Supplemental Information1

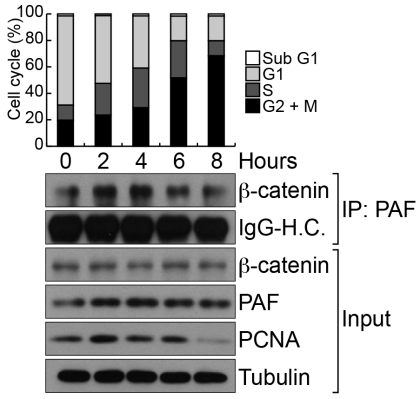
Supplemental Data (Figures S1 to S7)

Supplemental Figures (S1 to S7; ordered according to the main Figures to which each related in inventory)

Supplemental Experimental Procedures

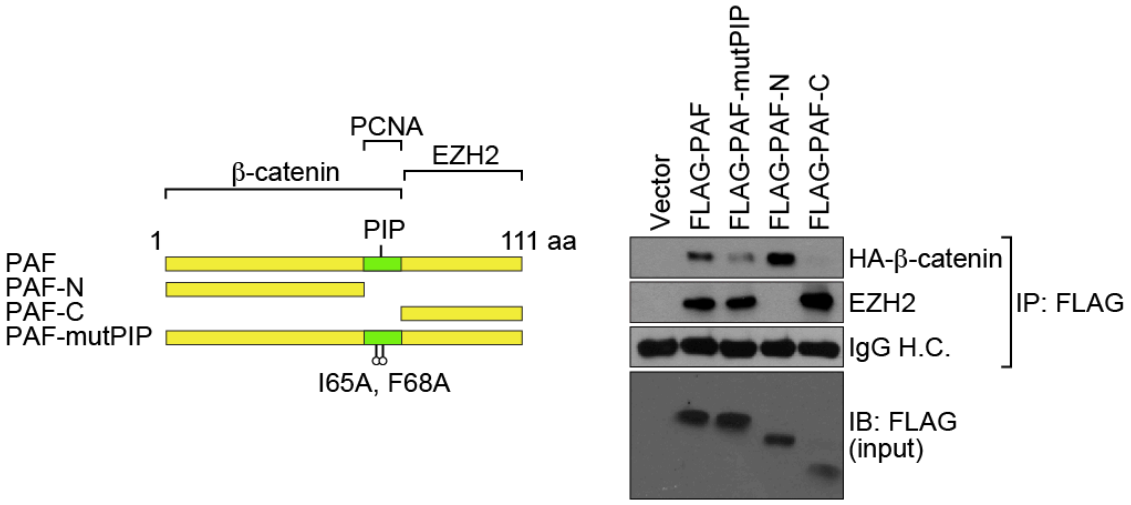
Supplemental References

Supplemental Figures



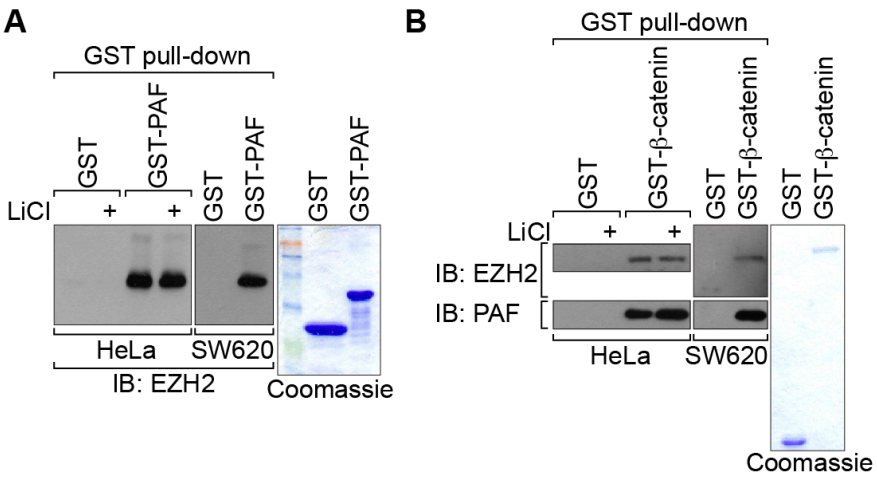
Supplemental Figure S1. Cell cycle-independent interaction between PAF and β-catenin.

SW620 cells were synchronized with thymidine double block method, and released for cell cycle progression, as we previously performed (Jung et al., 2013b). At each time point, cells were harvested for flow cytometry (upper panel) and co-IP assays (PAF; lower panel). Although PAF level in whole cell lysates (input) was slightly decreased at G2/M phase, the level of PAF-associated β-catenin was consistent with that of PAF (input). IgG-H.C.: immunoglobulin heavy chain. The representative results were shown (N = 2).



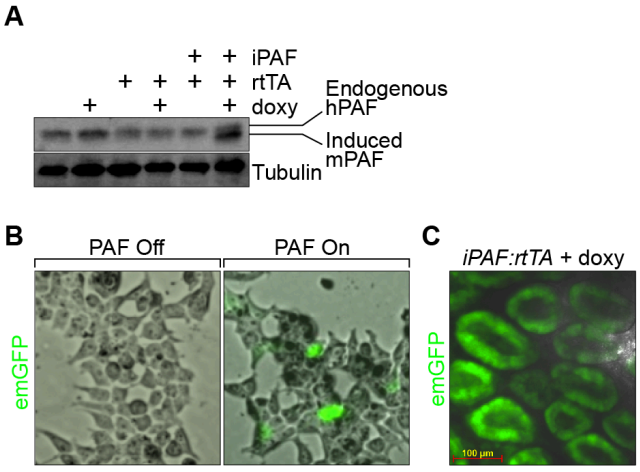
Supplemental Figure S2. Binding domain mapping of PAF for β-catenin and EZH2 interaction.

Several PAF mutants and HA-β-catenin plasmids were transiently transfected into 293T cells and analyzed for co-immunoprecipitation assays. C-terminal region of PAF did not bind to β-catenin. Also, PAF-mutPIP showed decreased interaction with β-catenin, which indicates that N-terminal and PIP regions are required for β-catenin interaction. Given that PIP motif is essential for PCNA interaction, these results suggest that β-catenin and PCNA compete each other for binding to PAF. Of note, EZH2 binds to C-terminal region of PAF.



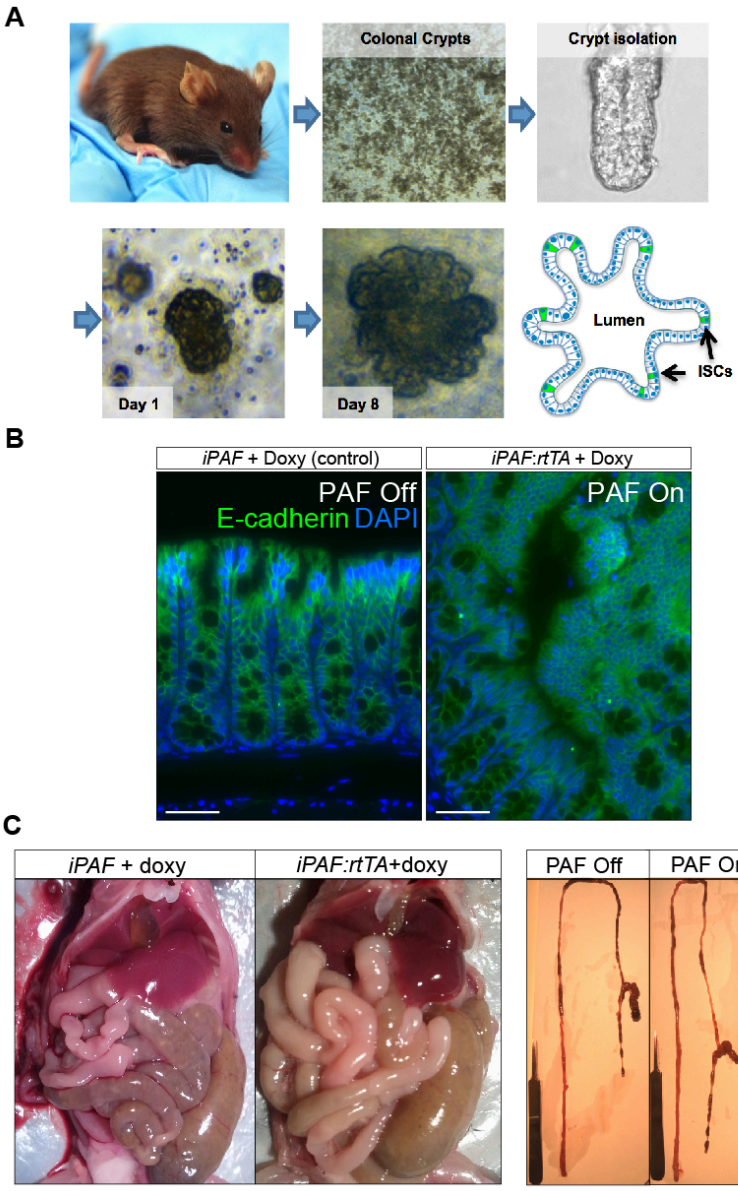
Supplemental Figure S3. *In vitro* interaction of EZH2 with PAF and β-catenin.

(A) GST (control) and GST-PAF proteins were used for GST pull-down assays with HeLa (LiCl, 25 mM, 4 hours) or SW620 cell lysates. Note that PAF-EZH2 interaction was not affected by LiCl treatment (activation of β-catenin).
(B) GST and GST-β-catenin protein were utilized for GST pull-down assays with HeLa (LiCl, 25 mM, 4 hours) or SW620 cell lysates.
Both GST-PAF and GST-β-catenin bound to EZH2, independently of LiCl in HeLa cells. Due to absence of post-translational modification of GST-proteins expressed in *E.coli*, these data suggest that post-translational modification of PAF or β-catenin is not necessary for EZH2 interaction.



Supplemental Figure S4 Conditional induction of PAF.

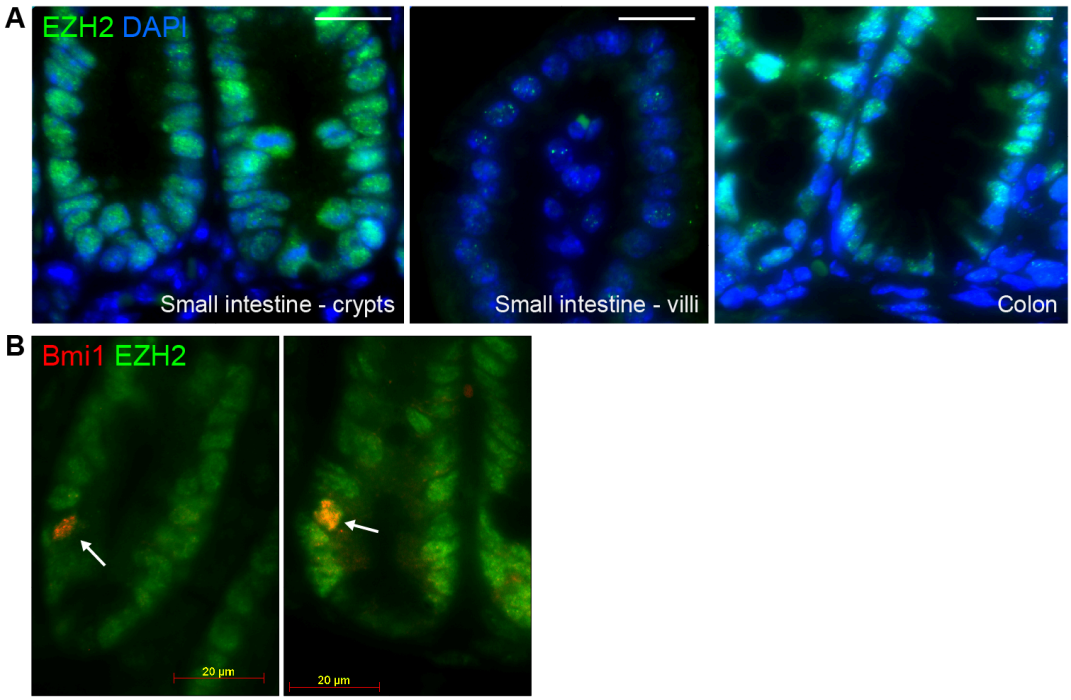
(**A**-**C**) PAF expression was conditionally induced by the combination of rtTA and doxycycline in various contexts. In 293T cells, transient transfection of rtTA combined with treatment with doxycycline (1 μg/ml, 36 h) successfully induced the expression of PAF (A) and emGFP (B) analyzed using IB and immunofluorescent staining, respectively. In *Rosa26-rtTA:iPAF* mice given doxycycline (2 mg/ml in drinking water for 4 days), expression of emGFP, a coexpression marker for PAF, was detected in the small intestine (C). The small intestine was fixed and visualized under an inverted fluorescent microscope (lumen: top view). Of note, no leakiness of PAF expression was detected.



Supplemental Figure S5 Intestinal epithelial cell hyperproliferation by PAF ectopic expression.

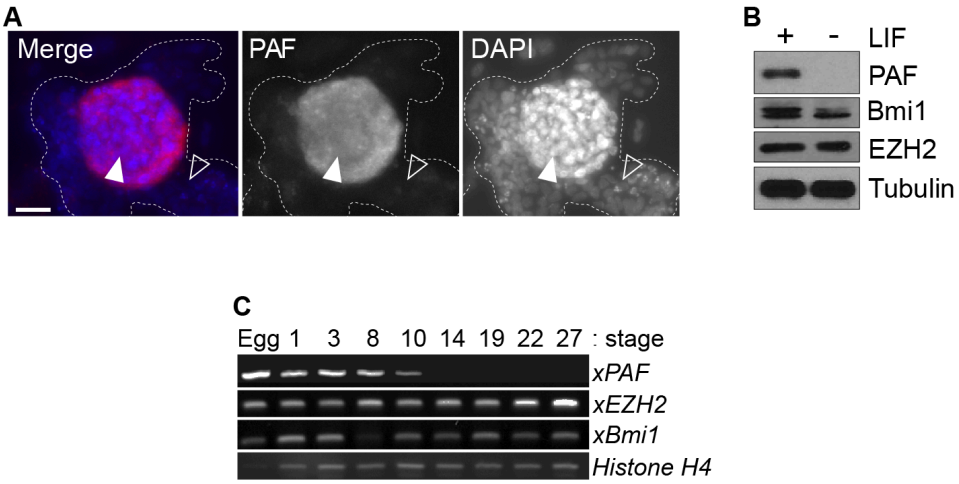
(**A**) Colonic crypt organoid culture system with minor modifications of previous procedures (Ootani et al., 2009), colonic crypts were isolated and cultured from *rtTA:iPAF* mice.
(**B**) Disorganized epithelial cell arrangement by PAF expression. *Villin-Cre:Rosa26-LSL-rtTA:iPAF* mice (experimental group) and *iPAF* mice (control)

were given doxycycline (2 mg/ml in drinking water, 2 months). Each tissue sample was immunostained for E-cadherin. Scale bars, 50 μm.
(**C**) Intestinal hypertrophy by ubiquitous PAF expression. *Rosa26-rtTA:iPAF* mice (experimental group) and *iPAF* mice (control) were given doxycycline (2 mg/ml in drinking water, 2 months). Macroscopically, PAF expression induced intestinal enlargement. The lengths of the intestines in control mice (*iPAF* + doxy) and mice with induced PAF expression (*Rosa26-rtTA:iPAF* + doxy) were compared (right panels).



Supplemental Figure S6. Expression of EZH2 and Bmi1 in intestinal crypts.

(A and B) Murine small intestine and colon samples were immunostained for EZH2 (A) and Bmi1 (B). EZH2 expression (green) was observed in epithelial cells in the crypts of both the small intestine and colon. (B) EZH2 is expressed the nucleus of intestinal epithelial cells including Bmi1 positive intestinal stem cells (arrows). However, EZH2 was not expressed in epithelial cells in villi (middle panel). These data suggest that EZH2 was not a limiting factor for PAF-induced activation of the β-catenin transcriptional complex in the murine intestine. Scale bar = 20 μm.



Supplemental Figure S7. Expression of PAF in self-renewing cells.

(**A**) PAF expression is limited to self-renewing cells. G4 mESCs were cultured under feeder-free conditions to induce spontaneous differentiation. Two days later, mESCs were stained with an anti-PAF antibody and DAPI. Upon plating mESCs on a feeder-free layer for spontaneous differentiation, cells partially differentiated, and this differentiation was initiated at the boundaries of each colony. Interestingly, PAF expression was rapidly lost in partially differentiated cells. Solid arrowheads, undifferentiated mESC; open arrowheads, differentiated mESC. Scale bar = 100 μm.
(**B**) PAF expression is lost in mESCs upon differentiation. mESCs were cultured on feeder cells with or without leukemia inhibitory factor (necessary for mESC self-renewal) for 4 days and then harvested for immunoblotting. After withdrawal of leukemia inhibitory factor, which is necessary for mESC self-renewal, expression of PAF was lost, whereas expression of polycomb group repressor complex (PRC) proteins, Bmi1 and EZH2, remained constant.
(**C**) PAF expression pattern during early embryogenesis. Whereas xPAF was highly expressed in the egg and throughout the early stage of blastulation, xPAF expression was completely lost at the gastrulation stage *X. laevis* embryos at each developmental stage were collected for semiquantitative RT-PCR analysis.

Supplemental Experimental Procedure

Gene expression analysis

For RNA extraction, samples were processed using TRIzol reagent (Invitrogen). One microgram of RNA was used for reverse transcription using SuperScript II (Invitrogen) and random hexamer. Next, cDNAs were used for gene expression analysis using semiquantitative RT-PCR or qRT-PCR. Quantification of qRT-PCR results was performed based on comparative $2^{-\Delta\Delta Ct}$ methods. The primer sequences used in this analysis are available upon request.

Mouse embryo whole-mount staining

Mouse embryos were fixed in 4% paraformaldehyde (PFA) and washed with phosphate-buffered saline. After 1 h of incubation in a blocking buffer (1% Triton X-100 and 10% fetal calf serum in phosphate-buffered saline), an anti-PAF antibody was added (1:250 dilution, overnight at 4°C). After a series of washing, a secondary antibody (horseradish peroxidase-conjugated anti-mouse; 1:500) was then added. Finally, a 3,3'-diaminobenzidine substrate reaction was performed to visualize PAF expression. For X-gal staining, *Axin2-LacZ* mouse embryos were collected and fixed in 4% PFA (5 min at 4°C) and incubated with X-gal for 2 h at 37°C. Next, the embryos were postfixed in 4% PFA for 10 min. Immunostained embryos were photographed using a dissection microscope (Zeiss).

Constructs

All constructs were generated from cDNA or open reading frame sources *via* polymerase chain reaction (PCR) and constructed into FLAG-pcDNA3.1, HA-pcDNA3.1, HA-pMSCV, FLAG-pMGIB, or pGEX-6T. nt-PAF, mutPIP-PAF, PAF deletion mutants, β-catenin deletion mutants, EZH2 mutants, and EZH2 F681I mutants were constructed using PCR-based mutagenesis. Akt and Myr-Akt constructs were kindly provided by Junjie Chen (MD Anderson Cancer Center).

Immunohistochemistry

Tissue samples were collected and fixed in 10% formalin and processed for paraffin embedding. Sectioned samples were immunostained according to standard protocols. Antibodies: PAF (Abcam and Santa Cruz Biotechnology), β-catenin (Cell Signaling), CD44 (BD Biosciences), and Ki67 (BD Biosciences). For X-gal staining of intestinal tissue samples from *BAT-gal* mice, the samples or embryos were fixed in 4% PFA (5 min at 4°C) and incubated with X-gal for 2 h. The microarrays were purchased from US Biomax (microarray panel CO242a, BC05023, and CO1002).

Reporter assays

The reporter plasmids pMegaTOPFLASH, pMegaFOPFLASH, c-Myc-luc, Siamois-luc, Cyclin D1-luc, FoxO-luc (6xDBE-luc), and Smad-binding element–

Luc were transiently transfected with pSV40-Renilla, as previously performed (Park et al., 2005). FoxO-luc. plasmid was kindly provided by Ann Brunet (Stanford University).

Immunoblot and immunoprecipitation assays

Whole cell lysates of mammalian cells were prepared and analyzed for IB and IP, as previously performed (Jung et al., 2013a). For immunoprecipitation, cell lysates were incubated with 20 μl of either HA- or FLAG-agarose beads. Immunoprecipitates were then washed with cell lysis buffer four times, eluted using an SDS sample buffer, and analyzed using immunoblotting. Information for antibodies is available in Supplemental Information. Antibodies: PAF (Abcam and Santa Cruz Biotechnology), Histone H4 (Santa Cruz Biotechnology), β-catenin (Cell Signaling), tubulin (Sigma), FLAG (M2; Sigma), hemagglutinin (HA-7: Sigma; 3F10: Roche), TCF/LEF1 (Cell Signaling), EZH2 (Cell Signaling), GST (Santa Cruz Biotechnology), Brg-1 (Santa Cruz Biotechnology), Sirt1 (Cell Signaling), PCNA (Abcam), EED (Millipore), Suz12 (Abcam), and Bmi1 (Abcam).

Transgenic animals

A TetO minimal promoter-*mPAF*-IRES-EmGFP-BGHpA DNA fragment was constructed in a pCR2.1 vector. DNA was then injected into the pronucleus of the zygotes to generate transgenic *iPAF* mice. Doxy-induced PAF expression and absence of expression leakiness were verified in four of nine founder mice. *iPAF* pups from three independent founder strains were utilized for analysis. *PAF* transgene expression was induced by doxy administration in the late generations crossed with C57BL/6 mice. For systemic induction of PAF, *Rosa26-rtTA* (Hochedlinger et al., 2005) (Gossen et al., 1995)*:iPAF* strains were administered doxy (2 mg/ml in 5% sucrose drinking water). To induce expression of PAF in a tissue-specific manner, *Villin-Cre* (el Marjou et al., 2004)*:Rosa26-LSL-rtTA* (Belteki et al., 2005)*:iPAF* strains were utilized. *Rosa26-rtTA*, *Villin-Cre*, *Rosa26-LSL-rtTA*, *Axin2-LacZ* (Lustig et al., 2002), and *BAT-gal* mice were purchased from The Jackson Laboratory. All mice were maintained according to institutional guidelines and Association for Assessment and Accreditation of Laboratory Animal Care International standards.

Cell synchronization

SW620 cells were synchronized using a double thymidine block. After removal of thymidine, cells were collected at each time point, and processed for cell cycle analysis. Briefly, cells were washed with phosphate-buffered saline, and fixed with ice-cold 70% ethanol. Then, cells were treated with 5 μg/μl RNase A and 50 μg/μl propidium iodine for 30 minutes at 37°C, analyzed using flow cytometer (C6, Accuri).

mESC culture

G4 mESCs were maintained in high-glucose Dulbecco's modified Eagle medium with 20% fetal bovine serum and 1000 U/ml leukemia inhibitory factor.

Supplemental References

Belteki, G., Haigh, J., Kabacs, N., Haigh, K., Sison, K., Costantini, F., Whitsett, J., Quaggin, S.E., and Nagy, A. (2005). Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. Nucleic Acids Res *33*, e51.

el Marjou, F., Janssen, K.P., Chang, B.H., Li, M., Hindie, V., Chan, L., Louvard, D., Chambon, P., Metzger, D., and Robine, S. (2004). Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. Genesis *39*, 186-193.

Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W., and Bujard, H. (1995). Transcriptional activation by tetracyclines in mammalian cells. Science *268*, 1766-1769.

Hochedlinger, K., Yamada, Y., Beard, C., and Jaenisch, R. (2005). Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell *121*, 465-477.

Jung, H.Y., Wang, X., Jun, S., and Park, J.I. (2013a). Dyrk2-associated EDD-DDB1-VprBP E3 ligase inhibits telomerase by TERT degradation. J Biol Chem *288*, 7252-7262.

Jung, H.Y., Wang, X., Jun, S., and Park, J.I. (2013b). Dyrk2-associated EDD-DDB1-VprBP E3 Ligase Inhibits Telomerase by TERT Degradation. J Biol Chem *288*, 7252-7262.

Lustig, B., Jerchow, B., Sachs, M., Weiler, S., Pietsch, T., Karsten, U., van de Wetering, M., Clevers, H., Schlag, P.M., Birchmeier, W.*, et al.* (2002). Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. Mol Cell Biol *22*, 1184-1193.

Ootani, A., Li, X., Sangiorgi, E., Ho, Q.T., Ueno, H., Toda, S., Sugihara, H., Fujimoto, K., Weissman, I.L., Capecchi, M.R.*, et al.* (2009). Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat Med *15*, 701-706.

Park, J.I., Kim, S.W., Lyons, J.P., Ji, H., Nguyen, T.T., Cho, K., Barton, M.C., Deroo, T., Vleminckx, K., Moon, R.T.*, et al.* (2005). Kaiso/p120-catenin and TCF/beta-catenin complexes coordinately regulate canonical Wnt gene targets. Developmental cell *8*, 843-854.