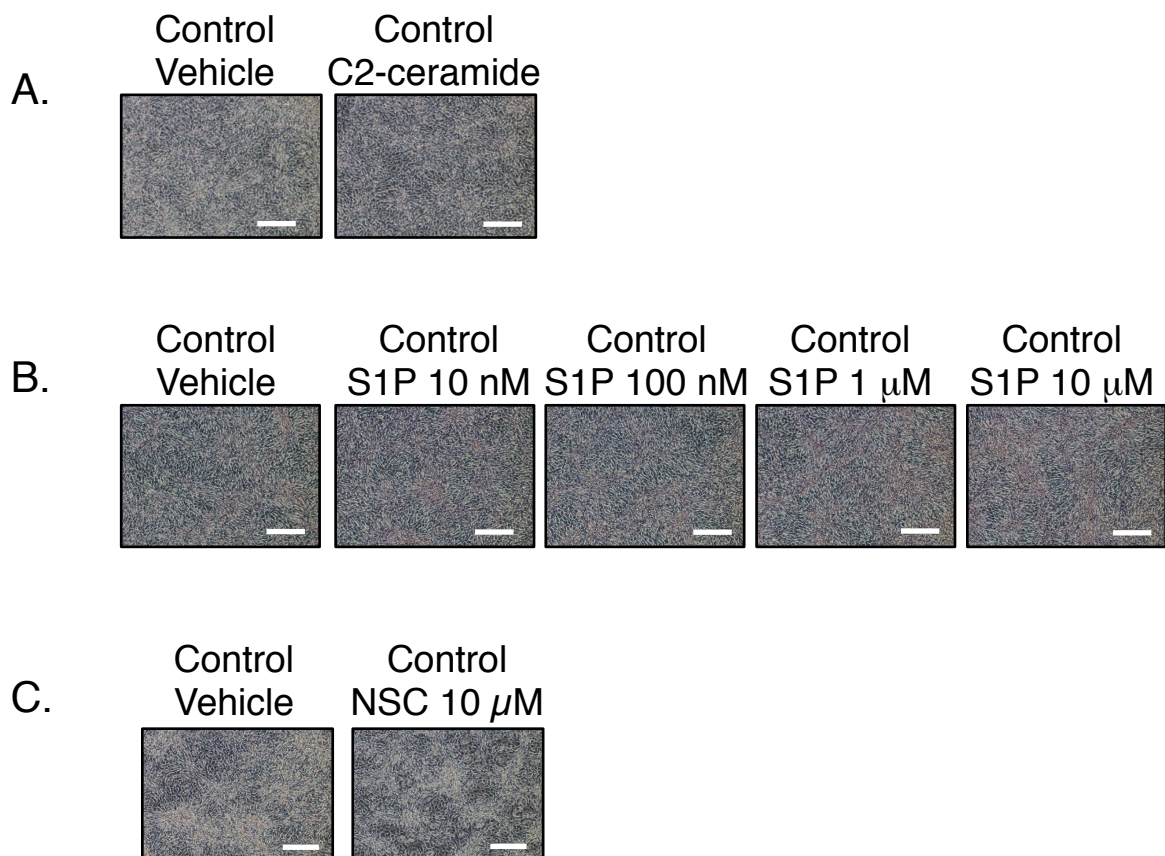


Supplemental Table S1. Primer sequences for Q-RT-PCR.

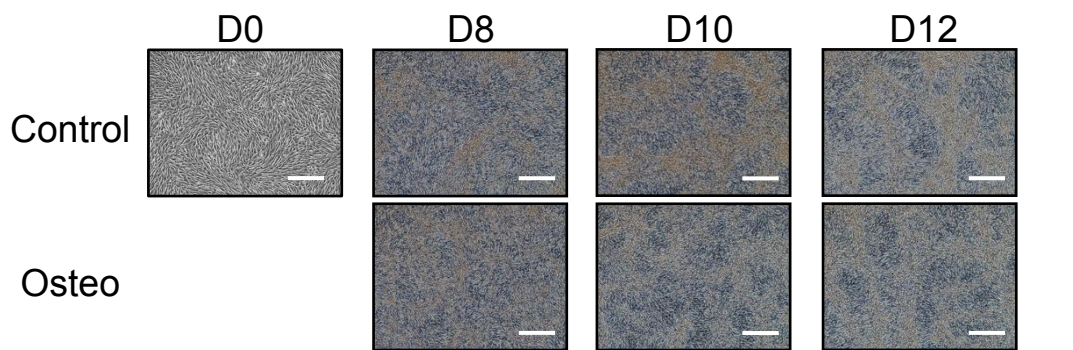
Primer pair (GenBank accession no.)	Sequence
aSMase (NM_001075383.2)	Forward – 5' TTT-TGA-TCA-ACT-CCA-CAG-ATC-C 3' Reverse – 5' GAA-TGT-GGC-CAA-TTA-TGT-GC 3'
SK1 (XM_002696204.3)	Forward – 5' GAA-CTA-TTA-CGC-CGG-GTA-CG 3' Reverse – 5' GGC-TGA-GCA-CAG-AGA-AGA-GG 3'
SK2 (XM_010815269.1)	Forward – 5' GTT-AGC-AGG-AGC-CGT-GAA-CC 3' Reverse – 5' GCA-GTA-AGG-AGC-AGT-TGA-GTA-GC 3'
RPL12 (NM_205797.1)	Forward – 5' CCA-GGC-AAC-TGG-TGA-TTG-G 3' Reverse – 5' TTG-ATG-ATC-AGG-GCA-GAA-GC 3'
PPIA (NM_178320.2)	Unknown – purchased from Primer Design

## Supplemental Figure S1

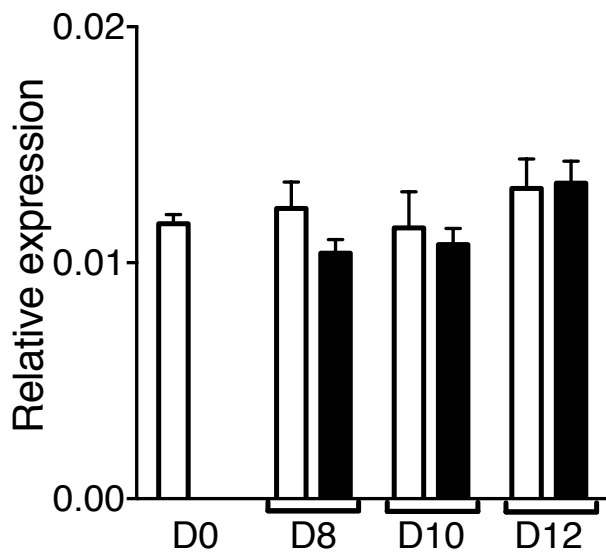


**Supplemental Figure S1: C2-Ceramide, S1P and NSC668394 do not affect VSMC cultured in control medium.** **A:** VSMC were cultured in control medium with or without C2-ceramide. At the time point corresponding to mid-mineralisation (Figure 3A) cells were stained with Alizarin Red. **B:** VSMC were cultured in control medium with the indicated concentrations of S1P. At the time point corresponding to early-mineralisation (Figure 3B) the cells were stained with Alizarin Red. **C:** VSMC were cultured in control medium with or without NSC668394 (10  $\mu$ M). At the time point corresponding to mid-mineralisation (Figure 6B) cells were stained with Alizarin Red. Photomicrographs are representative of 3 independent experiments; scale bar = 500  $\mu$ m in all.

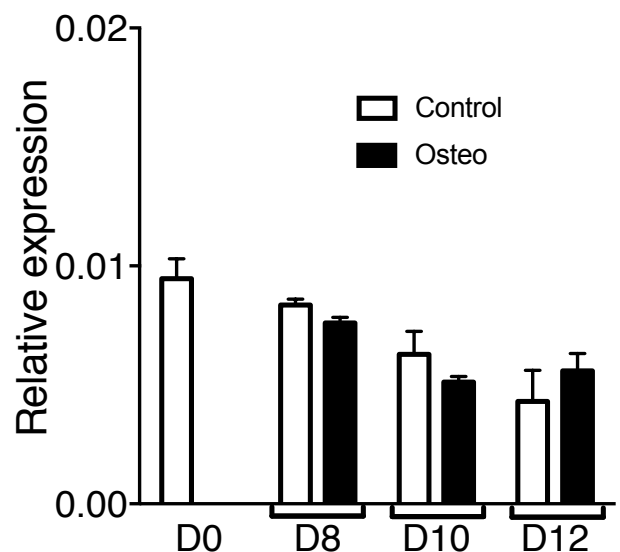
## Suppl Figure 2



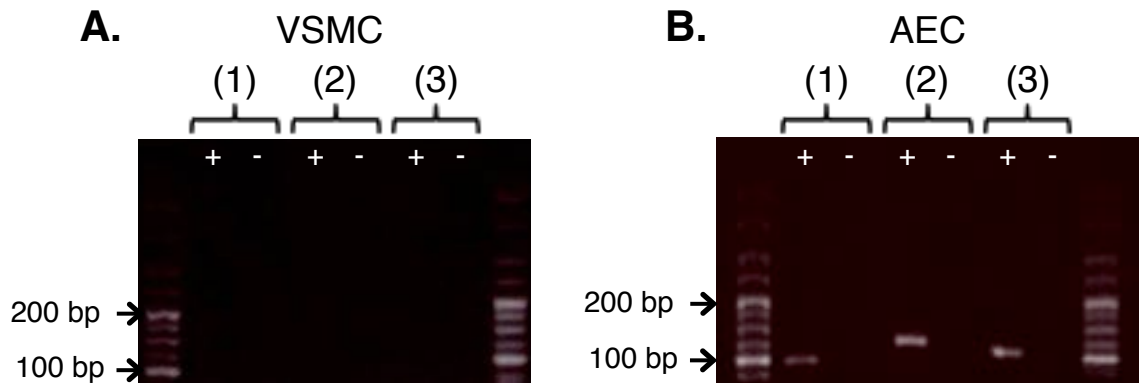
*SPHK1* (SK1)



*SPHK2* (SK2)



**Supplemental Figure S2: The mRNA expression of *SPHK1* and *SPHK2* does not change in non-mineralising VSMC cultured in osteogenic medium.** VSMC were cultured in control or osteogenic (Osteo) medium. At Day 0 (D0), day 8 (D8), day 10 (D10) and day 12 (D12) cells were stained with Alizarin Red or total RNA was extracted for quantification of target genes by qPCR. **A:** Representative photomicrographs following Alizarin Red staining. **B:** *SPHK1* and *SPHK2* expression. mRNA levels are shown relative to the house-keeping genes, PP1A and RPL12, as detailed in Methods. The data are mean $\pm$ SEM from 3 independent experiments.



**Supplemental Figure S3: nSMase2 was not detected in VSMC.** End-point PCR using three nSMase2 (GenBank accession number NM\_001192363.1) primer pairs; (1) Forward – 5' GGC-GTT-TGA-CAT-CAT-CTG-C 3', Reverse – 5' TAG-CGT-GTA-AAC-AGG-GAG-TGC 3'; (2) Forward – 5' CCT-ATC-ACT-GTT-ACC-CCA-ACG 3', Reverse – 5' TGT-GTG-CAG-GAG-ATG-TAC-CC 3'; (3) Forward – 5' CCC-TGT-TTA-CAC-GCT-ACA-AGG 3', Reverse – 5' CAC-ACT-TCC-TCA-TCA-TAG-AGA-CC 3'; on **A:** VSMC and **B:** aortic endothelial cells (AEC). For each primer pair, positive samples (+) and negative control samples (-) not containing the reverse transcriptase enzyme during cDNA synthesis were run. The predicted molecular weights of the amplified products are: nSMase2 (1) 90 bp, nSMase2 (2) 127 bp & nSMase2 (3) 109 bp.