Figure 1

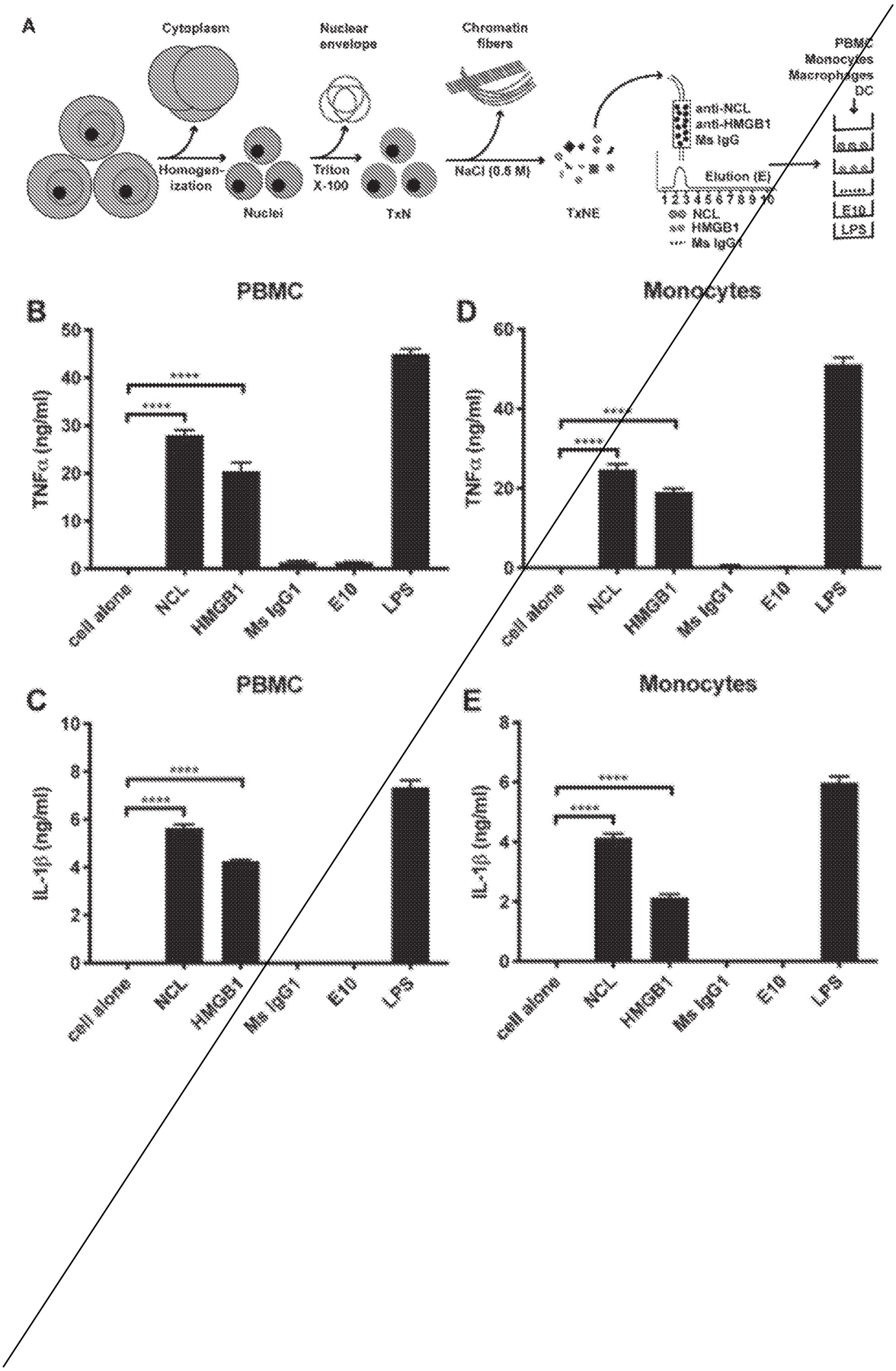

A
Cytoplasm
Nuclear envelope
Chromatin fibers
Homogen-ization
Nuclei
Triton X-100
TxN
NaCl (0.5 M)
TxNE
anti-NCL
anti-HMGB1
Ms IgG
Elution (E)
1 2 3 4 5 6 7 8 9 10
NCL
HMGB1
Ms IgG1
PBMC
Monocytes
Macrophages
DC
E10
LPS
B
PBMC
TNFα (ng/ml)
cell alone
NCL
HMGB1
Ms IgG1
E10
LPS
D
Monocytes
C
PBMC
IL-1β (ng/ml)
E
Monocytes

Figure 1 (continued)

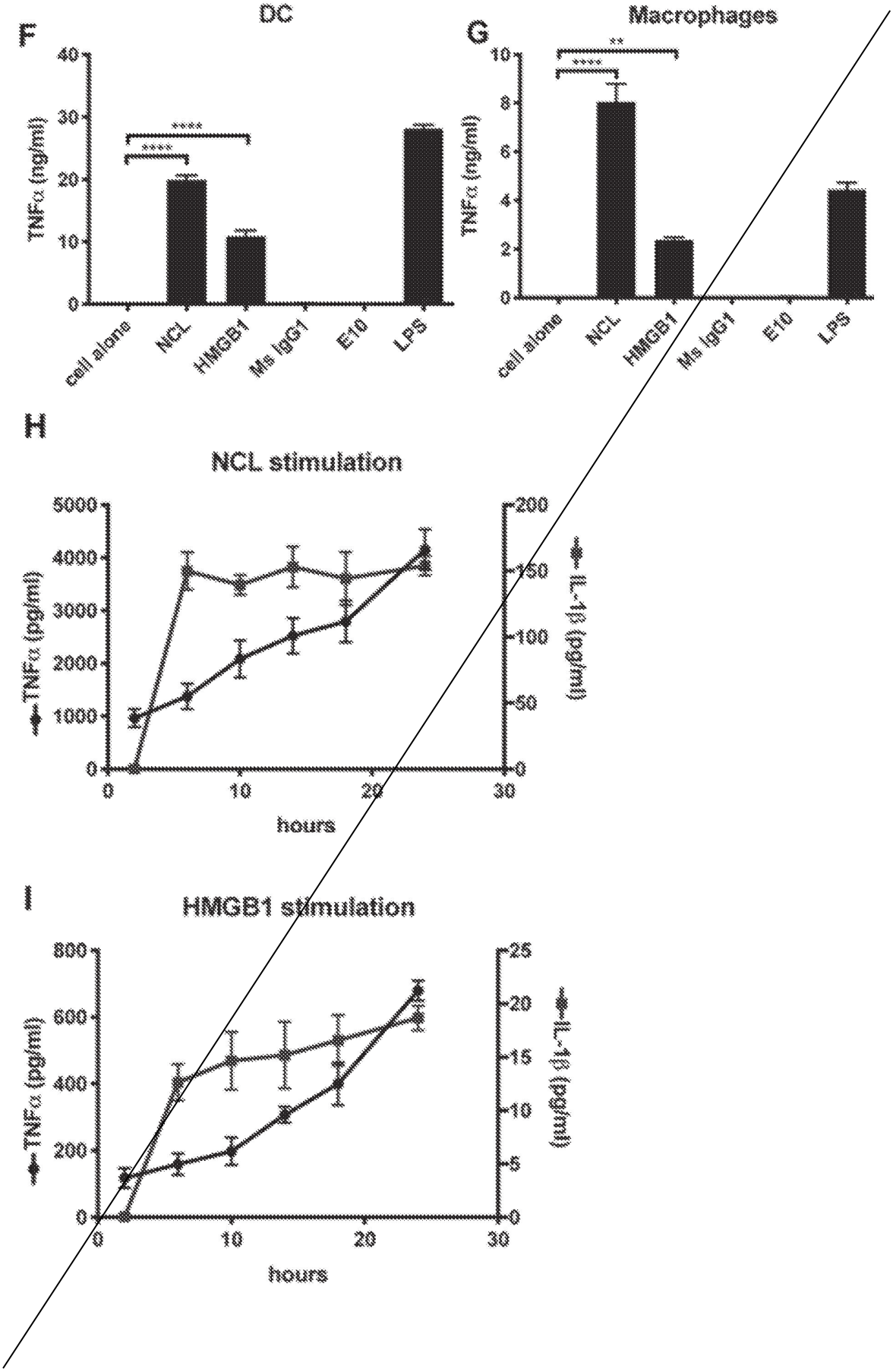


Figure 1 (continued)

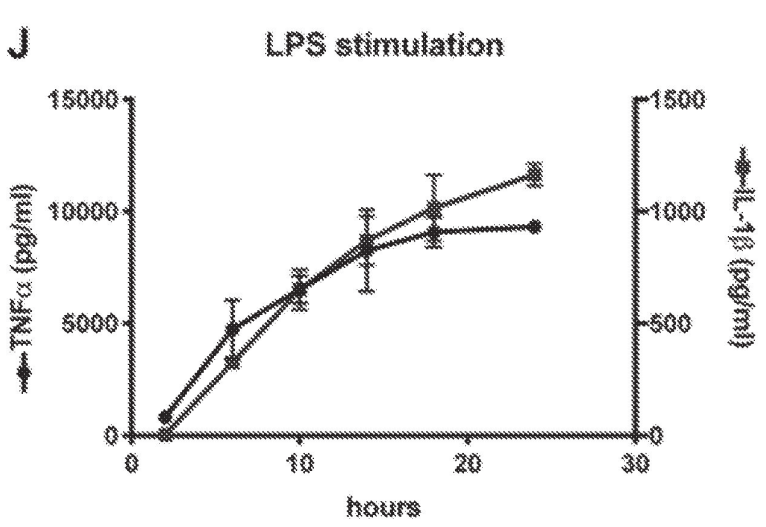
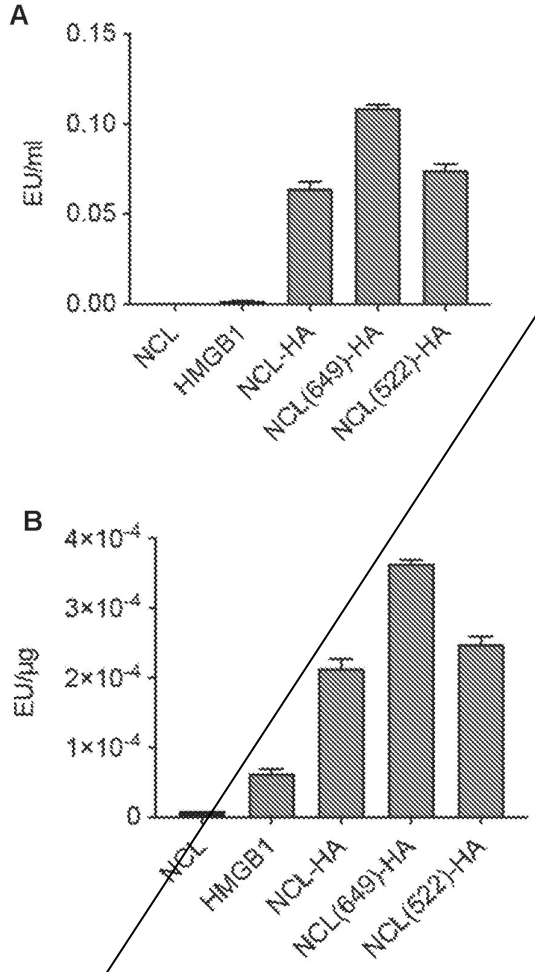


Figure 2



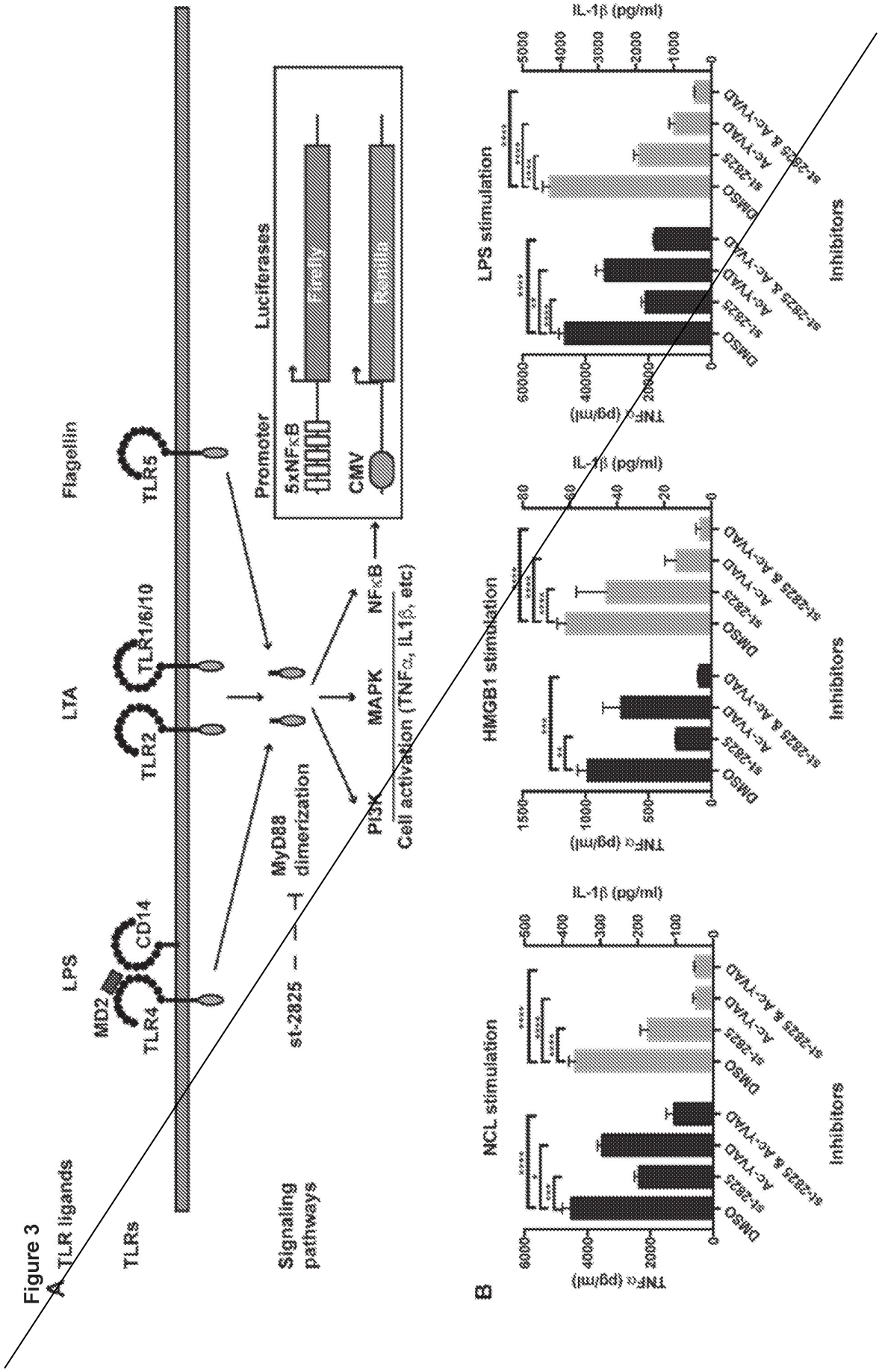


Figure 3
A
TLR ligands
TLRs
LPS
LTA
Flagellin
MD2
CD14
TLR4
TLR2
TLR1/6/10
TLR5
Signaling pathways
st-2825
MyD88 dimerization
PI3K
MAPK
NFκB
Cell activation (TNFα, IL1β, etc)
Promoter
Luciferases
5xNFκB
CMV
Firefly
Renilla
B
NCL stimulation
HMGB1 stimulation
LPS stimulation
IL-1β (pg/ml)
TNFα (pg/ml)
DMSO
st-2825
Ac-YVAD
st-2825 & Ac-YVAD
Inhibitors

Figure 3 (continued)

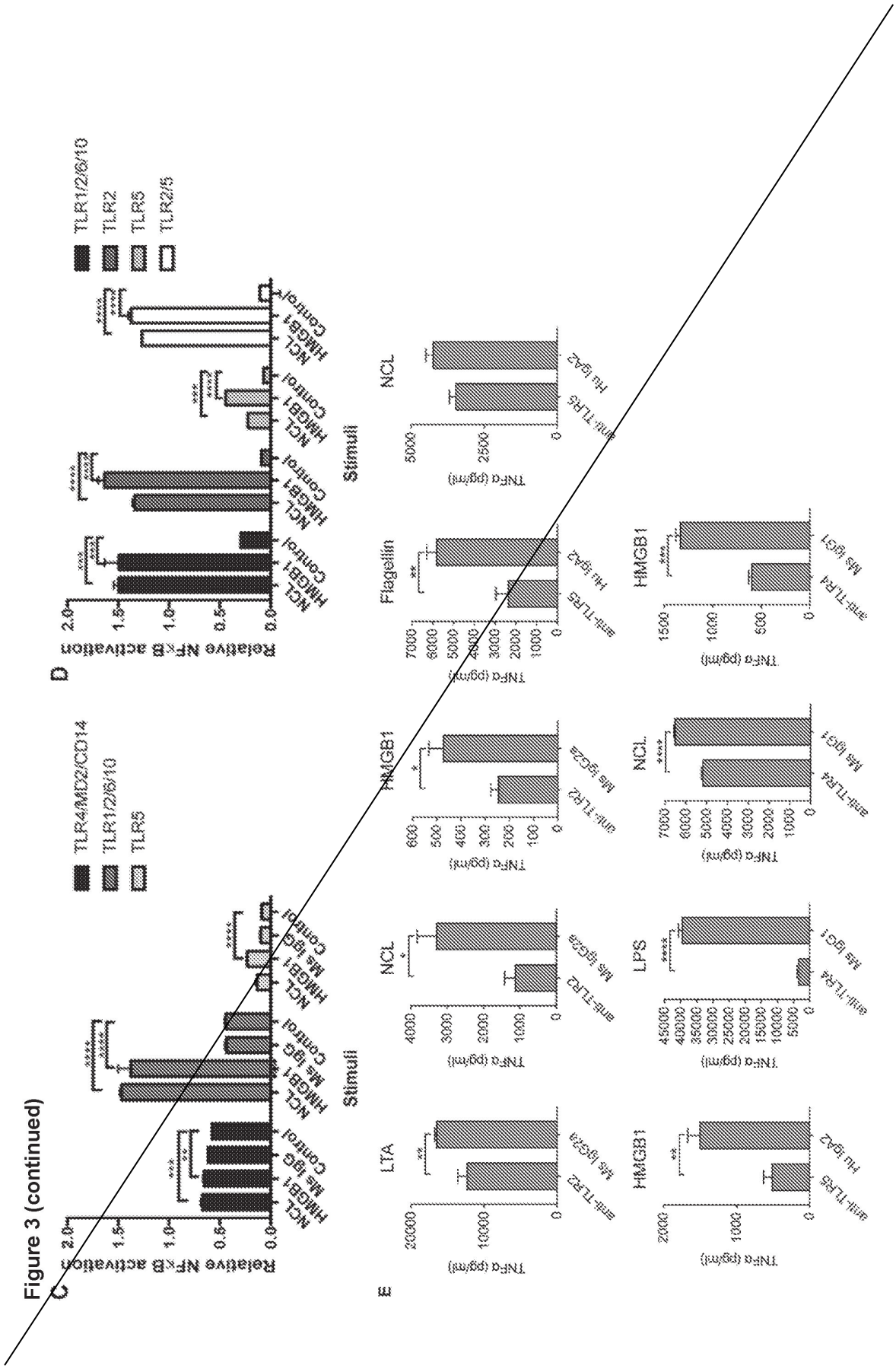


Figure 4

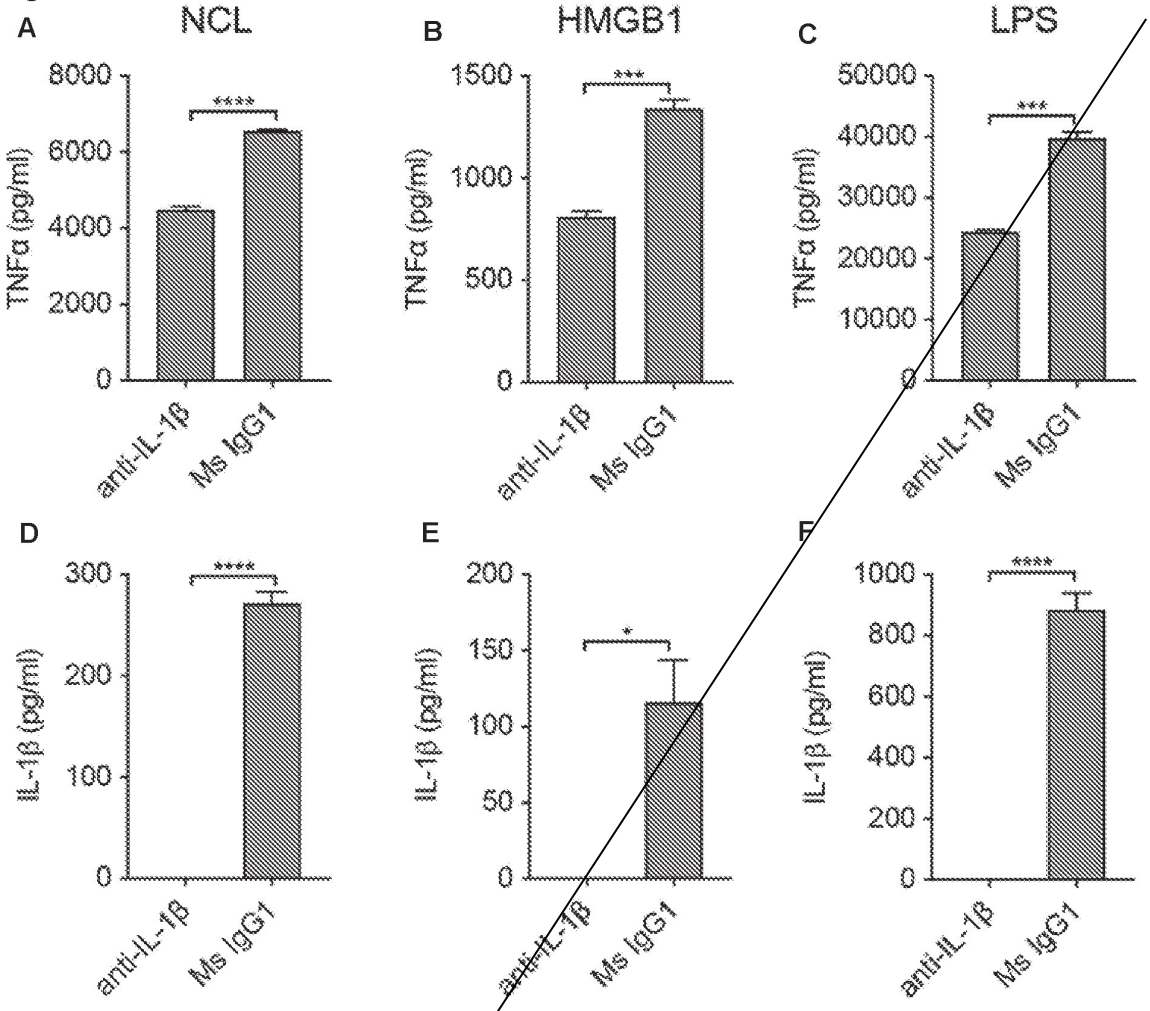


Figure 5

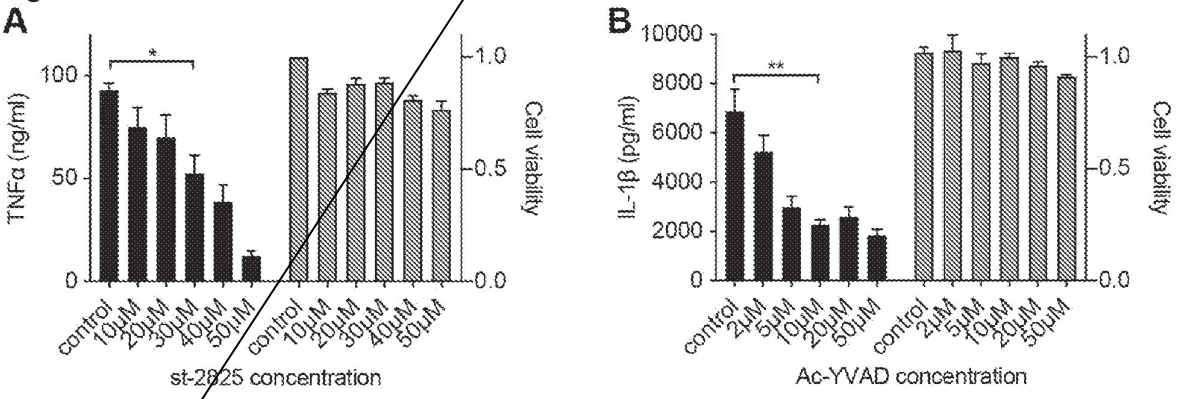


Figure 6

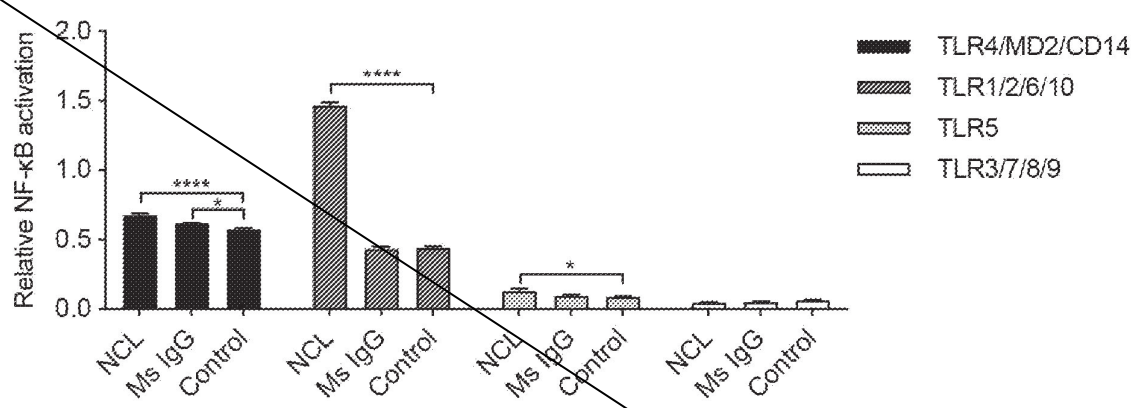


Figure 7

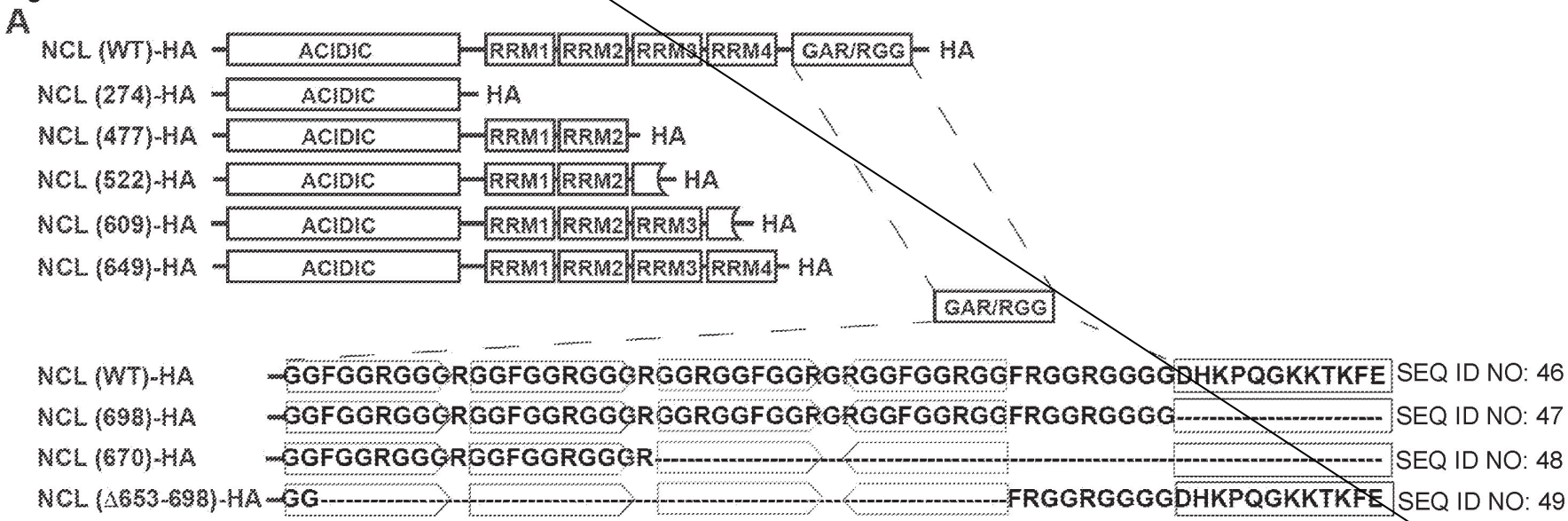


Figure 7 (continued)

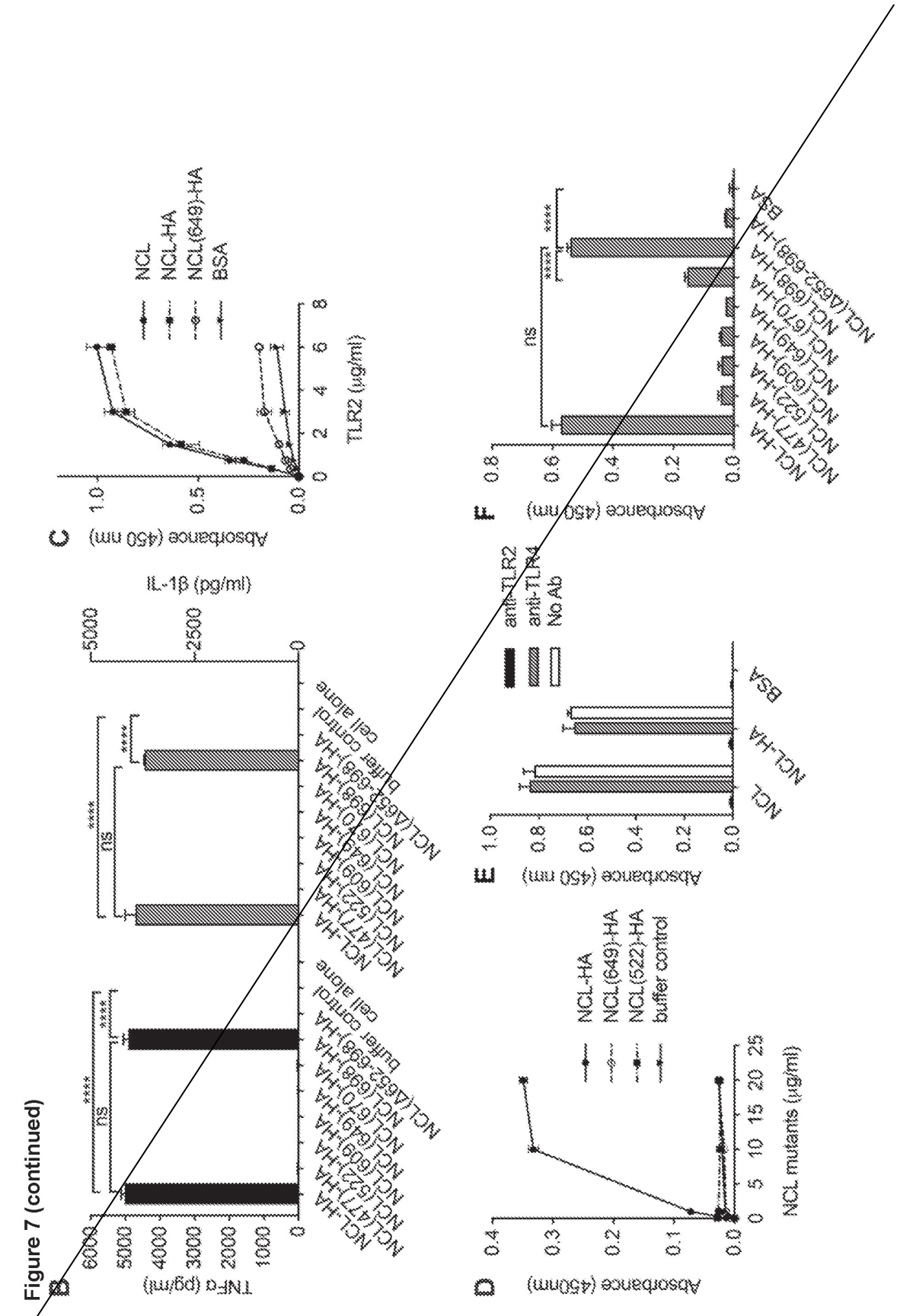


Figure 8

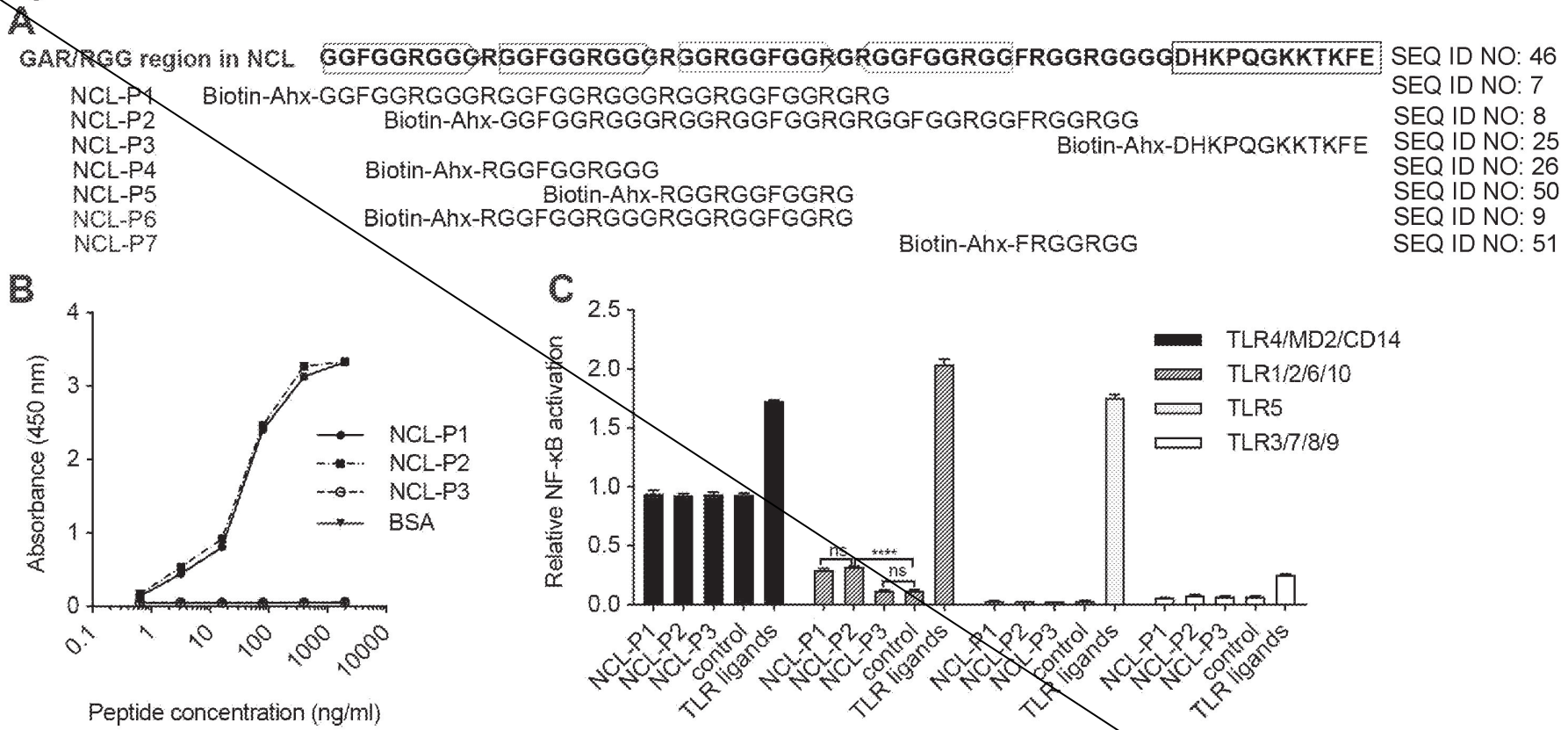

A
GAR/RGG region in NCL GGFGGRGGGRGGFGGRGGGRGGRGGFGGRGRGGFGGRGGFRGGRGGGGDHKPQGKKTKFE SEQ ID NO: 46
NCL-P1 Biotin-Ahx-GGFGGRGGGRGGFGGRGGGRGGRGGFGGRGRG SEQ ID NO: 7
NCL-P2 Biotin-Ahx-GGFGGRGGGRGGRGGFGGRGRGGFGGRGGFRGGRGG SEQ ID NO: 8
NCL-P3 Biotin-Ahx-DHKPQGKKTKFE SEQ ID NO: 25
NCL-P4 Biotin-Ahx-RGGFGGRGGG SEQ ID NO: 26
NCL-P5 Biotin-Ahx-RGGRGGFGGRG SEQ ID NO: 50
NCL-P6 Biotin-Ahx-RGGFGGRGGGRGGRGGFGGRG SEQ ID NO: 9
NCL-P7 Biotin-Ahx-FRGGRGG SEQ ID NO: 51
B
Absorbance (450 nm)
Peptide concentration (ng/ml)
NCL-P1
NCL-P2
NCL-P3
BSA
C
Relative NF-κB activation
TLR4/MD2/CD14
TLR1/2/6/10
TLR5
TLR3/7/8/9
ns
****
ns
NCL-P1
NCL-P2
NCL-P3
control
TLR ligands

Figure 8 (continued)

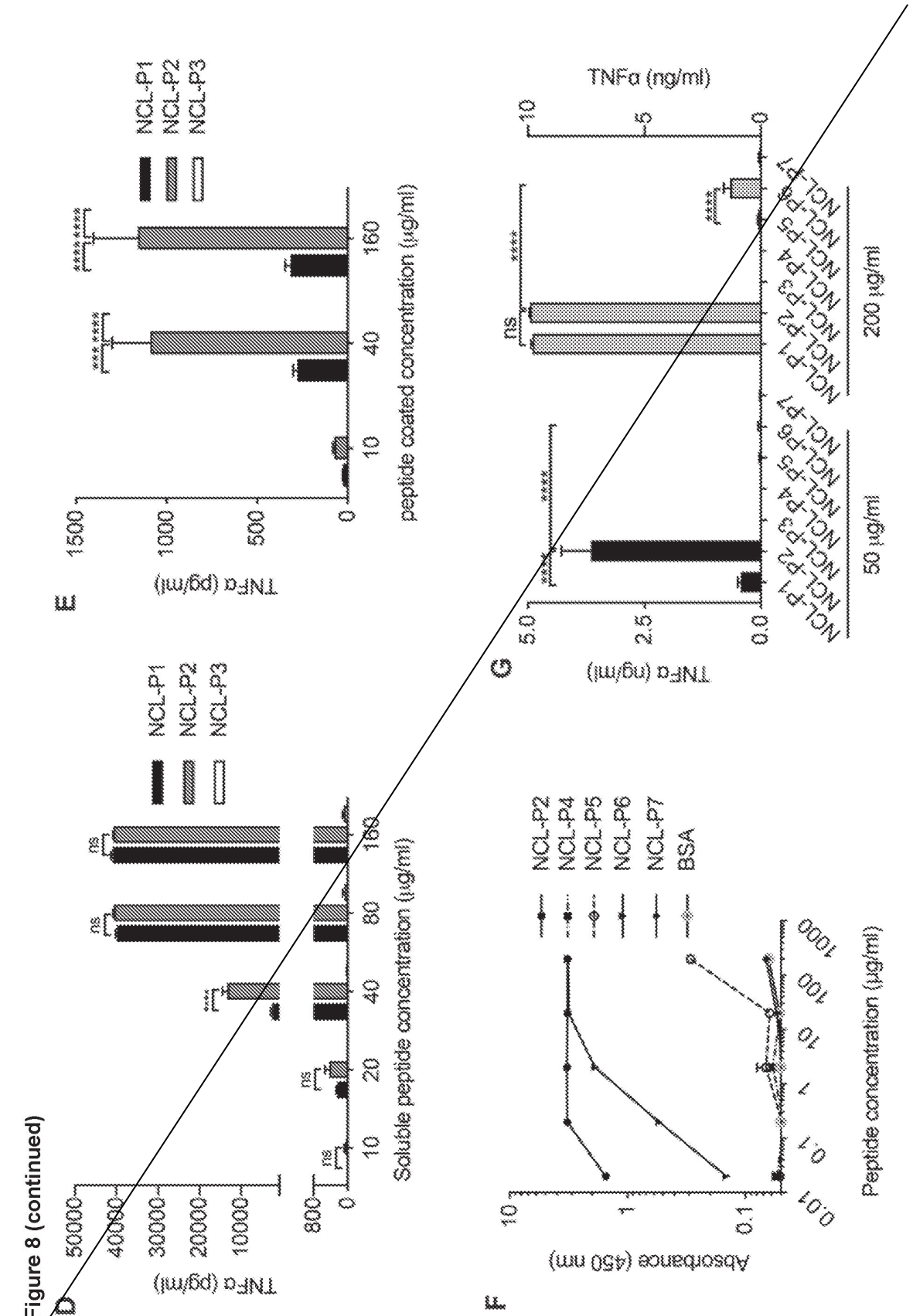


Figure 9

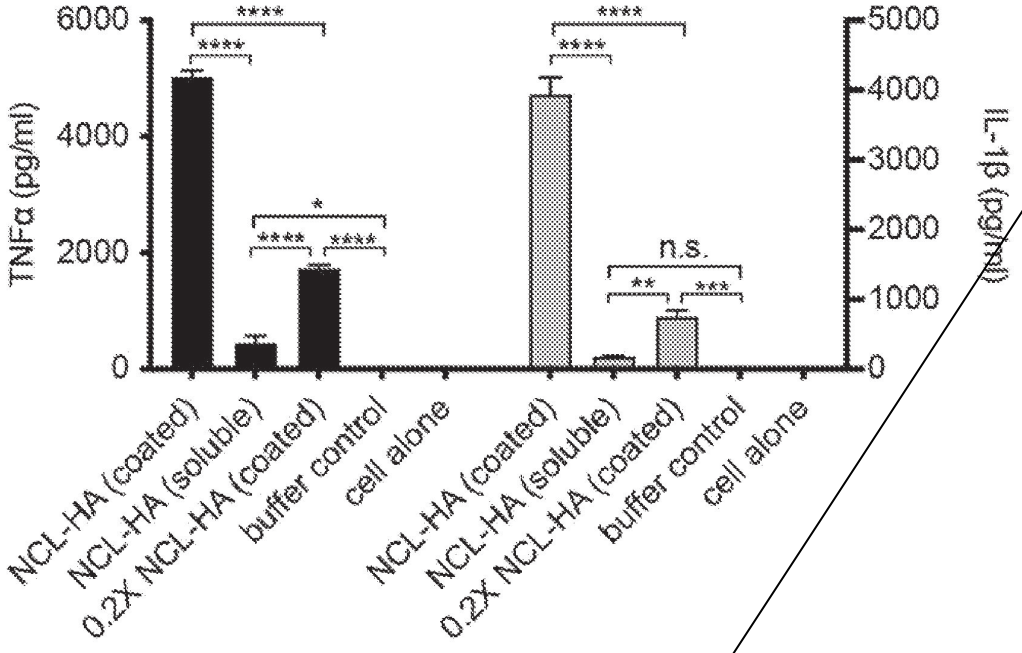


Figure 10

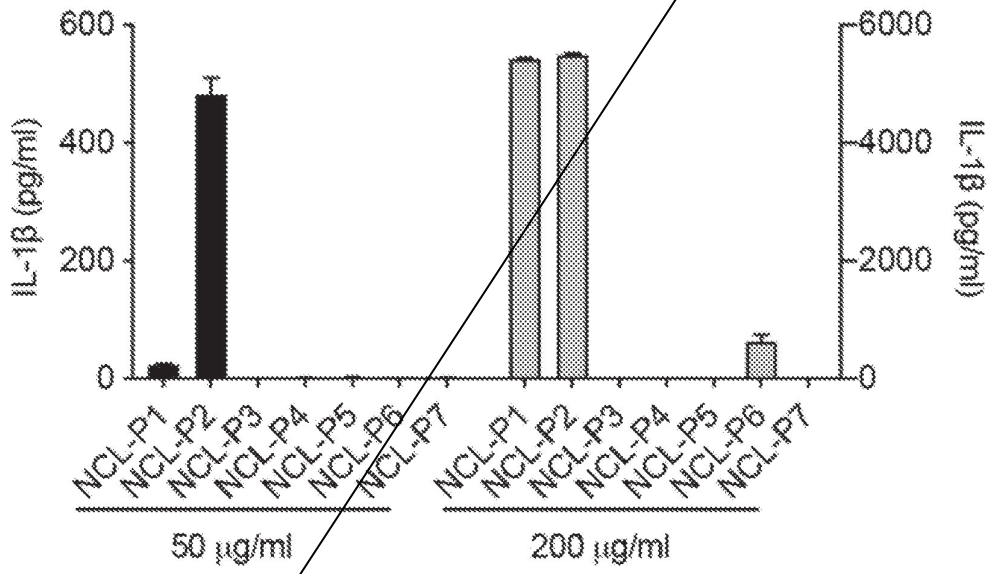


Figure 11

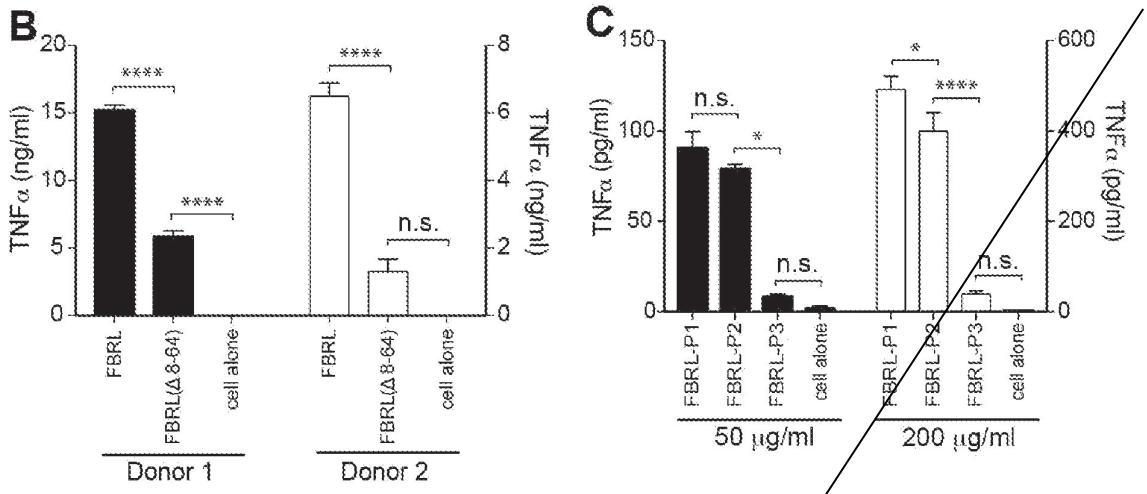
A

FBL-P1 FBL-P2 FBL-P3

MKPGFSP**RGGGFGGRGGFGDRGGRGG**RGG**FGGGRGRGGGFRGRG**RGG**GGGGGGGGGGGRG**
GGGFHSGGN**RGRGRGGKRG**NQSGKNVMVEPHRHEGVFICRGKEDALVTKNLVPGESVYGE
KRVSISEGDDKIEYRAWNPFRSKLAAAILGGVDQIHIKPGAKVLYLGAASGTTVSHVSDI
VGPDGLVYAVEFSHRSGRDLINLAKKRTNIIPVIEDARHPHKYRMLIAMVDVIFADVAQP
DQTRIVALNAHTFLRNGGHFVISIKANCIDSTASAEAVFASEVKKMQQENMKPQEQLTLE
PYERDHAVVVGVYRPPPKVKN

SEQ ID NO: 2

Figure 11 (continued)



D

MSFRGGGRGGFNRGGGGGGFNRGGSSNHFR
GGGGGGGGGNF**RGGGRGGFGRGGGRGG**FNK
GQDQGPPERVVLLGEFLHPCEDDIVCKCTT
DENKVPYFNAPVYLENKEQIGKVDEIFGQL
RDFYFSVKLSENMKASSFKKLQKFYIDPYK
LLPLQRFLPRPPGEKGPP**RGGGRGGRGGGR**
GGGGRGGGRGGGFRGGRGGGGGGFRGGRGG
GFRGRGH

SEQ ID NO: 3

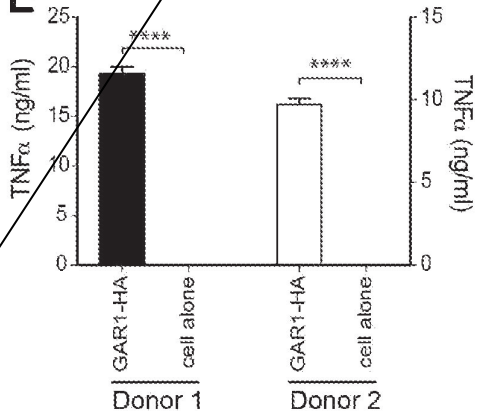


Figure 12

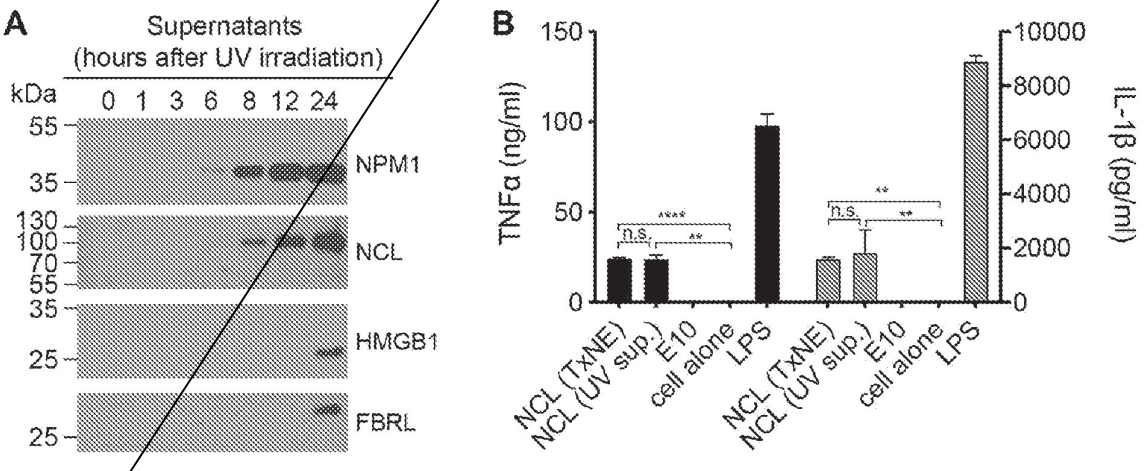


Figure 13

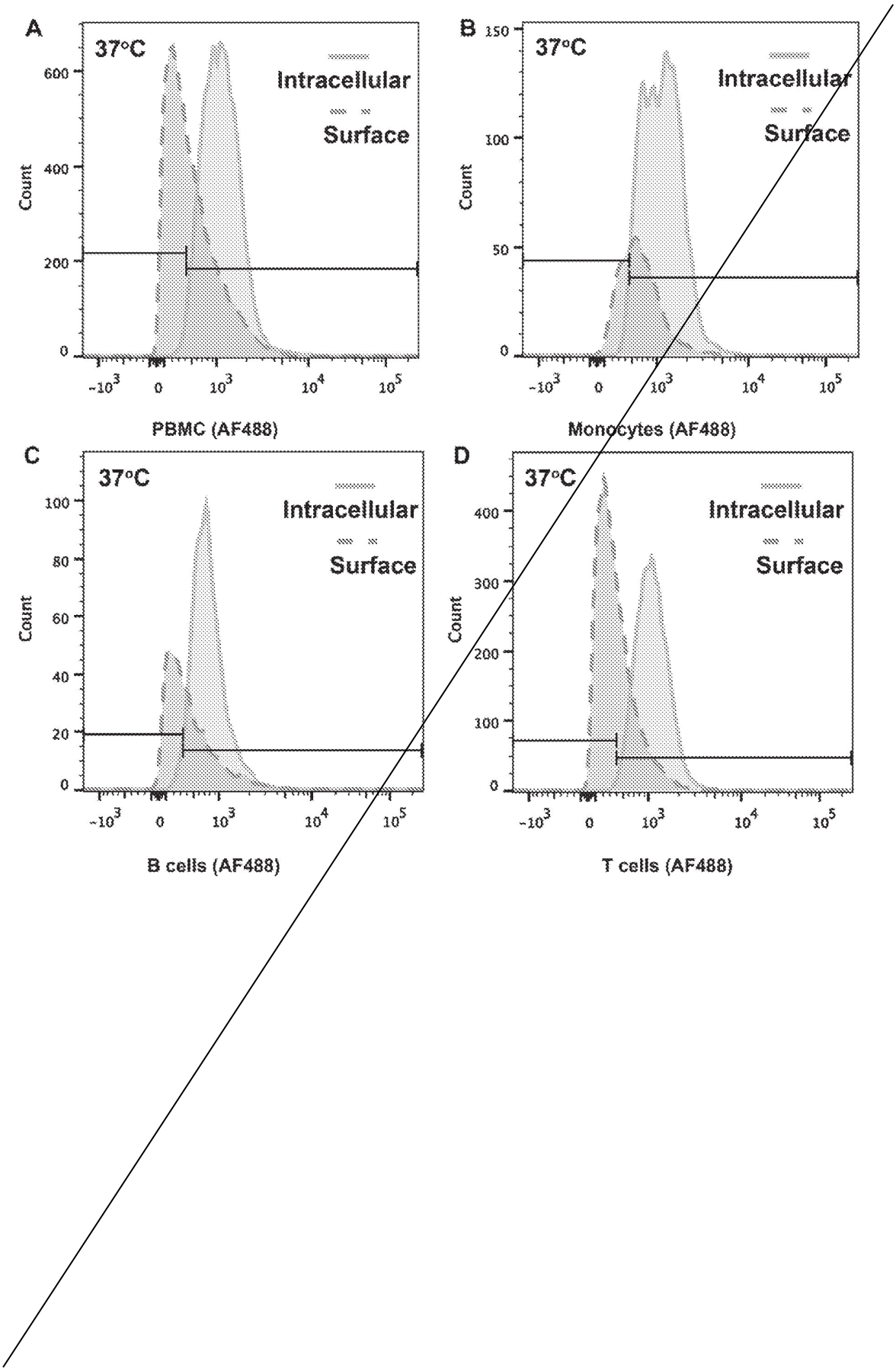

A
37°C
Intracellular
Surface
Count
PBMC (AF488)
B
37°C
Intracellular
Surface
Count
Monocytes (AF488)
C
37°C
Intracellular
Surface
Count
B cells (AF488)
D
37°C
Intracellular
Surface
Count
T cells (AF488)

Figure 13 (continued)

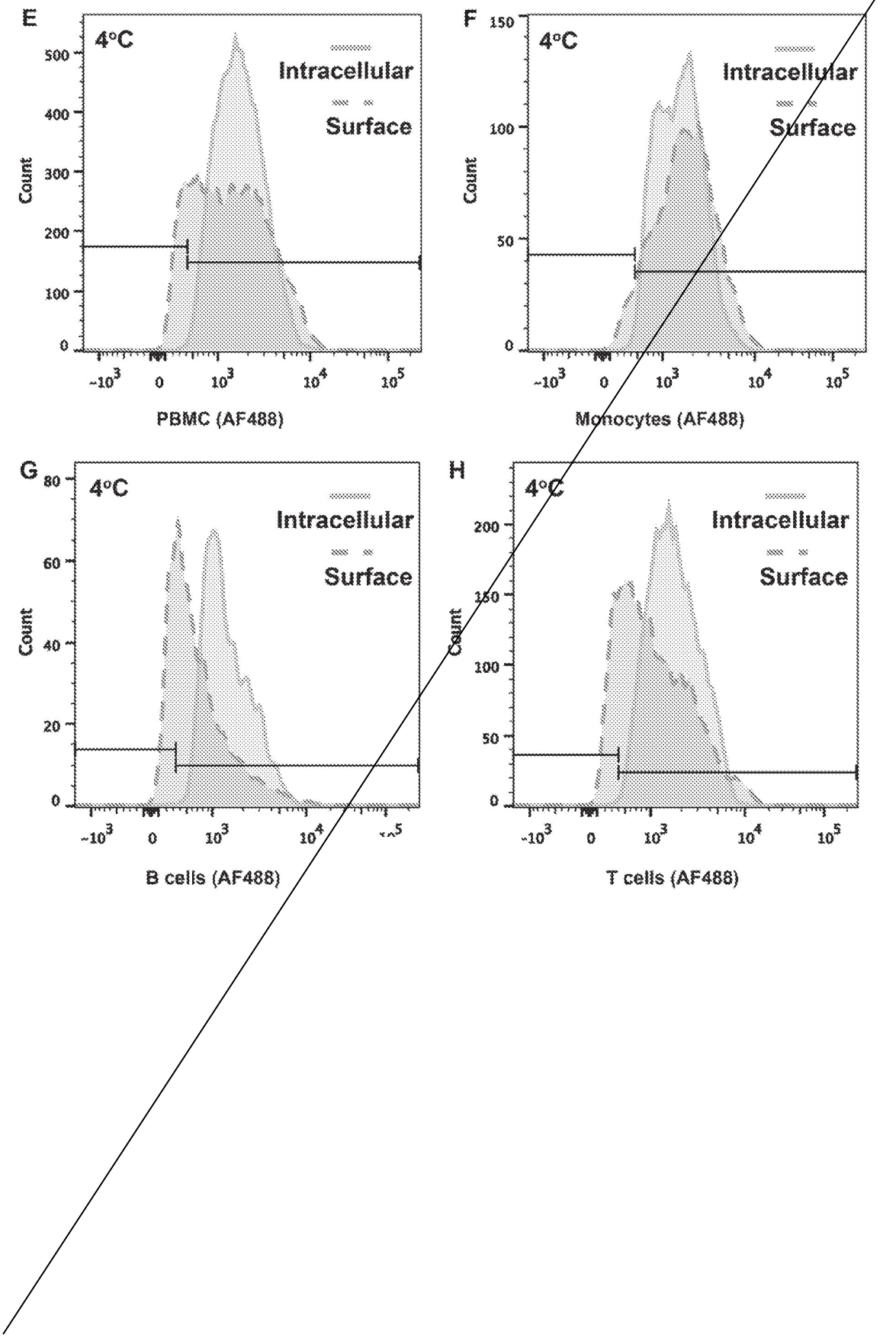


Figure 14

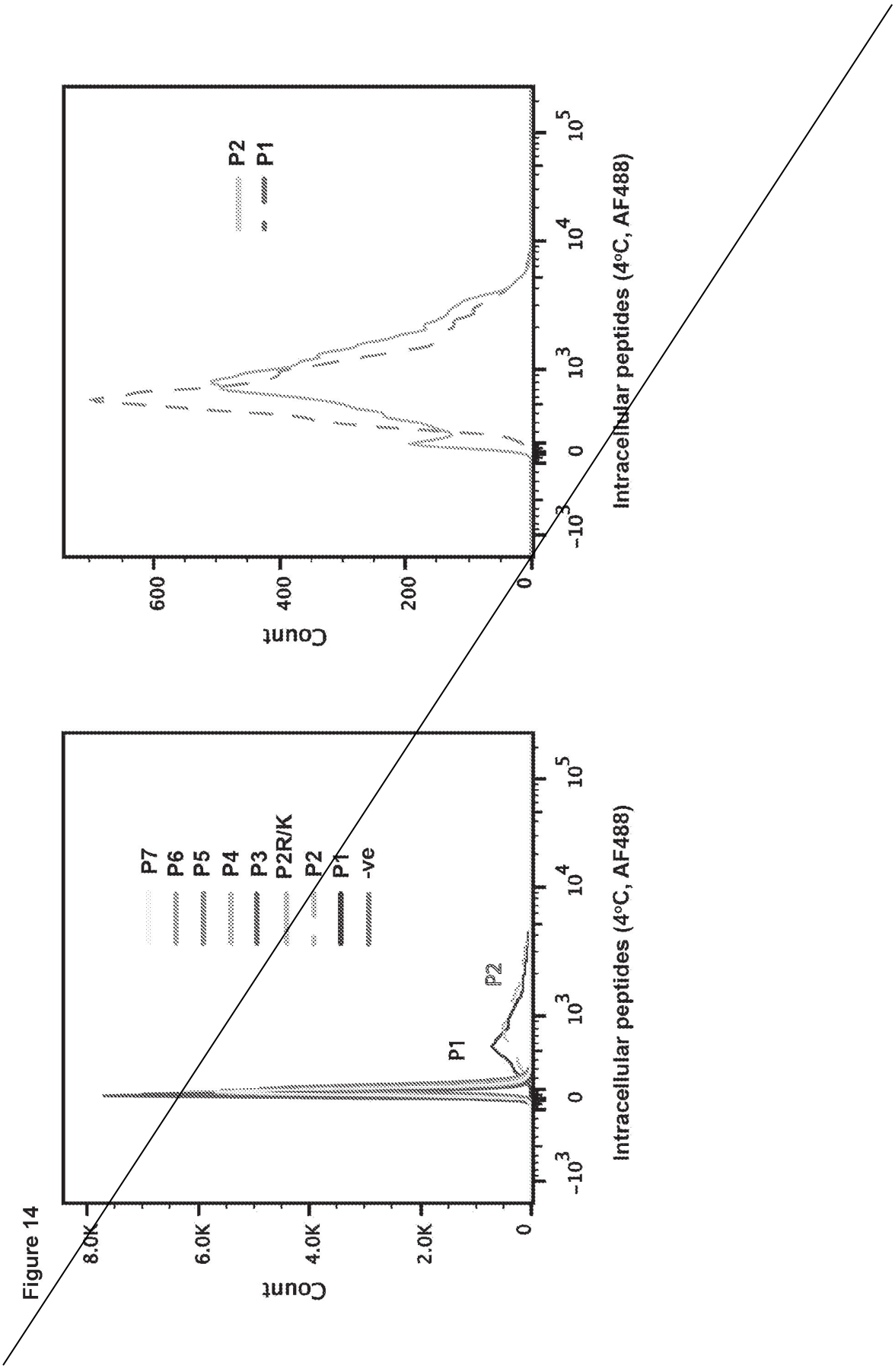

Count
8.0K
6.0K
4.0K
2.0K
0
P7
P6
P5
P4
P3
P2R/K
P2
P1
-ve
P1
P2
-10^3
0
10^3
10^4
10^5
Intracellular peptides (4°C, AF488)
Count
600
400
200
0
P2
P1
-10^3
0
10^3
10^4
10^5
Intracellular peptides (4°C, AF488)

Figure 15

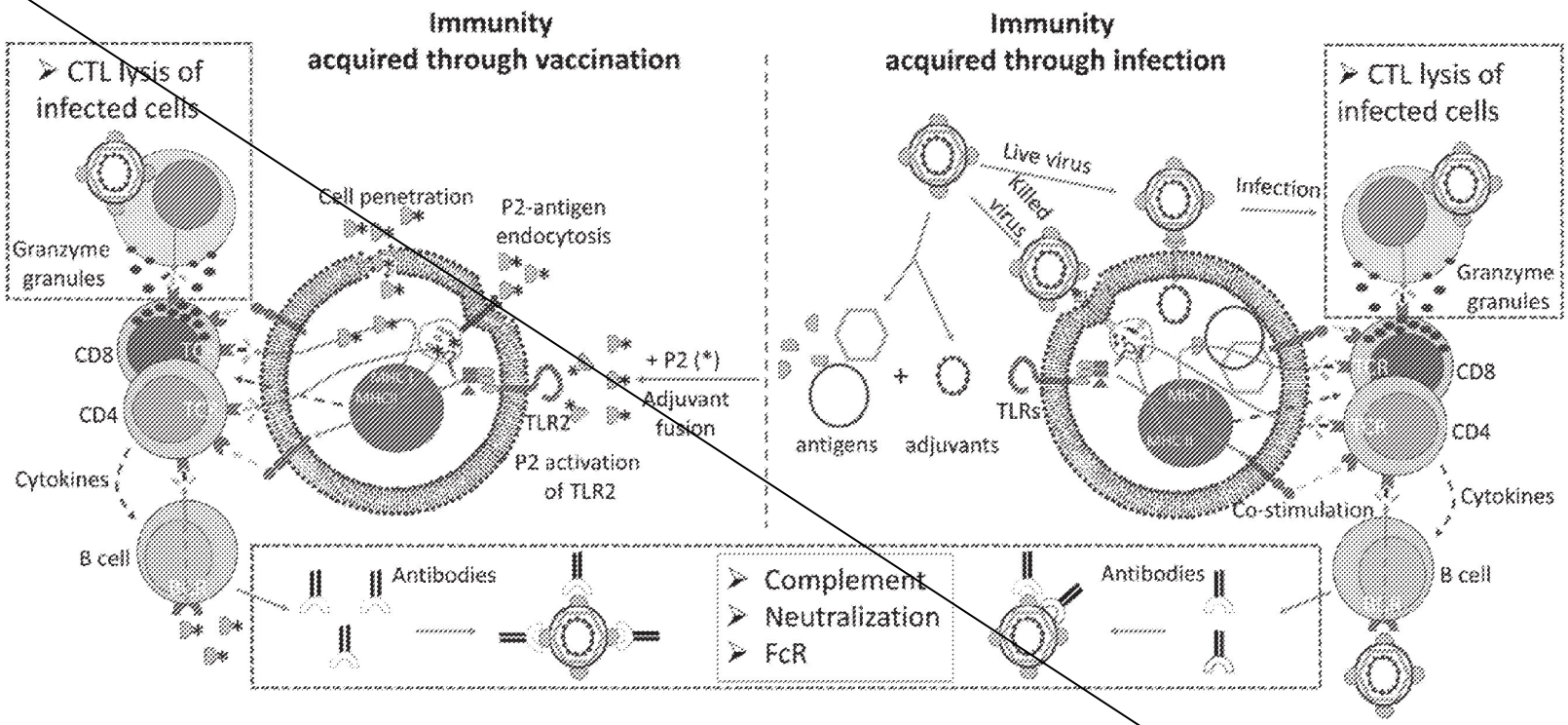

Immunity
acquired through vaccination
Immunity
acquired through infection
CTL lysis of infected cells
Granzyme granules
CD8
CD4
Cytokines
B cell
Cell penetration
P2-antigen endocytosis
+ P2 (*)
Adjuvant fusion
TLR2
P2 activation of TLR2
Antibodies
Complement
Neutralization
FcR
Live virus
Killed virus
Infection
antigens
adjuvants
TLRs
Co-stimulation
CTL lysis of infected cells
Granzyme granules
CD8
CD4
Cytokines
B cell
Antibodies

Figure 16

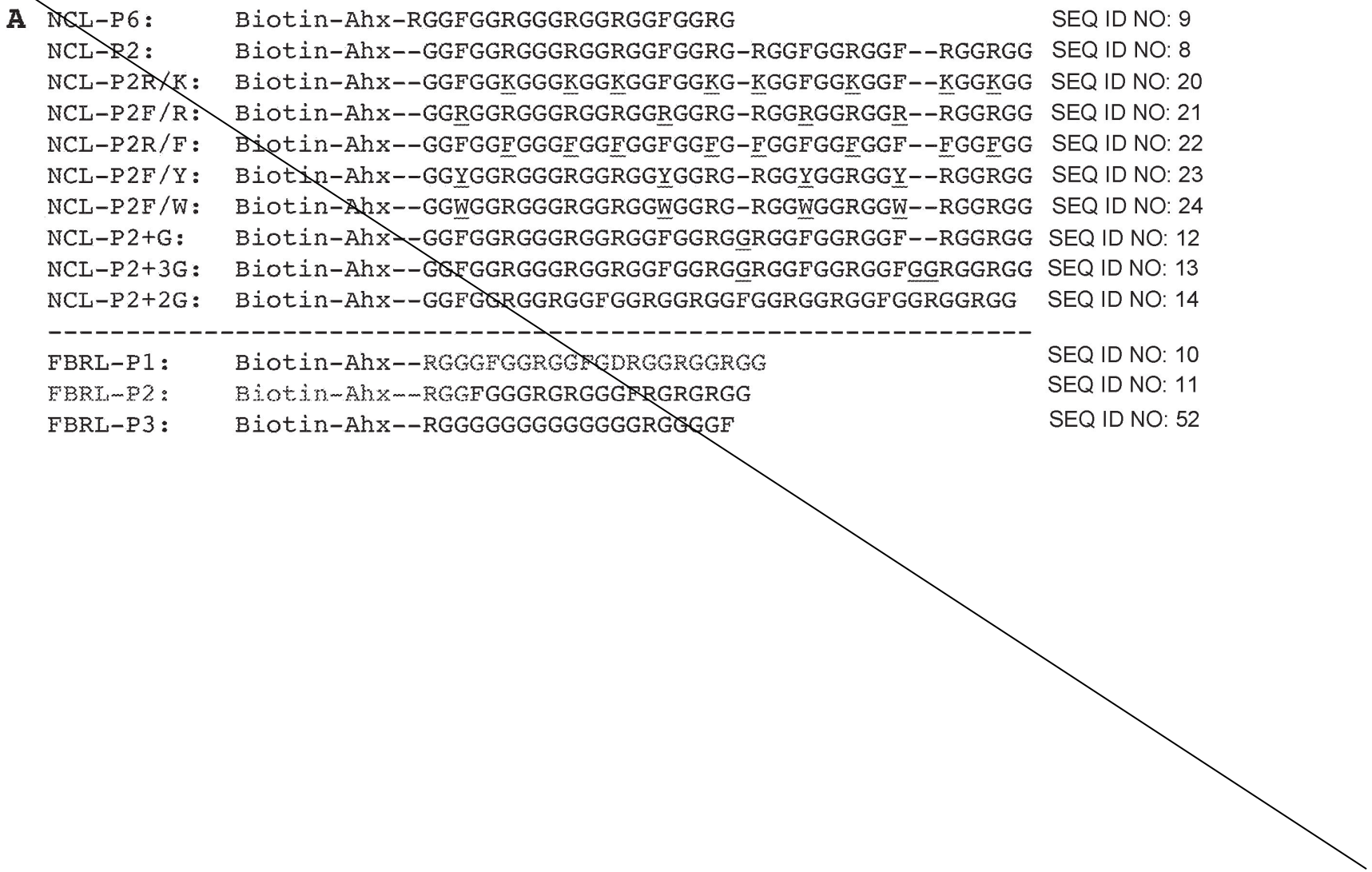
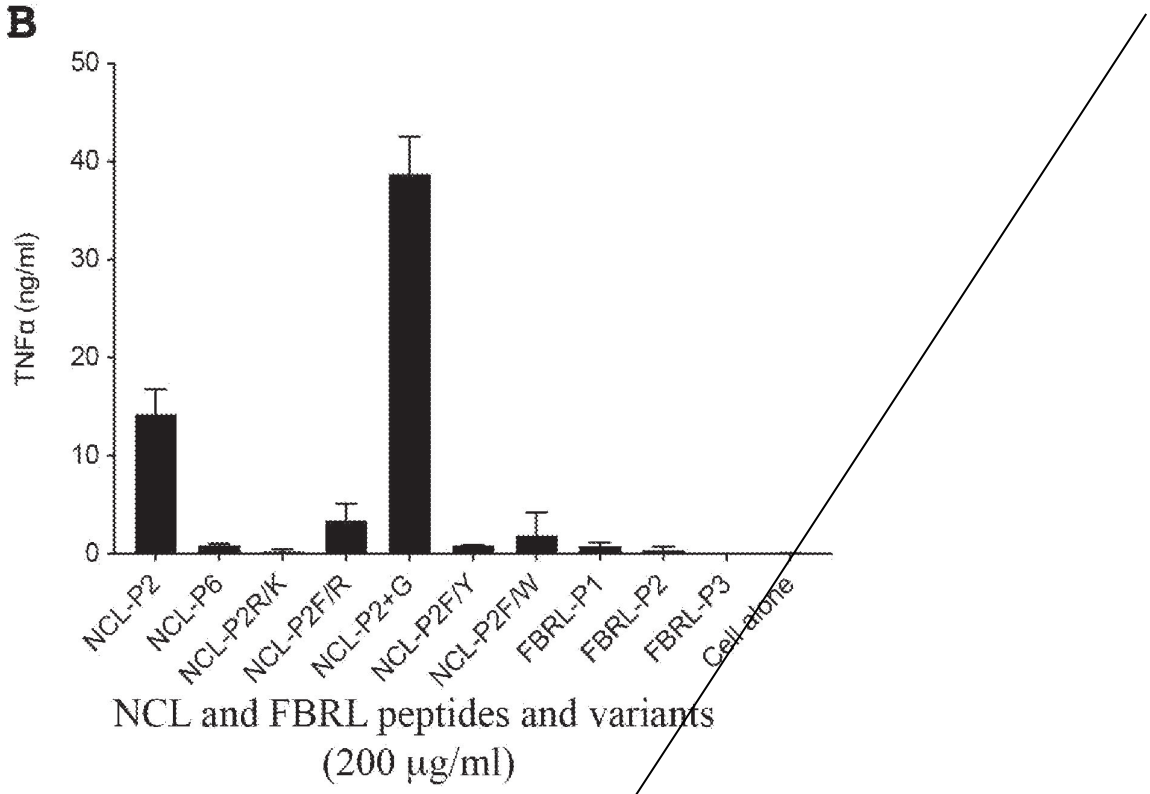

A
NCL-P6: Biotin-Ahx-RGGFGGRGGGRGGRGGFGGRG SEQ ID NO: 9
NCL-R2: Biotin-Ahx--GGFGGRGGGRGGRGGFGGRG-RGGFGGRGGF--RGGRGG SEQ ID NO: 8
NCL-P2R/K: Biotin-Ahx--GGFGGKGGGKGGKGGFGGKG-KGGFGGKGGF--KGGKGG SEQ ID NO: 20
NCL-P2F/R: Biotin-Ahx--GGRGGRGGGRGGRGGRGGRG-RGGRGGRGGR--RGGRGG SEQ ID NO: 21
NCL-P2R/F: Biotin-Ahx--GGFGGFGGGFGGFGGFGGFG-FGGFGGFGGF--FGGFGG SEQ ID NO: 22
NCL-P2F/Y: Biotin-Ahx--GGYGGRGGGRGGRGGYGGRG-RGGYGGRGGY--RGGRGG SEQ ID NO: 23
NCL-P2F/W: Biotin-Ahx--GGWGGRGGGRGGRGGWGGRG-RGGWGGRGGW--RGGRGG SEQ ID NO: 24
NCL-P2+G: Biotin-Ahx--GGFGGRGGGRGGRGGFGGRGGRGGFGGRGGF--RGGRGG SEQ ID NO: 12
NCL-P2+3G: Biotin-Ahx--GGFGGRGGGRGGRGGFGGRGGRGGFGGRGGFGGRGGRGG SEQ ID NO: 13
NCL-P2+2G: Biotin-Ahx--GGFGGRGGRGGFGGRGGRGGFGGRGGRGGFGGRGGRGG SEQ ID NO: 14
FBRL-P1: Biotin-Ahx--RGGGFGGRGGFGDRGGRGGRGG SEQ ID NO: 10
FBRL-P2: Biotin-Ahx--RGGFGGGRGRGGGFRGRGRGG SEQ ID NO: 11
FBRL-P3: Biotin-Ahx--RGGGGGGGGGGGGGRGGGGF SEQ ID NO: 52

Figure 16 (continued)



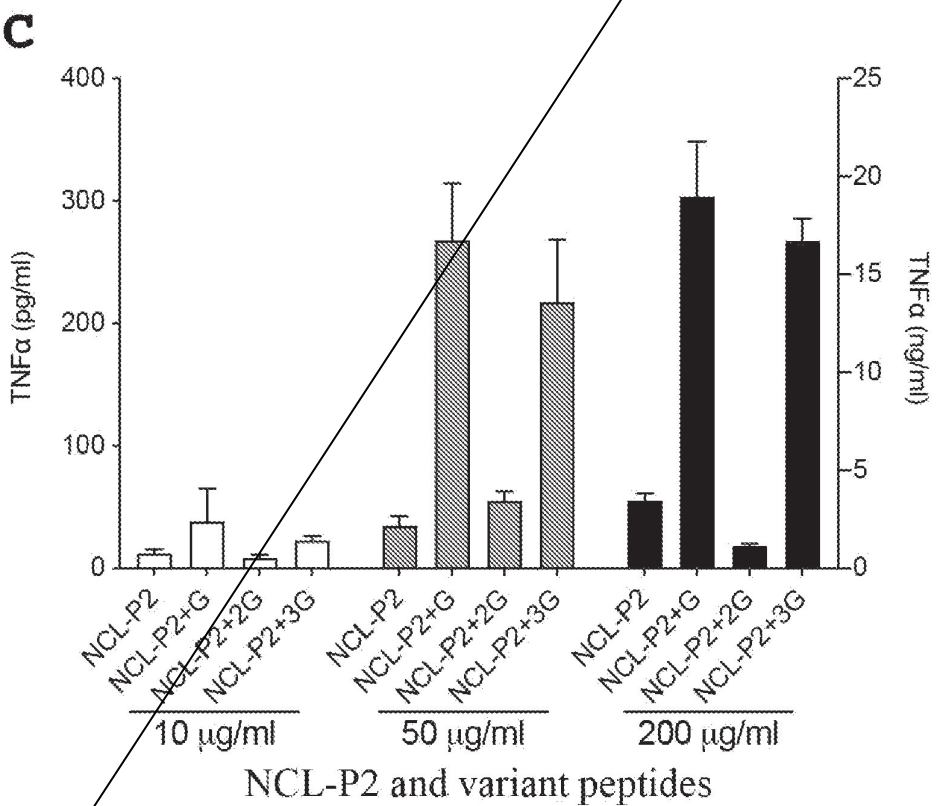


Figure 17

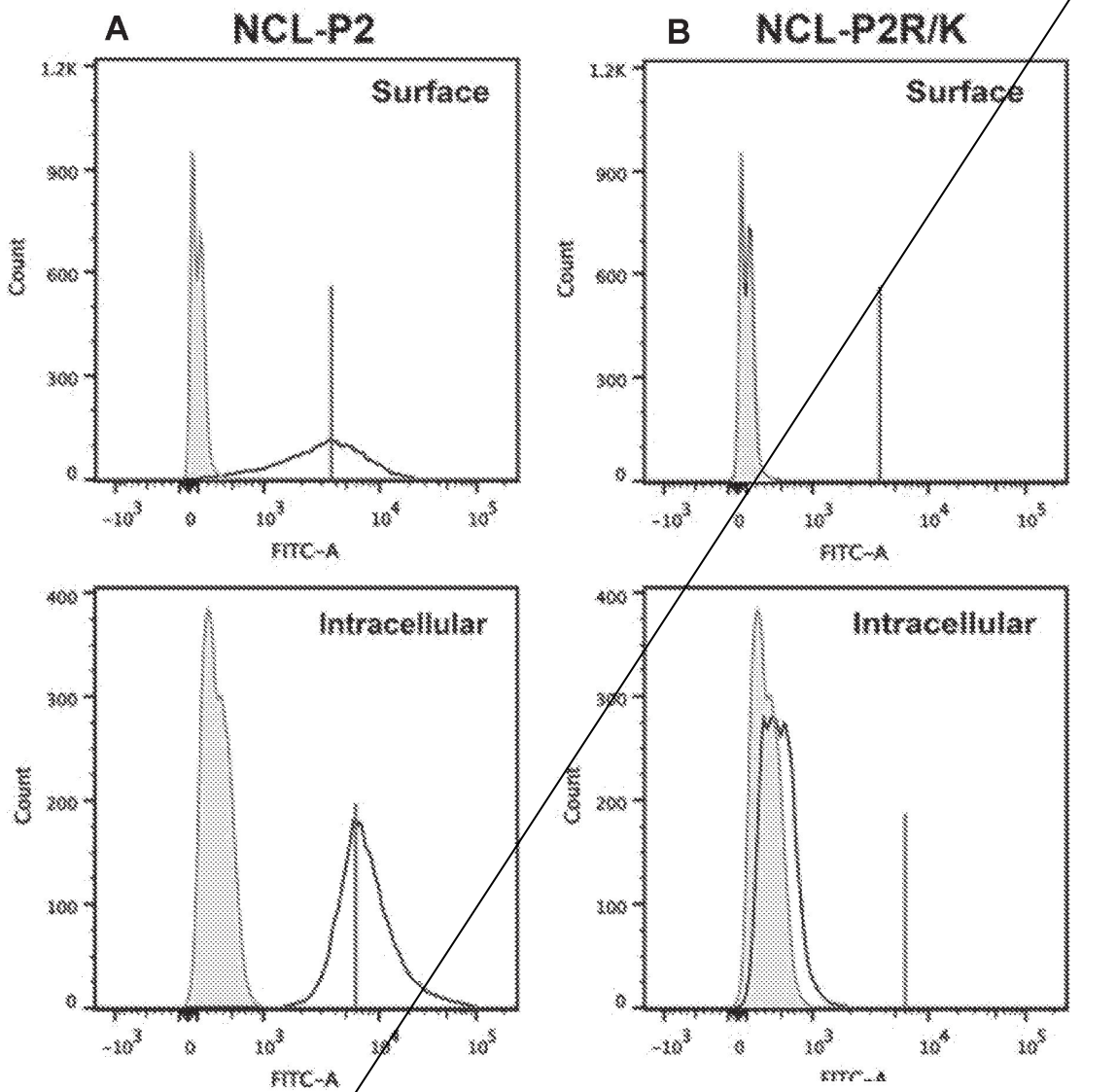

A
NCL-P2
Surface
Count
FITC-A
Intracellular
B
NCL-P2R/K
Surface
Intracellular

Figure 17 (continued)

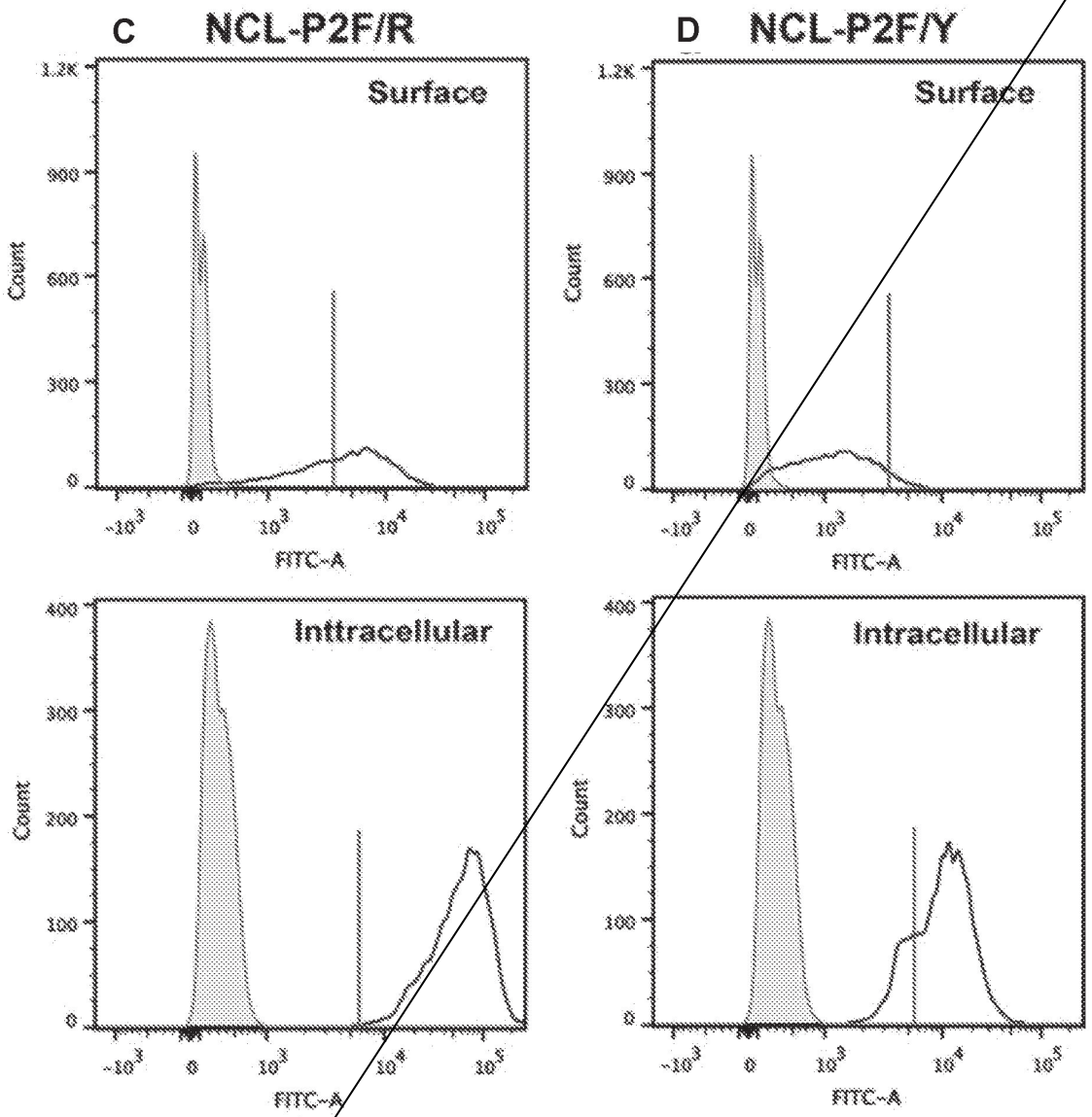


Figure 17 (continued)

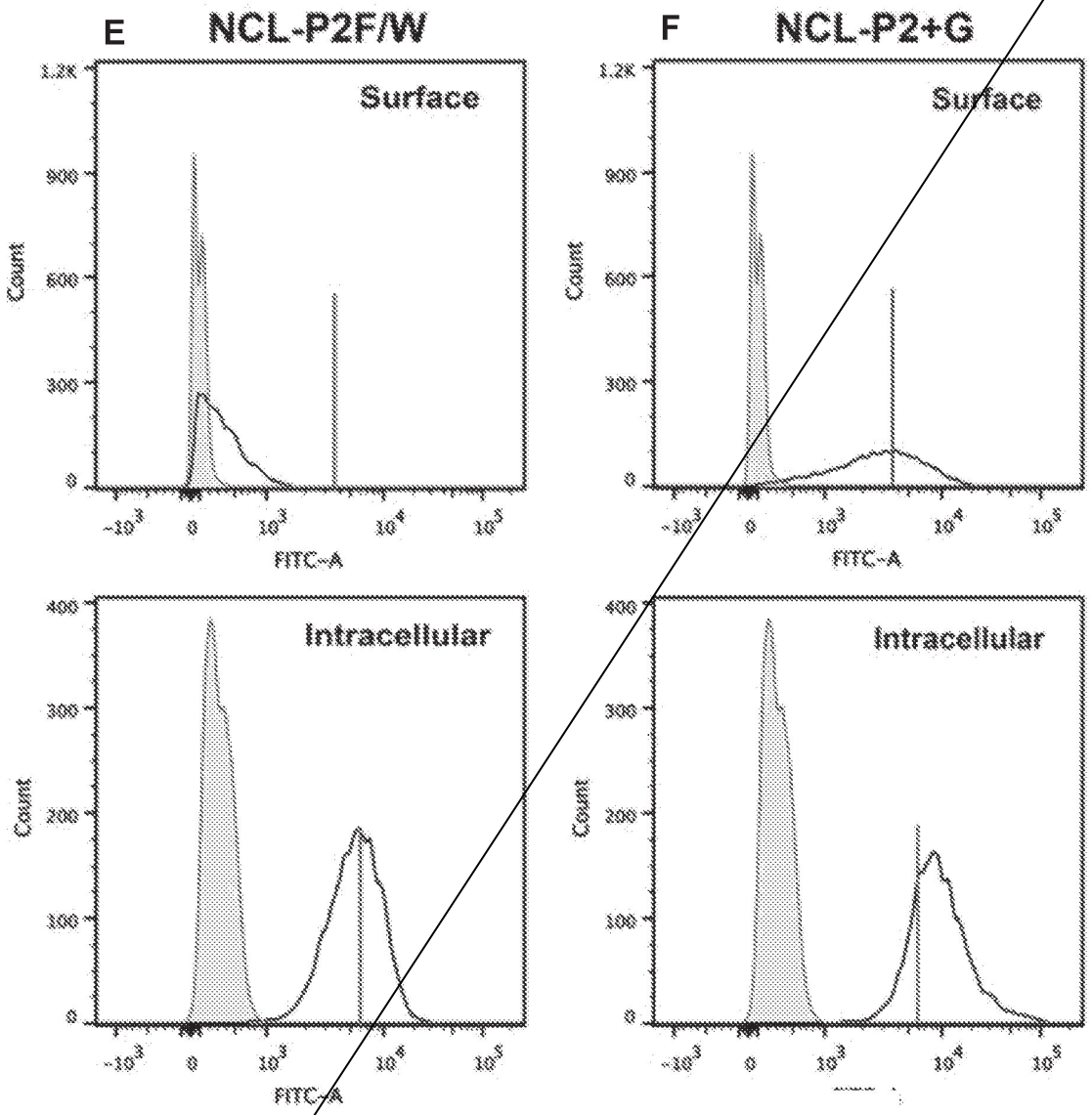
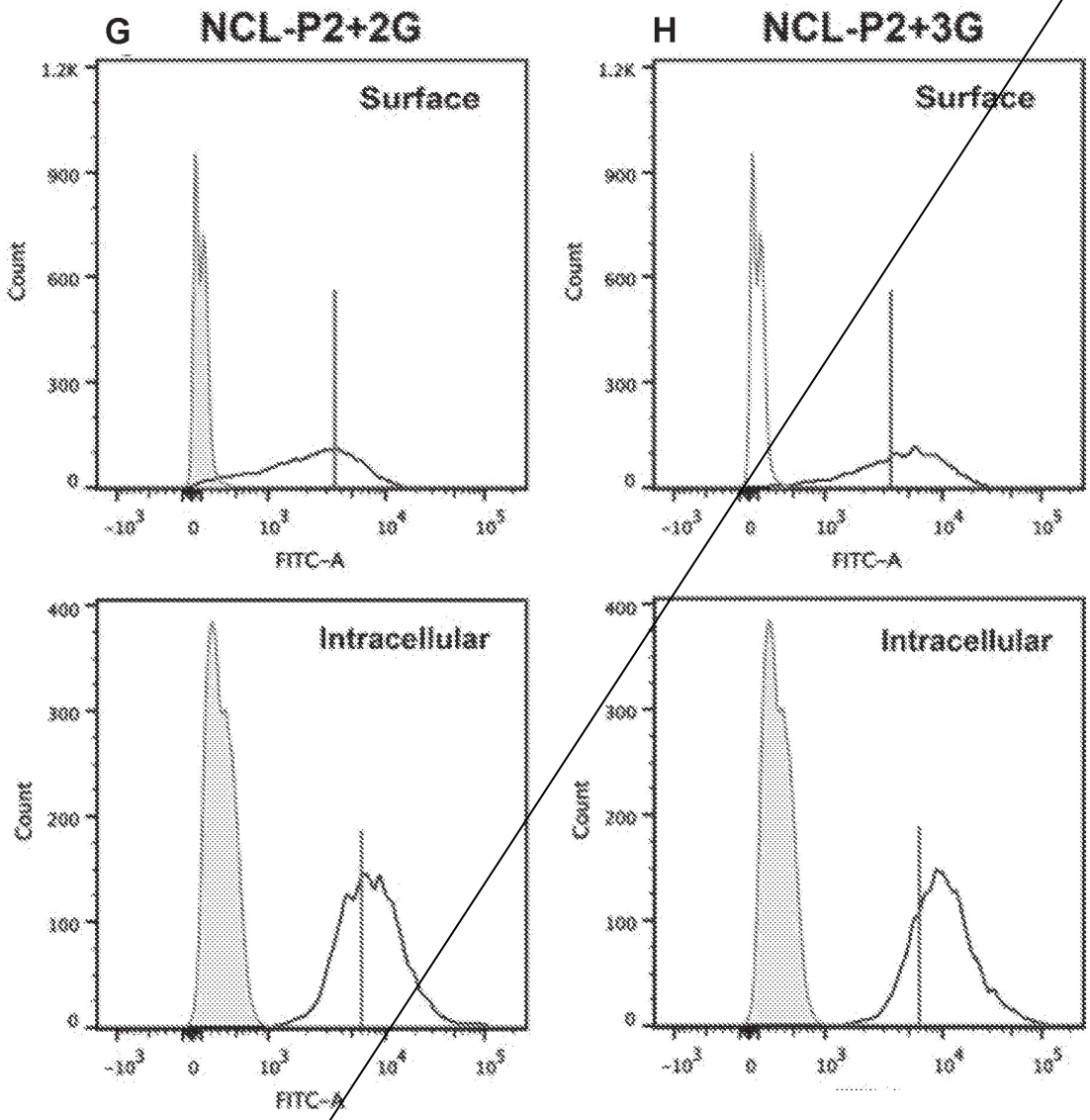


Figure 17 (continued)



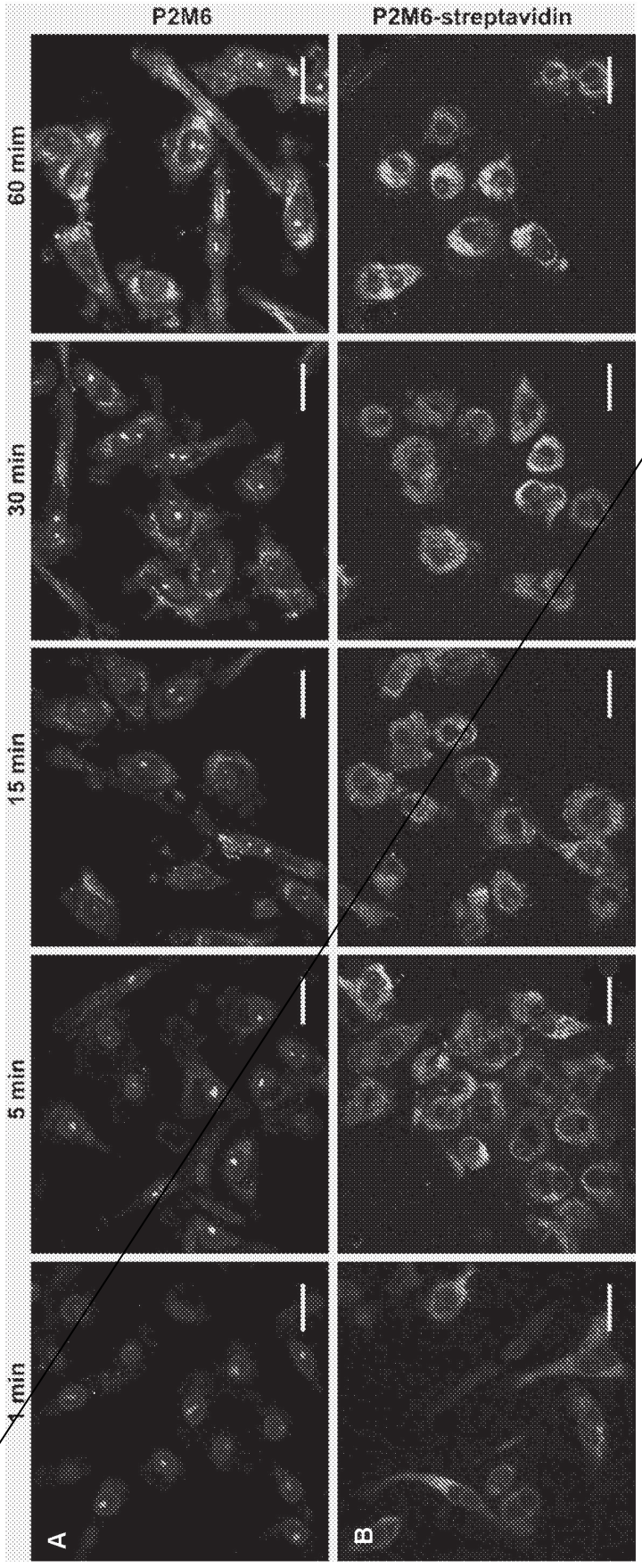


Figure 18

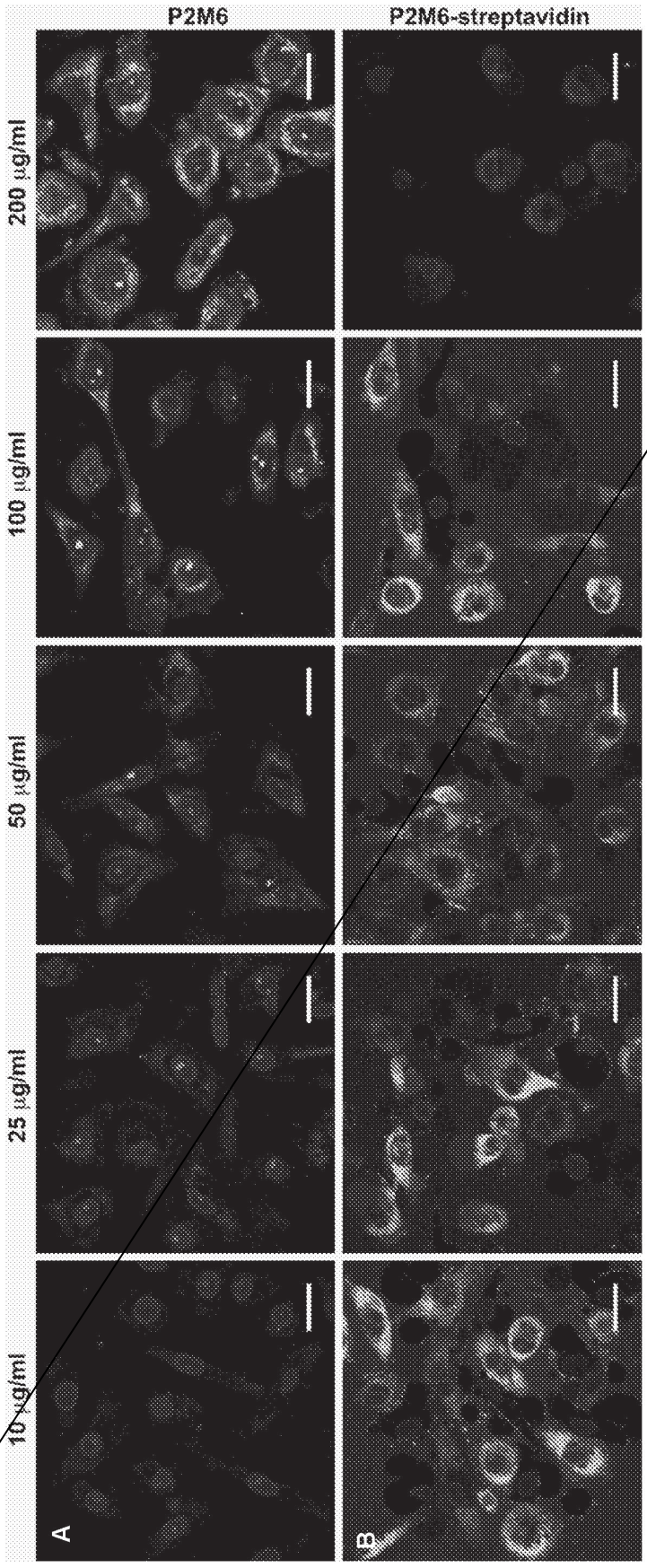

P2M6
P2M6-streptavidin
200 µg/ml
100 µg/ml
50 µg/ml
25 µg/ml
10 µg/ml
A
B

Figure 19

Figure 20

A

NCL-P2+G: Biotin-Ahx--GGFGGRGGGRGGRGGFGGRGGRGGFGGRGGFRGGRGG SEQ ID NO: 12
NCL-P2+G(F/I): Biotin-Ahx--GGIGGRGGGRGGRGGIGGRGGRGGIGGRGGIRGGRGG SEQ ID NO: 53
NCL-P2+G(F/L): Biotin-Ahx--GGLGGRGGGRGGRGGLGGRGGRGGLGGRGGLRGGRGG SEQ ID NO: 54
NCL-P2+G(G/A): Biotin-Ahx--GGFGARGGARGARGGFGARGARGGFGARGGFRGARGA SEQ ID NO: 55
NCL-P2+G(G/P): Biotin-Ahx--GGFGPRGGPRGPRGGFGPRGPRGGFGPRGGFRGPRGP SEQ ID NO: 56

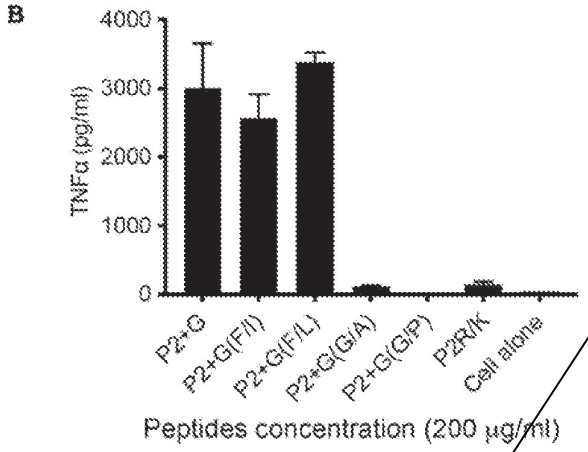
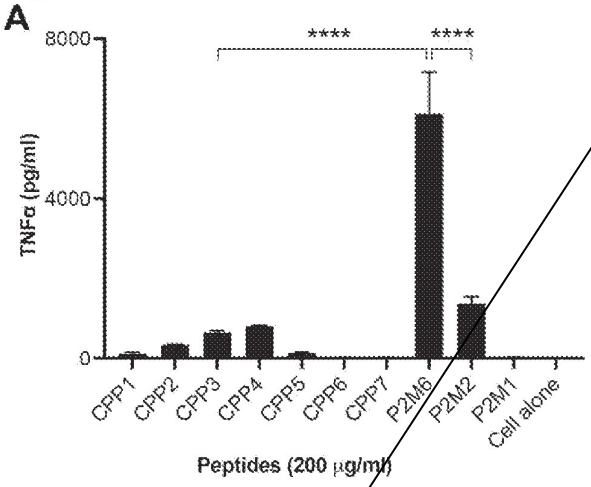


Figure 21



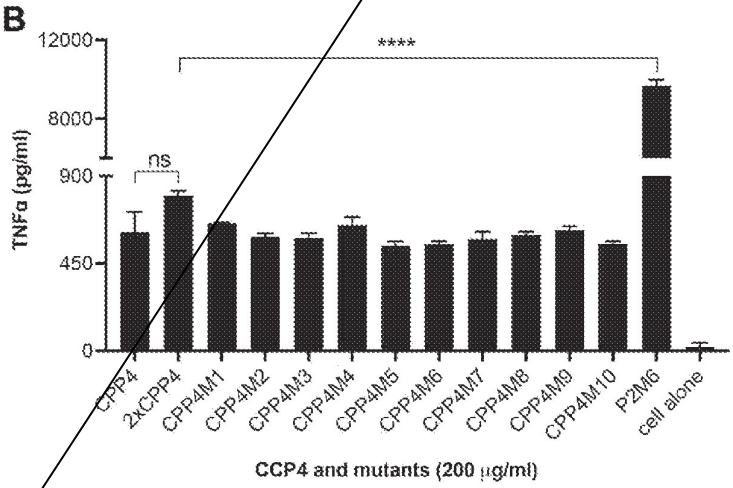


Figure 22

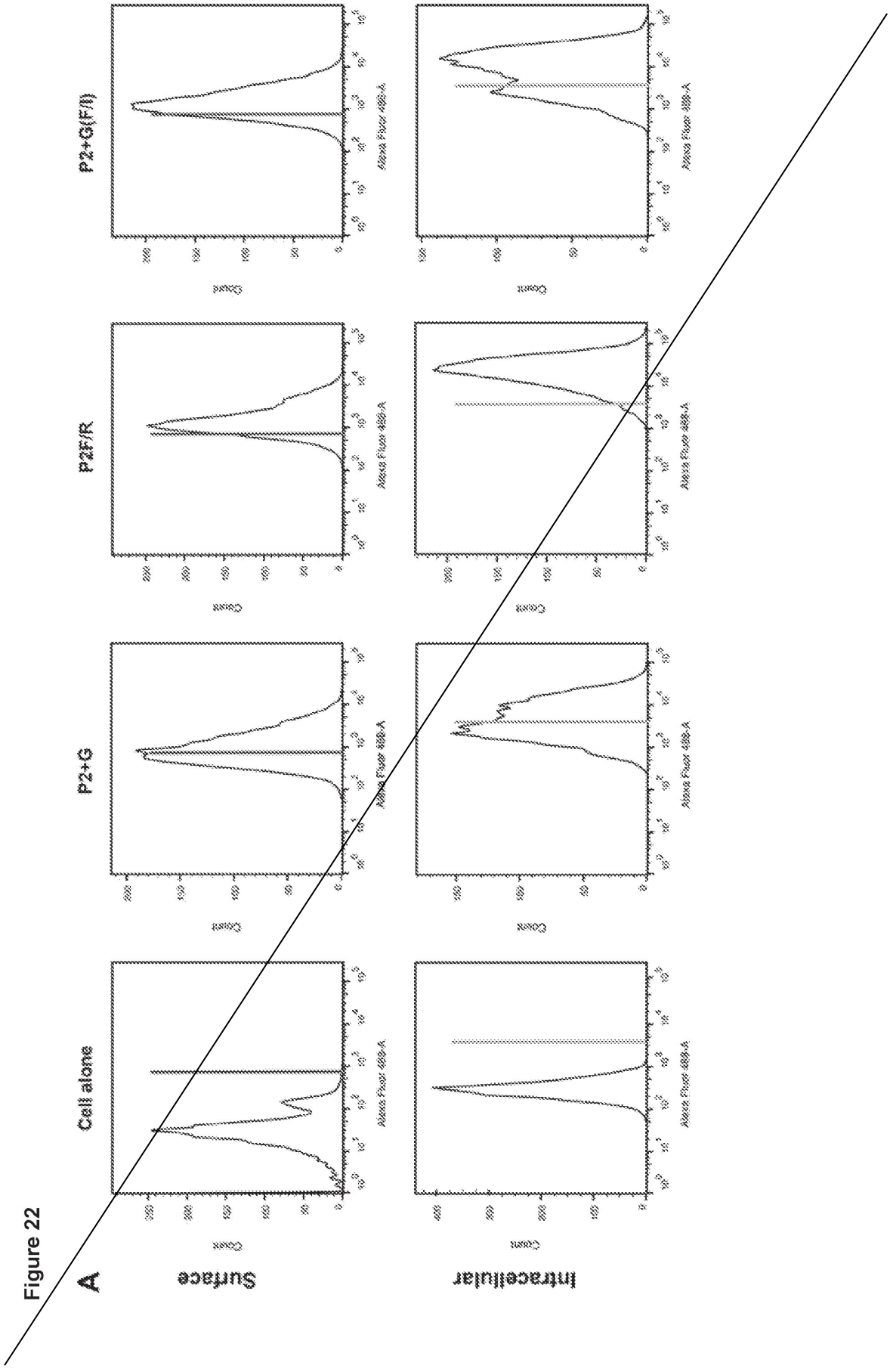

A
Surface
Intracellular
Cell alone
P2+G
P2F/R
P2+G(F/I)
Count
Alexa Fluor 488-A

Figure 22 (continued)

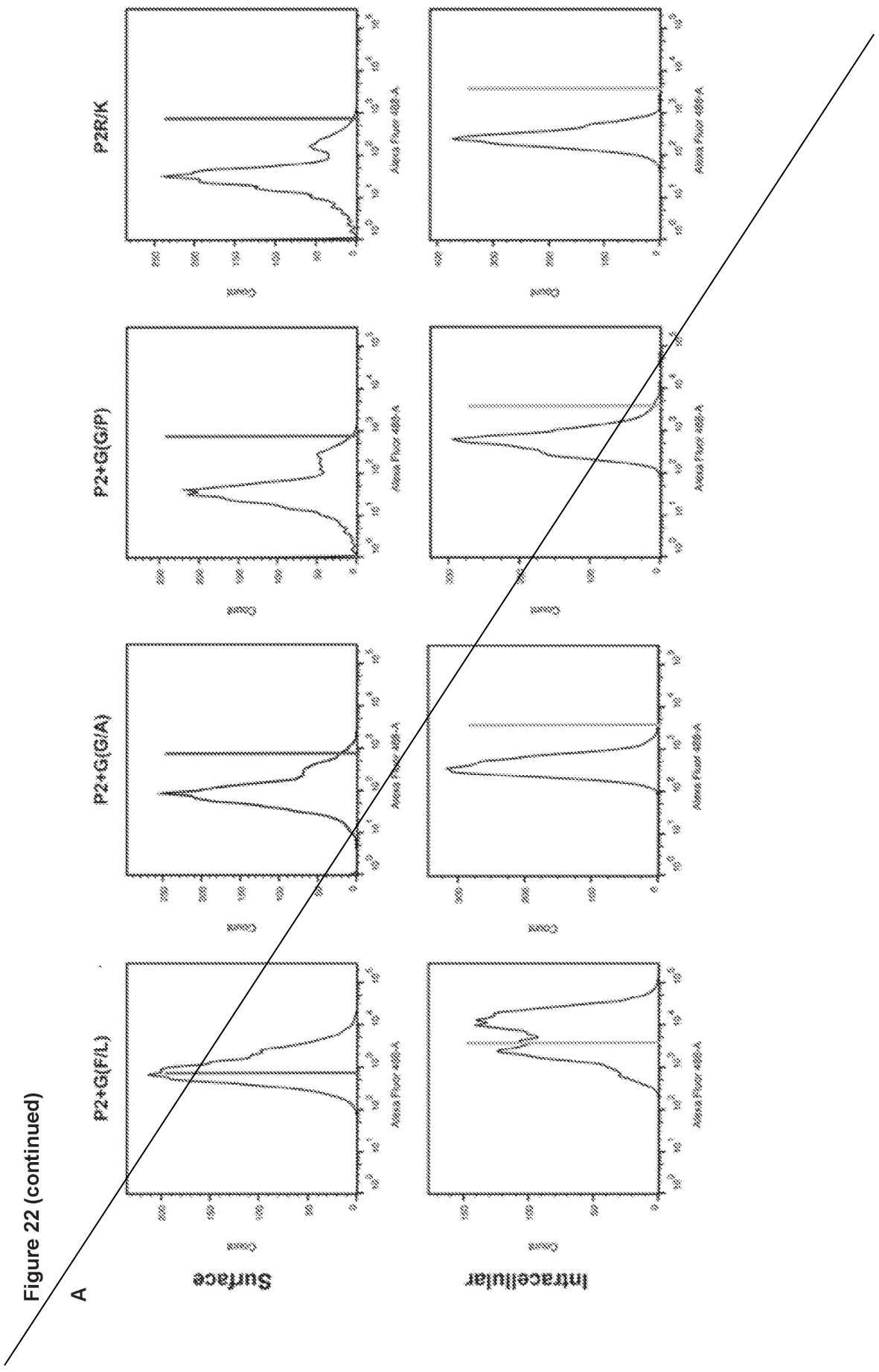


Figure 22 (continued)

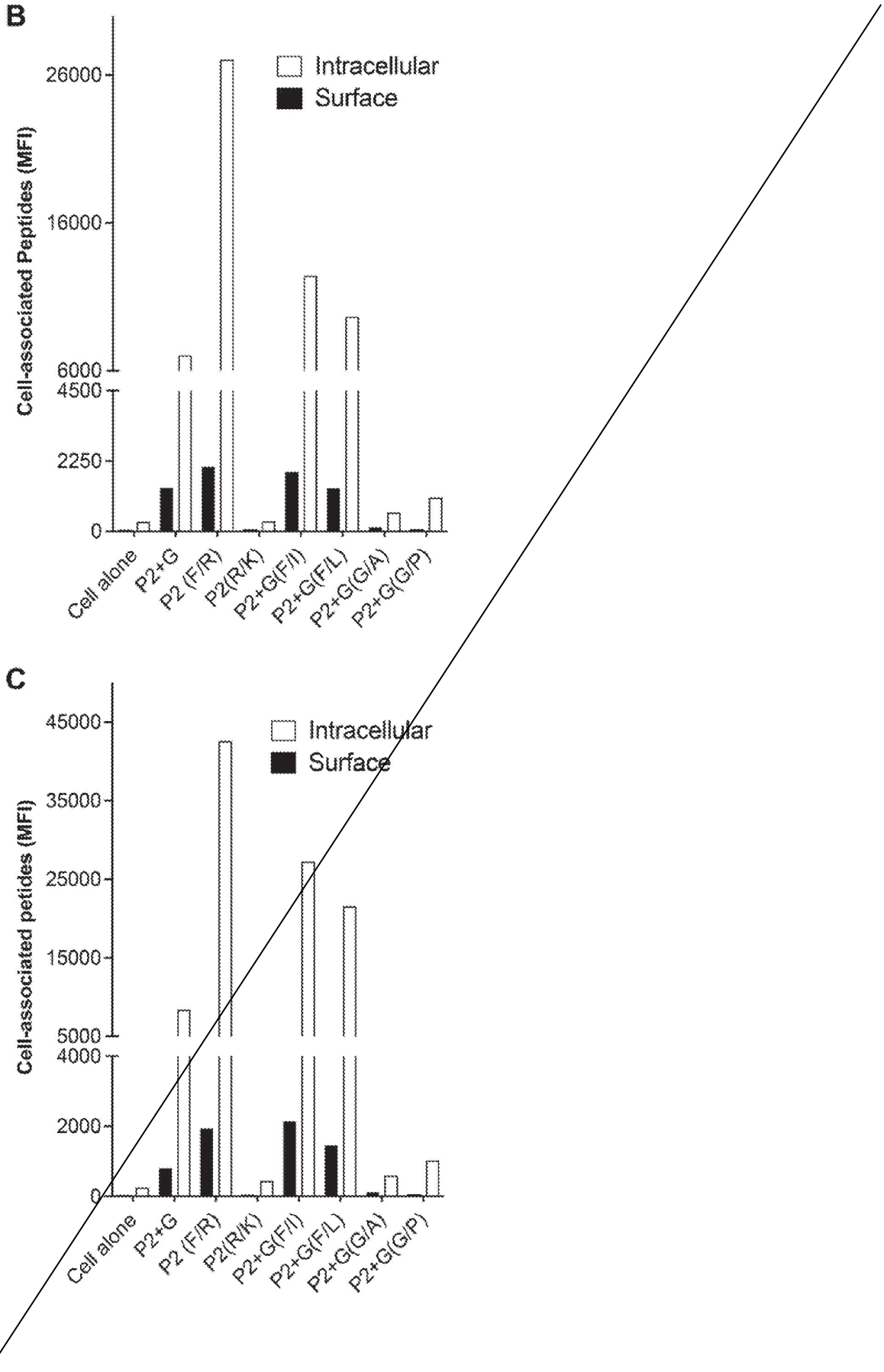


Figure 23

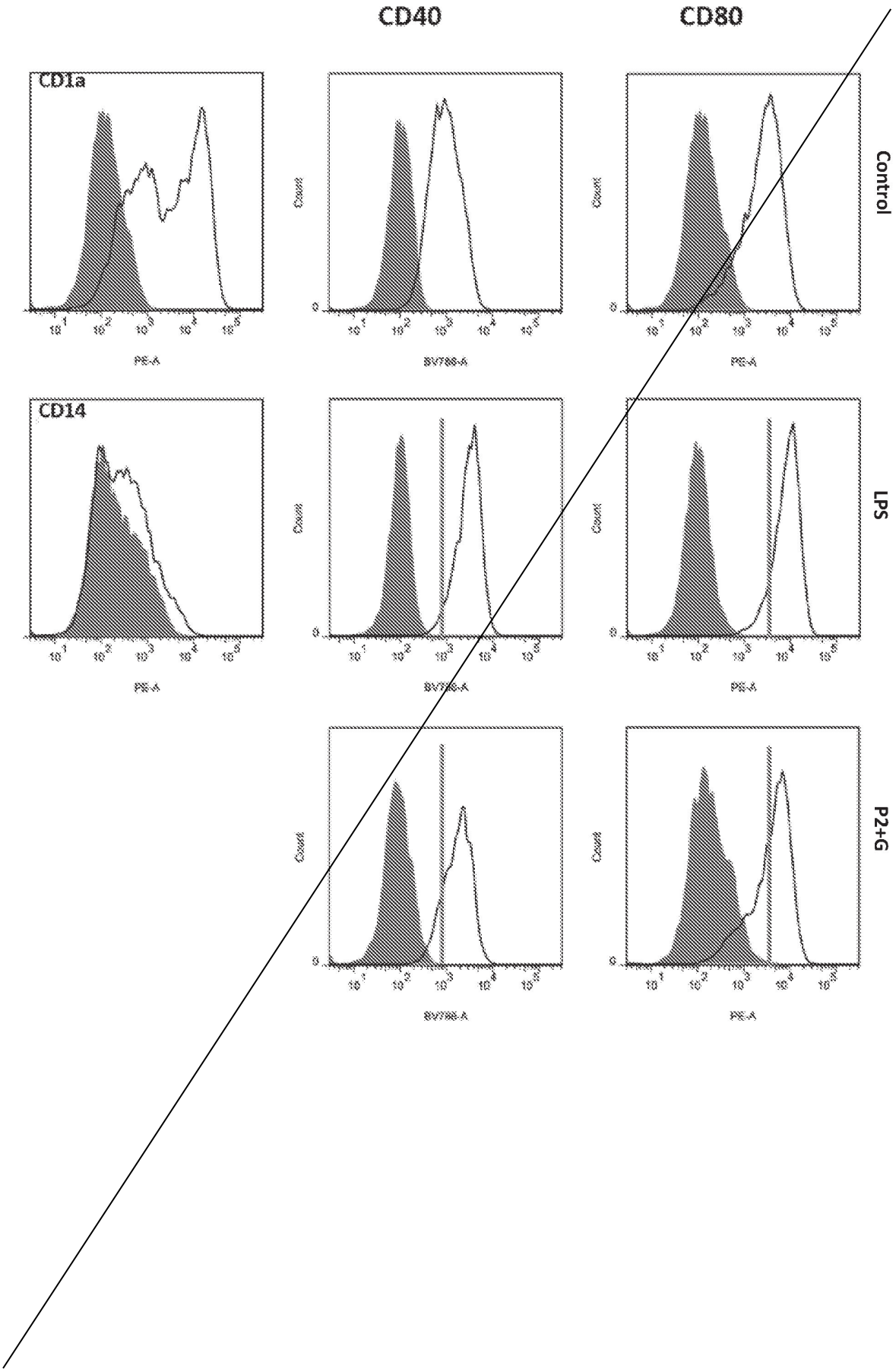


Figure 23 (continued)

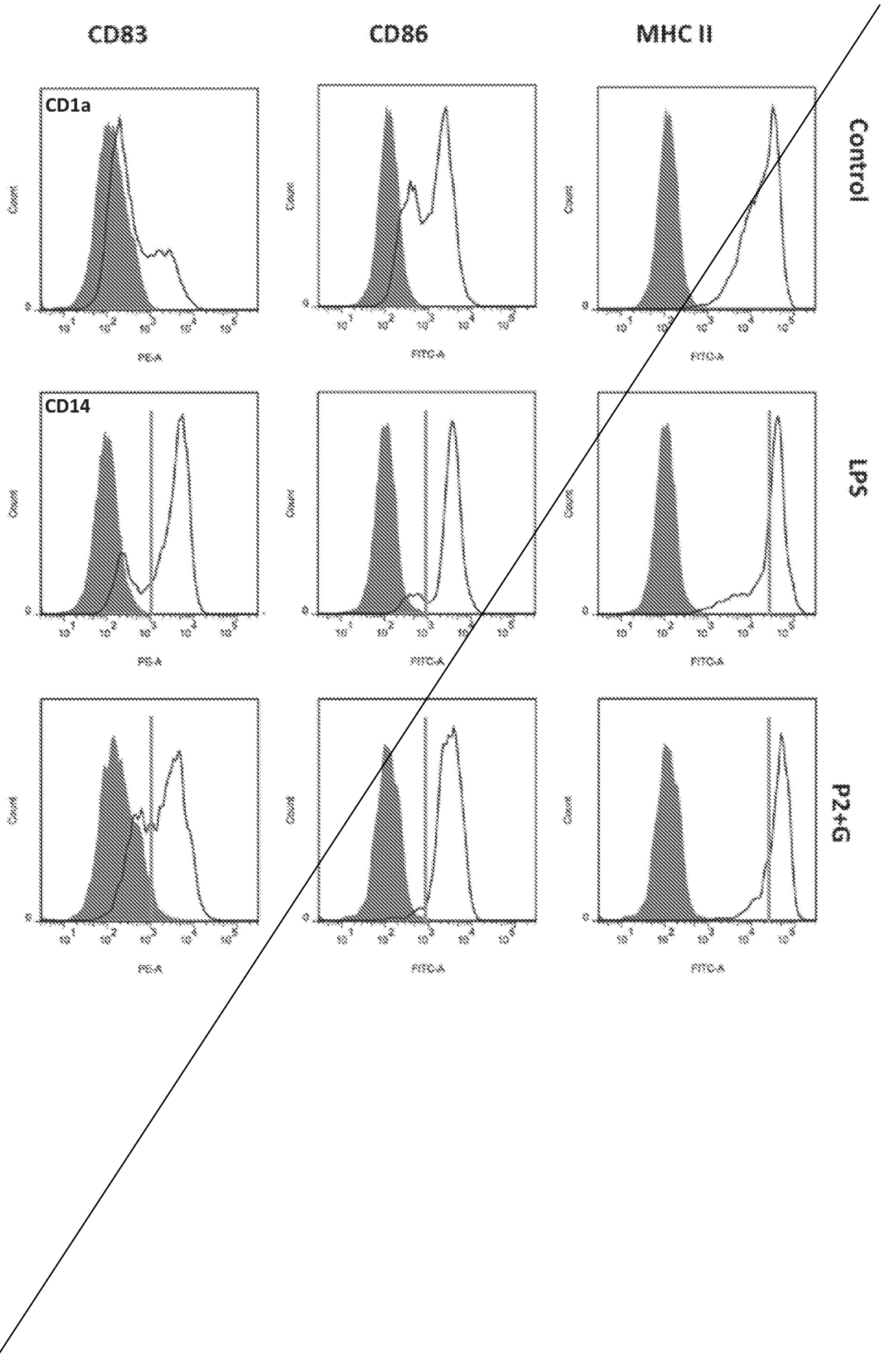
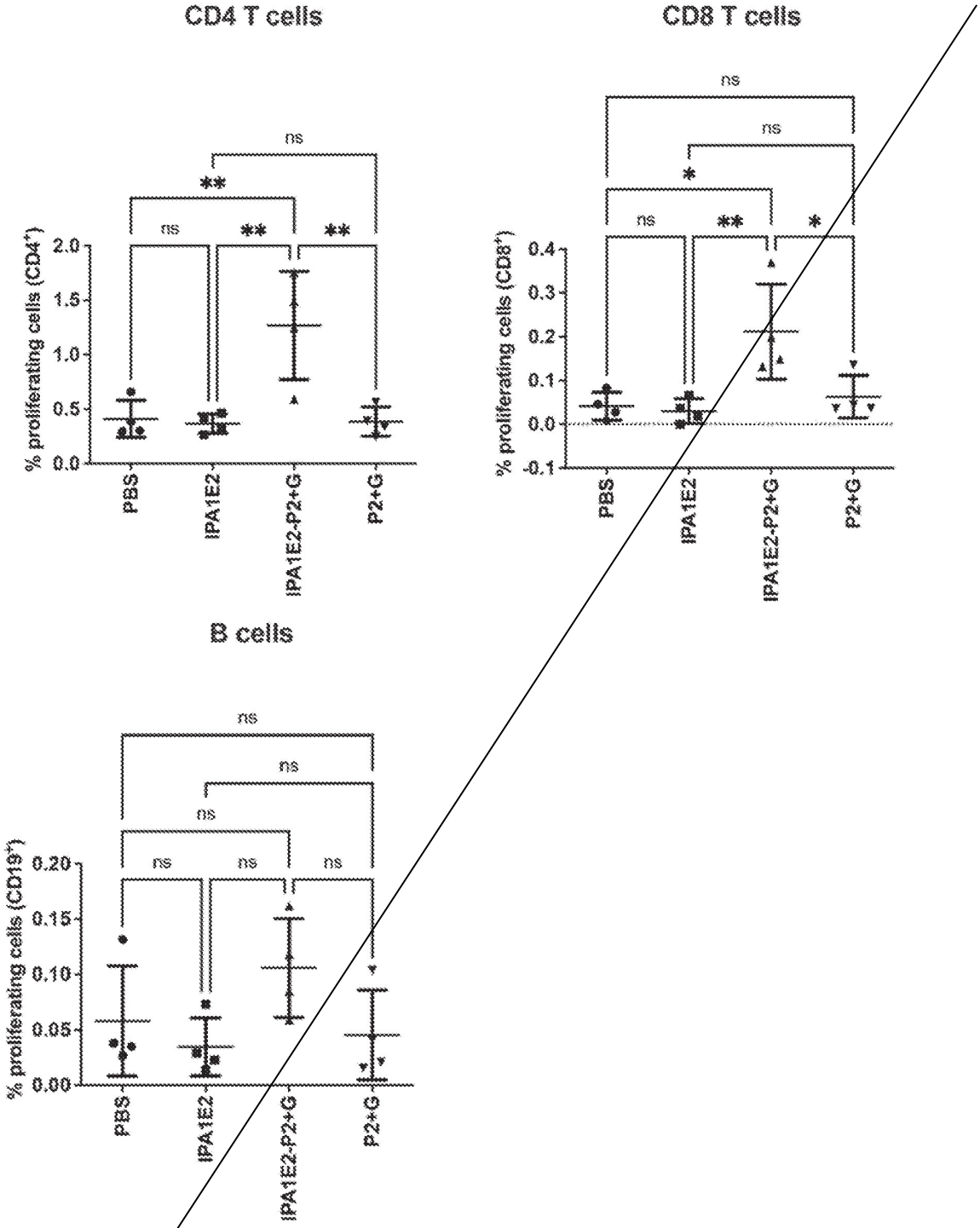


Figure 24



SEQ ID NO: 57	GQGQLVKRPKIMRFISHLWLALESANSEKR	IPA1E2
SEQ ID NO: 58	GQGQLVKRPKIMRFISHLWLALESANSEKR-P2+G	IPA1E2-P2+G
SEQ ID NO: 12	P2+G	P2+G

Figure 25

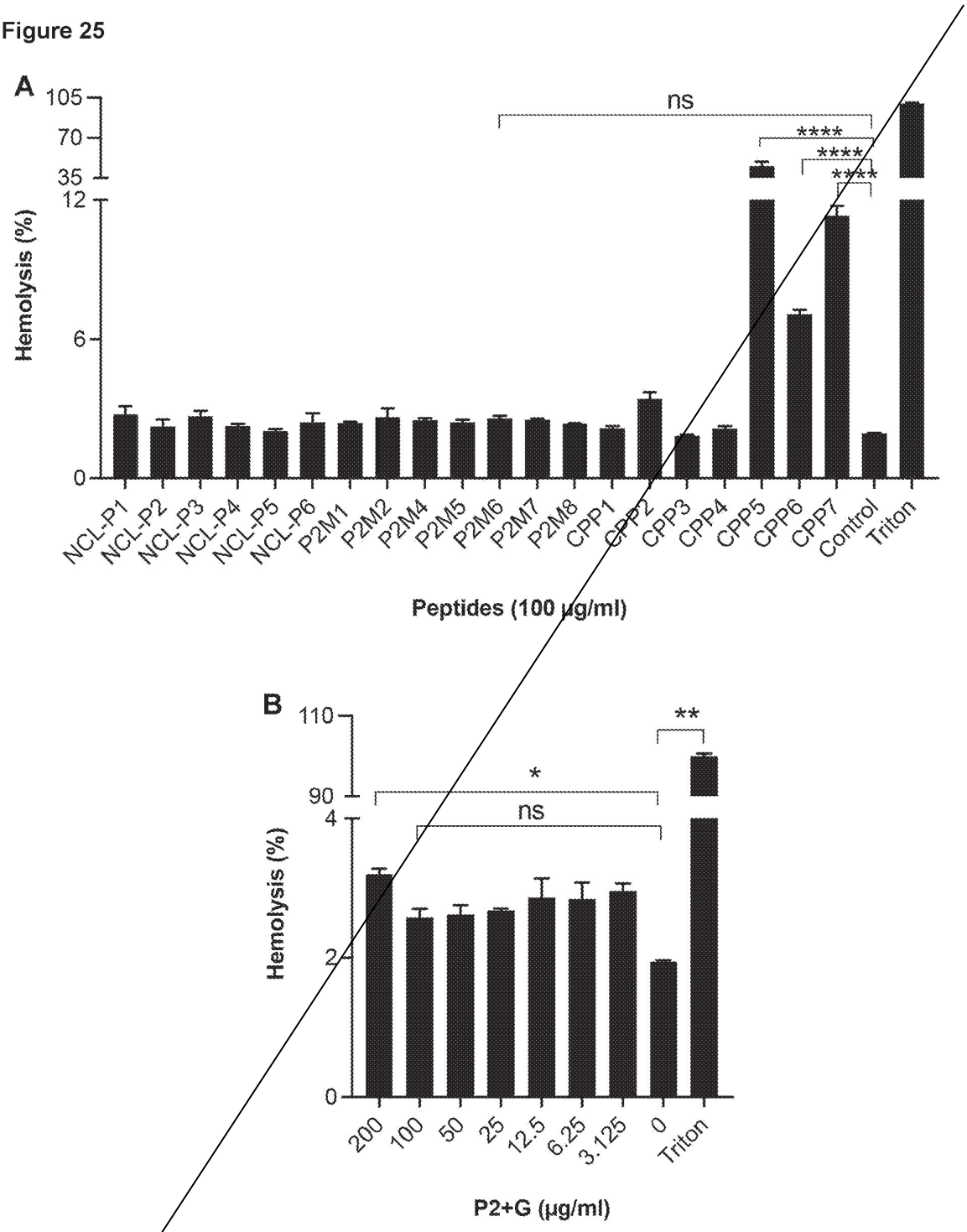

A
105
70
35
12
6
0
Hemolysis (%)
ns
****
****
****
NCL-P1
NCL-P2
NCL-P3
NCL-P4
NCL-P5
NCL-P6
P2M1
P2M2
P2M4
P2M5
P2M6
P2M7
P2M8
CPP1
CPP2
CPP3
CPP4
CPP5
CPP6
CPP7
Control
Triton
Peptides (100 µg/ml)
B
110
90
4
2
0
Hemolysis (%)
**
*
ns
200
100
50
25
12.5
6.25
3.125
0
Triton
P2+G (µg/ml)

Figure 26

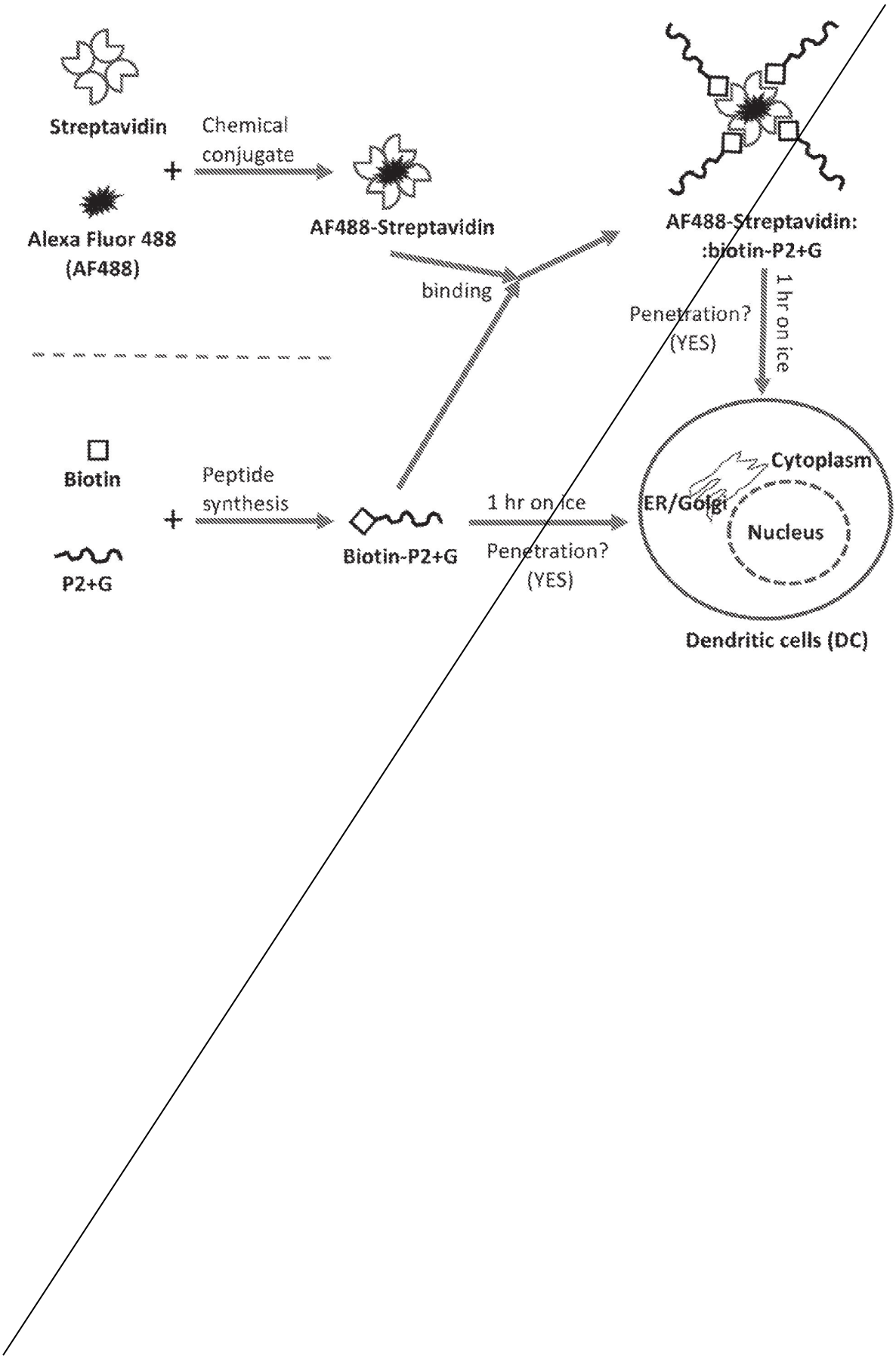

Streptavidin
Chemical conjugate
Alexa Fluor 488 (AF488)
AF488-Streptavidin
AF488-Streptavidin: :biotin-P2+G
binding
Penetration? (YES)
1 hr on ice
Biotin
Peptide synthesis
P2+G
Biotin-P2+G
1 hr on ice
Penetration? (YES)
Cytoplasm
ER/Golgi
Nucleus
Dendritic cells (DC)

Figure 1

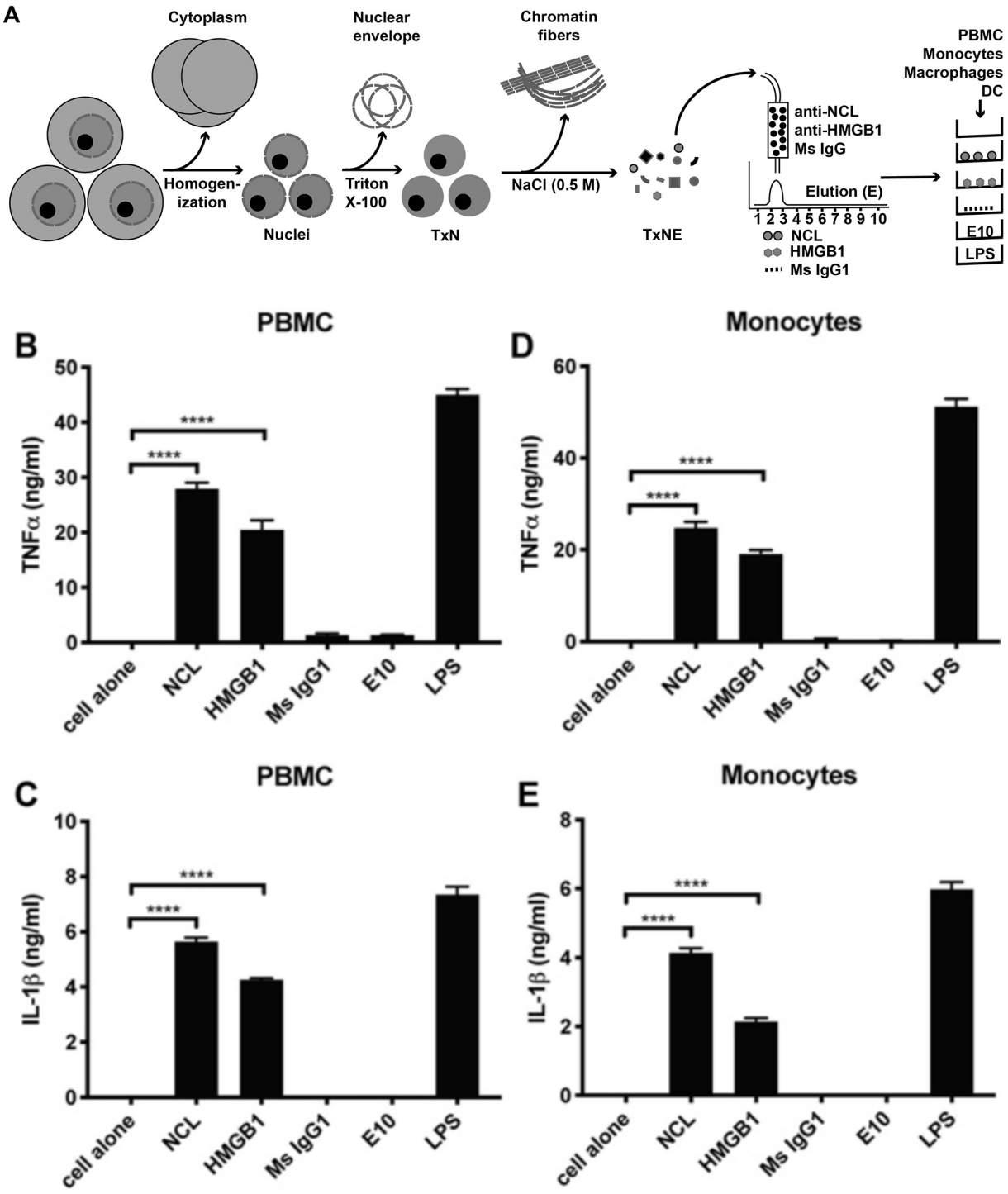

A
Cytoplasm
Nuclear envelope
Chromatin fibers
Homogen-ization
Nuclei
Triton X-100
TxN
NaCl (0.5 M)
TxNE
anti-NCL
anti-HMGB1
Ms IgG
Elution (E)
1 2 3 4 5 6 7 8 9 10
NCL
HMGB1
Ms IgG1
PBMC
Monocytes
Macrophages
DC
E10
LPS
B
PBMC
TNFα (ng/ml)
D
Monocytes
TNFα (ng/ml)
C
PBMC
IL-1β (ng/ml)
E
Monocytes
IL-1β (ng/ml)
****
cell alone
NCL
HMGB1
Ms IgG1
E10
LPS

Figure 1 (continued)

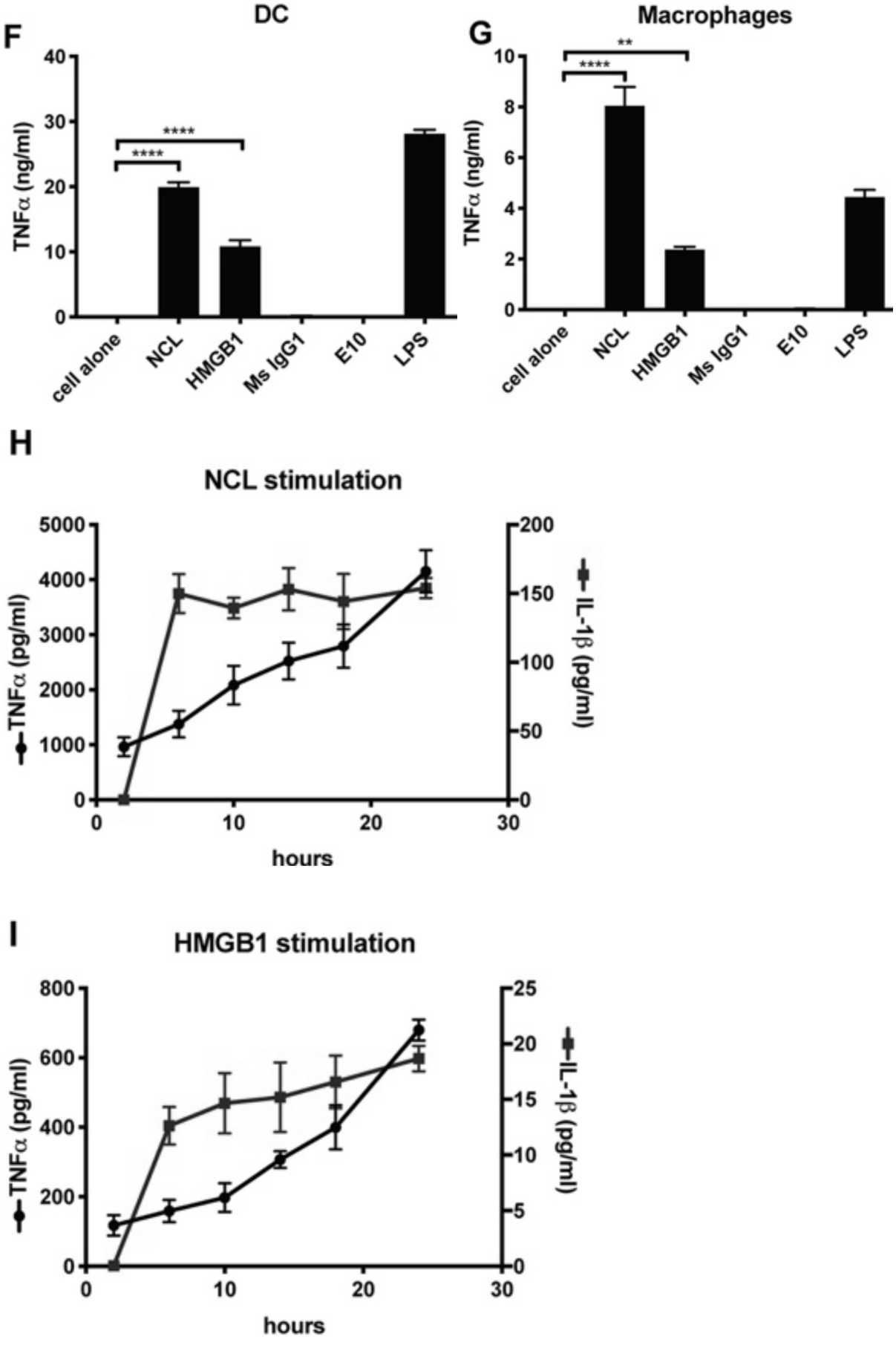


Figure 1 (continued)

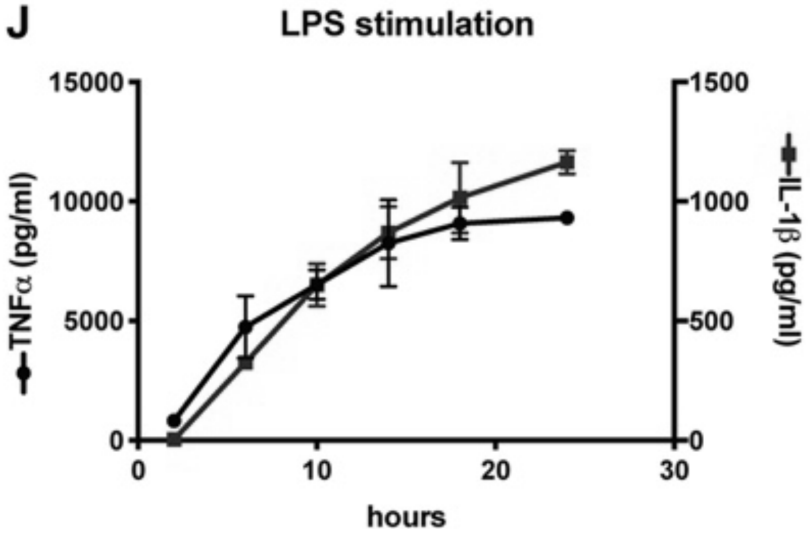


Figure 2

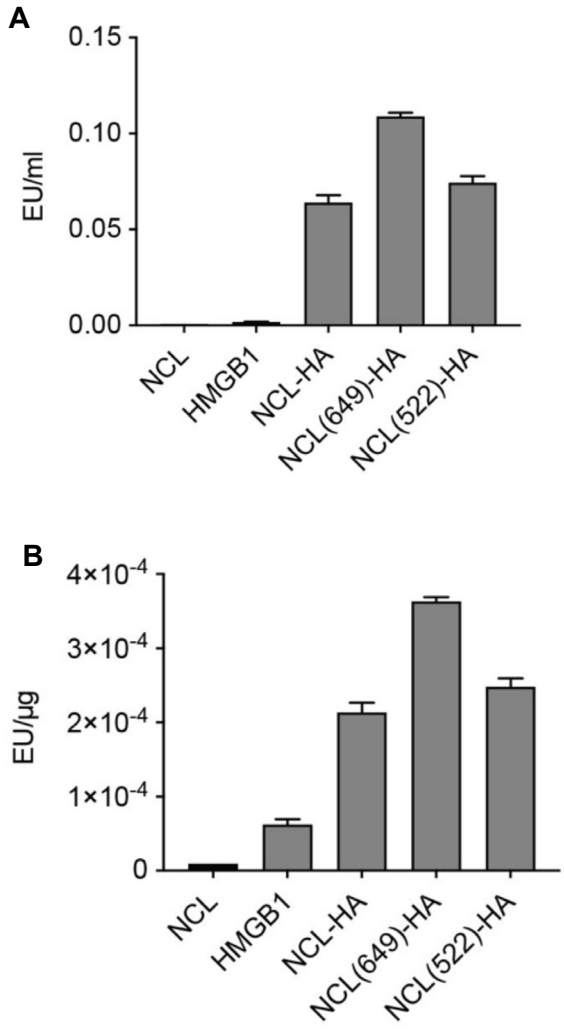


Figure 3

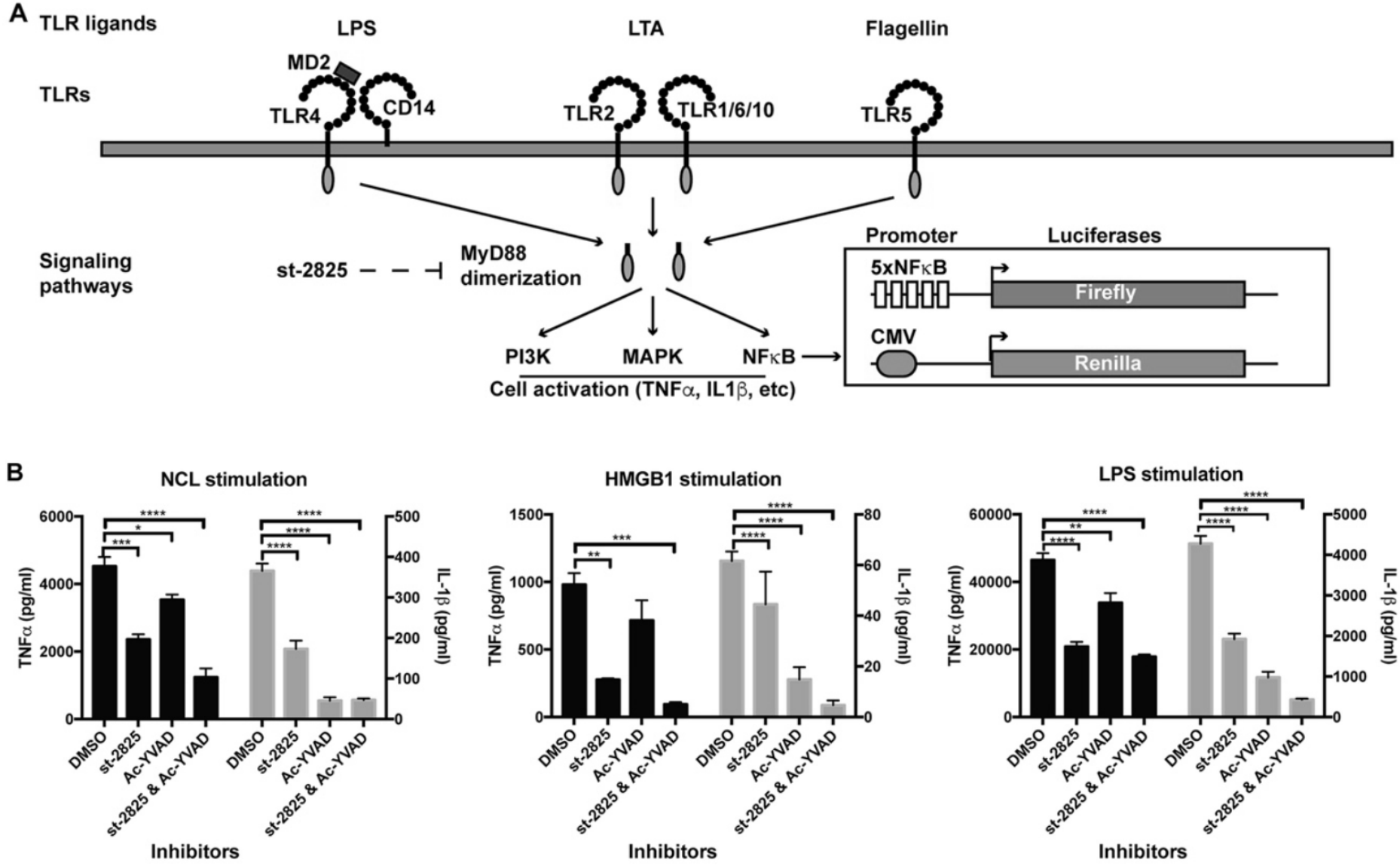

A
TLR ligands
LPS
LTA
Flagellin
TLRs
MD2
TLR4
CD14
TLR2
TLR1/6/10
TLR5
Signaling pathways
st-2825
MyD88 dimerization
Promoter
Luciferases
5xNFκB
Firefly
CMV
Renilla
PI3K
MAPK
NFκB
Cell activation (TNFα, IL1β, etc)
B
NCL stimulation
HMGB1 stimulation
LPS stimulation
TNFα (pg/ml)
IL-1β (pg/ml)
DMSO
st-2825
Ac-YVAD
st-2825 & Ac-YVAD
Inhibitors

Figure 3 (continued)

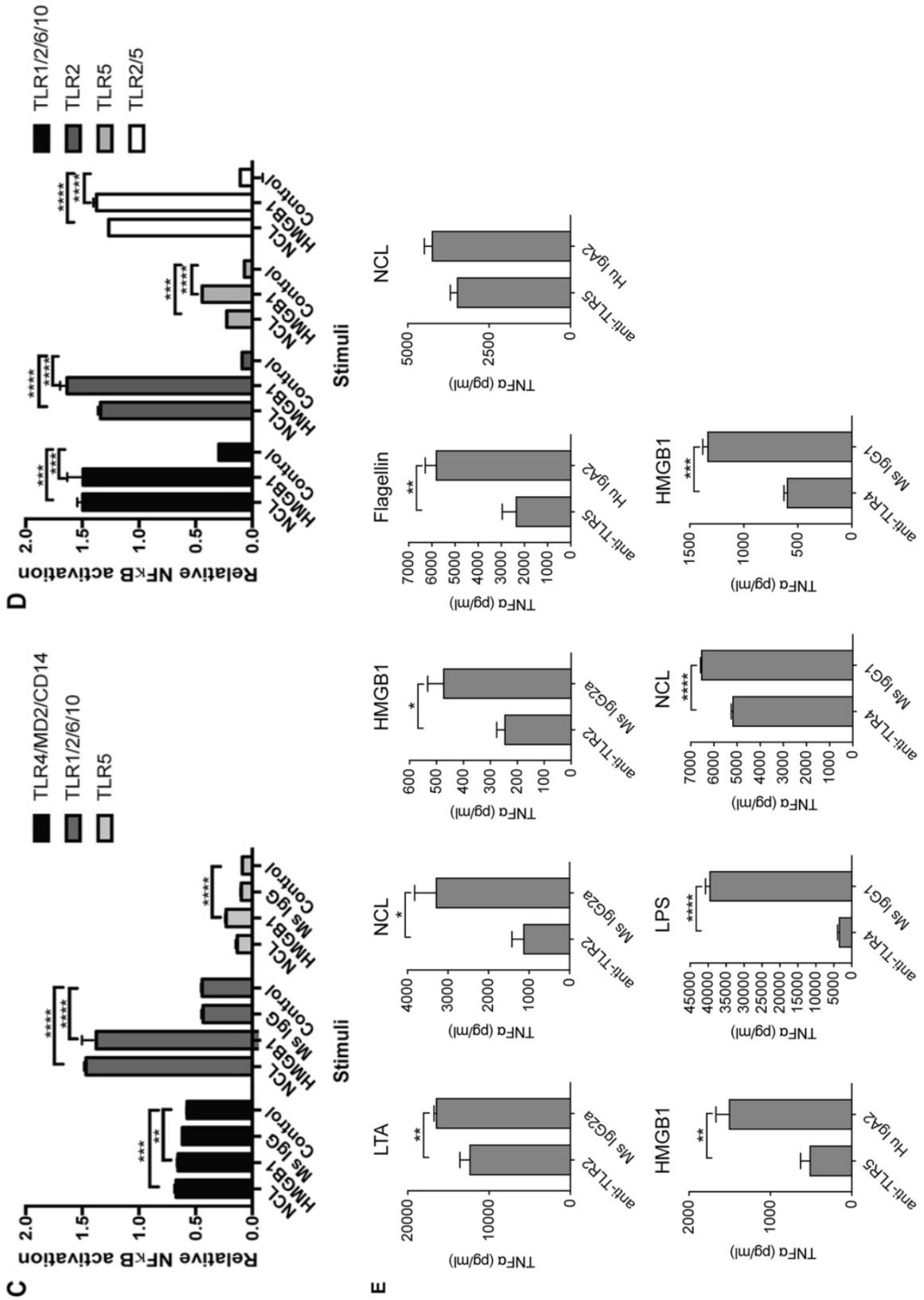


Figure 4

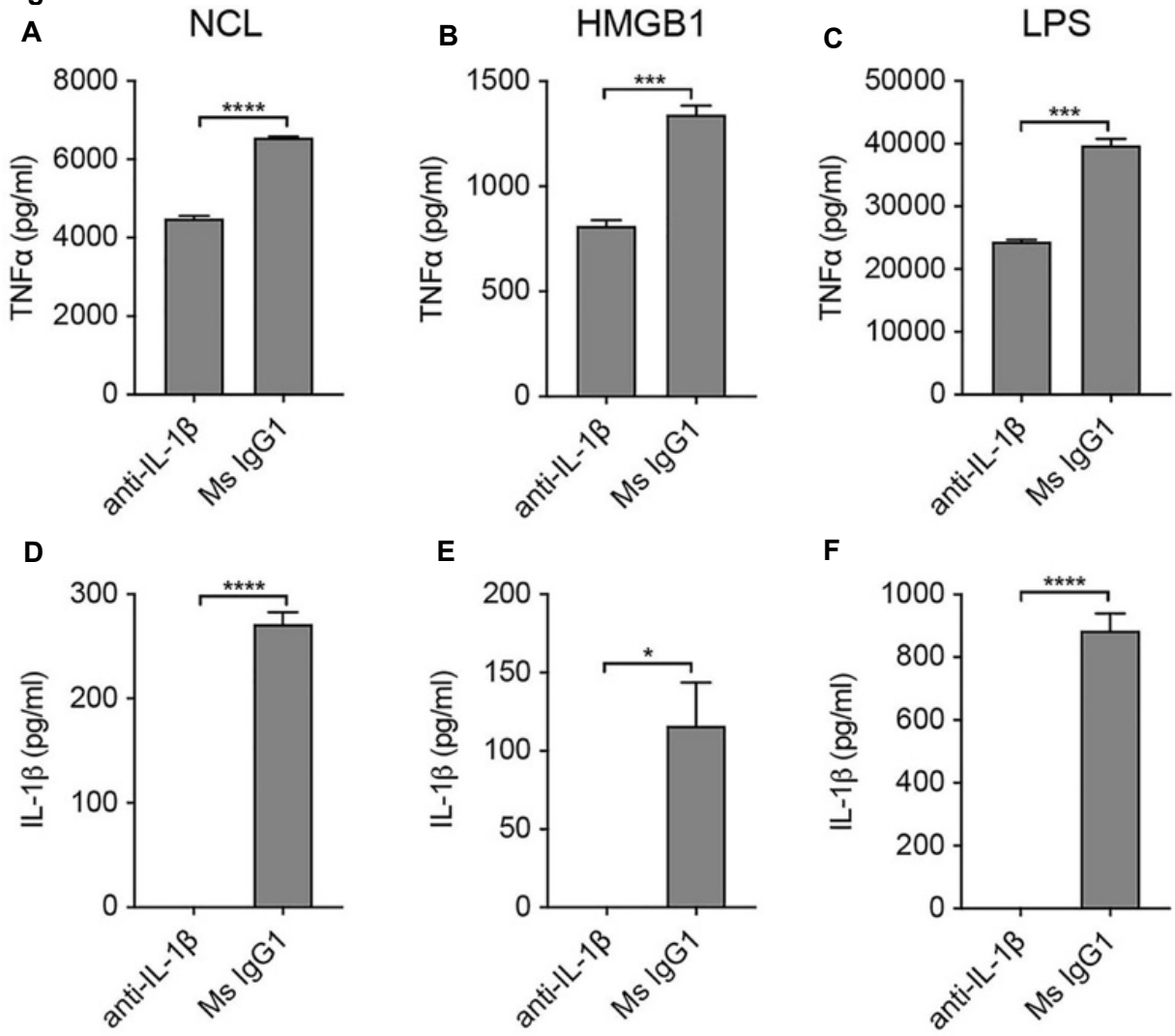


Figure 5

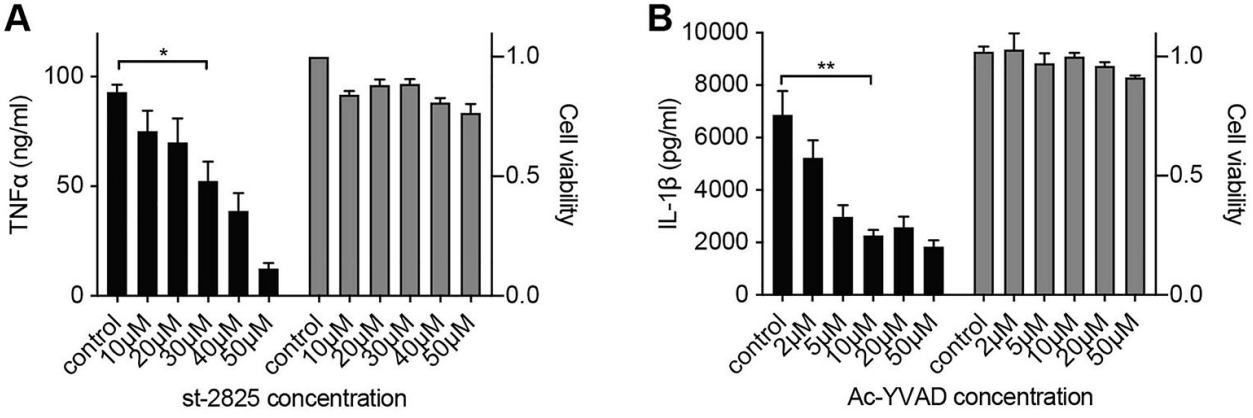


Figure 6

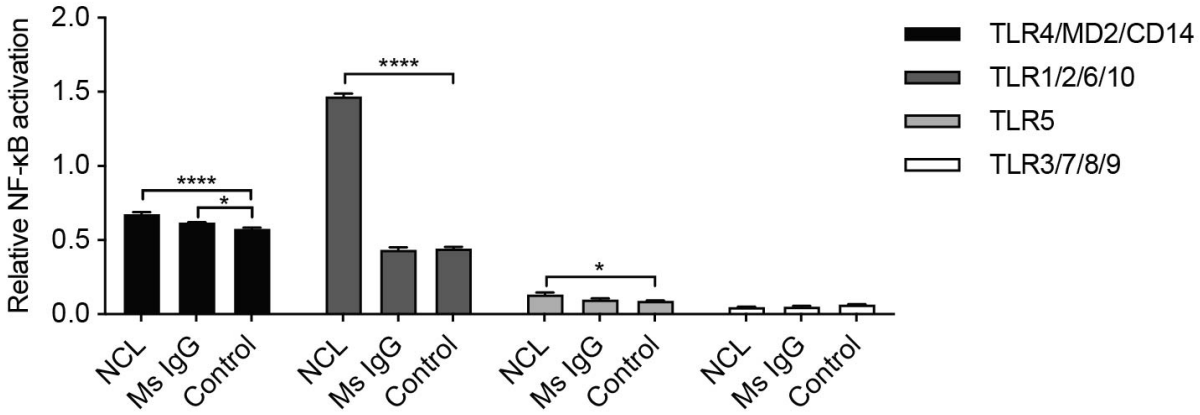


Figure 7

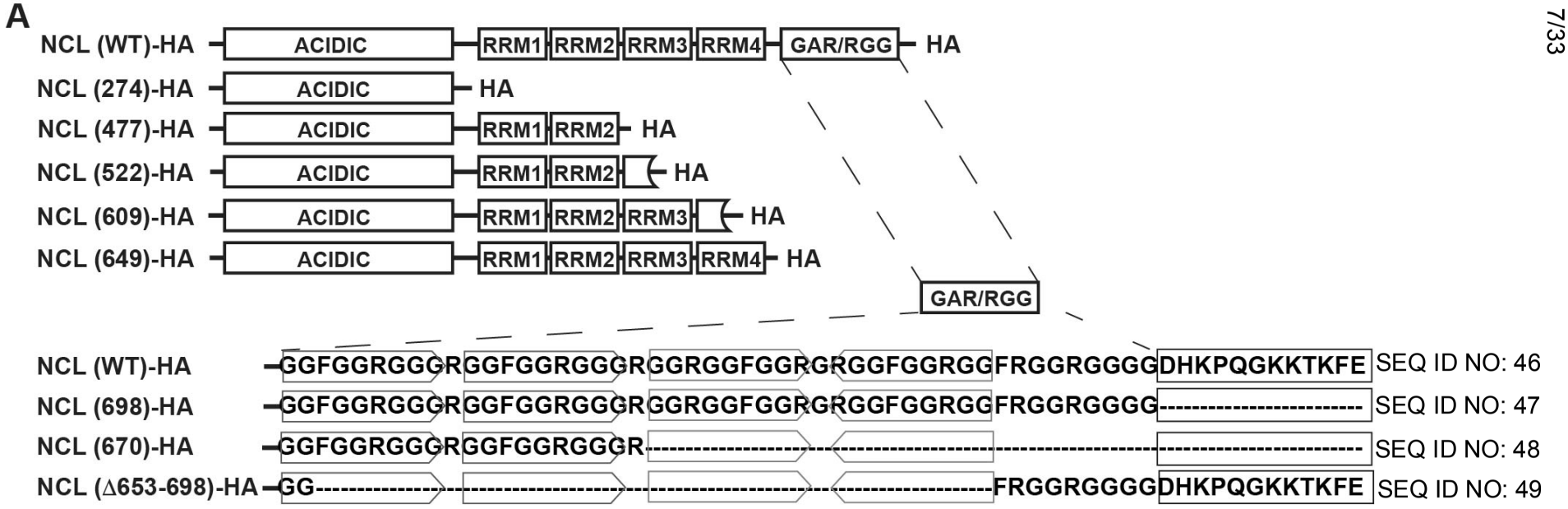


Figure 7 (continued)

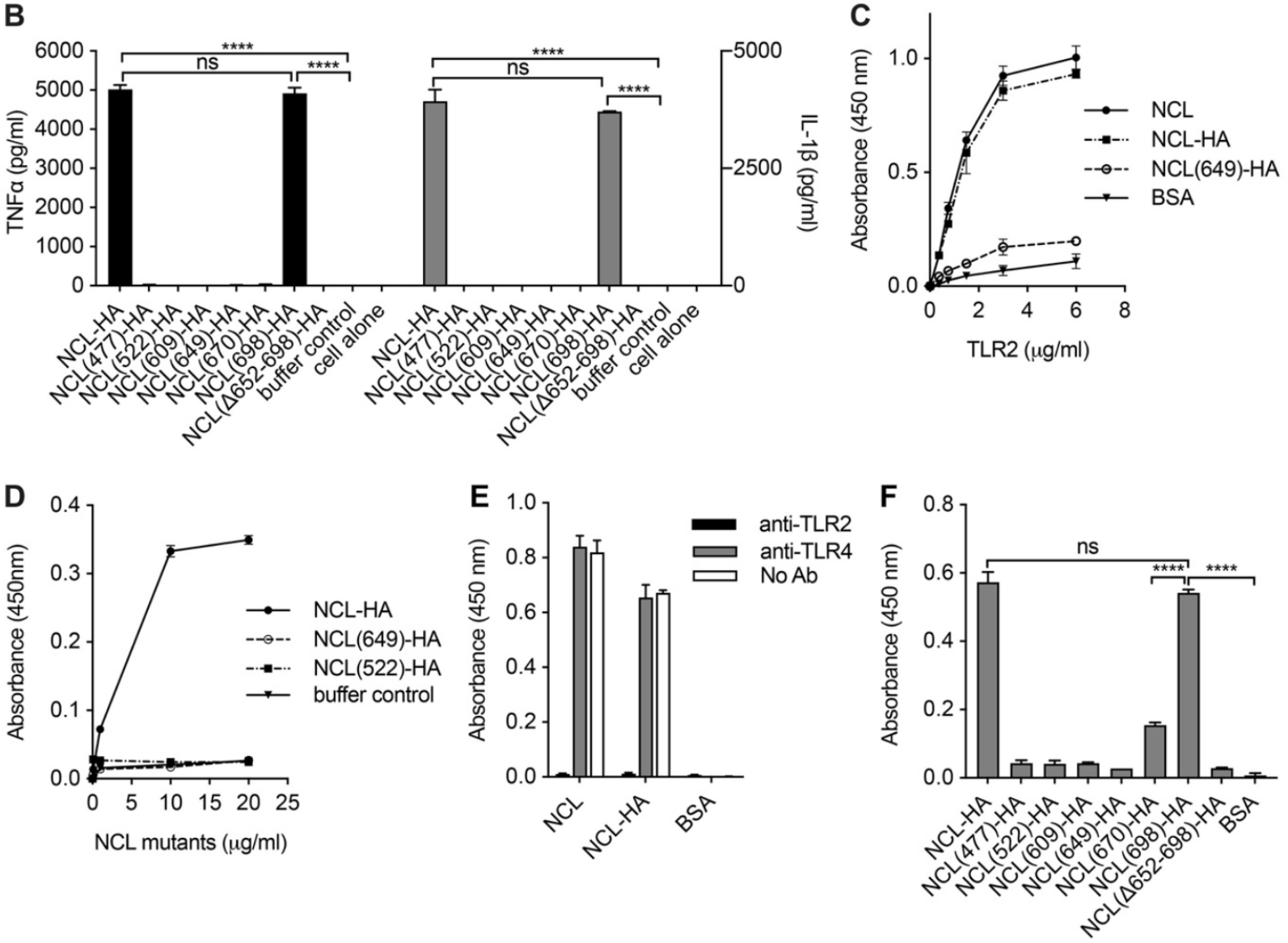
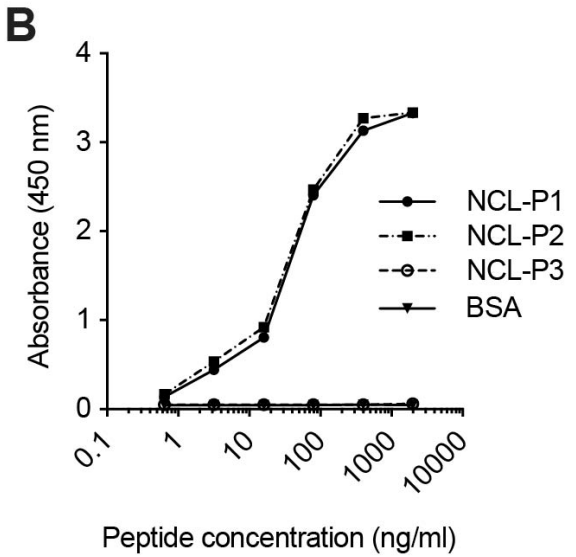


Figure 8

A

GAR/RGG region in NCL	**GGFGGRGGGRGGFGGRGGGRGGRGGFGGRGGRGGFGGRGGFRGGRGGGGDHKPQGKKTKFE**	SEQ ID NO: 46
NCL-P1	Biotin-Ahx-GGFGGRGGGRGGFGGRGGGRGGRGGFGGRGRG	SEQ ID NO: 7
NCL-P2	Biotin-Ahx-GGFGGRGGGRGGRGGFGGRGRGGFGGRGGFRGGRGG	SEQ ID NO: 8
NCL-P3	Biotin-Ahx-DHKPQGKKTKFE	SEQ ID NO: 25
NCL-P4	Biotin-Ahx-RGGFGGRGGG	SEQ ID NO: 26
NCL-P5	Biotin-Ahx-RGGRGGFGGRG	SEQ ID NO: 50
NCL-P6	Biotin-Ahx-RGGFGGRGGGRGGRGGFGGRG	SEQ ID NO: 9
NCL-P7	Biotin-Ahx-FRGGRGG	SEQ ID NO: 51



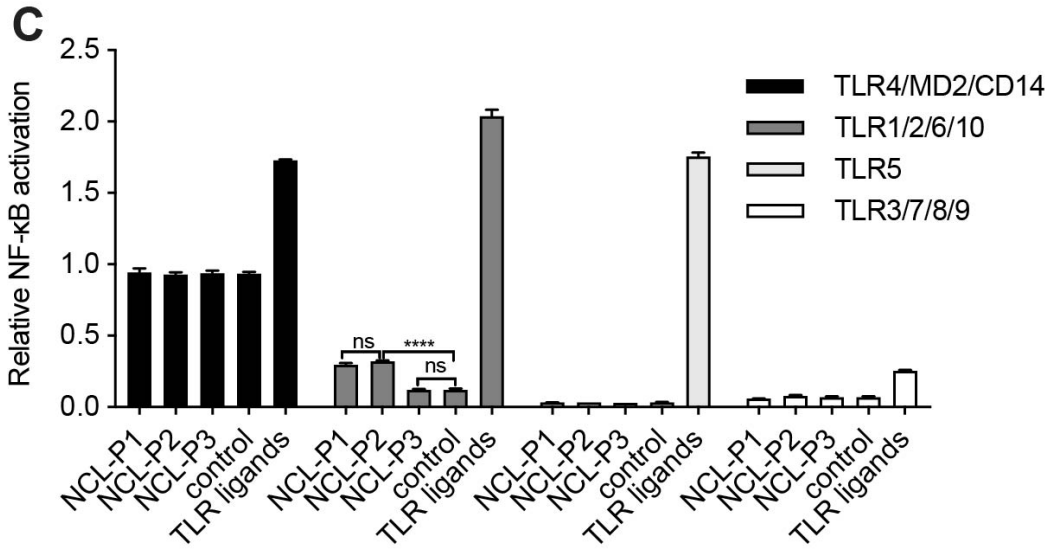


Figure 8 (continued)

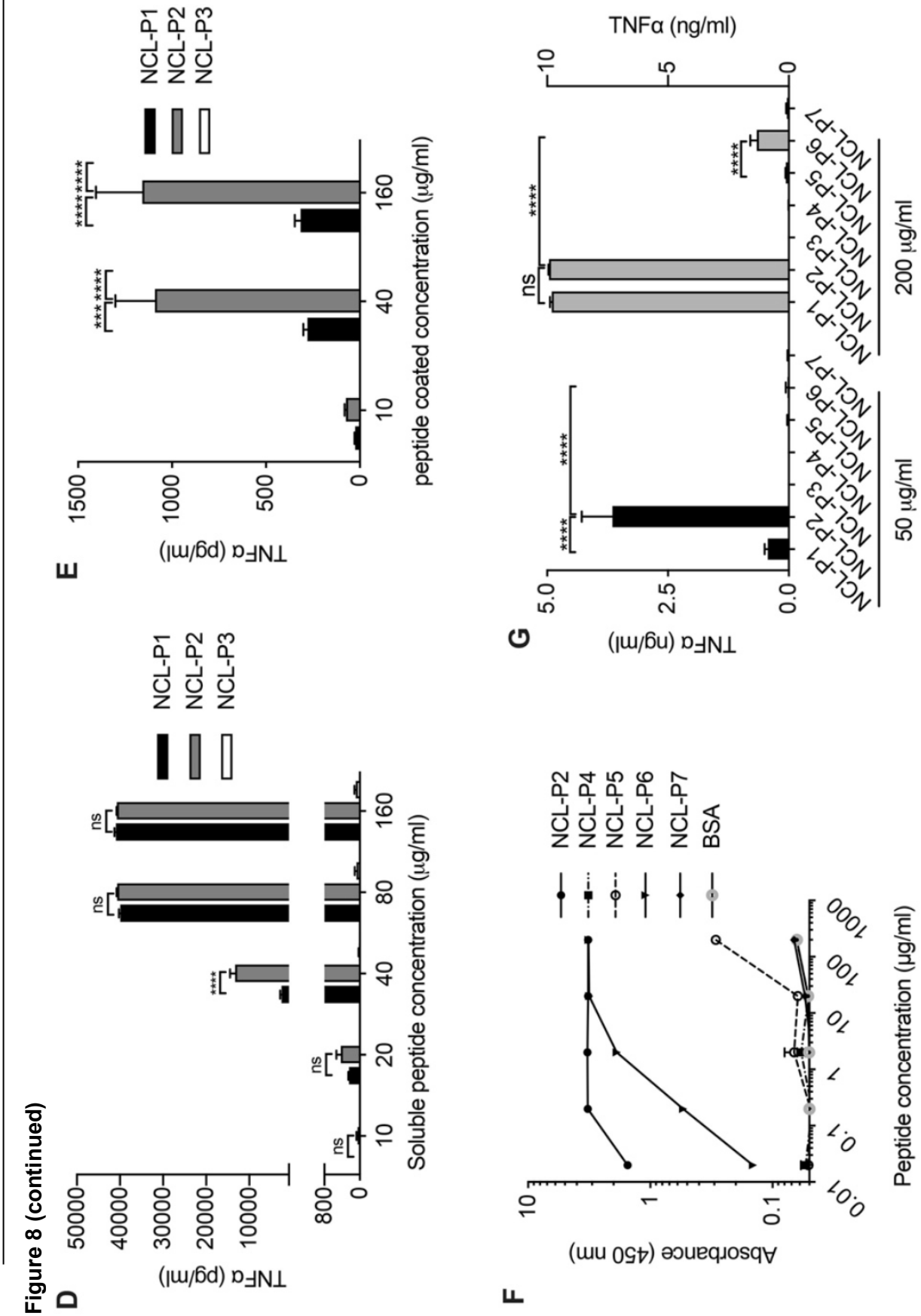


Figure 9

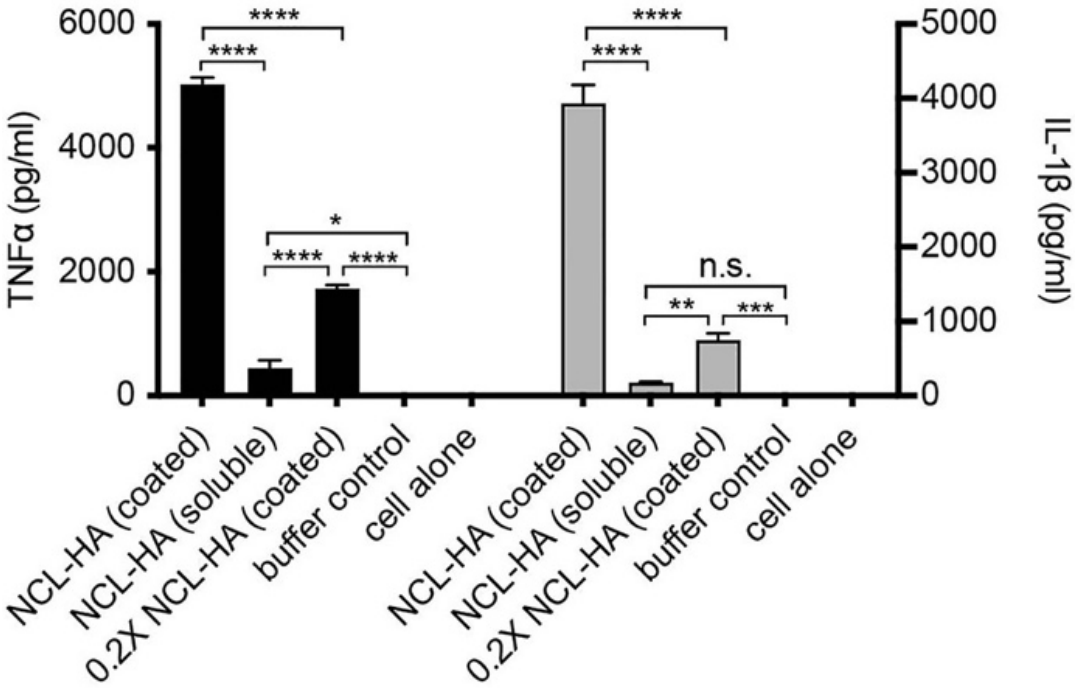


Figure 10

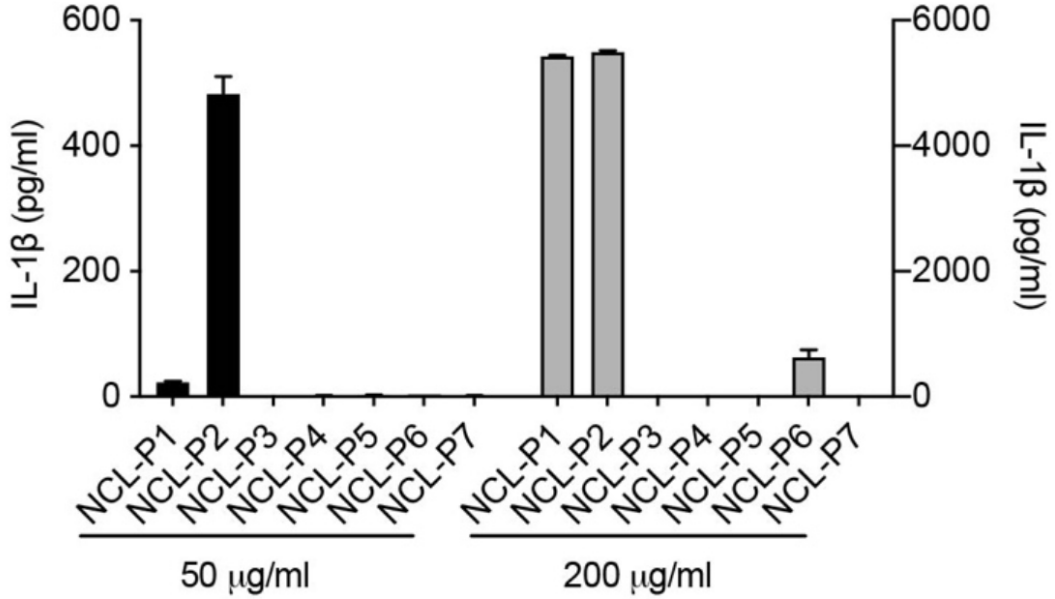


Figure 11

A

FBL-P1 FBL-P2 FBL-P3

MKPGFSP**RGGGFGGRGGFGDRGGRGG**RGG**FGGGRGRGGGFRGRG**RGG**GGGGGGGGGGGGRG**
GGGFHSGGN**RGRGRGGKRG**NQSGKNVMVEPHRHEGVFICRGKEDALVTKNLVPGESVYGE
KRVSISEGDDKIEYRAWNPFRSKLAAAILGGVDQIHIKPGAKVLYLGAASGTTVSHVSDI
VGPDGLVYAVEFSHRSGRDLINLAKKRTNIIPVIEDARHPHKYRMLIAMVDVIFADVAQP
DQTRIVALNAHTFLRNGGHFVISIKANCIDSTASAEAVFASEVKKMQQENMKPQEQLTLE
PYERDHAVVVGVYRPPPKVKN

SEQ ID NO: 2

Figure 11 (continued)

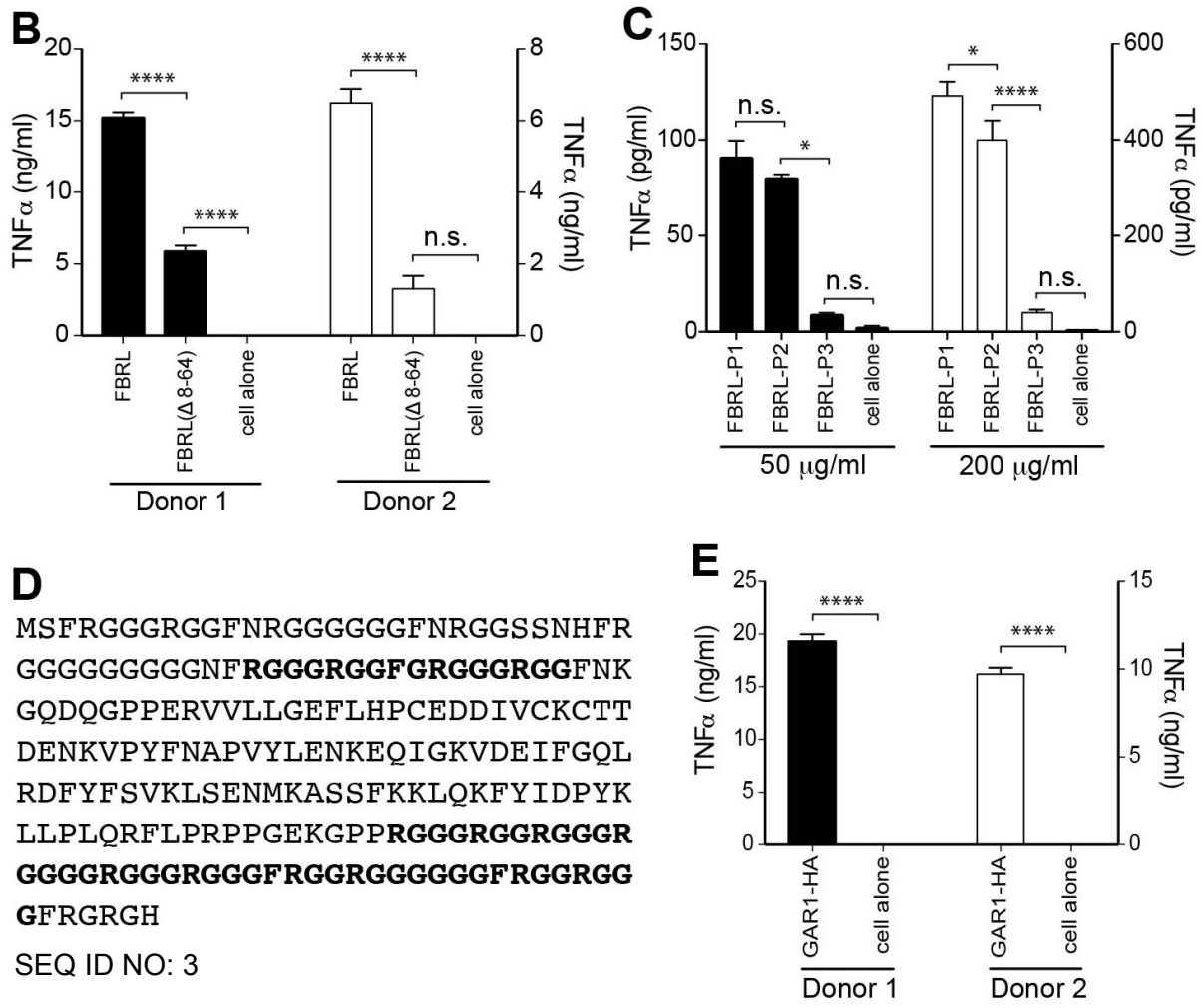


Figure 12

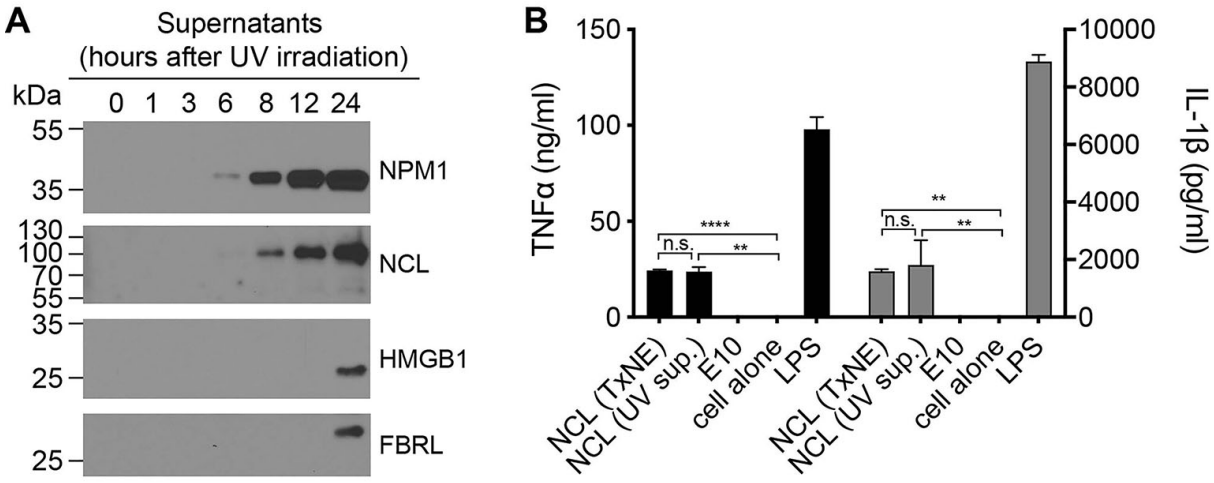


Figure 13

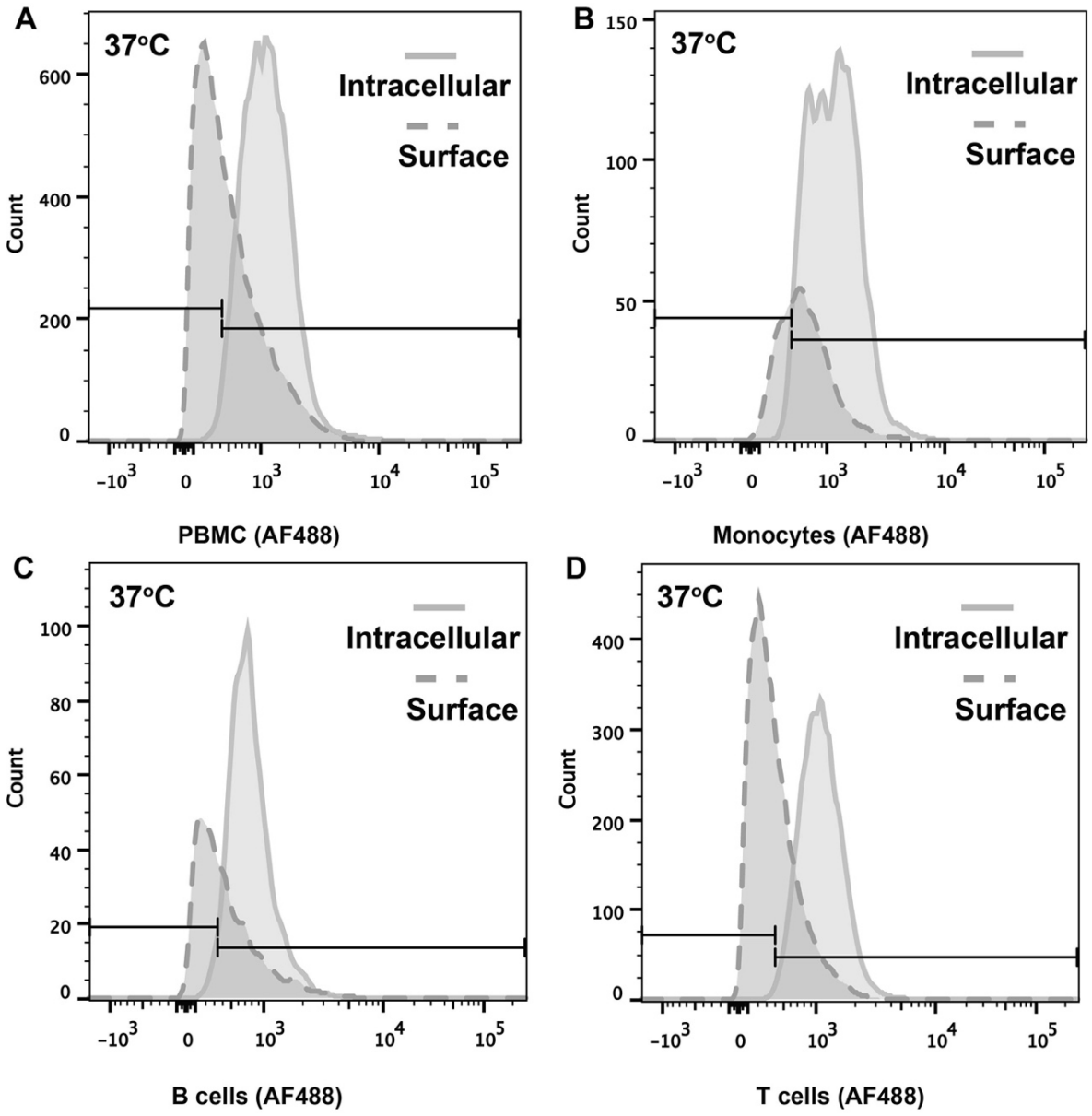

A
37°C
Intracellular
Surface
Count
PBMC (AF488)
B
37°C
Intracellular
Surface
Count
Monocytes (AF488)
C
37°C
Intracellular
Surface
Count
B cells (AF488)
D
37°C
Intracellular
Surface
Count
T cells (AF488)

Figure 13 (continued)

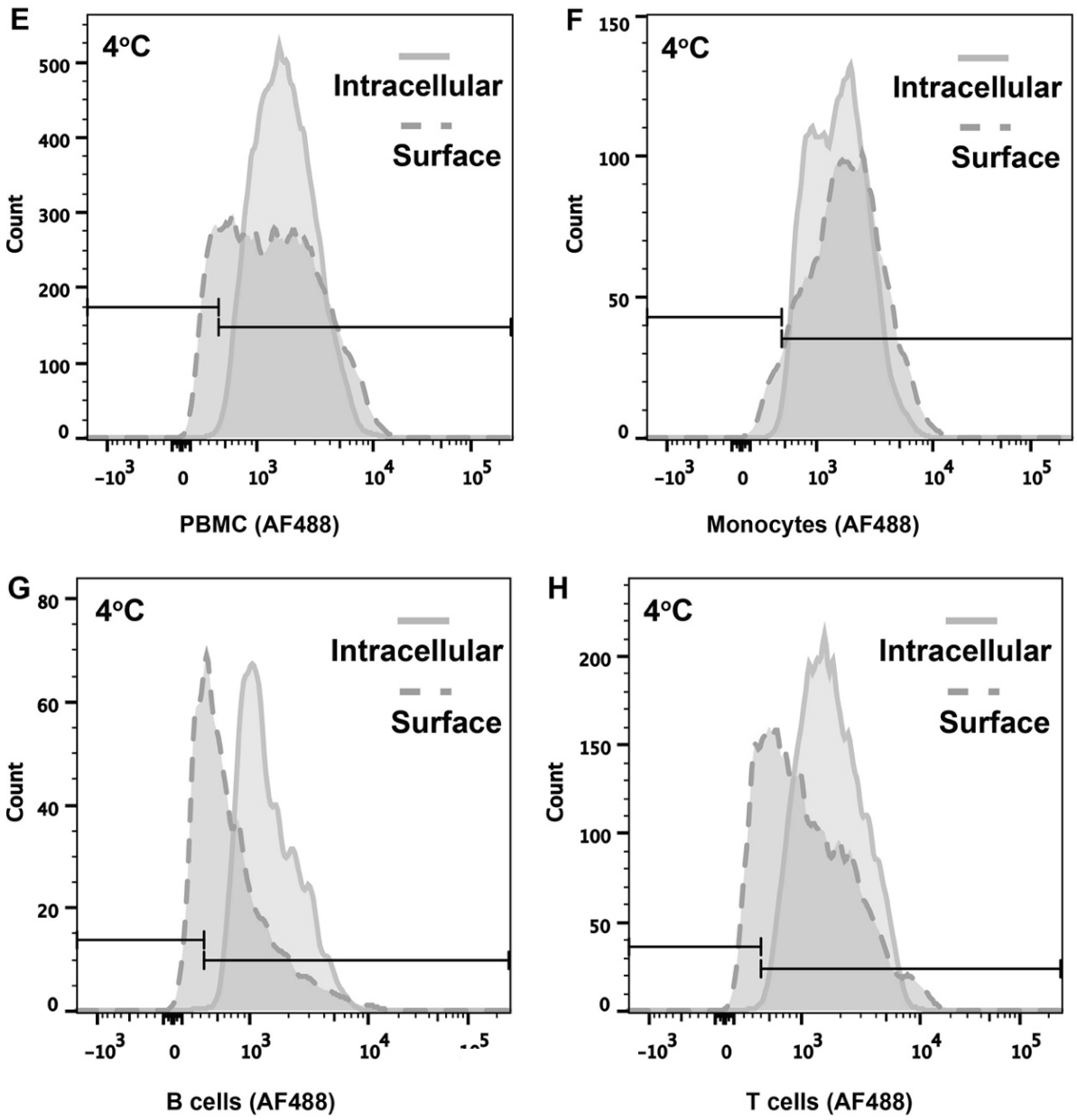
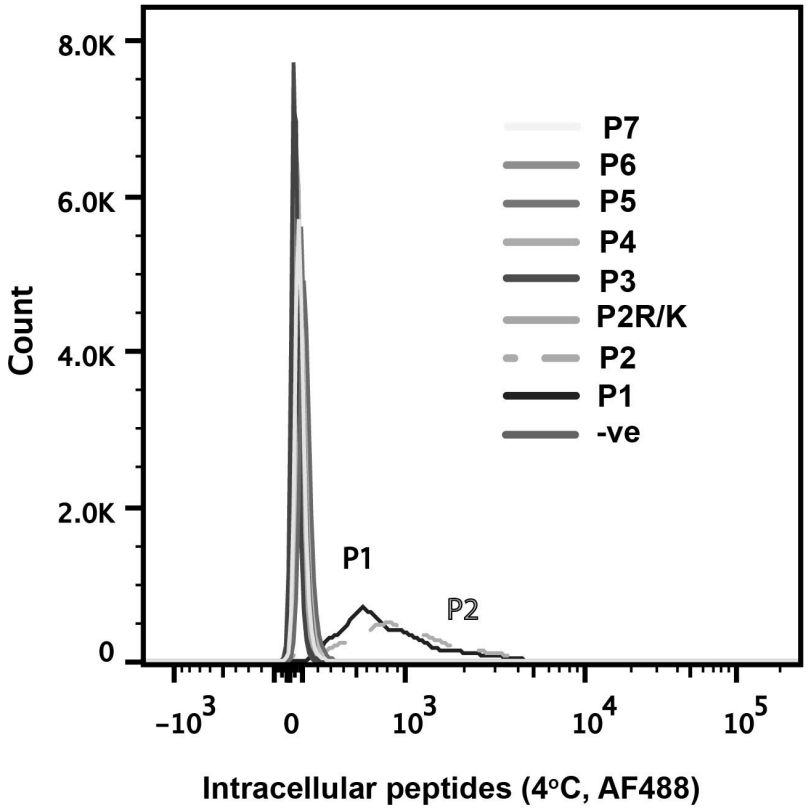


Figure 14


8.0K
6.0K
4.0K
2.0K
0
Count
P7
P6
P5
P4
P3
P2R/K
P2
P1
-ve
P1
P2
-10^3
0
10^3
10^4
10^5
Intracellular peptides (4°C, AF488)

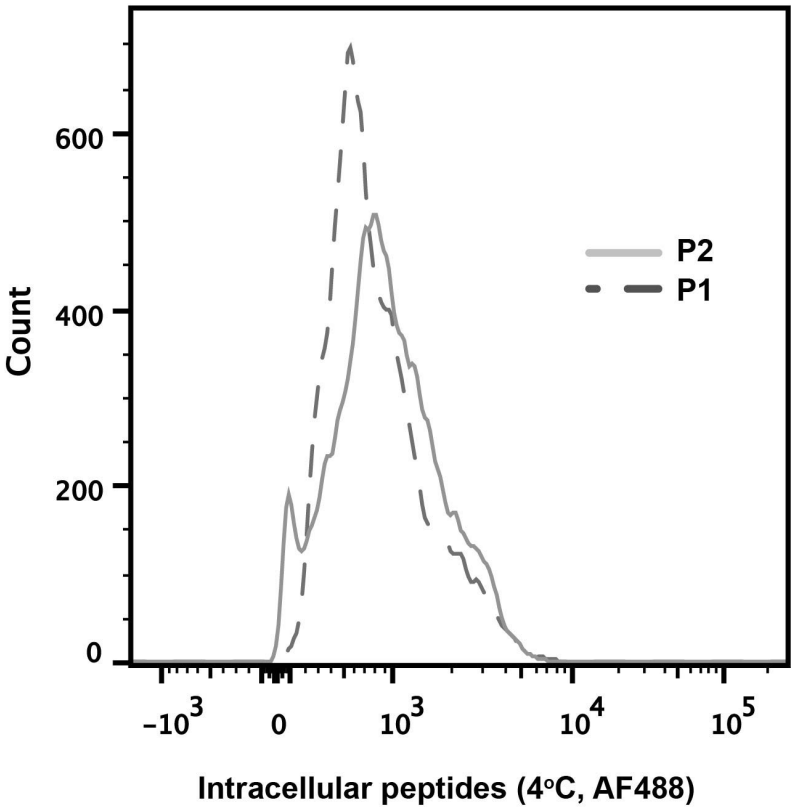

600
400
200
0
Count
P2
P1
-10^3
0
10^3
10^4
10^5
Intracellular peptides (4°C, AF488)

Figure 15

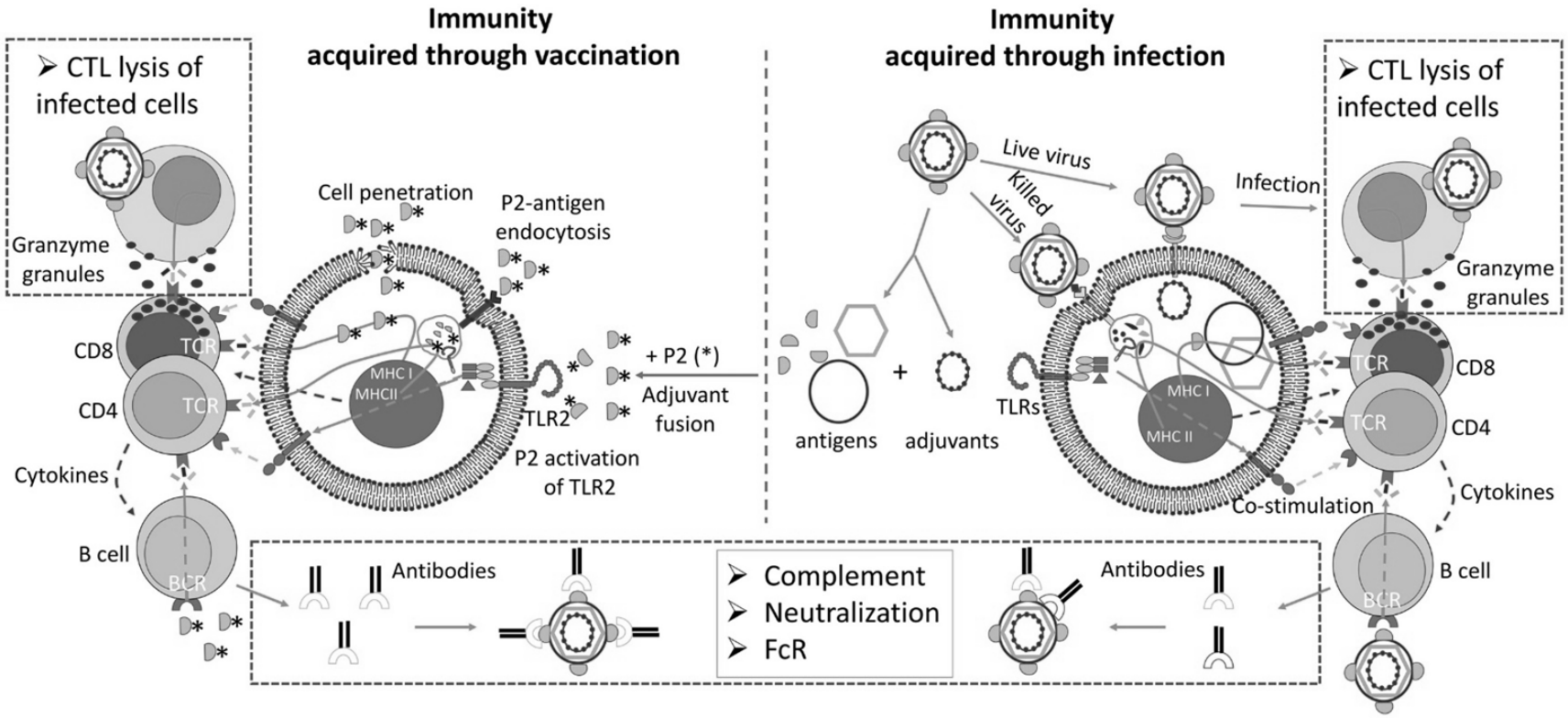
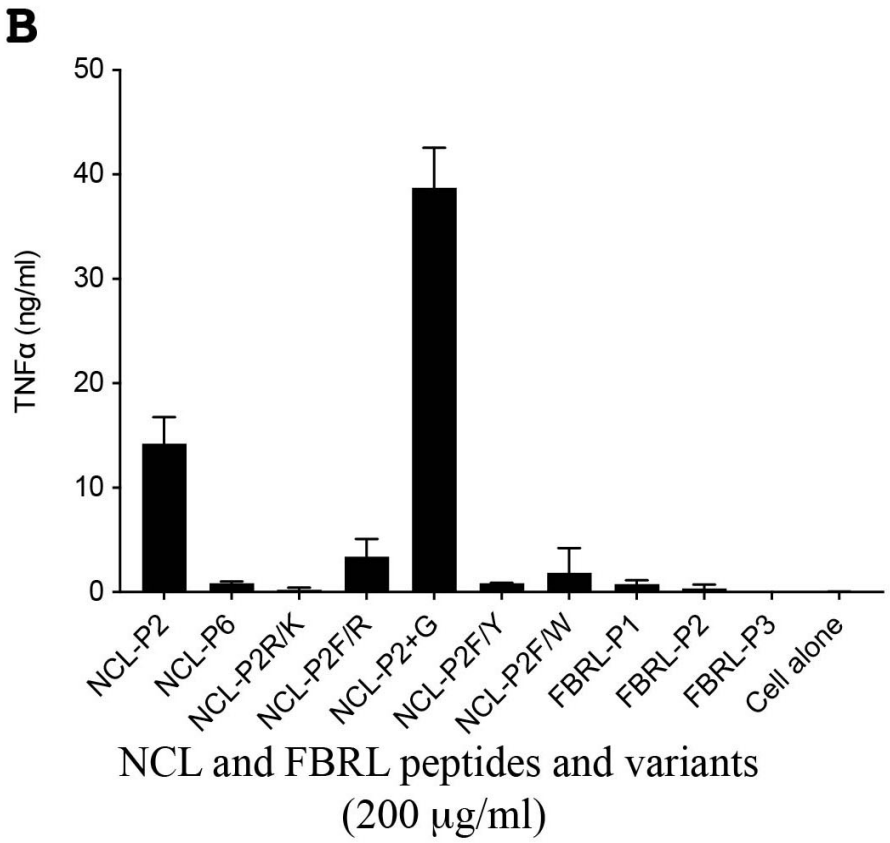

Immunity
acquired through vaccination
Immunity
acquired through infection
➢ CTL lysis of infected cells
Granzyme granules
CD8
TCR
CD4
TCR
Cytokines
B cell
BCR
Cell penetration
P2-antigen endocytosis
MHC I
MHCII
TLR2
+ P2 (*)
Adjuvant fusion
P2 activation of TLR2
Live virus
Killed virus
antigens
+
adjuvants
TLRs
Infection
MHC I
MHC II
Co-stimulation
➢ CTL lysis of infected cells
Granzyme granules
TCR
CD8
TCR
CD4
Cytokines
B cell
BCR
Antibodies
➢ Complement
➢ Neutralization
➢ FcR
Antibodies

Figure 16

A

NCL-P6:	Biotin-Ahx-RGGFGGRGGGRGGRGGFGGRG	SEQ ID NO: 9
NCL-P2:	Biotin-Ahx--GGFGGRGGGRGGRGGFGGRG-RGGFGGRGGF--RGGRGG	SEQ ID NO: 8
NCL-P2R/K:	Biotin-Ahx--GGFGGKGGGKGGKGGFGGKG-KGGFGGKGGF--KGGKGG	SEQ ID NO: 20
NCL-P2F/R:	Biotin-Ahx--GGRGGRGGGRGGRGGRGGRG-RGGRGGRGGR--RGGRGG	SEQ ID NO: 21
NCL-P2R/F:	Biotin-Ahx--GGFGGFGGGFGGFGGFGGFG-FGGFGGFGGF--FGGFGG	SEQ ID NO: 22
NCL-P2F/Y:	Biotin-Ahx--GGYGGRGGGRGGRGGYGGRG-RGGYGGRGGY--RGGRGG	SEQ ID NO: 23
NCL-P2F/W:	Biotin-Ahx--GGWGGRGGGRGGRGGWGGRG-RGGWGGRGGW--RGGRGG	SEQ ID NO: 24
NCL-P2+G:	Biotin-Ahx--GGFGGRGGGRGGRGGFGGRGGRGGFGGRGGF--RGGRGG	SEQ ID NO: 12
NCL-P2+3G:	Biotin-Ahx--GGFGGRGGGRGGRGGFGGRGGRGGFGGRGGFGGRGGRGG	SEQ ID NO: 13
NCL-P2+2G:	Biotin-Ahx--GGFGGRGGRGGFGGRGGRGGFGGRGGRGGFGGRGGRGG	SEQ ID NO: 14
---	---	
FBRL-P1:	Biotin-Ahx--RGGGFGGRGGFGDRGGRGGRGG	SEQ ID NO: 10
FBRL-P2:	Biotin-Ahx--RGGFGGGRGRGGGFRGRGRGG	SEQ ID NO: 11
FBRL-P3:	Biotin-Ahx--RGGGGGGGGGGGGGRGGGGF	SEQ ID NO: 52

Figure 16 (continued)



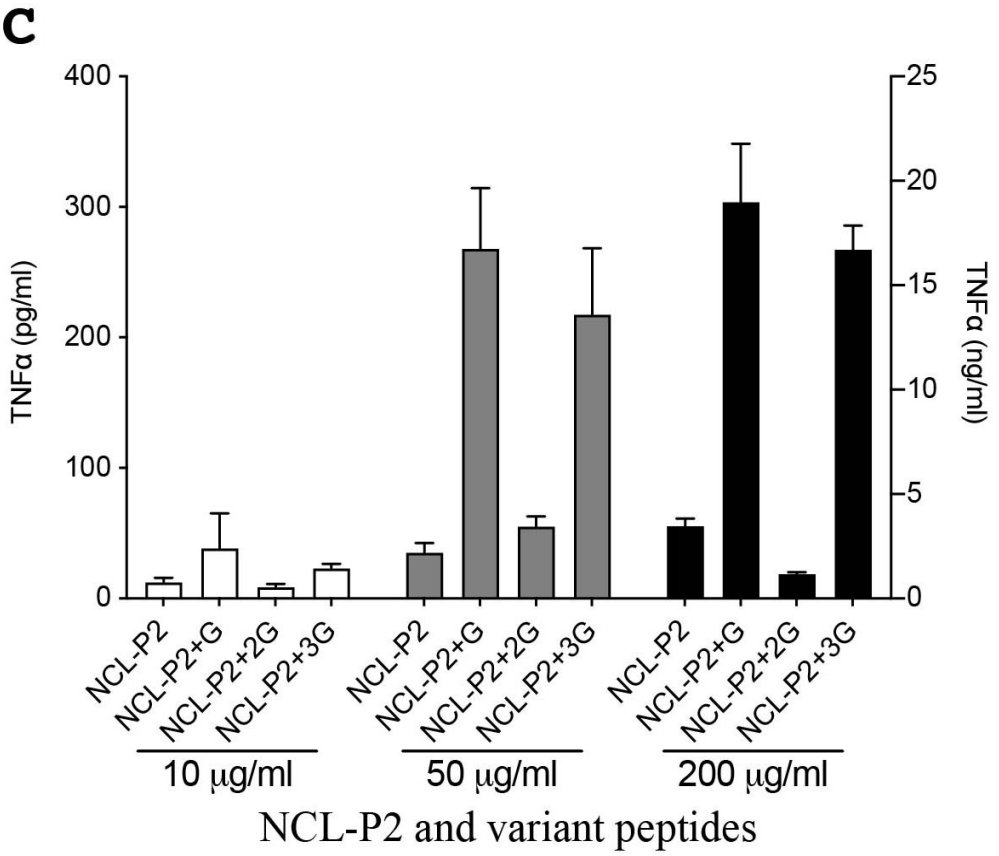


Figure 17

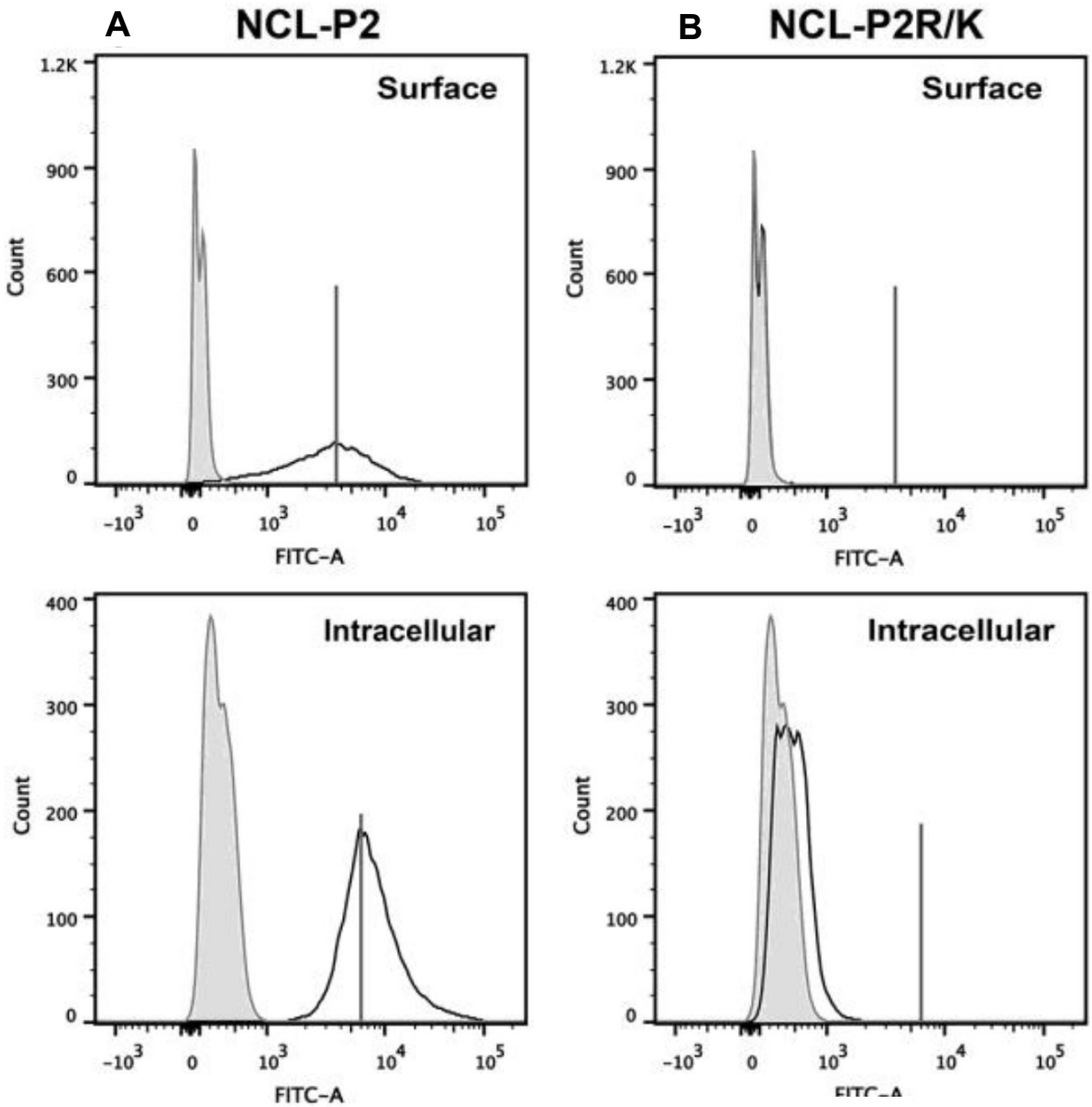

A
NCL-P2
B
NCL-P2R/K
Surface
Intracellular
Count
FITC-A
1.2K
900
600
300
0
400
200
100
-10^3
10^3
10^4
10^5

Figure 17 (continued)

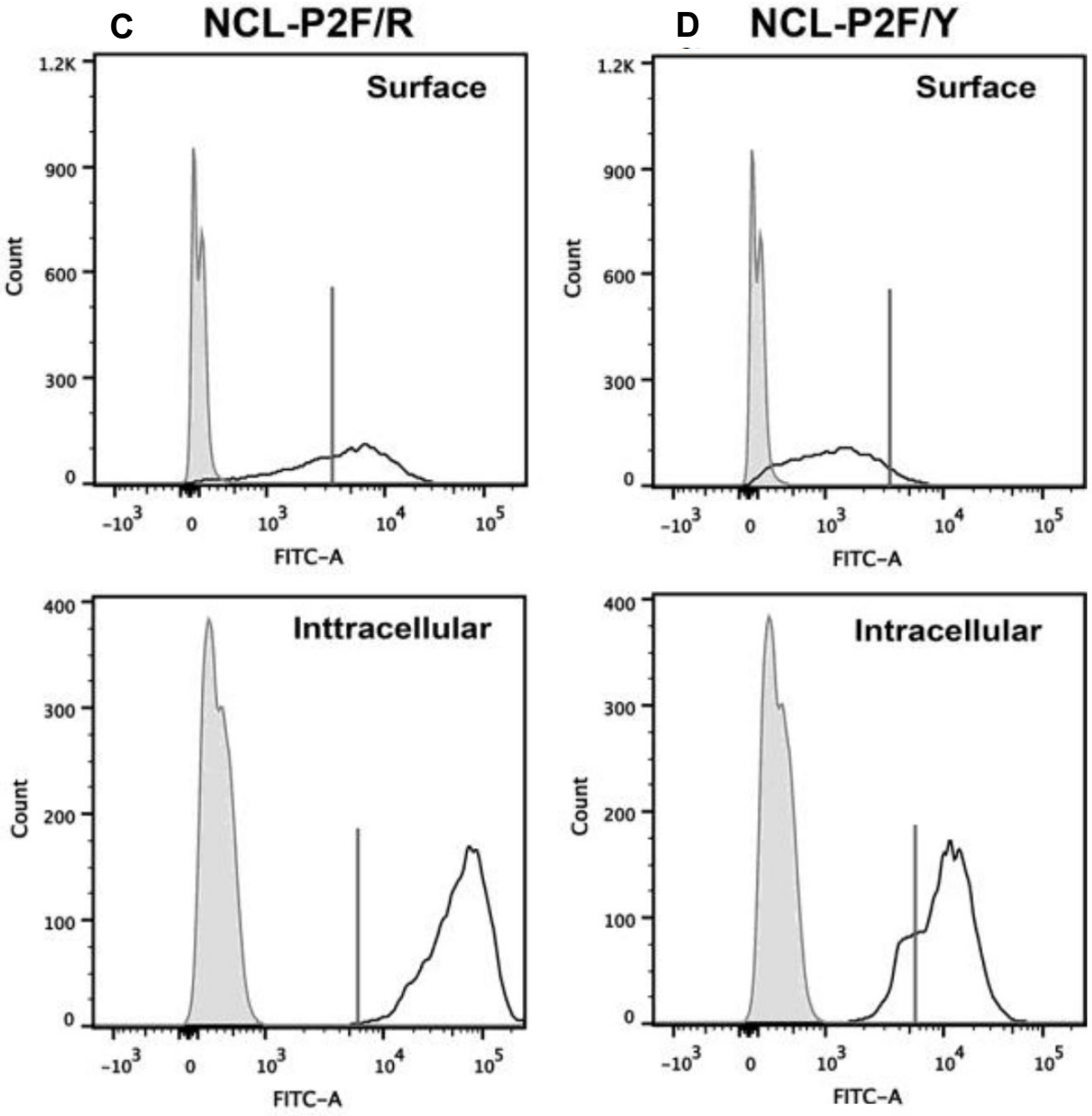


Figure 17 (continued)

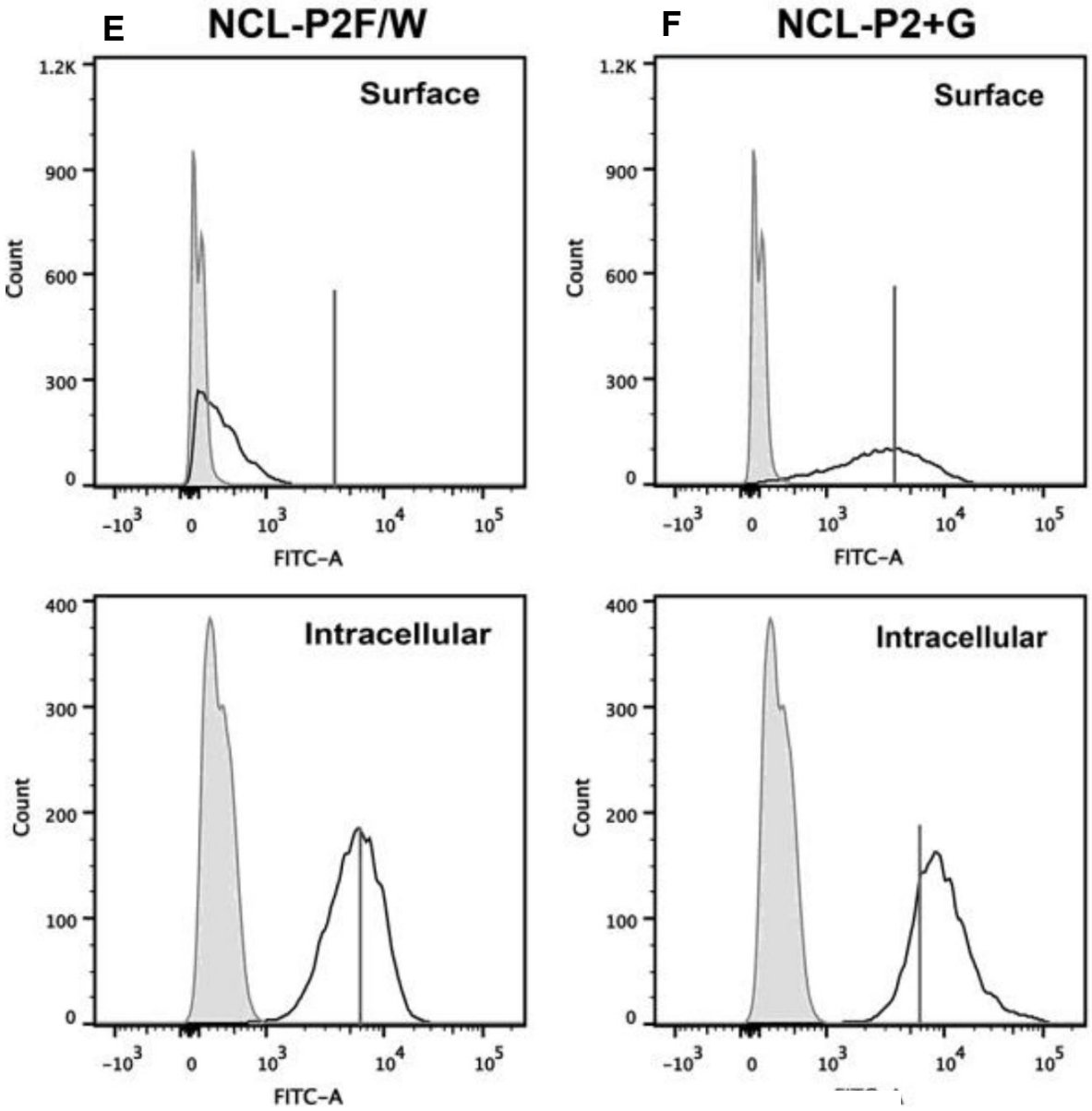
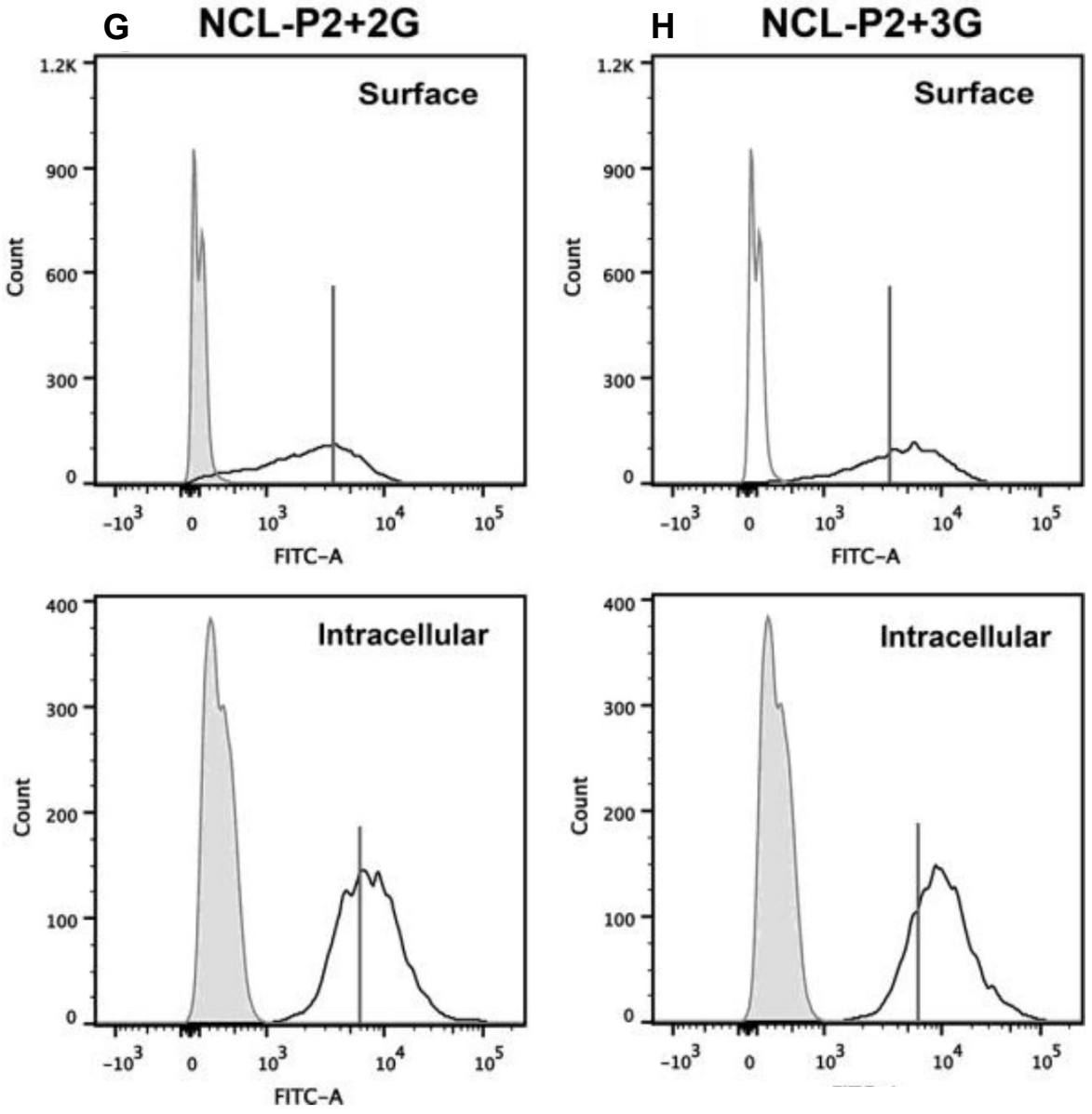


Figure 17 (continued)



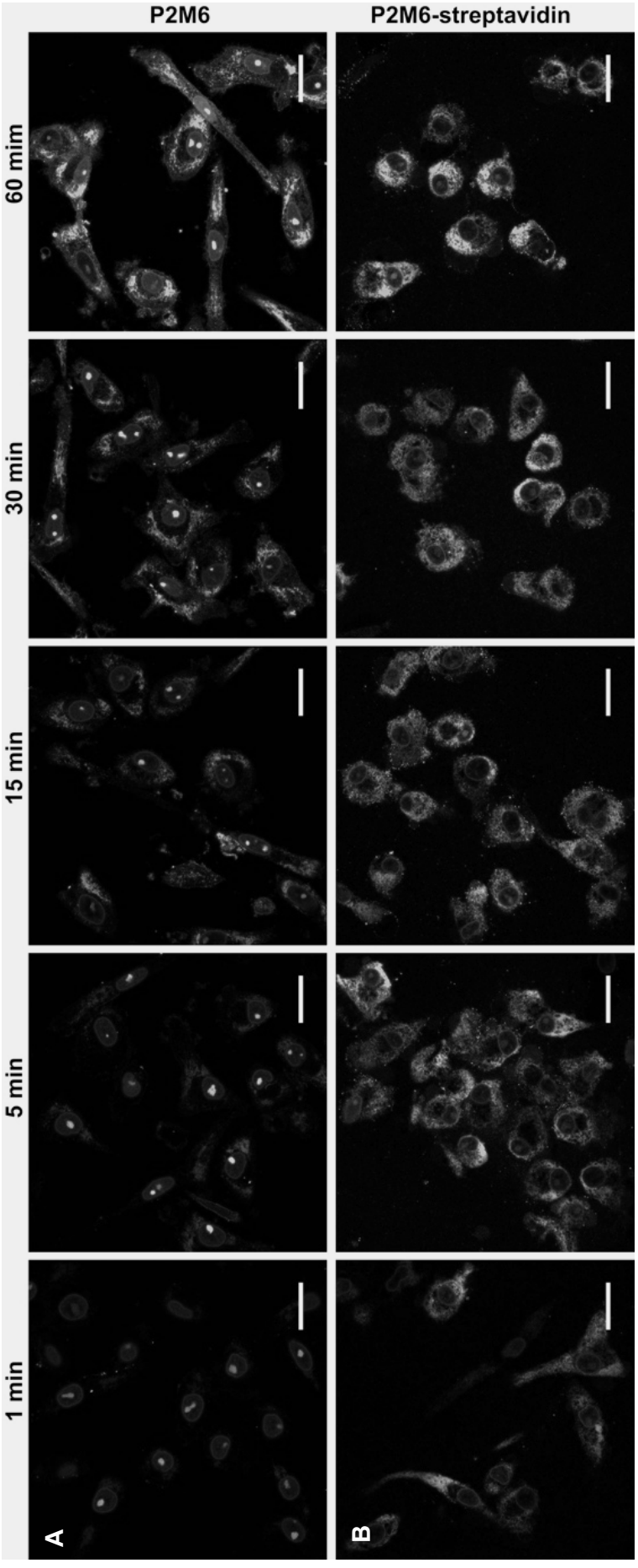


Figure 18

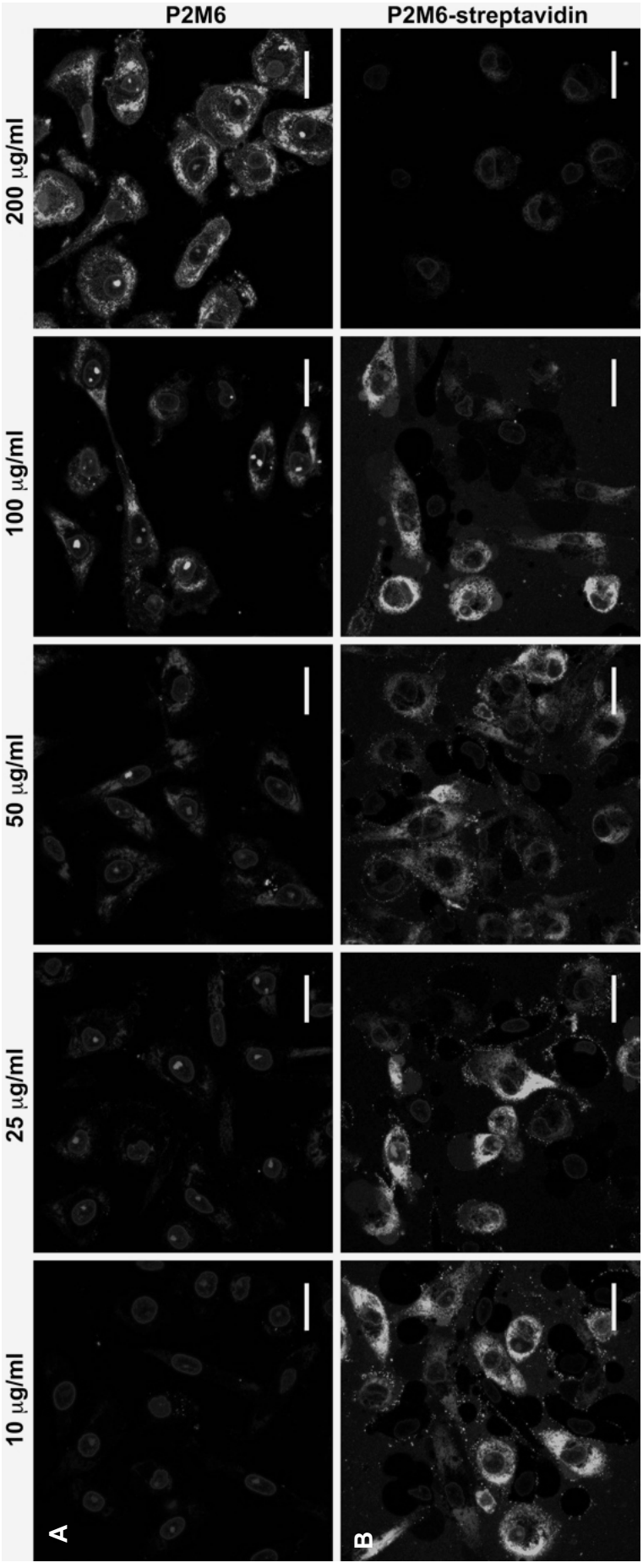

P2M6
P2M6-streptavidin
200 µg/ml
100 µg/ml
50 µg/ml
25 µg/ml
10 µg/ml
A
B

Figure 19

Figure 20

A

NCL-P2+G:	Biotin-Ahx--GGFGGRGGGRGGRGGFGGRGGRGGFGGRGGFRGGRGG	SEQ ID NO: 12
NCL-P2+G(F/I):	Biotin-Ahx--GGIGGRGGGRGGRGGIGGRGGRGGIGGRGGIRGGRGG	SEQ ID NO: 53
NCL-P2+G(F/L):	Biotin-Ahx--GGLGGRGGGRGGRGGLGGRGGRGGLGGRGGLRGGRGG	SEQ ID NO: 54
NCL-P2+G(G/A):	Biotin-Ahx--GGFGARGGARGARGGFGARGARGGFGARGGFRGARGA	SEQ ID NO: 55
NCL-P2+G(G/P):	Biotin-Ahx--GGFGPRGGPRGPRGGFGPRGPRGGFGPRGGFRGPRGP	SEQ ID NO: 56

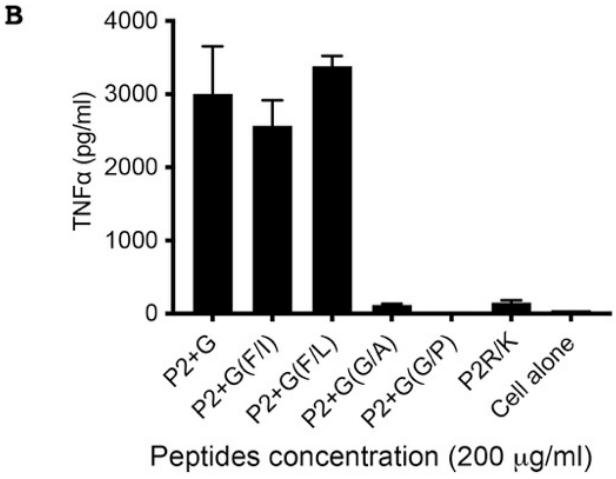
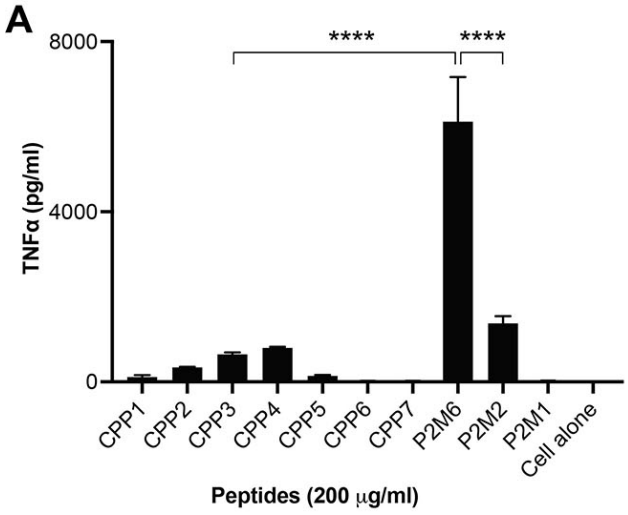


Figure 21



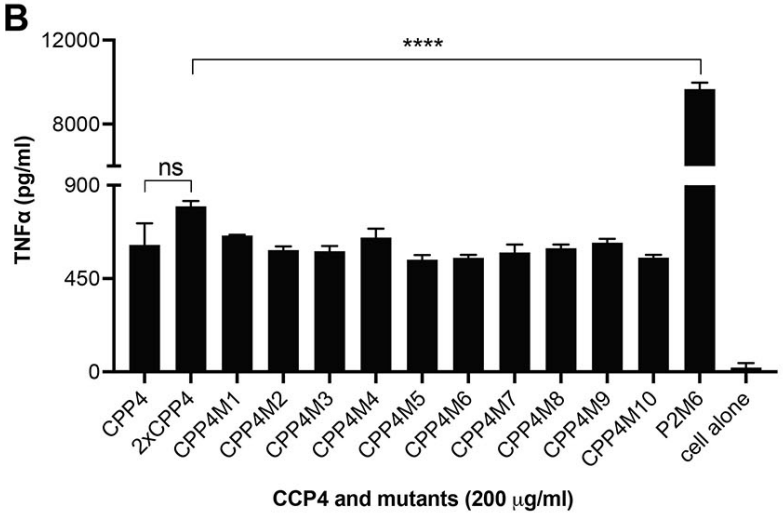


Figure 22

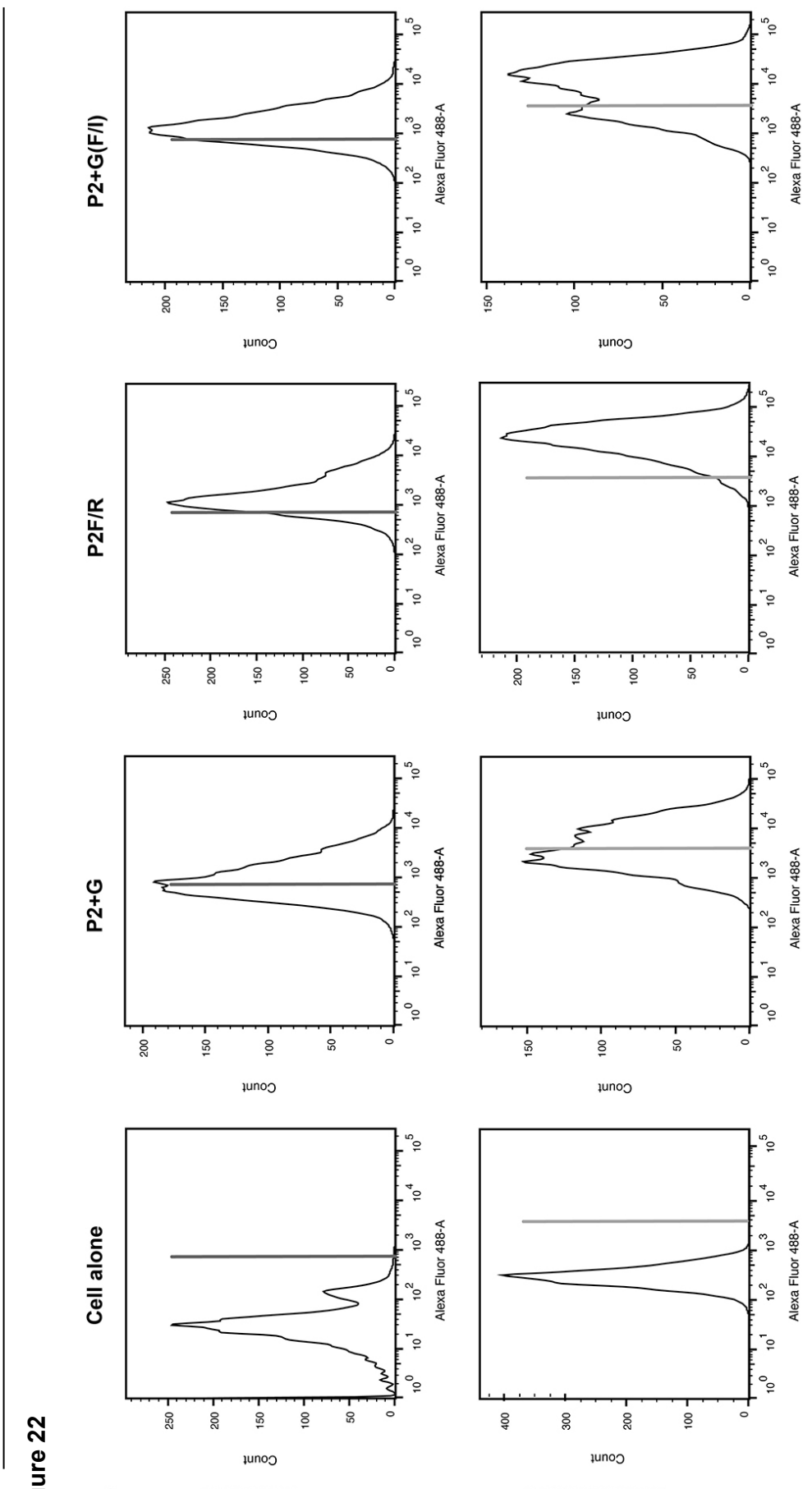

A
Surface
Intracellular
Cell alone
P2+G
P2F/R
P2+G(F/I)
Count
Alexa Fluor 488-A

Figure 22 (continued)

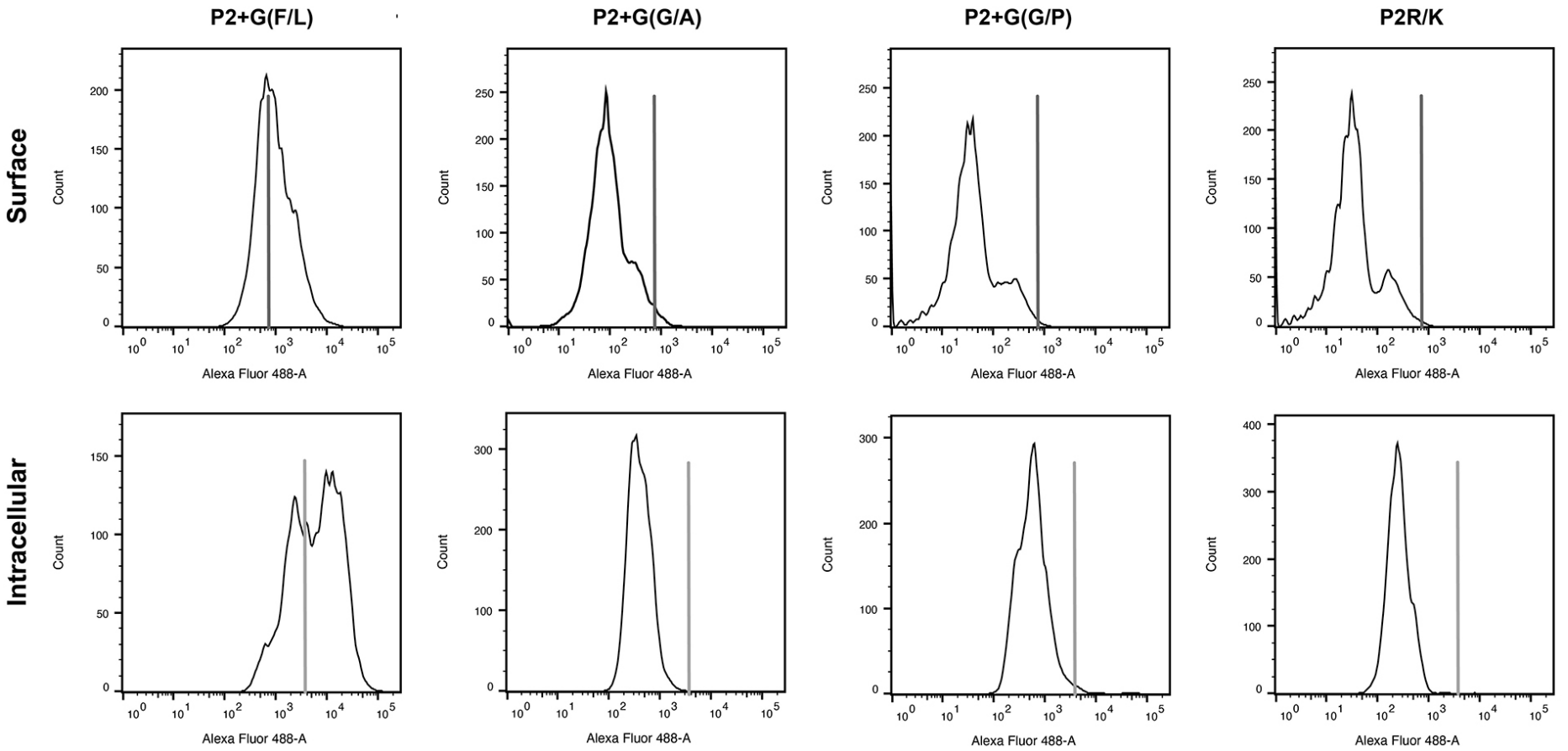
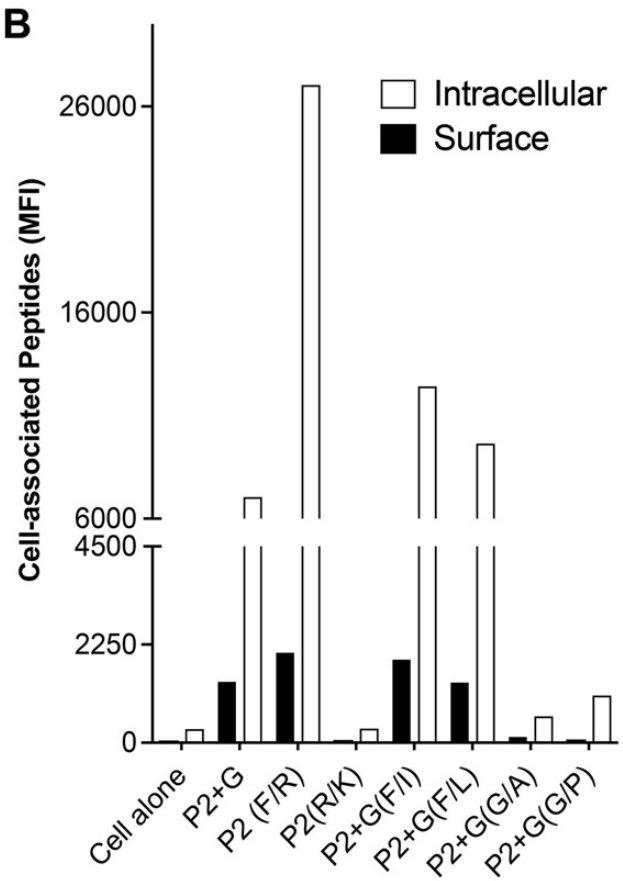


Figure 22 (continued)



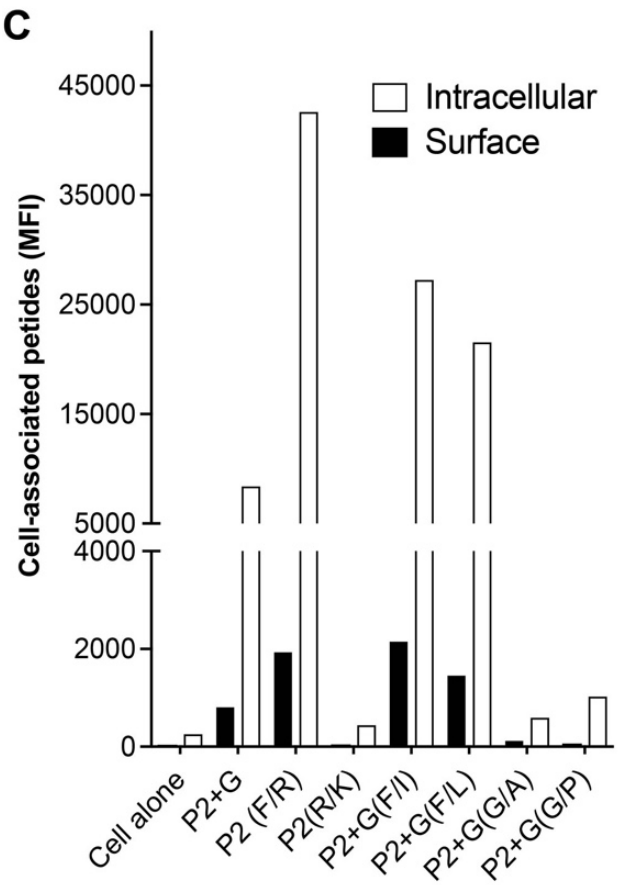


Figure 23

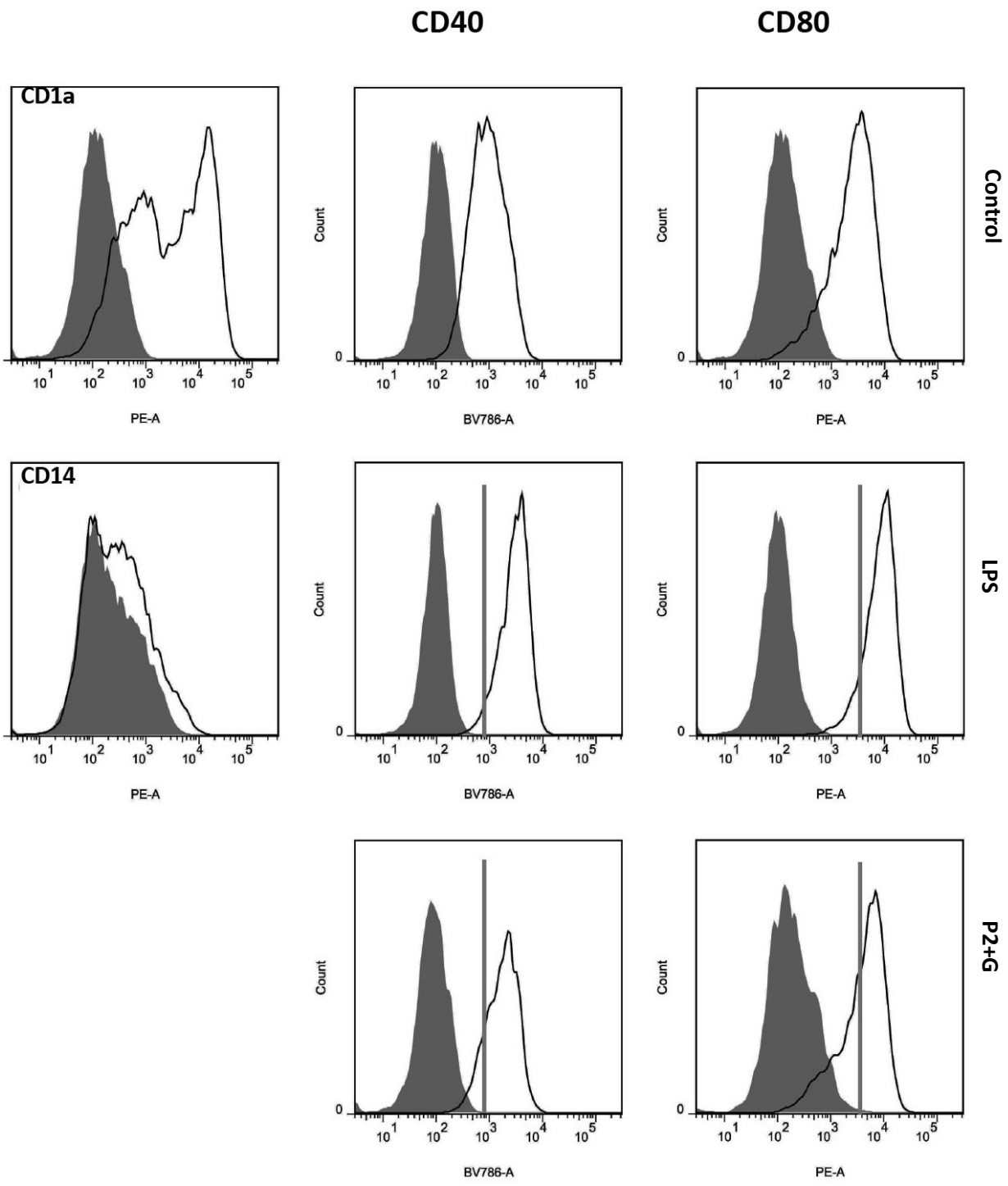

CD40
CD80
CD1a
CD14
Control
LPS
P2+G
Count
PE-A
BV786-A
0
10^1 10^2 10^3 10^4 10^5

Figure 23 (continued)

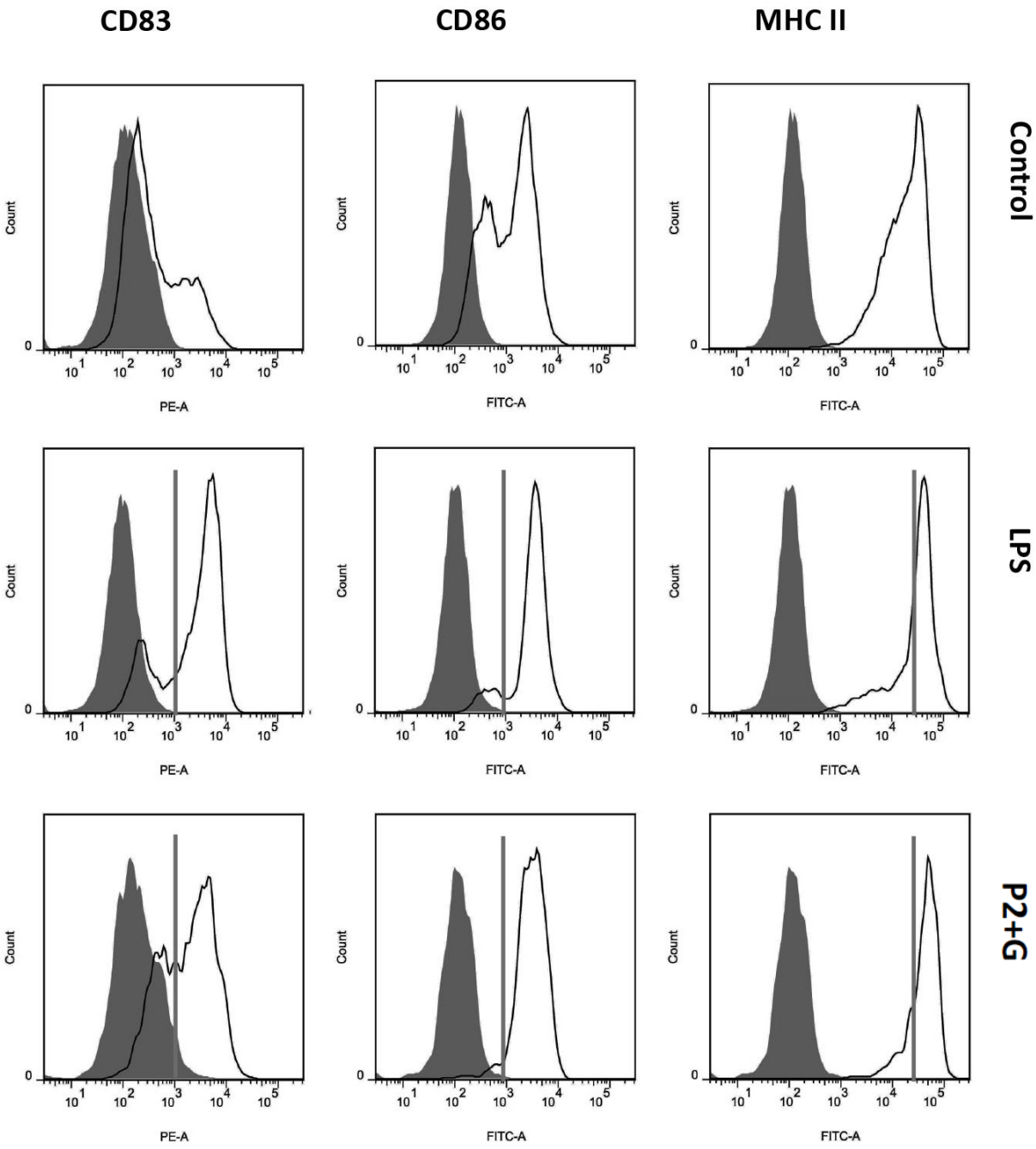
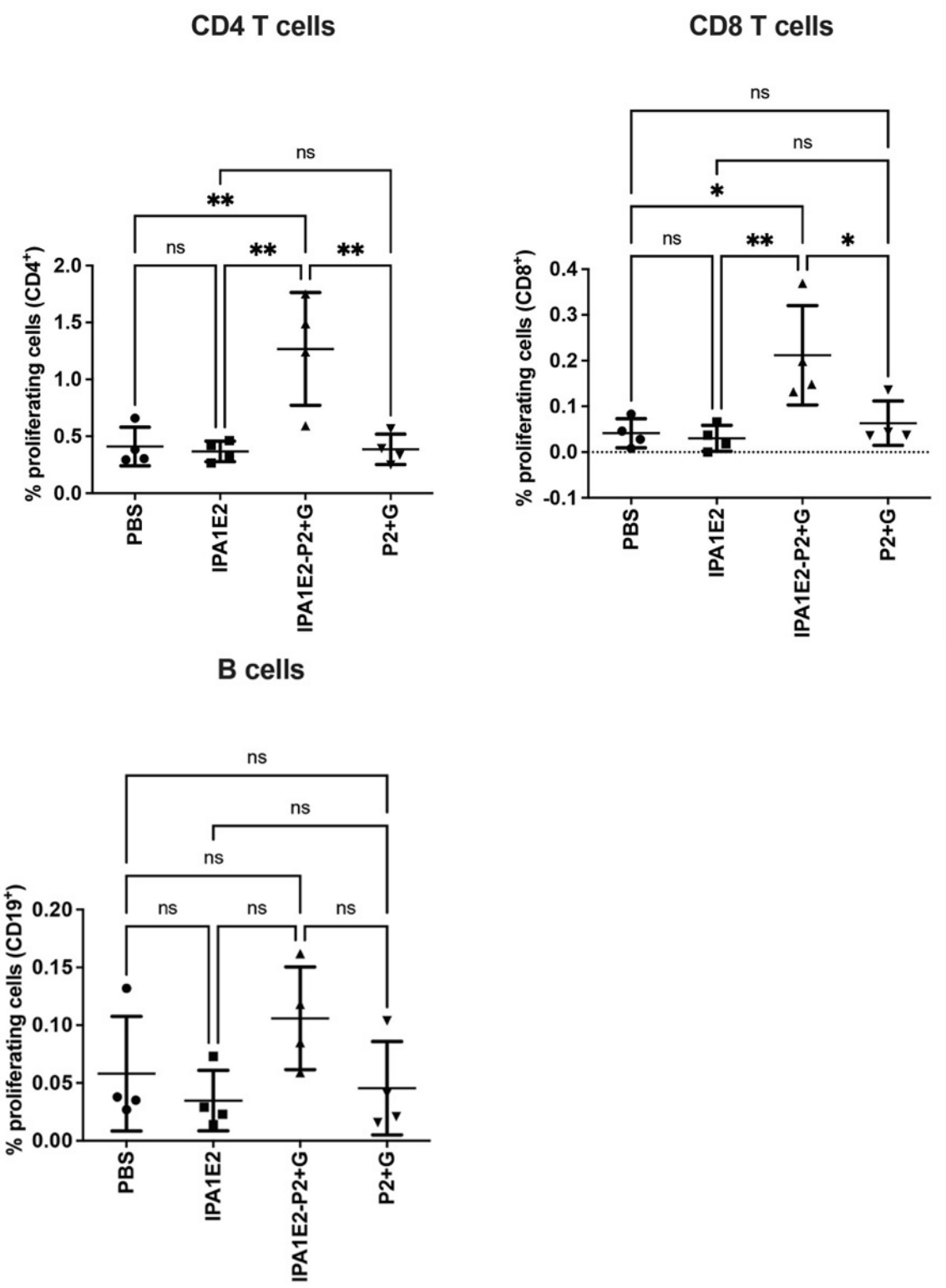


Figure 24



SEQ ID NO: 57	GQGQLVKRPKIMRFISHLWLALESANSEKR	IPA1E2
SEQ ID NO: 58	GQGQLVKRPKIMRFISHLWLALESANSEKR-P2+G	IPA1E2-P2+G
SEQ ID NO: 12	P2+G	P2+G

Figure 25

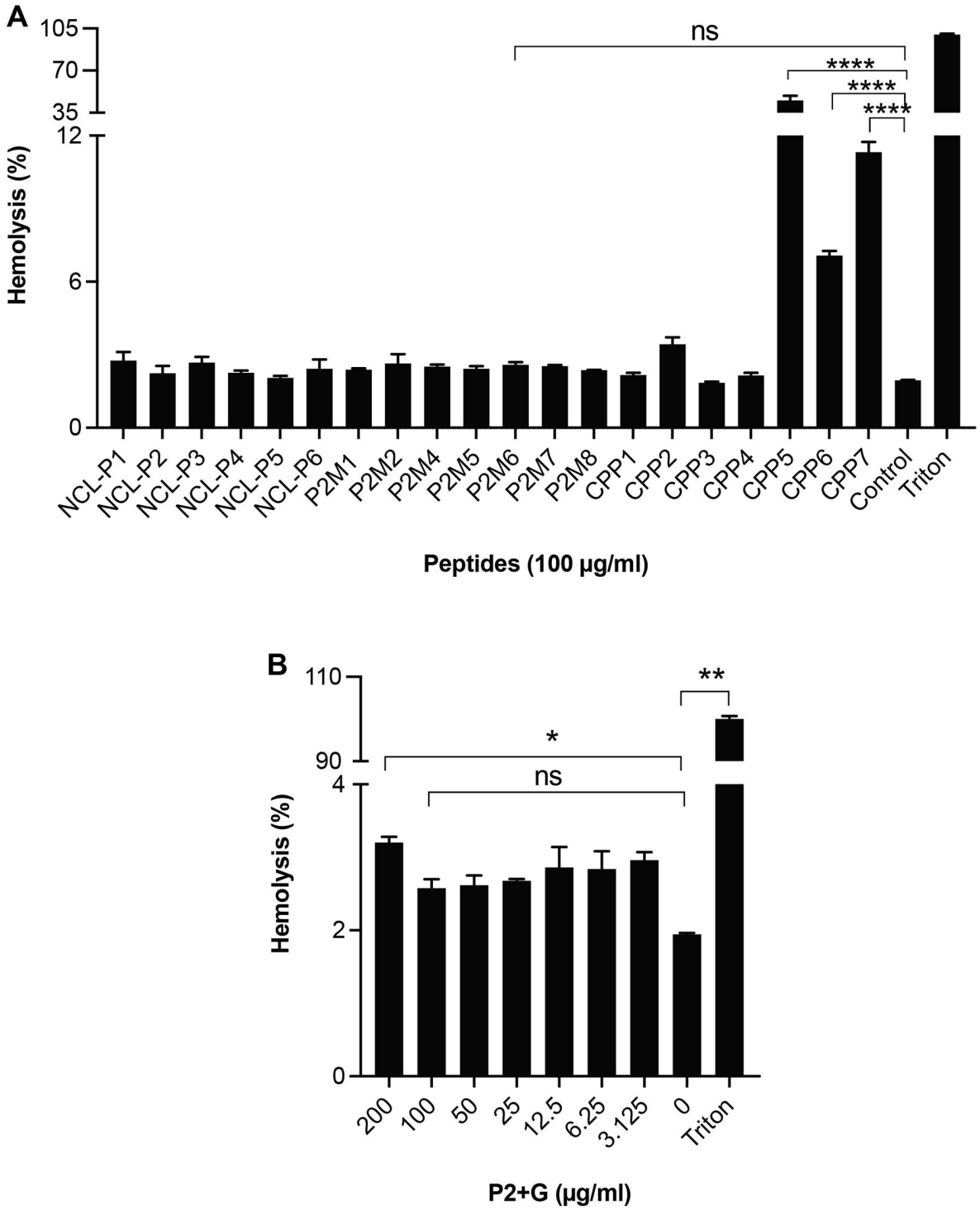
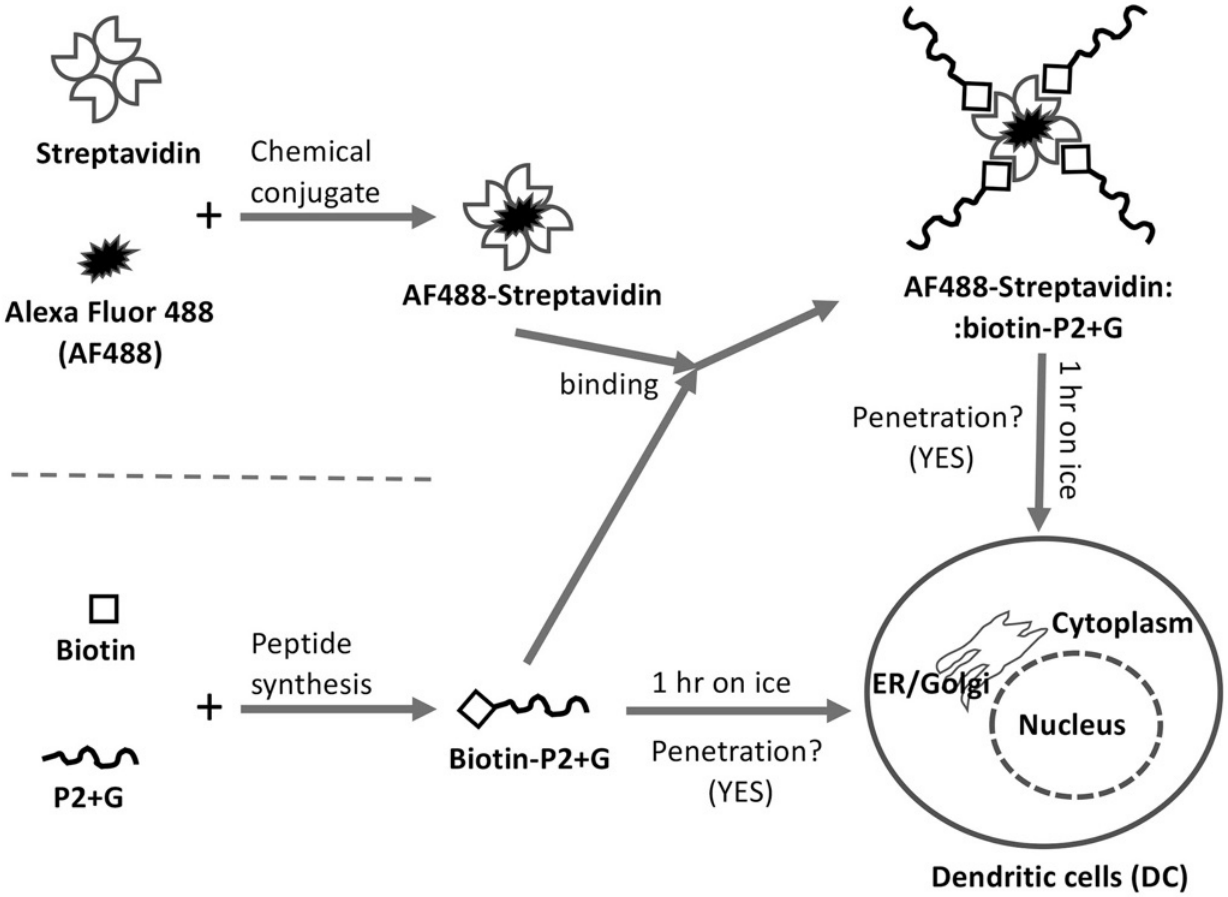

A
105
70
35
12
6
0
Hemolysis (%)
ns
****
****
****
NCL-P1
NCL-P2
NCL-P3
NCL-P4
NCL-P5
NCL-P6
P2M1
P2M2
P2M4
P2M5
P2M6
P2M7
P2M8
CPP1
CPP2
CPP3
CPP4
CPP5
CPP6
CPP7
Control
Triton
Peptides (100 µg/ml)
B
110
90
4
2
0
Hemolysis (%)
**
*
ns
200
100
50
25
12.5
6.25
3.125
0
Triton
P2+G (µg/ml)

Figure 26


Streptavidin
Chemical conjugate
Alexa Fluor 488 (AF488)
AF488-Streptavidin
AF488-Streptavidin: :biotin-P2+G
binding
Penetration? (YES)
1 hr on ice
Biotin
Peptide synthesis
P2+G
Biotin-P2+G
1 hr on ice
Penetration? (YES)
Cytoplasm
ER/Golgi
Nucleus
Dendritic cells (DC)