Appendix

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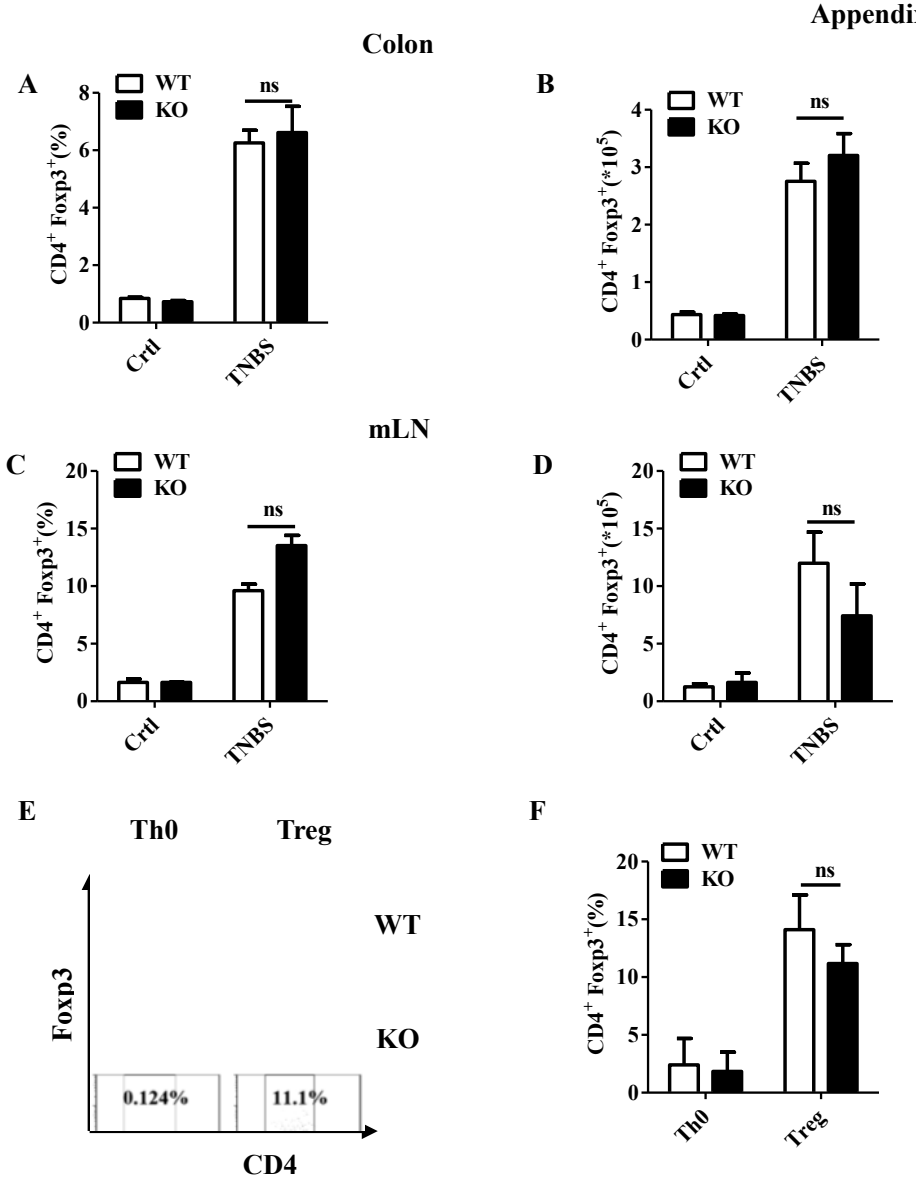
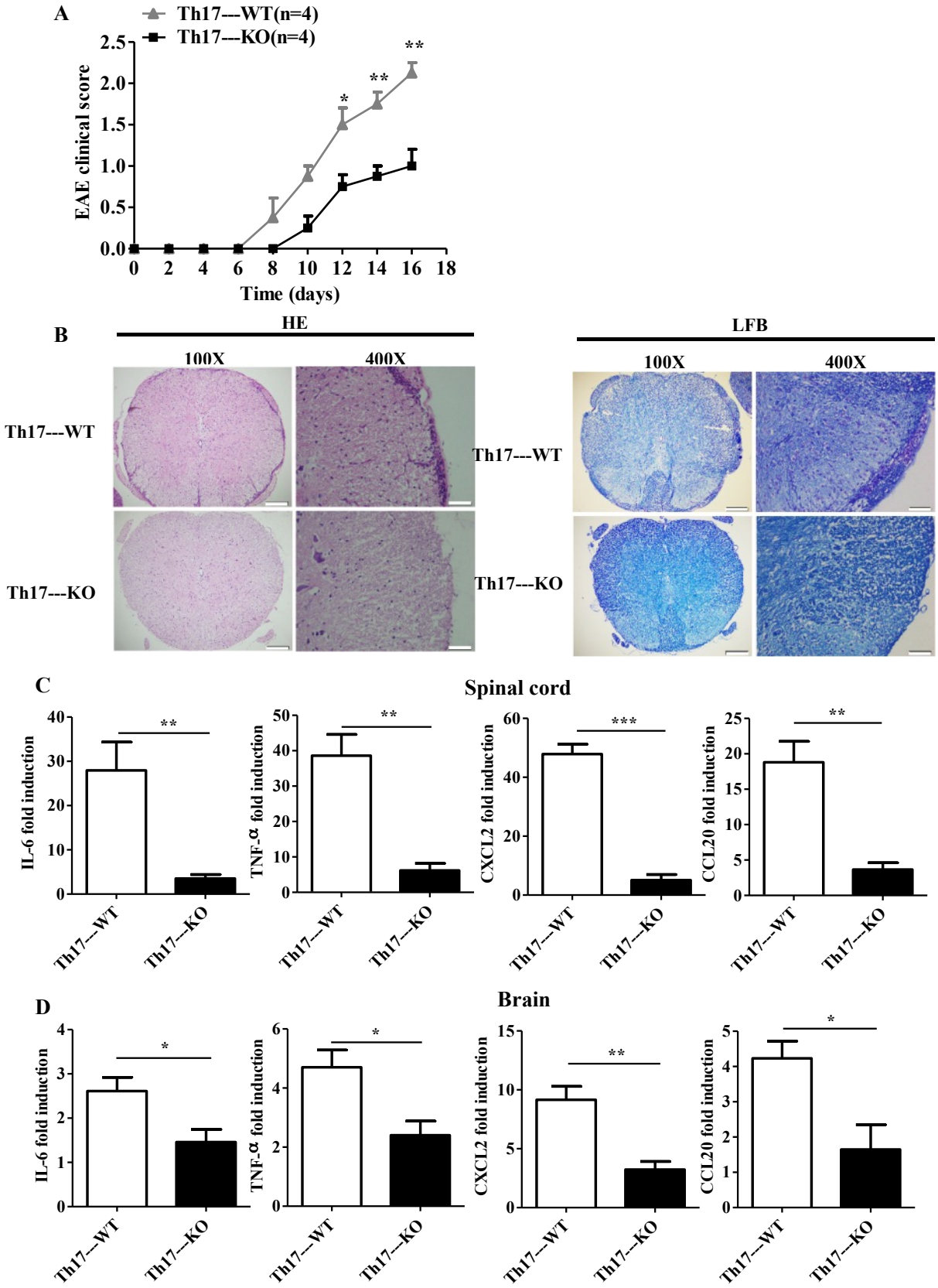
Appendix Figure S1



Appendix Figure S1. NDR1 deficiency does not affect Treg cell production in vivo and in vitro. A, B. WT and *Ndr1-KO* mice were presensitized with 1% TNBS, then were rectally injected with TNBS at a dose of 150 mg/kg body on day 7. Mice were euthanized on day 4, and colons were collected, then colonic propria cells were separated and staining with anti-mouse markers: CD4 and Foxp3, then were analyzed by flow cytometry, showing a summary graph of Treg cell frequency **(A)** and number **(B)**. **C, D.** The mesenteric lymph nodes cells were separated on day 4 and staining with anti-mouse markers: CD4 and Foxp3, then were analyzed by flow cytometry, showing a summary graph of Treg cell frequency **(C)** and number **(D)**.**E, F.** Naïve $CD4^+$ T cells ($CD44^{lo}CD62L^{hi}$) isolated from age- and sex-matched *Ndr1-KO* and control littermate (WT) mice were stimulated for 7d with anti-CD3 and anti-CD28 under Th0 or Treg conditions as described in the Materials and Methods section. Flow cytometry was used to measure the frequency of Foxp3 positive cells, and showing a representative plot **(E)** and a summary graph **(F)**. ns, not significant (unpaired, two-tailed students's t-test). Similar results were obtained in three (**A-D**) or two (**E, F**) independent experiments. Error bar are mean ± SEM values.

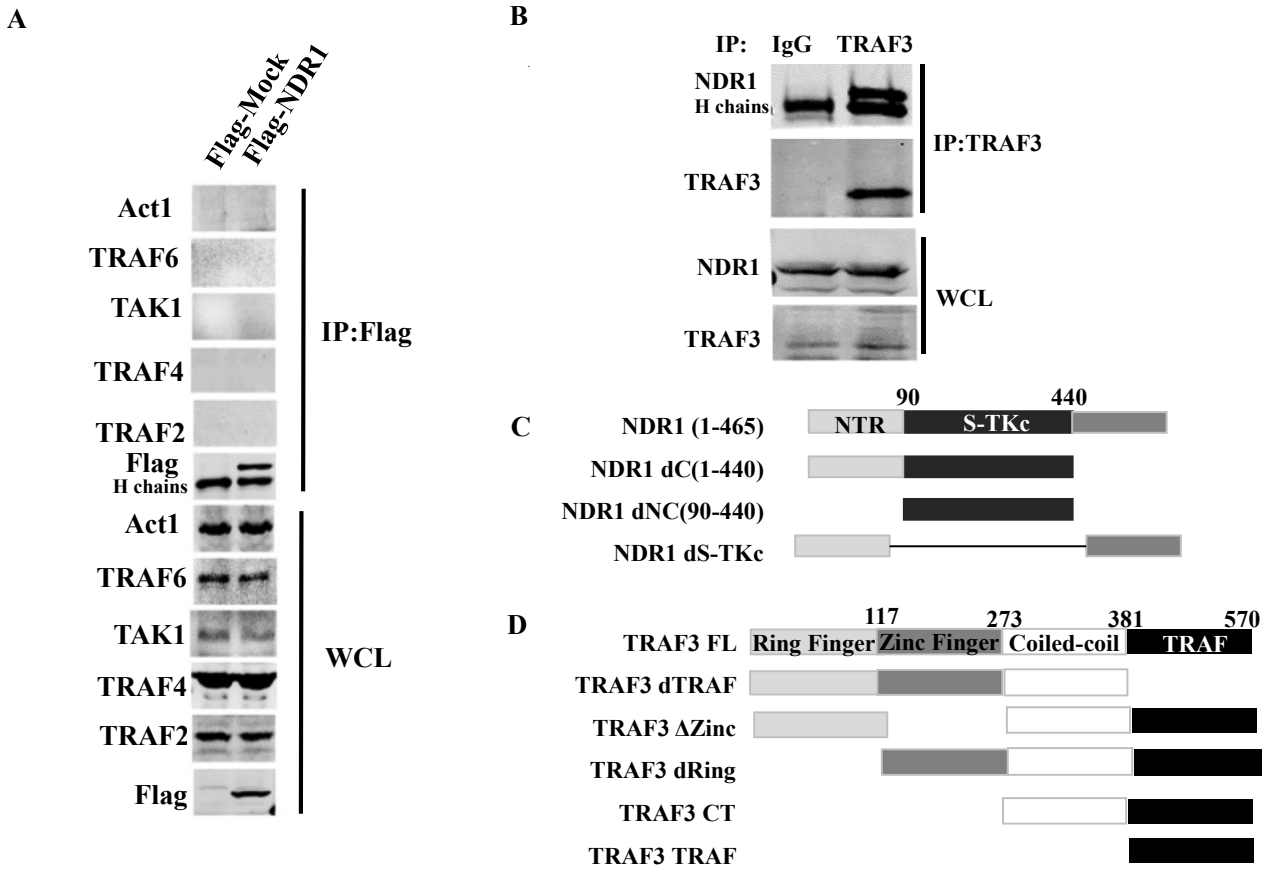
Appendix Figure S2



Appendix Figure S2. NDR1 deficiency restricts EAE by its promotion of IL-17 signaling.

A. EAE clinical scores of WT (n=4) or *Ndr1-KO* (n=4) mice adoptively transferred with MOG-specific Th17 T cells derived from MOG-immunized WT mice. **B.** Histology of the spinal cord was analyzed by hematoxylin and eosin (HE) or luxol fast blue (LFB) staining on day 16 after donor Th17 cell transfer. Scale bars for the left panel, 200 μm; Scale bars for the right panel, 50 μm. **C, D** *IL-6, TNF-α, CXCL2,* and *CCL20* mRNA in the spinal cords **(C)** or in the brains **(D)** were measured by real-time PCR on day16 after donor Th17 cell transfer. *P<0.05 and **P<0.01 (unpaired, two-tailed students's t-test). Error bar are mean ± SEM values.

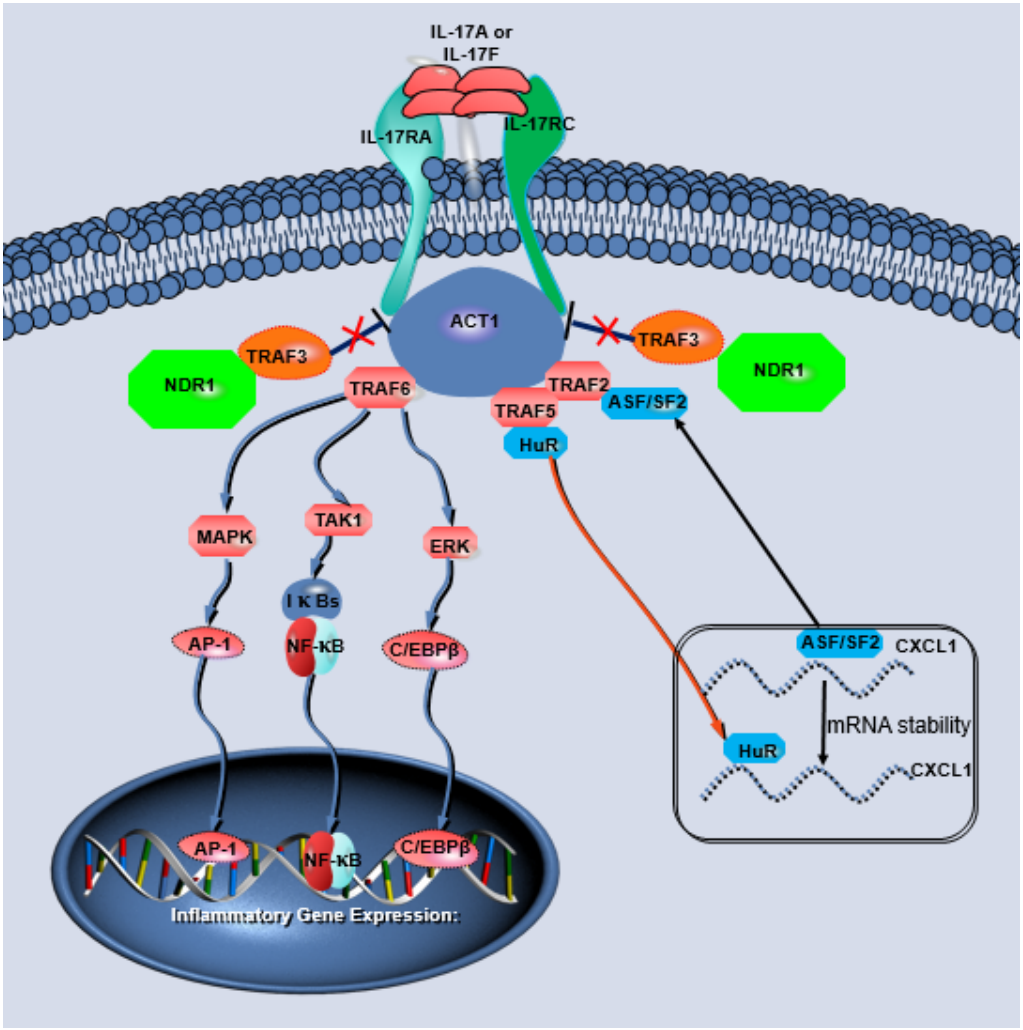
Appendix Figure S3



Appendix Figure S3. NDR1 specifically interacts with TRAF3 but not other events in IL-17 signaling. A. Whole cell lysates of HeLa cells stably expressing Flag-NDR1 or Flag-Mock were immunoprecipitated (IP) with anti-Flag, followed by immunoblotting (IB) with anti-Flag, anti-Act1, anti-TRAF6, anti-TAK, anti-TRAF4 or anti-TRAF2 anitibody. B. Whole cell lysates of WT MEFs were immunoprecipitated (IP) with anti-TRAF3 or control IgG, which was followed by immunoblotting (IB) with anti-TRAF3 or anti-NDR1.**C, D.** Schematic diagram of NDR1 deletion mutants **(C)** or TRAF3 deletion mutants **(D)**.

The data (A,B) are representative of three independent experiments.

Appendix Figure S4



Appendix Figure S4. Mechanism schematic diagram of NDR1 regulating IL-17 signaling. Upon binding of IL-17A or IL-17F to a dimeric receptor complex that consists of IL-17 receptor A (IL-17RA) and IL-17RC, IL-17R subunits recruit the adaptor protein Act1. Act1 mediates the K63-linked ubiquitylation of TRAF6, which results in the downstream activation of TAK1 and canonical NF-κB. In an alternative signaling pathway, Act1 recruits TRAF2 and TRAF5, sequesters the RNA-binding protein ASF/SF2 and recruits HuR to stabilize target mRNA transcripts. TRAF3 inhibits IL-17 signaling by competing with Act1 for binding to IL-17RA. NDR1 interacts with TRAF3 and interferes with the association of TRAF3 and IL-17R, resulting in increased formation of activation complex IL-17R-Act1, which is required for the downstream signaling and production of pro-inflammatory factors.

Appendix Table S1. Basic information of normal controls and UC patients from Xinhua Hospital

	Normal control n=29	UC n=34
Age (years)		
<=30	**0**	**3**
30-50	**2**	**10**
=>50	**27**	**21**
Gender		
Male	**14**	**20**
Female	**15**	**14**
Location		
Colon	**29**	**34**

Appendix Table S2. Primer for real-time PCR

human *gapdh* forward primer	5'-ATTCCACCCATGGCAAATTC-3'
human *gapdh* reverse primer	5'-GGATCTCGCTCCTGCAAGATG-3'
human *il-6* forward primer	5'-ATGAACTCCTTCTCCACAAGCGC-3'
human *il-6* reverse primer	5'-GGGAAGGCAGCAGGCAACAC-3'
human *cxcl2* forward primer	5'-CTCAAGAATGGGCAGAAAGC-3'
human *cxcl2* reverse primer	5'-AAACACATTAGGCGCAATCC-3'
human *ccl20* forward primer	5'-GCGCAAATCCAAAACAGACT-3'
human *ccl20* reverse primer	5'-CAAGTCCAGTGAGGCACAAA-3'
mouse *actin* forward primer	5'- CGTTGACATCCGTAAAGACC -3'
mouse *actin* reverse primer	5'-AACAGTCCGCCTAGAAGCAC -3'
mouse *il-6* forward primer	5'-AGTTGCCTTCTTGGGACTGA-3'
mouse *il-6* reverse primer	5'-TCCACGATTTCCCAGAGAAC-3'
mouse *cxcl1*forward primer	5'-CGCTTCTCTGTGCAGCGCTGCTGCT-3'
mouse *cxcl1* reverse primer	5'- AAGCCTCGCGACCATTCTTGAGTG-3'
mouse *cxcl2* forward primer	5'-CCTGGTTCAGAAAATCATCCA -3'
mouse *cxcl2* reverse primer	5'- CTTCCGTTGAGGGACAGC -3'
mouse *ccl20* forward primer	5'-AACTGGGTGAAAAGGGCTGT-3'
mouse *ccl20* reverse primer	5'-GTCCAATTCCATCCCAAAAA-3'
mouse *TNF-α* forward primer	5'-CTGGGACAGTGACCTGGACT -3'
mouse *TNF-α* reverse primer	5'-GCACCTCAGGGAAGAGTCTG -3'