

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

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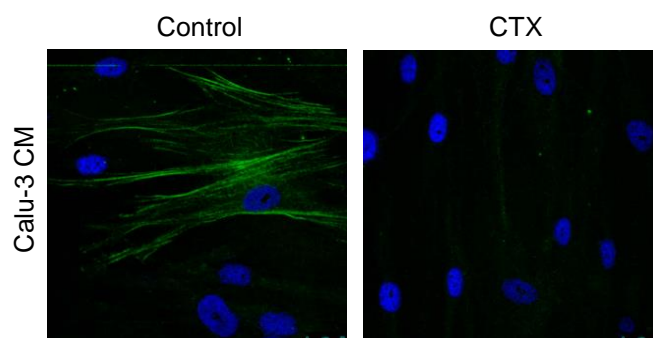


Figure S1. Myofibroblast differentiation in Calu-3 conditioned medium. Representative immunofluorescent images of MRC-5 cells pretreated with CTX (12.5 nM) for 2h and then, incubated in presence of tumor conditioned media from Calu-3 cells for 3 days. Green fluorescence indicates α -SMA-containing stress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

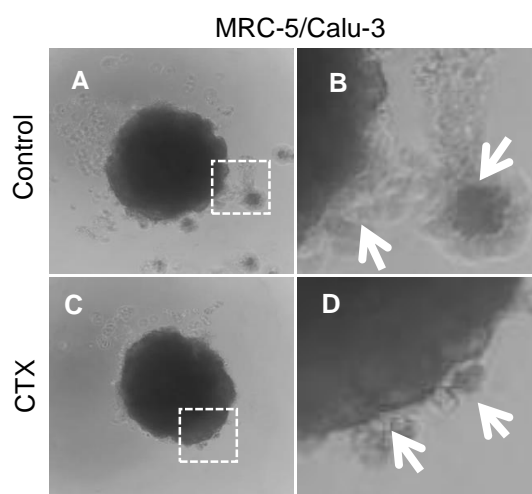
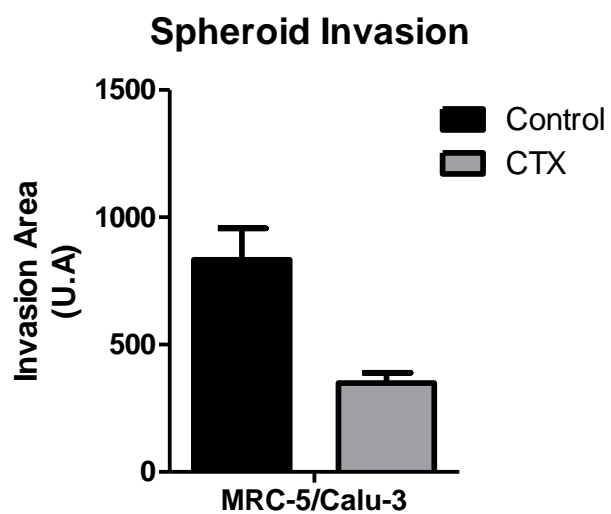


Figure S2. MRC-5/Calu-3 spheroid formation. MRC-5/Calu-3 spheroid formation was performed by hanging drop method. After 24 h cells aggregates formed compacted structures in the absence (A) or in the presence of CTX (C). A small amount of cancer cells did not incorporated into compacted cell aggregates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



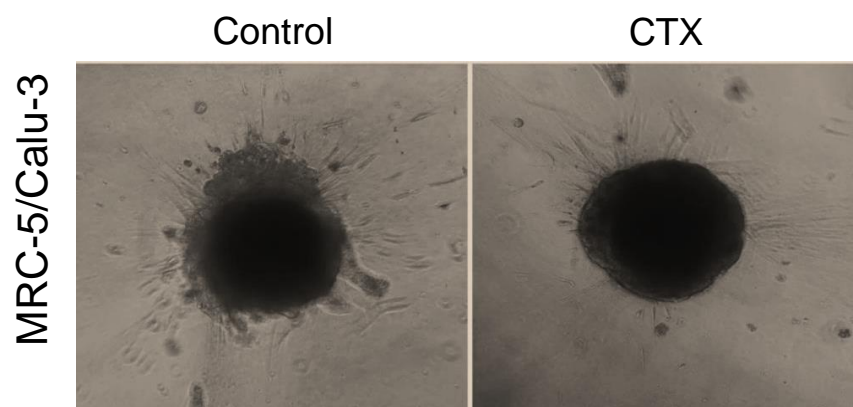


Figure S3. Invasion area of MRC-5/Calu-3 spheroids in 3D collagen gels. Spheroids MRC-5/Calu-3 constituted with 12.5nM CTX were embedded in collagen gel (1.2mg/mL) and cell invasion was photographed under phase-contrast microscopy up to 48h. Cell invasion area was measured and analyzed on ImageJ software.

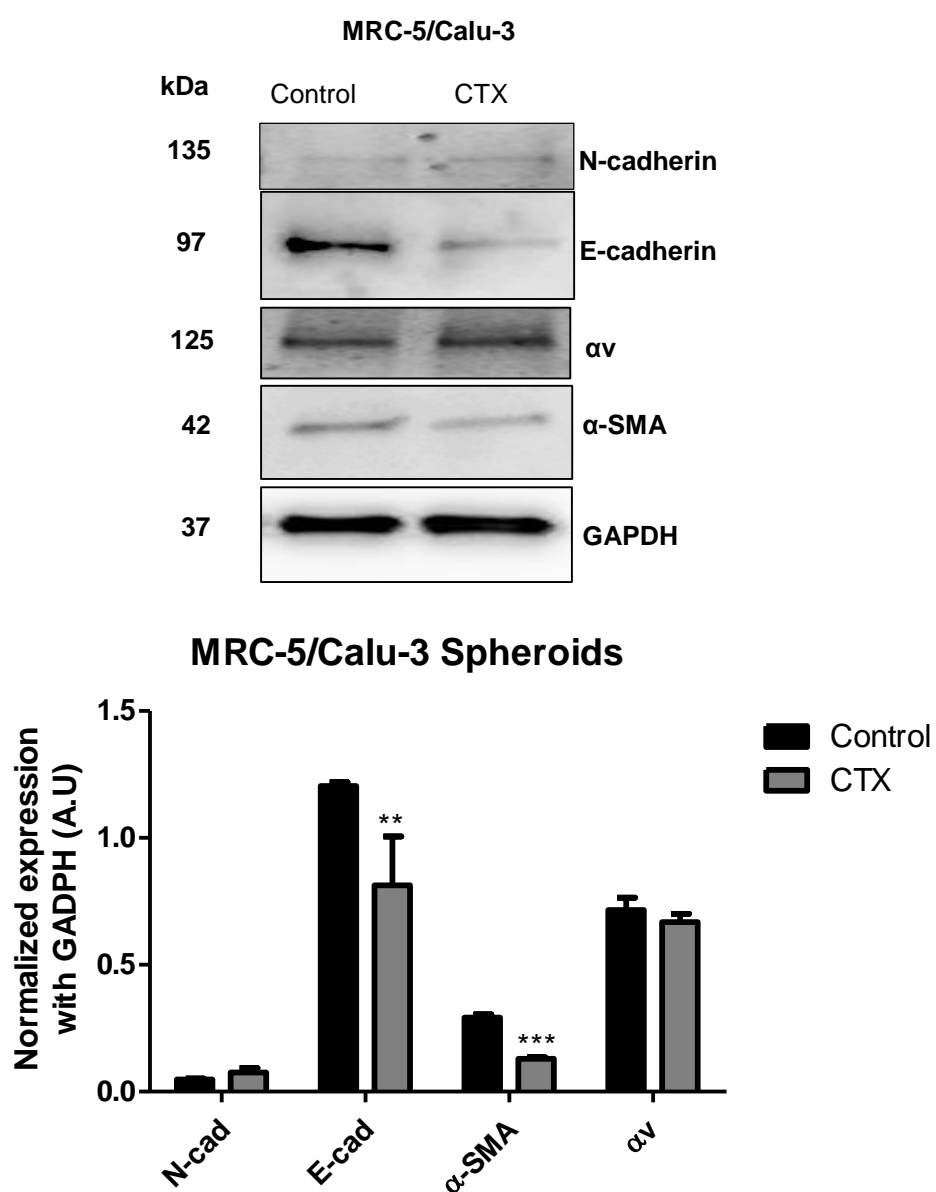


Figure S4. Expression of EMT-related proteins. After 3 days in culture, MRC-5/Calu-3 spheroids were lysated and analyzed on Western blot for E-cadherin, N-cadherin, α-SMA and GAPDH as loading control. *** $p < 0.001$ compared to control group. ** $p < 0.01$ compared to control group (n=4).