Introduction: Cancer remains the second leading cause of death worldwide. The research that drove most current cancer therapies focused primarily on the cancer cells themselves, when in reality a tumor includes a host of stromal cells which are constantly communicating with the local microenvironment. Through defining features such as hypoxia and stiffness, the tumor microenvironment (TME) can cause changes in the molecular signaling of a cell that result in tumorigenic phenotypes. This study aims to elucidate one such deleterious effect of the TME, specifically how the mechanical rigidity of the underlying matrix can regulate chromosomal instability in the form of tetraploidy. Tumors are inherently stiffer than normal tissue, and this feature has been shown to cause changes in cell growth and proliferation. Similarly, cell cycle errors have long been linked to chromosomal abnormalities, a phenotype common to 85% of solid cancers. The mechanisms by which tissue compliance regulates chromosomal instability remain unclear, but may provide an avenue for new cancer treatments. Here, we investigated the effects of substrata stiffness on tetraploidy in mammary epithelial cells.

Materials and Methods: Mammary epithelial cells were plated on polyacrylamide gels of varying compliance (Young's modulus of 130 Pa and 4020 Pa to mimic normal and tumor tissue, respectively) conjugated with fibronectin, an extracellular matrix protein that facilitates cell attachment to the substratum. Using epifluorescent microscopy, tetraploidy was easily observed as multinucleation. The signaling pathway controlling mechanical regulation of multinucleation was also investigated.

Results and Discussion: Mammary epithelial cells cultured on "stiff" substrata—representing tumor tissue—showed a nearly 14-fold increase in multinucleation compared to cells cultured on "soft" substrata, representing normal tissue. We found that multinucleation was regulated in part by signaling downstream of matrix metalloproteinase-3 (MMP-3), a protease responsible for remodeling of the extracellular matrix, and commonly found upregulated in cancer. This signaling depended on expression of the Rac1 splice variant, Rac1b, production of reactive oxygen species (ROS), and expression of Snail. Under all conditions, cells cultured on soft substrata maintained a low frequency of multinucleation.

Conclusions: Mouse mammary epithelial cells show increased levels of multinucleation with increasing substrata stiffness. This trend suggests that abnormal mechanical characteristics of the tumor microenvironment promote genomic instability in cancer. One responsible mechanism is induced by MMP-3; regulation of each pathway component shows a direct relationship to multinucleation. This pathway is inhibited on soft substrata, where multinucleation is low regardless of treatment conditions. Given this evidence, a drug that promotes the normalization of the microenvironment may be promising.