Chondrocytes within the growth plate (GP) are organized into anatomically well-defined horizontal zones, which reflect their distinct differentiation stages. Proliferative zone (PZ) chondrocytes acquire an elongated shape, align their mitotic spindle along the mediolateral (ML) axis of the bone, divide orthogonally to the direction of the growth and arrange into vertical columns directing the elongation process. Recently, members of the β1 integrin family have emerged as important orienting factors of the mitotic apparatus in epithelial tissues, but their role in cartilaginous growth plate in the long bones is unknown. Here, we show that β1 integrin function is pivotal for flattening and ML orientation of proliferative chondrocytes in the GP, which in turn dictates spindle alignment along the long axis of the cell.

Hind limbs from wt, β1fl/fl-Col2a1cre, β1fl/fl-Prx1cre and Col2a1-null mice at different time points were analyzed by IHC for polarity markers, β1 integrin and cytoskeletal components. Rib chondrocytes were isolated and stained with anti-α-tubulin and anti-pericentrin antibodies. The long and the short axes of chondrocytes, as well as the angle between the spindle axis and the proximodistal (PD) axis of the cartilage were measured. Cell orientation and shape indexes were determined. Atomic Force Microscopy (AFM) imaging and indentation tests were also performed.

During GP morphogenesis, aggregating MSCs were polygonal and non-oriented. Upon chondrogenic differentiation, wt GP chondrocytes exhibited moderate flattening and tend to align perpendicular to the PD axis. At E13, PZ chondrocytes further flattened and largely organized into lines along the ML axis. At E14, the first vertical stacks formed. Early spindles were randomly oriented at all stages, whereas late spindles were aligned along the long axis of the cell. In contrast, β1-deficient PZ chondrocytes were rounded, showed random orientation of both early and late spindles, and failed to organize into columns. On collagen II, both wt and β1-null primary chondrocytes remained round and displayed random spindle orientation relative to the substrate plane (SP). On fibronectin, wt cells were flattened and their spindle was aligned parallel to SP, while mutant cells did not spread, were rounded and aligned their spindle randomly. AFM revealed softening of the cartilage in β1- and Col2-deficient mice. Interestingly, PZ chondrocytes were able to flatten and orient properly in Col2a1-null mice, but they never form columns.

These data indicate that 1) β1 integrin-mediated matrix anchorage guides chondrocyte shape and polarity in the PZ; and 2) matrix stiffness modulates columnar arrangement.