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SUPPLEMENTARY INFORMATION

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Spatial confinement downsizes the inflammatory response of macrophages

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Supplementary Information

Spatial Confinement Downsizes the Inflammatory Response of Macrophages

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Legends for supplementary videos:

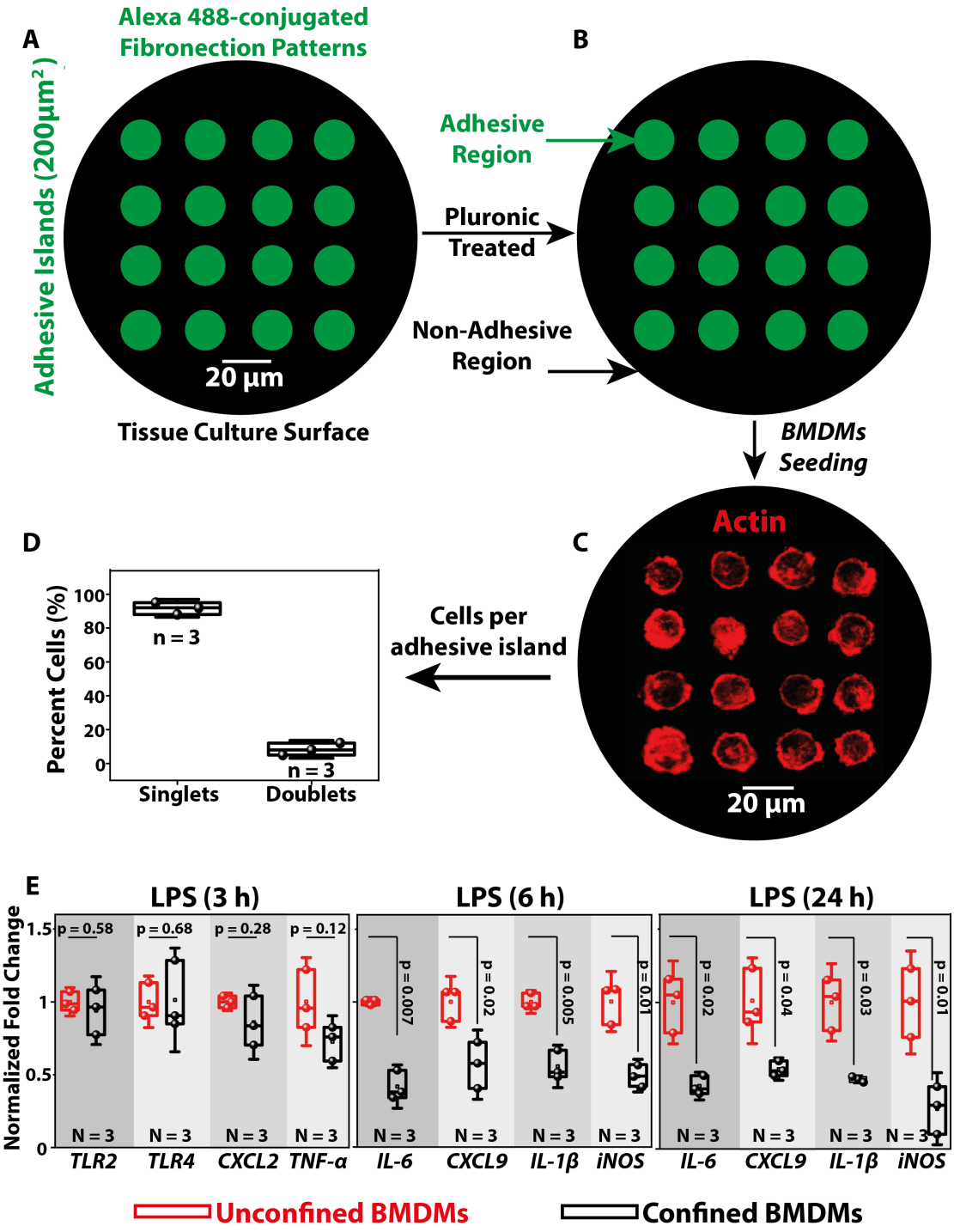
Supplementary Video 1: Video shows spreading of Control BMDMs. BMDMs were seeded on glass bottom dishes. 30 min after seeding, phase contrast images were captured every 10 min. The movie represents 990 min (frame interval of 10 min). Imaging was performed while maintaining the cells at 37 ^{o}C and 5% CO_2 conditions. Scale bar = 100 μm. Experiment was independently repeated three times.

Supplementary Video 2: Video shows spreading of LPS-treated BMDMs. BMDMs were seeded on glass bottom dishes. 30 min after seeding, LPS was added and phase contrast images were captured every 10 min. The movie represents 990 min (frame interval of 10 min). Scale bar = 100 μm. Live imaging was performed while maintaining the cells at 37 ^{o}C and 5% CO_2 conditions. Scale bar = 100 μm. Experiment was independently repeated three times.

Supplementary Video 3: Video shows spreading of IL-4/IL-13-treated BMDMs. BMDMs were seeded on glass bottom dishes. 30 min after seeding, IL-4/IL-13 was added and phase contrast images were captured every 10 min. The movie represents 990 min (frame interval of 10 min). Scale bar = 100 μm. Live imaging was performed while maintaining the cells at 37 ^{o}C and 5% CO_2 conditions. Scale bar = 100 μm. Experiment was independently repeated three times.

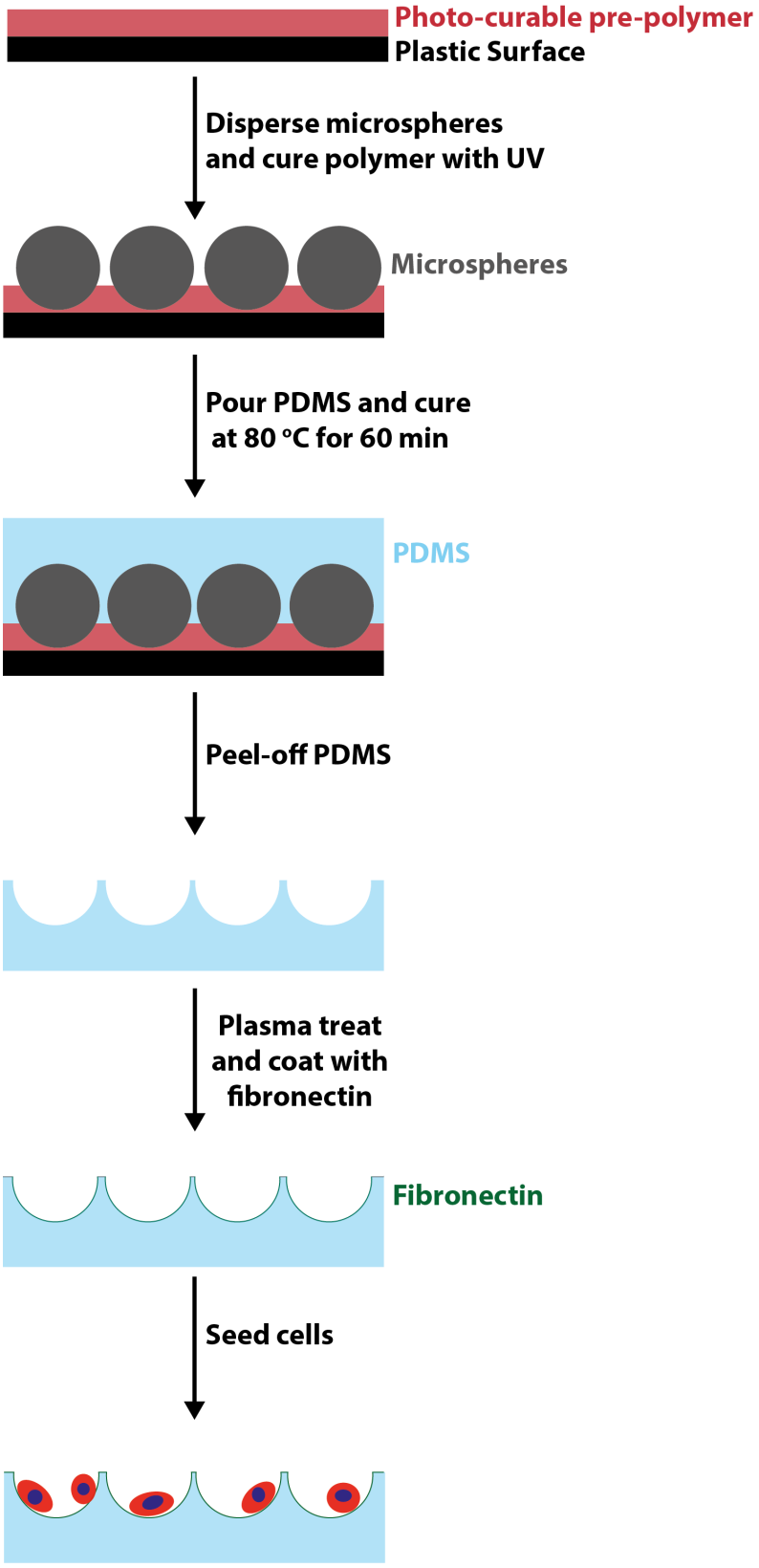
Supplementary Video 4: 3D reconstruction of Unconfined (UC) BMDM treated with LPS for 6 h and stained for nuclei, F-actin, G-actin and MRTF-A.

Supplementary Video 5: 3D reconstruction of Confined (CC) BMDMs treated with LPS for 6 h and stained for nuclei, F-actin, G-actin and MRTF-A.

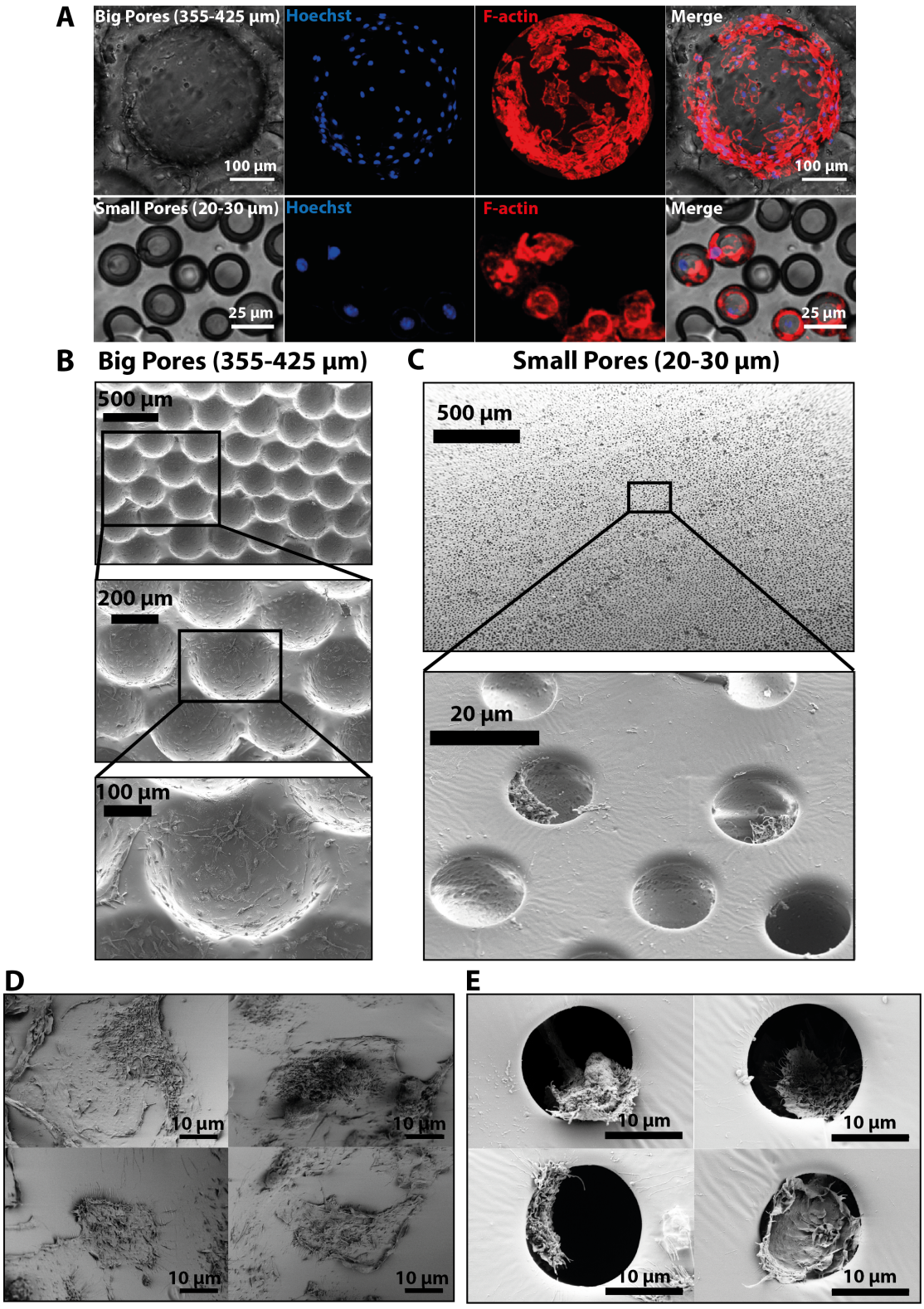


Supplementary Figure 1: Sculpting single macrophage confinement by micro-contact printing: (A) Alexa-488 labeled fibronectin coated circular micropatterned substrates of area ~200 μm^2 in a tissue culture plate. Scale bar = 20 μm. (B) Pluronic F-127 treatment for 30 min was done to passivate non-fibronectin coated regions. (C) Single confined on microfabricated patterns and stained with F-actin. (D) Box plots show the percentage of single cells obtained after seeding BMDMs on micropatterned

substrates. Data are pooled from n = 3 independent experiments. (E) Box plots show the expression levels of early and late responsive gene clusters in Unconfined (UC) and Confined (CC) BMDMs treated with LPS. Levels are normalized to the LPS-treated UCs. LPS treatment time is mentioned in the respective plots. N = number of biological replicates. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by a middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated three times with similar results.

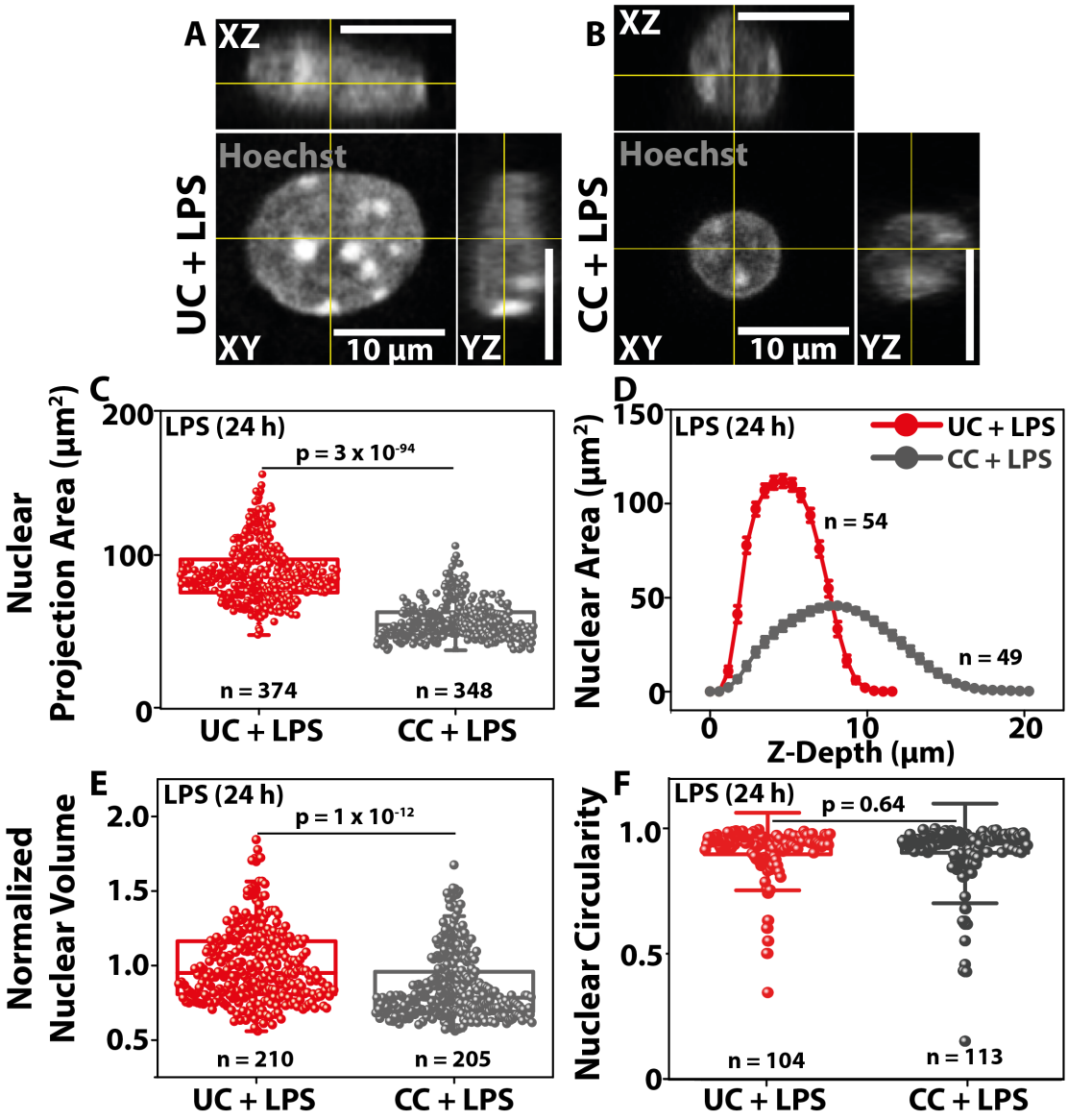


Supplementary Figure 2: Sculpting macrophage confinement by microwells of different pore sizes (big pores (355-425 µm) vs. small pores (20-30 µm)) to mimic the surface structure of porous implant materials.



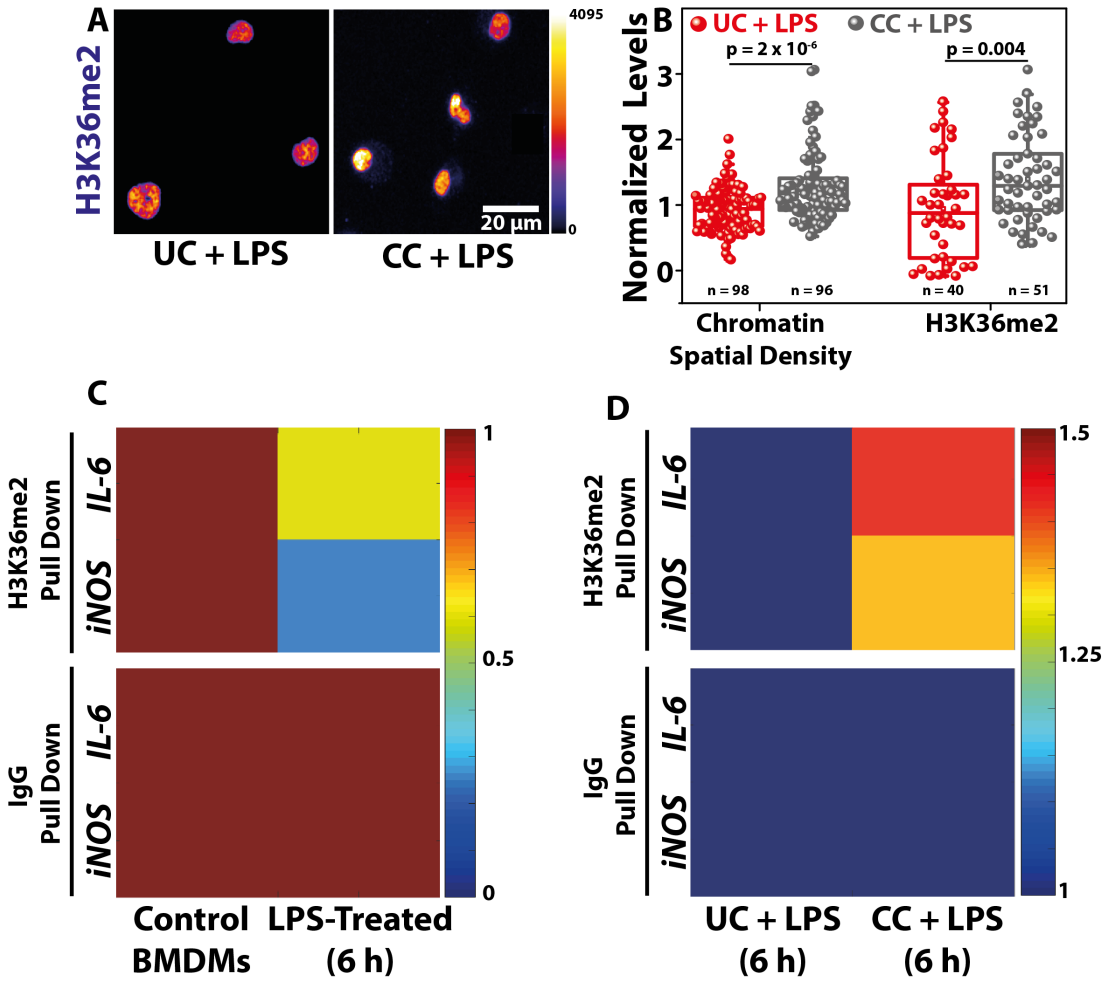
Supplementary Figure 3: (A) BMDMs cultured in microwells of different pore sizes (big pores (355-425 µm) vs. small pores (20-30 µm)) and stained for nuclei and F-actin. (B&C) Electron microscopy (EM) images of cells cultured in these microwells. EM

images were captured at an angle of 40 degree. Collage of EM images showing top view of different cells cultured in (D) big pores and (E) small pores. All the experiments were independently repeated three times with similar results.



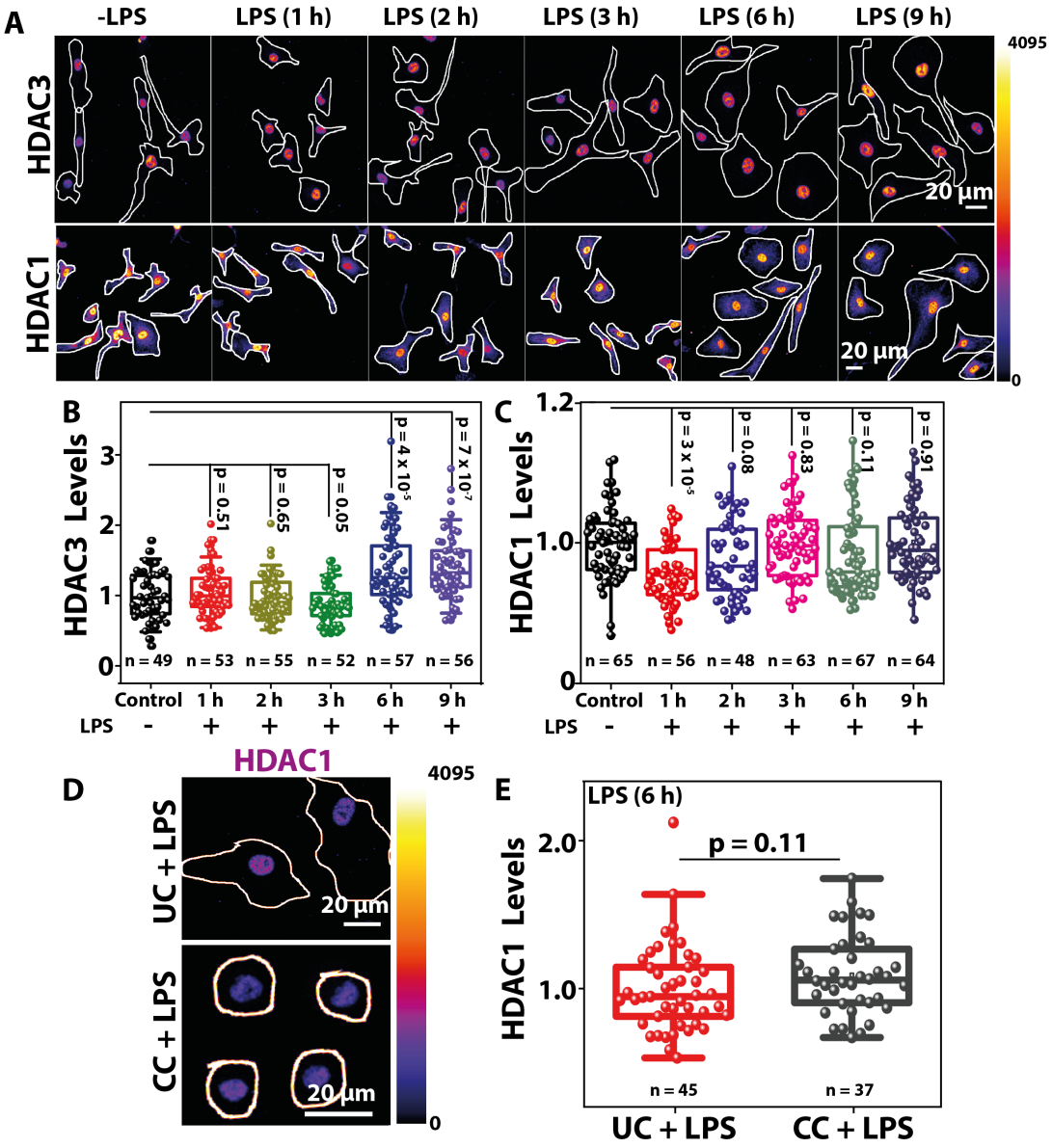
Supplementary Figure 4: Macrophage confinement regulates nuclear size: (A&B) Representative orthogonal views of nuclei in Unconfined (UC) and Confined (CC) BMDMs treated with LPS. Scale bar = 10 µm. (C) Box plots show nuclear projection areas in LPS-treated UCs and CCs. (D) Variation in nuclear area as a function of Z-depth between LPS-treated UCs and CCs. Data are shown as Mean ± S.E. (E) Box plots show nuclear volumes in LPS-treated UCs and CCs. Levels are normalized to the LPS-treated UCs. (F) Box plots show 2D nuclear circularity in LPS-treated UCs and CCs. Circularity of a cell is defined as $\frac{4\pi\,(area)}{(perimeter)^2}$. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by the middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the

experiments were independently repeated three times with similar results.



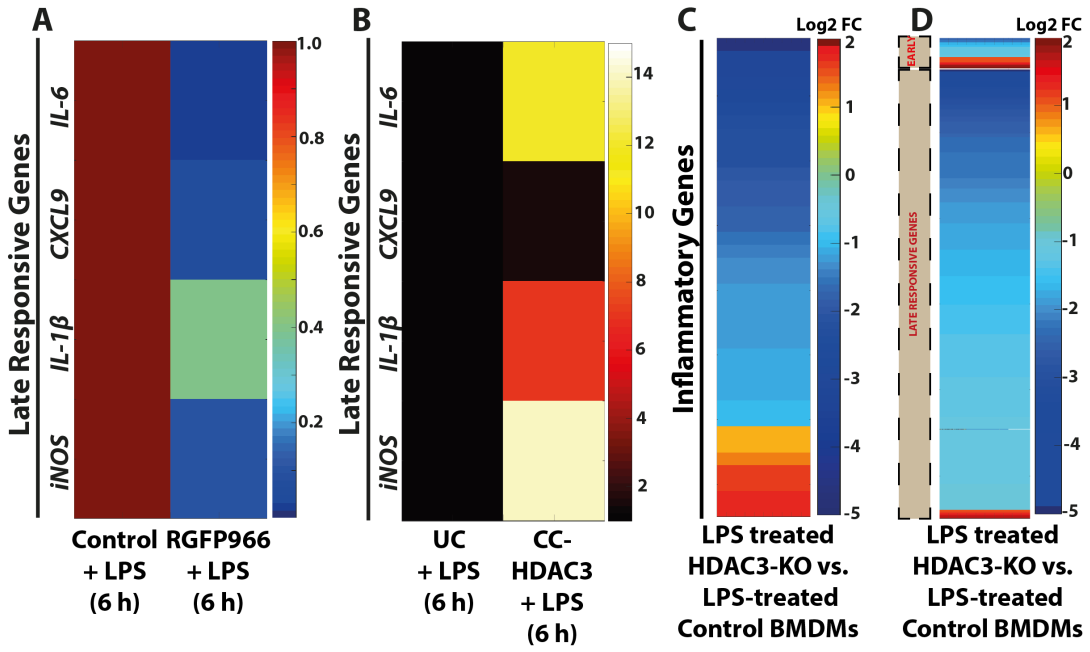
Supplementary Figure 5: Confinement regulates chromatin compaction and epigenetic modification: (A) Representative color-coded images of nuclei in LPS-treated Unconfined (UC) and Confined (CC) BMDMs stained for H3K36me2. Color-bar shows the pixel intensity values. Scale bar = 20 µm. (B) Box plots show the levels of chromatin average spatial density (total Hoechst33342 intensity per nuclear volume) and H3K36 di-methylation (H3K36me2) between LPS-treated UCs and CCs as normalized to LPS-treated UCs. The box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. Experiment was independently repeated three times. (C) Color-coded arrays show ChIP-qPCR analysis of H3K36me2 at the promoter region of *IL-6* and *iNOS* genes in Control and LPS-treated BMDMs. Levels are normalized to Control BMDMs. IgG served as a ChIP control. (D) Color-coded arrays show ChIP-qPCR analysis of H3K36me2 at the promoter region of *IL-6* and *iNOS*

genes in LPS-treated UCs and CCs. Levels are normalized to LPS-treated UCs. IgG served as a ChIP control. ChIP experiment was independently repeated twice with similar results.

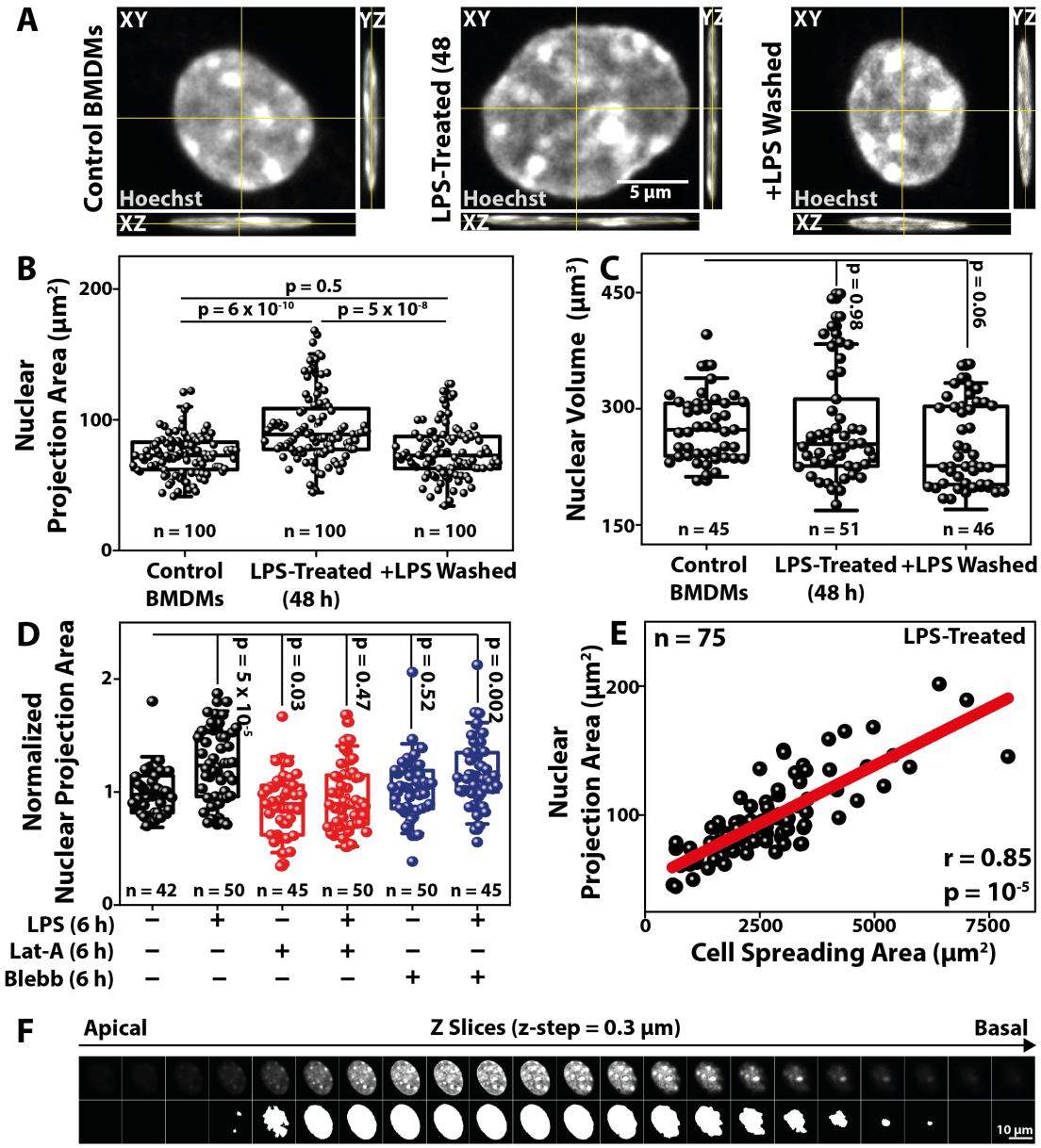


Supplementary Figure 6: HDAC3 expression, but not HDAC1 is dependent of cell confinement: (A) Color-coded representative images of Control and LPS-treated BMDMs for different periods of time and stained for HDAC3 and HDAC1. Color-bar shows the pixel intensity values. Cell edges are marked in white. Scale bar = 20 µm. Box plots show the time course of changes in levels of (B) HDAC3 and (C) HDAC1 in Control and LPS-treated BMDMs. Levels are normalized to the Control BMDMs. The box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. (D) Color-coded representative images of UCs and CCs treated with LPS for 6 h and stained for HDAC1. Cell edges are marked in white. Scale bar = 20 µm. (E) Box plots show levels of

HDAC1 in UCs and CCs treated with LPS for 6 h as normalized to the LPS-treated UCs. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated two time with similar results.

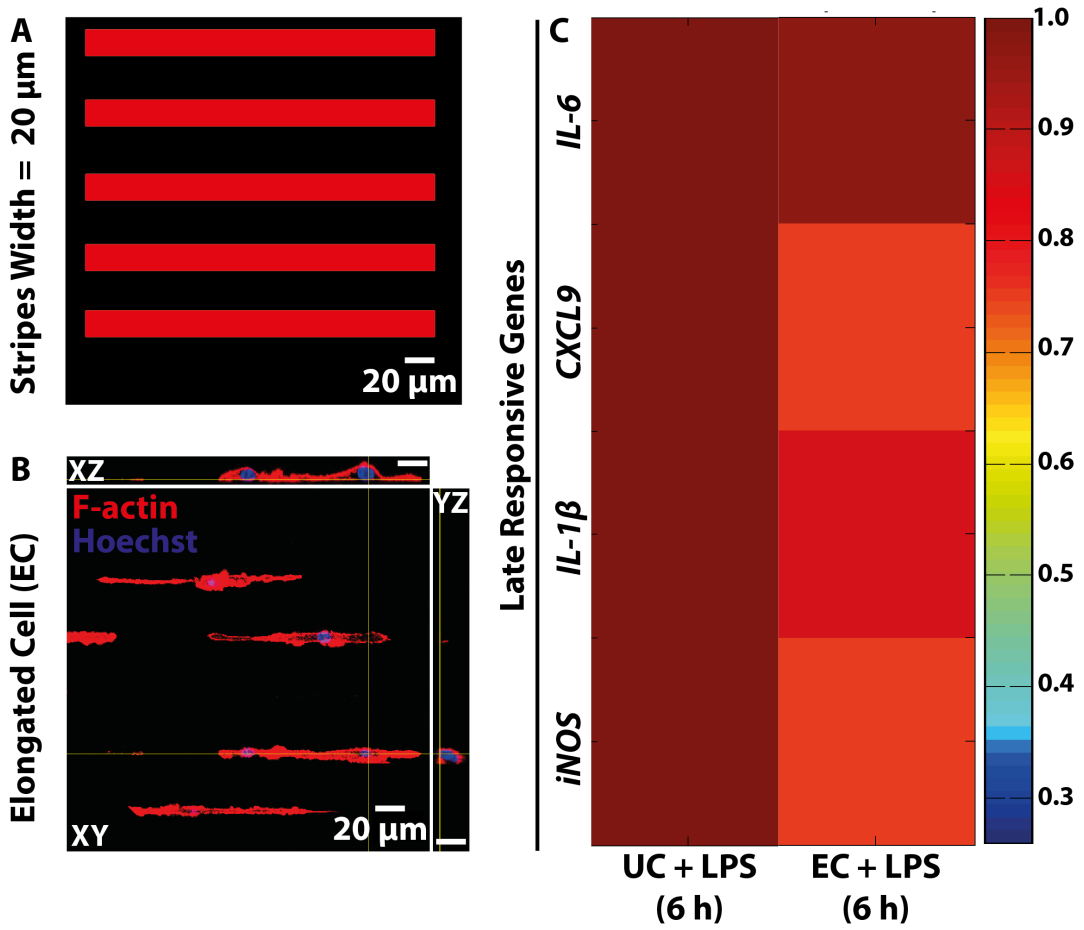


Supplementary Figure 7: Confinement-regulated HDAC3 activity is required for the expression of late-responsive genes: (A) Color-coded arrays show the expression levels of late-responsive genes, obtained by Real-Time PCR analysis, in Control and RGFP966 treated BMDMs, both treated with LPS. Levels are normalized to only LPS-treated BMDMs. (B) Color-coded arrays show the expression levels of late- responsive genes, obtained by Real-Time PCR analysis, in Unconfined Raw 264.7 macrophages (UCs) and HDAC3 overexpressing Raw 264.7 macrophages confined on micropatterns (CC-HDAC3), both treated with LPS. Levels are normalized to LPS-treated UCs. (C) Color-coded arrays show the log2 fold changes in the expression of known inflammatory genes in LPS-treated HDAC3-KO vs. LPS-treated Control BMDMs, plotted using previously published micro-array data (1). Refer to Supplementary data 1 for the gene list. (D) Color-coded arrays show the Log2 fold changes in the expression levels of all early and late responsive genes in LPS-treated HDAC3-KO cells vs. LPS-treated Control BMDMs. Refer to Supplementary data 1 for the gene list.

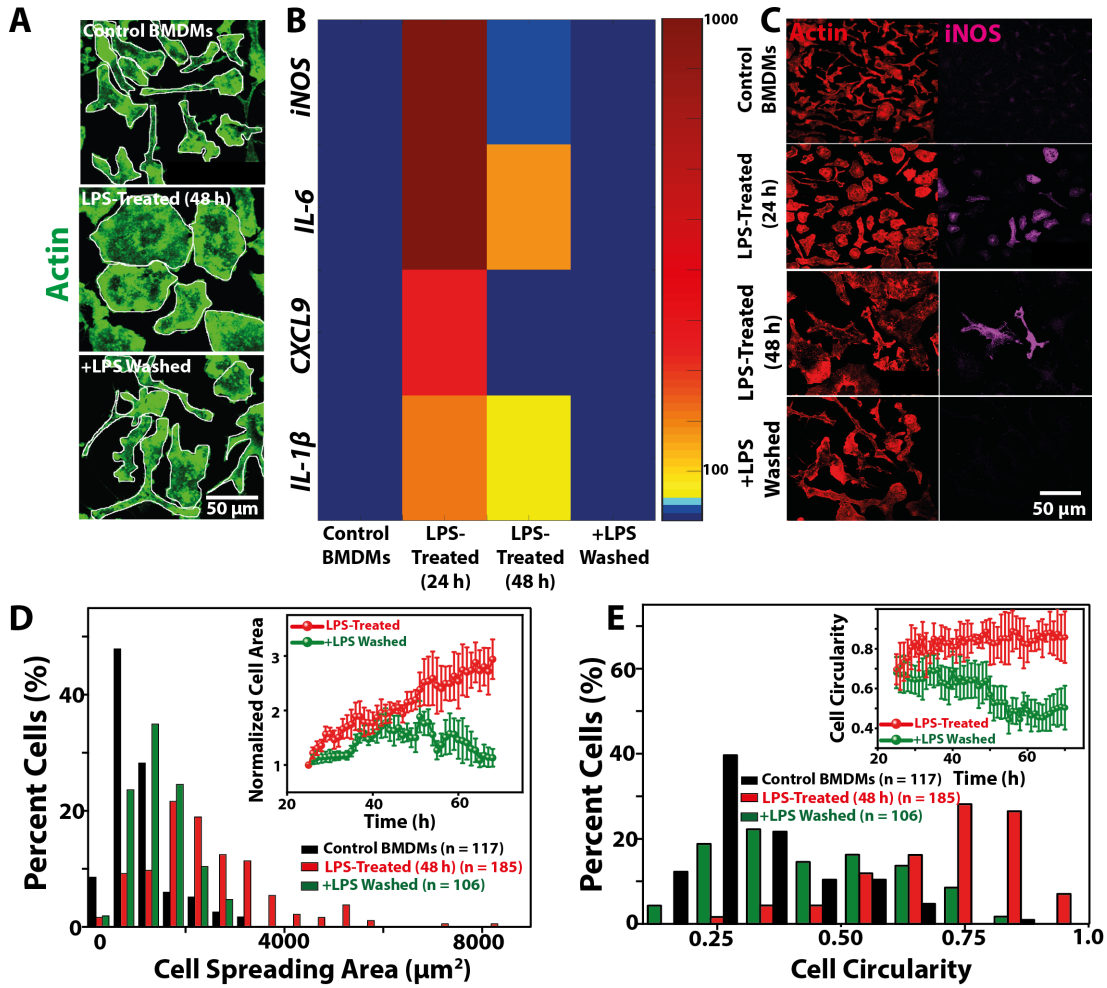


Supplementary Figure 8: Correlative changes in the cell and nuclear areas during M1 activation: (A) Orthogonal views of nuclei of Control, LPS-treated (48 h) and +LPS washed (BMDMs cultured first in medium supplemented with LPS for 24 h followed by culturing in medium without LPS for another 24 h) BMDMs. Scale bar = 5 µm. (B&C) Box plots show nuclear projection areas, and nuclear volumes in Control, LPS-treated (48 h) and +LPS washed BMDMs. (D) Box plots show normalized nuclear projection areas of BMDMs upon different drug treatments in the presence or absence of LPS. Levels are normalized to the untreated Control BMDMs. (E) Correlation plot between nuclear projection area and cell spreading area. 'r'= Pearson's correlation coefficient. (F) Z-sections of nucleus from basal to apical plane with a z-step size of

0.3 μm. Also, shown are the threshold images at different Z-sections used to quantify nuclear volume. Scale bar = 5 μm. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated three times with similar results.

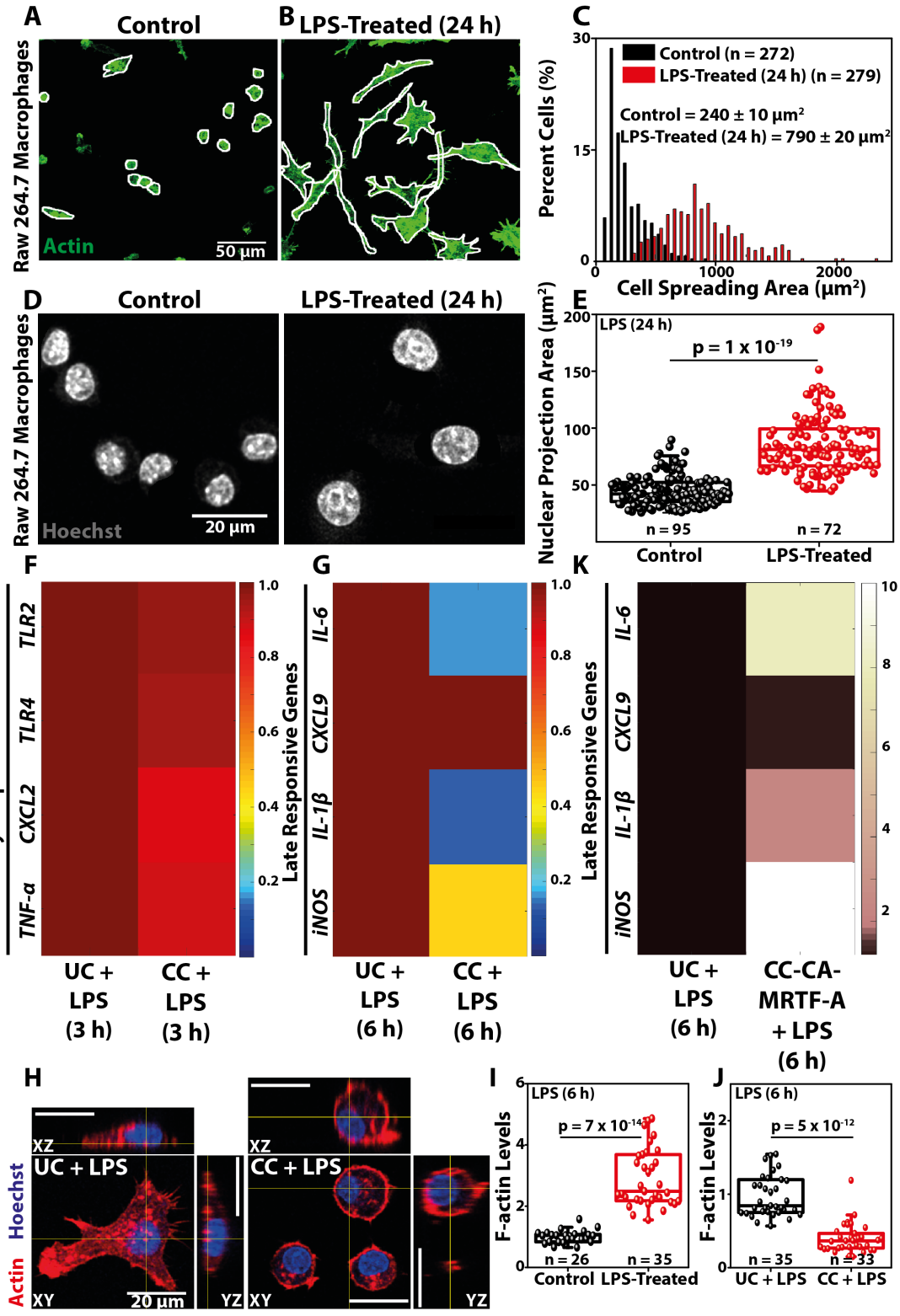


Supplementary Figure 9: Micro-stripes-imposed macrophage elongation has no effect on late-responsive gene expression: (A) Schematic showing Alexa-568 labeled fibronectin-coated micropatterned stripes (width = 20 µm) in a tissue culture plate to control BMDM elongation. Scale bar = 20 µm. (B) BMDMs cultured on stripes and stained for F-actin and nuclei. Scale bar = 20 µm. (C) Color-coded arrays show the expression levels of various late-responsive genes in Unconfined (UC) and Elongated (EC) BMDMs treated with LPS for 6 h. Levels are normalized to the LPS-treated UCs. Experiment was independently repeated twice with similar results.



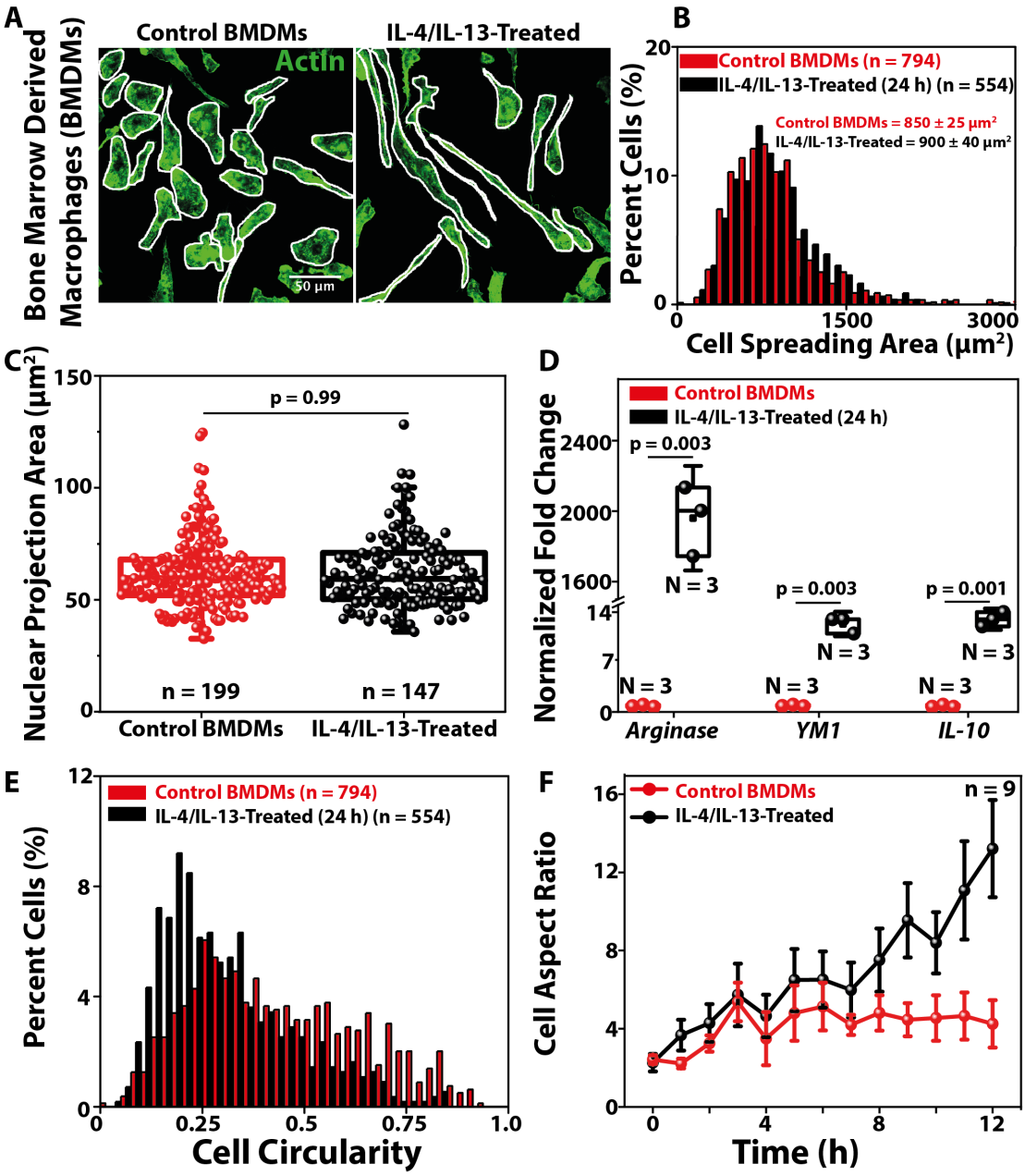
Supplementary Figure 10: Macrophage activation is transcriptionally and morphologically reversible: (A) Representative images showing Control, LPS-treated (48 h), +LPS washed (BMDMs cultured first in medium supplemented with LPS for 24 h followed by culturing in medium without LPS for another 24 h) BMDMs stained for F-actin. Cell edges are marked in white. (B) Color-coded arrays show the expression levels of pro-inflammatory genes in Control, LPS-treated, +LPS washed BMDMs. Levels are normalized to the untreated Control BMDMs. (C) Representative images showing Control, LPS-treated, +LPS washed BMDMs stained for F-actin and iNOS. (D) Normalized distributions of cell spreading areas of Control, LPS-treated and +LPS washed BMDMs. Inset shows the quantification of cell spreading areas in live LPS-treated and +LPS washed BMDMs. Data are shown as Mean ± S.E. (E) Normalized distributions of cell circularity of Control, LPS-treated and +LPS washed BMDMs. Inset shows the quantification of cell circularity in live LPS-treated and +LPS washed BMDMs. Data are shown as Mean ± S.E. Scale bar for all the images = 50 µm. n =

number of cells analyzed per condition. All the experiments were independently repeated three times with similar results.



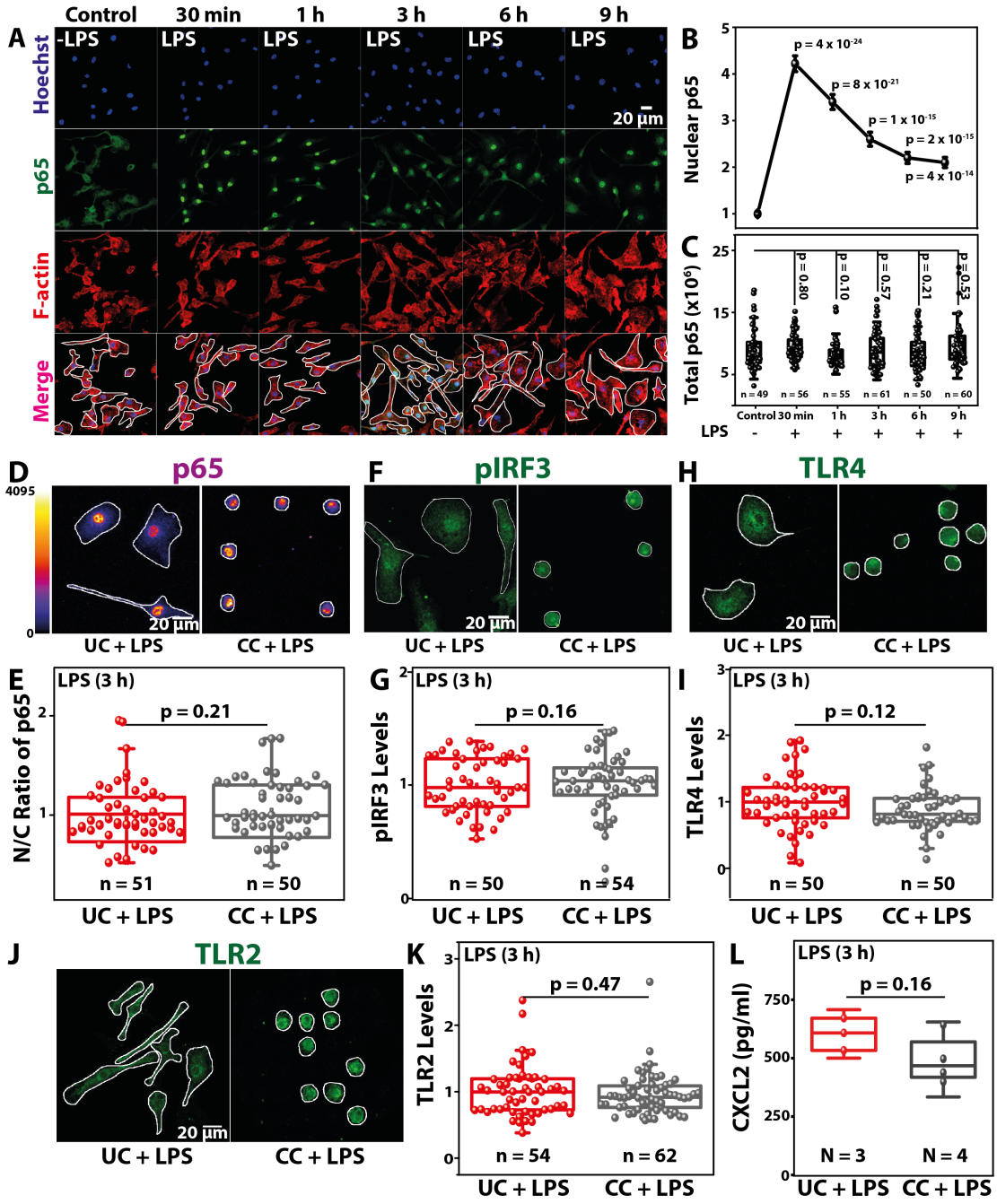
Supplementary Figure 11: M1 activation of RAW 264.7 macrophages is accompanied by changes in cell and nuclear size and is downsized by confinement: (A&B) Representative images showing Control and LPS-treated (24 h) RAW 264.7 macrophages stained for F-actin. Cell edges are marked in white. Scale bar = 50 µm.

(C) Normalized distributions of cell spreading areas of Control and LPS-treated RAW 264.7 macrophages. (D) Representative images and (E) quantification of nuclear projection areas in Control and LPS-treated RAW264.7 macrophages. Scale bar = 20 μm. (F&G) Color-coded arrays show the expression levels of early and late responsive genes in Unconfined (UC) and Confined (CC) RAW 264.7 macrophages treated with LPS. Levels are normalized to the LPS-treated UCs. (H) Representative orthogonal views of UCs and CCs on micropatterned substrates stained for F-actin and nuclei. Scale bar = 20 μm. (I) Box plots show levels of F-actin in Control and LPS-treated RAW 264.7 macrophages as normalized to the Control RAW 264.7 macrophages. (J) Box plots show levels of F-actin in UCs and CCs treated with LPS for 6 h as normalized to the LPS-treated UCs. (K) Color-coded arrays show the expression levels of late-responsive genes, obtained by Real-Time qPCR analysis, in Unconfined (UC) RAW 264.7 macrophages and constitutively active (CA) MRTF-A expressing plasmid transfected in RAW 264.7 macrophages confined on micropatterns (CC-CA-MRTF-A), both treated with LPS for 6 h. Levels are normalized to LPS-treated UCs. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated twice with similar results.



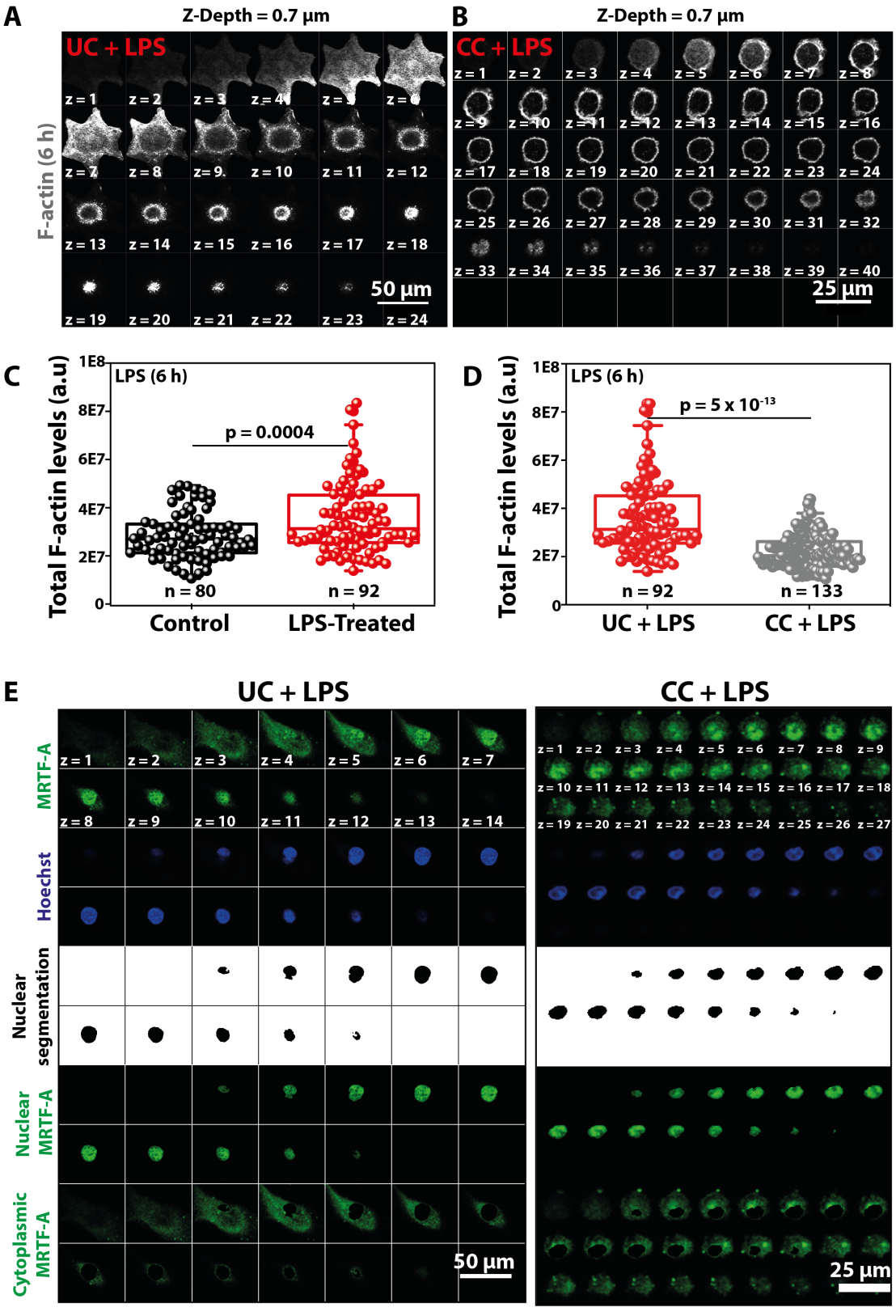
Supplementary Figure 12: Effect of IL-4/IL-13 induced M2 activation on cell and nuclear area in BMDMs: (A) Representative images of Control and IL-4/IL-13 treated (24 h) BMDMs, on fibronectin-coated substrate, stained for F-actin. Cell edges are marked in white. Scale bar = 50 µm. (B) Normalized distribution of cell spreading areas of Control and IL-4/IL-13 treated BMDMs. (C) Box plots show nuclear projection areas in Control and IL-4/IL-13 treated BMDMs. (D) Box plots show the expression levels of target genes between Control and IL-4/IL-13 treated BMDMs. (E) Normalized distribution of cell circularity of Control and IL-4/IL-13 treated BMDMs. Circularity of a cell is defined as $\frac{4\pi\,(area)}{(perimeter)^2}$. (F) Quantification of cell aspect ratios in live Control

and IL-4/IL-13 treated BMDMs. Data are shown as Mean ± S.E. Also refer to supplementary video 3. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition and N = number of biological replicates. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated twice with similar results.



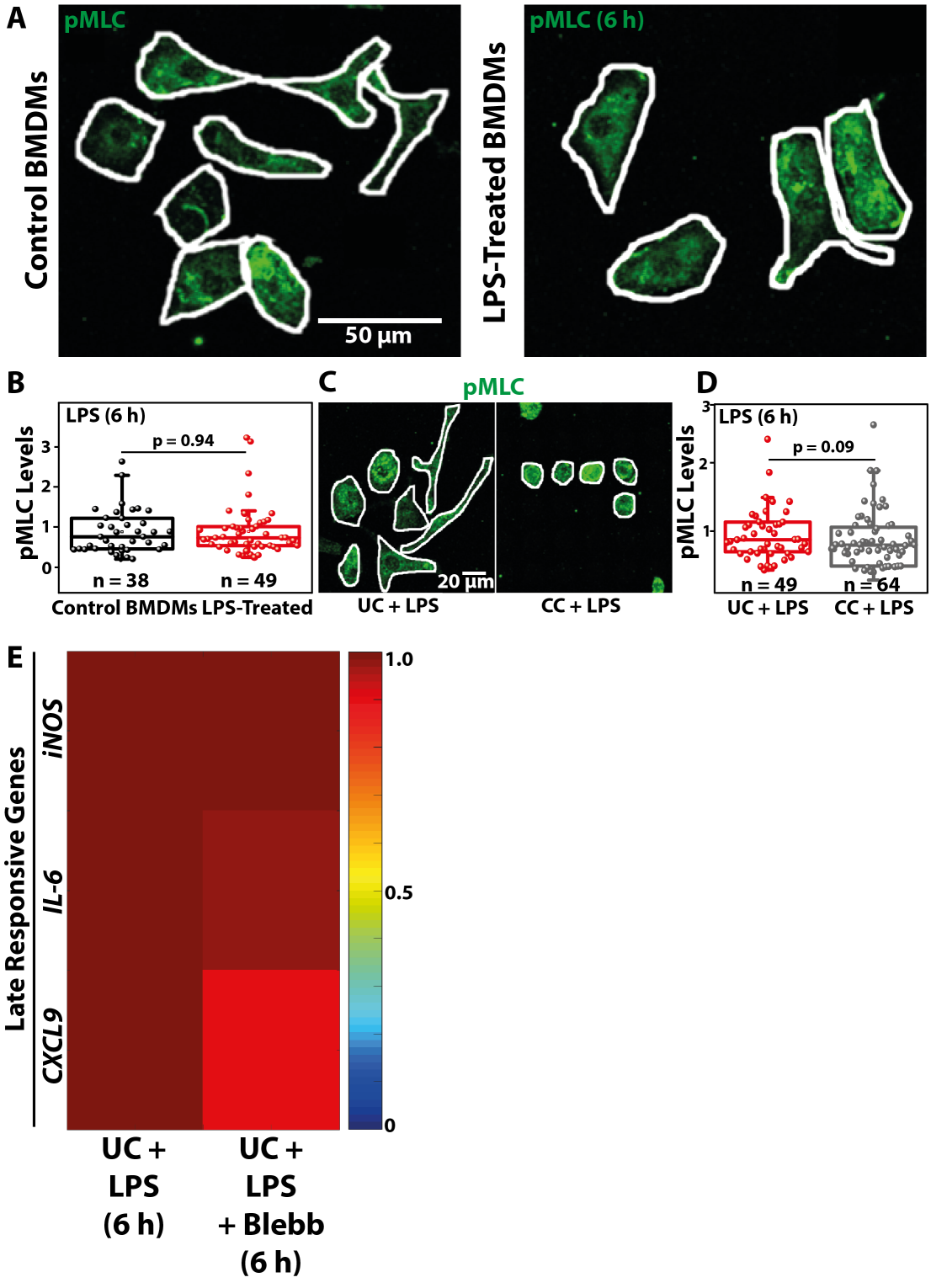
Supplementary Figure 13: Early signaling events during M1 activation are independent of macrophage confinement: (A) Representative images of Control and LPS-treated BMDMs for different periods of time stained for nuclei, p65 and F-actin. Cell edges are marked in white. (B) Time course of changes in nuclear p65 levels upon LPS treatment for different periods of time. Levels are normalized to the untreated Control BMDMs. Data are shown as Mean ± S.E. (C) Box plots show total levels of p65 in BMDMs upon LPS treatment for indicated periods of time. Representative images of Unconfined (UC) and Confined (CC) BMDMs treated with LPS for 3 h and

stained for (D) p65, (F) phospho-IRF3, (H) TLR4 and (J) TLR2. Cell edges are marked in white. Box plots shows levels of (E) N/C ratio of p65, (G) phospho-IRF3, (I) TLR4 and (K) TLR2 in UCs and CCs treated with LPS for 3 h as normalized to the LPS-treated UCs. (L) Box plots show the secreted levels of CXCL2 in UCs and CCs treated with LPS for 3 h. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. Scale bar for all the images = 20 μm. n = number of cells analyzed per condition and N = number of biological replicates. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated twice with similar results.



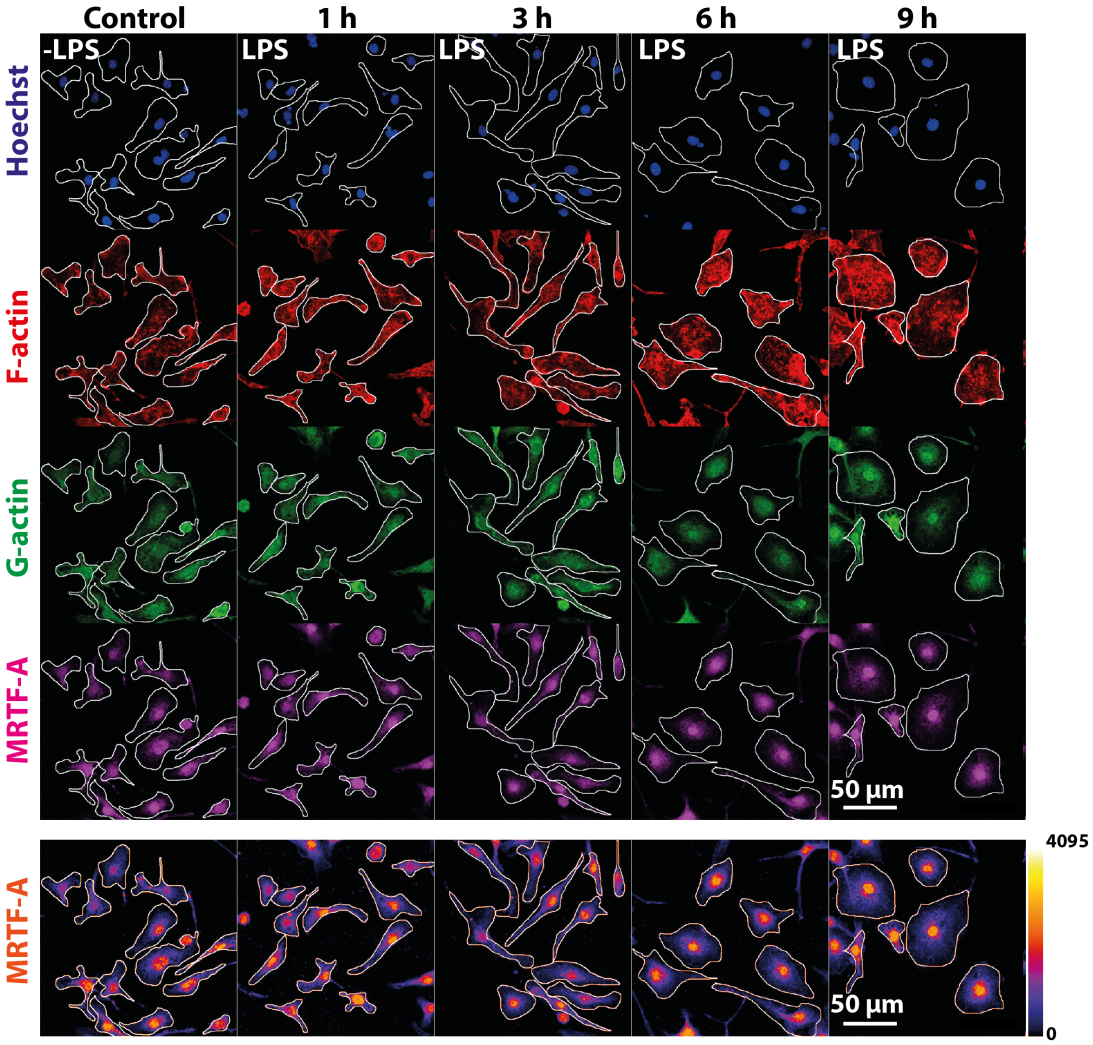
Supplementary Figure 14: Analysis and quantification of nuclear and cytoplasmic proteins: Detailed montage of F-actin organization and levels, at different Z-sections, between (A) Unconfined (UC) and (B) Confined (CC) BMDMs treated with LPS for 6

h. Also refer to supplementary videos 4 & 5. Scale bar = 50 μm. (C) Box plots show total levels of F-actin in Control and LPS-treated BMDMs. (D) Box plots show total levels of F-actin in LPS-treated UCs and CCs. (E) Confocal sections of BMDMs (from basal to apical plane) with a z-step size of 0.7 μm, stained for nucleus and MRTF-A in LPS-treated UCs and CCs. Also, shown are the segmented nuclear images at different Z-sections. Using these segmented nuclear images, levels of MRTF-A in the nuclear volume were quantified. Also, shown are the cytoplasmic levels of MRTF-A. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated atleast twice with similar results.

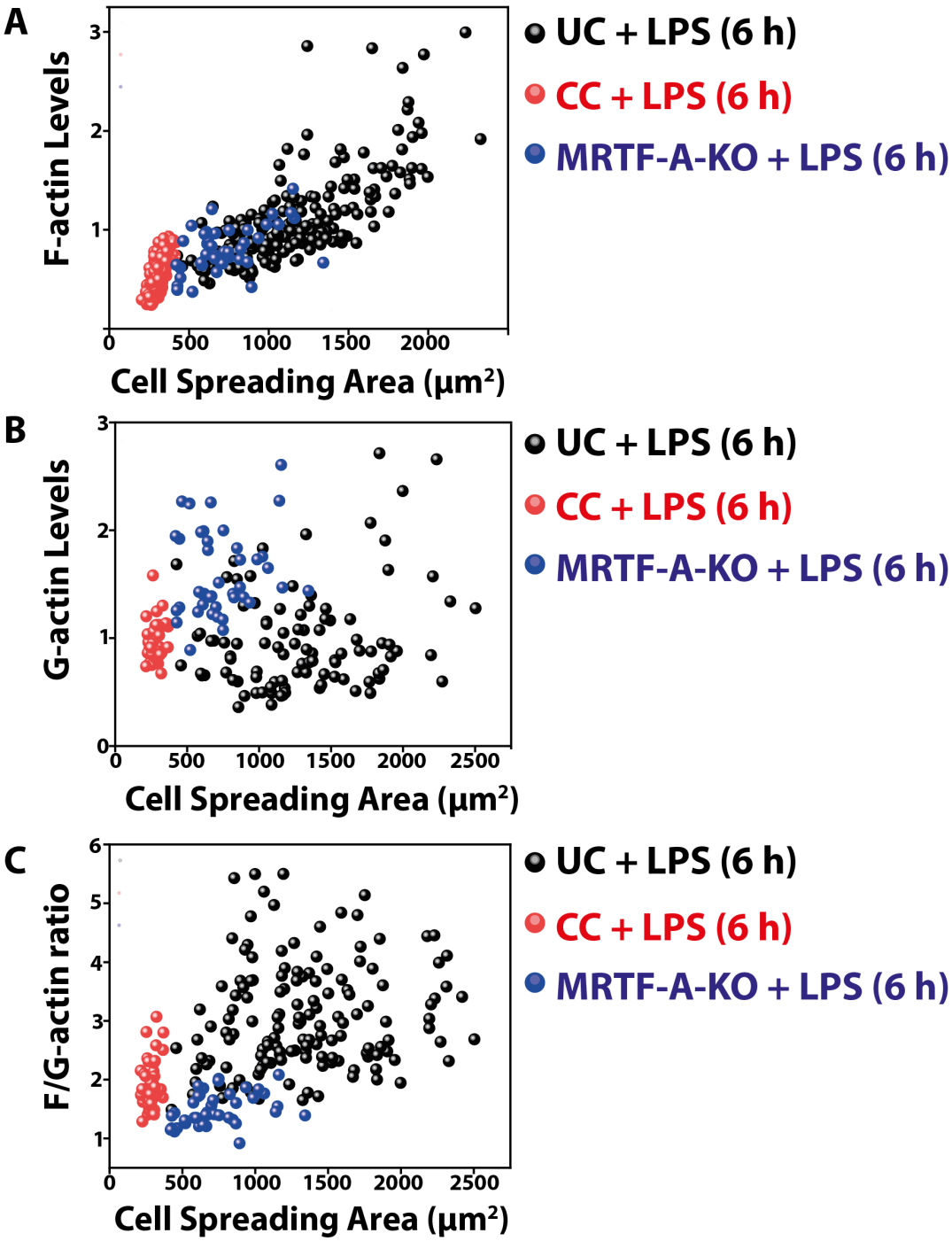


Supplementary Figure 15: Late-responsive gene expression is independent of myosin II contractility: (A) Representative images of Control and LPS-treated (6 h) BMDMs stained for phospho-myosin light chain (pMLC). Cell edges are marked in white. Scale bar = 50 µm. (B) Box plots show levels of p-MLC in Control and LPS-treated BMDMs as normalized to the Control BMDMs. (C) Representative images of

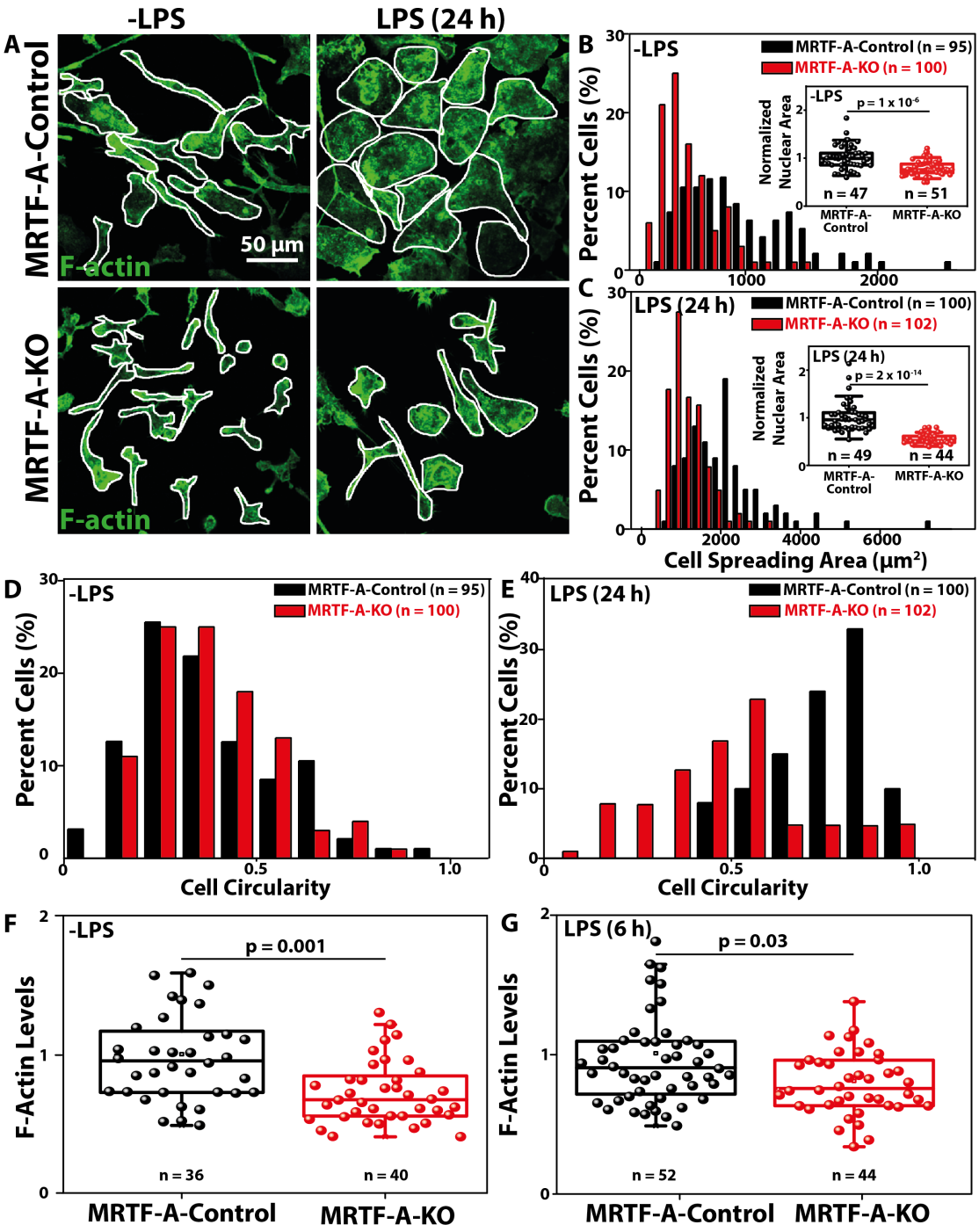
LPS-treated Unconfined (UC) and Confined (CC) BMDMs stained for pMLC. Cell edges are marked in white. Scale bar = 20 μm. (D) Box plots show levels of pMLC in LPS-treated UCs and CCs as normalized to the LPS-treated UCs. (E) Color-coded arrays show expression levels of late-responsive genes between LPS-treated UCs and Blebbistatin (Blebb) + LPS-treated UCs. Levels are normalized to LPS-treated UCs. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated twice with similar results.



Supplementary Figure 16: Representative images of Control and LPS-treated BMDMs, for different periods of time, stained for nuclei, F-actin, G-Actin and MRTF-A. Cell edges are marked in white. Scale bar = 50 µm. To better see MRTF-A nuclear levels, color-coded images of MRTF-A have also been added as lower image panel. Color-bar shows the pixel intensity values.

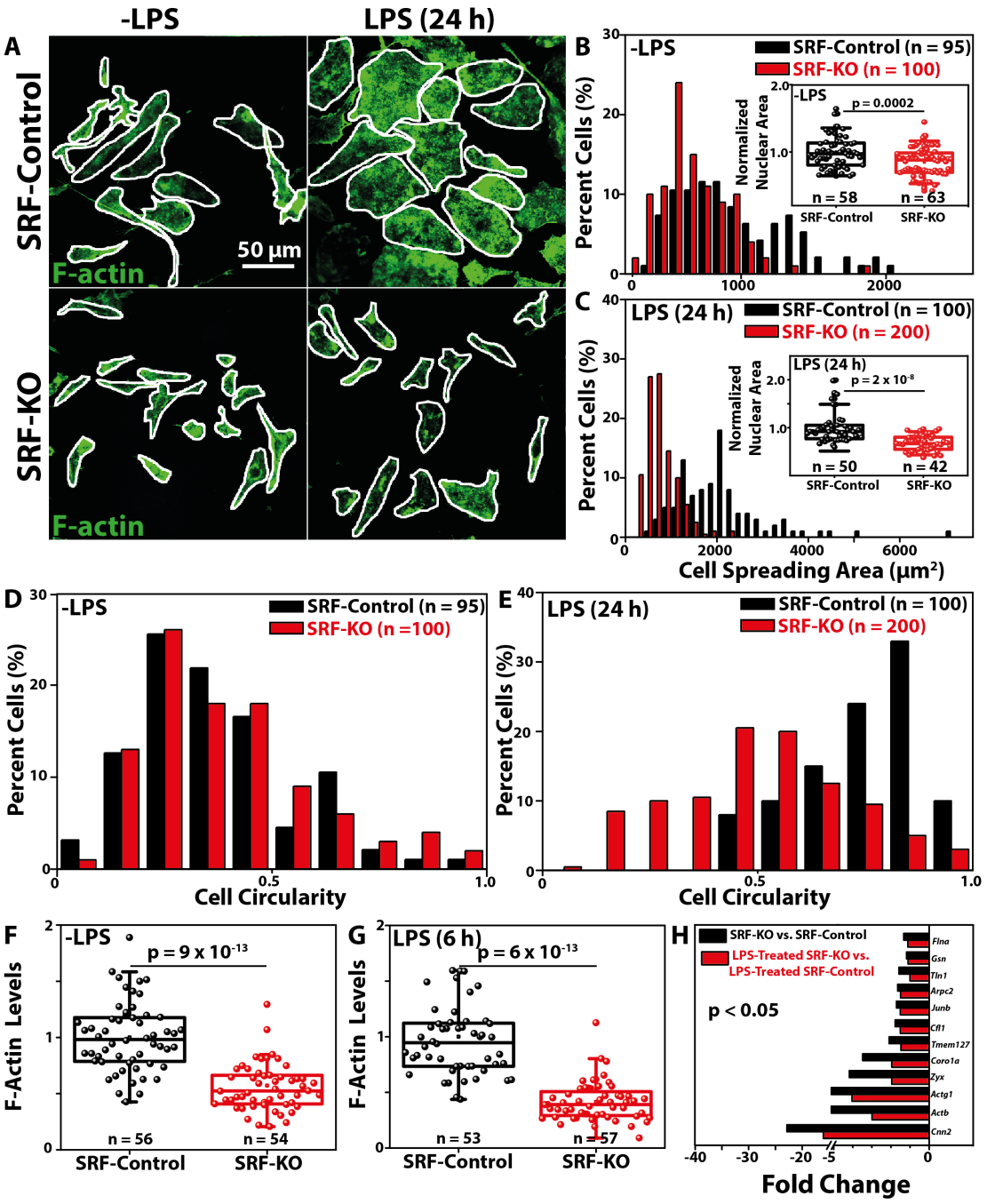


Supplementary Figure 17: Single cell analysis shows the levels of (A) F-actin, (B) G-actin and (C) F/G-actin ratio as a function of cell spreading area in Unconfined (UC), Confined (CC) and MRTF-A-KO BMDMs, all treated with LPS for 6 h.



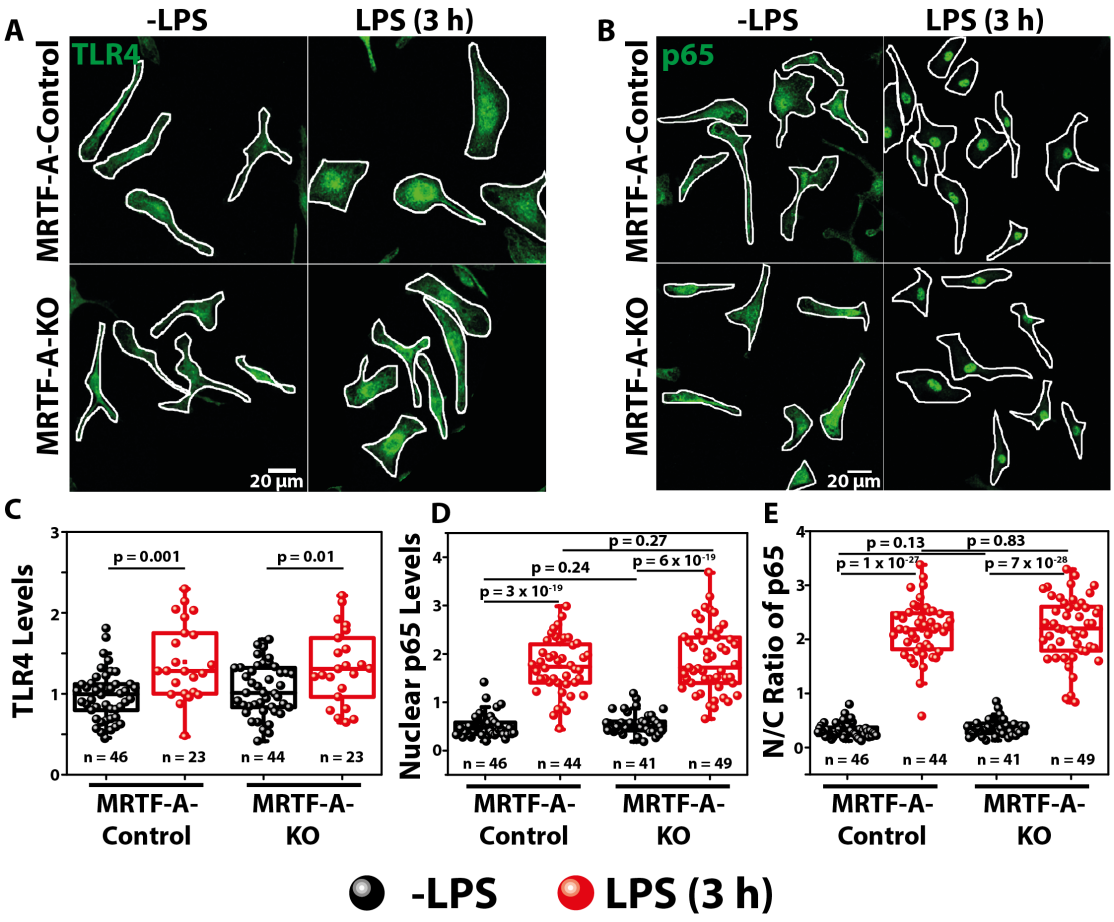
Supplementary Figure 18: MRTF-A KO macrophages show downsized cell spreading and actin polymerization: (A) Representative images of MRTF-A-Control and MRTF-A-KO BMDMs, before and after LPS treatment for 24 h, stained for F-actin. Cell edges are marked in white. Scale bar = 50 µm. (B&C) Normalized distributions of cell spreading areas, before and after LPS treatment for 24 h, of MRTF-A-Control and MRTF-A-KO BMDMs. Box plots in the insets show nuclear area in MRTF-A-Control and MRTF-A-KO BMDMs, before and after LPS treatment for 24

h, as normalized to MRTF-A-Control BMDMs. (D&E) Normalized distributions of cell circularity, before and after LPS treatment for 24 h, of MRTF-A-Control and MRTF-A-KO BMDMs. (F&G) Box plots show levels of F-actin between MRTF-A Control and MRTF-A-KO BMDMs, before and after LPS treatment for 6 h, as normalized to the MRTF-A-Control BMDMs. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated twice with similar results.

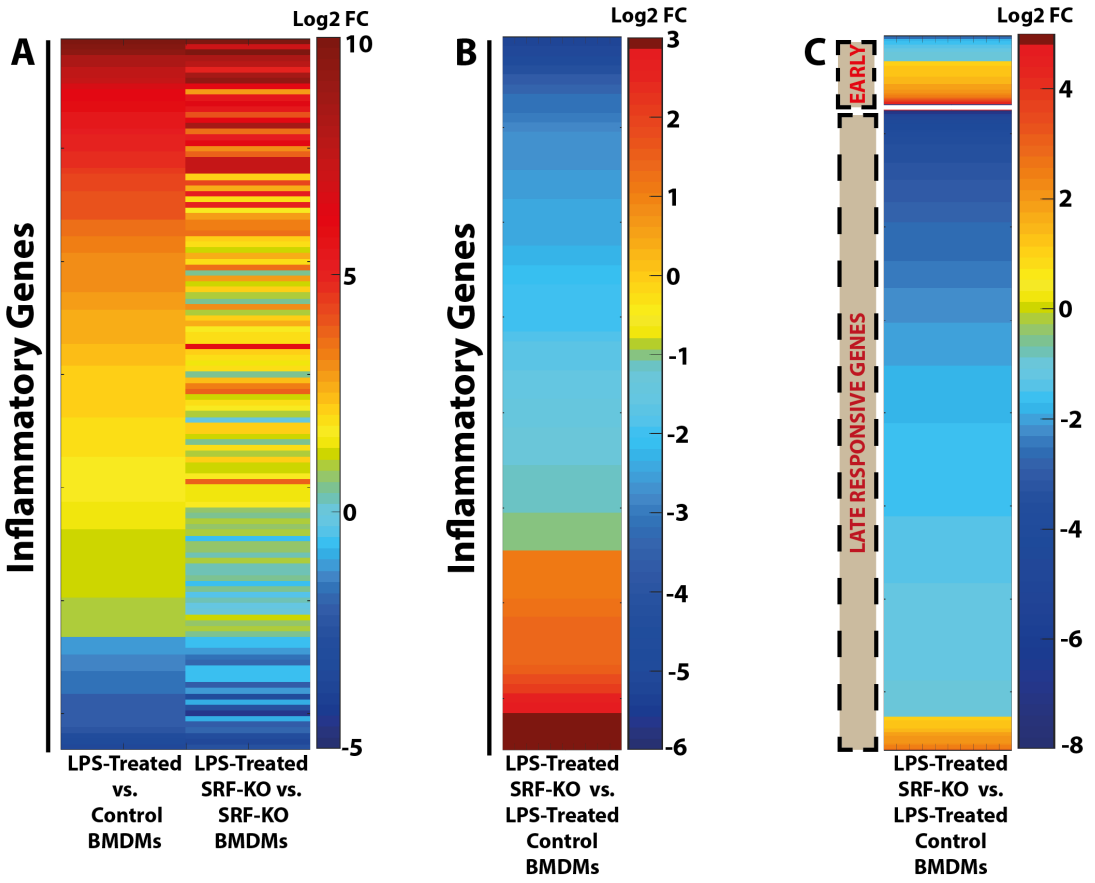


Supplementary Figure 19: SRF-KO macrophages show downsized cell spreading and actin polymerization: (A) Representative images of SRF-Control and SRF-KO BMDMs, before and after LPS treatment for 24 h, stained for F-actin. Cell edges are marked in white. Scale bar = 50 µm. (B&C) Normalized distributions of cell spreading areas, before and after LPS treatment for 24 h, of SRF-Control and SRF-KO BMDMs. Box plots in the insets show nuclear area in SRF-Control and SRF-KO BMDMs, before and after LPS treatment for 24 h, as normalized to SRF-Control BMDMs. (D&E) Normalized distributions of cell circularity, before and after LPS treatment for 24 h, of

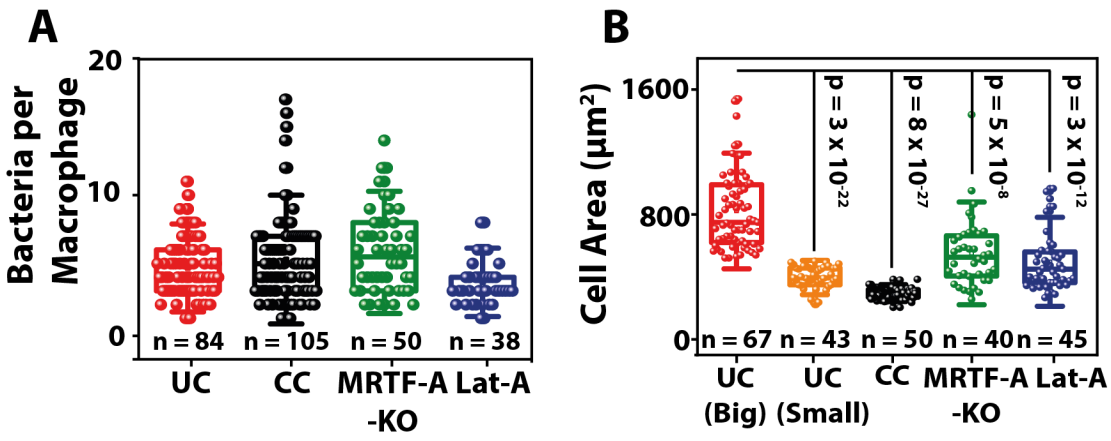
SRF-Control and SRF-KO BMDMs. (F&G) Box plots show levels of F-actin between SRF-Control and SRF-KO BMDMs, before and after LPS treatment for 6 h, as normalized to the SRF-Control BMDMs. (H) Differential expression of actin-related genes obtained from RNA-Seq data. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated twice with similar results.



Supplementary Figure 20: Early signaling events during M1 activation are not sensitive to MRTF-A Knockout: Representative images of MRTF-A-Control and MRTF-A-KO BMDMs, before and after LPS treatment for 3 h, stained for (A) TLR4 and (B) p65, respectively. Cell edges are marked in white. Scale bars for all the images = 20 µm. Box plots show the levels of (C) TLR4 and (D) nuclear p65 between MRTF-A-Control and MRTF-A-KO BMDMs, before and after LPS treatment for 3 h, as normalized to the untreated MRTF-A-Control cells. (E) Box plots show the nuclear-to-cytoplasmic ratio of p65 between MRTF-A-Control and MRTF-A-KO BMDMs, before and after LPS treatment for 3 h, as normalized to the untreated MRTF-A-Control cells. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated twice with similar results.



Supplementary Figure 21: SRF activity is required for efficient expression of pro-inflammatory late gene clusters: (A) Heat map shows the log2-fold change in the expression of known inflammatory genes in LPS-treated vs. Control BMDMs and LPS-treated SRF-KO vs. SRF-KO BMDMs, respectively. Refer to Supplementary data 3 for the gene list. (B) Heat map shows the log2-fold change in the expression of known inflammatory genes in LPS-treated SRF-KO vs. LPS-treated Control BMDMs. Refer to Supplementary data 3 for the gene list. (C) Heat map shows the Log2-fold change in the expression of all early and late responsive genes in LPS-treated SRF-KO vs. LPS-treated Control BMDMs. Refer to Supplementary data 3 for the gene list.



Supplementary Figure 22: Macrophage confinement has no effect on total bacteria per macrophage: (A) Box plots show the total number of bacteria per macrophage in UC, CC, MRTF-A-KO, and Latrunculin-A (Lat-A)-treated BMDMs. (B) Box plots show the cell spreading area of UC (Big), UC (Small), CC, MRTF-A-KO, and Latrunculin-A (Lat-A)-treated BMDMs. In all the box plots, the box represents 25th and 75th percentiles, median is denoted by middle horizontal line, mean is indicated by small open squares, and whiskers indicate SD. n = number of cells analyzed per condition. P-values were assessed with two-tailed Student's t-test. All the experiments were independently repeated three times with similar results.

Supplementary Table 1: Primer set for Gene expression analysis using Real-Time qPCR

GENE	FORWARD PRIMER	REVERSE PRIMER
HOUSE KEEPING GENE		
18S rRNA	AGAAACGGCTACCACATCCAA	GGGTCGGGAGTGGGTAATTT
M1 ACTIVATION-EARLY RESPONSIVE GENES		
TLR2	CGCCCTTTAAGCTGTGTCTC	CGTCAAAGAGCCTGAAGTGG
TLR4	CCAACATCATCCAGGAAGGC	GGACTTCTCAACCTTCTCAAG
CXCL2	GCCTGAAGACCCTGCCAAG	AACCAGGGGGGCTTCAGGG
TNF-α	ACGCTCTTCTGTCTACTGAAC	TTGTCTTTGAGATCCATGCC
M1 ACTIVATION-LATE RESPONSIVE GENES		
IL-1β	GATCCCAAGCAATACCCAAAG	CTTGTGCTCTGCTTGTGAGG
NOS2	GCAGCACTTGGATCAGGAAC	ACCATCTCCTGCATTTCTTCC
IL-6	AGCCAGAGTCCTTCAGAGAG	GTCCTTAGCCACTCCTTCTG
CXCL9	CTGTTCTTTTCCTCTTGGGCA	GGCAGGTTTGATCTCCGTTC
M2 ACTIVATION TARGET GENES		
Arginase-1	TGACTGAAGTAGACAAGCTGG	AGAGTTGGGTTCACTTCCATG
IL-10	AACATACTGCTAACCGACTCC	AAATCACTCTTCACTGCTCC
YM1	CTACTCCTCAGAACCGTCAG	GCATTTCCTTCACCAGAACAC

Supplementary Table 2: Primer set for ChIP analysis

GENE	FORWARD PRIMER	REVERSE PRIMER
IL-1β	AACGGAGGAGCCGTTGATATG	AGAGCATCTTCCTAATGC
NOS2	GCTGCCAGGGTCACAACTT	CCCAACACAAGACTCACCTTG
CXCL9	GAGCAGTGTGGAGTTCGAGG	TTATCTGCTGGCCTGAGAAGG
IL-6	CCCACCCTCCAACAAAGATT	GCTCCAGAGCAGAATGAGCTA

REFERENCES:

1. Chen X, *et al.* (2012) Requirement for the histone deacetylase Hdac3 for the inflammatory gene expression program in macrophages. *Proc Natl Acad Sci U S A* 109(42):E2865-2874.