Figure S1. **Recovery and inactivation of mFVIIa, mFVIIa-VEAY and mFVIIa-DVQ**
Recovery of mFVIIa, mFVIIa-DVQ or mFVIIa-VEAY was determined as follows: 1 µg/ml of recombinant protein was added to hemophilia A (HA) or B (HB) mouse or human plasma at room temperature and residual activity was determined at defined timepoints by aPTT, using a standard curve of plasma spiked with mFVIIa, mFVIIa-DVQ or mFVIIa-VEAY. Briefly, 25 µl of sample were incubated with an equal volume of aPTT reagent (Trinity Biotech, Co Wicklow, Ireland) for 3 min at 37° C, followed by the addition of 25 µl (for mouse plasmas) or 50 µl (for human plasmas) of 25 mM $CaCl_2$ prior to measuring the time to clot using a Start4 instrument (Diagnostica Stago, Parsippany, NJ). For determining the effect of antithrombin in residual activity, we used human antithrombin (AT)-deficient plasma (Affinity Biologicals, Ontario, Canada), 5 µg/ml of mFVIIa-VEAY and mFVIIa-DVQ and 50 µl of 25 mM $CaCl_2$ were used. The use of 5 µg/ml of each variant was necessary to reduce the initial clot times so that changes as a result of inactivation could be easily monitored. The graphs depict residual activity of mFVIIa, mFVIIa-VEAY and mFVIIa-DVQ following addition of 1µg/ml of recombinant protein in mouse hemophilia A (A) or B (B) plasma. Data were derived from at least 2 independent experiments and an asterisk denotes $P < .05$ (mFVIIa-DVQ) or $P < .01$ (mFVIIa-VEAY) compared to mFVIIa. (C) Clotting time (aPTT) following addition of 5 µg/ml of mFVIIa-VEAY and mFVIIA-DVQ in human AT deficient plasma. A grey box indicates the range of aPTT of human AT deficient plasma without the addition of protein. Data were derived from 3 independent experiments. All data and error bars are shown as average ± 1 standard deviation, respectively.

Figure S2. RNA from snap-frozen liver samples collected after week 14 post AAV administration was extracted using the RNeasy kit (Qiagen Inc., Valencia, CA)
Following reverse transcription of 100 ng of total RNA (Superscript III kit, Invitrogen), transcript levels for mFVII/mFVIIa/mFVIIa-VEAY were quantified with real-time PCR using appropriate primers (SABiosciences, Frederick, MD) and corrected for endogenous GAPDH transcript levels (Applied Biosystems, Foster City, CA). Both mFVIIa and mFVIIa-VEAY exhibited similar rates of amplification in real-time PCR. Three groups of mice are shown: untreated hemophilia A mice (HA), AAV-mFVIIa treated HA (HA-mFVIIa) and HA mice treated with low dose AAV-mFVIIa-VEAY (HA-mFVIIa-VEAY Low). Data are shown as mean ± 1 standard deviation (SD) of individual mice. Asterisk denotes $P < .007$ relative to HA mice treated with AAV-mFVIIa (HA-mFVIIa). Fold difference between HA-mFVIIa and HA-mFVIIa-VEAY (Low) is shown.

Figure S1

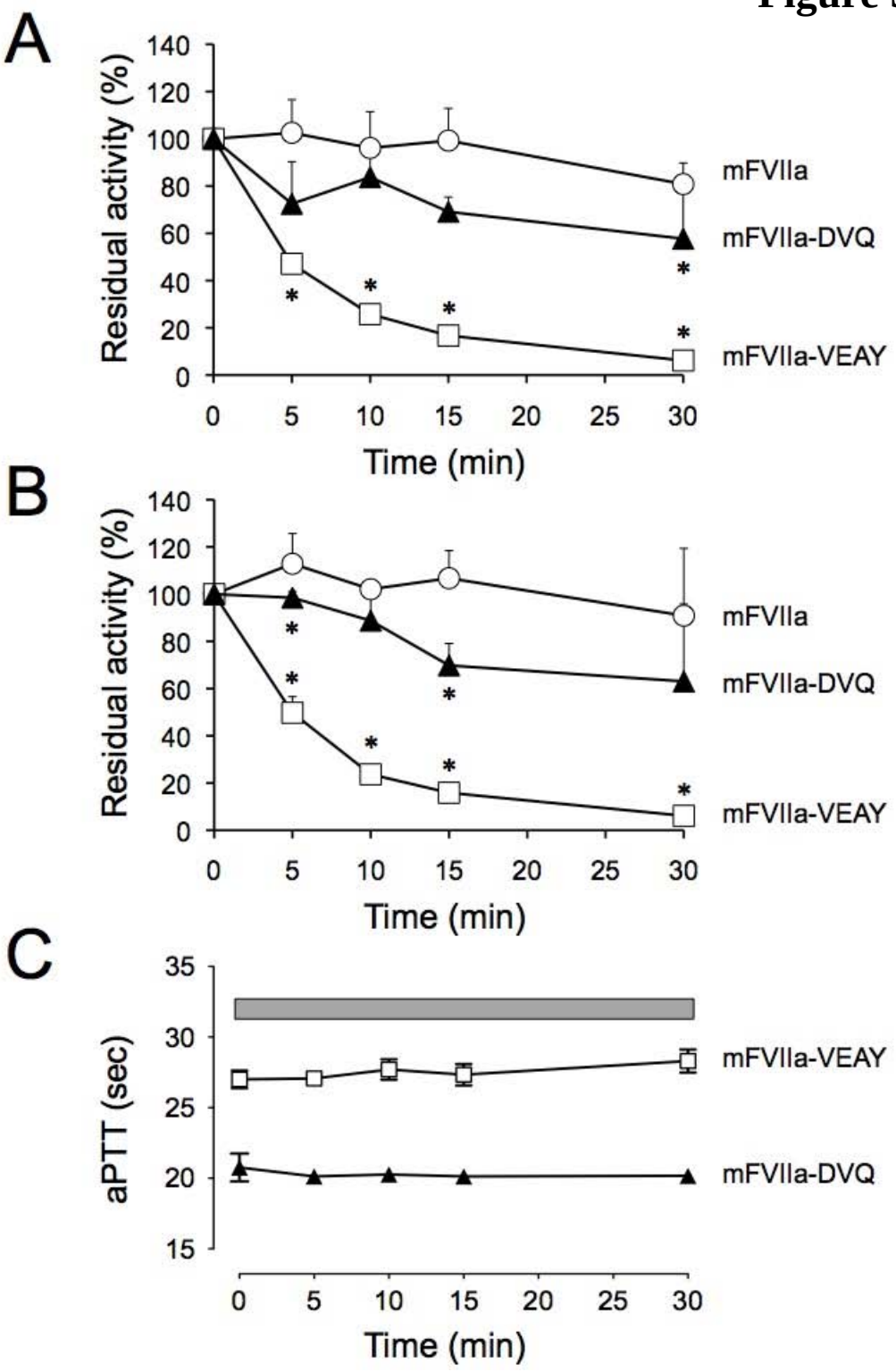
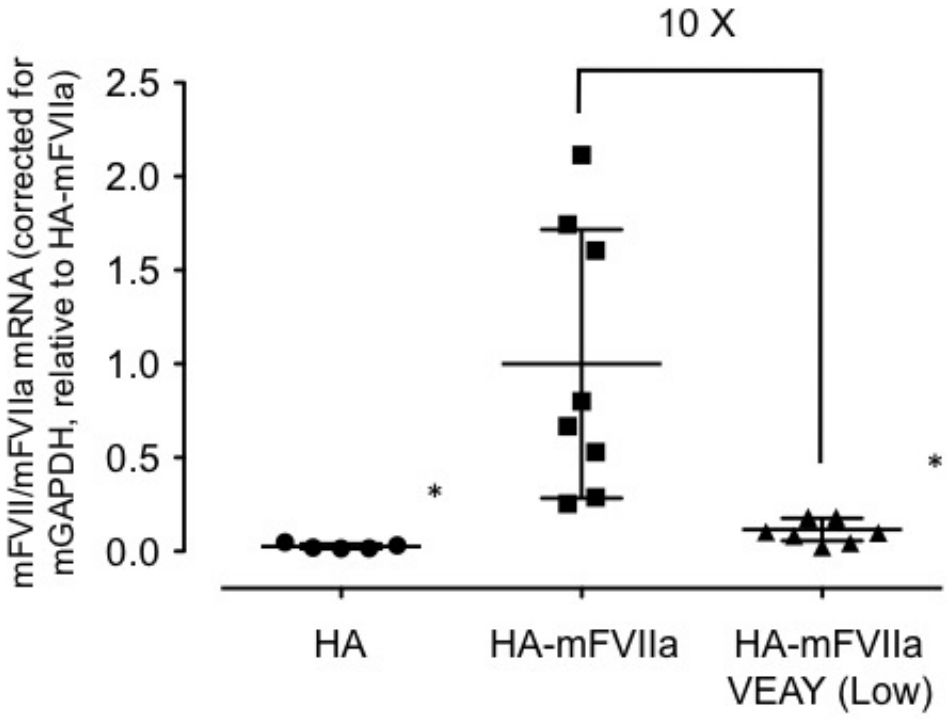

A
Residual activity (%)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (min)
mFVIIa
mFVIIa-DVQ
mFVIIa-VEAY
*
B
Residual activity (%)
Time (min)
mFVIIa
mFVIIa-DVQ
mFVIIa-VEAY
C
aPTT (sec)
35
30
25
20
15
Time (min)
mFVIIa-VEAY
mFVIIa-DVQ

Figure S2


10 X
mFVII/mFVIIa mRNA (corrected for mGAPDH, relative to HA-mFVIIa)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
HA
HA-mFVIIa
HA-mFVIIa VEAY (Low)