Supporting Information

D-CryptO: Deep learning-based analysis of colon organoid morphology from brightfield images

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Dose-dependent changes in organoid morphology

To further validate D-CryptO, we used it to assess the dose-dependent response of doxorubicin on opacity and budding following treatment at various concentrations. Doxorubicin is a chemotherapeutic that inhibits DNA and RNA synthesis and induces apoptosis.[1] We applied doxorubicin at concentrations of 50 µM, 5 µM, 0.5µM, 0.05 µM, and 0.005 µM **(Figure S1a)**. For opacity, the concentration at which 50% of organoids became opaque was 3.6 µM **(Figure S1b)**. For budding, the concentration at which 50% of the organoids still had budding structures was 39.8 µM **(Figure S1c)**. It is important to note that budding did not increase with higher dosages of doxorubicin. Instead, the percentage of non-viable organoids increased which was classified under the budding category. For diameter, the concentration at which 50% of the organoids had a reduction in diameter was 0.5 µM **(Figure S1d)**. Each parameter was impacted at different concentrations, indicating the importance of monitoring these features to assess drug toxicity.
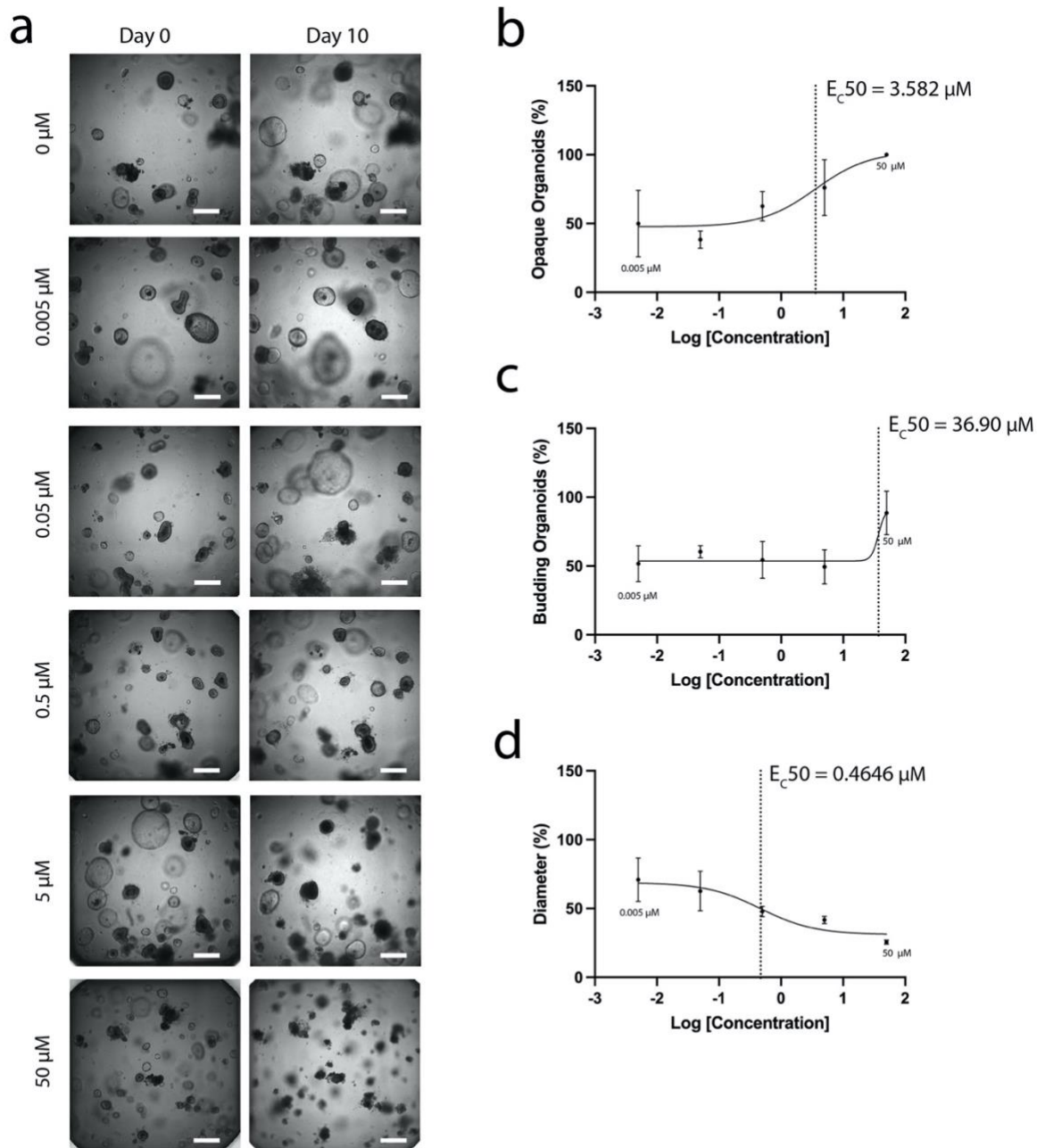
Supplementary Figure 1. Dose-dependent changes in organoid morphology. a, Brightfield images of organoids taken on Day 0 and Day 10 of drug treatment with doxorubicin at 5 different concentrations. Scale bar, 500 μm **b,** The percentage of opaque organoids following 10 days of treatment with increasing concentrations of doxorubicin. **c,** The percentage of budding organoids following 10 days of treatment with increasing concentrations of doxorubicin. **d,** The change in diameter following 10 days of treatment with increasing concentrations of doxorubicin.
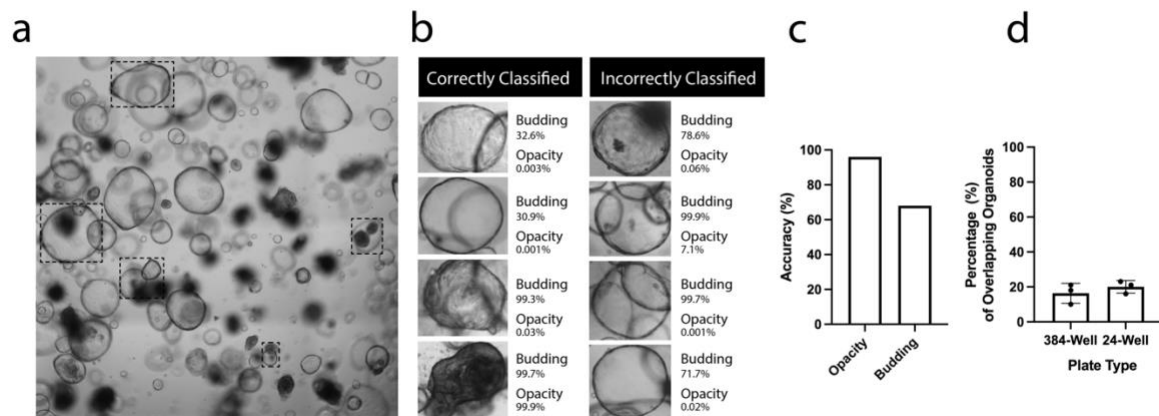
Supplementary Figure 2. D-CryptO performance on overlapping organoids. a, Examples of overlapping organoids. **b,** Correct and incorrect classifications by D-CryptO. **c,** D-CryptO accuracy when classifying overlapping organoids. **d,** Percentage of overlapping organoids in both 384 and 24-well plates.

References

[1] V. Malhotra, M. C. Perry, *Cancer Biol Ther* **2003**, *2*, DOI 10.4161/CBT.199.