Sh c ica i

Echinacea pupurea e ac s e i e de d i ic cell a a i b ac i a i f JNK, 38 MAPK a d NF-κB a h a s

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Af e e c e i g i fla a s i li, i a e DCs de g a a i , e l i g f a ige -ca i g cells T cell- i i g cells (D dek e al., 2013). Mea hile, ac i a ed DCs ca sec e e a di e sified a el f c ki es ha i i ia e ada i e i e- s ses i d ce le a ce (Ta a d O Neill, 2005). This a a- i ces has bee e ed be highl de e de he T ll-like ece s (TLRs)- edia ed i ge -ac i a ed ei ki ases (MAPKs) a d clea fac -κB (NF-κB) sig al a sd c i a h- a s, e s e TLR ag is s ch as bac e ial LPS (D li g e al., 2008). Acc la i g e ide ce s gges s ha EE a e i l ed i d la i g cell fa e, diffe e ia i a d e essi f s ecific i e- ela ed ge es i DCs (Wa g e al., 2006a; Be s e al., 2010), b he echa is f EE DCs a a i a d ac i a i is s ill bsc e. I his a e, e he ef e ai ed i es iga e he effec s f EE he a a i ces f b e a -de i ed de d i ic cells (BMDCs), a d he echa is s de l ig hese effec s e e als e a i ed. LPS, a ell-k s i l s f DC ac i a i , as sed as a si i e c l. We de s a ed ha EE c ld e b h he ic a d f c i al a a i f BMDCs a d EE- edia ed cell a a i i l es ac i a i f MAPKs a d NF-κB a h a s.

2. Materials and methods

2.1. Chemicals and reagents

E. purpurea e ac s (EE) sed i his s d e e chased f Sha d g Qil A i al Heal h C ., L d. Che ical c si i f EE e e: cich ic acid (3.045%), caf a ic acid (1.575%), chl ge ic acid (0.065%), d deca-2E, 4E, 8Z, 10E/Z- e ae ic acid is b la ide (1.635%). The e as de ec able e d i c a i a i as es ed b E d s ec (<0.10 e d i i s/ l). Li l saccha- ide (LPS, *Escherichia coli* 0111: B4, Ul a e) a d FITC-de a (40,000 Da) e e b ai ed f Sig a Che ical C . (S . L is, USA). G a l c e- ac hage c l -s i la i g fac (GM-CSF) a d i e le ki (IL)-4 e e chased f Pe Tech I c. (R ck Hill, USA). A i- se a ib dies FITC-CD11c, -CD40, -CD80, -CD83, -CD86 a d *anti*-NF-κB 65 e e chased f Bi lege d (Sa Dieg , USA). The ELISA ki s f IFN-γ, IL-12 70, IL-10 a d TGF-β1 e e f eBi scie ce (Sa Dieg , USA). A ib dies agai s h s h -ERK1/2, ERK1/2, h s h -JNK, JNK, h s h - 38 MAPK, 38 MAPK, La i B1, IκBα, β-ac i a d HRP-c j ga ed IgG e e b ai ed f Sa a C Bi ech (USA). I hibi s BAY 11-7082, SP600125 a d SB203580 e e b ai ed f Be i e Bi ech l g (Hai e , Chi a).

2.2. Bone marrow-derived dendritic cells (BMDCs)

C57BL/6 ice (fe ale, 6–8 eeks ld) e e b ai ed f Sha ghai SLAC Lab a A i al C a . BMDCs e e e a ed as e i sl des c ibed (Lee e al., 2010) i h difica i s. B iefl , he fe a d ibia f ice e e fl shed i h 5 l f c ld s e ile PBS. Af e l si g ed bl d cells, b e a cells e e i c ba ed a 37 °C i c le e RPMI edia s le e ed i h 10 g/ l GM-CSF, 10 g/ l IL-4, 10% FBS, 100 μg/ l s e ci , a d 100 U/ l e icilli . F esh edi as e laced e e he da , a d - adhe e cells e e a sfe ed e la es. Af e 7 da s f c l- e, -adhe e cells e e c llec ed a d he i f CD11c$^+$ (i e DC li eage-s ecific a ke) cell as >90% as e a i ed b fl c e (FACS).

2.3. Flow cytometry analysis

BMDCs (1 × 10^6 cells/ l) e e c l ed i 12- ell la es. E essi f cell s face lec les as de e i ed af e 48 h b ea e i h PBS, EE (400 μg/ l), LPS (50 g/ l), es ec i el . Cells f diffe e ea e g s e e c llec ed a d s s e- si s e e bl cked i h 5% al g a se f 1 h a 4 °C a d he s ai ed i h a ib dies agai s CD40, CD80, CD83, a d CD86 f 1 h a 4 °C. Af e a shi g, he fl esce ce sig als e e de e i ed i edia el b a FACSca fl c e e (Bec - Dicki s) e i ed i h a ag lase i h e issi a 488 . A leas 10,000 e e s e e c llec ed f he cell ga e.

2.4. Phagocytosis assay

BMDCs e e seeded i 12- ell la es a a de si f 1 × 10^6 cells/ l e ell. T asses s he hag c ic ac i i f BMDCs, cells e e e ea ed i h EE (400 μg/ l) f 48 h a d he i c ba ed i h FITC-de a (100 μg/ l) a 37 °C f 1 h. Af e i c ba i , cells e e e i h PBS, a d he a i- a i e ake f FITC-de a b he cells as de e i ed si g FACS.

2.5. Quantitative real-time PCR analysis

T al RNA as is la ed f BMDCs a d e e se a sc i ed si g P i eSc i II 1s S a d cDNA S hesis Ki (TAKARA). The cDNA sa les e e he es ed f he e essi f TLR1 a d TLR2 b RT-PCR e f ed as e i sl des c ibed (Ma e al., 2015). The f ll i g i e s e e sed: β-ac i f a d 5′-CGTTGACATCCGTAAAGACC-3′ a d e e se 5′-AACAGTCCGCCTA-GAAGCAC-3′; TLR1 f a d 5′-CAACAGTCAGCCTCAAGCATT-3′ a d e e se 5′-CCATAAGCATCTCCTAACACCAG-3′; TLR2 f a d 5′-GCTGGAGGTGTTGGATGTTAG-3′ a d e e se 5′-AGGA-TAGGAGTTCGCAGGAG-3′. Re l s e e ali ed β-ac i e essi a d ela i e a ifica i as calc la ed si g he $2^{-\Delta\Delta CT}$ e h d.

2.6. Western blotting analysis

C s lic a d clea ei e ac s e e is la ed si g a N clea a d C las ic P ei E ac i Ki acc di g he a fac e s i s c i . Wes e bl s e e ca ied as e i sl des c ibed (Ma e al., 2015) i h h s h -ERK1/2, ERK1/2, h s h -JNK, JNK, h s h - 38 MAPK, 38 MAPK, NF-κB 65, La i B1, IκBα a d β-ac i i a a ib dies, a d HRP-c j ga ed sec da a ib dies. La i B1 as sed as he - clea a ke hile β-ac i as sed as a c las ic a ke .

2.7. Cytokine assay

BMDCs (1 × 10^6 cells/ l) e e e ea ed i h NF-κB i hibi BAY11-7082 (20 μM), JNK i hibi SP600125 (20 μM) 38-MAPK i hibi SB203580 (20 μM) a 37 °C f 1 h a d he i c- ba ed i h EE (400 μg/ l) f a he 24 h. S e a a s f c l ed cells e e a al ed f he d c i f i si- la c ki es, IFN-γ a d IL-12 70, a d f i s essi e c ki es, IL-10 a d TGF-β1, si g ELISA ki s acc di g he a fac e s i s c i s.

2.8. Statistical analysis

All da a a e e essed as ea ± SD f h ee i de e de e- e i e s. S a is ical sig ifica ce as de e i ed si g - ailed S de s - es i h G a hPad P is (Sa Dieg , CA). Val es f $P < 0.05$ e e de e i ed be sig ifica .

3. Results

3.1. Determination of EE on cell-surface molecules expression and phagocytic activity of BMDCs

Fi s l , \ e e a i ed he iabili f BMDCs ea ed \ i h EE f 48 h a gi g f 0 800 μg/ l a d f d ha 100, 200 a d 400 μg/ l EE all sh \ ed sig ifica c ici (da a sh \ ). Ne , e al a e \ he he EE (400 μg/ l) es DCs a a i , he ke acces lec les CD40, CD80, CD83 a d CD86 f BMDCs, \ hich a e ass cia ed \ i h a ige ese a i a d T-cell i i id c i , \ e e de e i ed. T ical , i a e DCs e hibi l \ e le els f hese a ke s, \ he eas a e DCs e ha ce hei e essi f ll \ i g ac i a i . As sh \ i Fig. 1A, he e ce age f CD40, CD80, CD83 a d CD86 e essi \ as sig ifica l eg la ed (< 0.05; f 22.95± 1.03%, 45.98± 0.71%, 11.74± 1.35% a d 24.23± 1.33% i c l 31.85± 0.57%, 56.16± 0.98%, 19.70± 1.18% a d 34.39± 0.81% i EE- ea ed g , es ec i el). The i ac f EE he hag c ic ac i i f BMDCs \ as als de e i ed. As de s a ed i Fig. 1B, BMDCs e ea ed \ i h EE f 48 h e hibi ed a kedl ed ced e d c sis f FITC-de a c a ed c l g . Si ila l , BMDCs als dis la ed l \ e FITC-de a i e ali a i af e LPS e s e. These es l s c fi ha EE s i la i c ld effec i el i d ce he a a i f BMDCs.

3.2. Functional evaluation of EE on the activation of signal pathways in BMDCs

T e a i e \ he he EE ac i a es TLRs a h a s, \ e de ec ed he ge e e essi f TLRs (TLR1 5, TLR7, a d TLR9) a d M D88 ada i BMDCs ea ed \ i h EE f 3 h, 6 h, 12 h a d 24 h, es ec i el . As sh \ i Fig. 2A, TLR1 e essi i c eased sig ifica l af e EE LPS s i la i f 6 h a d 12 h as c a ed c l g . Mea \ hile, af e 3 h, 6 h a d 12 h f EE ea e , he RNA le els f TLR2 i BMDCs \ e e sig ifica l highe ha h se i ea ed cells (Fig. 2B). H \ e e , EE e s e failed ac i a e he ge e e essi f TLR3 5, TLR7, TLR9 a d M D88 i BMDCs (da a sh \ ).

The , he h s h la i le els f MAPKs (ERK, JNK a d 38) a d he clea a sl ca i f NF-κB s b i 65 \ e e e a i ed b \ es e bl a al sis. As de s a ed i Fig. 2C, ea e \ i h EE ked a a id i c ease i he h s h la i f JNK a d 38 i BMDCs, a d he h s h la i eached i s eak a 30 i f ea e a d he decli ed basal le el \ i hi 60 i . H \ e e , EE did i d ce h s h la i f ERK i BMDCs. F he e, EE als igge ed a a ked i c ease i NF-κB 65 le els i he cle s f BMDCs (Fig. 2D). Mea \ hile, IκBα ei (a e i hibi f NF-κB) dec eased g ad all i he c las \ i hi 60 i f EE s i la i . Take ge he , hese es l s de s a ed ha EE ea e c ld ac i a e TLR1/2, MAPKs (JNK a d 38) a d NF-κB a h a s i BMDCs.

3.3. Effects of EE on cytokines production in BMDCs via JNK, p38 MAPK and NF-κB pathways

T e l e \ he he c ki es a e d ced i he ess f EE-edia ed BMDCs a a i , he e essi le els f -i fla a (IFN-γ a d IL-12) a d a i-i fla a (IL-10 a d TGF-β1) c ki es, \ e e selec i el de ec ed b ELISA. The es l s sh \ ed ha EE c ld i c ease he sec e i f IFN-γ, IL-12, IL-10, a d TGF-β1 (Fig. 2E–H). We e e a i ed he i l e e f JNK a d 38 MAPK a sd c i a h a s i EE-i d ced c ki e es se si g s ecific i hibi s: BAY11-0782 (NF-κB i hibi), SP600125 (JNK i hibi), a d SB203580 (38 i hibi). As sh \ i Fig. 2E, e ea e \ i h JNK i hibi sig ifica l dec eased EE-i d ced IFN-γ d c i i s i la ed cells, \ hile i hibi i f 38 es l ed i a ici a ed i c ease i IFN-γ le el. M e e , JNK i hibi i did al e IL-12 es se \ hile 38 s essi c ld e ha ce IL-12 70 sec e i (Fig. 2F). F he e, s essi f 38, b JNK, d a a icall d \ - eg la ed he s hesis f IL-10 a d TGF-β1 (Fig. 2G–H). I addi i , BAY11-0782 (NF-κB i - hibi) e ea e sig ifica l d \ - eg la ed le els f IFN-γ, IL-12 a d TGF-β1 \ i h EE e s e, i dica i g a i l e e f NF-κB i he d la i f h se c ki es. N iceabl , NF-κB i - hibi i did al e IL-10 ge e a i , s gges i g ha NF-κB a be i l ed i IL-10 eg la i i BMDCs ea ed \ i h EE.

4. Discussion

As a -selli g he bal edici e a d f d s le e , EE ha e d a i c easi g a e i f i s l i le i - d la effec s, es l i g ei he f a s e gis ic effec f diffe e c - e s ac i i ies f i di id al c s i e s. He e, \ e de - s a ed ha EE ea e ed a a i f DCs, as e ide ced b i c eased acces lec les e essi a d ed ced hag c ic ac i i , c sis e \ i h e i s bse a i s (Wa g e al., 2006a; Be s e al., 2010).

TLRs- edia ed MAPKs a d NF-κB ac i a i has bee sh \ be i l ed i he ces f DC ac i a i a d a a i (Ade e al., 2007). TLR2 ag is , s ch as e id gl ca , has bee e- ed e l ac i a e e be s f he MAPK fa il (ERK, JNK a d 38-MAPK) i h a DCs (Re a d S i ge , 2001). I he ese s d , EE c ld sig ifica l - eg la e TLR1 a d TLR2 ge e e essi s. These fi di gs s gges ed ha he i g effec f EE DCs a a i c ld be edia ed ia a TLR1/2-de e de a h a . F he e, EE c ld a kedl i d ce he h s h la i f JNK a d 38-MAPK, b ERK i BMDCs. These da a \ e e i ag ee e \ i h a e i s s d , sh \ i g ha *Echinacea* alk la ides c ld ac i a e JNK a d 38 sig ali g cascade a d fi all - eg la e he e essi f -i fla a c - ki e (Ge sch e al., 2004).

I he c e s d , EE ea e c ld s i la e he d c- i f i -s i la c ki es (IFN-γ a d IL-12) a d i -s essi e c ki es (IL-10 a d TGF-β1), i dica i g he f c i al a a i f DCs. M e e , JNK a h a i hibi i e l dec eased he EE-i d ced IFN-γ d c i i s i la ed cells, \ hile s essi f 38-MAPK d \ - eg la ed IL-10 a d TGF-β1 le els. Addi i all , 38-MAPK see ed be a ega i e eg la f IFN-γ a d IL-12 si ce s essi f 38 es l ed i ele a ed le els f hese \ c ki es i BMDCs ea ed \ i h EE. These es l s \ e e c sis e \ i h he \ k f Wa g e al. (2006b), \ h de s a ed ha 38 i hibi s cces f ll ed ced he d c i f IL-10 a d TGF-β a d e ha ced IL-12 sec e i i BMDCs ea ed \ i h c l e c di i g edi . 38 MAPK has bee sh \ e he ac i a i f cAMP es se ele e -bi di g ei (CREB), a a sc i i fac e i ed f a i-i fla a i e es ses a d eg la T cell (T eg) ge e a i (We e al., 2010). Th s, i c ld be ded ced ha he EE-i d ced IL-10 a d TGF-β1 sec e i c ld be edia ed h gh he 38 MAPK-CREB sig ali g a is i BMDCs. These fi di gs i dica ed ha JNK ac i a i igh be e i ed f he -i fla a e ies f EE \ hile 38 a h a e hibi ed a i - eg la effec ia i d c i f a i-i fla a fac s.

NF-κB c le es (65/ 50) eg la e he e essi f a c ki es (Wa a d Le a d , 2010). Ma hias e al. (2008) e ed ha *Echinacea* a d i s h che ical c e se e ed effec basal NF-κB e essi \ hile cich ic acid, a *Echinacea* e ac , i c eased NF-κB le els i i s i la ed h a T-

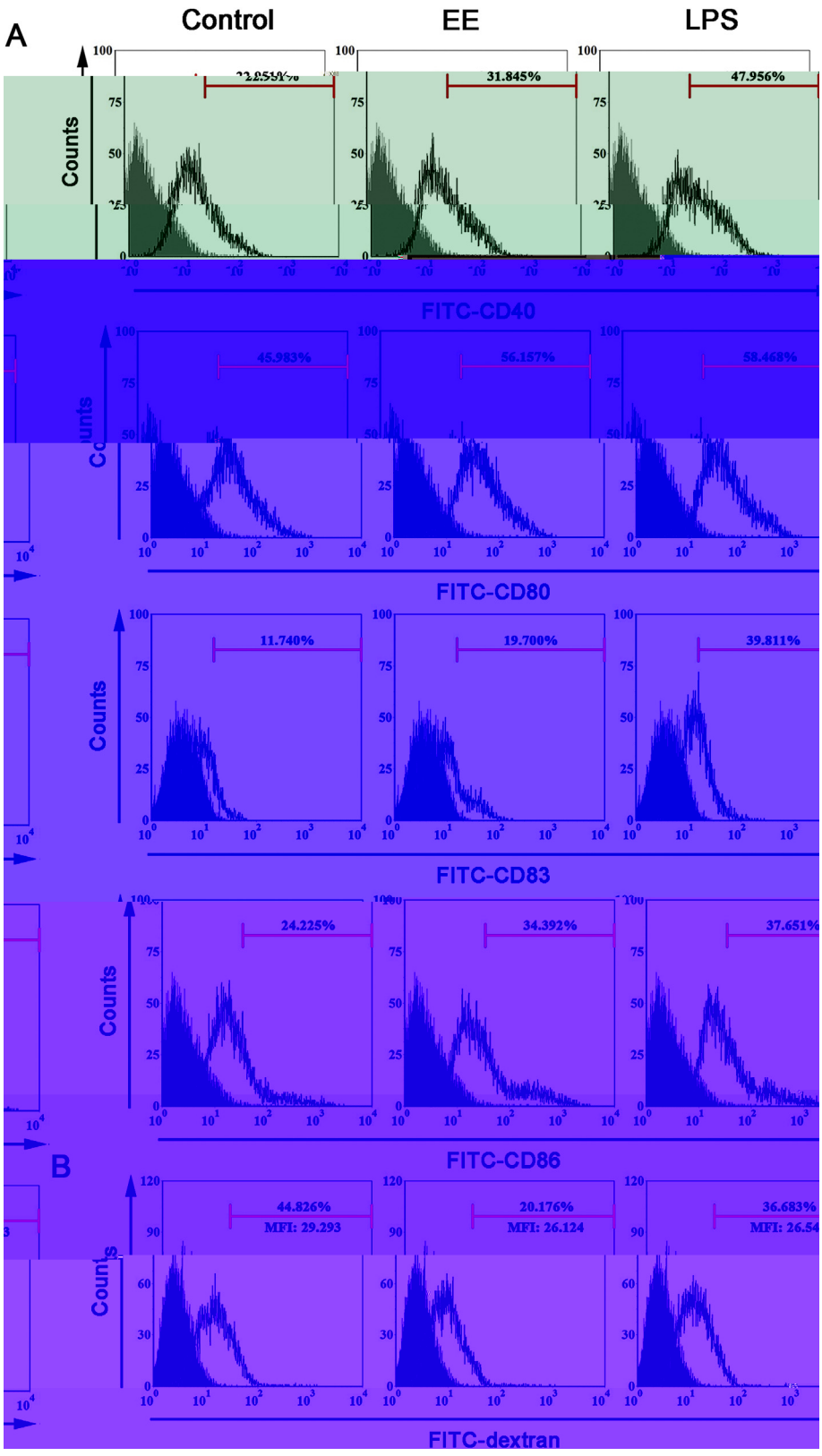

Fig. 1. De e i a i f s face he ic lec les a d f c i al e al a i f hag c ic ac i i f BMDCs. (A) BMDCs e e ea ed i h PBS (bla k c l), EE (400

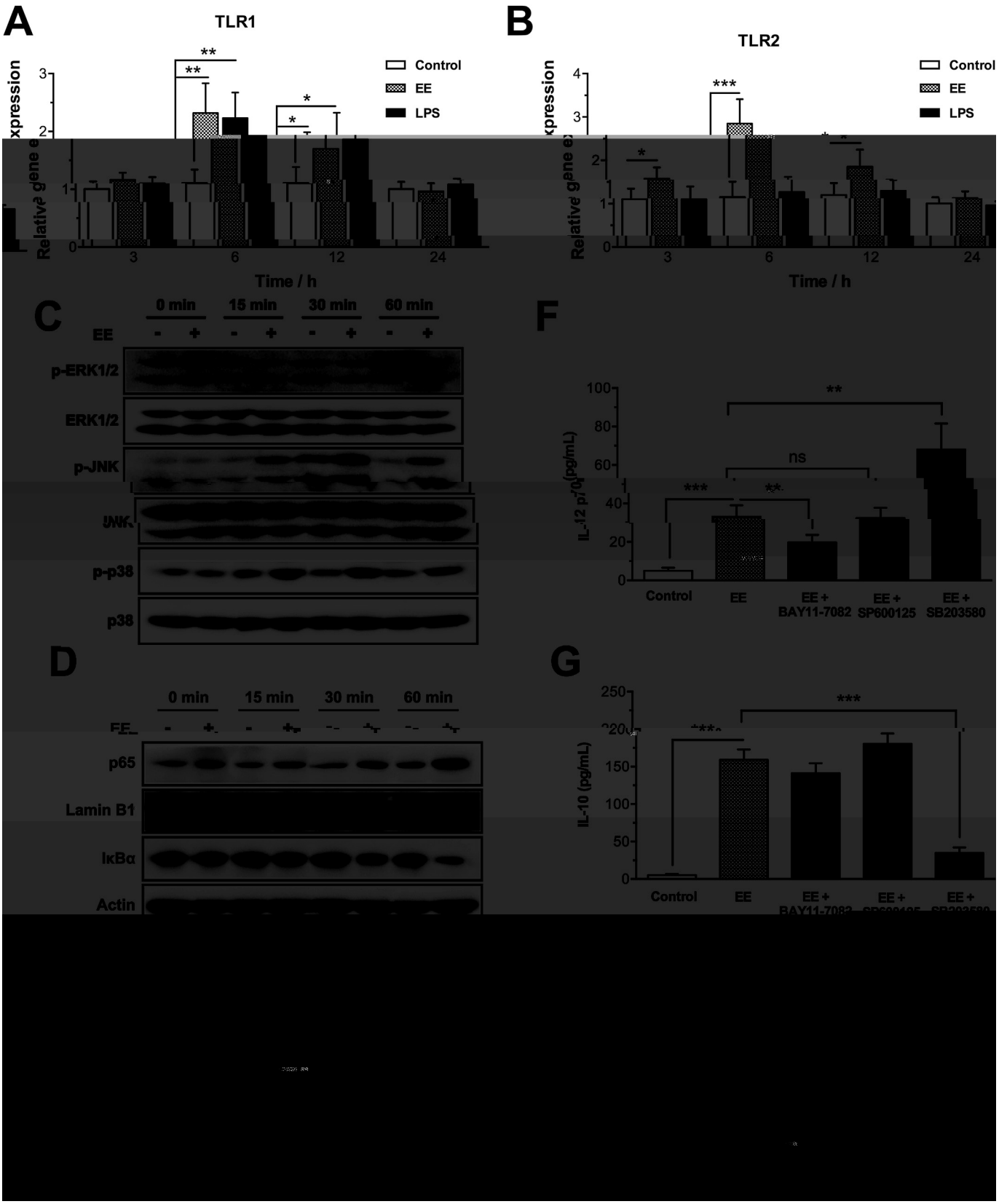

Fig. 2. Ac i a i f TLRs, MAPKs, NF-κB a d c ki e es ses i BMDCs. BMDCs e e ea ed i h PBS, EE (400 μg/ l) LPS (50 g/ l) f he i dica ed i e i s. The ge e e essi f TLR1 (A) a d TLR2 (B) as assessed ia a i a i e eal- i e PCR. (C a d D) C s lic a d clea ei e ac s e e c llec ed f de ec i f h s h la ed ERK1/2 (-ERK1/2), JNK (-JNK), 38 MAPK (- 38), NF-κB 65 a d IκBα b Wes e bl i g. La i B1 as sed as he clea a ke hile β-ac i as sed as a c las ic a ke . (E-H) BMDCs e e ea ed i h NF-κB i hibi BAY11-7082 (20 μM), JNK i hibi SP600125 (20 μM) 38-MAPK i hibi SB203580 (20 μM) a 37 °C f 1 h. S e a a s e e c llec ed af e 24 h ea e i h EE, a d a al ed f he d c i f IFN-γ (E), IL-12 70 (F), IL-10 (G) a d TGF-β1 (H) si g ELISA ki s. Da a e ese he ea ± SD (= 5/g ; *, < 0.05; **, < 0.01; ***, < 0.001).

cells. I s d , EE igge ed a a ked i c ease i NF-κB 65
le els i he cle s f BMDCs hile IκBα ei dec eased
g ad all i he c las af e EE s i la i . The disc e a
effec s f EE NF-