

# BONE AND CARTILAGE DEGENERATION IN MICE FOLLOWING LONG-DURATION SPACEFLIGHT: THE ROLE OF BONE MARROW STEM CELLS

Elizabeth A. Blaber, Olivia G. Stimpel, Margareth A. Cheng-Campbell, and Eduardo A.C. Almeida  
Space Biosciences Division, NASA Ames Research Center



## Background

Exposure to mechanical unloading during spaceflight is known to have significant degenerative effects on the musculoskeletal system. Our ongoing studies with mice in short-duration microgravity experiments have identified the failure of normal stem cell-based tissue regeneration, in addition to tissue degeneration, as a significant concern for long-duration spaceflight, especially in the mesenchymal and hematopoietic tissue lineages. Specifically, our laboratory has identified normal tissue regenerative processes, such as the formation of new bone, cartilage, immune cells, and blood from bone marrow mesenchymal stem cell precursors, as being particularly sensitive to the stresses of mechanical unloading in microgravity. The cellular mechanisms activated by microgravity unloading in mouse bone during spaceflight are not completely understood, but they include the inhibition of differentiation of marrow mesenchymal stem cells, via activation of cell cycle arrest in proliferative osteoprecursor cells on the bone surface. As we seek to explore beyond low Earth orbit, we need to understand the effects of long-duration mechanical unloading on physiological processes, including stem cell based tissue regeneration.

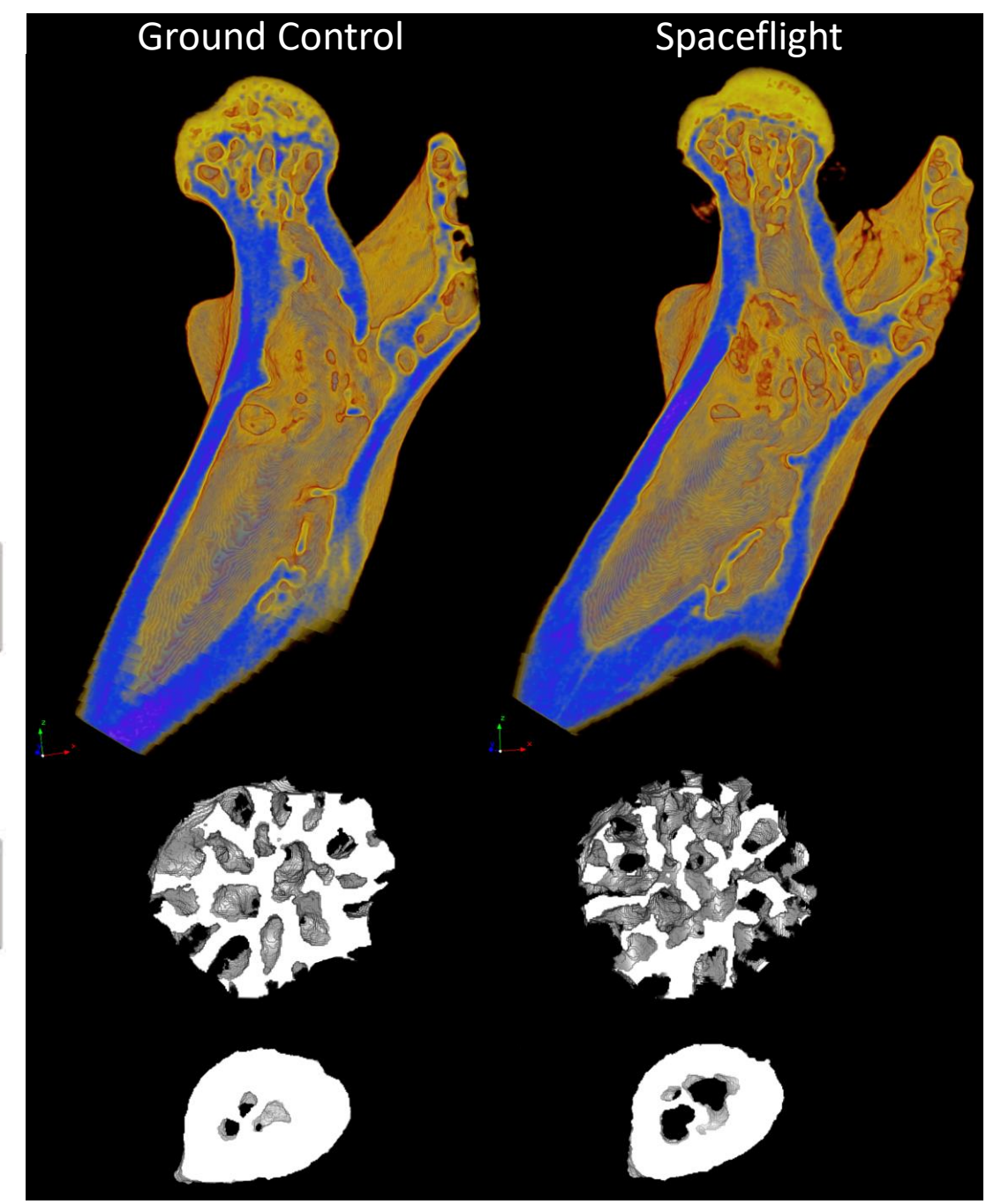
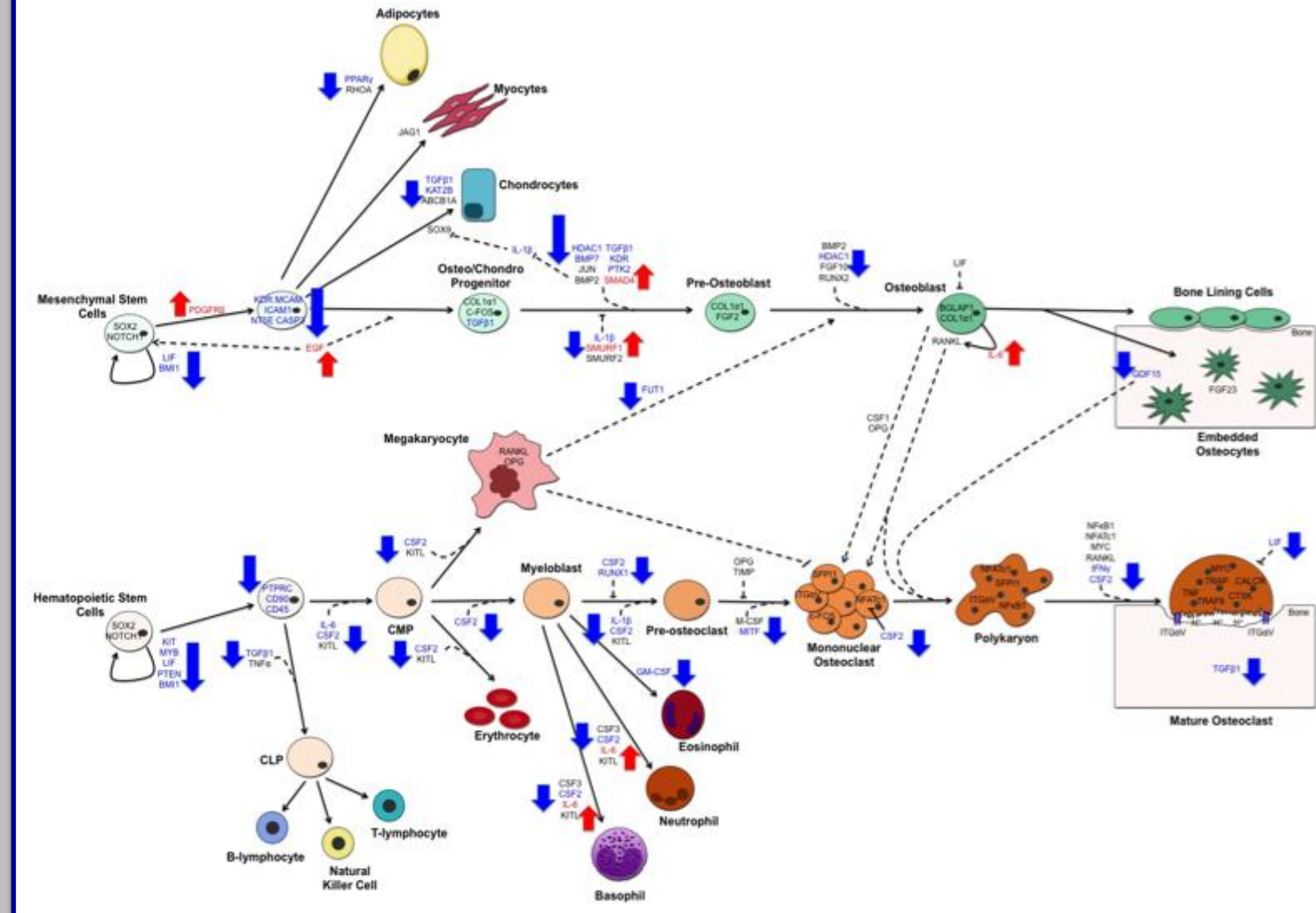


Figure 1: Spaceflight causes a broad down-regulation of key genes required for differentiation of mesenchymal and hematopoietic lineages into tissue specific cell types

Figure 2: MicroCT analysis of the proximal femur from 15-days spaceflight and corresponding ground controls.

## Hypothesis

We hypothesize that the inhibition of stem-cell based tissue regeneration in short duration spaceflight would continue during long-duration spaceflight resulting in significant tissue alterations.

## Methods

The Bion M1 and Rodent Research1 missions enabled, for the first time, the study of the effects of long-duration spaceflight on rodents.

The BionM1 mission consisted of seven 16-week-old C57BL/6 male mice flown on an unmanned Russian biosatellite for a period of 30 days. All mice were returned live and dissections were conducted 12-14 hours post landing. The Rodent Research-1 mission consisted of ten 16-week old female C57Bl/6J mice were exposed to 37 days of microgravity. Two mice from each group were

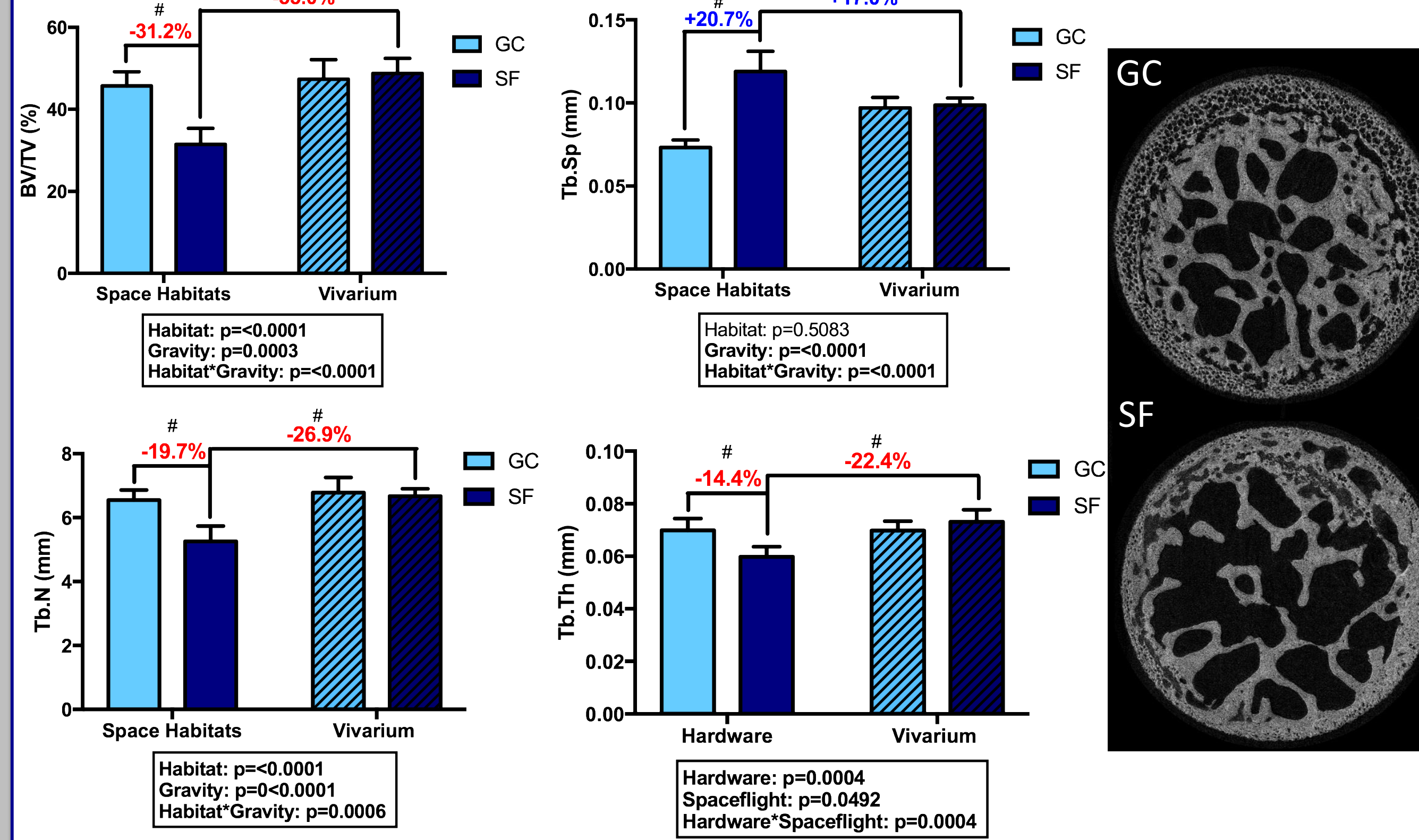


Figure 3. (Left) Rodent habitats flown on BionM1. (Above) NASA's Rodent Habitat (Photo Credit: NASA/Dominic Hart).

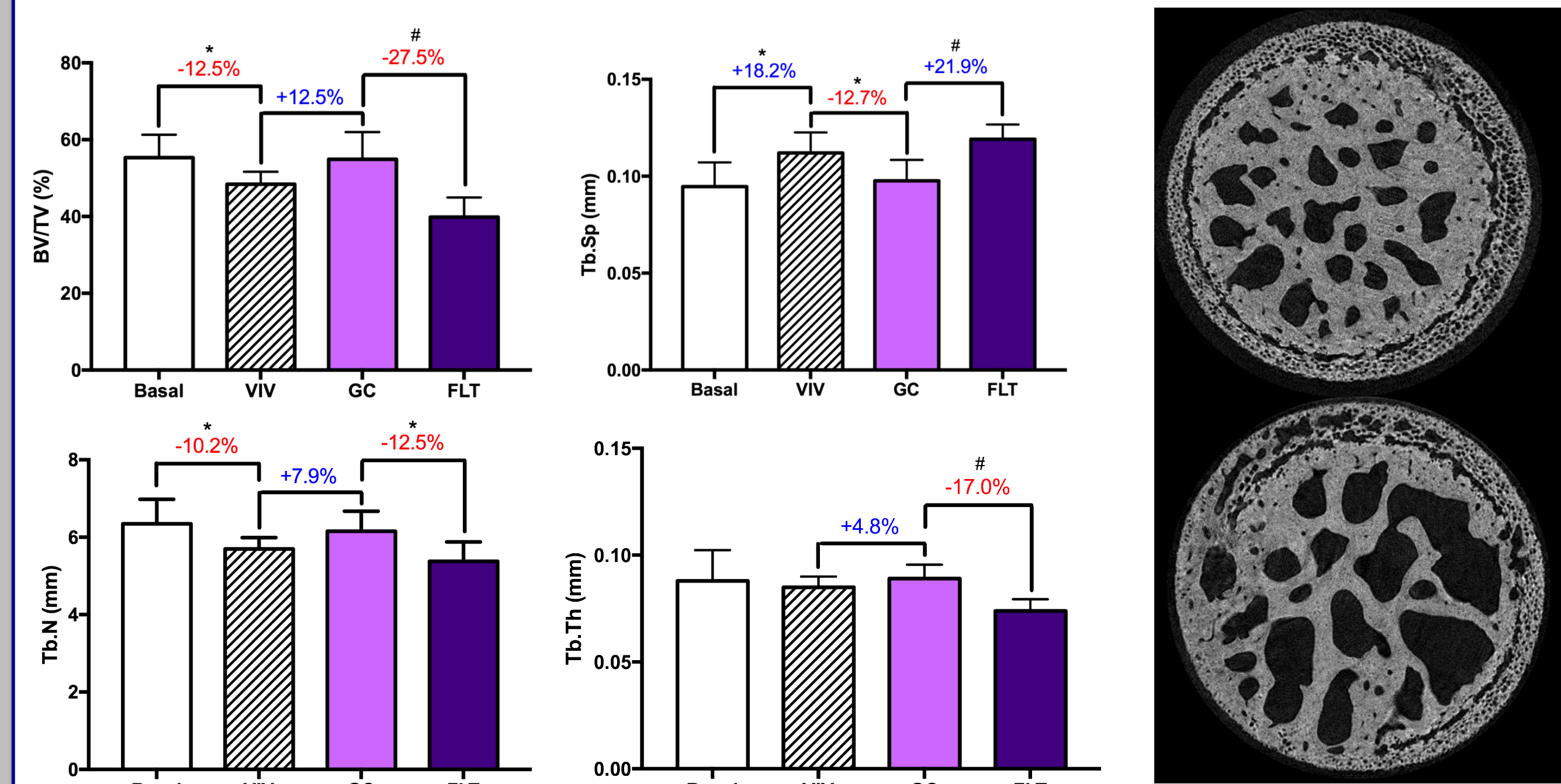
ethanized and dissection partially on orbit. Eight mice were euthanized and frozen for dissection on the ground. Analysis parameters: Micro-Computed Tomography, mechanical testing (three point bending), osteoblastogenesis and mineralized nodule formation, osteoclastogenesis and resorption assays, histology (TRAP staining and BrdU analysis), histomorphometry (bone formation rates, osteoclast activity) RT-qPCR analysis of key genes of interest, Affymetrix microarray whole genome expression profiling.

## Results

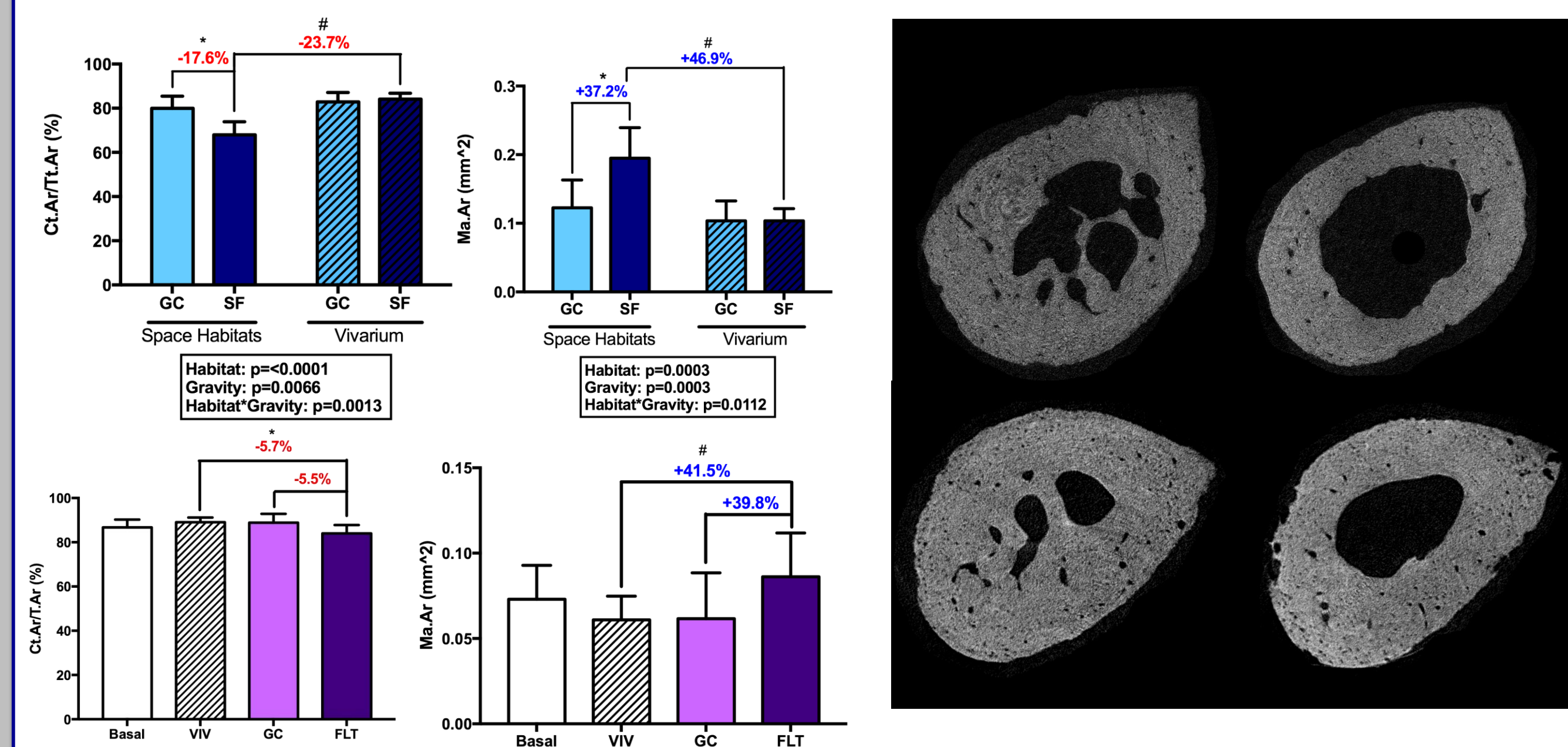
### MicroCT of the femoral head reveals significant bone loss in both male and female mice



MicroCT analysis of trabecular bone from the femoral head from male mice flown on BionM1 reveals significant bone loss due to both a decrease in trabecular number (Tb.N) and a decrease in trabecular thickness (Tb.Th).



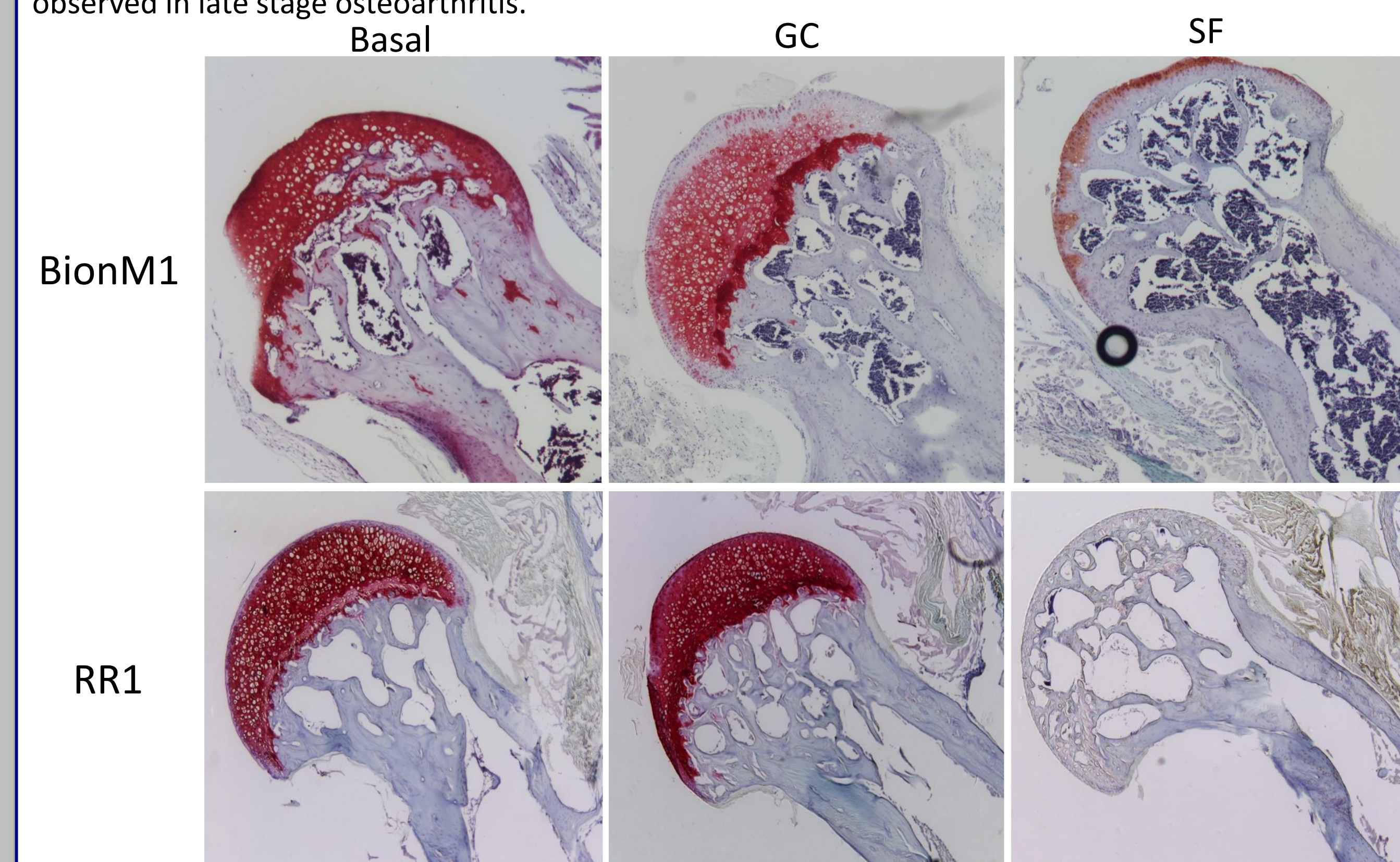
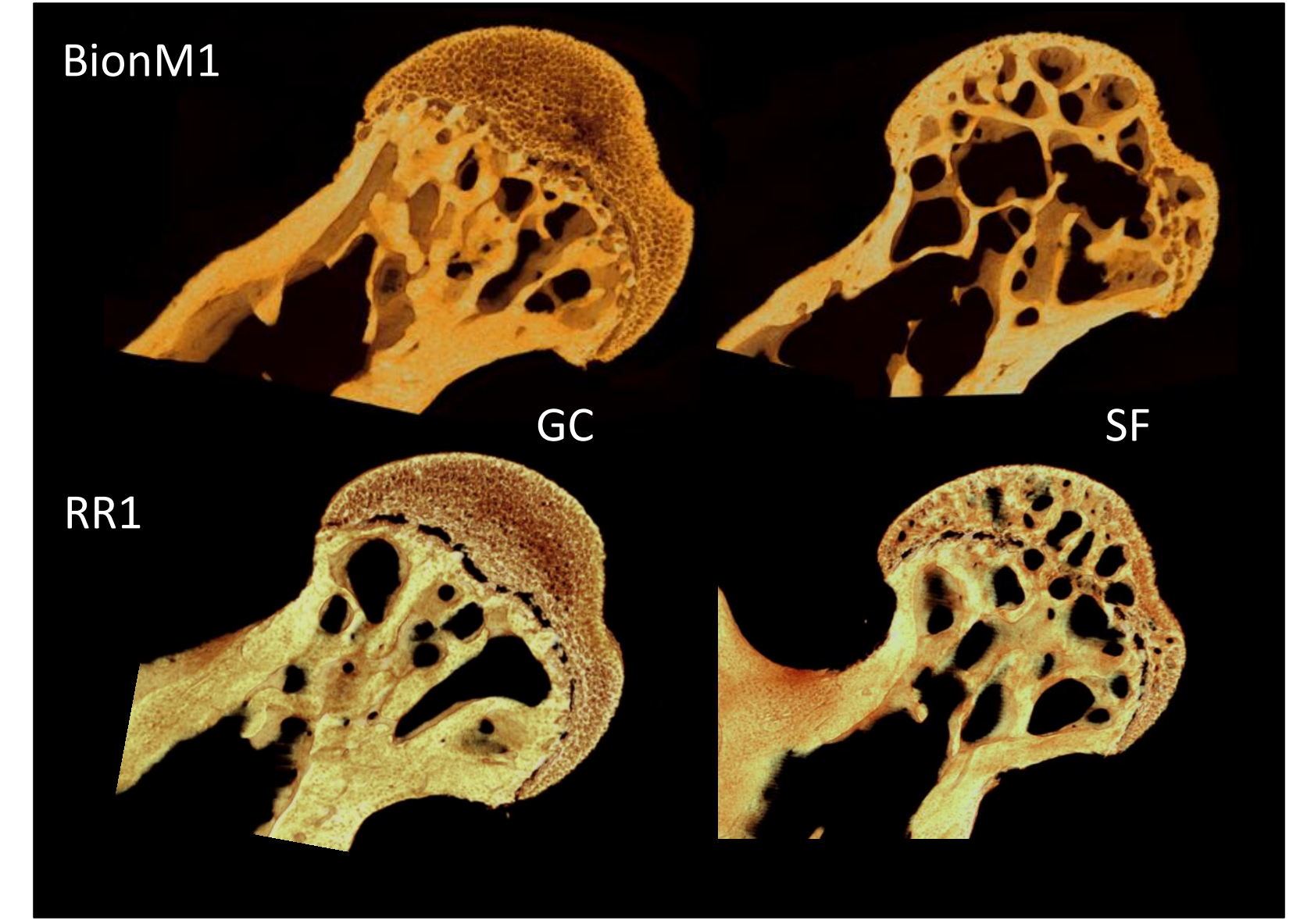
MicroCT analysis of trabecular bone from the femoral head from female mice flown on ISS reveals significant bone loss due mainly to a decrease in trabecular thickness (Tb.Th). Furthermore, ground control mice exhibited a trend for increased bone volume fraction potentially due to mechanical stimulation from the hardware design.



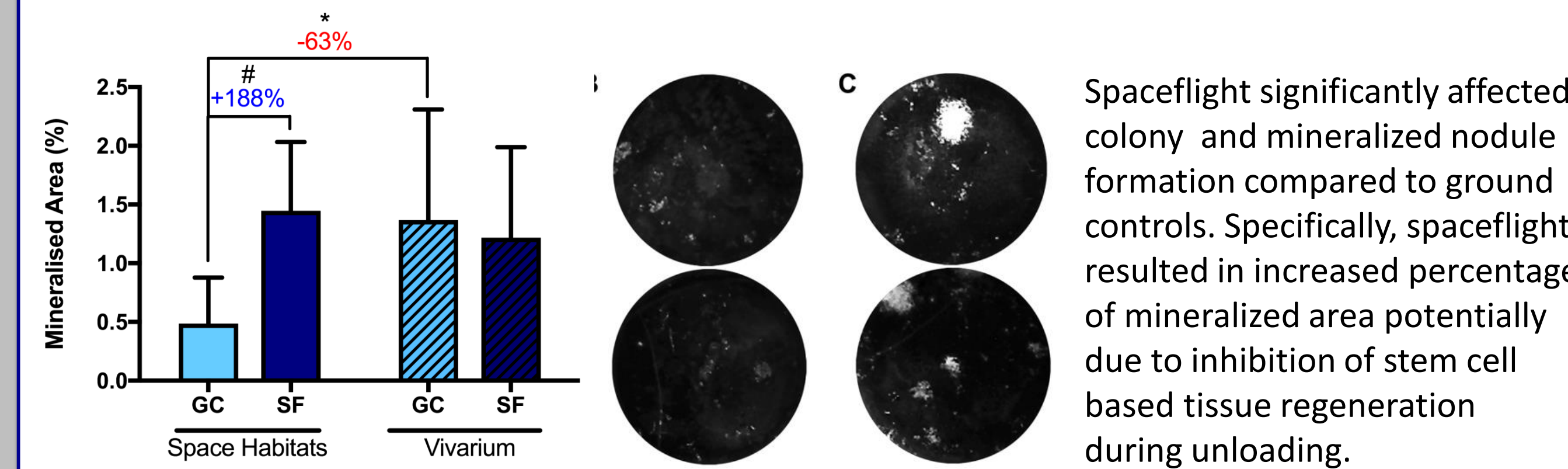
MicroCT analysis of the cortical bone in the femoral neck exhibits a significant decrease in cortical bone volume fraction and an increase in marrow area in both male and female mice exposed to long-duration spaceflight. This is indicative of a widening of the femoral neck and is similar to that which occurs during aging.

### Long duration spaceflight also causes significant alterations to the tissue structure of the femoral head

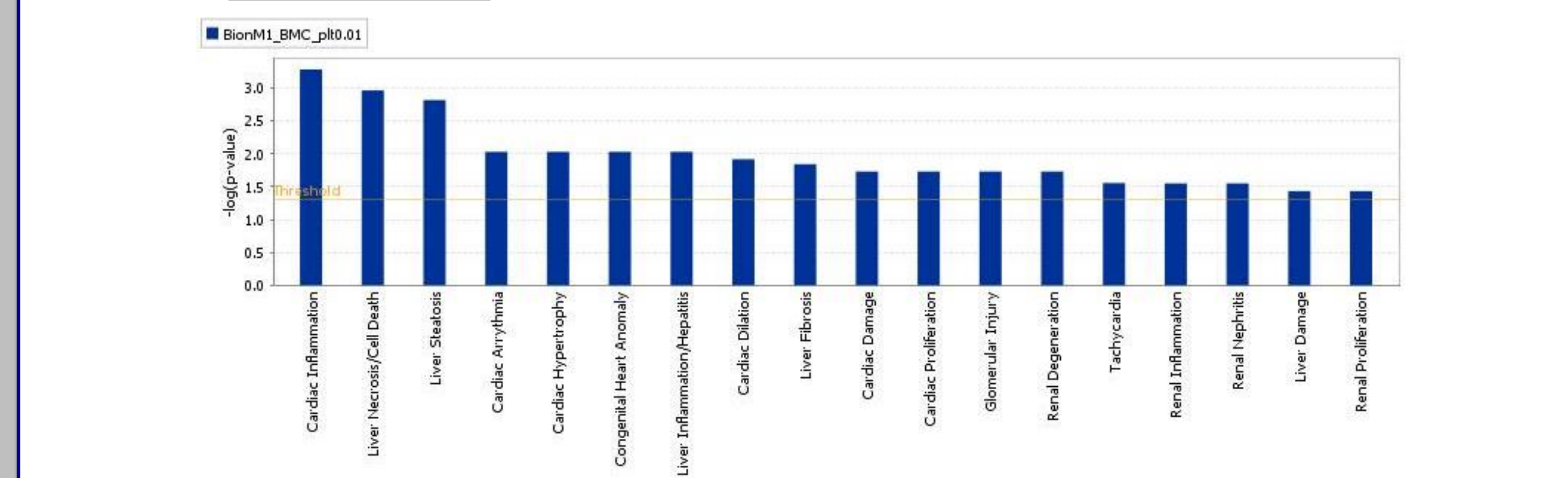
High resolution MicroCT analysis of the femoral head revealed significant alterations in tissue structure including disruption of the epiphyseal boundary in the femoral head, resulting in endochondral ossification and perforation of articular cartilage by bone. Immunohistochemical analysis and Saffranin-O staining reveal the extent of cartilage degeneration and invasion of bony structures following long-duration spaceflight. This phenotype is similar to that observed in late stage osteoarthritis.



### Stem Cell Differentiation Potential Following Mechanical Unloading



Spaceflight significantly affected colony and mineralized nodule formation compared to ground controls. Specifically, spaceflight resulted in increased percentage of mineralized area potentially due to inhibition of stem cell based tissue regeneration during unloading.



## Conclusions

Our results indicate that long duration mechanical unloading in spaceflight results in significant bone loss, disruption of the epiphyseal boundary and endochondral ossification of the femoral head, and an inhibition of stem cell based tissue regeneration, which, taken together, may indicate onset of an accelerated aging phenotype with signs of osteoarthritic disease.

**Acknowledgements:** This work was supported by a NASA Space Biology Grant NNN14ZTT001N-0063 to E. Almeida and NNN14ZTT001N-0062 to E. Blaber