Supplementary information

A bispecific antibody to factors IXa and X restores factor VIII hemostatic activity in a hemophilia A model

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Supplementary Figure 1  Surface plasmon resonance sensorgrams of the FIXa- and FX-specific variable arms of hBS23 binding to the antigens. (a, b) Changes in surface plasmon signal (RU: resonance units) of the FIXa-specific variable region after the injection of different concentrations of human FIX or FIXa as analyte. (c, d) Changes in surface plasmon signal of the FX-specific variable region after the injection of different concentrations of human FX or FXa as analyte. The average $K_D$ value calculated from two separate experiments was stated in each panel.
Supplementary Figure 2  PL-dependency of hBS23 activity on FIXa-catalyzed FX activation (FXa generation) in an enzymatic assay using purified coagulation factors. Data were collected in triplicate and expressed as the means ± s.d. (in many cases, the bars depicting s.d. are shorter than the height of the symbols).

Supplementary Figure 3  Effects of hBS23 and rhFVIII on a thrombin generation parameter, ETP, in FVIII-deficient plasma without and with inhibitors. Each plasma lot was from a single donor who had been confirmed as having severe hemophilia A without inhibitor or with inhibitors. Inhibitor titers of plasma with inhibitors (plasma with inhibitors 1 and 2) were 292 and 148 Bethesda units, respectively. Data were collected in triplicate for each plasma lot and expressed as the means ± s.d.
Supplementary Figure 4  Effects of hBS23 and rpoFVIII on APTT (a) and thrombin generation parameters, peak height (b) and ETP (c), in the pooled cynomolgus monkey plasma containing 300 μg ml\(^{-1}\) VIII-2236, a neutralizing antibody against FVIII with cross-reactivity to cynomolgus monkey FVIII but not to porcine FVIII. Data were collected in triplicate and expressed as the means ± s.d.