

Supplementary Materials for

In vitro responses to platelet-rich-plasma are associated with variable clinical outcomes in patients with knee osteoarthritis

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This file includes:

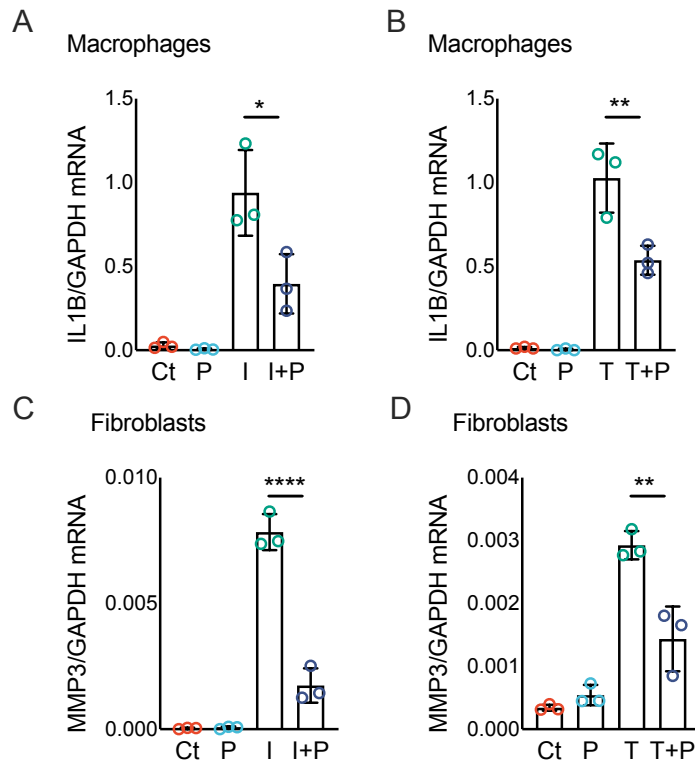
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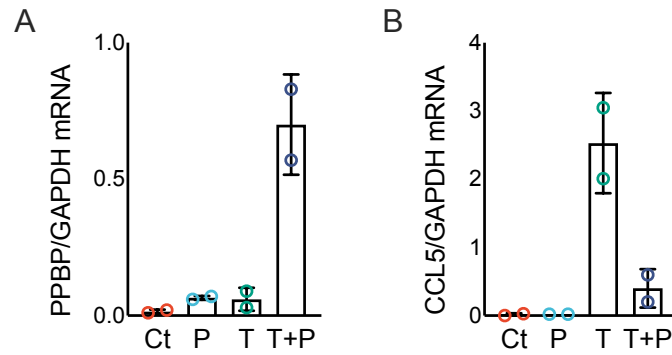
Table S3: RNA-seq analyses, differentially expressed genes in macrophages.
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Table S8: RNA-seq analyses, differentially expressed genes in fibroblasts.
Table S9: NanoString analyses, normalized counts in fibroblasts.
Table S10: QuSAGE pathway analyses in fibroblasts, PRP-vs-Control.
Table S11: QuSAGE pathway analyses in fibroblasts, TNF-vs-Control.
Table S12: QuSAGE pathway analyses in fibroblasts, TNF+PRP-vs-TNF.

SUPPLEMENTARY FIGURES

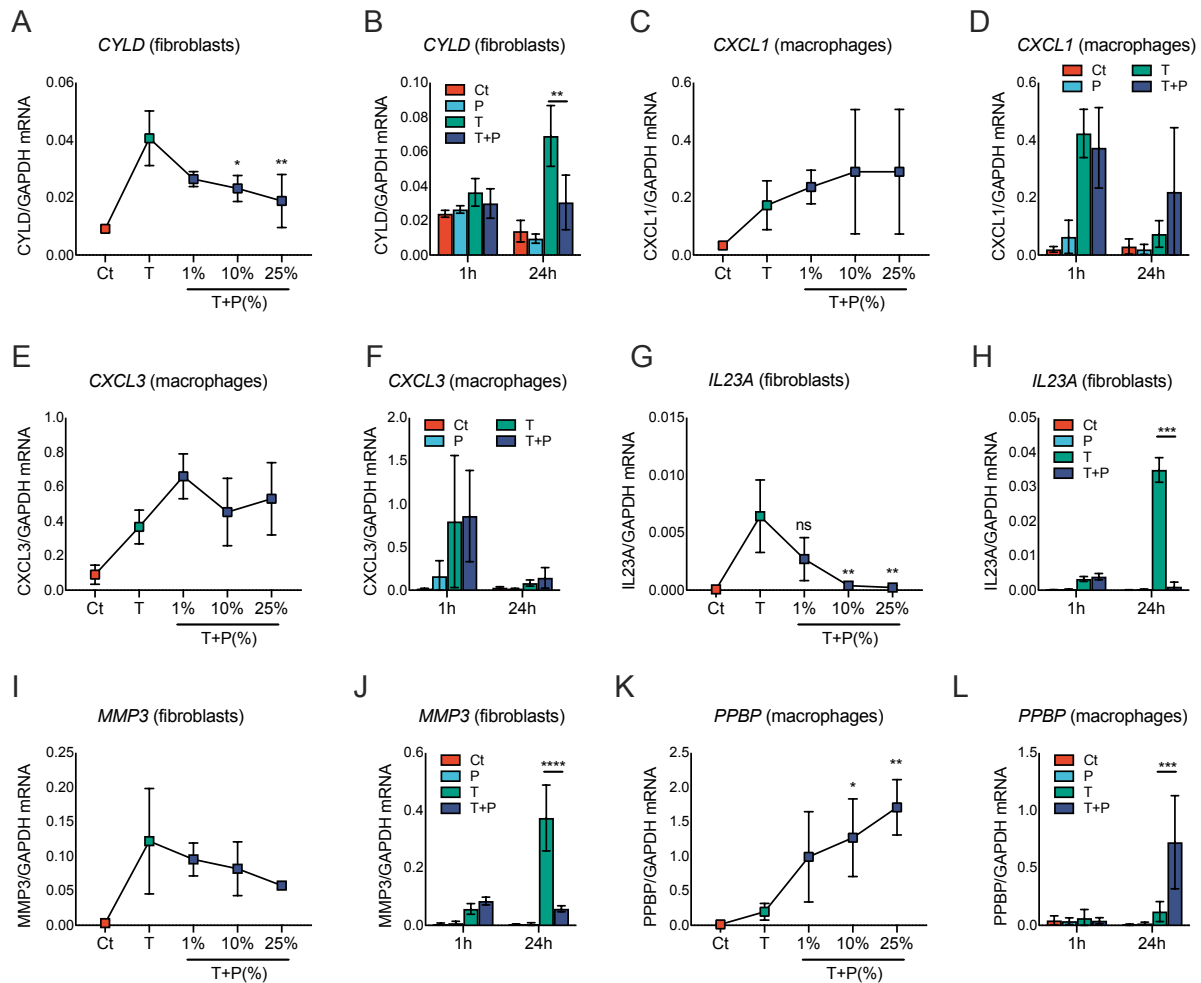
Supplementary Figure S1. PRP modulates the IL-1 β -induced responses in a coculture of macrophages and fibroblasts. (A) RTqPCR analyses of *IL1B* mRNA in macrophages left untreated (Ct) or treated with 10% PRP (P), 10ng/ml of IL1 β (I) or a combination of IL1 β +PRP (I+P) for 24h. (B) RTqPCR analyses of *IL1B* mRNA in macrophages left untreated (Ct) or treated with 10% PRP (P), 20ng/ml of TNF α (T) or a combination of TNF α +PRP (T+P) for 24h. (C) RTqPCR analyses of *MMP3* mRNA in fibroblasts left untreated (Ct) or treated with 10% PRP (P), 10ng/ml of IL1 β (I) or a combination of IL1 β +PRP (I+P) for 24h. (D) RTqPCR analyses of *MMP3* mRNA in fibroblasts left untreated (Ct) or treated with 10% PRP (P), 20ng/ml of TNF α (T) or a combination of TNF α +PRP (T+P) for 24h. Data is represented as the mean $\pm$ S.D (error bars) of 3 independent co-culture experiments, using macrophages and fibroblasts isolated from 3 different tissue donors and PRP prepared from 3 different subjects. *p<0.05, **p<0.01, ****p<0.0001 by ANOVA followed by Tukey's post-hoc test.



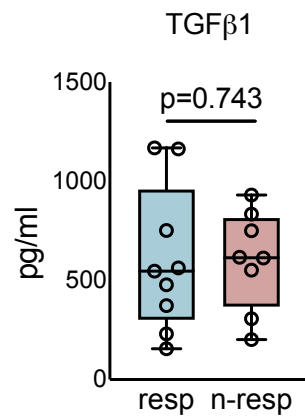
Supplementary Figure S2: PRP modulates the TNF α -induced responses in macrophages in monoculture. RTqPCR analyses of (A) *PPBP* and (B) *CCL5* mRNA in macrophage monocultures left untreated (Ct) or treated with 10% PRP (P), 20g/ml of TNF α (T) or a combination of TNF α +PRP (T+P) for 24h. Data is represented as the mean $\pm$ S.D (error bars) of 2 independent experiments, using macrophages isolated from 2 different tissue donors and PRP prepared from 2 different subjects.



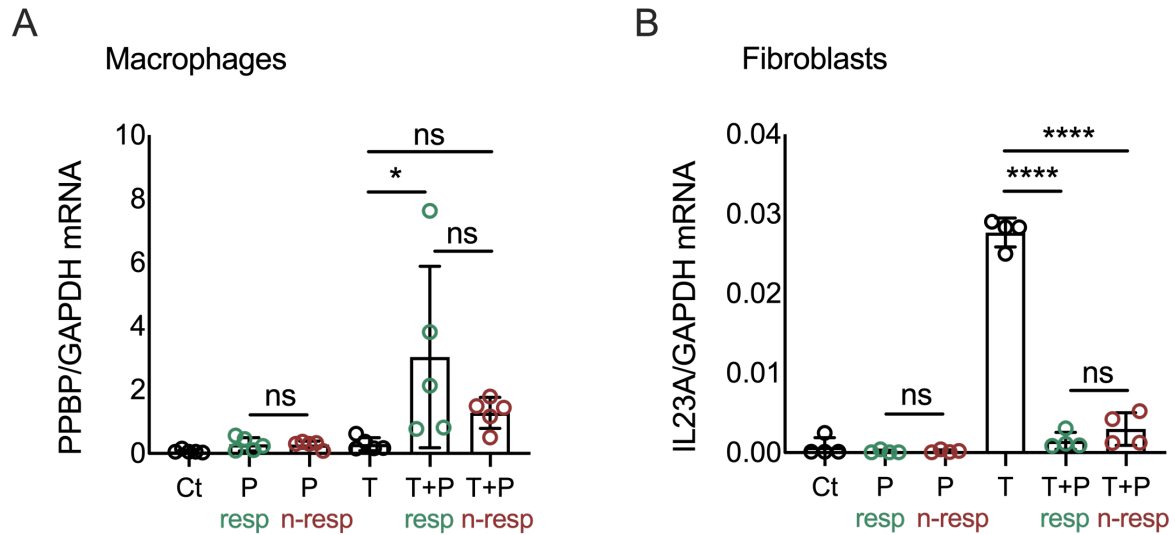
Supplementary Figure S3: Dose- and time-dependent responses in macrophages and fibroblasts. For dose-response experiments, cells were left untreated (Ct) or treated with TNF α (T) alone or combined with 1%, 10% and 25% of PRP (P) for 24 hours. For time-course experiments, cells were left untreated (Ct) or treated with TNF α (T) or 10% v:v PRP (P), alone or combined (T+P) for 1 and 24 hours. RTqPCR analyses of (A,B) *CYLD* in fibroblasts, (C,D) *CXCL1* in macrophages, (E,F) *CXCL3* in macrophages, (G,H) *IL23A* in fibroblasts, (I,J) *MMP3* in fibroblasts, and (K,L) *PPBP* in macrophages. Data are shown as mean $\pm$ S.D (error bars) of 3 independent experiments, using macrophages and fibroblasts isolated from 3 different tissue donors and PRP prepared from 3 different subjects. * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001 by ANOVA followed by Tukey's post-hoc test.



Supplementary Figure S4: TGF β 1 protein levels in PRP from responders and non-responders. The relative abundance of TGF β 1 protein in PRP samples from responders (resp, n=9) and non-responders (n-resp, n=8) was evaluated using ELISA assays. Data was analyzed by Mann-Whitney test.



Supplementary Figure S5: Comparison of the bioactivity of PRP from responders and non-responders. RTqPCR analyses of (A) *PPBP* mRNA in macrophages and (B) *IL23A* mRNA in fibroblasts left untreated (Ct) or treated with TNF α (T), PRP (P) or TNF α +PRP (T+P) for 24 h, using PRP from responders (resp) or non-responders (n-resp). RTqPCR data is represented as mean $\pm$ S.D (error bars) of 5 (macrophages) and 4 (fibroblasts) independent experiments. * p <0.05, **** p <0.0001 by ANOVA followed by Tukey's post-hoc test. ns = not significant.



Supplementary Table S1: Inclusion and exclusion criteria (HSS IRB # 2016-0267)

Inclusion criteria
Kellgren-Lawrence OA grade 1-3 based on plain radiographs
Age 30-75
Males and females
Exclusion criteria
BMI greater than 35
Diagnosis of inflammatory arthritis
Steroid injection within 3 months or hyaluronic acid injection within 6 months
Knee surgery within 1 year
Concurrent use of anti-platelet medications.
Varus malalignment- defined as femoral-tibial angle less than or equal to 0
Valgus malalignment- defined as femoral-tibial angle greater than 10
Evidence of prior complete meniscectomy based on MRI scan
Subchondral insufficiency fracture diagnosed based on bone marrow edema pattern on MRI

Supplementary Table S2: PCR primers and conditions

GENE	NCBI Ref Seq	Forward (5'>3')	Reverse (5'>3')	Anneal
<i>IL23A</i>	NM_016584.3	ACACATGGATCTAAGAGAAGAGGG	AGAGAAGGCTCCCCTGTGAA	60
<i>CCL5</i>	NM_002985.3	GAGGCTTCCCCTCACTATCC	CTCAAGTGATCCACCCACCT	60
<i>CXCL1</i>	NM_001511.4	CCAGCTCTTCCGCTCCTC	CACGGACGCTCCTGCTG	60
<i>CXCL3</i>	NM_002090.2	AGATACTGAACAAGGGGAGCAC	CCTTTCCAGCTGTCCCTAGA	60
<i>IL1B</i>	NM_000576.3	CTCAAGTGATCCACCCACCT	CTCAAGTGATCCACCCACCT	60
<i>MMP3</i>	NM_002422.5	CCCATATATGCCTGCTGTCC	ACAGGTTGATTCCTGGTCA	60
<i>PPBP</i>	NM_002704.3	CTTGCGAAAGGCAAAGAGG	TCAGTGTGGCTATCACTTCGAC	60
<i>CYLD</i>	NM_015247.2	TTGGTGTGGACATGGATAACCC	CTCCTTTCCTGCGTCACACT	60
<i>GAPDH</i>	NM_002046.7	ATCAAGAAGGTGGTGAAGCA	GTCGCTGTTGAAGTCAGAGGA	60

Supplementary Table S13: Comparison of responders (n=17) vs. non-responders (n=15) to PRP.

	responders	non-responders	p value*
Sex (male:female)	7:10	9:6	0.4795 ^a
Age (years, mean $\pm$ s.d.)	59.53 $\pm$ 8.91	57.07 $\pm$ 10.97	0.4890
BMI (kg/m², mean $\pm$ s.d.)	25.96 $\pm$ 4.11	24.77 $\pm$ 3.24	0.3824
K-L Score (mean $\pm$ s.d.)	1.88 $\pm$ 0.72	1.92 $\pm$ 0.86	0.8711
WORMS Score (mean $\pm$ s.d.)	35.00 $\pm$ 21.39	38.93 $\pm$ 25.76	0.6838
Platelets (x10³/ml, mean $\pm$ s.d.)	365.20 $\pm$ 71.26	386.00 $\pm$ 94.94	0.5124
Leukocytes (x10³/ml mean $\pm$ s.d.)	1.01 $\pm$ 0.59	1.36 $\pm$ 0.67	0.1226

*by *t*-test; ^aby Fisher's exact test

Supplementary Table S14: Luminex ELISA analyses comparing PRP from responders (resp) vs. non-responders (n-resp).

	N (valid:missing)		Median		Range		Test Statistics	
	resp	n-resp	resp	n-resp	resp	n-resp	Mann-Whitney U	exact P value
EGF	16:1	14:1	96.1	140.3	290.1	284.8	88	0.334
FGF2	16:1	15:0	468.6	451.3	477.6	387.7	111	0.74
IL1α	16:1	15:0	37.2	23.4	166.8	114.4	94.5	0.318
IL1β	16:1	15:0	1.0	1.6	13.8	8.9	91.5	0.264
IL1RA	16:1	14:1	1.6	30.3	331.8	478.7	94	0.473
IL6	17:0	15:0	0.0	3.2	17.6	23.2	107.5	0.455
IL7	15:2	13:2	8.7	3.1	17.8	16.7	50	0.029
IL8	16:1	14:1	5.2	7.0	12.5	40.1	96.5	0.525
IL10	16:1	15:0	0.8	0.0	56.9	508.2	107.5	0.626
MCP1	16:1	15:0	160.9	168.5	201.9	166.7	103	0.52
TNFα	17:0	15:0	28.3	27.4	33.6	36.8	113.5	0.602
VEGF	16:1	15:0	78.9	76.4	268.0	390.8	112.5	0.770