Supporting Information

Bromelain Surface Modification Increases the Diffusion of Silica Nanoparticles in the Tumor Extracellular Matrix

In memory of Katelyn Dallas Bravo (02/28/1999 - 06/17/2010)

*Alessandro Parodi[†,■,◊], Seth G. Haddix[†◊], Nima Taghipour[†], Shilpa Scaria[†], Francesca Taraballi[†,▲],Armando Cevenini[†,§,‡], Iman K. Yazdi[†,Ⱶ], Claudia Corbo[†,■], Roberto Palomba[†,■], Sm Z. Khaled[†], Jonathan O. Martinez[†,#], Brandon S. Brown[†,#], Lucas Isenhart[†], and Ennio Tasciotti[†]**

[†]Department of Nanomedicine, Houston Methodist Research Institute, Houston, Texas 77030, United States, [■]Fondazione SDN IRCCS, Naples, 80143, Italy, [§]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Naples, 80131, Italy, [‡]CEINGE-Biotecnologie Avanzate, s.c.a r.l., Naples, 80145, Italy, [Ⱶ]Department of Biomedical Engineering, University of Houston, Houston, Texas, 77204, United States, [#]The University of Texas Health Science Center at Houston, Houston, Texas, 77030, United States, and [▲]Pain Therapy Service, Fondazione IRCCS Policlinico San Matteo, Pavia, 27100, Italy.

[◊] These authors equally contributed to the manuscript

Address correspondence to etasciotti@houstonmethodist.org

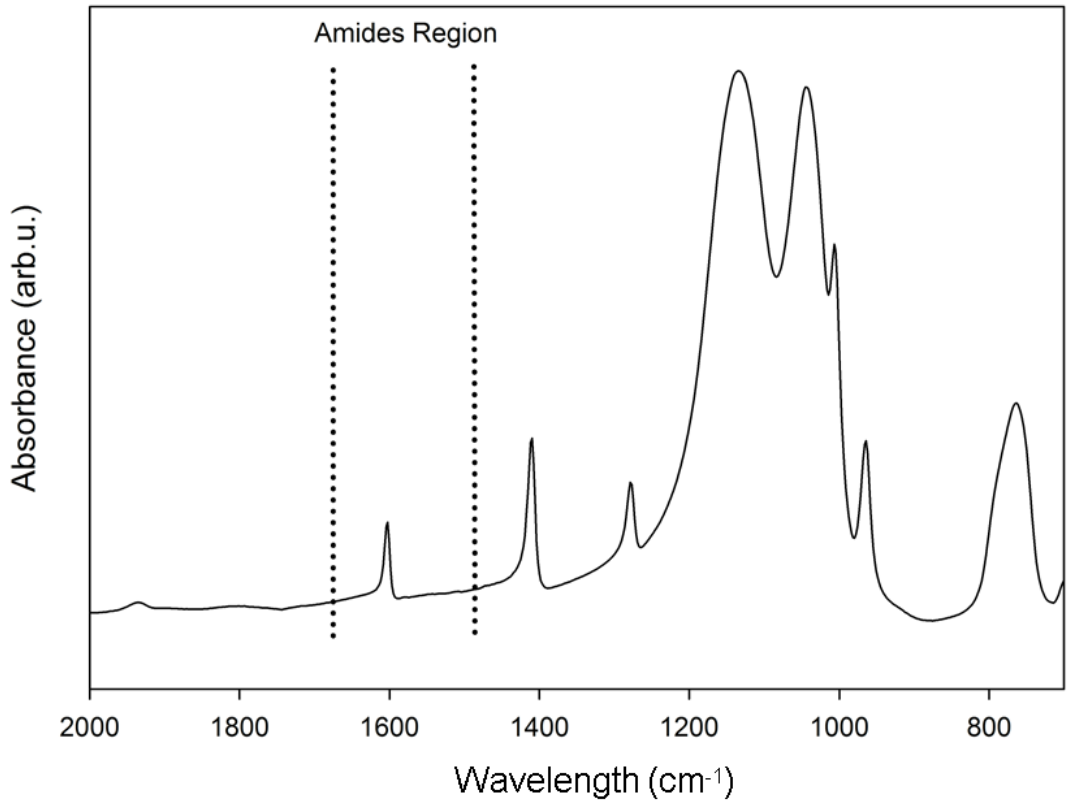


Figure S1. FTIR spectrum of Bromelain passively absorbed on MSN. The passive absorption of the enzyme to the MSN did not last after the washing procedure. The vibrational modes assignable to Bromelain in the Amides Region (highlighted between the dotted lines) are lacking.

FIGURE S2

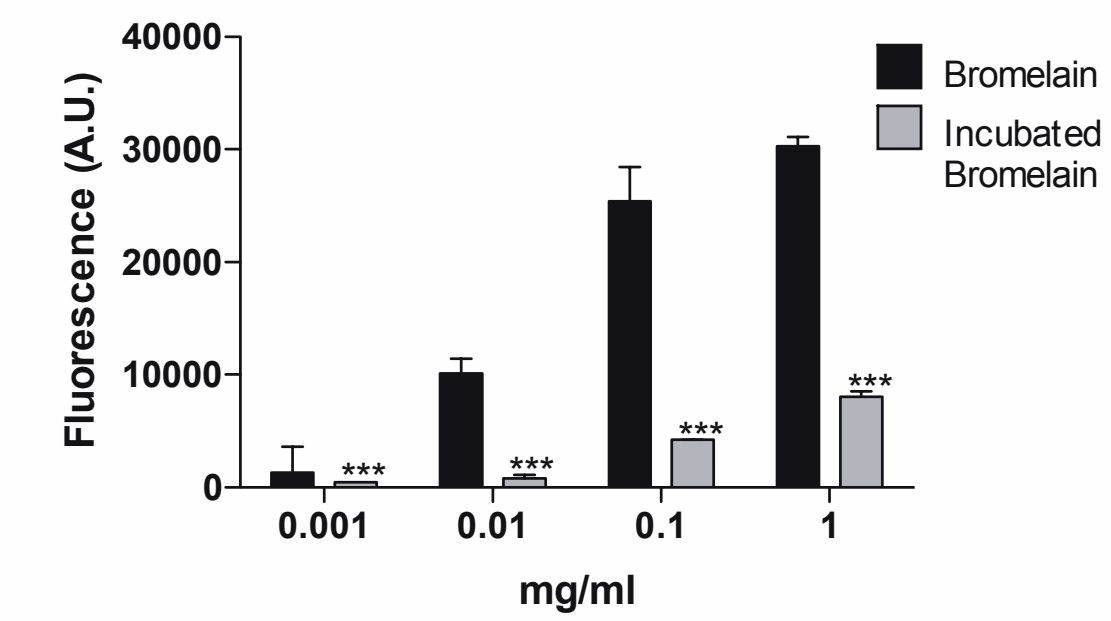


Figure S2. Matrigel degradation upon subjection to different concentrations of fresh Bromelain and Bromelain incubated at 37 °C for 24 h. ECM degradation was indirectly evaluated by measuring the release of FITC that had been previously embedded in the matrigel before polymerization. Due to its self-autodigestive effect, fresh Bromelain or newly modified Br-MSN was used for all experimentation.

FIGURE S3

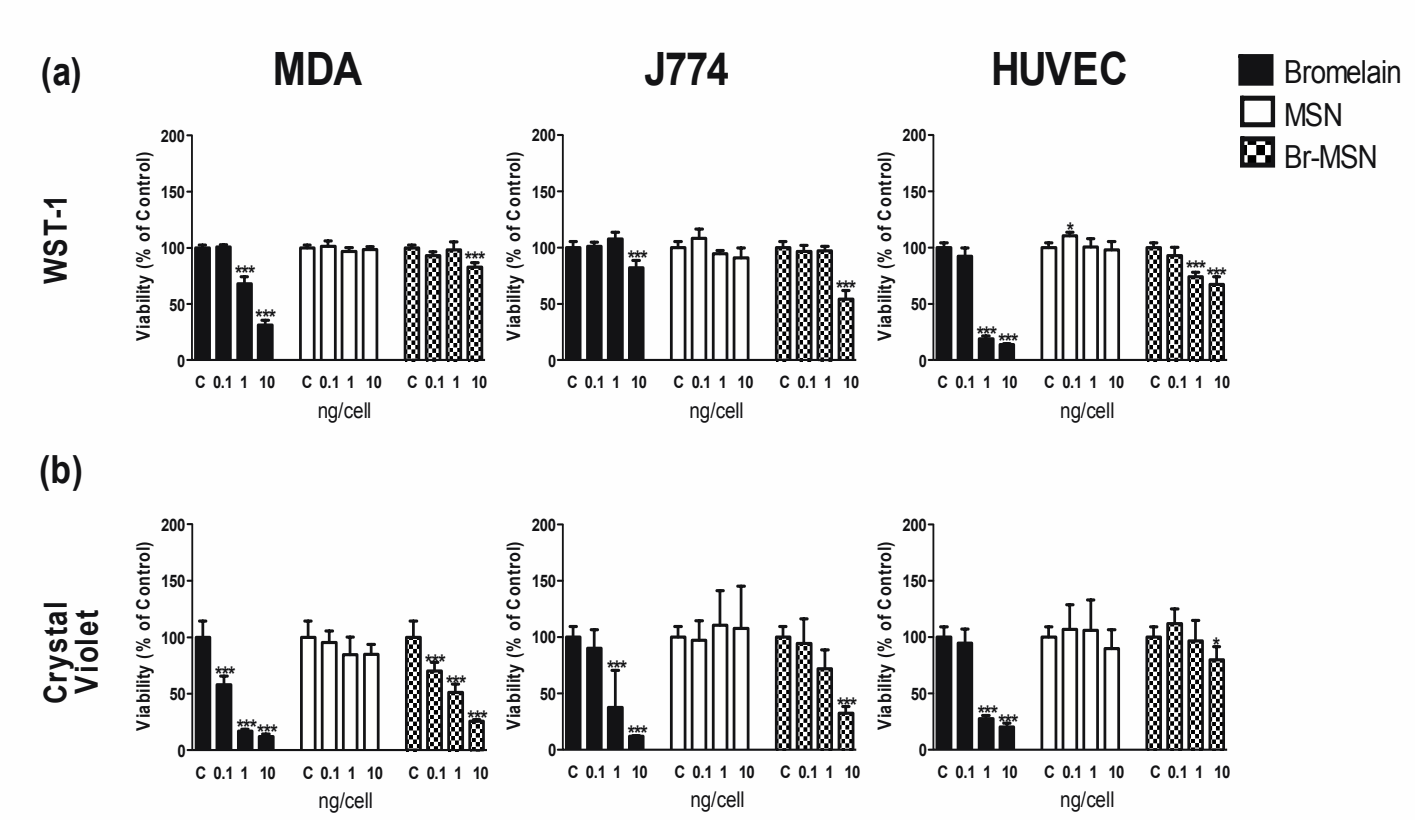


Figure S3. (a) Cell viability of MDA, J774 and HUVE cells measured with WST-1 assay after treatment for 24 h with pure Bromelain, MSN, and Br-MSN; (b) Cell viability of MDA, J774 and HUVE cells measured with Crystal Violet assay after treatment for 24 h with pure Bromelain, MSN, and Br-MSN.

FIGURE S4

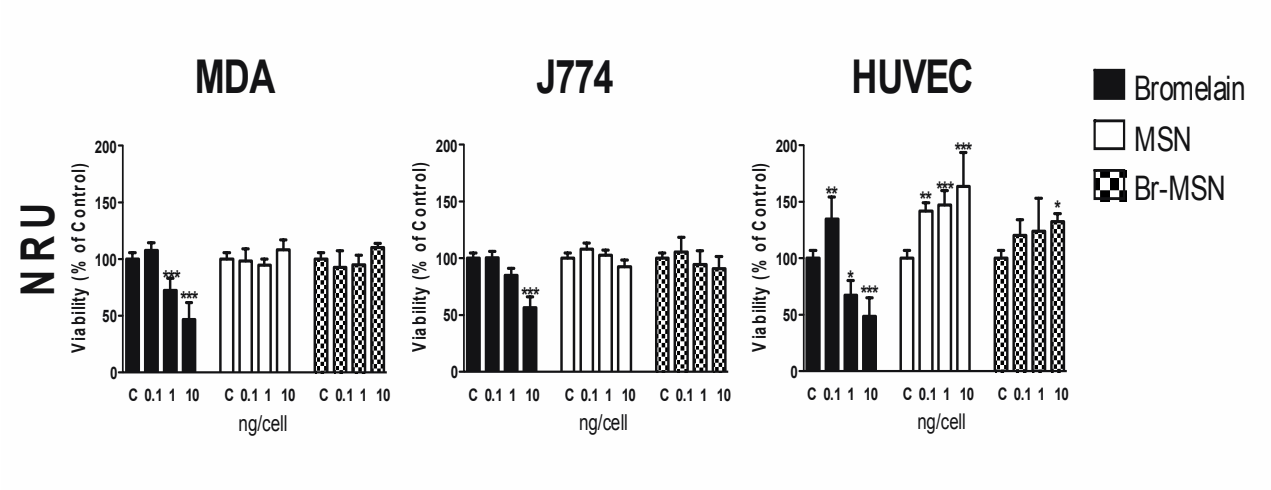


Figure S4. Evaluation of endolysosomal activity in MDA, J774 and HUVEC when exposed for 24 h to increasing concentrations of pure Bromelain, MSN, and Br-MSN as analyzed by NRU assay.

FIGURE S5

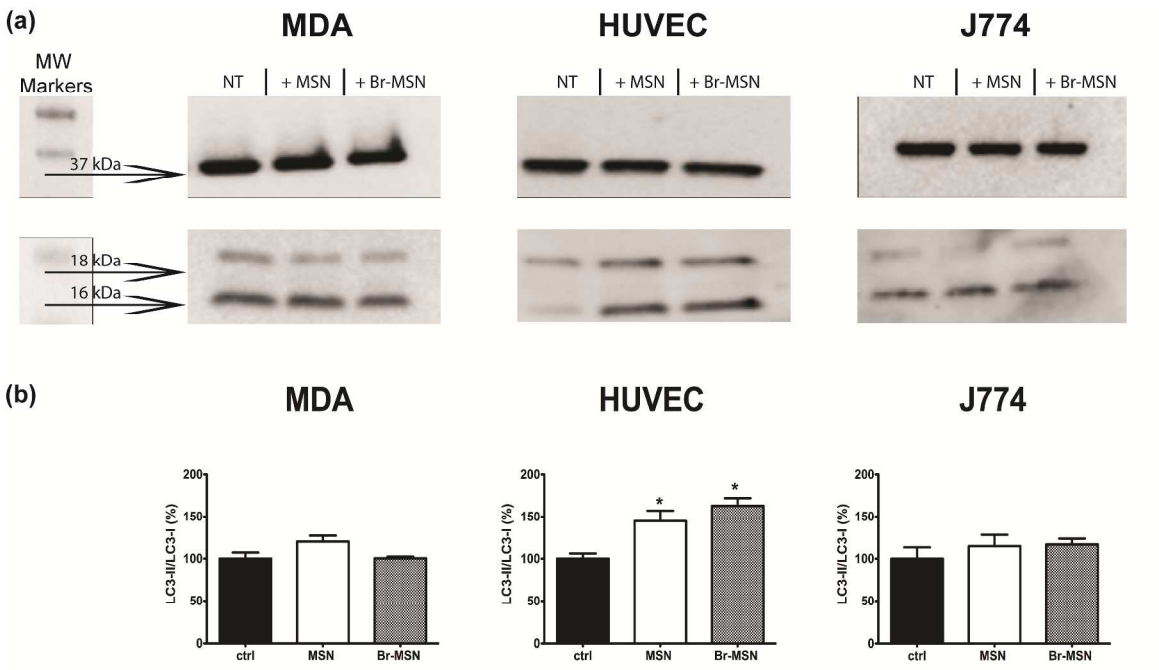
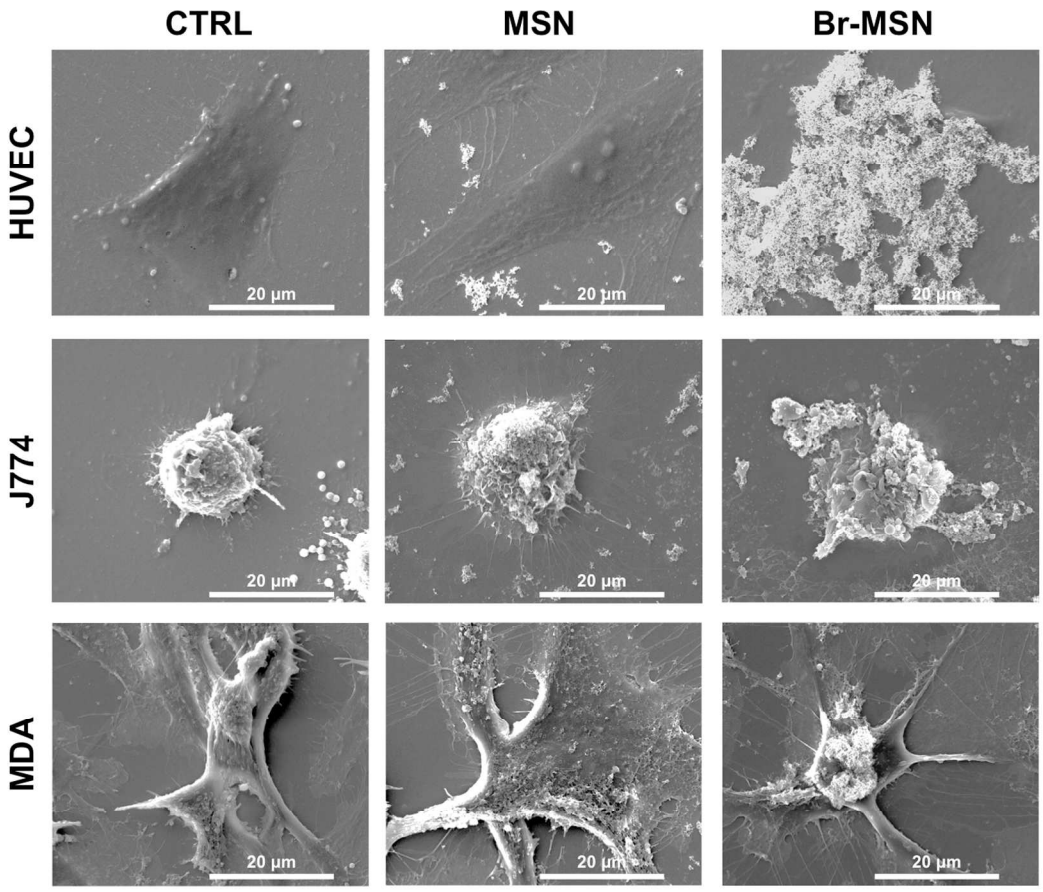


Figure S5. (a**)** Western blot analysis and (b) densitometric measurements of LC3-I and LC3-II in MDA, HUVEC and J774 cells. The analyses were performed on three independent samples; the results are shown as mean ± SD. An increase in the ratio of microtubule-associated protein 1 light chain 3-II to light chain 3-I (LC3II/LC3I) is shown in HUVEC cells as a consequence of a slight autophagic activation.

FIGURE S6



Remove (a), (b), (c) from caption as they are not in the figure

Figure S6. SEM micrographs of immediate cellular effects of Br-MSN on HUVEC, J774, and MDA cell lines. (a) SEM images of HUVEC internalization of MSN and Br-MSN, (b) J774 internalization of MSN and Br-MSN, and (c) MDA internalization of MSN and Br-MSN. Control group treatments (CTRL) of pure PBS in each cell line were also imaged. Br-MSN seem to locate on the surface of the selected cells to a greater extent than MSN after 15 min of treatment.

FIGURE S7

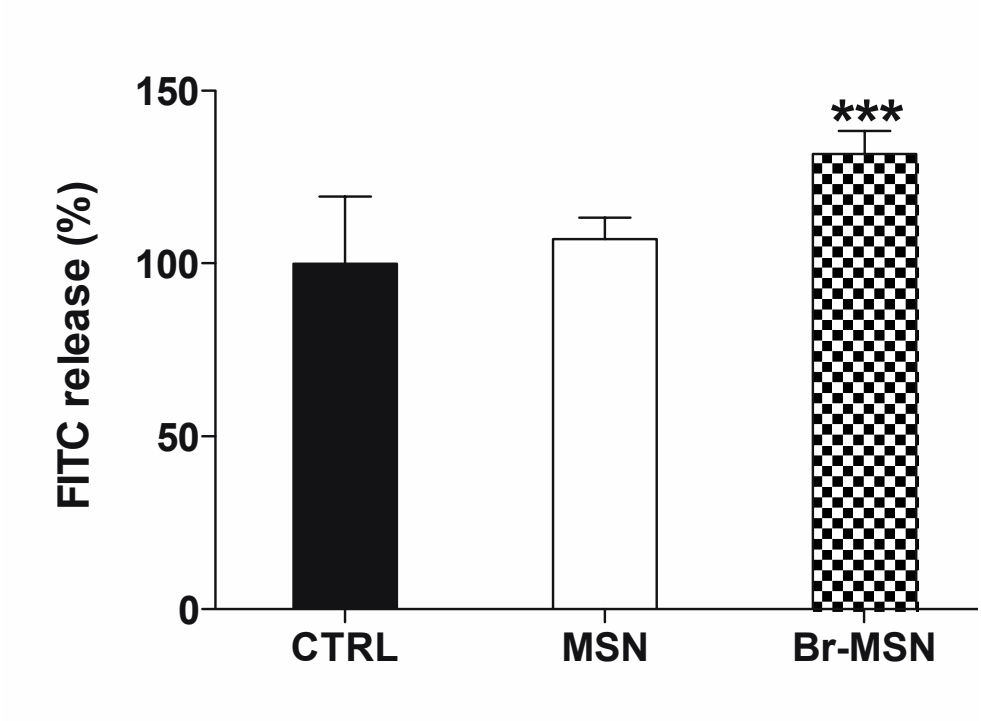


Figure S7. Degradation of gelatin when exposed to PBS (CTRL), MSN, and Br-MSN. Neither CTRL nor MSN showed a high degree of gelatin degradation, while Br-MSN resulted in approximately 25% more degradation compared to CTRL and MSN.

FIGURE S8

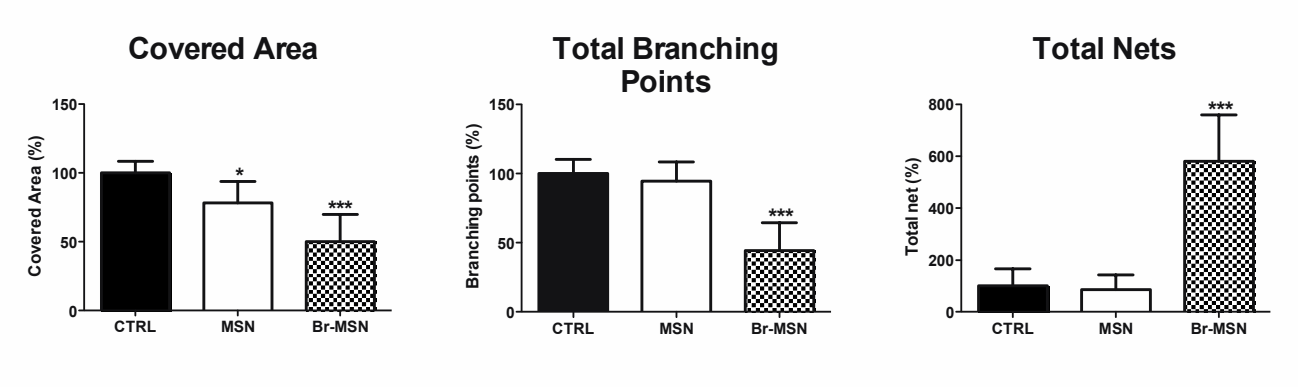


Figure S8. From left to right, image analysis of covered area, branching points, and total nets formed by HUVECs seeded on a matrigel bed and exposed to pure PBS (CTRL), MSN and Br-MSN. Br-MSN treatment significantly reduces the covered area and branching points of the capillary network. This is due to the degradation of the matrigel on which the cells are seeded. Biological factors that promote network formation are likely cleaved by Bromelain on Br-MSN, leading to these results. Strangely the total nets of the Br-MSN treatment increases. This may be an artifact of the image analysis software but must be investigated further.

FIGURE S9

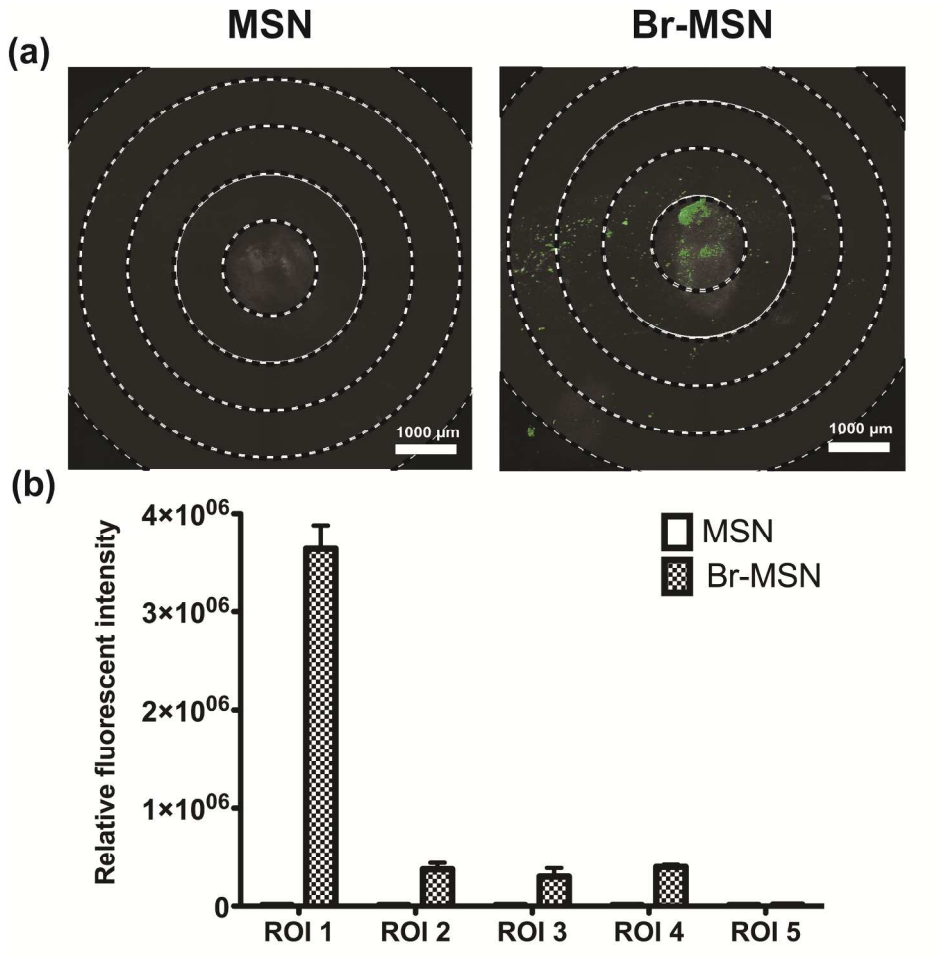


Figure S9. (a) Intravital confocal microscopic images of tumors after 24 h following intratumoral injection of MSN and Bromelain modified MSN. (b) Relative fluorescent intensity measurements in the 5 exclusive circular ROI (regions of interest) of selected Z-stacks.