MEASURING CELLULAR MECHANICS USING IMAGE ANALYSIS TECHNIQUES TO PROVIDE EVIDENCE FOR A MOLECULAR PATHWAY INVOLVING OBSCURIN. Stephanie N. Ouderkirk1, Kamrin D. Shultz1, Nathan T. Wright1, & Callie J. Miller2, 1James Madison University and 2Merck & Co. Inc. Obscurin activates ROCK via the RhoA pathway leading to an increase in actomyosin contractility and a decrease in actin filament polymerization, ultimately modulating cellular motility. The purpose of this study is to correlate changes in cellular area and motility, considering the presence of Obscurin and the introduction of various drugs affecting the RhoA pathway. This aims to improve our understanding of Obscurin’s potential role as a mechanosensing protein. Cellular area and motility are quantified using a robust, partially automated image analysis method. Utilizing this recently developed technique, we observed a decrease in cellular motility in the presence of Obscurin consistent with previous findings conducted in a different cell line. Additionally, we found that disruption of the actin cytoskeleton normally flattens cells, but the presence of Obscurin rescues the wild-type phenotype. Furthermore, we introduced a ROCK inhibitor known to increase cellular motility to impede the Obscurin RhoA pathway. However, we observed a decrease in motility in the presence of Obscurin consistent with our observations in Obscurin cells without the inhibitor. These findings suggest that Obscurin engages in multiple signaling pathways that influence cellular structure and motility beyond the RhoA pathway. Author contact: [ouderksn@dukes.jmu.edu](mailto:ouderksn@dukes.jmu.edu)