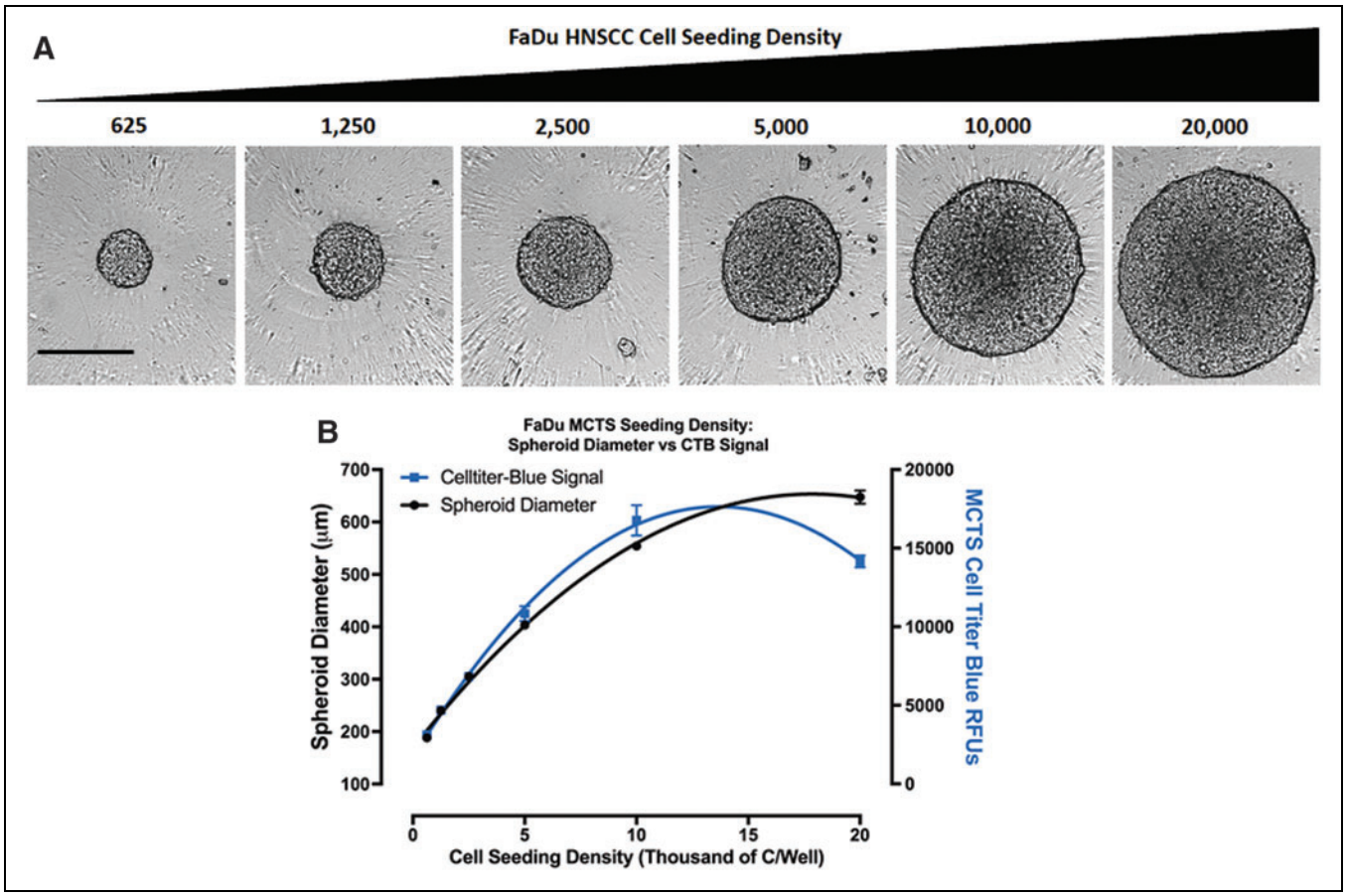
Supplementary Data



Supplementary Fig. S1. Correlation between FaDu cell seeding density MCTS diameter and CTB RFU Signals. **(A)** FaDu HNSCC cells were seeded at seeding densities ranging from 625 to 20,000 cells per well into 384-well ULA-plates, and after 24 h in culture 4×TL images of the MCTSs were acquired on the IXM. All scale bars represent 300 μm. **(B)** We measured the diameters of the FaDu MCTSs in the TL images using the line-scan tool and measured the CTB RFUs 4 h after reagent addition. The mean ± SD ($n=3$) of the FaDu MCTS diameter measurements in μm (black circle) and CTB RFU signals (blue square) from three wells for each seeding density are presented. The MCTS diameter measurements in μm are plotted on the *Y* axis on the left, and the CTB RFUs are plotted on the *Y* axis on the right. Representative experimental data from multiple independent experiments are shown. CTB, CellTiter-Blue®; HNSCC, head and neck squamous cell carcinoma; IXM, ImageXpress Micro; MCTS, multicellular tumor spheroid; RFU, relative fluorescent unit; SD, standard deviation; TL, transmitted light; ULA-plates, ultra-low attachment microtiter plates.