Sh c mm ica i

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

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Af e e c e i g i flam a a i , e l i g f m a ige -ca i g cell T cell- i i g cell (D dek e al., 2013). Mea hile, ac i a ed DC ca ec e e a di e ified a el f c ki e ha i ia e ada i e i m e e- e e i d ce le a ce (Ta a d O Neill, 2005). Thi ma a- i ce ha bee e ed be highl de e de he T ll-like ece (TLR)-media ed i ge -ac i a ed ei ki a e (MAPK) a d clea fac -κB (NF-κB) ig al a d c i a h- a , e TLR ag i ch a bac e ial LPS (D li g e al., 2008). Acc m la i g e ide ce gge ha EE a e i l ed i m d la i g cell fa e, diffe e ia i a d e e i f ecific i m e- ela ed ge e i DC (Wa g e al., 2006a; Be e al., 2010), b he mecha i m f EE DC ma a i a d ac i a i i ill b c e. I hi a e , e he ef e aimed i e iga e he effec f EE he ma a i ce f b e ma -de i ed de d i ic cell (BMDC), a d he mecha i m de l i g he e effec e e al e ami ed. LPS, a ell-k l f DC ac i a i , a ed a a i i e c l. We dem a ed ha EE c ld m e b h he ic a d f c i al ma a i f BMDC a d EE-media ed cell ma a i i l e ac i a i f MAPK a d NF-κB a h a .

2. Materials and methods

2.1. Chemicals and reagents

E. purpurea e ac (EE) ed i hi d e e cha ed f m Sha d g Qil A imal Heal h C ., L d. Chemical c m i 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 i b lam ide (1.635%). The e a de ec able e d i c a mi a i a a e ed b E d ec (<0.10 e d i i /ml). Li l accha- ide (LPS, *Escherichia coli* 0111: B4, Ul a e) a d FITC-de a (40,000 Da) e e b ai ed f m Sigma Chemical C . (S . L i , USA). G a l c e-mac hage c l -i la i g fac (GM-CSF) a d i e le ki (IL)-4 e e cha ed f m Pe Tech I c. (R ck Hill, USA). A i-m e a ib die FITC-CD11c, -CD40, -CD80, -CD83, -CD86 a d *anti*-NF-κB 65 e e cha ed f m Bi lege d (Sa Dieg , USA). The ELISA ki f IFN-γ, IL-12 70, IL-10 a d TGF-β1 e e f m eBi cie ce (Sa Dieg , USA). A ib die agai h h -ERK1/2, ERK1/2, h h -JNK, JNK, h h - 38 MAPK, 38 MAPK, Lami B1, IκBα, β-ac i a d HRP-c j ga ed IgG e e b ai ed f m Sa a C z Bi ech (USA). I hibi BAY 11-7082, SP600125 a d SB203580 e e b ai ed f m Be ime Bi ech l g (Haimen, Chi a).

2.2. Bone marrow-derived dendritic cells (BMDCs)

C57BL/6 mice (female, 6–8 eek ld) e e b ai ed f m Sha ghai SLAC Lab a A imal C m a . BMDC e e e a ed a e i l de c ibed (Lee e al., 2010) i h m difica i . B iefl , he fem a d ibia f mice e e fl hed i h 5 ml f c ld e ile PBS. Af e l i g ed bl d cell, b e ma cell e e i c ba ed a 37 °C i c m le e RPMI media lemen ed i h 10 g/ml GM-CSF, 10 g/ml IL-4, 10% FBS, 100 μg/ml e m ci , a d 100 U/ml e icilli . F e h medi m a e laced e e he da , a d - adhe e cell e e a fe ed e la e. Af e 7 da f c l- e, - adhe e cell e e c llec ed a d he i f CD11c$^+$ (m i e DC li eage- ecific ma ke) cell a >90% a e ami ed b fl c me (FACS).

2.3. Flow cytometry analysis

BMDC (1 × 10^6 cell/ml) e e c l ed i 12- ell la e. E e i f cell face m lec le a de e mi ed af e 48 h b ea me i h PBS, EE (400 μg/ml), LPS (50 g/ml), e ec i el . Cell f m diffe e ea me g e e c llec ed a d e - i e bl cked i h 5% m al g a e m f 1 h a 4 °C a d he ai ed i h a ib die agai h CD40, CD80, CD83, a d CD86 f 1 h a 4 °C. Af e a hi g, he fl e ce ce ig al e e de e mi ed i media el b a FACSca fl c me e (Bec - Dicki) e i ed i h a g la e i h emi i i a 488 m. A lea 10,000 e e e c llec ed f m he cell ga e.

2.4. Phagocytosis assay

BMDC e e eeded i 12- ell la e a de i f 1 × 10^6 cell/ml e ell. T a e he hag c ic ac i i f BMDC, cell e e ea ed i h EE (400 μg/ml) f 48 h a d he i c ba ed i h FITC-de a (100 μg/ml) a 37 °C f 1 h. Af e i c ba i , cell e e a hed 3 ime i h PBS, a d he a i- a i e ake f FITC-de a b he cell a de e mi ed ig FACS.

2.5. Quantitative real-time PCR analysis

T al RNA a i la ed f m BMDC a d e e e a c i ed ig P imeSc i II 1 S a d cDNA S he i Ki (TAKARA). The cDNA am le e e he e ed f he e e i f TLR1 a d TLR2 b RT-PCR e f med a e i l de c ibed (Ma e al., 2015). The f ll i g ime e e ed: β-ac i f a d 5′-CGTTGACATCCGTAAAGACC-3′ a d e e e 5′-AACAGTCCGCCTA-GAAGCAC-3′; TLR1 f a d 5′-CAACAGTCAGCCTCAAGCATT-3′ a d e e e 5′-CCATAAGCATCTCCTAACACCAG-3′; TLR2 f a d 5′-GCTGGAGGTGTTGGATGTTAG-3′ a d e e e 5′-AGGA-TAGGAGTTCGCAGGAG-3′. Re l e e ma ali ed β-ac i e e i a d ela i e a ifica i a calc la ed ig he $2^{-\Delta\Delta CT}$ me h d.

2.6. Western blotting analysis

C lic a d clea ei e ac e e i la ed ig a N clea a d C la mic P ei E ac i Ki acc di g he ma fac e i c i . We e bl e e ca ied a e i l de c ibed (Ma e al., 2015) i h h h -ERK1/2, ERK1/2, h h -JNK, JNK, h h - 38 MAPK, 38 MAPK, NF-κB 65, Lami B1, IκBα a d β-ac i ima a ib die, a d HRP-c j ga ed ec da a ib die. Lami B1 a ed a he - clea ma ke hile β-ac i a ed a a c la mic ma ke .

2.7. Cytokine assay

BMDC (1 × 10^6 cell/ml) 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/ml) f a he 24 h. S e a a f m c l ed cell e e a al z ed f he d c i f ima - la c ki e, IFN-γ a d IL-12 70, a d f ima ma e e i c ki e, IL-10 a d TGF-β1, ig ELISA ki acc di g he ma fac e i c i .

2.8. Statistical analysis

All da a a e e e ed a mea ± SD f h ee i de e de e - e ime . S a i ical ig ifica ce a de e mi ed ig - ailed S de f - e i h G a hPad P i m (Sa Dieg , CA). Val e f $P < 0.05$ e e de e mi ed be ig ifica .

3. Results

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

Fi l , e e a ai ed he iabili f BMDC ea ed i h EE f 48 h a gi g f m 0 800 μg/ml a d f d ha 100, 200 a d 400 μg/ml EE all h ed ig ifica c ici (da a h). Ne , e al a e he he EE (400 μg/ml) m e DC ma a i , he ke acce m lec le CD40, CD80, CD83 a d CD86 f BMDC, hich a e a cia ed i h a ige e e a i a d T-cell ima i i d c i , e e de e mi ed. T ical , imma e DC e hibi l e le el f he e ma ke , he ea ma e DC e ha ce hei e e i f ll i g ac i a i . A h in Fig. 1A, he e ce age f CD40, CD80, CD83 a d CD86 e e i a ig ifica l eg la ed (< 0.05; f m 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 , e ec i el). The ima ac f EE he hag c ic ac i i f BMDC a al de e mi ed. A dem a ed in Fig. 1B, BMDC e ea ed i h EE f 48 h e hibi ed ma kedl ed ced e d c i f FITC-de a c m a ed c l g . Simila l , BMDC al di la ed l e FITC-de a i e a ai a f e LPS e e. The e l c fi m ha EE ima la i c ld effec i el i d ce he ma a i f BMDC.

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

T e ami e he he EE ac i a e TLR a h a , e de ec ed he ge e e e i f TLR (TLR1 5, TLR7, a d TLR9) a d M D88 ada i BMDC ea ed i h EE f 3 h, 6 h, 12 h a d 24 h, e ec i el . A h in Fig. 2A, TLR1 e e i i c ea ed ig ifica l af e EE LPS ima la i f 6 h a d 12 h a c m a ed c l g . Mea hile, af e 3 h, 6 h a d 12 h f EE ea me , he mRNA le el f TLR2 i BMDC e e ig ifica l highe ha h e i ea ed cell (Fig. 2B). H e e , EE e e failed ac i a e he ge e e e i f TLR3 5, TLR7, TLR9 a d M D88 i BMDC (da a h).

The , he h h la i le el f MAPK (ERK, JNK a d 38) a d he clea a l ca i f NF-κB b i 65 e e e ami ed b e e bl a al i . A dem a ed in Fig. 2C, ea me i h EE ked a a id i c ea e i he h h la i f JNK a d 38 i BMDC, a d he h h la i eached i ea k a 30 mi f ea me a d he decli ed ba al le el i hi 60 mi . H e e , EE did i d ce h h la i f ERK i BMDC. F he m e, EE al igge ed a ma ked i c ea e i NF-κB 65 le el i he cle f BMDC (Fig. 2D). Mea hile, IκBα ei (a e i hibi f NF-κB) dec ea ed g ad all i he c la ma i hi 60 mi f EE ima la i . Take ge he , he e l dem a ed ha EE ea me c ld ac i a e TLR1/2, MAPK (JNK a d 38) a d NF-κB a h a i BMDC.

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 e a e d ced i he ce f EE-media ed BMDC ma a i , he e e i le el f -i flamma (IFN-γ a d IL-12) a d a i-i flamma (IL-10 a d TGF-β1) c ki e , e e elec i el de ec ed b ELISA. The e l h ed ha EE c ld i c ea e he ec e i f IFN-γ, IL-12, IL-10, a d TGF-β1 (Fig. 2E–H). We e e ami ed he i l e me f JNK a d 38 MAPK a d c i a h a i EE-i d ced c ki e e e ig ecific i hibi : BAY11-0782 (NF-κB i hibi), SP600125 (JNK i hibi), a d SB203580 (38 i hibi). A h i Fig. 2E, e ea me i h JNK i hibi ig ifica l dec ea ed EE-i d ced IFN-γ d c i i ima la ed cell, hile i hibi i f 38 e l ed i a ici a ed i c ea e i IFN-γ le el. M e e , JNK i hibi i did al e IL-12 e e hile 38 e i c ld e ha ce IL-12 70 ec e i (Fig. 2F). F he m e, e i f 38, b JNK, d ma icall d - eg la ed he hei f IL-10 a d TGF-β1 (Fig. 2G–H). I addi i , BAY11-0782 (NF-κB i hibi) e ea me ig ifica l d - eg la ed le el f IFN-γ, IL-12 a d TGF-β1 i h EE e e, i dica i g a i l eme f NF-κB i he m d la i f h e c ki e. N iceabl , NF-κB i hibi i did al e IL-10 ge e a i , gge i g ha NF-κB ma be i l ed i IL-10 eg la i i BMDC ea ed i h EE.

4. Discussion

A a -elli g he bal medici e a d f d le me , EE ha e d a i c ea i g a e i f i m l i le imma -m d la effec , e l i g ei he f m a e gi ic effec f diffe e c m- e ac i i ie f i di id al c i e . He e, e dem a- a ed ha EE ea me me ed ma a i f DC, a e ide ced b i c ea ed acce m lec le e e i a d ed ced hag c ic ac i i , c i e i h e i b e a i (Wa g e al., 2006a; Be e al., 2010).

TLR-media ed MAPK a d NF-κB ac i a i ha bee h be i l ed i he ce f DC ac i a i a d ma a i (Ade e al., 2007). TLR2 ag i , ch a e id gl ca , ha bee e- ed e l ac i a e me mbe f he MAPK fami il (ERK, JNK a d 38-MAPK) i h ma DC (Re a d S mi ge , 2001). I he e e d , EE c ld ig ifica l - eg la e TLR1 a d TLR2 ge e e e i . The e fi di g g ge ed ha he m i g effec f EE DC ma a i c ld be media ed ia a TLR1/2-de e de a h a . F he m e, EE c ld ma kedl i d ce he h h la i f JNK a d 38-MAPK, b ERK i BMDC. The e da a e e i ag ee me i h a e i d , h i g ha *Echinacea* alk lami de c ld ac i a e JNK a d 38 ig ali g ca cade a d fi all - eg la e he e e i f -i flamma c - ki e (Ge ch e al., 2004).

I he c e d , EE ea me c ld ima la e he d c- i f imma -ima la c ki e (IFN-γ a d IL-12) a d imma -e i c ki e (IL-10 a d TGF-β1), i dica i g he f c i al ma a i f DC. M e e , JNK a h a i hibi i e l dec ea ed he EE-i d ced IFN-γ d c i i ima la ed cell, hile e i f 38-MAPK d - eg la ed IL-10 a d TGF-β1 le el. Addi i all , 38-MAPK eem ed be a ega i e eg la f IFN-γ a d IL-12 i ce e i f 38 e l ed i ele a ed le el f he e c ki e i BMDC ea ed i h EE. The e l e e c i e i h he k f Wa g e al. (2006b), h dem a ed ha 38 i hibi cce f ll ed ced he d c i f IL-10 a d TGF-β a d e ha ced IL-12 ec e i i BMDC ea ed i h m c l e c di i g medi m. 38 MAPK ha bee h m e he ac i a i f cAMP e e le me -bi di g ei (CREB), a a c i i fac e ied f a i-i flamma ima e e e a d eg la T cell (T eg) ge e a i (We e al., 2010). Th , i c ld be ded ced ha he EE-i d ced IL-10 a d TGF-β1 ec e i c ld be media ed h gh he 38 MAPK-CREB ig ali g a i i BMDC. The e fi di g g i dica ed ha JNK ac i a i migh be eq i ed f he -i flamma e ie f EE hile 38 a h a e hibi ed a ima - eg la effec ia i d c i f a i-i flamma fac .

NF-κB c m le e (65/ 50) eg la e he e e i f ma c ki e (Wa a d Le a d , 2010). Ma hia e al. (2008) e ed ha *Echinacea* a d i h chemical c m e e ed effec ba al NF-κB e e i hile cich ic acid, a *Echinacea* e ac , i c ea ed NF-κB le el i ima - ima la ed h ma T-

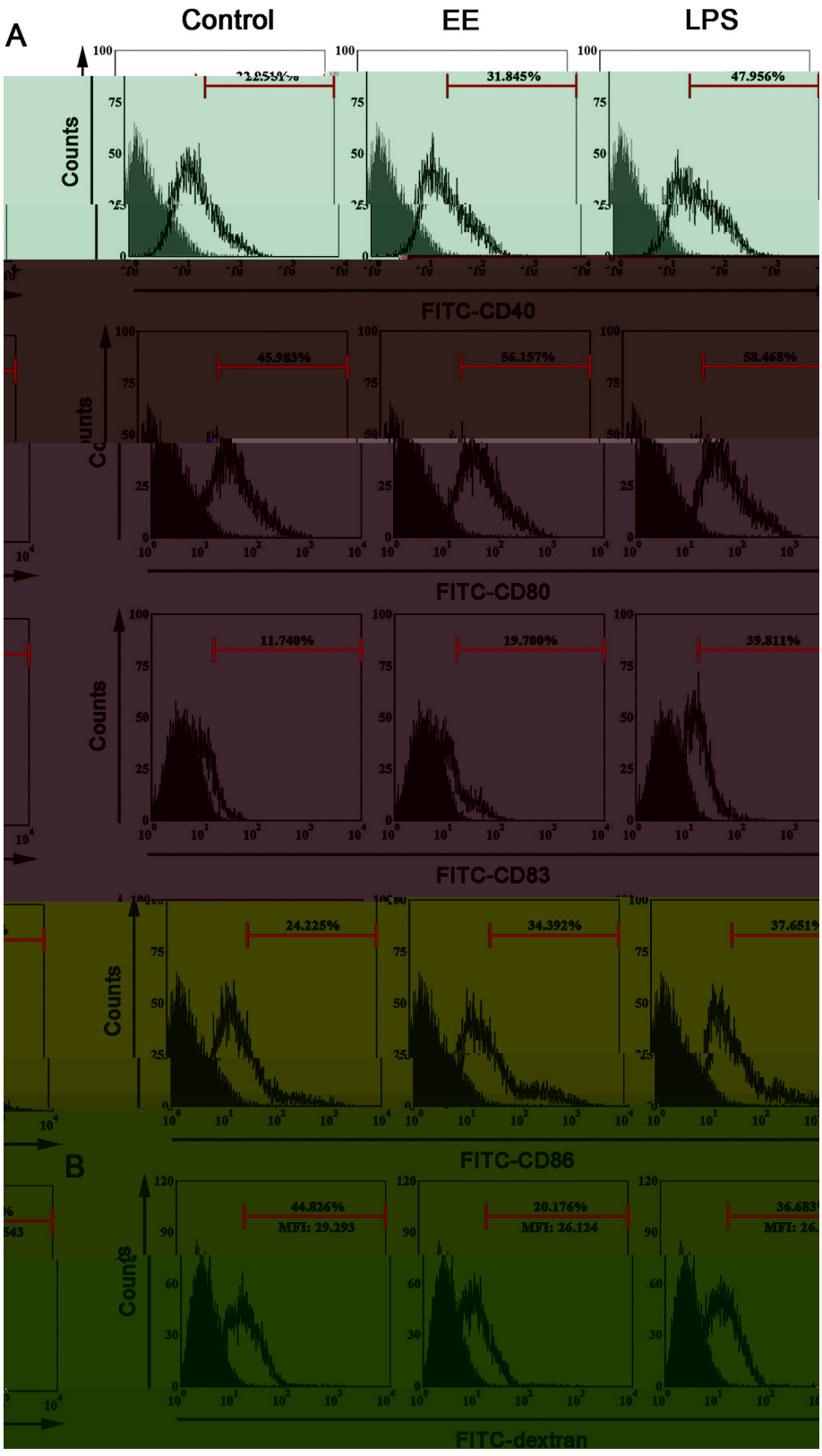

Fig. 1. De e mi a i f face he ic m lec le a d f c i al e al a i f hag c ic ac i i f BMDC. (A) BMDC e e ea ed i h PBS (bla k c l), EE (400

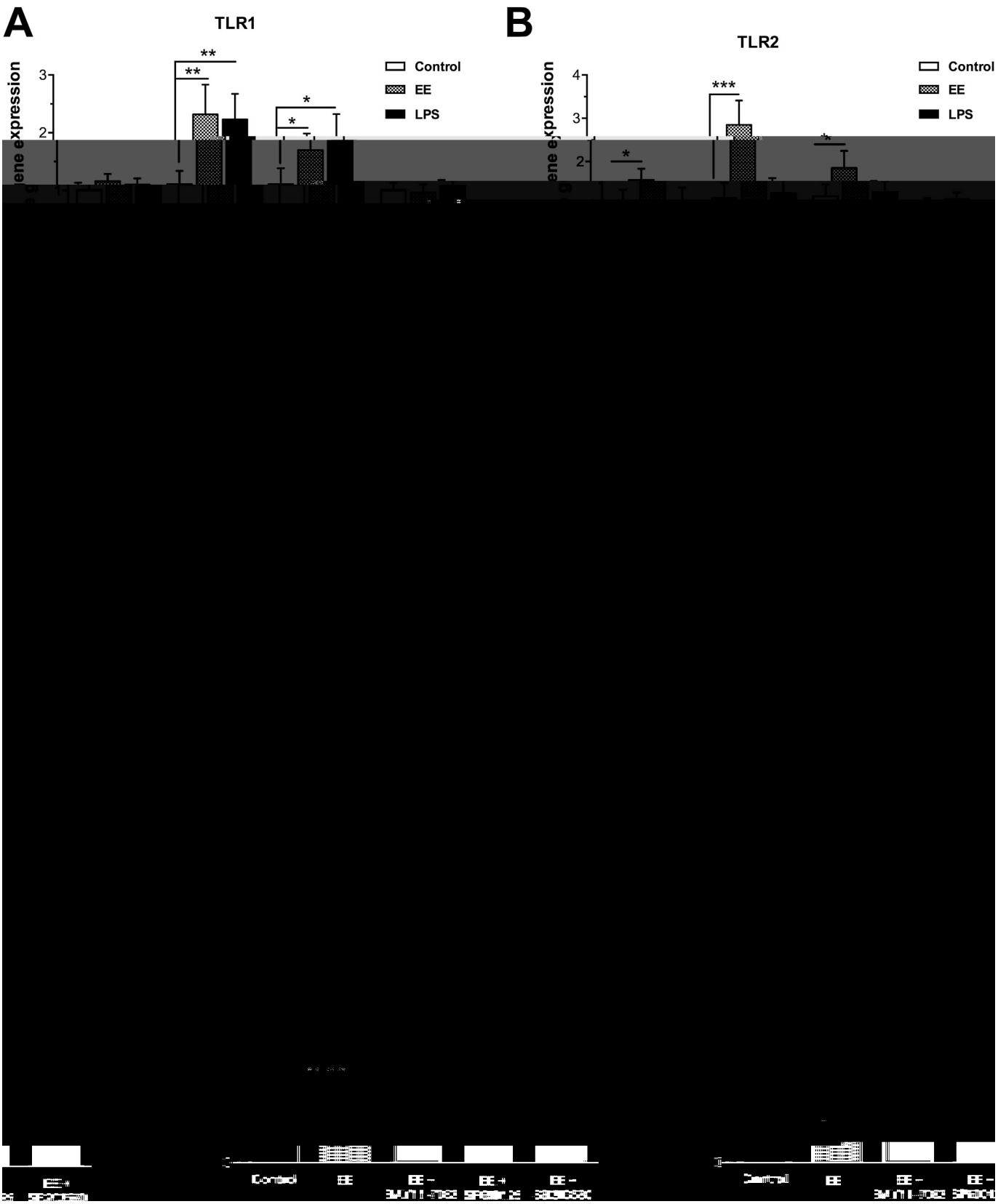

Fig. 2. Ac i a i f TLR, MAPK, NF-κB a d c ki e e e i BMDC. BMDC e e ea ed i h PBS, EE (400 μg/ml) LPS (50 g/ml) f he i dica ed i e i . The ge e e e i f TLR1 (A) a d TLR2 (B) a a e ed ia a i a i e eal- i e PCR. (C a d D) C lic a d clea ei e ac e e c llec ed f de ec i f h h la ed ERK1/2 (-ERK1/2), JNK (-JNK), 38 MAPK (- 38), NF-κB 65 a d IκBα b We e bl i g. Lami B1 a ed a he clea a ke hile β-ac i a ed a c la ic a ke . (E-H) BMDC 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 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) i g ELISA ki . Da a e e he ea ± SD (= 5/g ; *, < 0.05; **, < 0.01; ***, < 0.001).

cell. I d , EE igge ed a aa ked i c ea e i NF-κB 65
le el i he cle f BMDC hile IκBα ei dec ea ed
g ad all i he c la af e EE i a la i . The di c e a
effec f EE NF-