# Supporting Information

## Shiga toxin-B targeted gold nanorods for local photothermal treatment in oral cancer clinical samples

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## Supporting information items:

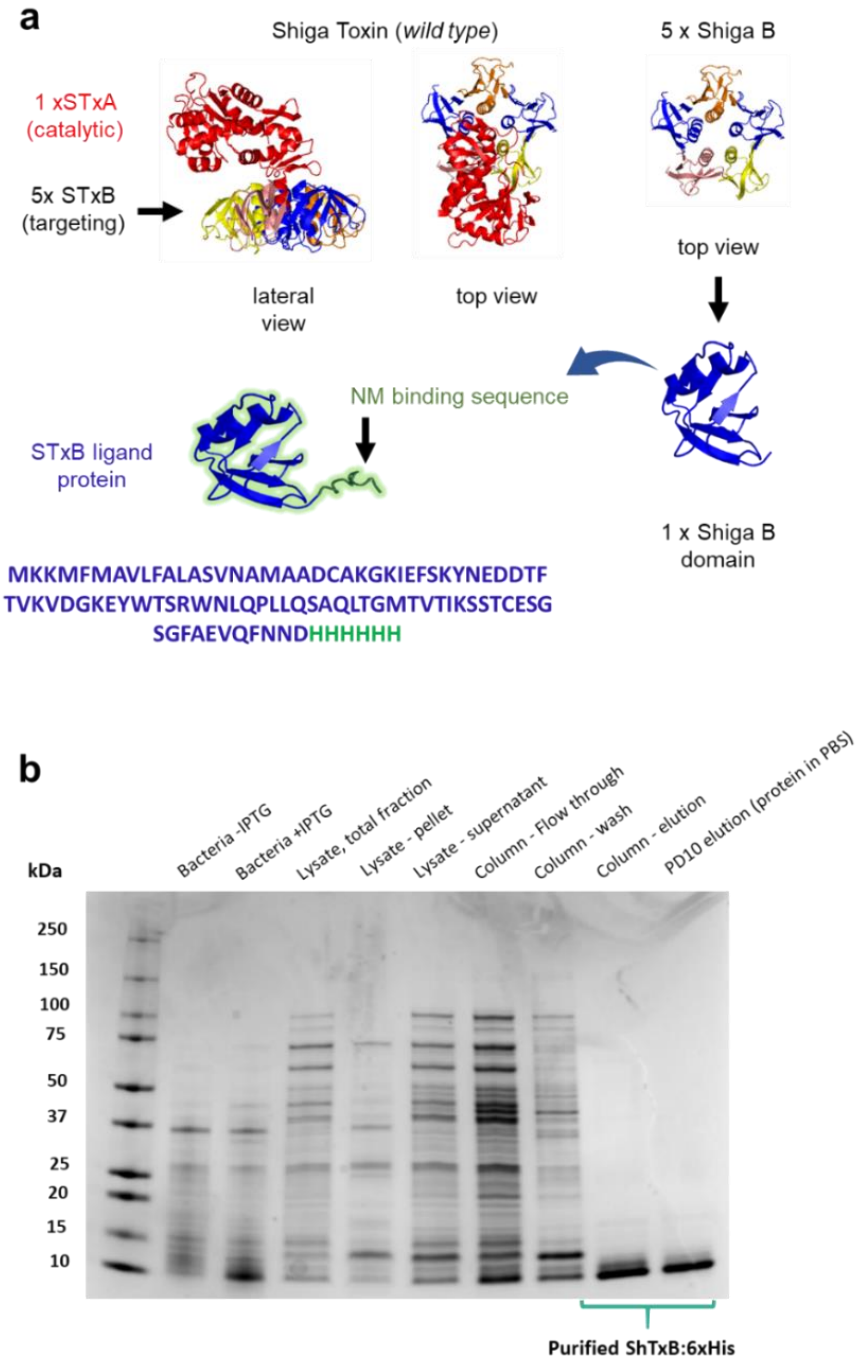


**Figure S1.** (a) Different domains in the structure of the Shiga toxin (ShTx). Single B domain (ShTxB) colored in blue attached to a 6 histidine cationic tail (green). (b) Coomassie-blue stained SDS-PAGE electrophoresis complete acrylamide gel of Fig 1d showing the different fractions of proteins obtained during the overexpression and purification steps of the StxB:6xHis protein.

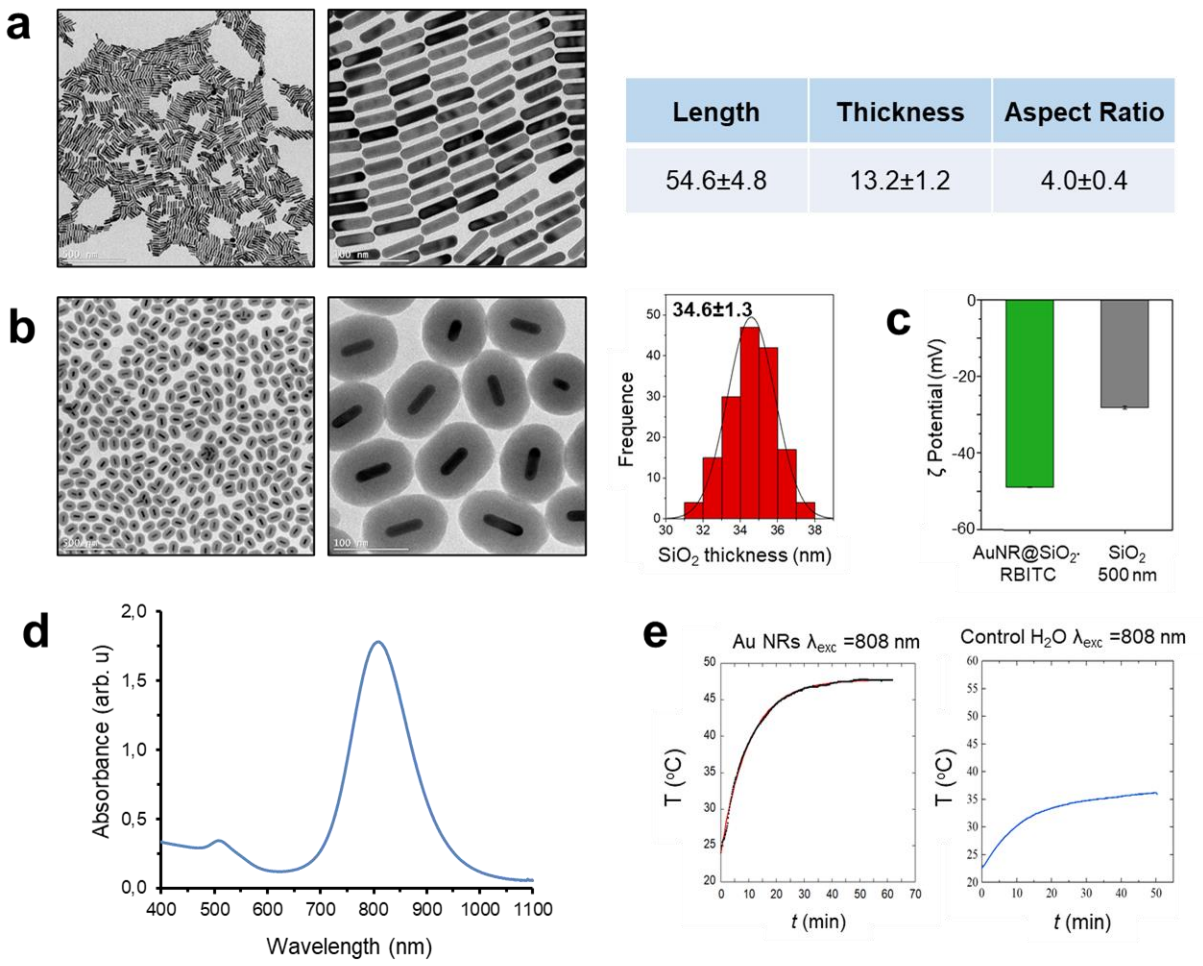


**Figure S2.** (a) Transmission electron microscopy (TEM) images and size (table, right; $n$ = 150) of the synthesized gold nanorods (AuNRs). (b) TEM images of the AuNRs after coating with the amorphous silica shells (AuNRs@$SiO_2$). The histogram on right shows shell thickness size distribution ($N$=150). (c) ζ Potential of AuNRs@$SiO_2$ in comparison to amorphous $SiO_2$ particles. (d) UV-visible absorption spectra of the synthesized AuNRs, with the longitudinal surface plasmon resonance (LSPR) peak at 810 nm. (e) AuNRs temperature rise upon near-infrared LD (λ=808 nm) irradiation (left), and in a control with water (right).

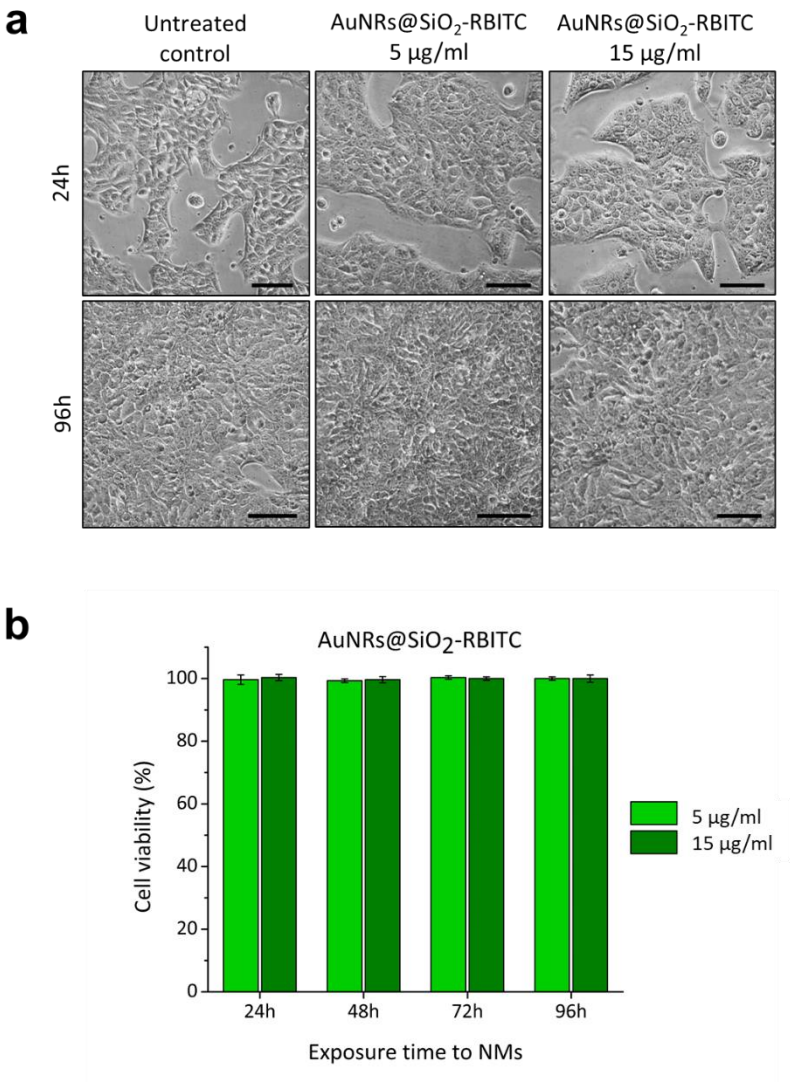


**Figure S3.** (a) Phase-contrast images of human pharynx epithelial carcinoma (Detroit) cells treated with 5-15 µg/mL of AuNRs@$SiO_2$:RBTIC after 24 and 96 h. (b) Cell viability study of these cells treated with 5-15 µg/mL of AuNRs@$SiO_2$:RBTIC after 24, 48, 72, and 96h.

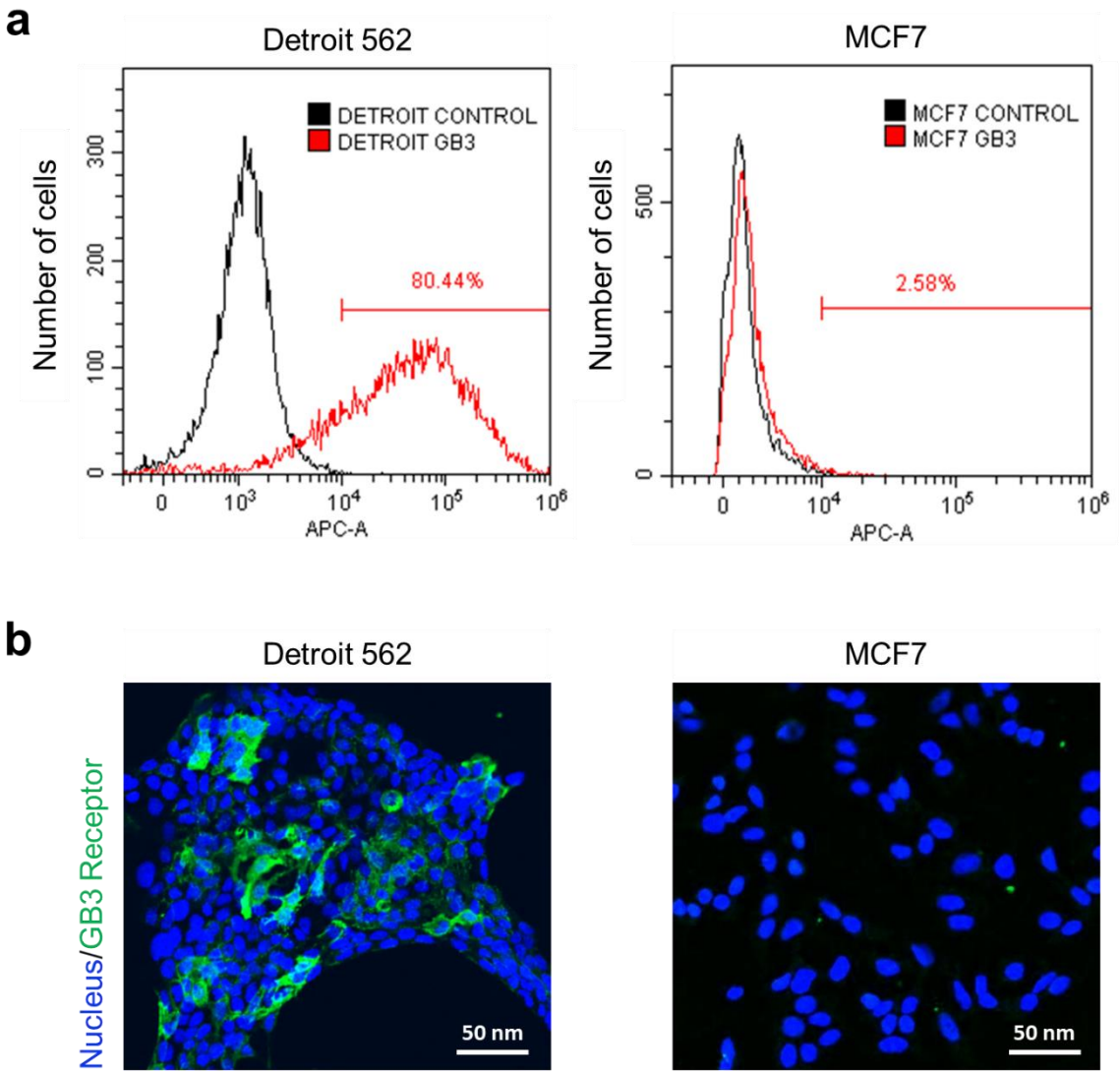


**Figure S4.** (a) Flow cytometric analysis of the GB3 expression on HNC cells (Detroit 562, left) and human breast adenocarcinoma (MCF7) that were used as GB3+ve and GB3-ve models, respectively. Plots show the quantification of the levels of GB3 expression per cell, in a total of 10.000 cells. (b) Confocal microscopy images of HNC and MCF7 cells immunostained for GB3 (green channel). Nuclei are stained with Hoechst (blue channel).

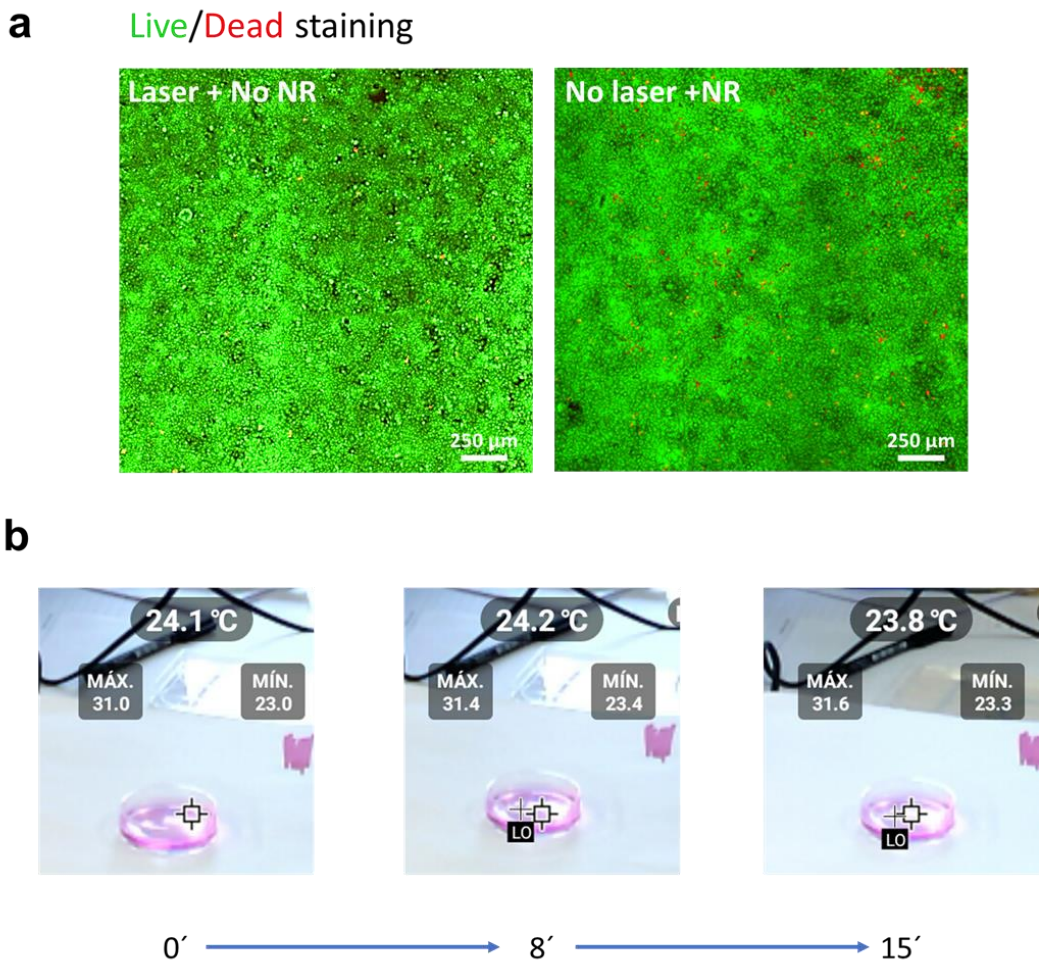


**Figure S5.** (a) Representative cultures of control GB3+ve HNC Detroit 562 cells irradiated with the LD but without AuNRs@$SiO_2$:RBTIC@ShTxB (left), or with the nanoparticles but without irradiation (right). Cells were stained with Acridine Orange (live cells, green channel) and ethidium bromide (dead cells, red channel). (b) Temperature monitorization in the culture media after 15 minutes of laser irradiation. No temperature rise was observed in the media during the irradiation procedure.

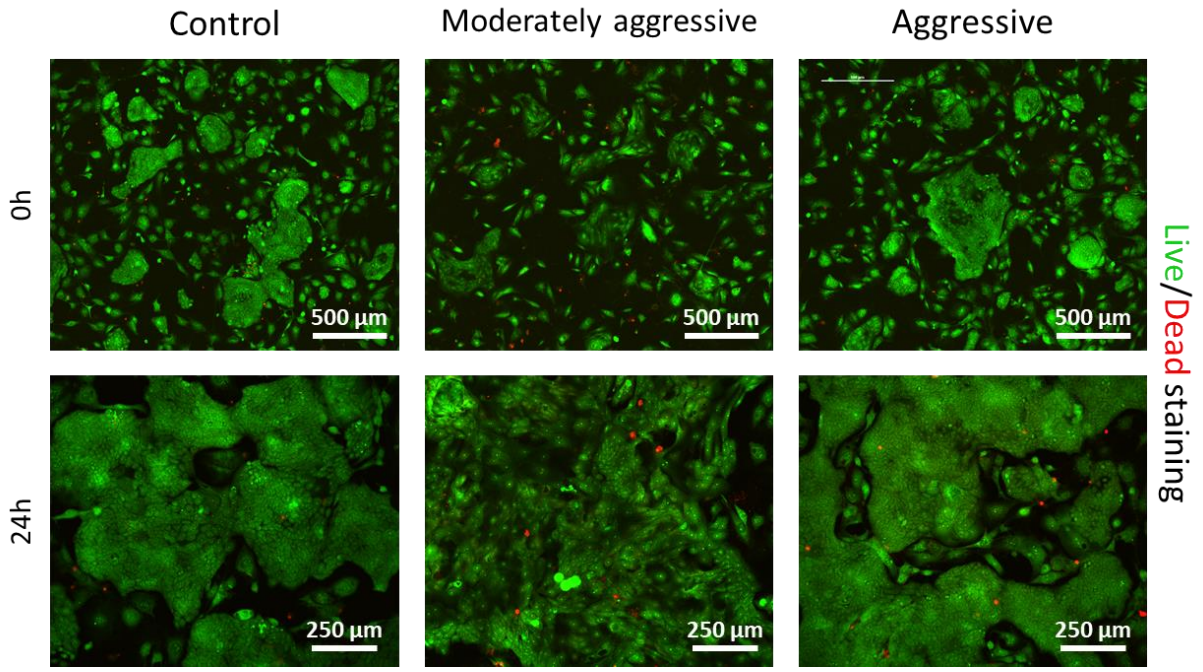


**Figure S6.** Confocal microscopy images of biopsy cells irradiated with an 808 nm LD but not treated with AuNRs@$SiO_2$@ShTxB. Cells were live/dead stained and observed immediately after irradiation (0 h) or after 24 h. Control cells were isolated from a healthy human biopsy (cheek inner mucosa). Human cancer cell cultures were grown from moderately aggressive, intermediate grade (MASCC) or aggressive, high grade (ASCC) biopsies of malignancies localized at the floor of the mouth and retromolar regions of the oral mucosa respectively.