

**Supplementary Fig. S2.** Distribution of the fluorescence in MCTSs formed from HNSCC cell lines prelabeled with CTO before cell seeding into Ultra-low Attachment plates. Before cell harvesting, counting, and seeding into ULA-plates, the five HNSCC cell lines were prelabeled with the CTO reagent according to the manufacturer's instructions. The five HNSCC cell lines were seeded at 2,500 cells per well in 384-well ULA-plates and cultured for 3 days, before  $4 \times$  images were acquired on the IXM in the TL and Texas Red channels. Grayscale TL and CTO fluorescent images are presented along with the corresponding pseudo-color pixel intensity visualizations and line-scan fluorescent intensity profiles. Representative images and data from multiple independent experiments are shown. All scale bars represent 300  $\mu$ m. CTO, CellTracker<sup>®</sup> Orange.