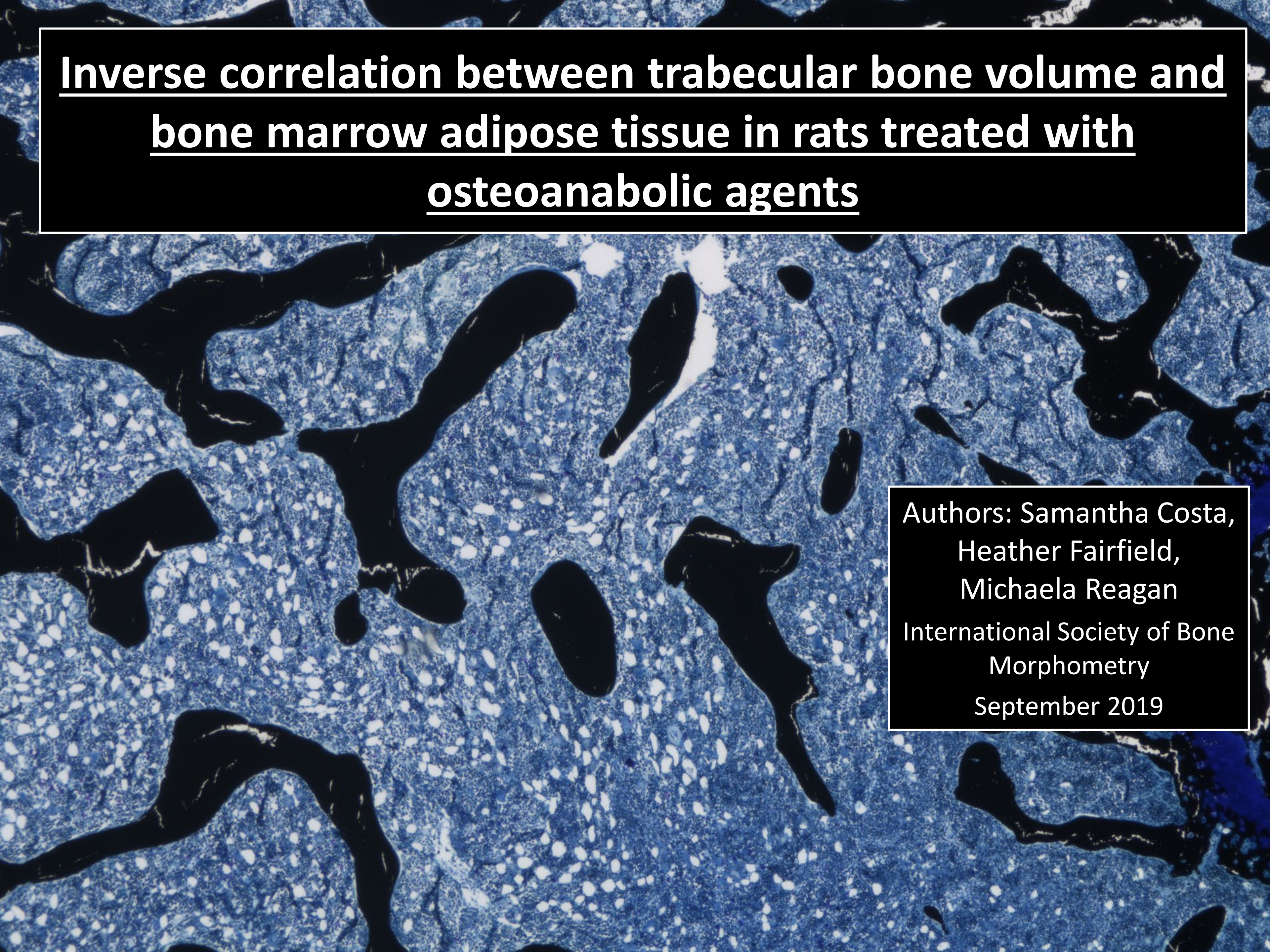

Inverse correlation between trabecular bone volume and bone marrow adipose tissue in rats treated with osteoanabolic agents

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International Society of Bone
Morphometry

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Conflicts of Interest and Funding

The authors have no conflicts of interests to declare

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Ominsky et al. 2015 provided the histology slides for this study.

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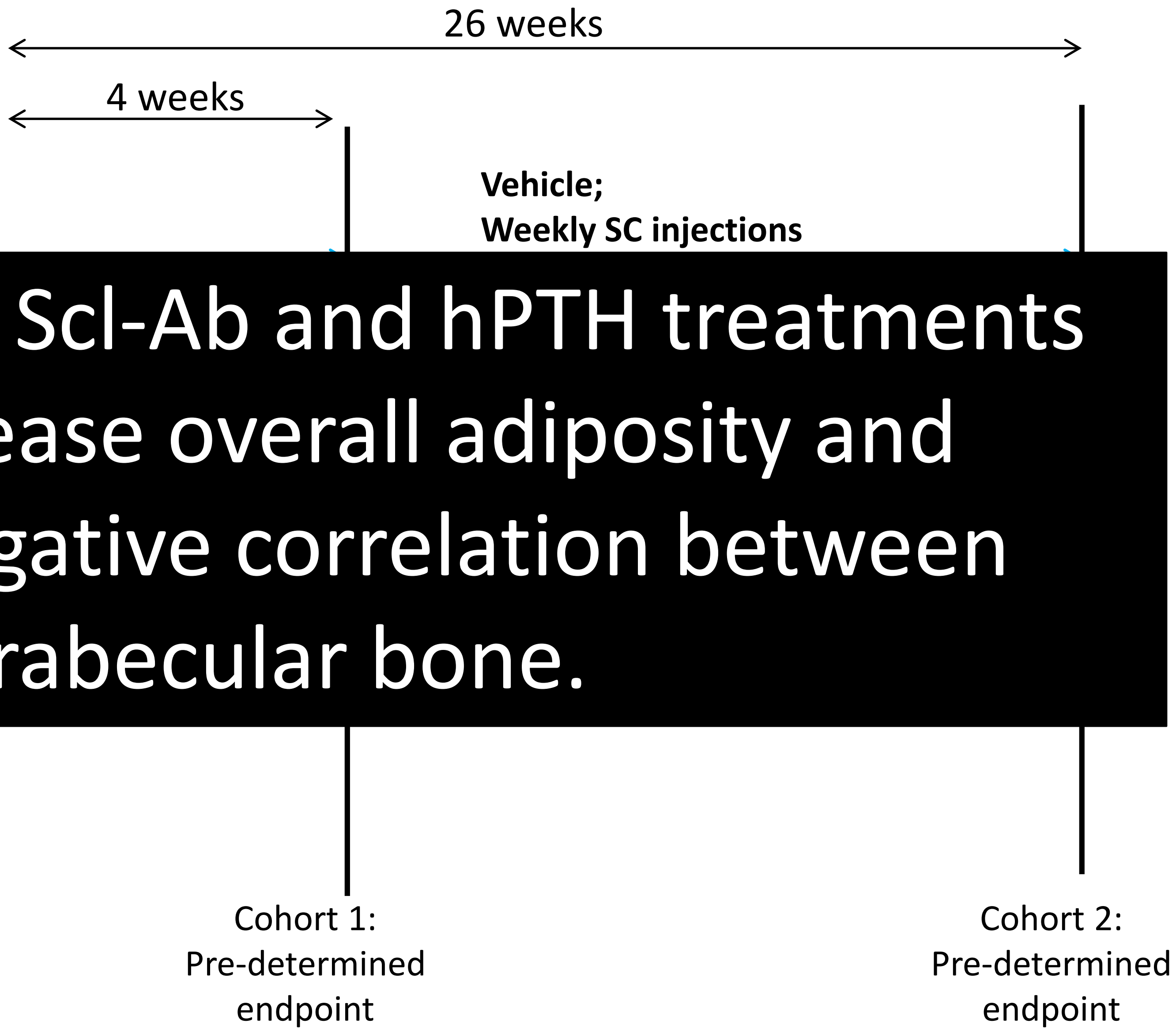
Outline

1. Background
2. Method Development: ImageJ Bone and BMAT Quantification
3. Effects of Sclerostin Antibody (Scl-Ab) and Human Parathyroid Hormone (hPTH) on bone and BMAT

Background-Original Experimental Design



26 weeks

4 weeks

Vehicle;
Weekly SC injections

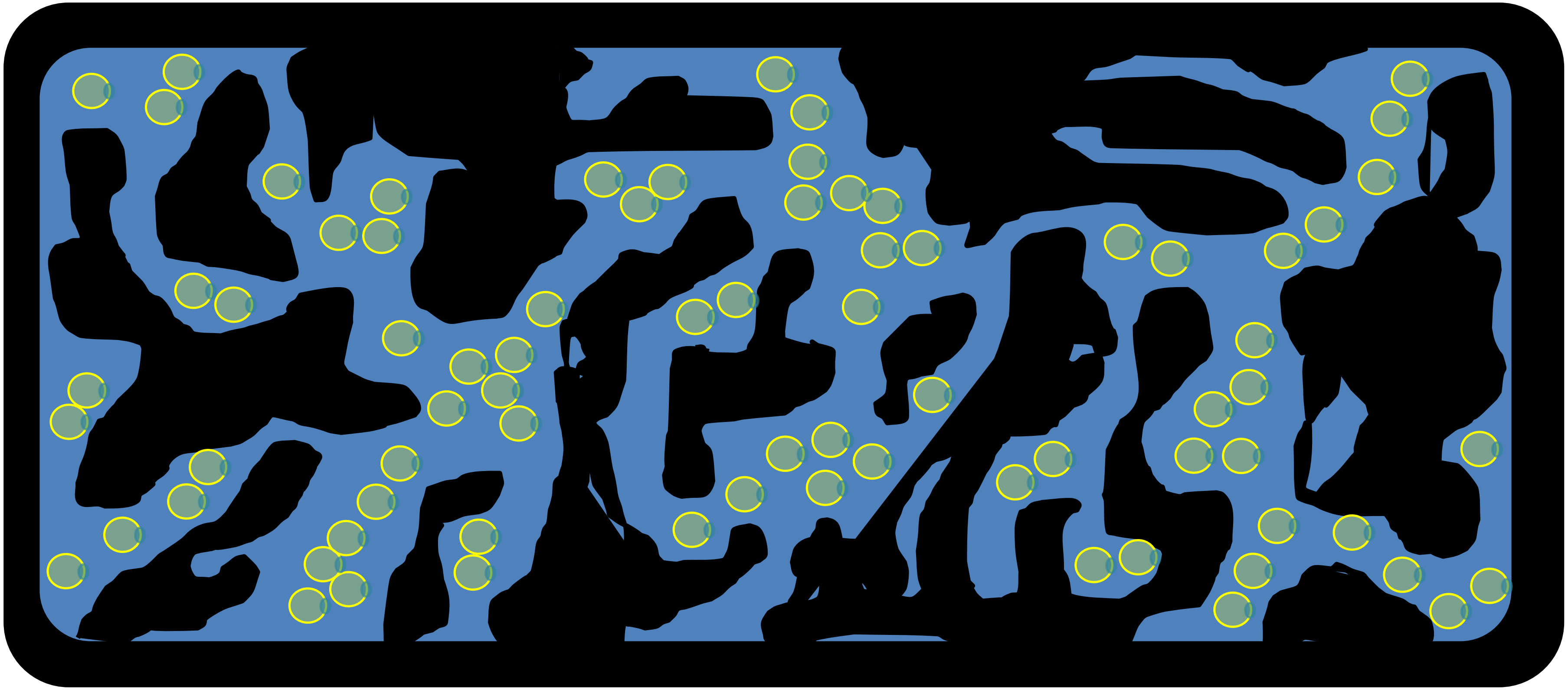
Hypothesis: Scl-Ab and hPTH treatments would decrease overall adiposity and induce a negative correlation between BMAT and trabecular bone.

Cohort 1:
Pre-determined
endpoint

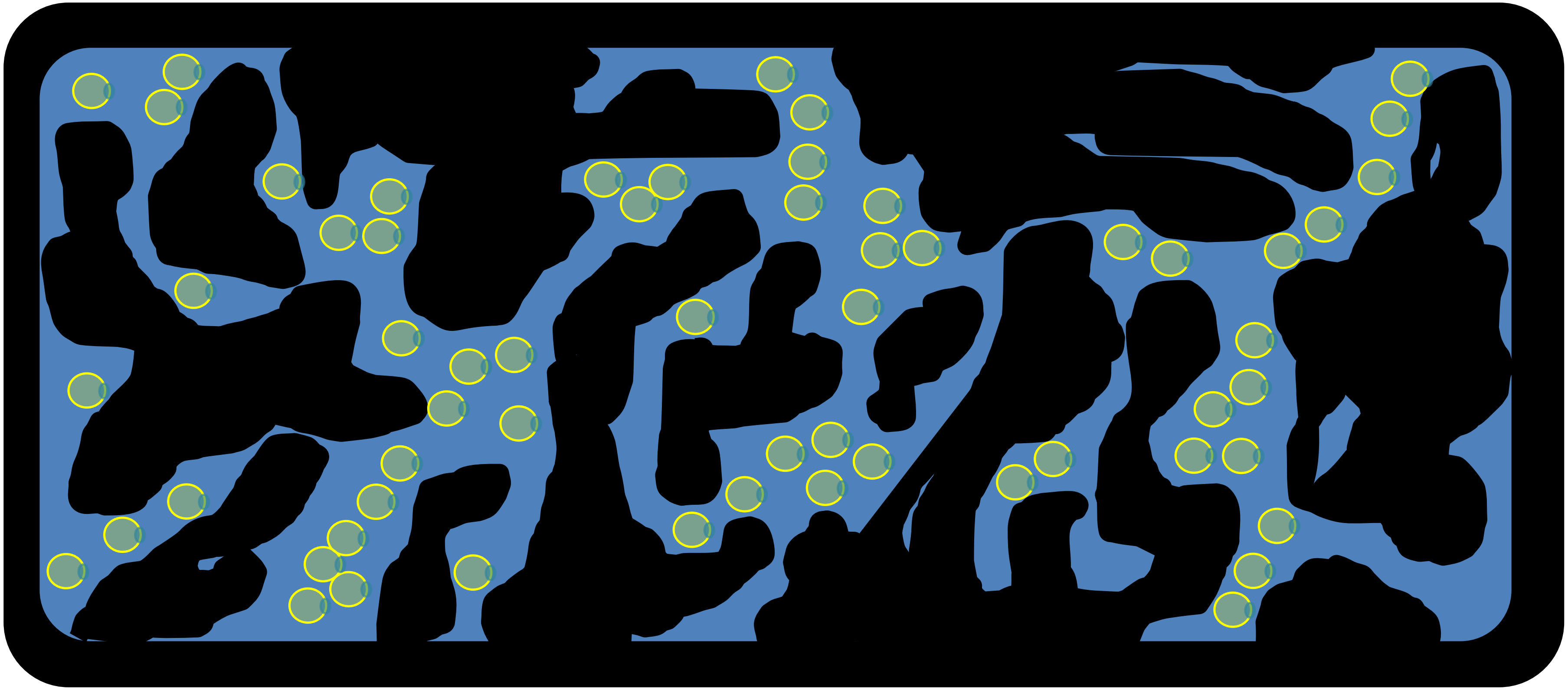
Cohort 2:
Pre-determined
endpoint

Ominsky et al. 2015, *Bone*

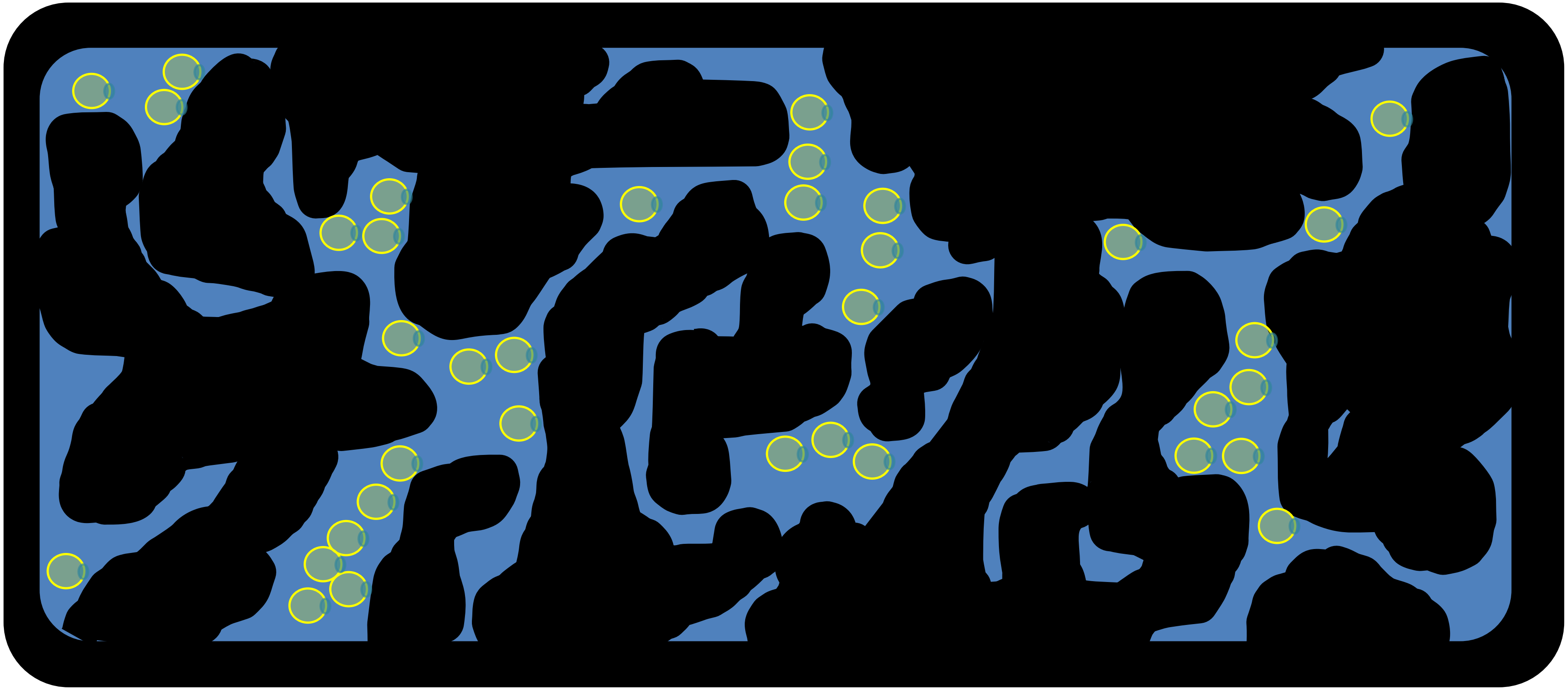
Increasing Trabecular Bone results in decreasing adiposity



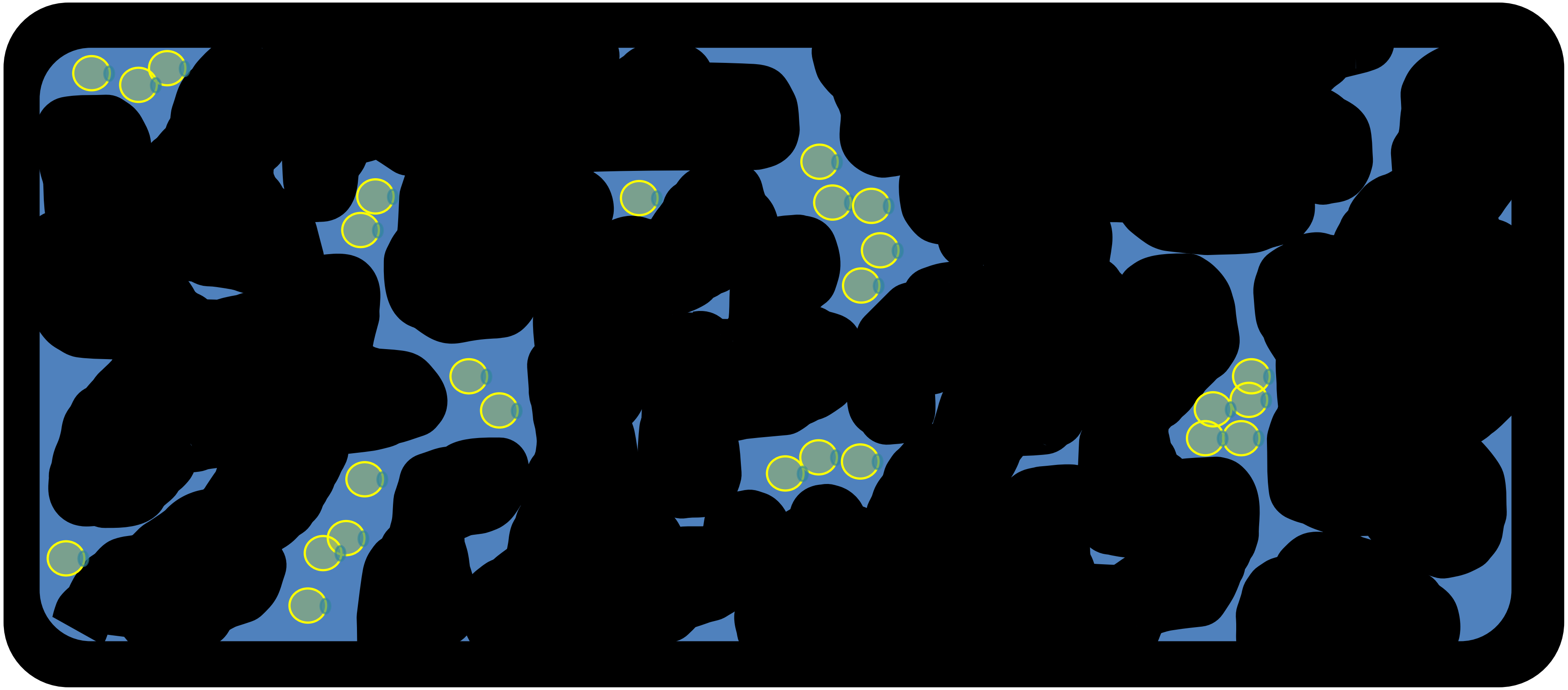
Increasing Trabecular Bone results in decreasing adiposity



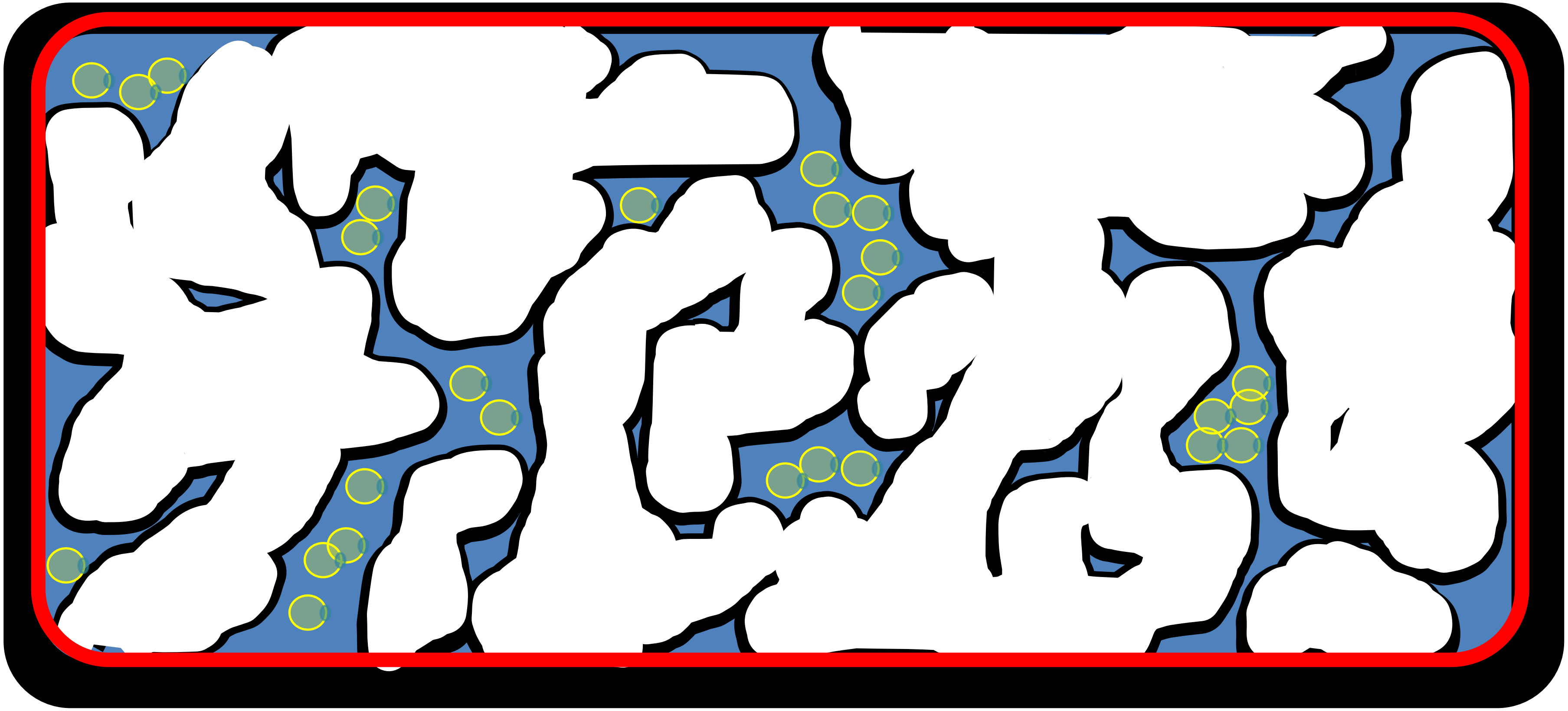
Increasing Trabecular Bone results in decreasing adiposity



Increasing Trabecular Bone results in decreasing adiposity



Bone-Independent Adiposity Analysis



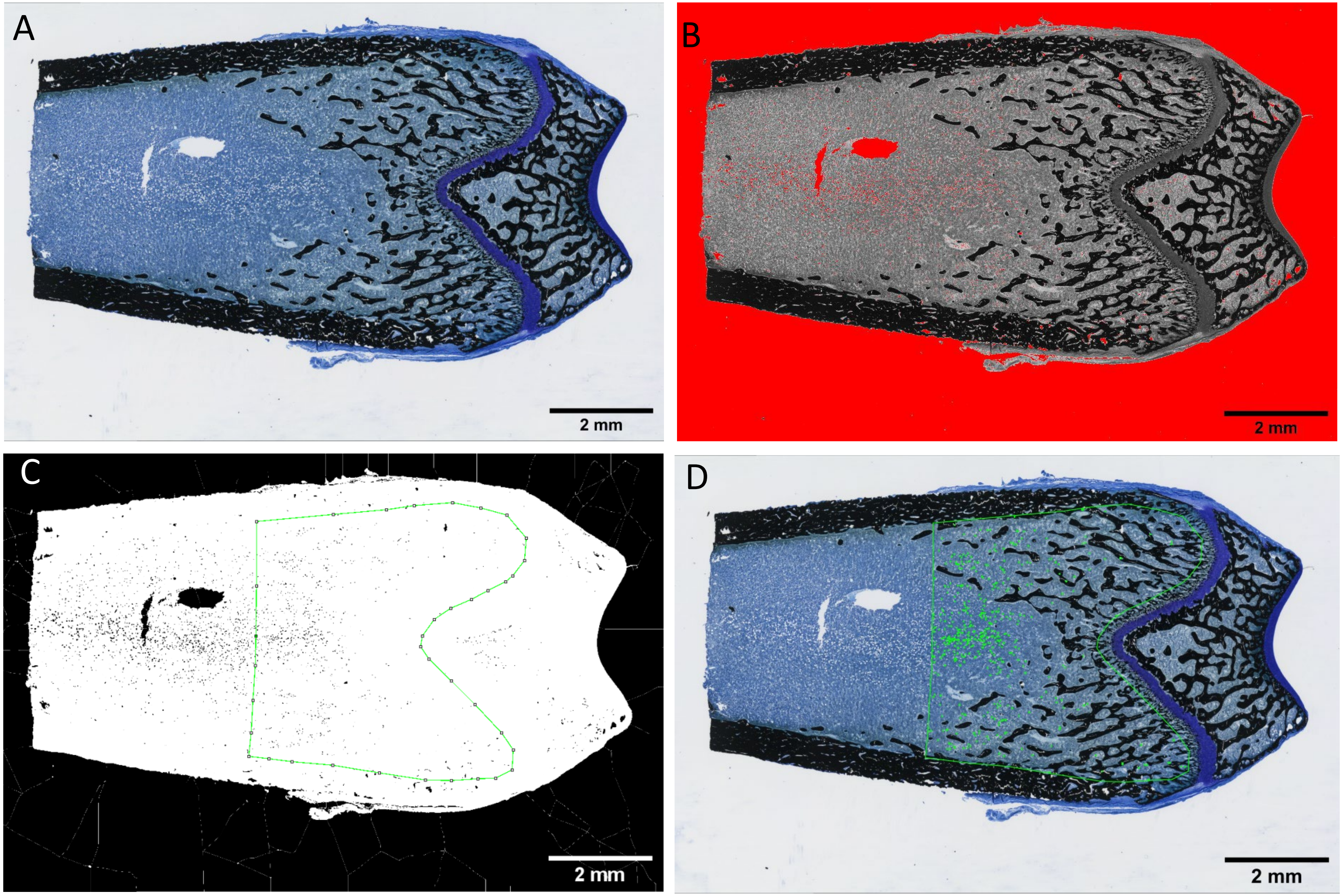
Tissue Area (T.Ar)= Trabecular Bone Area (Tb. B.Ar) + Marrow Area (Ma.Ar)

Marrow Area (Ma.Ar)= Tissue Area (T.Ar) - Trabecular Bone Area (Tb. B.Ar)

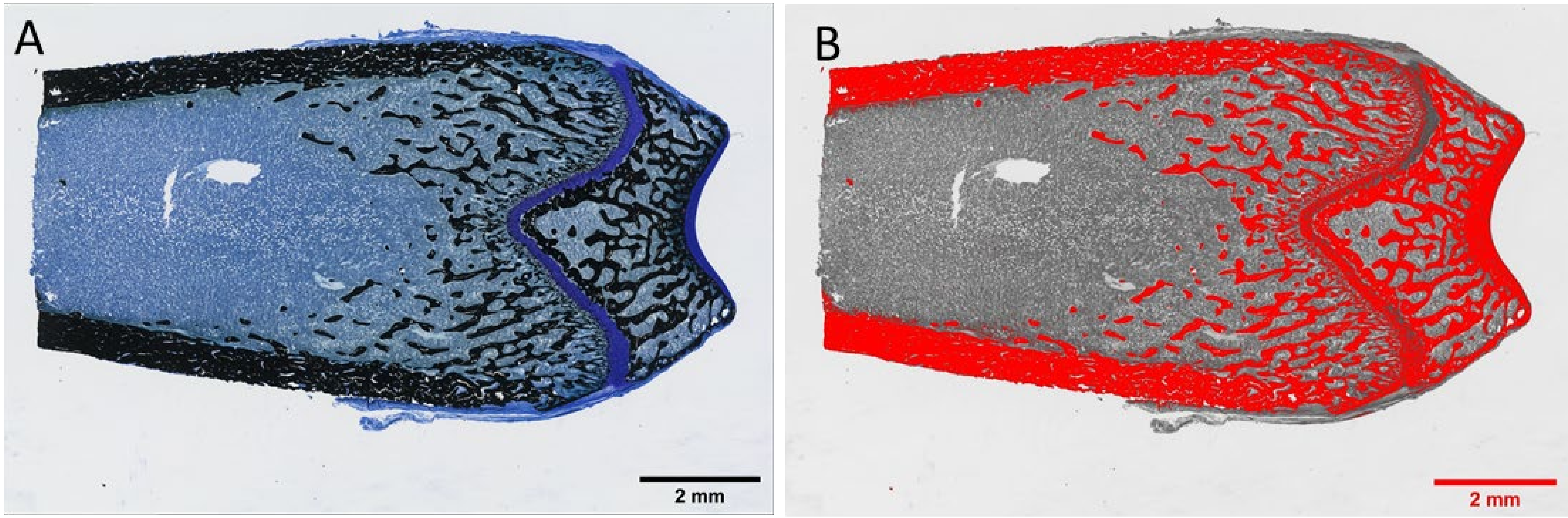
Methods Development

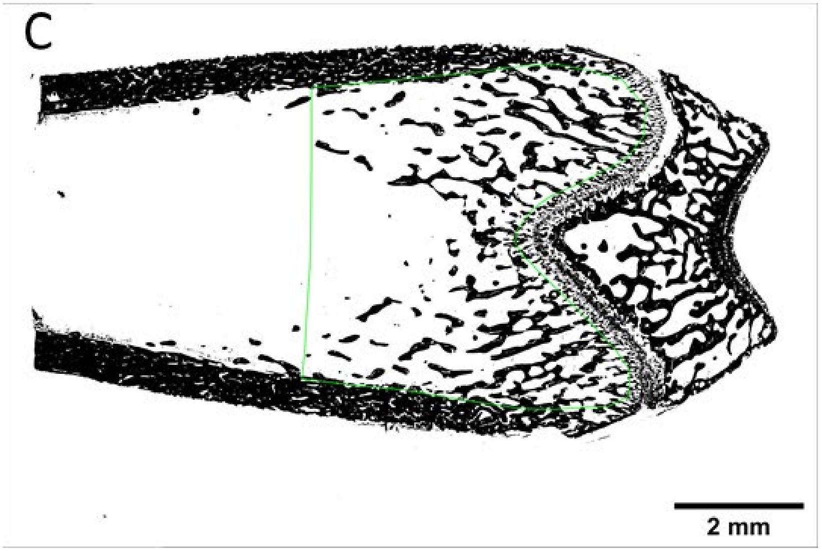
1. Developed ImageJ pipeline to test Scl-Ab and hPTH treatments effect on adiposity **(adiposity/tissue area, or adiposity/marrow area)** and bone.
2. Optimized parameters for adipocyte and trabecular bone quantification.

Adipocyte Quantification Method


A
2 mm
B
2 mm
C
2 mm
D
2 mm

Trabecular Bone Quantification Method

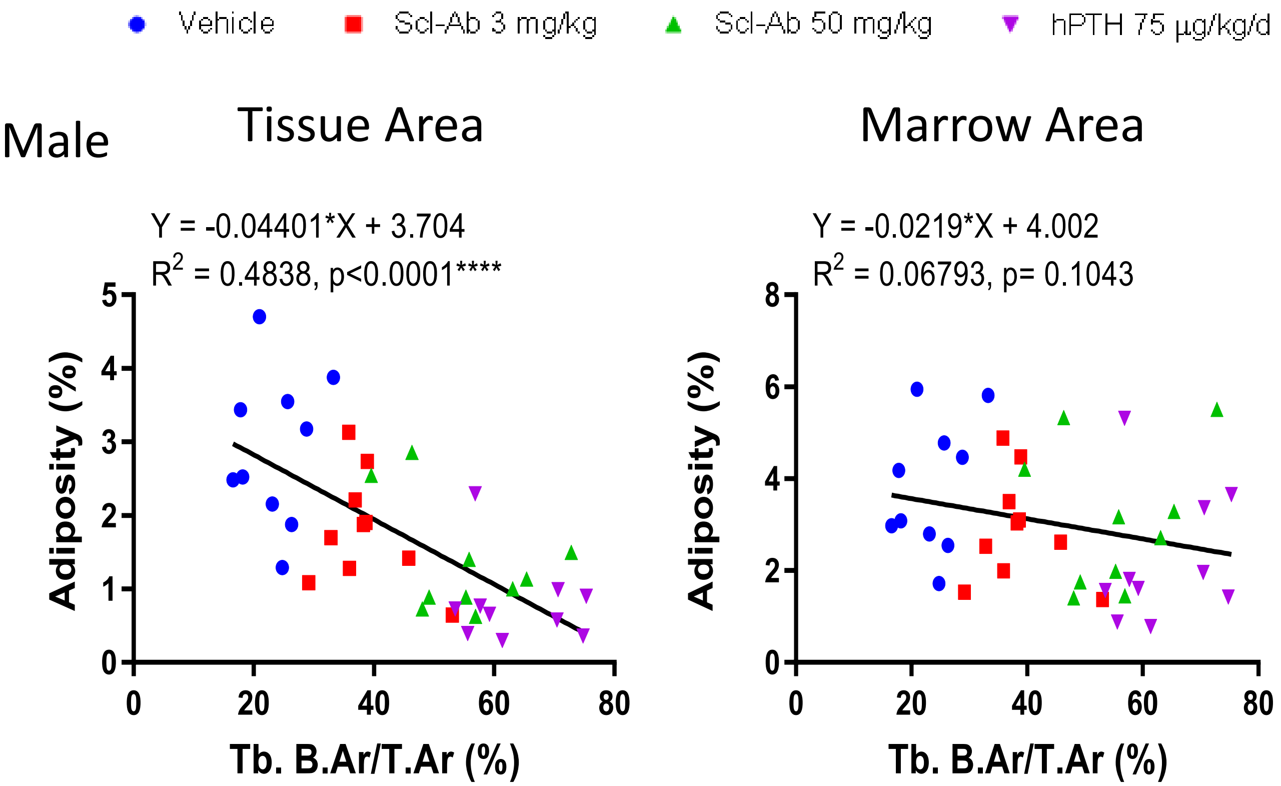




T.Ar= Tb. B.Ar + Ma.Ar

Ma.Ar= T.Ar - Tb. B.Ar

Scl-Ab and hPTH caused no inverse correlation between Ma.Ar adiposity and B.Ar after 26 weeks

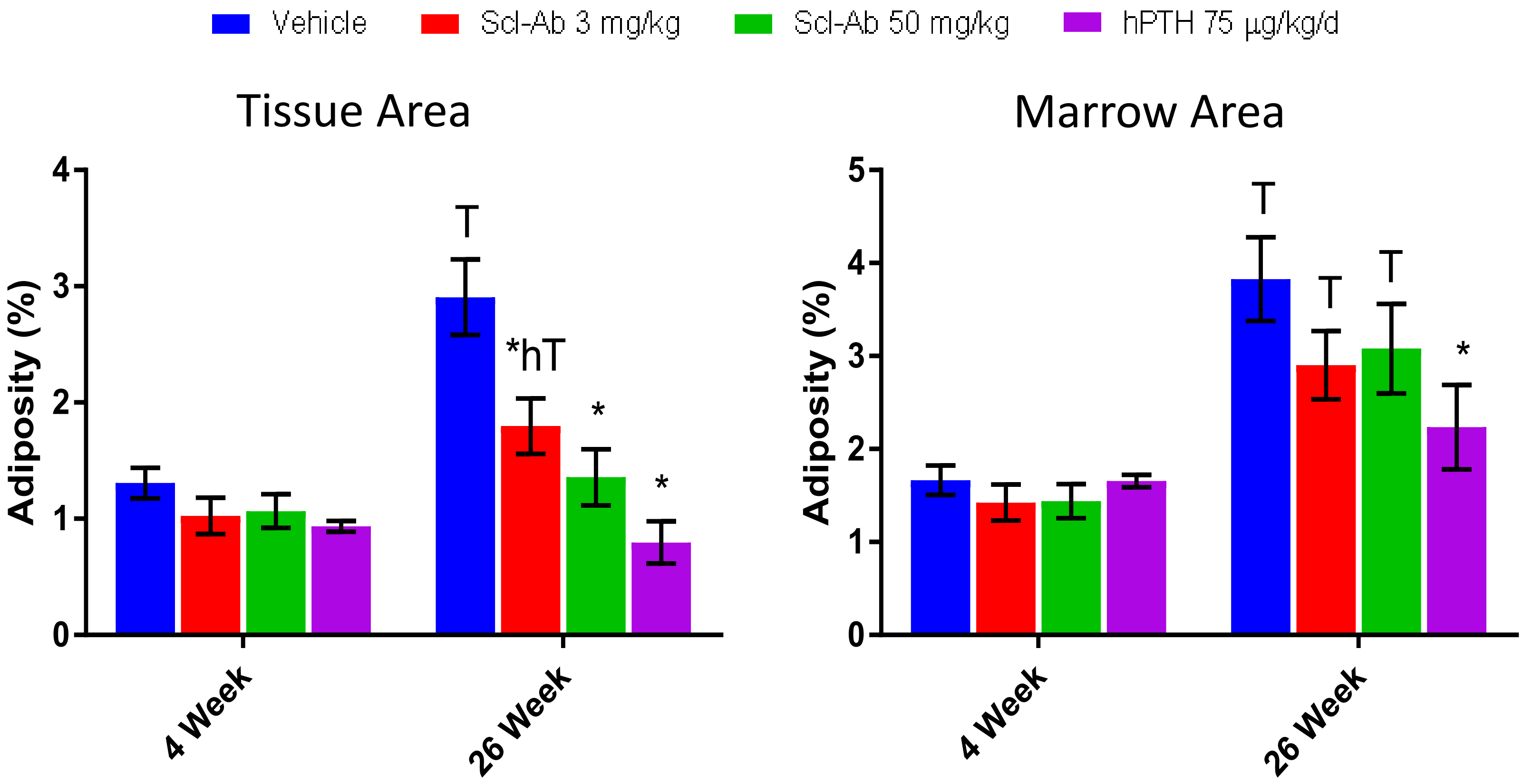


Collectively, the osteoanabolic treatments showed a significant inverse correlations in the tissue area in the males after 26 weeks

No inverse correlations within the marrow area were established at this time point

Osteoanabolic agents decreased T.Ar Adiposity more significantly than Ma.Ar after 26 weeks

Male

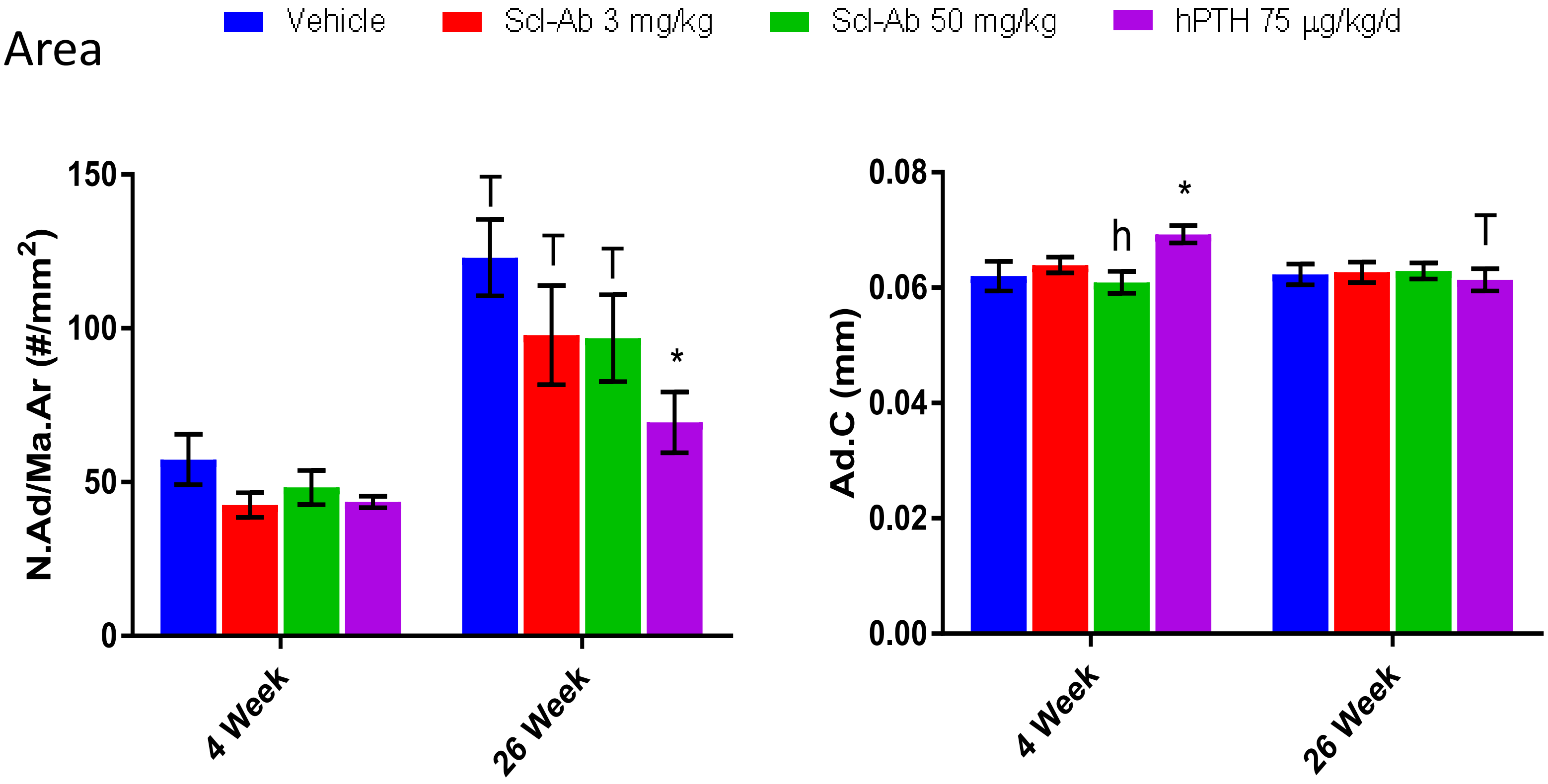


hPTH significantly decreased Ma.Ar adiposity after 26 weeks.

*p<0.05 vs. Vehicle; ^{h}p<0.05 vs. hPTH; ^{T}p<0.05, 4-week vs. 26-week; ^{A}p<0.05 Scl-Ab (3 mg/kg) vs. Scl-Ab (50 mg/kg). Data is shown as mean ± S.E.M. All analyses were performed as 2-way ANOVA + Tukey's/Sidak's multiple comparison tests.

hPTH decreased adipocyte number, but not size, in the Ma.Ar

Marrow Area
Male



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Conclusion

1. Bone marrow adiposity quantification depends on if adipose tissue is normalized to T.Ar or Ma.Ar
2. Chronic Scl-Ab and hPTH caused a significant inverse correlation between tissue area adiposity and trabecular bone area
3. Ma.Ar adiposity was decreased by trabecular bone accrual only after chronic treatments with hPTH in males
4. The ImageJ platform designed and used here is useful for histology quantification
    - multiple stains- H&E, Von Kossa tetrachrome, Trichrome
    - bone marrow adipose
    - trabecular bone
    - cortical bone
    - white adipose tissue

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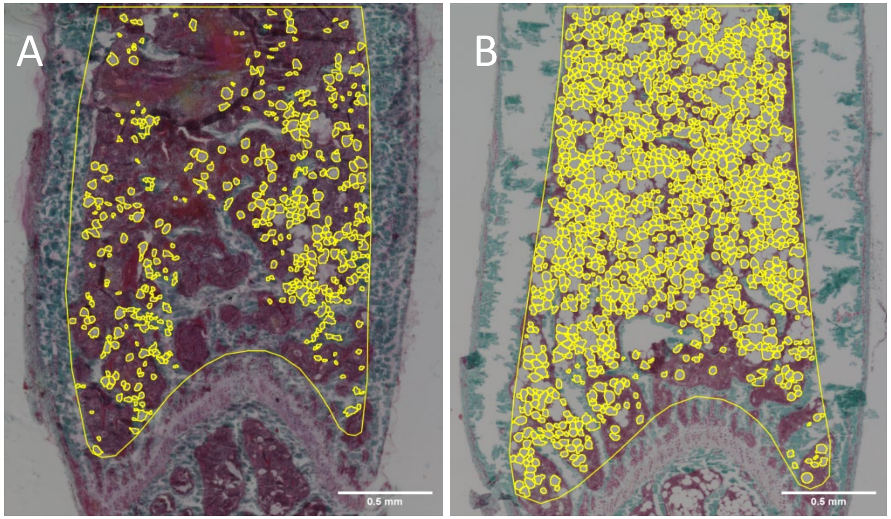
1811

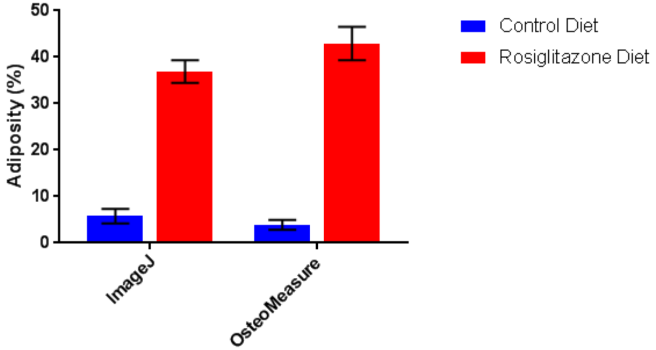
Reference

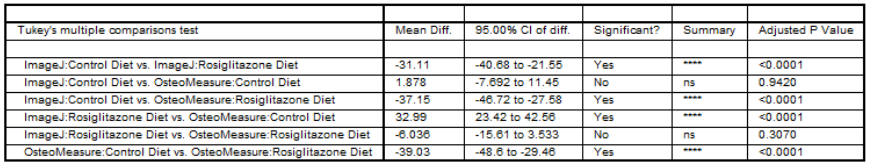
[1]M.S. Ominsky, D.L. Brown, G. Van, D. Cordover, E. Pacheco, E. Frazier, L. Cherepow, M. Higgins-Garn, J.I. Aguirre, T.J. Wronski, M. Stolina, L. Zhou, I. Pyrah, R.W. Boyce, Differential temporal effects of sclerostin antibody and parathyroid hormone on cancellous and cortical bone and quantitative differences in effects on the osteoblast lineage in young intact rats., Bone. 81 (2015) 380–391. doi:10.1016/j.bone.2015.08.007.

ImageJ Validation with OsteoMeasure



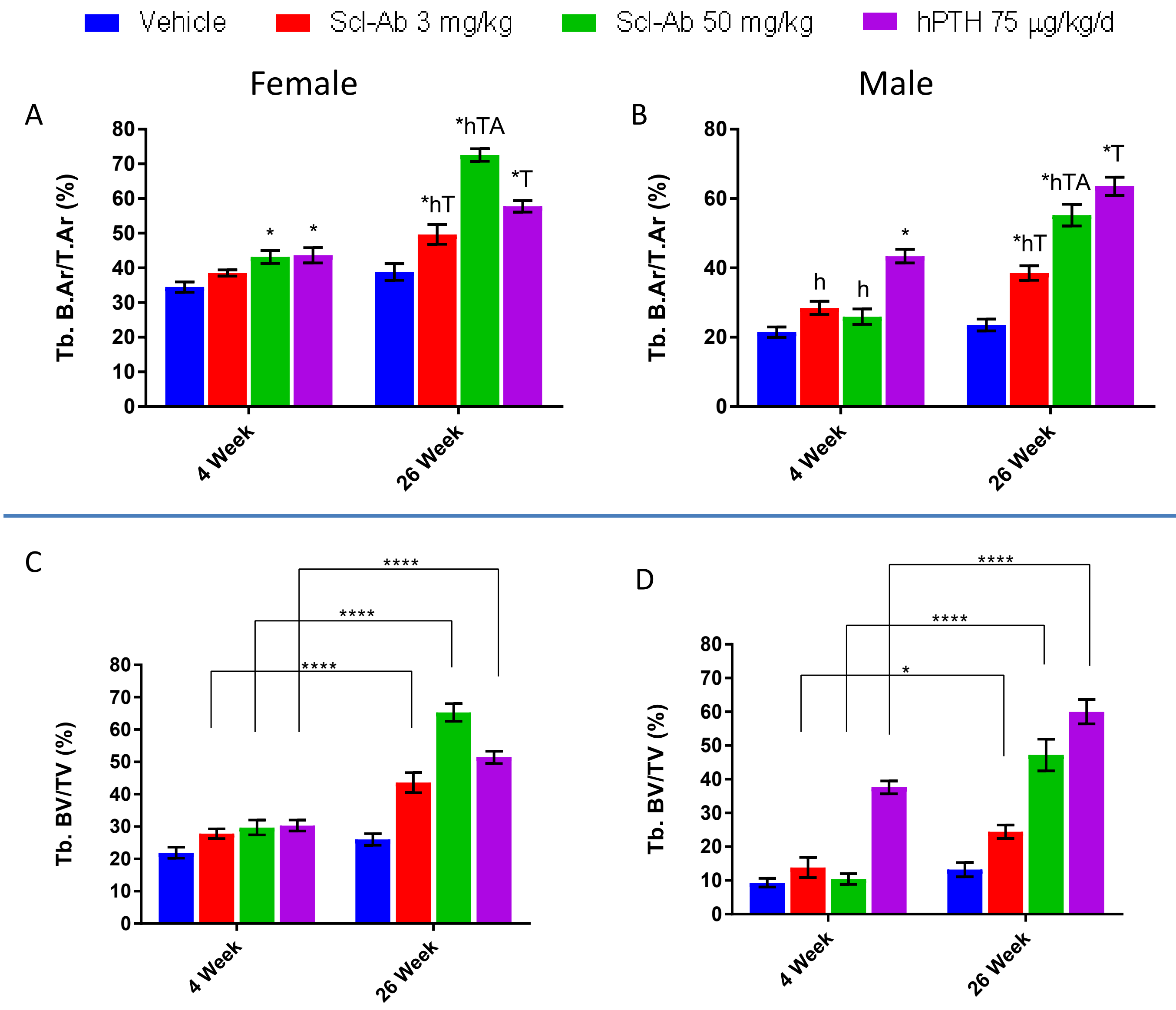
C





Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
ImageJ:Control Diet vs. ImageJ:Rosiglitazone Diet	-31.11	-40.68 to -21.55	Yes	****	<0.0001
ImageJ:Control Diet vs. OsteoMeasure:Control Diet	1.878	-7.692 to 11.45	No	ns	0.9420
ImageJ:Control Diet vs. OsteoMeasure:Rosiglitazone Diet	-37.15	-46.72 to -27.58	Yes	****	<0.0001
ImageJ:Rosiglitazone Diet vs. OsteoMeasure:Control Diet	32.99	23.42 to 42.56	Yes	****	<0.0001
ImageJ:Rosiglitazone Diet vs. OsteoMeasure:Rosiglitazone Diet	-6.036	-15.61 to 3.533	No	ns	0.3070
OsteoMeasure:Control Diet vs. OsteoMeasure:Rosiglitazone Diet	-39.03	-48.6 to -29.46	Yes	****	<0.0001

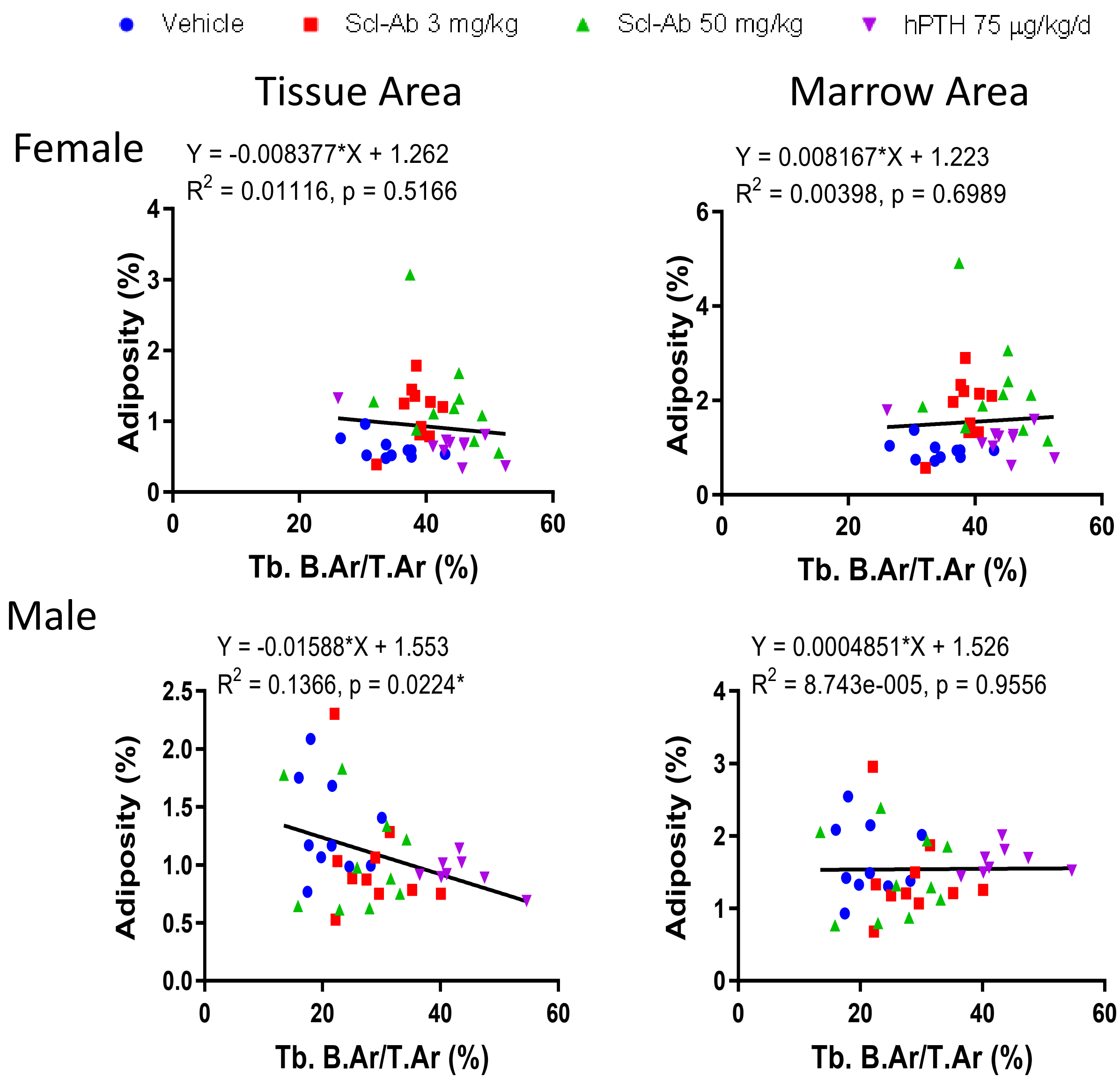
ImageJ Trabecular Bone Quantification Validation



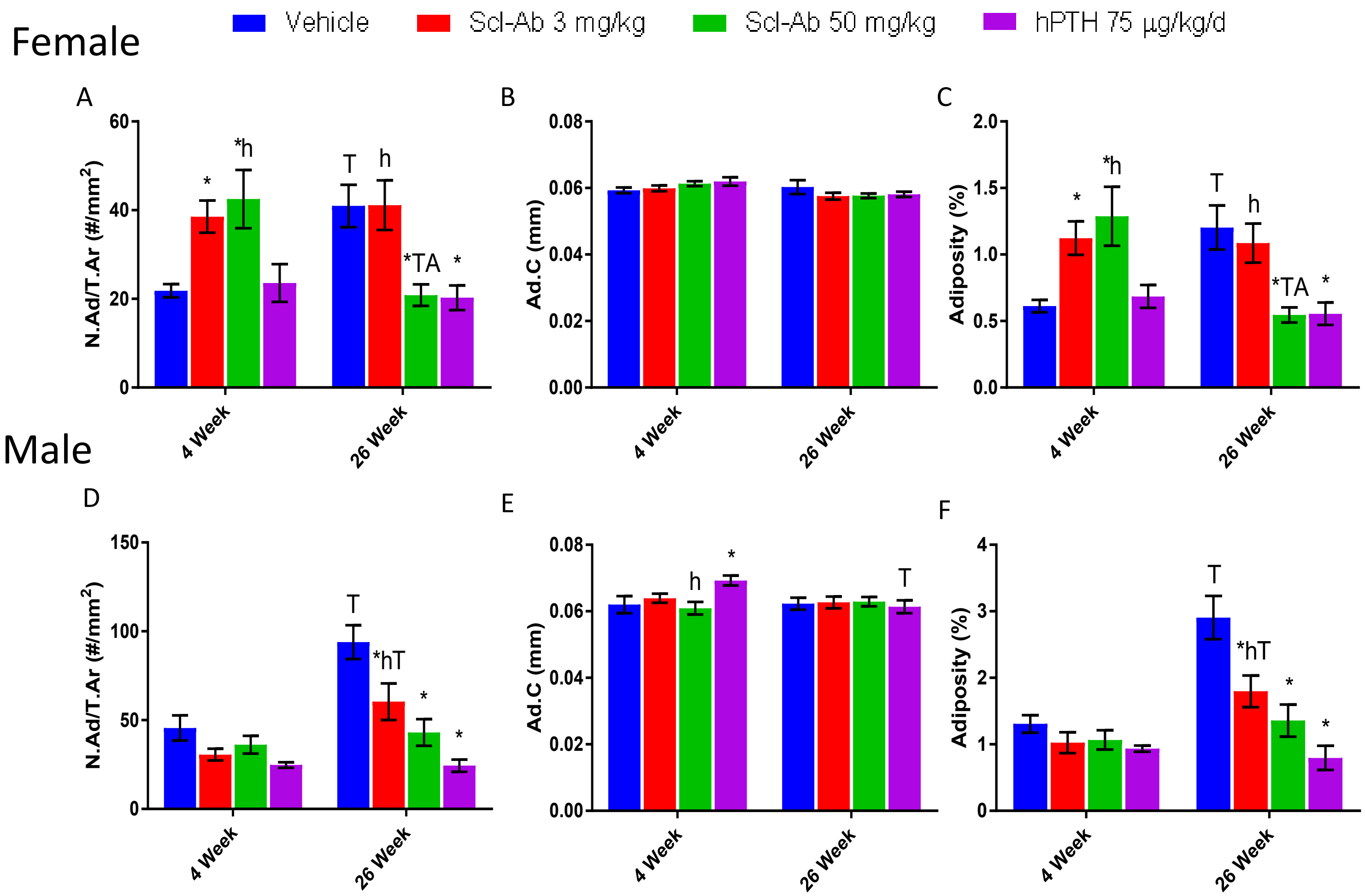
$*p<0.05$ vs. Vehicle; $^{h}p<0.05$ vs. hPTH; $^{T}p<0.05$, 4-week vs. 26-week; $^{A}p<0.05$ Scl-Ab (3 mg/kg) vs. Scl-Ab (50 mg/kg). Data is shown as mean ± S.E.M. All analyses were performed as 2-way ANOVA + Tukey's/Sidak's multiple comparison tests.

Reprinted from Ominsky *et al.* 2015[1]

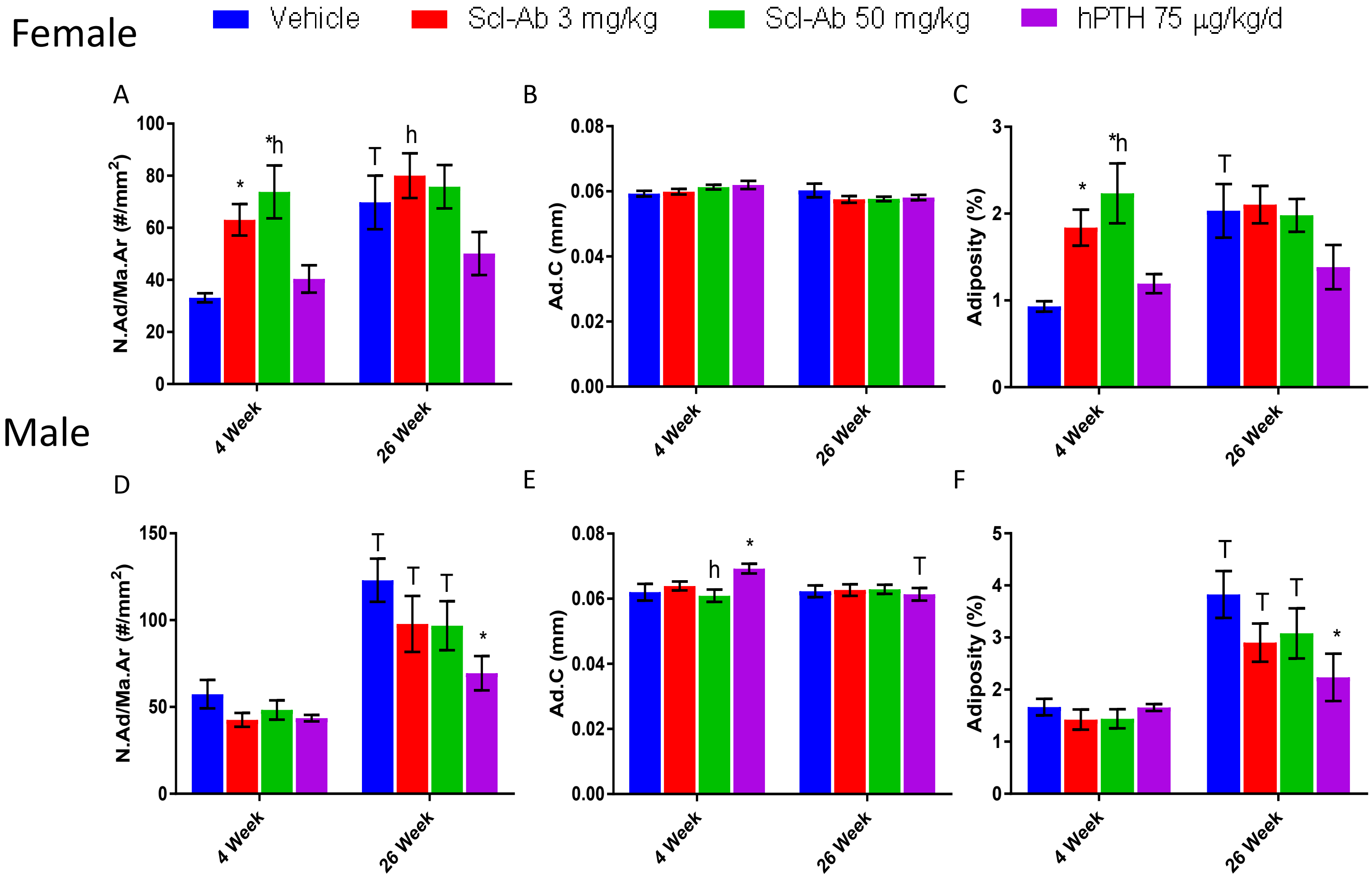
Adiposity Correlations to Tb. B.Ar/T.Ar after 4 weeks of treatment



Adipocyte parameters/T.Ar



Adipocyte parameters/Ma.Ar



		Week 4			**Week 26**		
		Tb. B.Ar/T.Ar Relative to Vehicle	T.Ar Adiposity Relative to Vehicle	Ma.Ar Adiposity Relative to Vehicle	Tb. B.Ar/T.Ar Relative to Vehicle	T.Ar Adiposity Relative to Vehicle	Ma.Ar Adiposity Relative to Vehicle
Males	Vehicle	1.000	1.000	1.000	1.000	1.000	1.000
	Scl-Ab 3 mg/kg	1.324	0.784	0.856	1.637	0.618	0.758
	Scl-Ab 50 mg/kg	1.206	0.815	0.865	2.348	0.467	0.805
	hPTH	2.019	0.716	0.995	2.700	0.274	0.584
Females	Vehicle	1.000	1.000	1.000	1.000	1.000	1.000
	Scl-Ab 3 mg/kg	1.117	1.834	1.974	1.279	0.903	1.035
	Scl-Ab 50 mg/kg	1.252	2.102	2.397	1.869	0.453	0.974
	hPTH	1.266	1.118	1.281	1.488	0.461	0.681