Additional Supplementary Methods

RNA-seq analysis

Total RNA was extracted from wild-type BMDM cells with and without ML-SA5 treatment, as well as from Mcoln1 knockout BMDM cells subjected to LPS stimulation.

Sample libraries were prepared from the purified mRNA, and paired-end sequencing of the constructed libraries was carried out at Sangon Biotech Co., Ltd. (Shanghai, China) using an Illumina HiSeq 2500 system. The quality of the raw reads was assessed and controlled using FastQC and Trimmomatic. The reads were aligned to the human reference genome GRCh37 using HISAT2 (version 1.12.4). Differentially expressed genes were identified using the DESeq2 package with standard settings. Subsequently, raw read counts were used to perform differential gene expression analysis with the DESeq2 package.

Gene set enrichment analysis (GSEA)

According to GSEA User Guider for RNA-seq data, DESeq2 was applied to perform the differential gene expression analysis. Parameters of log Fold change value and p adj value were selected to build pre-ranked list following the ranking method guide provided by Dr. Veronique Voisin (http://www.baderlab.org/CancerStemCellProject/VeroniqueVoisin/AdditionalResources/GSEA.

GSEA was performed based on the downloaded gene set collections using GSEA software (v4.1.3 and v4.3.3, https://www.gsea-msigdb.org/). Significance of the enrichment was calculated based on 1000 cycles of permutations.

Software and Packages were included in main and supplementary figure R files.