Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic Supplementary Informations

Simple droplet microfluidics platform for drug screening on cancer spheroids

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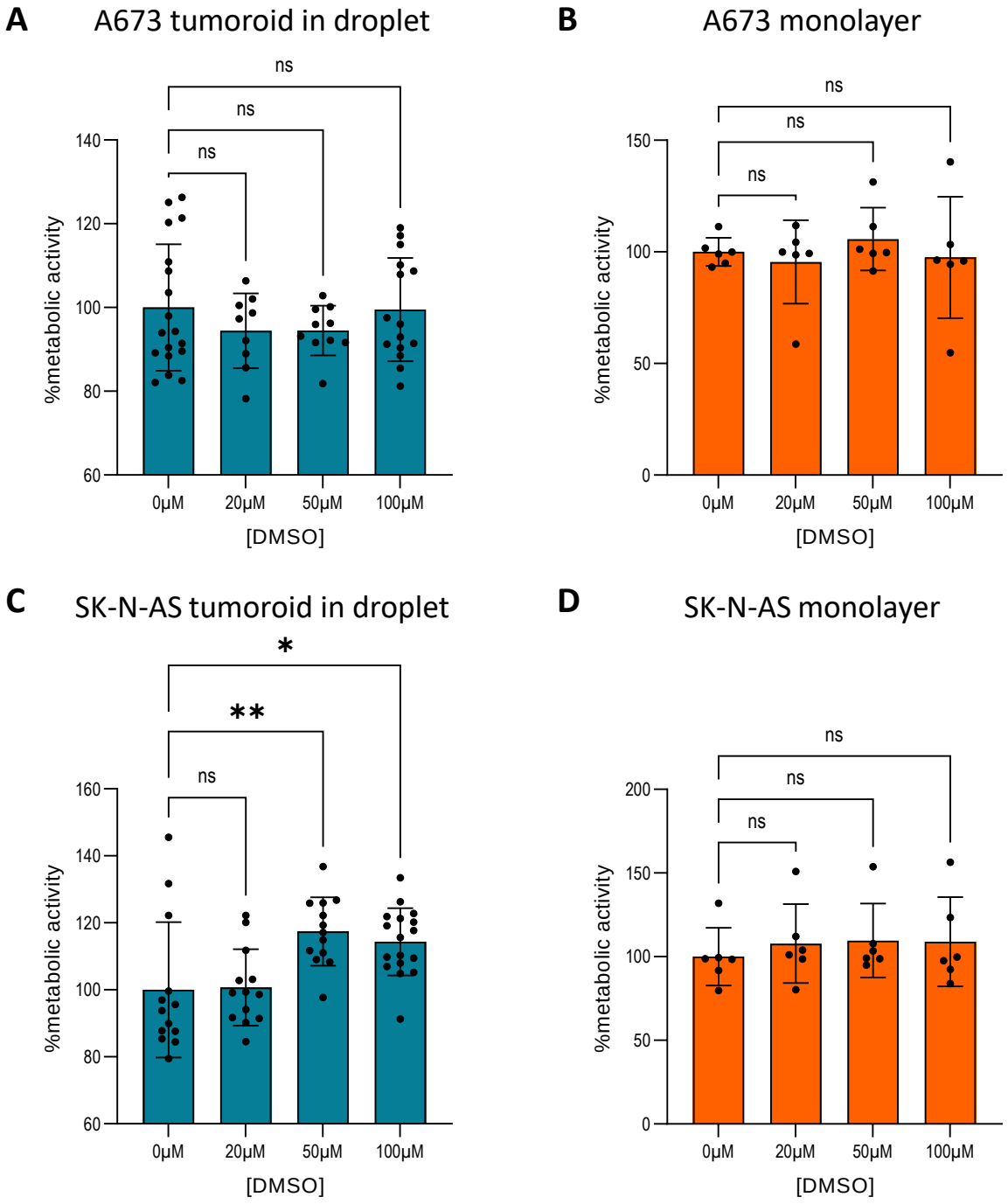

Fig. S1 Impact of DMSO on cells at different concentration for A) A673 tumoroid in droplet, B) A673 monolayer, C) SK-N-AS tumoroid in droplet and D) SK-N-AS monolayer. Conditions were similar as ones used with drug: cells were seeded for 24h and exposed to DMSO for 48h. Metabolic activity was determined using alamarBlue assay. Initial cell number was about 8,000 cells per well in monolayer and 350 cells per droplet in tumoroid. Each point represents one tumoroid or well. Errors bars represent SD on the mean. Data did not pass normality test, non-parametric Kruskal-Wallis test was performed using GraphPad Prism 9.3.1. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

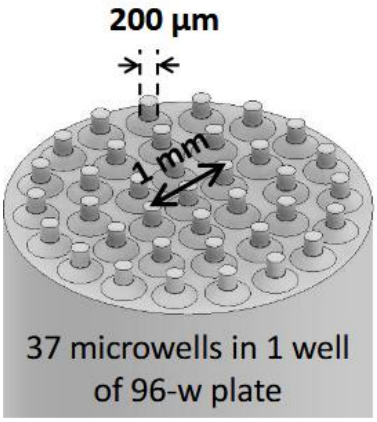

Fig. S2 Scheme of the stamp for agarose microwells production. 34 pillars of diameter 200µm are distributed over a cylinder fitting in a well of a 96-wells plate.

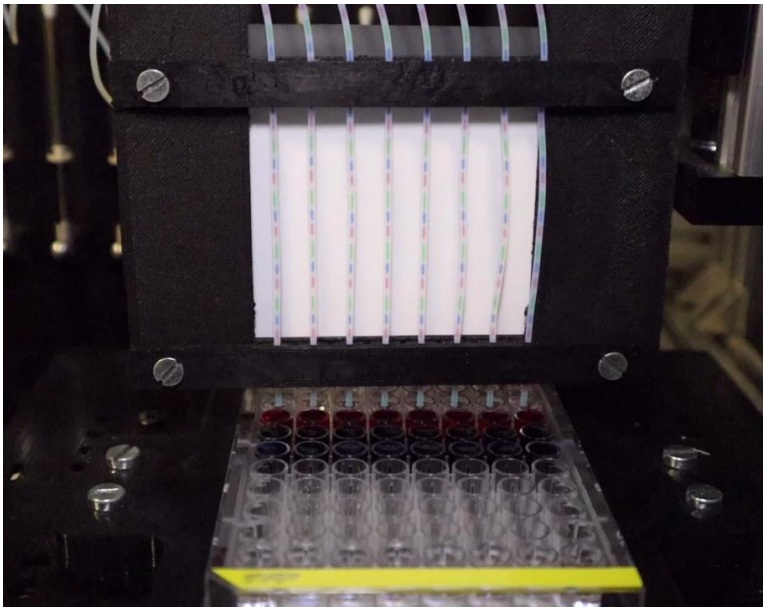

Fig. S3 (Caption) Video of the drug screening platform during filling of 8 tubes with colored droplets (food coloring in water). Real speed.

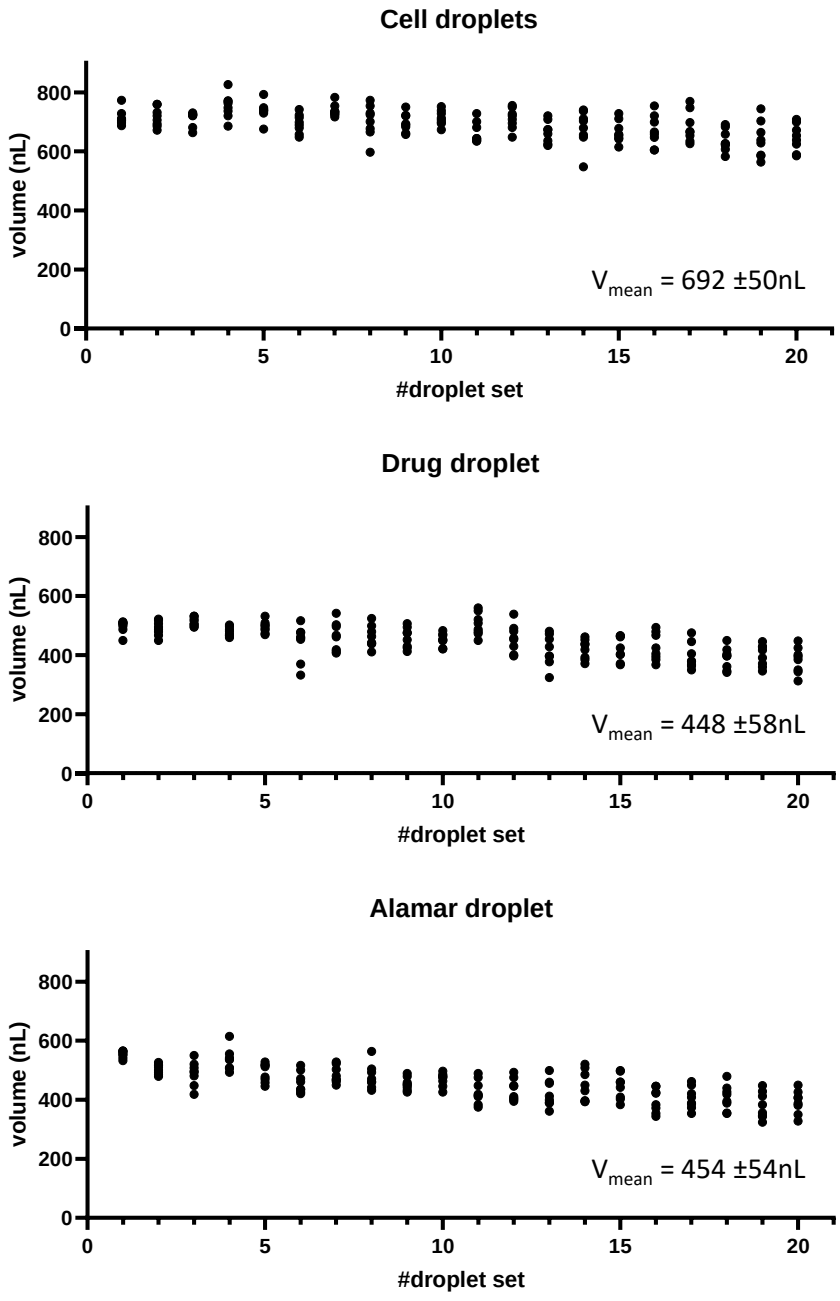

Fig. S4 Volumes of cell, drug and alamar droplets. Droplets were generated by pipetting 20 trains of colored droplets in each of the 8 tubes in parallel. Volumes were computed from the length of the droplets measured on fluorescence images of the tubes.

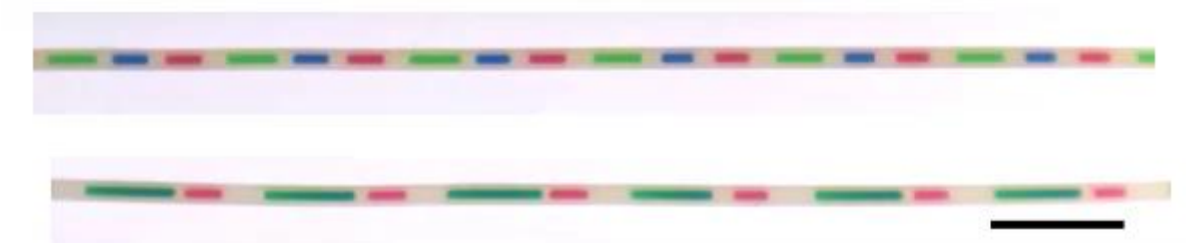

Fig. S5 (Caption) Video of the mergings of droplets. Top shows the first merging, bottom the second. Droplets were made of food coloring diluted in water. A fast flow (5µL/s) is applied, then a slow flow (0.3µL/s) to bring the droplets back to their initial position. This operation is repeated twice for each merging. Speed x10. Scale bar: 1cm.

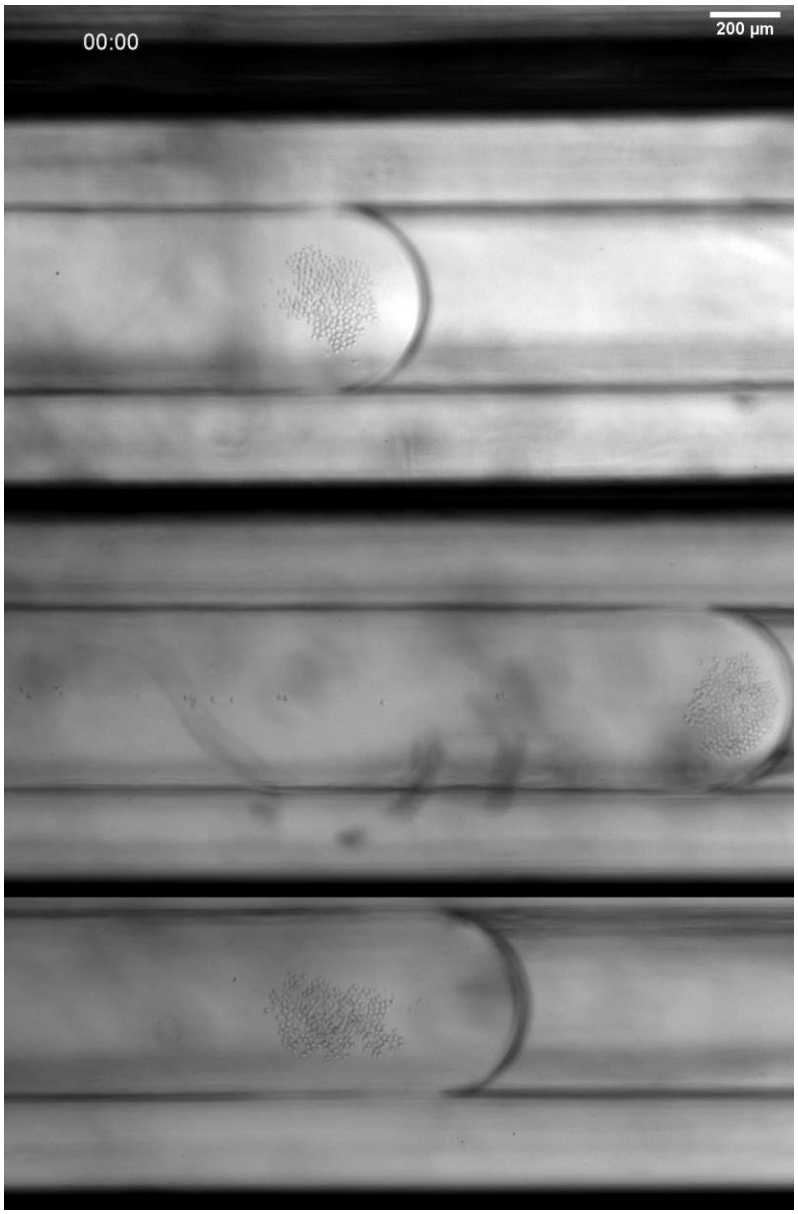

Fig. S6 (Caption) Video of tumoroid formation for 17h. Images were made every 1min for 4h, then every 20min in microscope (Nikon Ti, magnification 4x) with a controlled environment chamber (5%CO_2, 37°C).

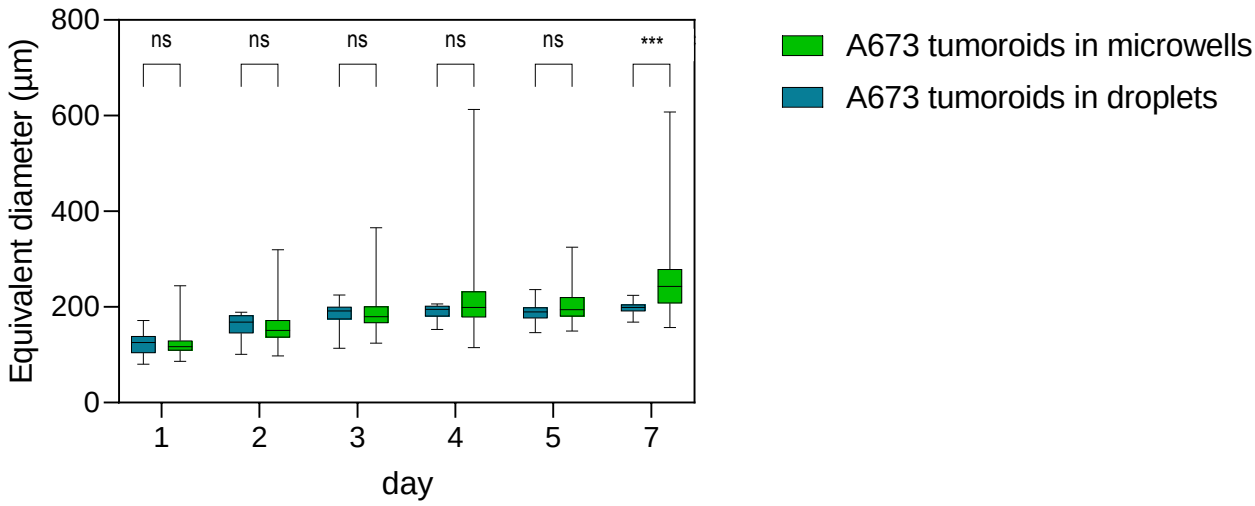

Fig. S7 Comparison of tumoroids diameter grown in droplets and in microwells. Lines represent the median, error bars the min and max values. Mann-Whitney test was performed using GraphPad Prism 9.3.1. ***p<0.001, **p<0.01, *p<0.05.

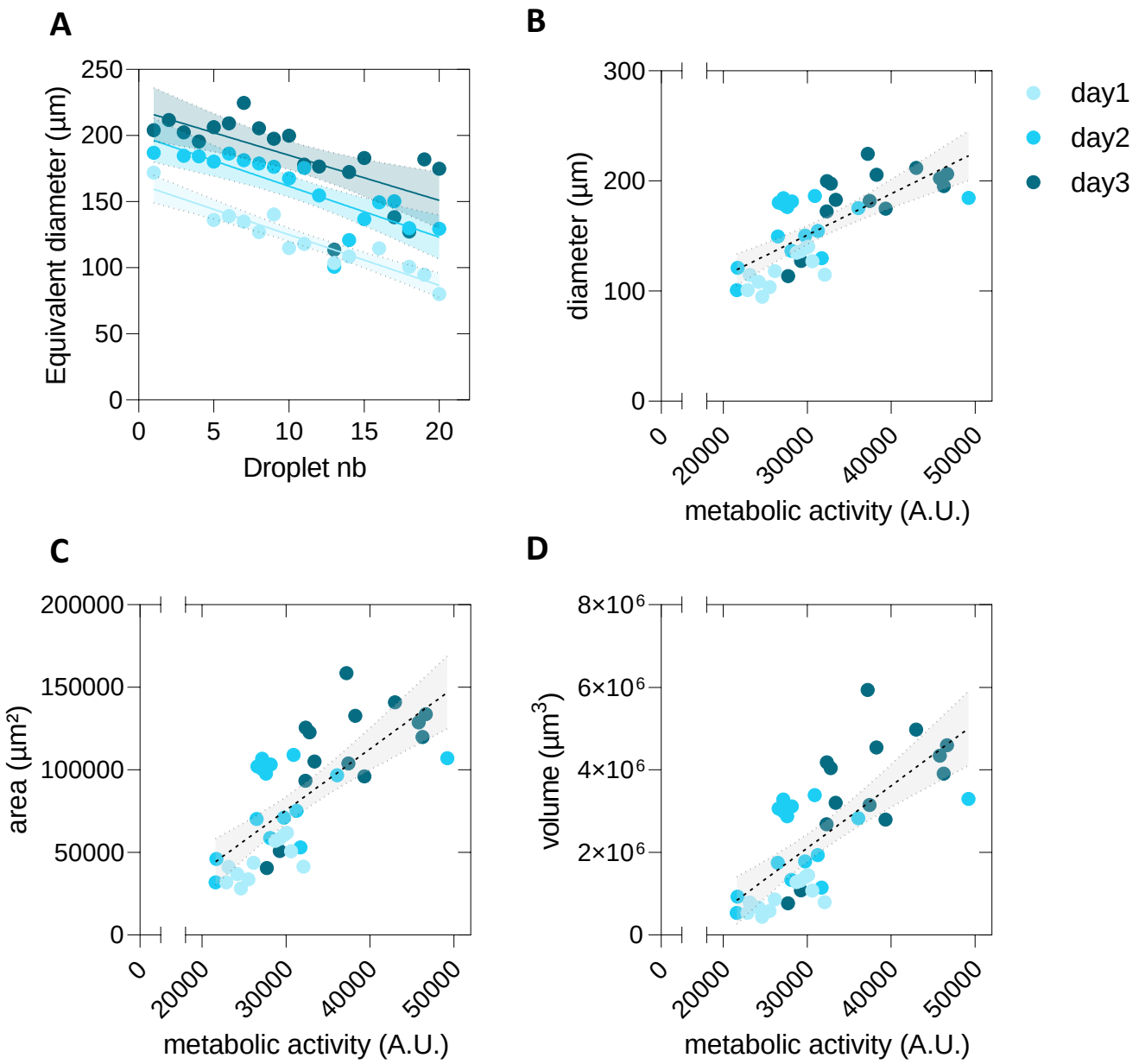

Fig. S8 A) Evolution of tumoroid diameter in droplets across the tube. Lines represent the linear regression with its 95% confidence bands (day1: R^2=0,8781, day2: R^2=0,6416, day3: R^2=0,4638). B) Diameter (R^2=0,5109), C) area (R^2=0,5175) and D) volume (R^2=0,5153) of tumoroids depending on their metabolic activity.

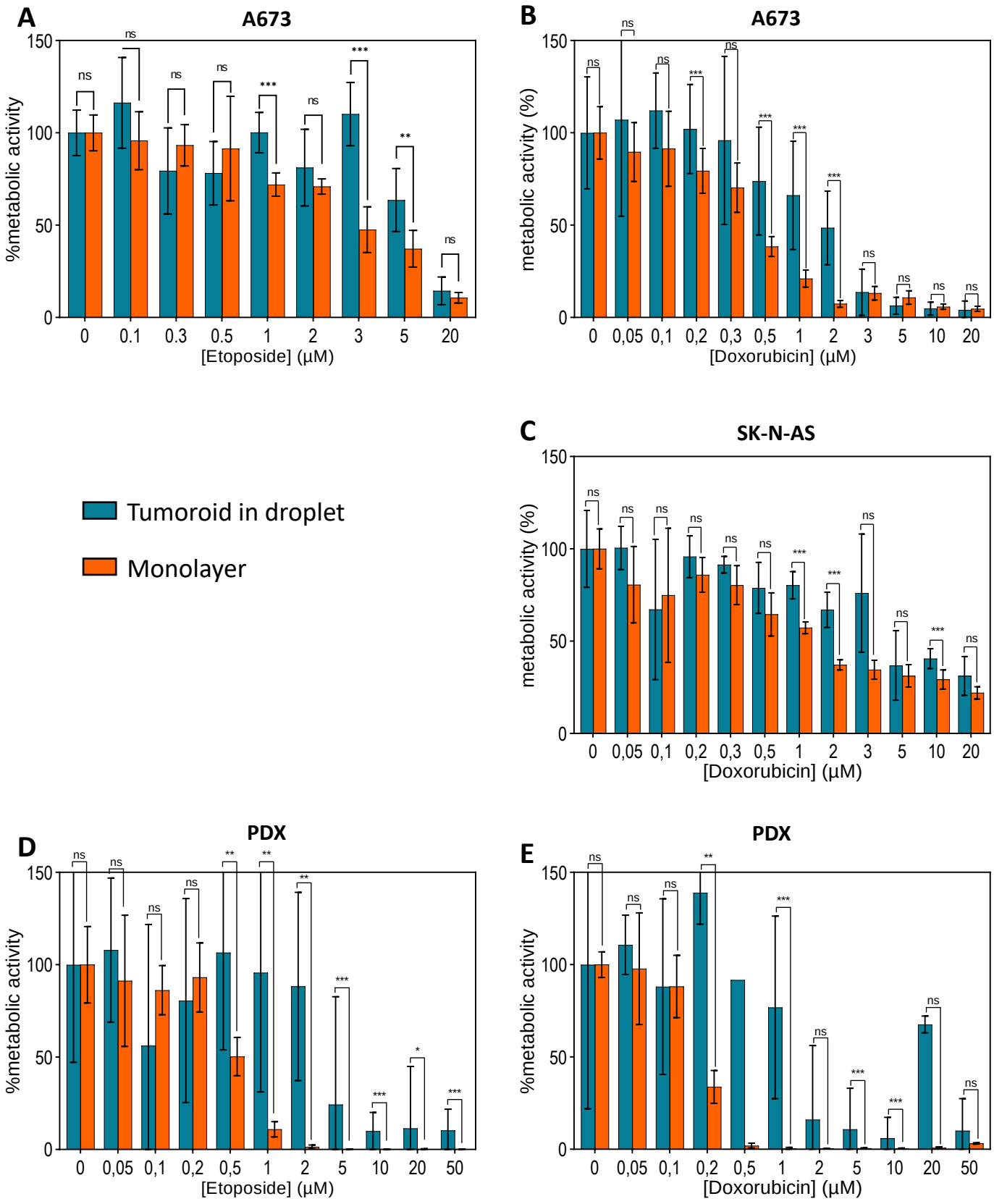

Fig. S9 Comparison of the impact of drug concentration on metabolic activity between tumoroids in droplet and monolayer culture. Metabolic activity was determined using alamarBlue assay. Initial cell number was about 8,000 cells per well in monolayer and 350 cells per droplet in tumoroid. Errors bars represent SD on the mean. Mann-Whitney test was performed using GraphPad Prism 9.3.1. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

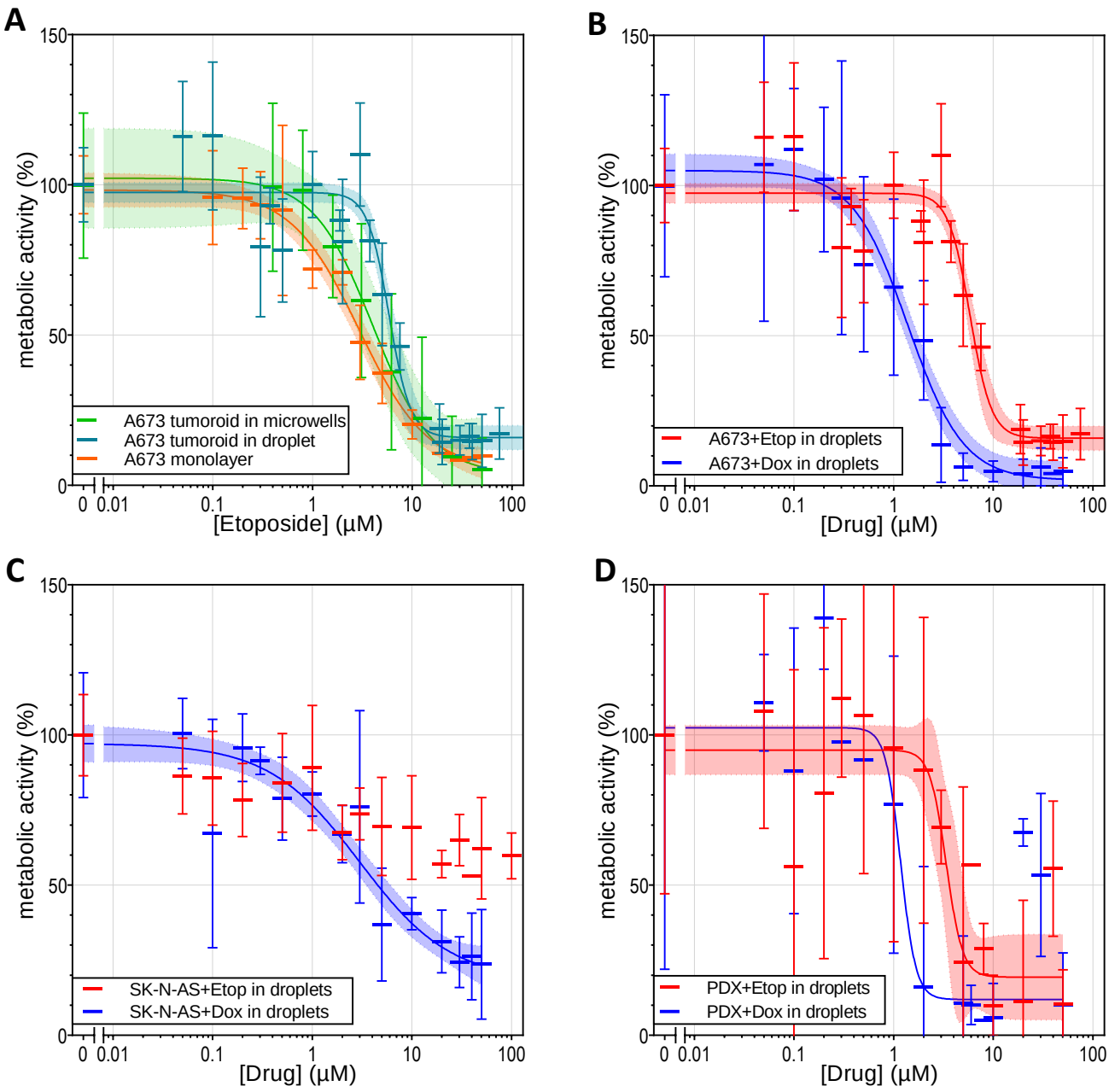

Fig. S10 A) Comparison of dose-response curves of A673 exposed to etoposide in mononayer (orange), tumoroids in microwells (green) and tumoroids in droplets (blue). B), C) & D) Comparison of dose-response curves of respectively A673, SK-N-AS and PDX exposed either to etoposide (red) or doxoruicin (blue). Cells were seeded for 24h and exposed to drug for 48h. Metabolic activity was determined using alamarBlue assay. Errors bars represent SD on the mean. Straight line represents the curve fitting to a 4-parameter sigmoid, with its 95% CI. Etop: etoposide, Dox: doxorubicin.

Cell line	Drug	Exposure time	Experiment design	Assay	IC50	Ref
A673	Etoposide	96h	0.5-1 10^4 cells/well, 96 wells plate	MTS	0,88µM	Boehme *et al.*[1]
		24h	200,000 cells/well, 6 wells plate	Flow cytometry (7-AAD & Annexin V-FITC)	>200µg/mL = >340µM	Chevalier *et al.*[2]
	Doxorubicin	96h	0.5-1 10^4 cells/well, 96 wells plate	MTS	27,18nM = 0,027µM	Boehme *et al.*[1]
		24h	200,000 cells/well, 6 wells plate	Flow cytometry (7-AAD & Annexin V-FITC)	2µg/mL = 3,7µM	Chevalier *et al.*[2]
SK-N-AS	Etoposide	48h	3 000 cells/well, 96 wells plate	MTT	80µM	Das *et al.*[3]
		48h	1 10^4 cells/well 96 wells plate	MTS	Not determined but similar, only 4 points	Day *et al.*[4]
		7 days	1 10^3 cells/well 6 wells plate	Flow cytometer (Caspase-GloTM3/7)	0,09µg/mL = 0,15µM	Harvey *et al.*[5]

Tab. S1 Some IC50 values from the literature.

References

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