Title: Flrt2 is involved in fine-tuning of osteoclast multinucleation

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Supplementary figure 1

Cloning of Flrt2 as a novel gene involved in late-stage osteoclast differentiation. Mouse bone marrow-derived monocytes were cultured with either MCSF + RANKL to differentiate into osteoclasts, or IL-4 + IL-3 to generate multinuclear giant cells (MGCs) which also undergo cell fusion, and gene expression was assessed in mononuclear macrophages (BMM2N) versus multinucleation-committed tetraploid osteoclasts (OC4N), or tetraploid MGCs (MGC4N). Because MGC fusion is dependent on STAT6-mediated IL-4 signaling, we also prepared Stat6−/− monocytes (Stat6−/−BMM2N) and cells cultured with IL-3 + IL-4 (Stat6−/−MGC2N) to filter out potentially cell fusion-independent MGC-associated genes. As a result, we identified the gene Flrt2. Monocytes (2N) and multinucleation-committed cells (4N) were sorted and total RNAs were isolated. Relative expression levels of Flrt2 in monocytes and multinucleation-committed cells are shown as mean ± SD of three independent experiments.
Supplementary figure 2

Western blot analysis of Flrt2 in lec-SUP and lec-WCL after treatment with Brefeldin A (BFA). Flrt2<sup>fl/fl</sup> BMMs cultured with M-CSF + RANKL for two days were treated with or without BFA (1 µg/ml) for 8 hrs, WCL and SUP were harvested, and pulled-down with Lectin. Western blot was performed using anti-Flrt2 antibody.
Supplementary figure 3

Total RNAs were isolated from Flrt2\textsuperscript{fl/fl} and Flrt2\textsuperscript{fl/fl} Mx1Cre BMMs cultured with M-CSF + RANKL for the indicated days, and expression of Flrt3 message was quantified by Q-PCR (left). Flrt2\textsuperscript{fl/fl} and Flrt2\textsuperscript{fl/fl} Mx1Cre BMMs retrovirally transduced with shFlrt3 or control vector were cultured with M-CSF + RANKL for three days, and knockdown of Flrt3 was determined by Q-PCR (middle). Cells were stained for TRAP. Frequency of TRAP\(^+\) hyper-multinucleated cells is shown (right). Scale bars represents 100 \(\mu\)m. Data are shown as mean \(\pm\) S.D. *; \(p<0.05\), **; \(p<0.01\), ***; \(p<0.001\).