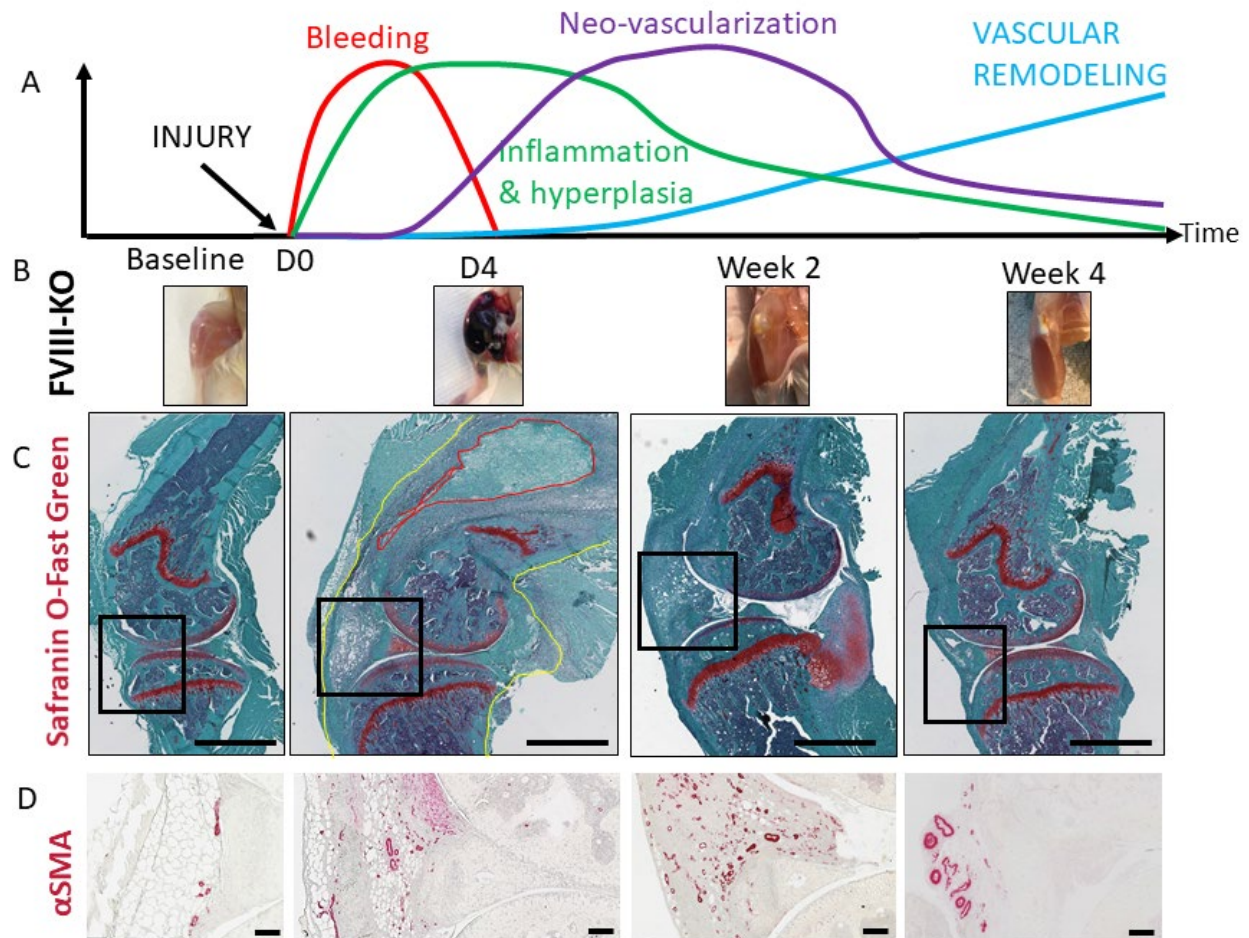


Supplemental data to Manuscript for JCI Insight

TAFI Deficiency Causes Maladaptive Vascular Remodeling After Hemophilic Joint Bleeding

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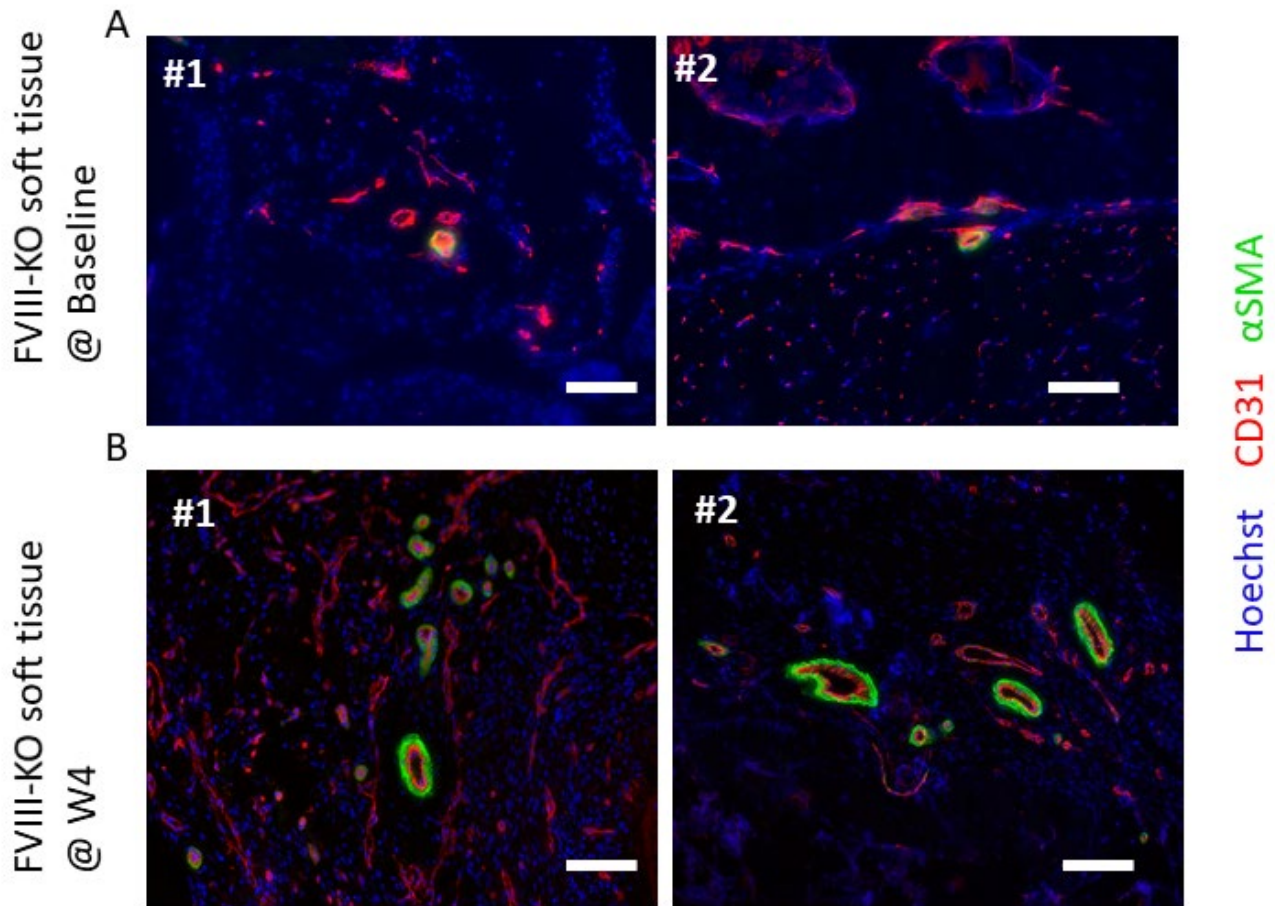
Supplemental Figures



Supplemental figure 1: Time-dependent intra-articular soft tissue and vascular changes caused by bleeding.

(A) Time course schematic of joint bleeding-induced tissue changes in BALB/c FVIII-KO mice, as previously established (1), with joint bleeding inducing synovial inflammation and hyperplasia that evokes a transient neovascular response that normalizes over time, while vascular remodeling into enlarged and distorted blood vessels progresses. (B) Representative pictures of the joints of FVIII-KO mice at the indicated time points after injury show that the intra- and peri-articular hematoma is largely resolved at week 2. (C) Representative Safranin-O Fast Green staining of injured joints showing soft tissue expansion and inflammation at day 4 and week 2,

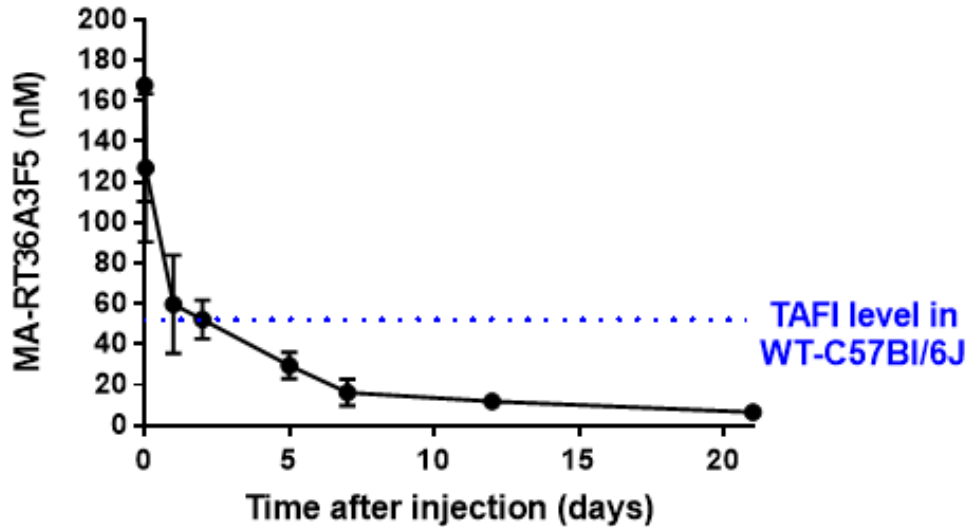
but to a much lesser extent at week 4 (scale bar = 1 mm). **(D)** Corresponding smooth muscle cell actin- α (α SMA) staining of the anterior meniscal areas indicating neovascularization is most profound at week 2, whereas excessive vascular remodeling into enlarged blood vessels persists beyond week 4 (scale bar= 100 μ m).



Supplemental figure 2: Vascular changes in the mouse joint after bleeding resemble those in Hemophilia patients.

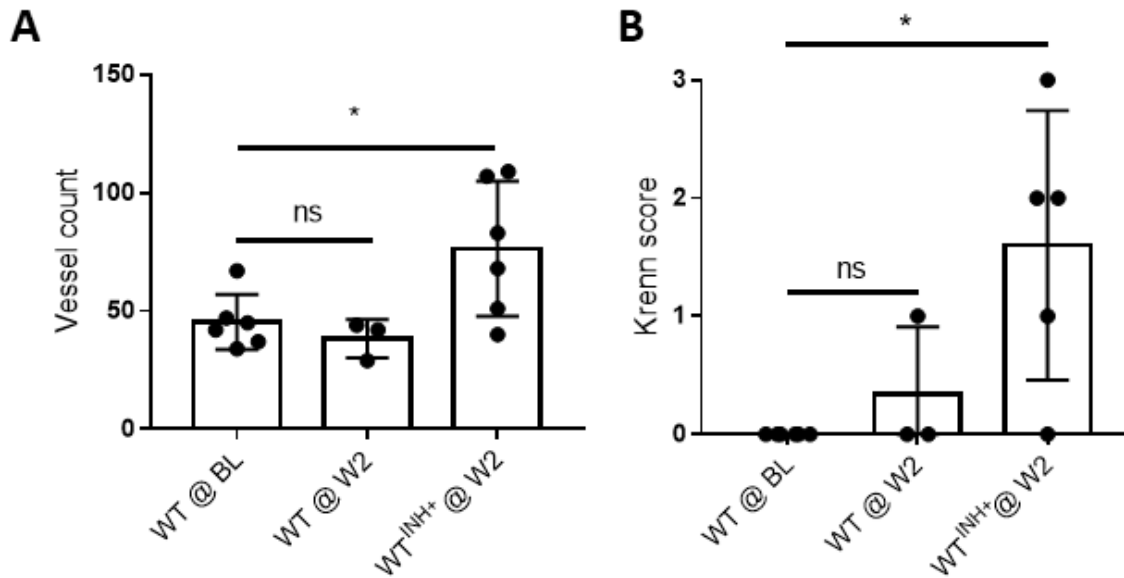
(A) Representative images of synovial samples from BALB/c FVIII-KO mice (numbered #1-2) at baseline and **(B)** W4 after injury stained for CD31 (red), α SMA (green), and nuclei (Hoechst, blue). At baseline small CD31⁺ α SMA⁻ and CD31⁺ α SMA⁺ vessels are observed in the soft tissue of the meniscus and circumventing the bone in FVIII-KO mice. At week 4 after injury, an increased

number of CD31⁺ blood vessels appear with abnormally enlarged (α SMA⁺) vessels. Original magnification 20x; scale bar = 100 μ m.



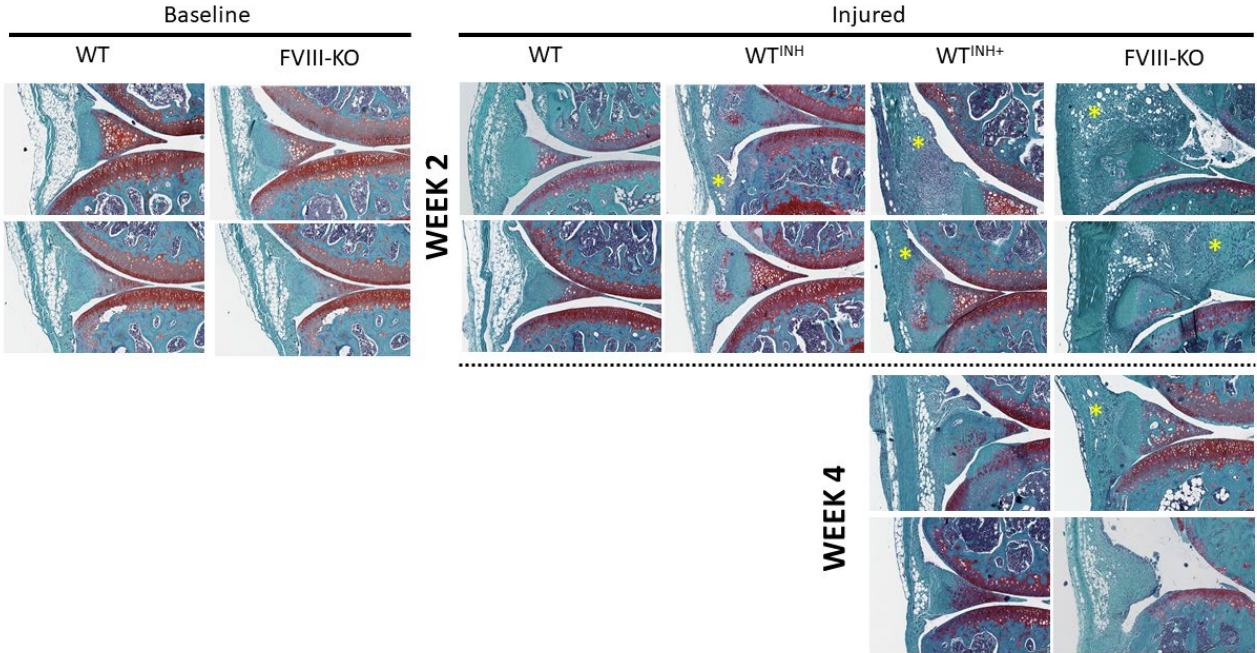
Supplemental figure 3: Pharmacokinetic profile of the inhibitory anti-TAFI antibody MA-RT36A3F5.

Plasma concentration-time profile of anti-mouse TAFI antibody, MA-RT36A3F5, in TAFI-KO mice after retro-orbital injection at a dosage of 7.5 mg/kg. The dosage of MA-RT36A3F5 was used in the WT^{INH+} model, together with an inhibitory anti-FVIII antibody, GMA-8015, at 0.25 mg/kg, to induce joint bleeding. GMA-8015 was previously shown to increase tail bleeding in WT mice up to 4 days after injection after which hemostasis returned to normal due to antibody clearing (2). MA-RT36A3F5 has an initial fast clearing rate resulting in a plasma level of 59.7 ± 24 nM at 24 hours after injection, after which the circulatory half-life was 3 days. MA-RT36A3F5 was raised against rat TAFI and was previously shown to cross-react with mouse TAFI and to inhibit mouse TAFIa with an IC₅₀ value of 0.44 molar fold ratio over TAFIa, achieving a maximal inhibition of 54% (3). These data indicate restored TAFI function after approximately one week.



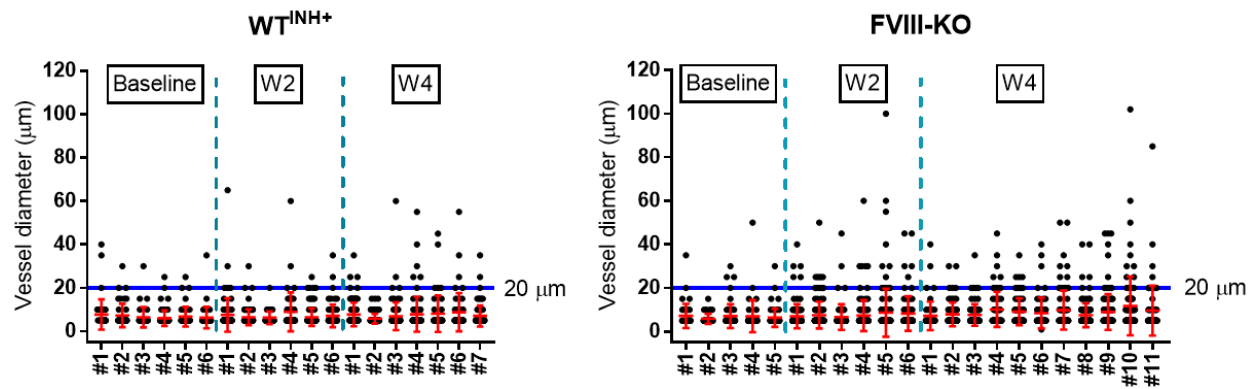
Supplemental figure 4: Hemostatically competent WT mice (BALB/c) display no neovascularization or synovial hyperplasia after joint injury.

(A) Total vessel count and (B) stromal proliferation (Krenn score; 0-6) in medial knee joint sections of BALB/c WT mice at baseline (BL; n=6) or WT (n=3) and WT^{INH+} (n=6) mice 2 weeks after injury. Note, injured WT mice did not receive the inhibitory anti-FVIII antibody, whereas WT^{INH+} received anti-FVIII antibody together with anti-TAFI antibody. Data for BL and WT^{INH+} were retrieved from Figure 2 as negative and positive reference, respectively. Data were analyzed using One-Way ANOVA with Tukey's multiple comparisons test (A) and Kruskal-Wallis with Dunn's multiple comparisons test (B). * p<0.05. ns denotes not significant.



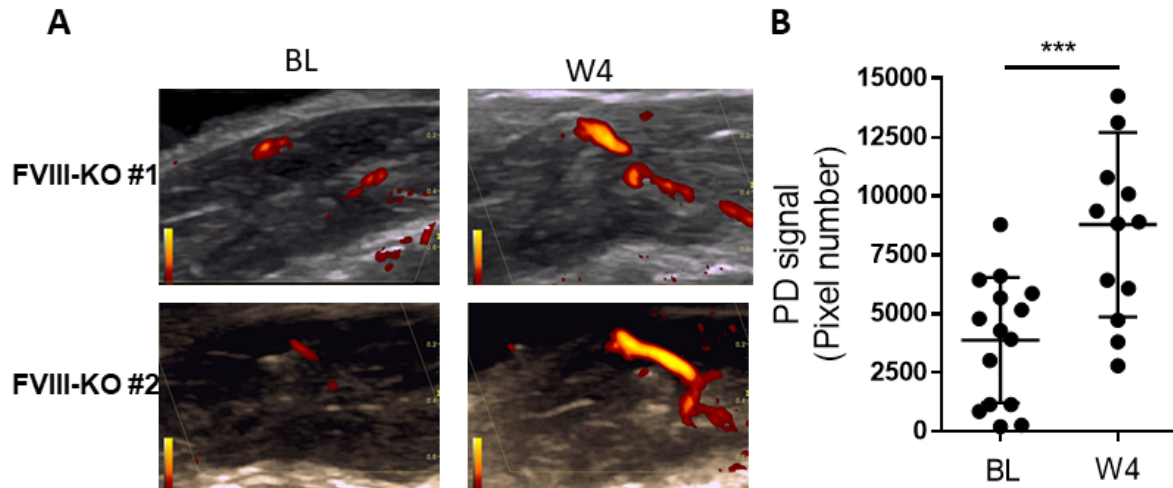
Supplemental figure 5: Histology of knee joints at baseline and after injury.

Representative Safranin-O Fast Green-stained knee joints before and at week 2 and 4 after injury of WT mice at baseline or injured WT, WT^{INH}, WT^{INH+} or FVIII-KO mice (all BALB/c, n= 2 per condition). WT^{INH} indicates WT mice injected with an inhibitory anti-FVIII antibody 2 hours prior to injury to induce transient acquired hemophilia A. WT^{INH+} indicates WT mice injected with a combination of an inhibitory anti-FVIII and anti-TAFI antibodies to induce FVIII-KO-like joint bleeding. Asterix indicates soft tissue proliferation and hypercellularity. Original magnification 40x.



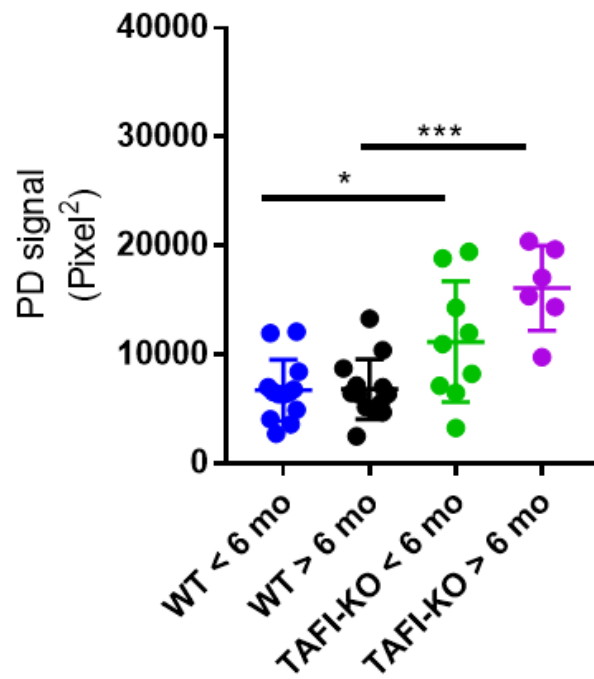
Supplemental figure 6: Individual vessel diameters in BALB/c WT^{INH+} and FVIII-KO mice at baseline and after injury.

Individual vessel diameters of each mouse at baseline (WT^{INH+}: n=6; FVIII-KO: n=5), at week 2 (W2) (WT^{INH+}: n=6; FVIII-KO: n=6) and at week 4 (W4) (WT^{INH+}: n=7; FVIII-KO: n=11). All mice are BALB/c.



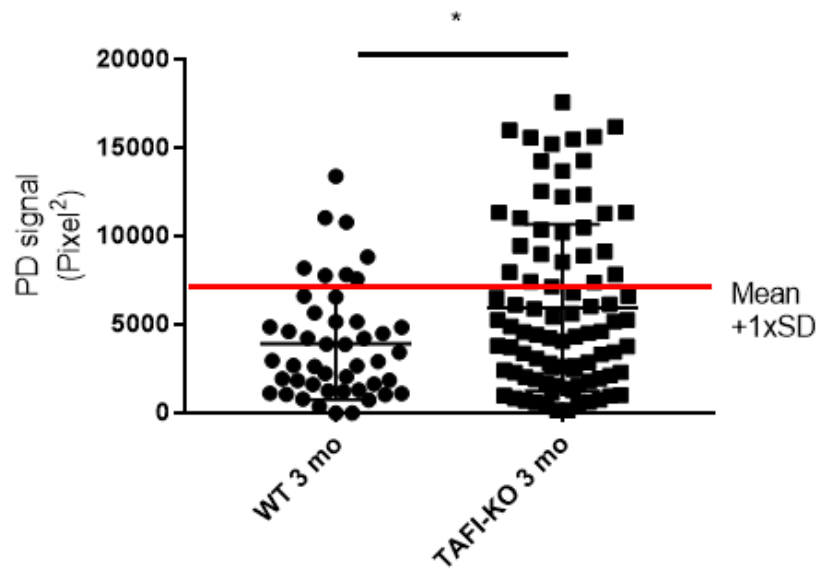
Supplemental figure 7: FVIII deficient mice display increased joint blood flow after bleeding.

(A) Representative power Doppler (PD) images (n=2 per time point) and **(B)** PD signal (pixel²; n=13-15 per time point) obtained from recording at the medial side of the joint of BALB/c FVIII-KO at baseline or 4 weeks after joint injury. *** p<0.001.



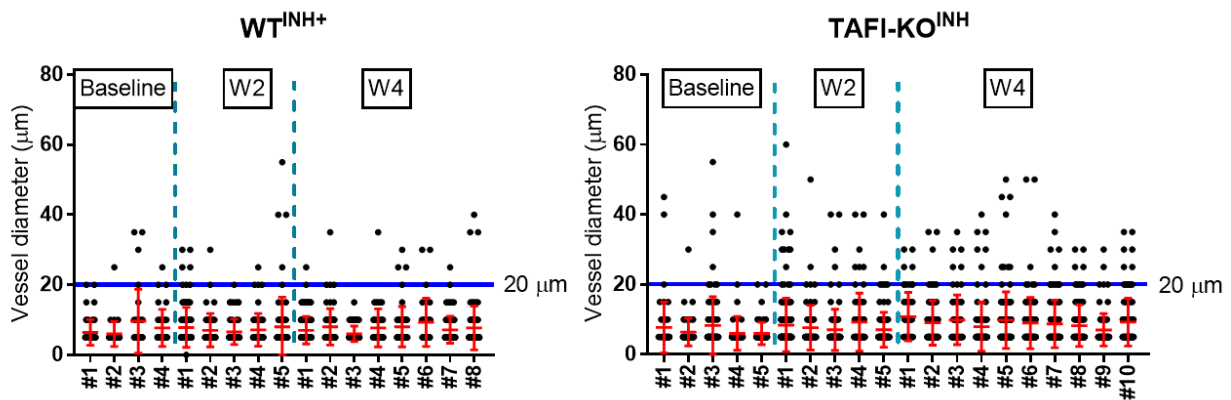
Supplemental figure 8: TAFI-KO mice from an independently generated and maintained colony display increased joint blood flow.

To verify that spontaneously increased joint blood flow is associated with TAFI deficiency, knee joints of TAFI-KO mice from an independently generated and maintained colony were analyzed by power Doppler (PD) (4). PD signal (pixel²; n=6-13) obtained from recording at the medial side of the joint of TAFI-KO mice and their WT controls were stratified into 2 age groups (under or above 6 months of age). * p<0.05; *** p<0.001.



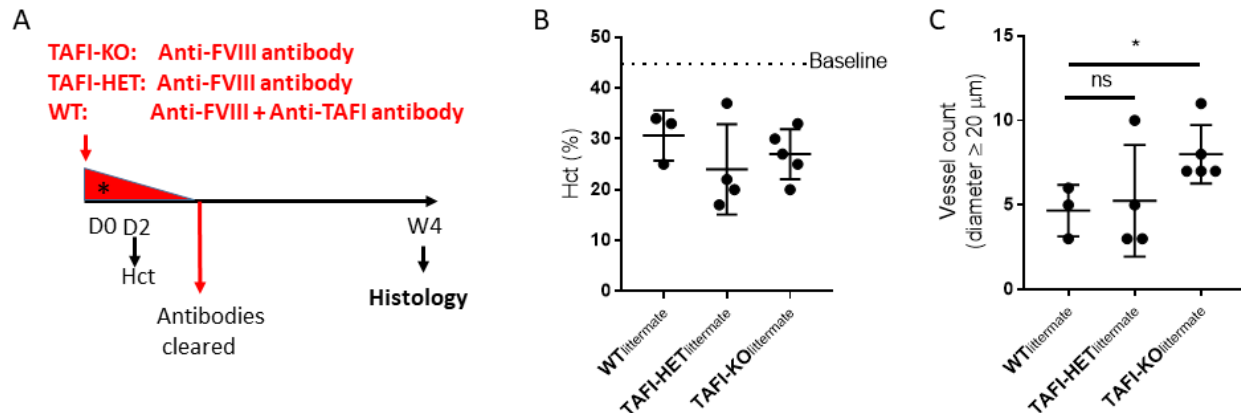
Supplemental figure 9: TAFI-KO mice display increased joint blood flow compared to WT control.

Power Doppler (PD) signal (pixel²; n=6-13) obtained from recording at the medial side of the uninjured joint of C57Bl/6J WT and TAFI-KO mice at 3 months of age. TAFI-KO mice were selected with a baseline PD signal lower than the “mean + SD” of the PD signal of WT in order to study vascularity changes induced by joint bleeding. * p<0.05.



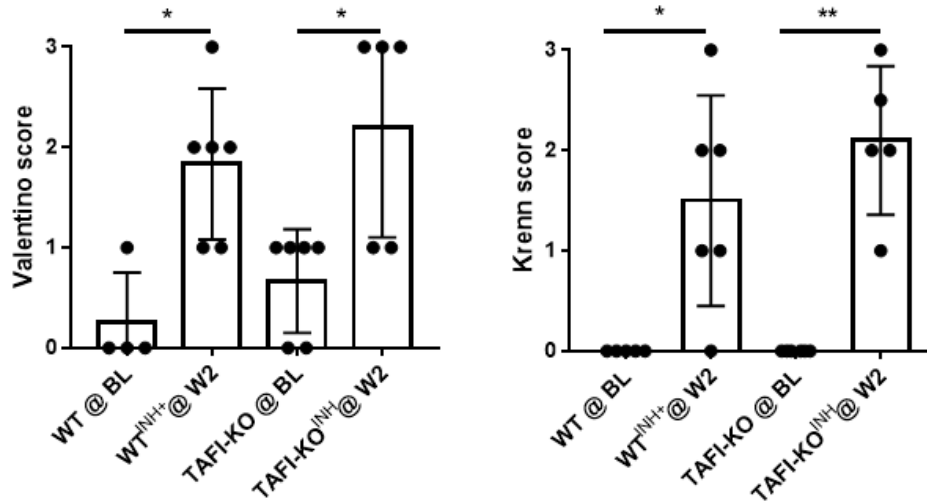
Supplemental figure 10: Individual vessel diameters in C57Bl/6J WT^{INH+} and TAFI-KO^{INH} mice at baseline and after injury.

Individual vessel diameters of each mouse at baseline (WT^{INH+}: n=4; TAFI-KO^{INH}: n=5), at week 2 (W2) (WT^{INH+}: n=5; TAFI-KO^{INH}: n=5) and at week 4 (W4) (WT^{INH+}: n=8; TAFI-KO^{INH}: n=10). All mice are C57Bl/6J.



Supplemental figure 11: Excessive vascular remodeling after joint bleeding in TAFI-KO mice compared to littermate TAFI-HET and WT controls.

(A) Schematic of the joint injury model comparing TAFI deficient mice with their heterozygous and WT littermate controls after hemophilic joint bleeding. Mice (n=3-5) were administered anti-FVIII antibody alone (TAFI-KO (TAFI^{-/-}) or TAFI-HET (TAFI^{+/-})) or together with an anti-TAFI antibody (WT^{INH+}). After antibody clearance, vascular remodeling was analyzed in WT, TAFI-HET and TAFI-KO mice at week 4 (W4). Asterix indicates time of joint injury at day 0 (D0). Hematocrit (Hct) was determined at day 2 (D2) post injury to assess joint bleeding severity. (B) Joint bleeding after injury in WT^{INH+} mice (n= 3) or TAFI-KO^{INH} (n=5) were similar. Joint bleeding was inferred from a post-injury drop in Hct at day 2 (D2) compared to baseline (BL; no-injury reference). (C) Vessel count with diameter ≥20 μm in medial knee joint sections of WT, TAFI-HET and TAFI-KO mice (n=3-5) at W4 after joint injury. Each point represents an individual mouse. Data were analyzed using Student's 2-tailed, unpaired t test per time point (C).



Supplemental figure 12: TAFI-KO^{INH} mice display increased soft tissue hyperplasia

(A) Synovial proliferation (Valentino score; 0-9) and (B) stromal proliferation (Krenn score; 0-6) in medial knee joint sections of C57Bl/6J WT mice at baseline (BL; n=4), C57Bl/6J TAFI-KO mice at baseline (BL; n=6), WT^{INH+} (n=6) and TAFI-KO^{INH+} (n=5) mice 2 weeks after injury. Data were analyzed using Kruskal-Wallis with Dunn's multiple comparisons test. * p<0.05; ** p<0.01.

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