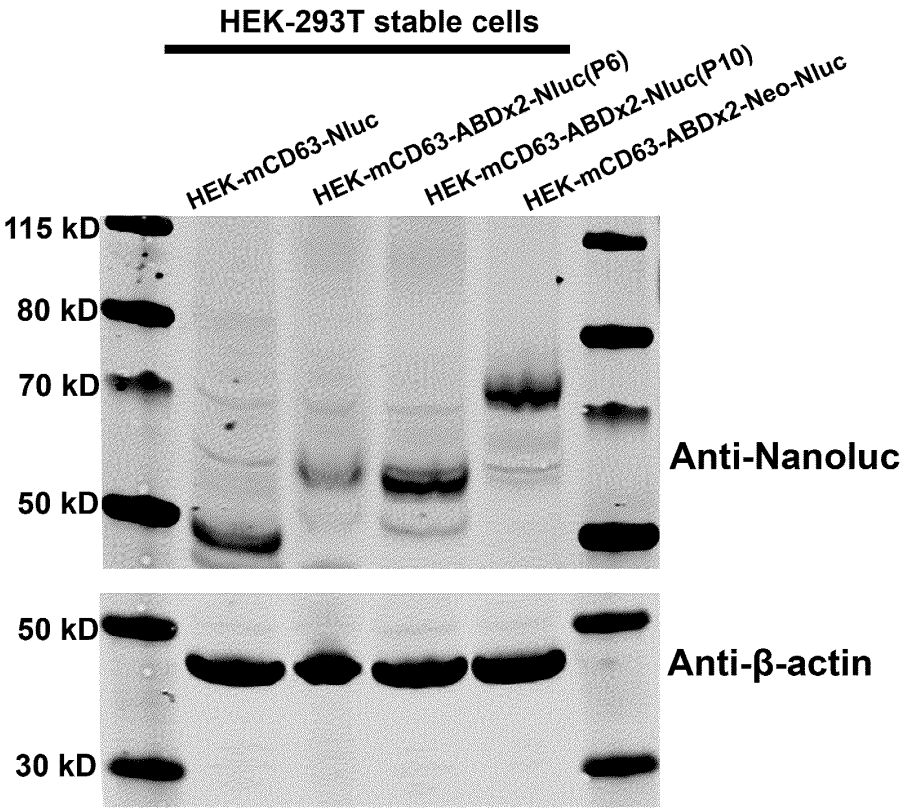

HEK-293T stable cells
HEK-mCD63-Nluc
HEK-mCD63-ABDx2-Nluc(P6)
HEK-mCD63-ABDx2-Nluc(P10)
HEK-mCD63-ABDx2-Neo-Nluc
115 kD
80 kD
70 kD
50 kD
Anti-Nanoluc
50 kD
30 kD
Anti-β-actin

FIG. 1

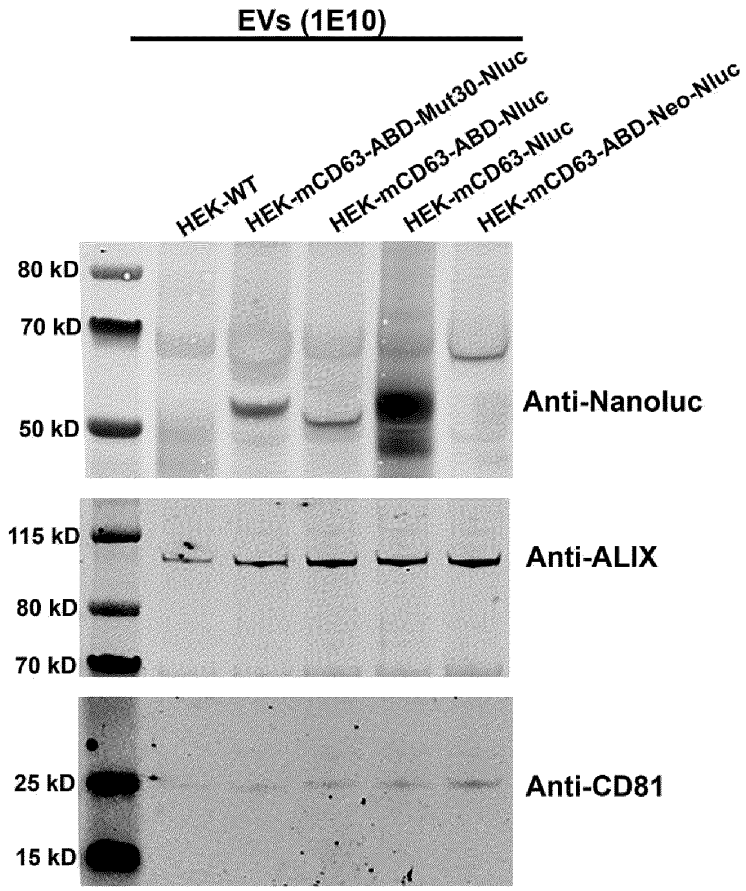

EVs (1E10)
HEK-WT
HEK-mCD63-ABD-Mut30-Nluc
HEK-mCD63-ABD-Nluc
HEK-mCD63-Nluc
HEK-mCD63-ABD-Neo-Nluc
80 kD
70 kD
50 kD
Anti-Nanoluc
115 kD
80 kD
70 kD
Anti-ALIX
25 kD
15 kD
Anti-CD81

FIG. 2

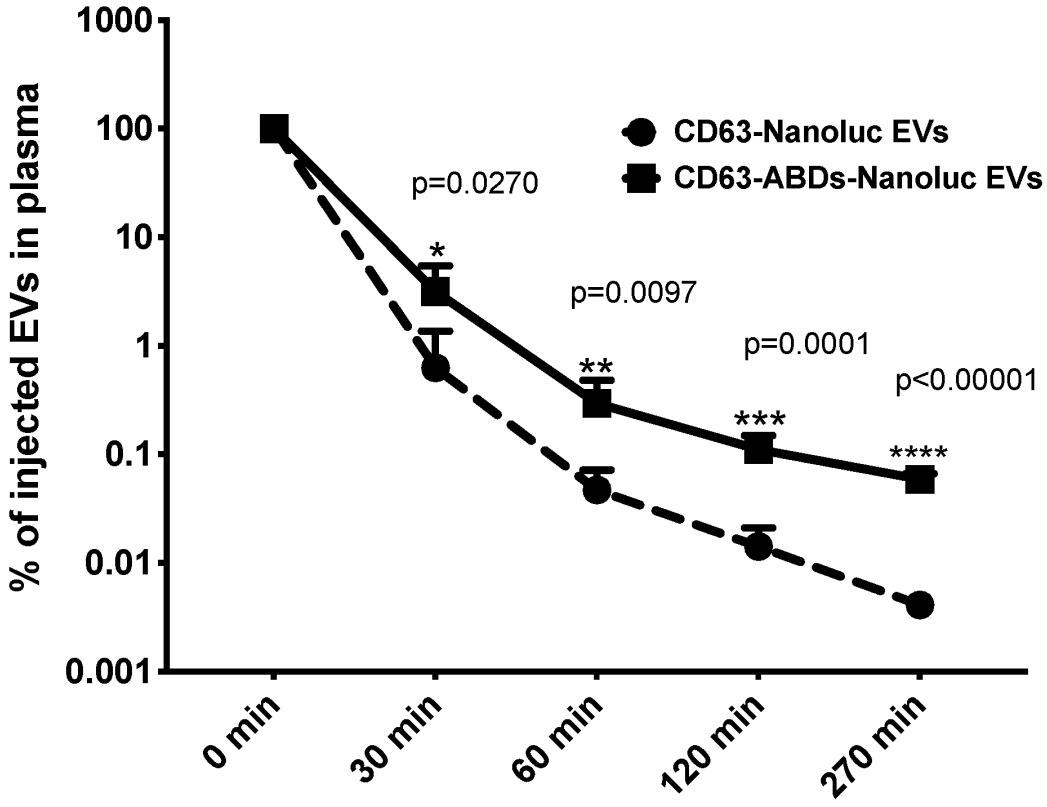

1000
100
10
1
0.1
0.01
0.001
% of injected EVs in plasma
CD63-Nanoluc EVs
CD63-ABDs-Nanoluc EVs
p=0.0270
*
p=0.0097
**
p=0.0001
***
p<0.00001
****
0 min
30 min
60 min
120 min
270 min

FIG. 3

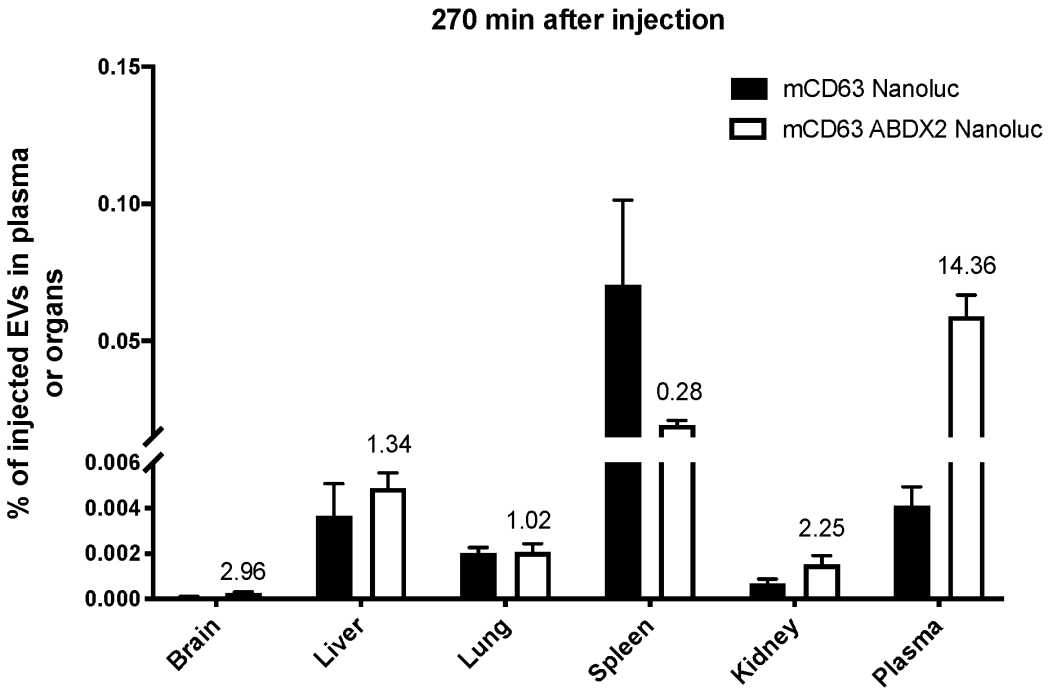

270 min after injection
% of injected EVs in plasma or organs
0.15
0.10
0.05
0.006
0.004
0.002
0.000
mCD63 Nanoluc
mCD63 ABDX2 Nanoluc
2.96
1.34
1.02
0.28
2.25
14.36
Brain
Liver
Lung
Spleen
Kidney
Plasma

FIG. 4

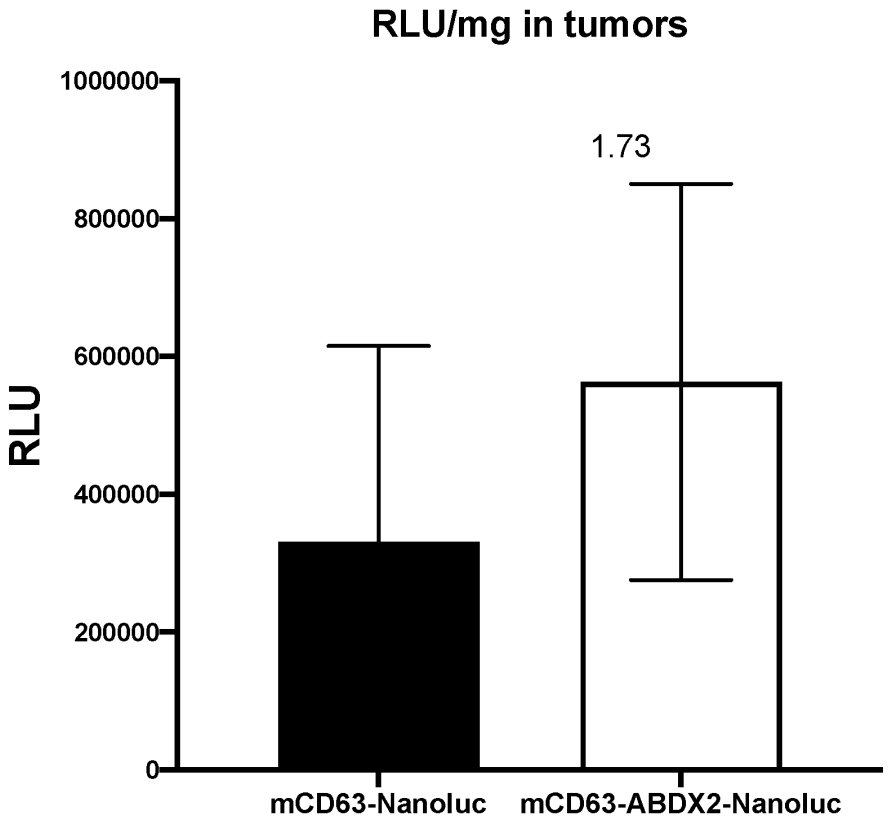

RLU/mg in tumors
1000000
800000
600000
400000
200000
0
RLU
1.73
mCD63-Nanoluc
mCD63-ABDX2-Nanoluc

FIG. 5

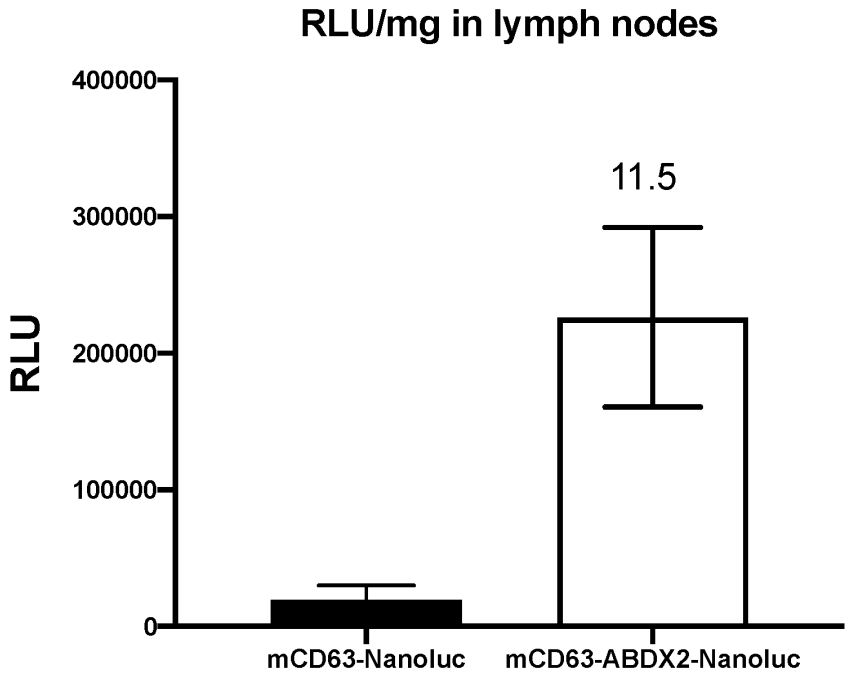

RLU/mg in lymph nodes
400000
300000
200000
100000
0
RLU
11.5
mCD63-Nanoluc
mCD63-ABDX2-Nanoluc

FIG. 6

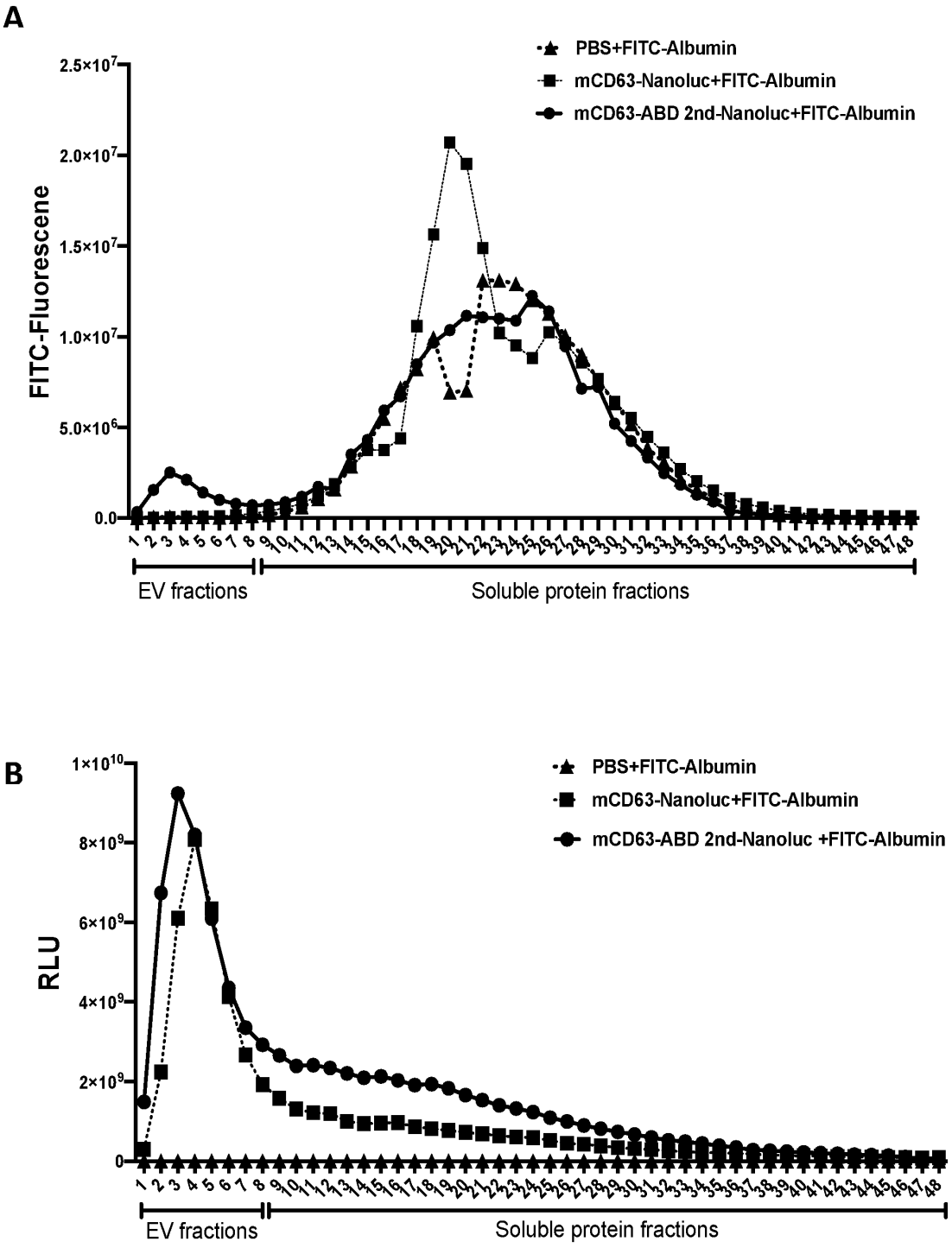

A
PBS+FITC-Albumin
mCD63-Nanoluc+FITC-Albumin
mCD63-ABD 2nd-Nanoluc+FITC-Albumin
FITC-Fluorescene
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
EV fractions
Soluble protein fractions
B
PBS+FITC-Albumin
mCD63-Nanoluc+FITC-Albumin
mCD63-ABD 2nd-Nanoluc +FITC-Albumin
RLU
1×10^10
8×10^9
6×10^9
4×10^9
2×10^9
0
EV fractions
Soluble protein fractions

FIG. 7

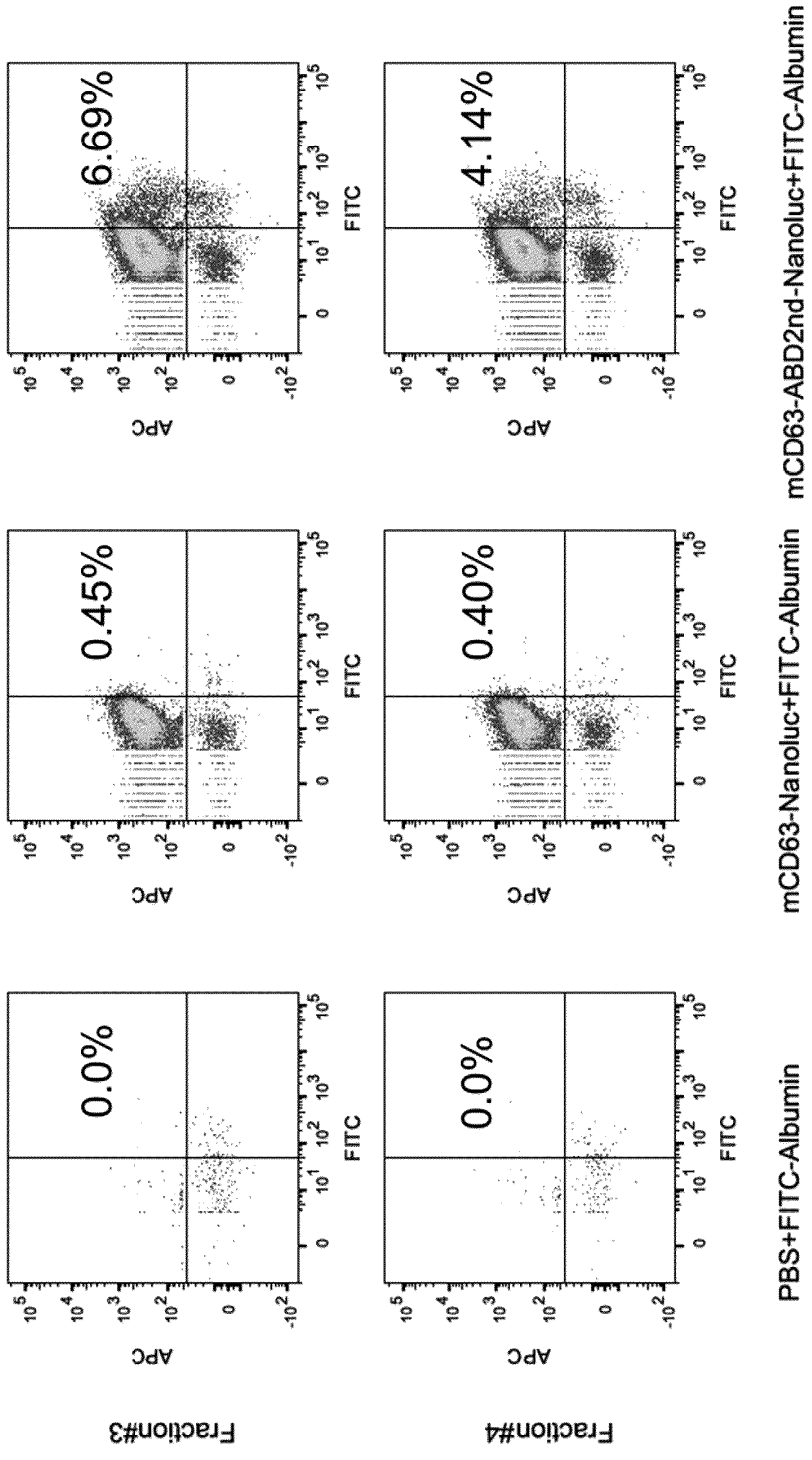

PBS+FITC-Albumin
mCD63-Nanoluc+FITC-Albumin
mCD63-ABD2nd-Nanoluc+FITC-Albumin
Fraction#3
Fraction#4
0.0%
0.0%
0.45%
0.40%
6.69%
4.14%
APC
FITC

FIG. 8

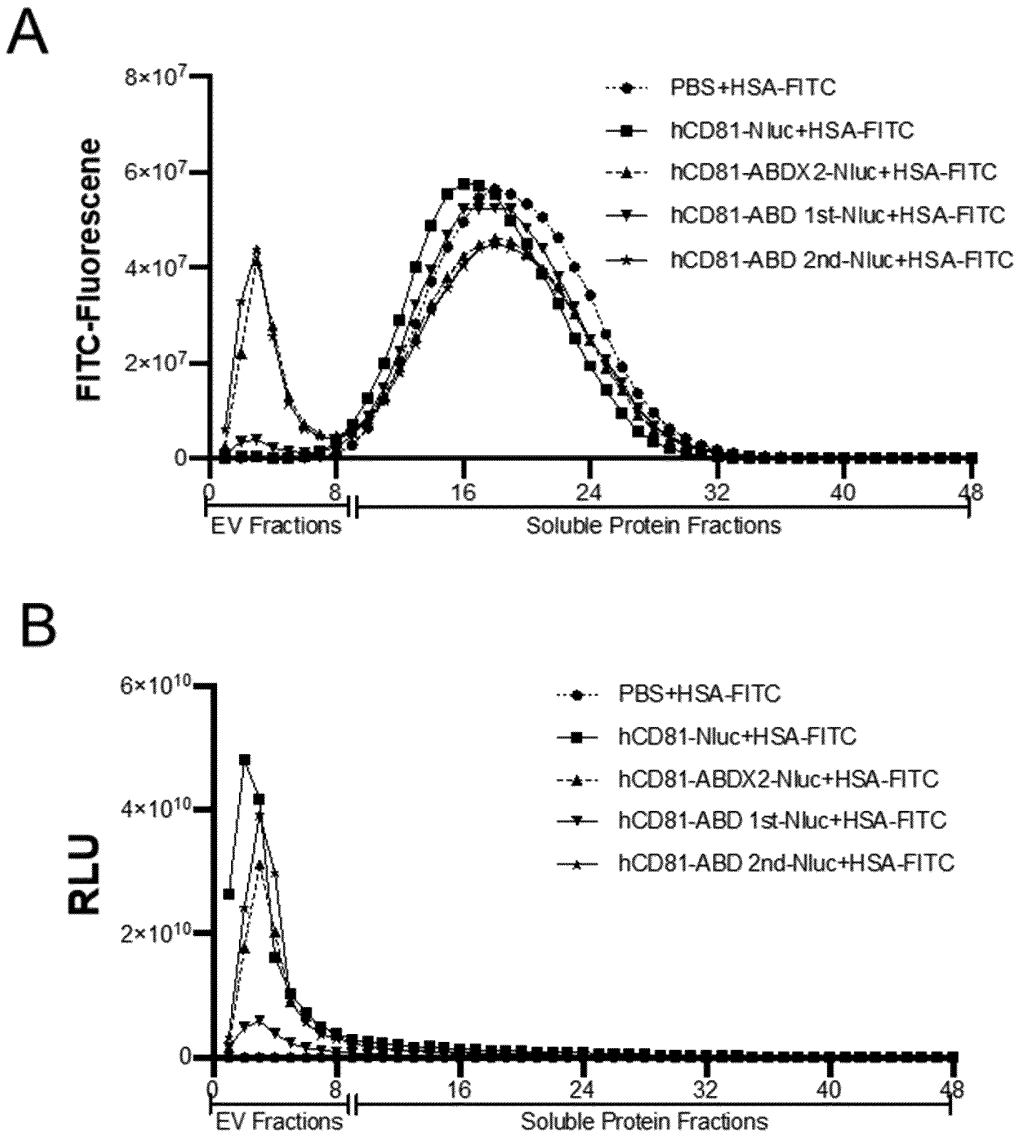

A
FITC-Fluorescene
8×10^7
6×10^7
4×10^7
2×10^7
0
PBS+HSA-FITC
hCD81-Nluc+HSA-FITC
hCD81-ABDX2-Nluc+HSA-FITC
hCD81-ABD 1st-Nluc+HSA-FITC
hCD81-ABD 2nd-Nluc+HSA-FITC
0 8 16 24 32 40 48
EV Fractions
Soluble Protein Fractions
B
RLU
6×10^10
4×10^10
2×10^10
0
PBS+HSA-FITC
hCD81-Nluc+HSA-FITC
hCD81-ABDX2-Nluc+HSA-FITC
hCD81-ABD 1st-Nluc+HSA-FITC
hCD81-ABD 2nd-Nluc+HSA-FITC
0 8 16 24 32 40 48
EV Fractions
Soluble Protein Fractions

FIG. 9

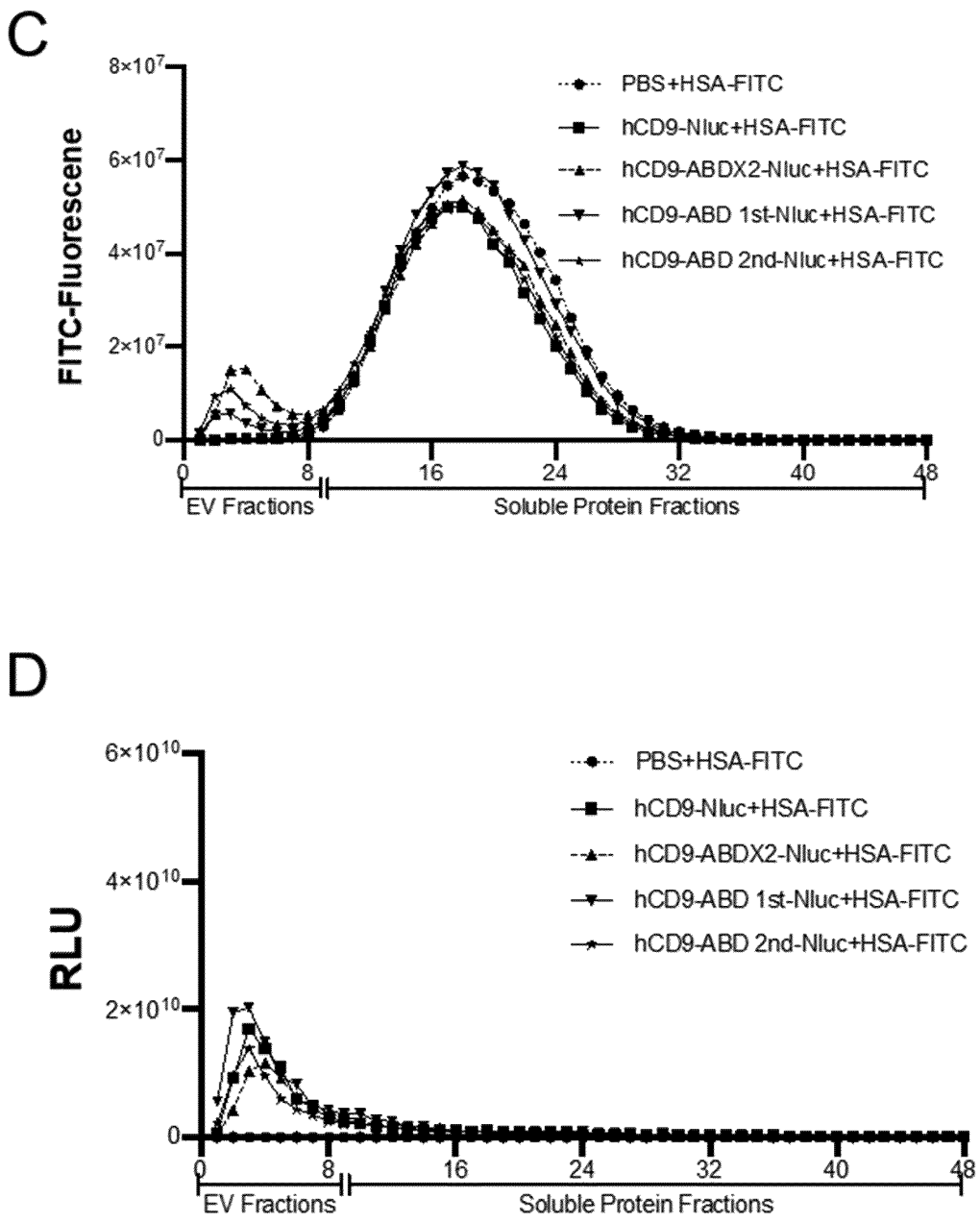
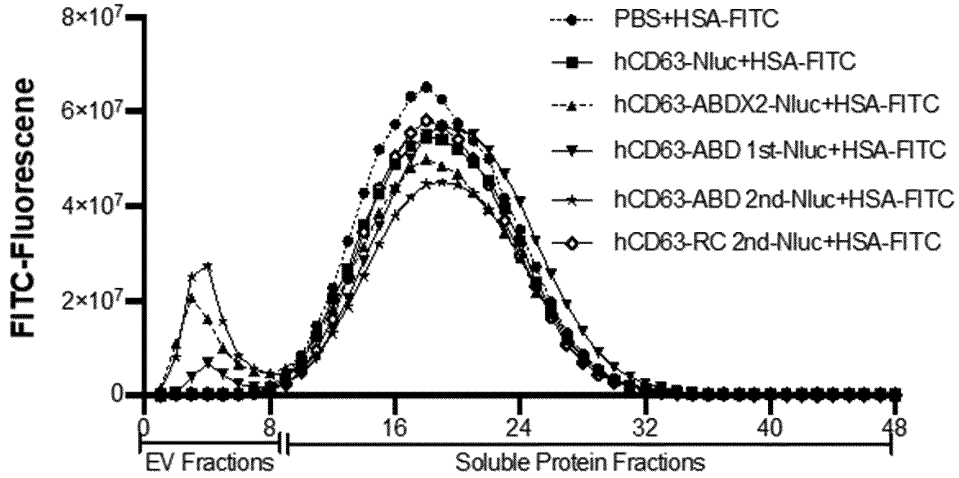

C
8×10^7
6×10^7
4×10^7
2×10^7
0
FITC-Fluorescene
PBS+HSA-FITC
hCD9-Nluc+HSA-FITC
hCD9-ABDX2-Nluc+HSA-FITC
hCD9-ABD 1st-Nluc+HSA-FITC
hCD9-ABD 2nd-Nluc+HSA-FITC
0
8
16
24
32
40
48
EV Fractions
Soluble Protein Fractions
D
6×10^10
4×10^10
2×10^10
0
RLU
PBS+HSA-FITC
hCD9-Nluc+HSA-FITC
hCD9-ABDX2-Nluc+HSA-FITC
hCD9-ABD 1st-Nluc+HSA-FITC
hCD9-ABD 2nd-Nluc+HSA-FITC
0
8
16
24
32
40
48
EV Fractions
Soluble Protein Fractions

FIG. 9 (cont.)

E


8×10^7
6×10^7
4×10^7
2×10^7
0
FITC-Fluorescene
PBS+HSA-FITC
hCD63-Nluc+HSA-FITC
hCD63-ABDX2-Nluc+HSA-FITC
hCD63-ABD 1st-Nluc+HSA-FITC
hCD63-ABD 2nd-Nluc+HSA-FITC
hCD63-RC 2nd-Nluc+HSA-FITC
0
8
16
24
32
40
48
EV Fractions
Soluble Protein Fractions

F

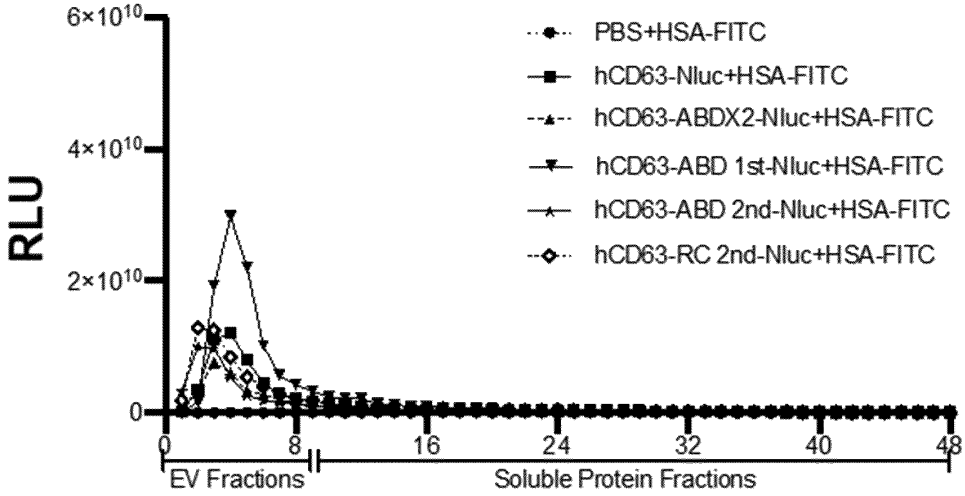

6×10^10
4×10^10
2×10^10
0
RLU
PBS+HSA-FITC
hCD63-Nluc+HSA-FITC
hCD63-ABDX2-Nluc+HSA-FITC
hCD63-ABD 1st-Nluc+HSA-FITC
hCD63-ABD 2nd-Nluc+HSA-FITC
hCD63-RC 2nd-Nluc+HSA-FITC
0
8
16
24
32
40
48
EV Fractions
Soluble Protein Fractions

FIG. 9 (cont.)

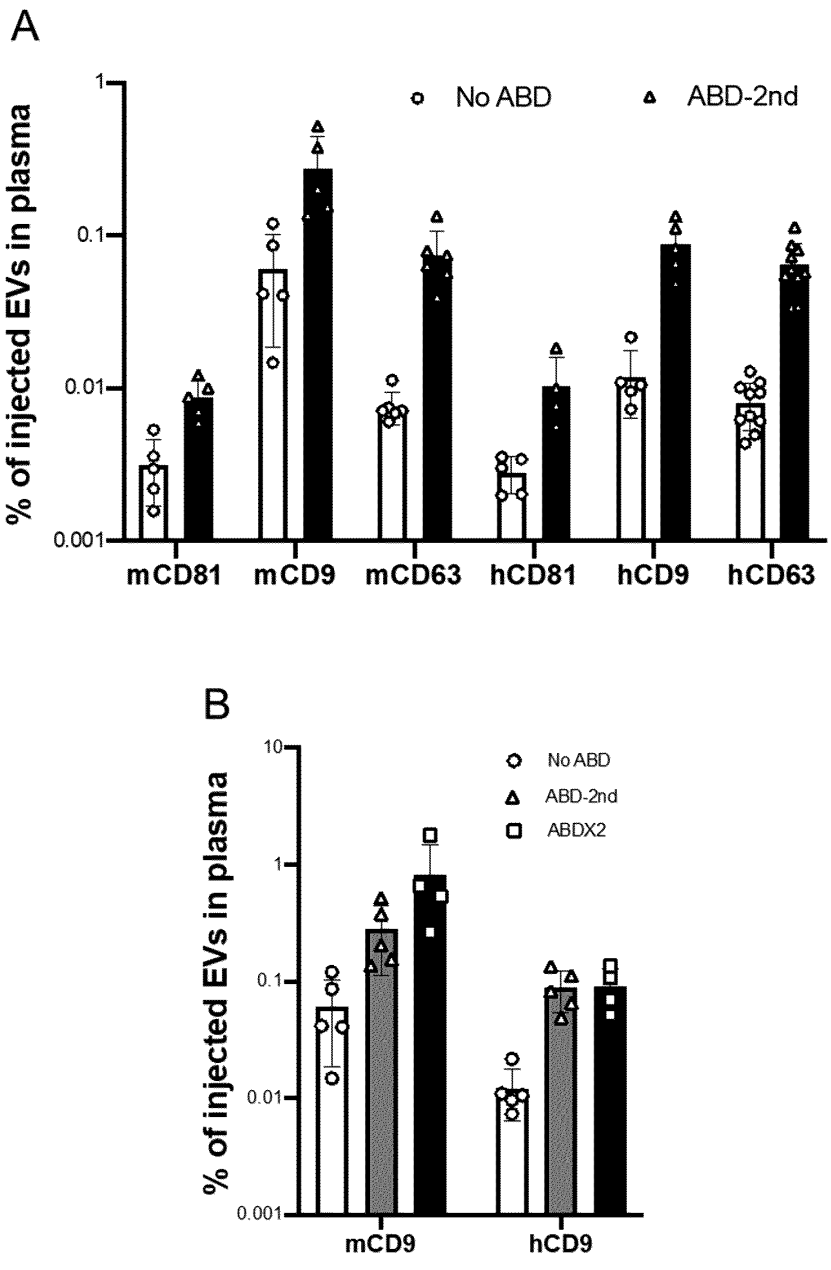

A
% of injected EVs in plasma
1
0.1
0.01
0.001
No ABD
ABD-2nd
mCD81
mCD9
mCD63
hCD81
hCD9
hCD63
B
% of injected EVs in plasma
10
1
0.1
0.01
0.001
No ABD
ABD-2nd
ABDX2
mCD9
hCD9

FIG. 10

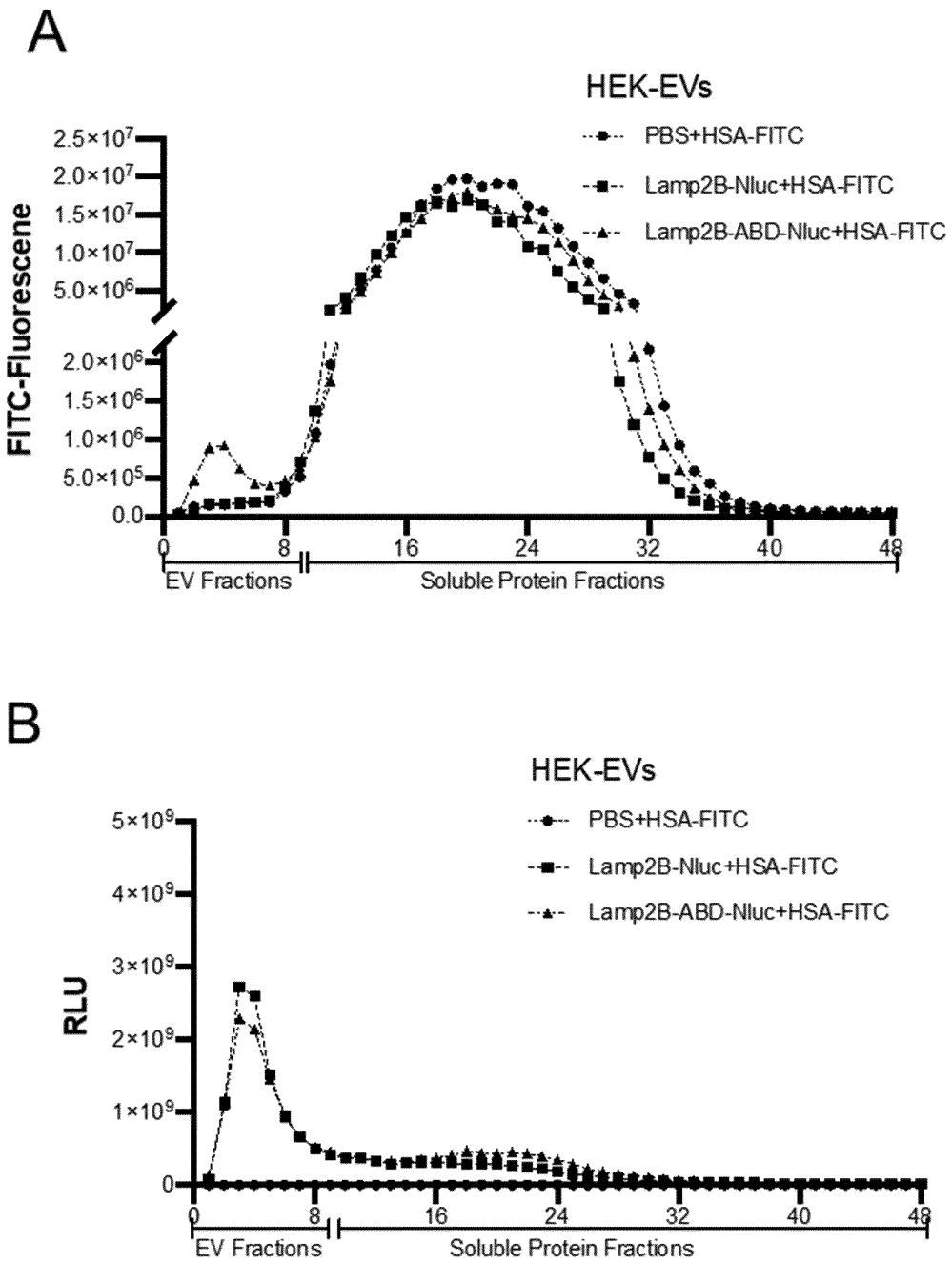

A
HEK-EVs
PBS+HSA-FITC
Lamp2B-Nluc+HSA-FITC
Lamp2B-ABD-Nluc+HSA-FITC
FITC-Fluorescene
2.5×10^7
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0.0
0
8
16
24
32
40
48
EV Fractions
Soluble Protein Fractions
B
HEK-EVs
PBS+HSA-FITC
Lamp2B-Nluc+HSA-FITC
Lamp2B-ABD-Nluc+HSA-FITC
RLU
5×10^9
4×10^9
3×10^9
2×10^9
1×10^9
0
0
8
16
24
32
40
48
EV Fractions
Soluble Protein Fractions

FIG. 11

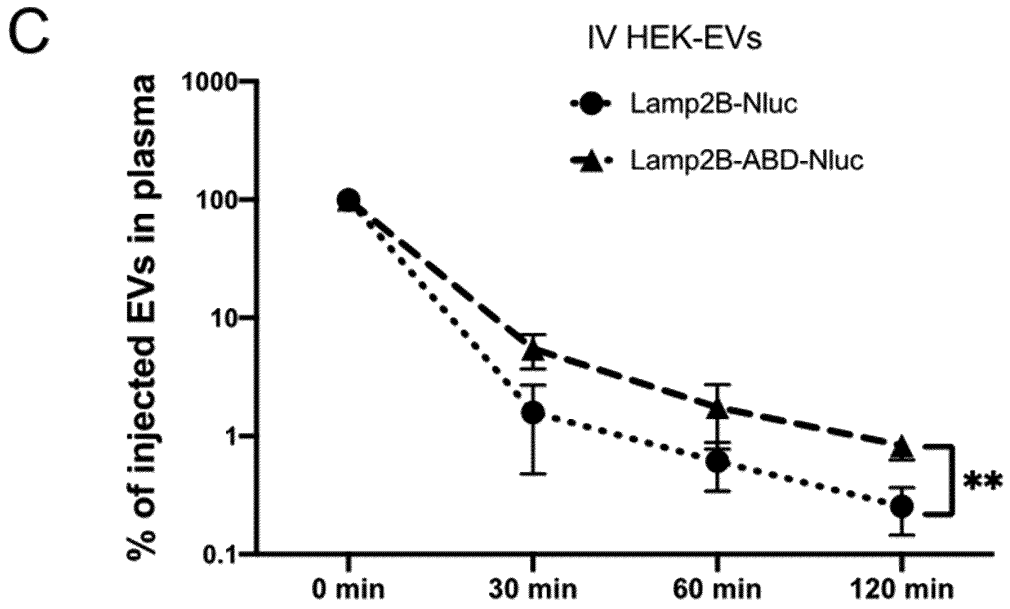

C
IV HEK-EVs
Lamp2B-Nluc
Lamp2B-ABD-Nluc
% of injected EVs in plasma
1000
100
10
1
0.1
0 min
30 min
60 min
120 min
**

FIG. 11 (cont.)

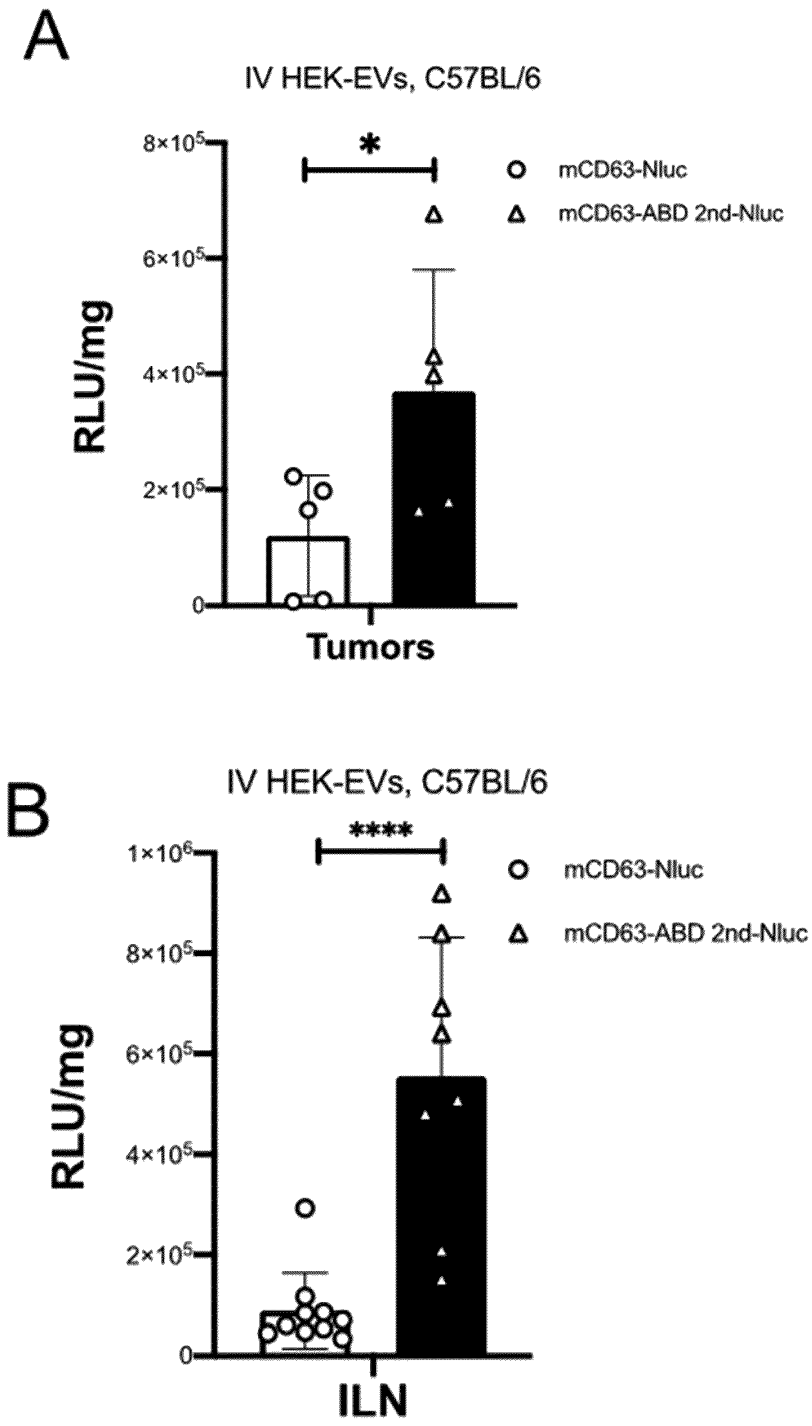

A
IV HEK-EVs, C57BL/6
*
mCD63-Nluc
mCD63-ABD 2nd-Nluc
RLU/mg
8×10^5
6×10^5
4×10^5
2×10^5
0
Tumors
B
IV HEK-EVs, C57BL/6
****
mCD63-Nluc
mCD63-ABD 2nd-Nluc
RLU/mg
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
ILN

FIG. 12

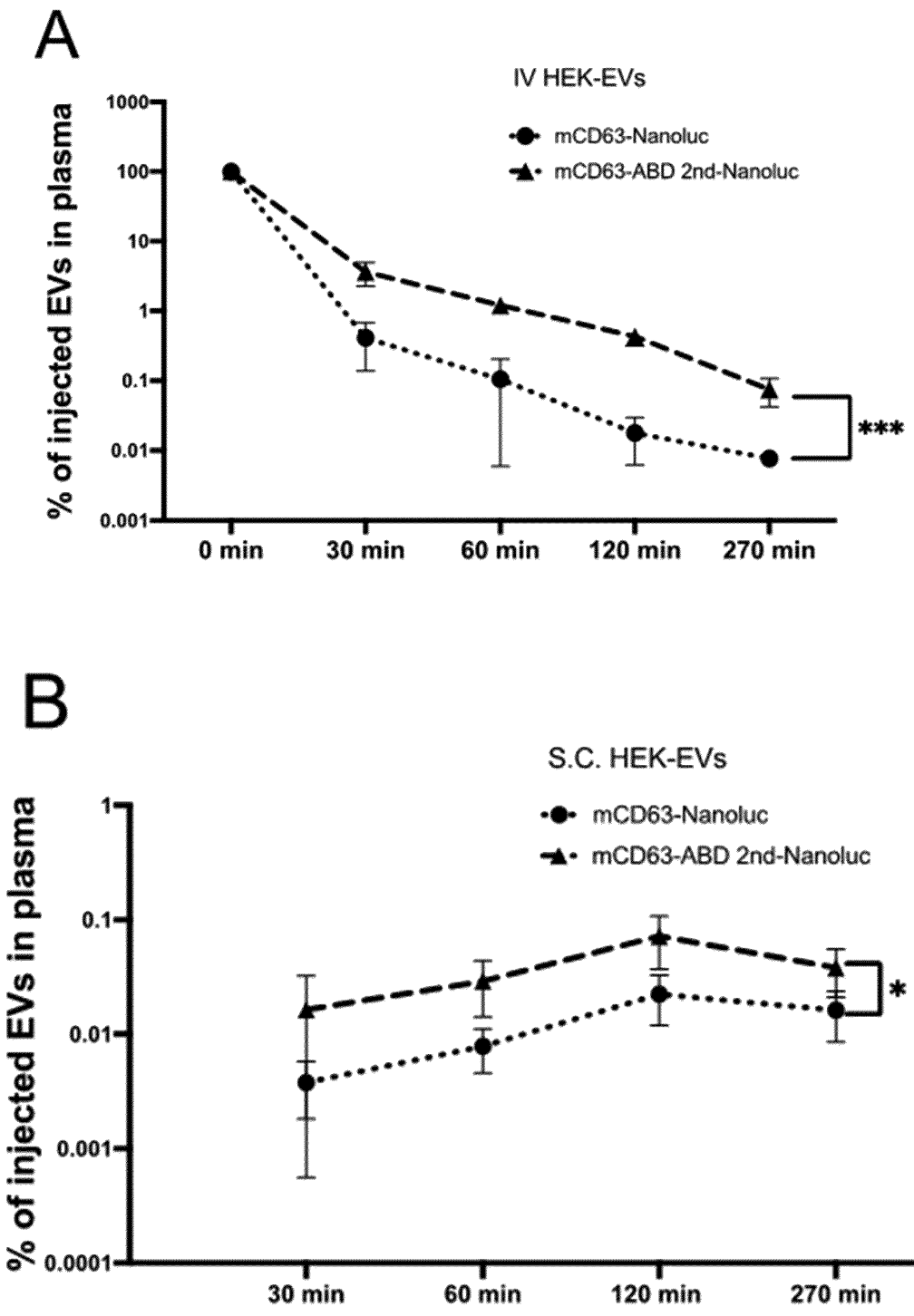

A
IV HEK-EVs
mCD63-Nanoluc
mCD63-ABD 2nd-Nanoluc
% of injected EVs in plasma
1000
100
10
1
0.1
0.01
0.001
***
0 min
30 min
60 min
120 min
270 min
B
S.C. HEK-EVs
mCD63-Nanoluc
mCD63-ABD 2nd-Nanoluc
% of injected EVs in plasma
1
0.1
0.01
0.001
0.0001
*
30 min
60 min
120 min
270 min

FIG. 13

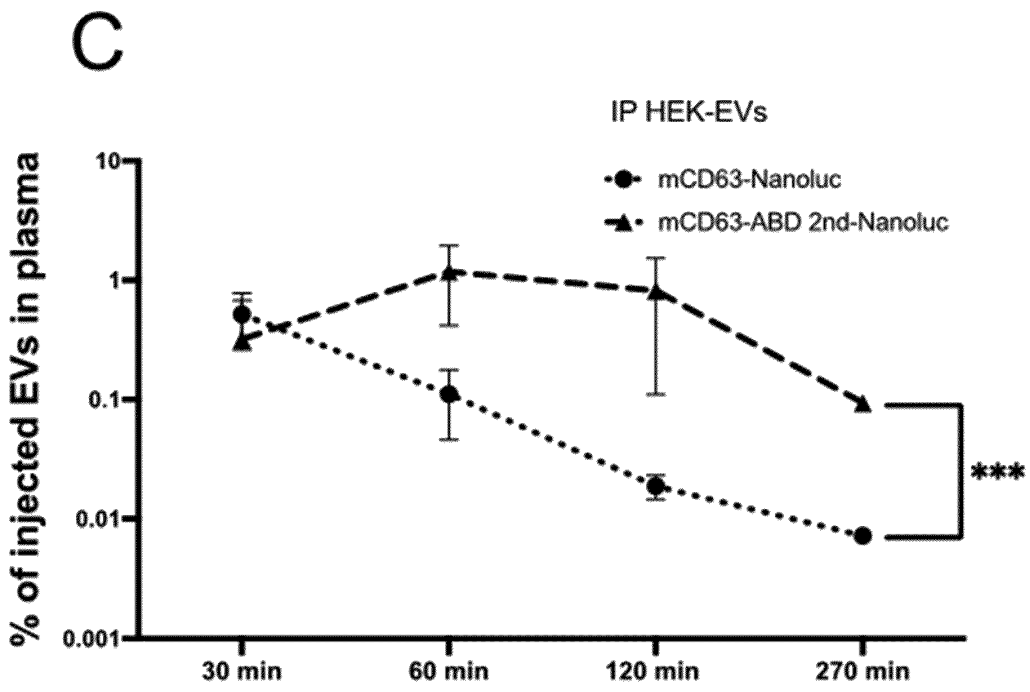

C
IP HEK-EVs
mCD63-Nanoluc
mCD63-ABD 2nd-Nanoluc
% of injected EVs in plasma
10
1
0.1
0.01
0.001
***
30 min
60 min
120 min
270 min

FIG. 13 (cont.)

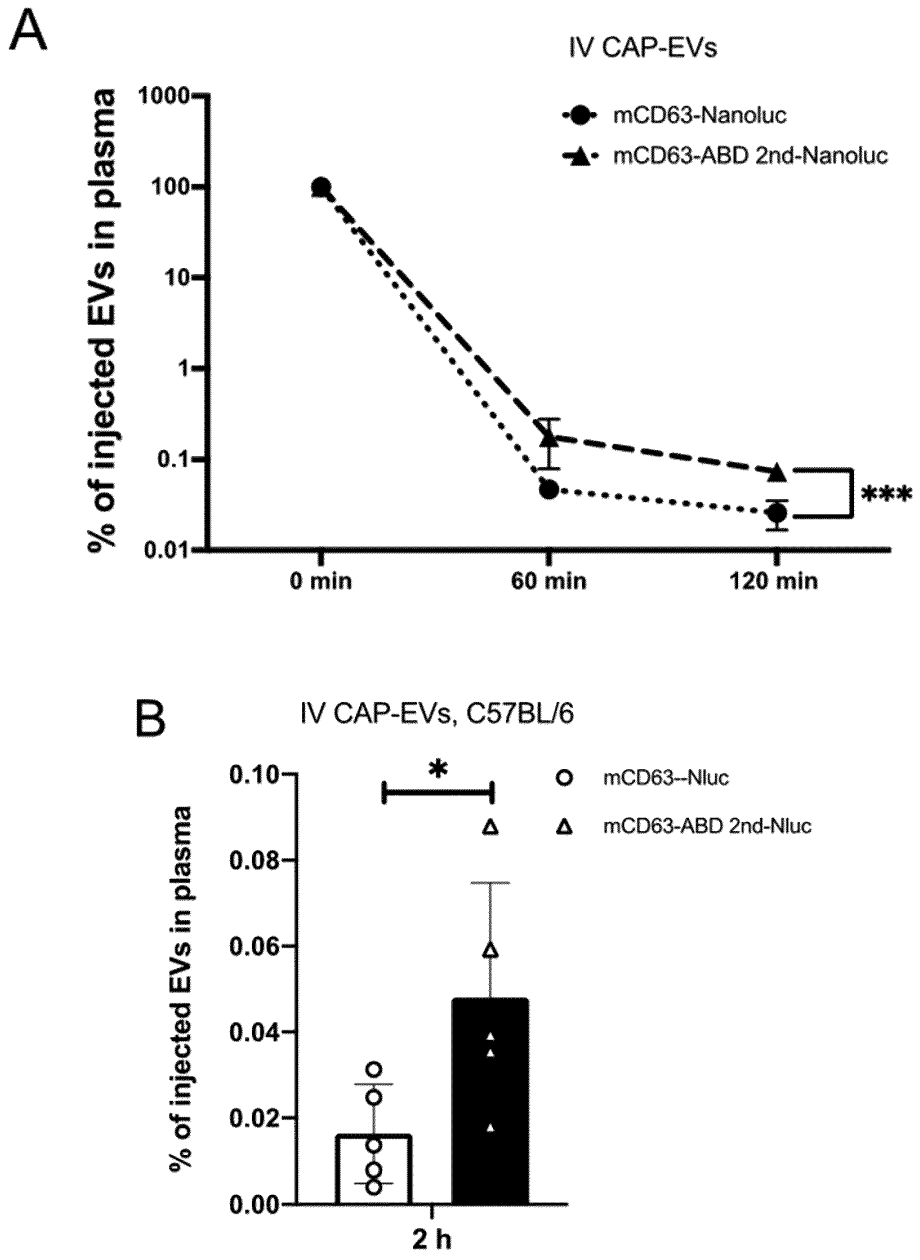

A
IV CAP-EVs
mCD63-Nanoluc
mCD63-ABD 2nd-Nanoluc
% of injected EVs in plasma
1000
100
10
1
0.1
0.01
***
0 min
60 min
120 min
B
IV CAP-EVs, C57BL/6
*
mCD63--Nluc
mCD63-ABD 2nd-Nluc
% of injected EVs in plasma
0.10
0.08
0.06
0.04
0.02
0.00
2 h

FIG. 14

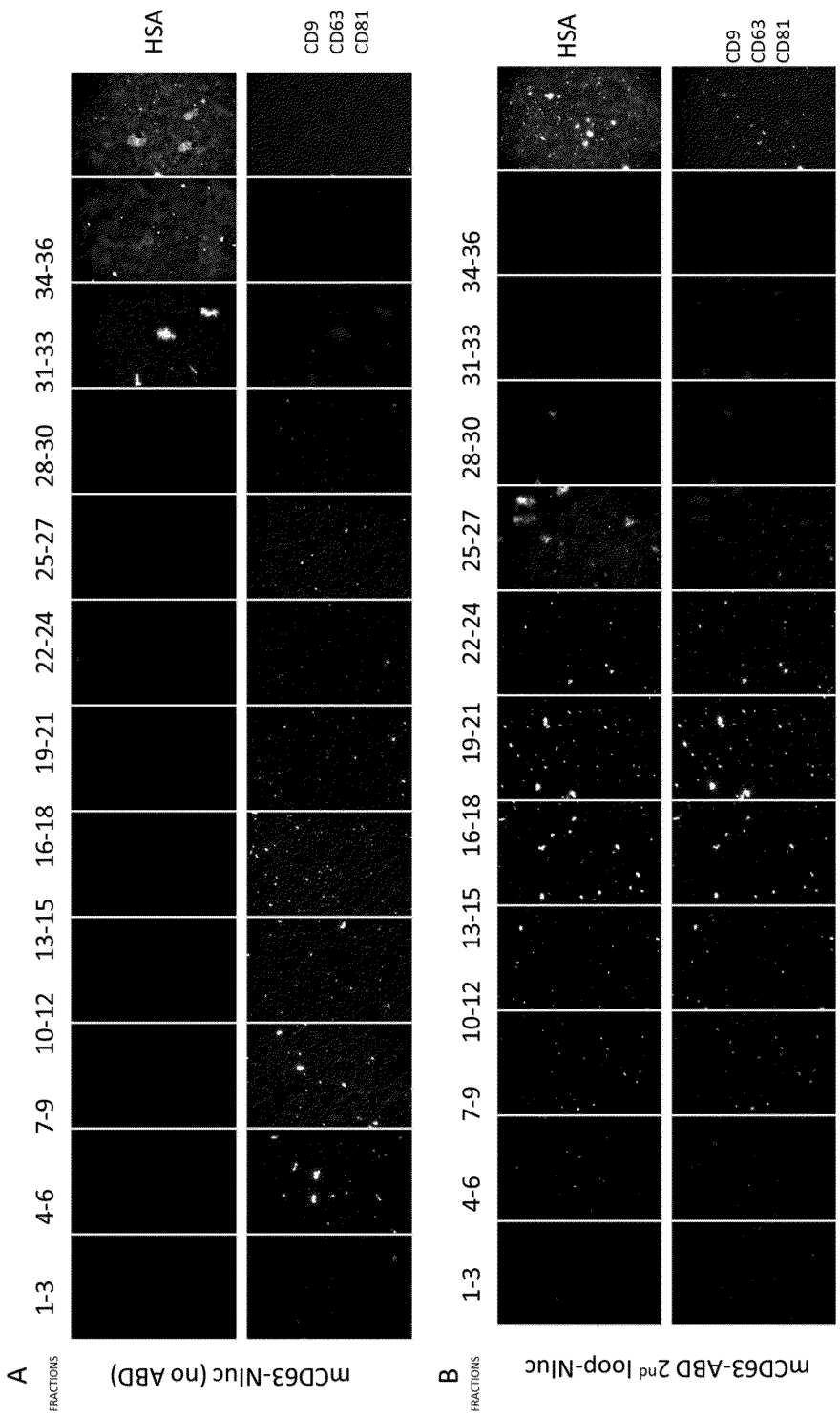

A
FRACTIONS
mCD63-Nluc (no ABD)
HSA
CD9
CD63
CD81
1-3
4-6
7-9
10-12
13-15
16-18
19-21
22-24
25-27
28-30
31-33
34-36
B
FRACTIONS
mCD63-ABD 2nd loop-Nluc
HSA
CD9
CD63
CD81
1-3
4-6
7-9
10-12
13-15
16-18
19-21
22-24
25-27
28-30
31-33
34-36

FIG. 15

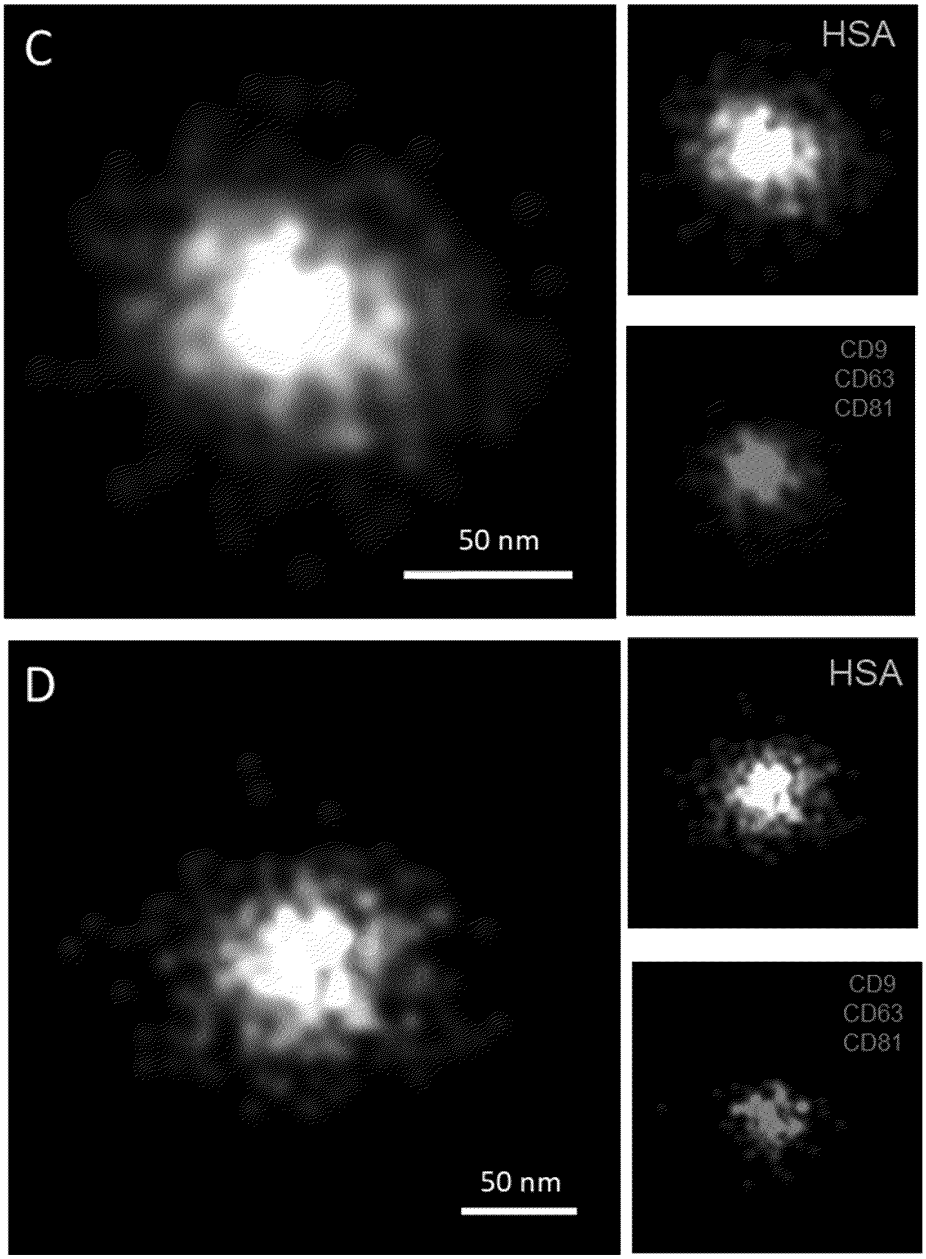


FIG. 15 (cont.)

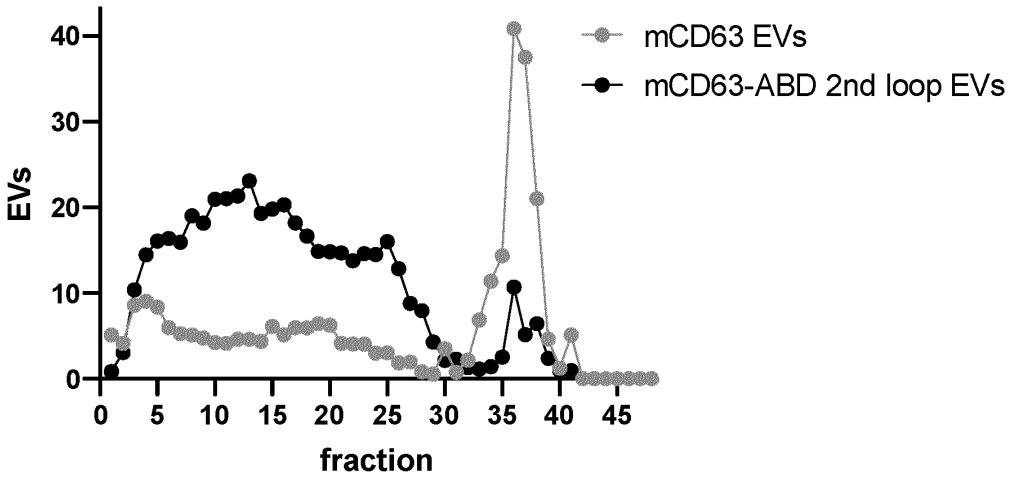

40
30
20
10
0
EVs
0
5
10
15
20
25
30
35
40
45
fraction
mCD63 EVs
mCD63-ABD 2nd loop EVs

FIG. 16

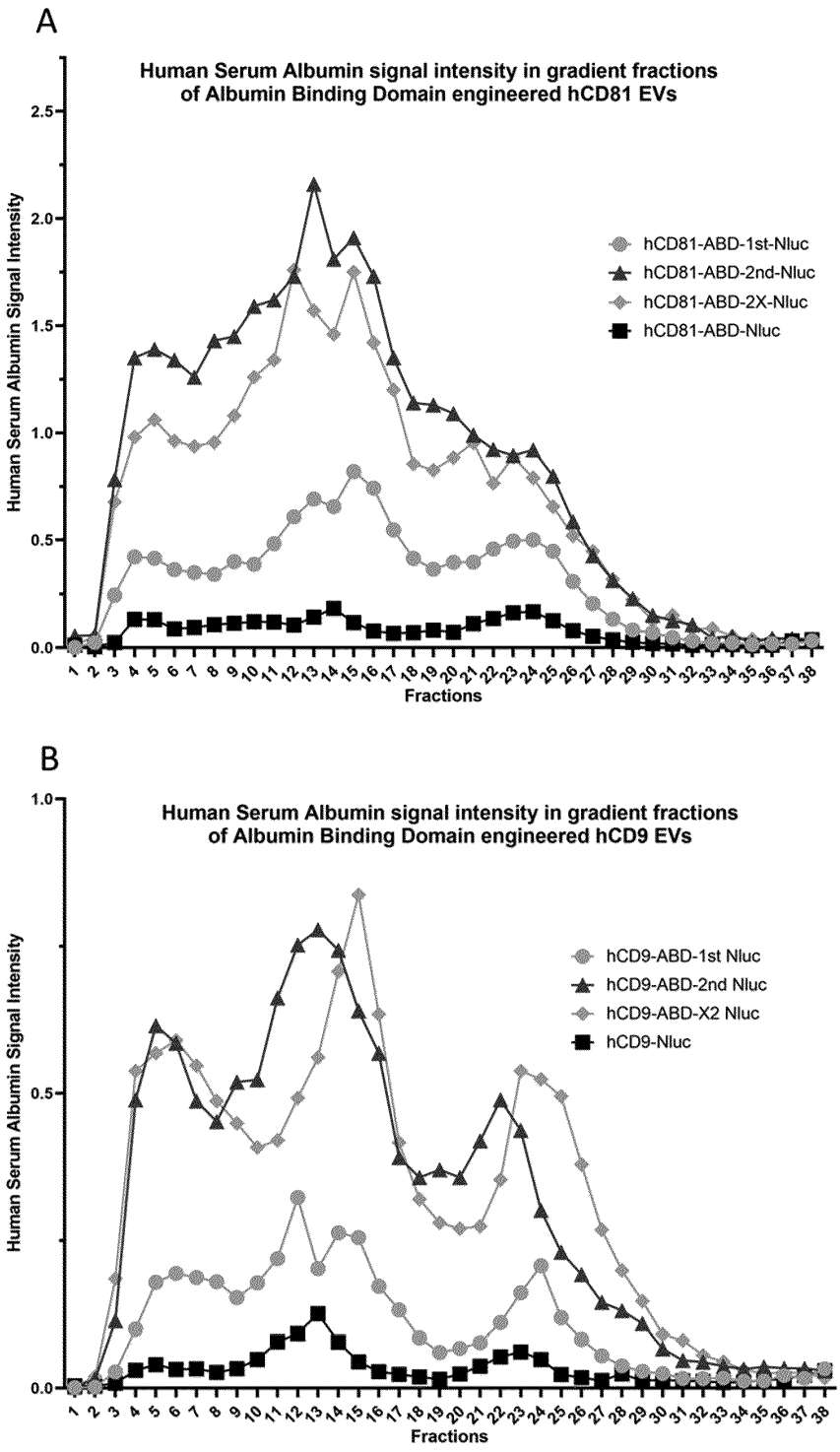
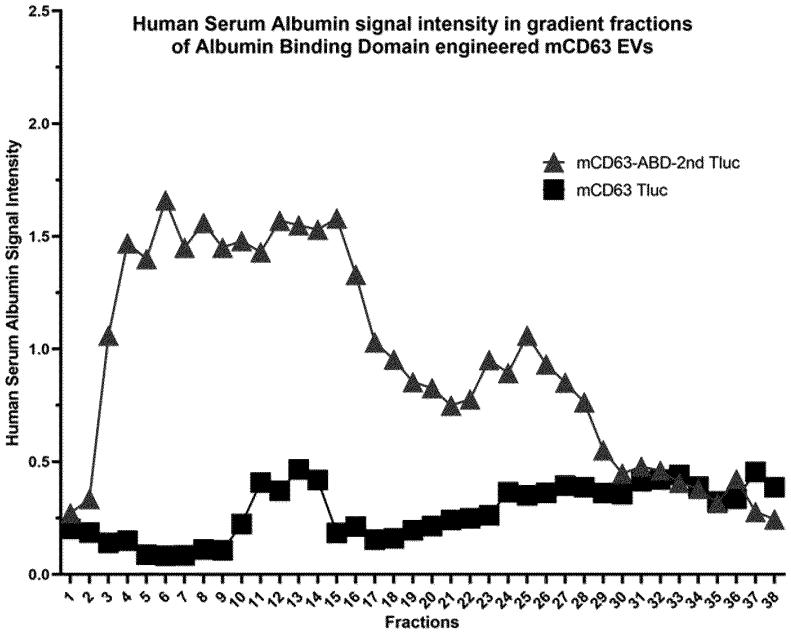

A
Human Serum Albumin signal intensity in gradient fractions of Albumin Binding Domain engineered hCD81 EVs
Human Serum Albumin Signal Intensity
Fractions
hCD81-ABD-1st-Nluc
hCD81-ABD-2nd-Nluc
hCD81-ABD-2X-Nluc
hCD81-ABD-Nluc
B
Human Serum Albumin signal intensity in gradient fractions of Albumin Binding Domain engineered hCD9 EVs
Human Serum Albumin Signal Intensity
Fractions
hCD9-ABD-1st Nluc
hCD9-ABD-2nd Nluc
hCD9-ABD-X2 Nluc
hCD9-Nluc

FIG. 17

C



D

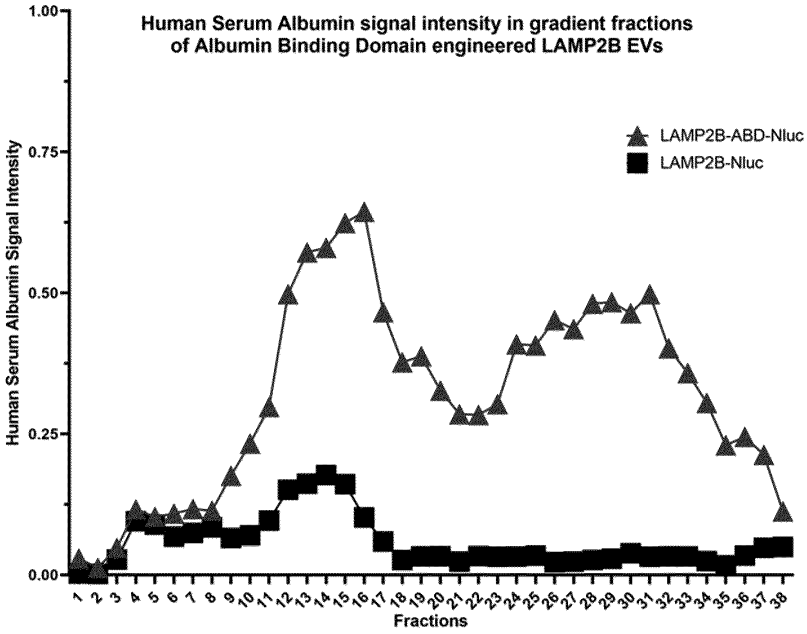


FIG. 17 (cont.)

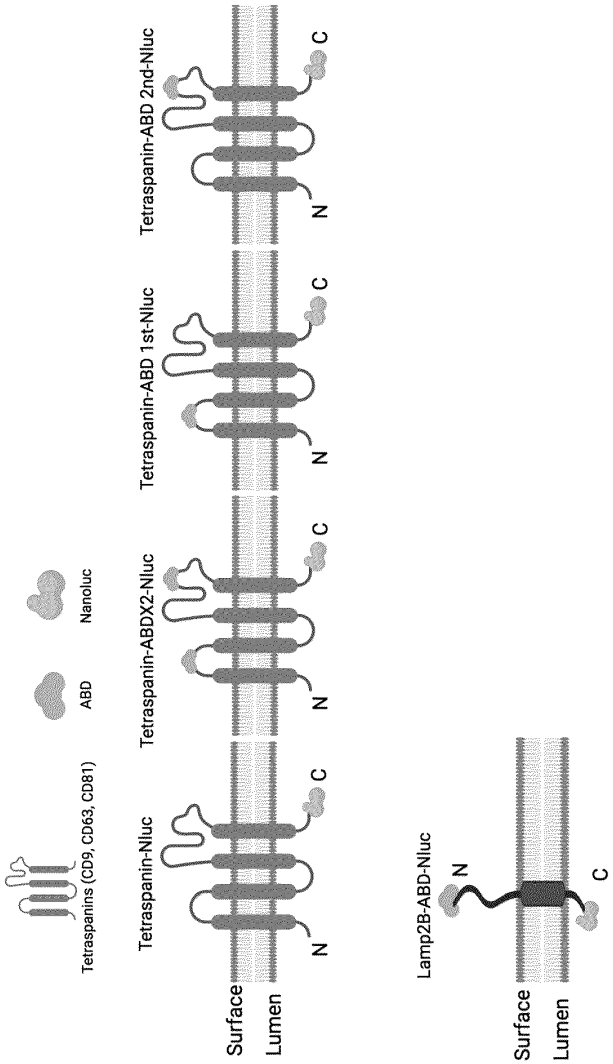

Tetraspanins (CD9, CD63, CD81)
ABD
Nanoluc
Tetraspanin-Nluc
Tetraspanin-ABDX2-Nluc
Tetraspanin-ABD 1st-Nluc
Tetraspanin-ABD 2nd-Nluc
Lamp2B-ABD-Nluc
N
C
Surface
Lumen

FIG. 18