

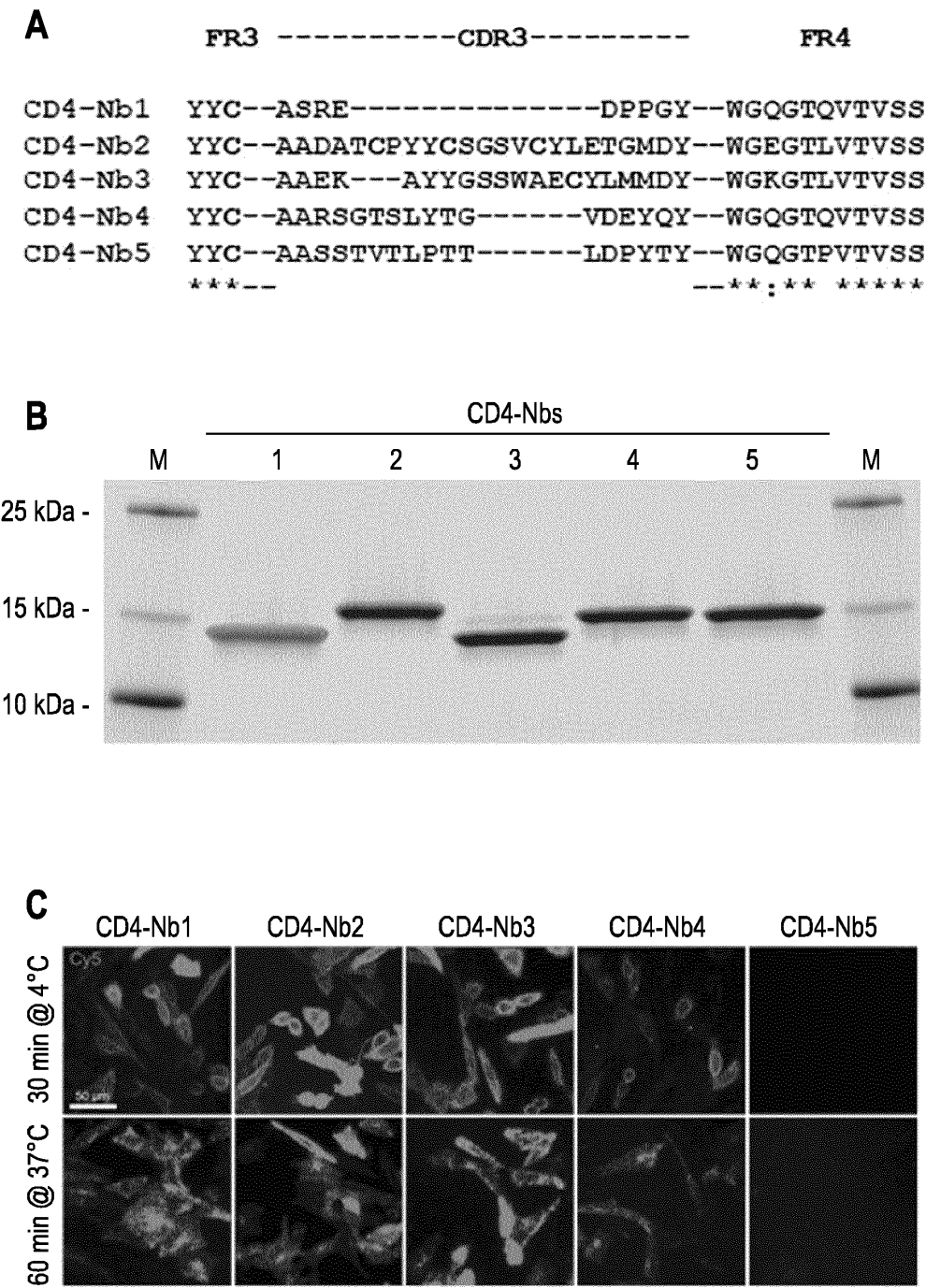


Fig. 1

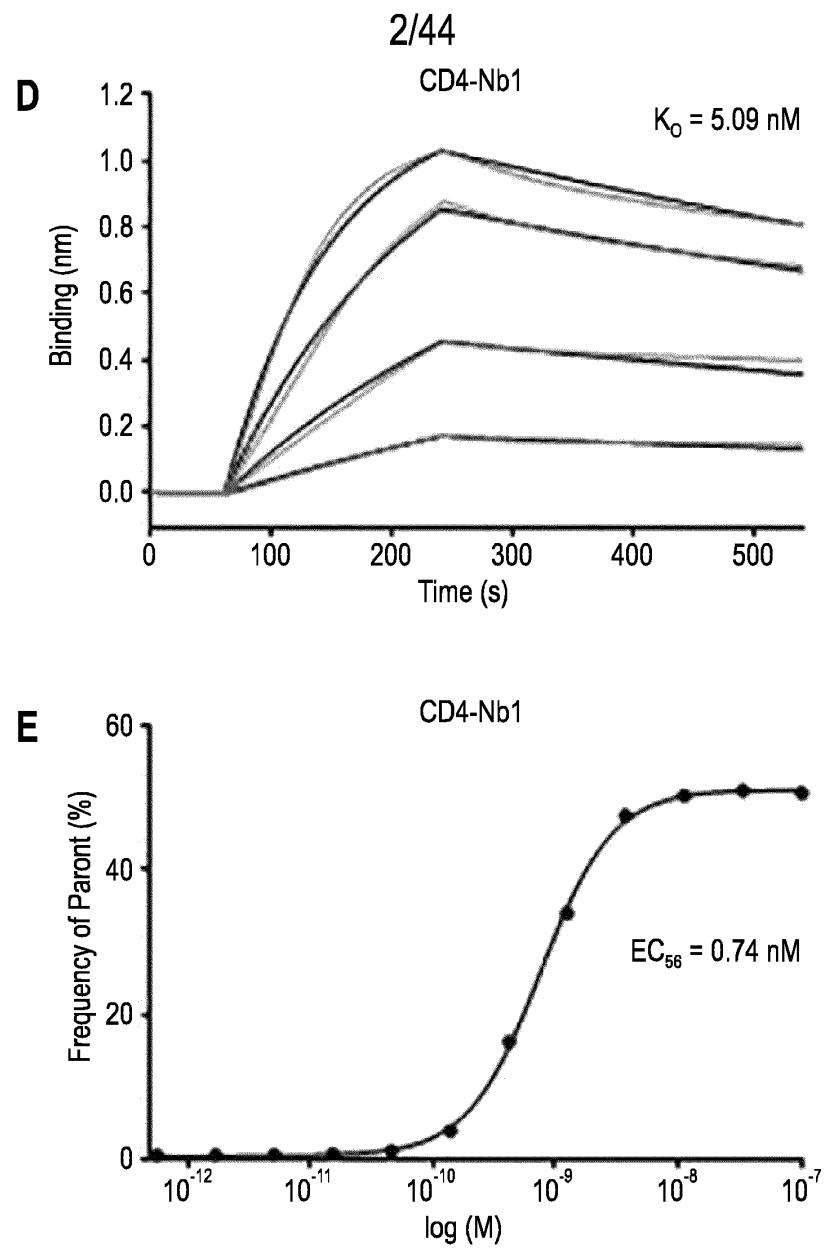


Table 1

	Dissociation constant K_D	k_{on} ($10^5 \text{ M}^{-1} \text{ s}^{-1}$)	k_{off} (10^{-4} s^{-1})	R^2	EC_{50}
CD4-Nb1	5.1 nM	1.21 ± 0.022	6.13 ± 0.27	0.996	0.74 nM
CD4-Nb2	6.5 nM	1.22 ± 0.015	7.95 ± 0.18	0.998	0.73 nM
CD4-Nb3	75.3 nM	0.82 ± 0.026	61.8 ± 2.00	0.983	533 nM
CD4-Nb4	135 nM	1.18 ± 0.014	160 ± 0.97	0.998	7.36 μM

Fig. 1

F

SEQ ID	-----Framework 1-----	--CDR1--	-----Framework 2----	---CDR2---	-----Framework 3-----	-----CDR3-----	-----Framework 4-----
No 10	EVQLVESGGGLVQPGGSLRLSCAA--SGFTFSKL--	AMSWHREPPGKGREWLAD--	IDS	SGDTTDYIA--SVKGRFTISRDN	AKNTLYIQMDSLKSEDTGVYICAS--RE--	DP	PG--YWGQGTQVTVSS
No 11	EVQLVESGGGSVQPGGSLRLSCGA--SGFDVYY--	ALGWFRQAPGKREGVSC--	I	ASDGSYYAD--SVKGRFTISRDN	AKNTVYLOMNSLKPEDTAVYICAA--DAT	CPYCGSGVCILETGM	--YWGEGTLVTVSS
No 12	HVQLVESGGGLVQPGGSLRLSCAV--SGFALEY--	ALGWFRQAPGKERERVAC--	MS	ASGGVINYSE--SVKGRFTISRDN	AKNTVYLQNTLKPEDTAVYICAA--EK--	AYGSSWAECYLMD	--YWGKGTIVTVSS

Fig. 1

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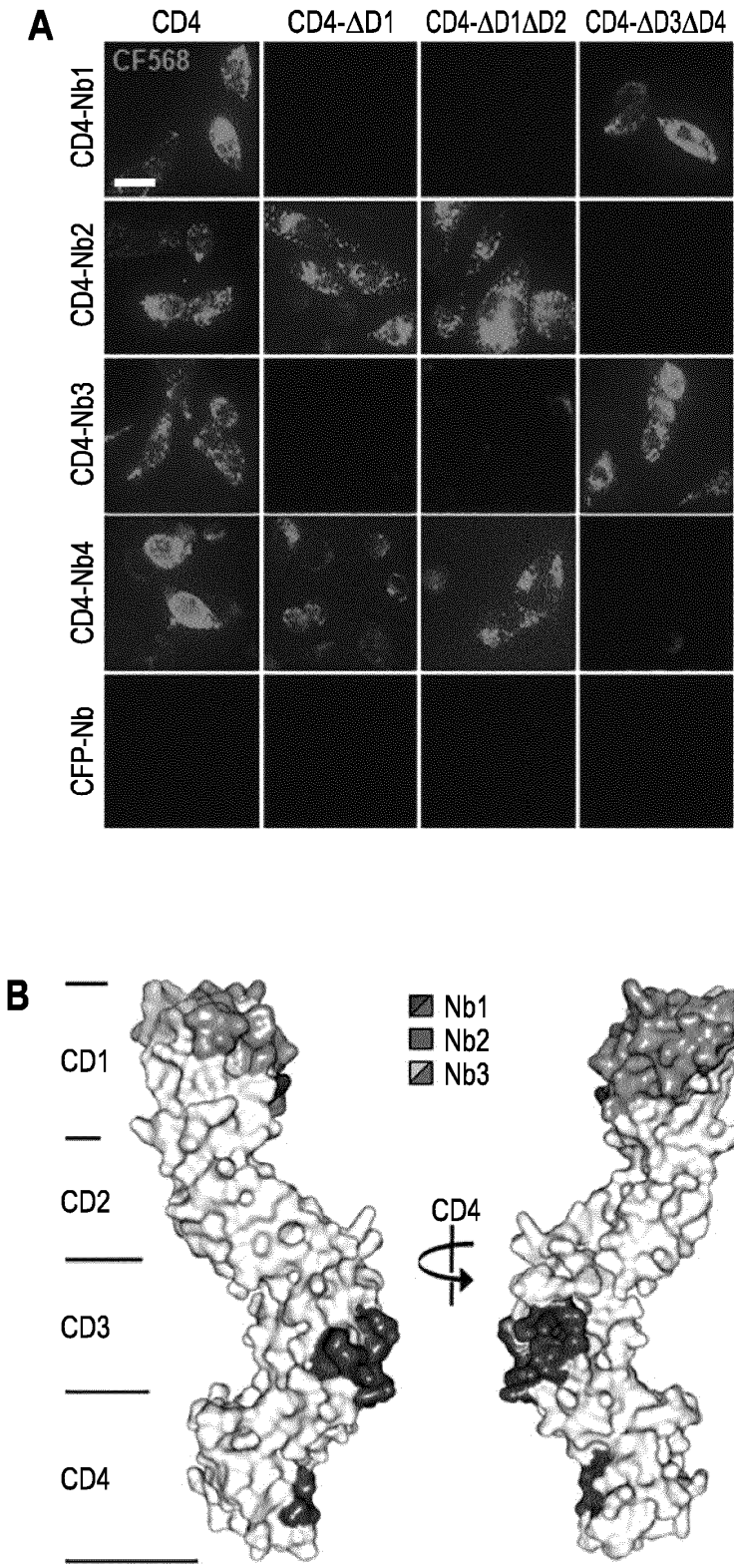


Fig. 2

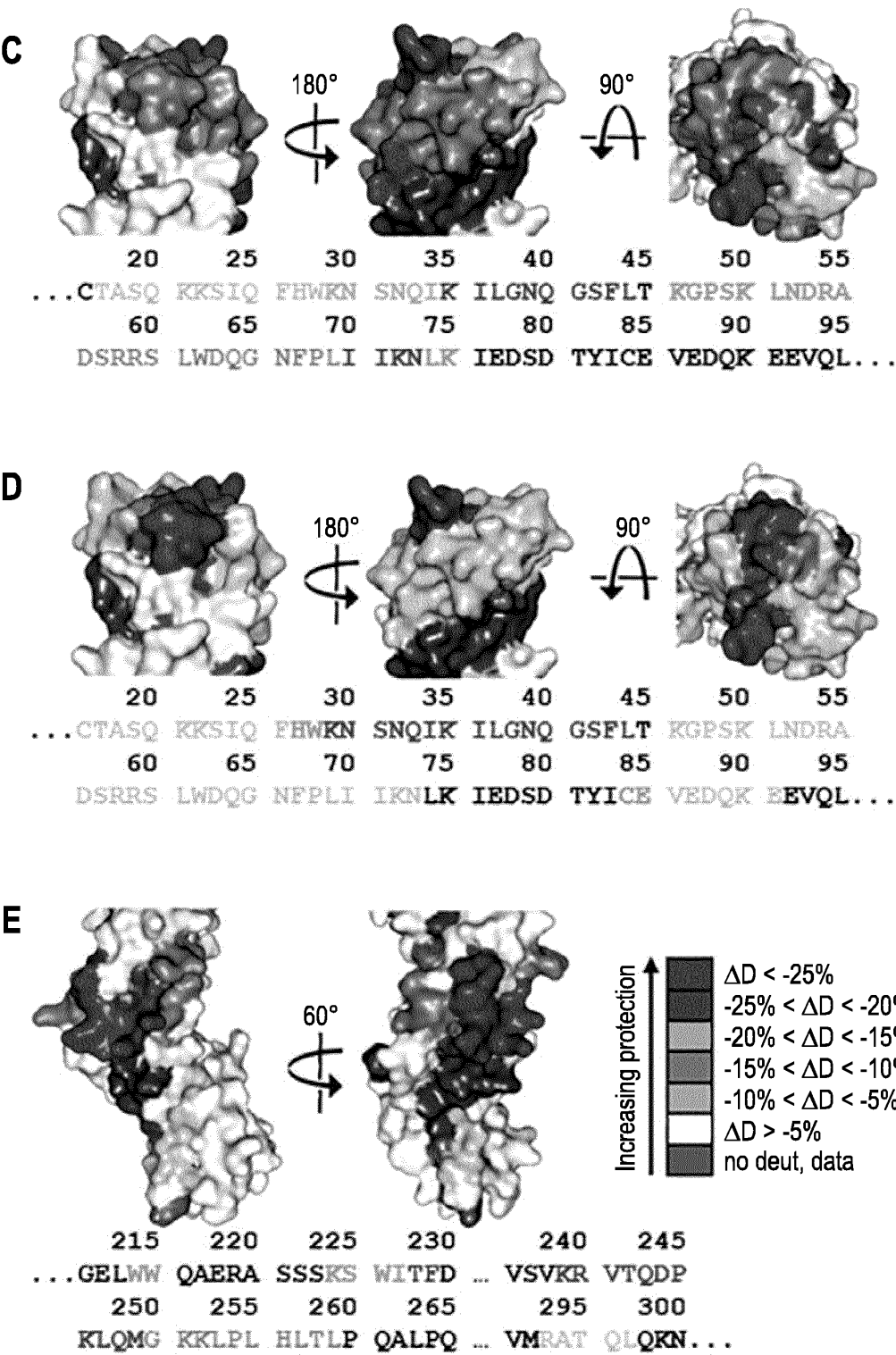


Fig. 2

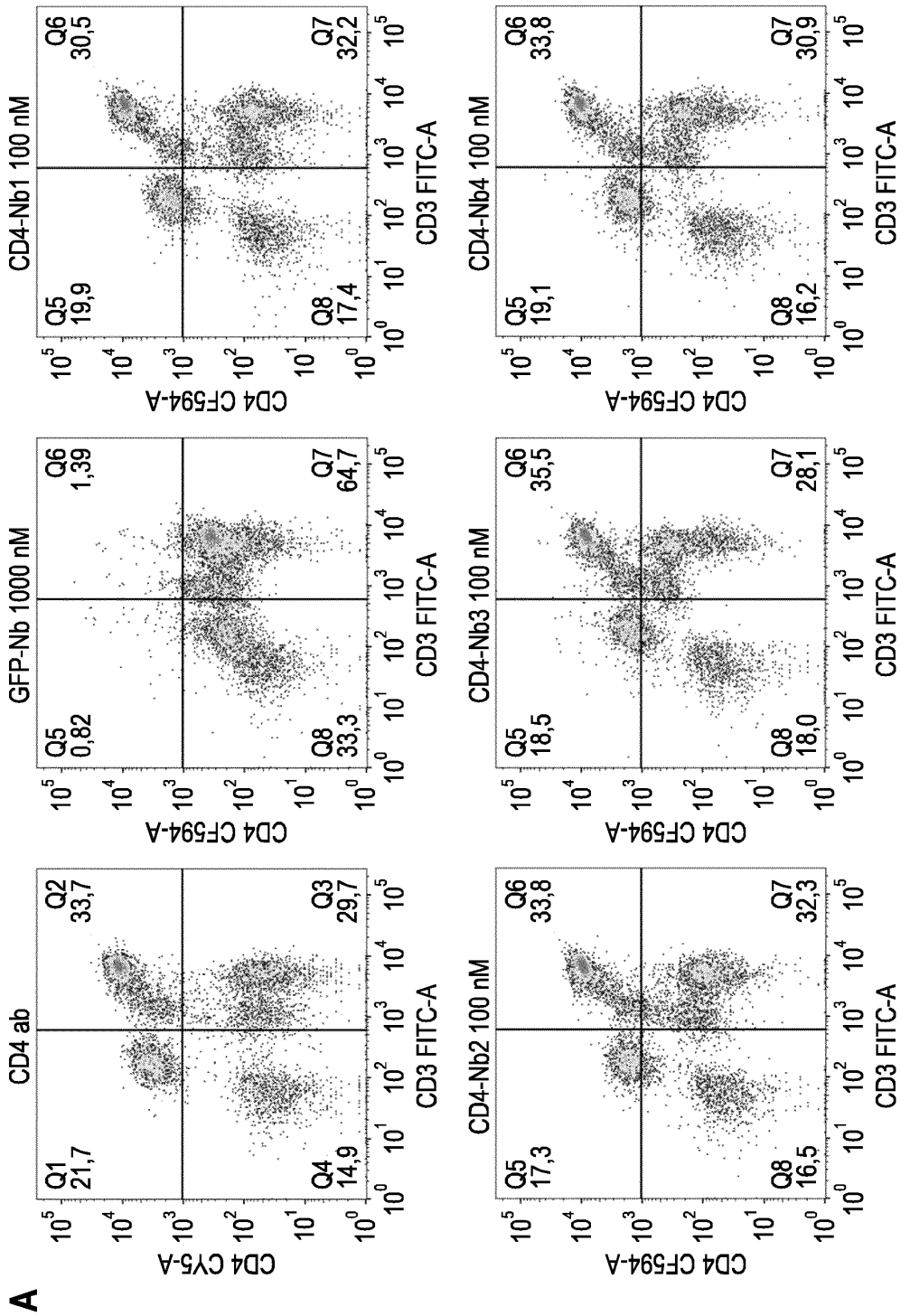


Fig. 3

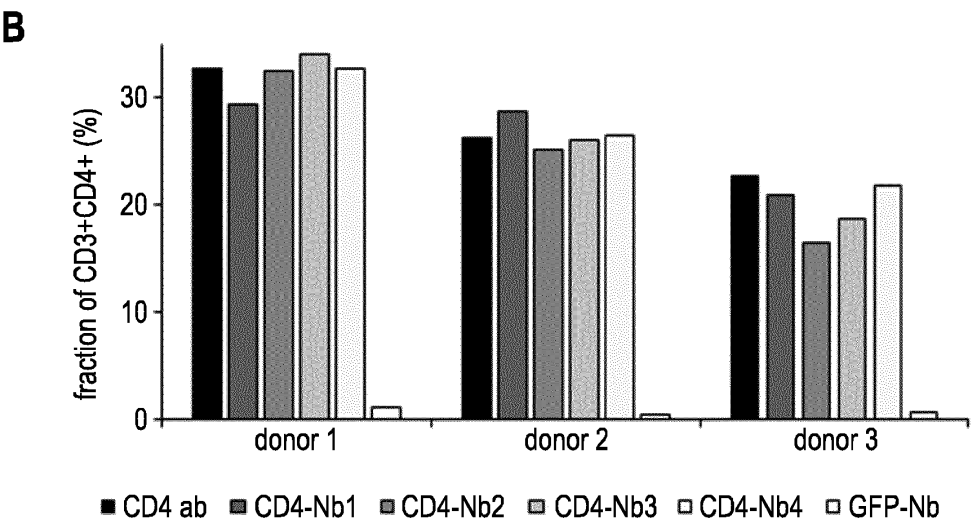


Table 2

		Frequency CD3+ CD4+ (%)		
	c (nM)	donor 1	donor 2	donor 3
anti-CD4 antibody	~1	32,7	26,2	22,8
CD4-Nb1	100	29,5	28,7	21
CD4-Nb2	100	32,5	25,2	16,5
CD4-Nb3	1000	34,1	26,1	18,6
CD4-Nb4	1000	32,7	26,4	21,9
GFP-Nb	1000	1,02	0,28	0,64

Fig. 3

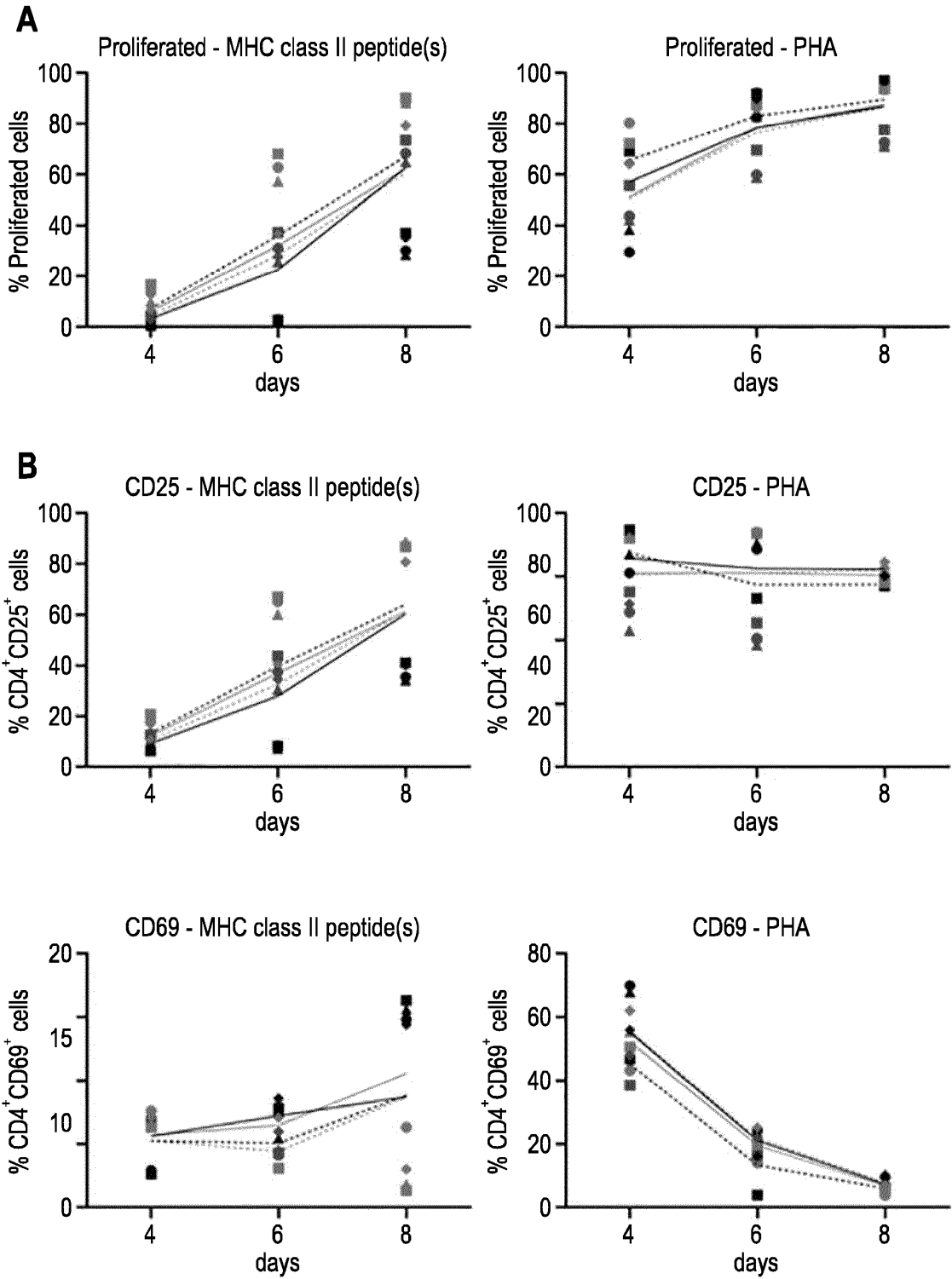


Fig. 4

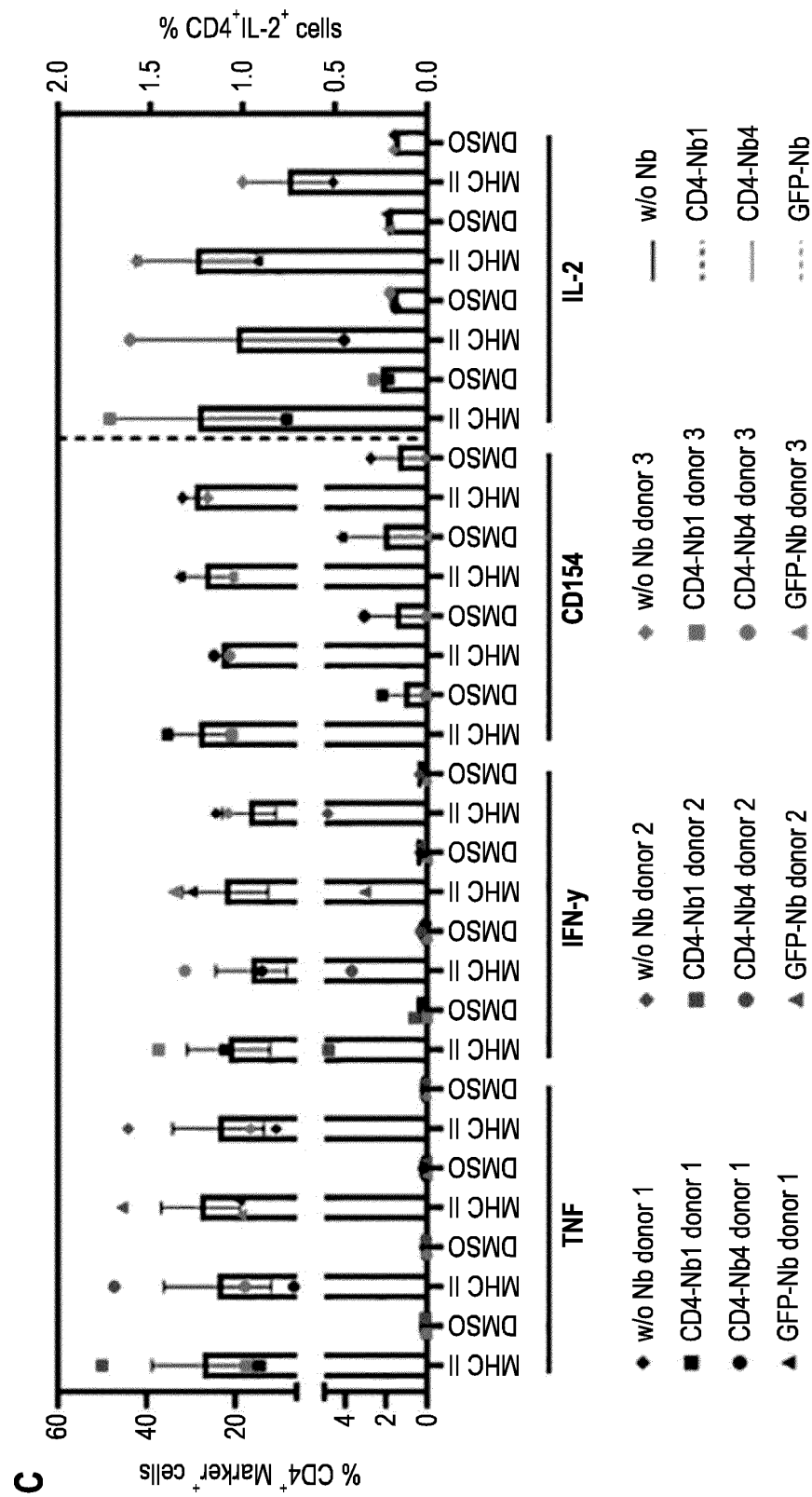


Fig. 4

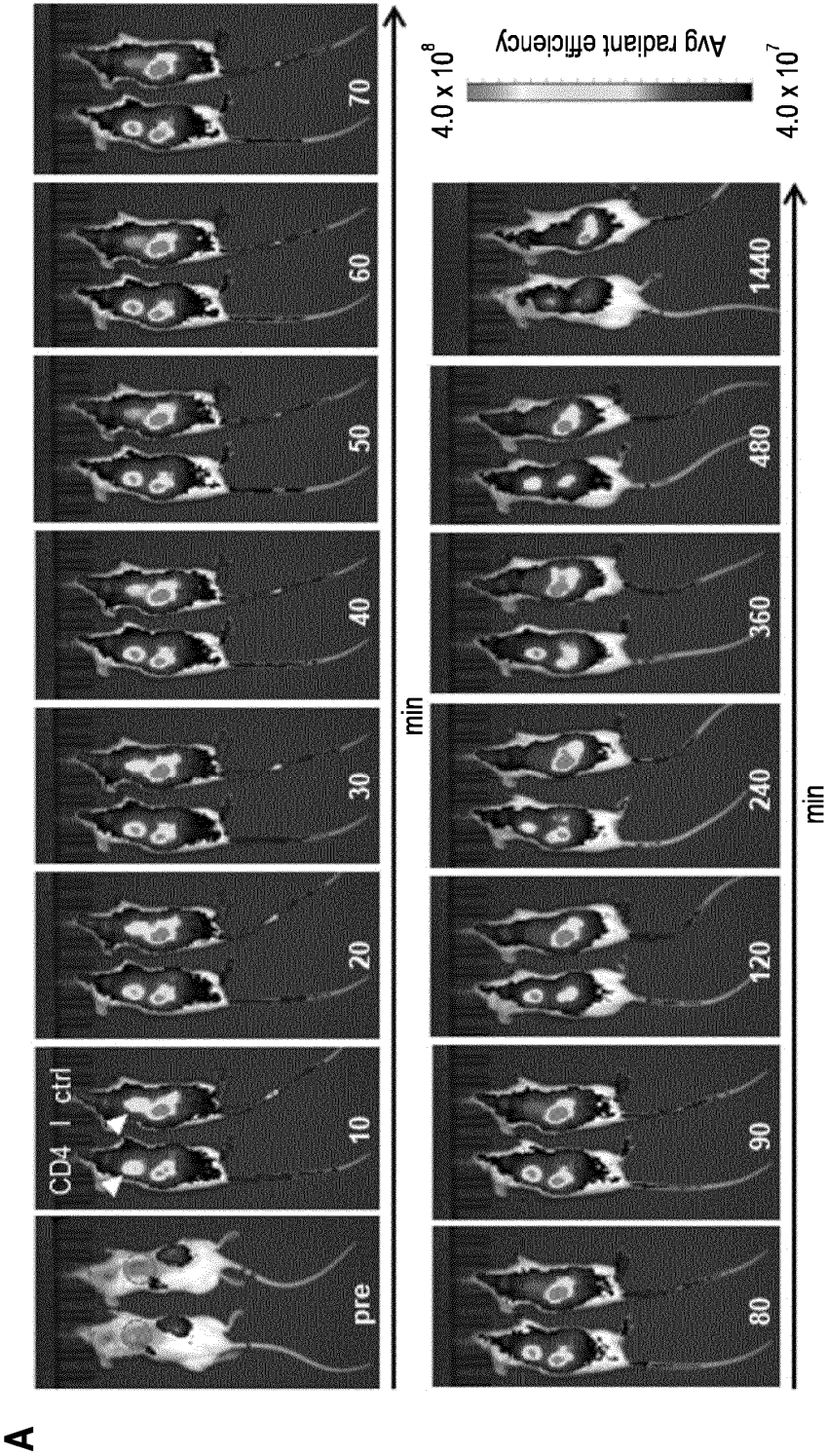


Fig. 5

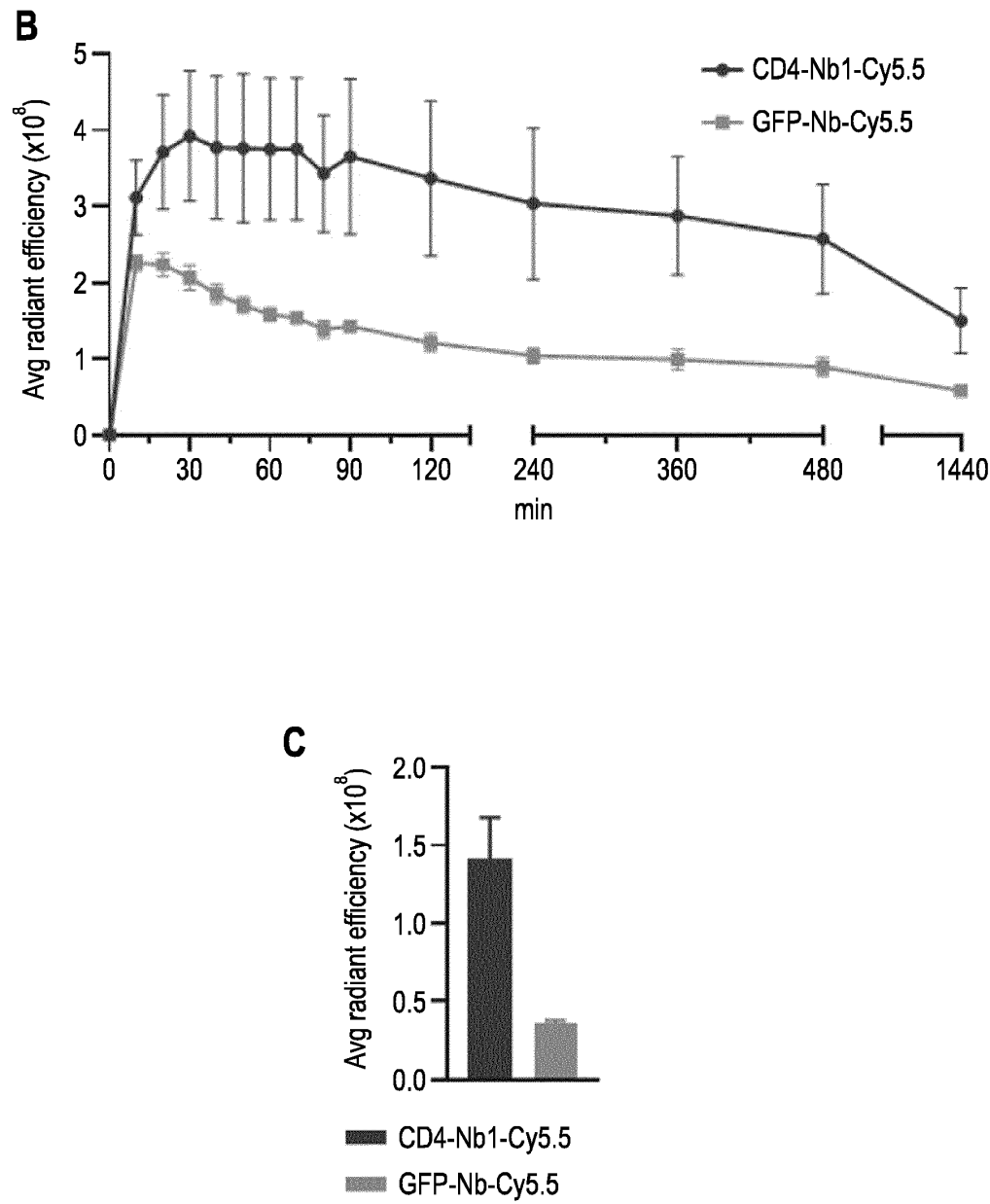


Fig. 5

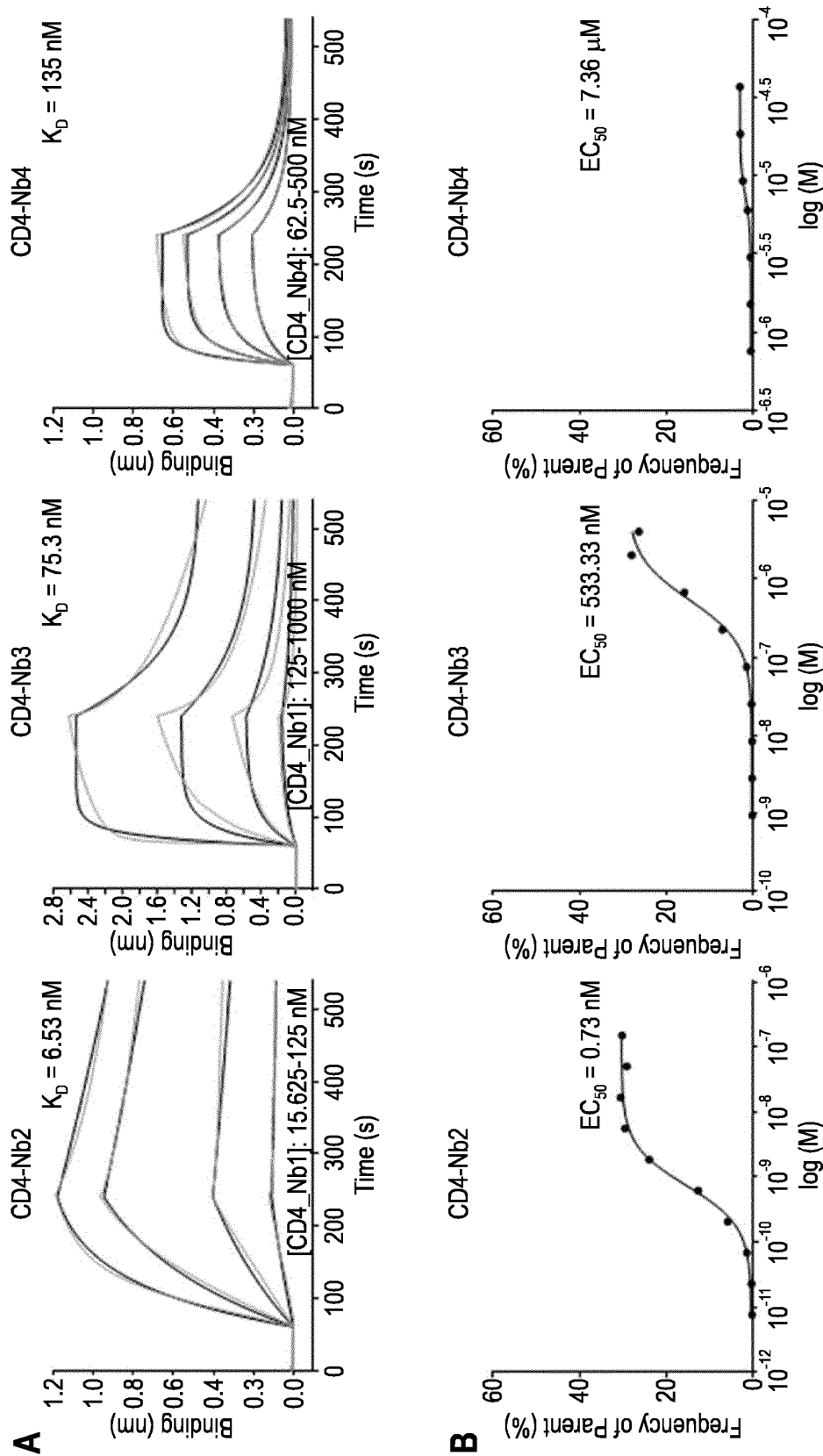


Fig. 6

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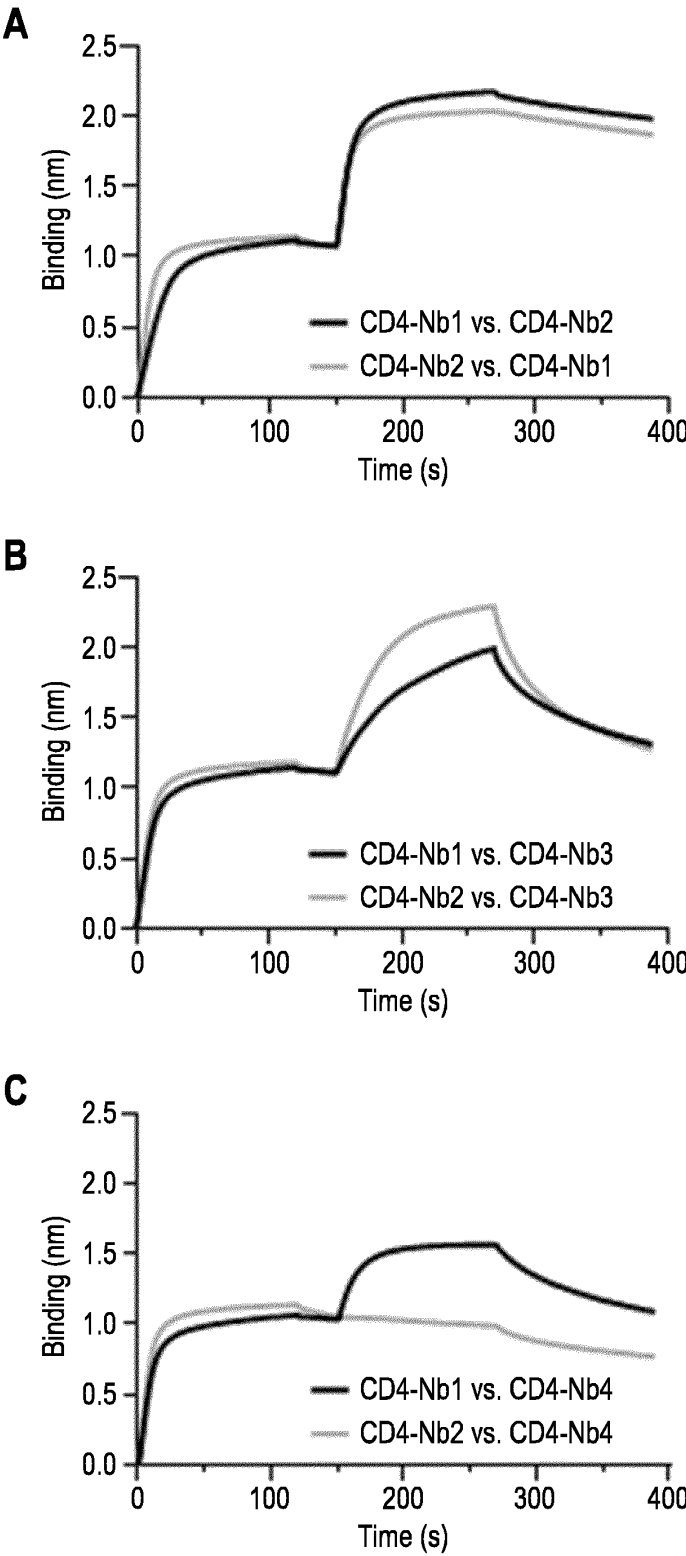


Fig. 7

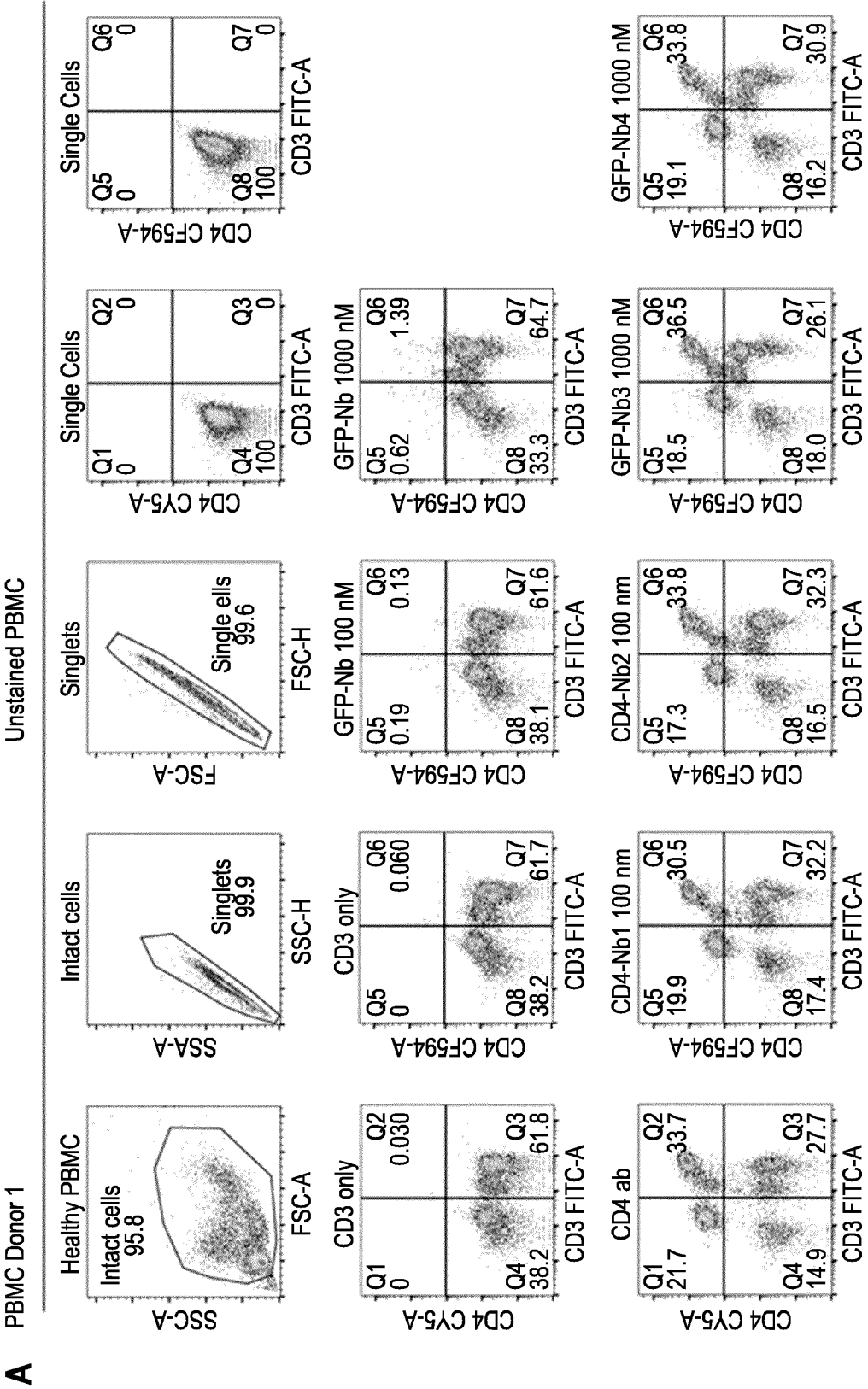


Fig. 8

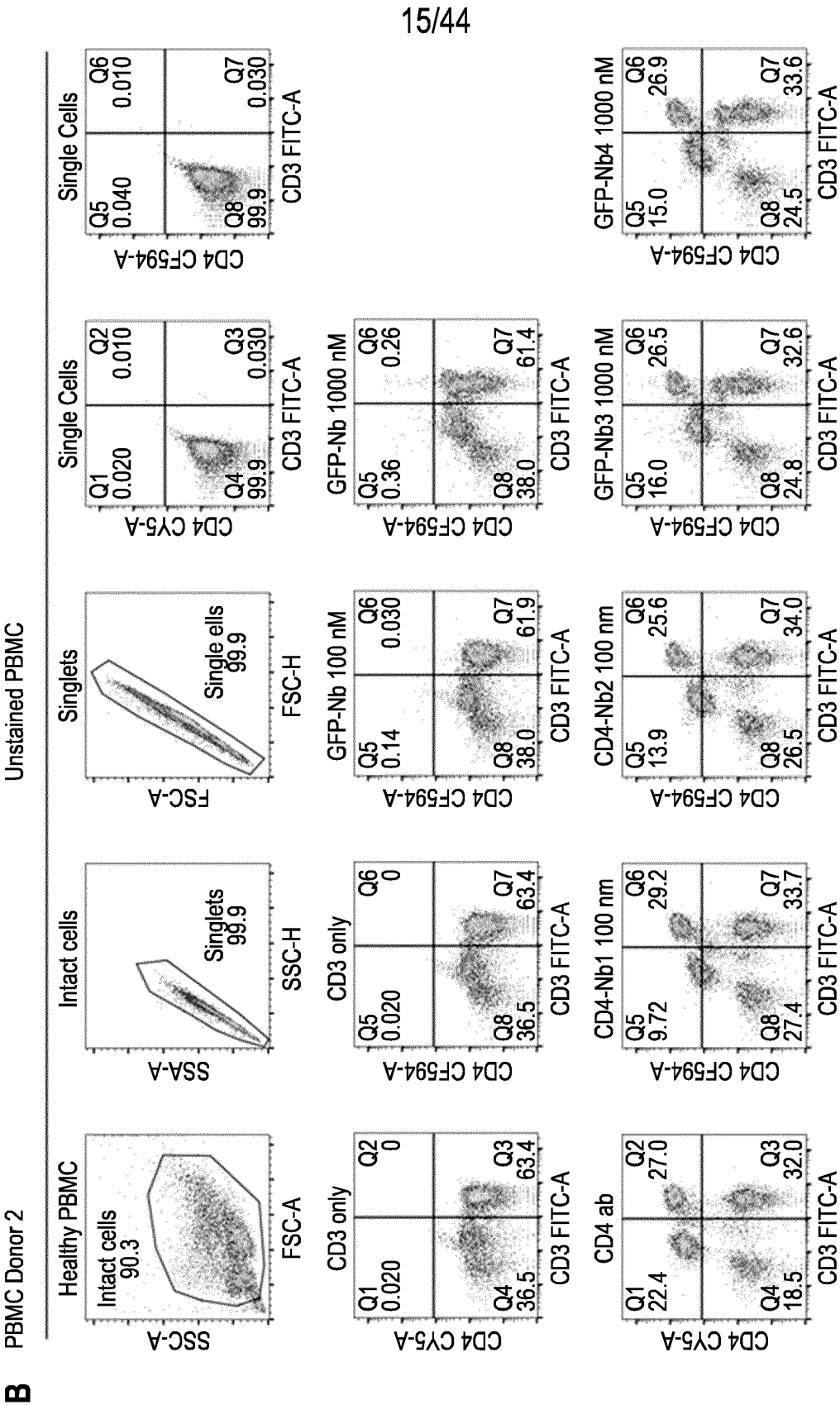


Fig. 8

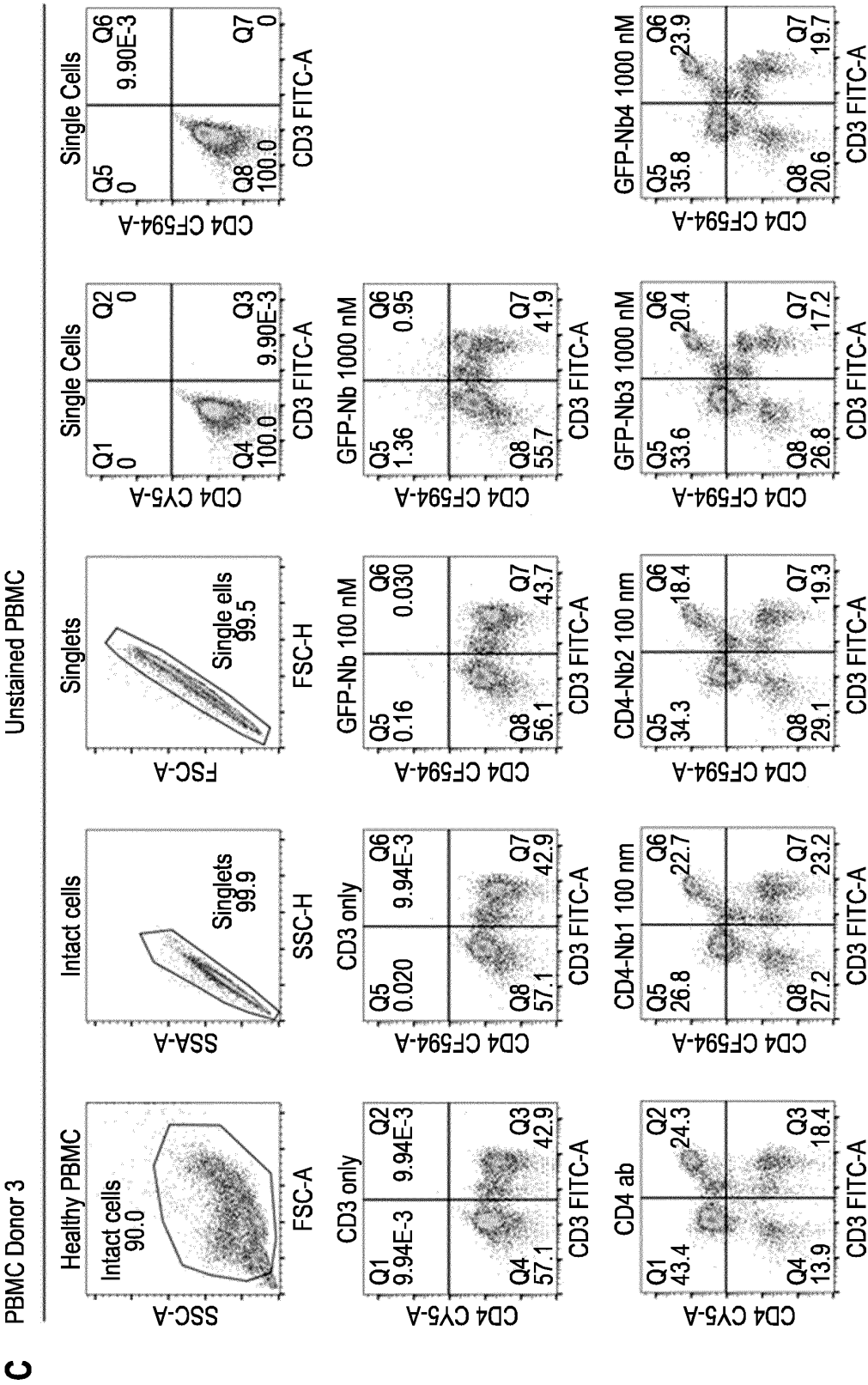
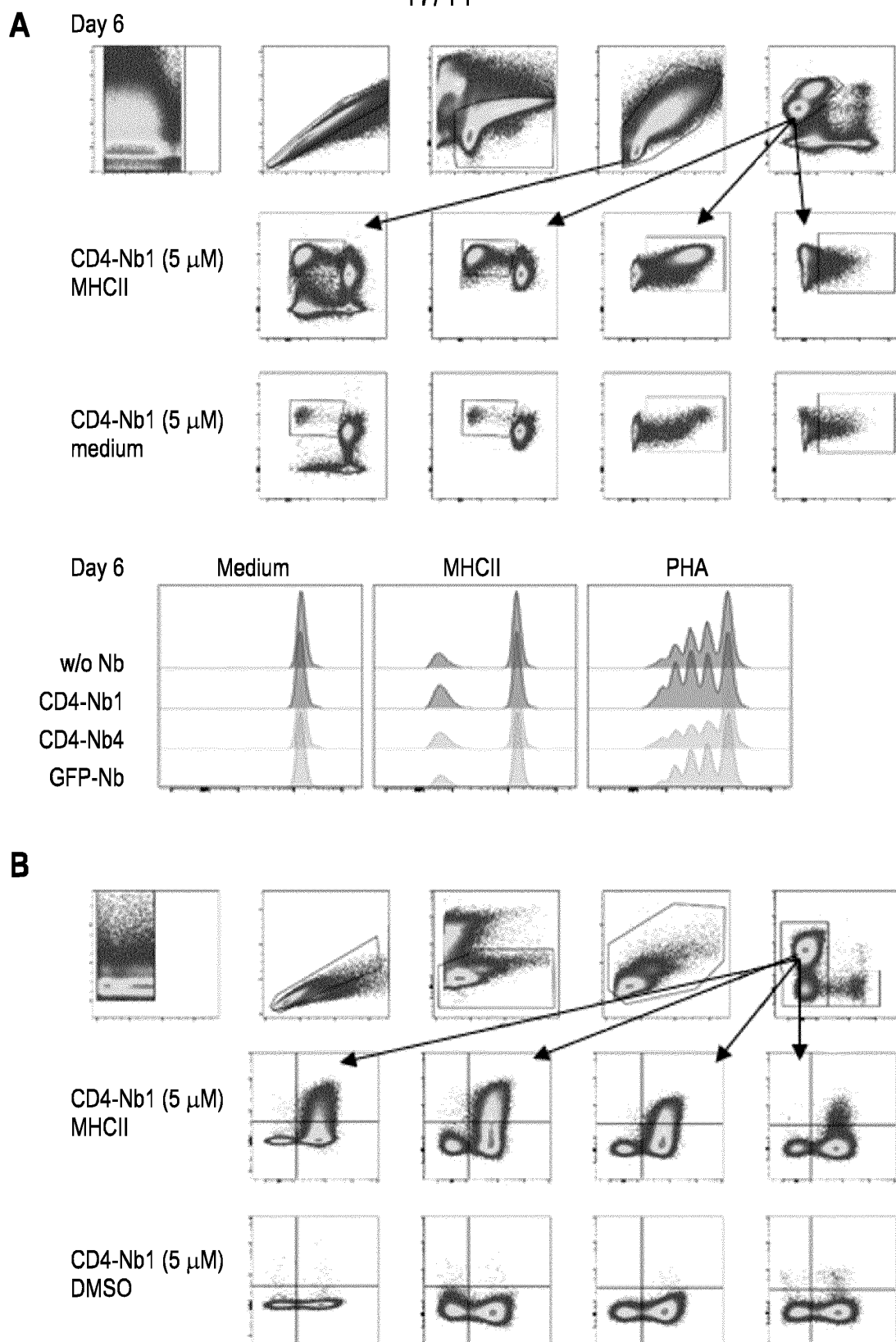


Fig. 8

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**Fig. 9**

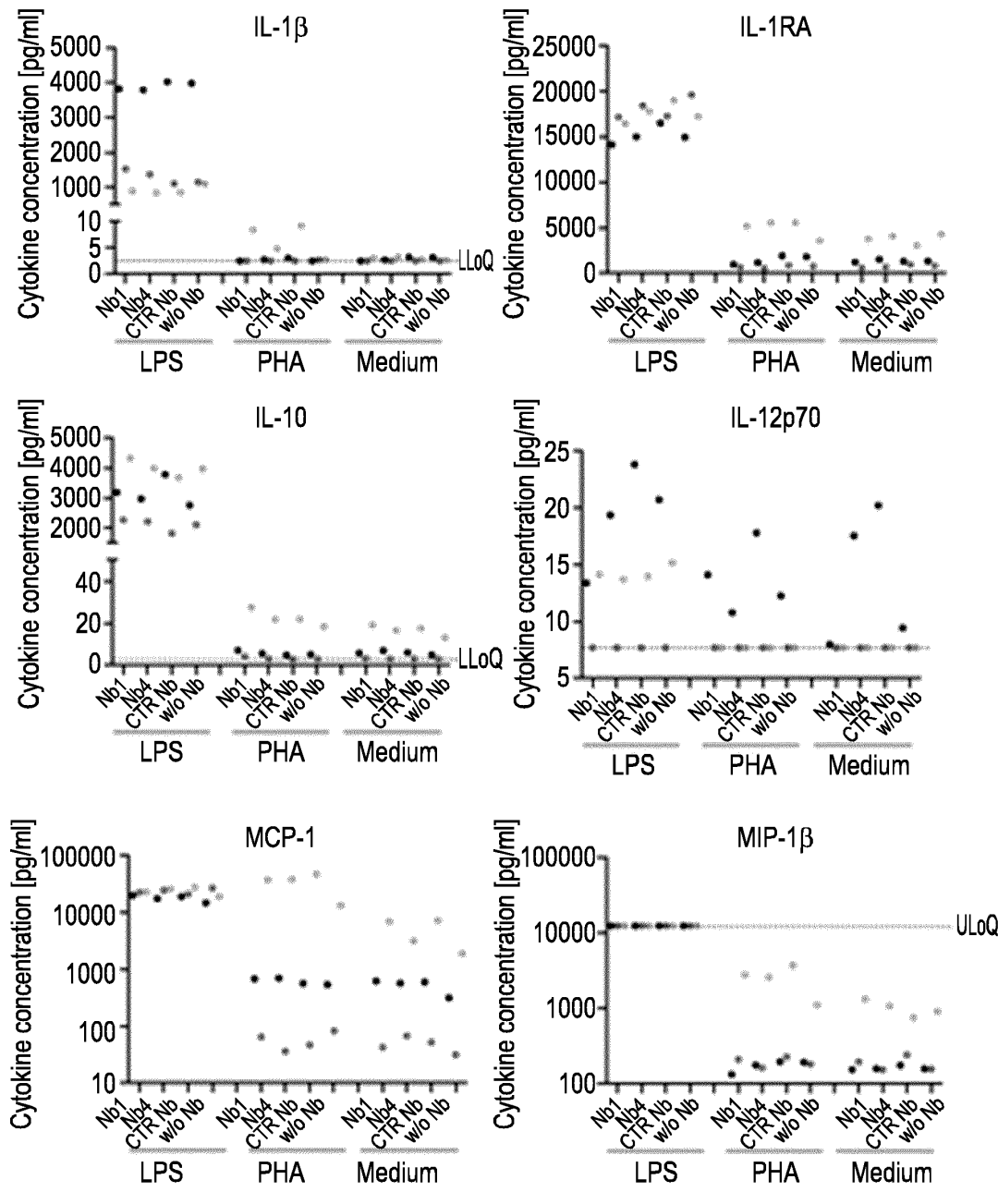


Fig. 10

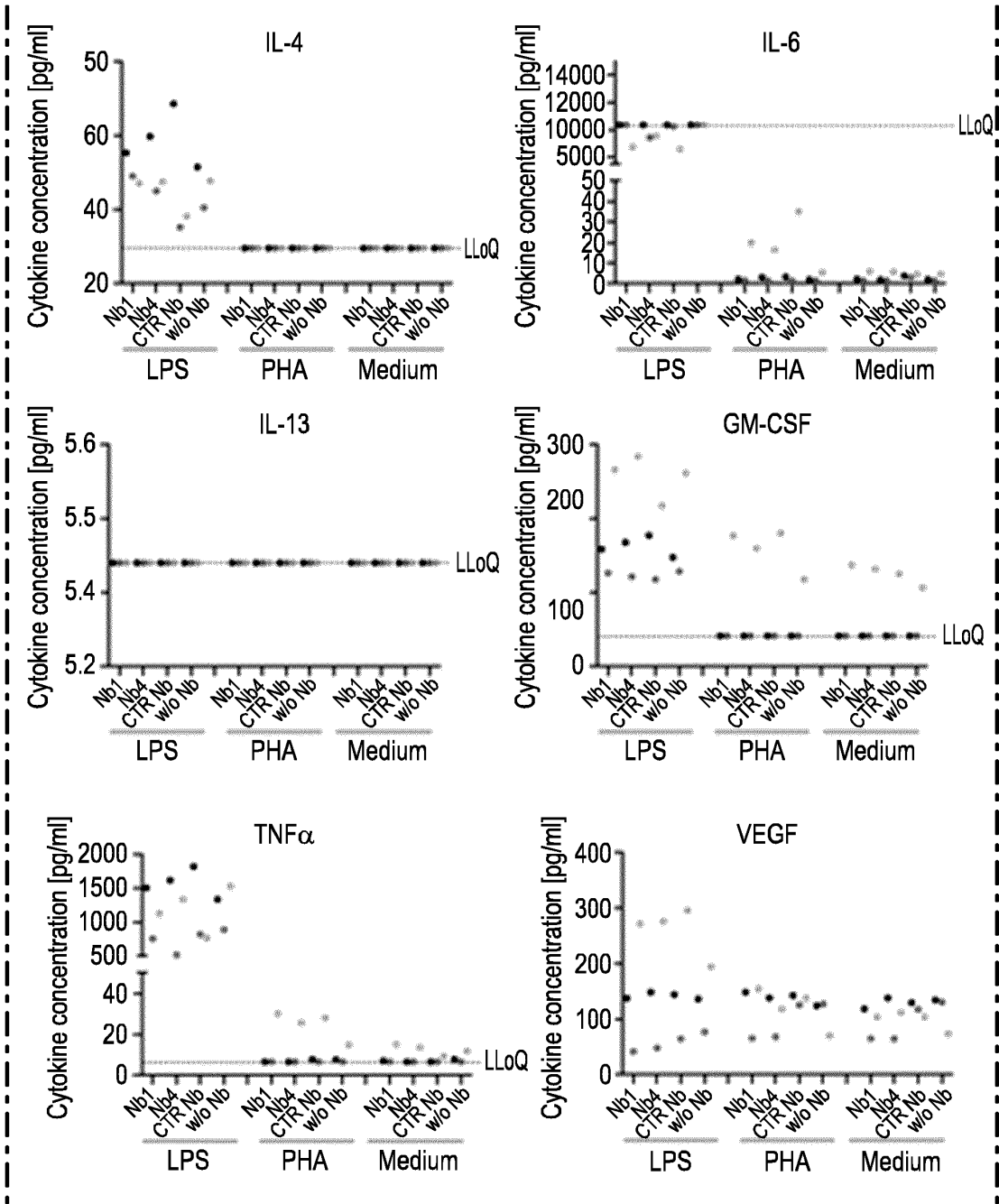


Fig. 10 continued

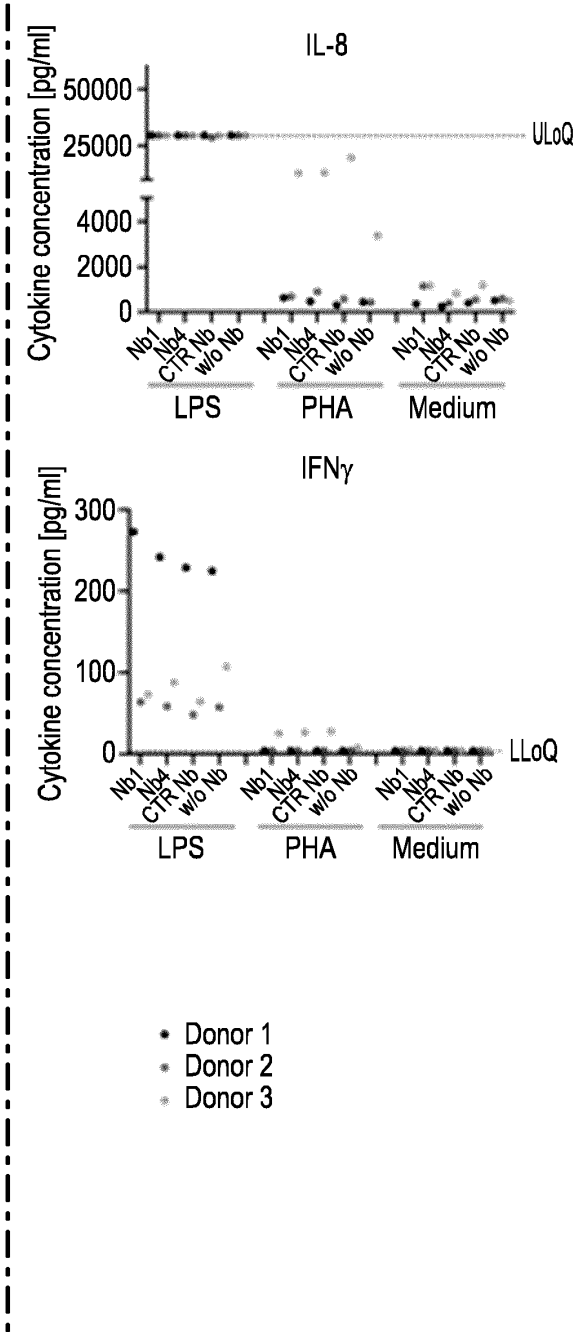


Fig. 10 continued

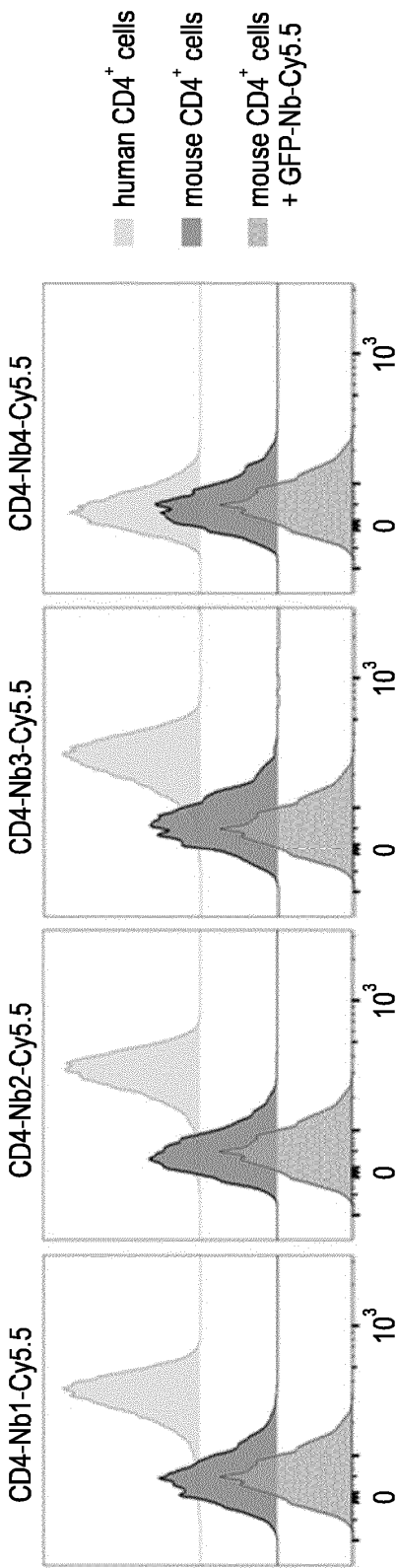


Fig. 11

A

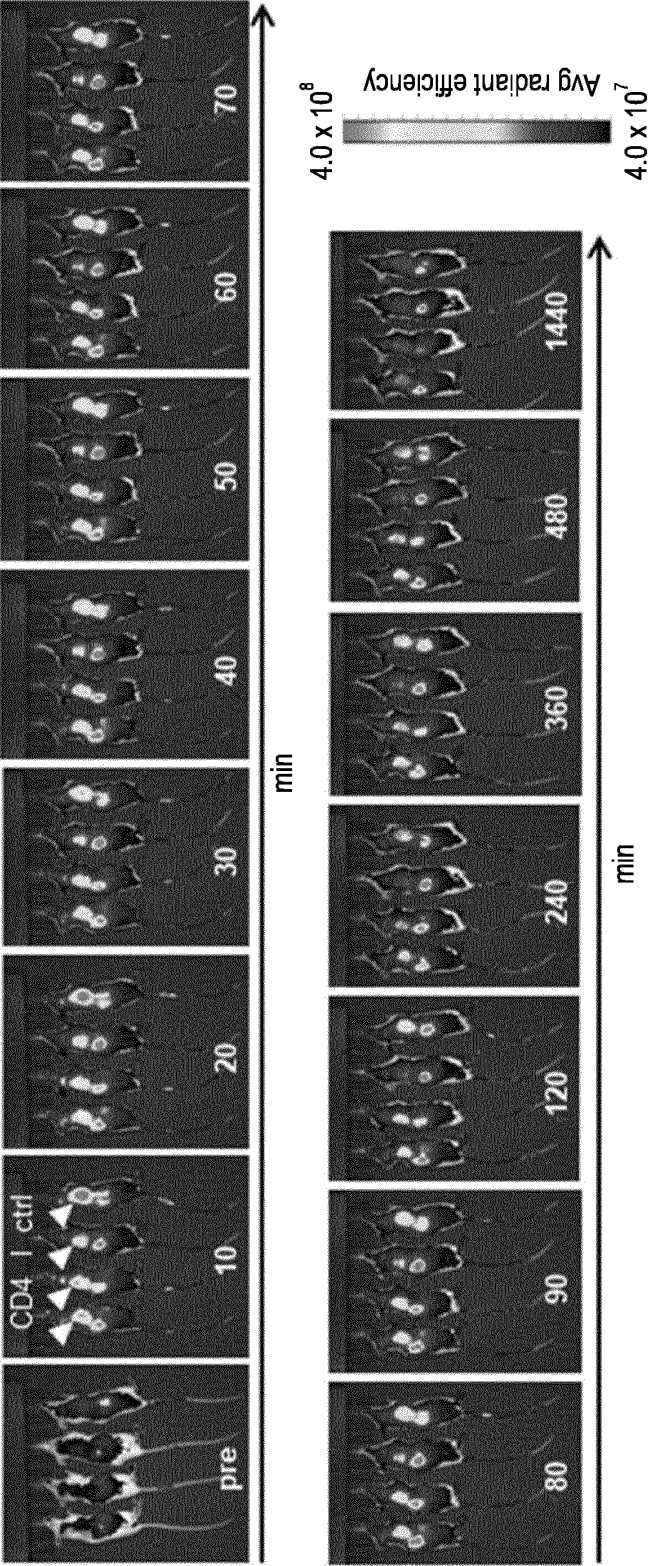


Fig. 12

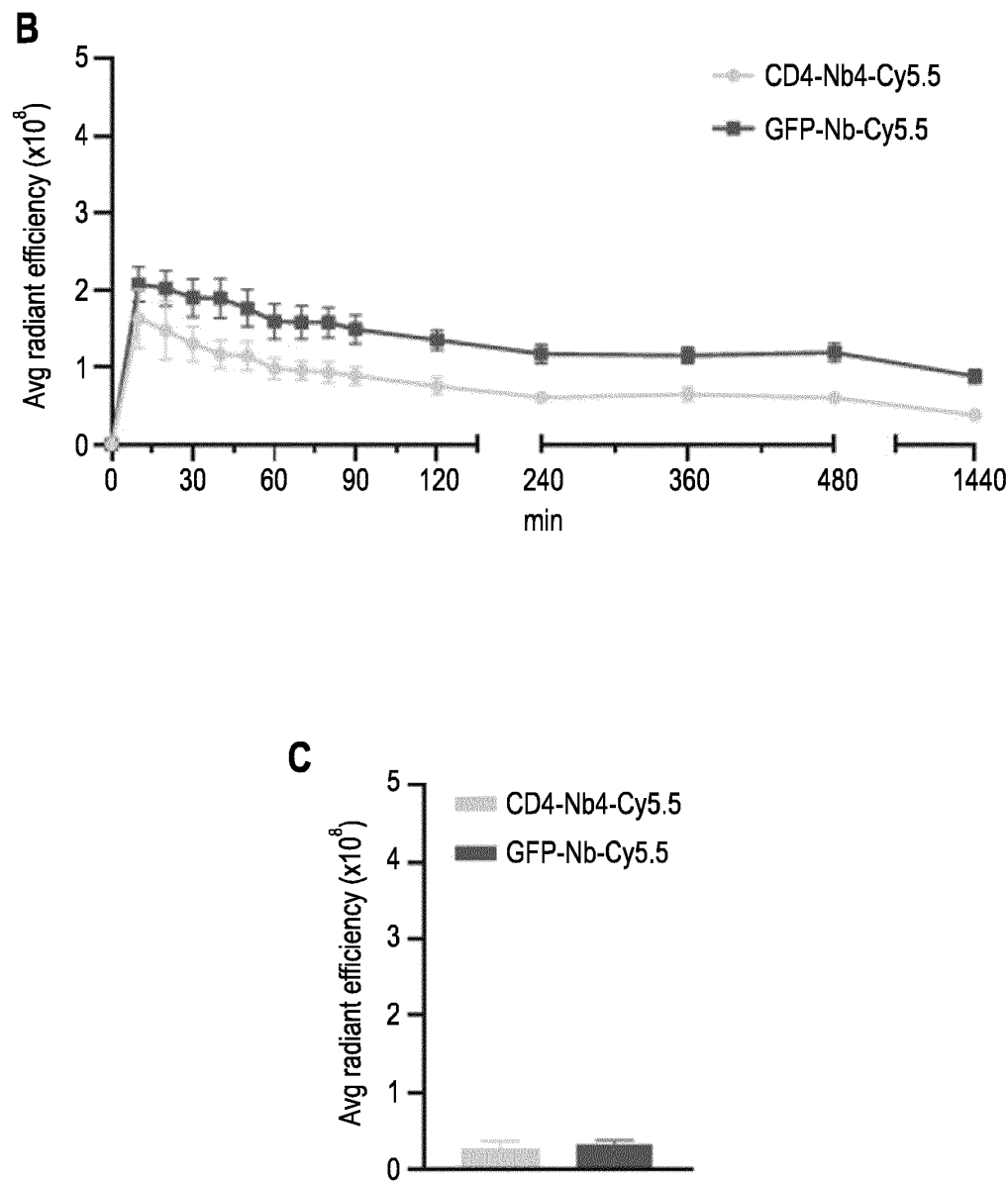


Fig. 12

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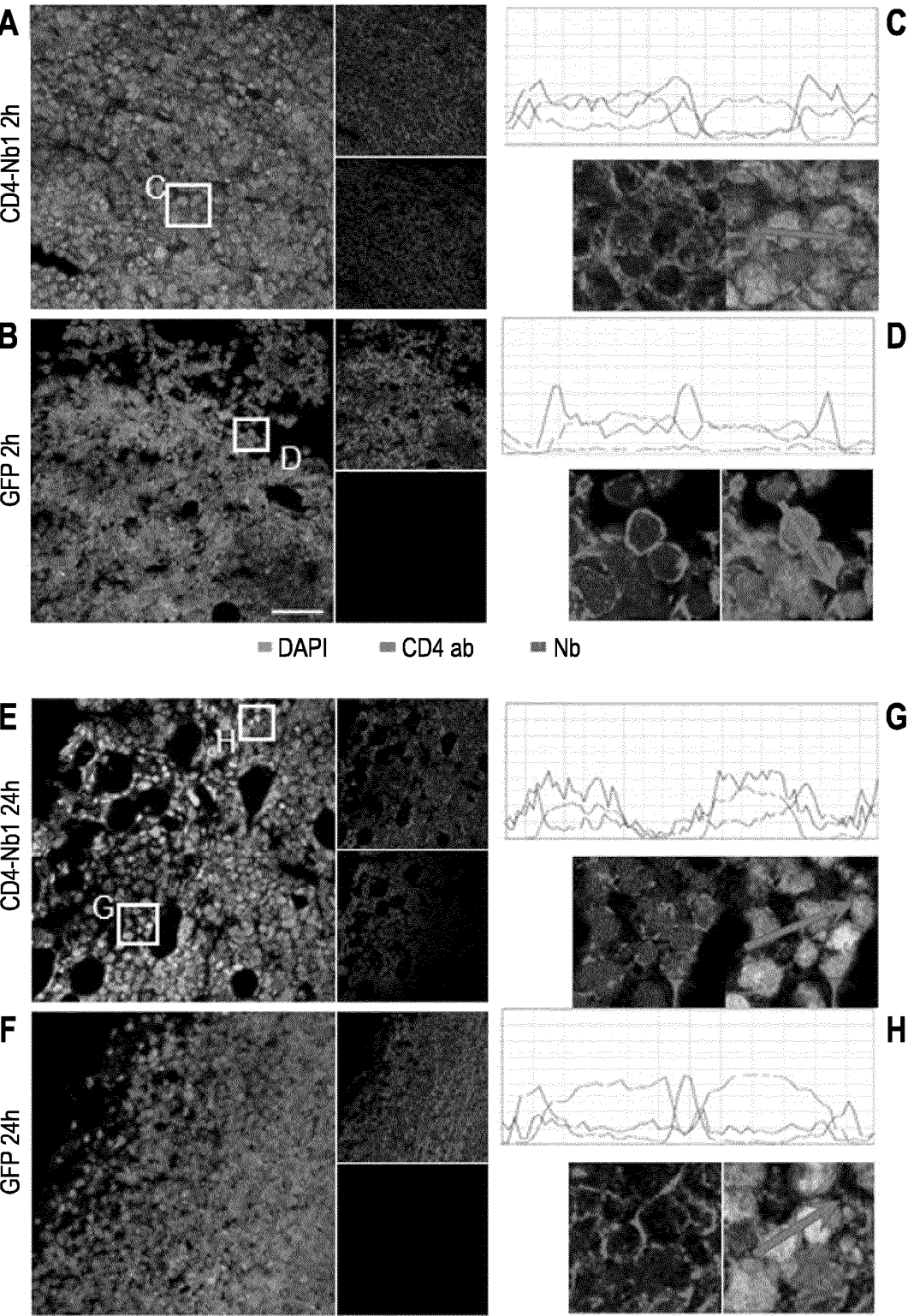


Fig. 13

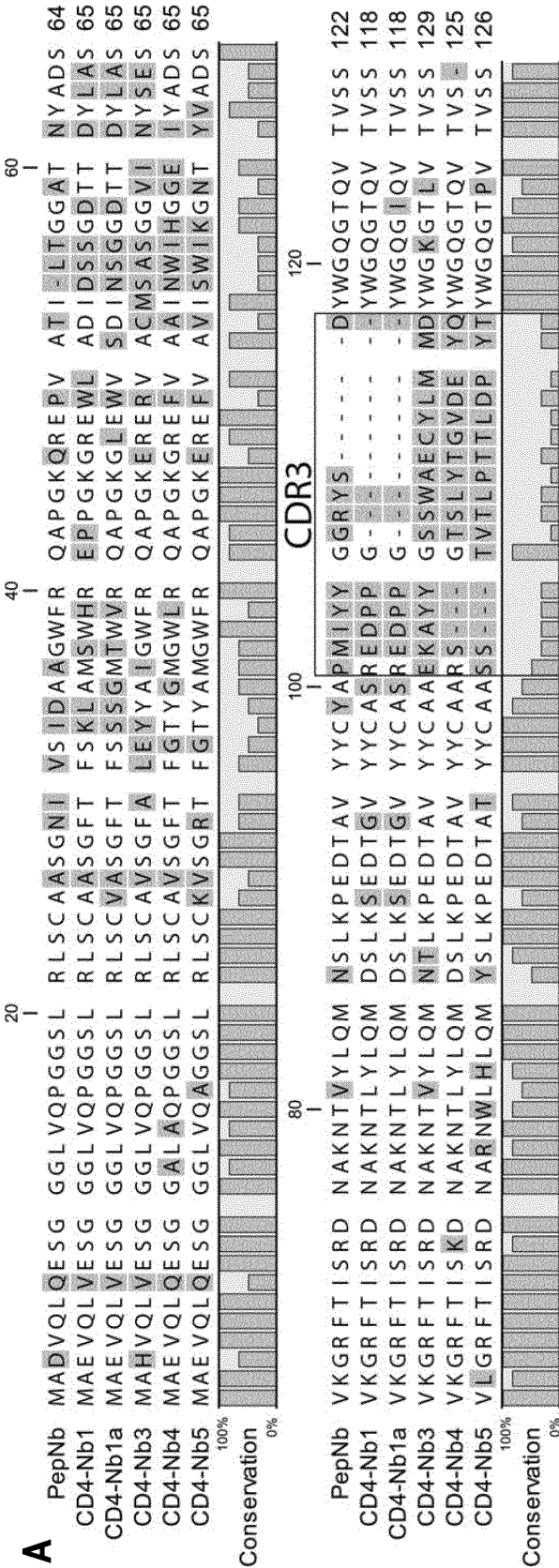


Fig. 14

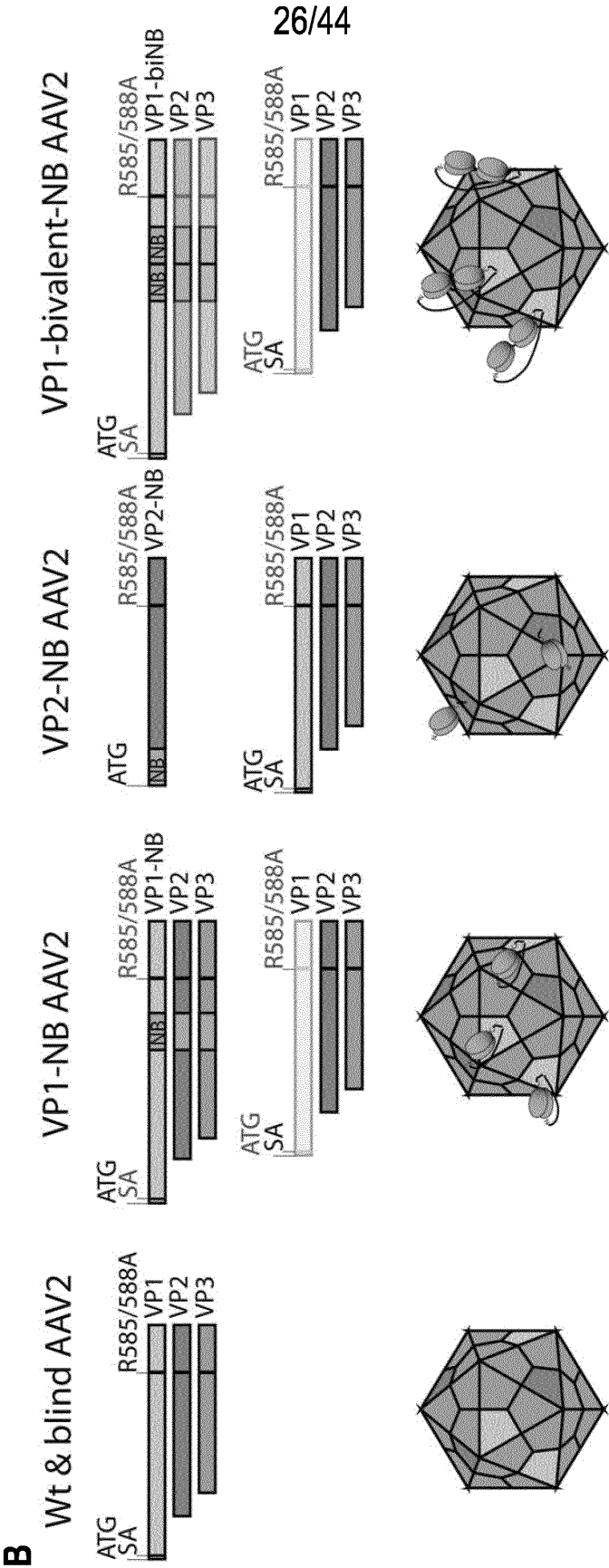


Fig. 14

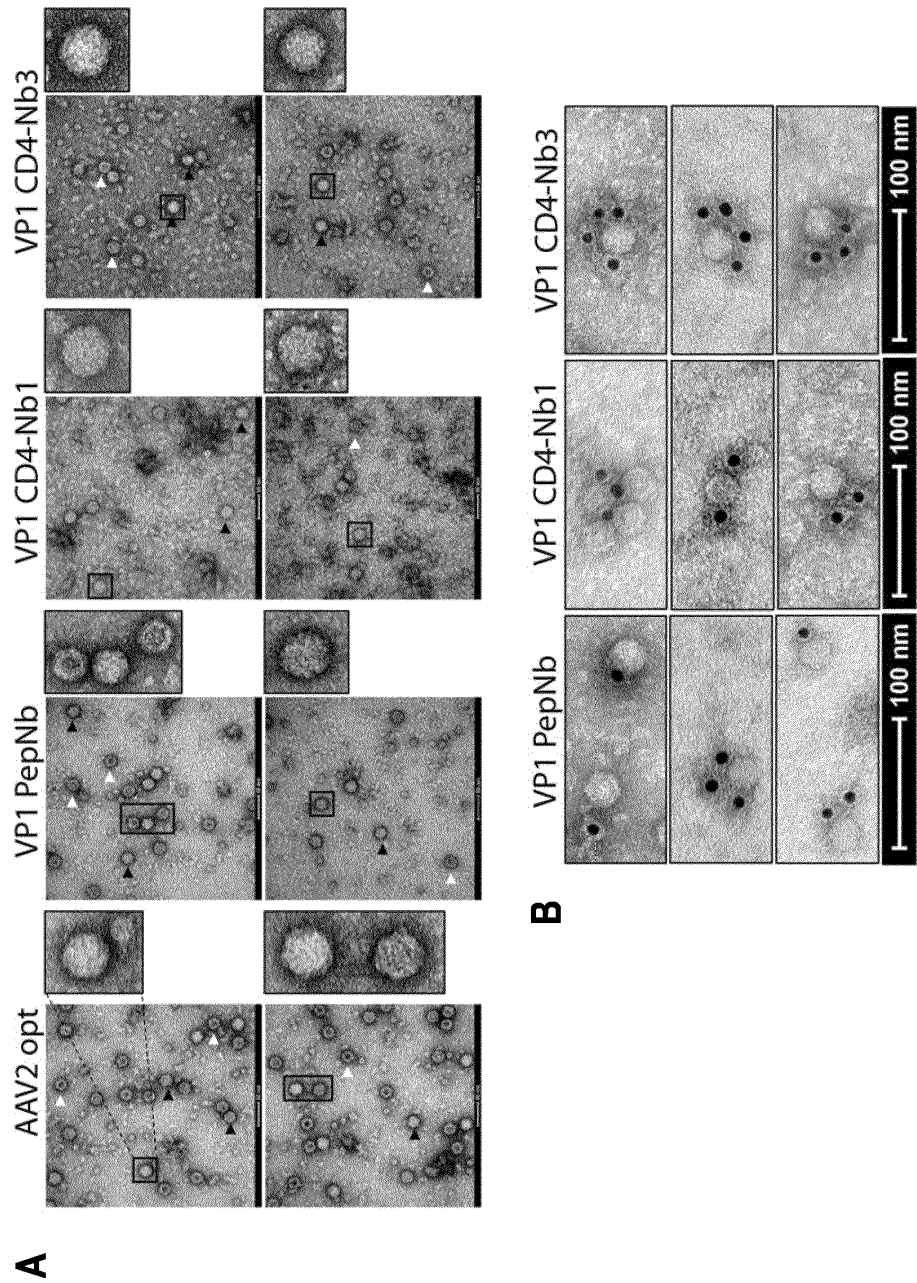


Fig. 15

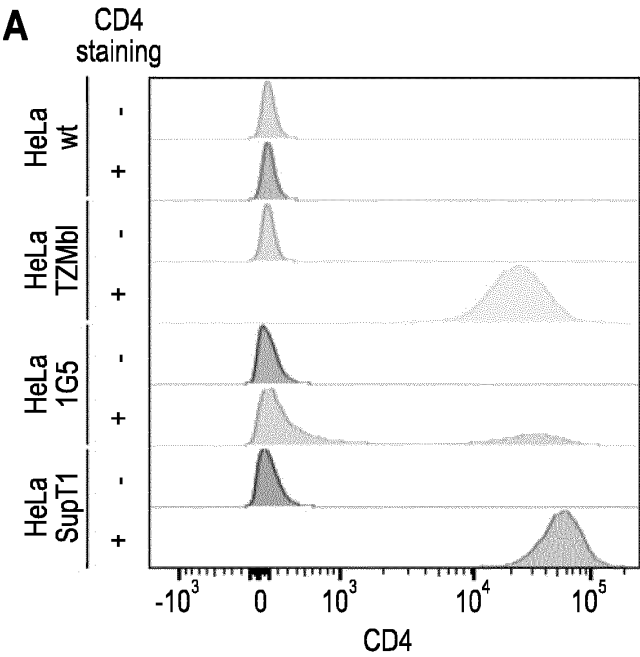


Fig. 16

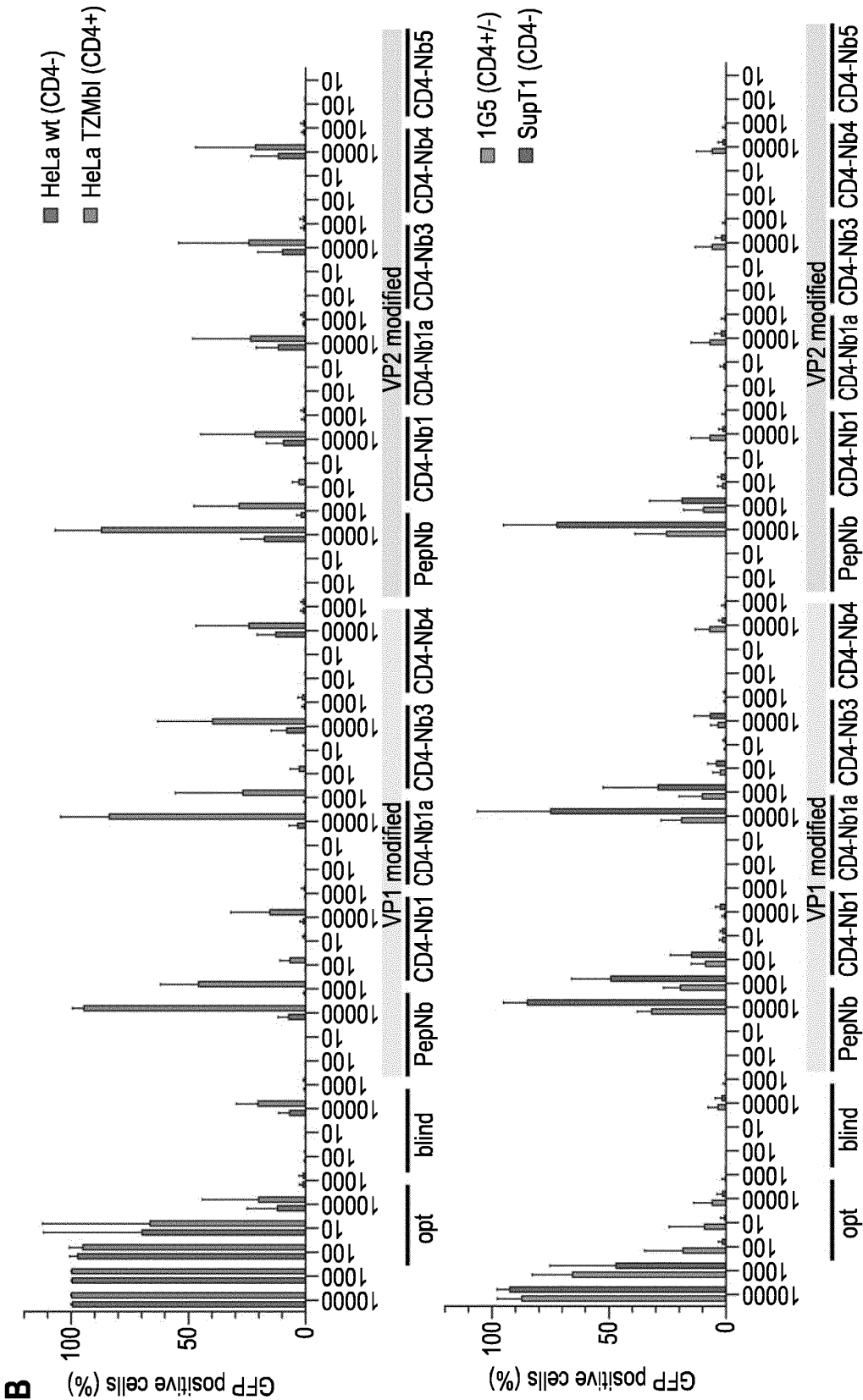


Fig. 16

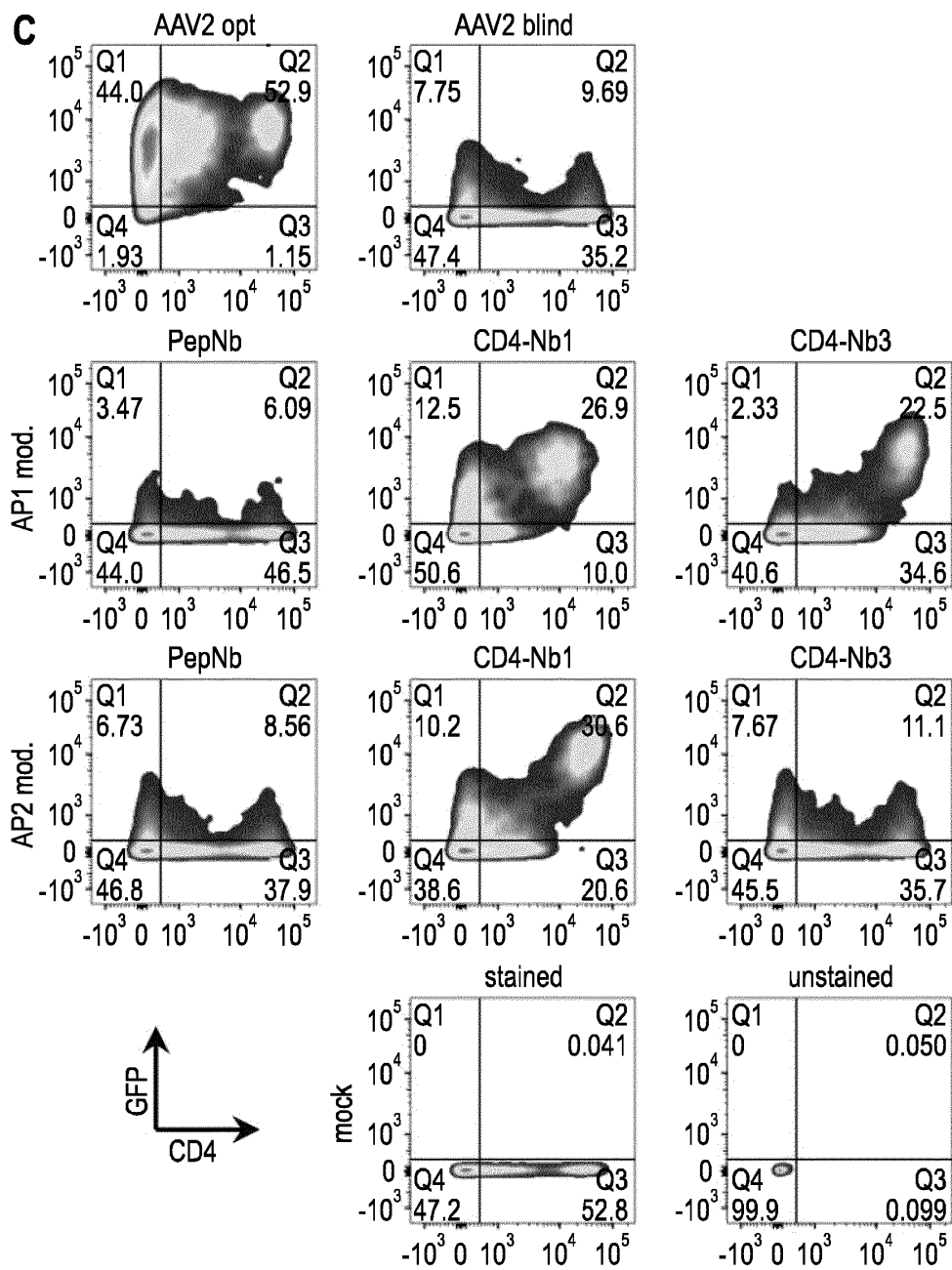


Fig. 16

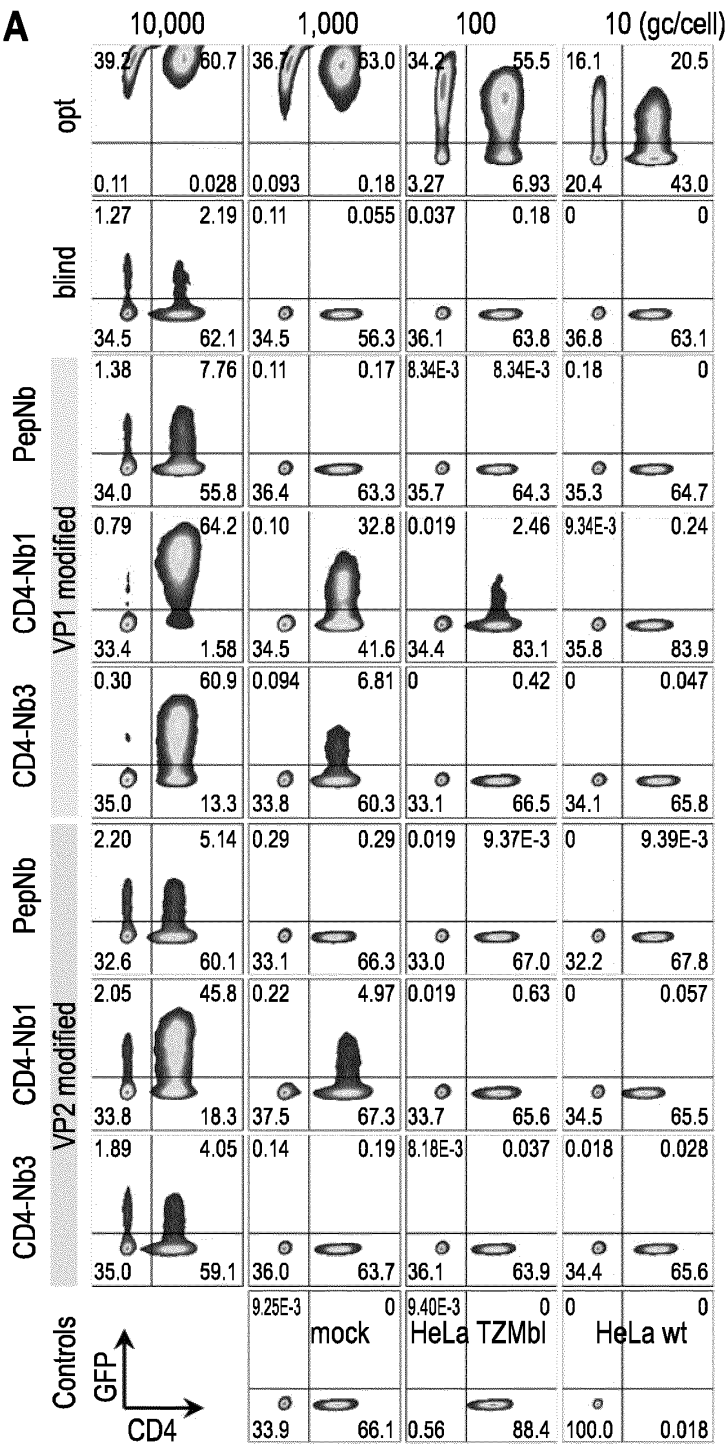


Fig. 17

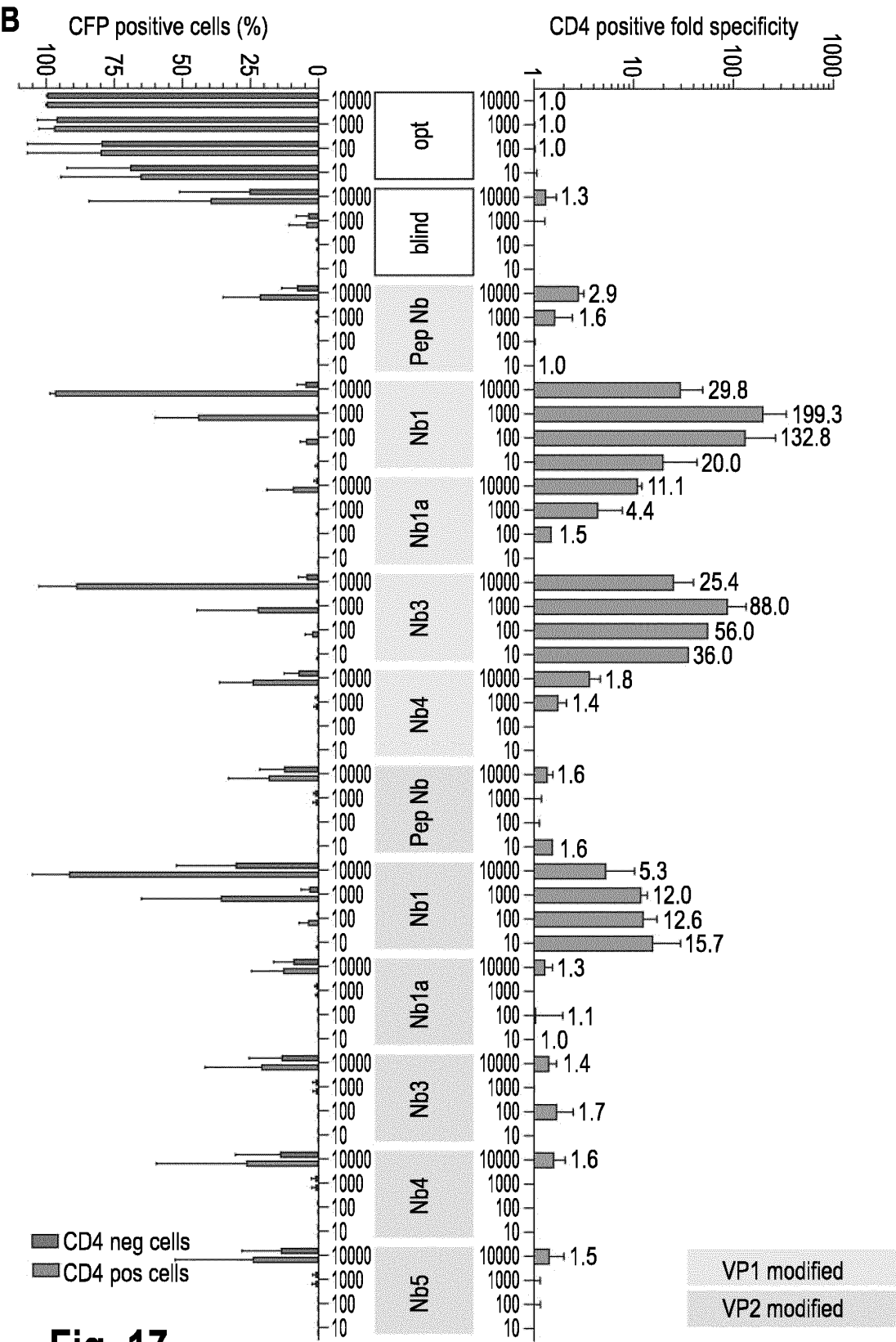
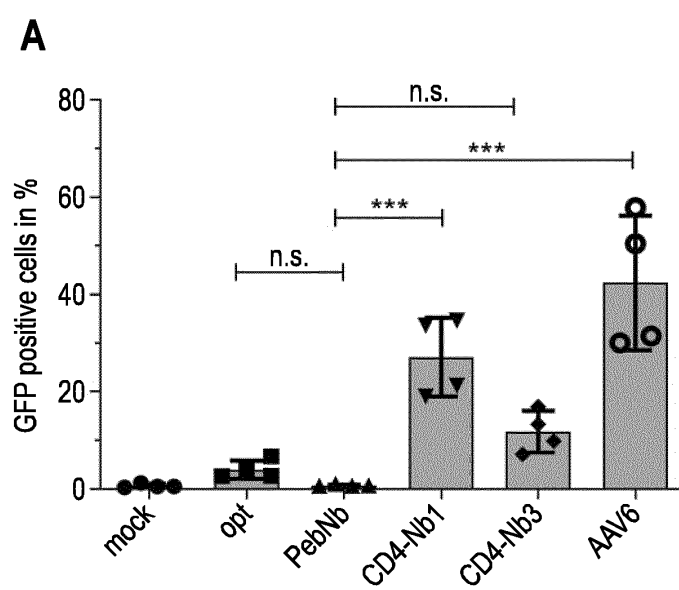


Fig. 17

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**Fig. 18**

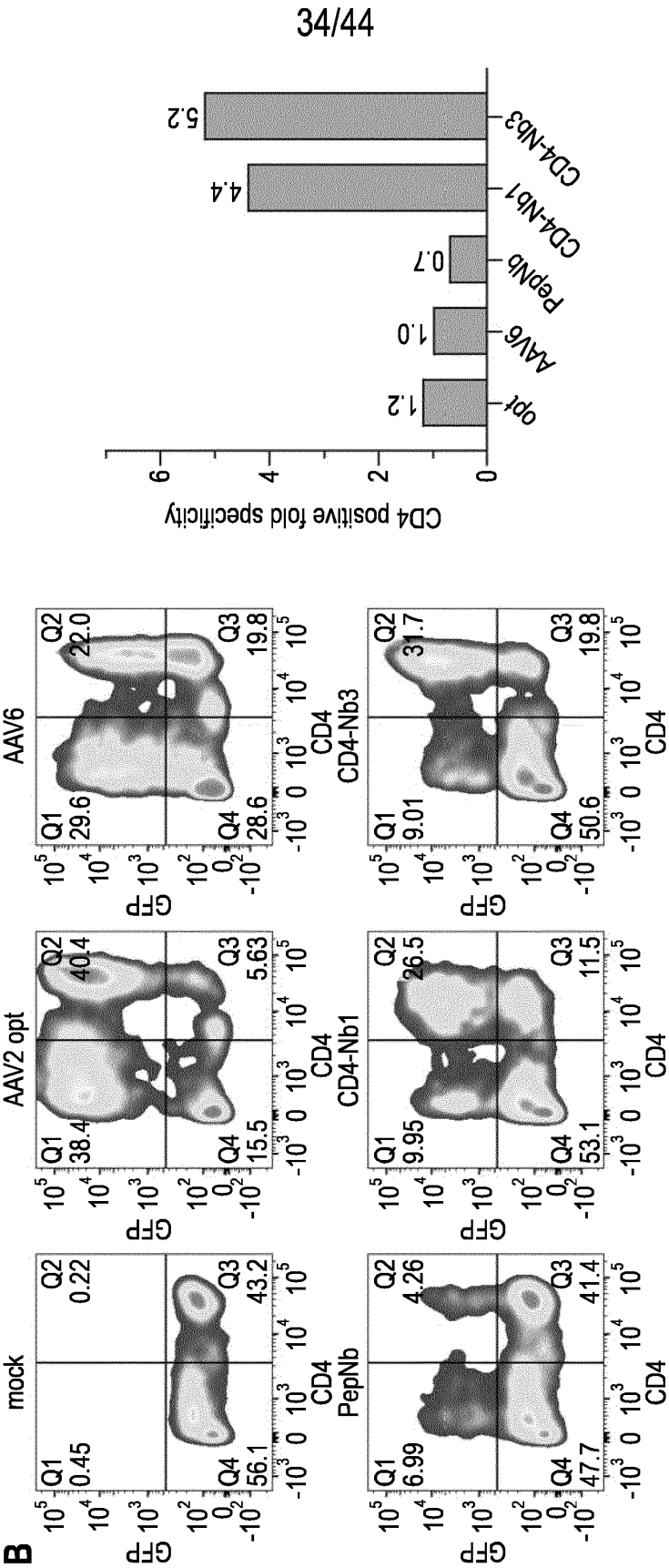


Fig. 18

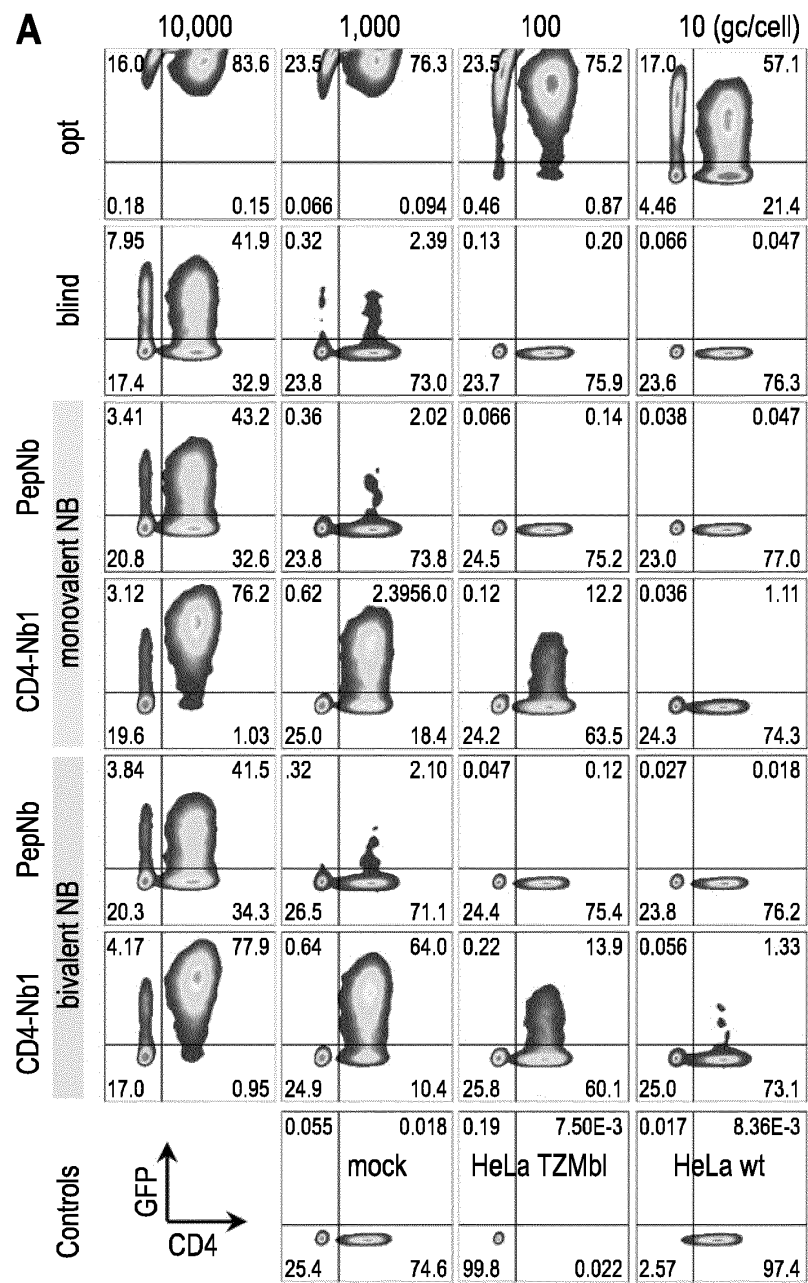


Fig. 19

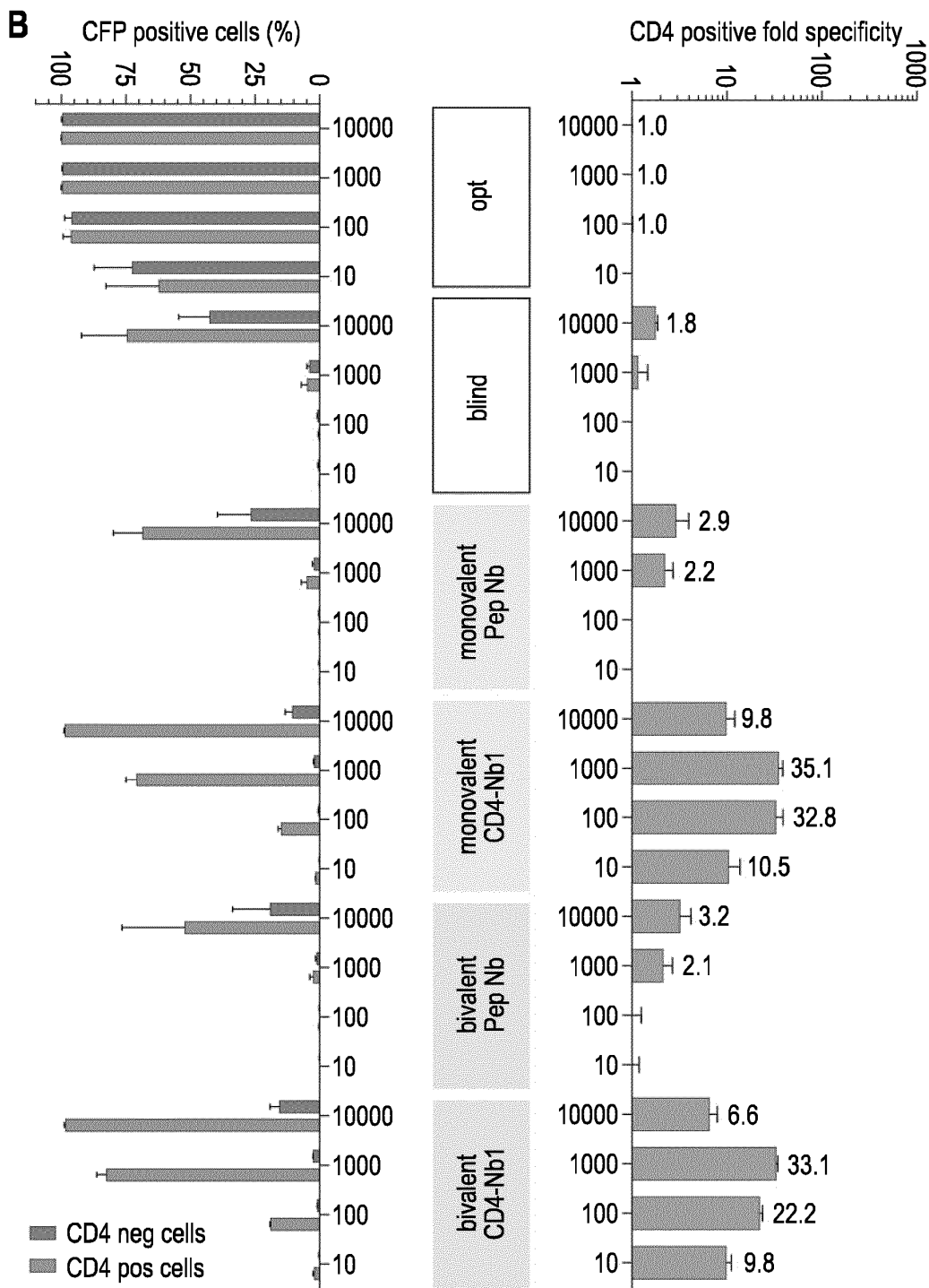


Fig. 19

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A

Name	Sequence 5'-3'	purpose
CALL001	GTCCTGGCTGCTCTTCTACAAGG	Nb library generation
CALL002	GGTACGTGCTGTTGAACTGTTCC	Nb library generation
FR1-1	CAT GGC NSA NGT GCA GCT GGT GGA NTC NGG NGG	Nb library generation
FR1-2	CAT GGC NSA NGT GCA GCT GCA GGA NTC NGG NGG	Nb library generation
FR1-3	CAT GGC NSA NGT GCA GCT GGT GGA NAG YGG NGG	Nb library generation
FR1-4	CAT GGC NSA NGT GCA GCT GCA GGA NAG YGG NGG	Nb library generation
FR1-ext1	GTAGGCCAGCCGGCCATGGCNSANGTGCAGCTGGTGG	Nb library generation
FR1-ext2	GTAGGCCAGCCGGCCATGGCNSANGTGCAGCTGCAGGA	Nb library generation
FR4-1	GAT GCG GCC GCN GAN GAN ACG GTG ACC NGN RYN CC	Nb library generation
FR4-2	GAT GCG GCC GCN GAN GAN ACG GTG ACC NGN GAN CC	Nb library generation
FR4-3	GAT GCG GCC GCN GAN GAN ACG GTG ACC NGR CTN CC	Nb library generation
FR4-4	GAT GCG GCC GCR CTN GAN ACG GTG ACC NGN RYN CC	Nb library generation
FR4-5	GAT GCG GCC GCR CTN GAN ACG GTG ACC NGN GAN CC	Nb library generation
FR4-6	GAT GCG GCC GCR CTN GAN ACG GTG ACC NGR CTN CC	Nb library generation
NGS fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATTGTTATTACTCGCGGCC	library PCR for NGS
NGS rev	GACTGGAGTTCCAGACGTGTGCTCTTCCGATCTGATCAGCTTCTGTTCTGCGGC	library PCR for NGS
hCD4 fwd	GGAGGATCCACAATGAACCGGGGAGTCCCTT	hCD4 expression
hCD4 rev	GGTCTCGAGTCAAATGGGGCTACATGTCTTCTGA	hCD4 expression
bsd fwd	ATACCCGGGGCCACCATGGCCAAGCCTTTGTCTC	hCD4 expression
bsd rev	TATGCGCGCTTAGCCCTCCACACATAACCAG	hCD4 expression
ΔD1 fwd	GCAGTCTCTCACTGGAGCAGCGGTTCCGATTGACTGCCAACTCTGACACC	CD4 ΔD1 expression
ΔD1 rev	GCGCACGCGATCAGGCATTCCCTGAGTGGCTGCTGGGAGG	CD4 ΔD1 expression
ΔD1ΔD2 fwd	GCAGTCTCTCACTGGAGCAGCGCTTTCCAGAAGGCCTCCAGCATAG	CD4 ΔD1ΔD2 expr.
ΔD1ΔD2 rev	GCGCACGCGATCAGGCATTCCCTGAGTGGCTGCTGGGAGG	CD4 ΔD1ΔD2 expr.
ΔD3ΔD4 fwd	CCCACATGGTCCACCCCG	CD4 ΔD3ΔD4 expr.
ΔD3ΔD4 rev	AGCTAGCACACGATGTCTATTTTG	CD4 ΔD3ΔD4 expr.
CD4-D1-4 f	ATACGTCTCAACTCTAAGAAAGTGGTGCTGGGCAAAAAAGG	CD4-D1-4 production
CD4-D1-4 r	TATGAATTCAGTGGTGATGGTGGTGGTGGGCGAGAACCTTGATGTTGGATTCC	CD4-D1-4 production

B

Cytokine	indicative for
Interleukin 1 β (IL-1β)	proinflammatory
Interleukin 1 receptor antagonist (IL-1RA)	antiinflammatory
Interleukin 4 (IL-4)	antiinflammatory
Interleukin 6 (IL-6)	pro-/antiinflammatory
Interleukin 8 (IL-8)	proinflammatory
Interleukin 10 (IL-10)	antiinflammatory
Interleukin 12 p70 (IL-12p70)	proinflammatory
Interleukin 13 (IL-13)	antiinflammatory
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	pro-/antiinflammatory
Interferon γ (IFNγ)	proinflammatory
Monocyte chemoattractant protein 1 (MCP-1)	proinflammatory
Macrophage inflammatory protein 1 β (MIP-1 β)	proinflammatory
Tumor necrosis factor α (TNF-α)	proinflammatory
Vascular endothelial growth factor (VEGF)	Wound healing factor

Fig. 20

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A) AAV2 VP1 Protein (blind and optimized):

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEADAAALE
 HDKAYDRQLDSGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTA PGKKRPVEHSP
 VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPPAAPSGLTNTMATGSGAPMADNNEGADGVG
 NSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFSPRD
 WQRLINNNWGRFPKRLNFKLFNIQVKEVTQNDGTTIANNLSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADV
 MVPQYGYLTNNGSQAVGRSSFYCLEYFSPQMLRTGNNTFTSYTFEDVPFHSSYAHQSGLDRMLNPLIDQFLYYLSR
 TNTPSGTITQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKVSADNNNSEFSWTGATKYHLNGRDSLVPNGP
 AMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEIRTNPVATEQYGSVSTNLQAGNAQAATADVNT
 QGVLPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPQILIKNTPVPANPSTTFSAAKFASFITQ
 YSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKS NVNVDFTVD TNGVYSEPRPIGTRFLTRNL*

B) Nanobody sequences in AAV VP1

VP1-PepNb

GGGGSGGGSGGGSGGGSGGGSGGGGSAMADVQLQESGGGLVQPGGSLRLSCAASGNIVSIDAAGWFRQAPG
 KQREP VATILTGGATNYADSVKGRFTISRDN AKNTVYLQMN SLKPEDTAVVYCYAPMIYYGGRYSDYWGQGTQVT
 VSSGGGGA

VP1-CD4-Nb4

GGGGSGGGSGGGSGGGSGGGSGGGGSMAEVQLQESGGALAQP GGSLRLSCAVSGFTFGTYGMGWLRQAPGK
 GREFVAAINWIHGGEIYADSVKGRFTISKDN AKNTLYLQMDSLKPEDTAVVYCAARSGTSLYTG VDEYQYWGQGT
 QVTVSSGGGGA

VP1-CD4-Nb1

GGGGSGGGSGGGSGGGSGGGSGGGGSMAEVQLVESGGGLVQPGGSLRLSCAASGFTFSKLAMSWHREPPGK
 GREWLADIDSSGDDTDY LASVKGRFTISRDN AKNTLYLQMDSLKSEDTGVYYCASREDPPGYWGQGTQVTVSSGGGGA

VP1-CD4-Nb3

GGGGSGGGSGGGSGGGSGGGSGGGGSMAHVQLVESGGGLVQPGGSLRLSCAVSGFALEYAIGWFRQAPGKE
 RERVACMSASGGVINYSESVKGRFTISRDN AKNTVYLQMN TLKPEDTAVVYCAAEKAYYGSSWAECYLMMDYWG
 KGTLVTVSSGGGGA

VP1-CD4-Nb1a

GGGGSGGGSGGGSGGGSGGGSGGGGSMAEVQLVESGGGLVQPGGSLRLSCVASGFTFSSSGMTWV
 RQAPGKGLEWVSDINSGDDTDY LASVKGRFTISRDN AKNTLYLQMDSLKSEDTGVYYCASREDPPGYWGQGIQV
 TVSSGGGGA

VP1-biCD4-Nb1

GGGGSGGGSGGGSGGGSGGGSGGGGSMAEVQLVESGGGLVQPGGSLRLSCAASGFTFSKLAMSWHREPPGK
 GREWLADIDSSGDDTDY LASVKGRFTISRDN AKNTLYLQMDSLKSEDTGVYYCASREDPPGYWGQGTQVTVSS**ASG**
GGGSGGGSGGGSGGGSGGGSGGGGSMAEVQLVESGGGLVQPGGSLRLSCAASGFTFSKLAMSWHREPPGKGRE
 WLADIDSSGDDTDY LASVKGRFTISRDN AKNTLYLQMDSLKSEDTGVYYCASREDPPGYWGQGTQVTVSSGGGGA

Fig. 21

VP1-biPepNB

GGGGSGGGSGGGSGGGSGGGSGGGSAMADVQLQESGGGLVQPGGSLRLSCAASGNIVSIDAAGWFRQAPGK
QREP VATILTGGATNYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCYAPMIYYGGRYSDYWGGGTQVTV
SS**ASGGGGSGGGSGGGSGGGSGGGSGGGG**SMAADVQLQESGGGLVQPGGSLRLSCAASGNIVSIDAAGWFRQAP
GKQREP VATILTGGATNYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCYAPMIYYGGRYSDYWGGGTQV
TVSS**GGGGA**

C) AAV2 VP2 Protein (blind and optimized):

MAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPPAAPSGLGTNTMATGSGAP
MADNNEGADGVGNSSGNWHCDSTWMGDRVITSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYF
DFNRFHCHFSRDPWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAH
QGCLPPFPADVFMVPQYGYLTNNNGSQAVGRSSFCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRM
NPLIDQFLYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKVSADNNNSEFSWTGATKYHL
NGRDSLNVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQAG
NAQAATADVNTQGVLPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPST
TFSAAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRFLTRNL*

D) Nanobody sequences in AAV VP2

VP2-PepNb

MADVQLQESGGGLVQPGGSLRLSCAASGNIVSIDAAGWFRQAPGKQREP VATILTGGATNYADSVKGRFTISRDN
AKNTVYLQMNSLKPEDTAVYYCYAPMIYYGGRYSDYWGGGTQVTVSS**RGGS GGSGGS GGSGGS**SR-VP2

VP2-CD4-Nb4

MAEVQLQESGGALAQPGGSLRLSCAVSGFTFGTYGMGWL RQAPGK GREFVA AINWIHGGEIYADSVKGRFTISKD
NAKNTLYLQMDSLKPEDTAVYYCAARSGTSLYTGVD EYQYWGQGTQVTVSS**RGGS GGSGGS GGSGGS**SR-VP2

VP2-CD4-Nb5

MAEVQLQESGGGLVQAGGSLRLSCKVSGRTFGTYAMGWFRQAPGKREFVAVISWIKGNTYVADSVLGRFTISRDN
NARNWLHLQMYSLKPEDTATYYCAASSTVLTPTTLDPTYWGGGTPVTVSS**RGGS GGSGGS GGSGGS**SR-VP2

VP2-CD4-Nb1

MAEVQLVESGGGLVQPGGSLRLSCAASGFTFSKLAMSWHREPPGKGREWLADIDSSGDTTDYLASVKGRFTISRDN
NAKNTLYLQMDSLKSEDTGVYYCASREDPPGYWGQGTQVTVSS**RGGS GGSGGS GGSGGS**SR-VP2

VP2-CD4-Nb3

MAHVQLVESGGGLVQPGGSLRLSCAVSGFALEYAIGWFRQAPGKERERVACMSASGGVINYESVKGRFTISRDN
NAKNTVYLQMNTLKPEDTAVYYCAA EKAYGSSWAECYLMMDYWGKGT LTVTVSS**RGGS GGSGGS GGSGGS**SR-VP2

VP2-CD4-Nb1a

MAEVQLVESGGGLVQPGGSLRLSCV ASGFTFSSSGMTWVRQAPGKGLEWVSDINSGGDTTDYLASVKGRFTISRDN
NAKNTLYLQMDSLKSEDTGVYYCASREDPPGYWGQGIQVTVSS**RGGS GGSGGS GGSGGS**SR-VP2

Fig. 21 continued

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Name	Sequence 5'-3'	Purpose
oMH16	cctTCTAGAAAtGGCTCCGGGAAAAAAG	ORF opt-blind VP2
oMH18	AACGGATCCTTACAGATTACGAGTCAG	ORF opt-blind VP2
oMH19	CAACGcACAAGCAGCTACCGCAGATG	SDM AAV2 cap blinding R585/588A
oMH20	CCTGcCTGGAGGTTGGTAGATACAG	SDM AAV2 cap blinding R585/588A
oMH22	AGTtCCTGTATTACTTGAGC	SDM AAV2 cap opt Y444F
oMH23	GGTCGATGAGAGGATTTCATG	SDM AAV2 cap opt Y444F
oMH24	CAACAACAGTGAATtCTCGTGGACTG	SDM AAV2 cap opt T491V/Y500F
oMH25	TTATCCGCAGATacCTTTGATACTCG	SDM AAV2 cap opt T491V/Y500F
oMH26	GATtCCTGACTCGTAATCTG	SDM AAV2 cap opt Y730F
oMH27	TGGTGCCAATGGGGCGAGGC	SDM AAV2 cap opt Y730F
oMH35	GCGAAGCTTgccgccATGGCAGAGGTG	CD4-VP2-Nb4 PCR
oMH36	TGCCTCGAGAGGAGACGGTGACCTGGG	CD4-VP2-Nb4 PCR
oMH37	GCGAAGCTTgccgccATGGCAGAGGTG	CD4-VP2-Nb5 PCR
oMH38	GGCCTCGAGaGGAACCGGTGACCGGAG	CD4-VP2-Nb5 PCR
oMH39	GCGAAGCTTgccgccATGGCTGAGGTG	CD4-VP2-Nb1 PCR
oMH40	GGCCTCGAGaTGAGACGGTGACCTGGG	CD4-VP2-Nb1 PCR
oMH41	GCGAAGCTTgccgccATGGCCACGTG	CD4-VP2-Nb3 PCR
oMH42	GGCCTCGAGaGGAGACGGTGACCAGGG	CD4-VP2-Nb3 PCR
oMH43	GCGAAGCTTgccgccATGGCCGAGGTG	CD4-VP2-Nb1a PCR
oMH44	GGCCTCGAGAGGAGACGGTGACCTGG	CD4-VP2-Nb1a PCR
oMH87	GCGAAGCTTgccgccatggctgacgtgcag	PepNb-VP2 PCR
oMH88	GCCTCGAGAGGAGACGGTGACCTggggtg	PepNb-VP2 PCR
oMH45	TCAGGTAaGGCTGCCGATGG	SDM VP1 start codon inactivation (sc)
oMH46	TTTAAATCATTTATTGTTCAAAGATGC	SDM VP1 start codon inactivation (sc)
oMH47	TCTTCcttATTGGCTCGAGG	SDM VP2/3 splice acceptor inactivation (SA)
oMH48	TAACCATCGGCAGCCATACC	SDM VP2/3 splice acceptor inactivation (SA)
oMH67	ACTTGGAGTGTTTGTCTGCTCAAGTAATACAGG	SDM NotI site implementation
oMH68	GCCGCggcggcgggcggcgcCTTCAGTTTTCTCAG	SDM NotI site implementation
oMH69	ggcggcgggcggcagcggcgggcggcagcggcgggcggcg gcagcggcgggcggcagcggcgggcggcagcggcggcgGCG	oligo annealing 5' linker block
oMH70	CGCgctgccgcgcgcgcgctgccgcgcgcgctgccgc cgccgcgctgccgcgcgcgcgctgccgcgcgcg	oligo annealing 5' linker block
oMH075	gcggcagcggcgggcggcgcgGcTATGGCTGAGGTGCAG	CD4-VP1-Nb1 PCR
oMH076	AACTGAAGcgcgccgccgccGcTtGAGACGGTGACCTG	CD4-VP1-Nb1 PCR
oMH077	gcggcagcggcgggcggcgcgGcTatGGCCGAGGTGCAG	CD4-VP1-Nb1a PCR
oMH078	AACTGAAGcgcgccgccgccGcTtGGAGACGGTGACCTG	CD4-VP1-Nb1a PCR
oMH079	gcggcagcggcgggcggcgcgGcTatGGCAGAGGTGCAG	CD4-VP1-Nb4 PCR
oMH080	AACTGAAGcgcgccgccgccGcTtGGAGACGGTGACCTG	CD4-VP1-Nb4 PCR
oMH083	gcggcagcggcgggcggcgcgGcTatGGCCACGTGCAG	CD4-VP1-Nb3 PCR
oMH084	AACTGAAGcgcgccgccgccGcTtGGAGACGGTGACCAG	CD4-VP1-Nb3 PCR

Fig. 22

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oMH095	gcggcagcggcggcggcggcagcGCtatggctgacgtgcag	PepNb-VP1 PCR
oMH096	AACTGAAGcgcgccgccgccGCtGGAGACGGTGAC	PepNb-VP1 PCR
oMH085	GCggcggcggcggcggcgcgCTTCAGTTTTC	overlap extension PCR cap gene
oMH086	AGGTACACATCTCTGTCCTGCCAGACCA	overlap extension PCR cap gene
oMH093	GCGATCTCGATGCTTTATTT	AAV qPCR SV40pA
oMH094	AGAGGGAGTGGA TAGTTT	AAV qPCR SV40pA

Fig. 22 continued

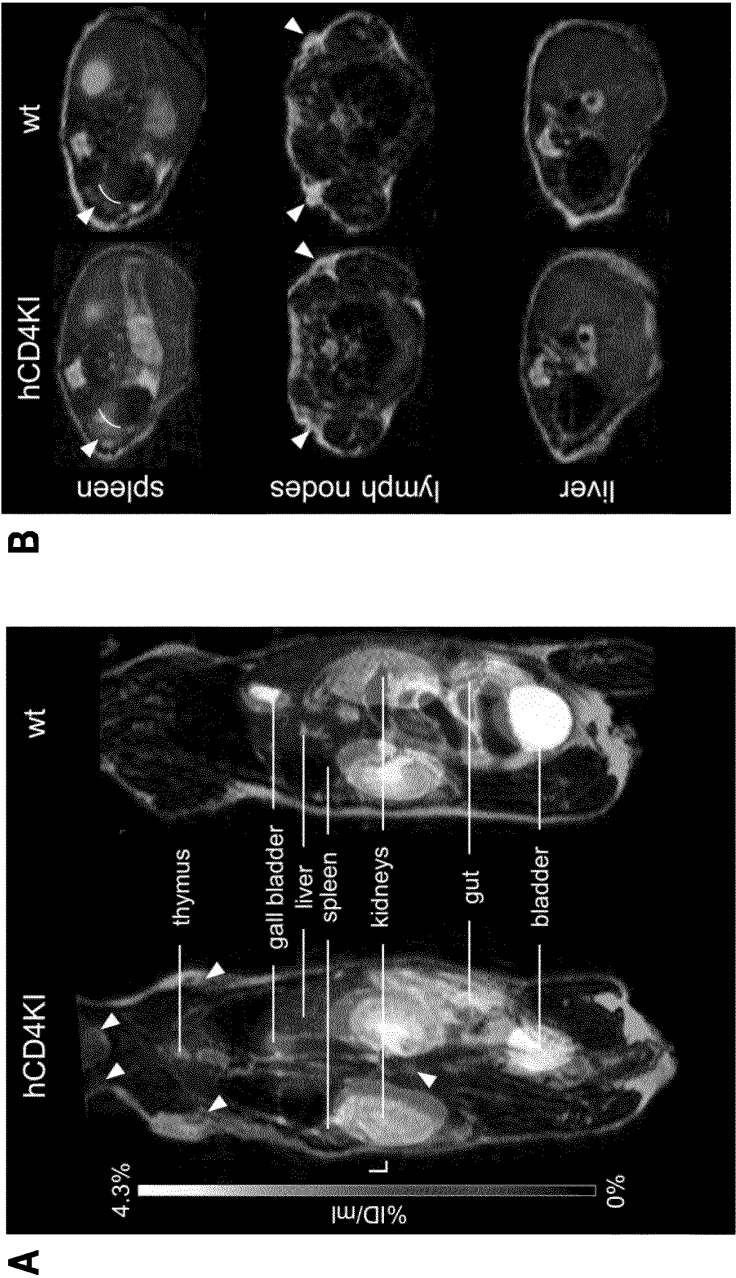


Fig. 23

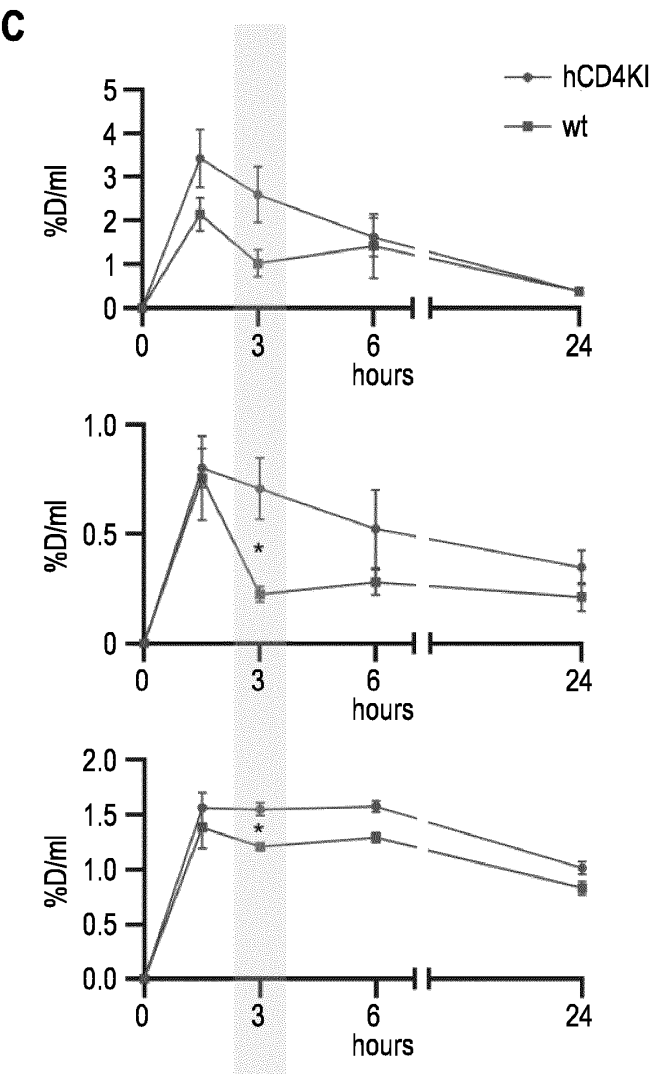
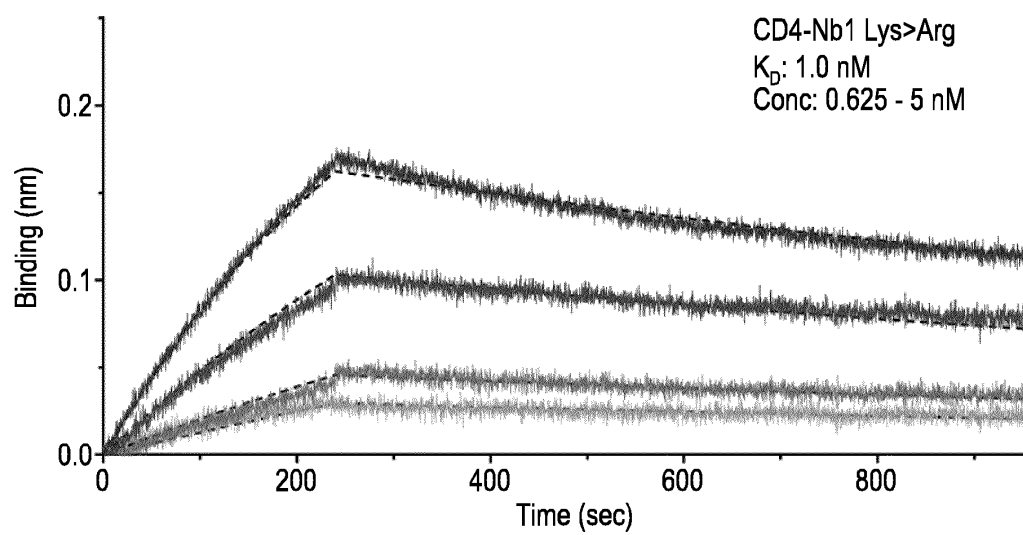


Fig. 23

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**Fig. 24**