Supplemental information

Identification of a regulatory pathway

governing TRAF1 via

an arthritis-associated non-coding variant

Qiang Wang, Marta Martínez-Bonet, Taehyeung Kim, Jeffrey A. Sparks, Kazuyoshi Ishigaki, Xiaoting Chen, Marc Sudman, Vitor Aguiar, Sangwan Sim, Marcos Chiñas Hernandez, Darren J. Chiu, Alexandra Wactor, Brian Wauford, Miranda C. Marion, Maria Gutierrez-Arcelus, John Bowes, Stephen Eyre, Ellen Nordal, Sampath Prahalad, Marite Rygg, Vibeke Videm, Soumya Raychaudhuri, Matthew T. Weirauch, Carl D. Langefeld, Susan D. Thompson, and Peter A. Nigrovic

Supplementary figures for:

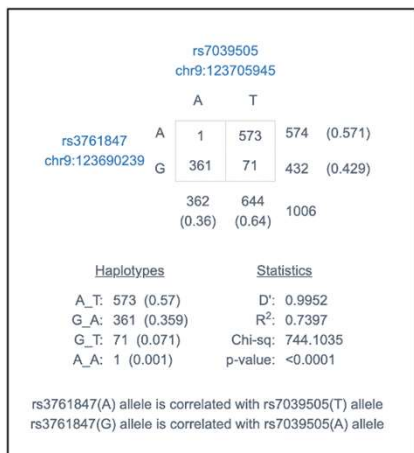## Identification of a regulatory pathway governing TRAF1 via an arthritis-associated non-coding variant

Qiang Wang[1,17], Marta Martínez-Bonet[2,3,17], Taehyeung Kim[1], Jeffrey A. Sparks[2], Kazuyoshi Ishigaki[2], Xiaoting Chen[4], Marc Sudman[4], Vitor Aguiar[1], Sangwan Sim[1], Marcos Chiñas Hernandez[1], Darren J. Chiu[2], Alexandra Wactor[1], Brian Wauford[1], Miranda C. Marion[5], Maria Gutierrez-Arcelus[1,4], John Bowes[6,7], Stephen Eyre[6,7], Ellen Nordal[8], Sampath Prahalad[9], Marite Rygg[10,11], Vibeke Videm[10], Soumya Raychaudhuri[2,6,12,13,14], Matthew T. Weirauch[4,15,16], Carl D. Langefeld[5], Susan D. Thompson[4,16], Peter A. Nigrovic[1,2,18]*

1. Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston MA, USA
2. Division of Rheumatology, Inflammation, and Immunity, Brigham and Women's Hospital, Harvard Medical School, Boston MA, USA
3. Laboratory of Immune-regulation, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid Spain
4. Center of Autoimmune Genomics and Etiology, Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
5. Department of Biostatistics and Data Science, and Center for Precision Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA
6. Centre for Genetics and Genomics Versus Arthritis, Centre for Musculoskeletal Research, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, The University of Manchester, Oxford Road, Manchester, UK.
7. NIHR Manchester Musculoskeletal Biomedical Research Unit, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, UK.
8. University Hospital of North Norway and UIT The Arctic University of Norway, Tromsø, Norway
9. Emory University Department of Pediatrics and Children's Healthcare of Atlanta, Atlanta, GA, USA
10. Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
11.Department of Pediatrics, St. Olav's University Hospital, Trondheim, Norway
12. Broad Institute of MIT and Harvard, Cambridge, MA, 02142, USA
13. Center for Data Science, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
14. Department of Biomedical Informatics, Harvard Medical School, Boston, MA, USA
15. Divisions of Human Genetics, Biomedical Informatics, and Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
16. Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA
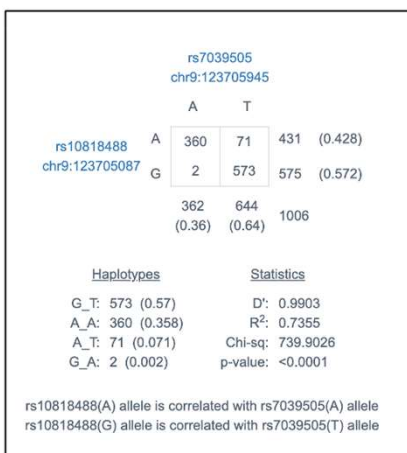17. These authors contributed equally
18. Lead contact

Correspondence: peter.nigrovic@childrens.harvard.edu

S. Figure 1

A

rs7039505
chr9:123705945

		A	T		
rs3761847	A	1	573	574	(0.571)
chr9:123690239	G	361	71	432	(0.429)
		362 (0.36)	644 (0.64)	1006	

Haplotypes	Statistics
A_T: 573 (0.57)	D': 0.9952
G_A: 361 (0.359)	R^2: 0.7397
G_T: 71 (0.071)	Chi-sq: 744.1035
A_A: 1 (0.001)	p-value: <0.0001

rs3761847(A) allele is correlated with rs7039505(T) allele
rs3761847(G) allele is correlated with rs7039505(A) allele

B

rs7039505
chr9:123705945

		A	T		
rs10818488	A	360	71	431	(0.428)
chr9:123705087	G	2	573	575	(0.572)
		362 (0.36)	644 (0.64)	1006	

Haplotypes	Statistics
G_T: 573 (0.57)	D': 0.9903
A_A: 360 (0.358)	R^2: 0.7355
A_T: 71 (0.071)	Chi-sq: 739.9026
G_A: 2 (0.002)	p-value: <0.0001

rs10818488(A) allele is correlated with rs7039505(A) allele
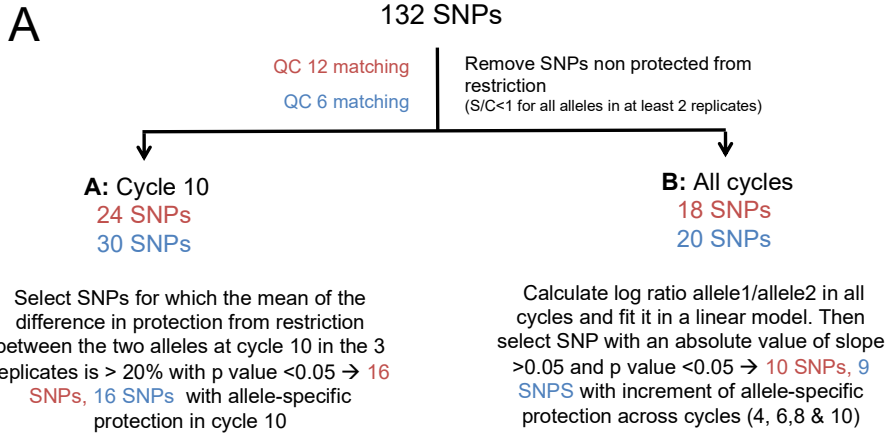rs10818488(G) allele is correlated with rs7039505(T) allele

Supplementary Figure 1. Linkage disequilibrium of rs7039505 with previously identified arthritis risk variants, related to Figure 1. (A) Rheumatoid arthritis, rs3761847. **(B)** Juvenile idiopathic arthritis, rs10818488.

S. Figure 2

A

132 SNPs

QC 12 matching
QC 6 matching

Remove SNPs non protected from restriction
(S/C<1 for all alleles in at least 2 replicates)

A: Cycle 10
24 SNPs
30 SNPs

Select SNPs for which the mean of the difference in protection from restriction between the two alleles at cycle 10 in the 3 replicates is > 20% with p value <0.05 → 16 SNPs, 16 SNPs with allele-specific protection in cycle 10

B: All cycles
18 SNPs
20 SNPs

Calculate log ratio allele1/allele2 in all cycles and fit it in a linear model. Then select SNP with an absolute value of slope >0.05 and p value <0.05 → 10 SNPs, 9 SNPS with increment of allele-specific protection across cycles (4, 6,8 & 10)

B

| QC 12 matching		QC 6 matching		Score
Aproach A	Aproach B	Aproach A	Aproach B	
rs7021880	rs7021880	rs7021880	rs7021880	4
rs7034653	rs7034653	rs7034653	rs7034653	4
rs758959	rs758959	rs758959	rs758959	4
rs6478484	rs6478484	rs6478484	rs6478484	4
rs7858209		rs7858209	rs7858209	3
rs3761849		rs3761849	rs3761849	3
rs7875829	rs7875829			2
rs9886724	rs9886724			2
rs10760129	rs10760129			2
		rs10985073	rs10985073	2
		rs1609810	rs1609810	2
rs1008381		rs1008381		2
rs10435844		rs10435844		2
rs7021206		rs7021206		2
	rs10760130		rs10760130	2
		rs10117059		1
		rs10739579		1
		rs1468671		1
		rs1860823		1
		rs4837804		1
rs10985102				1
rs7037195				1
rs2109896				1
rs4837799				1
	rs10739577			1
	rs10739581			1

Supplementary Figure 2. Selection of SNPs for experimental investigation from SNP-seq, related to Figure 3. (A) Schematic for analysis of SNP-seq NGS data. QC6 and QC12 matching refer to agreement of 6 or 12 nucleotides on each side of SNP from the NGS sequencing data with the original sequence. **(B)** Summary table of all the SNPs selected from SNP-seq based on one of the analysis approaches. The 11 SNPs highlighted in yellow were chosen for downstream validation. SNP, single nucleotide polymorphism; QC, quality control.

S. Figure 3

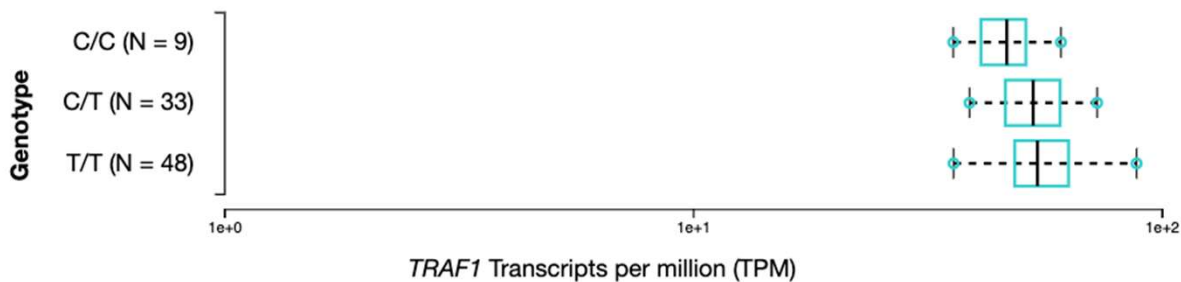A   **rs7034653 cis-eQTLs with TRAF1 in monocyte**

Monocyte

Raw P value:0.0098 Adj. P value: 0.9 Effect size: 0.4
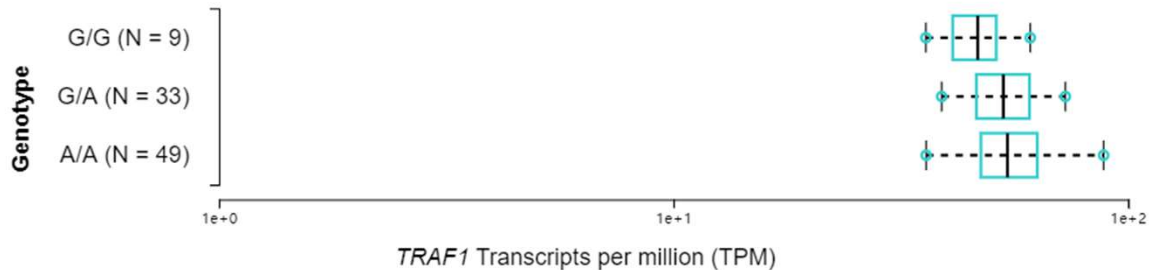
rs1609810 cis-eQTLs with TRAF1 in monocyte

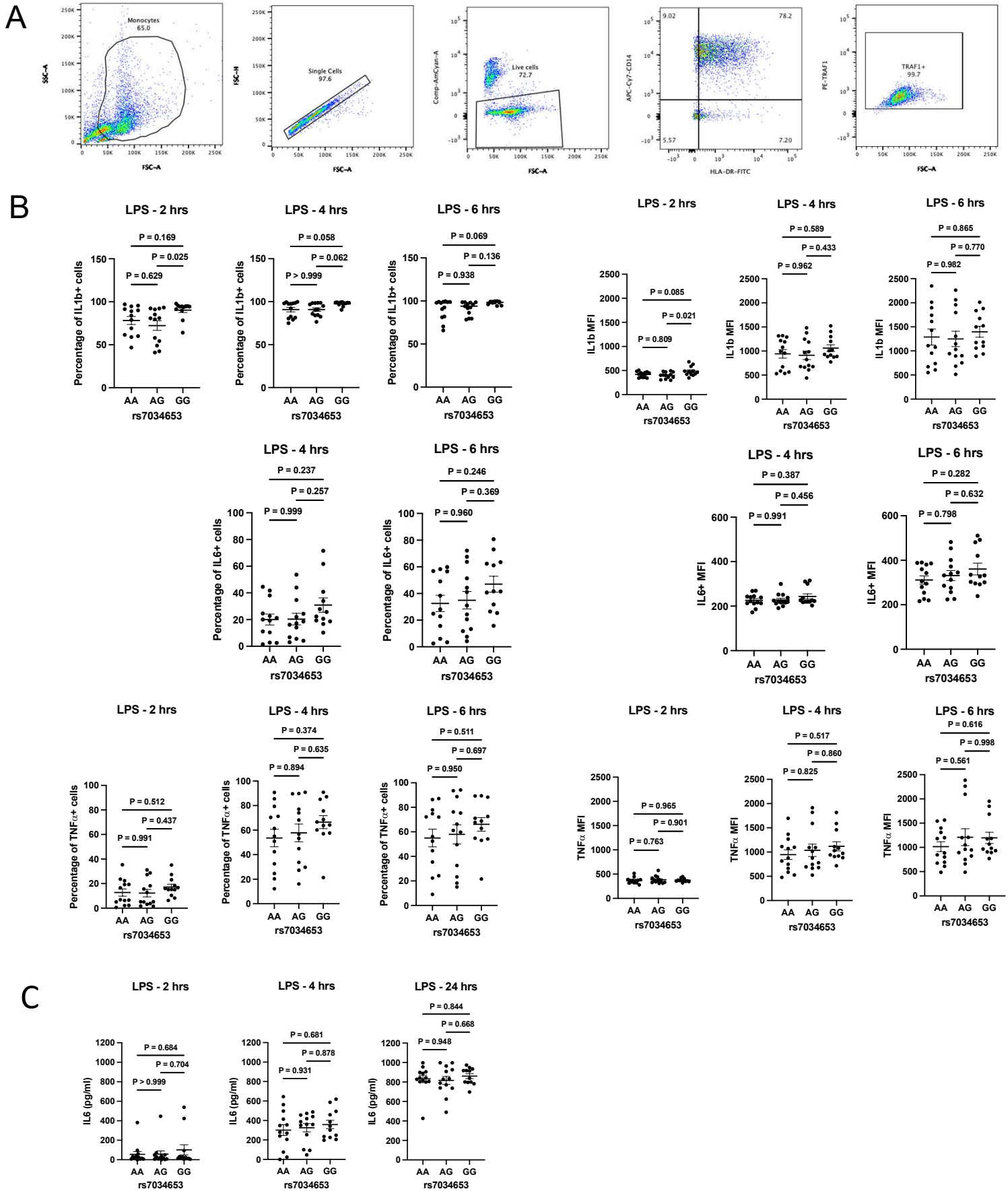Monocyte

Raw P value:0.008 Adj. P value: 0.86 Effect size: 0.41

B   **SNPs in cis-mQTLs with TRAF1 from BIOS QTL browser**

| P-value	SNP	SNP Chr.	SNP Chr. Position	CpG	CpG Chr.	CpG Chr. position	SNP Alleles	Assesed Allele	Z-score	Gene name	FDR
6.15E-229	rs7034653	9	123687372	cg21152671	9	123690610	A/G	G	32.3	TRAF1	0
5.55E-76	rs1014530	9	123685092	cg14064762	9	123688769	C/T	T	18.45	TRAF1	0
6.03E-56	rs1014530	9	123685092	cg15551881	9	123688691	C/T	T	15.76	TRAF1	0
1.66E-13	rs77617771	9	123729896	cg15551881	9	123688691	G/A	A	7.37	TRAF1	0
2.49E-06	rs10985143	9	123829010	cg15551881	9	123688691	C/G	G	4.71	TRAF1	0
3.14E-05	rs10739574	9	123594836	cg14064762	9	123688769	G/A	A	4.16	TRAF1	0.02

Supplementary Figure 3. Quantitative trait loci data, related to Figure 4. (A) Expression quantitative trait loci data for rs7034653 and rs1609810 in human monocytes. **(B)** SNPs in cis-methylation quantitative trait loci with TRAF1 from BIOS QTL browser. QTL, quantitative trait loci.

S. Figure 4

A

B

C

Supplementary Figure 4. Cytokine production in human monocytes as a function of genotype at rs7034653, related to Figure 4. (A) Gating strategy for human monocytes. **(B)** Purified monocytes from healthy human PBMCs were treated with LPS (100 ng/ml for 2, 4 or 6 hrs), and percentage of cytokine producing cells as well as MFI was assessed by intracellular staining for IL-1, IL-6, and TNF. **(C)** IL-6 secreted by LPS-treated human monocytes measured by ELISA. Each symbol represents one donor; n = 13, 13, 12 human donors of genotypes AA, AG, GG, respectively. Statistical analysis was performed using one-way ANOVA multiple comparisons.

S. Figure 5

A

B
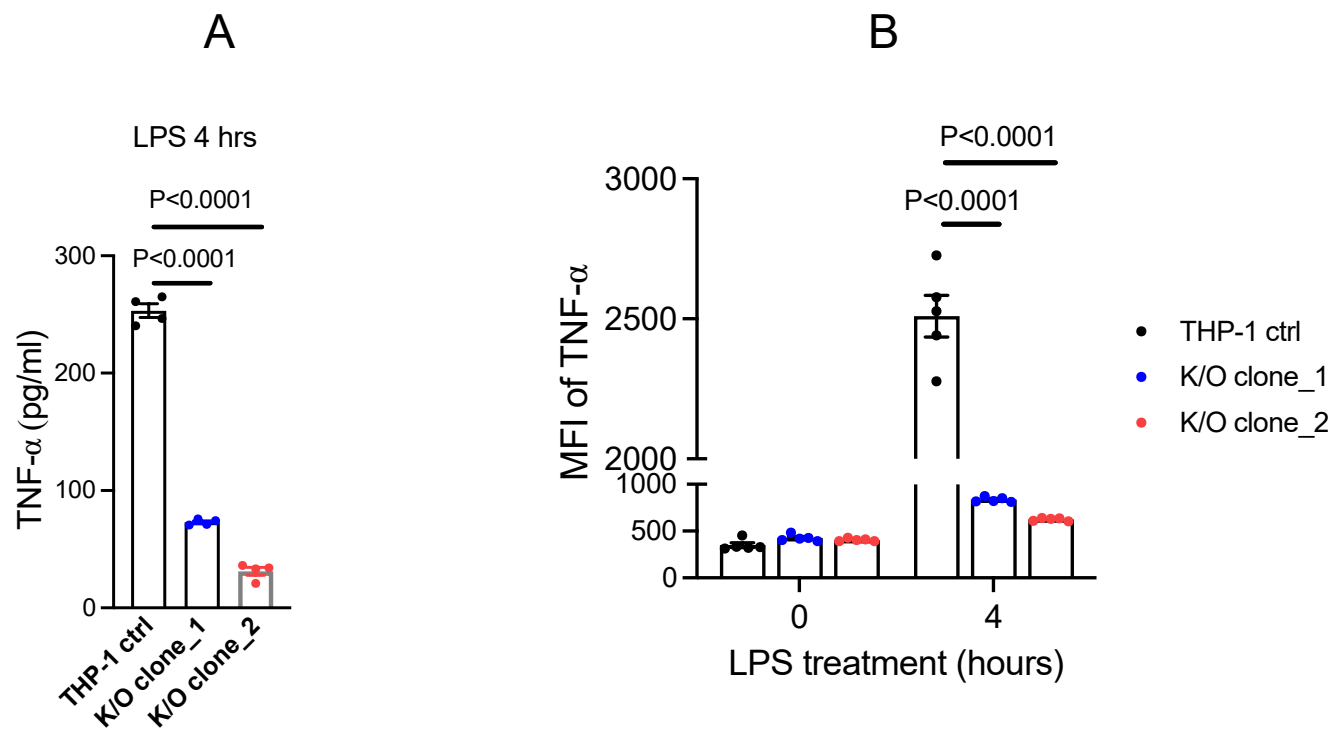
Supplementary Figure 5. Cytokine production by THP-1 cells deficient in FRA2, related to Figure 5. FRA2 knockout THP-1 clones show decreased TNF secretion **(A)** and intracellular production **(B)** compared with negative sgRNA-treated THP-1 control after 4 hours of LPS (100ng/ml) treatment (mean ± SEM, n = 4 (A), and n=5 (B)). Statistical analysis methods used were one-way ANOVA with multiple comparisons.