# GIRK3 Deletion Enhances Osteoblast Mineralization via Ifi202b

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INTRODUCTION: Bone mineral accrual depends on the coordinated differentiation of bone marrow stromal cells (BMSCs) into osteoblasts and subsequent mineralization. During aging, bone formation is outpaced by resorption, causing net bone loss and fragility. Our lab has previously identified G protein-gated inwardly rectifying $K^+$ channel 3 (GIRK3) as a negative regulator of bone mineral accrual in the adult murine skeleton, as whole-body and osteoblast-directed deletion of Girk3 increase bone mass and strength only after 18 weeks of age. High bone mass may be due, in part, to the fact BMSCs from Girk3$^{-/-}$ mice are more proliferative than littermate WT BMSCs. In this study, we sought to identify the molecular mechanisms underlying enhanced BMSC proliferation and mineralization in Girk3$^{-/-}$ mice.

METHODS: All animal research was performed in accordance with the NIH and the Institute of Laboratory Animal Resources, National Research Council guidelines, and approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. Bulk RNA-Sequencing was performed on calvaria of female 2- to 3-day-old WT and Girk3-/- mice and fresh flushed tibiae of male 24-week-old WT and Girk3-/- mice. Ifi202b expression was validated using qRT-PCR. BMSCs were then isolated from 4-week-old WT and Girk3$^{-/-}$ male and female mice and transfected with Ifi202b siRNA or control (pooled) siRNA. MTS assays were performed on proliferating cells two days after transfection, prior to addition of osteogenic medium. BMSCs were then cultured in osteogenic medium after siRNA transfection. Osteogenic differentiation was evaluated after 13 days using alizarin red. *In vitro* experiments were conducted a total of three times, with representative results shown.

RESULTS: Ifi202b was highly upregulated in calvaria of 2- to 3-day-old female Girk3$^{-/-}$ mice (**Fig 1A**), with nearly undetectable levels in WT tissues as indicated by normalized counts from RNA-Seq (**Fig 1B**). qRT-PCR confirmed elevated Ifi202b transcripts in neonatal Girk3$^{-/-}$ calvaria (**Fig 1C**). At 24 weeks of age, when high bone mass is evident in Girk3$^{-/-}$ compared to WT mice, Ifi202b expression was also increased in the flushed tibiae of male Girk3$^{-/-}$ mice (**Fig 1D-F**). These data suggested that elevated osteogenesis in the Girk3$^{-/-}$ skeleton may be related to expression of Ifi202b. To test this hypothesis, we transfected BMSCs from 4-week-old male WT and Girk3$^{-/-}$ mice with siRNA targeted to Ifi202b or control (nontargeting) siRNA. Ifi202b knockdown attenuated enhanced proliferation of Girk3$^{-/-}$ BMSCs (**Fig 1G**). As previously described, BMSCs from Girk3$^{-/-}$ mice also had elevated osteogenesis compared to WT BMSCs. siRNA-mediated knockdown of Ifi202b attenuated the rapid mineralization of Girk3$^{-/-}$ cells (**Fig 1H, I**).

DISCUSSION: Ifi202b is the top upregulated gene in two different RNA-sequencing experiments of Girk3$^{-/-}$ skeletal tissues. Ifi202b is minimally expressed in WT calvaria and tibial bone but is markedly elevated in Girk3$^{-/-}$ bone. Functional knockdown of Ifi202b reversed the enhanced proliferation and mineral deposition of Girk3$^{-/-}$ BMSCs *in vitro*. These data demonstrate that Ifi202b is a critical driver of enhanced mineralization in Girk3$^{-/-}$ mice and suggest that Ifi202b may be a novel regulator of osteoblast function and bone accrual.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings identify Ifi202b as a novel mediator of osteoblast proliferation and mineralization, linking interferon inducible signaling to skeletal homeostasis. Poor bone formation contributes to low bone mass and fragility. Targeting Ifi202b or its downstream pathways could provide a therapeutic strategy to enhance osteogenesis.

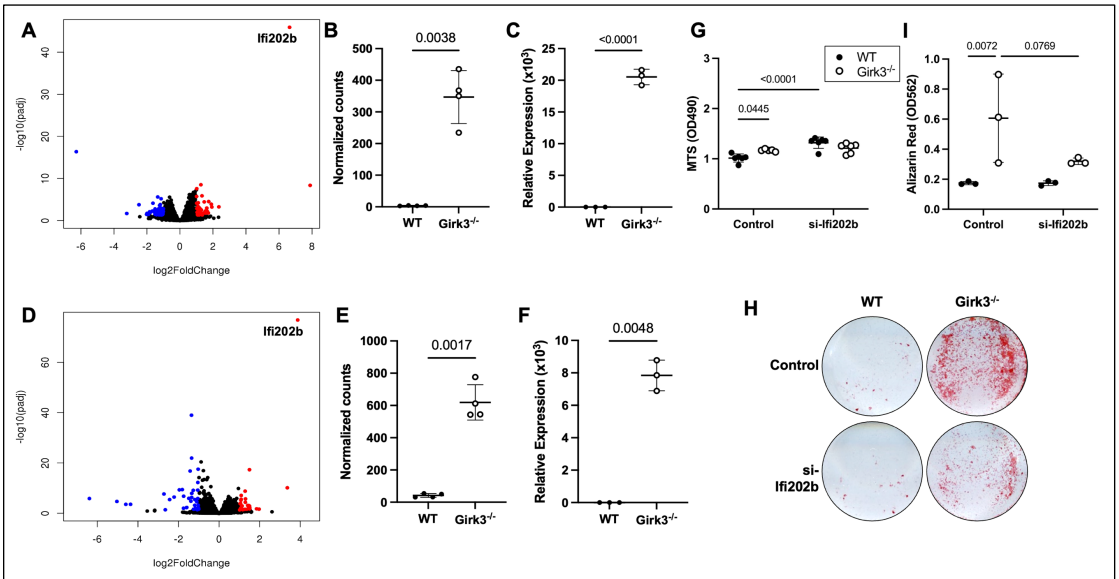


**Figure 1. Girk3 deletion increases expression of Ifi202b in skeletal tissues across the lifespan.** Bulk RNA-Sequencing was performed on calvaria of 2- to 3-day-old female WT and Girk3$^{-/-}$ mice (**A**) and normalized counts of Ifi202b were identified (**B**). Ifi202b expression was then validated in calvaria using qRT-PCR (**C**). Bulk RNA-Seq was also performed on the flushed tibiae of 24-week-old male WT and Girk3$^{-/-}$ mice (**D**), and Ifi202b counts were normalized (**E**). qRT-PCR (**F**) confirmed elevated Ifi202b expression in 24-week-old Girk3$^{-/-}$ BMSCs. MTS assays were then performed on BMSCs from 4-week-old male WT and Girk3$^{-/-}$ mice following siRNA transfection (**G**). siRNA-transfected BMSCs were also cultured in osteogenic medium for 13 days. Alizarin red staining was performed (**H**) and quantified (**I**). Statistics were performed using a two-way ANOVA with Tukey's post-hoc tests or Students *t*-test. Data are shown as mean ± standard deviation.