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Supplementary Materials for

Human interleukin-4–treated regulatory macrophages promote epithelial wound healing and reduce colitis in a mouse model

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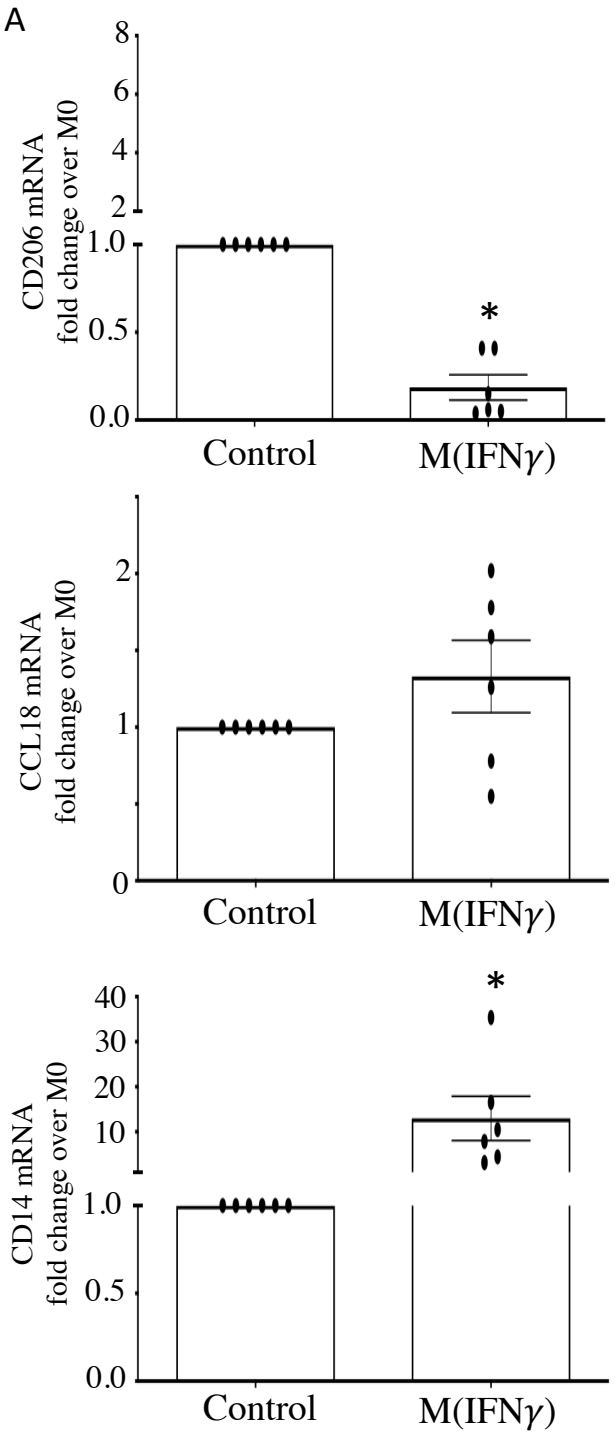
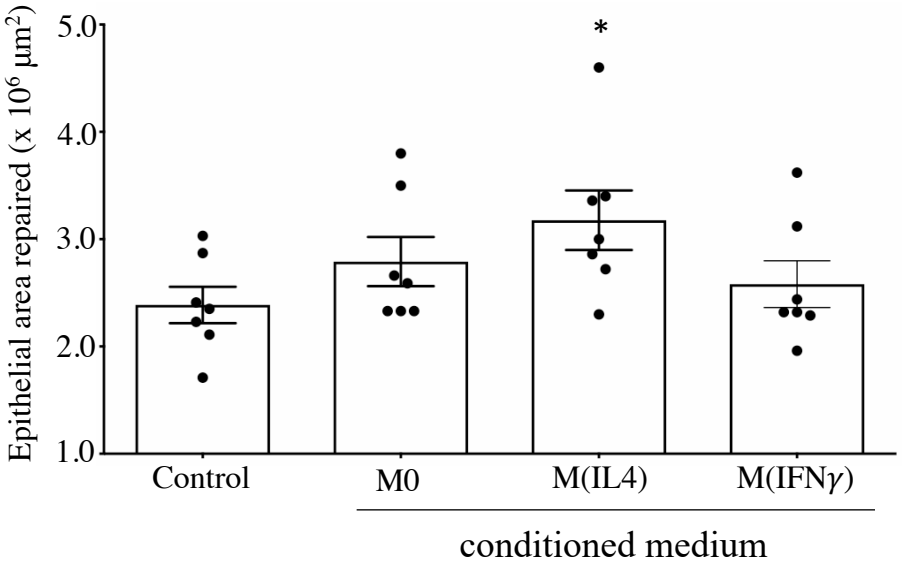
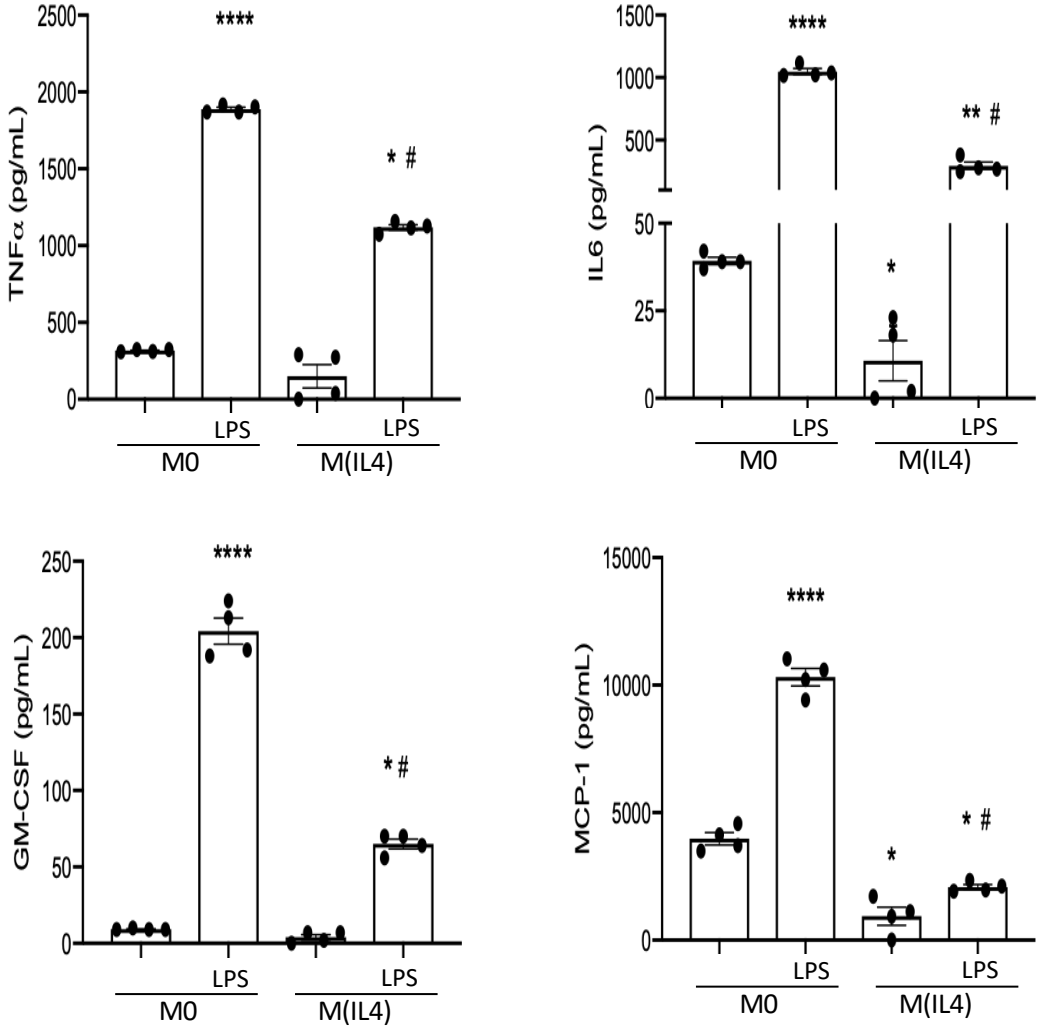

A
CD206 mRNA
fold change over M0
8
6
4
2
1.0
0.5
0.0
*
Control
M(IFNγ)
CCL18 mRNA
fold change over M0
2
1
0
Control
M(IFNγ)
CD14 mRNA
fold change over M0
40
30
20
10
1.0
0.5
0.0
*
Control
M(IFNγ)

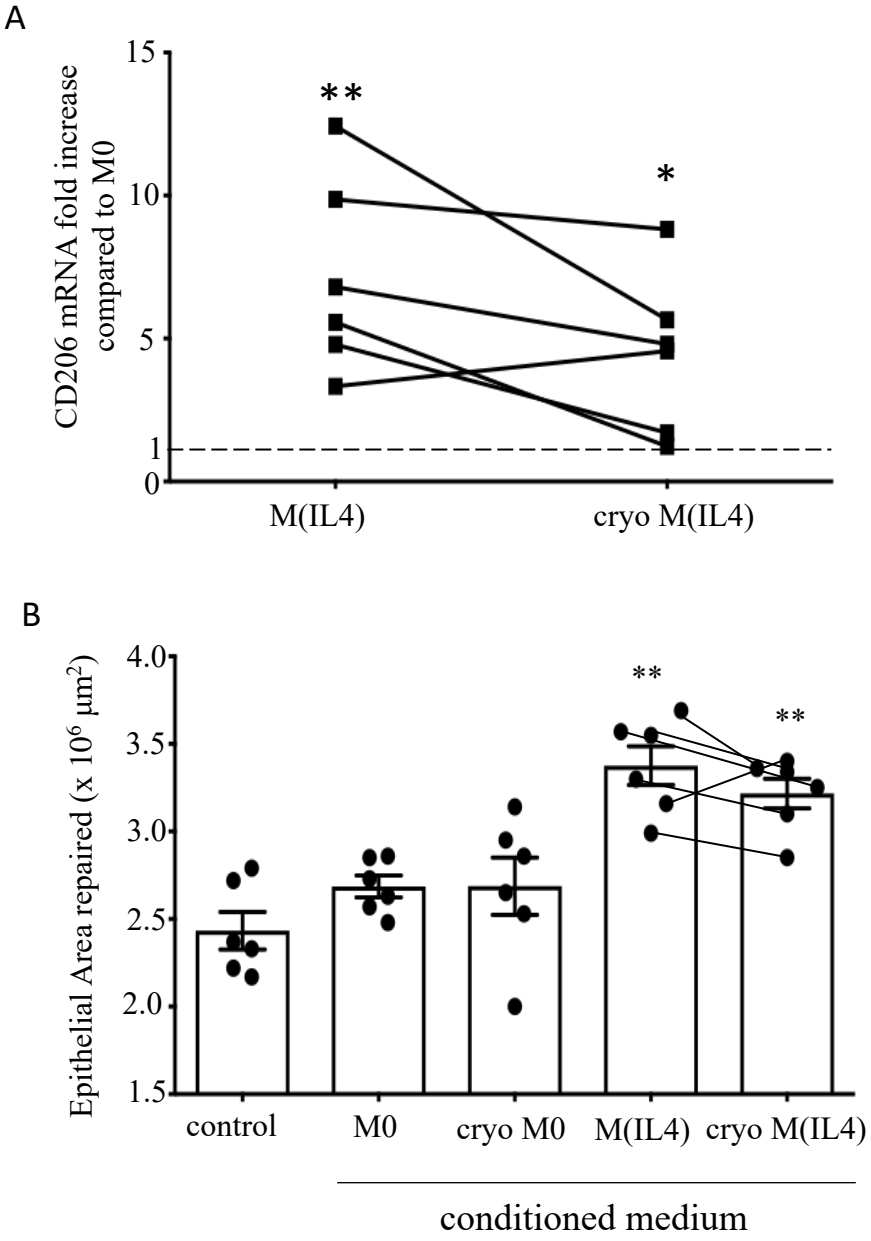
B



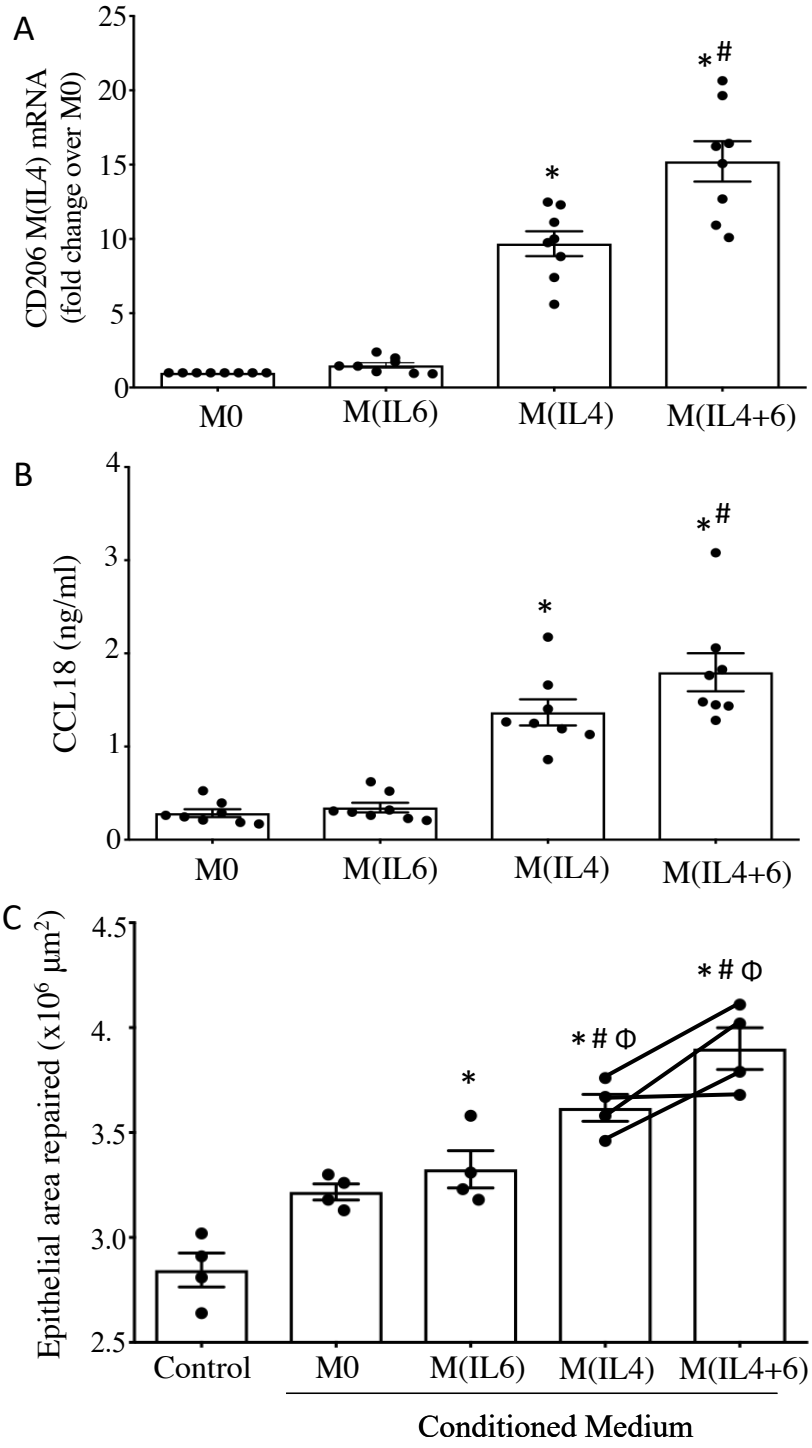
Supplementary Figure 1: Human IFNγ stimulated blood-derived macrophages do not express M(IL4) canonical markers or promote wound healing. Human blood-derived macrophages were stimulated with IFNγ (10 ng/ml; 48 h) and expression of (A) CD206, CCL18, and CD14 mRNA was measured as fold-increase compared to the unstimulated (M0) control (normalized to 1, see methods). Data are mean ± SEM; n=5; *, $p<0.05$ compared to M(0). (B) Confluent monolayers of the human Caco2 epithelial cell line were mechanically wounded, then received a 50% conditioned medium from M(0), M(IL4) of M(IFNγ). Forty-eight hours later the total area of epithelium from the wound margin was measured. Data are mean ± SEM; *, $p<0.05$ compared to control (culture medium only) and M(IL4) CM respectively by ANOVA followed by Tukey's test.



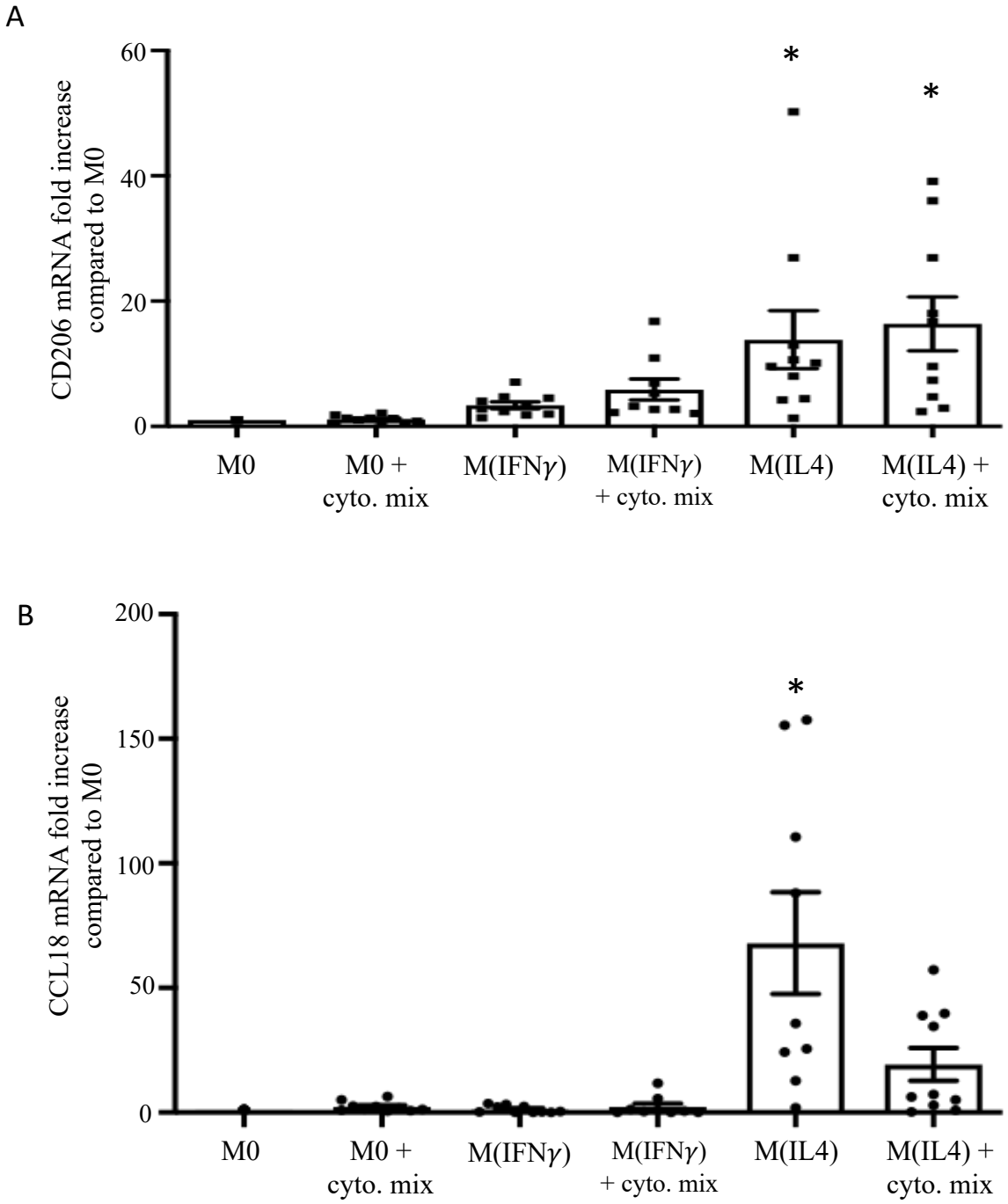
Supplementary Figure 2: Human M(IL4s) are hypo-responsive to LPS. Human blood-derived macrophages ($2.5x10^5$/ml) were treated with IL-4 (10 ng/ml, 48h) and then challenged with 10 ng/ml LPS and 24h later cell-free supernatants were collected and cytokines assessed in the 13 multi-plex cytokine Luminex array. Non-treated macrophages (M0) served as control (mean ± SEM; *, ** and *** $p<0.05$, 0.01 and 0.001 respectively compared to M0 only; #, $p<0.05$ compared to M0-LPS).



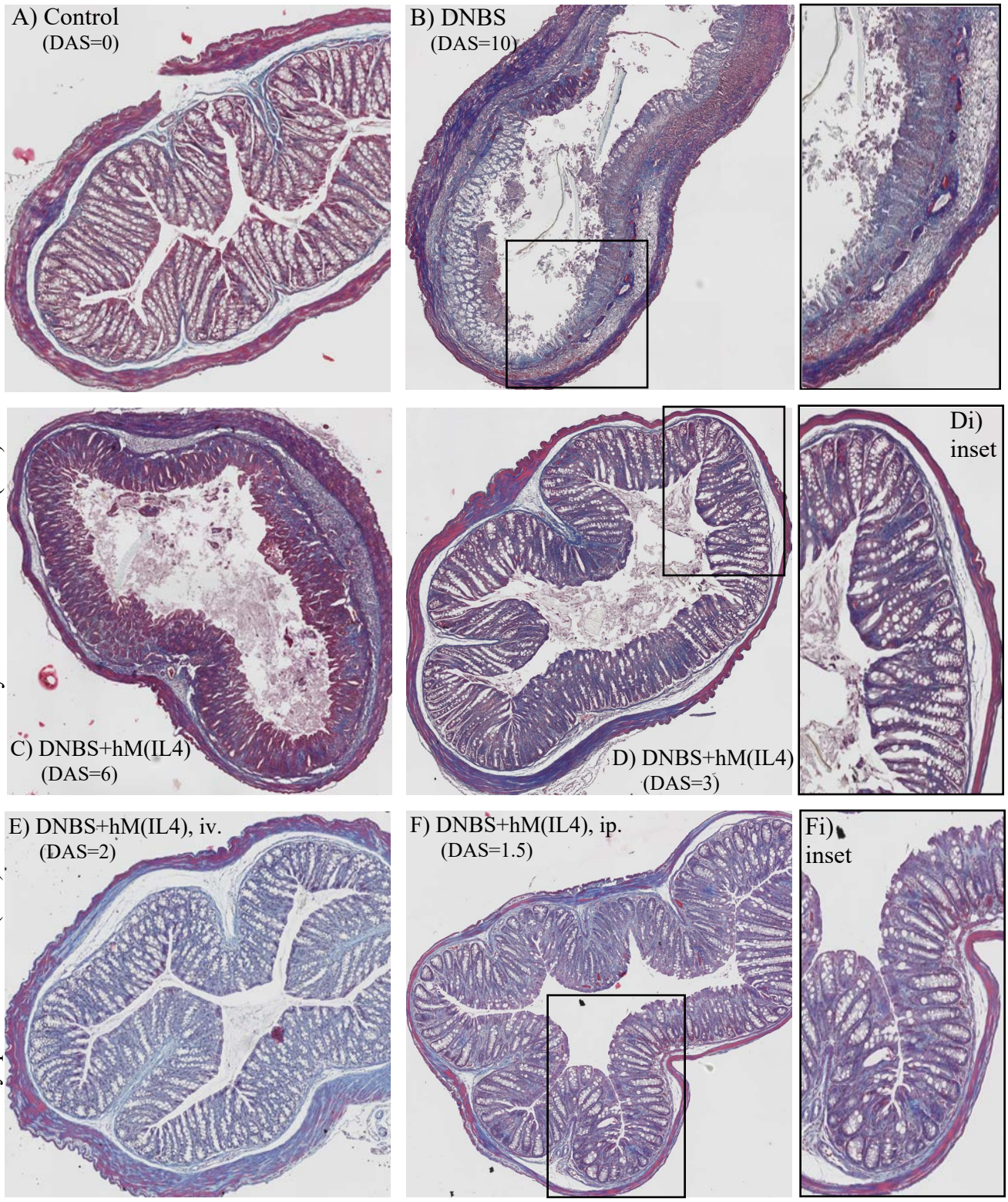
Supplementary Figure 3: Cryopreserved M(IL4)s maintained phenotype and function. Human blood-derived macrophages ($2x10^5$/ml) were converted to M(IL4)s (10 ng/ml IL-4, 48) and then cryopreserved (cryo) in liquid N_2 for at least 1 week. (A) Cells were retrieved and assessed for phenotype by qPCR for CD206. (B) Conditioned medium (CM) was collected from the cells after 24h of culture and diluted 1:1 in fresh medium and used in the *in vitro* would healing assay (Caco2 epithelia wounded and exposed to CM for 48h when total epithelial area repaired was measured). Lines connect data from freshly differentiated and cryo M(IL4) from the same healthy blood donor (mean ± SEM; **, $p<0.05$ compared to M(0); * $p<0.05$ compared to freshly differentiated M(IL4)s).



Supplementary Figure 4: IL-6 enhances the M(IL4) phenotype. Macrophages derived from blood monocytes of healthy individuals were stimulated with IL-4 (M(IL4)), IL-6 (M(IL6)) or both cytokines (M(IL4+6) (at 10 ng/ml) for 48h and (A) CD206 mRNA expression assessed and (B) conditioned medium (CM) collected for CCL18 measurement by ELISA (mean ± SEM; n=8; *, #, $p<0.05$ compared to M0 and M(IL4) respectively by ANOVA followed by Tukey's test). Panel C shows recovery of Caco2 epithelial cell monolayers after mechanical wounding and 24h of exposure to a 50% conditioned medium (CM) from the various cell populations (mean ± SEM; n=4; *, #, ϕ, $p<0.05$ compared to control (culture medium only), M0 CM and M(IL6) CM, respectively by ANOVA followed by Tukey's test).

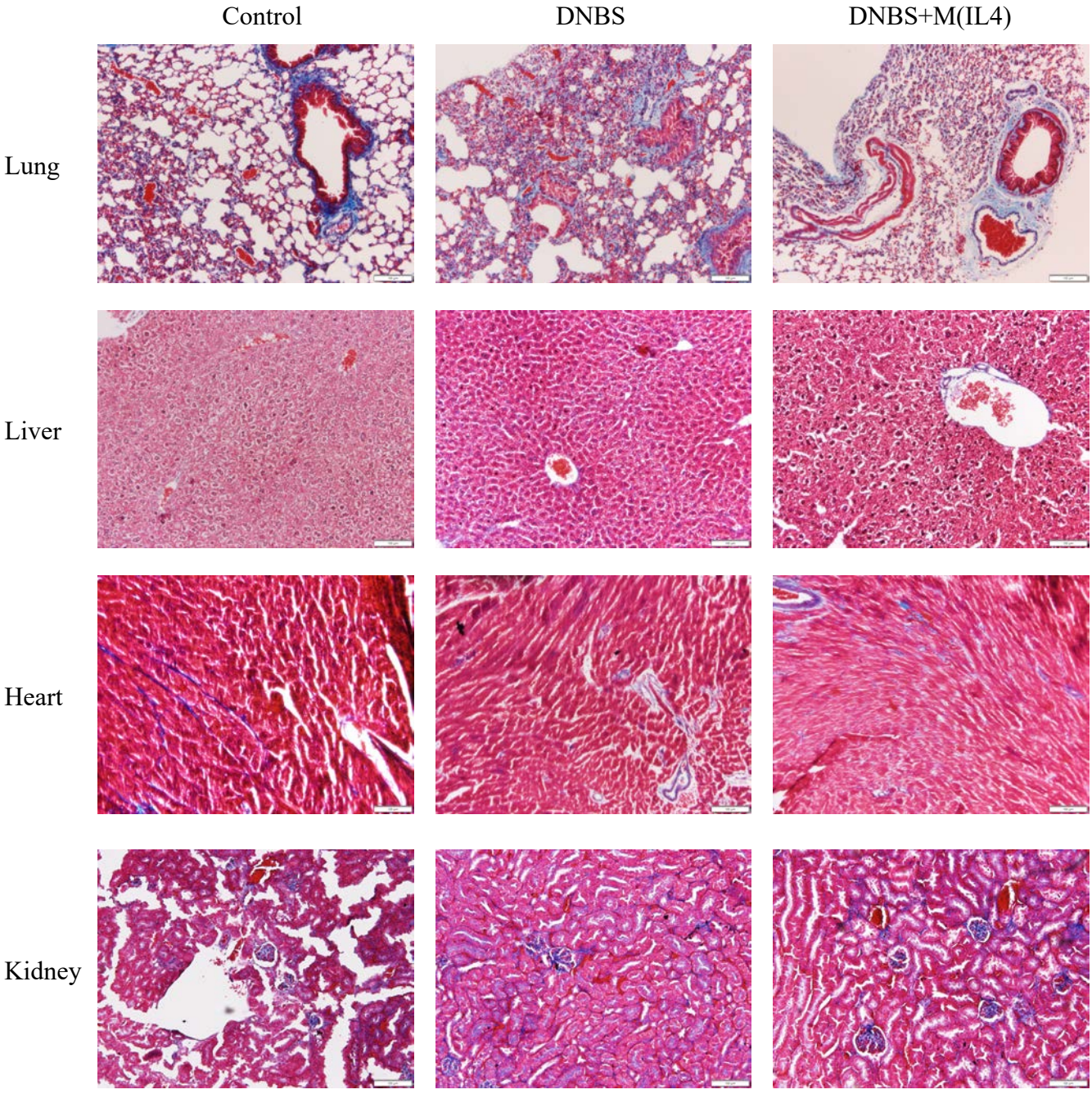


Supplementary Figure 5: An inflammatory cytokine mixture (cyto. mix) partially reduces the human M(IL-4)) phenotype. A cyto. mix consisting of TNFα, IFNγ, and IL-1β (all 10 ng/ml) was applied to monocyte-derived macrophages ($2x10^5$) from healthy donors for 48h. Cells were then rinsed and exposed to IL-4 or IFNγ (both 10 ng/ml) for a subsequent 48h, at which point qPCR was performed for (A) CCD206 and (B) CCL18 (mean ± SEM; *, $p<0.05$ compare to macrophages only (M0).

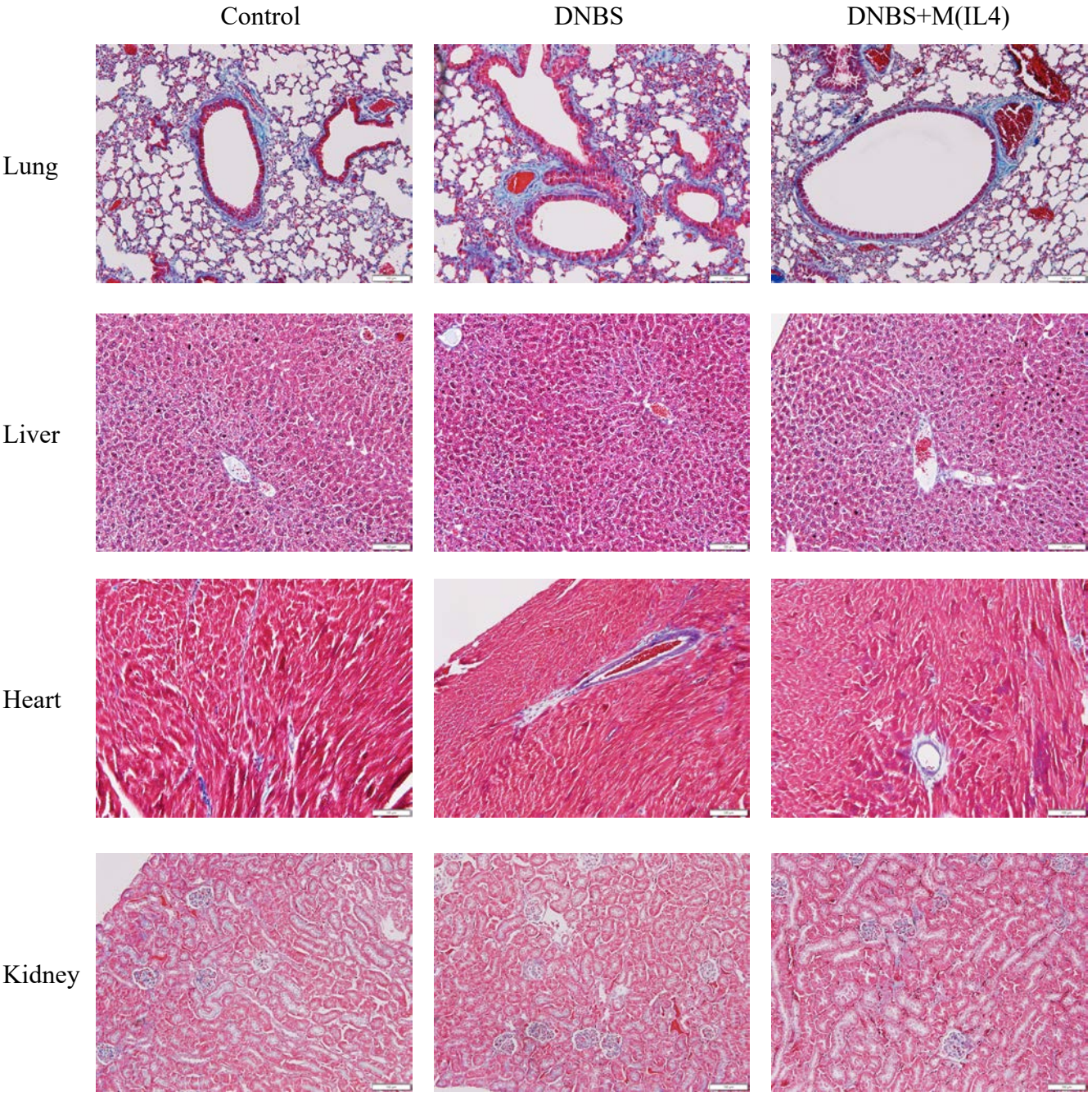


Supplementary Figure 6: Representative images of colonic histology. C57/bl6 *Rag1*$^{-/-}$ male mice were given 1x10^{6} freshly differentiated hM(IL4s) (C, D) via intraperitoneal (ip.) injection or cryopreserved hM(IL4)s intravenously (iv.) (E) or ip. (F) and 48h later received DNBS (5mg intra-rectal). Three-days post-DNBS, segments of mid-colon were formalin-fixed and subsequently sections were stained with Masson's Trichrome stain for collagen (DAS, macroscopic disease activity score of mouse from which section was obtained).

A



B



Supplementary Figure 7: Representative images of liver, lung, heart, and kidney histology of RAG$^{-/-}$ mice receiving M(IL4)s for 48h or 1 month prior to DNBS administration. C57/bl6 *Rag1*$^{-/-}$ male mice were given 1x10^{6} freshly differentiated hM(IL4s) via intraperitoneal injection (A) 3 days and (B) 1 month later received DNBS (5mg intra-rectal). Three-days post-DNBS, segments of the liver, lung, heart, and kidney were formalin-fixed and subsequently sections were stained with Masson's Trichrome stain for collagen.

Supplementary Table 1: Assessment of pro-fibrotic factors in cultured fibroblasts and murine colonic extracts

(A) *In vitro* analysis of CC18oc fibroblast responses			
Parameter \ condition	Culture medium	M(0) CM	M(IL4) CM)
cell count ($x10^5$)	2.62 ± 0.05	2.60 ± 0.03	2.64 ± 0.04
total protein of cells (ng/ml)	412 ± 13	554 ± 37	688 ± 68*
prolyl-4-hydrolase mRNA#	1	1.0 ± 0.2	1.1 ± 0.2
α-smooth muscle actin mRNA#	1	0.7 ± 0.1	1.0 ± 0.1
n	6	6	6

(B) Analysis of C57/BL6 *Rag1*$^{-/-}$ mouse colonic tissue				
Parameter \ condition	control	DNBS	DNBS + hM(IL4) ip.	DNBS + hM(IL4) iv.
prolyl-4-hydrolase mRNA†	1.0 ± 0.4	0.7 ± 0.3	0.5 ± 0.3	0.5 ± 0.3
α-smooth muscle actin mRNA†	1.0 ± 0.3	0.6 ± 0.2	0.5 ± 0.1	0.7 ± 0.2
collagen type III α1 mRNA†	1.0 ± 0.3	0.8 ± 0.3	0.4 ± 0.1	0.4 ± 0.2
Collagen (μg/mg tissue)	1.4 ± 0.1	0.7 ± 0.1*	1.1 ± 0.1	not done
n	2-4	4-6	4-6	4-6

(fibroblasts ($2.5x10^5$) were cultured for 24h with culture medium only or a 50% macrophage or IL-4-treated macrophage (M(IL4)) conditioned medium; #, fold change compared to culture medium only that was normalized to 1 in each experiment; male *Rag1*$^{-/-}$ mice were given $1x10^6$ hM(IL4)s either intraperitoneally (ip.) or by intravenous injection (iv.) and 48h later received dinitrobenzene sulphonic acid (DNBS; 5mg intra-rectally) and on necropsy 72h later portions of colon were excised for qPCR and collagen measurement; †, data are fold change relative to control that was normalized to 1; *$p<0.05$ compared to culture medium only or control).

Supplementary Table 2: Characteristics of patients with IBD from whom blood monocytes were obtained

Disease	State	Sex	Age	Location	Smoker	Diagnosis year
CD	Active	M	46	Unknown	Unknown	Unknown
CD	Active	M	35	Unknown	Unknown	Unknown
CD	Active	F	54	Ileocolonic	Current Smoker	2016
CD	Active	F	62	Colonic	Current Smoker	1998
CD	Active	F	40	Ileal	Non-smoker	2006
CD	Active	M	43	Colonic	Non-smoker	2017
CD	Active	M	46	Unknown	Non-smoker	2008
CD	Active	M	61	Ileocolonic	Non-smoker	2003
CD	Active	F	23	Unknown	Non-smoker	2012
CD	Active	M	67	Colonic	Unknown	2017
CD	Active	M	53	Unknown	Non-smoker	2012
CD	Active	M	59	Ileocolonic	Unknown	1986
CD	Inactive	F	46	Colonic	Non-smoker	2012
CD	Inactive	F	62	Unknown	Non-smoker	2010
CD	Inactive	M	32	Unknown	Unknown	Unknown
CD	Inactive	M	58	Unknown	Unknown	1977
CD	Inactive	M	65	Colonic	Ex smoker	2008
CD	Inactive	F	20	Ileocolonic	Non-smoker	2010
CD	Inactive	M	56	Unknown	Unknown	Unknown
CD	Inactive	F	27	Ileocolonic	Non-smoker	2015
CD	Inactive	F	34	Colonic	Unknown	2007
UC	Active	F	72	Unknown	Non-smoker	2017
UC	Active	F	23	Unknown	Non-smoker	2017
UC	Active	M	32	Left sided	Non-smoker	2011
UC	Active	M	31	Pancolitis	Ex smoker	2013
UC	Active	F	27	Unknown	Non-smoker	Unknown
UC	Active	F	58	Unknown	Ex smoker	2013
UC	Inactive	M	27	Left sided	Unknown	Unknown
UC	Inactive	M	65	Pancolitis	Ex smoker	1979
UC	Inactive	F	71	Unknown	Ex smoker	2016
UC	Inactive	M	87	Left sided	Ex smoker	1998
UC	Inactive	F	68	Ileocolonic	Ex smoker	2012
UC	Inactive	F	38	Left sided	Non-smoker	Unknown

(CD, Crohn's disease; F, female; M, male; UC, ulcerative colitis)

Supplementary Table 3: Sequences of qPCR primers used to assess human and mouse gene mRNA

Human				
Gene	**Name**	**Accession #**	**Forward**	**Reverse**
RN18S5	18s	NR_003286.2	ATGGCCGTTCTTAGTTGGTG	CGCTGAGCCAGTCAGTGTAG
CD14	CD14	NR_003286.2	GCCGCTGTGTAGGAAAGAAG	GCTGAGGTTCGGAGAAGTTG
TGFβ	Transforming growth factor-β	NM_000660.7	GGAAATTGAGGGCTTTCGCC	CGGTAGTGAACCCGTTGATGT
MRC1 (CD206)	Mannose receptor	NM_002438.3	GGCGGTGACCTCACAAGTAT	ACGAAGCCATTTGGTAAACG
CCL18	CCL18	NR_003286.2	CCCCAAGCCAGGTGTCATCCTC	GGGCCATTGCCCTGGCTCAG
ACTA2	α-smooth muscle actin	NM_001141945.2	TATCCCCGGGACTAAGACGG	CACCATCACCCCCTGATGTC
P4HA1	Prolyl-4-hydroxylase	NM_000917.3	CCGAGCTACAGTACATGACCC	TGGCTCATCTTTCCGTGCAA
Mouse				
Gene	**Name**	**Accession #**	**Forward**	**Reverse**
P4hal	Prolyl-4-hydroxylase	NM_011030.2	CTGAGGCGAGCCACCATTTC	CAGACAGCCAAGCACTTTTGC
Acta2	α-smooth muscle actin	NM_007392.2	CAGCCAGTCGCTGTCAGGAACC	ACCAGCGAAGCCGGCCTTACA
Col3a1	Collagen Type III, αI	NM_009930.2	GTCCGCCTGGTCCTCAGGGT	TGCCAGGAGCACCACTGGGT