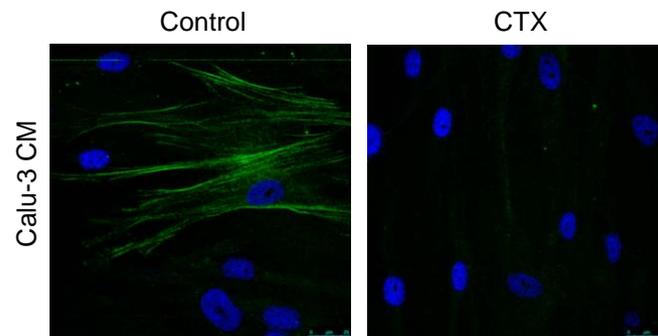
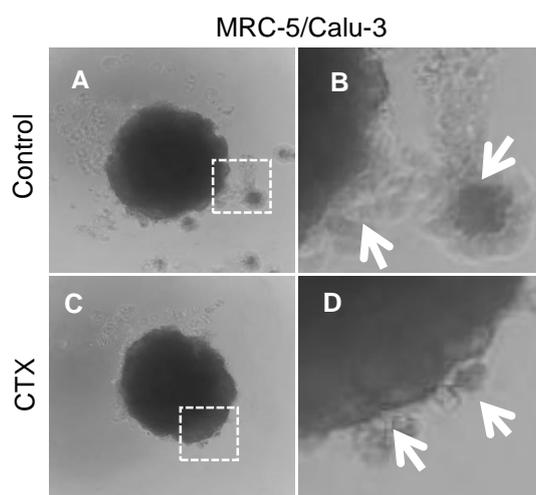


## Supplementary Materials: Crotoxin modulates events involved in epithelial-mesenchymal transition in 3D spheroid model

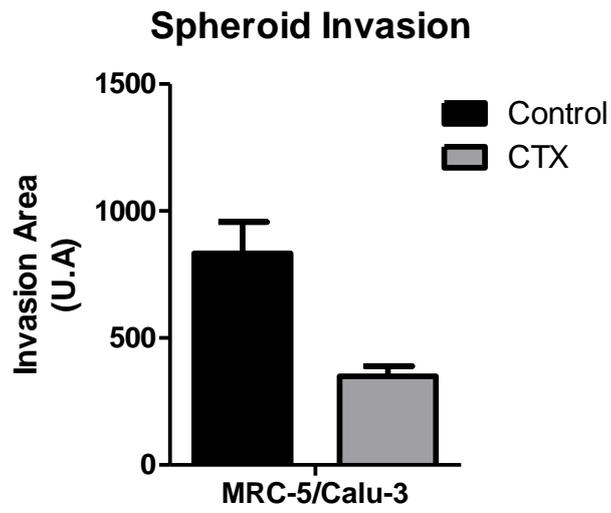
Ellen Emi Kato, Sandra Cocuzzo Sampaio

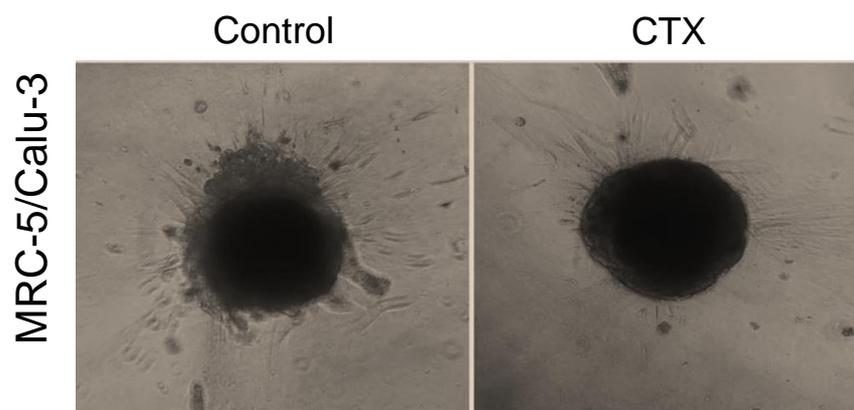


**Figure S1. Myofibroblast differentiation in Calu-3 conditioned medium.** Representative immunofluorescent images of MRC-5 cells pretreated with CTX (12.5 nM) for 2h and then, incubated in presence of tumor conditioned media from Calu-3 cells for 3 days. Green fluorescence indicates  $\alpha$ -SMA-containing stress fibers expression and blue fluorescence indicates the nuclei. Scale bar = 25  $\mu$ m.

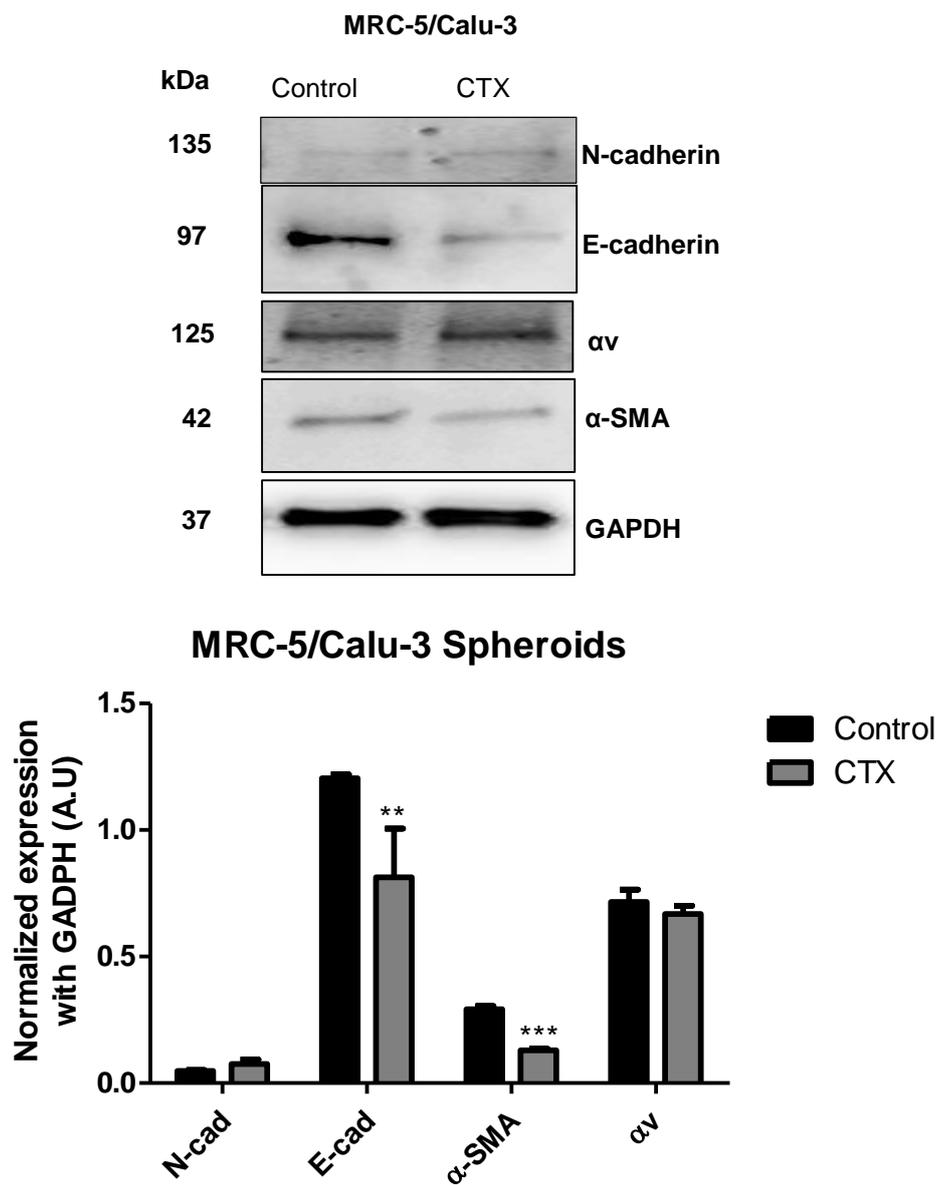


**Figure S2. MRC-5/Calu-3 spheroid formation.** MRC-5/Calu-3 spheroid formation was performed by hanging drop method. After 24 h cells aggregates formed compacted structures in the absence (A) or in the presence of CTX (C). A small amount of cancer cells did not incorporated into compacted cell aggregates (C and D)(arrow).





**Figure S3. Invasion area of MRC-5/Calu-3 spheroids in 3D collagen gels.** Spheroids MRC-5/Calu-3 constituted with 12.5nM CTX were embedded in collagen gel (1.2mg/mL) and cell invasion was photographed under phase-contrast microscopy up to 48h. Cell invasion area was measured and analyzed on ImageJ software.



**Figure S4. Expression of EMT-related proteins.** After 3 days in culture, MRC-5/Calu-3 spheroids were lysated and analyzed on Western blot for E-cadherin, N-cadherin, α-SMA and GAPDH as loading control. \*\*\* p<0.001 compared to control group. \*\* p<0.01 compared to control group (n=4).