Tkachenko et al, Supplemental Methods

To derive primary esophageal LSM cells, LSM bundles were isolated from human donor esophagi (under UCSD IRB exempt protocol 130835) followed by collagenase D digestion (Roche Mannheim, Germany). SoftSubstrates were coated with 1.6 µg/ml solution of collagen I (Advanced Biomatrix, San Diego, CA) in pH 7.4 PBS for 60 min at 37^0C followed by plating and culturing of cells. For morphological analyses, cells were cultured for 24-48 hours followed by fixation and staining for actin filaments with phalloidin-488 (Life Technologies, Grand Island, NY) and nuclei with DAPI (Vector, Burlingame, CA) using the manufacturers' protocols. Fluorescence micrographs were processed and analyzed using ImageJ (NIH). FACS analysis was performed in order to assess cell size on 0.8 and 24kPa matrixes as previously described (1). For transcriptional analyses, cells were harvested after 96 hours of culture followed by reverse transcription quantitative PCR analyses. Gene expression studies were repeated in technical duplicates to triplicates and in biologic triplicates.

To create stable cell lines expressing phospholamban (PLN), full-length PLN cDNA was cloned downstream of the Tet Responsive Element (TRE) of the HIV1 vector backbone plasmid, pLVX-Tight-Puro (Clontech, CA), which also expresses a puromycin-resistance gene driven by PGK promoter. Full-length PLN coding region was cloned by PCR using a human PLN cDNA containing plasmid as the template (OriGene Cat# RC202712). Esophageal SM cells were co-infected with LV-Tet-PLN and LV-Tet-On viruses at MOI of 10 each in the presence of polybrene (4 ug per ml). At 24 hours after LV infection, cells were fed with fresh medium containing puromycin (1 ug per ml) and G418 (200ug per ml) and resistant cells were continued in culture in low selection media using G418 and puromycin and passaged in smooth muscle cell media (ScienCell, San Diego, CA) with serum and growth factors. Cells were treated with 2ug/ml doxycycline for 72 hours prior to FACS analysis and PLN expression was verified using qPCR, immunoblotting, and immunohistochemistry (Figure 2b).

Reference

1. Banerjee I, Carrion K, Serrano R, Dyo J, Sasik R, Lund S, et al. Cyclic stretch of embryonic cardiomyocytes increases proliferation, growth, and expression while repressing Tgf-beta signaling. Journal of molecular and cellular cardiology. 2015 Feb;79:133-44. PubMed PMID: 25446186. Pubmed Central PMCID: 4302020.