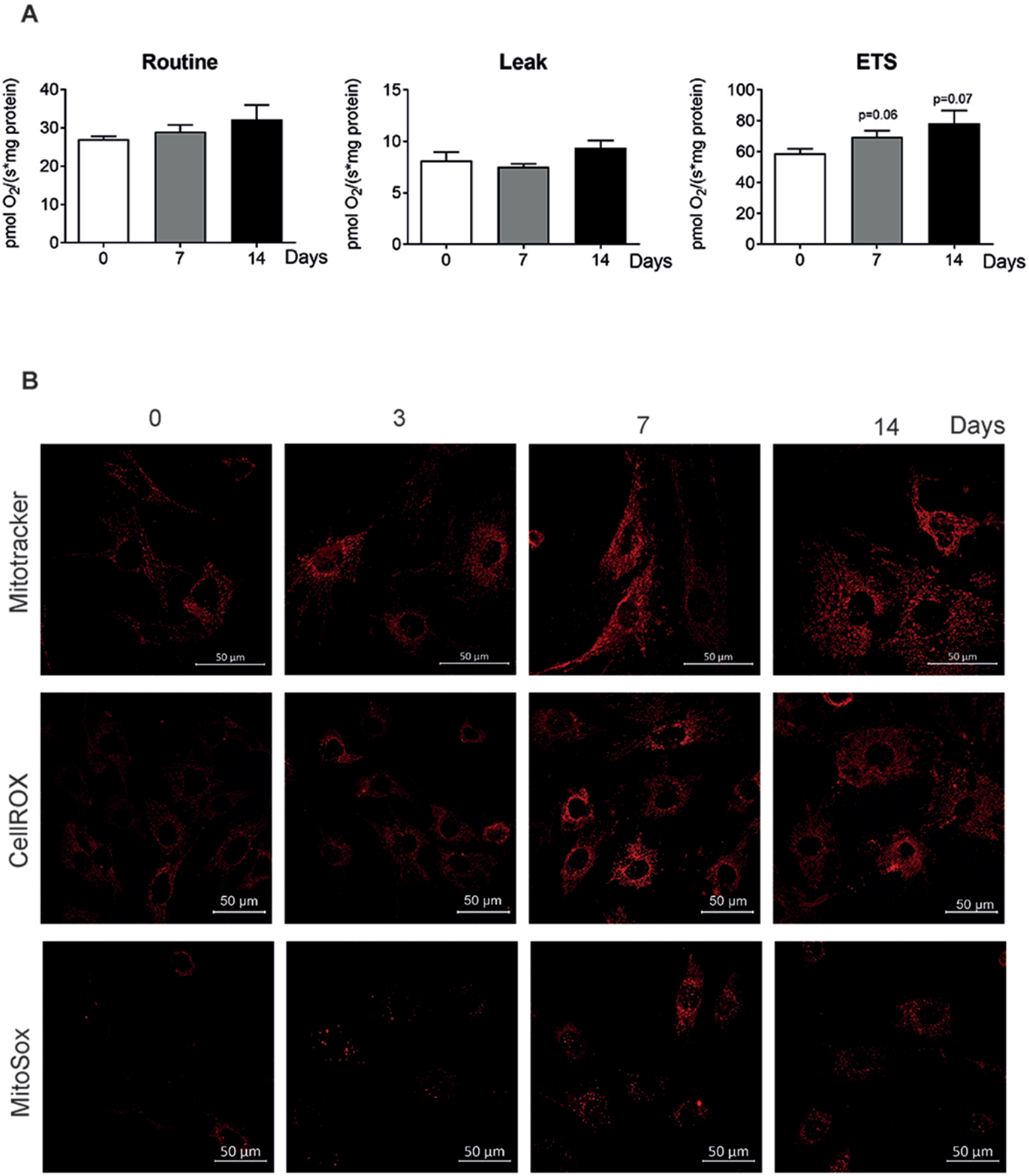
Supplementary Figure 1



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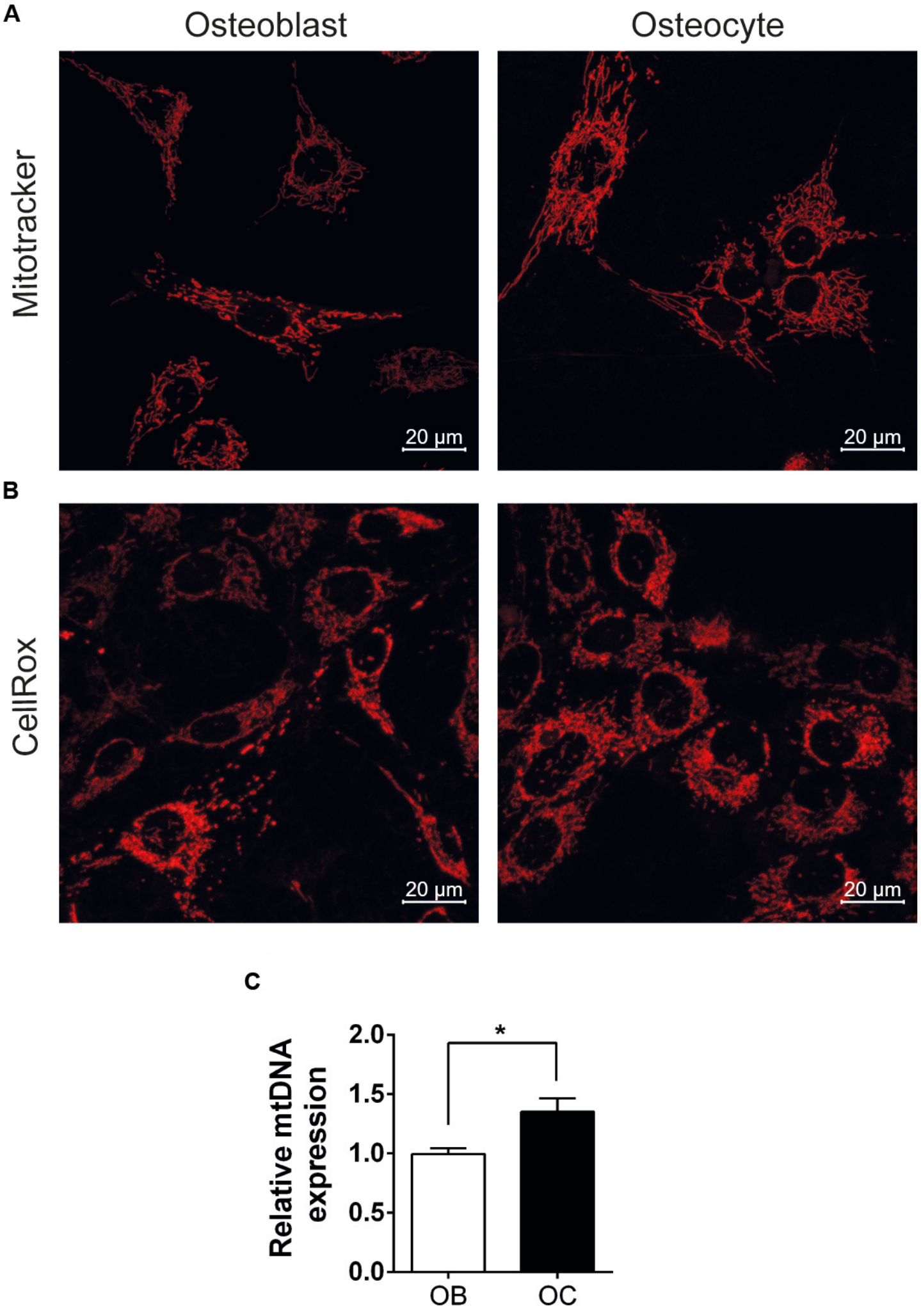

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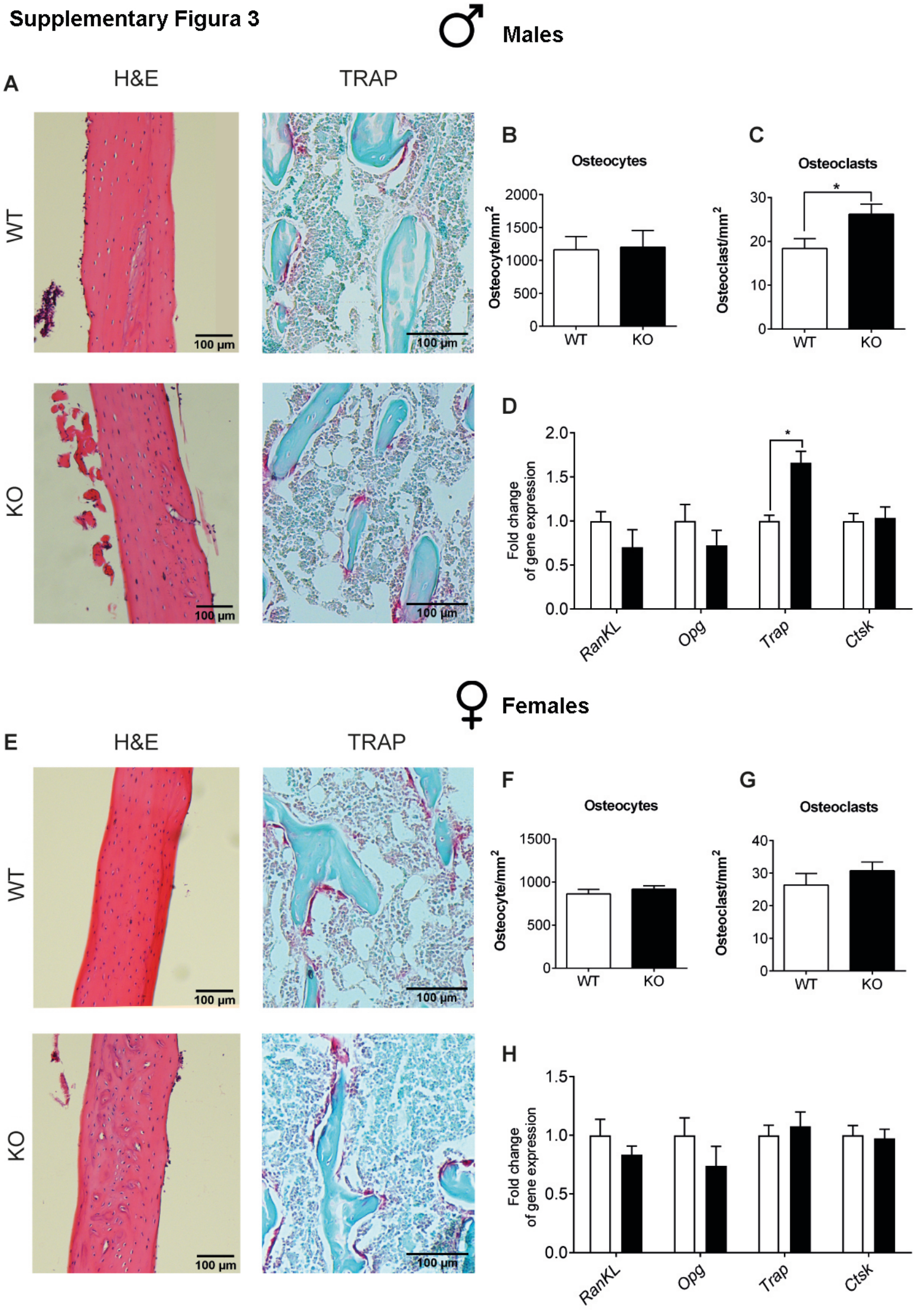

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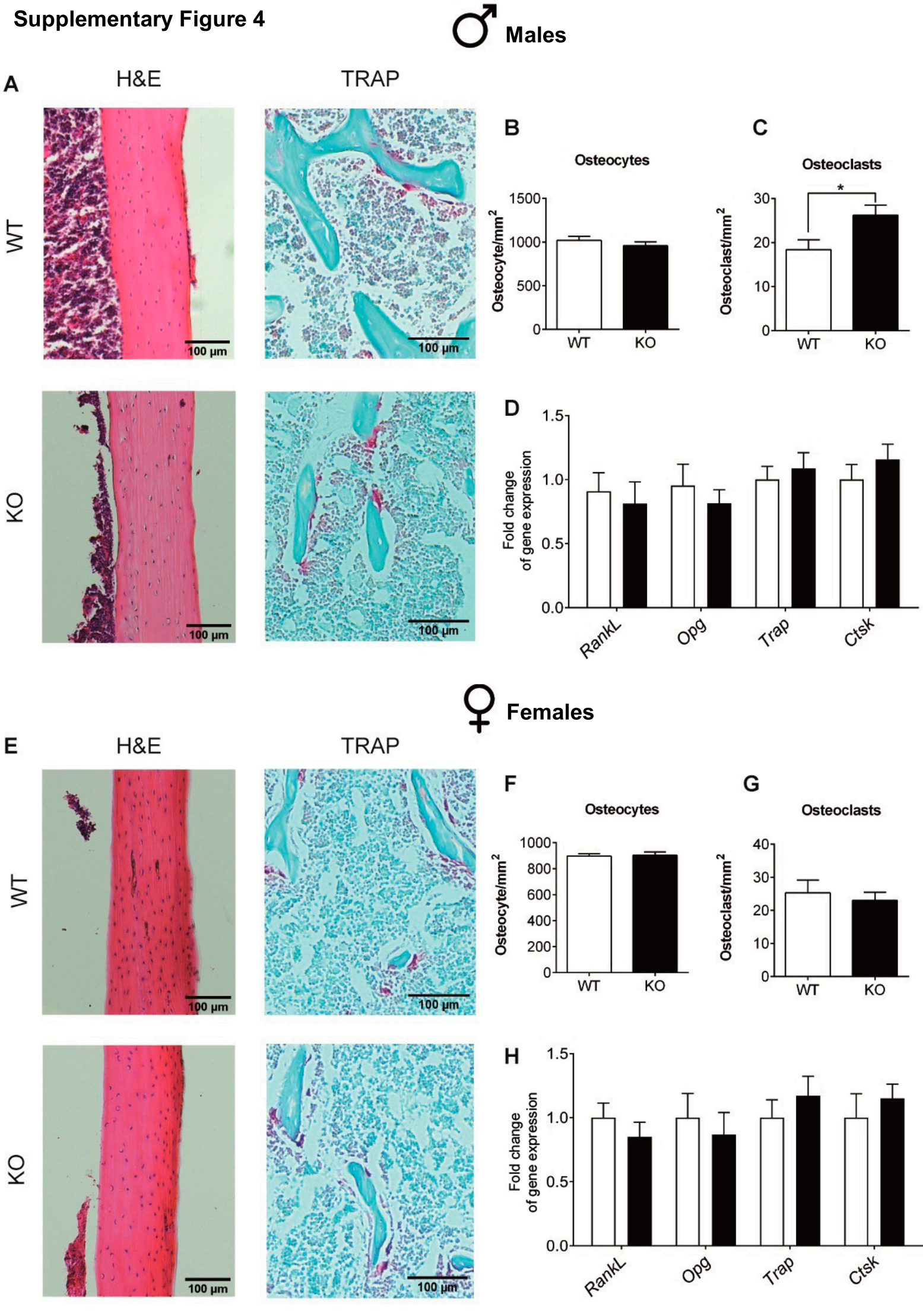

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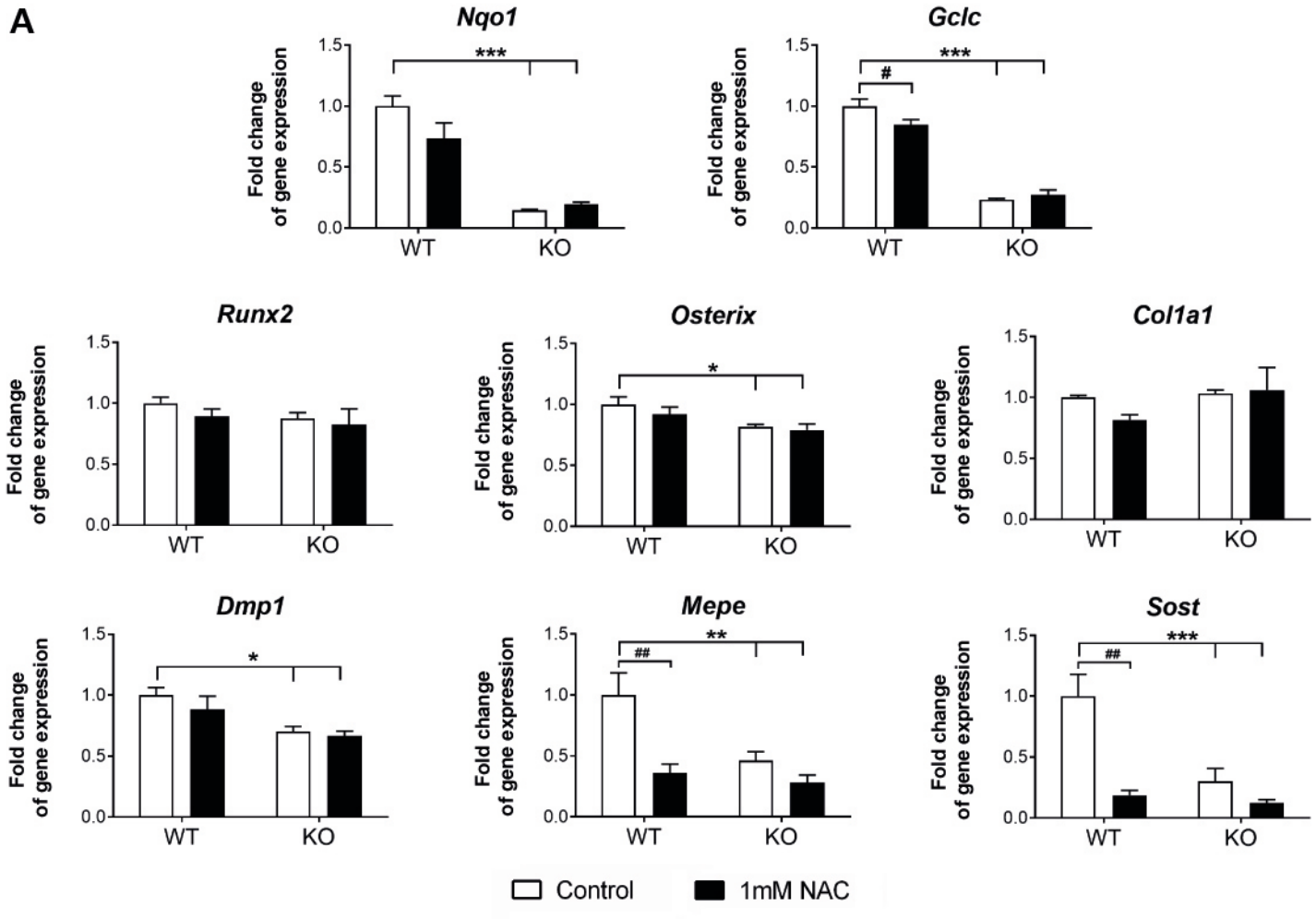

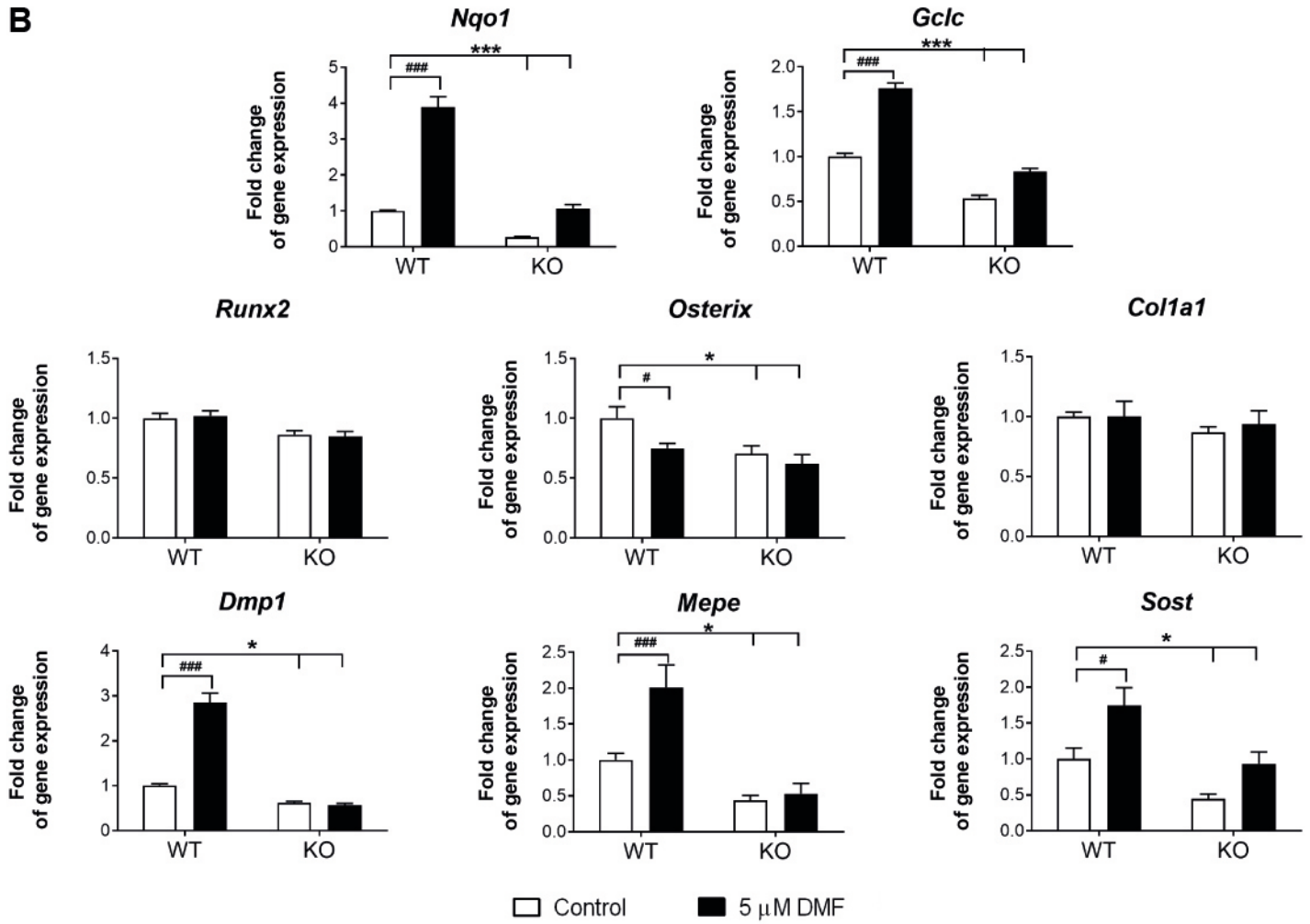

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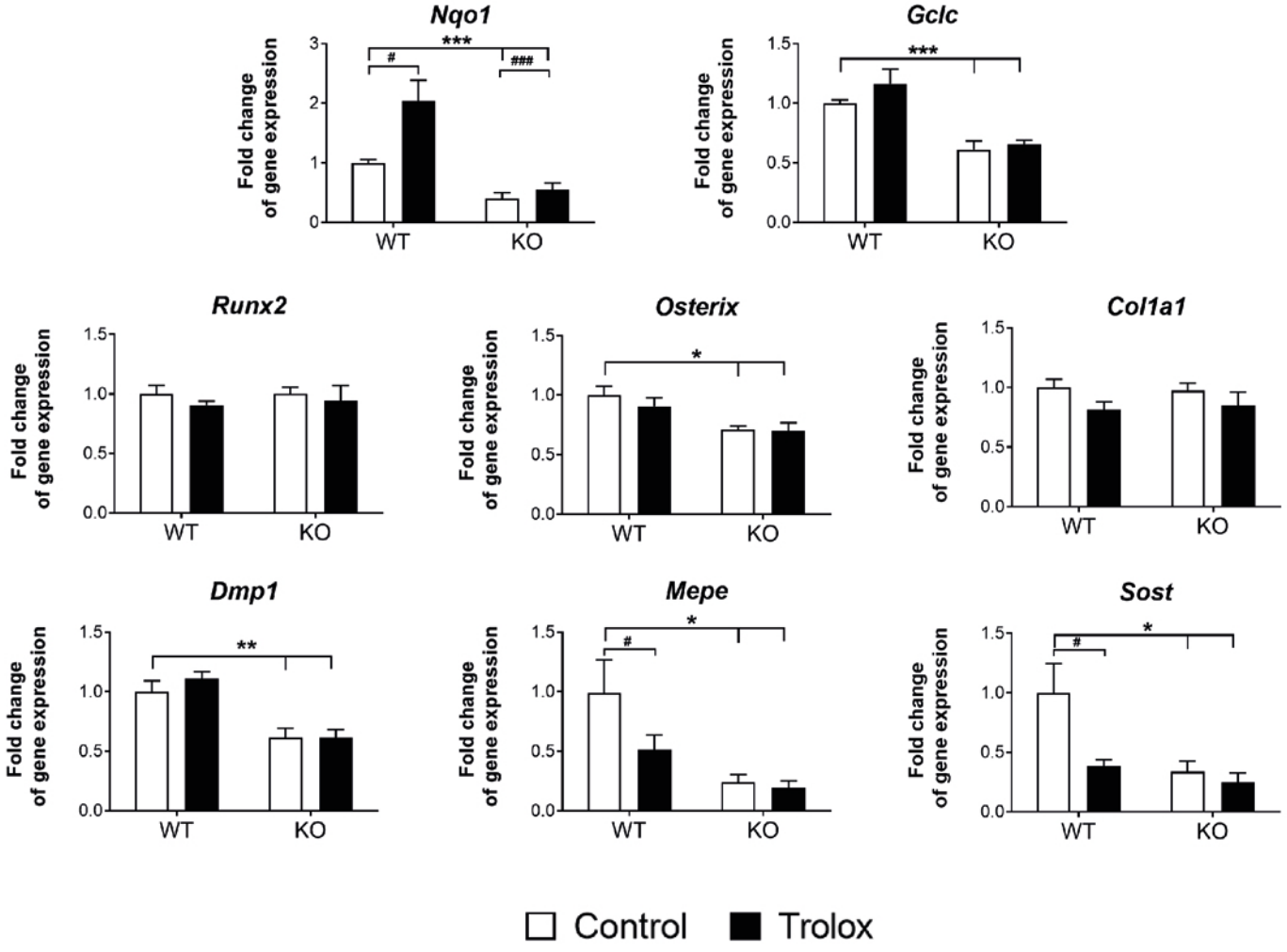

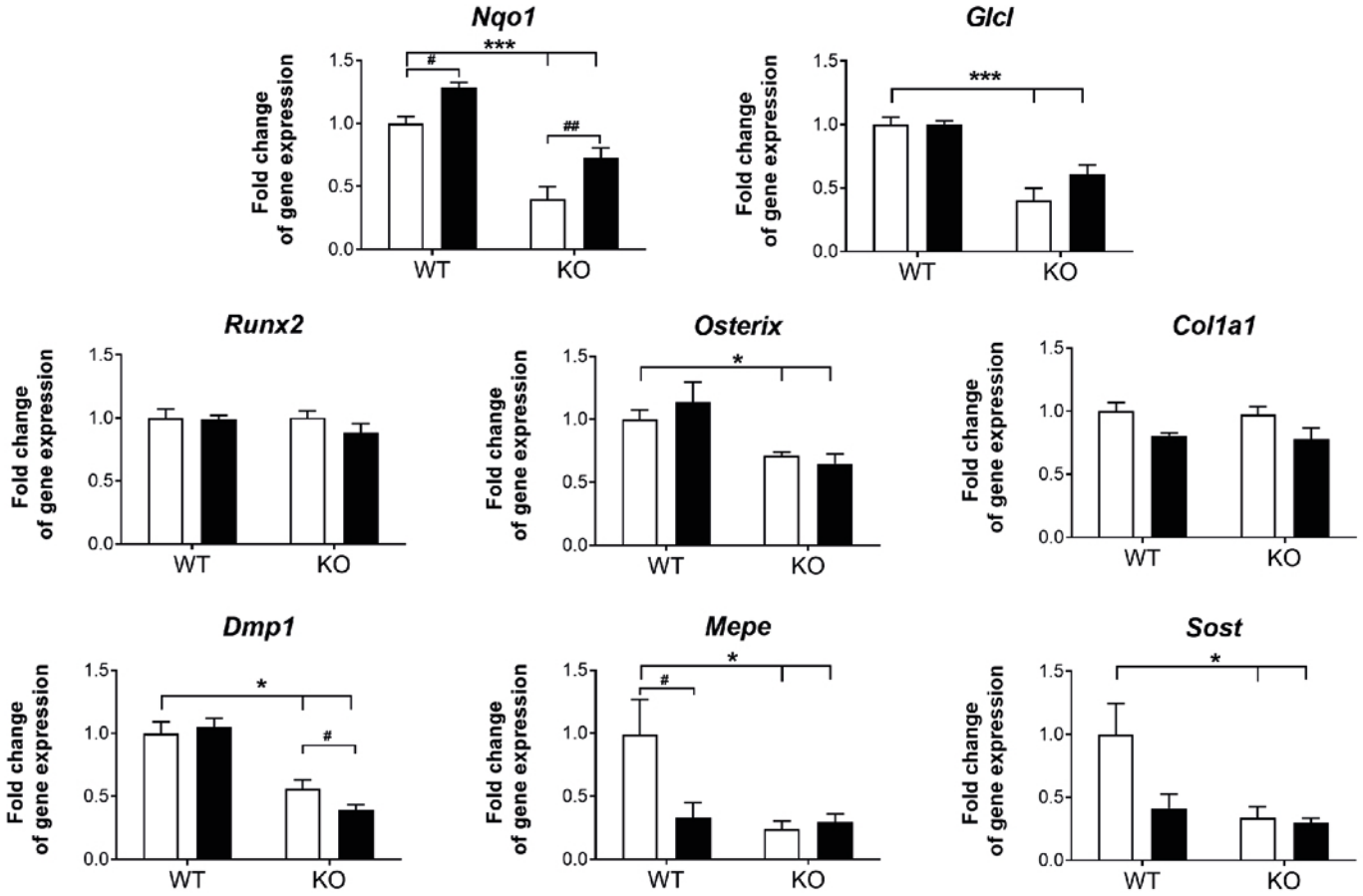

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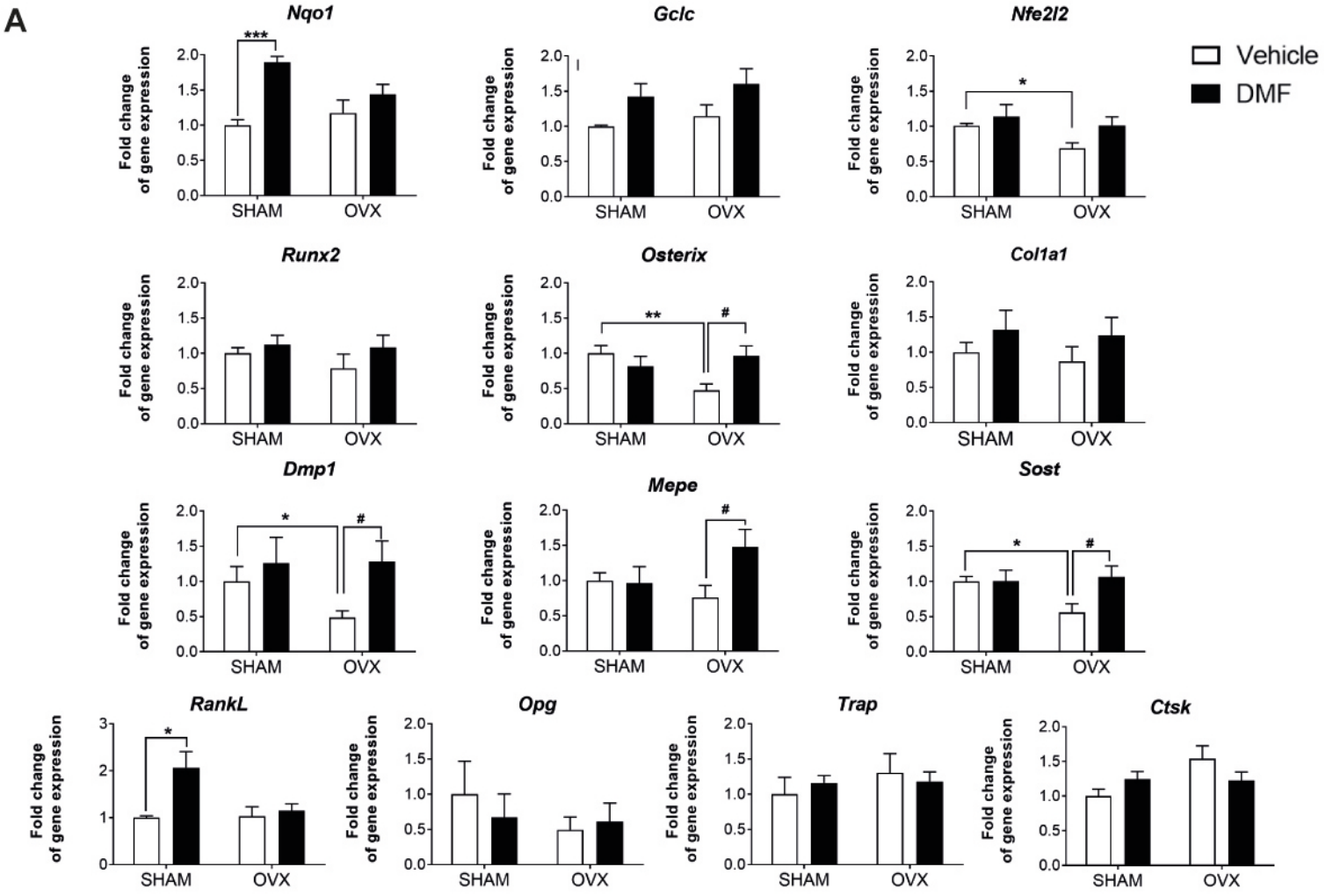
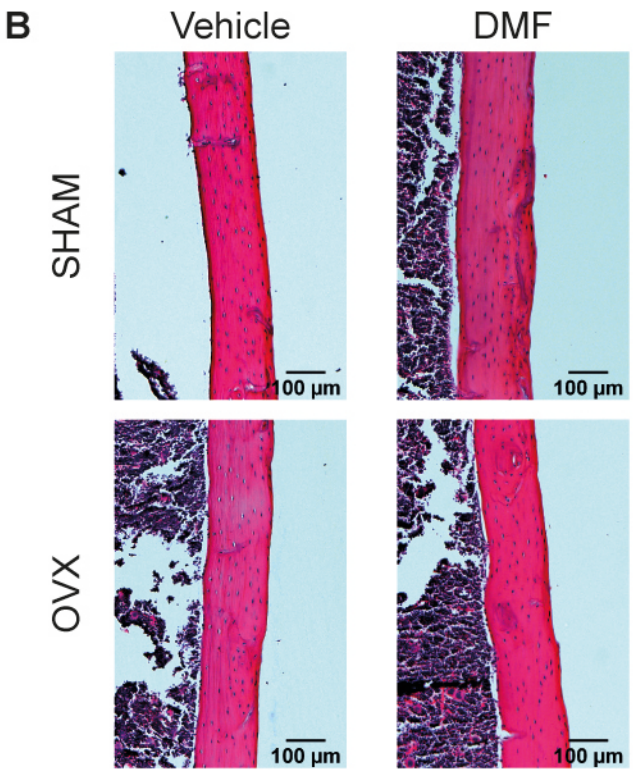
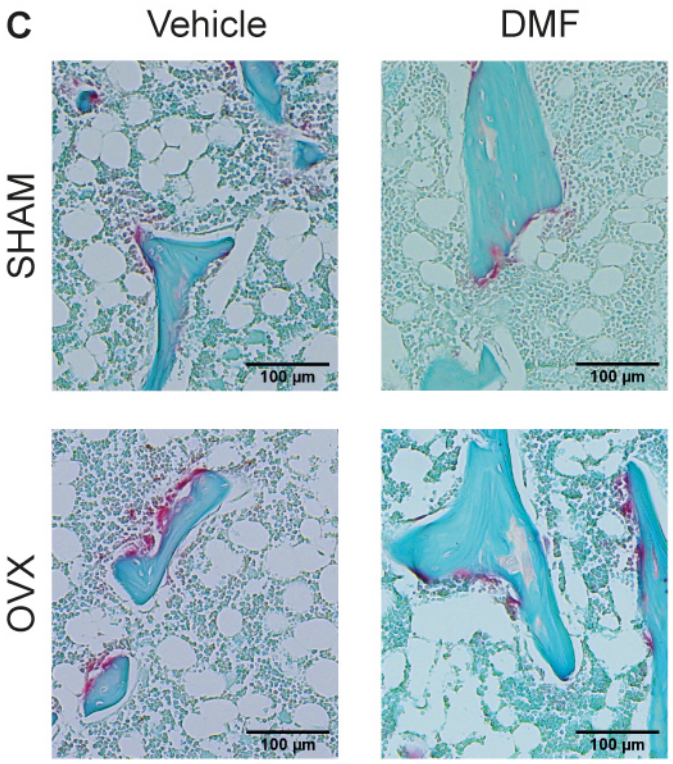
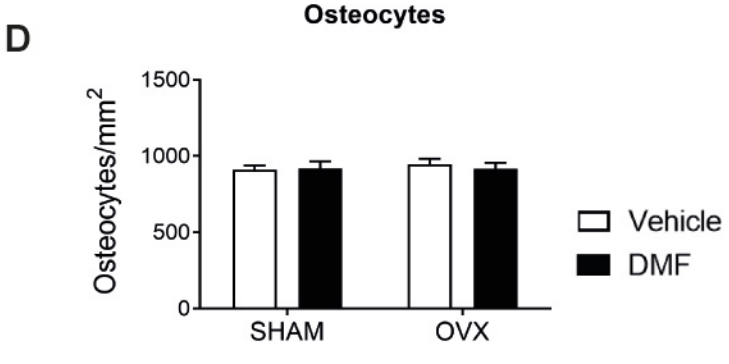
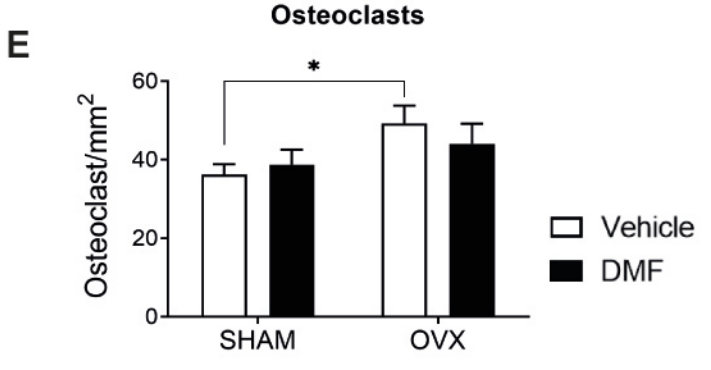


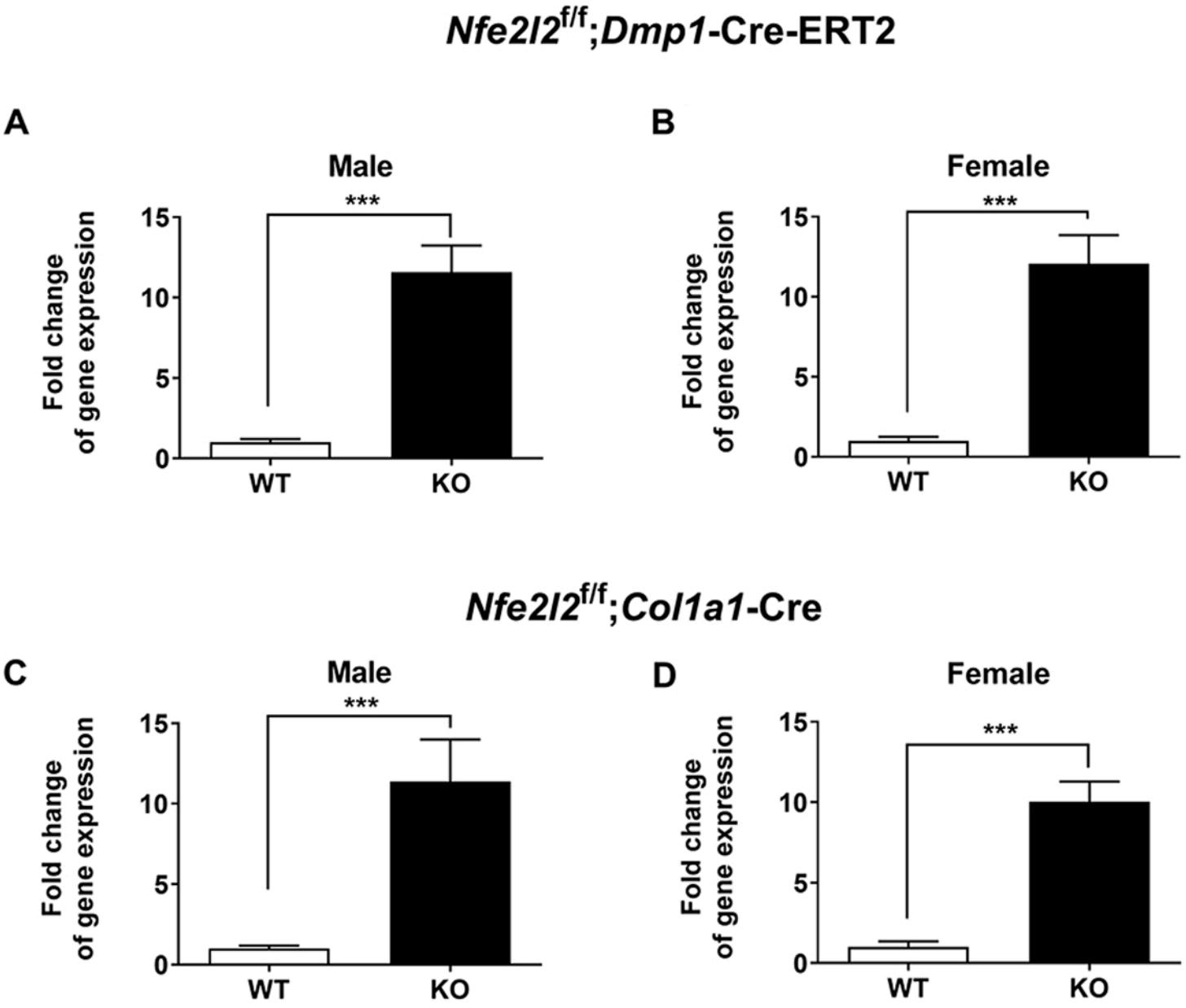








Supplementary Figure 8



SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Mitochondrial respiration and ROS levels during IDG-SW3 differentiation (A) Determination of routine oxygen consumption, leak respiration (uncoupled) and electron transfer capacity (ETS), and coupled respiration of IDG-SW3 undifferentiated and differentiated for 7 and 14 days. All results were expressed relative to protein content. Results are plotted as mean ± SEM of four to six independent experiments. (B) Visualization of mitochondria stained with 250 nM Mitotracker, total ROS stained with 5 µM CellROX Deep Red and mitochondrial superoxide stained with 2 µM MitoSOX in IDG-SW3 undifferentiated and differentiated for 3, 7 and 14 days. Mean ± SEM of 3 independent experiments. *p< 0.05, ** p < 0.01, *** or p < 0.001 using Student´s t-test.

Supplementary Figure 2. Mitochondrial content during osteocytic differentiation. (A) Visualization of mitochondria with Mitotracker Deep Red in murine osteoblasts and osteocytes. (B) Quantification of mtDNA in osteoblast and osteocyte primary cultures. Results are plotted as expression relative to osteoblasts (mean ± SEM; n=6).

Supplementary Figure 3. Histological analysis of femurs obtained from mice deficient of NRF2 in osteocytes. (A and E) Representative images of longitudinal sections of femur from male (A) and female (E) wild type (WT) and NRF2 knock-out (KO) mice stained with hematoxylin and eosin and TRAP. Images were taken at 4x magnification. (B and F) Osteocyte number per area of cortical bone in male (B) and female (F) wild type (WT) and *Nfe2l2f/f*;*Dmp1*-Cre-Ert2 knock-out (KO) mice. (C and G) Osteoclast number per area of trabecula determined by TRAP staining from male (C) and female (G) wild type (WT) and *Nfe2l2f/f*;*Dmp1*-Cre-Ert2 knock-out (KO) mice. (D and H) mRNA expression levels of relevant genes of osteoclast function from calvaria obtained from NRF2 conditional knock-out (*Nfe2l2f/f*;*Dmp1*-Cre-Ert2) and control

(*Nfe2l2f/f*) mice. mRNA expression levels were measured by RT-qPCR and normalized to *Tbp* expression. Results are plotted as mean ± SEM of seven to eleven independent animals. *p<0.05, **p<0.01, and ***p<0.001 using Student´s t-test.

Supplementary Figure 4. Histological analysis of femurs obtained from mice deficient of NRF2 in osteoprogenitors and osteoblasts. (A and E) Representative images of longitudinal sections of femur from male (A) and female (E) wild type (WT) and *Nfe2l2f/f*;*Col1a1*-Cre knockout (KO) mice stained with hematoxylin and eosin and TRAP. Images were taken at 4x magnification. (B and F) Osteocyte number per area of cortical bone in male (A) and female (E) wild type (WT) and *Nfe2l2f/f*;*Col1a1*-Cre knockout (KO) mice. (C and G) Osteoclast number per area of trabeculae determined by TRAP staining from male (C) and female (G) wild type (WT) and *Nfe2l2f/f*;*Col1a1*-Cre (KO) mice. (D and H) mRNA expression levels of relevant genes of osteoclast function in calvaria obtained from NRF2 conditional knock-out (*Nfe2l2f/f*;*Col1a1*-Cre) and control (*Nfe2l2f/f*) mice. mRNA expression levels were measured by RT-qPCR and normalized to *Tbp* expression. Results are plotted as mean ± SEM of seven to eleven independent animals. *p<0.05, **p<0.01, and ***p<0.001 using Student´s t-test

Supplementary Figure 5. Effect of N-acetyl-cysteine (NAC) and dimethyl fumarate (DMF) on osteocyte-specific gene expression. (A) Primary osteocytes from Nfe2l2fl/fl mice were infected with pMSCV-puro-Cre-ERT and treated with 1 µg/ml tamoxifen to induce Cre recombination. Osteocytes were cultured for 48 h in the presence or absence of 1 mM N-acetylcysteine (NAC, a ROS scavenger). (B) Primary osteocytes from Nfe2l2fl/fl mice were transduced with pMSCV-puro-Cre-ERT2, and treated with 1 µg/ml tamoxifen to induce Cre recombination. Osteocytes were cultured for 48 h in the presence or absence of 5 µM dimethyl fumarate (DMF). Results were plotted as expression relative to cells infected with the GFP

vector (mean ± SEM; n=10). *or #p < 0.05, ** or ## p < 0.01, *** or ### p < 0.001 using Student´s t-test. * refer to statistics between control WT and KO osteocytes. Similarly, # refers to significance between control WT and treated WT cells or between control KO and treated KO osteocytes.

Supplementary Figure 6. Effect of Trolox and Mitoquinone (MitoQ) on osteocyte-specific gene expression. (A) Primary osteocytes from *Nfe2l2fl/fl* mice were infected with pMSCV-puro-Cre-ERT and treated with 1µg/ml tamoxifen to induce Cre recombination. Osteocytes were cultured for 48 h in the presence or absence of 500µM Trolox. (B) Primary osteocytes from *Nfe2l2fl/fl* mice were transduced with pMSCV-puro-Cre-ERT2, and treated with 1µg/ml tamoxifen to induce Cre recombination. Osteocytes were cultured for 48 h in the presence or absence of 1µM MitoQ. Results were plotted as expression relative to cells infected with the GFP vector (mean ± SEM; n=8). *p < 0.05, ** p < 0.01, ***p < 0.001 using Student´s t-test. * refer to statistics between control WT and KO osteocytes. Similarly, # refers to significance between control WT and treated WT cells or between control KO and treated KO osteocytes.

Supplementary Figure 7. Dimethylfumarate (DMF) effect on bone gene expression and histology after ovariectomy. (A) mRNA levels in calvaria obtained from ovariectomized (OVX) and sham operated (SHAM) mice treated with 100 mg/kg of dimethylfumarate (DMF) or vehicle. mRNA expression levels were measured by RT-qPCR and normalized to Tbp expression. Results are plotted as mean ± SEM of seven to eleven independent animals. (B and C) Representative images of longitudinal sections of femur from OVX and SHAM mice treated with 100 mg/kg of DMF or vehicle stained with (B) hematoxylin and eosin or (C) TRAP. Images were taken at 4x and 10x magnification, respectively. (D) Osteocyte number per area of cortical bone in femur from OVX and SHAM mice treated with 100 mg/kg of DMF or

vehicle. Results are plotted as mean ± SEM of seven to eleven independent animals. (E) Osteoclast number per area of trabeculae determined by TRAP staining from OVX and SHAM mice treated with 100 mg/kg of DMF or vehicle. Results are plotted as mean ± SEM of seven to eleven independent animals. *P < 0.05, ** or ## P < 0.01, *** or ### P < 0.001 using Student´s t-test. * refer to statistics performed against SHAM mice treated with vehicle. Similarly, # refers to significance between OVX mice treated with vehicle or DMF.

Supplementary Figure 8. Cre expression levels in bone from NRF2 conditional knock-out (*Nfe2l2f/f*;*Dmp1*-Cre-Ert2 or *Nfe2l2f/f;Col1a1-Cre*) and control (*Nfe2l2f/f*) mice. mRNA expression levels were measured by RT-qPCR and normalized to *Tbp* expression. Results are plotted as mean ± SEM of nine to eleven independent animals. *p<0.05, **p<0.01, and ***p<0.001 using Student´s t-test.