PAQR11 modulates monocyte-to-macrophage differentiation and pathogenesis of rheumatoid arthritis

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Summary

During inflammation or tissue injury, pro-inflammatory mediators attract migratory monocytes to inflammatory sites and monocyte-to-macrophage differentiation occurs to activate macrophages. We report here that PAQR11, a member of the progesterone and AdipoQ receptor family, regulates monocyte-to-macrophage differentiation *in vitro* and *in vivo*. *Paqr11* gene was highly induced during monocyte-to-macrophage differentiation. Knockdown or deletion of *Paqr11* inhibited monocyte differentiation but had little effect on macrophage polarization. Mechanistically, PAQR11 promoted cell survival as apoptosis was increased by *Paqr11* knockdown or deletion. Activation of the MAPK signalling pathway was involved in the regulatory role of PAQR11 on monocyte differentiation and cell survival. C/EBPβ regulated the expression of *Paqr11* at the transcriptional level. In mice, deletion of *Paqr11* gene alleviated progression of collagen-induced rheumatoid arthritis. Thus, these results provide strong evidence that PAQR11 has a function in monocyte-to-macrophage differentiation and such function is related to autoimmune disease *in vivo*.

Keywords: apoptosis; C/EBPβ; monocyte-to-macrophage differentiation; PAQR11; rheumatoid arthritis.

Introduction

Monocytes, members of the mononuclear phagocyte system that comprises monocytes, macrophages and dendritic cells (DCs), play important roles in the innate and adaptive immune systems.[1] Monocytes represent 10% of leucocytes in human blood and 4% of leucocytes in mouse blood, linking inflammation to adaptive immune response.[2] One of the well-known functions of monocytes is as a reservoir of myeloid precursors for the renewal of tissue macrophages and DCs.[3,4] After birth, monocytes are derived from haematological precursors in the bone marrow and then enter the blood circulation. In a steady state, the monocytes have a half-life of approximately 1 day in the circulation, and then traffic into tissues and lymph nodes.[1] During inflammation or injury, a number of pro-inflammatory mediators including chemokines and complement components can attract migratory monocytes to inflammatory sites. One of the most important chemokines is monocyte chemo-attractant protein 1 (MCP1) which recruits Ly6C^{high} monocytes in mice and CD14^{high} monocytes in humans due to high expression of cell surface receptor CCR2.[4,5] During the process of recruitment and migration, monocyte-to-macrophage differentiation occurs to allow the cells to gain the properties of macrophages. The process of monocyte-to-macrophage differentiation involves changes in apoptosis, metabolism, cell shape and cell adherence.[1,5,6] After the differentiation, the mature cells adapt to local environment and function as resident macrophages. It is

Abbreviations: BMDM, bone marrow-derived macrophage; C/EBP, CCAAT/enhancer-binding protein; ChIP, chromatin immunoprecipitation; DAS-28, disease activity score; FACS, fluorescence-activated cell sorting; H&E staining, haematoxylin and eosin staining; IHC, immunohistochemistry; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor 1; PAQR, progestin and AdipoQ receptors; PBMC, peripheral blood mononuclear cell; PMA, phorbol 12-myristate 13-acetate; RA, rheumatoid arthritis; RT-qPCR, real-time quantitative polymerase chain reaction

noteworthy that the contribution of monocytes to resident macrophages differs in different tissues. Tissue macrophages in the intestine, dermis, heart and pancreas are mainly maintained by the circulating monocytes, while the macrophages in the liver and brain are not much affected by the monocytes.[7]

Although the process of monocyte-to-macrophage differentiation has been extensively investigated, the molecular mechanisms underlying the initiation and execution of the differentiation are still not completely understood. Recently, transcriptome and epigenome analyses revealed changes in inflammatory and metabolic pathways together with alterations in transcriptional programmes during monocyte-to-macrophage differentiation.[8,9] Among the transcription factors altered during monocyte-to-macrophage differentiation, CCAAT/enhancer-binding protein (C/EBP) family was found to have a dynamic changes, shown as an increase in *Cebpa* and reduction in *Cebpd* during the differentiation.[9] In addition, a number of molecules have been found to regulate monocyte-to-macrophage differentiation, such as AMPK and phosphatase PPM1A.[10–12]

Rheumatoid arthritis (RA) is a chronic and disabling disease associated with unrestrained inflammation in diarthrodial joints, affecting approximately 1% of the population worldwide.[13] Sustained influx of immune cells into the joints results in persistent synovitis, one of the hallmarks of RA.[14] In this setting, lymphocytes together with innate immune cells form a complex network that contributes to the damage of the joint and bone. In 40% of patients, the inflammatory infiltrate is constituted mainly by macrophages. Macrophages are important in RA progression by generating cytokines that enhance inflammation and contribute to destruction of cartilage and bone.[15] Macrophage colony-stimulating factor 1 (M-CSF) secreted by fibroblasts was also found to contribute to maturation of monocytes.[16] In addition, studies at the transcriptional level also demonstrated that macrophage maturation and polarization play functional roles in RA progression.[15] In addition, collagen-induced arthritis is a well-validated mouse model of rheumatoid arthritis, in which T cells response to collagen and recruit monocytes to result in severe inflammation.[17,18]

PAQR11, or called monocyte-to-macrophage differentiation-associated (MMD), is a member of progestin and AdipoQ receptor (PAQR) family.[19] PAQR11 is known as a Golgi apparatus scaffold protein to activate Ras signalling.[20] This gene was previously found in mature macrophages[21] and recently reported to be associated with macrophage activation *in vitro*.[22] In this study, we analysed the *in vitro* and *in vivo* function of PAQR11 in the regulation of monocyte-to-macrophage differentiation and found that PAQR11 modulated RA progression in mice.

Materials and methods

Reagents and antibodies

LPS, PMA, thioglycollate broth, and complete and incomplete Freund's adjuvant (CFA and IFA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). PD98059 was purchased from CSNpharm (Chicago, MI, USA). Mouse M-CSF was from Peprotech (Cranbury, NJ, USA). Human M-CSF, mouse IL-4, and IFN-γ were from Novoprotein (Shanghai, China). Immunization grade bovine type II collagen was from Chondrex (Seattle, WA, USA). The primary antibodies used in the immunoblotting assays were all from Cell Signaling Technology (Boston, MA, USA) as follows: C/EBPβ (1:1000, #3087s), phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204, 1:1000, #9101s), total p44/42 MAPK (EER1/2) (1:1000, #9102s), caspase-3 (1:1000, #9662s) and cleaved caspase-3 (Asp175) (1:1000, #9661s). Anti-α-tubulin antibody (1:3000, #T6199) was from Sigma-Aldrich. The secondary antibodies used in the immunoblotting assays were goat anti-mouse IgG (1:2000, Jackson, Philadelphia, PA, USA) and goat anti-rabbit IgG (1:2000, Jackson). The primary antibodies used in the flow cytometry were anti-mouse Ly-6C (HK1·4) APC, anti-mouse F4/80 (BM8) PE-efluor 610 and anti-mouse CD11b (M1/70) FITC from Thermo Fisher (Waltham, MA, USA). Annexin V FITC Apoptosis Detection Kit was purchased from BD Pharmingen (Franklin, NJ, USA). The antibodies used for immunohistochemistry were goat anti-mouse IL-1β (1:100) from R&D Systems (MI, USA) and rabbit anti-mouse F4/80 (1:200, #70076s) from Cell Signaling Technology.

Plasmids and transfection

Full-length *Paqr11* overexpression and shRNA plasmids were reported before.[20] C/EBPβ plasmid was purchased from Sino biological (Beijing, China). Full length of C/EBPβ was subcloned into the mammalian expression vector 3× Flag with a Flag epitope tag added to the N-terminus. C/EBPβ knockout plasmid was generated with LentiCRISPRV1 containing a sequence of sgRNA 5′-GGCCAACTTCTACTACGAGG-3′. Various lengths of *Paqr11* promoter were cloned from genome of HeLa cells by PCR and inserted into vector pGL3-basic. Vector pRL-TK was used to express Renilla luciferase for control. pMD2.G and psPAX2 plasmids were used for lentivirus packaging. HEK293T cells were transfected with polyethylenimine (PEI). Luciferase plasmids and *Paqr11* overexpression plasmid were transfected with Lonza Nucleofector into THP-1 cells. *Paqr11* knockdown, C/EBPβ knockout and C/EBPβ overexpression plasmids were transfected into THP-1 cells by lentivirus infection. Lentivirus was packaged with psPAX2-pMD2.G system. Each 10-mm dish of HEK293T cells was transfected with 7.5 μg of psPAX2, 3 μg of pMD2.G and 10 μg of shRNA-containing plasmid. The

cell culture medium was directly used to infect THP-1 cells for three times with medium changed every 24 h.

Cell isolation, differentiation and culture

HEK293T cells were cultured in DMEM with 10% FBS under 5% CO_2. THP-1 cells were purchased from cell bank of the Chinese Academy of Science, Shanghai, and kept in RPMI-1640 with 100 units/ml penicillin/streptomycin and 10% FBS at 37°, 5% CO_2. Human peripheral blood mononuclear cells (PBMCs) were separated by Percoll density gradient centrifugation from venous blood of healthy volunteers from Fudan University. Blood mixed with an equal amount of 1× PBS was added slightly on surface of 60% Percoll working solution. After centrifugation at 1000 *g* for 20 min, monocyte-rich layer was washed with PBS and then suspended in RPMI-1640. The cells were treated with 50 ng/ml of human M-CSF to induce monocyte differentiation. Mouse bone marrow-derived macrophages (BMDMs) were isolated from mouse leg bone marrow. In brief, muscle was scavenged from mouse legs, and bone marrow was flushed out with RPMI-1640. After red blood cells lysis, cells were cultured in RPMI-1640 with 10% FBS and 20 ng/ml M-CSF was added to induce differentiation. Polarization was induced after 6 days' differentiation with 100 ng/ml LPS and 50 ng/ml IFN-γ to M1-like or 20 ng/ml IL-4 to M2-like, respectively.

RNA isolation and RT-qPCR

RNA isolation, reverse transcription, RT-PCR and real-time PCR were carried out as described.[23] The sequence of primers for RT-PCR is shown in Table 1.

Western blotting

Protein isolation and Western blot were carried out as described.[20]

Flow cytometry

Cultured cells or primary cells were washed and suspended in PBS, then were diluted to 5×10^6 cells/ml. Cells were separated to 10^6 per tube and incubated with antibodies for 30 min, and washed with PBS twice. The cells were then suspended with 200 µl PBS for flow cytometry. The cells were also stained with propidium iodide (PI) for 5 min before cytometry. The flow cytometry results were analysed with FlowJo 7·6.

Dual-luciferase reporter assay

Dual-luciferase reporter assay kit was purchased from Promega. Different lengths of *Paqr11* promoter were cloned into pGL.3-basic. The plasmids pGL.3-basic and pRL-TK are cotransfected into THP-1 or HEK293T with a ratio 20:1. Twenty-four hours later, cells were lysed and luciferase activity was measured with the dual luciferase reporter assay system from Promega (Madison, WI, USA).

Chromatin immunoprecipitation and ChIP sequencing (ChIP-seq)

ChIP and ChIP-qPCR were carried out as others reported.[24,25] In brief, THP-1 transfected with Flag-tagged C/EBPβ was fixed with 1% (wt/vol) formaldehyde and subsequently quenched with glycine. After centrifugation, the cells were resuspended with lysis buffer and washed twice with washing buffer and shearing buffer to collect nucleus. The chromatin of nuclear pellets was sonicated to generate 200- to 1000-bp fragments. After sonication, the supernatant was collected and washed with conversion buffer. Supernatant was incubated with dynabeads for one hour for pre-clearance, with 5% as input and other 95% incubating with Flag-coated beads overnight. The beads were washed with RIPA buffer, high salt buffer and TE, followed by elution of DNA by fresh elution buffer containing RNase. Eluted DNA was incubated in 65° for 6 h for de-crosslinking. DNA was purified with DNA concentration kit (Tiangen, Beijing, China). In the ChIP-qPCR analyses, the values from the immunoprecipitated samples were normalized to those from the input DNA.

Animal studies

All animals were maintained and used in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, with an approval number 2010-AN-8. Mice were maintained on a 12-h light/dark cycle at 25°. Thioglycollate-recruited monocytes in the peritoneum were differentiated into mature macrophages. Mice were intraperitoneally injected with 1.5 ml of 3% thioglycollate. After 2–3 days of injection, the peritoneal exudate was isolated for RNA isolation or flow cytometry. RA mouse model was established as previously reported.[17] Type II collagen mixed with CFA was injected at the base of the tail. A boost was given on 14 days after the primary immunization with IFA and collagen. The mice were scored every 3 days. A third immunization was given to the mice when the scores were attenuated in 3 weeks after the second injection. The mice were killed 2 weeks after the third injection.

Haematoxylin and eosin (H&E) staining and immunohistochemistry

Murine paws were isolated from the mice, fixed overnight with 4% paraformaldehyde (PFA) for paraffin-embedding. For H&E staining, the tissue blocks were cut into at a

Table 1. Primers (5′-3′) used in PCR

Species	Gene name	F	R
Human being	Actin-b	TCCAAATATGAGATGCGTTGTT	TGCTATCACCTCCCCTGTGT
	Paqr11	TCTTTTTCTATCTCACAATGGGATT	CTGAAGTCCATCGGTGTTGTT
	Paqr10	CAGGATGGTGGAACACTGTC	ACGGAAGCCATAATCCAGAC
	Cd14	GTTCGGAAGACTTATCGACCAT	ACAAGGTTCTGGCGTGGT
	Cd36	TGGAACAGAGGCTGACAACTT	TTGATTTTGATAGATATGGGATGC
	Lox1	AGGACCAGCCTGATGAGAAG	AGGCAAAGGACCCCTAGAGT
	Cebpb	CGCTTACCTCGGCTACCA	ACGAGGAGGACGTGGAGAG
	Stat1	GGTACGAACTTCAGCAGCTTG	CCATGGGAAAACTGTCATCA
	Cebpa	GGAGCTGAGATCCCGACA	TTCTAAGGACAGGCGTGGAG
	Cebpd	GGACATAGGAGCGCAAAGAA	GCTTCTCTCGCAGTTTAGTGG
	Rela	TCATGAAGAAGAGTCCTTTCAGC	GGATGACGTAAAGGGATAGGG
	Brd4	AGAGTCGGAGAGCTCCAGTG	TGTCCAATGATTAGGCAGGA
	Spi1	CCACTGGAGGTGTCTGACG	CTGGTACAGGCGGATCTTCT
	P1	CCTTTCATCTTTACCACCAACT	GGGTAGTAAGAAGCCATTGGG
	P2	CTCCCGCACTGATGTTTTA	ATCTACCCGCACCCTC
	P3	GAAGCATGGAAAGCACCTCTCT	GCTCATTCGACCTACAGTAAAGC
	P4	GATTACAGGCATGAACCAC	TGTCGCTAAACCTAAACTCAG
	P5	CTCCTCCCCTTCCCCTCG	GCGGCTACGCTGCCATTC
	enhancer peak	CCAGAATCATTAAGGCAGGTACTT	TGCTGTAGAGGGTCCAGGTAG
	miR148-RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCAGTCACGT	
	miR200-RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATTATGAC	
	miR140-RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCGTCACCAA	
	miR148-F	TGTTTCAAGACATCACGTGACT	
	miR200-F	AGTAGTAATGGTCCGTCATAATCTC	
	miR140-F	GATGGUATCCCATTTTGGTGAC	
	miRNA-R	CTCAACTGGTGTCGTGGAGT	
Mouse	Actin-b	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA
	Paqr11	CAATTGCTACACACACGCATT	CCCGTAGATCCATGCTGTTATC
	Paqr10	CTGTGCCACCCATGCTTT	GTCGTCATCGGACAGGAAGT
	Adgre1	GGAGGACTTCTCCAAGCCTATT	AGGCCTCTCAGACTTCTGCTT
	Cd68	GACCTACATCAGAGCCCGAGT	CGCCATGAATGTCCACTG
	Itgam	AAAGAAACTGAAGCCTTTCTCG	AGCAACAAGCCAAGCACAC
	Arg1	GAATCTGCATGGGCAACC	GAATCCTGGTACATCTGGGAAC
	Cd206	CCACAGCATTGAGGAGTTTG	ACAGCTCATCATTTGGCTCA
	Ym1	GGTCTGAAAGACAAGAACACTGAG	GAGACCATGGCACTGAACG
	Il1b	AGTTGACGGACCCCAAAAG	AGCTGGATGCTCTCATCAGG
	Tnfa	CTGTAGCCCACGTCGTAGC	TTGAGATCCATGCCGTTG
	Mcp1	CATCCACGTGTTGGCTCA	GATCATCTTGCTGGTGAATGAGT
	Il6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA

thickness of 5μm and stained with H&E. For immunohistochemistry (IHC), the slides were processed by blocking with hydrogen peroxide, antigen retrieval, blocking with serum and then incubation with anti-IL-1β or anti-F4/80 antibodies. The secondary antibodies were applied after washing, followed by coloration with diaminobenzidine (DAB). The mounted slides were imaged using an Olympus BX51 microscope.

Statistical analysis

All the data were shown as mean ± SEM. The quantification results were analysed with *Student*'s *t*-test for comparison between two groups or ANOVA for comparison of more than two groups (In particular, one-way ANOVA was used for Figures 4F, 5F,G and two-way ANOVA was used for Figure 5B). Correlations between DAS-28 and gene expression in Figure 6B were analysed with Spearman's correlation coefficients. Values of $P < 0.05$ were considered statistically significant.

Results

Paqr11 expression is increased during monocyte-to-macrophage differentiation

To find changes during monocyte-to-macrophage differentiation, we mined an existing data set of PMA-

stimulated THP-1 differentiation (GSE53691), a common model to study monocyte differentiation.[26,27] We found 670 significantly upregulated genes and 559 downregulated genes with *P*-values < 0·005 and estimated absolute log2 fold change > 1 (Figure 1A). The top 10 upregulated genes were all related to cell adherence and inflammatory response, such as *Mmp1*, *Mmp8*, *Ccl20,* and *Il1b,* which were well known to be highly expressed during macrophage activation. To characterize the functional changes associated with macrophage differentiation, we performed GO enrichment analysis with DAVID 6.8.[28,29] The most frequent GO pathways were highly consistent with the GO analysis results of the upregulated genes (Figure 1B and Figure S1A), further indicating that the upregulated genes are involved in monocyte-to-macrophage differentiation.

GO analysis suggested that protein phosphorylation and cell proliferation were important in monocyte-to-macrophage differentiation (Figure 1B). PAQR11, a protein reported to mediate Ras signalling,[20] was one of the significantly upregulated genes (Figure 1A). We detected the changes of *Paqr11* in PMA-induced THP-1 cell differentiation. As shown in Figure 1C, *Paqr11* expression was increased over 30 times after three days' differentiation. We also analysed the change of *Paqr11* in monocyte differentiation induced by macrophage colony-stimulating factor (M-CSF). Both human peripheral blood mononuclear cells (PBMCs) and mouse bone marrow cells were stimulated by M-CSF, and we found that *Paqr11* expression was highly elevated during the differentiation (Figure 1D,E), indicating a positive correlation between *Paqr11* expression and monocyte-to-macrophage differentiation. As control experiments, we found that *Cd14* expression was reduced during M-CSF-induced differentiation in human PBMCs[30] (Figure 1D) and *Adgre1* expression was increased during the differentiation in mouse bone marrow cells[31] (Figure 1E).

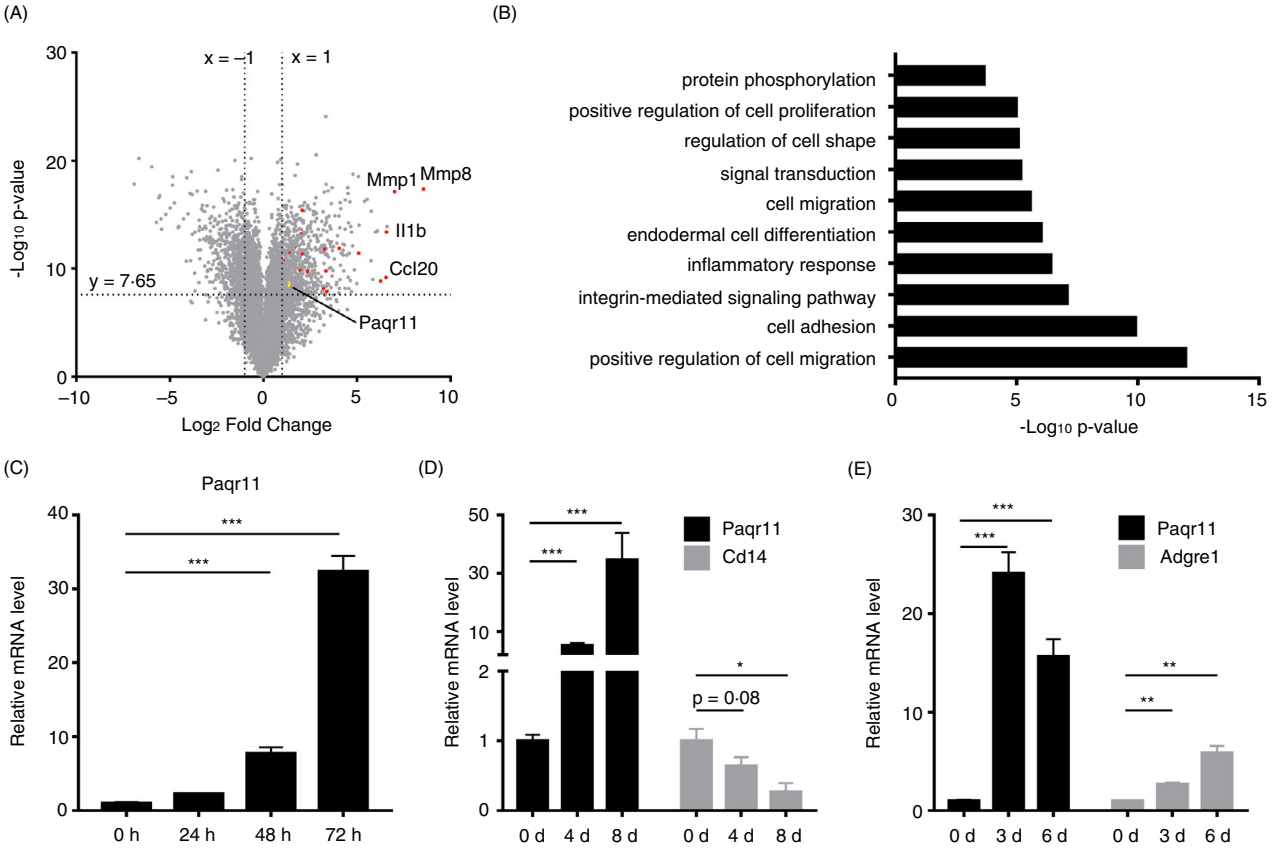


Figure 1. PAQR11 expression is increased during monocyte-to-macrophage differentiation. (A) Volcano plots to show RNA-Seq results of THP-1 cells with PMA stimulation for 3 days from GEO data set GSE53691. Red point, genes related to inflammation and cell adhesion from GEO data set GSE53691. Yellow point, *Paqr11*. (B) GO analysis of genes with fold change >2 or < −2 and $P < 0{\cdot}005$ ($n = 920$ genes). (C) *Paqr11* mRNA level in THP-1 cells during differentiation stimulated with 100ng/mL of PMA ($n = 3$ for each group). (D) *Paqr11* and *Cd14* mRNA level in human PBMCs stimulated by 50 ng/ml of M-CSF ($n = 3$ for each group). (E) *Paqr11* and *Adgre1* mRNA levels in mouse BMDMs stimulated by 20 ng/ml of M-CSF ($n = 3$ for each group). All mRNA expression levels are relative to β-actin. All the quantitative data are shown as mean ± SEM, * for $P < 0{\cdot}05$, ** for $P < 0{\cdot}01$ and *** for $P < 0{\cdot}001$.

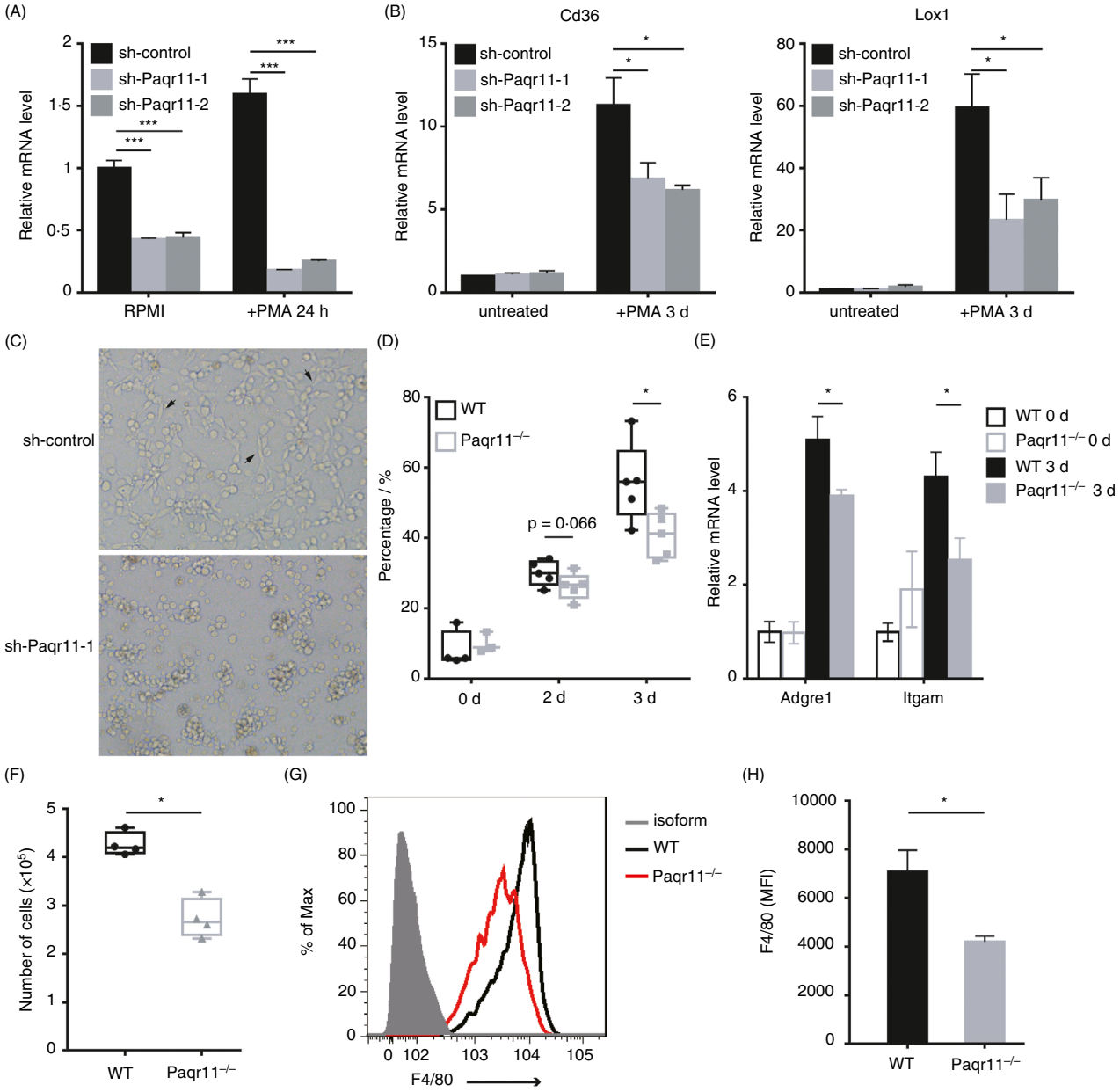


Figure 2. Knockdown or knockout of *Paqr11* attenuates monocyte-to-macrophage differentiation. (A) *Paqr11* mRNA level in THP-1 cells transfected with different shRNA-containing plasmids with or without PMA treatment (n = 3 for each group). (B) *Cd36* and *Lox1* mRNA levels in THP-1 cells transfected with different shRNA-containing plasmids with or without PMA treatment (n = 3 for each group). (C) Morphology of the cells as in B with PMA treatment for 24 h. The arrows indicate pseudopodium. (D) Percentage of $Ly6C^{-}/F4/80^{+}$ cells in $CD11b^{+}Ly\text{-}6G^{-}$ mononuclear cells detected by flow cytometry in peritoneal cells after injection of 3% thioglycollate in wild-type (WT) and *Paqr11*-deleted (*Paqr11*$^{-/-}$) mice (n = 3 at day 0, n = 5 at day 2 and day 3). (E) *Adgre1* and *Itgam* mRNA levels in peritoneal cells before and after thioglycollate induction in WT and *Paqr11*$^{-/-}$ mice (n = 5 for each group). (F) Numbers of mature macrophages derived from the bone marrows of WT and *Paqr11*$^{-/-}$ mice with M-CSF (20 ng/ml) stimulation for 6 d. Each group contained 2×10^6 cells before M-CSF treatment (n = 4 for each group). (G) Flow cytometry histogram of F4/80 expression of the cells as in F. (H) Mean fluorescence intensity of F4/80 of cells in F. All mRNA expression levels are relative to β-actin. All the quantitative data are shown as mean ± SEM, * for $P < 0{\cdot}05$ and *** for $P < 0{\cdot}001$.

Another gene in the PAQR family, PAQR10, was reported to have similar functions as PAQR11.[20] We found that *Paqr10* was not one of the 1,229 significantly changed genes (Figure 1A). We also found that *Paqr10* expression was only slightly altered during monocyte-to-macrophage differentiation, at a much lower degree than

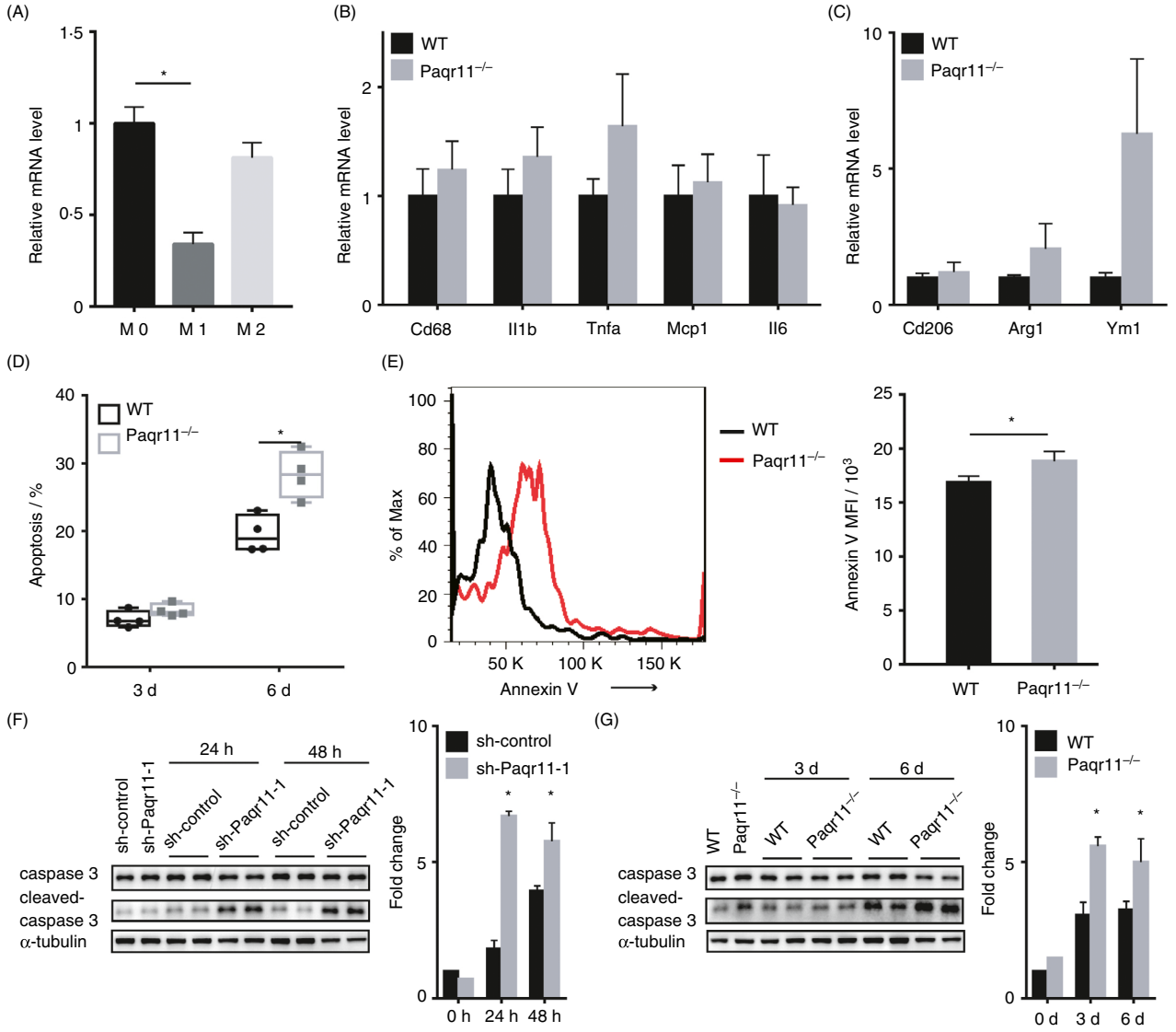


Figure 3. *Paqr11* deletion does not affect polarization but increases apoptosis of macrophages. (A) *Paqr11* mRNA level in M1-like and M2-like polarizations of BMDMs for 24 h ($n = 3$ for M0, $n = 6$ for M1-like and M2-like). (B, C) Gene expression of BMDMs from the wild-type (WT) and *Paqr11*$^{-/-}$mice during M1-like (for B) and M2-like (for C) polarization ($n = 4$ for B and $n = 5$ for C). (D) Percentage of Annexin V^{+} cells in BMDMs from WT and *Paqr11*$^{-/-}$ mice after differentiation for 3 days and 6 days ($n = 4$ for each group). (E) Flow cytometry histogram and quantification of Annexin V intensity of BMDMs from WT and *Paqr11*$^{-/-}$ mice after 6 days' differentiation ($n = 5$ for WT and $n = 4$ for *Paqr11*$^{-/-}$). (F) Western blotting to detect caspase-3 in THP-1 cells transfected with various shRNA plasmids and treated with or without PMA. The relative density of the Western blots is show in the right panel. (G) Western blotting to detect caspase-3 in mononuclear cells after M-CSF-induced differentiation in WT and *Paqr11*$^{-/-}$ mice. The relative density of the Western blots is shown in the right panel as normalized to α-tubulin. All mRNA expression levels are relative to β-actin. All the quantitative data are shown as mean ± SEM,* for $P < 0{\cdot}05$.

the changes in *Paqr11* (Figure S1C-E), indicating that PAQR10 is not likely playing a major role in macrophage differentiation.

Paqr11 knockdown/deletion attenuates monocyte-to-macrophage differentiation *in vitro* and *in vivo*

To investigate the function of PAQR11 in monocyte-to-macrophage differentiation, we constructed *Paqr11*-knocked down THP-1 cells (Figure 2A). Upon PMA stimulation, the expression of *Cd36* and *Lox1*, two THP-1 differentiation markers, was decreased in *Paqr11*-silenced cells (Figure 2B). Furthermore, the cell morphology was altered by *Paqr11* knockdown (Figure 2C). Due to a better adherent tendency, pseudopodium growth is commonly considered a feature of mature macrophages. We found that pseudopodium growth was severely reduced in *Paqr11* knockdown cells (Figure 2C). Collectively, these data demonstrated that *Paqr11* knockdown attenuated THP-1 differentiation.

We next analysed monocyte-to-macrophage differentiation using monocytes isolated from whole-body *Paqr11*-deleted mice (the generation of the mice will be described in a separate paper). Thioglycollate broth injection into the peritoneal cavity has been reported to study recruitment of circulating monocytes and differentiation of monocytes *in vivo*.[10,32] We injected thioglycollate into wild-type and *Paqr11*-deleted mice, and the peritoneal $Ly6C^{-}F4/80^{+}$ macrophages in $CD45^{+}CD11b^{+}Ly6G^{-}$ mononuclear cells were sorted in 2 or 3 days after the injection. As expected, the percentage of $Ly6C^{-}F4/80^{+}$ macrophages was significantly increased at day 2 and day 3 (Figure 2D). However, the percentage of $Ly6C^{-}F4/80^{+}$ macrophages was reduced by *Paqr11* deletion (Figure 2D). As a control experiment, we found that the expression of both *Adgre1* and *Cd11b*, two typical macrophage markers, was significantly elevated by thioglycollate injection (Figure 2E). The induction of both of the differentiation markers was also significantly reduced by *Paqr11* deletion (Figure 2E), consistent with the results of flow cytometry. We also analysed monocyte-to-macrophage differentiation upon M-CSF stimulation. The number of mature macrophages from $Paqr11^{-/-}$ mice was significantly smaller than that of wild-type mice after 6 days' stimulation by M-CSF (Figure 2F). FACS analysis revealed that bone marrow-derived macrophages (BMDMs) isolated from the $Paqr11^{-/-}$ mice had a reduced F4/80 fluorescence intensity (Figure 2G,H), suggesting insufficient maturation in *Paqr11*-deleted macrophages. Thus, these *in vitro* and *in vivo* data indicated that PAQR11 has a positive effect on monocyte-to-macrophage differentiation.

Deletion of *Paqr11* does not affect polarization but enhances apoptosis of macrophages

Macrophage polarization occurs after monocyte recruitment and maturation and is usually regulated by similar signals that affect monocyte-to-macrophage differentiation such as AMPKα1 signalling.[5,10,11,33] Previously, it was reported that *Paqr11* overexpression increased M1-like polarization in Raw264·7 cells.[22] We thus analysed the effect of *Paqr11* deletion on macrophage polarization. *Paqr11* transcript was decreased in M1-like polarization, while it was not altered in M2-like polarization (Figure 3A). However, we found no change of marker genes in M1-like polarization of BMDMs between the wild-type mice and $Paqr11^{-/-}$ mice (Figure 3B). In M2-like polarization, the expressions of *Arg*1 and *Cd206* were not altered, while *Ym1* was upregulated in *Paqr11*-deleted BMDMs (Figure 3C). Collectively, these data indicated that deletion of *Paqr11* does not have a profound effect on M1- or M2-like polarization of the macrophages.

In the absence of appropriate stimuli, monocytes undergo apoptosis, which is an important mechanism in regulating the numbers of monocytes and macrophages.[10,32,34] As deletion of *Paqr11*-attenuated monocyte-to-macrophage differentiation (Figure 2), we assessed whether PAQR11 had an effect on apoptosis of macrophages. Mouse BMDMs were isolated from the wild-type and $Paqr11^{-/-}$ mice and stained with Annexin V at the 6th day after M-CSF stimulation. The percentage of apoptotic cells was increased in *Paqr11*-deleted BMDMs as compared to the cells from the wild-type mice (Figure 3D). In addition, Annexin V signalling was elevated in *Paqr11*-deleted BMDMs (Figure 3E), further indicating an increase in apoptosis in *Paqr11*-deleted macrophages.

As apoptosis of monocytes/macrophages utilizes a caspase-3-dependent pathway,[34] we used Western blotting to analyse caspase-3 cleavage. In PMA-stimulated THP-1 cells, the protein intensity of cleaved caspase-3 was increased by *Paqr11* knockdown (Figure 3F). Furthermore, the level of cleaved caspase-3 was increased by *Paqr11* deletion in BMDMs upon M-CSF treatment (Figure 3G). These data therefore suggested that PAQR11 has a functional role in the prevention of macrophage apoptosis.

PAQR11-regulated p42/p44 MAPK phosphorylation promotes monocyte differentiation and cell survival

Previous studies have reported that monocyte-to-macrophage differentiation results in rewiring of MAPK signalling networks, and MAPK phosphorylation is a key regulator of the differentiation.[35–37] We also found that p42/p44 MAPK phosphorylation was increased during differentiation of THP-1 (Figure 4A), mouse BMDMs (Figure 4B) and human PBMCs (Figure 4C). As PAQR11 was previously reported to positively regulate Ras signalling regulation,[20] we investigated whether PAQR11 also had an effect on MAPK signalling in monocytes/macrophages. Knockdown of *Paqr11* in THP-1 drastically attenuated PMA-induced ERK1/2 phosphorylation (Figure 4D). Similarly, deletion of *Paqr11* in mouse BMDMs upon M-CSF stimulation reduced ERK1/2 phosphorylation (Figure 4E). As a control experiment, inhibition of ERK1/2 phosphorylation by PD98059, a MAPK inhibitor, could reduce PMA-induced THP-1 differentiation, similar to *Paqr11* knockdown (Figure 4F). In addition, treatment of mouse BMDMs with PD98059 reduced the number of mature macrophages and decreased the expression of *Adgre1* and *Itgam*, two markers of macrophage differentiation (Figure 4G). In cell morphology, PD98059 treatment also reduced the formation of pseudopodium in differentiated macrophages (Figure S2).

MAPK signalling pathway is commonly correlated with cell survival.[38,39] To analyse cell survival during monocyte differentiation, we removed M-CSF from BMDMs after one day's stimulation and PD98059 was added into the medium. Addition of PD98059 increased the level of

cleaved caspase-3 (Figure 4H). FACS analysis of BMDMs after 3 days' culture with DMSO or PD98059 revealed an increase in the number of apoptotic macrophages when MAPK pathway was inhibited by PD98059 (Figure 4I,J). These results therefore indicated that the MAPK signalling pathway regulated by PAQR11 plays a critical role in monocyte-to-macrophage differentiation and cell survival.

C/EBPβ regulates *Paqr11* expression during monocyte-to-macrophage differentiation

As we found that *Paqr11* expression was robustly elevated in 24 h after the initiation of monocyte differentiation (Figure 1), we next explored how *Paqr11* expression was regulated. At first, we identified 3 miRNAs that were predicted to target at *Paqr11* mRNA by TargetScan (www.targetscan.org) and these miRNAs were reported to downregulate PAQR11 transcript.[40,41] However, we found that all these three miRNAs were highly upregulated during monocyte-to-macrophage differentiation in THP-1 cells (Figure 5A), indicating that PAQR11 upregulation during monocyte differentiation was not likely caused by changes in these miRNAs.

To examine the expression of *Paqr11* at the transcriptional level, we performed a dual-luciferase reporter assay in THP-1 cells. We cotransfected THP-1 cells with a plasmid expressing Renilla luciferase and a plasmid of fly-luciferase reporter gene under the control of different lengths of the putative promoter region of *Paqr11* gene. Upon PMA stimulation, the −1500 bp to +100 bp of the putative promoter region of *Paqr11* gene had the highest luciferase activity among all the tested segments (Figure 5B), suggesting that the promoter of *Paqr11* is localized in this region.

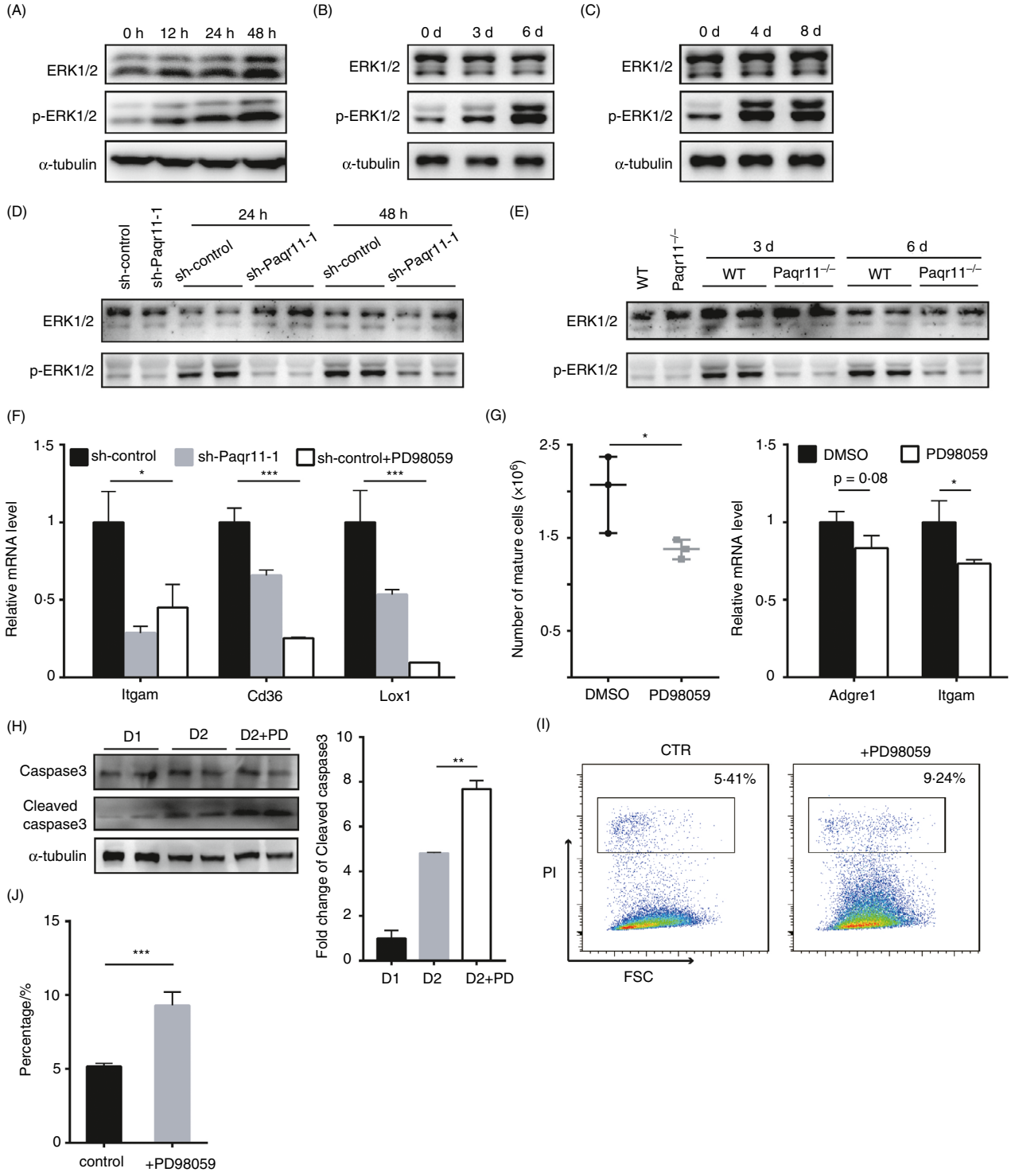
To identify which transcriptional factor plays a role in the transcriptional regulation of *Paqr11* gene, we analysed the expression of the predicted transcription factors during monocyte differentiation (Figure 5C, with the prediction performed using the websites http://alggen.lsi.upc.es and http://cistrome.org). We found that the trend of C/EBPβ gene expression was highly similar to the trend of *Paqr11* gene expression (Figure 5C). Motif analysis also revealed 4 potential binding sites of C/EBPβ in the promoter region of *Paqr11* gene (Figure S3A). We next examined whether C/EBPβ is a transcriptional factor that regulates *Paqr11* expression. The luciferase activity of *Paqr11* promoter (−1500/+100 bp) was elevated about 10 times by C/EBPβ overexpression (Figure 5D), while C/EBPβ had no effect on the promoter of −525/+100 bp (Figure S3B). ChIP-qPCR analysis further revealed that C/EBPβ could directly bind to the *Paqr11* promoter (Figure 5E).

As our data suggested that C/EBPβ is a transcriptional regulator of *Paqr11* expression, we hypothesized that changes in C/EBPβ expression would influence monocyte-to-macrophage differentiation. We found that deletion of C/EBPβ in THP-1 cells decreased *Paqr11* expression (Figure 5F). Meanwhile, the expression of *Cd36* and *Lox1* was decreased by C/EBPβ knockout (Figure 5F). *Paqr11* overexpression could partially rescue the inhibitory effect of C/EBPβ deletion on *Cd36* and *Lox1* expression (Figure 5F), indicating that PAQR11 functions downstream of C/EBPβ in regulating monocyte-to-macrophage differentiation. Furthermore, we found that C/EBPβ knockout also accelerated cell apoptosis shown as an increase in caspase-3 cleavage (Figure 5G), whereas overexpression of *Paqr11* partially attenuated the effect of C/EBPβ knockout (Figure 5G). Overall, these results revealed that C/EBPβ is upregulated during monocyte-to-macrophage differentiation and it modulates monocyte differentiation via transcriptional regulation of *Paqr11* expression.

Paqr11-deleted mice have reduced pathological characteristics of rheumatic arthritis

As macrophage recruitment and maturation participate in rheumatic arthritis (RA), we first analysed a data set of RA patients (GSE49604).[42] From this data set, we found that genes associated with macrophage maturation such as *Ccl2* and *Lox1* were increased in the synovial macrophages of the RA patients (Figure 6A). In addition, genes related to cell proliferation and inflammatory response such as

Figure 4. PAQR11 affects monocyte differentiation through ERK1/2 phosphorylation (A,B,C) ERK1/2 phosphorylation in THP-1 cells upon PMA treatment (100 ng/ml, for A), mouse BMDMs upon M-CSF treatment (20 ng/ml, for B) and human PBMCs upon M-CSF treatment (50 ng/ml, for C). (D) ERK1/2 phosphorylation during PMA-induced differentiation in THP-1 cells transfected with various shRNA plasmids. (E) ERK1/2 phosphorylation in BMDMs from the wild-type (WT) and *Paqr11*$^{-/-}$ mice. BMDMs were stimulated by M-CSF stimulation. (F) The mRNA levels of *Cd11b*, *Cd36* and *Lox1* in THP-1 cells upon PMA stimulation for 3 days. The cells were transfected with shRNA plasmids or treated with 10μM of PD98059 as indicated (n = 3 for each group). (G) Numbers of mature macrophages from 8 × 10^6 mouse BMDMs after 6 days' stimulation with 20 ng/ml of M-CSF. DMSO or 10 μM of PD98059 was added at day 3. The mRNA levels of *Adgre1* and *Itgam* are shown in the right panel (n = 3 for each group). (H) Western blotting of caspase-3 in BMDMs. The cells were treated with 50 ng/ml of M-CSF for one day and then treated with DMSO or 10 μM of PD98059 for one more day. The intensity quantification is shown in the right panel as normalized to α-tubulin. (I) Flow cytometry of BMDMs treated with DMSO or 10 μM of PD98059 for 3 days. The number indicates percentage of propidium Iodide-positive cells. (J) Quantification of the flow cytometry result of I (n = 5 for each group). All mRNA expression levels are relative to β-actin. All the quantitative data are shown as mean ± SEM, * for $P < 0{\cdot}05$, ** for $P < 0{\cdot}01$ and *** for $P < 0{\cdot}001$



Nras, Hras and Ccl20 were upregulated in the samples of the RA patients (Figure 6A). Interestingly, *Paqr11* was found to have an increased expression in the synovial macrophages of the RA patients (Figure 6A, $P = 0.025$ between the two groups). In addition, *Cebpb* is differentially expressed between the two groups (Figure 6A, $P = 0.011$). Further data mining was also used to investigate whether *Paqr11* expression was correlated with RA disease. From the data set GSE68689, we observed a positive correlation between *Paqr11* expression and disease activity measured as DAS28 score (Figure 6B). We also found that DAS28 score was correlated with PU.1

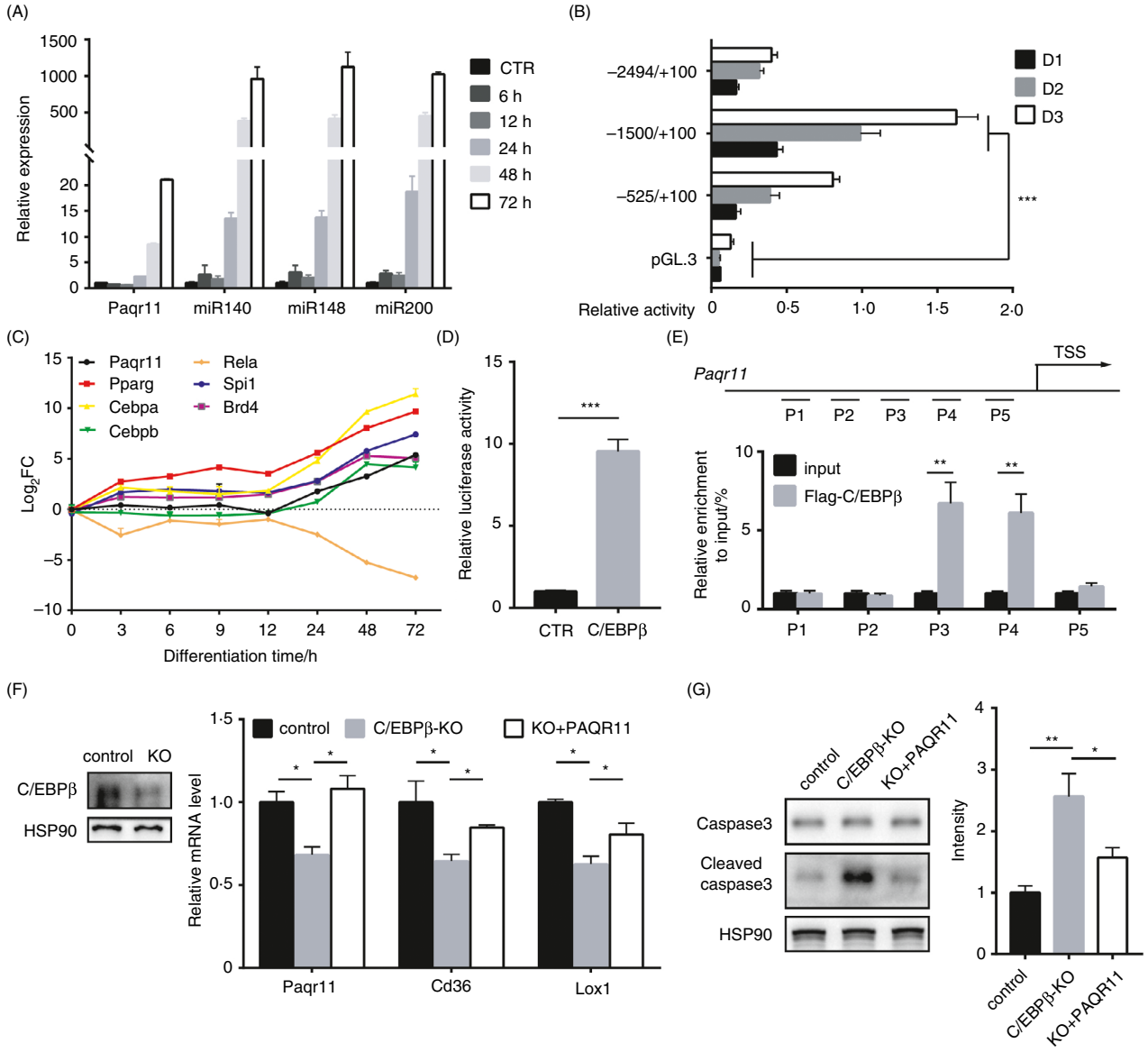


Figure 5. C/EBPβ regulates PAQR11 expression at the transcriptional level. (A) Expression of *Paqr11* and its targeting miRNAs at different times during PMA-induced THP-1 differentiation. (B) Dual-luciferase reporter assay in THP-1 cells treated with 100 ng/ml PMA for 3 days. Different lengths of putative *Paqr11* promoter were cloned into pGL3-basic plasmid and transiently expressed in the THP-1 cells. Relative luciferase activity was calculated by dividing the firefly luciferase activity by Renilla activity ($n = 4$ for each group). (C) The mRNA levels of a few transcription factors during THP-1 differentiation ($n = 5$ for each group). (D) Effect of C/EBPβ on luciferase activity of *Paqr11* promoter. HEK293T cells were transfected with pGL3-basic plasmid containing −1500/+100 *Paqr11* promoter with or without overexpression of C/EBPβ ($n = 3$ for each group). (E) ChIP-qPCR at different regions of *Paqr11* promoter in THP-1 overexpressing C/EBPβ. P1 to P5 were five different segments covering the region of −2500 bp to transcription start site (TSS) region ($n = 4$ for each group). (F) The mRNA levels of *Paqr11*, *Cd36* and *Lox1* in THP-1 cells with C/EBPβ knockout and PAQR11 overexpression as indicated ($n = 3$ for each group). Left, Western blotting of C/EBPβ to determine the efficiency of C/EBPβ knockout with CRISPR-Cas9 system. The cells were treated with PMA for 24 h as indicated. Note that a pool of CRISPR-Cas9-treated cells was used in this experiment. (G) Western blotting of caspase-3 with the cells as in F. Right, density quantification of the Western blots, normalized to HSP90. All mRNA expression levels are relative to β-actin. All the quantitative data are shown as mean ± SEM, * for $P < 0{\cdot}05$ and *** for $P < 0{\cdot}001$.

(Figure 6B), a well-known transcript factor regulating macrophage recruitment and maturation.[1] The other two data sets GSE10500 and GSE95588 also revealed an increased *Paqr11* expression in the synovial macrophages of the patients. Overall, these data indicated a potential role of PAQR11 in RA development.

To further investigate the functional role of PAQR11 in RA, we induced arthritis in wild-type and *Paqr11*$^{-/-}$ mice

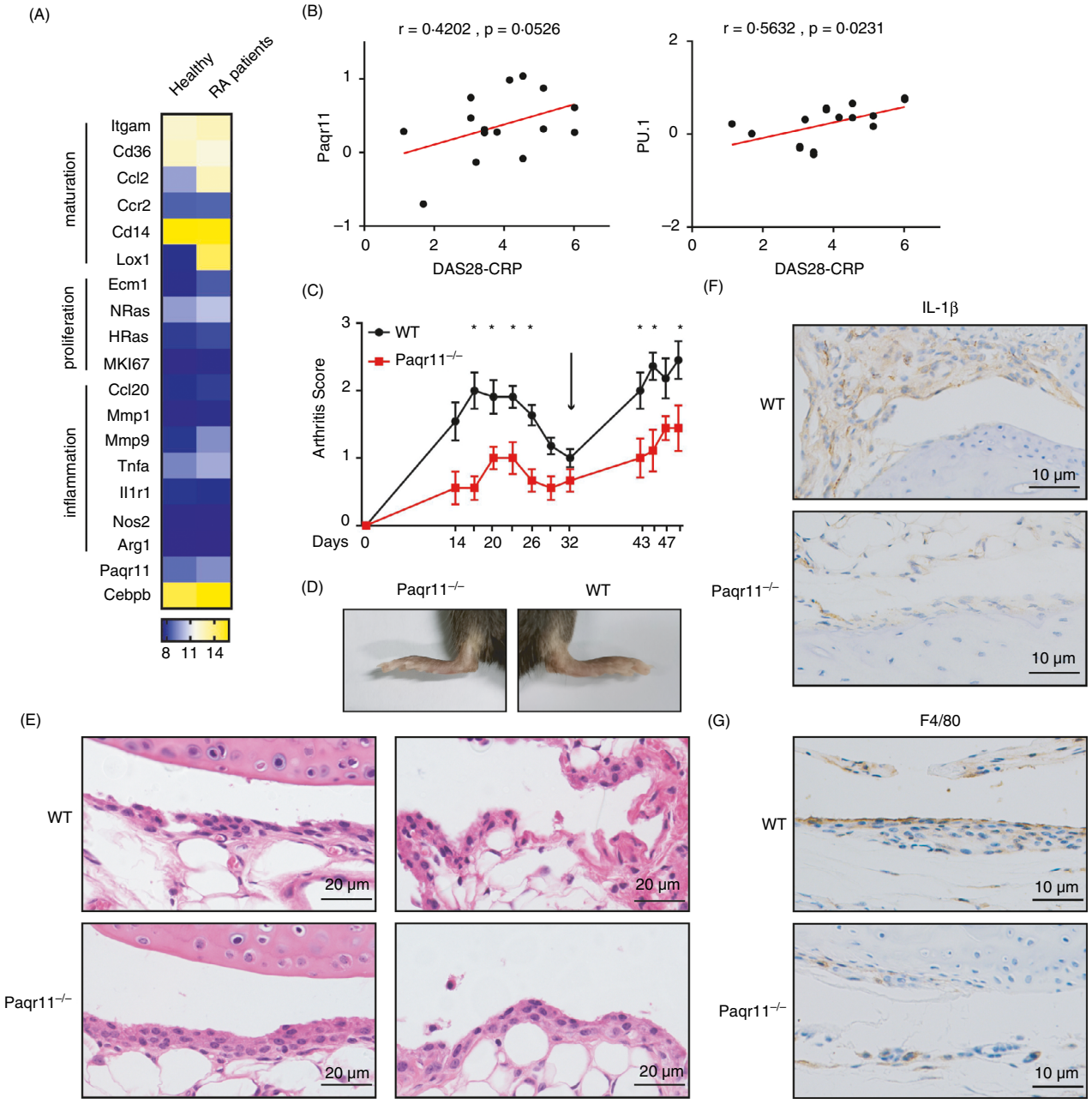


Figure 6. *Paqr11* deletion ameliorates pathogenesis of RA in mice. (A) Heatmap showing relative gene expression in synovial macrophages from the healthy and RA patients. Data are from GSE49604. (B) Correlation of *Paqr11* and PU.1 transcripts with disease activity score (DAS28-CRP). Data are from GSE68689. Correlation analyses performed using Spearman's correlation coefficients. (C) Arthritis clinical score in wild-type (n = 10) and *Paqr11*$^{-/-}$mice (n = 8) after second immunization. Arrows indicate the day of the third immunization. The data are shown as mean ± SEM, and * for $P < 0{\cdot}05$. (D) Representative images of the paws at day 49 post-immunization. (E) H&E staining of the joint and pad of mice. The images on the left are for articular cavity, and the images on the right are for synovium morphology. (F) Immunohistochemistry images of IL-1β at the joint of the mice. (G) Immunohistochemistry images of F4/80 of the mice.

with chicken type II collagen.[17] Clinical scores of arthritis and the symptoms were significantly reduced in *Paqr11*$^{-/-}$ mice as compared to the wild-type control (Figure 6C, D). Histological analysis revealed that the synovium structure in the *Paqr11*-deleted mice was more intact than that in the wild-type mice (Figure 6E), suggesting a reduction in tissue damage and inflammatory cell invasion. Immunohistochemistry analysis revealed that the expression of IL-1β, a classical pro-inflammatory cytokine, was reduced by *Paqr11* deletion (Figure 6F). In addition, the staining of F4/80 was reduced in the joint of *Paqr11*-deleted mice (Figure 6G), further indicating

that the infiltration of mature macrophages in the joint synovitis is attenuated in *Paqr11*$^{-/-}$ mice. Consistently, the size of inguinal lymph nodes was much smaller in *Paqr11*$^{-/-}$ mice than the wild-type controls (Figure S4). Collectively, these data demonstrated an *in vivo* function of PAQR11 in modulating the pathogenesis of RA.

Discussion

In summary, our studies indicated that PAQR11 is an active player in monocyte-to-macrophage differentiation through both *in vitro* and *in vivo* experiments. *Parq11* gene is highly induced during monocyte-to-macrophage differentiation in THP-1 and monocytes isolated from human blood or mouse bone marrow. PAQR11 has a stimulatory effect on monocyte-to-macrophage differentiation as knockdown/knockout of *Paqr11* inhibits the differentiation process. PAQR11 mainly impacts the monocyte-to-macrophage maturation process as it only has a small effect on macrophage polarization. Mechanistically, PAQR11 promotes cell survival as apoptosis is increased by *Paqr11* knockdown/knockout. Furthermore, activation of MAPK signalling pathway is involved in the regulatory role of PAQR11 on monocyte differentiation and cell survival. The transcription factor C/EBPβ is involved in the transcriptional regulation of *Paqr11* in monocytes. At the animal level, deletion of *Paqr11* gene alleviates RA progression. Thus, these data provided compelling evidence that PAQR11 has a function in monocyte-to-macrophage differentiation and such function is related to autoimmune disease *in vivo*.

Monocyte-to-macrophage differentiation is an early step of peripheral monocytes participating in an inflammatory response.[1] Because this progress seems like a 'transition state' in macrophage activation, many previous studies were focused on macrophage polarization or macrophage functions but not on macrophage maturation per se. In fact, most known molecules that affect macrophage maturation also play important roles in the processes that follow macrophage activation.[5,10,35,43] Here, we report that PAQR11 mainly affects monocyte-to-macrophage differentiation but not macrophage polarization, suggesting that the process of macrophage maturation can be functionally separated from macrophage polarization.

Numerous studies have explored the transcriptional regulation during monocyte-to-macrophage differentiation.[8,9,11] Studies at the epigenomics and transcriptomics levels have indicated that some of the transcription factors involved in monocyte-to-macrophage differentiation are also regulators of lipid metabolism such as PPARγ and C/EBPs.[8,9] We report here that C/EBPβ is involved in monocyte differentiation through PAQR11, thus providing a new insight about the functions of C/EBPβ in this process. However, it remains unclear whether the lipid metabolism regulated by C/EBPβ is required for macrophage differentiation. This is an issue that needs to be addressed in the future.

As an autoimmune disease is promoted by recruited immune cells but not resident ones, RA is a useful model to study macrophage differentiation. Although T cells are known to play a pivotal role in RA, macrophages are actively involved in the pathogenesis of RA.[44] Recruited macrophages recognize cytokines such as TNF-α and autoimmune antibodies secreted by adaptive immune cells, leading to production of cytokines such as interleukin-6 (IL-6) to exacerbate inflammation in the joints.[14,44] Therefore, inhibition of macrophage activation is an effective way for the treatment of RA, such as blocking IL-6 signalling pathway.[45] However, few therapies have targeted on the steps of macrophage recruitment and monocyte-to-macrophage differentiation that are upstream of macrophage activation. As we found that deletion of *Paqr11* ameliorates RA phenotype in the mice, inhibition of PAQR11-mediated signalling pathway can be considered as a potential strategy to combat against RA or other autoimmune diseases.

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Conflict of interest

We declare no conflict of interest.

Author contribution

Y.C. and Y.L. conceptualized the study, designed the study and wrote the paper. Y.L. performed the experiments. M.H., S.W., X.Y. and L.Z. provided technical assistance.

DATA AVAILABILITY STATEMENT

Not applicable.

References

1 Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol.* 2017; **17**(6):349–62.

2 Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol.* 2009; **27**:669–92.

3 Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunity* 1999; **11**(6):753–61.

4 Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol.* 2006; **7**(3):311–7.

5 Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat Rev Immunol.* 2011; **11**(11):762–74.

6 Gautier EL, Ivanov S, Lesnik P, Randolph GJ. Local apoptosis mediates clearance of macrophages from resolving inflammation in mice. *Blood* 2013; **122**(15):2714–22.

7 Ginhoux F, Guilliams M. Tissue-resident macrophage ontogeny and homeostasis. *Immunity* 2016; **44**(3):439–49.
8 Dong C, Zhao G, Zhong M, Yue Y, Wu L, Xiong S. RNA sequencing and transcriptomal analysis of human monocyte to macrophage differentiation. *Gene* 2013; **519**(2):279–87.
9 Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, Matarese F, *et al.* Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 2014; **345**(6204):1251086.
10 Zhang M, Zhu H, Ding Y, Liu Z, Cai Z, Zou MH. AMP-activated protein kinase alpha1 promotes atherogenesis by increasing monocyte-to-macrophage differentiation. *J Biol Chem.* 2017; **292**(19):7888–903.
11 Vasamsetti SB, Karnewar S, Kanugula AK, Thatipalli AR, Kumar JM, Kotamraju S. Metformin inhibits monocyte-to-macrophage differentiation via AMPK-mediated inhibition of STAT3 activation: potential role in atherosclerosis. *Diabetes* 2015; **64**:14.
12 Smith SR, Schaaf K, Rajabalee N, Wagner F, Duverger A, Kutsch O, *et al.* The phosphatase PPM1A controls monocyte-to-macrophage differentiation. *Sci Rep.* 2018; **8**(1):902.
13 Wang Y, Han CC, Cui D, Li Y, Ma Y, Wei W. Is macrophage polarization important in rheumatoid arthritis? *Int Immunopharmacol.* 2017; **50**:345–52.
14 Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nat Rev Rheumatol.* 2019; **15**(1):9–17.
15 Udalova IA, Mantovani A, Feldmann M. Macrophage heterogeneity in the context of rheumatoid arthritis. *Nat Rev Rheumatol.* 2016; **12**(8):472–85.
16 Hamilton JA, Filonzi EL, Ianches G. Regulation of macrophage colony-stimulating factor (M-CSF) production in cultured human synovial fibroblasts. *Growth Factors* 1993; **9**(2):157–65.
17 Inglis JJ, Simelyte E, McCann FE, Criado G, Williams RO. Protocol for the induction of arthritis in C57BL/6 mice. *Nat Protoc.* 2008; **3**(4):612–8.
18 Campbell IK, Rich MJ, Bischof RJ, Dunn AR, Grail D, Hamilton JA. Protection from collagen-induced arthritis in granulocyte-macrophage colony-stimulating factor-deficient mice. *J Immunol.* 1998; **161**(7):3639–44.
19 Tang YT, Hu T, Arterburn M, Boyle B, Bright JM, Emtage PC, *et al.* PAQR proteins: a novel membrane receptor family defined by an Ancient7-Transmembrane Pass Motif. *J Mol Evol.* 2005; **61**(3):372–80.
20 Jin T, Ding Q, Huang H, Xu D, Jiang Y, Zhou B, *et al.* PAQR10 and PAQR11 mediate Ras signaling in the Golgi apparatus. *Cell Res.* 2011; **22**(4):661–76.
21 Rehli M, Krause SW, Schwarzfischer L, Kreutz M, Andreesen R. Molecular cloning of a novel macrophage maturation-associated transcript encoding a protein with several potential transmembrane domains. *Biochem Biophys Res Commun.* 1995; **217**(2):661–7.
22 Liu C, Zheng J, Yin D, Xiang J, He F, Wang Y, *et al.* Monocyte to macrophage differentiation-associated (MMD) positively regulates ERK and Akt activation and TNF-a and NO production in macrophages. *Mol Biol Rep.* 2012; **39**:8.
23 Ding Q, Jin T, Wang Z, Chen Y. Catalase potentiates retinoic acid-induced THP-1 monocyte differentiation into macrophage through inhibition of peroxisome proliferator-activated receptor gamma. *J Leukoc Biol.* 2007; **81**(6):1568–76.
24 Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, *et al.* Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nat Commun.* 2016; **23**(7):11624.
25 Nelson JD, Denisenko O, Bomsztyk K. Protocol for the fast chromatin immunoprecipitation (ChIP) method. *Nat Protoc.* 2006; **1**(1):179–85.
26 Chanput W, Mes JJ, Wichers HJ. THP-1 cell line: an in vitro cell model for immune modulation approach. *Int Immunopharmacol.* 2014; **23**(1):37–45.
27 Daigneault M, Preston JA, Marriott HM, Whyte MK, Dockrell DH. The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. *PLoS One* 2010; **5**(1):e8668.
28 da Huang W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* 2009; **4**(1):44–57.
29 da Huang W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* 2009; **37**(1):1–13.
30 Nielsen MC, Andersen MN, Moller HJ. Monocyte isolation techniques significantly impact the phenotype of both isolated monocytes and derived macrophages in vitro. *Immunology* 2020; **159**(1):63–74.
31 Ray R, Rai V. Lysophosphatidic acid converts monocytes into macrophages in both mice and humans. *Blood* 2017; **129**(9):1177–83.
32 Zhang Y, Morgan MJ, Chen K, Choksi S, Liu ZG. Induction of autophagy is essential for monocyte-macrophage differentiation. *Blood* 2012; **119**(12):2895–905.
33 Sag D, Carling D, Stout RD, Suttles J. Adenosine 5'-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. *J Immunol.* 2008; **181**(12):8633–41.
34 Fahy RJ, Doseff AI, Wewers MD. Spontaneous human monocyte apoptosis utilizes a Caspase-3-dependent pathway that is blocked by endotoxin and is independent of caspase-1. *J Immunol.* 1999; **163**(4):8.
35 Bhattacharya A, Ghosh P, Prasad R, Ghosh A, Das K, Roy A, *et al.* MAP Kinase driven actomyosin rearrangement is a crucial regulator of monocyte to macrophage differentiation. *Cell Signal.* 2020; **10**(73):109691.
36 Fahling M, Persson AB, Klinger B, Benko E, Steege A, Kasim M, *et al.* Multilevel regulation of HIF-1 signaling by TTP. *Mol Biol Cell.* 2012; **23**(20):4129–41.
37 Richter E, Ventz K, Harms M, Mostertz J, Hochgrafe F. Induction of macrophage function in human THP-1 cells is associated with rewiring of MAPK signaling and activation of MAP3K7 (TAK1) protein kinase. *Front Cell Dev Biol.* 2016; **4**:21.
38 Kolch W. Coordinating ERK/MAPK signalling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol.* 2005; **6**(11):827–37.
39 Lavoie H, Gagnon J, Therrien M. ERK signalling: a master regulator of cell behaviour, life and fate. *Nat Rev Mol Cell Biol.* 2020; **21**(10):607–32.
40 Li W, He F. Monocyte to macrophage differentiation-associated (MMD) targeted by miR-140-5p regulates tumor growth in non-small cell lung cancer. *Biochem Biophys Res Commun.* 2014; **450**(1):844–50.
41 Tan X, Banerjee P, Guo HF, Ireland S, Pankova D, Ahn YH, *et al.* Epithelial-to-mesenchymal transition drives a pro-metastatic Golgi compaction process through scaffolding protein PAQR11. *J Clin Invest.* 2017; **127**(1):117–31.
42 You S, Yoo S, Choi S, Kim J, Park S, Ji JD, *et al.* Identification of key regulators for the migration and invasion of rheumatoid synoviocytes through a systems approach. *Proc Natl Acad Sci USA.* 2014;**111**:6.
43 Janssen WJ, Barthel L, Muldrow A, Oberley-Deegan RE, Kearns MT, Jakubzick C, *et al.* Fas determines differential fates of resident and recruited macrophages during resolution of acute lung injury. *Am J Respir Crit Care Med.* 2011; **184**(5):547–60.
44 Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. *Lancet* 2010; **376**:15.
45 Choy EH, Isenberg DA, Garrood T, Farrow S, Ioannou Y, Bird H, *et al.* Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial. *Arthritis Rheum.* 2002; **46**(12):3143–50.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (A) GO analysis of upregulated and down-regulated genes in during THP-1 differentiation with fold change > 2 meanwhile $P < 0{\cdot}005$. (B) Fold enrichment of GO terms in Figure 1B. (C, D, E) *Paqr11* and *Paqr10* mRNA level in THP-1 in C, mouse BMDMs in D and human PBMCs in E during monocyte differentiation ($n = 3$ for each group). All mRNA expression levels are relative to β-actin. Data are shown as mean ± SEM. * for $P < 0{\cdot}05$ and *** for $P < 0{\cdot}001$.

Figure S2. (A,B) Cell morphology and density of THP-1 treated with PMA for 1 day (A) and mouse BMDMs treated with M-CSF for 3 days (B) with DMSO or 10 μM PD98059. (C) Western blotting of ERK1/2 phosphorylation in differentiated THP-1 cells treated with 0, 2 and 10 μM of PD98059 as indicated.

Figure S3. (A) C/EBPβ motif analysis and potential binding sites at *Paqr11* promoter. (B) Effect of C/EBPβ on luciferase activity with pGL3-basic plasmid containing -525/+100 *Paqr11* promoter with or without overexpression of C/EBPβ-expressing plasmid ($n = 3$ for each group).

Figure S4. Inguinal lymph nodes of the mice as described in Figure 6C..