Characterization of Craniofacial Morphology of DMP-1 Promotor-driven Dicer Deficient Mouse

2024 Research Aid Awards (RAA)

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FollowUp Form

Award Information

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:

- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Title of Project:*
Characterization of Craniofacial Morphology of DMP-1 Promotor-driven Dicer Deficient Mouse

Award Type
Research Aid Award (RAA)

Period of AAOF Support
July 1, 2024 through June 30, 2025

Institution
The Board of Trustees of the University of Illinois

Names of principal advisor(s) / mentor(s), co-investigator(s) and consultant(s)
Phimon Atsawasuwan (mentor, Co-investigator)

Amount of Funding
$6,000.00

Summary/Abstract*

(Referring to your original abstract, summarize the findings of your project)

In summary, loss of Dicer in early-stage osteocytes led to significant alterations in the femoral and calvarial phenotypes at both microscopic and macroscopic levels as decreased trabecular thickness, decreased
trabecular bone mineral density, increased cortical bone thickness and increased cortical bone porosity in femurs and increased bone volume fraction in calvaria. These phenotypic alterations appear to change certain dimensions of calvaria shape and femoral presentation. The differences of certain bone parameters seem to be
more pronounced in male mice. Future directions should include further studies to elucidate the mechanisms and pathways that lead to dysmorphic bone phenotypes in the DicerKO group and use of an animal model closer to human tissue.

Comment: *Summary/Abstract is missing. Be objective and succinct, but give the orthodontic community a synopsis of the most important findings of your project and enter the information into the allotted space.*

Respond to the following questions:

Detailed results and inferences:*

If the work has been published, please attach a pdf of manuscript below by clicking "Upload a file".
OR
Use the text box below to describe in detail the results of your study. The intent is to share the knowledge you have generated with the AAOF and orthodontic community specifically and other who may benefit from your study. Table, Figures, Statistical Analysis, and interpretation of results should also be attached by clicking "Upload a file".

Final Report AAOF.pdf
See attached.

Comment: *Please include more details of the study's results within the allotted space. Be objective and succinct, but give the orthodontic community a synopsis of the most important findings. The final report should not simply be a poster or publication.*

Were the original, specific aims of the proposal realized?*

Yes these aims were realized. The aim of this study was to compare the calvarial and femoral bone phenotypes on both microscopic and macroscopic levels of the Dicer deficiency driven under DMP-1 promoter (Dicer KO) mice compared to their sex-matched and age-matched control mice (Dicer floxed and DMP-1 cre transgenic mice) using microcomputed tomography, morphometric analysis, shape analysis and histological sections under Hematoxylin and Eosin staining. Loss of Dicer in early-stage osteocytes lead to significant alterations in the femoral and calvarial phenotypes at both microscopic and macroscopic levels as decreased trabecular thickness, decreased trabecular bone mineral density, increased cortical bone thickness and increased cortical bone porosity in femurs and increased bone fraction in calvaria. These phenotypic alterations appear to change certain dimensions of calvaria shape leading to a distinct craniofacial

morphology in DicerKO mice. The differences of certain bone parameters seem to be more pronounced in male mice.

Comment: *Please list the aim(s) of the proposal.*

Were the results published?*

No

Have the results of this proposal been presented?*

Yes

To what extent have you used, or how do you intend to use, AAOF funding to further your career?*

All funds provided by the AAOF Research Aid Award have been used. I am extremely grateful for this Research Aid Award as these funds have provided me with the opportunity to pursue this project which will help us further understand the role of functional microRNA in osteocytes which has not been well studied. This new information will enhance our understanding of how these biomolecules control bone modeling during development. These funds also allowed me the opportunity to present this research via an e-poster at the 2026 AAO conference and at the 2026 UIC College of Dentistry Clinic and Research Day in which it won first place in the postgraduate student, basic science category.

Accounting: Were there any leftover funds?*

$0.00

Not Published

Are there plans to publish? If not, why not?*

We are collecting additional parts of the experiment and plan to publish the results in a peer-reviewed scientific journal soon. AAOF will be acknowledged in the publication. The manuscript will be submitted to the 2026 AAO resident award competition.

Presented

Please list titles, author or co-authors of these presentation/s, year and locations:*

The results have been presented at
1. Z Shaker, G Viana, S Lown, M Nahhas, A George, D Reed, C Nicholas, P Atsawasuwan. Bone Characterization of Dicer Deficiency Transgenic Mouse Driven by Dmp1 Promoter. 2026 University of Illinois Clinic and Research day. February 2026, UIC College of Dentistry, Chicago, IL. (won 1st place postgraduate student, basic science category).
2. Z Shaker, G Viana, S Lown, M Nahhas, A George, D Reed, C Nicholas, P Atsawasuwan. Bone Characterization of Dicer Deficiency Transgenic Mouse Driven by Dmp1 Promoter. 2026 Annual session of AAO. Orlando, FL.

Was AAOF support acknowledged?

If so, please describe:

Yes. The funding from AAOF was acknowledged in both presentations and will be acknowledged in future presentation.

Internal Review

Reviewer comments

summary/abstract added and approved via email by Dr. Rody-GR

Reviewer Status*

Approved

File Attachment Summary

Applicant File Uploads

- Final Report AAOF.pdf

Bone Characterization of Dicer Deficiency Transgenic Mouse Driven by Dmp1 Promoter

Histology:

Hematoxylin and Eosin(H &E) Staining was performed on both calvarial and femoral samples to visualize the thickness of cortical bone, suture patency and cell distribution. There were no apparent differences between the calvarial bone between all of the genotypes- both cortical thickness and sutural appearance appeared similar in all genotypic groups. There was increased cortical thickness and cortical bone porosity in the femoral cortical bone in the DicerKO group when compared to the control groups (Dicer floxed and DMP1cre groups). This phenotype appeared more pronounced in males. The DicerKO group also exhibited decreased trabecular thickness and density in their epiphyseal plates when compared to the control groups. (Figures 1-4)

Shape analysis:

Generalized Procrustes Analysis (GPA) was run, and a Procrustes ANOVA was conducted that revealed that genotype was a significant factor influencing shape and accounted for 9.2% of morphological variation. A Principal Components Analysis (PCA) was conducted and PC1-PC5 were selected for further evaluation as they each represented >5% of the total variation. Each PC represents a major axis of shape variation. Differences between genotypes were a significant source of variation on PC3 (9.2% of total variation; $p=0.002$). PC3 corresponds to anterior calvarial height and width. Post-hoc comparison was completed and revealed significant morphological differences between DicerKO and DMP1cre groups ($p<0.001$). Differences between age groups (4 and 8 weeks) were a significant source of variation along the first 4 PCs. There was a significant interaction between genotype and age ($p=0.002$), suggesting that genotypes had different calvarial shapes at different ages. There was also a significant interaction on the first 5 PCs between sex and age ($p=0.028$) suggesting that male and female groups had different morphological phenotypes/shapes at different ages, but interaction between genotype and sex was not significant ($p=0.054$). An age-matched comparison of centroid sizes was completed, and it was noted that there were no significant differences in centroid size between the genotypes in each age group ($p=0.902$), therefore supporting that the differences noted in the PCA is solely due to shape. (Figures 5 and 6).

Morphometric analyses: Post-hoc analyses including Bonferroni correction and Games-Howell tests were used and identified the following femoral and calvarial phenotypic differences between genotypes within the same age and sex groups.

Femoral Morphometric Analysis:

4 week females: DMP1cre exhibited a significantly smaller maximum femoral bone length($p=0.040$), smaller femoral mediolateral midshaft width ($p=0.002$) and larger femoral bone torsion ($p<0.001$) than DicerKO. No significant differences were noted in femoral biomechanical bone length or femoral epicondylar bone breadth between the genotypes. (Figure 7)

4-week males: DMP1cre had a significantly shorter maximum femoral bone length ($p<0.001$), significantly shorter femoral biomechanical bone length($p<0.001$), significantly narrower femoral mediolateral midshaft width($p=0.003$) and a significantly greater femoral bone torsion($p<0.001$) than DicerKO. DicerKO had than Dicer flox ($p=0.027$) and DMP1cre ($p<0.001$). No significant differences were noted in femoral epicondylar bone breadth between the genotypes. (Figure 7)

8-week females: The DicerKO group exhibited a significantly smaller femoral bone torsion than both control groups: Dicer flox ($p<0.001$), and DMP1cre ($p<0.001$). No other significant differences were noted. (Figure 8)

8-week males: The DicerKO group exhibited significantly smaller femoral bone torsion than the DMP1cre ($p<0.001$) group. No other significant differences were noted. (Figure 8)

Calvaria Morphometric Analysis:

4-week females: Dicer KO exhibited significantly smaller frontal($p=0.44$) and parietal ($p=0.27$) lengths than the DMP1cre group. The DicerKO group exhibited significantly smaller intermaxillary width than both control groups Dicer flox ($p<0.001$) and DMP1cre ($p<0.001$).(Figure 9)

4-week males: DicerKO group exhibited significantly smaller frontal length, parietal length and intermaxillary width than both control groups Dicer flox ($p=0.003$; $p<0.001$; $p<0.001$) and DMP1cre ($p=0.001$; $p<0.001$; $p<0.001$). DMP1cre exhibited a significantly narrower maximum width than DicerKO ($p=0.007$) and Dicer flox ($p=0.007$) groups. (Figure 9)

8-week females: Dicer KO group exhibited statistically smaller frontal length, parietal length and intermaxillary width when compared to both control groups Dicer flox ($p=0.003$; $p<0.001$; $p<0.001$) and DMP1cre ($p=0.001$; $p<0.001$; $p<0.001$). DMP1cre also exhibited a narrower maximum width than DicerKO ($p=0.007$) and Dicer flox ($p=0.007$). (Figure 10)

8-week males: DicerKO group exhibited a significantly shorter nasal length and intermaxillary width than both control groups: Dicer flox ($p=0.026$; $p<.001$) and DMP1cre ($p=0.009$; $p<0.001$). DicerKO mice showed a significantly decreased frontal and parietal length than DMP1cre ($p=0.003$; $p=0.044$). Coronal suture length appeared significantly greater in DicerKO ($p=0.006$) and Dicer flox ($p=0.004$) when compared to DMP1cre. (Figure 10)

Femoral Trabecular Microcomputed Tomography (microCT):

One-way ANOVAs and post hoc analyses including Bonferroni correction and Games-Howell tests were run to determine the effect of genotype within specific age and sex subgroups for all microCT analyses.

Female 4 weeks: DMP1cre group had significantly decreased tissue volume than the DicerKO group ($p=0.003$) No significant differences were noted in any other parameters. (Figure 11)

Male 4 weeks: DMP1cre group had significantly decreased tissue volume and trabecular number than DicerKO ($p<0.001$; $p=0.046$). The DicerKO group had a significantly elevated bone volume than the DMP1cre group ($p=0.029$). No other significant differences were observed. (Figure 11)

Female 8 weeks: Dicer KO group exhibited decreased trabecular thickness compared to the DMP1cre group ($p=0.049$). No other statistically significant differences were observed (Figure 12)

Male 8 weeks: The DicerKO group had a significantly lower bone volume fraction, bone mineral density, and bone volume than Dicer flox($p=0.017$; $p=0.021$; $p=0.030$) and DMP1cre ($p=0.004$; $p=0.006$; $p=0.048$). The DicerKO group exhibited a significantly decreased trabecular thickness than DMP1cre ($p=0.032$). No other significant differences were observed. (Figure 12)

Femoral Cortical Microcomputed Tomography:

One-way ANOVAs and post hoc analyses including Bonferroni correction and Games-Howell tests were run to determine the effect of genotype within specific age and sex subgroups for all microCT analyses.

Female 4 weeks: DicerKO group exhibits significantly greater cortical thickness than DMP1cre group ($p=0.009$). No other statistically significant differences were observed. (Figure 13)

Male 4 weeks: DicerKO group exhibited significantly elevated cortical thickness($p<0.001$), cortical bone area ($p<0.001$) and cortical bone porosity compared to DMP1cre group. DicerKO group exhibited significantly elevated cortical bone area ($p<0.001$) compared to the DMP1cre group. Dicer flox group exhibited significantly elevated subperiosteal area compared to DMP1cre ($p=0.021$). No other statistically significant differences were observed. (Figure 13)

Female 8 weeks: No statistically significant differences were observed in any parameters between these subgroups. (Figure 14)

Male 8 weeks: Dicer flox group exhibited significantly elevated subperiosteal area when compared to DMP1cre group ($p=0.021$). DicerKO group exhibited significantly increased cortical bone porosity than the DMP1cre ($p<0.001$) and Dicer flox (p-0.002) groups. No other statistically significant differences were observed. (Figure 14)

Calvaria Microcomputed Tomography:

One-way ANOVAs and post hoc analyses including Bonferroni correction and Games-Howell tests were run to determine the effect of genotype within specific age and sex subgroups for all microCT analyses.

Female 4 weeks; Female 8 weeks; Male 8 weeks: No significant differences were observed between genotypes in any parameters within these subgroups. (Figure 15, 16)

Males 4 weeks: DMP1cre group exhibited a significantly lower bone volume fraction and bone mineral density than DicerKO ($p=0.025$; $p=0.045$) and Dicer flox groups ($p=0.020$; $p=0.020$). No other statistically significant differences were observed. (Figure 15)

In summary, loss of Dicer in early-stage osteocytes led to significant alterations in the femoral and calvarial phenotypes at both microscopic and macroscopic levels as decreased trabecular thickness, decreased trabecular bone mineral density, increased cortical bone thickness and increased cortical bone porosity in femurs and increased bone volume fraction in calvaria. These phenotypic alterations appear to change certain dimensions of calvaria shape and femoral presentation. The differences of certain bone parameters seem to be more pronounced in male mice. Future directions should include further studies to elucidate the mechanisms and pathways that lead to dysmorphic bone phenotypes in the DicerKO group and use of an animal model closer to human tissue.

Figures:

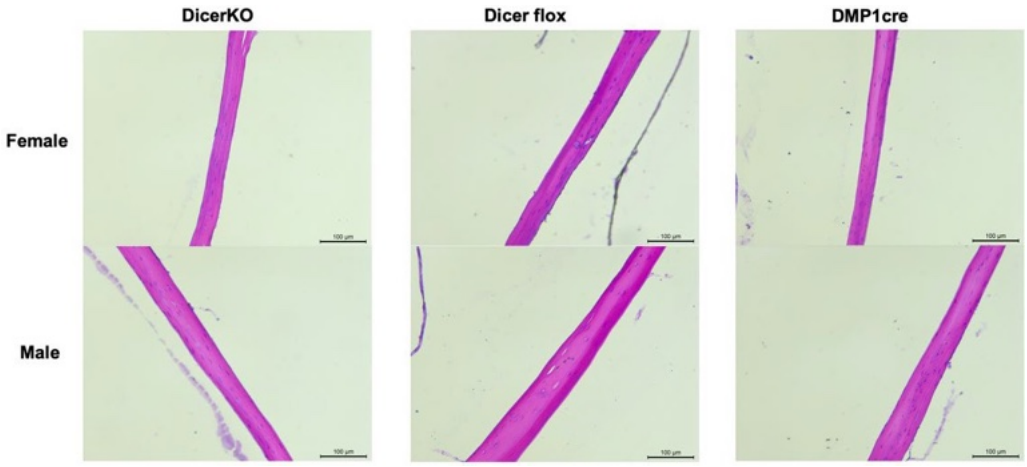

Figure 1: Photos of H &E stained sections of calvarial cortical bone. Photos were taken at 20x magnification. These images show minimal differences in genotype of calvarial cortical bone thickness. All genotypes and genders are shown. The measurement bar noted in the lower right corner of each image represents 100 µms. (See Appendix B for long description)

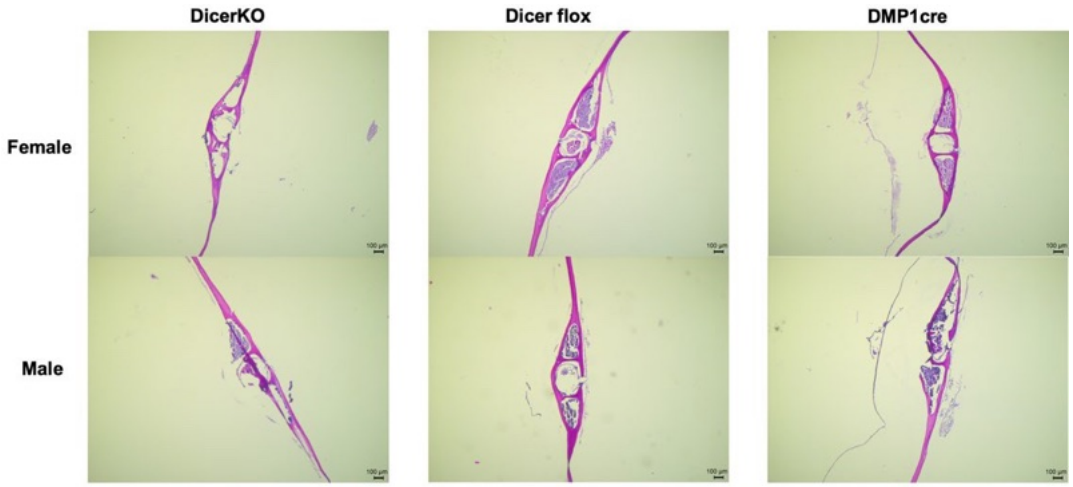

Figure 2: Photos of H & E stained sections of calvarial posterior frontal suture. Photos were taken at 4x magnification. These images show minimal differences in genotype of posterior frontal sutures. All genotypes and genders are shown. The measurement bar noted in the lower right corner of each image represents 100 µms. (See Appendix B for long description)

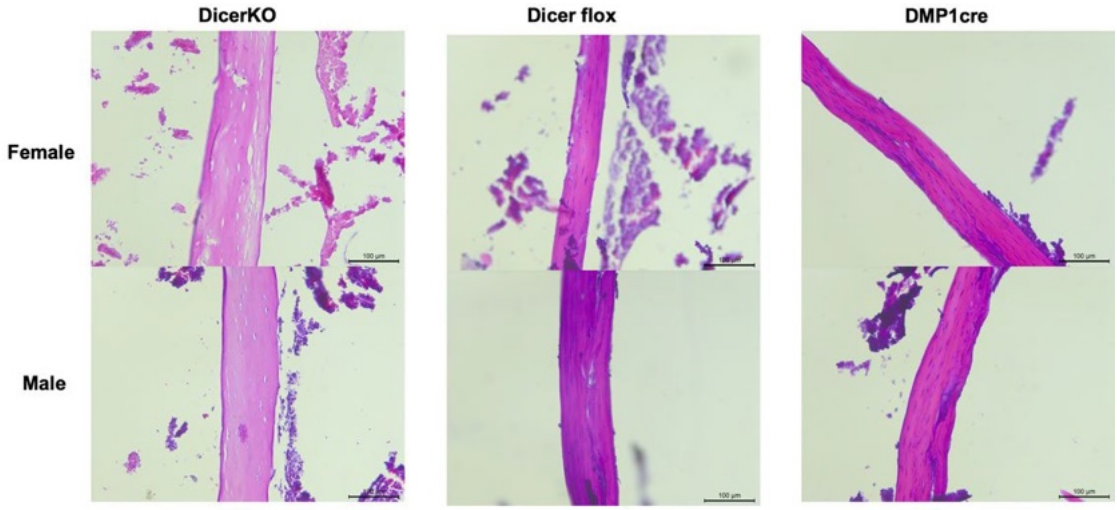

Figure 3: Photos of H &E stained sections of femoral cortical bone. Photos were taken at 20x magnification. These images show that the DicerKO group exhibits thicker cortical bone and increased bone porosity when compared to Dicer flox and DMP1cre groups. This appears to be more pronounced in males. All genotypes and genders are shown. The measurement bar noted in the lower right corner of each image represents 100 µms. (See Appendix B for long description)

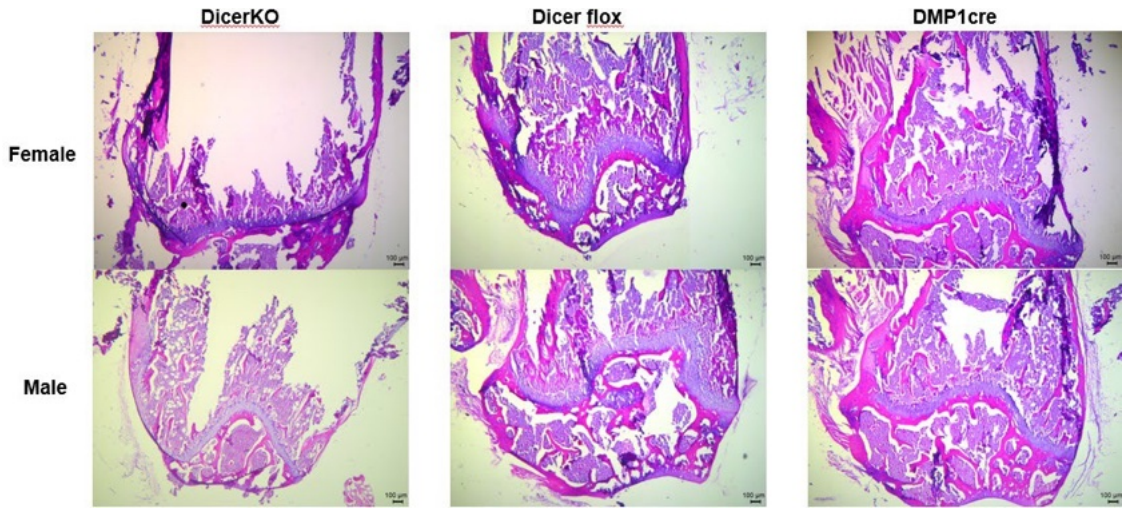

Figure 4: Photos of H &E stained sections of femoral epiphyseal (growth) plate. Photos were taken at 4x magnification. These images show decreased trabecular thickness and density in the DicerKO group when compared to the Dicer flox and DMP1cre groups. All genotypes and genders are shown. The measurement bar noted in the lower right corner of each image represents 100 µms. (See Appendix B for long description)

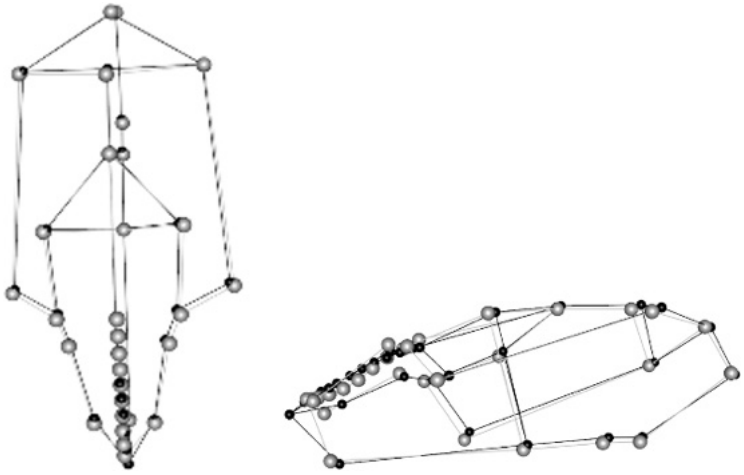

Figure 5: Schematic of the average ranges of the General Procrustes Analysis for variation in PC3 of the calvaria in DicerKO and DMP1cre groups. This schematic shows lateral and superior views of the average shapes of DicerKO (gray) and DMP1cre (black) samples on PC3. DicerKO and DMP1cre groups differed significantly on PC3. This shows minor but significant differences in cranial height and anterior width and length.

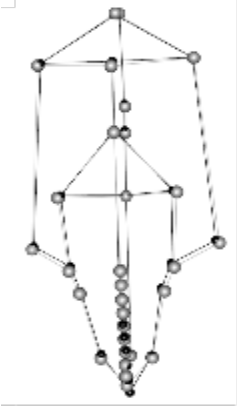

Figure 6: Schematic of the minimum and maximum ranges of the General Procrustes Analysis for variation in PC3 of the calvaria in all three genotypes. This schematic shows the superior view of samples contributing the greatest and least variation in PC3. This suggests that variations in PC3 are due to differences in anterior calvarial width and height.

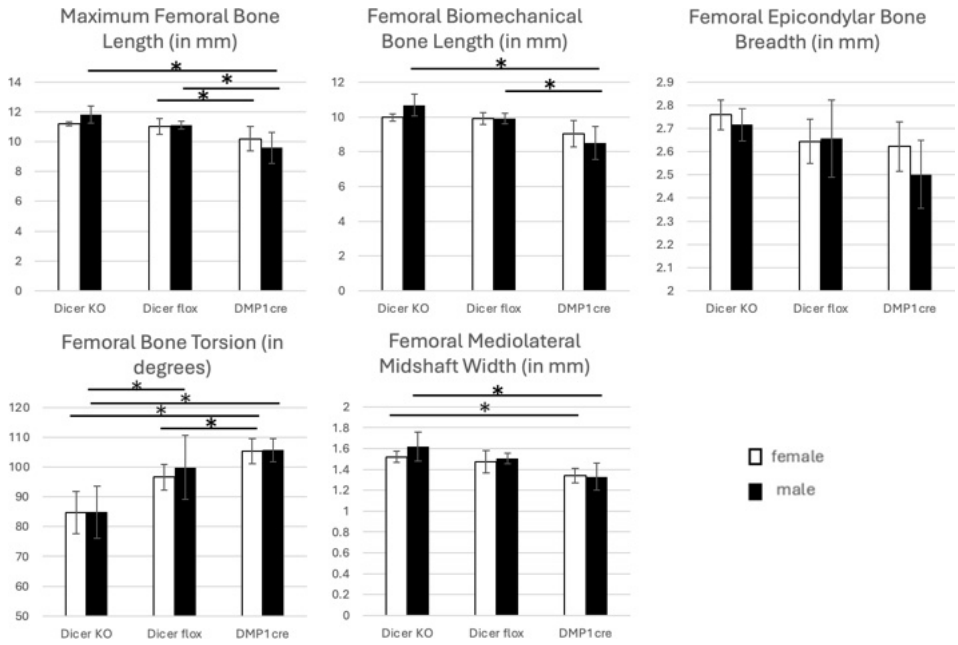

Figure 7: Comparison of femoral linear and angular morphometric measurements at 4 weeks amongst all genotypes. Differences between DicerKO, and DMP1cre are noted in most measurements. No significant differences were found amongst the genotypes in the femoral epicondylar bone breadth measurement. *P<0.05

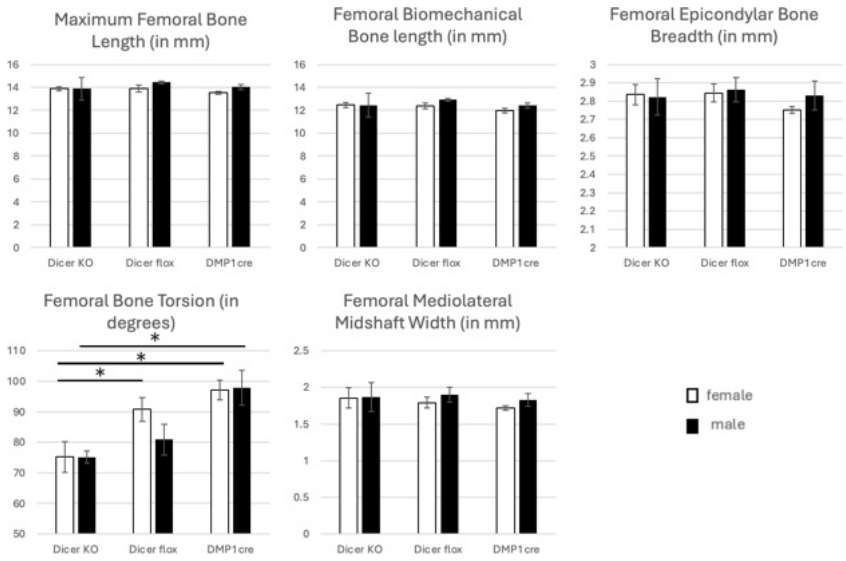

Figure 8: Comparison of femoral linear and angular morphometric measurements at 8 weeks amongst all genotypes. Differences between DicerKO, and the control groups are noted only in the femoral bone torsion measurements. No significant differences were found in any of the linear measurements. *P<0.05

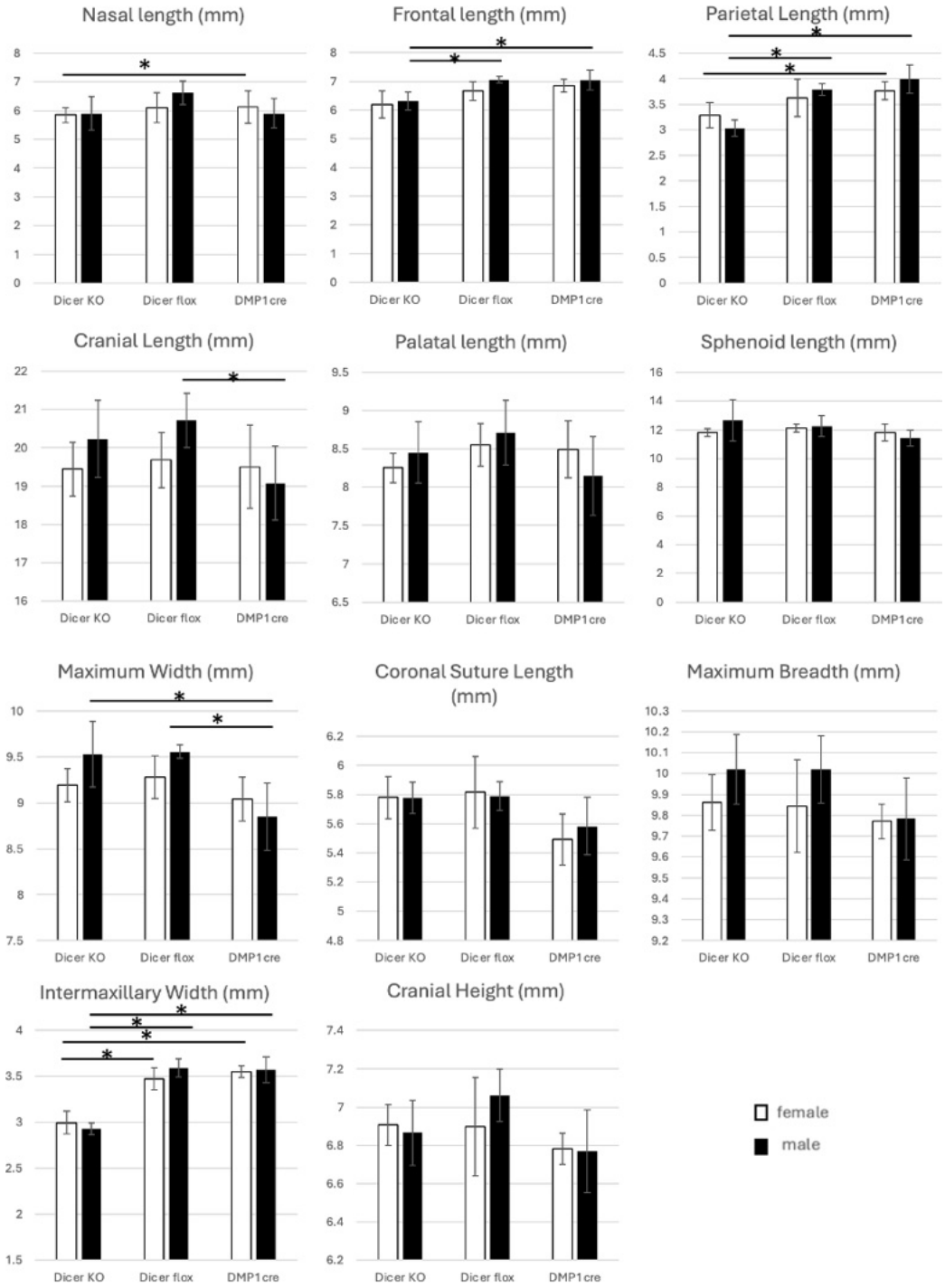

Figure 9: Comparison of linear measurements of calvaria at 4 weeks amongst all genotypes. Differences between DicerKO, and 1 or more control groups are noted in nasal length, frontal length, parietal length, maximum width and intermaxillary width measurements. *P<0.05

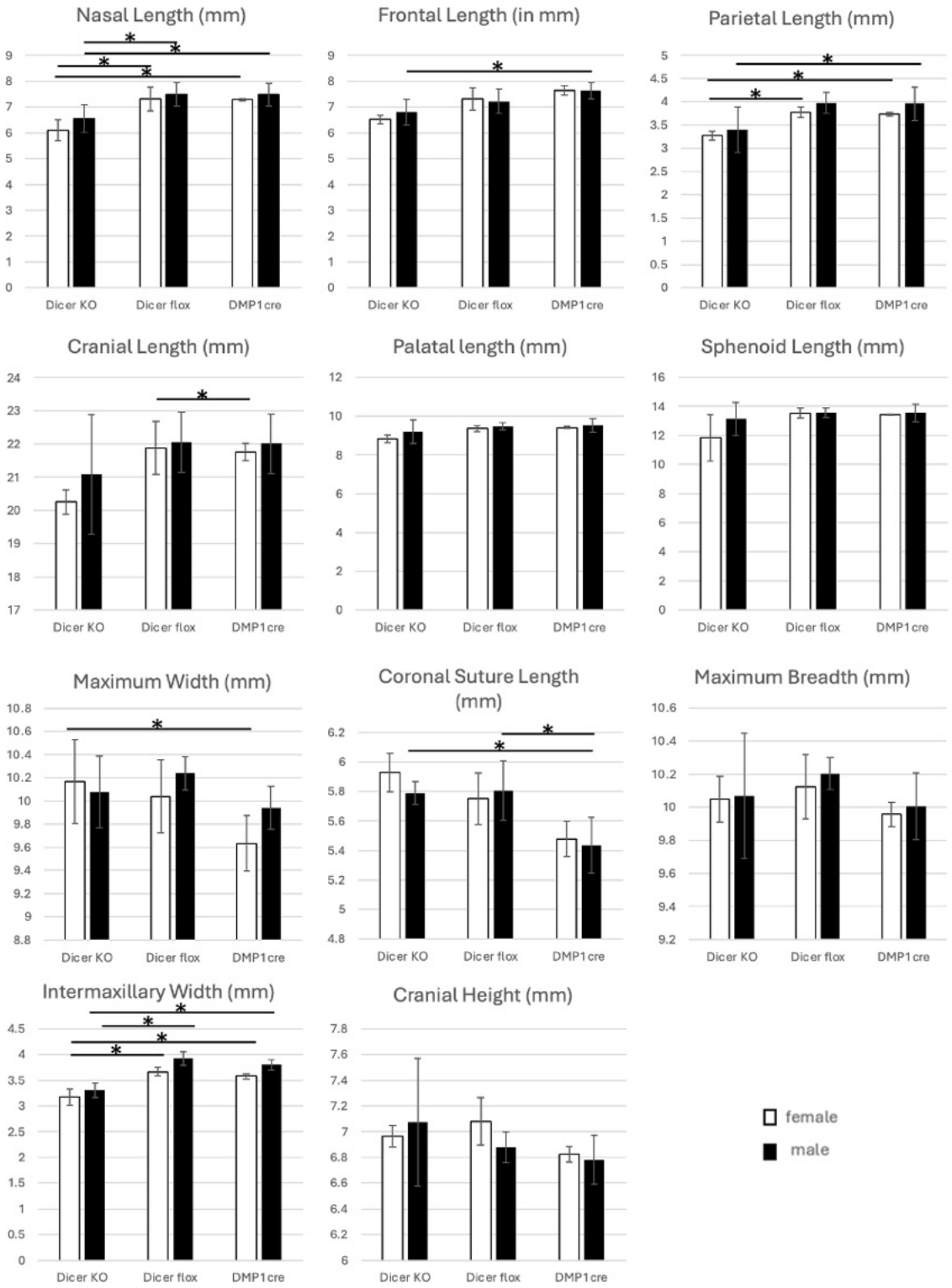

Figure 10: Comparison of linear measurements of calvaria at 8 weeks amongst all genotypes. Differences between DicerKO, and 1 or more control groups are noted in nasal length, frontal length, parietal length, maximum width, coronal suture length and intermaxillary width measurements. *P<0.05

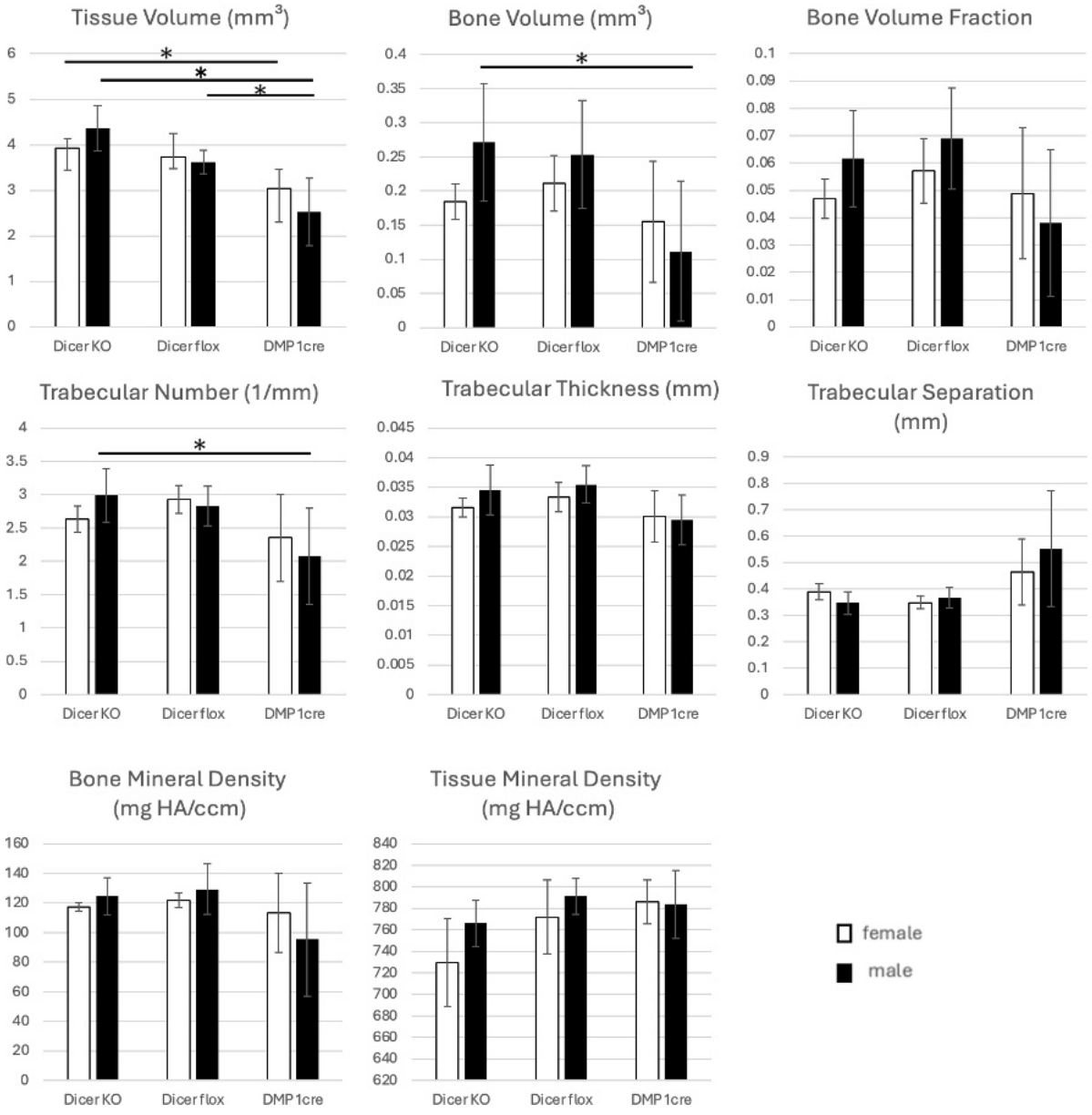

Figure 11: Comparison of femoral trabecular µCT parameters at 4 weeks amongst all genotypes. Differences between DicerKO, and 1 or more control groups are noted in tissue volume, bone volume and trabecular number parameters. These differences are more prevalent in males. *P<0.05

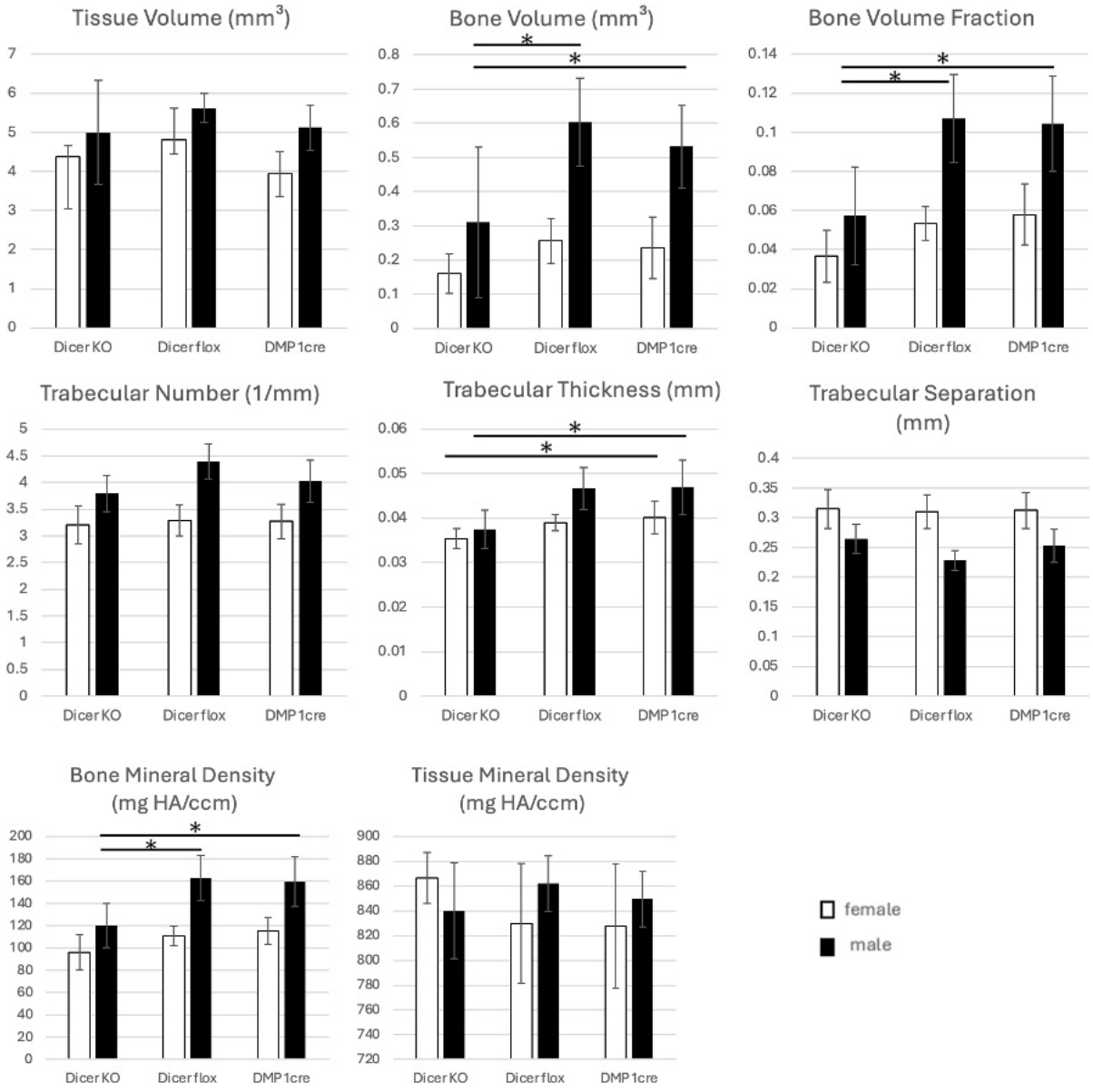

Figure 12: Comparison of femoral trabecular µCT parameters at 8 weeks amongst all genotypes. Differences between DicerKO, and 1 or more control groups are noted in bone volume, bone volume fraction, trabecular thickness and bone mineral density parameters. These differences are more pronounced in males. *P<0.05

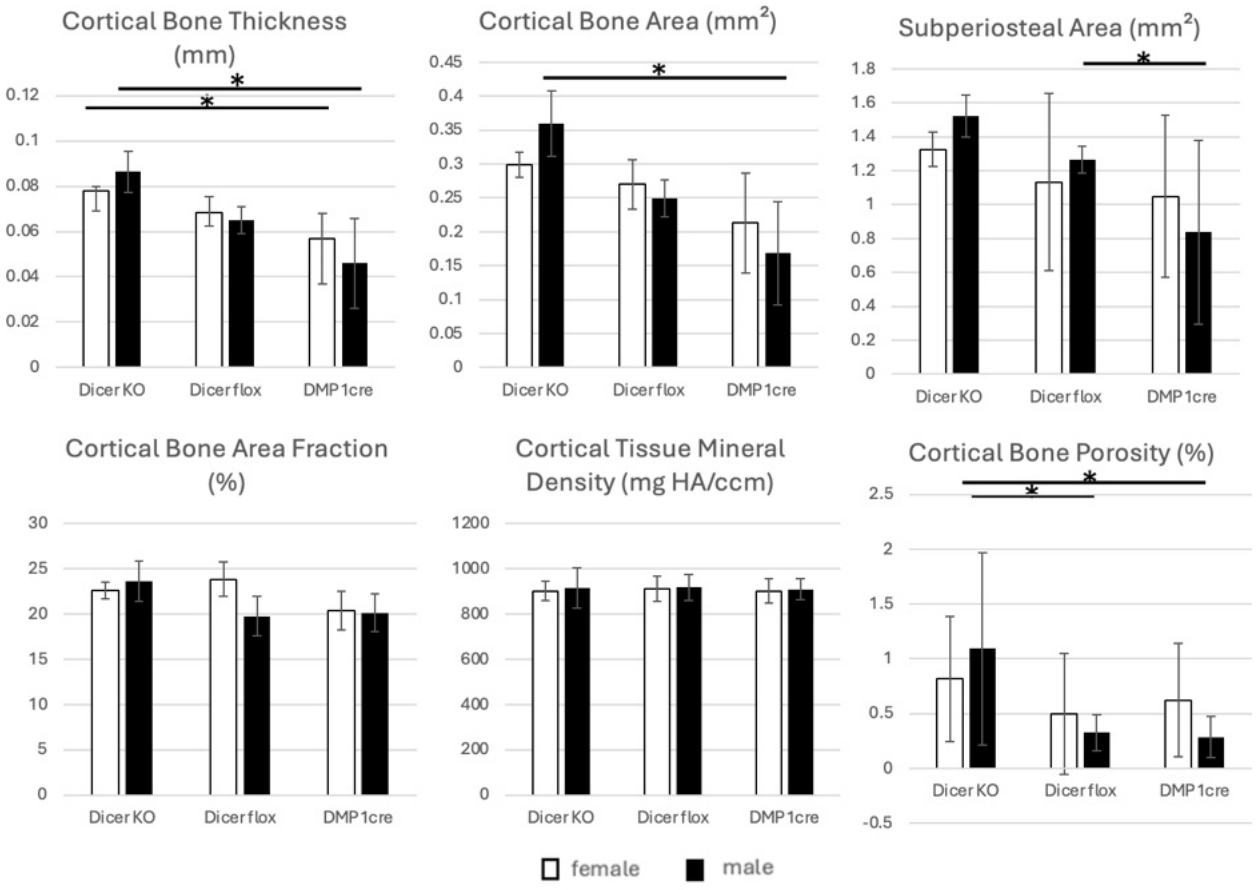

Figure 13: Comparison of femoral cortical µCT parameters at 4 weeks amongst all genotypes. Differences between DicerKO, and 1 or more control groups are noted in cortical bone thickness, area and porosity parameters. A significant difference is noted between the control groups in subperiosteal area. These significant differences are more prevalent in males. *P<0.05

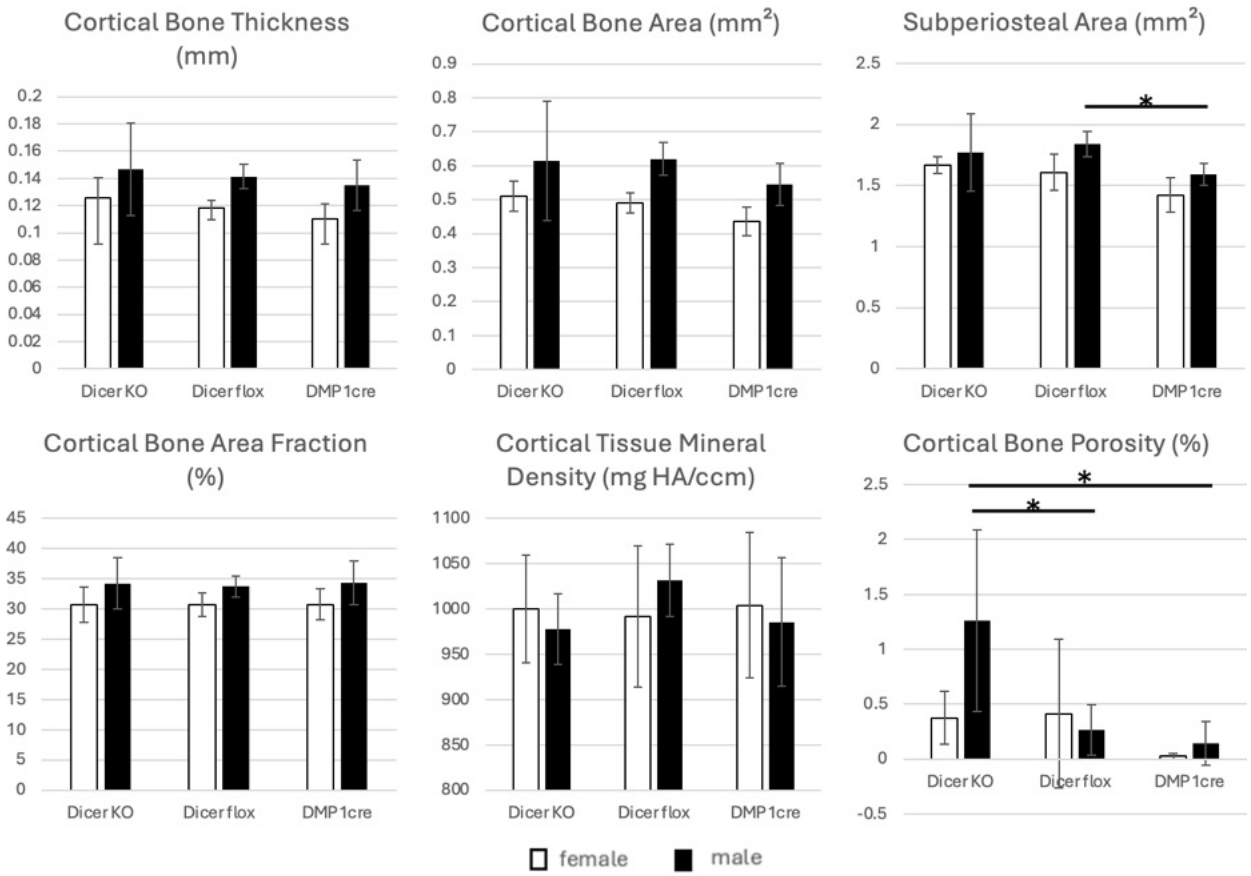

Figure 14: Comparison of femoral cortical µCT parameters at 8 weeks amongst all genotypes. Differences between DicerKO, and 1 or more control groups are noted in cortical bone porosity parameters in males. A significant difference is noted between the male control groups in subperiosteal area. *P<0.05

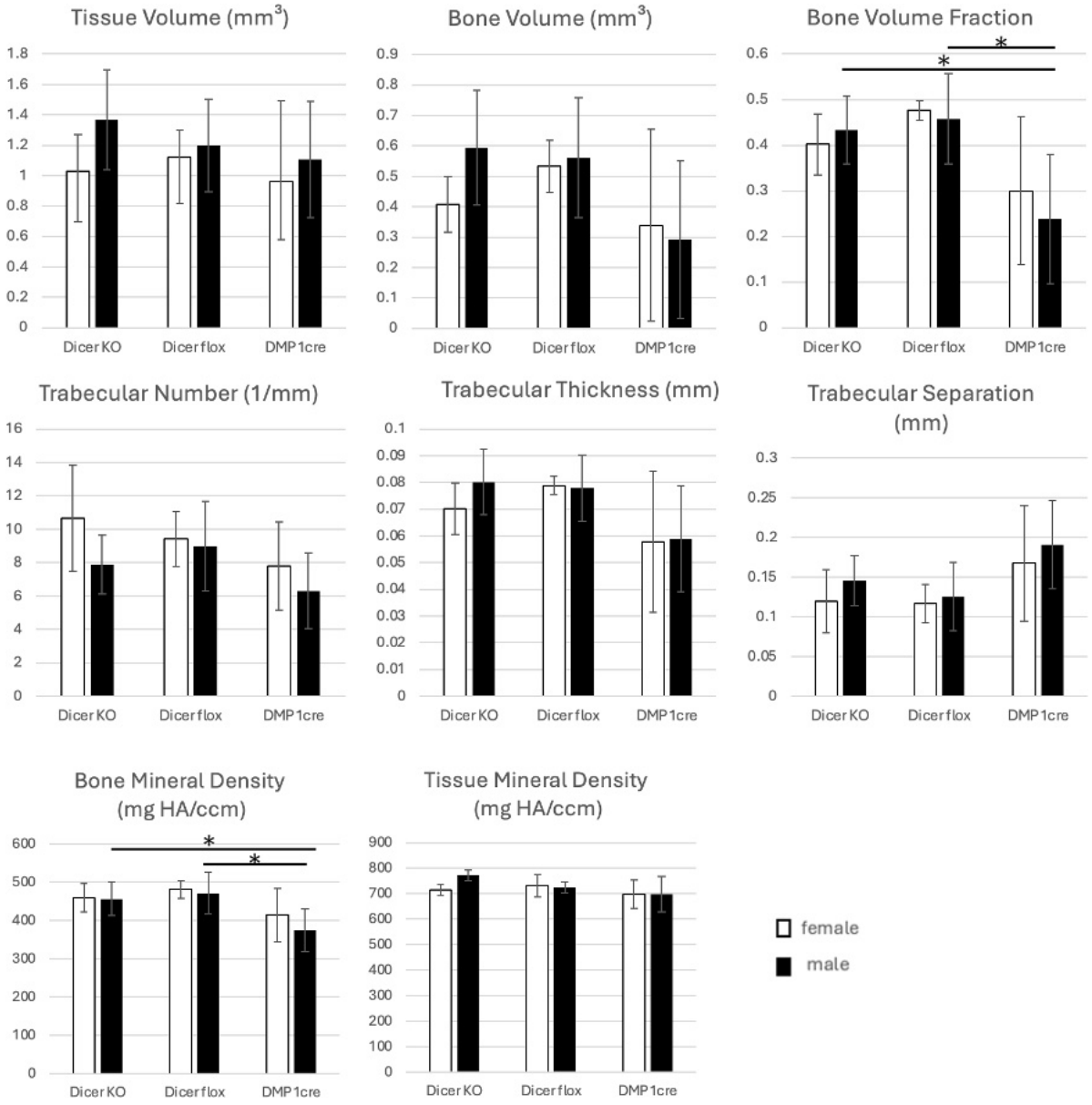

Figure 15: Comparison of trabecular parameters of calvaria at 4 weeks amongst all genotypes. Significant differences are noted in bone volume fraction and bone mineral density between Dicer flox and DMP1cre and DicerKO groups measurements. *P<0.05

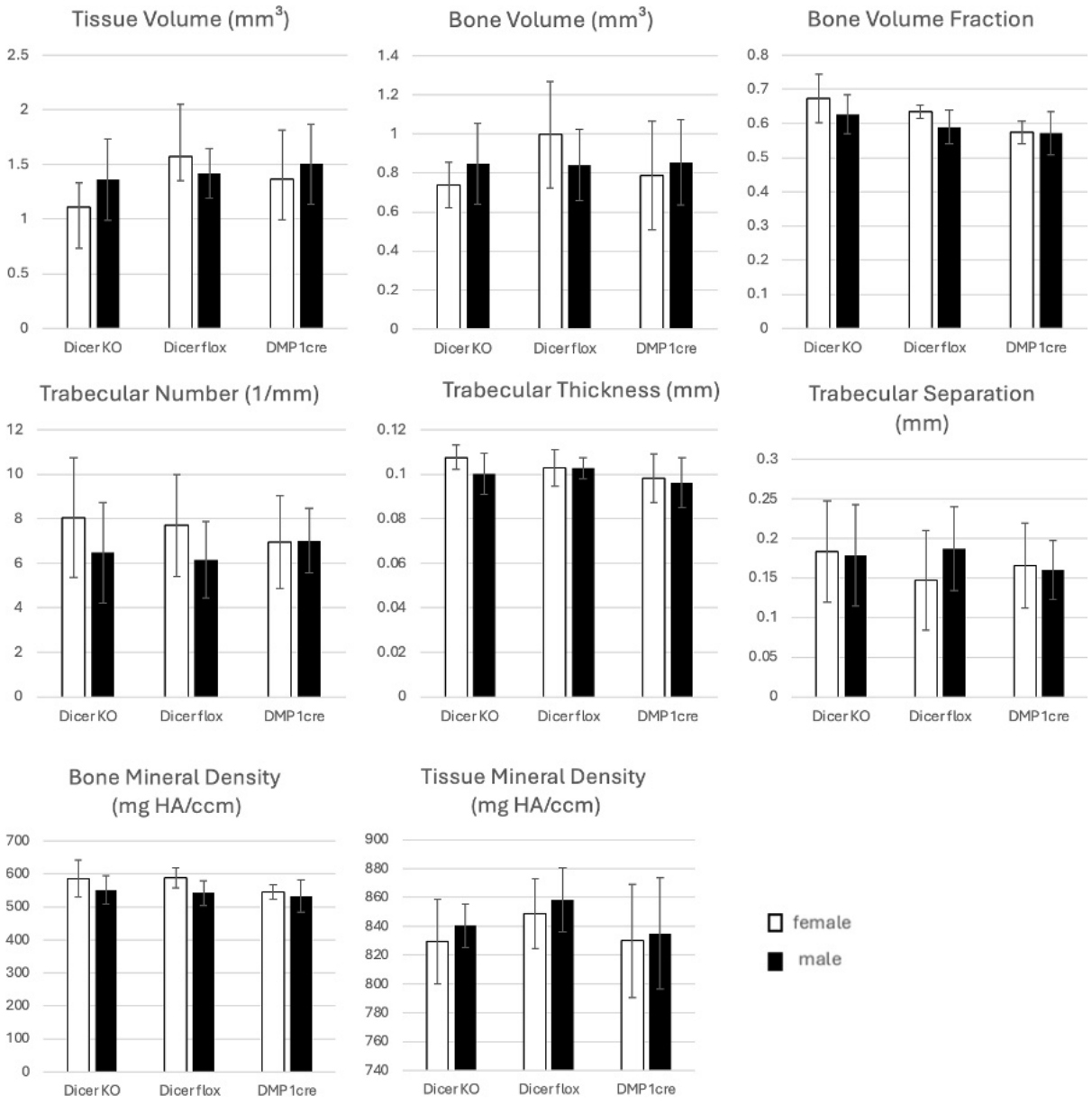

Figure 16: Comparison of trabecular parameters of calvaria at 8 weeks amongst all genotypes. No significant differences were noted in any of the parameters listed. *P<0.05