Automating high-throughput screens using patient-derived colorectal cancer organoids

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Introduction

Most potential oncology drugs fail at the later stages of the drug development pipeline and in clinical trials, despite having promising data for their efficacy in vitro. This high failure rate is partly due to insufficient predictive models being used to screen drug candidates in the early stages of drug discovery.

As such, there is a need to develop and utilize more representative models that are amendable for efficient testing of drug efficacy to discover new therapeutic targets.

3D cell models, specifically patient-derived organoids (PDOs), offer a promising solution to this problem. Studies show that patients and their derived organoids respond similarly to drugs, suggesting the therapeutic value of using PDOs to improve therapeutic outcomes. However, challenges commonly associated with using these organoids, such as assay reproducibility, ability to scale up, and cost have limited their widespread adoption as a primary screening method in drug discovery.

To address some of the hurdles associated with the use of PDOs in large scale screens, a semi-automated bioprocess has been developed for the controlled production of standardized PDOs at scale. Cultured PDOs were uniform in size, show high viability and were produced in repeatable batches in an assay-ready format.

Here, we develop an end-end, automated workflow starting with assay-ready CRC organoids.

Materials and methods
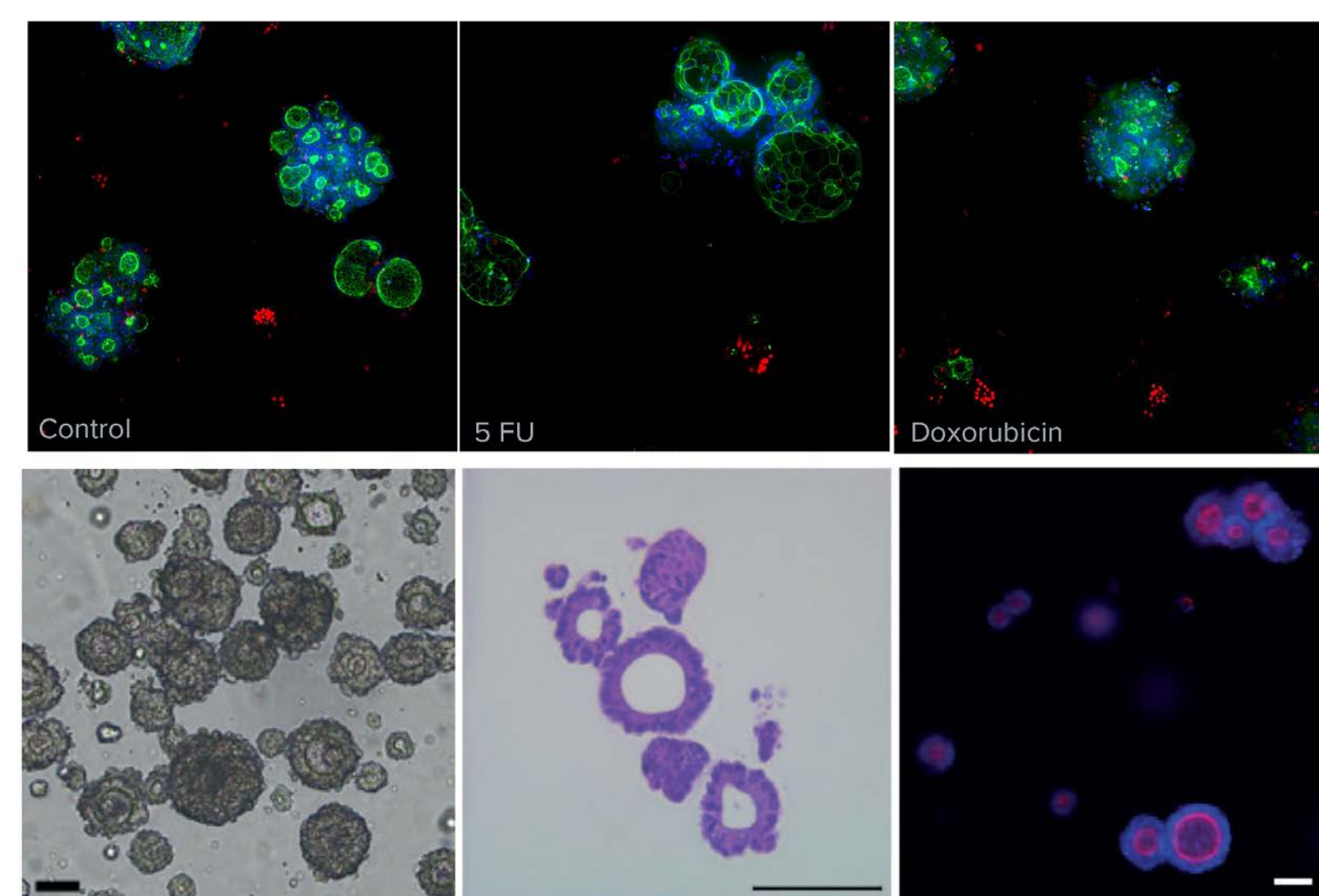
Top: Organoids (ISO68) treated with different compounds can results in varying phenotypic changes. Bottom: Representative images of the ISO38 colorectal cancer organoids. Left: Brightfield, middle: H&E, right: Fluorescent images (confocal), organoids are stained with a nuclear (blue) and cytoskeletal (red) marker.

CRC organoid culture

Colorectal cancer organoids (Line ISO38, Molecular Devices) were handled according to manufacturer's instructions. Briefly, organoids were thawed quickly at 37°C, gently resuspended, and washed in media. Organoids were resuspended in Matrigel and then seeded in a 384 well plate at 200 organoids per well either manually or with the Hamilton STAR liquid handler. For automated seeding, the organoid Matrigel suspension was prepared in three columns of a 96 well compound plate. The Hamilton STAR liquid handler was used to seed 10µl of suspension in each well of a 384 well plate using the multichannel probe. Columns 1–12 were seeded with automation, columns 13–24 were seeded manually. Organoids were incubated with media containing ROCK inhibitor for 48 hours to improve recovery. Organoids were then treated with selected compounds for 6 days at varying concentrations (approximately half-log dilutions or 4-fold) and in quadruplicates. Compound (highest concentration used): 5-Fluorouracil (5FU) (100µM), cisplatin (20µM), doxorubicin (60µM), romidepsin (10µM), trametinib (20µM).

Image acquisition and analysis
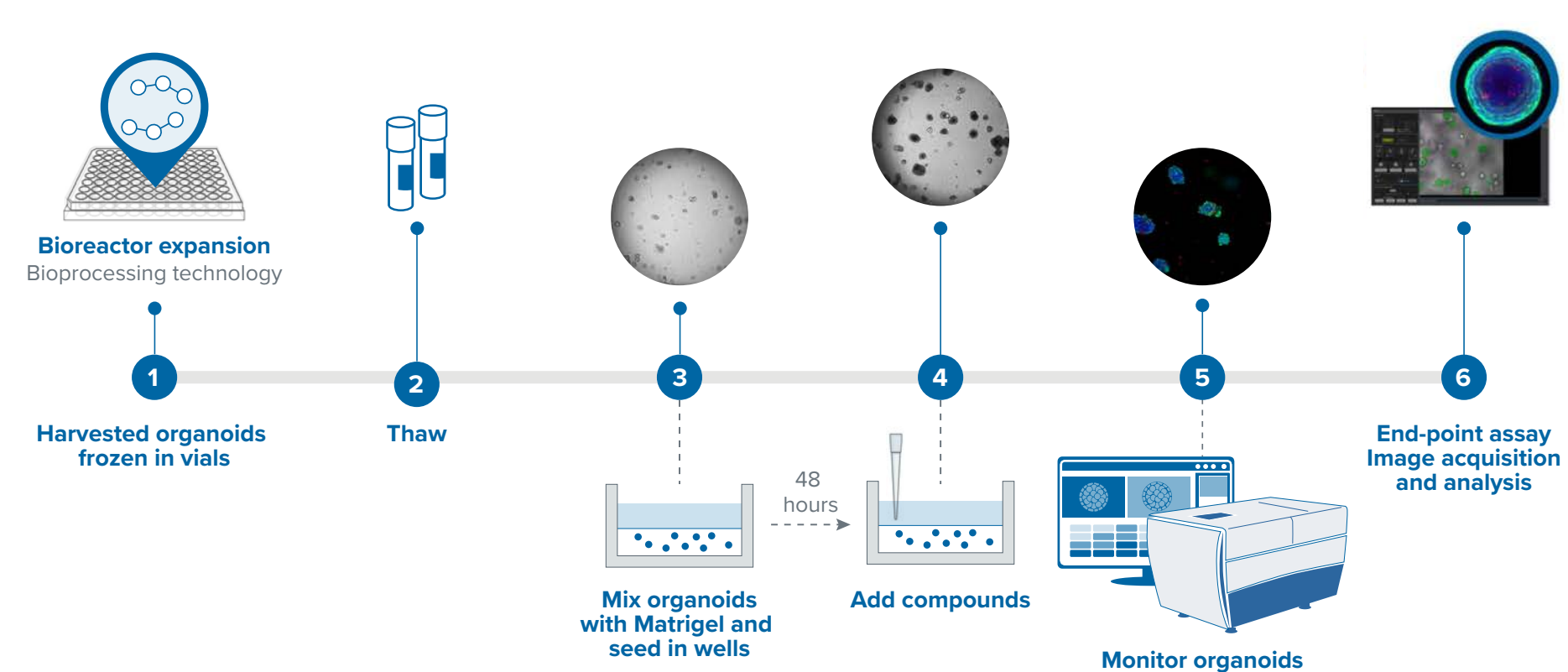
Workflow for using assay ready colorectal cancer organoids.

The effects of compound treatment was monitored over time using the ImageXpress® Micro Confocal System. CRC organoids were imaged using 4X objective, with z-stacks enabled. For viability assay, organoids were incubated with Hoechst, Calcein AM and ethidium homodimer for 2hrs at 37°C. Images were acquired on the ImageXpress Confocal HT.ai High-Content Imaging System using the 10X objective with Z-stacking.

IN Carta® Image Analysis Software was used to analyze images acquired during monitoring. A deep learning-based approach was used to create a model for organoid segmentation and analysis was carried out using 2D projection images. For analysis of growth over time, organoids were segmented using a user-trained model. The total organoid area on day 7 was normalized over the total organoid area pre-treatment to determine the amount of growth over time. For live/dead analysis, the intensity of Calcein AM dye (FITC channel) was divided by the intensity of ethidium homodimer dye per organoid.

Results

Automation setup for organoid seeding in 384-well plate

To automate organoid seeding in Matrigel, the liquid properties were optimized for the STAR liquid handler. To ensure minimum fluctuations in Matrigel temperature, the source plate containing the Matrigel organoid suspension was placed on a cold plate (IHECO CPAC) for the duration of the seeding process. The 384W destination plate and pipette tips were also pre-chilled before they were loaded on the deck.
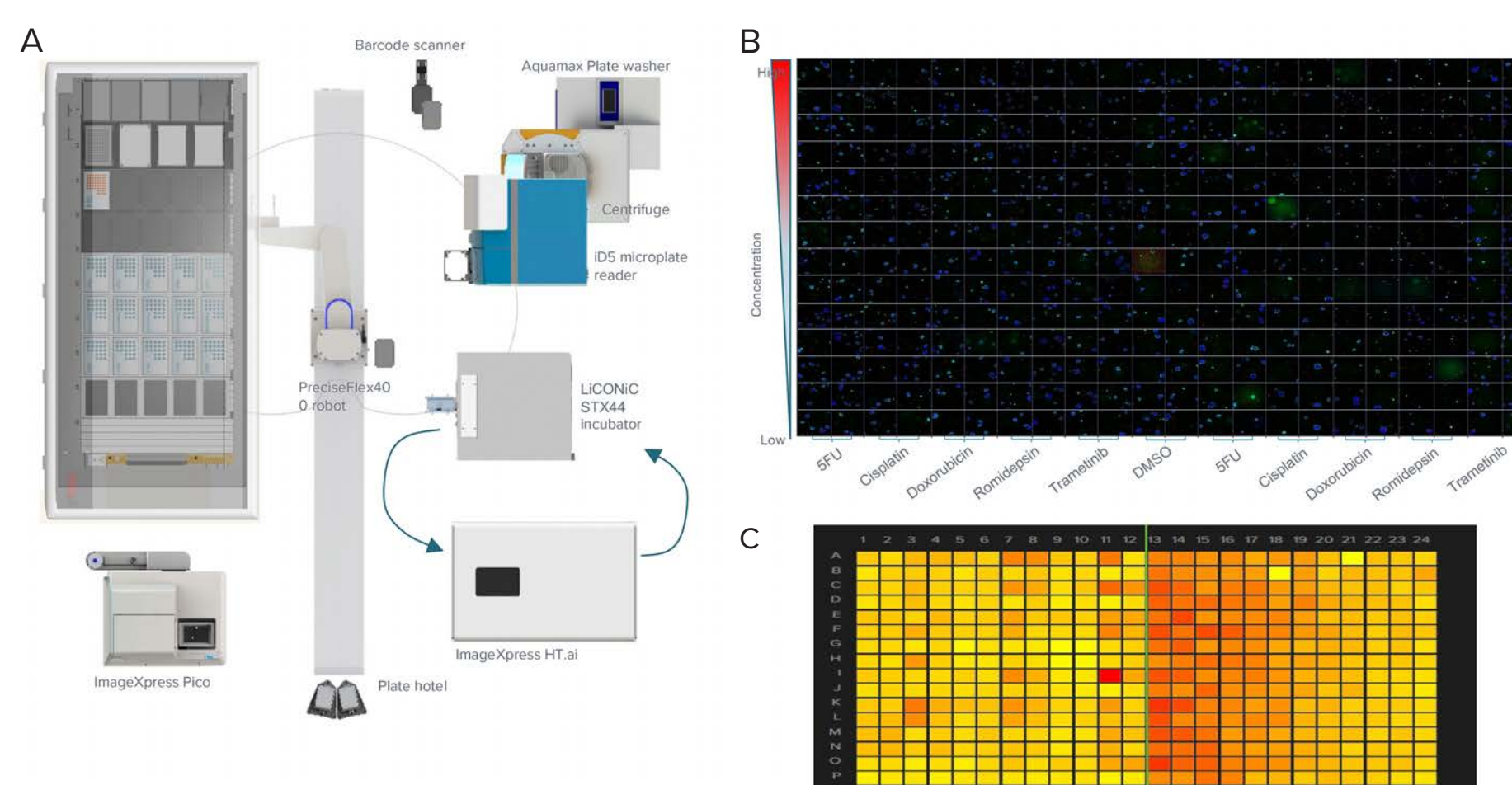

Figure 1. Layout of the automation work cell is illustrated in (A). The instruments are controlled by an integrated software that allows for set up of cell culture workflows. The curve arrows shown an example of the process to monitor cells in culture where plates are moved from the incubator to the ImageXpress Confocal HT.ai for imaging in brightfield and then back to the incubator. B) Assay setup in a 384well plate with four technical replicates for each condition. Compounds are added with the highest concentration in Row B (Border wells, columns 12 and 13 are controls). C) Organoids were seeded with the liquid handler (left half) or manually (right half). Shown here is a heat map representing the number of organoids per well. Organoids seeded with the liquid handler show a more homogenous distribution between wells while manual seeding show a gradient of organoid count from high to low (starting column 13).

AI-based imaged analysis for robust segmentation of label-free brightfield images

Automated image analysis is an integral part of an automation enabled platform. The ability to monitor cells and organoids in real time and to extract meaningful information is dependent on robust image analysis of label-free transmitted light images. Due to the meniscus effect and small seeding volume, organoids tend to settle at the corners of the well. This leads to shading artifacts in images acquired in brightfield which makes it challenging to segment and analyze the organoids.

Here, a deep-learning approach was used to identify the organoids for analysis using IN Carta image analysis software (Figure 2A). This method allows for robust segmentation of organoids at the side of the well and those beneath air bubbles (Figure 2B).
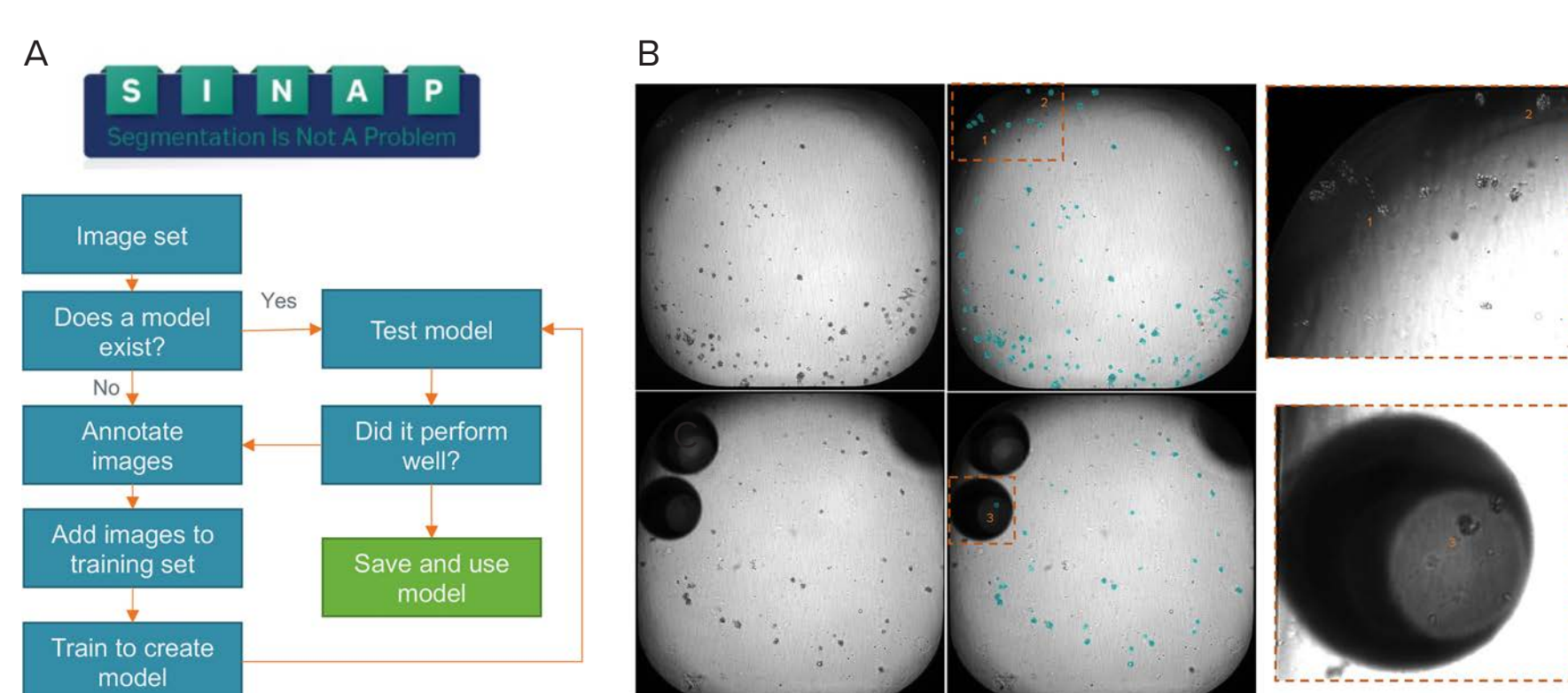

Figure 2. A) Overview of the SINAP workflow in IN Carta software used to generate a model for organoid segmentation. B) Examples of whole-well images of organoids and their respective segmentation masks (cyan). Inset in the figure is shown with adjusted brightness and contrast in order to bring out the organoids that are obscured by artifacts such as bubbles or edge effects. Note that the model can segment these "hard to see" organoids.

Compound effects on CRC organoid growth over time

Selected anti-cancer compounds were added to the organoids 48 hours after seeding and their effects on CRC growth over the next 6 days were monitored (Figure 3). Untreated organoids increase in size over time in culture. In contrast, organoids treated with romidepsin and trametinib show no obvious growth, suggesting that both these compounds have cytostatic effects on CRC organoids.
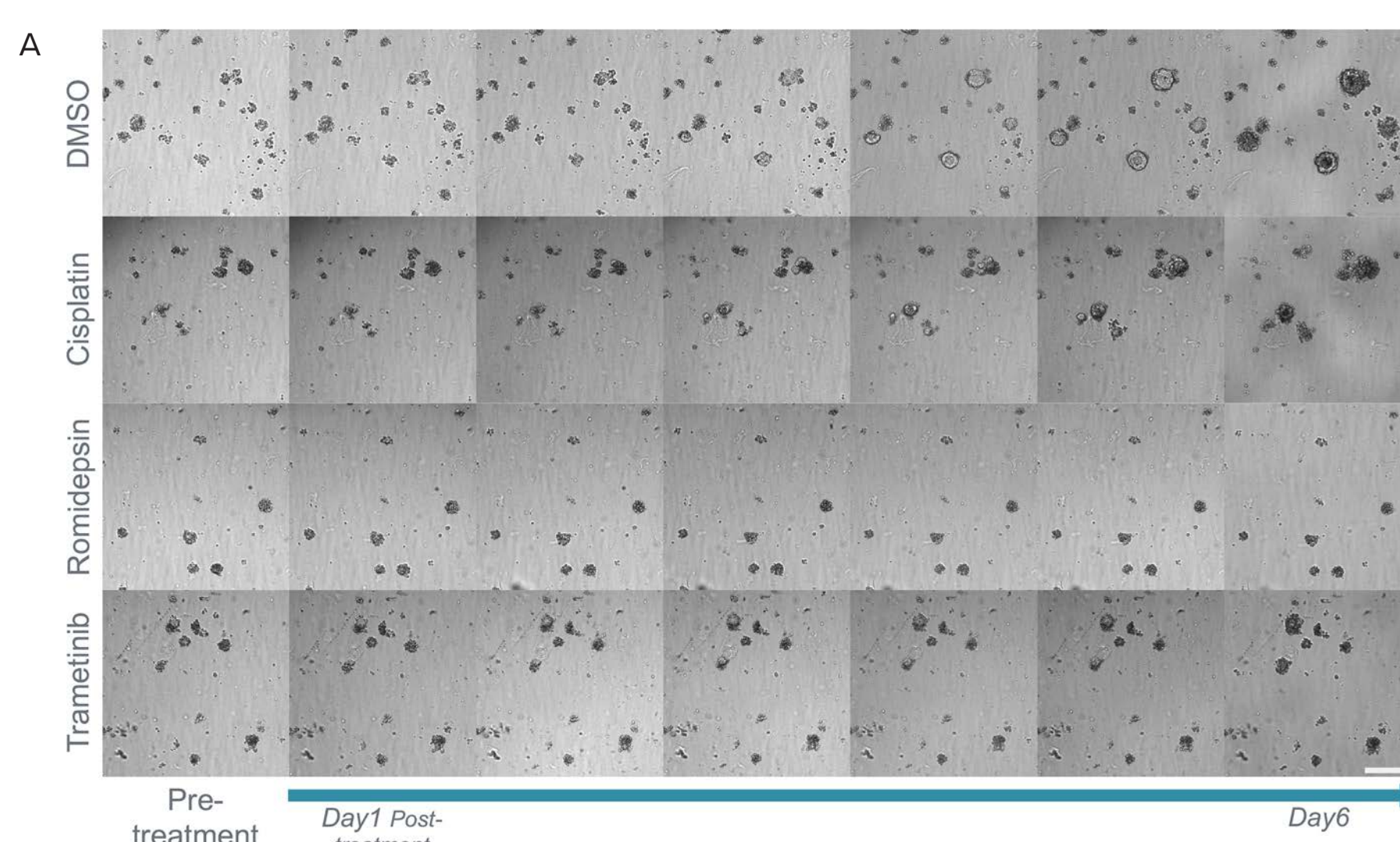

Figure 3. Effects of compounds of organoid growth over time A) Organoids were treated with 5FU, cisplatin, doxorubicin, romidepsin and trametinib at various concentrations and their effect on the CRC organoids were monitored over 6 days. Shown here are examples of CRC organoids treated at the highest compound concentrations. Scale = 200µm

Results

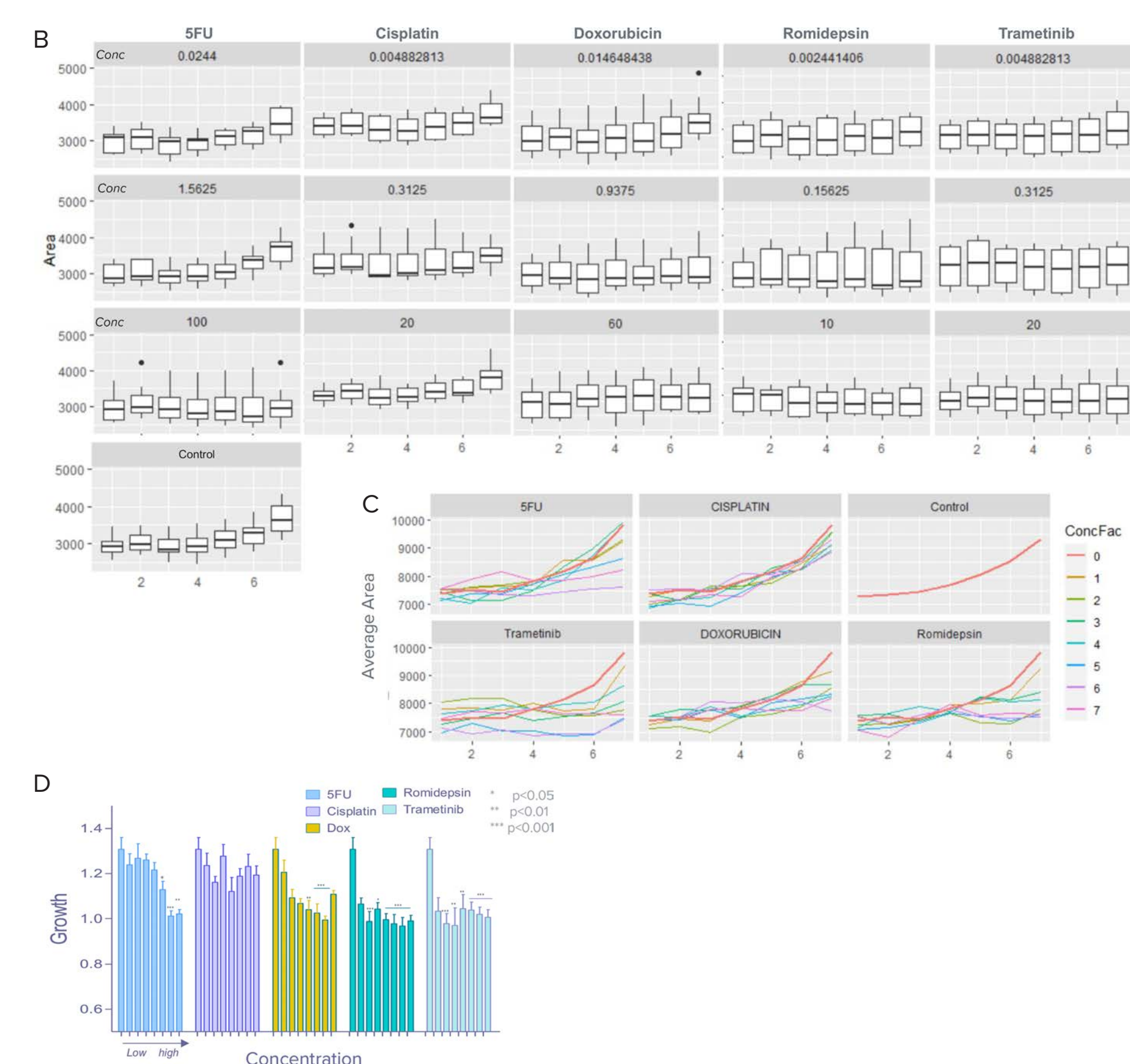### Compound effects on CRC organoid growth over time

Figure 3. B) Boxplot of organoid area in response to compounds at the indicated concentrations (in µM). Each column shows a compound at three different concentrations (lowest, middle and highest), time (days) is represented on the x-axis. Compounds are added on day two. Compared to the untreated organoids, romidepsin and trametinib treated organoids showed minimal growth. C) Average area of organoids are plotted against time (days). Colored lines represent the different compound concentrations (0 for control, 1 = lowest concentration, 7 = highest concentration). D) To quantify organoid growth over time, the total area of organoids on day 7 was normalized to the total area of pre-treated organoids. Control organoids show an average of ~30% growth. Growth inhibition was significant in doxorubicin, romidepsin, and trematinib treated organoids (based on 2-way ANOVA test).

Effects of anti-cancer compounds on organoid viability

To determine if the compounds affected cell viability, the organoids were stained with Calcein AM (live cell marker), Ethidium homodimer (dead cell marker) and Hoechst (nuclei dye). Organoids were imaged and analysis was carried out to quantify organoid viability.
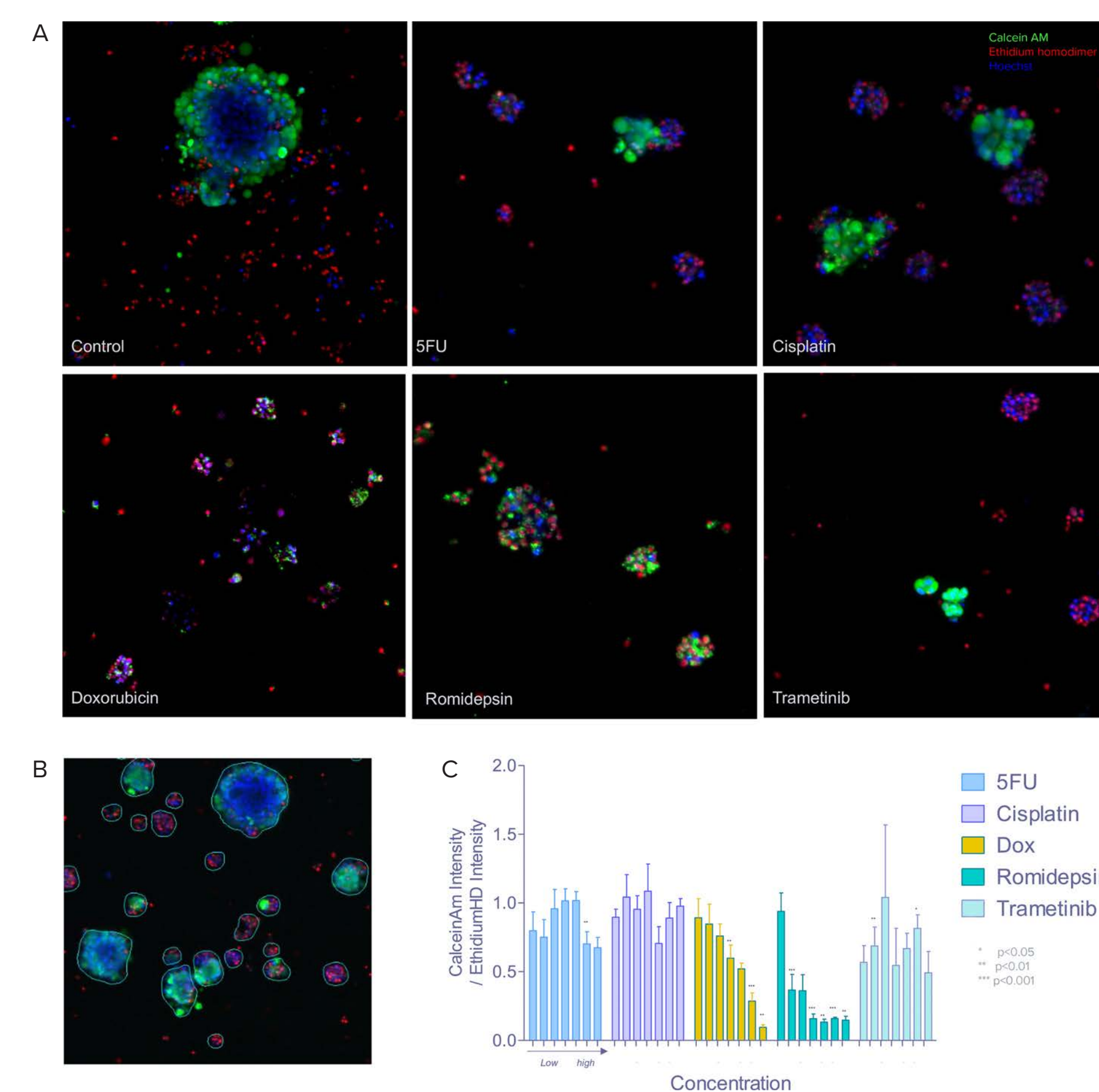

Figure 4. A) Viability assay was carried out on CRC organoids six days post treatment. Shown here are example images of organoids from the various treatment groups (treated with the highest concentrations). B) An example image with overlay of the segmentation mask shown as outlines. C) Viability was quantified by calculating the average ratio of Calcein AM to ethidium homodimer intensity per organoid. Bar graph here shows the data normalized to the controls (y-axis) vs concentration (x-axis, 1=lowest, 7=highest) grouped by compound. Two-way ANOVA was performed on unnormalized data, Bonferroni post-hoc test to compare treatments to DMSO.

Conclusions

- Our results support the use of PDOs for high-throughput assays such as compound screening. The availability of standardized, assay-ready PDOs provides significant time savings for screens using 3D models

- We show that an AI-based approach can be successfully used to generate robust segmentation for the analysis of label-free biological modes such as 3D organoids. The data obtained from monitoring gives mechanistic insights into compound effects on cancer organoids and supports studies on drug efficacy.