

Supplemental Figure 1. Analysis of the metaphyseal region in mice with sickle cell disease (A) Representative microCT image of an entire femoral bone with the metaphysis outlined in red. (B) Trabecular bone volume fraction in the metaphyseal region generated by microCT in C57BL/6 and CatK-/- mice that were reconstituted with AA or SS bone marrow. (AA bone marrow= black, SS bone marrow =red, n=3-7 mice per group) (C) Representative 3D heat maps of the trabecular thickness in the metaphyseal region in SS 3-month-old mice administered saline or E-64 for two months from 1 month of age. A pseudocolor scale of blue (0 mm) to red (0.2mm) depicts trabecular thickness (Scale bar 100 μ m) (D-E) Trabecular bone parameters of trabecular thickness and bone mineral density generated by microCT scans after E-64 treatment (SS=black, SS with E-64= red, n=6-9 mice per group). Data expressed as mean \pm SD. Statistical significance, * $p < 0.05$, determined by multiple t-tests with Holm-Sidak's post-hoc test. (Abbreviations: Tb. Th., Trabecular thickness; BMD, Bone Mineral Density)

Supplemental Figure 2. Bone mechanical testing parameters generated by four-point bending technique in 3-month-old AA and SS mice with or without treatment with E64. Based on displacement and force measurement graph and geometry data from microCT, ultimate stress (A) and stiffness (C) were calculated, and maximum force (B) was compared (n=5-8 per group).

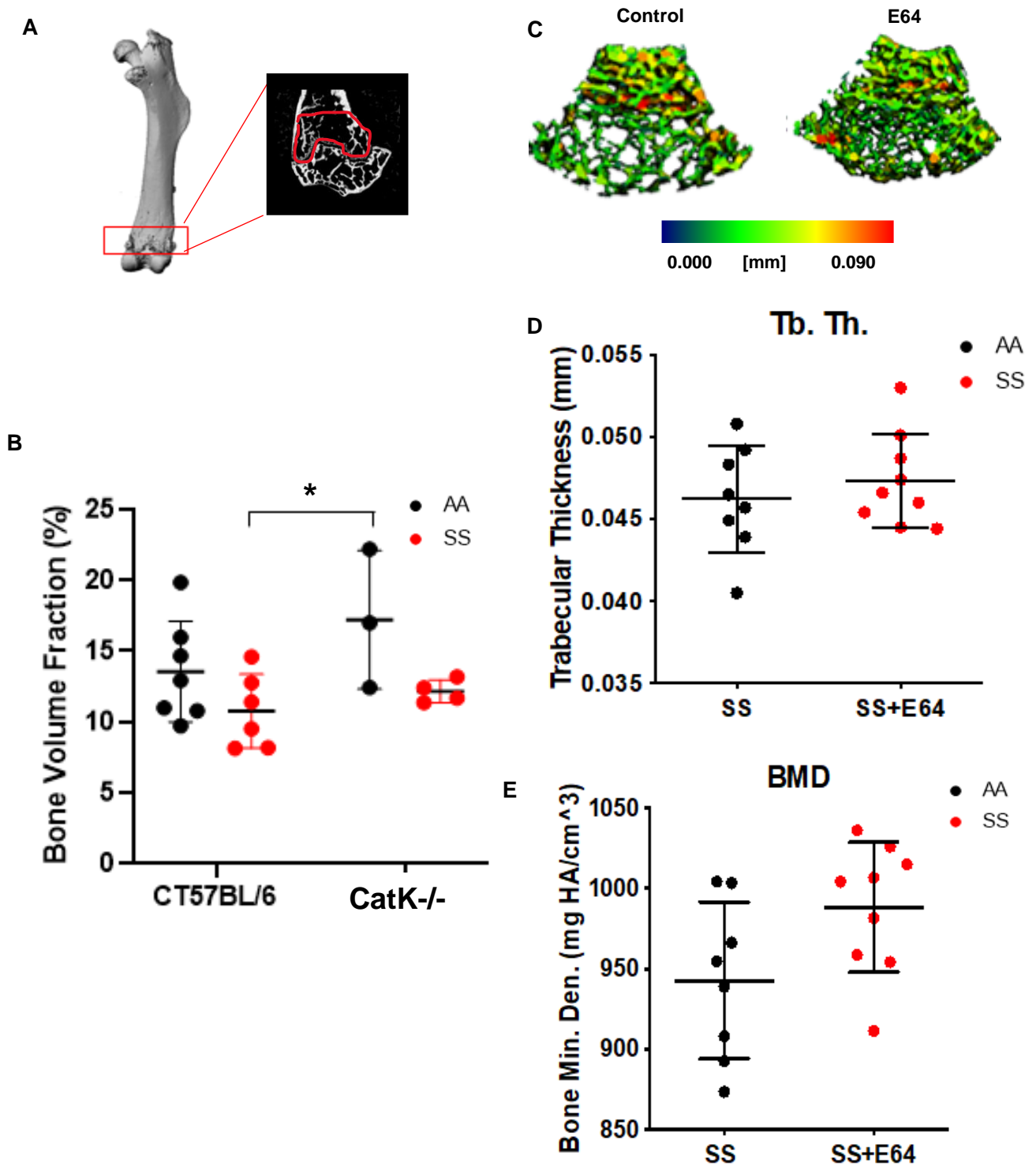
Supplemental Table 1. Summary of sex-specific means and significance generated for bone parameters in the distal epiphyseal region of AA, AS and SS mice at 3- and 5- months. Data expressed as means \pm SD. Statistical significance, * $p < 0.05$, determined by two-way ANOVA with Tukey's post-hoc test, n=4-6 mice per group. Abbreviations: Tb. Th., trabecular thickness; Tb. Sp., trabecular spacing; Conn. Density, connective density; BMD, bone mineral density; Tb. N., trabecular number.

Supplemental Table 2. Summary of sex-specific means and significance generated for bone parameters in the metaphyseal region of AA, AS and SS mice at 3- and 5- months. Data expressed as means \pm SD. Statistical significance, * $p < 0.05$, determined by two-way ANOVA with Tukey's post-hoc

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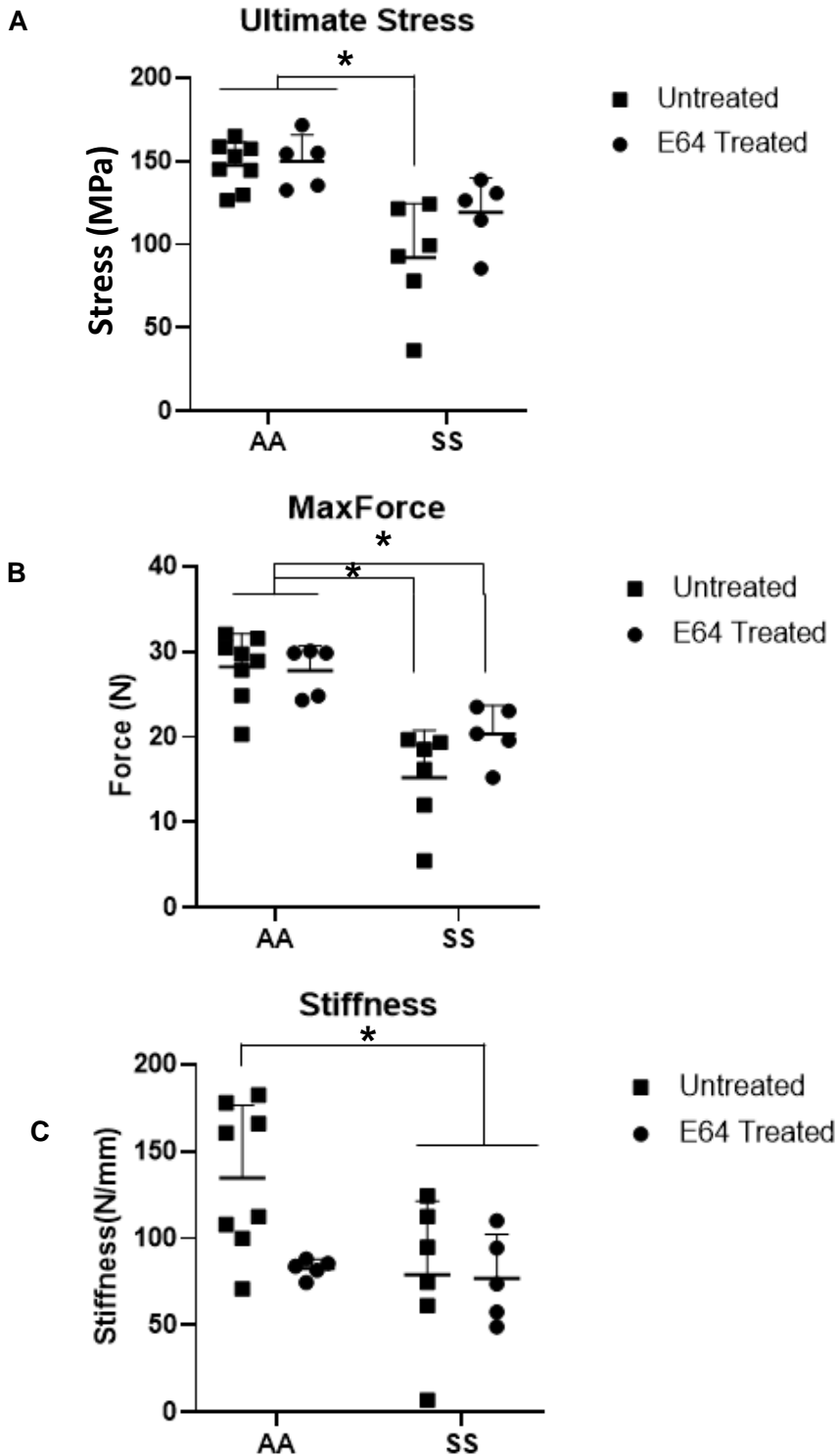
Supplemental Table 3. Summary of sex-specific means and significance generated for bone parameters in the mid-Diaphyseal region of AA, AS and SS mice at 3- and 5- months. Data expressed as means \pm SD. Statistical significance, * $p < 0.05$, determined by two-way ANOVA with Tukey's post-hoc test, n=4-6 mice per group. Abbreviations: Cort. Th., cortical thickness; BMD, bone mineral density; BA, bone area

Supplemental Figure 1



Supplemental Figure 1. Analysis of the metaphyseal region in mice with sickle cell disease

Supplemental Figure 2



Supplemental Figure 2. Bone mechanical testing parameters generated by four-point bending technique in 3-month-old AA and SS mice with or without treatment with E64.

Distal Epiphysis

		AA		AS		SS	
		Male	Female	Male	Female	Male	Female
Tb. Th. (mm)	3 mo	0.054 ±0.0020 ^a	0.056 ±0.0029 ^c	0.053 ±0.0019	0.052 ±0.0010	0.048 ±0.0013	0.048 ±0.0016
	5 mo	0.061 ±0.0052 ^a	0.065 ±0.0070 ^d	0.064 ±0.0054 ^a	0.061 ±0.0014	0.061 ±0.0078 ^{a,b}	0.056 ±0.0047
BMD (mg HA/cm³)	3 mo	1108 ±27.40	1116 ±22.29	1124 ±30.82	1123 ±31.10 ^c	1063 ±56.08	1137 ±27.68
	5 mo	1158 ±37.11 ^a	1143 ±17.76 ^{c,d}	1176 ±15.88 ^a	1175 ±15.10 ^d	1143 ±28.48	1094 ±28.99
Tb. Sp. (mm)	3 mo	0.129 ±0.00678	0.139 ±0.00335 ^c	0.118 ±0.00264	0.139 ±0.00487 ^c	0.120 ±0.00550	0.127 ±0.00494
	5 mo	0.128 ±0.0100	0.145 ±0.00282 ^{c,d}	0.127 ±0.00962	0.144 ±0.00346 ^{c,d}	0.128 ±0.0069	0.129 ±0.00494
Conn. Density (1/mm³)	3 mo	239.9 ±13.26 ^a	174.6 ±11.59 ^c	277.2 ±28.22 ^a	210.8 ±36.86 ^c	382.2 ±23.31	291.1 ±25.31
	5 mo	202.5 ±26.22 ^a	127.1 ±10.19 ^{c,d}	179.6 ±20.80 ^a	146.2 ±27.54 ^{c,d}	229.6 ±72.77 ^{a,b}	228.1 ±57.50 ^{c,d}
Tb. N. (1/mm)	3 mo	8.224 ±0.2361	8.148 ±0.1879	8.187 ±0.1411	8.119 ±0.1075	8.518 ±0.5017	8.242 ±0.1408
	5 mo	7.800 ±0.4036	8.164 ±0.3537	8.072 ±0.4569	7.871 ±0.2936	8.049 ±0.1775	8.280 ±0.2359
BV/TV (%)	3 mo	31.64 ±2.160	27.50 ±1.595	33.98 ±1.401 ^a	26.64 ±2.531	29.20 ±2.268	27.36 ±1.704
	5 mo	34.56 ±1.886	30.37 ±3.552	37.60 ±3.116 ^a	29.11 ±2.800	32.53 ±3.158	29.76 ±2.094

^ap <0.05 compared with 3 mo SS male, ^bp <0.05 compared with 5 mo SS male, ^cp <0.05 compared with 3 mo SS female, ^dp <0.05 compared with 5 mo SS female by determined by two-way ANOVA with Tukey's post-hoc

Supplemental Table 1. Summary of sex-specific means and significance generated for bone parameters in the distal epiphyseal region of AA, AS and SS mice at 3- and 5- months.

Metaphysis

		AA		AS		SS	
		Male	Female	Male	Female	Male	Female
Tb. Th. (mm)	3 mo	0.048 ±0.0018	0.049 ±0.0063	0.047 ±0.0013	0.045 ±0.0011	0.046 ±0.0018	0.045 ±0.0030
	5 mo	0.054 ±0.0051	0.046 ±0.0030	0.056 ±0.0085	0.044 ±0.0013	0.056 ±0.0030	0.045 ±0.0026
BMD (mg HA/cm³)	3 mo	951 ±76.52	976 ±79.78	1013 ±25.98	1008 ±20.11	975 ±55.52	974 ±46.19
	5 mo	1053 ±24.33	1012 ±60.39	1082 ±32.81	1052 ±40.65	1055 ±61.03	1015 ±58.56
Tb. Sp. (mm)	3 mo	0.165 ±0.00035	0.175 ±0.0100	0.162 ±0.00728	0.176 ±0.0187	0.142 ±0.0153	0.166 ±0.00141
	5 mo	0.220 ±0.0309 ^a	0.223 ±0.0199	0.220 ±0.0267	0.275 ±0.0206 ^c	0.222 ±0.0349 ^{a,b}	0.266 ±0.0606
Conn. Density (1/mm³)	3 mo	309.1 ±59.53 ^a	310.7 ±120.37 ^c	314.8 ±11.95 ^a	362.2 ±102.15	467.7 ±36.47	316.9 ±49.52
	5 mo	161.7 ±43.92 ^a	244.3 ±34.75 ^c	149.5 ±53.20 ^a	118.5 ±31.40 ^{c,d}	203.7 ±51.98 ^{a,b}	198.7 ±83.13 ^{c,d}
Tb. N. (1/mm)	3 mo	6.131 ±0.3995	5.750±0.4368	6.128 ±0.2723	5.672 ±0.5533	7.139 ±0.7783	6.337 ±0.03097
	5 mo	4.587 ±0.6318 ^a	4.485 ±0.3077 ^c	4.435 ±0.4294 ^a	3.762 ±0.2543 ^c	4.682 ±0.6898 ^{a,b}	4.289 ±1.084 ^{c,d}
BV/TV (%)	3 mo	19.14 ±1.460	21.95 ±5.798	17.23 ±1.533	20.40 ±6.362	22.47±1.961	20.12 ±5.211
	5 mo	17.80 ±4.073	14.77 ±3.127	16.98 ±4.247	9.31 ±0.4216 ^c	17.72 ±2.814	13.78 ±2.114

^ap <0.05 compared with 3 mo SS male, ^bp <0.05 compared with 5 mo SS male, ^cp <0.05 compared with 3 mo SS female, ^dp <0.05 compared with 5 mo SS female by determined by two-way ANOVA with Tukey's post-hoc

Supplemental Table 2. Summary of sex-specific means and significance generated for bone parameters in the metaphyseal region of AA, AS and SS mice at 3- and 5- months.

Mid-Diaphysis

		AA		AS		SS	
		Male	Female	Male	Female	Male	Female
Cort. Th. (mm)	3 mo	0.163 ±0.00806 ^a	0.159 ±0.0106 ^c	0.155 ±0.0122 ^a	0.152 ±0.0158 ^c	0.117 ±0.00992	0.122 ±0.00751
	5 mo	0.184 ±0.00517 ^{a,b}	0.186 ±0.0136 ^d	0.184 ±0.0103 ^{a,b}	0.167 ±0.00514 ^d	0.151 ±0.0050 ^{2a,b}	0.137 ±0.00654
BMD (mg HA/cm ³)	3 mo	1414 ±4.523 ^a	1399 ±41.28	1420 ±27.41 ^{a,b}	1418 ±13.67 ^c	1345 ±23.59	1368 ±12.35
	5 mo	1458 ±4.251 ^{a,b}	1461 ±42.89 ^{c,d}	1442 ±34.68 ^a	1467 ±4.978 ^{c,d}	1389 ±55.09	1398 ±24.86
BA (mm ²)	3 mo	0.534 ±0.0183 ^a	0.492 ±0.0307	0.515 ±0.0493 ^a	0.484 ±0.0246	0.399 ±0.0405	0.415 ±0.0232
	5 mo	0.666 ±0.0523 ^{a,b}	0.587 ±0.0513 ^{c,d}	0.657 ±0.0343 ^b	0.534 ±0.0157 ^c	0.555 ±0.0611	0.460 ±0.0663
TA (mm ²)	3 mo	1.296±0.3 953	1.212 ±0.05478	1.362 ±0.03019	1.263 ±0.1011	1.298 ±0.0630 0	1.318 ±0.06452
	5 mo	1.493±0.2 884	1.282 ±0.06568	1.590±0.2 064	1.281 ±0.1015	1.604±0 .2242	1.359 ±0.1908
BA/TA (%)	3 mo	39.90 ±1.628 ^{a,b}	41.39 ±3.897 ^c	38.29 ±2.195 ^a	38.31 ±2.751 ^c	29.40 ±2.331	31.42 ±0.9521
	5 mo	42.24 ±1.753 ^{a,b}	45.83 ±2.595 ^d	41.76 ±3.238 ^{a,b}	41.8 ±0.8662 ^d	34.13 ±1.267 ^{a,b}	34.1 ±3.118

^ap <0.05 compared with 3 mo SS male, ^bp <0.05 compared with 5 mo SS male, ^cp <0.05 compared with 3 mo SS female, ^dp <0.05 compared with 5 mo SS female by determined by two-way ANOVA with Tukey's post-hoc

Supplemental Table 3. Summary of sex-specific means and significance generated for bone parameters in the mid-diaphyseal region of AA, AS and SS mice at 3- and 5- months.