

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

Mutation	Name	theoretical mass <sup>b</sup> [Da]	measured mass <sup>c</sup> [Da]			differential mass [Da]				
			m <sub>1</sub>	m <sub>2</sub>	m <sub>3</sub>	m <sub>1</sub> -m <sub>th</sub>	m <sub>2</sub> -m <sub>th</sub>	m <sub>3</sub> -m <sub>th</sub>	m <sub>2</sub> -m <sub>1</sub>	m <sub>3</sub> -m <sub>1</sub>
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 <sup>a</sup> , N298D <sup>a</sup>	C175S deglyc	61491.9	61483.4	-	-	-8.5	-	-	-	-
C175S, N198Q, N298D <sup>a</sup>	3Q deglyc	61504.9	61500.5	63851.0	-	-4.4	2346.1	-	2350.5 <sup>d</sup>	-
C175S, N198D <sup>a</sup> , N298Q	4Q deglyc	61504.9	61498.3	63200.9	63362.9	-7.6	1696.0	1858.0	1703.6 <sup>d</sup>	1865.6 <sup>d</sup>

<sup>a</sup> expected Asn-to-Asp transition during PNGase F reaction

<sup>c</sup> only peaks > 20% signal intensity included

<sup>b</sup> molecular mass based on amino acid sequence

<sup>d</sup> 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.

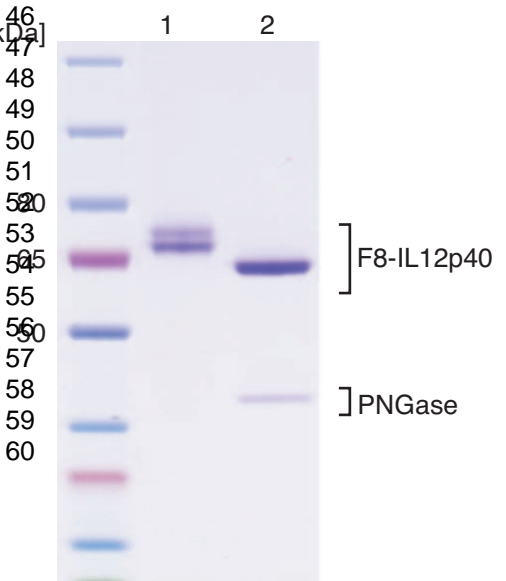
**SUPPLEMENTARY FIGURE 3:** Neuraminic acid (NeuNAc) quantification of F8-IL12p40 C175S variants and corresponding calibration curve.

**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.

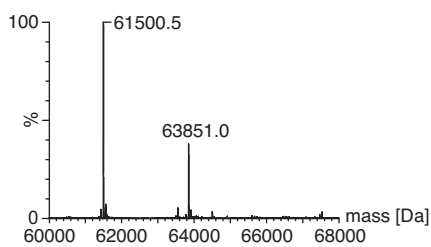
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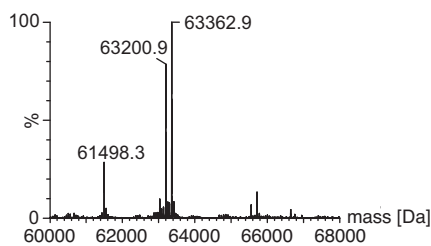




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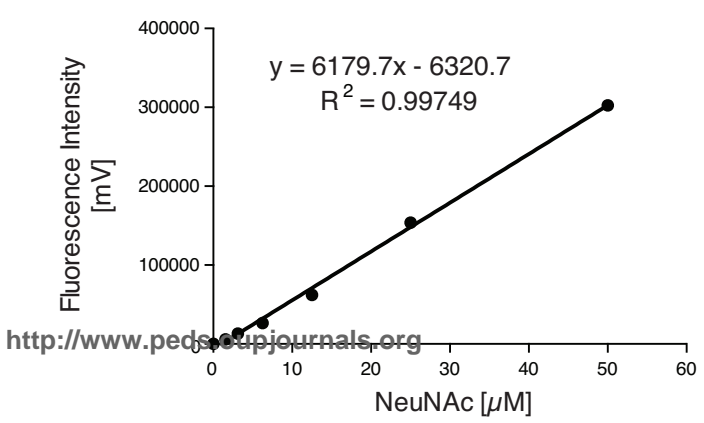
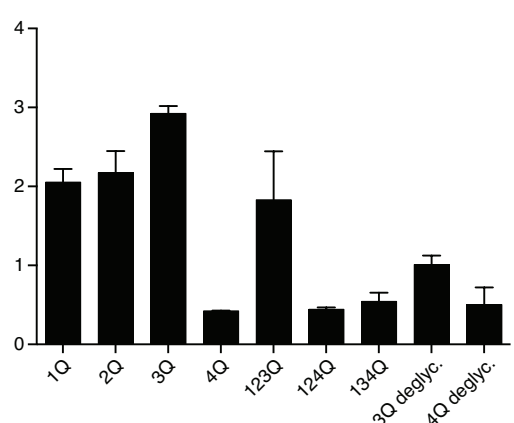
$m_1 = 61500.5$  Da: no glycan  $m_1 - m_{th} = -3.4$  Da  
 $m_2 = 63851.0$  Da: N298   $m_2 - m_1 = 2350.5$  Da



$m_1 = 61498.3$  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

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

GCTAGCGTCGACCATGGGCTGGAGCCTGATCCTCCTGTTCCCTCGTCGCTGTGGCTACAGGTGTGCACCTCGatgtgggagctggagaaagacgtttatgtttagaggtggactg
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TAG10CTGTTTACGATGAGCTGGGTCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGCTTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCG
GT11ACCATCTCCAGAGCAATTCGAAGAACACCGCTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAAGTACTCATTGTATCTTTT
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CC13CTTCAGGGCCAGTCAAGAGTGTAGCATGCCGTTTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGG
CAT14CCAGAGGTTTCACTGGCAGTGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCTGAAGATTTTGCAGTGTATTACTGTGTCAGCAGATGCGTGGTTCGGCC
GCC15ACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAATAGTGGAGCGGCCG

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

GCTAGCGTCGACCATGGGCTGGAGCCTGATCCTCCTGTTCCCTCGTCGCTGTGGCTACAGGTGTGCACCTCGatgtgggagctggagaaagacgtttatgtttagaggtggactg
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TC12ACTGGGGCCAGGAACTGGTCACCGTCTCGAGTGGCGGTAGCGGAGGGGAAATGTGTTGACGACAGCTCCAGGCACCTGTCTTGTCTCCAGGGAAAGAGCCAC
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CAT14CCAGAGGTTTCACTGGCAGTGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCTGAAGATTTTGCAGTGTATTACTGTGTCAGCAGATGCGTGGTTCGGCC
GCC15ACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAATAGTGGAGCGGCCG

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

GCTAGCGTCGACCATGGGCTGGAGCCTGATCCTCCTGTTCCCTCGTCGCTGTGGCTACAGGTGTGCACCTCGatgtgggagctggagaaagacgtttatgtttagaggtggactg
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GCC15ACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAATAGTGGAGCGGCCG

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

GCTAGCGTCGACCATGGGCTGGAGCCTGATCCTCCTGTTCCCTCGTCGCTGTGGCTACAGGTGTGCACCTCGatgtgggagctggagaaagacgtttatgtttagaggtggactg
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1230: NheI – signal peptide – IL12p40C175S123g – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI

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GT16ACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAAGTACTCATTTGTATCTTTT  
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CC17TCTCTGAGGCCAGTCCAGAGTGTAGCATGCCCCTTTTACCTGCTGACCCAGCAGAACTGGCCAGGCTCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGG  
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1240: NheI – signal peptide – IL12p40C175S124g – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI

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CC31TCTCTGAGGCCAGTCCAGAGTGTAGCATGCCCCTTTTACCTGCTGACCCAGCAGAACTGGCCAGGCTCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGG  
CA32CCAGACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTCACCTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCCAGCAGATGCGTGGTCGGCC  
GCCGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAATAGTGAGCGGCCG

1340: NheI – signal peptide – IL12p40C175S134g – L1(BahmHI) - F8VH(L2)F8VL – Stop – NotI

GCCTAGCGTCGACCATGGGCTGGAGCCTGATCCTCCTGTTCCCTCGTCGCTGTGGCTACAGGTTGCACTCGatgtgggagctggagaaagacgtttatgtttagaggtggactg  
ga35ccgatgccccctggagaacagtgcagctcacctgtgacacgcctgaagaagatgacatcacctggacctcagaccagagacatggagtcataaggctctggaaagacct  
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AGCTGTTTACGATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCTGGAGTGGGCTTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGCCGG  
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GA48CTGGGGCCAGGGAACCTGGTACCGTCTCGAGTGGCGGTAGCGGAGGGGAAATTTGTTGACGCAGCTCCAGGCACCTCTCTTTGTCTCCAGGGGAAAGGCCACC  
CT49TCTGCAGGGCCAGTCCAGAGTGTAGCATGCCGCTTTTACCTGTTACCAGCAGAACTGGCCAGGCTCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGC  
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CC51GTTTCGGCCAAGGGACCAAGGTGGAAATCAAATAGTGAGCGGCCG