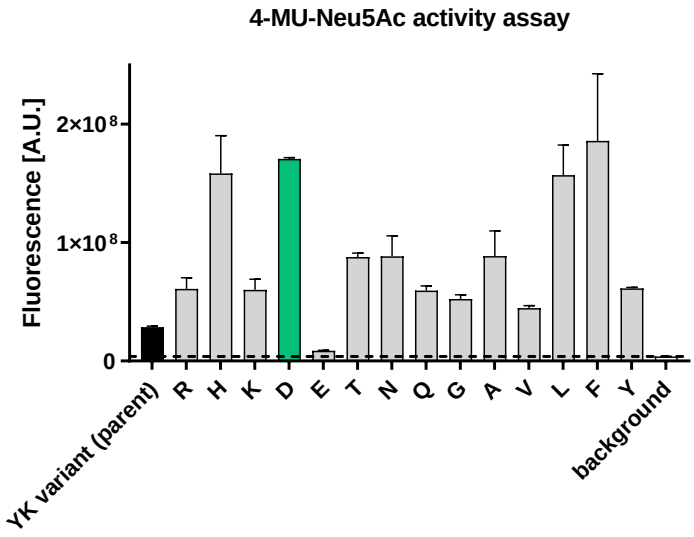
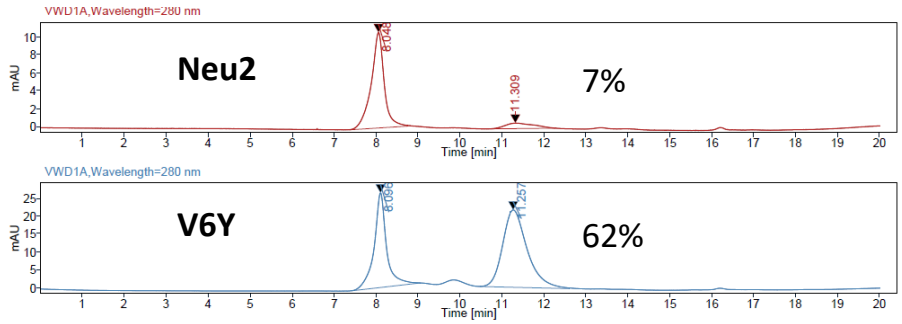
Supp Figure 1. N-terminal substitutions and impacts on enzyme activity and expression



N-terminal substitution	Protein-A yield (mg/L)	Mean enzyme activity (RFU)
Parent	17.3	2.87E+07
R	10.3	6.09E+07
H	3.0	1.58E+08
K	10.0	6.02E+07
D	**6.3**	**1.71E+08**
E	6.7	8.56E+06
S	-	-
T	4.7	8.79E+07
N	6.0	8.85E+07
Q	4.3	5.95E+07
G	6.7	5.23E+07
P	-	-
A	7.7	8.87E+07
V	8.7	4.46E+07
L	5.3	1.57E+08
F	3.3	1.86E+08
Y	0.7	6.13E+07

Top panel: Enzyme activity as measured by 4-MU-Neu5Ac substrate, using 0.5mM of substrate, and 2µg of each Neu2 variant per reaction. All variants were designed on parental Neu2 backbone with V6Y and I187K mutations "parent". Bottom table: Comparison of Protein-A expression yields of variants, N-terminal substitution of "D" achieved high enzyme activity, with moderately reduced expression yield.

Supp Figure 2. SEC-HPLC comparison of V6Y variant with Neu2 wildtype



SEC-HPLC chromatograms for Neu2 wildtype (Neu2-Fc) vs. Neu2 variant with V6Y mutation. Proteins were purified by 1-step Protein-A purification and analyzed on Superose 6 Increase 10/300 GL column (Cytiva). Estimated monomer percentages are indicated. Protein-A expression yield of Neu2 wildtype: 0.3mg/L. Protein-A expression yield of V6Y variant: 3.3mg/L.