

1 **Supplemental Materials and Methods**

2 **1. MicroCT Analysis of Trabecular Architecture**

3 To evaluate trabecular architecture of the distal femur, microCT was performed on fixed
4 bones, as described previously using a Scanco μ CT scanner and a 21- μ m voxel size (26, 32, 40,
5 41). The femur was placed in a holder and scanned non-destructively by using a Scanco μ CT
6 scanner (vivaCT 80; Scanco Medical AG, Bassersdorf, Switzerland) at 21 μ m isotropic voxel size
7 (the highest resolution) with X-ray source power of 55 kV and 145 μ A and integration time of 300
8 milliseconds. The trabecular microstructure of the distal femur was evaluated. The scanned grey-
9 scale images were processed by using a low-pass Gaussian filter ($\sigma = 0.8$, support = 1) to
10 remove noise, and a fixed threshold of 220 was used to extract the mineralized bone from soft
11 tissue and marrow phase. The reconstruction and 3D quantitative analyses were performed by
12 using software provided by Scanco. The same settings for scan and analysis were used for all
13 samples.

14 Scans were initiated at the distal end of the femur and extended to the center of the femur,
15 for a total of approximately 777 slices (~16.3 mm). Trabecular regions of interest consisted of 189
16 slices (~3.969 mm), beginning 0.5 mm proximal to the growth plate and continuing in a proximal
17 direction, were included in the bone analysis. Cortical regions of interest consisted of 100 slices
18 at the proximal end of the scans, located ~2.1 mm at the center of the femur, were also analyzed.
19 Cancellous bone was separated from the cortical regions by semi-automatically drawn contours.
20 The following 3D indices in the defined ROI were analyzed: bone volume (BV, mm^3), tissue
21 (cortical and marrow) volume (TV, mm^3), relative bone volume over total volume (BV/TV, %),
22 trabecular number (Tb.N, mm^{-1}), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp,
23 μm), connectivity density (Conn.Dn, mm^{-3}), structure model index (SMI, ranges from 0 to 3 with 0
24 = platelike and 3 = rodlike).

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26 2. Quantitative PCR.

27 The following osteoblastic and osteoclastic primers are purchased from ABI TaqMan Gene
28 Expression Assay: RANKL (Rn00589289_m1), TRAP (Rn00569608_m1), Intergrin β 3
29 (Rn01763790_m1), calcitonin receptor (CTR) (Rn00587525_m1), Runx2 (Rn01512296_m1),
30 osteocalcin (Rn00566386_g1), BSP (Rn00561414_m1), DKK1 (Rn01501538_g1), DKK2 (Rn0
31 1748499_m1), and sFRP1 (Rn01478472_m1). SOST primer is a customer-made one: SOST-
32 R: ATCTTTGGCGTCATAGGGATGGTG; SOST-F:CTTCAGGAATGATGCCACAGAGGT.

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34 **Supplemental Figure Legend**

35 **Figure 1. Cortical architecture of the femur midshaft after acute SCI. (A)** Representative
36 micro-CT 3D-images of cortical microarchitecture are displayed. **(B)** Cortical bone area (Ct.Ar),
37 total tissue area (Tt.Ar), the ratio of Ct.Ar and Tt.Ar (Ct.Ar/Tt.Ar), Cortical bone thickness (Ct.Th)
38 and medullary area. The percent change versus Sham group was calculated at 2- and 7-days
39 post-SCI for each outcome measurement and then was plotted in the graph. Data are expressed
40 as mean \pm SEM $n = 6-7$ animals per group. Significance of differences was determined by using
41 Student's *t*-test. * < 0.05 versus the indicated group.

Supplemental Table 1. The summarized data expressed in the original units of measurement by micro-CT

	2 days						
	BV/TV	Tb.N	Tb.Th	Tb.Sp	Conn-Dens.	SMI	Stiffness
Sham (n=7)	0.2646 ± 0.02668	3.863 ± 0.05561	0.0684 ± 0.006116	0.1906 ± 0.009115	96.29 ± 6.754	1.324 ± 0.2957	2023 ± 264.2
SCI (n=6)	0.2156 ± 0.01937	3.333 ± 0.1104	0.06242 ± 0.004569	0.2010 ± 0.01180	76.46 ± 4.982	1.674 ± 0.2030	1230 ± 237.0
	7 days						
	BV/TV	Tb.N	Tb.Th	Tb.Sp	Conn-Dens.	SMI	Stiffness
Sham (n=7)	0.2342 ± 0.01072	4.121 ± 0.2125	0.07398 ± 0.001806	0.2517 ± 0.01583	122.0 ± 7.797	1.708 ± 0.1011	2.208 ± 0.3343
SCI (n=6)	0.1668 ± 0.01936	3.468 ± 0.1941	0.06558 ± 0.002492	0.3127 ± 0.01663	68.69 ± 13.44	2.325 ± 0.2131	1.043 ± 0.3410

