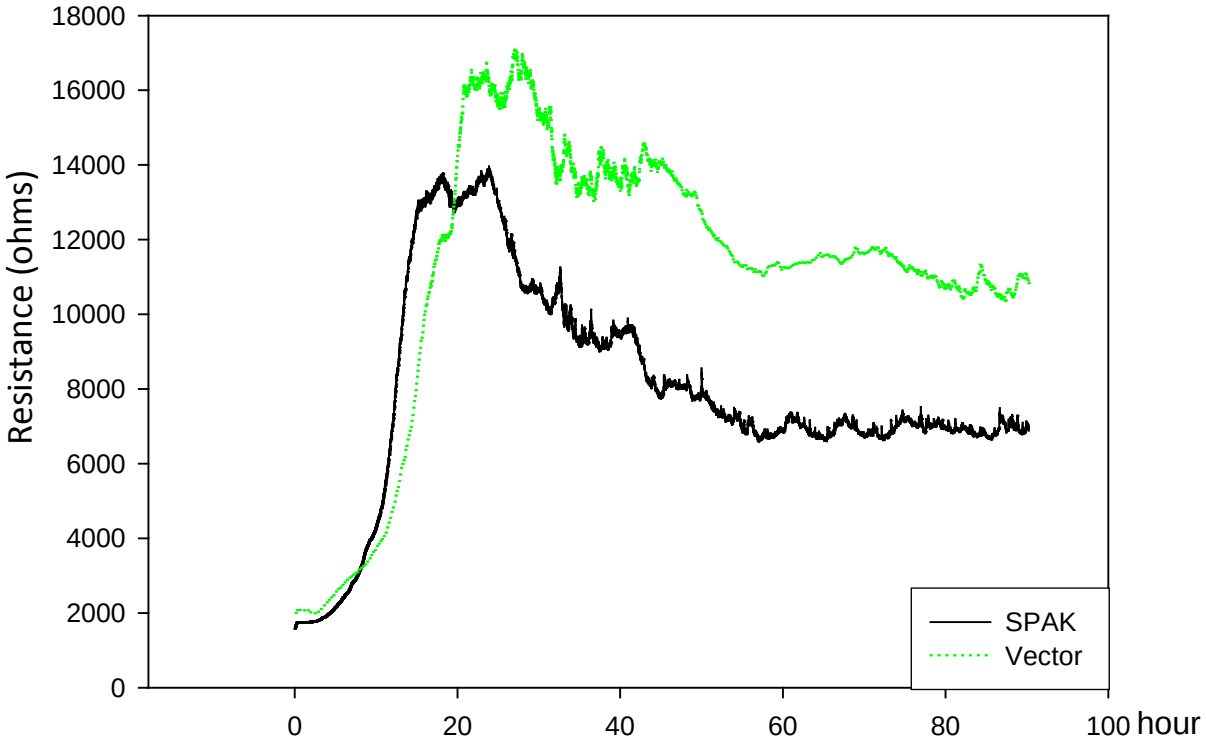
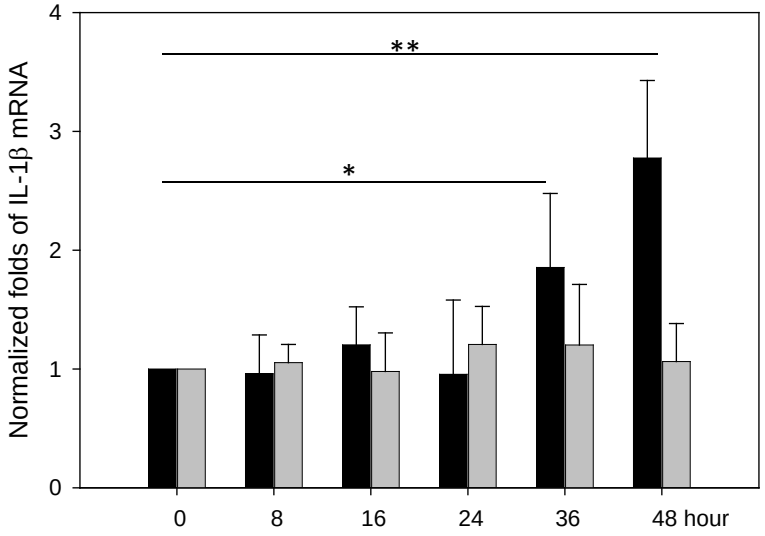
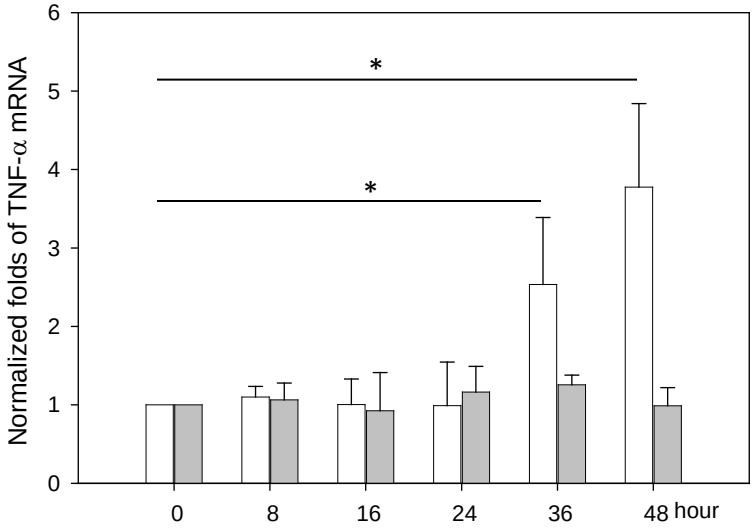
Supplemental data

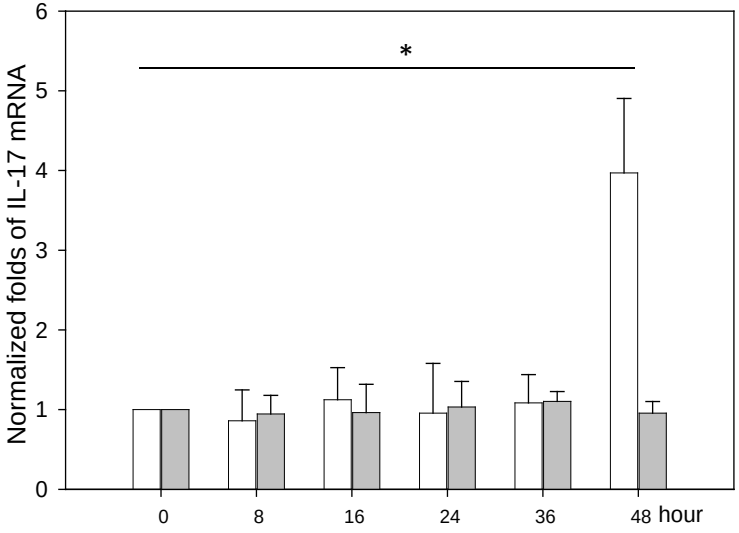


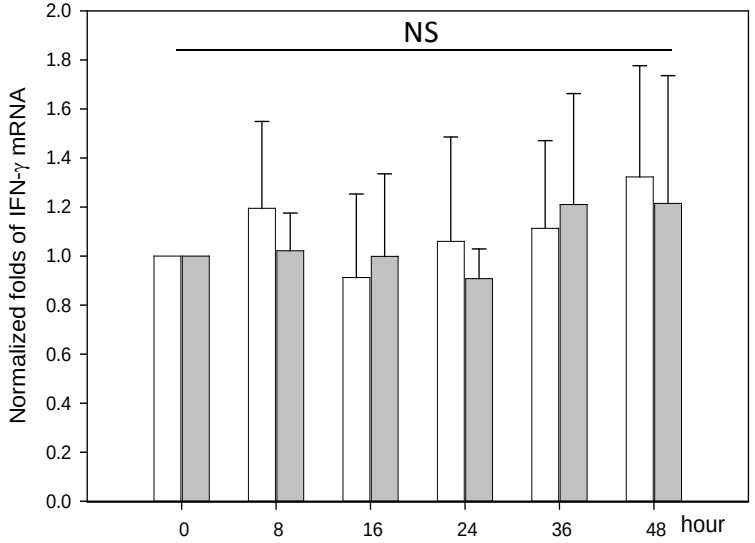
Supplemental figure S1. Electrophysiology assay. To determine whether SPAK over-expression primarily effect on inflammatory cytokine expression or barrier function, Caco2-BBE cells were transfected with SPAK or vector using electroporation by Neon transfection system (Invitrogen). The first group of cells were plated at $2.5*10^5$/well used for transepithelial resistance assay with electric cell-substrate impedance sensing (ECIS) system (Applied BioPhysics) that measures intestinal epithelial resistance in real time. SPAK and vector transfected Caco2-BBE cells showed similar degrees of exponential growth after plating of cells on the ECIS electrode. However, Caco2-BBE wild-type cells attained a maximal and plateau resistance higher than those of Caco-2-BBE cells overexpressing SPAK after 20 hours. The experiments were repeated three times. This suggests that SPAK overexpression by epithelial cells decreases *in vitro* barrier function.

Supplemental data



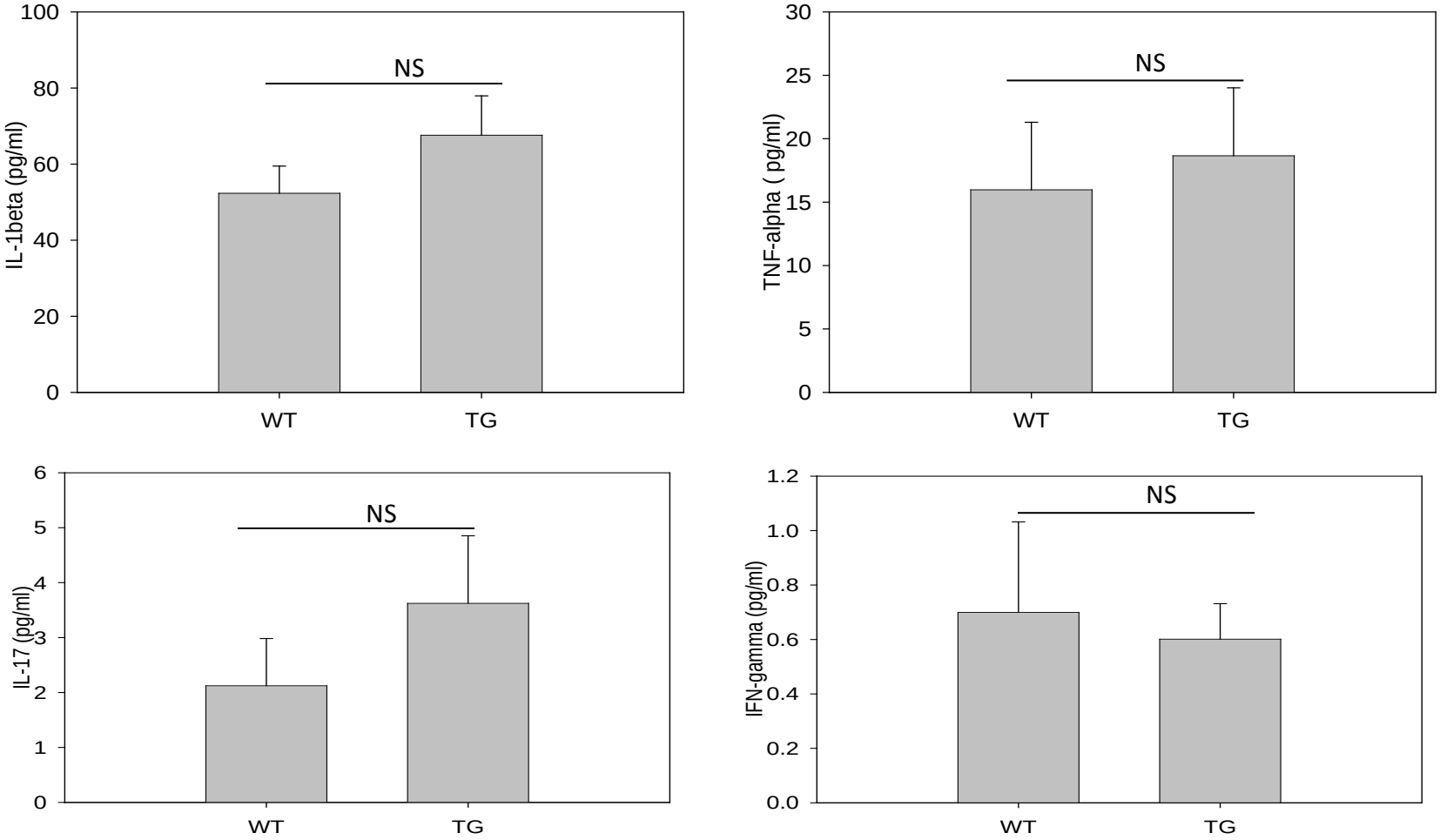






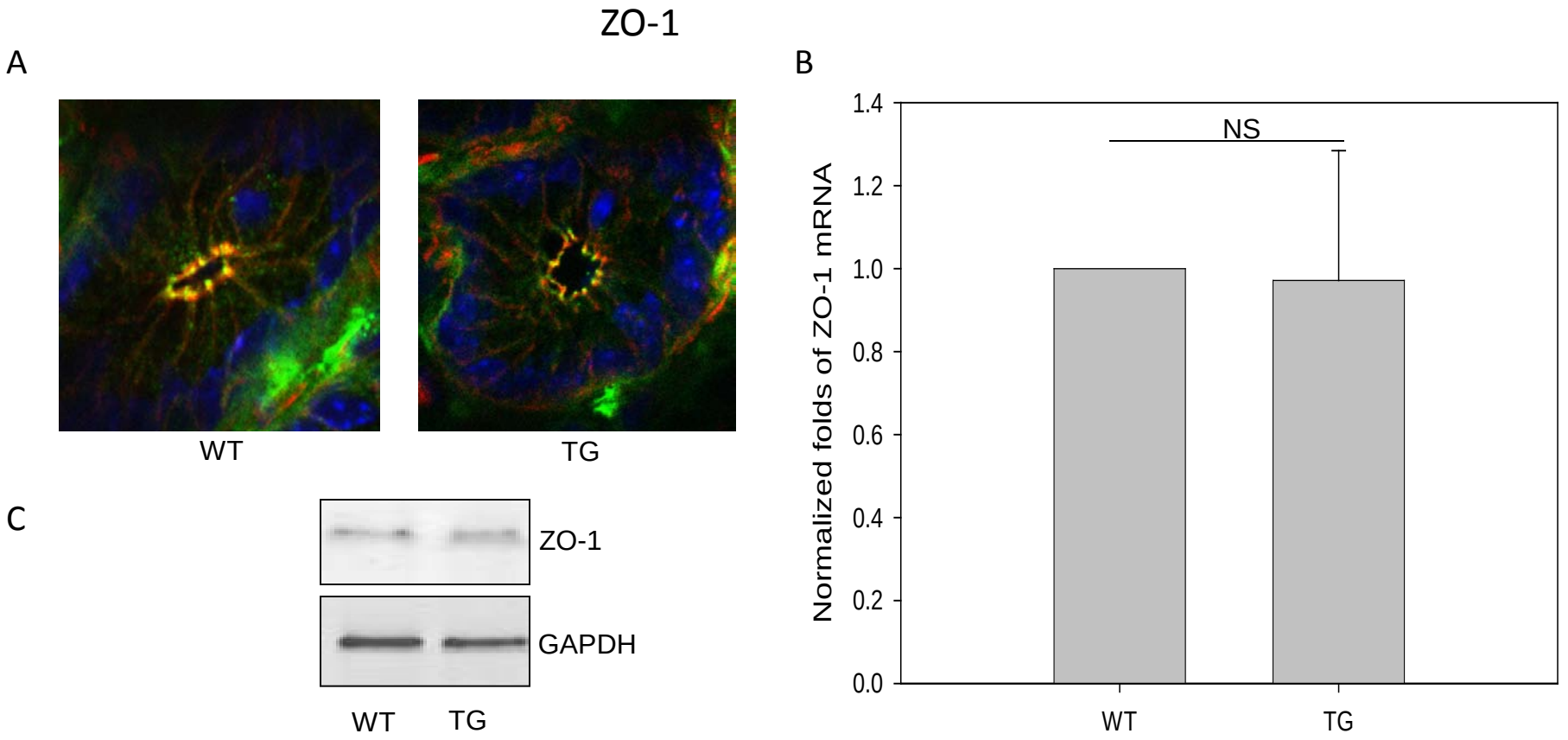
Supplemental figure S2. To determine whether the effect of SPAK over-expression is primarily on inflammatory cytokine expression or on barrier function. Caco2-BBE cells were transfected with SPAK or vector using electroporation by the Neon transfection system (Invitrogen). The cells were divided into different group, the first group of cells were used for resistance assay (supplemental figure s1), The second group of cells was plated in 6-well plates at $2.5*10^5$/well. Total RNA was prepared from these cells at different time points (0, 8, 16, 24, 36, 48 hours) and used for real-time PCR to determine the mRNA level of cytokines IL-1β, IFN-γ, TNF-α and IL-17. The supernatant from these wells were analyzed by ELISA for the same cytokines using the same time course (0, 8, 16, 24, 36, 48 hours). However, we did not see significant differences in cytokine expression at the mRNA level until 36 hours later. Additionally, we did not find any of these four inflammatory cytokines detectable by ELISA, which suggests any effect of SPAK on the intestinal barrier *in vitro* is not mediated by cytokine secretion (we added this information to our supplementary data). Together, the results suggest that SPAK primarily effects epithelial barrier function. Black solid bar represents data from SPAK transfected cells and gray solid bar represents data from vector transfected cells. NS: no significant, *p<0.05, ** p<0.01

Supplemental data



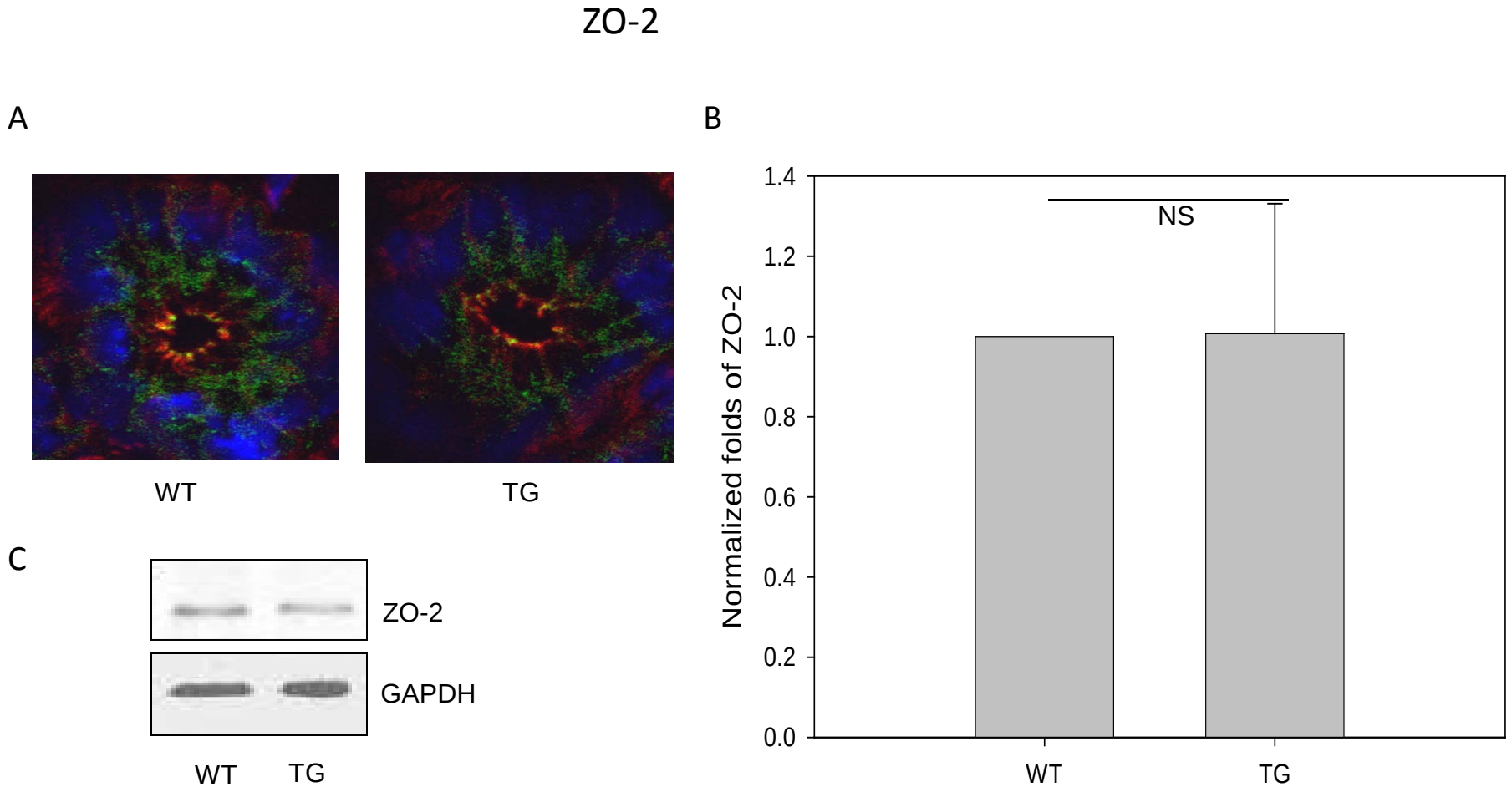
Supplemental figure S3. 200 mg of Colon tissue from WT littermate and TG mice were cultured for 12 hours, the supernatant were collected and analyzed by ELISA assay, no significant change in cytokine levels were observed for IL-1beta, TNF-alpha, IL-17 and IFN-gamma, which means th protein levels of pro-inflammatory cytokines did not rise above baseline in the absence of DSS treatment. In conclusion, intestinal barrier defect in TG mice is not caused by inflammatory cytokines tested. NS: No significant

Supplemental data



Supplemental figure S4. TG mice and WT littermate mice display no significant different expression of ZO-1 by immunoflurescrence, real time PCR and Western blot. NS: No significant

Supplemental data

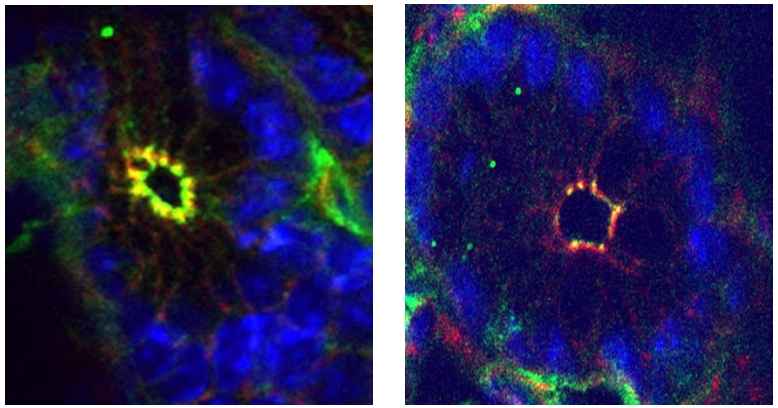


Supplemental figure S5. TG and WT littermate control mice display no significant different expression of ZO-2 by immunoflurescrence, real time PCR and Western blot. NS: No significant

Supplemental data

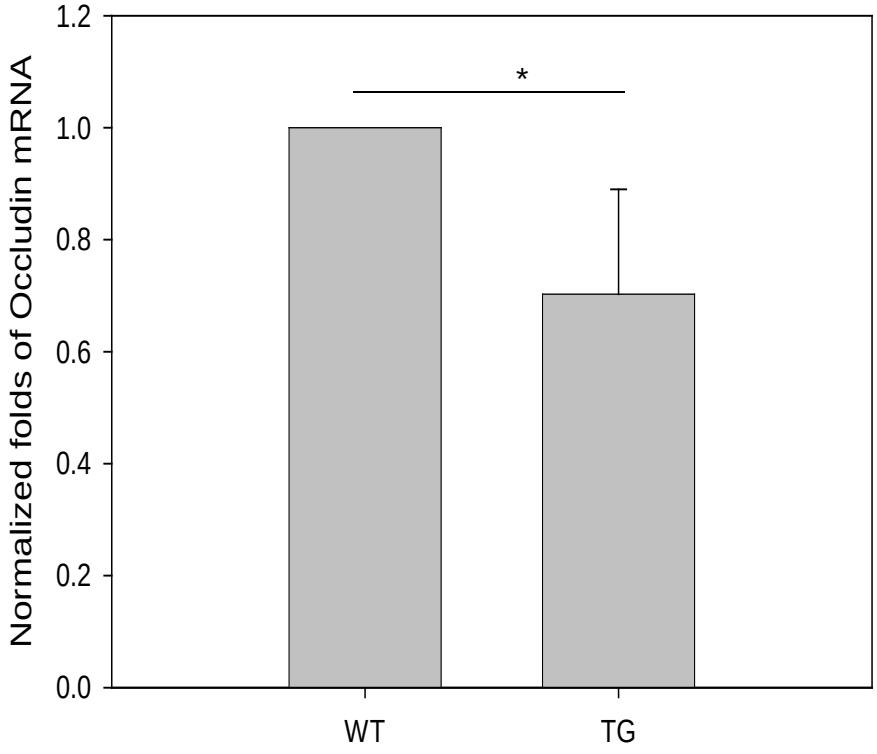
Occludin

A

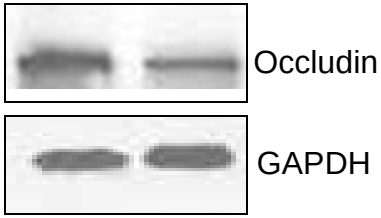


WT TG

B

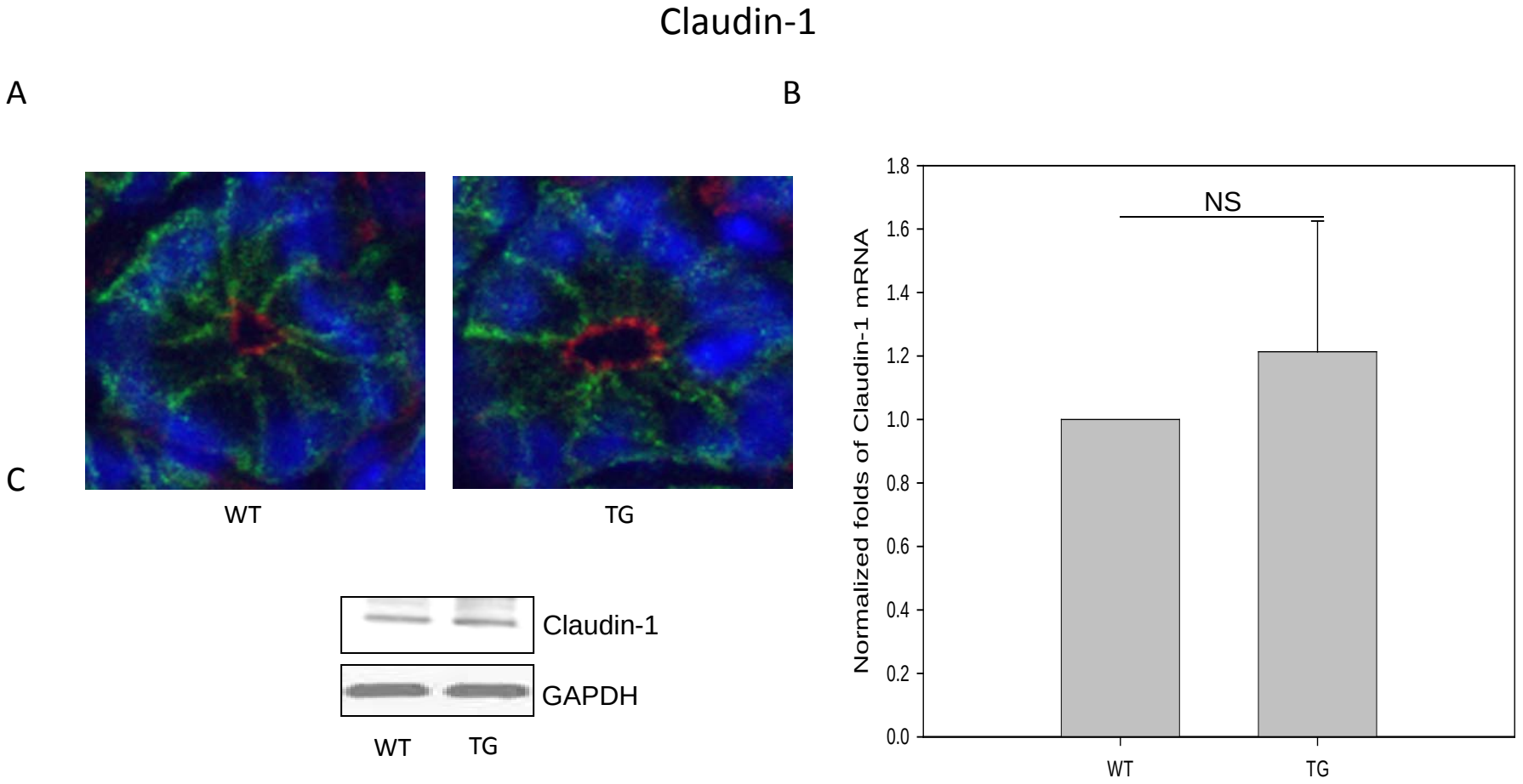


C



Supplemental figure S6. TG mice demonstrated significant decreased expression of tight junction protein occludin by immunoflurescrence, real time PCR and Western blot. * $p<0.05$

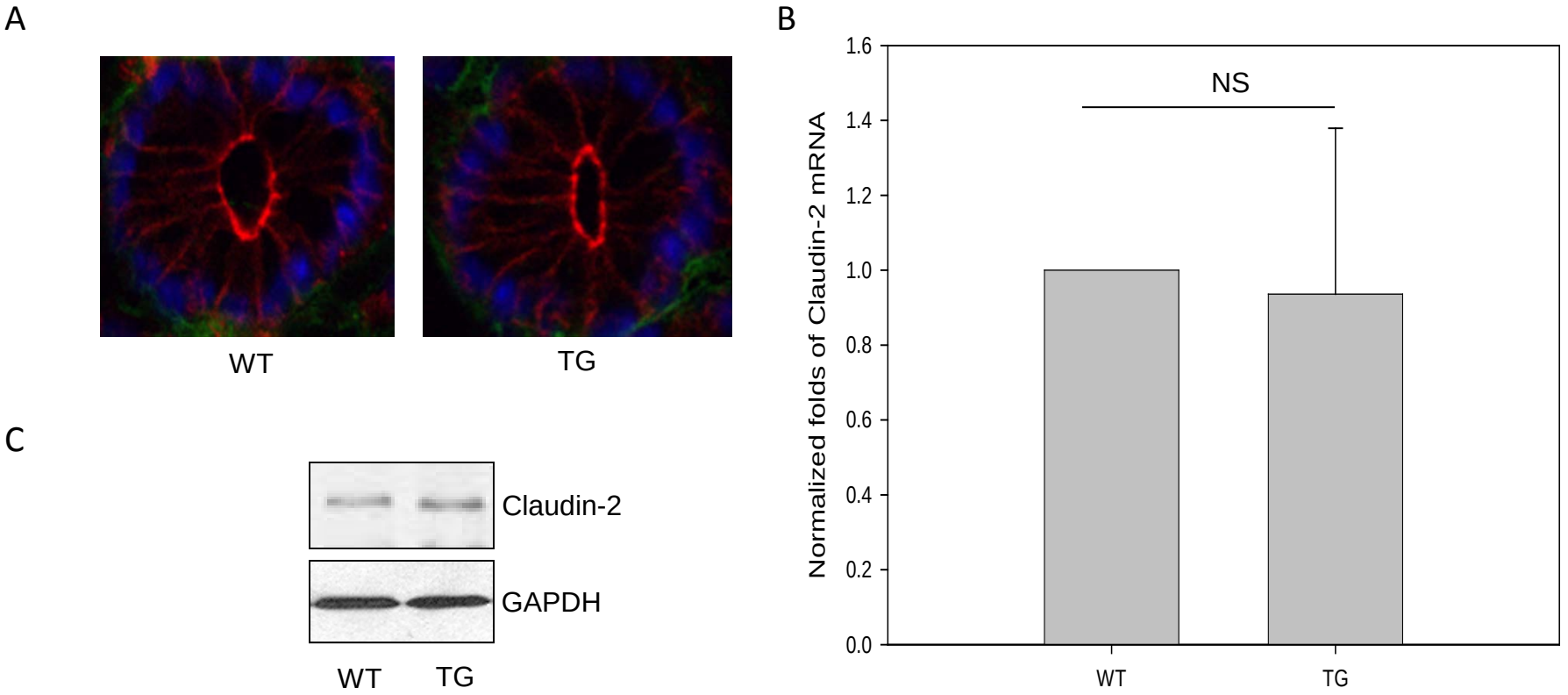
Supplemental data



Supplemental figure S7. TG and WT littermate control mice display no significant different expression of claudin-1 by immunoflurescrence, real time PCR and Western blot. NS: No significant

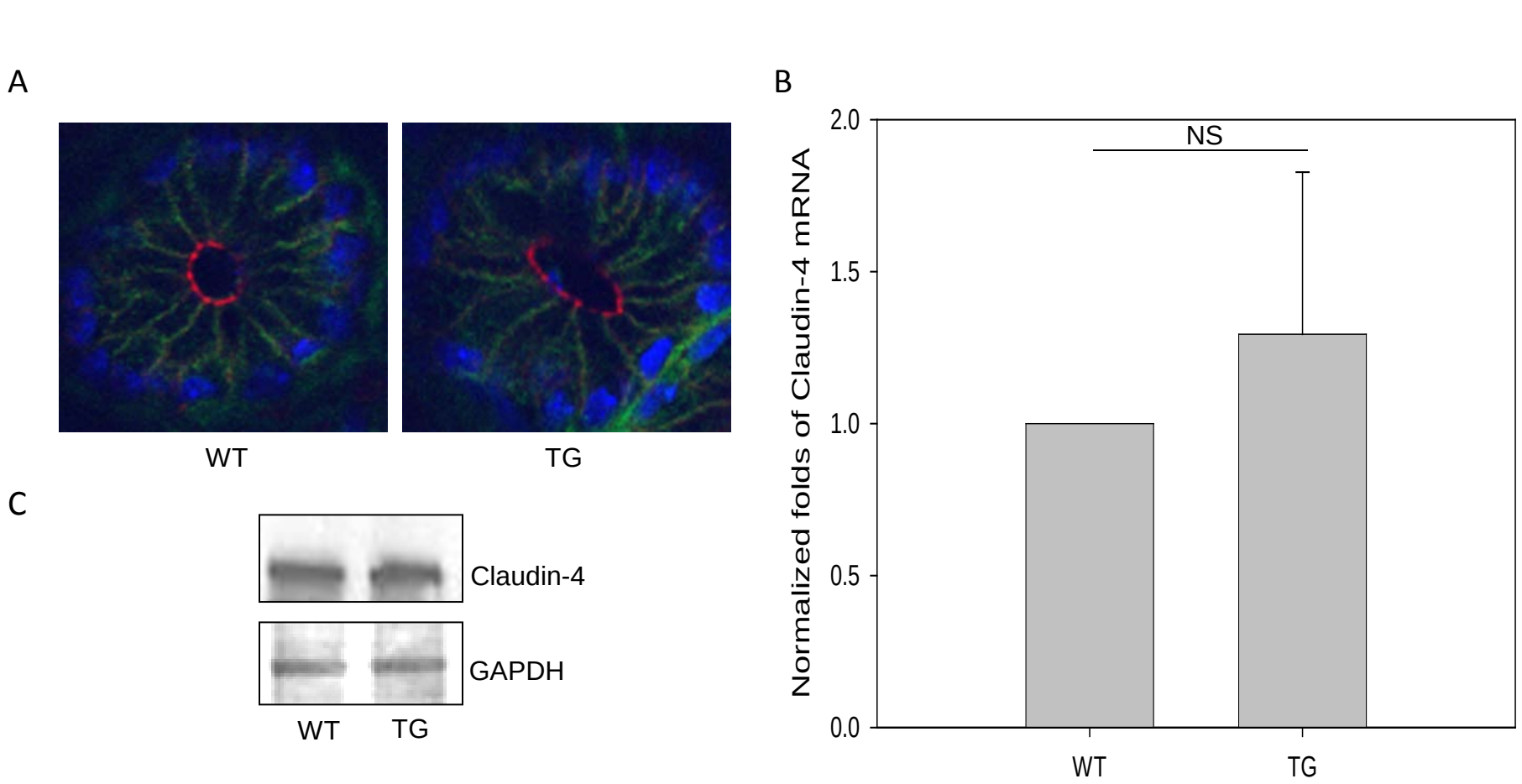
Supplemental data

Claudin-2



Supplemental figure S8. TG and WT littermate control mice display no significant different expression of claudin-2 by immunoflurescrence, real time PCR and Western blot. NS: No significant

Supplemental data



Supplemental figure S9. TG and WT littermate control mice display no significant different expression of claudin-4 by immunoflurescrence, real time PCR and Western blot. NS: No significant

Supplemental data

Supplemental table s1: Primers used for real-time PCR

Primers	Nucleotides Sequences
ZO-1For	CGAGTTGCAATGGTTAACGGA
ZO-1Rev	TCAGGATCAGGACGACTTACTGG
ZO-2For	ACTCCAGTCCCTATTCCTGAG
ZO-2Rev	GCTATTTCGATCCTCGCATTC
OccluFor	AATGTAGAGAAAGGTCCTGGTG
OccluRev	CCTTTAATTCCTGCACCA
Claud-1For	GCGCGATATTTCTTCTTGCAGG
Claud-1Rev	TTCGTACCTGGCATTGACTGG
Claud-2For	CTCCCTGGCCTGCATTATCTC
Claud-2Rev	ACCTGCTACCGCCACTCTGT
Claud-4For	GGCTGCTTTGCTGCAACTGTC
Claud-4Rev	GAGCCGTGGCACCTTACACG