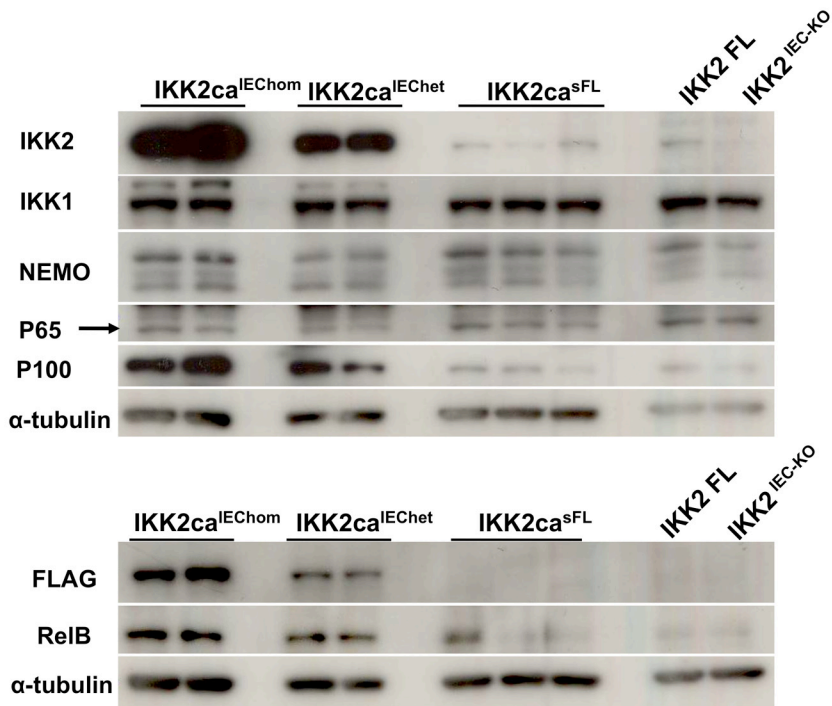
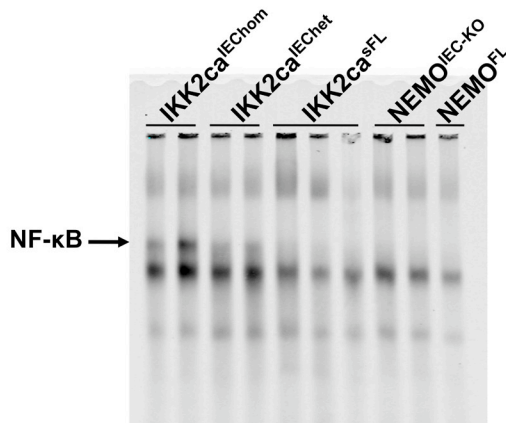
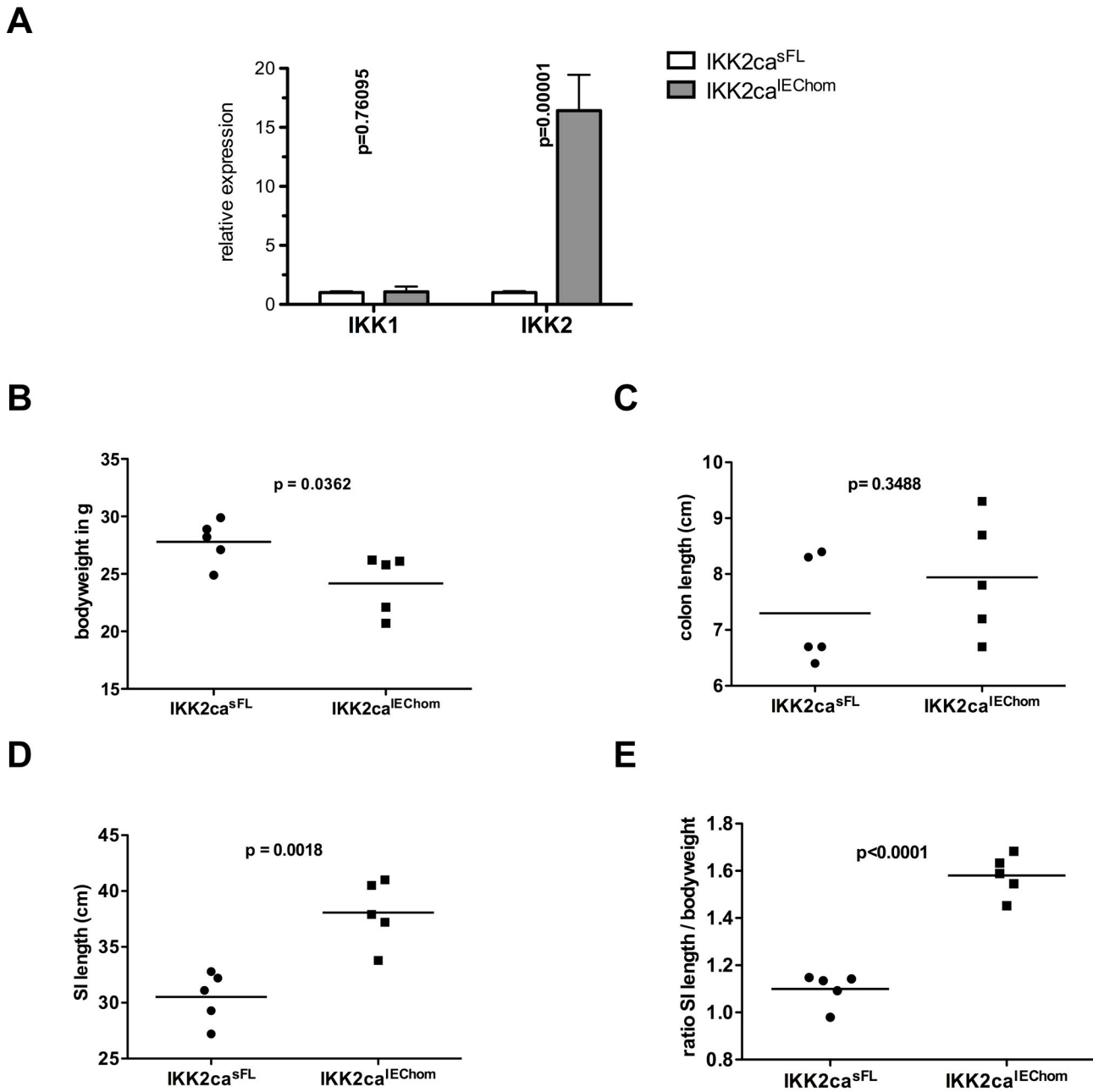


A**B****Supplementary Figure 1****Comparison of expression levels of IKK2 and NF- κ B pathway components in IECs from IKK2ca^{IEChom} and IKK2ca^{IEChet} mice**

A. WB analysis of protein extracts from small intestinal IECs depict IKK2 and IKK2ca only (anti-FLAG) in IKK2ca^{IEChom} and IKK2ca^{IEChet} mice. No difference in IKK1 or NEMO protein were detectable between genotypes. The protein abundance of RelB and p100, both targets of NF- κ B regulated transcription, was elevated in both IKK2ca^{IEChom} and IKK2ca^{IEChet} mice.

B. EMSA performed with nuclear extracts from small intestinal IECs reveals increased NF- κ B DNA-binding activity in IECs from IKK2ca^{IEChom} and IKK2ca^{IEChet} mice, as indicated by a shift in the NF- κ B probe (marked by an arrow).



Supplementary Figure 2

Expression of IKK2, body weight and intestinal length of IKK2ca^{IEChom} mice

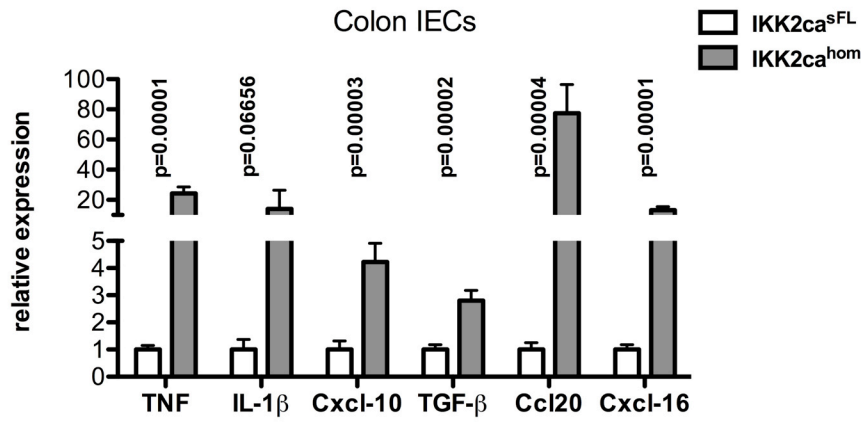
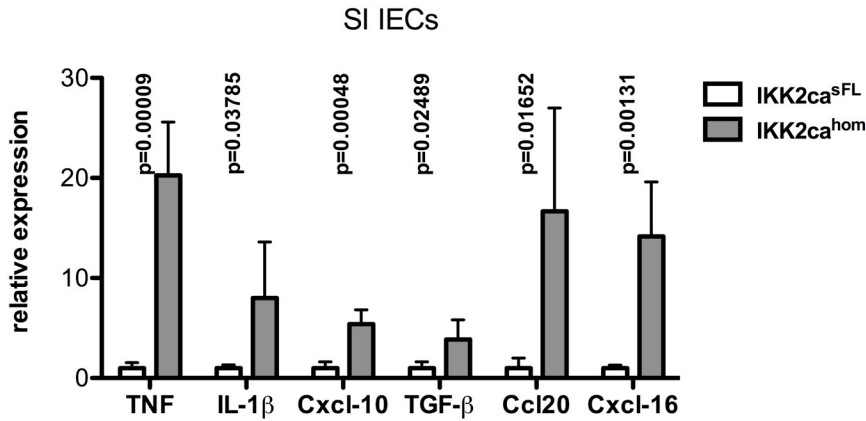
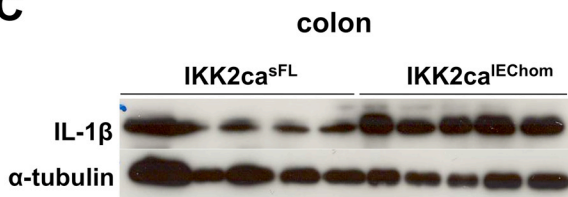
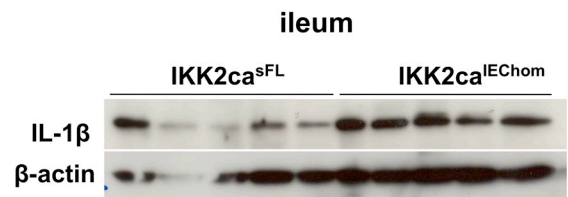
A. Quantitative RT-PCR showing elevated expression of IKK2 in colon IECs of IKK2ca^{IEChom} mice whereas IKK1 was not differentially expressed between genotypes (n = 5 per genotype; mRNA levels are presented as mean values \pm SD).

B. Reduced body weight of male IKK2ca^{IEChom} mice at 10 weeks of age (n = 5 per genotype; horizontal line represents mean value).

C. No statistically significant difference in colon lengths was observed between IKK2ca^{sFL} and IKK2ca^{IEChom} male mice at 10 weeks of age (n = 5 per genotype; horizontal line represents mean value).

D. The length of the small intestine was significantly longer in the same 10 week old male IKK2ca^{IEChom} mice when compared to IKK2ca^{sFL} animals (n = 5 per genotype; horizontal line represents mean value).

E. The relative length of the small intestine compared to the body weight of each mouse was significantly higher in IKK2ca^{IEChom} mice (n = 5 per genotype; horizontal line represents mean value)

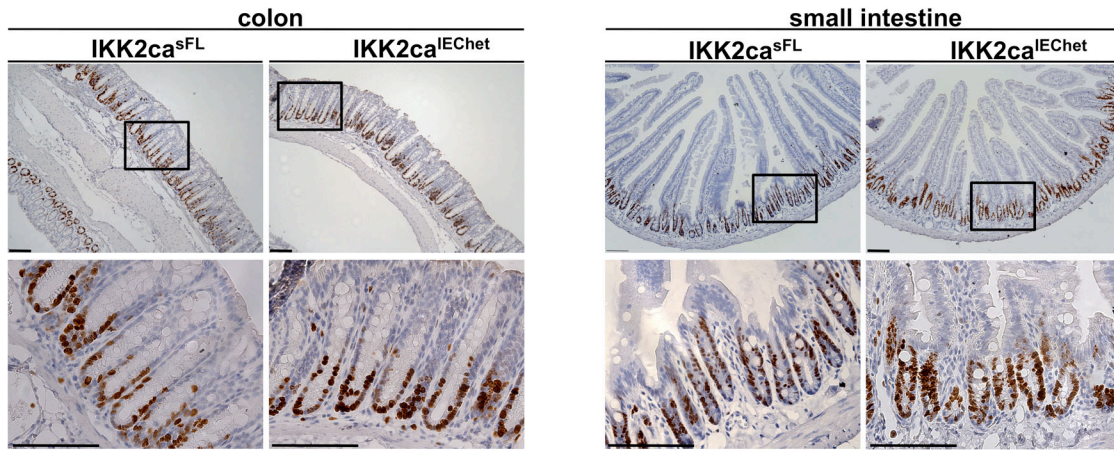
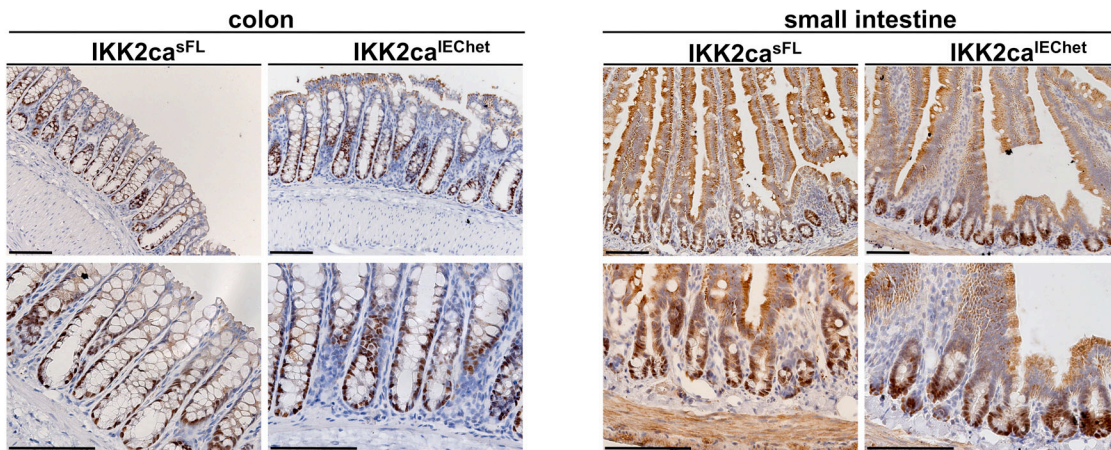
A**B****C****D****Supplementary Figure 3****Production of inflammatory mediators by intestinal epithelial cells of IKK2ca^{IEChom} mice**

A. Quantitative RT-PCR analysis shows significantly elevated expression levels of inflammatory mediators in colonic IECs from 8-week-old IKK2ca^{IEChom} animals. (n=5 per genotype; mRNA levels are presented as mean ± SD)

B. Inflammatory molecules are expressed at higher levels in primary small intestinal IECs derived from IKK2ca^{IEChom} mice when compared SI IECs from IKK2ca^{SFL} control animals. (n=5 per genotype; mRNA levels are presented as mean ± SD)

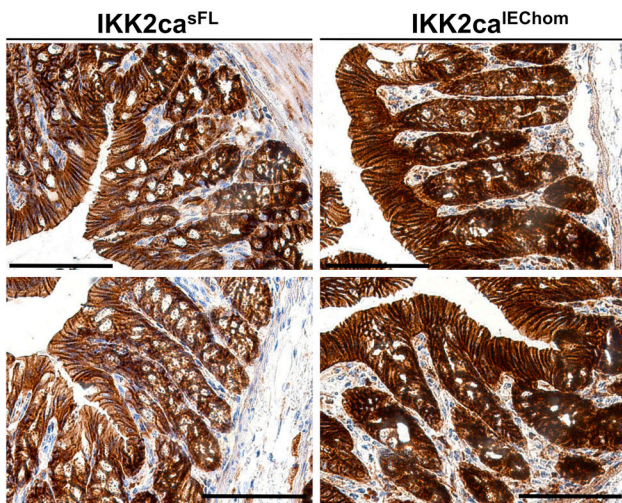
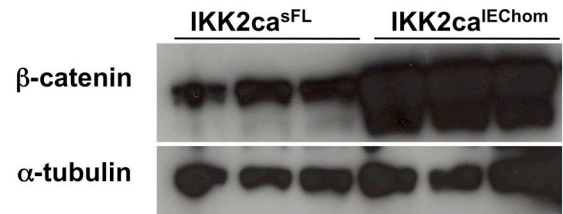
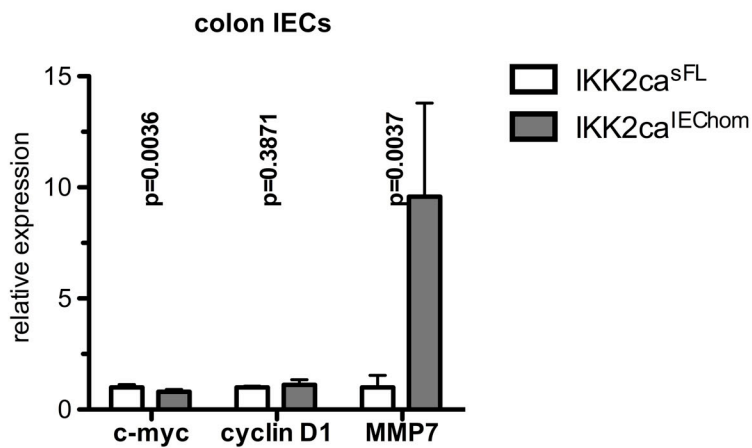
C. WB analysis for IL-1β on whole colon tissue extracts indicating an increased abundance of IL-1β protein in the colon of IKK2ca^{IEChom} mice.

D. Increased amount of IL-1β protein in ileal extracts derived from IKK2ca^{IEChom} mice as assessed by WB analysis.

A**B****Supplementary Figure 4****No differences in Ki67 and Sox9 expression in IECs between $IKK2ca^{sFL}$ and $IKK2ca^{IEChet}$ mice**

A. Ki67 immunohistochemical staining of sections from the colon and SI of 4-month-old animals did not reveal considerable differences between $IKK2ca^{IEChet}$ and $IKK2ca^{sFL}$ mice, consistent with the normal histology of these tissues as shown in figure 5B.

B. Sox9 immunohistochemical staining of sections from the colon and SI of 4-month-old animals did not reveal considerable differences between $IKK2ca^{IEChet}$ and $IKK2ca^{sFL}$ mice, consistent with the normal histology of these tissues as shown in figure 5B.

A**B****C****Supplementary Figure 5****Increased β-catenin levels in IKK2ca^{IEChom} mice**

A. Immunohistochemical staining showing increased abundance of β-catenin protein in the colonic epithelium of 9-week-old IKK2ca^{IEChom} mice when compared to IKK2ca^{FL} littermates. Pictures shown were taken from sections of IKK2ca^{FL} and IKK2ca^{IEChom} colons that were cut at the same thickness and were placed on the same glass slides to make sure identical conditions were used for the staining.

B. Immunoblot analysis of protein extracts from small intestinal IECs from IKK2ca^{IEChom} mice revealed higher levels of β-catenin compared to IKK2ca^{FL} IECs.

C. Quantitative RT-PCR analysis of selected Wnt target genes in colon IECs from IKK2ca^{FL} and IKK2ca^{IEChom} mice. (mRNA levels are presented as mean ± SD; n=5 per genotype).

All scale bars represent 50 μm.