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Supplementary Materials for

YAP-mediated mechanotransduction tunes the macrophage inflammatory response

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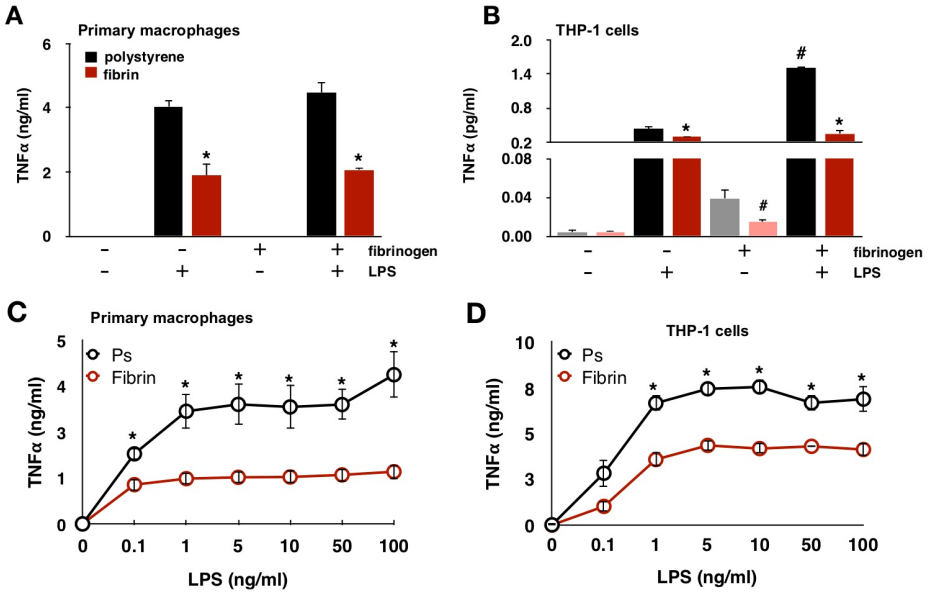

Fig. S1. Adhesion to fibrin hydrogels inhibits on inflammatory activation of macrophages. (**A**) TNFα secretion by human peripheral blood monocyte-derived macrophages and (**B**) PMA-treated THP-1 cells (right) cultured on 2 mg/ml fibrin gels or polystyrene, stimulated with 10 ng/ml ultrapure lipopolysaccharide (LPS) and/or 2 mg/ml soluble fibrinogen, or left unstimulated as a control, as measured by ELISA. (**C**) Secretion of TNFα by monocyte-derived macrophages cultured on 2 mg/ml fibrin gels or polystyrene, stimulated with the indicated concentrations of LPS and analyzed by ELISA (**D**) Secretion of TNFα by PMA-treated THP-1 cells cultured on 2 mg/ml fibrin gels or polystyrene, stimulated with the indicated concentrations of LPS and analyzed by ELISA. Data presented as mean ± SEM of 3 biological replicates and * $p<0.05$ when comparing fibrin vs. polystyrene with the same soluble condition and # $p<0.05$, when comparing fibrinogen vs. no fibrinogen, assessed by two-tailed Student's *t*-test.

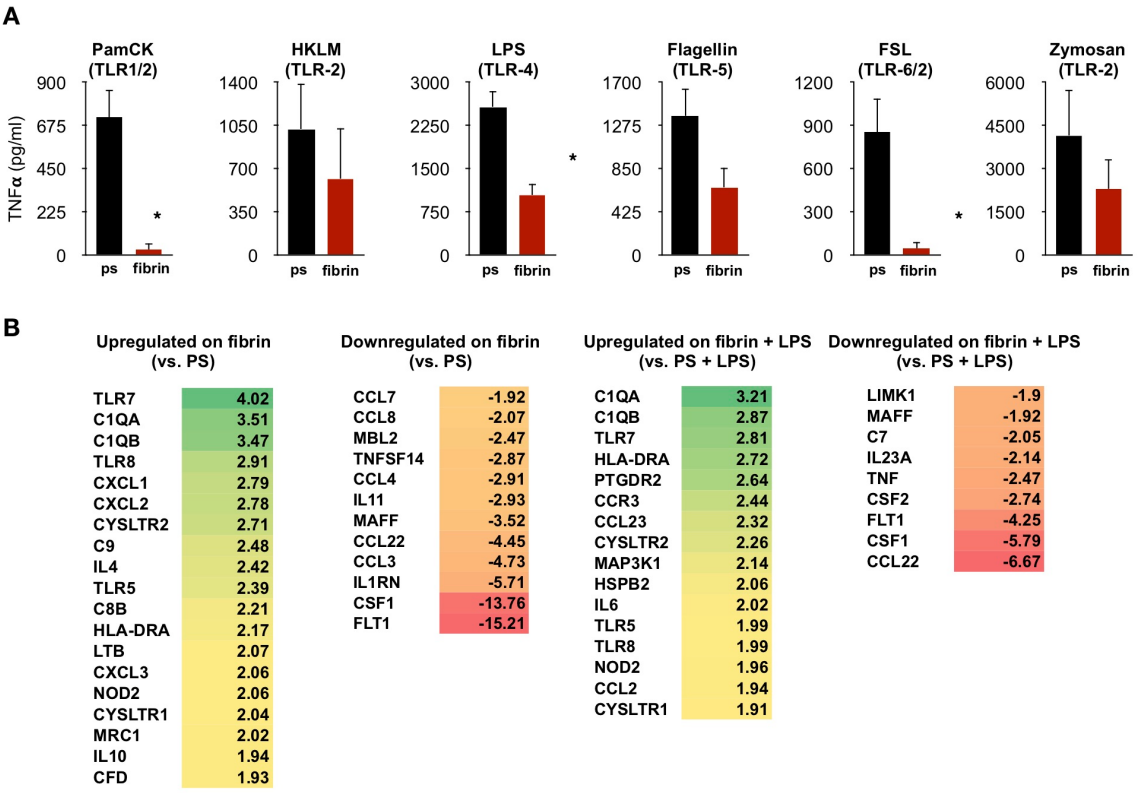

Fig. S2. Adhesion to fibrin broadly suppresses inflammatory activation of macrophages in response to Toll-like receptor (TLR) ligands. (**A**) Secretion of TNFα by monocyte-derived macrophages cultured on 2 mg/ml fibrin gels or polystyrene, stimulated with indicated TLR agonists, analyzed by ELISA. Values are the mean ± SEM of 3 biological replicates and * $p<0.05$ comparing fibrin to polystyrene, assessed by two-tailed student's *t*-test. (**B**) Gene expression of monocyte-derived macrophages cultured on 2 mg/ml fibrin gels or polystyrene, stimulated with LPS for 4h, analyzed using Nanostring inflammation gene expression panel. Values shown are the mean fold change across 2 biological replicates. Transcripts that were greater than 1.9 fold up or downregulated are shown.

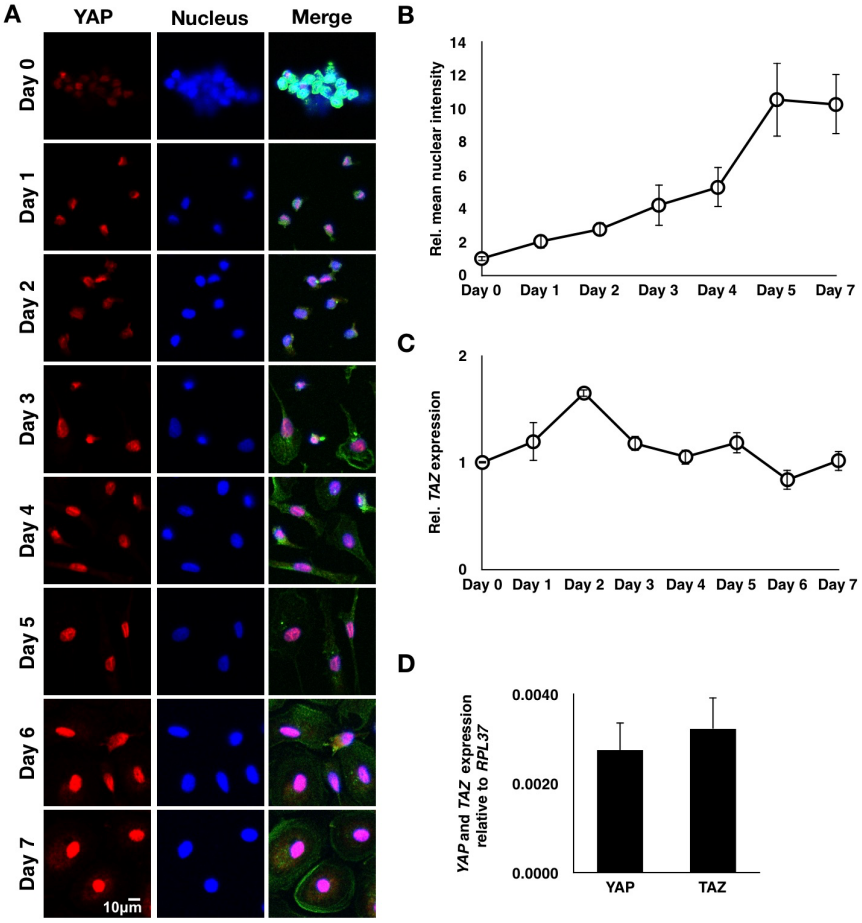

Fig. S3. YAP and TAZ expression with macrophage differentiation. (**A**) Confocal immunofluorescence images of YAP in monocytes cultured on glass with macrophage colony stimulating factor (M-CSF) to induce differentiation and stained for YAP during differentiation for seven days. (**B**) Graph shows average mean nuclear YAP intensity for two biological replicates normalized to Day 0**.** (**C**) Relative gene expression of *TAZ* in monocytes undergoing differentiation analyzed by qPCR. Graph shows mean expression for two biological replicates normalized to Day 0. (**D**) Gene expression of *YAP* and *TAZ* analyzed by qPCR in monocyte-derived macrophages differentiated on polystyrene for 7 days from three donors. Expression was normalized to *RPL37A* housekeeping gene. Values are the mean ± SEM of 3 biological replicates.

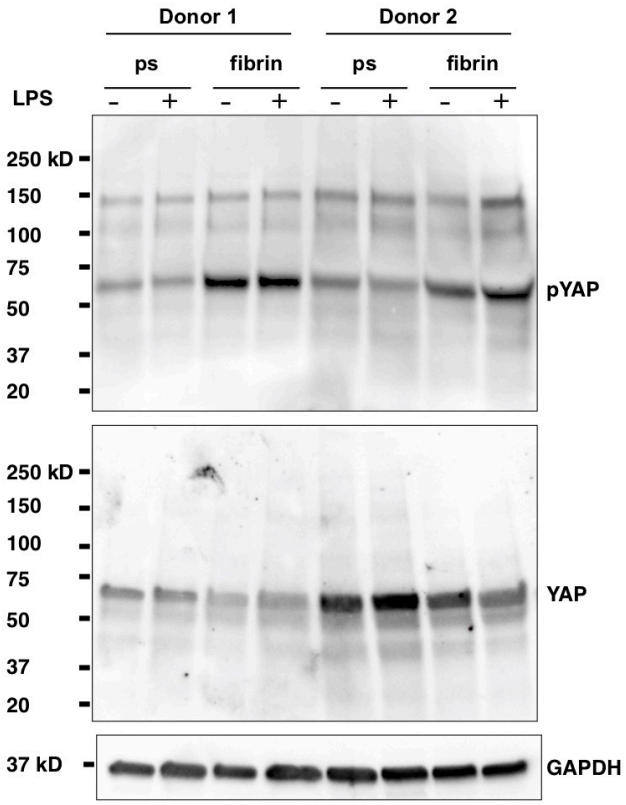

Fig. S4. Total and phosphorylated YAP is regulated by substrate. Representative complete Western blots of phosphorylated YAP (at serine 127, pYAP), total YAP, and GAPDH in monocyte-derived macrophages cultured on polystyrene or 2 mg/ml fibrin hydrogels for 24 h, and then stimulated with 10 ng/ml of LPS for 1 h. Two blood donors are shown.

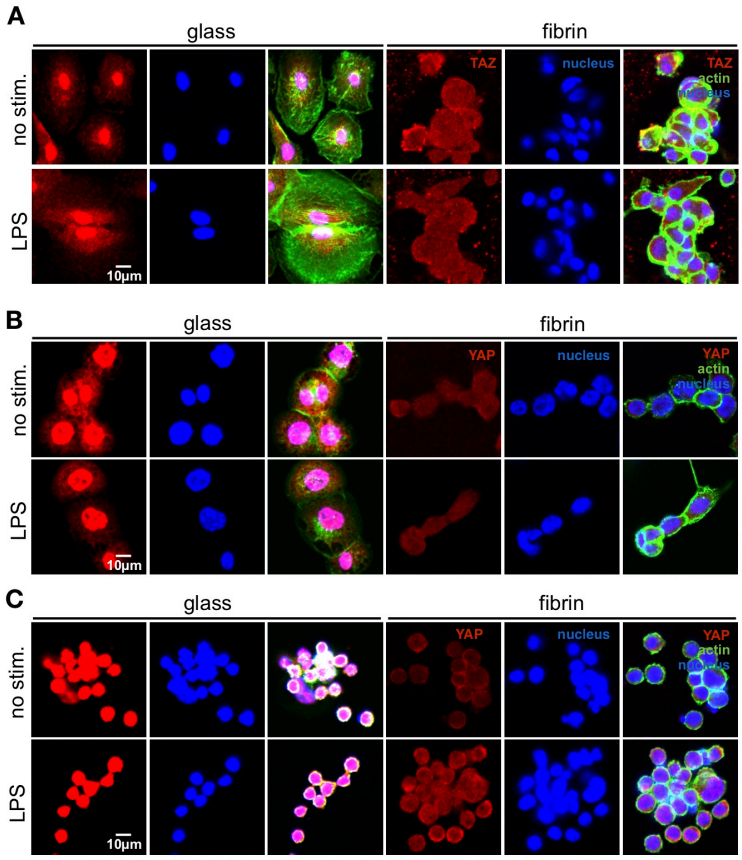

Fig. S5. TAZ localization in PBMC-derived macrophages and YAP localization in THP-1 and U937 human monocyte/macrophage cell lines. (**A**) Confocal immunofluorescence images of TAZ in monocyte-derived macrophages cultured on glass or 2 mg/ml fibrin for 24 h and stimulated with LPS for 1 h. Images are representative from 2 biological replicates. (**B**) Confocal immunofluorescence images of YAP in THP-1 cells cultured with phorbol myristate acetate (PMA) on 2 mg/ml fibrin gels or polystyrene for 24 h and then stimulated with LPS for 1 h (**C**) Confocal immunofluorescence images of YAP in U937 cells cultured with PMA on 2 mg/ml fibrin gels or polystyrene for 24 h and then stimulated with LPS for 1 h. Images are representative from 2 biological replicates.

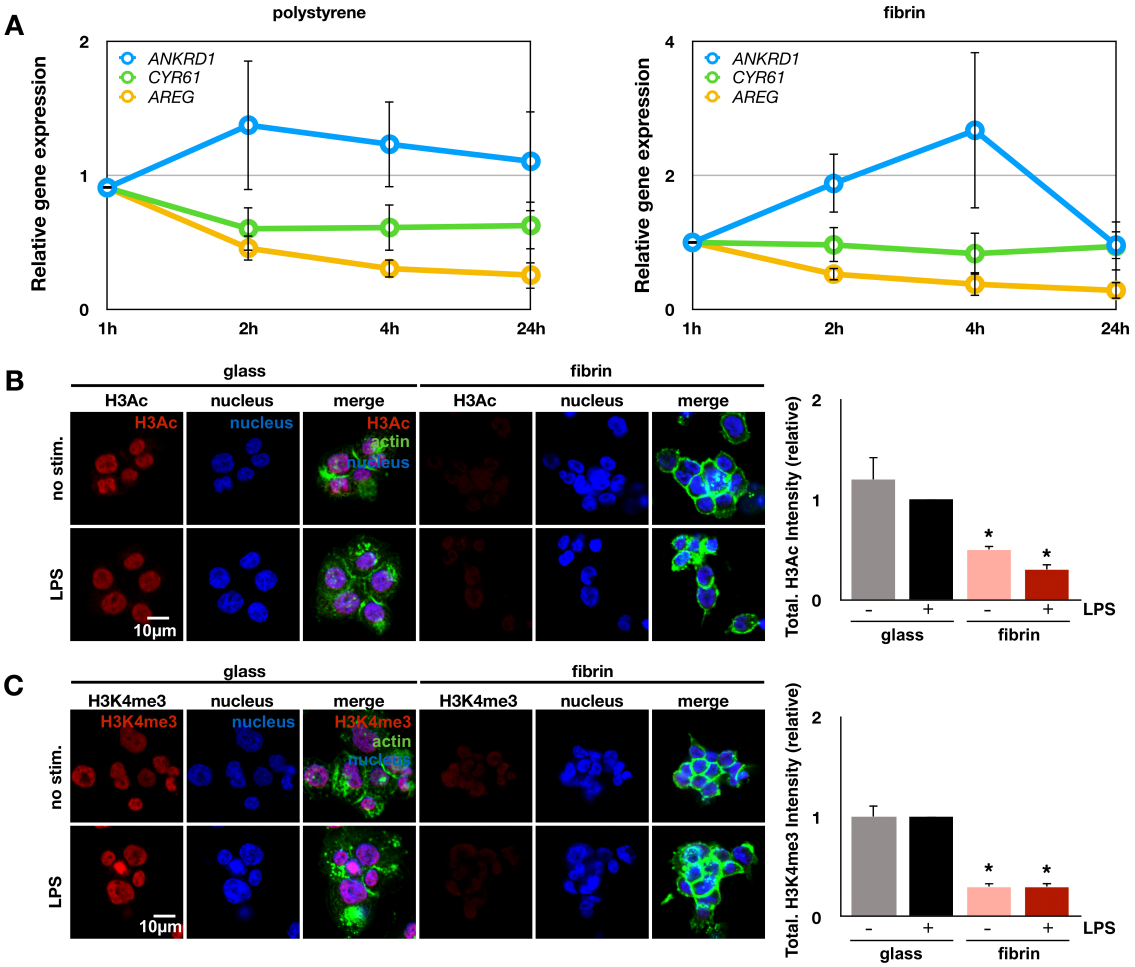

Fig. S6. Canonical YAP target gene expression and epigenetic modifications in macrophages cultured on polystyrene or glass and fibrin. (A) Expression of canonical YAP target genes including *ANKRD1, CYR61 and AREG* from monocyte-derived macrophages cultured on polystyrene or 2 mg/ml fibrin for 1, 2, 4 and 24 h. Values shown are expression normalized to *RPL37A* and then normalized to transcript level of the respective gene at 1 h. (**B**) Immunofluorescence confocal images of H3Ac in THP-1 cells treated with PMA and cultured on glass or 2 mg/ml fibrin for 42 h and stimulated with 10 ng/ml LPS for 6 h, and quantification of total intensity (right). (**C**) Immunofluorescence confocal images of H3K4me3 in THP-1 cells treated with PMA and cultured on glass or 2 mg/ml fibrin for 42 h and stimulated with 10 ng/ml LPS for 6 h, and quantification of total intensity (right). All images are representative across at least 3 biological replicates. For intensity, analysis was performed across 3 biological replicates and on at least 150 cells (for imaging). All the values are the mean ± SEM and * $p<0.05$ when comparing fibrin to glass assessed by two-tailed Student's *t*-test in (**B**) and (**C**).

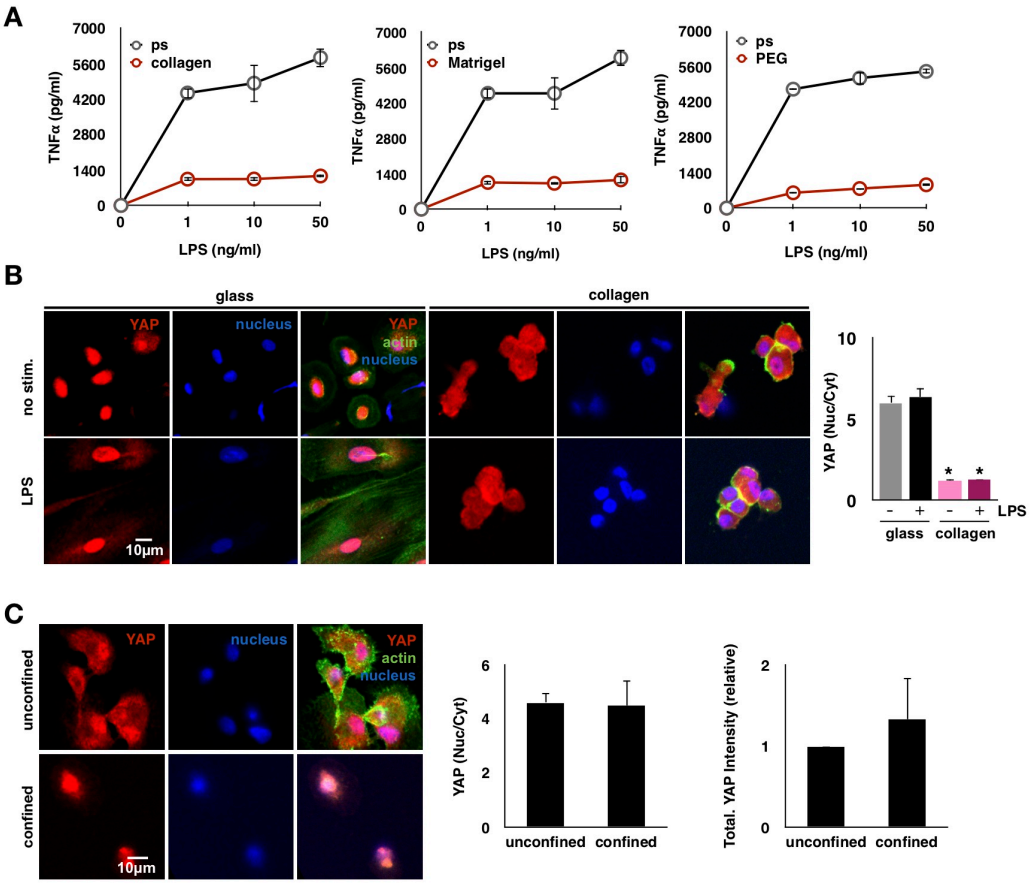

Fig. S7. Inflammatory response and YAP nuclear localization is regulated by hydrogel environments, but not micropatterning-induced adhesive confinement. (**A**) Secretion of TNFα by monocyte-derived macrophages cultured on 2 mg/ml of collagen, 3mg/ml Matrigel, or 5% polyethylene glycol (PEG) gels and stimulated with the indicated concentrations of LPS and analyzed by ELISA. Values are the mean ± SEM of 3 biological replicates for collagen and Matrigel and 2 replicates for PEG gels and * $p<0.05$ compared to cells seeded on polystyrene (ps) controls, assessed by two-tailed student's *t*-test. (**B**) Immunofluorescence confocal images of YAP (left) in monocyte-derived macrophages cultured on glass or 2 mg/ml collagen for 24 h and stimulated with 10 ng/ml LPS for 1 h, and quantification of nuclear/cytoplasmic ratio (right). Values are the mean ± SEM of two biological replicates and * $p<0.05$ compared to glass control, assessed by two-tailed Student's *t*-test. (**C**) Confocal images of YAP in monocyte-derived macrophages, cultured on flat (unconfined) and 10 x 10 μm (confined) micropatterned islands for 24 h and stained for YAP (left), and quantification of nuclear to cytoplasmic ratio and total intensity of YAP (right). Values are the mean ± SEM across 3 biological replicates.

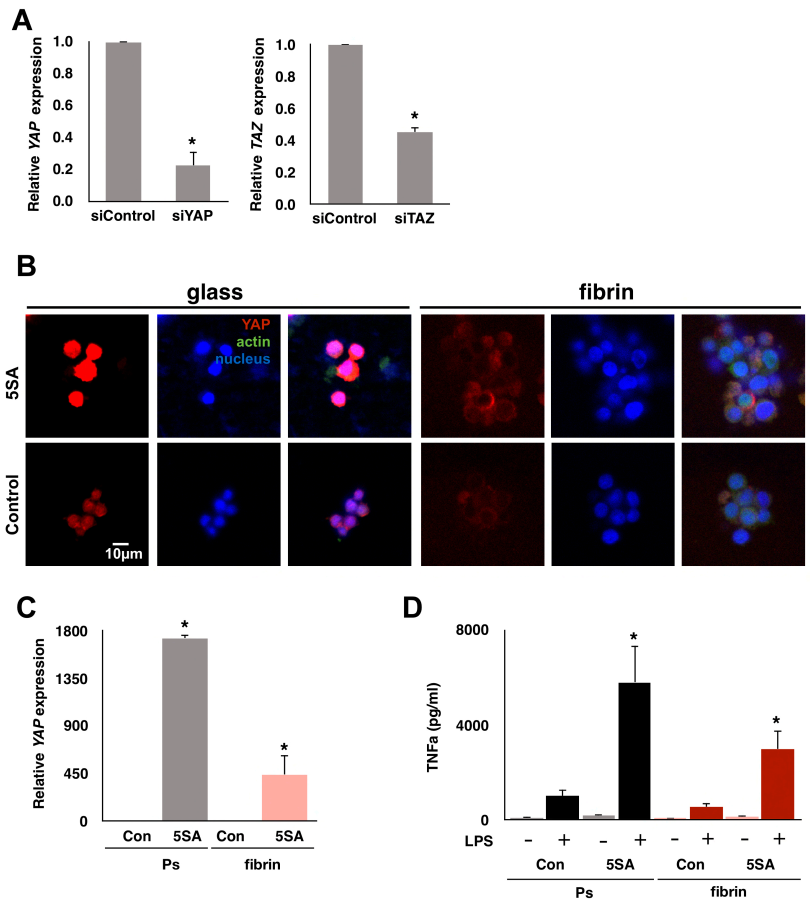

Fig. S8. Manipulation of YAP expression and activity. (**A**) Expression of *YAP* or *TAZ* gene analyzed by qPCR in monocyte-derived macrophages treated with (siYAP) or TAZ siRNA (siTAZ), respectively, relative to siControl. (**B**) Confocal images of YAP in YAP-5SA-expressing U937 cells cultured with PMA on glass or fibrin for 24 h. (**C**) YAP gene expression in conditions shown in (**B**) measured by qRT-PCR. Blank lentiviral vector was used as control. (**D**) Secretion of TNFα from cells in (**B**) stimulated with 10 ng/ml LPS, analyzed by ELISA. Values are mean ± SEM of 3 biological replicates and *p<0.05 when comparing YAP-5SA vs. control, assessed by two-tailed Student's *t*-test.

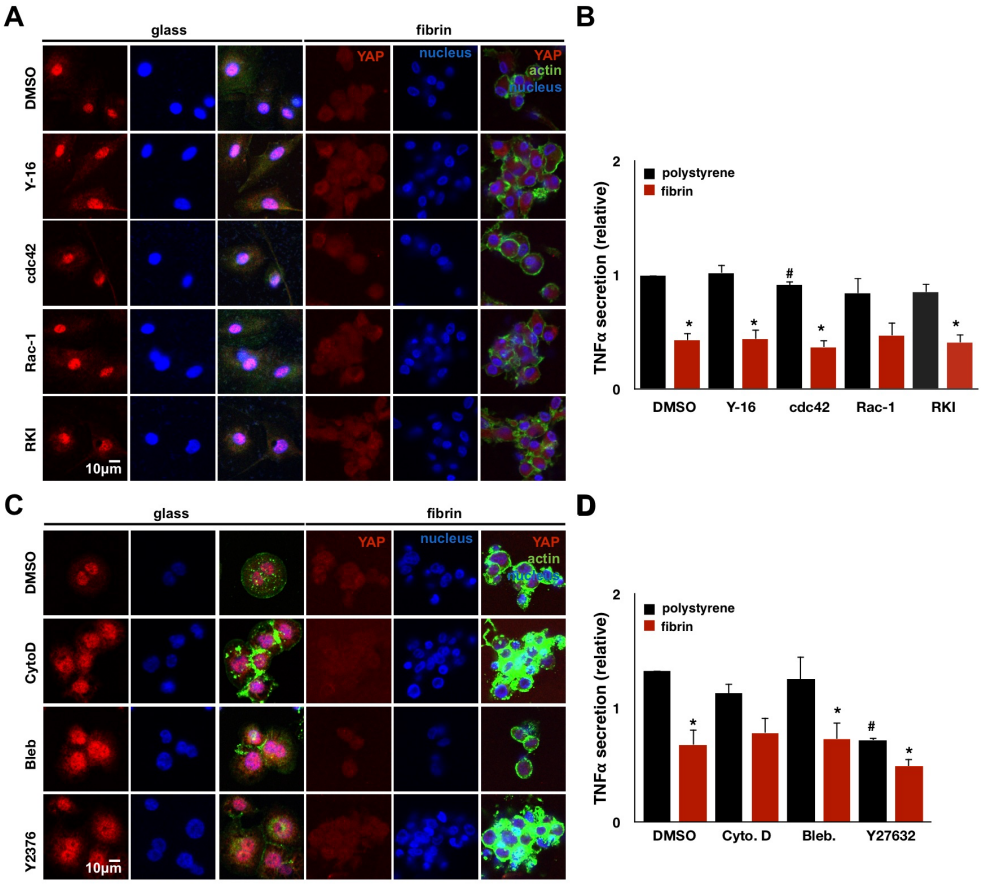

Fig. S9. Effect of Rho GTPase inhibitors and cytoskeletal drugs on YAP localization and inflammation. (**A**) Immunofluorescence confocal images of YAP in monocyte-derived macrophages, cultured on glass or 2 mg/ml fibrin hydrogel for 24 h and treated with indicated inhibitors of RhoA, Rac1, or Cdc42 and related signaling proteins for approximately 6 h. (**B**) Secretion of TNFα from cells in (**A**) except glass conditions were replaced with polystyrene, and stimulated with LPS for 6 h. (**C**) Immunofluorescence confocal images of YAP in PMA-treated THP-1 cells, cultured on glass or 2 mg/ml fibrin hydrogel for 24 h and treated with indicated cytoskeletal drugs for approximately 6 h (**D**) Secretion of TNFα secreted from cells in (**C**) except glass conditions were replaced with polystyrene, and stimulated with LPS for 6 h. Values are the mean ± SEM across 3 biological replicates and *$p<0.05$ comparing fibrin vs. glass or polystyrene and #$p<0.05$ comparing drug vs. DMSO, assessed by two-tailed Student's *t*-test.

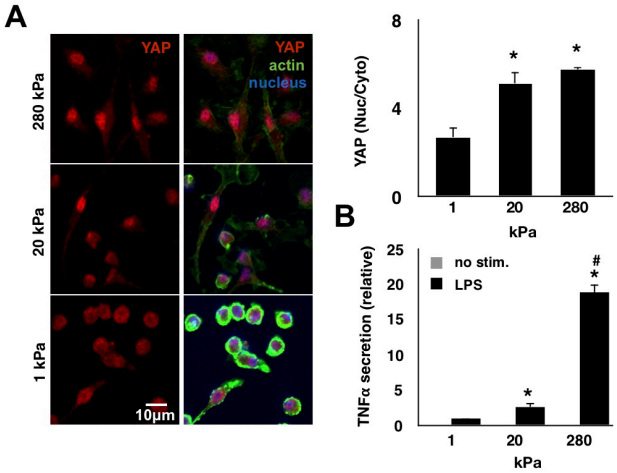

Fig. S10. Substrate stiffness modulates YAP nuclear localization and inflammation. (**A**) Immunofluorescence confocal images of YAP in mouse bone marrow derived - macrophages cultured on polyacrylamide (PA) gels of varying stiffness for 24 h and stimulated with LPS for 6 h (left) and quantification of nuclear to cytoplasmic ratio and total intensity (right). (**B**) Secretion of TNFα from cells in (A) and stimulated with 10 ng/ml of LPS for 6 h, analyzed by ELISA. Cytokines are quantified across 3 biological replicates. Immunostaining analysis was performed on at least 150 cells across 3 biological replicates at each time points. Values are the mean ± SEM and * $p<0.05$ when comparing 1 to 20 or 280 kPa and #$p<0.05$ when comparing 1 and 20 to 280 kPa assessed by two-tailed Student's *t*-test.

Supplementary Table 1: List of primers used in this study for qRT-PCR analysis.

Gene Name	Forward primer (5'-3')	Reverse primer (5'-3')
TNF	AGGCGCTCCCCAAGAAGACAGG	CAGCAGGCAGAAGAGCGTGGTG
IL6	AAGCCAGAGCTGTGCAGATGAGTA	CTTGGTCACCGACGTCCTGT
MCP1	CCCCAGTCCCTGCTGTTAT	TGGAATCCTGAACCCACTTC
IL10	TCATTCCCCAACCACTTCAT	GTAGAGACGGGGTTTCACCA
YAP	GCAGTTGGGAGCTGTTTCTC	GCCATGTTGTTGTCTGATCG
CYR61	TCACCCTTCTCCACTTGACC	AGTTTTGCTGCAGTCCTCGT
AREG	CTGGGAAGCGTGAACCATTTT	TCTGAGTAGTCATAGTCGGCTC
ANKRD1	GAACTGGTCACTGGAAAGAAGAATG	GGTGGGCTAGAAGTGTCTTCAGA
RPL37A	ATTGAAATCAGCCAGCACGC	AGGAACCACAGTGCCAGATCC

Supplementary Table 2: List of inhibitor drugs and their working concentrations used this study.

Inhibitor Drug	Working Concentration	Function
Cytochalasin D	10 μM	Potent inhibitor of actin polymerization
Blebbistatin	30 μM	Myosin inhibitor, specific for myosin II
Y-27632	30 μM	ROCK inhibitor
Rho II Y16	15 μM	Rho inhibitor II, targets RhoGEFs
Rac1	100 μM	Rac1 inhibitor, inhibits Rac1 GDP/GTP exchange activity
Cdc42 III	50 μM	Cdc42 inhibitor III, targets Cdc42 GEF