

Figure 1: **A** shows dose sensitivity to migration distance (plotted as area ratio) for two different drugs, Static and LLL-12, using multiple glioma cell lines and validated using Western Blots (**B**). In contrast, figure **C** shows no significant effect on migration for glioma cells on traditional, flat polystyrene using scratch assays. This demonstrates the artificial response of cells on flat 2-D surfaces and a more realistic response on a 3-D nanofiber substrate.

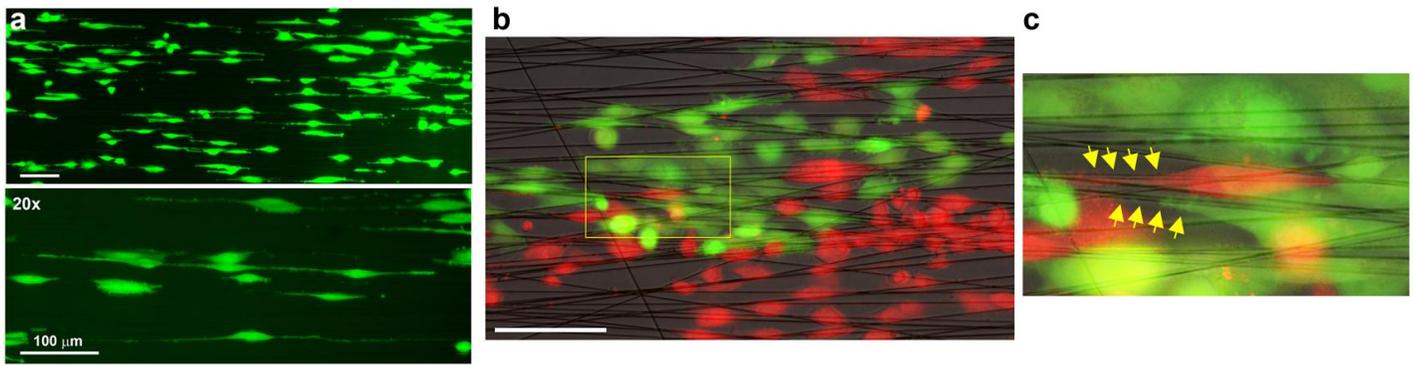


Figure 2: Nanofiber scaffolds can be used to co-culture tumor cells. A) Endothelial cells derived from a pediatric high-grade glioma on aligned nanofibers and labeled with calcein-AM. Representative images show the cells forming strings aligned horizontally along the nanofibers (Bars= 100 μ m). B) ECs as in (A) were labeled with CellTracker-CMRA and cultured on nanofibers. After 24h green-labeled glioma initiating cells were added to the ECs and cultured in EC medium for 24h. Cells were viable and motile during the co-culture (Bar=100 μ m, 20X). C) Magnification (40X) of boxed region in (B) shows a glioma cell (green) and an endothelial cell (red) stretching side by side (arrows) along parallel nanofibers.

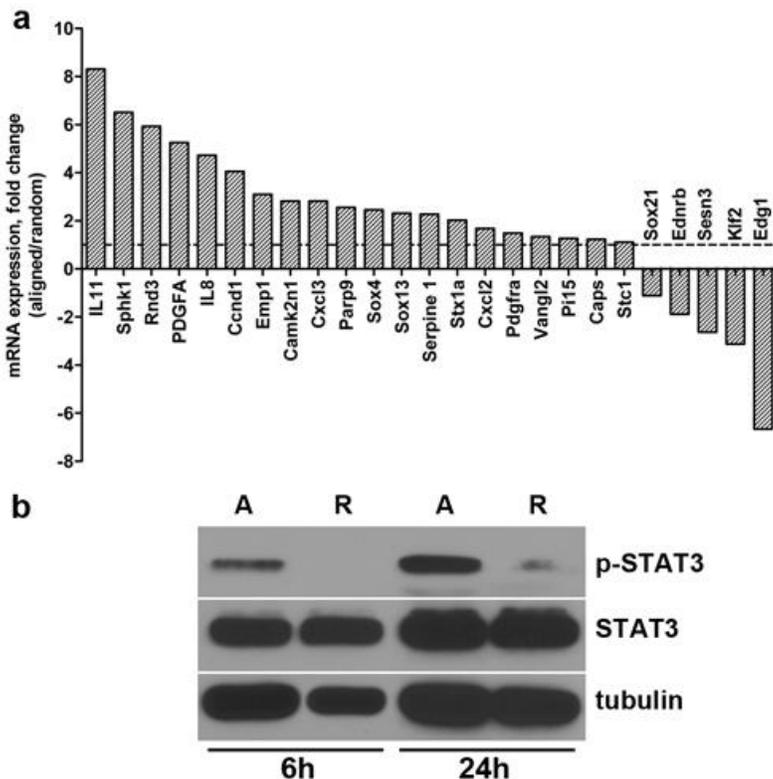


Figure 3: Glioma cell migration on aligned nanofibers correlates with activation of STAT3 signaling. **A)** Quantitative RT-PCR results showing differential gene expression levels in glioma cells cultured on aligned versus randomly-oriented nanofibers (selected genes are a subset of the microarray dataset GSE28167 analyzed by Viapiano lab and deposited at NCBI GEO). Most of the upregulated genes in the graph are known modulators or targets of JAK/STAT and are involved in positive regulation of cell migration. **B)** Dissociated U251 glioma cells (5×10^5 cells/ml) were plated on aligned (A) or randomly-oriented (R) nanofibers and collected 6h or 24h post attachment. Cells recovered from aligned fibers showed a substantial increase in Y⁷⁰⁵-phosphorylated STAT3 compared to cells recovered from random fibers.