Figure 1

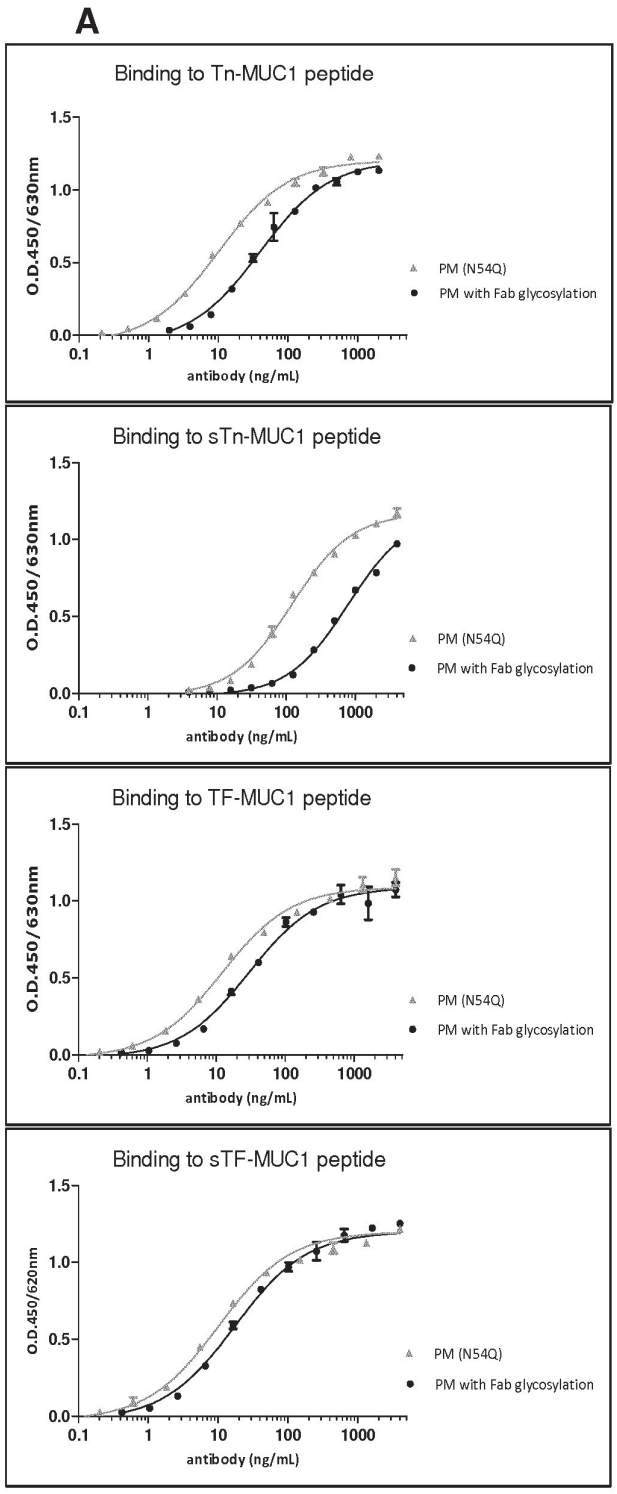
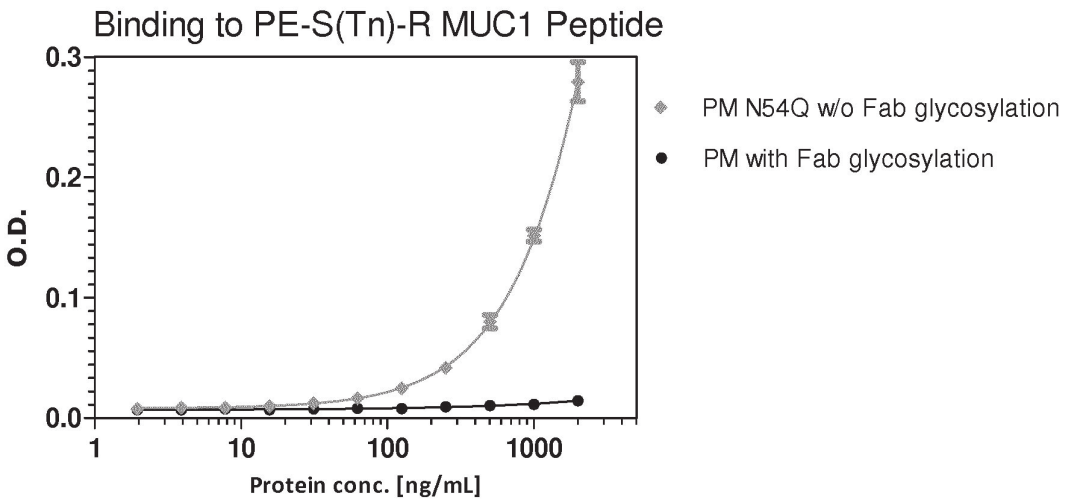

A
Binding to Tn-MUC1 peptide
O.D.450/630nm
1.5
1.0
0.5
0.0
0.1
1
10
100
1000
antibody (ng/mL)
PM (N54Q)
PM with Fab glycosylation
Binding to sTn-MUC1 peptide
O.D.450/630nm
antibody (ng/mL)
PM (N54Q)
PM with Fab glycosylation
Binding to TF-MUC1 peptide
O.D.450/630nm
antibody (ng/mL)
PM (N54Q)
PM with Fab glycosylation
Binding to sTF-MUC1 peptide
O.D.450/620nm
antibody (ng/mL)
PM (N54Q)
PM with Fab glycosylation

Figure 1 - continued

B



C

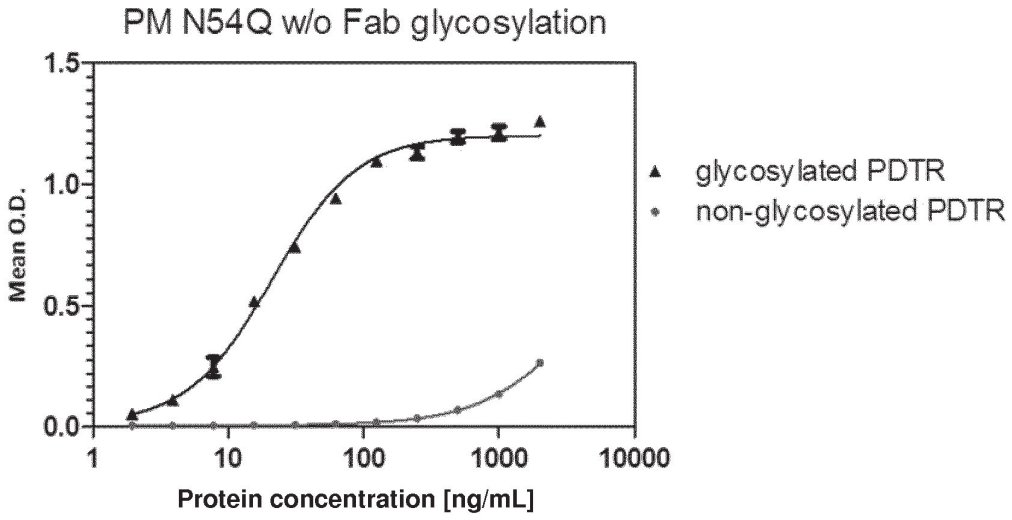


Figure 1 - continued

D

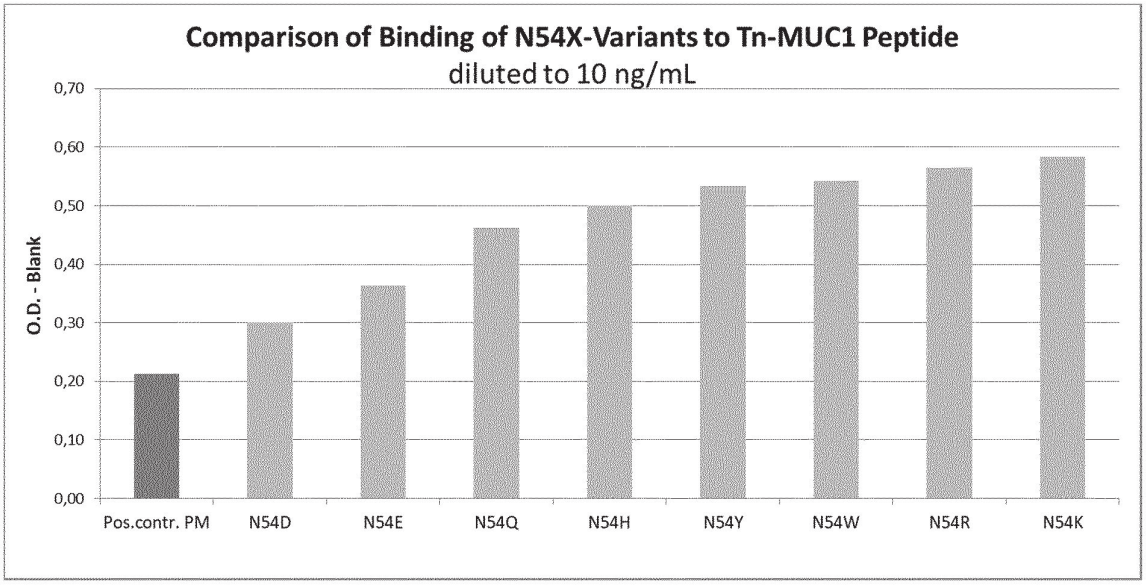


Figure 1 - continued

E

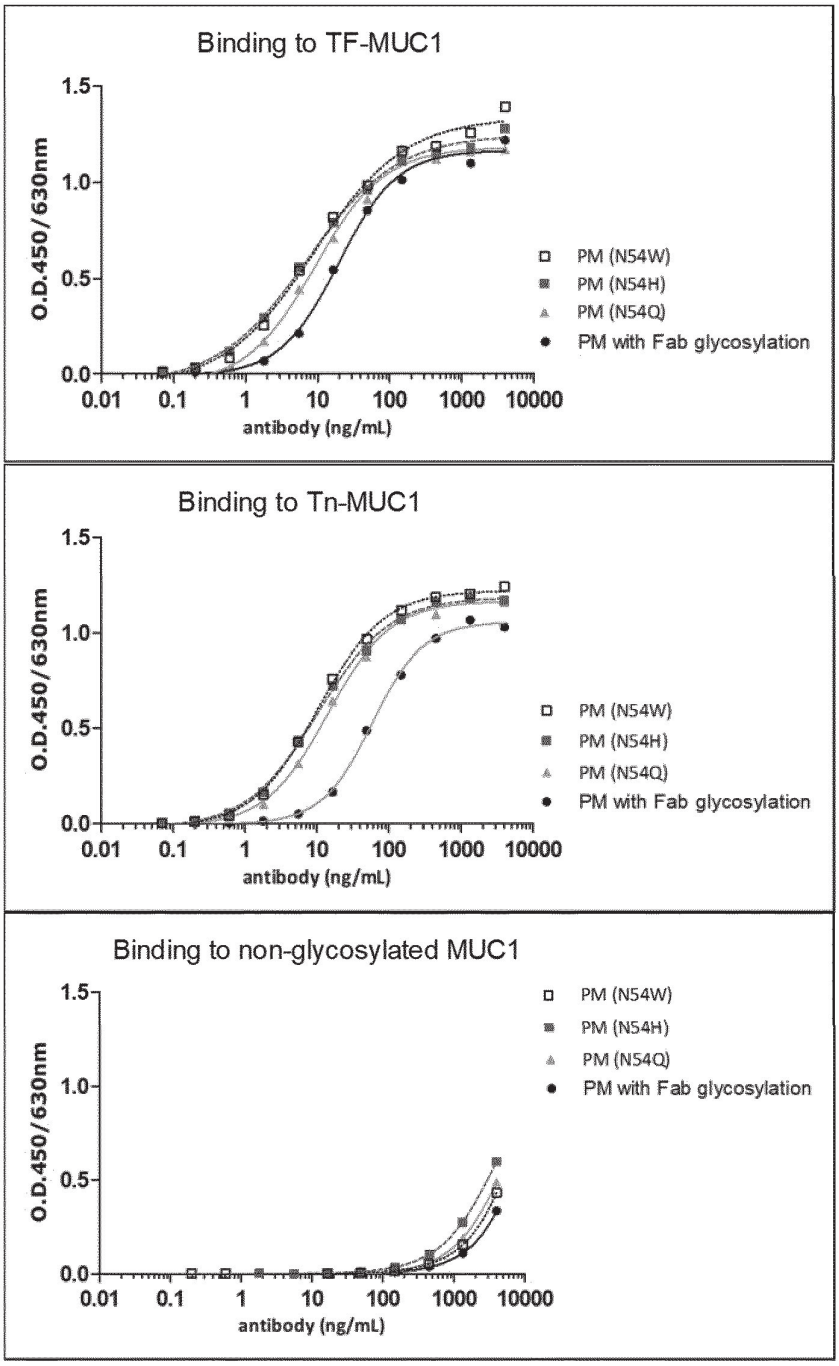


Figure 1 - continued

F

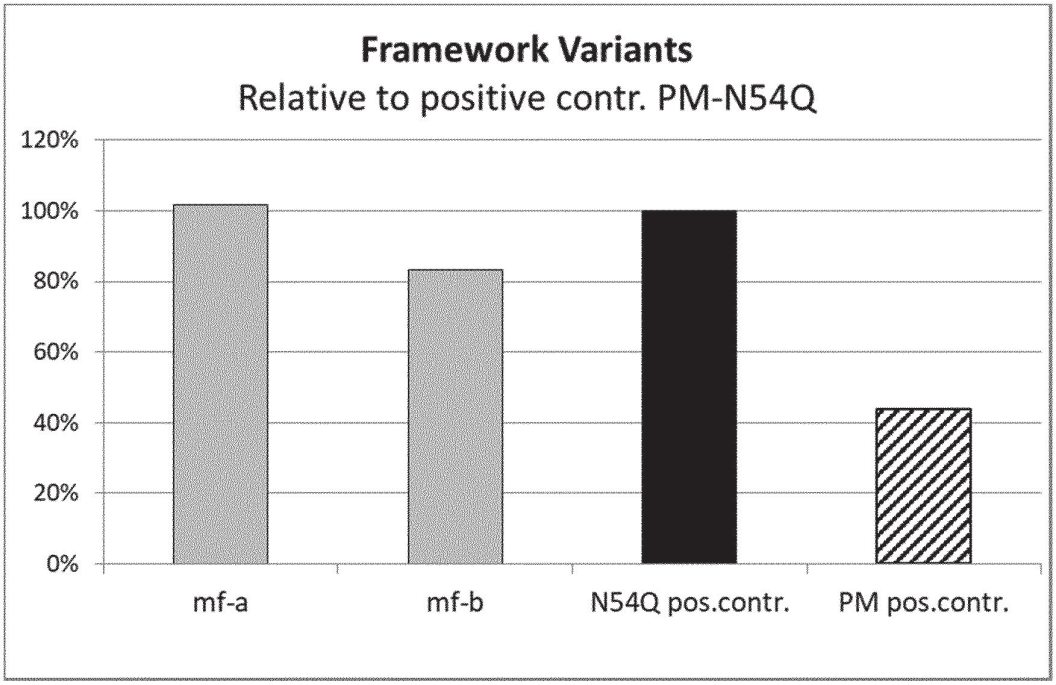


Figure 2

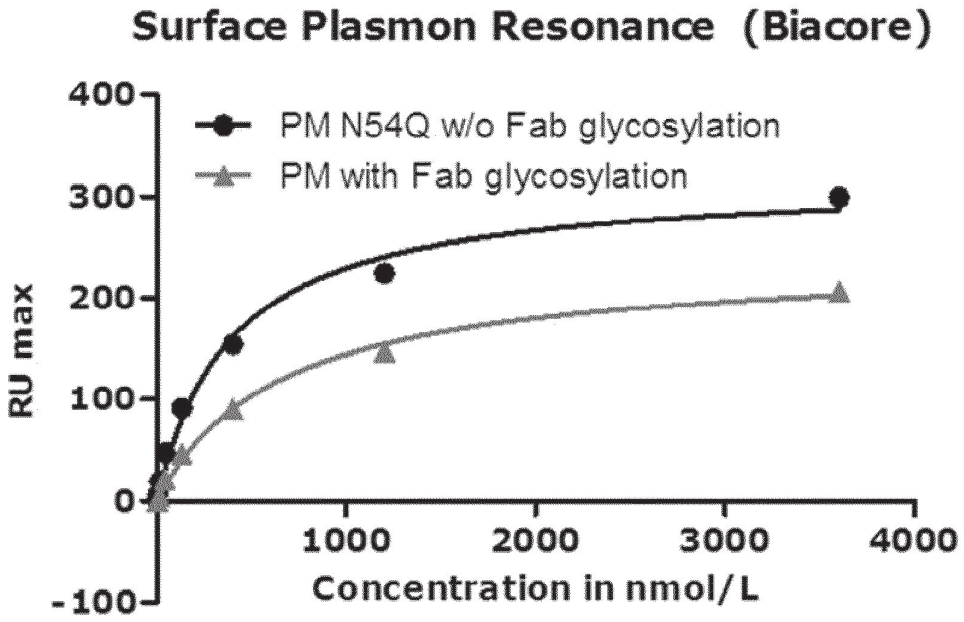


Figure 3

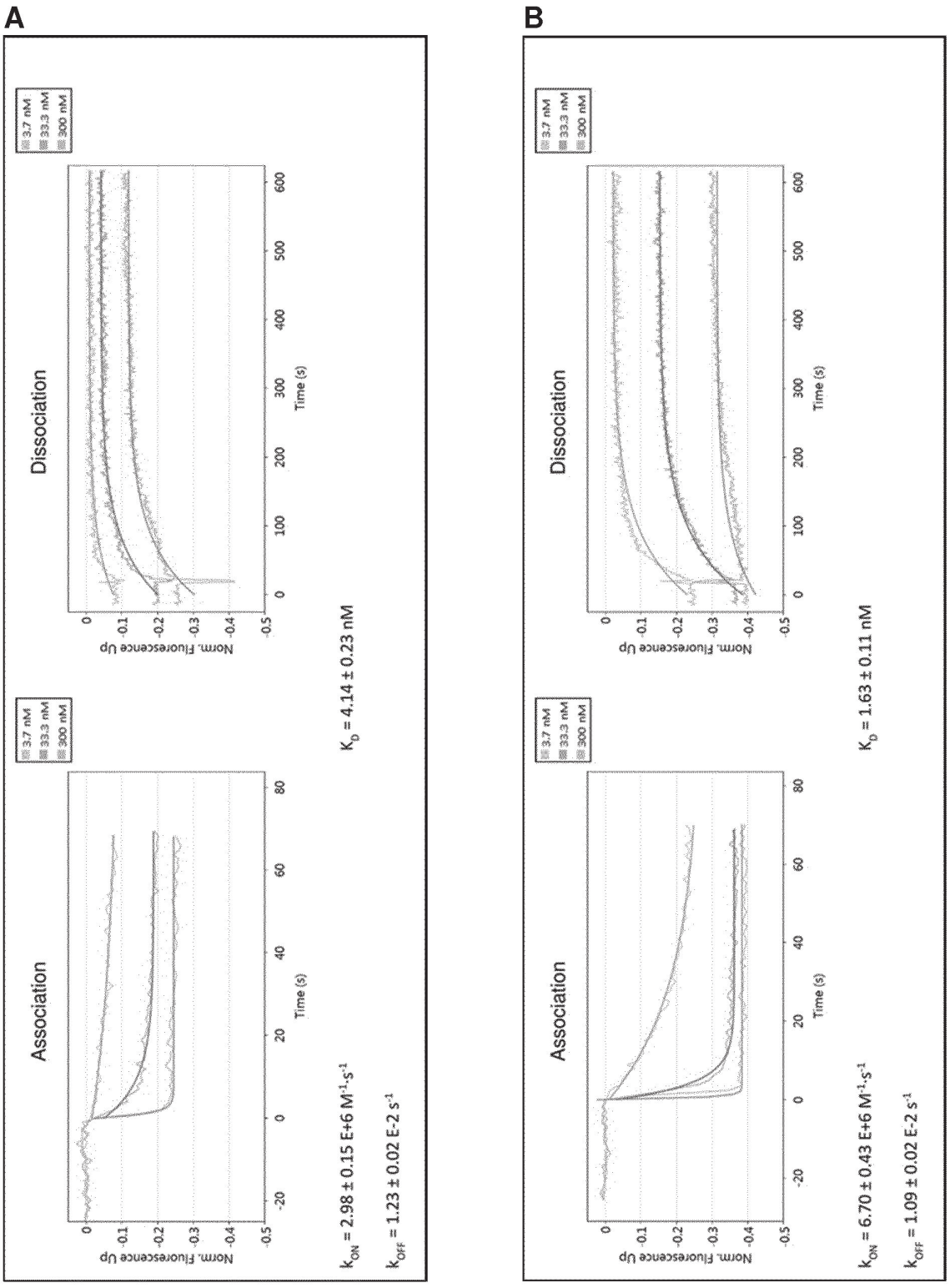

A
Association
Dissociation
3.7 nM
33.3 nM
300 nM
Norm. Fluorescence Up
Time (s)
K_D = 4.14 ± 0.23 nM
k_{ON} = 2.98 ± 0.15 E+6 M^{-1}·s^{-1}
k_{OFF} = 1.23 ± 0.02 E-2 s^{-1}
B
Association
Dissociation
3.7 nM
33.3 nM
300 nM
Norm. Fluorescence Up
Time (s)
K_D = 1.63 ± 0.11 nM
k_{ON} = 6.70 ± 0.43 E+6 M^{-1}·s^{-1}
k_{OFF} = 1.09 ± 0.02 E-2 s^{-1}

Figure 4

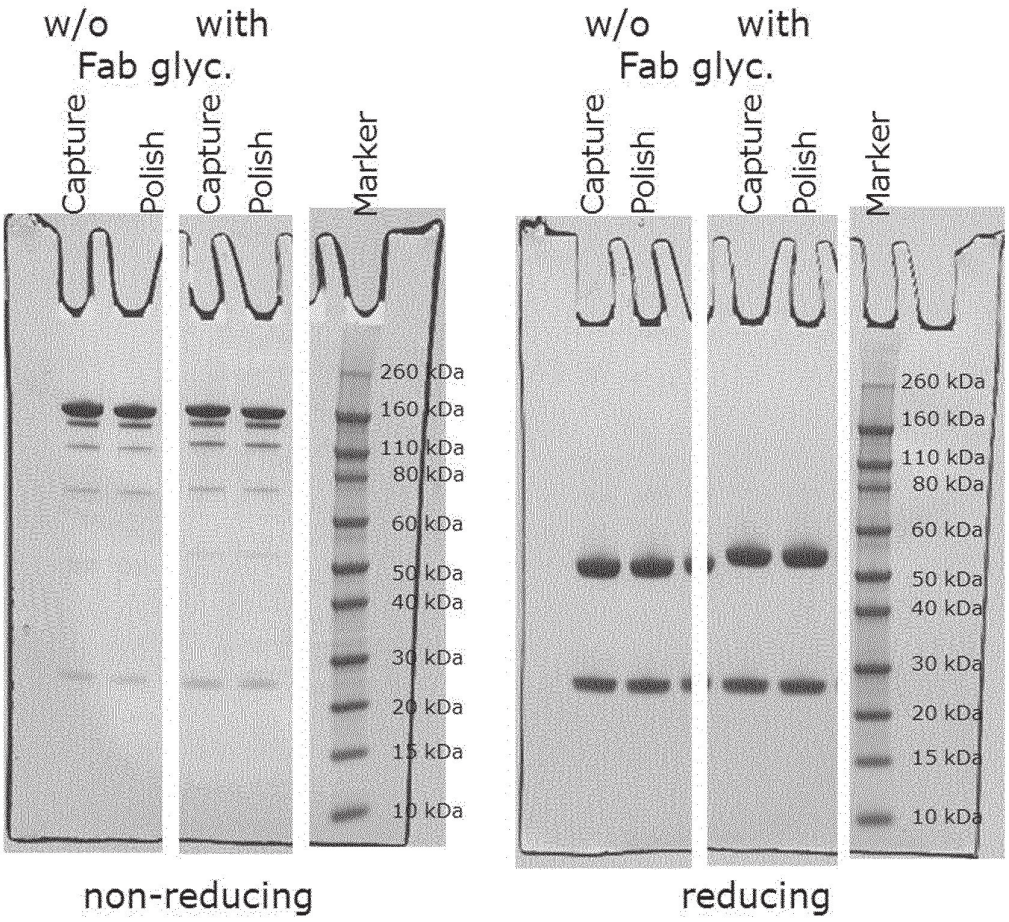


Figure 5

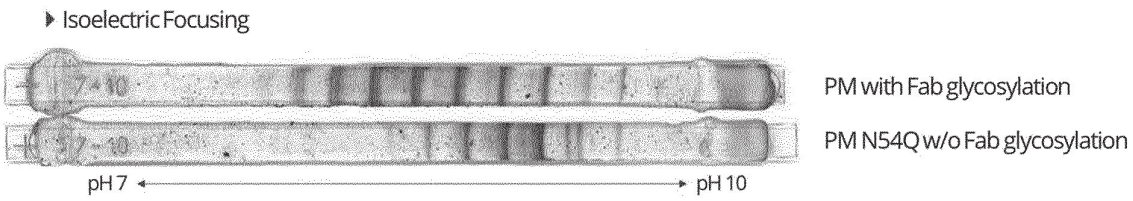


Figure 6

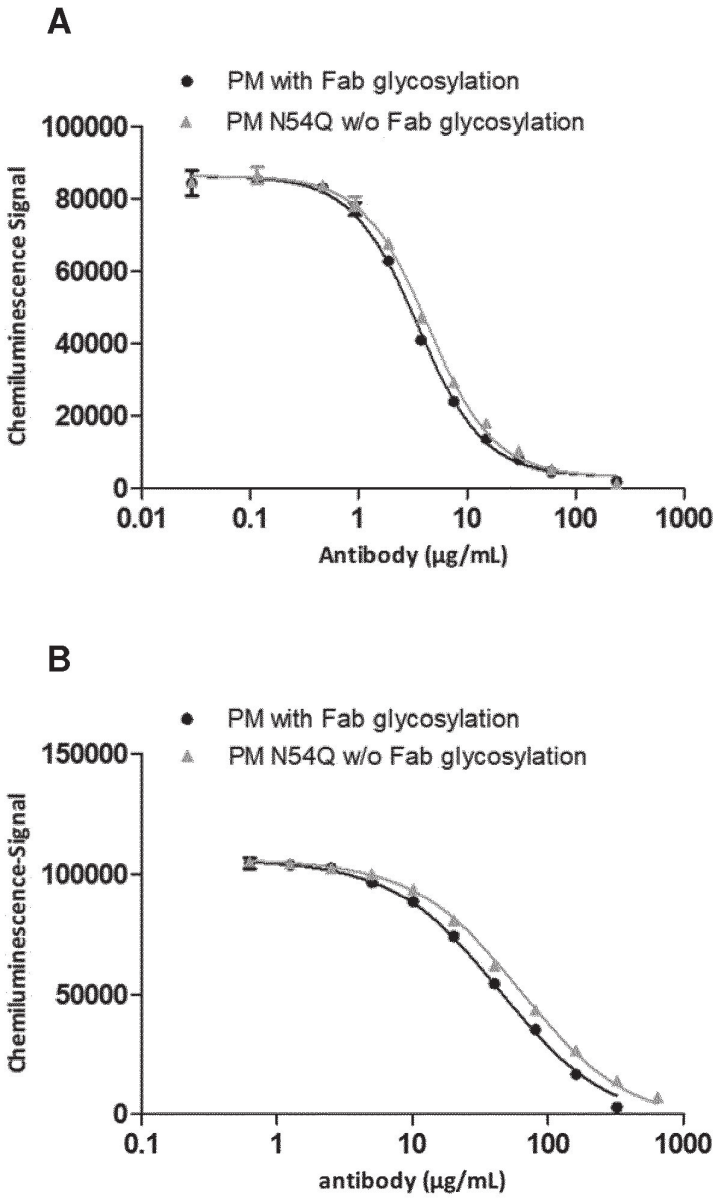
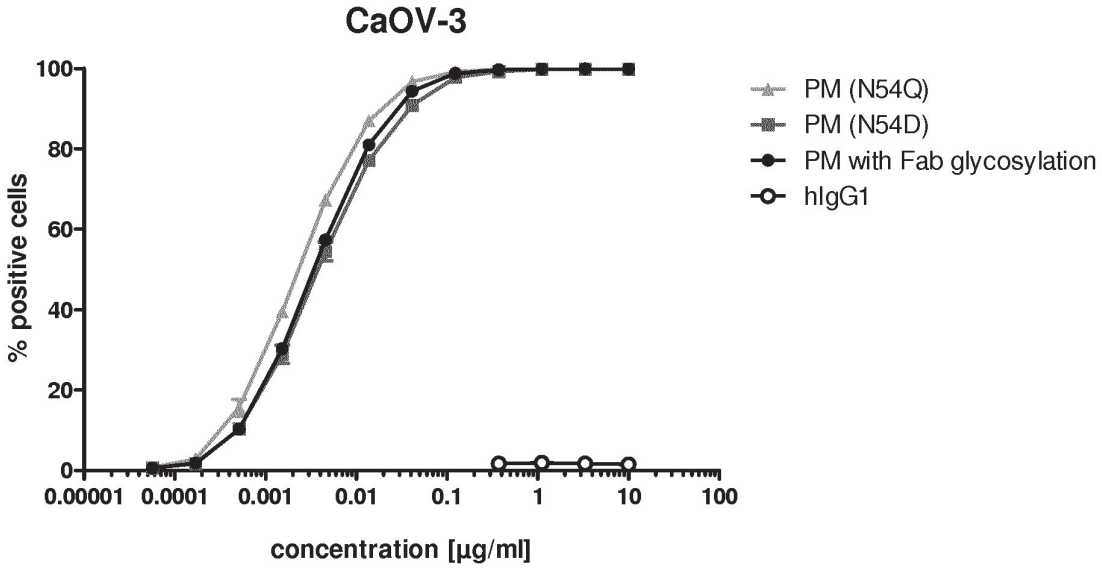

A
PM with Fab glycosylation
PM N54Q w/o Fab glycosylation
Chemiluminescence Signal
100000
80000
60000
40000
20000
0
0.01
0.1
1
10
100
1000
Antibody (µg/mL)
B
PM with Fab glycosylation
PM N54Q w/o Fab glycosylation
Chemiluminescence-Signal
150000
100000
50000
0
0.1
1
10
100
1000
antibody (µg/mL)

Figure 7

A


CaOV-3
% positive cells
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
concentration [µg/ml]
PM (N54Q)
PM (N54D)
PM with Fab glycosylation
hIgG1

B

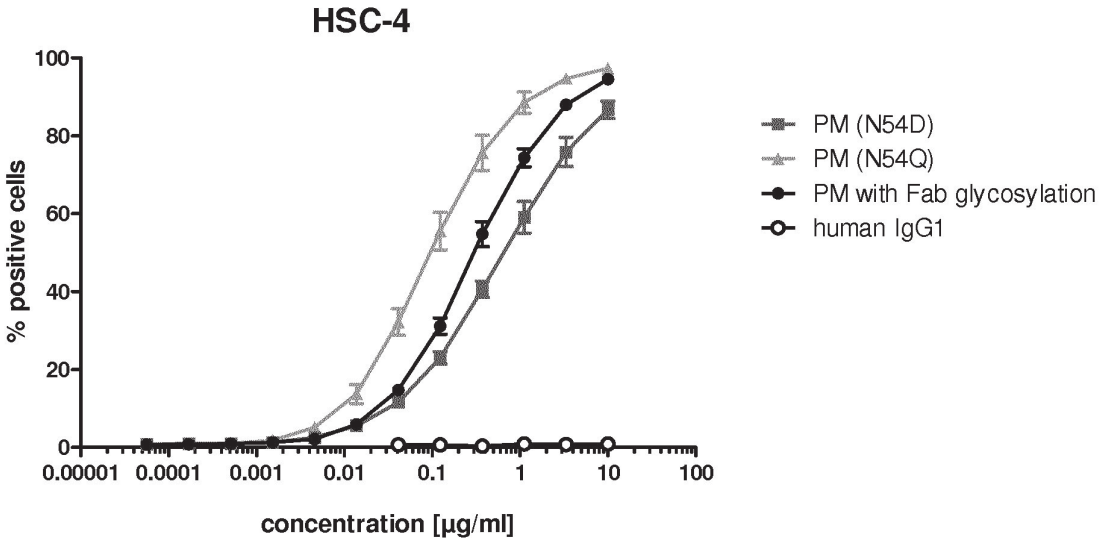

HSC-4
% positive cells
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
concentration [µg/ml]
PM (N54D)
PM (N54Q)
PM with Fab glycosylation
human IgG1

Figure 8

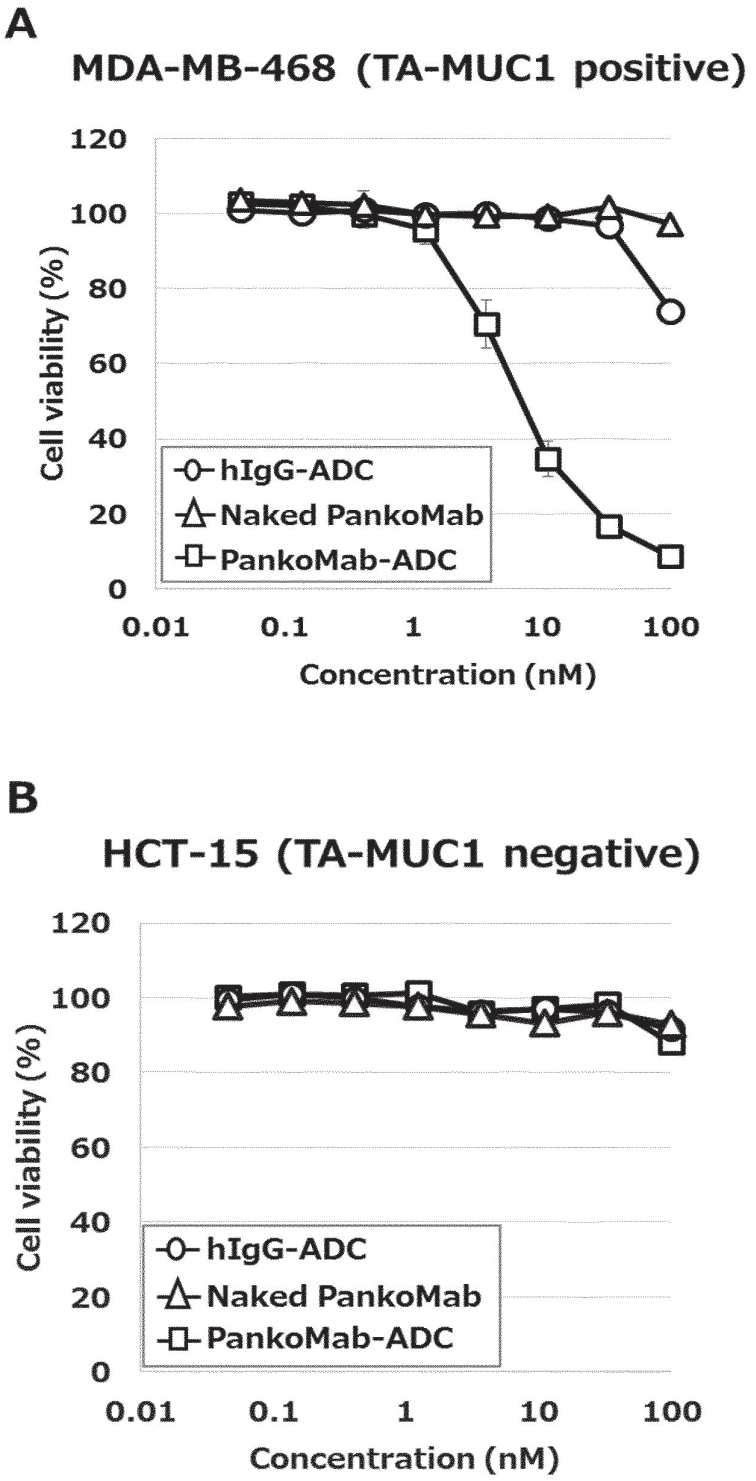

A
MDA-MB-468 (TA-MUC1 positive)
Cell viability (%)
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration (nM)
hIgG-ADC
Naked PankoMab
PankoMab-ADC
B
HCT-15 (TA-MUC1 negative)
Cell viability (%)
120
100
80
60
40
20
0
0.01
0.1
1
10
100
Concentration (nM)
hIgG-ADC
Naked PankoMab
PankoMab-ADC

Figure 8 – continued

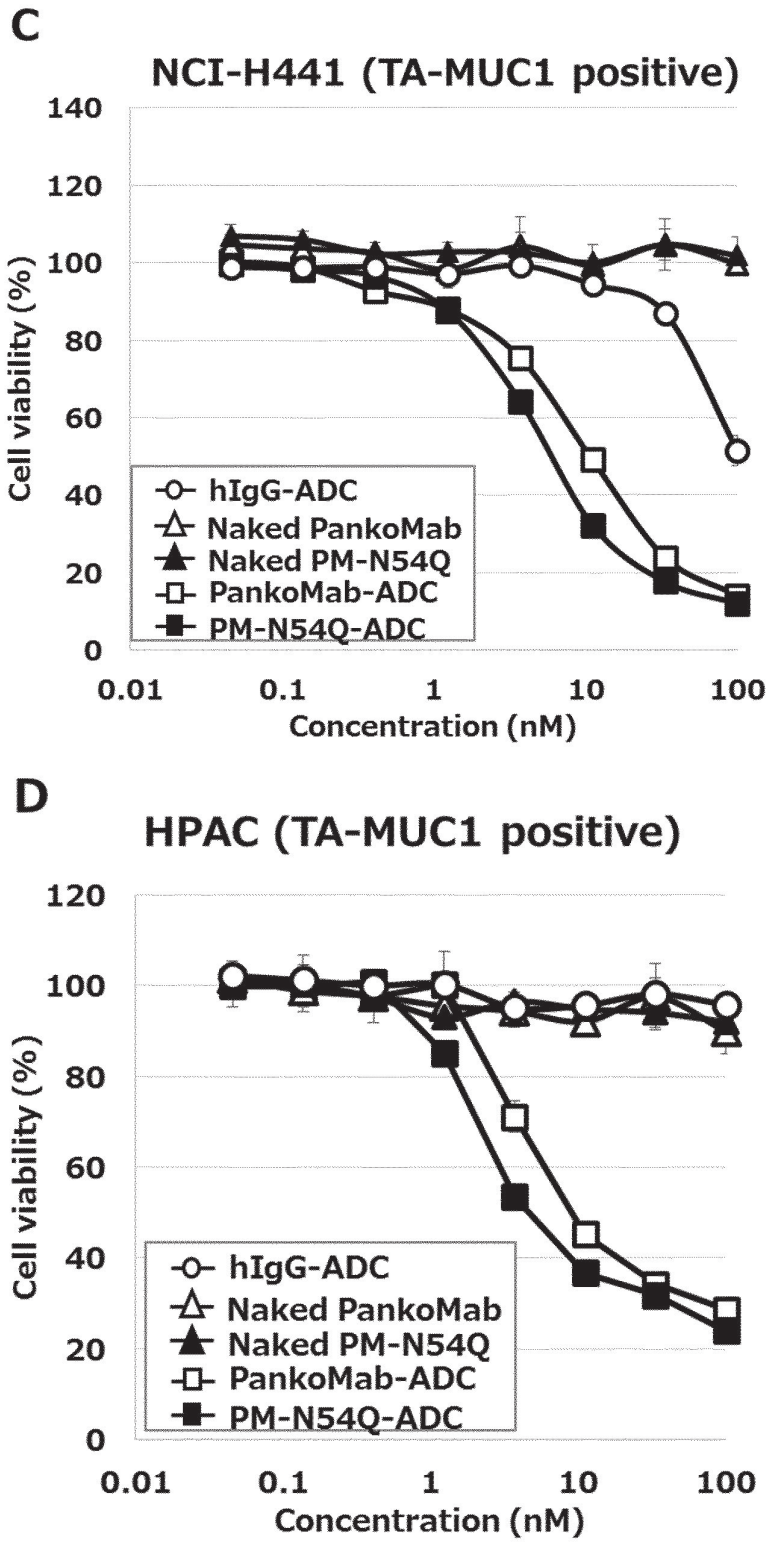


Figure 9

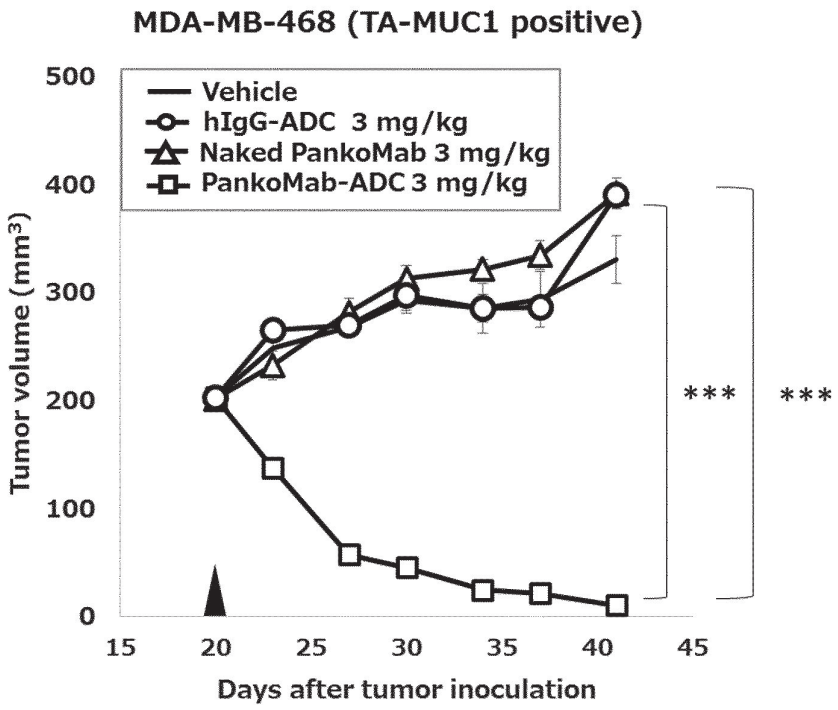


Figure 10

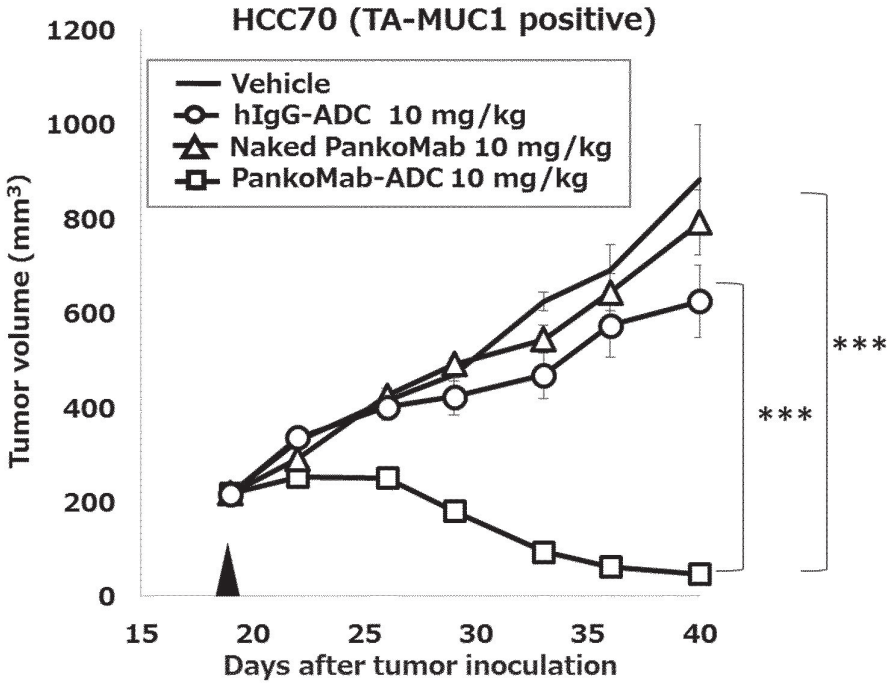


Figure 11

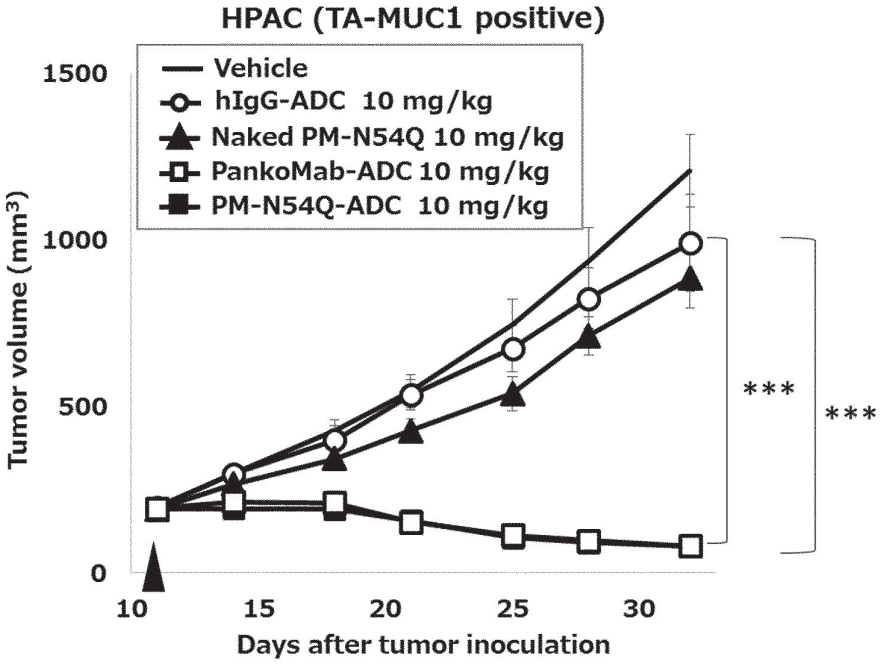


Figure 12

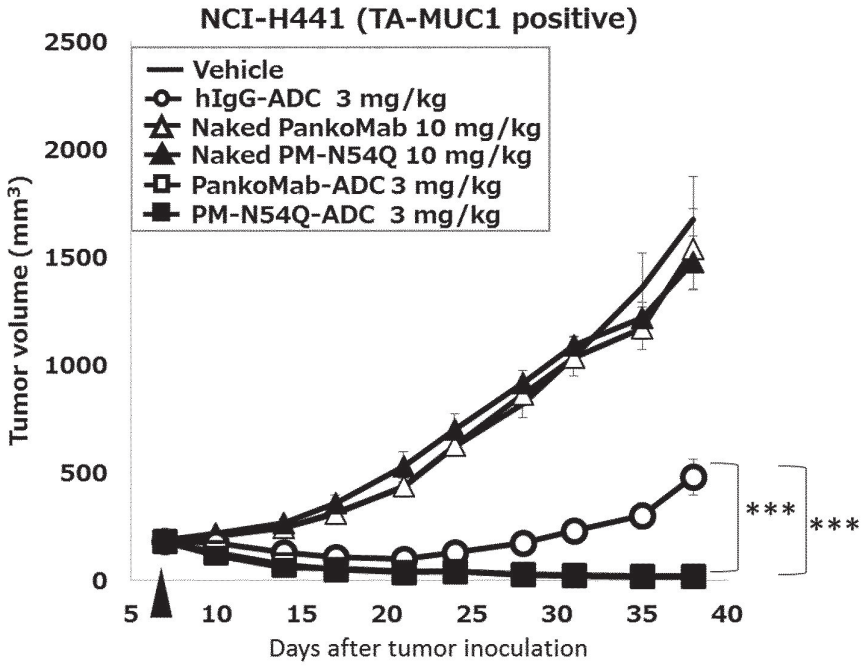


Figure 13

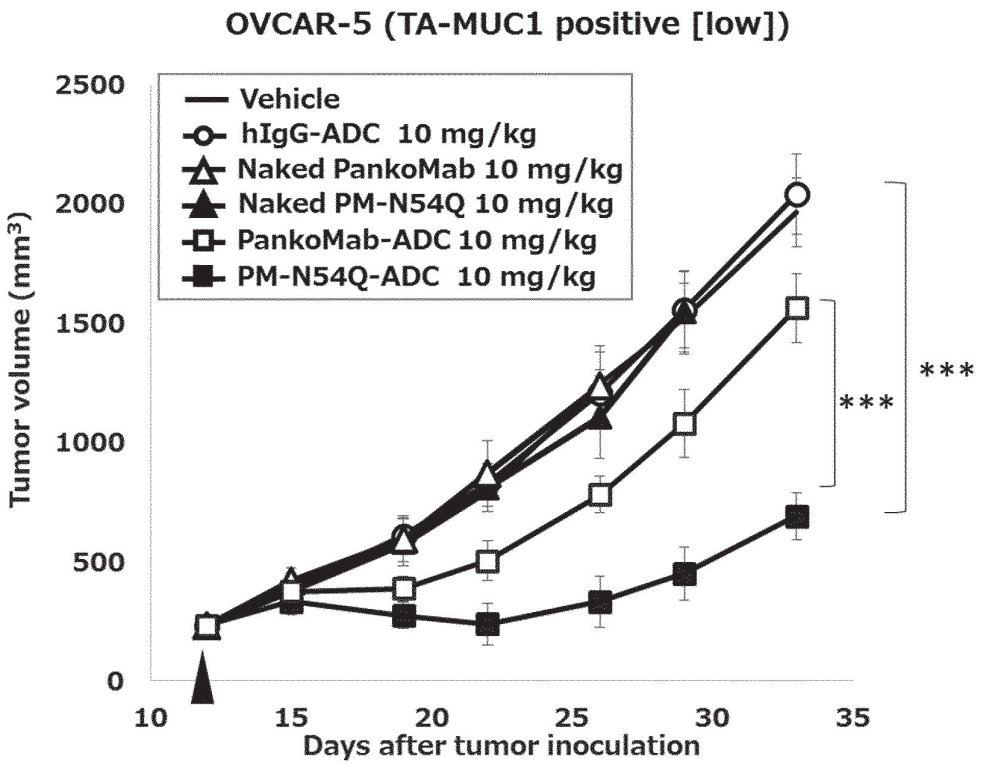


Figure 14

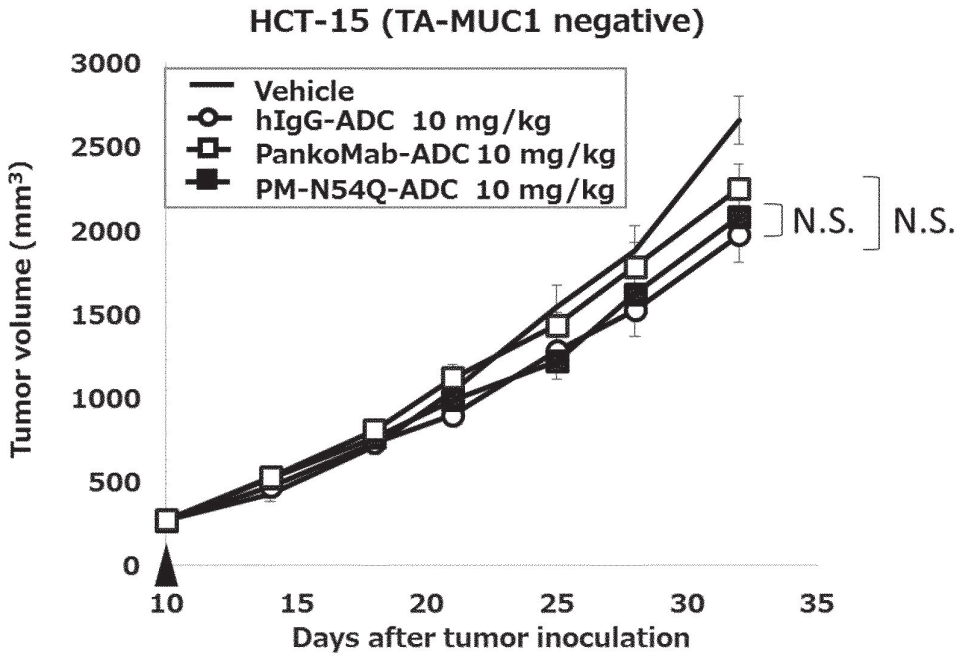


Figure 15

The amino acid sequence of the heavy chain of the humanized antibody N54Q (SEQ ID No: 22)

EVQLVESGGGLVQPGGSMRLSCVASGFPFSNYWMNWVRQAPGKGLEWVGEIRLKSN**Q**YTTHY
AESVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCTRHYYFDYWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEGLHN
HYTQKSLSLSPGK

variable region: aa 1-117, constant region: aa 118-447

Figure 16

The amino acid sequence of the light chain of the humanized antibodies N54Q and PankoMab (SEQ ID No: 16)

DIVMTQSPLSNPVTPGEPASISCRSSKSLLHSNGITYFFWYLQKPGQSPQLLIYQMSNLASG
VPDRFSGSGSGTDFTLRISRVEAEDVGVYYCAQNLELPPTFGQGTKVEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

variable region: aa 1-113, constant region: aa 114-219

Figure 17

The amino acid sequence of the heavy chain of the humanized antibody PankoMab (SEQ ID No: 19)

EVQLVESGGGLVQPGGSMRLSCVASGFPFSNYWMNWVRQAPGKGLEWVGEIRLKSN**N**YTTHY
AESVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCTRHYYFDYWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEGLHN
HYTQKSLSLSPGK

variable region: aa 1-117, constant region: aa 118-447

Figure 18

The amino acid sequence of the heavy chain of chimeric antibody PM N54Q (SEQ ID No: 23)

MKHLWFFLLLVAAPRWVLSEVQLVESGGGLVQPGGSMRLSCVASGFPFSNYWMNWVRQAPGK
GLEWVGEIRLKSNQYTTHYAESVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCTRHYYFD
YWGQGTLVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGV
HTFPAVLQSDLYTLSSSVTVPSSTWPSQTVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVP
EVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTKPREEQIN
STFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMA
KDKVSLTCMITNFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGN
TFTCSVLHEGLHNHHTEKSLSHSPGK

secretory signal sequence: aa 1-19, variable region: aa 20-136, constant region: aa 137-460

Figure 19

The amino acid sequence of the light chain of chimeric antibody PM N54Q (SEQ ID No: 21)

MVLQTQVFISLLLWISGAYGDIVMTQSPLSNPVTPGEPASISCRSSKSLLHSNGITYFFWYL
QKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLRISRVEAEDVGVYYCAQNLELPPTFG
QGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLN
SWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

secretory signal sequence: aa 1-20, variable region: aa 21-133, constant region: aa 134-239