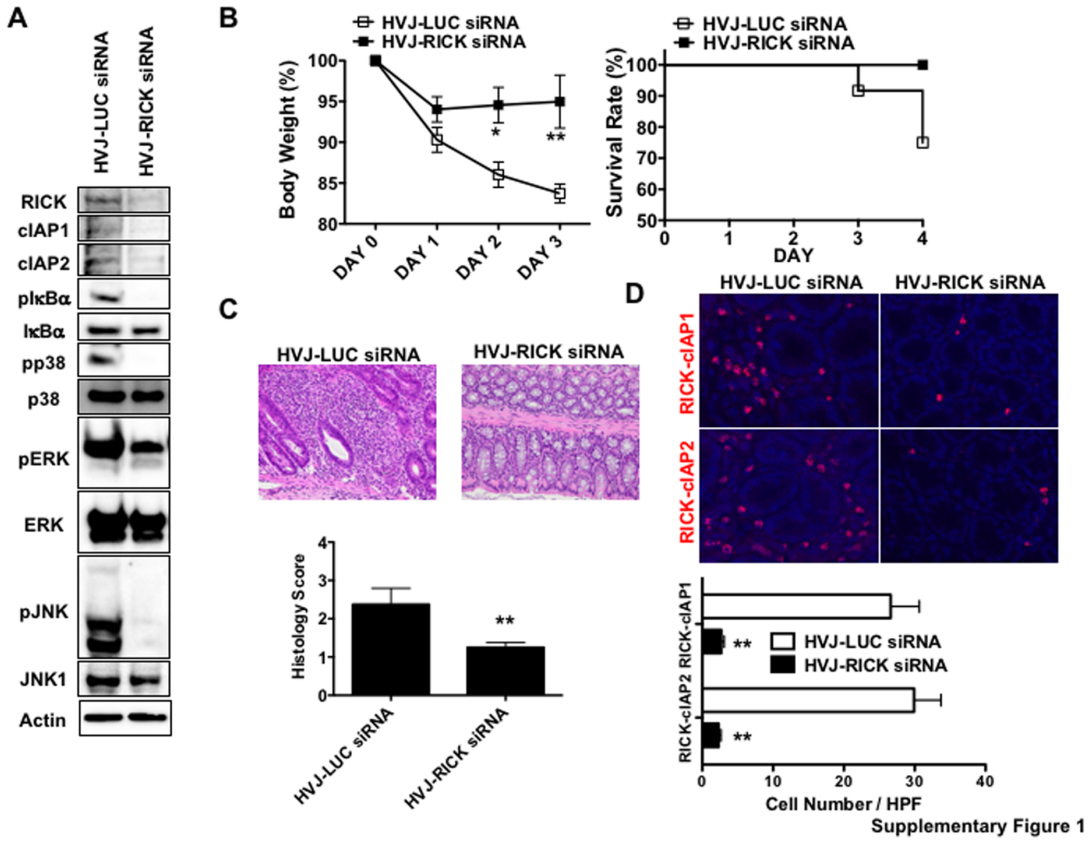
Supplementary Table 1. Clinical characteristics of patients.

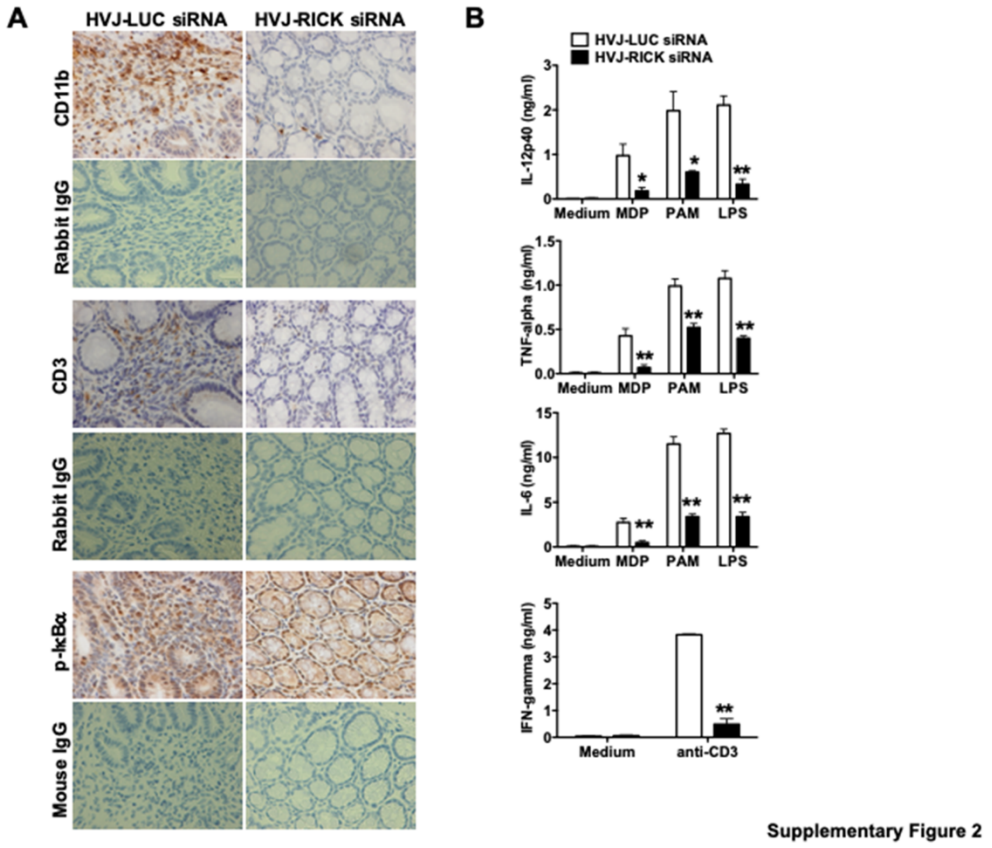
	Crohn's disease	Ulcerative colitis
Patient Number	28	118
Male / Female	17 / 11	62 / 56
Mean Age	37 (19-69)	47 (16-78)
Disease Type (number, %)		
ileal	5 (17.9)	
colonic	3 (10.7)	
ileocolonic	20 (71.4)	
proctitis		24 (20.3)
left sided colitis		37 (31.4)
pancolitis		57 (48.3)
Medication (number, %)		
treatment naive	0	12 (10.2)
5-aminosalicylic acid	26 (92.9)	95 (80.5)
prednisolone	4 (14.3)	20 (16.9)
TNF-alpha inhibitor	18 (64.3)	11 (9.3)
immunomodulator	7 (25.0)	21 (17.8)
calcineurin inhibitor	0	6 (5.1)
Remission / Active	3 / 25	49 / 69



Supplementary Figure 1. Prevention of trinitrobenzene sulfonic acid (TNBS)-colitis by administration of a receptor-interacting serine/threonine kinase (RICK) siRNA-expressing plasmid.

C57BL/10 mice were administered hemagglutinating virus of Japan-envelope (HVJ-E)-encapsulated receptor-interacting serine/threonine kinase (RICK)-siRNA expressing vector or control luciferase (LUC)-siRNA expressing vector via the intra-rectal route on days -2, -1, and 0 and then challenged with intra-rectal trinitrobenzene sulfonic acid (TNBS) on day 0. **(A)** Immuno-blots showing activation of pro-inflammatory signaling pathways in colonic lamina propria mononuclear cells (cLPMCs) in TNBS-treated mice on day 2. Protein lysates were prepared from cLPMCs isolated from the colon of TNBS-treated mice on day 2 and then subjected to immunoblotting. Activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) in the cLPMCs of TNBS-challenged mice were assessed by immunoblotting against the indicated components. **(B)** Changes of body weight in mice (n=6; each group) on day 3.

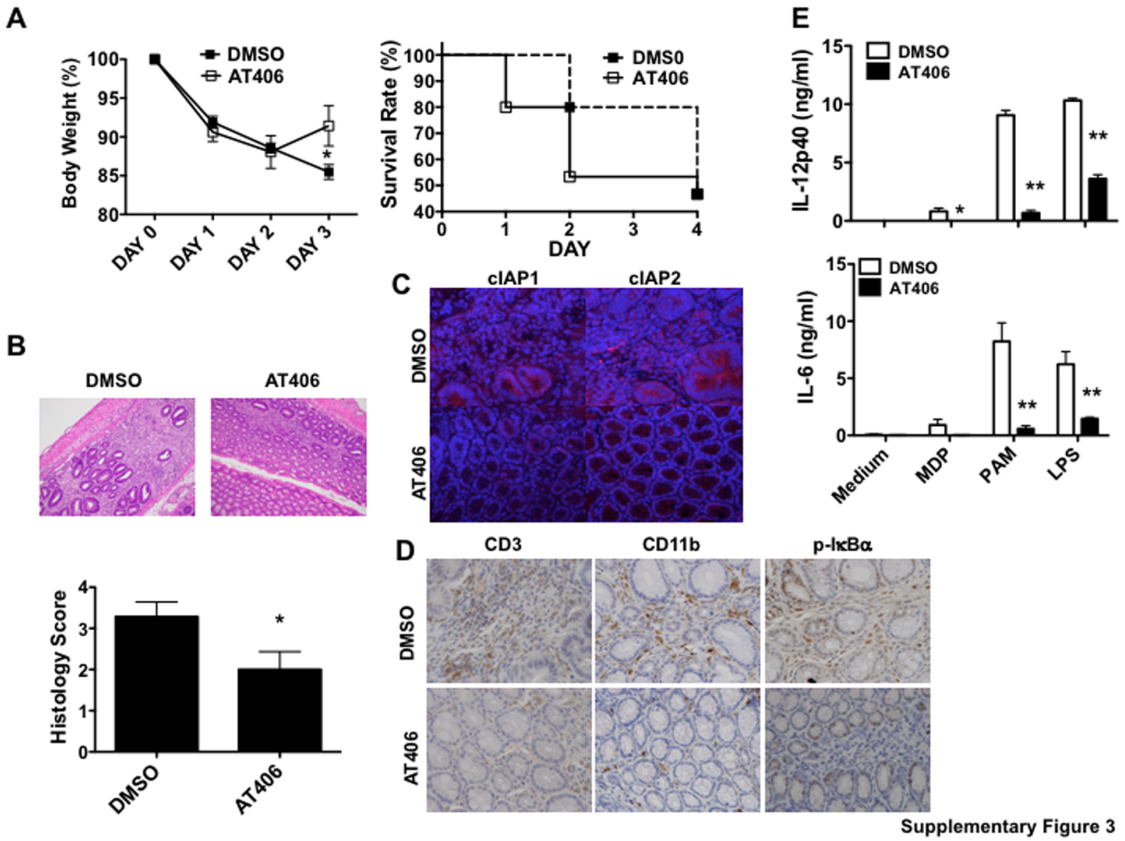
Results are expressed as mean ± SEM. *p<0.05, **p<0.01, as compared with control HVJ-LUC siRNA group. Results shown are the representative one of two independent experiments. Survival rates of TNBS-treated mice. Results shown were obtained from two independent experiments (control HVJ-LUC siRNA group; n=12, HVJ-RICK siRNA group; n=12). **(C)** Hematoxylin and eosin staining of colon tissue obtained from mice on day 4; pathology scores calculated from examination of tissues obtained from mice on day 4; results are expressed as mean ± SEM. Results shown were obtained from pool tissues from two independent experiments (control HVJ-LUC siRNA group; n=9, HVJ-RICK siRNA group; n=12). **p<0.01, as compared with control HVJ-LUC siRNA group. **(D)** The molecular interaction between cellular inhibitor of apoptosis protein 1 (cIAP1)-RICK or cIAP2-RICK in the colon tissue of mice challenged with TNBS on day 2; molecular interaction between cIAP1 or cIAP2 and RICK was visualized as red color; nuclei were stained with DAPI; magnification x1200. The number of cells positive for the interaction between cIAP1 or cIAP2 and RICK were determined by counting in high power fields (HPFs); results are expressed as mean ± SEM. **p<0.01, as compared with control HVJ-LUC siRNA group (n=5, in each group).



Supplementary Figure 2. Production of pro-inflammatory cytokines in C57BL/10 mice treated with intra-rectal administration of trinitrobenzene sulfonic acid (TNBS) and a receptor-interacting serine/threonine kinase (RICK) siRNA-expressing plasmid.

C57BL/10 mice were administered hemagglutinating virus of Japan-envelope (HVJ-E)-encapsulated receptor-interacting serine/threonine kinase (RICK)-siRNA expressing vector or control luciferase (LUC)-siRNA expressing vector via the intra-rectal route on days -2, -1, and 0 and then challenged with intra-rectal trinitrobenzene sulfonic acid (TNBS) on day 0. **(A)** Representative colon tissue stained with anti-CD11b Ab, anti-CD3 Ab, or anti-phospho-IκBα (pIκBα) Ab. Magnification x800. Expression of CD11b, CD3, and pIκBα in the colon tissue from mice challenged with TNBS 4 days before. Mouse IgG or rabbit IgG was used for isotype Ab staining. **(B)** Production of IL-12p40, TNF-α, IL-6, and IFN-γ by colonic lamina propria mononuclear cells (cLPMCs) isolated from TNBS-challenged mice on day 4; cLPMCs ($2x10^6$/ml) were stimulated with MDP, PAM,

LPS, or anti-CD3 mAb for 48 hours after which culture fluids were assayed for cytokine levels by ELISA, as indicated. Results are expressed as mean ± SEM. *$p<0.05$, **$p<0.01$, as compared with control HVJ-LUC siRNA group. Results shown are representative of data obtained in two experiments.

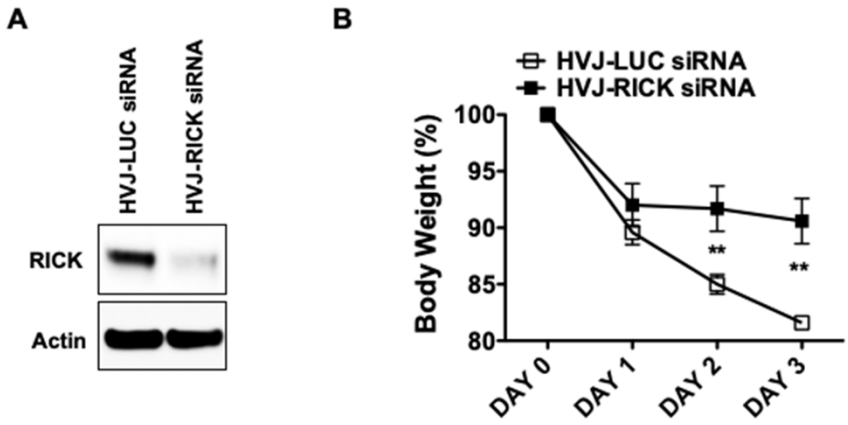


Supplementary Figure 3

Supplementary Figure 3. Attenuation of trinitrobenzene sulfonic acid (TNBS)-colitis by intra-peritoneal administration of AT406, a pan-IAP inhibitor.

C57BL/10 mice were administered AT406 (a pan-IAP inhibitor) or control DMSO via the intra-peritoneal route on days -2, -1, and 0 and then challenged with intra-rectal trinitrobenzene sulfonic acid (TNBS) on day 0. **(A)** Changes of body weight and survival rates in mice (n=15; each group). Results are expressed as mean ± SEM. *p<0.05, as compared with control DMSO group. Results shown are values from pooled data derived from two independent experiments. **(B)** Hematoxylin & eosin staining of colon tissue obtained from mice on day 4; pathology scores calculated from tissue obtained from mice on day 4; results are expressed as mean ± SEM. Results shown are a pool of two independent experiments. *p<0.05, as compared with control DMSO group. **(C)** Expression of cellular inhibitor of apoptosis protein 1 (cIAP1) and cIAP2 was examined by immunofluorescence analysis using colon samples obtained from TNBS-treated mice on day 4 (n=3,

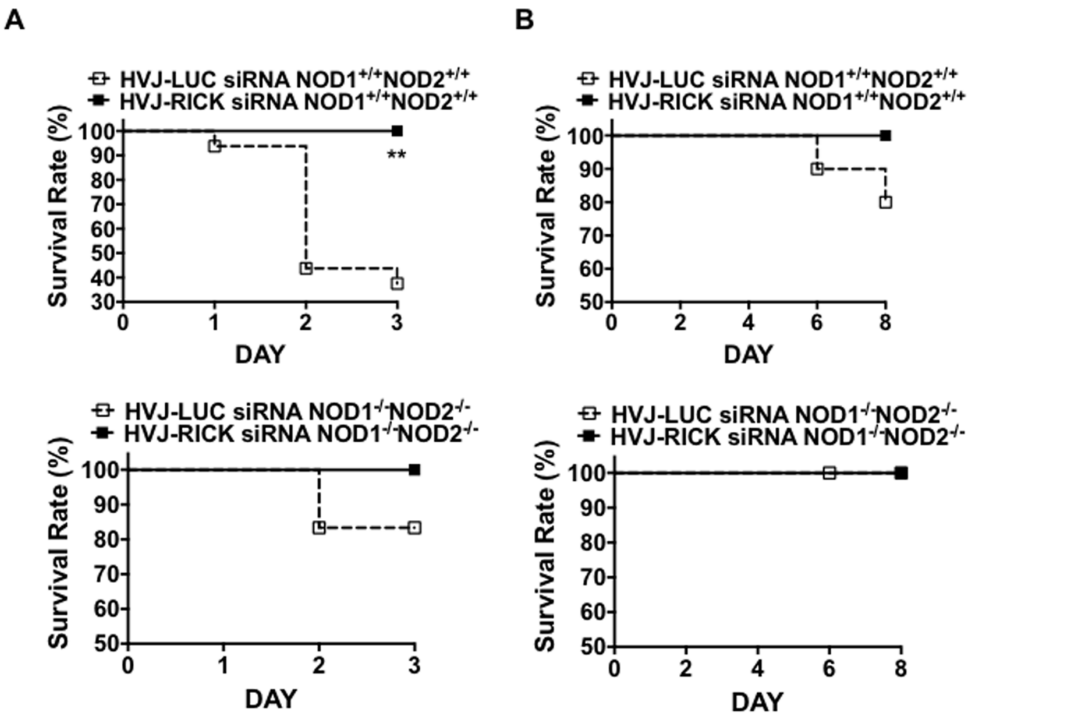
each group). Representative immunofluorescence stained with anti-cIAP1 Ab (red color) or anti-cIAP2 Ab (red color). Nuclei were stained with DAPI, magnification x800. **(D)** Representative colon tissue stained with anti-CD3 Ab, anti-CD11b Ab or anti-phospho-IκBα (pIκBα) Ab. Magnification x800. Expression of CD3, CD11b, and pIκBα in the colon tissue of mice challenged with TNBS on day 4. **(E)** Production of IL-12p40 and IL-6 by mesenteric lymph node (MLN) cells isolated from TNBS-challenged mice on day 4. MLN cells ($2x10^6$/ml) were stimulated with MDP, PAM or LPS for 48 hours after which culture fluids were assayed for cytokine levels by ELISA, as indicated. Results are expressed as mean $\pm$ SEM. *$p<0.05$, **$p<0.01$, as compared with DMSO group. Results shown are representative of two experiments.



Supplementary Figure 4

Supplementary Figure 4. Prevention of trinitrobenzene sulfonic acid (TNBS)-colitis by administration of a receptor-interacting serine/threonine kinase (RICK) siRNA-expressing plasmid in NOD2-deficient mice.

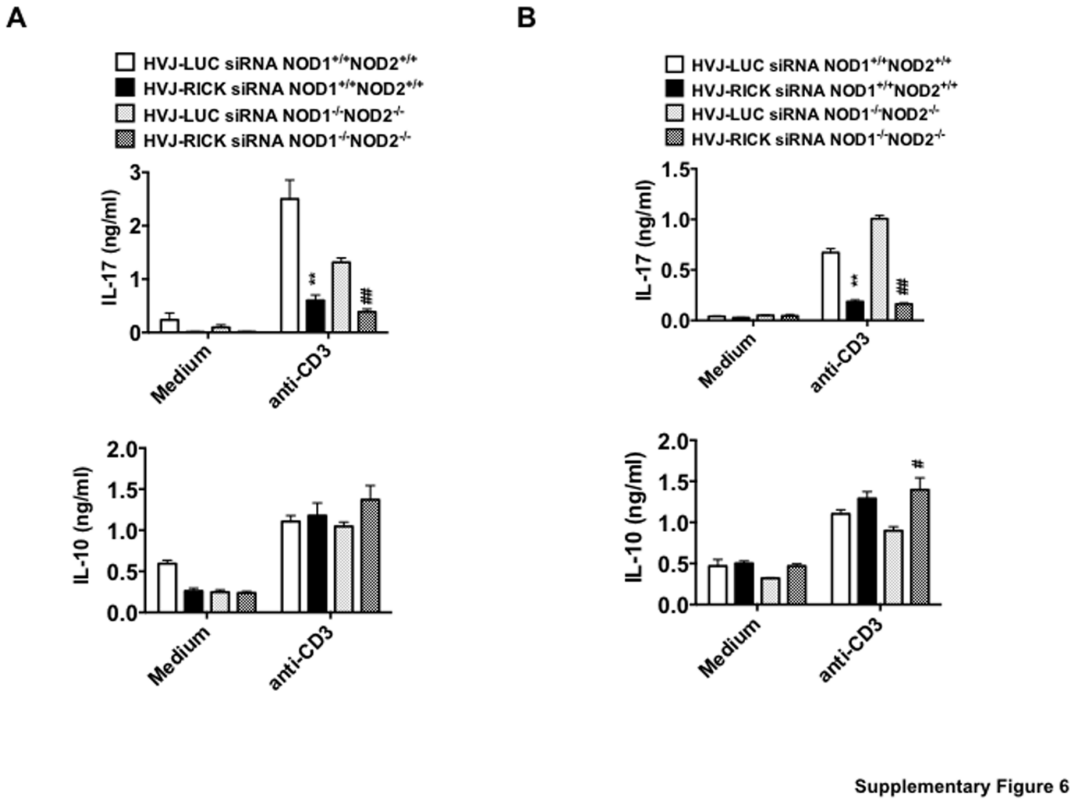
NOD2-deficient C57BL/6 mice were administered hemagglutinating virus of Japan-envelope (HVJ-E)-encapsulated receptor-interacting serine/threonine kinase (RICK) siRNA expressing vector or control luciferase (LUC) siRNA expressing vector via the intra-rectal route as described in Figure 1. **(A)** Expression of RICK in colonic lamina propria mononuclear cells (cLPMCs) in trinitrobenzene sulfonic acid (TNBS)-treated mice on day 3. Protein lysates were prepared from cLPMCs isolated from the colon of TNBS-treated mice on day 3 and then subjected to immunoblotting. **(B)** Changes of body weight in mice (n=5; each group). Results are expressed as mean $\pm$ SEM. **$p<0.01$, as compared with HVJ-LUC siRNA group.



Supplementary Figure 5. Survival rates of trinitrobenzene sulfonic acid or dextran sodium sulfate-challenged mice.

(A) NOD1 and NOD2-intact C57BL/6 mice ($NOD1^{+/+}NOD2^{+/+}$ mice) and NOD1 and NOD2-double deficient mice ($NOD1^{-/-}NOD2^{-/-}$ mice) were administered hemagglutinating virus of Japan-envelope (HVJ-E)-encapsulated receptor-interacting serine/threonine kinase (RICK)-siRNA expressing vector or control luciferase (LUC)-siRNA expressing vector via the intra-rectal route on days -2, -1, and 0 and then challenged with intra-rectal trinitrobenzene sulfonic acid (TNBS) on day 0 and 2. Survival rates are shown ($NOD1^{+/+}NOD2^{+/+}$ LUC-siRNA; n=16, $NOD1^{+/+}NOD2^{+/+}$ RICK-siRNA; n=10, $NOD1^{-/-}NOD2^{-/-}$ LUC-siRNA; n=12, $NOD1^{-/-}NOD2^{-/-}$ RICK-siRNA; n=7). **p<0.01, as compared with $NOD1^{+/+}NOD2^{+/+}$ HVJ-LUC siRNA group. **(B)** $NOD1^{+/+}NOD2^{+/+}$ mice and $NOD1^{-/-}NOD2^{-/-}$ mice were treated with dextran sodium sulfate (DSS, 4%) in the drinking water from day 0 to day 6. Mice were administered HVJ-E-encapsulated RICK-siRNA expressing vector or control LUC-siRNA expressing vector via the intra-rectal

route on days 0, 1, and 2. Survival rates are shown ($NOD1^{+/+}NOD2^{+/+}$ LUC-siRNA; n=10, $NOD1^{+/+}NOD2^{+/+}$ RICK-siRNA; n=10, $NOD1^{-/-}NOD2^{-/-}$ LUC-siRNA; n=10, $NOD1^{-/-}NOD2^{-/-}$ RICK-siRNA; n=8).

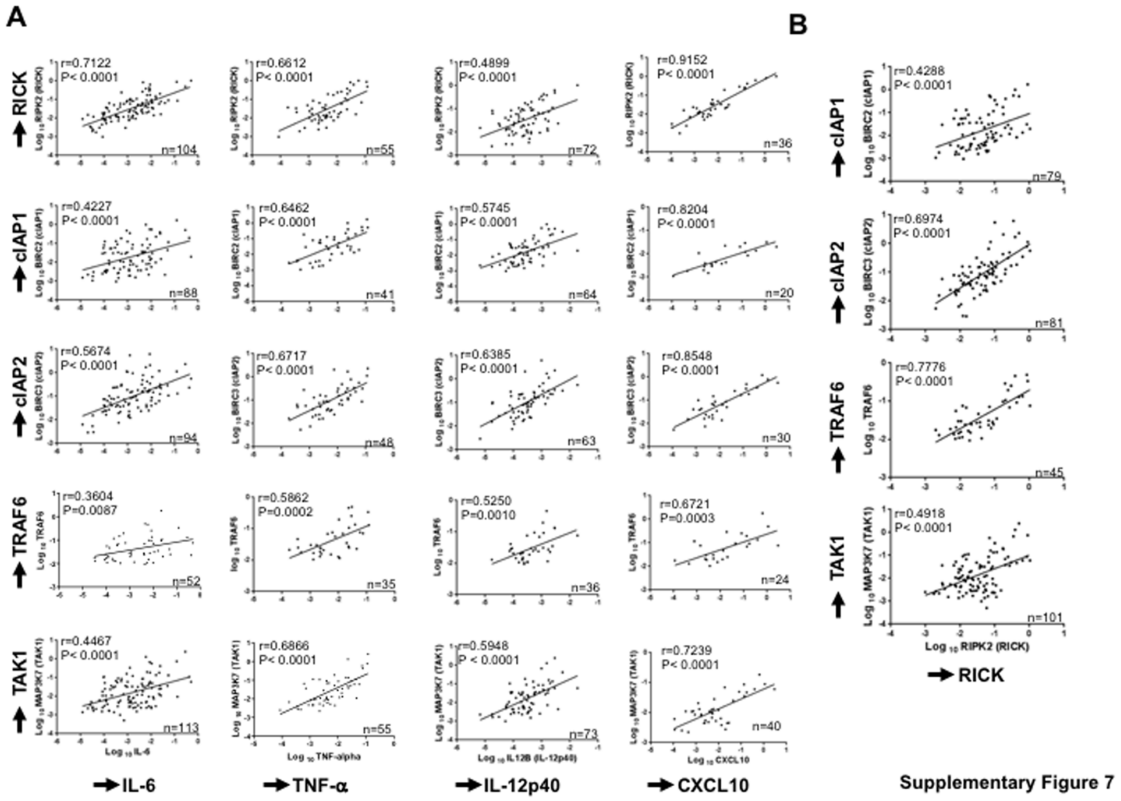


Supplementary Figure 6

Supplementary Figure 6. Production of IL-10 and IL-17 by colonic lamina propria mononuclear cells isolated from trinitrobenzene sulfonic acid or dextran sodium sulfate-challenged mice.

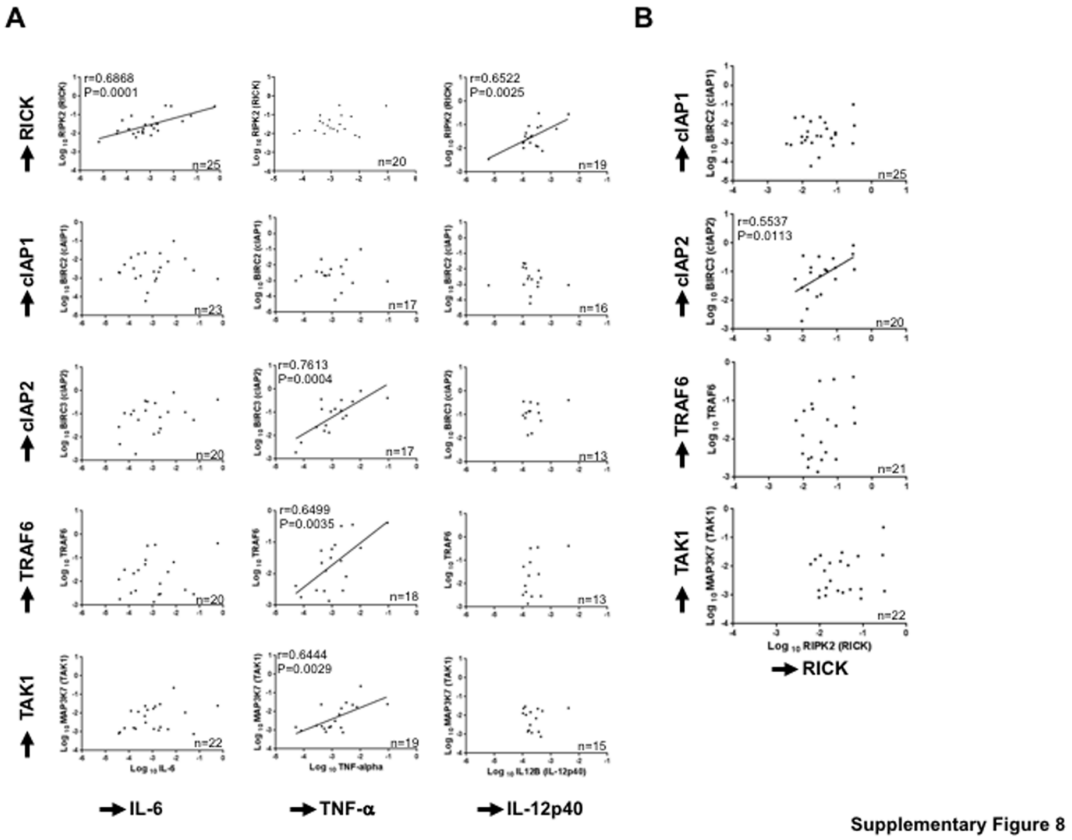
(A) NOD1 and NOD2-intact C57BL/6 mice ($NOD1^{+/+}NOD2^{+/+}$ mice) and NOD1 and NOD2-double deficient mice ($NOD1^{-/-}NOD2^{-/-}$ mice) were administered hemagglutinating virus of Japan-envelope (HVJ-E)-encapsulated receptor-interacting serine/threonine kinase (RICK)-siRNA expressing vector or control luciferase (LUC)-siRNA expressing vector via the intra-rectal route on days -2, -1, and 0 and then challenged with intra-rectal trinitrobenzene sulfonic acid (TNBS) on day 0 and 2. **(B)** $NOD1^{+/+}NOD2^{+/+}$ mice and $NOD1^{-/-}NOD2^{-/-}$ mice were treated with dextran sodium sulfate (DSS, 4%) in the drinking water from day 0 to day 6. Mice were administered HVJ-E-encapsulated RICK-siRNA expressing vector or control LUC-siRNA expressing vector via the intra-rectal route on days 0, 1, and 2. Production of IL-10 and IL-17 by colonic lamina propria mononuclear cells (cLPMCs) isolated from TNBS or DSS-challenged mice; cLPMCs ($1x10^6$/ml)

were stimulated with anti-CD3 mAb for 48 hours after which culture fluids were assayed for cytokine levels by ELISA, as indicated. Results are expressed as mean ± SEM. **$p<0.01$, as compared with $NOD1^{+/+}NOD2^{+/+}$ HVJ-LUC siRNA group. $^{\#}p<0.05$, $^{\#\#}p<0.01$, as compared with $NOD1^{-/-}NOD2^{-/-}$ HVJ-LUC siRNA group.



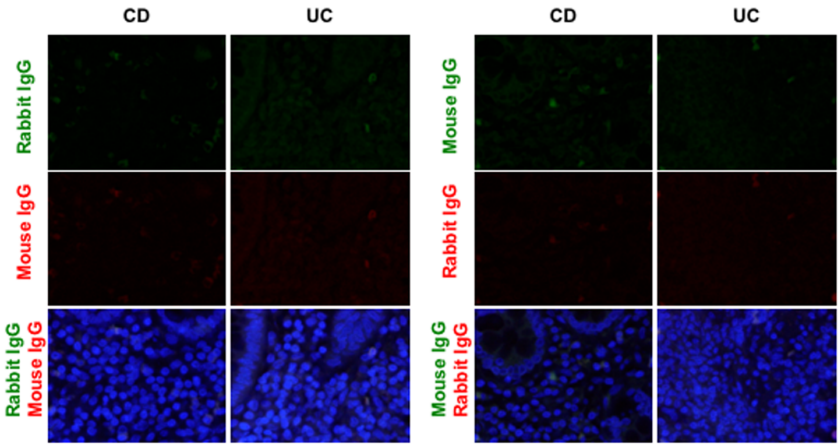
Supplementary Figure 7. Expression of pro-inflammatory cytokines is positively correlated to that of receptor-interacting serine/threonine kinase (RICK) and its associated molecules in patients with ulcerative colitis.

(A) Expression of pro-inflammatory cytokines and chemokine (IL-6, TNF-α, IL-12p40, and CXCL10) was measured by qPCR as described in Figure 3. Expression of receptor-interacting serine/threonine kinase (RICK), cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, TNF receptor-associated factor 6 (TRAF6), and TGF-β-activated kinase 1 (TAK1) was measured as described in Figure 3. Correlation analysis between each pro-inflammatory cytokine and RICK-associated molecules is shown. **(B)** Correlation analysis between RICK and cIAP1, cIAP2, TRAF6, and TAK1 is shown. The number associated with each data set shows the number of patients studied. Each dot represents a value of each patient sample. P values and Pearson r values are shown.



Supplementary Figure 8. Expression of pro-inflammatory cytokines is positively correlated to that of receptor-interacting serine/threonine kinase (RICK) and its associated molecules in patients with Crohn's disease.

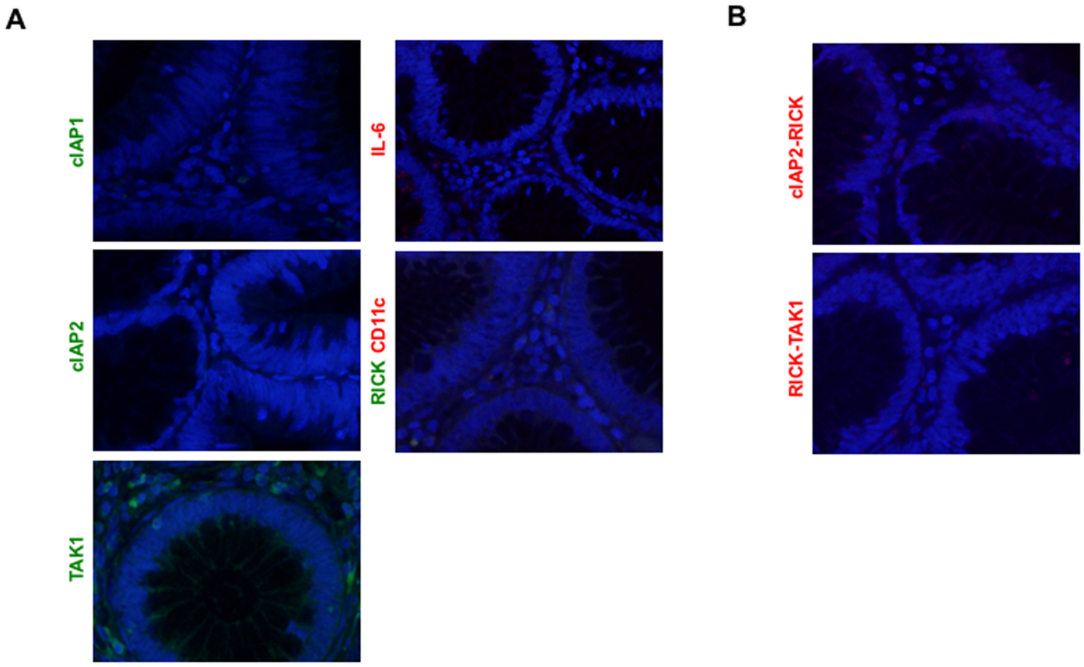
(A) Expression of pro-inflammatory cytokines (IL-6, TNF-α, IL-12p40) was measured by qPCR as described in Figure 3. Expression of receptor-interacting serine/threonine kinase (RICK), cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, TNF receptor-associated factor 6 (TRAF6), and TGF-β-activated kinase 1 (TAK1) was measured as described in Figure 3. Correlation analysis between each pro-inflammatory cytokine and RICK-associated molecules is shown. **(B)** Correlation analysis between RICK and cIAP1, cIAP2, TRAF6, and TAK1 is shown. The number associated with each data set shows the number of patients studied. Each dot represents a value of each patient sample. P values and Pearson r values are shown.



Supplementary Figure 9

Supplementary Figure 9. Isotype control antibody staining in surgical specimens obtained from Crohn's disease and ulcerative colitis patients.

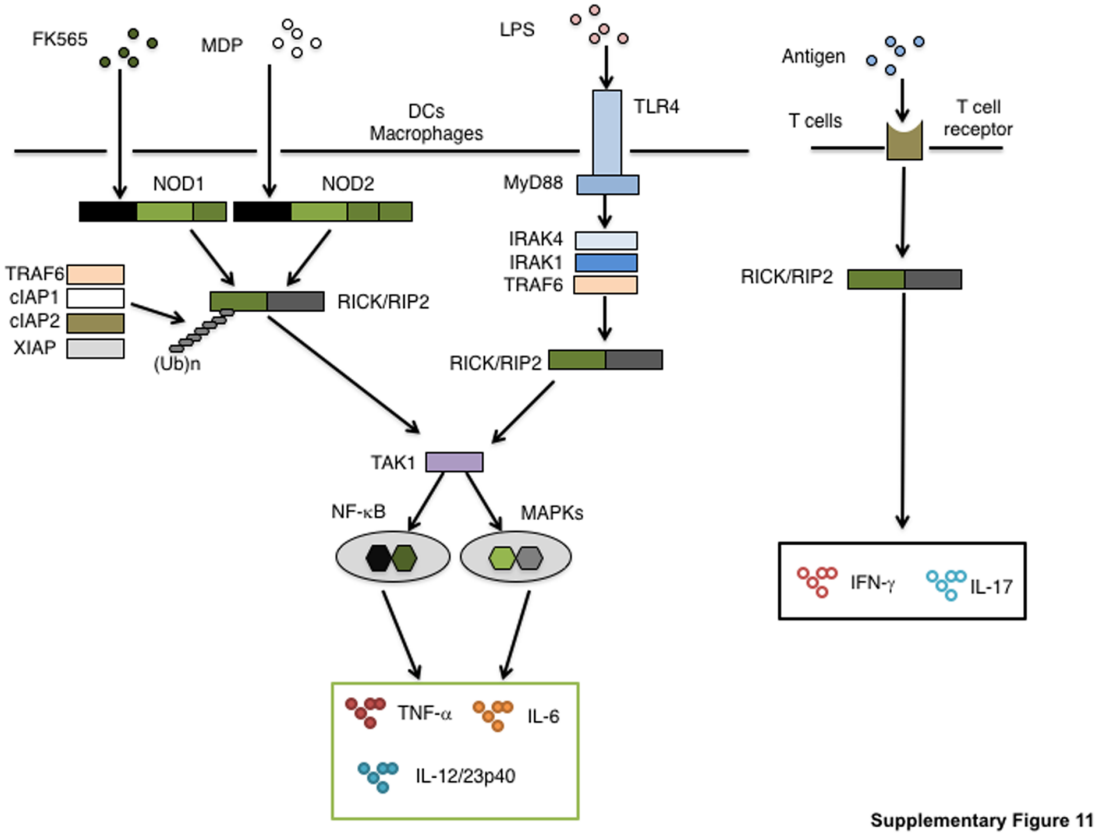
Surgical specimens obtained from Crohn's disease (CD) and ulcerative colitis (UC) patients were incubated with mouse IgG or rabbit IgG followed by the incubation with Alexa 488 or Alexa 546-conjugated anti-mouse IgG or rabbit IgG. The results shown are the representative one of two samples. Nuclei were stained with DAPI, magnification x1200.



Supplementary Figure 10

Supplementary Figure 10. Expression of IL-6, receptor-interacting serine/threonine kinase (RICK), cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, TGF-β-activated kinase 1 (TAK1) in control samples.

Non-tumorous portions of colorectal tumors were used as control samples. **(A)** Control samples were stained with Abs against cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, TGF-β-activated kinase 1 (TAK1), IL-6, receptor-interacting serine/threonine kinase (RICK), and CD11c as described in Figure 5 and Figure 6. **(B)** The molecular interaction between cIAP2 and RICK or RICK and TAK1 was analyzed by proximity ligation assays as described in Figure 7. The results shown are the representative one of two samples. Nuclei were stained with DAPI, magnification x1200.



Supplementary Figure 11. NOD1 and NOD2-independent RICK activation in experimental colitis and human inflammatory bowel disease.

See text for explanation. cIAPs, cellular inhibitor of apoptosis proteins; DC, dendritic cell; IRAK, interleukin-1 receptor-associated kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MDP, muramyl dipeptide; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; NOD, nucleotide-binding oligomerization domain; RICK, receptor-interacting serine/threonine kinase; TAK1, TGF-β-activated kinase 1; TLR, Toll-like receptor; TRAF6, TNF receptor-associated factor 6; XIAP, X-linked inhibitor of apoptosis protein.