pharmaceutics

# Supplementary Materials: A New Generation of Ultrasmall Nanoparticles Inducing Sensitization to Irradiation and Copper Depletion to Overcome Radioresistant and Invasive Cancers

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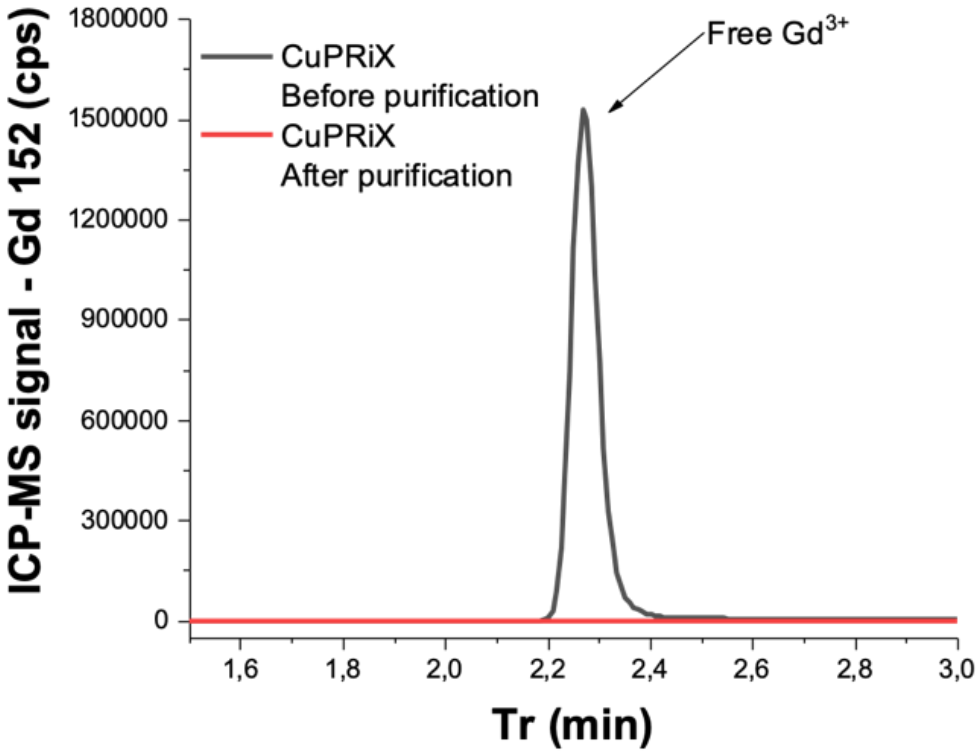


**Figure S1.** Comparison of free $Gd^{3+}$ ions level before and after purification followed by HPLC-ICP/MS.

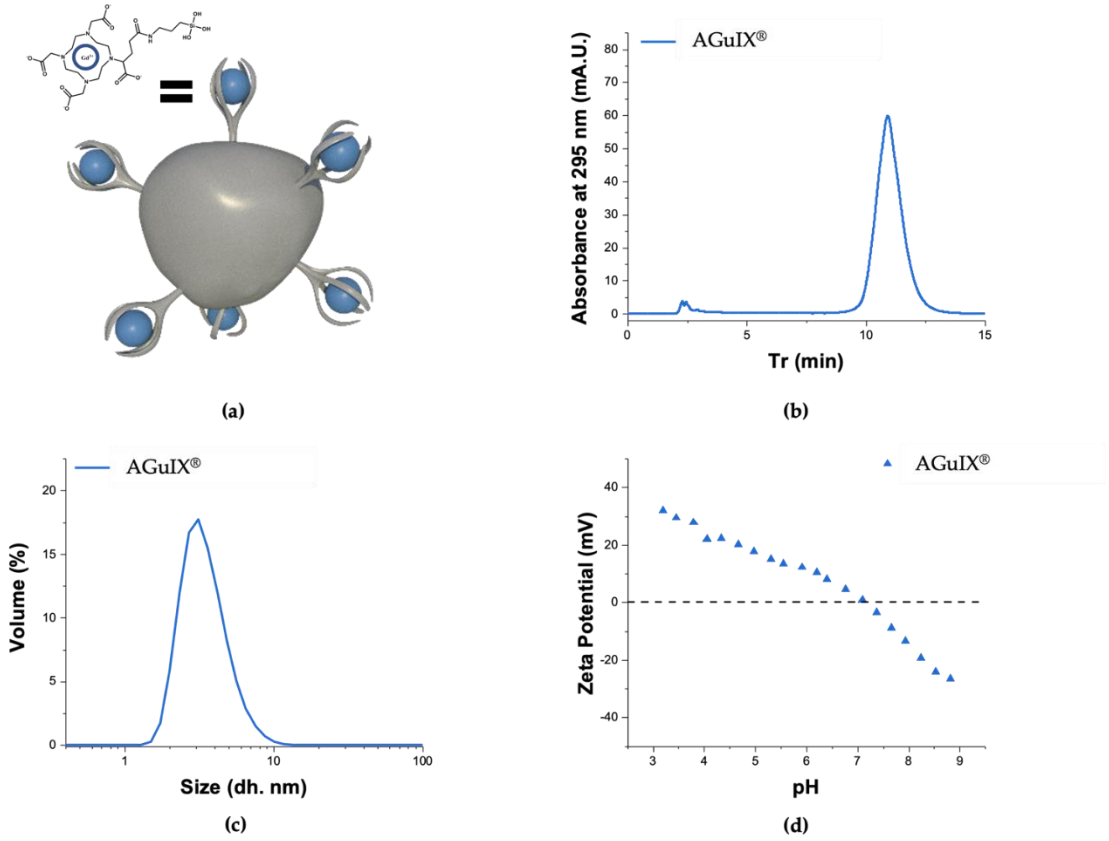


**Figure S2.** (a) Schematic representation of AGuIX® with the detailed structure of chelated DOTAGA groups; (b) HPLC-UV chromatograms of AGuIX® (10 µL, 100 g/L) recorded at 295 nm; (c) Hydrodynamic diameters distribution in volume as obtained by dynamic light scattering; (d) Zeta potential vs pH for AGuIX®.

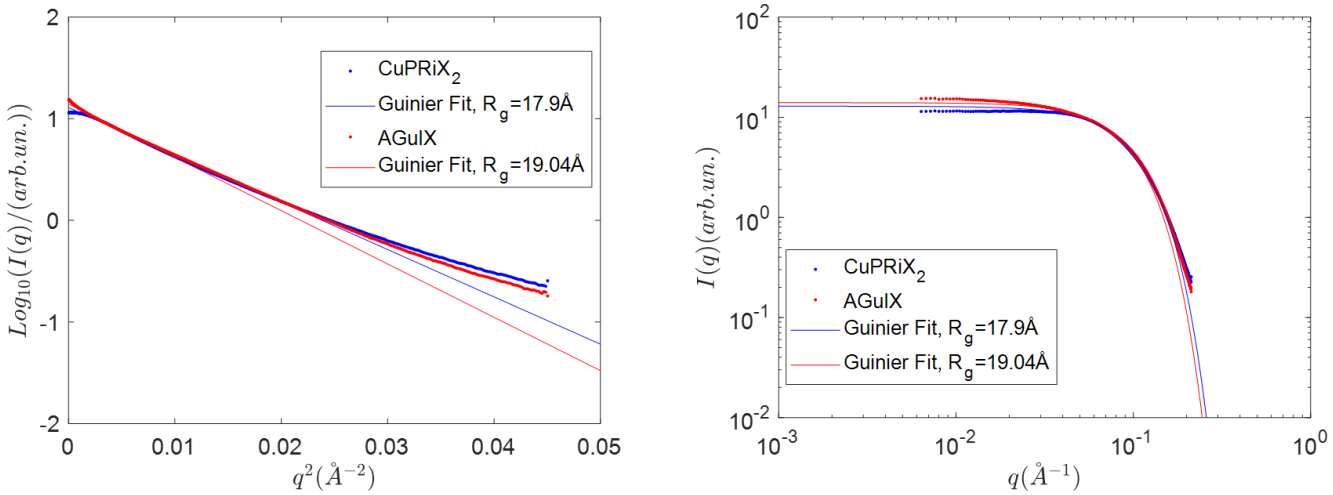


**Figure S3. Calculation of radius of gyration by SAXS.** (a) Guinier plots for $CuPRiX_2$ and AGuIX® hybrid nanoparticles. Both particles exhibit similar gyration radii. (b) Log-Log reprensentation using the same modelling as in (a), showing the deviation of the Guinier law in the high-$q$ range ($q > 0.2$ Å$^{-1}$) and in the low-$q$ range ($q < 3.10^{-3}$ Å$^{-1}$).

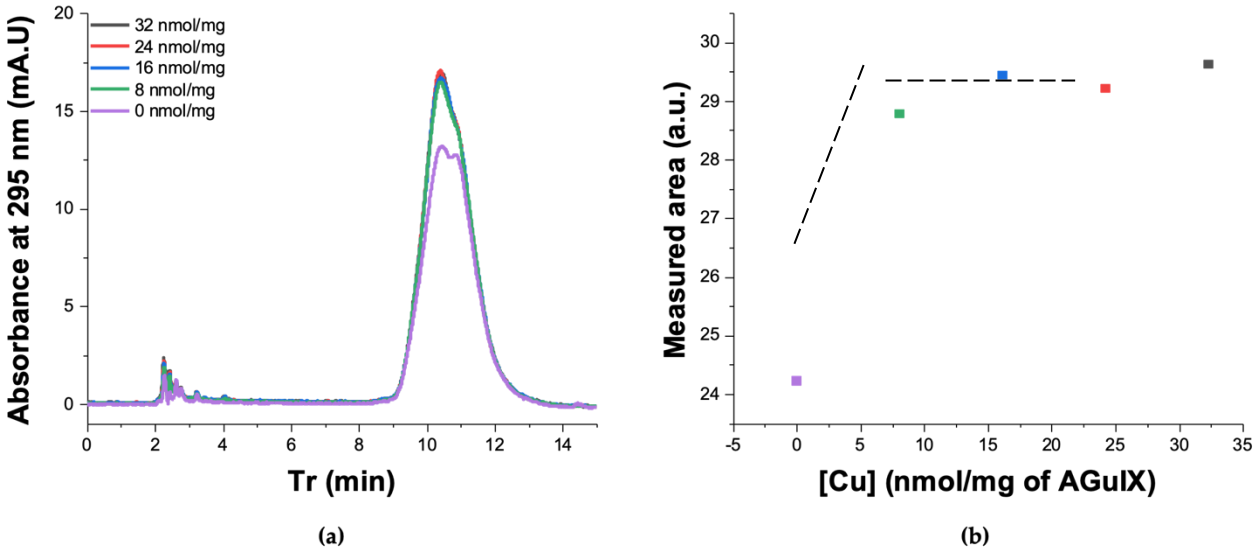


**Figure S4. Measurement of unchelated DOTAGA on AGuIX®.** (a) Chromatograms of samples with increasing amount of $Cu^{2+}$ per mg of AGuIX®. The increase in absorbance at 295 nm is due to the formation of DOTAGA@($Cu^{2+}$); (b) Measured area depending on the amount of $Cu^{2+}$. The onset of the absorbance plateau shows the amount of DOTAGA on AGuIX®.

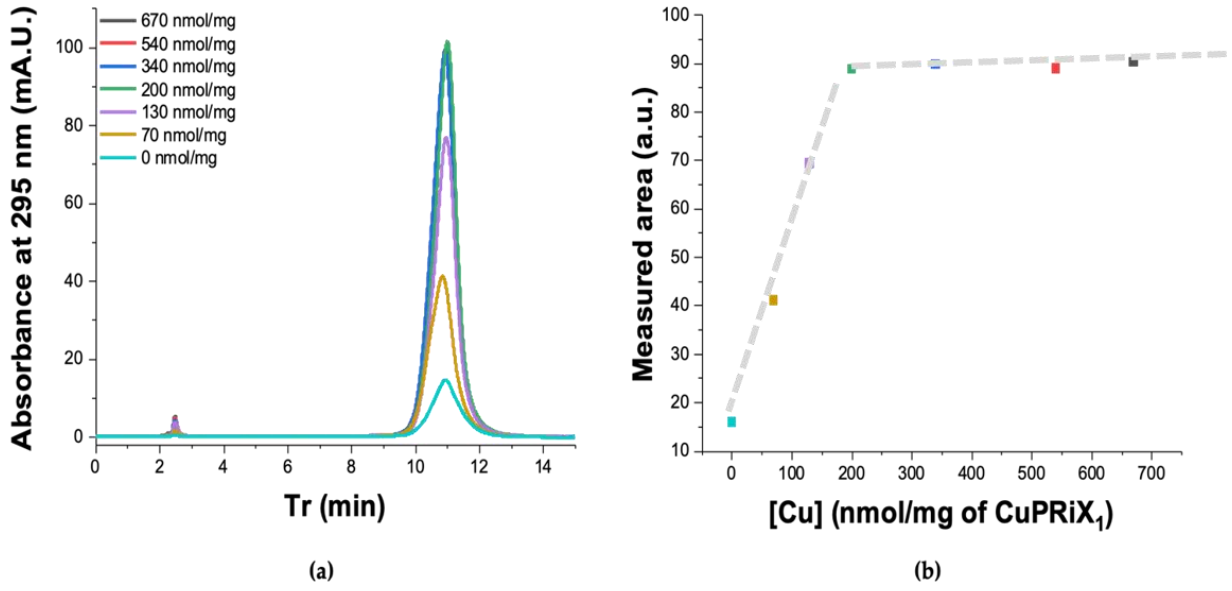


**Figure S5. Measurement of unchelated DOTAGA on CuPRiX1.** (a) Chromatograms of samples with increasing amount of $Cu^{2+}$ per mg of CuPRiX1. The increase in absorbance at 295 nm is due to the formation of DOTAGA@($Cu^{2+}$); (b) Measured area depending on the amount of $Cu^{2+}$. The slope change shows the amount of DOTAGA on CuPRiX1.

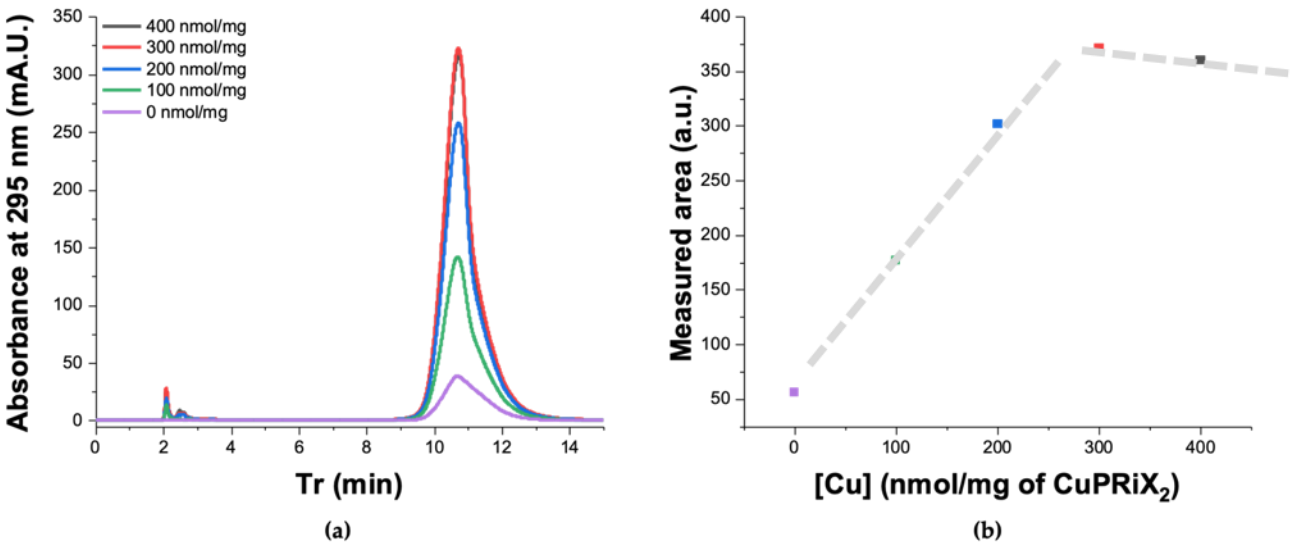


**Figure S6. Measurement of unchelated DOTAGA on CuPRiX2.** (a) Chromatograms of samples with increasing amount of $Cu^{2+}$ per mg of CuPRiX2. The increase in absorbance at 295 nm is due to the formation of DOTAGA@($Cu^{2+}$); (b) Measured area depending on the amount of $Cu^{2+}$. The slope change shows the amount of DOTAGA on CuPRiX2.

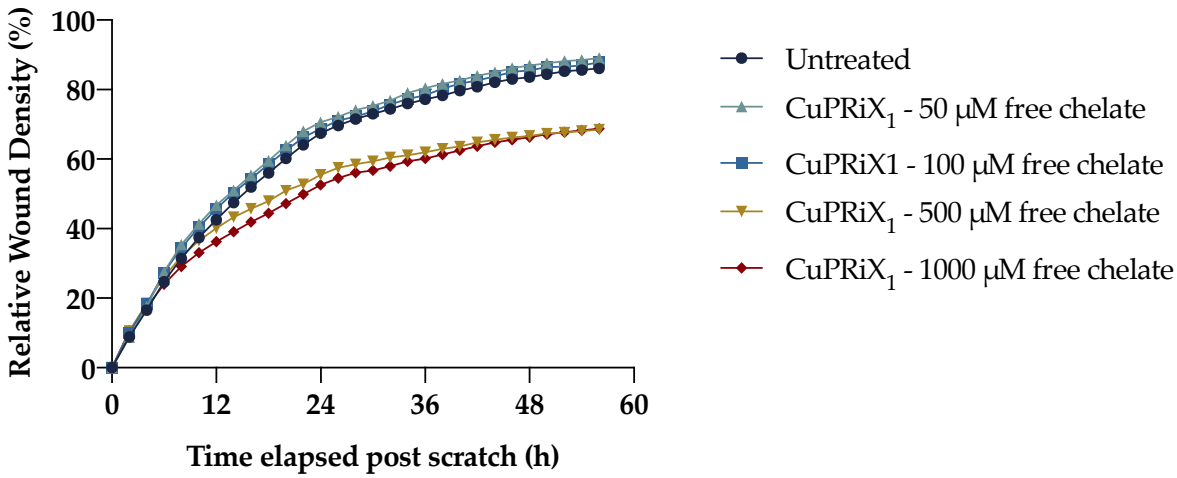


**Figure S7.** Effect of increasing concentrations of CuPRiX1 (0, 50, 100, 500, 1000 µM of uncomplexed chelate) on cell motility of A549 cells. Quantitative analysis of wound closure as a function of time. Relative wound density is a measure of the density of the wound region relative to the density of the cell region (%). Data are presented as the mean ± SEM (n=6).

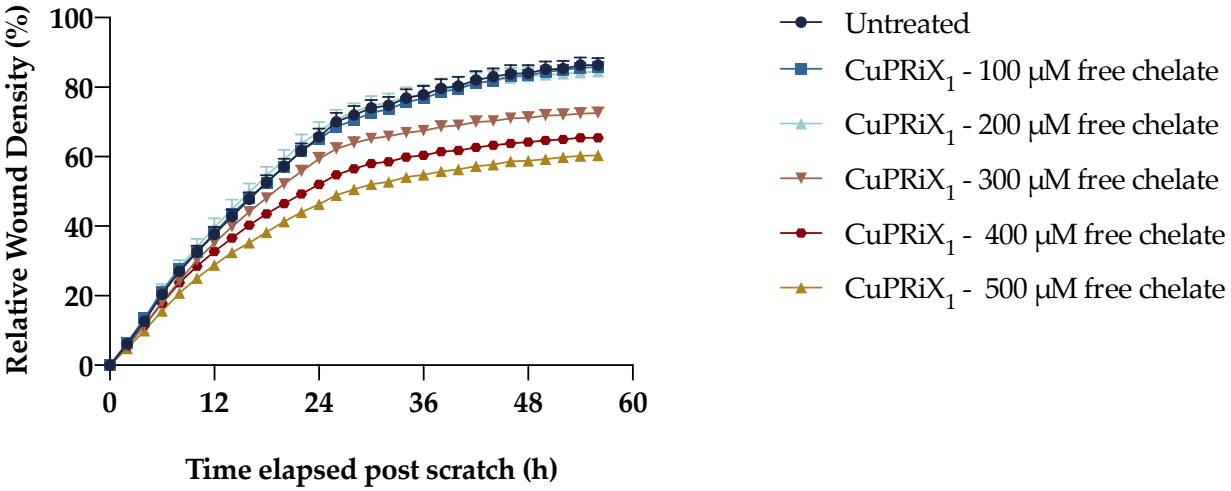


**Figure S8.** Effect of increasing concentrations of CuPRiX1 (0, 100, 200, 300, 400, and 500 µM of uncomplexed chelate) on cell motility of A549 cells. Quantitative analysis of wound closure as a function of time. Relative wound density is a measure of the density of the wound region relative to the density of the cell region (%). Data are presented as the mean ± SEM (n=6).

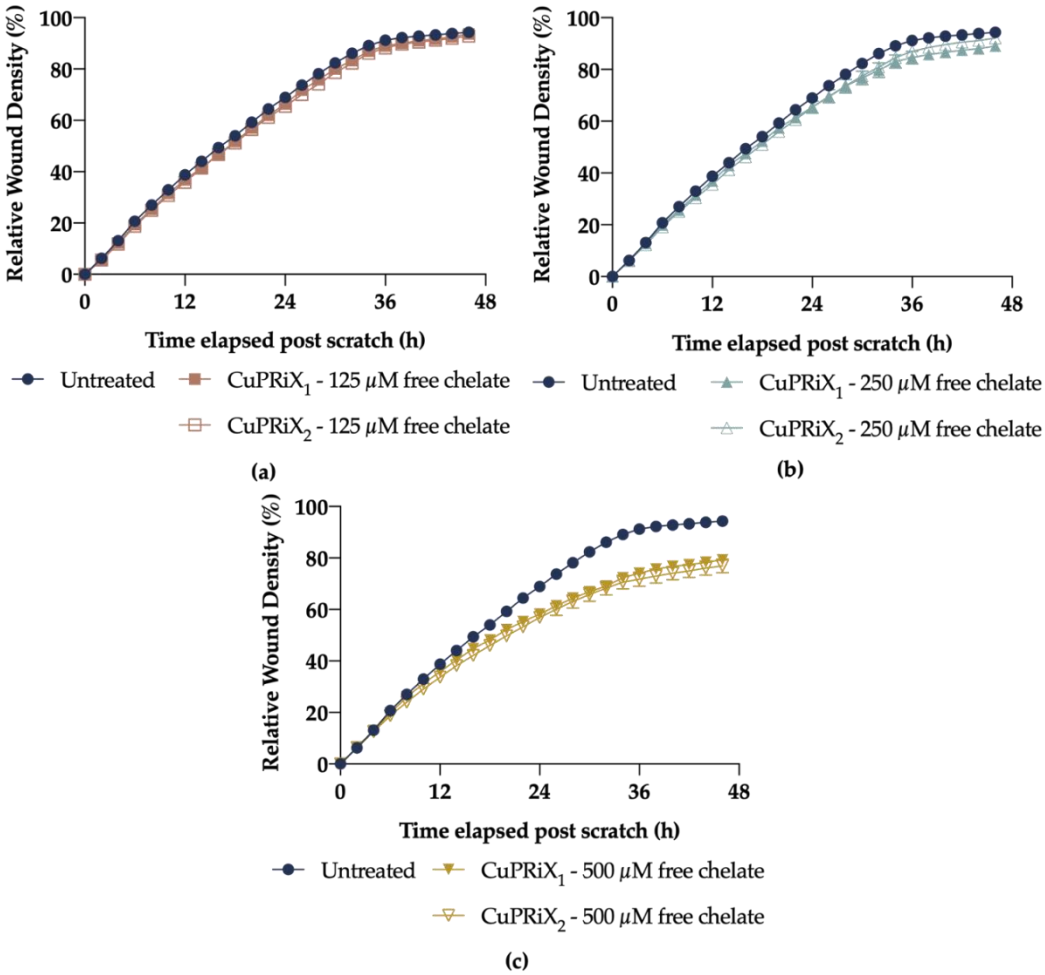


**Figure S9. Effect of CuPRiX1 and CuPRiX2 on cell motility of A549 cells.** (a) Treatment with 125 µM of uncomplexed chelate. (b) Treatment with 250 µM of uncomplexed chelate. (c) Treatment with 500 µM of uncomplexed chelate. Relative wound density is a measure of the density of the wound region relative to the density of the cell region (%). Data are presented as the mean ± SEM (n=6).

**Proliferation Assay**

A549 and SQ20B-CSCs cells were seeded in a 96-well plate at a density of 5,000 cells/well and incubated overnight at 37°C, 5% $CO_2$ to allow adhesion. Medium was removed and replaced with medium alone or containing CuPRiX$_1$ (500 µM of uncomplexed DOTAGA). The plates were placed in the IncuCyte ZOOM device and pictures were taken every 4 h for 48 h. Data were analyzed with the IncuCyte ZOOM software (v. 2018A), which allows quantification of cell surface coverage as confluence values.

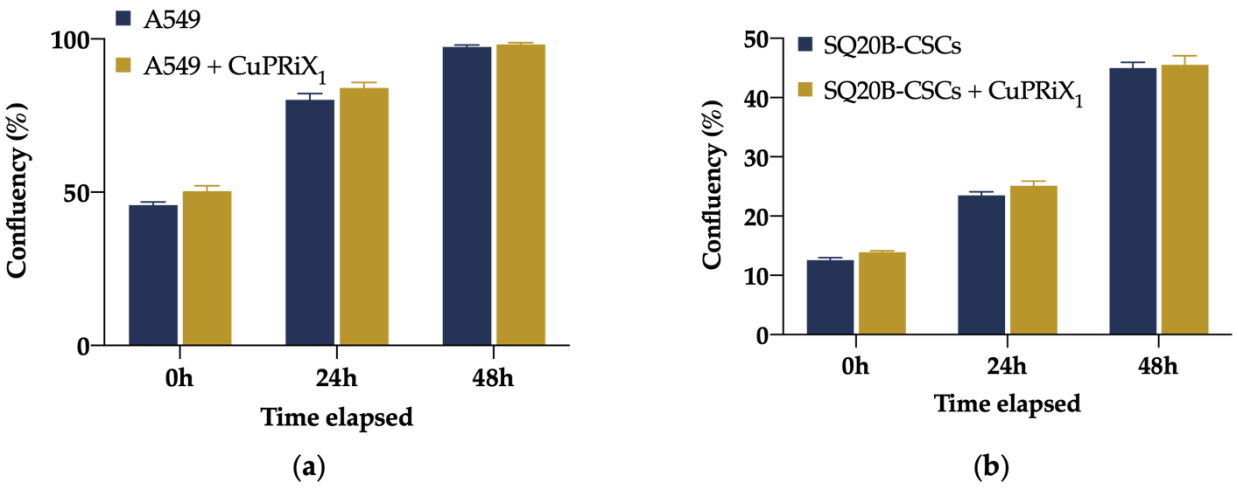


**Figure S10. Proliferation of A549 (a) and SQ20B-CSCs (b) cells after treatment with CuPRiX$_1$ (500 µM of free DOTAGA).** Cell proliferation was monitored with using IncuCyte ZOOM device, photos of the wells were taken every 6 hours for 48 h, and the percentage of cell confluency was expressed.