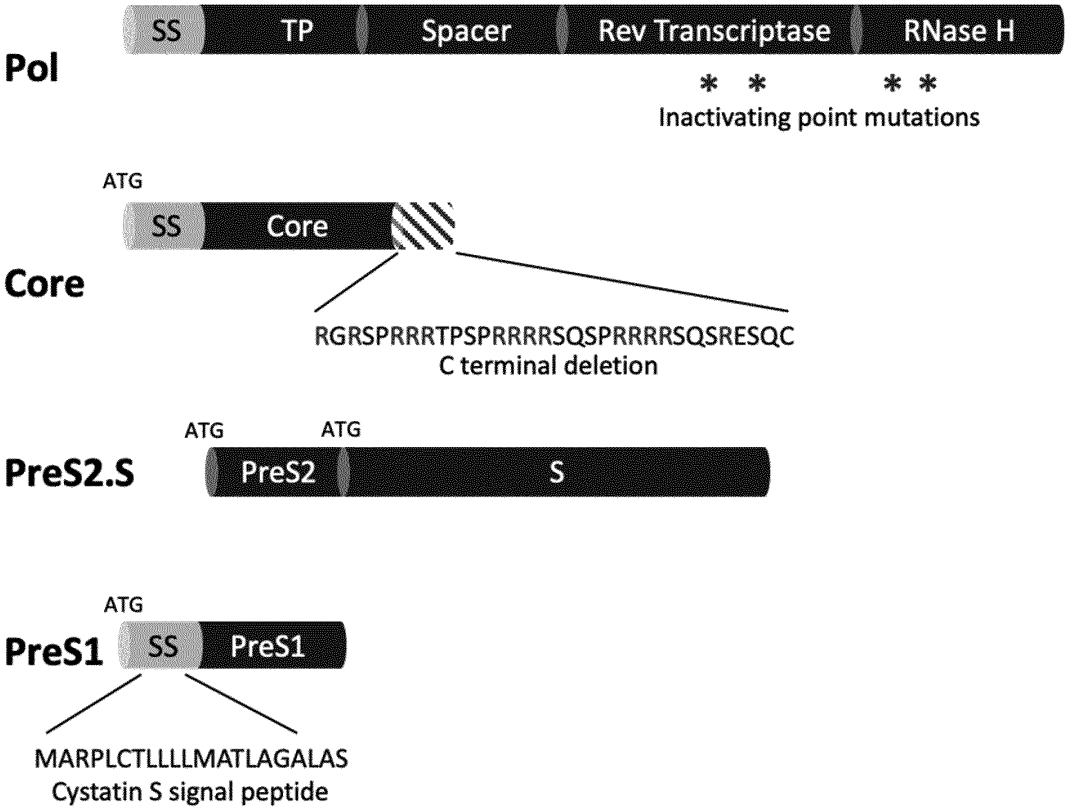
Pol
SS
TP
Spacer
Rev Transcriptase
RNase H
* *
* *
Inactivating point mutations
ATG
Core
SS
Core
RGRSPRRRTPSPRRRRSQSPRRRRSQSRESQC
C terminal deletion
PreS2.S
ATG
ATG
PreS2
S
PreS1
ATG
SS
PreS1
MARPLCTLLLLMATLAGALAS
Cystatin S signal peptide

FIG. 1A

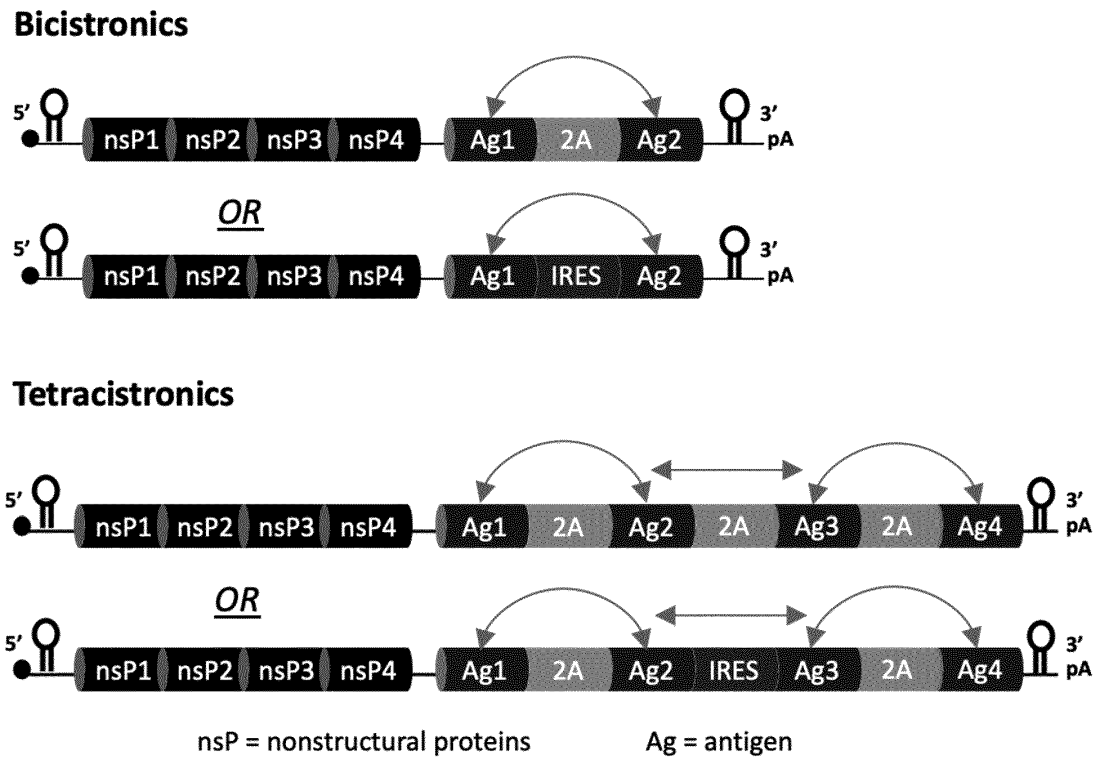
Bicistronics
5'
nsP1
nsP2
nsP3
nsP4
Ag1
2A
Ag2
3'
pA
OR
5'
nsP1
nsP2
nsP3
nsP4
Ag1
IRES
Ag2
3'
pA
Tetracistronics
5'
nsP1
nsP2
nsP3
nsP4
Ag1
2A
Ag2
2A
Ag3
2A
Ag4
3'
pA
OR
5'
nsP1
nsP2
nsP3
nsP4
Ag1
2A
Ag2
IRES
Ag3
2A
Ag4
3'
pA
nsP = nonstructural proteins
Ag = antigen

FIG. 1B

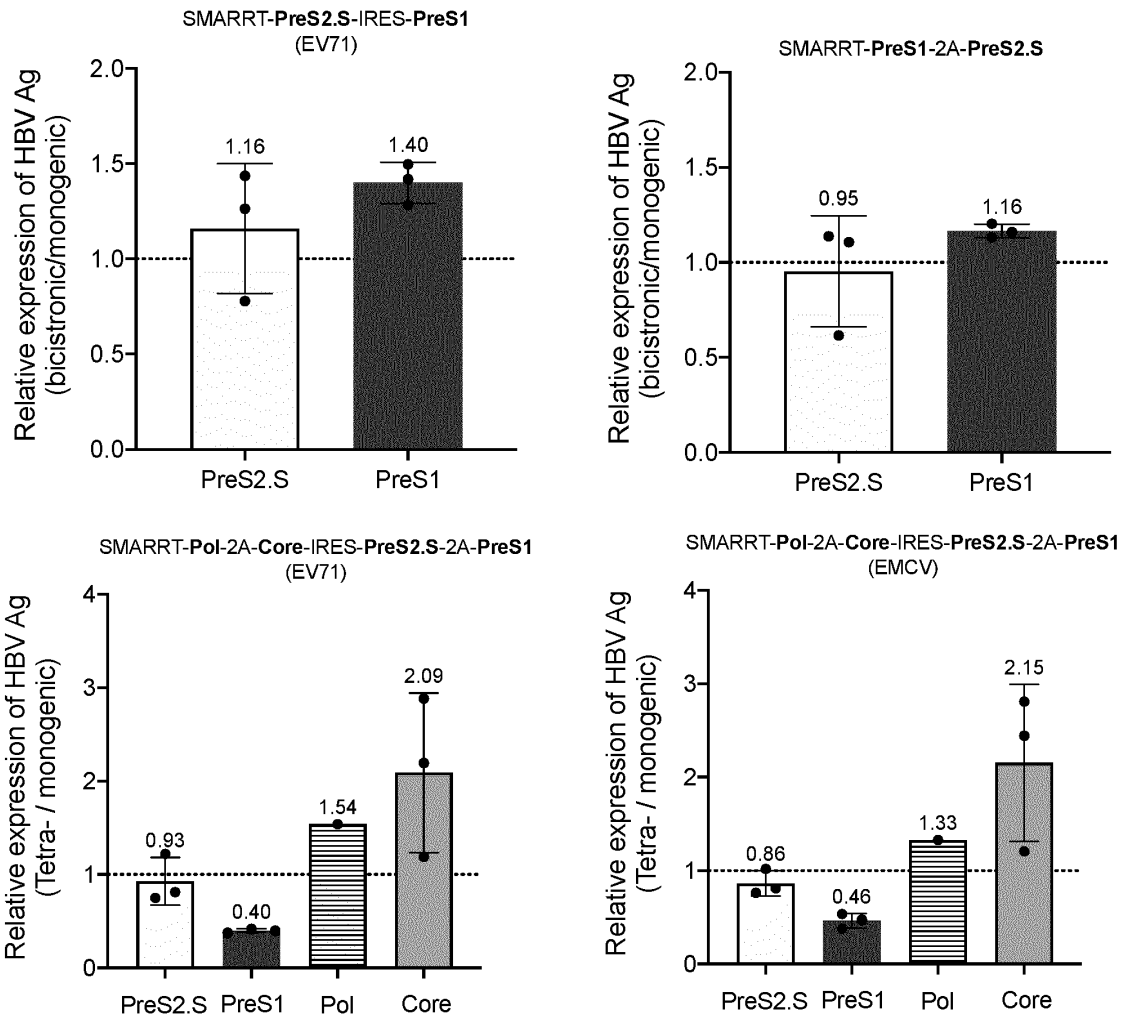
SMARRT-PreS2.S-IRES-PreS1
(EV71)
Relative expression of HBV Ag
(bicistronic/monogenic)
2.0
1.5
1.0
0.5
0.0
1.16
1.40
PreS2.S
PreS1
SMARRT-PreS1-2A-PreS2.S
Relative expression of HBV Ag
(bicistronic/monogenic)
0.95
1.16
PreS2.S
PreS1
SMARRT-Pol-2A-Core-IRES-PreS2.S-2A-PreS1
(EV71)
Relative expression of HBV Ag
(Tetra- / monogenic)
4
3
2
1
0
0.93
0.40
1.54
2.09
PreS2.S
PreS1
Pol
Core
SMARRT-Pol-2A-Core-IRES-PreS2.S-2A-PreS1
(EMCV)
Relative expression of HBV Ag
(Tetra- / monogenic)
0.86
0.46
1.33
2.15
PreS2.S
PreS1
Pol
Core

FIG. 2

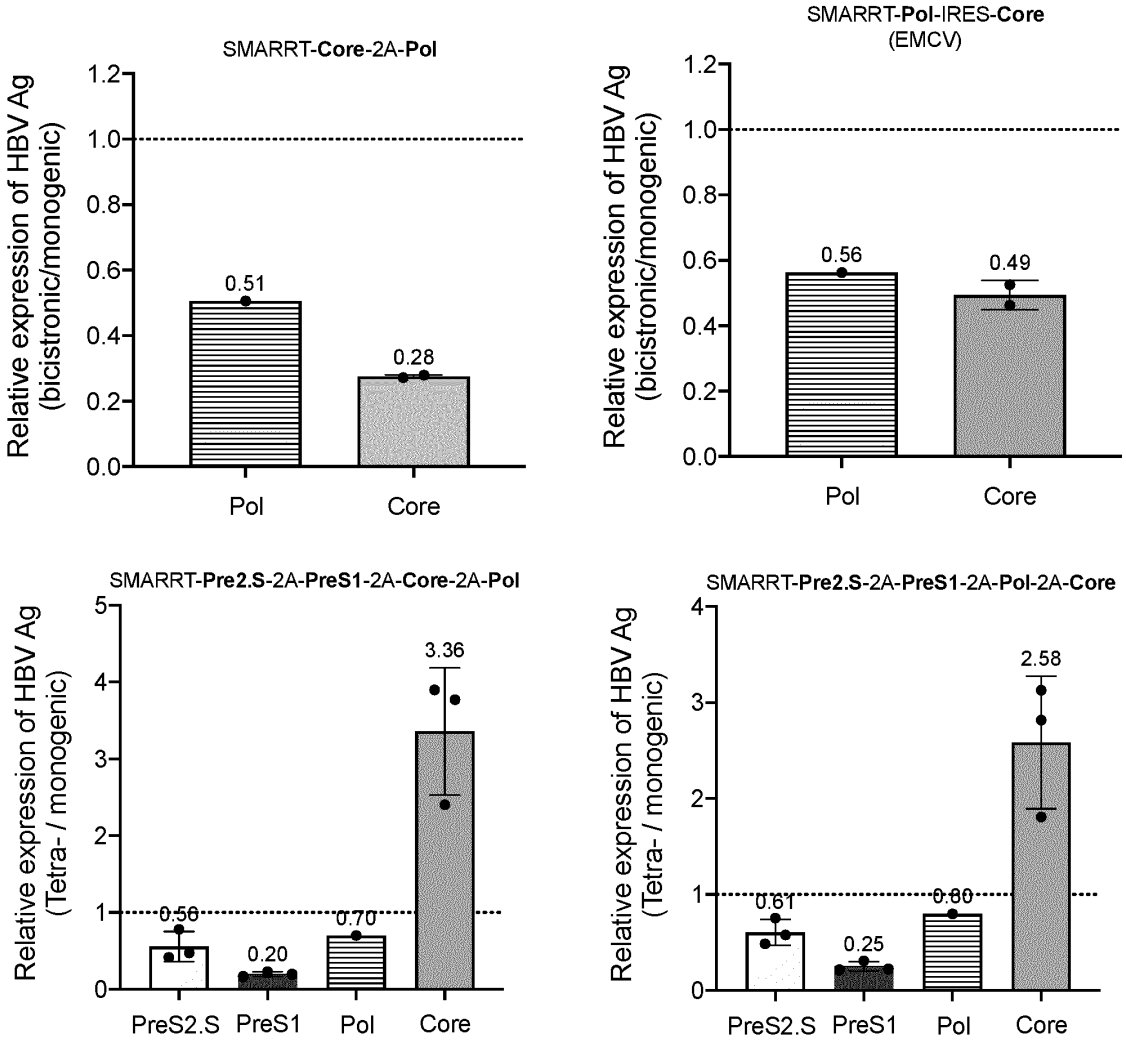

FIG. 2 continued

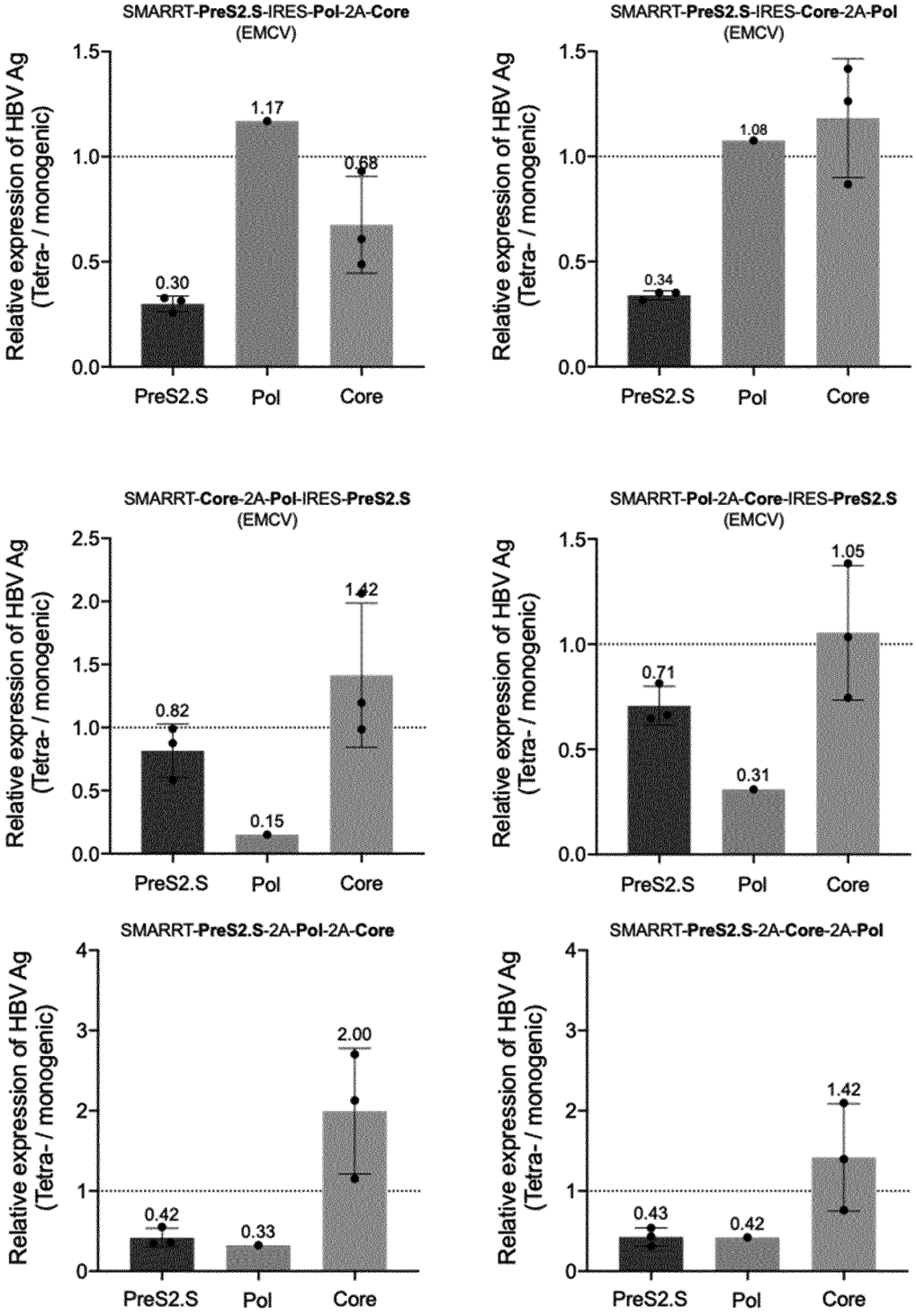
SMARRT-PreS2.S-IRES-Pol-2A-Core
(EMCV)
Relative expression of HBV Ag
(Tetra- / monogenic)
1.5
1.0
0.5
0.0
1.17
0.68
0.30
PreS2.S
Pol
Core
SMARRT-PreS2.S-IRES-Core-2A-Pol
(EMCV)
Relative expression of HBV Ag
(Tetra- / monogenic)
1.5
1.0
0.5
0.0
1.08
0.34
PreS2.S
Pol
Core
SMARRT-Core-2A-Pol-IRES-PreS2.S
(EMCV)
Relative expression of HBV Ag
(Tetra- / monogenic)
2.5
2.0
1.5
1.0
0.5
0.0
1.42
0.82
0.15
PreS2.S
Pol
Core
SMARRT-Pol-2A-Core-IRES-PreS2.S
(EMCV)
Relative expression of HBV Ag
(Tetra- / monogenic)
1.5
1.0
0.5
0.0
1.05
0.71
0.31
PreS2.S
Pol
Core
SMARRT-PreS2.S-2A-Pol-2A-Core
Relative expression of HBV Ag
(Tetra- / monogenic)
4
3
2
1
0
2.00
0.42
0.33
PreS2.S
Pol
Core
SMARRT-PreS2.S-2A-Core-2A-Pol
Relative expression of HBV Ag
(Tetra- / monogenic)
4
3
2
1
0
1.42
0.43
0.42
PreS2.S
Pol
Core

FIG. 3

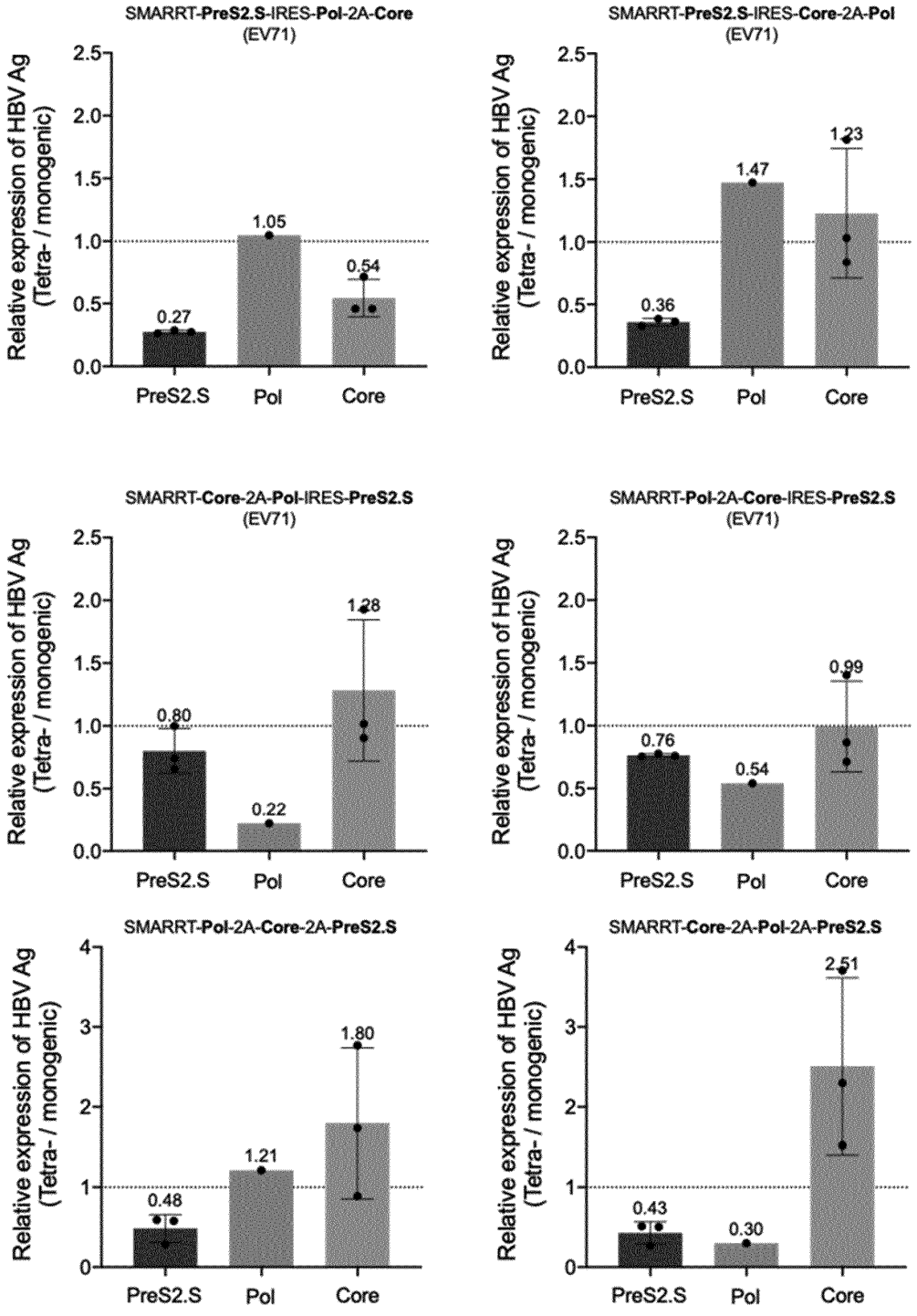

FIG. 3 continued

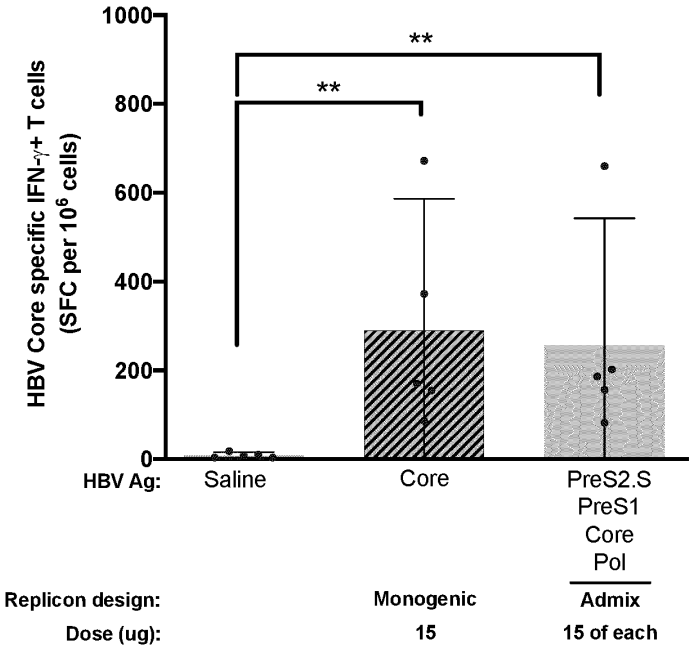
HBV Core specific IFN-γ+ T cells (SFC per 10^6 cells)
1000
800
600
400
200
0
**
**
HBV Ag:
Saline
Core
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 4A

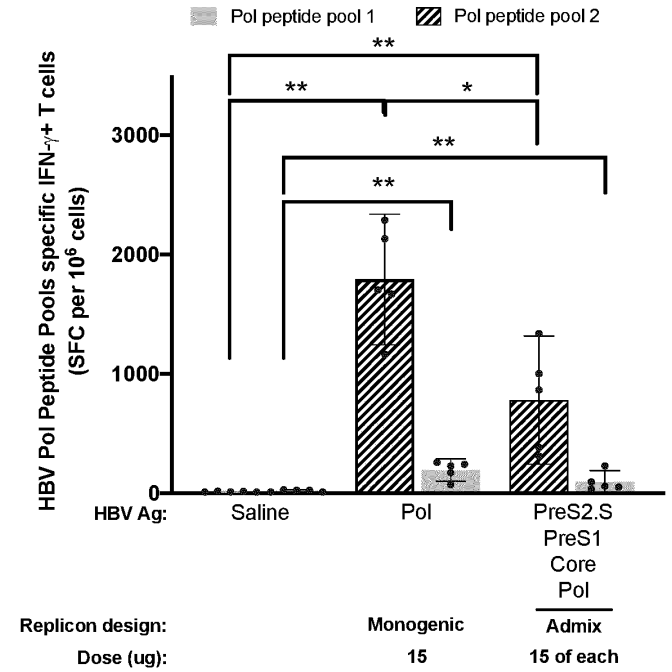
Pol peptide pool 1
Pol peptide pool 2
HBV Pol Peptide Pools specific IFN-γ+ T cells (SFC per 10^6 cells)
3000
2000
1000
0
**
**
*
**
**
HBV Ag:
Saline
Pol
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 4B

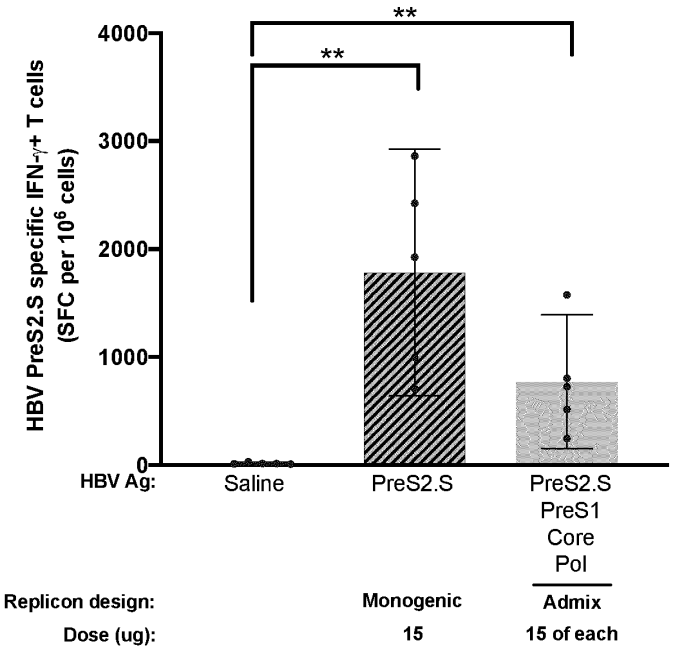
**
**
4000
3000
2000
1000
0
HBV PreS2.S specific IFN-γ+ T cells
(SFC per 10^6 cells)
HBV Ag:
Saline
PreS2.S
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 4C

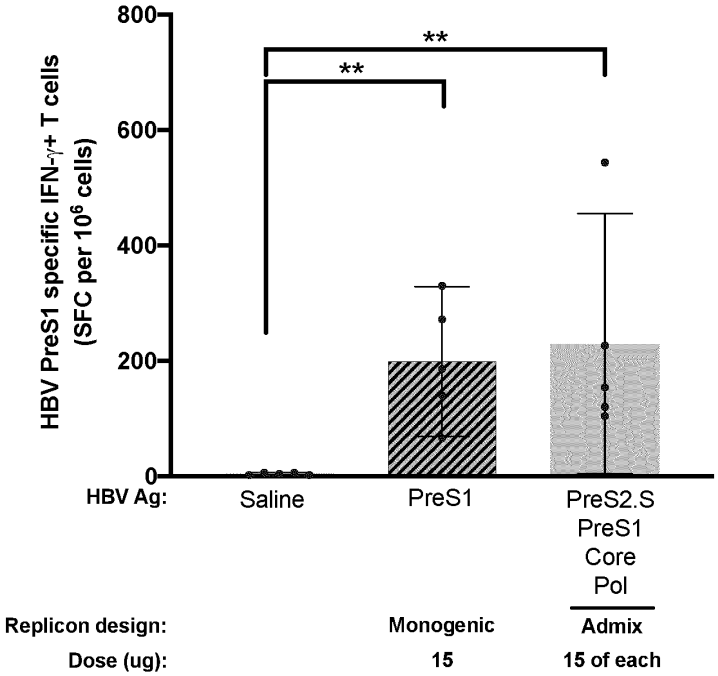
**
**
800
600
400
200
0
HBV PreS1 specific IFN-γ+ T cells
(SFC per 10^6 cells)
HBV Ag:
Saline
PreS1
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 4D

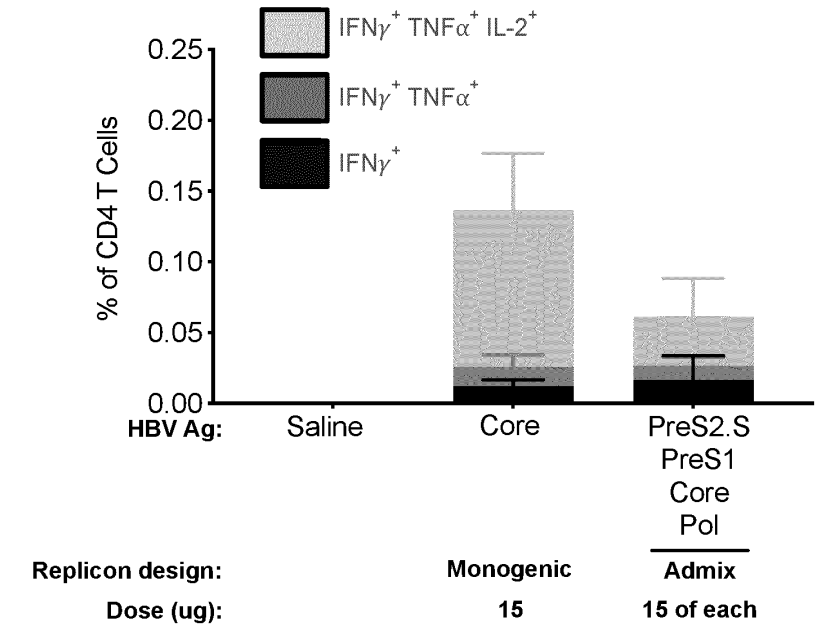
IFNγ+ TNFα+ IL-2+
IFNγ+ TNFα+
IFNγ+
% of CD4 T Cells
0.25
0.20
0.15
0.10
0.05
0.00
HBV Ag:
Saline
Core
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 5A

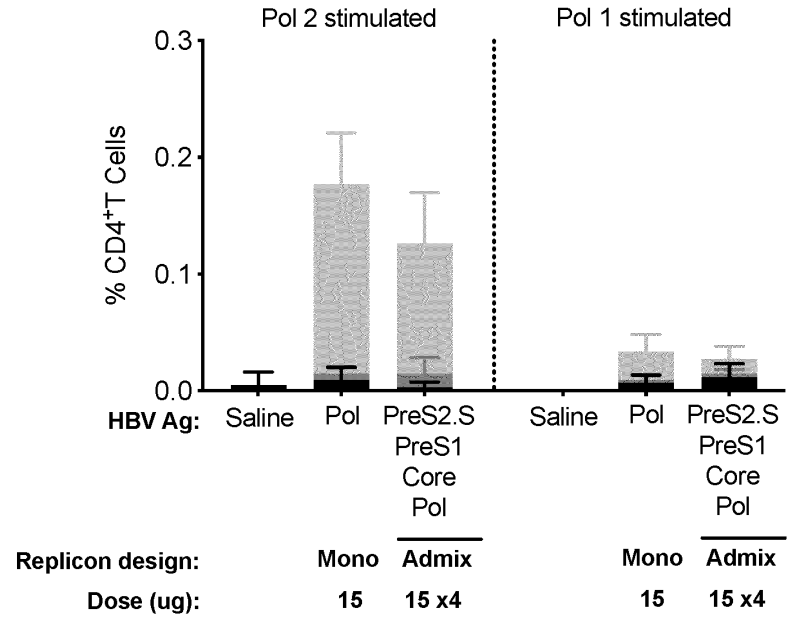
Pol 2 stimulated
Pol 1 stimulated
% CD4+T Cells
0.3
0.2
0.1
0.0
HBV Ag:
Saline
Pol
PreS2.S
PreS1
Core
Pol
Saline
Pol
PreS2.S
PreS1
Core
Pol
Replicon design:
Mono
Admix
Mono
Admix
Dose (ug):
15
15 x4
15
15 x4

FIG. 5B

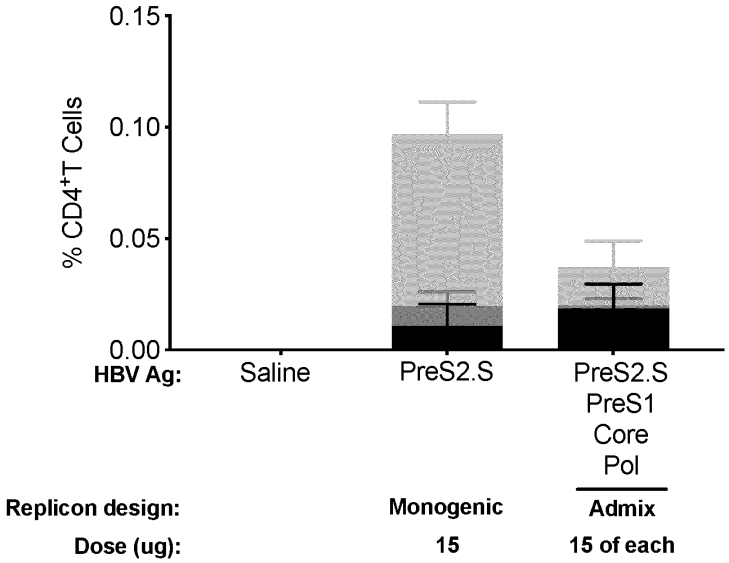
0.15
0.10
0.05
0.00
% CD4+T Cells
HBV Ag:
Saline
PreS2.S
PreS2.S PreS1 Core Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 5C

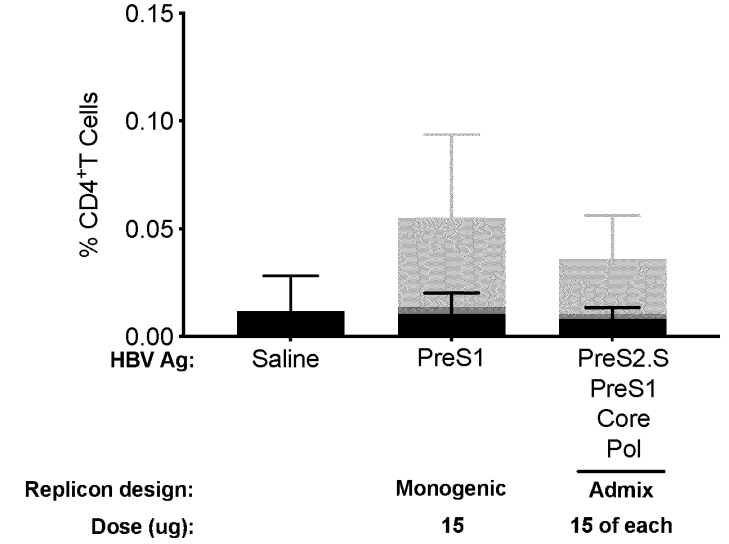
0.15
0.10
0.05
0.00
% CD4+T Cells
HBV Ag:
Saline
PreS1
PreS2.S PreS1 Core Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 5D

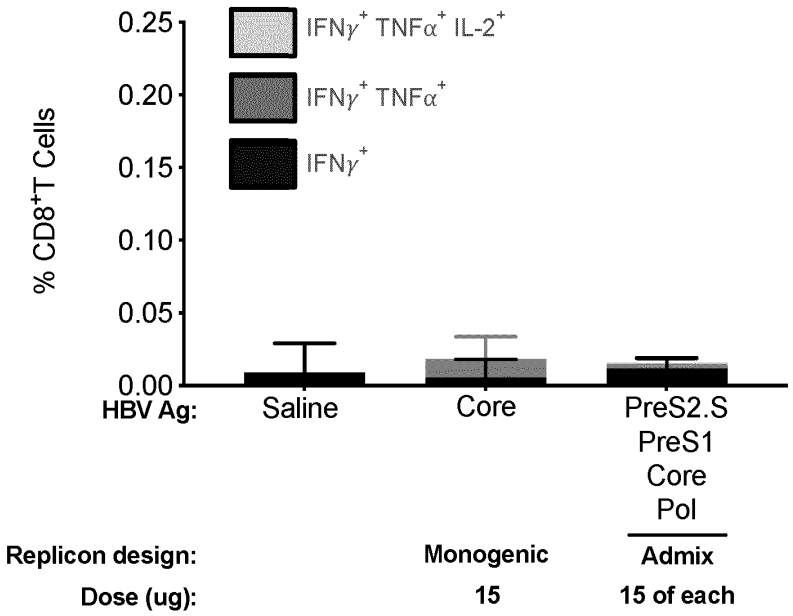
0.25
0.20
0.15
0.10
0.05
0.00
% CD8+T Cells
IFNγ+ TNFα+ IL-2+
IFNγ+ TNFα+
IFNγ+
HBV Ag:
Saline
Core
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 5E

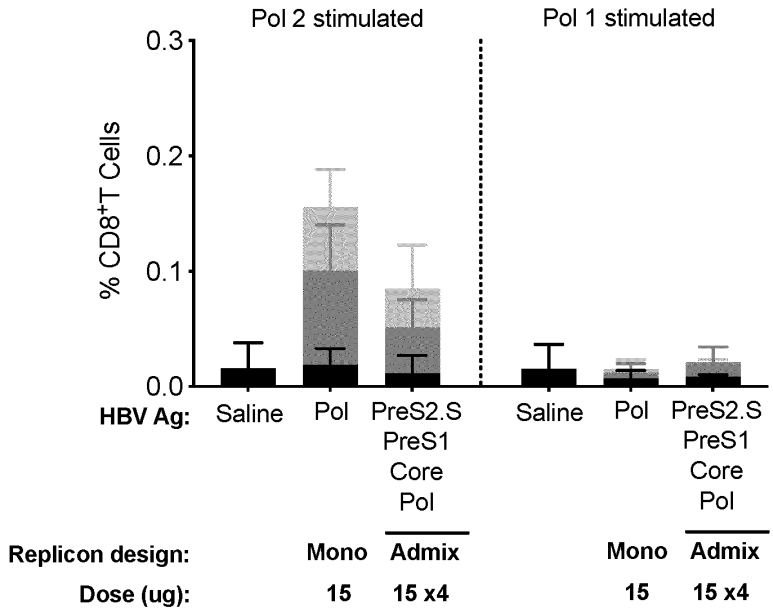
Pol 2 stimulated
Pol 1 stimulated
0.3
0.2
0.1
0.0
% CD8+T Cells
HBV Ag:
Saline
Pol
PreS2.S
PreS1
Core
Pol
Saline
Pol
PreS2.S
PreS1
Core
Pol
Replicon design:
Mono
Admix
Mono
Admix
Dose (ug):
15
15 x4
15
15 x4

FIG. 5F

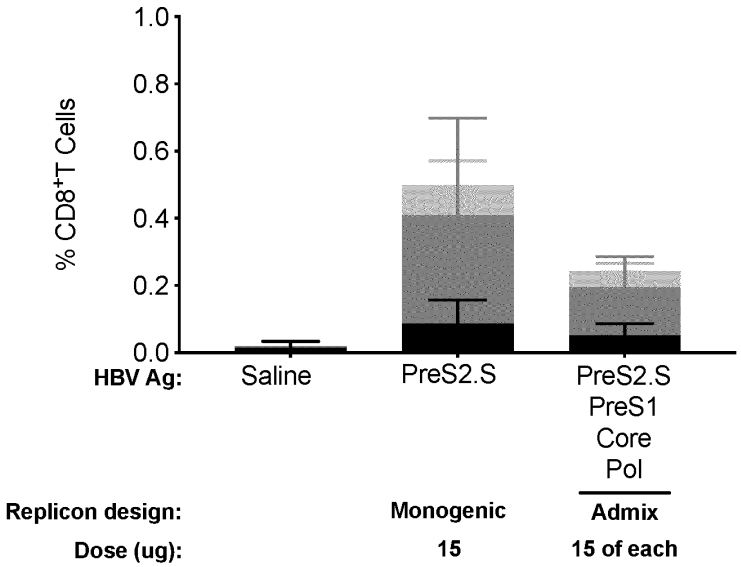
1.0
0.8
0.6
0.4
0.2
0.0
% CD8+T Cells
HBV Ag:
Saline
PreS2.S
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 5G

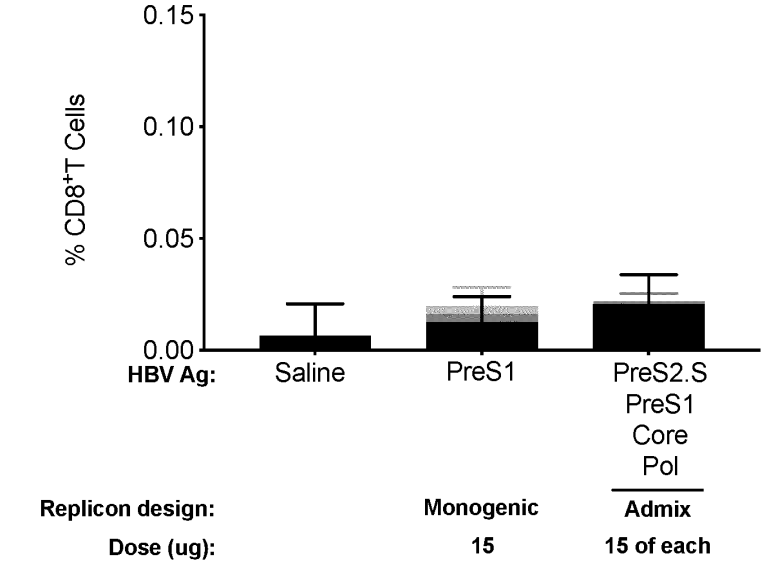
0.15
0.10
0.05
0.00
% CD8+T Cells
HBV Ag:
Saline
PreS1
PreS2.S
PreS1
Core
Pol
Replicon design:
Monogenic
Admix
Dose (ug):
15
15 of each

FIG. 5H

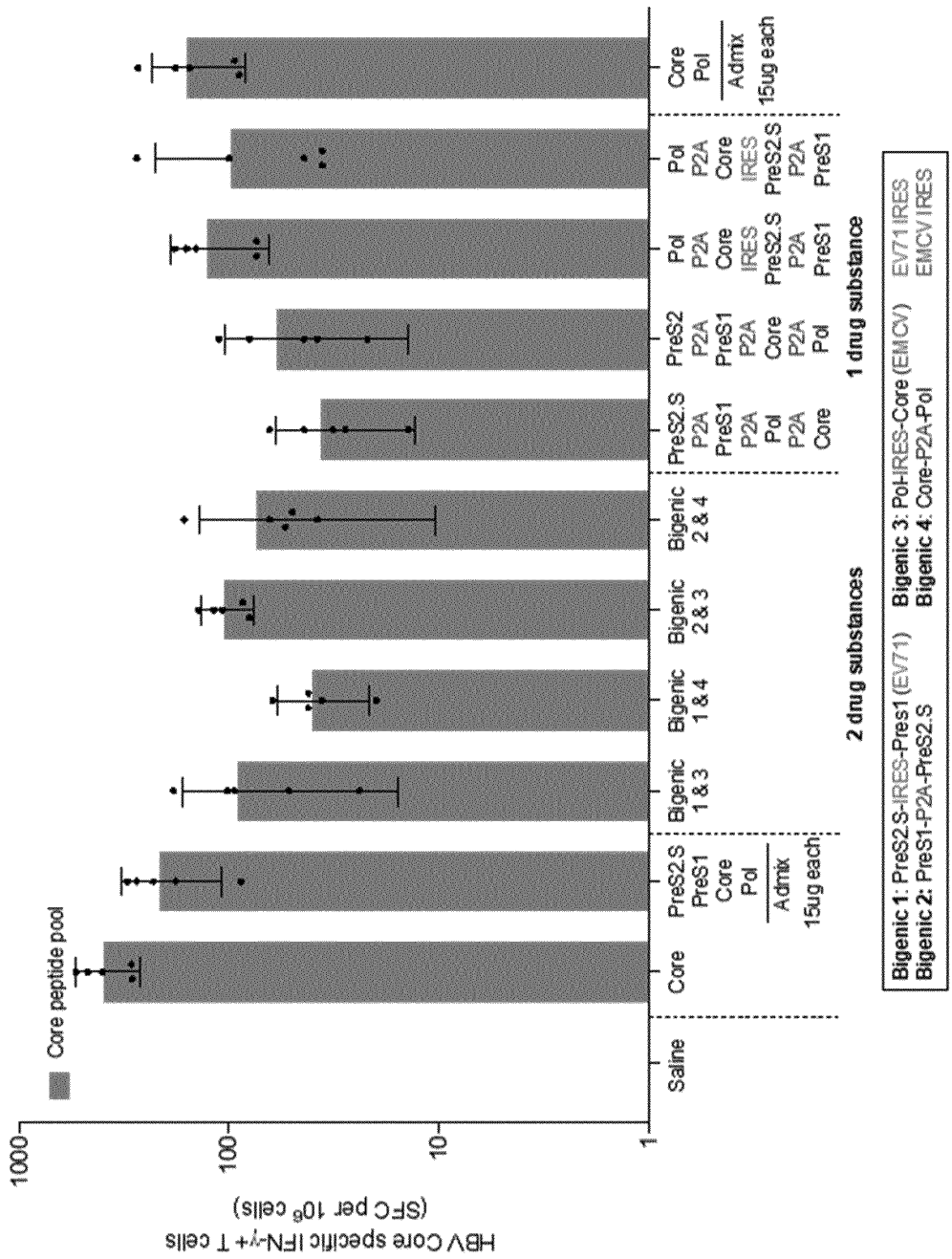
HBV Core specific IFN-γ+ T cells
(SFC per 10^6 cells)
1000
100
10
1
Core peptide pool
Saline
Core
PreS2.S PreS1 Core Pol Admix 15ug each
Bigenic 1&3
Bigenic 1&4
Bigenic 2&3
Bigenic 2&4
PreS2.S P2A PreS1 P2A Pol P2A Core
PreS2 P2A PreS1 P2A Core P2A Pol
Pol P2A Core IRES PreS2.S P2A PreS1
Pol P2A Core IRES PreS2.S P2A PreS1
Core Pol Admix 15ug each
2 drug substances
1 drug substance
Bigenic 1: PreS2.S-IRES-Pres1 (EV71)
Bigenic 2: PreS1-P2A-PreS2.S
Bigenic 3: Pol-IRES-Core (EMCV)
Bigenic 4: Core-P2A-Pol
EV71 IRES
EMCV IRES

FIG. 6A

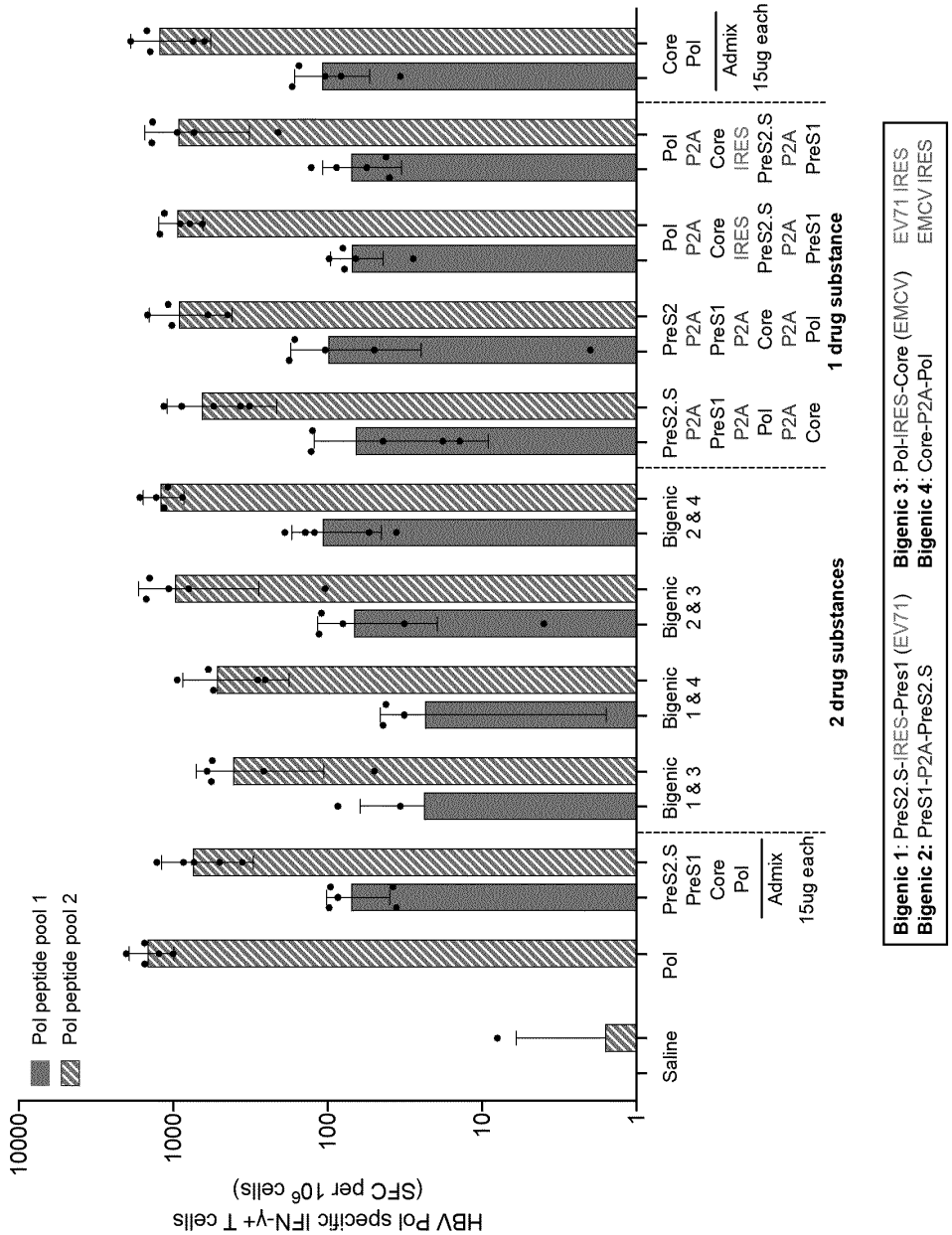
Pol peptide pool 1
Pol peptide pool 2
HBV Pol specific IFN-γ+ T cells
(SFC per 10^6 cells)
10000
1000
100
10
1
Saline
Pol
PreS2.S
PreS1
Core
Pol
Admix
15ug each
Bigenic 1 & 3
Bigenic 1 & 4
Bigenic 2 & 3
Bigenic 2 & 4
PreS2.S P2A PreS1 P2A Pol
PreS2.S P2A PreS1 P2A Core
PreS2 P2A PreS1 P2A Core
Pol P2A Core IRES PreS2.S
Pol P2A PreS1 IRES Core
Pol P2A PreS1 IRES PreS2.S
Pol P2A PreS1 IRES Core
Admix 15ug each
2 drug substances
1 drug substance
Bigenic 1: PreS2.S-IRES-Pres1 (EV71)
Bigenic 2: PreS1-P2A-PreS2.S
Bigenic 3: Pol-IRES-Core (EMCV)
Bigenic 4: Core-P2A-Pol
EV71 IRES
EMCV IRES

FIG. 6B

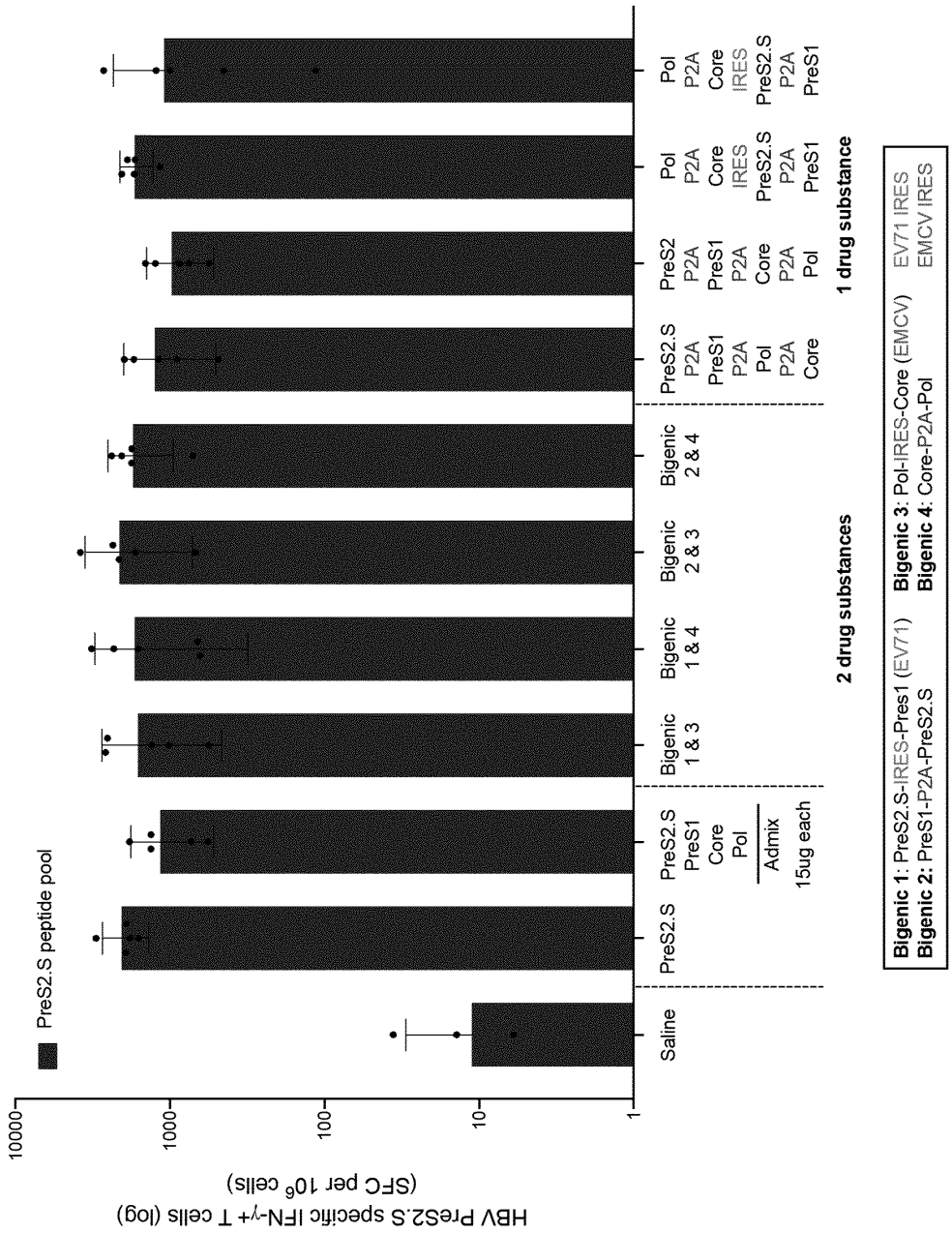
HBV PreS2.S specific IFN-γ+ T cells (log)
(SFC per 10^6 cells)
10000
1000
100
10
1
PreS2.S peptide pool
Saline
PreS2.S
PreS2.S
PreS1
Core
Pol
Admix
15ug each
Bigenic 1 & 3
Bigenic 1 & 4
Bigenic 2 & 3
Bigenic 2 & 4
PreS2.S P2A PreS1 P2A Pol P2A Core
PreS2 P2A PreS1 P2A Core P2A PreS2.S P2A Pol
Pol P2A Core IRES PreS2.S P2A PreS1
2 drug substances
1 drug substance
Bigenic 1: PreS2.S-IRES-Pres1 (EV71)
Bigenic 2: PreS1-P2A-PreS2.S
Bigenic 3: Pol-IRES-Core (EMCV)
Bigenic 4: Core-P2A-Pol
EV71 IRES
EMCV IRES

FIG. 6C

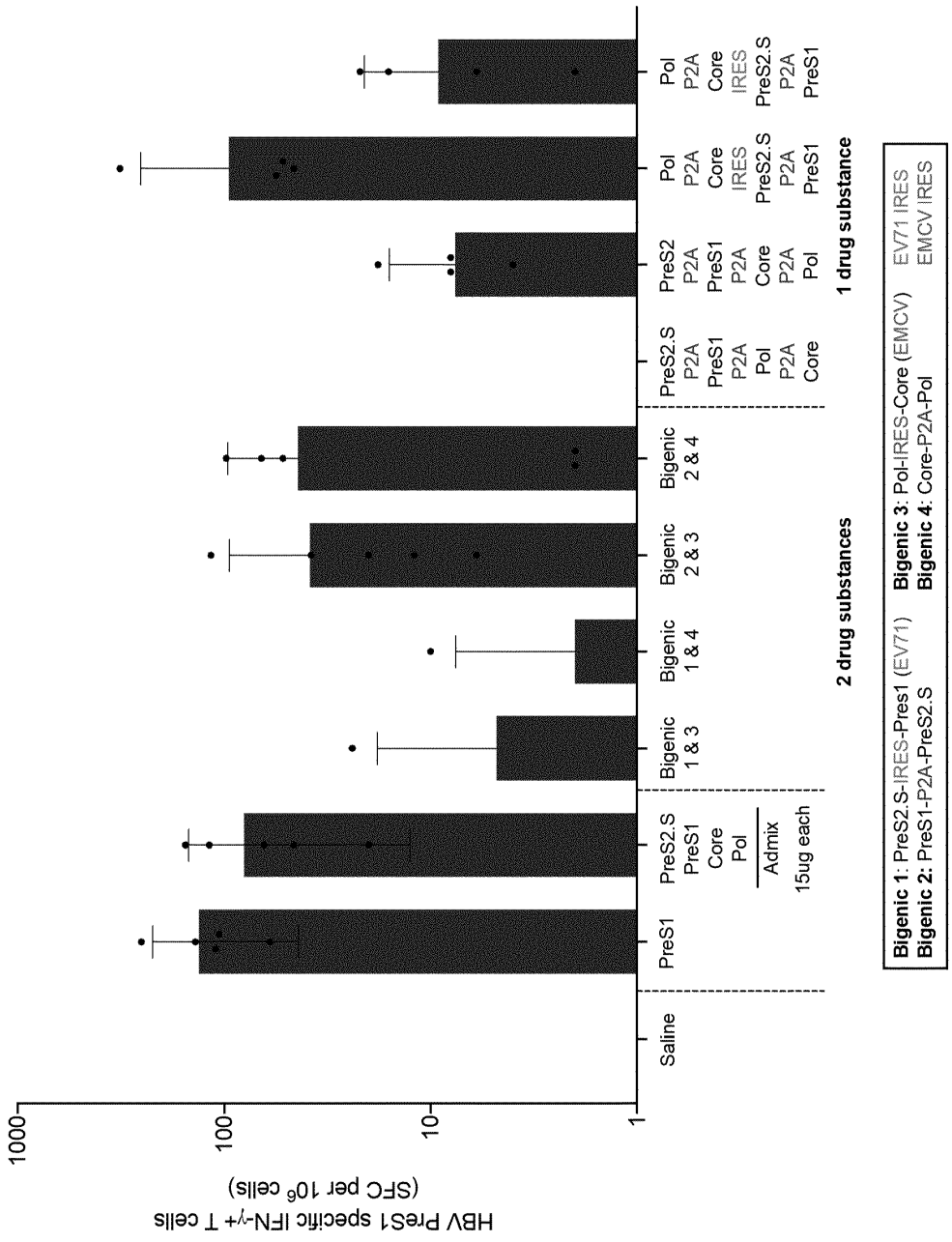
HBV PreS1 specific IFN-γ+ T cells
(SFC per 10^6 cells)
1000
100
10
1
Saline
PreS1
PreS2.S
PreS1
Core
Pol
Admix
15ug each
Bigenic 1 & 3
Bigenic 1 & 4
Bigenic 2 & 3
Bigenic 2 & 4
PreS2.S P2A PreS1 P2A Pol P2A Core
PreS2 P2A PreS1 P2A Core P2A Pol
Pol P2A Core IRES PreS2.S P2A PreS1
Pol P2A Core IRES PreS2.S P2A PreS1
2 drug substances
1 drug substance
Bigenic 1: PreS2.S-IRES-Pres1 (EV71)
Bigenic 2: PreS1-P2A-PreS2.S
Bigenic 3: Pol-IRES-Core (EMCV)
Bigenic 4: Core-P2A-Pol
EV71 IRES
EMCV IRES

FIG. 6D

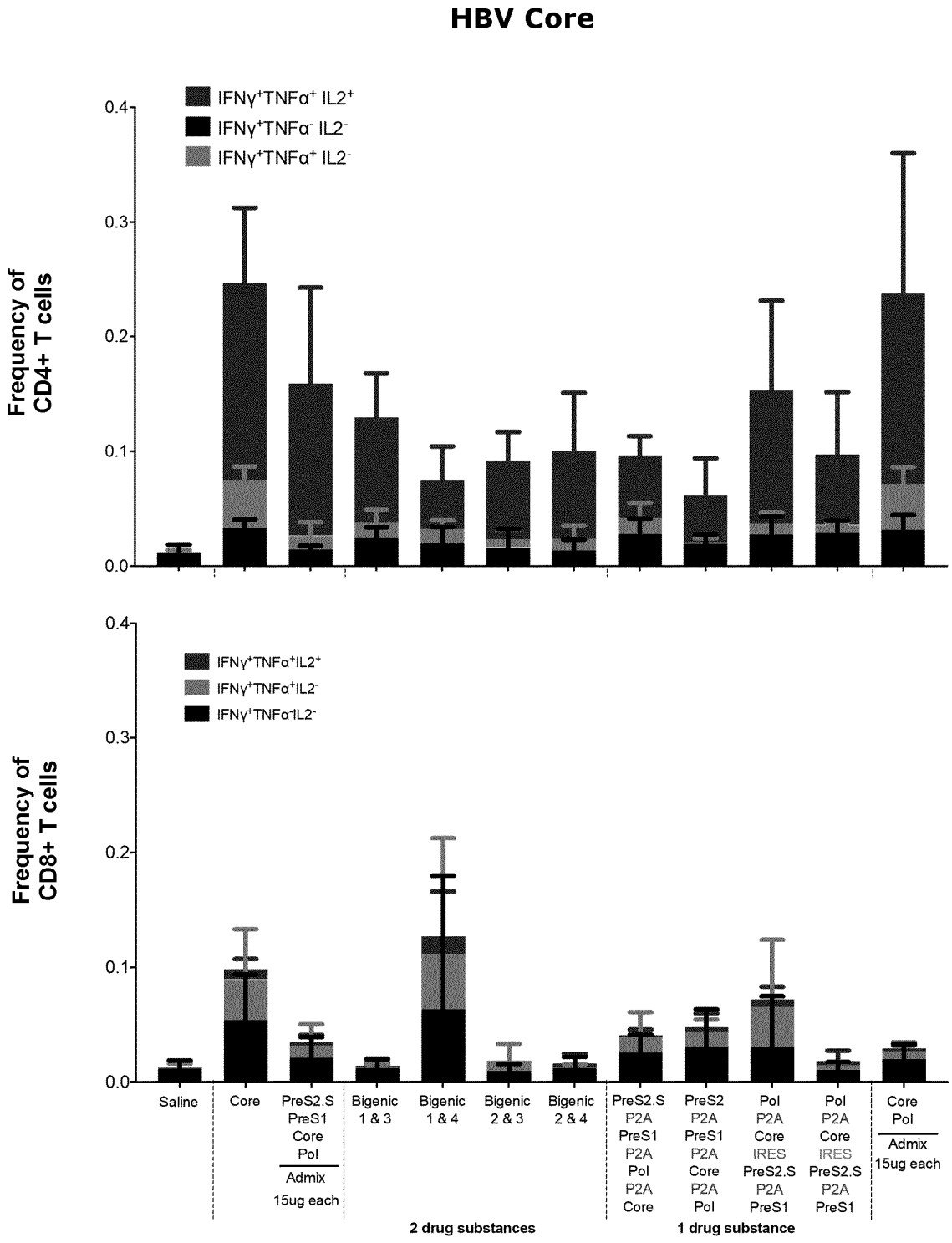
HBV Core
IFNγ+TNFα+ IL2+
IFNγ+TNFα- IL2-
IFNγ+TNFα+ IL2-
Frequency of CD4+ T cells
0.4
0.3
0.2
0.1
0.0
IFNγ+TNFα+IL2+
IFNγ+TNFα+IL2-
IFNγ+TNFα-IL2-
Frequency of CD8+ T cells
Saline
Core
PreS2.S PreS1 Core Pol
Admix 15ug each
Bigenic 1 & 3
Bigenic 1 & 4
Bigenic 2 & 3
Bigenic 2 & 4
PreS2.S P2A PreS1 P2A Pol P2A Core
PreS2 P2A PreS1 P2A Core P2A Pol
Pol P2A Core IRES PreS2.S P2A PreS1
Pol P2A Core IRES PreS2.S P2A PreS1
Core Pol
Admix 15ug each
2 drug substances
1 drug substance

FIG. 7A

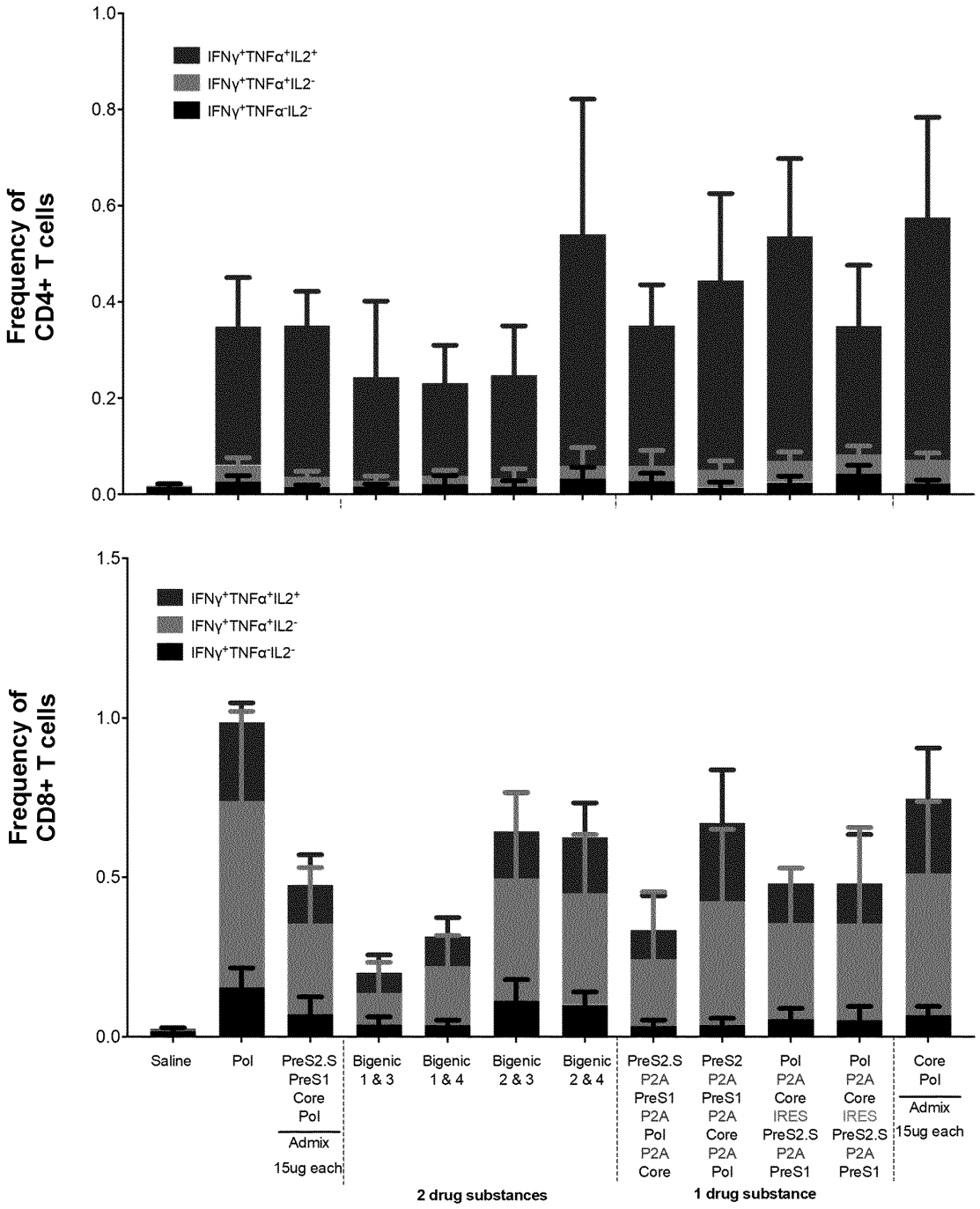
HBV Pol
Frequency of CD4+ T cells
1.0
0.8
0.6
0.4
0.2
0.0
IFNγ+TNFα+IL2+
IFNγ+TNFα+IL2-
IFNγ+TNFα-IL2-
Frequency of CD8+ T cells
1.5
1.0
0.5
0.0
IFNγ+TNFα+IL2+
IFNγ+TNFα+IL2-
IFNγ+TNFα-IL2-
Saline
Pol
PreS2.S PreS1 Core Pol
Admix 15ug each
Bigenic 1 & 3
Bigenic 1 & 4
Bigenic 2 & 3
Bigenic 2 & 4
PreS2.S P2A PreS1 P2A Pol P2A Core
PreS2 P2A PreS1 P2A Core P2A Pol
Pol P2A Core IRES PreS2.S P2A PreS1
Pol P2A Core IRES PreS2.S P2A PreS1
Core Pol
Admix 15ug each
2 drug substances
1 drug substance

FIG. 7B

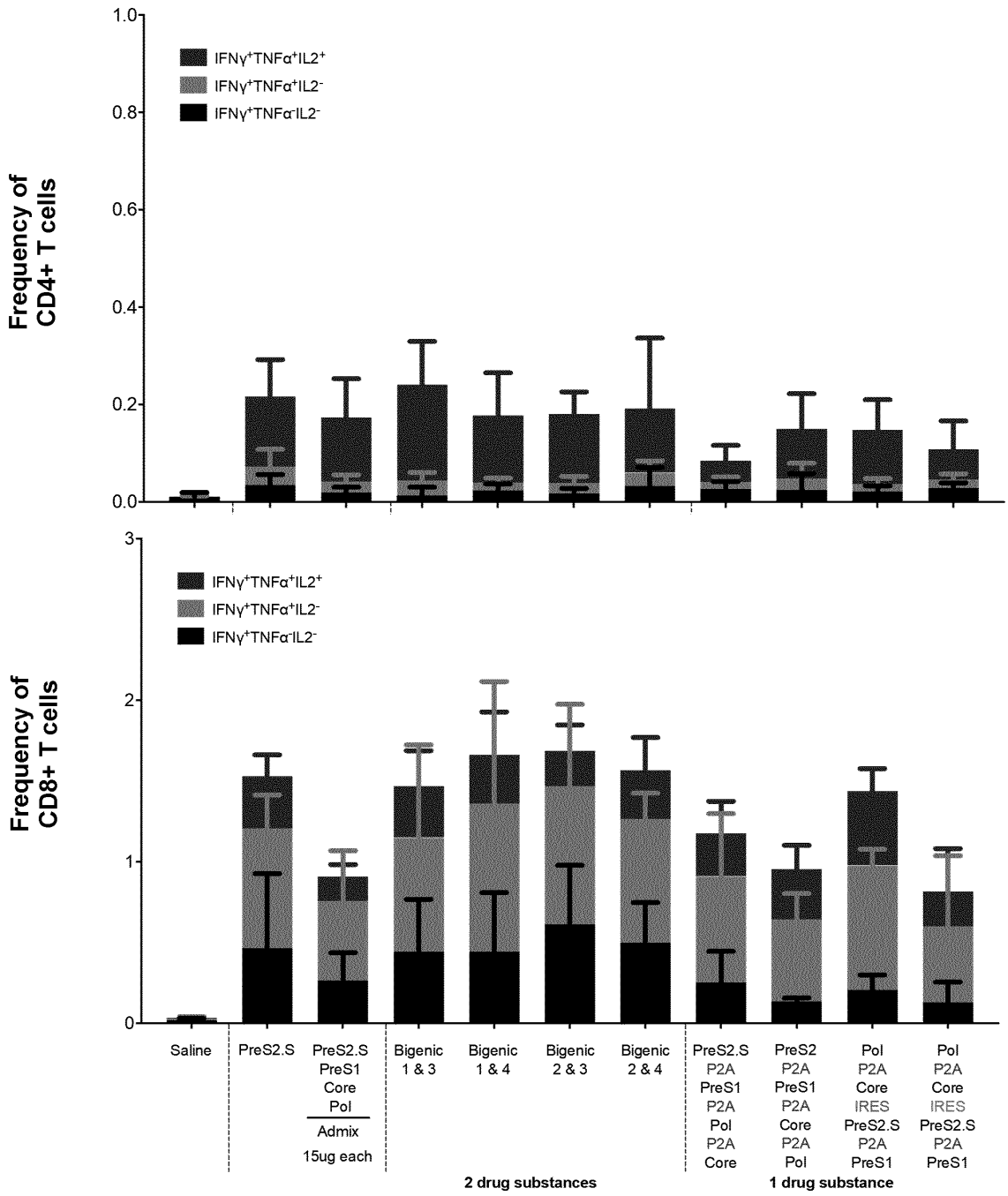
HBV PreS2.S
Frequency of CD4+ T cells
1.0
0.8
0.6
0.4
0.2
0.0
IFNγ+TNFα+IL2+
IFNγ+TNFα+IL2-
IFNγ+TNFα-IL2-
Frequency of CD8+ T cells
3
2
1
0
Saline
PreS2.S
PreS2.S PreS1 Core Pol
Admix
15ug each
Bigenic 1 & 3
Bigenic 1 & 4
Bigenic 2 & 3
Bigenic 2 & 4
PreS2.S P2A PreS1 P2A Pol P2A Core
PreS2 P2A PreS1 P2A Core P2A Pol
Pol P2A Core IRES PreS2.S P2A PreS1
Pol P2A Core IRES PreS2.S P2A PreS1
2 drug substances
1 drug substance

FIG. 7C

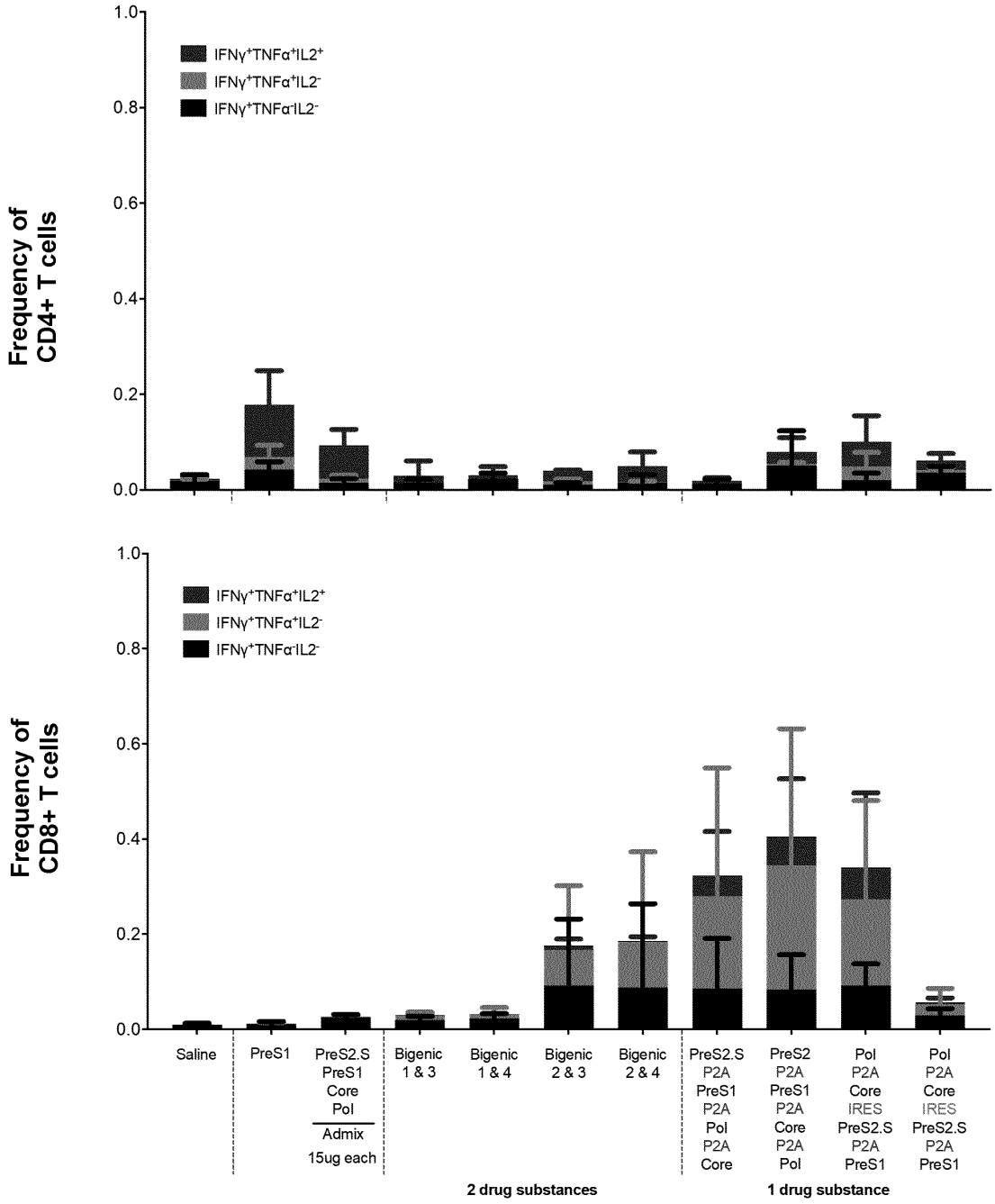
HBV PreS1
Frequency of CD4+ T cells
1.0
0.8
0.6
0.4
0.2
0.0
IFNγ+TNFα+IL2+
IFNγ+TNFα+IL2-
IFNγ+TNFα-IL2-
Frequency of CD8+ T cells
1.0
0.8
0.6
0.4
0.2
0.0
IFNγ+TNFα+IL2+
IFNγ+TNFα+IL2-
IFNγ+TNFα-IL2-
Saline
PreS1
PreS2.S PreS1 Core Pol
Admix 15ug each
Bigenic 1 & 3
Bigenic 1 & 4
Bigenic 2 & 3
Bigenic 2 & 4
PreS2.S P2A PreS1 P2A Pol P2A Core
PreS2 P2A PreS1 P2A Core P2A Pol
Pol P2A Core IRES PreS2.S P2A PreS1
Pol P2A Core IRES PreS2.S P2A PreS1
2 drug substances
1 drug substance

FIG. 7D