Expanded View Figures

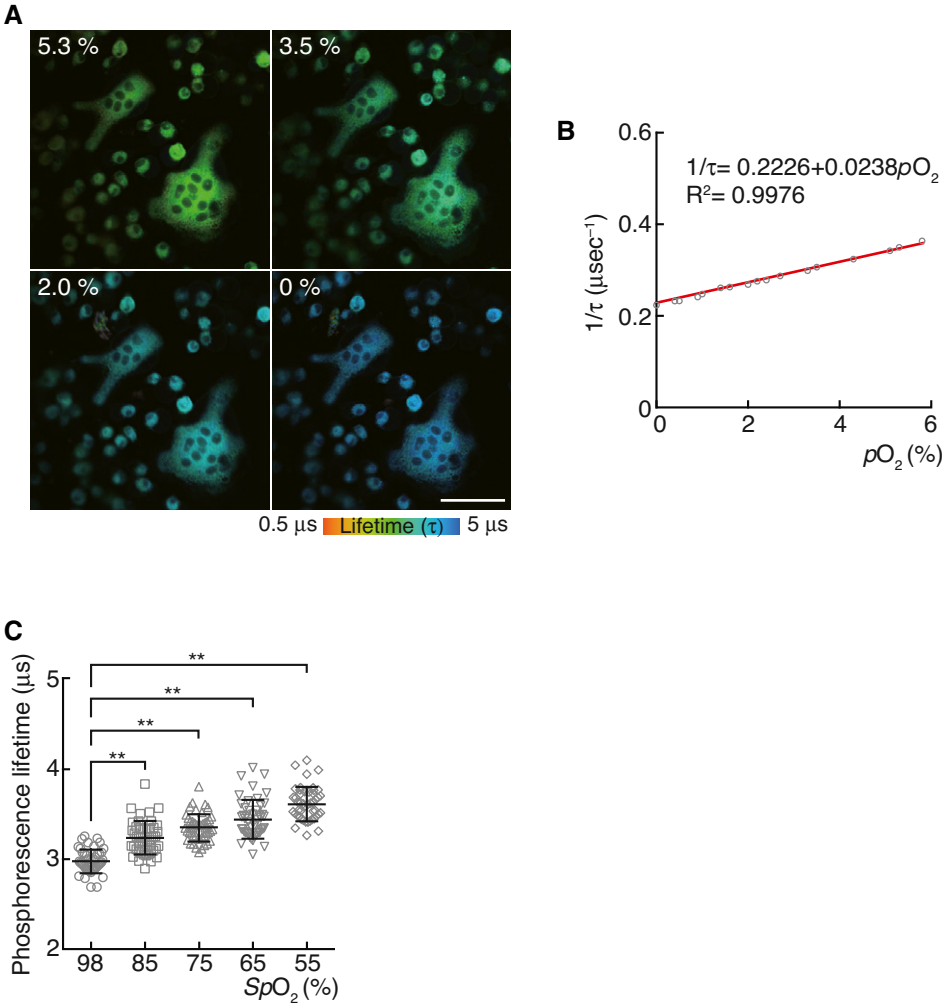

Figure EV1. Reciprocal plot of phosphorescence lifetime and oxygen concentration.

A PLIM images of *in vitro*-cultured osteoclasts under different conditions of oxygen concentration. Scale, 50 µm.

B The phosphorescence quenching due to dissolved oxygen in solution can be examined by the Stern–Volmer equation. The approximate line was constructed by a straight-line approximation, and an approximation formula and the coefficient of determination are shown.

C Phosphorescence lifetime in each osteoclast of the calvarial bone marrow of mice upon inhalation of normal (21% pO_2) and hypoxic (14% pO_2) air. The phosphorescence lifetime of each osteoclast was plotted (right, $n = 47$ from three mice for each SpO_2). Data denote mean ± s.e.m. $^{**}P < 0.01$ (ANOVA).

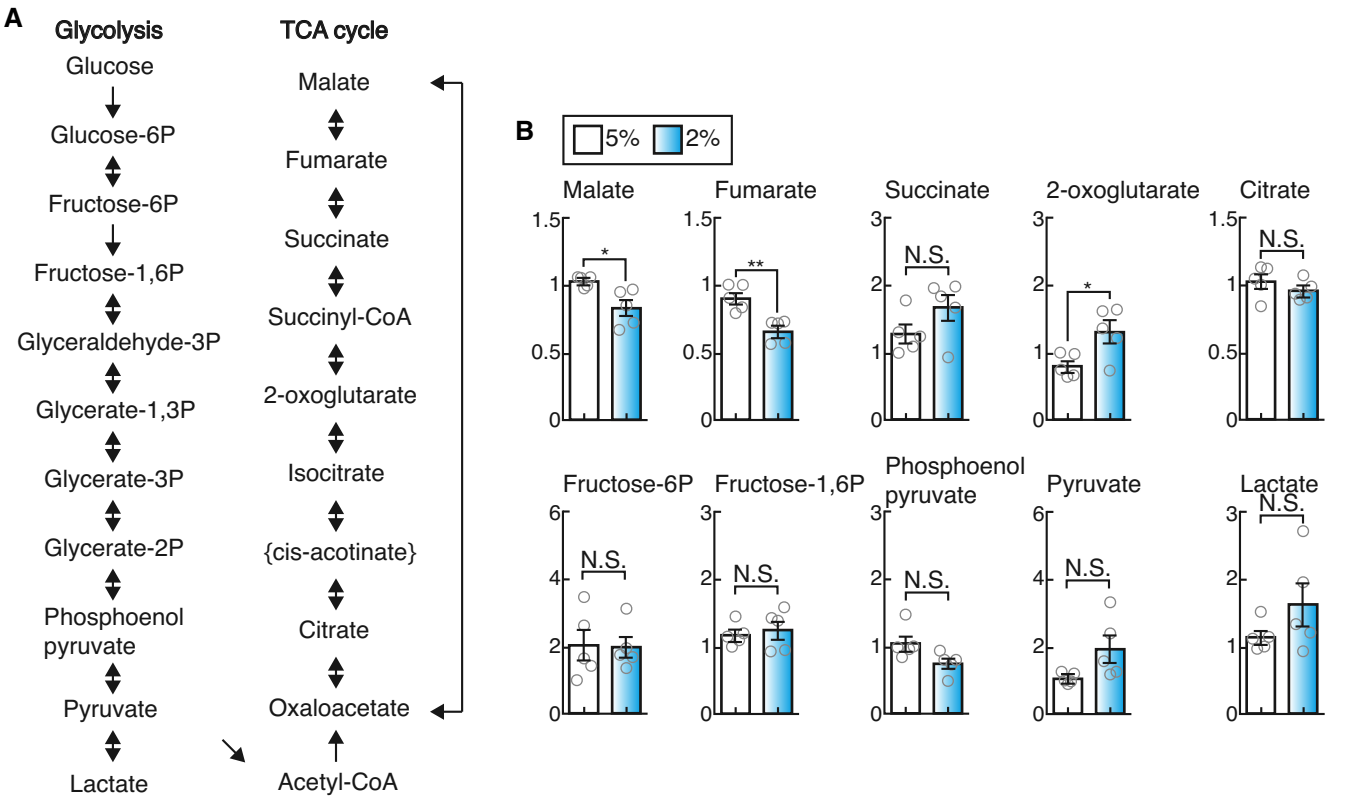

Figure EV2. Effect of physioxia and hypoxia on the levels of metabolites.

A Metabolites involved in glycolysis and the TCA cycle.

B Levels of metabolites involved in glycolysis and the TCA cycle. BMMs were cultured with 50 ng/ml RANKL in the presence of 10 ng/ml M-CSF for 2 days under 5% and 2% oxygen. Data denote mean ± s.e.m. *$P < 0.05$; **$P < 0.01$; NS, not significant ($n = 5$ biological replicates; t-test).

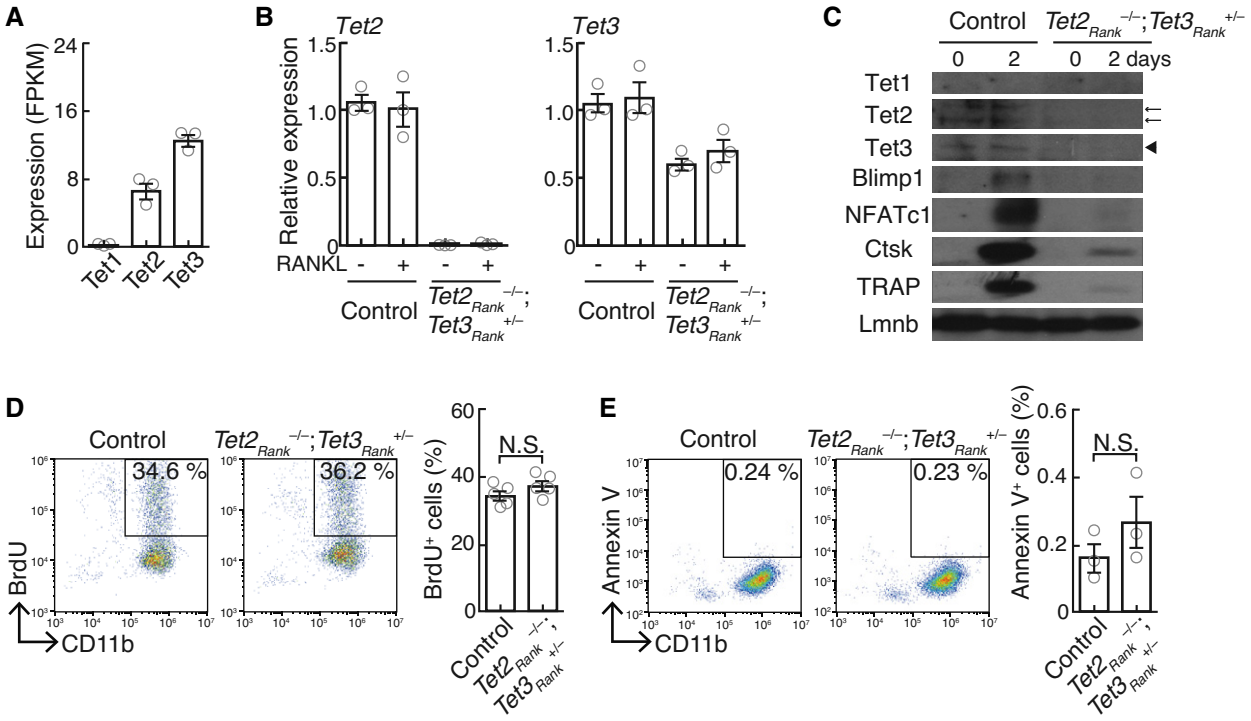

Figure EV3. Loss-of-function effect of *Tet2* and *Tet3* on cell proliferation, survival, and differentiation.

A mRNA expression of *Tet1*, *Tet2*, and *Tet3* in BMMs stimulated with RANKL for 0 and 2 days (RNA-seq analysis; $n = 3$ biological replicates). Data denote mean ± s.e.m.

B mRNA expression of *Tet2* and *Tet3* in control- and $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{+/-}$-derived BMMs cultured in the absence and presence of RANKL for 2 days (quantitative RT–PCR analysis; $n = 3$ biological replicates). Data denote mean ± s.e.m.

C Protein expression in control- and $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{+/-}$-derived BMMs cultured in the absence and presence of RANKL for 2 days. Arrows and arrowhead indicate Tet2 and Tet3 proteins, respectively.

D, E Percentage of BrdU-labeled CD11b$^+$ BMMs (D) and Annexin V$^+$ CD11b$^+$ BMMs (E) derived from control- and $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{+/-}$ mice. Data denote mean ± s.e.m. NS, not significant ($n = 5$ (D) and $n = 3$ (E) biological replicates; *t*-test).

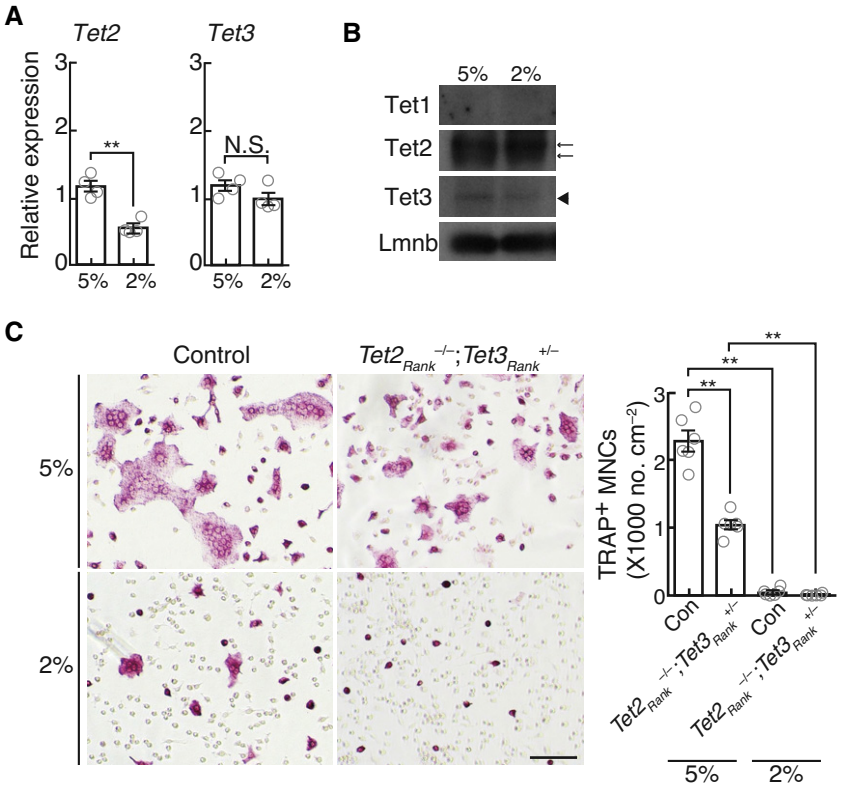

Figure EV4. Effect of hypoxia on the expression of *Tet2* and *Tet3*, and osteoclast differentiation.

A mRNA expression of *Tet2* and *Tet3* in BMMs stimulated with RANKL for 2 days under 5% and 2% oxygen. Data denote mean ± s.e.m. $^{**}P < 0.01$; NS, not significant ($n = 4$ biological replicates; *t*-test).

B Protein expression of Tet1, Tet2, and Tet3 in BMMs stimulated with RANKL for 2 days under 5% and 2% oxygen. Arrows and arrowhead indicate Tet2 and Tet3 proteins, respectively.

C Effect of *hypoxia* on osteoclastogenesis. TRAP-stained cells (left panel) and the number of TRAP-positive cells with more than three nuclei (right). Scale bar, 100 μm. Data denote mean ± s.e.m. $^{**}P < 0.01$ ($n = 6$ biological replicates; ANOVA).

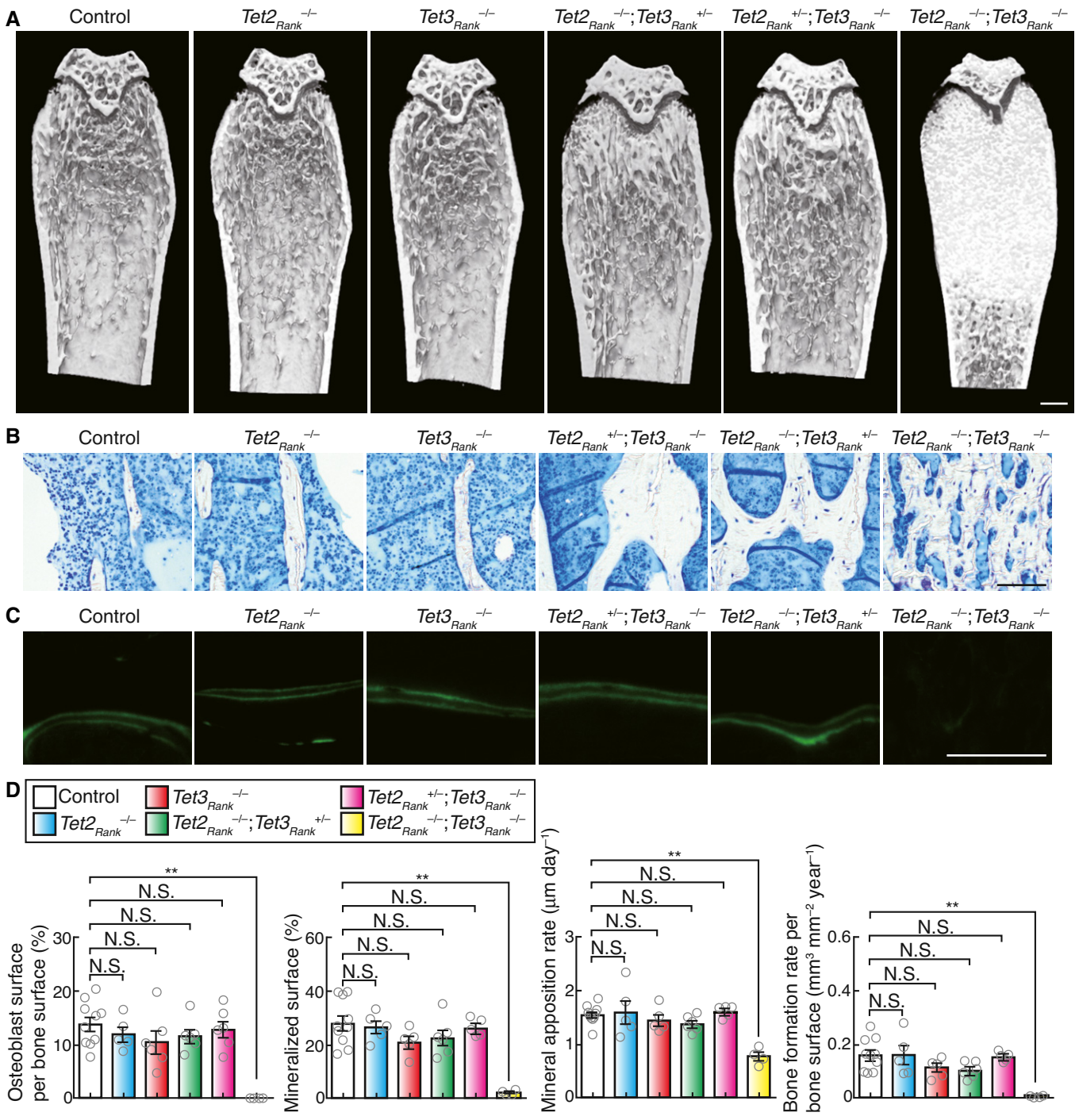

Figure EV5. Bone phenotype of osteoclast-specific *Tet2*- and *Tet3*-deficient mice.

A μCT analysis of the femurs from 10-week-old control, $Tet2_{Rank}^{-/-}$, $Tet3_{Rank}^{-/-}$, $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{+/-}$, $Tet2_{Rank}^{+/-}$; $Tet3a_{Rank}^{-/-}$ and $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{-/-}$ male mice (longitudinal view of the metaphyseal region). Scale, 0.5 mm.

B, C Histological analysis of the proximal tibias of 10-week-old control, $Tet2_{Rank}^{-/-}$, $Tet3_{Rank}^{-/-}$, $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{+/-}$, $Tet2_{Rank}^{+/-}$; $Tet3a_{Rank}^{-/-}$ and $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{-/-}$ male mice (C, toluidine blue staining; D, calcein labeling). Scale, 100 μm.

D Osteoblastic parameters of osteoclast-specific *Tet2*- and *Tet3*-deficient mice. Bone morphometric analysis of 10-week-old control ($n = 10$), $Tet2_{Rank}^{-/-}$ ($n = 5$), $Tet3_{Rank}^{-/-}$ ($n = 6$), $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{+/-}$ ($n = 6$), $Tet2_{Rank}^{+/-}$; $Tet3a_{Rank}^{-/-}$ ($n = 6$), and $Tet2_{Rank}^{-/-}$; $Tet3a_{Rank}^{-/-}$ ($n = 4$) male mice was performed. Data denote mean ± s.e.m. $^{**}P < 0.01$; NS, not significant (ANOVA).