Sclerosteosis is an autosomal recessive disease that is characterized by overgrowth of bone tissue and is linked to mutations in the gene encoding the secreted protein SOST. Sclerosteosis shares remarkable similarities with “high bone mass” diseases caused by “gain-of-function” mutations in the LRP5 gene, which encodes a co-receptor for Wnt signaling proteins. We show here that SOST antagonizes Wnt signaling in *Xenopus* embryos and mammalian cells by binding to the extracellular domain of the Wnt coreceptors LRP5 and LRP6 and disrupting Wnt-induced Frizzled-LRP complex formation. Our findings suggest that SOST is an antagonist for Wnt signaling and that the loss of SOST function likely leads to the hyperactivation of Wnt signaling that underlies bone overgrowth seen in sclerosteosis patients.

Sclerosteosis (1) and Van Buchem (2) disease are rare forms of autosomal recessive severe cranio-tubular hyperostoses. Both diseases are characterized by generalized overgrowth of bone tissue mostly manifested in cranial bones and in the diaphysis of tubular bones (3). Bone overgrowth appears as early as at the age of 5 years and becoming more prominent with time. Sclerosteosis is linked to a loss of function of the SOST gene product (4, 5), whereas Van Buchem disease is linked to a 52-kb deletion downstream of the SOST gene that causes down-regulation of SOST gene expression (6, 7).

The SOST gene encodes a secreted protein. During embryogenesis SOST expression is first detected in the mesenchyme at the sites of osteogenesis (8, 9), and SOST expression is confined specifically to osteoblasts and osteocytes postnatally (9, 10). The increased rate of bone formation and elevated levels of serum alkaline phosphatase and osteocalcin in (9, 10). The increased rate of bone formation and elevated levels of serum alkaline phosphatase and osteocalcin in (9, 10). The increased rate of bone formation and elevated levels of serum alkaline phosphatase and osteocalcin in (9, 10). The increased rate of bone formation and elevated levels of serum alkaline phosphatase and osteocalcin in (9, 10). The increased rate of bone formation and elevated levels of serum alkaline phosphatase and osteocalcin in (9, 10). The increased rate of bone formation and elevated levels of serum alkaline phosphatase and osteocalcin in (9, 10). The increased rate of bone formation and elevated levels of serum alkaline phosphatase and osteocalcin in (9, 10).

### MATERIALS AND METHODS

SOST cDNA was cloned by PCR using IMAGE cDNA clone 2380708 as the template. SOST/pcDNA3.1+ contains the full-length SOST. SOST-Myc/pcDNA5 and SOST-IgG/pcDNA3.1+ were generated by fusion at the 5′-end of full-length SOST cDNA without the stop codon with the 6X Myc epitope tag from CS2+MT or the IgG tag from IgG/pRK5 (21). Kremen2/pCS (22), DKK1-FLAG/CS2+ (23), LRP5N-Myc/pcDNA3, Xwnt8/CS2+ (19), LRP5N-Myc/pcDNA3, LGLRN-Myc/pcDNA3 (24), FzSCRD-IgG (21), TBR/pSp64T+ (25), LRP5/pcDNA3 (26), LRP6/pcDNA3.1 (27), LRP6N/CS2+ (28), Wnt1/LNCX (29), and PSecAP-MH (30) constructions have been described previously. Precipitation and immunoblotting were done similarly to Hsieh et al. (21). Fz-LRP6 complex formation (19), cytosolic β-catenin assay, and LRP6N-Fz5 complex disruption by SOST or DKK1 were performed as described previously (24).

**Xenopus** injection and RT-PCR were performed as described previously (24). Rat2 and HEK293T cells were maintained in high glucose Dulbecco's modified Eagle's medium with 10% newborn calf serum. Wnt1 conditioned medium (CM) was collected from retrovirally infected Rat2 cells. Other CMs were collected from transiently transfected HEK293T cells. TOPFlash reporter assays were performed using the Dual-Luciferase reporter assay system (Promega). HEK293T cells were seeded into 24-well plates 1 day before transfection. Cells were transfected with 100 ng of SuperTOPFlash reporter plasmid (31) and 10 ng of pRL-TK plasmid (Promega) with other plasmids as indicated in the experiment descriptions using Lipofectamine reagent (Invitrogen). DNA amounts were balanced with pcDNA3.1+ plasmid. Cell extracts were prepared 36 h after transfection and assayed sequentially for firefly and *Renilla* luciferase activity. Firefly luciferase readings were normalized against *Renilla* luciferase.

* This work was supported in part by National Institutes of Health Grant GM057603 (to X. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence may be addressed: Division of Neuroscience, Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115. Tel.: 617-919-2260 or 617-355-6885; Fax: 617-730-0243; E-mail: mikhail.semenov@childrens.harvard.edu or xi.he@childrens.harvard.edu.

‡ Partially supported by research fellowship from the Uehara Memorial Foundation.

¶ W. M. Keck Foundation Distinguished Young Scholar in Medical Research.

§ The abbreviations used are: BMP, bone morphogenetic protein; CM, conditioned medium; DKK, Dickkopf; Fz, Frizzled; HBM, high bone mass; LRP, low-density lipoprotein receptor-related protein; Xnr3, *Xenopus* nodal-related 3.
RESULTS

Xenopus axis duplication assay provides a sensitive and reliable way to test Wnt stimulatory and Wnt inhibitory activities (32). Injection of 5 pg of Xwnt8 or 50 pg of LRP6AN mRNA (an constitutively active mutant LRP6) into the ventral marginal zone of a four-cell Xenopus embryo caused ectopic axis formation in >80% of injected embryos (Fig. 1A). SOST mRNA ventral injection alone did not induce ectopic axis formation even when injected at a high dose (2000 pg/embryo). However, SOST mRNA efficiently blocked axis duplication by Xwnt8 mRNA (Fig. 1A). Thus, SOST antagonizes Xwnt8 activity. LRP6AN activated Wnt signaling in a Wnt-independent manner and was not inhibited by SOST (Fig. 1A). Two versions of SOST, an untagged SOST and a SOST with a carboxyl-terminal Myc tag, showed the same activity in antagonizing Wnt signaling in this assay (Fig. 1A). To ensure that SOST directly antagonizes Wnt signaling, we examined the ability of SOST to inhibit Xenopus nodal-related 3 (Xnr3) induction by Wnt8 in animal pole explants, because Xnr3 is a directly downstream target of Wnt signaling (32). SOST blocked Xnr3 activation by Xwnt8 but not by LRP6AN (Fig. 1B). SOST mRNA injection alone did not induce Xnr3 expression (Fig. 1B).

We also examined whether SOST possesses anti-BMP activity in Xenopus embryos (33, 34). Injection ventrally of 250 pg of truncated BMP receptor mRNA efficiently blocked BMP signaling in developing Xenopus embryos and caused trunk duplication in >80% of injected embryos. By contrast, SOST mRNA injection even at 2000 pg could not induce trunk axis duplication (Fig. 1C). Furthermore, dorsal injection of SOST mRNA (2000 pg/embryo) at the four-cell stage resulted in exaggerated anterior development such as enlargement of the cement gland (not shown), a phenotype similar to that observed by the injection of other Wnt antagonists (33). Thus, SOST shows potent anti-Wnt activity and does not exhibit anti-BMP activity in Xenopus embryos.

To test the ability of SOST to inhibit Wnt signaling in mammalian cells, we employed the SuperTOPflash reporter (31, 35), which is driven by T cell factor/lymphoid enhancer factor-binding elements and is responsive to Wnt signaling. The SuperTOPflash reporter was readily activated in HEK293T cells transfected with a Wnt1-expressing plasmid. Co-expression of a SOST expression plasmid inhibited Wnt signaling in a dose-dependent manner (Fig. 2A). To further study the SOST protein biochemically, we generated CM from HEK293T cells transfected with the SOST-Myc plasmid. We found that the majority of the SOST protein remains cell-associated (Fig. 2B). The major SOST isoform associated with cells and in the CM had an apparent molecular mass of 40 kDa as compared with the calculated molecular mass of 33 kDa. In addition, SOST isoforms with apparent molecular masses from 40 to 50 kDa and from 70 to 110 kDa were also detected in the CM (Fig. 2B). These higher molecular mass isoforms may be attributed to
post-translational modifications, because SOST has two predicted sites for N-glycosylation at Asn-53 and Asn-175 (www.w.cbs.dtu.dk/services/NetNGlyc/) and one site for O-linked glycosylation at Thr-55 (www.cbs.dtu.dk/services/NetOGlyc/) (36). We tested SOST CM for inhibition of Wnt signaling. Indeed, SOST CM inhibited cytosolic β-catenin accumulation induced by Wnt-1 CM in Rat2 cells (Fig. 2C). Thus, secreted SOST directly antagonizes Wnt1 activity extracellularly.

Similarities between sclerosteosis (associated with the loss of SOST function) and HBM disease linked to hyperactive LRP5 signaling suggest that SOST may antagonize Wnt signaling via direct binding to LRP5. To test the binding between SOST and LRP5 as well as LRP6, we produced CM containing a SOST-IgG fusion protein, which tags SOST with the constant region of immunoglobulin heavy chain, and the extracellular portion of LRP5 and LRP6 tagged with the Myc epitope (LRP5N-Myc and LRP6N-Myc). We found that SOST showed specific interactions with LRP5 and LRP6 but not with a similarly tagged low-density lipoprotein receptor (LDLRN-Myc) (Fig. 3A). Furthermore, when LDLRN-Myc was mixed in large excess together with LRP5N-Myc and LRP6N-Myc, SOST specifically bound to LRP5 and LRP6 (Fig. 3A). As an additional control for the binding specificity, we used Fz8CRD-IgG, which has a similar molecular mass to that of SOST-IgG. Despite much higher abundance in the CM, Fz8CRD-IgG was unable to precipitate any detectable amount of LRP5 or LRP6 protein (Fig. 3A).

To further demonstrate SOST-LRP5 and SOST-LRP6 interaction functionally, we examined whether SOST could inhibit LRP5 or LRP6 signaling activities. Although LRP5 and LRP6 overexpression alone showed little activation of Wnt signaling in the SuperTOPflash assay, LRP5 or LRP6 synergized robustly with Wnt1 (Fig. 3B). SOST efficiently inhibited signaling activated by Wnt1 plus LRP5 or Wnt1 plus LRP6 (Fig. 3B), showing that SOST functionally antagonizes signaling by LRP5 and LRP6. Formation of complexes between LRP5 or LRP6 and Frizzled (Fz) proteins in the presence of Wnt proteins has been proposed to be an initial step in Wnt signaling activation (19). As we showed previously (19), Fz8CRD-IgG showed no interaction with the extracellular domain of LRP6 (Figs. 3A and 4A), but the addition of Wnt1 CM induced the formation of complexes between Fz8 and LRP6 (Fig. 4A). The addition of CM containing either untagged or Myc-tagged SOST efficiently blocked such complex formation. CM containing alkaline phosphatase, used as a negative control, had no effect on complex formation (Fig. 4A). Thus, SOST appears to be able to disrupt Wnt-induced Fz-LRP6 complex formation.

We have shown that another Wnt antagonist, DKK1, also interacts with LRP5 and LRP6 and prevents Wnt-induced complex formation between LRP5/6 and Fz proteins (24). The anti-Wnt activity of DKK1 can be greatly enhanced by Kremen proteins, which also bind DKK1 (22). We thus compared the relationship between Kremen and SOST or DKK1 in Wnt signaling inhibition. We activated Wnt signaling by co-transfecting HEK293T cells with Wnt1-expressing plasmid (Fig. 2A), SOST-Myc protein is expressed and secreted into CM (Fig. 2B), and SOST CM inhibits cytosolic β-catenin stabilization induced by Wnt1 CM in Rat2 cells (Fig. 2C).

**Fig. 2. SOST inhibits Wnt signaling in mammalian cells.** A, SOST inhibits SuperTOPflash reporter expression activated by Wnt1. HEK293T cells were untransfected (lane 1) or transfected either with Wnt1-expressing plasmid (lane 2) or co-transfected with Wnt1 plus different amounts of the SOST-expressing plasmid (lanes 3–5). DNA amounts are shown in nanograms. B, SOST-Myc protein is expressed and secreted into CM in transiently transfected HEK293T cells. SOST protein associated with cells (lane 1) and secreted into CM (lane 4) were from untransfected cells. C, SOST CM inhibits cytosolic β-catenin stabilization induced by Wnt1 CM in Rat2 cells. Cells were either untreated (lane 1) or treated with Wnt1 CM (line 3) or Wnt1 CM plus SOST CM (lane 2). Cytosolic fractions were obtained and assayed for β-catenin.
single amino acid substitutions clustered in the first so-called triguingly, all known LRP5 mutations from HBM families are mutations of LRP5 are associated with HBM diseases (14, 15). In-
pseudoglioma syndrome (38), whereas “gain-of-function” muta-
LRP5 are associated with the recessive familial osteoporosis-
mediated by LRP5 and LRP6 plays a central role in mammalian

FIG. 3. SOST binds specifically to LRP5 and LRP6, and inhibits signaling by LRP5 and LRP6. A, LRPSN-Myc and LRPS6-Myc, but not LDLRN-Myc, were coprecipitated (IP) with SOST-IgG. None was coprecipitated with Fz8CRD-IgG. SOST-IgG CM (lanes 1–4) or Fz8CRD-IgG CM (lanes 5–8) was mixed with LRPSN-Myc CM (lanes 1 and 5), LRPS6-Myc (lanes 2 and 6), LDLRN-Myc (lanes 3 and 7), or LRPS6-Myc plus LRPSN-Myc plus LDLRN-Myc (lanes 4 and 8) and precipitated with protein G-agarose beads. Precipitates were immunoblotted with anti-Myc (section a) or anti-human IgG antibodies (section d). CM mixtures before precipitation were immunoblotted with anti-Myc (section b) or anti-human IgG antibodies (section c). Note that the SOST-IgG level in the input was much lower than that of Fz8CRD-IgG (section c). B, SOST inhibits SuperTOPflash reporter expression activated by Wnt1 plus LRP5 or Wnt1 plus LRP6. HEK293T cells were untransfected (lane 1) or transfected with Wnt1 plus the indicated plasmids (lanes 2–6). DNA amounts are shown in nanograms.

DISCUSSION

In this study we demonstrated that SOST, the product of the gene mutated in sclerosteosis and Van Buchem disease, is an antagonistic ligand for the Wnt coreceptors LRP5 and LRP6 and an inhibitor of the canonical Wnt/β-catenin signaling in both mammalian cells and Xenopus embryos. These findings not only expand the repertoire of secreted antagonistic ligands that bind to the Wnt coreceptors LRP5 and LRP6 but also have implications for our understanding of sclerosteosis, Van Buchem disease, and bone density diseases associated with LRP5 mutations such as HBM syndrome and osteoporosis.

It has become evident that the Wnt/β-catenin signaling mediated by LRP5 and LRP6 plays a central role in mammalian bone density regulation (13). Loss-of-function mutations of LRP5 are associated with the recessive familial osteoporosis-pseudoglioma syndrome (38), whereas “gain-of-function” mutations of LRP5 are associated with HBM diseases (14, 15). Intriguingly, all known LRP5 mutations from HBM families are single amino acid substitutions clustered in the first so-called YWTD β-propeller of the LRP5 extracellular domain (16). The molecular mechanism by which these mutations cause the increase of LRP5/β-catenin signaling during bone growth remains unclear. For one particular LRP5 mutation, G171V (glycine 171 mutated to valine), decreased inhibition of LRP5 by DKK1 was suggested to account for increased LRP5 signaling (14, 39). Because SOST is highly expressed in osteoblasts and osteocytes, and because a loss or down-regulation of SOST function in sclerosteosis and Van Buchem disease exhibits increased bone growth, SOST appears to be an endogenous negative regulator of bone growth, likely through the inhibition of LRP5 and LRP6 function. It will be interesting to examine whether LRP5 mutations associated with HBM disease result in compromised SOST inhibition of LRP5 function.

SOST shares 36% identity with WISE, which was shown to bind LRP6 and to activate or inhibit Wnt signaling in a context-dependent manner (20). These two closely related proteins share the “cysteine knot” domain that occupies the central part of proteins. One noticeable difference between SOST and WISE is that whereas SOST behaves exclusively as an antagonist for Wnt/LRP5/6 signaling in mammalian cells and Xenopus embryos, WISE alone can function as a weak agonist that activates β-catenin signaling to a limited extent (20). The mechanism and function of this Wnt agonist activity of WISE remains unclear. It is also of interest to compare SOST with the prototypic LRP5/6 antagonist DKK1 (33), which does not show any amino acid sequence similarity with SOST. Like DKK1 (24), SOST has the ability to disrupt Wnt1-induced Fz8-LRP6 complex formation in an in vitro assay, suggesting a potential mechanism for the observed SOST action. However, unlike DKK1, SOST inhibition of Wnt signaling is insensitive to the presence of Kremen2, a transmembrane protein that binds to DKK1 (22). Thus, although both are LRP5/6 ligands, the anti-Wnt activities of DKK1 and SOST can be differentially modulated by other co-factors.

Some studies suggest that SOST and WISE can bind BMP proteins and act as BMP antagonists (8–10, 40, 41). However,
in Xenopus embryo experiments neither SOST nor WISE exhibits any detectable antagonist activities toward BMP, although both strongly affect Wnt signaling. Indeed, whereas prototypic BMP antagonists such as Noggin and Chordin induce neural tissue and trunk formation (via inhibition of BMP signaling), SOST and WISE failed to do so even when they were expressed at high levels (Fig. 1C) (20). Thus, SOST and WISE may at best have weak BMP antagonist activities. Consistent with this notion, the binding affinities of SOST and WISE for BMPs are significantly weaker than those of Noggin and Chordin (8, 41). Further studies will be required to clarify whether SOST and WISE only function as Wnt/LRP5/6 antagonists or whether they function as antagonists for both Wnt and BMP signaling. Nonetheless, given the specific bone growth phenotypes of loss of SOST function and LRP5 mutations, modulating SOST and LRP5 interaction may be a potential therapeutic strategy for the treatment of bone density diseases such as osteoporosis.

Acknowledgments—We thank members of the Xi He laboratory for suggestions and help.

Addendum—While this manuscript was in preparation, Li et al. also reported that SOST can bind to LRP5/6 and function as a Wnt signaling antagonist (42).

REFERENCES
1. Beighton, P., Durr, L., and Hamersma, H. (1976) Ann. Intern. Med. 84, 393–397
2. van Buchem, F. S. P., Hadders, H. N., and Ubbens, R. (1955) Acta Radiol. (Stockh.) 44, 109–120
3. Hamersma, H., Gardner, J., and Beighton, P. (2003) Clin. Genet. 63, 192–197
4. Balemans, W., Ebeling, M., Patel, N., Van Hul, E., Olsen, P., Dioszegi, M., Laczà, C., Wuyts, W., Van Den Ende, J., Willems, P., Paes-Alves, A. F., Hill, S., Bueno, M., Ramos, F. J., Taconi, P., Dikkers, F. G., Stratakis, C., Lindpaintner, K., Vickery, B., Foernzler, D., and Van Hul, W. (2001) Hum. Mol. Genet. 10, 537–543
5. Brunkow, M. E., Gardner, J. C., Van Ness, J., Paepke, B. W., Kovacic, B. R., Proll, S., Skonier, J. E., Zhao, L., Sabo, P. J., Yu, F., Alisch, R. S., Gillett, L., Colbert, T., Taconi, P., Galas, D., Hamersma, H., Beighton, P., and Muligan, J. (2001) Am. J. Hum. Genet. 68, 577–589
6. Balemans, W., Patel, N., Ebeling, M., Van Hul, E., Wuyts, W., Laczà, C., Dioszegi, M., Dikkers, F. G., Hildering, P., Willems, P. J., Verheij, B. J., Lindpaintner, K., Vickery, B., Foernzler, D., and Van Hul, W. (2002) J. Med. Genet. 39, 91–97
7. Staehling-Hampton, K., Proll, S., Paepke, B. W., Zhao, L., Charmley, P., Brown, A., Gardner, J. C., Galas, D., Schatzman, R. C., Beighton, P., Papapoulos, S., Hamersma, H., and Brunkow, M. E. (2002) Am. J. Med. Genet. 110, 144–152
8. Kusui, N., Laurikkala, J., Imanishi, M., Ushui, H., Konishi, M., Miyake, A., Thesleff, J., and Itoh, N. (2000) J. Biol. Chem. 275, 24113–24117
9. Winkler, D. G., Sutherland, M. K., Geoghegan, J. C., Yu, C., Hayes, T., Skonier, J. E., Shepektor, D., Jonas, M., Kovacic, B. R., Staehling-Hampton, K., Appleby, M., and Latham, J. A. (2000) EMBO J. 19, 6267–6276
10. van Bezoë, R. L., Roelen, B. A., Visser, A., van der Veen-Pala, L., de Wilt, E., Karperien, M., Hamersma, H., Papapoulos, S. E., ten Dijke, P., and Lowik, C. W. (2004) J. Exp. Med. 199, 805–814
11. Wergedal, J. E., Veskovic, K., Hellan, M., Nyght, C., Balemans, W., Lihanati, C., Vanhoenacker, F. M., Tan, J., Baylink, D. J., and Van Hul, W. (2003) J. Clin. Endocrinol. Metab. 88, 5778–5783
12. Sutherland, M. K., Geoghegan, J. C., Yu, C., Turectt, E., Skonier, J. E., Winkler, D. G., and Latham, J. A. (2004) Bone 35, 828–835
13. Johnson, M. L., Harnish, K., Nusse, R., and Van Hul, W. (2004) J. Bone Miner. Res. 19, 1749–1757
14. Boyden, L. M., Mao, J., Belisky, J., Mitzner, L., Farhi, A., Mitsch, M. A., Wu, D., Isogawa, K., and Lifton, R. P. (2002) N. Engl. J. Med. 346, 1513–1521
15. Little, R. D., Carulli, J. P., Del Mastro, R. G., Dupuis, J., Osborne, M., Collet, F., Manning, S. P., Swain, P. M., Zhao, C., Eustace, B., Lappe, M. M., Spitzer, L., Zweier, S., Braunischweiger, K., Benczekou, Y., Ha, X., Adair, M. D., and jumping.
R. Chee, L. FitzGerald, M. G., Tulig, C., Caruso, A., Tseallas, N., Bawa, A., Franklin, R., McGuire, S., Noguez, X., Gong, G., Allen, K. M., Aniszowicz, A., Morales, A. J., Lomedico, P. T., Recker, S. M., Van Eerdewegh, P., Recker, R. R., and Johnson, M. L. (2002) Am. J. Hum. Genet. 70, 11–19

16. Van Wesenbeeck, L., Cleiren, E., Gram, J., Beals, R. K., Benichou, O., Scopelliti, D., Key, L., Benton, T., Bartels, C., Gong, Y., Warman, M. L., De Verneuil, M. C., Bollerslev, J., and Van Hul, W. (2003) Am. J. Hum. Genet. 72, 763–771

17. Wehrli, M., Dougan, S. T., Caldwell, K., O’Keefe, L., Schwartz, S., Vaizel-Ohayon, D., Scheijter, E., Tomlinson, A., and DiNardo, S. (2000) Nature 407, 527–530

18. Finson, K. L., Brennan, J., Monkley, S., Avery, B. J., and Skarnes, W. C. (2000) Nature 407, 538–538

19. Tamai, K., Semenov, M., Kato, Y., Spokony, R., Liu, C., Katsuyama, Y., Hess, J. F., Saint-Jeannet, J. P., and He, X. (2000) Nature 407, 535–538

20. Itasaki, N., Jones, C. M., Mercurio, S., Rowe, A., Domingos, P. M., Smith, J. C., and Ueno, N. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 664–667

21. Tamai, K., Zeng, X., Liu, C., Zhang, X., Harada, Y., Chang, Z., and He, X. (2004) Mol. Cell 13, 149–156

22. Mao, B., Wu, W., Davidson, G., Marhold, J., Blumenstock, C., and Niehrs, C. (2002) Development 129, 149–156

23. Harland, R., and Gerhart, J. (1997) Cell 89, 681–697

24. Tamai, K., Zeng, X., Liu, C., Zhang, X., Harada, Y., Chang, Z., and He, X. (2004) Mol. Cell 13, 149–156

25. Graff, J. M., Thies, R. S., Yang, J. A., and Hess, J. F. (1998) Cell 91, 10255–10259

26. Korinek, V., Barker, N., Morin, P. J., van Wissen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997) Science 275, 1784–1787

27. Julienius, K., Molgaard, A., Gupta, R., and Brunak, S. (2005) Glycobiology 15, 153–164

28. Chamorro, M. N., Schwartz, D. R., Venica, A., Brivanlou, A. H., Cho, K. R., and Varum, H. E. (2005) EMBO J. 24, 73–84

29. Munsterberg, A. E., Kitajewski, J., Bumcrot, D. A., McMahon, A. P., and Lassar, A. B. (1995) Genes Dev. 9, 2912–2922

30. Cheng, H. J., and Flanagan, J. G. (1994) Cell 77, 157–168

31. Veeman, M. T., Slusarski, D. C., Kaykas, A., Louie, S. H., and Moon, R. T. (2003) Curr. Biol. 13, 680–685

32. Zhang, Y., Wang, Y., Li, X., Zhang, J., Mao, J., Li, Z., Zheng, J., Li, L., Harris, S. E., and Wu, D. (2005) J. Biol. Chem. 280, 19883–19887

33. Suzuki, A., Thies, R. S., Yamaji, N., Song, J. J., Wozney, J. M., Murakami, K., and Ueno, N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10255–10259

34. Suzuki, A., Thies, R. S., Yamaji, N., Song, J. J., Wozney, J. M., Murakami, K., and Ueno, N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10255–10259

35. Korinek, V., Barker, N., Morin, P. J., van Wissen, D., de Weger, R., Kizler, K. W., Vogelstein, B., and Clevers, H. (1997) Science 275, 1784–1787

36. Julienius, K., Molgaard, A., Gupta, R., and Brunak, S. (2005) Glycobiology 15, 153–164

37. Chamorro, M. N., Schwartz, D. R., Venica, A., Brivanlou, A. H., Cho, K. R., and Varum, H. E. (2005) EMBO J. 24, 73–84

38. Gong, Y., Slei, R. B., Pakai, N., Rawadi, G., Roman-Roman, S., Regnato, A. M., Wang, H., Cundy, T., Glorieux, F. H., Lev, D., Zacharin, M., Oexle, K., Marcelino, J., Suwairi, W., Heeger, S., Sabatakos, G., Apte, S., Adkins, W. N., Allgrove, J., Arslan-Kirchner, M., Batch, J. A., Beighton, P., Black, G. C., Boles, R. G., Boun, L. M., Borrone, C., Brunner, H. G., Carle, G. F., Dallapiccola, B., De Pepe, A. M., Dingo, G., Halfhife, M. L., Hall, B., Hennekam, R. C. H., Hirose, T., Jans, A., Jupner, H., Kim, C. A., Keppler-Noreuil, K., Kohleschutter, A., LaCambe, D., Lambert, M., Lemyre, J., Letteboer, T., Peltonen, L., Ramesar, R. S., Romanengo, M., Sonner, H., Steinchen-Gersdorff, E., Steinmann, B., Sullivan, B., Superti-Furga, A., Swoboda, W., von den Bogaard, M. J., Van Hul, W., Vikkula, M., Votruba, M., Zabel, B., Garcia, T., Baron, R., Olsen, B. R., and Warman, M. L. (2001) Cell 107, 513–523

39. Zhang, Y., Wang, Y., Li, X., Zhang, J., Mao, J., Li, Z., Zheng, J., Li, L., Harris, S., and Wu, D. (2004) Mol. Cell. Biol. 24, 4677–4684

40. OSBRaughnressy, R. F., Yeu, W., Gauthier, J., Jahoda, C. A., and Christians, A. M. (2004) J. Invest. Dermatol. 123, 613–621

41. Laurikkala, J., Kassai, Y., Pakkaskarvi, L., Thesleff, I., and Itoh, N. (2003) Dev. Biol. 264, 91–105

42. Li, X., Zhang, Y., Kang, H., Liu, W., Liu, P., Zhang, J., Harris, S. E., and Wu, D. (2005) J. Biol. Chem. 280, 19883–19887

43. Zhang, Y., Wang, Y., Li, X., Zhang, J., Mao, J., Li, Z., Zheng, J., Li, L., Harris, S., and Wu, D. (2004) Mol. Cell. Biol. 24, 4677–4684
