Expanded View Figures

**Figure EV1.** Only deletion of both Gsk3α and Gsk3β leads to crypt-progenitor-cell phenotype.

A Small intestine of mice at day 6 after induction. Loss of Gsk3α and Gsk3β (AhCreER Gsk3αfl/fl Gsk3βfl/fl) leads to accumulation of nuclear β-catenin and upregulation of cMyc (arrows). The crypt-progenitor cell phenotype (red bar) is characterised by increased proliferation (BrdU) and perturbed differentiation/localisation of goblet and Paneth cells (Alcian Blue, Lysozyme respectively, arrows). Scale bar, 100 μm.

B Table shows cohort of AhCre Gsk3α Gsk3β mice aged until signs of intestinal tumour burden, genotypes as indicated. Note, mice homozygous for Gsk3β deletion, or with only one copy of GSK3α and GSK3β, did not develop intestinal tumours.

C An adenoma from an aged mouse deficient for 3 alleles of Gsk3α and Gsk3β (AhCre GSK3αfl/fl betafl/fl) showing that it developed after loss of the remaining GSK3β allele. Scale bar, 100 μm.

**Figure EV2.** Single copy activation of β-catenin only slowly transforms the intestine.

A Only activation of both alleles of β-catenin led to hyperproliferation in the small intestine. Proliferation of wild-type (WT), AhCreER Catnblox(ex3)/+ and AhCreER Catnblox(ex3)/lox(ex3) mice 5 days after induction was scored by counting the number of BrdU-positive cells/half-crypt. N ≥ 3 per group, at least 25 crypts per mouse were scored, P-value of one-sided Mann–Whitney U-test.

B Activation of one copy of β-catenin in an aged AhCreER Catnblox(ex3)/+ at day 25 post-induction with no phenotype in the colon. For comparison to a WT colon, see Appendix Fig S3. Scale bar, 100 μm.

C Activation of one copy of β-catenin in VilCreER Catnblox(ex3)/+ leads to the same crypt-progenitor cell phenotype (red bar) with similar kinetics as observed in AhCreER Catnblox(ex3)/+ mice. Scale bar, 100 μm.
Figure EV3. Rapid colonic phenotype in VilCreER Catnblox(ex3)/lox(ex3) and Apcfl/fl mice, but not in VilCreER Catnblox(ex3)/+ mice. Wild-type mice show very little nuclear β-catenin, and the expression of Sox9 is restricted to the bottom of the crypt. VilCreER Catnblox(ex3)/lox(ex3) show a similar phenotype. In contrast, VilCreER Catnblox(ex3)/lox(ex3) and VilCreER Apcfl/fl mice show increased nuclear β-catenin and high expression of Sox9. Scale bar, 100 μm. Mice were sampled at day 4 after induction.

Figure EV4. Complete loss of Gsk3 in Lgr5-positive stem cells leads to tumour formation in the small intestine and the colon. Immunofluorescence analysis shows intestinal lesions with accumulation of β-catenin and Sox9 in the small intestine and the colon, 25 days after induction. Scale bar, 50 μm.
Haploinsufficiency for E-cadherin lowers the threshold for Wnt activation of β-catenin mutation.

A. AhCre\textsuperscript{fl/+} Cdh1\textsuperscript{fl/+} at day 5 post-induction shows no intestinal phenotype. AhCre\textsuperscript{fl/+} Catnb\textsubscript{lox(ex3)/+} Cdh1\textsuperscript{fl/+} mice show increased proliferation (BrdU, red line) and accumulation of β-catenin and Sox9 in cells higher up the crypt-villus axis (arrows).

B. Increased proliferation as scored by BrdU\textsuperscript{+} cells per half-crypt. N ≥ 3, statistics: one-sided Mann-Whitney U-test.

Scale bar, 100 μm.