FIG 1

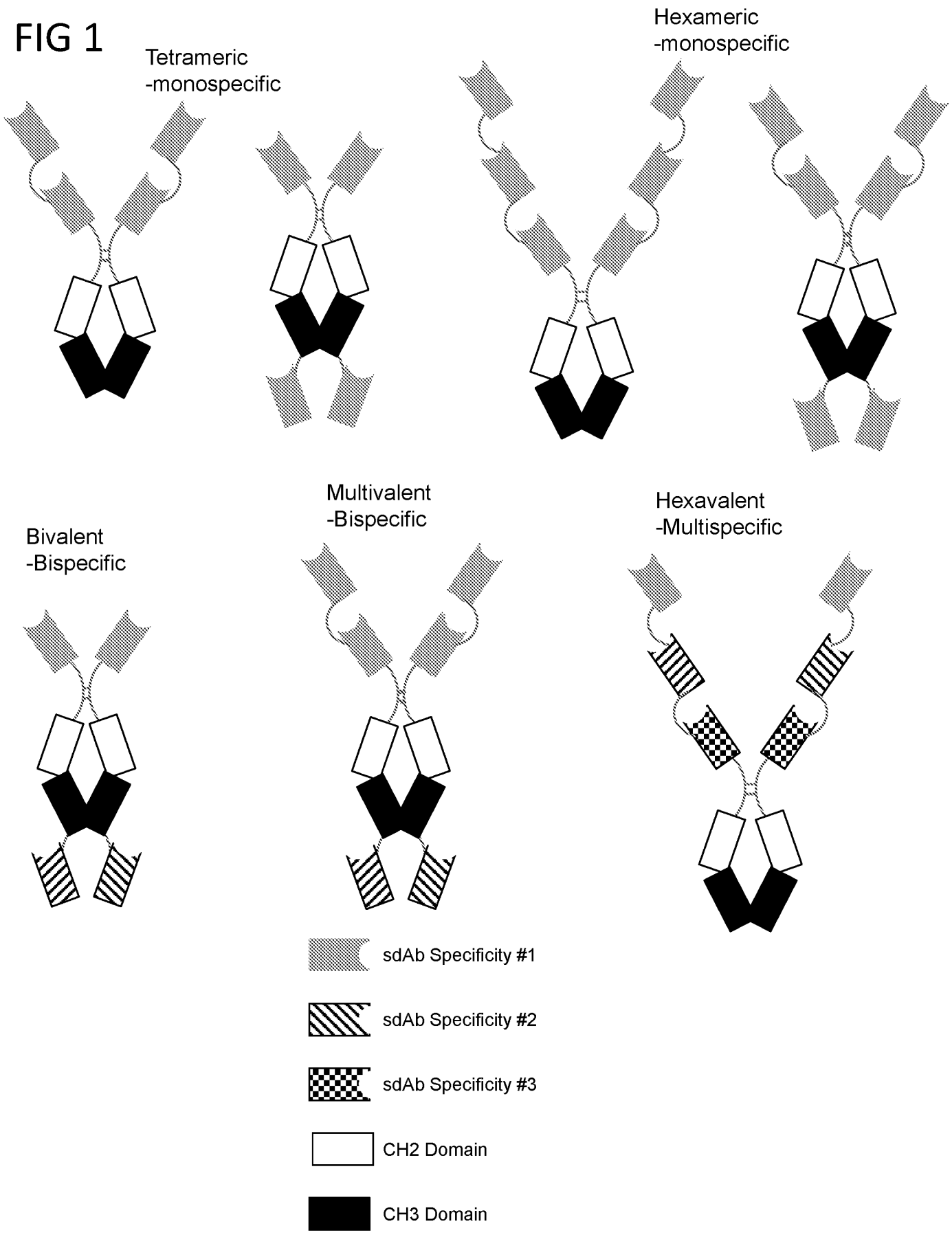

Tetrameric
-monospecific
Hexameric
-monospecific
Bivalent
-Bispecific
Multivalent
-Bispecific
Hexavalent
-Multispecific
sdAb Specificity #1
sdAb Specificity #2
sdAb Specificity #3
CH2 Domain
CH3 Domain

FIG 2A

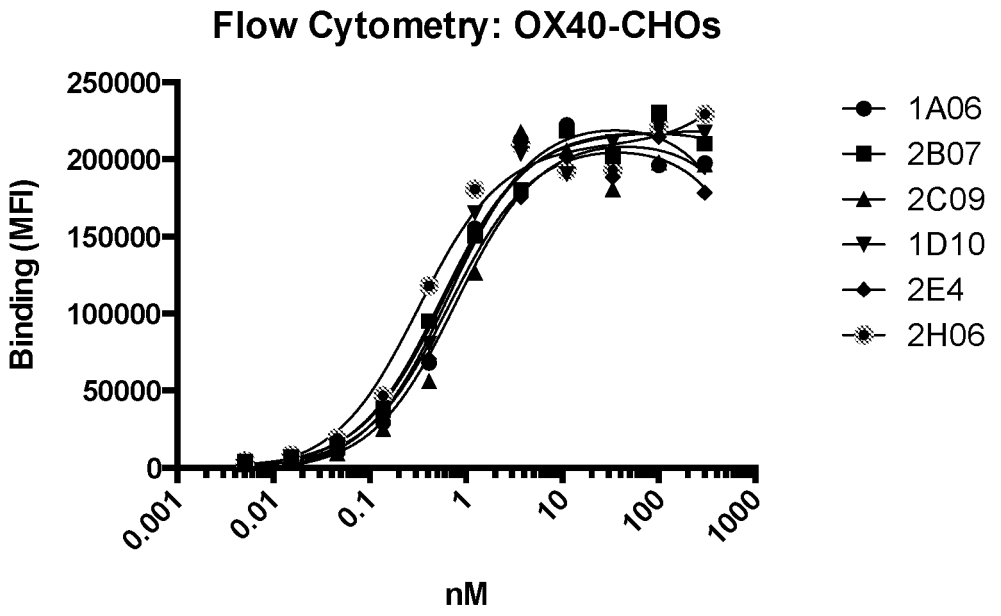

Flow Cytometry: OX40-CHOs
Binding (MFI)
250000
200000
150000
100000
50000
0
0.001
0.01
0.1
1
10
100
1000
nM
1A06
2B07
2C09
1D10
2E4
2H06

FIG 2B

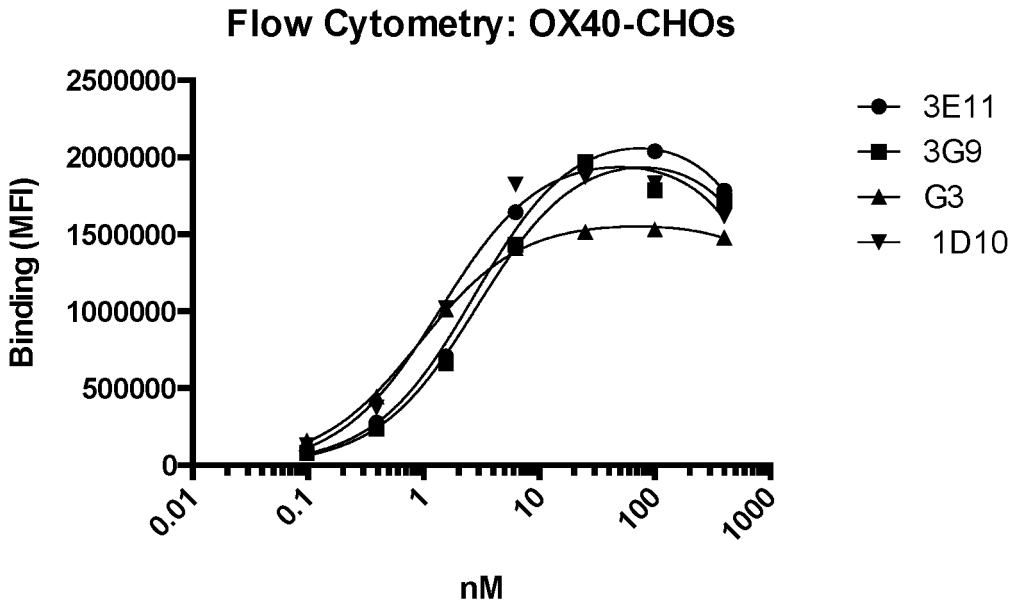

Flow Cytometry: OX40-CHOs
Binding (MFI)
2500000
2000000
1500000
1000000
500000
0
0.01
0.1
1
10
100
1000
nM
3E11
3G9
G3
1D10

FIG 3

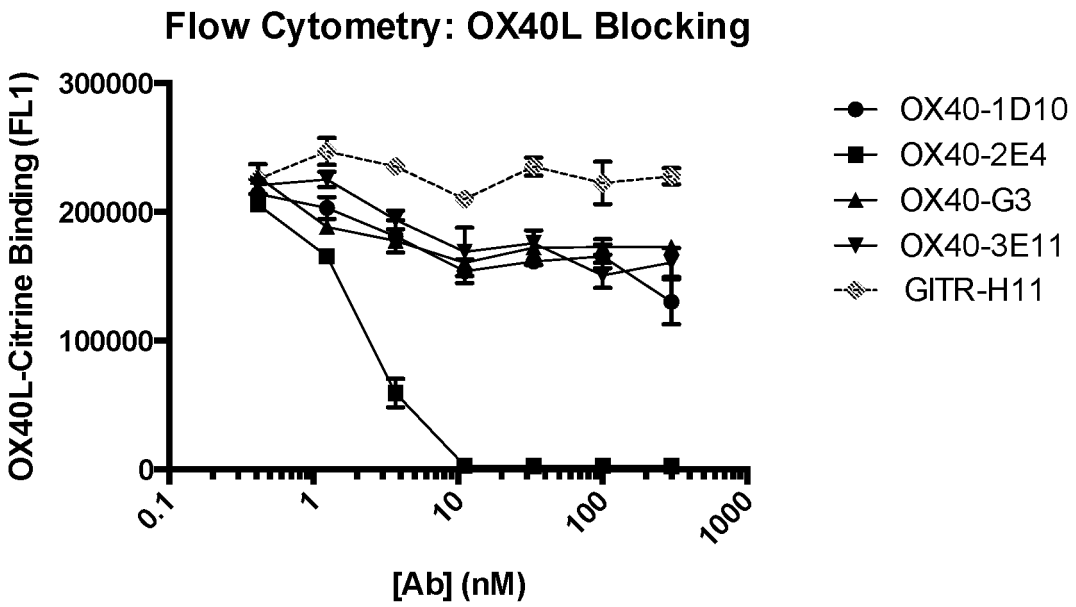

Flow Cytometry: OX40L Blocking
OX40L-Citrine Binding (FL1)
300000
200000
100000
0
0.1
1
10
100
1000
[Ab] (nM)
OX40-1D10
OX40-2E4
OX40-G3
OX40-3E11
GITR-H11

FIG 4

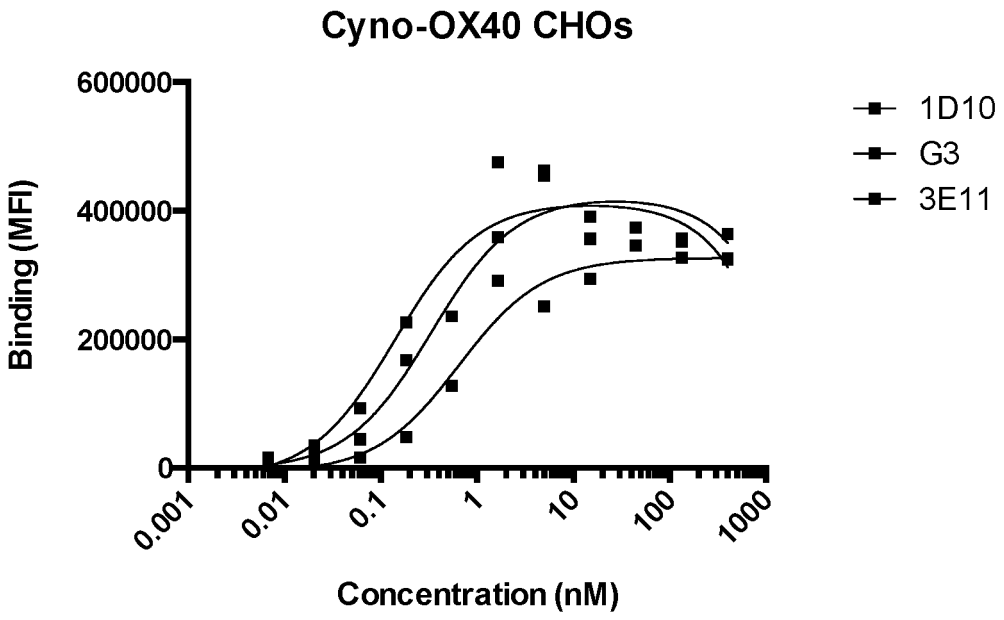

Cyno-OX40 CHOs
600000
400000
200000
0
Binding (MFI)
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)
1D10
G3
3E11

FIG 5A

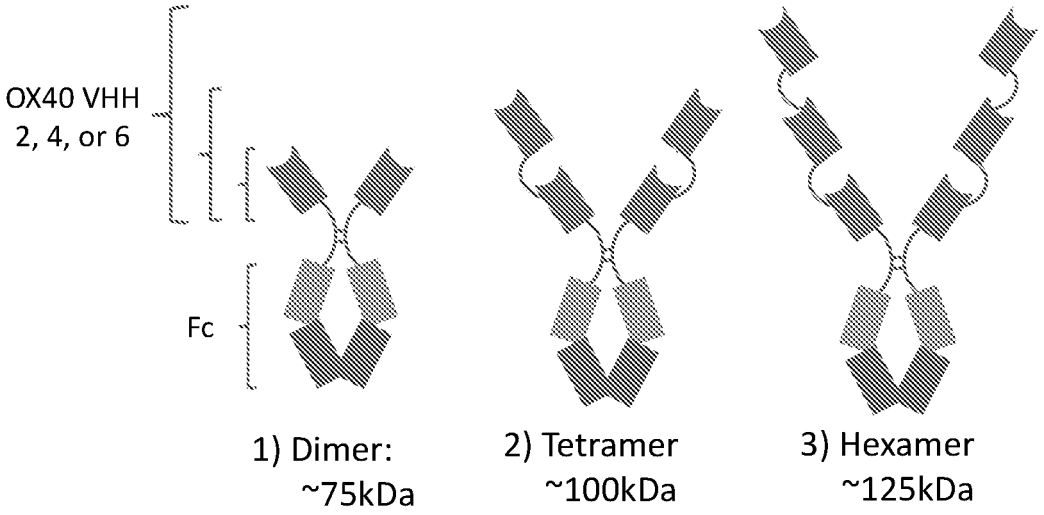

OX40 VHH
2, 4, or 6
Fc
1) Dimer:
~75kDa
2) Tetramer
~100kDa
3) Hexamer
~125kDa

FIG 5B

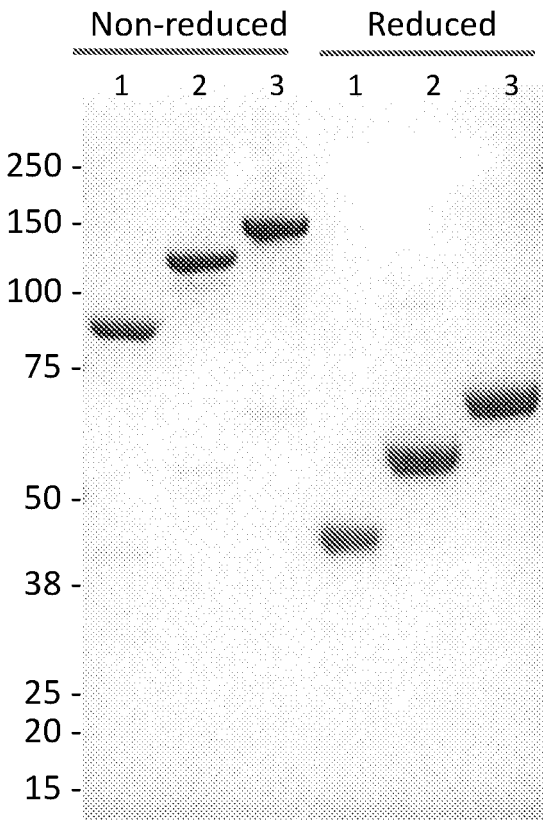

Non-reduced
Reduced
1
2
3
1
2
3
250
150
100
75
50
38
25
20
15

FIG 6

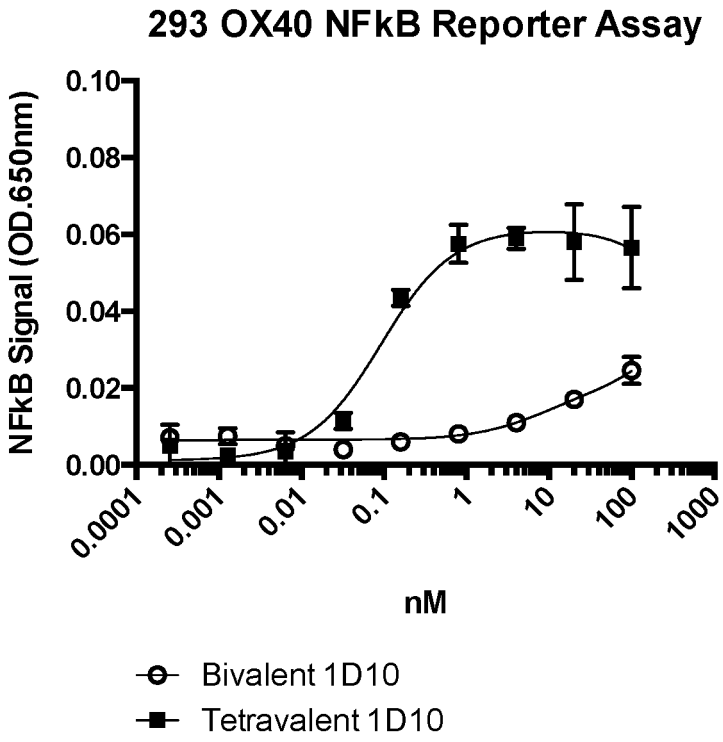

293 OX40 NFkB Reporter Assay
NFkB Signal (OD.650nm)
0.10
0.08
0.06
0.04
0.02
0.00
0.0001
0.001
0.01
0.1
1
10
100
1000
nM
Bivalent 1D10
Tetravalent 1D10

FIG 7A

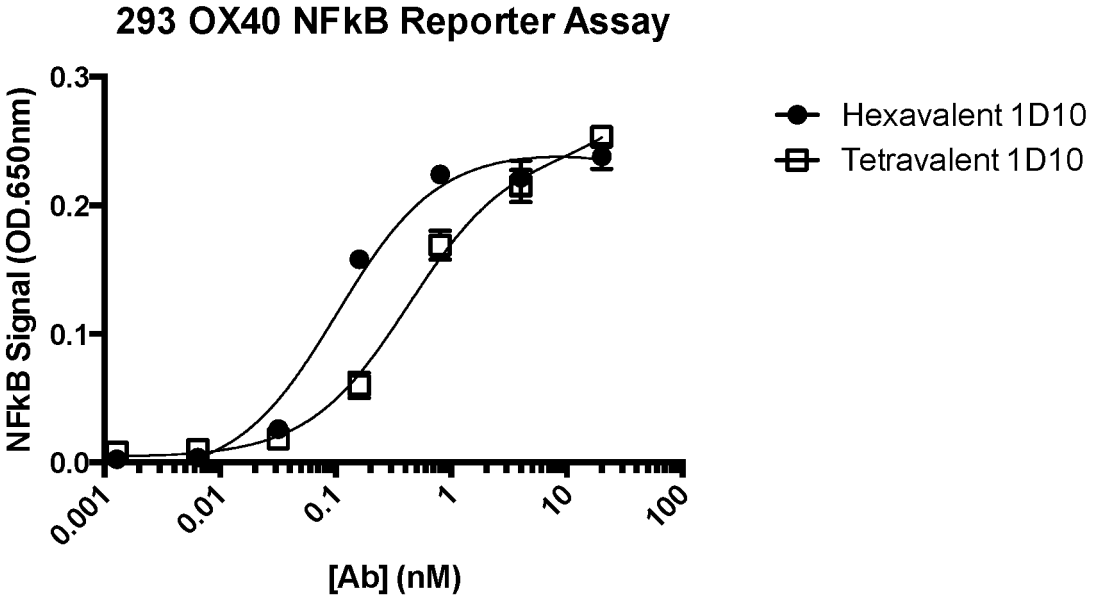

293 OX40 NFkB Reporter Assay
NFkB Signal (OD.650nm)
0.3
0.2
0.1
0.0
0.001
0.01
0.1
1
10
100
[Ab] (nM)
Hexavalent 1D10
Tetravalent 1D10

FIG 7B

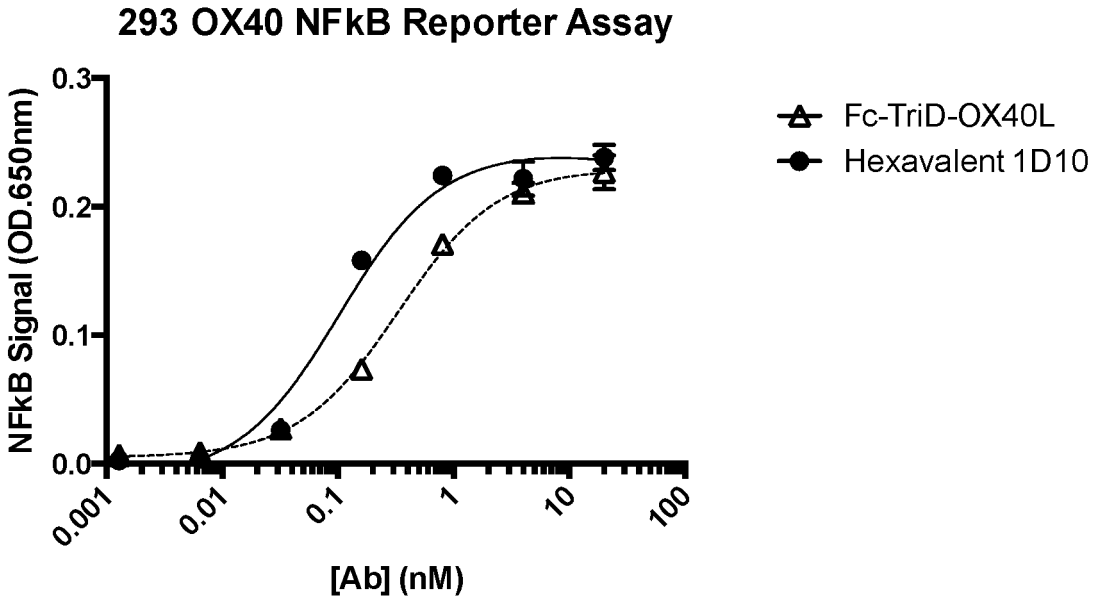
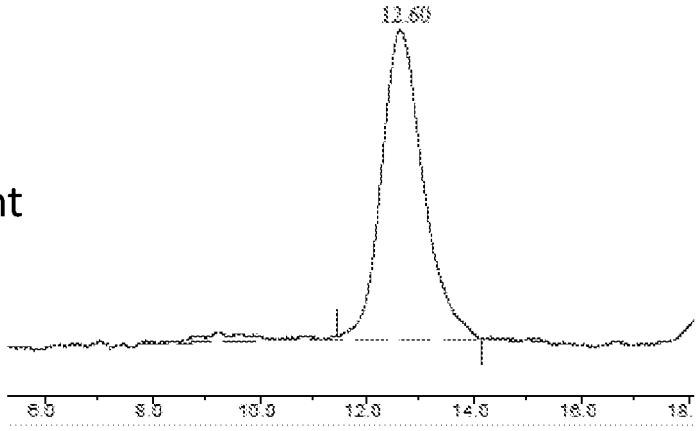

293 OX40 NFkB Reporter Assay
NFkB Signal (OD.650nm)
0.3
0.2
0.1
0.0
0.001
0.01
0.1
1
10
100
[Ab] (nM)
Fc-TriD-OX40L
Hexavalent 1D10

FIG 8A

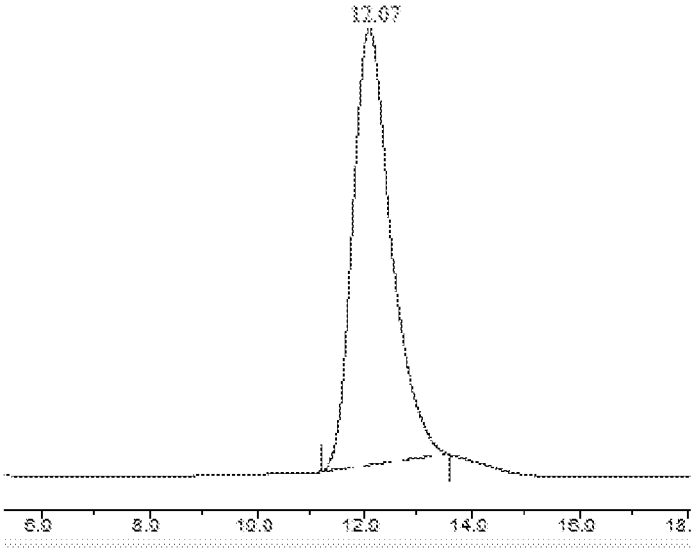
Tetravalent
1D10-Fc



12.60 = 139 kDa

FIG 8B

Hexavalent
1D10-Fc



12.07 = 177 kDa

FIG 9

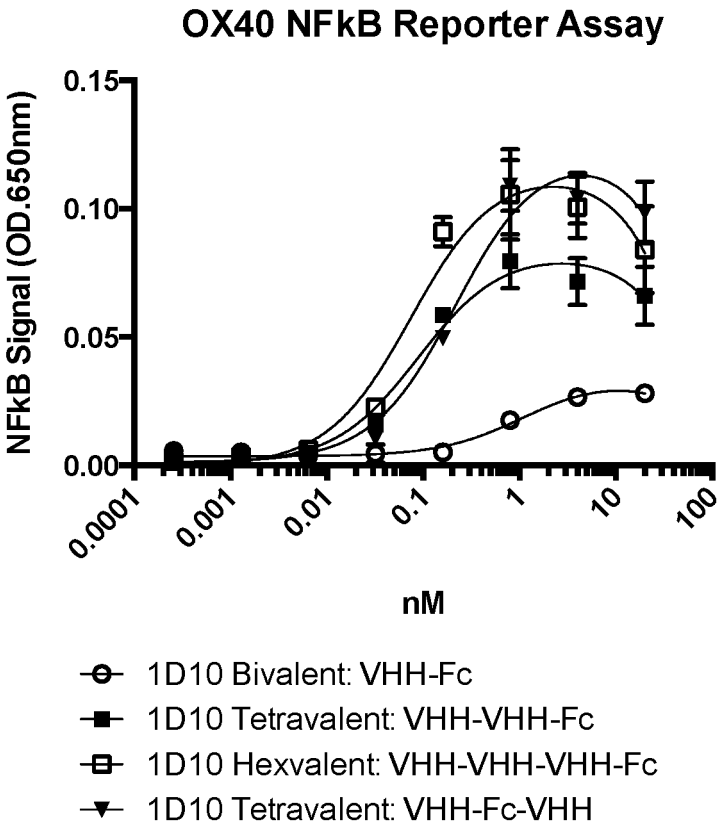
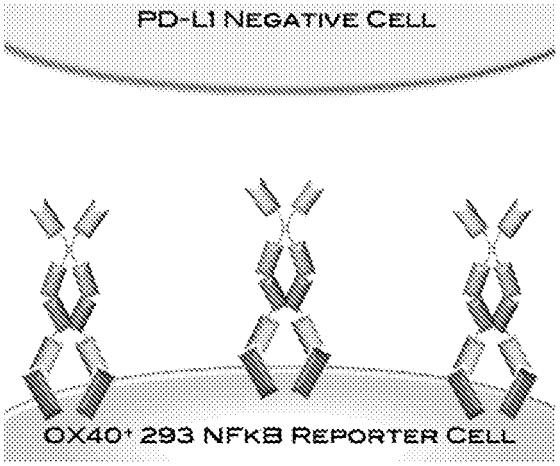
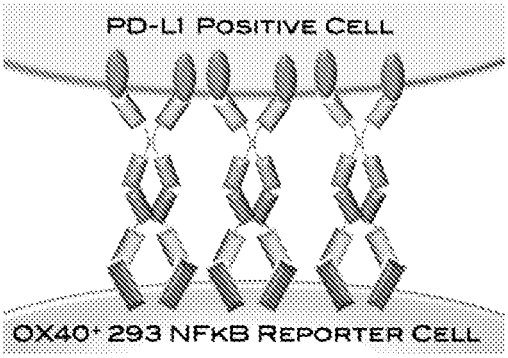

OX40 NFkB Reporter Assay
NFkB Signal (OD.650nm)
0.15
0.10
0.05
0.00
0.0001
0.001
0.01
0.1
1
10
100
nM
1D10 Bivalent: VHH-Fc
1D10 Tetravalent: VHH-VHH-Fc
1D10 Hexvalent: VHH-VHH-VHH-Fc
1D10 Tetravalent: VHH-Fc-VHH

FIG 10A



No OX40 signaling

FIG 10B



PDL1-dependent OX40 signaling

FIG 10C

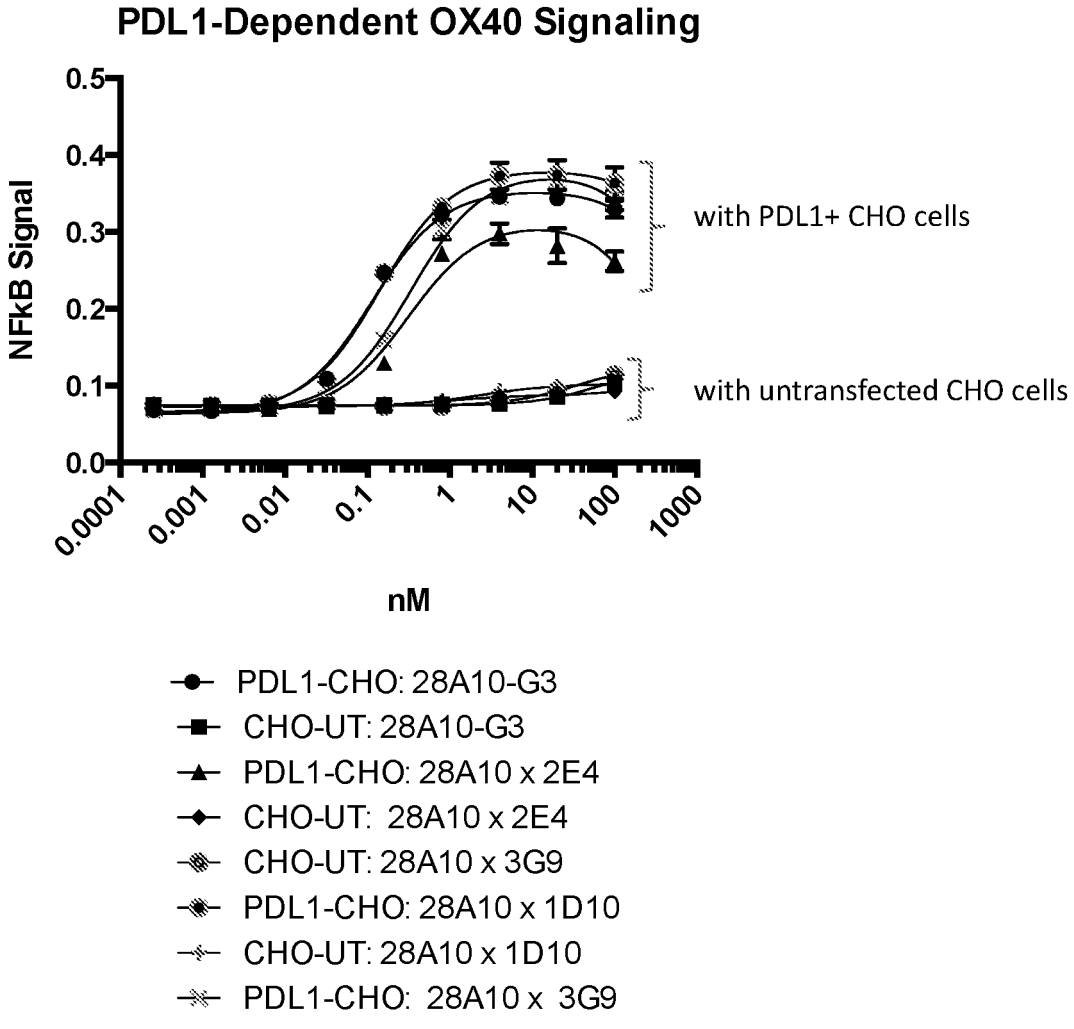


FIG 11

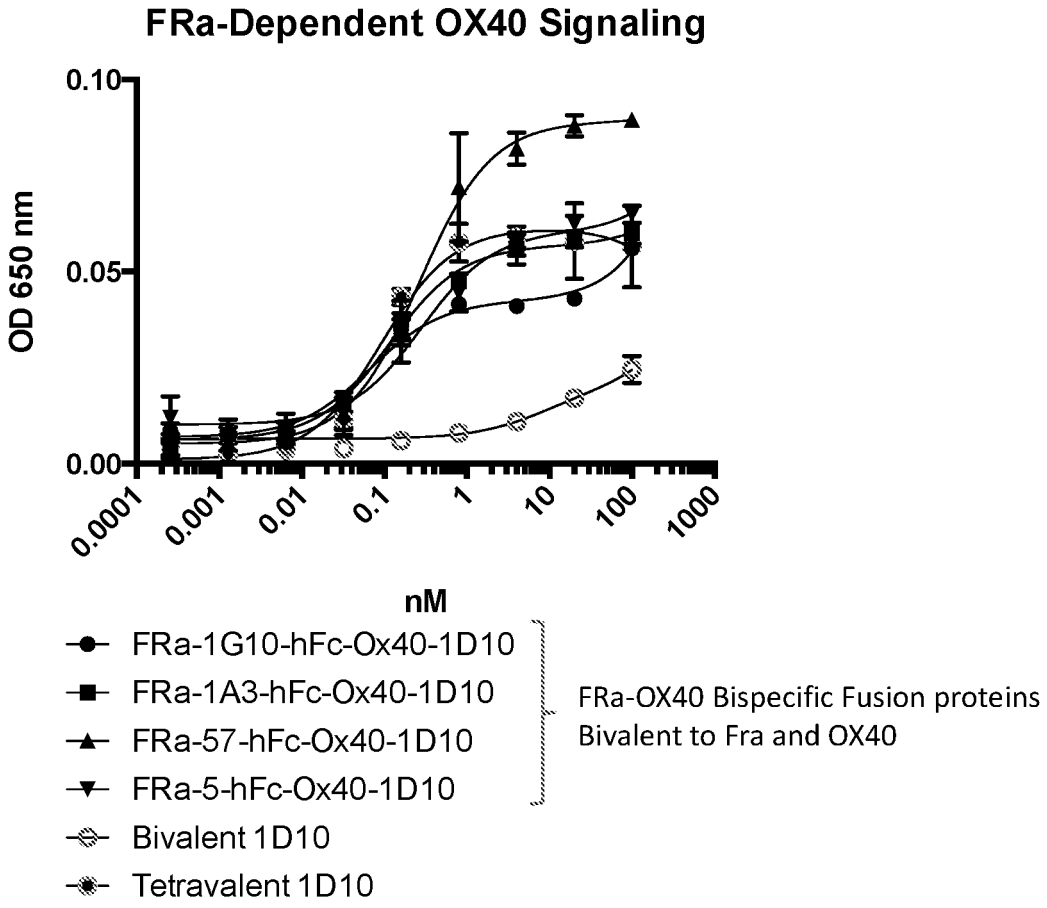

FRa-Dependent OX40 Signaling
OD 650 nm
0.10
0.05
0.00
0.0001
0.001
0.01
0.1
1
10
100
1000
nM
FRa-1G10-hFc-Ox40-1D10
FRa-1A3-hFc-Ox40-1D10
FRa-57-hFc-Ox40-1D10
FRa-5-hFc-Ox40-1D10
Bivalent 1D10
Tetravalent 1D10
FRa-OX40 Bispecific Fusion proteins
Bivalent to Fra and OX40

FIG 12A

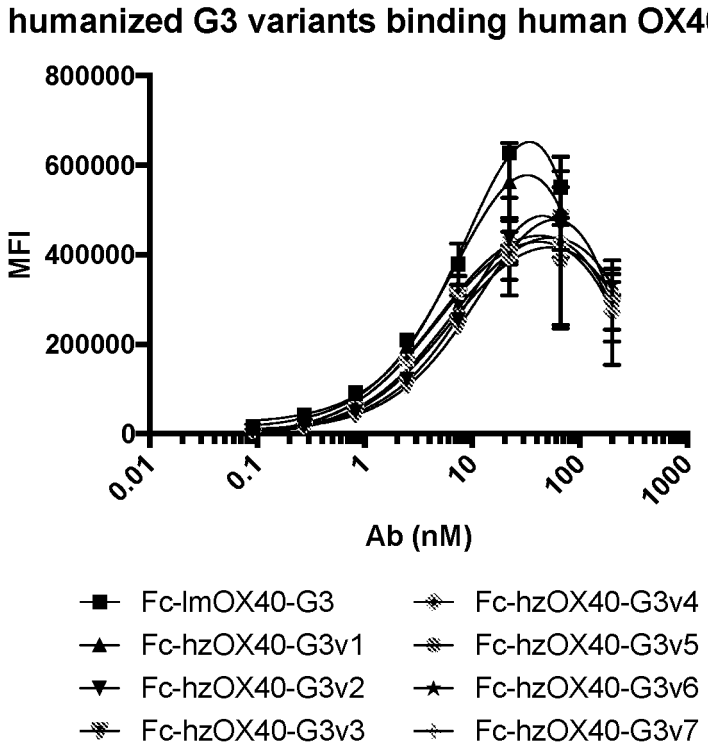


FIG 12B

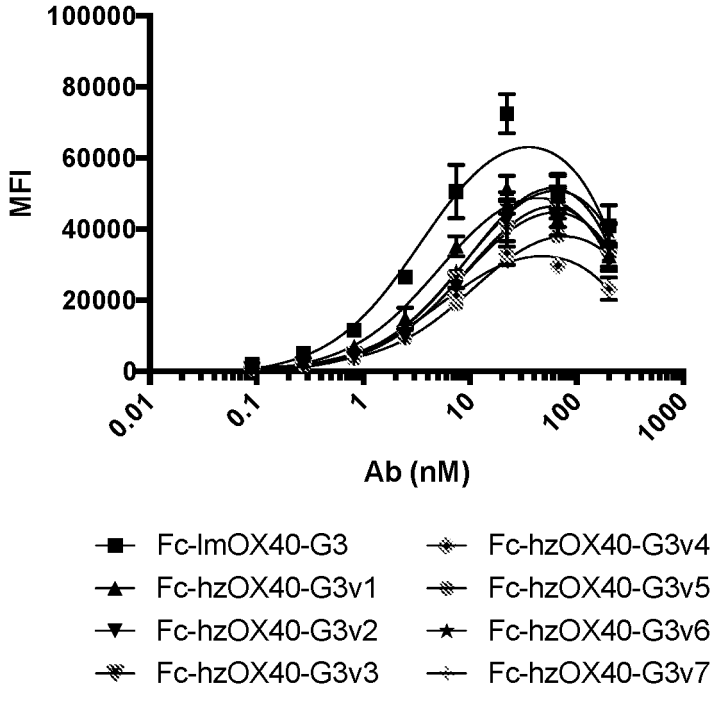


FIG 13A

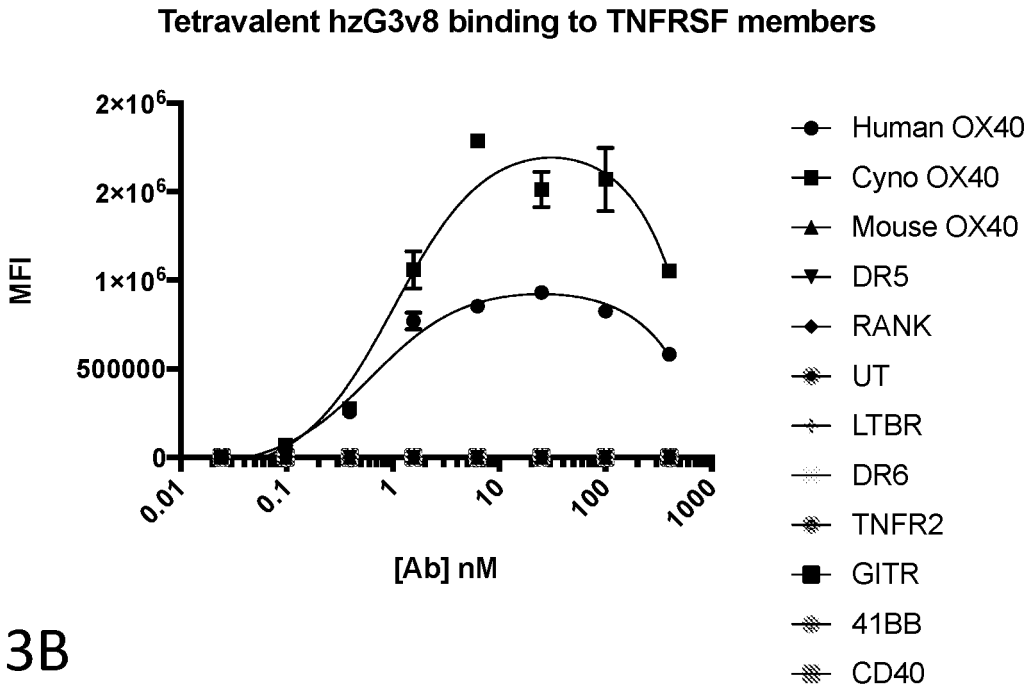


FIG 13B

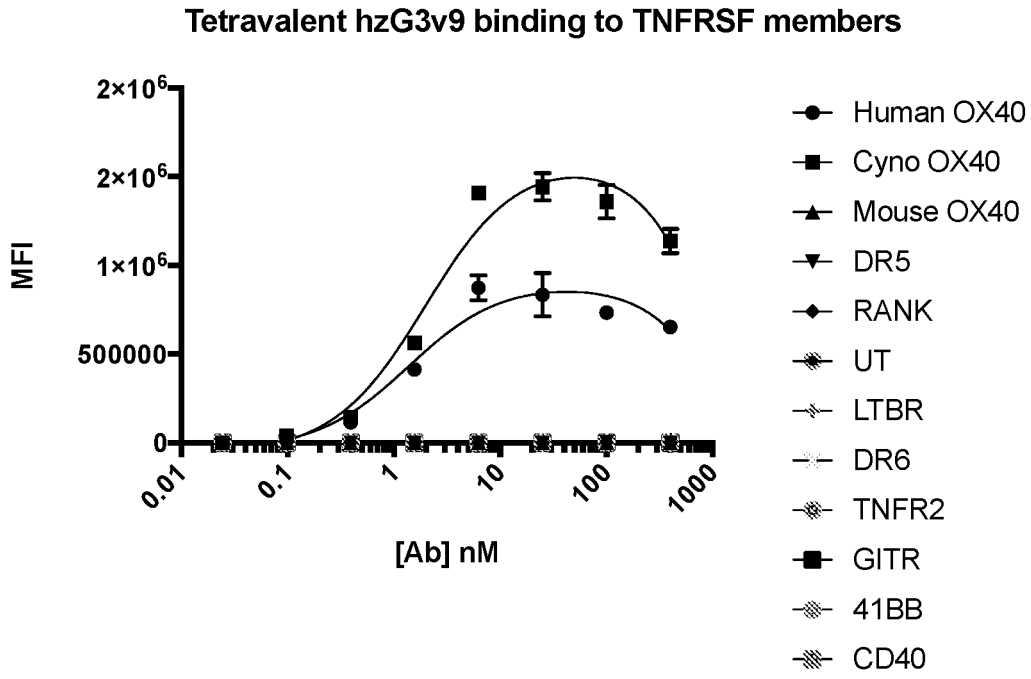


FIG 14A

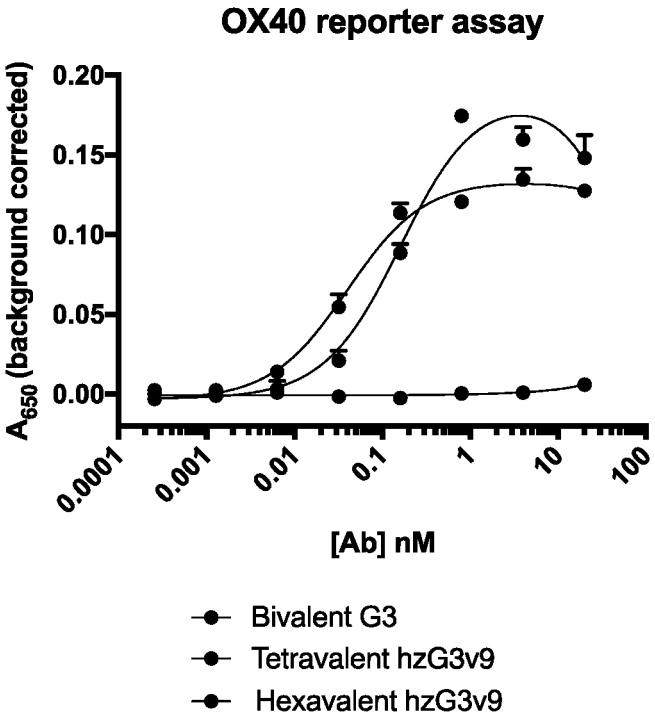


FIG 14B

