Supplementary Materials
Montilla et al., 2022

Figure S1.

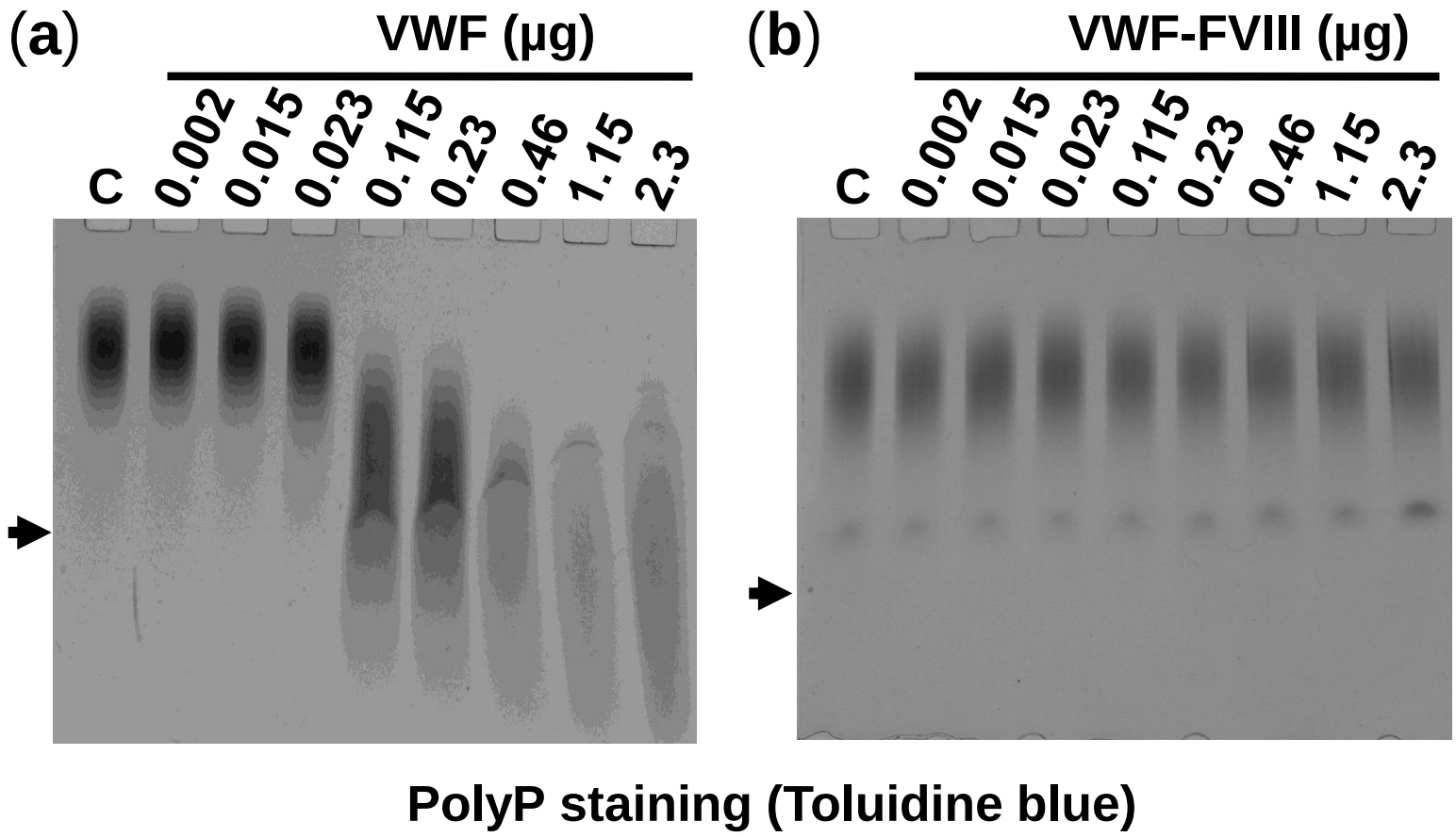

Figure S1. Urea-polyacrylamide gel electrophoresis of polyphosphate and von Willebrand Factor, in the absence or presence of factor VIII. Increasing amounts of von Willebrand Factor (Factor VIII-free) (**a**), or von Willebrand Factor co-purified with factor VIII (**b**), were incubated with 0.8 mM of isolated polyphosphate ($polyP_{65}$) as described in Methods. Samples were loaded onto urea-polyacrylamide gels, separated by electrophoresis, and stained using polyphosphate-specific staining (toluidine blue). Arrows show the mobility of von Willebrand factor on the gels. Representative experiments are shown (n=3).

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Figure S2.

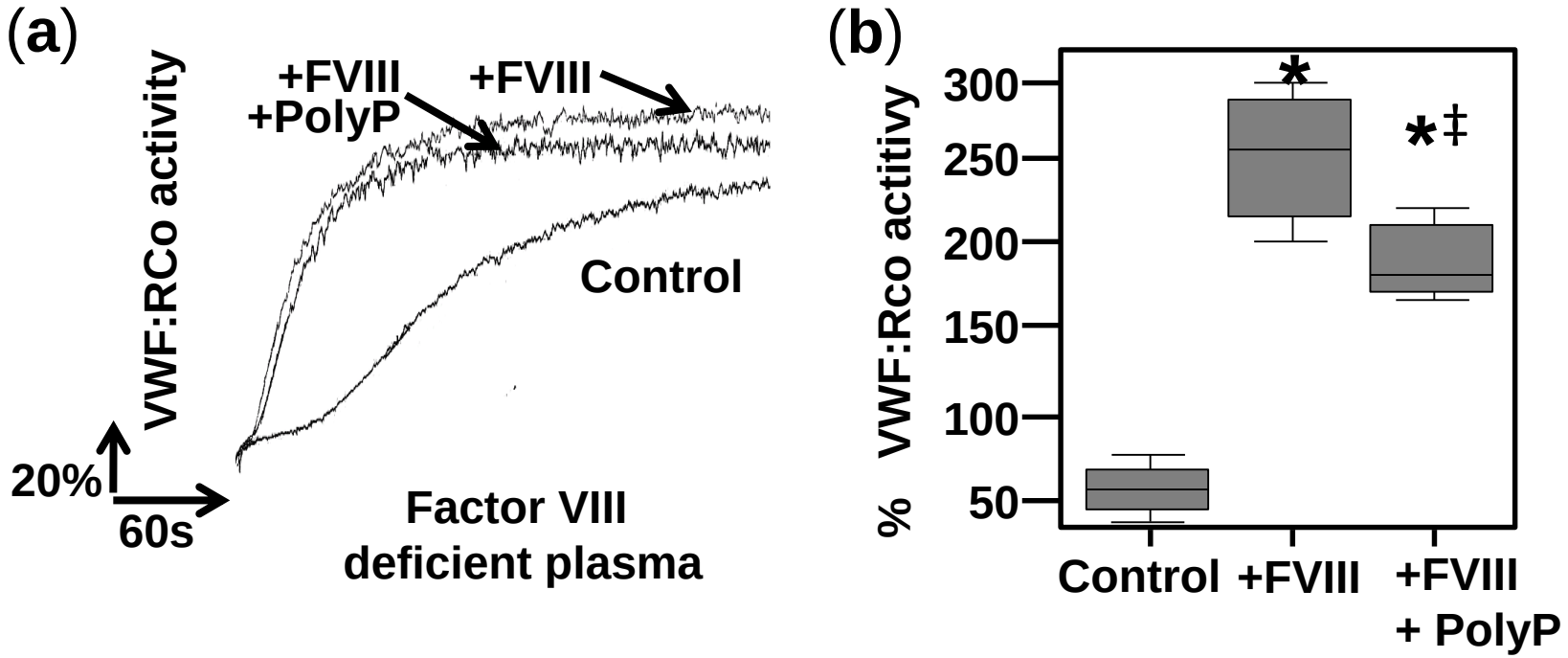

Figure S2. PolyP competes with factor VIII in the activation of von Willebrand Factor ristocetin cofactor activity. (**a**) Aggregation curves of fixed platelets in a ristocetin cofactor assay using factor VIII-deficient plasmas. Prior to the assay, 50 µl of plasmas (diluted 1:2) were incubated with isolated Factor VIII (+Factor VIII), or Factor VIII plus polyphosphate (+Factor VIII +polyP) (0.65 µg Factor VIII and 2.5 µM $polyP_{65}$ were used). Control samples were assayed without Factor VIII or $polyP_{65}$ addition. Representative experiments are shown (n=3). (**b**) Quantification of von Willebrand Factor ristocetin cofactor activity of the result shown in panel (**a**) in three different plasmas. Results are presented in a box-and-whiskers plot. Asterisk indicates a statistical difference of $p<.01$, determined by Mann-Whitney test, compared to the "Control" measurements. Symbol "‡" indicates a statistical difference of $p<.01$, determined by Mann-Whitney test, when Factor VIII is compared with Factor VIII plus polyphosphate. Measurements were performed using an automated aggregometer (Helena AggRAM).

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Figure S3.

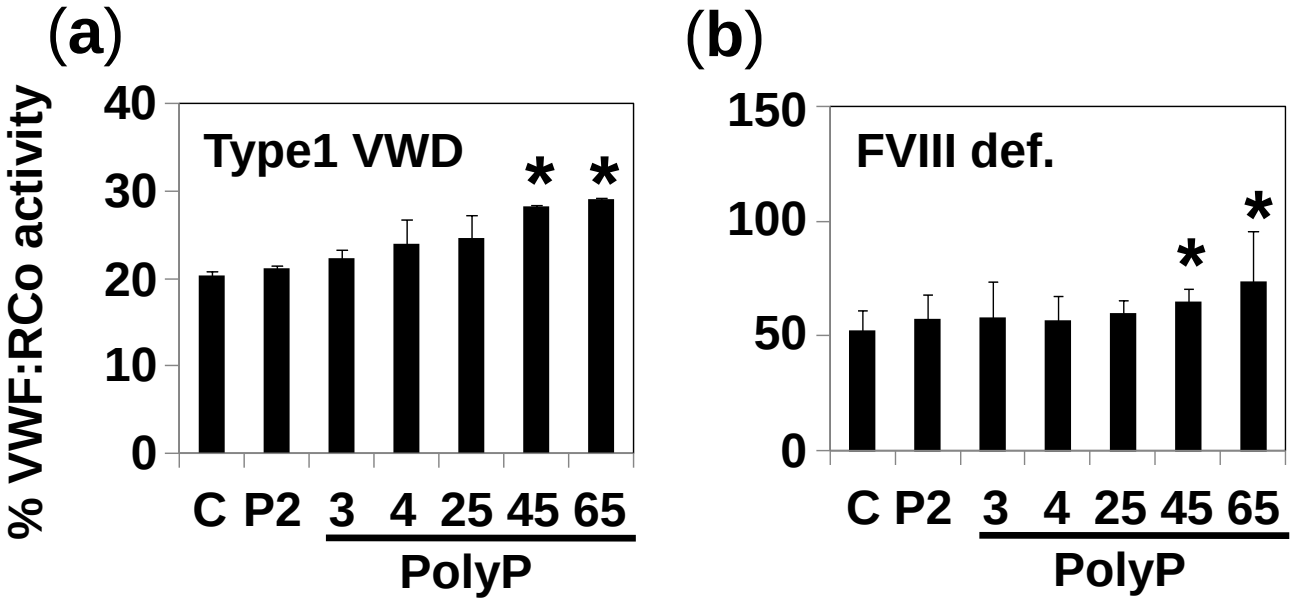

Figure S3. PolyP size determines the activation of von Willebrand Factor ristocetin cofactor activity. A ristocetin cofactor assay was performed using plasma from patients with (**a**) von Willebrand disease type 1, or (**b**) factor VIII-deficient plasmas, in the absence (control, "C") or in presence of 2.5 μM of pyrophosphate (P2), tripolyphosphate ($PolyP_3$), tetrapolyphosphate ($PolyP_4$), $PolyP_{25}$, $PolyP_{45}$, or $PolyP_{65}$. Results are expressed in percentages. Values represent the mean ±S.E from three separate experiments. PolyP and PPi concentrations are expressed in terms of phosphate residues.