

Effects of secondary factor VIII prophylaxis on joint and bone health in a hemophilia A mouse model

by Lara Monica, Joyce Kimenai, Olga Oleshko, Annika Klingberg, Jonas Fuener, Enrico Klotzsch, Korbinian Brand, Sander Botter, Sonja Werwitzke and Andreas Tiede

Received: July 1, 2026.

Accepted: October 8, 2026.

Citation: Lara Monica, Joyce Kimenai, Olga Oleshko, Annika Klingberg, Jonas Fuener, Enrico Klotzsch, Korbinian Brand, Sander Botter, Sonja Werwitzke and Andreas Tiede. Effects of secondary factor VIII prophylaxis on joint and bone health in a hemophilia A mouse model.

Haematologica. 2026 Oct 15. doi: 10.3324/haematol.2026.301495 [Epub ahead of print]

Publisher's Disclaimer.

E-publishing ahead of print is increasingly important for the rapid dissemination of science.

Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication.

E-publishing of this PDF file has been approved by the authors.

After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval, the final version of the manuscript will then appear in a regular issue of the journal.

All legal disclaimers that apply to the journal also pertain to this production process.

Effects of secondary factor VIII prophylaxis on joint and bone health in a hemophilia A mouse model

Lara Monica,^{1,2,3} Joyce Kimenai,^{4,5,6} Olga Oleshko,¹ Annika Klingberg,¹ Jonas Fünér,³ Enrico Klotzsch,^{7,8} Korbinian Brand,² Sander Botter,⁴ Sonja Werwitzke,² Andreas Tiede^{1,2}

1. Department of Hematology, Hemostasis, Oncology, and Cell Therapy, Hannover Medical School, Hannover, Germany
2. Institute of Clinical Chemistry and Central Laboratory, Hannover Medical School, Hannover, Germany
3. Preclinics GmbH, Potsdam, Germany
4. Swiss Center for Musculoskeletal Biobanking, Balgrist Campus AG, Zurich, Switzerland
5. Department of Health Science and Technology, ETH Zurich, Zurich, Switzerland
6. Department of Orthopedics, Balgrist University Hospital Zürich, University of Zurich, Zurich, Switzerland
7. Berlin Institute of Health (BIH) at Charité, Universitätsmedizin Berlin, BIH Center for Regenerative Therapies (BCRT), Berlin, Germany
8. Institute of Active Polymers, Helmholtz-Zentrum Hereon, 14513 Teltow, Germany

Correspondence

Lara Monica, Hematology, Hemostasis, Oncology, and Cell Therapy, Institute of Clinical Chemistry and Central Laboratory, Hannover Medical School, Carl Neuberg Str. 1, 30625 Hannover, Germany, and preclinics GmbH, Wetzlarer Str. 20, 14482 Potsdam. Tel.: + 49-331-24343350, email: lara.monica@stud.mh-hannover.de; lm@preclinics.com

Andreas Tiede (MD, PhD), Hematology, Hemostasis, Oncology, and Cell Therapy, Institute of Clinical Chemistry and Central Laboratory, Hannover Medical School, Carl Neuberg Str. 1, 30625 Hannover, Germany. Tel.: + 49-511-532-8488, fax: + 49-511-532-18524, email: tiede.andreas@mh-hannover.de

Disclosure of conflict of interest

L.M. received travel support from Octapharma. J.K. reports institutional grants for research and studies from Octapharma. O.O. reports institutional grants for research and studies from Biotest and Octapharma, and honoraria for lectures from CSL Behring and Roche/Chugai. E.K. reports a contractual agreement with Octapharma for data analysis services. S.B. reports institutional grants for research and studies from Octapharma, and honoraria for lectures or consultancy from Octapharma. S. W. reports institutional grants for research and studies from Biotest and Octa pharma, and honoraria for lectures or consultancy from Biotest and Stago. A.T. reports institutional grants for research and studies from Bayer, Biotest, Chugai, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, and Takeda, and honoraria for lectures or consultancy from Bayer, BioMarin, Biotest, Chugai, CSL Behring, Novo Nordisk, Octa pharma, Pfizer, Roche, Sobi, and Takeda.

Author contributions

L.M. and A.T. designed the study and wrote the manuscript. L.M., J.K., O.O., A.K., J.F., E.K., S.B., S.W. and A.T. collected, analyzed and interpreted data. All authors revised the manuscript for critical content. All authors approved of the final version of the manuscript.

Artificial intelligence declaration

ChatGPT-5.5 (OpenAI) was used to assist language editing. The authors reviewed and approved all AI-assisted edits.

Data sharing statement

For original data and material sharing agreements, please contact the corresponding authors (AT, LM).

Acknowledgements

The authors would like to thank Octapharma for financial support of this study. Lina Aires, Silvio Wuschko, and Larisa Belyanskaya are particularly acknowledged for their support of this research program. The authors also acknowledge the preclinics GmbH in vivo team and the MHH Hämostaseologie team for dedicated technical support throughout the execution of this study. Dr. med. vet. Simone Odaü is acknowledged for excellent veterinarian support.

Short title

Prophylaxis in Hemophilia A Mice

Key words (MeSH terms)

Hemophilia A • Hemorrhage • Hemarthrosis • Synovitis • Mice

Letter to the Editor

Hemophilic arthropathy remains a major cause of morbidity in patients with hemophilia A (HA), even with regular factor VIII (FVIII) prophylaxis.¹ While prophylaxis effectively reduces bleeding rates, its impact on long-term joint health is incompletely understood. Synovitis, progressive arthropathy, and low bone mineral density leading to osteoporosis still occur in a substantial proportion of patients receiving primary or secondary prophylaxis.^{2, 3}

The mechanisms underlying this incomplete protection remain poorly understood and a translationally relevant preclinical model is lacking. The FVIII knockout (F8^{-/-}) mouse model rapidly develops anti-drug antibodies (ADAs) that preclude long-term FVIII exposure.⁴ Immunodeficient alternatives avoid ADA formation but lack elements of adaptive or innate immunity that may themselves shape synovial inflammation and cartilage or bone remodeling after joint bleeding, limiting their suitability for studying hemophilic joint disease. We recently generated a HA double knockout model additionally lacking mature B cells (F8^{-/-}Ighm^{-/-}, DKO), which does not develop ADAs and permits long-term FVIII treatment with physiological pharmacokinetics.⁵ Here, we used this model to evaluate effects of secondary FVIII prophylaxis, defined as ongoing treatment initiated after an induced hemarthrosis, as an experimental approximation of clinical secondary prophylaxis that is usually defined as regular treatment after a first episode of joint bleeding but before arthropathy has developed.⁶

Thirty-six DKO mice stratified by sex were housed in single cages with access to a voluntary running wheel. Experiments were approved by the competent authority and were performed in accordance with relevant guidelines and the EU Directive 2010/63/EU. After 8 days of acclimatization, mice were allocated to three groups (Figure 1): group 1 (n=12) received no FVIII; group 2 (n=12) received a single dose of FVIII 200 IU/kg before injury; group 3 (n=12) received the same initial dose followed by secondary prophylaxis (100 IU/kg on days 1-4, then three times weekly). All mice underwent a unilateral needle-puncture injury to the left knee on day 1.⁷ Mice were sacrificed on days 4, 11, or 21 (n=4 per group and timepoint) for histological, hemostatic, and imaging endpoints. Untreated, uninjured mice served as baseline controls (n=8). Functional endpoints included knee circumference, measured using a caliper, and running behavior in the voluntary running wheel, expressed as daily total revolutions and maximum velocity. FVIII was measured using a chromogenic activity assay; thrombin generation was measured using a modified calibrated automated thrombogram assay (CAT, Stago, Düsseldorf, Germany) with human factor XIa (Life Technologies Corporation, Carlsbad, USA) diluted in MP reagent to 4.375 nM. Hind limbs were scanned by micro-CT (SkyScan 1176, Bruker; 8.86 µm voxel size), and trabecular bone volume fraction (BV/TV) and thickness (Tb.Th) were quantified in the proximal tibial metaphysis using a semi-automated pipeline (ImageJ/BoneJ) after excluding the cortical shell. Paraffin-embedded tissue sections were stained with Hematoxylin & Eosin or Safranin O. The predefined primary endpoint was the total joint score (TJS) developed by Valentino et al.,⁸ a semiquantitative joint damage score (range 0-10) assessed by three observers blinded to group assignment. Cartilage damage was additionally graded on Safranin O-stained sections using the OARSI score (range 0-6).⁹ TJS, knee measurements and running parameters were treated as normally distributed continuous variables for the purpose of statistical analysis and were expressed as mean

± standard deviation. Sample size was calculated to detect a 3-point TJS difference between groups 2 and 3 in a two-sided t-test (80% power, $\alpha=0.05$, requiring $n=7$ animals per group). To allow assessment of the temporal evolution of joint pathology, group sizes were increased to $n = 12$ animals per group, with analyses performed at three predefined time points (days 4, 11, and 21). TJS was analyzed using a two-way ANOVA of TJS with factors treatment group, day, and side (left = injured, right = uninjured), including interaction terms; post-hoc pairwise comparisons between groups were Tukey-adjusted. As a sensitivity analysis, a linear mixed-effects sensitivity analysis was additionally fitted with animal as a random intercept to account for the two knees scored per mouse; the between-animal variance was estimated at the boundary as ~ 0 (exact restricted likelihood-ratio test $P=1.000$), and fixed-effect and post-hoc estimates were essentially unchanged from the original ANOVA.

Baseline knee circumference was similar across groups. Following injury, knee swelling was pronounced and sustained in group 1, delayed and attenuated in group 2, and minimal in group 3 (Figure 2A). Running activity was correspondingly reduced on day 4 in group 1 (ratio to individual baseline: total revolutions 0.05 ± 0.05 , maximum velocity 0.50 ± 0.21), less affected in group 2, and virtually preserved in group 3 (Figure 2B).

Thrombin generation confirmed effective FVIII exposure: peak thrombin was substantially higher in group 3 than in groups 1 and 2 on days 4 and 11 (Figure 2C). FVIII activity was detectable in most group 3 animals on day 4 (one day after the last dose, median 7.8 IU/dl) and day 11 (two days after last dose, 4.1 IU/dl), falling below the limit of quantification by day 21 (three days after the last dose; Figure 2D). FVIII activity in groups 1 and 2 was always below quantification.

Histological signs of arthropathy as assessed by the TJS were the predefined primary endpoint of this study. At baseline, uninjured mice had a mean TJS of 2.9 ± 1.0 . On day 21, the injured side TJS in group 3 (5.0 ± 2.5) was significantly lower than that in group 2 (7.6 ± 0.5 , $p = 0.001$), rejecting the null hypothesis and demonstrating that FVIII prophylaxis was superior to single FVIII treatment in preventing arthropathy. ANOVA revealed that TJS was significantly influenced by treatment group and by side, with a significant group-by-side interaction (Supplement Table 1); pairwise contrasts confirmed lower scores in group 3 than in groups 1 and 2 on the injured side at day 21. Component-level analysis showed that prophylaxis reduced scores for erythrocytes (visible in the joint space or synovium), hemosiderin deposition, and cartilage erosion, whereas synovial hyperplasia was only partially reduced (Figure 3A). Representative histological images illustrate the marked synovitis present in the injured knees of groups 1 and 2, which was attenuated but not abolished in group 3 (Figure 3B).

This partial protection was reflected differentially across tissues. Cartilage damage, assessed by the OARSI score,⁹ persisted in a subset of group 3 animals despite prophylaxis, including two of four mice with marked damage at day 21 (Figure S1). In contrast, micro-CT of the tibial metaphysis showed that trabecular bone volume and thickness were fully preserved in group 3 at all timepoints, while groups 1 and 2 developed pronounced, predominantly ipsilateral trabecular bone loss (Figure S2). Secondary FVIII prophylaxis therefore fully protected trabecular bone but only partially protected synovium and cartilage.

This is, to our knowledge, the first evaluation of FVIII secondary prophylaxis in an HA mouse model that does not develop ADAs, allowing FVIII exposure over three weeks without the confounding

loss of activity seen in earlier F8^{-/-} models.⁴ Using a prophylaxis dosing regimen with frequent peaks and low troughs, reminiscent of classical prophylaxis with standard half-life FVIII concentrates, we found substantial but incomplete joint protection, closely mirroring the clinical experience: prophylactic factor replacement is superior to on-demand treatment,¹⁰ yet synovitis and progressive joint disease still develops in a substantial proportion of patients.^{10, 11}

The dissociation between complete bone protection and incomplete cartilage/synovial protection may reflect different exposure requirements. FVIII trough levels fell below quantification two to three days after each dose, which may be sufficient to preserve bone remodeling, consistent with a previous study,¹² but insufficient to fully prevent joint subclinical bleeding and its local consequences during key phases of tissue repair. A prior study using murine Fc-FVIII, which achieved higher trough levels than the standard-half-life FVIII used here, prevented synovitis more completely,¹³ supporting the hypothesis that sustained trough exposure, rather than peak levels, is the limiting factor for synovial protection. Iron released during hemarthrosis is also directly chondrotoxic via hydroxyl-radical formation,^{14, 15} which may contribute to cartilage damage independently of ongoing bleeding, a mechanism our model was not designed to test directly, but which offers an alternative explanation for the incomplete cartilage protection we observed.

Some limitations should be noted. The dosing regimen used here is an experimental model of secondary prophylaxis rather than a direct reproduction of clinical practice, and the absence of B cells in the DKO mouse model could independently influence synovial inflammation and bone remodeling. Group sizes were powered for the primary TJS endpoint but may not be sufficient to reliably quantify the amount of residual damage observed in group 3 or to correlate that to actual FVIII exposure. Additional measurements of inflammatory cytokines and transcriptomic analysis exploring systemic inflammation were done post hoc and are reported separately.

In conclusion, secondary FVIII prophylaxis in an inhibitor-free HA mouse model preserved physical activity and hemostasis and fully protected trabecular bone, but only partially protected against synovitis and cartilage damage. These findings support the evaluation of intensified or extended half-life FVIII regimens, and of adjunct anti-inflammatory strategies, to further reduce the residual burden of hemophilic arthropathy.

References

1. Chowdary P, Carcao M, Kenet G, Pipe SW. Haemophilia. *Lancet*. 2025;405(10480):736-750.
2. Lee A, Boyd SK, Kline G, Poon M-C. Premature changes in trabecular and cortical microarchitecture result in decreased bone strength in hemophilia. *Blood*. 2015;125(13):2160-2163.
3. Foppen W, van der Schaaf IC, Beek FJA, Mali W, Fischer K. MRI predicts 5-year joint bleeding and development of arthropathy on radiographs in hemophilia. *Blood Adv*. 2020;4(1):113-121.
4. Hausl C, Maier E, Schwarz HP, et al. Long-term persistence of anti-factor VIII antibody-secreting cells in hemophilic mice after treatment with human factor VIII. *Thromb Haemost*. 2002;87(5):840-845.
5. Monica L, Oleshko O, Aires L, et al. Characterization of a factor VIII/immunoglobulin heavy chain mu double-knockout mouse model of hemophilia A for long-term exposure to factor VIII proteins. *Res Pract Thromb Haemost*. 2026;10(1):103277.
6. Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, Van Den Berg HM, Srivastava A. Definitions in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost*. 2014;12(11):1935-1939.
7. Hakobyan N, Valentino LA, Cong L, et al. Haemarthrosis model in mice: BSS - Bleeding Severity Score assessment system. *Haemophilia*. 2016;22(5):790-798.
8. Valentino LA, Hakobyan N. Histological changes in murine haemophilic synovitis: a quantitative grading system to assess blood-induced synovitis. *Haemophilia*. 2006;12(6):654-662.
9. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage*. 2010;18 Suppl 3:S17-23.
10. Melchiorre D, Milia AF, Linari S, et al. RANK-RANKL-OPG in hemophilic arthropathy: from clinical and imaging diagnosis to histopathology. *J Rheumatol*. 2012;39(8):1678-1686.
11. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med*. 2007;357(6):535-544.
12. Lau AG, Sun J, Hannah WB, et al. Joint bleeding in factor <sc>VIII</sc> deficient mice causes an acute loss of trabecular bone and calcification of joint soft tissues which is prevented with aggressive factor replacement. *Haemophilia*. 2014;20(5):716-722.
13. Chumappumkal Joseph B, De Pablo-Moreno JA, Falah N, Lora Cacho M, Von Drygalski A. Defective lymphangiogenesis and iron removal after hemarthrosis in factor VIII-deficient mice are rectified with therapeutic factor VIII administration-implications for joint health. *J Thromb Haemost*. 2025;24(1):88-98.
14. Lee AJ, Gangi LR, Zandkarimi F, Stockwell BR, Hung CT. Red blood cell exposure increases chondrocyte susceptibility to oxidative stress following hemarthrosis. *Osteoarthritis Cartilage*. 2023; 31(10):1365-1376.
15. Hooiveld MJ, Roosendaal G, van den Berg HM, Bijlsma JW, Lafeber FP. Haemoglobin-derived iron-dependent hydroxyl radical formation in blood-induced joint damage: an in vitro study. *Rheumatology (Oxford)*. 2003; 42(6):784-790.

Figure legends

Figure 1: Experimental design and projected FVIII activity according to treatment group.

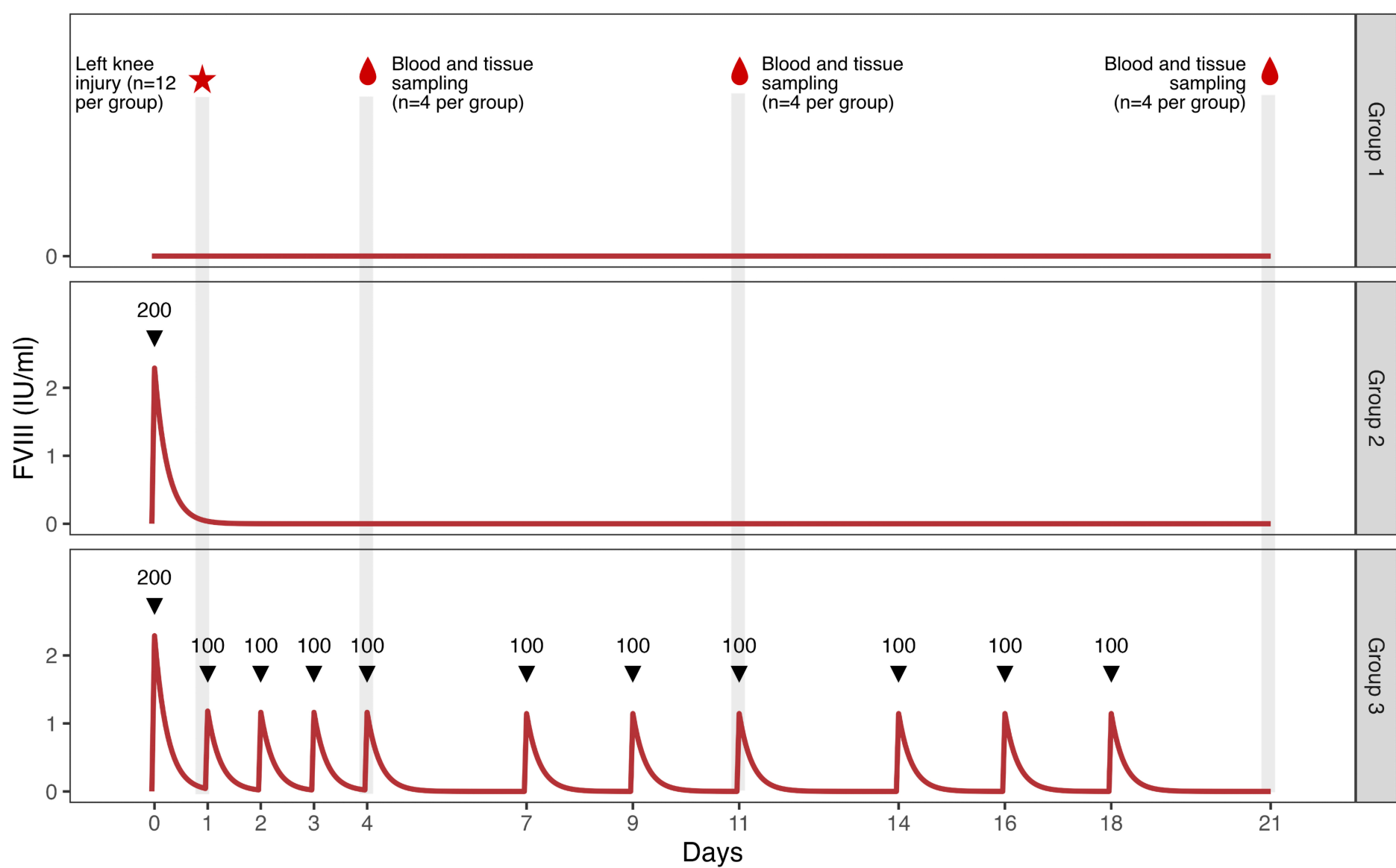
Experimental schedule: mice received no FVIII (group 1, n=12), a single FVIII dose before injury (group 2, n=12), or FVIII prophylaxis (group 3, n=12), and underwent unilateral knee injury on day 1; sacrifice for histology, hemostasis, and imaging occurred on days 4, 11, and 21 (n=4 per group and timepoint). Projected FVIII activity is derived from a prior pharmacokinetic study.³

Figure 2: Functional and hemostatic outcomes by treatment group

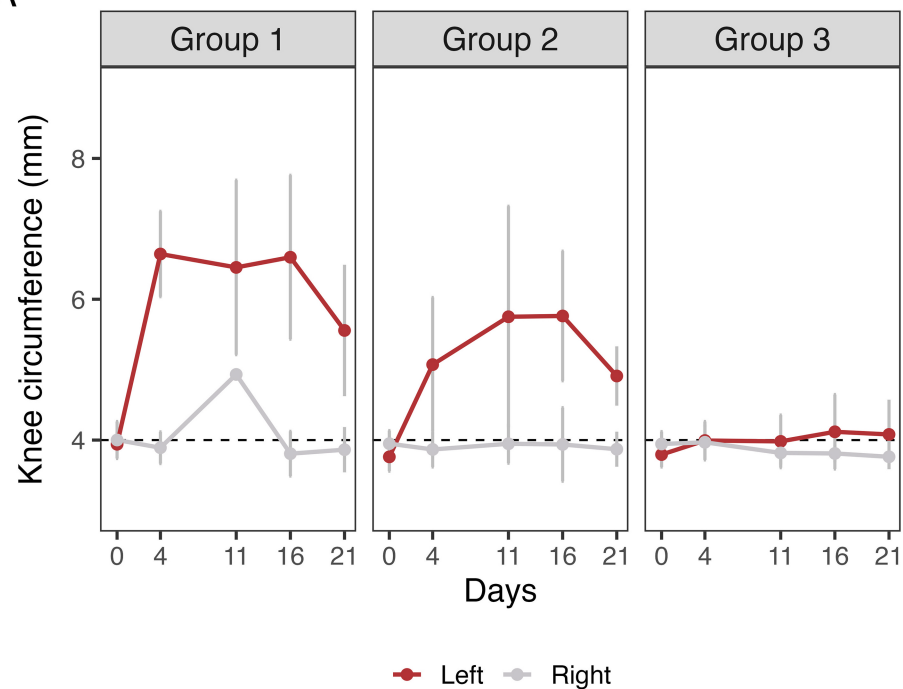
(A) Knee circumference of injured (left) and uninjured (right) knees over time. **(B)** Running-wheel total revolutions and maximum velocity, normalized to each animal's own pre-injury baseline. **(C)** Peak thrombin generation (FXIa-triggered) in pooled plasma by group and day. **(D)** FVIII activity (chromogenic assay) in individual plasma samples with median (solid line); dashed line indicates the limit of quantification (5 IU/dl); BLOQ, below limit of quantification. Data in panels (A) to (C) are mean $\pm$ standard deviation.

Figure 3: Histological joint pathology (primary endpoint).

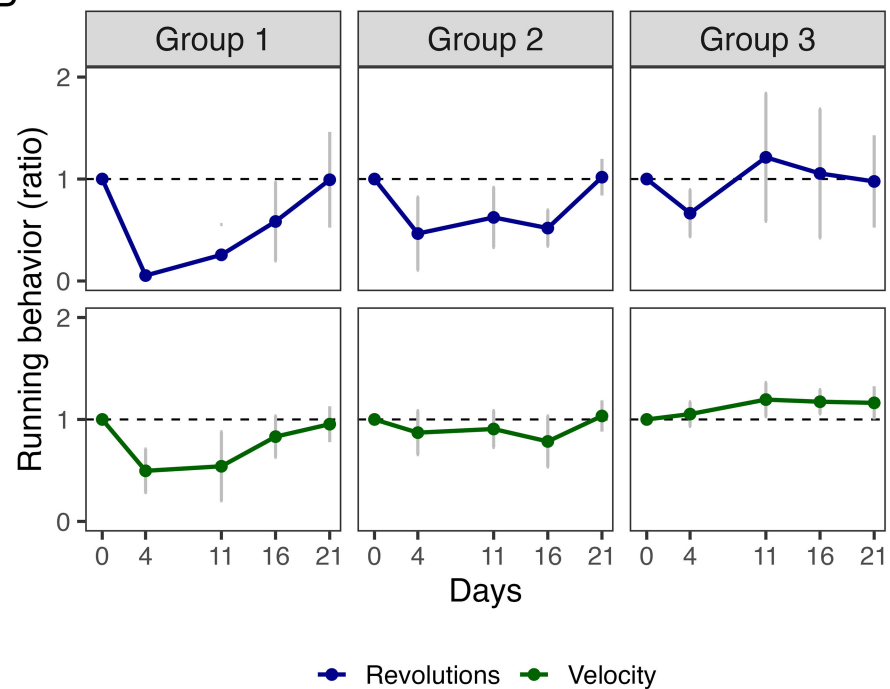
(A) Mean total joint score (TJS) per component (synovial hyperplasia, vascularity, hemosiderin, erythrocytes, villus formation, cartilage erosion) by group and day, for the injured (left, top) and uninjured (right, bottom) knee. **(B)** Representative H&E-stained histological sections of injured and contralateral knees from groups 1-3 on day 21.



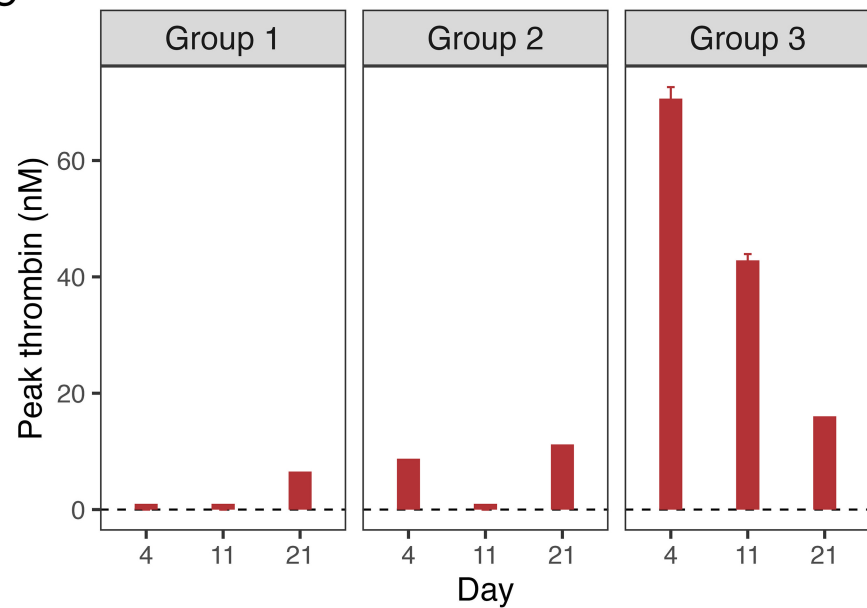
A



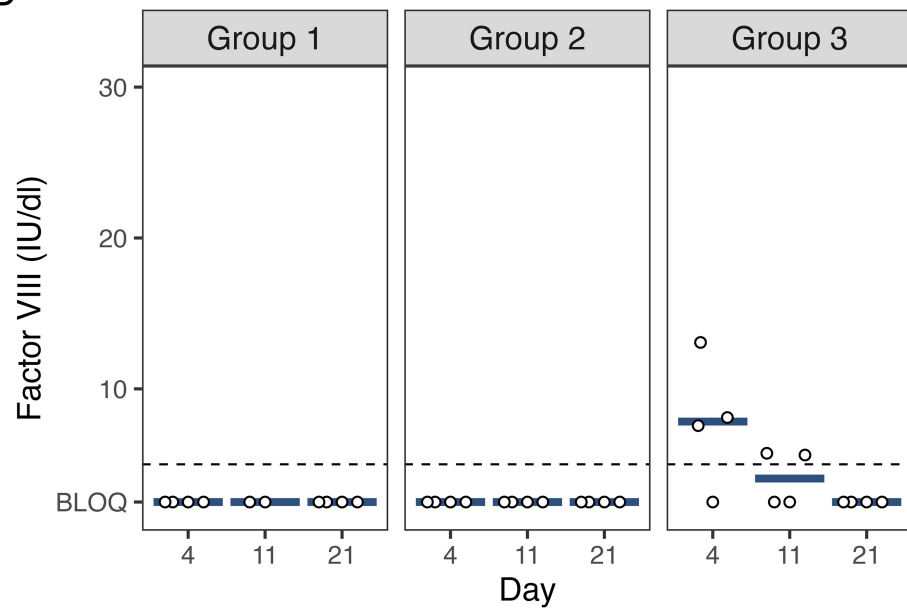
B

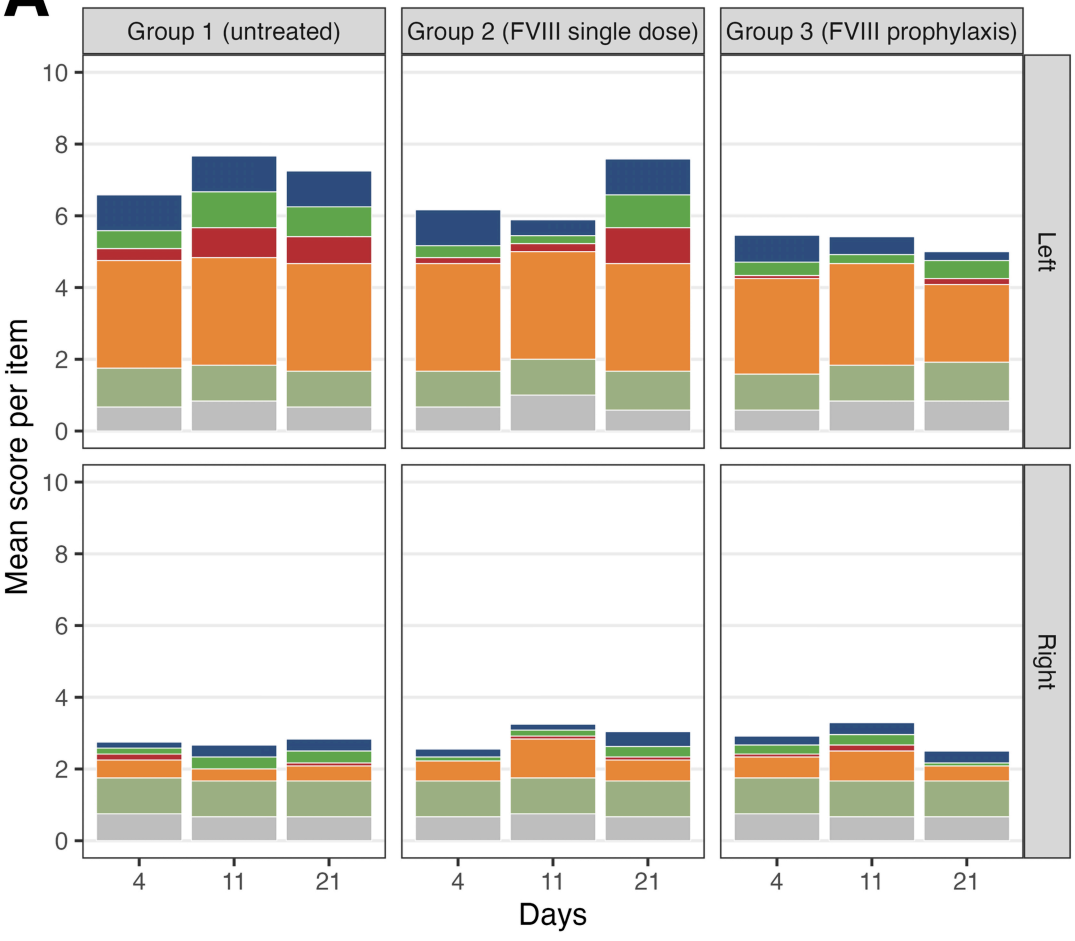
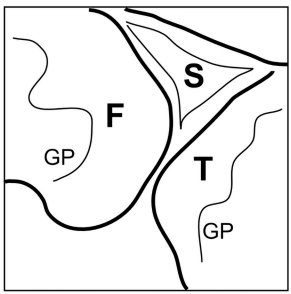
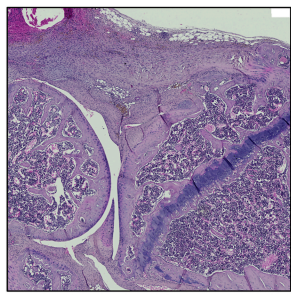
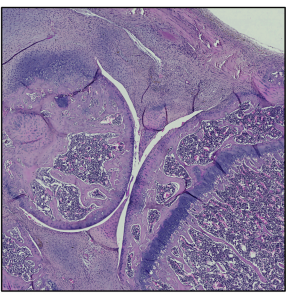
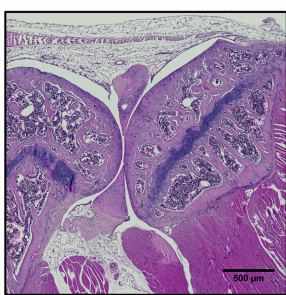
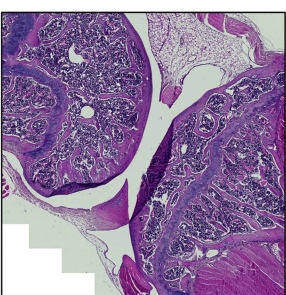
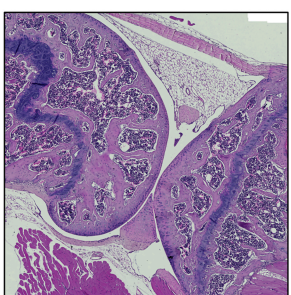
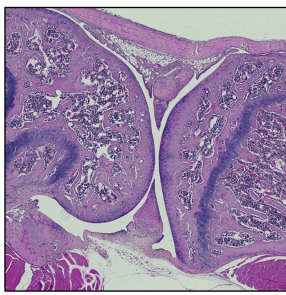


C



D



A**B****Group 1 (ID06, d 21)****Group 2 (ID23, d 21)****Group 3 (ID36, d 21)**

Left

Right

Supplementary Appendix

Effects of secondary factor VIII prophylaxis on joint and bone health in a hemophilia A mouse model

Lara Monica,^{1,2,3} Joyce Kimenai,^{4,5,6} Olga Oleshko,¹ Annika Klingberg,¹ Jonas Föner,³ Enrico Klotzsch,^{7,8} Korbinian Brand,² Sander Botter,⁴ Sonja Werwitzke,² Andreas Tiede^{1,2}

1. Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
2. Institute of Clinical Chemistry and Central Laboratory, Hannover Medical School, Hannover, Germany
3. Preclinics GmbH, Potsdam, Germany
4. Swiss Center for Musculoskeletal Biobanking, Balgrist Campus AG, Zurich, Switzerland
5. Department of Health Science and Technology, ETH Zurich, Zurich, Switzerland
6. Department of Orthopedics, Balgrist University Hospital Zürich, University of Zurich, Zurich, Switzerland
7. Berlin Institute of Health (BIH) at Charité, Universitätsmedizin Berlin, BIH Center for Regenerative Therapies (BCRT), Berlin, Germany
8. Institute of Active Polymers, Helmholtz-Zentrum Hereon, 14513 Teltow, Germany

Correspondence

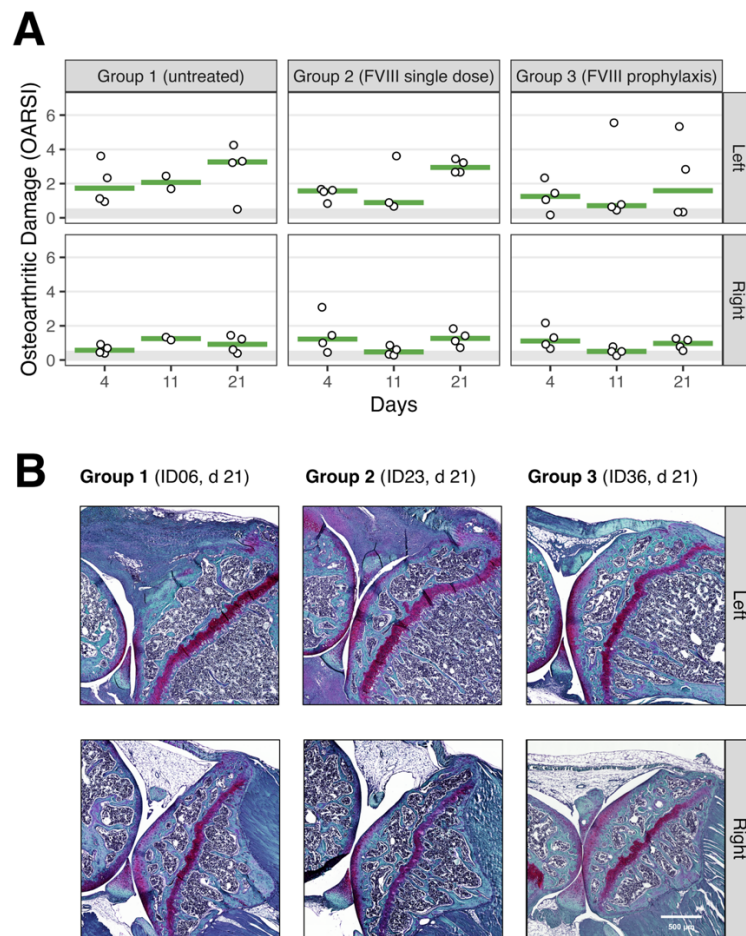
Lara Monica, Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Institute of Clinical Chemistry and Central Laboratory, Hannover Medical School, Carl Neuberg Str. 1, 30625 Hannover, Germany, and preclinics GmbH, Wetzlarer Str. 20, 14482 Potsdam. Tel.: + 49-331-24343350, email: lara.monica@stud.mh-hannover.de; lm@preclinics.com

Andreas Tiede (MD, PhD), Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Institute of Clinical Chemistry and Central Laboratory, Hannover Medical School, Carl Neuberg Str. 1, 30625 Hannover, Germany. Tel.: + 49-511-532-8488, fax: + 49-511-532-18524, email: tiede.andreas@mh-hannover.de

Supplement Table 1

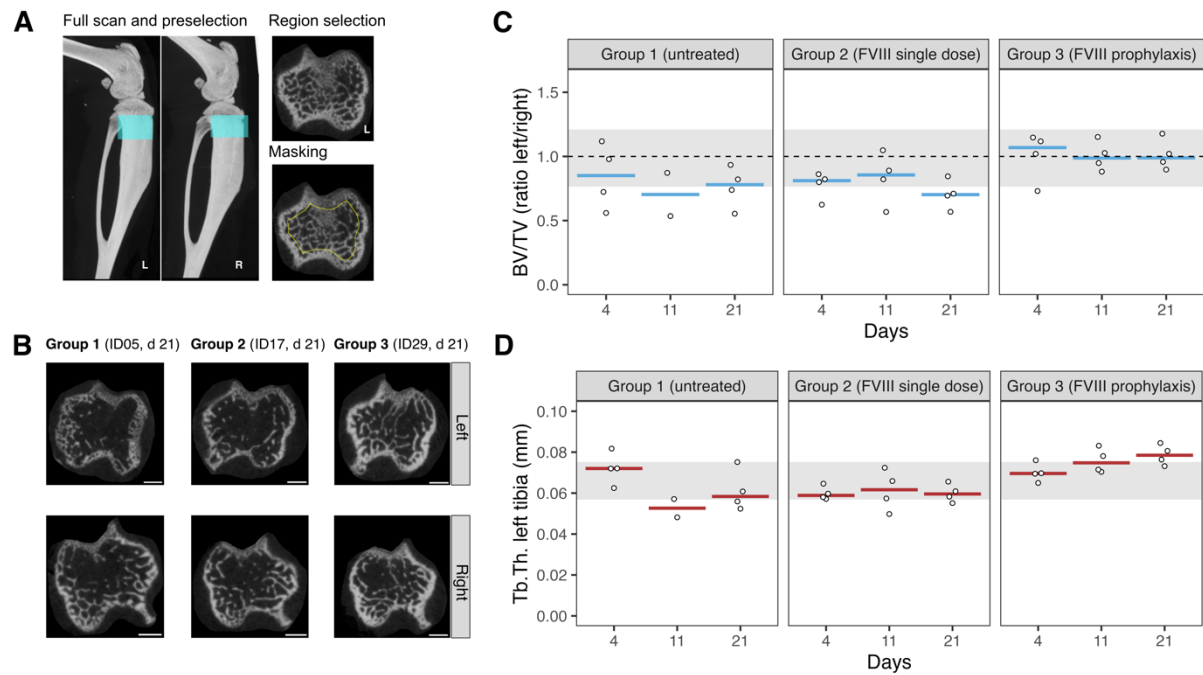
Two-way ANOVA of TJS with factors treatment group (1 = untreated, 2 = FVIII single dose, 3 = FVIII prophylaxis), day (4, 11, 21 after injury), and side (left = injured, right = uninjured), including interaction terms; post-hoc pairwise comparisons between groups (Tukey-adjusted) are shown for significant comparisons only.

Main effects					
Term	df	Sum Sq	Mean Sq	F value	P value
Side	1	189.61	189.61	231.989	<0.001
Group	2	9.132	4.566	5.587	0.007
Day	2	0.509	0.255	0.312	0.734
Interactions					
Group × side	2	10.556	5.278	6.458	0.003
Group × day	4	4.533	1.133	1.387	0.253
Day × side	2	1.478	0.739	0.904	0.412
Group × day × side	4	3.12	0.78	0.954	0.441
Residuals	48	39.231	0.817	–	–
Tukey-adjusted post-hoc contrasts (p<0.05)					
Contrast (injured side)	df	Estimate	SE	T ratio	P value
Group 1 – Group 3, day 11	48	2.25	0.783	2.874	0.016
Group 1 – Group 3, day 21	48	2.25	0.639	3.520	0.003
Group 2 – Group 3, day 21	48	2.58	0.639	4.041	0.001



Supplement Figure 1: Cartilage damage (OARS) score).

(A) Mean OARS score of the injured (left, top) and uninjured (right, bottom) knee by group and day; shaded area indicates the mean of baseline control animals. (B) Representative Safranin-O-stained histological sections from groups 1-3 on day 21.



Supplement Figure 2: Micro-CT analysis of the proximal metaphysis of the tibia

(A) Analysis strategy, VOI pre-selection and masking. (B) Representative examples of day 21. (C) Trabecular bone fraction (left / right knee ratio) and (D) Mean trabecular thickness (Tb.Th.) of the left tibia. White dots show the ratios of individual biological replicates ($n=4$ per group and time point, except for group 1 on day 11 with $n=2$). Bars represent median values. The grey shaded area represents the reference interval obtained in 4 uninjured animals (minimum and maximum).