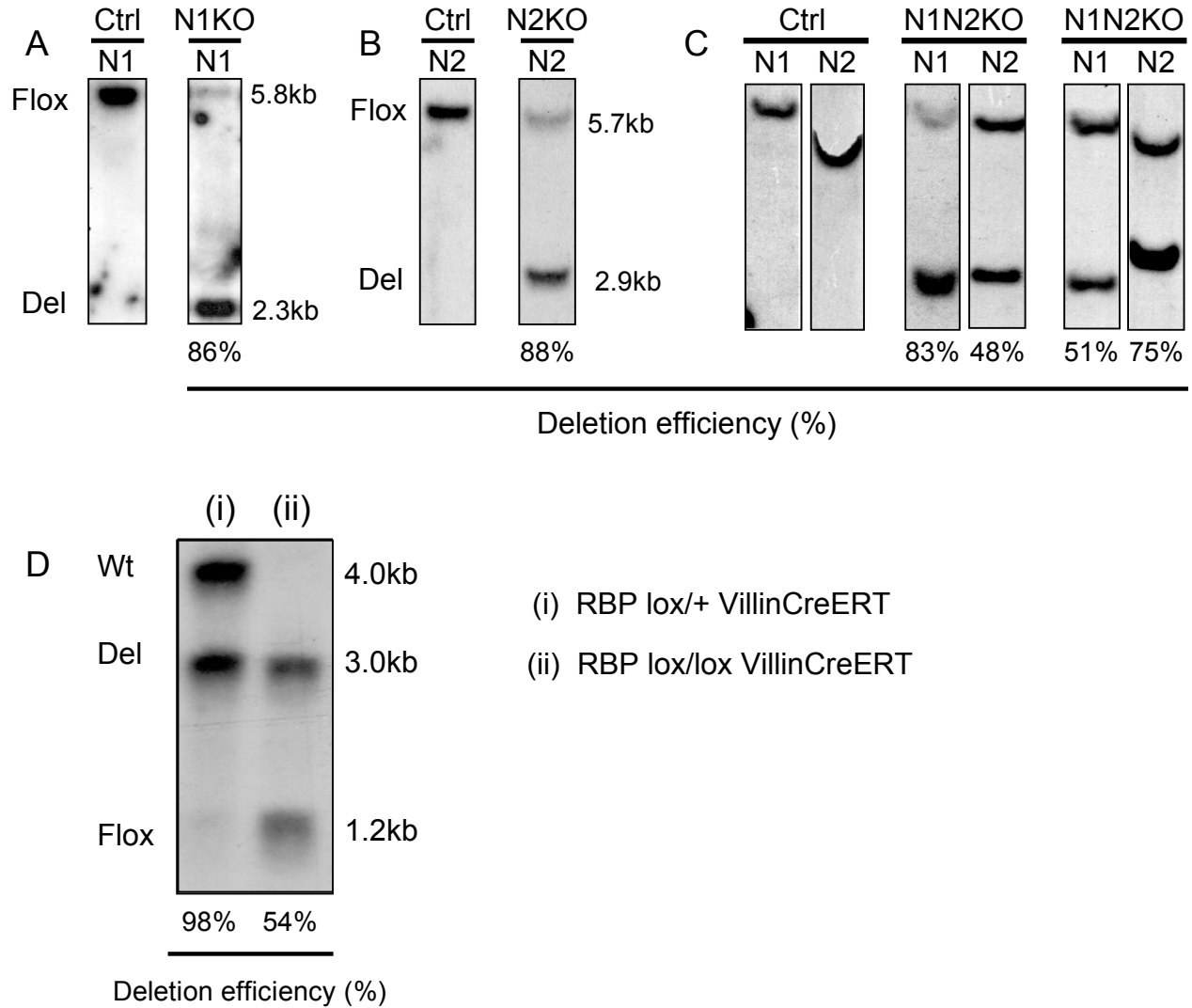


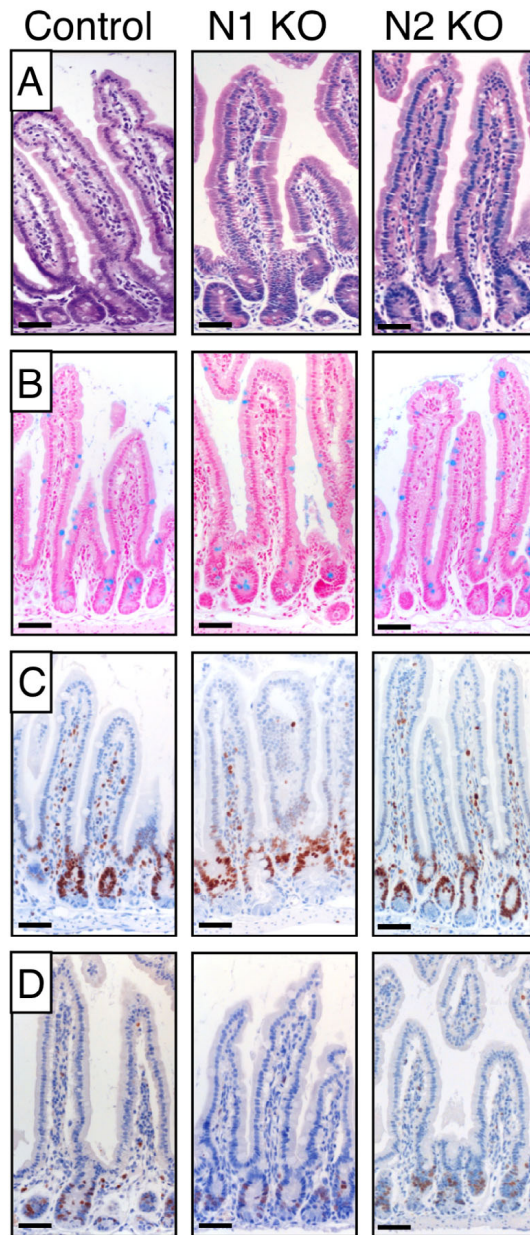
Supplementary information



Supplementary Figure 1

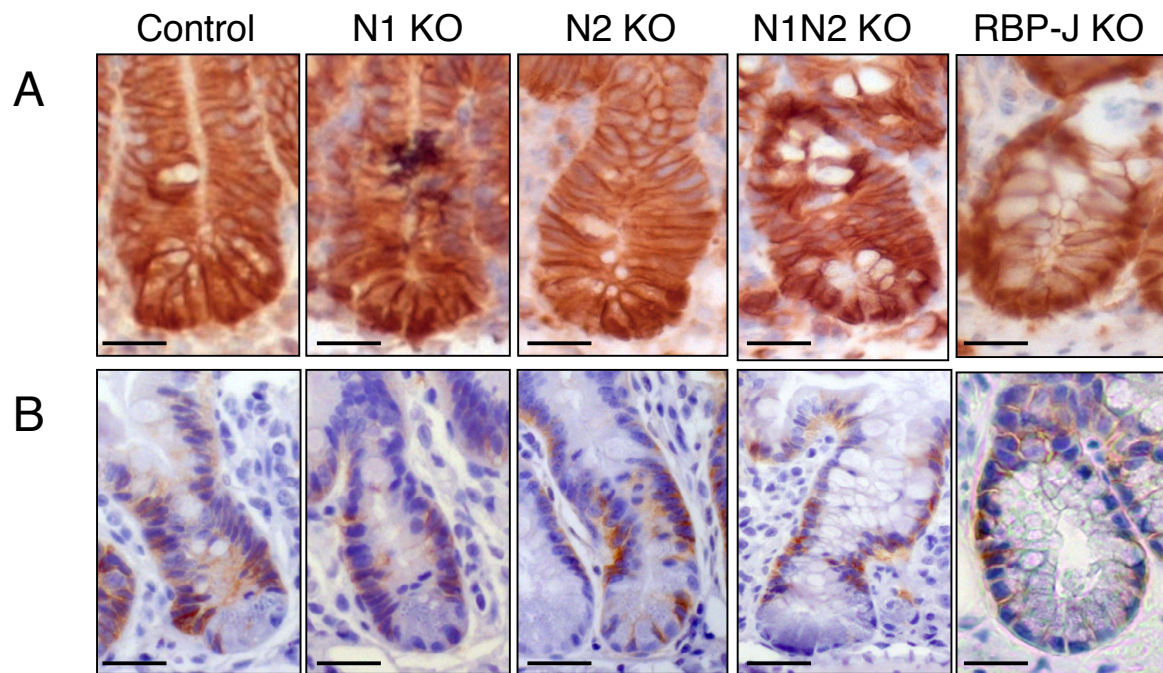
Southern blot analysis of genomic DNA from the small intestine of litter mate controls (Ctrl) and (A) gut specific Notch1 knockout mice (N1KO), (B) gut specific Notch2 knockout mice (N2KO), (C) two Notch1Notch2 double knockout mice (N1N2KO) and (D) gene-targeted mice for the RBP-J gene. (i) and (ii): corresponding genotypes; N1 and

N2: Southern blot analysis of the corresponding loci; Wt: wild type allele, Flox: floxed alleles and Del: inactivated alleles of the corresponding gene-targeted mice. The deletion efficiency of individual alleles is indicated and has been quantified using a Phosphorimager. The deletion efficiency of one RBP-J allele in the genetic background of a wild type allele is very efficient (98%). These mice do not have a phenotype. However, inactivation of RBP-J in mice carrying homozygously floxed alleles results in the conversion of crypt progenitor cells into post mitotic goblet cells (Figure 2). The deletion efficiency of 50% for the floxed RBP-J alleles is sufficient to cause this dramatic phenotype, which suggests a counter selection against the complete loss of RBP-J function. Mice with gut-specific gene inactivation of either only Notch1 or Notch2 do not show any phenotype despite a good deletion efficiency (86% and 88%). Mice with a simultaneous inactivation of Notch1 and Notch2 exhibit a reduced deletion efficiency, which is nevertheless sufficient to recapitulate the phenotype of the RBP-J gene-targeted mice.



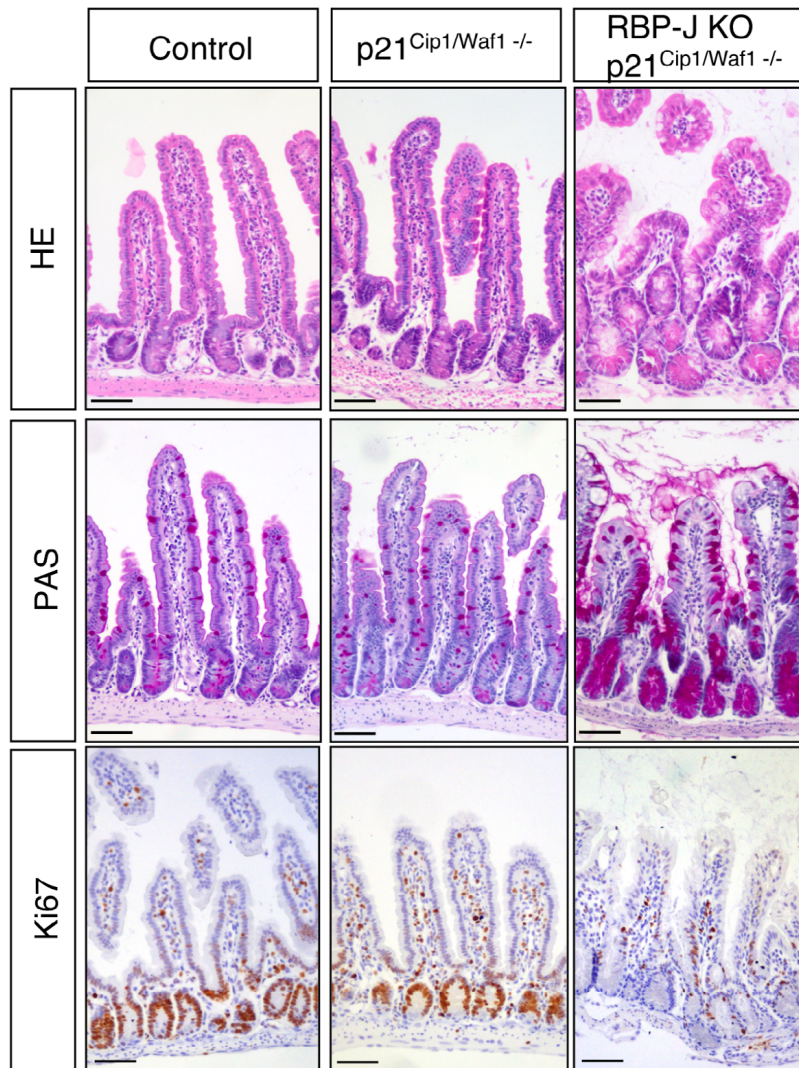
Supplementary Figure 2

Redundant function of Notch1 and Notch2 signaling within the small intestine. Representative sections of the small intestine from control, N1 KO, and N2 KO stained with (A) hematoxylin and eosin (H&E), (B) with Alcian Blue (AB) for goblet cell identification, (C) with antibodies against Ki67 and (D) BrdU to identify proliferating crypt progenitors. Scale bars = 50µm.



Supplementary Figure 3

Loss of Notch signaling does not change β -catenin and wnt target gene expression in crypts of the small intestine. Immunohistochemical analysis of small intestine sections derived from control, N1 KO, N2 KO, N1N2 KO, and RBP-J KO mice stained with antibodies against (A) β -catenin and (B) CD44. Scale bars = 25 μ m.



Supplementary Figure 4

Histological analysis of small intestines derived from RBP-J KO/ p21^{Cip1/Waf1} -/- double deficient mice. Small intestine sections from RBP-J KO/ p21^{Cip1/Waf1} -/-, p21^{Cip1/Waf1} -/- and wild type mice were analyzed by HE, PAS, and Ki67 immunostaining. Scale bars = 50 μ m.

Methods

Animals

The following intestine specific gene-targeted mice were generated by crossing floxed Notch1 (Radtke et al., 1999), floxed Notch2 (Besseyrias et al., 2007) or floxed RBP-J (Han et al., 2002) mice, with mice carrying the *vil-Cre-ERT2* transgene (el Marjou et al., 2004). The Cre-ERT2 recombinase was activated by intraperitoneal (i.p.) injection of tamoxifen (10mg/kg body weight) into 10-14 day old mice for 5 consecutive days. Mice deficient for *p21^{Cip1/Waf1}* (Brugarolas et al., 1995; Deng et al., 1995) or for *p27^{Kip1}* (Fero et al., 1996) were crossed with floxed RBP-J *vil-Cre-ERT2* mice or injected with 30µmol/kg of dibenzazepine i.p. (DBZ, (Milano et al., 2004)). Two hours prior to sacrifice, floxed and control mice were injected with BrdU i.p. (100 µg/g of body weight).

Immunohistochemistry, antibodies and in situ hybridization

Following antibodies were used: mouse anti-Ki67 (1:100, Novocastra), rat anti-BrdU (1:500, Oxford Biotech), mouse anti-β-catenin (1:50, Transduction Labs), rat anti-CD44 (1:250, Bender MedSystems), mouse anti-p27^{Kip1} (1:250, Transduction Labs), goat anti-p57^{Kip2} (1:100, Santa Cruz), guinea-pig anti-Hes1 (1:200, a kind gift from Prof. R. Kageyama, University of Kyoto) and rat anti-Hes1 (1:200, MBL int.). The Envision+ kit (DakoCytomation) was used as a secondary reagent for mouse antibodies. For anti-goat or anti-guinea pig antibodies, a bridge was created using a biotinylated rabbit-anti-goat (1:600, Vector) or an anti-guinea pig polyclonal antibody (1:300, Jackson ImmunoResearch). To reveal these biotinylated antibodies, streptavidin-HRP (1:1000, Amersham), was used. The secondary antibody for the rat antibody was goat-anti-rat Ig

(1:250, Biosource) directly coupled to horseradish peroxidase (HRP). Slides were developed using DAB and counterstained with hematoxylin. *In situ* hybridization were performed as described (Moorman et al., 2001). The probes used for the *in situ* hybridization were as described (Schroder and Gossler, 2002).

Laser Capture Microdissection and Quantitative RT-PCR

Notch1/Notch2 and RBP-J deficient mice as well as littermate controls, were sacrificed and the intestinal tracts removed, flushed with cold RNase free PBS, embedded and frozen in OCT (Sakura). Ten μm cryo-sections were cut and placed on special membrane-based slides for laser microdissection (Leica Microsystems). Slides were thawed for 5 sec and dipped in 70% EtOH for 1 minute. Sections were counterstained with eosin and washed out with 70% EtOH for 30 seconds with slight agitation. This was followed by a 30 sec incubation in 95% EtOH then 1 minute in 100% EtOH. Finally, sections were dehydrated in xylol for 2 minutes. Crypts from KO and wt animals were laser cut under a Nikon Eclipse TE200 microscope and tissue fragments were collected. RNA was extracted from the microdissected crypts and isolated using the RNAeasy Mini kit (Qiagen) following the manufacturer's instructions. RNA amplification was performed using the MessageAMPTM II aRNA Amplification kit (Ambion) following the manufacturer's instructions. For reverse transcription, M-MLV Reverse Transcriptase kit (Invitrogen) was used. Gene expression was assessed using the TaqMan real-time PCR method. The primers were obtained from Applied Biosystems. Data were analyzed using standard statistical software (Chi square test). $P < 0.05$ was regarded as significant.

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