Supplementary Information (SI) for Nanoscale Advances.

Bone-derived Nanoparticles (BNPs) enhanced Osteogenic Differentiation via Notch Signaling

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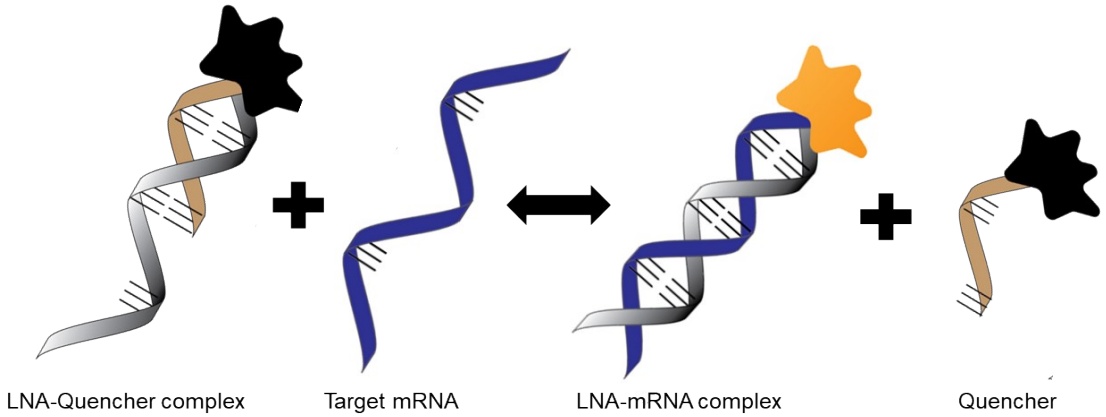

Fig. S1 Illustration of LNA/DNA nanobiosensor. The LNA/DNA nanobiosensor is a complex of LNA donor and quencher probe. The fluorophore at the 5' of LNA donor probe is quenched due to close proximity. In the presence of target mRNA sequence, the LNA donor sequence is displaced from the quencher to bind to the target sequence, allowing the fluorophore to fluorescence.

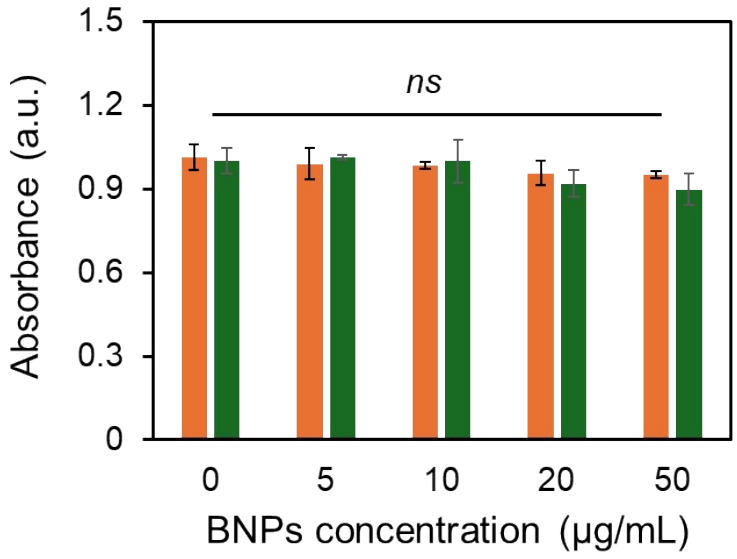

Fig. S2 Effects of internalized BNPs on cell viability. MSCs were treated with BNPs with the concentration of 0, 5, 10, 20, and 50 μg/mL. After 14 days of incubation, the absorbance at 450 nm was read using fluorescent microplate reader (BioTek, Synergy 2). Data expressed as mean± s.e.m. (n=3).

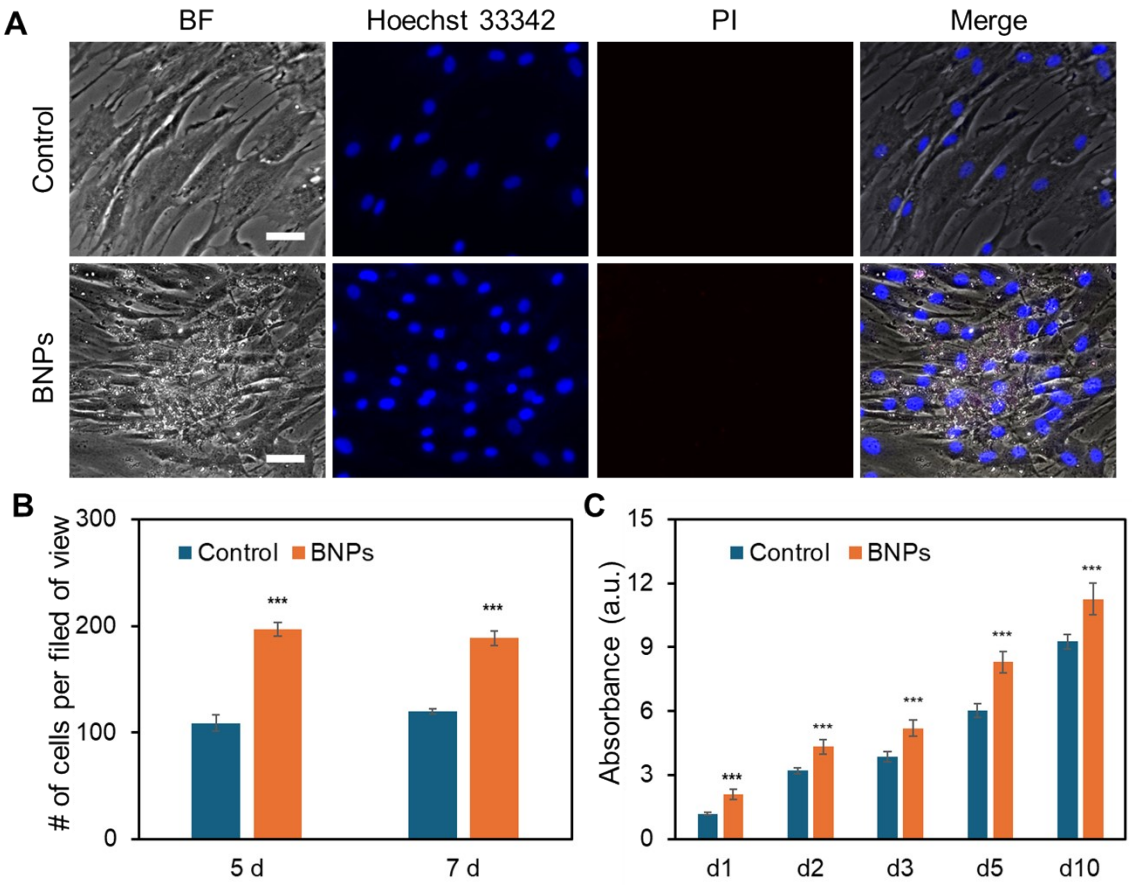

Fig. S3. Effects of BNPs on MSCs viability. **(A)** Representative bright field and fluorescence images of MSCs under control and BNPs treated group after 5 days of incubation. Cells were stained with propidium iodide (PI, red), and Hoechst 33342 (blue), respectively. Scale bar: 50 μm. **(B)** Quantification and comparison of cell numbers with and without BNPs co-culturing after 5 days and 7 days, respectively. Cell numbers were calculated using a MATLAB program by counting the number of nucleuses in each field of view. At least 10 images were quantified for each condition. **(C)** Cumulative absorbance using cck-8 proliferation assay after 1, 2, 3, 5, and 10 days of incubation. Data are expressed as mean± s.e.m. (n=3, ***, P<0.001).

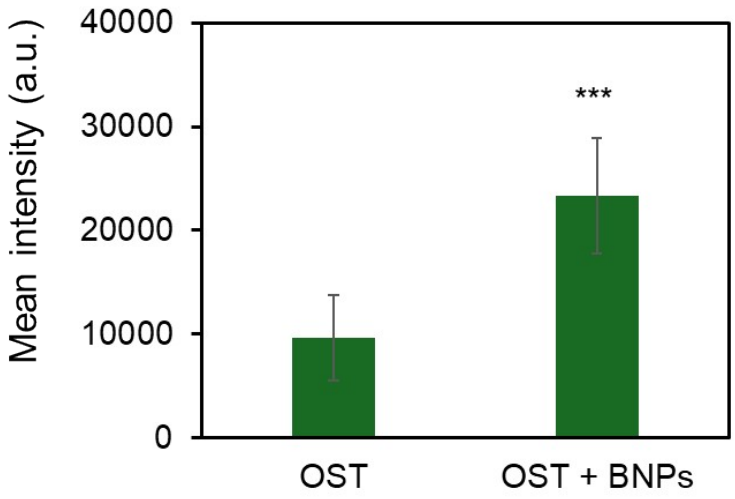

Fig. S4 Comparison of ALP intensity of MSCs after 7 days of osteogenic induction. Cells were treated with BNPs (20 μg/mL) and cultured in osteogenic induction medium. At least 100 cells were analyzed for each condition. Data expressed as mean± s.e.m. (n=3).

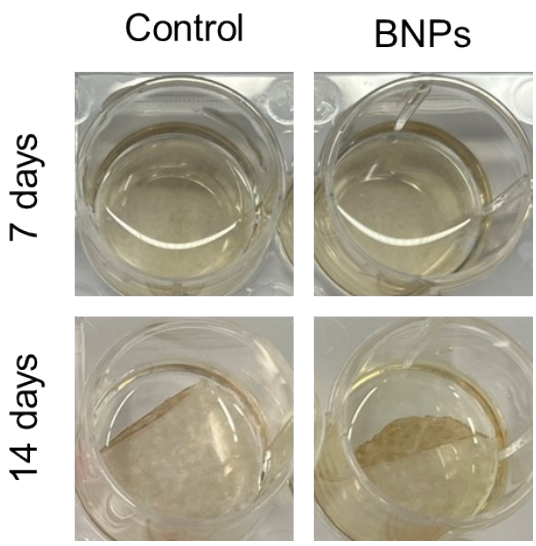

Fig. S5 Images of MSCs in 12-well plate after 7 days and 14 days of culturing in basal medium. For BNPs treated groups, MSCs were treated with BNPs at the concentration of 20 μg/mL overnight for internalization. Alizarin Red Staining showed no calcium deposition observed when MSCs cultured in basal medium.

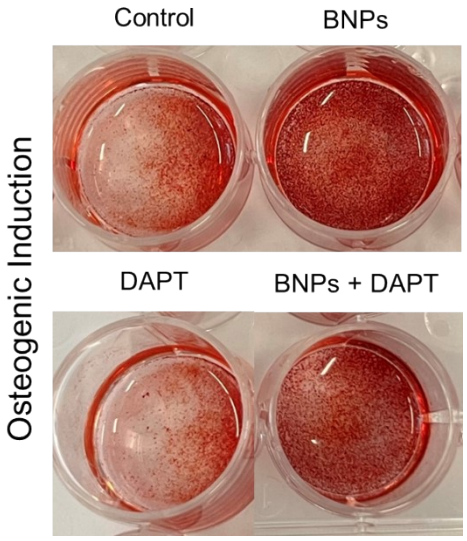

Fig. S6 Images of MSCs in 12-well plate after 14 days of osteogenic induction. For BNPs treated groups, MSCs were treated with BNPs at the concentration of 20 μg/mL overnight for internalization. DAPT (20 μM) were added to MSCs for Notch inhibition.

Tab. S1. LNA/DNA probes and quencher sequences

Name		Sequence (5'-3')	Fluorophore
Dll4 mRNA	Donor	+AA +GG +GC +AG +TT +GG +AG +AG +GG +TT	/56-FAM
	Quencher	+TT +CC +CG +TC +AA	/3-Iowa BlackFQ
	Target	AA CC CT CT CC AA CT GC CC TT	

* + represents LNA monomer