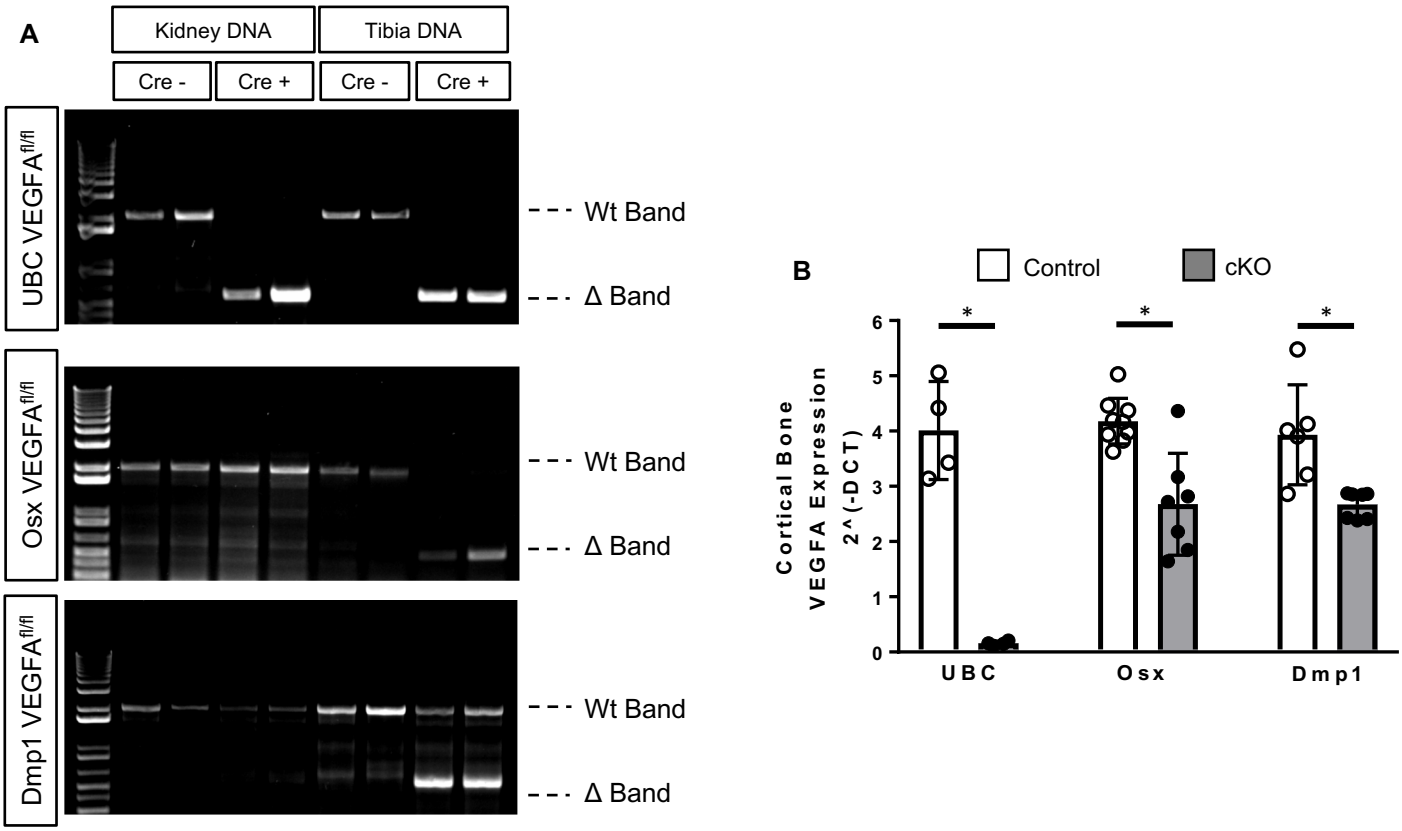
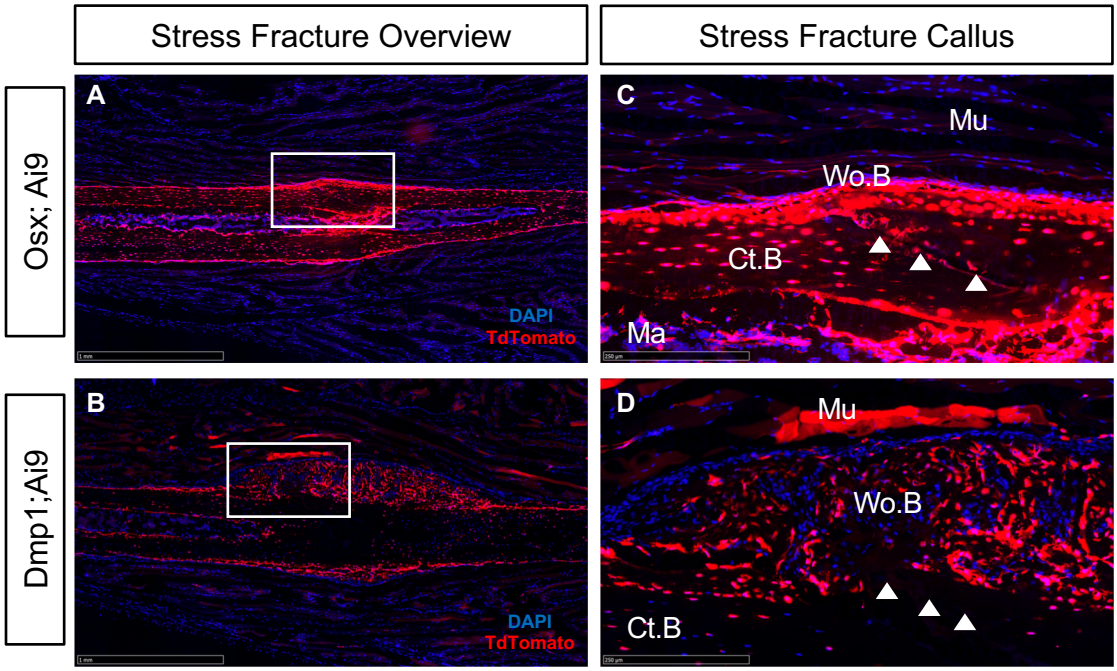
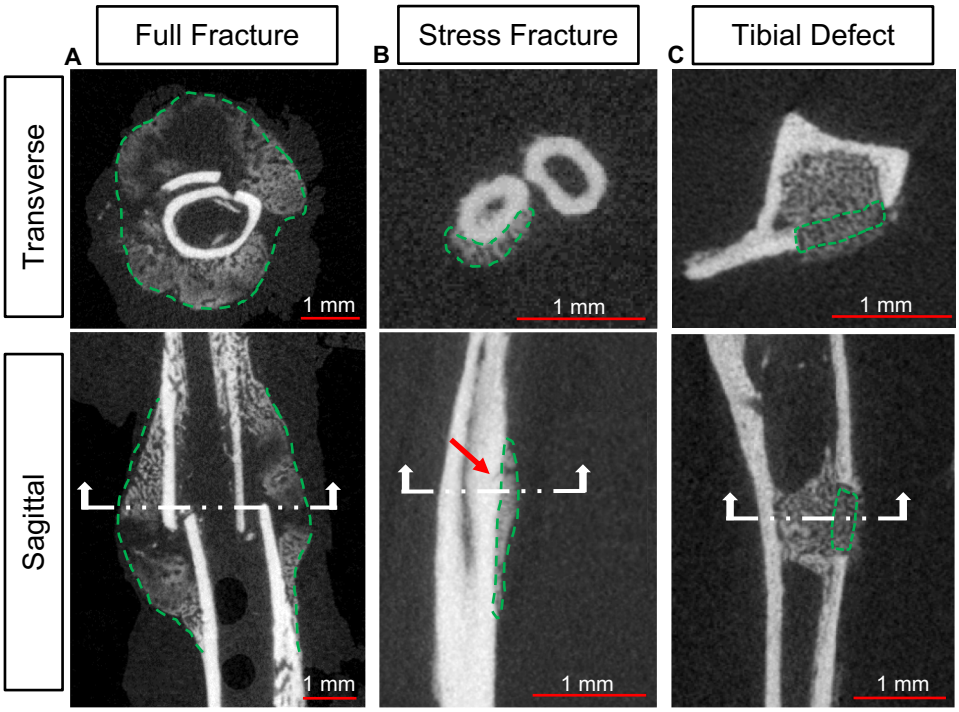


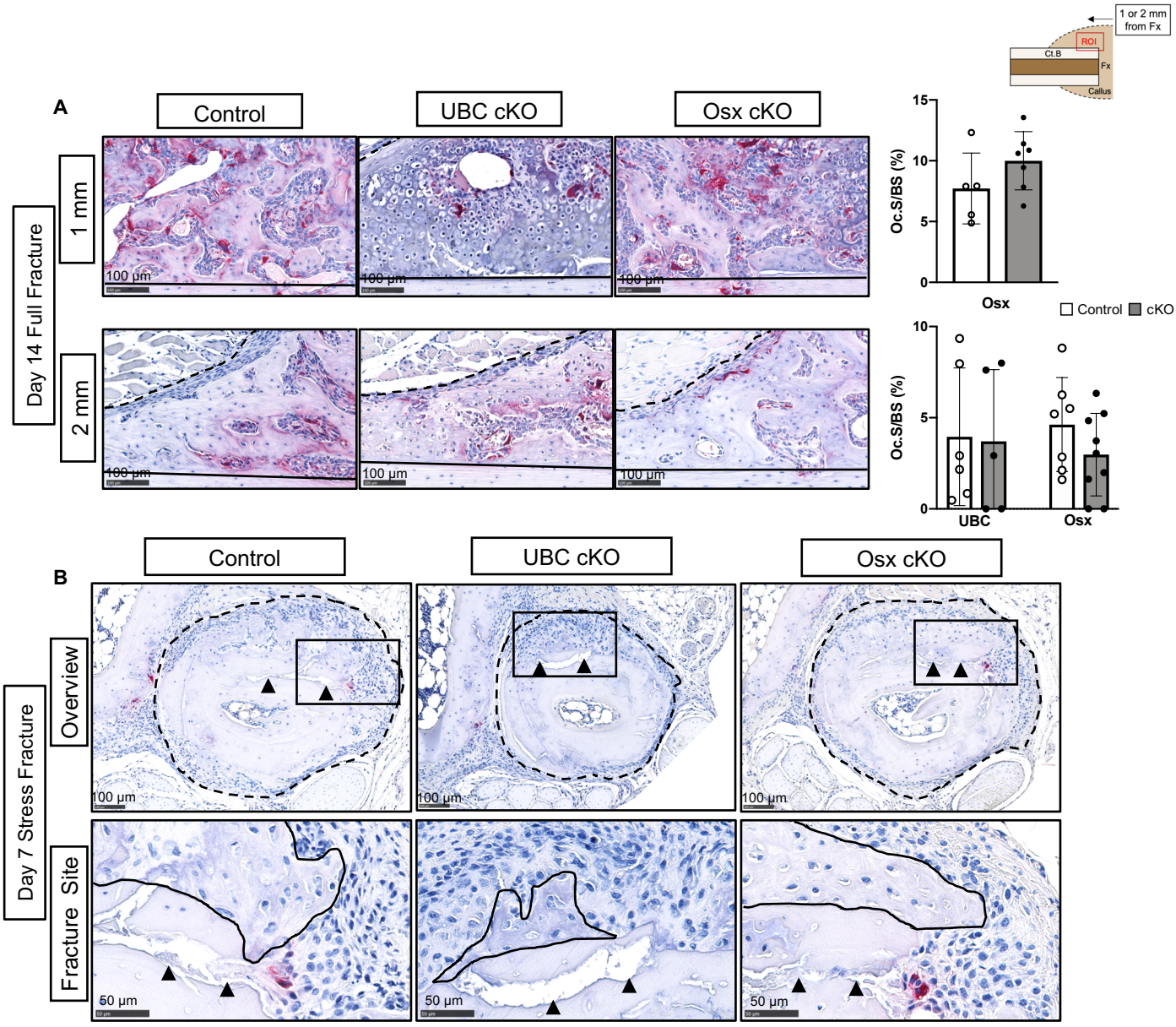
Supplemental Figure 1. Inducible Cre lines show spatial targeting of tissues and different efficiencies for VEGFA deletion in cortical bone. DNA recombination for exon 3 of the VEGFA gene was assayed for by PCR using DNA extracted from kidney and tibia digests from Cre-ERT2$^{+/-}$ VEGFA$^{fl/fl}$ mice receiving 3 weeks of tamoxifen. Two mice from each genotype are represented. **A)** UBC cKO (Cre^{+}) animals showed a shorter amplicon of 560 base pairs (Δ band) in kidney and tibia DNA, thereby showing VEGFA recombination in these tissues. On the other hand, UBC Control (Cre-) mice only showed a larger amplicon of 2160 base pairs (Wt band), indicative of no recombination at exon 3. Osx cKO animals showed a shorter amplicon at 560 base pairs in DNA from tibia but not kidney. Dmp1 cKO animals show a shorter amplicon at 560 base pairs in DNA from tibia but not kidney. **B)** qPCR for VEGFA mRNA expression in diaphyseal cortical bone following 3 weeks of tamoxifen. All 3 inducible Cre lines showed significantly reduced VEGFA expression (unpaired t-test; *$p<0.05$) in cKO versus control animals.



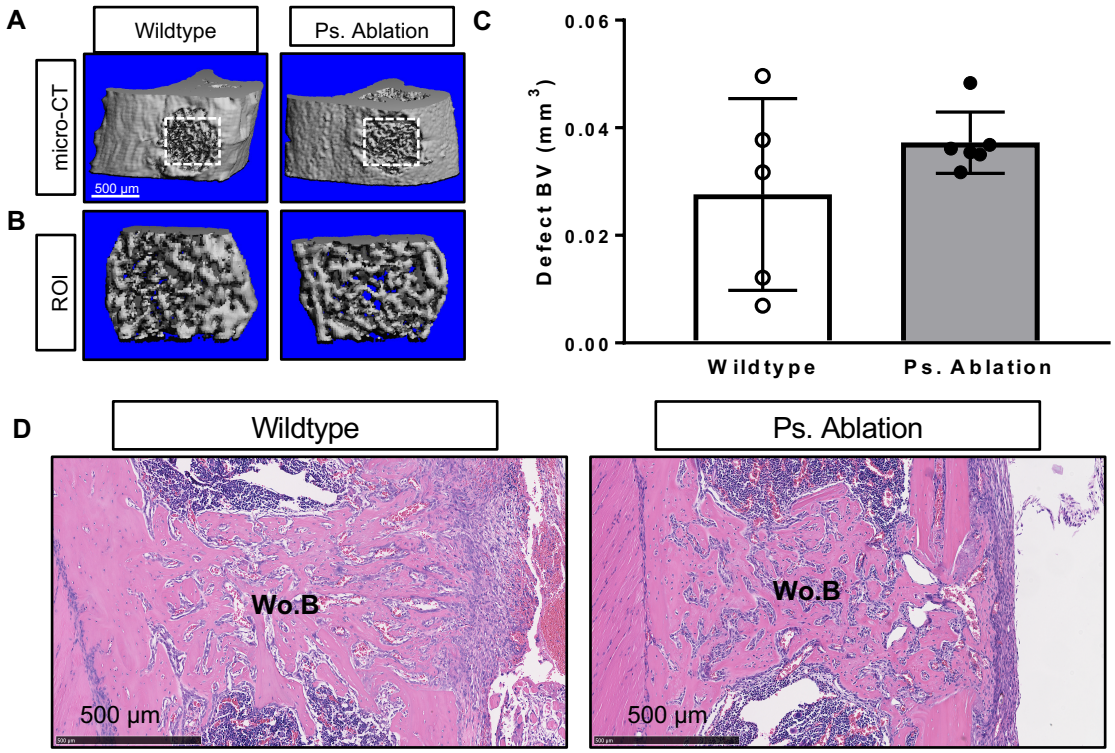
Supplemental Figure 2. Inducible conditional Cre specificity during stress fracture healing. TdTomato reporter images (20x) counterstained with DAPI demonstrate spatial inducible Cre activation in Cre-ERT2 Ai9 VEGFA$^{wt/wt}$ mice following tamoxifen schedule as shown in Figure 1B. **A-B)** Tdtomato stress fracture calluses at day 10. White box represents 20x magnification of callus region near stress fracture crack. **C-D)** Magnified callus of Osx and Dmp1 Ai9 mice near the stress fracture line (white arrow heads). All inducible Cre's showed activation in the woven bone region of the callus. Dmp1 also showed some tdTomato expression in muscle. Abbreviations: Wo.B = woven bone; Mu = muscle; Ct.B = cortical bone; Ma = marrow.



Supplemental Figure 3. Region of interest (ROI) for micro-CT analysis. Transverse sections represent injury midpoints where peak bone formation for each bone repair model occurs. Transverse section locations are noted on sagittal sections by white dashed line. **A)** Bone formation at day 14 following full fracture was assessed by contouring the mineralized periosteal callus (dashed green lines) 600 slices around the fracture midpoint. This volume was then thresholded to 183 per mille to calculate full fracture bone volume (BV). **B)** Bone formation was assessed at day 7 following stress fracture by contouring the entire periosteal callus (dashed green lines) around the fracture midpoint (red arrow). This volume was then thresholded to 150 per mille to calculate stress fracture bone volume (BV). **C:** Bone formation was assessed at day 7 following cortical defect by contouring the intracortical space (dashed green lines) on 55 slices centered within the defect site. This volume was then thresholded to 150 per mille to calculate cortical defect bone volume (BV). Note how new woven bone formation in the full fracture and stress fracture model is periosteal. In contrast, almost all the new bone in the cortical defect model is intramedullary.



Supplemental Figure 4. TRAP+ Osteoclasts Aren't Significantly Altered by Inducible Conditional Loss of VEGFA During Full Fracture and Stress Fracture Bone Formation. A) TRAP+ multinucleated cells on callus woven bone surfaces were quantified and normalized to bone surface perimeter at regions 1 mm (transition zone) and 2 mm (woven bone only) from the full fracture site. Day 14 UBC cKO fracture calluses had no osteoclasts on woven bone tissue at the 1mm fracture site and no significant differences in osteoclast number (N.Oc; data not shown) or osteoclast surface (Oc.S) per bone surface (BS) versus controls at the 2mm site. Osx cKO fracture calluses at the same timepoint had no significant differences versus controls in N.Oc/BS (data not shown) or Oc.S/BS at either the 1mm or 2mm site. **B)** Both UBC and Osx stress fracture specimens at day 7 had no TRAP+ multinucleated cells on woven bone tissue (black outline). There were a few TRAP+ multinucleated cells at the fracture site (black arrow heads) or on the adjacent radius periosteum in each section. No differences due to loss of conditional VEGFA were noted.



Supplemental Figure 5. Periosteal ablation doesn't affect cortical defect repair. A) 3D reconstruction of representative tibias at day 7 showed new bone formation at defect site (white box) regardless of whether the periosteum was left intact (Wildtype) or stripped (Ps. Ablation) at the time of injury. Reconstruction of thresholded bone tissue that was quantified within the defect site. **C)** Thresholded bone volume (BV) within cortical defect site (ROI in Supplemental Figure 3C) after 7 days of healing showed no differences in defect bone formation between experimental groups **D)** Magnified H&E sections depicting woven bone formation (Wo.B) at the defect site regardless of whether the periosteum was left intact (Wildtype) or not (Ps. Ablation).

Supplemental Table 1. Stress Fracture Loading Parameters

Calibration Mice

Mouse Line	*No. mice*	*Calibration: Ultimate Force (N)*	*Calibration: Cyclic Displacement Increase to Failure (mm)*	*Calibration: Cycles to Failure (Cycles)*
UBC VEGFA Ai9	13 (6M and 7F)	4.40 ± 0.585	1.07 ± 0.266	7062 ± 6282
Osx VEGFA Ai9	13 (7M and 6F)	4.03 ± 0.670	0.955 ± 0.210	4345 ± 4852
DMP1 VEGFA Ai9	18 (10M and 8F)	4.14 ± 0.508	1.05 ± 0.253	3191 ± 4101

Experimental Mice

Genotype	*No. mice*	*Peak Force (N)*	*Displacement Increase to Stress Fracture (mm)*	*Cycles to Stress Fracture (Cycles)*
UBC Control	20	3.3	0.54	4827 ± 3991
UBC KO	15	3.3	0.54	**8264 ± 6166 b**
Osx Control	33	3.03	0.48	5013 ± 4382
Osx KO	20	3.03	0.48	3967 ± 3951
Dmp1 Control	5	3.11	0.53	5490 ± 4681
Dmp1 KO	9	3.11	0.53	3863 ± 5916

b = $p < 0.10$ due to genotype by unpaired t-test

Supplemental Table 2. Vessel Density of Stress Fracture

Genotype	***No. mice***	***Day 5 Callus Vessel Density (# Endomucin$^+$/mm^2)***	***Day 5 Woven Bone Vessel Density (# Endomucin$^+$/mm^2)***
UBC Control	5	486.3 ± 52.64	172.8 ± 117.2
UBC cKO	3	**358.7 ± 123.2 a**	**58.45 ± 11.58 a**
Osx Control	3	353 ± 70.93	251.3 ± 86.91
Osx cKO	4	**429.9 ± 223.8 b**	208.1 ± 162.2
Genotype	***No. mice***	***Day 7 Callus Vessel Density (# Endomucin$^+$/mm^2)***	***Day 7 Woven Bone Vessel Density (# Endomucin$^+$/mm2)***
UBC Control	6	376.1 ± 82.53	217.3 ± 136.4
UBC cKO	4	**307.8 ± 102.6 a**	**22.55 ± 16.68 a,c**
Osx Control	6	334.4 ± 77.27	236.8 ± 120
Osx cKO	6	**365.9 ± 98.77 b**	159.2 ± 132.8

a = $p < 0.05$ due to genotype on 2-way ANOVA (genotype + time)
b = $p < 0.05$ due to interaction on 2-way ANOVA (genotype + time)
c = $p < 0.05$ by Sidak Multiple Comparisons Post Hoc Test

Supplemental Table 3. Femur Cortical Bone Properties

Genotype	*No. mice*	*Tt. Ar (mm^2)*	*Ct.Ar (mm^2)*	*Ma.Ar (mm^2)*	*vBMD (mgHA/cm3)*	*TMD (mgHA/cm3)*
UBC Control	3	9.71 ± 0.458	5.25 ± 0.292	4.47 ± 0.175	508 ± 3.70	1001 ± 11.6
UBC cKO	3	10.9 ± 1.97	5.53 ± 0.870	5.37 ± 1.11	481 ± 25.1	1004 ± 16.8
Osx Control	3	9.73 ± 0.745	5.49 ± 0.466	4.24 ± 0.279	541 ± 8.78	1019 ± 8.42
Osx cKO	3	**8.14 ± 0.555 d**	**4.68 ± 0.122 d**	3.46 ± 0.465	532 ± 31.1	**981 ± 14.3 a**
Dmp1 Control	3	8.37 ± 0.460	4.27 ± 0.321	4.10 ± 0.304	447 ± 32.4	943 ± 23.8
Dmp1 cKO	3	8.87 ± 0.639	4.75 ± 0.390	4.12 ± 0.260	**507 ± 10.3 a**	**1010 ± 26.0 d**

a = $p < 0.05$ due to genotype by unpaired t-test
d = $p < 0.10$ due to genotype by Mann-Whitney test