Supplemental Experimental Procedures and figures

Monoclonal antibodies, flow cytometry and cell sorting

For mouse experiments, we used the following monoclonal antibodies: FITC-conjugated anti-F4/80 (clone BM8, eBioscience), PE-conjugated anti-F4/80 (clone BM8, BioLegend), PE-conjugated anti-MHC class II (clone M5/114.15.2, BD Biosciences), PerCP-Cy5.5 conjugated anti-CD80 (clone 16-10A1, BD Biosciences), PE-Cy7-conjugated anti-Ly6C (clone AL-21, BD Biosciences), APC-conjugated anti-CD115 (clone AFS98, BioLegend), APC-Cy7-conjugated anti-CD11b (clone M1/70 BioLegend), V450-conjugated anti-Ly6G (clone 1A8, BD Biosciences). Antibodies for human PBMCs were purchased from BD Biosciences. We used FITC-conjugated anti-HLA-DR (clone TU36), FITC-conjugated anti-CD3 (clone UCHT1), PE-conjugated anti-CD2 (clone RPA-2.10), PE-conjugated anti-CD56 (clone B159), PE-conjugated anti-CD19 (clone HIB19), PE-Cy7-conjugated anti-CD14 (clone M5E2), APC-conjugated anti-CD16 (clone B73.1), APC-Cy7-conjugated anti-CD16 (clone 3G8) and V450-conjugated anti-CD19 (clone HIB19). Different cell types analyzed in supplementary figure 2 were isolated from fresh whole blood samples of active RA patients (inclusion criteria: ACR>4; DAS28>4; no Prednisolone). Briefly, blood samples were lysed with EL buffer (Qiagen, Hilden, Germany) at 4°C according to the instruction of the manufacturer. Granulocytes and B-lymphocytes were isolated separately using CD15 and CD19 MicroBeads, respectively (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany), and the automated separation system Auto-MACS (Miltenyi Biotec). CD15-depleted cell fractions were used to stain for NK cells (CD56-PE, clone B159; BD Biosciences), T cells (CD3-APC, clone UCHT1; BD Biosciences), T helper cells (CD4-PE-Cy5, RPA-T4; BD Biosciences). Propidium iodide was used to exclude dead cells. Using a

FACSAria cell sorter (BD Biosciences), NK and CD4 cells were isolated in parallel with purities and viabilities of >99%. After sorting, cells were centrifuged and pellets were lyzed in RLT-buffer (Qiagen) containing 1% mercaptoethanol. Lysates were stored at -80°C. The Ethics Committee of the Medical Faculty of Charité Universitätsmedizin Berlin approved the study.

Microarray hybridization and data analysis

RNA quality control was ensured with Bioanalyzer (Agilent Technologies) and RNA quantification with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The generation of biotinylated anti-sense cRNA was performed with 100 ng of total RNA using the Affymetrix IVT Express protocol (3' IVT Express 2010 technical manual P/N 702646 Rev8). Following fragmentation 15 µg of cRNA were hybridized for 16 h at 45 °C on GeneChip Mouse 430_2 arrays in the Affymetrix oven 645. GeneChips were washed and stained in the Affymetrix Fluidic station 450 with HWS kit. Chips were scanned using the Affymetrix GeneChip scanner 3000 7G and the data were generated with the Affymetrix Expression Console software v 1.2.1. Data were analyzed using BioRetis database (http://www.bioretis-analysis.de) with High Performance Chip Data Analysis (HPCDA). The differentially expressed probe-sets were filtered with the default query parameters as described in more detail {Biesen, 2008, Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus}. Shortly, these parameters use a Signal threshold, the detection p-value, and the present call to detect the present genes. The normally default used Welch t tests with the log 2 of fold changes (SLRs) could not be used in these one vs. one chip analyses, therefore the Increase/Decrease change call, calculated by Affymetrix GCOS software (GeneChips) with a non-parametrical test was the only

possibility to obtain significant differentially expressed lists of present genes. Nevertheless in one vs. one chip analyses it is much more unreliable to get a correct list of significant genes. Therefore we reduced the gene list, obtained with BioRetis default parameters with a fold change (FC) and an extended Signal threshold or difference as shown in detail at DOI: 10.13140/RG.2.1.2547.5923 (also shown are results of all other comparisons BTC251-256). For hierarchical cluster analysis, we used the program Genes@Work {Califano, 2000, Analysis of gene expression microarrays for phenotype classification} with gene vectors for normalization, Pearson w/mean for similarity measure and center of mass as cluster type. The chip data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number (GSE77159).

Experimental osteoporosis model

After body weight measurements, 8-weeks old C57BL/6 female mice were anesthetized and underwent bilateral ovariectomy via the dorsal approach. Sham-operated mice had the ovaries exteriorized but not removed. Uterine weight was determined at sacrifice to verify the successful removal of the ovaries. Mice were subjected to microtomography analysis until day 16.

1. Biesen R, Demir C, Barkhudarova F, et al. Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus. Arthritis Rheum. 2008; 58: 1136-45.

2. Califano A, Stolovitzky G, Tu Y. Analysis of gene expression microarrays for phenotype classification. Proc Int Conf Intell Syst Mol Biol. 2000; 8: 75-85.

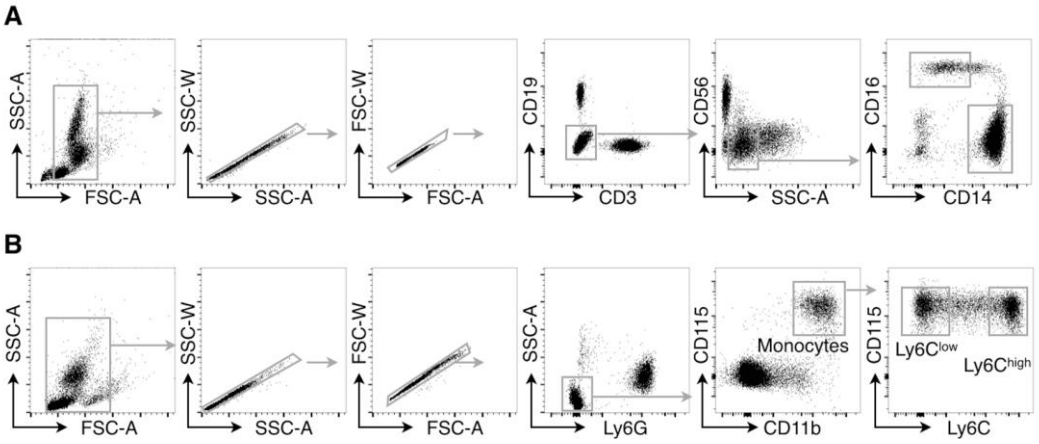

Figure S1. Gating strategy for flow cytometry analyses of circulating monocytes. Representative gating strategies for FACS staining of human (A) and mouse (B) monocyte subsets from the blood are shown. Cells were first gated for singlets. (**A**) Human monocytes were $CD19^{-}$, $CD3^{-}$, $CD56^{-}$, SSC^{low}. Classical and non-classical monocytes were further defined as $CD14^{high}CD16^{low}$ and $CD14^{low}CD16^{high}$, respectively. (**B**) Mouse monocyte subsets were defined as $Ly6G^{-}$, SSC^{low}, $CD11b^{+}$, $CD115^{+}$, and $Ly6C^{high}$ or $Ly6C^{low}$.

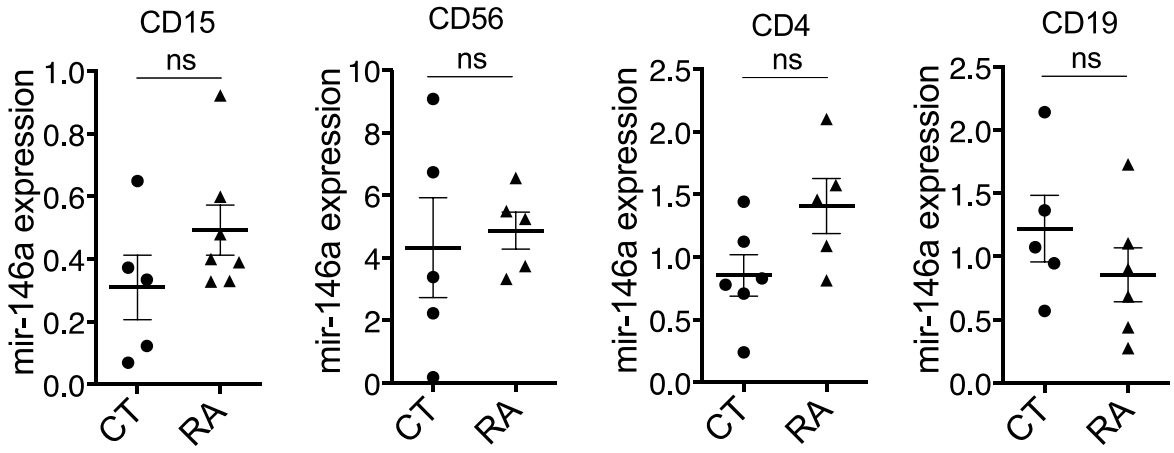

Figure S2. miR-146a expression levels in human systemic subsets cells in healthy or RA donors. Different immune cell types were FACS sorted from the blood of either healthy donors (CT, black circles) or patients with rheumatoid arthritis (RA, black triangles). Relative miR-146a expression was quantified using RT-qPCR in granulocytes (CD15), NK cells (CD56), T helper cells (CD4) and B cells (CD19). Data are presented as mean ± SEM of 3-8 biological replicates corresponding to either individual (human). Two-tailed Mann-Whitney test was used. ns: not significant.

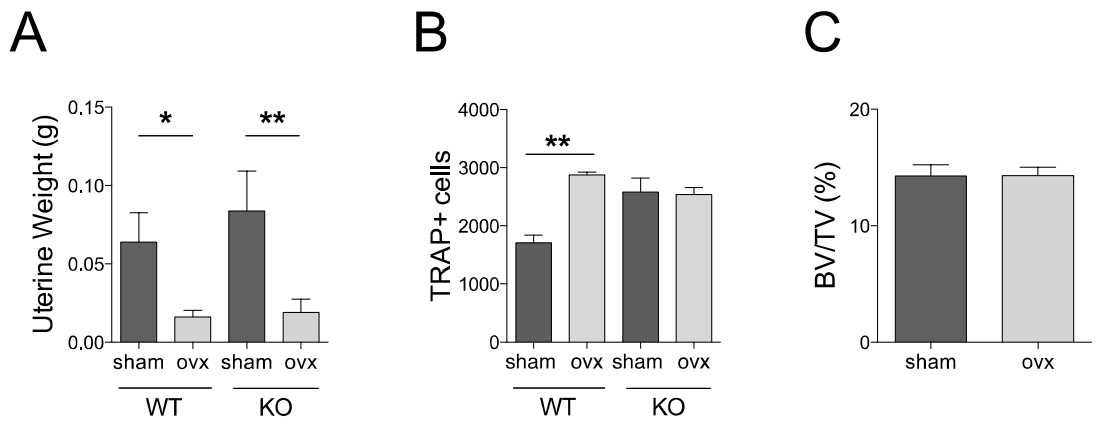

Figure S3. miR-146a deficiency presents no impact in a non-inflammatory model of bone remodeling. miR-146a$^{-/-}$ (KO) mice and wild type littermates (WT) were ovariectomized (OVX), and sacrificed at 16 days post-ovariectomy (n=5-7 mice/group). Controls were sham-ovariectomized. Uterine were weighted at sacrifice for OVX control quality (**A**). CD11b^{+} splenic precursors were isolated at sacrifice and cultivated in osteoclast differentiation medium. TRAP-positive multinucleated cell count was performed at 7 days after RANKL stimulation (**B**). Micro-computed tomography analysis was performed at day 15 for 3-dimensional reconstruction of the ankle bones and quantification of trabecular micro-architecture of proximal femur bone, here represented bone volume parameter (**C**) * $p<0.05$, ** $p<0.01$, ordinary one-way ANOVA t-test.

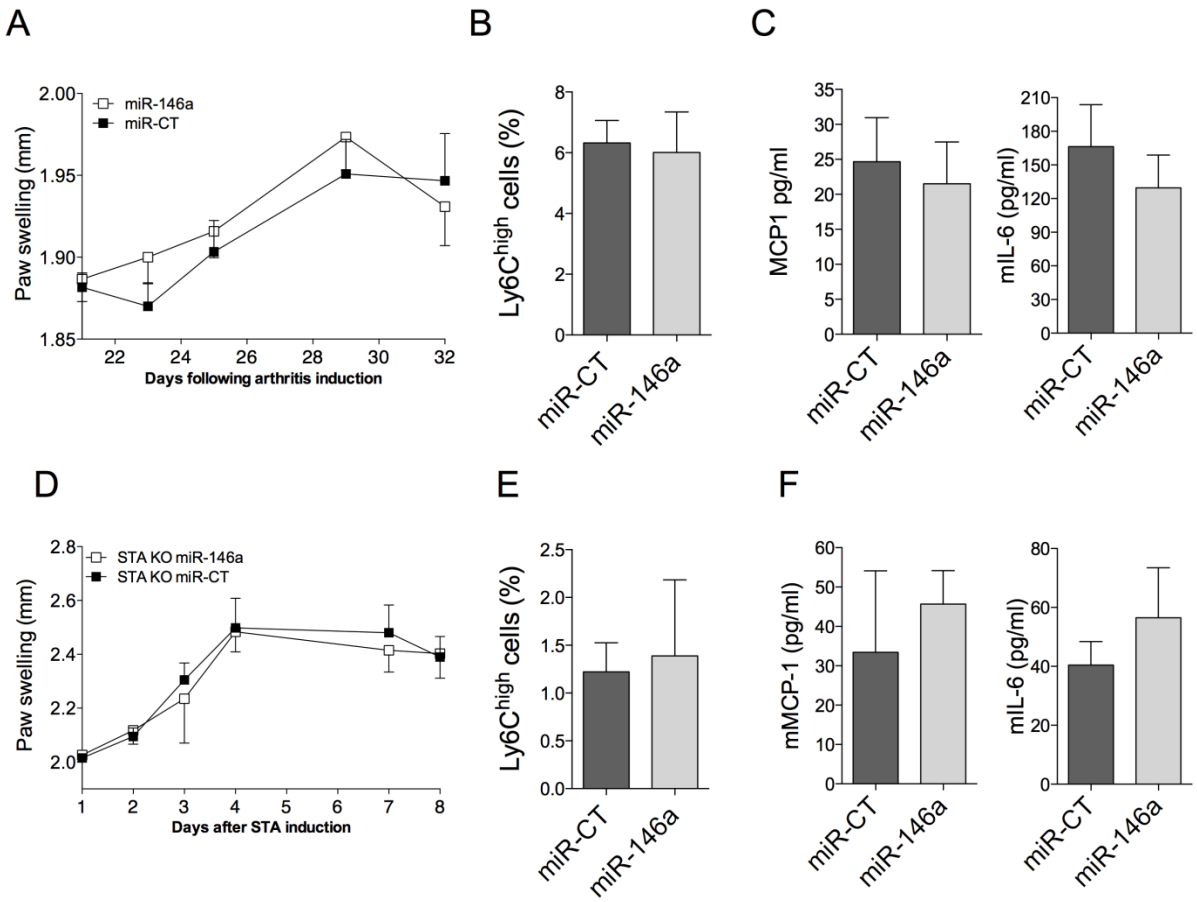

Figure S4. Enforced expression of miR-146a in Ly6C^{high} monocytes was not effective in reducing arthritis severity and inflammation. DBA/1 mice were immunized with bovine type II collagen and boosted on day 21 to induce collagen-induced arthritis (CIA) (A-C). miR-146a$^{-/-}$ (KO) mice were injected i.p. on day 0 and 1 with K/BxN serum to induce serum-transfer arthritis (STA) (D-F). The DMAPAP/DOPE cationic liposome was formulated with either miR-146a mimics (miR-146a) or non-targeting miRNA mimics (miR-CT) and injected (0.5mg/kg) thrice at D19, D25 and D32 for CIA, and twice at D4 and D7 for STA arthritis. Mice were sacrificed 16 or 11 days after the first injection (n=3-4 mice/group) for CIA and STA respectively. Arthritis severity was monitored for CIA (**A**) and STA (**D**), number of Ly6C^{high} monocytes was monitored by FACS for CIA (**B**) and STA (**E**), and pro-inflammatory cytokine MCP1 and IL-6 for CIA (**C**) and STA (**F**). Differences between groups were compared using Mann-Whitney t-test.

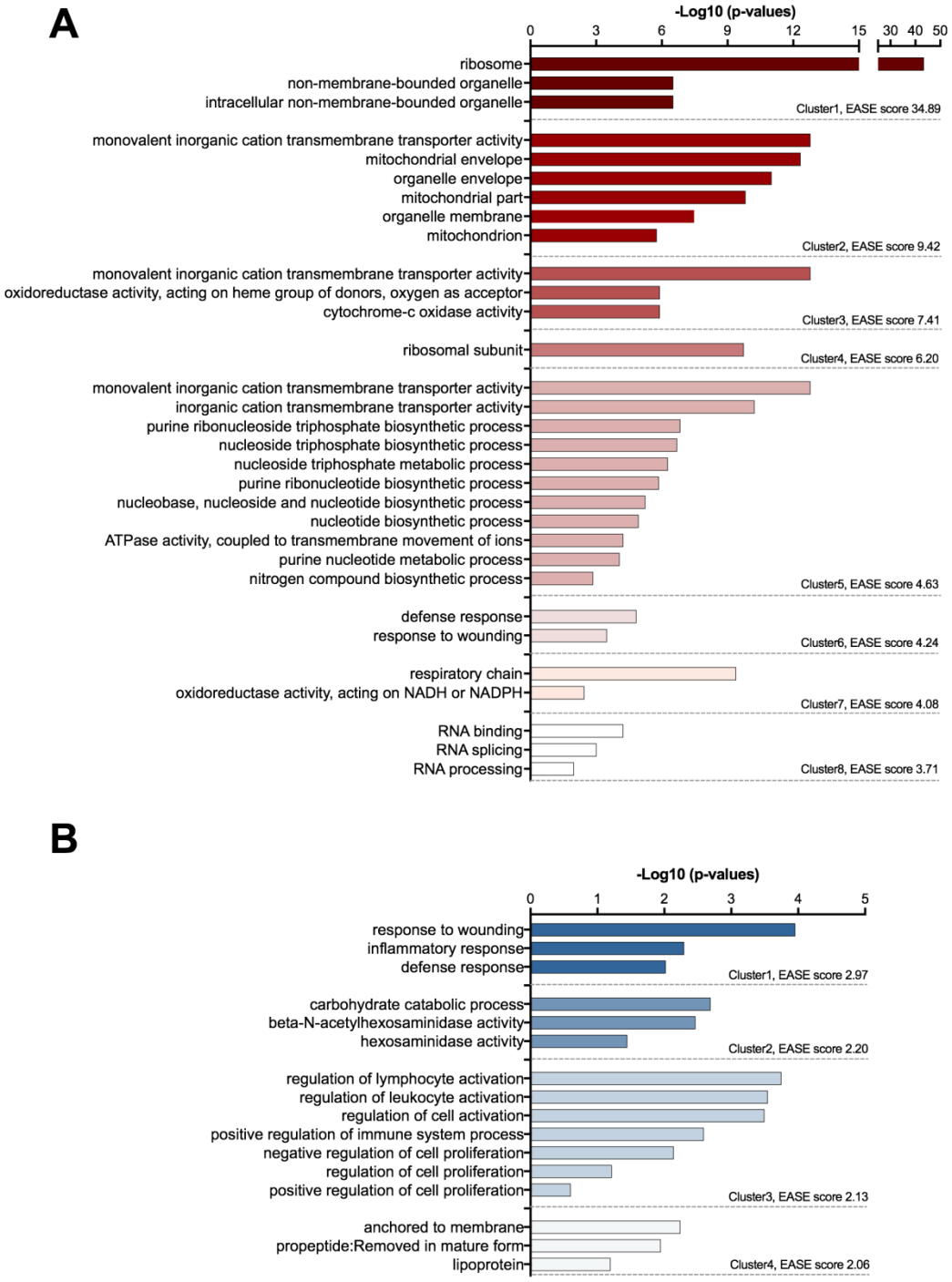

Figure S5 : Gene ontology (GO) categories enriched among the genes deregulated in miR-146a KO versus WT Ly6C^{high} and Ly6C^{low} monocytes. Biological processes that are modulated in connection with miR-146a KO in Ly6C^{high} and Ly6C^{low} subsets were identified using the annotation tool DAVID and related processes clustered using the functional annotation clustering tool. (**A**) Processes in Ly6C^{high} miR-146a KO versus WT with the highest enrichment scores included items related to ribosome, mitochondria, nucleotide process, defense response and RNA processing. (**B**) Processes in Ly6C^{low} miR-146a KO versus WT regulated gene clusters with highest enrichment scores were related to inflammatory response, carbohydrate catabolic process, regulation of cell activation and proliferation and lipoprotein.

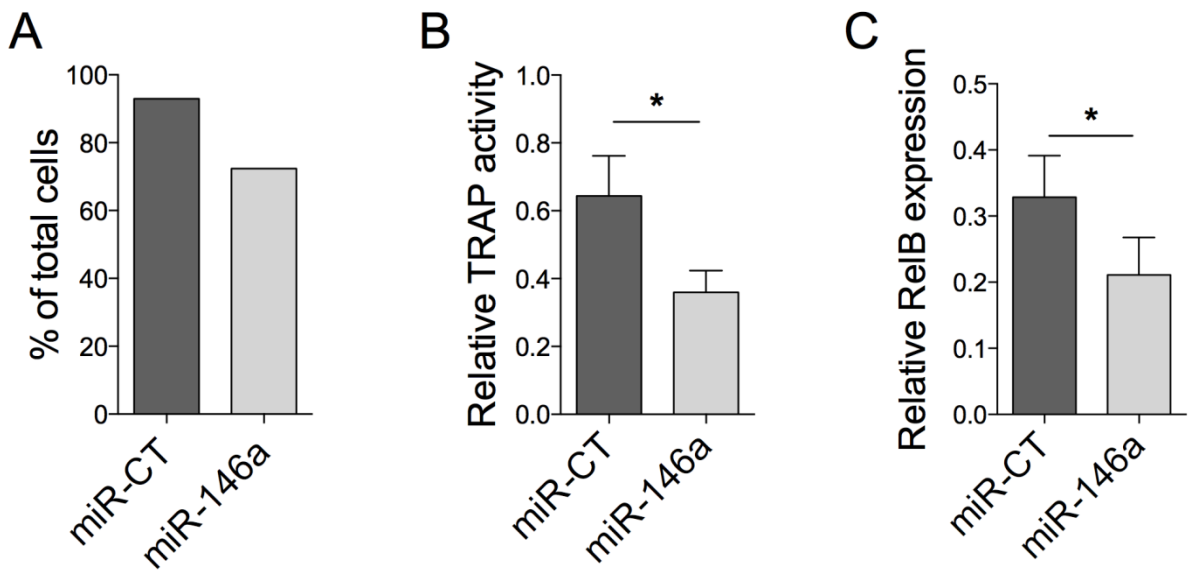

Figure S6. Enforced expression of miR-146a either in vivo in Ly6C^{high} monocytes or ex vivo in bone marrow OCP decreased osteoclast formation. Bone marrow osteoclast precursors from collagen-induced arthritic mice were either transfected in vivo (**A**) or ex vivo (**B**) with miR-146a (grey bar) or control (black bar) mimic-containing lipoplexes, and cultivated in osteoclast differentiation medium for 7 days. Number of TRAP-positive multinucleated osteoclasts was quantified using automated fluorescence imaging (n=20 areas/well). (**C**) Expression levels of *RelB* were quantified in bone marrow precursors from miR-146a$^{-/-}$ (KO) with miR-146a (grey bar) or control miRNA (black bar) mimic-containing lipoplexes transfected in vivo. Data are presented as mean ± SEM and differences between groups were compared using Mann-Whitney t-test. * $p<0.05$.