Expanded View Figures

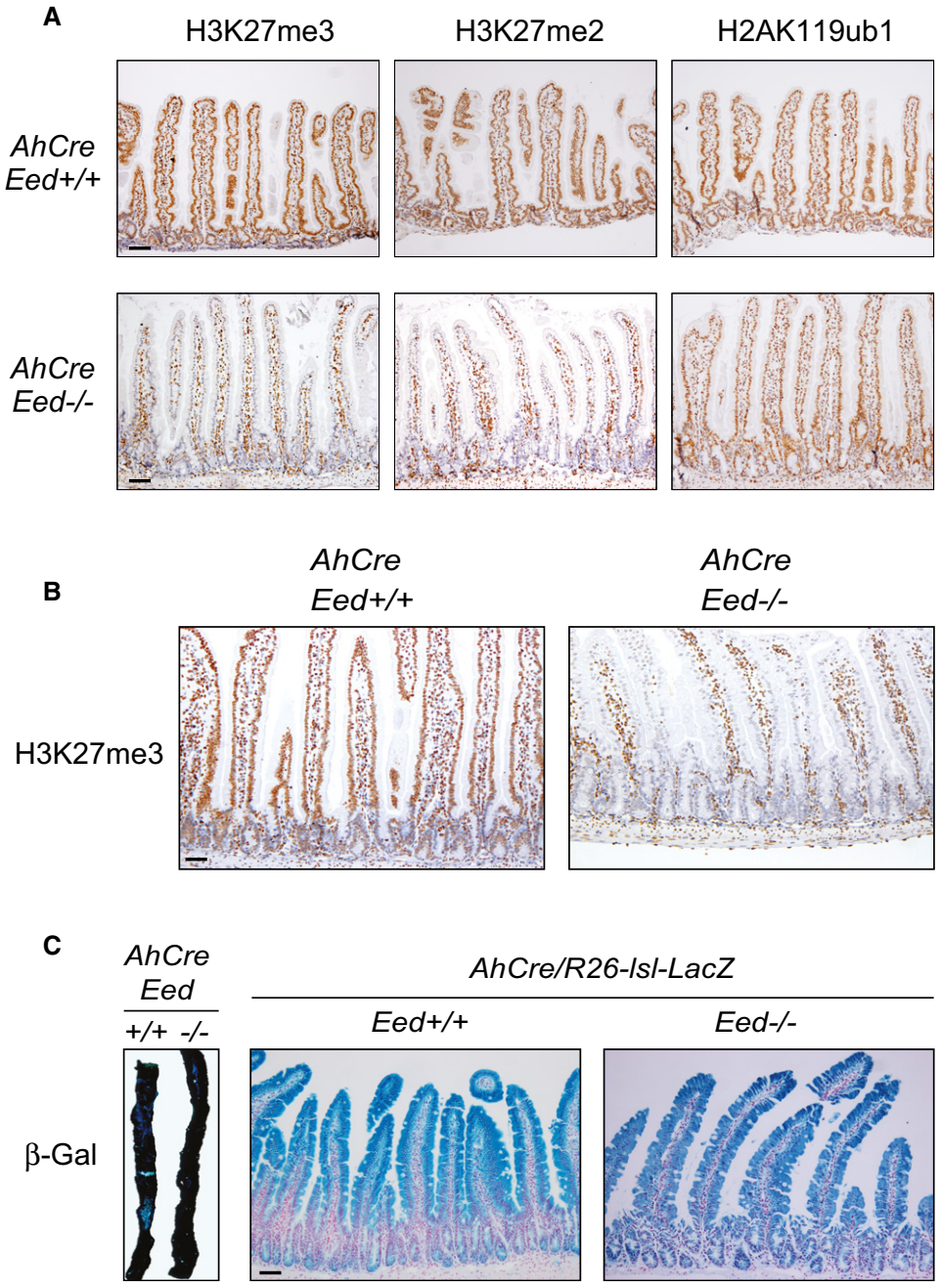

Figure EV1. Loss of *Eed* affects H3K27 methylation.

A Wider fields of the same immunohistochemistry staining presented in Fig 1A, performed on small intestinal sections from *AhCre Eed*$^{+/+}$ and *AhCre Eed*$^{fl/fl}$ mice injected with β-naphthoflavone and sacrificed after 15 days, using the indicated antibodies.

B Immunohistochemistry of small intestinal sections from *AhCre Eed*$^{+/+}$ and *AhCre Eed*$^{fl/fl}$ mice using an H3K27me3-specific antibody, analysed 30 days after β-naphthoflavone injection (n = 5).

C Whole intestine isolated from *AhCre Eed*$^{+/+}$ and *AhCre Eed*$^{fl/fl}$ crossed with knock-in mice expressing *Rosa26*-driven Cre-inducible LacZ transgene stained (left panels) for β-galactosidase activity, 30 days after the first β-naphthoflavone administration. Sections of the β-galactosidase-stained small intestines are presented in the right panels (n = 4).

Data information: All scale bars represent 100 μm.

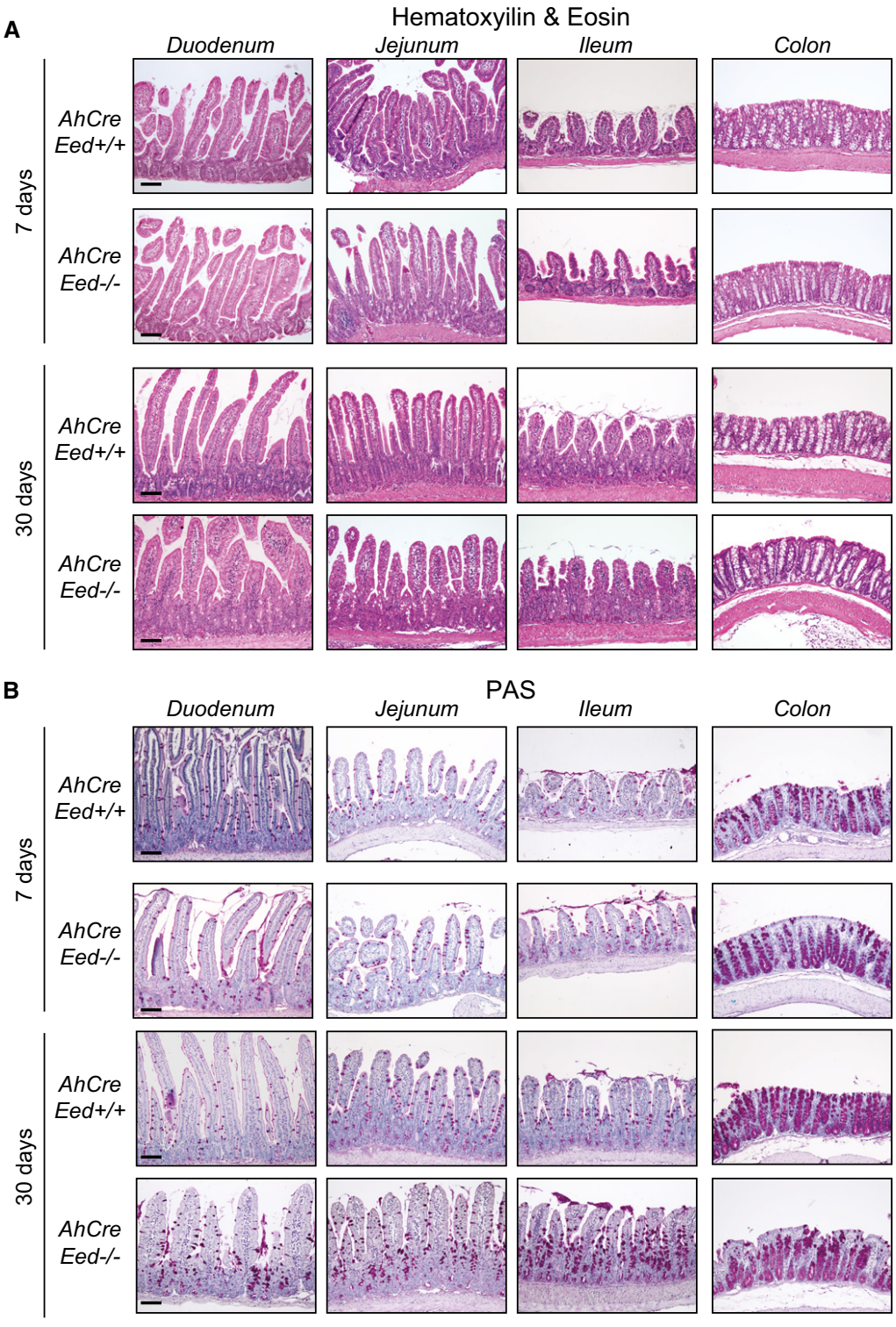

Figure EV2. *Eed* loss perturbs intestinal architecture and increases goblet cell numbers.

AhCre Eed$^{+/+}$ and *AhCre Eed*$^{fl/fl}$ mice were sacrificed 7 or 30 days after β-naphthoflavone administration.

A Haematoxylin and eosin staining of sections prepared from the different intestinal tracts.

B PAS staining performed on sections prepared from the different intestinal tracts.

Data information: All scale bars represent 100 μm.

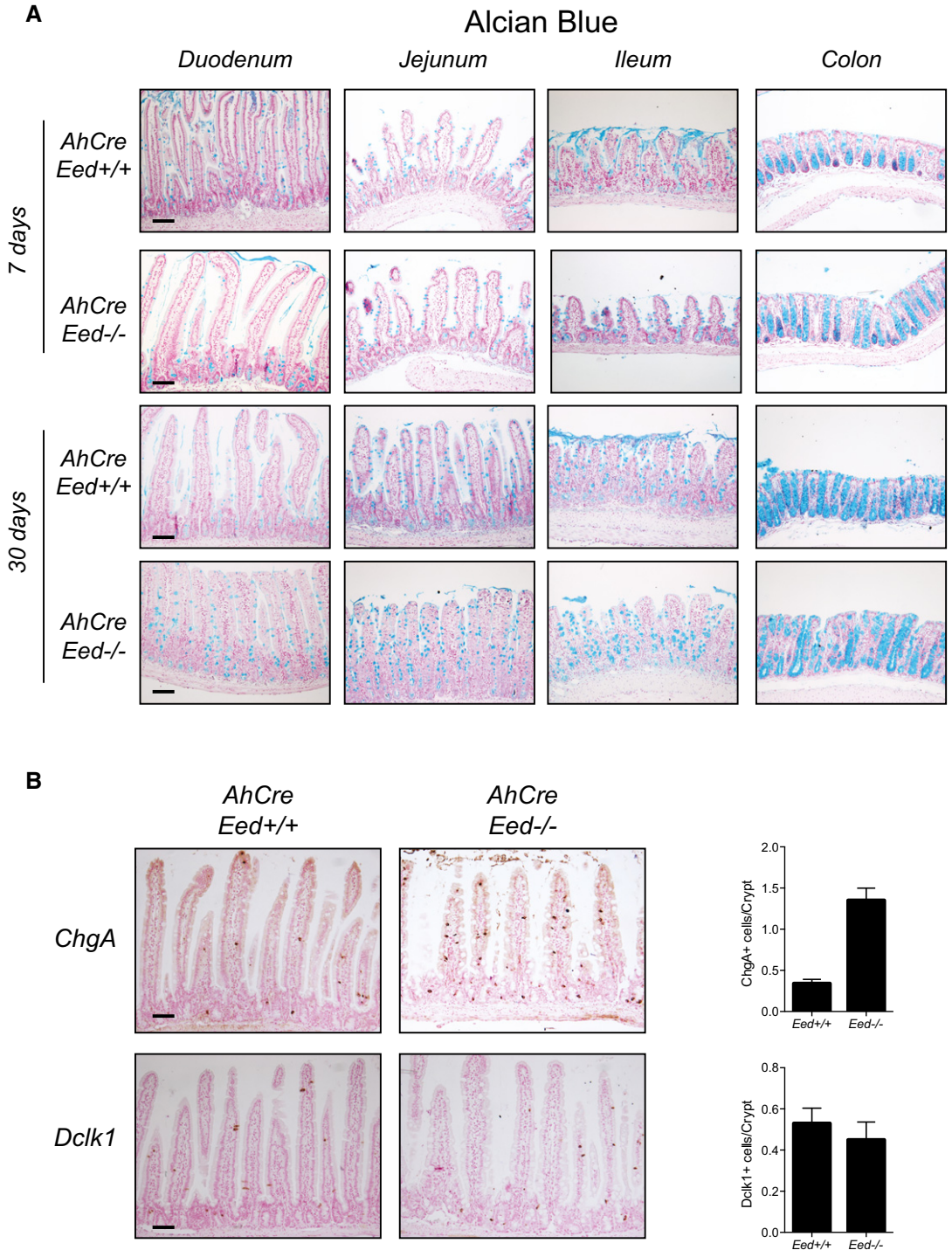

Figure EV3. *Eed* loss increases the number of goblet and enteroendocrine cells.

A Alcian blue staining on sections prepared from the different intestinal tracts. *AhCre Eed*$^{+/+}$ and *AhCre Eed*$^{fl/fl}$ mice were sacrificed 7 or 30 days after β-naphthoflavone administration.

B Immunohistochemistry analyses of small intestinal sections from *AhCre Eed*$^{+/+}$ and *AhCre Eed*$^{fl/fl}$ mice isolated 15 days after β-naphthoflavone administration using ChgA (an enteroendocrine marker)- and Dclk1 (a tuft cell marker)-specific antibodies. Quantifications of positive cells per crypt for each staining are presented on the right (mean ± SD). More than 100 crypts were scored for each condition.

Data information: All scale bars represent 100 μm.

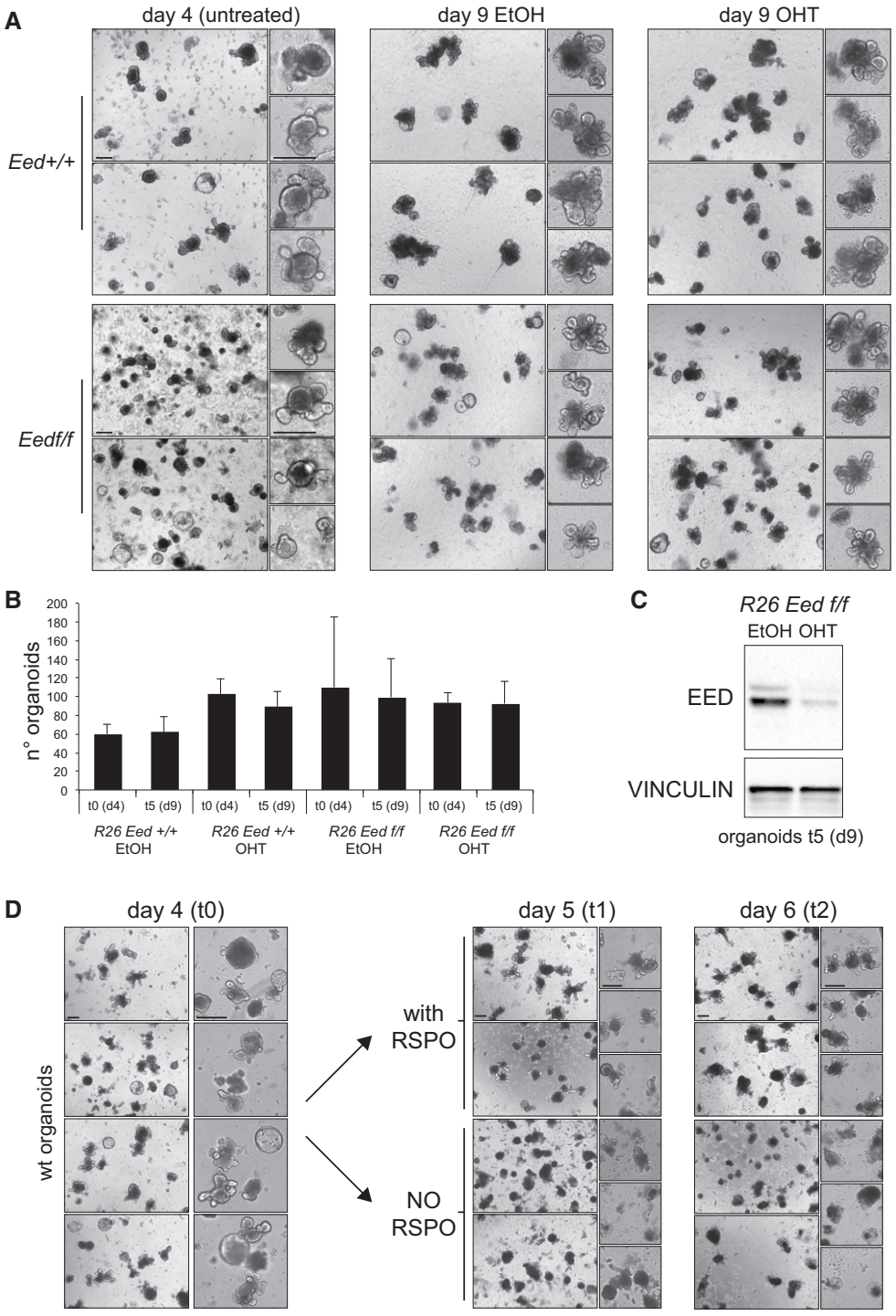

Figure EV4. Loss of *Eed* does not alter general organoids homeostasis.

A *In vitro* organoids formation using crypts isolated from *Rosa26 CreER*^{T2} *Eed*$^{+/+}$ and *Eed*$^{fl/fl}$ mice treated after 4 days with 4-OHT or EtOH (as control). Organoid homeostasis was monitored for additional 5 days (day 9).

B Quantifications of the analysis presented in (A) after 5 days of OHT or EtOH treatment (mean ± SD; n = 3).

C Western blot analysis of the organoids presented in (A) at day 9 using EED-specific antibodies. Vinculin is presented as a loading control.

D *In vitro* organoids formation using crypts isolated from wild-type mice. RSPO1 was removed after 4 days (t0) and the effects on organoids homeostasis were monitored 24 and 48 h after RSPO1 removal (t1 and t2, right panels).

Data information: All scale bars represent 200 μm.
Source data are available online for this figure.

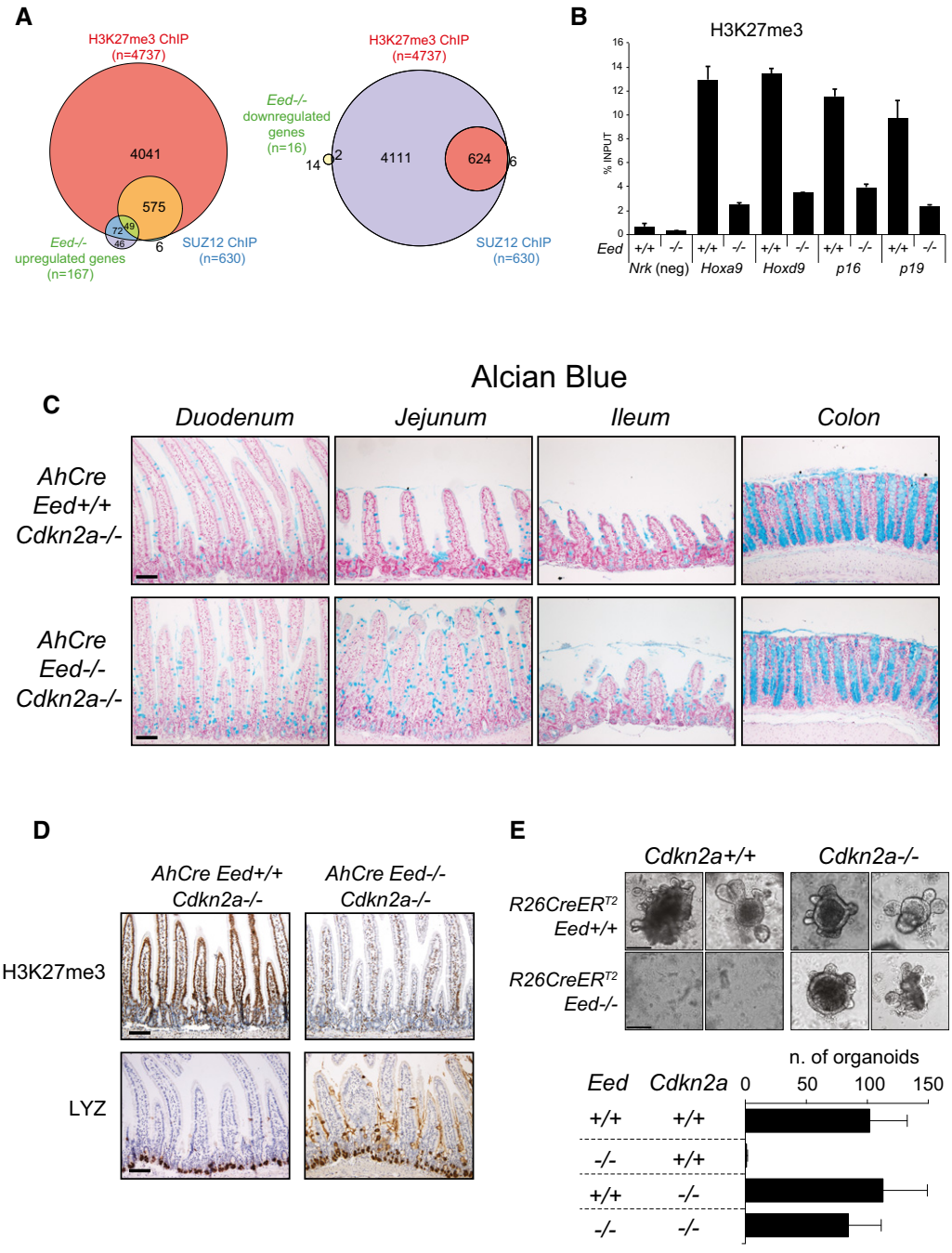

Figure EV5. PRC2 directly maintains transcriptional repression, and *Eed* loss increases goblet cell differentiation, independently from *Cdkn2a* activation.

A Venn diagrams showing the extent of overlap between SUZ12 occupancy and H3K27me3 deposition in wild-type crypts with respect to the upregulated and downregulated genes in $Eed^{-/-}$ crypts.

B ChIP analyses for the indicated gene promoters using an H3K27me3-specific antibody in crypts isolated from *AhCre* $Eed^{+/+}$ and *AhCre* $Eed^{fl/fl}$ mice 15 days after β-naphthoflavone administration. Primers amplifying the *Nrk* gene body are presented as negative PRC2 target.

C Alcian blue staining of sections prepared from different intestinal tracts isolated from *AhCre* $Cdkn2a^{-/-}$ $Eed^{+/+}$ and *AhCre* $Cdkn2a^{-/-}$ $Eed^{fl/fl}$ mice 15 days after the first β-naphthoflavone administration.

D Immunohistochemical analysis of small intestinal sections, using antibodies specific for H3K27me3 and lysozyme (LYZ, a Paneth cell marker) in *AhCre* $Cdkn2a^{-/-}$ $Eed^{+/+}$ and *AhCre* $Cdkn2a^{-/-}$ $Eed^{fl/fl}$ mice 15 days after the first β-naphthoflavone administration.

E *In vitro* organoid formation using crypts isolated from *Rosa26* $CreER^{T2}$ $Cdkn2a^{+/+}$ $Eed^{+/+}$ or $Eed^{fl/fl}$ and *Rosa26* $CreER^{T2}$ $Cdkn2a^{-/-}$ $Eed^{+/+}$ or $Eed^{fl/fl}$ mice 9 days after the first tamoxifen injection (mean ± SD).

Data information: All scale bars represent 100 μm.

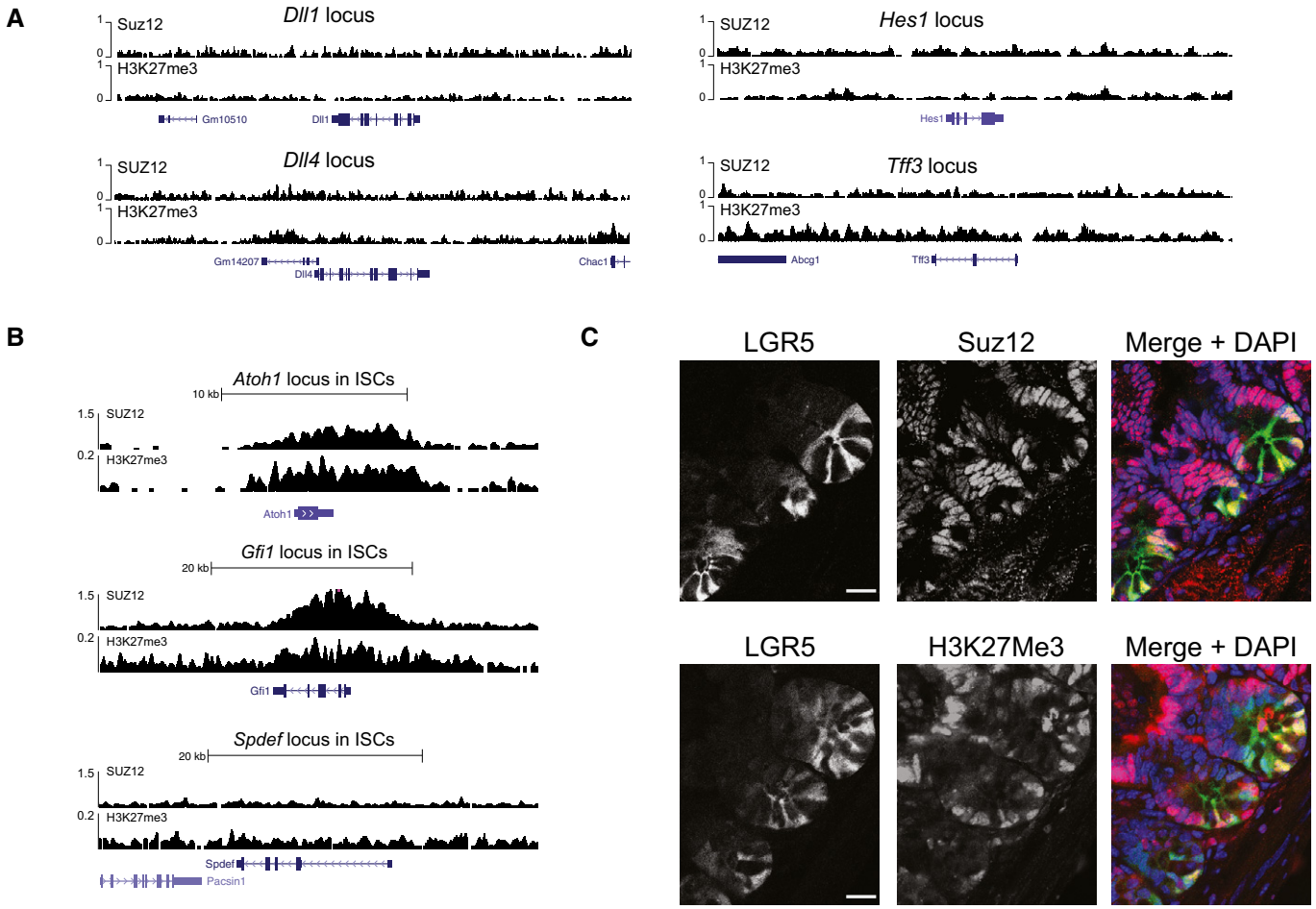

Figure EV6. PRC2 is active in intestinal stem cells.

A, B Genomic snapshots of the indicated genomic loci for SUZ12 occupancy and H3K27me3 deposition in intestinal crypts (A) or ISCs (B).

C SUZ12 and H3K27me3 immunofluorescence staining (red) and Lgr5-eGFP expression (green) in near-native agarose-embedded small intestinal sections. Scale bar represents 25 μm.