

Supplementary Materials:

1. HPLC and MS spectra 2

1.1. Peptide-PEG SDS-PAGE 7

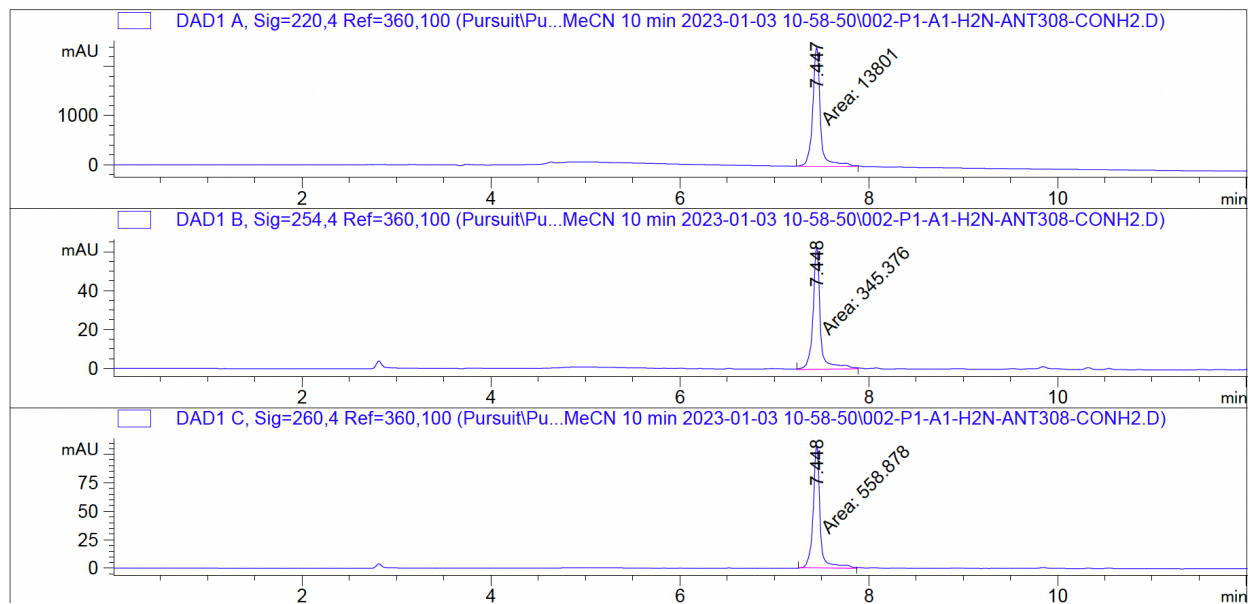
1.2. Additional Peptide Sequences..... 8

1.3. Plasma Stability T Cell Activation Study 9

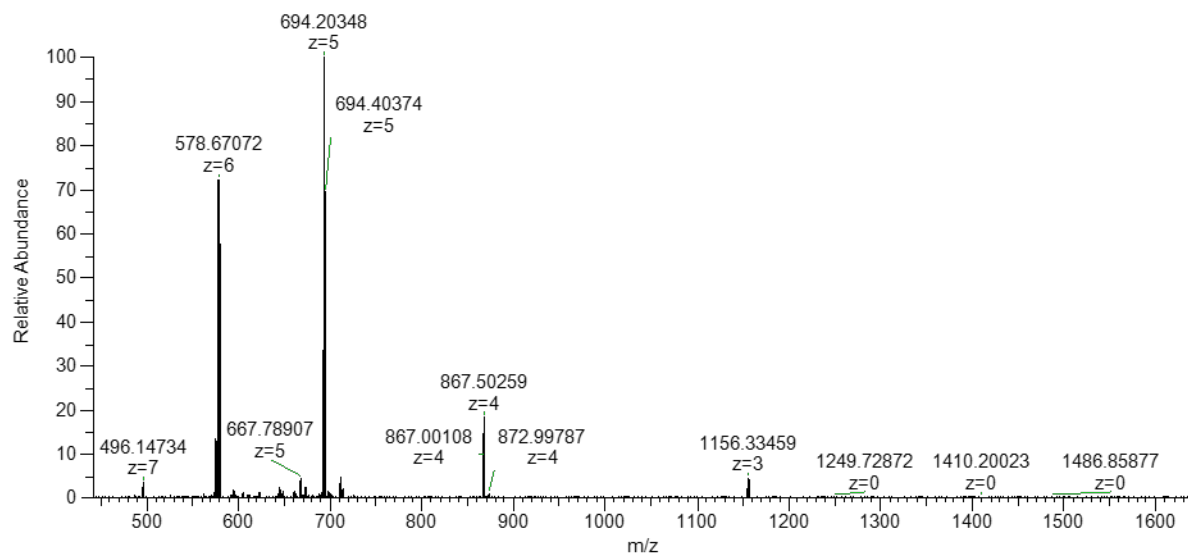
1. HPLC and MS Spectra

Peptide	MW	6M ⁶⁺ calc	6M ⁶⁺ exp	5M ⁵⁺ calc	5M ⁵⁺ exp	4M ⁴⁺ calc	4M ⁴⁺ exp	3M ³⁺ calc	3M ³⁺ exp
ANT308	3466.16	578.70	578.67	694.23	694.20	867.55	867.50	1156.39	1156.33
AcANT308	3505.98	585.71	585.67	702.66	702.60	878.07	878.00	1170.42	1170.33
ANT308K	3620.27	604.39	604.35	725.06	725.02	906.07	906.02	1207.76	1207.70
ANT308C1 3C17	3425.86	571.98	572.15	686.18	686.58	857.47	857.72	1142.96	1143.30
ANT308C1 3C17stp	3530.23	589.38	589.33	707.05	706.99	883.56	883.49	1177.75	1177.65

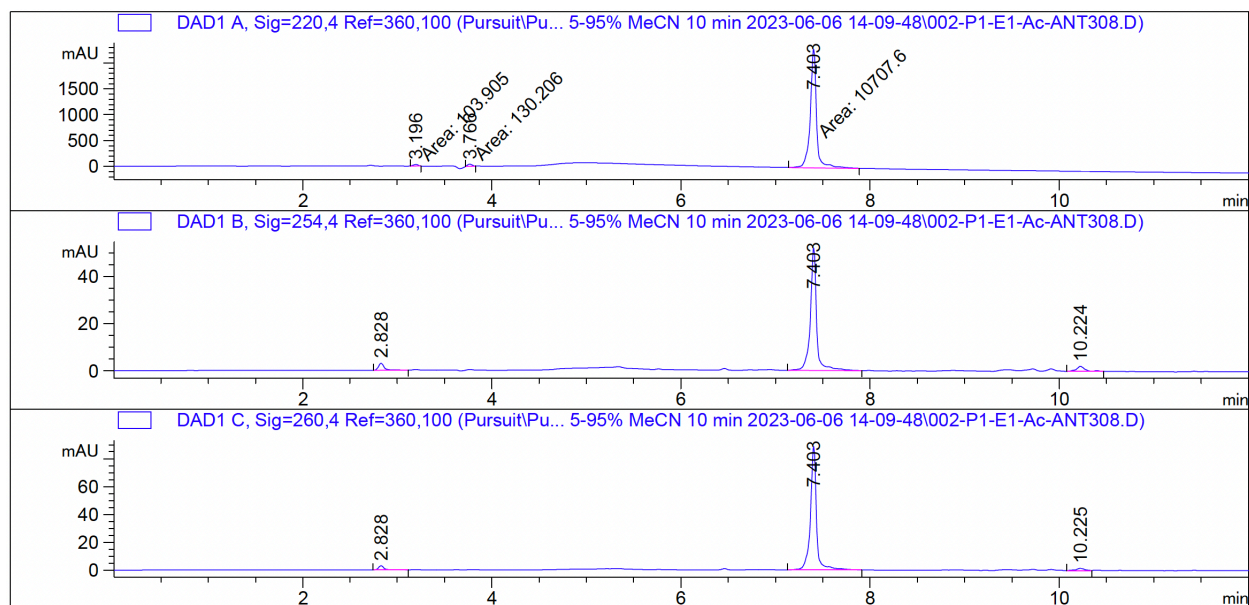
ANT308



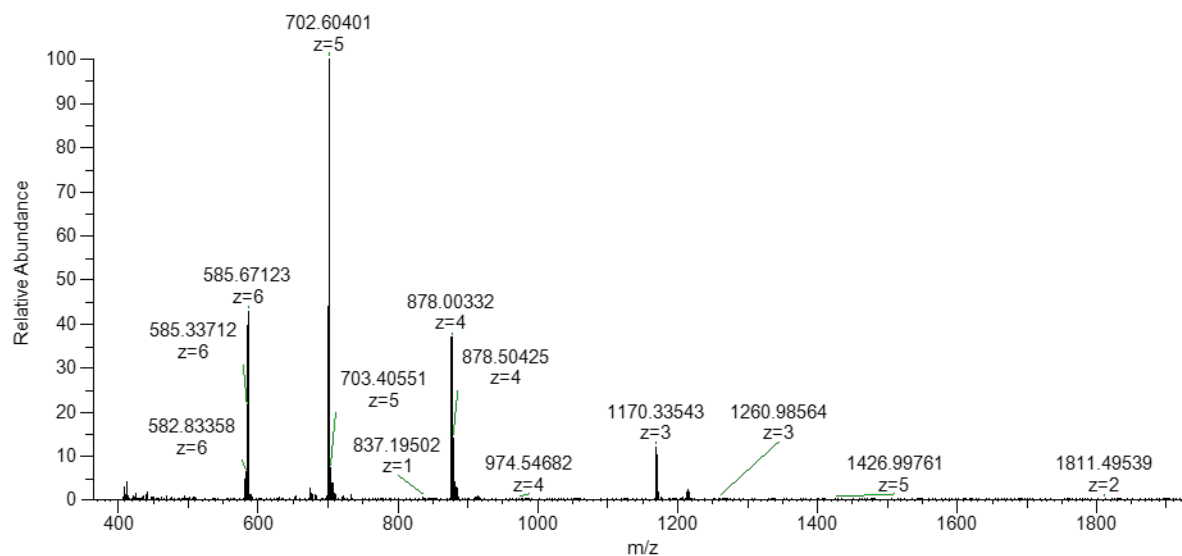
EX2995 #18-536 RT: 0.16-4.68 AV: 519 NL: 6.39E+007
T: FTMS + p ESI Full ms [300.0000-4000.0000]



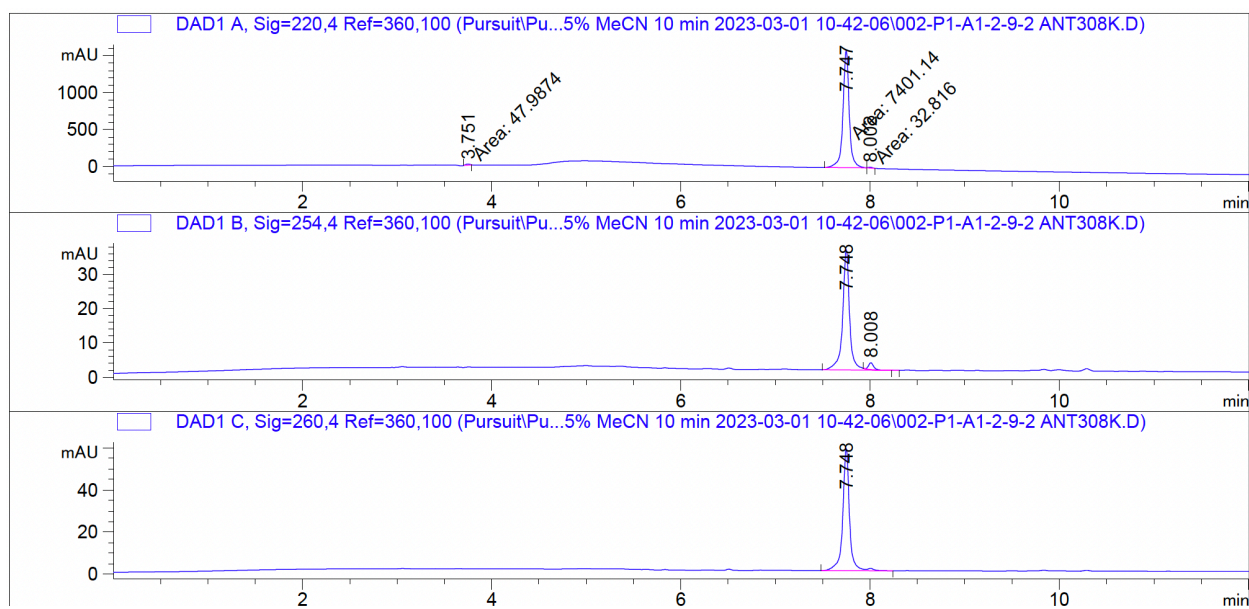
Ac-ANT308

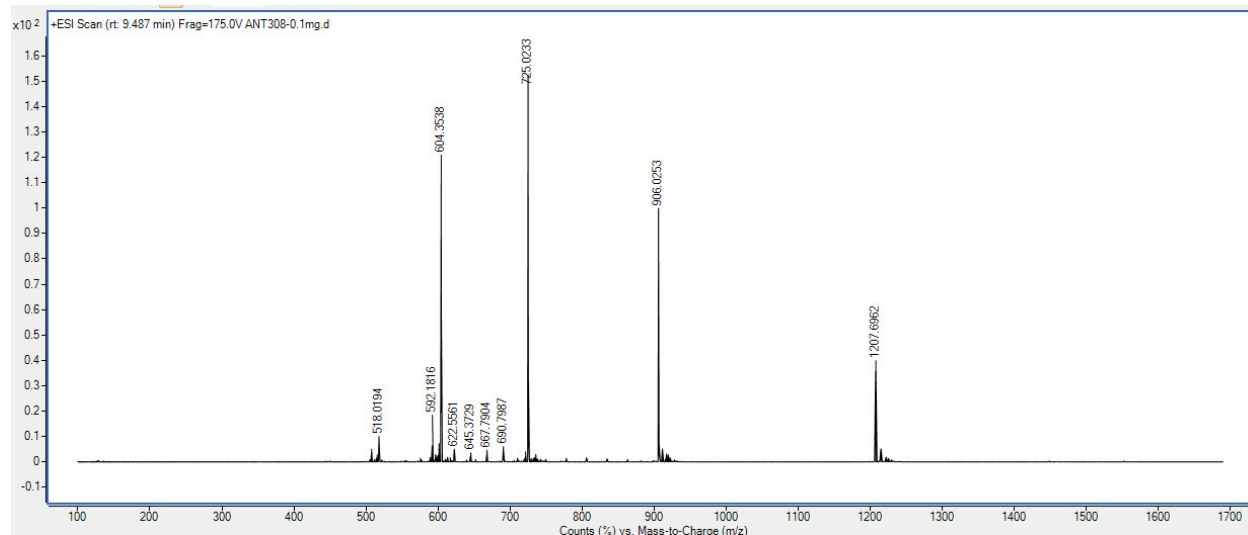


EX28423 #3-91 RT: 0.03-0.79 AV: 89 NL: 6.01E+007
T: FTMS + p ESI Full ms [400.0000-4000.0000]

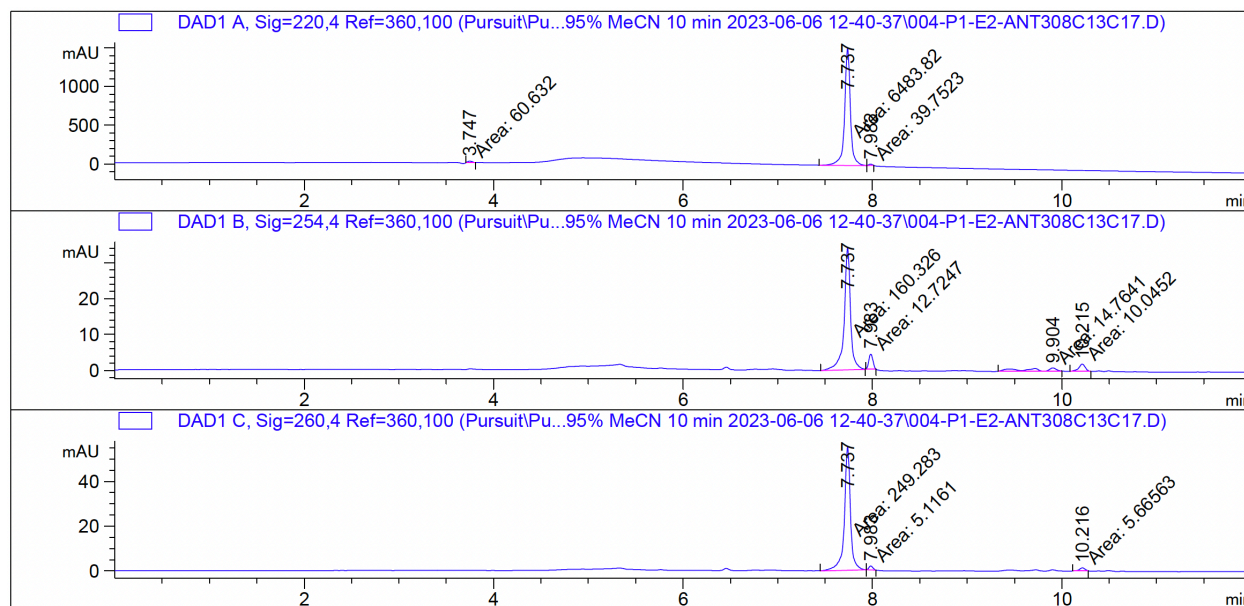


ANT308K_{N3}

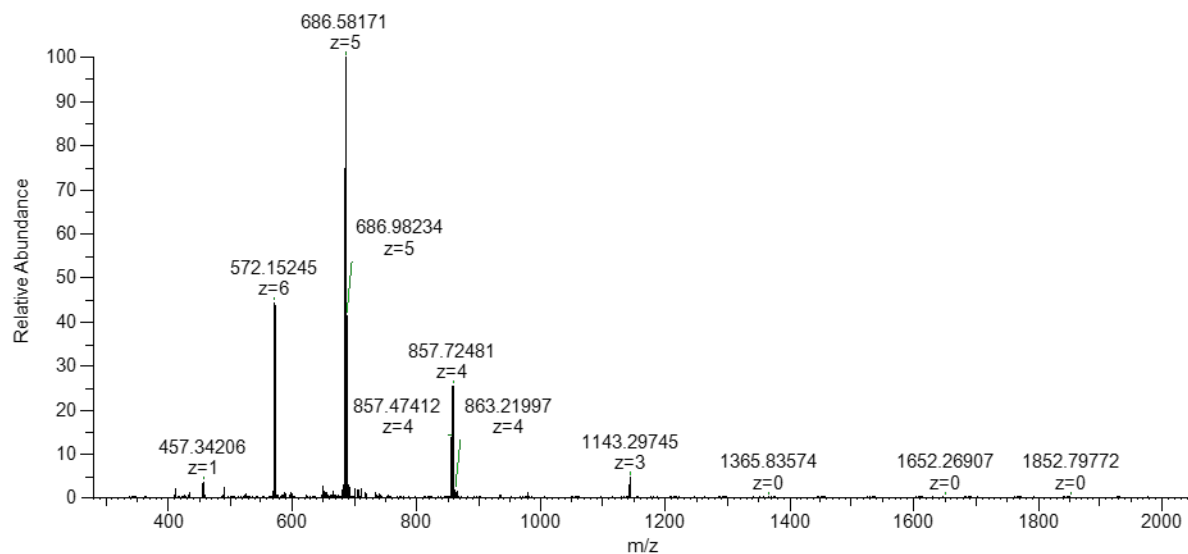




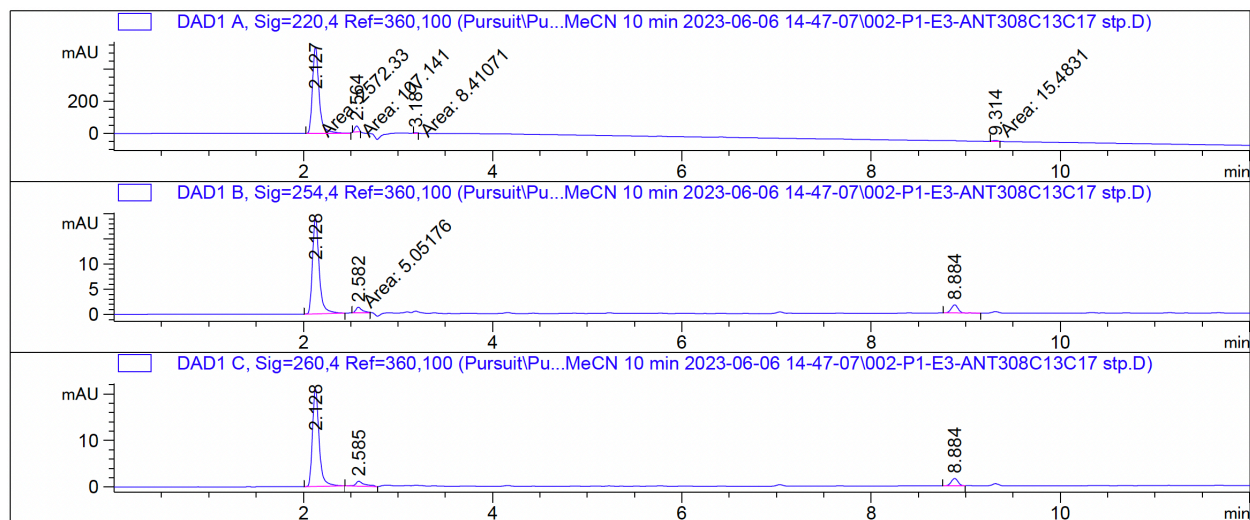
ANT308C13C17



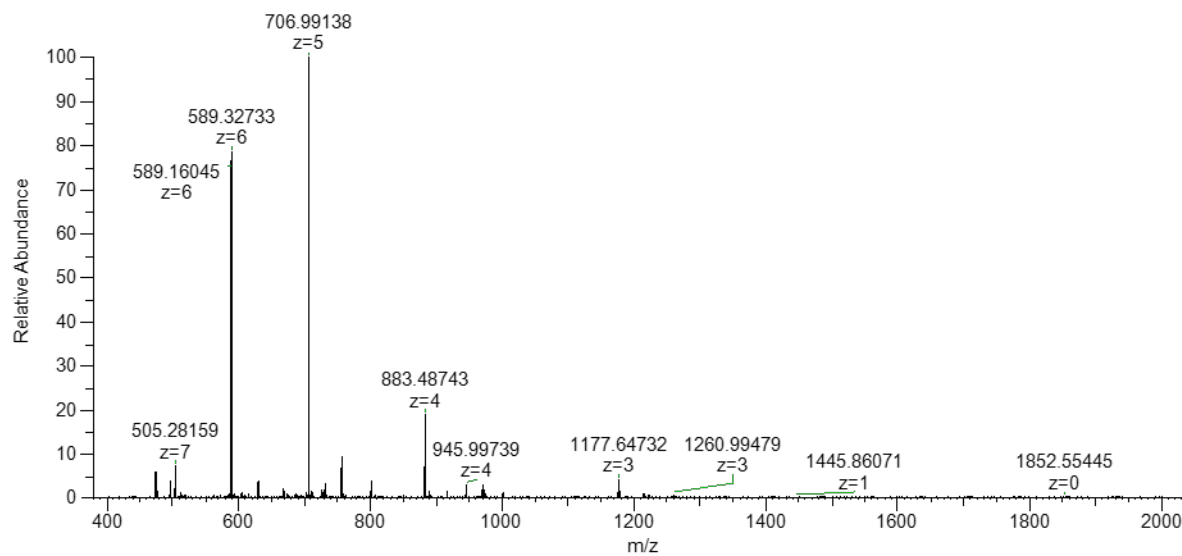
EX3248_20210908122621 #3-54 RT: 0.03-0.47 AV: 52 NL: 5.43E7
T: FTMS + p ESI Full ms [300.0000-2000.0000]



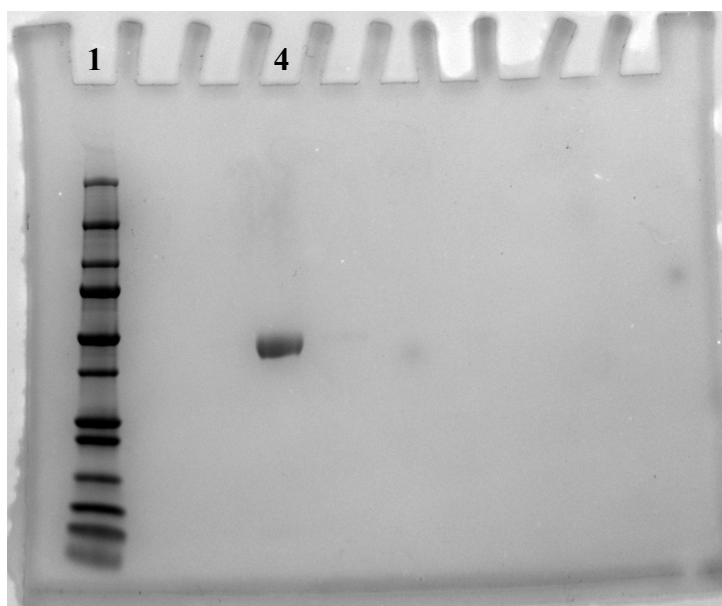
ANT308C13C17 stp



EX3291 #3-126 RT: 0.03-1.1 AV: 124 NL: 3.92E7
T: FTMS + p ESI Full ms [400.0000-2000.0000]



1.1 Peptide-PEG Analysis and Characterization



Lane	Sample
1	ladder
4	ANT308-PEG

1.2 Additional Peptide Sequences

Peptide controls used in the murine studies were purchased from RS Synthesis (Louisville, KY):

Fully SCRAM: YDDHTKLKINTARLVSAQNVFNMLKYR-CONH₂

1.3 Plasma Stability T Cell Activation Study

200 μ M ANT308 or 200 μ M ANT308K-PEG were mixed with healthy donors' plasma at 1:1 ratio, followed by incubation at 37°C. Aliquots (150 μ L) were collected at 24 hrs, 48 hrs, 72 hrs, and 96 hrs and stored at -80°C before pooled human T cells activation assay. Assays were performed with three different donor's plasma.

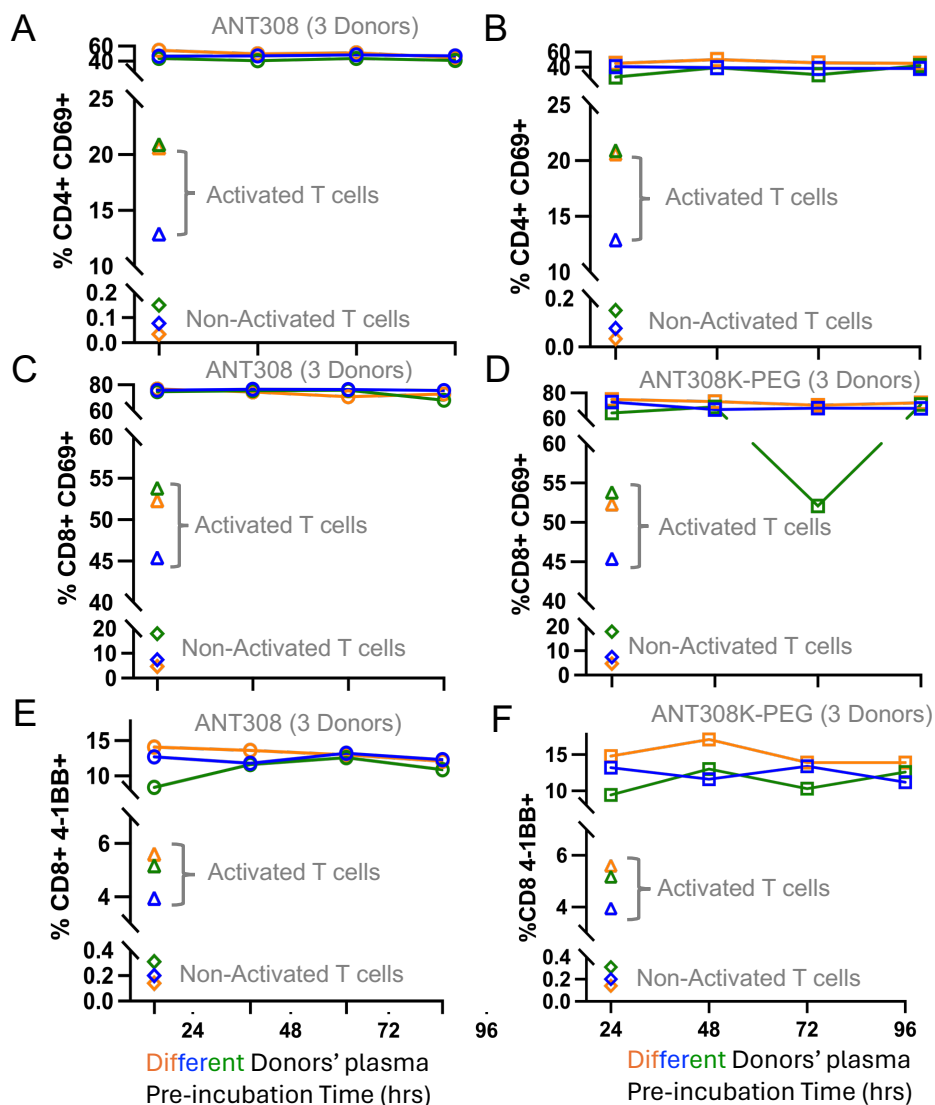


Figure S1A-F: Plasma stability and human T cells activation. After incubation with healthy donors' plasma at 37°C for 24hrs, 48hrs, 72hrs, and 96hrs. 10 μ M ANT308 (A,C,E) or 10 μ M ANT308K-PEG (B,D,F) were directly mixed with isolated & pooled human T cells with the

presence of α CD3/CD28 activator and IL-2. 48hrs following activation, CD4 or CD9 T cell subsets were examined for CD69 and 4-1BB expressions.