Supplementary Information

Differentiation Regulation of Mesenchymal Stem Cells via Autophagy induced by Structurally-different Silica Based Nanobiomaterials

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Additional data:

Cell viability: The viability of MSCs was evaluated by MTT assay. MSCs were cultured into a 24-well plate with an initial density of 1×10^4 cells/cm^2. The particle suspension of DMSN, MSN and SSN was added into each well. The final concentration of particles was adjusted to 50 μg/mL. After culturing for 4 and 7 days, the culture medium containing the particles was removed. 100μL of MTT (5 mg/mL) and 0.9 mL of fresh culture medium was added to each well and incubated for another 4 h at 37 °C. After that, the culture medium containing MTT was removed and 500μL of dimethyl sulfoxide (DMSO) was added to each well. Finally, the optical density of the solution was measured at 490 nm by spectrophotometric microplate reader (Bio-Rad 680).

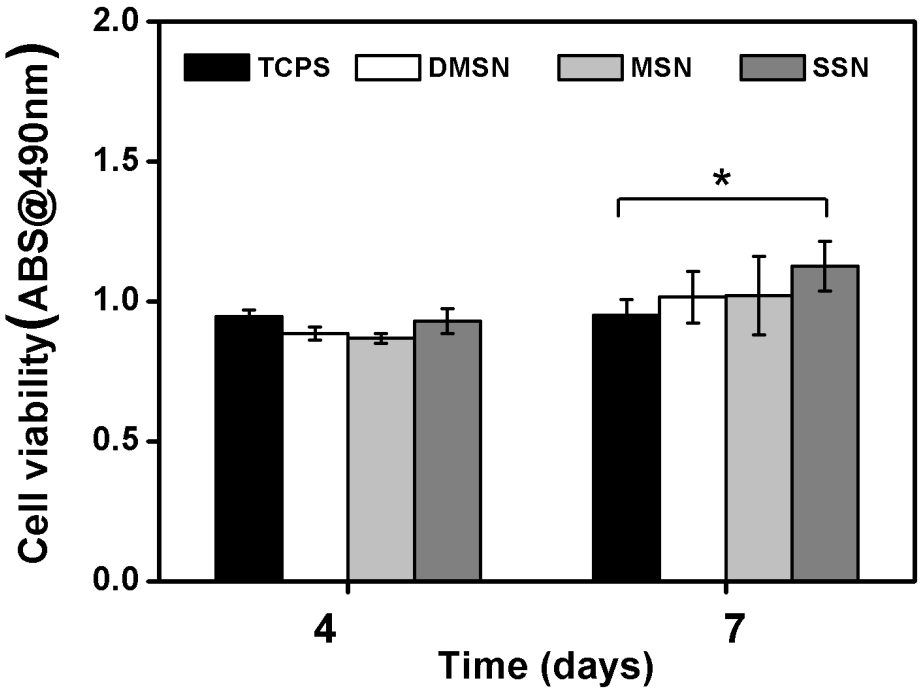

Fig. S1 Cell viability of MSCs treated with different SNPs and normal culture medium for 4 and 7 days, respectively. Error bars represent means ± SD, n=3, *$p < 0.05$.

In this study, MTT assay was performed to evaluate the viability of MSCs when cultured with different nanoparticles, SSN displayed the highest cell ability among all groups after culturing for 7 days (Figure S1). However, there was no significant difference in cell viability between SSN and DMSN or between SSN and MSN.

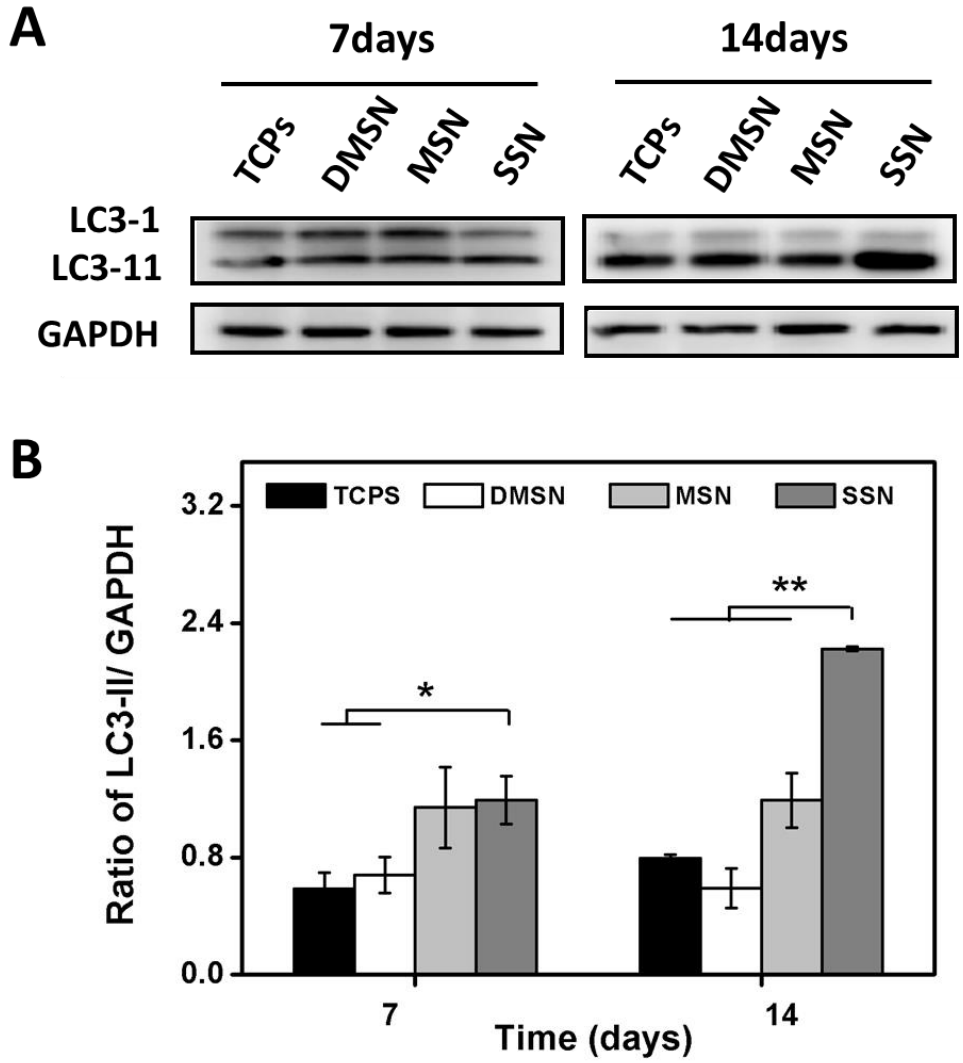

Fig. S2 Representative western blotting （A）and quantitative analysis (B) of LC3β-I and LC3β-II expression in MSCs treated with different SNPs and normal culture medium for 7 and 14 days, respectively. Error bars represent means ± SD, n = 3,*p < 0.05 and **p < 0.01. Western Blot was performed to evaluate the expression level of autophagy-associated proteins (LC3) in MSCs stimulated by different nanoparticles within 14 days. As shown in Figure S2, the SSN group showed higher expression of LC3-II in MSCs than DMSN, MSN and control group (TCPS), It was noted that SSN showed a significant higher (*P<0.05,**P<0.01) expression of LC3-II in MSCs compared with MSN, DMSN and TCPS after culturing for 7 and 14 days.

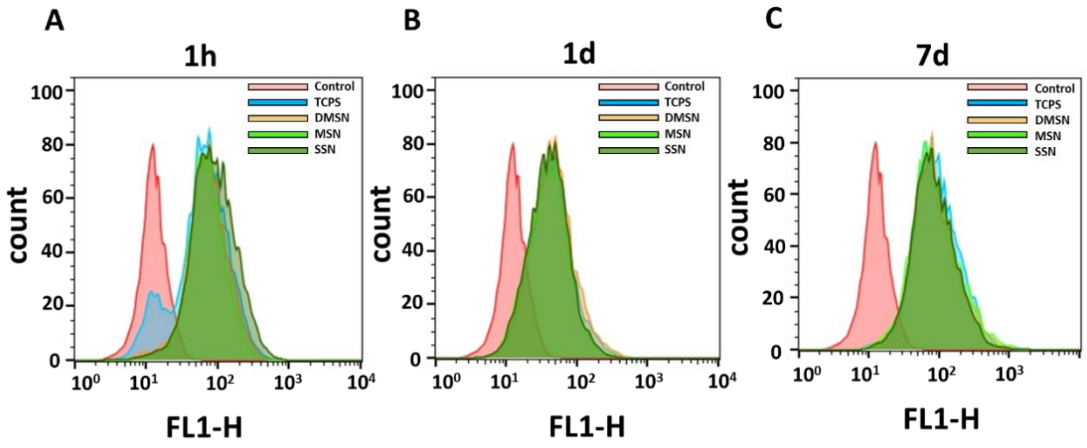

Fig. S3 Intracellular calcium ion concentration in MSCs treated with different SNPs and normal culture medium for 1 h, 1 day and 7 days, respectively.

Perturbation of calcium flux is an established mechanism for mTOR-independent autophagy induction. Therefore, calcium flux in MSCs incubated with different nanoparticles was tested by flow cytometry. As shown in Figure S3, MSCs treated by DMSN, MSN and SSN displayed higher calcium flux compared to TCPS after 1h. However, there was no obvious difference in calcium flux among DMSN, MSN and SSN. A similar trend was found when the incubation time was extended to 1 day and 7 days. The results indicated that the contribution of calcium flux to the autophagic activity of MSCs when treated with different nanoparticle is negligible.

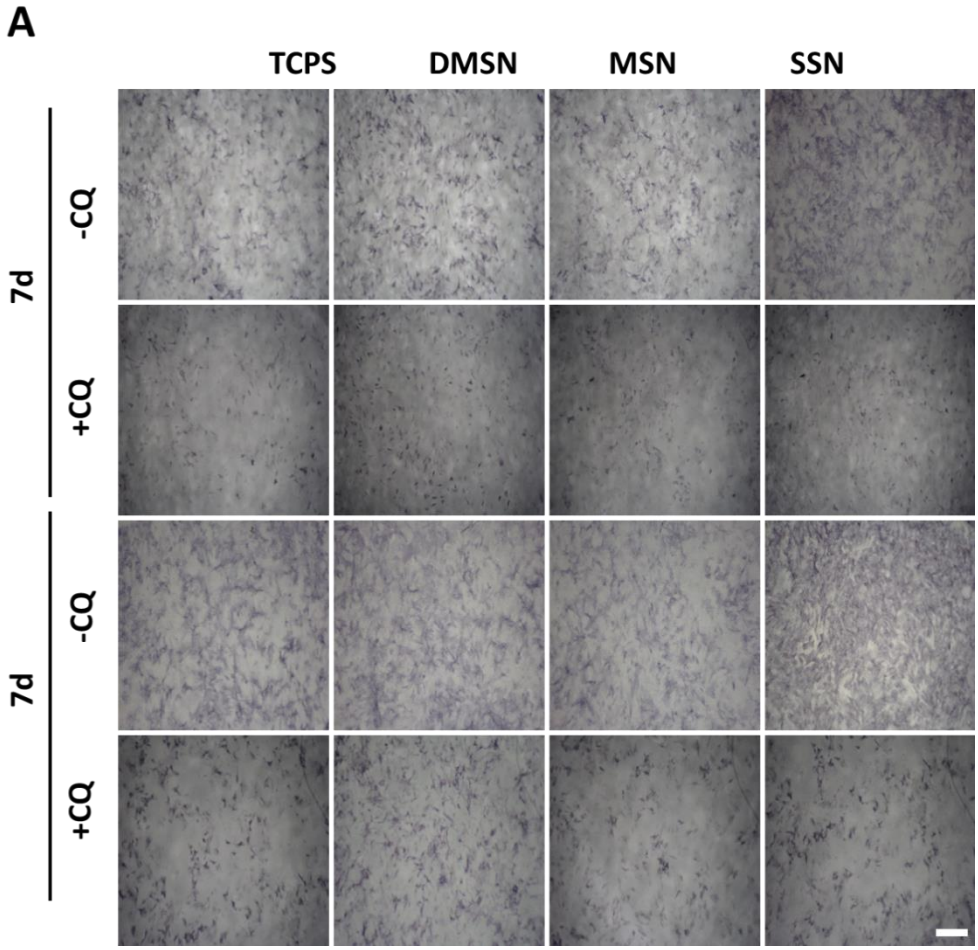

Fig. S4 ALP staining of MSCs treated by different SNPs and normal culture medium (with or without CQ) for 7 and 14 days, respectively. Scare bar = 1mm.

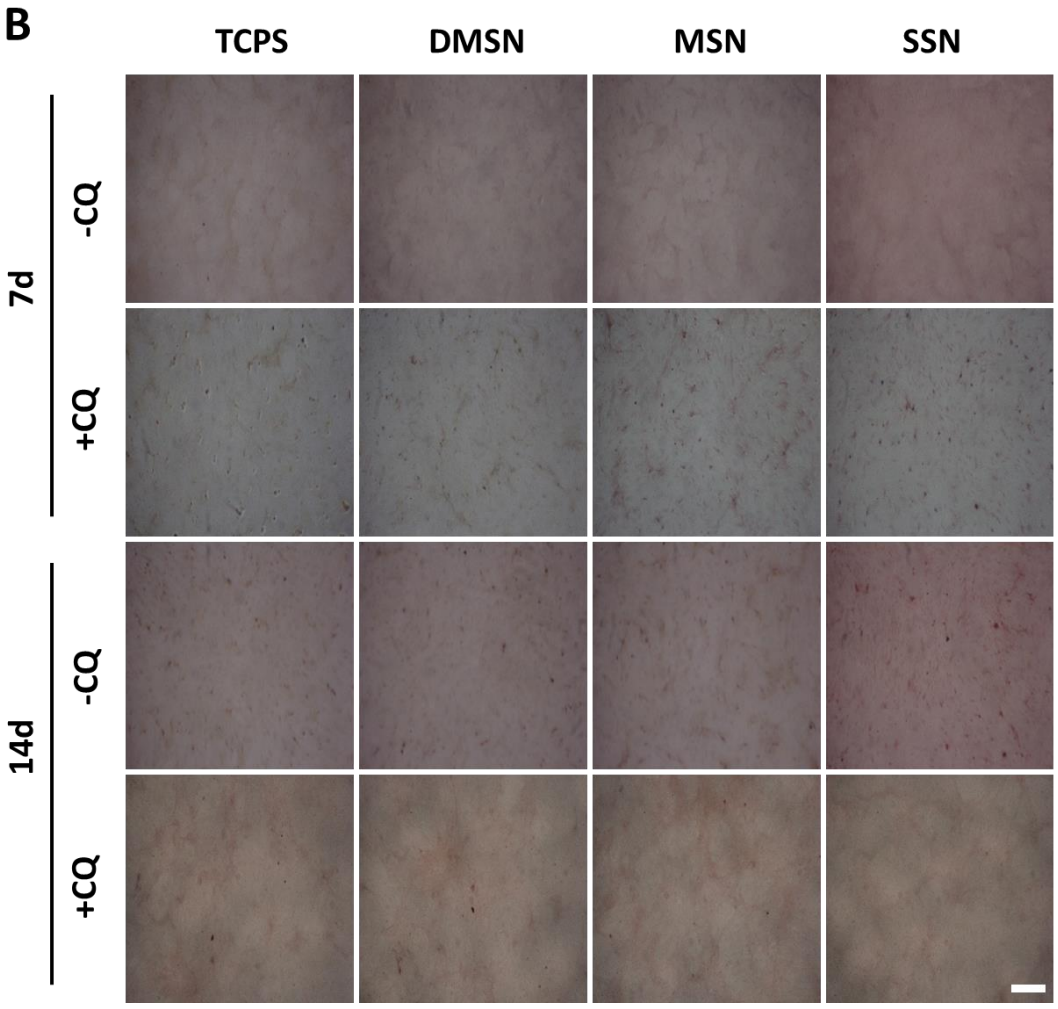

Fig. S5 Alizarin Red S (ARS) staining of MSCs treated by different SNPs and normal culture medium (with or without CQ) for 14 and 21 days, respectively. Scale bar = 1mm.

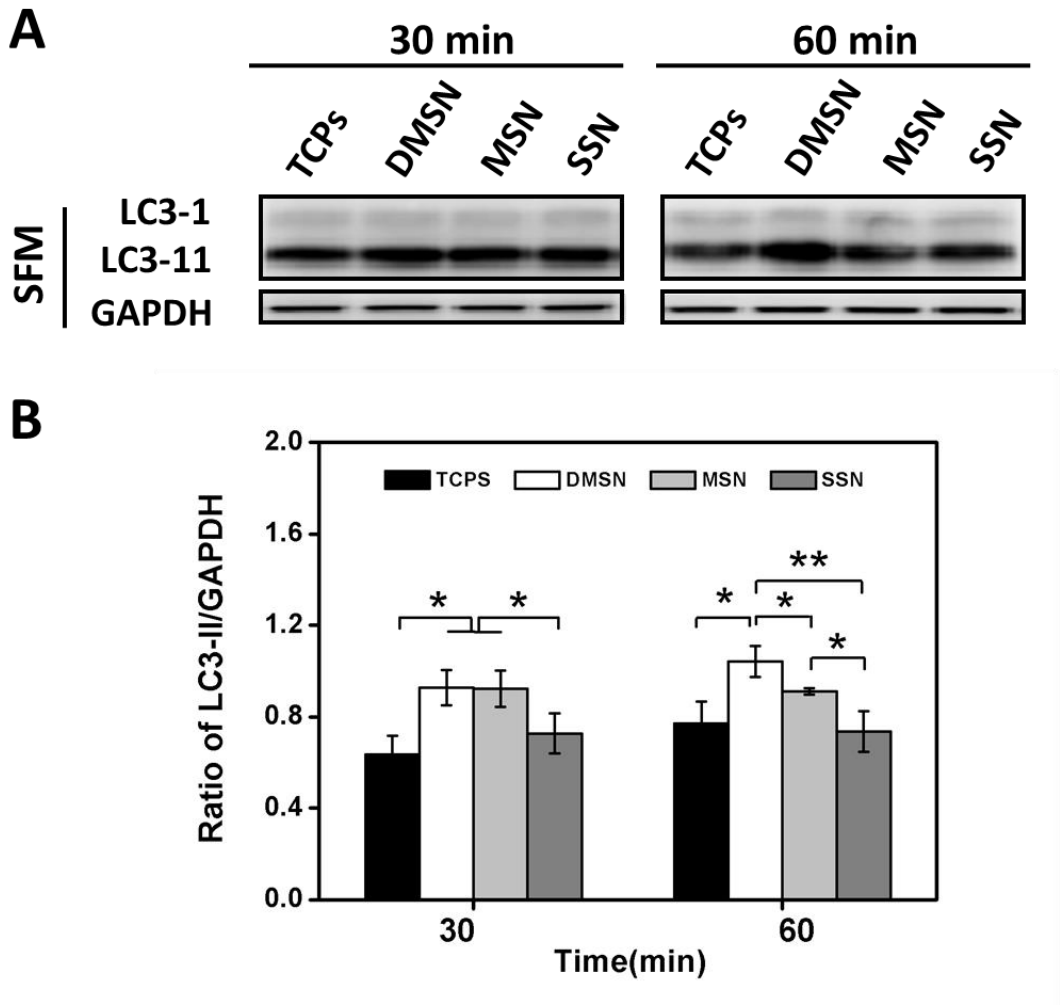

Fig. S6. Representative western blotting (A) and quantitative analysis (B) of LC3-I and LC3-II expression in MSCs treated with different SNPs or serum-free medium(SFM) for 30 min and 60 min. Error bars represent means ± SD, n=3, **p < 0.01, *p < 0.05.

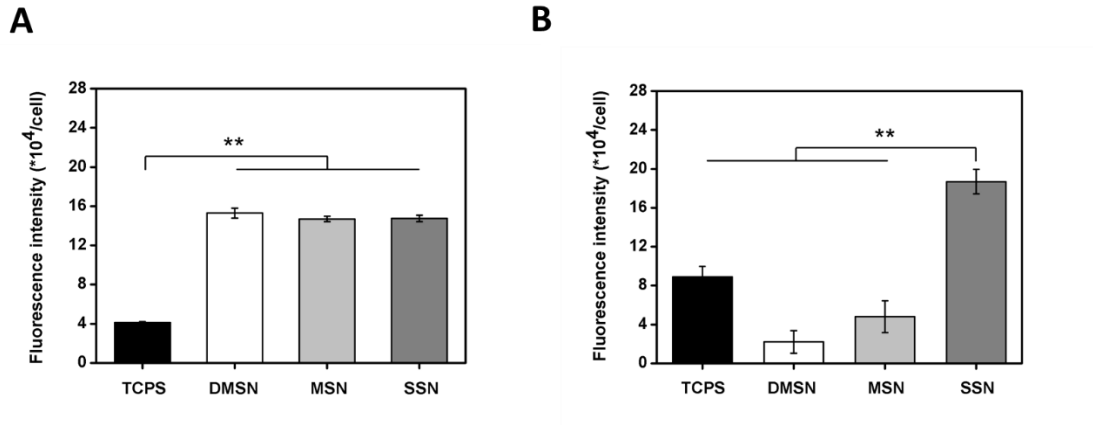

Fig. S7 the fluorescence intensity. Cell viability of MSCs treated with different SNPs and normal culture medium for 1 days, respectively. (A) Endosomes was labeled by FM1-43 (green). (B) Green fluorescence represents FITC-labeled Autophagosome. Error bars represent means ± SD, n=3, **p < 0.01.

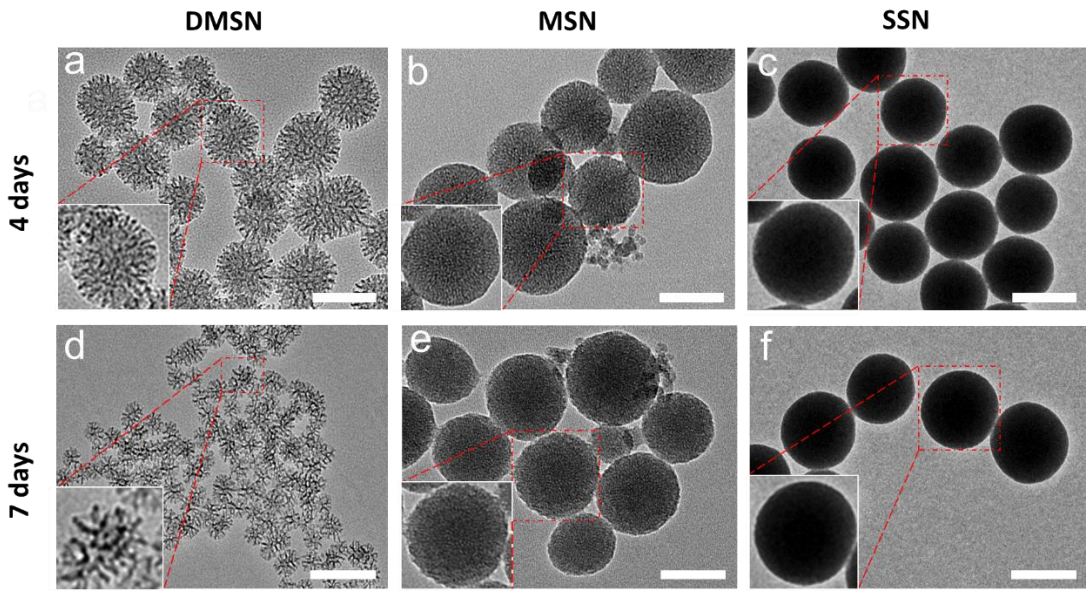

Fig. S8 the stability of these nanoparticles. These nanoparticles treated and soaked in the normal culture medium for 4 and 7 days, respectively. Morphological characterizations of the as-prepared materials, including TEM images of (a, d) DMSN, (b, e) MSN, and (c, f) SSN, (Scale bar: 100 nm).

To investigate the stability and solubility of these nanoparticles, all samples were incubated in normal culture medium (DMEM with low glucose supplemented with 10% FBS (bovine serum)) for 7 days and then monitored with TEM. Firstly, it was observed that DMSN showed relatively faster degradation after 7 days of incubation and the outermost structures were first degraded. However, the degradation of MSN was much slower, indicated by the partial deformation of MSNs after incubating for 7 days. In comparison, only minimal degradation was observed for SSM as its structure remained almost intact. Moreover, no visible precipitate was observed for the three nanoparticles throughout the incubation period, immediately suggesting their excellent dispersibility

in a biologically-relevant environment.

Table S1 Real-time polymerase chain reaction primers used in this study.

Target gene	Gene Bank (Accession no.)	Primers	Product size (bp)
COLI	NM_053304.1	CCTGAGCCAGCAGATTGA TCCGCTCTTCCAGTCAG	106
OPN	M99252	GACAGCAACGGGAAGACC CAGGCTGGCTTTGGAACT	216
OCN	XM006232594.2	AGATTGTTGGGGCACAAGGT CCTTCAGCAGGGAAACCGAT	191
Runx2	NM_053470.2	GCCGTAGAGAGCAGGGAAGAC CTGGCTTGGATTAGGGAGTCAC	150
OPG	RNU94330	GCCCAGACGAGATTGAGAG CAGACTGTGGGTGACGGTT	173
GAPDH	AF106860.2	GGCATTGCTCTCAATGACAA TGTGAGGGAGATGCTCAGTG	223