Estrogen receptor α- (ERα), but not ERβ-signaling, is crucially involved in mechanostimulation of bone fracture healing by whole-body vibration

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Abstract

- Mechanostimulation by low-magnitude high frequency vibration (LMHFV) provoke anabolic effects on the intact skeleton
- Experimental studies revealed that, during bone fracture healing, the effect of whole-body vibration is profoundly influenced by the estrogen status.
- LMHFV significantly improved fracture healing in ovariectomized (OVX) mice and was significantly reduced in non-OVX mice
- ERα and ERβ were differentially expressed in the callus depending on the estrogen status

Estrogen receptors

- ERα
  Breast, Uterus, Hypophyse, Hypothalamus
- is considered the most essential receptor for mediating estrogen effects on bone
- Crucial for anabolic effects of mechanical loading
- ERβ
  Bone, Vascular System, Prostate, Ovary, Lungs, Brain
- reverses or inhibits ERα-mediated gene transcription

- both ERs can exert signaling both in the presence and absence of their ligand estrogen
  the activated genes are highly dependent on the receptor subtype

Estrogenes in Osteoporosis

- Significant reduction of fractures

- Not used anymore due to:
- risk-benefit negative:
  increased breast cancer risk
  higher thrombosis risk

SERM

- Raloxifen

  (Recker RRe et al. Long-term raloxifene for postmenopausal osteoporosis; Kim, K. et al. Osteoporos Int (2014) Comparative cost-effectiveness of bazedoxifene and raloxifene in the treatment of postmenopausal osteoporosis in Europe, using the FRAX algorithm)

- Bazedoxifen

  (Biskobing D.M. Update on bazedoxifene: A novel selective estrogen receptor modulator; Palacios S. Efficacy and safety of bazedoxifene, a novel selective estrogen receptor modulator for the prevention and treatment of postmenopausal osteoporosis)

- IND: prevention and treatment of osteoporosis in postmenopausal women
- Agonistic effects on bone tissue and lipid metabolism
- Antagonistic effects on breast and uterus

Material & Methods

- At the age of 12 weeks female mice received either a bilateral ovariectomy (OVX) or were sham-operated, and femur osteotomy was performed 4 weeks after OVX/sham operation.
- Estrogen serum levels were determined at day 21 after surgery using an estradiol ELISA.
- the osteotomy was created at the right femur diaphysis using a 0.4 mm gigli wire saw (RISystem, Davos, Switzerland) and stabilized by a semi-rigid external fixator (RISystem)
- Half of all mice received LMHFV. All mice were sacrificed 21 days after surgery using an isoflurane overdose.

Table 1
Overview about experimental groups and number of used mice per group.

genotype	WT				ERα-KO				ERβ-KO			
sham-OVX	6	8			6	8			8	6		
OVX			7	8			6	7			9	7
sham-vibration	6		7		6		6		8		9	
vibration		8		8		8		7		6		7

LMHVF

- Mice were placed on custom-made vibration platforms for 20 min per day for 5 days per week, starting with the third postoperative day.
- Vibration settings were 0.3 g sinusoidal peak-to-peak acceleration and 45 Hz frequency. The amplitude and frequency were continuously recorded using integrated accelerometers at the platform.

Biomechanical testing and µCT analysis

- Fractured femurs were explanted after euthanasia and subjected to a non-destructive three-point bending test
- a force of maximal 4 N was applied on top of the cranio-lateral callus side and deflection was measured using a materials testing machine. Flexural rigidity was calculated from the slope of the linear region of the force-deflection curve

- After biomechanical testing, bones were fixed in 4% paraformaldehyde and scanned in a µCT device to determine the apparent bone mineral density

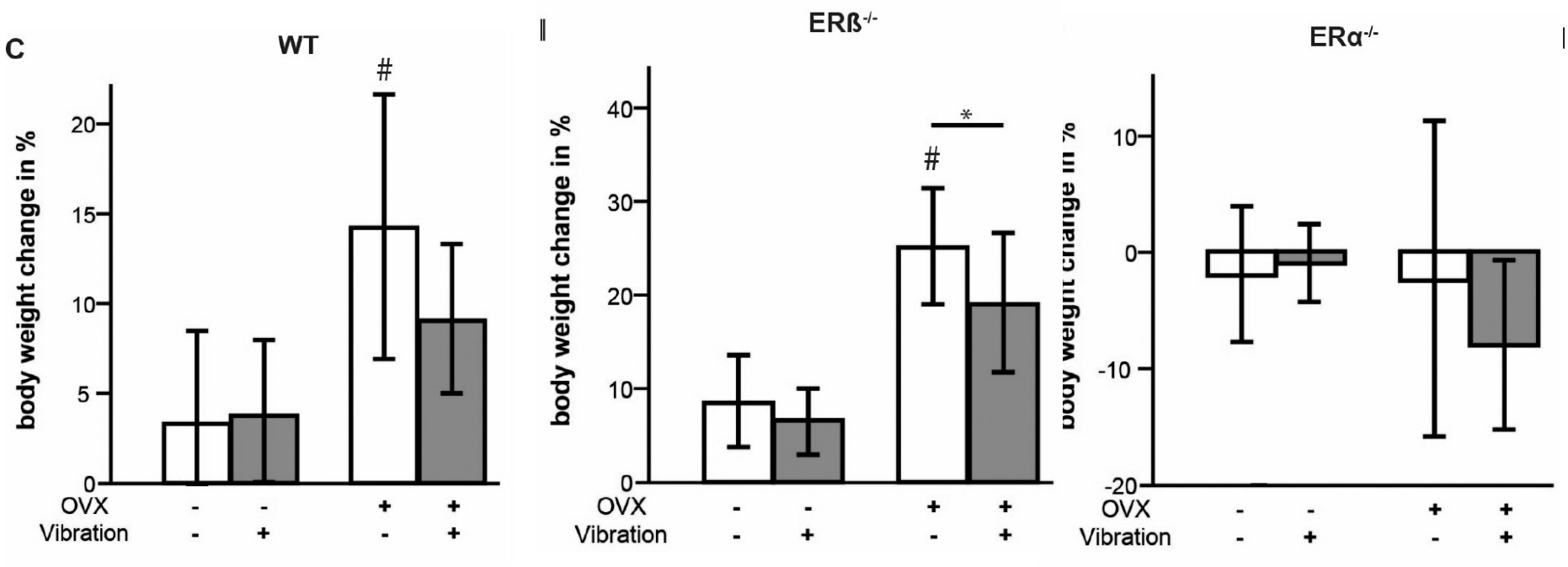
Histomorphometry and immunohistochemistry

- Longitudinal sections of 7 µm were stained with Giemsa or Safranin O for histomorphometric tissue quantification.
- The number and surface of osteoblasts were determined using Toluidine blue staining.
- The number and surface of osteoclasts were determined using tartrate-resistant alkaline phosphatase (TRAP) staining.
- ERα and ERβ expression were detected using a primary antibody against ERα and ERβ, and secondary antibodies against rabbit IgG were used for immunohistochemistry

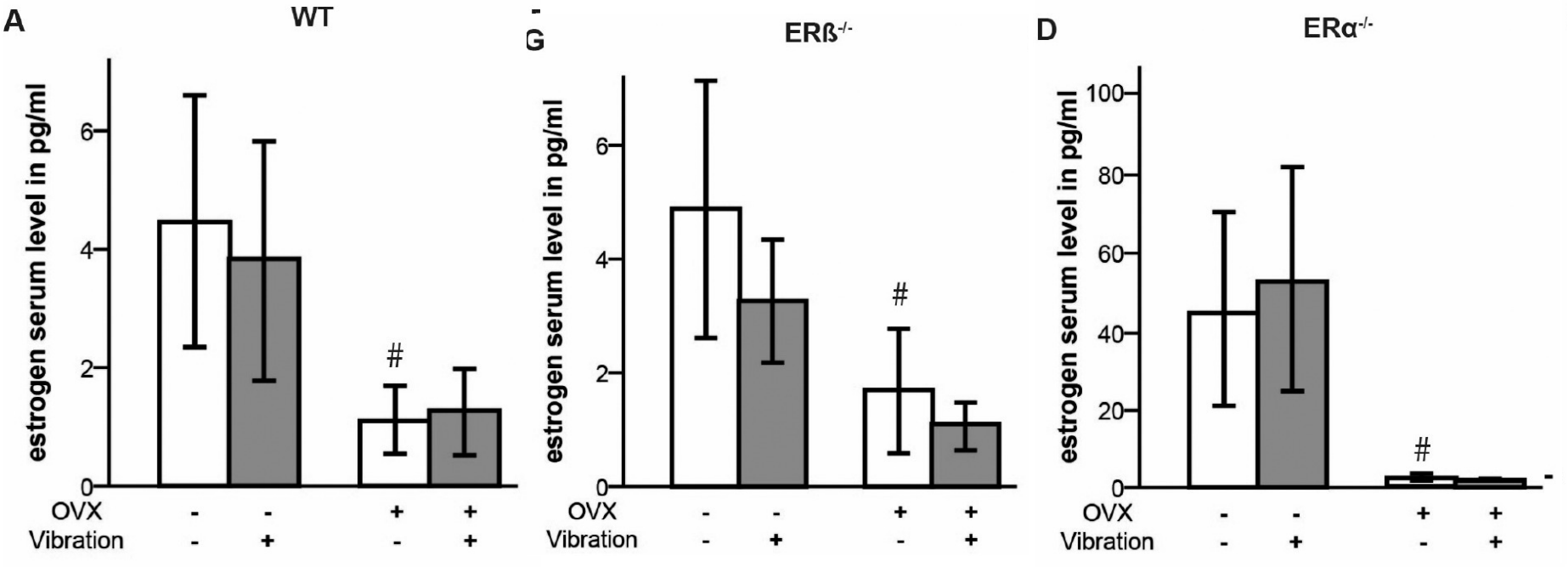
Expression of ERs in the fracture callus

- ERβ-KO mice displayed a striking increase in ERα expression in almost all cells of the fracture callus
- WT mice displayed only low amount of the receptor, mostly on osteoclasts.
- only non-vibrated OVX mice additionally displayed some ERβ expression in osteoblasts

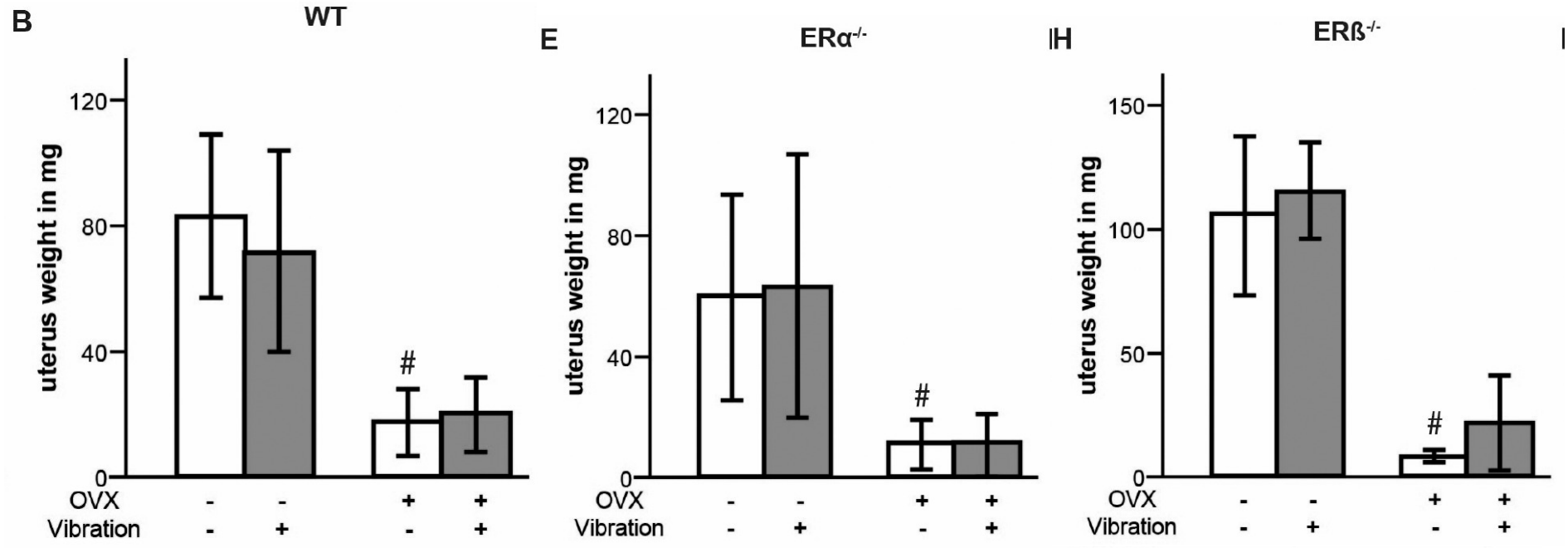
Body weight change



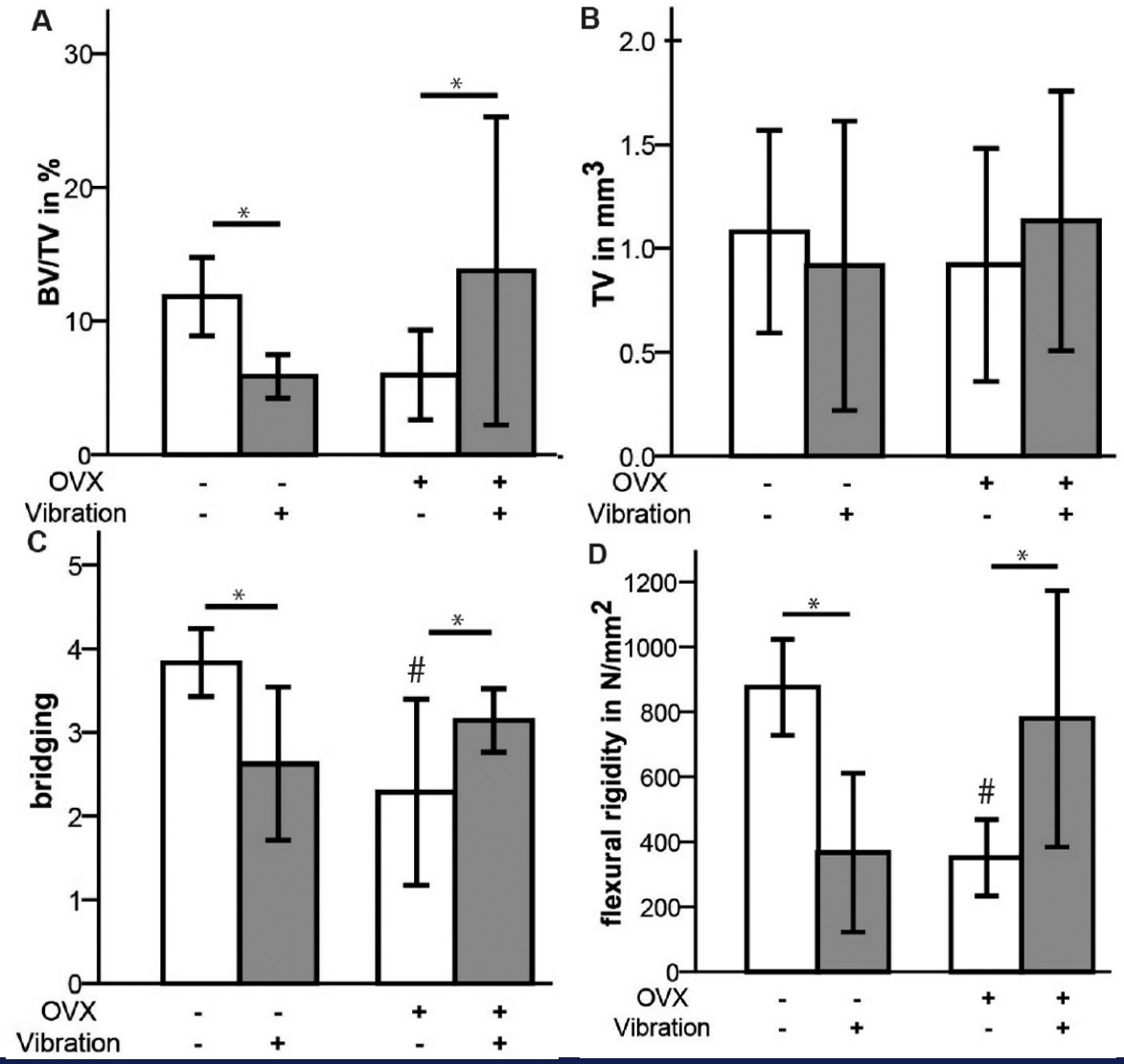
Estrogen serum level



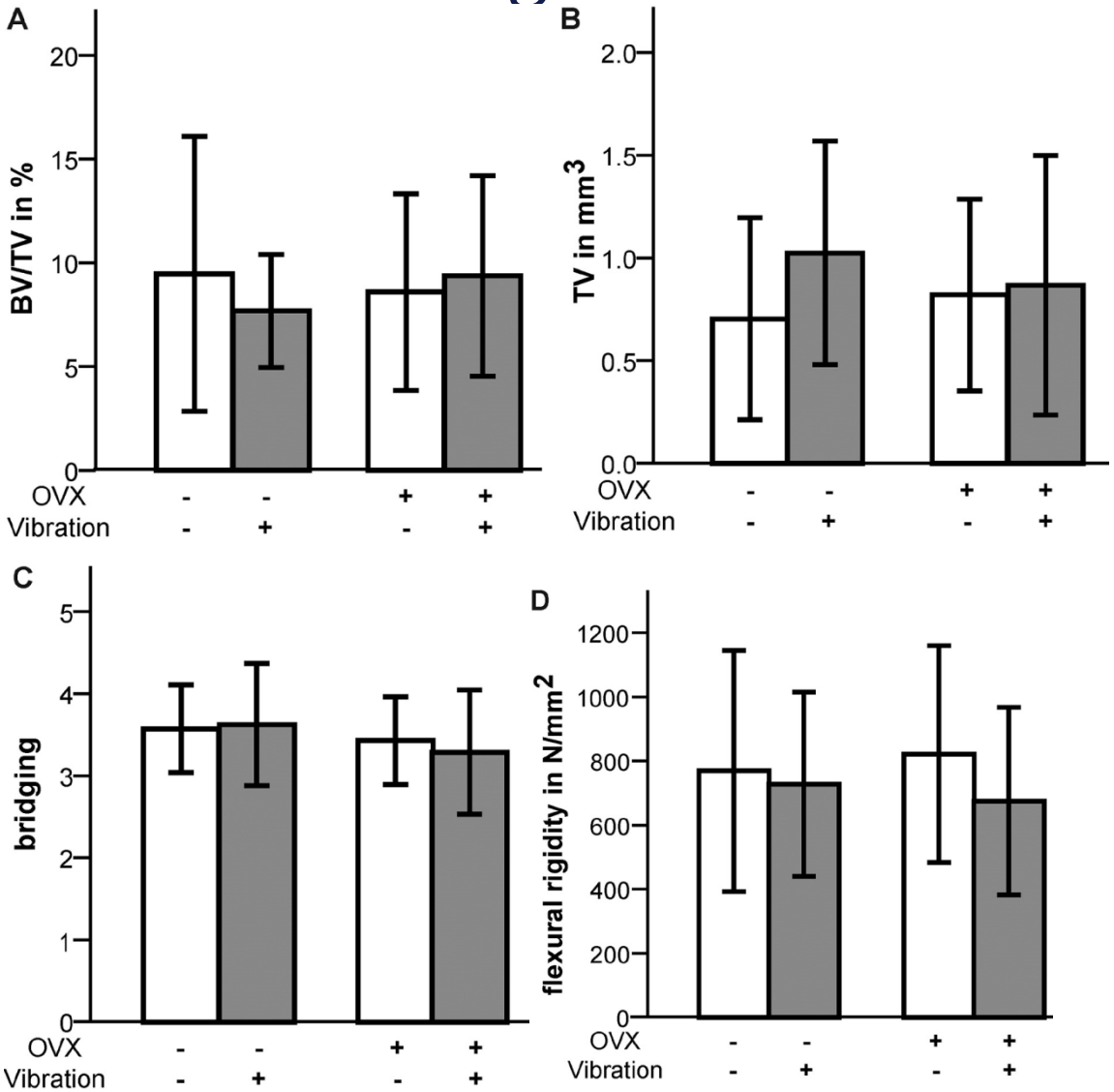
Uterus weight



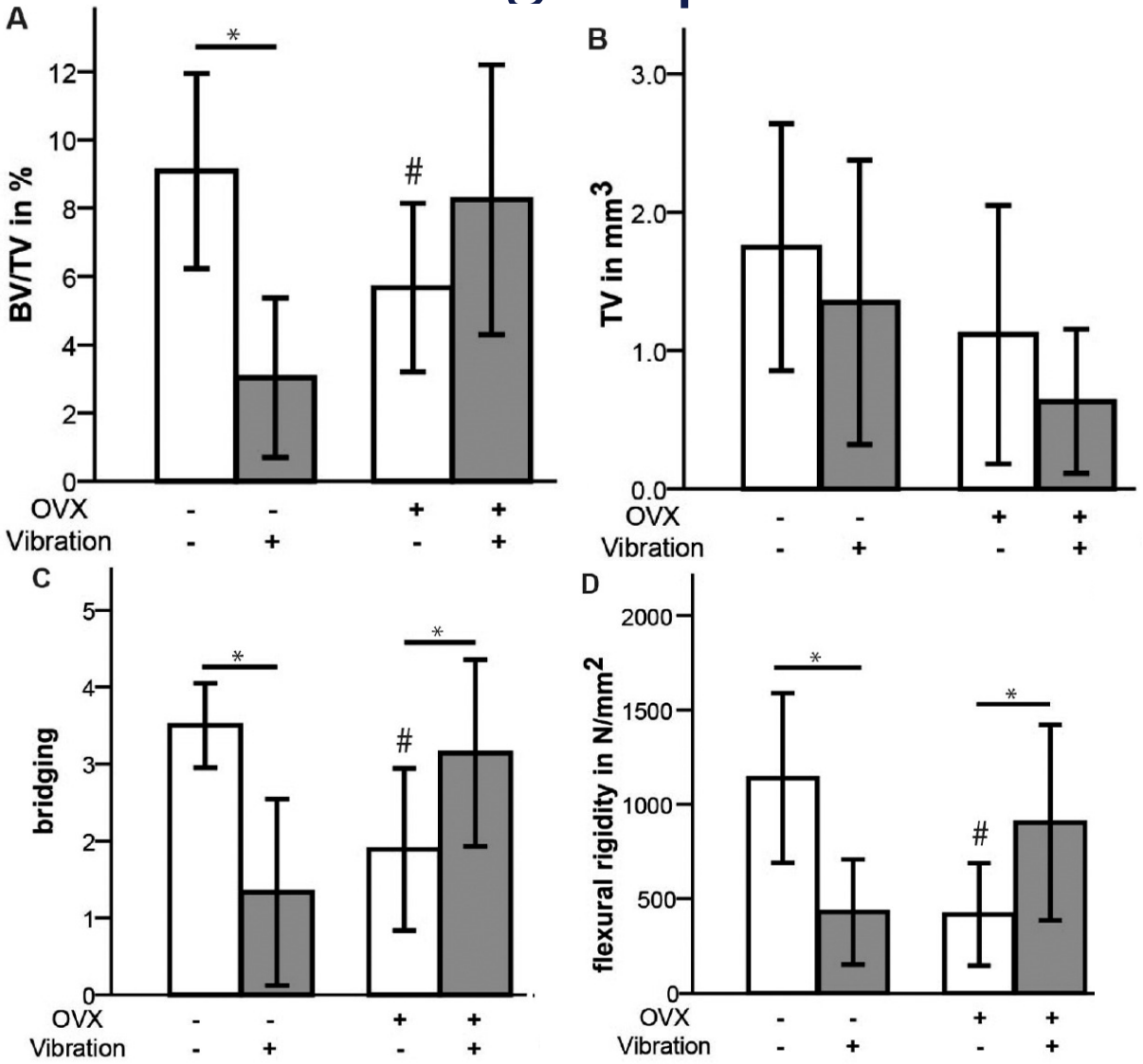
Bone healing WT



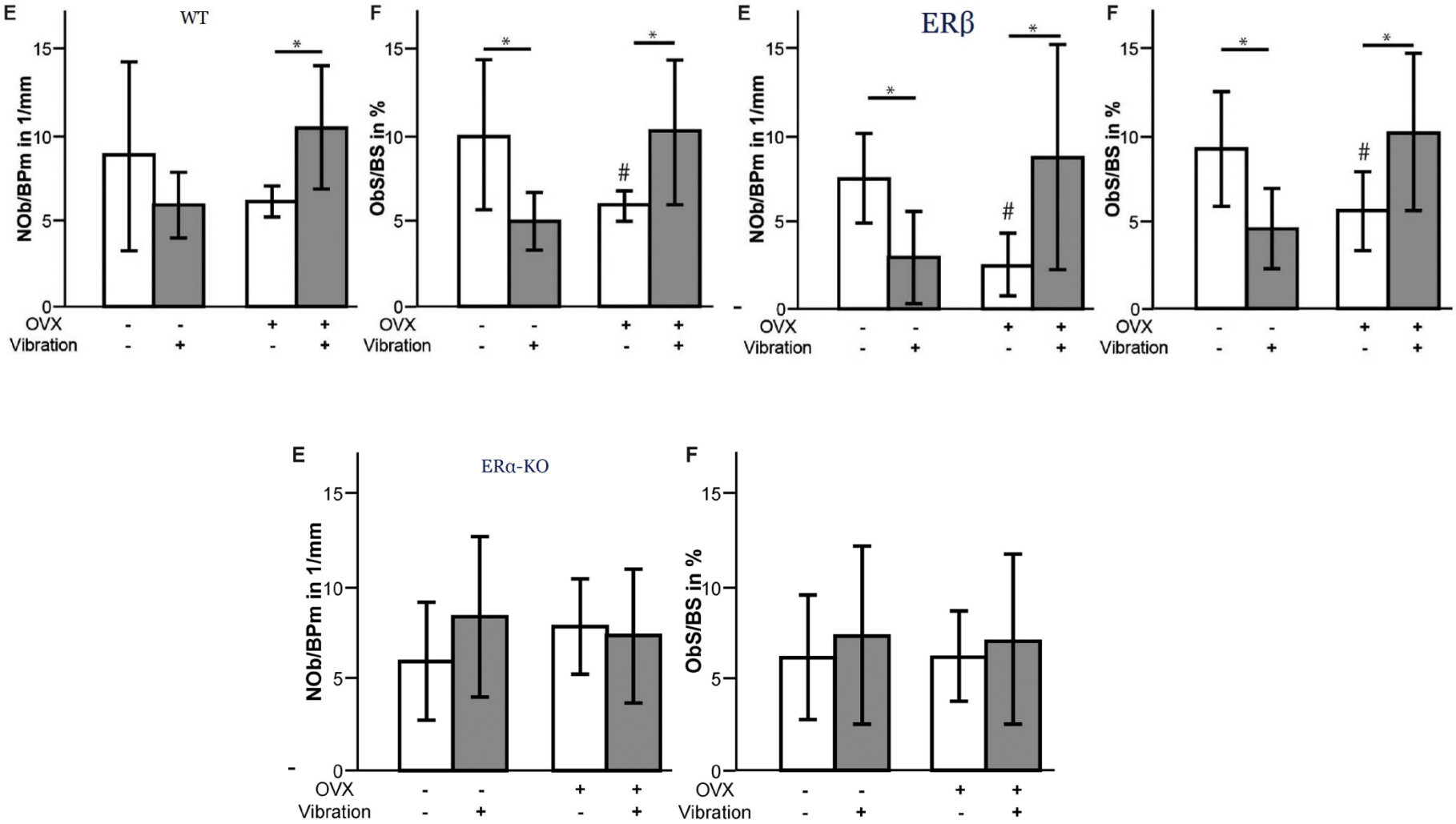
Bone healing ERα



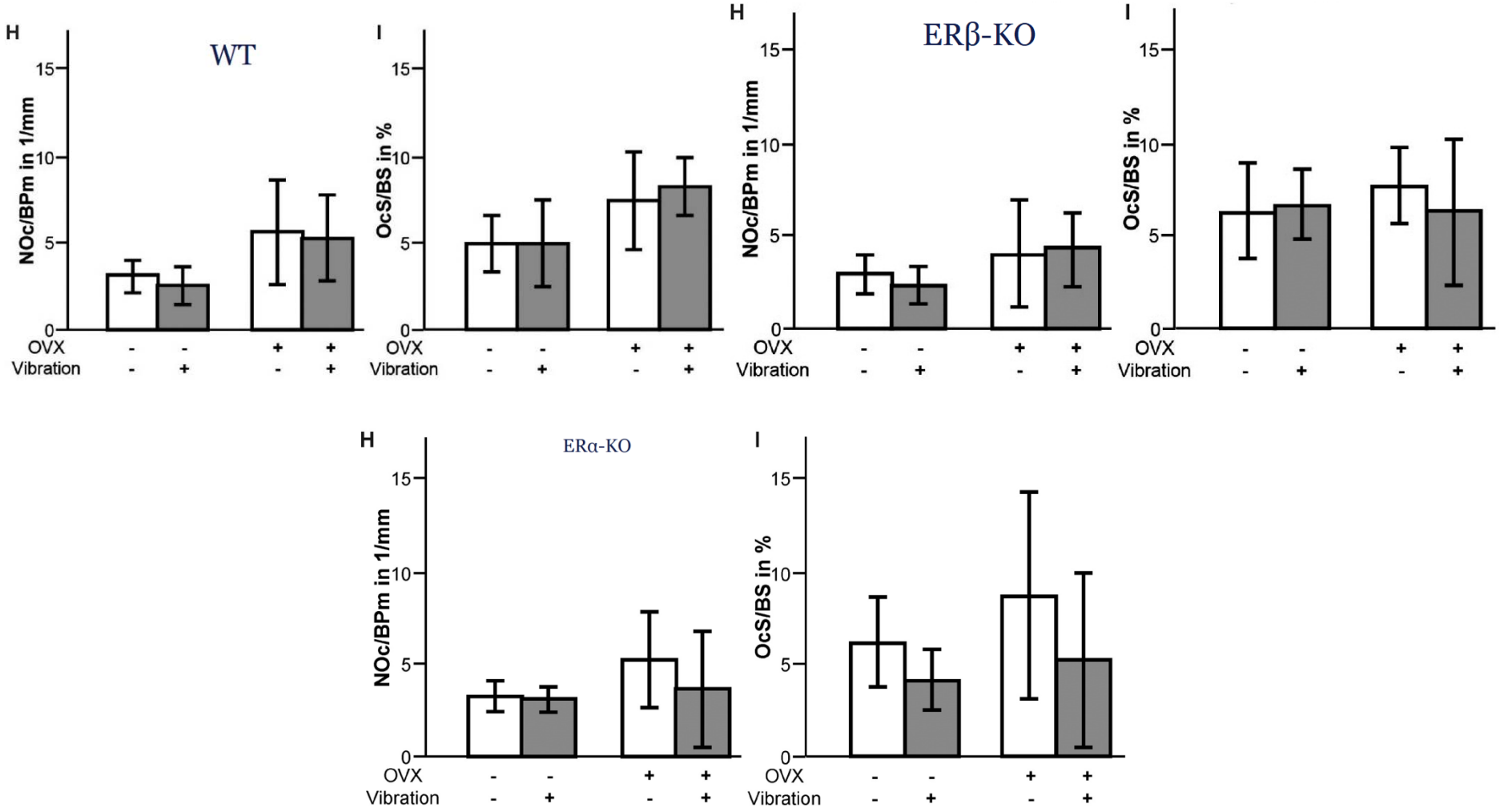
Bone healing ERβ



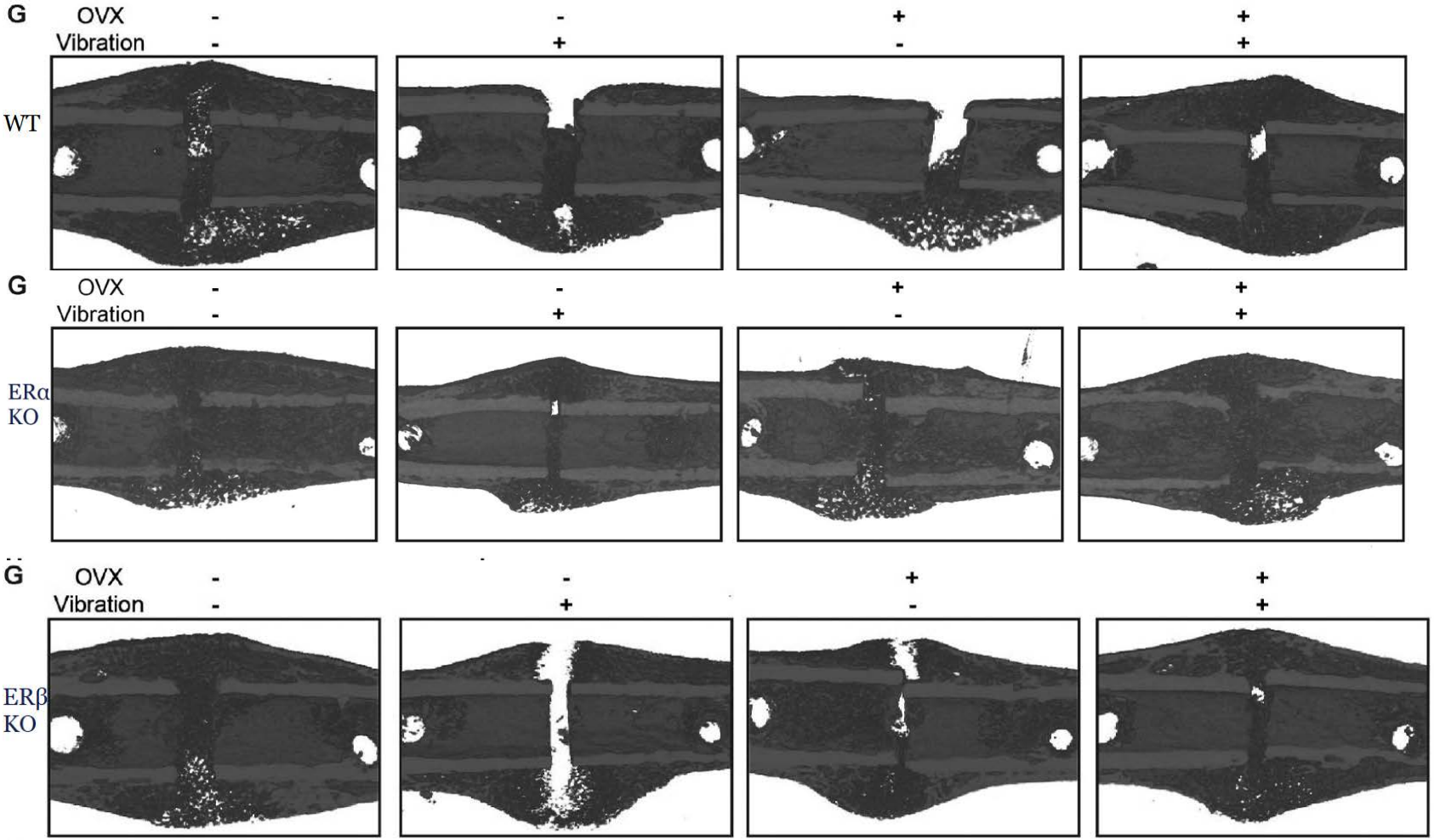
Osteoblasts



Osteoclasts

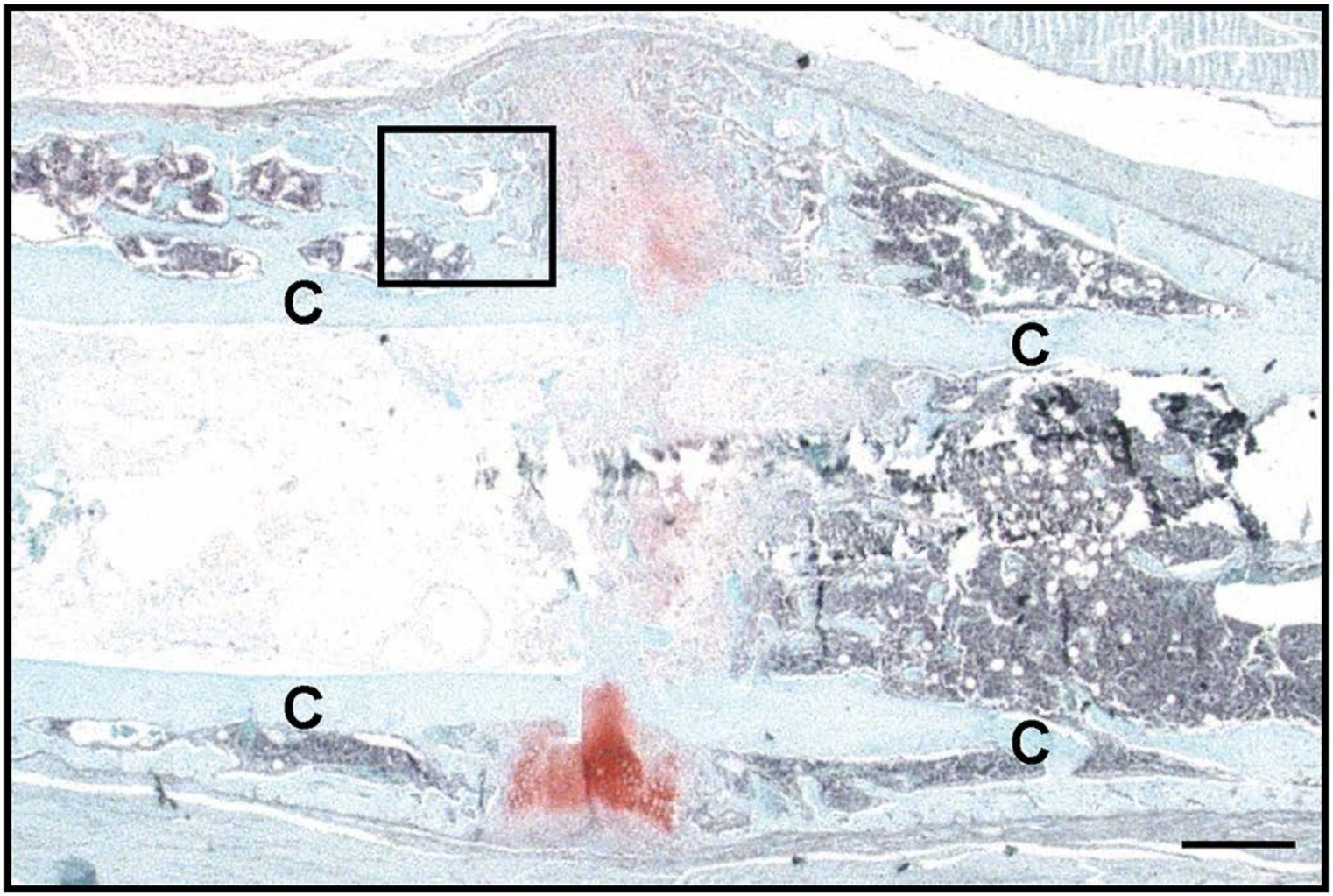


Bridging

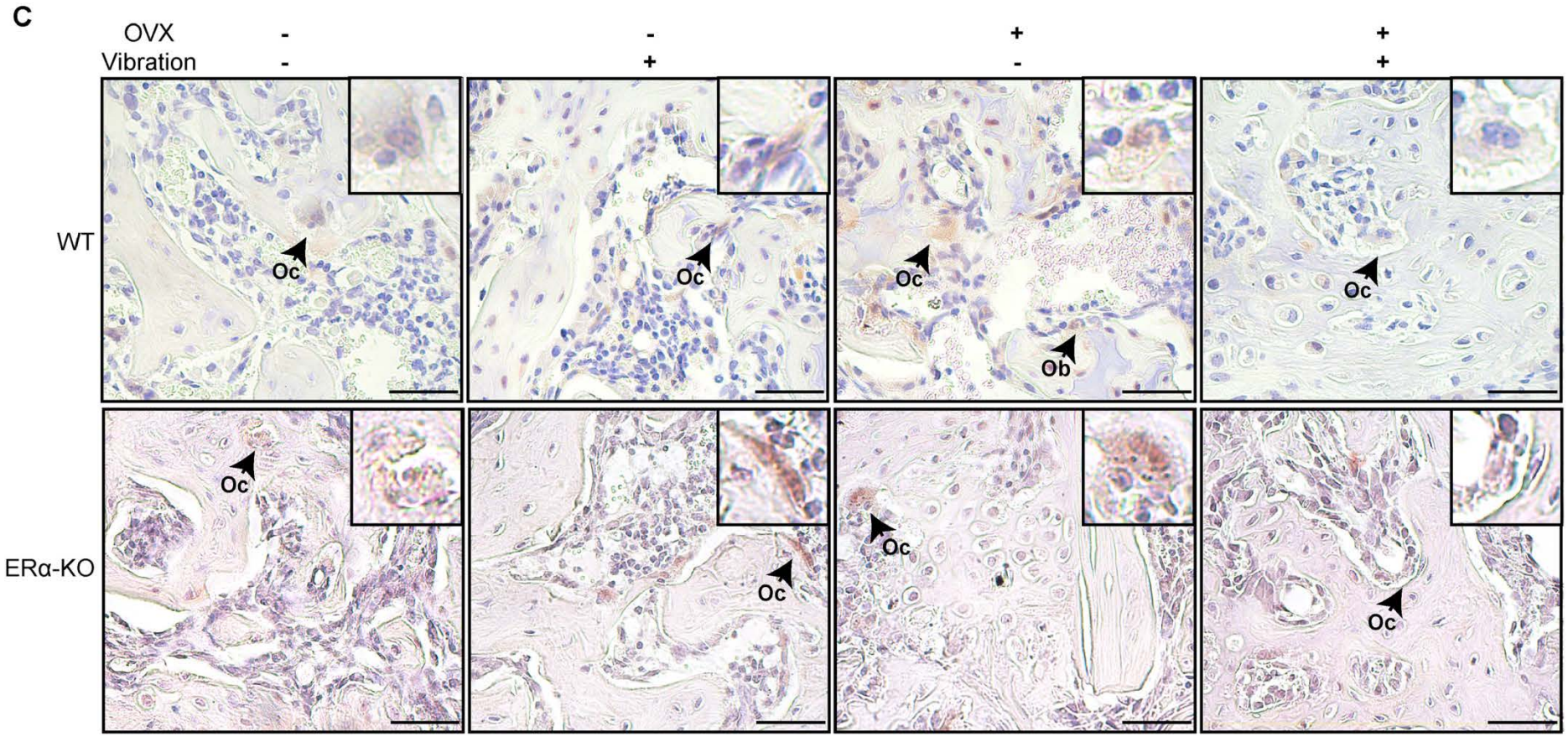

G
OVX - - + +
Vibration - + - +
WT
G
OVX - - + +
Vibration - + - +
ERα KO
G
OVX - - + +
Vibration - + - +
ERβ KO

Histology (1)

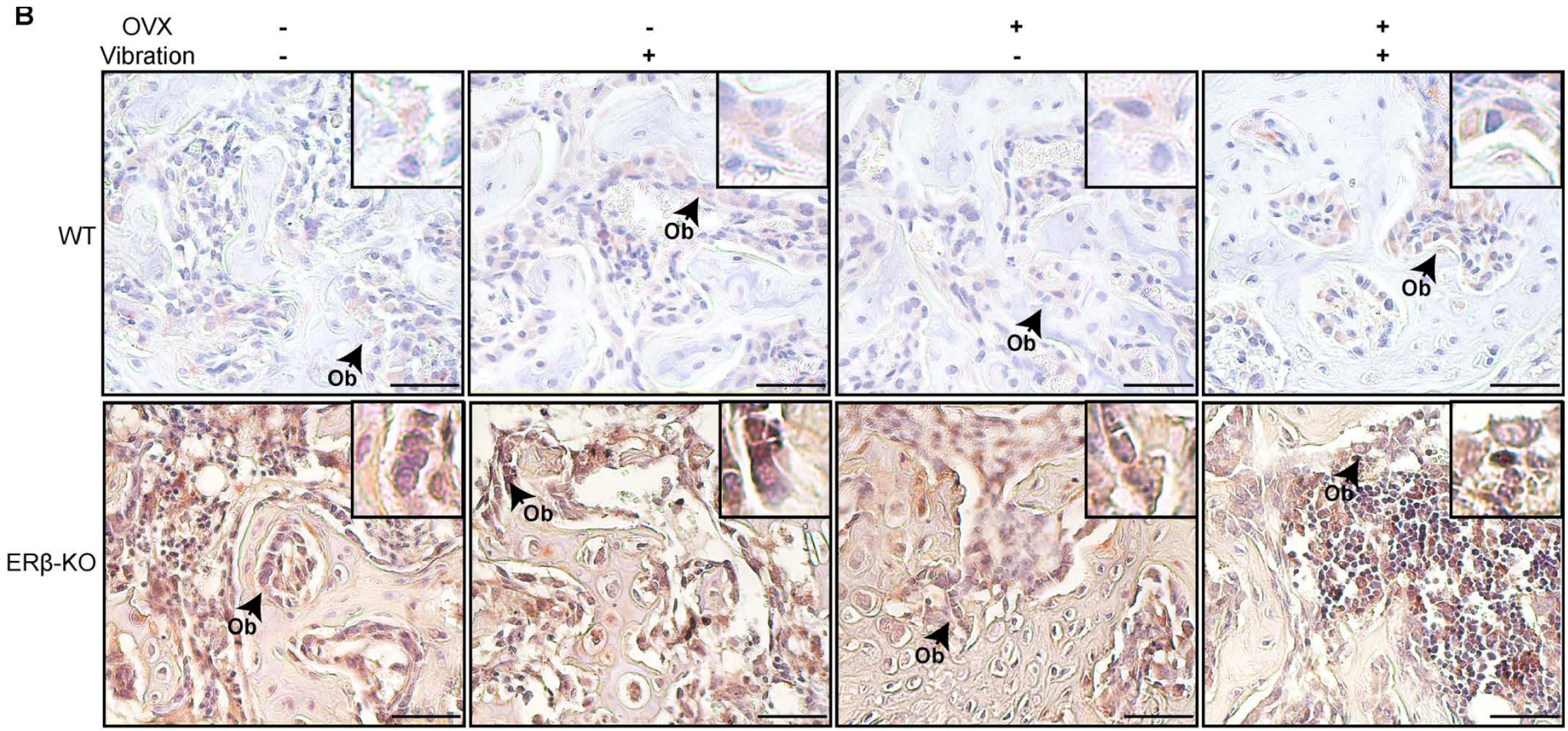
A



Histology (2)



Histology (3)



Discussion

- the results suggest a critical role of ERα-, but not of ERβ-signaling in the effects of mechanostimulation of bone fracture healing.
- to date, there are no other studies that investigated the influence of ERα on bone healing, and only one study that investigated fracture healing in ERβ- KO mice.
- BUT healing was unaffected at a later stage they conclude that under normal healing conditions in young, healthy mice, both ERs are not crucial for fracture healing.
- These results are against our expectations, since both ERs were known to significantly influence the endochondral ossification process during long bone growth, which is recapitulated during fracture healing.