

Supplementary Information

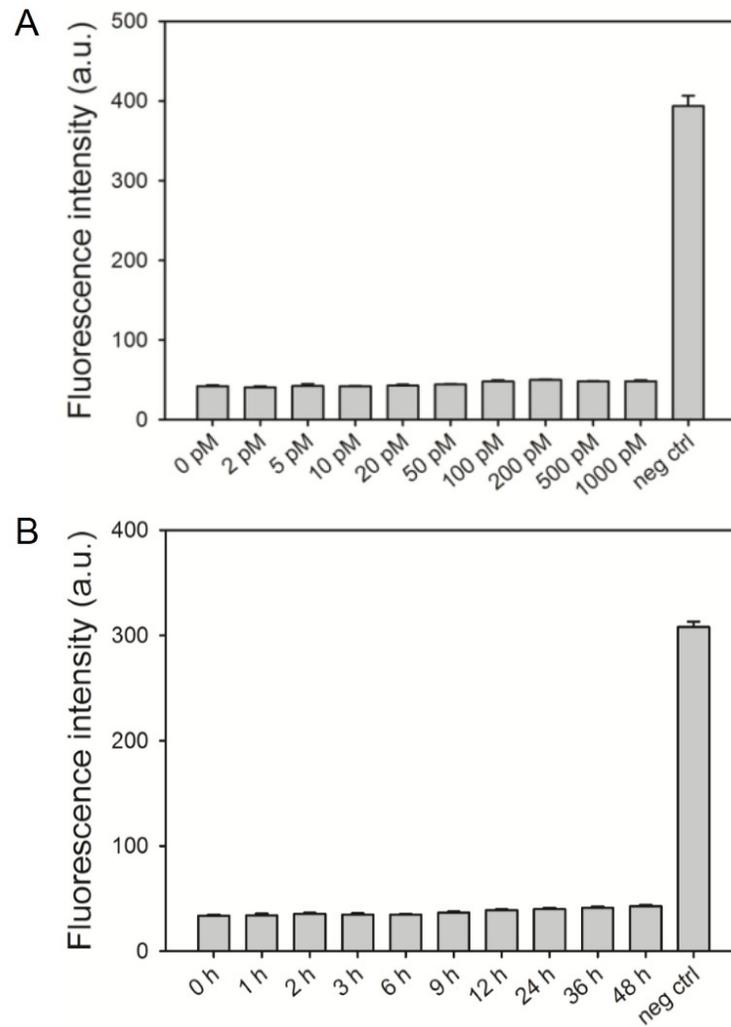
**Labeling Human Mesenchymal Stem Cells with Gold Nanocages for *in vitro* and *in vivo* Tracking by Two-Photon Microscopy and Photoacoustic Microscopy**

Yu Shrike Zhang<sup>†</sup>, Yu Wang<sup>§</sup>, Lidai Wang<sup>§</sup>, Yucai Wang<sup>†</sup>, Xin Cai<sup>§</sup>, Chi Zhang<sup>§</sup>,  
Lihong V. Wang<sup>§,\*</sup>, and Younan Xia<sup>†,\*</sup>

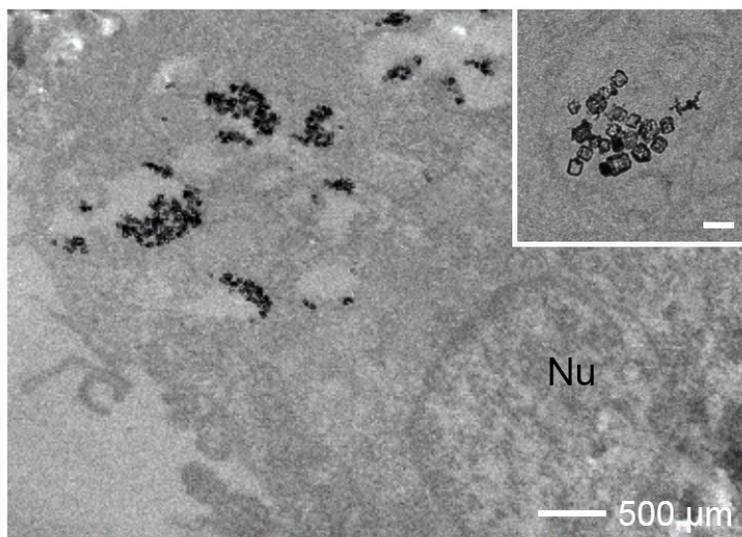
<sup>†</sup>The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332, USA

<sup>§</sup>Department of Biomedical Engineering, Washington University in St. Louis, One Brookings Drive, St. Louis, MO 63130, USA

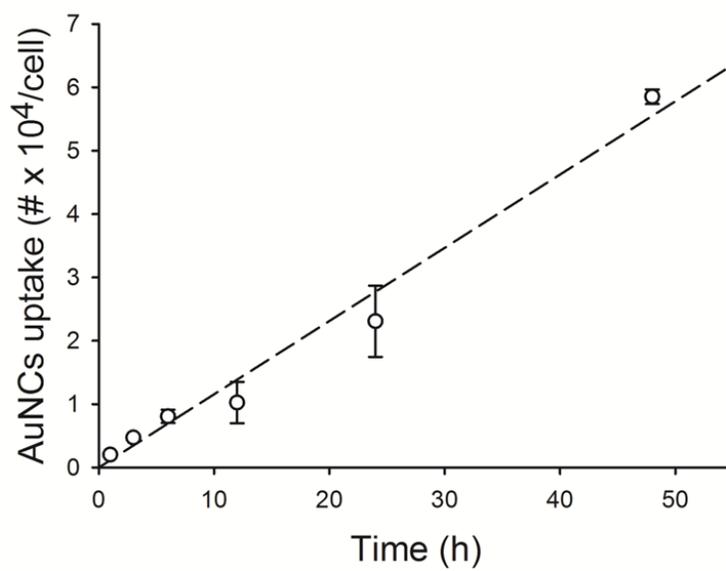
\*Corresponding authors. E-mail: younan.xia@bme.gatech.edu (for gold nanocages, cell culture and two-photon microscopy); lhwang@seas.wustl.edu (for photoacoustic microscopy).



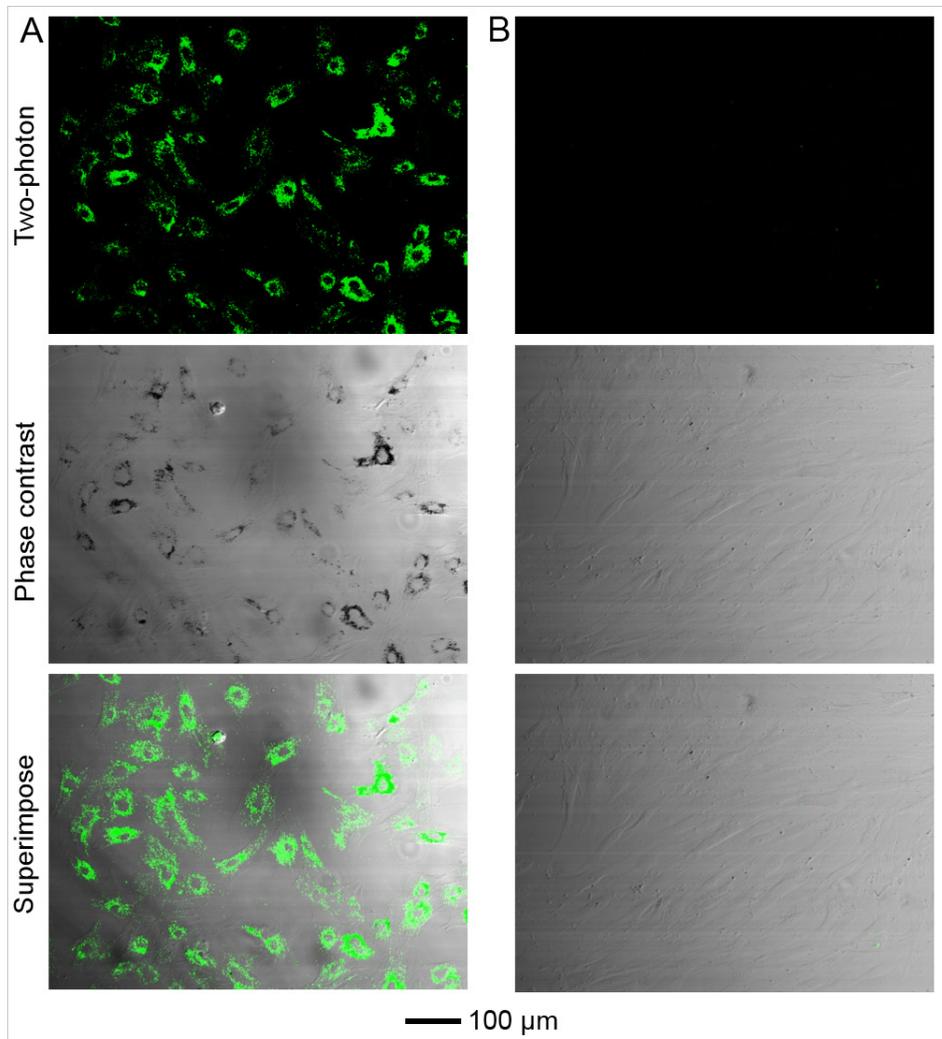
**Figure S1.** Plots showing membrane integrity of hMSCs incubated with AuNCs at various concentrations and for different periods of time, as quantified by the release of glucose-6-phosphate dehydrogenase (G6PD) from the cells into the surrounding medium.



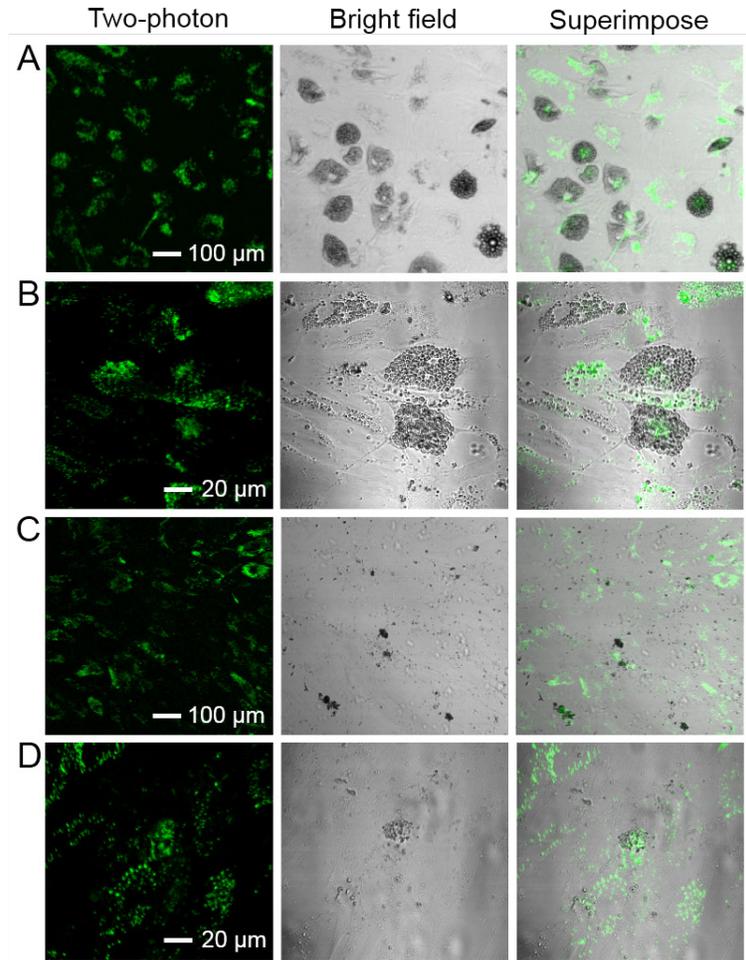
**Figure S2.** TEM image showing the typical distribution of AuNCs in an hMSC. ‘Nu’ refers to the nucleus of the cell. The inset is a magnified image showing the morphology of AuNCs in the cell; scale bar: 100 nm.



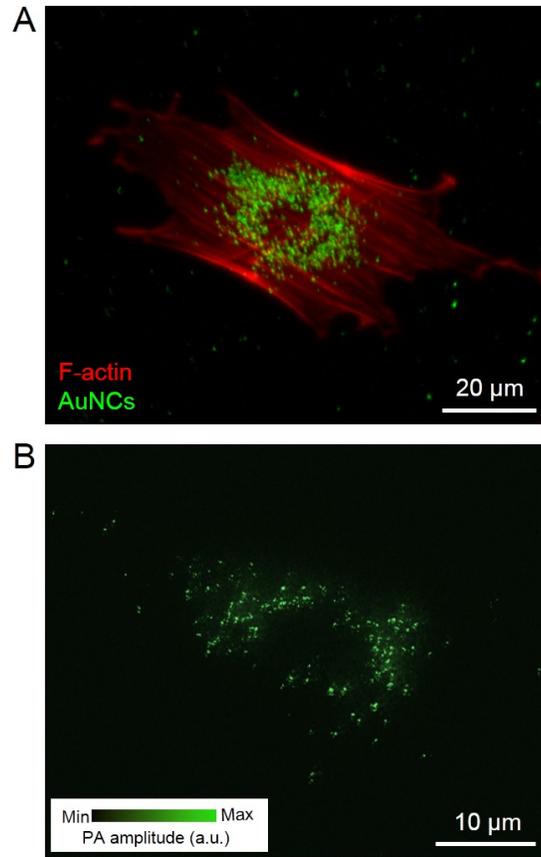
**Figure S3.** Quantification of the uptake of AuNCs by hMSCs *versus* the incubation time by ICP-MS. The concentration of AuNCs was 25 pM.



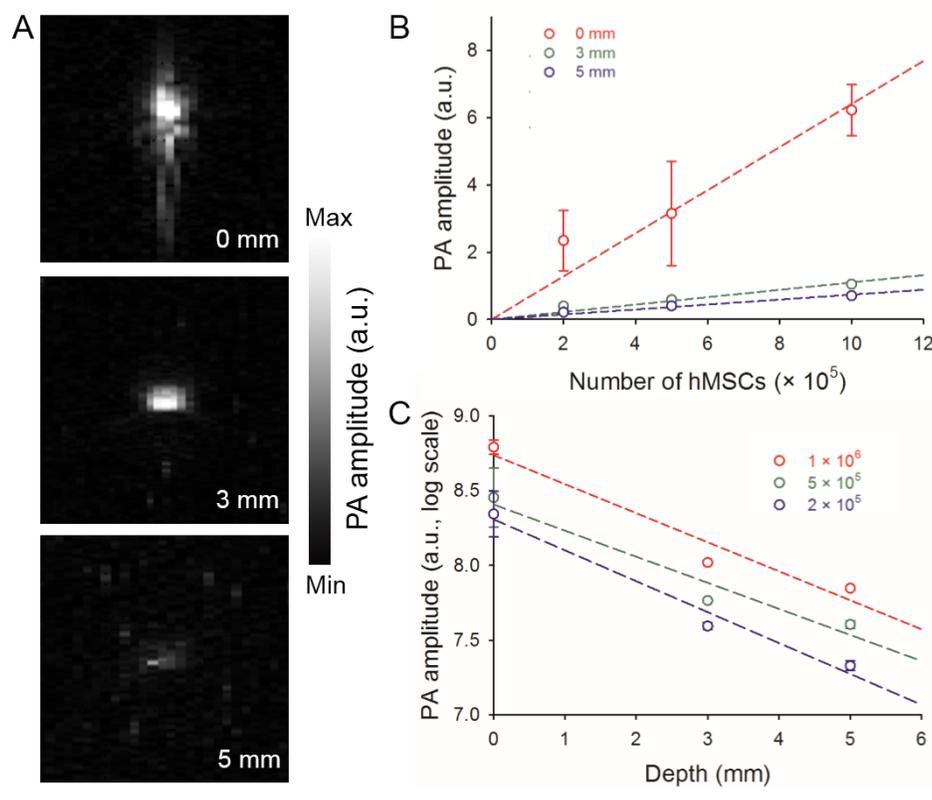
**Figure S4.** (A) Two-photon and phase contrast images of samples after the hMSCs pre-labeled with AuNCs had been cultured in a proliferation medium for 28 days. (B) Two-photon and phase contrast images of the hMSCs cultured in the medium collected from the cultures of cells pre-labeled with AuNCs. Note that the laser power was increased to 5% (c.f. **Figure 3C** where the laser power was 2%) when imaging these cells for better observation due to the decreased amount of AuNCs in the cells after 28 days of culture.



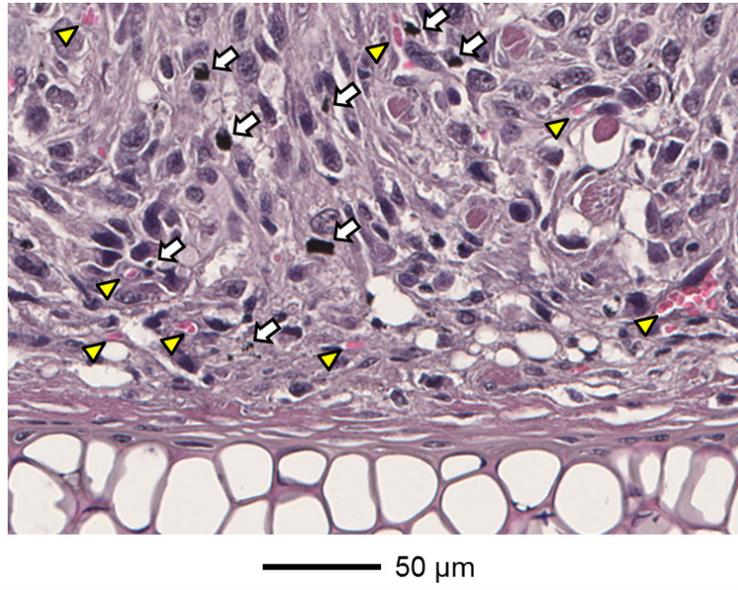
**Figure S5.** Two-photon and phase contrast micrographs showing AuNCs-labeled hMSCs after induction for (A, B) adipogenesis and (C, D) osteogenesis for 28 days.



**Figure S6.** (A) Two-photon image showing an hMSC labeled with AuNCs, where f-actin was stained in red by rhodamine-phalloidin. (B) A high-resolution photoacoustic (PA) image showing an hMSC labeled with AuNCs.



**Figure S7.** (A) Photoacoustic (PA) images of hMSCs labeled with AuNCs ( $1 \times 10^6$ ) embedded in chicken tissues at different depths. (B) A plot showing the relationship between the PA amplitude and the number of hMSCs labeled with AuNCs, at different depths. (C) A plot showing the relationship between the PA amplitude and the depth, at different numbers of hMSCs labeled with AuNCs.



**Figure S8.** An optical micrograph showing a hematoxylin and eosin stained tumor section, where the blood vessels are indicated by yellow arrowheads and AuNCs-carrying hMSCs by white arrows.