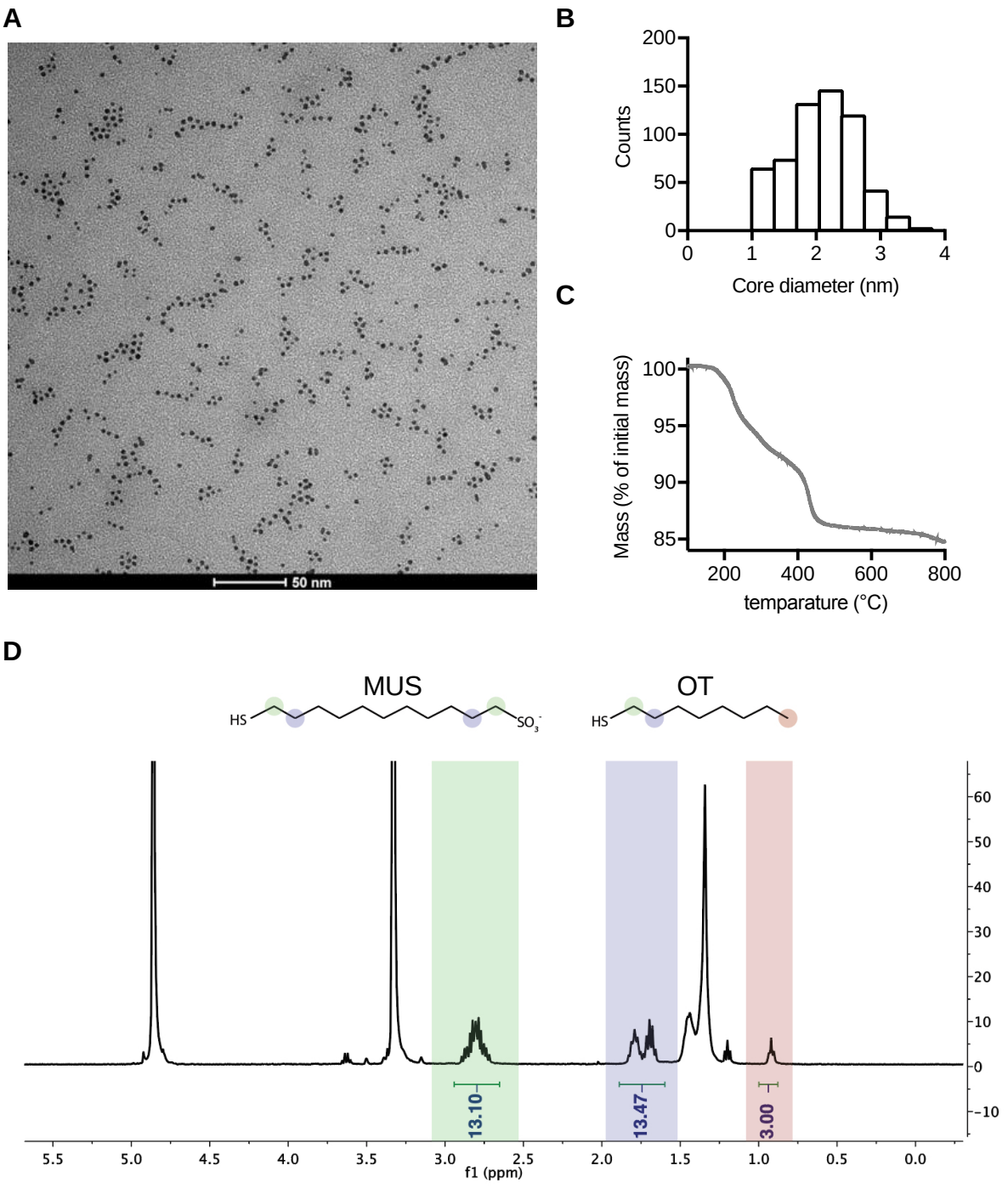
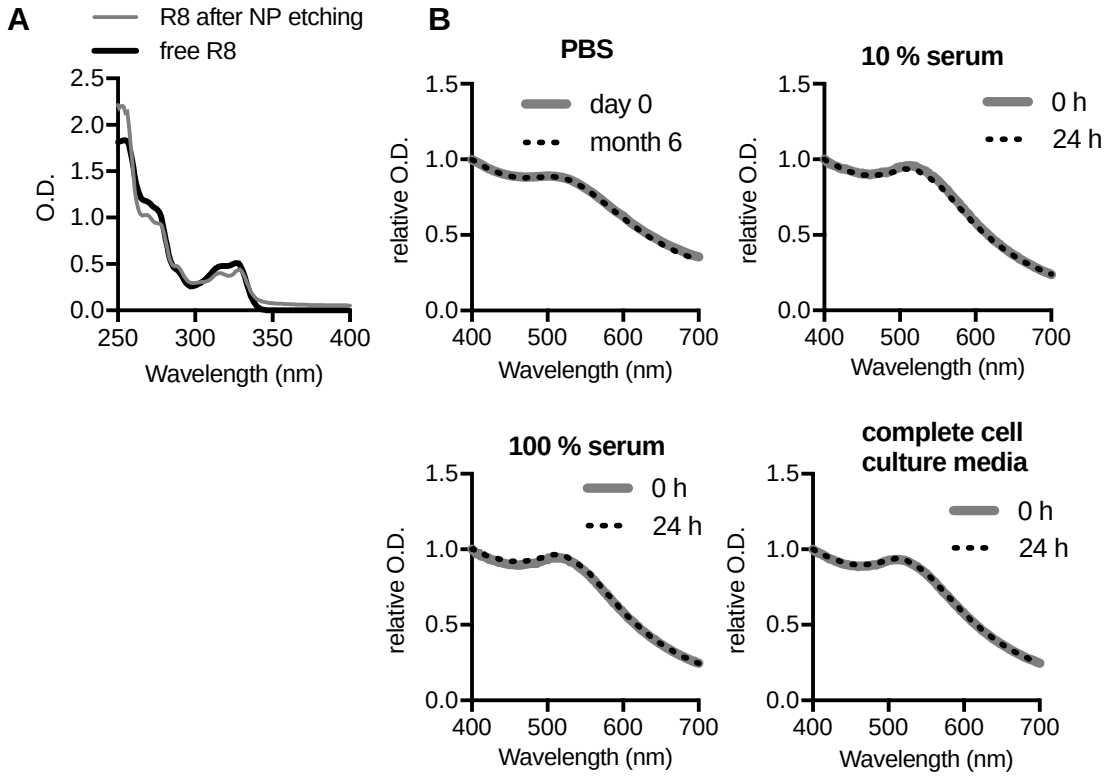
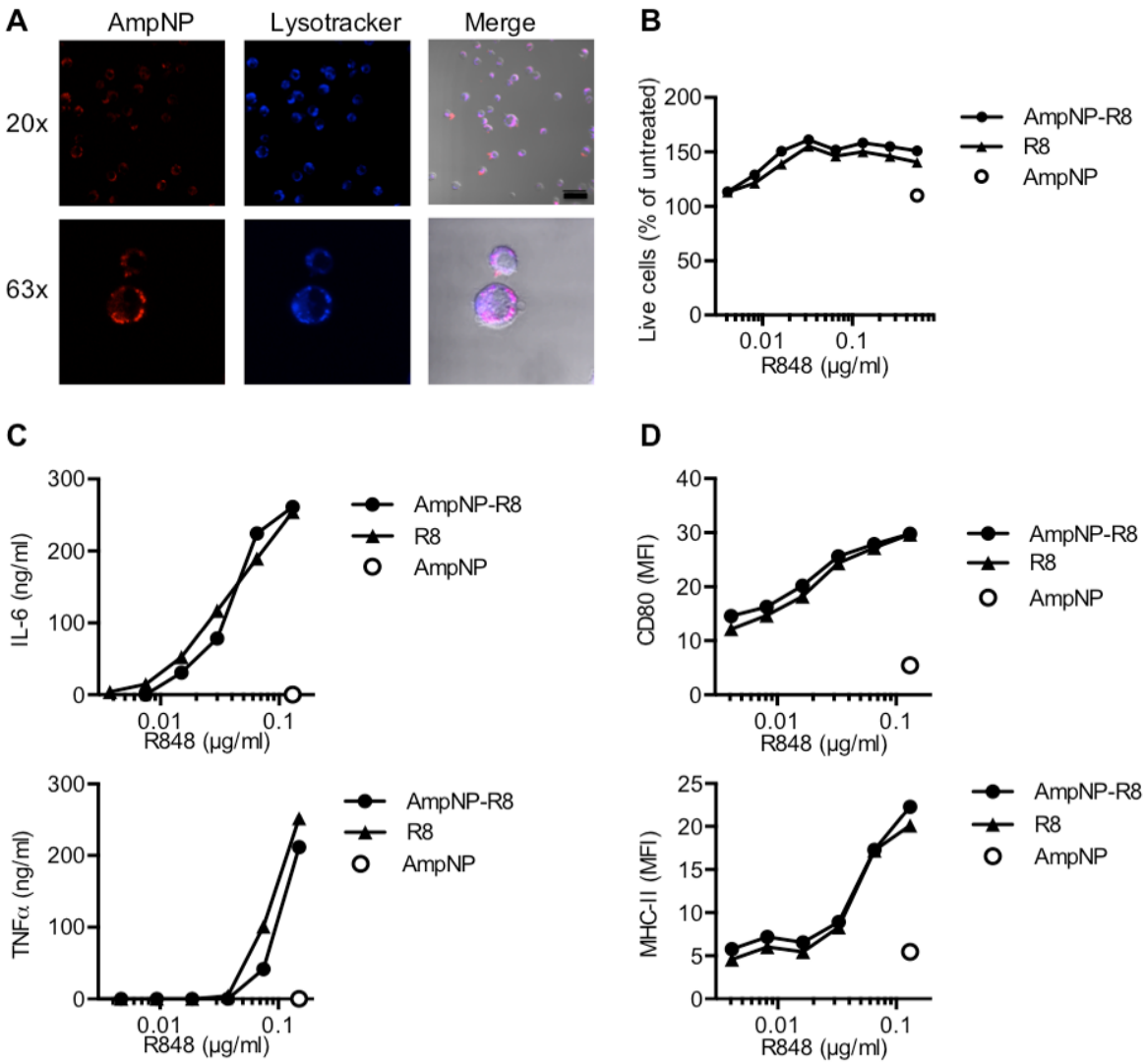


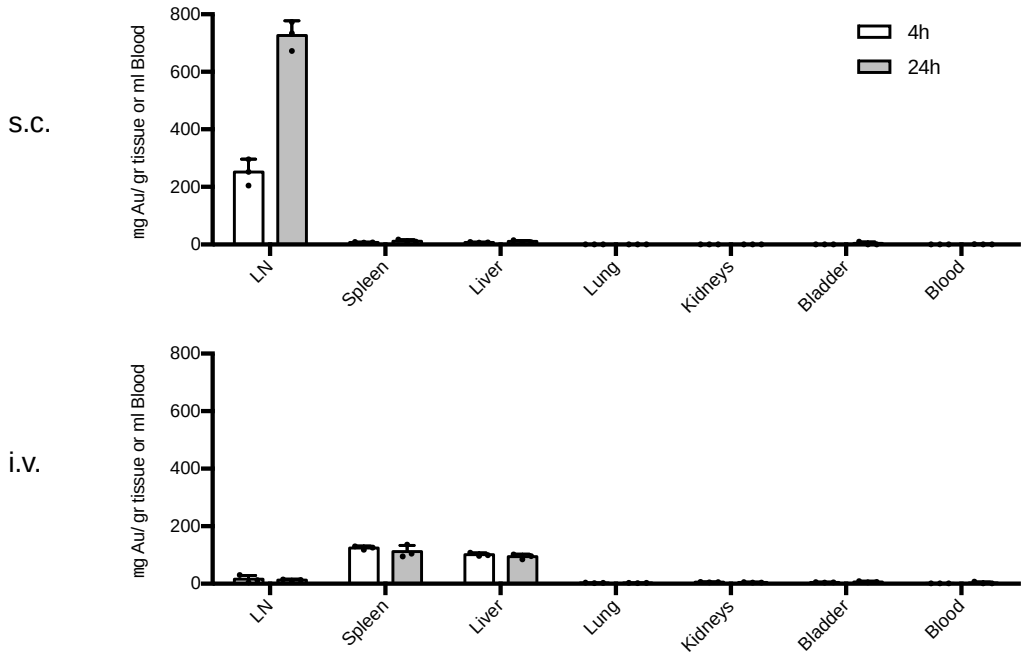
Supplementary Figure 1: AmpNP characterization.
(A) Representative TEM micrograph of AmpNP. (B) Gold core diameter histogram of AmpNP obtained by TEM analysis. (C) Organic mass percentage of ligand shell compared to metallic core of the AmpNP is determined by thermogravimetric analysis (TGA). (D) Ligand ratio of MUS and OT is calculated based on NMR spectroscopy. Peaks for corresponding protons are highlighted with the same color in the chemical structure of MUS and OT above. Graphs are representative of one batch from the three used for this study.



Supplementary Figure 2: AmpNP-R848 were stable for at least 6 months.
(A) Absorbance spectra of R848 before and after conjugation to AmpNPs. The overlapping spectra indicates the lack of structural alteration of drug even after being contact with AmpNPs. (B) Absorbance spectra of AmpNP-R848 at day of synthesis (day 0) and after 6-month storage at 4°C. Also, AmpNPs were shown to be stable in number of challenging conditions such as in sera and cell culture media even after 24 h incubation at 37 °C. Optical density (O.D.) at 400 nm was normalized to 1. Graphs are representative of at least 2 independent experiments.

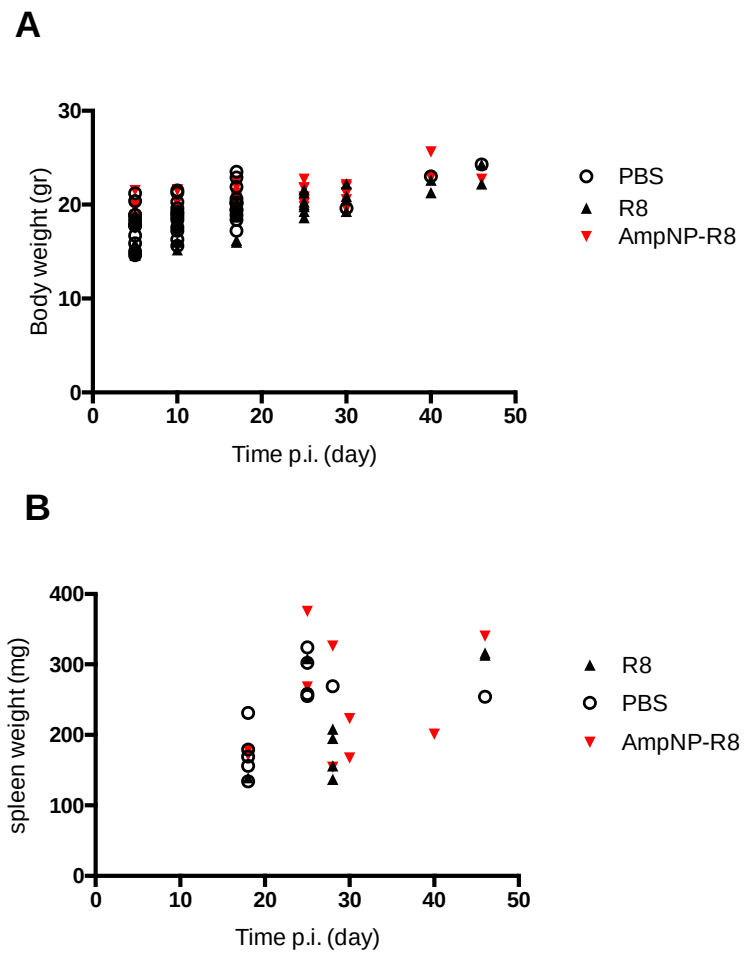


Supplementary Figure 3: R848 cargo on AmpNP is immunologically active.
(A) Live cell imaging at 20x and 63x magnification of J774.1 macrophages after a 4-h incubation with 3 μg/ml of BODIPY-labeled AmpNP (red). Blue: Lysotracker (endosomal marker). Scale bar: 50 μm. (B, C and D) Bone marrow-derived dendritic cells were incubated for 24 h with increasing concentrations of AmpNP loaded with R848 (AmpNP-R8). Equivalent amounts of free R848 (R8) or unloaded AmpNP (AmpNP) were used as control. (B) Cell viability. (C) Concentration of cytokines in the supernatant measured by ELISA. (D) Median fluorescence intensity (MFI) of the surface maturation markers of dendritic cells measured by flow cytometry. Graphs are representative of 3 independent experiments.



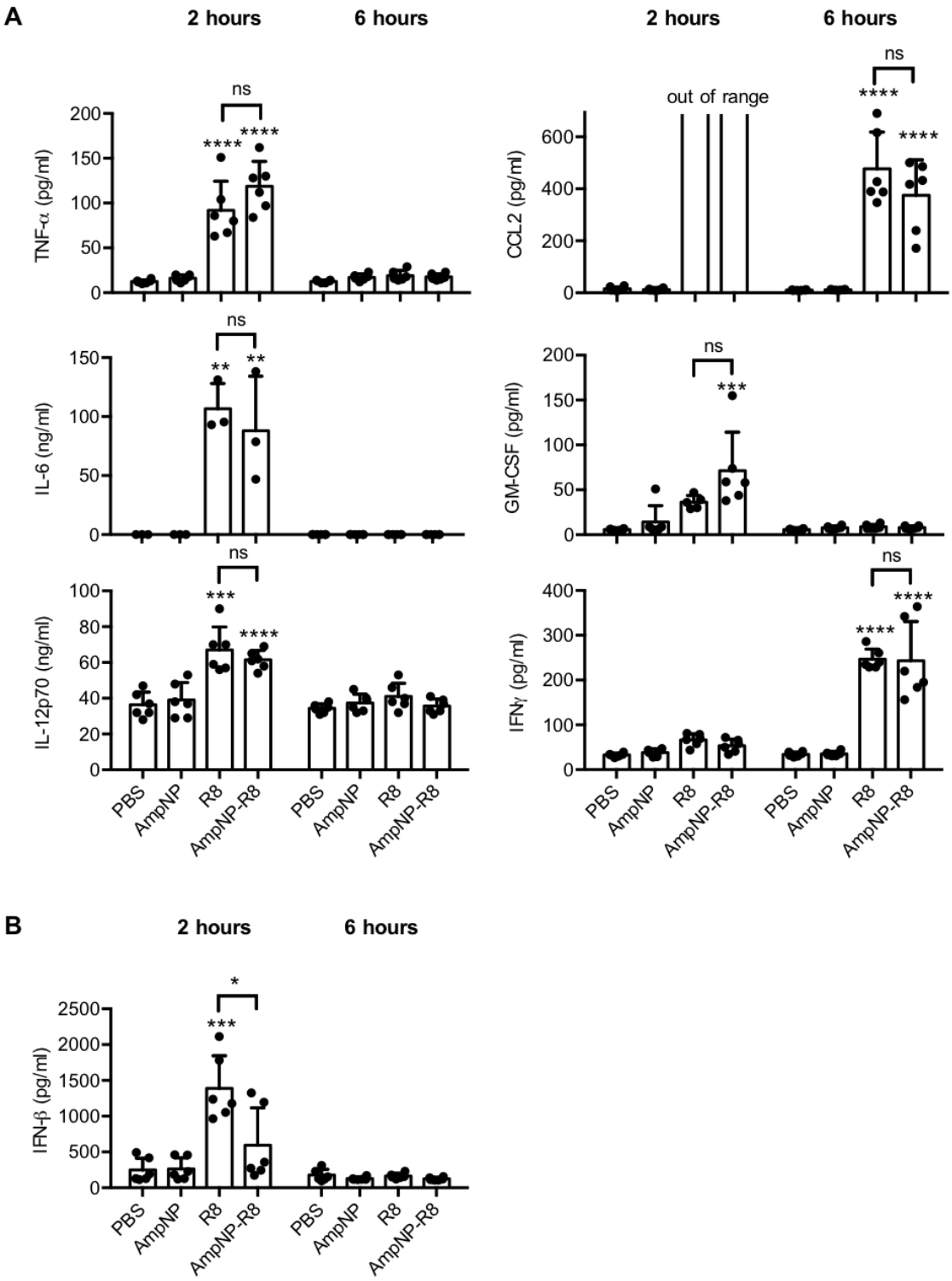
Supplementary Figure 4: AmpNP accumulated in the lymph nodes following s.c. injection.

Mice were injected s.c. in the tail base or i.v. with 300 µg AmpNP. Quantification of the gold content by ICP-MS in tissues 4 h and 24 h post injection. Each dot represents one mouse. Bars represent the mean ± SD of the pooled data.



Supplementary Figure 5: Body and spleen weight of tumor-bearing mice treated with Amp-NP-R848.

CT26 tumor-bearing mice with a tumor volume of 200 mm3 were injected 5 times at 3-day intervals s.c. in the inguinal area with AmpNP-R8 (R848 dose 6 μg) or an equivalent dose of free R848. (A) Body weight during the experiment. (B) Spleen weight at the time of sacrifice. Each dot represents one mouse. One representative experiment from 2 independent experiments is shown.



Supplementary Figure 6: AmpNP-R848 induced a Th1 cytokine profile.
Mice were injected with AmpNP-R8, free R848 or AmpNP as in figure 2. (A-B) Concentration of cytokines in the serum of mice 2 or 6 h post injection was measured by a bead-based immunoassay. Data were pooled from 2 independent experiments. Each dot represents one mouse. Bars represent the mean ± SD of the pooled data. Asterisks without brackets indicate significant difference to the untreated group using one-way ANOVA followed by Dunnett's multiple comparison test. Asterisk with brackets indicates significant difference using unpaired two-tailed Student's T-test. ns: not significant.