Abstract
Endometriosis can cause pelvic pain and infertility in women arising from aberrant growth of endometrial-like lesions in the pelvic cavity. Here we studied the expression of gap junction (GJ) genes, which are implicated in cell invasiveness in benign and malignant conditions. Single-cell analysis revealed that primary endometrial stromal cells expressed lower GJ expression in endometriosis samples with loss of heterogeneity in advanced disease.

Introduction
Endometriosis is a disease that is commonly misdiagnosed or left untreated. Endometriosis is a highly invasive disease leading to growth of endometrial-like benign lesions in the pelvic cavity, which in some cases have been attributed to be precursors to some ovarian cancer subtypes. Whether the invasive process in endometriosis is mediated by intercellular communication through gap junction channels is unclear. Gap junctions serve as channels that allow for the import and export of metabolites between adjacent cells. To begin to understand the role of gap junctions in cell-cell communication in endometriosis, we examined the expression of gap junction genes in endometrial biopsies from women with and without endometriosis at the single cell level.

Materials and Methods

Materials:
- C1 Single Cell Auto Prep System (Fluidigm) – BASiC Core
- BioMark Microfluidic PCR (Fluidigm) – BASiC Core
- Primary Endometrial stromal cells

Methods:
To begin, we obtained human endometrial samples from two patients with endometriosis and one subject who did not have the disease. Primary endometrial stromal cells were obtained and expanded in culture. The cells were prepared for loading into the C1 IFC chip (Figure below). Single cells were separated in the C1, and microscope images were then taken to verify single cell capture (image below). Corresponding single-cell cDNA was synthesized and preamplified in the C1. Once completed, the amplicons were harvested underneath a post-PCR fume hood using a multi-channel pipette onto a harvesting plate. The amplicons were then analyzed by microfluidic PCR in the Biomark HD system.

Results
Loss of gap junction (GJ) gene expression was observed in endometriosis patient samples compared to control (heat maps below; n=60 single cells each). There was a further decrease in expression and cellular heterogeneity in stage 3/4 compared to Stage 1/2 endometriosis (heat maps below). This loss of heterogeneity is reflected in the subgroup of cells in the upper right corner of the heat maps showing high GJ expression in control and lower expression in endometriosis.

Conclusion
The loss of gap junction expression and heterogeneity may be an indicator as to why endometriosis lesions are able to proliferate outside the uterus (please see poster presentation by Taryn Olivas et al). According to previous research, the loss of gap junctions is correlated to other types of disease (Norstrand, D., et al). This loss of heterogeneity may affect cell-cell communication during the development of endometriosis.

Acknowledgements
-Bianca Gonzalez is a summer student funded by the U54 grant (U54 CA217297). * Fe Maria Pena contributed to the work equally
-C1 and Biomark analysis was performed in the BASiC Core, UT-Health SA

References