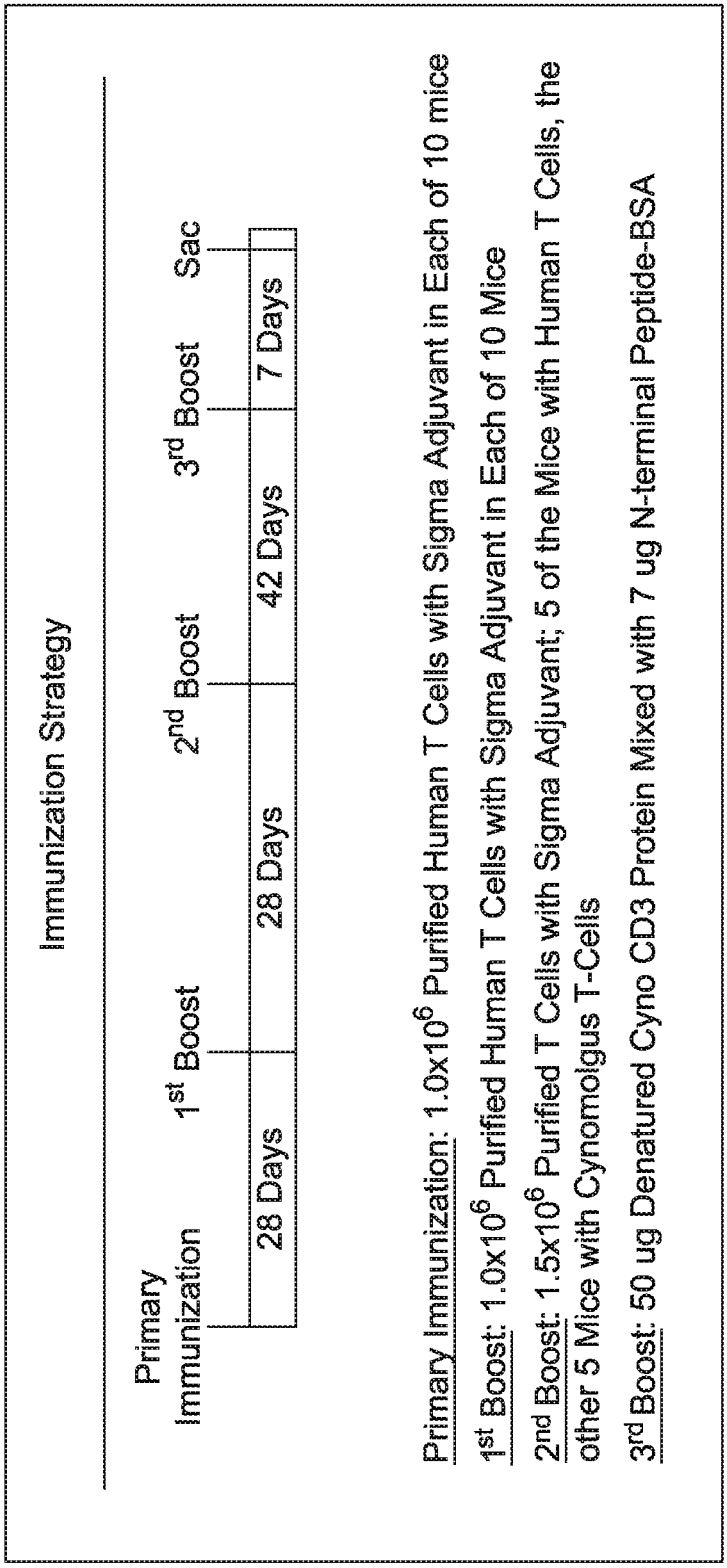

Immunization Strategy
Primary Immunization
1st Boost
2nd Boost
3rd Boost
Sac
28 Days
28 Days
42 Days
7 Days
Primary Immunization: 1.0x10^6 Purified Human T Cells with Sigma Adjuvant in Each of 10 mice
1st Boost: 1.0x10^6 Purified Human T Cells with Sigma Adjuvant in Each of 10 Mice
2nd Boost: 1.5x10^6 Purified T Cells with Sigma Adjuvant; 5 of the Mice with Human T Cells, the other 5 Mice with Cynomolgus T-Cells
3rd Boost: 50 ug Denatured Cyno CD3 Protein Mixed with 7 ug N-terminal Peptide-BSA

Figure 1

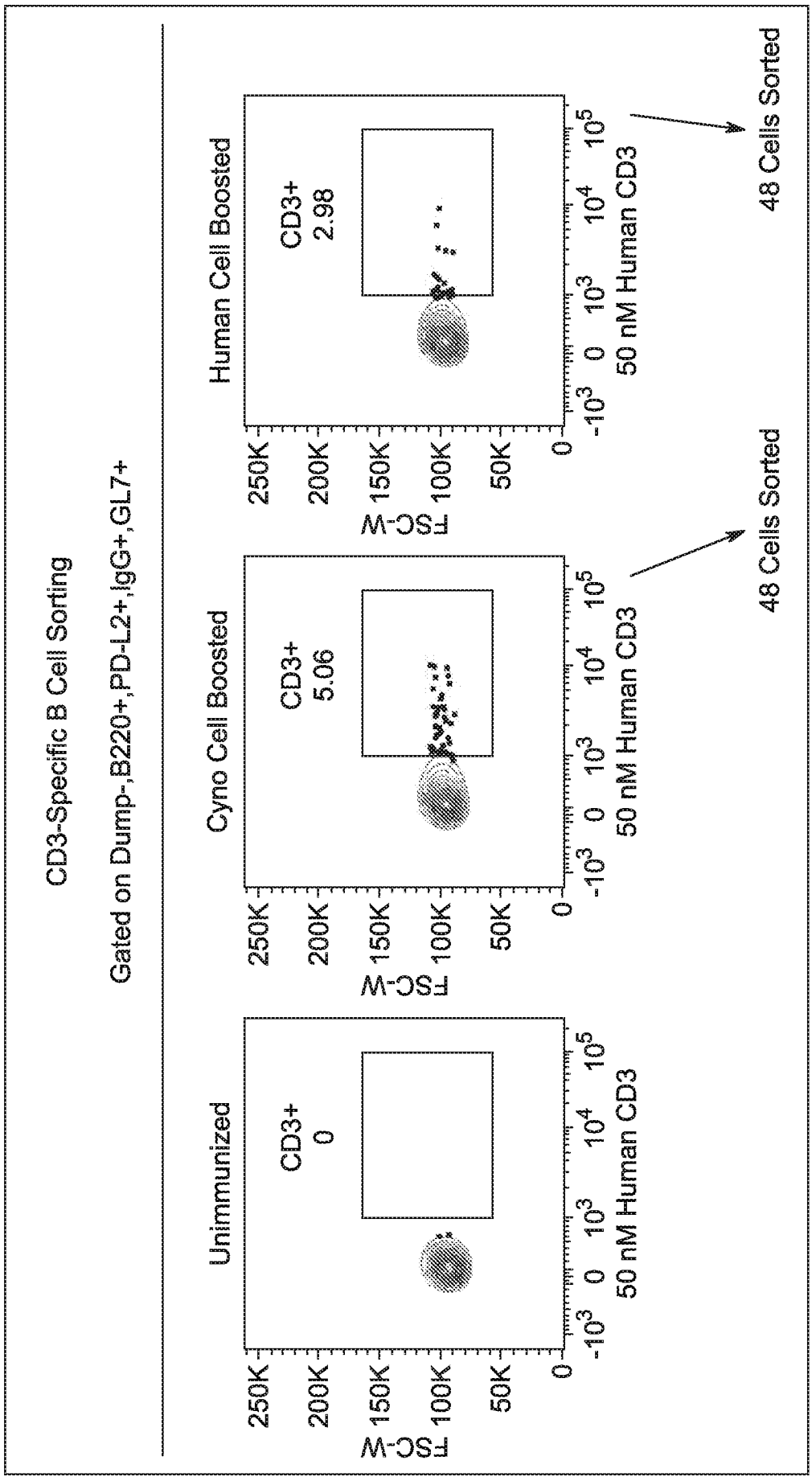

CD3-Specific B Cell Sorting
Gated on Dump-,B220+,PD-L2+,IgG+,GL7+
Unimmunized
CD3+
0
Cyno Cell Boosted
CD3+
5.06
Human Cell Boosted
CD3+
2.98
FSC-W
250K
200K
150K
100K
50K
0
-10^3 0 10^3 10^4 10^5
50 nM Human CD3
48 Cells Sorted
48 Cells Sorted

Figure 2

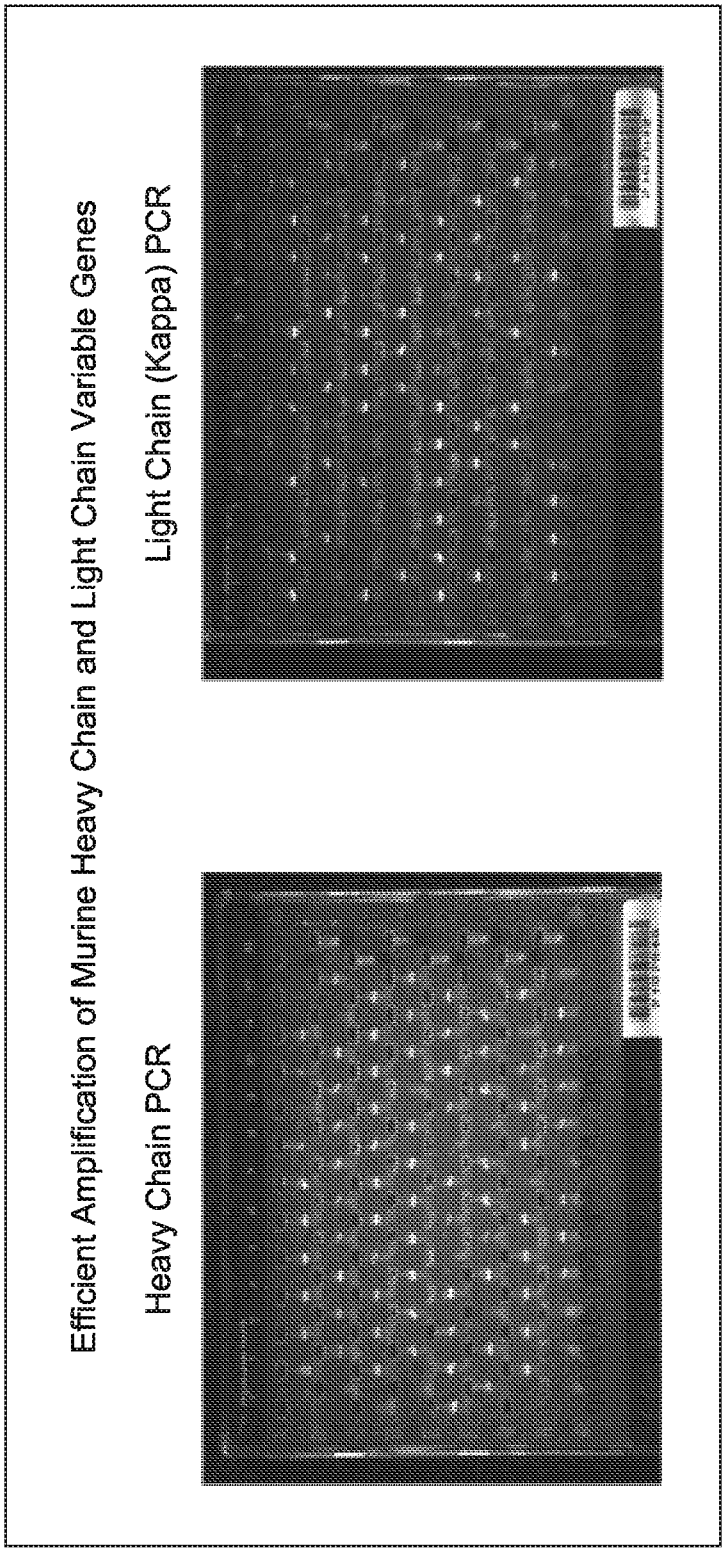

Efficient Amplification of Murine Heavy Chain and Light Chain Variable Genes
Heavy Chain PCR
Light Chain (Kappa) PCR

Figure 3

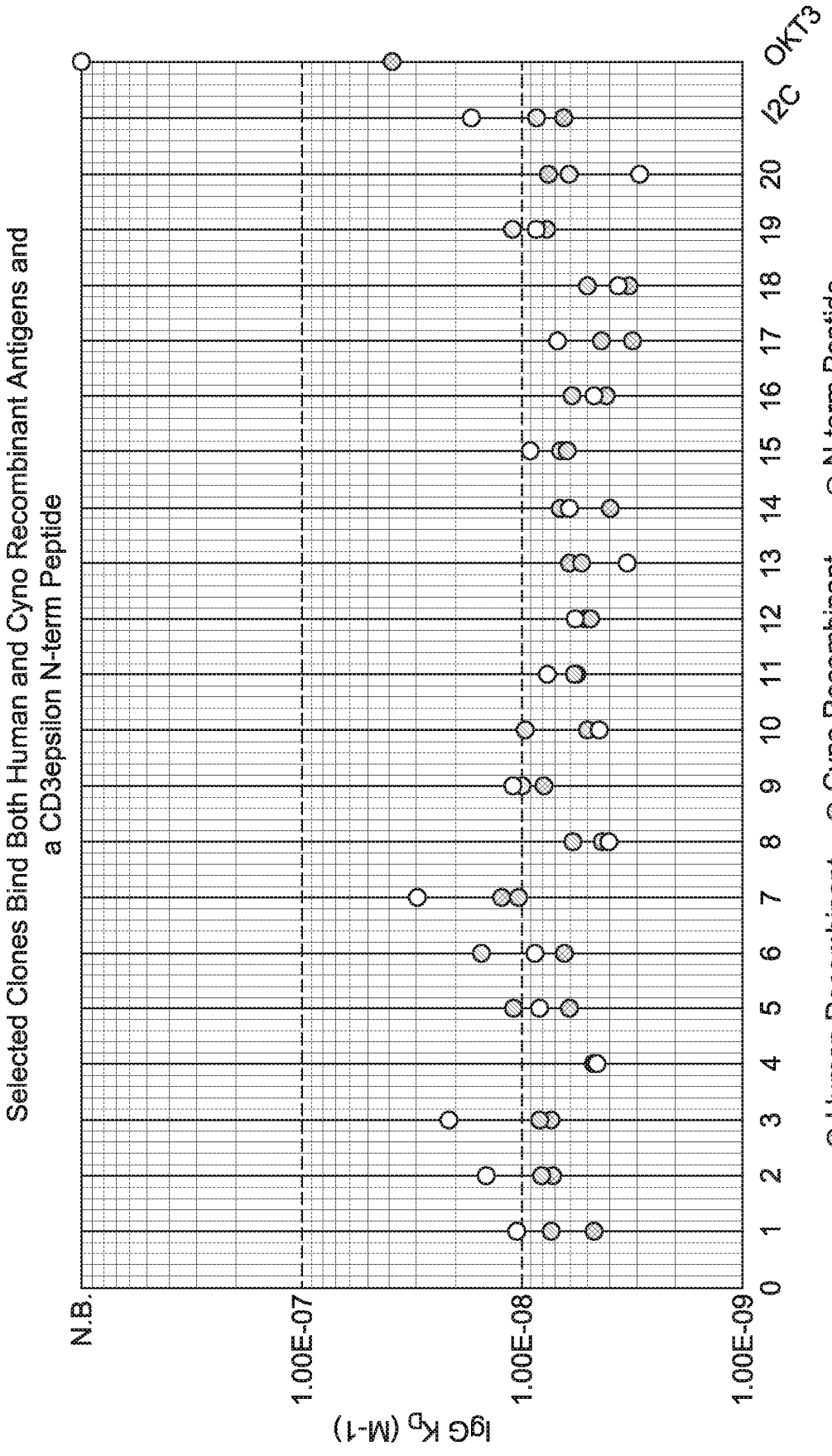

Selected Clones Bind Both Human and Cyno Recombinant Antigens and a CD3epsilon N-term Peptide
IgG K_D (M-1)
N.B.
1.00E-07
1.00E-08
1.00E-09
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
$I2C$ OKT_3
Human Recombinant
Cyno Recombinant
N-term Peptide

Figure 4A

Clones with Related Sequences Comprise ~85% of the Binding Response

Clone ID	VH Germ line	VH CDR1	VH CDR2	VH FR3	VH CDR3	VL Germ line	VL CDR3
ADI-29538	V1-125B	YTFTTYYIH	WIYPGNVNAKYNEKFKG	KATLTADKSSSTAYMQLSSLTSEDSAVYFC	ARDDGYYFDY	VK08-021	KQSYNLRT
ADI-29535	V1-125B	YTFTSYYIH	WIYPGNVNTKYNENFKD	KATLTADKSSSTAYMHLSSLTSEDSAVYFC	ARDDGYYFDY	VK08-021	KQSYNLRT
ADI-15505	V1-125B	YTFISYYIH	WIYPGNVNTKYNEKFKA	KATLTADKSSSTAYMQLSSLTSEDSAVYFC	ARDDNYSFAY	VK08-021	KQSYNLRT
ADI-15506	V1-125E	YAFTSYYIH	WIYLGDGSTNYNEKFKG	KTTLTADKSSSTAYMLLSSLTSEDSATYFC	ARDDSYYFDY	VK08-021	KQSYSLRT
ADI-29540	V1-125B	YTFTSYYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMQLSSLTSEDSAVYFC	ARDGDYFFDY	VK08-021	KQSYNLRT
ADI-29539	V1-125B	YTFTSYYIH	WIYPGNVNTQYNEKFKG	KATLTADKSSSTTYMQLSSLTSEDSAVYFC	ARDGDYYFDY	VK08-021	KQSYNLRT
ADI-15507	V1-125B	YTFTRYYIH	WIYPGNVNTKYNEKFKG	KATLSADKSSSTAYMQLSSLTSEDSAVYFC	ARDGDYYFDY	VK08-021	KQSYNLRT
ADI-15508	V1-125B	YTFTSYYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMQLSSLTSEDSAVYFC	ARDGDYYFDY	VK08-021	KQSYNLRT
ADI-15509	V1-125B	YTFTSYYIH	WIYPGNVNTKYNEKFKG	KATLTADTSSSTAYMQLSSLTSEDSAVYFC	ARDGNYFFDV	VK08-021	KQSYNLRT
ADI-15510	V1-125B	YTFTSYYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMQLSSLTSEDSAIYFC	ARDGNYFFDV	VK08-021	KQSYNLRT
ADI-29537	V1-125B	YTFTSYYIH	WIFPGNVNTKYNEKFKG	KATLTADKSSSTAYMQLSSLTSEDSAVYFC	ARDGNYSFAY	VK08-021	KQSYNLRT
ADI-15511	V1-125B	NTFTSSYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTVYMQLSSLTSEDSAVYFC	ARDGSSYYFDY	VK08-021	KQSYNLRT
ADI-15512	V1-125E	YTFTSYYIH	WIYPGDGSTKYNEKFKG	KTTLTADKSSSTAYMLLSSLTSEDSAIYFC	ARDGSYYFDY	VK08-021	KQSYSLRT
ADI-15515	V1-125B	YTFTSYYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTVYMQLSSLTSEDSAVYFC	ARDYGYYFDY	VK08-021	KQSYNLRT
ADI-15514	V1-125E	YTFTSYYIH	WIYPGDGSTKYNEKFKG	KTTLTADKSSSTAYMLLSSLTSEDSAIYFC	ARDYGYYFDY	VK08-021	KQSYNLRT
ADI-15516	V1-125B	YTFTSSYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMHLSSLTSEDSAVYFC	ARDYGYYFDY	VK08-021	KQSYNLRT
ADI-15517	V1-125B	YTFTSYYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMQLSSLTSEDSAVYFC	ARDYGYYFGS	VK08-021	KQSYNLRT
ADI-16513	VH14-1	FNIKDYYMH	WIDPENGNTIYDPKFQG	KASITADTSSNTAYLQLSSLTSEDTAVYYC	ARDGYARYYFDY	VK08-021	KQSYNLRT
ADI-29536	V1-125E	YTFTNYYIH	WIYPEDGNTKYNEKFKG	KTTLTADKSSSTAYMLLSSLTSEDSAIYFC	ARNNGSSYAMDY	VK08-021	KQSYNLRT
ADI-29534	V1-039A	FNIKDYYMH	WIDPENGNTIYDPKFQG	KASITADTSSNTAYLQLSSLTSEDTAVYYC	GRDGNYFFDY	VK08-021	KQSYNLRT
ADI-15518	V1-125B	YTFTSYYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTVYMQLSSLTSEDSAVYFC	GRDYYGSYYFDN	VK08-021	KQSYNLRT

Figure 4B

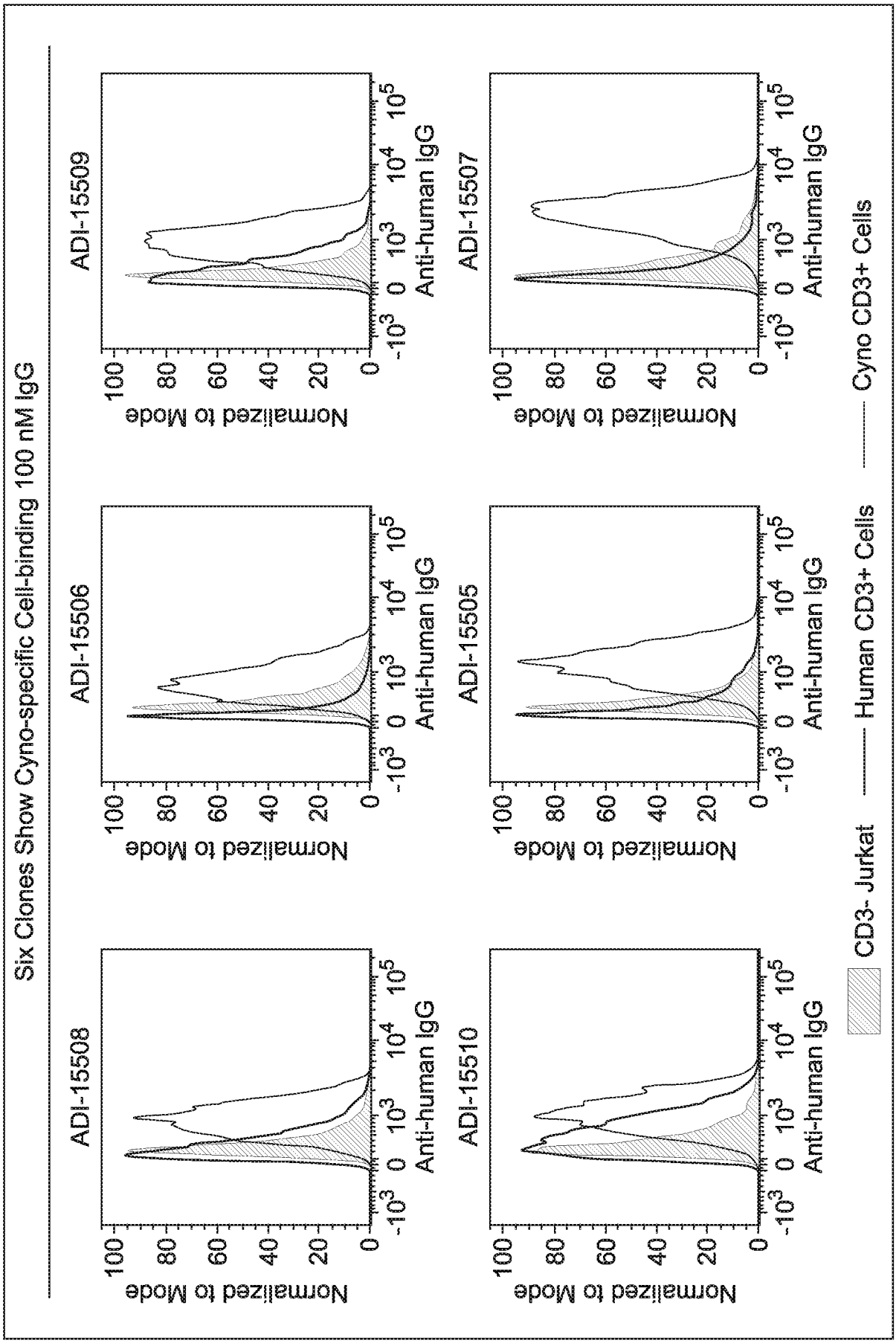

Six Clones Show Cyno-specific Cell-binding 100 nM IgG
ADI-15508
ADI-15506
ADI-15509
ADI-15505
ADI-15507
ADI-15510
Normalized to Mode
Anti-human IgG
CD3- Jurkat
Human CD3+ Cells
Cyno CD3+ Cells

Figure 5

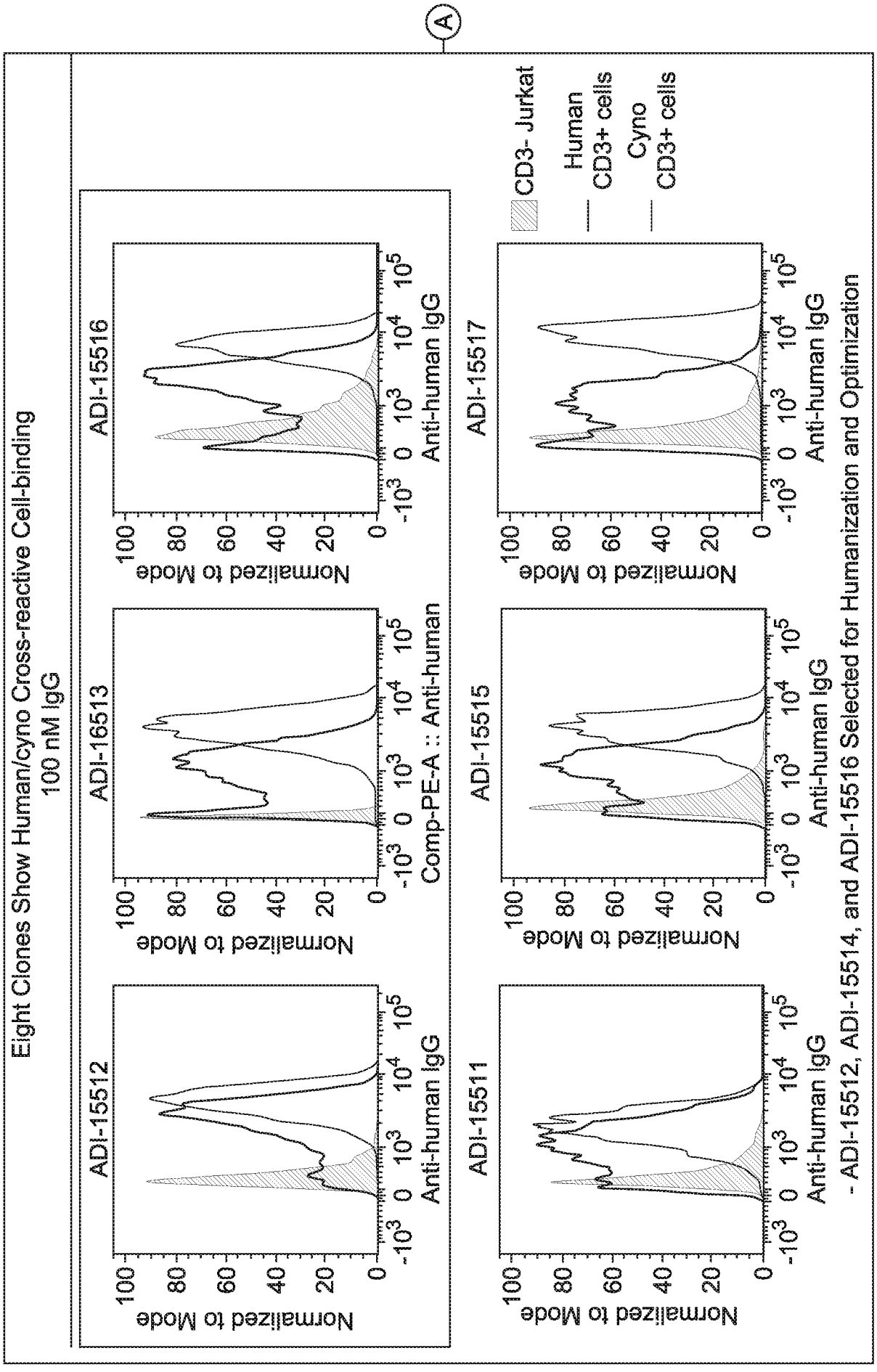

A
Eight Clones Show Human/cyno Cross-reactive Cell-binding
100 nM IgG
ADI-15512
ADI-16513
ADI-15516
ADI-15511
ADI-15515
ADI-15517
Normalized to Mode
Anti-human IgG
Comp-PE-A :: Anti-human
CD3- Jurkat
Human CD3+ cells
Cyno CD3+ cells
- ADI-15512, ADI-15514, and ADI-15516 Selected for Humanization and Optimization

Figure 6

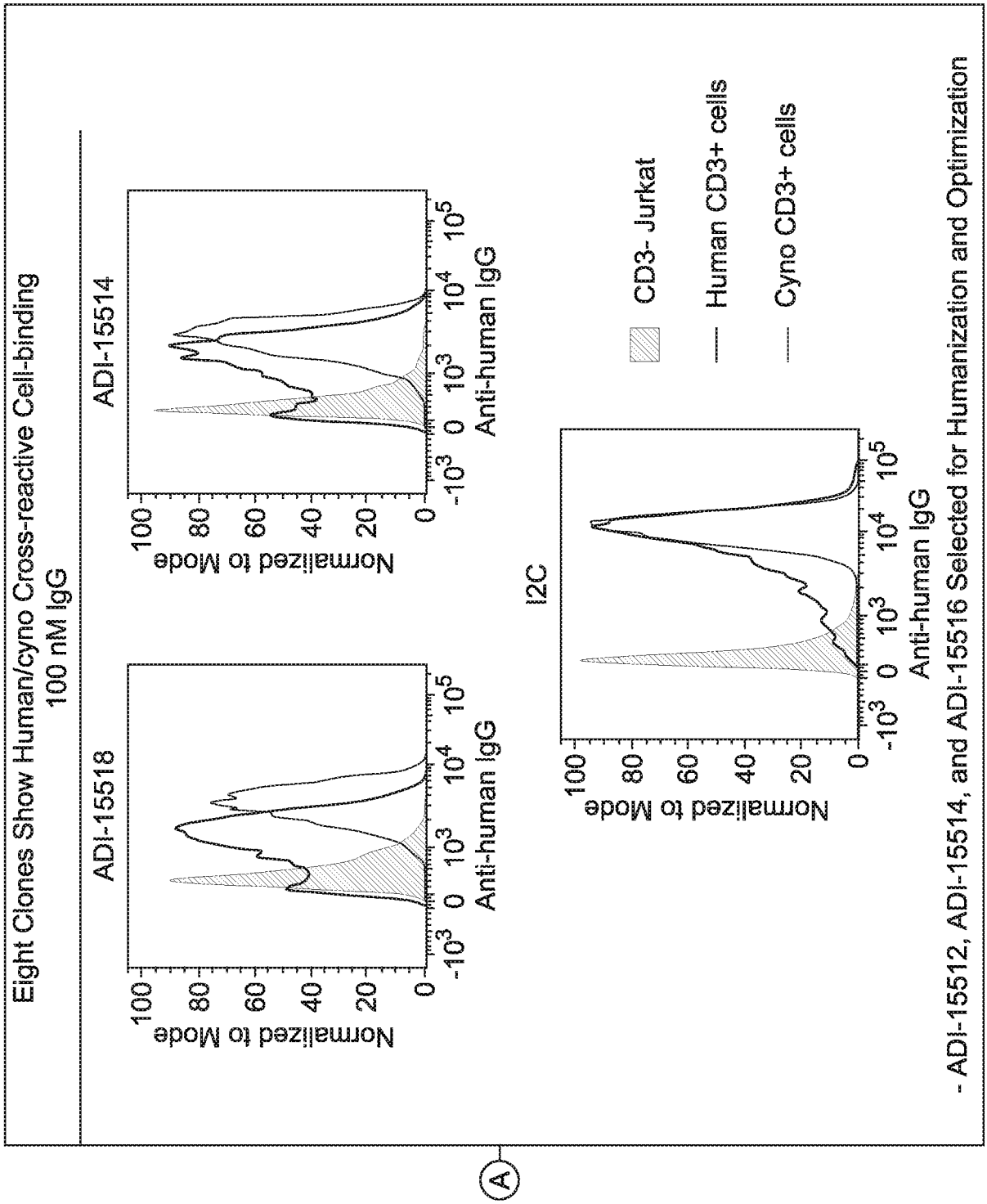


Figure 6 (Continued)

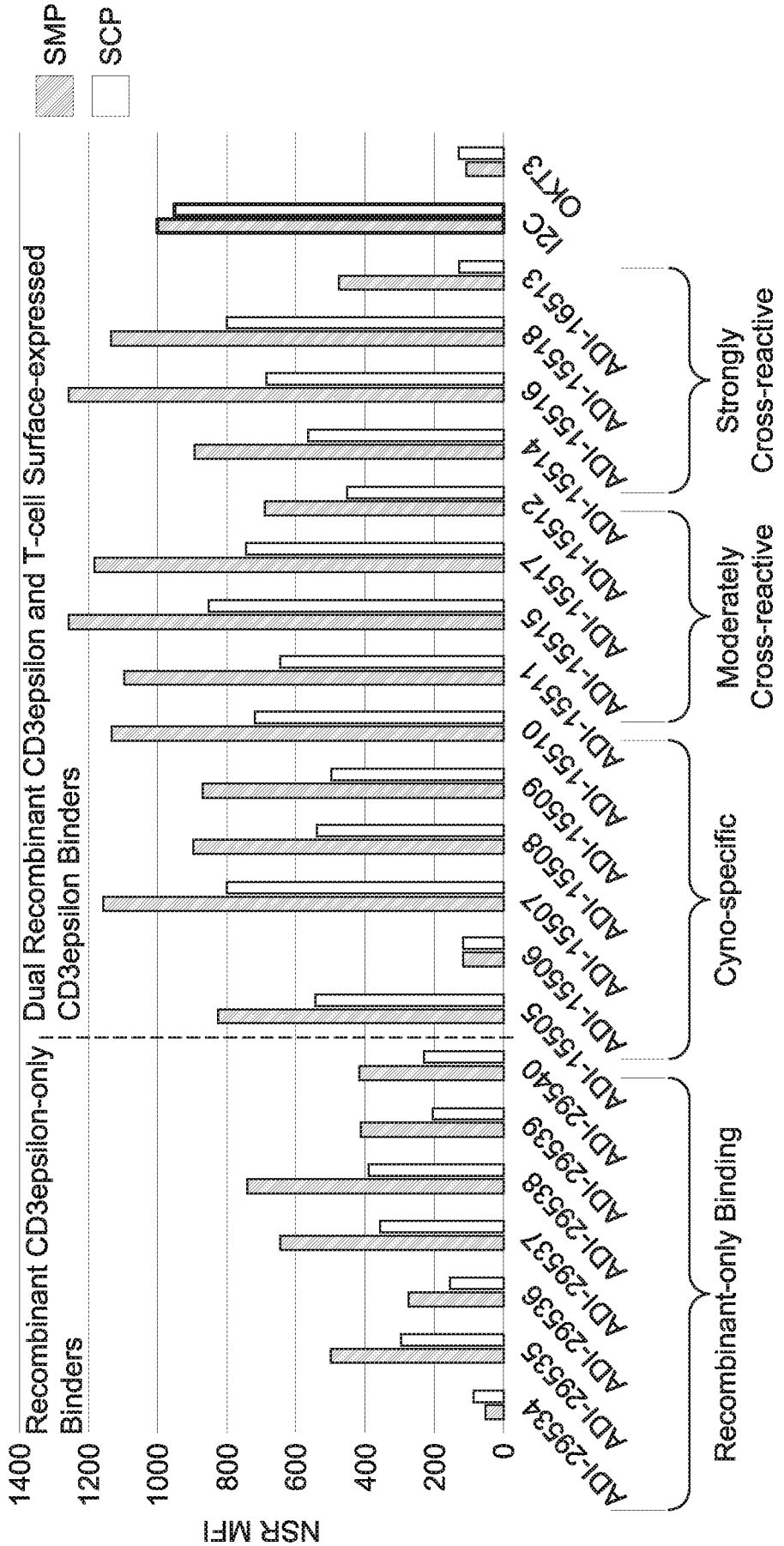

All Human/cyno CD3epsilon Cross-reactive IgGs, Including I2C, have Medium NSR Reactivity (MFIs ~400-1200)
SMP
SCP
NSR MFI
1400
1200
1000
800
600
400
200
0
Recombinant CD3epsilon-only Binders
Dual Recombinant CD3epsilon and T-cell Surface-expressed CD3epsilon Binders
ADI-29534
ADI-29535
ADI-29536
ADI-29537
ADI-29538
ADI-29539
ADI-29540
ADI-15505
ADI-15506
ADI-15507
ADI-15508
ADI-15509
ADI-15510
ADI-15511
ADI-15515
ADI-15517
ADI-15512
ADI-15514
ADI-15516
ADI-15518
ADI-16513
I2C
OKT3
Recombinant-only Binding
Cyno-specific
Moderately Cross-reactive
Strongly Cross-reactive

Figure 7

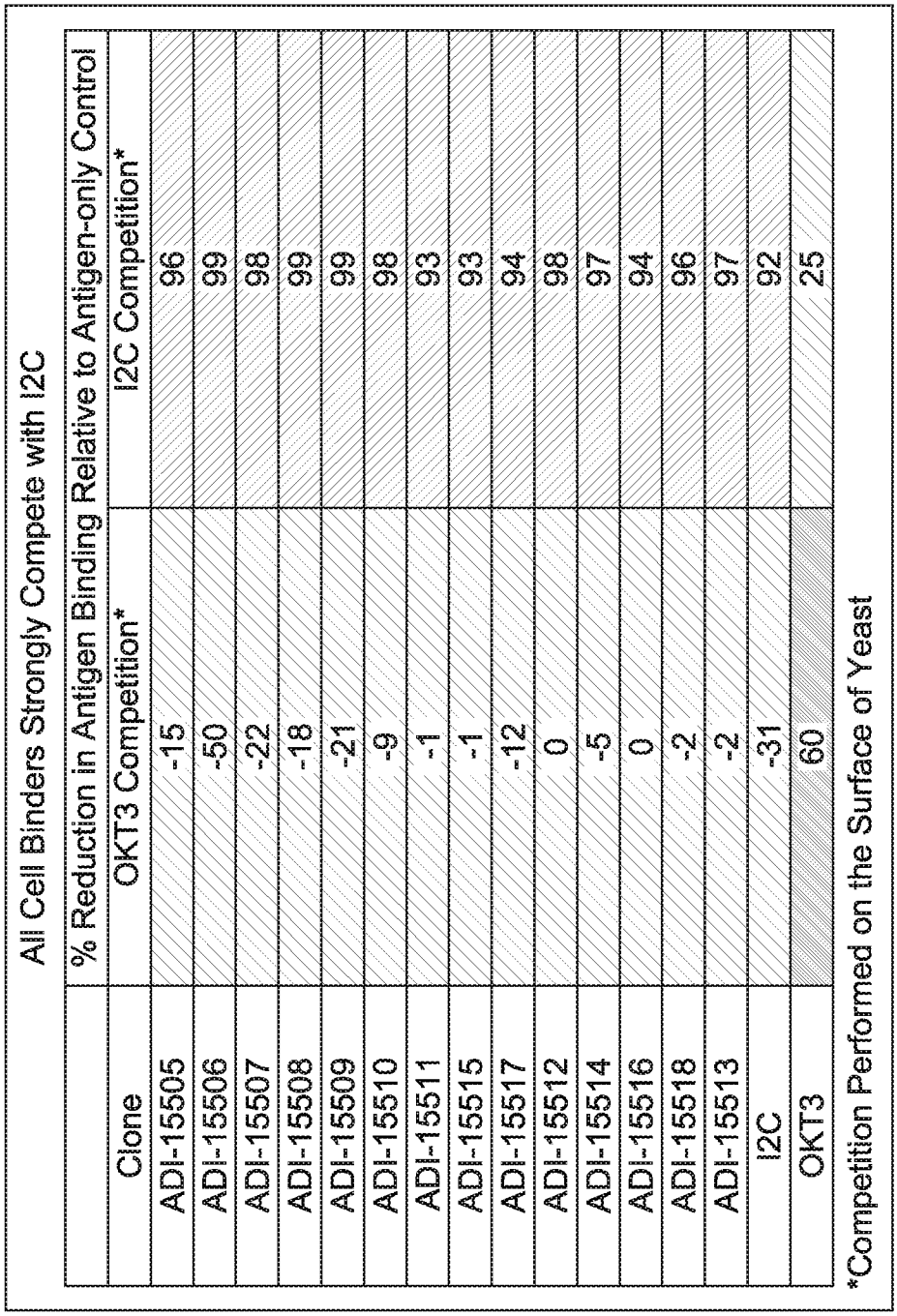
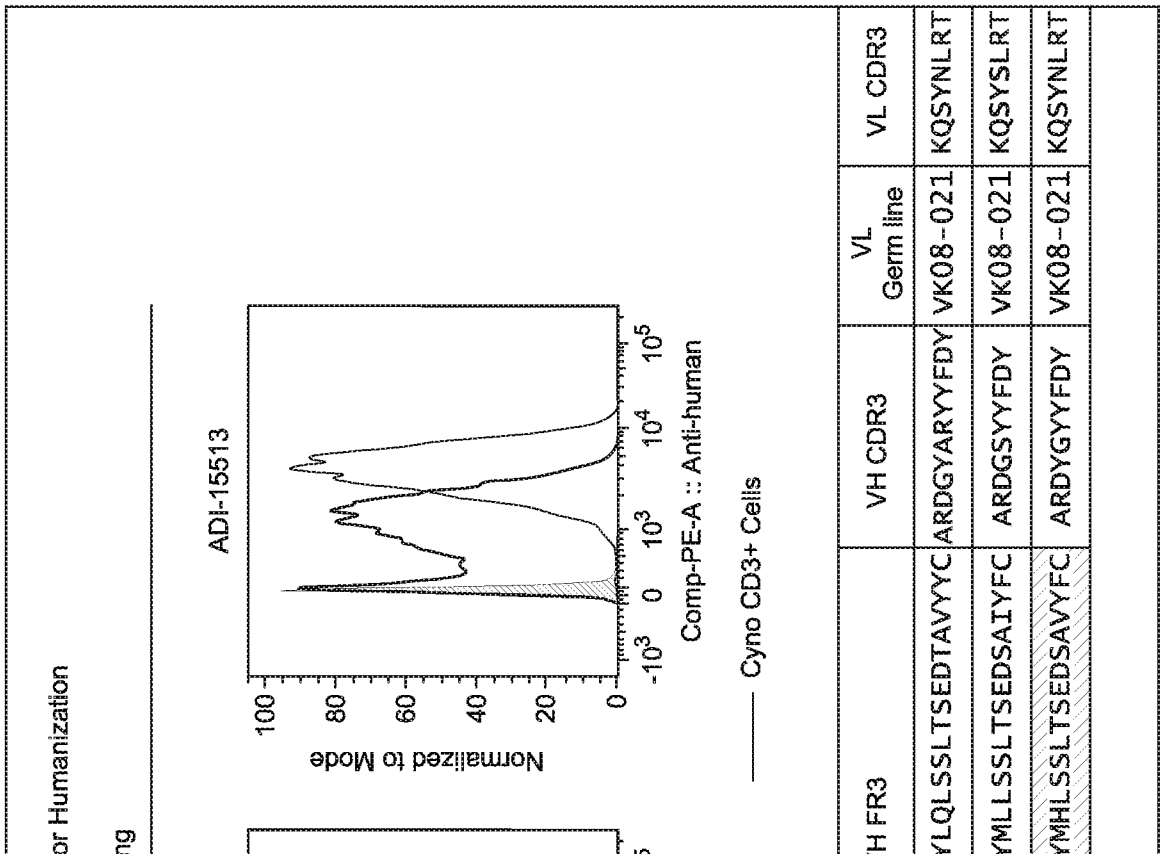

All Cell Binders Strongly Compete with I2C
% Reduction in Antigen Binding Relative to Antigen-only Control
Clone
OKT3 Competition*
I2C Competition*
ADI-15505 -15 96
ADI-15506 -50 99
ADI-15507 -22 98
ADI-15508 -18 99
ADI-15509 -21 99
ADI-15510 -9 98
ADI-15511 -1 93
ADI-15515 -1 93
ADI-15517 -12 94
ADI-15512 0 98
ADI-15514 -5 97
ADI-15516 0 94
ADI-15518 -2 96
ADI-15513 -2 97
I2C -31 92
OKT3 60 25
*Competition Performed on the Surface of Yeast

Figure 8



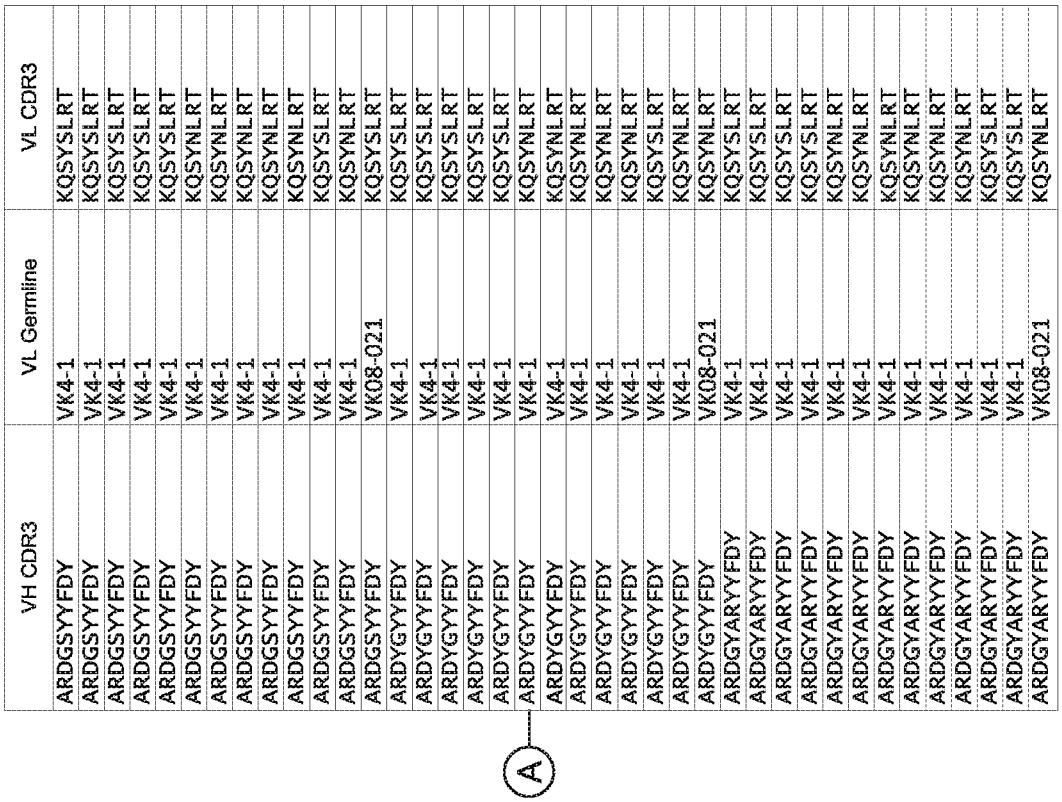
Clone ID	VH Germline	VH CDR1	VH CDR2	VH FR3	VH CDR3	VL Germ line	VL CDR3
ADI-16513	V1-039A	FNIKDYYMH	WIDPENGNTIYDPKFQG	KASITADTSSNTAYLQLSSLTSEDTAVYYC	ARDGYARYYFDY	VK08-021	KQSYNLRT
ADI-15512	V1-125E	YTFTSYYIH	WIYPGDGSTKYNEKFKG	KTTLTADKSSSTAYMLLSSLTSEDSAIYFC	ARDGSYYFDY	VK08-021	KQSYSLRT
ADI-15516	V1-125B	YTFTSSYIH	WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMHLSSLTSEDSAVYFC	ARDYGYYFDY	VK08-021	KQSYNLRT

Figure 9

Notes	Clone ID	VH Germline	VH FR1	VH CDR1	VH FR2	VH CDR2	VH FR3
	ADI-18562	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWMG	WIYPGDGSTKYNEKFKG	RVTMTRDTSTSTVYMELSSLRSEDTAVYYC
	ADI-18564	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWIG	WIYPGDGSTKYNEKFKG	RVTMTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18565	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWMG	WIYPGDGSTKYNEKFKG	RVTMTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18566	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWIG	WIYPGDGSTKYNEKFKG	RTTLTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18567	VH1-46	QVQLVQSGAEVKKPGSSVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWIG	WIYPGDGSTKYNEKFKG	RTTLTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18568	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWMG	WIYPGDGSTKYNEKFKG	RVTMTRDTSTSTVYMELSSLRSEDTAVYYC
	ADI-18570	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWIG	WIYPGDGSTKYNEKFKG	RVTMTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18571	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWMG	WIYPGDGSTKYNEKFKG	RVTMTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18572	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWIG	WIYPGDGSTKYNEKFKG	RTTLTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18573	VH1-46	QVQLVQSGAEVKKPGSSVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWIG	WIYPGDGSTKYNEKFKG	RTTLTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18563	VH1-69	QVQLVQSGAEVKKPGSSVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWMG	WIYPGDGSTKYNEKFKG	RVTITADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18569	VH1-69	QVQLVQSGAEVKKPGSSVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWMG	WIYPGDGSTKYNEKFKG	RVTITADKSTSTAYMELSSLRSEDTAVYYC
Chimeric	ADI-15512	V1-125E	EVQLEQSGPELVKPGASVKMSCKASG	YTFTSYYIH	WVKQRPGQGLEWIG	WIYPGDGSTKYNEKFKG	KTTLTADKSSSTAYMLLSSLTSEDSAIYFC
	ADI-18574	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQRLEWMG	WIYPGNVNTKYNEKFKG	RVTITRDTSASTAYMELSSLRSEDTAVYYC
	ADI-18575	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWIG	WIYPGNVNTKYNEKFKG	RATLTADKSASTAYMHLSSLRSEDTAVYYC
	ADI-18576	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQRLEWIG	WIYPGNVNTKYNEKFKG	RVTITADKSASTAYMELSSLRSEDTAVYYC
	ADI-18578	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWIG	WIYPGNVNTKYNEKFKG	RVTMTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18579	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWIG	WIYPGNVNTKYNEKFKG	RATLTADKSTSTAYMHLSSLRSEDTAVYYC
	ADI-18580	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQRLEWMG	WIYPGNVNTKYNEKFKG	RVTITRDTSASTAYMELSSLRSEDTAVYYC
	ADI-18581	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWIG	WIYPGNVNTKYNEKFKG	RATLTADKSASTAYMHLSSLRSEDTAVYYC
	ADI-18582	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQRLEWIG	WIYPGNVNTKYNEKFKG	RVTITADKSASTAYMELSSLRSEDTAVYYC
	ADI-18584	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWIG	WIYPGNVNTKYNEKFKG	RVTMTADKSTSTAYMELSSLRSEDTAVYYC
	ADI-18585	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWIG	WIYPGNVNTKYNEKFKG	RATLTADKSTSTAYMHLSSLRSEDTAVYYC
	ADI-18577	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWMG	WIYPGNVNTKYNEKFKG	RVTMTRDTSTSTVYMELSSLRSEDTAVYYC
	ADI-18583	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWMG	WIYPGNVNTKYNEKFKG	RVTMTRDTSTSTVYMELSSLRSEDTAVYYC
Chimeric	ADI-15516	V1-125B	EVQLEESGPELVRPGASVRISCKASG	YTFTSSYIH	WVKQRPGQGLEWIG	WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMHLSSLTSEDSAVYFC
	ADI-18588	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQGLEWIG	WIDPENGNTIYDPKFQG	RATITADTSTNTAYMELSSLRSEDTAVYYC
	ADI-18589	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWMG	WIDPENGNTIYDPKFQG	RVTITRDTSASTAYMELSSLRSEDTAVYYC
	ADI-18590	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWIG	WIDPENGNTIYDPKFQG	RVTITADTSASTAYMELSSLRSEDTAVYYC
	ADI-18591	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWIG	WIDPENGNTIYDPKFQG	RATITADTSANTAYMELSSLRSEDTAVYYC
	ADI-18593	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQGLEWIG	WIDPENGNTIYDPKFQG	RATITADTSTNTAYMELSSLRSEDTAVYYC
	ADI-18594	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWMG	WIDPENGNTIYDPKFQG	RVTITRDTSASTAYMELSSLRSEDTAVYYC
	ADI-18595	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWIG	WIDPENGNTIYDPKFQG	RVTITADTSASTAYMELSSLRSEDTAVYYC
	ADI-18596	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWIG	WIDPENGNTIYDPKFQG	RATITADTSANTAYMELSSLRSEDTAVYYC
	ADI-18597	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQGLEWIG	WIDPENGNTIYDPKFQG	RVTMTADTSTSTAYMELSSLRSEDTAVYYC
	ADI-18592	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQGLEWMG	WIDPENGNTIYDPKFQG	RVTMTRDTSTSTVYMELSSLRSEDTAVYYC
	ADI-18587	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQGLEWIG	WIDPENGNTIYDPKFQG	RVTMTADTSTSTAYMELSSLRSEDTAVYYC
	ADI-18586	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQGLEWMG	WIDPENGNTIYDPKFQG	RVTMTRDTSTSTVYMELSSLRSEDTAVYYC
Chimeric	ADI-16513	V1-039A	QVQLEQSGAELVRPGALVKLSCKASG	FNIKDYYMH	WVKQRPEQGLEWIG	WIDPENGNTIYDPKFQG	KASITADTSSNTAYLQLSSLTSEDTAVYYC

A

Figure 10A



VH CDR3	VL Germline	VL CDR3
ARDGSYYFDY	VK4-1	KQSYSLRT
ARDGSYYFDY	VK4-1	KQSYSLRT
ARDGSYYFDY	VK4-1	KQSYSLRT
ARDGSYYFDY	VK4-1	KQSYSLRT
ARDGSYYFDY	VK4-1	KQSYSLRT
ARDGSYYFDY	VK4-1	KQSYNLRT
ARDGSYYFDY	VK4-1	KQSYNLRT
ARDGSYYFDY	VK4-1	KQSYNLRT
ARDGSYYFDY	VK4-1	KQSYNLRT
ARDGSYYFDY	VK4-1	KQSYNLRT
ARDGSYYFDY	VK4-1	KQSYSLRT
ARDGSYYFDY	VK4-1	KQSYNLRT
ARDGSYYFDY	VK08-021	KQSYSLRT
ARDYGYYFDY	VK4-1	KQSYSLRT
ARDYGYYFDY	VK4-1	KQSYSLRT
ARDYGYYFDY	VK4-1	KQSYSLRT
ARDYGYYFDY	VK4-1	KQSYSLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYSLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDYGYYFDY	VK4-1	KQSYNLRT
ARDGYARYYFDY	VK4-1	KQSYSLRT
ARDGYARYYFDY	VK4-1	KQSYSLRT
ARDGYARYYFDY	VK4-1	KQSYSLRT
ARDGYARYYFDY	VK4-1	KQSYSLRT
ARDGYARYYFDY	VK4-1	KQSYNLRT
ARDGYARYYFDY	VK4-1	KQSYNLRT
ARDGYARYYFDY	VK4-1	KQSYNLRT
ARDGYARYYFDY	VK4-1	KQSYNLRT
ARDGYARYYFDY	VK4-1	KQSYNLRT
ARDGYARYYFDY	VK4-1	KQSYSLRT
ARDGYARYYFDY	VK4-1	KQSYSLRT
ARDGYARYYFDY	VK08-021	KQSYNLRT

Figure 10A (Continued)

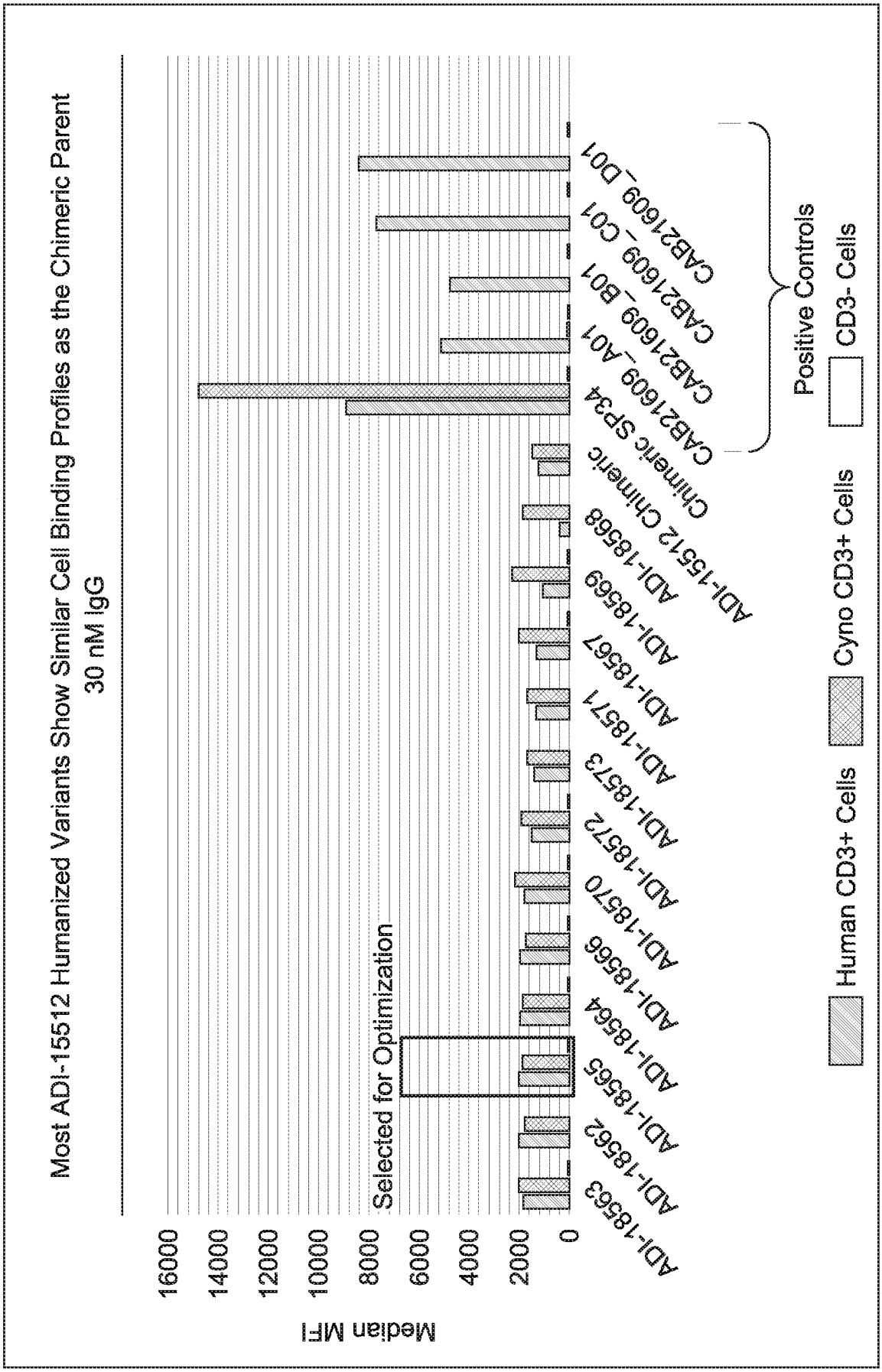

Most ADI-15512 Humanized Variants Show Similar Cell Binding Profiles as the Chimeric Parent
30 nM IgG
Median MFI
16000
14000
12000
10000
8000
6000
4000
2000
0
Selected for Optimization
ADI-18563
ADI-18562
ADI-18565
ADI-18564
ADI-18566
ADI-18570
ADI-18572
ADI-18573
ADI-18571
ADI-18567
ADI-18569
ADI-18568
ADI-15512 Chimeric
Chimeric SP34
CAB21609_A01
CAB21609_B01
CAB21609_C01
CAB21609_D01
Positive Controls
Human CD3+ Cells
Cyno CD3+ Cells
CD3- Cells

Figure 10B

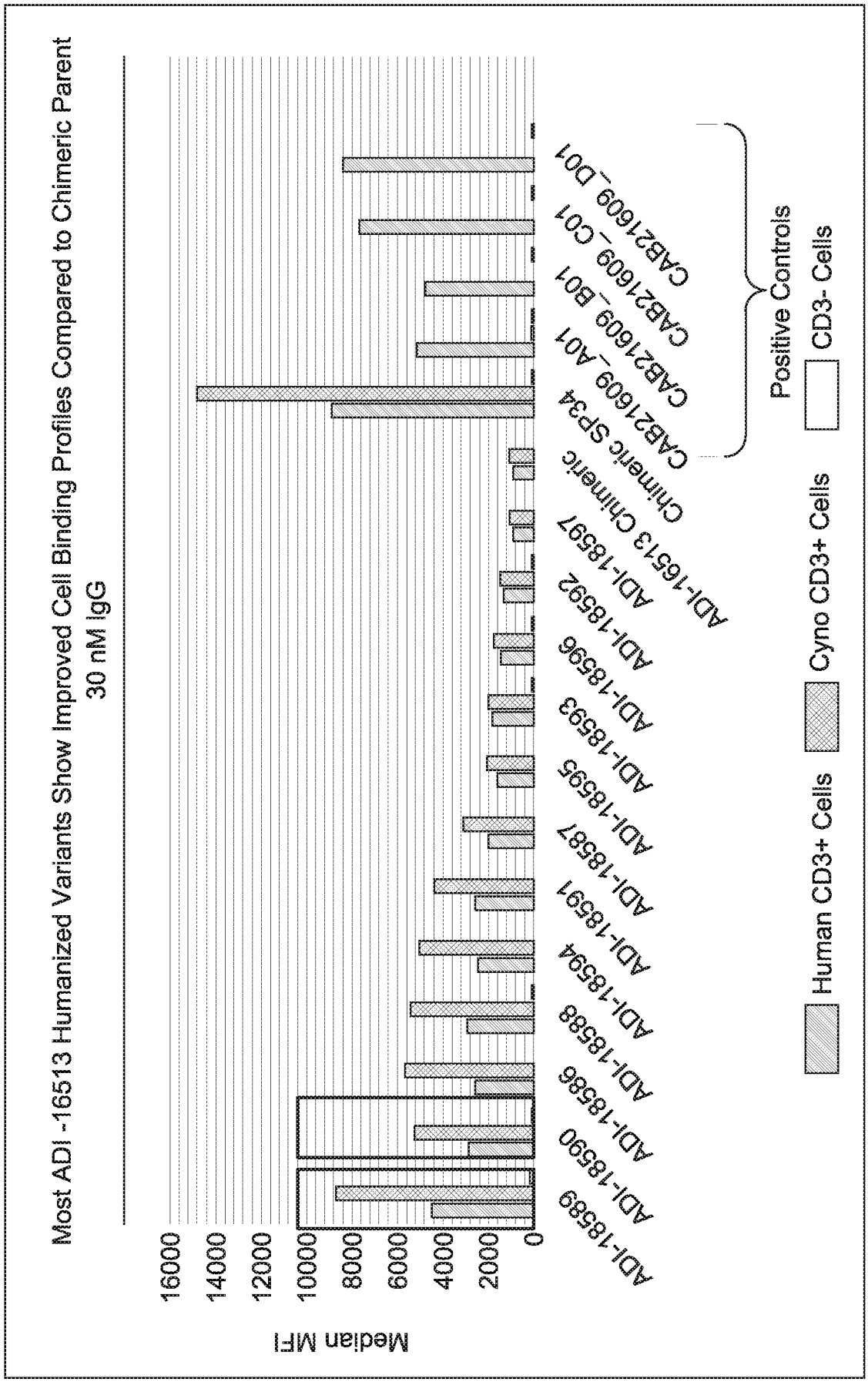

Most ADI -16513 Humanized Variants Show Improved Cell Binding Profiles Compared to Chimeric Parent
30 nM IgG
Median MFI
16000
14000
12000
10000
8000
6000
4000
2000
0
ADI-18589
ADI-18590
ADI-18586
ADI-18588
ADI-18594
ADI-18591
ADI-18587
ADI-18595
ADI-18593
ADI-18596
ADI-18592
ADI-18597
ADI-16513 Chimeric
CAB21609_A01
CAB21609_B01
CAB21609_C01
CAB21609_D01
Positive Controls
Human CD3+ Cells
Cyno CD3+ Cells
CD3- Cells

Figure 10C

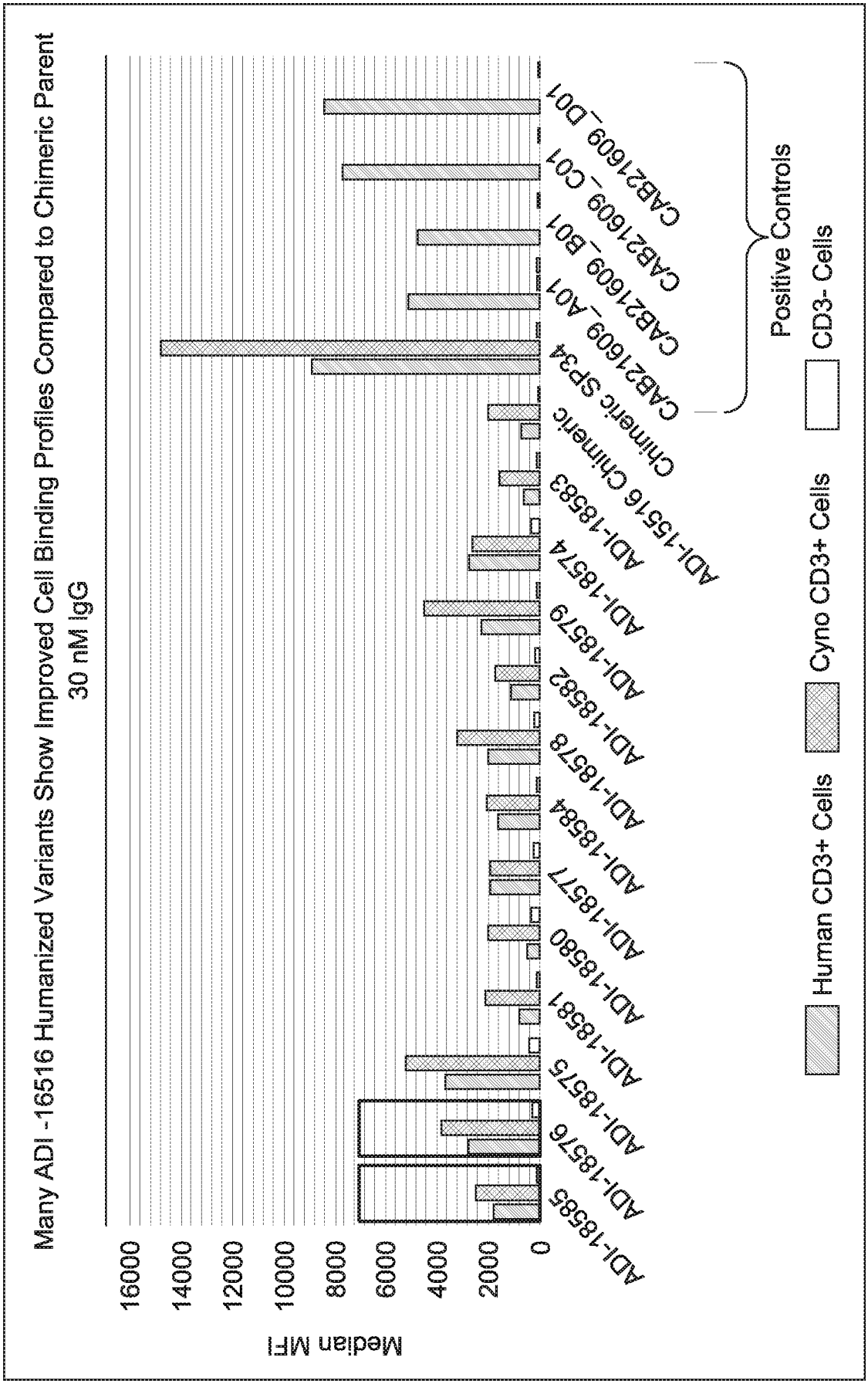

Many ADI -16516 Humanized Variants Show Improved Cell Binding Profiles Compared to Chimeric Parent
30 nM IgG
Median MFI
16000
14000
12000
10000
8000
6000
4000
2000
0
ADI-18585
ADI-18576
ADI-18575
ADI-18581
ADI-18580
ADI-18577
ADI-18584
ADI-18578
ADI-18582
ADI-18579
ADI-18574
ADI-18583
ADI-15516 Chimeric
Chimeric SP34
CAB21609_A01
CAB21609_B01
CAB21609_C01
CAB21609_D01
Positive Controls
Human CD3+ Cells
Cyno CD3+ Cells
CD3- Cells

Figure 10D

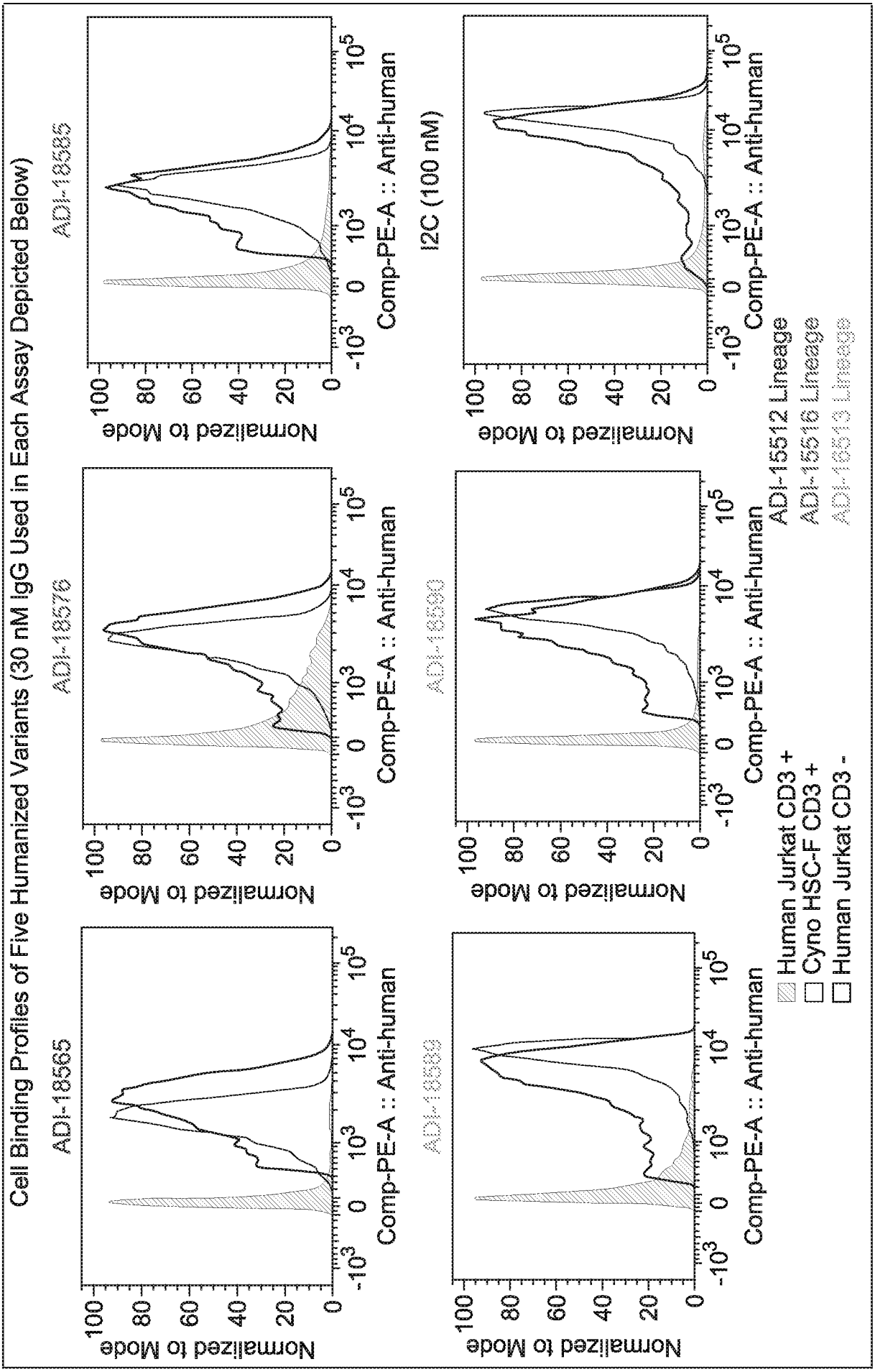

Cell Binding Profiles of Five Humanized Variants (30 nM IgG Used in Each Assay Depicted Below)
ADI-18565
ADI-18576
ADI-18585
ADI-18588
ADI-18590
I2C (100 nM)
Normalized to Mode
Comp-PE-A :: Anti-human
Human Jurkat CD3 +
Cyno HSC-F CD3 +
Human Jurkat CD3 -
ADI-15512 Lineage
ADI-15516 Lineage
ADI-16513 Lineage

Figure 11A

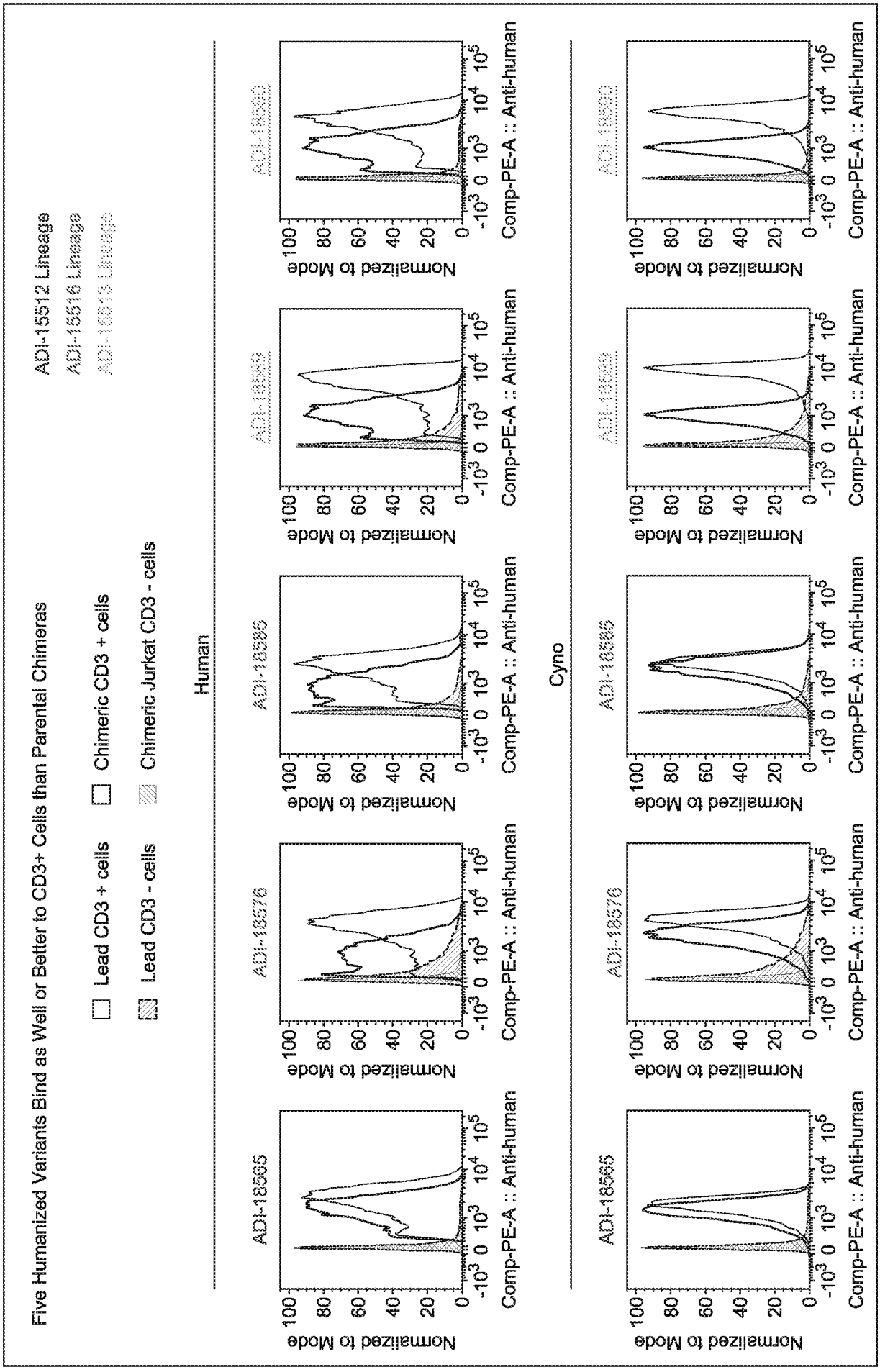

Five Humanized Variants Bind as Well or Better to CD3+ Cells than Parental Chimeras
Lead CD3 + cells
Chimeric CD3 + cells
Lead CD3 - cells
Chimeric Jurkat CD3 - cells
ADI-15512 Lineage
ADI-15516 Lineage
ADI-15813 Lineage
Human
Cyno
ADI-18565
ADI-18576
ADI-18585
ADI-18589
ADI-18590
Normalized to Mode
Comp-PE-A :: Anti-human

Figure 11B

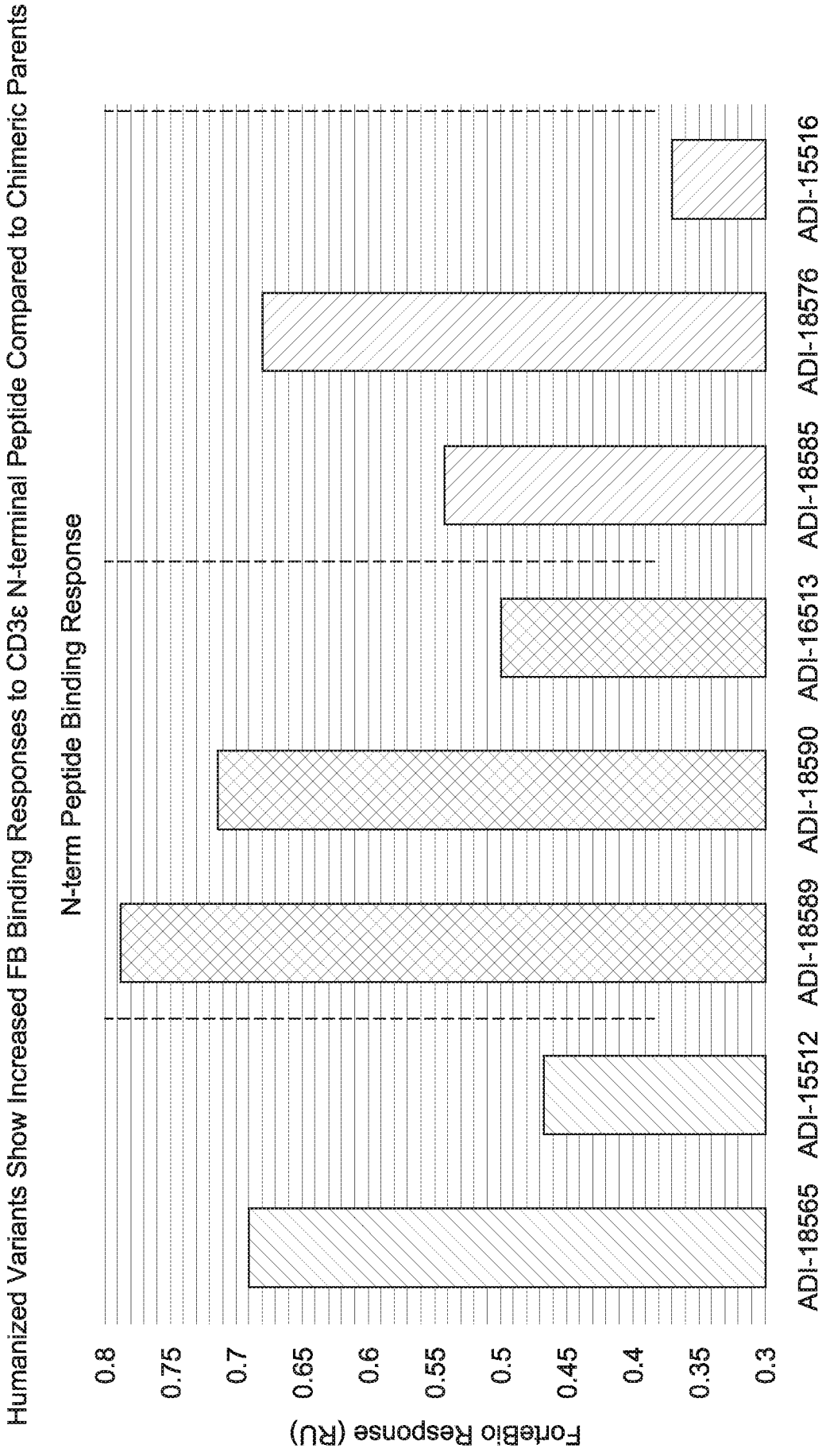

Humanized Variants Show Increased FB Binding Responses to CD3ε N-terminal Peptide Compared to Chimeric Parents
N-term Peptide Binding Response
ForteBio Response (RU)
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
ADI-18565
ADI-15512
ADI-18589
ADI-18590
ADI-16513
ADI-18585
ADI-18576
ADI-15516

Figure 11C

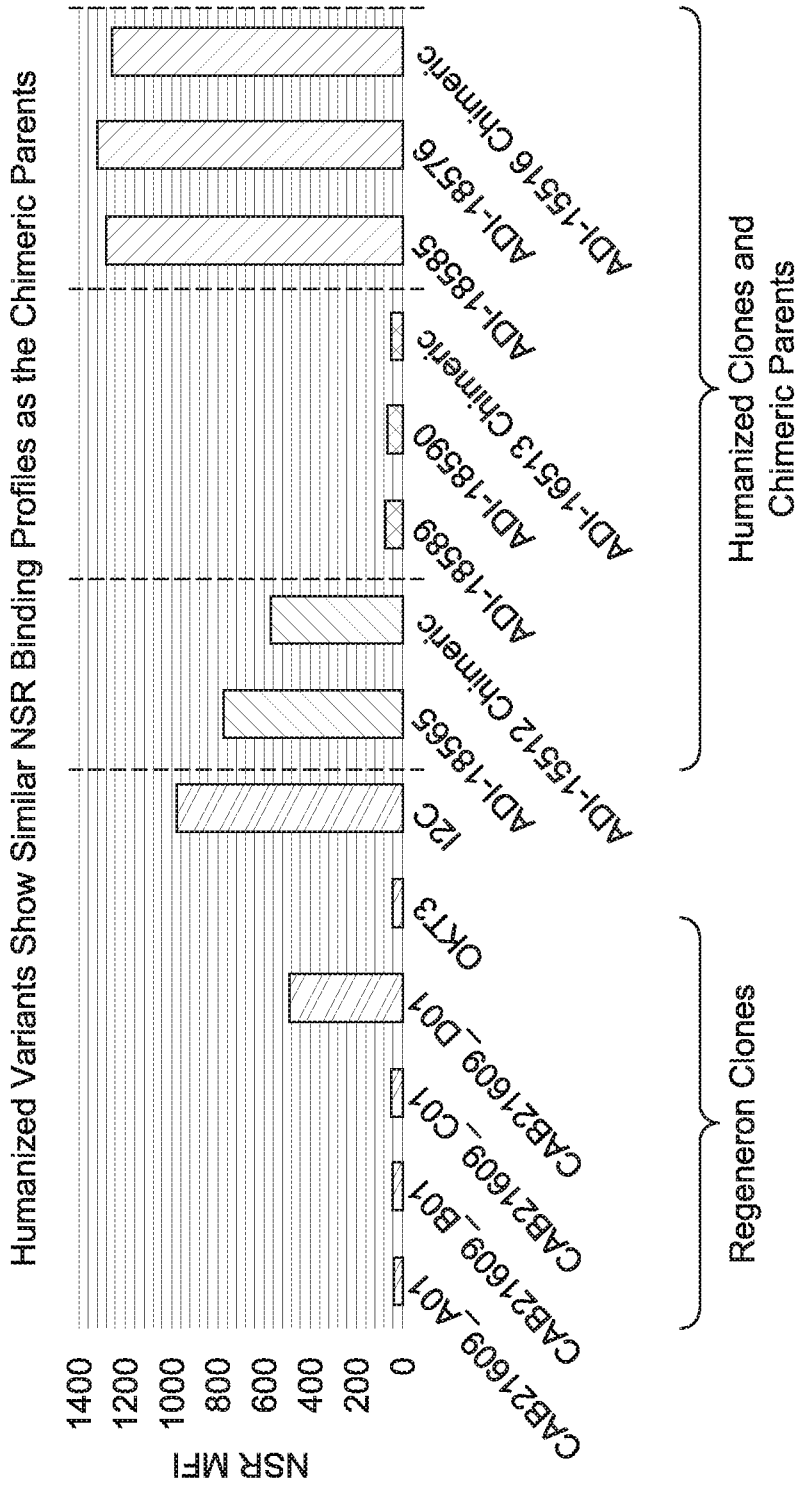

Humanized Variants Show Similar NSR Binding Profiles as the Chimeric Parents
NSR MFI
1400
1200
1000
800
600
400
200
0
CAB21609_A01
CAB21609_B01
CAB21609_C01
CAB21609_D01
OKT3
I2C
ADI-18565
ADI-15512 Chimeric
ADI-18589
ADI-18590
ADI-16513 Chimeric
ADI-18585
ADI-18576
ADI-15516 Chimeric
Regeneron Clones
Humanized Clones and
Chimeric Parents

Figure 12A

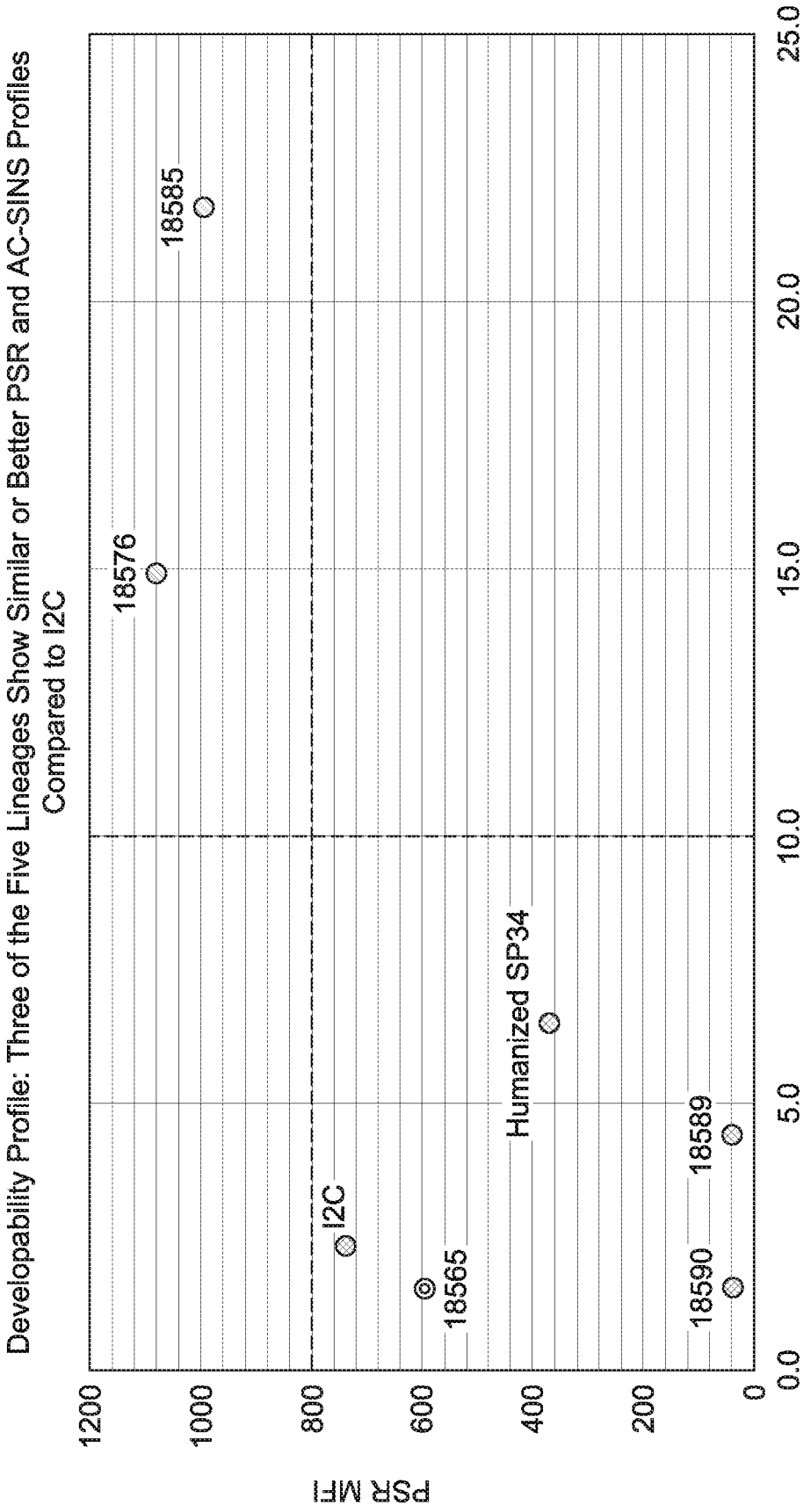

Developability Profile: Three of the Five Lineages Show Similar or Better PSR and AC-SINS Profiles Compared to I2C
PSR MFI
AC-SINS (Δλmax)
0
200
400
600
800
1000
1200
0.0
5.0
10.0
15.0
20.0
25.0
18590
18589
Humanized SP34
18565
I2C
18576
18585

Figure 12B

Sequences of Humanized Clones Selected for Optimization and their Respective Parents

	Clone ID	VH Germ line	VH FR1	VH CDR1	VH FR2
Lead	ADI-18565	VH1-46	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSYYIH	WVRQAPGQGLEWMG
Parent	ADI-15512	V1-125E	EVQLEQSGPELVKPGASVKMSCKASG	YTFTSYYIH	WVKQRPGQGLEWIG
Lead	ADI-18589	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWMG
Lead	ADI-18590	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	FNIKDYYMH	WVRQAPGQRLEWIG
Parent	ADI-16513	V1-039A	QVQLEQSGAELVRPGALVKLSCKASG	FNIKDYYMH	WVKQRPEQGLEWIG
Lead	ADI-18576	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQRLEWIG
Lead	ADI-18585	VH1-3	QVQLVQSGAEVKKPGASVKVSCKASG	YTFTSSYIH	WVRQAPGQGLEWIG
Parent	ADI-15516	V1-125B	EVQLEESGPELVRPGASVRISCKASG	YTFTSSYIH	WVKQRPGQGLEWIG

Ⓐ

	Clone ID	VL Germ line	VL FR1	VL CDR1
Lead	ADI-18565	VK4-1	DIVMTQSPDSLAVSLGERATINC	KSSQSLLNSRTRKNYLA
Parent	ADI-15512	VK08-021	DIQLTQSPSSLAVSAGEKVTMSC	KSSQSLLNSRTRKNYLA
Lead	ADI-18589	VK4-1	DIVMTQSPDSLAVSLGERATINC	KSSQSLLNSRTRKNYLA
Lead	ADI-18590	VK4-1	DIVMTQSPDSLAVSLGERATINC	KSSQSLLNSRTRKNYLA
Parent	ADI-16513	VK08-021	DIQLTQSPSSLAVSAGEKVTMSC	KSSQSLLNSRTRKNYLA
Lead	ADI-18576	VK4-1	DIVMTQSPDSLAVSLGERATINC	KSSQSLLNSRTRKNYLA
Lead	ADI-18585	VK4-1	DIVMTQSPDSLAVSLGERATINC	KSSQSLLNSRTRKNYLA
Parent	ADI-15516	VK08-021	DIVLTQSPSSLAVSAGEKVTMSC	KSSQSLLNSRTRKNYLA

Figure 13

Sequences of Humanized Clones Selected for Optimization and their Respective Parents		
VH CDR2	VH FR3	VH CDR3
WIYPGDGSTKYNEKFKG	RVTMTADKSTSTAYMELSSLRSEDTAVYYC	ARDGSYYFDY
WIYPGDGSTKYNEKFKG	KTTLTADKSSSTAYMLLSSLTSEDSAIYFC	ARDGSYYFDY
WIDPENGNTIYDPKFQG	RVTITRDTSASTAYMELSSLRSEDTAVYYC	ARDGYARYYFDY
WIDPENGNTIYDPKFQG	RVTITADTSASTAYMELSSLRSEDTAVYYC	ARDGYARYYFDY
WIDPENGNTIYDPKFQG	KASITADTSSNTAYLQLSSLTSEDTAVYYC	ARDGYARYYFDY
WIYPGNVNTKYNEKFKG	RVTITADKSASTAYMELSSLRSEDTAVYYC	ARDYGYYFDY
WIYPGNVNTKYNEKFKG	RATLTADKSTSTAYMHLSSLRSEDTAVYYC	ARDYGYYFDY
WIYPGNVNTKYNEKFKG	KATLTADKSSSTAYMHLSSLTSEDSAVYFC	ARDYGYYFDY

Ⓐ

VL FR2	VL CDR2	VL FR3	VL CDR3
WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEOVAVYYC	KQSYSLRT
WYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVQAEOLAVYYC	KQSYSLRT
WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	KQSYSLRT
WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	KQSYSLRT
WYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC	KQSYNLRT
WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	KQSYSLRT
WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	KQSYNLRT
WYQQKAGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC	KQSYNLRT

Figure 13 (Continued)

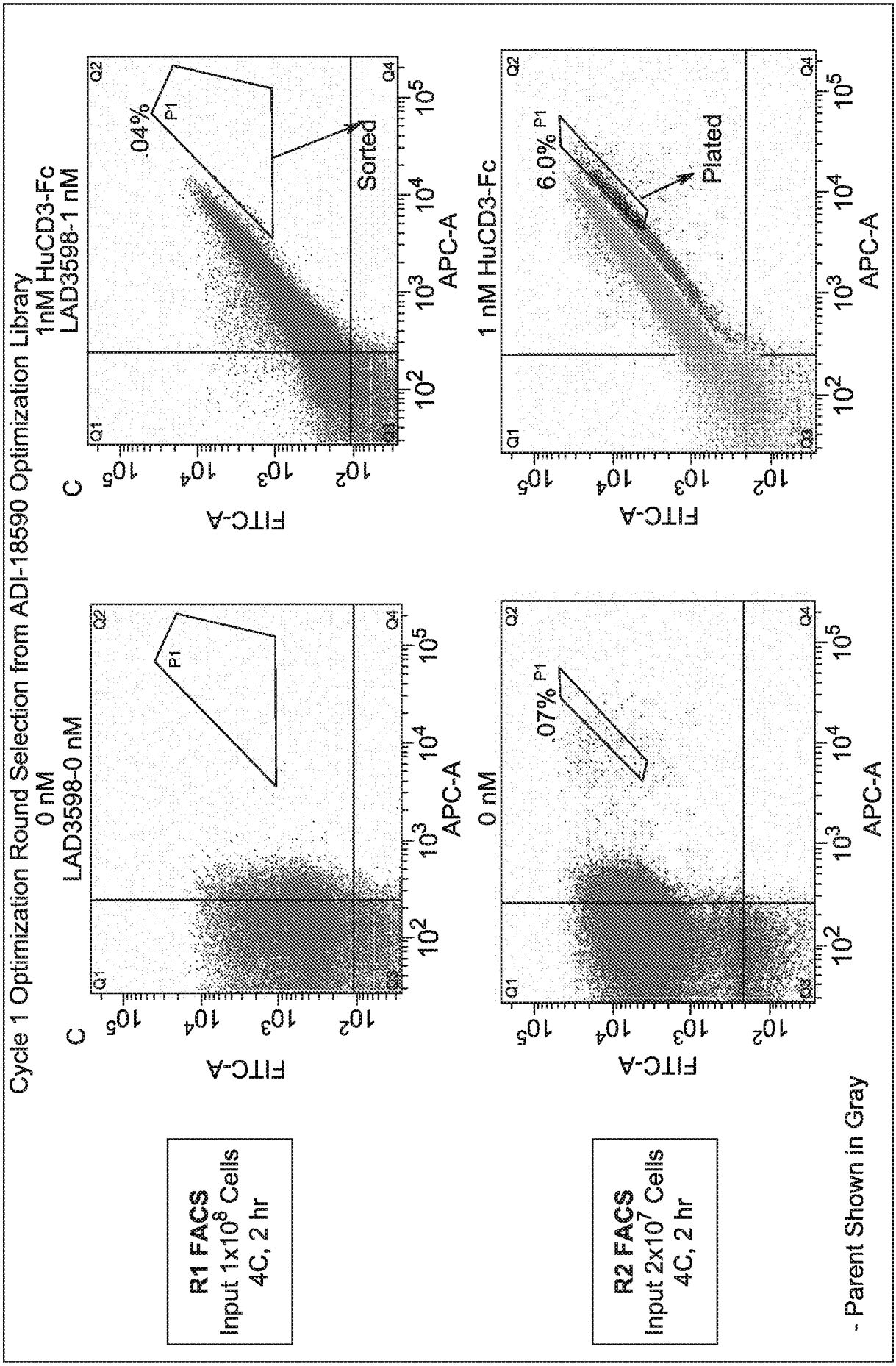

Cycle 1 Optimization Round Selection from ADI-18590 Optimization Library
0 nM
LAD3598-0 nM
1nM HuCD3-Fc
LAD3598-1 nM
1 nM HuCD3-Fc
0 nM
C
FITC-A
APC-A
P1
Q1
Q2
Q3
Q4
.04%
Sorted
6.0%
Plated
.07%
R1 FACS
Input 1x10^8 Cells
4C, 2 hr
R2 FACS
Input 2x10^7 Cells
4C, 2 hr
- Parent Shown in Gray

Figure 14

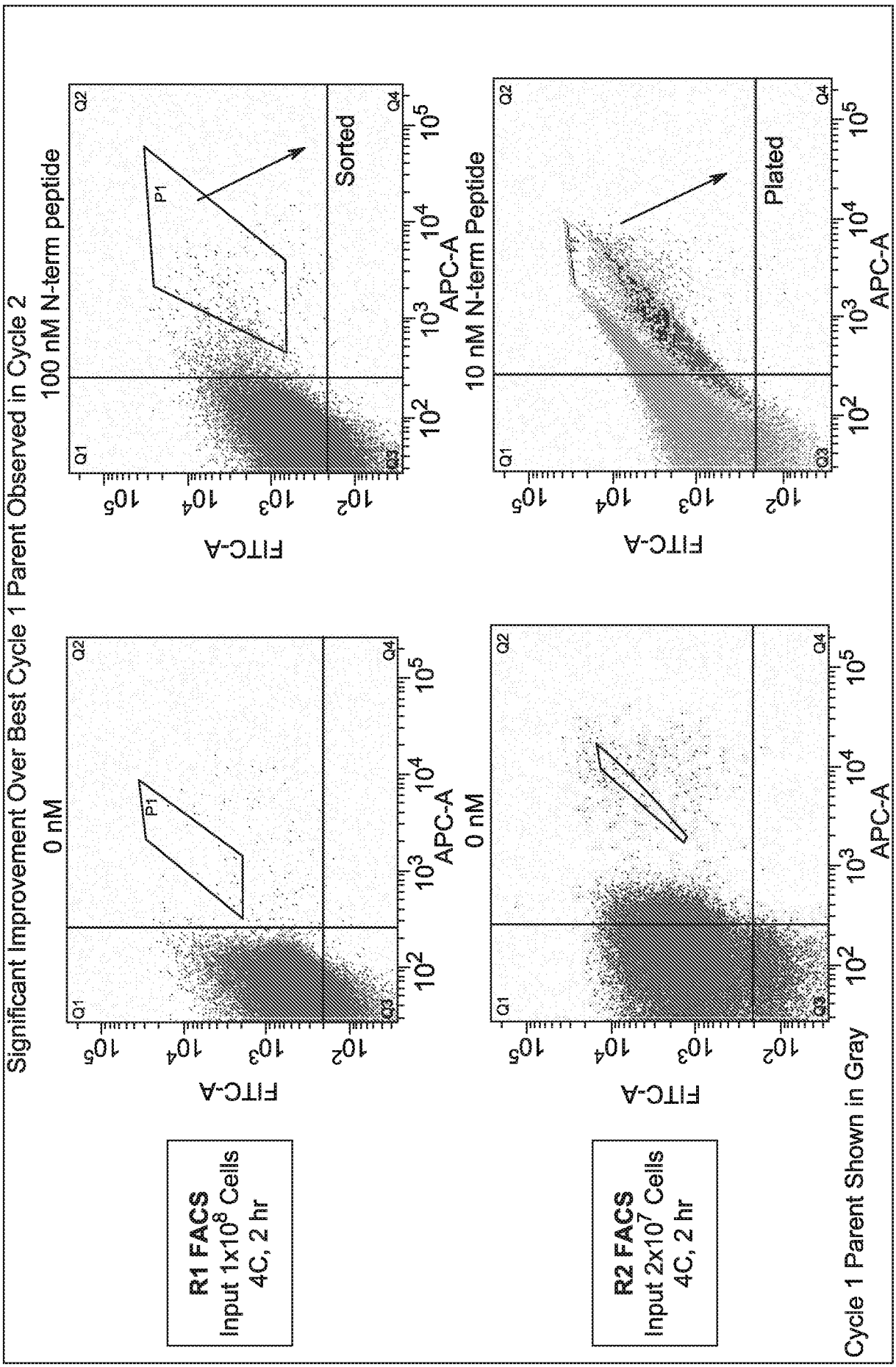

Significant Improvement Over Best Cycle 1 Parent Observed in Cycle 2
100 nM N-term peptide
Sorted
10 nM N-term Peptide
Plated
0 nM
0 nM
R1 FACS
Input 1x10^8 Cells
4C, 2 hr
R2 FACS
Input 2x10^7 Cells
4C, 2 hr
FITC-A
APC-A
P1
Q1
Q2
Q3
Q4
Cycle 1 Parent Shown in Gray

Figure 15

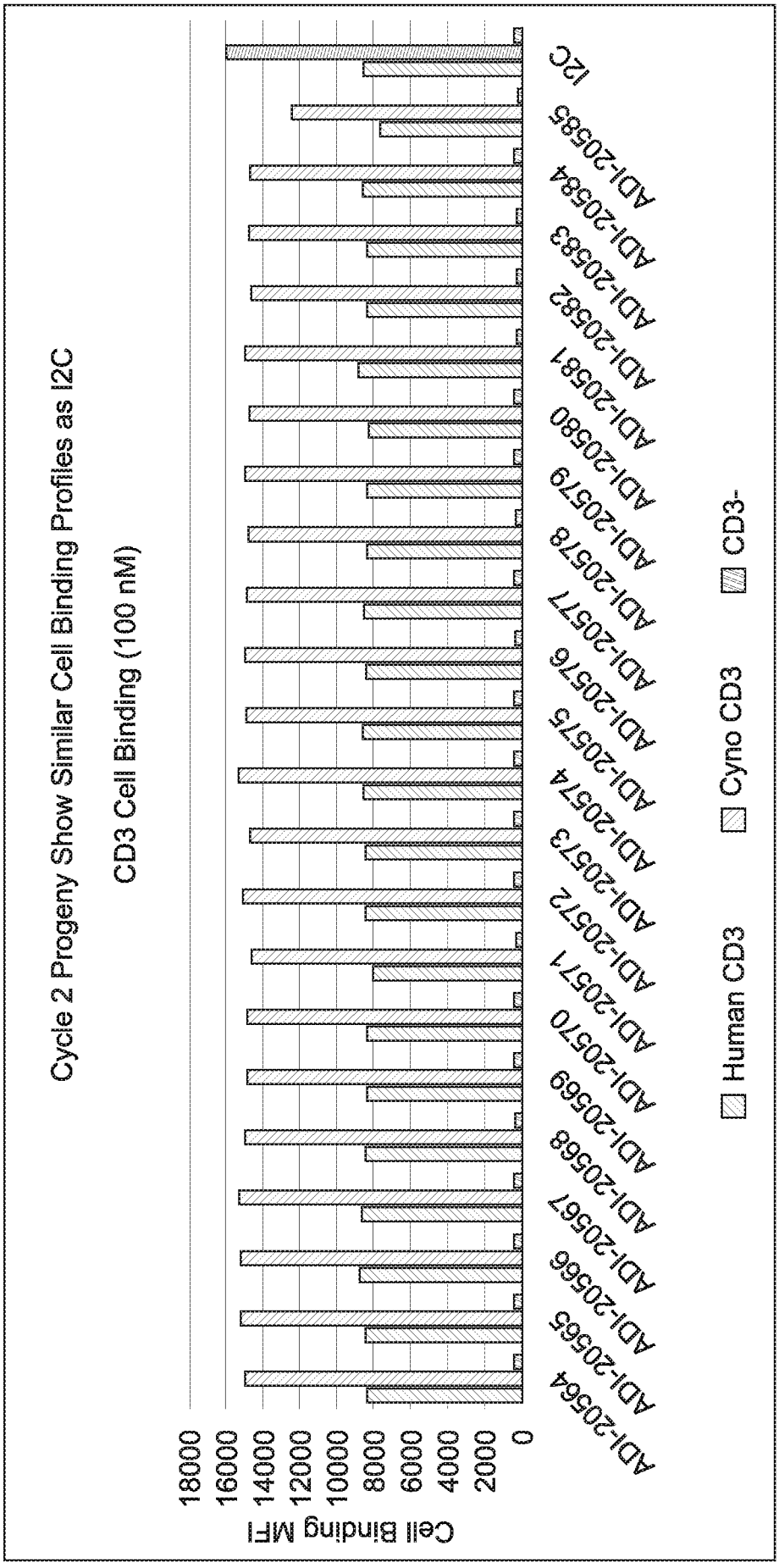

Cycle 2 Progeny Show Similar Cell Binding Profiles as I2C
CD3 Cell Binding (100 nM)
Cell Binding MFI
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
ADI-20564
ADI-20565
ADI-20566
ADI-20567
ADI-20568
ADI-20569
ADI-20570
ADI-20571
ADI-20572
ADI-20573
ADI-20574
ADI-20575
ADI-20576
ADI-20577
ADI-20578
ADI-20579
ADI-20580
ADI-20581
ADI-20582
ADI-20583
ADI-20584
ADI-20585
I2C
Human CD3
Cyno CD3
CD3-

Figure 16

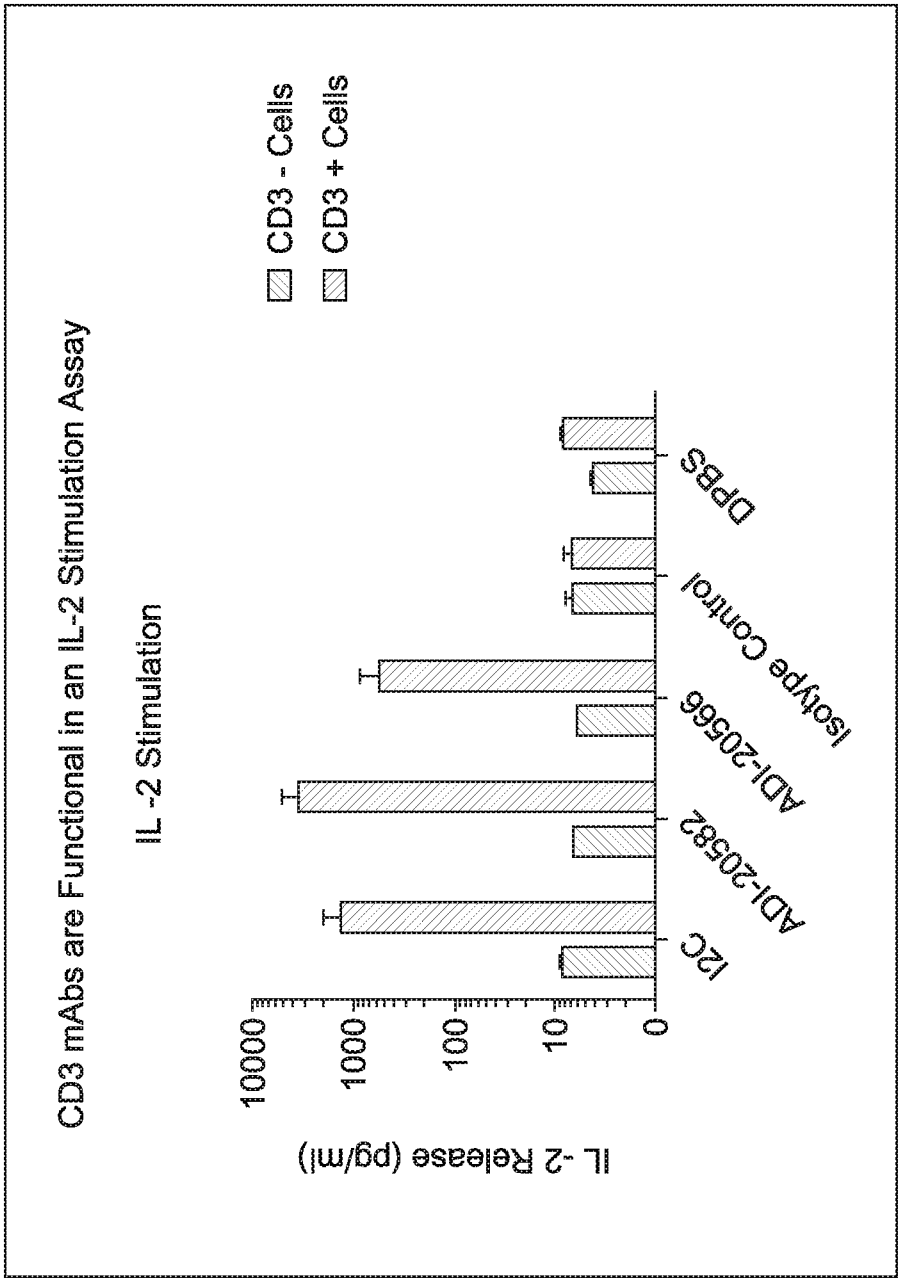

CD3 mAbs are Functional in an IL-2 Stimulation Assay
IL -2 Stimulation
CD3 - Cells
CD3 + Cells
IL -2 Release (pg/ml)
10000
1000
100
10
0
I2C
ADI-20582
ADI-20566
Isotype Control
DPBS

Figure 17

Recombinant Heterodimeric CD3 Fc Fusion Antigens
Hu QDGNEEMGGIT...
Cy QDGNEEMGSIT...
α β
ε γ
δ ε
TCR/CD3 complex
Hu CD3εδ Fc
Cy CD3εδ Fc
Hu CD3ε Fc
QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVCENCMEMD
GGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK
Hu CD3δ Fc-HIS
FKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMCQSCVELDGGSDKTHTCPPCPAPELLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKHHHHHH
Cy CD3ε Fc
QDGNEEMGSITQTPYQVSISGTTVILTCSQHLGSEAQWQHNGKNKEDSGDRLFLPEFSEMEQSGYYVCYPRGSNPEDASHHLYLKARVCENCMEMD
GGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK
Cy CD3δ Fc-HIS
FKIPVEELEDRVFVNCNTSVTWVEGTVGTLLTNNTRLDLGKRILDPRGIYRCNGTDIYKDKESAVQVHYRMCQNCVELDPGSDKTHTCPPCPAPELLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKHHHHHH

Figure 18

Parent	Lineage	Selection Type	Theoretical Diversity	Transformation Efficiency
ADI-20580	ADI-18589	H3 Oligo Mutagenesis	$>10^4$	$2x10^6$

H3 NNK Diversification Scheme

DTAVYYCXXDGYARYYFDYWGQGTLVTVSS	DTAVYYCARDXYARXYFDYWGQGTLVTVSS
DTAVYYCXRXGYARYYFDYWGQGTLVTVSS	DTAVYYCARDXYARYXFDYWGQGTLVTVSS
DTAVYYCXRDXYARYYFDYWGQGTLVTVSS	DTAVYYCARDXYARYYXDYWGQGTLVTVSS
DTAVYYCXRDGXARYYFDYWGQGTLVTVSS	DTAVYYCARDXYARYYFXYWGQGTLVTVSS
DTAVYYCXRDGYXRYYFDYWGQGTLVTVSS	DTAVYYCARDXYARYYFDXWGQGTLVTVSS
DTAVYYCXRDGYAXYYFDYWGQGTLVTVSS	DTAVYYCARDGXXRYYFDYWGQGTLVTVSS
DTAVYYCXRDGYARXYFDYWGQGTLVTVSS	DTAVYYCARDGXAXYYFDYWGQGTLVTVSS
DTAVYYCXRDGYARYXFDYWGQGTLVTVSS	DTAVYYCARDGXARXYFDYWGQGTLVTVSS
DTAVYYCXRDGYARYYXDYWGQGTLVTVSS	DTAVYYCARDGXARYXFDYWGQGTLVTVSS
DTAVYYCXRDGYARYYFXYWGQGTLVTVSS	DTAVYYCARDGXARYYXDYWGQGTLVTVSS
DTAVYYCXRDGYARYYFDXWGQGTLVTVSS	DTAVYYCARDGXARYYFXYWGQGTLVTVSS
DTAVYYCAXXGYARYYFDYWGQGTLVTVSS	DTAVYYCARDGXARYYFDXWGQGTLVTVSS
DTAVYYCAXDXYARYYFDYWGQGTLVTVSS	DTAVYYCARDGYXXYYFDYWGQGTLVTVSS
DTAVYYCAXDGXARYYFDYWGQGTLVTVSS	DTAVYYCARDGYXRXYFDYWGQGTLVTVSS
DTAVYYCAXDGYXRYYFDYWGQGTLVTVSS	DTAVYYCARDGYXRYXFDYWGQGTLVTVSS
DTAVYYCAXDGYAXYYFDYWGQGTLVTVSS	DTAVYYCARDGYXRYYXDYWGQGTLVTVSS
DTAVYYCAXDGYARXYFDYWGQGTLVTVSS	DTAVYYCARDGYXRYYFXYWGQGTLVTVSS
DTAVYYCAXDGYARYXFDYWGQGTLVTVSS	DTAVYYCARDGYXRYYFDXWGQGTLVTVSS
DTAVYYCAXDGYARYYXDYWGQGTLVTVSS	DTAVYYCARDGYAXXYFDYWGQGTLVTVSS
DTAVYYCAXDGYARYYFXYWGQGTLVTVSS	DTAVYYCARDGYAXYXFDYWGQGTLVTVSS
DTAVYYCAXDGYARYYFDXWGQGTLVTVSS	DTAVYYCARDGYAXYYXDYWGQGTLVTVSS
DTAVYYCARXXYARYYFDYWGQGTLVTVSS	DTAVYYCARDGYAXYYFXYWGQGTLVTVSS
DTAVYYCARXGXARYYFDYWGQGTLVTVSS	DTAVYYCARDGYAXYYFDXWGQGTLVTVSS
DTAVYYCARXGYXRYYFDYWGQGTLVTVSS	DTAVYYCARDGYARXXFDYWGQGTLVTVSS
DTAVYYCARXGYAXYYFDYWGQGTLVTVSS	DTAVYYCARDGYARXYXDYWGQGTLVTVSS
DTAVYYCARXGYARXYFDYWGQGTLVTVSS	DTAVYYCARDGYARXYFXYWGQGTLVTVSS
DTAVYYCARXGYARYXFDYWGQGTLVTVSS	DTAVYYCARDGYARXYFDXWGQGTLVTVSS
DTAVYYCARXGYARYYXDYWGQGTLVTVSS	DTAVYYCARDGYARYXXDYWGQGTLVTVSS
DTAVYYCARXGYARYYFXYWGQGTLVTVSS	DTAVYYCARDGYARYXFXYWGQGTLVTVSS
DTAVYYCARXGYARYYFDXWGQGTLVTVSS	DTAVYYCARDGYARYXFDXWGQGTLVTVSS
DTAVYYCARDXXARYYFDYWGQGTLVTVSS	DTAVYYCARDGYARYYXXYWGQGTLVTVSS
DTAVYYCARDXYXRYYFDYWGQGTLVTVSS	DTAVYYCARDGYARYYXDXWGQGTLVTVSS
DTAVYYCARDXYAXYYFDYWGQGTLVTVSS	DTAVYYCARDGYARYYFXXWGQGTLVTVSS

Figure 19

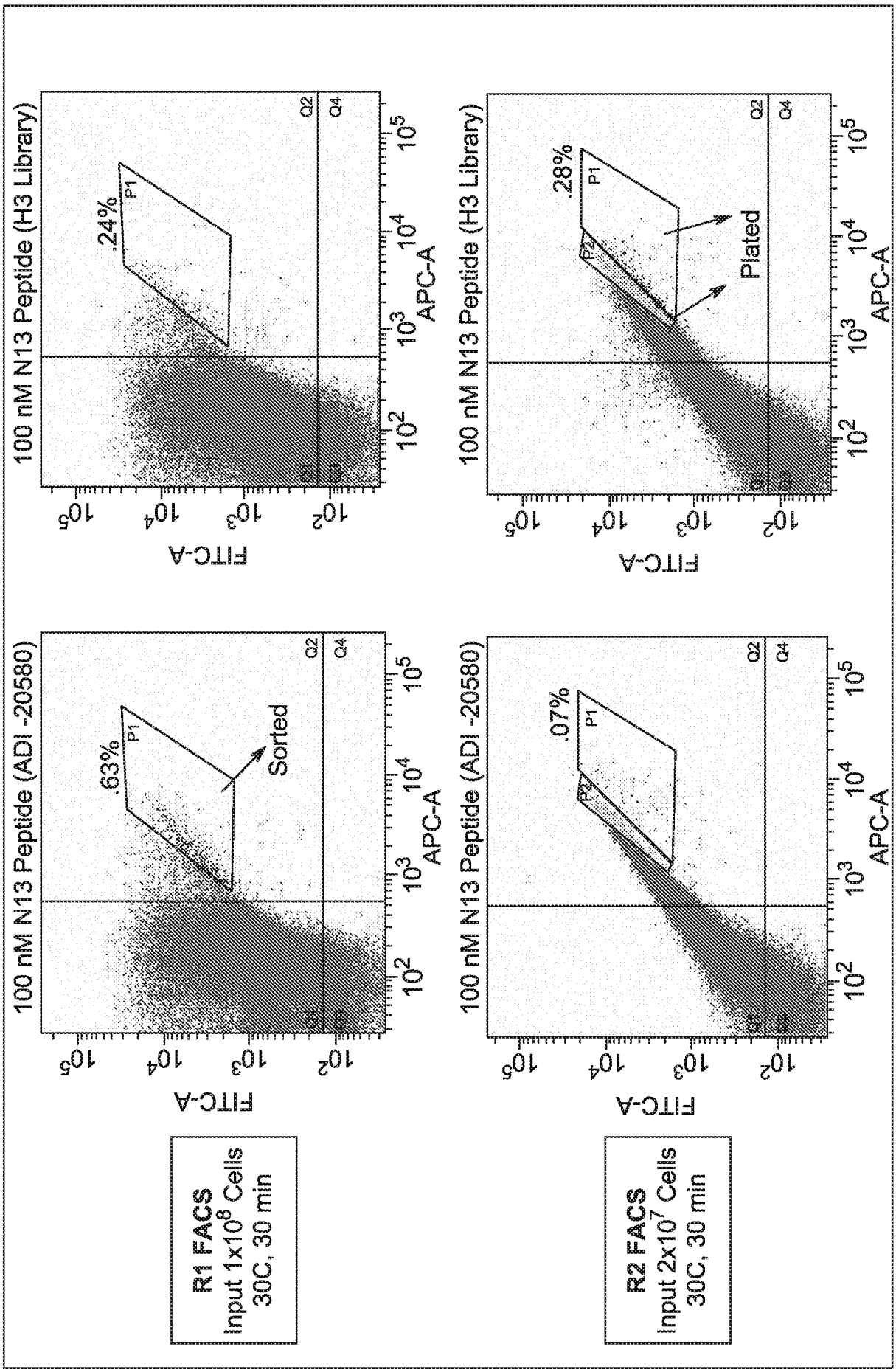

100 nM N13 Peptide (H3 Library)
100 nM N13 Peptide (ADI -20580)
FITC-A
APC-A
P1
P2
.24%
.63%
.28%
.07%
Sorted
Plated
Q2
Q4
R1 FACS
Input 1x10^8 Cells
30C, 30 min
R2 FACS
Input 2x10^7 Cells
30C, 30 min

Figure 20

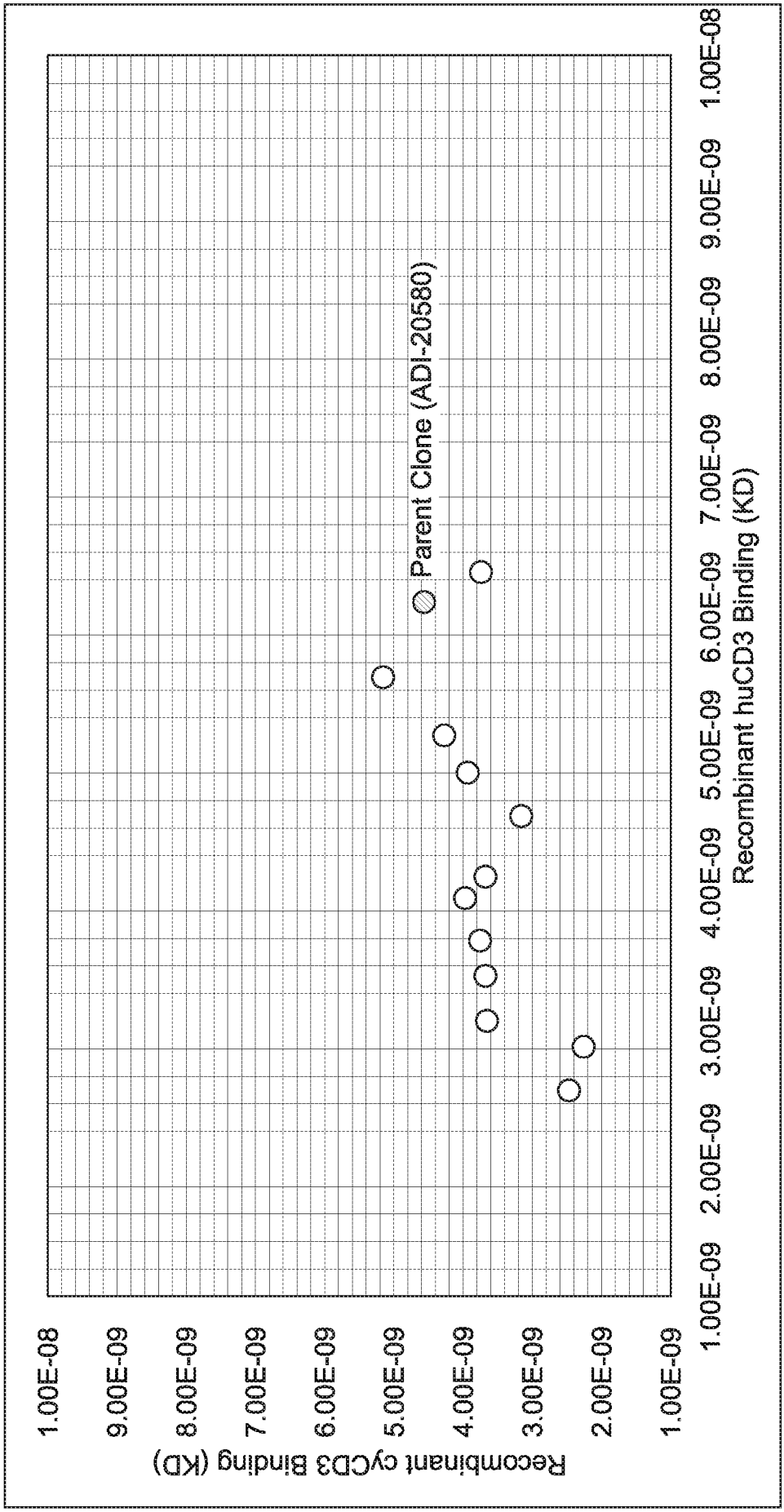

Recombinant cyCD3 Binding (KD)
1.00E-08
9.00E-09
8.00E-09
7.00E-09
6.00E-09
5.00E-09
4.00E-09
3.00E-09
2.00E-09
1.00E-09
Parent Clone (ADI-20580)
1.00E-09
2.00E-09
3.00E-09
4.00E-09
5.00E-09
6.00E-09
7.00E-09
8.00E-09
9.00E-09
1.00E-08
Recombinant huCD3 Binding (KD)

Figure 21

ADI ID	Type	H3	Repeats	K_D Cy CD3εδ Fc (M)	Jurkat CD3+ (Median MFI)	Jurkat CD3- (Median MFI)	Cy HSC-F (Median MFI)	PSR Score	Fab Tm (°C)
ADI-20580	Parent	ARDGYARYYFDY	50	3.0E-09	5829	220	11651	0.45	N.D.
ADI-21959	Progeny	ARDGYGRYYFDY	64	7.8E-09	7735	159	11765	0.28	81.5
ADI-21961	Progeny	ARDGYARYYYDY	32	4.0E-09	7123	162	11681	0.23	80.0
ADI-21967	Progeny	ARDGYARYFFDY	2	7.9E-09	7227	147	11808	0.19	79.5
ADI-21969	Progeny	GSDGYARYYFDY	1	1.3E-08	1175	70	1977	0.16	69.0
ADI-21970	Progeny	ARDGYGRYFFDY	1	2.9E-09	7059	170	12091	0.29	81.0
ADI-21960	Progeny	ARDGYARYYFDV	3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ADI-21962	Progeny	SRDGYARYYFDY	2	1.2E-08	7072	137	11697	0.14	76.0
ADI-21963	Progeny	ARDGYARYYFDI	1	6.0E-09	6844	141	11676	0.17	81.5
ADI-21964	Progeny	NRDGYARYYYDY	1	8.6E-09	7354	148	12083	0.22	72.0
ADI-21965	Progeny	ARDGYGRYYFDA	1	1.0E-09	7110	146	12038	0.28	82.0
ADI-21966	Progeny	ARDGYARYFFDN	1	1.6E-09	6952	157	11501	0.20	77.0
ADI-21968	Progeny	ARDGYGRYYFDR	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ADI-21973	Rational	ARDGYGRYFYDY	N.A.	3.4E-09	7466	190	12573	0.30	81.5
ADI-21978	Rational	ARDGYGRYFYDV	N.A.	2.2E-09	7432	187	12230	0.29	81.5
ADI-21974	Rational	ARDGYGRYFFDV	N.A.	2.7E-09	7268	170	11486	0.29	82.5
ADI-21971	Rational	ARDGYGRYYYDY	N.A.	1.7E-09	7297	180	12398	0.27	81.5
ADI-21972	Rational	ARDGYGRYYYDV	N.A.	3.8E-09	7569	183	12670	0.30	83.0

Figure 22

ADI-21978 Potential Degradation Motifs

ADI ID	CDR H2	CDR H3	CDR L1	Fab KD Cy CD3εδ Fc (nM)
ADI-21978 (ADI-22523)	WIDLENANTIYDPKFQG	ARDGYGRYFYDV	KSSQSLLNSRTGKNYLA	2.1
ADI-23667	WIDLENANTIYDPKFQG	ARDGYGRYFYDV	KSSQSLLNSRTGKNYLA	2.3
ADI-23668	WIDLENGNTIYDAKFQG	ARDGYGRYFYDV	KSSQSLLNSRTGKNYLA	1.5
ADI-23669	WIDLENGNTIYDPKFQG	ARDAYGRYFYDV	KSSQSLLNSRTGKNYLA	4.1
ADI-23660	WIDLENGNTIYDAKFQG	ARDGYGRYFYDV	KSSQSLLNARTGKNYLA	1.7

ADI ID	CDR H2	CDR H3	CDR L1	Fab KD Cy CD3εδ Fc (nM)
ADI-21978 (ADI-22523)	WIDLENGNTIYDPKFQG	ARDGYGRYFYDV	KSSQSLLNSRTGKNYLA	3.4
ADI-25133	WIDLENANTIYDAKFQG	ARDAYGRYFYDV	KSSQSLLNARTGKNYLA	4.2

Figure 23A

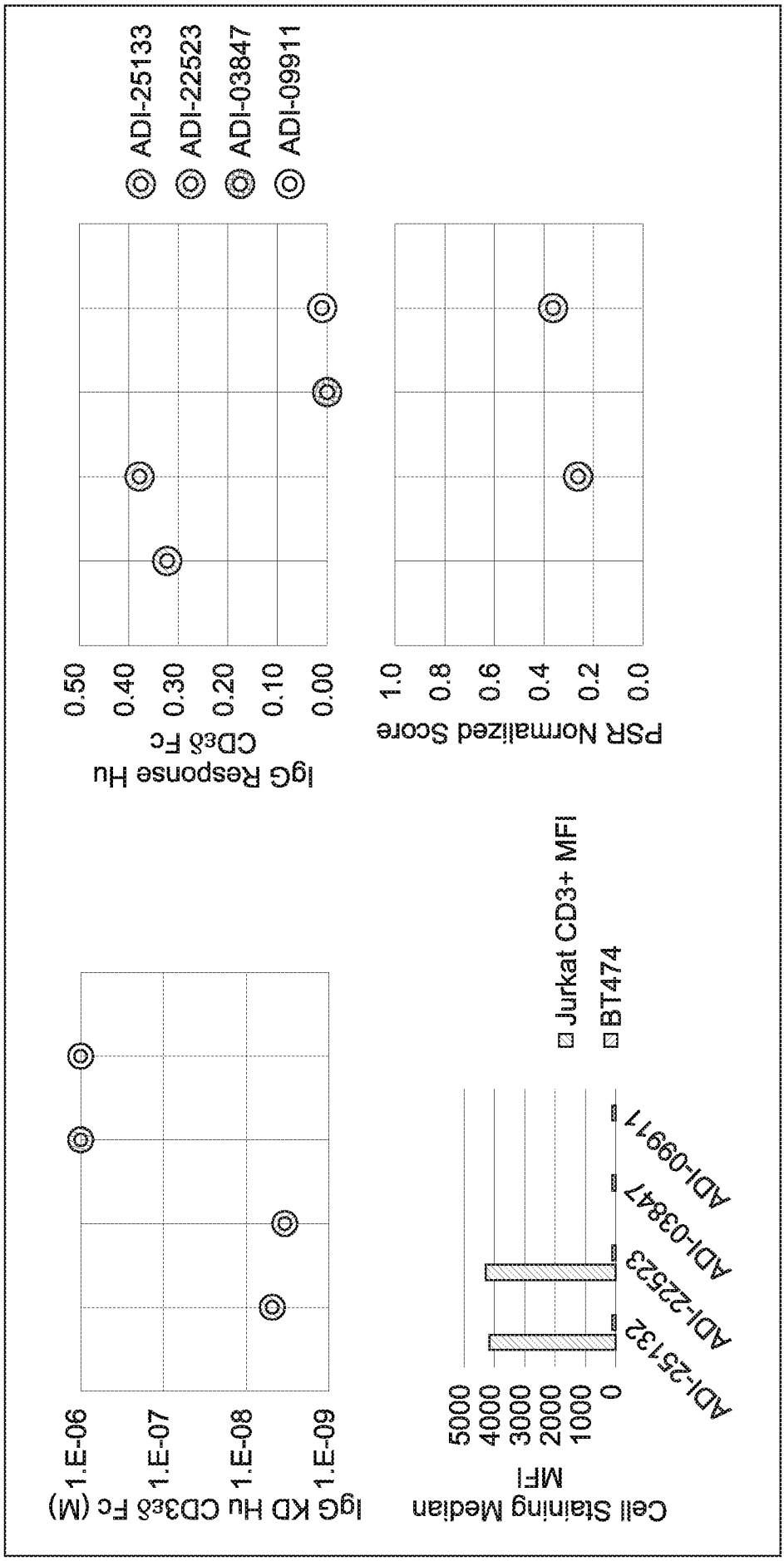

ADI-25133
ADI-22523
ADI-03847
ADI-09911
0.50
0.40
0.30
0.20
0.10
0.00
IgG Response Hu CDεδ Fc
1.0
0.8
0.6
0.4
0.2
0.0
PSR Normalized Score
1.E-06
1.E-07
1.E-08
1.E-09
IgG KD Hu CD3εδ Fc (M)
Jurkat CD3+ MFI
BT474
5000
4000
3000
2000
1000
0
Cell Staining Median MFI
ADI-25132
ADI-22523
ADI-03847
ADI-09911

Figure 23B

Parent	Lineage	Selection Type	Theoretical Diversity
ADI-21978 (ADI-22523)	ADI-18589	H3 Oligo Mutagenesus	$<10^4$

ADI-21978(ADI-22523) FR3-CDR H3-FR4

EDTAVYYCARDGYGRYFYDVWGQGTLVTVS

EDTAVYYCXRXGYGRYFYDVWGQGTLVTVS
EDTAVYYCXRDXYGRYFYDVWGQGTLVTVS
EDTAVYYCAXGYGRYFYDVWGQGTLVTVS
EDTAVYYCAXDXYGRYFYDVWGQGTLVTVS
EDTAVYYCARXXYGRYFYDVWGQGTLVTVS
EDTAVYYCARXGXGRYFYDVWGQGTLVTVS
EDTAVYYCARXGYXRYFYDVWGQGTLVTVS
EDTAVYYCARXGYGXYFYDVWGQGTLVTVS
EDTAVYYCARXGYGRXFYDVWGQGTLVTVS
EDTAVYYCARXGYGRYXYDVWGQGTLVTVS
EDTAVYYCARXGYGRYFXDVWGQGTLVTVS
EDTAVYYCARXGYGRYFYXVWGQGTLVTVS
EDTAVYYCARXGYGRYFYDXWGQGTLVTVS
EDTAVYYCARDXXGRYFYDVWGQGTLVTVS
EDTAVYYCARDXYXRYFYDVWGQGTLVTVS
EDTAVYYCARDXYGXYFYDVWGQGTLVTVS
EDTAVYYCARDXYGRXFYDVWGQGTLVTVS
EDTAVYYCARDXYGRYXYDVWGQGTLVTVS
EDTAVYYCARDXYGRYFXDVWGQGTLVTVS
EDTAVYYCARDXYGRYFYXVWGQGTLVTVS
EDTAVYYCARDXYGRYFYDXWGQGTLVTVS

Selection

Novel CD3 Binder ADI -21978 (ADI-22523) H3 Mutagenesis Progeny

Clone ID	CDR H3	KD Hu CD3εδ Fc (M)	Response Hu CD3εδ Fc
ADI-21978	ARDGYGRYFYDV	3.3E-09	0.25
ADI-26907	GRDAYGRYFYDV	3.8E-09	0.18
ADI-29607	ARDAYGRYFYRV	4.1E-09	0.15
ADI-29608	PRDAYGRYFYDV	4.3E-09	0.20
ADI-29609	ARDAYGRYFYDG	6.4E-09	0.27
ADI-29610	ARDRYGAYFYDV	6.7E-09	0.26
ADI-29611	ARDRYGRYFYDV	7.3E-09	0.27
ADI-29613	ARDSYGRYFYDV	7.8E-09	0.28
ADI-29614	ARDVYGRYLYDV	9.9E-09	0.14
ADI-26912	ARDVLGRYFYDV	1.1E-08	0.18
ADI-26913	ARDQYGRYFYDV	1.2E-08	0.27
ADI-29617	ARDVYRRYFYDV	1.2E-08	0.15
ADI-29618	VRDLRGRYFYDV	1.3E-08	0.15
ADI-26916	ARDVYGRYFYDL	1.3E-08	0.22
ADI-26917	ARDAYGGYFYDV	2.1E-08	0.22
ADI-26918	ARDNYGGYFYDV	2.5E-08	0.17

Figure 24

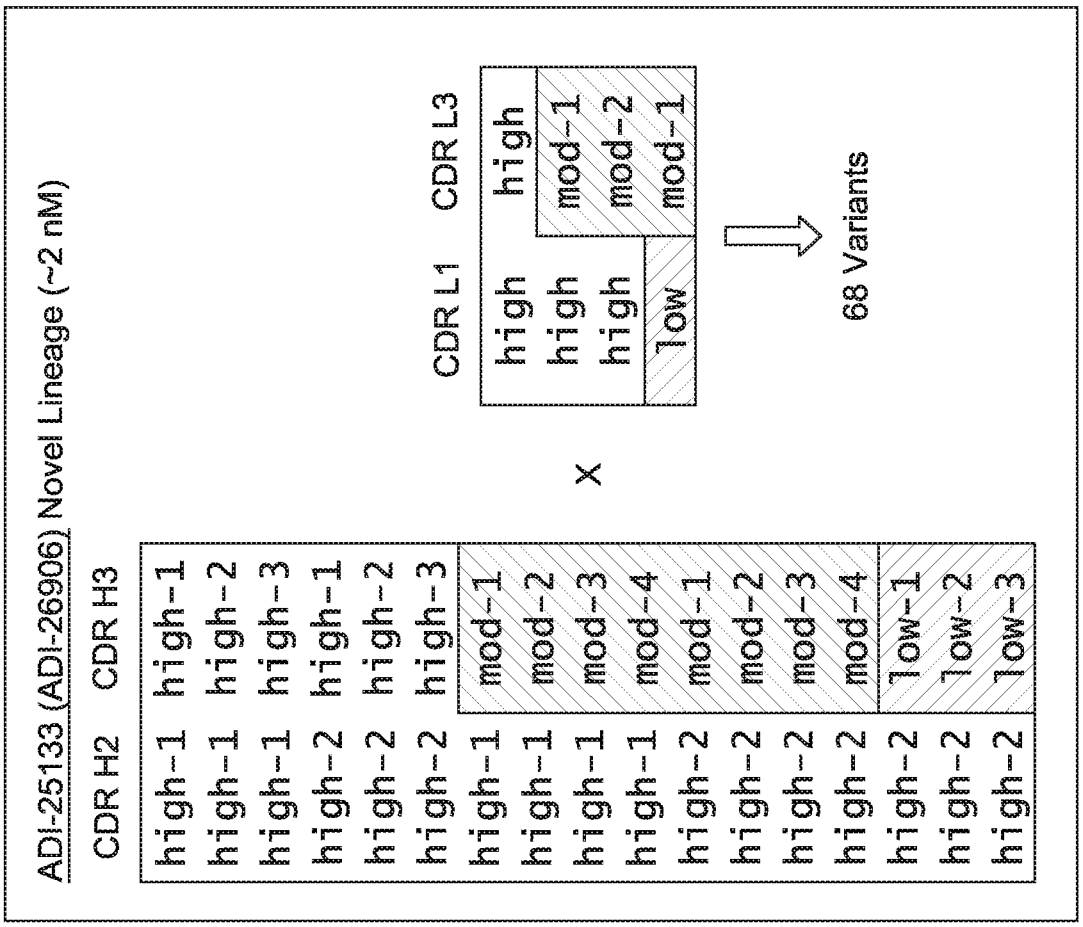

ADI-25133 (ADI-26906) Novel Lineage (~2 nM)
CDR H2
CDR H3
high-1 high-1
high-1 high-2
high-1 high-3
high-2 high-1
high-2 high-2
high-2 high-3
high-1 mod-1
high-1 mod-2
high-1 mod-3
high-1 mod-4
high-2 mod-1
high-2 mod-2
high-2 mod-3
high-2 mod-4
high-2 low-1
high-2 low-2
high-2 low-3
X
CDR L1
CDR L3
high high
high mod-1
high mod-2
low mod-1
68 Variants

Figure 25A

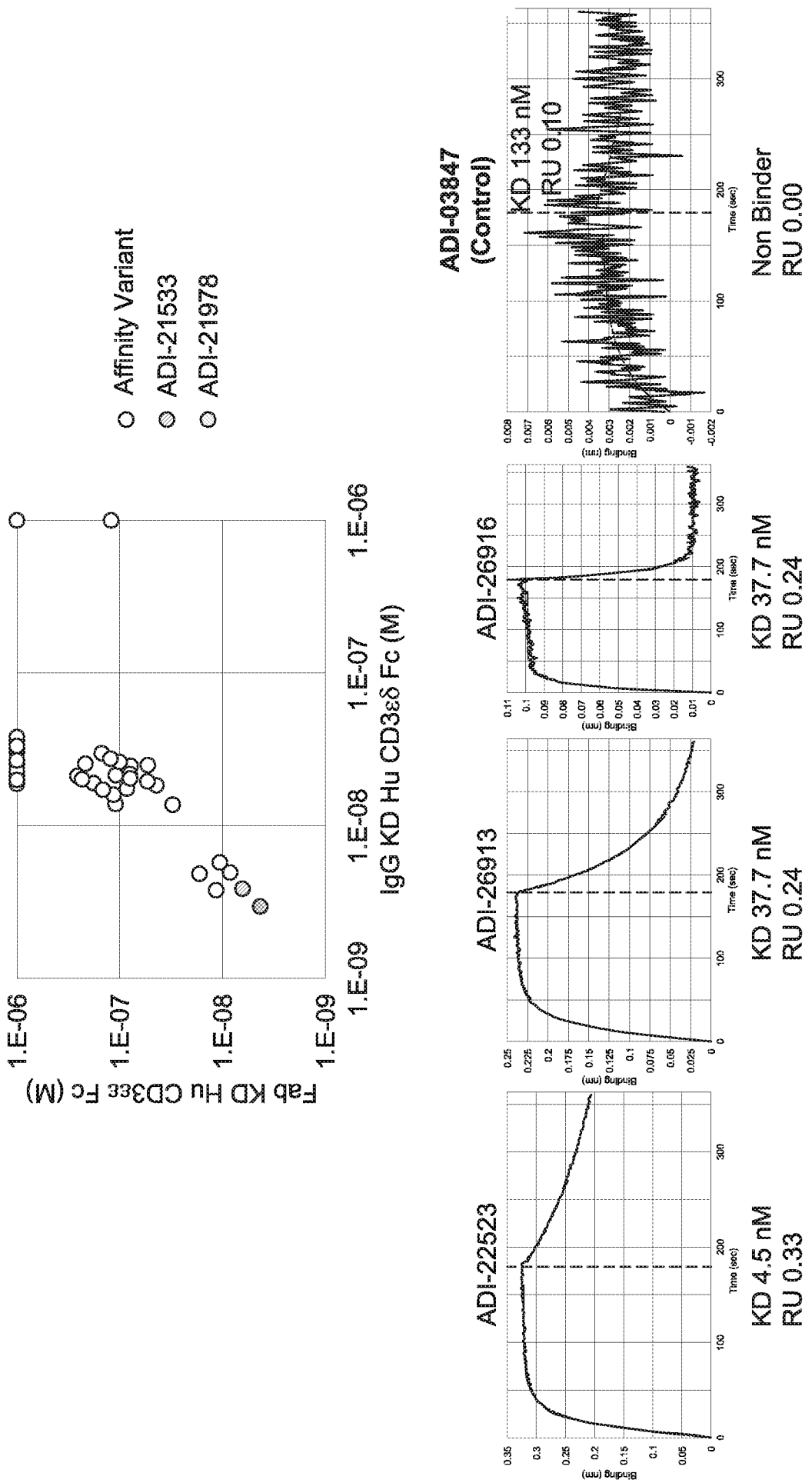

Affinity Variant
ADI-21533
ADI-21978
Fab KD Hu CD3εε Fc (M)
1.E-06
1.E-07
1.E-08
1.E-09
IgG KD Hu CD3εδ Fc (M)
ADI-03847 (Control)
KD 133 nM
RU 0.10
Non Binder
RU 0.00
ADI-26916
KD 37.7 nM
RU 0.24
ADI-26913
KD 37.7 nM
RU 0.24
ADI-22523
KD 4.5 nM
RU 0.33
Binding (nm)
Time (sec)

Figure 25B

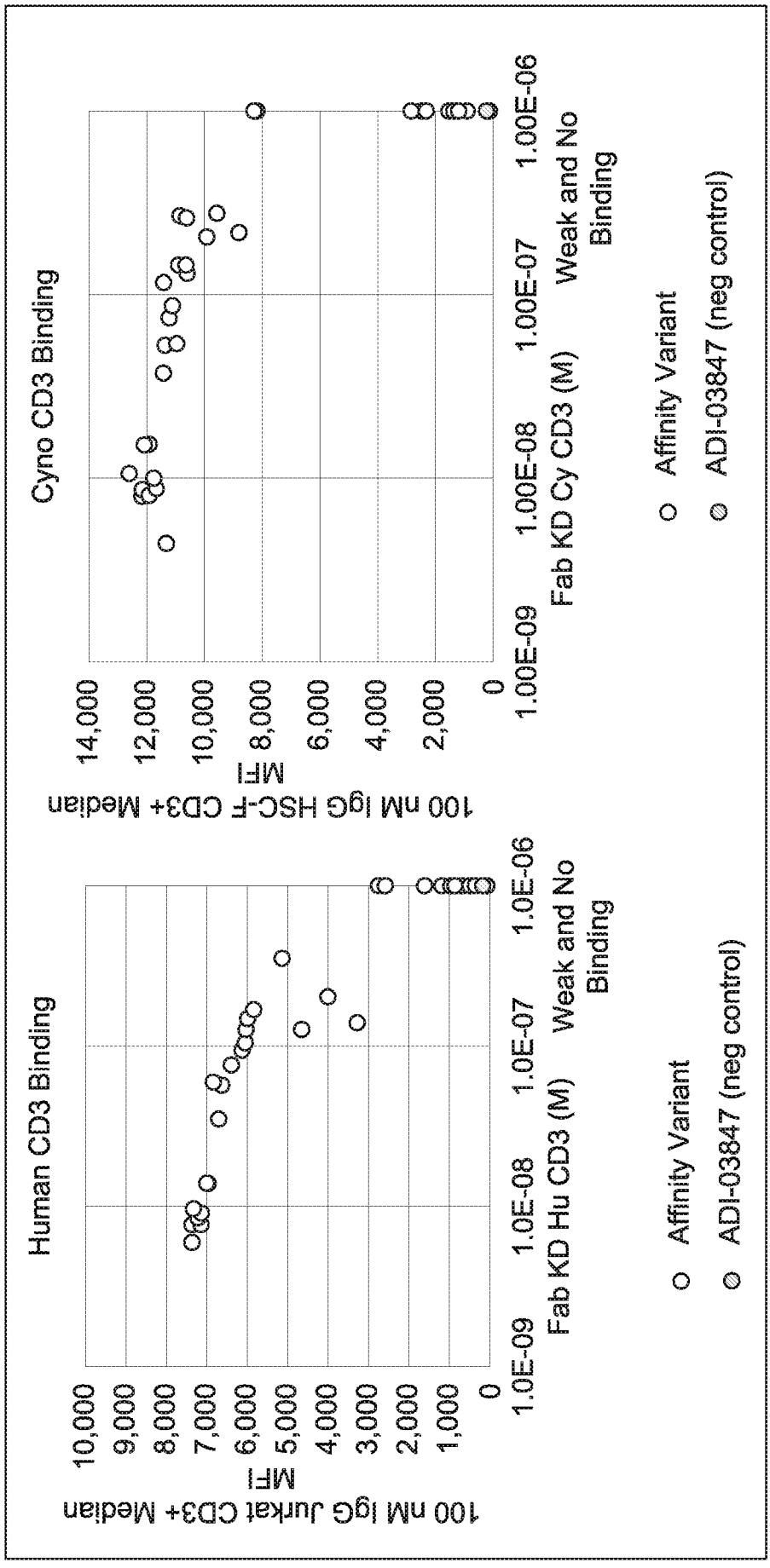

Human CD3 Binding
100 nM IgG Jurkat CD3+ Median MFI
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
1.0E-09
1.0E-08
1.0E-07
1.0E-06
Fab KD Hu CD3 (M)
Weak and No Binding
Affinity Variant
ADI-03847 (neg control)
Cyno CD3 Binding
100 nM IgG HSC-F CD3+ Median MFI
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
1.00E-09
1.00E-08
1.00E-07
1.00E-06
Fab KD Cy CD3 (M)
Weak and No Binding
Affinity Variant
ADI-03847 (neg control)

Figure 25C

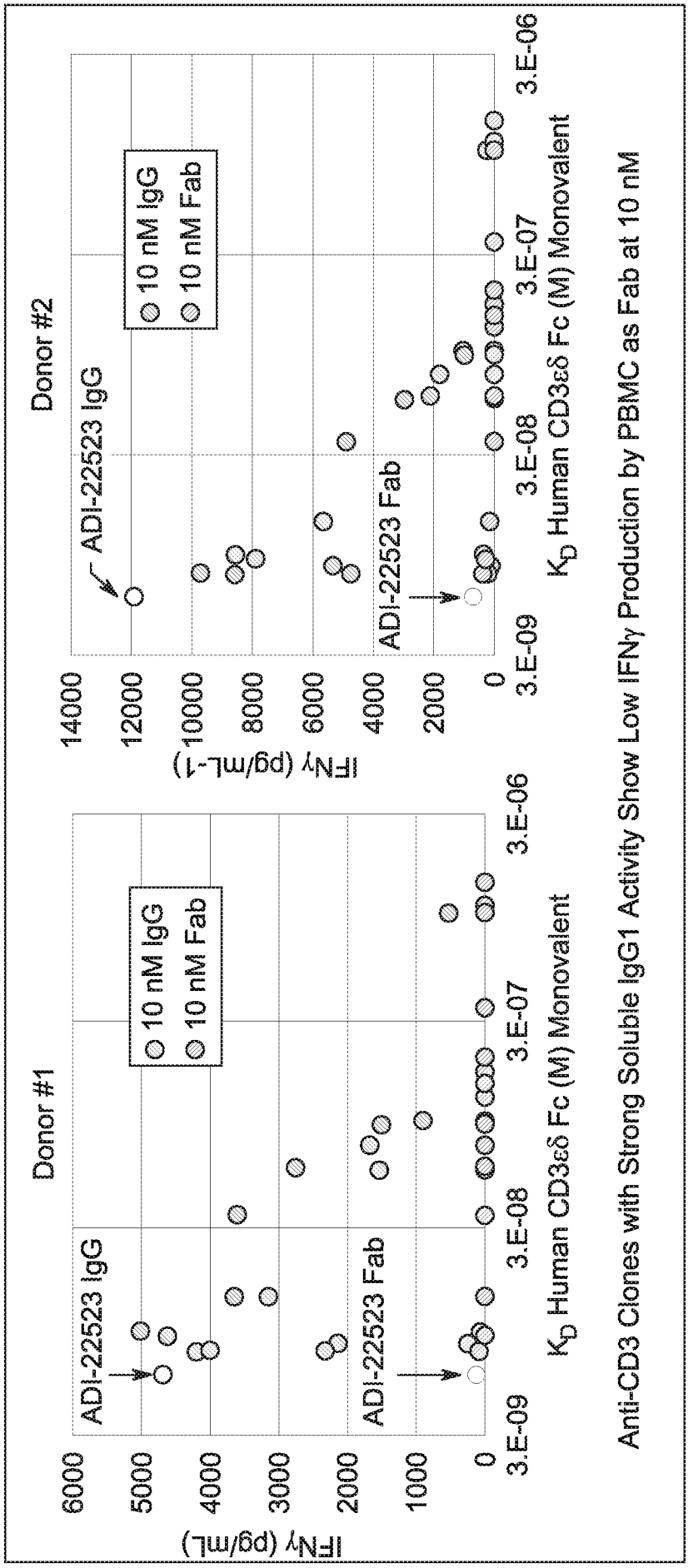
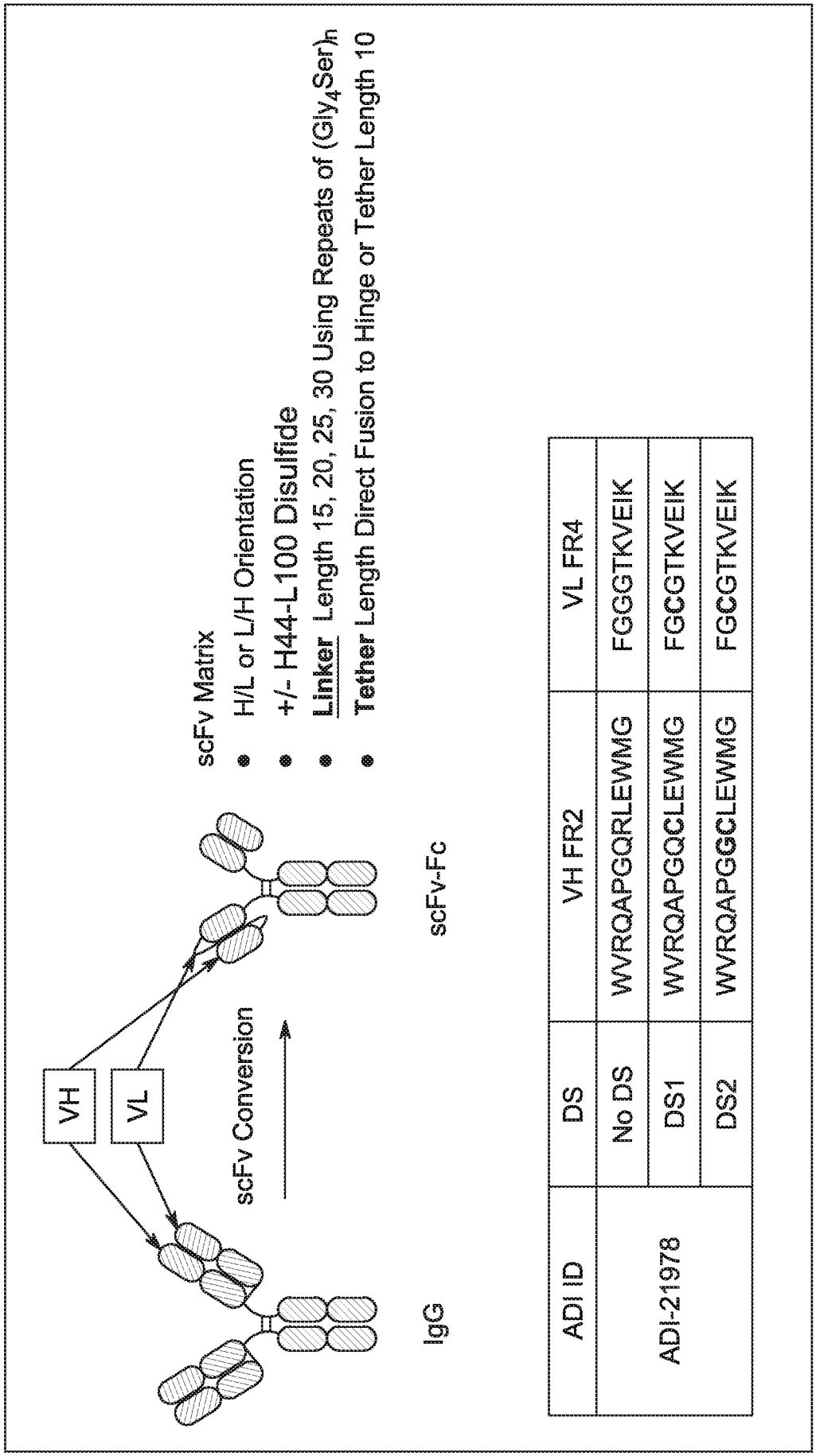

Anti-CD3 Clones with Strong Soluble IgG1 Activity Show Low IFNγ Production by PBMC as Fab at 10 nM
Donor #1
Donor #2
IFNγ (pg/mL)
IFNγ (pg/mL-1)
ADI-22523 IgG
ADI-22523 Fab
10 nM IgG
10 nM Fab
KD Human CD3εδ Fc (M) Monovalent
3.E-09
3.E-08
3.E-07
3.E-06
0
1000
2000
3000
4000
5000
6000
2000
4000
6000
8000
10000
12000
14000

Figure 25D



ADI ID	DS	VH FR2	VL FR4
ADI-21978	No DS	WVRQAPGQRLEWMG	FGGGTKVEIK
	DS1	WVRQAPGQCLEWMG	FGCGTKVEIK
	DS2	WVRQAPGGCLEWMG	FGCGTKVEIK

Figure 26A

Parent	Type	#	KD Hu CD3εδ Fc (M)	Fold Reduction	KD Cy CD3εδ Fc (M)	Fold Reduction
ADI-21978	IgG	1	2.7E-09	N/A	1.9E-09	N/A
	scFv-Fc	15	5.9E-09	2.2	4.8E-09	2.5
	scFv-Fc (DS1)	13	6.0E-09	2.6	6.9E-08	3.6
	scFv-Fc (DS2)	1	7.2E-09	2.7	6.4E-09	3.4

Figure 26B

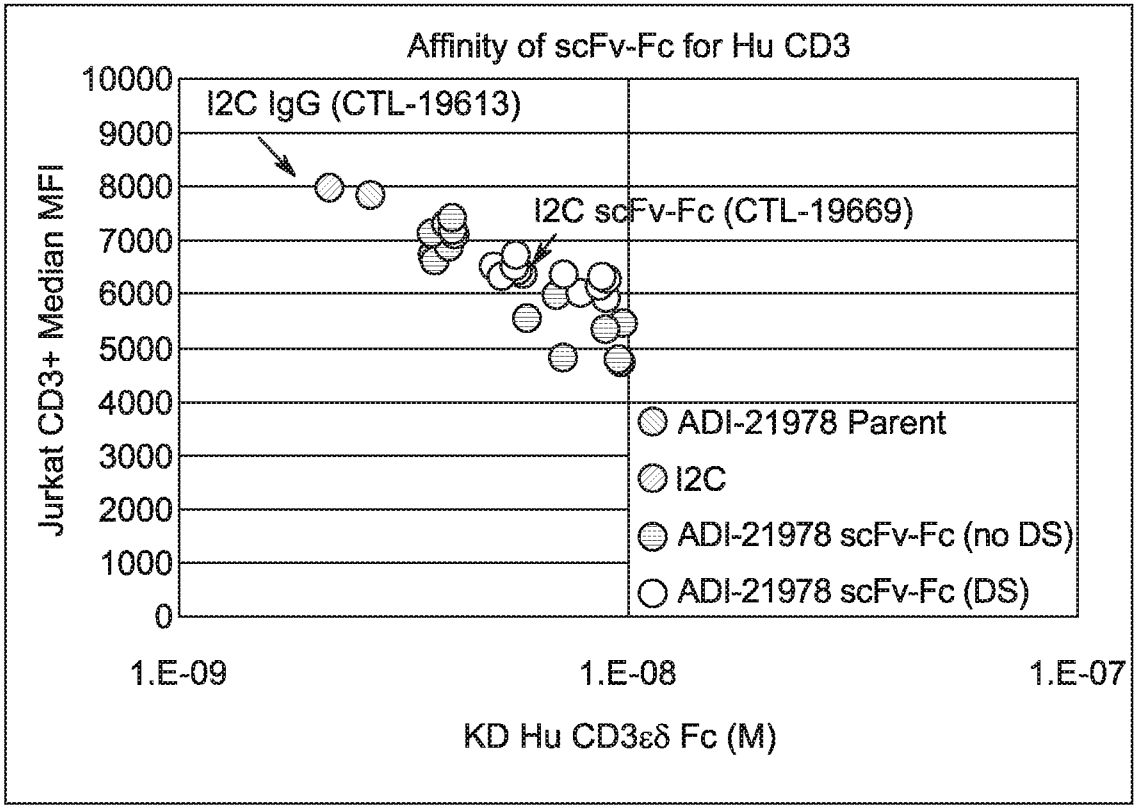


Figure 26C

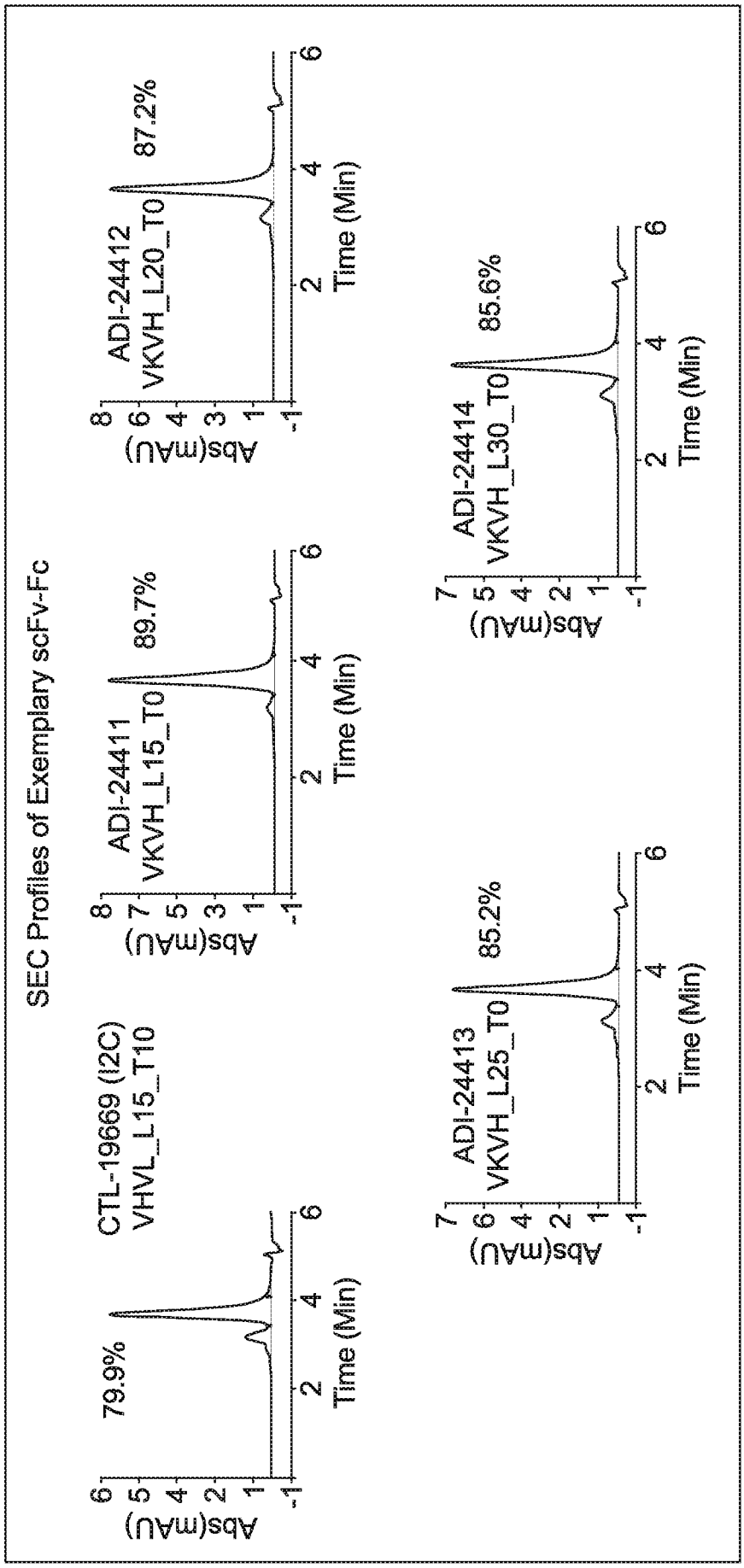

SEC Profiles of Exemplary scFv-Fc
CTL-19669 (I2C)
VHVL_L15_T10
79.9%
ADI-24411
VKVH_L15_T0
89.7%
ADI-24412
VKVH_L20_T0
87.2%
ADI-24413
VKVH_L25_T0
85.2%
ADI-24414
VKVH_L30_T0
85.6%
Abs(mAU)
Time (Min)

Figure 26D

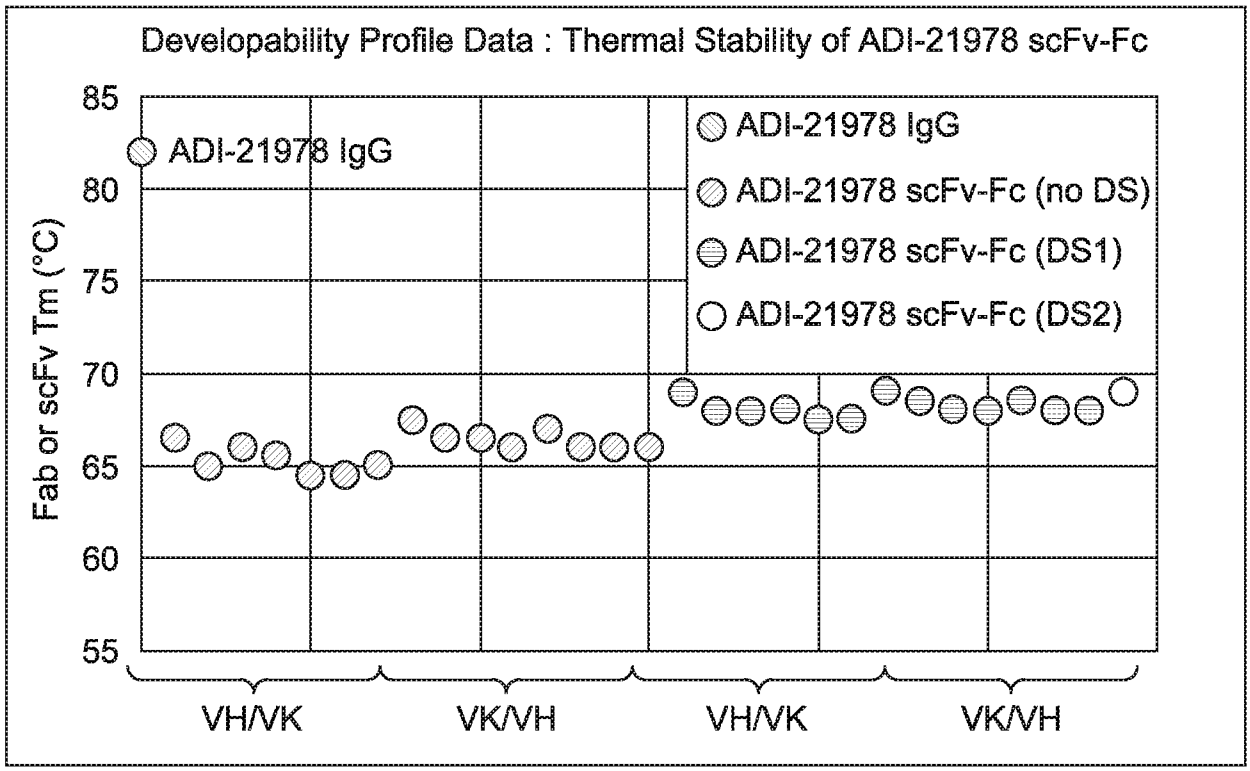


Figure 26E

Developability Profile Data

Parent	Type	#	DSF Tm (°C)	ΔTM Fab/scFv	ΔTM +/-DS
ADI-21978	IgG	1	82.0	N/A	N/A
	scFv-Fc	15	65.9	-16.1	N/A
	scFv-Fc (DS1)	13	68.2	-13.8	+2.3
	scFv-Fc (DS2)	1	69.0	-13.0	+3.1

Figure 26F

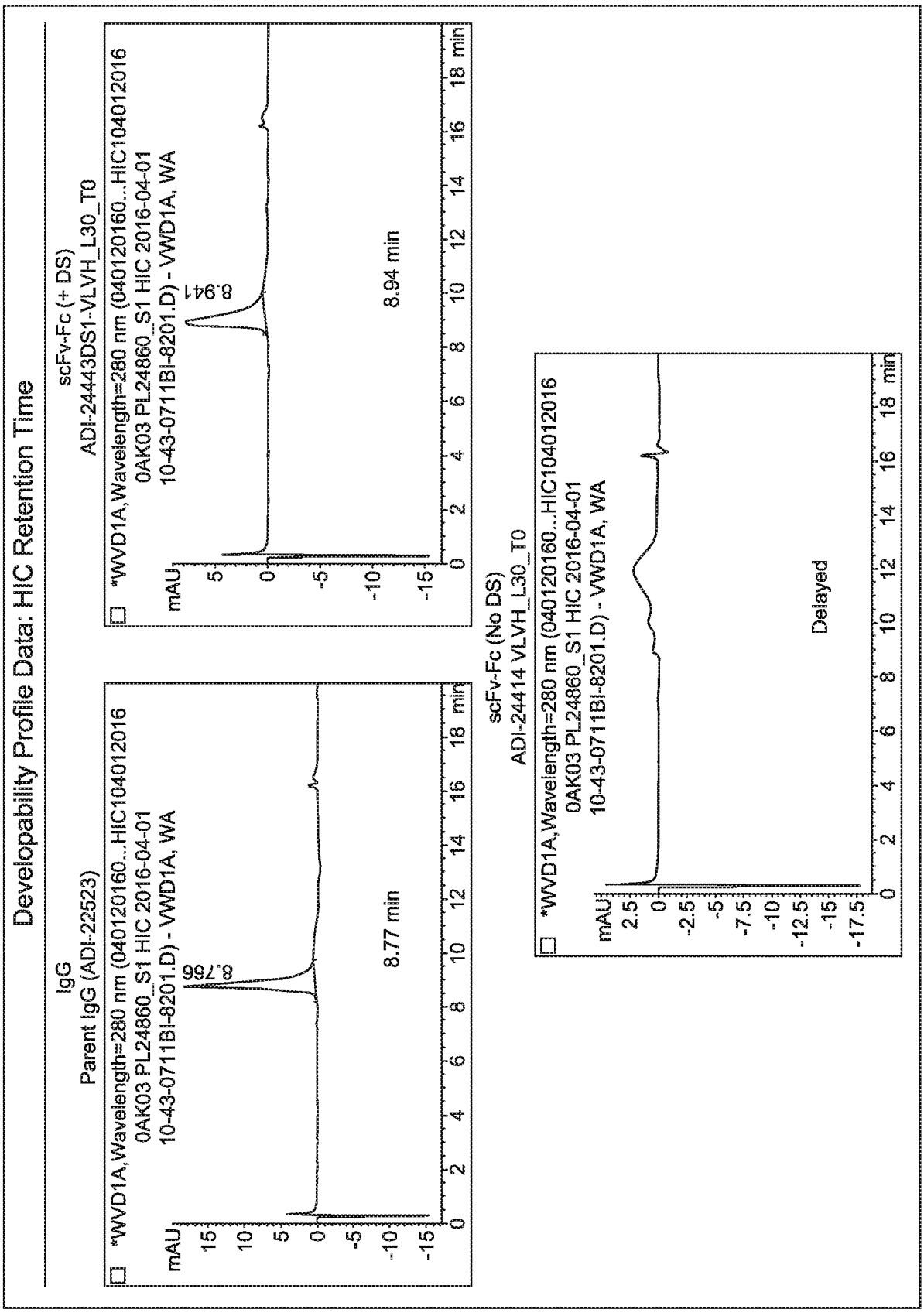

Developability Profile Data: HIC Retention Time
IgG
Parent IgG (ADI-22523)
*WVD1A,Wavelength=280 nm (040120160...HIC104012016
0AK03 PL24860_S1 HIC 2016-04-01
10-43-0711BI-8201.D) - VWD1A, WA
mAU
8.766
8.77 min
min
scFv-Fc (+ DS)
ADI-24443DS1-VLVH_L30_T0
*WVD1A,Wavelength=280 nm (040120160...HIC104012016
0AK03 PL24860_S1 HIC 2016-04-01
10-43-0711BI-8201.D) - VWD1A, WA
mAU
8.941
8.94 min
min
scFv-Fc (No DS)
ADI-24414 VLVH_L30_T0
*WVD1A,Wavelength=280 nm (040120160...HIC104012016
0AK03 PL24860_S1 HIC 2016-04-01
10-43-0711BI-8201.D) - VWD1A, WA
mAU
Delayed
min

Figure 26G

HC Design	Germline	FR Mutations	Proteing Sequence
HuSP34-HC1	VH3-23	0	EVQLLESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVSRIRSKYNNYATYY ADSVKDRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
HuSP34-HC2	VH3-23	3	EVQLLESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKSTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
HuSP34-HC3	VH3-72	0	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYY ADSVKDRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS
HuSP34-HC4	VH3-72	1	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

LC Design	Germline	FR Mutations	Proteing Sequence
HuSP34-LC1	VL8-61	0	QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWYQQTPGQAPRTLIYGTNKRAPGVPDR FSGSILGNKAALTITGAQADDESDYYCALWYSNLWVFGGGTKLTVL
HuSP34-LC2	VL8-61	1	QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWYQQTPGQAPRTLIGGTNKRAPGVPDR FSGSILGNKAALTITGAQADDESDYYCALWYSNLWVFGGGTKLTVL
HuSP34-LC3	VL8-61	5	QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPGVPDR FSGSLLGDKAALTITGAQADDESDYYCALWYSNLWVFGGGTKLTVL
HuSP34-LC4	VL7-46	0	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPGQAPRTLIYGTNKRAPWTPAR FSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL
HuSP34-LC5	VL7-46	6	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPAR FSGSLLGDKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL
HuSP34-LC6	VH3-15	0	EIVMTQSPATLSVSPGERATLSCRSSTGAVTTSNYANWVQQKPGQAPRLLIYGTNKRAPGIPAR FSGSGSGTEFTLTISSLQSEDFAVYYCALWYSNLWVFGGGTKVEIK
HuSP34-LC7	VH3-15	6	EIVVTQSPATLSVSPGERATLSCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPAR FSGSGSGDEATLTISSLQSEDFAVYYCALWYSNLWVFGGGTKVEIK

Mutations Occurring with Respect to Human Germline Highlighted in **RED**

Figure 27

Type	HC	LC	ADI ID	Fab K_D Hu CDεδ Fc (M)	Fab K_D Cy CDεδ Fc (M)	Jurkat CD3+ 100nM Fab (MFI)	Jurkat CD3- 100nM Fab (MFI)	HSC-F 100 nM Fab (MFI)	Fab Tm (°C)	AC-SINS Δλmax (nm)	PSR Norm. MFI
ChSP34				5.8E-09	8.7E-09	1404.9	77.7	11806.1	60.5	9.9	1125.3
HuSP34	HuSP34-HC3	HuSP34-LC5		3.6E-09	4.9E-09	1992.6	79.1	11843.6	N.D.	26.5	885.8
	HuSP34-HC4	HuSP34-LC7		1.8E-08	2.1E-08	982.1	103.6	10735.0	N.D.	18.3	N.D.
	HuSP34-HC2	HuSP34-LC3	ADI-16606	5.8E-09	8.0E-09	1628.9	80.5	11885.9	60.5	5.1	400.2
	HuSP34-HC2	HuSP34-LC5		5.3E-09	7.5E-09	1918.0	102.2	12048.5	N.D.	12.3	N.D.
	HuSP34-HC4	HuSP34-LC5		5.9E-09	7.1E-09	1713.6	84.0	11435.5	N.D.	20.0	720.1

Figure 27 (Continued)

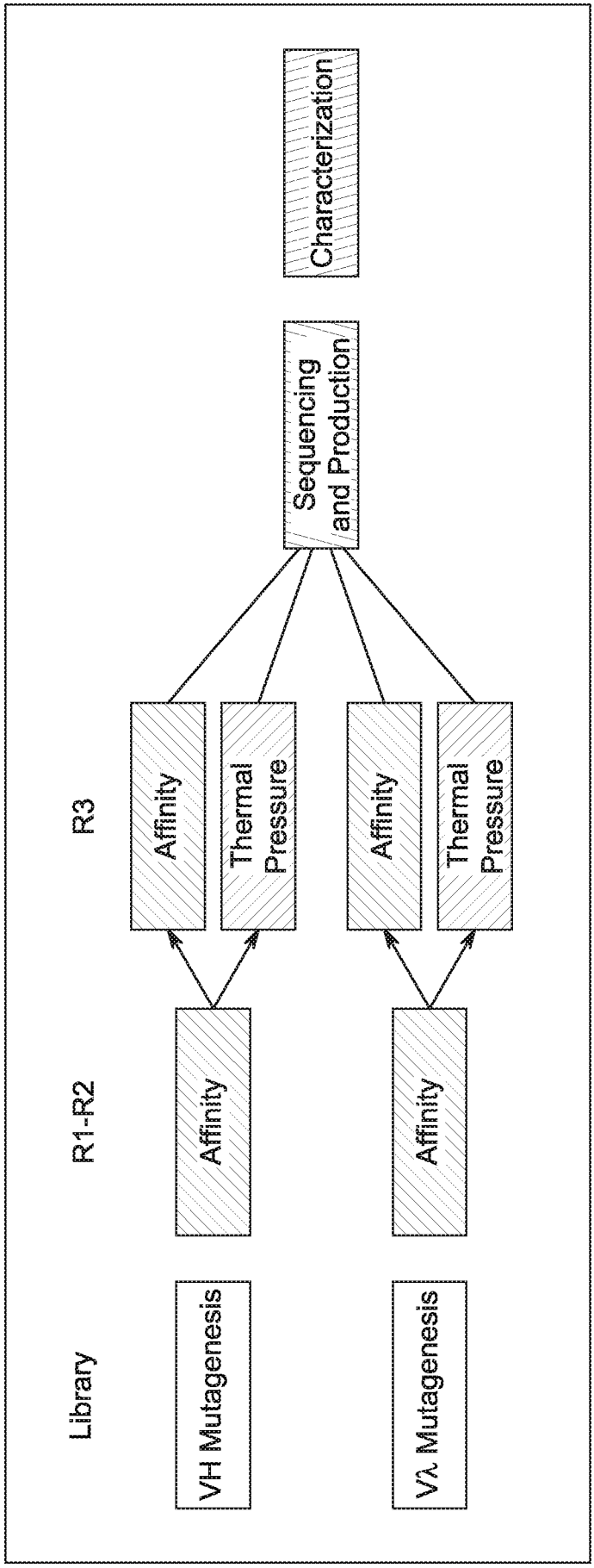

Library
R1-R2
R3
VH Mutagenesis
Vλ Mutagenesis
Affinity
Affinity
Affinity
Thermal Pressure
Affinity
Thermal Pressure
Sequencing and Production
Characterization

Figure 28A

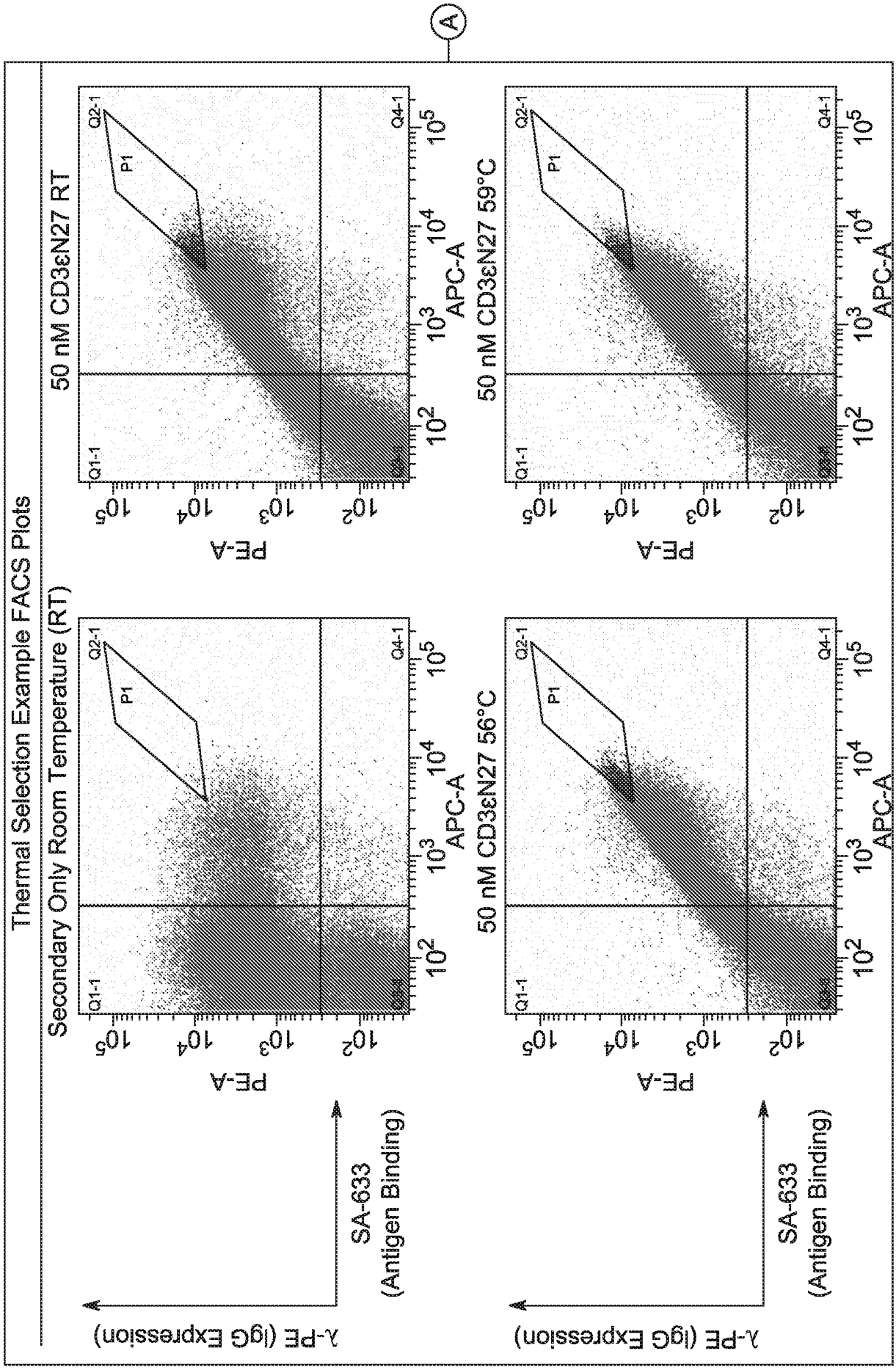

Thermal Selection Example FACS Plots
Secondary Only Room Temperature (RT)
50 nM CD3εN27 RT
50 nM CD3εN27 59°C
50 nM CD3εN27 56°C
λ-PE (IgG Expression)
SA-633 (Antigen Binding)
PE-A
APC-A
Q1-1
Q2-1
Q3-1
Q4-1
P1
A

Figure 28B

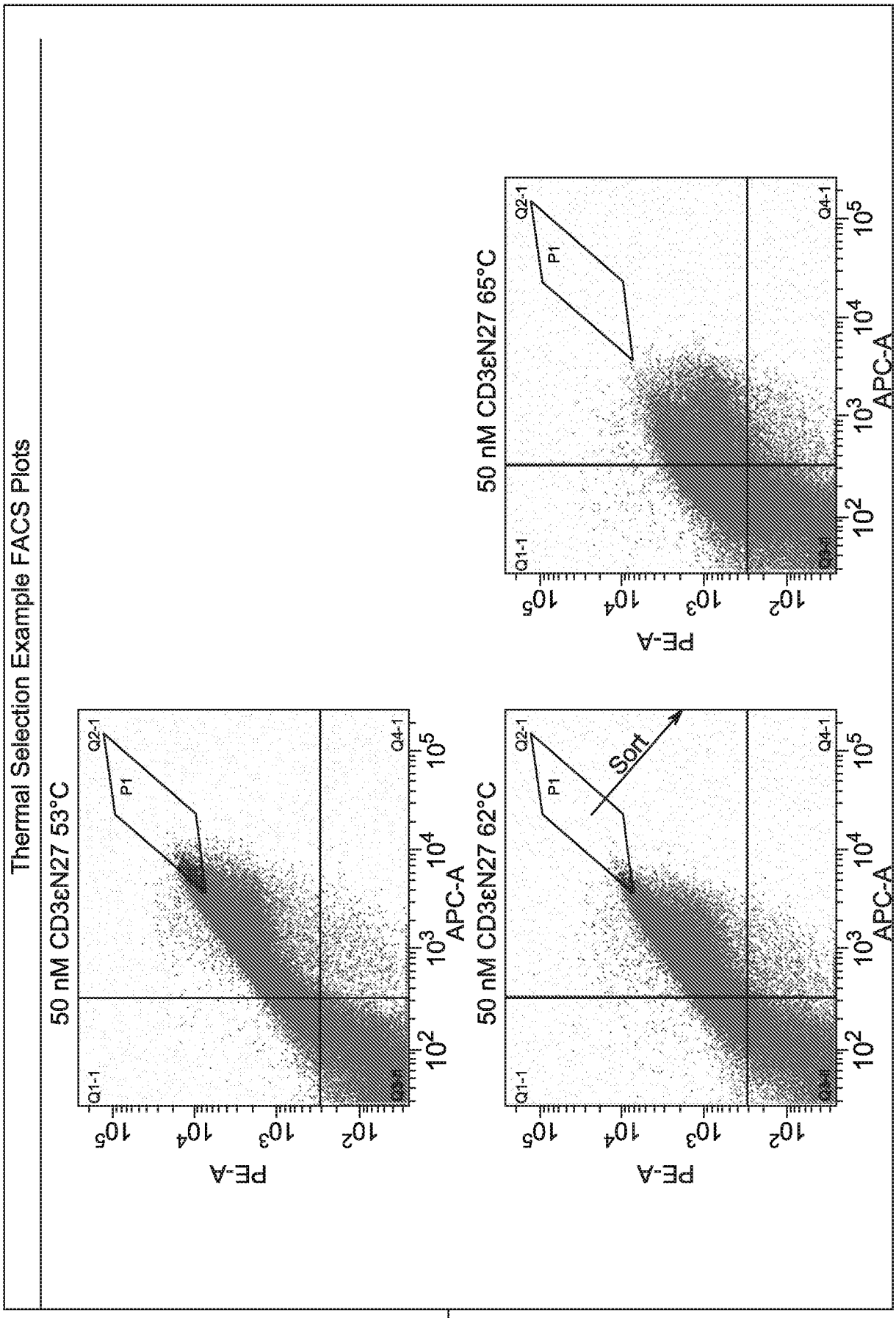


Figure 28B (Continued)

VA Mutagenesis Affinity-pressured Output Sequencing

Parent	VAmut	Repeats	VL-FR1	VL-CDR L1	VL-FR2	VL-CDR L2	VL-FR3	VL-CDR L3	VL-FR4
ADI-16606	Parent	2	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	L95R	45	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	R24G,L95R	9	QTVVTQEPSFSVSPGGTVTLTC	GSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	T27A,L95R	7	QTVVTQEPSFSVSPGGTVTLTC	RSSAGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	F11L,L95R	6	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	K70R,L95R	4	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	T2A,L95R	4	QAVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	T2A,T27I,L95R	4	QAVVTQEPSFSVSPGGTVTLTC	RSSIGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	S30CG,L95R	2	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTGNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	T39A,A43T,L95R	2	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQAPGQTPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	N52S,L95R	2	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTSKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	R24G,K53R	1	QTVVTQEPSFSVSPGGTVTLTC	GSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNRRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	Q37R,L95R	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVRQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	T74I,L95R	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALIITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	K53R,S93N	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNRRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYNNLWV	FGGGTKLTVL
	R24K,L95R	1	QTVVTQEPSFSVSPGGTVTLTC	KSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	L95H	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNHWV	FGGGTKLTVL
	K53R,L95R	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNRRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL

VH Mutagenesis Affinity-pressured Output Sequencing

Parent	VHmut	Repeats	VH-FR1	VH-CDR H1	VH-FR2	VH-CDR H2	VH-FR3	VH-CDR H3	VH-FR4
ADI-16606	Parent	80	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	G96D, G99R	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHDNFRNSYVSWFAY	WGQGTLVTVSS
	N30D, Y102H	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFDTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAH	WGQGTLVTVSS
	Y100bH	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSHVSWFAY	WGQGTLVTVSS
	L5V, N54D, Y100bH	1	EVQLVESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNDYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSHVSWFAY	WGQGTLVTVSS
	Y102H	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSHVSWFAH	WGQGTLVTVSS
	N30D	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFDTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	L5S	1	EVQLSESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	T31A	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNAYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS

Figure 28C

Vλ Mutagenesis Thermal-pressured Output Sequencing

Parent	VλMut	Repeats	VL-FR1	VL-CDR L1	VL-FR2	VL-CDR L2	VL-FR3	VL-CDR L3	VL-FR4
ADI-16606	Parent	2	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	L95R	20	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	T27A, L95R	10	QTVVTQEPSFSVSPGGTVTLTC	RSSAGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	F11L, L95R	9	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	R24G, L95R	6	QTVVTQEPSFSVSPGGTVTLTC	GSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	F11L, S93N	6	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYNNLWV	FGGGTKLTVL
	T2A, T27I, L95R	6	QAVVTQEPSFSVSPGGTVTLTC	RSSIGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	F11L	4	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	F11L, R24G	2	QTVVTQEPSLSVSPGGTVTLTC	GSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	F11L, T74I, S84A	2	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALITITGAQADDEADYYC	ALWYSNLWV	FGGGTKLTVL
	Q1R, F11L, S93N	2	RTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYNNLWV	FGGGTKLTVL
	S93N	2	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYNNLWV	FGGGTKLTVL
	R24G, N52D, D69G	2	QTVVTQEPSFSVSPGGTVTLTC	GSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTDKRAP	GVPDRFSGSLLGGKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	T2P	2	QPVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	T2A, L95R	2	QAVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	R24G	2	QTVVTQEPSFSVSPGGTVTLTC	GSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	Q1R, R24G, T74I	1	RTVVTQEPSFSVSPGGTVTLTC	GSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALIITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	F11L, S12A, N52D, K53Q, A72T	1	QTVVTQEPSLAVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTDQRAP	GVPDRFSGSLLGDKATLTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	T2A, P8Q, T27I, L95R	1	QAVVTQEQSFSVSPGGTVTLTC	RSSIGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	F11L, R24G, S25P	1	QTVVTQEPSLSVSPGGTVTLTC	GPSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	T39A, K53Q, S84A	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQAPGQAPRGLIG	GTNQRAP	GVPDRFSGSLLGDKAALTITGAQADDEADYYC	ALWYSNLWV	FGGGTKLTVL
	A78V, L95R	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGVQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	N52D, A78V	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTDKRAP	GVPDRFSGSLLGDKAALTITGVQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	A72V	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAVLTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	S84A	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDEADYYC	ALWYSNLWV	FGGGTKLTVL
	P8T, F11L, N52D, D81E	1	QTVVTQETSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTDKRAP	GVPDRFSGSLLGDKAALTITGAQAEDESDYYC	ALWYSNLWV	FGGGTKLTVL
	T74I, L95R	1	QTVVTQEPSFSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALITITGAQADDESDYYC	ALWYSNRWV	FGGGTKLTVL
	R24G, T27A	1	QTVVTQEPSFSVSPGGTVTLTC	GSSAGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYSNLWV	FGGGTKLTVL
	F11L, S84A	1	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDEADYYC	ALWYSNLWV	FGGGTKLTVL
	T2P, T30bA, T39A, K53Q, S93N	1	QPVVTQEPSFSVSPGGTVTLTC	RSSTGAVTASNYAN	WVQQAPGQAPRGLIG	GTNQRAP	GVPDRFSGSLLGDKAALTITGAQADDESDYYC	ALWYNNLWV	FGGGTKLTVL
	F11L, N52D, D81E	1	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTDKRAP	GVPDRFSGSLLGDKAALTITGAQAEDESDYYC	ALWYSNLWV	FGGGTKLTVL
	F11L, S84A, S93N	1	QTVVTQEPSLSVSPGGTVTLTC	RSSTGAVTTSNYAN	WVQQTPGQAPRGLIG	GTNKRAP	GVPDRFSGSLLGDKAALTITGAQADDEADYYC	ALWYNNLWV	FGGGTKLTVL

Figure 28D

VH Mutagenesis Thermal-pressured Output Sequencing

Parent	VHMut	Repeats	VH-FR1	VH-CDR H1	VH-FR2	VH-CDR H2	VH-FR3	VH-CDR H3	VH-FR4
ADI-16606	Parent	78	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	N30D	4	EVQLLESGGGLVQPGGSLRLSCAASG	FTFDTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	V100cI	3	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYISWFAY	WGQGTLVTVSS
	L5S, N30S	3	EVQLSESGGGLVQPGGSLRLSCAASG	FTFSTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	G96S	2	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHSNFGNSYVSWFAY	WGQGTLVTVSS
	V48I	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWIA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	N82aD	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMDSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	N54D	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNDYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	N30D, D72N	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFDTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRNDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSYVSWFAY	WGQGTLVTVSS
	V100cS	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSSVSWFAY	WGQGTLVTVSS
	V100cH	1	EVQLLESGGGLVQPGGSLRLSCAASG	FTFNTYAMN	WVRQAPGKGLEWVA	RIRSKYNNYATYYADSVKD	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNSHVSWFAY	WGQGTLVTVSS

Figure 28D (Continued)

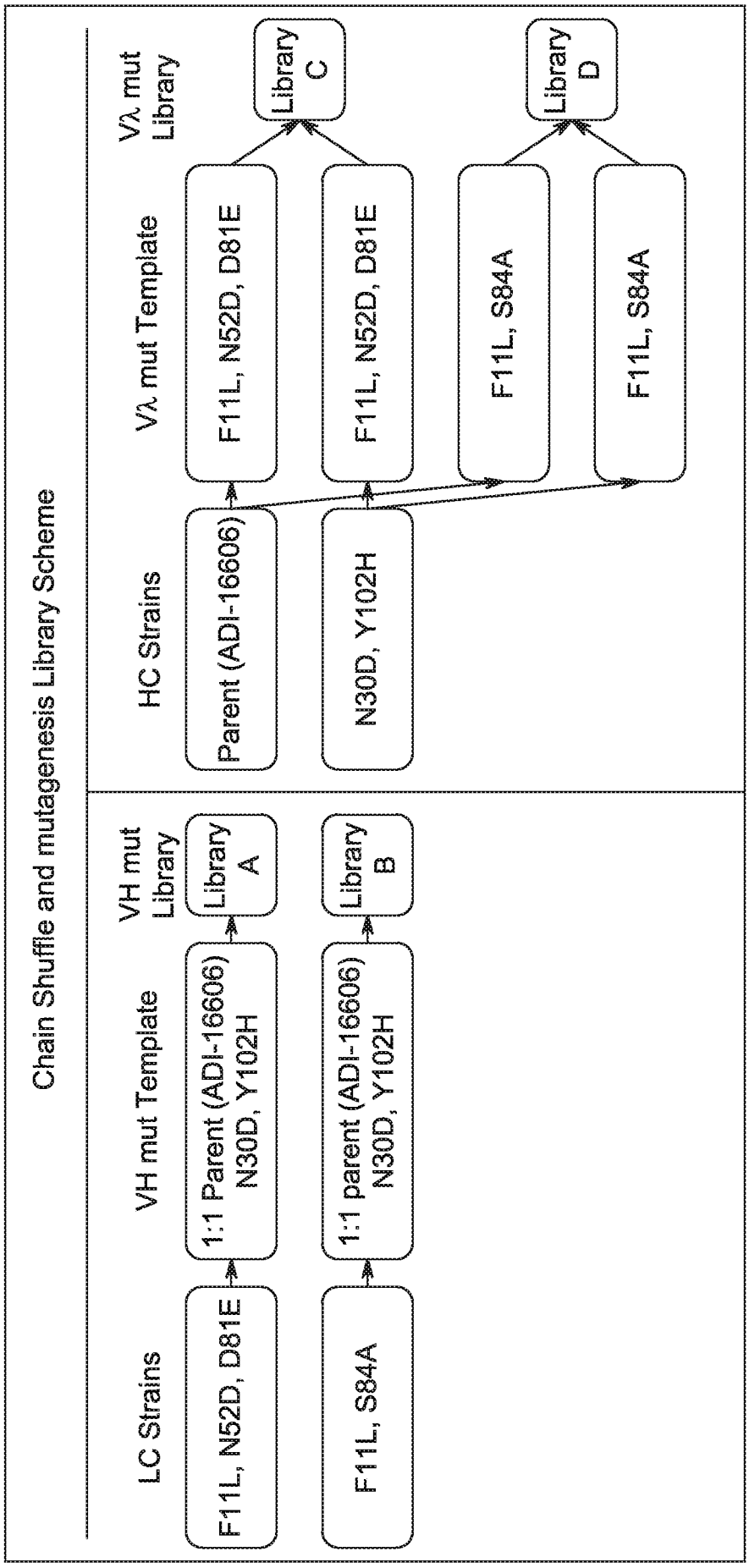

Chain Shuffle and mutagenesis Library Scheme
LC Strains
VH mut Template
VH mut Library
HC Strains
Vλ mut Template
Vλ mut Library
F11L, N52D, D81E
1:1 Parent (ADI-16606) N30D, Y102H
Library A
F11L, S84A
1:1 parent (ADI-16606) N30D, Y102H
Library B
Parent (ADI-16606)
F11L, N52D, D81E
Library C
N30D, Y102H
F11L, N52D, D81E
F11L, S84A
Library D
F11L, S84A

Figure 29A

Parents for Cycle 2 Optimization -Binding and Developability Profile Data

Name	VH mut	Vλ mut	Fab Tm (°C)	IgG Hu CD3εδ Fc-HIS KD (M)	IgG Cy CD3εγ Fc-HIS RU (M)	Jurkat CD3+ MFI	Jurkat CD3- MFI	Jurkat FOB	Cyno HSC-F MFI
CTL-19613			67.5	2.1E-09	2.7E-09	5101	150	34	11654
ADI-16606			61.5	3.5E-09	4.9E-09				
ADI-29601	Parent	F11L, N52D, D81E	66.0	4.5E-09	6.1E-09	5369	92	59	12368
ADI-29602	Parent	F11L, S84A	66.5	4.0E-09	5.3E-09	5737	113	51	12230
ADI-29603	N30D, Y102H	Parent	61.5	3.0E-09	3.5E-09				

Figure 29B

ADI-20587
K_D4.1 nM, Tm 66.5°C
ADI-20607
K_D1.1 nM, Tm 66.0°C
ADI-20590
K_D4.3 nM, Tm 65.5°C
28X Vk Rational Combinations
X
3X HC
Stability
Expression
PSR
Affinity
Variant
VL Chain
Muts Versus ADI-16606
ADI-16606
QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPGVPDRFSGSLLGDKAALTITGAQADDESDYYCALWYSNLWVFGGGTKLTVL
LC1 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALIITGAQADDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T74I
LC2 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALIITGVQADDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T74I, A78V
LC3 QTVVTQEPSLSVSPGGTVTLTCGSSAGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALIITGAQADDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T74I, T27A
LC4 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALIITGAQAEDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T74I, D81E
LC5 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALIITGAQADDEADYYCALWYNNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T74I, S93N
LC6 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALIITGAQADDEADYYCVLWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T74I, A89V
LC7 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALIITGAQADDEADYYCALWYSNHWVFGGGTKLTVL F11L, R24G, N52D, S84A, T74I, L95H
LC8 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGVQADDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, A78V
LC9 QTVVTQEPSLSVSPGGTVTLTCGSSAGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGVQADDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, A78V, T27A
LC10 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGVQAEDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, A78V, D81E
LC11 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGVQADDEADYYCALWYNNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, A78V, S93N
LC12 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGVQADDEADYYCVLWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, A78V, A89V
LC13 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGVQADDEADYYCALWYSNHWVFGGGTKLTVL F11L, R24G, N52D, S84A, A78V, L95H
LC14 QTVVTQEPSLSVSPGGTVTLTCGSSAGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T27A
LC15 QTVVTQEPSLSVSPGGTVTLTCGSSAGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T27A, D81E
LC16 QTVVTQEPSLSVSPGGTVTLTCGSSAGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCALWYNNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T27A, S93N
LC17 QTVVTQEPSLSVSPGGTVTLTCGSSAGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCVLWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, T27A, A89V
LC18 QTVVTQEPSLSVSPGGTVTLTCGSSAGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCALWYSNHWVFGGGTKLTVL F11L, R24G, N52D, S84A, T27A, L95H
LC19 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, D81E
LC20 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCALWYNNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, D81E, S93N
LC21 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCVLWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, D81E, A89V
LC22 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQAEDEADYYCALWYSNHWVFGGGTKLTVL F11L, R24G, N52D, S84A, D81E, L95H
LC23 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCALWYNNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, S93N
LC24 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCVLWYNNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, S93N, A89V
LC25 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCALWYNNHWVFGGGTKLTVL F11L, R24G, N52D, S84A, S93N, L95H
LC26 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCVLWYSNLWVFGGGTKLTVL F11L, R24G, N52D, S84A, A89V
LC27 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCVLWYSNHWVFGGGTKLTVL F11L, R24G, N52D, S84A, A89V, L95H
LC28 QTVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAPRGLIGGTDKRAPGVPDRFSGSLLGDKAALTITGAQADDEADYYCALWYSNHWVFGGGTKLTVL F11L, R24G, N52D, S84A, L95H

Figure 30

HuSP34 ADI-21952 Potential Degradation Motifs				
ADI-ID	VH FR3	VH CDR H13	Fab KD Hu CD3εδ-Fc (nM)	Fab KD Cy CD3εδ-Fc (nM)
ADI-21952	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNGYVSWFAH	4.5	3.4
ADI-23633	RFTISRDDSKSTLYLQMESLRAEDTAVYYC	VRHGNFGNGYVSWFAH	4.2	4.1
ADI-23634	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNEYVSWFAH	18.0	12.0
ADI-23636	RFTISRDDSKSTLYLQMNALRAEDTAVYYC	VRHGNFGNGYVSWFAH	3.9	4.1
ADI-23637	RFTISRDDSKSTLYLQMNSLRAEDTAVYYC	VRHGNFGNAYVSWFAH	21.0	13.0

Figure 31

ADI-21952 CDR H3 Designs

DTAVYYCVRHGNFGNGYVSWFAHWGQGTLV
DTAVYYCXRHGNFGXGYVSWFAHWGQGTLV
DTAVYYCXRHGNFGNXYVSWFAHWGQGTLV
DTAVYYCVXHGNFGXGYVSWFAHWGQGTLV
DTAVYYCVXHGNFGNXYVSWFAHWGQGTLV
DTAVYYCVRXGNFGXGYVSWFAHWGQGTLV
DTAVYYCVRXGNFGNXYVSWFAHWGQGTLV
DTAVYYCVRHXNFGXGYVSWFAHWGQGTLV
DTAVYYCVRHXNFGNXYVSWFAHWGQGTLV
DTAVYYCVRHGXFGXGYVSWFAHWGQGTLV
DTAVYYCVRHGXFGNXYVSWFAHWGQGTLV
DTAVYYCVRHGNXGXGYVSWFAHWGQGTLV
DTAVYYCVRHGNXGNXYVSWFAHWGQGTLV
DTAVYYCVRHGNFXXGYVSWFAHWGQGTLV
DTAVYYCVRHGNFXNXYVSWFAHWGQGTLV
DTAVYYCVRHGNFGXGXVSWFAHWGQGTLV
DTAVYYCVRHGNFGXGYXSWFAHWGQGTLV
DTAVYYCVRHGNFGXGYVXWFAHWGQGTLV
DTAVYYCVRHGNFGXGYVSXFAHWGQGTLV
DTAVYYCVRHGNFGXGYVSWXAHWGQGTLV
DTAVYYCVRHGNFGXGYVSWFXHWGQGTLV
DTAVYYCVRHGNFGXGYVSWFAXWGQGTLV
DTAVYYCVRHGNFGNXYVSWFAHWGQGTLV
DTAVYYCVRHGNFGNXYVXWFAHWGQGTLV
DTAVYYCVRHGNFGNXYVSXFAHWGQGTLV
DTAVYYCVRHGNFGNXYVSWXAHWGQGTLV
DTAVYYCVRHGNFGNXYVSWFXHWGQGTLV
DTAVYYCVRHGNFGNXYVSWFAXWGQGTLV

Parent	Selection Type	Theoretical Diversity
ADI-21952	H3 Oligo Mutagenesis	$<10^4$

Selection

HuSP34 ADI-21952 H3 Mutagenesis Progeny

Clone ID	CDR H3	KD Hu CD3εδ Fc (M)	PSR Score
ADI-23633	VRHGNFGNGYVSWFAH	3.1E-09	0.19
ADI-29716	VRHGNFGGGYVSWFAH	1.9E-09	0.21
ADI-29717	VRHGNFGNSYVSWFAH	3.4E-09	0.27
ADI-29718	VRHGNFGGGYVAWFAH	2.0E-09	0.19
ADI-29719	VRHGNFQGGYVSWFAH	2.5E-09	0.09
ADI-29720	VRHGNVGGGYVSWFAH	2.0E-09	0.00
ADI-29721	VRHDNFGAGYVSWFAH	5.9E-09	0.00
ADI-29722	VRHGNFGVGYVSWFAS	6.3E-09	0.00
ADI-29723	VRHGNFGNYAVSWFAH	3.2E-09	0.09
ADI-29724	VRHGSFGNHIVSWFAH	4.9E-08	0.00

Figure 32

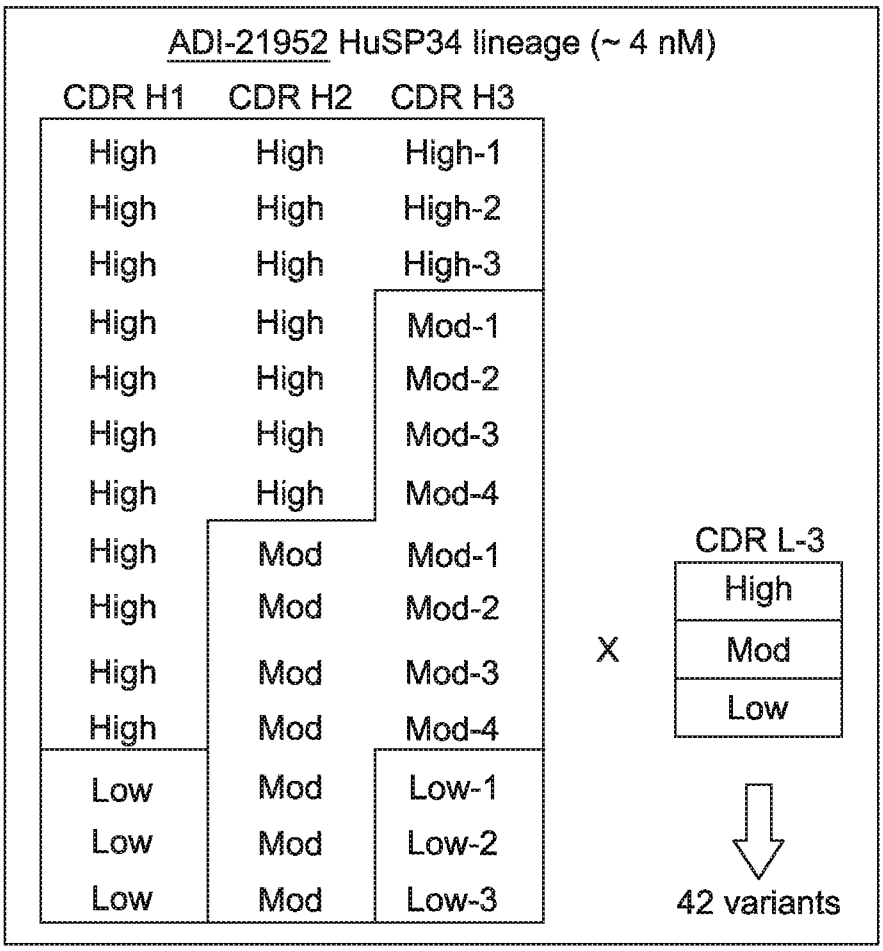


Figure 33A

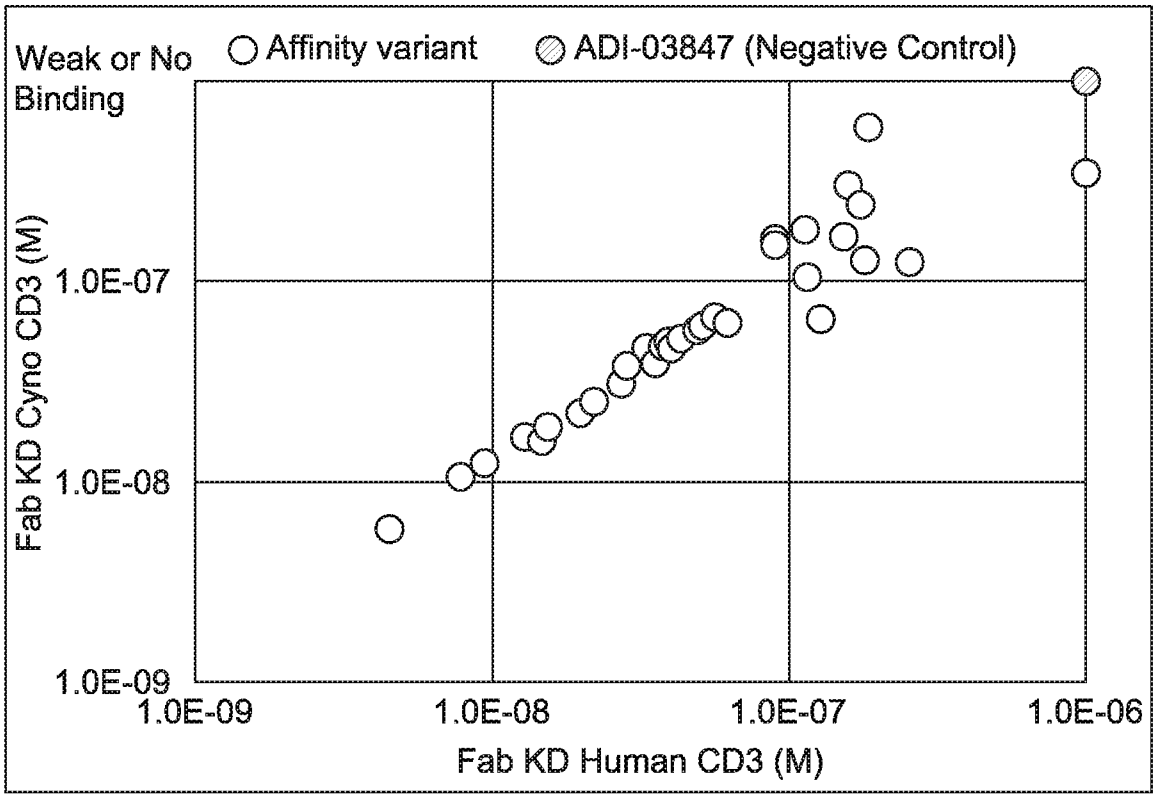


Figure 33B

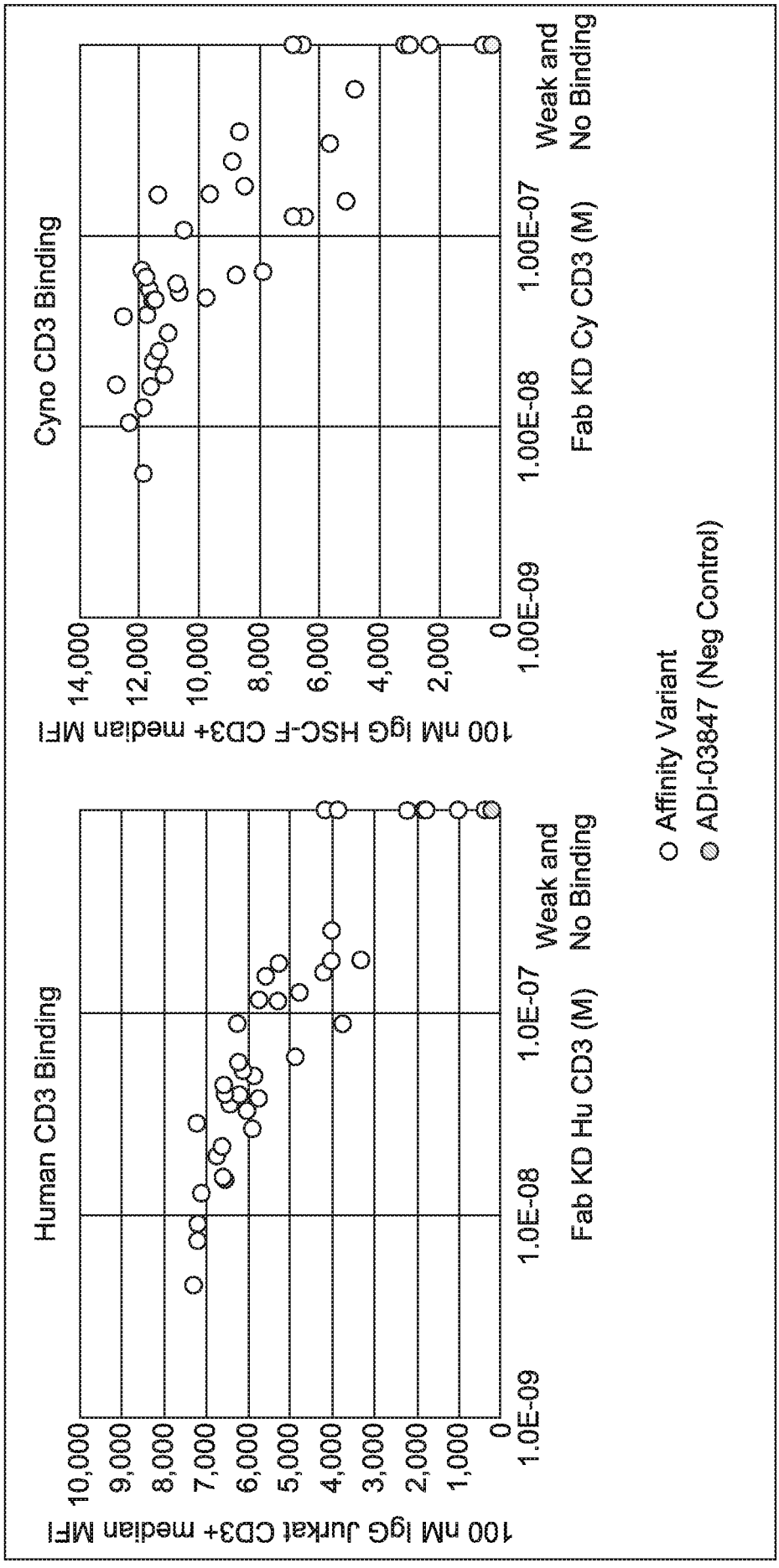

Human CD3 Binding
100 nM IgG Jurkat CD3+ median MFI
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
1.0E-09
1.0E-08
1.0E-07
Fab KD Hu CD3 (M)
Weak and No Binding
Cyno CD3 Binding
100 nM IgG HSC-F CD3+ median MFI
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
1.00E-09
1.00E-08
1.00E-07
Fab KD Cy CD3 (M)
Weak and No Binding
Affinity Variant
ADI-03847 (Neg Control)

Figure 33C

Parent	Type	#	KD Hu CD3εδ Fc (M)	Fold Reduction	KD Cy CD3εδ Fc (M)	Fold Reduction
ADI-21952	IgG	1	2.7E-09	N/A	2.5E-09	N/A
	scFv-Fc	15	4.9E-09	1.8	4.3E-09	1.7
	scFv-Fc (DS1)	15	2.0E-08	7.4	2.1E-08	8.4
	scFv-Fc (DS2)	2	2.1E-08	7.8	2.3E-08	9.2

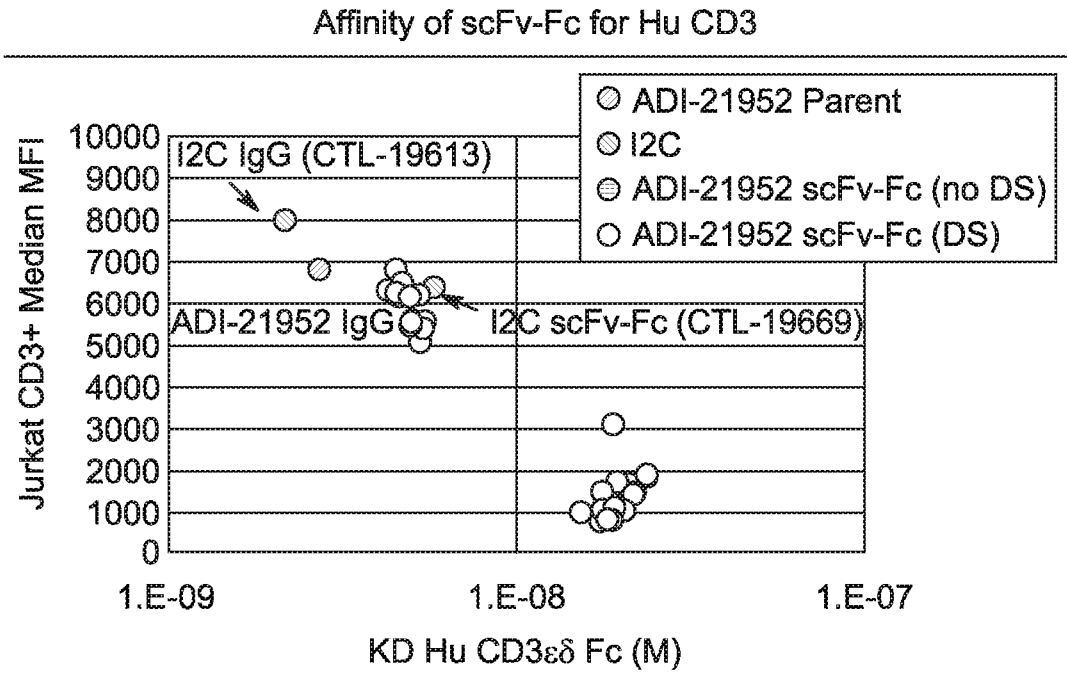


Figure 34A

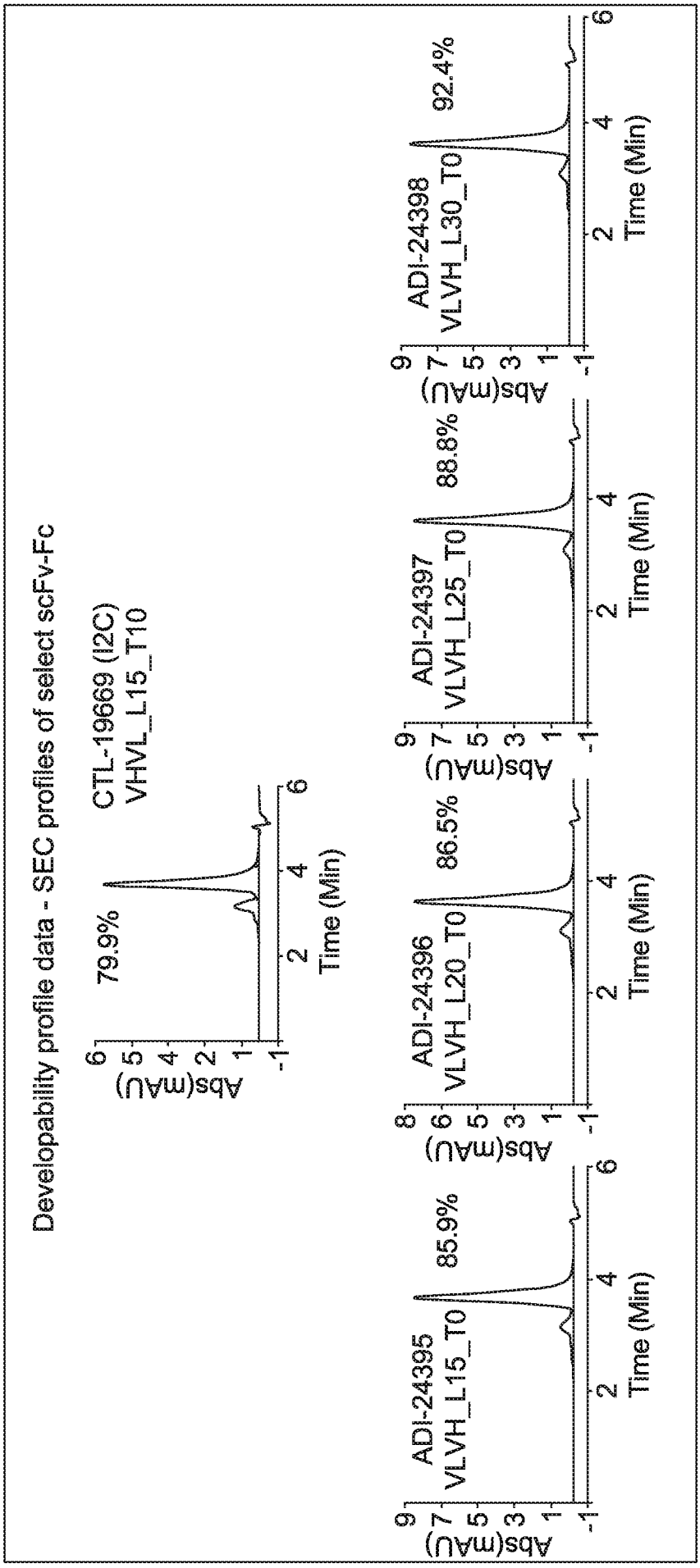

Developability profile data - SEC profiles of select scFv-Fc
CTL-19669 (I2C)
VHVL_L15_T10
79.9%
Abs(mAU)
Time (Min)
ADI-24395
VLVH_L15_T0
85.9%
ADI-24396
VLVH_L20_T0
86.5%
ADI-24397
VLVH_L25_T0
88.8%
ADI-24398
VLVH_L30_T0
92.4%

Figure 34B

Developability Profile Data

Parent	Type	#	DSF Tm (°C)	ΔTM Fab/scFv	ΔTM +/-DS
ADI-21952	IgG	1	69.5	N/A	N/A
	scFv-Fc	15	59.7	-9.8	N/A
	scFv-Fc (DS1)	15	63.7	-5.8	+4.0
	scFv-Fc (DS2)	2	65.0	-4.8	+5.3

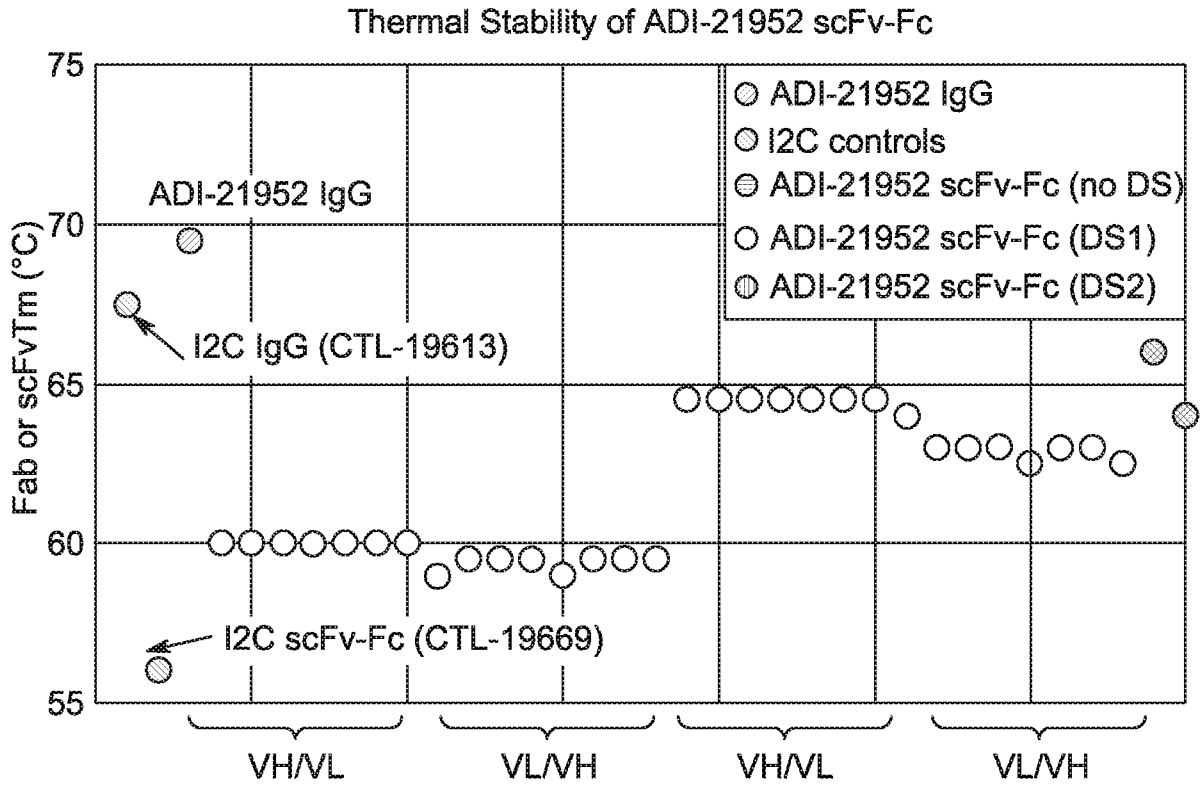


Figure 34C

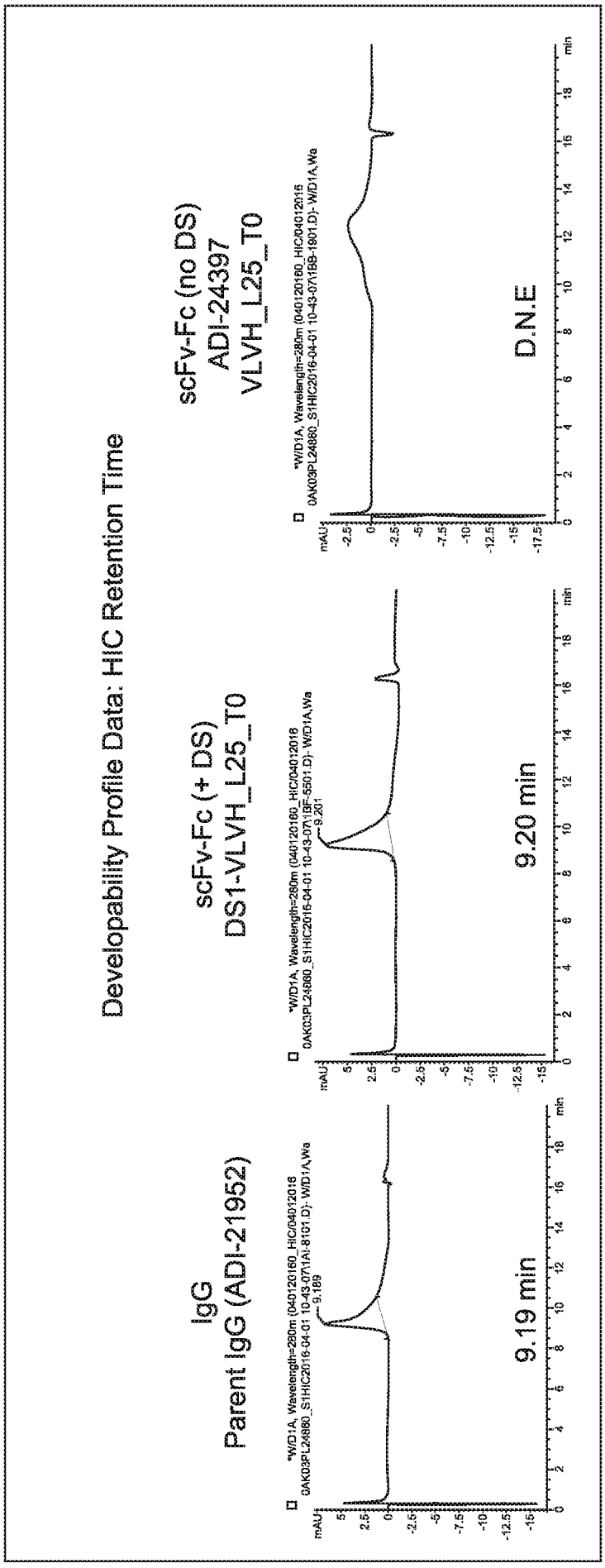

Developability Profile Data: HIC Retention Time
IgG
Parent IgG (ADI-21952)
9.19 min
scFv-Fc (+ DS)
DS1-VLVH_L25_T0
9.20 min
scFv-Fc (no DS)
ADI-24397
VLVH_L25_T0
D.N.E
mAU
min

Figure 34D

Binding and Developability Profile Data

ADI ID	VH CDR H3 sequence	BiaCore Fab K_D (M) Hu CD3ed-Fc Monovalent	BiaCore Fab K_D (M) Cy CD3ed-Fc Monovalent	ForteBio IgG K_D (M) Hu CD3ed-Fc Avid	ForteBio IgG K_D (M) Cy CD3ed-Fc Avid	ForteBio IgG K_D (M) Hu CD28-Fc Avid	Jurkat CD3+ median MFI
CTL-19672	ARDGYSRYYFDY	9.69E-10	1.05E-09	1.46E-10	1.39E-10	N.B.	5626
ADI-22523	ARDGYGRYFYDV	1.64E-09	1.39E-09	1.83E-10	1.66E-10	N.B.	6058
ADI-26906	ARDAYGRYFYDV	3.52E-09	3.34E-09	2.36E-10	1.98E-10	N.B.	5825
ADI-26907	GRDAYGRYFYDV	4.46E-09	3.87E-09	2.57E-10	2.32E-10	N.B.	5937
ADI-26908	ARDAYGRYFYDV	5.18E-09	4.49E-09	2.74E-10	2.32E-10	N.B.	5612
ADI-26910	ARDRYGAYFYDV	7.62E-09	6.98E-09	3.07E-10	2.68E-10	N.B.	5467
ADI-26913	ARDQYGRYFYDV	2.92E-08	2.72E-08	5.03E-10	4.28E-10	N.B.	5275
ADI-26915	ARDVLGRYFYDV	W.B.	W.B.	1.94E-09	3.06E-09	N.B.	1310
ADI-26919	ARDAYGRYFYDV	5.56E-08	4.05E-08	5.07E-10	4.40E-10	N.B.	4448
ADI-26920	GRDAYGRYFYDV	5.15E-08	5.11E-08	5.86E-10	5.76E-10	N.B.	4987
ADI-26921	ARDAYGRYFYDV	4.61E-08	3.84E-08	6.16E-10	5.35E-10	N.B.	5202
ADI-26943	ARDAYGGYFYDV	W.B.	W.B.	4.09E-09	2.50E-09	N.B.	1083
ADI-26954	ARDNYGGYFYDV	N.B.	N.B.	N.B.	N.B.	N.B.	31
CTL-19613	VRHGNFGNSYTSYWAY	1.43E-09	1.62E-09	1.67E-10	1.59E-10	N.B.	6137
ADI-21952	VRHGNFGNGYVSWFAH	4.13E-09	5.04E-09	7.41E-10	7.42E-10	N.B.	5724
ADI-26955	VRHGNFQGGYVSWFAH	3.28E-09	3.81E-09	2.97E-10	3.05E-10	N.B.	5200
ADI-26956	VRHGNFGGGYVSWFAW	3.77E-09	4.31E-09	2.33E-10	2.34E-10	N.B.	5222
ADI-26962	VRHGNFGEGYVSWFAH	2.39E-08	2.67E-08	3.94E-10	3.87E-10	N.B.	4014
ADI-26978	VRHGNFGGAYVSWFAH	2.92E-08	2.93E-08	5.41E-10	5.30E-10	N.B.	2956
ADI-26983	VRHGNFGGGYVSWFAW	2.03E-08	2.25E-08	N.D.	N.D.	N.B.	4513
ADI-26994	VRHGNVGGGYVSWFAH	W.B.	W.B.	3.26E-09	2.23E-09	N.B.	750
ADI-18965	GRDWDGAIRVLDY	N.B.	N.B.	N.B.	N.B.	N.B.	20

CTL-19672 is 38E4	Highlighted Mutations to ADI-22523 H3 in **RED**	W.B. = Weak-binder
CTL-19613 is I2C	Highlighted Mutations to SP34 H3 in **BLUE**: VRHGNFGNSYVSWFAY	N.B. = Non-binder
VH1-3/VK4-1 Negative Control		Multiple Concentration Global Fitting

100 nM IgG

N.D. = Not Determined

Figure 35A

Cy HSC-F Median MFI	Poly-Specificity Reagent (PSR) Score (0-1)	BVP ELISA Fold Over Background (FOB)	HIC Retention Time (min)	AC-SINS Δλmax (nm)	SEC (% mono)	Fab Tm (°C)	HEK titer (mg/L)
12831	0.71	22.8	8.1	25.2	98.0	74.5	38.2
12276	0.40	13.6	8.3	19.3	94.8	74.5	147.4
12000	0.29	9.4	8.3	11.2	98.1	81.5	163.6
11751	0.23	7.4	8.4	10.4	98.0	73.5	204.3
11831	0.23	10.1	8.3	9.0	97.8	81.5	172.8
11887	0.30	5.1	8.3	8.2	97.9	81.5	173.4
11471	0.10	3	8.2	2.6	98.1	81.0	190.4
6165	0.00	1.3	8.2	0.8	98.1	79.0	180.3
9742	0.05	3.7	8.5	6.8	94.7	82.0	123.5
11962	0.02	2.9	8.5	7.0	97.2	75.5	93.2
11723	0.06	3.3	8.5	5.5	93.8	82.0	175.7
7180	0.00	1.3	8.8	0.9	96.7	75.5	127.6
23	0.00	1.2	8.6	0.3	97.6	79.0	176.6
13240	0.71	26	9.6	20.0	93.1	60.0	104.8
13125	0.43	14.8	9.8	7.0	94.9	72.5	34.1
12893	0.26	2.8	9.1	0.4	97.2	71.5	207.6
11775	0.24	5.8	9.9	0.3	97.5	69.5	136.0
12297	0.10	3.3	9.2	0.1	93.4	67.5	96.6
11524	0.10	5.6	9.2	0.4	96.2	69.5	156.4
12504	0.11	N.D.	N.D.	0.7	96.3	69.0	154.2
3555	0.10	1.6	9.1	0.7	96.3	70.5	198.5
25	0.14	N.D.	9.5	N.D.	97.4	73.0	69.7

100 nM IgG

Clean PSR: 0.00 -0.10	N.D. = Not Determined	Clean to Low HIC: < 10.5 min	ΔλMax < 5 nM Implies Low Self-interaction
Low PSR: 0.10-0.33	Clean BVP: FOB < 2.1 Implies Clean	Medium HIC: ≥ 10.5 and < 11.5 min	ΔλMax ≥ 5 nm and < 20 nM Implies Medium Self-interaction
Medium PSR: 0.33 -0.66	Medium BVP: FOB ≥ 2.1 and < 6.5		
High PSR: 0.66 -1.00	High BVP: FOB ≥ 6.5 Implies High BVP Reactivity	High HIC: ≥ 11.5 Min	ΔλMax ≥ 20 nM Implies high Self-interaction

Titer < 50 mg/L

Figure 35A (Continued)

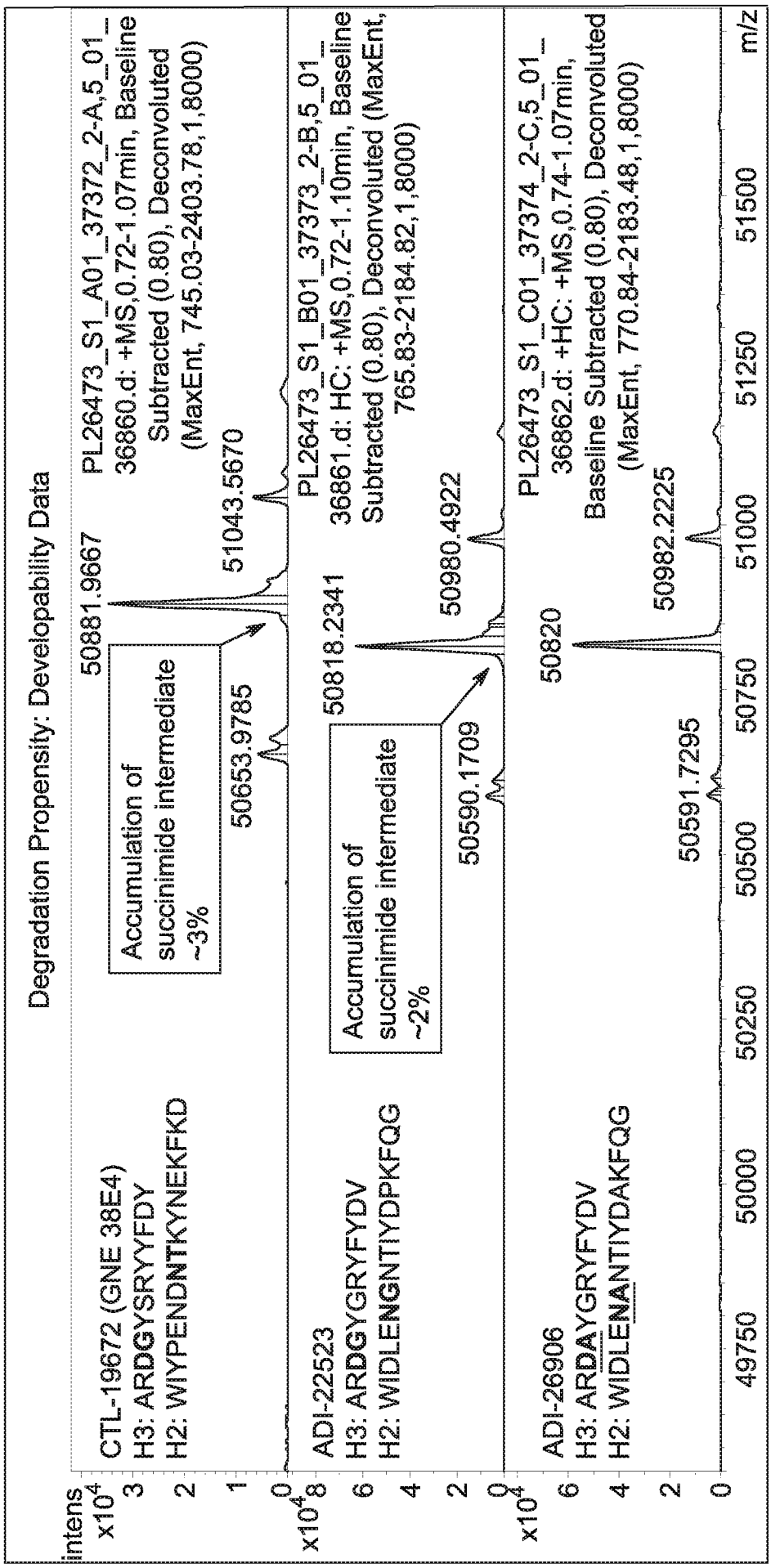

Degradation Propensity: Developability Data
intens
CTL-19672 (GNE 38E4)
H3: ARDGYSRYYFDY
H2: WIYPENDNTKYNEKFKD
ADI-22523
H3: ARDGYGRYFYDV
H2: WIDLENGNTIYDPKFQG
ADI-26906
H3: ARDAYGRYFYDV
H2: WIDLENANTIYDAKFQG
50881.9667
51043.5670
50653.9785
50818.2341
50980.4922
50590.1709
50820
50982.2225
50591.7295
Accumulation of succinimide intermediate ~3%
Accumulation of succinimide intermediate ~2%
PL26473_S1_A01_37372_2-A,5_01_36860.d: +MS,0.72-1.07min, Baseline Subtracted (0.80), Deconvoluted (MaxEnt, 745.03-2403.78,1,8000)
PL26473_S1_B01_37373_2-B,5_01_36861.d: HC: +MS,0.72-1.10min, Baseline Subtracted (0.80), Deconvoluted (MaxEnt, 765.83-2184.82,1,8000)
PL26473_S1_C01_37374_2-C,5_01_36862.d: +HC: +MS,0.74-1.07min, Baseline Subtracted (0.80), Deconvoluted (MaxEnt, 770.84-2183.48,1,8000)
m/z

Figure 35B

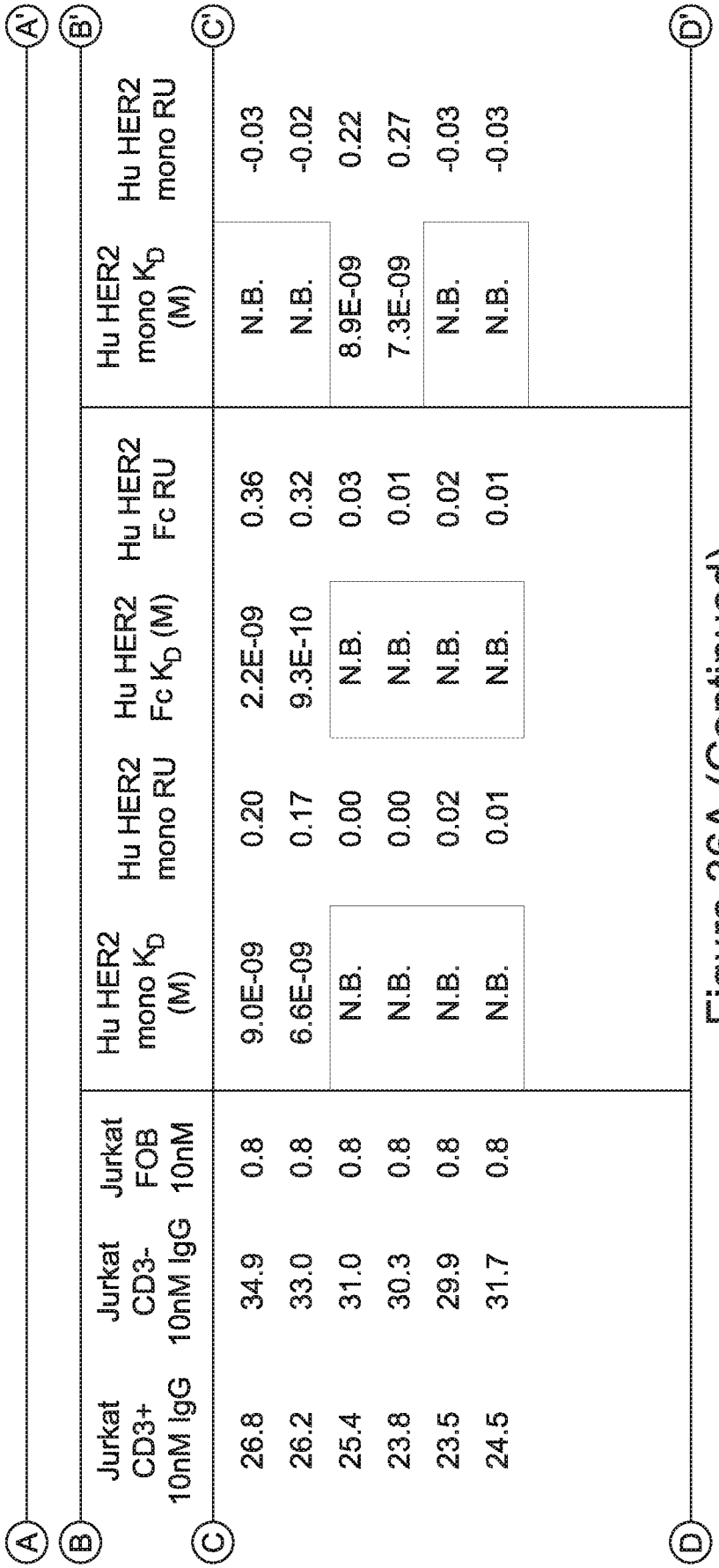
Properties of Monospecific TAA scFv-Fc: Binding and Developability profile data

Name	Target	Format	Design	Titer (mg/L)	Tm (°C)	AC-SINS	HIC Retention Time (min)	SEC
Trastuzumab	HER2	scFv-Fc	IgG4 scFv-Fc H/L, L20, T5	227.9	N.D.	N.D.	N.D.	97.0
Trastuzumab	HER2	scFv-Fc	IgG4 scFv-Fc L/H, L20, T5	208.0	61.5	5.1	10.9	97.8
Lintuzumab	CD33	scFv-Fc	IgG4 scFv-Fc H/L, L20, T5	117.2	N.D.	N.D.	N.D.	98.2
Lintuzumab	CD33	scFv-Fc	IgG4 scFv-Fc L/H, L20, T5	169.2	60.0	4.0	11.8	97.1
ADI-03847	HEL	scFv-Fc	IgG4 scFv-Fc H/L, L20, T5	16.0	N.D.	N.D.	N.D.	98.8
ADI-03847	HEL	scFv-Fc	IgG4 scFv-Fc L/H, L20, T5	76.3	55.5	2.4	D.N.E.	95.1

Selected for Bispecific Assembly

D.N.E. = Does not Elute

Figure 36A



Jurkat CD3+ 10nM IgG	Jurkat CD3- 10nM IgG	Jurkat FOB 10nM	Hu HER2 mono K_D (M)	Hu HER2 mono RU	Hu HER2 Fc K_D (M)	Hu HER2 Fc RU	Hu HER2 mono K_D (M)	Hu HER2 mono RU
26.8	34.9	0.8	9.0E-09	0.20	2.2E-09	0.36	N.B.	-0.03
26.2	33.0	0.8	6.6E-09	0.17	9.3E-10	0.32	N.B.	-0.02
25.4	31.0	0.8	N.B.	0.00	N.B.	0.03	8.9E-09	0.22
23.8	30.3	0.8	N.B.	0.00	N.B.	0.01	7.3E-09	0.27
23.5	29.9	0.8	N.B.	0.02	N.B.	0.02	N.B.	-0.03
24.5	31.7	0.8	N.B.	0.01	N.B.	0.01	N.B.	-0.03

Figure 36A (Continued)

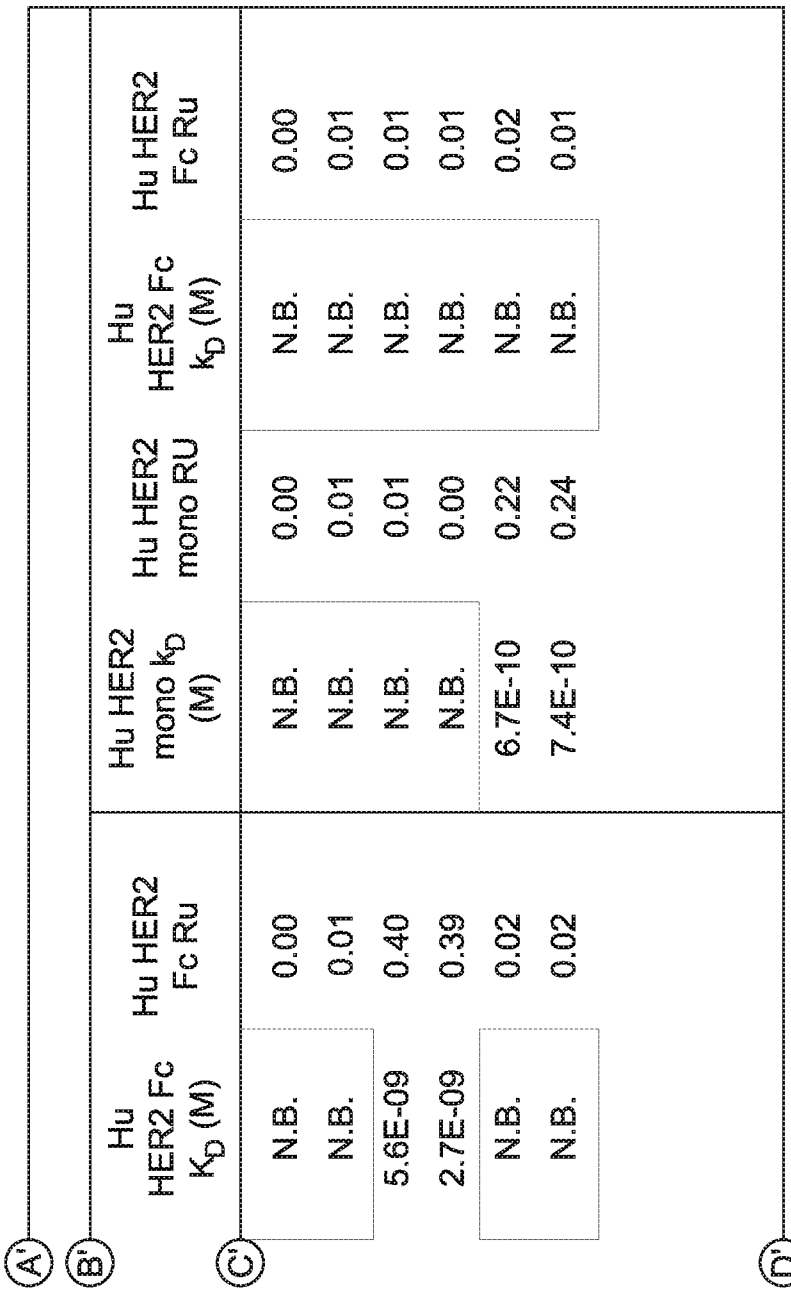
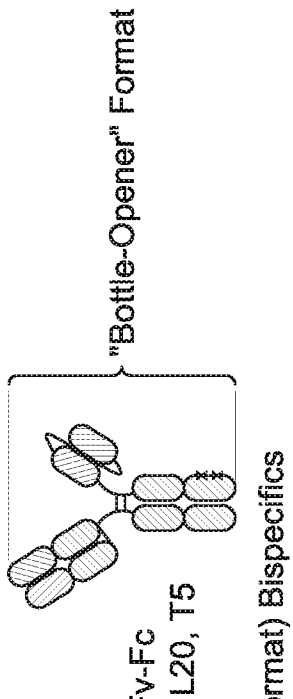


Figure 36A (Continued)



Bispecific Characteristics

- IgG4 Ser228Phe
- His435->Arg, Tyr436->Phe Mutations (X)

Purification of Fab/scFv-Fc Heterodimer (Aka "Bottle-Opener" Format) Bispecifics

Figure 36B

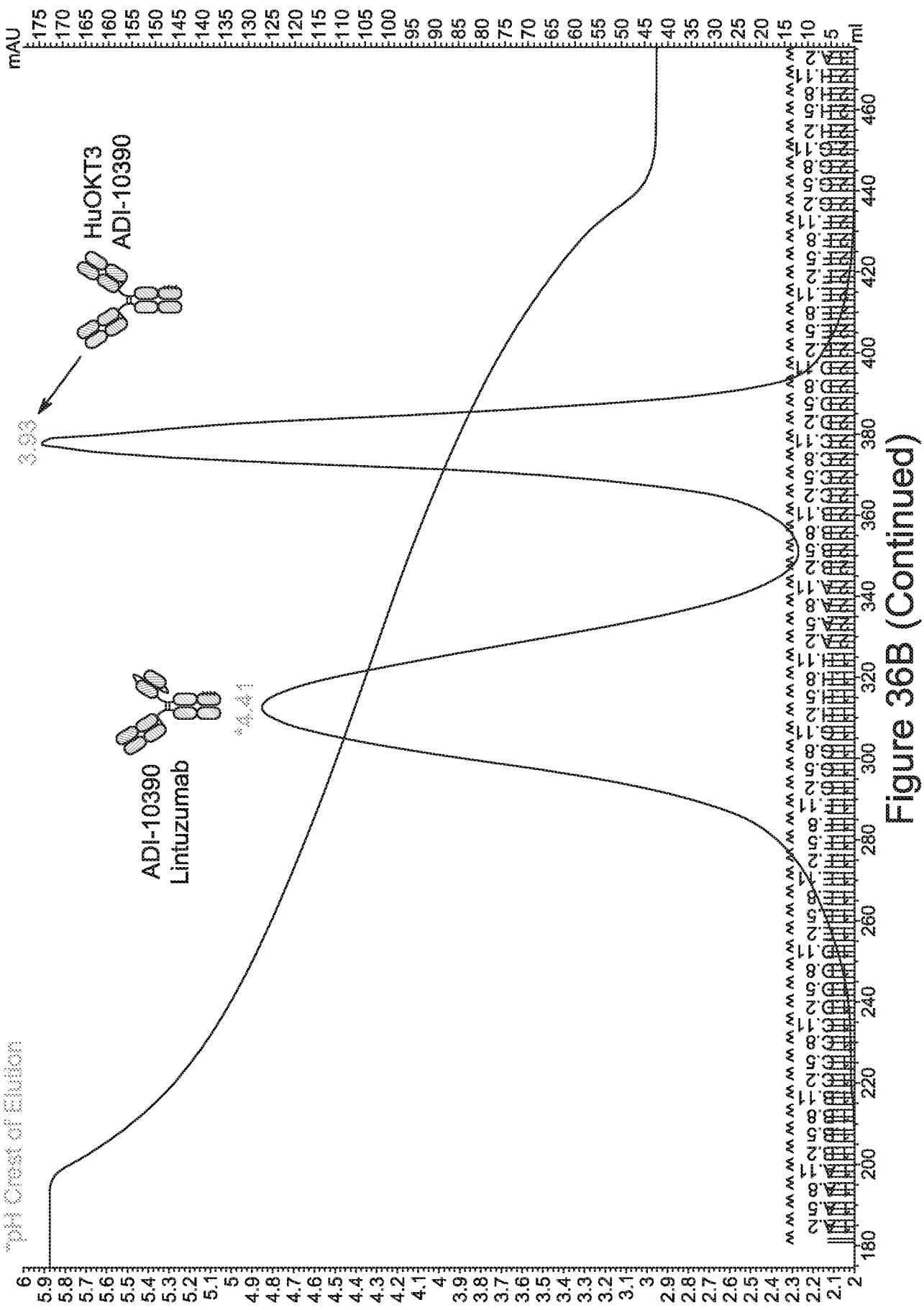


Figure 36B (Continued)

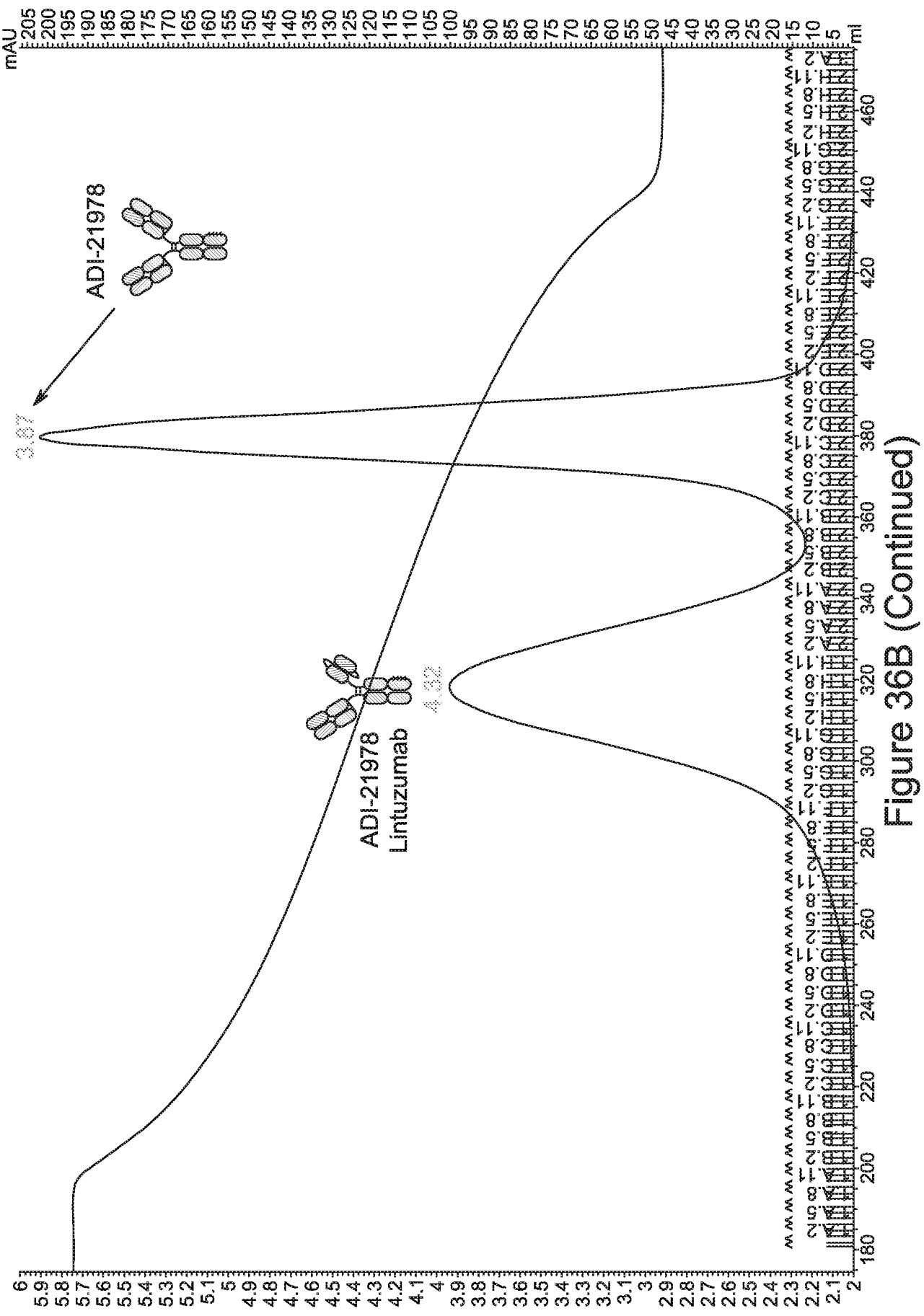


Figure 36B (Continued)

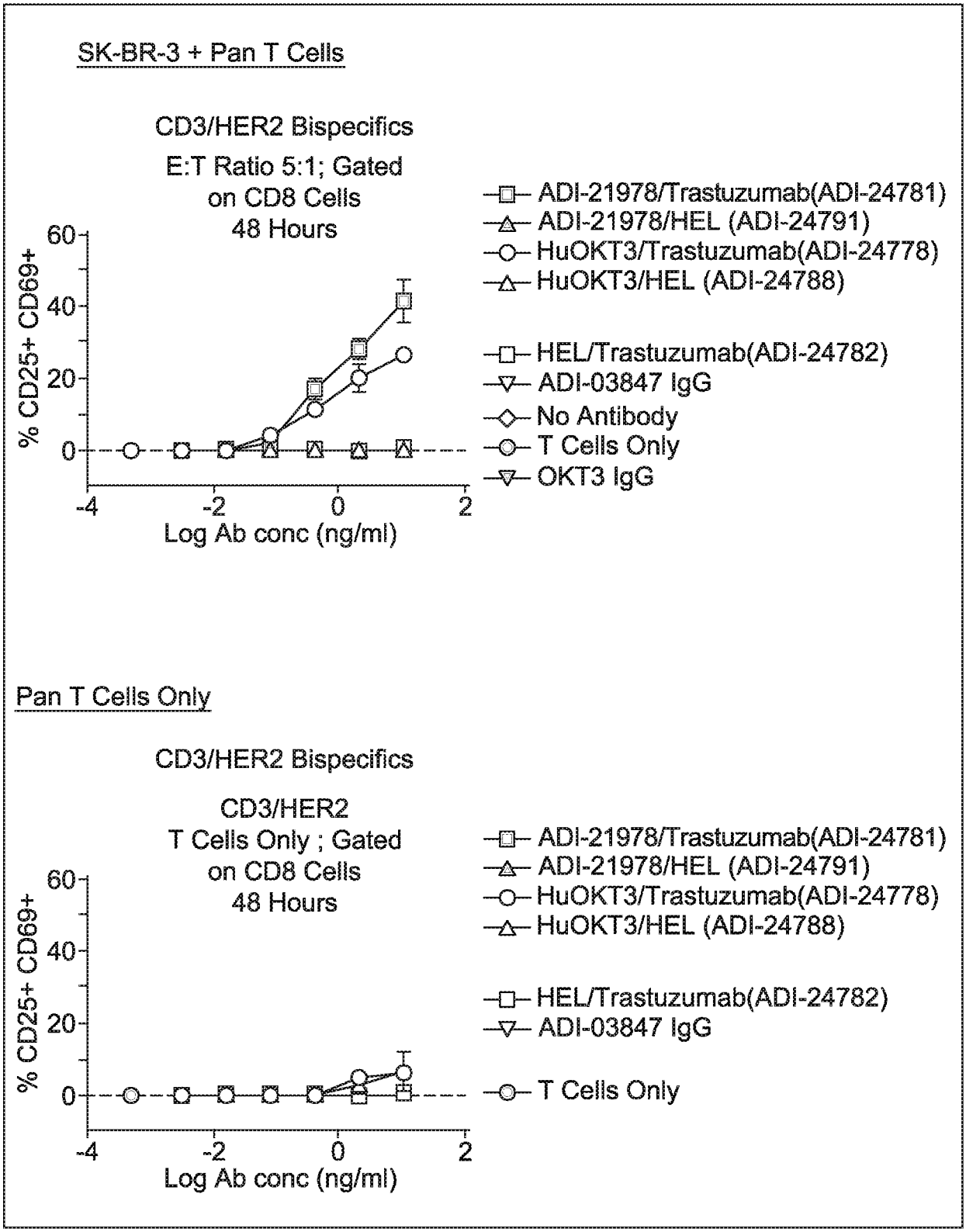
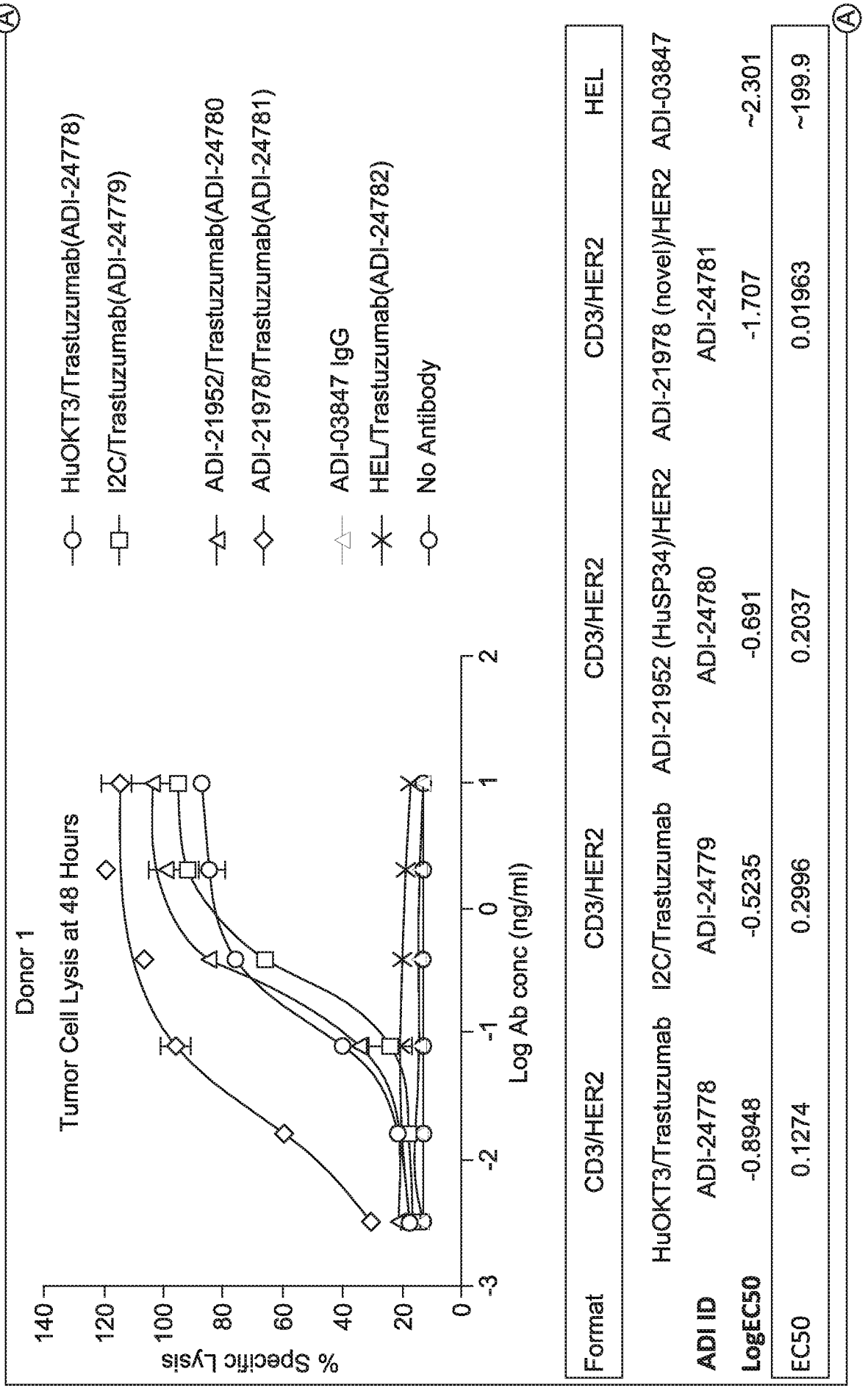
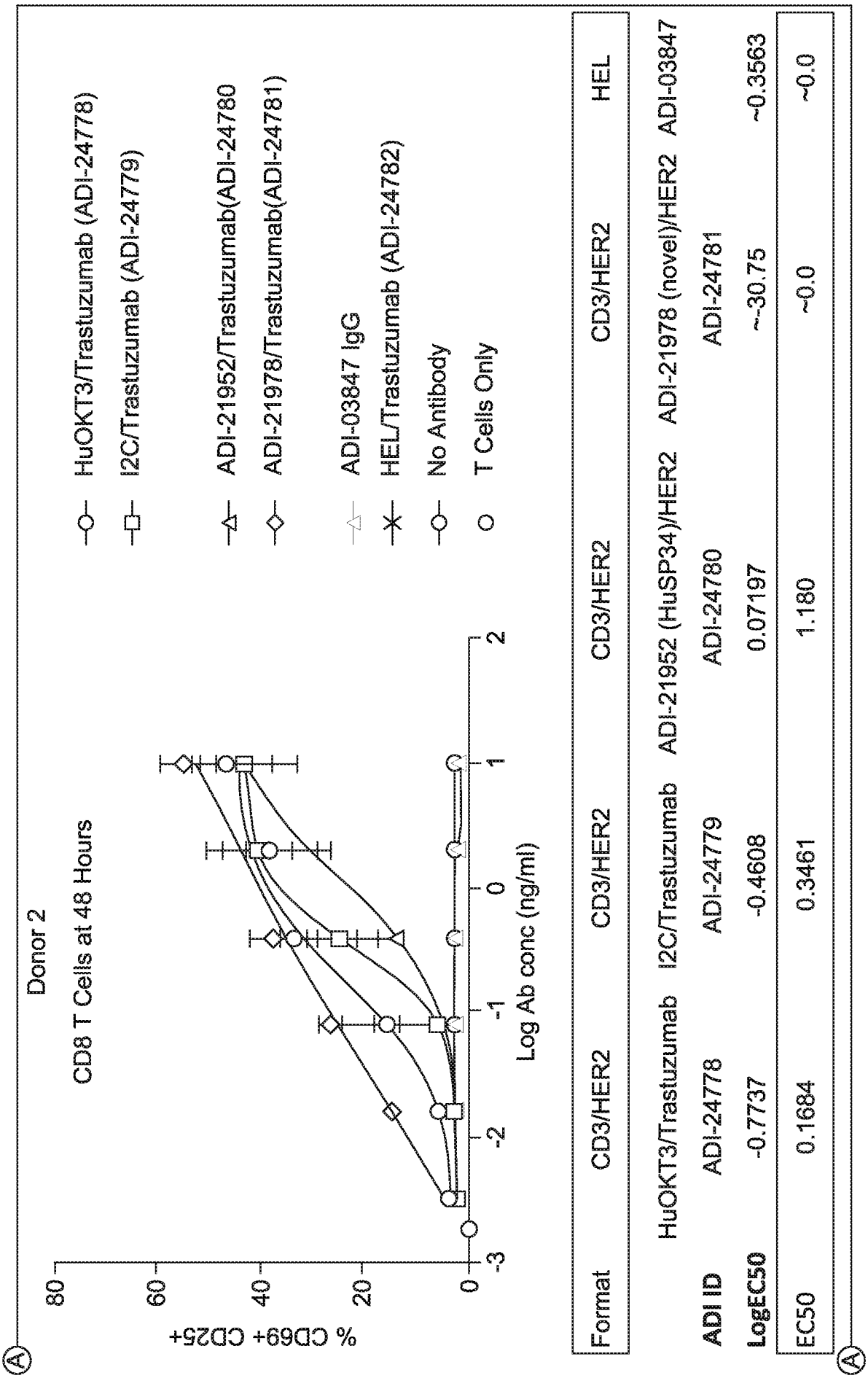

SK-BR-3 + Pan T Cells
CD3/HER2 Bispecifics
E:T Ratio 5:1; Gated
on CD8 Cells
48 Hours
% CD25+ CD69+
60
40
20
0
-4
-2
0
2
Log Ab conc (ng/ml)
ADI-21978/Trastuzumab(ADI-24781)
ADI-21978/HEL (ADI-24791)
HuOKT3/Trastuzumab(ADI-24778)
HuOKT3/HEL (ADI-24788)
HEL/Trastuzumab(ADI-24782)
ADI-03847 IgG
No Antibody
T Cells Only
OKT3 IgG
Pan T Cells Only
CD3/HER2 Bispecifics
CD3/HER2
T Cells Only ; Gated
on CD8 Cells
48 Hours
% CD25+ CD69+
60
40
20
0
-4
-2
0
2
Log Ab conc (ng/ml)
ADI-21978/Trastuzumab(ADI-24781)
ADI-21978/HEL (ADI-24791)
HuOKT3/Trastuzumab(ADI-24778)
HuOKT3/HEL (ADI-24788)
HEL/Trastuzumab(ADI-24782)
ADI-03847 IgG
T Cells Only

Figure 36C



Format	CD3/HER2	CD3/HER2	CD3/HER2	CD3/HER2	HEL
	HuOKT3/Trastuzumab	I2C/Trastuzumab	ADI-21952 (HuSP34)/HER2	ADI-21978 (novel)/HER2	ADI-03847
ADI ID	ADI-24778	ADI-24779	ADI-24780	ADI-24781	
LogEC50	-0.8948	-0.5235	-0.691	-1.707	~2.301
EC50	0.1274	0.2996	0.2037	0.01963	~199.9

Figure 37



Format	CD3/HER2	CD3/HER2	CD3/HER2	CD3/HER2	HEL
	HuOKT3/Trastuzumab	I2C/Trastuzumab	ADI-21952 (HuSP34)/HER2	ADI-21978 (novel)/HER2	ADI-03847
ADI ID	ADI-24778	ADI-24779	ADI-24780	ADI-24781	
LogEC50	-0.7737	-0.4608	0.07197	~-30.75	~0.3563
EC50	0.1684	0.3461	1.180	~0.0	~0.0

Figure 37 (Continued)

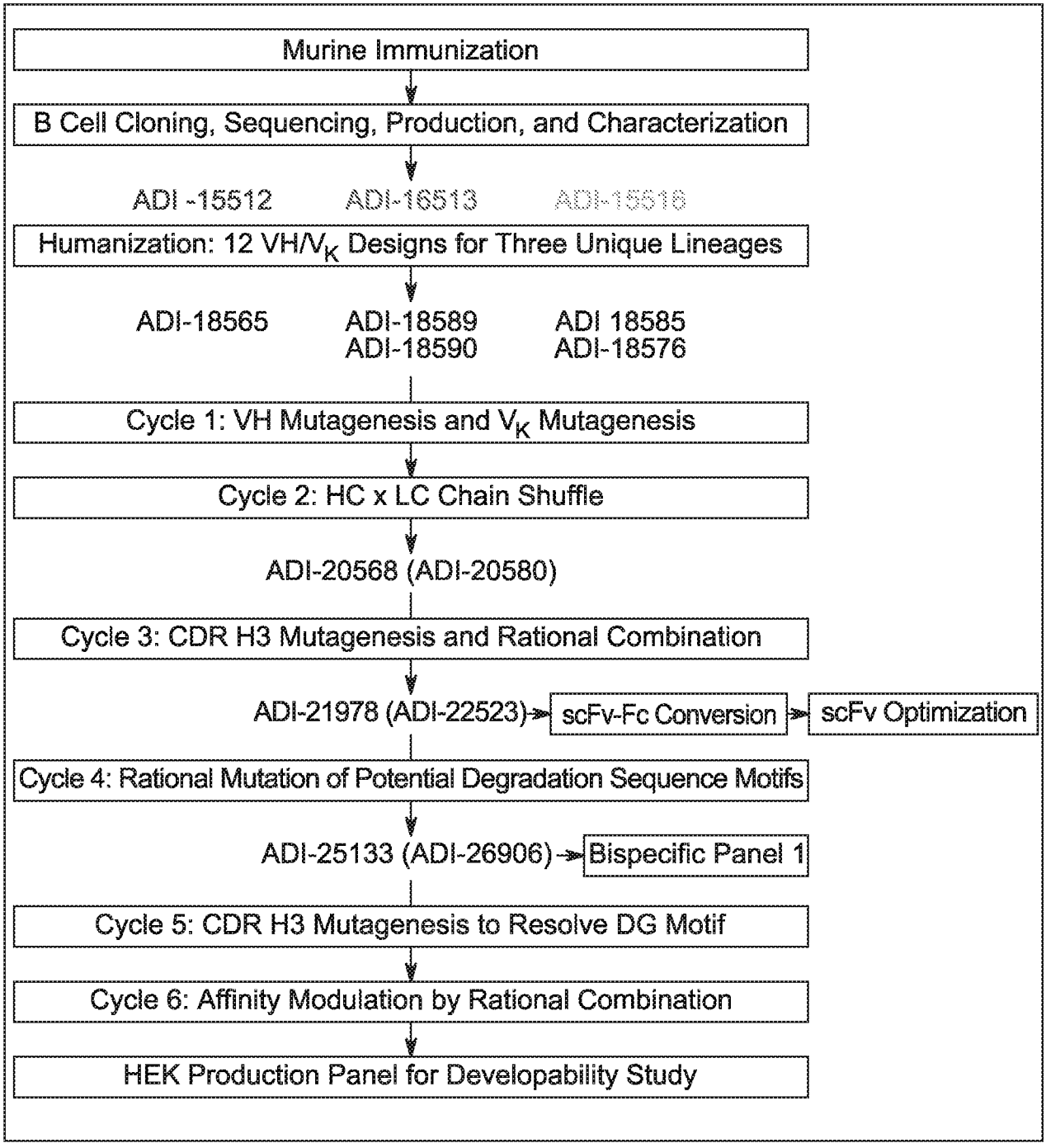

Murine Immunization
B Cell Cloning, Sequencing, Production, and Characterization
ADI -15512
ADI-16513
ADI-15516
Humanization: 12 VH/VK Designs for Three Unique Lineages
ADI-18565
ADI-18589
ADI-18590
ADI 18585
ADI-18576
Cycle 1: VH Mutagenesis and VK Mutagenesis
Cycle 2: HC x LC Chain Shuffle
ADI-20568 (ADI-20580)
Cycle 3: CDR H3 Mutagenesis and Rational Combination
ADI-21978 (ADI-22523)
scFv-Fc Conversion
scFv Optimization
Cycle 4: Rational Mutation of Potential Degradation Sequence Motifs
ADI-25133 (ADI-26906)
Bispecific Panel 1
Cycle 5: CDR H3 Mutagenesis to Resolve DG Motif
Cycle 6: Affinity Modulation by Rational Combination
HEK Production Panel for Developability Study

Figure 38A

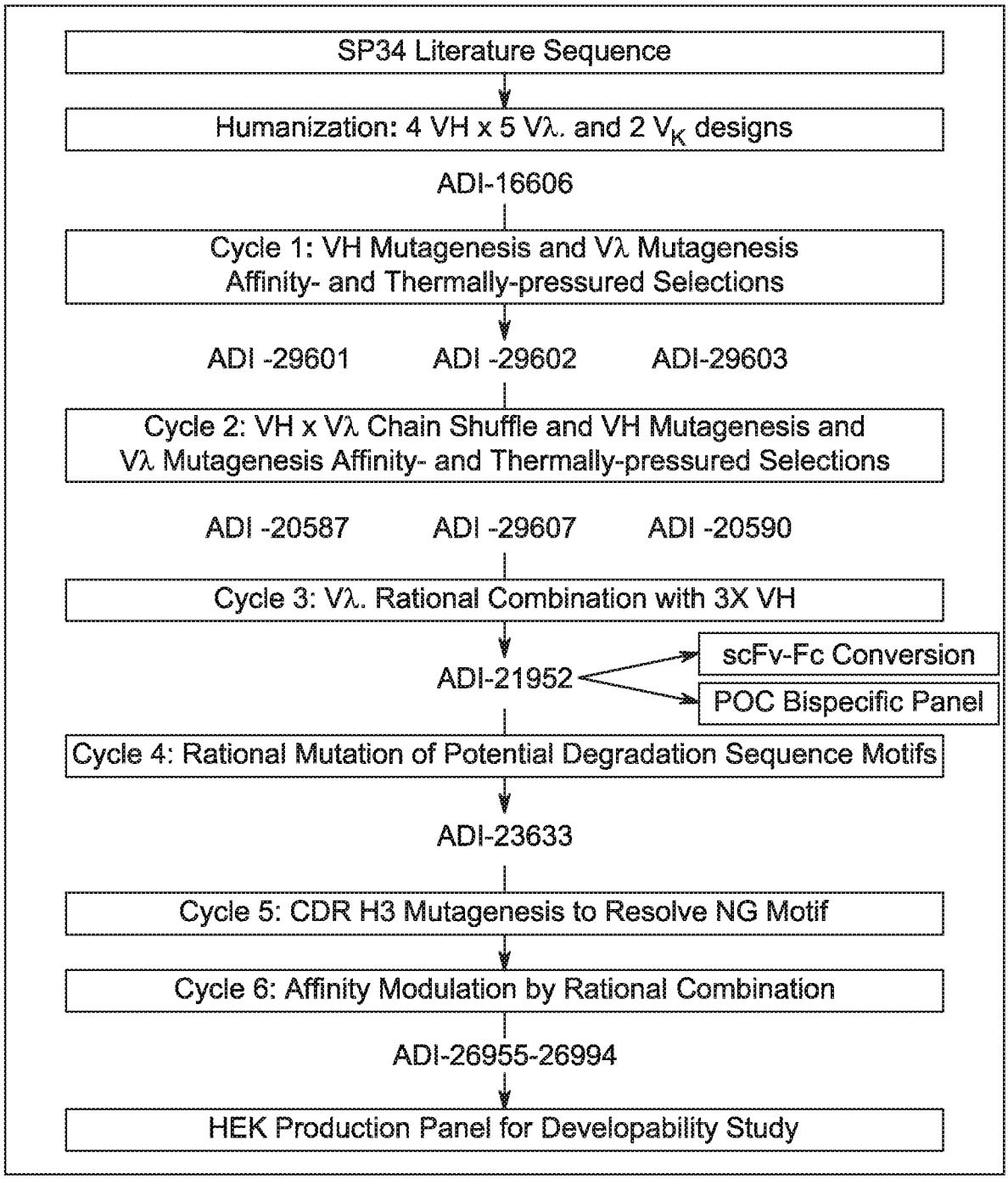

SP34 Literature Sequence
Humanization: 4 VH x 5 Vλ. and 2 V_K designs
ADI-16606
Cycle 1: VH Mutagenesis and Vλ Mutagenesis
Affinity- and Thermally-pressured Selections
ADI -29601
ADI -29602
ADI-29603
Cycle 2: VH x Vλ Chain Shuffle and VH Mutagenesis and
Vλ Mutagenesis Affinity- and Thermally-pressured Selections
ADI -20587
ADI -29607
ADI -20590
Cycle 3: Vλ. Rational Combination with 3X VH
ADI-21952
scFv-Fc Conversion
POC Bispecific Panel
Cycle 4: Rational Mutation of Potential Degradation Sequence Motifs
ADI-23633
Cycle 5: CDR H3 Mutagenesis to Resolve NG Motif
Cycle 6: Affinity Modulation by Rational Combination
ADI-26955-26994
HEK Production Panel for Developability Study

Figure 38B