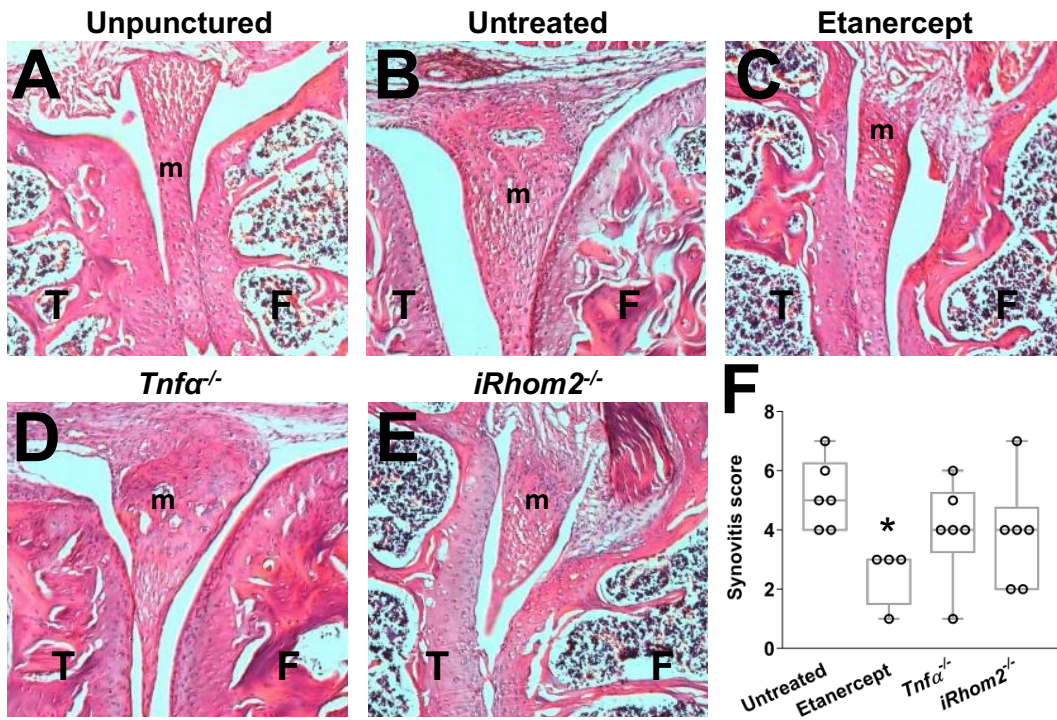


**Supplementary Material for the manuscript entitled
“Blood-induced bone loss in a mouse model of hemophilic arthropathy is
prevented by blocking the iRhom2/ADAM17/TNF α pathway”
by Haxaire et al.**

Suppl. Figure S1



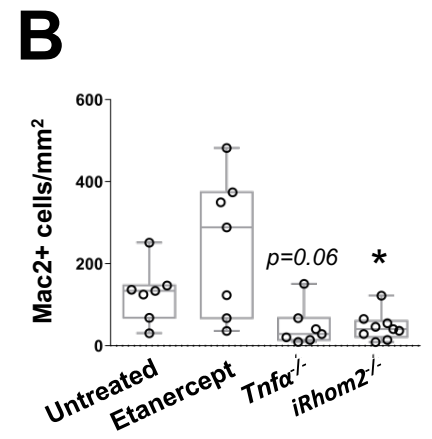
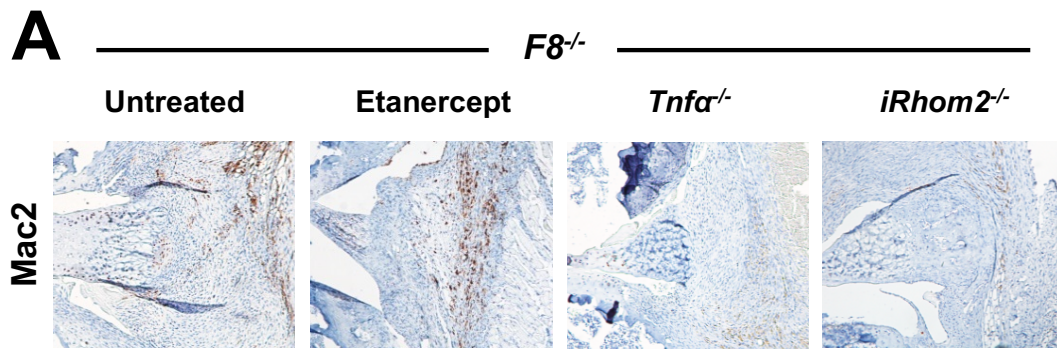
J

***F8*^{-/-}**

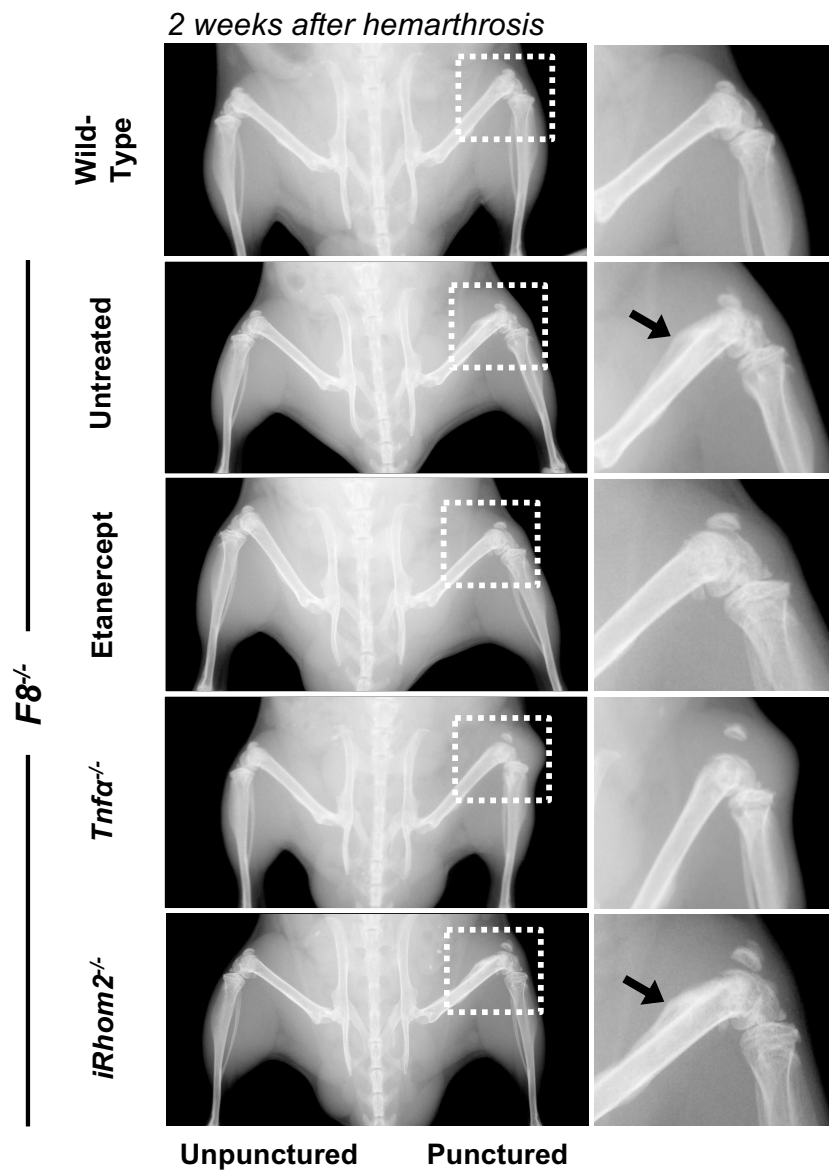
		Untreated	Etanercept	<i>TNFα</i> ^{-/-}	<i>iRhom2</i> ^{-/-}
Enlargement of the synovial lining cell layer (0-3)	2 weeks	2.5 ± 0.22	1.6 ± 0.4	2 ± 0	2.3 ± 0.2
	12 weeks	1.5 ± 0.3	1 ± 0	1 ± 0.2	0.6 ± 0.2
Density of the resident cells (0-3)	2 weeks	3 ± 0	2 ± 0.5	2.5 ± 0.3	1.9 ± 0.2
	12 weeks	1.8 ± 0.1	0.75 ± 0.2	1.6 ± 0.2	1.5 ± 0.3
Inflammation infiltrate (0-3)	2 weeks	3 ± 0	1.7 ± 0.4	2.3 ± 0.3	2 ± 0.1
	12 weeks	1.8 ± 0.1	0.75 ± 0.4	1.3 ± 0.3	1.6 ± 0.3
Total score	2 weeks	8.5 ± 0.2	5.3 ± 1	6.8 ± 0.6	6.2 ± 0.4
	12 weeks	5.1 ± 0.5	2.5 ± 0.4	4 ± 0.7	3.8 ± 0.7

* Values are shown as mean ± SEM

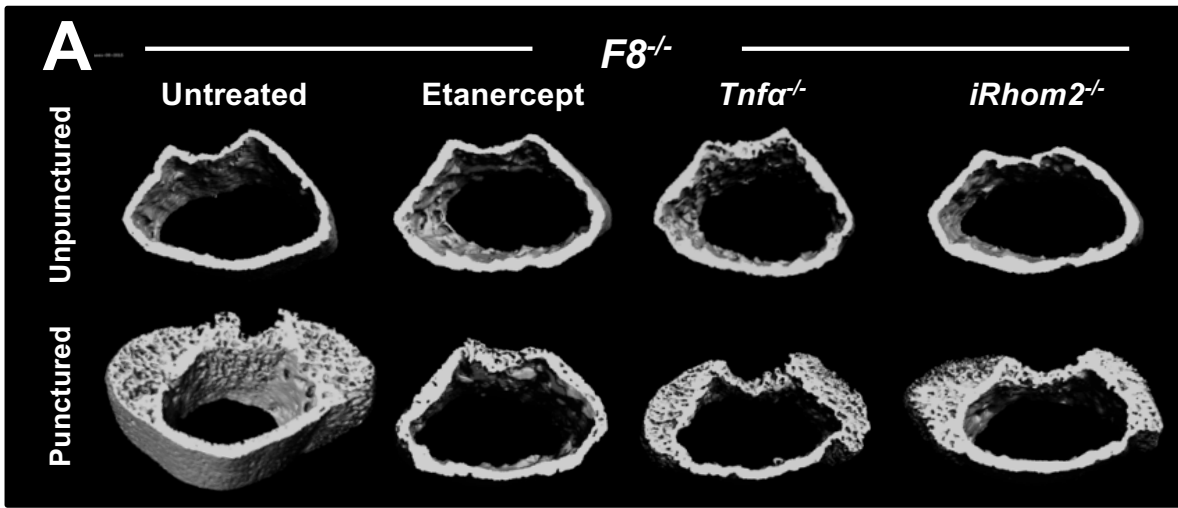
Suppl. Figure S2



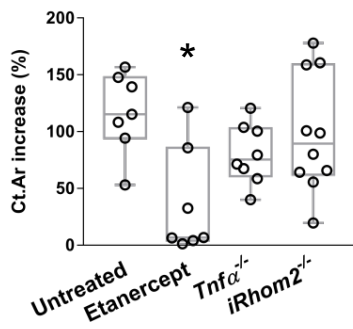
Suppl. Figure S3



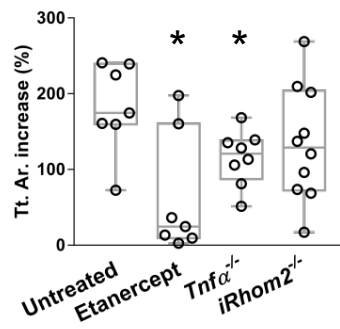
Suppl. Figure S4



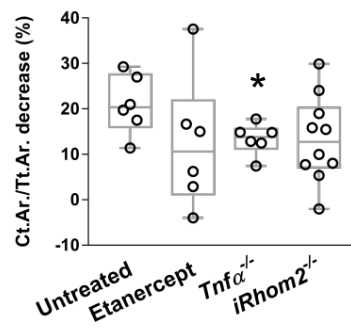
B



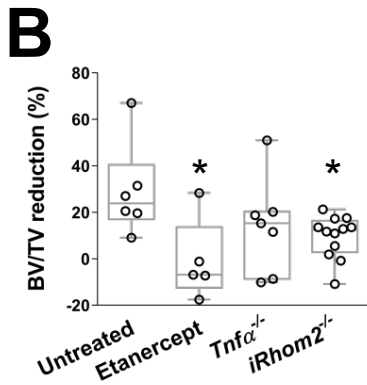
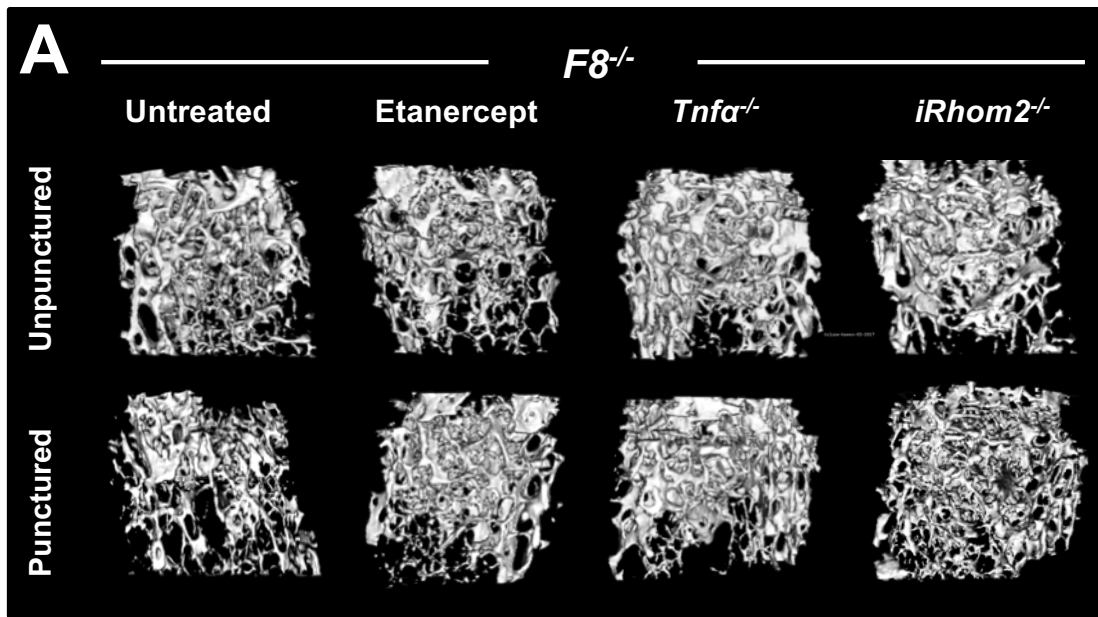
C



D



Suppl. Figure S5



Suppl. Table S1

F8^{-/-}

		Untreated		Etanercept		<i>TNFα</i> ^{-/-}		<i>iRhom2</i> ^{-/-}	
<i>Bone parameters*</i>		<i>Left</i>	<i>Right</i>	<i>Left</i>	<i>Right</i>	<i>Left</i>	<i>Right</i>	<i>Left</i>	<i>Right</i>
BV/TV (%)	<i>2 weeks</i>	0.112 ±0.016	0.03 ±0.007	0.125 ±0.01	0.07 ±0.009	0.131 ±0.011	0.065 ±0.007	0.128 ±0.012	0.066 ±0.011
	<i>12 weeks</i>	0.142 ±0.002	0.101 ±0.011	0.116 ±0.017	0.113 ±0.011	0.127 ±0.015	0.116 ±0.015	0.115 ±0.007	0.104 ±0.005
Tb.N (1/mm)	<i>2 weeks</i>	4.65 ±0.2	2.84 ±0.26	5.01 ±0.27	4.16 ±0.08	4.55 ±0.21	3.79 ±0.18	5.03 ±0.32	4.09 ±0.3
	<i>12 weeks</i>	4.21 ±0.17	3.05 ±0.46	4.28 ±0.1	3.92 ±0.24	4.08 ±0.19	3.49 ±0.23	4.31 ±0.14	3.96 ±0.14
Tb.Sp (μm)	<i>2 weeks</i>	0.217 ±0.01	0.376 ±0.03	0.202 ±0.01	0.239 ±0.004	0.226 ±0.01	0.267 ±0.01	0.204 ±0.01	0.254 ±0.02
	<i>12 weeks</i>	0.243 ±0.012	0.285 ±0.008	0.233 ±0.007	0.26 ±0.02	0.247 ±0.01	0.295 ±0.02	0.233 ±0.01	0.254 ±0.01
Tb.Th (μm)	<i>2 weeks</i>	0.042 ±0.002	0.033 ±0.001	0.046 ±0.005	0.037 ±0.003	0.045 ±0.002	0.039 ±0.002	0.042 ±0.002	0.037 ±0.002
	<i>12 weeks</i>	0.052 ±0.001	0.069 ±0.018	0.046 ±0.004	0.048 ±0.004	0.048 ±0.002	0.05 ±0.002	0.045 ±0.001	0.046 ±0.001

* Values are shown as mean ± SEM

Left, femur from unpunctured control knee; *Right*, femur from punctured knee.*F8^{-/-}* Untreated: 2 weeks n=8, 12 weeks n=6;Etanercept-treated *F8^{-/-}* mice: 2 weeks n=5, 12 weeks n=9;*F8^{-/-} / Tnf α ^{-/-}* mice: 2 weeks n=7, 12 weeks n=7;*F8^{-/-} / iRhom2^{-/-}* mice: 2 weeks n=10, 12 weeks n=11.

Bone volume/tissue volume (BV/TV); Trabecular number (Tb.Nb); Trabecular separation (Tb.Sp); Trabecular Thickness (Tb.Th).

Supplementary Figure legends

Supplementary Figure S1. Modified Krenn scoring system to measure synovial inflammation in joint sections of needle punctured mice 2 and 12 weeks after hemarthrosis. A-E) Representative section of joints from unpunctured control joints of $F8^{-/-}$ mice (A) or from needle puncture-injured $F8^{-/-}$ animals that were either untreated (B), treated with etanercept (C), or $F8^{-/-}Tnf\alpha^{-/-}$ (D) or $F8^{-/-}iRhom2^{-/-}$ mice (E) 12 weeks after hemarthrosis. All the images were taken with an Eclipse Ni-E Microscope (Nikon, Tokyo, Japan) at 10x magnification using an Andor Zyla camera (Andor Technology Ltd, Andor, Belfast, Northern) and analyzed using Nikon NIS Element Software. F) Modified Krenn scores of the synovial inflammation seen in joint sections at 2 weeks (see also Figure 3) or 12 weeks after needle puncture injury in $F8^{-/-}$ mice that were untreated, etanercept-treated, or lacked $Tnf\alpha^{-/-}$ or $iRhom2^{-/-}$. Enlargement of the synovial lining cell layer and density of the resident cells were scored (from 0 to 3) as initially described⁴⁶⁻⁴⁸. Inflammation was graded as 0= absent, 1= mild (few scattered lymphocytes and monocytes), 2= moderate (diffuse monocytes and lymphocytes, scattered granulocytes), 3= dense diffuse inflammatory infiltrate, predominantly consisting of lymphomonocytes and some granulocytes. The images shown are representative for the average synovial inflammation observed in each treatment group (T, tibia; F, femur; m, meniscus).

Supplementary Figure S2. Immunohistochemical analysis of Mac2 staining. A) Representative images of the synovium stained with the macrophage marker Mac2+ are shown. B) Quantification of the staining for Mac2 showed a significant decrease in $F8^{-/-}$ mice that lacked $Tnf\alpha$ (47, ± 19 cells/mm²; n=7) or $iRhom2$ (44, ± 11 cells/mm²; n=9) compared to $F8^{-/-}$ mice (127, ± 26 cells/mm²; n=7), but not in etanercept-treated mice (238, ± 64 cells/mm²; n=7). * indicates $p < 0.05$ vs. untreated $F8^{-/-}$ mice.

Supplementary Figure S3. Evaluation of the development of osteopenia 12 weeks after hemarthrosis in $F8^{-/-}$ mice. A) Representative micro-CT images of the femoral trabecular bone from unpunctured controls (upper panel) or punctured knees (lower panel) in untreated, etanercept-treated, or in $F8^{-/-}Tnf\alpha^{-/-}$ or $F8^{-/-}iRhom2^{-/-}$ double knockout mice. B, C) Quantification of the bone volume to total volume fraction (BV/TV) is indicated as percent reduction compared to the unpunctured control knee; 29 \pm 8% for $F8^{-/-}$ untreated mice (n=6), 0.9 \pm 7% for etanercept-treated $F8^{-/-}$ mice (n=9), 8.9 \pm 5% for $F8^{-/-}Tnf\alpha^{-/-}$ mice (n=7) and 8.2 \pm 3% for $F8^{-/-}iRhom2^{-/-}$ mice (n=11). * indicates $p \leq 0.05$ vs untreated $F8^{-/-}$ mice.

Supplementary Figure S4. Faxitron (X-ray) analyses of knee joints of $F8^{-/-}$ mice 2 weeks after hemarthrosis. The right knee was subjected to needle puncture

hemarthrosis, whereas the left knee in each animal served as control in wild type mice, $F8^{-/-}$ mice, $F8^{-/-}$ mice treated with etanercept, or $F8^{-/-}$ mice an additional lacking $TNF\alpha$ or $iRhom2$.

Supplementary Figure S5. Micro-CT analysis of the cortical thickening observed in the distal femur of $F8^{-/-}$ mice following needle puncture injury. Micro-CT 3D reconstruction of cortical bone in the distal femur 2 weeks after needle puncture injury in $F8^{-/-}$ mice. A) Representative micro-CT images of the femoral cortical bone from unpunctured controls (upper panel) or punctured knees (lower panel) in untreated, etanercept-treated, or in $F8^{-/-}/Tnf\alpha^{-/-}$ or $F8^{-/-}/iRhom2^{-/-}$ double knockout mice. B – D) Quantification of the cortical parameters: cortical area (Ct. Ar, B), total cross-sectional area (Tt. Ar., C) and cortical area fraction (Ct. Ar/Tt. Ar, D) of the punctured joints relative to the unpunctured control joint in each animal. Data are indicated as percent increase in (B) and (C), and percent decrease in (D) compared to the unpunctured control knee, Ct. Ar, Tt. Ar and Ct. Ar/Tt. Ar values are 116, $\pm 13\%$, 181, $\pm 22\%$ and 20, $\pm 2\%$, respectively, for $F8^{-/-}$ untreated mice (n=7); 42, $\pm 20\%$, 72, $\pm 34\%$ and 12, $\pm 6\%$ for etanercept-treated $F8^{-/-}$ mice (n=6); 80, $\pm 9\%$, 115, $\pm 13\%$ and 13, $\pm 1\%$ for $F8^{-/-}/Tnf\alpha^{-/-}$ mice (n=8) and 98, $\pm 16\%$, 134, $\pm 24\%$ and 13, $\pm 3\%$ for $F8^{-/-}/iRhom2^{-/-}$ mice (n=10). * indicates $p \leq 0.05$ vs untreated $F8^{-/-}$ mice.

Supplementary Table S1. Quantification of the trabecular bone parameters in the femur of the unpunctured (left) and punctured (right) knee in untreated, etanercept-treated $F8^{-/-}$ or $F8^{-/-}/Tnf\alpha^{-/-}$ or $F8^{-/-}/iRhom2^{-/-}$ mice. Analysis was performed by Micro-CT at 2 and 12 weeks after hemarthrosis.