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THE ROLE OF α-ACTININ IN OSTEOBLAST SURVIVAL SIGNALING AND MECHANOTRANSDUCTION

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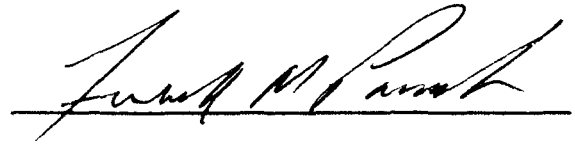

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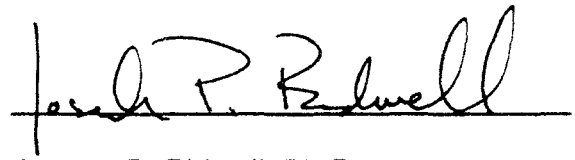

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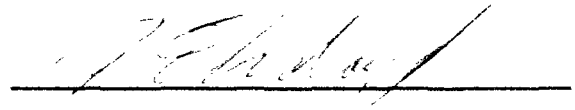

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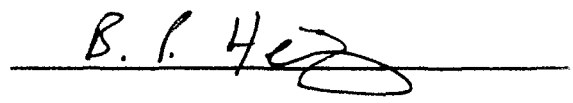

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ABSTRACT

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THE ROLE OF α-ACTININ IN OSTEOBLAST SURVIVAL SIGNALING AND MECHANOTRANSDUCTION

Advances in cell culture and molecular biology techniques have allowed for effective ways to understand the cellular processes that underlie physiological phenomena. In this work, the use of the topoisomerase inhibitor etoposide was explored as a means to increase adenoviral infection efficiency. Using this technology, a truncated form of α-actinin, an actin bundling protein localized to focal adhesions, was expressed. Expression of this protein, termed ROD-GFP, disrupted focal adhesion organization and signaling capabilities without causing a complete collapse of the actin cytoskeleton. This represents a novel and effective way to assess the role of focal adhesions in cellular processes separate from dramatic changes in cell shape or architecture. Cells expressing ROD-GFP were more sensitive to apoptotic stimuli, due to decreased expression of anti-apoptotic proteins and inhibited survival signaling in response to tumor necrosis factor-α. Additionally, varying effects were observed on the ability of cells to transduce mechanical signals when expressing ROD-GFP. Specifically, p38 and extracellular signal-related kinase phosphorylation in response to fluid shear stress were inhibited in cells expressing ROD-GFP. However, many other mechanical signaling pathways were unaffected by ROD-GFP-induced focal

adhesion disruption. In summary, this works presents an improved method to introduce a novel protein into cultured osteoblasts, which has allowed for a molecular and biochemical analysis of the role of α-actinin in many cellular processes important in osteoblast physiology.

Fredrick M. Pavalko, Ph.D. - Chair

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