

Expanded View Figures

Figure EV1. *Nedd4l* deficiency promotes IL-17-induced inflammatory cytokine and chemokine expression.

- A, B Immunoblot analysis of NEDD4L in HeLa cells silenced with scramble or human *Nedd4l*-specific siRNA(A), and in MEFs cells (B).
- C, D Real-time PCR analysis of *Il-6*, *Cxcl2*, and *Ccl20* mRNA expression in *Nedd4l*^{+/+}- and *Nedd4l*-deficient heterozygous (*Nedd4l*^{+/-}) MEFs following stimulation with IL-17 (C) or IL-17F (D) (100 ng/ml) (*n* = 3 technical replicates).
- E, F Immunoblot analysis of NEDD4L in *Nedd4l*^{+/+} and *Nedd4l*^{-/-} primary lung epithelial cells (E), and in *Nedd4l*^{+/+} and *Nedd4l*^{+/-} primary astrocytes (F).

Data information: Data are shown as the mean ± S.E.M. of technical replicates. All experiments were performed two times, and representative data are shown. Source data are available online for this figure.

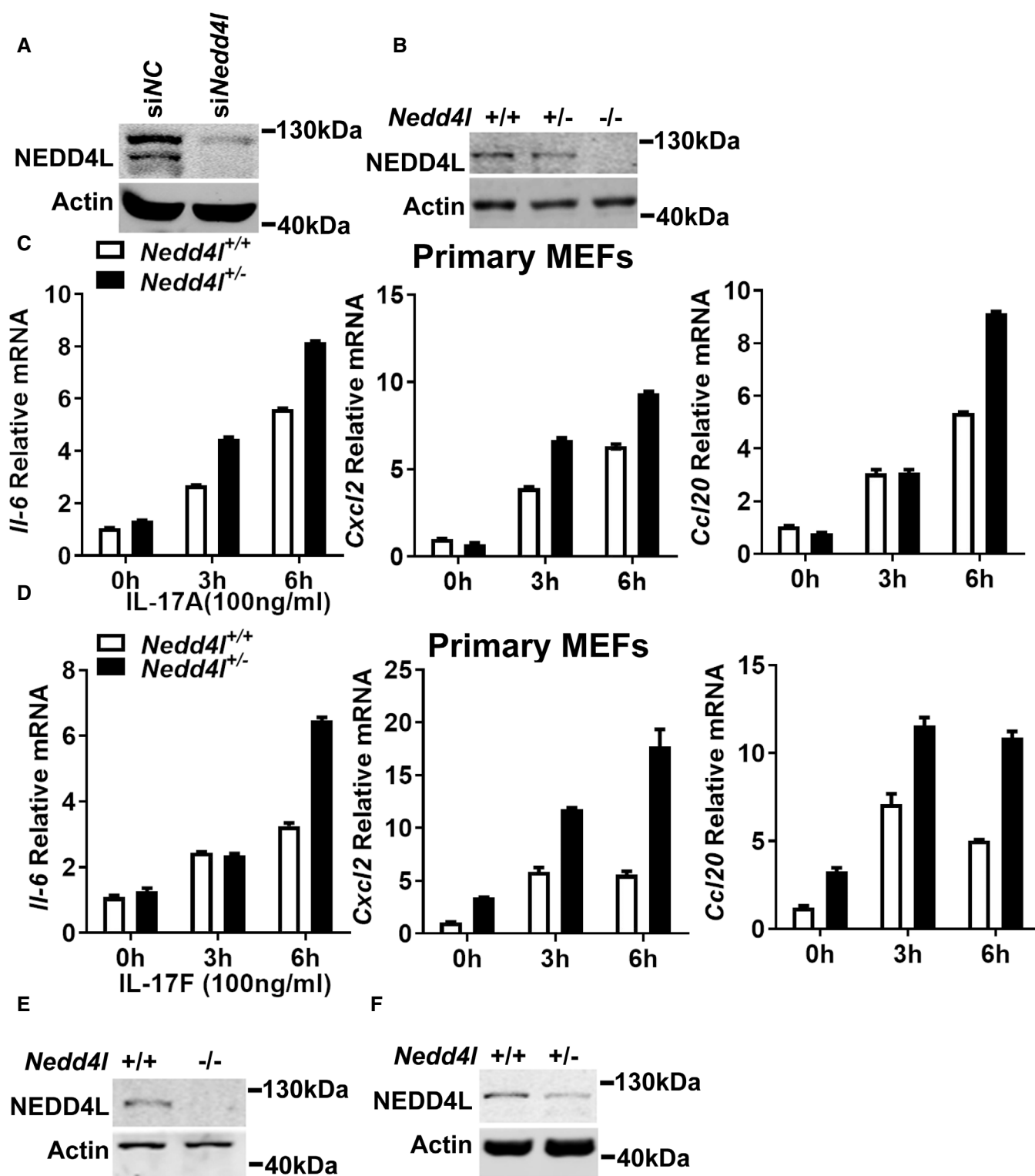


Figure EV1.

Figure EV2. *Nedd4l* deficiency promotes TNF- α - and IL-1 α -induced inflammatory cytokine and chemokine expression.

- A, B HeLa cells were transfected with *Nedd4l* siRNA (*siNedd4l*) or control RNA (*siNC*). *Il-6*, *Cxcl2*, and *Ccl20* mRNA expression in HeLa cells transfected with *Nedd4l* siRNA (*siNedd4l*) or control RNA (*siNC*) and stimulated with TNF- α (50 ng/ml) (A) and IL-1 α (10 ng/ml) (B) for the indicated time ($n = 3$ technical replicates).
- C, D Real-time PCR analysis of *Il-6*, *Cxcl2*, and *Ccl20* mRNA expression in *Nedd4l*^{+/+}- and *Nedd4l*-deficient heterozygous (*Nedd4l*^{+/-}) MEFs following stimulation with TNF- α (50 ng/ml) (C) and IL-1 α (10 ng/ml) (D) for the indicated time ($n = 3$ technical replicates).
- E Immunoblot analysis of NEDD4L in peritoneal mesothelial cells isolated from *Nedd4l*^{+/+} and *Nedd4l*^{+/-} mice.

Data information: Data are shown as the mean $\pm$ S.E.M. of technical replicates. All experiments were performed two times, and representative data are shown. Source data are available online for this figure.

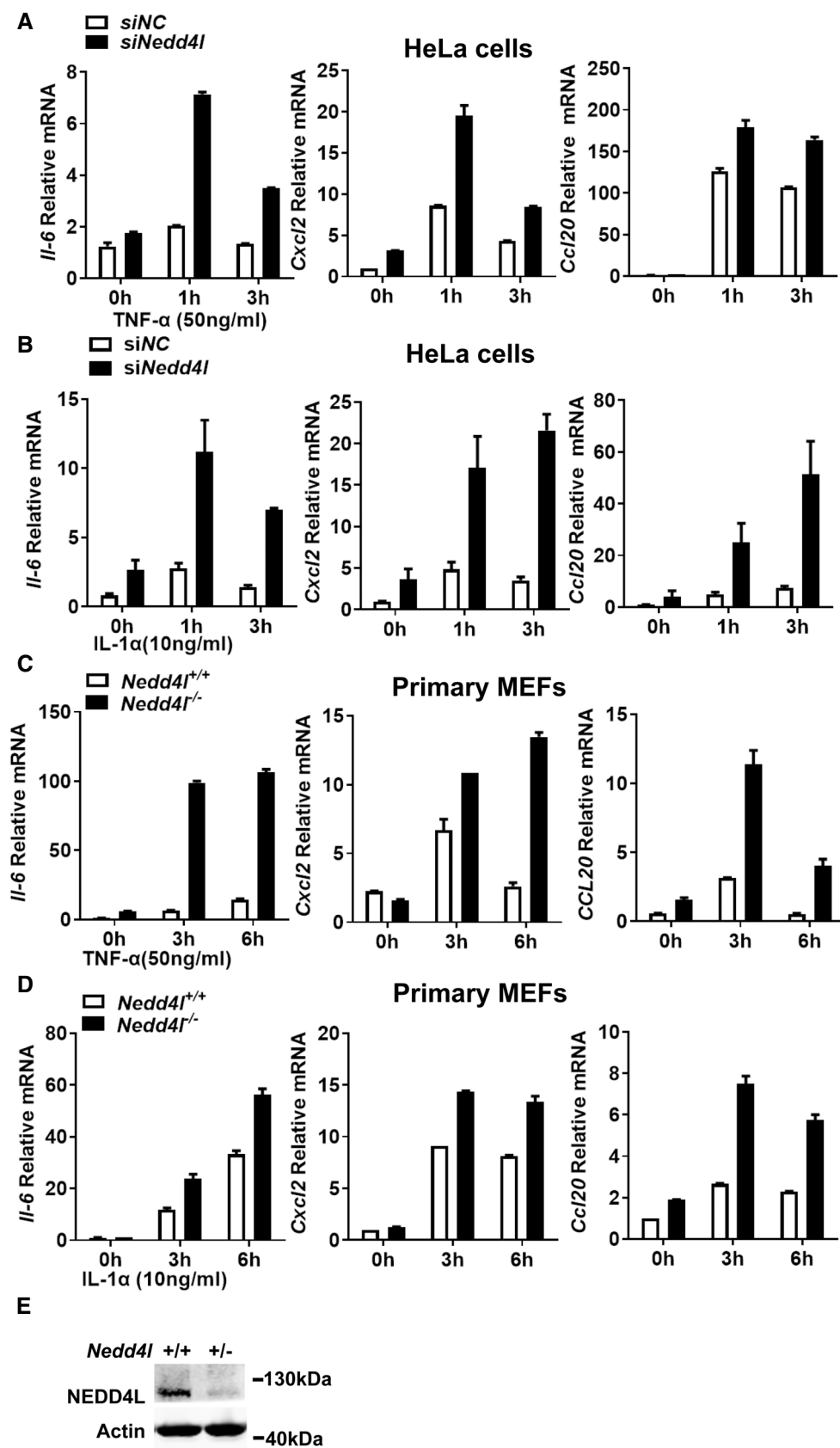


Figure EV2.

Figure EV3. *Nedd4l* deficiency promotes the IL-17-, TNF- α -, and IL-1 α -induced P38, NF- κ B activation.

- A–D Immunoblot analysis of phosphorylated p38 (P-P38), total P38, phosphorylated p65 subunit of NF- κ B (P-P65), total p65, MEKK2, NEDD4L, and actin in HeLa cells, or *Nedd4l*^{+/+} and *Nedd4l*^{-/-} MEFs treated with 50 ng/ml TNF- α (A, C) or 10 ng/ml IL-1 α (B, D) for the indicated time.
- E Immunoblot analysis of phosphorylated NF- κ B p65 (P-P65), total P65, MEKK2, NEDD4L, and actin in HeLa cells transfected with control RNA (siNC) and *Nedd4l* siRNA (si*Nedd4l*) followed by overexpression of Myc-tagged Null, Myc-tagged NEDD4L, or Myc-tagged NEDD4L-C942A (Myc-tagged NEDD4L-CA), and stimulated with IL-17 (50 ng/ml) for the indicated time.
- F Immunoblot analysis of HA-tagged MEKK2, Myc-tagged NEDD4L, and actin in HEK293T cells transfected with the indicated plasmids with anti-HA, anti-Myc-tag, or anti-actin antibody.

Data information: Numbers between two blots indicate the densitometry of phosphorylated proteins relative to that of total proteins, respectively. All experiments were performed two times, and representative data are shown.

Source data are available online for this figure.

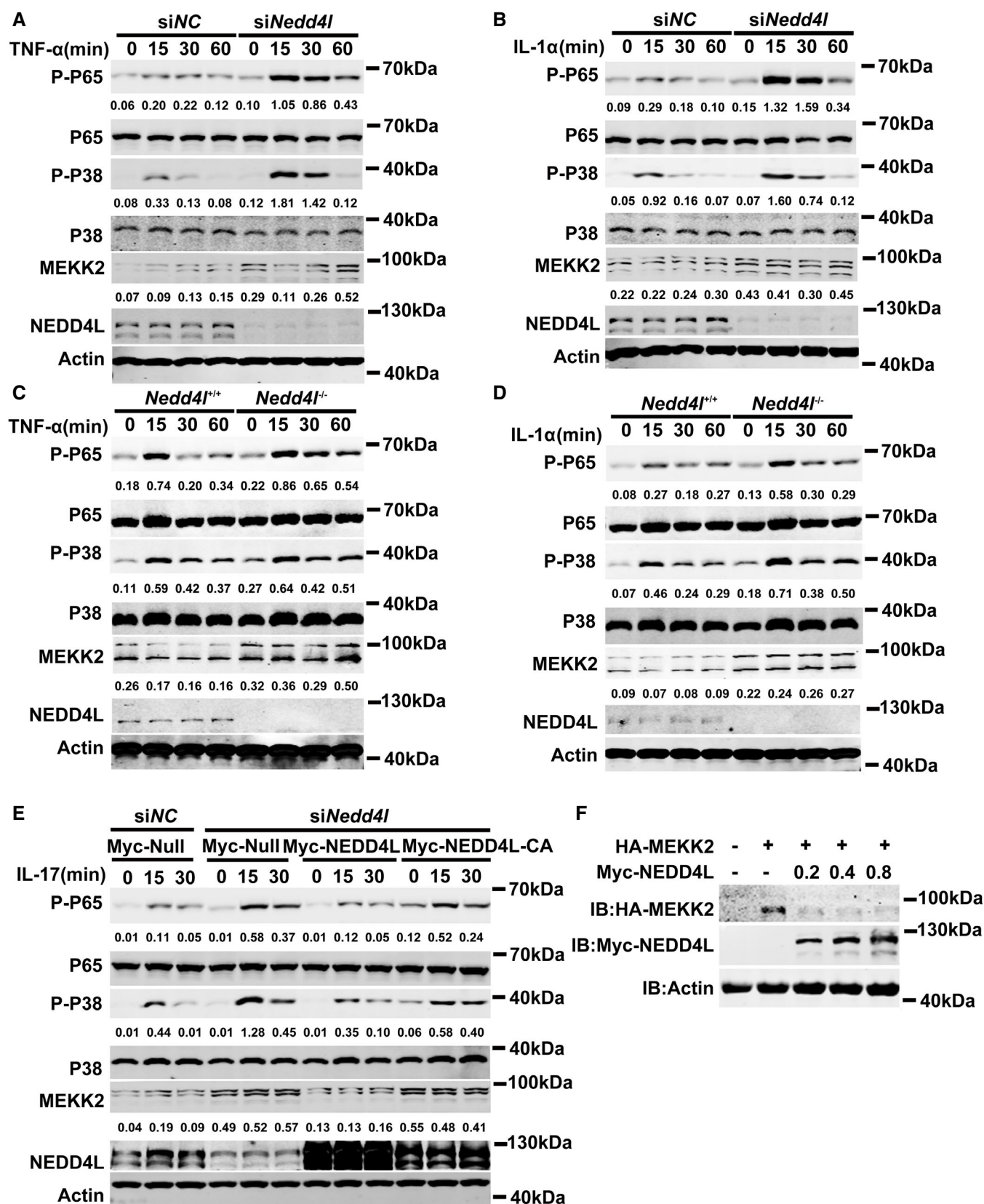


Figure EV3.

Figure EV4. *Nedd4l* deficiency promotes IL-17-induced inflammatory cytokine and chemokine expression dependent on MEKK2.

- A Immunoblot analysis of NEDD4L and MEKK2 immunoprecipitated with anti-MEKK2 antibody from lysates of HeLa cells treated with 20 μ M MG-132 for 6 h and then 50 ng/ml IL-17 for the indicated time.
- B Schematic diagram of wild-type and mutant NEDD4L constructs.
- C Immunoblot analysis of phosphorylated NF- κ B p65 (P-P65), total P65, MEKK2, NEDD4L, Smurf1, and actin transfected with control RNA (siNC), *Nedd4l* siRNA (si*Nedd4l*), *Smurf1* siRNA (si*Smurf1*) or *Nedd4l* siRNA plus *Smurf1* siRNA (si*Nedd4l* + si*Smurf1*), and stimulated with IL-17 (50 ng/ml) for the indicated time.
- D Real-time PCR analysis of *Il-6*, *Cxcl2*, and *Ccl20* mRNA expression in HeLa cells treated as in (C) ($n = 3$ technical replicates).
- E Real-time PCR analysis of *Il-6*, *Cxcl2*, and *Ccl20* mRNA expression in HeLa cells transfected with control RNA (siNC) or *Nedd4l* siRNA (si*Nedd4l*), followed by being pre-treated with BAY 61–3,606 (10 μ M) and stimulated with IL-17 (50 ng/ml) for the indicated time ($n = 3$ technical replicates).
- F Immunoblot analysis of Ser520 phosphorylation of MEKK2 (P-MEKK2), total Flag-tagged MEKK2, and actin in HEK293T cells transfected with Flag-tagged Null, Flag-tagged MEKK2, and mutant MEKK2.
- G Immunoblot analysis of Ser520 phosphorylation of MEKK2 (P-MEKK2) immunoprecipitated from HEK293T cells transfected with Flag-tagged Null, or Flag-tagged MEKK2, and then incubated with or without calf intestinal alkaline phosphatase time (CIAP) for 30 min.
- H Immunoblot analysis of phosphorylated NF- κ B p65 (P-P65), total P65, MEKK2, and actin in HeLa cells transfected with control RNA (siNC), *Mekk2* siRNA (si*Mekk2*) followed by overexpression of Flag-tagged Null, Flag-tagged MEKK2, or Flag-tagged MEKK2-S520A (Flag-tagged MEKK2-SA), and stimulated with IL-17 (50 ng/ml) for the indicated time.
- I Schematic diagram of wild-type and mutant MEKK2 constructs.
- J Immunoblot analysis of Flag-tagged and mutant MEKK2 co-immunoprecipitated with anti-Flag antibody from lysates of HEK293T cells co-transfected with plasmids expressing HA-tagged TAK1 and Flag-tagged wild-type or mutant MEKK2. Asterisk (*) indicates the target band.

Data information: Data are shown as the mean $\pm$ S.E.M. of technical replicates. All experiments were performed two times, and representative data are shown. Source data are available online for this figure.

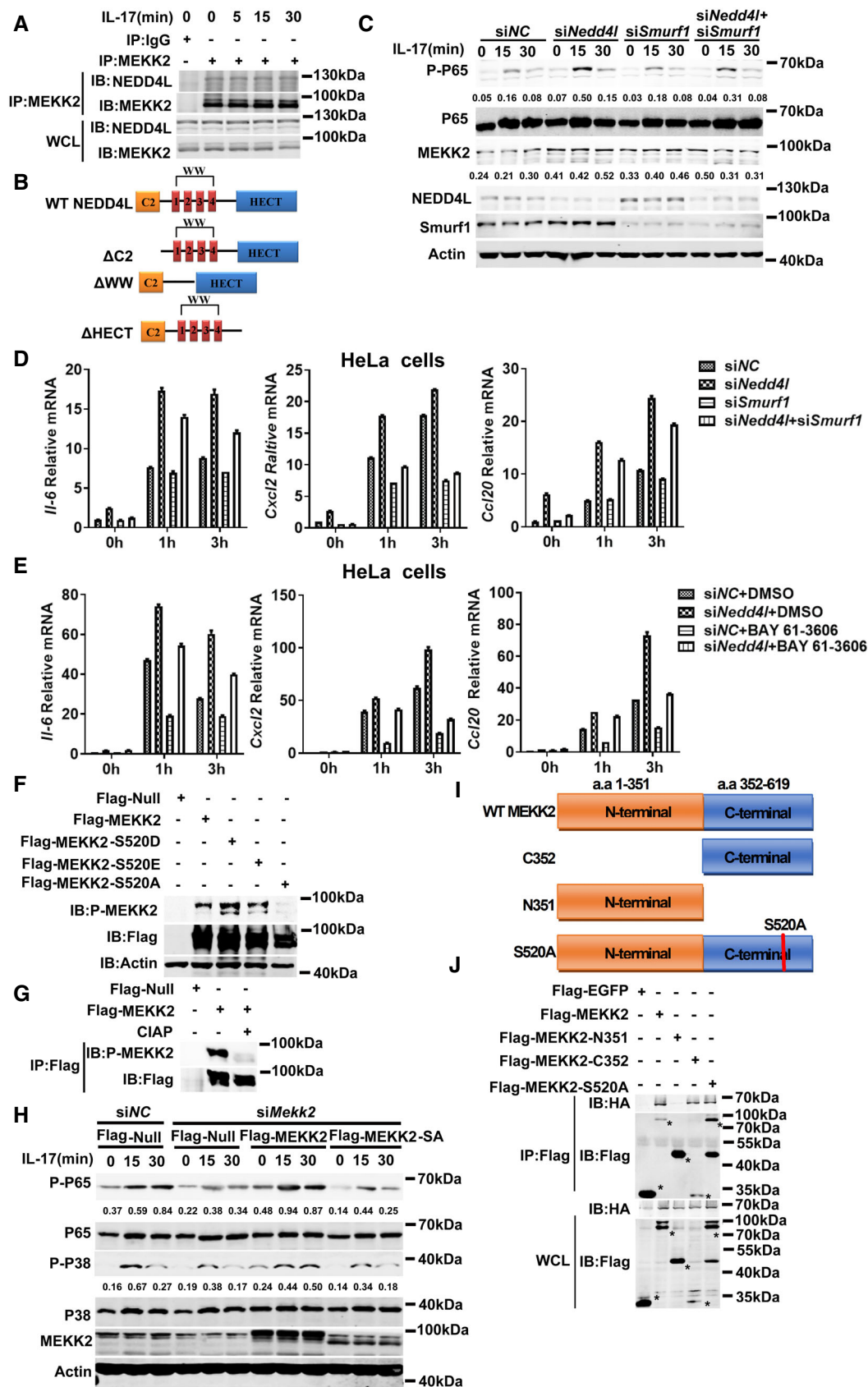


Figure EV4.

Figure EV5. *Nedd4l* deficiency promotes MOG₃₅₋₅₅-induced EAE.

- A The mean clinical scores for *Nedd4l*^{+/+} and *Nedd4l*^{+/-} mice (*Nedd4l*^{+/+} *n* = 5 biological replicates and *Nedd4l*^{+/-} *n* = 5 biological replicates) induced by being immunized with MOG₃₅₋₅₅ were assessed according to the standards described in the Materials and Methods from day 0 to day 18.
- B Histology of the spinal cord was analyzed by H&E (left panel) or LFB (right panel) staining on day 18 after the second MOG₃₅₋₅₅ immunization. Scale bars (a whole spinal cord, 100×), 500 μm; scale bars (a portion of the spinal cord, 200×), 200 μm.
- C, D Flow cytometric analysis of immune cells (including CD45⁺CD4⁺ T cells, CD45⁺CD8⁺ T cells, CD45⁺CD19⁺ B cells, and CD45⁺CD11b⁺ myeloid cells), infiltrated to the spinal cord and brain of EAE-induced *Nedd4l*^{+/+} and *Nedd4l*^{+/-} mice at day 18 after immunization (*Nedd4l*^{+/+} *n* = 5 biological replicates and *Nedd4l*^{+/-} *n* = 5 biological replicates). Data are presented as representative plots.
- E Summary graph of the percentages of immune cells in the CNS, including CD45⁺CD4⁺ T cells, CD45⁺CD8⁺ T cells, CD45⁺CD19⁺ B cells, CD45⁺CD11b^{high}, and CD45⁺CD11b^{low} myeloid cells. CNS-infiltrating cells isolated from mice treated as in (A) were stained with the appropriate antibodies (*Nedd4l*^{+/+} *n* = 5 biological replicates and *Nedd4l*^{+/-} *n* = 5 biological replicates).
- F Summary graph of the percentages of CD11b⁺Gr-1⁺ neutrophils in the CD45⁺CD11b^{high} CNS. CNS-infiltrating cells isolated from mice treated as in (A) were stained with the appropriate antibodies (*Nedd4l*^{+/+} *n* = 5 biological replicates and *Nedd4l*^{+/-} *n* = 5 biological replicates).
- G *Il-6*, *Cxcl2*, *Cccl20* mRNA, IL-17A, and IFN γ mRNA in the CNS tissues (including brain and spinal cords) were measured by real-time PCR on day 14 after the second MOG₃₅₋₅₅ immunization (*Nedd4l*^{+/+} *n* = 5 biological replicates and *Nedd4l*^{+/-} *n* = 5 biological replicates).

Data information: Data are shown as the mean $\pm$ S.E.M. of biological replicates. All experiments were performed two times, and representative data are shown. Significant differences were tested using a two-tailed Mann–Whitney *U* test (A) or a two-tailed Student's *t*-test (E, F, G). **P* < 0.05 and ***P* < 0.001. NS, no significance.

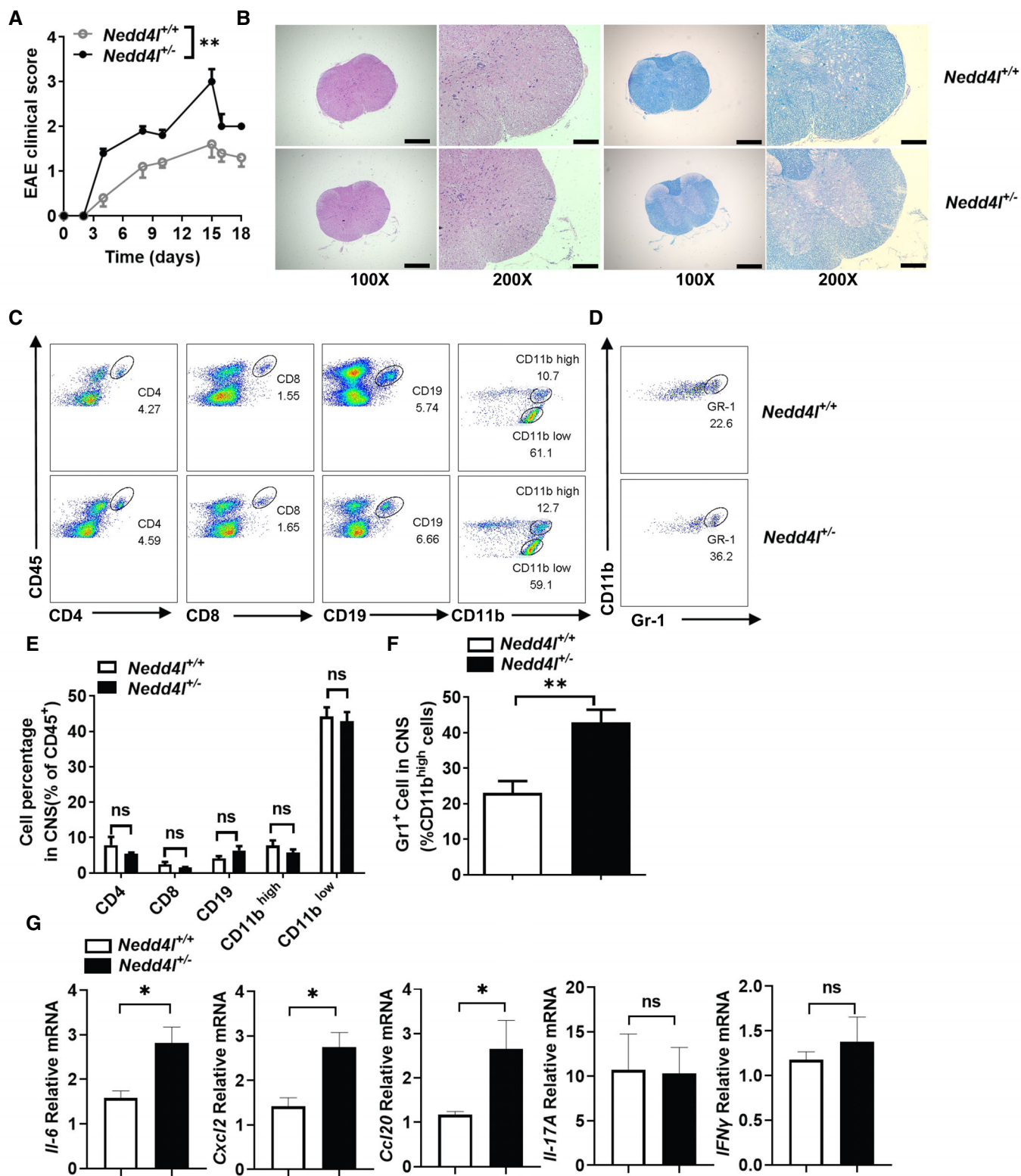


Figure EV5.