Supplementary Fig. S1 Higher resolution images of micrographs shown in Fig. 5 of the main article. Immunostaining of knee joint sections of wild-type and hemophilia mice administered with varying doses of recombinant human factor VIIa (rhFVIIa)-eptacog beta. Wild-type (WT) and FVIII−/− mice were administered with three different doses, 90, 250, and 500 μg/kg, of rhFVIIa-eptacog beta intravenously via the tail vein. Mice were killed at varying time intervals after Evans Blue (EB) administration; knee joints were excised and fixed in Excel Plus fixative. Fixed tissues were processed for tissue sectioning and immunostaining of human FVIIa and counter stained with hematoxylin. (A–D) Immunostaining of tissue sections for hFVIIa from tissue samples obtained at 3 hours (A), 24 hours (B), 3 days (C), and 7 days (D) following EB administration. Top, tissue sections from FVIII−/− mice; bottom, tissue sections from wild-type mice. Within the panel, top images were obtained at 4× magnification. Bottom images depict 40× magnification focused on the boxed area. (E) Immunostaining of hFVIIa in knee joint sections of FVIII−/− and WT mice that did not receive rhFVIIa (controls). BM, bone marrow; CH, chondrocytes; S, synovium.
Supplementary Fig. S1 (Continued).
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Supplementary Fig. S2 Hematoxylin and eosin stain (H&E) and iron staining of knee joint tissue sections from wild-type and FVIII−/− mice. Knee joint tissue sections prepared from tissues harvested from wild-type and FVIII−/− mice were stained with H&E stain (A) and Prussian Blue solution (prepared by mixing equal volumes of 3% potassium ferrocyanide and 3% hydrochloric acid; Iron Stain Kit, Stat Lab) for iron (B). Sections stained for iron were counterstained with Nuclear Fast Red solution. Unstained iron control slides provided in the kit were used as a positive control to verify histologic technique and reagent reactivity. Magnification: top panel 4×; bottom panel, 40×.