Supplementary information

Table S1. Primer sequence used for gentyping of LDLR and LOX-1 KO mice.

LDLR primers:

oIMR$_{5284}$, 5'- GTCTGCGAATAGGCTGGTGTAAGCCATAGC-3'; (Primer-3, P-3)

oIMR$_{5285}$, 5'- GATTGGGAAGACAATAGCAGGCATGC -3'; (Primer-E, P-E)

oIMR$_{5286}$, 5'- TTTGAACTCAGGACTTCGTGCTCTGGCAGC -3'; (Primer-4, P-4)

LOX-1 primers:

LOX-1-1485F-2, 5'-CCTCGTGCTTTACGGTATCGCC-3'

LOX-1(genetype)Del1064-F,5'-CCTTCAAGACGGAGCTGTGTTCGCTG-3'

LOX-1(genotype)-R, 5'-CCAGGTCAGGCAGGAAGCATCATTTA-3'

ABCG1 primers:

G1 E, 5'-GGGATCTCTGGGAAATTCAACAGTG-3'

G1ET, 5'-GTGAGCAGAGCTTCTGGTAGCAAAC-3'

Neo, 5'-GGGCCAGCTCATTCCTCCCACTCAT-3'

Table S2. Taqman probe ID.

c-Fos mRNA (*c-Fos*, Mm00487425_m1)
NFATc1 mRNA (*Nfatc1,* Mm00479445_m1)
TRAP mRNA (*Acp5,* Mm00475698_m1)
cathepsin K mRNA (*Ctsk* , Mm00484039_m1)
DC-STAMP mRNA (*Dcstamp*, Mm04209236_m1)
OC-STAMP mRNA (*Ocstamp*, Mm00512445_m1)
ATA6v0d2 mRNA (*Atp6v0d2,* Mm01222963_m1)
ABCA1 mRNA (*Abca1,* Mm00442646_m1)
ABCA2 mRNA (*Abca2,* Mm00431553_m1)
ABCA3 mRNA (*Abca3,* Mm00550501_m1)
ABCA4 mRNA (*Abca4,* Mm00492035_m1)
ABCB1 mRNA (*Abcb1α,* Mm00440761_m1)
ABCB1 mRNA (*Abcb1β,* Mm00440736_m1)
ABCB4 mRNA (*Abcb4,* Mm00435630_m1)
ABCG1 mRNA (*Abcg1,* Mm00437390_m1)
ABCG4 mRNA (*Abcg4,* Mm00507247_m1)
LXRα mRNA (*Lxr-α*, Mm00443451_ml)
LXRβ mRNA (*Lxr-β*, Mm00437265_ml)
18S rRNA (Mm03928990_g1)

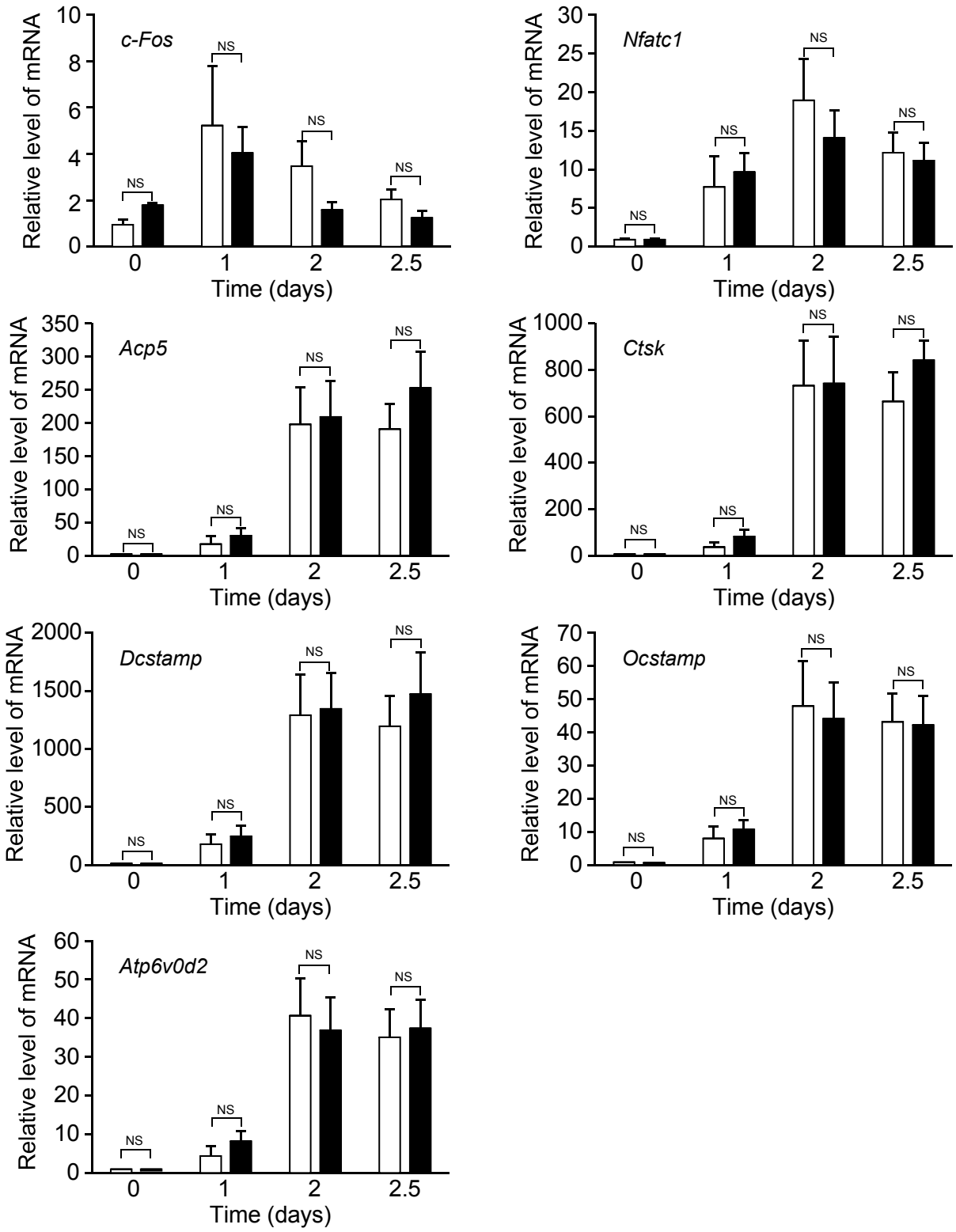

Fig. S1. Expression of osteoclast differentiation-related genes in *LDLR1/LOX-1* double knockout (dKO) OCLs. OCL-precursors from wild-type (WT, open bars) and *LDLR1/LOX-1* dKO (closed bars) bone marrow cells were cultured with sRANKL (10 ng/ml) and M-CSF (20 ng/ml) for the indicated days. After culture, total RNA was prepared, and the synthesized cDNA was subjected to quantitative real-time RT-PCR to determine the mRNA levels of c-Fos (*c-Fos*), NFATc1 (*Nfatc1*), TRAP (*Acp5*), cathepsin K (*Ctsk*), DC-STAMP (*Dcstamp*), OC-STAMP (*Ocstamp*) and ATP6v0d2 (*Atp6v0d2*) during osteoclastogenesis. The values represent the mean ± SD (n = 3). *NS* means that the difference was not significant between the cultures of WT and dKO OCLs on each day.

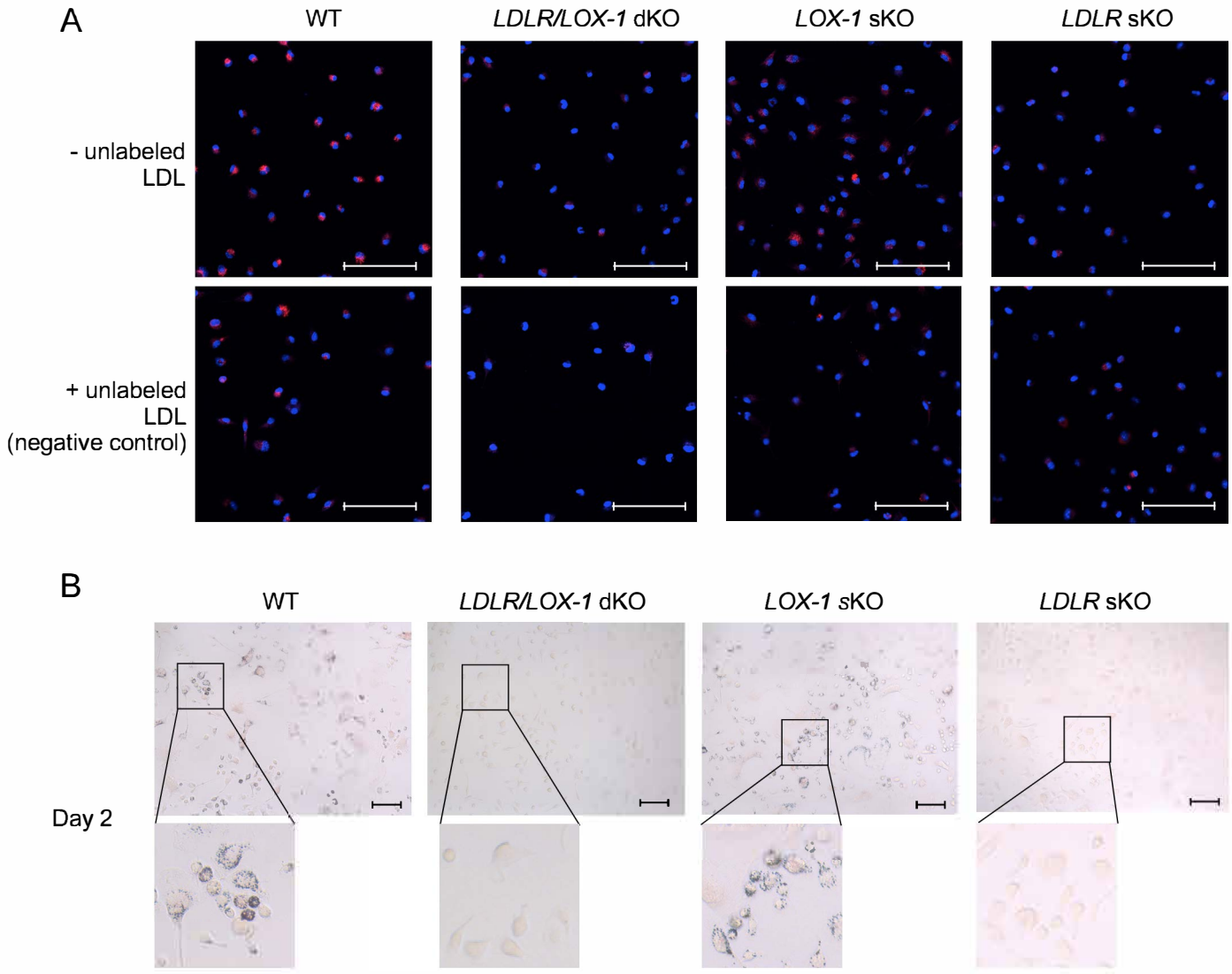

Fig. S2. Uptake of LDL into osteoclastic cells f om WTand *LDLR1 LOX-1* d O OCL-precursors and the accumulation of neut al lipids, including choleste ol, in the cells. (A) After OCL-precursors of WT, *LDLR/LOX-1* double KO (dKO), *LOX-1* single KO (sKO) and *LDLR* sKO mice were starved for exogenous LDL overnight, the cells were incubated with pHrodo^{TM} Red-labeled LDL for 3 h prior to counterstaining the nuclei. For the negative control, cells were pretreated with unlabeled LDL for 1 h. Scale bars, 100 µm. (B) For Oil Red O staining for intracellular neutral lipids, including cholesterol, the OCL-precursors were treated with M-CSF and sRANKL for 2 days and stained with Oil Red O solution. The scale bars (upper panels in (B)) indicate 100 µm, and the square areas were expanded (lower panels). The experiments were performed in triplicate, and reproducibility was confirmed.

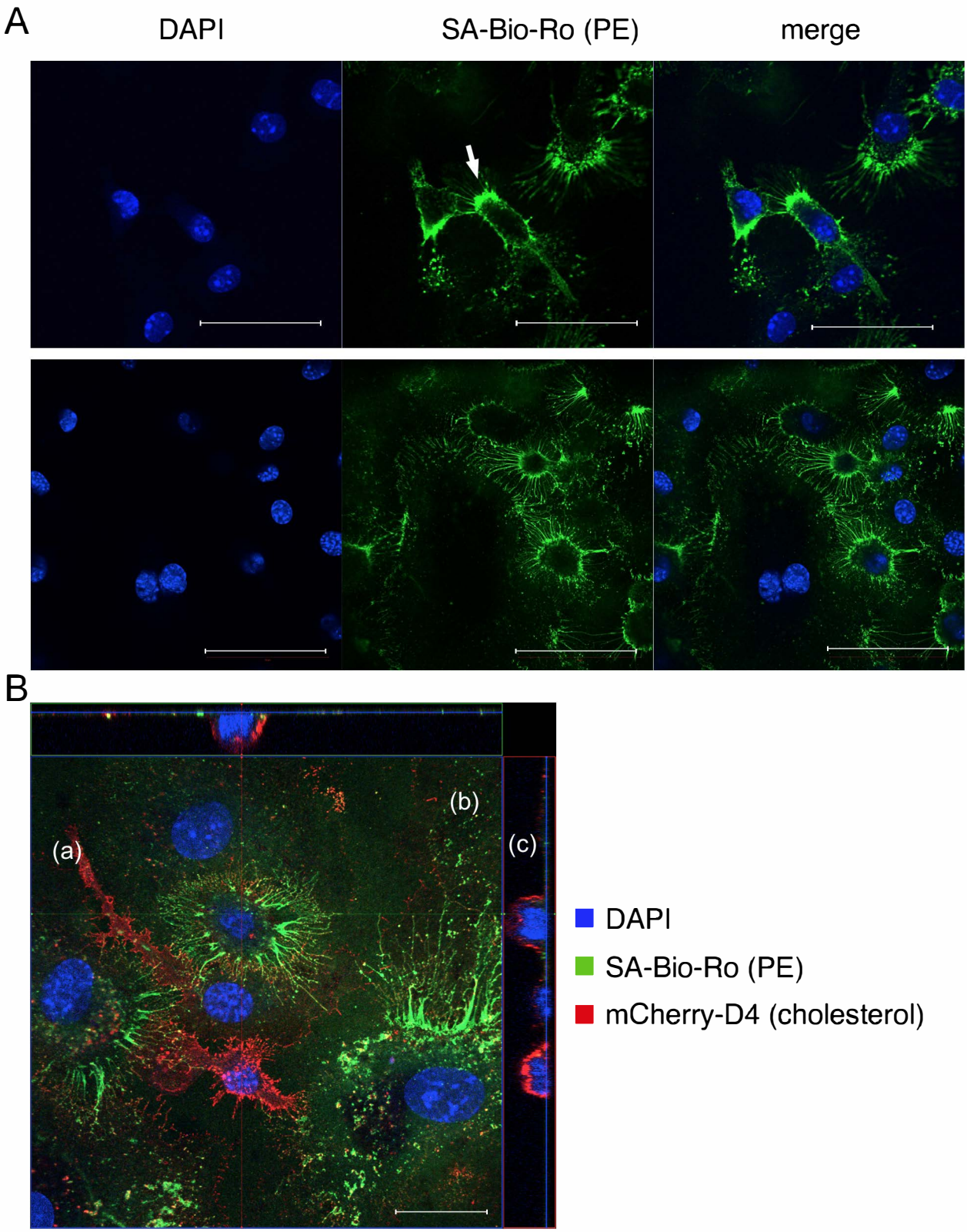

Fig. S3. Distributions of PE and cholesterol on the cell surface of osteoclastic cells. WT OCLs cultured with M-CSF and sRANKL for 2 days were incubated with SA-Bio-Ro for 30 min, and thereafter, the cells were fixed and immunostained by FITC-conjugated anti-SA antibody. The nuclei of the OCLs were counterstained with DAPI. The cells were observed by confocal laser microscopy. Scale bar, 20 µm. (B) The OCLs were simultaneously incubated with both SA-Bio-Ro and His-tag-mCherry-D4. After fixation, the cells were immunostained with anti-SA antibody. Thirty-five-slice images from the bottom to the top of the cells were obtained using a Z-stack method of confocal laser microscopy, and then, the images were reconstructed. (a), XY-image. (b) and (c), XZ-images. Scale bar, 10 µm.

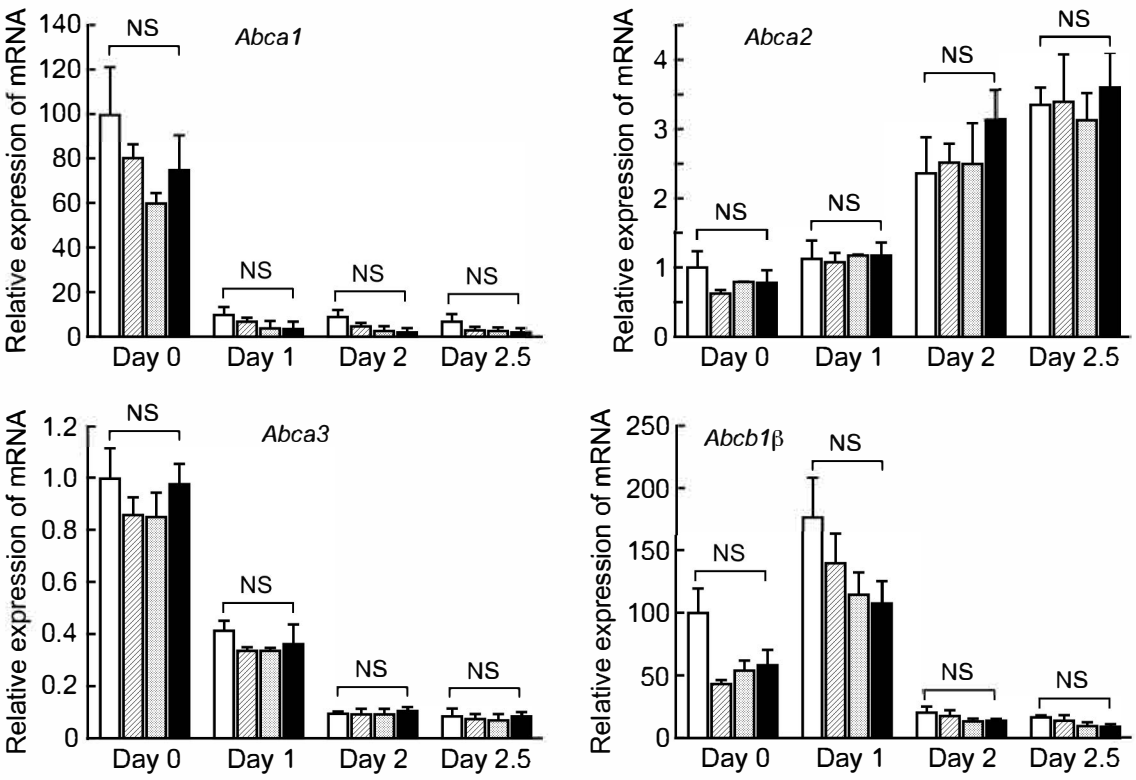

Fig. S4. mRNA expression of ABC transporters, in addition to ABCB4 and ABCG1, in OCLs with four different genotypes. Osteoclast precursors from WT (open bars), *LOX-1* single KO (sKO, hatched bars), *LDLR* sKO (dotted bars) and *LDLR1/LOX-1* dKO (closed bars) bone marrow cells were cultured with sRANKL (10 ng/ml) and M-CSF (20 ng/ml) for the indicated days. After culture, total RNA was prepared, and the synthesized cDNA was subjected to quantitative real-time RT-PCR to determine the mRNA levels of ABCA1 (*Abca1*), ABCA2 (*Abca2*), ABCA3 (*Abca3*) and ABCB1 (*Abcb1*) during osteoclastogenesis. The experiments were performed in triplicate, and reproducibility was confirmed. The values represent the mean ± SD (n = 3). *NS* means that the difference was not significant between the cultures of WT cells and each KO OCLs of each other genotype on each day.

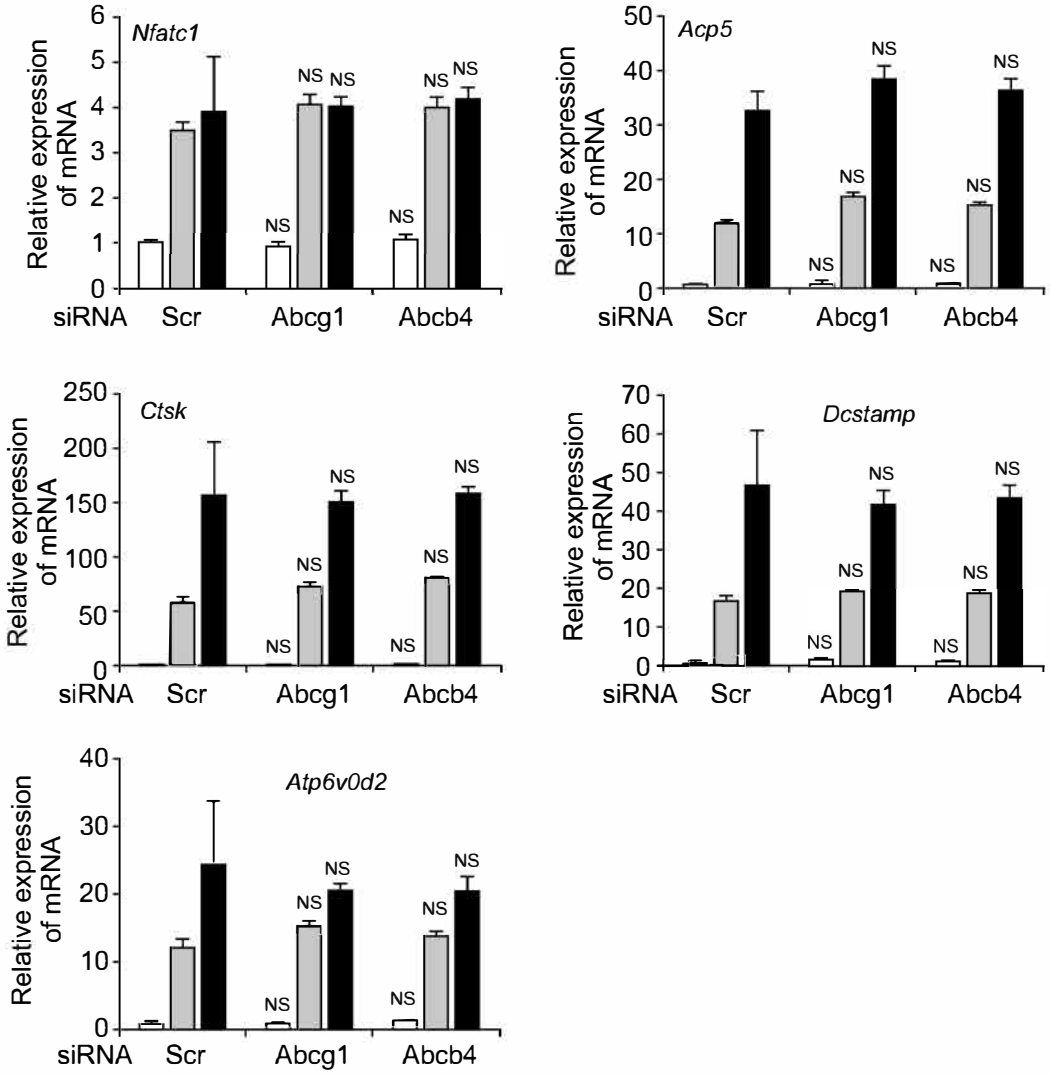

Fig. S5. Effect of *Abcg1* and *Abcb4* mRNA knockdown on mRNA expression of osteoclast-related molecules. WT OCL-precursors were electroporated with scramble RNA (Ser, 1 μM, negative control), *Abcg*1 siRNA (1 μM) or *Abcb4* siRNA (1 μM). After transduction of the siRNAs, the OCL-precursors were cultured with M-CSF and sRANKL. After culturing for one day (open bars), 2 days (gray bars) and 3 days (closed bars), the mRNA levels of *Nfatc1, Acp5, Ctsk, Dcstamp* and *Atp6v0d2* were determined by quantitative real-time RT-PCR. The experiments were performed in triplicate, and reproducibility was confirmed. The values represent the mean ± SD (n = 3). **P* < 0.05 *vs*. culture of osteoclastic cells transduced by scramble RNA on each day. *NS* means that the difference was not significant.

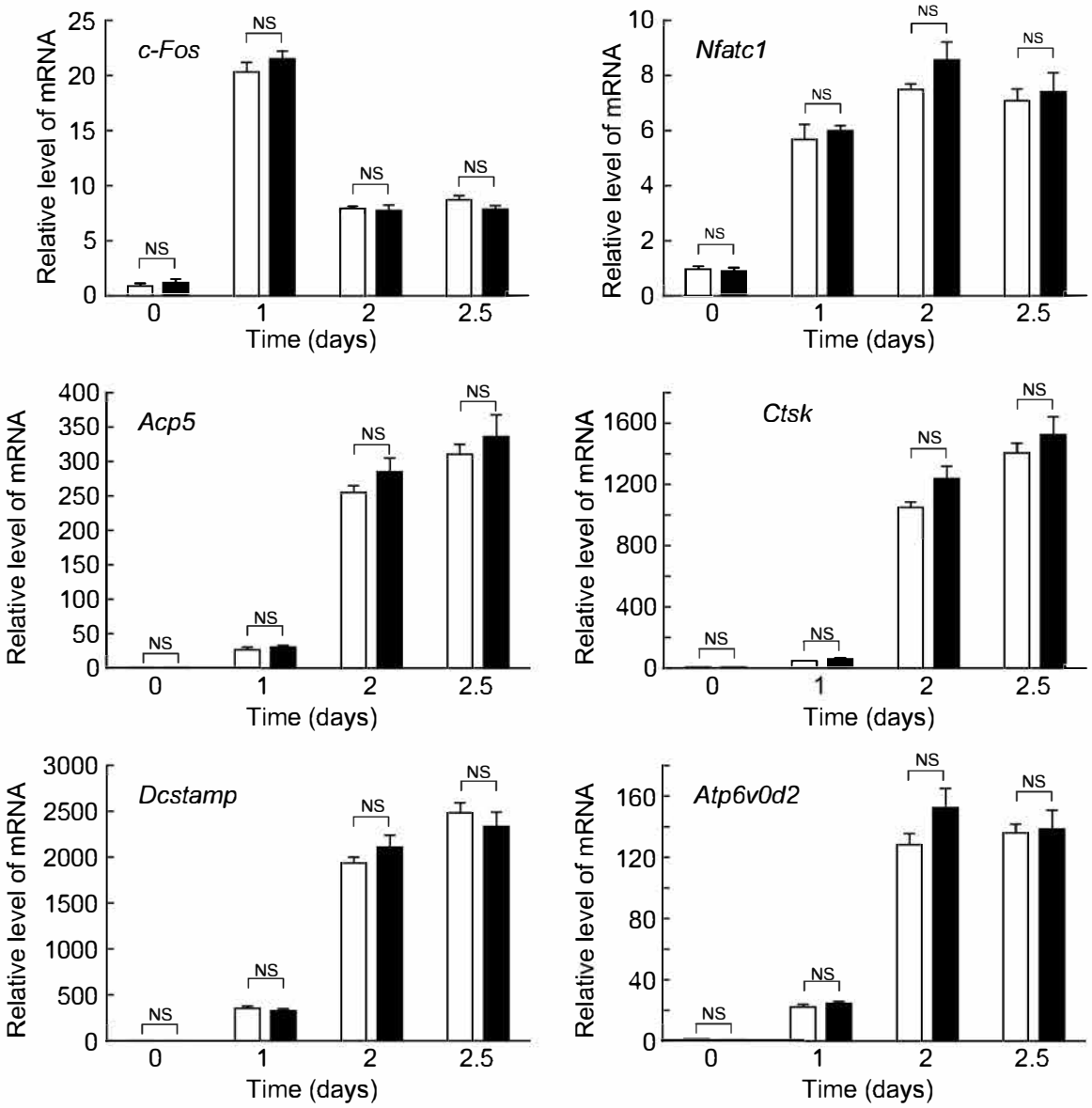

Fig. S6. Exp ession of osteoclast differentiation-related genes in *Abcg1* sKO OCLs. OCL-precursors from WT (open bars) and *ABCG1* sKO (closed bars) bone marrow cells were cultured with sRANKL (10 ng/ml) and M-CSF (20 ng/ml) for the indicated days. After culture, total RNA was prepared, and the synthesized cDNA was subjected to quantitative real-time RT-PCR to determine the mRNA levels of *c-Fos*, *Nfatc1*, *Acp5*, *Dcstamp* and *Atp6v0d2* during osteoclastogenesis. The experiments were performed in triplicate, and reproducibility was confirmed. The values represent the mean ± SD (n = 3). *NS* means that the difference was not significant between the cultures of WT cells and dKO OCLs on each day.

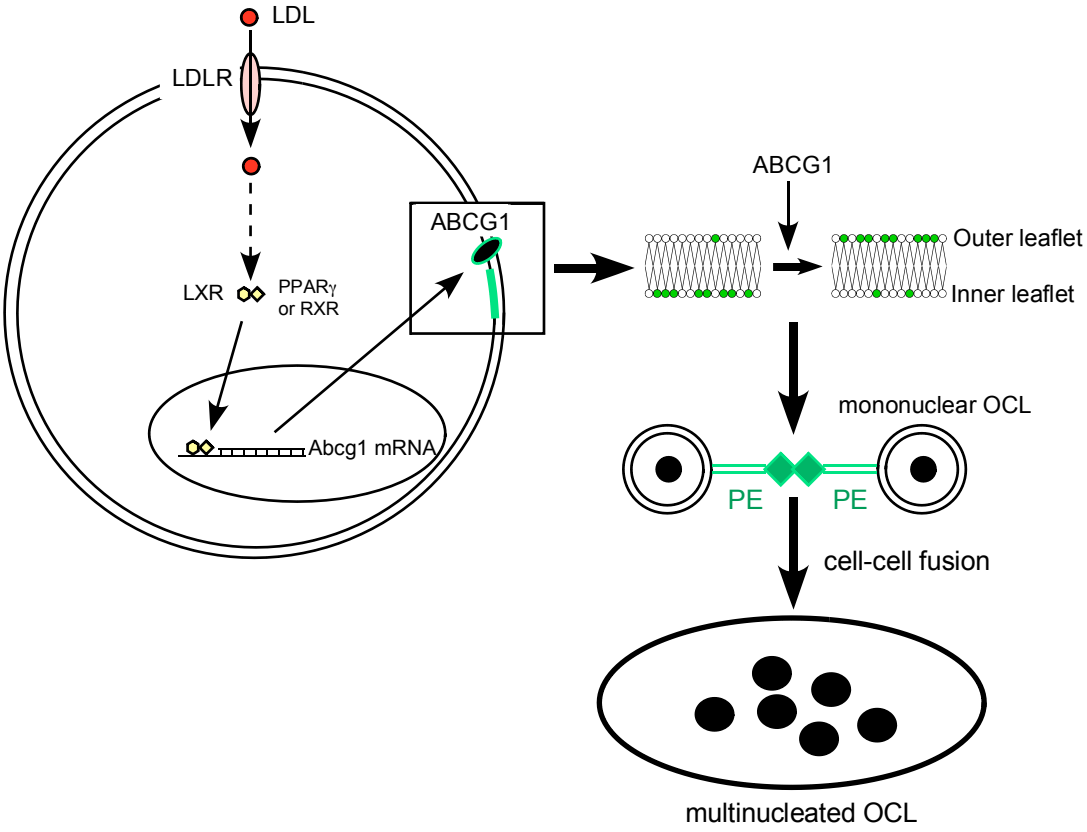

Fig. S7. Scheme of sequential cellular events from LDL-uptake mediated by LDLR up to the cell-cell fusion in osteoclast-like cells *in vitro*.