Supplemental material

Huang et al., https://doi.org/10.1084/jem.20180210

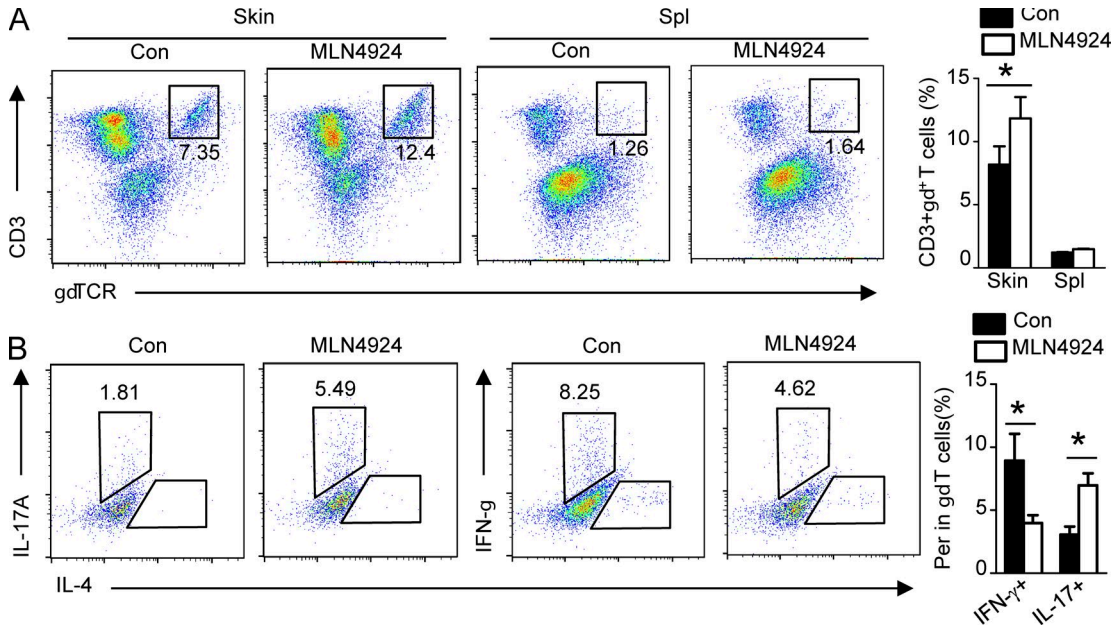

Figure S1. **MLN4924 enhances the frequent of IL-17-producting γδT cells under inflammatory condition. (A)** Representative plots and bar graphs represented flow cytometry analysis of γδT cells localized at skin and Spl of mice treated with MLN4924. **(B)** Flow cytometry analysis of IFN-γ and IL-17 positive cells gating with γδT cells in the skin of mice treated with MLN4924. Data are representative of three independent experiments. Error bars show mean ± SEM. Significance was determined by two-tailed Student's *t* test (*, P < 0.05).

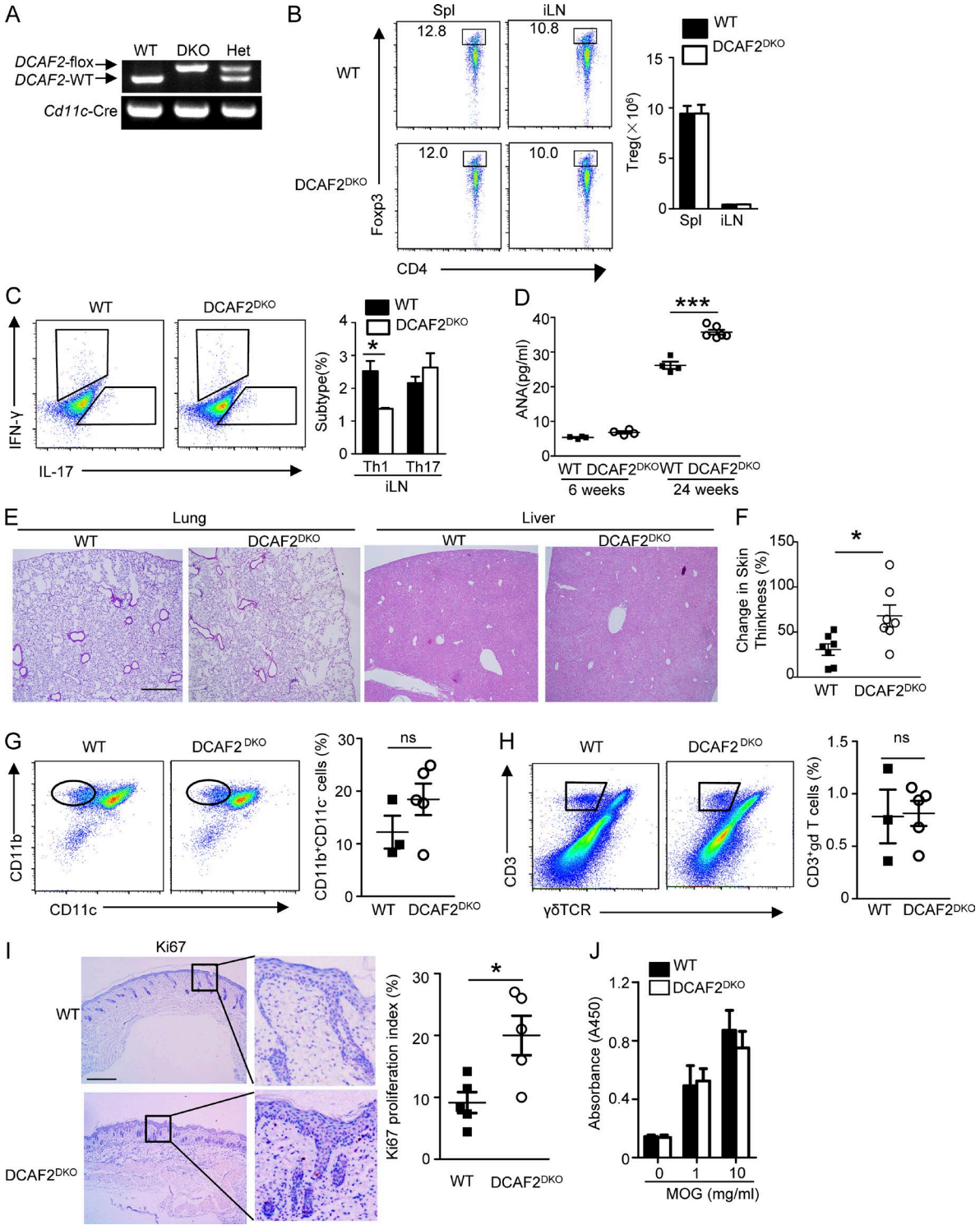

Figure S2. **DCAF2 deficiency promotes autoimmune symptom in psoriasis mice or nontreated aging mice. (A)** Genotyping PCR of DCAF2-flox and WT alleles or the *CD11c*-Cre. **(B)** The absolute cell numbers of T reg cells in Spl or iLN. **(C)** Flow cytometric analysis of the percentage of IFN-γ– and IL-17–producing $CD4^+$ T cells in the iLN of 6-wk-old WT and $DCAF2^{DKO}$ mice. **(D)** The ANA antibody in serum of young (6 wk) or older (24 wk) mice was examined by ELISA. **(E)** H&E staining of the indicated tissue sections from 6-wk-old WT and $DCAF2^{DKO}$ mice. Bar, 200 µm. **(F)** WT and $DCAF2^{DKO}$ mice (n = 8/group) were treated with Aldara for 6 d. Plots graphs represented percent change in skin thickness. **(G–H)** Plots and summary graphs represented flow cytometry analysis of inflamed skin of psoriasis mice. Cells were gated on $CD45^+CD11b^+CD11c^-$ or $CD45^+CD3^+\gamma\delta TCR^-$ for the presence of skin-infiltrating myeloid and αβT cells. **(I)** Immunohistochemical detection of the proliferation marker Ki67 in keratinocytes from psoriatic WT and $DCAF2^{DKO}$ mice (brown staining). Ki67 proliferation index (%) calculated as Ki67-positive layer cells divided by total basal layer cells × 100. Bar, 200 µm. **(J)** WT and $DCAF2^{DKO}$ mice were subjected to MOG35-55–induced EAE (n = 3/group). Cell proliferation assays of splenic T cells were evaluated by CCK8 from day 15 EAE-induced mice, stimulated in vitro with the indicated concentrations of MOG-peptide. Data are representative of three independent experiments. Error bars show mean ± SEM. Significance was determined by two-tailed Student's t test (*, $P < 0.05$; ***, $P < 0.005$; ns, not significant).

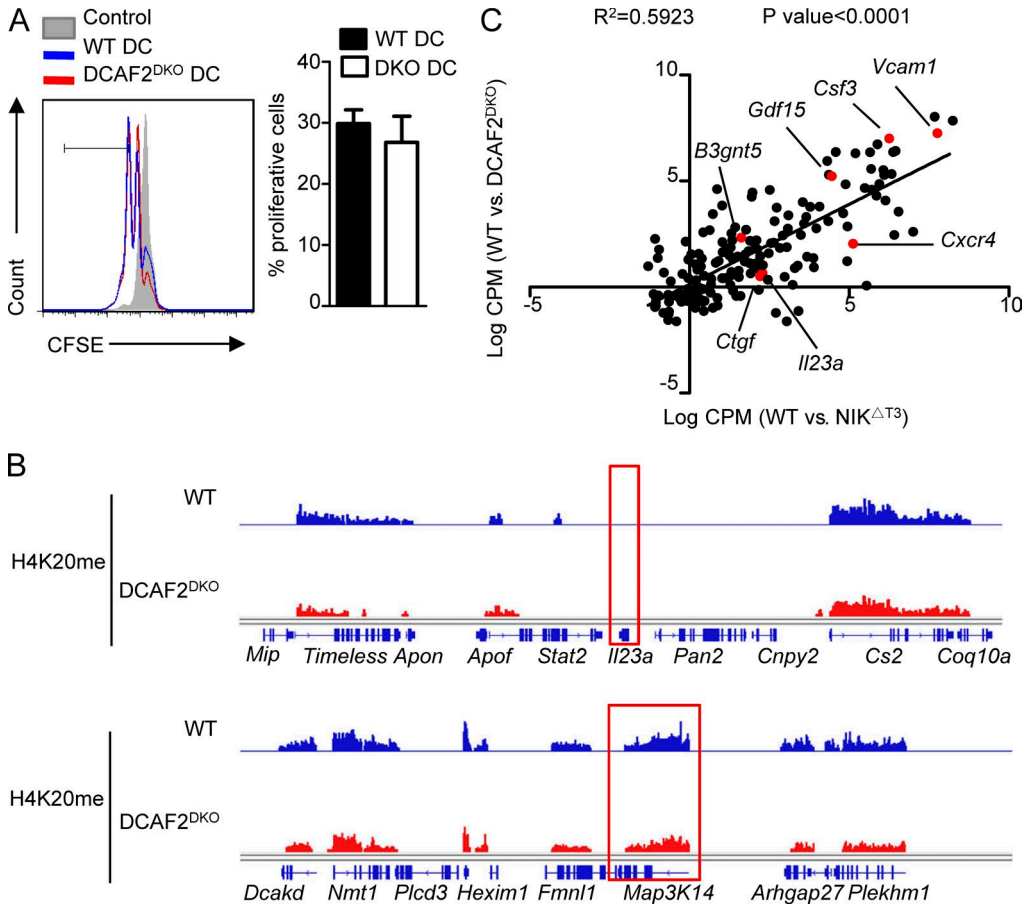

Figure S3. **DCAF2 deficiency regulated noncanonical NF-κB, but not required for its capacity of antigen presentation. (A)** Flow cytometric analysis to measure the proliferation of CFSE-labeled OTII T cells incubated with either medium control or OVA-pulsed WT and DCAF2^{DKO} Spl DCs. **(B)** Snapshot of the H4K20me1 ChIP-Seq signal at the *Il23a* and *Map3k14*. The arrow indicates the location of the ChIP primer pairs. **(C)** Correlation analysis of overlapped DEGs expression between WT versus NIK$^{\Delta T3}$ and WT versus DCAF2 KO BMDCs. Noncanonical NF-κB targeted genes were labeled as red plots. Data in A are representative of three independent experiments. Error bars show mean ± SEM. Significance was determined by two-tailed Student's *t* test.

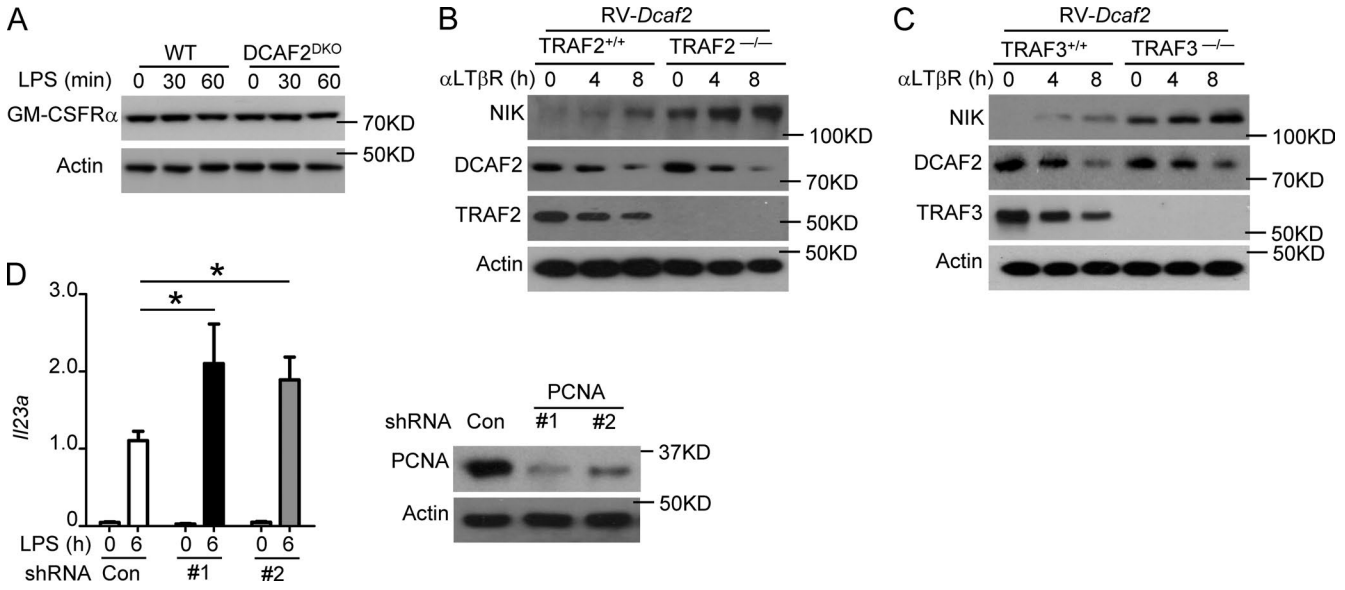

Figure S4. **DCAF2 negatively regulates NIK stability independent of TRAF2 or TRAF3. (A)** IB analyses of GM-CSFRa and actin levels in total lysates of WT and DCAF2 KO BMDCs. **(B and C)** WT, TRAF2- (B) or TRAF3-deficient (C) MEFs reconstituted with WT DCAF2 with retroviral vectors. IB analysis of the indicated proteins in reconstituted MEFs stimulated with aLTbR. **(D)** WT BM cells were incubated with GM-CSF, and infected with pGIPZ lentiviral vectors encoding a nonsilencing control shRNA (C) or two different Pcna-specific shRNAs at 24 h. After 7 d, GFP⁺ cells were sorted with FACS and then stimulated with LPS as indication. qRT-PCR analysis of the *Il23a* expression. Whole-cell lysates was subjected to IB to detect the PCNA level. Data are representative of three independent experiments. Error bars show mean ± SEM. Significance was determined by two-tailed Student's *t* test (*, P < 0.05).

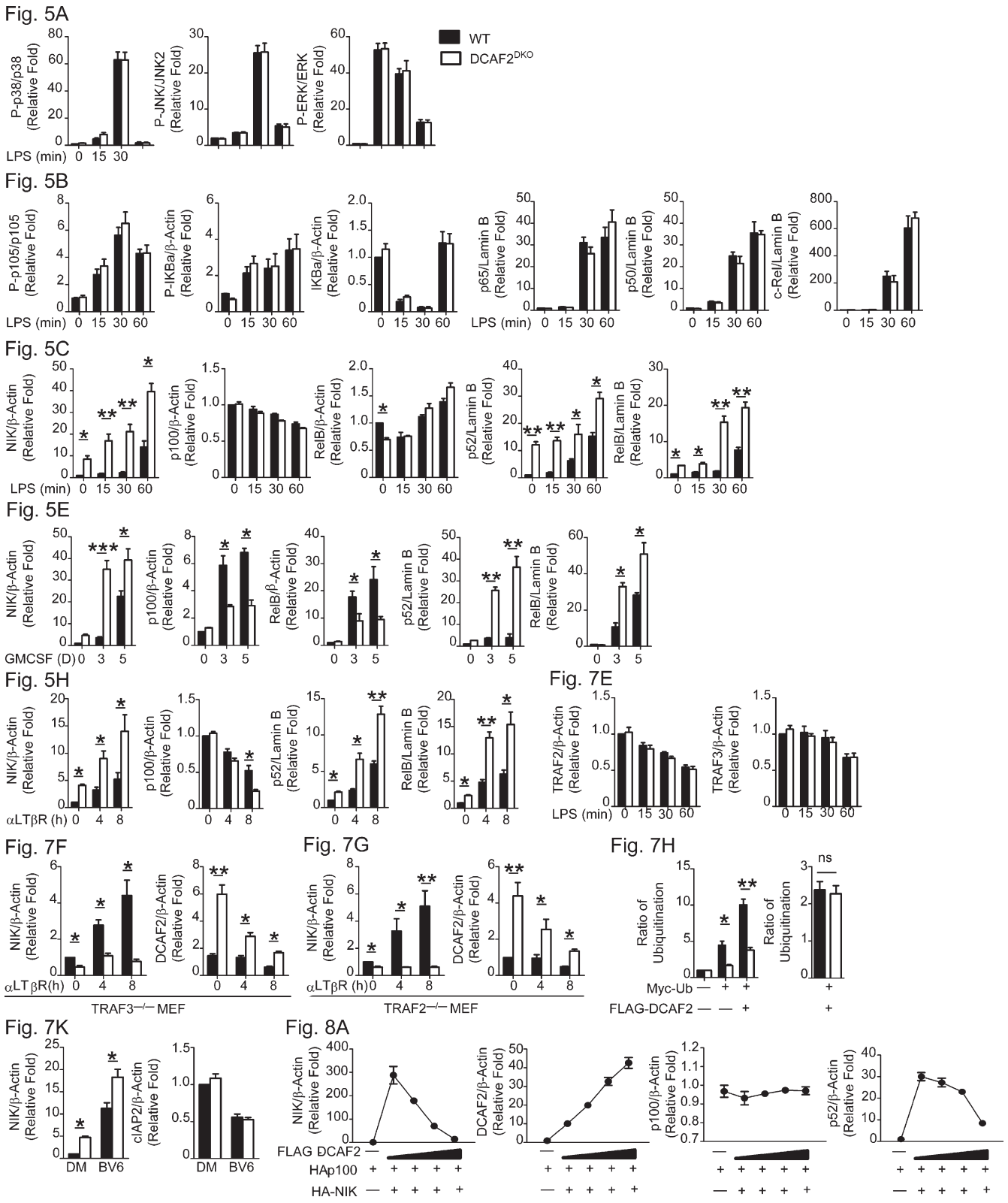

Figure S5. **The relative density of Western blot result.** In IB and IP experiments, the relative density of bands was measured using ImageJ for at least three times. Data are representative of three independent experiments. Error bars show mean ± SEM. Significance was determined by two-tailed Student's *t* test (*, P < 0.05; **, P < 0.01; ***, P < 0.005).