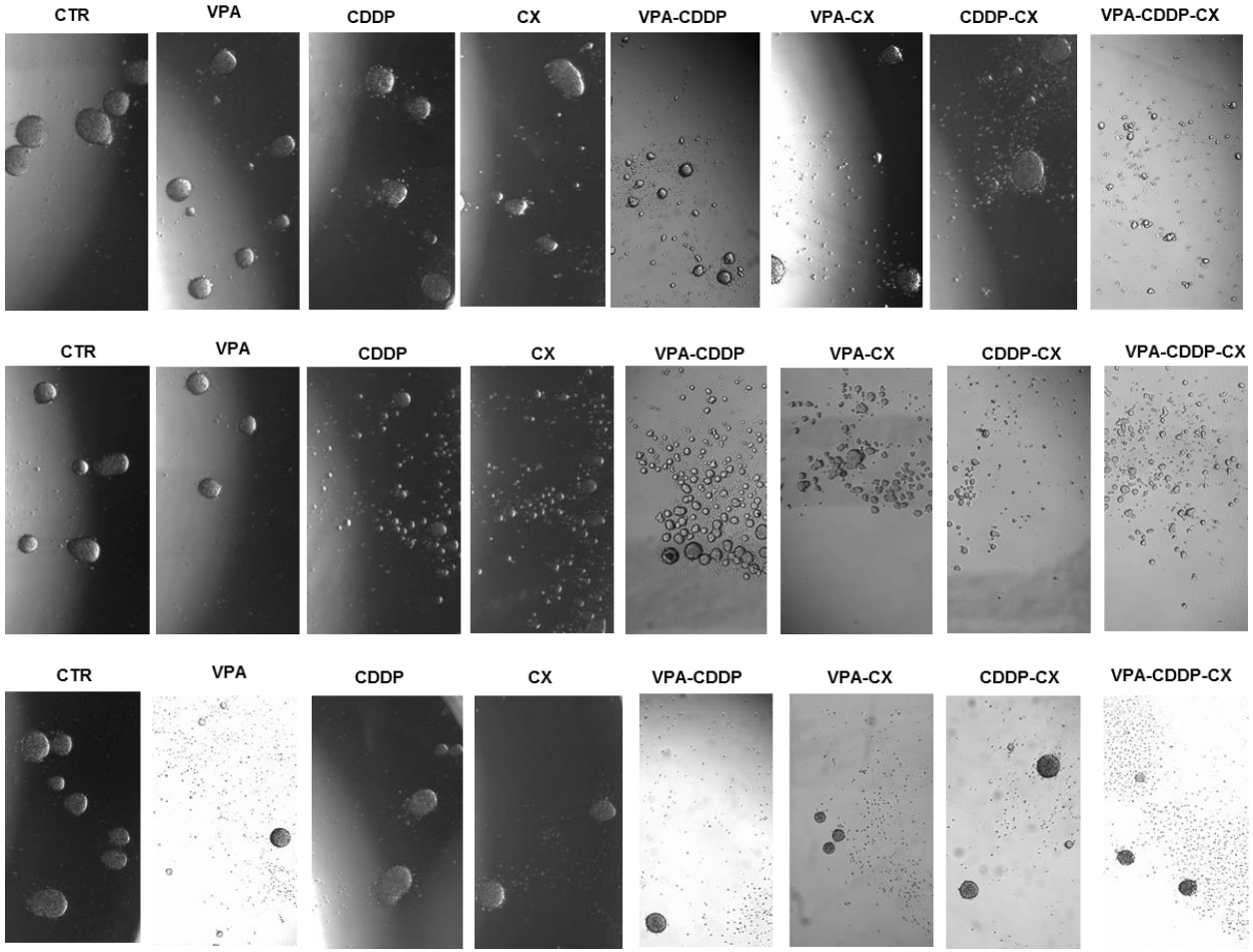
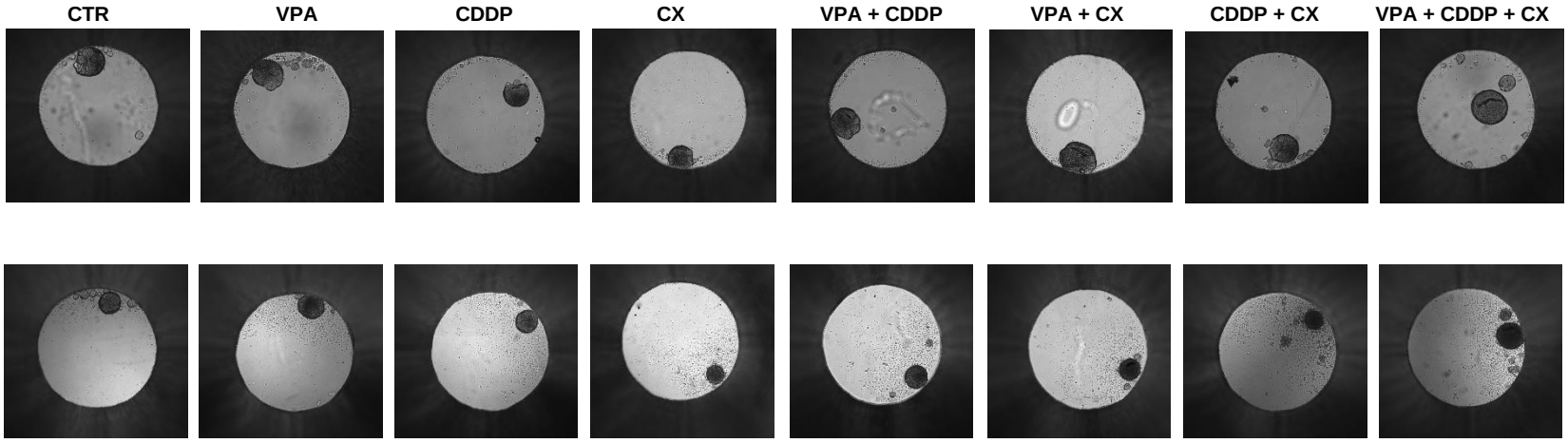
Cal27



Spheroids were cultured in Sphere Medium (DMEM/F12 supplemented with BSA, glucose, heparin, FGF, EGF, neuronal cell culture B27, insulin). The cells (40,000 cells/mL) were plated in low-attachment multi-well plates and treated with indicated drugs. Spheres A (1st generation sphere formation): cells plated in low-attached plate in sphere medium and concomitantly treated, to investigate the capacity of treatment to prevent/reduce tumor formation; Spheres B (2nd generation sphere formation): cells were grown for 72 h, then disaggregated and plated again in the presence of drugs, to evaluate the impact of treatment to prevent/reduce more aggressive tumors; Spheres C (pre-formed spheres): spheres allowed to grow for 72 h without any treatment and then treated, to evaluate the capacity of treatment to induce tumor regression.

InSphero microtissues Cal27



Cal27 cells were cultured as microtissues by the GravityPLUS™ Hanging Drop System (InSphero AG, Wagistrasse, Switzerland). Cells were plated following instructions from InSphero kit and untreated or treated with drugs, as indicated. Within 2-4 days, a single microtissue forms in each drop. Once the microtissues are formed they can easily be harvested into the GravityTRAP™ plate with a simple media addition step. 3D microtissues were maintained in the incubator and after 72 hours images from each microtissue was taken.