

	Relative specific two-stage chromogenic activity	Relative specific one-stage clotting activity
FVIII WT	1.0	1.0
Lys1967Ile	0.7	0.7
Lys1967Ala	0.9	1.1
Lys1968Ala	1.2	1.9

Table S2. Specific two-stage and one-stage activities of the FVIII variants relative to FVIII WT. The FVIII antigen concentration and two-stage chromogenic activity were assessed as described in van den Biggelaar *et al.* (33) with the exception that anti-heavy chain antibody CLB-CAg9 was employed to assess the antigen concentration instead of CLB-CAg12. In the one-stage clotting assay, FVIII WT and variants thereof were reconstituted in FVIII deficient plasma (Siemens Healthcare Diagnostics, Marburg, Germany). The clotting time was assessed on a Sysmex CA-1500 Coagulation Analyzer (Sysmex Corporation, Kobe, Japan) employing Actin-FSL (Siemens Healthcare Diagnostics, Marburg, Germany). Specific activities were obtained by dividing the results of the one-stage and two-stage assays with the antigen concentration.