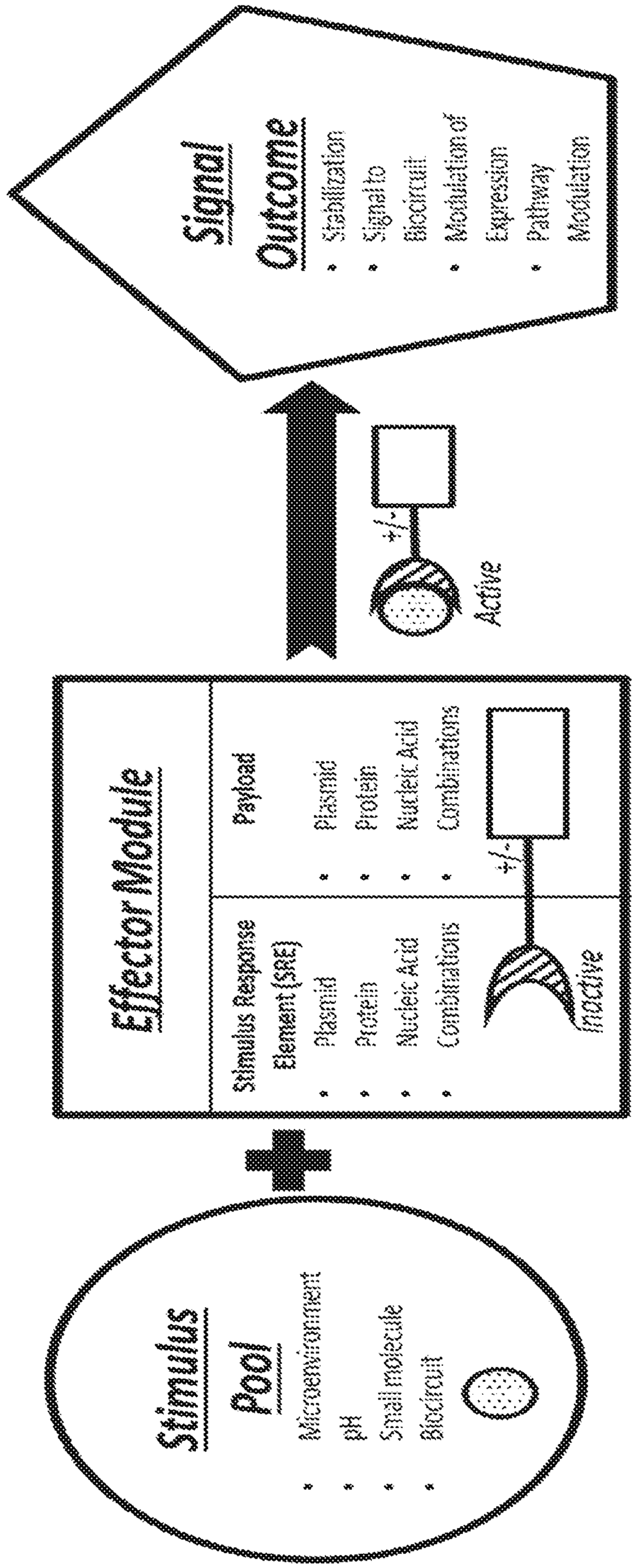
Figure 1


Stimulus Pool
Microenvironment
pH
Small molecule
Biocircuit
Effector Module
Stimulus Response Element (SRE)
Plasmid
Protein
Nucleic Acid
Combinations
Payload
Plasmid
Protein
Nucleic Acid
Combinations
+/-
Inactive
+/-
Active
Signal Outcome
Stabilization
Signal to Biocircuit
Modulation of Expression Pathway Modulation

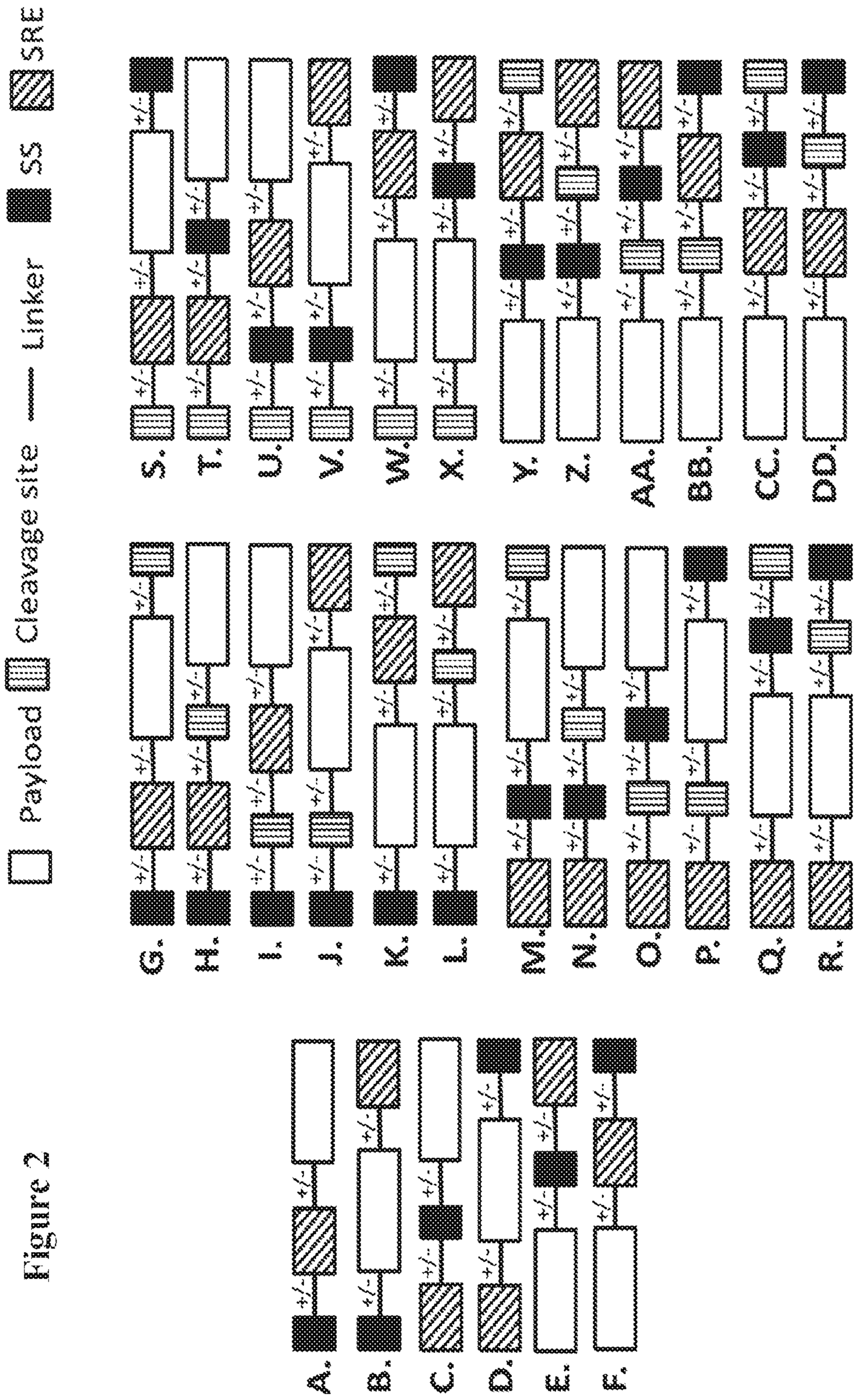

SRE
SS
Linker
Cleavage site
Payload
+/-
A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.
L.
M.
N.
O.
P.
Q.
R.
S.
T.
U.
V.
W.
X.
Y.
Z.
AA.
BB.
CC.
DD.

Figure 2

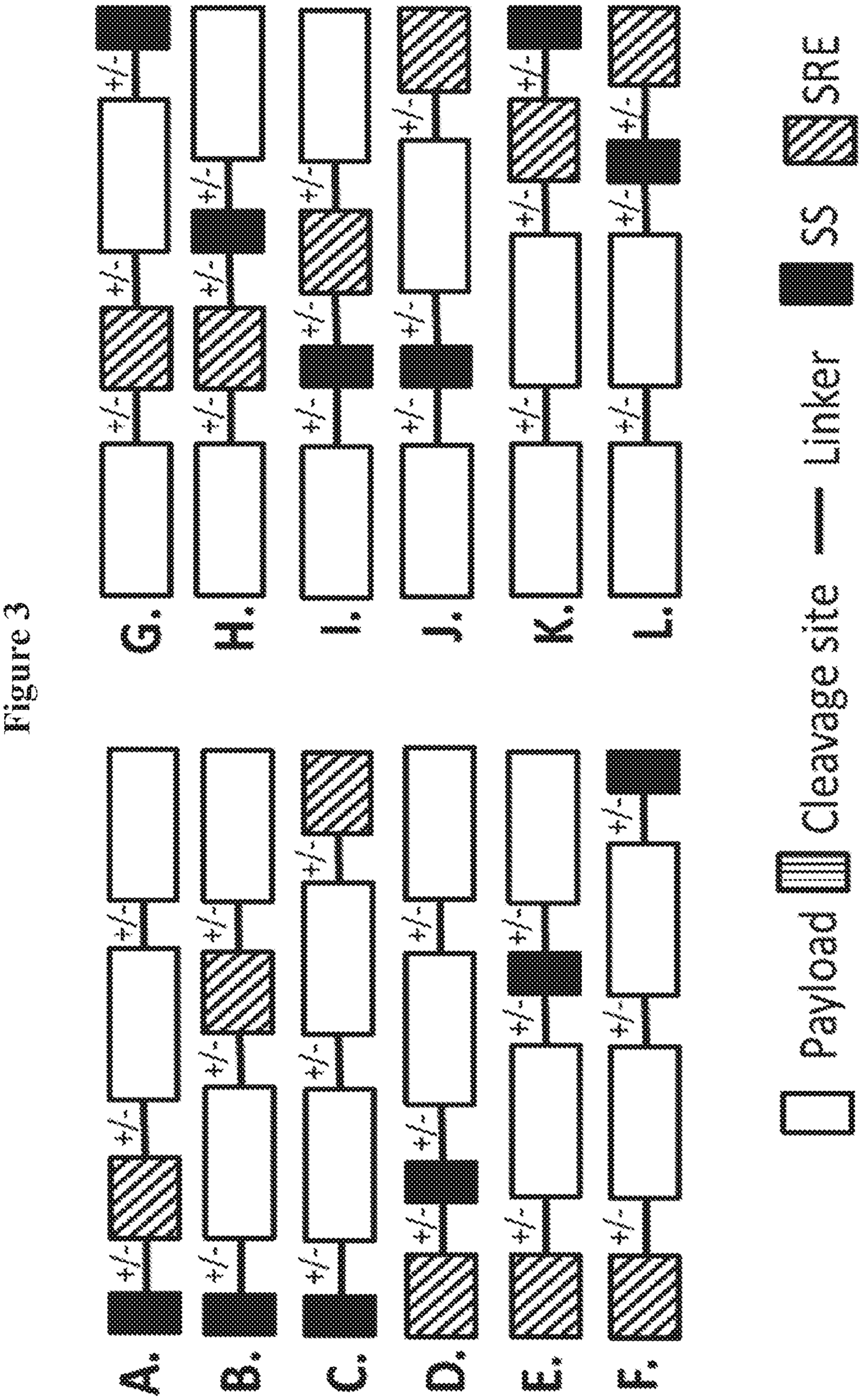


Figure 3

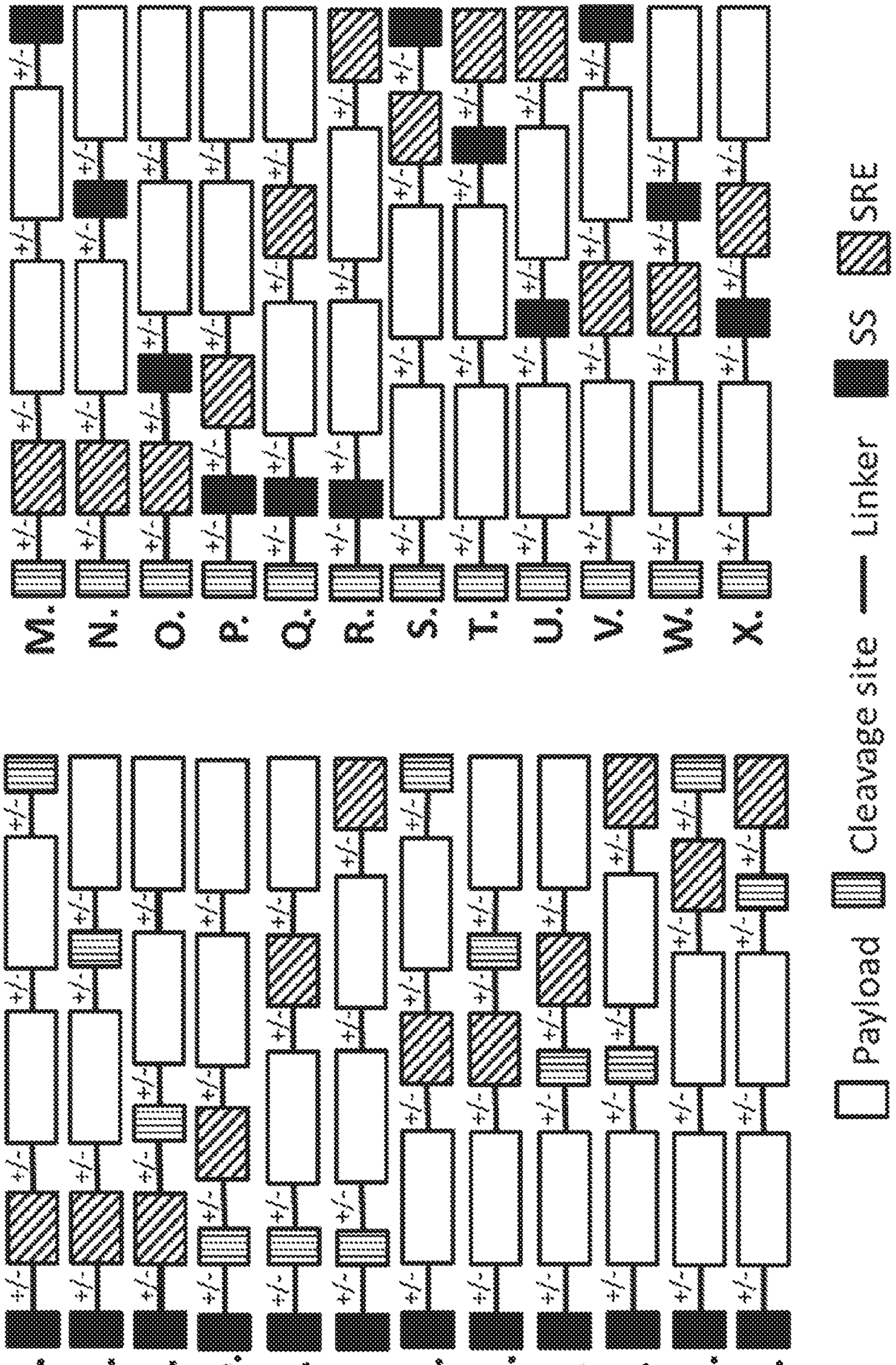
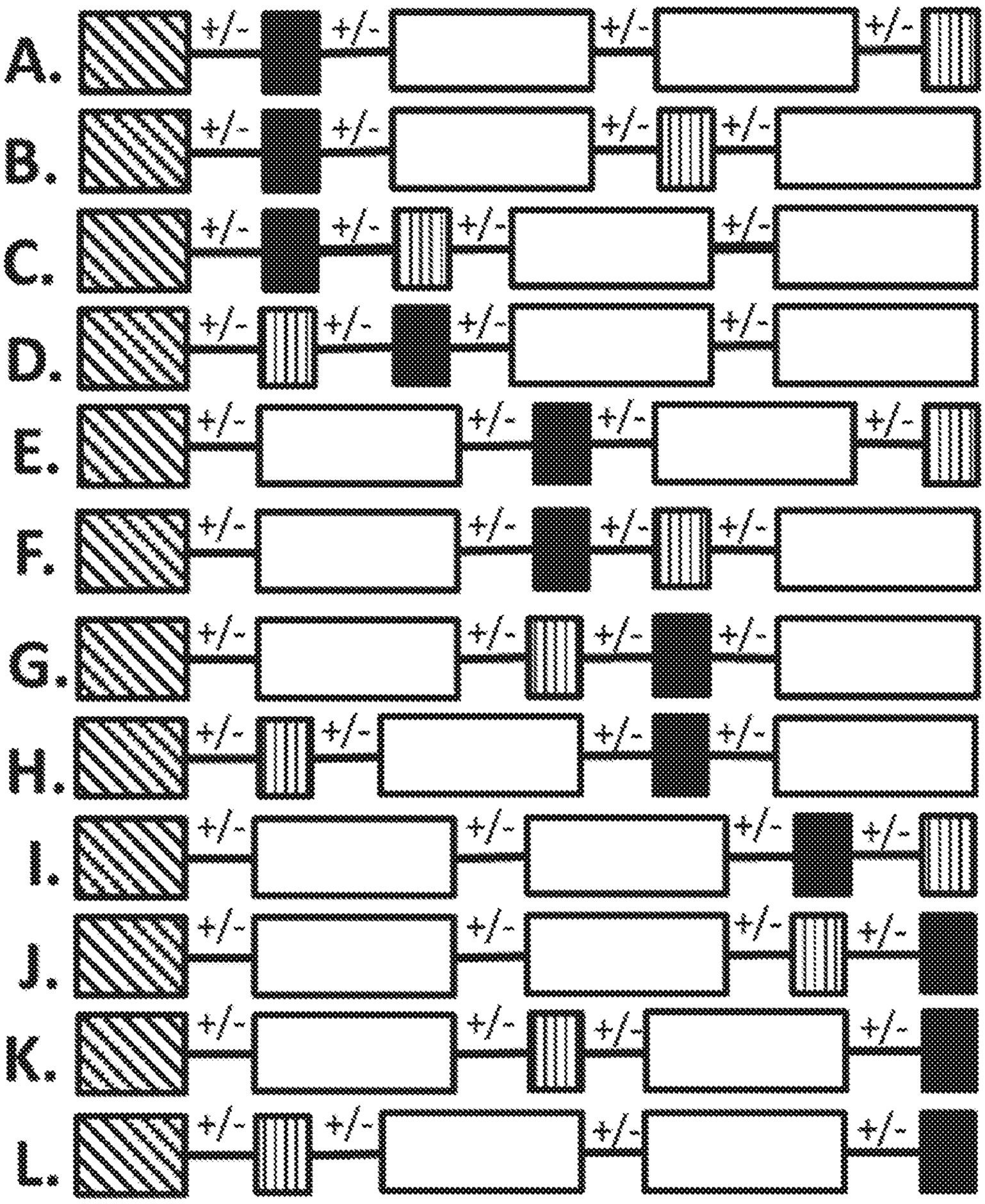

M.
N.
O.
P.
Q.
R.
S.
T.
U.
V.
W.
X.
Payload
Linker
SS
SRE
Cleavage site
A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.
L.

Fig. 4

Figure 5



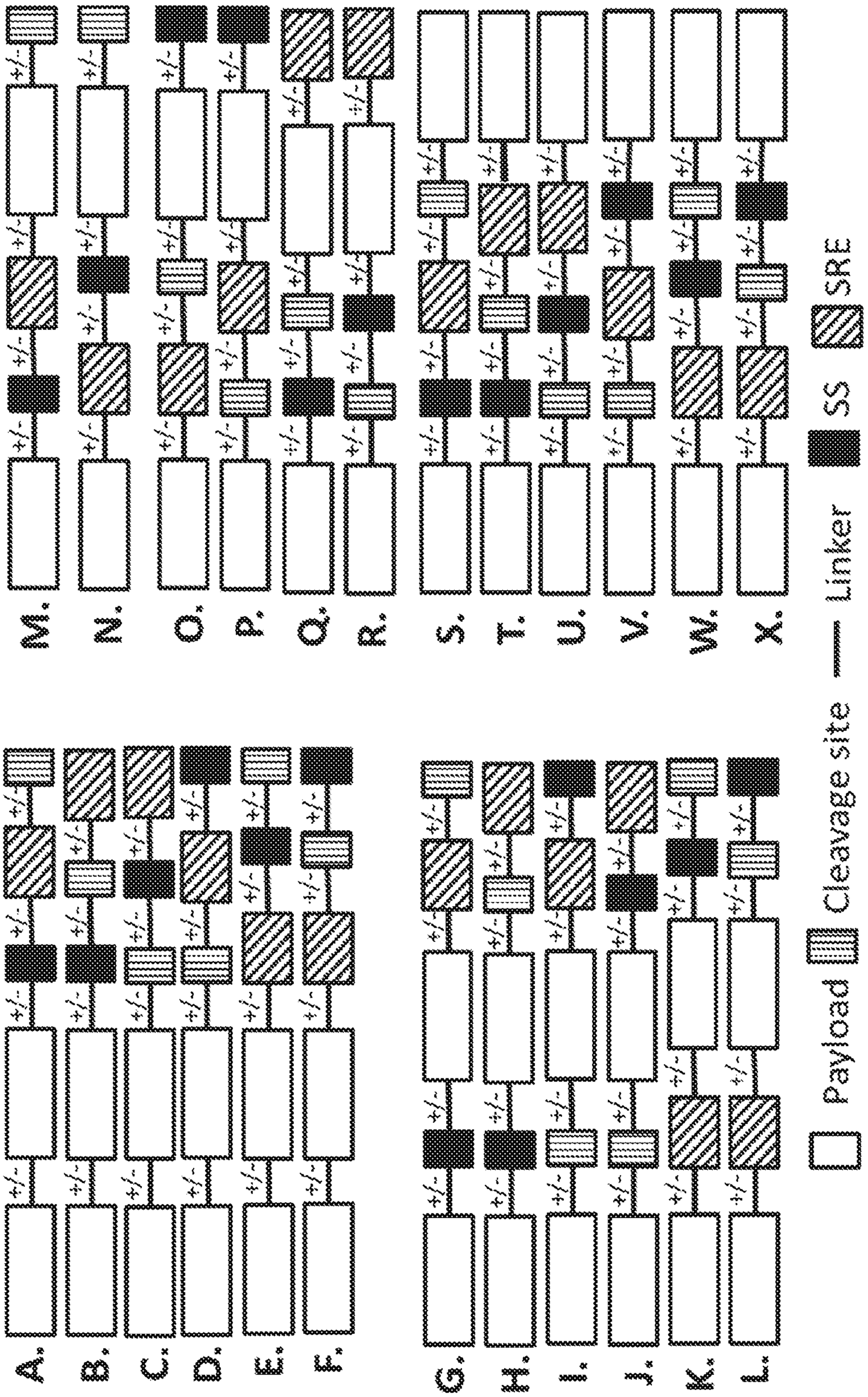

M.
N.
O.
P.
Q.
R.
S.
T.
U.
V.
W.
X.
Linker
SS
SRE
Payload
Cleavage site
A.
B.
C.
D.
E.
F.
G.
H.
I.
J.
K.
L.

Figure 6

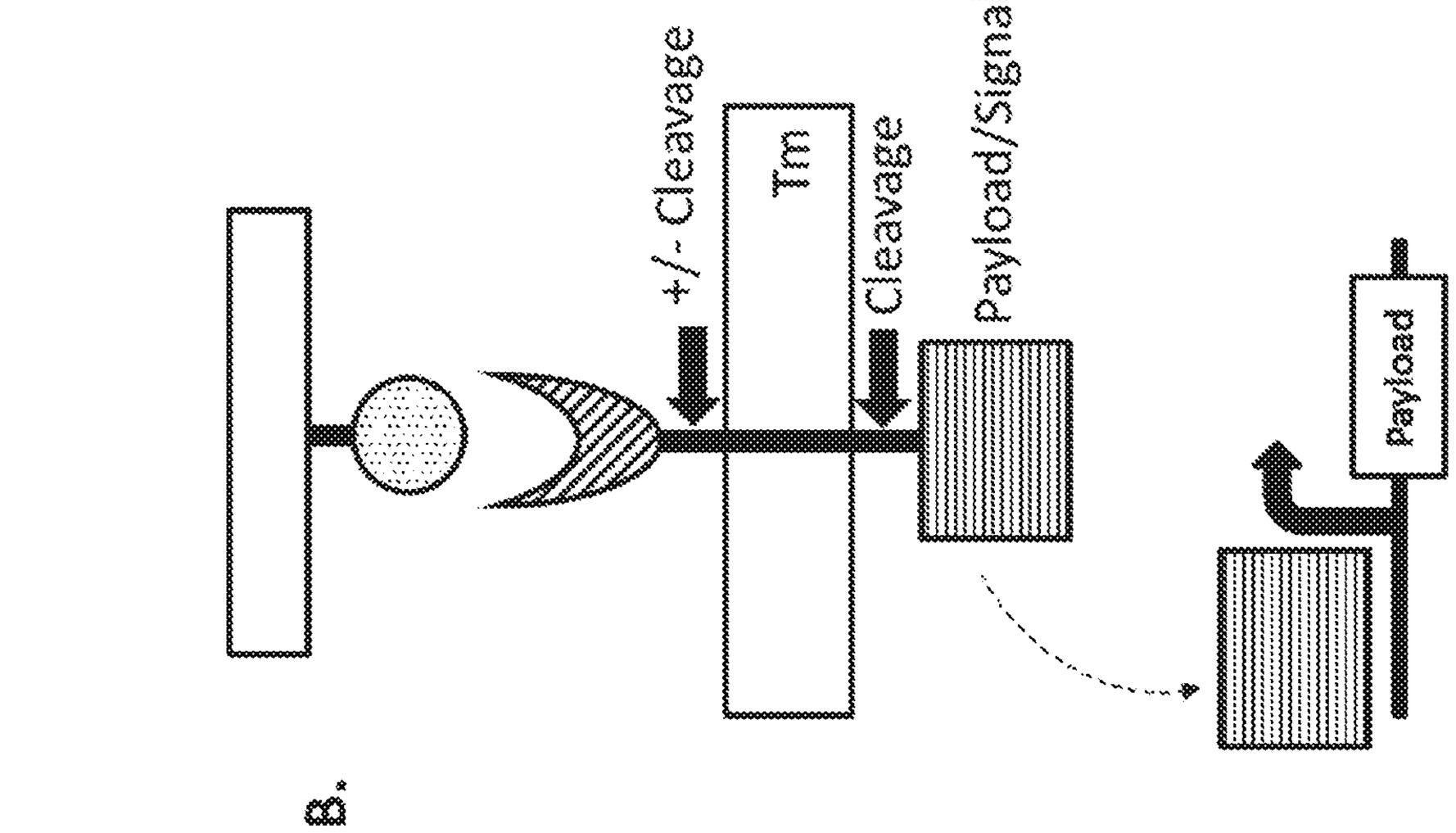
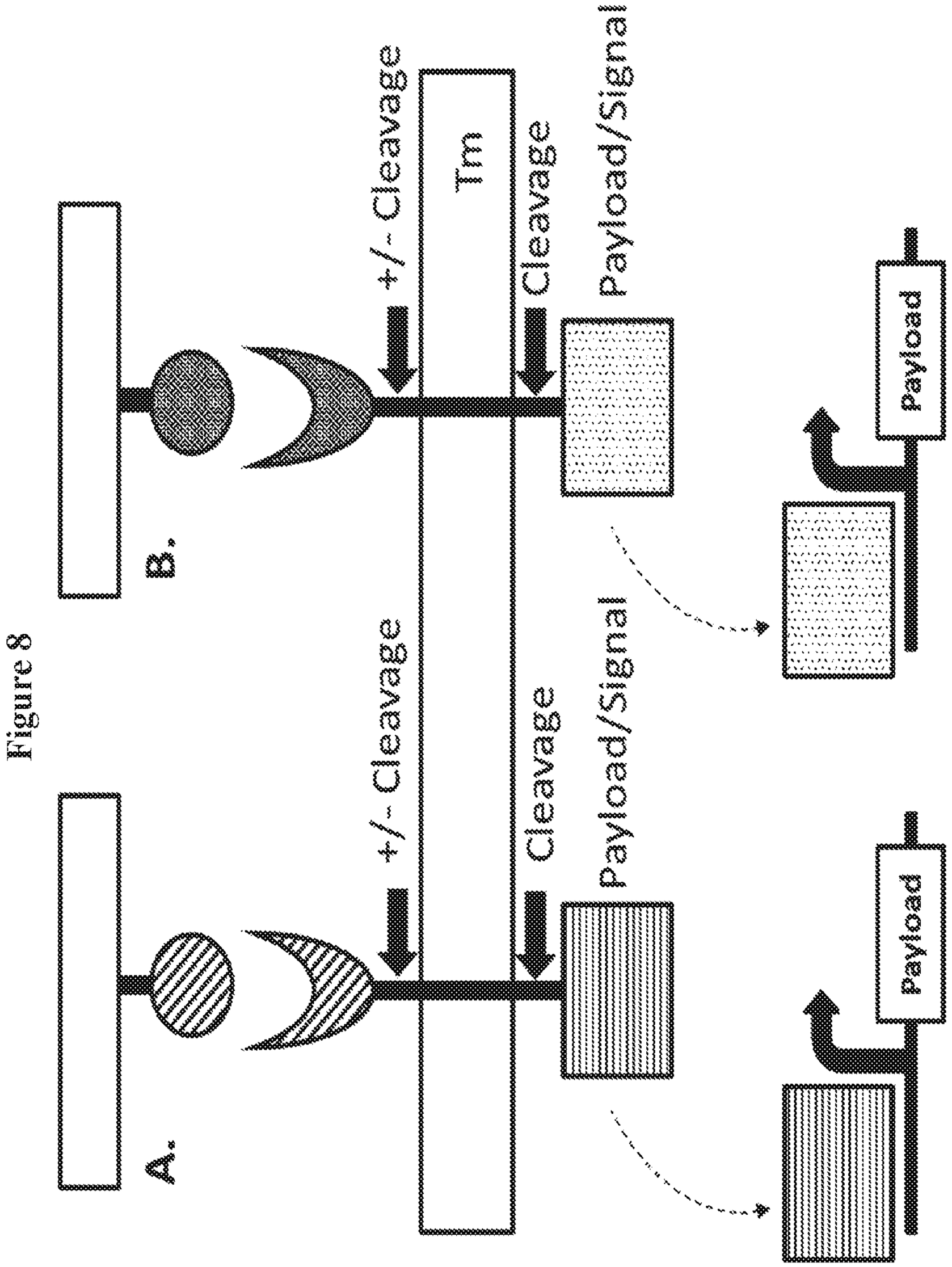


Figure 7


Figure 8
a.
+/- Cleavage
Tm
Cleavage
Payload/Signal
Payload
b.
+/- Cleavage
Tm
Cleavage
Payload/Signal
Payload

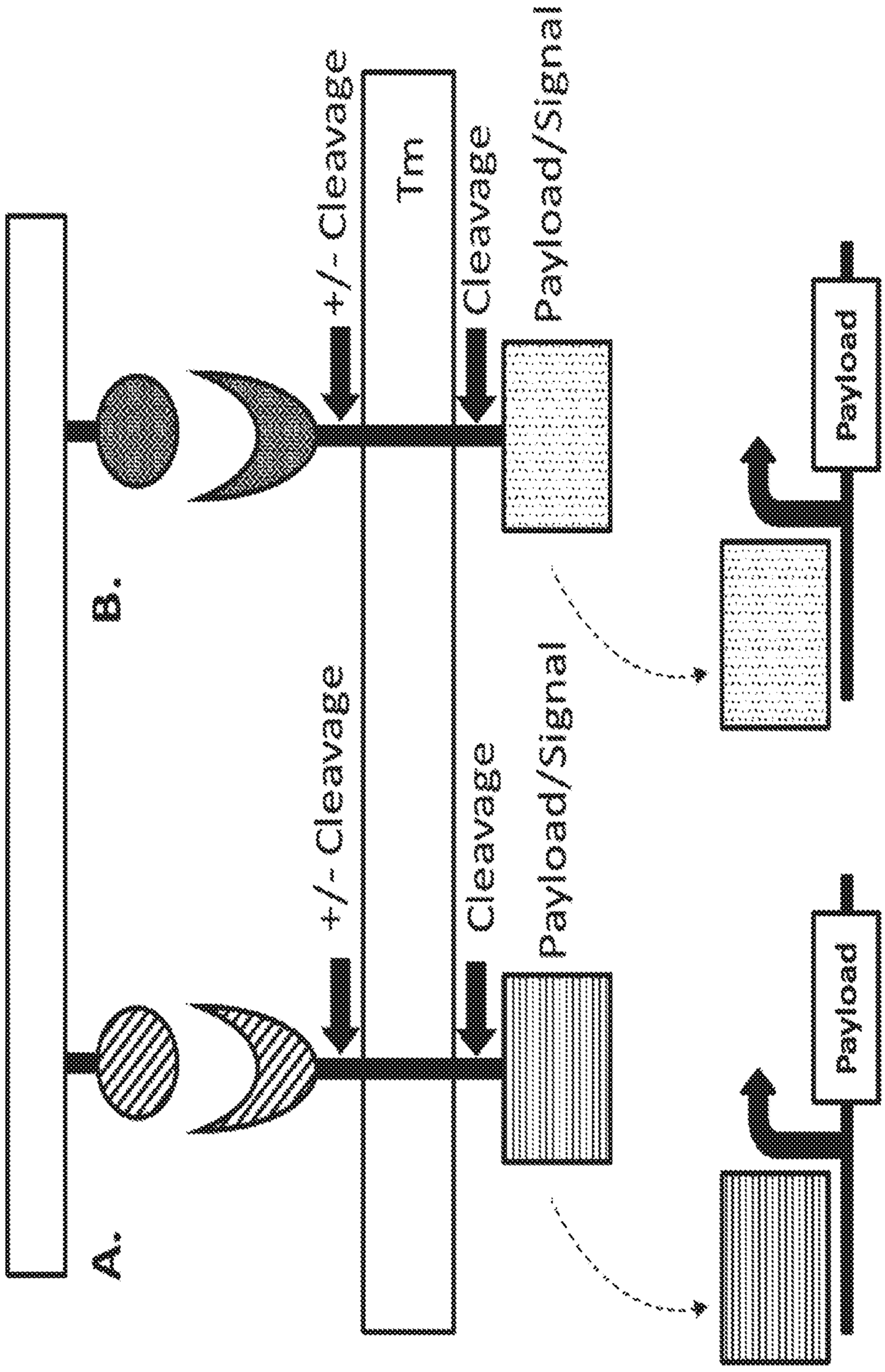

a.
+/- Cleavage
Tm
Cleavage
Payload/Signal
Payload
b.
+/- Cleavage
Cleavage
Payload/Signal
payload

Figure 9

Figure 10

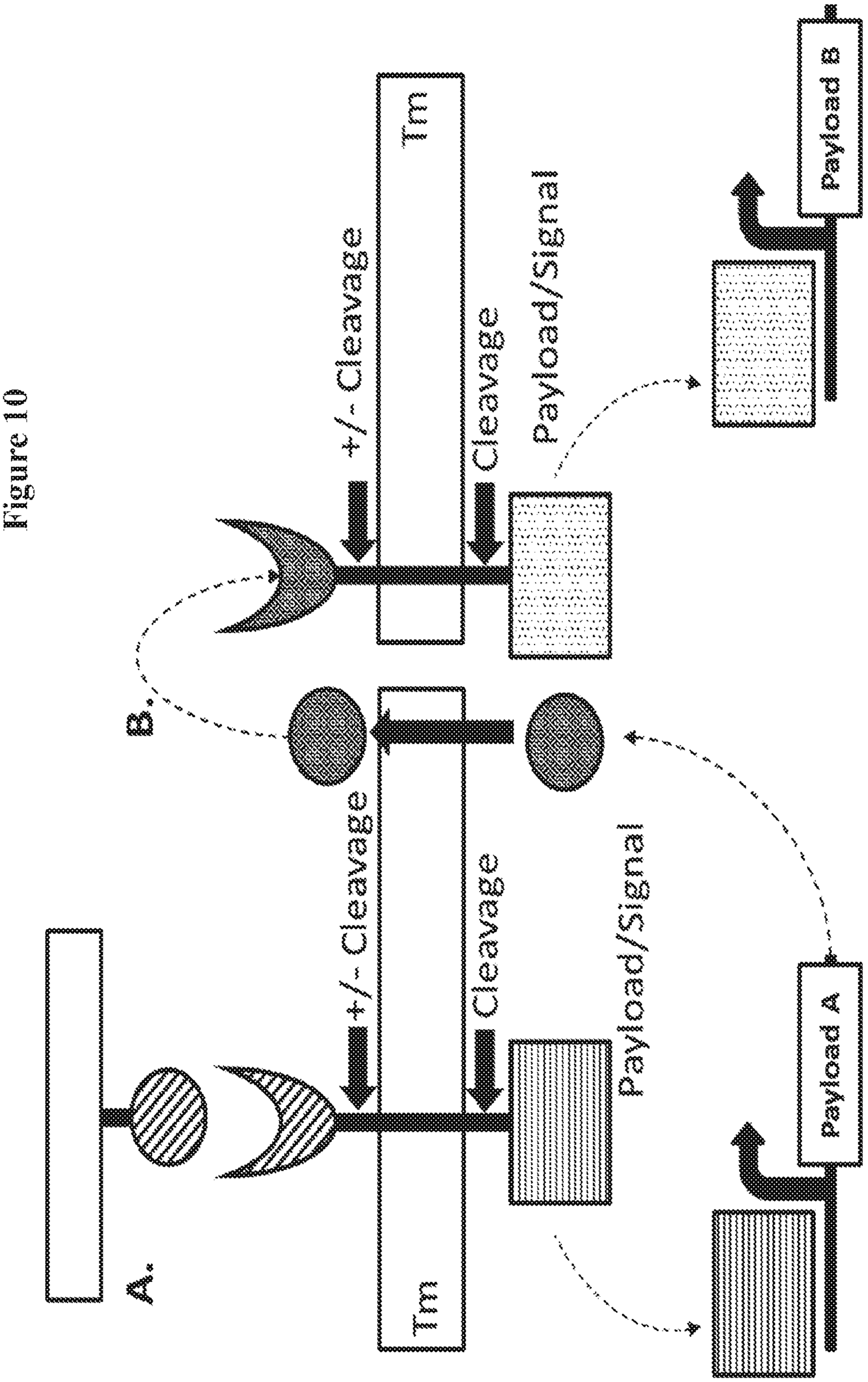

A.
Tm
+/- Cleavage
Cleavage
Payload/Signal
Payload A
B.
Tm
+/- Cleavage
Cleavage
Payload/Signal
Payload B

Figure 11

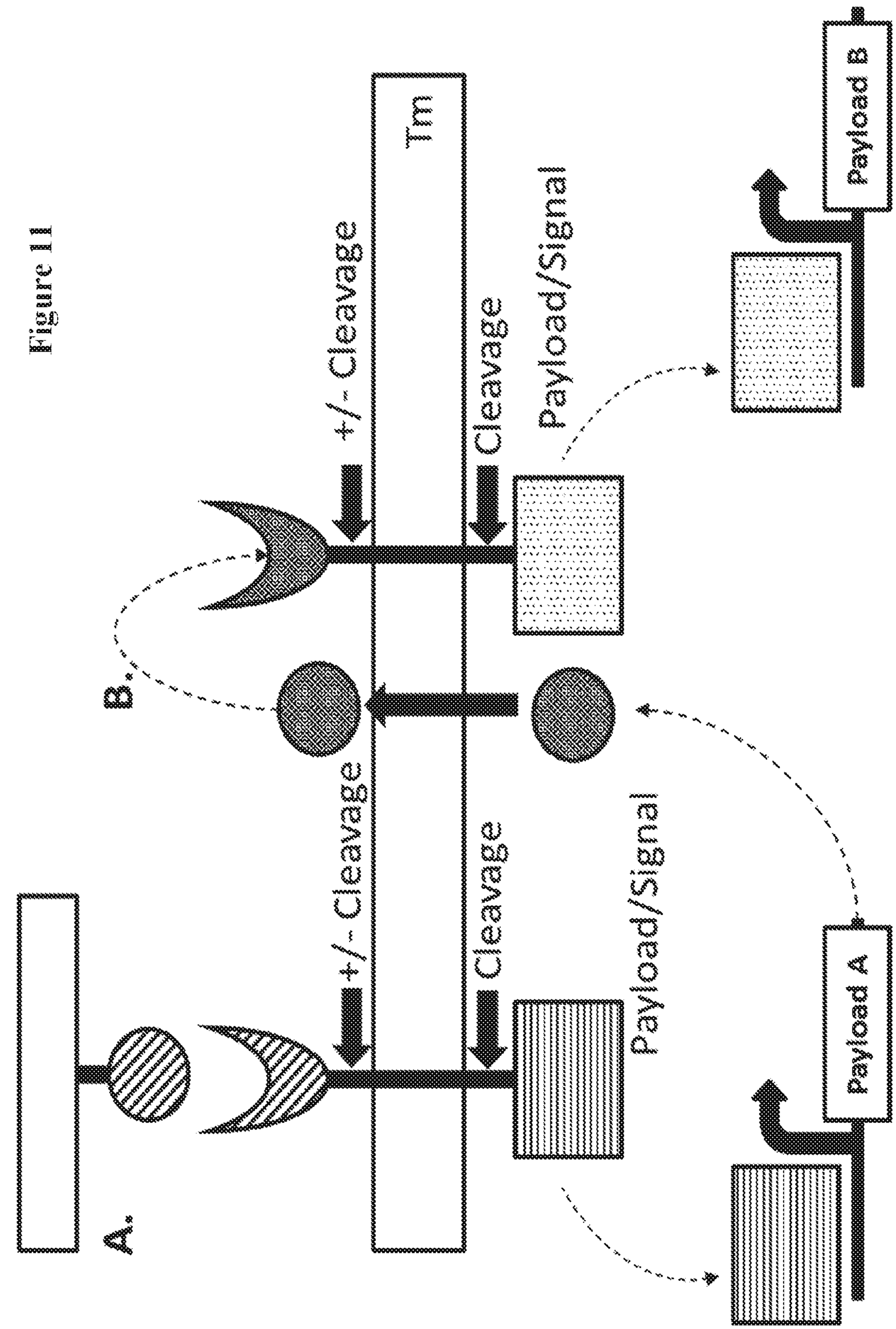

Payload A
Payload/Signal
+/- Cleavage
Tm
Cleavage
Payload/Signal
Payload A
Payload/Signal
+/- Cleavage
Cleavage
Payload/Signal
Payload A

Figure 12

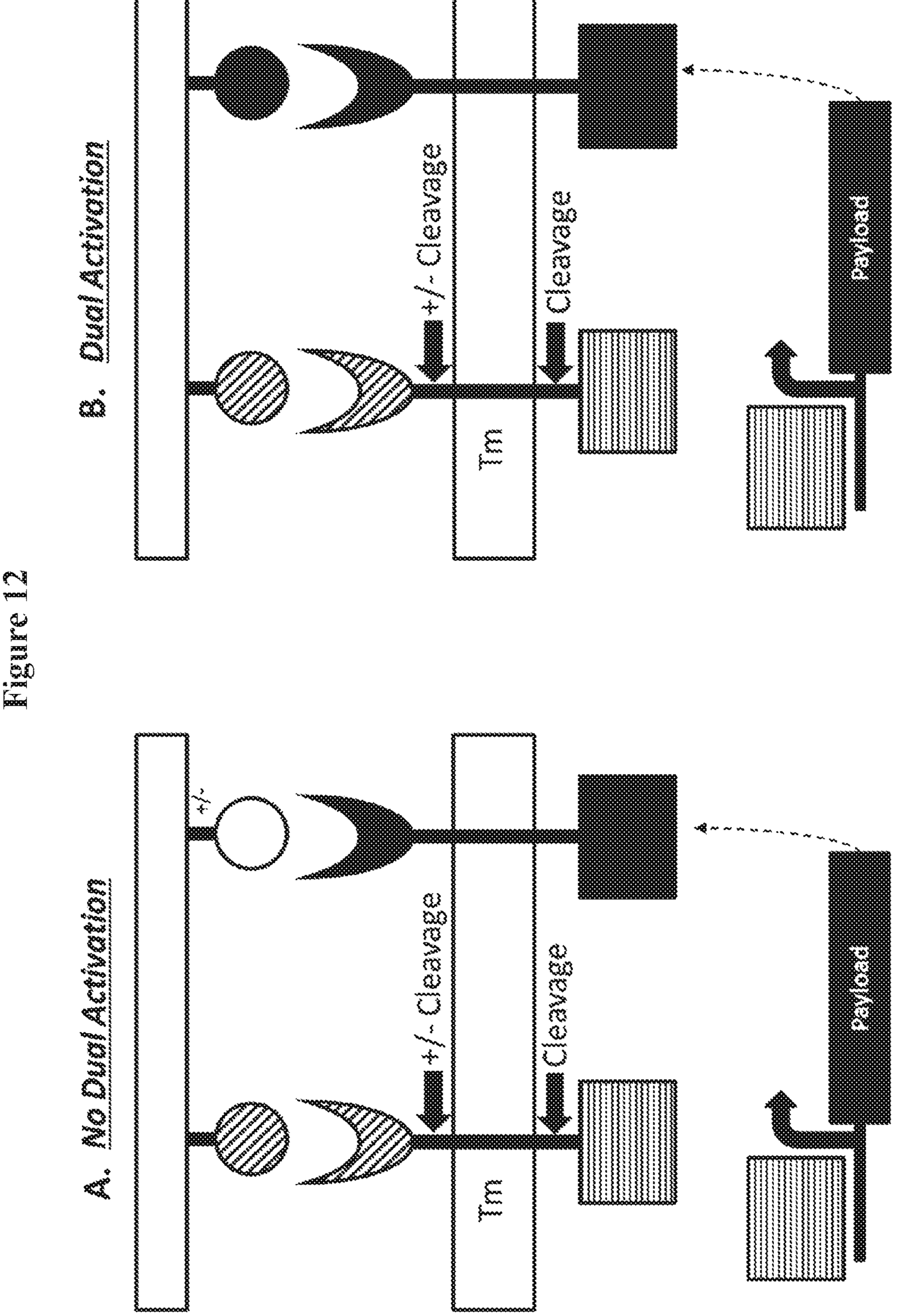

A. No Dual Activation
+/-
+/- Cleavage
Cleavage
Tm
Payload
B. Dual Activation
+/-
+/- Cleavage
Cleavage
Tm
Payload

Figure 13

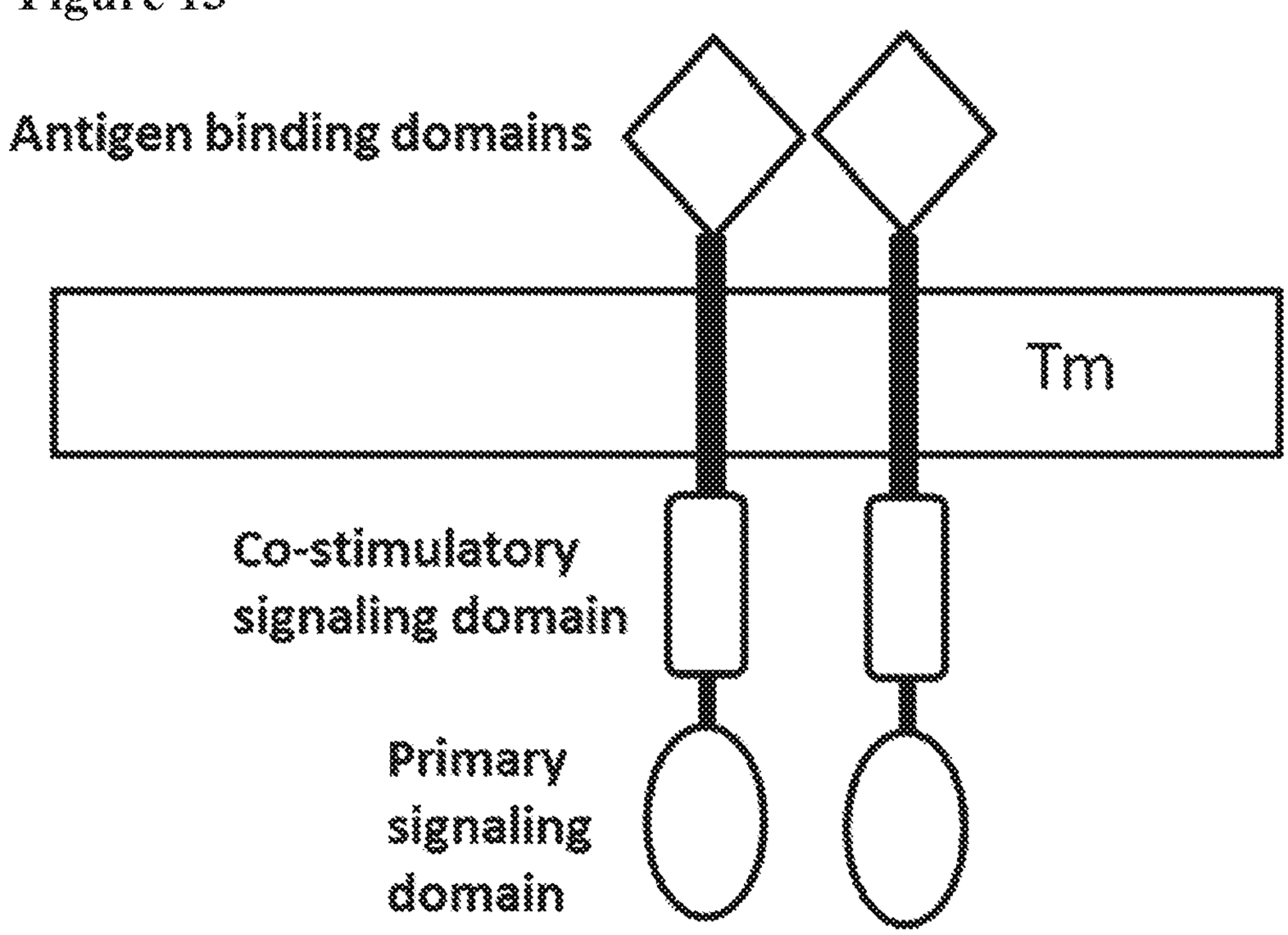


Figure 14

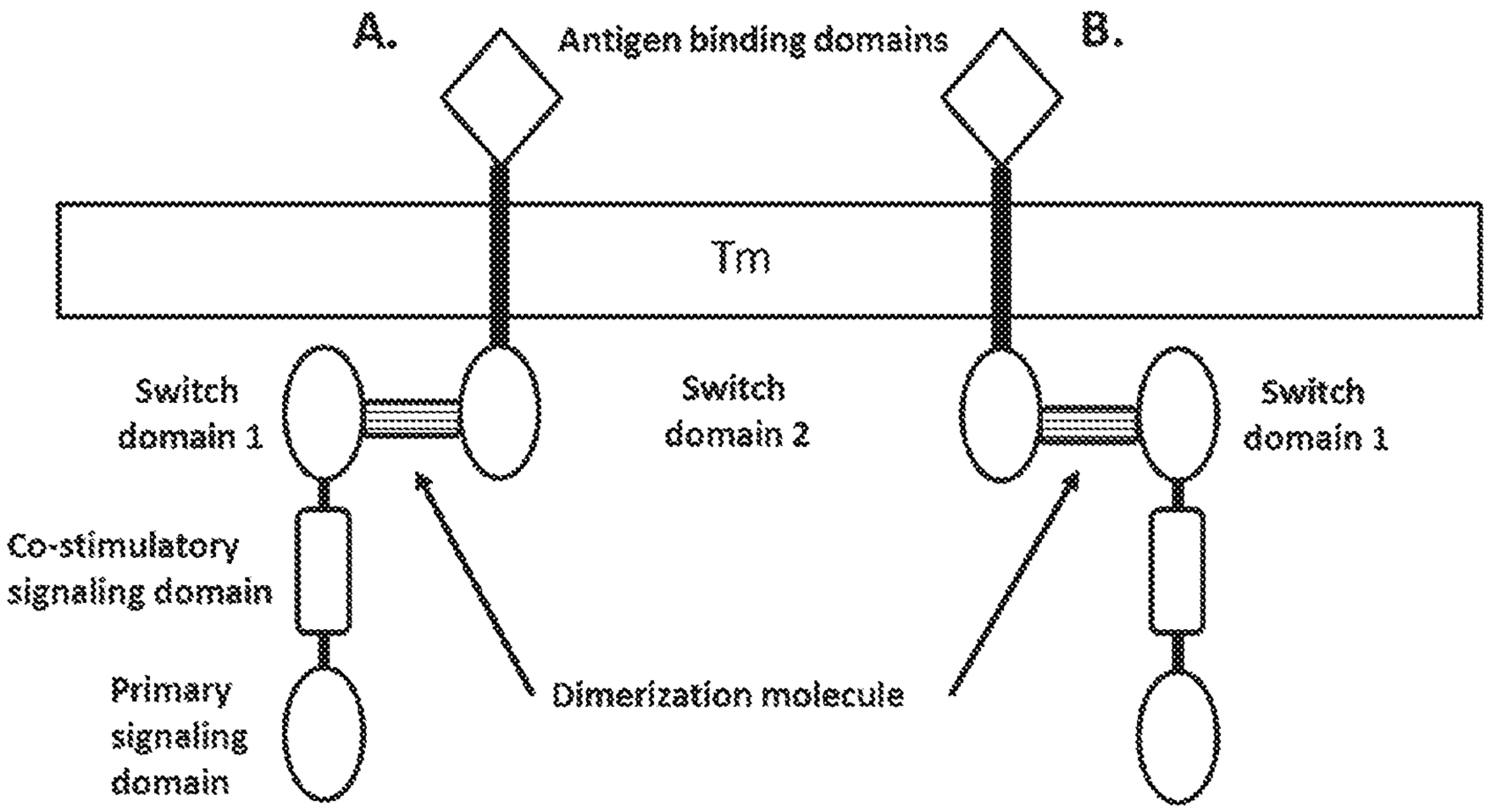
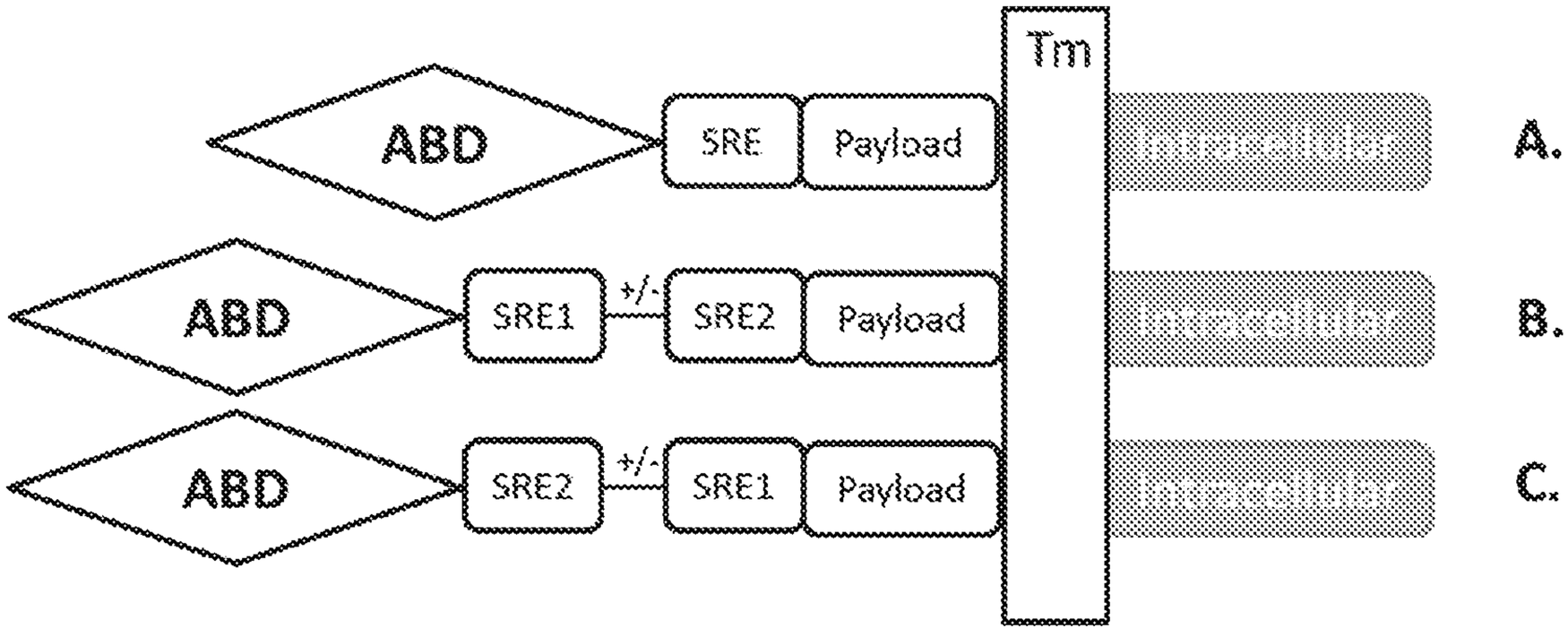


Figure 15


Tm
ABD
SRE
Payload
A.
ABD
SRE1
+/-
SRE2
Payload
B.
ABD
SRE2
+/-
SRE1
Payload
C.

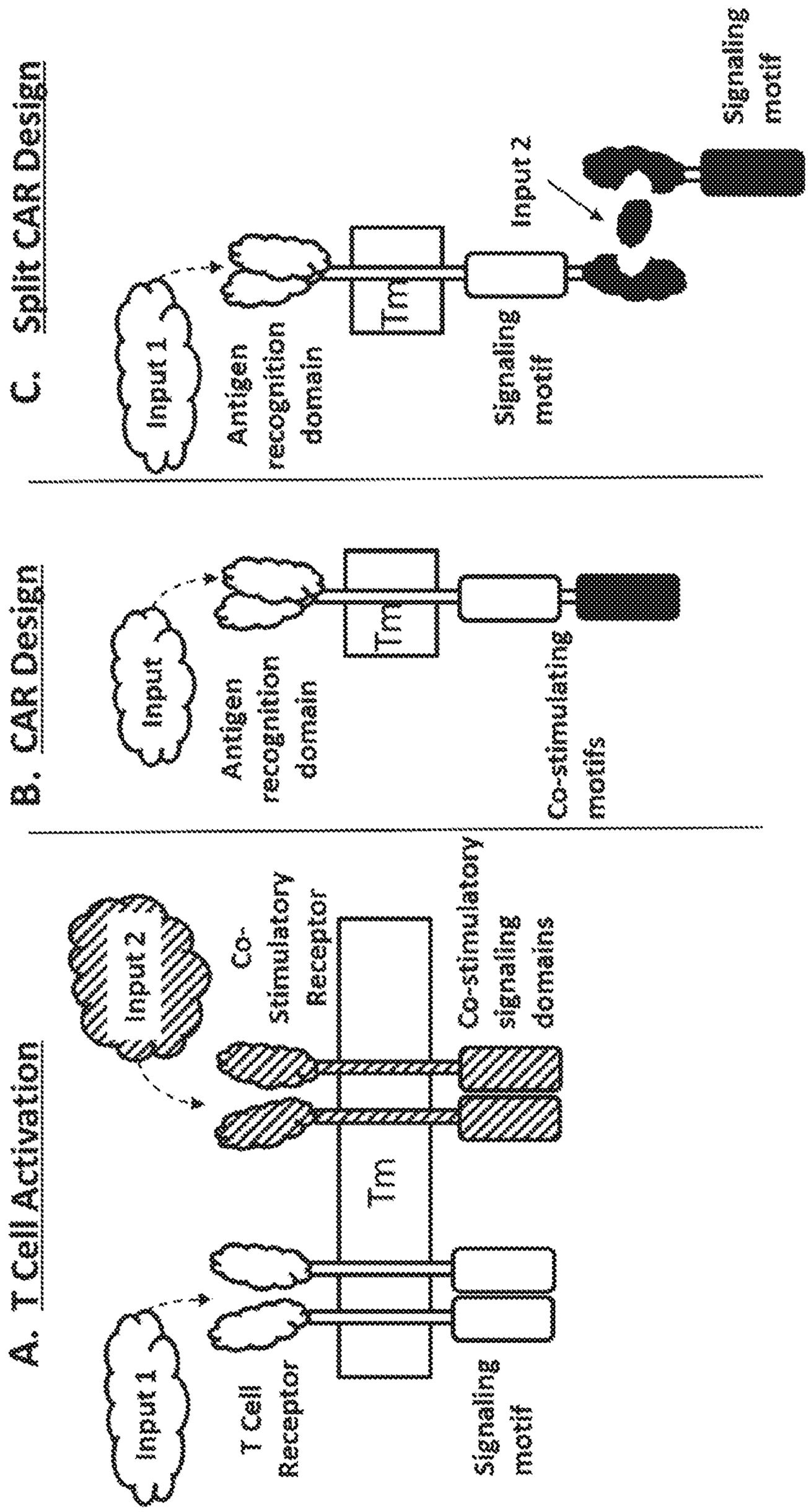

A. T cell Activation
Input 1
T Cell Receptor
Input 2
Co-Stimulatory Receptor
Tm
Signaling motif
Co-stimulatory signaling domains
B. CAR Design
Input 1
Antigen recognition domain
Tm
Co-stimulating motifs
C. Split CAR Design
Input 1
Antigen recognition domain
Tm
Signaling motif
Input 2
Signaling motif

Figure 16

Figure 17

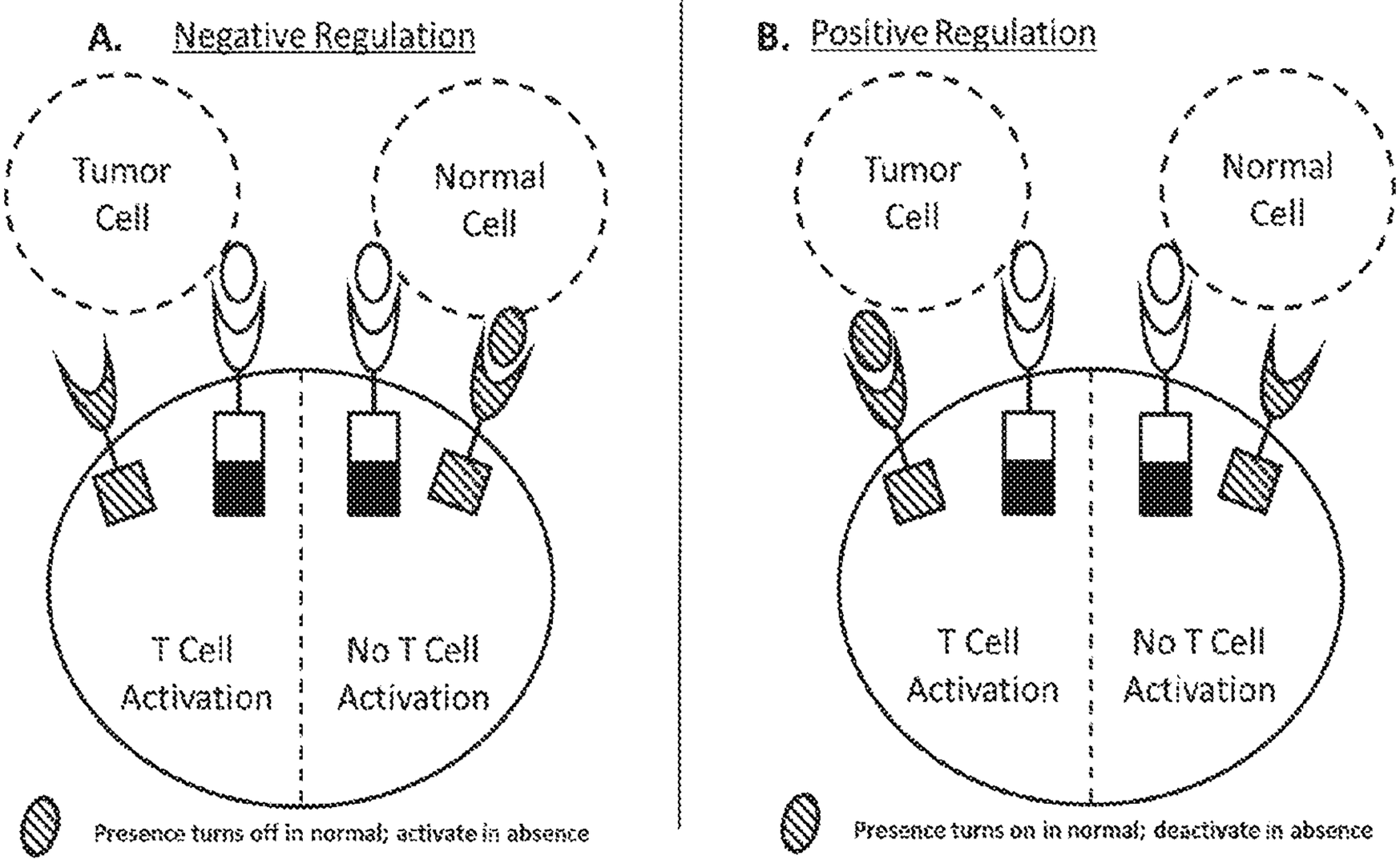


Figure 18

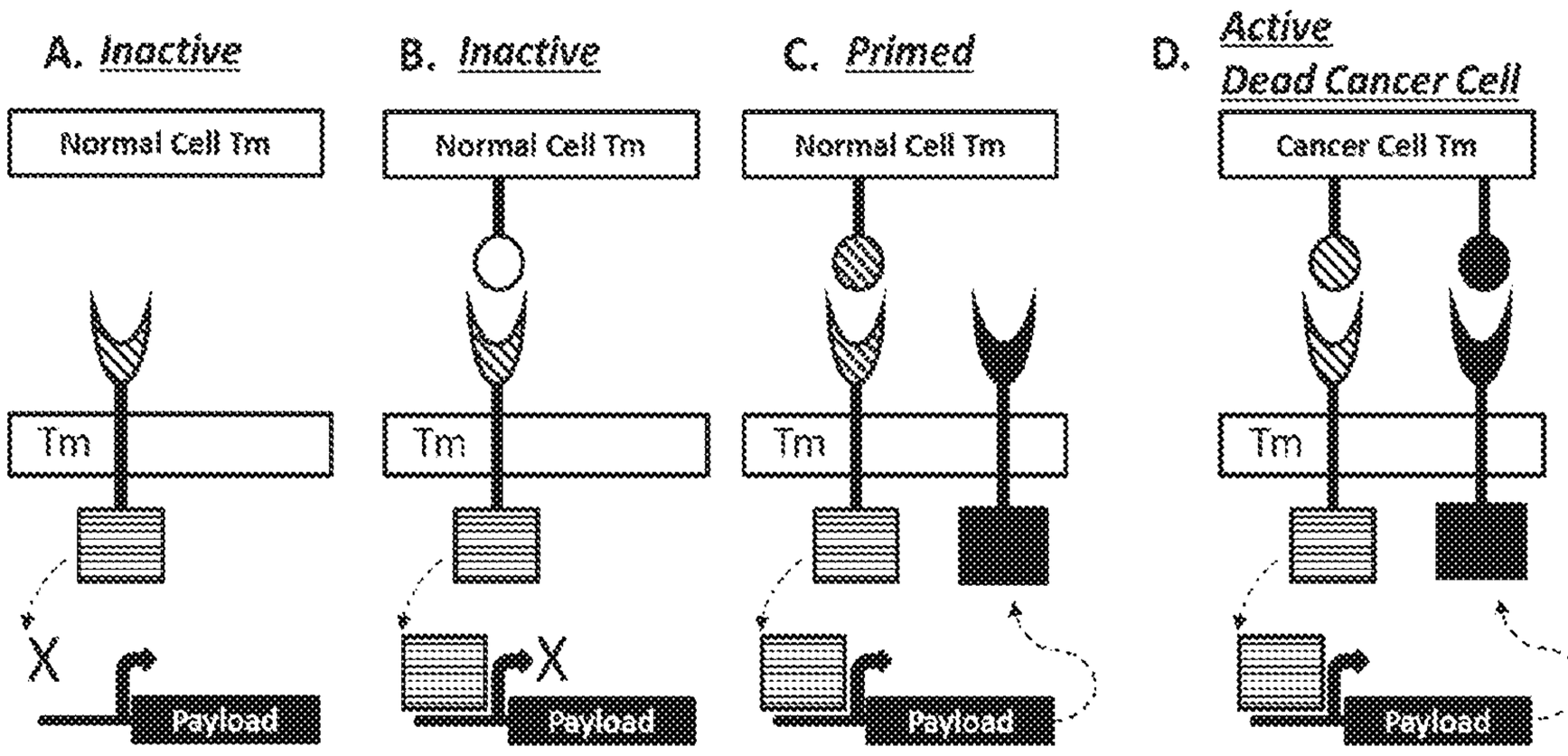
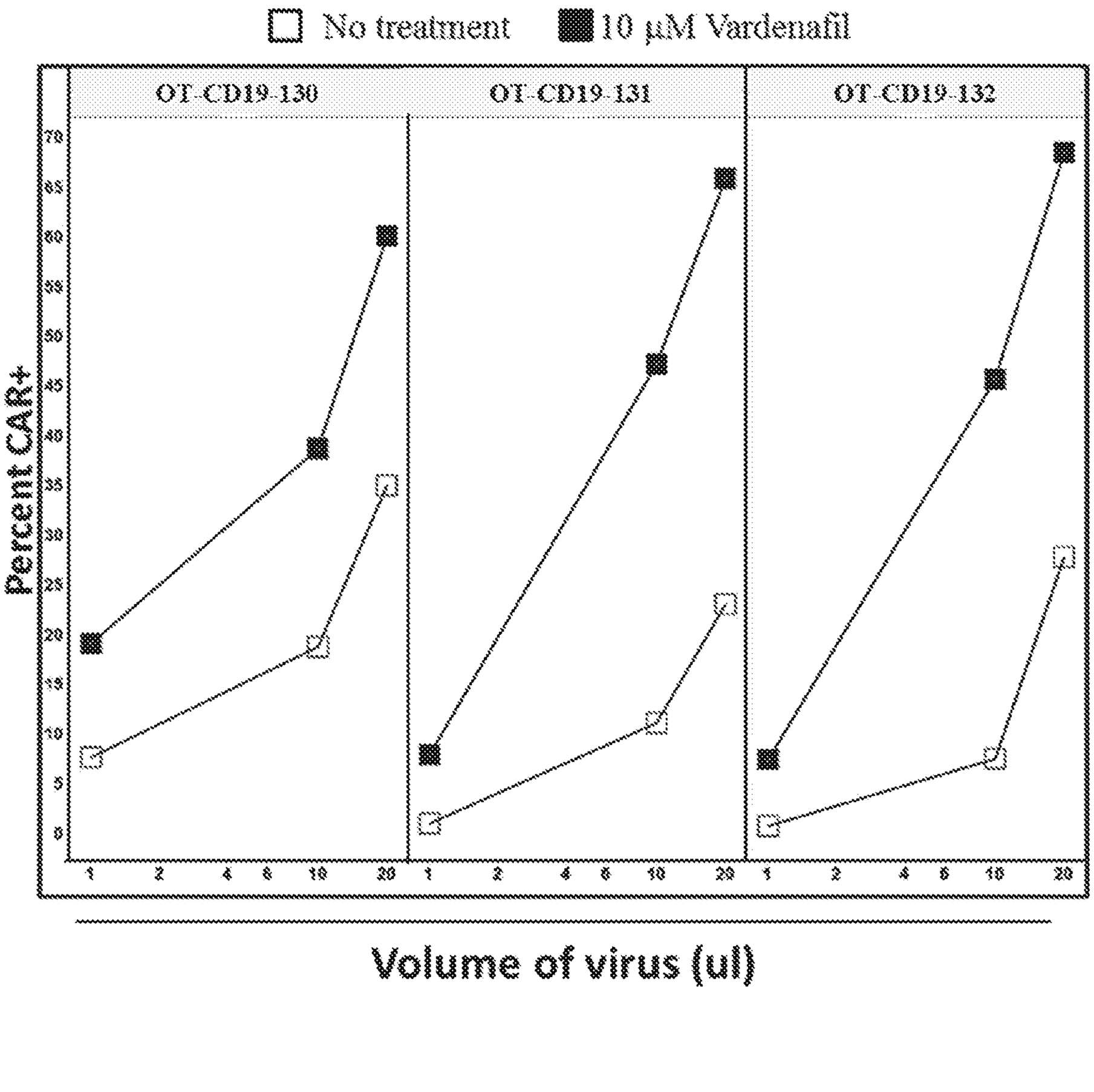


Figure 19A


No treatment
10 μM Vardenafil
OT-CD19-130
OT-CD19-131
OT-CD19-132
Percent CAR+
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
1
2
4
6
10
20
Volume of virus (ul)

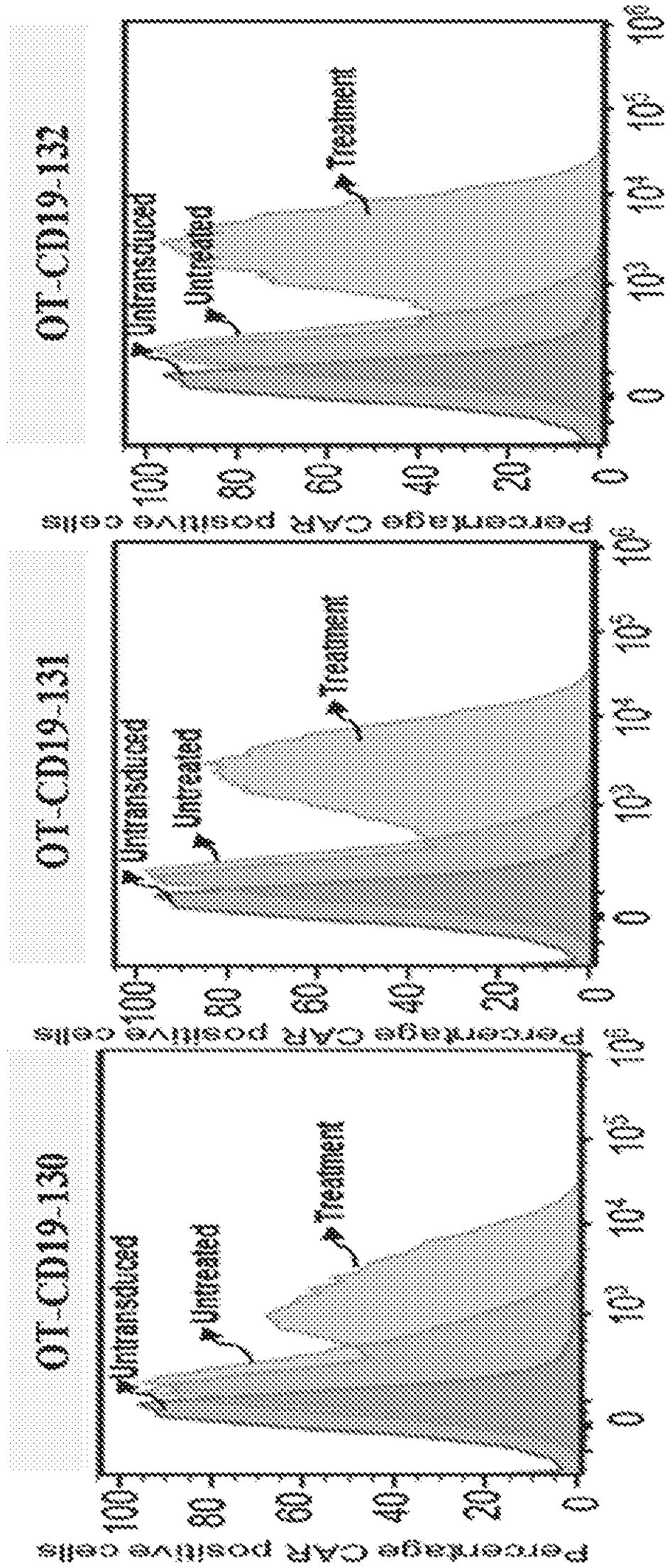

Percentage CAR positive cells
0
20
40
60
80
100
0
10³
10⁴
10⁵
Untransduced
Untreated
Treatment
OT-CD19-132
OT-CD19-131
OT-CD19-130

Figure 19B

Figure 19C

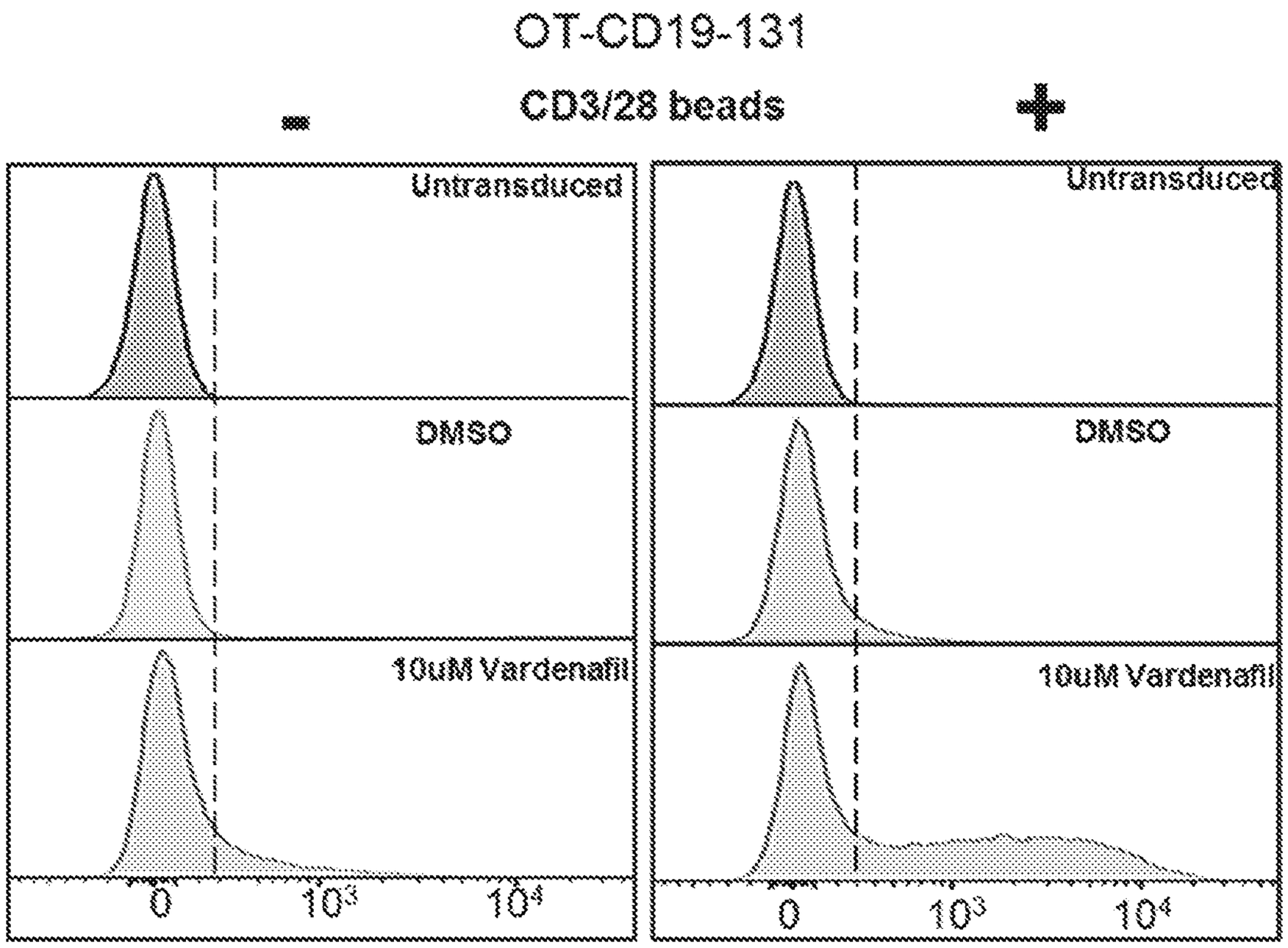
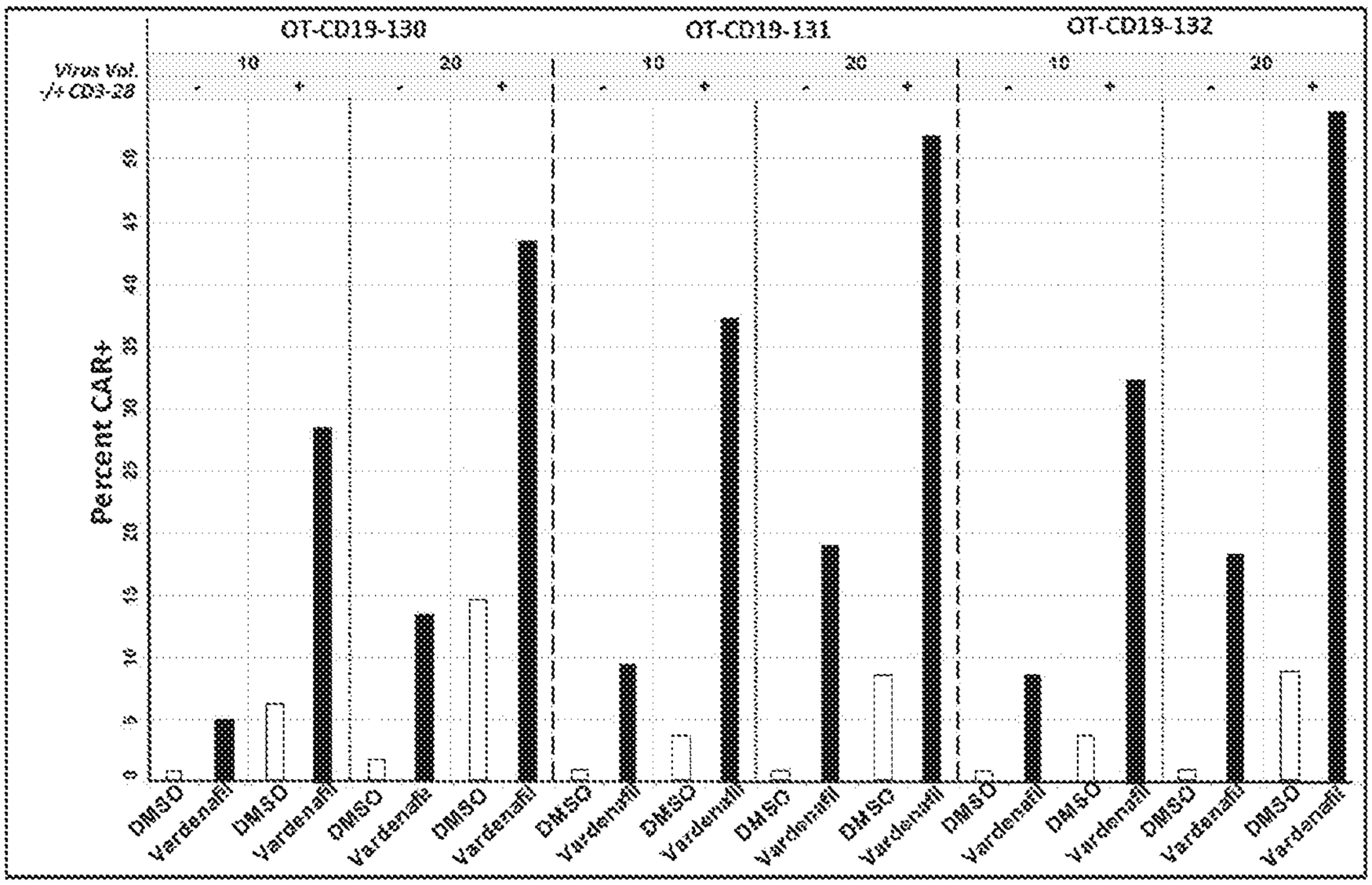

OT-CD19-131
CD3/28 beads
-
+
Untransduced
DMSO
10uM Vardenafil
Untransduced
DMSO
10uM Vardenafil
0
10^3
10^4
0
10^3
10^4

Figure 19D


OT-CD19-130
OT-CD19-131
OT-CD19-132
Virus Vol.
-/+ CD3-28
10
20
-
+
Percent CAR+
0
5
10
15
20
25
30
35
40
45
50
DMSO
Vardenafil

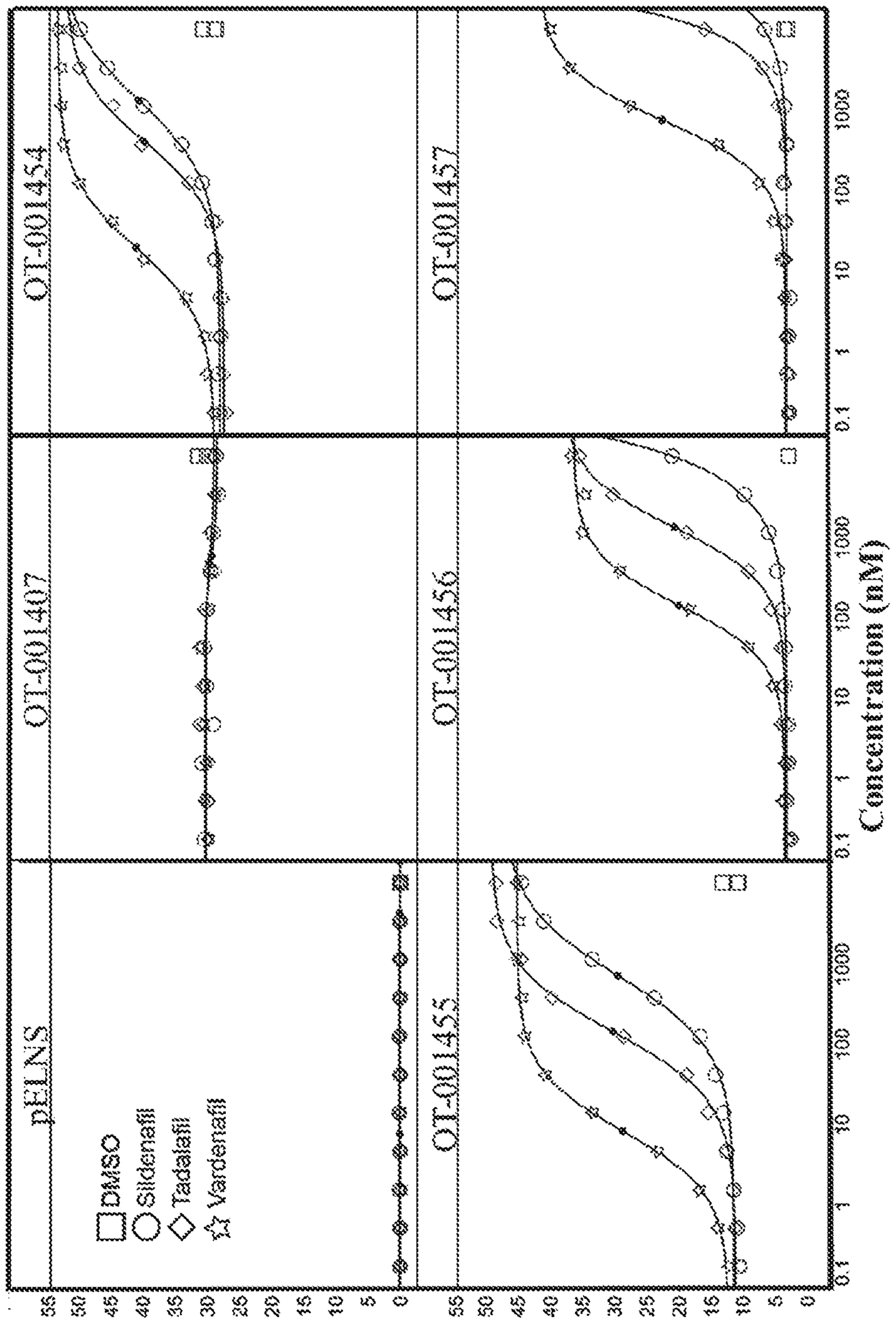

OT-001454
OT-001457
OT-001407
OT-001456
pELNS
OT-001455
DMSO
Sildenafil
Tadalafil
Vardenafil
0.1
1
10
100
1000
Concentration (nM)
Sinusoidal/Live CAR+:
Fold of Parent

Figure 19E

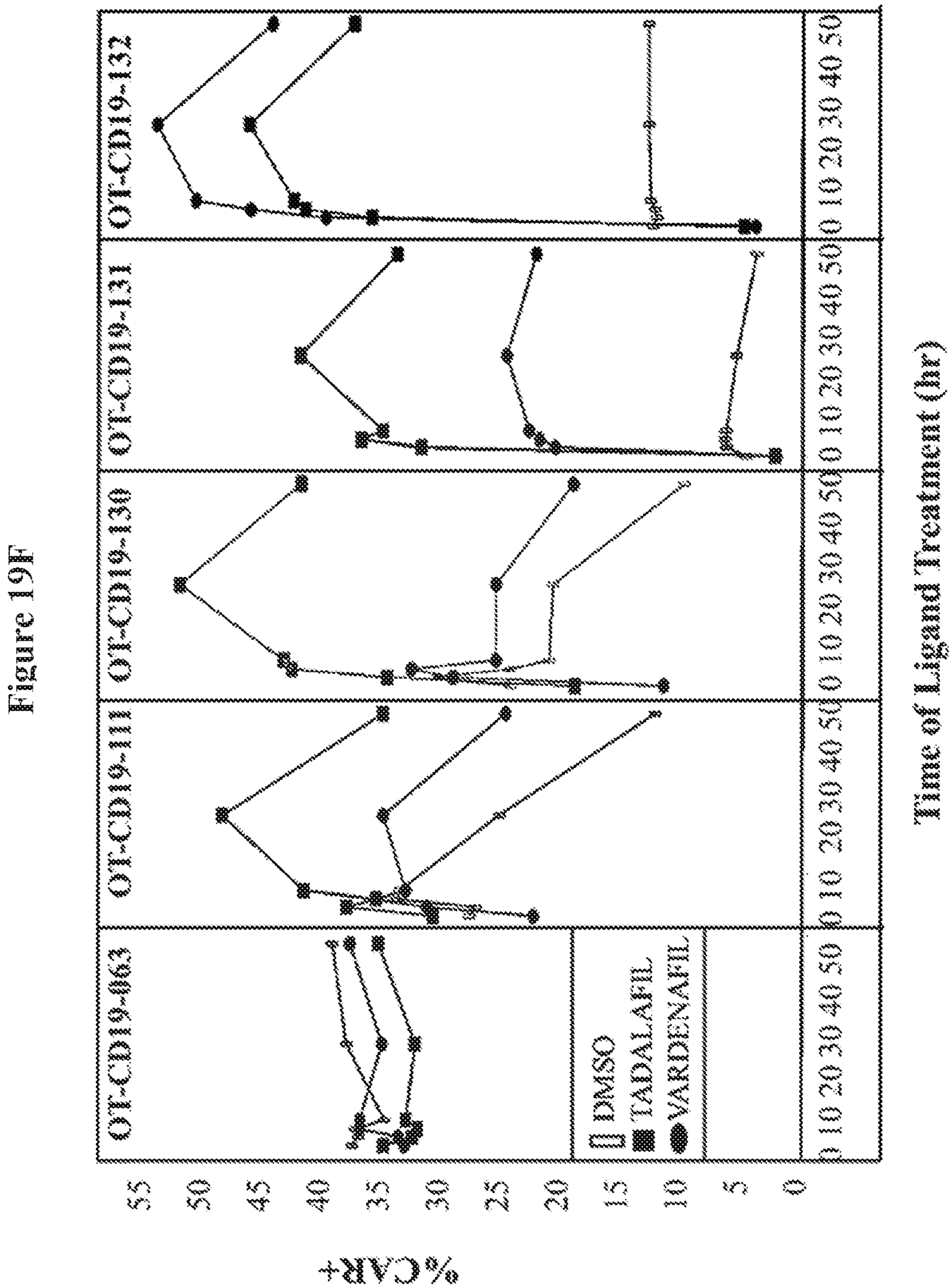

OT-CD19-132
OT-CD19-131
OT-CD19-130
OT-CD19-111
OT-CD19-463
0 10 20 30 40 50
DMSO
TADALAFIL
VARDENAFIL
%CAR+
Time of Ligand Treatment (hr)

Figure 19F

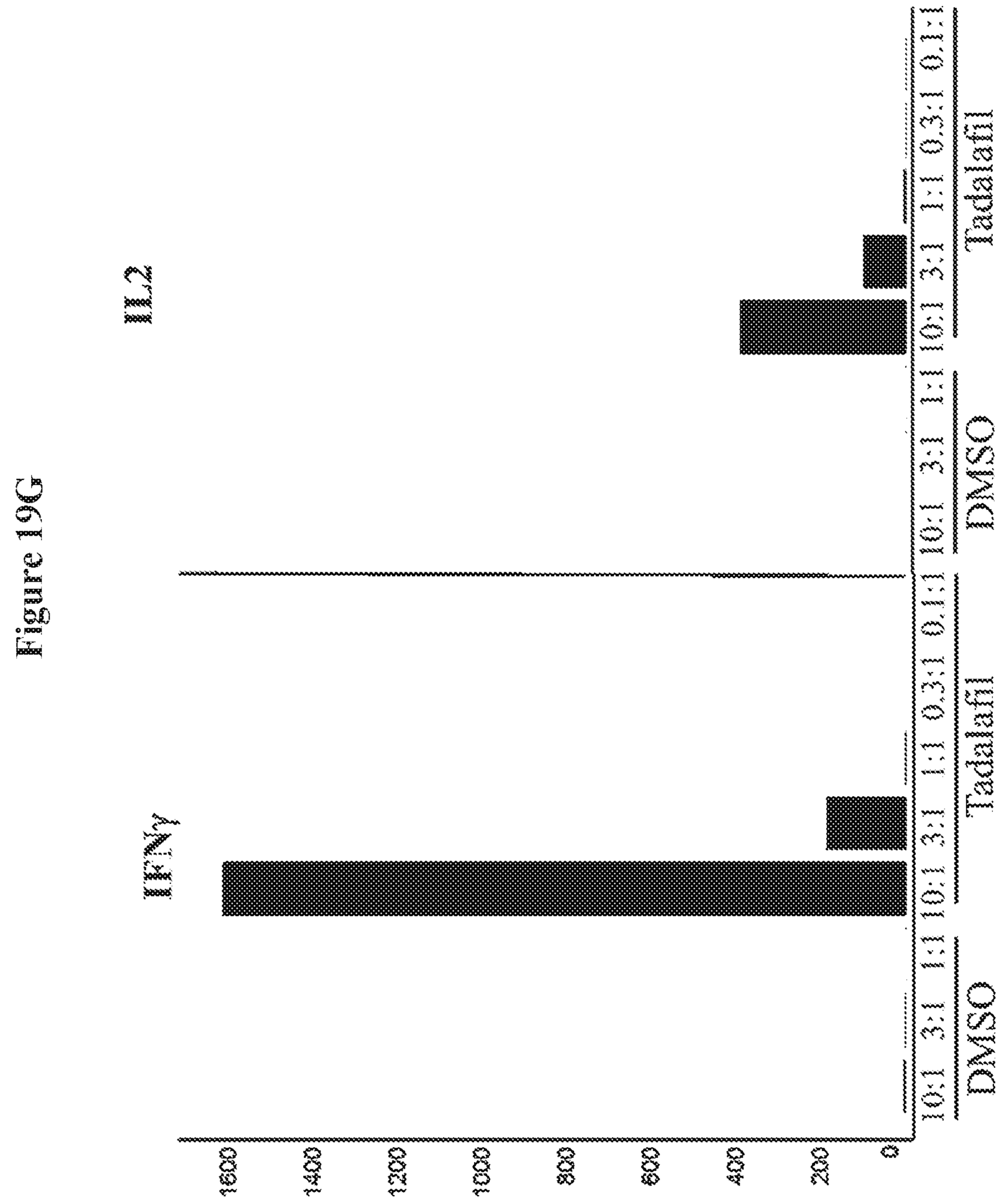

IL2
IFNγ
Avg (Final adjusted concentration (pg/ml)
1600
1400
1200
1000
800
600
400
200
0
10:1 3:1 1:1 10:1 3:1 1:1 0.3:1 0.1:1
DMSO
Tadalafil
10:1 3:1 1:1 10:1 3:1 1:1 0.3:1 0.1:1
DMSO
Tadalafil

Figure 19G

Figure 19H

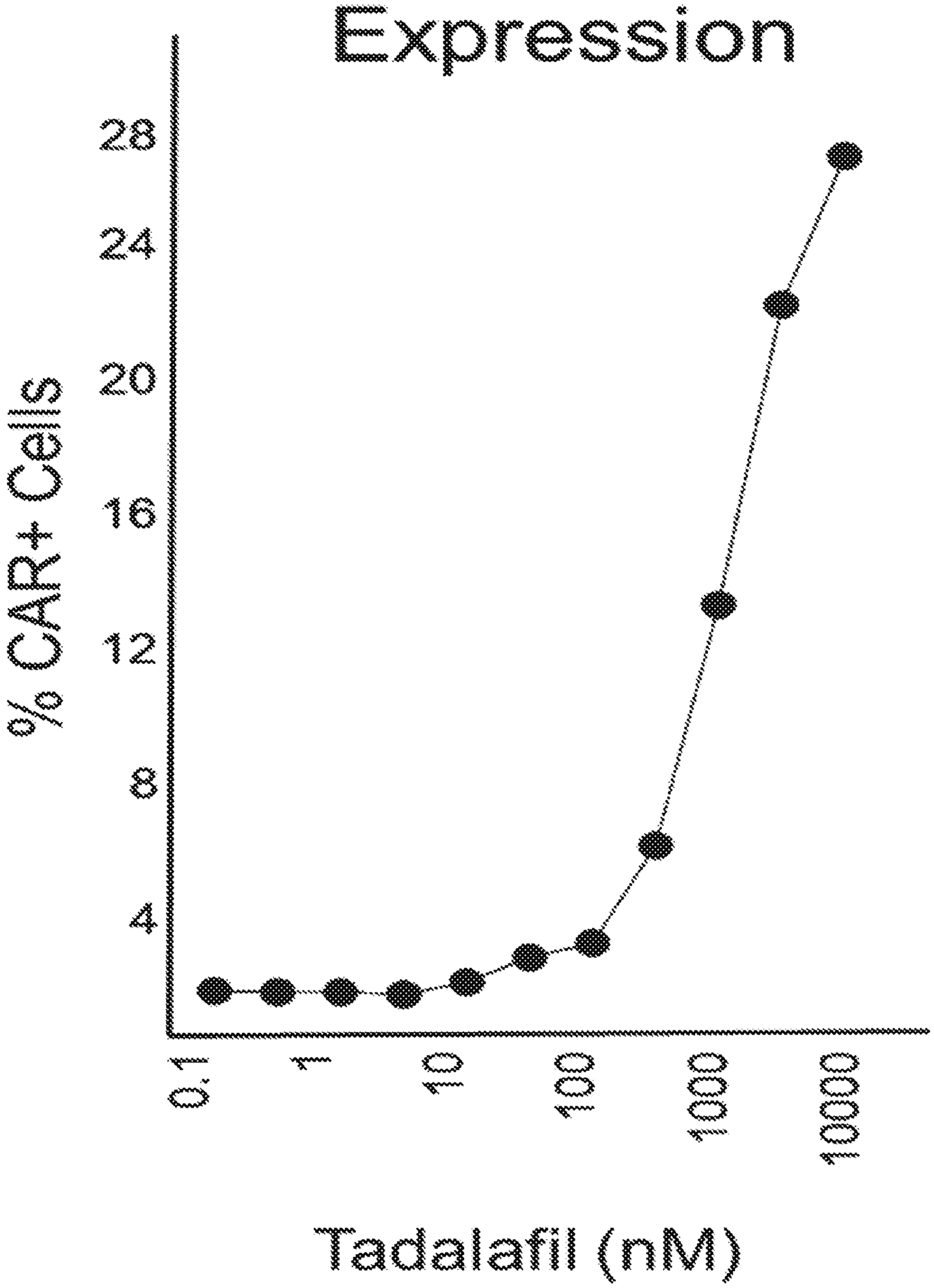

CAR
Expression
% CAR+ Cells
4
8
12
16
20
24
28
0.1
1
10
100
1000
Tadalafil (nM)

Figure 19I

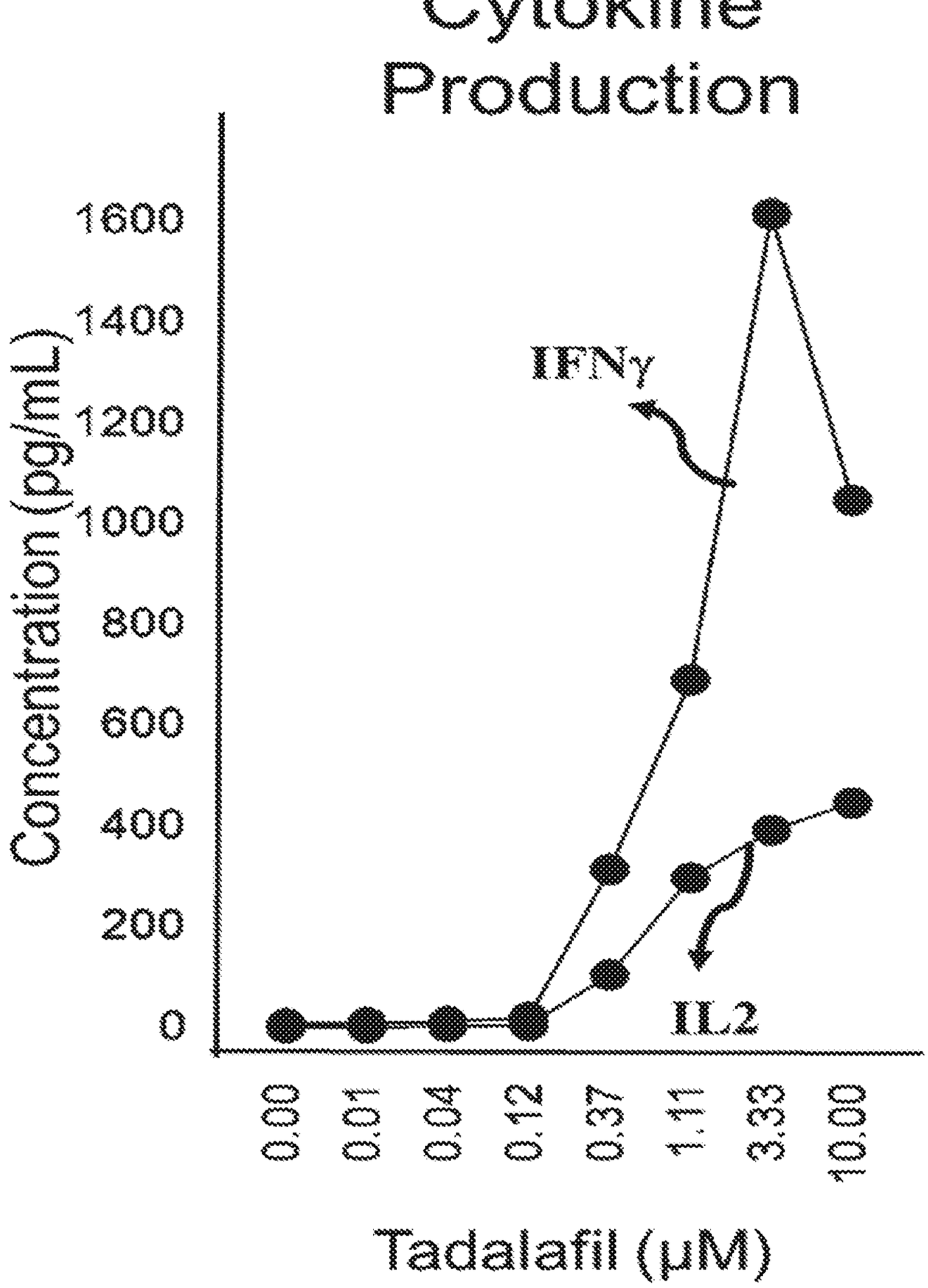

Cytokine Production
Concentration (pg/ml)
1600
1400
1200
1000
800
600
400
200
0
IFNγ
IL2
0.00
0.01
0.04
0.12
0.37
1.11
3.33
10.00
Tadalafil (μM)

Figure 19J

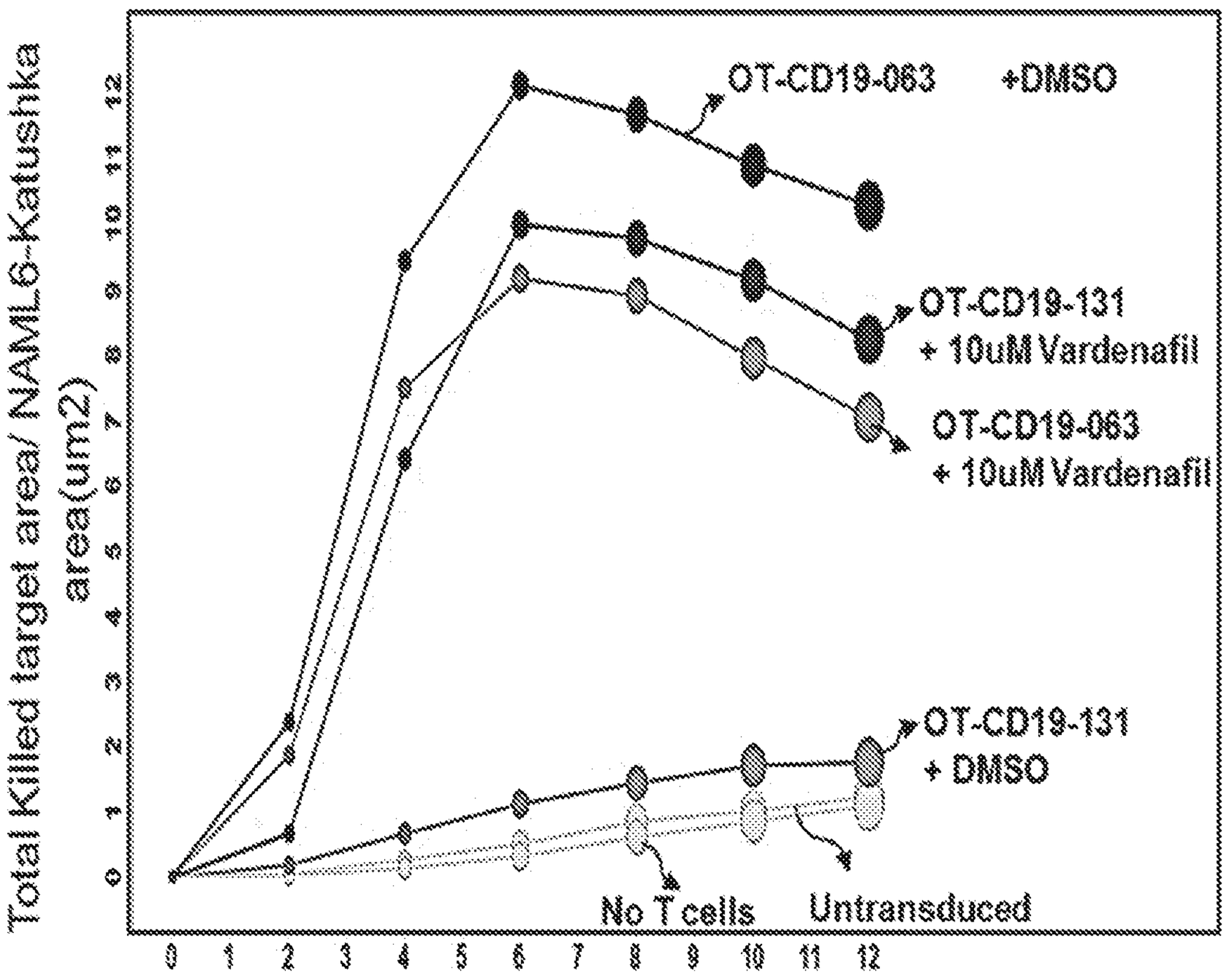

Total Killed target area/ NAML6-Katushka area(um2)
0
1
2
3
4
5
6
7
8
9
10
11
12
OT-CD19-063 +DMSO
OT-CD19-131 + 10uM Vardenafil
OT-CD19-063 + 10uM Vardenafil
OT-CD19-131 + DMSO
No T cells
Untransduced

Figure 19K

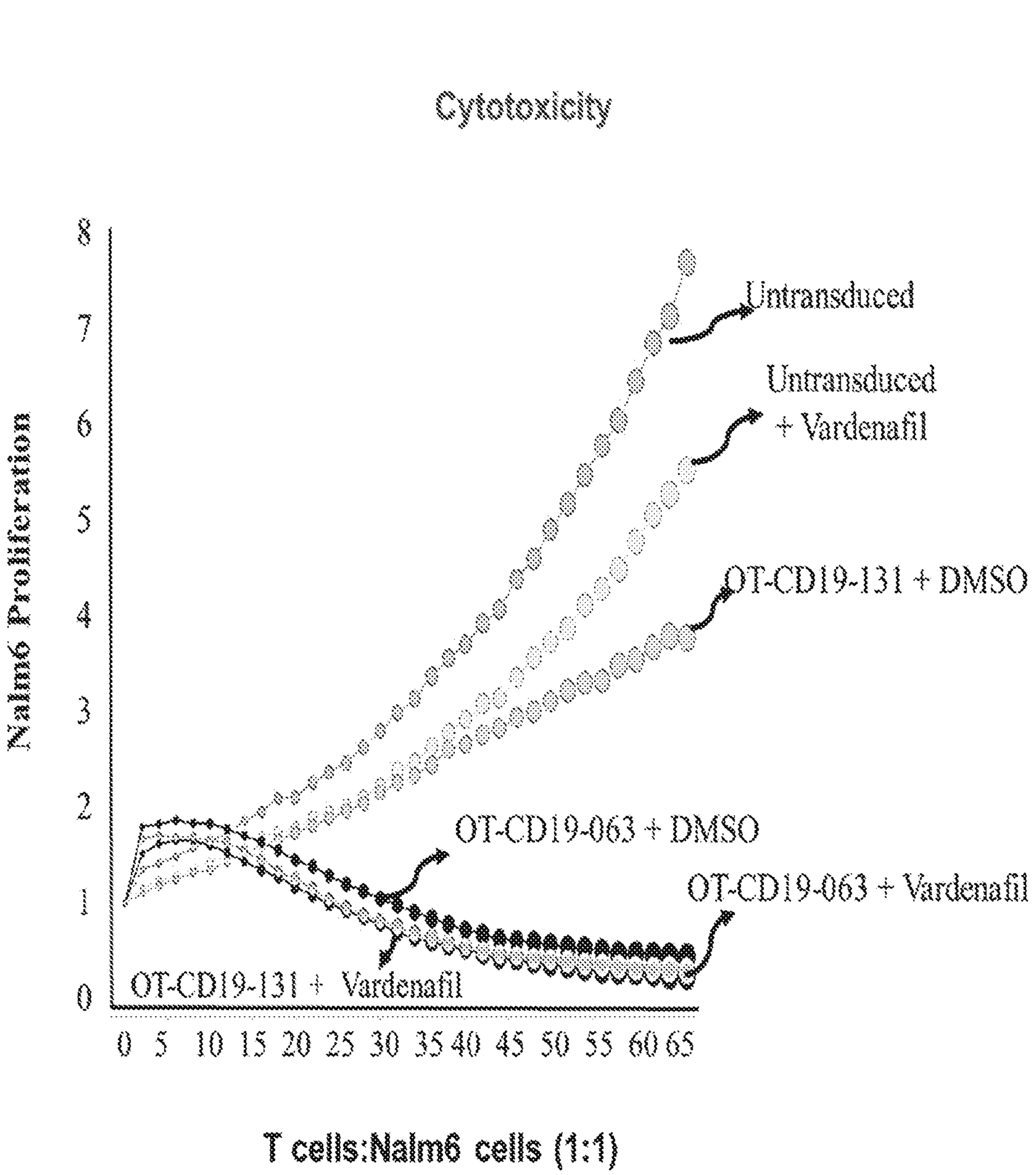

Cytotoxicity
Nalm6 Proliferation
0
1
2
3
4
5
6
7
8
0 5 10 15 20 25 30 35 40 45 50 55 60 65
Untransduced
Untransduced + Vardenafil
OT-CD19-131 + DMSO
OT-CD19-063 + DMSO
OT-CD19-063 + Vardenafil
OT-CD19-131 + Vardenafil
T cells:Nalm6 cells (1:1)

Figure 20A

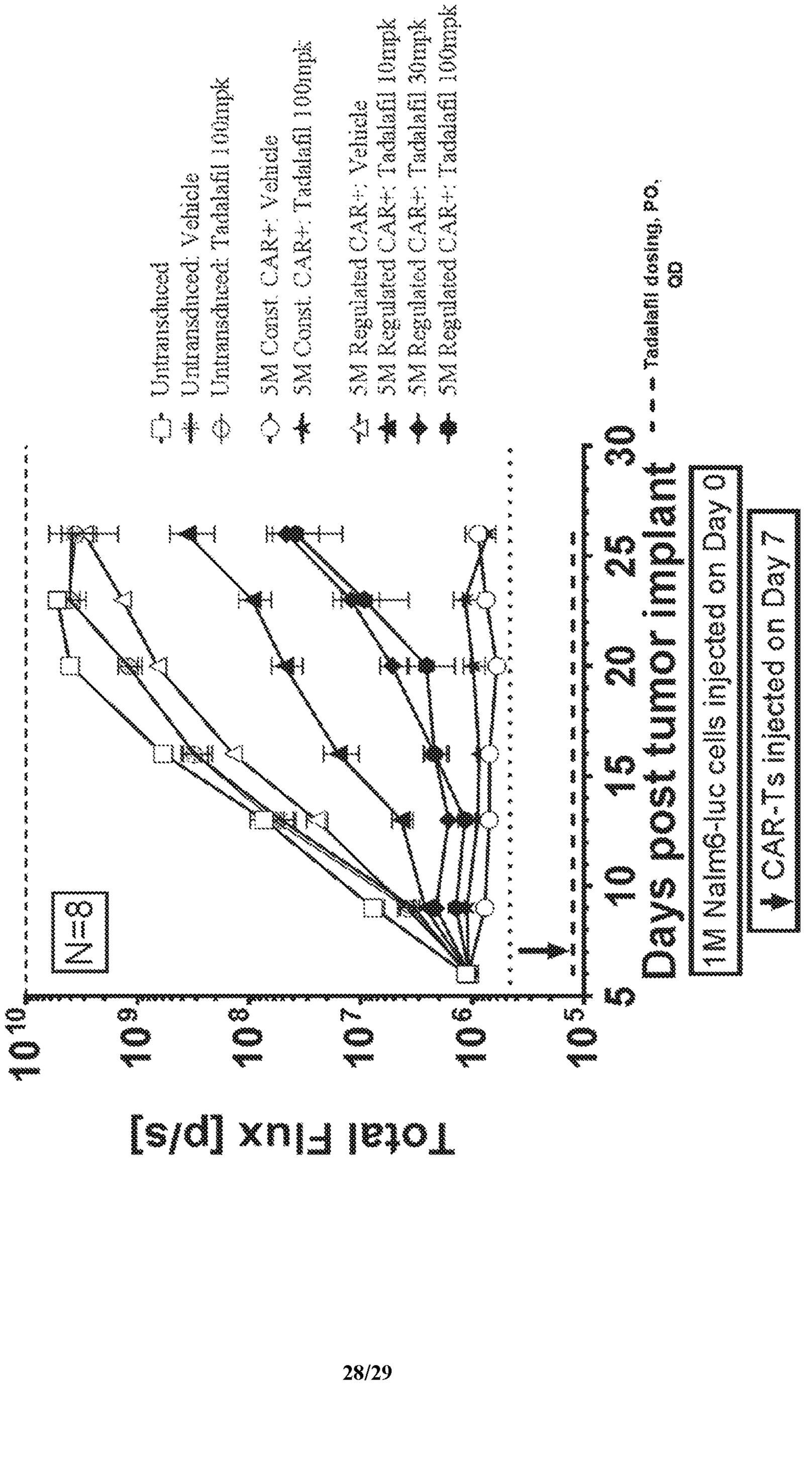

Untransduced
Untransduced Vehicle
Untransduced Tadalafil 100mpk
SM Const CAR+ Vehicle
SM Const CAR+ Tadalafil 100mpk
SM Regulated CAR+ Vehicle
SM Regulated CAR+ Tadalafil 10mpk
SM Regulated CAR+ Tadalafil 30mpk
SM Regulated CAR+ Tadalafil 100mpk
1M Nalm6-luc cells injected on Day 0
Tadalafil dosing, PO, qd
CAR-Ts injected on Day 7
Days post tumor implantation
N=8
Total Flux [p/s]
1e+05
1e+06
1e+07
1e+08

Figure 20B

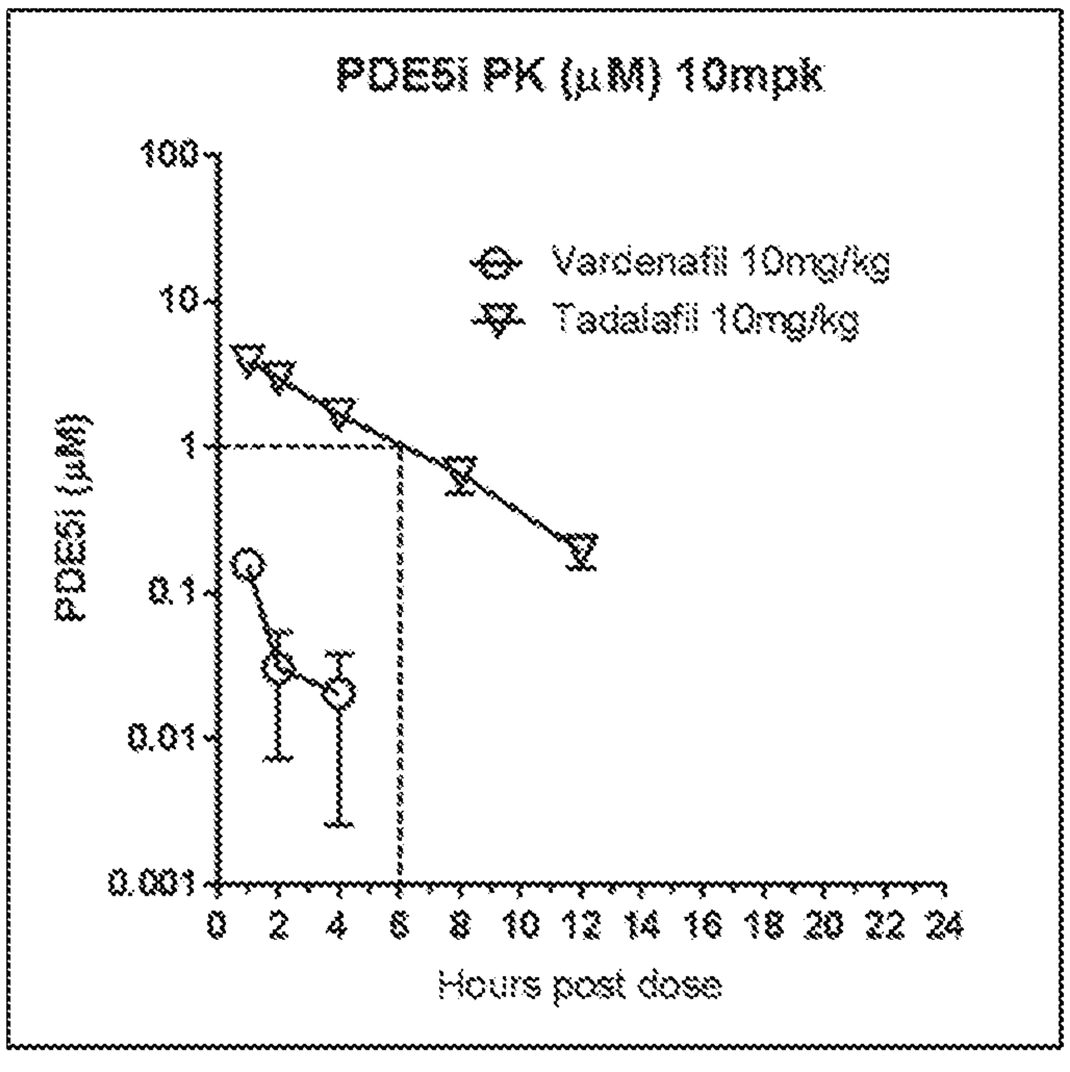


Figure 20C

