

Supporting Information

siRNA Delivery Using Dithiocarbamate-Anchored Oligonucleotides on Gold Nanorods

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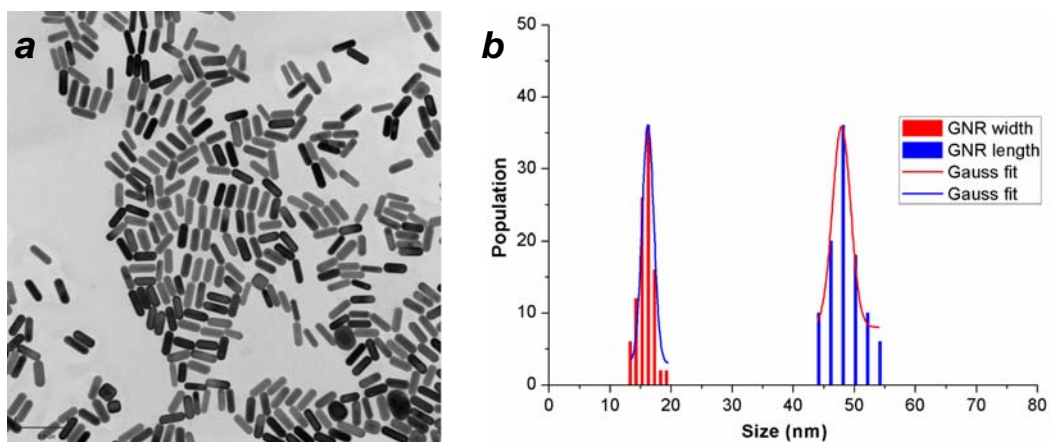


Figure S1. Size analysis of citrate-stabilized GNRs. (a) TEM image (scale bar =100 nm); (b) statistical distribution of width (16.1 ± 1.2 nm) and length (48.2 ± 2.6 nm; $N = 100$).

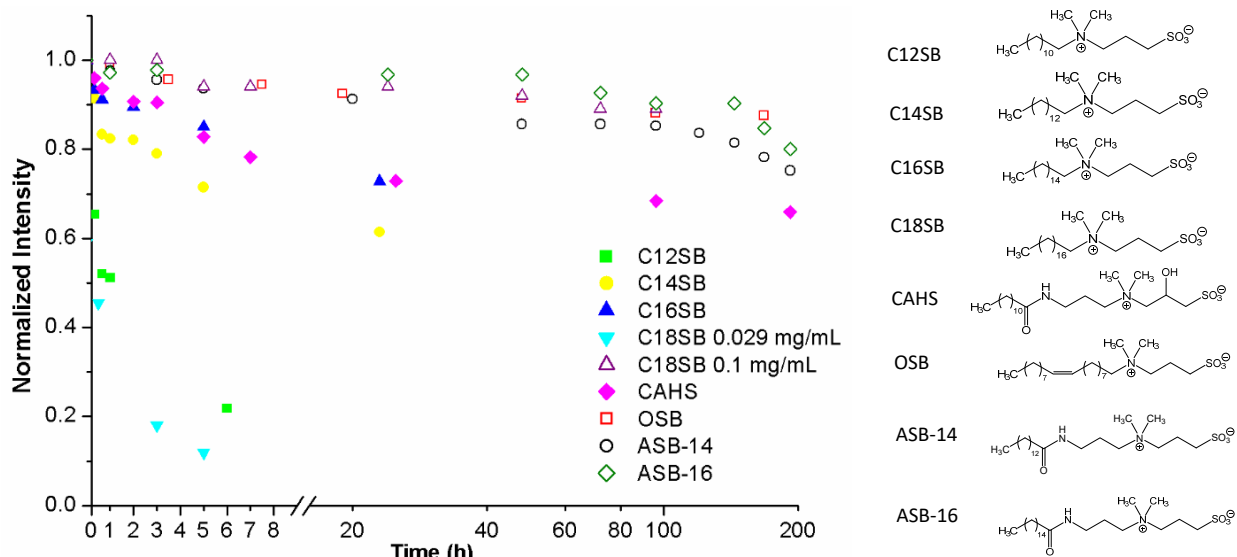


Figure S2. Dispersion stability of sulfobetaine (SB)-stabilized GNRs in 1 M NaCl over a 1-week period, as a function of surfactant structure. Surfactant concentrations at 2.1 mg/mL, unless otherwise noted.

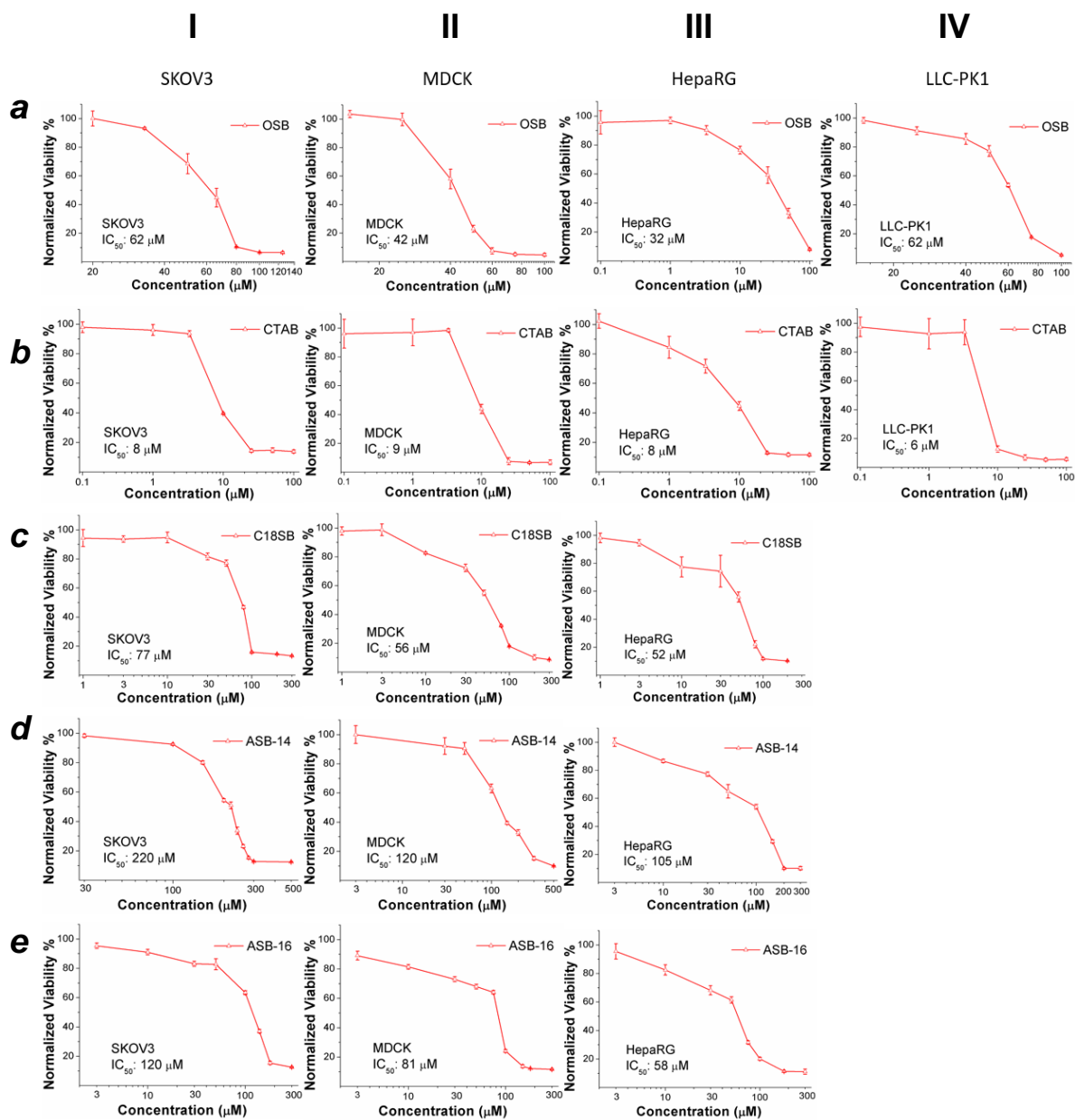


Figure S3. Cell viabilities (with IC₅₀ values) of CTAB and various sulfobetaines by MTT assay, using different cell lines. Rows a–e: OSB, CTAB, C18SB, ASB-14, and ASB-16. Columns I–IV: SKOV-3, MDCK, HepaRG, and LLC-PK1 cells.

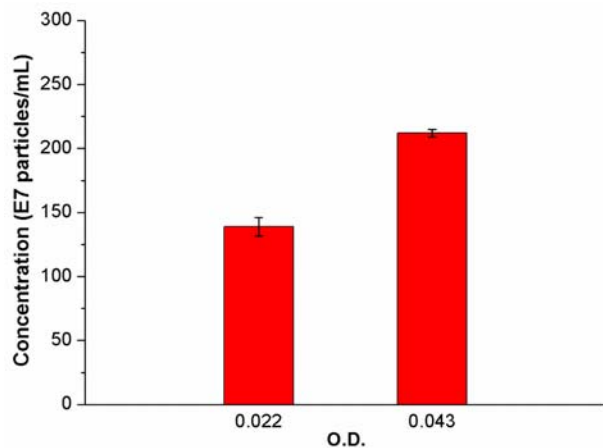


Figure S4. OSB-GNR counts as a function of optical density (absorbance at λ_{\max} ; cf. Figure 2a), determined by nanoparticle tracking analysis (NTA). Particle counts based on a minimum of 2000 particles, and calibrated against a suspension of standardized 100-nm polystyrene beads.⁴⁹ A GNR dispersion with an initial O.D. of 1.1 was diluted 25- and 50-fold using particle-free water prior to NTA (final O.D. values shown). Error represents one standard deviation of three separate trials. The concentration of OSB-GNRs at O.D. 1.0 is estimated to be 5.1×10^{10} particles/mL, or 85 pM.

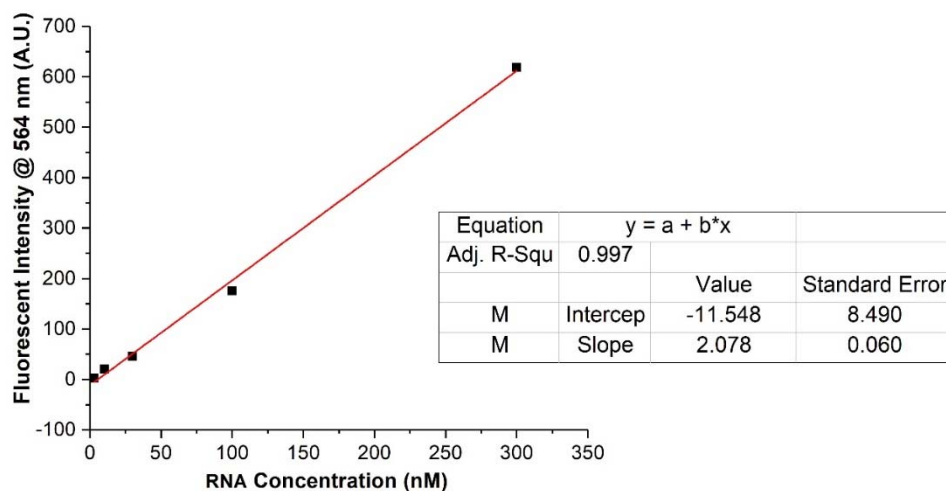


Figure S5. Standard linear curve for eGFP antisense RNA, based on Dy547 fluorescence ($\lambda_{\text{em}} = 564$ nm) with serial dilution of Dy547-eGFP antisense RNA (300 nM to 3 nM). The fluorescence intensity for the experimental data point was 25.8, corresponding to 14.7 nM antisense RNA released from GNR-siRNA carriers (64 pM).

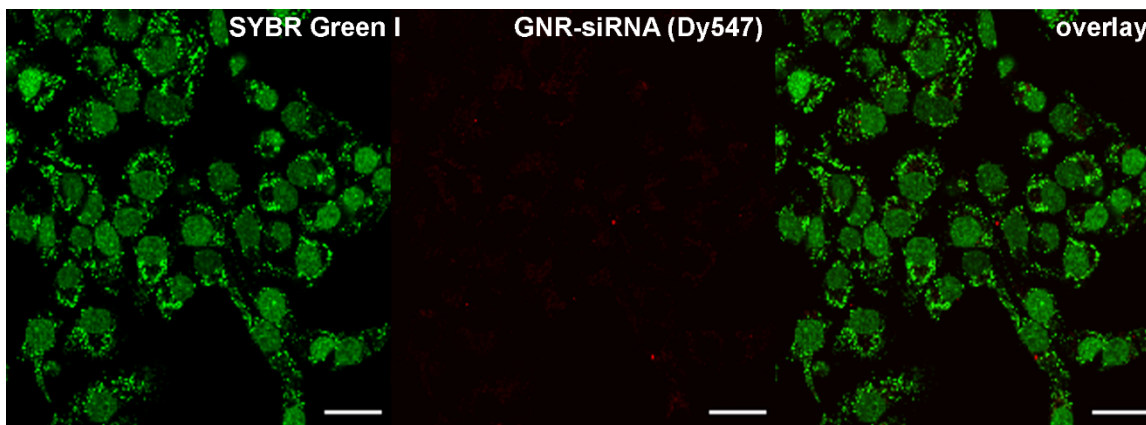


Figure S6. Confocal fluorescence microscopy of wild-type SKOV-3 cells after 24-h incubation with GNR-siRNA labelled with Dy547 (85 pM), using HS-mPEG as a co-adsorbate. Bar = 30 μ m.

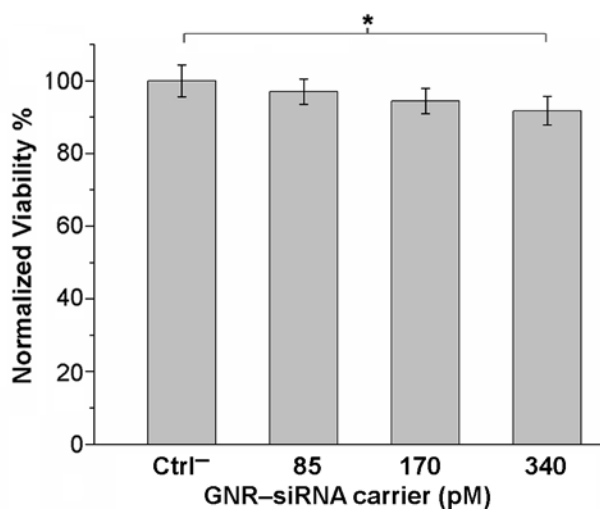


Figure S7. Cytotoxicity analysis of SKOV-3 cells by MTT assay after 24-hour exposure to GNR-siRNA carriers, without laser irradiation ($N = 3$). * $p = 0.06$ (Student's t -test, two-tailed null hypothesis) or 0.095 (one-way ANOVA, posthoc Tukey). Carriers were prepared using DTC-siRNA labelled with HS-PEG-folate.

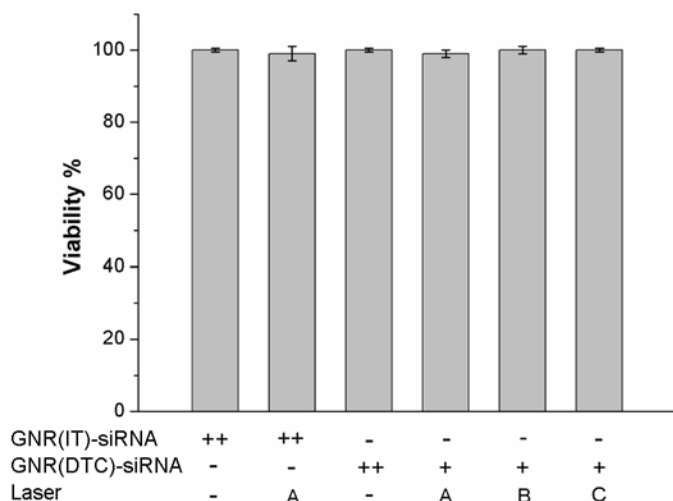


Figure S8. Short-term viability of SKOV-3 cells after a 24-hour incubation with GNR-siRNA carriers followed by laser treatment, based on Trypan Blue exclusion assay. GNR-siRNA carriers were prepared using DTC-siRNA or iminothiolate (IT)-modified siRNA. GNR concentrations of 85 pM (+) or 0.34 nM (++) were used. (A) 15-min irradiation by CW laser (808 nm, 1.33 W/cm²); (B) 3-min irradiation with stationary fs-pulsed laser (800 nm, 7.5 nJ/pulse, 1.2 W/cm²); (C) 3-min irradiation with scanning fs-pulsed laser (800 nm, 1.5 nJ/pulse, 0.6 W/cm²).

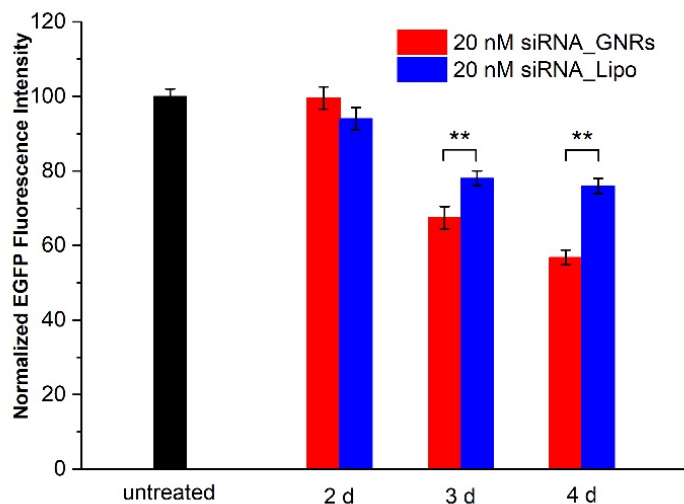


Figure S9. eGFP knockdown using GNR-siRNA carriers at 85 pM (DTC-anchored, equivalent to 20 nM siRNA) and stationary fs-pulsed laser irradiation (1.2 W/cm²), two to four days after treatment (red bars). Knockdown using unmodified siRNA duplexes at 20 nM and Lipofectamine RNAiMAX (blue bars) performed as positive control. ** $p < 0.01$ ($N=3$).

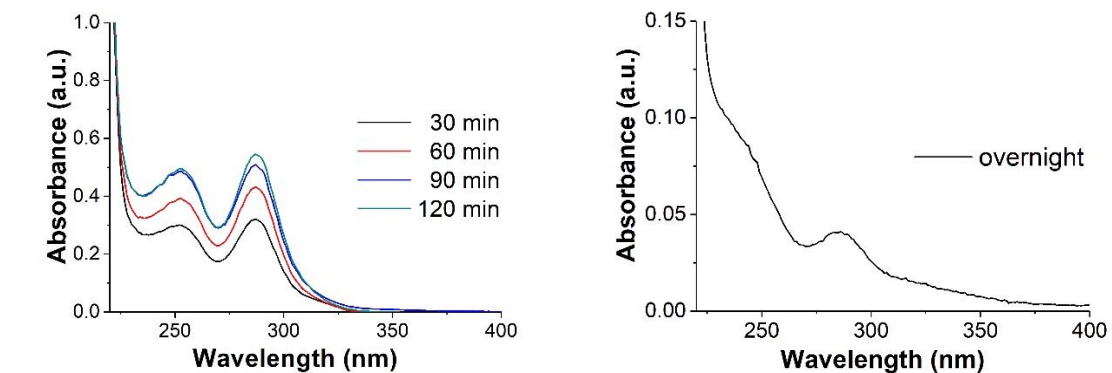


Figure S10. UV-visible spectra for monitoring in situ DTC formation, using mPEG-NH₂. *Left*, 80 μ M mPEG-NH₂ treated with CS₂ in methanol, over a 2-hour period; *right*, 50 μ M mPEG-NH₂ in borate buffer (pH 9.5) treated with saturated aqueous CS₂ solution, after 12 hours.

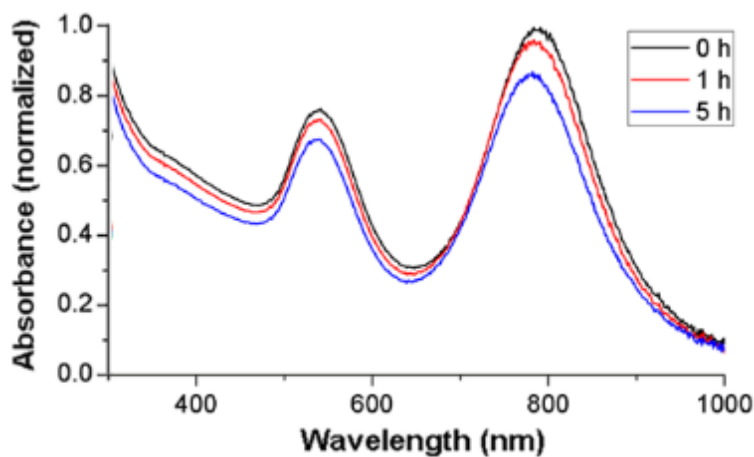


Figure S11. Dispersion stability of GNRs (no Au overgrowth) coated with mPEG-DTC, after treatment with 20 mM mercaptoethanol.

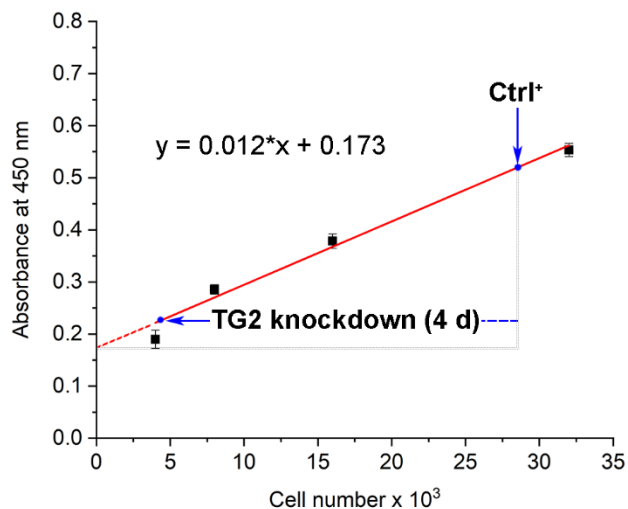


Figure S12. Standard (linear) curve for TG2-specific enzyme activity, as a function of SKOV-3 cell count (two-fold serial dilution from 3.2×10^4 cells). The linear fit was applied toward an estimate in the relative reduction in TG2 activity by SKOV-3 cells, 4 days after treatment with GNR-siRNA carriers and scanning fs-pulsed laser irradiation. The mean absorbance value for the control group (Ctrl⁺) was 0.519, corresponding to 2.9×10^3 cells. The mean absorbance value for the experimental group (TG2 knockdown) was 0.227, corresponding to a 84% knockdown in TG2 activity.

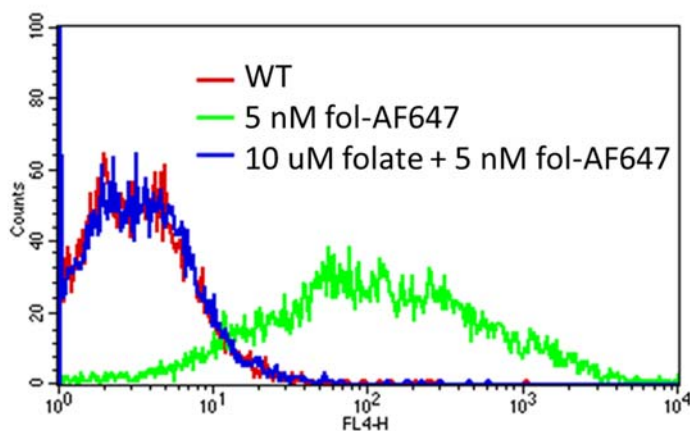


Figure S13. Flow cytometry analysis for folate receptor expression on SKOV-3 cells, cultured in folate-deficient media. WT = wild-type SKOV-3 cells without dye labelling (red); SKOV-3 cells treated with 5 nM folate-AF647 (green), incubated for 30 min at room temperature prior to analysis, using 635-nm laser excitation and 661/16 nm emission filter. Competition experiment (blue): SKOV-3 cells treated with 10 μ M folic acid for 5 min at room temperature, followed by incubation with 5 nM folate-AF647 for 30 min at room temperature.

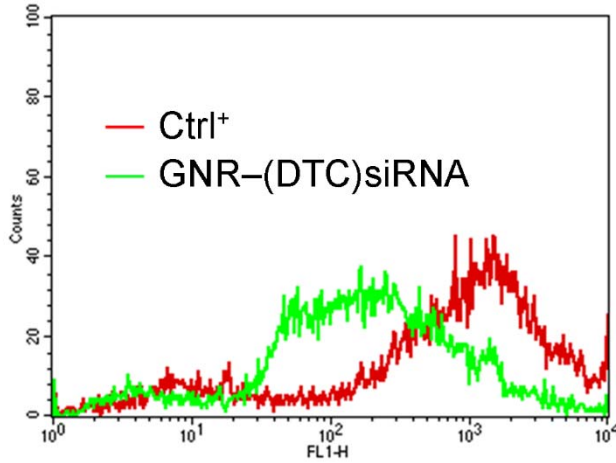


Figure S14. Representative flow cytometry data for quantitative eGFP expression in SKOV-3 cells, 4 days following no treatment (Ctrl⁺; *red*) or treatment with 85 pM GNR-siRNA labelled with HS-PEG-folate plus scanning fs-pulsed laser irradiation at 800 nm (*green*). Mean fluorescence values after gating are 1543.5 (*red*) and 428.2 (*green*), the latter corresponding with a 72% reduction in eGFP production.