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Supplemental Information

Enhanced Factor IX Activity following Administration of AAV5-R338L “Padua” Factor IX versus AAV5 WT Human Factor IX in NHPs

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Supplementary Methods

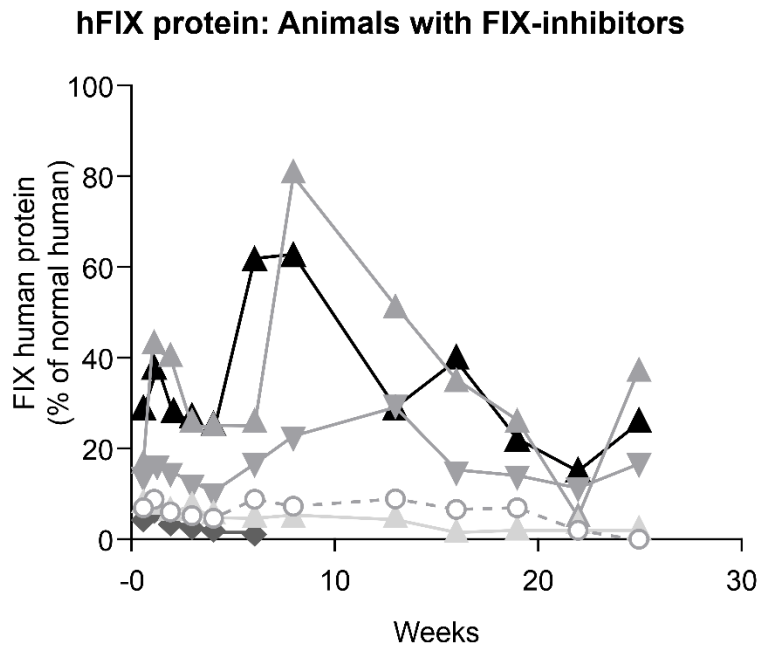
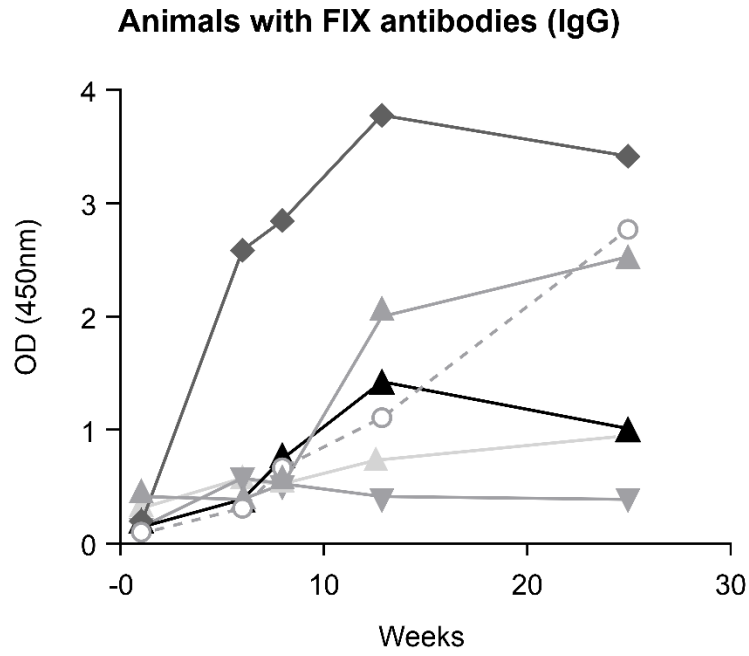
Detection of anti-hFIX Abs

Antibodies against hFIX were analyzed at different time points during the study. In brief, native hFIX protein was immobilized to the wells of an immunoabsorbent assay plate and blocking reagents were added. Serum, plasma, and control samples were loaded to allow the binding of hFIX-specific immunoglobulins to the immobilized antigen and hFIX-specific bound IgG was detected using HRP conjugated anti-monkey IgG.

In silico immunogenicity analyses

Potential immunogenicity risk of *FIX*-Padua was assessed by performing an *in-silico* analysis.^{52,53} The global and regional immunogenic potential was examined using an *in-silico* platform for epitope identification and prediction (EpiMatrix system developed by Epivax, Inc) for both Class I (all nucleated cells) and Class II (antigen presenting cells) HLA and compared to the full-length wild type human *FIX* sequence.

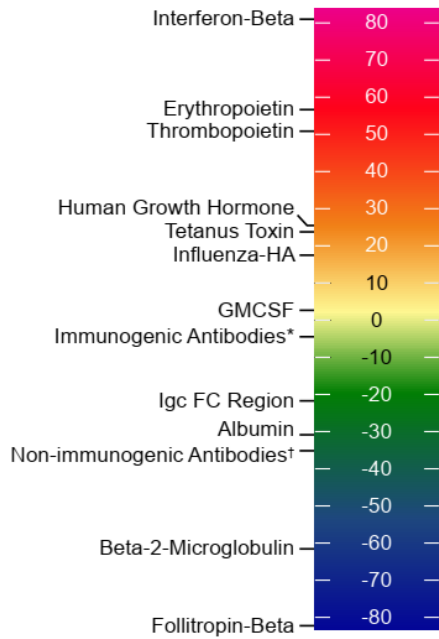
Supplementary Figure 1. Detection of cynomolgus antibodies against human FIX in AAV-injected NHPs.



- 844: AMT-060 5×10^{12}
- ▲ 847: AMT-061 5×10^{12}
- ▲ 851: AMT-061 2.5×10^{13}
- ▼ 852: AMT-061 2.5×10^{13}
- ◆ 863: AMT-061 2.5×10^{13}
- ▲ 856: AMT-061 9×10^{13}

Supplementary Figure 2. Class I and II immunogenicity scoring

Class II Immunogenicity Scale



*ADA in >5% exposed patients
 †ADA in <5% exposed patients

Class I:

Protein label	Class I epimatrix score
WT Factor IX, by 9	6.11
Padua Factor IX, by 9	7.62
WT Factor IX, by 10	7.81
Padua Factor IX, by 10	9.35

Class II:

Protein label	Length	Assessments	EpiMatrix hits	EpiMatrix score
WT Factor IX	461	4,077	170	-25.49
Padua Factor IX	461	4,077	170	-25.47

Padua Factor IX (-25.47)
 WT Factor IX (-25.49)

EpiMatrix predicted excess/shortfall in aggregate immunogenicity relative to a random protein standard