The molecular mechanisms that transduce the osteoblast response to physical forces in the bone microenvironment are poorly understood. Here, we used genetic and pharmacological experiments to determine whether the polycystins PC1 and PC2 (encoded by \textit{Pkd1} and \textit{Pkd2}) and the transcriptional coactivator TAZ form a mechanosensing complex in osteoblasts. Compound-heterozygous mice lacking 1 copy of \textit{Pkd1} and \textit{Taz} exhibited additive decrements in bone mass, impaired osteoblast-mediated bone formation, and enhanced bone marrow fat accumulation. Bone marrow stromal cells and osteoblasts derived from these mice showed impaired osteoblastogenesis and enhanced adipogenesis. Increased extracellular matrix stiffness and application of mechanical stretch to multipotent mesenchymal cells stimulated the nuclear translocation of the PC1 C-terminal tail/TAZ (PC1-CTT/TAZ) complex, leading to increased runt-related transcription factor 2–mediated (Runx2-mediated) osteogenic and decreased PPARγ-dependent adipogenic gene expression. Using structure-based virtual screening, we identified a compound predicted to bind to PC2 in the PC1:PC2 C-terminal tail region with helix:helix interaction. This molecule stimulated polycystin- and TAZ-dependent osteoblastogenesis and inhibited adipogenesis. Thus, we show that polycystins and TAZ integrate at the molecular level to reciprocally regulate osteoblast and adipocyte differentiation, indicating that the polycystins/TAZ complex may be a potential therapeutic target to increase bone mass.
Polycystin-1 interacts with TAZ to stimulate osteoblastogenesis and inhibit adipogenesis

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The molecular mechanisms that transduce the osteoblast response to physical forces in the bone microenvironment are poorly understood. Here, we used genetic and pharmacological experiments to determine whether the polycystins PC1 and PC2 (encoded by Pkd1 and Pkd2) and the transcriptional coactivator TAZ form a mechanosensing complex in osteoblasts. Compound-heterozygous mice lacking 1 copy of Pkd1 and Taz exhibited additive decrements in bone mass, impaired osteoblast-mediated bone formation, and enhanced bone marrow fat accumulation. Bone marrow stromal cells and osteoblasts derived from these mice showed impaired osteoblastogenesis and enhanced adipogenesis. Increased extracellular matrix stiffness and application of mechanical stretch to multipotent mesenchymal cells stimulated the nuclear translocation of the PC1 C-terminal tail/TAZ (PC1-CTT/TAZ) complex, leading to increased runt-related transcription factor 2–mediated (Runx2-mediated) osteogenic and decreased PPARγ-dependent adipogenic gene expression. Using structure-based virtual screening, we identified a compound predicted to bind to PC2 in the PC1:PC2 C-terminal tail region with helix:helix interaction. This molecule stimulated polycystin- and TAZ-dependent osteoblastogenesis and inhibited adipogenesis. Thus, we show that polycystins and TAZ integrate at the molecular level to reciprocally regulate osteoblast and adipocyte differentiation, indicating that the polycystins/TAZ complex may be a potential therapeutic target to increase bone mass.

Introduction

Skeletal loading and unloading have major effects on skeletal homeostasis. In contrast to postmenopausal osteoporosis, in which bone resorption plays a major role in bone loss, and age-related osteoporosis and conditions of skeletal unloading, such as occurs due to microgravity, immobility, and/or sarcopenia, a decrease in osteoblast-mediated bone formation accompanied by a gain of marrow fat underlies the pathogenesis of osteopenia (1–4). On the other hand, mechanical loading from exercise, vibration, or pulsed electromagnetic fields leads to a gain in bone mass due to stimulation of osteoblastogenesis and inhibition of bone marrow adipogenesis (5–7). The molecular identity of the physiologically relevant mechanosensor in bone, and the cellular mechanisms responsible for the reciprocal control of osteoblastogenesis and adipogenesis during skeletal unloading, are uncertain (8–10).

Polycystin-1 (PC1, encoded by the PKDI gene) and polycystin-2 (PC2, encoded by the PKD2 gene) form a prototypic mechanosensing complex in the kidney and other tissues. PC1, an adhesion-like receptor (11, 12), and PC2, a nonselective ion channel (13), interact through their respective coiled-coil domains to form a complex that senses fluid-shear stress in renal epithelial cells (14–17). Inactivating mutations of either PKD1 or PKD2 cause renal cystic disease in mice (18, 19) and autosomal dominant polycystic kidney disease (ADPKD) in humans (20, 21).

Polycystins are also expressed in other tissues (22, 23), and several extrarenal functions are being recognized (23, 24), including in the skeleton (25, 26), where recent evidence shows that the polycystin complex plays an important role in osteoblastogenesis to control bone formation (27–29). In this regard, osteoblast-specific deletion of Pkd1 or Pkd2 resulted in osteopenia, reduced runt-related transcription factor 2–dependent (Runx2-dependent) osteoblastogenesis, and impaired bone mechanosensing responses to in vivo mechanical loading in mice (30–32). While loss of Pkd1 and Pkd2 have concordant effects on osteoblast-mediated bone formation, they exhibit opposite effects on bone marrow adipogenesis (30–32). Indeed, loss of Pkd1 increases PPARγ-dependent adipogenesis, leading to increased bone marrow fat, while loss of Pkd2 results in decreased adipogenesis and suppression of both Runx2 and PPARγ. The discordant effects on adipogenesis suggest that PC1 and PC2 functions can be uncoupled and that alternative, PC2-independent pathways link PC1 to adipogenesis (30–32).

TAZ (transcriptional coactivator with a PDZ-binding domain; also known as WW domain–containing transcription regulator 1, or WWTR1), a downstream effector of the Hippo pathway, is a candidate for linking PC1 to adipogenesis. TAZ is regulated by extracellular mechanical stimuli that involve cytoskeletal-dependent nuclear shuffling in response to alterations in extracellular matrix.
stiffness (33–35). There is evidence that PC1 and TAZ may interact to cause polycystic kidney disease through a common pathway. Global Ta z knockout and conditional deletion of Taz from the kidney lead to cystic kidney disease in mice (36, 37); and in renal epithelial cells, TAZ binds to the PC1 C-terminal tail (PC1-CTT) to facilitate nuclear translocation (38–40), and binds to PC2-CTT to enhance PC2 degradation (41). TAZ also differentially regulates osteoblastogenesis and adipogenesis (42). Nuclear translocation of Taz in osteoblasts leads to increased osteoblast-mediated bone formation and decreased bone marrow adipogenesis (45); deletion of taz in zebrafish impairs bone development (42), and Taz+/− mice have small stature and ossification defects (36). These observations raise the possibility that TAZ may be an essential component of the polycystin mechanosensing pathway in bone that regulates bone mass through the reciprocal control of osteoblastogenesis and adipogenesis.

In the current study, we investigated whether TAZ mediates the differential effects of PC1 on osteoblastogenesis and adipogenesis in both in vivo mouse genetic models and in vitro cell culture models. We found that PC1-CTT interacts with TAZ in osteoblasts to integrate 2 distinct mechanosensing pathways, leading to stimulation of Runx2-mediated osteoblastogenesis and inhibition of PPARγ-mediated adipogenesis in the skeleton. Furthermore, using 3D ensemble docking algorithms, we identify a small molecule, predicted to bind to PC2 in the PC1:PC2 interacting region, that stimulated osteoblastogenesis and inhibited adipogenesis in vitro and in vivo. Hence, the polycystins/TAZ complex is a potential target for pharmaceutical development of small molecules to mimic mechanical loading to increase bone mass.

Results

Additive effects of combined Taz and Pkd1 deficiency on bone mass and lineage commitment. We explored genetic interactions between PC1 and TAZ in the skeleton by characterizing the effects of single- and compound-heterozygous loss of Pkd1 and Taz on bone mineral density (BMD). This was accomplished by crossing of heterozygous Taz+/− mice with heterozygous Pkd1+/− mice to create double-heterozygous Taz+/−;Pkd1+/− animals. These mice were born at the expected Mendelian frequency, and all genotypes had normal survival, gross appearance, and body weight indistinguishable from those of WT mice over the period of study. Neither single- nor compound-mutant mice developed cystic kidney disease by gross and histological inspection of the kidneys (data not shown).

Adult male and female Pkd1+/− mice exhibited a low BMD by dual-energy x-ray absorptiometry scan analysis. This was due to a reduction in trabecular bone volume (13.2%), cortical bone thickness (9.2%), and impaired osteoblast-mediated mineral apposition rates (26.6%), as previously reported in 6-week-old mice (46). In contrast, neither male nor female Taz−/− mice had abnormalities in bone mass, bone structure, and bone formation rate compared with WT mice (Figure 1). However, combined loss of 1 allele of Taz and Pkd1 resulted in additive reductions in BMD, as evidenced by the 21% reduction in BMD in the double-heterozygous Taz−/−;Pkd1−/− mice (Figure 1A). Micro-CT analysis revealed that double-heterozygous Taz−/−;Pkd1−/− mice had greater loss in both trabecular (26.3%) and cortical bone (19.4%) than did single-heterozygous mice (Figure 1B). These reductions in bone volume were associated with a significant decrease in mineral apposition rates in single-heterozygous Taz−/− and Pkd1−/− mice compared with age-matched WT mice and an even greater reduction (44.6%) in double-heterozygous Taz−/−;Pkd1−/− mice (Figure 1C).

Finally, bone marrow exhibited an increased percentage of fat cells in single-heterozygous Pkd1−/− mice compared with age-matched WT mice and even greater increments in double-heterozygous Taz−/−;Pkd1−/− mice. Consistent with low-turnover osteopenia with a reduction in osteoclast activity, histomorphometric analysis of the femurs by tartrate-resistant acid phosphatase (TRAP) immunostaining showed reduced osteoclast surface per bone surface in Pkd1−/− and Taz−/−; Pkd1−/− mice compared with WT and Taz−/− mice (Figure 1E).

To investigate whether combined TAZ and Pkd1 deficiency resulted in additive effects on gene expression profiles in bone, we examined by real-time reverse transcription PCR (RT-PCR) the expression levels of a panel of osteoblast-, osteoclast-, and chondrocyte-related mRNAs in the femurs of 8-week-old WT, heterozygous Taz+/−, heterozygous Pkd1+/−, and double-heterozygous Taz+/−;Pkd1+/− mice (Table 1). Single-heterozygous Taz+/− showed normal bone gene expression profiles except 50% reduction of Taz transcripts (Table 1). However, bone derived from single-heterozygous Pkd1+/− mice had measurable reductions in osteocalcin, osteopontin, osteoprotegerin (Opg), RANK ligand (Rankl), Mmp13, sclerostin (Sost), and Dmp1 mRNA levels in comparison with WT mice. Significantly greater reductions of osteoblasts and osteocyte-related genes were observed in double-heterozygous Taz+/−;Pkd1+/− mice for osteocalcin, Rankl, Mmp13, Sost, and Dmp1. Bone expression of Trap and Mmp9, markers of bone resorption, were reduced in heterozygous Pkd1+/− mice and to a greater extent in double-heterozygous Taz+/−;Pkd1+/− mice compared with WT littermates (Table 1), suggesting that bone loss was mainly due to decreased osteoblast-mediated bone formation. These findings suggest that a low bone formation rate rather than increased bone resorption accounts for the low BMD and bone volume of femurs in single-heterozygous Pkd1+/− and double-heterozygous Taz+/−;Pkd1+/− deficient mice. Pparg, an adipocyte transcription factor, and adipocyte markers, including lipoprotein lipase (Lpl) and adipocyte fatty acid-binding protein 2 (aP2), were significantly increased in femurs of heterozygous Pkd1+/− mice and to a greater extent in double-heterozygous Taz+/−;Pkd1+/− mice compared with WT littermates (Table 1). Transcripts of chondrocyte-related genes did not differ between single- and double-heterozygous Taz+/−;Pkd1+/− mice (Table 1). Thus, Taz deficiency and Pkd1 deficiency are additive on both osteogenesis and adipogenesis.

Additive effects of combined Taz and Pkd1 deficiency on osteoblast differentiation. To explore the mechanism underlying the increase in osteoblastogenesis and decreased adipogenesis in double-heterozygous Taz+/−;Pkd1+/− mice, we performed bone marrow-derived stromal cell (BMSC) cultures derived from age-matched WT, heterozygous Taz+/−, heterozygous Pkd1+/−, and double-heterozygous Taz+/−;Pkd1+/− mice grown in osteoblastic differenti-
Figure 1. Effects of combined Pkd1 and Taz deficiency on bone mass in 8-week-old mice. (A) BMD in femurs by dual-energy x-ray absorptiometry scan. (B) Micro-CT analysis of distal femoral metaphysis and midshaft diaphysis. (C) Periosteal mineral apposition rate (MAR) by calcein double labeling. (D) OsO4 staining of decalcified tibiae by micro-CT analysis. (E) TRAP staining (red color) for osteoclast activity. Data are presented as the mean ± SD from 6–8 individual mice (n = 6–8). P values were determined by 1-way ANOVA with Newman-Keuls multiple-comparisons test. *Significant difference from WT control mice, #significant difference from Taz+/– mice, & significant difference from Pkd1+/– mice at P < 0.05, respectively. BV/TV, bone volume density; Ct.Th, cortical thickness; Ad.V/Ma.V, adipocyte volume per marrow volume; Ad.N, adipocyte number; Oc.S/BS, osteoclast surface per bone surface.
Table 1. Gene expression profiles in femurs from 8-week-old mice by real-time quantitative reverse transcription PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession no.</th>
<th>Taz+/–</th>
<th>Pkd1+/–</th>
<th>Taz+/–;Pkd1+/–</th>
<th>P value</th>
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<td>Osteoblast lineage</td>
<td></td>
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</tr>
<tr>
<td>Pkd1</td>
<td>NM_013630.2</td>
<td>0.97 ± 0.19</td>
<td>0.50 ± 0.17</td>
<td>0.48 ± 0.08</td>
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<td>Taz</td>
<td>NM_133784.3</td>
<td>0.52 ± 0.14</td>
<td>1.02 ± 0.32</td>
<td>0.50 ± 0.13</td>
<td>&lt;0.0001</td>
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<tr>
<td>Runx2-II</td>
<td>NM_009820.5</td>
<td>1.05 ± 0.22</td>
<td>0.74 ± 0.08</td>
<td>0.60 ± 0.16</td>
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<tr>
<td>Osteocalcin</td>
<td>NM_007541.2</td>
<td>0.99 ± 0.26</td>
<td>0.73 ± 0.08</td>
<td>0.47 ± 0.06</td>
<td>&lt;0.0001</td>
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<tr>
<td>Osteopontin</td>
<td>AF515708.1</td>
<td>0.95 ± 0.25</td>
<td>0.72 ± 0.12</td>
<td>0.49 ± 0.11</td>
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<tr>
<td>Mmp13</td>
<td>NM_008607.2</td>
<td>0.93 ± 0.15</td>
<td>0.63 ± 0.16</td>
<td>0.41 ± 0.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Opq</td>
<td>NM_008764.3</td>
<td>0.94 ± 0.24</td>
<td>0.64 ± 0.14</td>
<td>0.67 ± 0.16</td>
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<td>Rankl</td>
<td>NM_011613.3</td>
<td>0.94 ± 0.27</td>
<td>0.60 ± 0.23</td>
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<td>Sost</td>
<td>NM_024449.6</td>
<td>0.99 ± 0.16</td>
<td>0.72 ± 0.14</td>
<td>0.55 ± 0.09</td>
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<td>Dmp1</td>
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<td>1.01 ± 0.12</td>
<td>0.73 ± 0.16</td>
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<td>Wnt10b</td>
<td>U61970.1</td>
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<td>Axin2</td>
<td>AF205898.1</td>
<td>1.13 ± 0.24</td>
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<td>Fzd2</td>
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<td>Osteoclast</td>
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<td>Tg</td>
<td>NM_007388.3</td>
<td>0.93 ± 0.16</td>
<td>0.65 ± 0.06</td>
<td>0.56 ± 0.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mmp9</td>
<td>NM_013599.3</td>
<td>0.95 ± 0.24</td>
<td>0.71 ± 0.05</td>
<td>0.47 ± 0.08</td>
<td>0.0001</td>
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<tr>
<td>Chondrocyte</td>
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<tr>
<td>Type II collagen</td>
<td>NM_031163.3</td>
<td>1.02 ± 0.45</td>
<td>0.91 ± 0.27</td>
<td>0.94 ± 0.13</td>
<td>0.6916</td>
</tr>
<tr>
<td>Vegfa</td>
<td>NM_009505.4</td>
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<td>1.03 ± 0.24</td>
<td>1.05 ± 0.21</td>
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<td>Adipocyte</td>
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<tr>
<td>Pparg</td>
<td>NM_009505.4</td>
<td>0.99 ± 0.24</td>
<td>1.51 ± 0.39</td>
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<td>aP2</td>
<td>NM_024406.2</td>
<td>1.26 ± 0.29</td>
<td>1.71 ± 0.28</td>
<td>2.59 ± 0.84</td>
<td>&lt;0.0001</td>
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<tr>
<td>Lpl</td>
<td>NM_008509.2</td>
<td>1.17 ± 0.20</td>
<td>1.57 ± 0.22</td>
<td>2.19 ± 0.73</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Data are mean ± SD from 6 femurs of 8-week-old individual mice (n = 6) and are expressed as the fold changes relative to the housekeeping gene cyclophilin A subsequently normalized to control mice. P values refer to the differences between group means by 1-way ANOVA. If the differences between the means are statistically significant (i.e., P < 0.05), the group means are compared by Newman-Keuls multiple-comparisons test. *Significant difference from WT control mice, †significant difference from Taz+/– mice, ‡significant difference from Pkd1+/– mice at P < 0.05, respectively.

In significant reductions in osteogenic markers such as Runx2 and osteocalcin and enhanced adipogenic markers such as Pparg and aP2 in vitro (Supplemental Figure 1A; supplemental material available online with this article; https://doi.org/10.1172/JCI93725DS1). In contrast, Pkd2-deficient osteoblasts exhibited coordinated reductions in both osteogenic and adipogenic markers during culture under osteogenic conditions (Supplemental Figure 1B). To verify these results, and to remove any confounding effects of the in vivo environment on the phenotype of osteoblasts isolated from knockout mice, we used adenovirus-CMV-iCre (Ad-CMV-iCre) and freshly isolated primary osteoblasts derived from the floxed Pkd1fl/fl and Pkd2fl/fl mice to examine whether loss of polycystins modulates osteoblast and adipocyte differentiation. We found that incubation of primary Pkd1fl/fl and Pkd2fl/fl osteoblasts with Ad-CMV-iCre ex vivo resulted in a 65% decrease in Pkd1 and Pkd2 message expression compared with Ad-CMV-null vector controls. The reduction in Pkd1 and Pkd2 transcripts resulted in significant reductions of osteogenic markers, such as Runx2 and alkaline phosphatase (ALP) (Supplemental Figure 1C). However, Ad-CMV-iCre-mediated deletion of Pkd1 resulted in significant increases of adipogenic markers, such as Pparg and aP2 (Supplemental Figure 1C), whereas Ad-CMV-iCre-mediated deletion of Pkd2 ex vivo resulted in decreased adipogenic markers (Supplemental Figure 1D), consistent with observations in conditional Pkd1fl/fl and Pkd2fl/fl mice as we previously reported (30–32).

Evidence for a functional link between polycystins and TAZ in vitro. We assessed changes in TAZ protein expression and phosphorylation in Pkd1- and Pkd2-deficient osteoblasts. We found that the level of total TAZ protein was slightly decreased, but TAZ phosphorylation at Ser 89, which leads to TAZ cytoplasmic sequestration (47), was increased in both Pkd1- and Pkd2-deficient osteoblasts (Figure 3A and B). Consistent with reductions in functional TAZ protein levels, we observed that TAZ-mediated activation of TEAD reporter, a measure of TAZ transcriptional activity, was markedly decreased in both Pkd1- and Pkd2-deficient osteoblasts (Figure 3C). Although both Pkd1 and Pkd2 deficiencies lead to decreased TEAD reporter activities, they may differentially regulate TAZ.

To explore TAZ interactions with PC1 and PC2, we overexpressed FLAG-tagged TAZ and full-length PC1 and PC2 in HEK-293T cells and performed coimmunoprecipitation (co-IP) with an anti-FLAG antibody. We found that TAZ forms a complex with PC1 and PC2, as evidenced by their co-IP (Figure 3D). We also substituted PC1-CTT for the full-length PC1 in these studies. We found that PC1-CTT was sufficient for binding to TAZ, since overexpression of PC1-CTT brought down TAZ and PC2 in the IP complex with an anti-myc antibody for PC1-CTT (Figure 3D). To test whether polycystins regulate TAZ function, we cotrans-
fected either full-length PC1 or PC1-CTT and full-length PC2 along with TEAD reporter constructs into C3H10T1/2 cells. We found that overexpression of either full-length PC1 or PC1-CTT along with full-length PC2 markedly increased TAZ-induced activation of TEAD activity (Figure 3E), suggesting a functional link between polycystins and TAZ signaling. These findings are also consistent with the observations that PC2 increases PC1 C-terminal cleavage in COS-7 cells (48, 49), and subsequently enhances consistent with the observations that PC2 increases PC1 C-terminal cleavage in COS-7 cells (48, 49), and subsequently enhances PC1-CTT nuclear translocation and TAZ signaling pathway.

**PC1 C-tail interacts with TAZ to coregulate Runx2 and PPARγ activities.** Next, we examined whether the PC1-CTT/TAZ complex coregulates Runx2 and PPARγ activities (33, 42). It is known that the PC1-CTT binds to TAZ, which is a coactivator for Runx2 and a corepressor for PPARγ activity (42, 44), but the effects of the PC1-CTT/TAZ complex on Runx2 and PPARγ activities have not been studied. Thus, we overexpressed FLAG-tagged TAZ, PC1-CTT, and Runx2 or Pparg in HEK-293T cells and performed co-IP with an anti-FLAG antibody (Figure 4, A and B). We found that TAZ was sufficient for binding to PC1-CTT, since overexpression of PC1-CTT brought down TAZ in the IP complex. We also found that TAZ and PC1-CTT form a complex with either Runx2 or PPARγ, as evidenced by their co-IP (Figure 4, A and B). Overexpression of TAZ, PC1-CTT, and Runx2 or PPARγ in multipotent C3H10T1/2 mesenchymal cells found that TAZ stimulated osteocalcin (Oc) promoter activity and was enhanced by PC1-CTT, and overexpression of Runx2. In contrast, we observed that TAZ inhibits PPARγ stimulation of aP2 promoter activity, and this effect was further suppressed by overexpression of PC1-CTT (Figure 4, C and D). These findings suggest that PC1-CTT modulates TAZ function through its binding to TAZ, which coactivates Runx2-mediated gene transcription and corepresses PPARγ-induced gene expression.

To examine the effects of PC1 C-tail cleavage on Oc or aP2 promoter reporter activities, we overexpressed membrane-bound PCI-CTT construct (26, 50) along with Oc or aP2 reporters in C3H10T1/2 cells. Overexpression of PC1-CTT stimulated Oc promoter reporter activities, but suppressed aP2 promoter reporter activities (Figure 4, E and F). As previously reported (51), PC1-CTT is released by a γ-secretase. We found that DAPT, a γ-secretase inhibitor, blocked the effects of PC1-CTT to stimulate Oc promoter, and reversed the inhibitory effect on aP2 promoter activities (Figure 4, E and F). These findings suggest that cleavage and release of the PC1 C-tail from the membrane form that translocates to the nucleus play an essential role in the regulation of targeting gene transcription.

Next, we investigated the co-occupancy of endogenous Oc or aP2 promoter by PC1-CTT, TAZ, and Runx2 or PPARγ complex in C3H10T1/2 cells. OSE2 is a key osteoblast-specific cis-acting element in the proximal Oc promoter (52); Runx2 was shown to bind the OSE2 site and regulates mouse Oc promoter activity (53). Therefore, we examined whether PC1-CTT, TAZ, and Runx2 form a complex that binds the region of the proximal Oc promoter that contains the OSE2 site. Quantitative chromatin immunoprecipitation (ChIP) analyses using anti-FLAG for TAZ, anti-myc for PC1-CTT, and anti-Runx2 identified that TAZ, PC1-CTT, and Runx2 were specifically recruited to a region of the proximal Oc promoter containing the known OSE2 cis-element that is known to bind to Runx2 (Figure 4G). There was an approximately 3- to 7-fold increase in the ratio of the promoter sequence versus the coding region sequence in the anti-FLAG, anti-myc, or anti-Runx2 group compared with the IgG control group by quantitative real-time PCR. To establish that TAZ and PC1-CTT suppress PPARγ binding to the consensus ARE6 site in the proximal aP2 promoter (54), we performed quantitative ChIP analysis using an anti-FLAG for TAZ, an anti-myc for PC1-CTT, and an anti-PPARγ antibody. Real-time PCR on the immunoprecipitated DNA fragments with primers to amplify the region spanning the ARE6 sites con-
firmed that TAZ, PC1-CTT, and PPARγ were specifically recruited to the segment of the proximal aP2 promoter containing the ARE6 site that is known to bind PPARγ (Figure 4H). There was an approximately 3- to 7-fold increase in the ratio of the promoter sequence versus the coding region sequence in the anti-FLAG, anti-myc, or anti-PPARγ group compared with the IgG control group by quantitative real-time PCR. Together, these findings indicate that PC1-CTT and TAZ colocalize to the proximal aP2 promoter to enhance Runx2-mediated osteocalcin gene transcription, and colocalize to the proximal aP2 promoter to enhance PPARγ-mediated aP2 gene transcription.

Matrix stiffness regulates Oc or aP2 promoter activities by PC1-CTT and TAZ nuclear translocation. Previous publications showed that matrix stiffness has an important impact on osteogenesis and adipogenesis (33). We examined whether matrix stiffness affects PC1-CTT and TAZ nuclear translocation to regulate osteogenic Oc promoter reporter and adipogenic aP2 promoter reporter activities in C3H10T1/2 cells transfected with PC1-CTT. Using C-terminal FLAG-tagged full-length PC1 construct, we found that hard matrix promotes γ-secretase and PC1-CTT cleavage, whereas soft matrix inhibits γ-secretase and PC1-CTT cleavage (Figure 5, A and B). Using membrane-bound PC1-CTT construct, we observed that hard matrix (40 kPa) promotes PC1-CTT and TAZ translocation to the nucleus (Figure 5, C and D) and increases Oc promoter reporter activity (Figure 5E), whereas soft matrix (0.5 kPa) attenuates PC1-CTT and TAZ translocation to nucleus (Figure 5, C and D) and enhances aP2 promoter reporter activity (Figure 5F). In addition, DAPT, a γ-secretase inhibitor, blocked the effects of the transfected PC1-CTT to stimulate Oc promoter reporter, and reversed the inhibitory effect on aP2 promoter reporter activities (Figure 5, E and F). We observed that the TEAD, Oc, and aP2 promoter activity responded similarly in C3H10T1/2 cells expressing endogenous PC1 as compared with cells transfected with PC1-CTT (Supplemental Figure 2, A–C). In addition, real-time RT-PCR showed that soft matrix inhibited the expression of the osteogenic genes Runx2, Alpl, and osteocalcin but promoted the expression of the adipogenic markers Pparg and aP2 in C3H10T1/2 cells, whereas hard matrix had the opposite effect on gene expression, which was reversed by treatment with the γ-secretase inhibitor.
(DAPT) (Supplemental Figure 2D). These findings suggest that matrix stiffness regulates cleavage, release, and nuclear translocation of PC1-CTT from plasma membrane in the regulation of targeting gene transcription.

Mechanical stretch regulates Oc or aP2 promoter activities by PC1-CTT and TAZ nuclear translocation. Previous studies show that mechanical stretch promotes osteogenesis (55–57) and inhibits adipogenesis (1, 5, 58). We examined whether stretch affects PC1-CTT cleavage and TAZ nuclear translocation to regulate osteogenic Oc promoter reporter and adipogenic aP2 promoter reporter activities in C3H10T1/2 cells. Using C-terminal FLAG-tagged full-length PCI, we found that stretch stimulates γ-secretase activity and PC1-CTT cleavage, whereas the γ-secretase inhibitor DAPT abolished stretch-induced PC1-CTT cleavage (Figure 6, A and B). Using membrane-bound PC1-CTT, we observed that stretch promotes PC1-CTT and TAZ translocation to the nucleus (Figure 6, C and D) and increases TEAD and Oc promoter reporter activities (Figure 6, E and F), whereas DAPT blocks PCI-
predicted to bind to the PC2:PC1 interacting region. From this screen of compounds predicted to modulate protein:protein interactions, Zinc01442821 (4-phenyl-1H-pyrrole-3-carboxylic acid, designated MS for molecular staple) was the best compound predicted to bind to the $\alpha$ regions of PC2, as shown in Figure 7A. In the computational model, Zinc01442821 binding forms hydrophobic interactions with Val880 and Leu881 that are essential for the PC1-PC2 coiled-coil stabilization and also with charged PC2 residues Arg877, Arg878, and Lys874, i.e., with the central region of the KRRE cluster that is essential to the PC1:PC2 interactions (17).

To assess engagement of Zinc01442821 with polycystins and TAZ, we performed co-IP assays using HEK-293T cell lysates (Figure 6, C and D) and enhances $aP2$ promoter reporter activity (Figure 6G). Real-time RT-PCR confirmed that stretch enhanced the expression of the osteogenic genes $Runx2$, $Alpl$, and osteocalcin but inhibited expression of the adipogenic markers $Pparg$ and $aP2$ (Supplemental Figure 3). These findings suggest that mechanical stretch stimulates osteogenesis through regulation of PC1-CTT and TAZ nuclear translocation.

**Discovery of a small molecule that activates PC1/PC2/TAZ signaling.** We constructed a 3D model of the PC1:PC2 coiled-coil structure based on previous published structural information (17) and performed docking calculations (59, 60) to identify compounds predicted to bind to the PC2:PC1 interacting region. From this screen of compounds predicted to modulate protein:protein interactions, Zinc01442821 (4-phenyl-1H-pyrrole-3-carboxylic acid, designated MS for molecular staple) was the best compound predicted to bind to the $\alpha$ regions of PC2, as shown in Figure 7A. In the computational model, Zinc01442821 binding forms hydrophobic interactions with Val880 and Leu881 that are essential for the PC1-PC2 coiled-coil stabilization and also with charged PC2 residues Arg877, Arg878, and Lys874, i.e., with the central region of the KRRE cluster that is essential to the PC1:PC2 interactions (Figure 7B) (17).

To assess engagement of Zinc01442821 with polycystins and TAZ, we performed co-IP assays using HEK-293T cell lysates.
intracellular calcium and TAZ-mediated activation of the TEAD reporter. We observed that Zinc01442821 significantly increased the peak value of Fluo-4 intensity and intracellular calcium levels (Figure 8A), consistent with activation of PC1/PC2 signaling. In addition, Zinc01442821 significantly increased TEAD reporter activity, consistent with enhanced TAZ nuclear translocation (Figure 8B) in MC3T3-E1 osteoblastic cells. The above co-IP data and these signaling responses suggest that Zinc01442821 may interact with PC2 to enhance calcium signaling as well as disrupt PC1/TAZ interactions to promote TAZ signaling.

Next, we examined the dose-dependent effects of Zinc01442821 on osteoblastogenesis and adipogenesis in cotransfected with GFP-tagged full-length PC1, full-length PC2, and FLAG-tagged TAZ. Cells were treated with either Zinc01442821, its inactive analog MS4 (see below), or the DMSO vehicle control. We found that the FLAG antibody coprecipitated PC1, PC2, and TAZ in vehicle-treated cultures, consistent with the above studies showing that PC1, PC2, and TAZ form a trimeric complex (Figure 3). The addition of Zinc01442821 (10 μM) attenuated the incorporation of TAZ into this trimeric complex (Figure 7, C–E), but enhanced the co-IP of PC1 and PC2 complexes (Figure 7, F–H), consistent with stabilization of PC1 and PC2 interactions.

Using an MC3T3-E1 osteoblastic cell line that expresses endogenous PC1/PC2, we tested the effects of Zinc01442821 on intracellular calcium and TAZ-mediated activation of the TEAD reporter. We observed that Zinc01442821 significantly increased the peak value of Fluo-4 intensity and intracellular calcium levels (Figure 8A), consistent with activation of PC1/PC2 signaling. In addition, Zinc01442821 significantly increased TEAD reporter activity, consistent with enhanced TAZ nuclear translocation (Figure 8B) in MC3T3-E1 osteoblastic cells. The above co-IP data and these signaling responses suggest that Zinc01442821 may interact with PC2 to enhance calcium signaling as well as disrupt PC1/TAZ interactions to promote TAZ signaling.

Next, we examined the dose-dependent effects of Zinc01442821 on osteoblastogenesis and adipogenesis in
MC3T3-E1 osteoblasts in vitro. Zinc01442821 (about 0.5–10 μM) dose-dependently increased ALPL activity and calcium deposition during osteogenic cultures (Figure 8, C and D). Also, real-time RT-PCR analysis revealed that Zinc01442821 at 10 μM concentration markedly stimulated the expression of Runx2 and its downstream gene osteocalcin, but significantly attenuated the expression of Pparg and its downstream gene aP2 (Figure 8E). To determine that PC1 and PC2 are mediating the response to Zinc01442821 in osteoblasts, we derived primary osteoblasts from Pkd1- and Pkd2-null mice. We found that primary osteoblasts responded to Zinc01442821, but Pkd1- and Pkd2-deficient osteoblasts lost Zinc01442821 stimulation of intracellular calcium (Figure 8F) and TEAD reporter activity (Figure 8G).

Finally, it is well known that the acute administration of bone active agents, such as parathyroid hormone, can lead to alterations in osteoblast-related gene expression within hours to days (61). Therefore, we administered Zinc01442821 to WT mice and assessed its effects on the expression of osteoblast and adipocyte markers in bone after 72 hours. Consistent with the in vitro osteoblast culture data, treatment of mice with Zinc01442821 (100 mg/kg) twice daily for 3 days significantly upregulated the expression of Runx2 message and its downstream gene osteocalcin, but inhibited the expression of Pparg message and its downstream gene aP2 (Figure 8H). These findings suggest that Zinc01442821 modulates PC1/PC2/TAZ signaling to regulate osteoblastogenesis and adipogenesis both in vitro and in vivo.

In vitro functional assays of Zinc01442821 analogs. We also synthesized 5 close analogs of Zinc01442821 (designated as MS1–MS5). MS1 is a methyl ester of Zinc01442821, MS2 is an N-methyl analog of MS1, and MS3, MS4, MS5 have the position of the nitrogen in this scaffold is important for function. Indeed, MS4, unlike Zinc01442821, failed to inhibit PC1/PC2/TAZ trimeric complex formation (Figure 7, C and D).

Discussion
In this study, we identify novel interactions between polycystins and TAZ in osteoblasts that create a molecular mechanism whereby the skeleton senses mechanical loading to regulate bone mass. PC1 forms a mechanosensing complex with PC2 (10, 31, 32), and also interacts with TAZ (33, 42) to regulate bone mass through the reciprocal control of osteoblastogenesis and adipogenesis. Accordingly, mice with compound deletion of Pkd1 and Taz exhibit additive effects to reduce bone mass through reduced osteoblastogenesis and increased bone marrow adipogenesis. An interaction between PC1 and TAZ was demonstrated by co-IP studies. Additional in vitro studies show that PC1-CTT and TAZ translocate to the nucleus to activate Runx2 and inhibit PPARγ-dependent gene transcription in response to matrix stiffness and mechanical stretch. Moreover, we discovered a novel small molecule that binds to PC2, enhances TAZ-mediated transcription, and mimics the effects of mechanical loading to stimulate osteoblastogenesis and inhibit adipogenesis in vitro.

Loss-of-function double-heterozygous mice indicate genetic interactions between PC1 and TAZ. Compound-heterozygous Pkd1+/– and Taz+/– mice exhibit additive effects to promote bone loss, reduce osteoblast-mediated bone formation, and increase bone marrow fat. Bone marrow stromal cell cultures derived from compound-heterozygous Pkd1+/– and Taz+/– mice confirm decreased osteogenesis and increased adipogenesis in vitro. In addition, in primary osteoblast cultures derived from Pkd1nullnull, we observed reduced osteoblast markers and increased adipogenic markers. In contrast, primary osteoblast culture from Pkd2nullnull mice exhibited a concordant decrease in both osteoblast and adipocyte gene expression. Ex vivo adenoviral Cre-mediated deletion of Pkd1 in primary osteoblasts isolated from Pkd1nullnull mice also resulted in impaired osteoblast differentiation and increased adipogenic markers, whereas adenoviral Cre-mediated deletion of Pkd2 resulted in a concordant decrease in both osteogenic and adipogenic markers. These cell culture studies are consistent with our prior finding that conditional deletion of Pkd1 in the osteoblast lineage in mice results in inhibition of osteoblastogenesis and stimulation of adipogenesis in vivo, whereas conditional deletion of Pkd2 results in concordant inhibition of both osteoblastogenesis and adipogenesis (30, 32).

Gain-of-function studies demonstrate that PC1-CTT and TAZ stimulate osteoblast and inhibit adipocyte differentiation, respectively. Transfection of PCI-CTT, TAZ, or Runx2 individually stimulates Oc promoter activity, and cotransfection of all 3 constructs exhibits additive effects on osteocalcin gene transcription in C3H10T1/2 cells. Similarly, transfection of PCI-CTT, TAZ, or Pparg individually suppresses aP2 promoter activity, and cotransfection of all 3 constructs results in additive suppression of aP2 gene transcription in C3H10T1/2 cells. We also found that both hard matrix and mechanical stretch stimulated the polycystins/TAZ mechanosensing signaling complex in vitro, consistent with the possibility that this complex may integrate the response to different physical forces in the bone microenvironment (31, 33). Since these in vitro methods of mechanical loading are nonphysiologic, additional studies to explore the response to mechanical loading in compound Pkd1 and TAZ heterozygous mutant mouse models are needed to further test our hypothesis in vivo. Furthermore, we found that pharmacological activation of PC1/
TC2/TAZ signaling by Zinc01442821 increased intracellular calcium levels and induced TAZ activation of the TEAD promoter in osteoblasts, in agreement with the effect of extracellular matrix (ECM) stiffness in inducing nuclear translocation of TAZ (33, 62). Zinc01442821 also promoted osteoblastogenesis and inhibited adipogenesis in osteoblast cultures. Finally, short-term administration to mice stimulates osteoblastic and inhibits adipocyte differentiation in vivo. The mice were treated with i.p. injection of Zinc01442821 (100 mg/kg) or vehicle control twice a day for 3 days. (A) Intracellular [Ca2+]i response induced by Zinc01442821 (10 μM) in MC3T3-E1 osteoblastic cells. (B) Zinc01442821-stimulated (10 μM) TAZ-mediated activation of TEAD reporter activities in MC3T3-E1 osteoblastic cells. (C) Dose-dependent effects of Zinc01442821 on ALPL activity at day 15. (D) Dose-dependent effects of Zinc01442821 on mineralization accumulation by alizarin red S staining at day 21. (E) Effects of Zinc01442821 (10 μM) on the expression of a panel of osteoblast and adipocyte gene markers in MC3T3-E1 cultures at day 21. (F and G) [Ca2+]i response (F) and TAZ-mediated activation of TEAD reporter activities (G) in WT and polycystin-deficient osteoblasts. (H) Effects of Zinc01442821 on osteogenic and adipogenic markers in femurs from vehicle control- and Zinc01442821-treated mice. Data are expressed as the mean ± SD from 3 independent experiments (n = 3). P values were determined by 1-way ANOVA with Newman-Keuls multiple-comparisons test or unpaired t test. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control group.
Osteoblastic cell cultures. Immortalized Pkd1null/null, Pkd2null/null, and MC3T3-E1 osteoblastic cell lines (ATCC) were cultured in α-MEM containing 10% FBS and 1% P/S as previously described (31, 32). For intracellular calcium measurements, Fluo-4 or Fluo-4 AM (Invitrogen) was used according to the manufacturer’s instructions (25). For TAZ signaling study, the immortalized Pkd1 null/null and Pkd2 null/null cells were cultured in the osteogenic differentiation media for 48 hours. Then the cells were lysed with 150 μl of T-PER with 1× Halt protease inhibitor and 1 mM PMSF per well. After 30-second sonication 3 times, total cell lysates were centrifuged at 13,000 g for 10 minutes, and supernatants were stored at –80°C until use for the following Western blot analysis.

**Mechanical cell stretch.** C3H10T1/2 (ATCC) cells were plated onto 6-well plates with Collagen Type I surface (Flexcell International) for 24 hours and then subjected to a 6-hour stretch regimen by software-controlled vacuum applied to a loading station housed in a humidified 5% CO₂ incubator at 37°C (Flexcell FX-4000T, Flex-
nuclear translocation in mesenchymal stem cells. γ-secretase, PPAR-α, and PPARγ mediated Oc promoter activities through PC1-CTT and TAZ adipogenesis in bone marrow. Mechanical force and hard matrix regulate Runx2-secretase activity, cleavage of PC1-CTT, and PC1-CTT and TAZ nuclear translocation at a completion of 6 hours of stretch. The cells subjected to 6 hours of stretch (70).

**Figure 10. Schematic of PC1 and TAZ interaction in regulation of osteogenesis and adipogenesis in bone marrow.** Mechanical force and hard matrix regulate Runx2-mediated Oc and PPARγ-mediated aP2 promoter activities through PC1-CTT and TAZ nuclear translocation in mesenchymal stem cells.

Cell International). Each cycle consisted of 0.5 seconds of stretch (10%) and 0.5 seconds of relaxation for a total of 15 cycles per minute, which achieves the maximum effect on osteogenic gene expression in BMSCs and C3H10T1/2 cells as previously described for osteogenesis (55–57). The replicate control plates consisted of cells grown on the same flexible surface but not subjected to mechanical stretch. We immediately collected cell samples for the measurements of γ-secretase activity, cleavage of PC1-CTT, and PC1-CTT and TAZ nuclear translocation at a completion of 6 hours of stretch. The cells were processed for luciferase activity assays after 18 hours when subjected to 6 hours of stretch (70).

**γ-Secretase assay.** Cellular γ-secretase activities were quantified using an assay based on a previous report (71). Briefly, C3H10T1/2 cells were harvested in cell lysis buffer A containing 20 mM HEPES (pH 7.0), 150 mM KCl, 2 mM EDTA, 1% [3-(cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO; wt/vol; Sigma-Aldrich), and Protease Inhibitor Cocktail (Thermo Fisher Scientific). Lysates were centrifuged at 9,300 g for 1 minute at 4°C to remove nuclei and large cell debris. Supernatants were collected, and the protein concentration was quantified. Fifty micrograms of protein was added to an opaque 96-well plate (Nunclon; Nunc) and made to a final volume of 200 μl with assay buffer containing 50 mM Tris-HCl (pH 6.8), 2 mM EDTA, and 0.25% CHAPSO (wt/vol); 10 mM of fluorogenic amyloid β-peptide precursor protein–derived probe acting as a γ-secretase substrate (Sigma-Aldrich) was added to the plate. Controls used were the peptide probe and cell lysates alone. Samples were incubated at 37°C for 18 hours in the dark. Fluorescence was measured using a Synergy H4 Hybrid Reader (BioTek Instruments Inc.), with excitation wavelength at 340 nm and emission wavelength at 460 nm. Background fluorescence of the peptide probe was subtracted from all readings.

**Computational small-molecule discovery.** Zinc01442821 (4-phenyl-1H-pyrrole-3-carboxylic acid, C11H9NO2) was identified by a computationally driven, structure-based, and virtual docking screening, following approaches previously described (59, 60). Briefly, we used the PCI:PC2 coiled-coil model of Zhu et al. (17) to perform a docking screening of 1,000 diverse fragment-like molecules from the ZINC database using the MOE docking facility (Chemical Computing Group) version 2015, in an induced-fit setting. Zinc01442821 ranks among the top molecules predicted to interact with residues in the binding pocket of PC2 in which PCI binds. This compound was selected based on the number of similar compounds and potential chemical modifications that can be identified in the ZINC database for follow-up screening.

To induce osteoblastic differentiation, the MC3T3-E1 cells were plated at a density of 2 × 10^4 cells per well in a 12-well plate and 4 × 10^5 cells per well in a 6-well plate and grown up to 21 days in osteogenic medium in the presence of Zinc01442821 (0, 0.5, 1, and 10 μM). ALPL activity and alizarin red S histochemical staining for mineralization were performed as previously described (30, 32). Total DNA content was measured with a PicoGreen dsDNA quantitation reagent and kit (Molecular Probes). Protein concentrations of the supernatant were determined with a Bio-Rad protein assay kit.

**Real-time quantitative RT-PCR and Western blot analysis.** For real-time quantitative RT-PCR, 1.0 μg total RNA isolated from either the long bone of 6-week-old mice or BMSCs cultured for 8 days in differentiation media was reverse transcribed as previously described (30, 32). PCR reactions contained 20 ng template (cDNA), 375 nM each forward and reverse primers, and 1× EvaGreen Supermix (Bio-Rad) in 10 μl. The threshold cycle (Ct) of tested-gene product from the indicated genotype were determined with a Bio-Rad protein assay kit.

**For Western blot analysis, protein concentrations of the supernatant were determined with a Bio-Rad protein assay kit (Bio-Rad). Equal quantities of protein were subjected to 4%–12% Bis-Tris or 3%–8% Tris-Acetate gradient gels (Invitrogen) and were analyzed with standard Western blot protocols as previously described (30, 32). Polycystin-1 antibody (7E12, sc-130554), polycystin-1 antibody (C-20, sc-10372), polycystin-2 antibody (H-280, sc-25749), and polycystin-2 antibody (YCE2, sc-47734) were purchased from Santa Cruz Biotechnology. Puriﬁed mouse TAZ antibody (no. 560236) was purchased from BD Biosciences. Phosphorylated TAZ (Ser 89, sc-47610) and β-actin (sc-7778) antibodies were from Santa Cruz Biotechnology. The intensity of bands was quantiﬁed using ImageJ software (NIH; http://rsb.info.nih.gov/ij/).

**Transient transfection, promoter reporter activity, and co-IP.** C3H10T1/2 cells were cultured in minimum essential medium (MEM)
containing 10% FBS and 1% P/S. To examine the effect of PCI-CTT and TAZ interaction on Runx2 or PPARγ activity, 5 × 10^6 C3H10T1/2 cells were transfected with 1.0 μg of PCI-CTT, 1.0 μg of TAZ, and/or 1.0 μg of Runx2 or Ppaγ expression constructs in combination with 3.0 μg of mouse osteocalcin or α2P promoter luciferase reporter and 0.6 μg of Renilla luciferase–null as internal control plasmid by electrotransfection using a Cell Line optimal transfection kit according to the manufacturer’s protocol (Amaxa Inc.). A total of 6.6 μg of plasmid DNAs was used for each electrotransfection. The transfected cells were plated in 12-well plates and harvested 32 hours after transfection. Cells were lysed in 1× reporter lysis buffer, a luciferase assay was performed using a dual luciferase assay kit (Promega), and activity was measured with a Synergy H4 Hybrid Reader. To test the effect of PCI C-tail cleavage on Oc or α2P reporter activity, the transfected cells were treated with vehicle (DMSO) control or 50 μM DAPT (Sigma-Aldrich; a γ-secretase inhibitor) for the last 12 hours and harvested 36 hours after transfection.

For co-IP analyses, 5 × 10^6 HEK-293T cells were transfected with either 3.0 μg of FLAG-tagged TAZ, 3.0 μg of GFP-tagged full-length PCI, and 3.0 μg of myc-tagged full-length PCI (Baltimore PKD Core Center) or 3.0 μg of FLAG-tagged TAZ, 3.0 μg of myc-tagged PCI-CTT, and 3.0 μg of Runx2 or Ppaγ expression constructs for 48 hours. The transfected cells were washed once with ice-cold 1× PBS and added to appropriate ice-cold 1× IP lysis buffer (Pierce Biotechnology) containing 1× protease inhibitors. The cellular lysates were centrifuged at approximately 13,000 g for 10 minutes to pellet the cell debris at 4°C, and the supernatants were collected to a new tube for protein concentration determination. Fifty microliters of either anti-FLAG, anti-myc, or anti-GFP magnetic beads was added to aliquots of the lysis supernatant as suggested in the manufacturer’s protocol (Sigma-Aldrich). The mixtures were incubated with gentle agitation for 2 hours or overnight at 4°C. The magnetic beads were washed 3 times with 1 ml of cold lysis buffer and suspended in 30 μl of Laemmli’s sample buffer. The supernatant samples were boiled for 3–5 minutes and collected for further analysis. Ten microliters of the sample per lane was loaded in either 3%–8% Tris-Acetate or 4%–12% Bis-Tris gradient gels for Western blot analysis. Anti-PPARγ, anti-PC2, and anti-TC (D24E4, 8418) were purchased from Cell Signaling Technology. Anti-DDDDK Pulse-IT Motif (Sigma-Aldrich) antibody (MA1-91878) was purchased from Pierce Biotechnology. Lamin A/C antibody was purchased from Santa Cruz Biotechnology. Signals were detected using HRP-conjugated secondary antibodies (Santa Cruz Biotechnology) and an enhanced chemiluminescence detection kit (ECL Plus Western Blotting Detection Reagents, GE Healthcare).

**Statistics.** We evaluated differences between 2 groups by unpaired t test, and between multiple groups by 1-way ANOVA with Newman-Keuls multiple-comparisons test. All values are expressed as means ± SD. All computations were performed using commercial biostatistics software (GraphPad Software Inc.).

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