Expanded View Figures

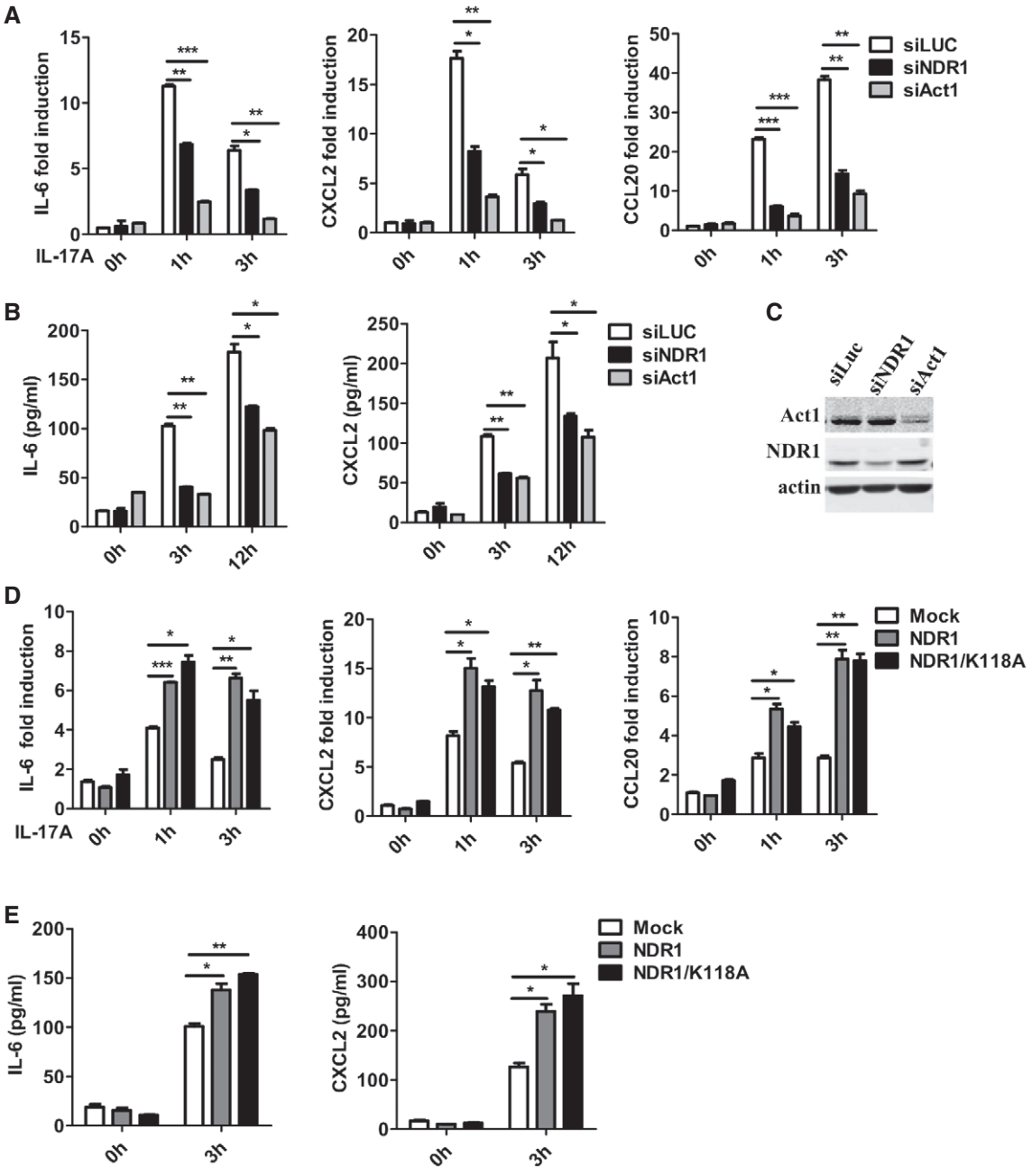

Figure EV1. NDR1 promotes the IL-17-induced expression of pro-inflammatory cytokines in HeLa cells in a kinase activity-independent manner.

A–C HeLa cells were transfected with NDR1 siRNA, Act1 siRNA (positive control) or luciferase siRNA (negative control) and then stimulated with IL-17 (50 ng/ml) for 0, 1, 3, or 12 h. The mRNA levels and production of *IL-6*, *CXCL2*, and *CCL20* were analyzed by real-time PCR (A) and ELISA (B), respectively. The efficiency of knockdown was detected by Western blot (C).

D, E HeLa cells were transfected with mock, NDR1, or NDR1/K118A plasmids and then stimulated with IL-17 (50 ng/ml) for the indicated times. The induction of *IL-6*, *CXCL2*, and *CCL20* mRNA expression were analyzed by real-time PCR (D) and ELISA (E), respectively.

Data information: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ (unpaired, two-tailed Student's *t*-test). Similar results were obtained in three (A–C) or two (D, E) independent experiments. Error bars are mean ± SEM values.
Source data are available online for this figure.

Figure EV2. NDR1 promotes the IL-17F-induced expression of pro-inflammatory cytokines.

A WT and *Ndr1*-KO MEFs transfected with a retrovirus encoding mock, Flag-NDR1, or Flag-NDR1/K118A were treated with IL-17 (100 ng/ml) for 0 or 6 h. The mRNA levels of *IL-6*, *CXCL2*, and *CCL20* were analyzed by real-time PCR.

B, C WT and *Ndr1*-KO MEFs (B) or primary astrocytes (C) were treated with IL-17F (100 ng/ml) for the indicated times, and the induction of *IL-6*, *CXCL2*, *CCL20*, and *CXCL1* mRNA expression was analyzed by real-time PCR.

D HeLa cells were transfected with NDR1 siRNA or control siRNA and then were treated with IL-17F (50 ng/ml) for 0, 1, or 3 h, and the induction of *IL-6*, *CXCL2*, and *CCL20* mRNA expression was analyzed by real-time PCR.

E, F WT and *Ndr1*-KO MEFs were treated with TNF-α (20 ng/ml) (D) or IL-1β (10 ng/ml) (E) for the indicated times, and the induction of *IL-6*, *CXCL2*, *CCL20*, and *CXCL1* mRNA expression was analyzed by real-time PCR.

Data information: $*P < 0.05$, $**P < 0.01$ (unpaired, two-tailed Student's *t*-test). Similar results were obtained in at least two independent experiments. Error bars are mean ± SEM values.

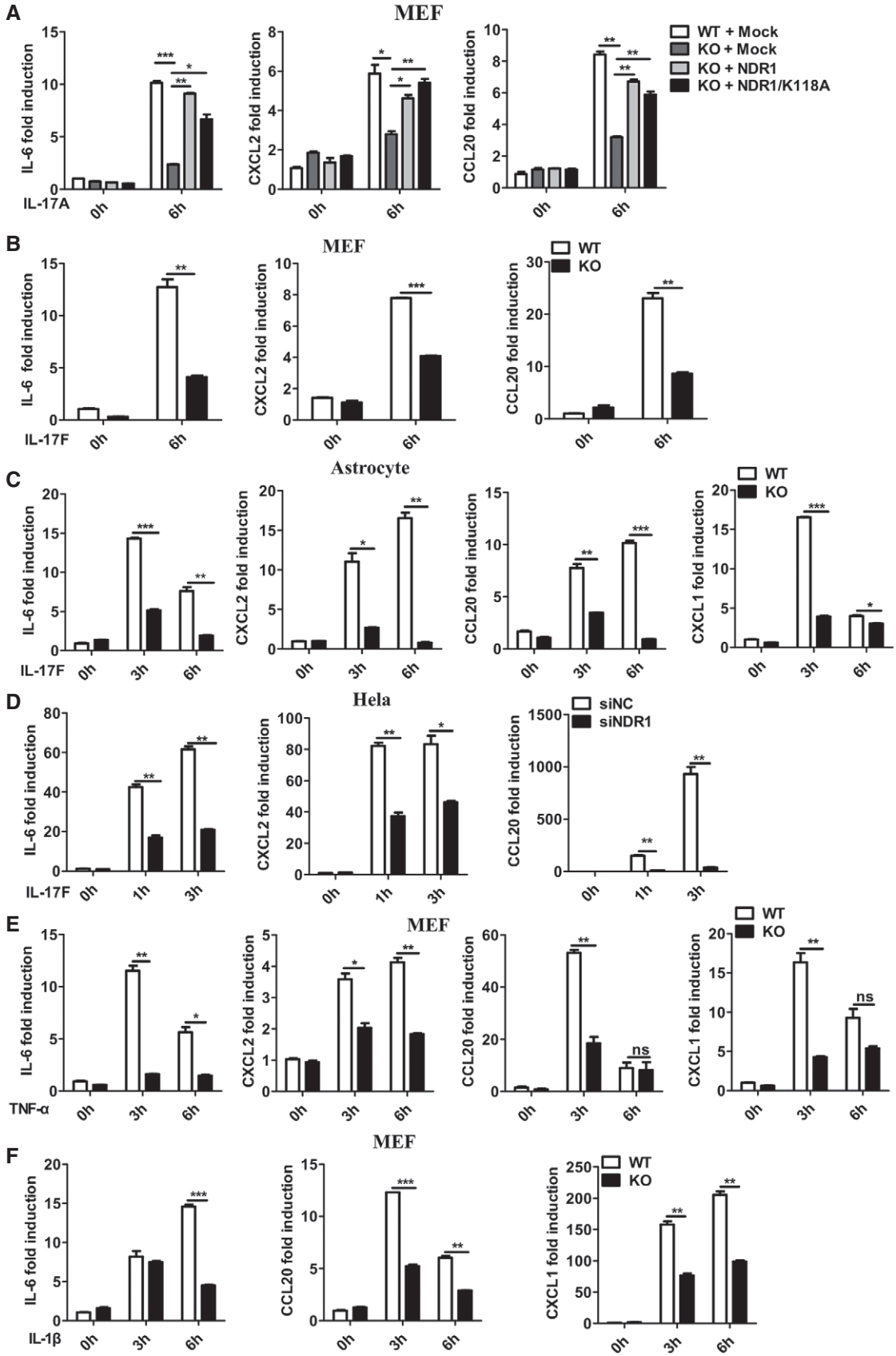

Figure EV2.

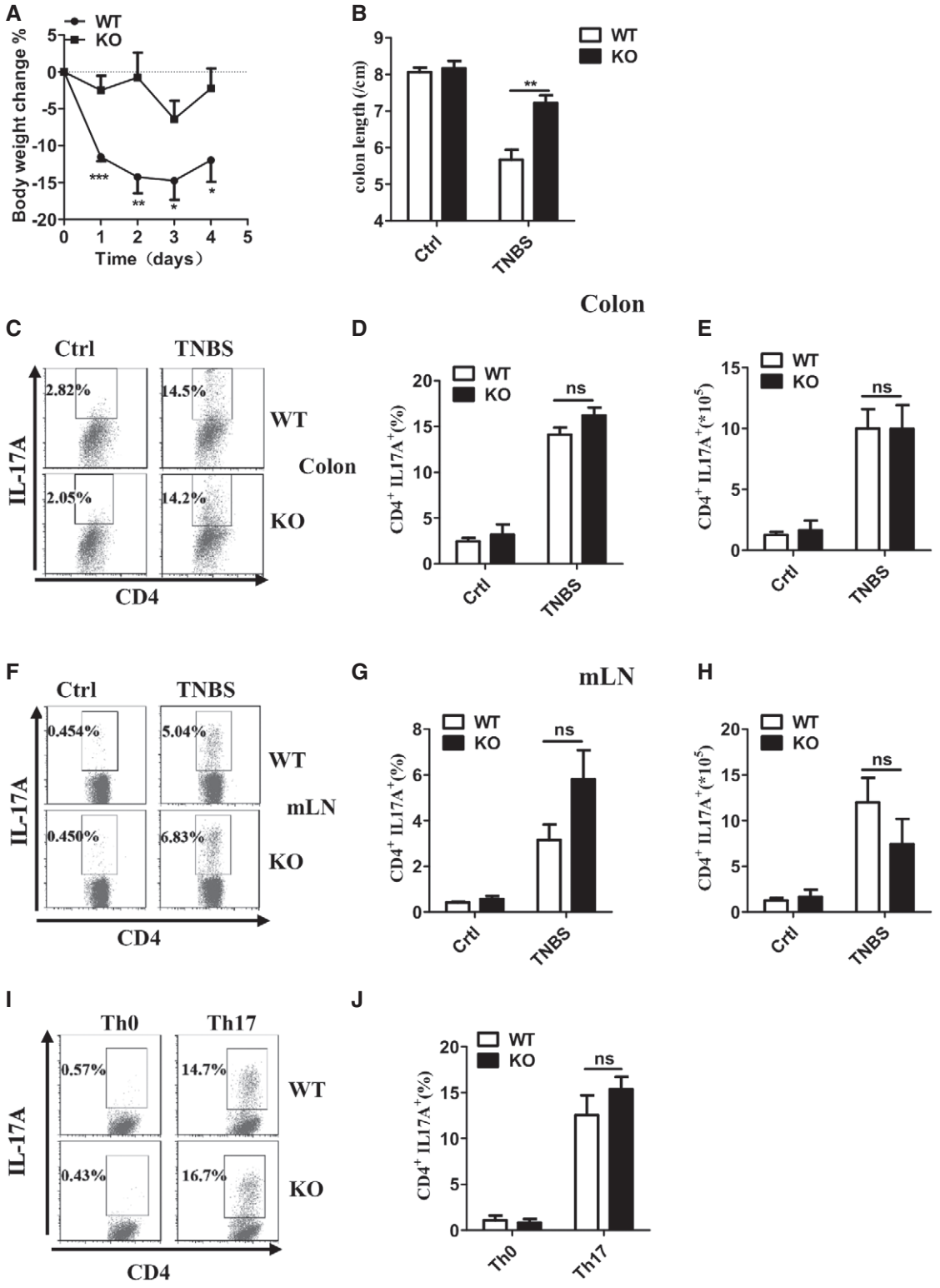
A
WT
KO
Body weight change %
Time (days)
B
WT
KO
colon length (/cm)
Ctrl
TNBS
Colon
C
Ctrl
TNBS
2.82%
14.5%
2.05%
14.2%
WT
KO
Colon
IL-17A
CD4
D
WT
KO
ns
CD4+ IL17A+(%)
Crtl
TNBS
E
WT
KO
ns
CD4+ IL17A+(*10^5)
Crtl
TNBS
F
Ctrl
TNBS
0.454%
5.04%
0.450%
6.83%
WT
KO
mLN
IL-17A
CD4
G
mLN
WT
KO
ns
CD4+ IL17A+(%)
Crtl
TNBS
H
WT
KO
ns
CD4+ IL17A+(*10^5)
Crtl
TNBS
I
Th0
Th17
0.57%
14.7%
0.43%
16.7%
WT
KO
IL-17A
CD4
J
WT
KO
ns
CD4+ IL17A+(%)
Th0
Th17

Figure EV3.

Figure EV3. NDR1 deficiency does not affect Th17 cell production *in vivo* and vitro.

A, B WT (n = 6) and *Ndr1*-KO (n = 5) mice were presensitized with 1% TNBS and then were rectally injected with TNBS at a dose of 150 mg/kg body weight on day 7. Changes in body weight (A) and colon length (B) were measured in WT and *Ndr1*-KO mice with TNBS for a total of 4 days. Mice were euthanized on day 4.

C–E Colons were collected on day 4; then, colonic propria cells were separated and stained with anti-mouse markers CD4 and IL-17A and then analyzed by flow cytometry. A representative plot of Th17 cell frequency (C) and a summary graph of Th17 cell frequency (D) and number (E) were showed.

F–H The mesenteric lymph node cells were treated as in Fig 3C. A representative plot of Th17 cell frequency (F) and a summary graph of Th17 cell frequency (G) and number (H) were showed.

I, J Naive $CD4^+$ T cells ($CD44^{lo}CD62L^{hi}$) isolated from *Ndr1*-KO mice and control littermates (WT) were stimulated for 7 days with anti-CD3 and anti-CD28 under Th0 or Th17 conditions as described in the Materials and Methods section. Flow cytometry was used to measure the frequency of IL-17-producing cells, which is shown a representative plot (I) and a summary graph (J).

Data information: ns, not significant, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ (unpaired, two-tailed Student's t-test). Similar results were obtained in three independent experiments. Error bars are mean ± SEM values.

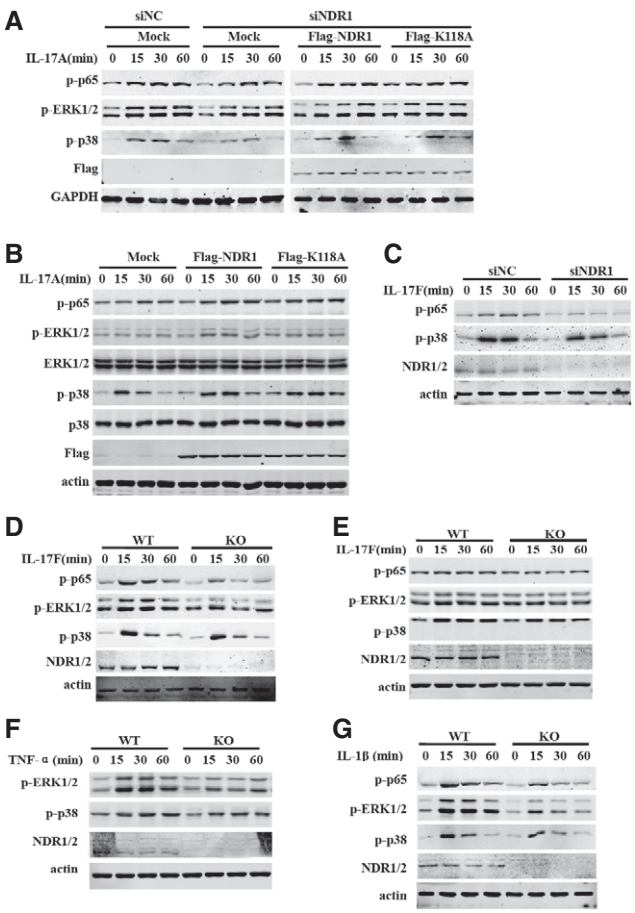

Figure EV4. NDR1 promotes IL-17A and IL-17F signaling.

A HeLa cells were transfected with NDR1 siRNA or control siRNA for 24 h and then were transfected with empty vectors or plasmids encoding Flag-NDR1 or Flag-NDR1/K118A for 48 h. The cells were stimulated with 50 ng/ml IL-17 for 0, 15, 30, or 60 min, and the whole cell lysates were immunoblotted with the indicated antibodies.

B HeLa cells were transfected with mock or plasmids encoding Flag-NDR1 or Flag-NDR1/K118A and then stimulated with IL-17 (50 ng/ml) for 0, 15, 30, or 60 min. Whole cell lysates were immunoblotted with the indicated antibodies.

C HeLa cells were transfected with NDR1 siRNA or control siRNA and then were treated with IL-17F (50 ng/ml) for 0, 15, 30, or 60 min. Whole cell lysates were immunoblotted with the indicated antibodies.

D, E WT and *Ndr1*-KO MEFs (D) or primary astrocytes (E) were treated with IL-17F (100 ng/ml) for 0, 15, 30, or 60 min. Whole cell lysates were immunoblotted with the indicated antibodies.

F, G WT and *Ndr1*-KO MEFs were treated with TNF-α (20 ng/ml) (F) or IL-1β (10 ng/ml) (G) for 0, 15, 30, or 60 min. Whole cell lysates were immunoblotted with the indicated antibodies.

Data information: Similar results were obtained in three (A, B) or two (C–G) independent experiments.
Source data are available online for this figure.

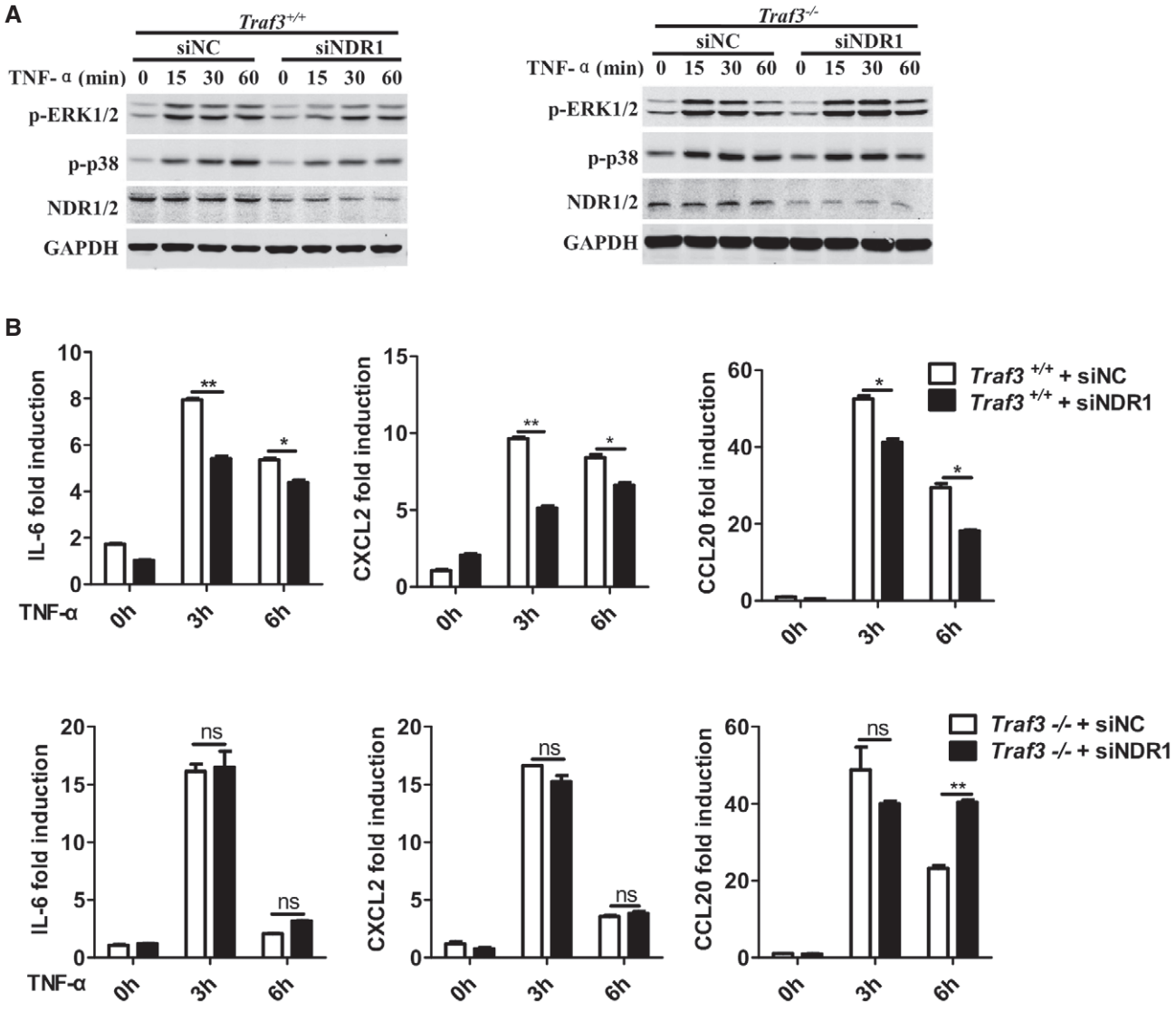

Figure EV5. NDR1 promotes TNF-α signaling via targeting TRAF3.

A Wild-type (*Traf3*$^{+/+}$) and TRAF3-deficient (*Traf3*$^{-/-}$) MEFs were transfected with NDR1 siRNA or control siRNA and then stimulated with TNF-α (20 ng/ml) for the indicated times. Whole lysates were subjected to Western blot.

B Real-time PCR analysis of *IL-6*, *CXCL2*, and *CCL20* mRNA expression in MEFs treated as described in (A). *$P < 0.05$ and **$P < 0.01$ (unpaired, two-tailed Student's *t*-test). Similar results were obtained in two independent experiments. Error bars are mean ± SEM values.

Source data are available online for this figure.