Skeletal diseases caused by mutations in PTH1R show aberrant differentiation of skeletal progenitors due to dysregulation of DEPTOR

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Alterations in the balance between skeletogenesis and adipogenesis is a pathogenic feature in multiple skeletal disorders. Clinically, enhanced bone marrow adiposity in bones impairs mobility and increases fracture risk, reducing the quality of life of patients. The molecular mechanism that underlies the balance between skeletogenesis and adipogenesis is not completely understood but alterations in skeletal progenitor cells' differentiation pathway plays a key role. We recently demonstrated that parathyroid hormone (PTH)/PTH-related peptide (PTHrP) control the levels of DEPTOR, an inhibitor of the mechanistic target of rapamycin (mTOR), and that DEPTOR levels are altered in different skeletal diseases. Here, we show that mutations in the PTH receptor-1 (PTH1R) alter the differentiation of skeletal progenitors in two different skeletal genetic disorders and lead to accumulation of fat or cartilage in bones. Mechanistically, DEPTOR controls the subcellular localization of TAZ (transcriptional co-activator with a PDZ-binding domain), a transcriptional regulator that governs skeletal stem cells differentiation into either bone and fat. We show that DEPTOR regulation of TAZ localization is achieved through the control of Dishevelled2 (DVL2) phosphorylation and that it ultimately translates into the transcriptional control of PTH and WNT target genes. Depending on nutrient availability, DEPTOR directly interacts with PTH1R to regulate PTH/PTHrP signaling or it forms a complex with TAZ, to prevent its translocation to the nucleus and therefore inhibit its transcriptional activity. Our data point DEPTOR as a key molecule in skeletal progenitor differentiation; its dysregulation under pathologic conditions results in aberrant bone/fat balance.

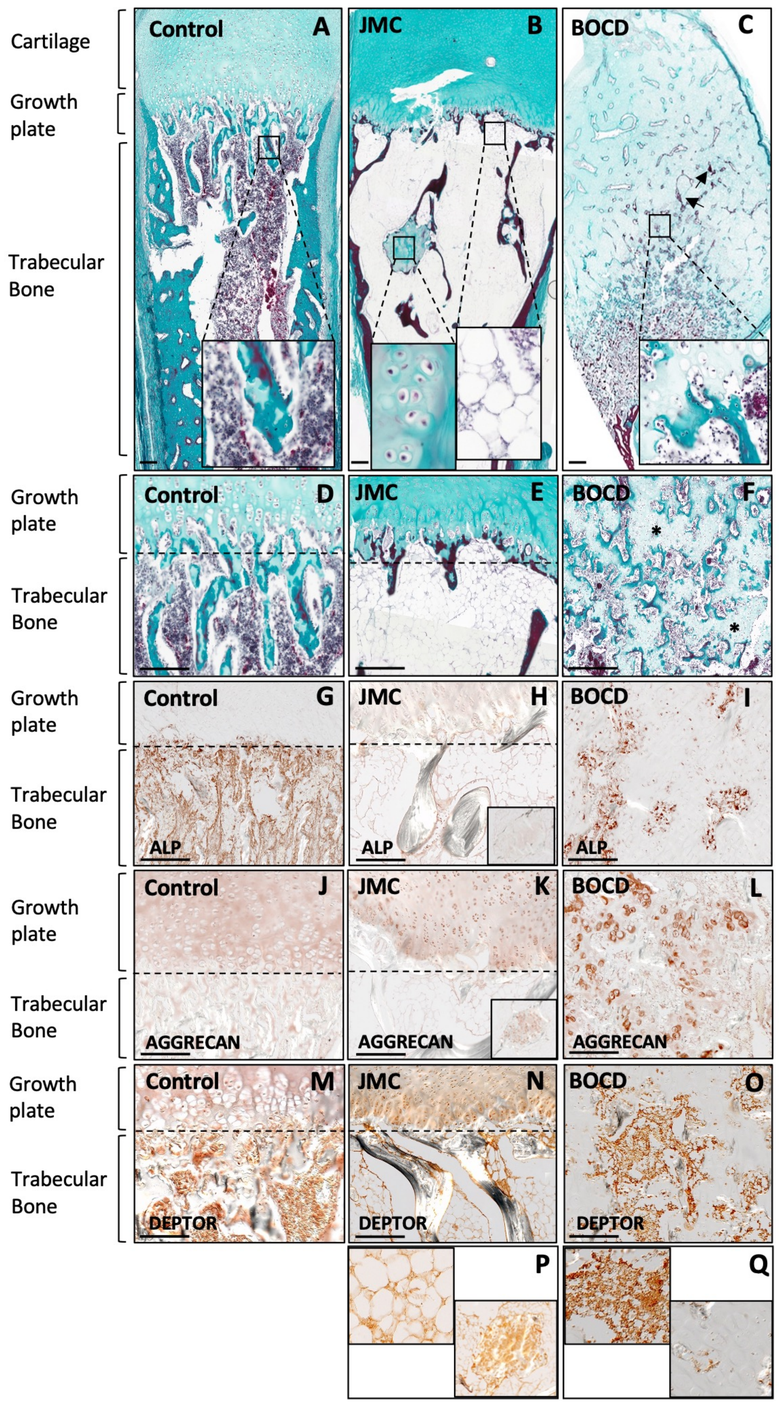

Figure 1. JMC and BOCD patients show aberrant differentiation of skeletal progenitors. (A-F) Masson Goldners staining of Control (A, D), JMC (B, E) and BOCD (C, F). Note the accumulation of fat and the small region of cartilage in the trabecular bone of JMC patients (magnification in B), and the invasion of chondrocytes in the trabecular bone of BOCD patients (magnification in C). Arrows point to calcified islands, asterisks indicate undermineralized trabecular bone. (G-I) Immunolocalization of ALP of Control (G), JMC (H) and BOCD (I). (J-L) Immunolocalization of AGGRECAN in Control (J), JMC (K) and BOCD (L). ALP staining demonstrates decreased bone mass in JMC and BOCD patients; AGGRECAN immunolocalization confirms the presence of cartilage in JMC and BOCD trabecular bone. (M-Q) Immunolocalization of DEPTOR in Control (M), JMC (N, P) and BOCD (O, Q). JMC: Jansen Methaphyseal Chondrodysplasia; BOCD: Blomstrand Chondrodysplasia; ALP: Alkaline phosphatase. Scale bars, 100 μm.

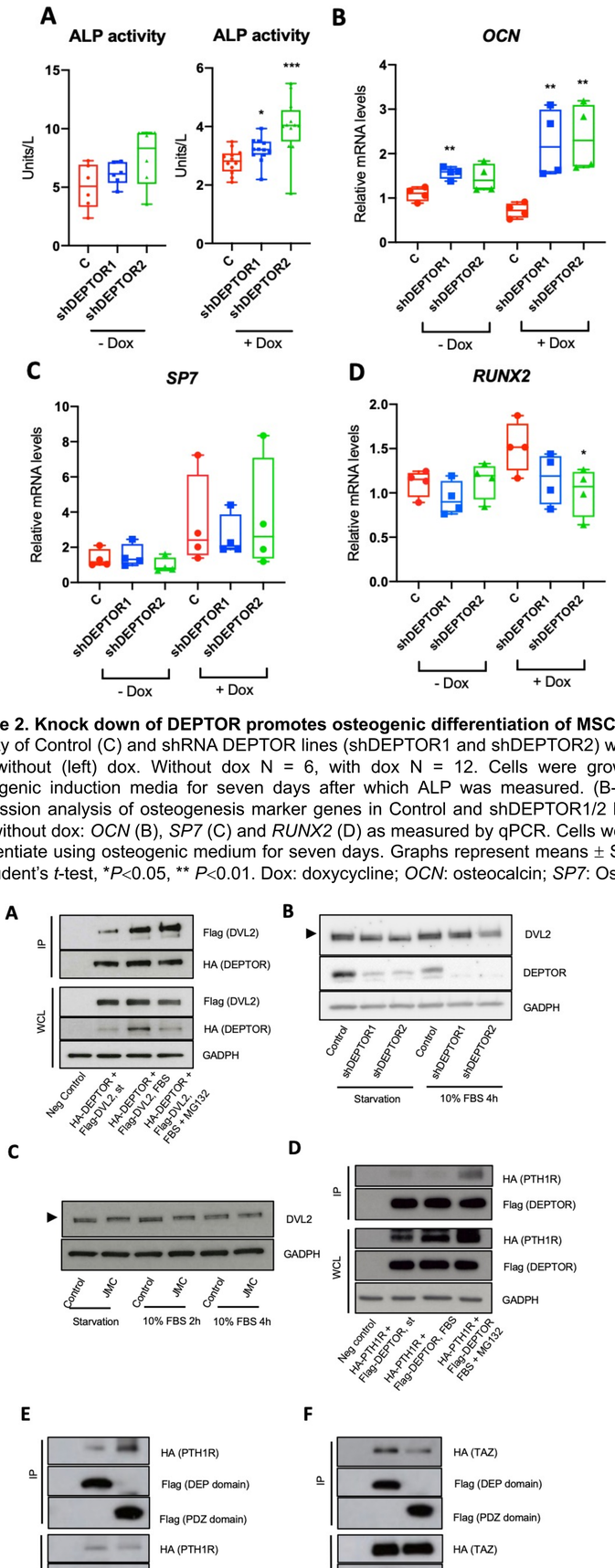

Figure 2. Knock down of DEPTOR promotes osteogenic differentiation of MSC. (A) ALP activity of Control (C) and shRNA DEPTOR lines (shDEPTOR1 and shDEPTOR2) with (right) and without (left) dox. Without dox N = 6, with dox N = 12. Cells were grown using osteogenic induction media for seven days after which ALP was measured. (B-D) Gene expression analysis of osteogenesis marker genes in Control and shDEPTOR1/2 lines with and without dox: *OCN* (B), *SP7* (C) and *RUNX2* (D) as measured by qPCR. Cells were left to differentiate using osteogenic medium for seven days. Graphs represent means ± SEM. N = 4. Student's *t*-test, **P*<0.05, ** *P*<0.01. Dox: doxycycline; *OCN*: osteocalcin; *SP7*: Osterix.

Figure 4. DEPTOR-DVL2 interaction regulates TAZ shuttling between nucleus and cytoplasm. (A) Co-IP of DEPTOR and DVL2. N-terminal HA tagged DEPTOR and N-terminal Flag tagged DVL2 were cotransfected into HEK293T cells. After transfection cells were starved O/N and then treated with 10% FBS alone or in combination with MG132 for four hours. DEPTOR was pulled down using anti-HA magnetic beads and the interaction with DVL2 was detected by western blot using an anti-Flag antibody. (B) Western blots of Control and shDEPTOR1/2 lines. Cells were starved O/N and then treated with 10% FBS for four hours. (C) Western blot of DVL2 in Control and JMC fibroblasts. Cells were starved O/N and then treated with 10% FBS for two and four hours. (D) Co-IP of DEPTOR and PTH1R. N-terminal HA tagged PTH1R and N-terminal Flag tagged DEPTOR were cotransfected into HEK293T cells. After transfection cells were starved O/N and then treated with 10% FBS alone or in combination with MG132 for four hours. DEPTOR was pulled down using anti-Flag magnetic beads and the interaction with PTH1R was detected by western blot using an anti-HA antibody. (E-F) Co-IP of PTH1R or TAZ and DEPTOR DEP and PDZ domains. (E) N-terminal HA tagged PTH1R and N-terminal Flag tagged DEP or PDZ domains were cotransfected into HEK293T cells. After transfection cells were starved O/N and then treated with 10% FBS for four hours. DEP and PDZ domains were pulled down using anti-Flag magnetic beads and the interaction with PTH1R was detected by western blot using an anti-HA antibody. (F) N-terminal HA tagged TAZ and N-terminal Flag tagged DEP or PDZ domains were cotransfected into HEK293T cells. After transfection cells were starved O/N. DEP and PDZ domains were pulled down using anti-Flag magnetic beads and the interaction with TAZ was detected by western blot using an anti-HA antibody.

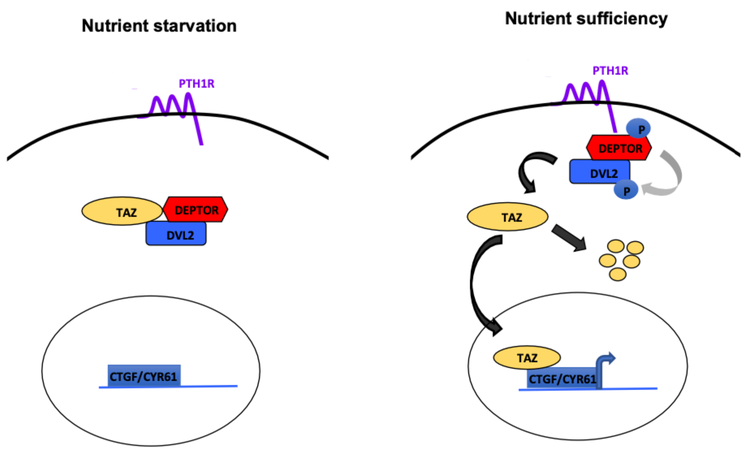

Figure 5. Schematic representation of the mode of action of DEPTOR over TAZ, DVL2 and PTH1R. Under starvation, DEPTOR forms a complex with DVL2 that retains TAZ in the cytoplasm, preventing its transcriptional activity. In the presence of serum, DEPTOR-DVL2 release TAZ and interact with PTH1R; TAZ is translocated to the nucleus to control the expression of its target genes.

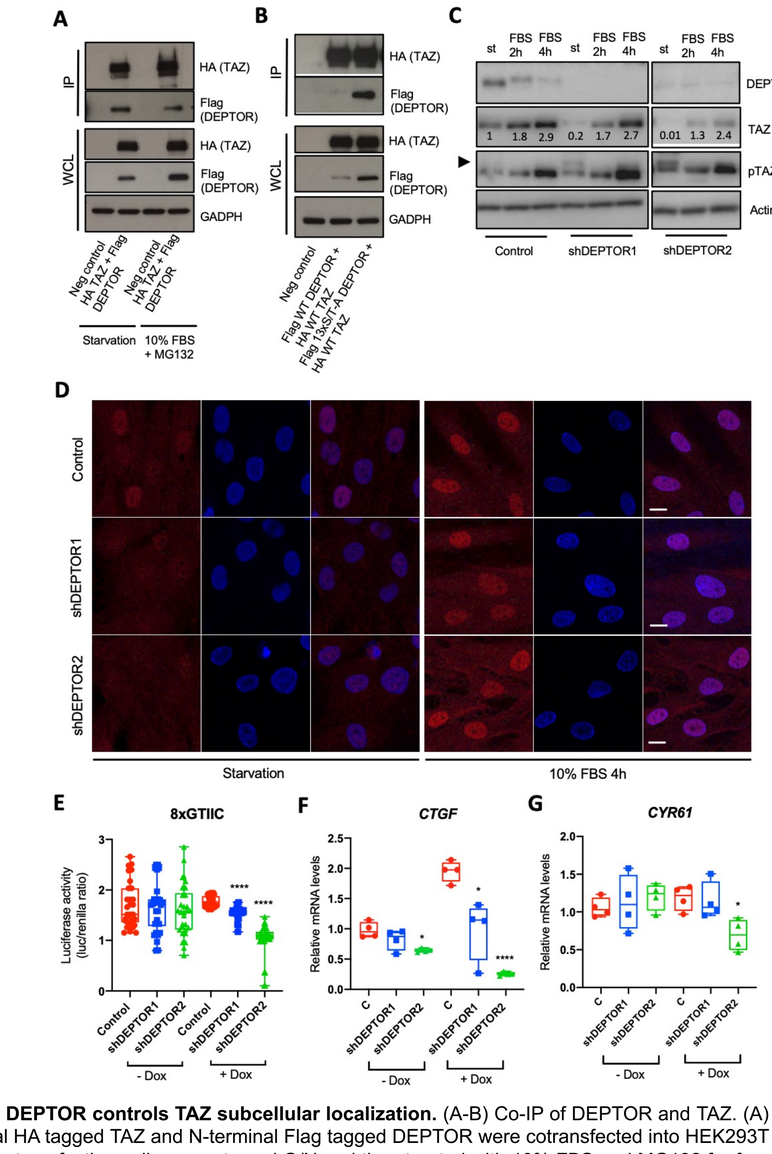

Figure 3. DEPTOR controls TAZ subcellular localization. (A-B) Co-IP of DEPTOR and TAZ. (A) N-terminal HA tagged TAZ and N-terminal Flag tagged DEPTOR were cotransfected into HEK293T cells. After transfection cells were starved O/N and then treated with 10% FBS and MG132 for four hours. TAZ was pulled down using anti-HA magnetic beads and the interaction with DEPTOR was detected by western blot using an anti-Flag antibody. (B) N-terminal HA tagged TAZ and N-terminal Flag tagged 13xS/T-A DEPTOR were cotransfected into HEK293T cells. After transfection cells were starved O/N. IP was performed as in (A). (C) Western blots of Control and shDEPTOR1/2 lines. Cells were starved O/N and then treated with 10% FBS for two and four hours. Numbers indicate quantification of bands using Fiji. (D) Immunofluorescence of TAZ in Control and shDEPTOR1/2 lines under starvation and after four hours 10% FBS treatment. (E) Luciferase reporter assay of YAP/TAZ reporter (8xGTIIC) in Control and shDEPTOR1/2 lines under starvation. Graphs represent means ± SEM. N = 30. Student's *t*-test, *****P*<0.0001. (F-G) Gene expression analysis of YAP/TAZ target genes CTGF (F) and CYR61 (G) under starvation, with and without dox as measured by qPCR. Graphs represent means ± SEM. N = 4. Student's *t*-test, **P*<0.05, *****P*<0.0001. Dox: doxycycline. Scale bars: 15 μm.

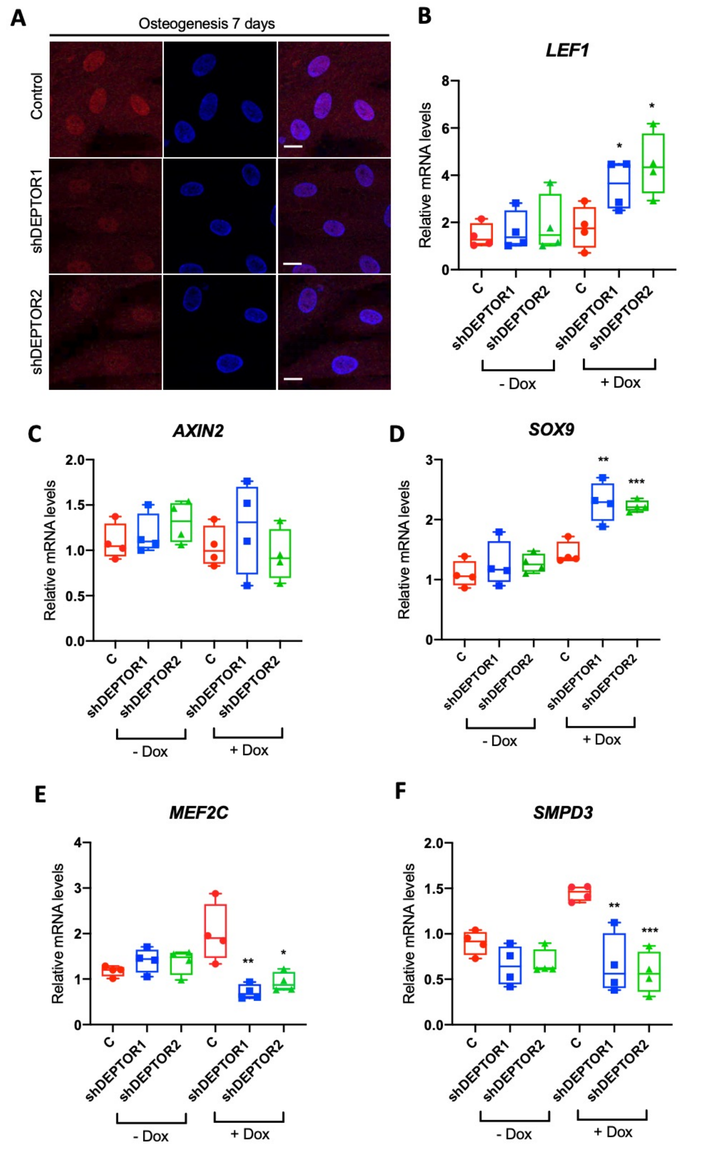

Figure 6. DEPTOR regulates WNT and PTH signaling pathways. (A) Immunofluorescence of TAZ in Control and shDEPTOR1/2 lines after seven days of osteogenic differentiation. (B-F) Gene expression analysis of b- catenin target genes *LEF1* (B), *AXIN2* (C) and *SOX9* (D) after seven days of osteogenic differentiation with and without dox as measured by qPCR. (E- F) Gene expression analysis of the PTH-regulated genes *MEF2C* (E) and *SMPD3* (F). Graphs represent means ± SEM. N = 4. Student's *t*-test, **P*<0.05, ***P*<0.01, *** *P*<0.001. Scale bars: 15 μm.

Conclusions

1. Mutations in PTH1R compromise skeletal stem cells differentiation resulting in the dysregulation of the balance between bone and fat formation
2. DEPTOR interacts with TAZ through DVL2 and controls its subcellular localization under different environmental conditions
3. DEPTOR regulation of TAZ ultimately results in changes in the expression of PTH and WNT target genes
4. DEPTOR dysregulation under several pathological conditions results in aberrant bone/fat balance

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