Supplementary Material

**Supplementary Table S1.** **Sequences of the real-time qPCR primers.**

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| ***Genes*** | **Annealing T (°C)** | **Sequences** |
| *β2-microglobulin* | 60 | Fw: 5'-TGGTGCTTGTCTCACTGACC-3'  Rv: 5'-GTATGTTGGCTTCCCATTC-3' |
| *Cathepsin-k* | 55 | Fw: 5'-CCTCTCTTGGTCTCCATACA-3'  Rv: 5'-ATCTCTCTGTACCCTCTGCA-3' |
| *Ctr* | 60 | Fw: 5'-ACCGACGAGCAACGCCTACGC-3'  Rv: 5'-GCCTTCACAGCCTTCAGGTAC-3' |
| *Mmp-9* | 55 | Fw: 5'-CTGTCCAGACCAAGGGTACAGCCT-3'  Rv: 5'-GTGGTATAGTGGGACACATAGTGG-3' |
| *Nfatc1* | 60 | Fw: 5'-CATGCAGCCATCATCGA-3'  Rv: 5'-TGGGATGTGAACTCGGAAGAC-3' |
| *Pla2g1b* | 62 | Fw: 5'-CCCCAGTGGACGACTTAGACA-3'  Rv: 5'-TCCAGCTTCTTGGCCTGACT-3' |
| *Pla2g2a* | 57 | Fw: 5'-TACAAGCGCCTGGAGAAAAG-3'  Rv: 5'-TTATCGCACTGACACA-3' |
| *Pla2g2c* | 66 | Fw: 5'-TTGCCATCTTCCTTGTCTTCATC-3'  Rv: 5'-CATCCTCTGGAACTGCCAGAA-3' |
| *Pla2g2d* | 66 | Fw: 5'-ATCTCCCAGGGCACTATCCA-3'  Rv: 5'-CCTCCTTGTCACAAGCACACA-3' |
| *Pla2g2e* | 62 | Fw: 5'-CCCAAGCTGGAAAAGTACCTCTT-3'  Rv: 5'-CGCTGGCAAGCCGTTCT-3' |
| *Pla2g2f* | 66 | Fw: 5'-TCAGGGCCTCTCCCTCTAAAA-3'  Rv: 5'-GGACTGCGATGGCAAAGAAT-3' |
| *Pla2g3* | 64 | Fw: 5'-TGGCCCAAAACATCAAAGTG-3'  Rv: 5'-GGCTTGATCTGGTGCTCACA-3' |
| *Pla2g4a* | 57 | Fw: 5'-CAGCTCTCAGGATTCCTTCGA-3'  Rv: 5'-TCATATATTCGTTCAAATTCATCTGGAT-3' |
| *Pla2g5* | 58 | Fw: 5'-CCCCAAGGATGGCACTGAT-3'  Rv: 5'-GCACAGTCTTTTTCCTCCAGTTG-3' |
| *Pla2g6* | 62 | Fw: 5'-TCCATGAGTACAATCAGGACATGA-3'  Rv: 5'-AGAAACGACTATGGAGAGTTTCTTCAC-3' |
| *Pla2g10* | 58 | Fw: 5'-CGATCTCCGATGGCTTACAT-3'  Rv: 5'-TTGGCATTTGTTCTCTGCTG-3' |
| *Pla2g12a* | 62 | Fw: 5'-CGCTCGGACTATCTCAGAACGT-3'  Rv: 5'-AAATGGATGACGCTGTCAAAGA-3' |
| *Pla2g12b* | 64 | Fw: 5'-CTGGGCTTTGTCTCCAACGT-3'  Rv: 5'-GGTCCACACGGTGTTGAACA-3' |
| *Pla2g16* | 57 | Fw: 5'-TACAGGCTGACCAGCGAGAACT-3'  Rv: 5'-CCACTCCAGCGATGCCTACCG-3' |
| *Trap* | 55 | Fw: 5'-AAATCACTCTTTAAGACCAG-3'  Rv: 5'-TTATTGAATAGCAGTGACAG-3' |

Fw, forward; Rv, reverse

**Supplementary Table S2. Modulation of PLA2 mRNA levels upon sPLA2-IIA interference in RAW264.7 macrophages.** RAW264.7 cells were interfered with si‒NT or si‒sPLA2-IIA siRNAs for 72 h (see Section 2 for details). Then, the mRNA levels of the indicated PLA2s were measured, by real-time qPCR, and expressed as fold of *si‒NT RAW264.7* *cells* (italic numbers). Subsequently, interfered cells were treated without (w/o) or with 30 ng/mL RANKL (RANKL) for further 48-72 h, and the PLA2 levels were quantified and expressed as fold of si‒NT cells treated without RANKL (w/o si‒NT).

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| --- | --- | --- | --- | --- | --- | --- |
|  | ***RAW264.7 cells*** | | **w/o** | | **RANKL** | |
| **PLA2s** | *si‒NT* | *si‒sPLA2-IIA* | si‒NT | si‒sPLA2-IIA | si‒NT | si‒sPLA2-IIA |
| *Pla2g2a* | *1.00* | *0.48 ±0.01\*\*\** | 1.00 | 0.93 ±0.06 | 2.15 ±0.11 | 1.64 ±0.21§ |
| *Pla2g2c* | *1.00* | *1.04 ±0.05* | 1.00 | 1.11 ±0.08 | 2.94 ±0.38 | 3.36 ±0.55 |
| *Pla2g2e* | *1.00* | *1.11 ±0.04* | 1.00 | 1.24 ±0.15 | 4.28 ±1.00 | 4.89 ±0.31 |
| *Pla2g5* | *1.00* | *0.81 ±0.04\** | 1.00 | 1.07 ±0.21 | 3.61 ±0.14 | 3.00 ±0.22§ |
|  |  |  |  |  |  |  |
| *Pla2g4a* | *1.00* | *1.13 ±0.02* | 1.00 | 1.00 ±0.07 | 0.63 ±0.18 | 0.71 ±0.09 |
| *Pla2g6* | *1.00* | *1.13 ±0.01* | 1.00 | 1.05 ±0.04 | 0.60 ±0.04 | 0.56 ±0.07 |

Data of interfered cells are expressed as fold of *si‒NT* *RAW264.7 cells*, and are means ±SEM of at least three independent experiments. \**p* < 0.05; \*\*\**p* < 0.001 versus correspondent *si‒NT RAW264.7 cells,* by pairedStudent’s *t*-tests. Data of differentiated cells are expressed as fold of si-NT cells treated without RANKL (w/o si-NT), and are means ±SEM of at least three independent experiments. §*p* < 0.05 versus correspondent si‒NT cells treated with RANKL (RANKL), by one-way ANOVA.

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**Supplementary Figure S1. Secreted PLA2 activity measured in RAW264.7-cell lysates.** RAW264.7 cells were treated without (w/o) or with 15 ng/mL RANKL (R) for 72 h. Secreted PLA2 activity was measured in cell lysates (250 μg) in the presence of the indicated concentrations of LY311727 (LY) or with DMSO as vehicle (DMSO). **(A)** Absorbance-time graph of PLA2 activity. Data are means ± range of a representative experiment. **(B)** Dose-response curve of LY311727 inhibition of sPLA2 activity derived from the graph on the left, to extrapolate an apparent IC50 value.

**Supplementary Figure S2. Effects of PLA2 inhibitors on the osteoclast differentiation of RAW264.7 macrophages.** RAW264.7 cells were treated in the absence (w/o) or presence of 15-30 ng/mL RANKL for 72-96 h, with the indicated PLA2 inhibitors (20 μM Inhib-I, 1 μM BEL, 2 μM cPLA2–Inh., 20 μM MAFP) or with DMSO as carrier (‒). The differentiation markers were quantified by real-time qPCR, and normalized using *β2-microglobulin* expression, as the housekeeping gene. Data are means ± SEM of five independent experiments. \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.005 versus RANKL (paired Student’s *t*-tests).



**Supplementary Figure S3. Effects of PLA2 inhibitors on the osteoclast fusion of RAW264.7 macrophages.** RAW264.7 cells were treated in the absence (w/o) or presence of 15-30 ng/mL RANKL for 72-96 h, with the indicated PLA2 inhibitors (20 μM Inhib-I, 1 μM BEL, 2 μM cPLA2–Inh., 20 μM MAFP) or with DMSO as carrier. Osteoclast syncytium formation was determined as number of nuclei/cell, by fluorescence microscopy. Data are means ± SE of four independent experiments. \**p* < 0.05; \*\**p* < 0.01 versus corresponding RANKL (paired Student’s *t*-tests).



**Supplementary Figure S4. Osteoclasts from *Pla2g2a*-ko OCP show impaired *ex-vivo* osteoclastogenesis.** The M-CSF–expanded OCP were further treated with 20 ng/mL M-CSF alone (w/o) or with 20 ng/mL M-CSF and 2.5 ng/mL RANKL (RANKL) for 3-6 days for RNA extraction, or 4-5 days for immunofluorescence analysis. **(A)** The differentiation markers were quantified in RANKL-treated cells by real-time qPCR, and normalized using *β2-microglobulin* expression, as the housekeeping gene. Data are expressed as fold of differentiated wt OCP, and are shown as means ± SEM of ten age- and sex-matched mice for each genotype. **(B-C)** Osteoclast syncytium formation was determined as number of nuclei/cell, by fluorescence microscopy. Data are means ± SE of ten age- and sex-matched mice for each genotype. \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.005 versus correspondent RANKL wt (unpaired Student’s *t*-tests).



**Supplementary Figure S5. Inhibitors of sPLA2-IIA impair the RANKL-induced osteoclastogenesis of wt OCP.** The M-CSF–expanded OCP were further treated with 20 ng/mL M-CSF and 2.5 ng/mL RANKL, in presence of the indicated sPLA2-IIA inhibitors (20 μM Inhib-I; 40 μM KH064) or with DMSO as carrier (‒), for 6 days for RNA extraction, or 5 days for immunofluorescence analysis. **(A-E)** The differentiation markers were quantified by real-time qPCR, and normalized using *β2-microglobulin* expression, as the housekeeping gene. Data are expressed as fold of correspondent RANKL, and are means ± SEM of three age- and sex-matched mice for each genotype. The mRNA levels from RANKL *Pla2g2a*-ko, expressed as fold of RANKL wt, were: 0.09 ±0.01 for *Nfatc1*; 0.07 ±0.01 for *Cathepsin-k*; 0.12 ±0.03 for *Mmp-9*; 0.11 ±0.01 for *Trap*; 0.003 ±0.001 for *Ctr*. **(F-G)** Osteoclast syncytium formation was determined as number of nuclei/cell, under fluorescence microscopy. Data are means ± SE of three age- and sex-matched mice for each genotype. \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.005 versus RANKL wt (one-way ANOVA, **A-E**; or unpaired Student’s *t*-tests, **F-G**).





**Supplementary Figure S6. Inhibitors of sPLA2-IIA reduce the RANKL-induced formation of TRAP-positive osteoclasts from wt OPC.** The M-CSF–expanded OCP were further treated with 20 ng/mL M-CSF and 2.5 ng/mL RANKL, in presence of the indicated sPLA2-IIA inhibitors (20 μM Inhib-I; 40 μM KH064) or with DMSO as carrier (‒), for 5 days for TRAP staining. The quantification of TRAP-positive cells (TRAP+) is shown as means ± SEM of three age- and sex-matched mice for each genotype, analyzed in triplicates. \**p* < 0.05; \*\*\**p* < 0.005 versus DMSO (‒) wt (one-way ANOVA).

**Supplementary Figure S7. BPB selectively inhibits RANKL-induced marker transcription of RAW264.7 macrophages.** RAW264.7 cells were treated without (w/o) or with of 30 ng/mL RANKL for 72 h, in presence of 10 nM BPB or with DMSO as carrier (‒). **(A-B)** The differentiation markers were quantified by real-time qPCR, and normalized using *β2-microglobulin* expression, as the housekeeping gene. Data are expressed as fold of RANKL, and are means ± SEM of six independent experiments. **(C)** Osteoclast syncytium formation was determined as number of nuclei/cell, by fluorescence microscopy. Data are means ± SE of four independent experiments. \*\**p* < 0.01; \*\*\**p* < 0.005 versus correspondent RANKL (paired Student’s *t*-tests).



**Supplementary Figure S8. SB203580 selectively inhibits RANKL-induced osteoclast fusion of murine primary precursors.** The M-CSF–expanded OCP were further treated with 20 ng/mL M-CSF and 2.5 ng/mL RANKL, in presence of 1 µM SB203580 or with DMSO as carrier (‒), for 4 days for RNA extraction, or 6 days for TRAP staining. **(A-E)** The differentiation markers were quantified by real-time qPCR, and normalized using *β2-microglobulin* expression, as the housekeeping gene. Data are expressed as fold of correspondent RANKL, and are means ± SEM of three age- and sex-matched mice for each genotype. **(F)** The quantification of TRAP-positive cells (TRAP+) is shown as means ± SEM of five age- and sex-matched mice for each genotype, performed in triplicates. \**p* < 0.05; \*\*\**p* < 0.005 versus correspondent DMSO (one-way ANOVA).

