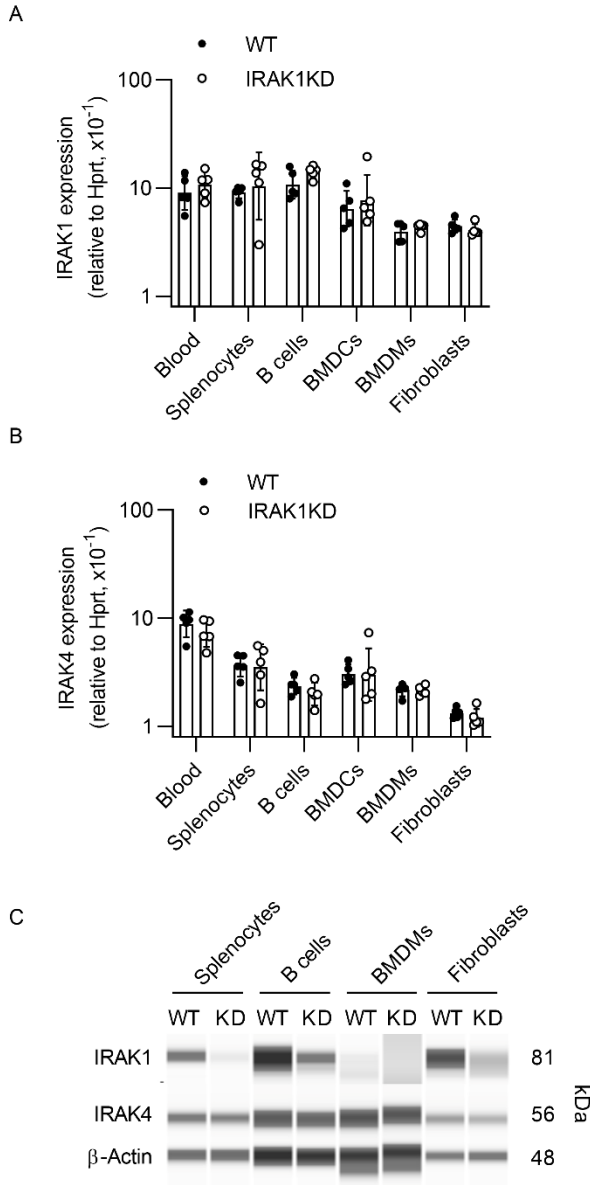


1 Supplemental Data

2 Supplemental Figures

3 Supplemental Figure 1: Expression of IRAK1 across multiple cell types from WT and IRAK1KD
4 mice

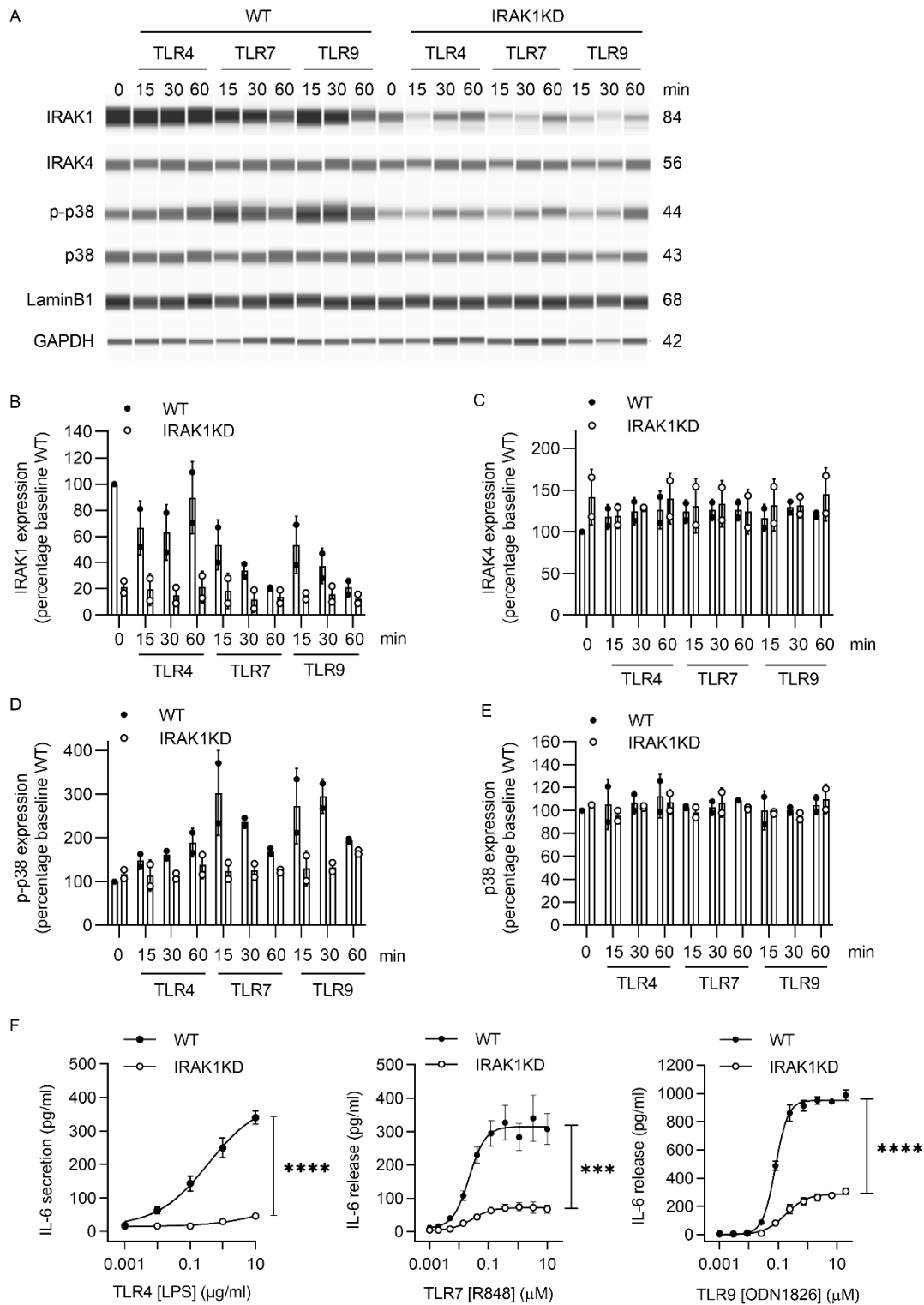


6 Expression of (A) IRAK1 and (B) IRAK4 transcript in indicated cell types. Data points are individual
7 mice, geomeans \pm SD, unpaired t-test. (C) IRAK1 and IRAK4 protein expression in different cell
8 types. Data are from five pooled mice per lane.

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15 **(A)** Protein expression in B cells from WT and IRAK1KD following stimulation with TLR ligands (TLR4:

16 1 mg/ml LPS; TLR7: 1 mM R848; TLR9: 1 mM ODN1826). Expression of **(B)** IRAK1, **(C)** IRAK4, **(D)** p-

17 p38 and **(E)** p38, normalised to protein loading control and relative to WT baseline. Data are

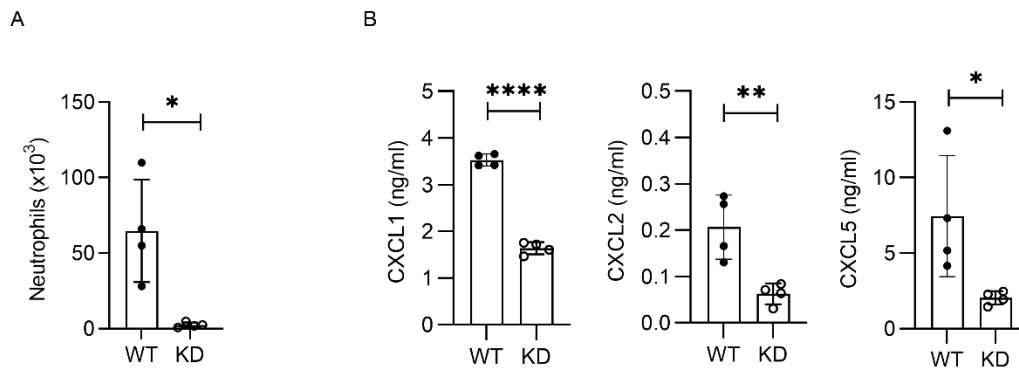
18 representative **(A)** or means \pm SD **(B-E)** of two independent experiments, B cells were pooled from five

19 WT and IRAK1KD mice. (F) IL-6 secretion upon TLR4, TLR7 and TLR9 stimulation. Data points
20 represent means of five individual mice with \pm SD; one representative of 2-3 independent experiments,
21 unpaired t-test.

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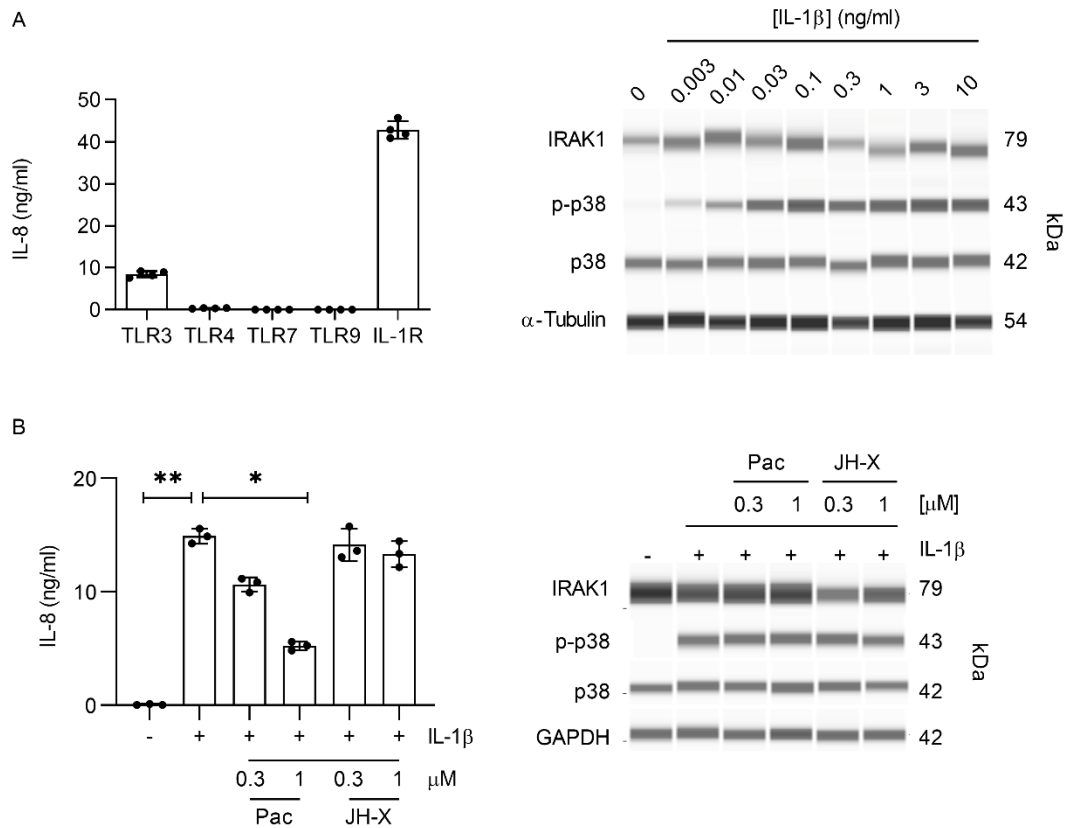
24 **Supplemental Figure 3: Reduced neutrophil recruitment in IRAK1KD mice after *in vivo* IL-**
25 **1 β challenge**



27 **(A)** Total counts of infiltrating neutrophils and **(B)** Chemokine secretion in air pouch exudates 6 h after
28 injection of IL-1 β . Data points are individual mice, means \pm SD. One representative of two independent
29 experiments, unpaired t-test.

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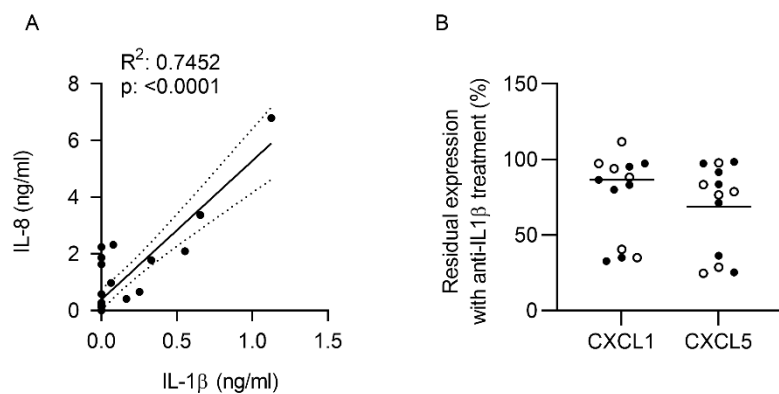
34 **Supplemental Figure 4: Responses of human RA synovial fibroblasts to TLR and IL-**
 35 **1 β stimulation**
 36



38 **(A)** Left, IL-8 secretion, bars represent means of quadruplicate measurements \pm SD. Right, protein
 39 expression by capillary Western Blot from human RA synovial fibroblasts following IL-1 β stimulation.
 40 One representative of two independent experiments. **(B)** Left, IL-8 secretion upon IL-1 β stimulation (0.1
 41 ng/ml for 15 min, i.e. EC80) and IRAK1 inhibitor treatment, bars represent means of triplicate
 42 measurements \pm SD, Kruskal-Wallis test with Dunn's post-test. Right, protein expression by capillary
 43 Western Blot from human RA synovial fibroblasts. One representative of two independent experiments.

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47 **Supplemental Figure 5: Analysis of human synovial fluids**



49 **(A)** IL-8 and IL-1 β protein in individual human RA synovial fluids. Pearson correlation, dotted lines
50 represent 95% confidence interval, **(B)** Residual expression of CXCL1 or CXCL5 from WT synovial
51 fibroblasts treated with RA (n=7 patients, filled symbols) or gout (n=6 patients, open symbols) synovial
52 fluids in presence of anti-IL1 β , compared to untreated WT synovial fibroblasts; data from Figure 6B.
53 Horizontal bars, medians.
54

55 **Supplemental Tables**56 **Supplemental Table 1: Immune cell counts of IRAK1KD and WT mice**

Thymus		Total	CD4-CD8-	CD4+CD8+	CD4+	CD8+
	WT	79.4 ± 20.6	3.9 ± 1.9	59.8 ± 8.6	9.2 ± 5.7	2.3 ± 1.2
	IRAK1KD	95.8 ± 27.6	7.8 ± 5.2	65.4 ± 18.6	16.6 ± 7.6	4.4 ± 2.5
Spleen		Total	CD4 ⁺	CD8 ⁺	CD4 ⁺ CD8 ⁻ CD25 ⁺ Foxp3 ⁺	CD3 ⁺ γδTCR ⁺
	WT	54.4 ± 10	11.3 ± 3.6	8.0 ± 2.0	1.3 ± 0.3	0.3 ± 0.1
	IRAK1KD	46.3 ± 18.3	9.1 ± 3.9	6.7 ± 2.6	1.1 ± 0.5	0.3 ± 0.1
		CD11b ⁺ Ly6G/C ^{low}	CD11b ⁺ Ly6G/C ^{dim}	CD11b ⁺ Ly6G/C ^{high}	CD11c ^{dim} CD11b ⁻ B220 ⁺ pDC	CD11c ^{high} CD11b ⁻ B220 ⁻ cDC
	WT	2.0 ± 0.5	0.8 ± 0.1	1.0 ± 0.5	0.7 ± 0.1	0.2 ± 0.1
IRAK1KD	1.9 ± 0.6	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.3 ± 0.1	
MLN		Total	CD4 ⁺	CD8 ⁺	CD19 ⁺	CD11c ^{dim} CD11b ⁻ B220 ⁺ pDC
	WT	12.8 ± 6.0	5.8 ± 3.9	3.8 ± 1.6	4.1 ± 1.9	0.1 ± 0.1
	IRAK1KD	15.0 ± 4.7	6.8 ± 2.2	4.4 ± 1.2	4.6 ± 1.6	0.1 ± 0.04
BM		Total	B220 ⁺ CD43 ⁺ CD19 ⁺ proB	B220 ⁺ CD43 ⁻ IgM ⁻ preB	B220 ⁺ CD43 ⁻ IgM ⁺ Immature B	B220 ^{high} CD43 ⁻ IgM ⁺ mature B
	WT	24.2 ± 13.8	0.6 ± 0.2	2.9 ± 0.8	1.0 ± 0.4	2.1 ± 0.6
	IRAK1KD	22.2 ± 4.4	0.4 ± 0.08	2.1 ± 0.7	0.8 ± 0.4	1.3 ± 0.6

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58 Counts [$\times 10^6$] of cell subsets in different compartments as evaluated by flow cytometry. MLN,
59 mesenteric lymph nodes, BM, bone marrow. Means \pm SD of 8 mice.

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62 **Supplemental Table 2: Antibodies**

Flow cytometry		
Antigen	Clone	Company
CD3	145-2c11	eBioscience
CD4	RM4-5	BD Biosciences
CD8a	53-6.7	eBioscience
CD11b	M1/70	eBioscience
CD11c	HL3	BD Biosciences
CD16/32	2.4G2	eBioscience
CD19	1D3	BD Biosciences
CD21/35	7E9	Biolegend
CD23	B3B4	Biolegend
CD25	PC61.5/BC96	eBioscience
CD43	S7	BD Biosciences
CD45	30-F11	Biolegend
CD45R / B220	RA3-6B2	Biolegend
c-Kit	2B8	eBioscience
F4/80	BM8	eBioscience
Fc ϵ R1	MAR-1	eBioscience
FoxP3	FJK-16S	eBioscience
GR-1 / Ly6G/C	Rb6-8C5	BD Biosciences
GL-7	GL-7	Biolegend
IgM	11/41	eBioscience
Ly6C	HK1.4	Biolegend
Ly6G	1A8	Biolegend
PDCA-1	927	Biolegend
TCR $\gamma\delta$	UC7-13D5	eBioscience
F4/80	Cl:A3-1	AbD Serotec
Immunohistochemistry		
Ly-6B2	7/4	AbD Serotec
F4/80	Cl:A3-1 AbD	AbD Serotec
Rabbit-anti-rat IgG	polyclonal	Vector Laboratories
Protein detection (WES)		
Anti-IRAK1	D51G7	Cell Signaling
Anti-pIRAK4 (Thr345/Ser346)	D6D7	Cell Signaling
Anti-IRAK4	2H9	Abcam
Anti-GAPDH	D16H11	Cell Signaling
Anti-p38 MAPK	#9212	Cell Signaling
Anti-Phospho-p38 (Thr180/Tyr182)	#9211	Cell Signaling
Anti-Phospho-NF- κ B p65 (Ser536)	93H1	Cell Signaling
Anti-Lamin B1	AF8525	R&D Systems
Anti-alpha Tubulin	11H10	Cell Signaling
Anti- β Actin	AC-15	Cell Signaling
Anti-rabbit-HRP detection module for WES	DM-001	ProteinSimple
Anti-mouse-HRP detection module for WES	DM-002	ProteinSimple

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64 All monoclonal antibodies are directed against mouse antigens unless indicated otherwise.

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66 **Supplemental Table 3: Conditions for cytokine release from WT and IRAK1KD cells.**

	TLR ligand concentrations for cytokine release					
	TLR3 (PolyIC*)	TLR4 (LPS)	TLR7 (R848)	TLR7 (ssRNA*)	TLR9 (ODN1585)	TLR9 (ODN1826)
BMDCs (IFNα)						
WT (EC ₅₀)	10 \pm 0.47 (2)	>10,000 (2)	-	0.69 \pm 0.63 (13)	160 \pm 81 (6)	-
IRAK1KD (EC ₅₀)	10 \pm 0.31 (3)	>10,000 (3)	-	>30 (14)	>10,000 (6)	-
For Fig. 1	30	1000	-	1	1000	
BMDCs (IL-6)						
WT (EC ₅₀)	5.5 \pm 6.1 (2)	120 \pm 130 (2)	-	0.57 \pm 0.26 (14)	-	3.8 \pm 5.6 (5)
IRAK1KD (EC ₅₀)	10.6 \pm 0.77 (3)	100 \pm 70 (3)	-	>30 (10)	-	4.0 \pm 6.5 (7)
For Fig. 1	30	1000	-	3	-	100
B cells (IL-6)						
WT (EC ₅₀)	>30 (2)	390 \pm 330 (10)	21 \pm 5 (6)	-	-	105 \pm 37 (10)
IRAK1KD (EC ₅₀)	>30 (2)	>10,000 (10)	27 \pm 16 (6)	-	-	306 \pm 175 (10)
For Fig. 1	30	1000	1000	-	-	1000
BMDMs (IL-6)						
WT (EC ₅₀)	>30 (7)	0.55 \pm 0.27 (8)	-	0.45 \pm 0.20 (6)	-	15 \pm 4 (7)
For Fig. 1	30	10	-	1	-	100

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68 Specific cells were isolated as indicated in Materials and Methods and stimulated with TLR agonists:
69 TLR3 (PolyIC*, μ g/ml), TLR4 (LPS, ng/ml), TLR7 (R848, nM), TLR7 (ssRNA*, μ g/ml), TLR9 (ODN1585,
70 nM) and TLR9 (ODN1826, nM). ODN1585 (i.e. CpG-A) was only used for elicitation of IFN α . The
71 optimal TLR7 agonist for robust IFN α secretion was ssRNA/DOTAP and used for all BMDC
72 experiments. Since B cells respond only weakly to ssRNA/DOTAP, R848 was instead used. Effective
73 potency (EC₅₀) values are geomeans \pm SD of (n) separate experiments with each dataset performed in
74 duplicates. The concentrations of TLR ligands for each cell type used in Figure 1 were selected based
75 on an approximate EC₉₀ for the respective WT cell type or using a concentration that gave the maximum
76 stimulated response. *: presence of DOTAP (25 μ g/ml).

77

78 **Supplemental Table 4: qPCR Primer sequences**

Taqman primers and probes	Cxcl10	Mm99999072_m1
	Mx1	Mm01218004_m1
	Oasl1	Mm00455082_m1
	Rasd2	Mm00491265_m1
	Irf7	Mm00516791_g1
	Ifi44	Mm00505670_m1
	Ifi44l	Mm00518988_m1
	Hprt	Mm00446968_m1
	IRAK1	Mm00434254_m1
	IRAK4	Mm00459443_m1
Primers for SYBRgreen	Cxcl1	Fwd: 5'-GTG TTG CCC TCA GGG CC-3'
		Rev: 5'-GCC TCG CGA CCA TTC TTG-3'
	Cxcl2	Fwd: 5'-ACG CCC CCA GGA CCC-3'
		Rev: 5'-CTT TTT GAC CGC CCT TGA GA-3'
	Cxcl5	Fwd: 5'-CTC GCC ATT CAT GCG GAT-3'
		Rev: 5'-CTT CAG CTA GAT GCT GCG GC-3'
	Ccl2	Fwd: 5'-TGG CTC AGC CAG ATG CAG T-3'
		Rev: 5'-TTG GGA TCA TCT TGC TGG TG-3'
	IL1b	Fwd: 5'-TGG CAA CTG TTC CTG-3'
		Rev: 5'-GGA AGC AGC CCT TCA TCT TT-3'
	b2m	Fwd: 5'-CCG AAC ATA CTG AAC TGC TAC GTA A-3'
		Rev: 5'-CCC GTT CTT AGC ATT TGG A-3'

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84 **Supplemental Table 5: Characteristics of patients for knee joint immunohistochemistry**

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Patient	Sex	Age	Description
RA 1	M	65	Thickening of synovial layer, inflammatory infiltrate
RA 2	M	71	Hyperplastic synovial membranes, infiltrations. Krenn synovitis score 7
RA 3	F	58	Hyperplastic synovial membrane, infiltrated. Krenn synovitis score 9
Non-inflamed 1	F	55	Single-layered synovial membrane, no infiltrate
Non-inflamed 2	M	71	Fibrotic connective tissue, no inflammation
Non-inflamed 3	M	73	Fibrotic scar tissue with histiocytic reaction

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