of myeloid cells in intestinal tissues, cytokine secretion from myeloid cells is decreased. JAK signaling, through the regulation of the inhibitory mediators IL-10, IL-4, IL-22 and TSLP, is required for the optimal decrease in pro-inflammatory cytokines when these chronic PRR-exposed myeloid cells are restimulated with PRR ligands or pathogenic bacteria. Consequently, JAK inhibition in intestinal myeloid cells results in increased pro-inflammatory cytokines upon exposure to live pathogenic bacteria.