SUPPLEMENTARY DATA

Isolation of compounds from *Cimicifuga*

The EtOAc-soluble fraction (308 g) of the rhizomes of *C. yunnanensis* was subjected to silica gel column chromatography (cc) and eluted with $CHCl_3$-MeOH (100:0, 50:1, 20:1, 10:1, 0:100) to afford fractions A (68.3 g), B (13.2 g), C (92.5 g) and D (42.7 g). Fraction C was successive subjected to silica gel cc and eluted with $CHCl_3$-MeOH (30:1 to 10:1) to afford four fractions (C.1-C.4). Fraction C.3 (24.6 g) was further resolved into five fractions (C.3.1-C.3.5) by eluting on a RP-18 column (MeOH-H_2O, gradient from 50:50 to 100:0). Fraction C.3.3 (9.6 g) was further subjected to repeated silica gel cc, eluted with $CHCl_3$-Me_2CO (gradient from 20:1 to 10:1) to yield **KCY03** (120.6 mg), **04** (80.2 mg), **05** (4.3 mg), **06** (20.1 mg), **14** (58.7 mg), **31** (7.6 mg), **44** (12.3 mg), and **49** (13.4 mg). **KCY08** (135.4 mg) and **37** (10.2 mg) were isolated from fraction C.3.2 (4.2 g) by conducting silica gel cc, eluting with $CHCl_3$-Me_2CO (gradient from 15:1 to 8:1).

The EtOAc-soluble fraction (158 g) of the roots of *C. foetida* was chromatographed over a silica gel column and eluted with $CHCl_3$-MeOH (100:0, 50:1, 20:1, 10:1, 0:100) to afford fractions A (13.4 g), B (8.7 g), C (34.5 g), and D (37.2 g). Fraction B (8.7 g) was subjected to cc on silica gel. Gradient elution with $CHCl_3$-MeOH (60:1 to 40:1) gave fractions B.1, B.2 and B.3. Fraction B.2 (3.8 g) was chromatographed on an RP-18 column (MeOH-H_2O, gradient from 70:30 to 100:0) and then purified on Sephadex LH-20 (MeOH) to afford **KHF06** (13.1 mg), **07** (21.5 mg), **08** (11.4 mg), **13** (9.7 mg), **21** (17.2 mg), **22** (14.3 mg), **27**(8.4 mg) and **30** (7.6 mg). Fraction C (34.5 g) was subjected to cc on silica gel and eluted

with $CHCl_3$-MeOH (gradient from 40:1 to 20:1) to yield fractions C.1, C.2 and C.3. Fraction C.2 (2.6 g) was subjected to silica gel cc ($CHCl_3$-Me_2CO, 4:1), then RP-18 (MeOH-H_2O, 60:40) to yield **KHF17** (11.2 mg), **24** (4.3 mg), **29** (3.7 mg), **41** (5.6 mg), and **51** (3.8 mg). Fraction C.3 (16.3 g) was chromatographed on silica gel cc ($CHCl_3$-Me_2CO, gradient from 10:1 to 5:1); then RP-18 (MeOH-H_2O, 60:40) to afford fractions C.3.1-C.3.4. Fraction C.3.2 (2.5 g) was subjected to cc on an RP-18 column (MeOH-H_2O, 60:40), then purified on a Sephadex LH-20 column (MeOH) to afford **KHF16** (45.3 mg), **18** (11.5 mg) and **38** (23.5 mg).

The EtOAc extract (313.6 g) of the aerial parts of *C. foetida* was subjected to silica gel cc, eluted with $CHCl_3$-MeOH (100:0, 50:1, 20:1, 10:1) to afford fractions A (43.2 g), B (23.4 g), C (78.5 g) and D (91.3 g). Fraction B (23.4 g) was divided into four sub-fractions (B.1-B.4) after performing silica gel cc, eluted with $CHCl_3$-MeOH (gradient from 60:1 to 40:1). **KYY11** (8.9 mg), **14** (5.6 mg), and **27** (12.3 mg) were purified from fraction **B.2** (8.1 g) by conducting cc on RP-18 (MeOH-H_2O, 70:30) and then Sephadex LH-20 (MeOH). Fraction **C** (78.5 g) was fractionated into three sub-fractions (**C.1-C.3**) by performing silica gel, eluted with $CHCl_3$-MeOH (gradient from 40:1 to 20:1). Fraction **C.2** (16.6 g) was subjected to silica gel cc ($CHCl_3$-Me_2CO, 10:1), then RP-18 (MeOH-H_2O, 60:40) to yield **KYY04** (21.4 mg), **08** (16.4 mg), **15** (14.3 mg), **18** (8.9 mg), **20** (45.3 mg), **33** (7.6 mg), **41** (23.1 mg) and **56** (18.3 mg).

The EtOAc extract (13.1 g) of the roots of *C. dahurica* was subjected to silica gel cc, eluted with $CHCl_3$-MeOH (100:0, 50:1, 20:1, 10:1) to afford fractions A (0.4 g), B (2.8 g), C (5.5 g) and D (3.3 g). Fraction B was subjected to repeated silica gel

cc, eluted with $CHCl_3$-Me_2CO (gradient from 20:1 to 10:1) and then repeated semi-preparative HPLC (eluted with CH_3CN-H_2O, gradient from 60:40 to 85:15) to yield **KXG42** (3.5 mg). **KXG106** (2.8 mg), **113** (3.2 mg), and **115** (3.5 mg), were purified from fraction C by conducting silica gel cc, eluting with $CHCl_3$-Me_2CO (10:1), followed by repeated semi-preparative HPLC (eluted with CH_3CN-H_2O, gradient from 50:50 to 80:20).

The EtOAc extract (12.8 g) of the roots of *C. heracleifolia* was subjected to silica gel cc, eluted with $CHCl_3$-MeOH (100:0, 50:1, 20:1, 10:1) to afford fractions A (0.5 g), B (2.3 g), C (4.9 g) and D (2.8 g). Fraction C was subjected to repeated silica gel cc, eluted with $CHCl_3$-Me_2CO (10:1) and then repeated semi-preparative HPLC (eluted with CH_3CN-H_2O, gradient from 50:50 to 80:20) to yield **KSY38** (2.7 mg).

The names and chemical structures of compounds isolated from *Cimicifuga*

Serial number	Name	Structure
KCY03	Actein	OAc, O, O, O, OH, H, HO, HO, OH, O, O
KCY04	23-*epi*-26-deoxyactein	OAc, O, O, O, H, HO, HO, OH, O, O
KCY05	26-methylacteinol	OAc, O, O, O, OCH3, HO

KCY06	24-*O*-acetyl-25-anhydroshengmanol-3-*O*-*β*-D-xylopyranoside	
KCY08	cimigenol-3-*O*-*β*-D-xylopyranoside	
KCY14	25-*O*-acetylcimigenol-3-*O*-*β*-D-xylopyranoside	
KCY31	Cimicifugoside H1	
KCY37	15*α*-hydrofoetidol-3-*O*-*β*-D-xylopyranoside	
KCY44	12*β*-*O*-acetyl-25-anhydrocimigenol-3-*O*-*β*-D-xylopyranoside	
KCY49	25-chlorodeoxycimigenol-3-*O*-*β*-D-xylopyranoside	
KHF06	24-*O*-acetylhydroshengmanol-3-*O*-*β*-D-xylopyranoside	
KHF07	23-*O*-acetylshengmanol-3-*O*-*β*-D-xylopyranoside	

KHF08	Cimiaceroside B	
KHF13	12*β*-hydroxycimigenol-3-*O*-*β*-D-xylopyranoside	
KHF16	24-*O*-acetylisodahurinol-3-*O*-*β*-D-xylopyranoside	
KHF17	12*β*,25-*O*-diacetylcimigenol-3-*O*-*β*-D-xylopyranoside	
KHF18	Asiaticoside A	
KHF21	26-deoxyacteinol	
KHF22	Acteinol	
KHF24	4′,25-*O*-diacetylcimigenol-3-*O*-*β*-D-xylopyranoside	
KHF27	12*β*-*O*-acetylcimigenol	

KHF29	2′-*O*-(*E*)-2-butenoyl-25-*O*-acetylcimigenol-3-*O*-*β*-D-xylopyanoside	
KHF30	3′-*O*-acetylactein	
KHF38	cimigenol-3-*O*-*α*-L-arabinoside	
KHF41	3′-*O*-acetyl-23-*epi*-26-deoxyactein	
KHF51	2′,25-*O*-diacetylcimigenol-3-*O*-*β*-D-xylopyranosid	
KYY04	2′-*O*-acetylcimigenol-3-*O*-*α*-L-arabinoside	
KYY08	2′,24-*O*-diacetylisodahurinol-3-*O*-*α*-L-arabinoside	
KYY11	12*β*-hydroxy-25-anhydrocimigenol	

KYY14	Isodahurinol	
KYY18	25-anhydrocimigenol-3-*O*-*α*-L-arabinopyranoside	
KYY20	25-*O*-acetylcimigenol-3-*O*-*α*-L-arabinoside	
KYY27	Cimiracemonol B	
KYY33	2′,24-*O*-diacetoxy-25-anhydroshengmanol-3-*O*-*α*-L-arabinopyranoside	
KYY41	4′,23-*O*-diacetylshengmanol-3-*O*-*α*-L-arabinopyranoside	
KYY15	24-*O*-acetylisodahurinol-3-*O*-*α*-L-arabinoside	

KYY56	23-*O*-acetylshengmanol-3-*O*-*α*-L-arabinopyranoside	
KXG42	Acerinol	
KXG106	2′-*O*-(*E*)-2-butenoylcimigenol-3-*O*-*α*-L-arabinopyranoside	
KXG113	3′,25-*O*-diacetylcimigenol-3-*O*-*α*-L-arabinopyranoside	
KXG115	4′,25-*O*-diacetylcimigenol-3-O-α-L-arabinopyranoside	
KSY38	2′,4′-*O*-diacetylcimigenol-3-*O*-*α*-L-arabinopyranoside	

SUPPLEMENTARY Figure Legend

Figure S1. KHF16 decreases the protein expression levels of multiple cell cycle and apoptosis regulators in MDA-MB-468 and SW527

The legend is same with Figure 5. The quantification data is listed below each band.

Figure S2.. KHF16 blocks the TNFα-induced NF-κB signaling pathway and

anti-apoptosis protein expression in MDA-MB-468 and SW527

The legend is same with Figure 6. The quantification data is listed below each band.

Figure S3. RelA knockdown reduces the efficacy of KHF16 in MDA-MB-468 and SW527

A. RelA was knocked down in MDA-MB-468 as measured by WB. RelA siRNA pool contains two target sequences: #1: 5′-GCCCUAUCCCUUUACGUCAdTdT-3′, #2: 5′-UCCAGUGUGUGAAGAAGCGdTdT-3′.
B. RelA was silenced in MDA-MB-468 for 24 h. The cells were treated with KHF16 (10 or 20 μM) for 24 h. The cell viability was measured by the Sulforhodamine B (SRB) assay. *P < 0.05 (Student's t-test).
C. RelA was silenced in MDA-MB-468 for 24 h. The cell viability was measured after the cells were treated with KHF16 KHF16 (10 μM) for 8 or 24 h.
D. RelA was knocked down in SW527 as measured by WB.
E. RelA was silenced in SW527 for 24 h. The cell viability was measured after the cells were treated with KHF16 (10 or 20 μM) for 24 h.
F. RelA was silenced in SW527 for 24 h. The cell viability was measured after the cells were treated with KHF16 (10 μM) for 8 or 24 h.

Figure S4. KHF16 is not toxic to mouse splenocytes but inhibits the NF-κB pathway dependent immune response

A. KHF16 is not toxic to mouse splenocytes. Cell viability of mouse splenocytes were performed with the Cell Counting Kit-8 (Dojindo) according to the manufacturer's guidelines after the cells were treated with KHF16 (5-20 μM for 4-24 h). FVB mice were maintained in a specific pathogen–free facility, and all animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Kunming Institute of Zoology. Primary splenocytes were isolated from the spleen of

8-week female mice using the mouse 1× Lymphocyte Separation Medium (DKW33-R0100). Blood red cells were deleted using the 1× RBC Lysis Buffer Solution (eBioscience). Splenocytes were cultured in RPMI-1640 medium (Gibco) supplemented with 10% (vol/vol) FBS and 100 U/ml penicillin and 0.1 mg/mL streptomycin (Biological Industries).

B. KHF16 completely blocked the induction of IFNγ by PMA/Ionomycin in mouse splenocytes. SYBR Select Master Mix (Life technologies) and a 7900HT Fast Real-time PCR System (Life Technologies) were used for quantitative RT-PCR. Total RNA was prepared for mouse splenocytes and subjected to qRT-PCR using gene-specific primers: mouse IFNγ forward primer, 5′-GCGTCATTGAATCACACCTG -3′; reverse primer, 5′-TGAGCTCATTGAATGCTTGG -3′); mouse β-actin (forward primer, 5′-AGGTCATCACTATTGGCAACGA-3′; reverse primer, 5′-CACTTCATGATGGAATTGAATGTAGTT-3′). Mouse splenocytes were treated with or without KHF16 (10 μM) for 4 h. The cells were treated with or without PMA (20 nM) plus ionomycin (1 μM) along with KHF16 (10μM) for 4 h. Total RNA was extracted for qRT-PCR. *$P < 0.05$ (Student's t-test).

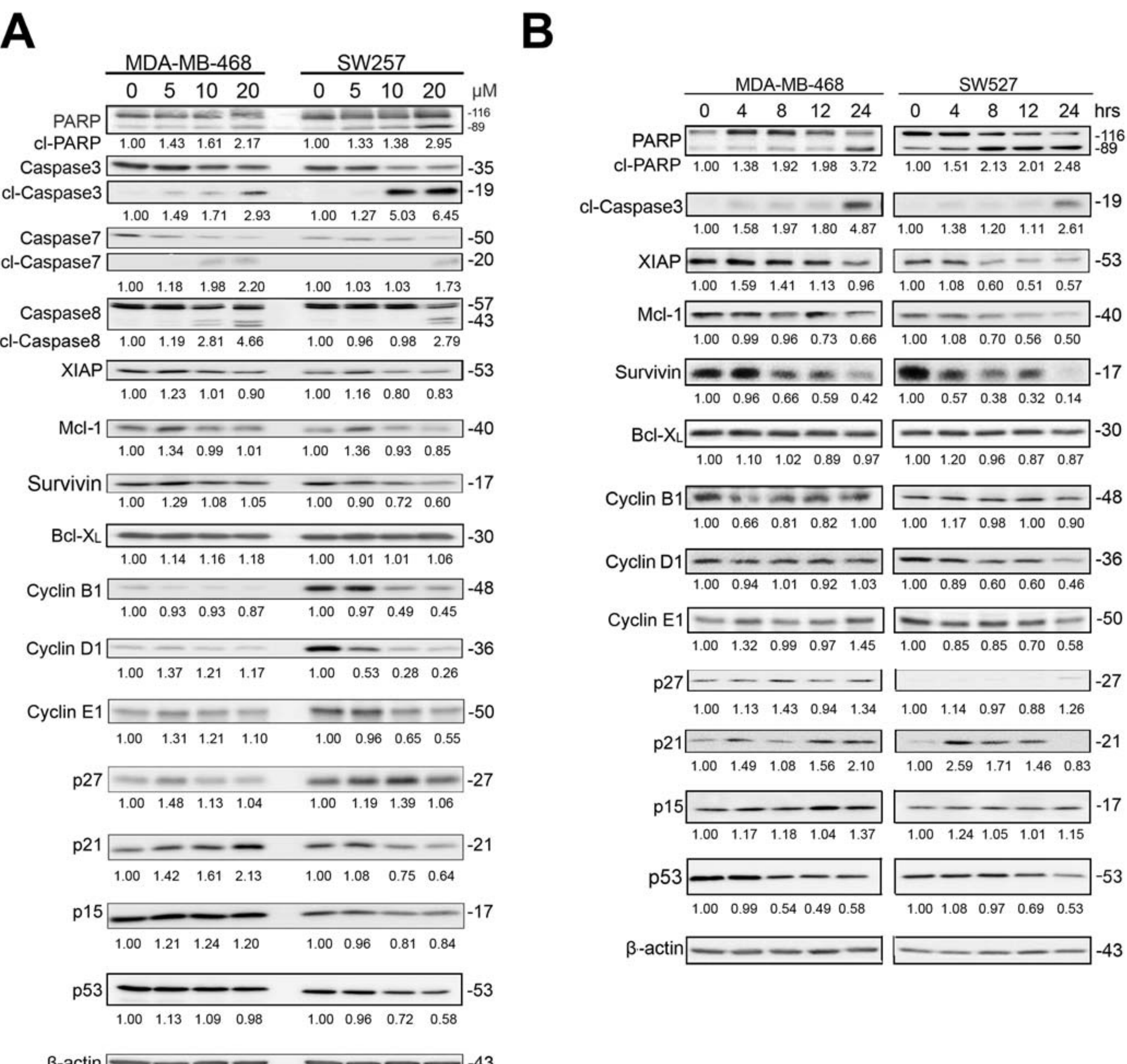

Figure S1

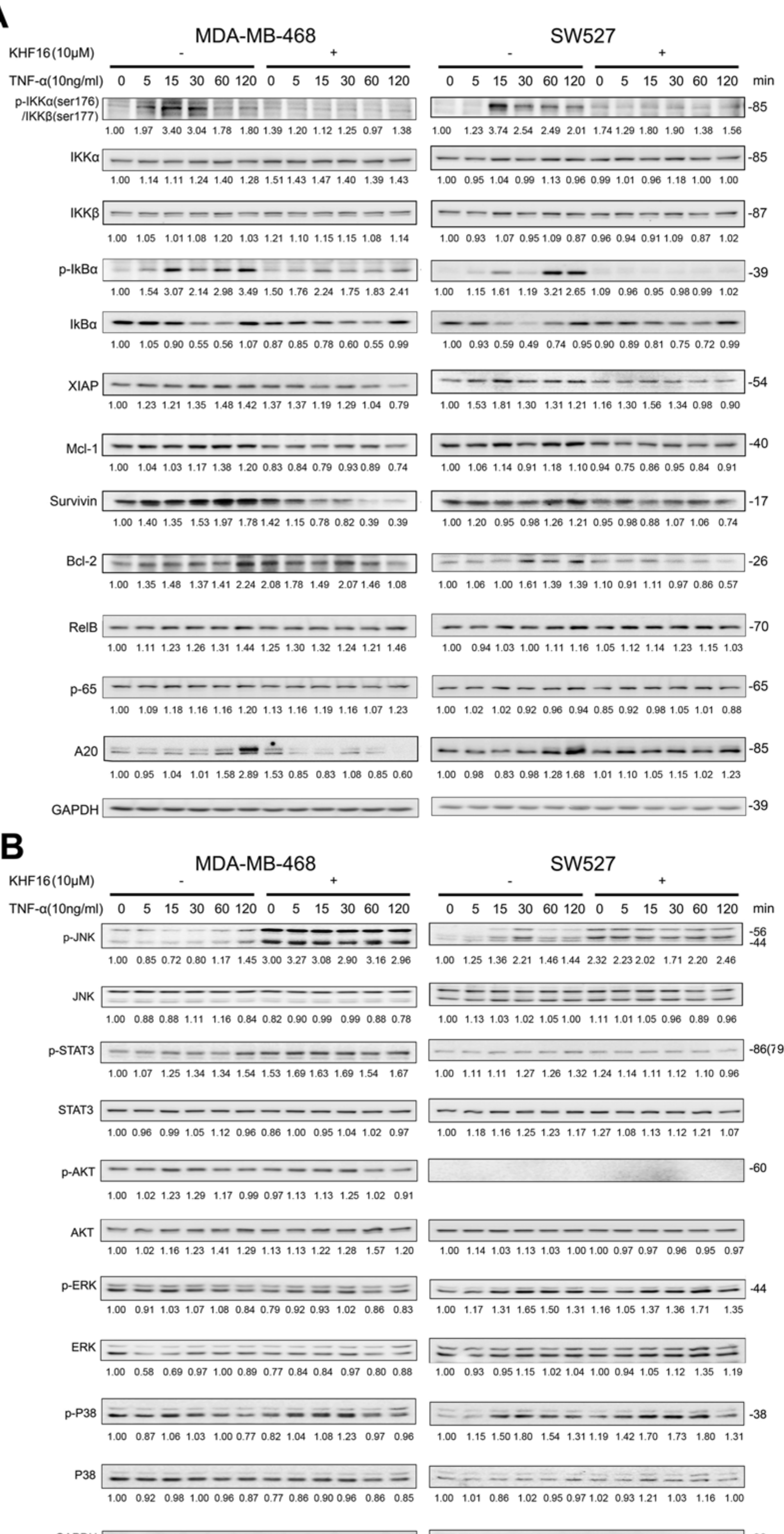

Figure S2

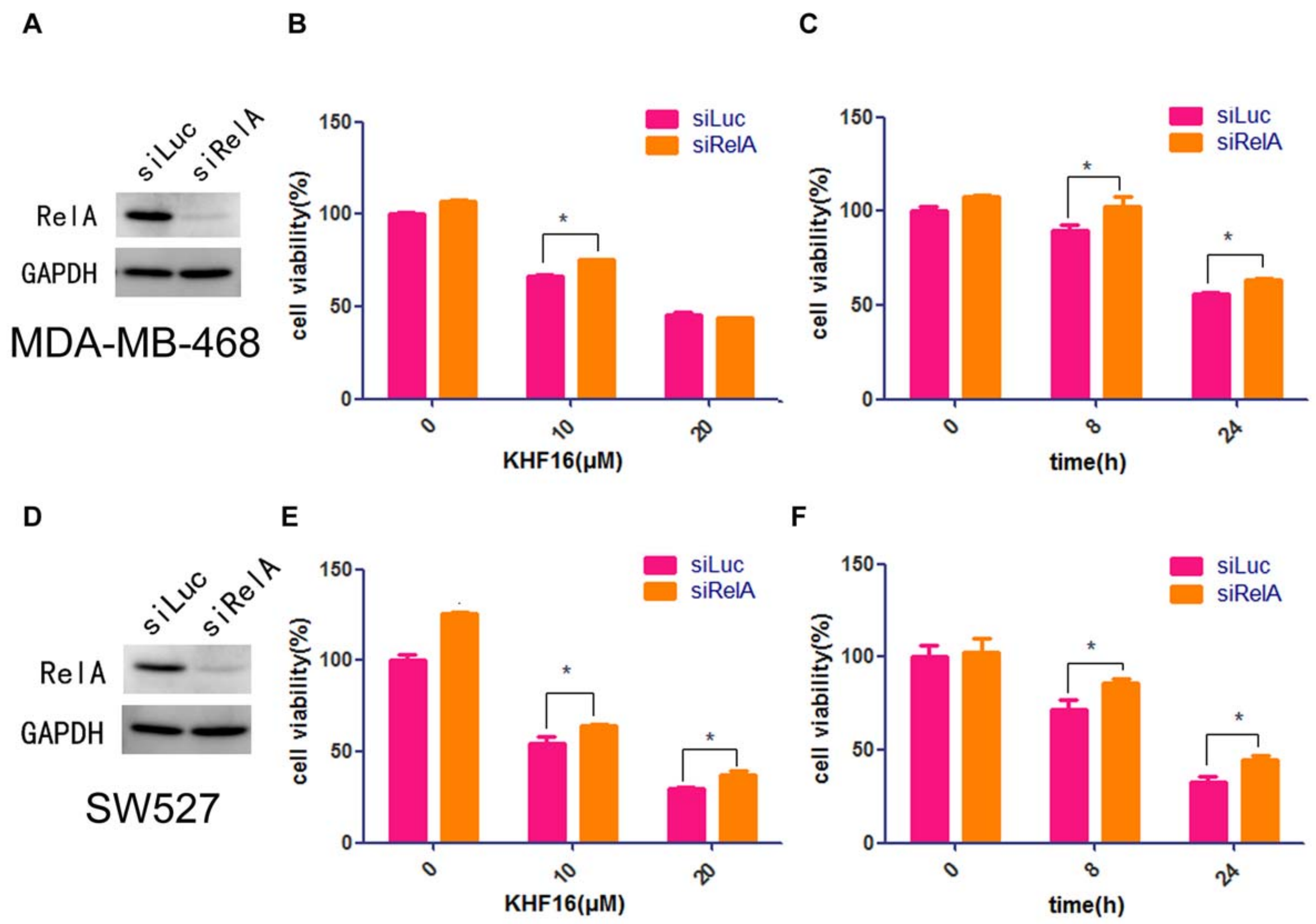

Figure S3

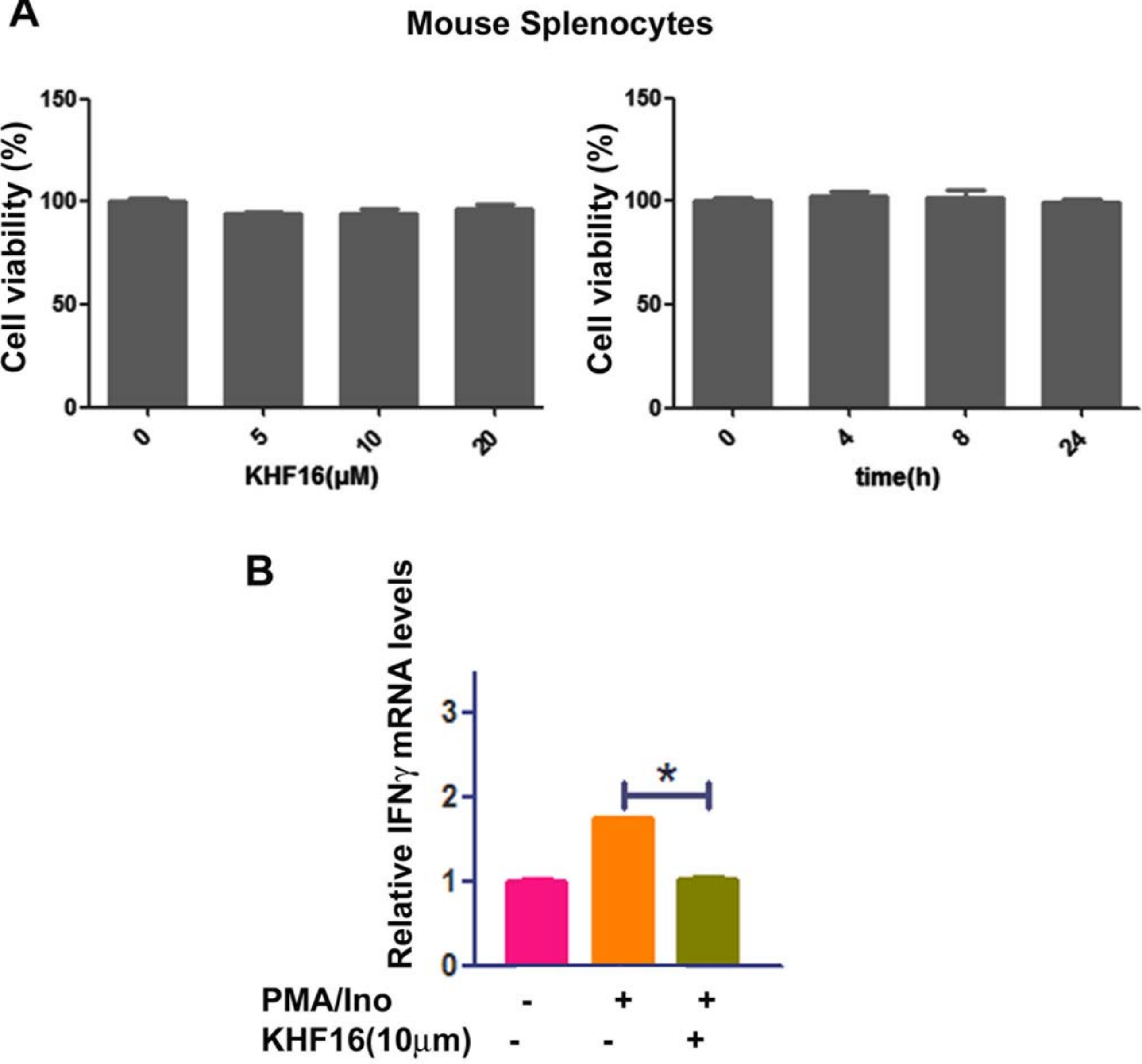

Figure S4