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STEM CELLS

A WNTer Revisit: New Faces of β-Catenin and TCFs in Pluripotency

Kazuhide Watanabe and Xing Dai*

New evidence has revealed interesting aspects of how the Wnt–β-catenin pathway controls self-renewal and lineage differentiation of pluripotent embryonic stem cells. Although Wnt–β-catenin signaling is dispensable for the self-renewal of naive mouse embryonic stem cells, it facilitates their expansion and resistance to differentiation through an unconventional dual mechanism involving the transcriptional repressor T cell factor (TCF) 3 and the transcriptional activator TCF1.

Embryonic stem cells (ESCs), such as those derived from mouse and human blastocysts (*1*, *2*), are pluripotent and can give rise to any cell type in the body. Understanding how the interplay between intracellular transcriptional networks and extracellular signaling cues governs the pluripotent state of ESCs (*3*, *4*) could provide insight into the basic mechanisms of mammalian development and direct the potential uses of ESCs in regenerative medicine. Extensive evidence implicates a positive, context-dependent role of Wnt signaling in self-renewal of ESCs (*5*). For example, chemical inhibition or genetic deletion of glycogen synthase kinase 3 (GSK3), an inhibitor of the Wnt pathway, promotes the self-renewal of ESCs (*6*–*9*). Moreover, several Wnts seem to support the maintenance of an undifferentiated state in ESCs (*8*, *10*–*12*). Given the diverse molecular responses elicited by GSK3 inhibition and Wnt ligands (*13*, *14*), one might question whether these effects in ESCs require the canonical Wnt signaling pathway, which uses β-catenin and transcription factors of the lymphoid enhancer factor (LEF) and T cell factor (TCF) families (LEF/TCF) as downstream effectors (*15*), or whether they are mediated through β-catenin– and LEF/TCF-independent mechanisms. Furthermore, several studies have yielded inconsistent results regarding the role of β-catenin in ESC self-renewal (*16*–*18*), and LEF/TCF-independent functions have been proposed (*19*). Is β-catenin's canonical mode of action in Wnt signaling still at play in ESCs, or do alternative mechanisms take center stage? Recent work (*12*, *20*–*22*) offers insights into these issues and challenges some of the prevailing notions regarding β-catenin and TCF functions in Wnt signaling and ESC biology.

As illustrated by ten Berge *et al*. (*12*), Wnt signals prevent differentiation of ESCs into epiblast stem cells and facilitate the derivation of ESC lines from mice. However, the question of whether β-catenin is required for ESC self-renewal arises from the apparently normal early development, including blastocyst formation, of β-catenin–null mice (*23*). Analysis of different β-catenin–deficient ESC lines has not always supported a stemness-promoting role for β-catenin (*16*–*18*). Lyashenko *et al*. and Wray *et al*. have independently established and characterized new β-catenin–deficient ESC lines using Cre-mediated recombination to eliminate adaptation or commitment of ESCs during the selection process (*20*, *21*). Both groups showed that when ESCs are cultured in appropriate conditions (*3*, *9*), the loss of β-catenin does not alter self-renewal expression of genes encoding pluripotency factors. However, in serum-free media, β-catenin–deficient cells exhibit a near-obligatory need for leukemia inhibitory factor (LIF) and, to a lesser extent, mitogen-activated protein kinase (MAPK) inhibition to maintain clonogenicity and stemness. β-catenin deletion abolishes the ability of GSK3 inhibition to prevent exit from the so-called naïve pluripotent state. Together, these results demonstrate that β-catenin is not required for ESC self-renewal per se, but it is an important (although not exclusive) mediator of the effect of GSK3 inhibition in resisting early differentiation (Fig. 1) (*21*). This notion is consistent with the finding that the Wnt3a ligand can substitute for GSK3 inhibition in supporting ESC self-renewal when LIF is present and MAPK is inhibited, as reported by ten Berge *et al*. (*12*). Hence, the apparently different conclusions drawn from various studies probably reflect differences in derivation and culturing conditions that affect the intrinsic state of the cell lines under study. Depending on the strain or species of origin of ESC lines and the exact signaling milieu they are in, ESCs may be in a naive ground state of pluripotency corresponding to that of the inner cell mass of blastocysts, or they may more closely resemble epiblast stem cells, which are in a primed pluripotent state with an increased readiness to differentiate (*3*, *24*, *25*). Because inner cell mass–to–epiblast development spans several days, there may be more intermediate states than are operationally defined. As our understanding of the cellular heterogeneity and resolution within an overall pluripotency framework improves, previously unknown aspects of β-catenin's function in pluripotency may unfold.

In canonical Wnt signaling, cytoplasmic β-catenin is stabilized and translocates to the nucleus, where it facilitates LEF/TCF-dependent gene expression, in part through its C-terminal transcriptional activation (CTA) domain (*26*). Reconstitution experiments carried out by Wray *et al*. revealed that the CTA domain is not required for β-catenin's ability to rescue ESC self-renewal in response to GSK inhibition (*21*). This opens at least two possibilities regarding β-catenin's role in ESC self-renewal: (i) It acts through nontranscription mechanisms such as cell adhesion, or (ii) it regulates transcription, but via noncanonical mechanisms.

In addition to its role as an effector of canonical Wnt signaling, β-catenin is also a component of adherens junctions, where it links cadherins to actin filaments through α-catenin (*27*). Loss of β-catenin in vivo induces increased abundance of plakoglobin, another cadherin-associated protein that can compensate for β-catenin in cell adhesion (*23*). Hence, β-catenin loss-of-function phenotypes have often been attributed to its signaling role. In self-renewing ESCs, β-catenin ablation leads to cell adhesion defects; however, they are subtle and transient, owing to partial compensation from plakoglobin, and do not seem to affect ESC self-renewal (*20*, *21*). During in vitro differentiation (embryoid body formation) of ESCs, adhesion defects become prominent, possibly due to decreased abundance of plakoglobin (*20*). Because β-catenin lacking the CTA domain can rescue defects in endoderm and neuronal differentiation induced by β-catenin deficiency, Lyashenko *et al*. concluded that the adhesion function of β-catenin, rather than its ability to regulate LEF/TCF-dependent signaling, is more

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important in these processes (Fig. 1) (*20*). Although these correlation-based claims are reasonable, they warrant additional evidence that more directly links β-catenin–mediated cell adhesion to these lineage differentiation processes. A lack of requirement for the CTA domain alone is insufficient to favor cell adhesion function and exclude the involvement of LEF/TCF-dependent mechanisms in β-catenin action. Other regions in β-catenin possess transactivation function or interact with transcriptional coactivators, such as Bcl9 and Pygopus (*28*, *29*). Moreover, as illustrated by additional studies from Wray *et al*., β-catenin displays CTA-independent signaling activity that involves derepression of TCF3. We also urge caution in interpreting findings based on the use of transgenes for the obvious reason of nonphysiological abundance and possible intracellular mislocalization of the encoded proteins. Because different dosages of β-catenin can elicit different biological outcomes (*30*), a finer dissection of the relative contribution of cell adhesion and signaling mechanisms to β-catenin function awaits the deletion of the CTA domain from the endogenous β-catenin locus (and additional mutations of this locus), as well as a quantitative assessment of how these genetic alterations affect endoderm and neuron formation.

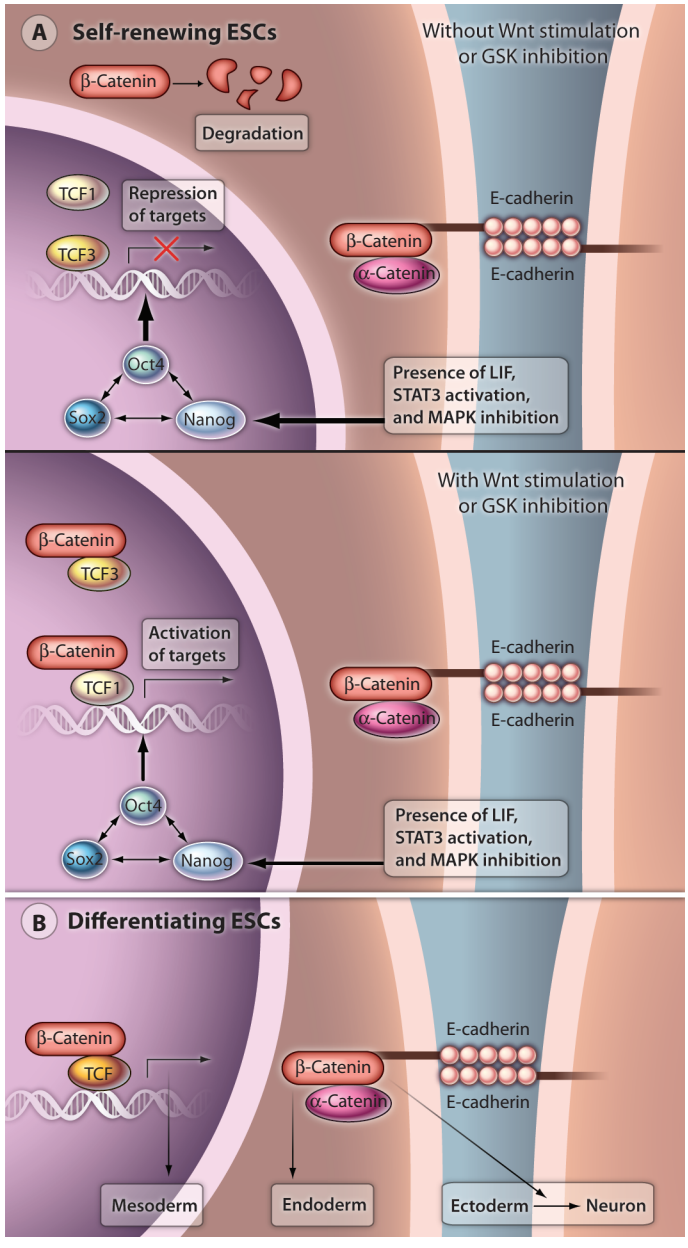

Fig. 1. Model of the Wnt–β-catenin/TCF pathway in ESC self-renewal and differentiation. (**A**) In self-renewing ESCs, TCF/LEF target genes are bound by transcriptional repressor TCF3, which limits their activation by the core regulatory circuit to ensure differentiation competence (top). Upon Wnt stimulation or GSK3 inhibition (middle), β-catenin is stabilized and enters the nucleus to remove TCF3 repression and facilitate the activation of target genes by transcriptional activator TCF1. This enables ESCs to maintain self-renewal and resist differentiation when other pluripotency- or self-renewal–promoting influences—such as the presence of LIF, activation of signal transducer and activator of transcription 3 (STAT3), or MAPK inhibition—are lacking. (**B**) During differentiation, β-catenin at cell-cell junctions appears critical for ESCs to give rise to definitive endoderm or neurons (through neuroectoderm), whereas its signaling role is important for mesoderm formation.

An answer as to whether β-catenin uses noncanonical mechanisms to regulate transcription in ESCs comes from focused studies of TCF3 by Wray *et al*. and Yi *et al*. (*21*, *22*). Of the four LEF/TCF transcription factors, TCF3 is the most abundant in ESCs (*22*, *31*) and co-occupies a set of target promoters with the core group of Oct4, Sox2, and Nanog pluripotency factors (*32*). A twofold overexpression of TCF3 suppresses ESC self-renewal, an effect that is counteracted by the addition of Wnt3a (*22*). Ablation of TCF3 replaces the need for GSK3 inhibition in supporting ESC self-renewal and delaying differentiation regardless of the presence or absence of β-catenin (*21*, *22*). An emerging consensus is that TCF3 restricts ESC self-renewal by acting as a β-catenin–independent transcriptional repressor of genes encoding factors that promote self-renewal and pluripotency to restrict ESC self-renewal (Fig. 1) (*21*, *22*, *31*, *33*, *34*). Upon GSK3 inhibition or Wnt3a stimulation, this repressor function is abrogated in a β-catenin–dependent manner, facilitating the self-renewal of ESCs. Evidence is, in part, provided by the failure of ESC lines with a form of TCF3 lacking the β-catenin binding domain (*21*, *22*) to self-renew in response to GSK3 inhibition or Wnt3a stimulation.

Does Wnt-stabilized β-catenin convert TCF3 from a repressor to an activator, a scenario in line with the classical model of Wnt–β-catenin signaling (*35*, *36*), or is a distinct TCF factor responsible for Wnt-induced activation of LEF/TCF target genes in ESCs? Bioinformatic studies by Yi *et al*. suggest the latter (*22*). Wnt3a stimulation and TCF3 deletion in ESCs converge on the regulation of a common set of genes, a substantial portion of which is also under the influence of the core pluripotency factors Oct4 and Nanog (*22*, *33*). Depletion of TCF1 reduces Wnt-mediated gene expression and self-renewal, highlighting TCF1 as a pivotal mediator of Wnt-induced transcriptional activation in ESCs. Functional contributions to Wnt3a-stimulated ESC self-renewal and target expression have been identified from both β-catenin–dependent derepression of TCF3 and TCF1-mediated activation. Hence, it appears that a balance between the repressor function of TCF3 and the activator function of TCF1 is critical in controlling ESC self-renewal and that Wnt–β-catenin signaling tips this balance by both countering TCF3 repression and facilitating TCF1 activation. That two different TCF factors oppose each other in

determining ESC fate is somewhat reminiscent of a familiar theme in Wnt biology, namely, the opposing effects of dominant-negative and full-length LEF/TCF isoforms in normal and cancer cells (*37*). Whether somatic stem cells use a similar mechanism to control their fate is an intriguing issue that merits future investigation. Along this line, we note that during the lineage progression of adult hair follicle stem cells, TCF3 and TCF4 are present in quiescent bulge stem cells, whereas LEF1 is active in committed hair follicle progenitor cells (*38*–*40*).

Does TCF1 physically displace TCF3 from Wnt target promoters upon signaling activation? Alternatively, there may not be distinct TCF1-on-target or TCF3-on-target ESC populations; instead, in any given ESC, both TCF1 and TCF3 dynamically associate and dissociate with the same set of target promoters, but Wnt-stabilized β-catenin may alter the equilibrium to stabilize TCF1 occupancy. The genetic and molecular data from Yi *et al*. do not distinguish between these possibilities. It would be necessary to examine TCF3 and TCF1 occupancy at target promoters with and without Wnt stimulation, preferably over a time scale that allows the visualization of dynamic factor exchange (*41*). Do TCF3 and TCF1 associate with distinct transcriptional regulatory complexes, such as corepressor and coactivator complexes, respectively, in ESCs? Regardless of the underlying biochemical mechanisms, work by Yi *et al*. and Wray *et al*. extends our current thinking on how β-catenin–LEF/TCF complexes regulate transcription. In particular, their observation that disruption of TCF3 repression mediated by Wnt–β-catenin requires the β-catenin–interacting domain in TCF3 offers a clue to the underlying biochemical basis. Wnt signals induce the phosphorylation of *Xenopus* TCF3 (xTCF3) in a β-catenin– and homeodomain-interacting protein kinase 2 (HIPK2)–dependent manner, leading to xTCF3 dissociation from target promoters (*42*, *43*). In contrast, xTCF1 does not undergo Wnt-HIPK2–mediated phosphorylation and remains bound to its target promoter upon HIPK2 overexpression (*43*), providing a possible biochemical explanation for the differential effects of β-catenin on TCF1 and TCF3 function. It will be interesting to test whether these findings in *Xenopus* are applicable to Wnt–β-catenin regulation of TCF3 function in ESCs, and possibly somatic stem cells, such as those in the hair follicle bulge. If so, perhaps the textbook model of canonical Wnt signaling will need to be expanded to include a β-catenin–assisted, HIPK2-mediated TCF3-TCF1 switch.

An intriguing paradox arises from recent and previous studies, regarding why TCF3 is abundant in pluritent ESCs if its role is to restrict self-renewal (*22*, *33*, *39*). One explanation is that it builds an intrinsic mechanism that confers to the cells competence to differentiate in response to appropriate cues. Controlling the expression of genes encoding self-renewal or pluripotency factors through opposing forces—including TCF3, TCF1, and the core pluripotency circuitry—may be advantageous in coordinating self-renewal with differentiation as well as in providing sensitivity and robustness to extracellular signals such as Wnts.

References and Notes

1. M. J. Evans, M. H. Kaufman, Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**, 154–156 (1981).
2. J. A. Thomson, J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, J. M. Jones, Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145–1147 (1998).
3. J. H. Hanna, K. Saha, R. Jaenisch, Pluripotency and cellular reprogramming: Facts, hypotheses, unresolved issues. *Cell* **143**, 508–525 (2010).
4. M. F. Pera, P. P. Tam, Extrinsic regulation of pluripotent stem cells. *Nature* **465**, 713–720 (2010).
5. T. Miki, S.-y. Yasuda, M. Kahn, Wnt/β-catenin signaling in embryonic stem cell self-renewal and somatic cell reprogramming. *Stem Cell Rev. Rep.* 10.1007/s12015-011-9275-1 (2011).
6. B. W. Doble, S. Patel, G. A. Wood, L. K. Kockeritz, J. R. Woodgett, Functional redundancy of GSK-3α and GSK-3β in Wnt/β-catenin signaling shown by using an allelic series of embryonic stem cell lines. *Dev. Cell* **12**, 957–971 (2007).
7. J. Hanna, S. Markoulaki, M. Mitalipova, A. W. Cheng, J. P. Cassady, J. Staerk, B. W. Carey, C. J. Lengner, R. Foreman, J. Love, Q. Gao, J. Kim, R. Jaenisch, Metastable pluripotent states in NOD-mouse-derived ESCs. *Cell Stem Cell* **4**, 513–524 (2009).
8. N. Sato, L. Meijer, L. Skaltsounis, P. Greengard, A. H. Brivanlou, Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat. Med.* **10**, 55–63 (2004).
9. Q. L. Ying, J. Wray, J. Nichols, L. Batlle-Morera, B. Doble, J. Woodgett, P. Cohen, A. Smith, The ground state of embryonic stem cell self-renewal. *Nature* **453**, 519–523 (2008).
10. J. Hao, T. G. Li, X. Qi, D. F. Zhao, G. Q. Zhao, WNT/β-catenin pathway up-regulates Stat3 and converges on LIF to prevent differentiation of mouse embryonic stem cells. *Dev. Biol.* **290**, 81–91 (2006).
11. D. K. Singla, D. J. Schneider, M. M. LeWinter, B. E. Sobel, wnt3a but not wnt11 supports self-renewal of embryonic stem cells. *Biochem. Biophys. Res. Commun.* **345**, 789–795 (2006).
12. D. T. Berge, D. Kurek, T. Blauwkamp, W. Koole, A. Maas, E. Eroglu, R. K. Siu, R. Nusse, Embryonic stem cells require Wnt proteins to prevent differentiation to epiblast stem cells. *Nat. Cell Biol.* **13**, 1070–1075 (2011).
13. B. W. Doble, J. R. Woodgett, GSK-3: Tricks of the trade for a multi-tasking kinase. *J. Cell Sci.* **116**, 1175–1186 (2003).
14. R. van Amerongen, R. Nusse, Towards an integrated view of Wnt signaling in development. *Development* **136**, 3205–3214 (2009).
15. H. Clevers, Wnt/β-catenin signaling in development and disease. *Cell* **127**, 469–480 (2006).
16. R. Anton, H. A. Kestler, M. Kühl, β-catenin signaling contributes to stemness and regulates early differentiation in murine embryonic stem cells. *FEBS Lett.* **581**, 5247–5254 (2007).
17. F. Soncin, L. Mohamet, D. Eckardt, S. Ritson, A. M. Eastham, N. Bobola, A. Russell, S. Davies, R. Kemler, C. L. Merry, C. M. Ward, Abrogation of E-cadherin-mediated cell-cell contact in mouse embryonic stem cells results in reversible LIF-independent self-renewal. *Stem Cells* **27**, 2069–2080 (2009).
18. R. T. Wagner, X. Xu, F. Yi, B. J. Merrill, A. J. Cooney, Canonical Wnt/β-catenin regulation of liver receptor homolog-1 mediates pluripotency gene expression. *Stem Cells* **28**, 1794–1804 (2010).
19. K. F. Kelly, D. Y. Ng, G. Jayakumaran, G. A. Wood, H. Koide, B. W. Doble, β-catenin enhances Oct-4 activity and reinforces pluripotency through a TCF-independent mechanism. *Cell Stem Cell* **8**, 214–227 (2011).
20. N. Lyashenko, M. Winter, D. Migliorini, T. Biechele, R. T. Moon, C. Hartmann, Differential requirement for the dual functions of β-catenin in embryonic stem cell self-renewal and germ layer formation. *Nat. Cell Biol.* **13**, 753–761 (2011).
21. J. Wray, T. Kalkan, S. Gomez-Lopez, D. Eckardt, A. Cook, R. Kemler, A. Smith, Inhibition of glycogen synthase kinase-3 alleviates Tcf3 repression of the pluripotency network and increases embryonic stem cell resistance to differentiation. *Nat. Cell Biol.* **13**, 838–845 (2011).
22. F. Yi, L. Pereira, J. A. Hoffman, B. R. Shy, C. M. Yuen, D. R. Liu, B. J. Merrill, Opposing effects of Tcf3 and Tcf1 control Wnt stimulation of embryonic stem cell self-renewal. *Nat. Cell Biol.* **13**, 762–770 (2011).
23. J. Huelsken, R. Vogel, V. Brinkmann, B. Erdmann, C. Birchmeier, W. Birchmeier, Requirement for β-catenin in anterior-posterior axis formation in mice. *J. Cell Biol.* **148**, 567–578 (2000).
24. P. J. Tesar, J. G. Chenoweth, F. A. Brook, T. J. Davies, E. P. Evans, D. L. Mack, R. L. Gardner, R. D. McKay, New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448**, 196–199 (2007).
25. J. Nichols, A. Smith, Naive and primed pluripotent states. *Cell Stem Cell* **4**, 487–492 (2009).
26. M. van de Wetering, R. Cavallo, D. Dooijes, M. van Beest, J. van Es, J. Loureiro, A. Ypma, D. Hursh, T. Jones, A. Bejsovec, M. Peifer, M. Mortin, H. Clevers, Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene *dTCF*. *Cell* **88**, 789–799 (1997).
27. H. Aberle, H. Schwartz, R. Kemler, Cadherin-catenin complex: Protein interactions and their implications for cadherin function. *J. Cell. Biochem.* **61**, 514–523 (1996).
28. S. C. Hsu, J. Galceran, R. Grosschedl, Modulation of transcriptional regulation by LEF-1 in response to Wnt-1 signaling and association with β-catenin. *Mol. Cell. Biol.* **18**, 4807–4818 (1998).
29. S. Jessen, B. Gu, X. Dai, Pygopus and the Wnt signaling pathway: A diverse set of connections. *Bioessays* **30**, 448–456 (2008).

30. W. E. Lowry, C. Blanpain, J. A. Nowak, G. Guasch, L. Lewis, E. Fuchs, Defining the impact of β-catenin/Tcf transactivation on epithelial stem cells. *Genes Dev.* **19**, 1596–1611 (2005).
31. L. Pereira, F. Yi, B. J. Merrill, Repression of Nanog gene transcription by Tcf3 limits embryonic stem cell self-renewal. *Mol. Cell. Biol.* **26**, 7479–7491 (2006).
32. M. F. Cole, S. E. Johnstone, J. J. Newman, M. H. Kagey, R. A. Young, Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells. *Genes Dev.* **22**, 746–755 (2008).
33. F. Yi, L. Pereira, B. J. Merrill, Tcf3 functions as a steady-state limiter of transcriptional programs of mouse embryonic stem cell self-renewal. *Stem Cells* **26**, 1951–1960 (2008).
34. G. Guo, Y. Huang, P. Humphreys, X. Wang, A. Smith, A *PiggyBac*-based recessive screening method to identify pluripotency regulators. *PLoS ONE* **6**, e18189 (2011).
35. M. Bienz, TCF: Transcriptional activator or repressor? *Curr. Opin. Cell Biol.* **10**, 366–372 (1998).
36. H. Brantjes, N. Barker, J. van Es, H. Clevers, TCF: Lady Justice casting the final verdict on the outcome of Wnt signalling. *Biol. Chem.* **383**, 255–261 (2002).
37. K. Hovanes, T. W. Li, J. E. Munguia, T. Truong, T. Milovanovic, J. Lawrence Marsh, R. F. Holcombe, M. L. Waterman, β-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. *Nat. Genet.* **28**, 53–57 (2001).
38. H. Nguyen, B. J. Merrill, L. Polak, M. Nikolova, M. Rendl, T. M. Shaver, H. A. Pasolli, E. Fuchs, Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia. *Nat. Genet.* **41**, 1068–1075 (2009).
39. B. J. Merrill, U. Gat, R. DasGupta, E. Fuchs, Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin. *Genes Dev.* **15**, 1688–1705 (2001).
40. R. DasGupta, E. Fuchs, Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development* **126**, 4557–4568 (1999).
41. J. Sierra, T. Yoshida, C. A. Joazeiro, K. A. Jones, The APC tumor suppressor counteracts β-catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev.* **20**, 586–600 (2006).
42. H. Hikasa, J. Ezan, K. Itoh, X. Li, M. W. Klymkowsky, S. Y. Sokol, Regulation of TCF3 by Wnt-dependent phosphorylation during vertebrate axis specification. *Dev. Cell* **19**, 521–532 (2010).
43. H. Hikasa, S. Y. Sokol, Phosphorylation of TCF proteins by homeodomain-interacting protein kinase 2. *J. Biol. Chem.* **286**, 12093–12100 (2011).
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