Figure 1

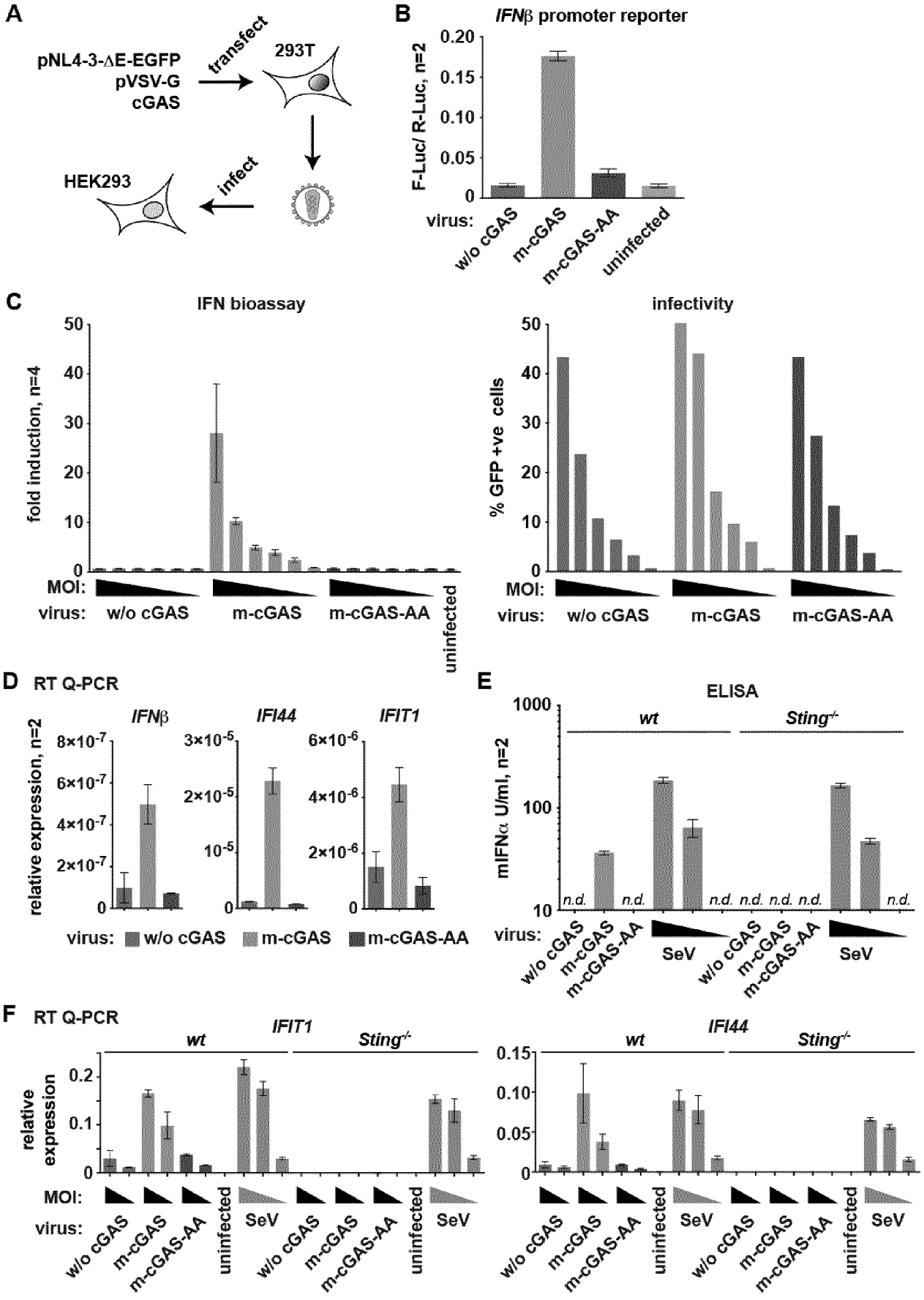

A
pNL4-3-ΔE-EGFP
pVSV-G
cGAS
transfect
293T
HEK293
infect
B
IFNβ promoter reporter
F-Luc/ R-Luc, n=2
virus:
w/o cGAS
m-cGAS
m-cGAS-AA
uninfected
C
IFN bioassay
fold induction, n=4
MOI:
virus:
w/o cGAS
m-cGAS
m-cGAS-AA
uninfected
infectivity
% GFP +ve cells
D
RT Q-PCR
IFNβ
IFI44
IFIT1
relative expression, n=2
E
ELISA
wt
Sting-/-
mIFNα U/ml, n=2
n.d.
SeV
F
RT Q-PCR
IFIT1
IFI44
relative expression

Figure 2

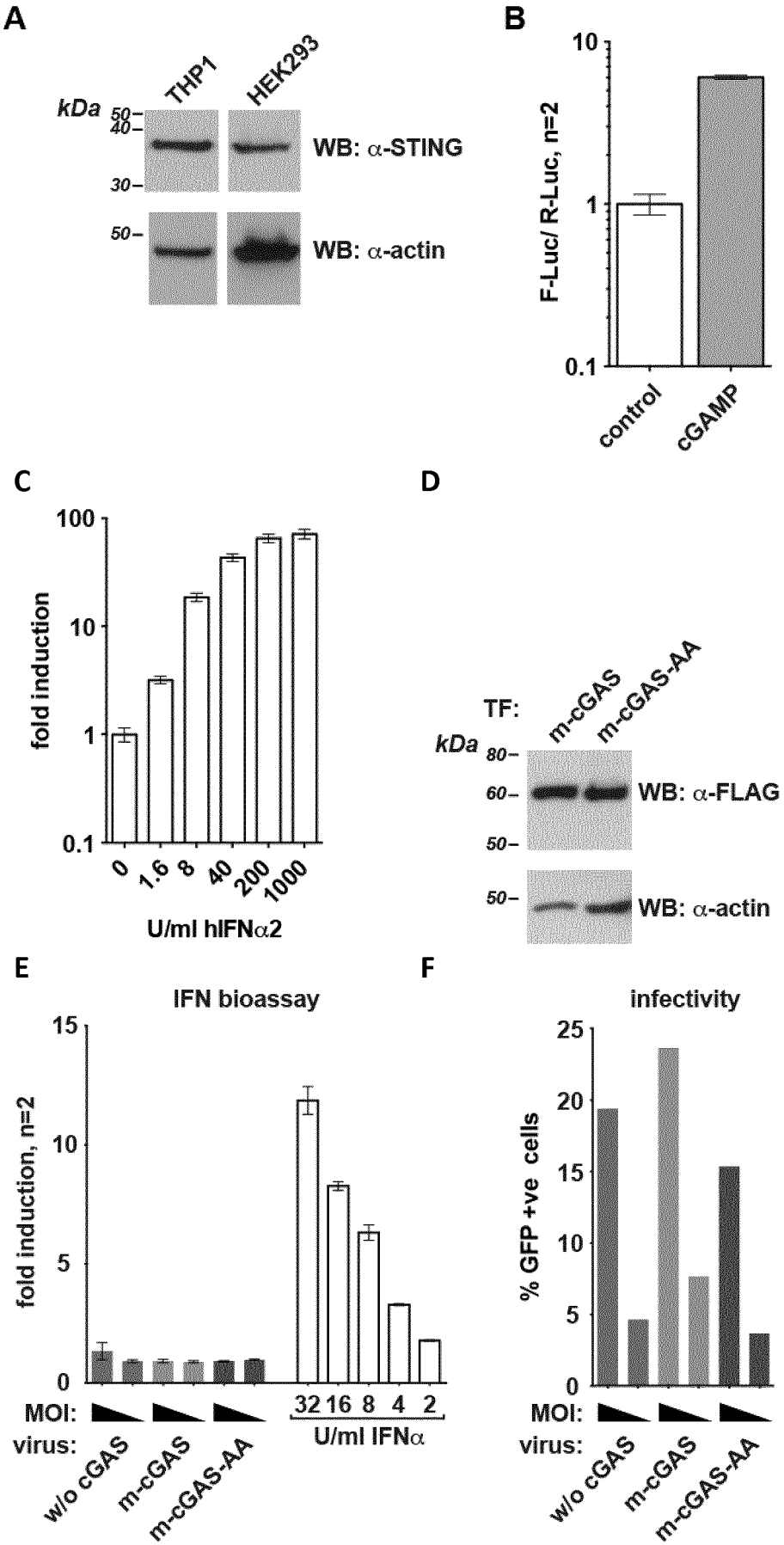

A
THP1
HEK293
kDa
50
40
30
WB: α-STING
50
WB: α-actin
B
F-Luc/ R-Luc, n=2
10
1
0.1
control
cGAMP
C
fold induction
100
10
1
0.1
0
1.6
8
40
200
1000
U/ml hIFNα2
D
TF:
m-cGAS
m-cGAS-AA
kDa
80
60
50
WB: α-FLAG
50
WB: α-actin
E
IFN bioassay
fold induction, n=2
15
10
5
0
MOI:
virus:
w/o cGAS
m-cGAS
m-cGAS-AA
32 16 8 4 2
U/ml IFNα
F
infectivity
% GFP +ve cells
25
20
15
10
5
0
MOI:
virus:
w/o cGAS
m-cGAS
m-cGAS-AA

Figure 3

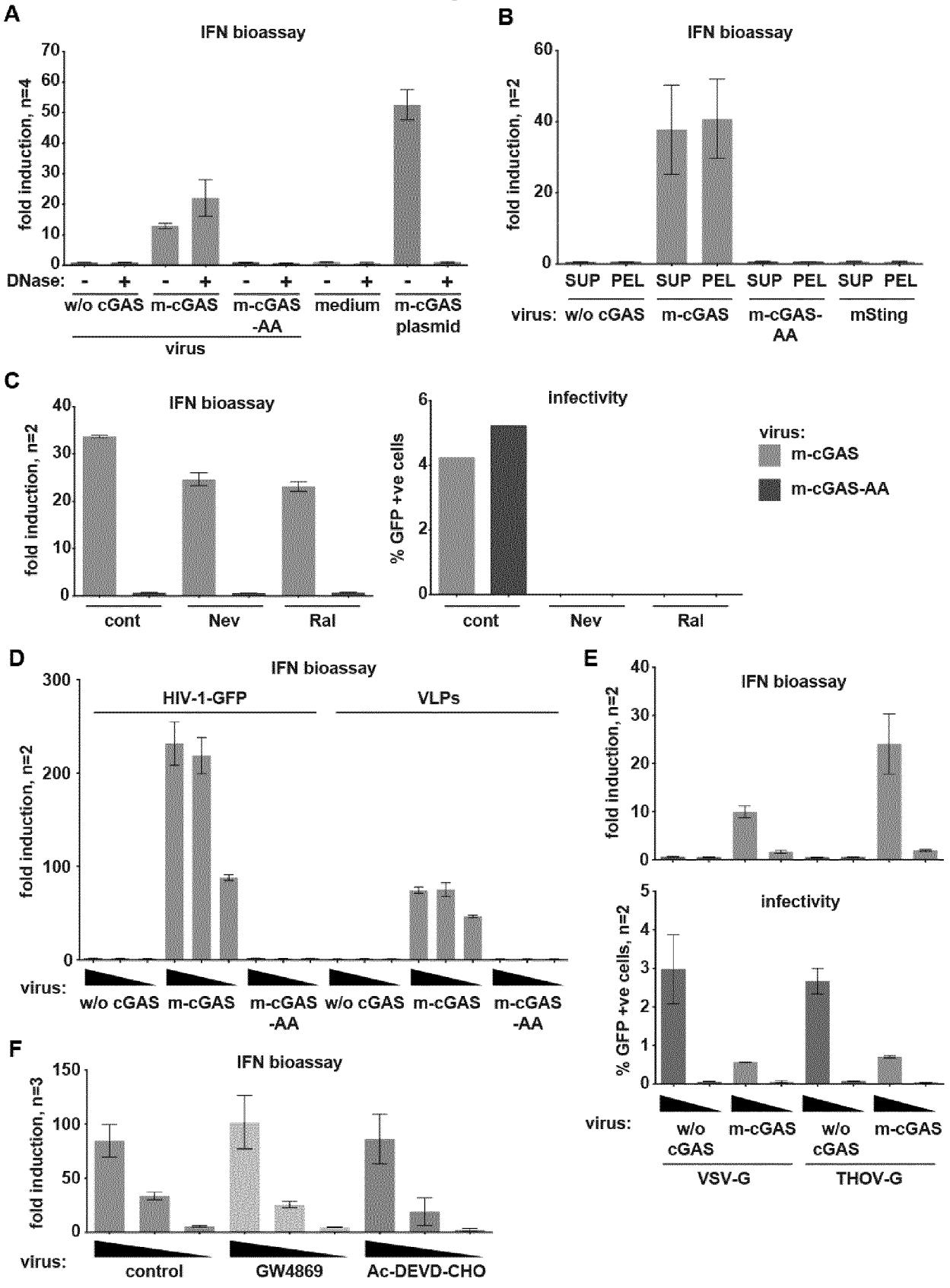

A
IFN bioassay
fold induction, n=4
DNase:
w/o cGAS
m-cGAS
m-cGAS -AA
medium
m-cGAS plasmid
virus
B
IFN bioassay
fold induction, n=2
SUP PEL SUP PEL SUP PEL SUP PEL
virus: w/o cGAS
m-cGAS
m-cGAS-AA
mSting
C
IFN bioassay
fold induction, n=2
cont
Nev
Ral
infectivity
% GFP +ve cells
virus:
m-cGAS
m-cGAS-AA
D
IFN bioassay
HIV-1-GFP
VLPs
fold induction, n=2
virus:
w/o cGAS
m-cGAS
m-cGAS -AA
E
IFN bioassay
fold induction, n=2
infectivity
% GFP +ve cells, n=2
virus:
w/o cGAS
m-cGAS
VSV-G
THOV-G
F
IFN bioassay
fold induction, n=3
virus:
control
GW4869
Ac-DEVD-CHO

Figure 4

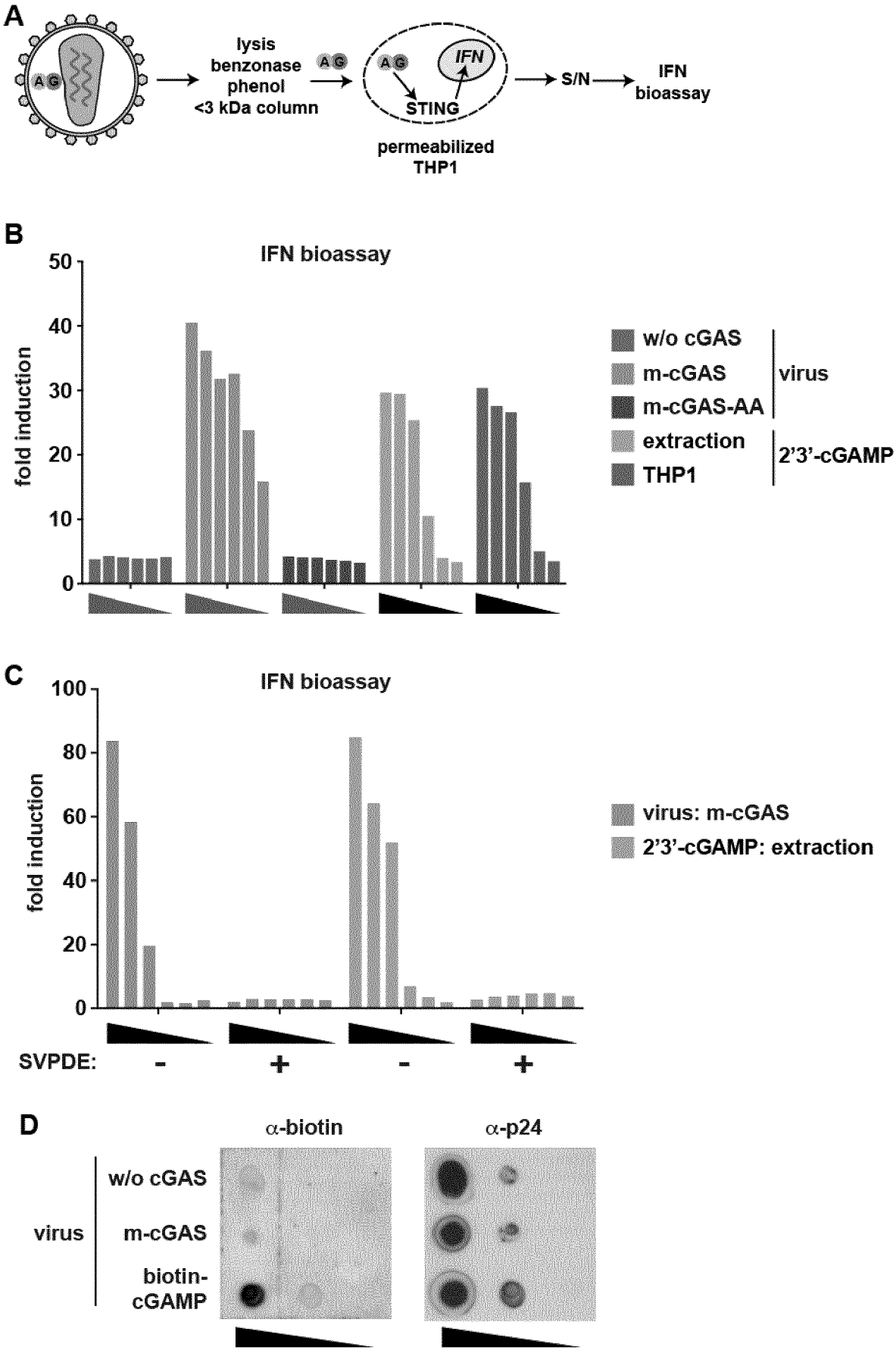

A
A G
lysis
benzonase
phenol
<3 kDa column
A G
A G
IFN
STING
permeabilized
THP1
S/N
IFN
bioassay
B
IFN bioassay
fold induction
50
40
30
20
10
0
w/o cGAS
m-cGAS
m-cGAS-AA
virus
extraction
THP1
2'3'-cGAMP
C
IFN bioassay
fold induction
100
80
60
40
20
0
virus: m-cGAS
2'3'-cGAMP: extraction
SVPDE:
-
+
-
+
D
α-biotin
α-p24
virus
w/o cGAS
m-cGAS
biotin-
cGAMP

Figure 5

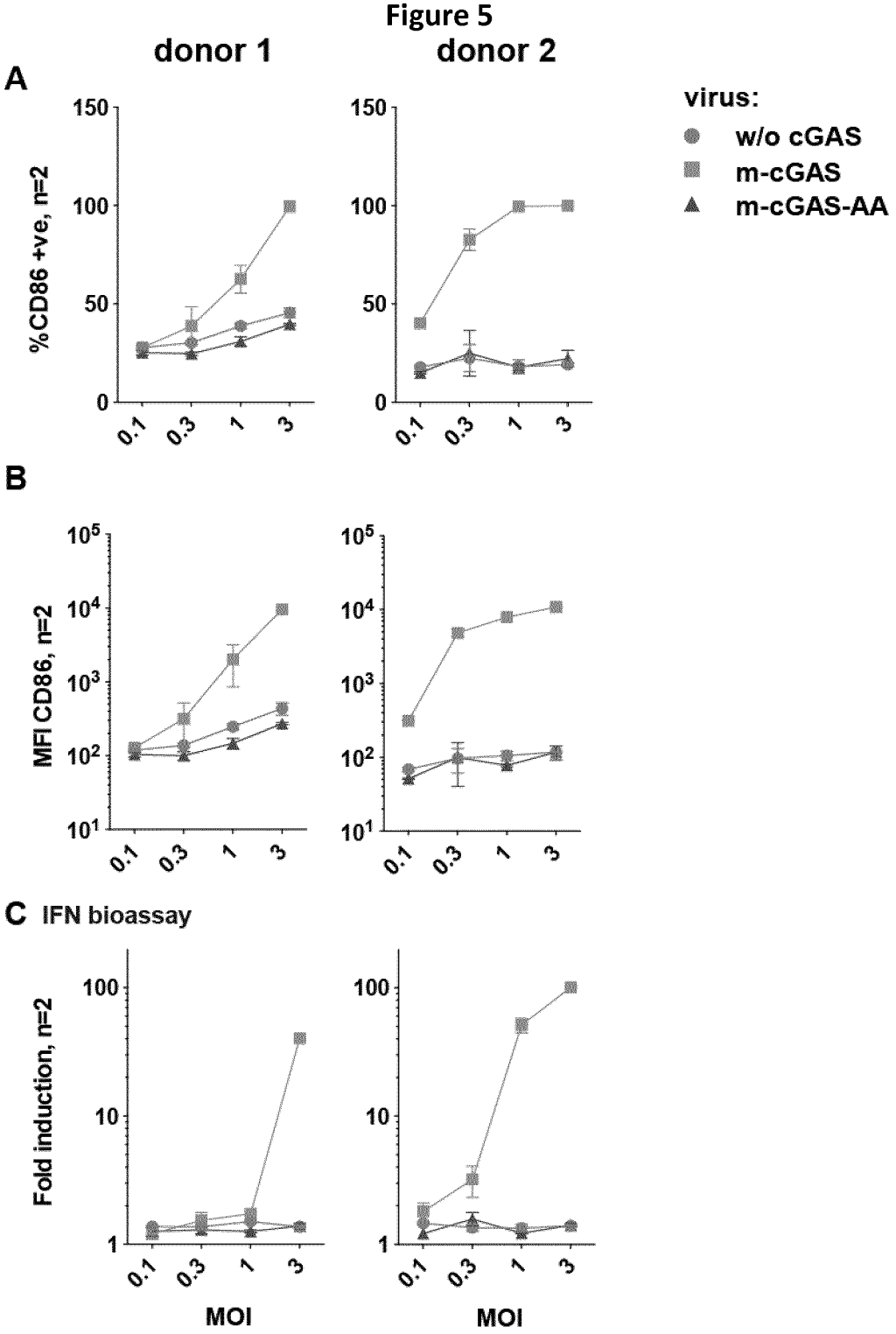

donor 1
donor 2
A
%CD86 +ve, n=2
150
100
50
0
0.1
0.3
1
3
virus:
w/o cGAS
m-cGAS
m-cGAS-AA
B
MFI CD86, n=2
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
C IFN bioassay
Fold induction, n=2
100
10
1
MOI

**Figure 6**

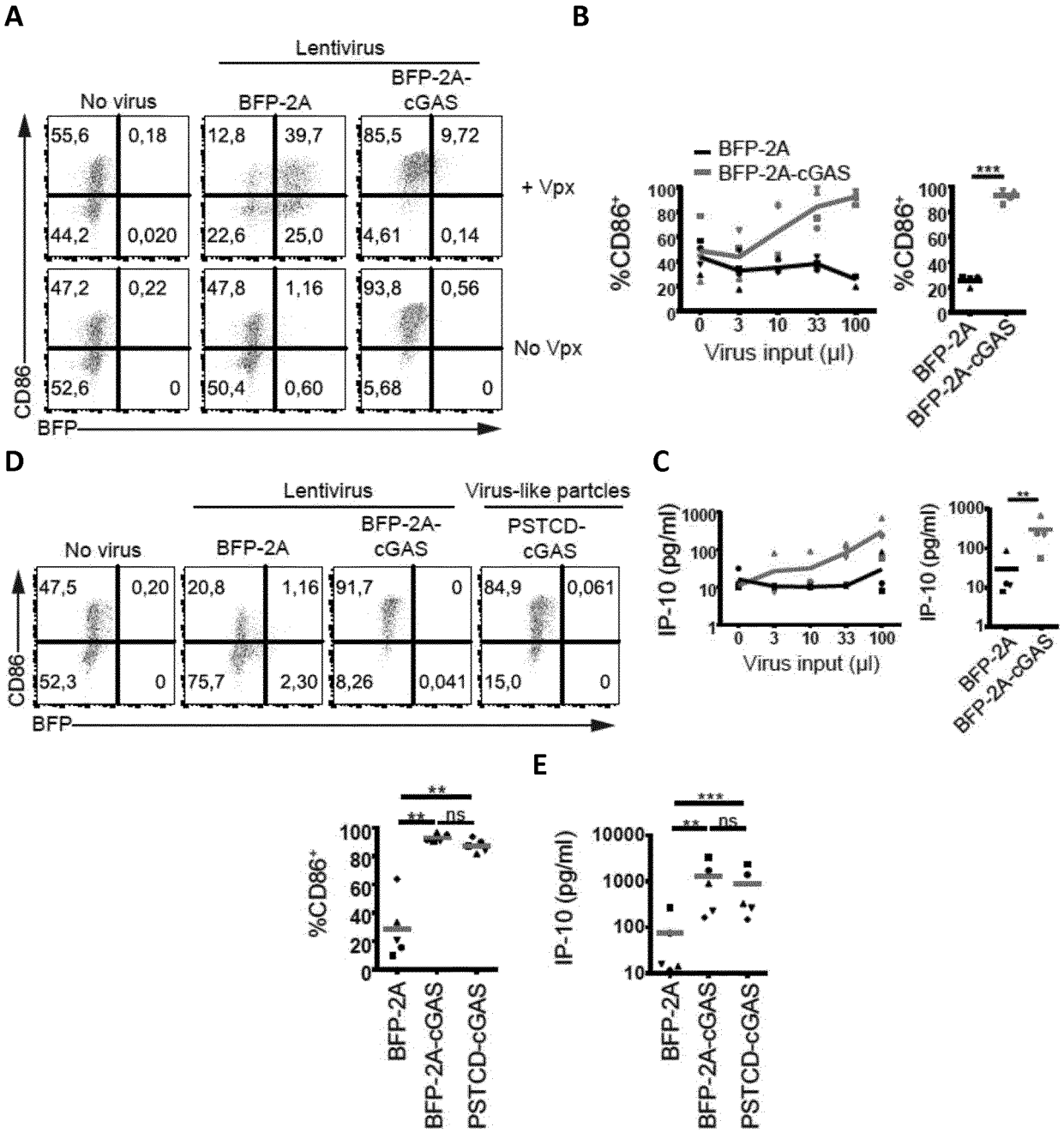

A
B
Lentivirus
No virus
BFP-2A
BFP-2A-cGAS
55,6
0,18
12,8
39,7
85,5
9,72
+ Vpx
44,2
0,020
22,6
25,0
4,61
0,14
47,2
0,22
47,8
1,16
93,8
0,56
No Vpx
52,6
0
50,4
0,60
5,68
0
CD86
BFP
BFP-2A
BFP-2A-cGAS
%CD86+
Virus input (µl)
0 3 10 33 100
***
D
C
Lentivirus
Virus-like partcles
No virus
BFP-2A
BFP-2A-cGAS
PSTCD-cGAS
47,5
0,20
20,8
1,16
91,7
0
84,9
0,061
52,3
0
75,7
2,30
8,26
0,041
15,0
0
IP-10 (pg/ml)
**
E
ns
PSTCD-cGAS

**Figure 7**

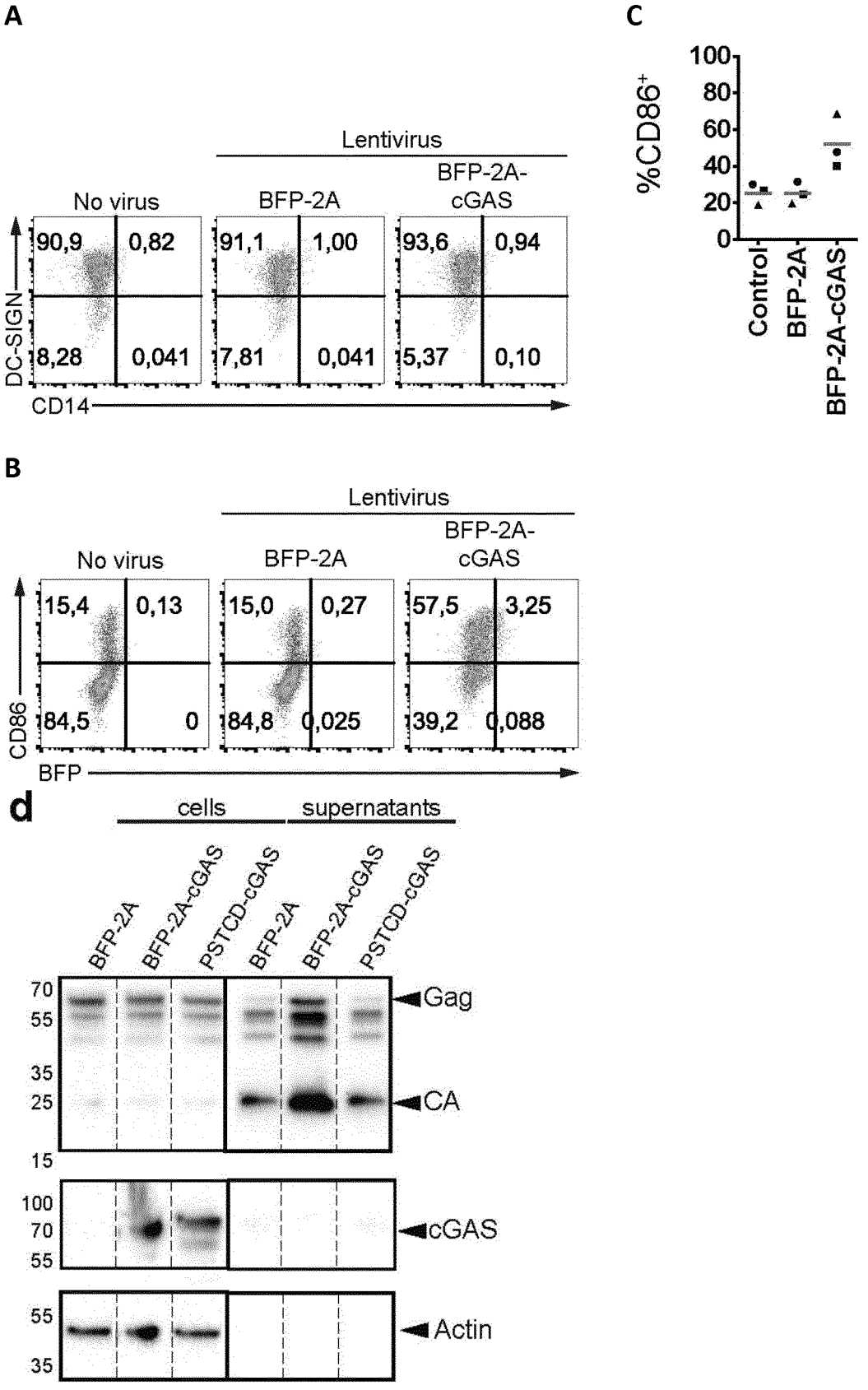

A
Lentivirus
No virus
BFP-2A
BFP-2A-cGAS
90,9
0,82
91,1
1,00
93,6
0,94
8,28
0,041
7,81
0,041
5,37
0,10
DC-SIGN
CD14
C
%CD86+
100
80
60
40
20
0
Control
BFP-2A
BFP-2A-cGAS
B
Lentivirus
No virus
BFP-2A
BFP-2A-cGAS
15,4
0,13
15,0
0,27
57,5
3,25
84,5
0
84,8
0,025
39,2
0,088
CD86
BFP
d
cells
supernatants
BFP-2A
BFP-2A-cGAS
PSTCD-cGAS
BFP-2A
BFP-2A-cGAS
PSTCD-cGAS
70
55
35
25
15
Gag
CA
100
70
55
cGAS
55
35
Actin

**Figure 8**

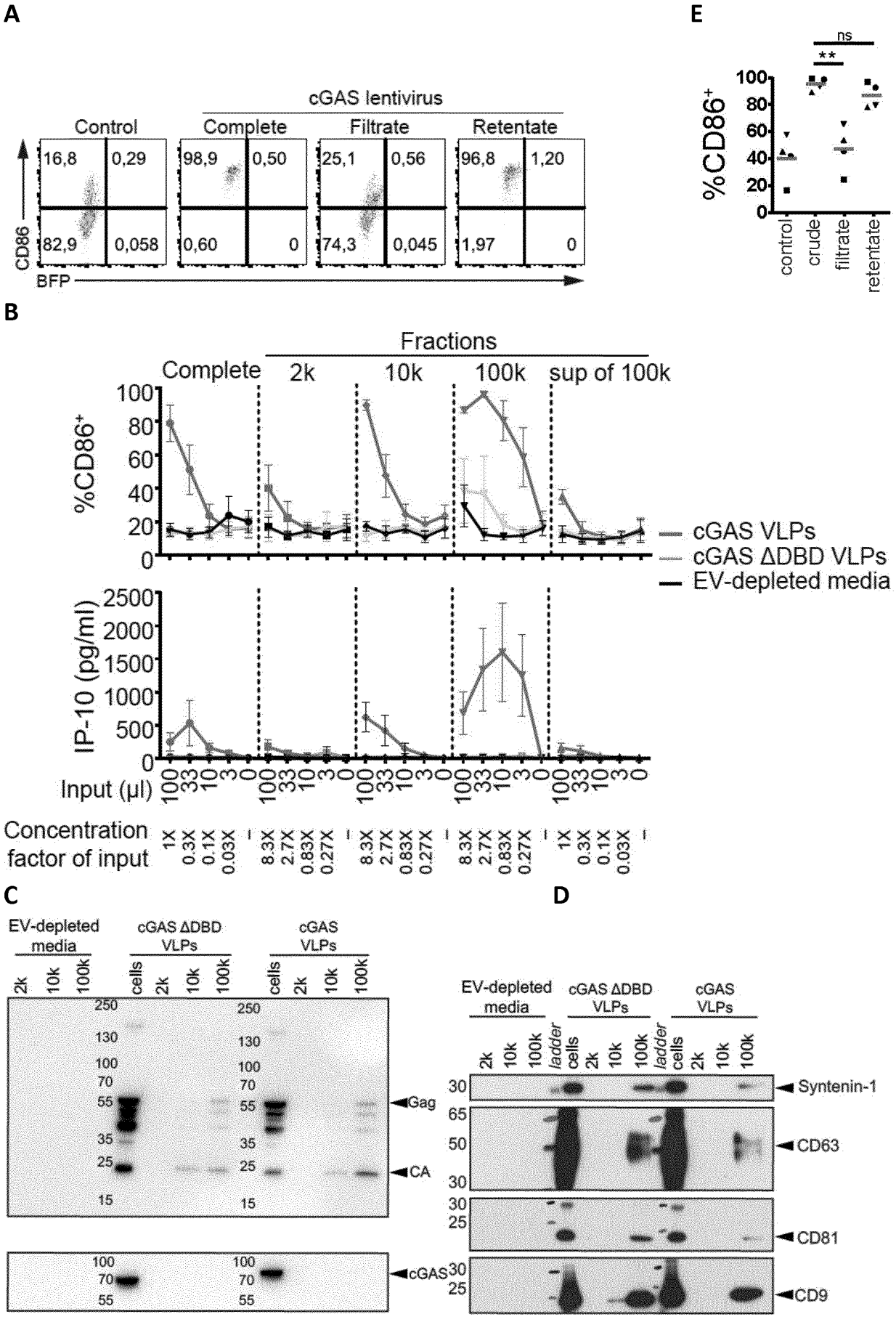
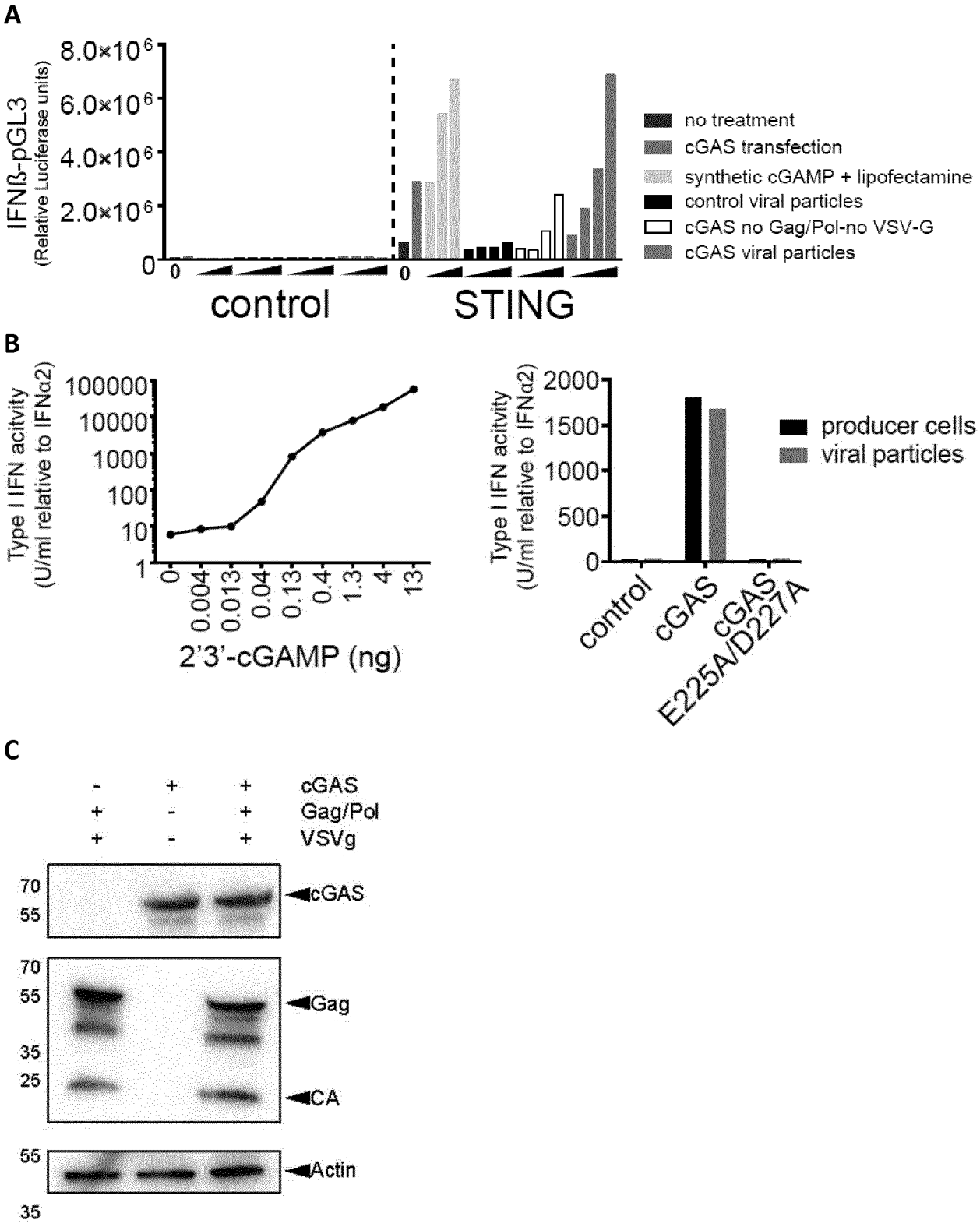
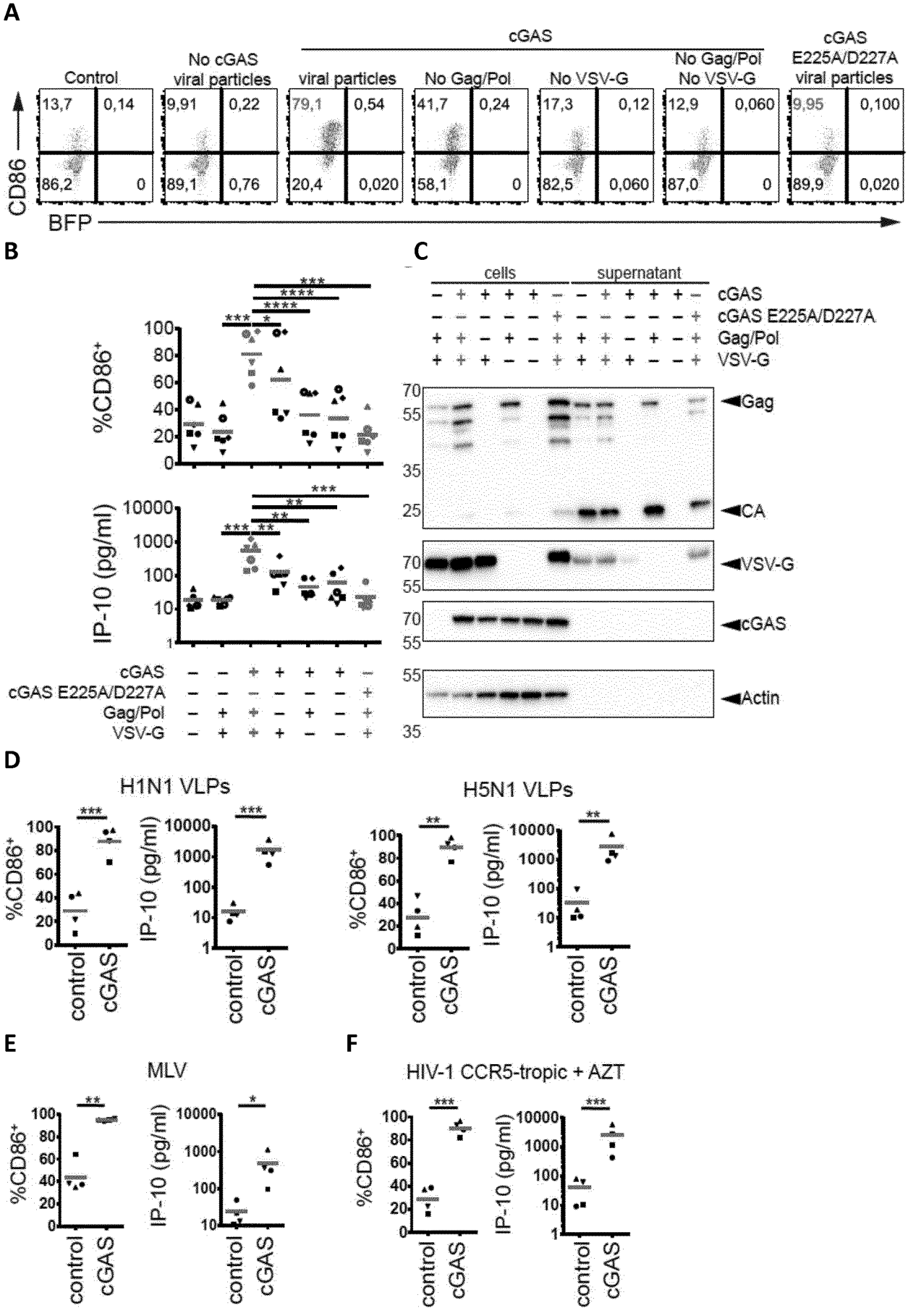

A
cGAS lentivirus
Control
Complete
Filtrate
Retentate
16,8
0,29
82,9
0,058
98,9
0,50
0,60
0
25,1
0,56
74,3
0,045
96,8
1,20
1,97
0
CD86
BFP
E
ns
**
%CD86+
control
crude
filtrate
retentate
B
Fractions
Complete
2k
10k
100k
sup of 100k
%CD86+
cGAS VLPs
cGAS ΔDBD VLPs
EV-depleted media
IP-10 (pg/ml)
Input (µl)
Concentration factor of input
C
EV-depleted media
cGAS ΔDBD VLPs
cGAS VLPs
cells
Gag
CA
cGAS
D
ladder
Syntenin-1
CD63
CD81
CD9

**Figure 9**


A
IFNß-pGL3
(Relative Luciferase units)
8.0×10^6
6.0×10^6
4.0×10^6
2.0×10^6
0
0
0
control
STING
no treatment
cGAS transfection
synthetic cGAMP + lipofectamine
control viral particles
cGAS no Gag/Pol-no VSV-G
cGAS viral particles
B
Type I IFN acitvity
(U/ml relative to IFNα2)
100000
10000
1000
100
10
1
0
0.004
0.013
0.04
0.13
0.4
1.3
4
13
2'3'-cGAMP (ng)
Type I IFN acitvity
(U/ml relative to IFNα2)
2000
1500
1000
500
0
control
cGAS
cGAS
E225A/D227A
producer cells
viral particles
C
- + + cGAS
+ - + Gag/Pol
+ - + VSVg
70
55
cGAS
70
55
35
25
Gag
CA
55
35
Actin

## Figure 10


A
Control
No cGAS viral particles
cGAS
viral particles
No Gag/Pol
No VSV-G
No Gag/Pol No VSV-G
cGAS E225A/D227A viral particles
13,7
0,14
9,91
0,22
79,1
0,54
41,7
0,24
17,3
0,12
12,9
0,060
9,95
0,100
86,2
0
89,1
0,76
20,4
0,020
58,1
0
82,5
0,060
87,0
0
89,9
0,020
CD86
BFP
B
%CD86+
IP-10 (pg/ml)
cGAS
cGAS E225A/D227A
Gag/Pol
VSV-G
C
cells
supernatant
cGAS
cGAS E225A/D227A
Gag/Pol
VSV-G
Gag
CA
VSV-G
cGAS
Actin
D
H1N1 VLPs
H5N1 VLPs
%CD86+
IP-10 (pg/ml)
control
cGAS
E
MLV
F
HIV-1 CCR5-tropic + AZT

## Figure 11

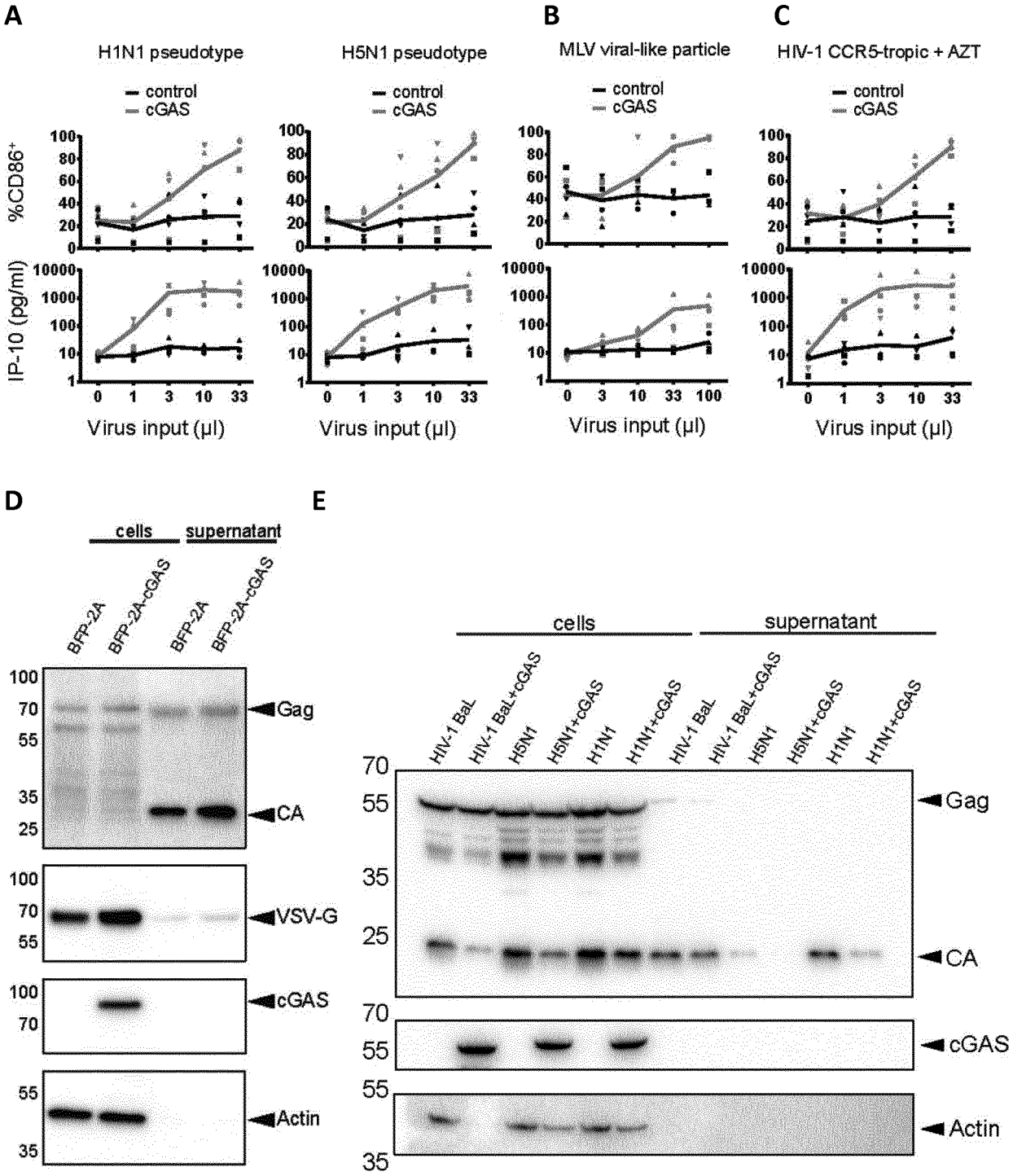

A
B
C
H1N1 pseudotype
H5N1 pseudotype
MLV viral-like particle
HIV-1 CCR5-tropic + AZT
control
cGAS
%CD86+
IP-10 (pg/ml)
Virus input (µl)
D
E
cells
supernatant
BFP-2A
BFP-2A-cGAS
Gag
CA
VSV-G
cGAS
Actin
HIV-1 BaL
HIV-1 BaL+cGAS
H5N1
H5N1+cGAS
H1N1
H1N1+cGAS

**Figure 12**

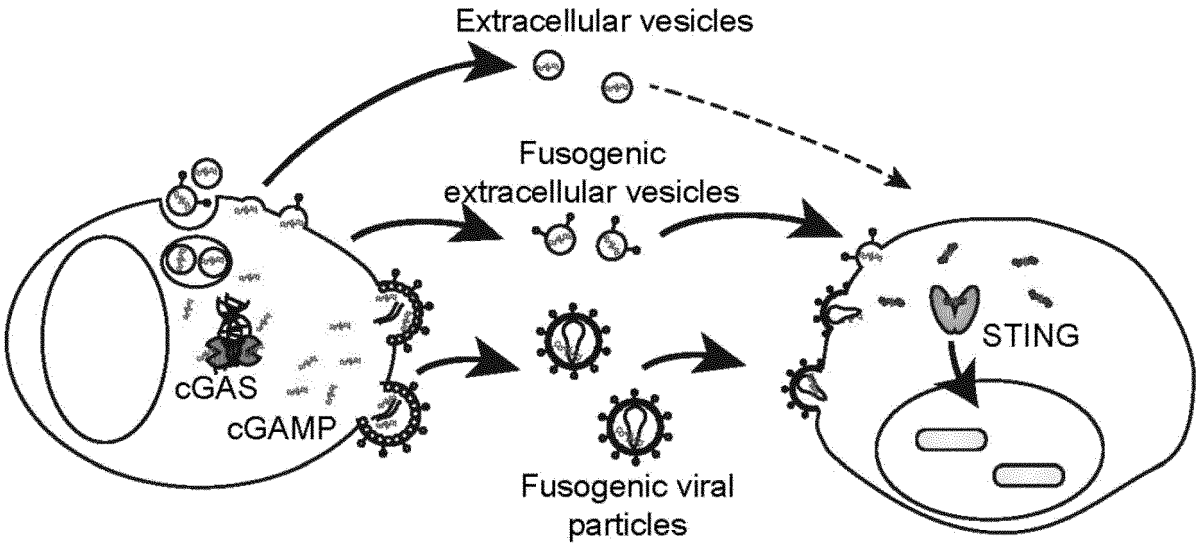

Extracellular vesicles
Fusogenic
extracellular vesicles
cGAS
cGAMP
Fusogenic viral
particles
STING

Figure 13

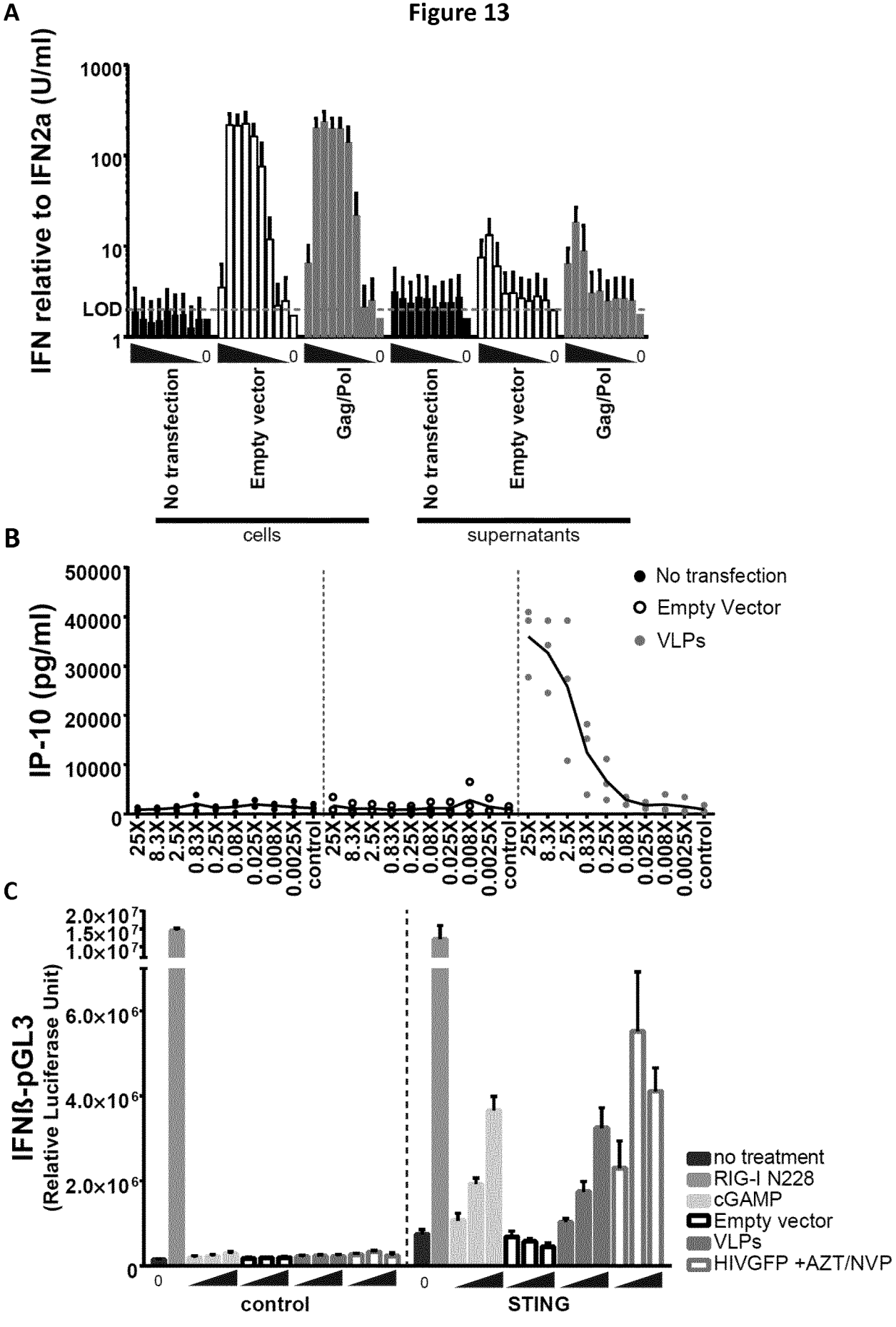

A
IFN relative to IFN2a (U/ml)
1000
100
10
LOD
1
0
No transfection
Empty vector
Gag/Pol
cells
supernatants
B
IP-10 (pg/ml)
50000
40000
30000
20000
10000
No transfection
Empty Vector
VLPs
25X
8.3X
2.5X
0.83X
0.25X
0.08X
0.025X
0.008X
0.0025X
control
C
IFNß-pGL3
(Relative Luciferase Unit)
2.0×10⁷
1.5×10⁷
1.0×10⁷
6.0×10⁶
4.0×10⁶
2.0×10⁶
control
STING
no treatment
RIG-I N228
cGAMP
Empty vector
VLPs
HIVGFP +AZT/NVP

**Figure 14**

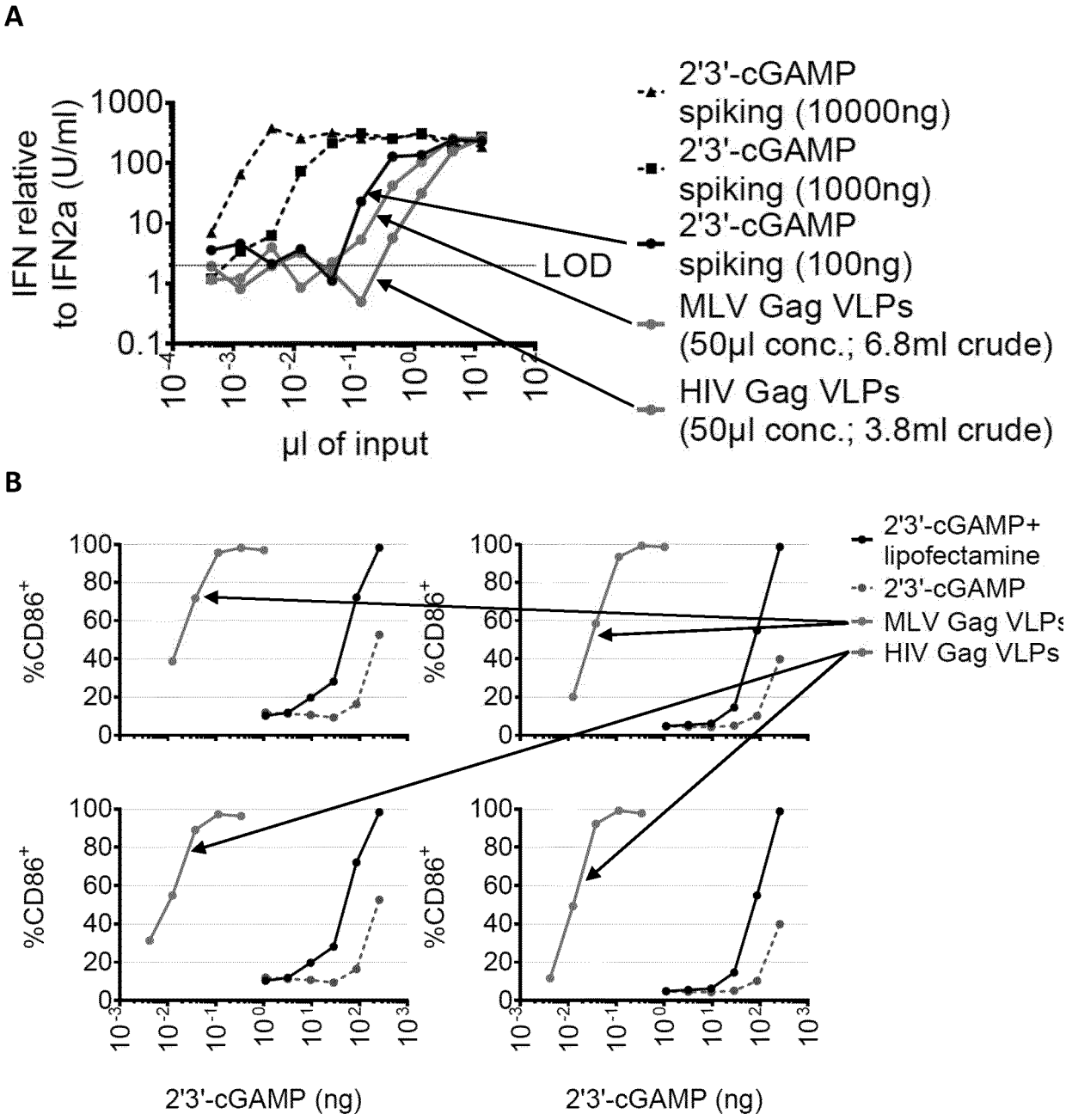

A
IFN relative to IFN2a (U/ml)
1000
100
10
1
0.1
LOD
µl of input
2'3'-cGAMP spiking (10000ng)
2'3'-cGAMP spiking (1000ng)
2'3'-cGAMP spiking (100ng)
MLV Gag VLPs (50µl conc.; 6.8ml crude)
HIV Gag VLPs (50µl conc.; 3.8ml crude)
B
%CD86+
2'3'-cGAMP+ lipofectamine
2'3'-cGAMP
MLV Gag VLPs
HIV Gag VLPs
2'3'-cGAMP (ng)
2'3'-cGAMP (ng)

**FIG 15A**

| | | | |
|---|---|---|---|
| 1- | dose 1 | Ova-cGAMP-VLP | |
| 2- | dose 1/3 | Ova-cGAMP-VLP | |
| 3- | dose 1/9 | Ova-cGAMP-VLP | |
| 4- | dose 1 | cGAMP-VLP | |
| 5- | 20µg | Ova protein | + 10µg cGAMP |
| 6- | 20µg | Ova protein | + 40µg CpG |
| 7- | | PBS | |

**FIG 15B**

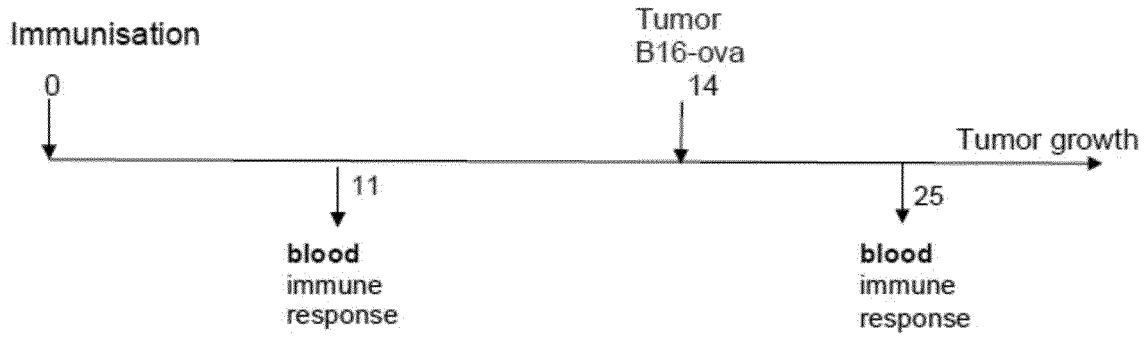



**FIG 15C**

Before tumor

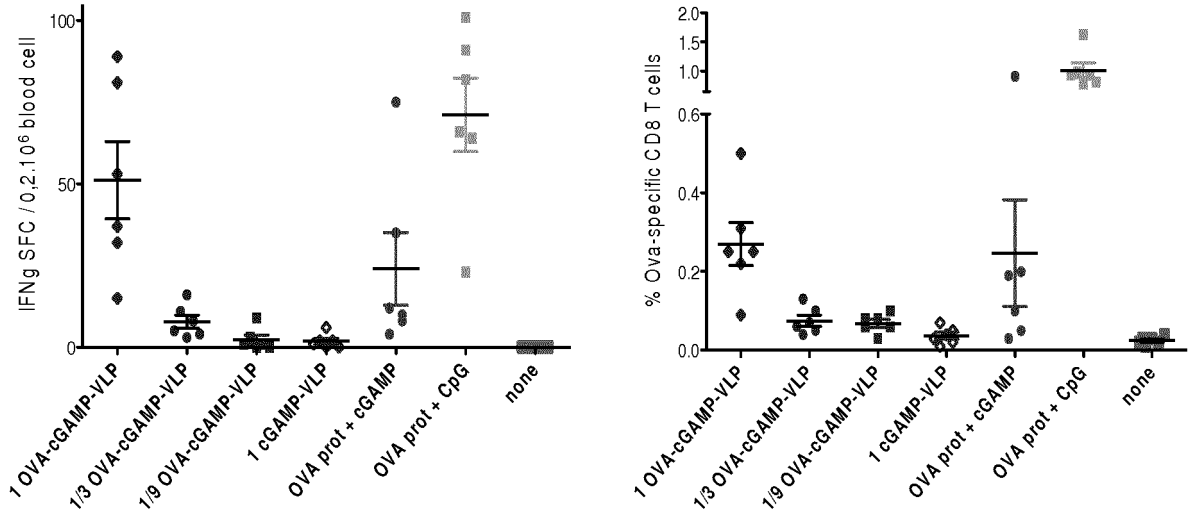


FIG 15D

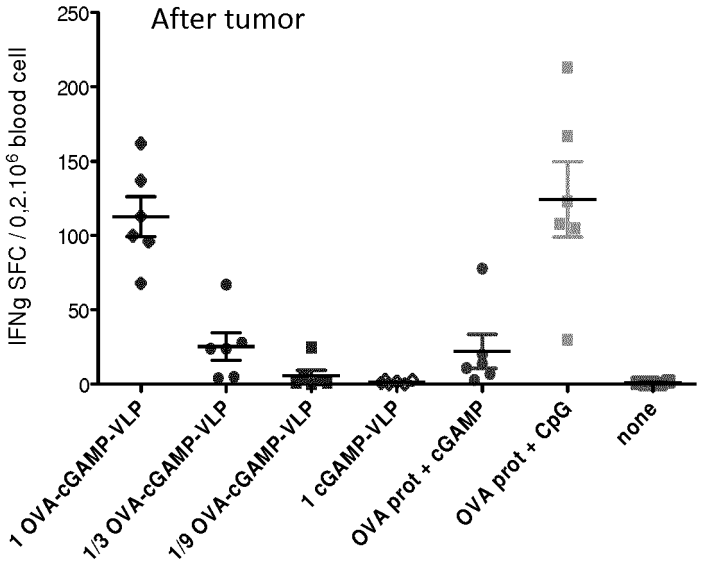



FIG 15E

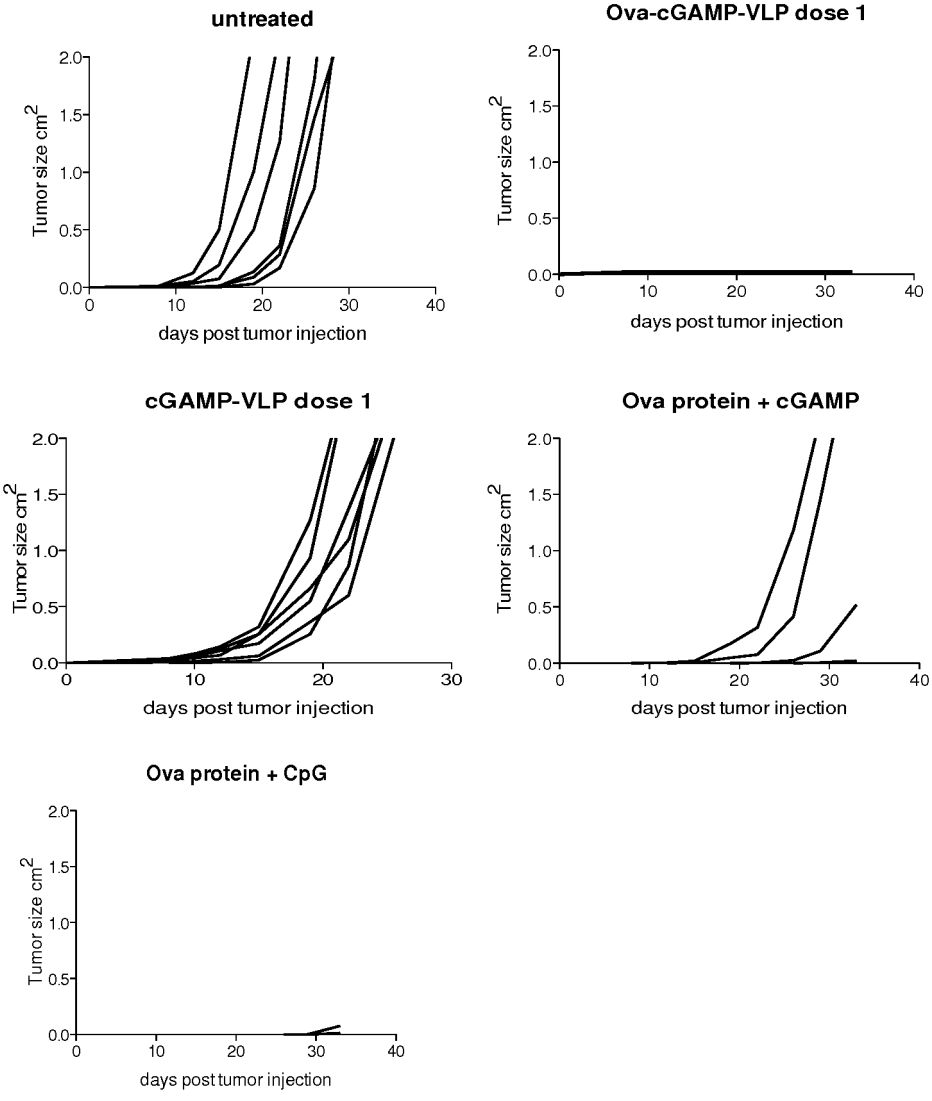


FIG 15F

| | | |
|---|---|---|
| 1- | dose 1 | cGAMP-VLP |
| 2- | dose 1/3 | cGAMP-VLP |
| 3- | dose 1/9 | cGAMP-VLP |
| 4- | | PBS |

FIG 15G

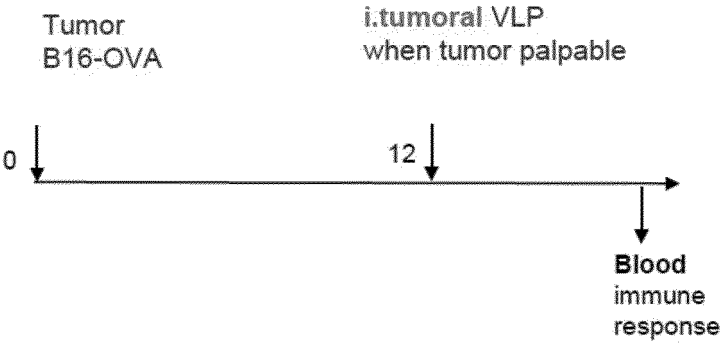
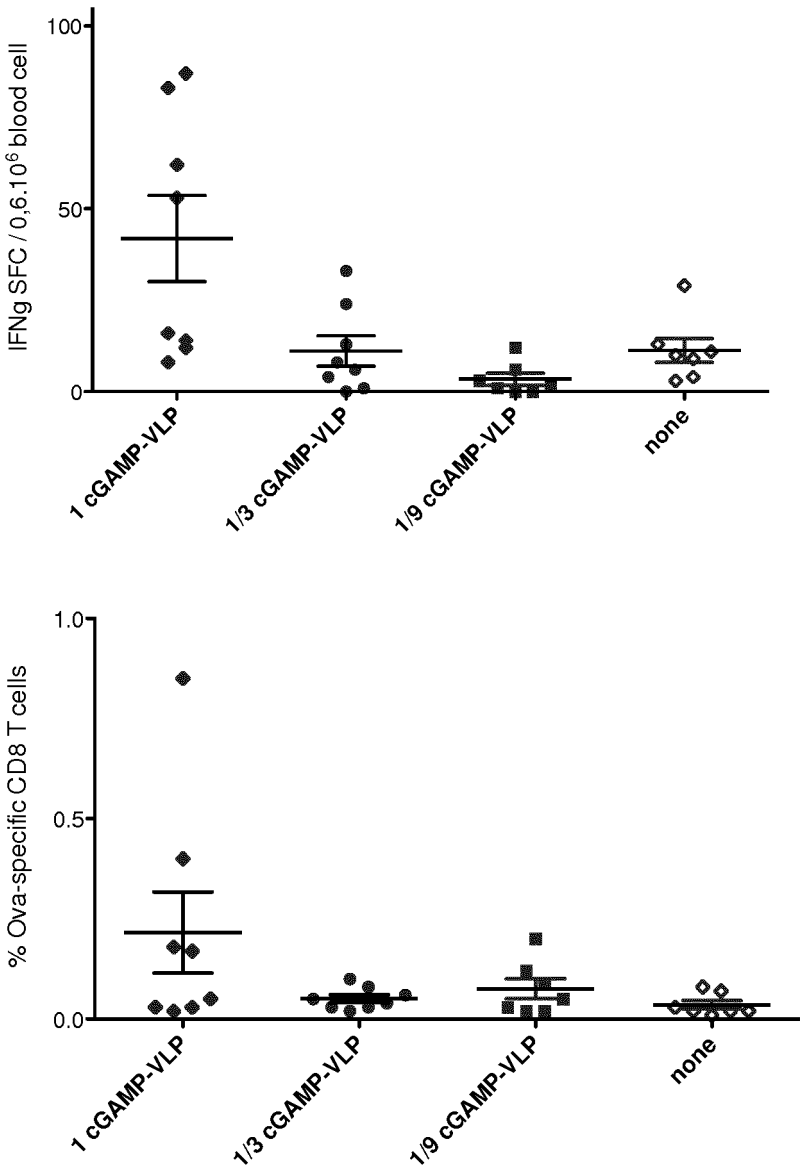



FIG 15H



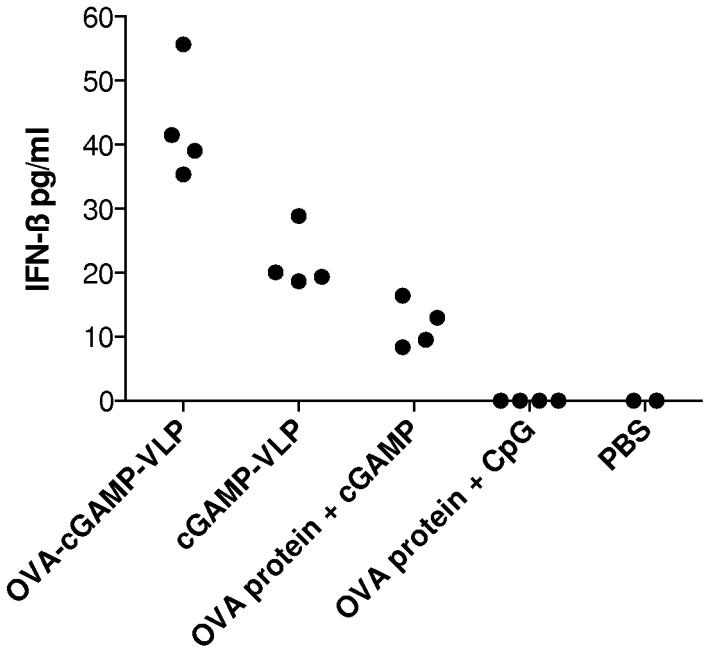



FIGURE 16A

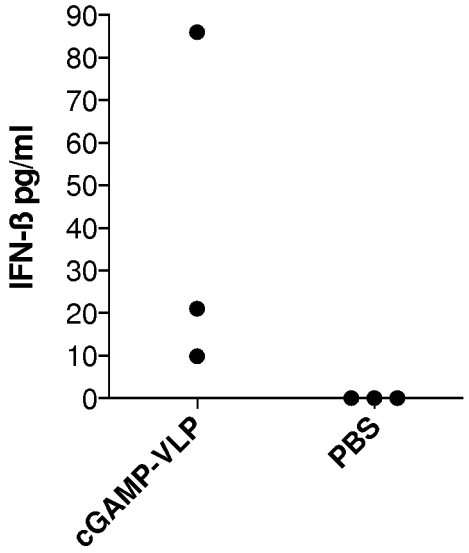



FIGURE 16B

| | n= | | | Dose injected |
|---|---|---|---|---|
| 1- | 6 | dose 1 | Ova-cGAMP-VLP | 4,5ng (p30) |
| 2- | 6 | dose 1/3 | Ova-cGAMP-VLP | 1,5ng (p30) |
| 3- | 6 | dose 1 | Ova-VLP | 4,5ng (p30) |
| 4- | 6 | dose 1/3 | Ova-VLP | 1,5ng (p30) |
| 5- | 6 | 20µg | Ova protein | + 10µg cGAMP |
| 6- | 6 | 20µg | Ova protein | + 40µg CpG |
| 7- | 6 | | PBS | |

**FIGURE 17A**

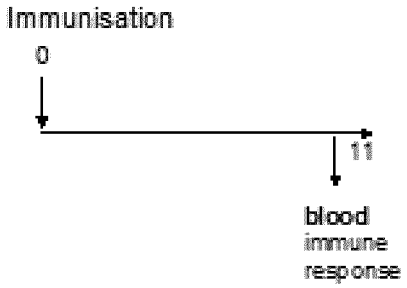



**FIGURE 17B**

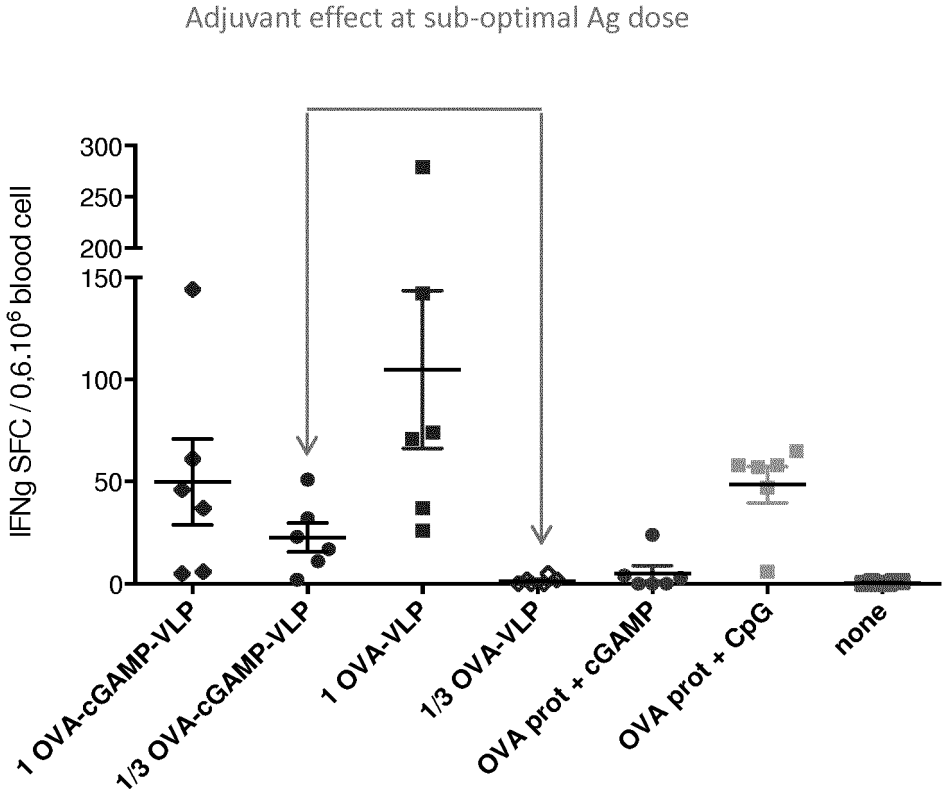



**FIGURE 17C**