Phlpp1, But Not Phlpp2, Regulates Endochondral Ossification

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INTRODUCTION: Endochondral ossification is a complex, dynamic process controlled by signaling factors, hormones, and mechanical forces. The proliferation and hypertrophy of chondrocytes in the epiphyseal growth plate drives appendicular growth. Numerous intracellular and extracellular factors regulate chondrocyte proliferation, maturation, and terminal differentiation, and thereby bone lengthening. Pleckstrin homology (PH) domain and leucine rich repeat phosphatases 1 and 2 (Phlpp1 and Phlpp2) control cell proliferation and survival through posttranslational modification. Phlpp1 has been the focus of numerous developmental and disease processes in the musculoskeletal system, and controls chondrocyte proliferation and expansion of the postnatal growth plate. Phlpp1 and Phlpp2 are highly homologous, but here is a paucity of research on the role of Phlpp2 in musculoskeletal development.

METHODS: All animal research was performed according to the NIH and the Institute of Laboratory Animal Resources, National Research Council guidelines, and the Mayo Clinic Institutional Animal Care and Use Committee. Male wildtype (WT), Phlpp1$^{-/-}$, Phlpp2$^{-/-}$, and Phlpp1$^{-/-}$;Phlpp2$^{-/-}$ (P1/P2$^{-/-}$) mice were aged to two weeks. Radiographs were performed and tibia length was determined. Tibiae were then decalcified, dehydrated, and embedded in paraffin for Safranin O / Fast Green staining. Mice were also aged to 5 days old, then primary chondrocytes were isolated and submitted for bulk RNA-Sequencing, and phospho- and total proteomic analysis.

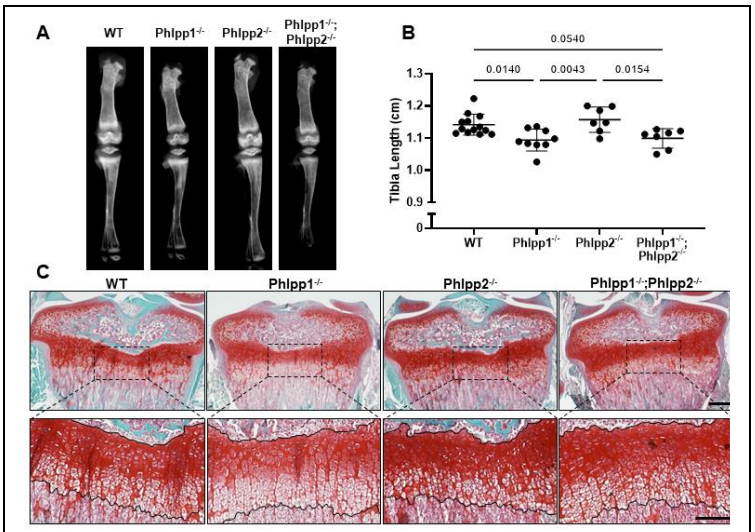

Figure 1. Phlpp2 deletion does not alter limb length. (**A**) Radiographs of WT, Phlpp1$^{-/-}$, Phlpp2$^{-/-}$, and P1/P2$^{-/-}$ mice reveal that (**B**) Phlpp1$^{-/-}$ and P1/P2$^{-/-}$, but not Phlpp2$^{-/-}$ mice, have shorter tibiae. (**C**) Phlpp1$^{-/-}$ and P1/P2$^{-/-}$ mice also have expanded tibial epiphyseal growth plates.

RESULTS SECTION: Male Phlpp1$^{-/-}$ and P1/P2$^{-/-}$ mice had shorter tibiae compared to WT littermates at 2 weeks of age. By contrast, Phlpp2$^{-/-}$ had equivalent limb length to WTs. Phlpp1$^{-/-}$ and P1/P2$^{-/-}$ animals also had expanded tibial epiphyseal growth plates (**Fig 1**). Bulk RNA-Seq revealed only modest changes in the transcriptome of Phlpp1$^{-/-}$, Phlpp2$^{-/-}$, and P1/P2$^{-/-}$ chondrocytes. The total transcriptome and phospho-proteome of Phlpp1$^{-/-}$ chondrocytes was similar to P1/P2$^{-/-}$ chondrocytes, while WT and Phlpp2$^{-/-}$ chondrocytes were more similar to each other. Phlpp1$^{-/-}$ chondrocytes were the most differentially phosphorylated. Upregulated pathways in the Phlpp1$^{-/-}$ chondrocyte transcriptome include positive regulation of transcription and cell proliferation (**Fig 2**).

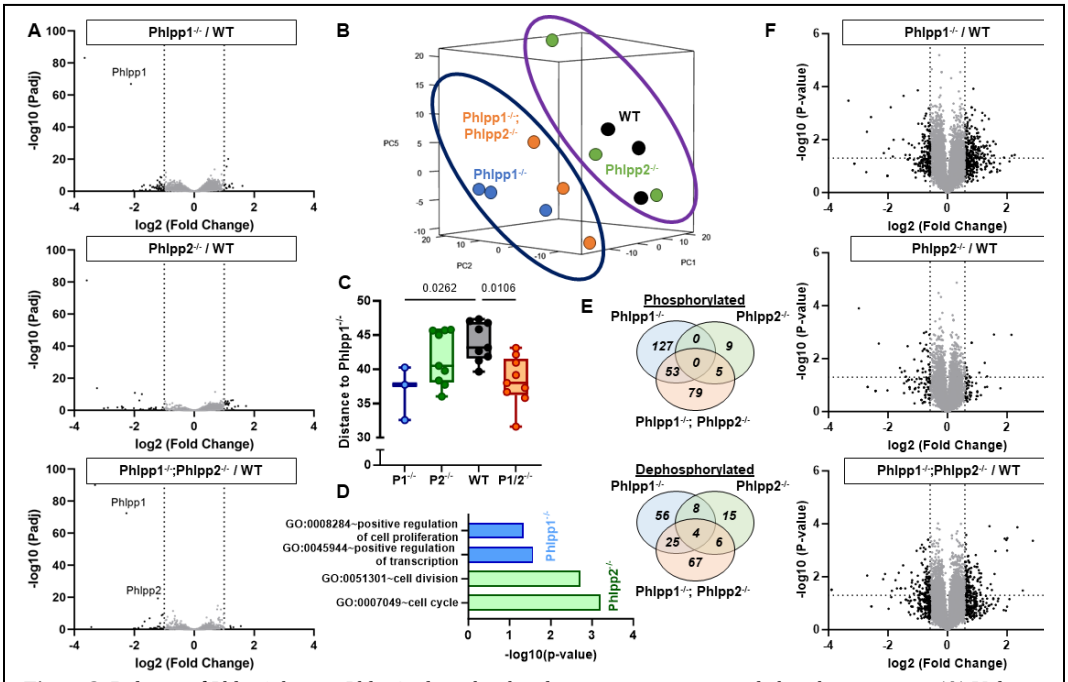

Figure 2. Deletion of Phlpp1, but not Phlpp2, alters the chondrocyte transcriptome and phospho-proteome. (**A**) Volcano plots demonstrate the modest differential expression of Phlpp2$^{-/-}$ compared to WT chondrocytes compared to Phlpp1$^{-/-}$ and P1/P2$^{-/-}$. (**B**) PCA plots and (**C**) distance analysis show similar clustering of Phlpp1$^{-/-}$ and P1/P2$^{-/-}$ chondrocytes. (**D**) GO-term analysis of upregulated differentially expressed genes include positive regulation of proliferation in Phlpp1$^{-/-}$ and genes involved in cell cycle in Phlpp2$^{-/-}$ chondrocytes. (**E**) Phospho-proteomics reveals greater phosphorylation and (**F**) similarity of volcano plots between Phlpp1$^{-/-}$ and P1/P2$^{-/-}$ chondrocytes.

DISCUSSION: Understanding the processes driving endochondral ossification is critical to develop regenerative therapies and treat developmental diseases. These data demonstrate that Phlpp2 does not regulate endochondral ossification. In addition to similar limb length, the transcriptome, proteome, and phospho-proteome of Phlpp2$^{-/-}$ chondrocytes is comparable to WT chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Phlpp1, but not Phlpp2, is a relevant therapeutic target for endochondral ossification.