

940 **Supplementary figures:**

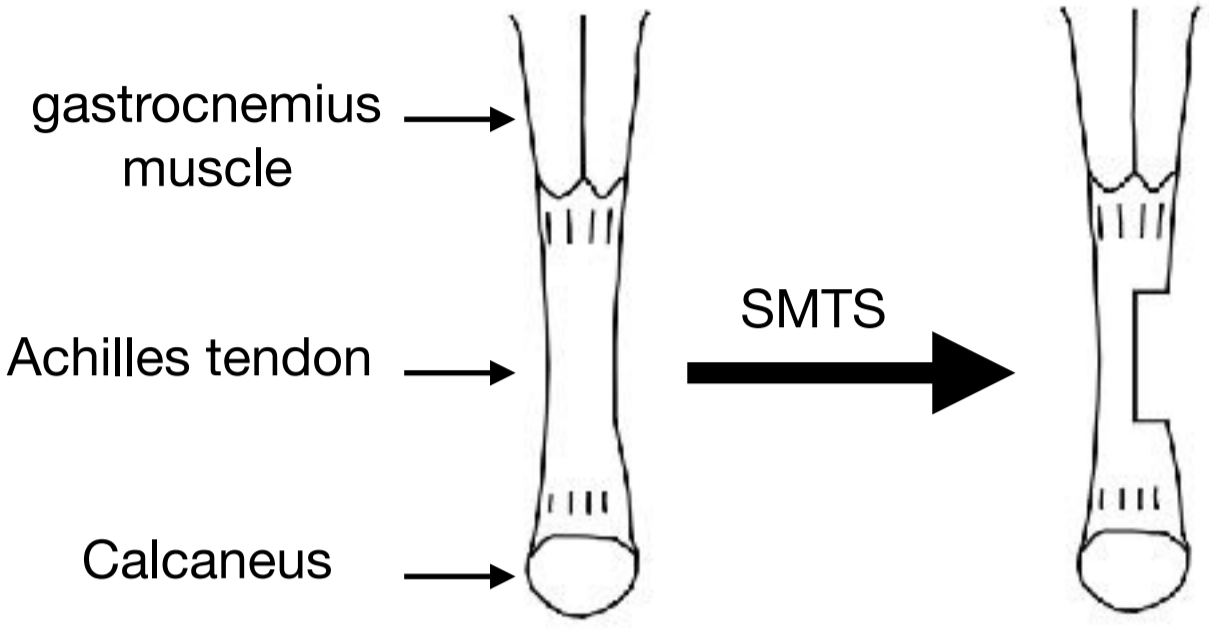
941 **Supplemental Figure 1:** Diagram of semi-transection of Achilles tendon surgery.

942

943 The SMTS surgery was performed in the middle of Achilles tendon body distant to Posterior
944 Calcaneal Tuberosity (PCT, the site of Achilles tendon attachment to calcaneus) to induce a
945 destabilized enthesopathy on Achilles tendon enthesis.

946

Supplemental Figure 1



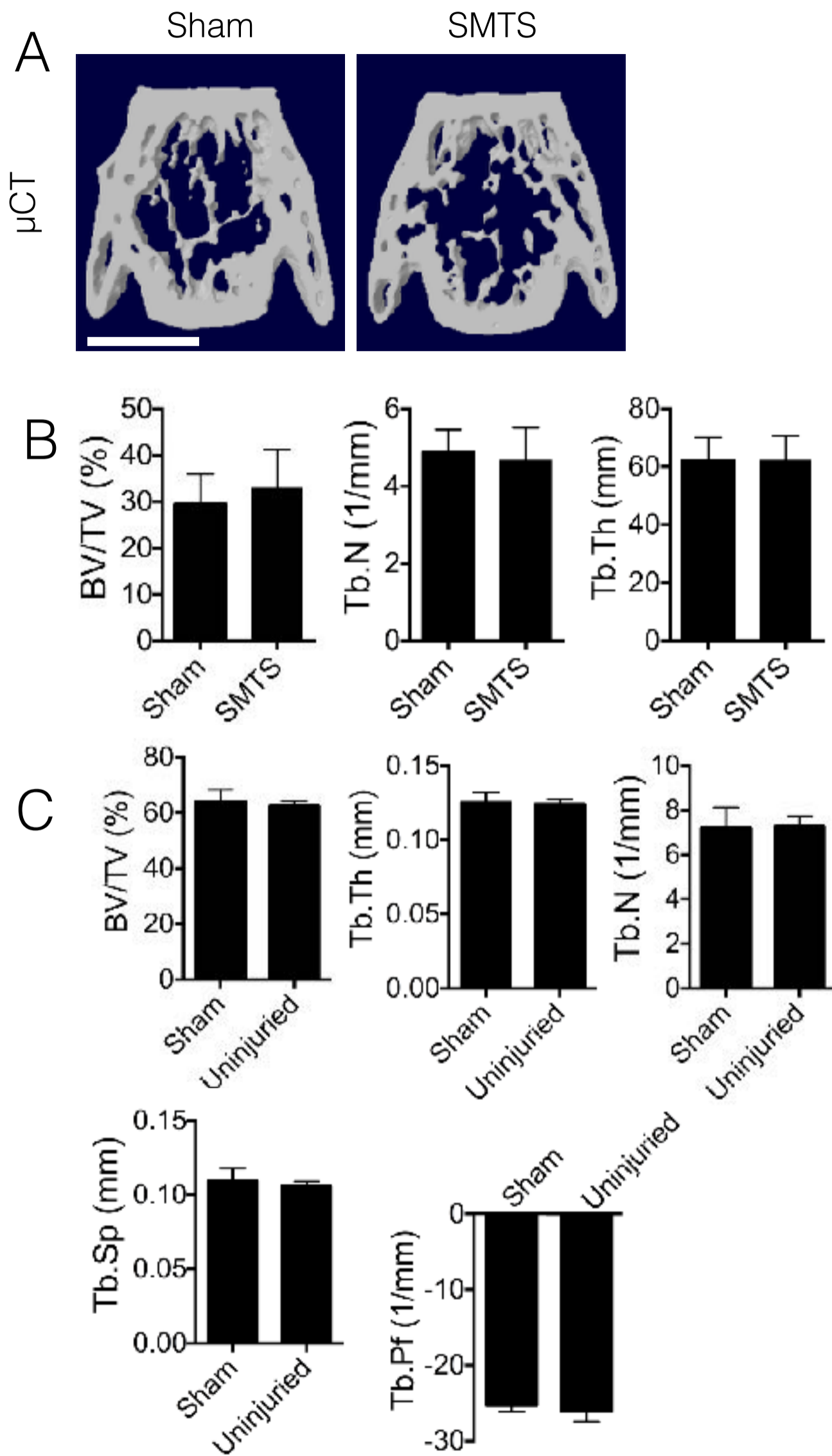
947 **Supplemental Figure 2:** High levels of active TGF- β did not alter bone turnover at a distant site
948 or induce enthesopathy at the uninjured enthesis

949

950 **(A)** Micro CT images of the L4 vertebral bodies and **(B)** quantification of structural parameters in
951 mice 8 weeks after sham or SMTS operation. Scale bar, 1mm. **(C)** Quantitative analysis of
952 BV/TV, Tb.Th, Tb.N, Tb.Sp and Tb.Pf of the contralateral (uninjured) Achilles tendon enthesis 8
953 weeks after SMTS determined by μ CT analysis relative to sham operated mice. Data shown as
954 Mean \pm SEM. n = 10.

955

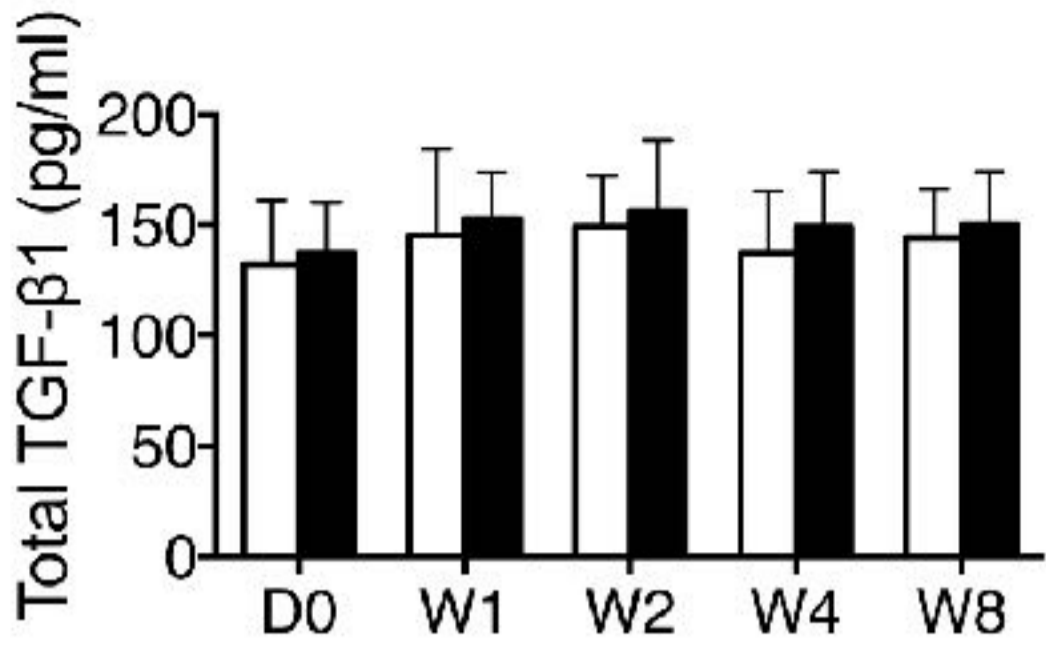
Supplemental Figure 2



956 **Supplemental Figure 3:** No significance was seen in total TGF- β 1 in peripheral serum by
957 ELISA.

958
959 Quantitative analysis of total TGF- β 1 in peripheral serum of Sham (open bar) and SMTS (black
960 bar) operated mice by ELISA. Data shown as Mean \pm SEM. n = 10. *P < 0.05 compared to the
961 sham group.

Supplemental Figure 3



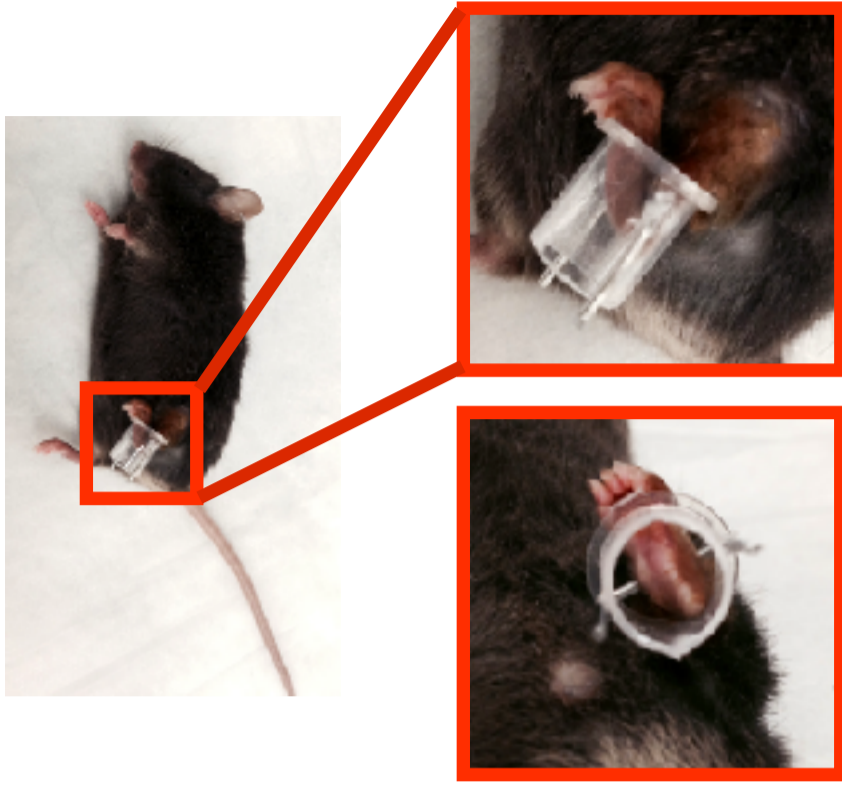
962 **Supplemental Figure 4:** Dorsiflexion immobilization device were applied on the left feet of mice.

963

964 (A) Devices were applied on mice to maintain a dorsiflexion position for 12 hours every day for 4
965 or 8 weeks as noted in results. (B, D) Immunostaining and (C, E) quantification of (B, C)
966 MMP13⁺ cells and (D, E) TUNEL⁺ cells of mid-Achilles tendon body of mice 8 weeks after sham
967 or DI. Scale bars, 200µm. Data shown as Mean ± SEM. n = 10. *P < 0.05 compared to the
968 sham group.

Supplemental Figure 4

A

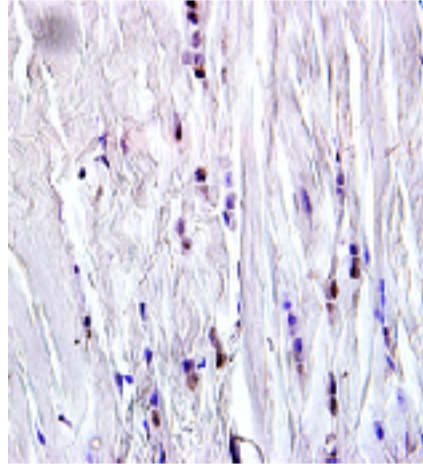
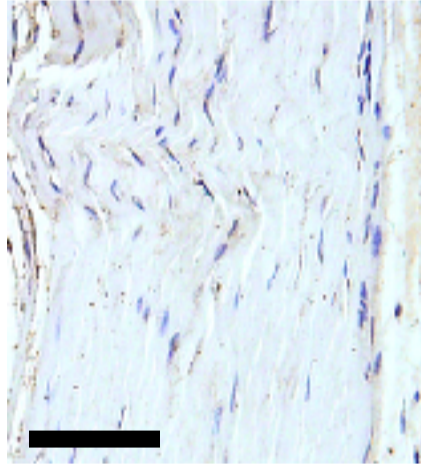


B

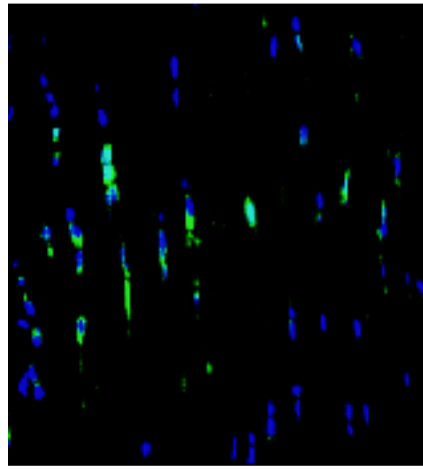
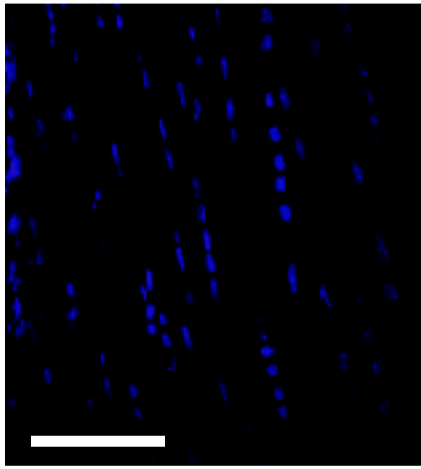
Sham

DI

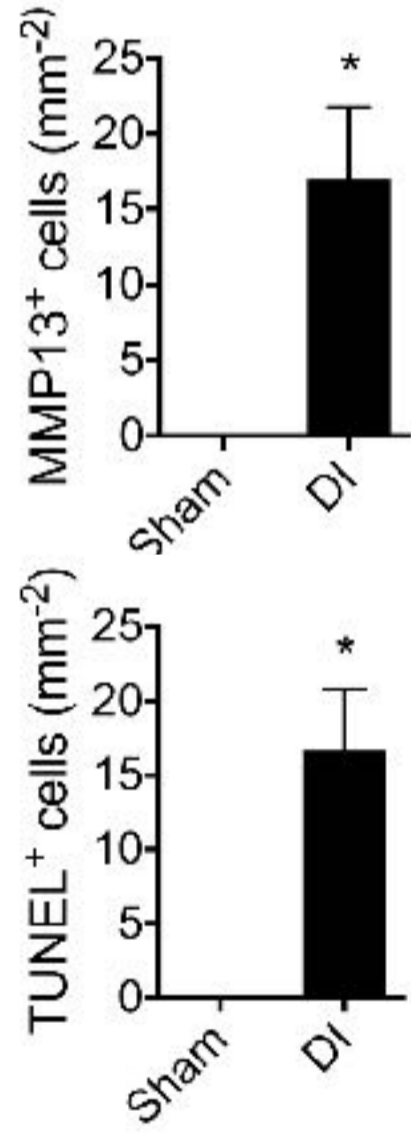
MMP13



DAPI/TUNEL



C



969 **Supplemental Figure 5:** Upregulated TGF- β signaling is associated with trabecular bone
970 changes in DI enthesopathy mice

971

972 **(A)** Micro CT images of the PCT (sagittal view). Scale bar, 500 μ m. **(B)** Quantitative analysis of
973 BV/TV, Tb.Th, Tb.N, Tb.Sp and Tb.Pf in PCT determined by μ CT analysis. **(C)** TRAP staining
974 (magenta) in mouse PCT bone marrow. Scale bar, 200 μ m. **(D)** Quantitative analysis of TRAP⁺
975 osteoclast surface (OCS) per Bone Surface (BS). **(E)** Immunofluorescent staining of pSmad2/3⁺
976 cells (red) in mouse PCT bone marrow. Scale bar, 200 μ m. **(F)** Quantitative analysis of the
977 number of pSmad2/3⁺ cells per bone marrow area (mm²). **(G)** Quantitative analysis of active
978 TGF- β 1 in serum by ELISA. **B**, Bone; **BM**, bone marrow. **W4**, four weeks after DI; **W8**, eight
979 weeks after DI. Data shown as Mean \pm SEM. n = 10. *P < 0.05 compared to the sham group.

Supplemental Figure 5

W4

W8

A

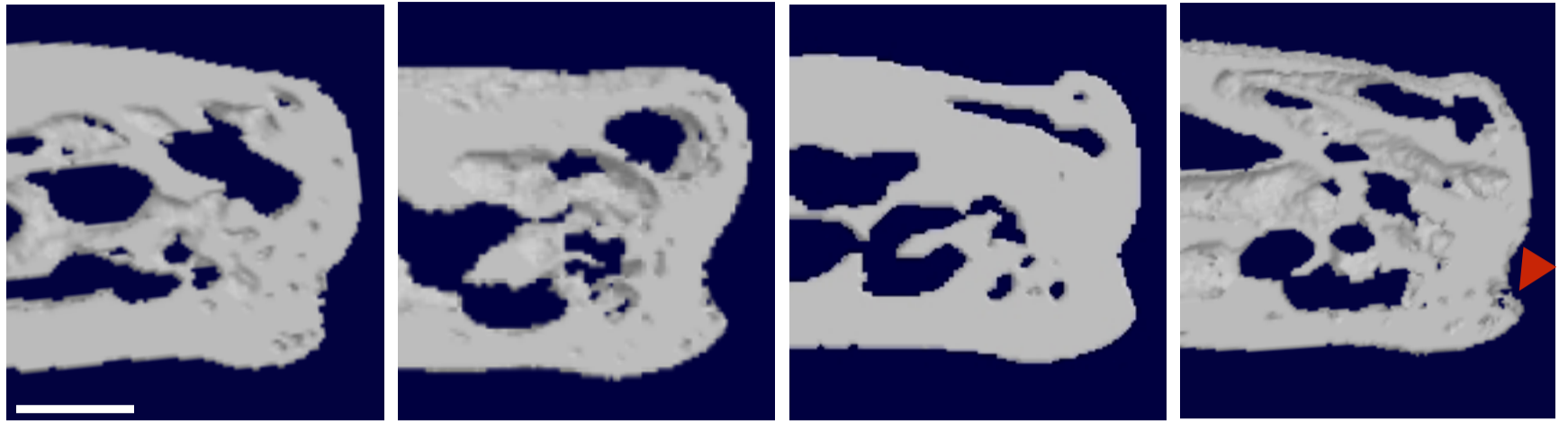
Sham

DI

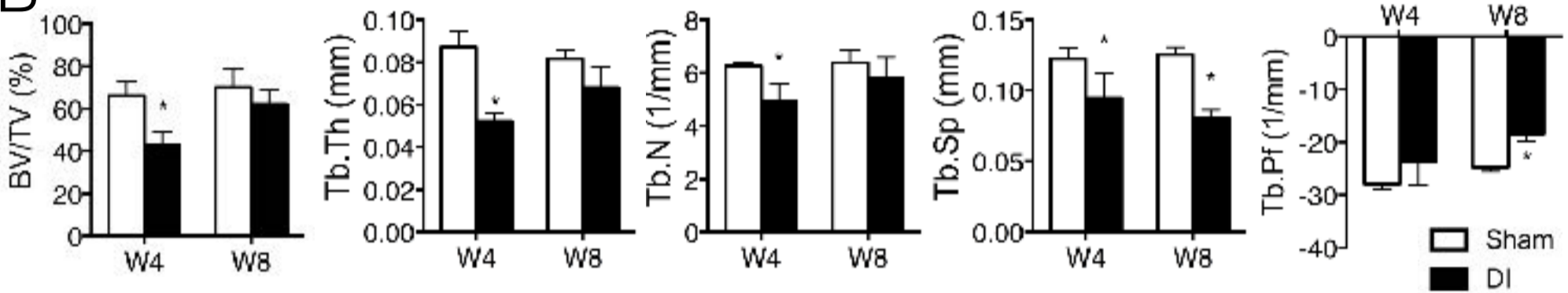
Sham

DI

μCT

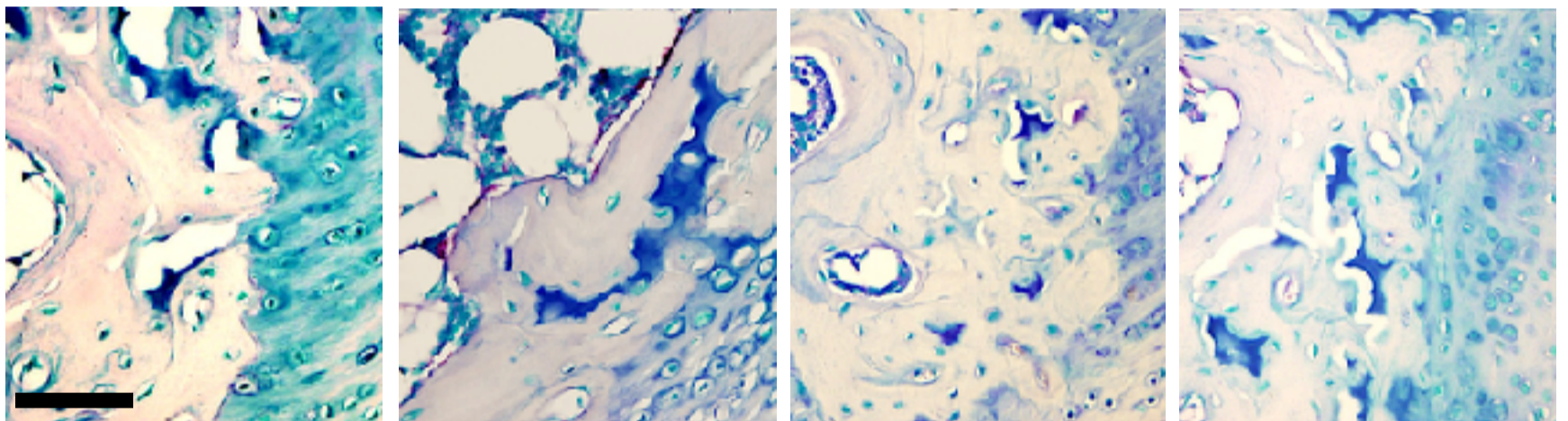


B



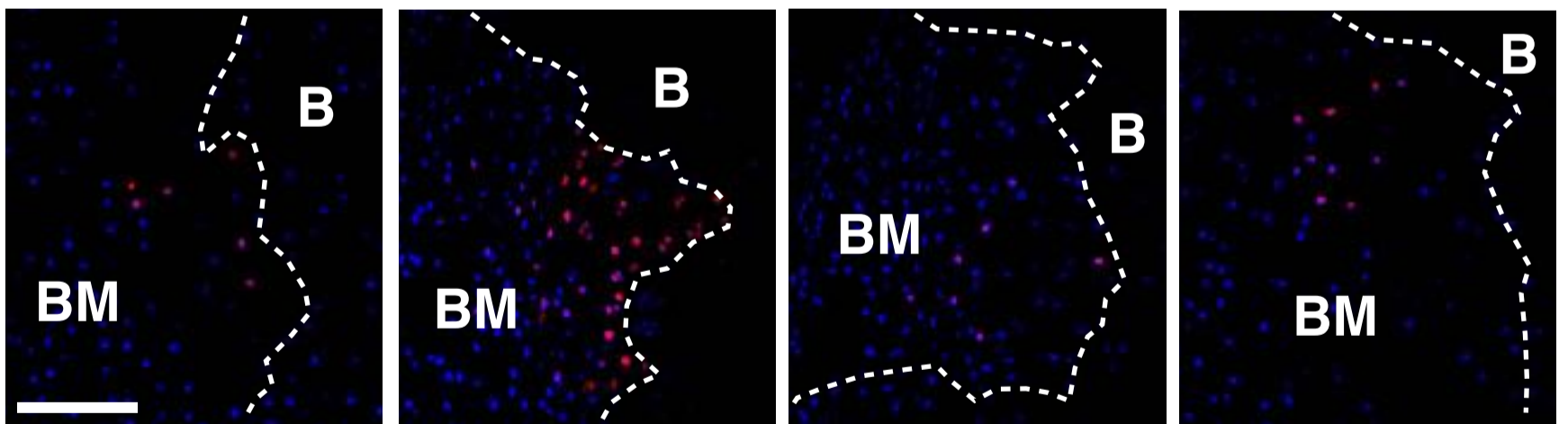
C

TRAP

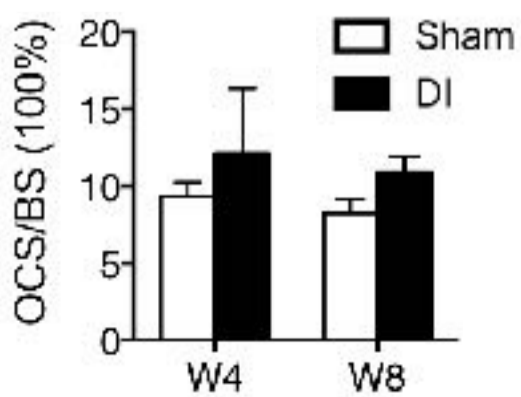


E

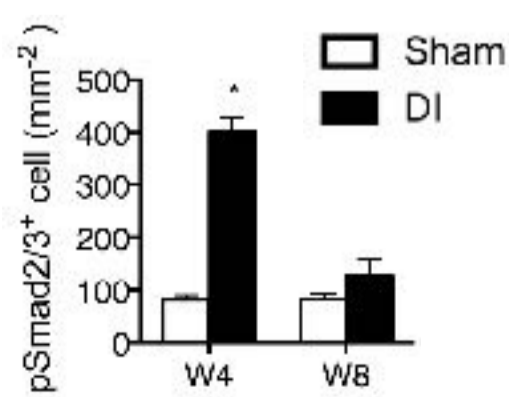
pSmad2/3/DAPI



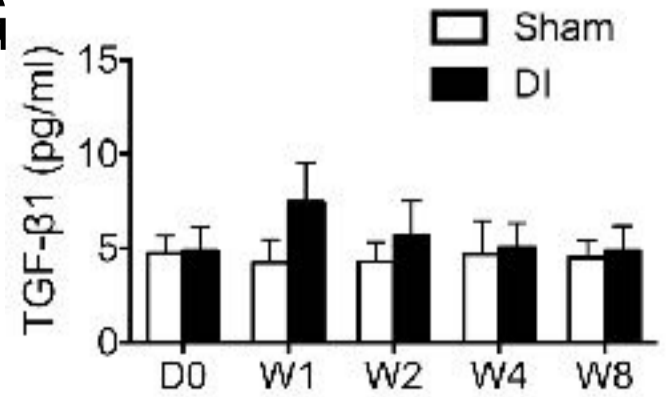
D



F



G

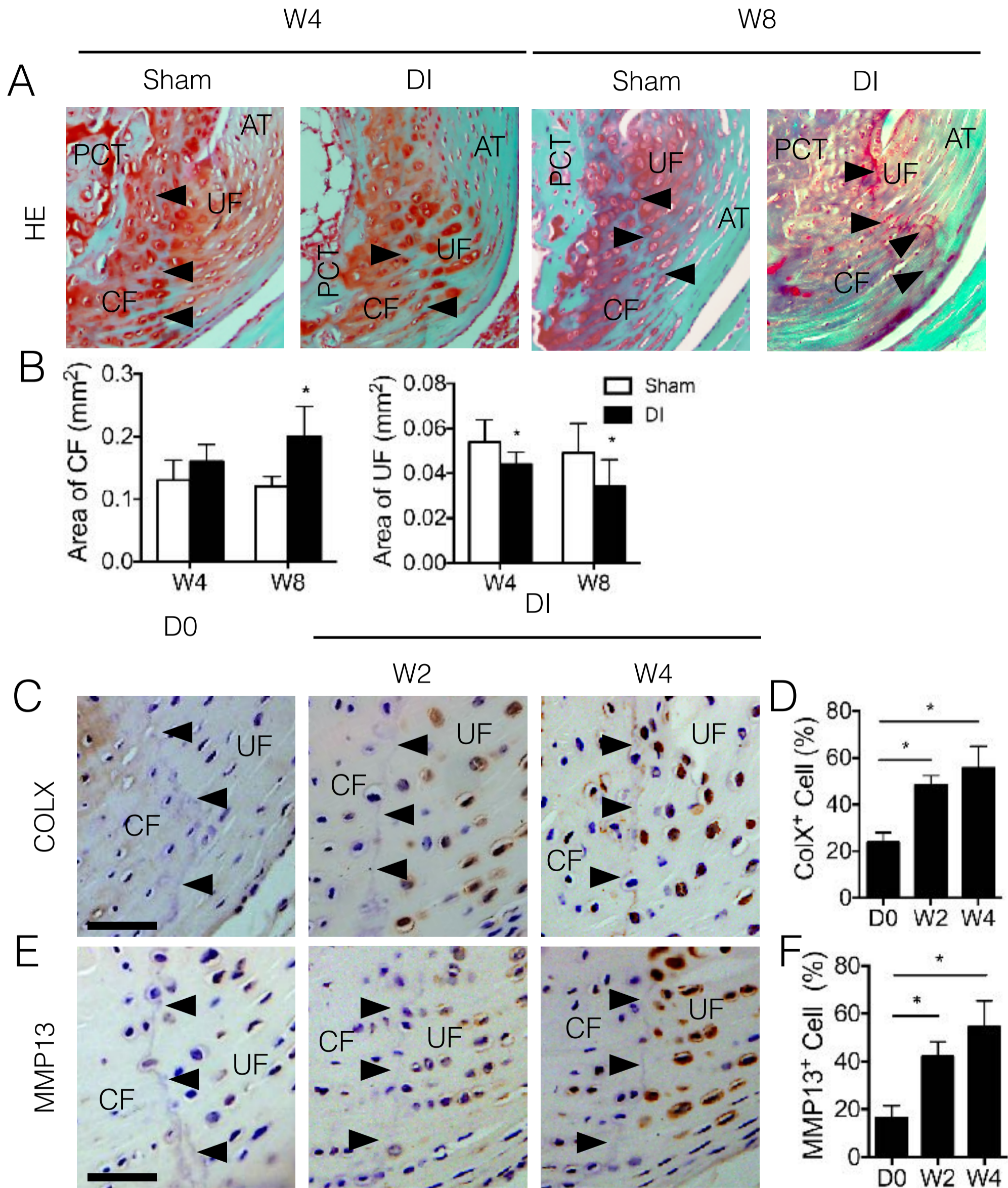


980 **Supplemental Figure 6:** Fibrocartilage displays mineralization in Achilles tendon enthesis.

981

982 (A) SOFG staining of Achilles tendon enthesis. Black triangles indicate tidemarks. To better
983 illustrate tidemarks, we used dotted lines in main figures. Scale bar, 200 μm . (B) Quantitative
984 analysis of area of CF and UF. (C, E) Immunohistochemical and (D, F) quantitative analysis of
985 (C, D) COLX⁺ cells and (E, F) MMP13⁺ cells in fibrocartilage of mouse Achilles tendon enthesis
986 after SMTS surgery. Black triangles indicate tide mark. Scale bars, 150 μm . D0, prior to SMTS
987 surgery; W2, two weeks after SMTS surgery; W4, four weeks after SMTS surgery; W8, eight
988 weeks after SMTS surgery. Data shown as Mean \pm SEM. n = 10. *P < 0.05 compared to the
989 sham or D0 group.

Supplemental Figure 6



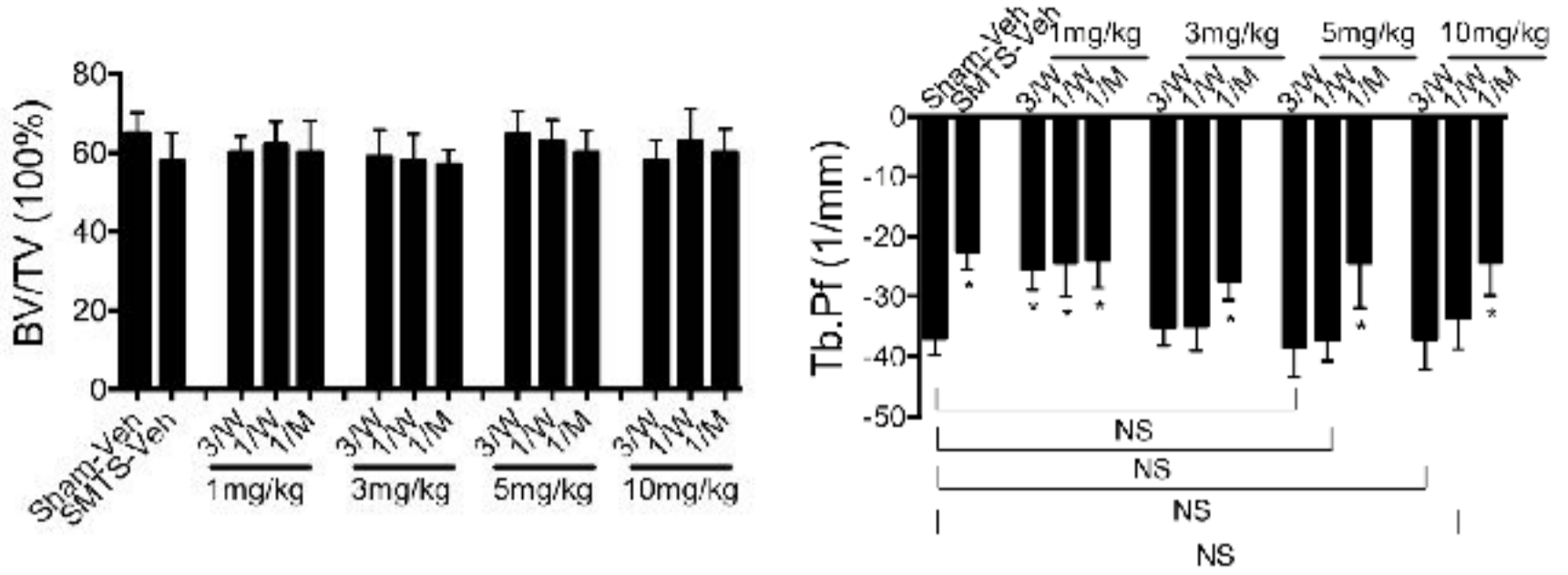
990 **Supplemental Figure 7:** The dosage of TGF- β antibody is optimized by screening experiments.

991

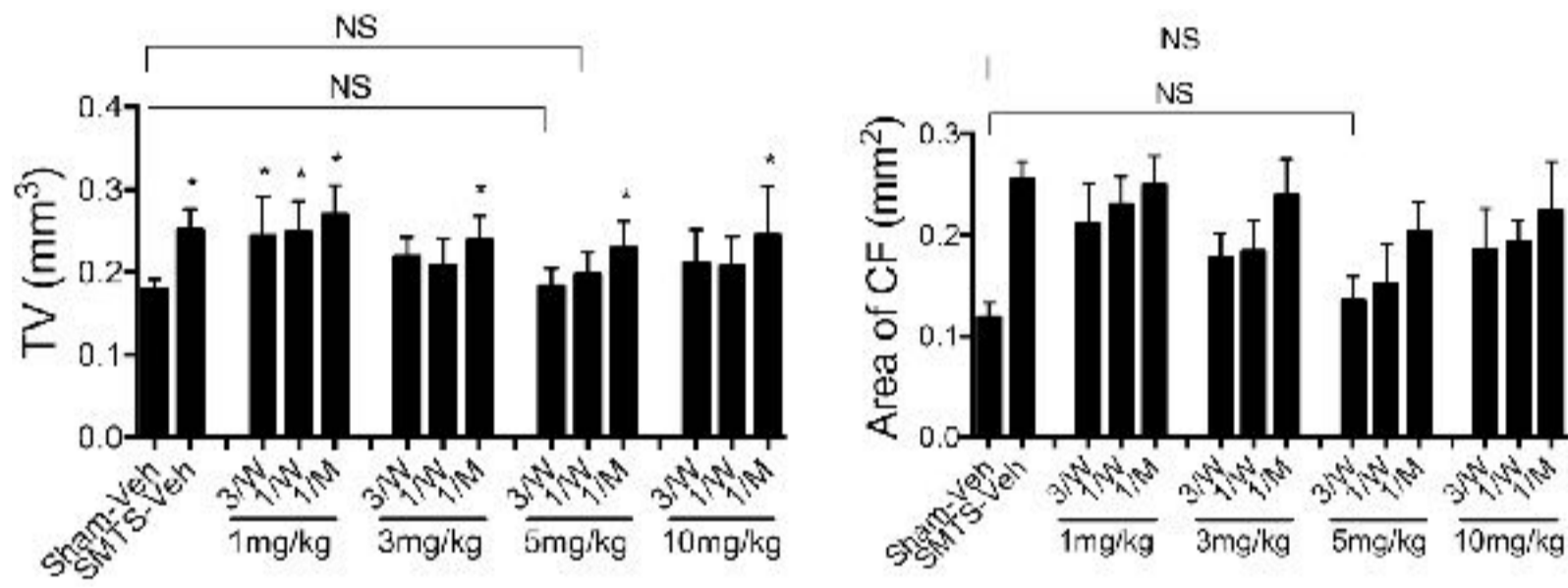
992 (A) Quantitative analysis of BV/TV and Tb.Pf in PCT determined by μ CT analysis of sham or
993 SMTS operated mice treated with different doses (1, 3, 5, 10 mg/kg) of TGF- β antibody (1D11)
994 or vehicle antibody (13C4) intraperitoneally three times a week (3/W), once a week (1/W) and
995 once a month (1/M) for 30 d. (B) Quantitative analysis of area of CF and UF at Achilles enthesis.
996 Data shown as Mean \pm SEM. n = 10. *P < 0.05 compared to the Sham-Vehicle group.
997

Supplemental Figure 7

A



B

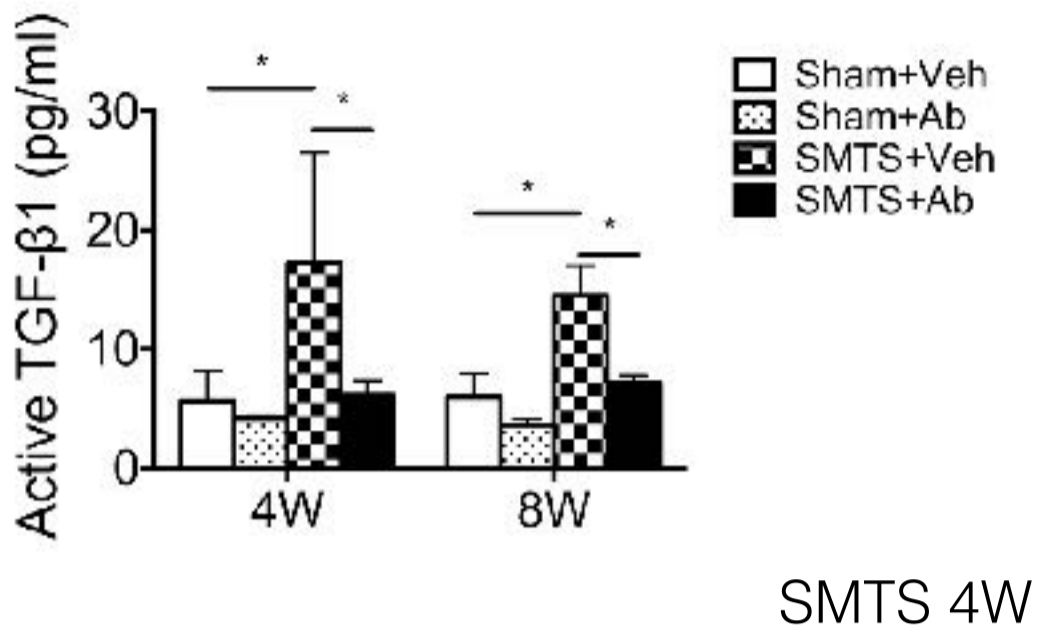


998 **Supplemental Figure 8:** 1D11 inhibits TGF- β signaling in enthesis.
999

1000 (A) Quantitative analysis of active TGF- β 1 in peripheral serum of antibody (1D11) or vehicle
1001 (13C4) treated mice 4 or 8 weeks after sham or SMTS operation by ELISA. (B, D)
1002 Immunohistochemical and (C, E) quantitative analysis of pSmad2/3⁺ cells (brown) in mouse (B,
1003 C) Achilles tendon enthesis fibrocartilage and (D, E) PCT bone. Scar bars, 50 μ m. Data shown
1004 as Mean \pm SEM. n = 10. *P < 0.05 compared between groups or to the sham group.

Supplemental Figure 8

A

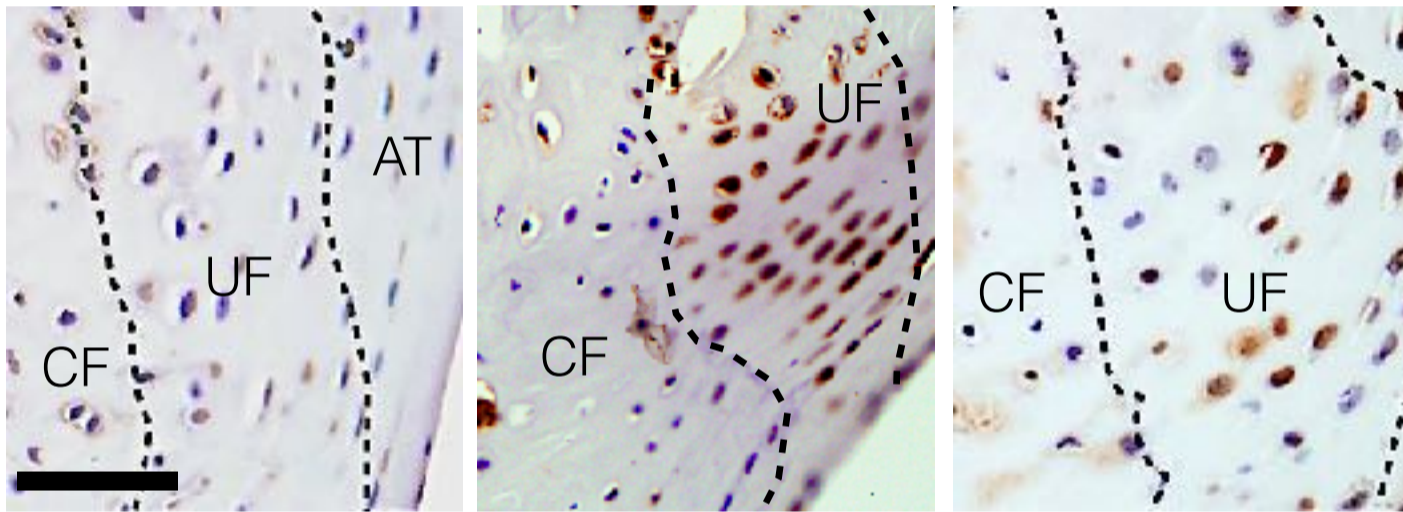


Sham + Veh

Veh

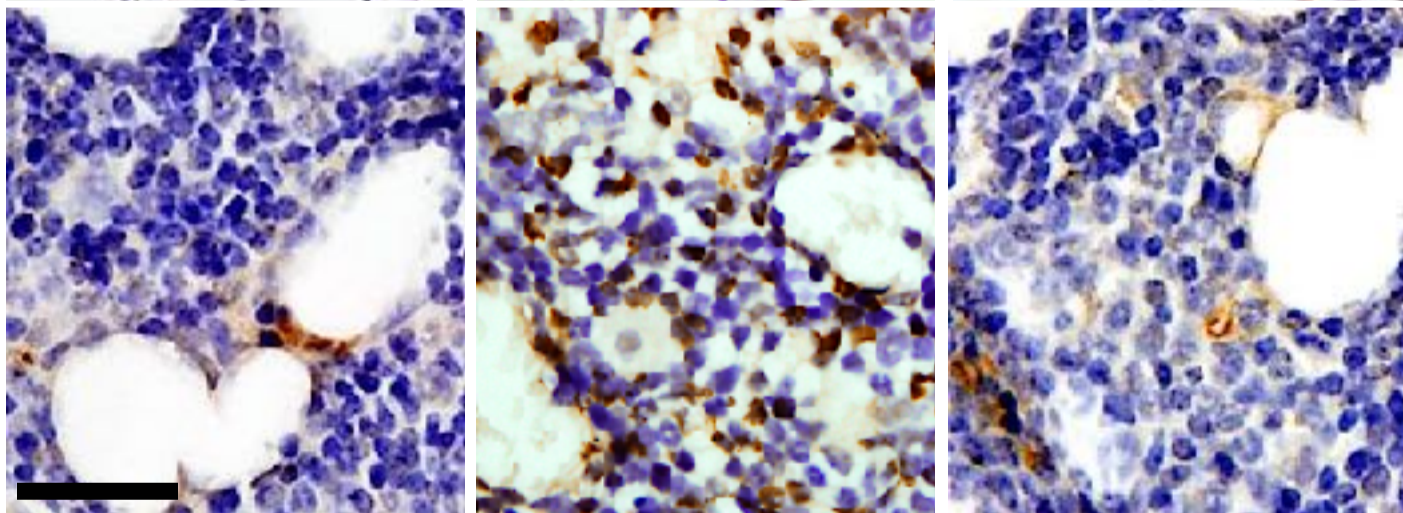
Ab

B



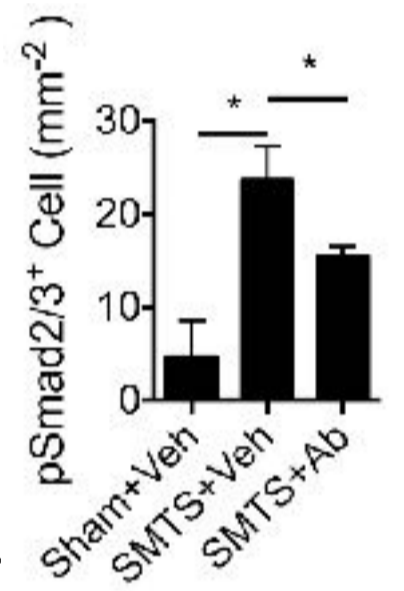
pSmad2/3

D

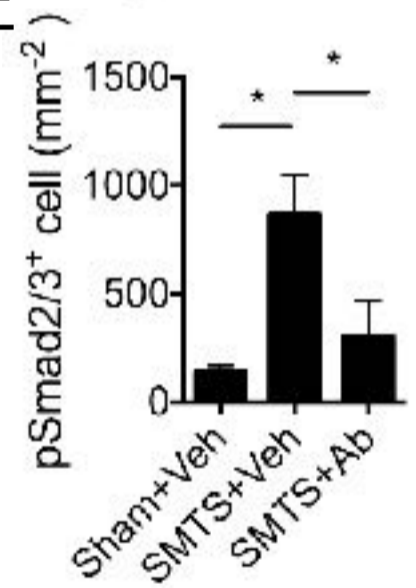


pSmad2/3

C



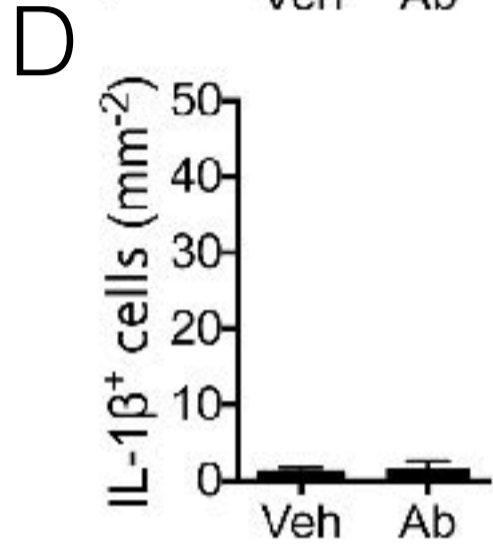
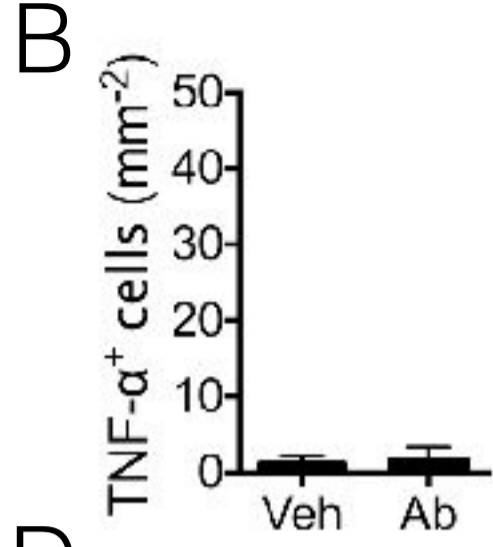
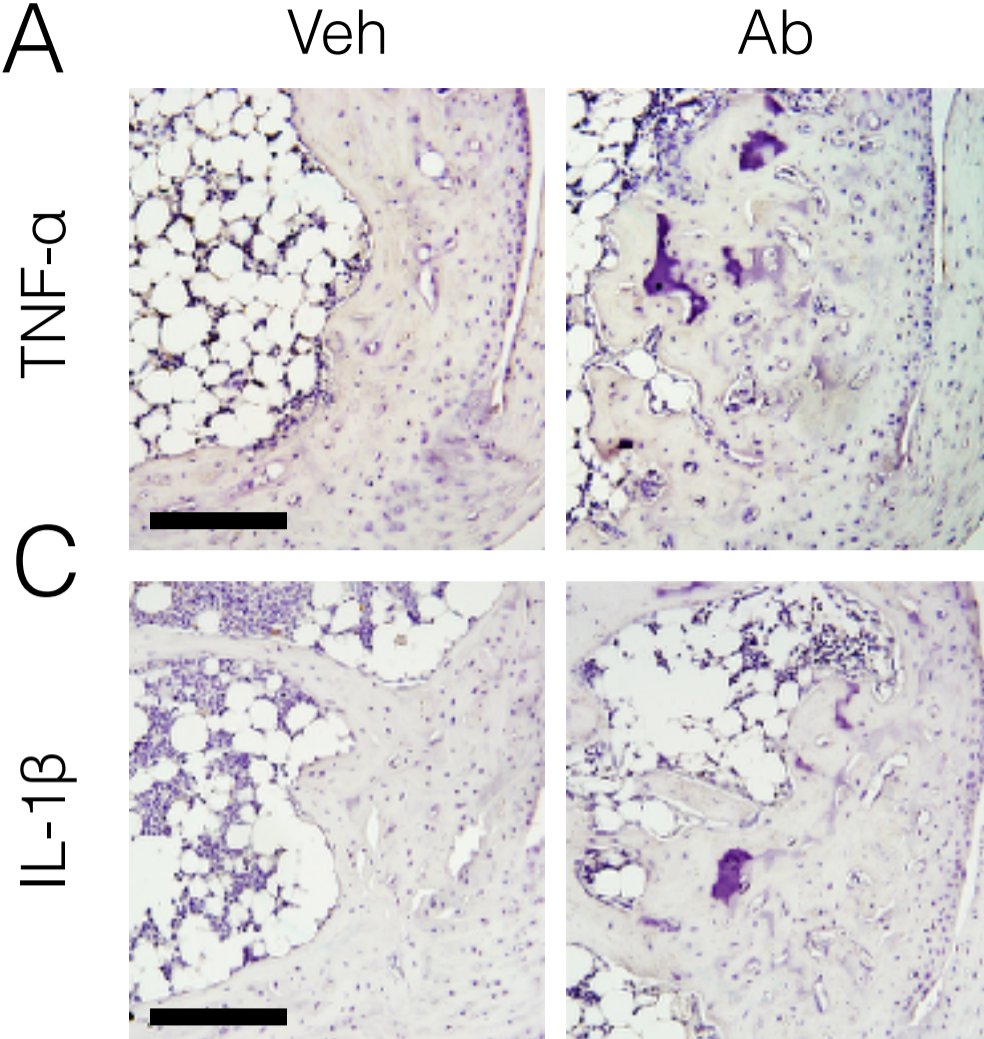
E



1005 **Supplemental Figure 9:** No significances in inflammatory cytokine levels between vehicle and
1006 antibody treated SMTS mice at week 8

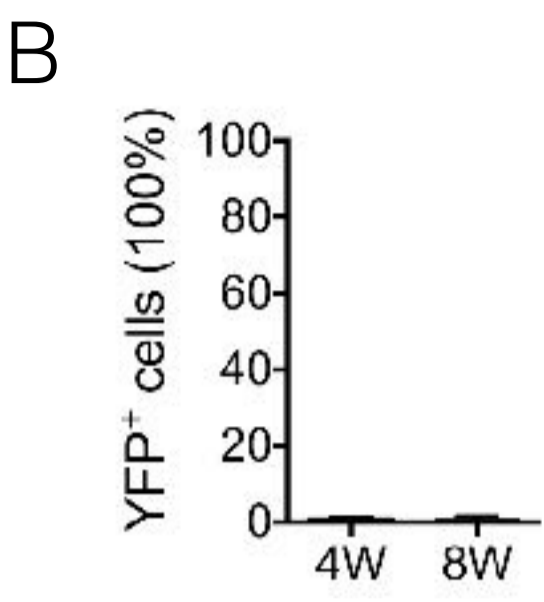
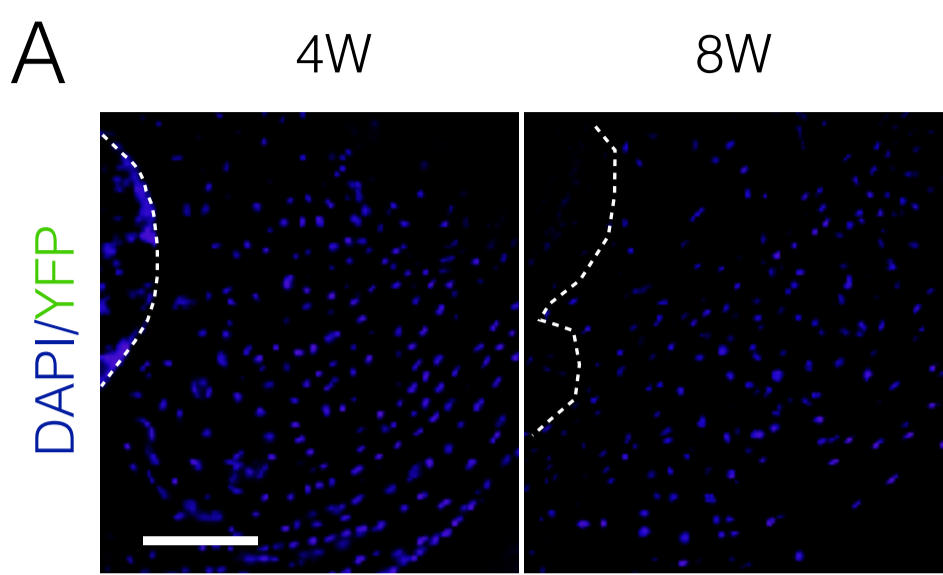
1007
1008 **(A, C)** Immunostaining of **(A)** TNF- α ⁺ and **(C)** IL-1 β ⁺ cells in the SMTS Achilles tendon enthesis
1009 (8 weeks) treated with vehicle or antibody and **(B, D)** quantitation. Scale bars, 250 μ m, n = 10.
1010

Supplemental Figure 9



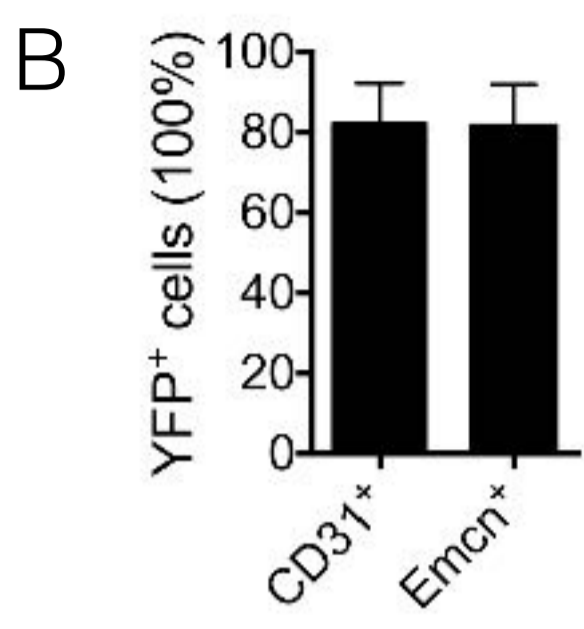
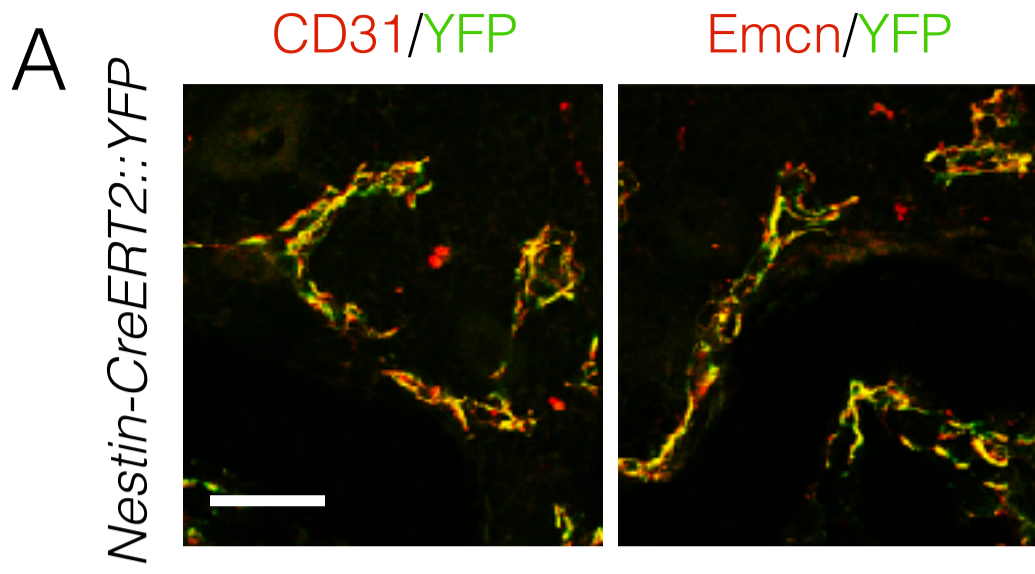
1011 **Supplemental Figure 10:** Enthesis is not derived from Nestin lineage cells in adult mice
1012
1013 **(A)** Immunostaining and **(B)** quantification of YFP⁺ cells (green) in Achilles tendons of 3 month
1014 old *Nestin-creERT2::YFP* mice injected with tamoxifen weekly for 4 and 8 weeks. Scale bars,
1015 200µm, n = 10.

Supplemental Figure 10



1016 **Supplemental Figure 11:** Vessels were formed at the tendon semi-transection site
1017
1018 **(A, left)** CD31⁺ cells (red), YFP⁺ cells (green) and **(A, right)** Emcn⁺ cells (red), YFP⁺ cells
1019 (green) and **(B)** quantitation in the tendon semi-transection site of *Nestin-creERT2::EYFP^{flox/flox}*
1020 mice 8 weeks post SMTS. Scale bar, 100μm, n = 10.

Supplemental Figure 11



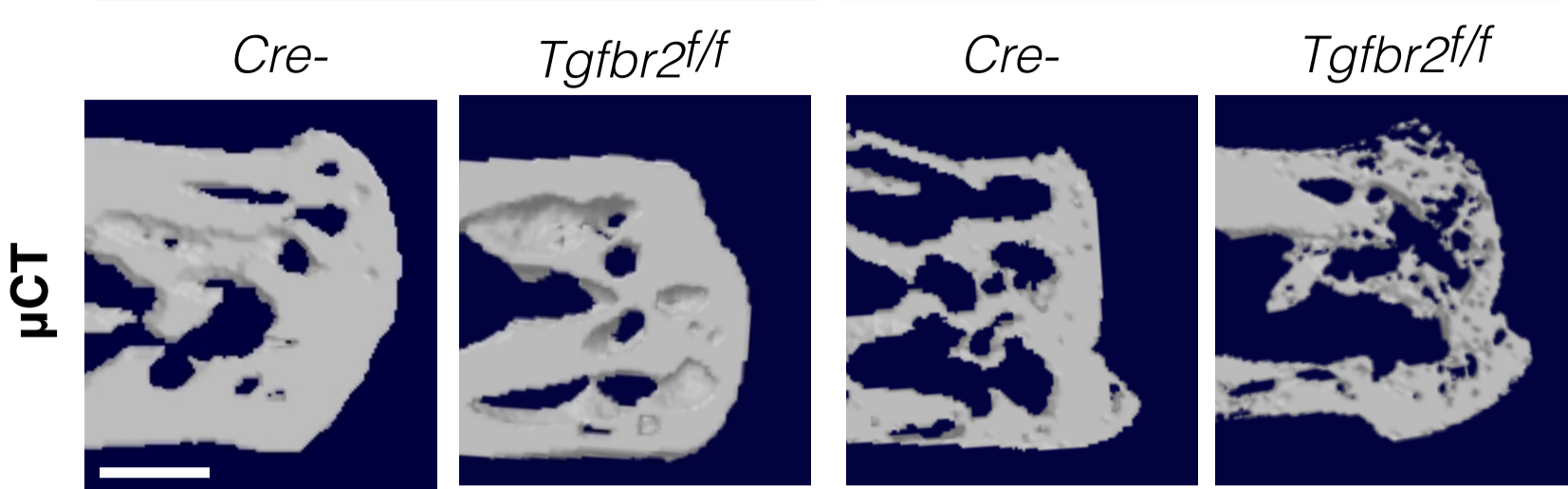
1021 **Supplemental Figure 12:** No significant differences between tamoxifen treated *Nestin-creERT2*
1022 mice (*Cre*) mice and vehicle treated *Nestin-creERT2::Tgfr2^{ff}* (*Tgfr2^{ff}*) mice.
1023
1024 (A) Micro CT images and (B) quantifications of PCT of tamoxifen treated *Nestin-creERT2* mice
1025 (*Cre*) mice and vehicle treated *Nestin-creERT2::Tgfr2^{flx/flx}* (*Tgfr2^{ff}*) mice. Scale bars, 500µm.
1026 Data shown as Mean ± SEM. n = 10. *P < 0.05.

Supplemental Figure 12

Sham 8W

SMTS 8W

A



B

