Expanded View Figures

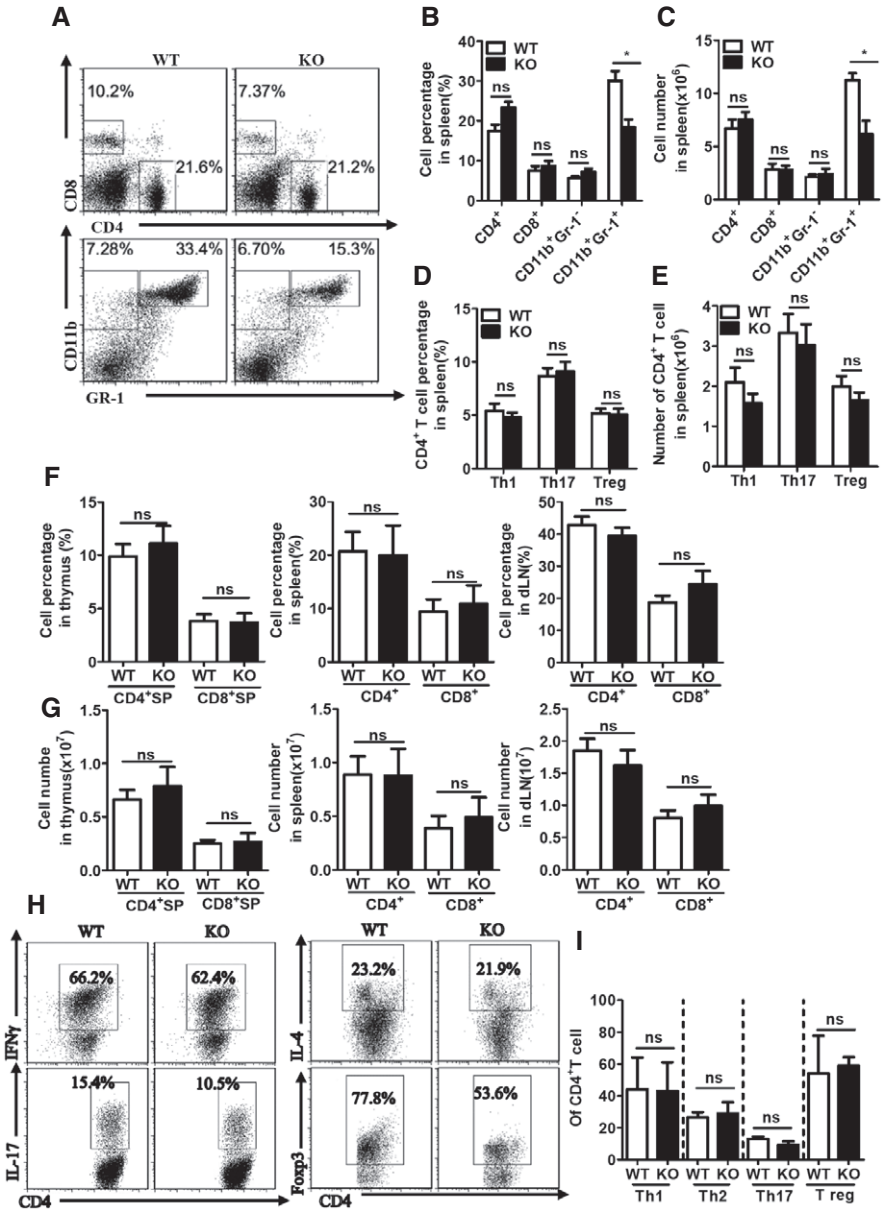

Figure EV1. RKIP deficiency has no effect on T cell development, polarization, and immune cell composition in the spleen from EAE mice.

A–C Immune cells isolated from spleen of day 31 MOG_{35-55}-immunized WT and *RKIP-KO* mice were stained with mouse anti-CD4, anti-CD8, anti-CD11b, and anti-Gr-1 antibodies and analyzed by flow cytometry (FACS; WT $n = 6$, KO $n = 5$). Data are presented as the representative plot (A), summary graph of the percentages of cells (B), and the absolute numbers of cells (C).

D, E T-helper cells isolated from spleen of day 31 MOG_{35-55}-immunized WT and *RKIP-KO* mice were fixed and permeabilized, and the $CD4^+$ T cells were analyzed by flow cytometry to measure intracellular IFN-γ, IL-17, and Foxp3. Data are presented in summary graphs of percentages (D) and absolute cell numbers (E) (WT $n = 6$, KO $n = 5$).

F, G T cells from thymus, spleen, and dLNs of WT and *RKIP-KO* mice ($n = 5$/per group) were analyzed with anti-CD4 and anti-CD8 staining.

H, I The naïve $CD4^+$ T cells were polarized under the Th1, Th2, Th17, or Treg condition, respectively, and 5 days later, the $CD4^+$ T cells were fixed and permeabilized, followed by flow cytometry to measure intracellular IFN-γ, IL-4, IL-17, and Foxp3. Data are presented as the representative plot (H) and summary graph of the percentages of cells (I), Th1 ($n = 4$), Th2 ($n = 2$), Th17 ($n = 4$), and Treg ($n = 2$).

Data information: $^*P < 0.05$; ns, no significant difference (unpaired, two-tailed Student's *t*-test). Data are representative of three independent experiments with similar results. Data are means ± SEM values.

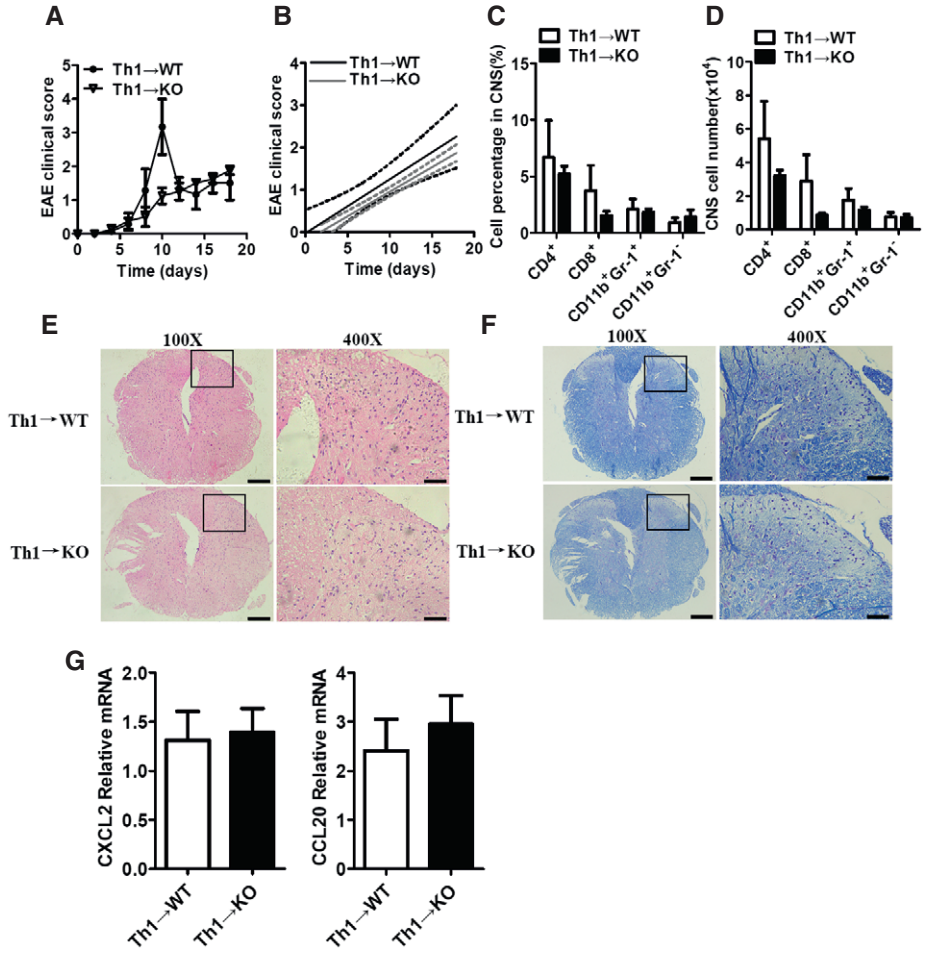

Figure EV2. RKIP deficiency does not affect Th1 cells passive transferring-induced EAE.

A The mean EAE clinical scores of γ-irradiated (5Gylc) WT and *RKIP-KO* mice (WT n = 3, KO n = 4) reconstituted with WT MOG_{35-55}-pre-sensitized Th1 cells were determined from day 0 to day 18 after Th1-cell transfer.

B Linear regression curves of (A) dashed lines indicate the 95% confidence intervals of the regression lines.

C, D Summary graph of the percentages of cells (C) and the absolute numbers of cells (D) in the CNS. CNS-infiltrating cells isolated from mice treated as in (A) on day 18 after the Th1-cell transfer was stained with the appropriate antibody.

E, F Histology of the spinal cord from mice reconstituted with Th1 cells was analyzed by H&E (E) and LFB (F) staining of spinal cord sections from mice reconstituted with Th1 cells. Scale bars (a whole spinal cord, 100X), 200 μm; scale bars (a portion of the spinal cord, 400X), 50 μm. Typical stainings in the black box region were magnified and shown in 400X panel.

G Real-time PCR analysis of *CXCL2* and *CCL20* mRNA expression in the CNS tissues of WT and *RKIP-KO* mice treated as in (A).

Data information: Data are representative of two independent experiments with similar results.

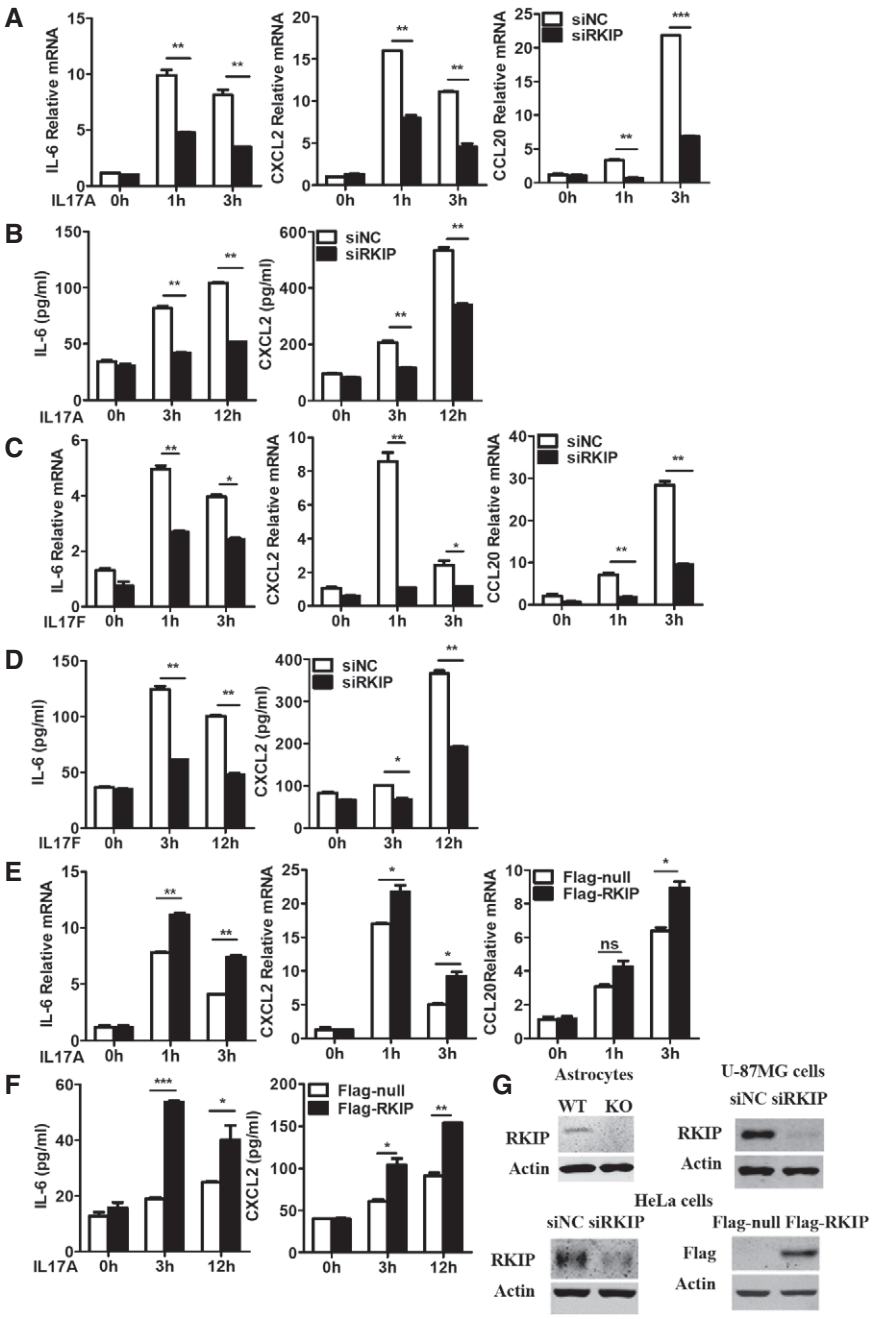

Figure EV3. RKIP positively regulates IL-17A/F-induced proinflammatory cytokine and chemokine expression in HeLa cells.

A, B HeLa cells were transfected with RKIP-specific siRNA (siRKIP) or negative control siRNA (siNC), and then, the cells stimulated with IL-17A (50 ng ml^{-1}) for the indicated time. IL-6, CXCL2, and CCL20 mRNA expression (A) and protein production (B) were analyzed by real-time PCR and ELISA, respectively.

C, D HeLa cells were transfected with RKIP-specific siRNA (siRKIP) or negative control siRNA (siNC), and then, the cells were stimulated with IL-17F (50 ng ml^{-1}) for the indicated time. IL-6, CXCL2, and CCL20 mRNA expression (C) and protein production (D) were analyzed by real-time PCR and ELISA, respectively.

E, F Flag-tagged RKIP-expressing or control mock (Flag-tagged null) plasmid-transfected HeLa cells were stimulated with IL-17A (50 ng ml^{-1}) for indicated time.

G Immunoblot analysis of RKIP protein expression in all of knockout, knockdown, and overexpression experiments.

Data information: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; ns, no significant difference (unpaired, two-tailed Student's *t*-test). Data are representative of three independent experiments with similar results. Data are means ± SEM values.

Source data are available online for this figure.

Figure EV4. RKIP silencing has no effect on TNF-α-, IL-1β-, and LPS-induced proinflammatory cytokine and chemokine expression in HeLa cells.

A–C HeLa cells were transfected with RKIP-specific siRNA (siRKIP) or negative control siRNA (siNC), and then, the cells stimulated with TNF-α (20 ng ml^{-1}) (A), IL-1β (20 ng ml^{-1}) (B), and LPS (100 ng ml^{-1}) (C) for the indicated time. IL-6, CXCL2, and CCL20 mRNA expression was analyzed by real-time PCR.

D HeLa cells were transfected with Flag-tagged RKIP or Flag-null control plasmids and then treated with 50 ng ml^{-1} IL-17A for the indicated time. The WCLs were subsequently immunoblotted with the indicated antibodies.

E–G HeLa cells were transfected with RKIP-specific siRNA (siRKIP) or negative control siRNA (siNC) and treated with TNF-α (20 ng ml^{-1}) (E), IL-1β (20 ng ml^{-1}) (F), and LPS (100 ng ml^{-1}) (G) for the indicated time, and then, the WCLs were immunoblotted with the indicated antibodies.

Data information: ns, no significant difference (unpaired, two-tailed Student's *t*-test). Data are representative of three independent experiments with similar results. Data are means ± SEM values.

Source data are available online for this figure.

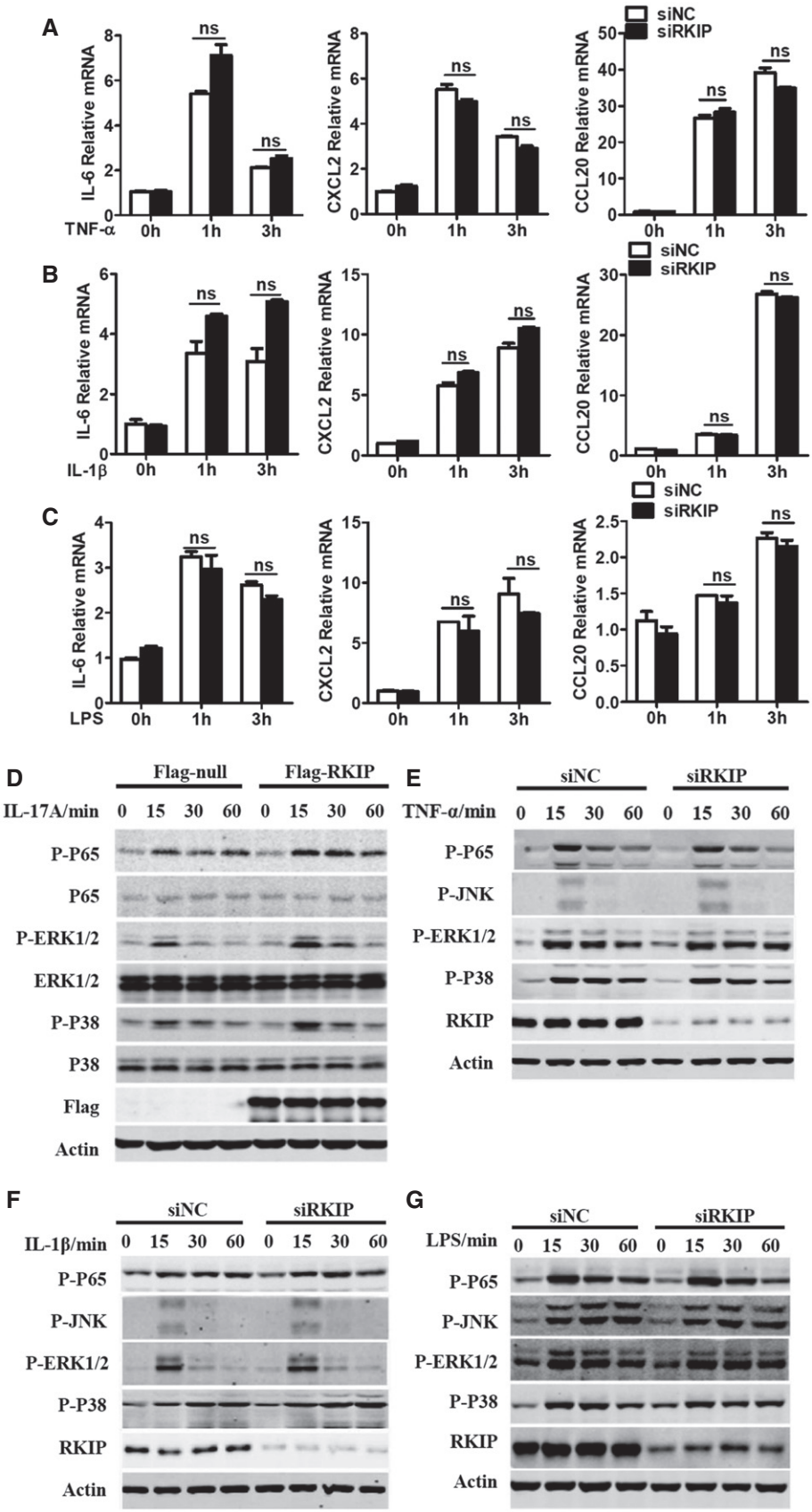

Figure EV4.

Figure EV5. RKIP interacts with Act1 and IL-17RA and promotes the formation of IL-17RA-Act1 complex.

A Schematic diagram of Act1 deletion mutants. Flag-tagged null, Flag-tagged full-length Act1, Flag-tagged Act1-SEFIR (400–555), or Flag-tagged Act1-ΔSEFIR (1–400) mutants were co-expressed with Myc-tagged RKIP in HEK293T cells, and the lysates from the transfected cells were immunoprecipitated (IP) with anti-Flag beads and then subjected to immunoblot analysis with the indicated antibodies. Asterisk (*) indicates the target band.

B Schematic diagram of IL-17RA deletion mutants. HA-tagged null, HA-tagged full-length IL-17RA, HA-tagged IL-17RA-ΔTiLL (Δ532–553), HA-tagged IL-17RA-ΔDistal (1–553), HA-tagged IL-17RA-ΔSEFIR (Δ379–536), or HA-tagged IL-17RA-SEFIR (379–536) mutants were co-expressed with Myc-tagged RKIP in HEK293T cells, and the lysates from the transfected cells were immunoprecipitated (IP) with anti-HA beads and then subjected to immunoblot analysis with the indicated antibodies. Asterisk (*) indicates the target band.

C, D HEK293T cells were transfected with HA-tagged IL-17RA and Flag-tagged Act1 with or without HA-tagged RKIP (C) or with or without RKIP-specific siRNA (D). The lysates from the transfected cells were immunoprecipitated (IP) with anti-Flag beads and then subjected to immunoblot analysis with the indicated antibodies.

E siRKIP- or siNC-transfected Hela cells were pretreated with TNF-α (20 ng ml^{-1}) for 1 h, followed by treatment (horizontal axes) with actinomycin D (5 μg ml^{-1}) alone or actinomycin D plus IL-17 (25 ng ml^{-1}) for the indicated time and then were subjected to real-time PCR analysis of CXCL1 mRNA expression. $*P < 0.05$ (unpaired, two-tailed Student's *t*-test). Data are representative of three independent experiments with similar results. Data are means ± SEM values.

Source data are available online for this figure.

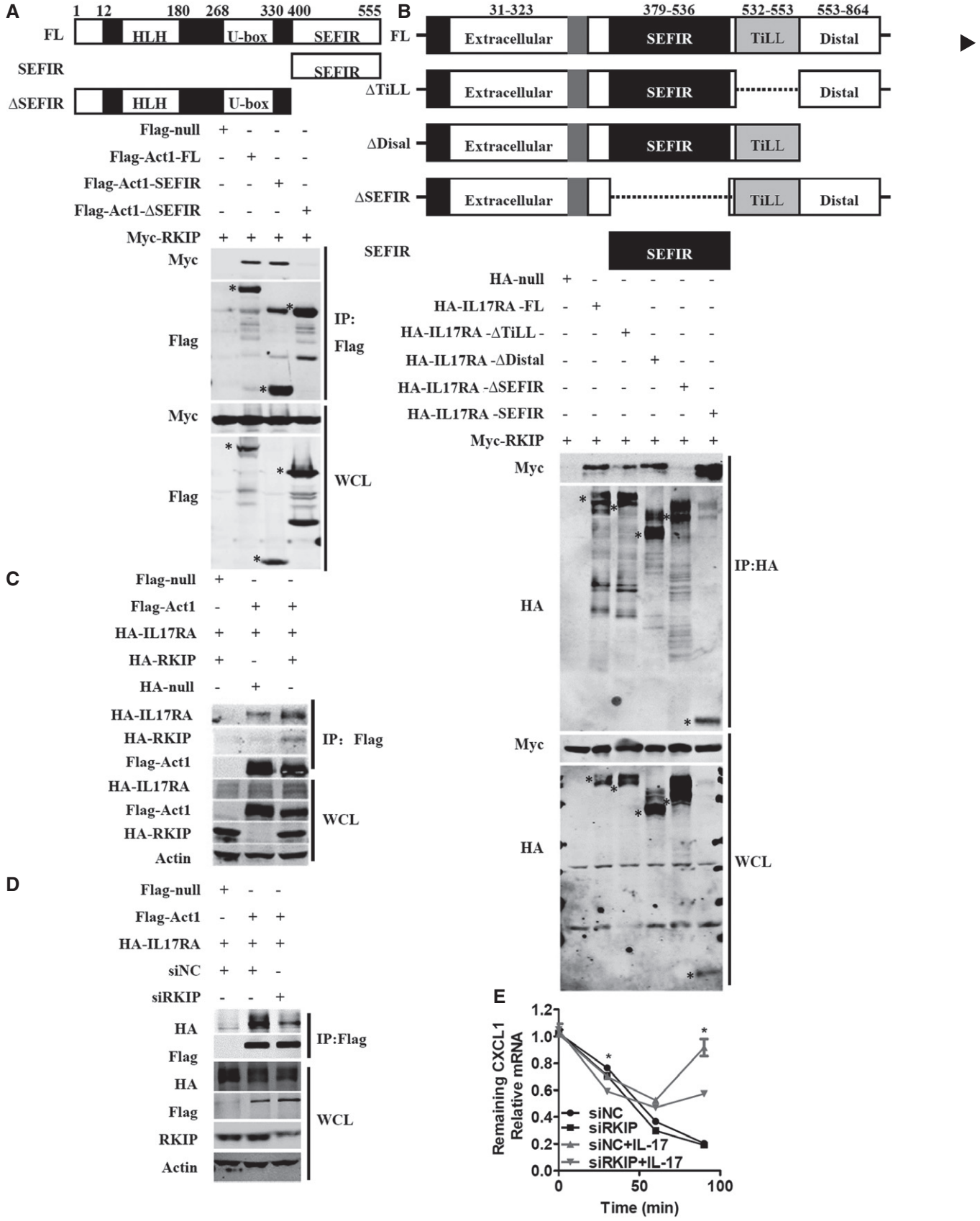

Figure EV5.

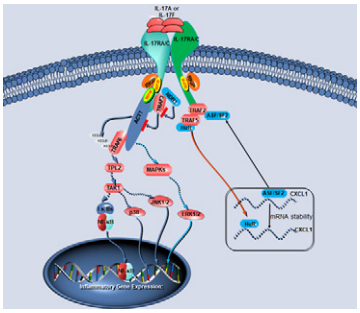

Figure EV6. Mechanism schematic diagram of RKIP regulating IL-17R signaling.

Upon binding with IL-17A or IL-17F, IL-17R cytoplasm domain recruits the adaptor protein Act1. RKIP functions as an important adaptor protein to promote Act1 to IL-17R in a SEFIR domain-dependent manner. Activated Act1 mediates the downstream signaling in a TRAF6-dependent or TRAF6-independent manner to regulate signaling transduction and gene expression of cytokines or chemokines.