YMTHE, Volume *29*

Supplemental Information

Early Phase Clinical Immunogenicity of Valoctocogene Roxaparvovec, an AAV5-Mediated Gene Therapy for Hemophilia A

Brian R. Long, Philippe Veron, Klaudia Kuranda, Romain Hardet, Nina Mitchell, Gregory M. Hayes, Wing Yen Wong, Kelly Lau, Mingjin Li, M. Benjamin Hock, Stephen J. Zoog, Christian Vettermann, Federico Mingozzi, and Becky Schweighardt

Figures S1. Longitudinal FVIII activity, ALT values, corticosteroid use, and incidence of AAV5 capsid specific and FVIII specific cellular immunity by IFN-γ FluoroSpot assay following BMN 270 dose administration. Instances of FluoroSpot assay positive results (Symbols, SFU/10^6 PBMC) are plotted along the left Y-axis. ALT values (yellow line, U/L) and FVIII activity measured by chromogenic substrate assay (blue line, IU/dL) are plotted along the right Y-axis. Cellular immune response detected by IFN-γ secretion following stimulation with 2 AAV5 peptide pools and 4 FVIII peptide pools are displayed as symbols by time point when assay results were positive (≥50 SFU/10^6 PBMC) according to the symbol key at bottom. Corticosteroid dose and duration are indicated by the green box below the plot. Table below each plot shows positive FluoroSpot results by time point when positive for each peptide pool stimulation. SFU values ≥600 SFU are plotted at the top of the scale and displayed in the table. N indicates time points for which PBMC were assayed and returned a negative result. Blank spaces indicate where no results were obtained due to too few cells or poor cell viability.

Figure S1

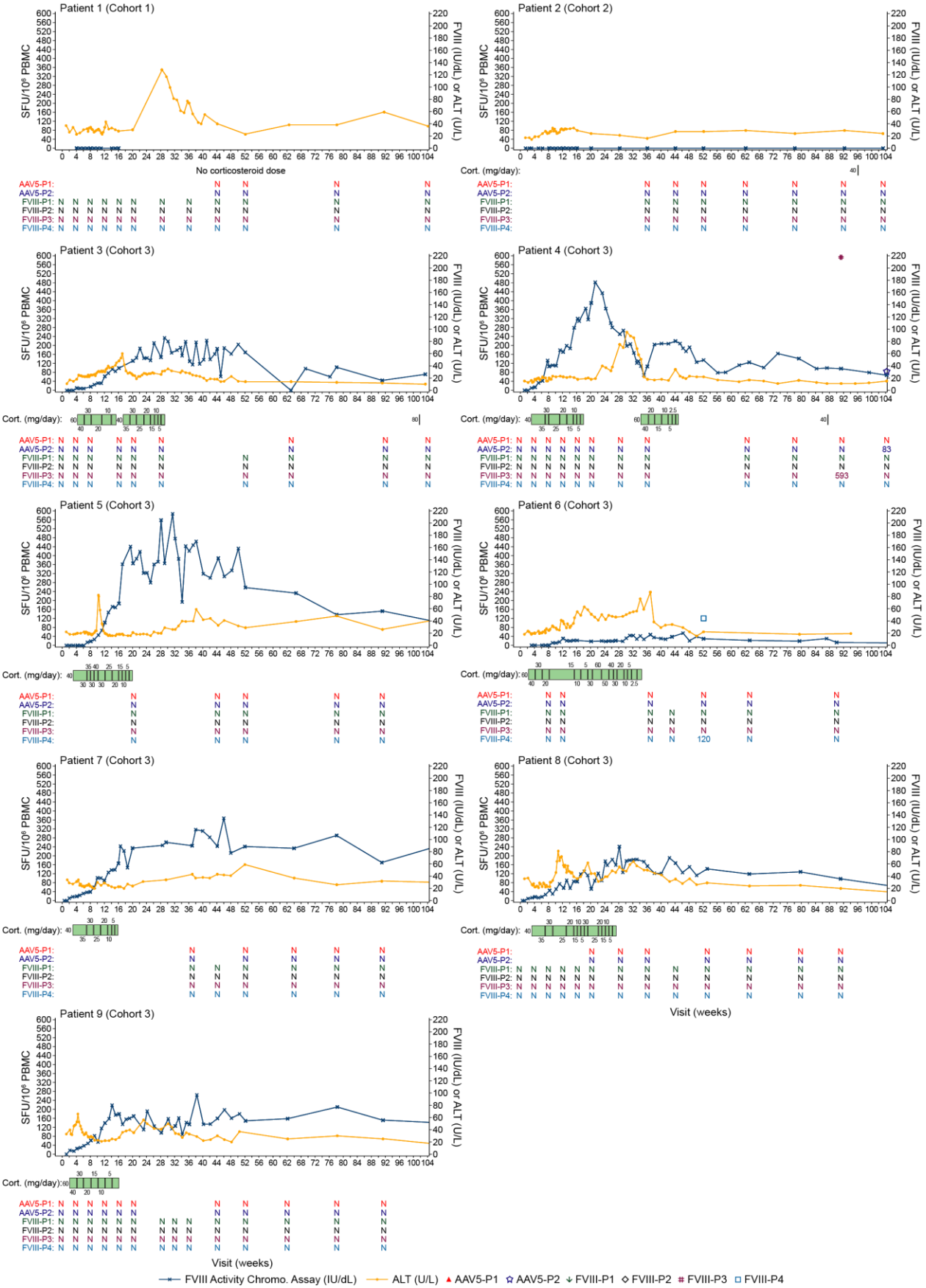
Patient 1 (Cohort 1)
Patient 2 (Cohort 2)
Patient 3 (Cohort 3)
Patient 4 (Cohort 3)
Patient 5 (Cohort 3)
Patient 6 (Cohort 3)
Patient 7 (Cohort 3)
Patient 8 (Cohort 3)
Patient 9 (Cohort 3)
SFU/10⁶ PBMC
FVIII (IU/dL) or ALT (U/L)
No corticosteroid dose
Cort. (mg/day):
AAV5-P1:
AAV5-P2:
FVIII-P1:
FVIII-P2:
FVIII-P3:
FVIII-P4:
Visit (weeks)
FVIII Activity Chromo. Assay (IU/dL)
ALT (U/L)
AAV5-P1
AAV5-P2
FVIII-P1
FVIII-P2
FVIII-P3
FVIII-P4

Figure S1 cont.

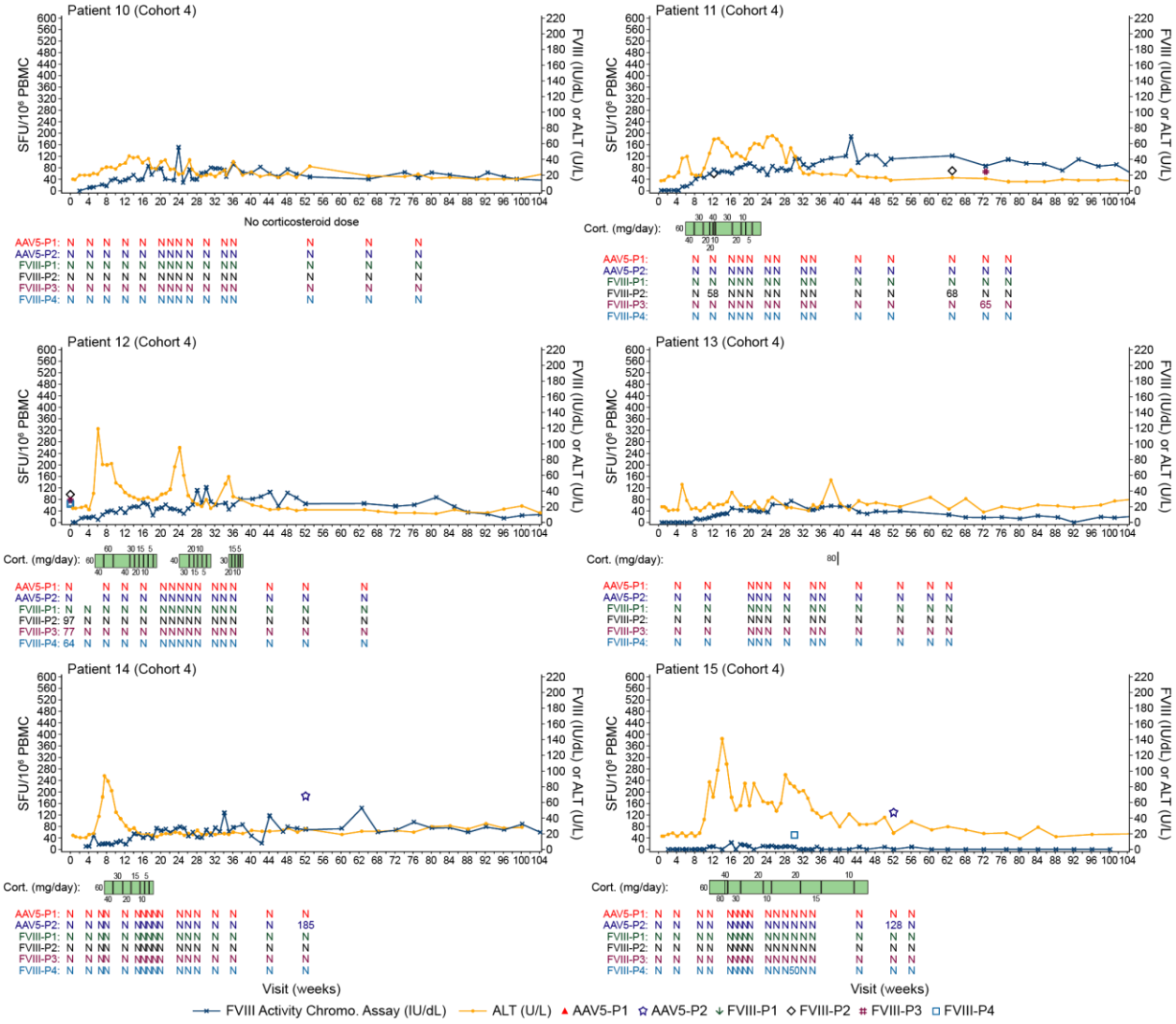

Figure S2. Longitudinal FVIII activity, ALT values, corticosteroid use, and incidence of AAV5 capsid specific and FVIII specific cellular immunity by TNF-α FluoroSpot assay following BMN 270 dose administration. Instances of FluoroSpot assay positive results (Symbols, SFU/10^6 PBMC) are plotted along the left Y-axis. FVIII activity measured by chromogenic substrate assay (blue line, IU/dL) and ALT values (yellow line, U/L) are plotted along the right Y-axis. Cellular immune response detected by TNF-α secretion following stimulation with 2 AAV5 peptide pools and 4 FVIII peptide pools are displayed as symbols by time point when assay results were positive (≥50 SFU/10^6 PBMC) according to the symbol key at bottom. Corticosteroid dose and duration are indicated by the green box below the plot. Table below each plot shows positive FluoroSpot results by time point when positive for each peptide pool stimulation. SFU values that were too numerous to count were interpolated at 5,000 SFU/10^6 PBMC and are plotted at the top of the scale and displayed as T in the table. N indicates time points for which PBMC were assayed and returned a negative result. Blank spaces indicate where no results were obtained due to too few cells or poor cell viability.

Figure S2

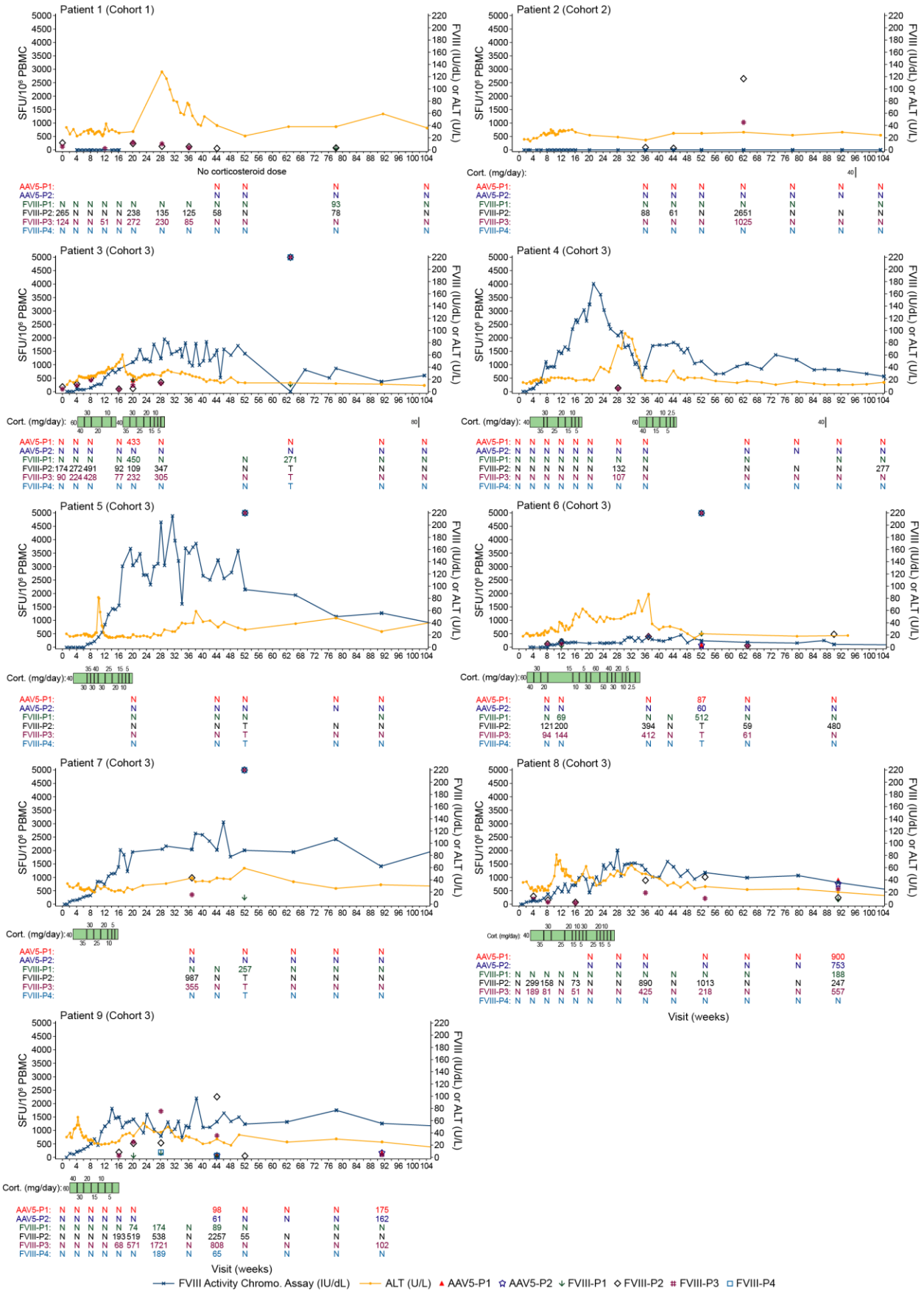
Patient 1 (Cohort 1)
Patient 2 (Cohort 2)
Patient 3 (Cohort 3)
Patient 4 (Cohort 3)
Patient 5 (Cohort 3)
Patient 6 (Cohort 3)
Patient 7 (Cohort 3)
Patient 8 (Cohort 3)
Patient 9 (Cohort 3)
SFU/10⁶ PBMC
FVIII (IU/dL) or ALT (U/L)
No corticosteroid dose
Cort. (mg/day):
AAV5-P1:
AAV5-P2:
FVIII-P1:
FVIII-P2:
FVIII-P3:
FVIII-P4:
Visit (weeks)
FVIII Activity Chromo. Assay (IU/dL)
ALT (U/L)
AAV5-P1
AAV5-P2
FVIII-P1
FVIII-P2
FVIII-P3
FVIII-P4

Figure S2 cont.

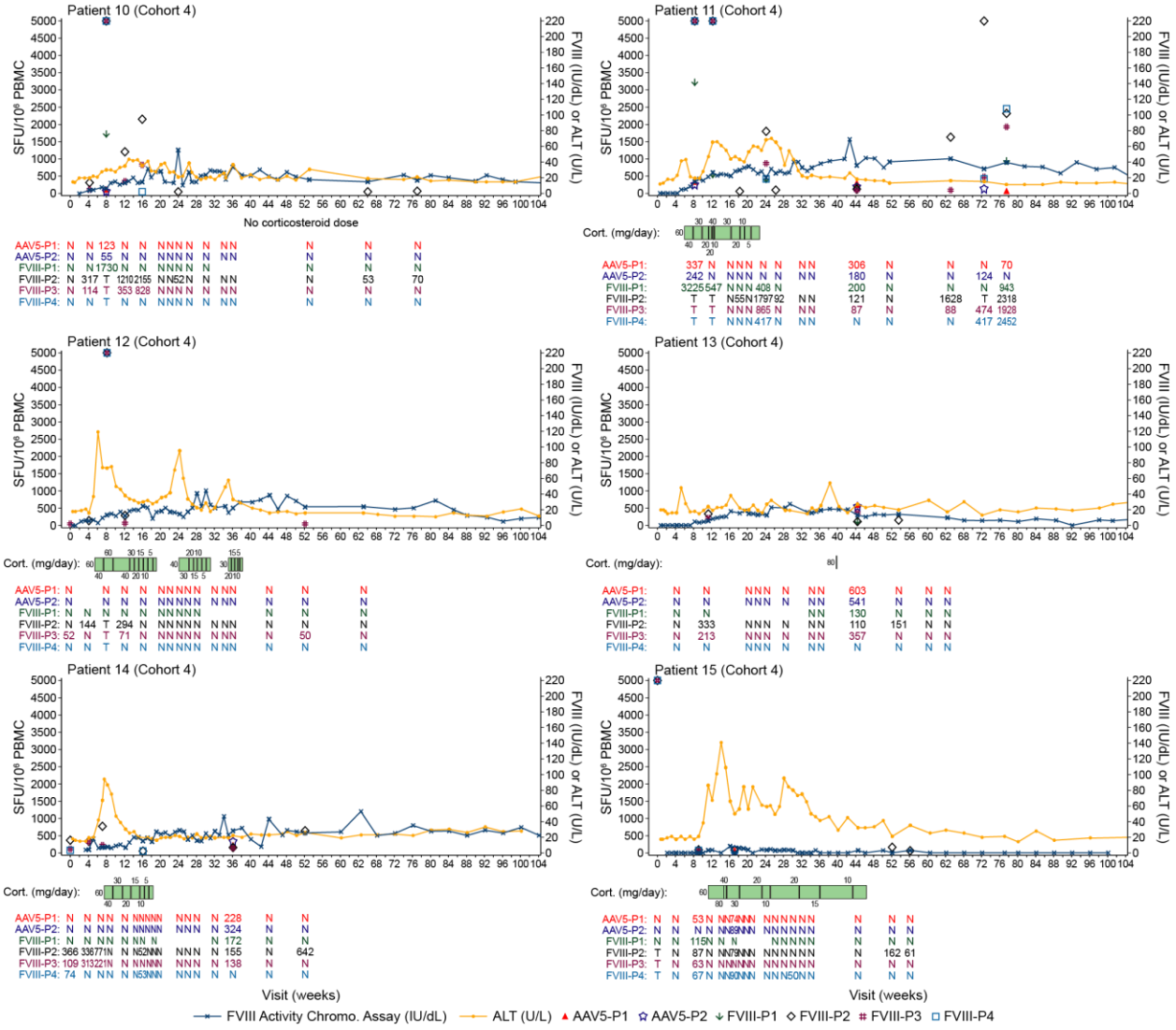

Figure S3. Analyte secretion in response to AAV5 and FVIII-SQ peptide pools at a single time point in healthy donors. T cell responses were measured by the secretion of two different analytes (IFN-γ in panel A and TNF-α in panel B) and is expressed as SFU/10^6 total PBMC in 15 healthy donors stimulated with AAV5 and FVIII-SQ peptide pools. Overlapping peptide pools (15mers overlapping by 10aa) of AAV5 capsid protein VP1 and the vector transgene product (TG) were custom synthesized to >70% purity. Due to the size of the protein sequences, the AAV5 VP1 sequence (UniProt: Q9YIJ1) was split into 2 peptide pools (AAV5 pool 1 and AAV5 pool 2) and the transgene product sequence was split into 4 peptide pools (F8 pool 1, F8 pool 2, F8 pool 3, and F8 pool 4), with each pool containing approximately 70 peptides each.