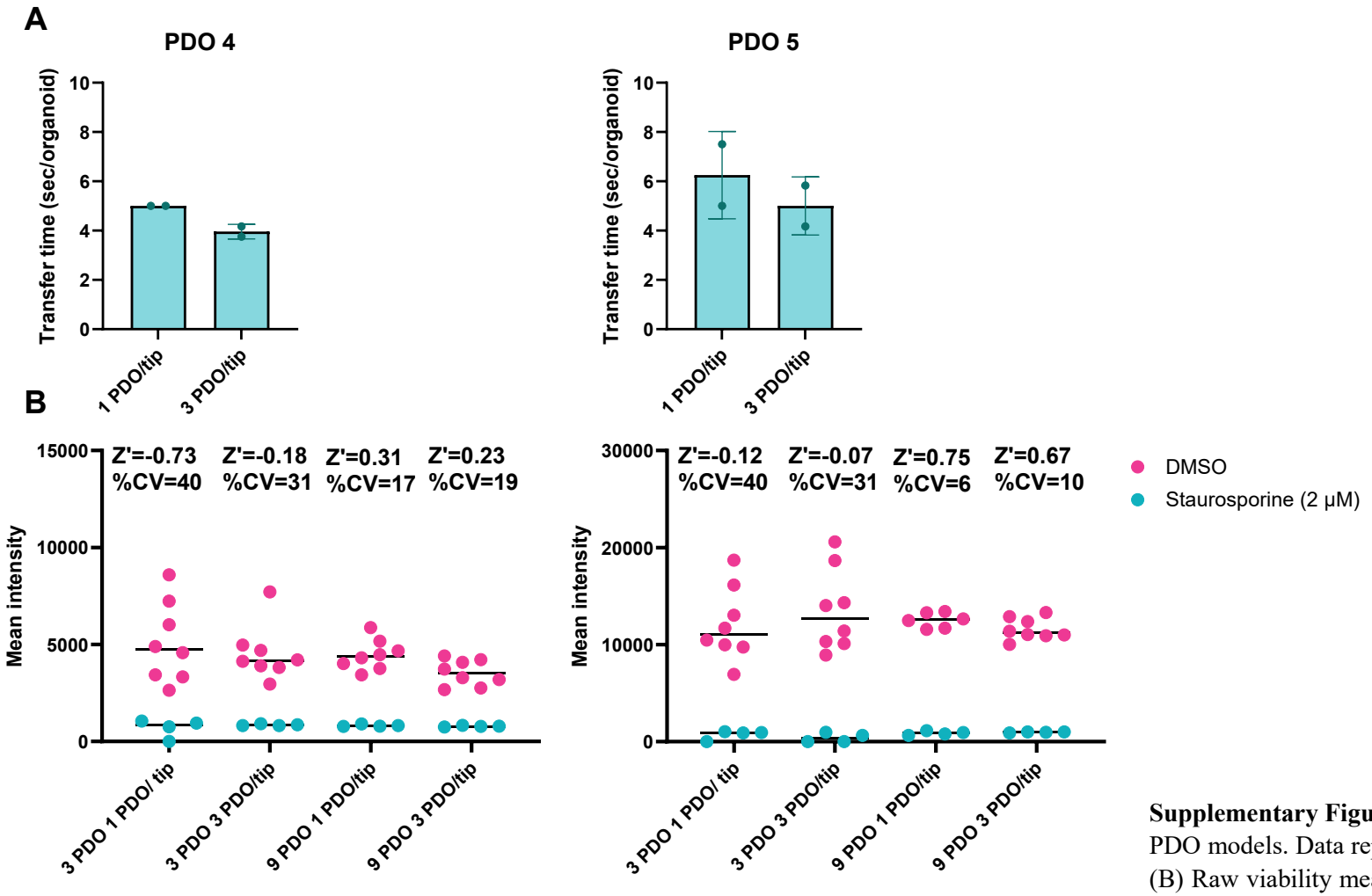
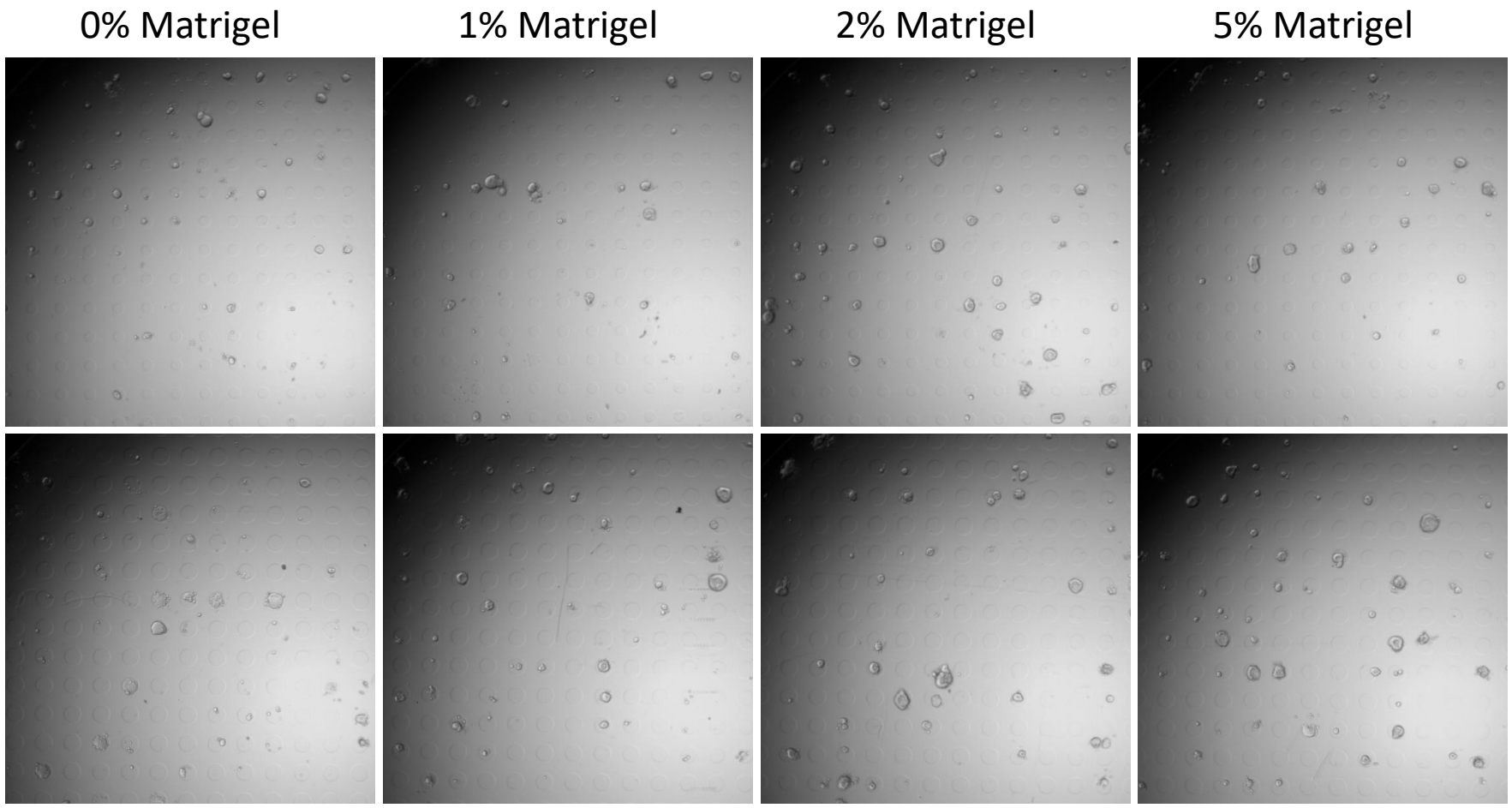
Supplementary Figure 1



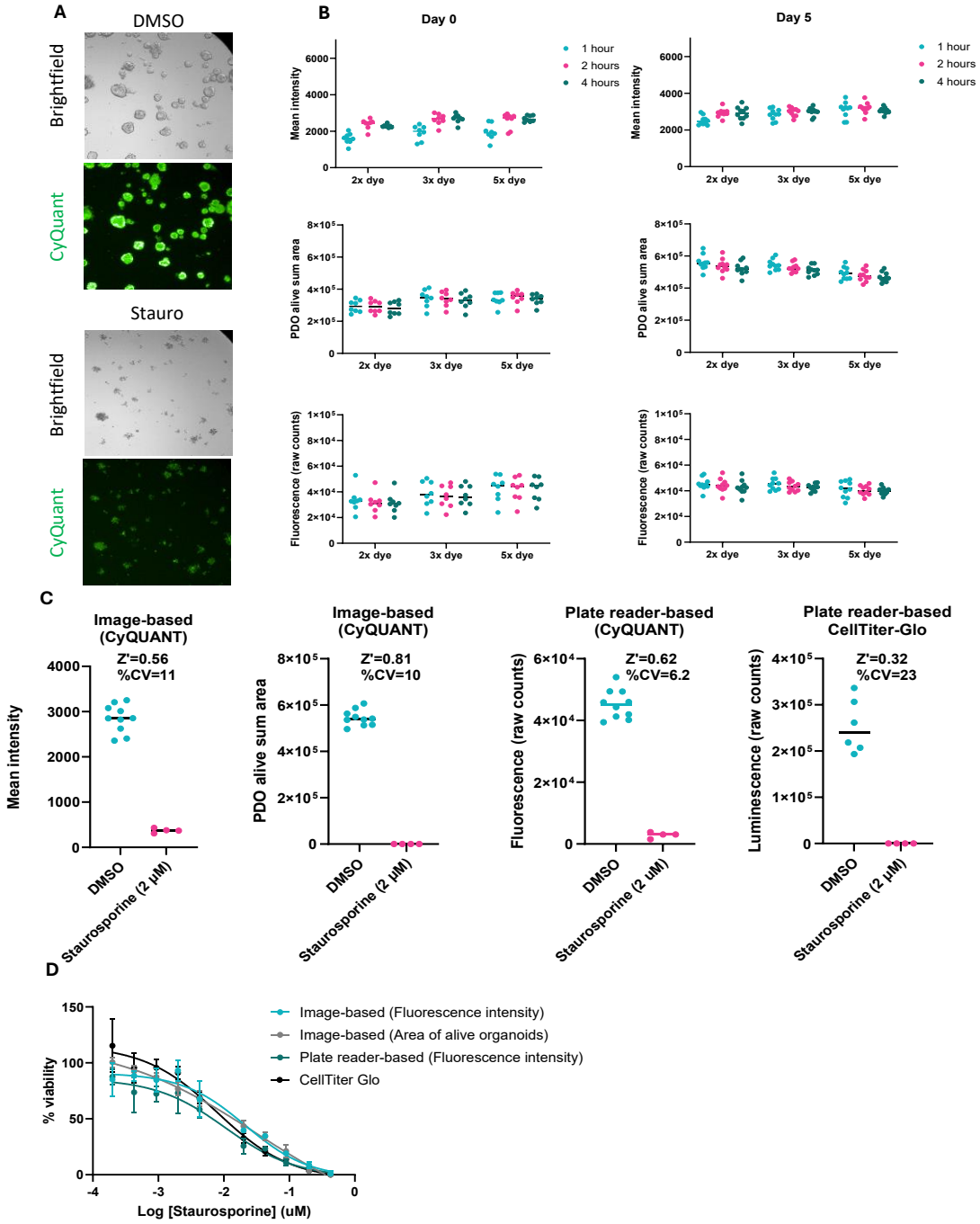
Supplementary Figure 1. (A) PDO transfer time using 1 or 3 PDOs per tip for two PDO models. Data represents mean and standard deviation of biological duplicates. (B) Raw viability measures of positive and negative control wells for CRC PDO 4 and CRC PDO 5. Data points represent mean and standard deviation of technical replicates, numbers indicate Z'-factor and %CV respectively.

Supplementary Figure 2



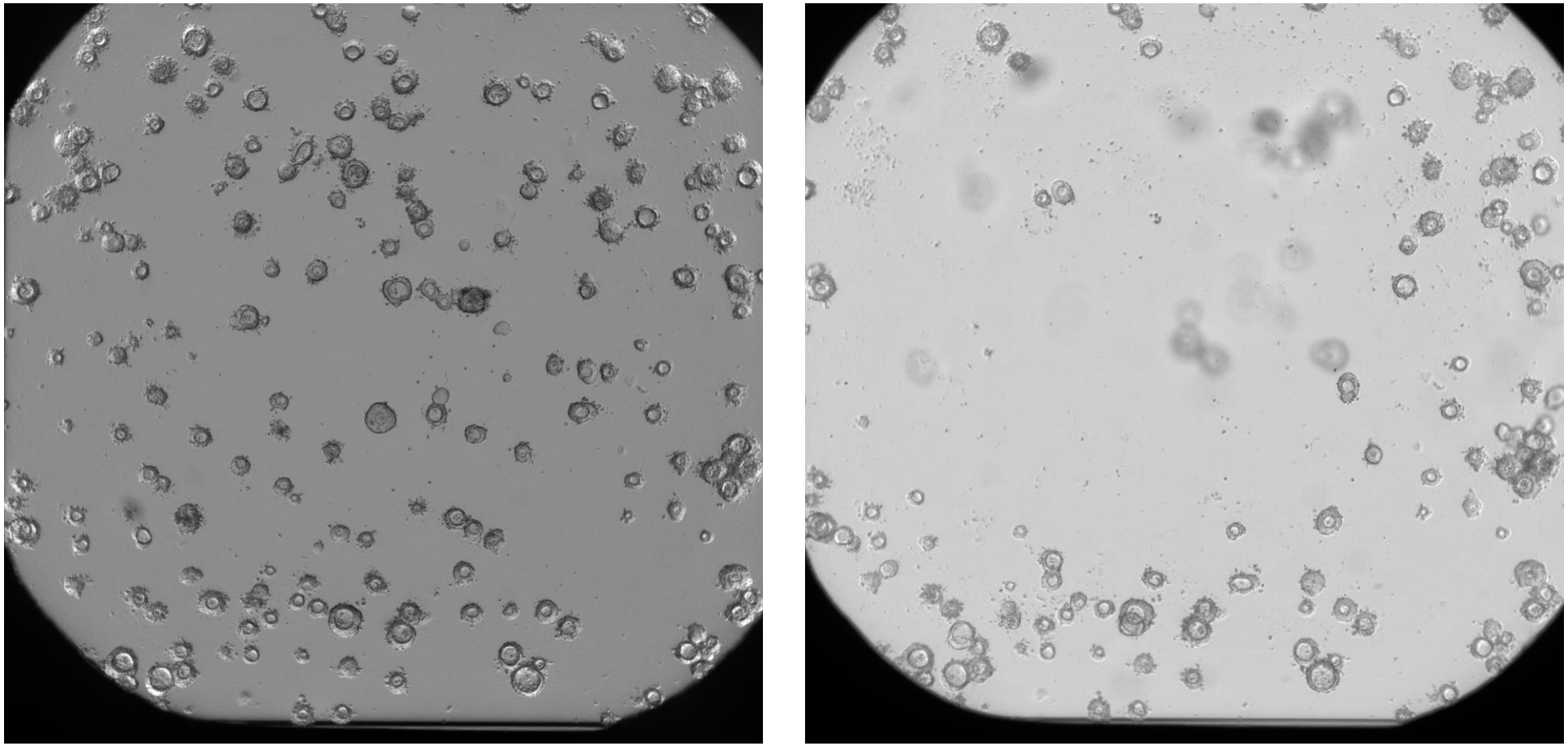
Supplementary Figure 2. Distribution of PDOs upon transfer with YCH to 384-well screening plate. Representative brightfield images of PDO distribution within a well with different ECM percentages; 0,1,2 or 5% at 5x magnification.

Supplementary Figure 3



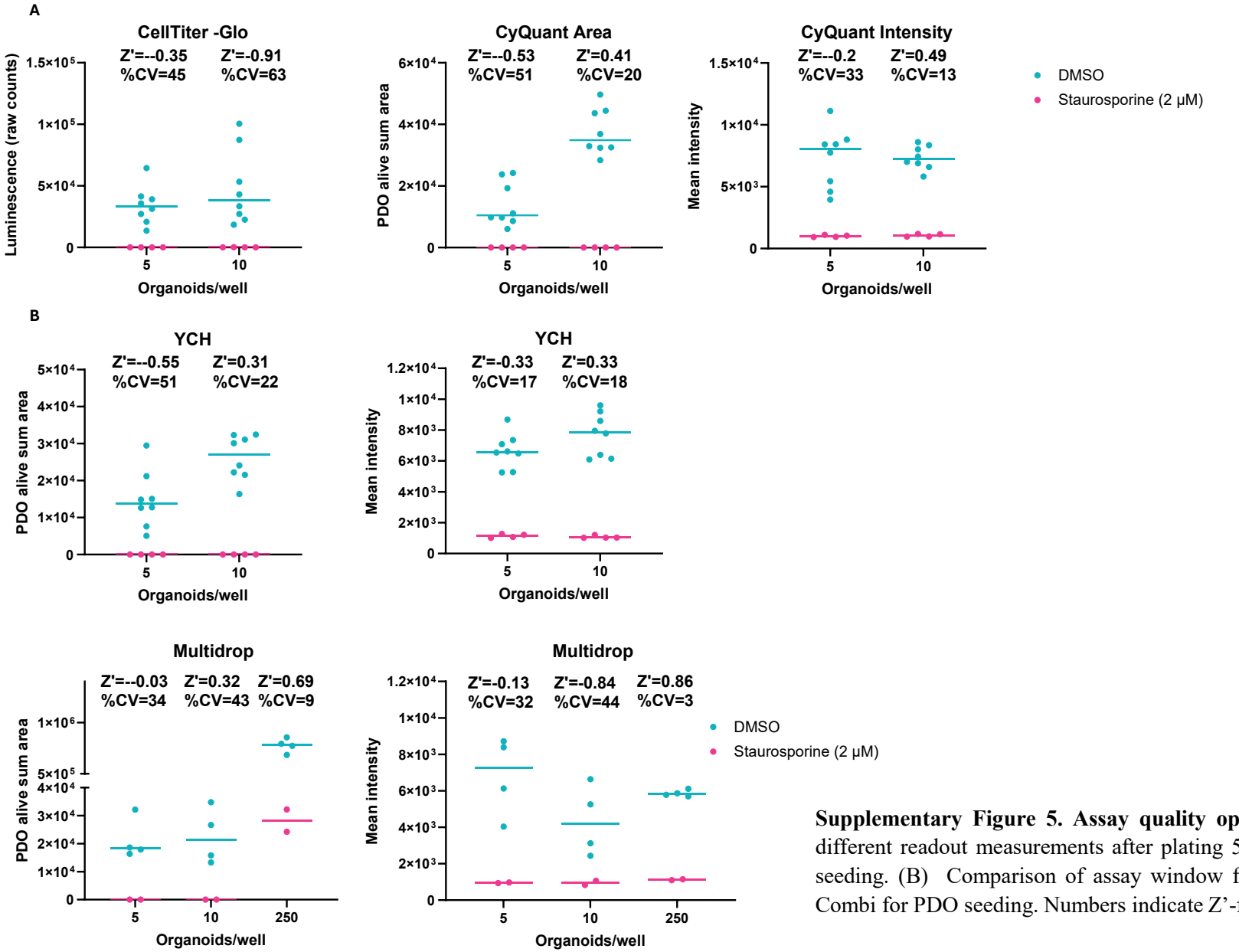
Supplementary Figure 3. CyQUANT image-based readout optimization using 250 PDOs per well. (A) Representative images of PDOs 5 days after incubation with DMSO (0.1%) or Staurosporine. (B) CyQUANT measurements at day 0 and day 5 in PDOs using different incubation times and dye concentrations. (C) Assay window (positive (staurosporine) and negative (DMSO) control wells) for different CyQUANT measurements and CellTiter-Glo measurement. Numbers indicate Z'-factor and %CV respectively. (D) Staurosporine response curves for different readout measurements in one PDO model. Data represents the mean from technical replicates (n=10 DMSO and n=4 Staurosporine).

Supplementary Figure 4



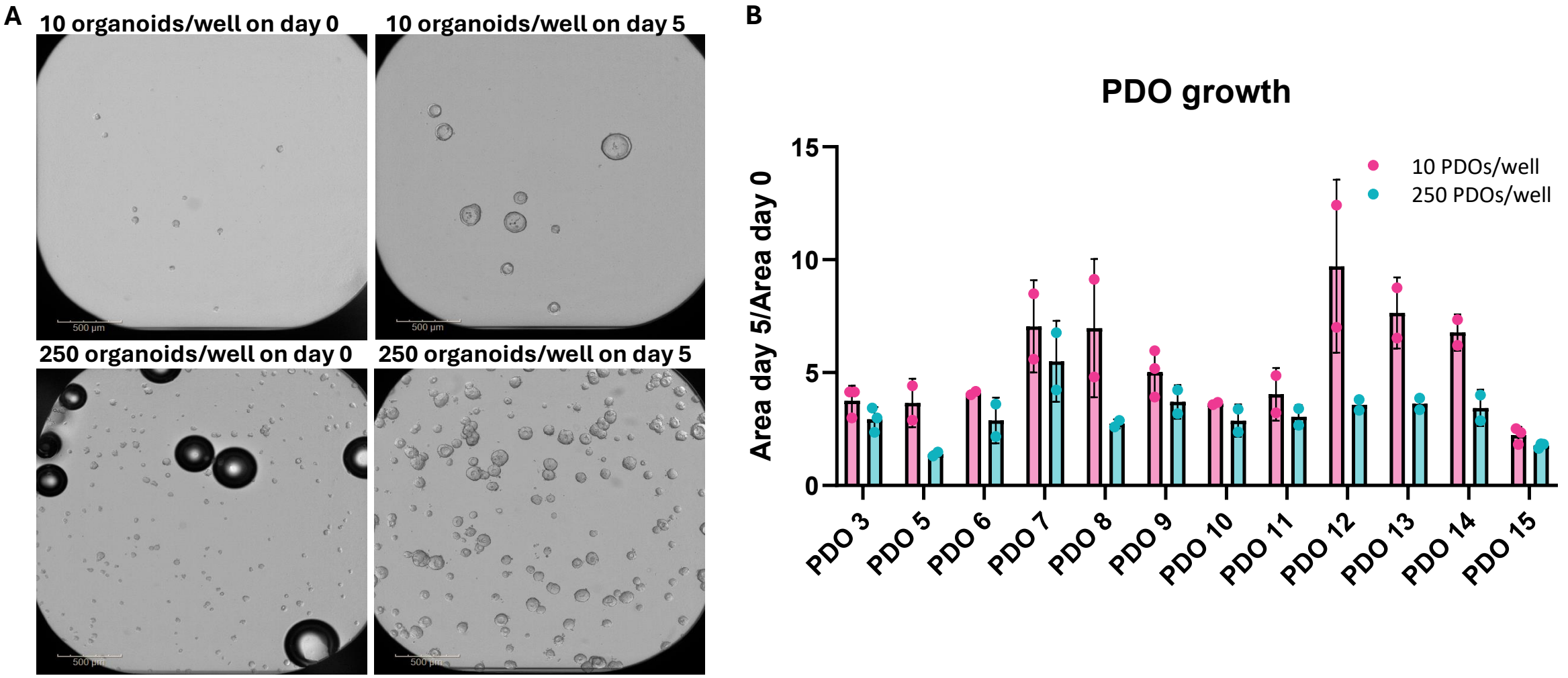
Supplementary Figure 4: PDO detachment upon CyQUANT addition using the Multidrop Combi dispenser. PDOs in 384 well-plate before (left) and after (right) addition of CyQUANT solution using the Multidrop Combi dispenser.

Supplementary Figure 5



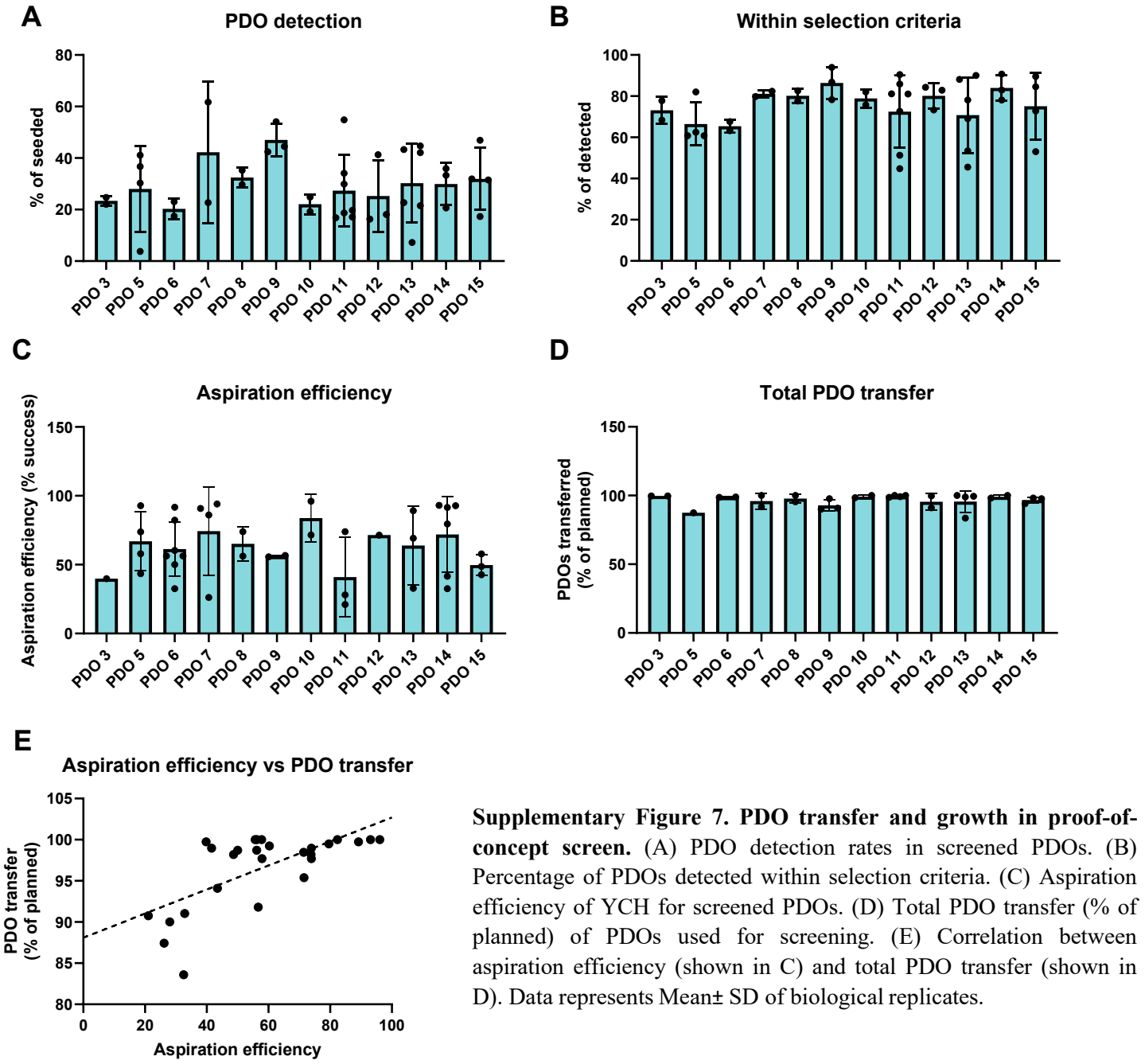
Supplementary Figure 5. Assay quality optimization for low PDO numbers. (A) Assay window different readout measurements after plating 5-10 PDOs per well using the Multidrop Combi for PDO seeding. (B) Comparison of assay window for CyQUANT measurements using YCH and Multidrop Combi for PDO seeding. Numbers indicate Z'-factor and %CV respectively.

Supplementary Figure 6



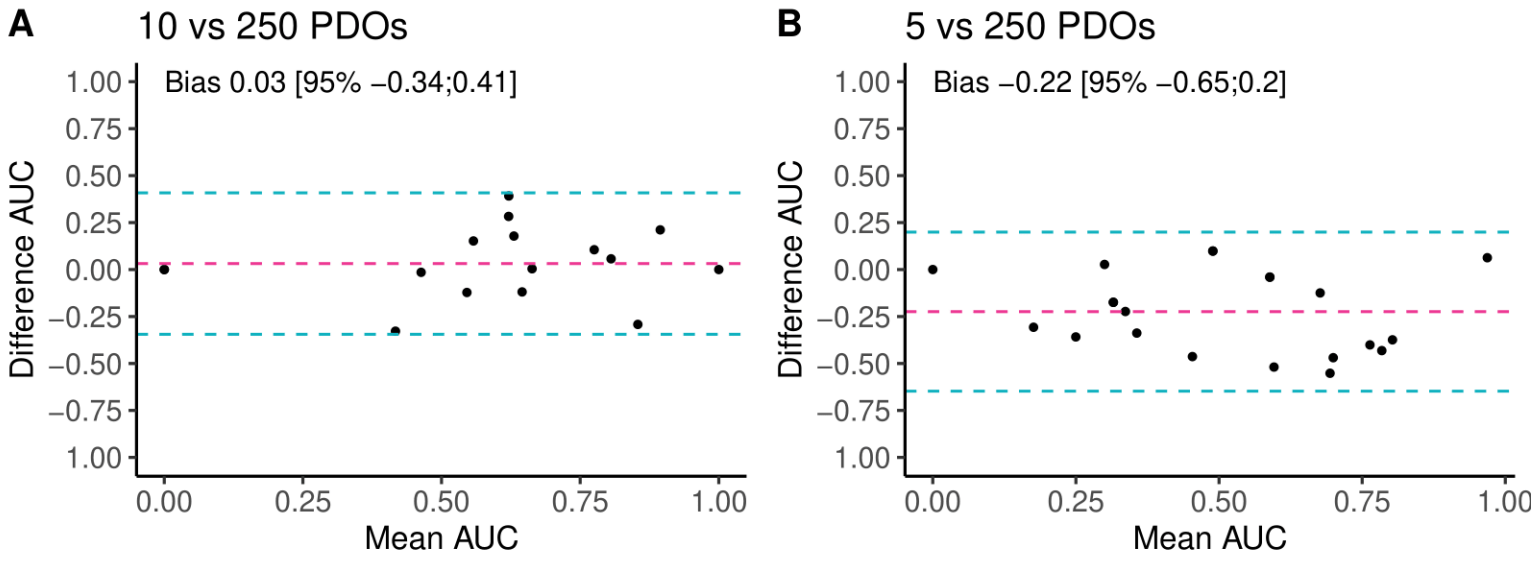
Supplementary Figure 6. PDO growth of 10 organoids per well and 250 organoids per well. (A) Images of PDO growth compared between day 0 (left) and day 5 (right) of 10 organoids per well (top) and 250 organoids per well (bottom). (B) PDO growth for PDOs, directly comparing 10 and 250 PDOs per well. Data represents Mean± SD of biological replicates.

Supplementary Figure 7



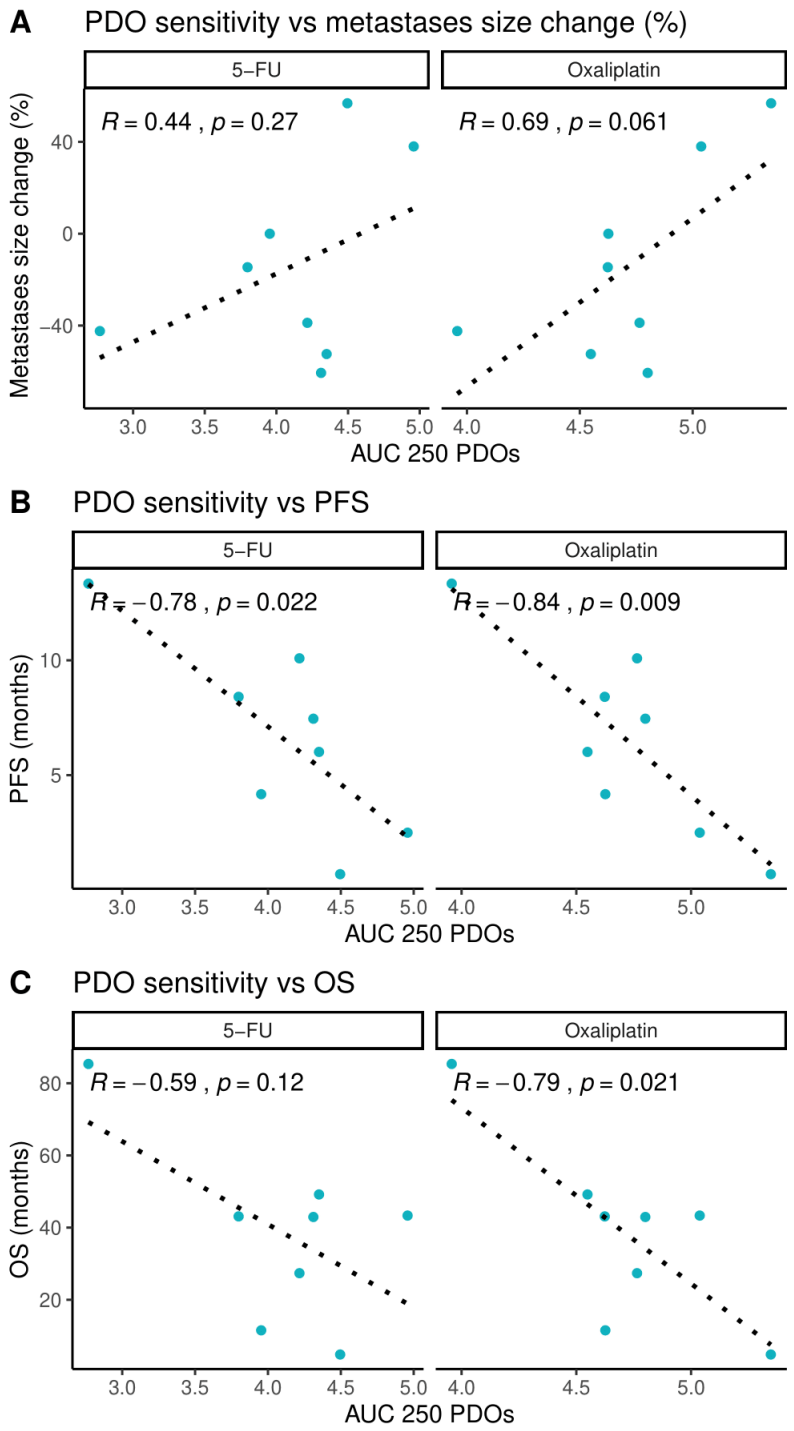
Supplementary Figure 7. PDO transfer and growth in proof-of-concept screen. (A) PDO detection rates in screened PDOs. (B) Percentage of PDOs detected within selection criteria. (C) Aspiration efficiency of YCH for screened PDOs. (D) Total PDO transfer (% of planned) of PDOs used for screening. (E) Correlation between aspiration efficiency (shown in C) and total PDO transfer (shown in D). Data represents Mean± SD of biological replicates.

Supplementary Figure 8



Supplementary Figure 8. Bland-Altman plots of PDO response to 5-FU and oxaliplatin with different seeding densities (A) The difference between normalized AUCs of drug screens using 10 versus 250 PDOs per well. (B) The difference between normalized AUCs of drug screens using 10 versus 250 PDOs per well. Data represents bias (mean difference) and 95% limits of agreement.

Supplementary Figure 9



Supplementary Figure 9. Correlation of PDO responses using 250 PDOs per well and size change metastatic lesions, PFS and OS of patients treated with 5-FU and oxaliplatin combination treatment. (A) Correlation between tumor size change and intensity of PDO responses to 5-FU and oxaliplatin. (B) Correlation between patient progression-free survival and CyQUANT intensity of PDO responses to 5-FU and oxaliplatin. (C) Correlation between patient overall survival and CyQUANT intensity of PDO responses to 5-FU and oxaliplatin. Data points represent different PDOs. Measurements shown are mean of different biological replicates. AUCs are based on mean CyQUANT intensity drug response curves.

Supplementary Table 1

Supplementary Table 1. List of PDOs used in this study linked to corresponding experiments and figures. The table includes the corresponding HUB codes and mutational status, if relevant.

PDO number	HUB codes	Used in Figure(s)	Mutations, if relevant
PDO 1	HUB-02-B2-006	2, 3, 4, 5, 6, 7, sup. fig. 3, sup. fig. 5	BRAF mutant
PDO 2	HUB-02-C2-055	6	
PDO 3	HUB-02-C2-154-IV	7, sup. fig. 7	KRAS wild-type (WT)
PDO 4	HUB-HS-02-C2-M18-00204-I	Sup. fig. 1	
PDO 5	HUB-02-C2-023	Sup. fig. 1	
PDO 6	HUB-02-B2-164	Sup. fig. 7	
PDO 7	HUB-02-C2-019	8, sup. fig 7	
PDO 8	HUB-HS-02-C2-M18-00166-I	Sup. fig. 7	
PDO 9	HUB-HS-02-C2-M19-00708-I	8, sup. fig. 7	
PDO 10	HUB-HS-02-C2-M19-00801-I	8, sup. fig. 7	
PDO 11	HUB-02-C2-000	8, sup. fig. 7	
PDO 12	HUB-HS-02-C2-M20-00016-II	Sup. fig. 7	
PDO 13	HUB-HS-02-C2-M21-00115-I	8, sup. fig. 7	
PDO 14	HUB-02-C2-163	8, sup. fig. 7	
PDO 15	HUB-HS-02-C2-M22-00136-I	8	
PDO 16	HUB-HS-02-C2-M23-00025-I	8	
PDO 17	HUB-HS-02-C2-M23-00044-I	8	
PDO 18	HUB-HS-02-C2-H23-10002-I	8	
PDO 19	HUB-HS-02-C2-M22-00097-I	8	
PDO 20	HUB-HS-02-C2-H23-10014-I	8	
PDO 21	HUB-HS-02-C2-M21-00137-I	8	
PDO 22	HUB-HS-02-C2-H23-10026-I	8	
PDO 23	HUB-HS-02-C2-M22-00076-I	8	
PDO 24	HUB-HS-02-C2-M22-00097-I	8	
PDO 25	HUB-HS-02-C2-M23-00047-I	8	
PDO 26	HUB-HS-02-C2-M23-00061-I	8	

Supplementary Table 2

Supplementary Table 1. List of PDOs used in this study linked to corresponding experiments and figures. The table includes the corresponding HUB codes and mutational status, if relevant.

Drug	Figure	Source	Concentration range
5-FU	6	15596885 (UMCU)	PDO1: IC10:2.60 µM IC30:33.2 µM IC50: 164.2 µM PDO2: IC10:3.25 µM IC30:23.3 µM IC50: 80.0 µM
Panitumumab	7	15343561 (UMCU)	30,000 – 0.11444 ng/ml (4-fold)
5-FU	8 and 9 (10 PDO per well)	15596885 (UMCU)	800-0.33 µM (7-fold)
Oxaliplatin	8 (10 PDO per well)	15532585 (UMCU)	300-2.22 µM (5-fold)
5-FU	8 and Sup. Fig. 9 (250 PDO per well)	15596885 (UMCU)	900-0.05 µM (3-fold)
Oxaliplatin	8 and Sup. Fig. 9 (250 PDO per well)	15532585 (UMCU)	500-0.03 µM (3-fold)
5-FU	8 (5 PDO per well)	15596885 (UMCU)	900-1.44 µM (5-fold)
Staurosporine	Sup. Fig. 3	Merck Life Science N. V. (37095)	0.43-0.0002 µM (2.2-fold)