

Titanium dioxide nanoparticles affect osteoblasts derived exosomes cargo and impairs osteogenic differentiation of human mesenchymal stem cells

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Supplementary Information

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Titanium dioxide nanoparticles (TiO ₂ NPs)		
Sample	Average size	Polydispersity index (PDI)
TiO ₂ 0 Hours	247,50 ± 13,3 nm	0,77 ± 0,06
TiO ₂ 24 Hours	113,03 ± 1,1 nm	0,18 ± 0,01
TiO ₂ αMEM 10% FBS	143,43 ± 2,2 nm	0,24 ± 0,01

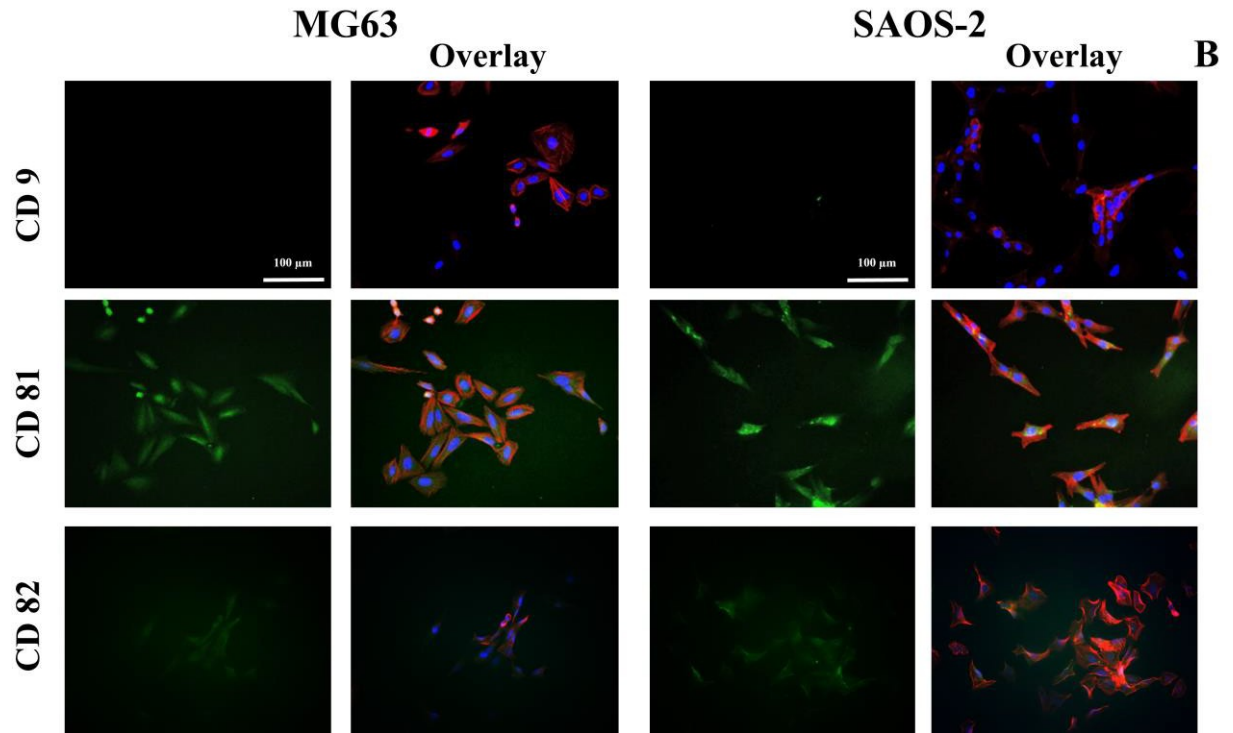
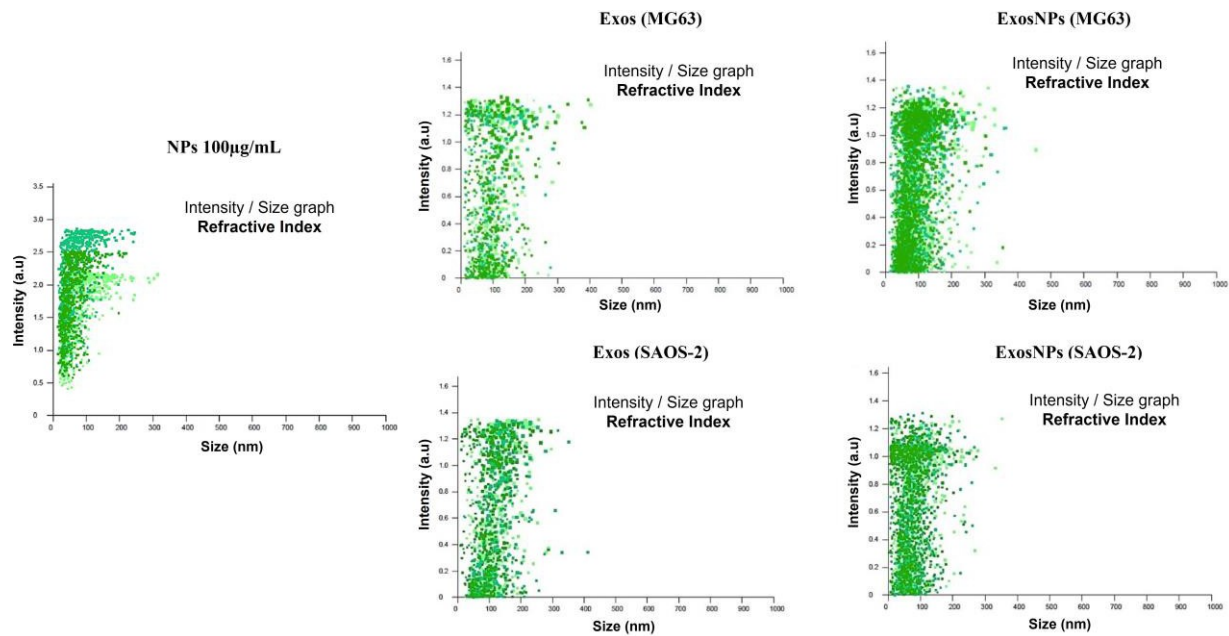
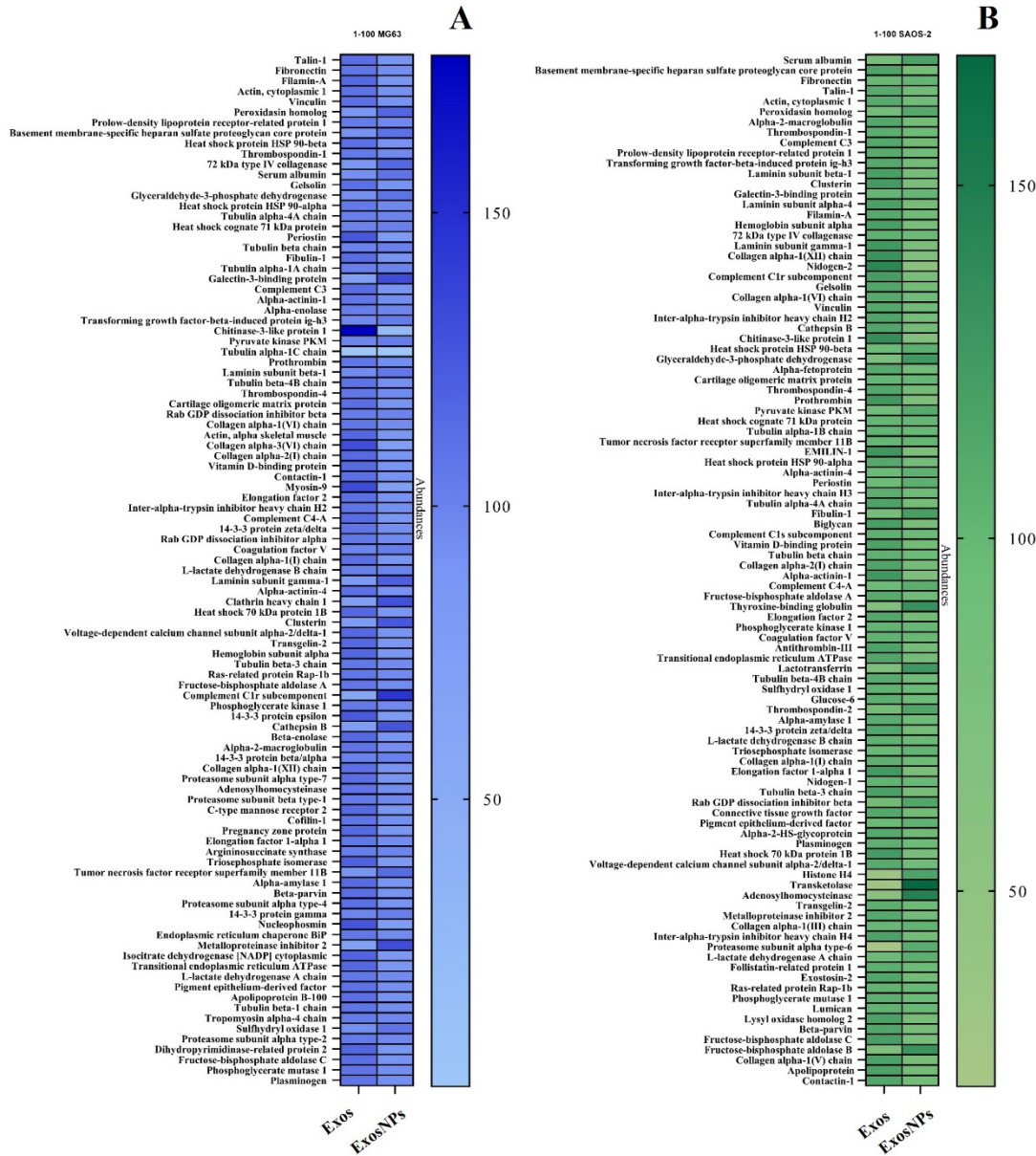


Figure of Complementary Information. 1: TiO₂ NPs and definition of Exos markers suitable for osteoblast cell lines: Table with the average size and polydispersity index of TiO₂ NPs in water and culture medium that was evaluated by dynamic light scattering (A). Fluorescence micrographs of MG63 and SAOS-2 cells were marked in green for CD9, CD81, and CD82 (B), F-actin in red, and nucleus in blue (scale bar: 100 μm). The experiment was performed with a n = 3.



Complementary Information Figure. 2: Nanoparticle Tracking Analysis (NTA) for identifying TiO₂ NPs in Exos: NTA showing the refractive index of TiO₂ NPs diluted in water (100 µg/mL); Exos obtained from Immature osteoblasts (ExosMG63) and osteoblasts (ExosSAOS2) without interaction with TiO₂ NPs (control); Exos obtained from immature osteoblasts (ExosNPs (MG63)) and osteoblasts (ExosNPs (SAOS-2)) after interaction with TiO₂ NPs for 72 hours. The experiment was performed with a n = 3.



Complementary Information Figure. 3 TiO₂ nanoparticles alter the charge of exosomes derived from MG63 and SAOS-2: (A) Heat map of identified proteins from three replicated samples of Exos derived from MG63 and SAOS-2 exposed or not to TiO₂ NPs by LC-MS/MS (Abundance = amount of peptides). The samples were analyzed in the Uniprot database for taxonomic selection for Homo sapiens. Data are presented as mean \pm sd. Statistical significance was calculated by one-way ANOVA and the unpaired Student's *t*-test (**P* < 0.01, significant difference between control (Exos) and treatment (Exos/NPs), *n* = 3)).