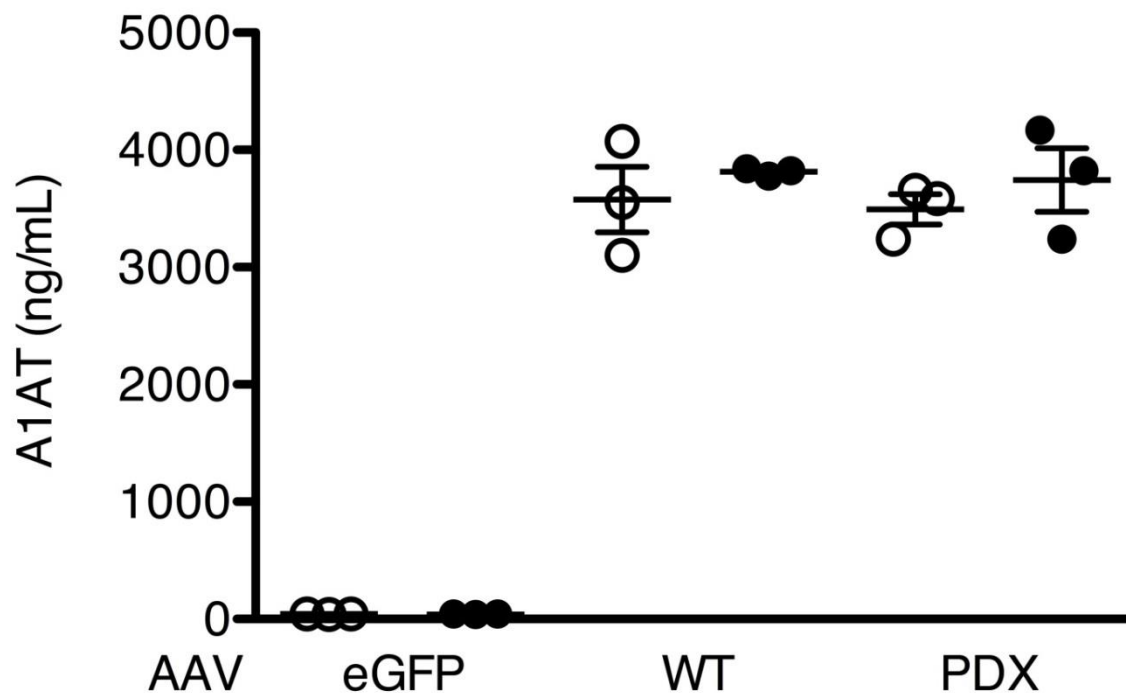
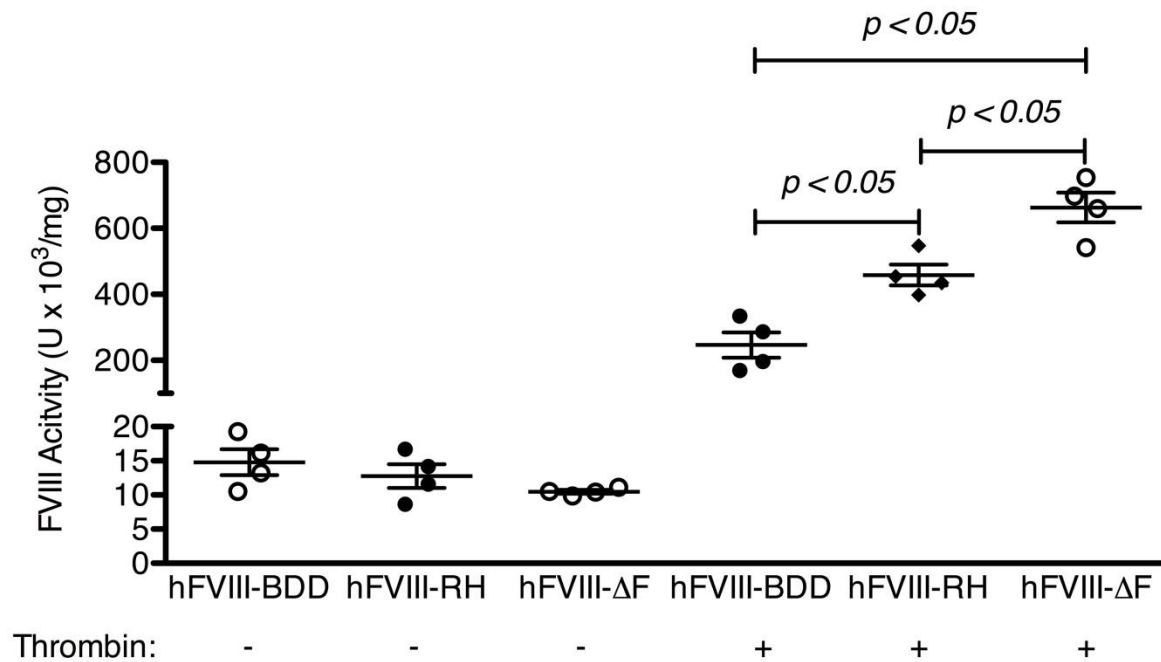
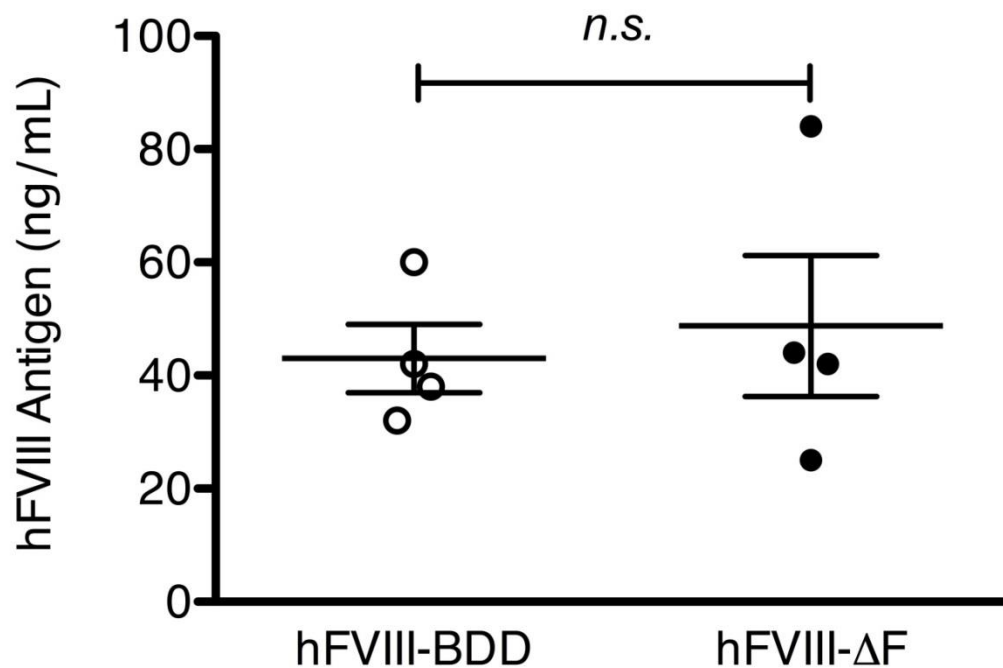




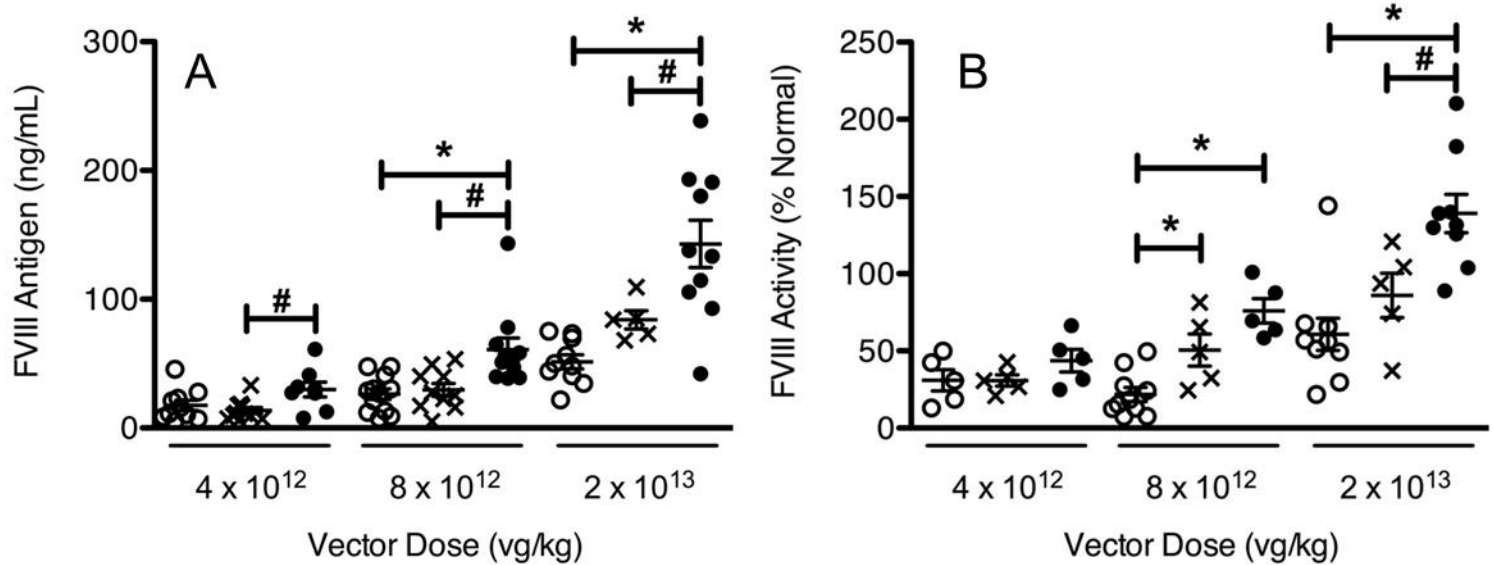
Supplementary Figure 1: Alpha-1-antitrypsin (A1AT) levels in transduced cell media. A1AT levels determined by ELISA after transduction with the indicated vector (adeno-associated viral (AAV)-eGFP, AAV-A1AT-wild type (WT), or or AAV-A1AT-Portland (PDX) of either factor (F) VIII-BDD (open) or FVIII-ΔF (closed) expressing BHK cells. Each point represents a distinct transduction. Horizontal markers in whisker plots represent the mean and 1-standard error.



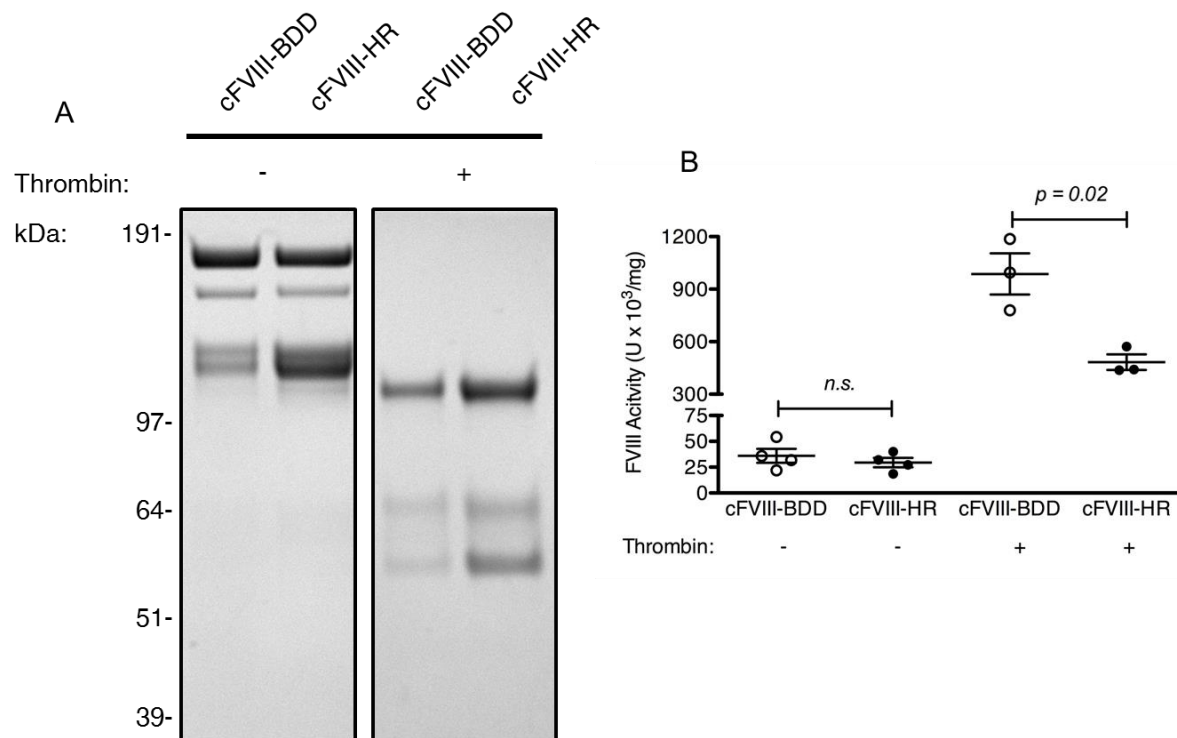
Supplementary Figure 2: Clotting activity of human factor (F) VIII-BDD, FVIII-R1645H, and FVIII-ΔF. Human FVIII clotting activity was determined by 1-stage or 2-stage clotting assay. Each point represents a distinct dilution. Similar results obtained with at least two distinct protein preparations. p-values determined by ANOVA with Bonferroni post-test. Horizontal markers in whisker plots represent the mean and 1-standard error.



Supplementary Figure 3: Human (h) factor (F) VIII-BDD and hFVIII-ΔF antigen levels after protein infusion in hemophilia A mice utilized in the tail-clip challenge experiment shown in Figure 5. FVIII antigen levels determined after 30 minutes. Each point represents a single animal. Horizontal markers in whisker plots represent the mean and 1-standard error.



Supplementary Figure 4: Expression of human (h) factor (F) VIII variants following Adeno-Associated Viral serotype 8 (AAV8)-mediated liver gene transfer in severe hemophilia A mice. Three dosing-cohorts of mice received either AAV-hFVIII-BDD (*open circles*), AAV-hFVIII-R1645H (*exes*), and AAV-hFVIII-ΔF (*closed circles*) with a liver-specific promoter at the indicated vector-doses and circulating hFVIII antigen (A) and activity (B), as determined by chromogenic assay, was measured 4-8 weeks after vector infusion. * and # indicate a p-values < 0.05 as determined by ANOVA with Bonferroni post-test within each dosing cohort for comparing hFVIII-ΔF with hFVIII-BDD and hFVIII-R1645H respectively. Horizontal markers in whisker plots represent the mean and 1-standard error.



Supplementary Figure 5: Characterization of recombinant canine factor (F) VIII-H1645R compared to canine FVIII-BDD. (A) SDS-PAGE analysis of 3-8 μg of canine FVIII-H1645R and canine FVIII-BDD staining with Coomassie blue before (-) or after (+) activation with 20 nM thrombin. cFVIII-H1645R is 40% single-chain compared to the 70% single-chain of cFVIII-BDD as determined by densitometry analysis. (C) cFVIII clotting activity was determined by 1-stage or 2-stage clotting assays. Each point represents a distinct dilution. Horizontal markers in whisker plots represent the mean and 1-standard error.

Supplementary Table 1: Summary data of the efficacy of AAV8-cFVIII encoding canine FVIII-ΔF compared to unmodified BDD^{*} in male dogs with severe hemophilia A

Dog	cFVIII Transgene	Vector Dose (x 10 ¹² vg/kg)	Age at time of administration (years)	Weight (kg)	Peak cFVIII Activity (%)	Plateau cFVIII Activity [†] (%)	Plateau WBCT [†] (min)
Cuddles	ΔF	6	2.7	30	3.7	2.5 ± 0.4	16 ± 2
O89	ΔF	6	3.7	30	1.9	1.3 ± 0.3	17 ± 2
L51*	BDD	20	0.4	14	1.5	0.7 ± 0.5	19 ± 2
M66*	BDD	20	1.3	22	2.9	1.7 ± 0.8	17 ± 3
M06*	BDD	40	0.5	20	9.3	7.8 ± 2.7	16 ± 3
M50*	BDD	40	0.6	17	2.9	3.1 ± 1.5	12 ± 2

* Previously published data (reference 5)

[†] Mean ± SD

Abbreviations: AAV8, adeno-associated viral serotype 8; BDD, B-domain deleted (FVIII-SQ); cFVIII, canine factor VIII; WBCT, whole blood clotting time.

