

## Supplementary materials

# Preclinical Photodynamic Therapy Targeting Blood Vessels with AGuIX<sup>®</sup> Theranostic Nanoparticles

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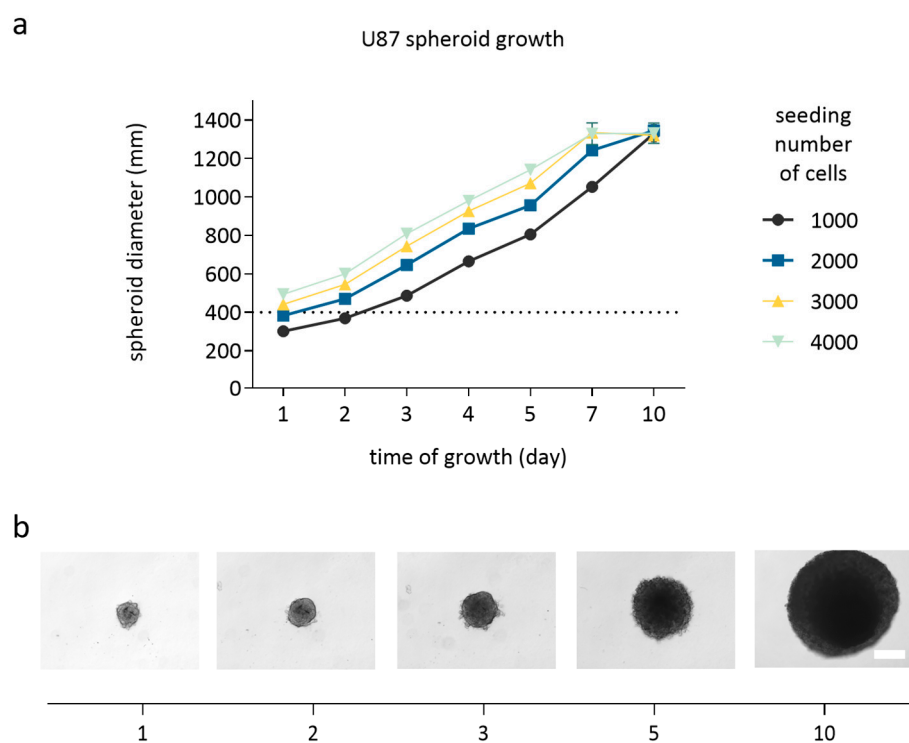
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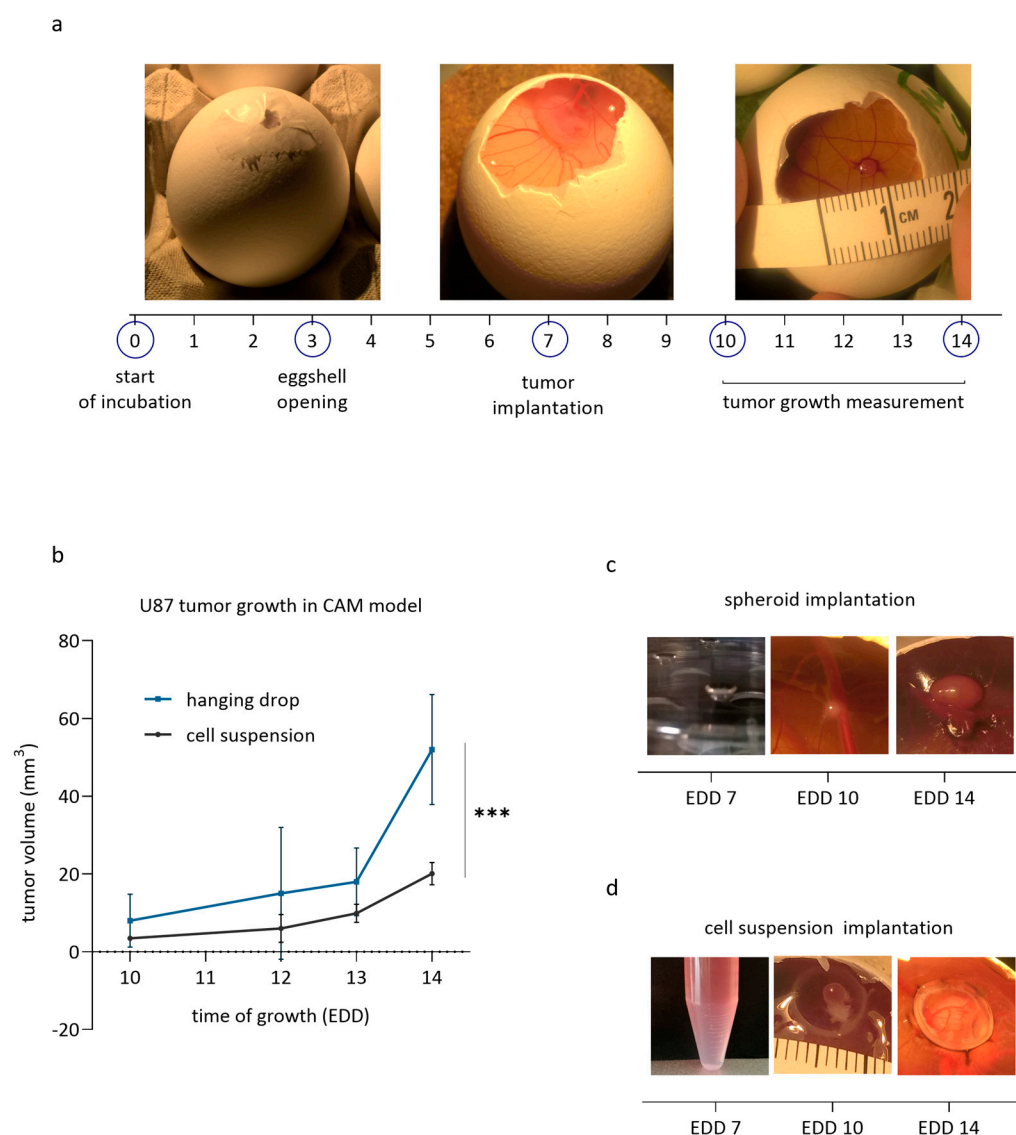
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To test the photodynamic effect in the 3D *in vitro* glioblastoma model, human U87 glioblastoma cells were used to form reproducible 3D spheroids. The seeding density was optimized at 1000 to 1500 cells/well to obtain spheroids with a diameter of 500  $\mu\text{m}$  on day 3 after seeding. The spheroids were maintained for ten days. As measured by diameter, spheroids started with 400 reaching 1100  $\mu\text{m}$  at day 10. The spheroids formed compact round structures on day 1, which increased in size over time (Figure S1).



**Supplementary Figure S1.** Optimization of the 3D spheroid model of cultured glioblastoma U87 cells. Spheroid kinetics and morphology of U87. **(a)** Growth kinetics represented in the diameter of the spheroid over 10 days at a seeding density (1000-4000 cells/well)  $N=3$  **(b)**. Representative images of U87 spheroids grown at the optimal seeding density of 1500 cells between days 1 and 10. The scale bar represents 300  $\mu\text{m}$ .



**Supplementary Figure S2.** Optimization of the chicken CAM model of U87 human glioblastoma tumors. **(a)** Cultivation protocol that presents the time frames for model maintenance. Growth curves of U87 tumors growing in CAM for different implantation methods. **(b)** Representative images of the stages of the hanging drop and cell suspension implantation methods in CAM. Photographs show a model in different stages of cultivation (**c,d**).  $n=3$ ,  $***P<0.001$  A statistically significant difference was observed in tumor sizes on day 14 between hanging drop vs cell suspension implantation methods.