Differential regulation of β-catenin-mediated transcription via N- and C-terminal co-factors governs identity of murine intestinal epithelial stem cells

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The homeostasis of the gut epithelium relies upon continuous renewal and proliferation of crypt-resident intestinal epithelial stem cells (IESCs). Wnt/β-catenin signaling is required for IESC maintenance, however, it remains unclear how this pathway selectively governs the identity and proliferative decisions of IESCs. Here, we took advantage of knock-in mice harboring transgenic β-catenin alleles with mutations that specifically impair the recruitment of N- or C-terminal transcriptional co-factors. We show that C-terminally-recruited transcriptional co-factors of β-catenin act as all-or-nothing regulators of Wnt-target gene expression. Blocking their interactions with β-catenin rapidly induces loss of IESCs and intestinal homeostasis. Conversely, N-terminally recruited co-factors fine-tune β-catenin’s transcriptional output to ensure proper self-renewal and proliferative behaviour of IESCs. Impairment of N-terminal interactions triggers transient hyperproliferation of IESCs, eventually resulting in exhaustion of the self-renewing stem cell pool. IESC mis-differentiation, accompanied by unfolded protein response stress and immune infiltration, results in a process resembling aberrant “villisation” of intestinal crypts. Our data suggest that IESC-specific Wnt/β-catenin output requires selective modulation of gene expression by transcriptional co-factors.
Intestinal epithelial stem cells (IESCs) fuel the perpetual replenishment of the epithelial monolayer that lines the gut. They divide on average once a day to give rise to cycling progenitors (transit amplifiers, TA), which undergo several rounds of cell division before differentiating into cells of the absorptive (enterocytes) and the secretory lineage (Paneth, goblet, tuft, and enteroendocrine cells). Wnt/β-catenin signalling is required for the maintenance of the IESC pool. However, to date, the gene regulatory networks governing the behavior of IESCs remain elusive. The activity level of the Wnt pathway alone cannot explain the cell-type-specific proliferative rate and self-renewal ability of IESCs, TAs, and Paneth cells. Hence, differential regulation of Wnt-target gene expression may determine the identity of the self-renewing pool of IESCs.

A common extracellular signal may be translated into distinct cellular responses, and fate decisions, via the expression of specific cell-autonomous effectors. In the context of Wnt signalling, transcriptional co-factors that interact with β-catenin have been repeatedly implicated in tissue- and cell type-specific modulation of Wnt responses. C-terminal β-catenin-interactors, such as CREB-binding protein (CBP) and p300, as well as members of the Mediator and of the RNA polymerase II-associated factor 1 (PAFI) complexes, are promiscuous co-activators that interact, besides with β-catenin, with several other transcription factors. Conversely, B–cell lymphoma 9 (BCL9) and the paralog BCL9-like (BCL9L) specifically bind an N-terminal moiety of β-catenin, and act as tissue-specific transcriptional effectors. While dispensable for normal intestinal homeostasis, BCL9/9L are required for intestinal regeneration upon insults, indicating an essential role of these mutations for the maintenance of intestinal homeostasis. Using mouse models of colorectal cancer, we previously generated transgenic β-catenin (Ctnnb1) alleles that harboring mutations that prevent interactions with N- or C-terminal β-catenin’s co-factors, we show how differential regulation of Wnt/β-catenin signalling does govern IESC identity and fate. While C-terminal co-factors act as an all-or-nothing switch for Wnt-target gene expression, N-terminal co-factors are responsible for selective transcriptional modulation ensuring proper proliferation and self-renewal of IESCs.

Results

Attenuation of N- vs. C-terminal β-catenin transcriptional outputs in the intestinal epithelium has distinct phenotypic impacts. We previously generated transgenic β-catenin (Ctnnb1) alleles harboring mutations that prevent interactions with N- or C-terminal transcriptional co-factors (NTFs and CTFs, respectively). The ΔC164A mutation abrogates interaction with NTFs, while the ΔC truncation abrogates the interaction with the CTFs. To overcome the embryonic lethality of these alleles, we used compound heterozygous mice carrying one mutant and one conditional β-catenin allele (Supplementary Fig. 1a). Similarly, to constitutively hemizygous Ctnnb1\\text{KO}/\\text{wt} mice, Ctnnb1\\text{ΔC164A}\text{fl}\text{ox} and Ctnnb1\\text{ΔCfl}\text{ox} animals show no overt abnormalities, indicating haplosufficiency of β-catenin for the maintenance of intestinal homeostasis. Combination with the villin-CreERT2 driver enables the inducible deletion of the conditional β-catenin allele (Ctnnb1\\text{fl}\text{ox}) specifically in the intestinal epithelium, thus leaving the Wnt-transcriptional outputs solely under the control of mutant β-catenin (D164A or ΔC). While villin-CreERT2;Ctnnb1\\text{wt}\text{fl}\text{ox} (control) mice are viable and indistinguishable from homozygous wild-type (wt) animals, the sole presence of mutant β-catenin is lethal. Villin-CreERT2;Ctnnb1\\text{ΔC164A}\text{fl}\text{ox} (ΔC) animals exhibit atrophic crypts and reach humane endpoint 4 days after CreERT2 induction (4d post-induction (pi)) (Supplementary Fig. 1b). This is in accordance with our previous results in villin-CreERT2;Ctnnb1\\text{D164A}\text{fl}\text{ox} animals, which express double mutant (dm) β-catenin harboring both N- and C-terminal mutations. Villin-CreERT2;Ctnnb1\\text{ΔC164A}\text{fl}\text{ox} (D164A) animals only reach humane endpoint at 7d pi, and suffer from severe colitis (Supplementary Fig. 1c). Thus, neither C- nor N-terminally mutated β-catenin is haplosufficient in the intestinal epithelium, as these mutants are not able to substitute for the wt allele. However, the distinct phenotypic impacts of these mutations suggest different roles of the C- and N-terminal branches of β-catenin-mediated transcriptional outputs, which we set out to investigate.

We confirmed full recombination of the floxed β-catenin allele in the mutant intestinal epithelium 2d pi (Supplementary Fig. 1d). Consequently, and consistent with the rapid turnover of intestinal epithelial cells, we observed depletion of wt β-catenin protein from crypts 2d pi (Supplementary Fig. 1e). Thus, 2d pi, crypts of ΔC, D164A, and dm animals only harbor mutant β-catenin, which is present at cytosolic and nuclear levels comparable to those of wt β-catenin (Supplementary Fig. 1f). Importantly, crypt and villus integrity remained unaltered. Indeed, as previously reported for the β-catenin-dm animal, the observed phenotype is entirely connected to transcriptional outputs, and not attributable to loss of epithelial adhesiveness, as in the case of complete β-catenin loss. In fact, mutant β-catenin co-localizes with epithelial cell adhesion molecule (Epcam) at the cell membrane (Supplementary Fig. 1g).

We performed RNA sequencing of bulk preparations of small intestinal epithelium isolated 2d pi from control, ΔC, D164A, dm, and KO animals. Principal component analysis indicated surprising differences in impairing N- versus C-terminal interactions on the epithelial transcriptome (Fig. 1b). The differentially expressed genes (DEGs, logFC > [2]; \( p < 0.05 \)) of ΔC animals broadly overlapped with those of dm and full knock-out (KO) animals, indicating that C-terminally mutated β-catenin is not able to sustain Wnt signalling levels required for proper renewal of the intestinal epithelium (Fig. 1c and Supplementary Fig. 2a). Indeed, attenuating C-terminal β-catenin transcriptional outputs in the intestinal epithelium causes a loss of IESCs, and attrition of the crypts (Fig. 1d), gene set enrichment analysis (GSEA, Supplementary Fig. 2b), as well as protein stainings and RNA in situ hybridization (Fig. 1e–g). Downregulation of bona fide Wnt target genes in ΔC, dm, and KO animals 2d pi was confirmed by qRT-PCR (Supplementary Fig. 2c).

Contrary to what was observed in ΔC mice, the transcriptomic changes induced in β-catenin-D164A animals (i.e., N-terminal mutant) only minimally overlapped with those induced by the loss of β-catenin (Fig. 1c and Supplementary Fig. 2a). Of note, the exclusivity of DEGs in D164A-mutants can be partially attributed to a D164A-specific enrichment of genes expressed by infiltrating immune cells (Supplementary Fig. 2d). As opposed to what we observed in ΔC crypts, the expression of Wnt targets, IESC genes and proliferation markers, was significantly increased in D164A crypts 2d pi (Fig. 1e, f, and Supplementary Fig. 2c). Moreover, D164A mutant crypts displayed an increase of the Lgr5 + area, as shown by single-molecule fluorescent in situ hybridization (smFISH), indicating an expansion of the IESC compartment.
Furthermore, both the presence of Ki67+ cells at the bottom of D164A crypts (white arrows in Fig. 1e, and evidenced in Supplementary Fig. 2f) and upregulated Myc expression at crypt base (Fig. 1h) indicate increased IESC proliferation. These results indicate that preventing β-catenin’s interactions to CTFs completely represses the Wnt-outputs, including proliferation and IESC-associated genes, hence compromising stem cell maintenance. On the contrary, attenuating β-catenin’s N-terminal transcriptional outputs increases the proliferation of IESCs and results in an expansion of the stem cell compartment.

Of note, despite the villin-driven CreERT2 being active along the gastrointestinal epithelium, we found neither morphologic nor proliferative changes in the colonic epithelium of β-catenin mutant animals at 2 or 4d pi (Supplementary Fig. 3a, b). This is most likely due to the slower renewal dynamics of the colonic...
epithelium20. Moreover, high E-cadherin levels in the colon act as a sink for \( \beta \)-catenin, delaying the effects of introduced mutations21. We therefore focused our investigation on the small intestinal epithelium.

Intestinal crypts lacking the output via N-terminal recruited co-factors exhibit stem cell loss and secretory hyperplasia 4d pi.

We had not observed any overt phenotype of goblet cells or Paneth cells in \( \Delta C \) or D164A animals 2d pi (Supplementary Fig. 3c, d). Unexpectedly, 4d pi, crypt morphology in D164A was profoundly altered by the appearance of large granular cells that lacked expression of Wnt-target genes (Axin2), as well as proliferative (Ki67) and IESC (Olfm4) markers (Fig. 2a). These cells express the secretory lineage marker Sox9 (Fig. 2a), and are double positive for lysozyme (Lyz) and mucins (stained by alcian blue, AB) (Fig. 2b). Moreover, we observed Lyz+ cells mis-localized to the villus of D164A animals (Fig. 2b). These cells are reminiscent of intermediate cells, which share both Paneth (lysozyme) and goblet cell traits (mucins), are the result of stem cell mis-differentiation, and have been observed upon experimental perturbation of Notch, Wnt, or EGF signalling in the intestinal epithelium22–26. However, the molecular mechanisms underlying their appearance in the tissue is largely unknown. Interestingly, a shift towards secretory lineage differentiation was observed upon deletion of N-terminal co-factors BCL9/9L in APCmin tumors15, suggesting that NTFs might repress specification of the secretory lineage.

Taken together, the results presented so far indicate that, similar to the complete deletion of \( \beta \)-catenin, blocking its CTF recruitment rapidly caused a lethal loss of stem cells (Fig. 2C). On the other hand, impairing interaction with BCL9/9L triggers transient hyperproliferation of IESCs, followed by their aberrant differentiation. The profoundly distinct cellular dynamics leading to crypt collapse upon impairment of N- vs. C-terminal \( \beta \)-catenin outputs, thus suggest independent roles of these transcriptional branches in maintaining IESC homeostasis.

Hyperproliferative IESCs expressing only Ctnnb1\(^{D164A}\) have an increased ability to form organoids. Recent work indicated that intestinal organoids could not be established from BCL9/9L-deficient crypts isolated 4d pi15. However, we hypothesized that expansion of the proliferative IESC compartment in D164A crypts observed 2d pi would translate into increased organoid formation rate. To test this, we devised an organoid formation assay (Fig. 3a): we isolated intestinal crypts from villin-CreERT\(^2\), Ctnnb1\(^{wt/fox}\) (control) and villin-CreERT\(^2\), Ctnnb1\(^{D164A/fox}\) (D164A) animals 0, 2, and 4d post CreERT\(^2\) induction, seeded equal amounts in Matrigel and quantified the number of organoids formed 7 days later. Strikingly, D164A crypts isolated 2d pi exhibited a 1.6-fold increase in organoid formation rate compared to non-induced (Ctnnb1\(^{D164A/fox}\)) crypts, and a 10-fold increase compared to the respective control (Fig. 3b). Morphologically, organoids derived from hyperproliferative crypts showed increased cell proliferation and budding (Fig. 3c). Consistent with the transient nature of the hyperproliferation observed in vivo, organoid lines obtained from D164A crypts 2d pi cannot be maintained in culture, and died upon first passaging (post splitting panels, Fig. 3c). Crypts isolated from D164A animals 4d pi failed to establish organoids in vitro, in agreement with the loss of stem cell and proliferation markers observed in the tissue (Fig. 3b). In sum, these results in vitro recapitulate and functionally underscore our observations in vivo.

We wondered why control crypts isolated 2d and 4d pi showed a drastic reduction in organoid formation rate compared to unrecombined crypts (Fig. 3b). We found that, in contrast to the in vivo situation, \( \beta \)-catenin is not haplosufficient in vitro: crypts isolated from constitutively hemizygous mice (Ctnnb1\(^{K0/wt}\)), which show no overt phenotype, fail to establish intestinal organoids (Supplementary Fig. 3a). Moreover, crypts isolated from Ctnnb1\(^{AC/fox}\) and Ctnnb1\(^{dm/fox}\) animals also fail to grow in vitro (Supplementary Fig. 4a), indicating that two copies of transcriptionally active \( \beta \)-catenin alleles are necessary to maintain Wnt-target gene expression required by intestinal crypts. We established organoid cultures from non-injected villin-CreERT\(^2\), Ctnnb1\(^{wt/fox}\) (control) and villin-CreERT\(^2\), Ctnnb1\(^{D164A/fox}\) (D164A) animals, and induced recombination in vitro. In contrast to organoids lacking the villin-CreERT\(^2\) driver, both control and D164A organoids died within 7 days after 4OHT addition, and quickly lost expression of markers indicative of Wnt signalling, stemness or proliferation (Supplementary Fig. 4b–d). This behavior could not be rescued by the addition of Wnt3a-conditioned medium to the culture prior to 4OHT addition (Supplementary Fig. 4e). The differential Wnt/\( \beta \)-catenin-signalling requirements of intestinal crypts in vitro might be due to the fact that organoids lack a mesenchymal support niche, and that they closely resemble a regenerative, rather than a homeostatic state of intestinal crypts.

Myc-E2F-driven proliferation leads to exhaustion of the stem cell pool in N-terminal mutant (D164A) animals. To dissect the gene regulatory networks underlying the transient hyperproliferation of IESCs triggered by impaired \( \beta \)-catenin-NTF interactions, we performed droplet-based single-cell RNA sequencing (scRNAseq) of small intestinal crypts isolated from control and D164A animals at 0, 2, and 4d pi. We focused our analysis on the gene expression changes occurring over time in stem cells and early progenitors (SCEP cells), which we annotated manually based on marker gene expression (Supplementary Fig. 5a, b). Differential gene expression analysis confirmed our previous results, indicating that IESCs and proliferation markers (e.g., Olfm4 and Mki67) are upregulated in D164A SCEP cells 2d pi.
compared to controls, while their expression is lost 4d pi (Supplementary Fig. 5c, d). To disentangle the transcriptomic effects caused by impaired N-terminal interactions from those arising due to the acute loss of the conditional β-catenin allele, we devised a normalization procedure using the control time series data (Methods). The resulting normalized data thus represent the transcriptomic changes occurring in SCEP cells, over time, upon impairing β-catenin-NTF interactions.

Confirming the data presented above, D164A SCEP cells exhibited transient upregulation of IESC and proliferation markers 2d pi (Olfm4, Mki67, Ccnd1), followed by increased expression of differentiation markers 4d pi (Ada, Apoa4, Apoa1, Axin2, E-cadherin).
This is highlighted by diffusion maps as a shift along the stem cell differentiation continuum: SCEP cells isolated 2d pi have a significantly more "stem-like" distribution, whilst SCEP cells from D164A crypts 4d pi are more shifted towards differentiation (Fig. 4a, b). We also identified intermediate secretory cells in D164A within SCEP cells 4d pi by their co-expression of Paneth and goblet cell features (Supplementary Fig. 5e).

The upregulation of stem cell markers 2d pi was accompanied by a significant increase in the mean expression levels of cell cycle-related genes (Fig. 4c). Of note, this behavior of D164A SCEP cells was not observed in other cell types with

**Fig. 3 Hyperproliferative IESCs expressing only Ctnnb1D164A have an increased ability to form organoids.** a Experimental design of organoid formation assay. b Organoid formation rate (in %) of control (villin-CreERT2;Ctnnb1wt/flx) and mutant (villin-CreERT2;Ctnnb1D164A/flx) crypts isolated 0, 2 and 4d pi. ****p < 0.0001, **p = 0.0088, *p = 0.0207, as calculated by unpaired, two-tailed Student's T-test (n = 3). Barplot indicates mean rate. c Brightfield images of control and mutant organoids formed from crypts isolated 2d pi grown in Matrigel for 10 days. Insets show higher magnification. Representative images of three biological replicates. Scale bars, 20 μM. d Confocal images of crypts isolated 2d pi and grown in Matrigel for 7 days. EdU staining shows proliferating cells. E-cadherin (cell shape) and DAPI (nuclei) as counterstain. Scale bar, 20 μM. Representative images of three biological replicates.
Fig. 4 Myc-E2F-driven proliferation is followed by stress signalling and loss of stem cells in N-terminal mutant (D164A) crypts. a Diffusion map of stem cells and early progenitors (SCEP) cells isolated from D164A (villin-CreERT2;Ctnnb1D164A/fl, n = 3) crypts 0, 2 and 4d pi and normalized by respective control (villin-CreERT2;Ctnnb1wt/fl, n = 3). Cells (dots) colored by timepoint after Cre induction (days pi). Diffusion component 1 (DC1) represents differentiation pseudotime. On the right, cells colored by their average expression of stem cell (top) and differentiation marker genes (bottom). b Distribution of DC1 score in normalized D164A SCEP cells over time (**p < 2.2e−16, two-sided Wilcoxon test). c Distribution of cell cycle score (average expression of cycling genes) in normalized D164A SCEPs over time (**p < 2.2e−16, two-sided Wilcoxon test). d Expression profile of stem cell and proliferation marker genes in normalized SCEP cells ordered along supervised pseudotime (psupertime). Cells (dots) colored by timepoint. e Gene set expression analysis (GSEA) on time-varying genes in SCEPs across timepoints. Normalized enrichment score (y-axis) over time (x-axis) of MSigDB gene sets. Dot size indicates the size of the enriched gene set. Dot color indicates p value of enrichment, as calculated by the hypergeometric test. Selected gene sets labeled (identifiers in the text). f Distribution of predicted model responses of logistic regression showing a shift of normalized SCEP cells 2d pi from intestinal epithelial stem cells (IESC) to transit amplifier (TA) traits. ***p < 2.2e−16, as calculated by two-sided Kolmogorov-Smirnov test.
active Wnt signalling, such as in Paneth cells or enterocytes (Supplementary Fig. 5f). Supervised pseudotime analysis confirmed that stem cell and proliferation markers exhibit a similar expression profile over time, increasing 2d pi, and subsequently dropping 4d pi (Fig. 4d). We had observed increased Myc expression in D164A crypts 2d pi (Fig. 1h), so we therefore asked whether Myc-driven proliferation was inducing increased cell cycle rate of SCEP cells. Indeed, at 2d pi, Myc-dependent Wnt-targets were significantly enriched in DEGs between 0d and 2d pi (Myc-dependent APC-targets obtained from ref. 30). MisSigDB code M1757, normalized enrichment score (NES) = 1.68, p < 0.05) (Fig. 4e). Moreover, the signature of E2 factor (E2F)—a major cell cycle regulator, and a Myc-target—was significantly enriched (M5901, NES = 1.56, p < 0.05) together with genes involved in cell cycle (M14460, NES = 1.65, p < 0.05) and G2M checkpoint (M5901, NES = 1.72, p < 0.05) (Fig. 4d). These proliferation signatures were all significantly depleted 4d pi (NES < −1, p < 0.05), reflecting an abrupt proliferative arrest (Fig. 4c, d).

We wondered whether the observed Myc-E2F-driven proliferation burst induced an en-bloc conversion of D164A IESCs to TAs, which are known to exhibit higher expression of proliferative Wnt targets. As our mouse models don’t harbor any IESCs reporter (e.g., Lgr5-EGFP) that enables the identification of stem cells, we tested this hypothesis computationally. We trained a logistic regression model on TAs and IESCs obtained from a publicly available single-cell dataset of murine small intestinal epithelium, and tested SCEP cells from 0d and 2d pi against this model. Comparison of the distribution of predicted model responses from the two timepoints shows a significant shift of 2d pi cells towards TA traits (Fig. 4F). Cumulatively, our results indicate that impairing β-catenin-NTF interactions transiently activates a Myc-E2F-driven proliferation program in IESCs, which results in exhaustion of the self-renewing IESC pool.

Cell-intrinsic and environmental stress signals induce mis-differentiation. GSEA of time-varying genes in SCEP cells suggests an increase in stress signaling in crypts upon impairment of β-catenin-NTF interactions. Indeed, key mediators of the unfolded protein response (UPR)—Xbp1, Creb3, and Creb3l3—are feature among the most strongly upregulated transcription factors in D164A SCEP cells over time (Supplementary Fig. 5h). We confirmed the ectopic expression of Creb3l3 in D164A crypts 4d pi using smFISH (Fig. 5a). Creb3l3 is a strongly zonated gene; its expression increases along the crypt-villus axis, with peak expression at the villus tip. Moreover, expression of the UPR markers Hspa5 and Ddit3 in crypts 2d pi was significantly upregulated in D164A compared to controls, as independently measured by qRT-PCR (Fig. 5b). Finally, consistent with activation of UPR, genes involved in translation initiation and ribosomal function are significantly downregulated in D164A crypts (M27686, NES = −2.52, p < 0.05 and M189, NES = −3.26, p < 0.001, respectively) (Fig. 4e).

In addition to genes involved in cell-intrinsic stress, we also found cytokine-induced stress genes strongly upregulated in D164A SCEP cells over time. Indeed, signatures of Tumor Necrosis Factor α (TNFα) and Interferon γ (IFNγ) signalling were significantly enriched (M5913, NES = −2.32, p < 0.001 and M5890, NES = −2.08, p < 0.001, respectively). Moreover, the cytokine-induced genes Ifi7, Fos, Jun, and Jund featured among the most upregulated transcription factors according to our supervised pseudotime analysis (Supplementary Fig. 5h). Interestingly, we found a time-dependent upregulation of genes involved in endogenous antigen presentation (M16750, NES = 3.01, p < 0.001), specifically genes encoding for components of the MHC class II complexes (Figs. 4e and 5c).

Our scRNAseq data thus suggest that the crosstalk between the crypt epithelial and immune compartments increases upon impairment of β-catenin-NTF interactions. We profiled the intestinal immune infiltrate by flow cytometry and found that the number of CD45+ cells was more than doubled in D164A animals over time, but not in controls (Fig. 5e). Indeed, immunofluorescence analysis indicates a substantial infiltration of CD45+ cells in the pericryptic zone, as well as within crypts, of N-terminal mutant animals (Fig. 5d). Specifically, leukocyte profiling revealed a significant increase of CD4+ T cells, MHCII+ and MHCII− monocytes, as well as neutrophils, in D164A animals. Of note, ΔC mutants show unaltered CD45+ cell count and immune population composition at 2d pi (Fig. 5e and Supplementary Fig. 6a). It has recently been shown that T-cell-derived TNFα, IFNγ and interleukin 17 (IL-17) inhibit IESC renewal, and that excess interferon-γ signalling induces mis-differentiation towards the secretory fate. Upon restimulation with PMA and ionomycin, we observed a substantial, albeit not significant increase in TNFα, IFNγ and IL-17 secretion from CD4+ and CD8+ T cells isolated from D164A animals 4d pi (Supplementary Fig. 6b).

We asked whether the influx of immune cells was caused by a breach of the intestinal epithelial barrier, rather than cytokine-driven signals. To assay intestinal permeability, we administered fluorescein isothiocyanate-conjugated dextran (FITC-dextran) by oral gavage to ΔC, D164A and control mice 2d and 4d pi. Consistent with our previous observations indicating intact epithelial integrity, we found no significant difference (p > 0.01) in FITC-dextran concentration in the plasma of mutant animals (Fig. 5g).

Altogether, these results suggest that, following their hyper-proliferation induced by impaired β-catenin-NTF interactions, IESCs are subjected to combination of cell-intrinsic (UPR) and environmental (cytokines) stresses, which inhibit their self-renewal. Notably, antigen presentation is intrinsically linked to UPR, as Xbp1 is also a regulator of MHC class II genes. Loss of stem cells and aberrant differentiation of the crypt, accompanied by increasing immune infiltration, inevitably result in failure of epithelial homeostasis.

The chromatin landscape of D164A crypts reflects the transition to proliferative arrest and mis-differentiation. Our analyses revealed rapid and profound transcriptomic and morphological changes in the crypts upon impairment of β-catenin-NTF interactions (D164A mutant). We reasoned that profiling the chromatin landscape of D164A crypts would provide insight into the gene regulatory mechanisms responsible for such drastic reprogramming of epithelial cells. To this end, we performed Assay for Transposase-Accessible Chromatin using sequencing (ATACSeq) of control and D164A crypts isolated 2d pi. Differential peak analysis revealed that 1469 ATAC-peaks are differentially accessible (logFC > |1| & p < 0.01) in D164A crypts, compared to control crypts (Fig. 6a, b). We subjected these peaks to motif analysis with HOMER, and found a significant enrichment (Benjamini-corrected q-value < 0.001) of motifs associated with transcription factors involved in intestinal differentiation, such as hepatocyte nuclear factors (HNF4α,1,1b), ETS-related transcription factors (EHH, ELF3,5), Caudal Type Homeobox (Cdtx2,4), GATA-binding factors and Krüppel-like factors (Supplementary Fig. 7a). These findings correlate with increased expression of secretory and absorptive lineage markers in D164A crypts seen at 4d pi. Moreover, peaks annotated to Wnt target genes...
(Tcf7l1/2, Axin2, Fzd7, Sp5), Wnt-inhibitors (Tle3, Trabdb2b, Wif1, Dab2ip, Amer3), and cell cycle genes (E2f1, Cenpf, Cebpe) figure among the lost peaks (Fig. 6b). These results indicate that the chromatin landscape 2d pi preludes the proliferative arrest and secretory hyperplasia observed in N-terminal mutants (D164A) 4d pi. Indeed, we found that a significant fraction of gene sets overrepresented in D164A vs control crypts according to our ATAC-peak analysis (2d pi) were also overrepresented in the transcriptome of D164A mutant crypts (4d pi) (Supplementary Fig. 7b).

JNK signalling mediates major chromatin remodeling in D164A crypts. In line with our scRNAseq results the analysis of the ATACseq data revealed a strong enrichment of motifs bound by transcription factors downstream of the c-Jun N-terminal
kinases (JNK) signalling, namely the AP-1 transcription factors Fra1/2, Fos, FosL2, JunB, Atf3, and BATF (Fig. 6c). Cumulatively, these motifs were enriched in 220 out of the 600 gained peaks in D164A (37%), including peaks mapped to Fos, as well as Mapk8 (JNK) and the closely related Mapk13 (SAPK4), indicating robust JNK-mediated activation of gene expression (Fig. 6d and Supplementary Fig. 7a). We validated JNK pathway activation by immunofluorescence, which reveals strong nuclear accumulation of phosphorylated JNK (pJNK) in D164A, but not in control crypts 4d pi (Fig. 6e). Similar to Creb3l3, the expression of transcription factors Fos and JunB is normally restricted to enterocytes at the villus tip33,40. However, in D164A animals 4d pi, Fos, and JunB transcripts are ectopically expressed in crypts (Fig. 6f), corroborating the ATAC-Seq and scRNASeq results. Moreover, the transmembrane mucin glycoprotein Muc13, which was independently shown to be induced by JNK activity41, also figure among the gained peaks in D164A mutant crypts (Fig. 6b), in line with increased alcin blue positivity of hyperplastic and mis-differentiated intermediate cells 4d pi. Altogether, these results indicate that, following lack of β-catenin-NTF interactions, JNK signalling is activated. While mis-differentiation of IESCs upon perturbation of niche signalling has been previously described, the JNK pathway hasn’t, so far, been implicated in the loss of IESCs.

Bcl9/9L are essential upon acute reduction in β-catenin levels. We wondered why the sole presence of β-catenin-D164A leads to stem cell exhaustion, while the loss of BCL9/9L only transiently downregulates IESC markers13–16. Possibly, this discrepancy is due to the fact that, in our model, the impairment of β-catenin-NTF interactions occurs concomitantly with an acute reduction in β-catenin levels (recombination of the floxed wt allele). Indeed, while dispensable for intestinal epithelial homeostasis, BCL9/9L are required when homeostasis is challenged, e.g., upon derepression of Wnt signalling by LGK974 inhibition15, DSS treatment13 or irradiation15. Supporting this notion, simultaneous deletion of BCL9/9L and one copy of β-catenin in villin-CreERT2;Ctnnb1fl/fl;Bcl9lfl/ox;Ctnnb1D164A/ox animals, leads to humane endpoint 6d pi (Fig. 7a). Morphologically, we observed crypt atrophy, mesenchymal thickening, and villus shortening, as well as crypts reminiscent of the secretory hyperplasia observed in D164A animals (Fig. 7b). In the presence of two copies of β-catenin, loss of BCL9 and BCL9L leaves the intestinal morphology unaltered (Fig. 7b).

Cumulatively, our data indicate that transcriptional co-factors selectively modulate β-catenin-mediated Wnt-target gene expression. We propose a model in which C-terminal co-activators govern basal Wnt signalling levels. Impairing their activity leads to loss of stem cells and proliferative arrest. In contrast, N-terminal co-factors maintain stem cell state. Blocking their contribution results in hyperproliferation and aberrant differentiation of IESCs (Fig. 7c).

Discussion

We have previously shown that the C- and N-terminal transcriptional outputs of Wnt/β-catenin signalling have distinct and independent functions during embryonic development8. In this study, we aimed to uncover their specific contributions to the maintenance of adult IESC homeostasis. We show that the cellular dynamics of crypt collapse upon impairment of β-catenin-BCL9/9L interactions are profoundly distinct from the rapid crypt atrophy observed upon β-catenin deletion or impairment of interactions with C-terminally recruited co-factors. Our data suggest that C-terminally recruited co-activators act as a binary on-off-switch of transcription, and are therefore essential for the basal β-catenin-mediated transcription of Wnt-target genes. Conversely, N-terminal co-factors fine-tune β-catenin’s transcriptional output to the levels required for proper proliferation and self-renewal of IESCs. The results presented herein provide evidence that differential regulation of β-catenin transcriptional outputs preserves the narrow window of Wnt-pathway activity required to govern the identity and fate of IESCs. This selective transcriptional modulation may be key in preserving “just-right” levels of Wnt signalling, which have been implicated in IESC homeostasis both physiological and in tumor context42.

Compelling evidence has recently been provided that the intestinal IESC niche is readily regenerated upon perturbations, as differentiated cells can revert to stem cells43–46. Epithelial plasticity can thus mask the effects of loss function studies of proteins, such as BCL9/9L, whose contribution to intestinal homeostasis only becomes apparent when the epithelial stem cell niche is challenged. While increasing attention has been devoted to the role of these proteins in maintaining colorectal cancer stemness, the function of β-catenin-NTF interactions in healthy intestinal homeostasis has been overlooked. Our data suggest that N-terminal co-factors selectively restrain Wnt/β-catenin outputs that promote Myc-E2F-driven proliferation, thereby ensuring the maintenance of a self-renewing pool of IESCs. Indeed, in N-terminal mutant crypts, excessive proliferation of IESCs rapidly led to the exhaustion of the stem cell pool. Concomitantly with robust JNK pathway activation, the crypts undergo a profound mis-differentiation and “villisation”. Indeed, we show ectopic crypt expression of gene programs characteristic of enterocytes at the villus tip33,40, where terminally differentiated cells are eliminated by apoptosis. Mounting inflammation and colitis eventually result in a lethal loss of intestinal function. Interestingly, a proliferative subset of IESCs has recently been described to function as unconventional antigen-presenting cells, orchestrating interactions with immune cells in the intestine via expression of MHC class II complexes34. On the other hand, T-cell-derived signals...
are able to inhibit self-renewal and induce differentiation of IESC\textsuperscript{34–36}. It is tempting to speculate that cytokine-induced terminal differentiation of stem cells, possibly via the JNK pathway, is an in-built mechanism to prevent their abnormal proliferation. However, further studies should dissect the role of immune cell-derived signals for the maintenance of intestinal homeostasis in both physiological and perturbed conditions.

**Methods**

**Mice.** The Ctnnb1-flox allele\textsuperscript{47} was combined with the Ctnnb1-delC, Ctnnb1-dm or Ctnnb1-D164A\textsuperscript{8}, and bred into the VillinCre-ERT2 mouse strain (The Jackson Laboratory) to generate villin-Cre\textsuperscript{ERT2};Ctnnb1\textsuperscript{wt/fl} (control) villin-Cre\textsuperscript{ERT2};Ctnnb1\textsuperscript{fl/fl} (KO), villin-Cre\textsuperscript{ERT2};Ctnnb1\textsuperscript{dm/fl} (dm), villin-Cre\textsuperscript{ERT2};Ctnnb1\textsuperscript{ΔC/fl} (ΔC). In these mice, the conditional β-catenin allele can be deleted specifically in the intestinal epithelium by tamoxifen-inducible VillinCre-ER\textsuperscript{ERT2}-mediated recombination, leaving

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**Supplementary Table:**

| Motif | TF | pV |
|-------|----|----|
| GTGGTCTCCT | Junb | 1e-57 |
| GCATCAAA | Fra2 | 1e-56 |
| TGAACACTC | AP-1 | 1e-55 |
| GGTATAGGC | Fra1 | 1e-53 |
| AGGCATTTC | Atf3 | 1e-53 |
| GATTTTAC | Fos | 1e-52 |
| GTGTAATTGA | Fos2 | 1e-40 |
| TGAACACTC | Jun-AP1 | 1e-32 |
| HNF4a | 1e-29 |
Fig. 6 JNK signalling mediates major chromatin remodeling and triggers the expression of villus tip genes in D164A crypts. a Venn diagram of measured ATACseq peaks in crypts isolated from control (villin-CreERT2;Ctnnb1wt/fox, n = 3) and D164A (villin-CreERT2;Ctnnb1D164A/fox, n = 3) animals. Timepoint: 2d pi. Differential peak analysis indicates that 600 peaks are gained (logFC > 1 & p < 0.01) and 869 are lost (logFC < −1 & p < 0.01, as calculated by edgeR’s exact test) in D164A crypts. b Volcano plot of ATACseq peaks in control and mutant crypts. Gained peaks (logFC > 1 & p < 0.01) evidenced in red box. Lost peaks (logFC < −1 & p < 0.01) evidenced in gray box. Selected peaks are labeled. P-values calculated with edgeR’s exact test. c Table of the 10 most significantly enriched transcription factor (TF) binding motifs in differential peaks obtained from HOMER motif analysis (hypergeometric test). d Combined ATACSeq tracks of control and D164A counts. Black arrows show gained peaks with AP-1 binding motif annotated to Mapk8/JNK and Mapk13/SAP1. e Immunostaining of phospho-JNK in control and D164A duodenal sections. E-cadherin (cell shape) as counterstain. Timepoint: 4d pi. Scale bar, 20 μM. Representative images of 3 biological replicates. f smFISH of Fos and JunB mRNA in control and D164A duodenal sections. DAPI (nuclei) as counterstain. Insets show higher magnification. Arrows indicate single mRNA molecules visible as dots. Timepoint: 4d pi. Scale bar, 20 μM. Representative images of three biological replicates.

Fig. 7 Selective regulation β-catenin transcriptional outputs govern the identity of the intestinal epithelial stem cells. a Survival plot of villin-CreERT2;Ctnnb1wt/fox (control, n = 3), villin-CreERT2;Ctnnb1D164A/fox (D164A, n = 5), villin-CreERT2;Bcl9fl/fl;Bcl9Lfl/fl;Ctnnb1wt/fox (n = 3) and villin-CreERT2;Bcl9fl/fl;Bcl9Lfl/fl;Ctnnb1wt/wt animals (n = 3). ****p < 0.0001 as calculated by two-sided log-rank (Mantel-Cox) test. b Histology sections of villin-CreERT2;Bcl9fl/fl;Bcl9Lfl/fl;Ctnnb1wt/fox and villin-CreERT2;Bcl9fl/fl;Bcl9Lfl/fl;Ctnnb1wt/wt animals. Concomitant deletion of Bcl9, Bcl9L, and one copy of β-catenin induces crypt atrophy and villus shortening. Timepoint: 6d pi. Scale bar, 20 μM. Representative images of three biological replicates. c Schematics of the proposed model. C-terminal co-activators govern basal Wnt signaling and preventing their activity leads to loss of stem cells and proliferative arrest. Contrary, N-terminal co-factors act as stem cell specifiers. Blocking their contribution results in hyperproliferation and aberrant differentiation of intestinal epithelial stem cells. IESC intestinal epithelial stem cell, TA transit amplification cell.
Wnt/β-catenin-mediated transduction under the sole control of mutant β-catenin (wt in control animals). The Cmm1t-D164A allele was detected with the primers 5′-GCCCTGTCCGTGCTCTGCCACCTGC-3′ and 5′-GGTGCTCCTGGTGTGGAAGGCTT-3′, yielding an amplicon of the size 475 bp for the wild-type and 628 bp for mutant allele4. Cmm1t-ΔC allele was determined using primers 5′-GTTGACGCTGCTGACAGGCTG-3′ and 5′-TGGGCTGTTGCTCCAGATCGTCC-3′, which generate an amplicon of size 345 bp for the wild-type and 415 bp for mutant allele5. The presence of the villin-CreERT2 allele was detected with the primers 5′-CAAATCCCTGCTGAGGGC-3′ and 5′-GGCCAATCCATCTGAGTCT-3′, which generate a 220 bp product5. To induce Cre-ER(T2)-mediated recombination, tamoxifen (Sigma, 80 mg/kg) was injected intraperitoneally on 2 consecutive days. Concomitant with the detection of β-catenin allele was confirmed for every mouse included in this study by PCR with the primers 5′-AAGGTAGATGAGTAAGGTGTT-3′ (RM41), 5′-CACCAGCTGGCTCTGGTATT-3′ (RM42) and 5′-TACACTATTGGAACAGGAAG-3′, generating products of 221 bp for the wild-type allele, 324 bp for the floxed allele and 500 bp for the floxed allele5 (Supplementary Fig. 1d). To eliminate B2 and B2b, previously published6 conditional alleles were transfected with the vector containing the Cre-ER(T2) allele (villin-CreERT2), together with the Cmm1t-flx allele (villin-CreERT2: B2bflxed: B2bflxed: Cmm1tflx). All genotyping primer sequences can be found in Supplementary Table 1.

Immunohistochemistry. Diced duodenum was flushed thoroughly in ice-cold PBS, then cut in 1 cm pieces and fixed in 4% PFA in PBS overnight at 4 °C. After repeated washing in PBS, tissues were dehydrated in a spin tissue processor, embedded in paraffin, and cut in 8 μm sections. Deparaffinized tissue sections were subjected to antigen retrieval in 2 mM sodium citrate and 1.6 mM citric acid, pH 6, for 20 min in a steamer. Sections were washed with PBS (0.1% Tween 20 in PBS) and blocked for 30 min at RT in blocking buffer (5% BSA, 5% heat-inactivated normal goat serum in PBST). Nuclei were stained with DAPI (Sigma, D5602). Sections were then incubated in a 3% aqueous solution of acetic acid for 3 min and then incubated in a 1:1000 dilution of biotinylated secondary antibody for 1 h at RT and then visualized with VEC-Axin2 (Sigma), which is a commercially available antibody against the Ser33/34-phosphorylated form of Axin2, a component of the Wnt/β-catenin signaling complex. Sections were co-optained with 20× objective. The presence of the Kanton of Zurich, Switzerland. All animals were kept on a C57BL/6 background. Mice were 8–12 weeks old at the time of treatments and cell isolations. Mice of both sexes were used in all experiments and littermates were used as controls.

Quantitative real-time PCR. mRNA in situ hybridization was performed with the Biosearch Technologies (see Supplementary Data 1) and coupled to Cy5 (Myc, Fox, Creb3E3) or TMR (Lgr5, JunB) as described30. The intestinal sections were dissected, fixed, embedded in OCT and cut into 10 μm sections. Deparaffinized sections were pre-hybridized for 2 h on a roller and then hybridized overnight at 37 °C in a probe solution consisting of 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate), 1% Tween 20 and 1% blocking reagent. Hybridization was performed on a Leica THUNDER 3D Live Cell Imaging system, using the following THUNDER Computational Clearing Settings in with the LAS-X software: Feature Scale (nm): 350, Strength (%): 98, Deconvolution settings: Auto and Optimization: High. The of Lgr5− area per crypt was manually quantified using Fiji. We quantified at least 8 crypts from 2 to 3 different mice per condition. Barplots were generated on GraphPad Prism. The unpaired Student’s T test function in GraphPad Prism was used to analyze the significance of two-group comparisons.

Cryopreservation and culture. Duodenal crypts were isolated and cultured as described30 with minor modifications. The duodenum was dissected out, flushed thoroughly, opened longitudinally, and then cut in 1–2 mm stripes. After repeated washing in ice-cold PBS, the duodenal fragments were incubated for 20 min in Gentle Cell Dissociation Reagent (Stemcell Technologies). Crypts were gradually released from the tissue during four rounds of washing in 0.2% BSA and filtering through a 70 micron filter, generating 4 fractions. Only fractions 3 and 4 containing intestinal crypts were used for downstream experiments (organoids or single-cell suspension). Crypts were seeded in 30 μL Matrigel (Corning) domes and cultured in IntestinCell (Stemcell Technologies). Upon passaging, the culture medium was supplemented with 1 μM Rho-associated protein kinase (ROCK) inhibitor (Y27632, Millipore). Recommodation was induced with 100 nM 4-hydroxytamoxifen (4OHT, Sigma) added to the culture medium upon splitting. Wnt3a-conditioned medium was obtained as described29. For the organoid formation assay, mice have sacrificed 0, 2 or 4 day post infection. Crypts were seeded in equal amounts (500 crypts) in 5 x 5 μl Matrigel droplets. Organoids were quantified manually 7 day post crypt seeding and micrographs were collected using the AmScope 5.2. software. For Edu staining, crypts were seeded on glass-bottomed 8-well chambered slides (Thermo Fisher Scientific, Lab-Tek II chambered coverslips). After 20μl droplets were added, Edu incorporation (154532) and 20 μg/ml Click67 Kit (baseclick) prior to overnight incubation with anti-Ecadherin (BS Transfection Lab) antibody for cell shape staining. Imaging was performed on a Visirion CSU-W1 spinning disk confocal microscope with a CFI PlanFluor ×20 objective.

Double histology for intermediate cells. Deparaffinized tissue sections were incubated with hydrogen peroxide for 5 min to block endogenous peroxidase, then incubated for 30 min in blocking buffer (5% BSA, 5% heat-inactivated normal goat serum in PBST) for 30 min. After overnight incubation with primary anti-Ly5 antibody (Dako), sections were repeatedly washed in PBST and incubated with biotynilated secondary antibody for 1 h at RT and then visualized with VEC-TASTAIN ABC HRP Kit (Vector Laboratories) as indicated by the manufacturer. Sections were then incubated in a 3% aqueous solution of acetic acid for 3 min and then mucins were stained with alcin blue (1 g in 100 ml 3% acetic acid) for 15 min. Hematoxylin (50% aqueous solution) staining was then carried out according to standard procedure. Sections were imaged on Leica THUNDER 3D Live Cell Imaging system.

RNase in situ hybridization. Duodenum was flushed thoroughly in ice-cold PBS, then cut in 1 cm pieces and fixed under gentle agitation in 10% formalin for 20 h at room temperature. Axi32 mRNA in situ hybridization was performed with the ACD RNase kit (ACDrbio), according to the manufacturer’s instructions. Sections were imaged on a Vectors 3.0 Automated Quantitative Pathology Imaging System (Vector Laboratories) using the quantification method54. Protein concentration was determined with the BCA assay. Protein lysates were boiled with NuPAGE Sample Denaturing Agent (4×) and Reducing Agent (10×) (Invitrogen), separated on NuPAGE 4%–12% Bis-Tris gels and transferred on nitrocellulose membranes. HRP-conjugated secondary antibodies were used to detect anti-β-catenin (Novus NPB1-3239), mouse monoclonal anti-β-actin (Santa Cruz Biotechnology, sc-7778), and mouse monoclonal anti-lamin A/C (ebioscience, 14-9847-82) were visualized with WesternBright Quantum HRP substrate (advantana). Uncropped blots are visualized in the source data file.

Quantitative real-time PCR. Cmm1t-driven gene expression and reverse transcription with the cDNA synthesis kit (Takara Bio Inc.) according to the manufacturer’s instructions. One nanogram of total RNA was used. Expression of genes of interest was quantified by qRT-PCR using the Applied Biosystems SYBR Green Kit monitored by the QuantStudio3 system (Applied Biosystems). Samples were measured in technical replicate and average cycle threshold values were normalized to GAPDH using the ΔCT method52.
Leukocyte isolation for flow cytometry. Control (n = 5), AC (n = 3) and D164A (n = 5) mice were sacrificed 2d or 4d pi. The duodenum was open longitudinally, washed with PBS and the peripheral blood was removed. Pieces were weighted and incubated in HBSS with 10% FCS, 100 U/ml penicillin/streptomycin and 5 mM EDTA at 37 °C in a shaking incubator. Tissues were then digested at 37 °C for 30 min with 15 mM Heps, an equal mixture of 250 U/ml type IV and type VIII collagenase (Sigma-Aldrich), and 0.05 mg/ml DNase I in RPMI-1640 medium supplemented with 50% FCS and 100 U/ml penicillin/streptomycin. Cells were then layered onto a 40/80% Percoll gradient, centrifuged, and the interface was washed in PBS. Total leukocyte counts were determined by adding Bright Absolute Counting Beads (Life Technologies) to each sample before flow cytometry for normalization to tissue weight. For surface staining, cells were stained in PBS with 0.5% BSA with the fixable viability dye eFluor 780 (1:1000, eBioscience) and a combination of the following antibodies (1:200): anti-mouse B220 (RA3-6B2), Ly121 (M1/70), CD11c (N418), CD4 (RM-4), CD45 (30-F11), F4/80 (BM8), LyGn (IAB), Ly6C (HK1.4), CD103 (2E7), CD8 (53-67), F4/80 (M88), MHC-II (M5/114.15.2), TCR-β (H57-59.7), all from BioLegend. Fc block (anti-CRD/CD32, Affymetrix) was included to minimize nonspecific antibody binding. For intracellular cytokine staining of T cells, cells were incubated for 3.15 h in complete IMDM containing 0.1% FBS, 10% FCS, 100 U/ml penicillin/streptomycin, 5 μM gentamicin, 2 mM GlutaMax, and 100 μg/ml of anti-CD16/32 (2.4G2). Cells were then washed thoroughly in ice-cold PBS, then opened longitudinally and incubated for 20 min in 10 mM inosine (dissociation buffer 2) (30 mM EDTA in PBS) at 37 °C under gentle agitation. The epithelium was released by vortexing, pelleted by centrifugation at 4 °C for 5 min at 1000 x g, and resuspended in 500 μL TRI Reagent (Sigma). RNA was extracted by phenol-chloroform precipitation. DNase treatment was carried out with a DNA-free DNA Removal Kit (Invitrogen) according to the manufacturer’s instructions. The library preparation was performed with the Illumina NextSeq500 and the TruSeq RNA Kit. RNA sequencing was performed on the Illumina NextSeq500 by the Functional Genomics Centre Zurich (FGCZ).

Computational RNA sequencing analysis. Reads were quality-checked with FastQC34. Reads alignment to the reference genome Mus_musculus.GRCh38.95 was performed using the Support Users for SHERI script integration (SUSHI) script with the RSEMApp application. Pairwise comparisons were performed with the SUSHI application EdgeRApp (based on edger46). Principal component analysis and filtering of differentially expressed genes (DEGs, logFC > 2, p < 0.01) were performed on R (version 3.6.1). The package pheatmap45 was used to generate the heatmap of normalized FPKM. We performed GSEA48 on significantly differentially expressed genes (logFC > 2, p < 0.01) using the Bioconductor package gsea with default parameters49. Genes were ranked based on p-value, and taking into account directionality of the fold change with the formula ranking = −log10(P_value)/log2FC (obtained from the blogpost http://genomespot.blogspot.co.at/2016/04/how-to-generate-rank-file-from-gene.html). The Hallmarks gene set collection from the Molecular Signatures Database50 was imported in R with the package sigvfdr. Cell type enrichment analysis of DEGs across conditions was performed on the web-based tool EnrichR51. The analysis was performed independently for ATACSeq library preparation, following the protocol by the manufacturer’s instructions. The library preparation was performed with the Illumina NextSeq500 and the TruSeq RNA Kit. RNA sequencing was performed on the Illumina NextSeq500 by the Functional Genomics Centre Zurich (FGCZ).

Computational analysis of scRNAseq data. Dimensionality reduction and clustering. Reads were quality-checked with FastQC. Raw data processing of fastq files was performed with the ZUM pipeline53 using the MAK and MAK-CHARLAWARE tools. The MAK pipeline was extended to include the single cell scRNAseq pipeline that was performed with Seurat on R 3.2.164. Cells were filtered based on mitochondrial gene content, unique molecular identifier (UMI) counts were log-normalized according to default Seurat settings. Scaling was performed on the variable genes (FindVariableFeatures, parameters: x.low.cutoff = 0.0125, x.high.cutoff = 3, y.cutoff = 0.5) resulting in 100% mito UMI. Principal component analysis (PCA) was performed with Seurat on R 3.2.164. The merged and joint analysis was performed with the package conos65. Cells were clustered with the Leiden community method (resolution = 1.3). The joint graph (in PCA space) was embedded in UMAP space and converted to a Seurat object. The stem cell and early progenitor clusters were subtyped and the log-normalized UMI counts were exported and used for the normalization algorithm and downstream analysis.

Normalization algorithm. Analysis of the control timecourse data revealed that the acute loss of one β-catenin allele only caused a slight and transient downregulation of Wnt-targets, proliferation, and stem markers, which then returned to normal expression levels by 4d pi. Thus, we devised a normalization of the mutant time-course data to disentangle these effects from those induced by impaired N-terminal interactions, which were the focus of our investigation. For every timepoint (0, 2, and 4 pi), we divided the D164A single-cell expression (from data slot) of each gene by the mean expression of the corresponding gene in the control with the formula: In[exp(D164A)/mean(exp(control))]. The resulting three normalized D164A sparse matrices (one per timepoint) were used to create Seurat objects, which were merged, scaled, and visualized in UMAP space using dimensions 1:10.

Diffusion maps and pseudotime analysis. For diffusion maps and supervised pseudotime analysis, the normalized Seurat object was converted in a SingleCellExperiment. Diffusion maps were generated with the Bioconductor package diffusionmap66 using default parameters. Scores in the diffusion component 1 were compared using the Wilcoxon test (alternative: two-sided) in R base. Supervised pseudotime analysis was performed with the Bioconductor package psrptime29 on all genes or on mouse transcription factors only, setting scale to FALSE.

Cell cycle scoring and classification. Cell cycle scoring was performed with the CellCycleScoring algorithm from Seurat, using cell cycle-related genes from28, Wilcoxen test (alternative: two-sided) was used to compare G2M scores. To assign a measure of similarity to ISECs or TAs transcriptomic profiles, we trained a logistic regression model (modified version of the multinomial regression of MatchCoRe27) using ISECs and TA markers extracted from a publicly available single-cell data set52. Upon collecting the estimated probabilities of TA class memberships for each timepoint separately, we compared these probability distributions and tested the significance of the difference using the Kolmogorov-Smirnov test (alternative: two-sided).

Gene set enrichment analysis. We used the Seurat FindMarkers function (default parameters min.pct 0.25 and log.threshold 0.25) to compute differentially expressed genes between timepoints in our normalized D164A stem cell and early progenitor dataset. We then ranked genes with the formula ranking = −log10(P_value estad)/log2FC and performed GSEA30 on R using the Bioconductor package gsea with default parameters49. The following gene set collections were imported in R with the package sigvfdr: Hallmarks53, Chemical and Genetic Perturbations (various contributions), KEGG58,59, and gene ontology biological process60. Plots were generated with the R package ggplot271.
calculated with edgeR. Statistical peaks between sample groups were identified using the exact test function in edgeR. Only peaks with a log fold change >1 and an FDR p-value < 0.05 were considered as differentially accessible for further analysis. HOMER was used for peak annotation and motif enrichment analysis. Motifs with Benjamini-corrected q-value < 0.001 were considered significantly enriched. BigWig files of control (n = 3) and D164A (n = 3) were combined into a single track for visualization in Integrative Genomics Viewer (IGV). Genes associated with a differentially accessible peak were ranked with the formula ranking index = −log10(Pvalue) × sign(logFC) and used for GSEA with the Bioconductor package fsgsea with default parameters. The significance of overlap between genes sets overrepresented in scRNAseq and in ATACseq was quantified with hypergeometric test. Plots were generated with the R package ggplot2.

Statistical analysis. Statistical analysis and visualization were performed using R (Version 3.6.0, R Foundation for Statistical Computing Vienna, Austria), R Studio and Prism 8.2.0. Statistical significance tests were performed as described in each figure legend. Unless stated otherwise all tests were significant with p value < 0.05. p values >0.05 were defined as not significant and are not presented in the graphs. The p values provided were adjusted for multiple testing hypothesis according to the particular statistical method mentioned in the figure legend and in the corresponding part describing the computational analysis.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Data generated in this study have been deposited in the GEO database under the accession codes: GSE148941, GSE148942, GSE148940. The remaining data are available within the Article, Supplementary Information, or available from the authors upon request. Source data are provided with this paper.

Code availability
The code used in this study is available at the public repository https://github.com/cocoborelli/betacat (https://doi.org/10.5281/zenodo.4461476).

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Author contributions
C.B. designed and performed experiments, collected, analyzed and interpreted data and wrote the manuscript. T.V. initiated and conceived the research, designed and performed experiments, interpreted data, and wrote the manuscript. K.H., K.V., and G.M. assisted with the experiments. K.H., A.L., and I.V.R. helped with the bioinformatic analysis. A.G. and I.C.A. performed leukocyte profiling and intestinal permeability assay. G.H. and A.E.M. discussed the data and assisted with manuscript preparation. A.G.M. supported the research. K.B. initiated and supported the research, discussed the data, and assisted with manuscript preparation.

Competing interests
The authors declare no competing interests.

Additional information
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