Supplementary Table I. Overview of theoretical and measured molecular masses of F8-IL12p40 C175S mutants and deglycosylated variants investigated by LC-MS.

		theoretical mass[b] [Da]	measured mass[c] [Da]			differential mass [Da]				
Mutation	Name	m_{th}	m_1	m_2	m_3	m_1-m_{th}	m_2-m_{th}	m_3-m_{th}	m_2-m_1	m_3-m_1
C175S, N25Q	1Q	61503.9	65546.0	65707.2	-	4042.1	4203.3	-	161.2	-
C175S, N110Q	2Q	61503.9	65381.7	65706.9	-	3877.8	4203.0	-	325.2	-
C175S, N198Q	3Q	61503.9	63843.3	-	-	2339.4	-	-	-	-
C175S, N298Q	4Q	61503.9	63193.4	63355.6	-	1689.5	1851.7	-	162.2	-
C175S, N25Q, N110Q, N198Q	123Q	61532.0	63873.3	-	-	2341.3	-	-	-	-
C175S, N25Q, N110Q, N298Q	124Q	61532.0	63223.8	63384.5	-	1691.8	1852.5	-	160.7	-
C175S, N25Q, N198Q, N298Q	134Q	61532.0	61521.4	-	-	-10.6	-	-	-	-
C175S, N25Q, N110Q, N198Q, N298Q	1234Q	61546.0	61539.0	-	-	-7.0	-	-	-	-
C175S	C175S	61489.9	65539.7	65702.4	-	4049.8	4212.5	-	162.7	-
C175S; N198D[a], N298D[a]	C175S deglyc	61491.9	61483.4	-	-	-8.5	-	-	-	-
C175S, N198Q, N298D[a]	3Q deglyc	61504.9	61500.5	63851.0	-	-4.4	2346.1	-	2350.5[d]	-
C175S, N198D[a], N298Q	4Q deglyc	61504.9	61498.3	63200.9	63362.9	-7.6	1696.0	1858.0	1703.6[d]	1865.6[d]

[a] expected Asn-to-Asp transition during PNGase F reaction
[b] molecular mass based on amino acid sequence
[c] only peaks > 20% signal intensity included
[d] used to elucidate putative glycan structures

Supplementary Information

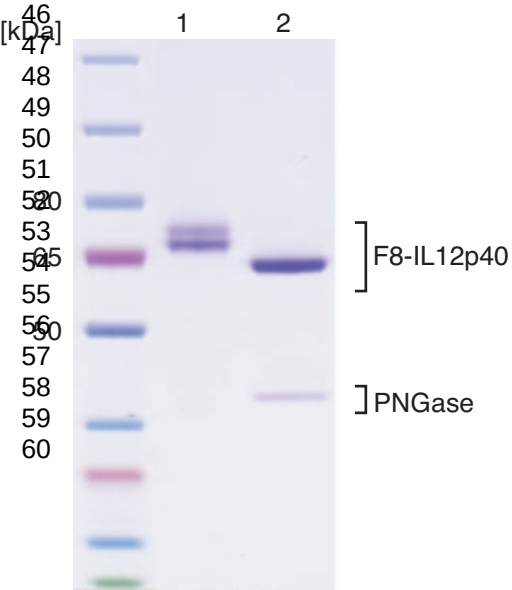
SUPPLEMENTARY FIGURE 1: SDS-PAGE analysis of 1) F8-IL12p40 C175S and 2) enzymatically deglycosylated F8-IL12p40 C175S after denaturation and reduction.

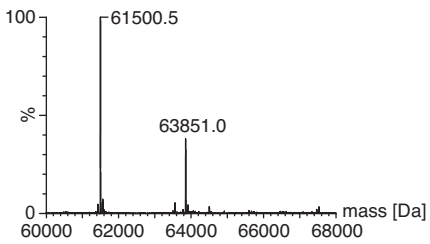
SUPPLEMENTARY FIGURE 2: ESI/LC-MS mass spectra of F8-IL12p40 C175S mutants 3Q (N198Q) and 4Q (N298Q) after partial deglycosylation with PNGase F and putative N-glycan structures predicted from the observed mass differences.

SUPPLEMENTARY FIGURE 3: Neuraminic acid (NeuNAc) quantification of F8-IL12p40 C175S variants and corresponding calibration curve.

SUPPLEMENTARY FIGURE 4A: DNA Sequences of F8-IL12p40 C175S and the mutants 1Q, 2Q, 3Q.

SUPPLEMENTARY FIGURE 4B: DNA Sequences of F8-IL12p40 C175S mutants 4Q, 123Q, 124Q and 134Q.

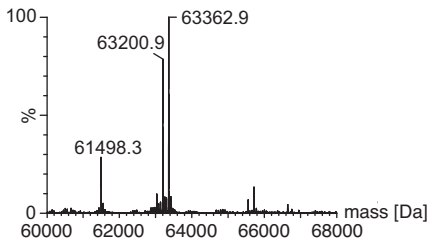




3Q deglyc.

m_1 = 61500.5 Da: no glycan $m_1 - m_{th}$ = -3.4 Da

m_2 = 63851.0 Da: N298 $m_2 - m_1$ = 2350.5 Da

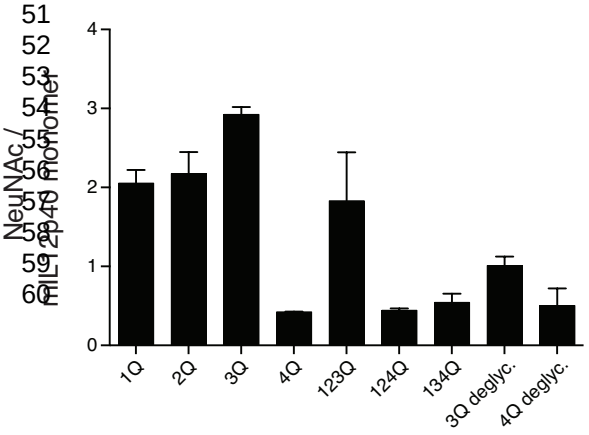


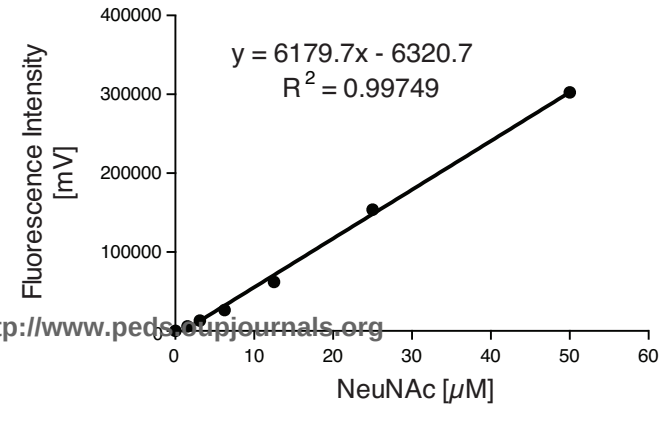
4Q deglyc.

m_1 = 61498.3 Da: no glycan $m_1 - m_{th}$ = -5.6 Da

m_2 = 63200.9 Da: N198 $m_2 - m_1$ = 1702.6 Da

m_3 = 63362.9 Da: N198 $m_3 - m_1$ = 1864.6 Da





F8-IL12p40 C175S: NheI – signal peptide – IL12p40C175S – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI

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1C: NheI – signal peptide – IL12p40C175S1g – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI

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2C: NheI – signal peptide – IL12p40C175S2g – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI

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3C: NheI – signal peptide – IL12p40C175S3g – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI

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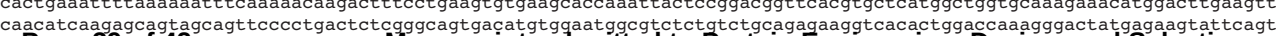

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1345: NheI – signal peptide – IL12p40C175S134g – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI