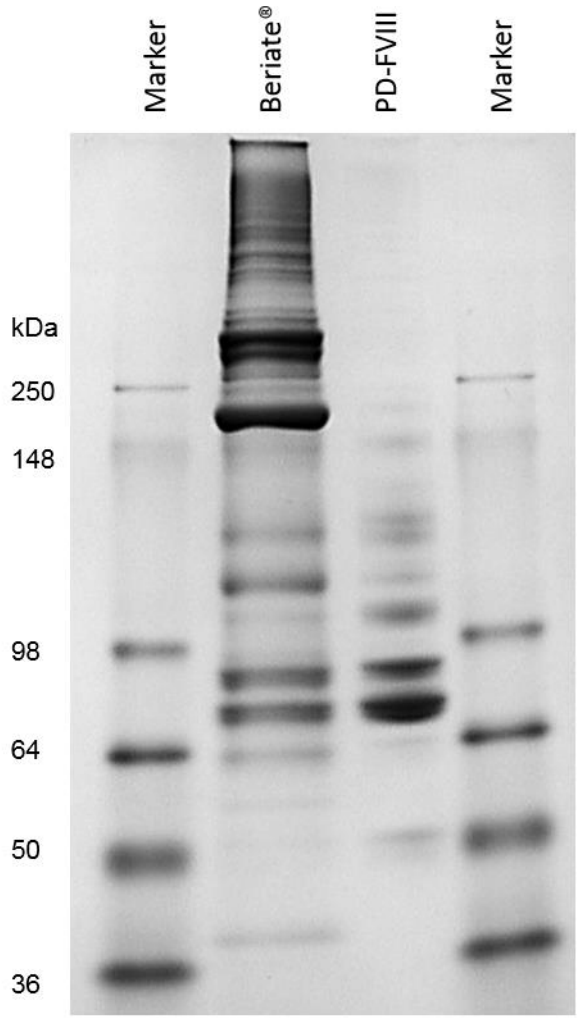
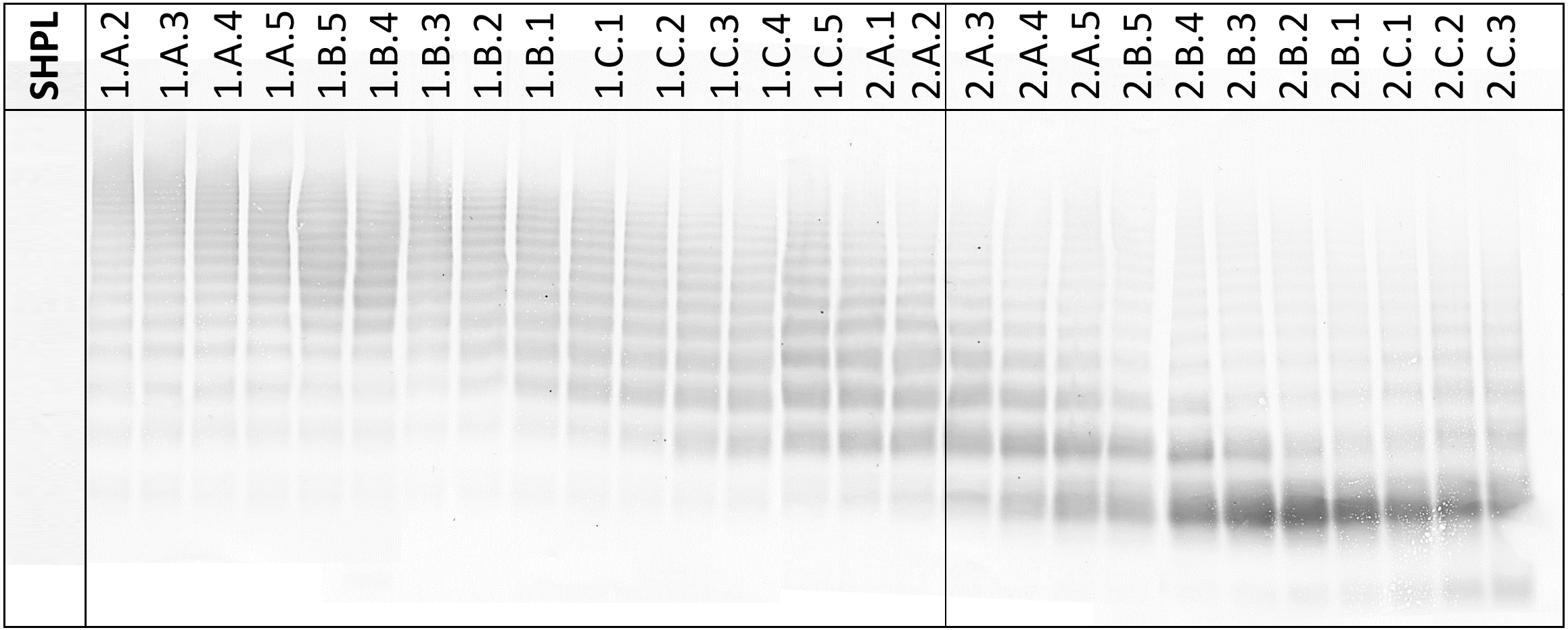
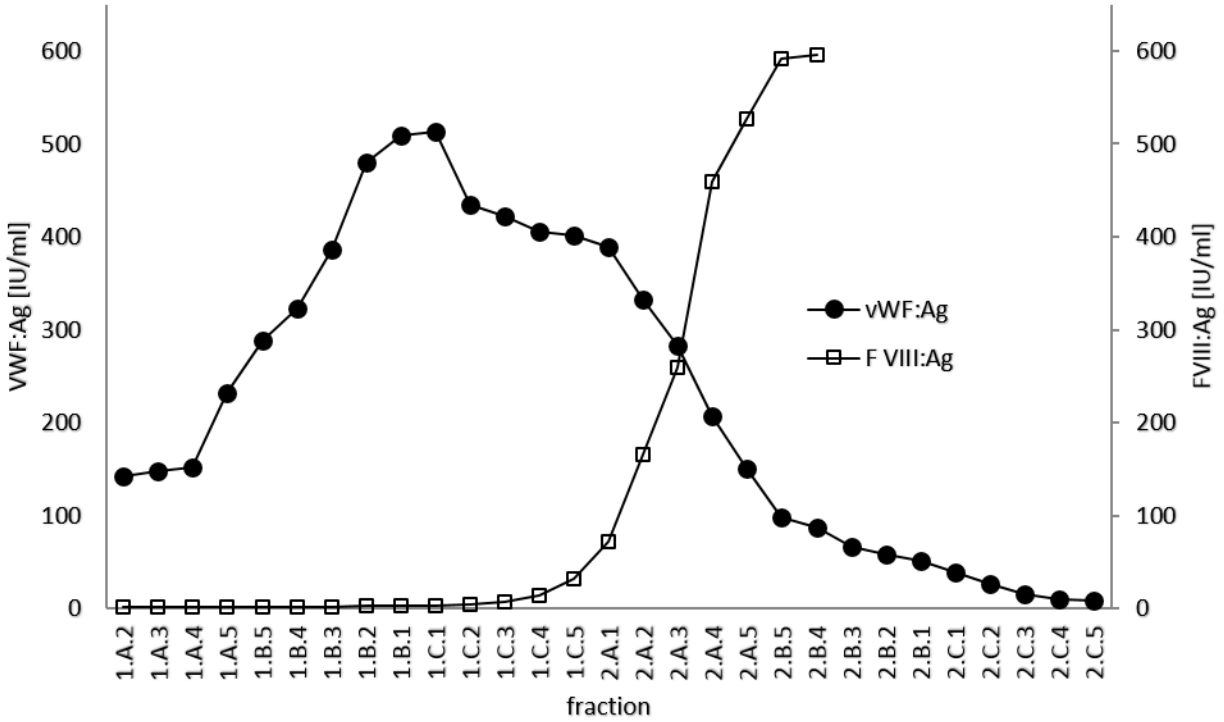


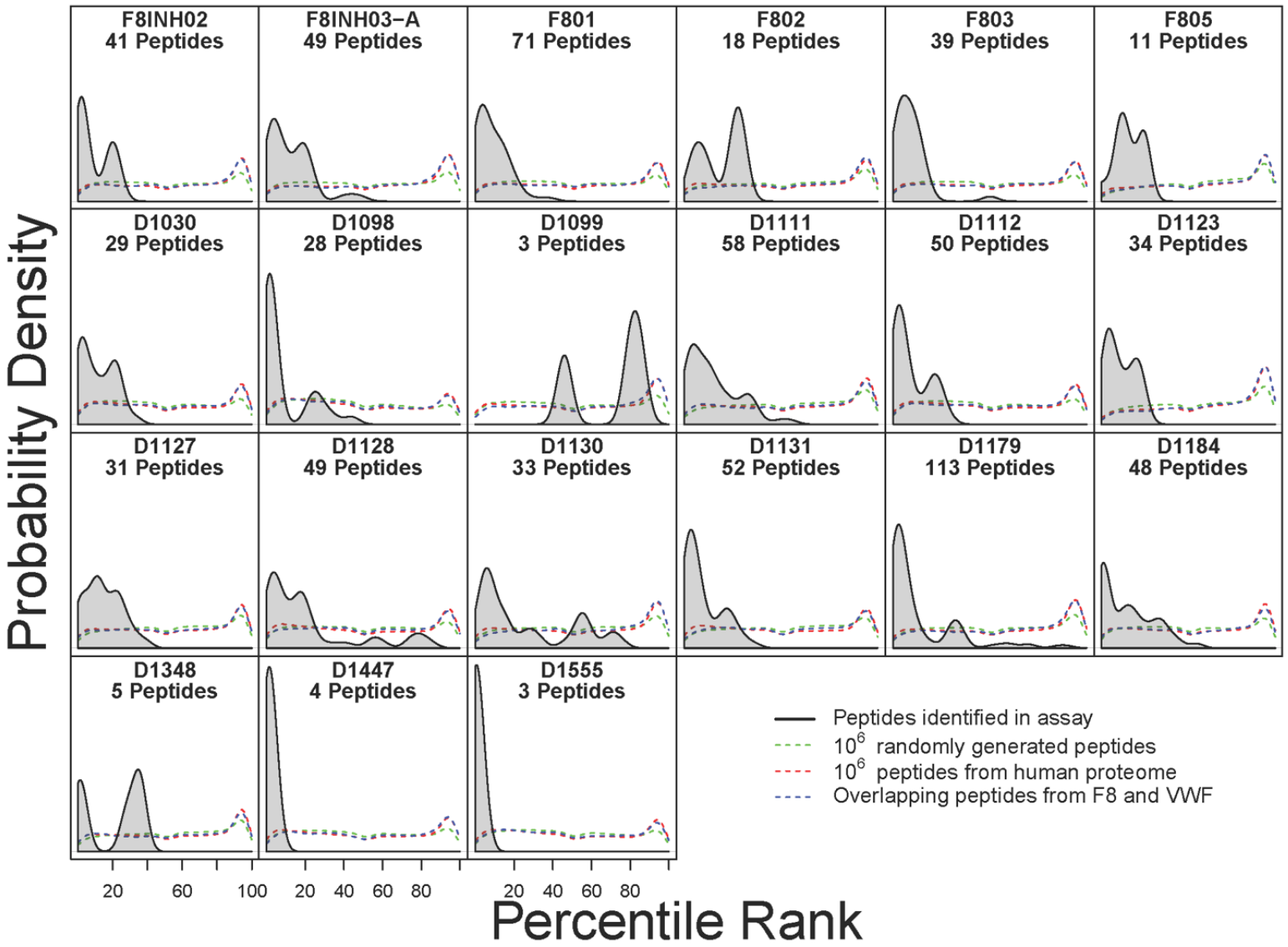
Supplementary Figure 1: SDS-PAGE of Beriate®, a pdFVIII concentrate containing VWF, and VIIISelect™ affinity step eluate (GE Healthcare, Uppsala, Sweden), designated as PD-FVIII (without VWF). Tris-Glycine PAGE, 8-16% (Novex); load 12 IU FVIII, chromogenic activity (Coamatic®); GelCode™ Blue Stain (Thermo Scientific).



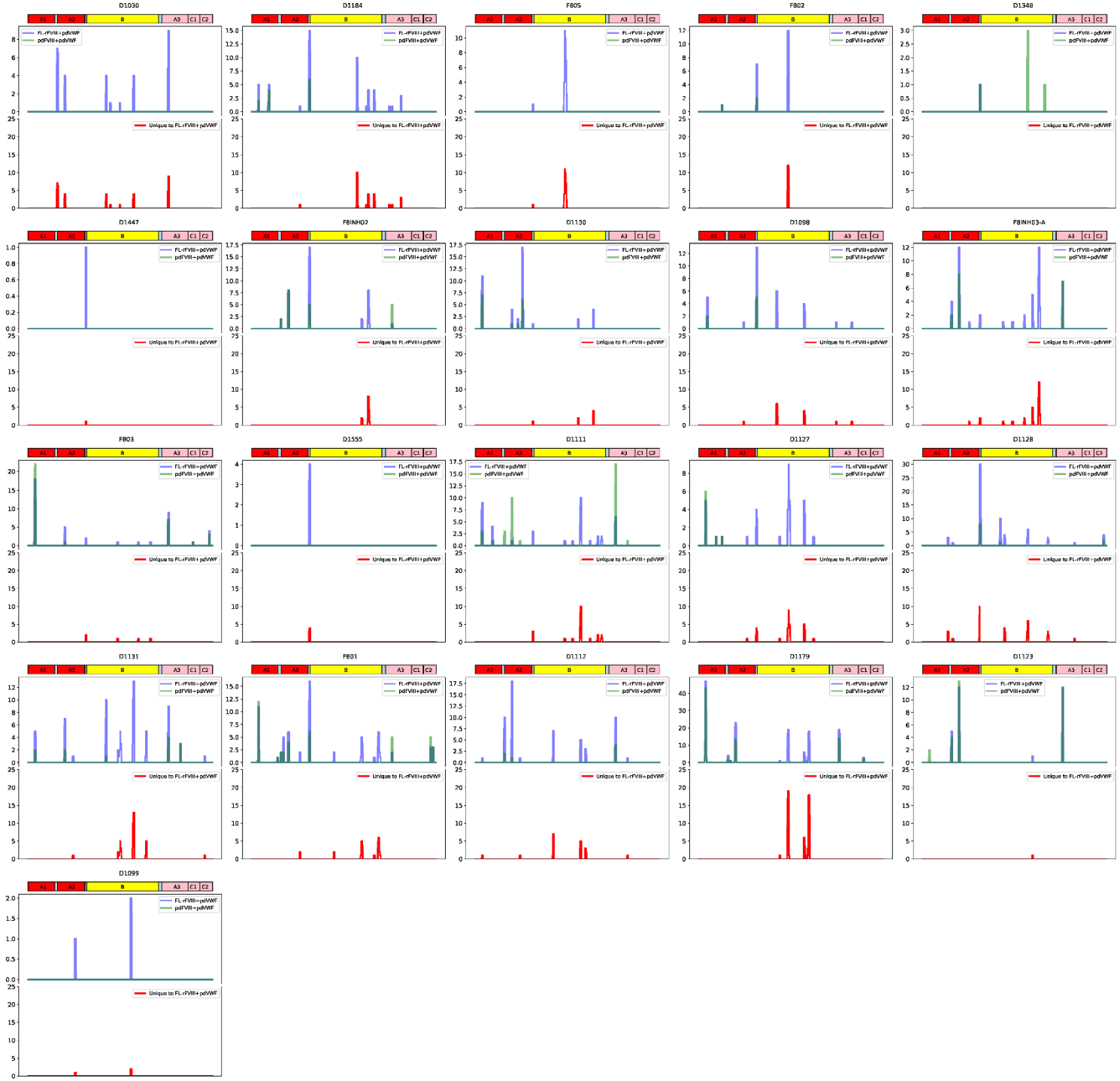
Supplementary Figure 2. Western blot of VWF multimers. Fractions 1.A.2 to 2.A.2 were collected and used as pdVWF. Standard human plasma and samples (fractions of size exclusion chromatography step) were loaded in a 1% agarose gel (Biorad), each 2 IU VWF:Ag (0.1 IU/mL). Sample and electrophoresis buffer: Tris-Glycine/SDS (Novex). Protein was transferred to a nitrocellulose membrane (Amershan), detected by rabbit anti-human-VWF (Dako) and stained with anti- rabbit-IgG conjugated with alkaline phosphatase (Sigma).



Supplementary Figure 3: Separation of PD-FVIII bound to PD-VWF through size exclusion chromatography step (HiPrep Sephacryl S500, GE Healthcare); HEPES buffer was added 400 nM calcium chloride. PdVWF fractions were collected, fractions containing FVIII and VWF were discarded. VWF:Ag and FVIII:Ag were detected by commercial ELISA Kits (vWF Ag® Testkit, SIEMENS Heathineers, Marburg (Germany); Anti-Human Factor VIII:C, Cedarlane, Hornby, Ontario (Canada)).



Supplementary Figure 4. Affinities of FVIII peptides identified on MoDCs. The distribution of percentile rank scores predicted by netMHCIIpan3.2 for all peptides found in the MAPPs assay for each donor (black/grey fill), compared to the distribution of percentile rank scores for 1 x 106 random strings of 15 amino acids (green), 1 x 106 random 15-mers from the human proteome (red), and all 15- mers from FVIII and VWF (blue).



Supplementary Figure 5. Comparison of FVIII peptides identified in a MAPPs assay following incubation of MoDCs with FL-rFVIII + pdVWF or pdFVIII + pdVWF. Frequencies of peptides identified in the MAPPs assay for each donor following incubation of APCs with FL-rFVIII + pdVWF (blue) or pdFVIII + pdVWF (red). Lower panel (green) shows location and frequencies of peptides identified following incubation with FL- rFVIII + pdVWF where no peptides were found after incubation with pdFVIII+ pdVWF.

Study ID	Mutation	Severity	Inhibitor	Race	Factor products used
F8INH02	Intron 22	severe	Past	White	Novoseven, Hemofil M
F8INH03-A	missense / c.6147G>T (p.Lys2049Asn)	severe	Current	White	Alphanate, Novoseven, FEIBA, Hemofil M, Kogenate, Xyntha
F801	Intron 22	severe	No history at time of procedure	White	Advate, Recombinate, Adynovate, Koate
F802	Intron 22	severe	No	White	Eloctate, Recombinate, Xyntha, Afstyla, Koate
F803	missense / c.7033T>C (p.Cys2345Arg)	severe	No	White	Hemofil M, Monarc-M, Koate
F805	Intron 22	severe	No	White	Kogenate (new patient to UNC, don't have factor product history, but has been on Kogenate since around 2008 per patient report)

Supplementary Table 1: HA patient information