SUPPLEMENTARY METHODS

Phylum level qPCR. DNA was extracted according to the indicated method by using a stool DNA mini kit (GeneAll, Seoul, Korea). The supernatant of 100 mg of stool in PBS was collected for DNA isolation after homogenization and centrifugation. The isolated DNA was used as a template and genes for 16S rRNA were amplified using two bacteria phylum specific primers (*Bacteroidetes*, 5'-GTT TAA TTC GAT GAT ACG CGA G-3', 5'-TTA ASC CGA CAC CTC ACG G-3'; *Firmicutes*, 5'-GGA GYA TGT GGT TTA ATT CGA AGC A-3', 5'-AGC TGA CGA CAA CCA TGC AC-3'; *Actinobacteria*, 5'-TGT AGC GGT GGA ATG CGC-3', 5'-AAT TAA GCC ACA TGC TCC GCT-3'; *Verrucomicrobia*, *5'*-TCA KGT CAG TAT GGC CCT TAT-3', 5'-CAG TTT TYA GGA TTT CCT CCG CC-3'; Alpha-proteobacteria, 5'-CIA GTG TAG AGG TGA AAT T-3', 5'-CCC CGT CAA TTC CTT TGA GTT-3'; Gamma-proteobacteria, 5'-TCG TCA GCT CGT GTY GTG A-3', 5'-CGT AAG GGC CAT GAT G-3'; *Beta-proteobacteria*, 5'-AAC GCG AAA AAC CTT ACC TAC C-3', 5'-TGC CCT TTC GTA GCA ACT AGT G-3'; *Epsilon-proteobacteria*, 5'-TAG GCT TGA CAT TGA TAG AAT C-3', 5'-CTT ACG AAG GCA GTC TCC TTA-3').

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. A. CMT93 mouse cells were treated with human EGF (50 ng/mL) for 24 h. Cellular lysate protein was assessed by using western blot analysis. **B-C**. Six-week-old female mice were pretreated twice with vehicle, EcN, or EGF-EcN over 7 days (n = 12–15). The mice were then exposed to 3% DSS for 5 days to induce colitis. The 16S rRNA of the gut microbiota was analyzed at the phylum level on the 10^{th} day after DSS treatment (B). Based on each phylum level, the F/B ratio was compared between groups (C). Results are shown as mean values ± SEM and different letters in the graph represent a significant difference between groups ($p < 0.05$ using one-way ANOVA with the Newman-Keuls *post hoc* test).

Figure S2. Actions of post-injury treatment with EGF-secreting EcN in DSS-induced colitis. Six-week-old female mice were treated twice with vehicle or EGF-secreting EcN after DSS exposure (n = 5). 7 **A.** Schematic overview of the EGF-secreting EcN treatment after DSS-induced colitis. **B**. Mouse body weight was monitored at indicated times after DSS exposure. The asterisks in the graph represent significant differences from mass changes in DSS treatment group at each time point ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ using two-tailed unpaired Student's *t*-test). **C-D.** Changes in colon length were measured on the 13^{th} day after DSS treatment. The asterisks in box-and-whisker plot (min to max) (D) represent significant differences between two groups ($^{*}p < 0.05$ using two-tailed unpaired Student's *t*-test). **E.** Representative hematoxylin/eosin staining of the intestinal lesions observed under a microscope (100× magnification); scale bar, 100 μm. **F–G.** Histopathological scores (F) and ulcer area levels (G) compared between the two groups. Significant difference from the DSS alone-treatment group indicated by asterisks (F and G, * $p <$

0.05, ** $p < 0.01$ using two-tailed unpaired Student's *t*-test).

Figure S3. Effects of EcN on murine gut barrier-linked markers. Six-week-old female mice were treated twice with vehicle or EcN (10^9 cfu) over 7 days (n = 12–15). **A.** Gut epithelia stained for SOX-9 (A), p-EGFR (B), and goblet cell/mucin production (C) (original magnification 400×; scale bar(s), 100μm). A quantitative comparison is shown in the right graph. The asterisks in box-and-whisker plot (min to max) represent significant differences between two groups ($^{*} p < 0.05$, $^{**} p < 0.01$, $^{***} p < 0.001$ using two-tailed unpaired Student's *t*-test).

Figure S4. Involvement of EGFR signaling in recombinant bacteria-mediated protection. Eight-week-old female C57BL/6 mice were treated twice with 3% DSS after oral gavages with 1 × 10^9 EcN or EGF-EcN. Mice were then injected (I.P.) with EGFR inhibitor (1 mg AG1478/mouse, Selleckchem, Houston, TX, USA) twice at two-day intervals before and after the last day of DSS exposure. **A**. Histopathological scores. Results are shown as mean values ± SEM and asterisks representing significant differences between two groups ($^{*} p < 0.05$, $^{**} p < 0.01$, $^{***} p < 0.001$ using two-tailed unpaired Student's *t*-test). **B**. Ulcer area. The asterisks in box-and-whisker plot (min to max) represent significant differences between two groups ($^{*} p < 0.05$, $^{**} p < 0.01$, $^{***} p < 0.001$ using two-tailed unpaired Student's *t*-test).

Figure S5. Effects of post-injury treatment with EGF-EcN on NSAID-induced ulcerative injuries. Ten-week-old male mice (n = 12) were treated with 30 mg/kg of indomethacin *via* gavage and then twice inoculated with EcN and EGF-EcN (10^9 cfu) over 48 h. **A.** Tissues were observed in "Swiss rolls" of small intestines and examined under a stereoscopic microscope. The white

arrows indicate hemorrhages along the line of intestines. **B and C.** Tissue analysis showing H&E patterns (B, the upper panel), pathological severity score (B, the lower graph), and ulcer area (C).

Fig. S1

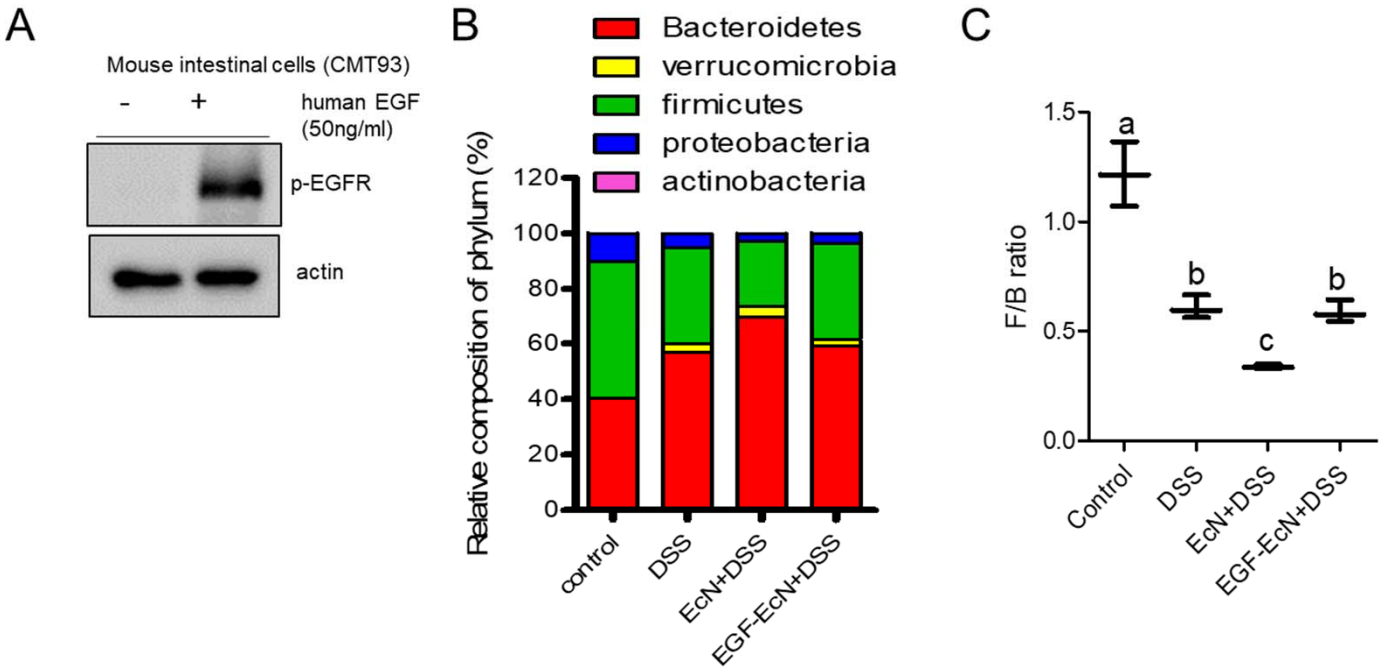

A
Mouse intestinal cells (CMT93)
-
+
human EGF
(50ng/ml)
p-EGFR
actin
B
Bacteroidetes
verrucomicrobia
firmicutes
proteobacteria
actinobacteria
Relative composition of phylum (%)
120
100
80
60
40
20
0
control
DSS
EcN+DSS
EGF-EcN+DSS
C
F/B ratio
1.5
1.0
0.5
0.0
a
b
c
b
Control
DSS
EcN+DSS
EGF-EcN+DSS

Fig. S2

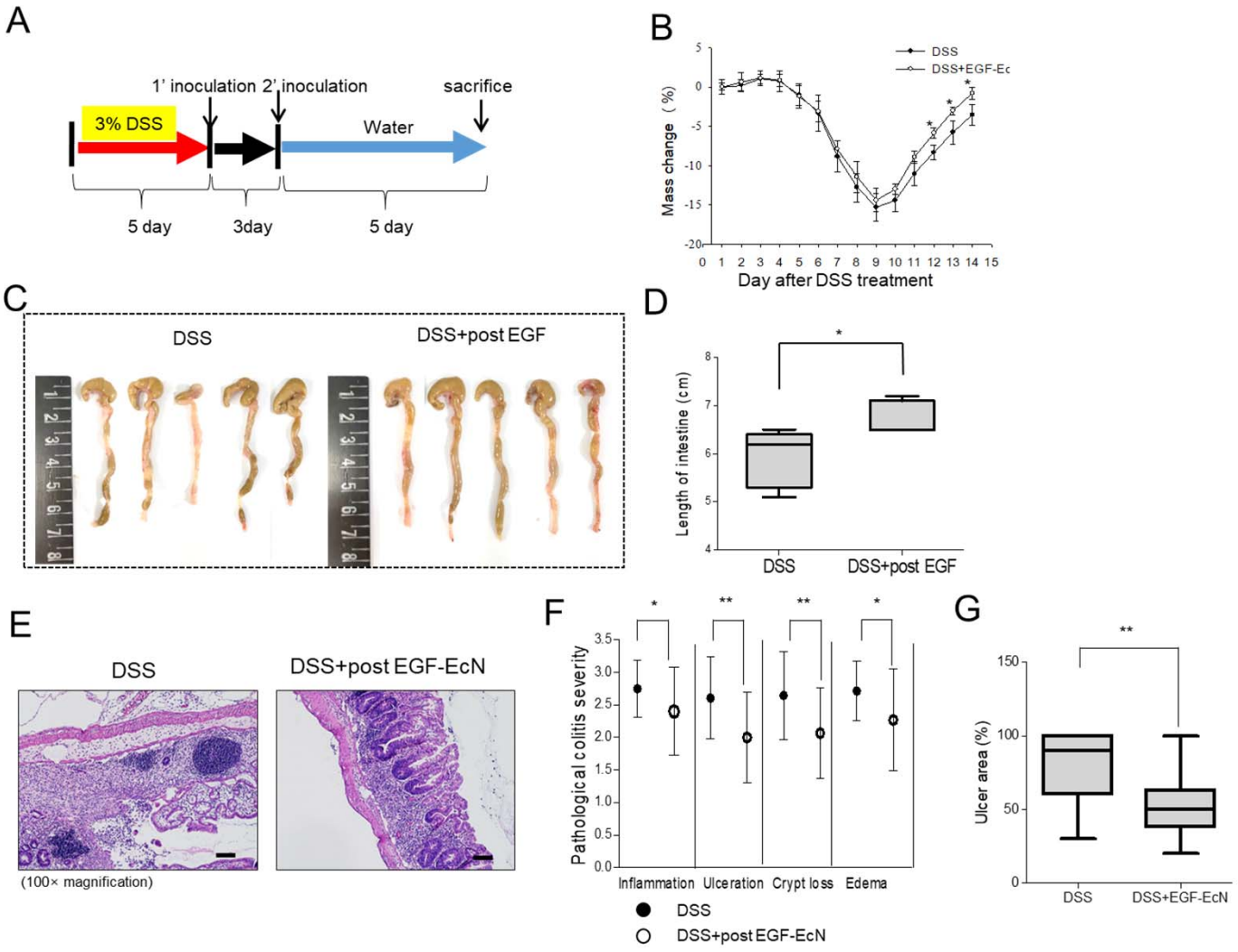

A
1' inoculation
2' inoculation
sacrifice
3% DSS
Water
5 day
3day
5 day
B
DSS
DSS+EGF-Ec
Mass change (%)
Day after DSS treatment
C
DSS
DSS+post EGF
D
Length of intestine (cm)
DSS
DSS+post EGF
E
DSS
DSS+post EGF-EcN
(100× magnification)
F
Pathological colitis severity
Inflammation
Ulceration
Crypt loss
Edema
DSS
DSS+post EGF-EcN
G
Ulcer area (%)
DSS
DSS+EGF-EcN

Fig. S3

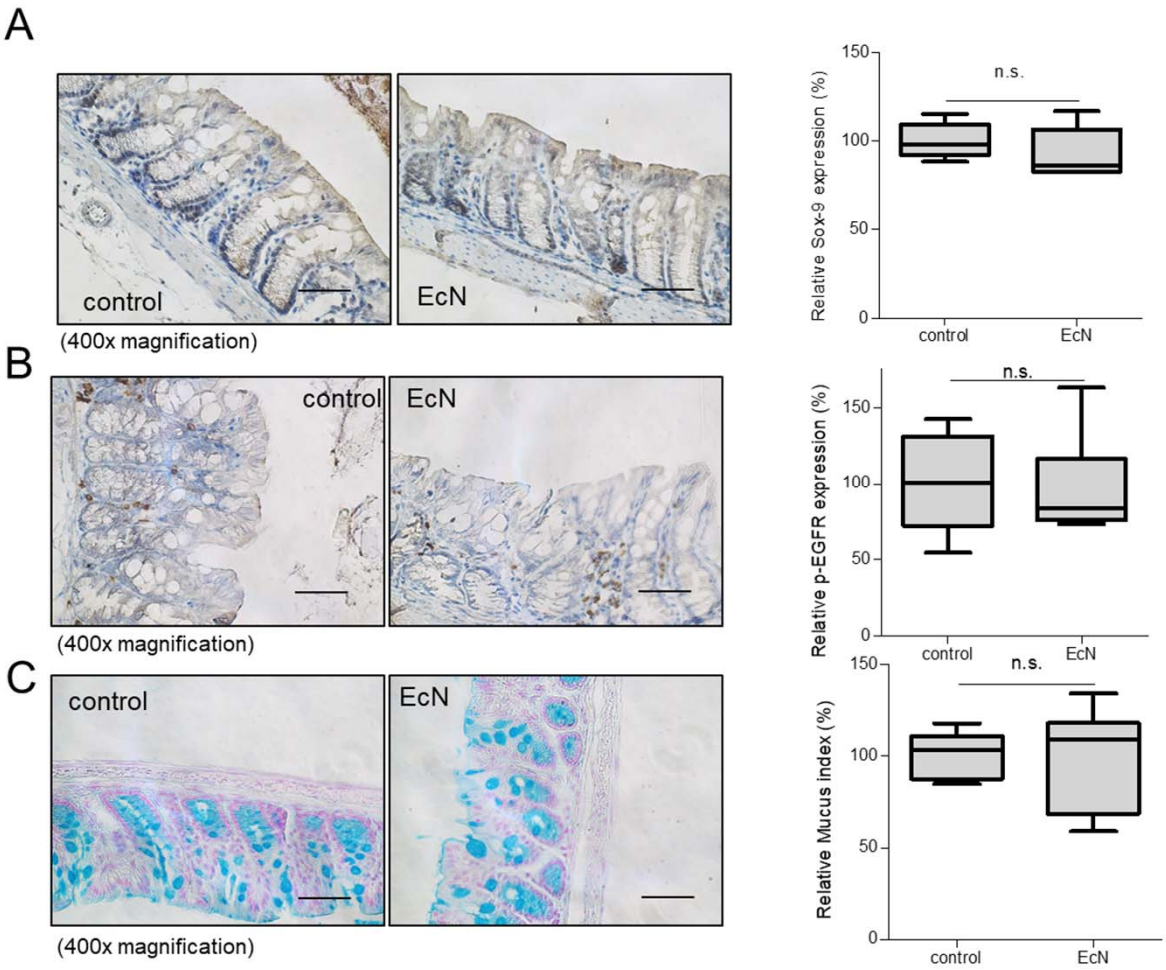

A
control
EcN
(400x magnification)
B
control
EcN
(400x magnification)
C
control
EcN
(400x magnification)
Relative Sox-9 expression (%)
n.s.
150
100
50
0
control
EcN
Relative p-EGFR expression (%)
n.s.
150
100
50
0
control
EcN
Relative Mucus index (%)
n.s.
150
100
50
0
control
EcN

Fig. S4

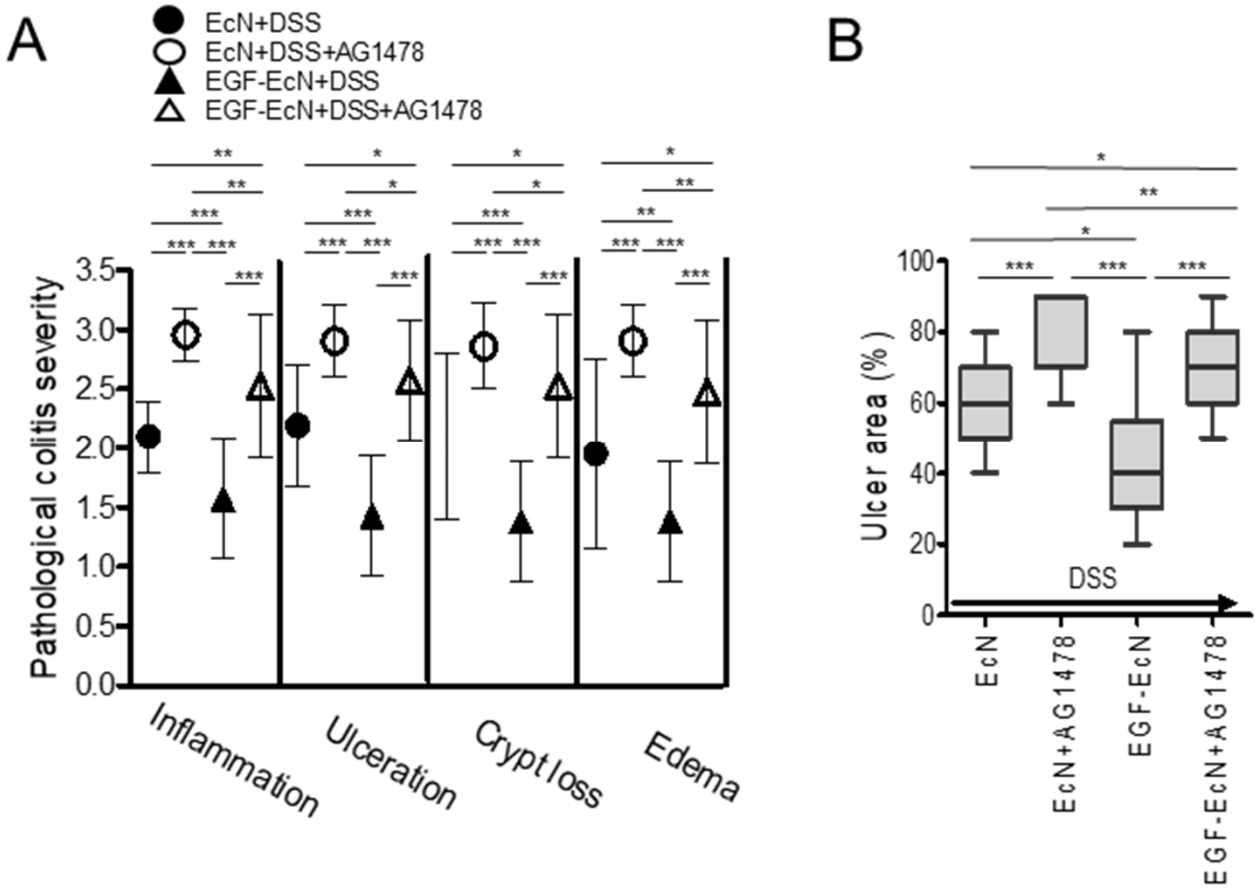
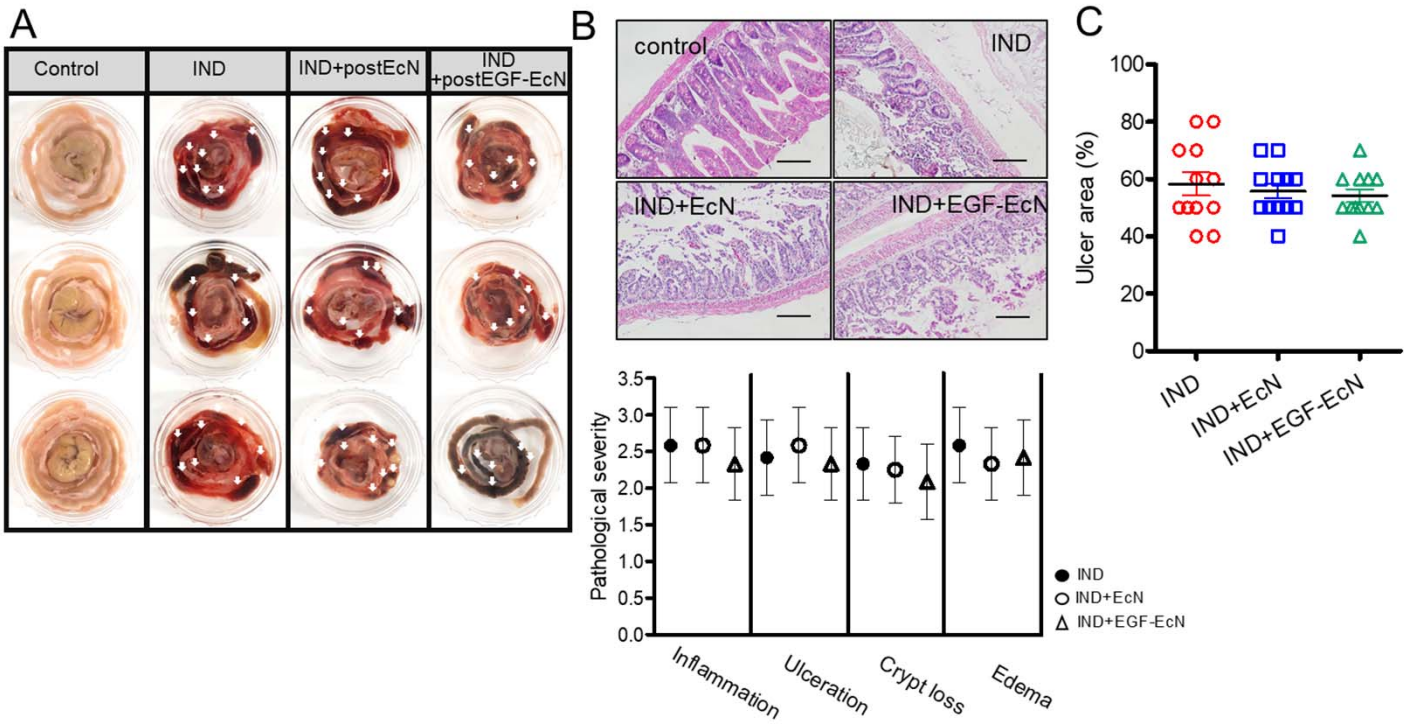

A
EcN+DSS
EcN+DSS+AG1478
EGF-EcN+DSS
EGF-EcN+DSS+AG1478
Pathological colitis severity
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Inflammation
Ulceration
Crypt loss
Edema
B
Ulcer area (%)
100
80
60
40
20
0
DSS
EcN
EcN+AG1478
EGF-EcN
EGF-EcN+AG1478

Fig. S5


A
Control
IND
IND+postEcN
IND +postEGF-EcN
B
control
IND
IND+EcN
IND+EGF-EcN
Pathological severity
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Inflammation
Ulceration
Crypt loss
Edema
IND
IND+EcN
IND+EGF-EcN
C
Ulcer area (%)
100
80
60
40
20
0
IND
IND+EcN
IND+EGF-EcN