Supporting Information

Novel treatment for glioblastoma delivered by a radiation responsive and radiopaque hydrogel

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Contents list

Supplemental figures

Experimental Section

1. *Materials*

Gold (III) chloride trihydrate, sodium borohydride, poly(bis(4-carboxyphenoxy)phosphazene) disodium salt (PCPP, 1 MDa), L-glutathione reduced (GSH), selenocystamine dihydrochloride, hexamethylenediamine, doxorubicin and albumin-fluorescein conjugate were purchased from Sigma-Aldrich (St. Louis, MO). Quisinostat dihydrochloride was purchased from Selleckchem (Houston, TX). HepG2, U251, Renca, and SVEC4-10 cell lines were purchased from ATCC (Manassas, VA). LIVE/DEAD assay kits were purchased from Life Technologies Invitrogen (Grand Island, NY). Cells were cultured in Dubecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin (10000 units/mL) from Life Technologies Invitrogen (Grand Island, NY). Athymic nude mice were obtained from Charles River Labs (stock #490).

2. *Cell based high throughput death receptor screen*

High throughput screening (HTS) was performed by the HTS core at the University of Pennsylvania. U251 DR5-Luc cells were plated at $2.0x10^3$ cells/well across ten 384 well plates. Plates were then incubated in a humidified environment at 37 ^oC and 5% CO_2 for twenty-four hours. Compounds from the Selleckchem Bioactive Compound Library I was added to each well at 100 nM (approximately 3200 compounds). Tunicamycin (100 nM) and DMSO (0.1% v/v) served a positive and negative plate controls. Brite-Lite (PerkinElmer, Waltham, MA) reagent was added all plates twenty-four hours following the addition of compound, and plates were assayed on Envision Xcite (PerkinElmer) plate reader. The mean (μ) and standard deviation (σ) for each plate was used to transform total flux for each compound (x_i) into z-scores using the formula $z = (x_i-\mu/\sigma)$. Calculation of Z-prime for this screening assay was based upon the formula set forth by Zhang et al utilizing tunicamycin and DMSO as a positive and negative controls respectively.[1] Positive hits were identified as compounds that produced z-prime values greater than tunicamycin. Reporter activity was validated following compound incubation and bioluminescence imaging on the IVIS spectrum (PerkinElmer).

3. *Western blot analysis*

Western blot analysis was performed as previously described.[2]

4. *Compound dilution studies and cell viability*

Cells were plated at density of 7.5-1.0x10^4 cells/well in 96 well plates using their respective media. Plates were then incubated in a humidified environment at 37 ^oC and 5% CO_2 for twenty-four hours. An eleven-point dilution series of positive hits from our HTS screen was added to each plate. Cells treated with 0.1% DMSO served as a vehicle control. Plates were incubated with compound for seventy-two hours at 37 ^oC and 5% CO_2, followed by addition of Cell Titer Glo® (Promega). Bio-luminescent readings were obtained using a Synergy HT luminometer (BioTek, Winooski, VT). Raw bio-luminescent values were normalized to their vehicle treated controls, and then fit to a modified Hill Equation using OriginPro 8 (OriginLab, Northampton, MA) in order to calculate IC_{50} values. All dilution series experiments were performed as biological triplicates.

5. Sphere formation assay

T4213 and NS039 cells were plated at 2000 cells/well in six well plates in the presence of 0.1% DMSO or 1.0 □M Quisinostat in biological triplicate for twelve days. On day twelve, individual wells were imaged on a Lionheart Fx microscope at 4X magnification following addition of NucBlue™ (Hoechst 33342, Invitrogen). Circular blue objects greater than 50 □m in diameter and less than 750 □M were counted as neurospheres. Differences in sphere formation between cells and treatments were assessed using a one-way ANOVA followed by post-hoc Tukey's test in GraphPad Prism 8. P-values less than 0.05 were considered significant.

6. Primary brain tumor specimens

As previously described,[3] the use of primary tumor tissue was coordinated by the University of Pennsylvania Tumor Tissue/Biospecimen Bank following ethical and technical guidelines on the use of human samples for biomedical research purposes. Patient glioblastoma tissues were collected at the Hospital of the University of Pennsylvania after informed patient consent under a protocol approved by the University of Pennsylvania's Institutional Review Board. All patient samples were de-identified before processing. Mutational status of clinical glioblastoma tissue was made available from a routinely performed clinical sequencing panel.

7. Brain tumor organoids, compound incubation, and viability

Glioblastoma organoids (GBOs) were generated from primary brain tumor as previously described.[3] Briefly, primary brain tumor specimens were minced into approximately 1 mm^3 pieces following clinical confirmation of pathologic diagnosis and incubated in GBO medium

containing 50 % DMEM:F12 (Thermo Fisher Scientific), 50 % Neurobasal (Thermo Fisher Scientific), 1X GlutaMax (Thermo Fisher Scientific), 1X NEAAs (Thermo Fisher Scientific), 1X PenStrep (Thermo Fisher Scientific), 1X N2 supplement (Thermo Fisher Scientific), 1X B27 w/o vitamin A supplement (Thermo Fisher Scientific), 1X 2-mercaptoethanol (Thermo Fisher Scientific), and 2.5 µg/ml human insulin (Sigma) per well and placed on an orbital shaker rotating at 120 rpm within a 37 °C, 5 % CO_2, and 90 % humidity sterile incubator.

Organoids would generally form 2-4 weeks following initial culturing. Organoids were then incubated in media containing either vehicle (0.1% DMSO) or 1.0 µM quisinostat with media changes every two days for seven days total. Post-treatment images were taken on brightfield microscope and samples were subsequently fixed in 4% paraformaldehyde x 30 minutes for immunofluorescence analysis.

8. Immunofluorescence

As previously described, serial tissue sections (20 µm) were sliced using a cryostat (Leica, CM3050S), and melted onto charged slides (Thermo Fisher Scientific). Slides were dried at room temperature and stored at -20 °C until ready for immunohistology. For immunofluorescence staining, the tissue sections were outlined with a hydrophobic pen (Vector Laboratories) and washed with TBS containing 0.1 % Tween-20 (v/v). Non-specific binding was blocked using a solution containing 10 % donkey serum (v/v), 0.5 % Triton X-100 (v/v), 1 % BSA (w/v), 0.1 % gelatin (w/v), and 22.52 mg/ml glycine in tris-buffered saline-Tween (TBST) for 1 hour at room temperature. The tissue sections were incubated with primary antibodies against Ki67 (BD Biosciences Cat# 550609) and Cleaved-Caspase 3 (Cell Signaling Technology CAT# 9661) diluted in TBST with 5 % donkey serum (v/v) and 0.1% Triton X-100 (v/v) overnight at 4 °C. After washing in TBST, the tissue sections were incubated with secondary antibodies (Alexa Fluor 555 and 647 respectively) diluted in TBST with 5 % donkey serum (v/v) and 0.1 % Triton X-100 (v/v) for 1.5 hours at room temperature. After washing with TBST, sections were incubated with TrueBlack reagent (Biotium) diluted 1:20 in 70 % ethanol for 1 minute to block autofluorescence due to lipofuscin and blood components. After washing with DPBS, slides were mounted in mounting solution (Vector Laboratories), cover-slipped, and sealed with nail polish.

GBOs were imaged on a Zeiss LSM 710 confocal microscope (Zeiss) using a 20X objective with Zen 2 software (Zeiss). Images were quantified and analyzed using ImageJ software. Graphical and statistical analysis was performed using GraphPad Prism 8 software.

9. Orthotopic tumor implantation and treatment with quisinostat

All animal work was approved by the Institute for Animal Care and Use Committee at the University of Pennsylvania (# 805893). T4213 cells were stably modified using a lentiviral vector containing GFP and Luciferase (Biosettia, San Diego, CA). Cells were resuspended in serum free media, and then $3.0x10^5$ cells were stereotactically injected into the right striatum of five-week female athymic nude mice (Charles River Labs). Tumor bioluminescence was quantified on the IVIS spectrum (PerkinElmer) following intraperitoneal administration of D-Luciferin (Goldbio, St Louis MO) two days following cell implantation. In a separate experiment, mice underwent serial bioluminescence imaging following orthotopic T4213 implantation. Mice were assigned to different treatment groups such that the total flux (photons/s) was equivalent between groups ($6.4x10^6$ photons/s). Treatment was initiated immediately following bioluminescent imaging, and consisted of once daily intraperitoneal administration of 2-Hydroxypropyl-β-cyclodextrin (vehicle, Cayman Chemical, Ann Arbor MI) or 10 mg/kg Quisinostat (Cayman Chemical) for a total of eight doses (Figure S3). Mice were given a seventy-two-hour break between treatments if they demonstrated weight loss, dehydration, or lethargy (Figure 4B). Bioluminescent imaging was repeated seventy-two hours after the last treatment was administered. Mice were weighed two to three times per week throughout all experiments until they met criteria for humane euthanasia (loss of 20% of body weight, neurologic deficits). Following human euthanasia brains were carefully removed and stored in 10 % Neutral Buffered Formalin at 4^{o}C. Select brains were sent to the Cancer Histology Core at the University of Pennsylvania for paraffin embedding followed by hematoxylin and eosin staining. Serial bioluminescence was assessed using a one-way repeated measures ANOVA followed by a post-hoc Tukey test. Post-treatment total flux was background corrected, and the percent change in total flux was analyzed using a Mann-Whitney U test. Both analyses were performed in GraphPad Prism 8, and p-values less than 0.05 were considered significant.

10. Gold nanoparticle synthesis

The gold nanocrystals were synthesized via a modification of the method of Turkevich by reduction of gold (III) chloride with sodium borohydride and subsequent surface modification with a stabilizing ligand.[4] $AuCl_3.6H_2O$ was dissolved in DI H_2O (8 $mg.mL^{-1}$, 100 mL) under stirring. A freshly prepared solution of $NaBH_4$ (5 $mg.mL^{-1}$, 2 mL DI H_2O) was dropped to the gold chloride solution and allowed to stir at room temperature for 20 min. Then, a solution of the capping GSH (5.2 $mg.mL^{-1}$, 1 mL DI H_2O) was added to the mixture, which was further transferred to 50 mL plastic tubes and incubated at 4 °C overnight. Concentration and

purification of AuNP were performed using molecular weight cut off tubes (10 kDa MWCO tubes), centrifuged at 4 k rpm, rinsed trice with DI H_2O to afford the purified AuNP. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed on a Spectro Genesis ICP to determine the final gold concentration of the stock solution.

11. Se-PCPP hydrogel formulation

Se-PCPP hydrogel: A mixture of 1 mL PCPP (4 $mg.mL^{-1}$, PBS) and 10.3 mg Na_2HPO_4 were mixed together and pH was adjusted to 7.4 by addition of HCl (1 M). Next, 1.4 µL of selenocystamine dihydrochloride (110 $mg.mL^{-1}$, PBS) were added to the mixture and vortexed for 30 seconds. The mixture was dropping into 10 mL of a $CaCl_2$ (8.8%, DI H_2O) and left to react under vigorous stirring for 20 minutes at room temperature. Finally, the solution was transferred to conical tubes and centrifuged at 2 k rpm for 8 min. The supernatant was discarded, and the pellet was re-suspended in DI water. After repeating this process three times, the Se-PCPP solution was re-suspended in DI water, vortexed at 14 k rpm and stored at 4 °C. The final concentration of AuNP in the sample was determined using ICP-OES performed on a Spectro Genesis ICP.

Cargo loaded Se-PCPP hydrogel: For the cargo loaded Se-PCPP hydrogel, the PCPP (4 $mg.mL^{-1}$, PBS) solution was mixed with the desired amount of cargo (solutions of doxorubicin, FITC-BSA or quisinostat at 1 $mg.mL^{-1}$) and 10.3 mg Na_2HPO_4. The total volume was completed to 2 mL with PBS, pH was adjusted to 7.4 by addition of HCl (1 M) and incubated for 10 minutes before cross-linking with selenocystamine dihydrochloride as described above. The final drug concentration in the sample were determined using UV-Vis spectroscopy by determining the loading efficiency (LE), calculated as follows: %LE = ([stock drug] - [non-loaded drug] / [stock drug])*100.

C-PCPP hydrogel: The non-X-ray sensitive hydrogel was synthesized following the same procedure as described above, using a solution of hexamethylenediamine (65 $mg.mL^{-1}$) for cross-linking and hydrogel formation, in place of selenocystamine.

12. UV-Vis spectroscopy

The UV-vis absorption spectrum of GSH-AuNP and Se-PCPP hydrogels were recorded on a UV/visible spectrophotometer (Thermo Fisher Scientific, USA) after dilution in DI water.

13. Transmission electron microscopy (TEM)

For air-dried samples, six microliters of hydrogel suspension in DI H_2O ([P] = 0.5 $mg.mL^{-1}$) were deposited onto a copper grid and the sample was air-dried for a minimum of 24 h prior image acquisition. For embedded samples, hydrogels were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde, embedded, cut into sections of 60 nm thickness and mounted onto copper grids. TEM images were acquired at 80 keV using a JEM 1010 microscope (JEOL, Tokyo, Japan) with an AMT NanoSprint500 5 Megapixel (AMT, Woburn (MA), USA).

14. Scanning electron microscopy (SEM)

Samples were freeze-dried shortly before the acquisition of SEM images using a Quanta 250 (FEI, Hillsboro, OR) at near atmospheric pressure, with a gaseous secondary electron detector.

15. Fourier transform infra-red (FT-IR)

5 µL samples were ground with 100 mg KBr and pressed into pellet; the transmission spectrum of Se-PCPP hydrogel was collected on a JASCO FT/IR-480 PLUS.

16. Inductively coupled plasma optical emission spectroscopy (ICP-OES)

Gold and phosphorous concentrations in Se-PCPP hydrogels were determined using ICP-OES (Spectro Genesis ICP). The samples were prepared by dissolving 10 µl of sample stock solution in 1 mL of aqua regia and making the final volume to 6 mL with DI water.

17. Vial inversion

The gel characteristics of the Se-PCPP hydrogel were initially assessed by performing the vial inversion method. Briefly, the samples were transferred to vials and allowed to stand reversed for one hour. All mixtures that remained unchanged and without dropping were considered as having the characteristics of a gel.

18. Rheology

The hydrogels were sonicated for 30 minutes in an ultrasound bath before loading onto the rheometer. The rheometer experiments were conducted using a parallel plate geometry, a gap size of 100 µm, and the following measurements were carried out at 25°C: frequency sweep (0.01–100 Hz, 0.2% strain), time sweep (0.2% strain, 10 Hz), strain sweep (0.01–500% strain, 10 Hz), continuous flow (shear rates from 0 to 50 s^{-1} over 2 min 30 s), and cyclic strain (low: 0.2% strain, high: 500% strain, 10 Hz).

19. Injection force

The hydrogels were sonicated for 30 minutes in an ultrasound bath before loading 50 µL of the sample into a 1 mL syringe assembled with a 25-gauge needle. The syringe was placed into an Instron 5848 microtester (Norwood, MA) and the load cell was placed in close vicinity to the plunger before initiating the experiment with a flow rate of 2 mL/hr while recording the maximum force applied during hydrogel extrusion over time. Testing was carried out in air and repeated 6 times.

20. X-ray irradiation

The radiation dose delivered to the sample is quantified in gray (Gy) with the relation of 1 Gy = 1 $J.kg^{-1}$. The X-ray irradiation was performed on an X-RAD 320ix irradiator (PXi, North Branford, CT) operated at 320 kV, 12.5 mA, focal spot of 8 mm^2 and with an aluminum filter of 2 mm delivering a dosage rate of 2.65 Gy/minute. In brief, the Se-PCPP hydrogel was sampled into 1 mL tube and placed in the irradiator chamber for the desired time depending onto the X-ray dose to be delivered.

21. Hydrogels degradation and cargo release experiments

Se-PCPP hydrogel ([Au] = 0.2 $mg.mL^{-1}$) was suspended into 1 mL of freshly prepared PBS/10 % FBS in micro-centrifuge tubes and centrifuged at 4 k rpm for 8 minutes. Samples were irradiated with the desired X-ray dose and incubated at 37 °C for the overall experiment time. At the desired time point, samples were vortexed, centrifuged at 4 k rpm for 8 minutes and the supernatant was gently collected for quantification of the released cargo by ICP-OES or UV-vis spectroscopy. 1 mL of freshly prepared incubation medium was rapidly added to each sample, all were incubated at 37 °C until the next time point.

22. Cell culture

HepG2, Renca, SVEC4-10, U251 and NS039 cell lines were cultured according to the supplier's instructions, and all experiments were performed in triplicate.

LIVE/DEAD assay was performed to assess the biocompatibility of Se-PCPP hydrogel. Cells were plated in 6 wells plate at a density of 100 k cells per well. After 24 h incubation, the cells were treated with Se-PCPP hydrogel at concentrations of 0, 0.125, 0.25 and 0.5 mg/mL. After 8 hours of incubation at 37 °C, 5% CO_2 for 24 h, the cells were washed twice with DBPS and incubated for another 20 min with 400 µL of LIVE/DEAD cocktail (0.025% stock Calcein and 0.1% stock Ethidium-1 homodimer in DPBS). Three images of four different areas of each well were acquired (excitation/emission filter pairs, 495/515 nm, and 528/ 617 nm, for Calcein or Ethidium Homodimer-1, respectively). Cell counts for each dye in each image was performed using a custom MATLAB code, and the ratio of dead to living cells normalized to control was calculated as the viability measurement.

MTS assay was performed to assess the cytotoxicity of the drug released from the hydrogel on GBM cells U251 and NS039 by adaptation of previously reported procedures.[5,6] Cells were plated in 96 wells plate at a density of 5 k cells per well and incubated at 37 °C, 5% CO_2 for 24 h. In the meantime, the drug loaded hydrogel was incubated in cell medium, at 37 °C, for 24 h post X-ray irradiation (0 Gy for drug elution or 60 Gy for X-ray triggered drug release). Then, the drug containing supernatants were used to treat GBM cells for 48 h at 37 °C, 5% CO_2. Cells were finally rinsed with PBS once, and incubated with MTS solution (20 µL stock solution in 100 µL cell medium) 30 min prior absorbance measurement at 490 nm with a plate reader (Synergy H1, BioTek, VT).

23. Computed tomography (CT) phantom imaging

Samples of different Au/P ratio were prepared into 250 µL vial tubes and secured in a plastic rack. A MiLabs µ-CT was operated at a tube voltage of 55 kV with tube current of 190 µA and exposure of 75 ms to acquire the CT images. Slices of 100 µm thickness with an increment of 0.1 mm were reconstructed using the algorithm provided by the manufacturer. Image analysis was performed using OsiriX (v.3.7.1 64-bit software). A circular ROI was drawn on the coronal view of each tube and the mean attenuation value for the ROI of five slices per tube was recorded and normalized to the value of the non-loaded Se-PCPP hydrogel. The reported attenuation value for each concentration correspond to the average of three samples of that same concentration.

24. In vivo rodent model of xenografted GBM

All the mouse work was performed under an approved protocol (Institutional Animal Care and Use Committee, IACUC) at the University of Pennsylvania (# 805893). NS039 cells were resuspended in PBS, and then $1.0x10^6$ cells were subcutaneously implanted on the right flank of athymic nude mice and allowed to grow to a volume of approximately 100 mm^3. Daily monitoring of the mice was performed, ensuring the cage cleanliness, adequate water and food as well as good mouse activity. Both tumor growth and mice weight were measured every other day. Tumor length and width were measured with an electronic caliper and the volume was determined as $V = (length*width^2)/2$. Tumors were allowed to grow up to $V = 15\ cm^3$ as per IACUC standards, after which animals were sacrificed.

25. Intra-tumoral injection

All mice were first anesthetized with isoflurane, then were administered an intra-tumoral injection of 50 µL of treatment as follows: Group 1: untreated (n=5), Group 2: radiotherapy (n=6), Group 3 radiotherapy + hydrogel (n=6), Group 4 hydrogel + drug (n=6), Group 5 hydrogel + drug + radiotherapy (n=6). Hydrogel concentration [Au] = 8 $mg.mL^{-1}$; Drug concentration [QS] = 0.5 $mg.mL^{-1}$; radiotherapy dose XR = 10 Gy.

26. In vivo CT imaging and radiotherapy

In vivo CT: The mice were first anesthetized with isoflurane, then CT images were acquired on a SARRP (Gulmay Medical, Inc, Camberley, United Kingdom) cone-beam CT at 50 kVp, 0.5 mA, and 1440 projections were used to reconstruct the images using with the algorithm provided by the manufacturer. Image analysis was performed using OsiriX (v.3.7.1 64-bit software). ROI was measured using the ROI segmentation tool to delineate the hydrogel from surrounding tissues, the hydrogel was reconstructed in 3D volume and ROI was measured as the ROI values normalized to the muscle, and summed across the hydrogel.

In vivo radiotherapy: 24 h post-injection of the hydrogel, the mice (groups 3, 4 and 5) were anesthetized with isoflurane and were administered an X-ray dose of 10 Gy focused onto the tumor, delivered with a SARRP (Gulmay Medical, Inc, Camberley, United Kingdom), through a 17 mm diameter collimator mounted with a 0.15 mm copper filter and at a distance of 35 cm separating the mouse to the source.

27. Survival analysis of xenografted mice

The survival time until the mice reached the maximum tumor growth as per IACUC standards, was calculated from the date of cell implantation (day -20). Mice were sacrificed if showing signs of distress, including excessive weight loss (> 20%), lethargy, tumor metastasis, in accordance with IACUC standards.

28. Organs harvesting and ex vivo investigations

The mice were first euthanized with CO_2, followed by cervical dislocation as per IACUC recommendations. Then the abdominal cavity was opened to perform left ventricle perfusion with 20 mL of cold PBS and finally, dissection was performed to harvest the major organs (heart, lungs, liver, spleen, kidneys) and the tumor. All organs were washed with cold PBS, transferred to 10 % buffered formalin solution, cut to small pieces keeping aside one sample for histopathological investigation and the remaining for gold quantification.

Ex vivo biodistribution of gold: organ pieces were digested by 1 mL HNO_3 for 17 h at 75 °C and one more hour at 75° C with 250 µL HCl. All solutions were diluted with DI H_2O to a final volume of 6 mL, centrifuged (1 krpm, 10 min) and filtered prior gold quantification using ICP-OES.

Histopathological tissue analysis: organ slice was fixed in 10 % buffered formalin solution and kept at – 4°C until further processing with sequential dehydration in increasing concentrations of ethanol and storage at -20°C. Finally, organ slices were embedded in paraffin, sectioned, stained with hematoxylin and eosin dyes (H&E) and analyzed with a slide scanner Aperio CS-O (Leica Biosystems).

29. Statistical analysis

Each experiment was performed at least in three replicates, unless stated otherwise. A one-way ANOVA test was performed on the Excel software for statistical analysis, and the data were considered non-statistically significant for $p > 0.05$.

Supplemental figures

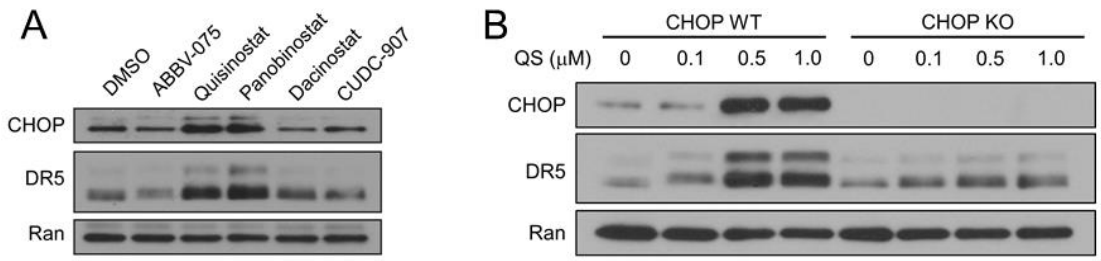

Figure S1: Results of Western blotting for the effects of A) the lead compounds on CHOP and DR5, and B) dose dependent effects of quisinostat on CHOP and DR5.

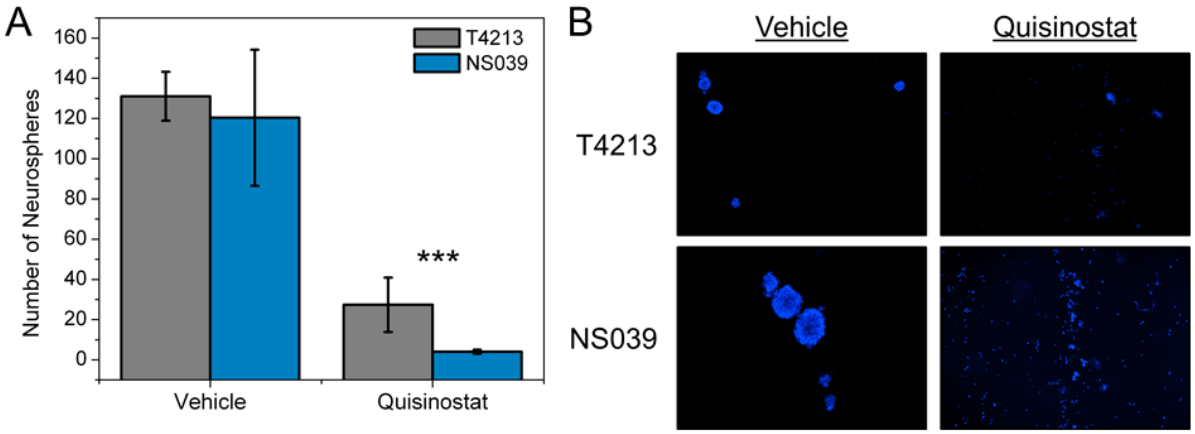

Figure S2: Effect of quisinostat on glioblastoma neurosphere and organoid formation. Comparison of the effect either vehicle or 1.0 μM quisinostat treatment on neurospheres by A) analysis of numbers of neurospheres of over 50 μm in diameter that are formed from the cells, B) representative images of neurospheres after treatment at 10x magnification.

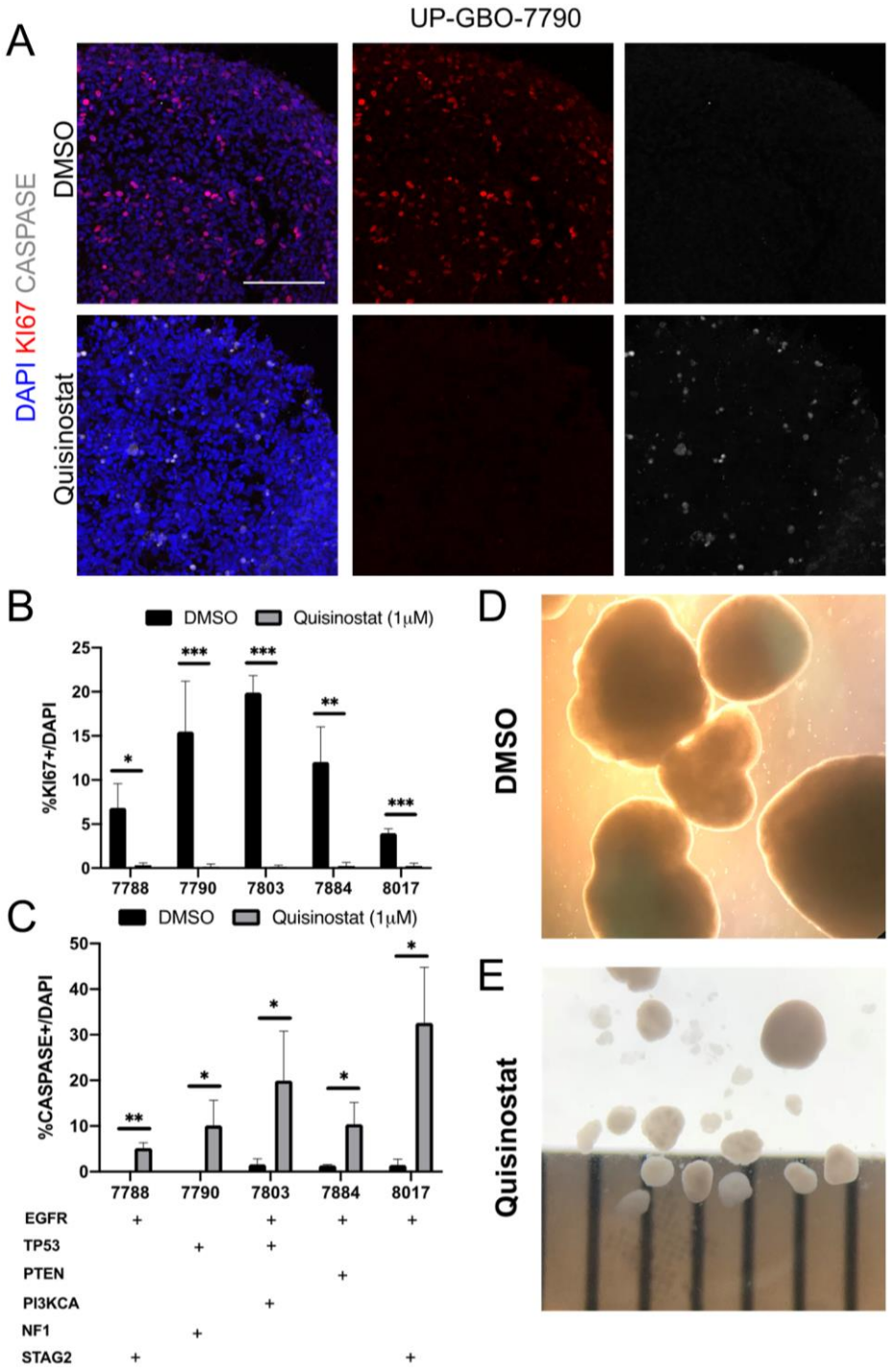

Figure S3: A) An example of fluorescence microscopy of DAPI, Ki67 and caspase staining on patient derived glioblastoma organoids (GBOs) after treatment with DMSO or quisinostat, scale bar 500 µm. Quantification of the ratio of fluorescence comparing B) Ki67 to DAPI, or C) caspase to DAPI for several GBOs. Morphology of GBOs after treatment with D) DMSO or E) quisinostat. *, ** and *** indicate a significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.005$ respectively.

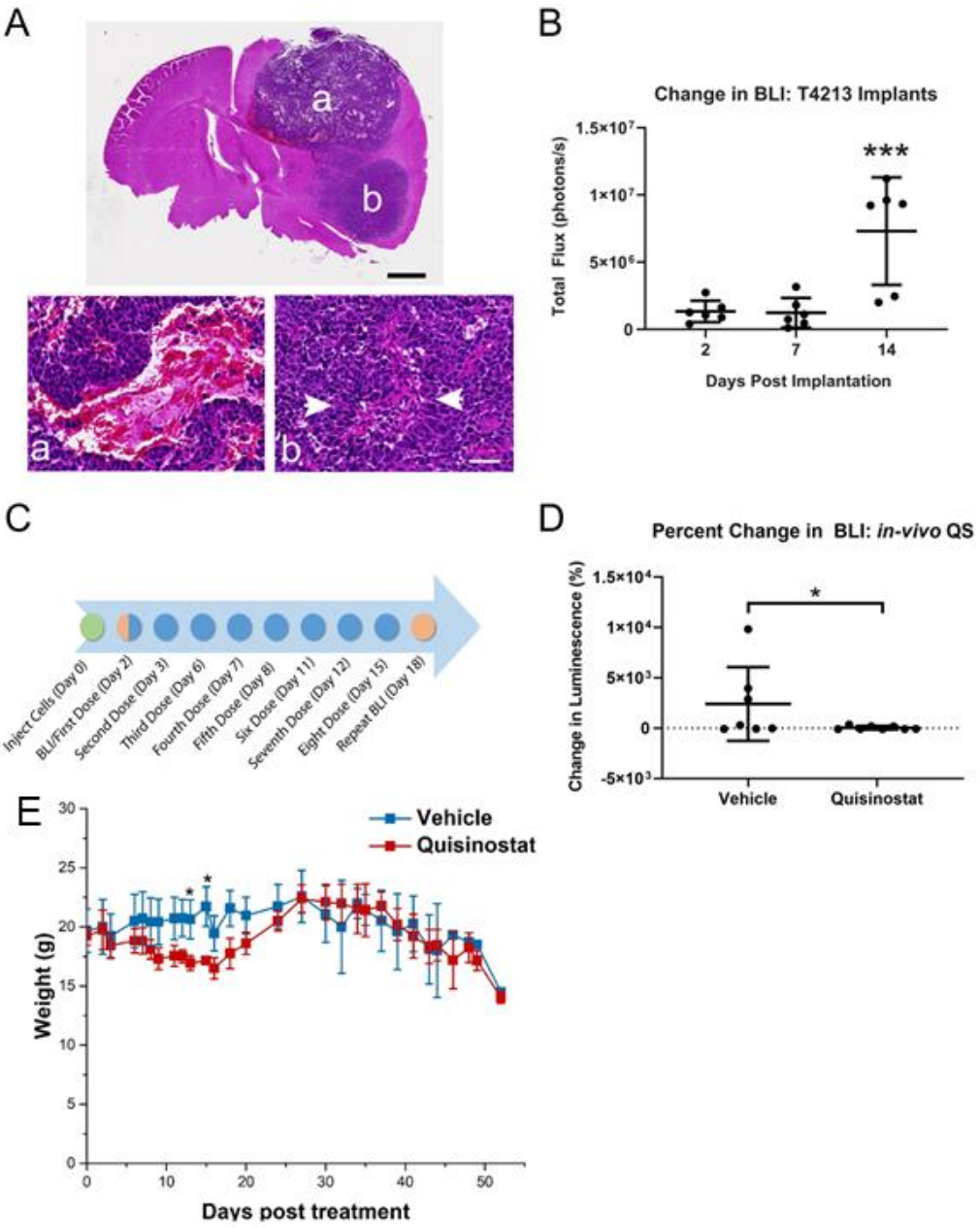

Figure S4: A) Representative photo of the aggressive lesion due to luciferase labelled T4213 implantation after H&E staining, and B) corresponding BLI signal increase in the implants over time. C) Timeline of the *in vivo* protocol for cell implantation and lesion follow up by BLI. D) Comparison of the *in vivo* effects of either vehicle of quisinostat on the lesion by BLI, and E) on mice weight. * and *** indicate a significant difference at $p < 0.05$ and $p < 0.005$ respectively.

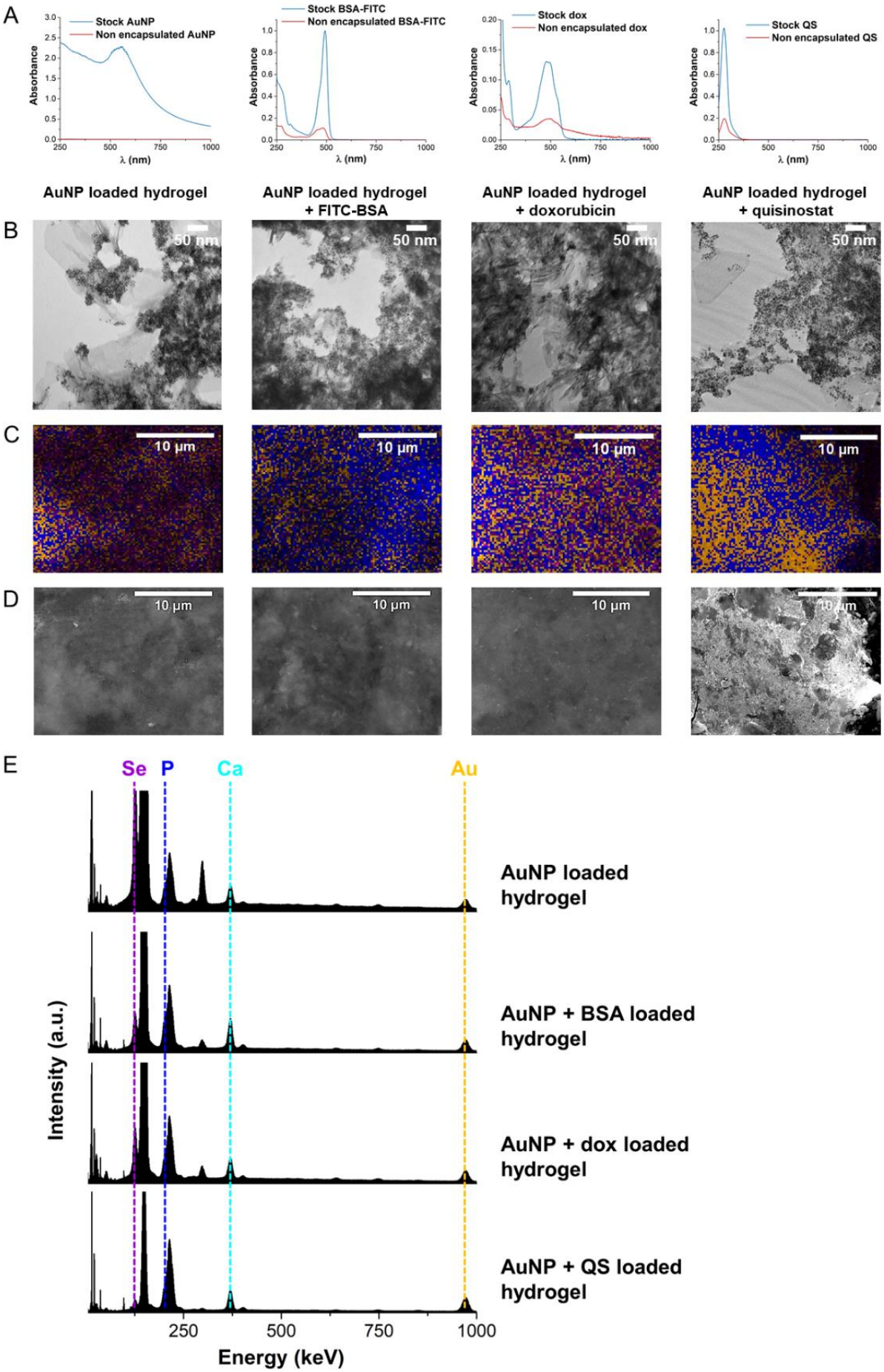
A
Stock AuNP
Non encapsulated AuNP
Stock BSA-FITC
Non encapsulated BSA-FITC
Stock dox
Non encapsulated dox
Stock QS
Non encapsulated QS
Absorbance
λ (nm)
AuNP loaded hydrogel
AuNP loaded hydrogel + FITC-BSA
AuNP loaded hydrogel + doxorubicin
AuNP loaded hydrogel + quisinostat
B
50 nm
C
10 µm
D
10 µm
E
Se
P
Ca
Au
AuNP loaded hydrogel
AuNP + BSA loaded hydrogel
AuNP + dox loaded hydrogel
AuNP + QS loaded hydrogel
Intensity (a.u.)
Energy (keV)

Figure S5: Structural characterizations of the Se-PCPP hydrogel with various loadings. Characterization of Se-PCPP hydrogel with various loadings, AuNP, FITC-BSA, doxorubicin and quisinostat respectively from left to right. A) Quantification of the drug loading efficiency by comparing the absorbance of the non-encapsulated drug to the stock solution by UV-Vis spectroscopy. Analysis of the bulk hydrogel by B) TEM, C) elemental mapping of P (*blue*), Au (*yellow*) and Se (*violet*) on a copper grid (Cu pics unlabeled), and D) SEM of the corresponding area. E) EDX spectra of Se-PCPP hydrogel with various loadings, AuNP, FITC-BSA, doxorubicin and quisinostat respectively from top to bottom.

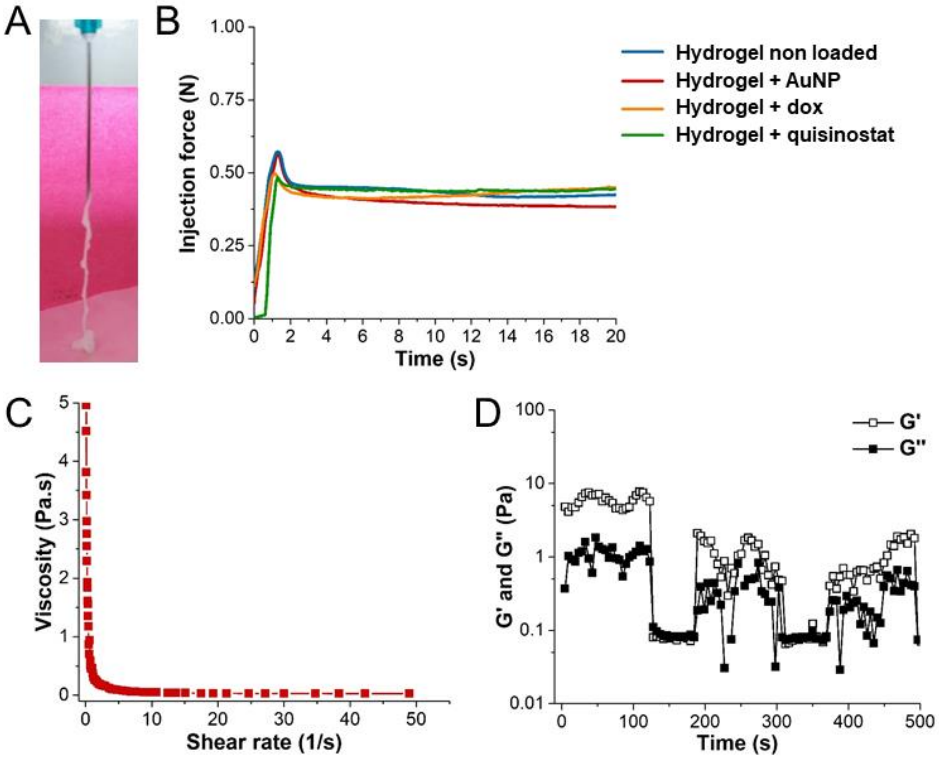

Figure S6: Mechanical tests on the Se-PCPP hydrogel and influence of the various loadings. A) Picture of the non-loaded Se-PCPP hydrogel during the injection process. B) Measurement of the injection force of the hydrogel with various loadings. Rheological evaluation of the C) shear-thinning and D) self-healing abilities of the Se-PCPP hydrogel. G' and G" correspond to the storage modulus and loss modulus respectively, with a low strain of 0.2% strain and a high strain of: 500% strain, 10 Hz.

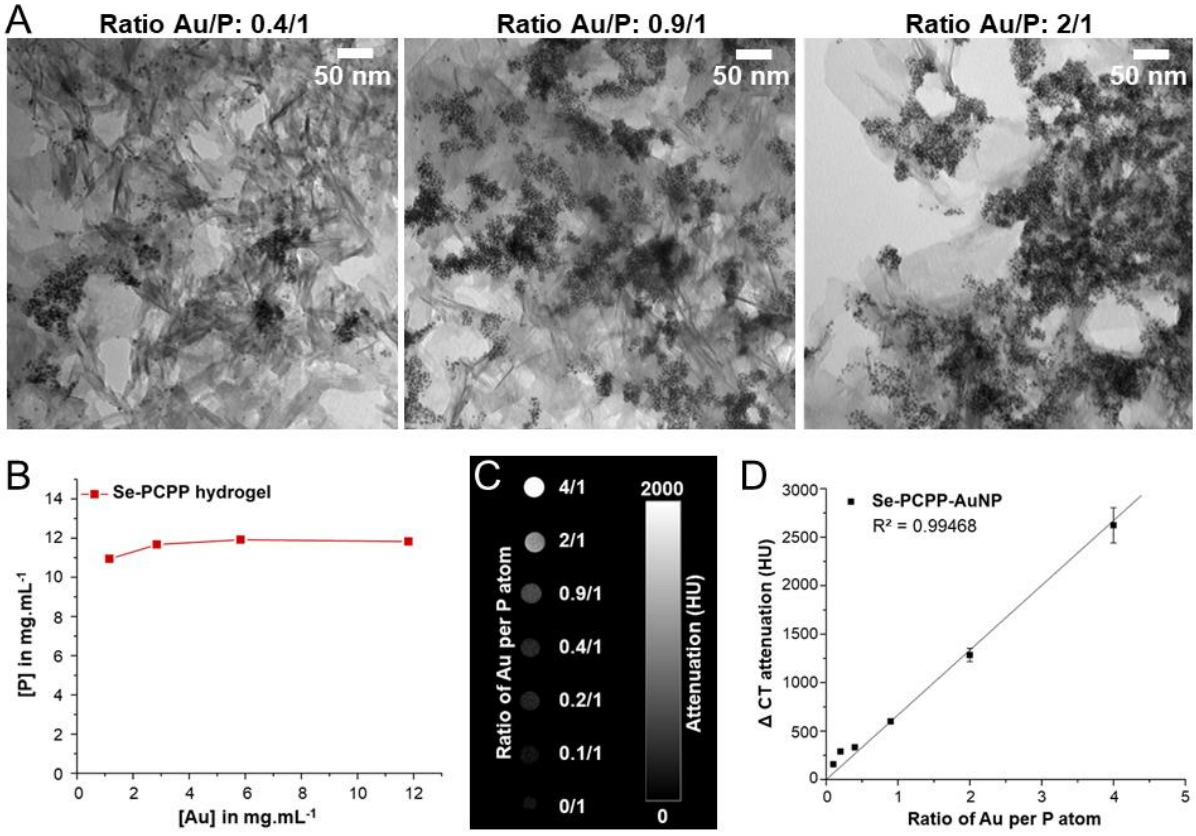

Figure S7: Increasing the loading of AuNP in the Se-PCPP hydrogel for contrast enhancement in CT. A) TEM of the Se-PCPP hydrogel loaded with increasing amounts of AuNP from left to right. B) Increasing the gold concentration in the Se-PCPP hydrogel. C) Contrast enhancement of the hydrogel with increasing AuNP concentration, and D) linear correlation of the CT attenuation to the Au per P ratio of the hydrogel (error bars representing the attenuation variation among triplicate samples– where not visible, the error bars are concealed by the data marker).

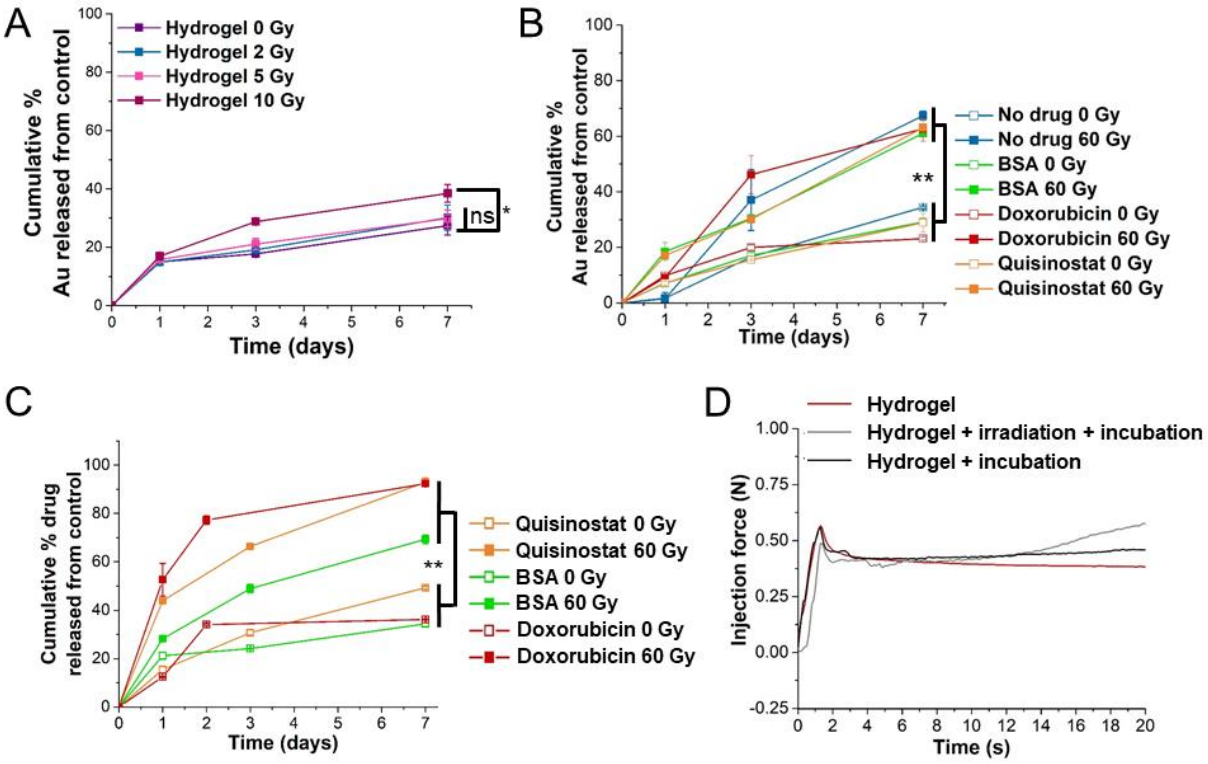

Figure S8: Influence of X-rays on the release rate of payloads from hydrogel and its mechanical properties. Quantification of the released gold from the hydrogel irradiated with A) low X-ray doses of 0 Gy (*violet*) or 2 Gy (*blue*) or 5 Gy (*pink*) or 10 Gy (*purple*). B) High X-ray dose of 60 Gy (*filled*) compared to mock irradiation (*empty*). C) Quantification of the released drug from the Se-PCPP hydrogel containing quisinostat (*orange*), doxorubicin (*red*) or FITC-BSA (*green*) at high X-ray dose of 60 Gy (*filled*) compared to mock irradiation (*empty*). D) Loss of flowability of the irradiated hydrogel (*grey*) compared to hydrogel stored at room temperature (*red*) or 37 °C (*black*). 'ns' indicates the non-significant difference with $p > 0.05$ while * and ** indicate a significant difference at $p < 0.05$ and $p < 0.01$ respectively.

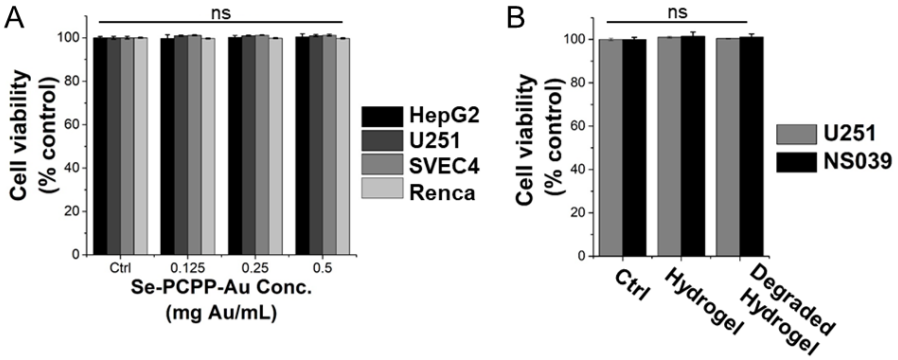

Figure S9: A) Viability of cell lines determined by LIVE-DEAD assay upon 8 hours treatment with media conditioned with the AuNP-loaded Se-PCPP hydrogel. B) Viability of glioblastoma

cell lines exposed to media conditioned with AuNP-loaded Se-PCPP hydrogel, with and without X-ray degradation. 'ns' indicates the non-significant difference with $p > 0.05$.

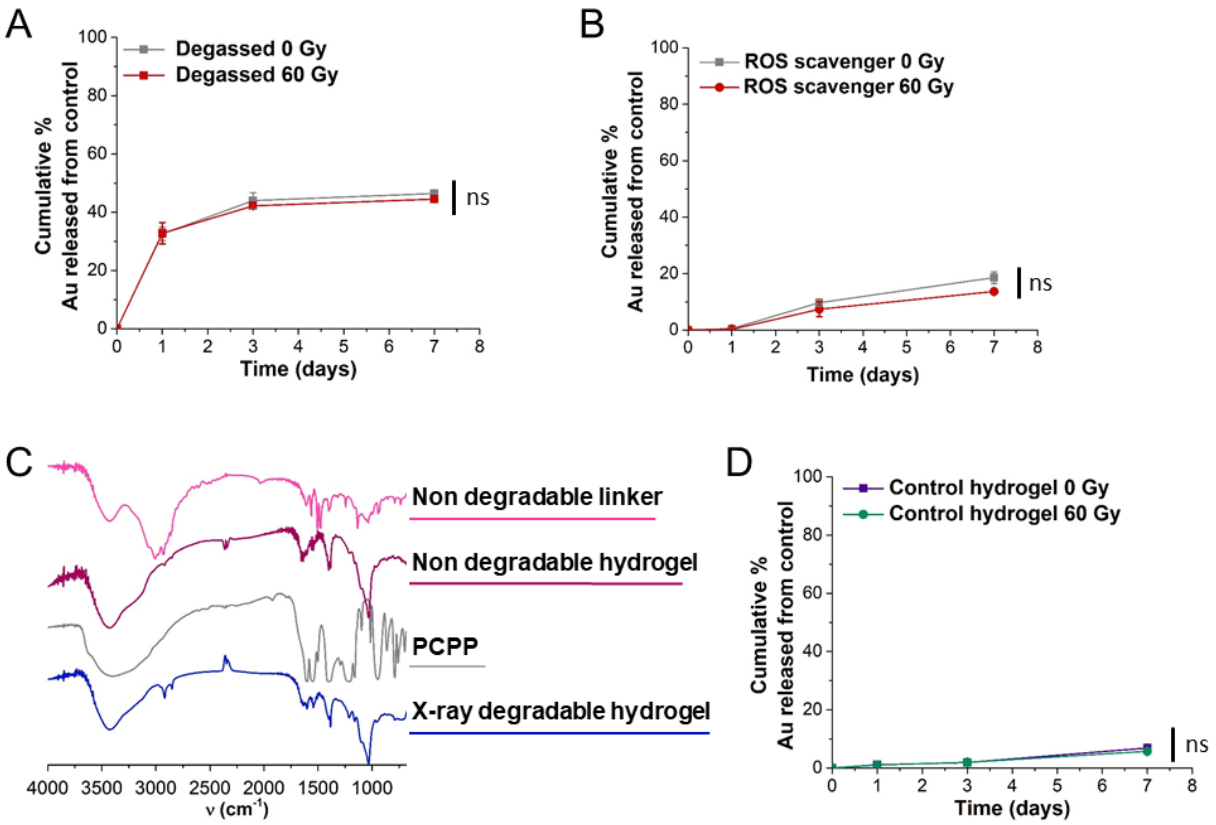

Figure S10: Investigation of the mechanism of radiation triggered degradation of the Se-PCPP hydrogel. Quantification of the gold release from the Se-PCPP hydrogel after irradiation with either 0 Gy (grey) or 60 Gy (red) in A) a degassed medium and B) a ROS scavenging medium. Probing the role of selenocystamine degradation on hydrogel radiation responsiveness *via* comparison with a control non-radiation sensitive cross-linker: C) chemical composition confirmation by FT-IR and D) quantification of the gold release from the non-radiation sensitive hydrogel. 'ns' indicates the non-significant difference with $p > 0.05$.

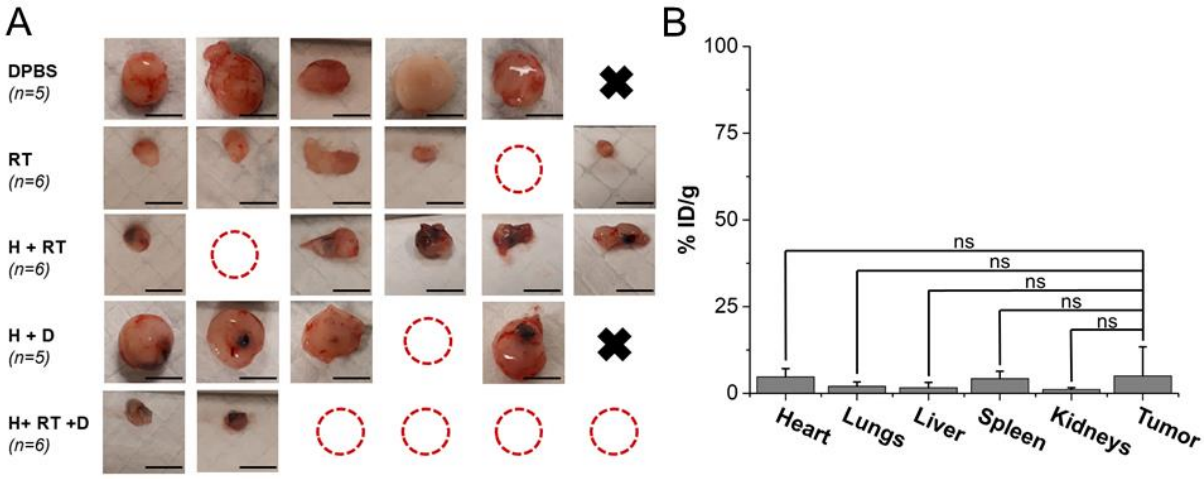

Figure S11: A) Photographs of the tumors after harvesting; scale bar 1 cm; the black cross indicates the mouse died before treatment administration. B) Biodistribution in major organs of the AuNP released from the Se-PCPP hydrogel in group 'H+drug+RT'. 'ns' indicates the non-significant difference with $p > 0.05$.

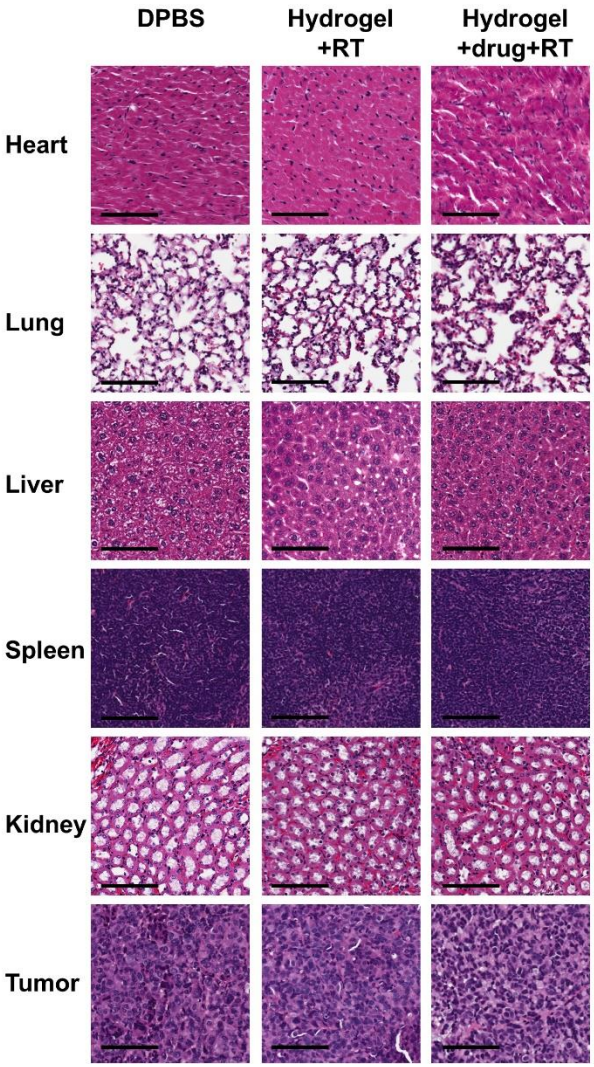

Figure S12: Histopathological investigation comparing representative H&E stained micrographs from the control group (*left*) to the effect of the drug loaded Se-PCPP hydrogel (*right*) on major organs, *i.e.* heart, lung, liver, spleen, kidney, and the tumor. The scale bars correspond to 200 µm.

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