Targeting actin-bundling protein L-plastin as an anabolic therapy for bone loss

Xiaoqun Li1,2*, Lipeng Wang3, Biaotong Huang4, Yanqi Gu5,6*, Ying Luo7, Xin Zhi1, Yan Hu1, Hao Zhang1, Zhengrong Gu8, Jin Cui1, Liehu Cao9, Jiawei Guo1, Yajun Wang1, Qirong Zhou1, Hao Jiang1, Chao Fang1, Weizong Weng1, Xiaofei Chen61§, Xiao Chen1,9§, Jiacan Su1,48§

The actin-bundling protein L-plastin (LPL) mediates the resorption activity of osteoclasts, but its therapeutic potential in pathological bone loss remains unexplored. Here, we report that LPL knockout mice show increased bone mass and cortical thickness with more mononuclear tartrate-resistant acid phosphatase–positive cells, osteoblasts, CD31hiEmcnhi endothelial vessels, and fewer multinuclear osteoclasts in the bone marrow and periosteum. LPL deletion impeded preosteoclasts fusion by inhibiting filopodia formation and increased the number of preosteoclasts, which release platelet-derived growth factor-BB to promote CD31hiEmcnhi vessel growth and bone formation. LPL expression is regulated by the phosphatidylinositol 3-kinase/AKT/specific protein 1 axis in response to receptor activator of nuclear factor–κB ligand. Furthermore, we identified an LPL inhibitor, oroxylin A, that could maintain bone mass in ovariectomy-induced osteoporosis and accelerate bone fracture healing in mice. In conclusion, we showed that LPL regulates osteoclasts fusion, and targeting LPL serves as a novel anabolic therapy for pathological bone loss.

**INTRODUCTION**

L-plastin (LPL), also known as plastin 2, is a member of the actin-bundling plastin family (1). LPL is expressed exclusively in myeloid lineage cells, including macrophages and lymphocytes, while the other homologous isoforms in mammals, I-plastin and T-plastin, are expressed in the intestine, kidney, and solid tissues (1, 2). It is characterized by two actin-binding domains that bind distinct actin filaments to form tight actin bundles, increasing the stability of actin-based structures, such as podosomes and filopodia, in cytoskeleton reorganization (3, 4).

Bone homeostasis is delicately orchestrated by the osteoclasts and osteoblasts through continuous bone modeling and remodeling (5). Osteoclasts are multinucleated cells differentiating from monocytes/macrophages in response to macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor–κB ligand (RANKL) (6). Before fusing into multinuclear mature osteoclasts, tartrate-resistant acid phosphatase–positive (TRAP+) mononuclear preosteoclasts release platelet-derived growth factor-BB (PDGF-BB) to stimulate CD31hiEmcnhi vessel growth, a special subtype of vessel coupling angiogenesis and osteogenesis in the bone (7). Increasing the number of preosteoclasts to induce PDGF-BB secretion promotes angiogenesis and bone formation, which ameliorates pathological bone loss (7).

LPL participates in osteoclastogenesis and bone resorption. Its phosphorylation promotes the formation of sealing ring in osteoclasts in the early phase via the actin-bundling process, and its inhibition leads to substantially compromised bone resorption in vitro (8). In vivo, the bone mass of LPL knockout (LPL−/−) mice is significantly increased because of the impaired bone resorption activity of osteoclasts (9). The bone formation rate is not attenuated despite the impaired bone resorption, and a growing trend is even noted in 12-week-old mice (9). These observations suggest that the role of LPL is not limited to bone resorption, but its mechanisms of action remain unclear.

Osteoclasts are multinuclear cells that arise through cell fusion, in a multistep process mediated by rearrangement of the actin cytoskeleton (10). This reorganization is mediated by the formation of actin-rich structure filopodia and podosomes (11, 12). In macrophages and osteoclasts, filopodia sense external stimuli, aid migration, and initiate the cell fusion (11, 12). Meanwhile, podosomes participate in cell migration, adhesion, and belt-like sealing zone formation for osteoclast maturation (11, 12). LPL mediates filopodia formation in renal podocytes and cooperates with other bundling proteins to form filopodia in cancer cells (3, 13). The inhibition of LPL by nanobody or oxidation diminishes the actin-bundling proteins to form filopodia in cancer cells (4, 14).

In this study, we found that LPL deletion inhibited preosteoclast fusion, and the subsequently increased numbers of preosteoclasts released PDGF-BB to induce the formation of CD31hiEmcnhi vessels and bone formation. LPL−/− in C57BL/6 mice impeded preosteoclast fusion by suppressing filopodia formation, and LPL expression level was regulated by the phosphatidylinositol 3-kinase (PI3K)/AKT/ specific protein 1 (SP1) axis in response to RANKL. We identified oroxylin A as an LPL inhibitor and demonstrated that it significantly ameliorated bone loss by inhibiting osteoclastogenesis.
RESULTS

**LPL**−/− promotes bone formation and impairs bone resorption

To explore the roles of plastin proteins in osteoclastogenesis, we first performed quantitative polymerase chain reaction (qPCR) to determine the mRNA levels of plastin family members including I-plastin, T-plastin, and L-plasin on days 0, 3, and 6 during osteoclast differentiation induced by M-CSF/RANKL. Compared to I-plastin and T-plastin, the transcription of LPL was significantly up-regulated on day 3 and decreased on day 6 when multinuclear osteoclasts emerged. By contrast, I-plastin and T-plastin levels decreased gradually during osteoclastogenesis (fig. S1). To further examine the roles of LPL in bone metabolism, we generated LPL−/− mice (fig. S2, A and B). The LPL−/− mice showed comparable body weight, length, and organ development to wild-type (WT) controls within 8 weeks after birth (fig. S2, C and F). Tooth eruption at 4 weeks old was normal (fig. S2D). Increased bone mass in the distal femur was observed in LPL−/− mice at 8 weeks (fig. S2E). Micro–computed tomography (micro-CT) showed that the bone mineral density (BMD), number of trabeculae (Tb.N.), and bone volume per tissue volume (BV/TV) were significantly increased in LPL−/− mice compared to WT controls (Fig. 1, A and B). In contrast to a previous report (9), we found that LPL−/− mice also showed a slight increase in cortical thickness (Fig. 1, A and C). Immunofluorescence staining of osteocalcin (OCN) showed an elevated number of trabecular osteoblasts in LPL−/− mice (Fig. 1, D and E). In addition, calcein double staining indicated that LPL−/− mice showed enhanced bone formation in both the trabecular bone and cortical bone relative to their WT littermates (Fig. 1, F to I). These results suggest that LPL−/− promotes bone formation and impairs bone resorption.

**LPL**−/− promotes angiogenesis by increasing preosteoclasts

To investigate osteoclast development, we analyzed sections of the femora from 8-week-old WT and LPL−/− mice by TRAP staining. LPL−/− mice showed comparable numbers of TRAP+ osteoclasts on the trabecular bone as WT mice (Fig. 2, A and B). There were fewer multinuclear TRAP+ osteoclasts on the trabecular bone of LPL−/− mice than in WT mice (Fig. 2, A and B). Periosteal TRAP+ cells were mainly unfused mononuclear cells (7), and we showed that mononuclear
TRAP^+^ cells on the surface of the periosteal bone in LPL−/− mice were more abundant relative to the control WT mice (Fig. 2, A and C). Preosteoclasts induce the formation of CD31^hi^Emcn^hi^ vessels (7). Then, we performed angiography to examine the vessels in femora. The vessel volume and surface area were markedly higher in the LPL−/− mice than their WT littermates (Fig. 2, D and E). Immunofluorescence staining showed that the LPL−/− mice had more CD31^hi^Emcn^hi^ vessels in the bone marrow and periosteum than WT controls (Fig. 2, F and G). Flow cytometry showed an increase in CD31^hi^Emcn^hi^ cells in the bone marrow in LPL−/− mice compared to their WT littermates (4.67% versus 2.07%, respectively) (Fig. 2H). Preosteoclasts couple angiogenesis and osteogenesis by secreting PDGF-BB (7). Next, we assessed the distribution of PDGF-BB/TRAP^+^ cells in the femur. Immunofluorescence staining showed that in both the trabecular bone and periosteum of LPL−/− mice, large numbers of TRAP^+^ cells were positive for PDGF-BB, but fewer of these cells were observed in control mice (Fig. 2, I and J). In addition, the serum concentrations of PDGF-BB and vascular endothelial growth factor (VEGF) were significantly higher in the LPL−/− group compared to the control group (Fig. 2B). Meanwhile, we silenced LPL in RAW264.7 cells, and the cells were induced with M-CSF and RANKL (fig. S3A). The results were consistent with those of BMMs (fig. S3, B and C).

We used a double fluorescence method to investigate the effects of LPL−/− on preosteoclast fusion (15). The preosteoclasts were separated into two groups: One was labeled with Hoechst (blue fluorescent nuclear dye), and the other was labeled with DiI (red...
fluorescent cell membrane dye). Then, the cells from the two groups were cocultured. Fused cells showed a blue nucleus and red membrane under fluorescence microscopy (Fig. 3C). The membrane merge rate was calculated as described previously (16). After induction with M-CSF and RANKL for 3 days, we observed fewer fused cells and a significantly lower membrane merge rate in the LPL−/− group compared to WT controls (Fig. 3C). Meanwhile, the results of qPCR indicated that the transcription of osteoclast fusion–related genes, including CD9, CD44, and CD47, were significantly downregulated in the LPL−/− group on day 3, indicating impaired osteoclast fusion (Fig. 3E). Cell fusion usually begins from filopodia mediated by actin reorganization (11). On day 3 of osteoclast induction, we observed significantly inhibited filopodia formation by confocal fluorescence staining of LPL and F-actin in LPL−/− mice compared to their WT littermates (Fig. 3D). Consistent results were shown in LPL-silenced RAW 264.7 cells (fig. S3D). In addition, we assessed the function of the formed multinuclear osteoclasts and found a decrease in F-actin ring formation and resorption pits in the LPL−/− group, indicating reduced osteoclast resorptive function (fig. S3, E and F).

To detect PDGF-BB secretion during osteoclastogenesis after LPL deletion, we conducted enzyme-linked immunosorbent assay (ELISA) to examine the PDGF-BB level in culture medium. The PDGF-BB level reached a peak on day 3 and then decreased slightly on day 6 (Fig. 3F). Significantly higher levels of PDGF-BB in culture medium were found on days 3 and 6 in the LPL−/− group compared to WT controls (Fig. 3F). To further explore the ability of vessel formation, we performed the Matrigel tube formation assay. Results showed that conditioned medium of BMMs from WT mice induced endothelial progenitor cell (EPC) tube formation, whereas conditioned medium of BMMs from LPL−/− mice showed more significant tube formation (fig. S3G). Western blotting analyses showed that the expression level of cathepsin K (CK), a marker expressed in mature osteoclasts and preosteoclasts, was significantly lower on days 3 and 6 in the LPL−/− group compared to the control group (Fig. 3G). To exclude the effect of apoptosis, we performed Hoechst staining to explore apoptosis during osteoclastogenesis after LPL deletion. The results indicated that the apoptotic rate of the LPL−/− group showed no significant difference compared with the WT group (fig. S3H). Together, these results indicate that LPL is required for the formation of filopodia and preosteoclast fusion.

**LPL-mediated preosteoclast fusion is regulated via the PI3K/AKT/SP1 pathway**

To explore the molecular mechanisms of LPL regulation during osteoclastogenesis, we searched for the DNA binding motif sequence of the LPL promoter in the TRANSFAC database (http://genexplain.com/transfac/). The results indicated that SP1, an important transcription factor during osteoclastogenesis, could bind to the LPL promoter and regulate LPL transcription (17). Then, we predicted the potential transcription factor binding sites of SP1 via the Jaspar database (http://jaspar.genereg.net/) and designed the top five predicted sequences on the positive-sense strand (table S1). Then, a chromatin immunoprecipitation (ChIP) assay was performed.
The results showed that SP1 markedly bound to the LPL promoter on sequence 1 (TCACCACCCCC) at a high level (Fig. 4A). To further confirm the role of SP1 in regulating LPL expression, we performed the luciferase reporter assay. The results showed that SP1 promoted the transcription of LPL, whereas the transcription was significantly down-regulated when we mutated the binding sites from 5'-TCACCACCCCC-3' into 5'-GACATGTTATA-3' (Fig. 4B). These results indicated that SP1 was a major transcription factor regulating LPL expression, and the transcription factor binding site sequence was TCACCACCCCC.

The PI3K/Akt signaling pathway regulates the fusion of osteoclasts, and SP1 is a downstream transcription factor of the PI3K/AKT pathway (18, 19). Therefore, we further investigated the relationships among LPL, SP1, and the PI3K/Akt pathway under the stimulation of RANKL. After inhibiting PI3K phosphorylation with LY294002 or AKT phosphorylation with perifosine, the expression levels of SP1 and LPL were significantly down-regulated (Fig. 4, C and D). Inhibition of SP1 by mithramycin A resulted in a reduction in expression of LPL (Fig. 4, C and D). We further performed the immunofluorescent staining. The expression of SP1 and LPL could be inhibited by the PI3K and AKT phosphorylation inhibitors, and the expression of LPL could be inhibited by the SP1 inhibitor (fig. S4, A to C). These results demonstrated that the expression of LPL was regulated by the PI3K/AKT/SP1 axis.

To further explore the role of the PI3K/AKT/SP1/LPL axis in the formation of filopodia and fusion of osteoclast precursors, we examined the number of filopodia and membrane merge rates after treatment with LY294002, perifosine, and mithramycin A. After treatment with LY294002, perifosine, and mithramycin A, the number of filopodia was significantly reduced compared to the control group in both BMMs and RAW264.7 cells (Fig. 4, E and F, and fig. S4, D and E). The membrane merge rates were also suppressed after treatment with these inhibitors (Fig. 4, G and H). Notably, independent inhibition of phosphorylated PI3K (p-PI3K), p-AKT, and SP1 axis was adequate to prevent filopodia formation and preosteoclast fusion with M-CSF/RANKL stimulation. The results of immunofluorescent studies of p-PI3K, p-AKT, SP1, and LPL were consistent with the Western blotting results (fig. S4, A to C). Together, these findings indicate that LPL expression is regulated by the PI3K/AKT/SP1 axis in osteoclastogenesis.

Oroxylin A targets LPL and inhibits preosteoclast fusion

LPL deletion prevented the fusion of osteoclast precursors and increased angiogenesis, osteogenesis, and bone formation. Targeting LPL has the potential for anabolic therapy to treat pathological bone loss. We used biological chromatography, a highly sensitive and high-throughput approach for screening underlying compounds for LPL. Recombinant LPL is immobilized on the chromatographic stationary phase to achieve high-specificity screening of active compounds. In this study, we established an online two-dimensional (2D) LPL-immobilized biological chromatography/C18 column/time-of-flight mass spectrometry (TOFMS) system for high-throughput

**Fig. 4. LPL mediates preosteoclast fusion via the PI3K/AKT/SP1 pathway.** (A) ChIP of LPL and SP1 (sequence 1). IgG, immunoglobulin G. (B) Luciferase activity of LPL with SP1 overexpression (OE) and mutation of SP1-LPL–binding sites. (C and D) Western blotting and quantitative analyses of the phosphorylation of PI3K, AKT, SP1, and LPL with PI3K inhibitor (perifosine), AKT inhibitor (LY294002), and SP1 inhibitor (mithramycin A). n = 5 per group. (E) Confocal fluorescence images of BMMs treated with perifosine, LY294002, or mithramycin A. Scale bar, 20 µm. (F) Quantification of filopodia with the above inhibitors. n = 6 per group. (G) Double fluorescence analyses of BMM fusion with perifosine, LY294002, or mithramycin A. Scale bar, 50 µm. (H) Quantification of membrane merge rates with the above inhibitors. n = 6 per group.

*P < 0.05, **P < 0.01, and ***P < 0.001.
screening of ligands targeting LPL from herbal extracts based on our previous studies (fig. S5A) (20, 21). From the 3D spectra of identified components in Radix Scutellariae extract, we found that oroxylin A showed a significant retention on LPL biological chromatographic column (Fig. 5A, left), indicating that oroxylin A had a strong binding affinity with LPL. This was confirmed using the authentic standard of oroxylin A (Fig. 5A, right). To further validate the affinity of oroxylin A with LPL, we performed surface plasmon resonance (SPR) assay to directly monitor the kinetic parameters and calculate the affinity dissociation constant ($K_d$). The results showed that oroxylin A bound to recombinant LPL protein with a $K_D$ of 9.223 μM (Fig. 5B). Drug affinity-responsive target stability experiments further demonstrated the combination of oroxylin A and LPL (fig. S5B). Thus, oroxylin A was identified as a potential lead compound targeting LPL.

Then, we examined the effects of oroxylin A on osteoclastogenesis. After treatment with oroxylin A, the numbers of TRAP+ preosteoclasts were comparable in the two groups on the 3rd day, whereas multinuclear osteoclast formation was significantly decreased relative to the control group in both BMMs and RAW264.7 cells (Fig. 5, C and D, and fig. S6, A and B). On day 3 after induction, the number of filopodia was significantly decreased after incubation with oroxylin A (Fig. 5E and fig. S6C). As expected, double fluorescence assay showed that oroxylin A treatment inhibited cell fusion on day 3 after induction by M-CSF/RANKL (Fig. 5F). The qPCR results further showed that the transcription of osteoclast fusion–related genes, including CD9, CD44, and CD47, significantly decreased after oroxylin A treatment on day 3 (Fig. 5G). Then, we examined the function of osteoclasts and demonstrated that oroxylin A (10 μM) significantly inhibited F-actin ring formation and their resorption activity (fig. S6, D and E). Next, we measured the PDGF-BB level in the cell medium after treatment with oroxylin A. As shown in Fig. 5H, oroxylin A administration increased the level of PDGF-BB compared to the control group. Similar to LPL deletion, oroxylin A treatment enhanced tube formation effect compared with the control group (fig. S6F). Then, we explored the expression of CK by Western blotting analyses after oroxylin A treatment. The expression of CK was significantly lower after induction on days 3 and 6 with oroxylin A intervention (Fig. 5I). The results of Hoechst staining showed that the apoptotic rate of oroxylin A treatment group showed no difference compared with the control group, indicating that the inhibitory effect of oroxylin A

![Fig. 5. Oroyxlin targets LPL and inhibits preosteoclast fusion.](http://advances.sciencemag.org/)

(A) Fast screening of oroxylin A as a novel LPL inhibitor by the 2D LPL protein biological chromatography/C18 column/TOFMS system. Left plot: 70% ethanol extract of Radix Scutellariae. Right plot: Authentic standard of oroxylin A. MPTS, mercaptopropyltrimethoxysilane. (B) SPR analyses of binding between oroxylin A and LPL immobilized on a CMS chip. The equilibrium dissociation constant ($K_d$) was 9.223 μM. RU, response unit. (C) TRAP staining on day 3 after oroxylin A treatment with quantification of TRAP+ cells. Scale bar, 50 μm. n = 6 per group. (D) TRAP staining on day 6 after oroxylin A treatment with quantification of osteoclasts. Scale bar, 200 μm. n = 6 per group. (E) Confocal fluorescence images of BMMs on day 3 after oroxylin A treatment with quantification of filopodia. Scale bar, 20 μm. n = 6 per group. (F) Representative images of double fluorescence analyses for BMMs fusion on day 3 after oroxylin A treatment with quantification of the membrane merge rate. Scale bar, 50 μm. n = 6 per group. (G) qPCR analysis of CD9, CD44, and CD47. (H) Supernatant PDGF-BB levels on days 1, 3, and 6 after oroxylin A treatment. n = 6 per group. *P < 0.05, **P < 0.01, ***P < 0.001.
was not due to apoptosis (fig. S6G). Together, oroxylin A targets LPL, inhibiting the fusion of osteoclast precursors and promoting PDGF-BB secretion.

**Targeting LPL attenuates bone loss in OVX mice and promotes bone fracture healing**

As LPL deletion promoted angiogenesis and bone formation, we speculated that targeting LPL may have therapeutic potential for bone loss disorders. We first used OVX mice to assess the effects of LPL deletion on estrogen deficiency–induced bone loss. Both bone loss and metatrophia were observed to confirm successful surgery (fig. S9A). The trabecular bone mass and thickness of the bone cortex were significantly reduced after ovariectomy, and LPL<sup>−/−</sup> attenuated the bone loss in the OVX model (fig. S9, B and C).

To investigate the roles of oroxylin A on pathological bone loss, mice received an intraperitoneal injection of oroxylin A (50 mg/kg) daily for 8 weeks after OVX surgery. The bone mass was preserved as shown by the increased BMD, Tb.N, and BV/TV compared to untreated OVX mice (Fig. 6, A and B). The bone cortex thickness was maintained after oroxylin A treatment (Fig. 6, A and C). The results of histomorphological analyses also showed protective effects of oroxylin A on bone loss (Fig. 6, D and E).

Next, we examined angiogenesis. Consistent with previous findings (7), bone marrow vascularization was decreased after OVX, and the vessel volume and surface were restored after oroxylin A administration (Fig. 6, F and G). Immunofluorescence staining showed that CD31<sup>hi</sup>Emcn<sup>hi</sup> vessels were decreased in both the trabecular bone and periosteal bone after OVX surgery (Fig. 6, H and I), and oroxylin A administration reversed the decrease of CD31<sup>hi</sup>Emcn<sup>hi</sup> vessels (Fig. 6, H and I). Flow cytometry showed that the reduction of CD31<sup>hi</sup>Emcn<sup>hi</sup> endothelial cells in the bone marrow after ovariectomy was also reversed after oroxylin A treatment (Fig. 6J). To further confirm the effects of oroxylin A on promoting the formation of CD31<sup>hi</sup>Emcn<sup>hi</sup> vessels, we determined the levels of PDGF-BB and VEGF in serum. After intraperitoneal injection of oroxylin A for 8 weeks, the serum PDGF-BB and VEGF levels were significantly upregulated compared to the OVX group (Fig. 6N). The calcine double staining indicated that oroxylin A enhanced bone formation activity in both the trabecular bone and cortical bone after ovariectomy (Fig. 6, K to M). The serum-decreased levels of TRACP5b and CTX-1

![Fig. 6. Oxyxylin attenuates bone loss in OVX mice.](http://advances.sciencemag.org/)
indicated that oroxylin A impeded bone resorption (Fig. 6O), while increased levels of OCN implied that oroxylin A enhanced bone formation in the OVX model (Fig. 6P).

As bone fracture repair was associated with extensive angiogenesis, we hypothesized that targeting LPL could promote bone fracture healing. Oroxynin A (50 mg/kg) was administered daily by intraperitoneal injection to 12-week-old male mice with an open fracture at the midshaft of the femur. After 21 days of treatment, micro-CT showed higher BMD, BV/TV, and union rate compared to the control mice in the oroxylin A treatment group (fig. S8, A to C). Biomechanical studies showed higher maximum load and stiffness in the oroxylin A treatment group compared to control mice (fig. S8D). Indirect (secondary) bone fracture healing is the most common form of fracture healing with primary soft callus formation, followed by endochondral ossification and intramembranous bone healing (22). To explore how oroxylin A promoted fracture healing, we analyzed the formation of soft callus by safranin O and fast green staining on days 7, 14, and 21 after fracture. We found that the formation of soft callus on days 7 and 21 showed no difference between the two groups (fig. S8F). However, a significant reduction of soft callus area was observed on day 14 in the oroxylin A treatment group (fig. S8, E and F). We further calculated the relative callus mineralization at the fracture site on days 7, 14, and 21 after fracture to evaluate the bone formation activity after oroxylin A treatment. The results showed that relative callus mineralization increased on days 14 and 21 after treatment (fig. S8G), indicating enhanced endochondral ossification. We then explored whether this anabolic effect was preceded by an increase in preosteoclasts and CD31<sup>hi</sup>Emcn<sup>hi</sup> vessels. TRAP staining showed that, after treatment with oroxylin A, the number of TRAP<sup>+</sup> mononuclear preosteoclasts increased on day 14 (fig. S8H). In addition, more CD31<sup>hi</sup>Emcn<sup>hi</sup> vessels were observed after oroxylin A treatment on day 14 (fig. S8I). Together, oroxylin A promotes secondary ossification after fracture by increasing preosteoclast number and blood vessel formation. Together, these results suggest that oroxylin A is a therapeutic candidate for preventing pathological bone loss, including osteoporosis, and promoting bone fracture healing.

**DISCUSSION**

At present, over 200 million people suffer from osteoporosis around the world (23). The loss of bone mass results in increased fracture risk and mortality (23). It has been suggested that one in five men and one in three women over 50 years old will experience osteoporotic fractures (23). Clinically, antiresorptive drugs are routinely used to treat osteoporosis, but inhibition of bone resorption also disturbs bone remodeling and suppresses de novo bone formation (24). Serious adverse effects, including osteonecrosis of the jaw and atypical bone fracture, have been reported after long-term application of antiresorptive drugs (24). Teriparatide, a recombinant parathyroid hormone, is currently the main anabolic therapeutic option in clinical practice (24). Anti-sclerostin antibody is a new option for bone formation, and further clinical investigations are needed to evaluate its benefits and risks (24). In recent years, anabolic therapeutic strategies for pathological bone loss have attracted attention (25–27).

LPL is an actin-bundling protein that is indispensable for actin cytoskeleton organization (1). The dynamic adjustment of the cytoskeleton is a prerequisite for fundamental functions of eukaryotic cells, such as motility and adhesion (28). Physiologically expressed in hematopoietic cell lineages, LPL contributes to the maturation and activation of macrophages and T cells (2, 29), and some non-hematopoietic cancer cells express LPL (3, 14). As LPL is specifically expressed in myeloid lineage cells, global knockout would not affect mesenchymal stromal cells functions, indicating that increased osteoblast differentiation and bone formation are secondary to osteoclastogenesis changes.

It was recently reported that LPL is involved in osteoclastogenesis and bone resorption. The inhibition or loss of LPL leads to impaired sealing zone formation in mature osteoclasts and resorptive activity in vivo and in vitro (8, 9). LPL<sup>−/−</sup> mice show significantly reduced bone resorption but unaltered bone formation and even a growing trend of bone formation at 12 weeks (9). These observations suggest that LPL may function through mechanisms other than interfering with bone resorption. In the present study, we found a special dual effect of LPL on bone resorption and formation. LPL deletion reduced bone resorption but increased bone formation. Similar results have been reported for CK inhibitor for the treatment of osteoporosis (30).

Appropriate bone formation originates from osteogenesis mediated by osteoblasts and conducive milieu created by adjacent tissues in the bone, including vascular endothelium (31). CD31<sup>hi</sup>Emcn<sup>hi</sup> vessels couple angiogenesis and osteogenesis (31). A decrease in CD31<sup>hi</sup>Emcn<sup>hi</sup> vessel number and the concomitant decline of osteoprogenitors are observed in aged mice, and prevention of CD31<sup>hi</sup>Emcn<sup>hi</sup> vessel reduction alleviates bone loss (31). Monocytes/macrophages differentiate and fuse into mature osteoclasts upon M-CSF and RANKL stimulation. Before fusion into multinucleated osteoclasts, preosteoclasts are mononuclear TRAP<sup>+</sup> cells and secrete PDGF-BB to promote CD31<sup>hi</sup>Emcn<sup>hi</sup> vessel formation (7). Here, we reported an increase in preosteoclasts in both the trabecular bone and cortical bone in LPL<sup>−/−</sup> mice. These observations indicated that the deletion of LPL promotes PDGF-BB secretion, angiogenesis, and coupled bone formation.

Cell-cell fusion is vital in many biological activities, including fertilization, tumorigenesis, and immune responses (32). Regardless of the cell type, similar underlying fusion steps are observed, including chemotaxis, migration, cell adhesion, cytoskeletal rearrangement, and membrane merging, and the principal requirement of fusion is to increase cell contact (32). Filopodia promote cell fusion (33). During osteoclastogenesis, two actin-rich “protruding” structures are observed to promote preosteoclast contact, i.e., filopodia extending from the mononuclear cells and podosomes of large multinucleated cells (11, 12). Filopodia serve as “antennae” that search for other fusion-competent cells with which to fuse. The contact of filopodia from different cells initiates fusion, and a bridge enriched with filopodia is further established between fusing cells for subsequent membrane fusion (34). The abundance of filopodia also ensures the massive surface area for cell contact to start fusion. After mature osteoclasts form, the filopodia disappear (11). The podosome forms a sealing zone, the site for bone resorption in osteoclasts (35). In addition, the podosomes merging from the sealing belt of large multinucleated cells also mediate osteoclast fusion (11). LPL regulates filopodia formation, and the inhibition of LPL by specific nanobodies or oxidation block filopodia formation (4, 14). Here, we showed that filopodia initiated preosteoclast fusion and that LPL deletion inhibited filopodia formation and thus preosteoclast fusion. However, LPL<sup>−/−</sup> did not disturb podosome formation and cell fusion.
formation in mature osteoclasts (9). Knockout of the fusion-specific factor, dendrocyte-expressed seven transmembrane protein, leads to complete abrogation of osteoclast fusion and osteopetrosis in mice (36). LPL deletion partially inhibited preosteoclast fusion and did not completely suppress mature osteoclast formation, resulting in less interference on bone remodeling.

With regard to the molecular mechanism of LPL regulation, we showed that the expression of LPL in preosteoclasts is increased and the level is regulated by the PI3K/AKT/SP1 pathway after RANKL stimulation. The specific inhibition of phosphorylation of PI3K and AKT, as well as SP1, led to reduced LPL expression and filopodia formation. LPL has a calcium-dependent switch helix and two F-actin binding sites, and the binding of \( \text{Ca}^{2+} \) inhibits the actin-bundling activity of LPL (37). Another mechanism regulating LPL function is the phosphorylation of Ser, which activates LPL and is essential for T cell activation and tumor metastasis (38, 39). The phosphorylation of LPL also contributes to actin assembly and sealing zone formation in mature osteoclasts (8). However, the mechanism underlying LPL activation during osteoclast fusion is unknown, and further research is required.

In this study, we found that oroxylin A targeted LPL. On the basis of our previously reported method, we developed an online 2D LPL immobilized biological chromatography/C18 column/TOFMS system to screen herbal extracts for ligands targeting LPL (20, 21). We identified and confirmed that oroxylin A, an active ingredient derived from Radix Scutellariae, targets LPL. Oxyxylin A treatment blocked osteoclast fusion by inhibiting filopodia formation, attenuated bone loss in OVX mice, and promoted bone fracture healing. The administration of oroxylin A suppressed osteoclastogenesis and bone resorption with markedly increased angiogenesis and bone formation in OVX mice. In contrast to antiresorptive agents, oroxylin A decreased osteoclastogenesis and bone resorption while increasing angiogenesis and bone formation in OVX mice. We also found that oroxylin A also promoted bone fracture healing and improve the biomechanical properties of the bone in mice. Indirect bone fracture healing is the most common form of fracture healing with primary soft callus formation, followed by enchondral ossification and intramembranous bone healing. In general, four steps are involved in fracture repair: hematoma formation (days 1 to 5), fibrocartilaginous callus formation (days 5 to 11), bony callus formation (days 11 to 28), and bone remodeling (day 18 onward, lasting months to years) (40). We found that oroxylin A treatment did not affect the formation of soft callus but promoted secondary ossification by increasing preosteoclasts number and blood vessel formation. Notably, oroxylin A enhanced BMD and increased maximum load and stiffness of the bone.

LPL loss leads to deficiencies in immune cells and impaired host defense in mice (2, 29). Higher titers of anti-LPL antibodies are detected in the sera of patients with systemic lupus erythematosus (SLE), indicating a strong association between LPL and this disease (41). The bone accrual benefits and potential side effects of the LPL-targeting agent deserve further exploration. Together, these results suggest that targeting LPL has the potential to treat pathological bone loss as anabolic therapy (Fig. 7).

**MATERIALS AND METHODS**

**Study design**

This study was performed to explore the roles of LPL in the formation of osteoclasts and bone remodeling, as well as the therapeutic potential of compounds targeting LPL for pathological bone loss. We generated LPL^{-/-} mice and determined the numbers of mononuclear TRAP^{+} cells, multinuclear osteoclasts, osteoblasts, and CD31^{hi}Emcn^{hi} endothelial vessels in the trabecular bone and cortical bone by immunofluorescence and histomorphometric analyses. Serum markers of osteoclastogenesis and osteogenesis were examined by ELISA. The therapeutic potential of LPL targeting in pathological bone loss was evaluated in murine OVX and bone fracture models. Samples were divided into different groups randomly, and littermates were used as controls. At least five samples were used for statistical analyses in a blinded manner. The experiments were approved by Shanghai Model Organisms [SCXK (Shanghai) 2017-0010 and SYXK (Shanghai) 2017-0012], and Institutional Animal Care and Use Committee guidelines were followed with animal subjects. For in vitro experiments, at least three independent experiments were conducted. All data were analyzed using GraphPad Prism software.
Quantitative polymerase chain reaction

Total RNA was extracted using TRIzol reagent (Ambion), and the PrimeScript RT Reagent Kit with guide DNA Eraser (RR047A, Takara) was used for reverse transcription. I-plastin, LPL, and T-plastin expressions were detected using a Taq Green premix Ex Tag kit (RR420A, Takara) with a real-time PCR system (LightCycler 480, Roche). The primers used for PCR were as follows: I-plastin, 5′-AGGAGGAGCATTTCGCAACT-3′ (forward) and 5′-GGTGACGAGCCTGTTCACC-3′ (reverse); LPL, 5′-TCCGTTGCTGACGAA-GAAATG-3′ (forward) and 5′-GCCTGCTAAGGCTTGATTTCCA-3′ (reverse); 1-plastin, 5′-GGAGACCACCAGATTCTCCAAA-3′ (forward) and 5′-GCAGTGCCATATTGCTTCTTG-3′ (reverse); LPL (sequence 1), 5′-TGGCCTGGCTTCTTAC-3′ (forward) and 5′-AGGCCCTGCTACGGCAATT-3′ (reverse); LPL (sequence 2), 5′-TCAGGGATAAAGGAGATGG-3′ (forward) and 5′-CTGATTTGTCGGCTGGTA-3′ (reverse); LPL (sequence 3), 5′-CGTGGGAAATCTGGTCTGT-3′ (forward) and 5′-GGTTTACCGCGGAAAGGT-3′ (reverse); CD9, 5′-ATCCGGTCAAGAGGATTGAG-3′ (forward) and 5′-GCCATGATCCATAAGCAGCA-3′ (reverse); CD44, 5′-TGTATTTGATGAATCTGCCG-3′ (forward) and 5′-CAGTCCGGGAGATCTGAGC-3′ (reverse); CD47, 5′-TGTGGGAAACTACTGTGC-3′ (forward) and 5′-CGTTGGCTTTTCAGCCTAT-3′ (reverse).

Mouse models

LPL-knockout mice on C57BL/6 background were generated with the CRISPR-Cas9 gene editing by the Shanghai Model Organisms (fig. S2A). First, guide RNAs (gRNAs) were designed flanking exon 4 of the LPL gene (gRNA 1, 5′-GCGGCCACCTGGGGCGCAGG-3′; gRNA 2, 5′-CAAATGGGTACCTCCTGGTAC-3′). Then, the Cas9 mRNA and gRNA were microinjected into mouse zygotes. The F0 generation pups were detected by PCR using the following primers: P1, 5′-CAGGAGACCTCAAACGCAACC-3′; P2, 5′-GCAAAACCTTGTTTGATT-3′ (reverse); P3, 5′-CGTGGGGAAATCTGGTCTGT-3′ (forward) and 5′-GGTTTACCGCGGAAAGGT-3′ (reverse); CD9, 5′-ATCCGGTCAAGAGGATTGAG-3′ (forward) and 5′-GCCATGATCCATAAGCAGCA-3′ (reverse); CD44, 5′-TGTATTTGATGAATCTGCCG-3′ (forward) and 5′-CAGTCCGGGAGATCTGAGC-3′ (reverse); CD47, 5′-TGTGGGAAACTACTGTGC-3′ (forward) and 5′-CGTTGGCTTTTCAGCCTAT-3′ (reverse).

Immunofluorescence and histomorphometry

For immunofluorescence analyses of CD31 and endomucin (Emcn), femora were removed from the mice and fixed in 4% paraformaldehyde at 4°C for 3 days. Then, the femora were decalcified with EDTA (0.5 M; pH 7.4) for 3 days with continued turbulence and washed three times with phosphate-buffered saline (PBS). Sections of 30 μm in thickness were prepared for immunofluorescence staining. Sample sections were treated with 0.3% Triton X-100 for 20 min and then blocked with 5% donkey serum for 20 min. Rat anti-EMCN antibody (1:100; sc-65495, Santa Cruz Biotechnology) and rabbit anti-CD31 antibody (1:20; ab28364, Abcam) were incubated with the samples at room temperature for 2 hours. The samples were washed three times with PBS and incubated with Alexa Fluor 488 donkey anti-rabbit immunoglobulin G (IgG; 1:200; 711-545-152, the Jackson laboratory) and Alexa Fluor 594 donkey anti-rat IgG (1:200; 712-585-150, the Jackson laboratory) for 2 hours at room temperature. Quantification of CD31hiEMCNhi vessels was performed as described previously (7). For the immunofluorescence of OCN, PDGF-BB, and TRAP, femora were removed from the mice and fixed in 4% paraformaldehyde at 4°C for 3 days. Then, the femora were decalcified by EDTA (0.5 M; pH 7.4) for 3 weeks at room temperature. Sections (3 μm in thickness) were prepared and incubated with antibodies against OCN (1:50; 23418-1-AP, Protein Tech), PDGF-BB (1:50; ab34074, Abcam), and TRAP [1:50; 32694, Signalway Antibody (SAAB) overnight at 4°C. Then, the sections were incubated with secondary antibodies for 2 hours at room temperature. For bone histomorphometry, femora were removed from the mice and fixed in 4% paraformaldehyde at 4°C for 3 days. Then, they were decalcified with EDTA (0.5 M; pH 7.4) for 3 weeks at room temperature. Sections (3 μm in thickness) were stained with hematoxylin and eosin and TRAP.

Enzyme-linked immunosorbent assay

The serum levels of CTX-1, TRACP-5b, OCN, PDGF-BB, and VEGF were examined by ELISA using a CTX-1 ELISA kit (NBP2-69074, Novus), TRACP-5b ELISA kit (EK7661, SAB), OCN ELISA kit (NBP2-68151, Novus), PDGF-BB ELISA kit (ab224879, Abcam), and VEGF ELISA kit (ab100751, Abcam). The expression levels of PDGF-BB in conditioned media were determined using a PDGF-BB ELISA kit (ab224879, Abcam). All ELISAs were performed in accordance with the manufacturer’s instructions.

Micro-CT and x-ray analyses

Quantitative micro-CT analyses were performed using a high-resolution low-dose x-ray scanner (SkyScan 1076, Bruker). Femora were removed from the mice and fixed in 70% ethanol overnight before micro-CT analyses. The scanner was set at a resolution of 8 μm per pixel, a voltage of 80 kV, and a current of 124 μA. The 3D models were analyzed by CTAn and CTVol. Two hundred section planes below the growth plate were observed. The trabecular bone of the bone marrow was analyzed for BMD, Tb.N, and BV/TV. The cortical bone was analyzed by determining the cortical thickness. The tooth eruption of the mice was determined by x-ray analyses.

Flow cytometry

CD31hiEMCNhi cells in the bone were detected by flow cytometry. The femora were first removed from the mice without the epiphysis region. Then, the muscles and periosteum were removed from the bone. The samples were crushed and digested to obtain suspensions of bone marrow cells. The cells were incubated with phycoerythrin-Cy7 anti-mouse CD31 antibody (102417, BioLegend), Emcn monoclonal antibody [eBioV.7C7 (V.7C7), ebioscience], eFlour 660 viability dye, ebioscience (50585182, Invitrogen), fluorescein isothiocyanate (FITC) anti-mouse TER-119/erythroid cells antibody
polymerized Matrigel. Cells were then treated with conditioned medium. After incubation at 37°C for 4 hours, we stained the cells with Calcein AM (C2012-0.1 ml, Beyotime) and detected the tube formation by a fluorescent microscope.

**Osteoclastic resorption assay**

Osteoclastic resorption assay was performed to explore the effects of LPL on the function of osteoclasts. Briefly, mature osteoclasts were digested and seeded onto bone biomimetic synthetic plates (Corning) with M-CSF (30 ng/ml) and RANKL (100 ng/ml). The plates were flamed 3 times with 0.5% sodium hypochlorite after an additional 2 days in culture. The resorbing area was calculated using the Image J software.

**In vitro assay for osteoclast fusion**

Cells were flushed out of the bone marrow cavity from the femora of 4-week-old mice and cultured for 3 days in α-MEM supplemented with 10% FBS and M-CSF (50 ng/ml). Then, the adherent cells were washed three times with PBS and seeded onto 12-well plates. After incubation with M-CSF (30 ng/ml) and RANKL (100 ng/ml) for 3 days, cells were labeled with the blue fluorescent nuclear dye Hoechst (H3569, Invitrogen) or red fluorescent cell membrane dye CM-Dil (C7000, Invitrogen) and incubated for 10 min at room temperature. The two groups of cells were put together onto the plate for 2 hours, the media were removed, and fluorescence microscopy was performed. The membrane merge rate was determined using the ImageJ as described previously (16).

**In vitro assay of filopodia and F-actin ring formation**

Bone marrow cells were flushed out of the bone marrow cavity from the femora of 4-week-old mice and cultured in α-MEM with 10% FBS and M-CSF (50 ng/ml) for 3 days. The adherent cells were washed three times with PBS and seeded onto 12-well plates. After incubation with M-CSF (30 ng/ml) and RANKL (100 ng/ml) for 3 days, cells were labeled with phalloidin (HY-D1059, Invitrogen) and the LPL in the cells was detected. After incubation with M-CSF (30 ng/ml) and RANKL (100 ng/ml) for 6 days, cells were labeled with phalloidin (HY-D1059; MCE), and the F-actin rings were analyzed.

**Luciferase assay**

A LPL promoter fragment (1000 base pairs, spanning from −1000 to +0 relative to the transcription start site of NC_000080.6) was amplified by PCR and cloned into the Kpn I and Hind III sites of PHY-803 to construct the reporter vectors. The nucleotide sequences of the DNA fragments cloned in the reporter vectors were confirmed by Sanger sequencing. The resultant vectors were cotransfected with the SP1 overexpression vector into mouse B16 cells by the Neon Transfection System (Invitrogen). In the mutation group, the predicted binding sequence in promoter fragment 5′-TCACCACCCCCC-3′ was mutated into 5′-GACATTTATA-3′ to construct the reporter vectors. Twenty-four hours after transfection, cellular extracts were prepared and measured for luciferase activity using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s instructions.

**Western blotting**

The primary antibodies for Western blotting analyses were rabbit anti-P13K (1:1000; ab191606, Abcam), rabbit anti–p-P13K (1:500;
loading buffer, followed by heating at 95°C for 10 min. Western blotting analyses were performed to examine the degradation levels of LPL in each sample.

**Bone fracture model**
Bone fracture surgery was performed as described previously (44). Briefly, 12-week-old male C57BL/6 mice were anesthetized with 4% chloral hydrate. The right femur was exposed, and a needle was inserted into the bone marrow cavity of the femur parallel to its length. A single cut was made with a cutting wheel in the middle of the femur. Muscles were placed over the surgery site, and the skin was closed. Mice were intraperitoneally injected with oroxylin A (50 mg/kg) daily for 3 weeks, and then the femurs were harvested for analyses.

**Ovariectomy-induced bone loss model**
Eight-week-old C57BL/6 mice were anesthetized with 4% chloral hydrate and subjected to sham operation or bilateral ovariectomy. Mice were injected with or without oroxylin A (50 mg/kg) daily for 8 weeks. Then, the femurs were harvested for analyses. Serum was obtained after centrifugation of blood samples (3000 rpm for 10 min) and stored at −80°C for ELISA.

**Biomechanical analyses**
Biomechanical analyses were performed as described previously (44). A precision electromagnetic-based load frame (ElectroForce 3200, EnduraTEC) was used for analyses of maximum load (in newton) and stiffness (in newton per square millimeter) of the bone.

**Statistical analyses**
All data are presented as the means ± SD or SEM. Comparisons between two groups were performed using the two-tailed Student’s t test. The union rate in bone fracture models was evaluated by the Fisher’s exact test. Comparisons among multiple groups were analyzed by the one-way analysis of variance (ANOVA). The data were analyzed using the GraphPad Prism software, and P < 0.05 was taken to indicate statistical significance.

**SUPPLEMENTARY MATERIALS**
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/47/eabb7135/DC1

**REFERENCES AND NOTES**
1. H. Shimiomiya, Plastin family of actin-bundling proteins: Its functions in leukocytes, neurons, intestines, and cancer. Int. J. Cell Biol. 2012, 213492 (2012).
2. S. C. Morley, The actin-bundling protein L-plastin supports T-cell motility and activation. Immunol. Rev. 256, 48–62 (2013).
3. I. Van Audenhove, M. Denert, C. Boucherie, L. Pieters, M. Cornelissen, J. Gettemans, Fascin rigidity and L-plastin flexibility cooperate in cancer cell invadopodia and filopodia. J. Biol. Chem. 291, 9148–9160 (2016).
4. V. Delanote, B. Vanloo, M. Catillon, E. Friederich, J. Vandekerckhove, J. Gettemans, An alpaca single-domain antibody blocks filopodia formation by obstructing L-plastin-mediated F-actin bundling. FASEB J. 24, 105–118 (2010).
5. E. Seeman, Bone modeling and remodeling. Crit. Rev. Eukaryot. Gene Expr. 19, 219–233 (2009).
6. C. E. Jaumonneau, G. I. Perchin, J. T. Muller, E. Mass, T. Lazarov, J. Eitler, M. Rauner, V. K. Yadav, L. Crozet, M. Bohm, P. L. Lohyer, G. Karsenty, C. Waskow, F. Geissmann, Developmental origin, functional maintenance and genetic rescue of osteoclasts. Nature 568, 541–545 (2019).
7. H. Xie, Z. Cui, L. Wang, Z. Xia, Y. Hu, L. Xian, C. Li, L. Xie, J. Crane, M. Wan, G. Zhen, Q. Biao, B. Yu, W. Chang, T. Qiu, M. Pickarski, L. T. Duong, J. J. Windle, X. Luo, E. Xiao, C. Cao, PDSF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. Nat. Med. 20, 1270–1278 (2014).
of H. Tks5-dependent formation of L.
K.
actin-based functions of L-plastin.

on O.
Anal. Chem.
X.
The fusion process of
Hannemann, B.
Loots, J.
G.
Loots, J.

12
Sci. Transl. Med.
507
13

Onken, S.
94
235–263 (2014).

10.1126/sciadv.abb7135
Published 18 November 2020

Acknowledgments: We thank the Shanghai Model Organisms for constructing the LPL−/−
mice. The English in this manuscript has been approved by at least two professional editors, both are native speakers of English. For a certificate, please visit: www.textcheck.com/
certificate/X1gkG5fFunding: This work was supported by the National Key Research and Development Plan (2018YFC0101500), National Natural Science Foundation of China (NSF) Key Research Program in Aging (19479204), National Natural Science Foundation of China (18717419, 18710109, 81501052, 81701364, 81901426, 81703526, and 81732921), Municipal Human Resources Development Program for Outstanding Leaders in Medical Disciplines in Shanghai (2017BR011), Science and Technology Support Project in Biomedical Field of Shanghai Science and Technology Innovation Program (1843190300), Shanghai Baoshan District Science and Technology Commission Special Funds for Scientific and Technological Innovation (17E-33), Scientific Research Project of Shanghai Municipal Health and Family Planning Commission (201640156 and 201740237), Shanghai Sailing Program (19YF144700), Natural Science Foundation of Shanghai (19ZB1419600), and Shanghai Rising-Star Program (grant no. 19QJ1411500). Author contributions: Conceptualization: Xiaofei Chen, Xiao Chen, and J.S. Methodology: X.L., L.W., and J.S. Formal analysis: X.L., L.W., B.H., and J.S. Funding acquisition: J.C., Xiaofei Chen, Xiao Chen, and J.S. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data associated with this study are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 12 March 2020
Accepted 1 October 2020
Published 18 November 2020
10.1126/sciadv.abb7135

Citation: X. Li, L. Wang, B. Huang, Y. Gu, Y. Luo, X. Zhi, Y. Hu, H. Zhang, Z. Gu, J. Cui, L. Cao, J. Guo, Y. Wang, Q. Zhou, H. Jiang, C. Fang, W. Weng, X. Chen, X. Chen, J. Su. Targeting actin-bundling protein L-plastin as an anabolic therapy for bone loss. Sci. Adv. 6, eabb7135 (2020).

8. M. A. Chellaiah, T. Ma, S. Majumdar, L-plastin phosphorylation regulates the early phase of sealing ring formation by actin bundling process in mouse osteoclasts. Exp. Cell Res. 372, 73–82 (2018).

9. M. A. Chellaiah, M. C. Moorer, S. Majumdar, H. Aljohani, S. C. Morley, Y. Yingling, J. P. Stains, L-plastin deficiency produces increased trabecular bone due to attenuation of sealing ring formation and osteoclast dysfunction. Bone Res. 8, 3 (2020).

10. M. Pereira, E. Petretto, S. Gordon, J. H. J. D. Basset, G. R. Williams, J. Behmoraas, Common signalling pathways in macrophage and osteoclast multinucleation. J. Cell Sci. 131, jcs216267 (2018).

11. R. L. Song, X. Z. Liu, J. Q. Zhu, J. M. Zhang, G. Gao, H. Y. Zhao, A. Z. Sheng, Y. Yuan, J. H. Gu, H. Zou, Q. C. Wang, Z. P. Liu, New roles of filopodia and podosomes in the differentiation and fusion process of osteoclasts. Genet. Mol. Res. 13, 4776–4787 (2014).

12. T. Oikawa, M. Oyama, H. Kozuka-Hata, S. Uehara, N. Udagawa, H. Saya, K. Matsuo, Tks5-dependent formation of circumferential podosomes/invadopodia mediates cell–cell fusion. J. Cell Biol. 197, 553–568 (2011).

13. L. K. Schenk, A. Möller-Kerut, R. Klosowski, D. Wolters, E. Schaffner-Reckinger, T. Weide, H. Pavestad, B. Vollenbroeker, Actinsensit II regulates phosphorylation of actin-associated proteins in human podocytes. FASEB J. 31, 5019–5035 (2017).

14. E. Balta, R. Haridt, J. Liang, H. Kirchgessner, C. O rich, B. Jahraus, S. Hillmer, S. Meuer, K. Hübner, G. H. Wabnitz, Y. Samstag, Spatial oxidation of L-plastin downmodulates actin-based functions of macrophage lineage and L-plastin. Anal. Chem. 84, 67–74 (2012).

15. A. M. Phillips, Overview of the fracture healing cascade. Injury 36, 55–57 (2005).

16. E. C. D. M. Neto, A. Kumar, N. A. Shadick, A. M. Michon, P. Matsudaira, R. B. Eaton, P. Kumar, P. H. Schur, Antibodies to T- and L-isotypes of the cytoskeletal protein, fimbrin, in patients with systemic lupus erythematosus. J. Clin. Invest. 90, 1037–1042 (1992).

17. S. Iwamoto, N. Toyama, T. Kusumbe, S. Samstag, Spatial oxidation of cytoskeletal protein, fimbrin, by a novel target for the immunosuppressive drug desamethasone in primary human T cells. Eur. J. Immunol. 41, 3157–3169 (2011).

18. G. H. Wabnitz, F. Michealke, C. Stober, H. Kirchgessner, B. Jahraus, D. H. van den Boomen, Y. Samstag, L-plastin phosphorylation: A novel target for the immunosuppressive drug desamethasone in primary human T cells. Eur. J. Immunol. 41, 3157–3169 (2011).

19. S. Iwamoto, N. Toyama, T. Kusumbe, S. Samstag, Spatial oxidation of cytoskeletal protein, fimbrin, by a novel target for the immunosuppressive drug desamethasone in primary human T cells. Eur. J. Immunol. 41, 3157–3169 (2011).
Targeting actin-bundling protein L-plastin as an anabolic therapy for bone loss
Xiaoqun Li, Lipeng Wang, Biaotong Huang, Yanqiu Gu, Ying Luo, Xin Zhi, Yan Hu, Hao Zhang, Zhengrong Gu, Jin Cui, Liethu Cao, Jiawei Guo, Yajun Wang, Qirong Zhou, Hao Jiang, Chao Fang, Weizong Weng, Xiaofei Chen, Xiao Chen and Jiacan Su

Sci Adv 6 (47), eabb7135.
DOI: 10.1126/sciadv.eabb7135

ARTICLE TOOLS http://advances.sciencemag.org/content/6/47/eabb7135

SUPPLEMENTARY MATERIALS http://advances.sciencemag.org/content/suppl/2020/11/16/6.47.eabb7135.DC1

REFERENCES This article cites 43 articles, 9 of which you can access for free
http://advances.sciencemag.org/content/6/47/eabb7135#BIBL

PERMISSIONS http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service