Actin Regulators, Cell-Matrix Adhesions, and Cellular Morphodynamics in 3D.

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The actin cytoskeleton is a dynamic entity that integrates intra- and extracellular cues, generates forces that are transmitted to the extracellular matrix (ECM), and defines the cellular morphodynamic output. However, to date, our understanding of the complex interplay between actin regulators, and how they alter cell-matrix adhesions and morphodynamics, is based on cells imaged in a mechanically, dimensionally, and biochemically artefactual environment (e.g., coverslips). To overcome these limitations, we developed a comprehensive experimental approach that combines custom high-resolution light-sheet microscopes [1-5] and computer vision to quantitatively evaluate single cells in reconstituted ECM environments. More specifically, we systematically knocked-out a diverse set of actin regulators using CRISPR-Cas9, and evaluated the effect of each on cell spreading, cellular branching, and adhesion size and dynamics for cells embedded in stroma-like ECM environments. Early results suggest that cells require a delicate balance between actin polymerization and depolymerization to generate traction forces and properly spread, and that these effects are more evident when cells are placed in mechanically soft 3D ECM environments. These results are explained by the altered cell-matrix adhesions, disrupted integrin-actin coupling, and the imbalance between the protrusive capacity of the underlying actin network and Myosin II contractility (potentially inflicted by altered matrix geometry surrounding the cells). Together these results emphasize the importance of the cellular environment, and suggest that subtle phenotypes may have been overlooked throughout the past several decades due to inappropriate contextual ECM cues.

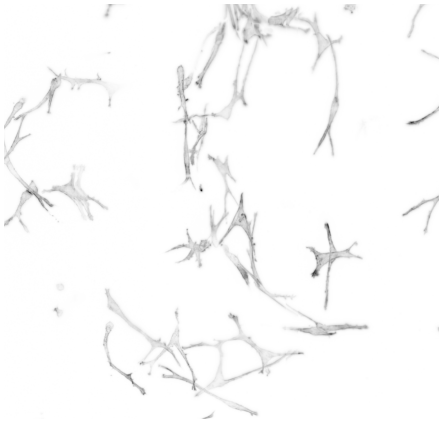

Figure 1: CAPZB knockout cells in 3D collagen environment.

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