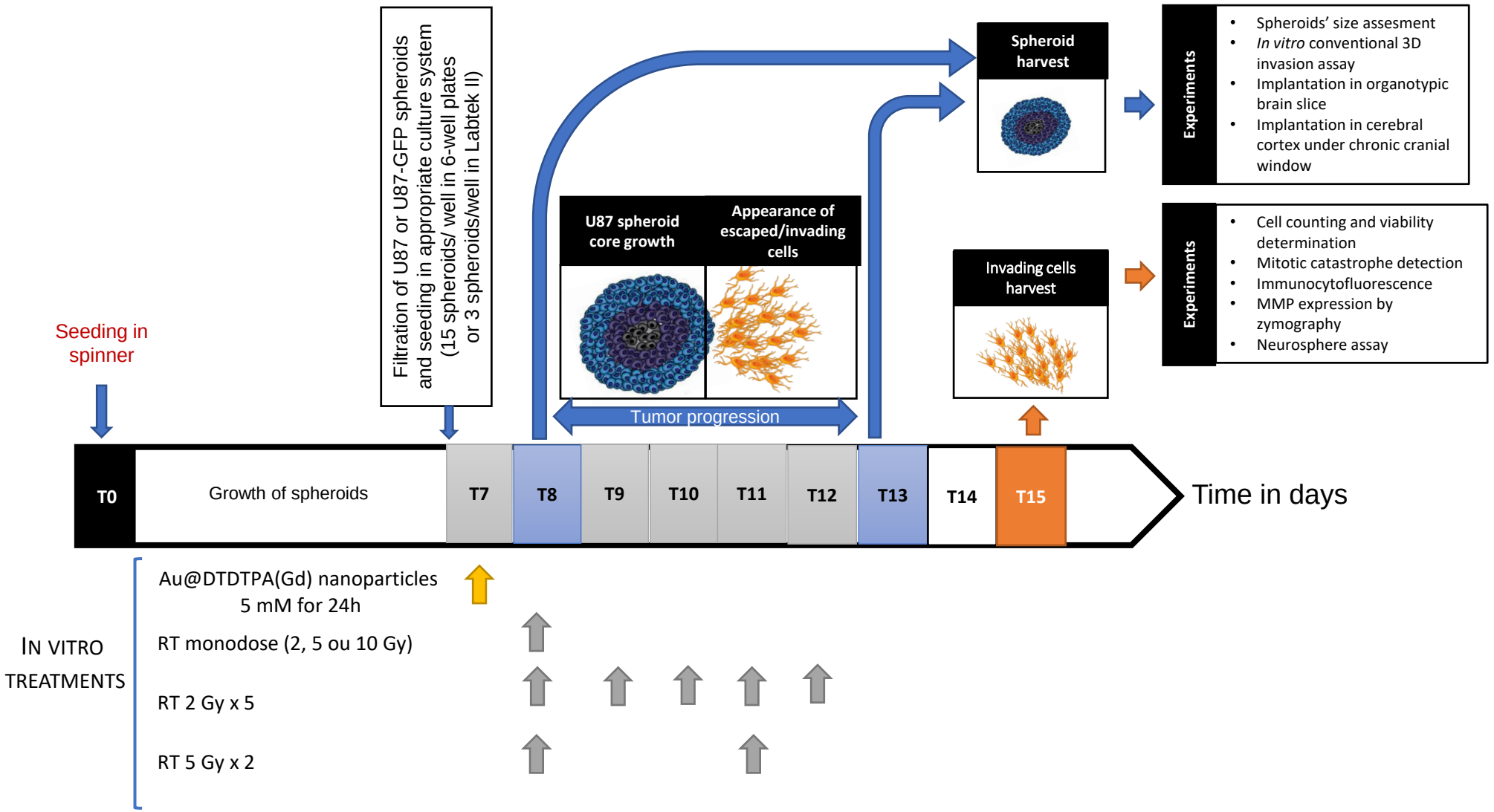
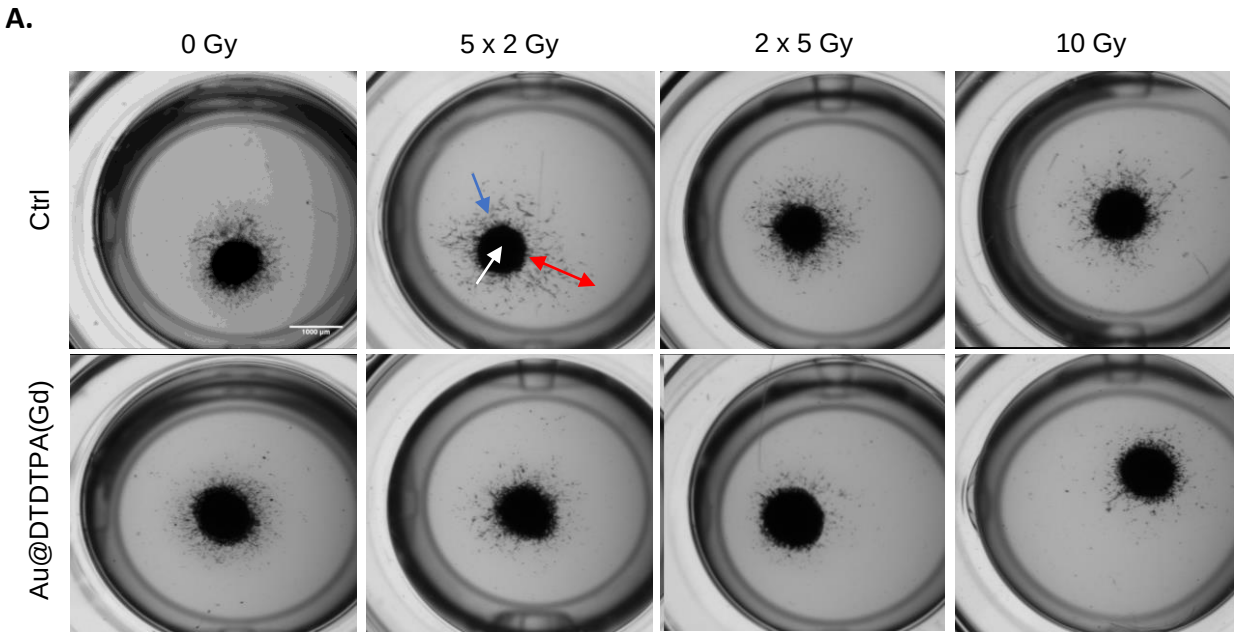
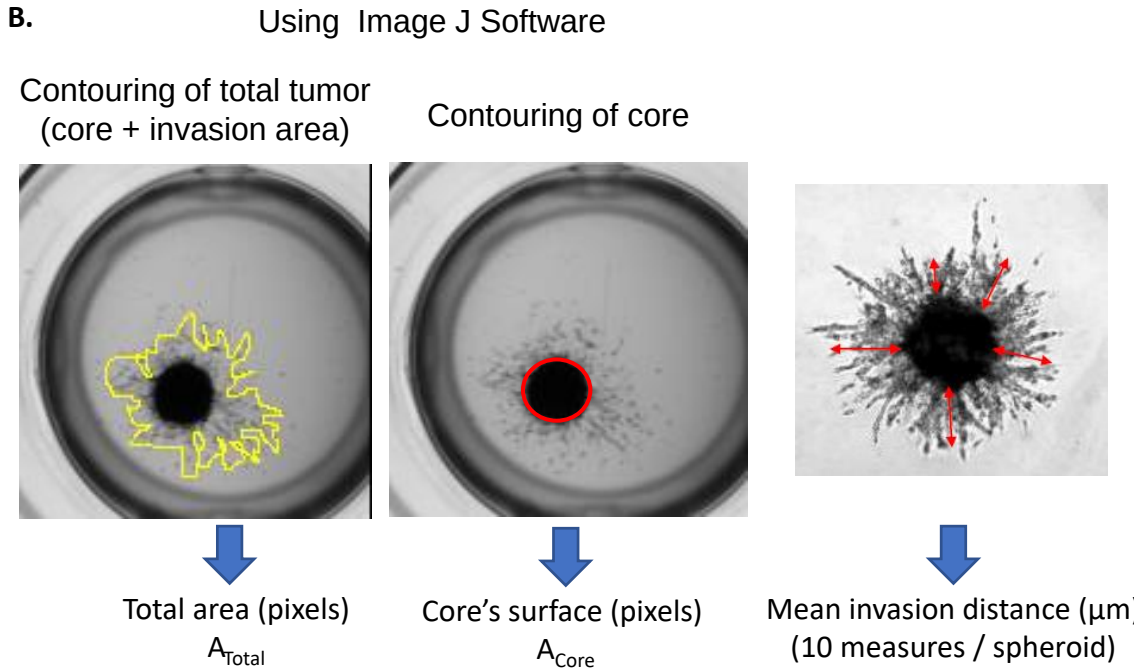
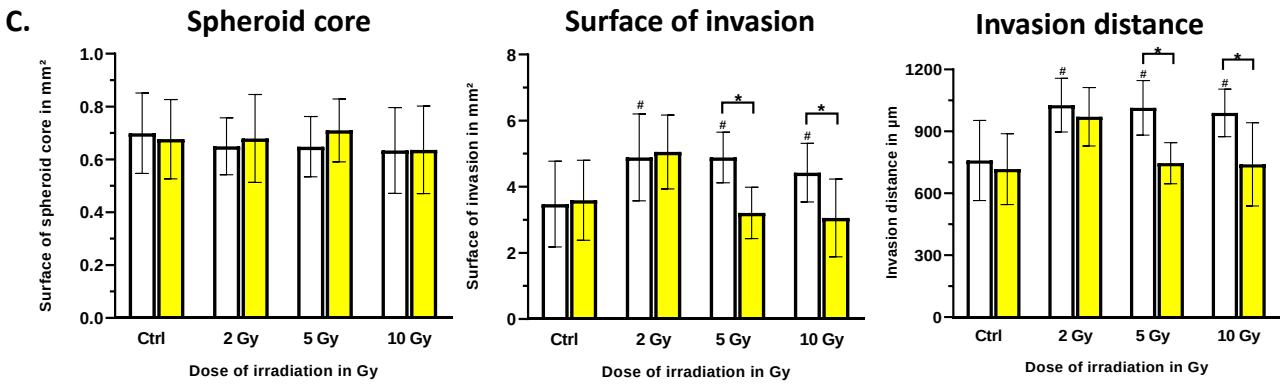
Supplementary Methods 1 : Graphical representation of the experimental process for in vitro assays.



Supplementary Methods 2 : Conventional 3D invasion assays and calculation of invasion surface.







Calculation of invasion surface (mm^2)

$$\frac{(A_{Total} - A_{core}) \times 10.58^2}{10^6}$$

Conventional 3D invasion assays. **(A)** U87 spheroids were harvested at T8 or T13 after treatments and were seeded in 6-well culture plates (one spheroid per well): each one was embedded in a mixture of Matrigel®, complete culture medium enriched with hyaluronic acid. Culture dishes were maintained at 37°C for 96h to allow the tumor progression (*i.e.* the growth of tumor core and the appearance of infiltration). **The white arrow represents the spheroid's core. The blue arrow represents the cell invasion. The red arrow represents the distance of invasion. (B)** Using ImageJ software, the total area of the tumor (manual yellow delineation) and the core area (red delineation) were determined 4 days after seeding for each spheroid, allowing to calculate the "invasion surface" (1 pixel = 10.58 µm x 10.58 µm). In parallel, mean invasion distance was determined (10 measures / spheroid). **(C)** Surface of spheroid core (mm^2), surface of invasion (mm^2) and invasion distance (µm) have been plotted in histograms.

Supplementary Methods 3: Analysis of Gene Expression by RT-qPCR.

RNA extraction and cDNA synthesis

Total RNAs were extracted and purified with RNeasy Plus Universal Mini Kit (Qiagen) according to manufacturer's recommendations. Treatment with gDNA Eliminator Solution, to ensure elimination of genomic DNA, was applied during the extraction step. Quantity of RNA extracts was measured with a NanoDrop™ One^{C} Spectrophotometer (ThermoFisher) and quality was checked by agarose gel electrophoresis. Next, 250 ng of RNA of each sample was used for reverse-transcription with the Verso cDNA Synthesis kit (Thermo Scientific) following the manufacturer's instructions.

Reference genes, target gene and primers

5 reference genes were selected : actin beta *Act* (NM_001101.3), beta 2 microglobulin *B2M* (NM_004048.2), glyceraldehyde-3-phosphate dehydrogenase *GAPDH* (NM_002046.3), 60s ribosomal protein L32 *RPL32* (NM_000994.4), 18S ribosomal RNA *RS18* (NM 022551.2). The target gene was SRY-box transcription factor 2 *SOX2* (NM_003106.4). A melt curve analysis was performed at the end to check the specificity of the amplified products and the PCR efficiency of the primer sets was 90% to 110%. *GAPDH* and *RPL32* were identified as the most stable genes according RefFinder web-tool (Xie *et al,* 2012). The list of reference and targeted genes is given in Table 1.

Analysis of Gene Expression by RT-qPCR

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed with qPCRBIO SyGreen Blue Mix according to manufacturer instructions (PCRBIOSYSTEMS), in a Biorad CFX96 Touch thermocycler using the Bio-Rad CFX Maestro 2.3 software. Relative gene expression was calculated using the 2(-Delta Delta C(T)) Method *(Livak and Schmittgen, 2001)*

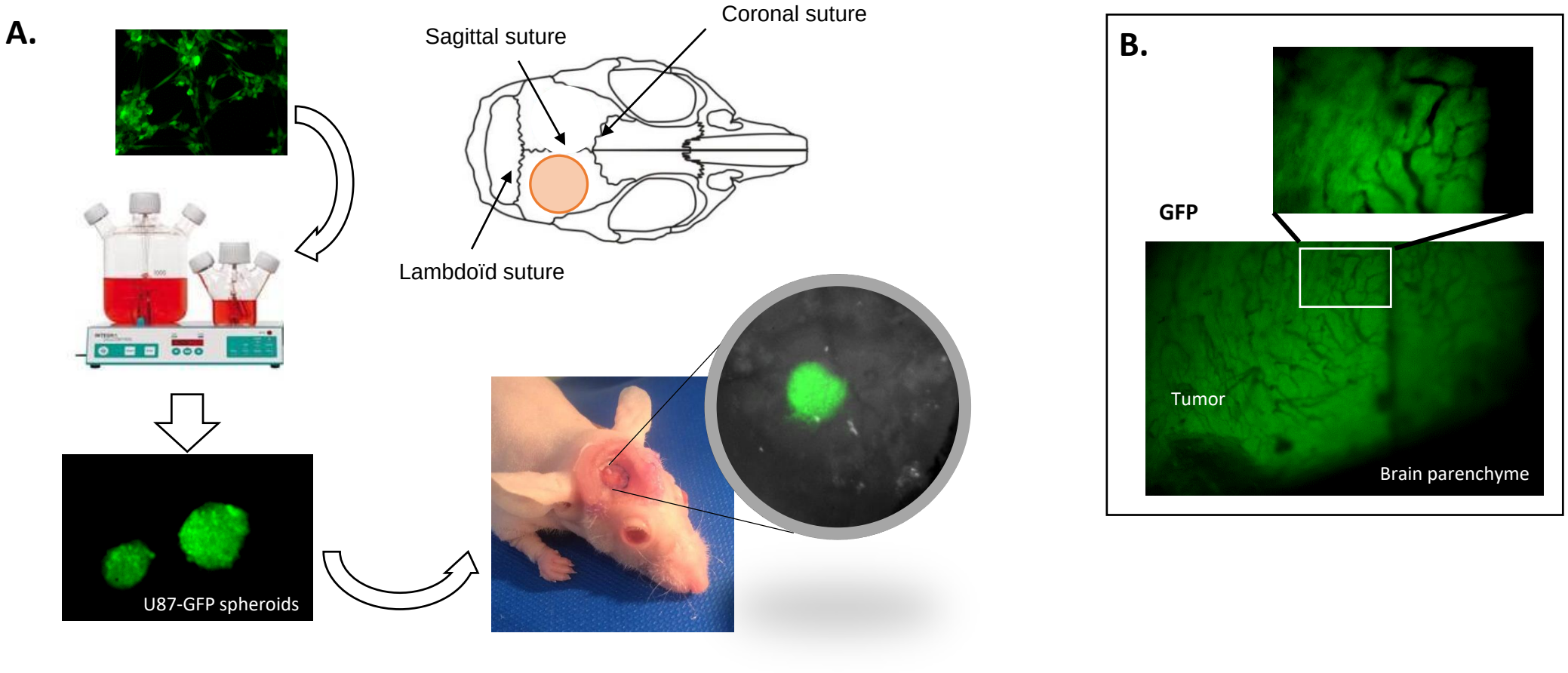
Table 1: Primer sequences used for qRT-PCR analysis genes.

Gene	Name	Accession number[1]	Forward Primer (5'-3')	Reverse primer (5'-3')	Annealing temperature (°C)	Amplicon size (bp)	Reference
Act	actin beta	NM_001101.3[1]	TCCCTGGAGAAGAGCTACGA	GTAGTTTCGTGGATGCCACA	60	131	Zcharia *et al*, 2009
B2M	beta 2 microglobulin	NM_004048.2[1]	ATCCATCCGACATTGAAGTTG	GGCAGGCATACTCATCTTTTTC	60	150	Zhou *et al*, 2014
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	NM_002046.3[1]	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC	60	258	Zhou *et al*, 2014
RPL32	60S ribosomal protein L32	NM_000994.4[1]	CATTGGTTATGGAAGCAACAAA	TTCTTGGAGGAAACATTGTGAG	60	150	Campion *et al*, 2021
RS18	18S ribosomal RNA	NM_022551.2[1]	GCAGAATCCACGCCAGTACAA	GCCAGTGGTCTTGGTGTGCT	60	208	Campion et al, 2021
SOX2	SRY-box transcription factor 2	NM_003106.4[1]	TTTCACGTTTGCAACTGTCC	AGTCTCCAAGCGACGAAAAA	60	189	

[1] NCBI accession number

Campion O, Thevenard Devy J, Billottet C, *et al.* Biomedicines. 2021;9;9(10):1430.
F Xie, P Xiao, D Chen, *et al*.. Plant molecular biology 2012;80 (1), 75-84.
Livak KJ, Schmittgen TD. Methods. 2001;25(4):402-8.
Zcharia E, Jia J, Zhang X, *et al.* PLoS ONE 2009:4(4): e5181.
Zhou ZJ, Zhang JF, Xia P. PLoS One. 2014;18;9(2):e88892.

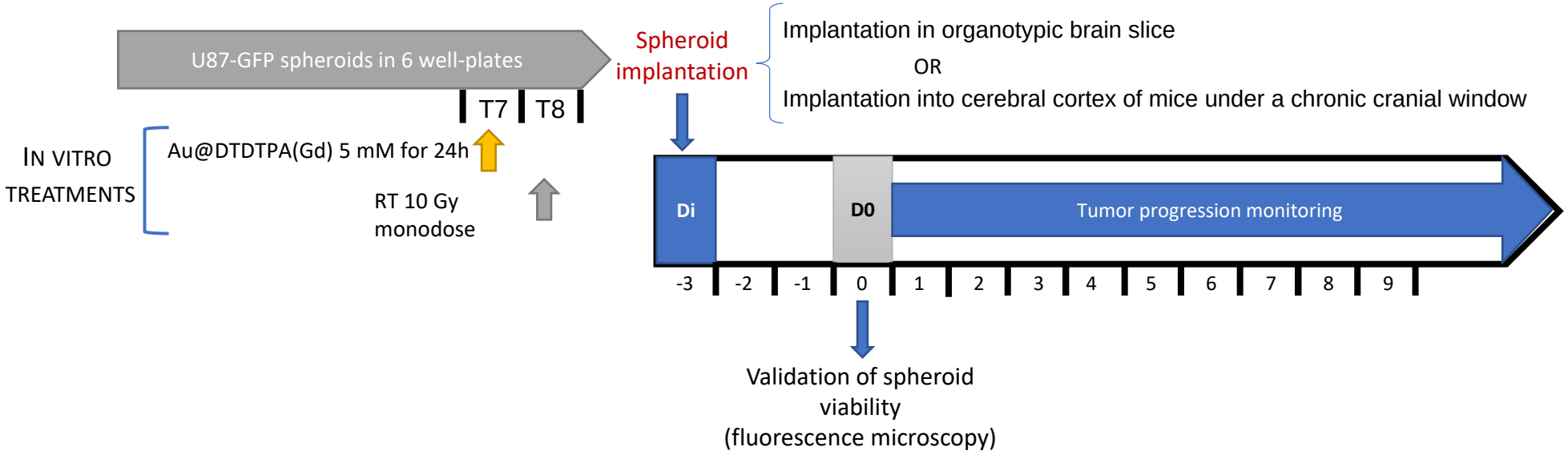
Supplementary Methods 4: The model of U87-GFP spheroid under chronic cranial window



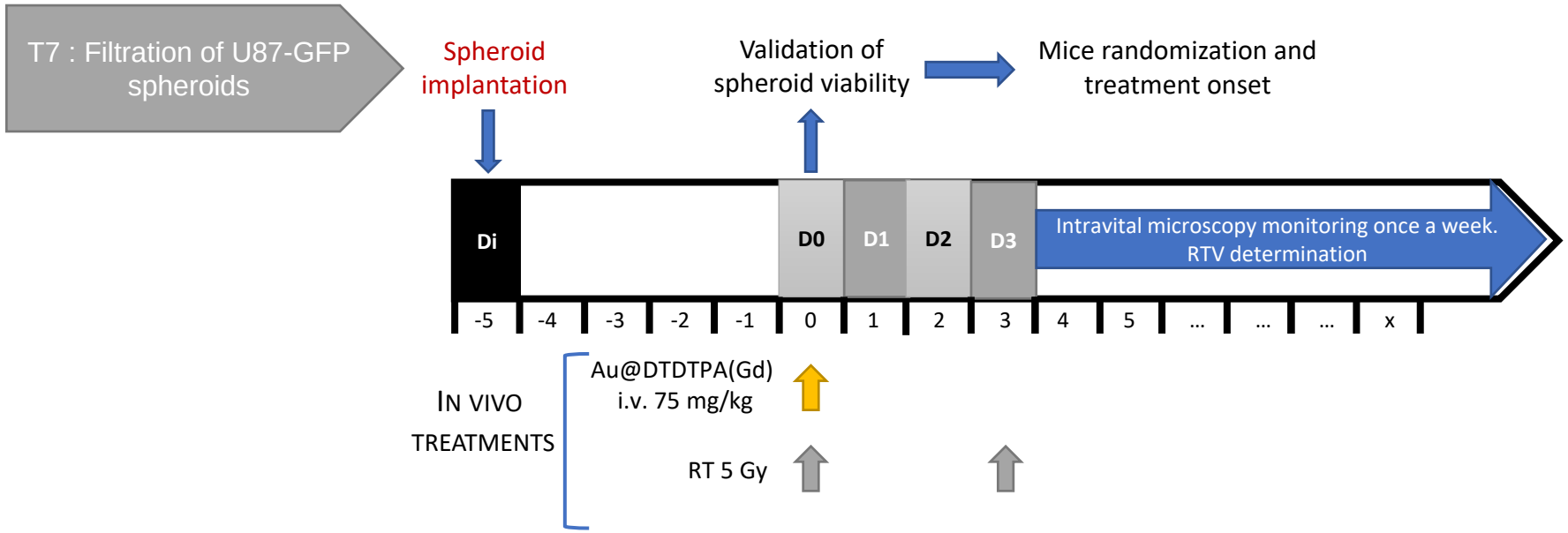
(A) For *in vivo* experiments, we used an intracerebral xenograft model of U87-GFP spheroids under chronic cranial windows: the model was adapted from previously published protocols[1]. Three to five days after implantation, we verified by intravital microscopy the presence of fluorescence signals for GFP (Exc 395 nm/Em 504 nm) in the brain. **(B)** The presence of a rich vascular network (in black) in U87-GFP tumor testifies to the viability of the grafted spheroid.

[1]*Li Y, Baran U, Wang RK. Plos One. 2014;9(11):e113658 ; Holtmaat A, Bonhoeffer T, Chow DK, et al.. Nat Protoc. 2009;4(8):1128-44. ; Mostany R, Portera-Cailliau C. J Vis Exp JoVE. 2008;(12).*

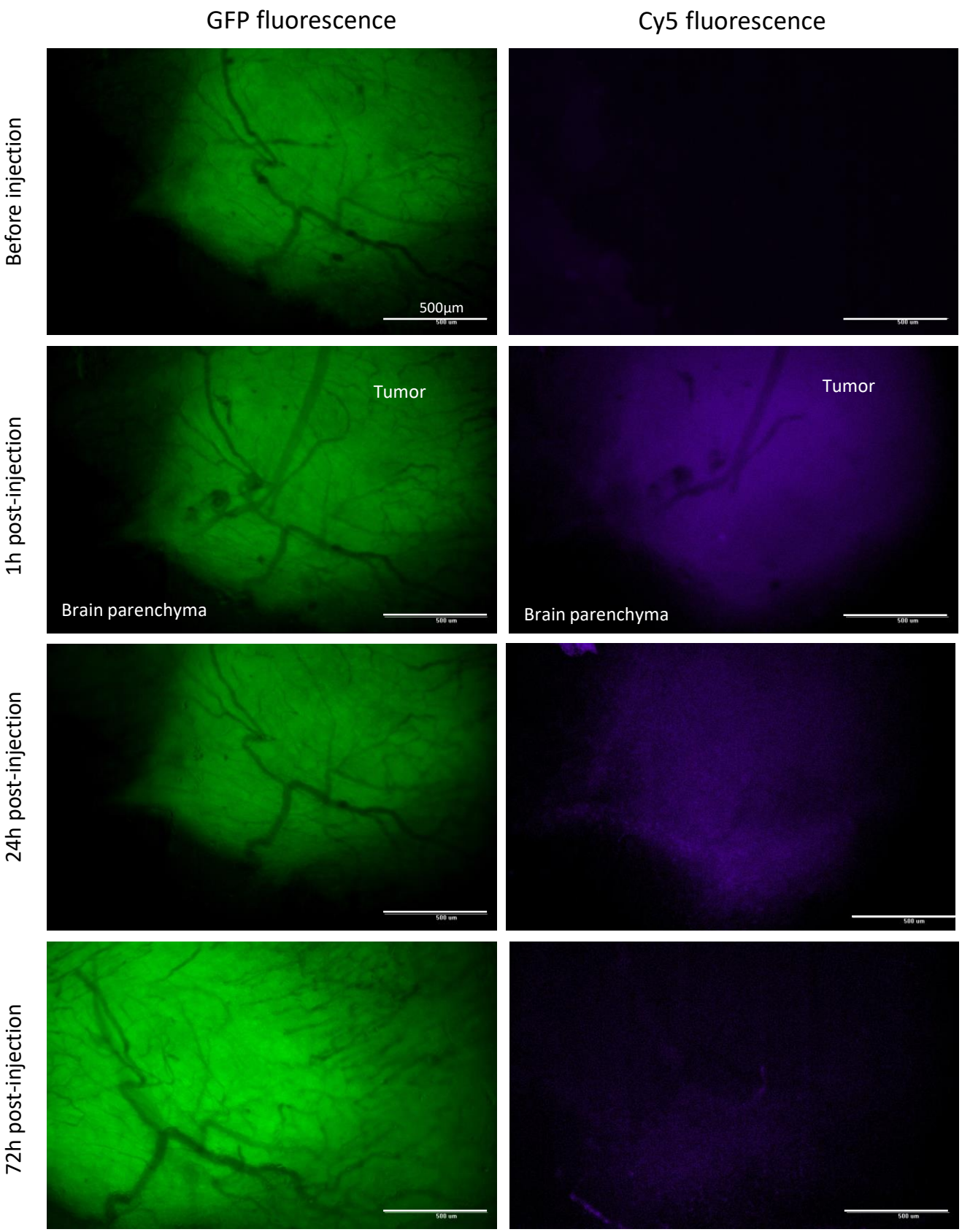
Supplementary Methods 5: Graphical representation of the experimental process for exploring invasion properties using complemenary models.



Supplementary Methods 6: Graphical representation of the experimental process for *in vivo* evaluation of treatment efficacy.

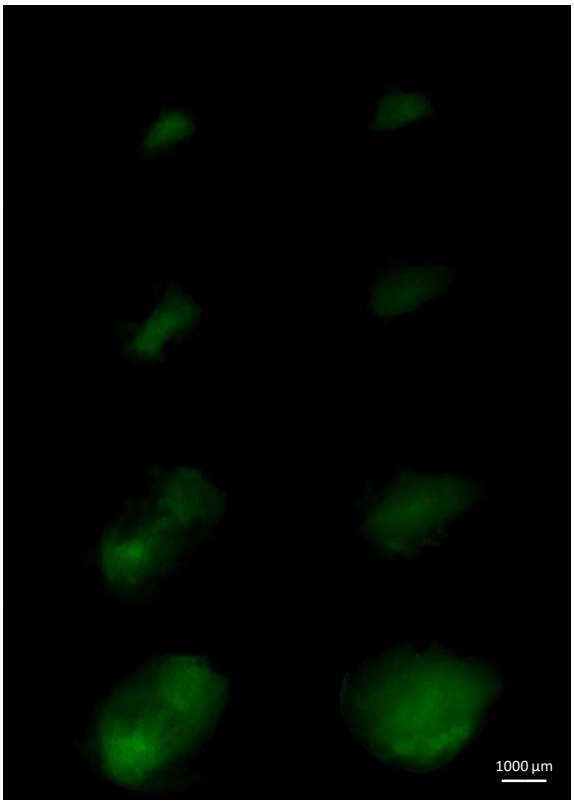


Supplementary Figure 1



Intracerebral distribution of Au@DTDTPA(Gd) nanoparticles using an intracerebral xenograft model of U87-GFP spheroids under chronic cranial windows. Mice with a viable U87-GFP spheroid received intravenous injection of Cy5-labelled Au@DTDTPA(Gd) nanoparticles (75 µg/g). Intravital microscopy allowed to observe the presence of fluorescence signals for GFP (Exc 395 nm/Em 504 nm) and Cy5 (Exc 650 nm/Em 670 nm) in the brain. Nanoparticles reached the brain tumor as soon as 10 min after i.v. injection, and intense fluorescence signal was maintained for 24h, while it slowly decreased until complete disappearence at 96h. One hour after injection, both signals overlapped very well, suggesting that Au@DTDTPA(Gd) nanoparticles were distributed throughout the tumor. The scale bar is 500µm.

Supplementary Figure 2



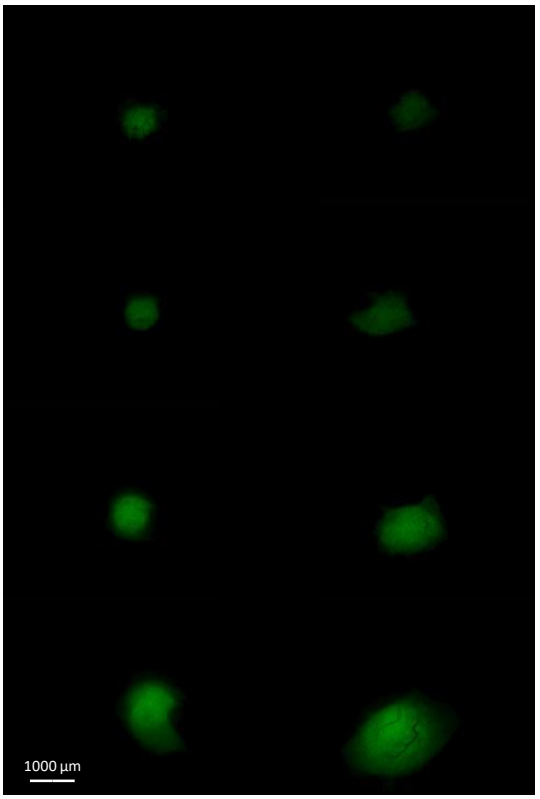
RT 2 X 5 Gy

D0

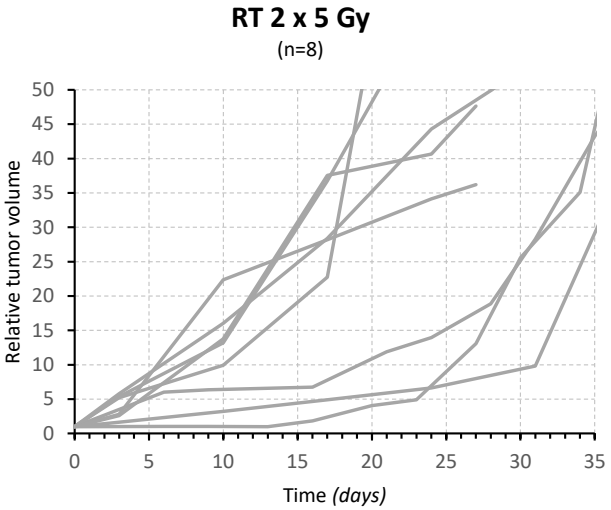
D3

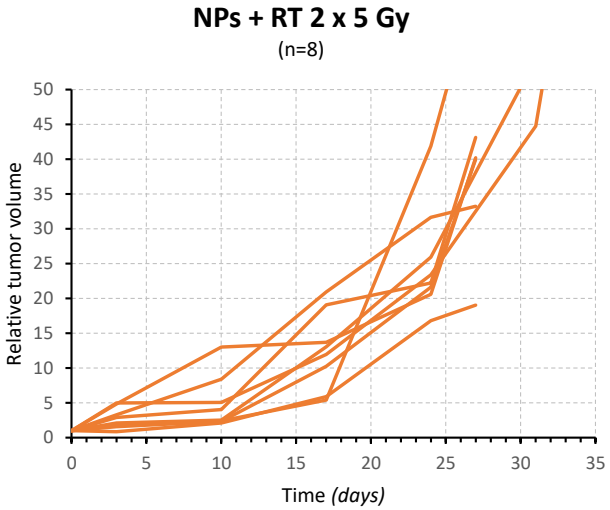
D10

D17



Au@DTDTPA(Gd) +RT 2 X 5 Gy



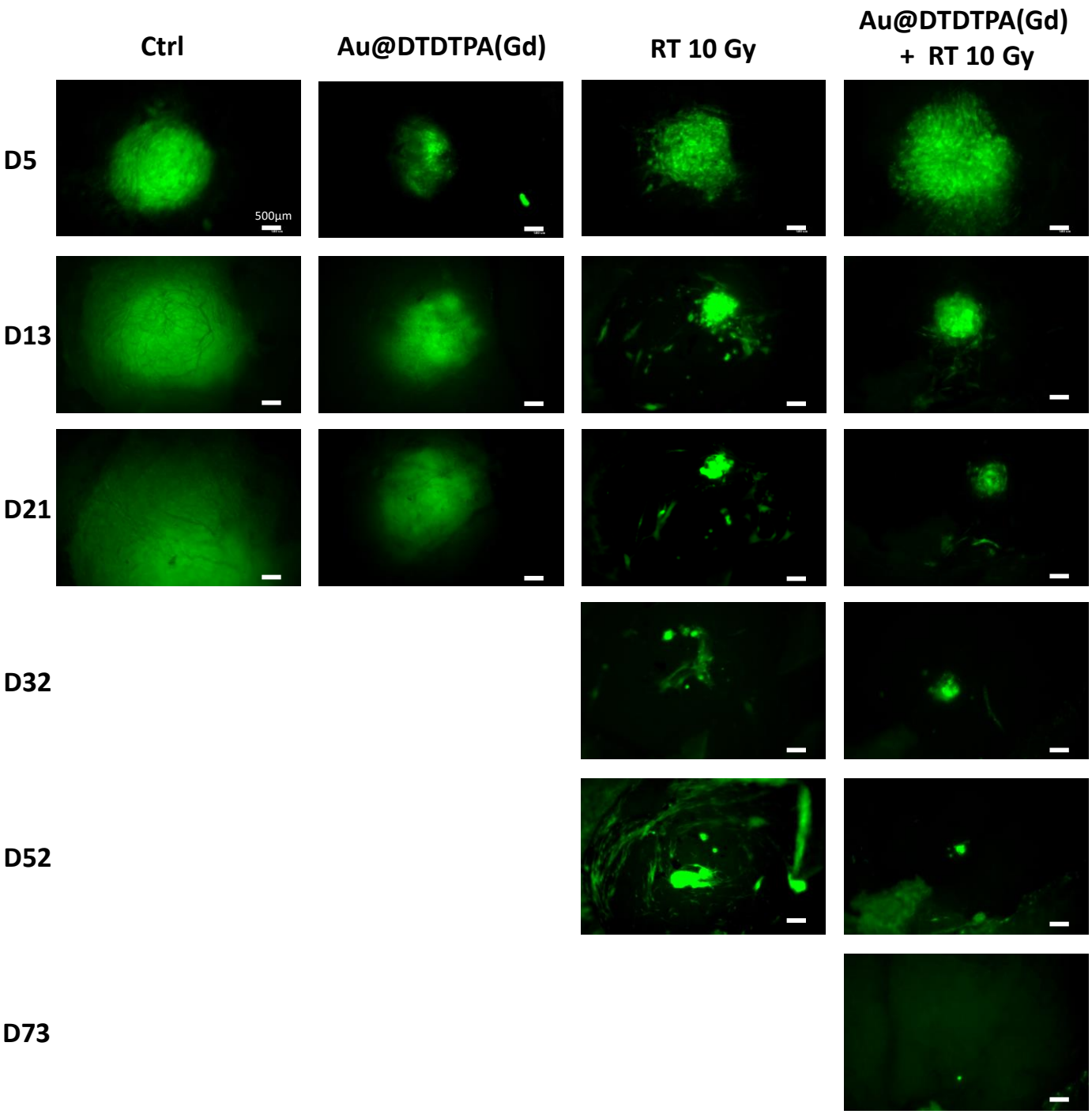


***In vivo* evaluation of the effects of treatments on tumor progression.**

A) For *in vivo* testings, the model of U87-GFP spheroid under the chronic cranial window was relevant to evaluate the radioresponse of brain tumors *in vivo*, because it allowed for long-term monitoring of tumor growth and spread. Nevertheless, as soon as 3 days after treatment (D3), the contouring of tumors and the volume determination appeared more difficult in some of RT-receiving mice (left panel), because of areas of tumor extension not so bright and more blurred than in group receiving Au@DTDTPA(Gd) + RT (right panel). The scale bar is 1000 µm. *N.B.* D0 corresponds to the treatment onset.

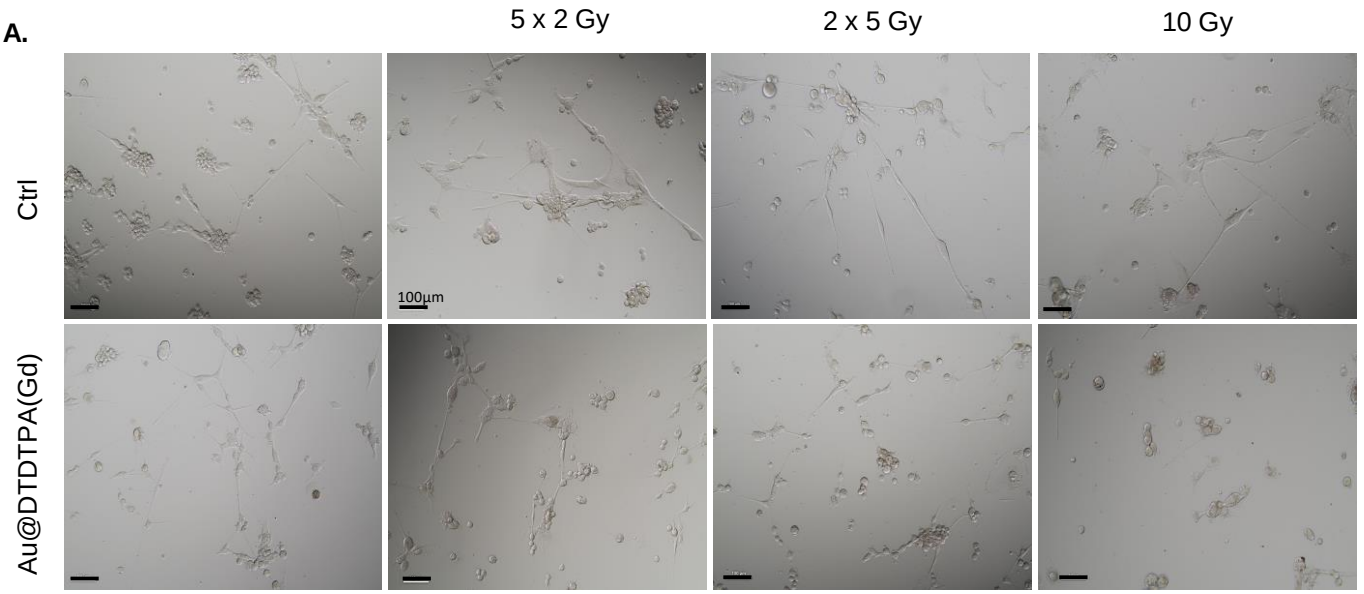
B) Based on the recorded fluorescence images, tumor volumes were calculated and the tumor growth curve was plotted for each mouse in each treatment group (RT vs NPs + RT). We noticed a more heterogeneous behavior in the response of RT-treated mice.

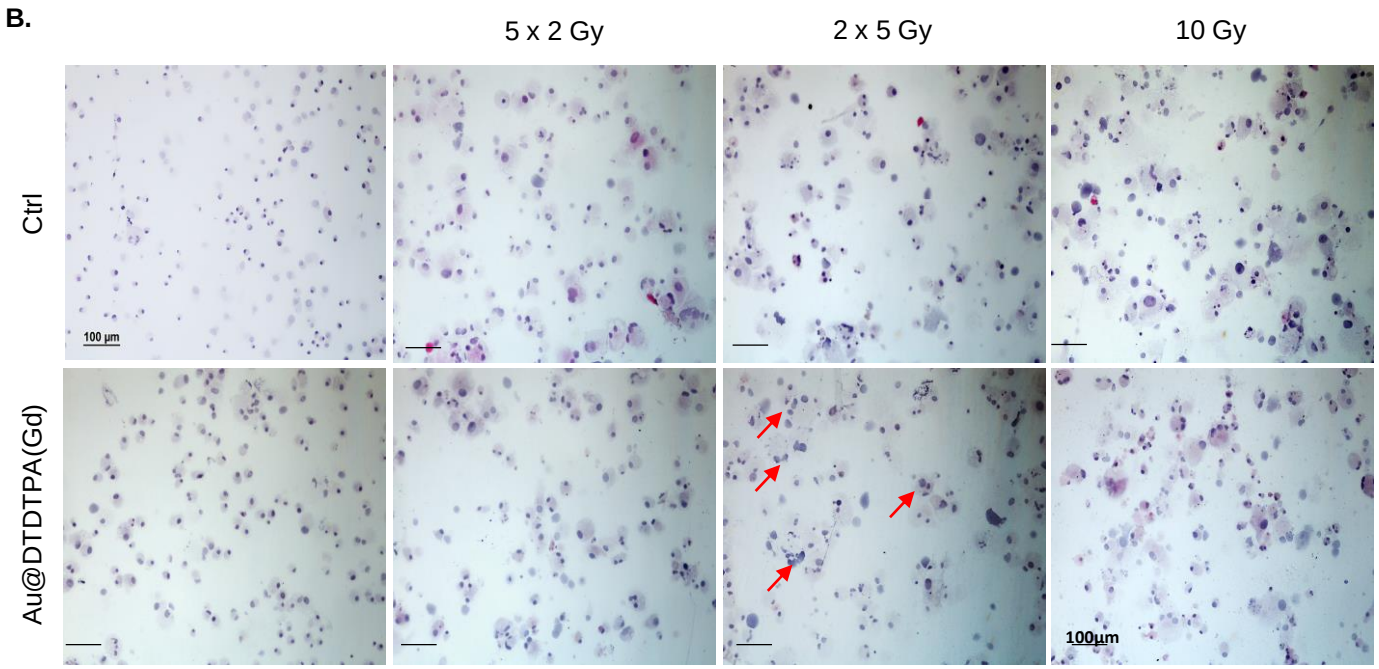
Supplementary Figure 3



To address the invasion of U87 cells after treatments, *in vitro* explorations were completed with an hybrid *in vitro*/*in vivo* model, that consisted in *in vitro* treatment of U87-GFP spheroids and then the implantation of the treated spheroids into brain parenchyma under a cranial window. This model allowed for long-term monitoring by intravital microscopy of glioblastoma cells invasiveness in brain environment. Untreated spheroids and NPs-treated spheroids resulted in rapid growth of tumor mass, forcing the sacrifice of mice before day 30 of follow-up. Ten Gy-irradiation induced a dramatic reduction of tumor mass until it almost disappeared but isolated tumor cells escaped from the tumor bulk and progressively disseminated into the brain parenchyma, causing disease symptoms (considered as end points implying euthanisia) after 75 days. When spheroids were treated by 10 Gy-irradiation plus Au@DTDTPA(Gd) nanoparticles, the tumor cells disappeared, allowing the animals to remain alive until the end of the study (> 4 months). The scale bar is 500 µm. *N.B.* D0 corresponds to the day of spheroid implantation. Intravital microscopy observations began on D5.

Supplementary Figure 4





(A) *In vitro* expriments were conducted to focus on invading cells that have escaped from U87 spheroids. To do that, 15 U87 spheroids (~ 500 µm in diameter) were seeded per well in 6-well plates and were exposed or not to various treatments, *i.e.* Au@DTDTP(Gd) 5 mM and/or Radiotherapy with different regimens (5 x 2 Gy ; 2 x 5 Gy; 10 Gy). Spheroids gave rise invading/escaping tumor cells. These invading cells were more or less able to form new clusters all over the culture well and clusters could be linked together by isolated cells that emitted membrane extensions. The scale bar is 100µm. **(B)** To assess treatment-induced mitotic catastrophe (MC), we performed hematoxyllin and eosin staining of invading cells to detect morphologically abnormal nuclei (*i.e.* micro- and multinucleation) that allow the determination of the MC rate for each therapeutic condition. The scale bar is 100µm.