Supporting Information

Tumor Stiffening, a Key Determinant of Tumor Progression, is Reversed by Nanomaterial-Induced Photothermal Therapy

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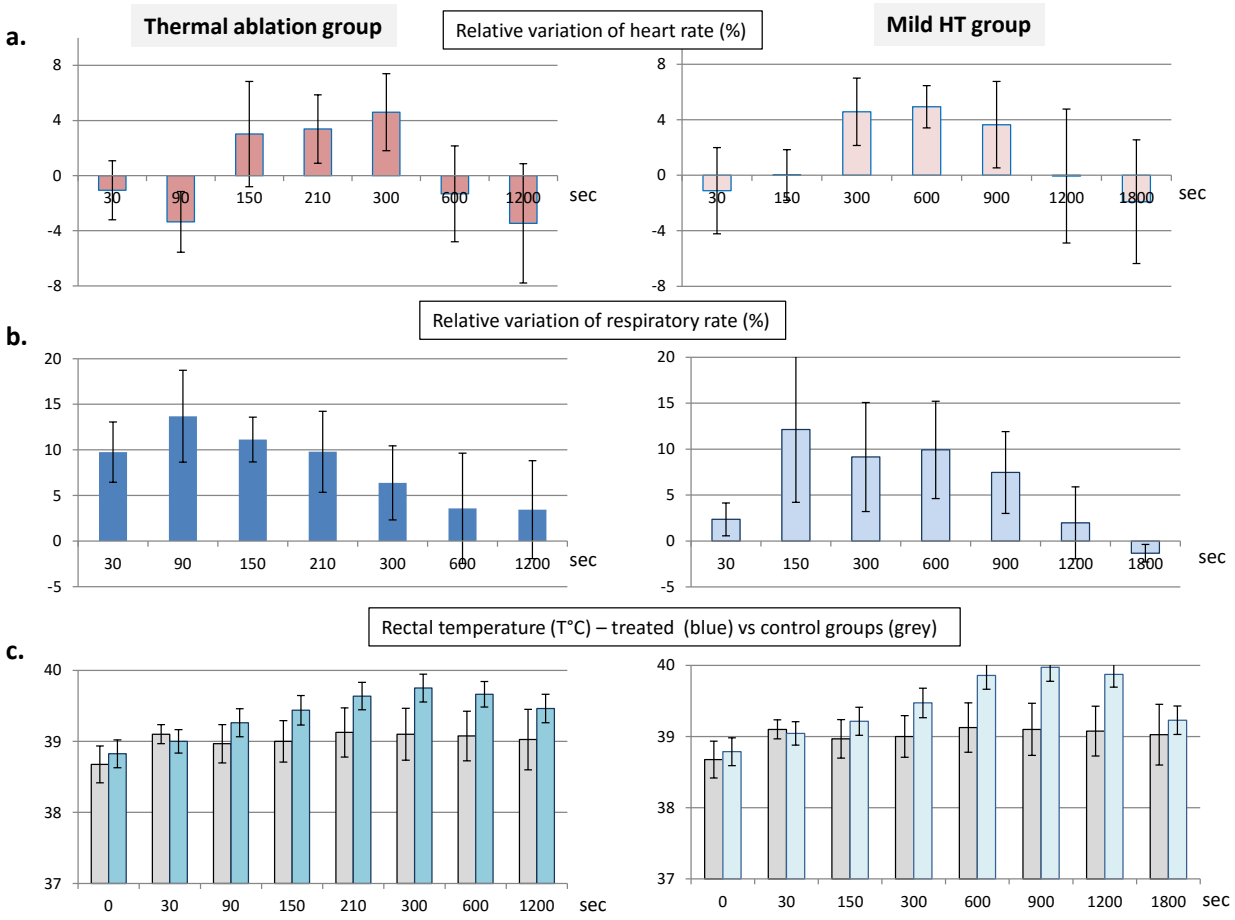

Figure S1: **Monitoring of physiological parameters during mild hyperthermia (mild HT) and thermal ablation (TA) treatments** (t=0 corresponds to the start of laser irradiation, duration of the irradiation, 180 s for TA and 900 s for mild HT). a. Relative variation of heart rate in comparison to the pretreatment level for CNT-injected irradiated mice (left: thermal ablation group (n=12), right: mild hyperthermia (n=6)). b. Relative variation of respiratory rate in comparison the pretreatment level. c. Rectal temperature for CNT-injected irradiated mice (blue) and non-CNT injected irradiated control mice (grey). Error bars represent the standard errors of the mean (SEM).

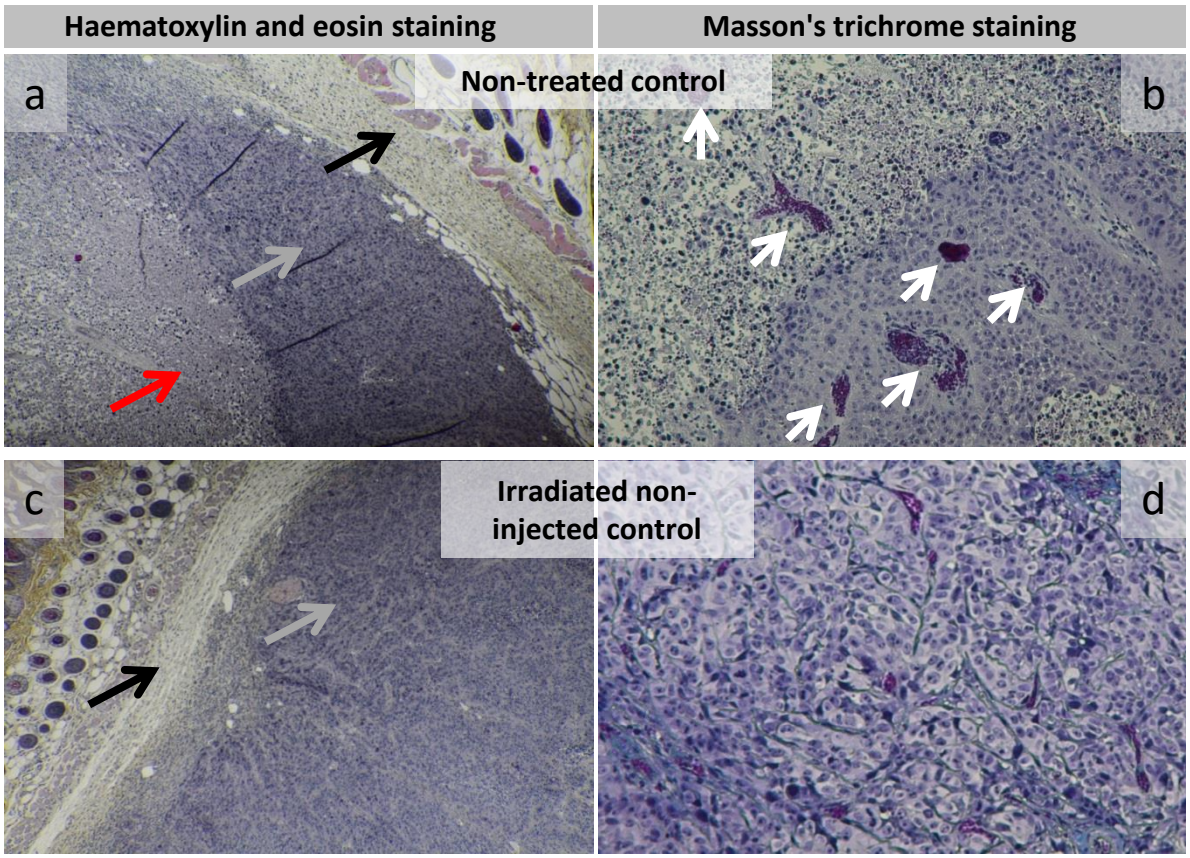

Figure S2. **Histological analysis of control groups.** Control untreated tumor (a-b) featuring a necrotic core (red arrow), a layer of viable cells (gray arrow) and a collagen-rich stroma (black arrow) (a). Tumor vasculature observed at higher magnification (white arrows) (b). Control tumor non-injected with CNTs but laser exposed at the same set-up of mild hyperthermia (c-d) presenting viable tumoral tissue (gray arrow) surrounded by collagen-rich stroma (black arrow) (c). Higher magnification showing viable mitotic tumor cells (d).

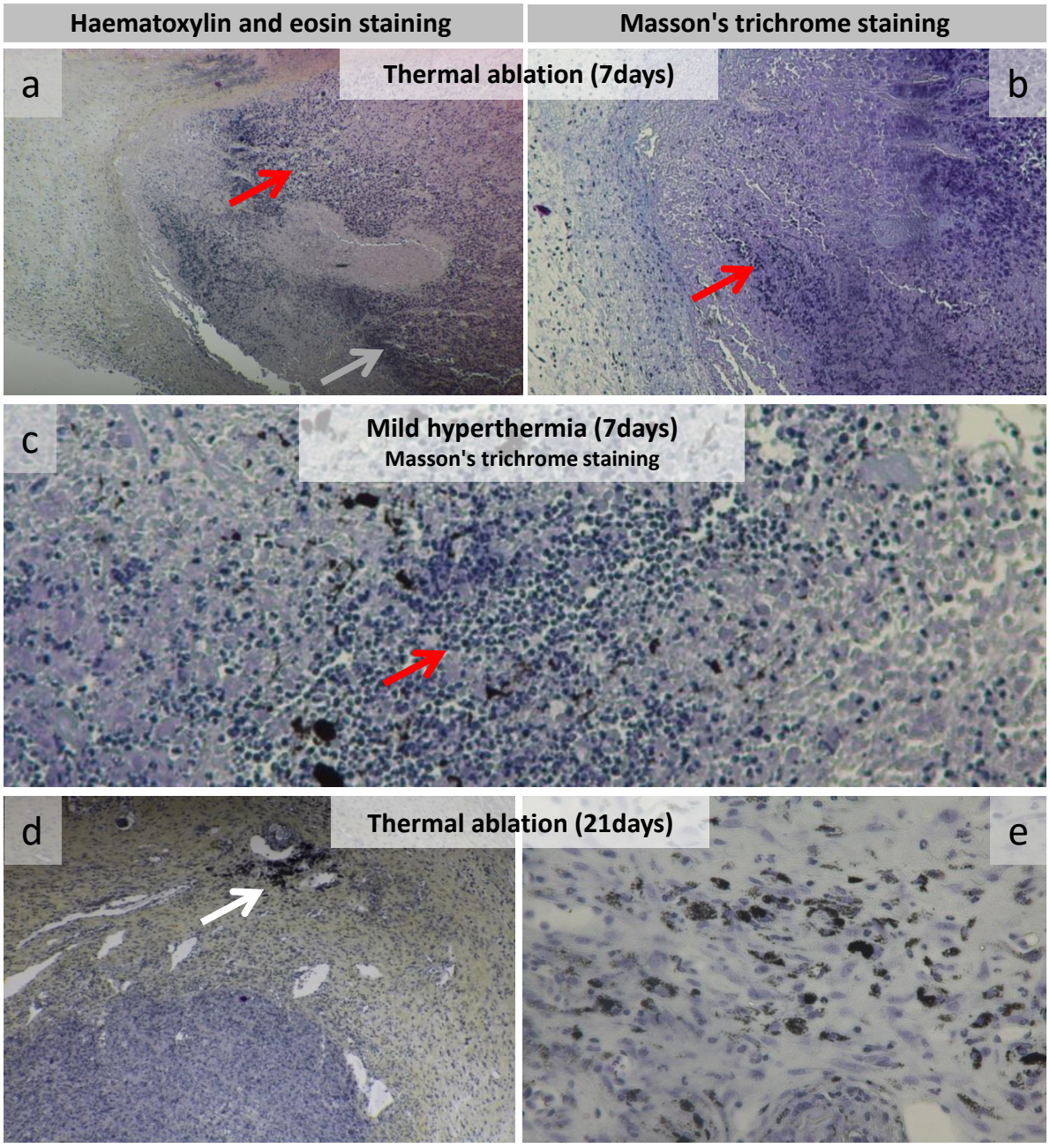

Figure S3: **Histological analysis of mild hyperthermia and thermal ablation treated groups.** Tumor 7 days following thermal ablation (a-b) featuring an extended necrotic zone (red arrow) and a small viable zone (gray arrow) (a). Necrotic tissue (red arrow) at higher magnification (b). Tumor 7 days following mild hyperthermia presented an extended necrotic zone (red arrow) (c). Tumors treated by thermal ablation at day 21 (d-e). CNTs visualized near collagen fibers (white arrow) (d) and in the tumor mass presenting a reduced cell density and featuring inflammatory cells (e). At day 21, most CNTs are internalized into cells.

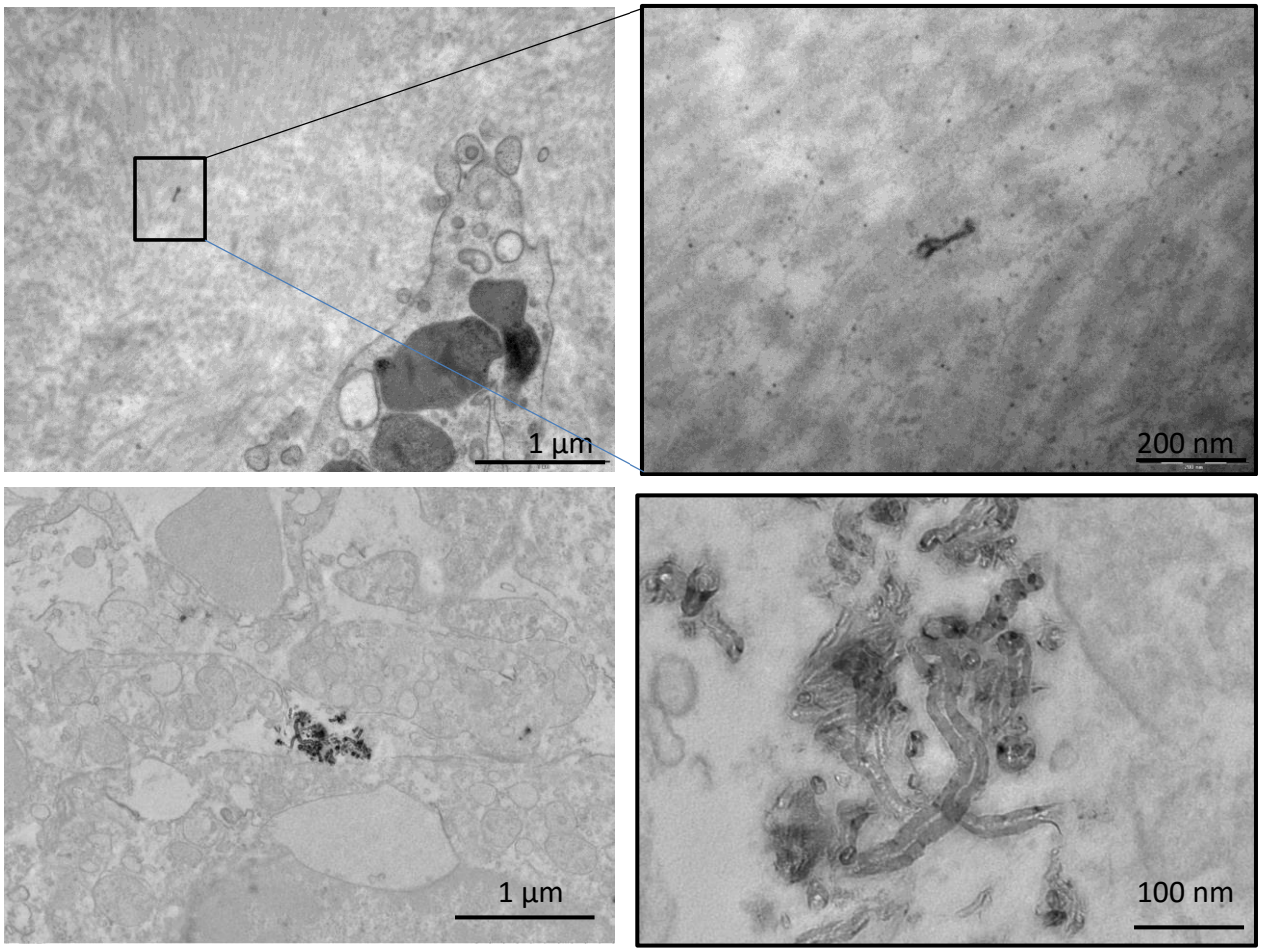

Figure S4: **TEM analysis of CNT-injected non-irradiated tumor one hour following injection of CNTs.** Up – Example of one CNT along collagen fibers in the tumor stroma. The panel on the right is a magnification of the area featured on the left. Bottom – CNT aggregate located in the extracellular space.

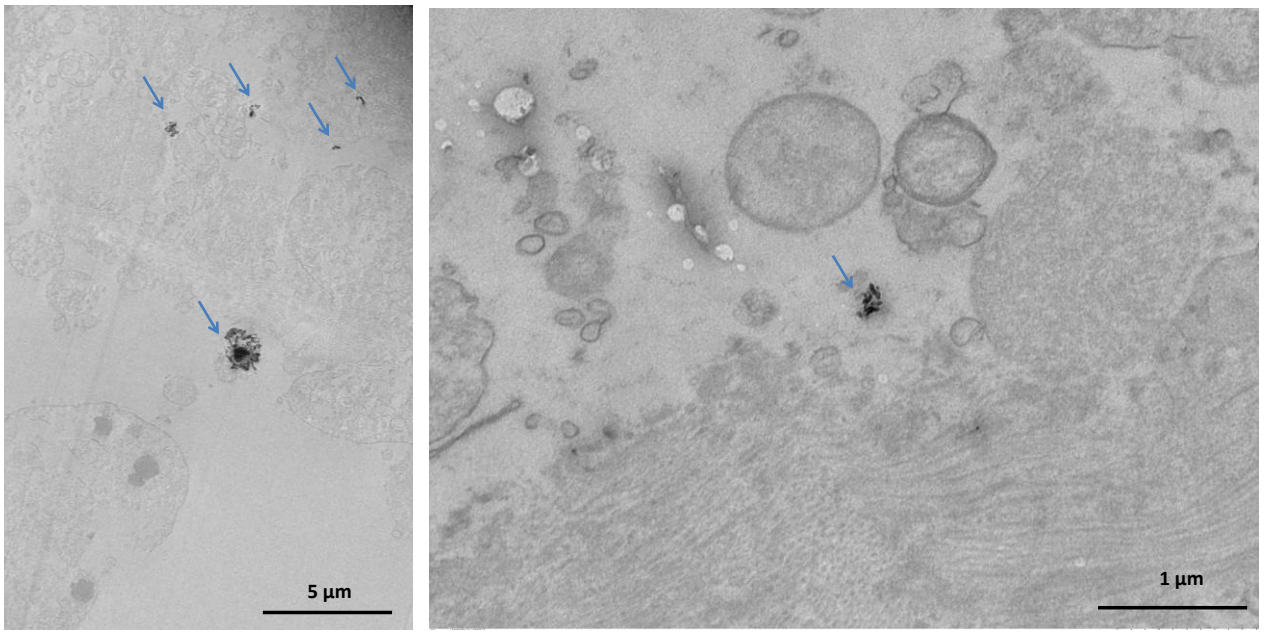

Figure S5: **TEM micrographs of CNT-injected irradiated tumor one hour following thermal ablation treatment.** CNT aggregates (blue arrows) are surrounded by highly damaged extracellular matrix and cells. Collagen matrix distant from CNT aggregate remains intact.

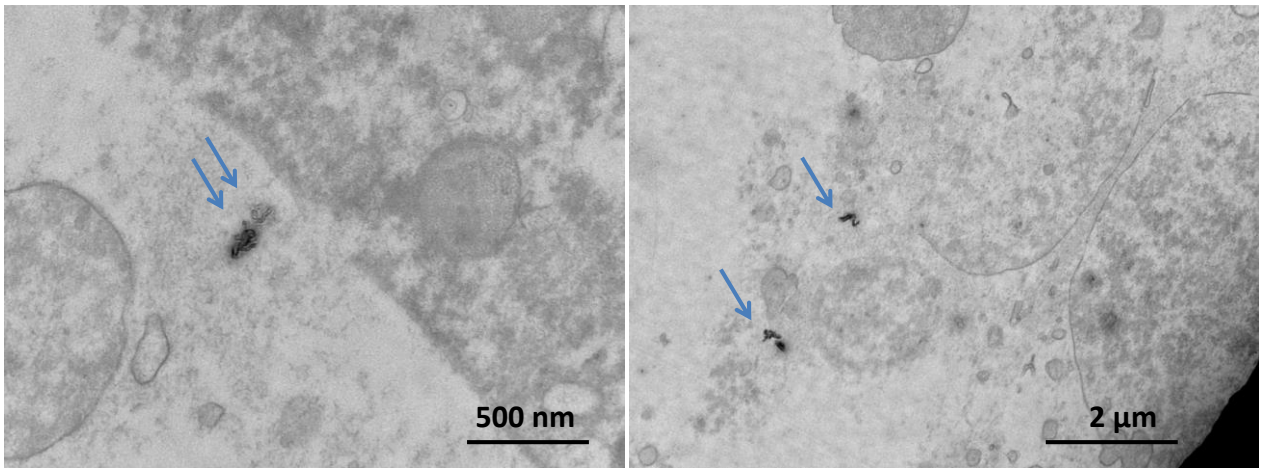

Figure S6: **TEM micrographs of CNT-injected irradiated tumor one hour following mild hyperthermia treatment.** CNT aggregates (blue arrows) are surrounded by cells debris.

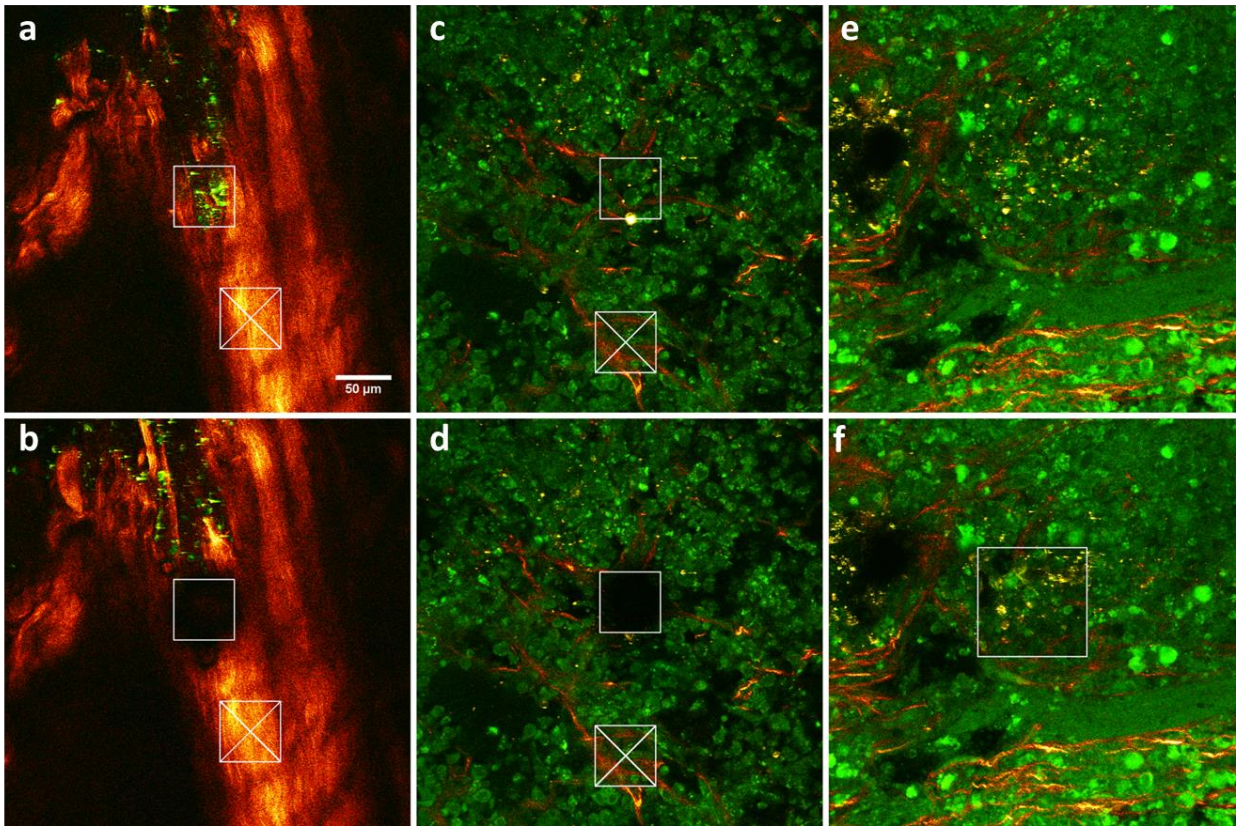

Figure S7: **Two photon microscopy images of sheep tendon collagen (a-b) and tumor slices (c-f): laser zoom scanning effect on collagen network denaturation and tissue integrity in presence of CNTs.** SHG and TPEF contrasts were Red Hot and Green LUT for collagen and CNTs, respectively in sheep tendon (a-b). Alternatively, TPEF contrasts were Red Hot for collagen and CNTs while cells were in Green LUT in tumor slices (c-f). For all images, white squares represent regions of interest (ROI) with and without CNTs (empty and crossed squares, respectively). (a, c, e, f) Images acquired under standard acquisition settings (20 mW zoom 1×). (b and d) Images acquired following real time photothermal therapy (15 seconds 18× zoom scanning at 40 mW applied in the two ROIs). Collagen denaturation (b) and cell damage (d), observed as dark areas, occurred exclusively in the presence of CNTs in empty squares while no changes were observed in the absence of CNTs (crossed squares). No cell damage was observed in the presence of CNTs (empty square) under standard acquisition settings (20 mW zoom 4x 5 seconds) (f).