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Supplemental Figures

Figure S1. Generation of intestinal epithelial-specific STAT5 knock out mice ($STAT5^{IEC\ KO}$).
A. $STAT5^{IEC\ KO}$ mice were generated using cre-mediated recombination to delete the *stat5* locus in IECs.
B. Genotypes of *villin*-cre *Stat5* floxed mice were determined by PCR.

Figure S2.
A. Mice were treated with 3% DSS for 7 days and then followed by 14 days of water. Weight gain and loss percentage were evaluated. Results were expressed as the mean ± SEM (n = 5 or 6), * $p < 0.01$ versus $STAT5^{IEC\ WT}$ mice.
B. The tyrosine phosphorylation of STAT5 (pSTAT5) was determined by IH in the inflamed colonic crypts and proliferating crypt cells in $STAT5^{IEC\ KO}$ and littermate control mice exposed to DSS for 7 days, n = 7. Original magnification, × 100, bar = 100 μm.

Figure S3. Mice were treated with 3% DSS for 5 days and then followed by 5 days of water.
A. Regulatory T cells ($CD4^+CD25^+Foxp3^+$), activated $CD4^+$ T cells ($CD4^+CD44^{hi}CD62L^{lo}$), and macrophages ($CD4^-CD11C^-CD11b^+F4/80^+$) in MLNs were determined by FACS. Representative scatter graphs were shown.
B, C. Paraffin-embedded colonic sections were labeled with F4/80 and Foxp3. F4/80 and Foxp3 positive cells appeared brown, n = 5. Original magnification, × 400; bar = 100 μm.
D. MLNs were imaged and weighed, open circles represent MLNs.

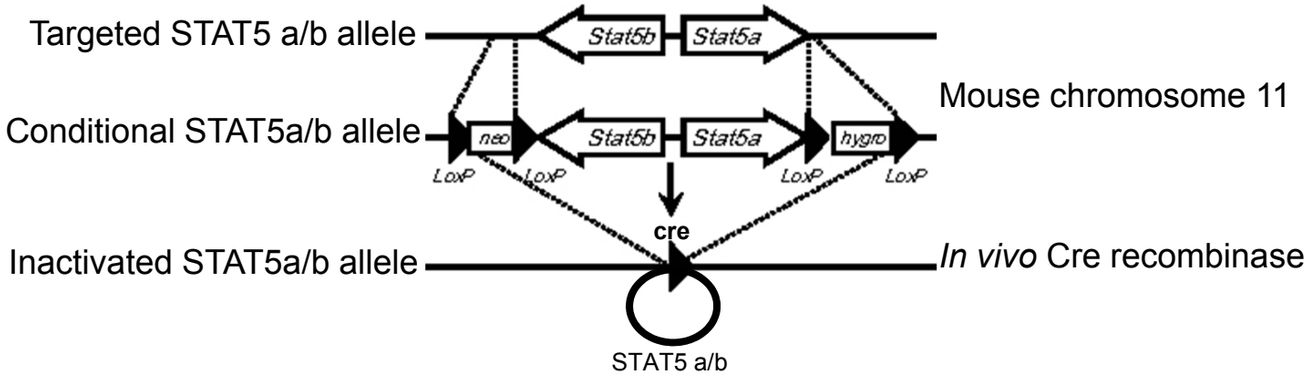
Figure S4. IEC apoptosis and proliferation in $STAT5^{IEC\ KO}$ mice.
A, B. IEC apoptosis was detected by cleaved Caspase-3 immunohistochemistry staining (A), and IEC proliferation was determined using BrdU incorporation measured by immunohistochemistry staining (B); original magnification, × 400, bar = 100 μm. Results were expressed as the mean ± SEM (n = 7).

Figure S5.
A. Colonic IECs were isolated from $STAT5^{IEC\ WT}$ and $STAT5^{IEC\ KO}$ mice, ZO-1, -2 and -3, and occludin were measured with WB. HT-29 IEC monolayers on transwell filters, with and without stat5 RNAi, were exposed to IFNγ (10 ng/ml) induction for 18 hrs and then TNFα (10 ng/ml) stimulation for 12 hrs.
B. HT-29 cell monolayers on the transwell inserts were immunostained with ZO-1 & 3, n = 3. Original magnification is × 600, bar = 100 μm.
C. Nuclear proteins (NE) and cytosolic proteins (CE) were isolated from HT-29 monolayers on the transwell inserts, pSTAT5 and STAT5 abundances in NE and CE were measured by WB, The representative result is shown from three repeated experiments.

Supplemental Table: PCR Primers.

Fig S1

A


Targeted STAT5 a/b allele
Stat5b
Stat5a
Mouse chromosome 11
Conditional STAT5a/b allele
neo
Stat5b
Stat5a
hygro
LoxP
LoxP
LoxP
LoxP
cre
Inactivated STAT5a/b allele
In vivo Cre recombinase
STAT5 a/b

B

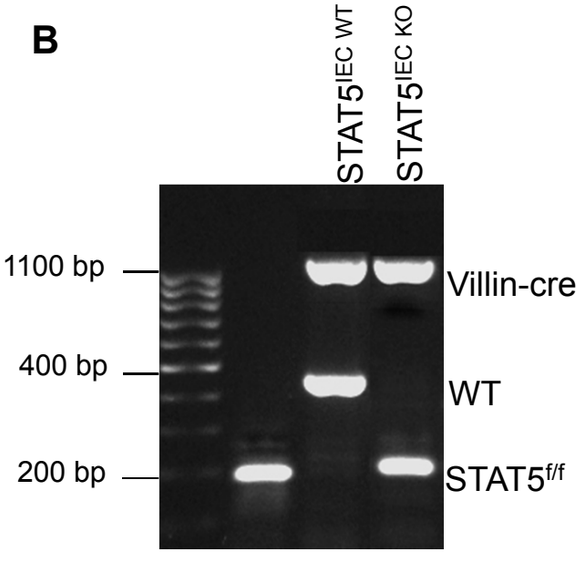
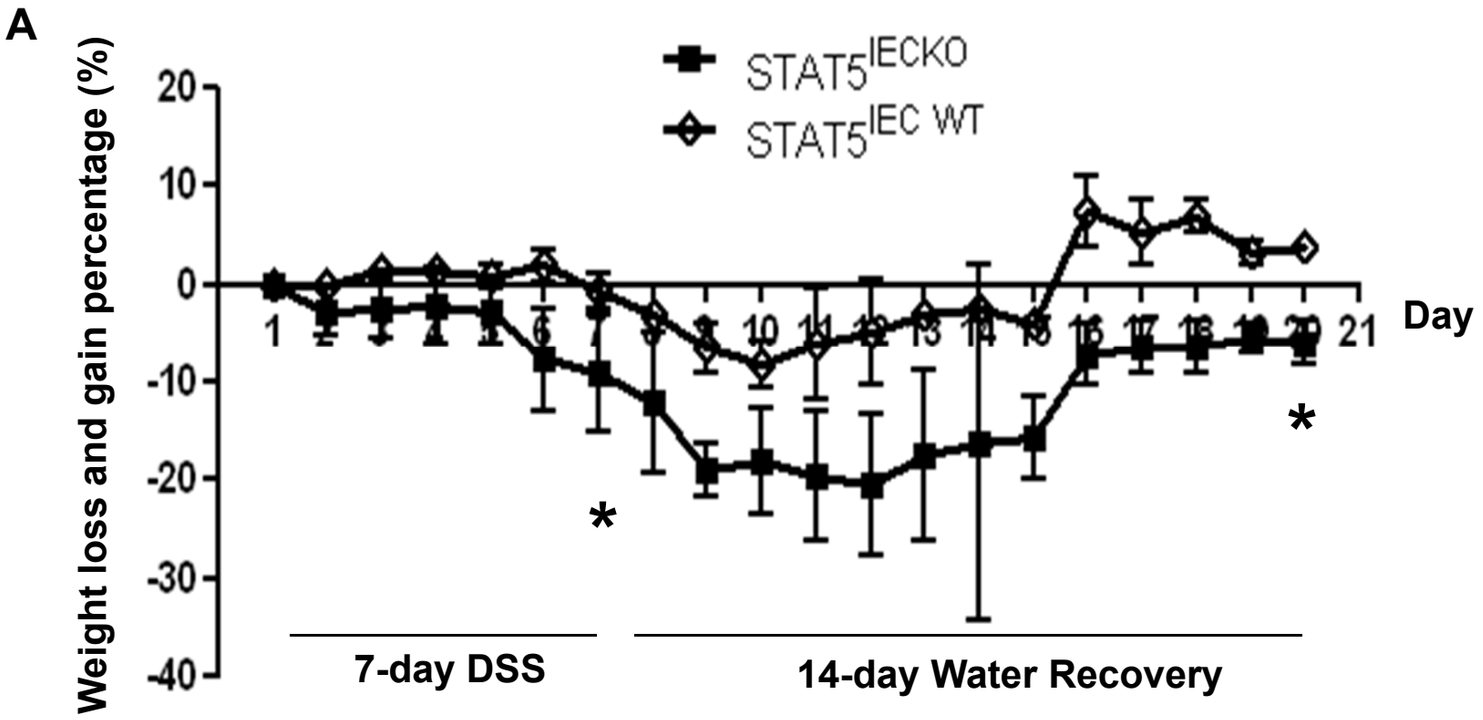

STAT5IEC WT
STAT5IEC KO
1100 bp
400 bp
200 bp
Villin-cre
WT
STAT5f/f

Fig S2


A
STAT5IECKO
STAT5IEC WT
Weight loss and gain percentage (%)
20
10
0
-10
-20
-30
-40
1
21
Day
*
*
7-day DSS
14-day Water Recovery

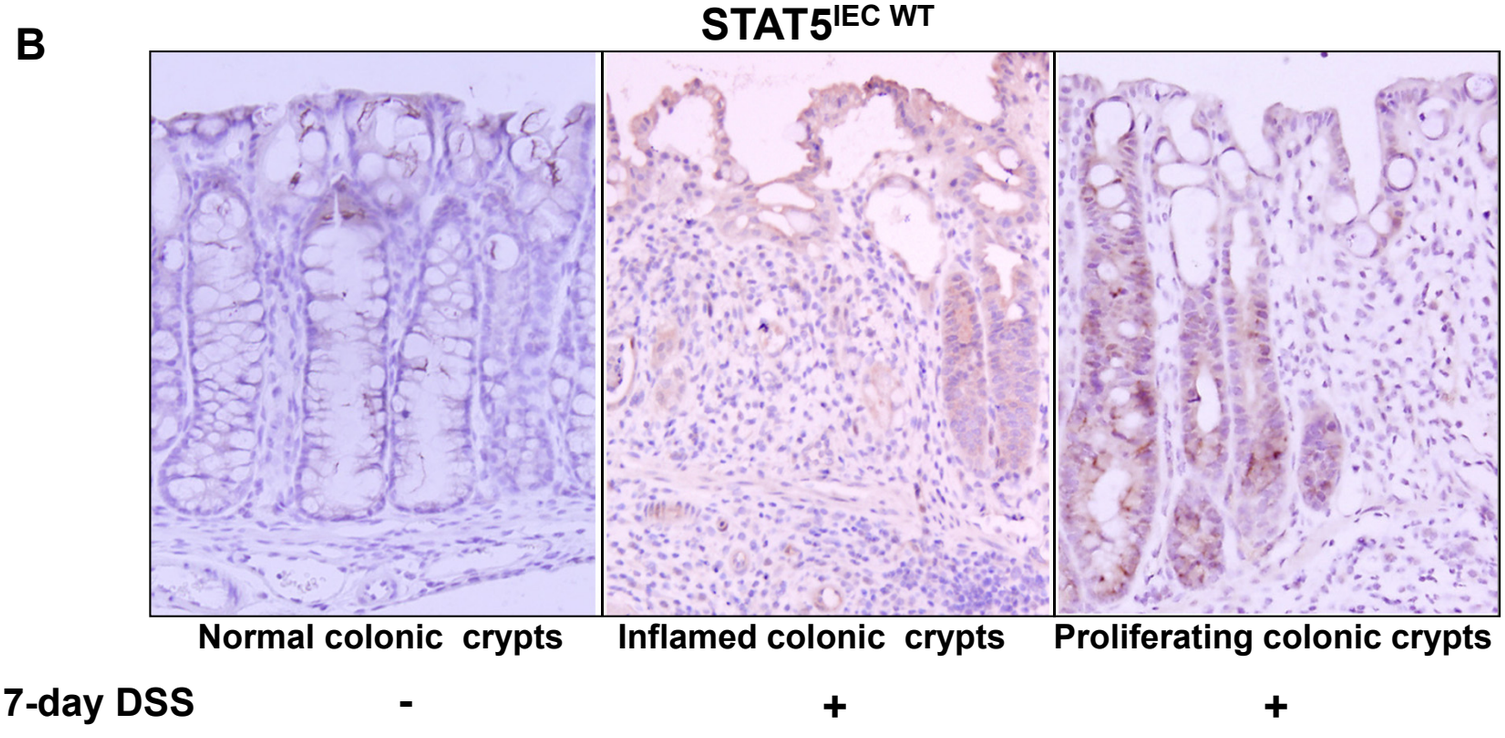

B
STAT5IEC WT
Normal colonic crypts
Inflamed colonic crypts
Proliferating colonic crypts
7-day DSS
-
+
+

Fig S3

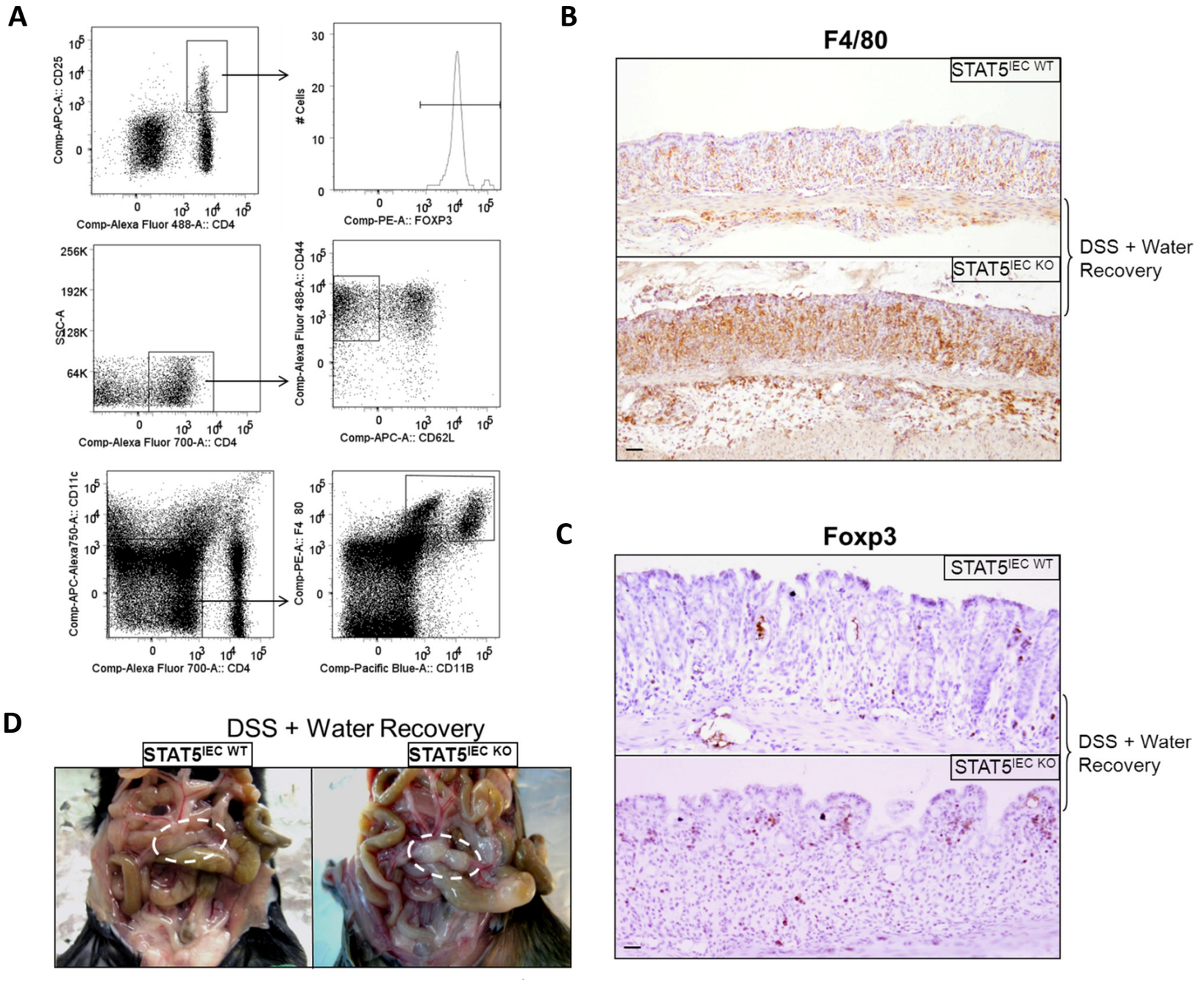
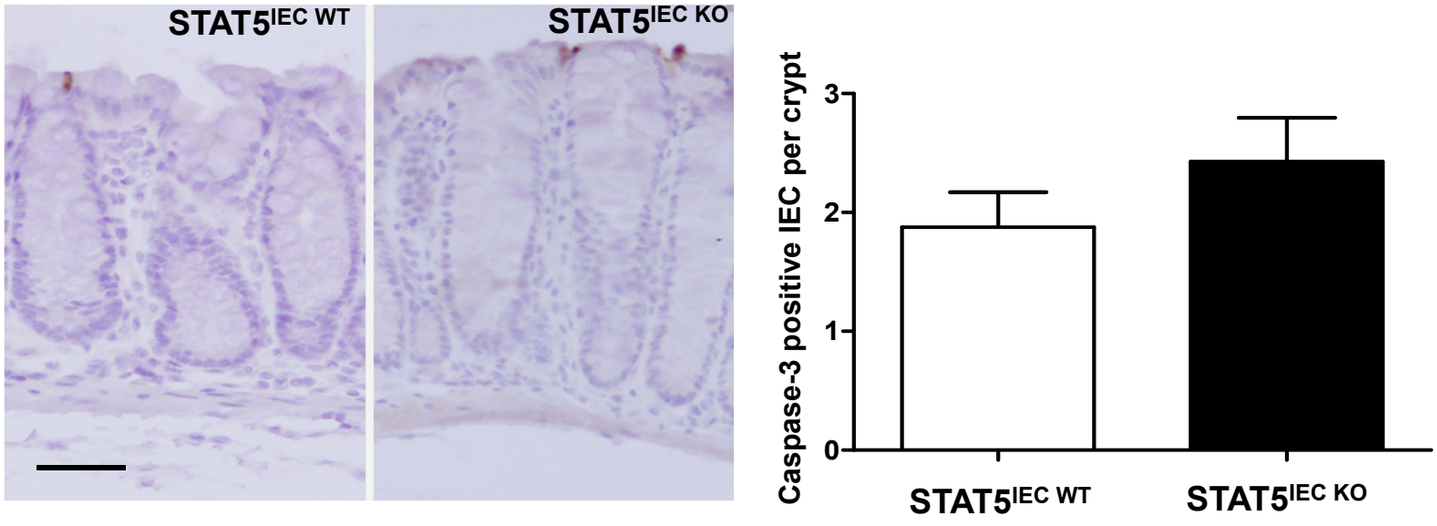

A
Comp-APC-A:: CD25
Comp-Alexa Fluor 488-A:: CD4
Cells
Comp-PE-A:: FOXP3
SSC-A
Comp-Alexa Fluor 700-A:: CD4
Comp-Alexa Fluor 488-A:: CD44
Comp-APC-A:: CD62L
Comp-APC-Alexa750-A:: CD11c
Comp-Alexa Fluor 700-A:: CD4
Comp-PE-A:: F4 80
Comp-Pacific Blue-A:: CD11B
B
F4/80
STAT5IEC WT
STAT5IEC KO
DSS + Water Recovery
C
Foxp3
STAT5IEC WT
STAT5IEC KO
DSS + Water Recovery
D
DSS + Water Recovery
STAT5IEC WT
STAT5IEC KO

Fig S4

A


STAT5IEC WT
STAT5IEC KO
Caspase-3 positive IEC per crypt
0
1
2
3
STAT5IEC WT
STAT5IEC KO

B

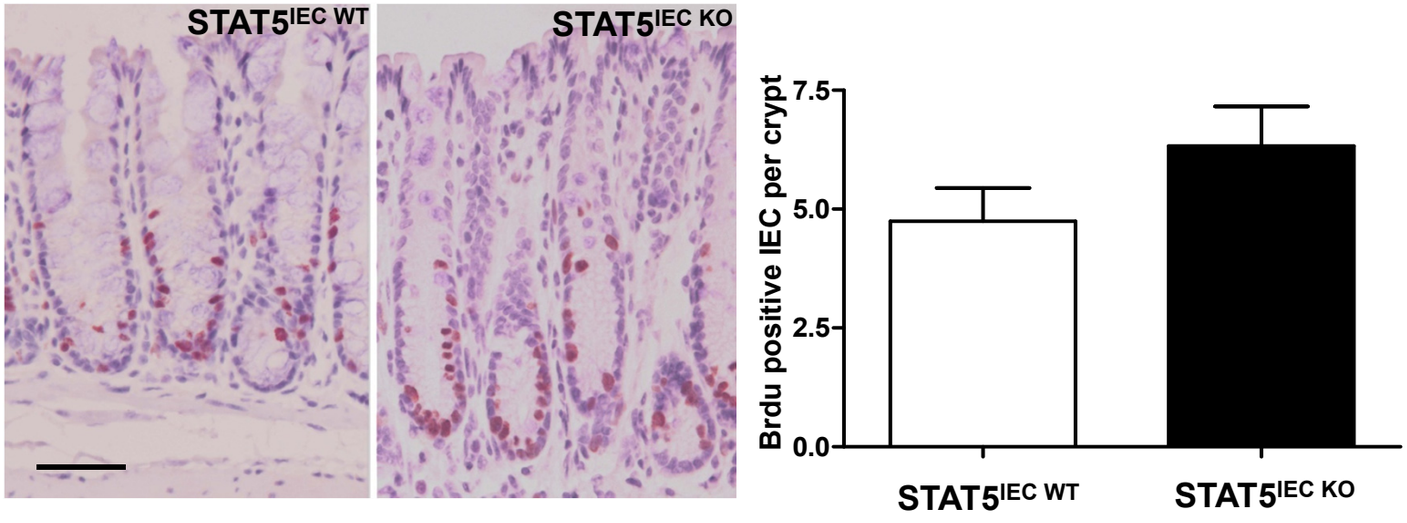
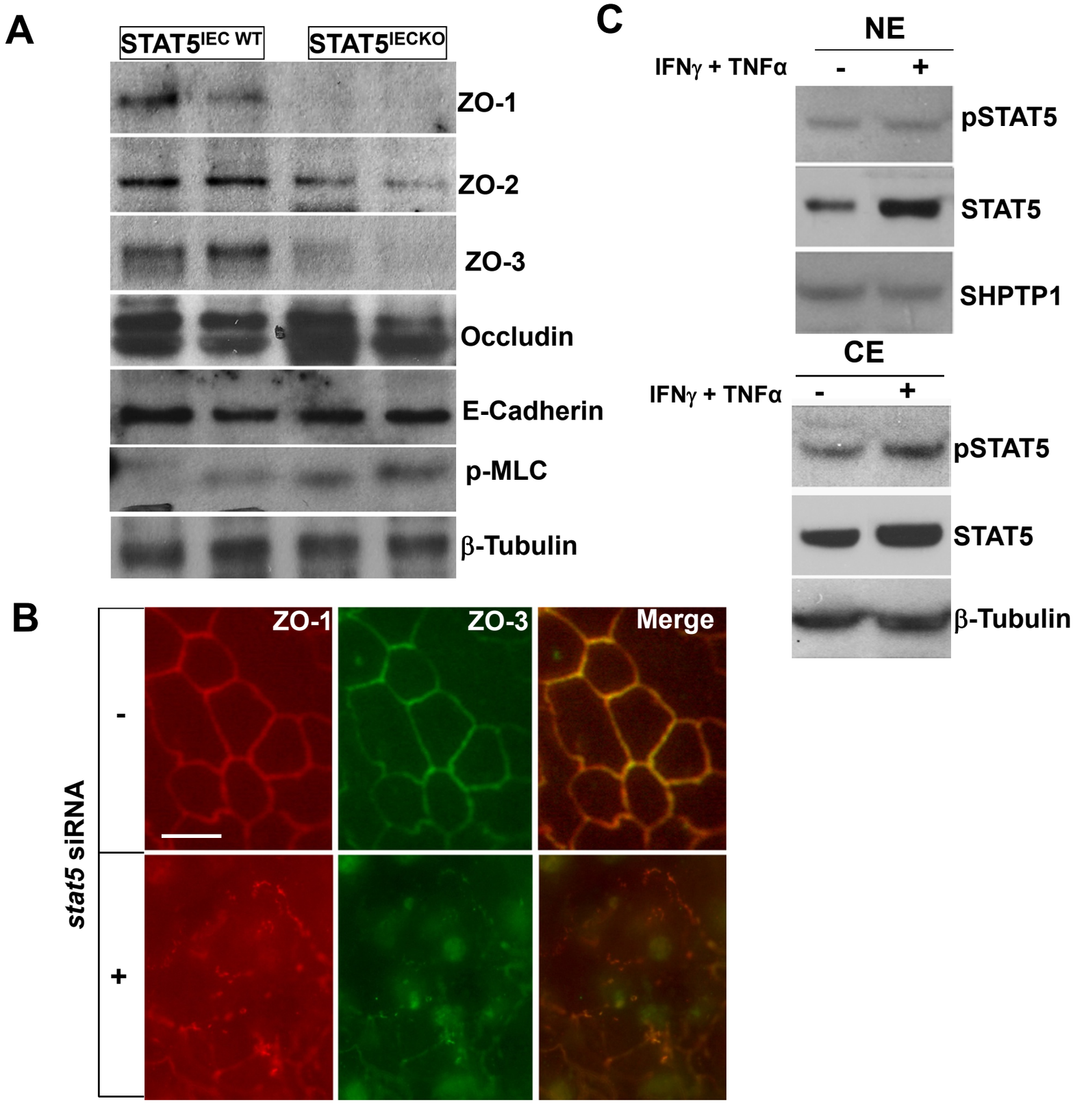

STAT5IEC WT
STAT5IEC KO
Brdu positive IEC per crypt
0.0
2.5
5.0
7.5
STAT5IEC WT
STAT5IEC KO

Fig S5


A
STAT5IEC WT
STAT5IECKO
ZO-1
ZO-2
ZO-3
Occludin
E-Cadherin
p-MLC
β-Tubulin
C
NE
IFNγ + TNFα
-
+
pSTAT5
STAT5
SHPTP1
CE
IFNγ + TNFα
-
+
pSTAT5
STAT5
β-Tubulin
B
ZO-1
ZO-3
Merge
stat5 siRNA
-
+

Supplemental Table: PCR Primers and EMSA Oliogonucleotide Sequences (Denning et al, 2007; Han et al, 2011; Han et al, 2009; Lewkowich et al, 2008; Shivakumar et al, 2004; Villarino et al, 2008)**.**

Primers	Sequences
GAPDH	Forward: 5'-GGTGGGTGGTCCAAGGTTTC-3' Reverse: 5'-TGGTTTGACAATGAATACGGCTAC-3'
β actin	Forward: 5'- CCCTAAGGCCAACCGTGAA -3' Reverse: 5'- CAGCCTGGATGGCTACGTACA -3'
TNFα	Forward: 5' - AATGGCCTCCCTCTCATCAGTT - 3' Reverse: 5' - CCACTTGGTGGTTTGCTACGA - 3'
IFNγ	Forward: 5' - GGCTGTCCCTGAAAGAAAGC - 3' Reverse: 5' - GAGCGAGTTATTTGTCATTCGG - 3'
IL-6	Forward: 5' - CAAAGCCAGAGTCCTTCAGAGAGATAC - 3' Reverse: 5' - GGATGGTCTTGGTCCTTAGCCAC - 3'
IL-10	Forward: 5' - TGGTTTCTCTTCCCAAGACC - 3' Reverse: 5' - CCCTTTGCTATGGTGTCCTT - 3'
IL-17	Forward: 5' - TTTAACTCCCTTGGCGCAAAA - 3' Reverse: 5' - CTTTCCCTCCGCATTGACAC - 3'
IL-23	Forward: 5' - CATGGGGCTATCAGGGAGTA - 3' Reverse: 5' - GACCCACAAGGACTCAAGGA - 3'
Long MLCK	Forward: 5' - AAGTCATGGATGGAAGCCAGGTCA- 3' Reverse: 5' - AATCTCATTCCCGTCGTGAAGCCA- 3'
NF-κB	Sense: 5- AGT TGA GGG GAC TTT CCC AGG C-3 Anti-sense: 3-TCA ACT CCC CTG AAA GGG TCC G-5
κB binding	Sense: 5-CAA CTT CAG GAG CTT CCC AGC CGA G-3 Anti-sense: 3-GTT GAA GTC CTC GAA GGG TCG GCT C-5

Supplemental References

Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B (2007) Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol 8**:** 1086-1094

Han X, Mann E, Gilbert S, Guan Y, Steinbrecher KA, Montrose MH, Cohen MB (2011) Loss of guanylyl cyclase C (GCC) signaling leads to dysfunctional intestinal barrier. PLoS One 6**:** e16139

Han X, Ren X, Jurickova I, Groschwitz K, Pasternak BA, Xu H, Wilson TA, Hogan SP, Denson LA (2009) Regulation of intestinal barrier function by signal transducer and activator of transcription 5b. Gut 58**:** 49-58

Lewkowich IP, Lajoie S, Clark JR, Herman NS, Sproles AA, Wills-Karp M (2008) Allergen uptake, activation, and IL-23 production by pulmonary myeloid DCs drives airway hyperresponsiveness in asthma-susceptible mice. PLoS One 3**:** e3879

Shivakumar P, Campbell KM, Sabla GE, Miethke A, Tiao G, McNeal MM, Ward RL, Bezerra JA (2004) Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFN-gamma in experimental biliary atresia. J Clin Invest 114**:** 322-329

Villarino AV, Artis D, Bezbradica JS, Miller O, Saris CJ, Joyce S, Hunter CA (2008) IL-27R deficiency delays the onset of colitis and protects from helminth-induced pathology in a model of chronic IBD. Int Immunol 20**:** 739-752