

Expanded View Figures

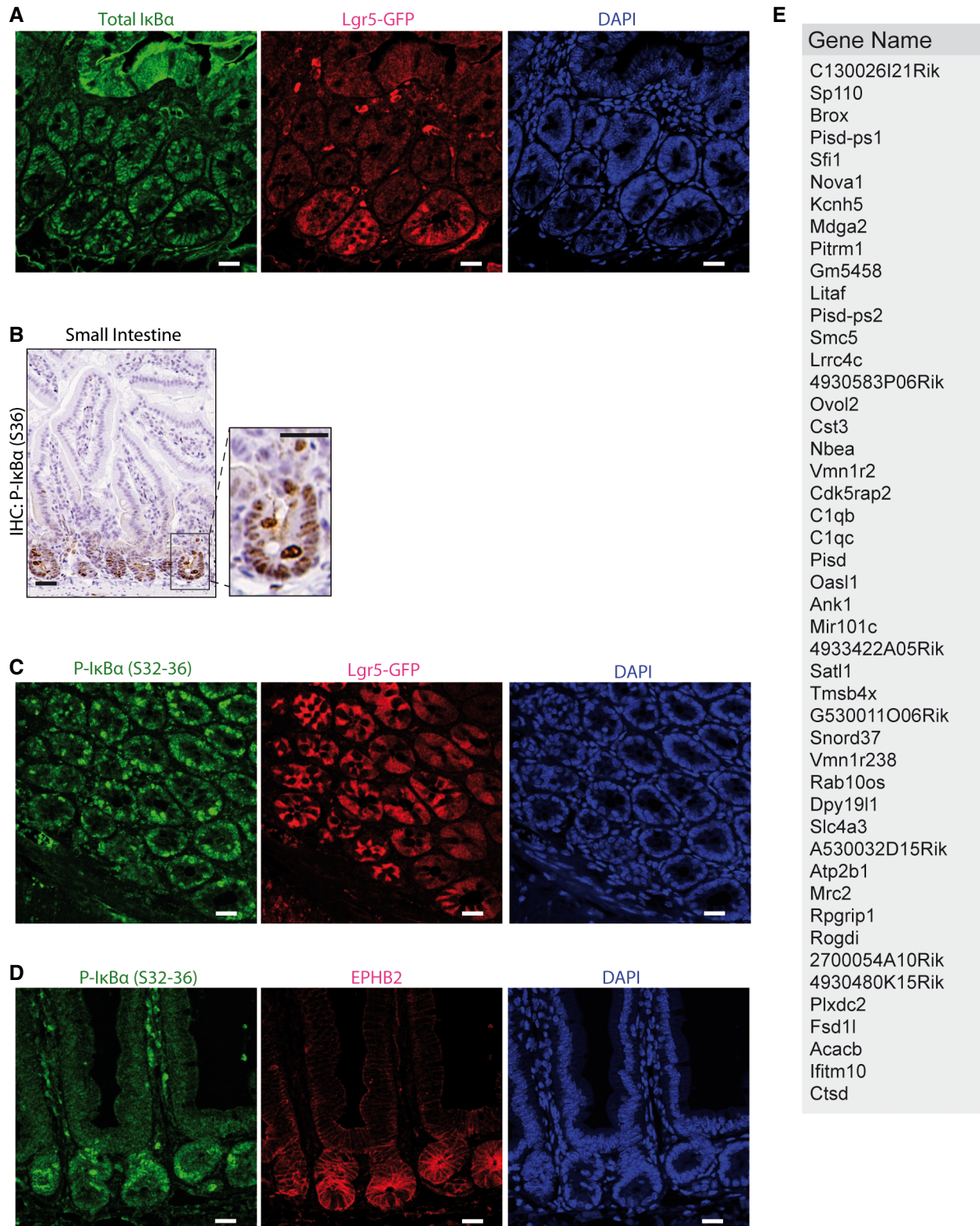


Figure EV1.

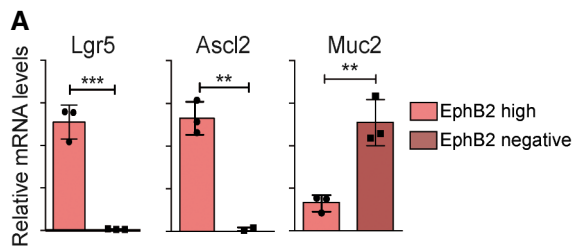
Figure EV1. P-I κ B α localizes in the nucleus of intestinal crypt cells and binds chromatin at specific genes.

A IHC analysis of P-I κ B α (S36) in intestinal mouse sections.

B–D IF analysis in sections from murine small intestine of 2-month-old Lgr5-GFP reporter mice (B and C) and WT mice (D).

E Table indicating the genes identified in I κ B α ChIP-sequencing of P6 WT intestinal cells with $P_{adj} < 0.05$. $n = 4$ 2-month-old WT mice were analyzed.

Data information: Scale bars, in A, B, C, and D, 50 μ m.

**Figure EV2. I κ B α KO intestines showed alterations in ISC population.**

A qPCR analysis of EphB2-high and EphB2-negative sorted cells from P6 WT intestine. Three technical replicates were analyzed. Bars represent mean values \pm standard error of the mean (s.e.m.); P values were derived from an unpaired t -test, two-tailed, *** P -value < 0.0005 , ** P -value < 0.005 .

B Table indicating the ISC genes differentially expressed between I κ B α WT and KO EphB2-high sorted cells (RNA-seq, $n = 3$ WT and $n = 3$ KO), including genes of ISC signature [43] (in orange).

B DEG (ISC) KO/WT

GENE	log2FC	p-value	p-adj
Tert	-3.474	0.0000	0.0000
Dclk1	-3.412	0.0083	0.0458
Id2	-2.409	0.0000	0.0000
Rnf32	-1.608	0.0136	0.0670
Ascl2	-1.128	0.0015	0.0117
Olfm4	-1.011	0.0022	0.0155
Foxp4	-0.878	0.0000	0.0000
Sox4	-0.807	0.0026	0.0178
Lgr5	-0.670	0.0017	0.0126
Sox9	-0.635	0.0057	0.0337
Cdx2	-0.361	0.0145	0.0700
Trim28	-0.259	0.0191	0.0858
Lrig1	0.402	0.0096	0.0511
Prom1	0.906	0.0000	0.0000
Cd44	1.559	0.0000	0.0000
Runx1	2.974	0.0000	0.0001

*Genes in ISC signature (Muñoz et al, 2012)

Figure EV3. p65 and H3K27me3 ChIP-sequencing analysis showed no differences along genomic regions.

A Representation of p65 distribution (from ChIP-sequencing, $n = 2$ WT and $n = 2$ KO) in the indicated genomic regions.

B Graph showing the distribution of H3K27me3 peaks in the indicated genomic locations in I κ B α WT and I κ B α KO.

C Graph showing the chromosomal distribution of H3K27me3 peaks in I κ B α WT and I κ B α KO.

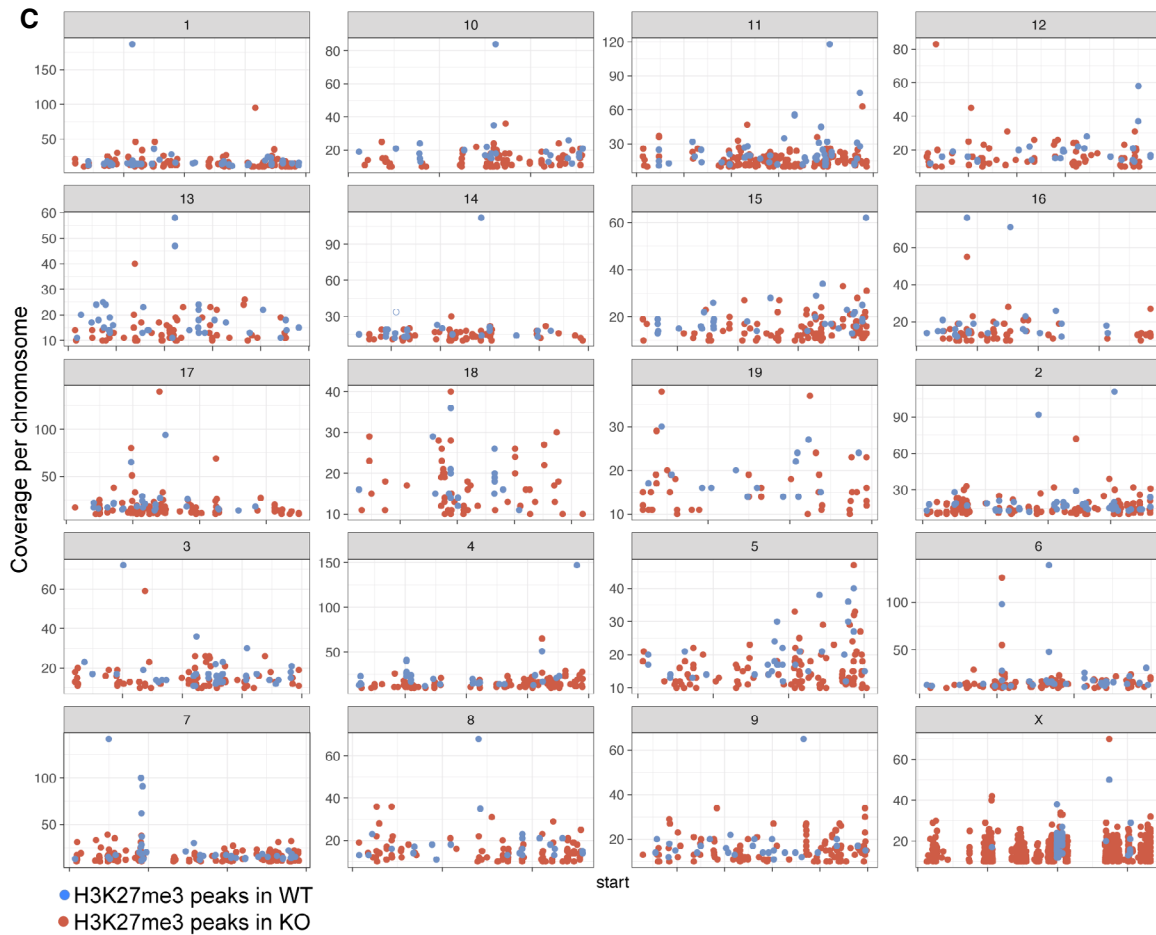
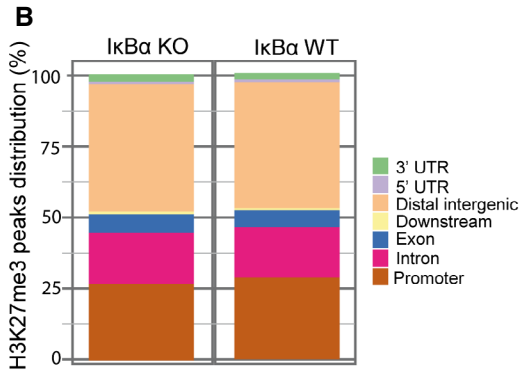
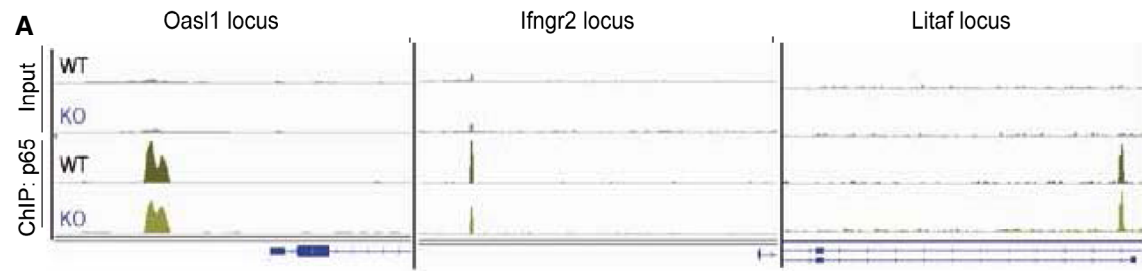


Figure EV3.

Figure EV4. P-I κ B α is expressed in organoid pockets, and its deficiency leads to transcriptional alterations in differentiation markers and pathways, which are NF- κ B- and Wnt/ β -catenin-independent.

- A IF analysis of P-I κ B α in WT organoids.
- B, C Selection of differentially expressed genes associated with intestinal lineages (B) and molecular pathways (C) identified by microarray assay from WT and I κ B α KO-derived organoids. Five different organoids per genotype were analyzed, which were generated from at least 2 different mice per genotype.
- D Validation of relevant genes by qPCR. Three technical replicates of a minimum of two organoids per condition were analyzed. Bars represent mean values \pm standard error of the mean (s.e.m.); *P* values were derived from an unpaired *t*-test, two-tailed, *****P*-value < 0.0001, ****P*-value < 0.0005, ***P*-value < 0.005.
- E Representative stereoscopic images of I κ B α KO organoids treated as indicated.
- F Table indicating the Wnt/ β -catenin target genes [44] differentially expressed between I κ B α WT and KO organoids (expression microarray, *n* = 5 WT and *n* = 5 KO). In red, Wnt target ISC genes.

Data information: Scale bars, in A and E, 50 μ m.

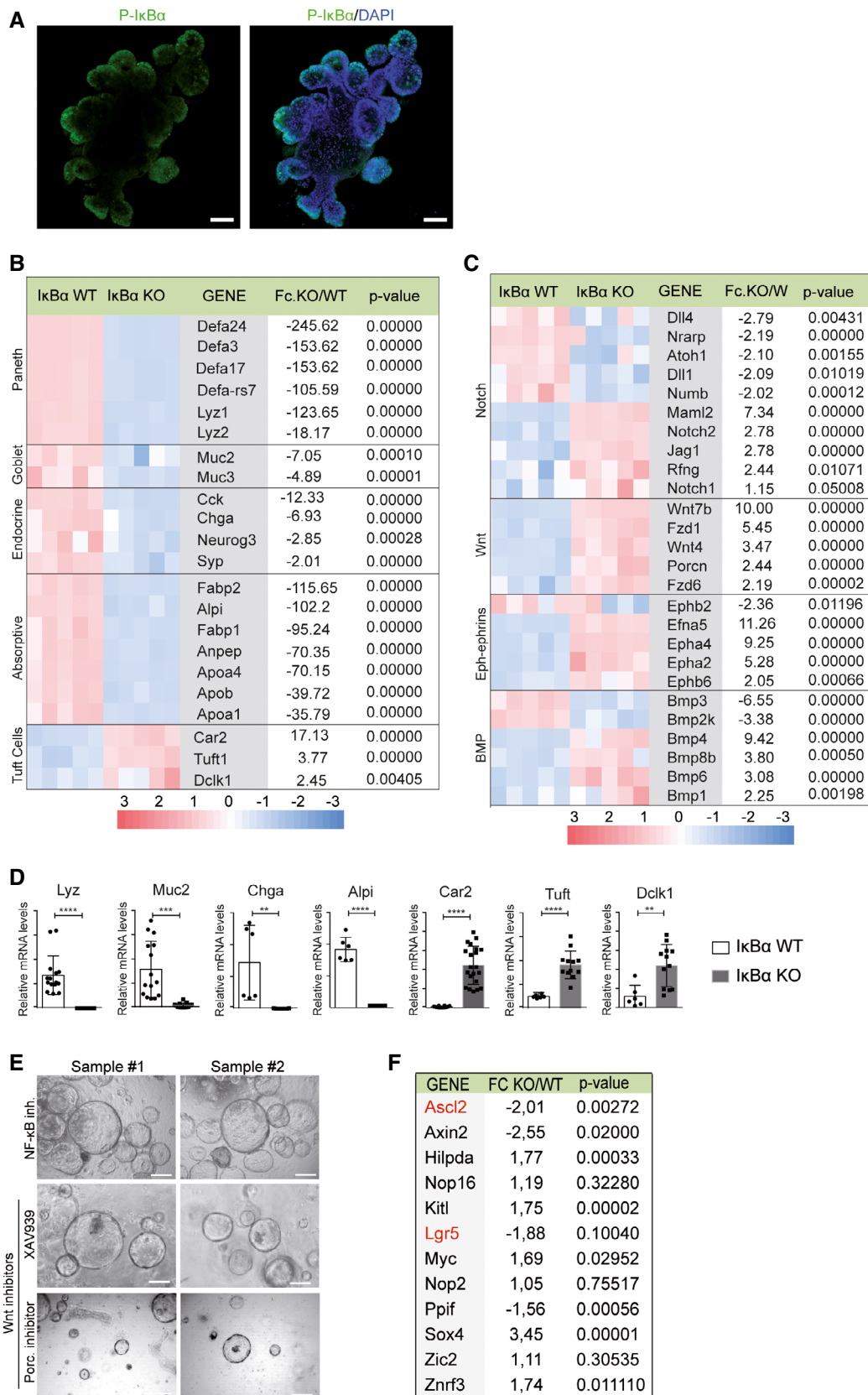


Figure EV4.

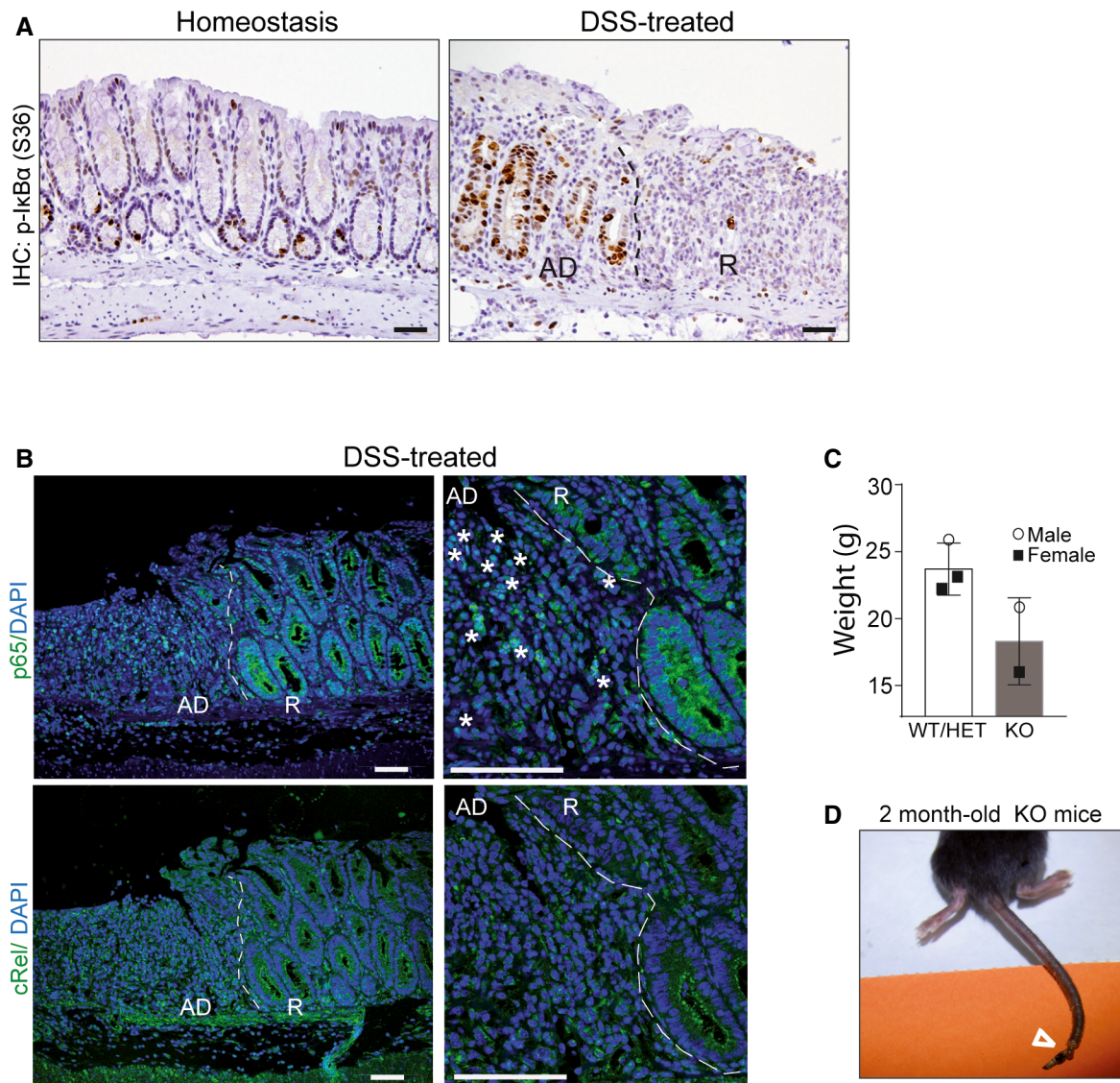


Figure EV5. P-IκBα is lost in damaged areas, where NF-κB pathway is active, and accumulated in regenerating crypts.

- A IHC analysis of P-IκBα (S36) in the colonic tissue of control and DSS-treated mice including acute damaged (AD) areas and recovery (R) areas.
- B IF analysis of the NF-κB subunits p65/RelA and c-Rel in areas of acute damage and recovery. Asterisks indicate the presence of nuclear p65 in acute damage areas adjacent to the recovery region. The pictures show acute damaged (AD) areas and recovery (R) areas.
- C, D Graph indicating the reduced weight (A) and representative image of the skin defects (see arrowhead) (D) observed in the 2-month-old IκBα KO mice. Bars represent mean values ± standard error of the mean (s.e.m.).

Data information: Scale bars, in A and B, 50 μm.