Innervation by the sensory nervous system plays a key role in skeletal development and orchestration of bone remodeling and regeneration. However, it is unclear how and in which bone cells sensory nerves act to control these processes. Here, we present a microfluidic coculture system comprising dorsal root ganglion (DRG) neurons and mesenchymal stem cells (MSCs), which more faithfully represents the in vivo scenario of bone sensory innervation. We report that DRG neurons promote the osteogenic differentiation capacity of MSCs, by mediating an increase in alkaline phosphatase activity and upregulation of osteoblast-specific genes. Furthermore, we show that DRG neurons have a positively impact on Cx43 levels in MSCs during osteoblastogenesis, especially at an early stage of this process. Conversely, we described a negative impact of DRG neurons on MSCs-N-cadherin expression in MSCs at a later stage of the process. Finally, we demonstrate the cytoplasmic accumulation and translocation of β-catenin into the nucleus, and the subsequently Lynhoid Enhancer Binding Factor 1—responsive transcriptional activation of downstream genes in cocultured MSCs. Together, our study provides strong evidence that the direct interaction of DRG neurons with MSCs in a bone-like microenvironment leads to an enhancement of osteoblast differentiation potential of MSCs. The osteogenic effect of DRG neurons on MSCs is mediated through the regulation of Cx43 and N-cadherin expression and activation of the canonical/β-catenin Wnt signaling pathway.

Comment [A1]: Here, the sentence has been revised to clearly indicate that accumulation and translocation of β-catenin are being referred to.

Comment [A2]: A compound modifier contains 2 or more words, which act together as one adjective and are connected by hyphens. Hyphens are used with these terms so that their meaning is understood clearly.

Comment [A3]: The text alongside has been revised to lowercase as this is not a proper noun.