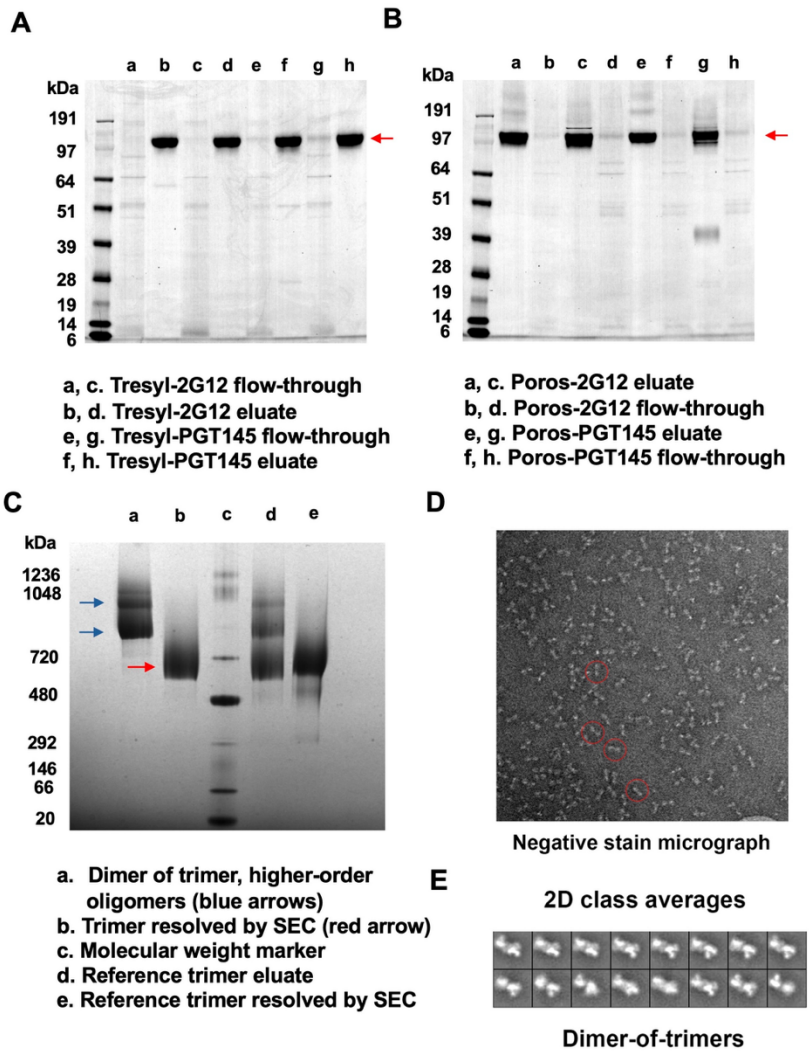
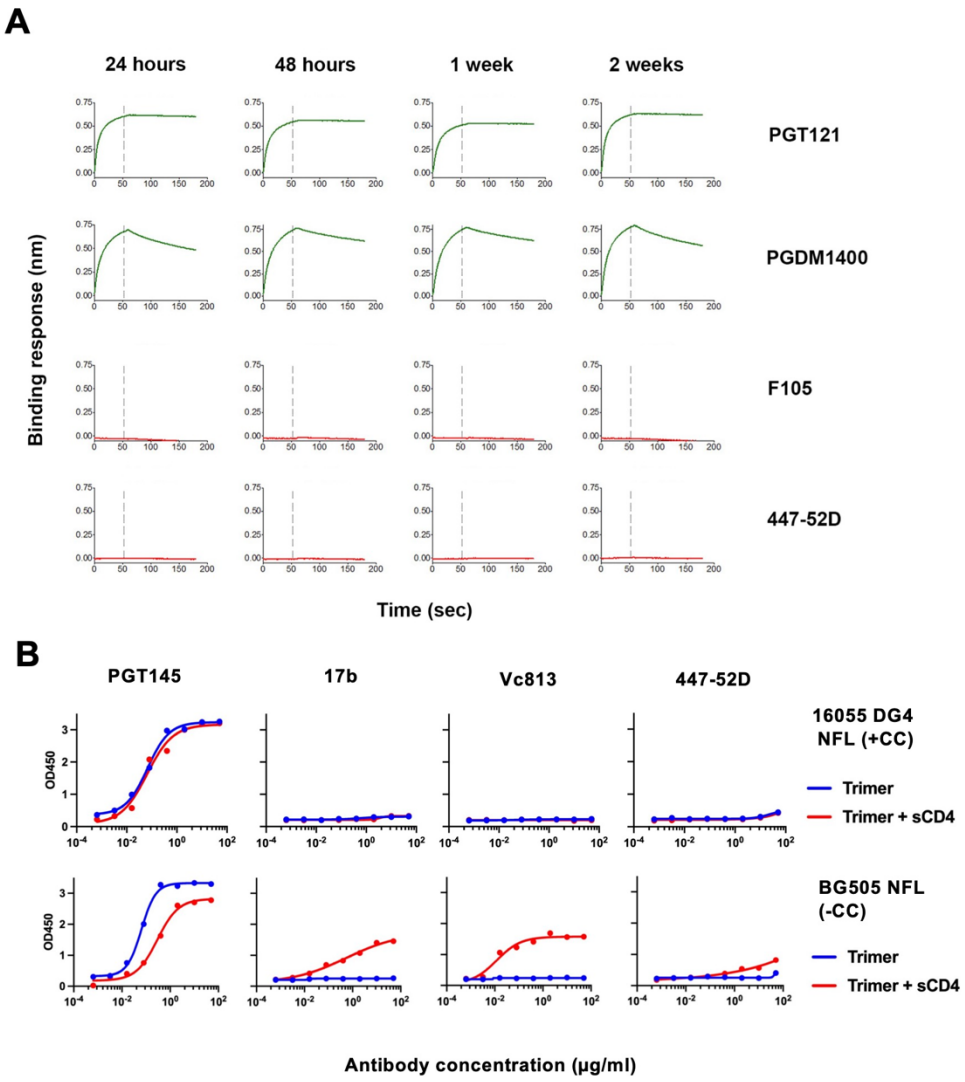
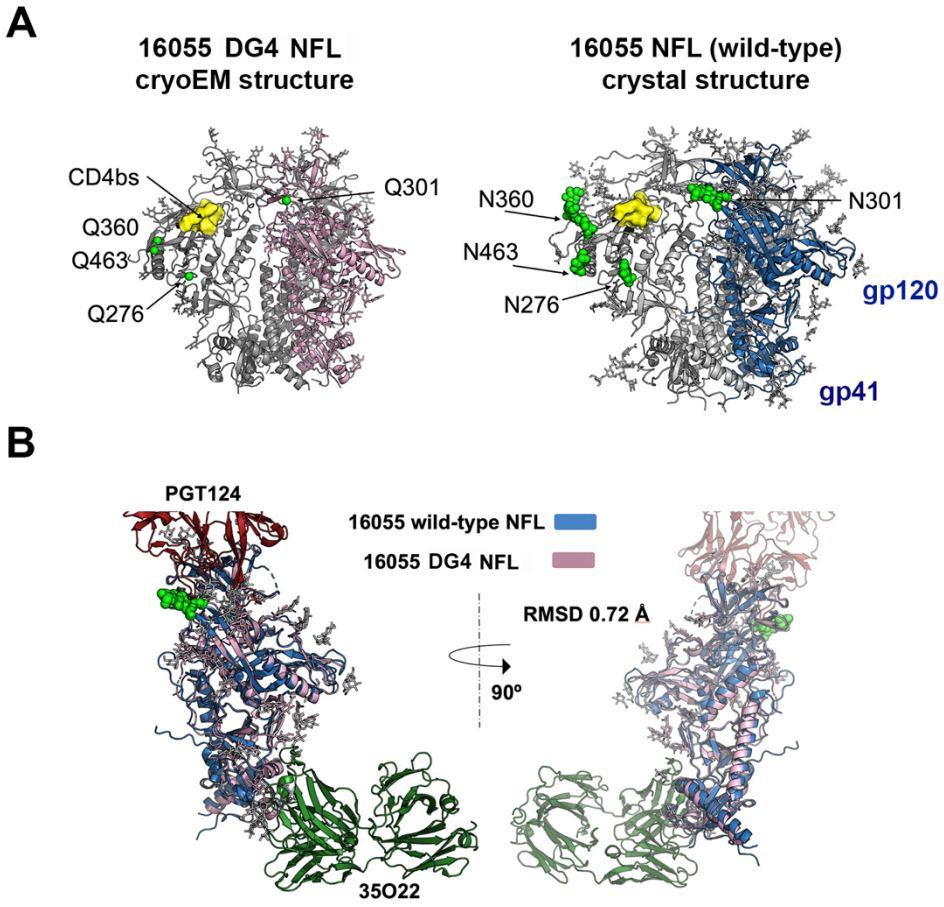


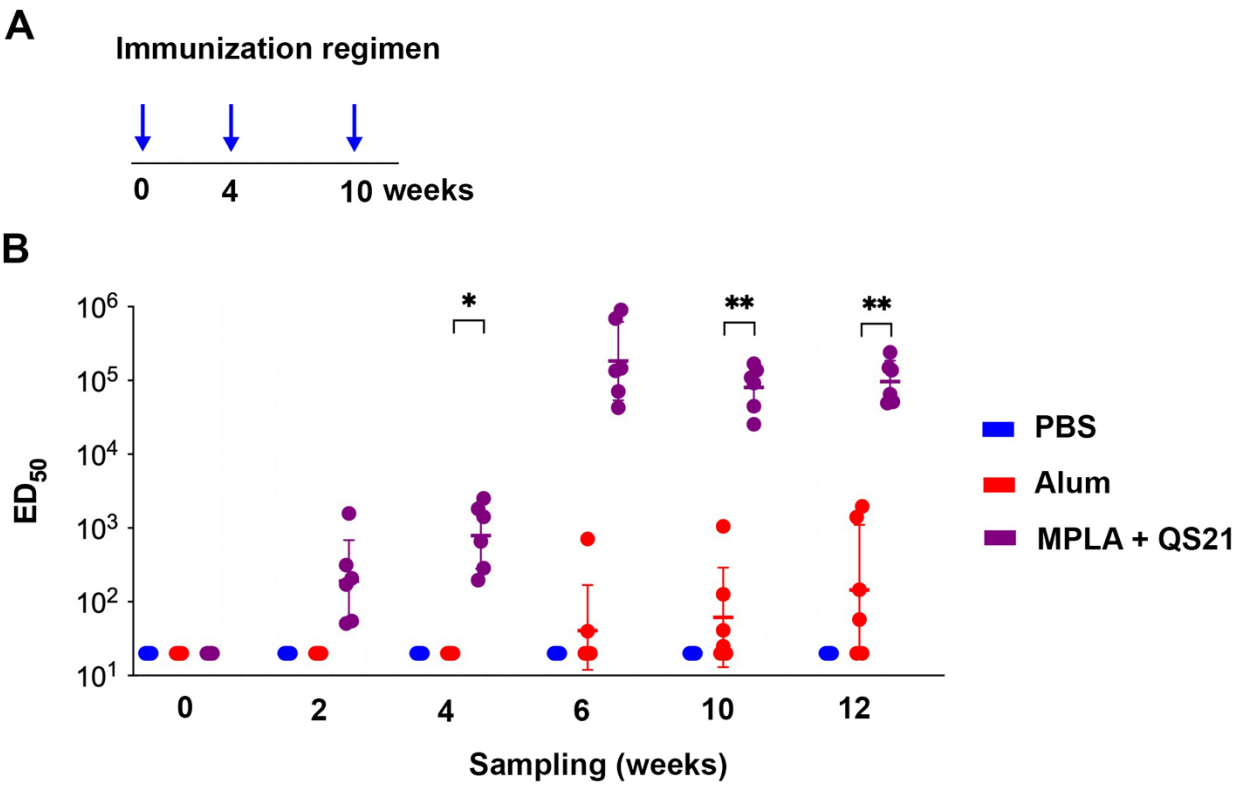
Supplementary Figure 1. Evaluation of bNAb-resin combinations by SDS PAGE (panel A, Tresyl and panel B, Poros) for purification of 16055 DG4 NFL (red arrow). All resins captured the trimer effectively with detectable trimer at ~130 kD in the flow-through for the Poros-coupled bNAbs. Unidentified lower molecular weight bands were detected in the eluate with the Poros-PGT145 resin. (C) BN-PAGE of 16055 DG4 NFL engineering run trimer resolved by SEC (lane a, dimer of trimer and higher-order oligomers, blue arrows; lane b, trimer, red arrow) compared to reference standard 16055 NFL trimer expressed in 293F cells (lane d, overall trimer eluate; lane e, trimer resolved by SEC). (D) A representative negative stain EM micrograph of the minor dimer-of-trimer fraction (circled in red) resolved from the major trimer fraction by SEC. (E) 2D class averages of the individual dimer-of-trimer particles formed by tail-to-tail association.



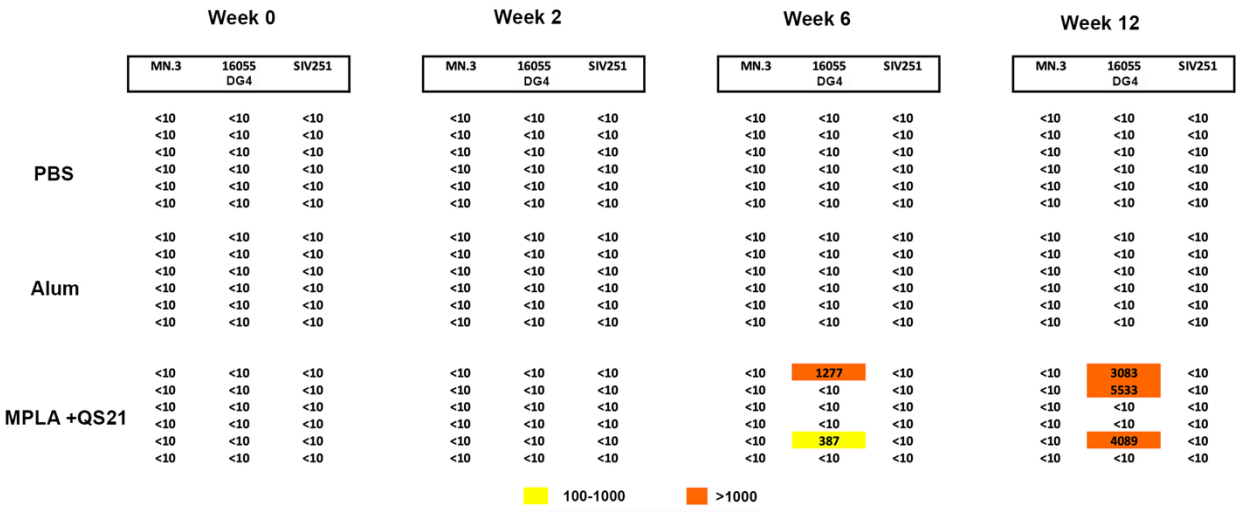
Supplementary Figure 2. (A) 16055 DG4 NFL maintains a favorable antigenic profile for at least 2 weeks at 4°C. BLI binding response (nm) for bNAbs (PGT121 - V3-base region; PGDM1400-V2-apex; each green) and non-neutralizing mAbs (F105 – CD4bs and 447-52D – V3 'tip'; red) is shown at various time points analyzed. (B) ELISA binding data of the 16055 DG4 NFL trimer (+CC, top panel) compared to BG505 NFL trimer (-CC, bottom panel) without (blue) and with (red) incubation with sCD4. The17b and Vc813 mAbs recognize formation of the induced bridging following interaction with CD4 ; 447D recognizes the tip of V3 exposed by CD4-induced conformational changes; the PGT145 bNAb recognizes well-folded trimers with an intact trimer apex that are not affected by interaction with CD4.



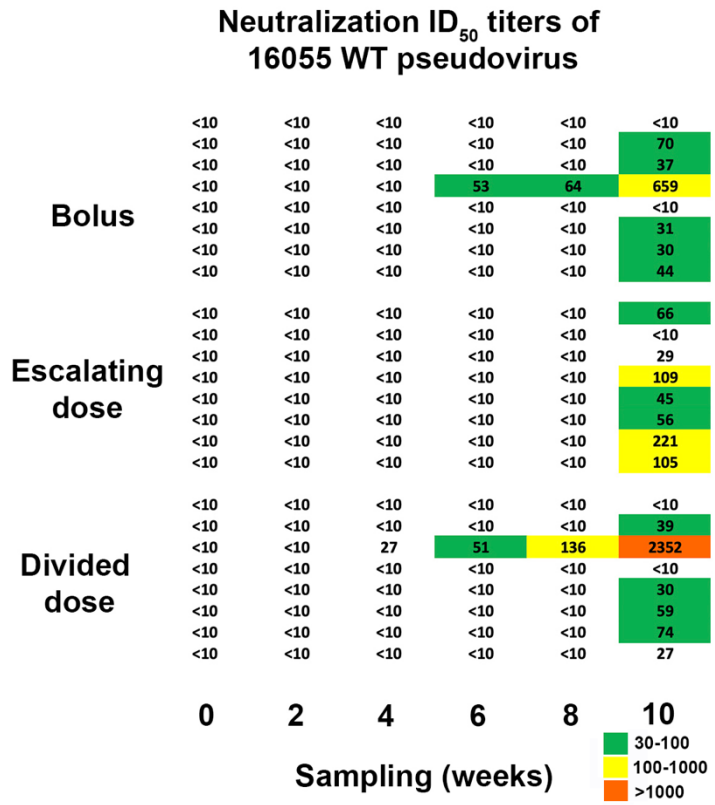
Supplementary Figure 3. (A) CryoEM structure of the 16055 DG4 NFL (left; gp140, light pink; PDB code 9BE9) compared to the crystal structure of fully glycosylated 16055 NFL (right; gp140, blue; PDB code 5UM8). N-glycans at positions 276, 301, 360 and 463 (green) and CD4bs (yellow) are highlighted. (B) Superposition of both the structures, RMSD of 0.72 Å. The N-glycan at position 301 (green) and mAbs PGT124, 35O22 from the crystal structure are shown.



Supplementary Figure 4. (A) Schematic representation of the regimen using 16055 DG4 NFL trimer in mice with selected adjuvants. Bleeds were collected from mice in all groups at weeks 0, 2, 4, 6, 8 and 10. (B) The geometric mean (ED_{50}) of trimer-specific binding titers was plotted at the designated bleed sampling time points. Mice immunized with trimer plus MPLA+QS21 adjuvant elicited robust binding titers that were statistically significantly higher than mice immunized with trimer in Alum adjuvant. No binding responses were detected in mice inoculated without adjuvant (PBS, blue).



Supplementary Figure 5. Neutralization ID_{50} titers of serum from mice immunized with 16055 DG4 NFL trimer with PBS, Alum or MPLA + QS21 adjuvant. Neutralization was only observed in three out of six mice immunized with the MPLA + QS21 adjuvant.



Supplementary Figure 6. Neutralization ID_{50} titers of rabbit serum against the 16055 wild type pseudo-virus. Rabbits from all groups elicited antibodies that modestly neutralized this virus at 10 weeks after immunization by the three different regimens with the same total dose of trimer (100 μg).

Supplementary Table 1. Summary of 16055 DG4 NFL Manufacturing lots

Description	Lot Number	Purpose
Demonstration Lot	NFL-P006-9	Process Demonstration and Characterization
Engineering Lot	DGP.NFL.ER01	Reference Standard Reference Standard Stability Viral Clearance Preclinical studies
cGMP Lot	DGP.NFL.01	Substance Stability Product Stability Clinical Trial Material

Supplementary Table 2. Comparison of Upstream Manufacturing Process Parameters for Demonstration, Engineering and cGMP batches of 16055 DG4 NFL Trimer

	Demonstration (Lot NFL-P006-9)	**Engineering (Lot DGP.NFL.ER01)**	**GMP (Lot DGP.NFL.01)**
Cell bank	MCB	MCB	MCB
Media	Cell Expansion: CD OptiCHO™+ 6mM GlutaMax™ Production: CD OptiCHO™+ 2mM GlutaMax™	Cell Expansion: CD OptiCHO™+ 6mM GlutaMax™ Production: CD OptiCHO™+ 2mM GlutaMax™	Cell Expansion: CD OptiCHO™+ 6mM GlutaMax™ Production: CD OptiCHO™+ 2mM GlutaMax™
Shake Flask Expansion Total Culture Volume (mL); P = expansion passage number	P1: 12 P2: 120 P3: 900 P4: 7200 P5: 9600	P1: 14 P2: 220 P3: 2000 P4: 7200 P5: 9600	P1: 10.5 P2: 87 P3: 860 P4: 9600
Shake Flask Expansion Duration (Days)	11	11	12
Shake Flask Expansion Total Cells on Final Day	5.06E+10	3.72E+10	4.99E+10
Bioreactor Cell Expansion Culture Volume (L)	140	150	150.5
Bioreactor Cell Expansion Duration (Days)	4	4	4
Bioreactor Post-transfection Supplement	- Efficient Feed™ A + Yeastolate at Days 0,2,4,6 - 0.25 mM GlutaMax™ at Days 2,4,6 - -Addition of glucose to 2.5 g/L when concentration below 2 g/L - Addition of valproic acid on Day 1 post Electroporation - Addition of 7.5% sodium bicarbonate for pH control	- Efficient Feed™ A + Yeastolate at Days 0,2,4,6 - 0.25 mM GlutaMax™ at Days 2,4,6 - -Addition of glucose to 2.5 g/L when concentration below 2 g/L - Addition of valproic acid on Day 1 post Electroporation - Addition of 7.5% sodium bicarbonate for pH control	- Efficient Feed A™ + Yeastolate at Days 0,2,4,6 - 0.25 mM GlutaMax™ at Days 2,4,6 - -Addition of glucose to 2.5 g/L when concentration below 2 g/L - Addition of valproic acid on Day 1 post Electroporation - Addition of 7.5% sodium bicarbonate for pH control
Bioreactor Production Target Scale (L)	50	70	70
Bioreactor Production Duration (Days)	8	8	8
Inoculum Weight added (L)	5.5	6.8	7.7
Final Bioreactor Weight-Pre-clarification Harvest volume (L)	56.6	74.6	69.1
Average Viable Cell Density ($x10^6$ viable cells/mL), Production Period	7.51	9.02	6.45
Harvest Titer (g/L)	0.044	0.038	0.037

Supplementary Table 3. Comparison of Downstream Manufacturing Process Parameters for Demonstration, Engineering and cGMP batches of 16055 DG4 NFL Trimer

Parameter	Demo Run NFL-P006-9	Engineering Run DGP.NFL.ER01	cGMP Run DGP.NFL.01
Harvest Filtrate Volume (L)	23.6	72.35	75.4
Harvest Filtrate Titer (g/L)	0.044	0.038	0.037
300 kD MWCO UFDF Retentate Volume (L)	5.17	15.24	13.38[a]
300 kD MWCO UFDF Retentate Concentration (g/L)	N/A	N/A	0.185
300 kD MWCO UFDF Step Yield	N/A	N/A	88.73%
Viral Inactivation Triton X-100 Addition (kg)	0.270	0.790	0.844
Viral Inactivation Final Volume (L)	5.36	16.03	16.378
Tresyl –PGT145 Chromatography Volume (L)	2.45	2.35	2.35
Tresyl –PGT145 Chromatography Pool Concentration (g/L)	0.345	1.09	0.92
Tresyl –PGT145 Chromatography Step Yield	84.52%	85.4%	87.34%
100 kD MWCO UFDF Retentate Volume (L)	2.68	2.85	2.954
100 kD MWCO UFDF Concentration (g/L)	0.27	0.75	0.64
100 kD MWCO UFDF Step Yield	86.0%	91.3 %	87.44%
MabSelect SuRe Chromatography Flow-through Volume (L)	2.68	3.44	3.236
MabSelect SuRe Chromatography Concentration (g/L)	0.27	0.60	0.53
MabSelect SuRe Chromatography Step Yield	100.0%	96.2%	90.72%
Nuvia cPrime Chromatography Flow-through Volume (L)	10.49	18.94	39.9[b]
Nuvia cPrime Chromatography Step Concentration (g/L)	0.068	0.080	0.04
Nuvia CPrime Chromatography Step Yield	93.3%	83.0%	93.06%
Nanofiltrate Net Weight (L)	9.66	19.45	39.25[c]
Nanofiltrate Protein Concentration (g/L)	0.068	0.07	0.04
Nanofiltrate Step Yield	100%	90.0%	98.37%
UFDF 30 kD MWCO Retentate Volume (L)	0.209	0.58	1.67
UFDF 30 kD MWCO Concentration (g/L)	2.95	2.29	0.72
Superdex 200PG Chromatography Volume (L)	94.3%	97.0%	76.6%
Superdex 200PG Chromatography Concentration (g/L)	0.640	1.940	5.808
Superdex 200PG Chromatography Step Yield	61.0%	75.0%	72.5%
Final UFDF 30 kD MWCO Retentate Volume (L)	0.448	1.152	1.532
Final UFDF 30 kD MWCO Concentration (g/L)	1.0[c]	0.8[c]	0.63[d]
Final UFDF 30 kD MWCO Step Yield	92.5%	96.9%	110.97%
Bulk Fill Final Volume (L)	0.349	1.020	1.031
Bulk Fill Concentration (g/L)	1.1	0.8	0.6
Overall Process Yield	35.2%	30.6%	19.9%

[a] Volume is the UFDF1 Retentate Pre-Flush Volume,

[b] Additional volumes of the buffer/WFI were necessary to achieve required pH/ conductivity,

[c] Volume is the Nanofiltrate Pre-Flush Volume,

[d] Concentration taken from UFDF4 in-process sample

Supplementary Table 4

A 16055 DG4 NFL Drug Substance Release testing

Quality Attribute	Test	Specification	Result
Quality	pH USP <791>	7.5 ± 0.5	7.5
	Appearance USP <631> USP <790> EP 8.0	Clear to slightly opalescent, colorless to slightly yellow solution, essentially free of visible particulates	Clarity: Translucent/ Opalescent Color: Colorless Phase: Liquid No Particulates observed
Strength	Concentration (UV-Vis A280)	0.8 ± 0.2 mg/mL	0.6 mg/mL
Purity/Identity	SE-UPLC	Report Results (%Main Peak, %HMW, %LMW)*	92.5 % Main peak, 4.8 % HMW 1.3 % LMW
Identity	SDS-PAGE	Comparable to Reference (Reduced and Non-Reduced)	Comparable to Reference (Reduced and Non-Reduced)
Potency	BLI Binding (Antibodies: PGT145 / F105)	Report results (K_D) and binding profile is comparable to Reference	PGT145 Antibody: k_a (1/Ms) = 2.4E+05 k_d (1/s) = 1.2E-03 K_D = 5.0 nM
			F105 Antibody: K_D = Minimal binding
Safety	Residual HCP-ELISA	≤ 100 ppm	< 2.6 ng/mg
	Residual Protein A-ELISA	Report Results	< 0.28 ng/mL
	Residual IgG-ELISA	Report Results	< 1.3 ng/mL
	Endotoxin USP <85>	< 560 EU/mL	< 0.1 EU/mL
	Bioburden USP <61>	≤ 1 CFU/mL*	TAMC: <1 CFU/mL TYMC: <1 CFU/mL
	qPCR for CHO DNA	< 1 ng/mg	< 1.05E-03 ng/mg
	qPCR Residual Plasmid DNA	< 1.5E+08 copies/mL	7.86E+03 copies/mL
	RT-PCR (Calicivirus 2117)	Not detected	Pass

* HMW, High Molecular Weight; LMW, Low Molecular Weight; CFU, Colony Forming Unit

B 16055 DG4 NFL Trimer Drug Product Release testing

Quality Attribute	Test	Specification	Result

Quality	pH USP <791>	7.5 ± 0.5	7.5
	Appearance USP <631> USP <790> EP 8.0	Clear to slightly opalescent, colorless to slightly yellow solution, essentially free of visible particulates	Clarity: Translucent/ Opalescent Color: Colorless Phase: Liquid No Particulates observed
Strength	Concentration (UV-Vis A280)	0.6 ± 0.1 mg/mL	0.6 mg/mL
Purity/Identity	SE-UPLC	Report Results (%Main Peak, %HMW, %LMW)	93.1% Main peak, 4.2 % HMW 2.7 % LMW
Identity	SDS-PAGE	Comparable to Reference (Reduced and Non-Reduced)	Comparable to Reference (Reduced and Non-Reduced)
Potency	BLI Binding (Antibodies: PGT145 / F105)	Report results (K_D) and binding profile is comparable to Reference	Comparable to Reference: PGT145 Antibody: K_D = 4.6 nM
			F105 Antibody: K_D = Minimal binding
Safety	Particulate matter USP <788>	≥10 µm: ≤ 6000 particles per vial ≥25 µm: ≤ 600 particles per vial	≥10µm: 21 particles per vial ≥25µm: 2 particles per vial
	Endotoxin USP <85> EP 2.6.14 JP 4.01 11325/20090	< 560 EU/mL	< 3 EU/mL
	Sterility USP <71> EP 2.6.1 JP 4.06 10204	No growth	No growth

Supplementary Table 5. 16055 DG4 NFL stability testing

A. Testing at -80 ± 10°C

Quality Attribute	Test	Specification	Testing interval*
Safety	Container closure USP <1207>	No dye ingress	B
	Sterility USP <71> EP 2.6.1 JP 4.06	No growth	C
	Particulate matter USP <788>	≥10 µm: ≤ 6000 particles per vial ≥25 µm: ≤ 600 particles per vial	C
Quality	pH USP <791>	7.5 ± 0.5	A
	Appearance USP <631> USP <790> EP 8.0	Clear to slightly opalescent, colorless to slightly yellow solution, essentially free of visible particulates	A
Strength	Concentration (UV-Vis A280)	0.6 ± 0.1 mg/mL	A
Purity/Identity	SE-UPLC	Report Results (%Main Peak, %HMW, %LMW)	A
Identity	SDS-PAGE	Comparable to Reference (Reduced and Non-Reduced)	A
Potency	BLI Binding (Antibodies: PGT145 / F105)	Report results (K_D) and binding profile is comparable to Reference	A

*A = 0, 3, 6, 9, 12, 18, 24, 36, 48, and 60 months at -80 ± 10°C
B= 12, 24, 36, and 48 months at -80 ± 10°C
C= 0, 60 months at -80 ± 10°C
The T-zero time point is equivalent to the release testing that occurred after the completion of the fill by the fill/finish manufacturer.

B. Testing at 5 ± 3°C

Quality Attribute	Test	Specification	Testing interval*
Quality	pH USP <791>	7.5 ± 0.5	E
	Appearance USP <631> USP <790> EP 8.0	Clear to slightly opalescent, colorless to slightly yellow solution, essentially free of visible particulates	E
Strength	Concentration (UV-Vis A280)	0.6 ± 0.1 mg/mL	E

Purity/Identity	SE-UPLC	Report Results (%Main Peak, %HMW, %LMW)	E
Identity	SDS-PAGE	Comparable to Reference (Reduced and Non-Reduced)	E
Potency	BLI Binding (Antibodies: PGT145 / F105)	Report results (K_D) and binding profile is comparable to Reference	E

*E = 2 weeks, 1 month at 5 ± 3°C

C. Testing at 25 ± 2°C

Quality Attribute	Test	Specification	Testing interval*
Quality	pH USP <791>	7.5 ± 0.5	F
	Appearance USP <631> USP <790> EP 8.0	Clear to slightly opalescent, colorless to slightly yellow solution, essentially free of visible particulates	F
Strength	Concentration (UV-Vis A280)	0.6 ± 0.1 mg/mL	F
Purity/Identity	SE-UPLC	Report Results (%Main Peak, %HMW, %LMW)	F
Identity	SDS-PAGE	Comparable to Reference (Reduced and Non-Reduced)	F
Potency	BLI Binding (Antibodies: PGT145 / F105)	Report results (K_D) and binding profile is comparable to Reference	F

*F = 2 weeks at 25 ± 2°C

Supplementary Table 6. Cryo-EM data collection, refinement, and validation statistics

	16055 DG4 NFL (EMD-44474) (PDB 9BE9)
Data collection and processing	
Microscope	FEI Titan Krios
Magnification	130,000x
Voltage (kV)	300
Electron exposure (e–/Å^2)	48
Defocus range (μm)	-0.7 to -2.0
Detector	Gatan K2 Summit
Recording mode	Counting
Pixel size (Å)	1.045
Symmetry imposed	C3
Micrographs (no.)	3,416
Initial particle images (no.)	307,993
Final particle images (no.)	91,115
Map resolution (Å)	3.8
FSC threshold	0.143
Map sharpening *B* factor (Å^2)	-167
Map pixel size (Å)	1.045
Map resolution range (Å)	2.8-4.4
Refinement	
Initial model used (PDB code)	5UM8
Model resolution (Å)	4.0
FSC threshold	0.5
Model resolution range (Å)	2.8-4.4
EMRinger score	2.50
Model composition	
Non-hydrogen atoms	14,253
Protein residues	1,656
Ligands	78
Mean *B* factors (Å^2)	
Protein	56.49
Ligand	60.62
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.359
Validation	
MolProbity score	1.14
Clashscore	1.17
Poor rotamers (%)	0.20
Ramachandran plot	
Favored (%)	95.54
Allowed (%)	4.46
Disallowed (%)	0.00
Cβ outliers (%)	0.00

Supplementary Table 7. Immunization regimen

Group	#[a]	Immunization regimen	Week 0	Week 2	Week 4	Week 6	Week 8	Total dose
1	8	Bolus	50 μg	-	-	-	50 μg	100 μg
2	8	Escalating dose [b]	0.5 μg, 1.0 μg, 2.5 μg	5.0 μg, 12.0 μg, 29.0 μg	-	-	50 μg	100 μg
3	8	Divided dose	20 μg	20 μg	20 μg	20 μg	20 μg	100 μg

[a] Number of animals per group

[b] Priming immunizations for Group 2 were performed on days 0, 2, 4, 7, 9 and 11, respectively.

Supplementary Table 8. Summary of Test results for Tresyl-PGT145 resin made at 5.7 L scale.

Parameter	Acceptance Criteria	Results
Particle size	≥80% (40-90 μm)	95%
Ligand density (gel)	5-10 mg/mL gel	6.7 mg/mL gel
Binding Capacity (gel)	≥0.3 mg /mL gel	0.95 mg/mL
Leaching of antibody PGT145	≤ 2 μg /mL	0.035 μg /mL
Endotoxin (supernatant)	≤ 2 EU/mL	< 0.6 EU/mL
Bioburden (gel)	≤ 100 CFU/g	0 CFU/g