Cell Reports, Volume *43*

Supplemental information

Lactic acid induces transcriptional repression of macrophage inflammatory response via histone acetylation

Weiwei Shi, Tiffany J. Cassmann, Aditya Vijay Bhagwate, Taro Hitosugi, and W.K. Eddie Ip

Location of ATAC-seq regions	Associated gene	Length (bp)	qPCR primer forward	qPCR primer reverse
chr5:29935610-29936397	*Il6*	788	5’-GTTCCACTGTGGTTTTAAGCAG-3’	5'-ACATTTGTTTAGTCAGCCAGC-3'
chr11:44372219-44373279	*Il12b*	1061	5’-TGCACCCACTCAGCCAATAG-3’	5’-GTCAGTGACAGTGCATCCCT-3’
chr11:78919748-78920034	*Nos2*	287	5’-GCTGAGCTGACTTTGGGGAC-3’	5’-CCAATAAAGCATTCACACATGGC-3’
chr1:150108293-150109047	*Ptgs2*	755	5'-AAGAGTCAGAACTTATTACCTCAGT-3'	5’-TTGGGATTTTCAAGACAGGGT-3’
chr15:101254000-101254390	*Nr4a1*	391	5’-GGGCGCCGCTATTTTTAGCC-3’	5’-TGACGCGCGCCCATTGA-3’

Table S1. ATAC-seq regions differentially altered by lactate and their primer sequences for ChIP-qPCR.

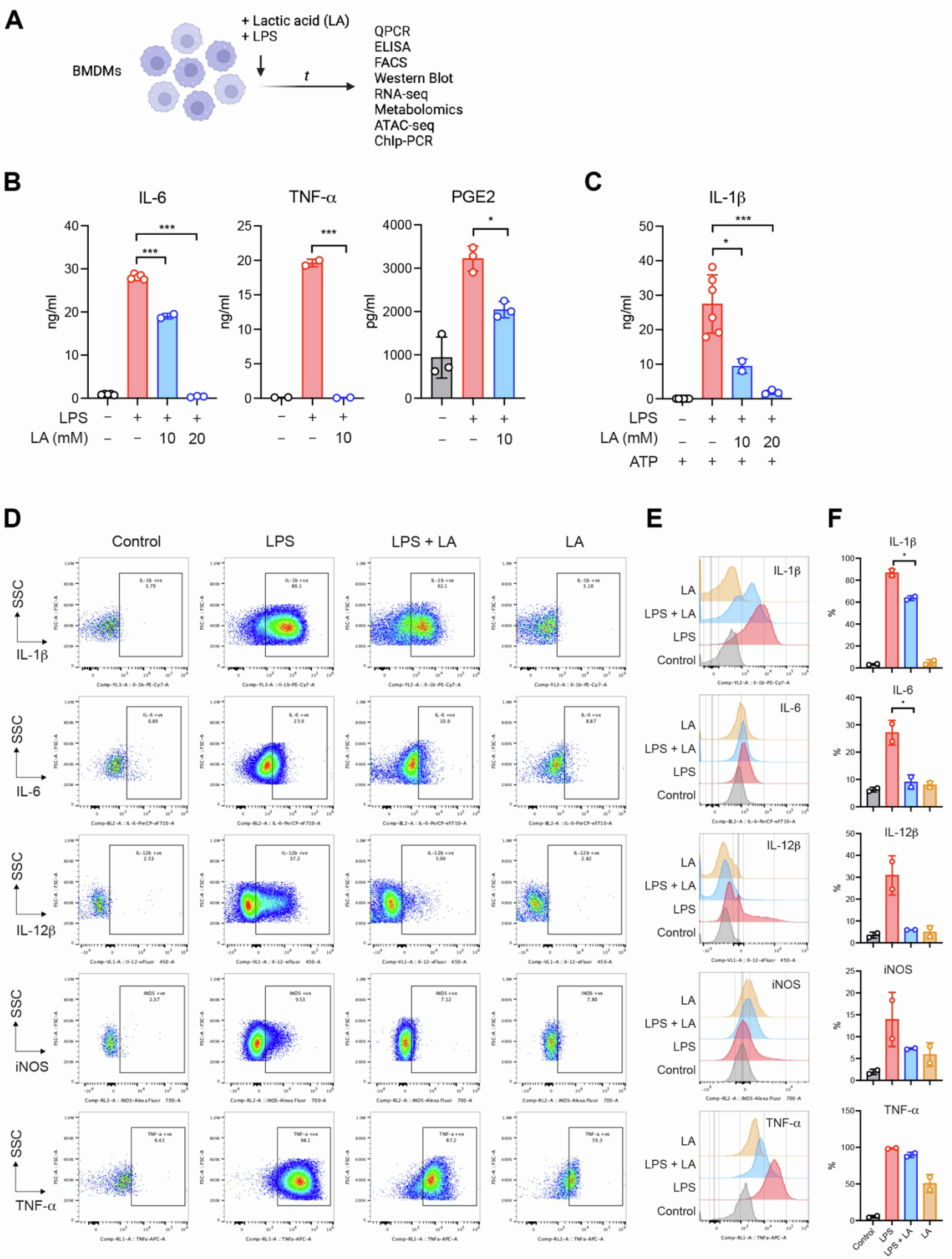

Figure S1. Inhibition of pro-inflammatory response by lactic acid in macrophages after LPS stimulation.

(**A**) Experimental approach to assess lactic acid effect on macrophage pro-inflammatory function. Created with BioRender.com. (**B** and **C**) Pro-inflammatory cytokine or PGE2 secretion in BMDMs stimulated without (control) or with LPS in the presence or absence of lactic acid (LA) for 3 h (**B**) and followed by ATP stimulation for 30 min for IL-1β secretion (**C**). (**D**-**F**) Intracellular cytokine production in BMDMs stimulated as in (**B**). Data are representative as mean ± SD of replicates (B, C, F). One-way ANOVA (**B**, **C**, **F**) followed by Tukey's post-test: *$p < 0.05$, **$p < 0.001$, ***$p < 0.001$. Related to Figure 1.

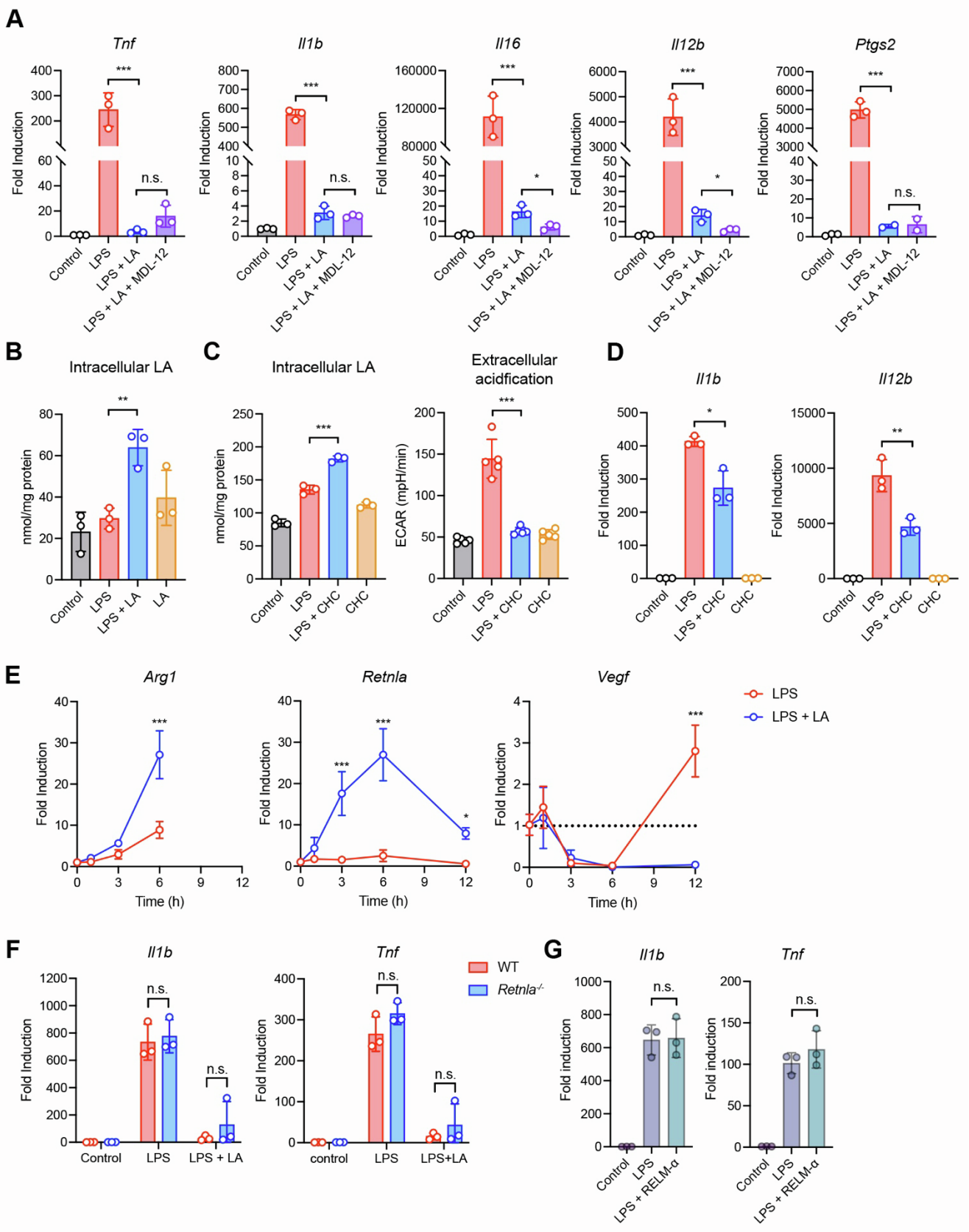

Figure S2. Anti-inflammatory effect of lactic acid requires lactic acid influx or intracellular accumulation and is independent of M2 gene expression in macrophages after LPS stimulation. (**A**) Pro-inflammatory gene expression in BMDMs stimulated without (control) or with LPS in the presence or absence of lactic acid (LA) or LA together with MDL-12 for 3 h. (**B**) Intracellular lactic acid by targeted

metabolomics in BMDMs stimulated as in (**A**). (**C**) Intracellular lactic acid and ECAR in BMDMs stimulated without (control) or with LPS in the presence or absence of CHC for 3 h. (**D**) Pro-inflammatory gene expression in BMDMs stimulated as in (**C**). (**E**) M2 gene expression in BMDMs stimulated as in (A) for the indicated times. (**F**) Pro-inflammatory gene expression in wild-type or *Relnta*$^{-/-}$ BMDMs stimulated as in (**A**). (**G**) Pro-inflammatory gene expression in BMDMs stimulated without (control) or with LPS in the presence or absence of RELM-α for 6 h. Data are representative as mean ± SD of triplicates. One-way or two-way ANOVA (**E** and **F**) followed by Tukey's or Sidak's post-test: * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$, n.s. = not significant. Related to Figure 1 and Figure 2.

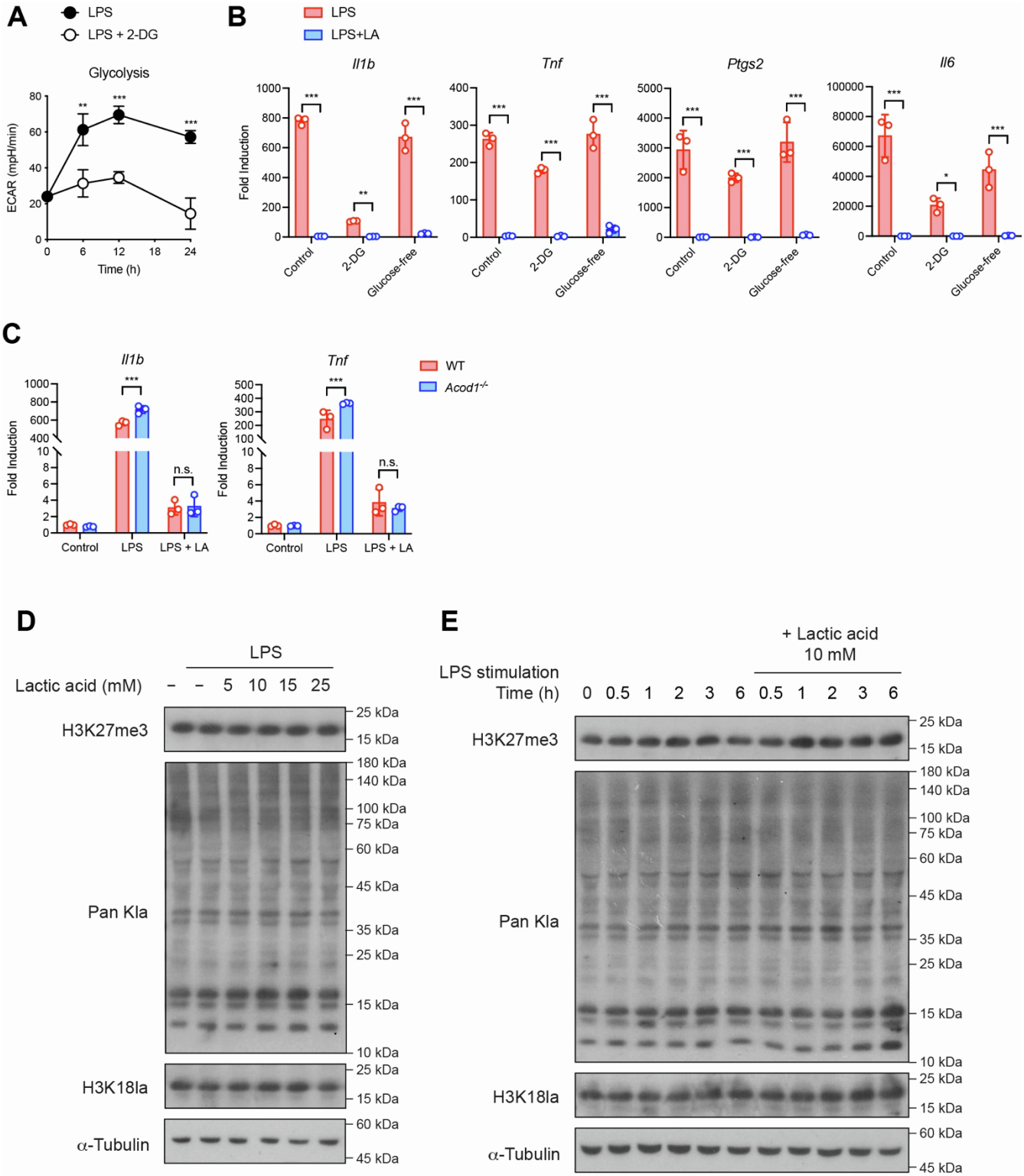

Figure S3. Lactic acid does not require glucose uptake or itaconate production for its anti-inflammatory effect and has no effect on H3K27 tri-methylation and histone pan- and H3K18 lactylation during early phase of LPS stimulation.

(**A**) ECAR of Seahorse analysis in BMDMs stimulated with LPS in the presence of absence of 2-DG for the indicated times. (**B**) Pro-inflammatory gene expression in BMDMs cultured in complete medium (control) or glucose-free medium, or complete medium containing 2-DG and stimulated with LPS in the presence or absence of lactic acid (LA) at 10 mM for 3 h. (**C**) Pro-inflammatory gene expression in wild-type or *Acod1*$^{-/-}$ BMDMs stimulated as in (**B**). (**D** and **E**) Total protein lactylation (Pan-Kla), H3K18 lactylation (H3K18la) and H3K27 tri-methylation (H3K27me3) in BMDMs stimulated with LPS in the presence or absence of LA at the indicated concentrations (**D**) or 10 mM (**E**) for 3 h (**D**) or the indicated times (**E**). Data are representative as mean ± SD of triplicates (**A**-**C**). Two-way ANOVA followed by Sidak's post-test (**A**-**C**): * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$, n.s. = not significant. Related to Figure 3 and Figure 4.

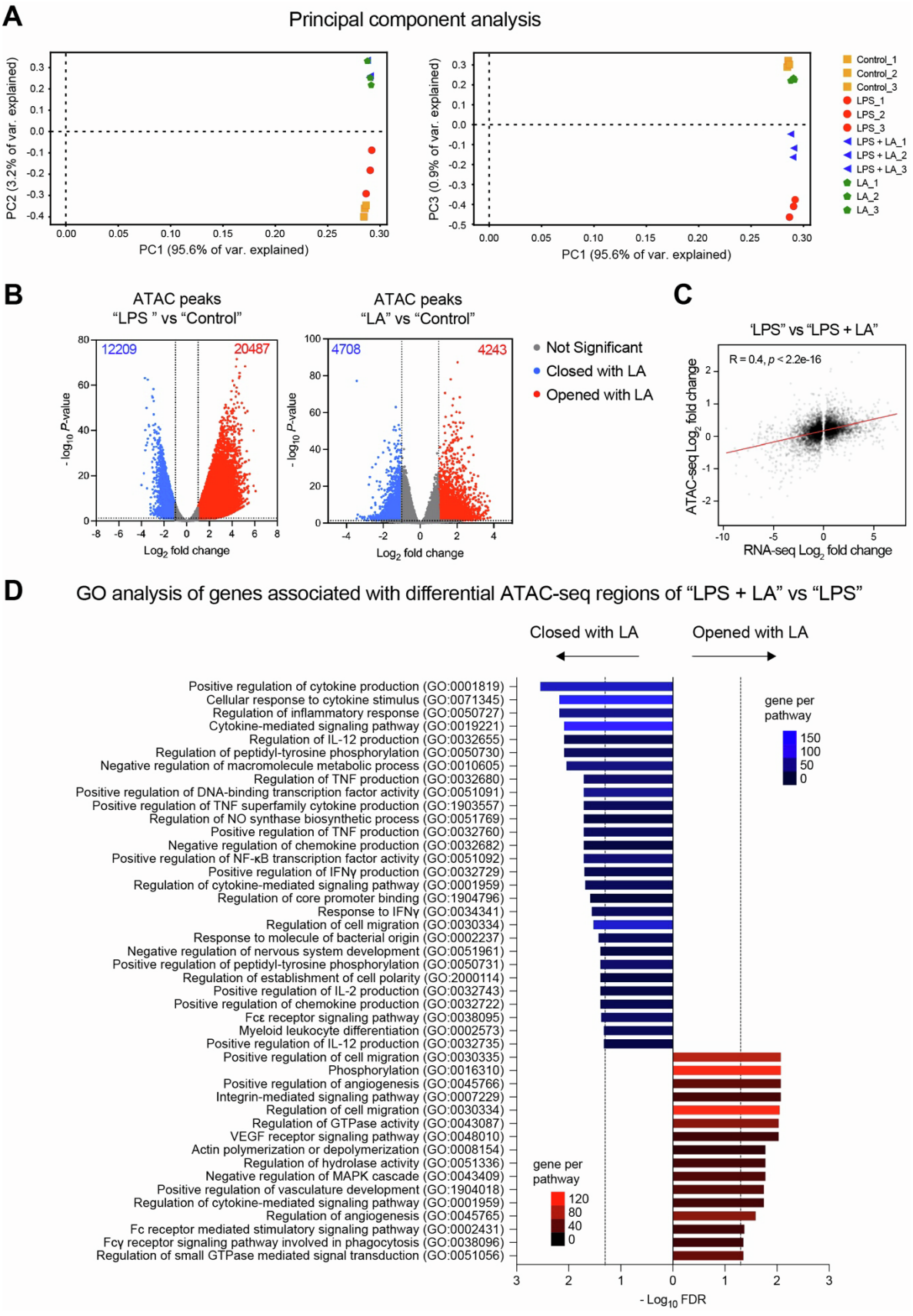

Figure S4. ATAC-seq analysis for lactic acid effect in macrophages during LPS stimulation.

(**A**). PCA plot of ATAC-seq data from BMDMs stimulated without (control) or with LPS in the presence or absence of lactic acid (LA) at 10 mM for 3 h showing clusters of samples. (**B**) Volcano plot from ATAC-seq data as in (**A**) showing cutoffs (Log_2 fold change ≥ or ≤ 1 and FDR ≤ 0.05) used to identify differential accessibility regions in BMDMs stimulated without or with LPS (LPS *vs* Control) or LA (LA *vs* Control). (**C**) Correlation between ATAC-seq Log2 fold change and RNA-seq Log2 cold change in BMDMs stimulated with LPS in the presence or absence of LA ("LPS + LA" vs "LPS"). (**D**) GO analysis of differential accessibility regions identified in Figure 5B ("LPS + LA" vs "LPS") showing biological processes enriched in accessibility regions closed or opened by LA in BMDMs stimulated with LPS. Related to Figure 5.

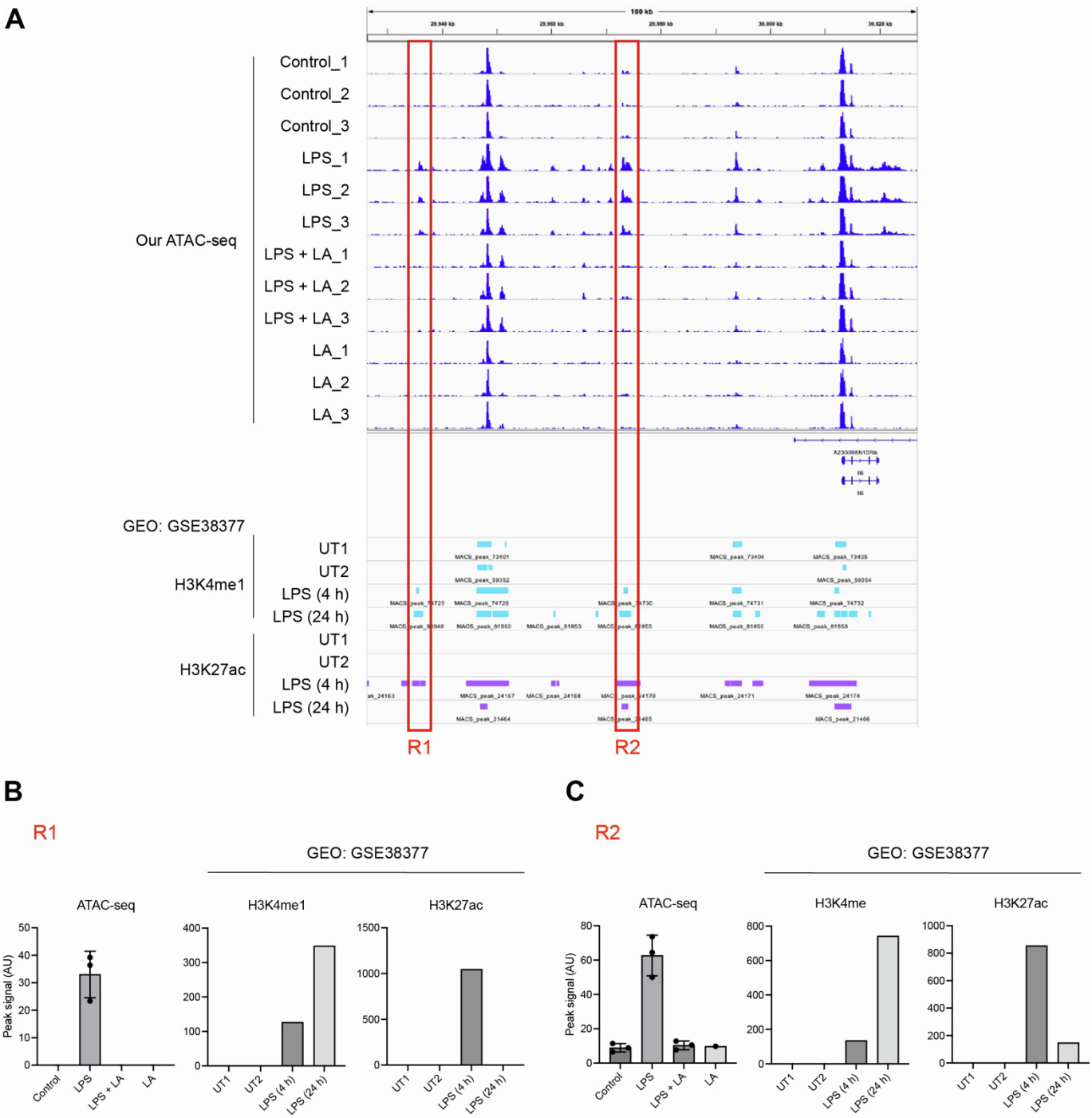

Figure S5. Lactic acid-reduced chromatin accessible regions in *Il6* locus in LPS-stimulated macrophages are associated with published LPS-induced enhancers.
(**A**) Gene tracks of ATAC-seq data at *Il6* locus aligned with published H3K4me1 and H3K27ac ChIP-seq data from BMDMs untreated (UT1 and UT2) or stimulated with LPS for 4 or 24 h (GEO: GSE38377). DNA regions boxed in red (R1 and R2) showing differential regions closed by lactic acid (LA). (**B** and **C**) Peak signals from our ATAC-seq data and the published ChIP-seq data in DNA region R1 (**B**) and R2 (**C**). Related to Figure 6.

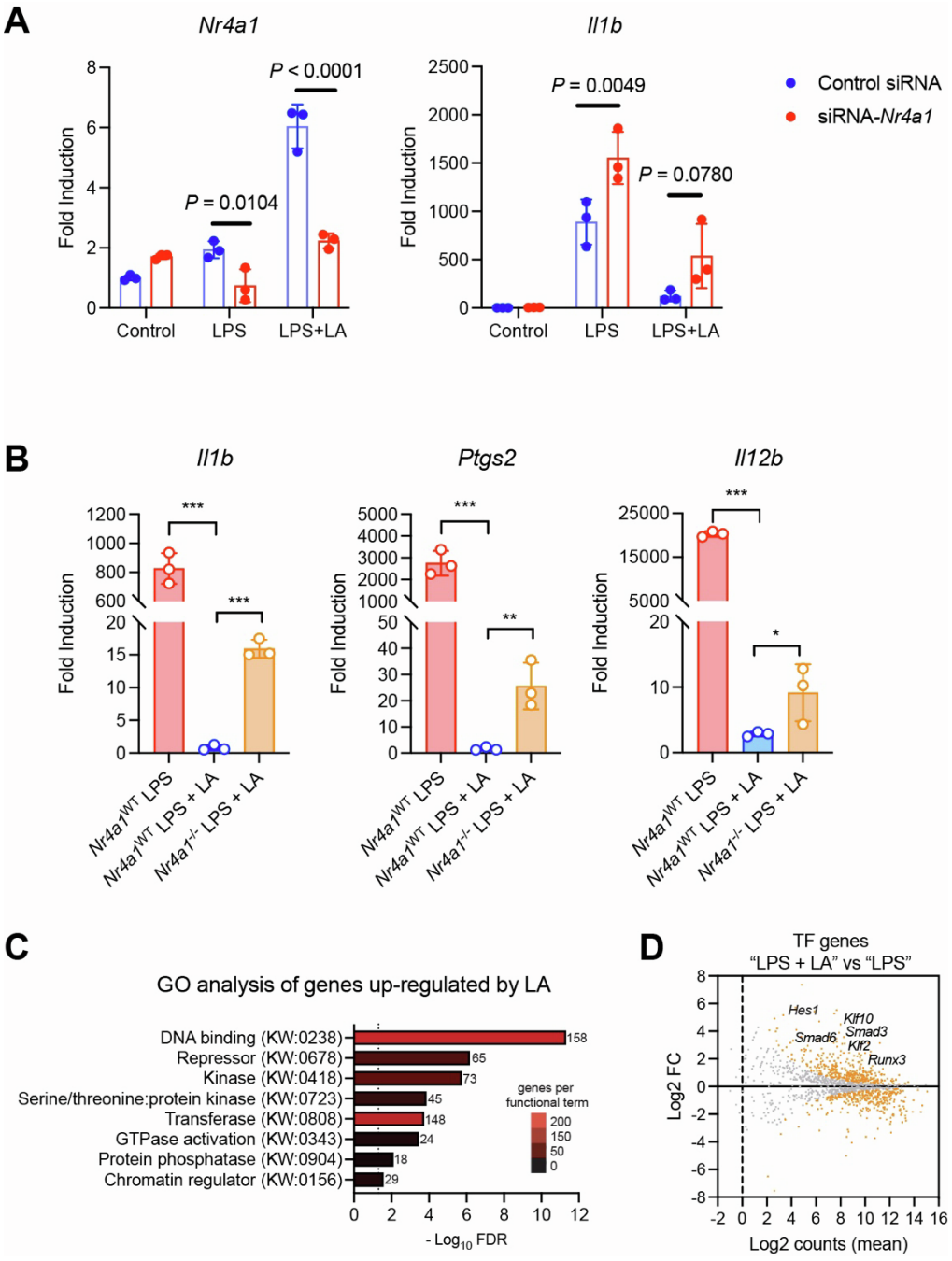

Figure S6. Partial loss of immunosuppression by lactic acid in NR4A1-deficient macrophages and RNA-seq analysis of genes induced by lactic acid.

(**A**) *Nr4a1* and *Il1b* expression in Raw 264.7 cells with siRNA for *Nr4a1* or control siRNA stimulated without (control) or with LPS in the presence or absence of lactic acid (LA) at 10 mM for 3 h. (**B**) Pro-inflammatory expression in *Nr4a1*$^{-/-}$ BMDMs stimulated as in (**A**) (**C**) GO analysis of RNA-seq data showing molecular functions enriched in gene up-regulated by LA in BMDMs stimulated as in (**A**). (**D**) MA plot of RNA-seq data showing TF gene expression in BMDMs stimulated as in (**A**). Differentially expressed TF genes (FDR ≤ 0.05) are in orange. Data are represented as mean ± SD of triplicates (**A** and **B**). One-way (**B**) or two-way ANOVA (**A**) followed by Tukey's or Sidak's post-test: * $p < 0.05$, **$p < 0.001$, ***$p < 0.001$. Related to Figure 7.

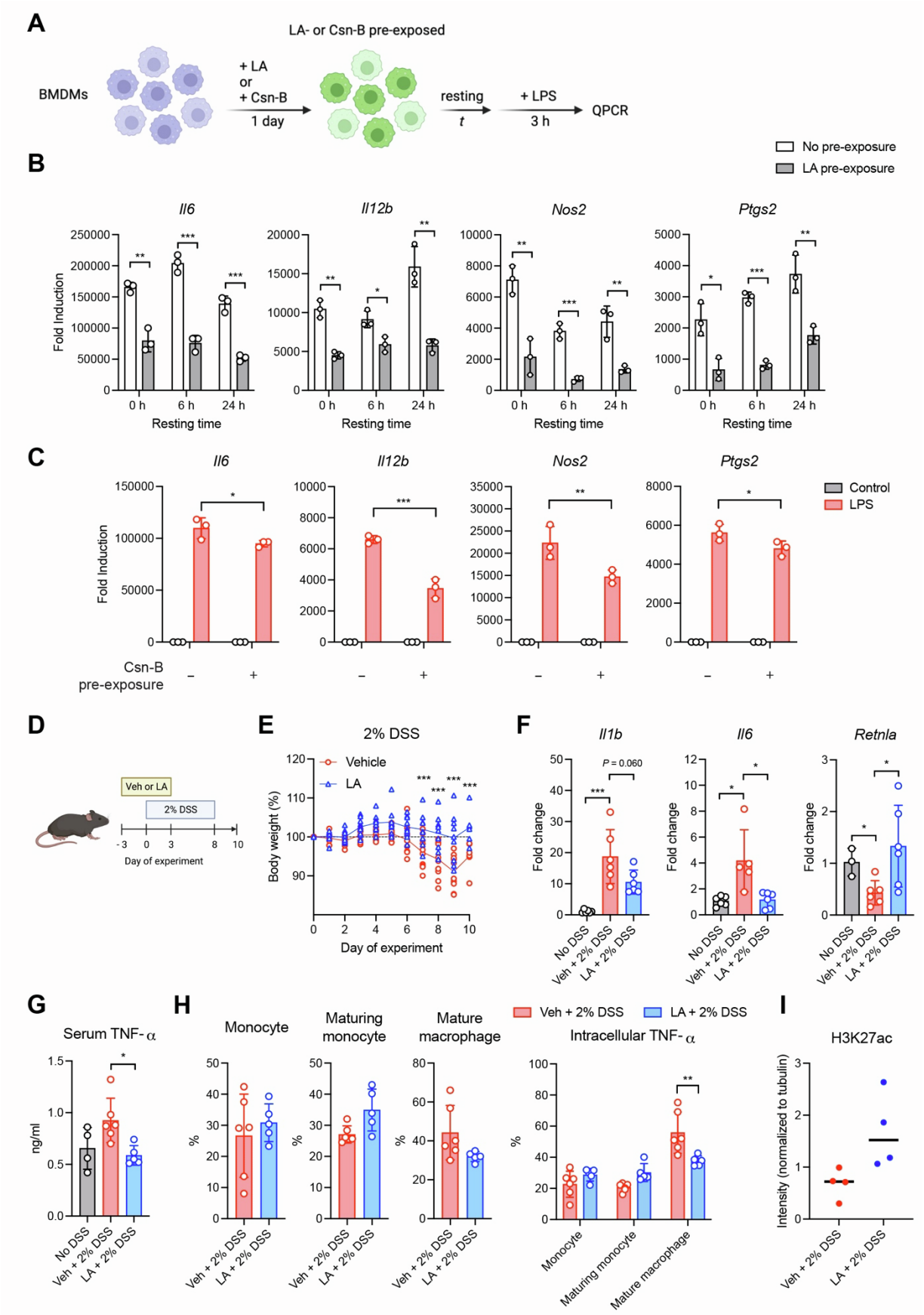

Figure S7. Long-term immunosuppression of macrophage pro-inflammatory response by lactic acid and NR4A1 agonist.

(**A**) Experimental approach to assess the long-term effect of lactic acid on macrophage pro-inflammatory function. (**B**) LPS tolerance in BMDMs pre-exposed to LA for 24 h and rested for the indicated times prior to LPS stimulation for 3 h. (**C**) LPS tolerance in BMDMs pre-exposed to Csn-B for 24 h prior to LPS stimulation for 3 h. (**D**) Experimental approach to assess the lactic acid effect in mouse DSS-induced colitis model. (**E**) Body weight in mice following treatment with 2% DSS after receiving PBS (vehicle; n = 10) or LA (n = 9) via enema. (**F** and **G**) Pro-inflammatory expression in colon tissues and serum at day 10 following treatment as in (**E**). (**H**) Frequency of monocyte, maturing monocyte and mature macrophage populations and their TNF-α producing cells in the colon lamina propria at day 10 following treatment as in (**E**). (**I**) Histone H3K27 acetylation in colon lamina propria cells isolated as in (**H**). Data are represented as mean ± SD of triplicates (**B** and **C**), mean ± SEM of tested animals (**E**), or mean ± SD of tested animals (**F**-**H**). One-way (**F** and **G**) or two-way ANOVA (**B**, **C**, **E**, and **H**) followed by Tukey's or Sidak's post-test: * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$. Created with BioRender.com (**A** and **D**). Related to Figure 7