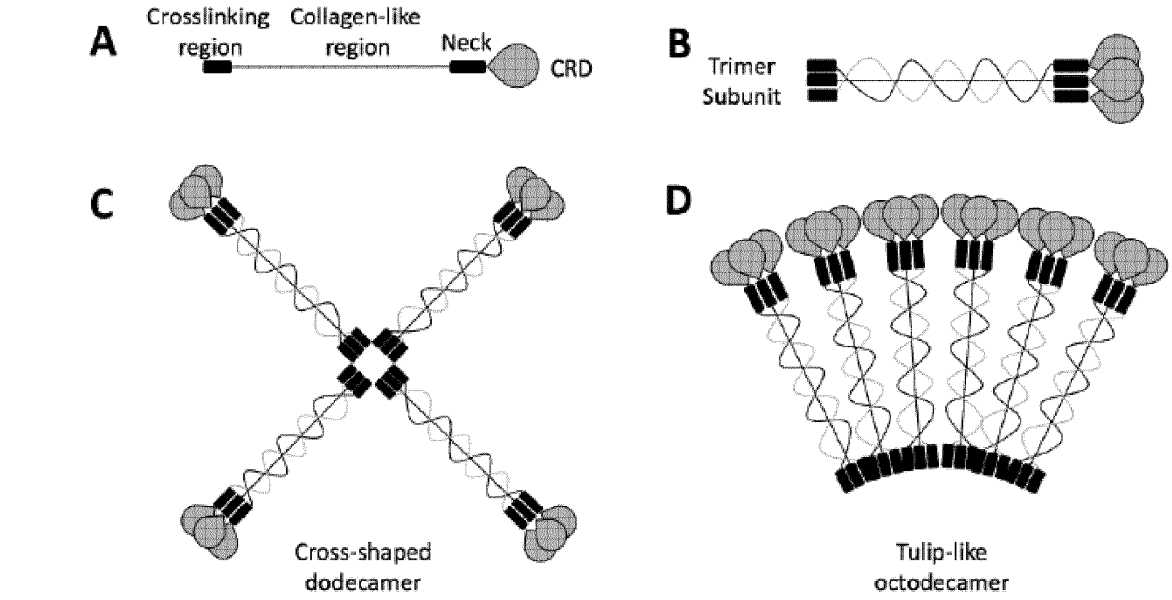

A
Crosslinking region
Collagen-like region
Neck
CRD
B
Trimer Subunit
C
Cross-shaped dodecamer
D
Tulip-like octodecamer

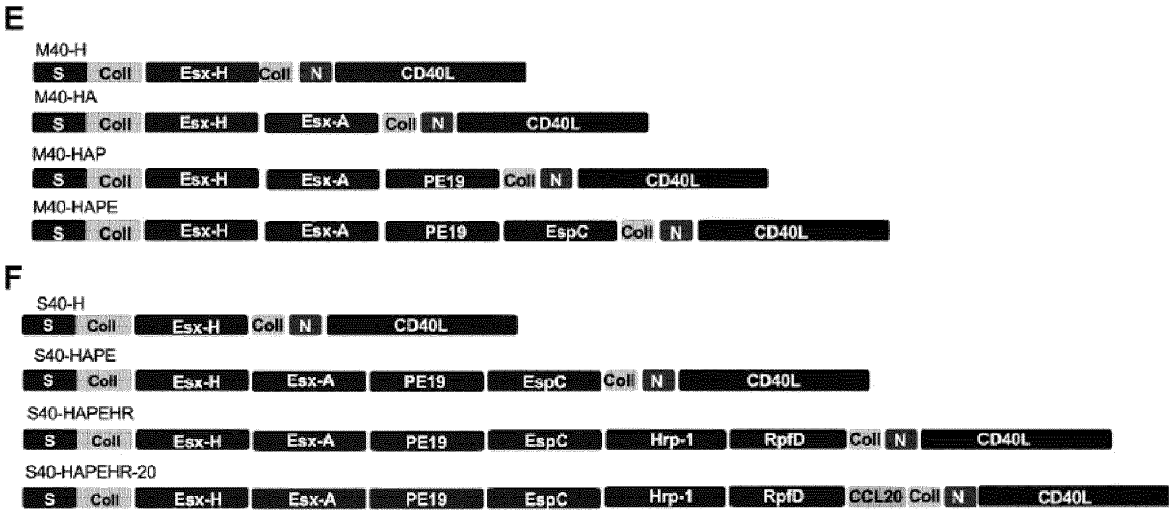

E
M40-H
S
Coll
Esx-H
Coll
N
CD40L
M40-HA
S
Coll
Esx-H
Esx-A
Coll
N
CD40L
M40-HAP
S
Coll
Esx-H
Esx-A
PE19
Coll
N
CD40L
M40-HAPE
S
Coll
Esx-H
Esx-A
PE19
EspC
Coll
N
CD40L
F
S40-H
S
Coll
Esx-H
Coll
N
CD40L
S40-HAPE
S
Coll
Esx-H
Esx-A
PE19
EspC
Coll
N
CD40L
S40-HAPEHR
S
Coll
Esx-H
Esx-A
PE19
EspC
Hrp-1
RpfD
Coll
N
CD40L
S40-HAPEHR-20
S
Coll
Esx-H
Esx-A
PE19
EspC
Hrp-1
RpfD
CCL20
Coll
N
CD40L

Figure 1

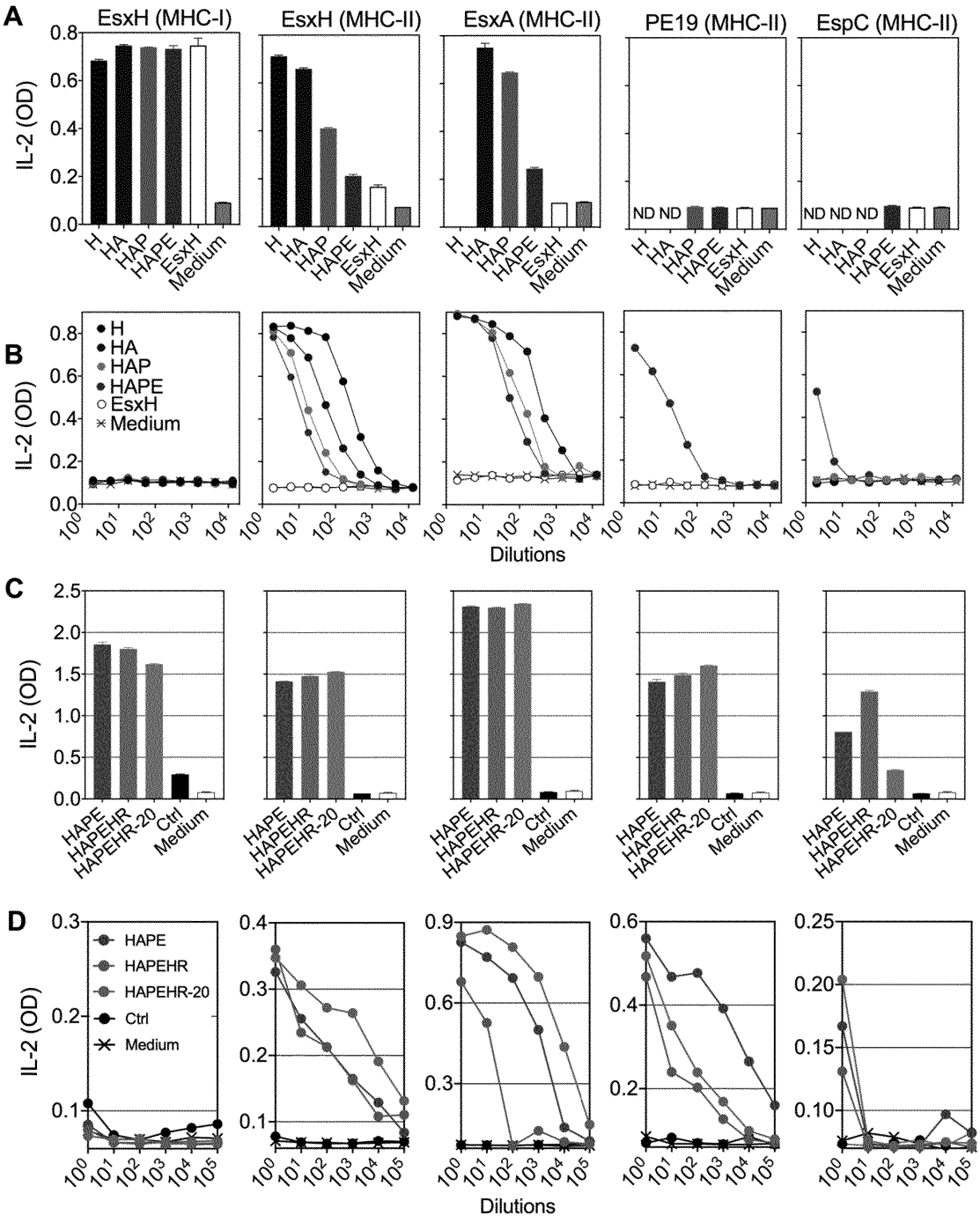
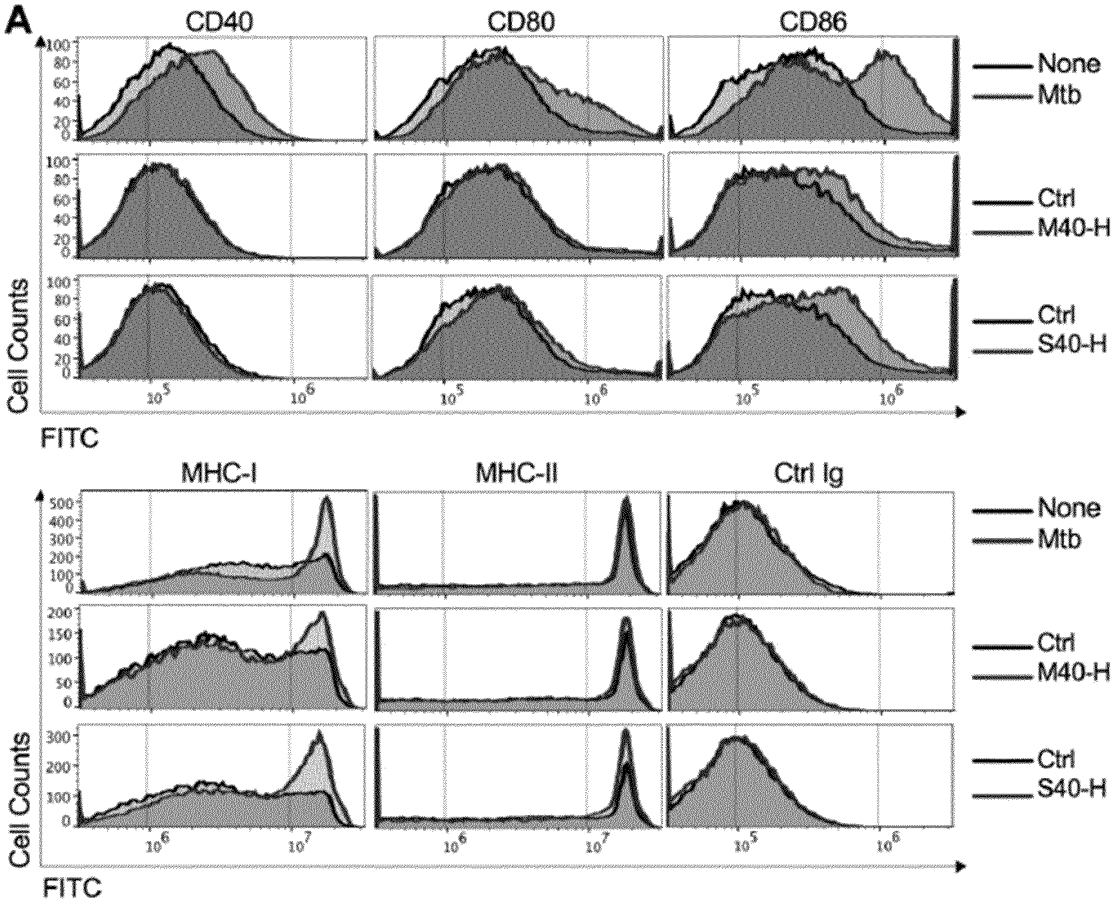

A
EsxH (MHC-I)
EsxH (MHC-II)
EsxA (MHC-II)
PE19 (MHC-II)
EspC (MHC-II)
IL-2 (OD)
ND ND
ND ND ND
H
HA
HAP
HAPE
EsxH
Medium
B
Dilutions
C
HAPEHR
HAPEHR-20
Ctrl
D
Dilutions

Figure 2


A
CD40
CD80
CD86
None
Mtb
Ctrl
M40-H
Ctrl
S40-H
Cell Counts
FITC
MHC-I
MHC-II
Ctrl Ig
None
Mtb
Ctrl
M40-H
Ctrl
S40-H
Cell Counts
FITC

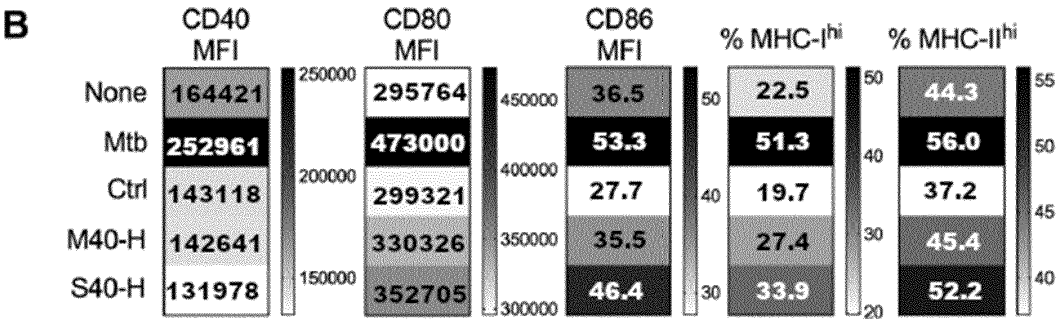

B
CD40 MFI
CD80 MFI
CD86 MFI
% MHC-I^hi
% MHC-II^hi
None 164421 295764 36.5 22.5 44.3
Mtb 252961 473000 53.3 51.3 56.0
Ctrl 143118 299321 27.7 19.7 37.2
M40-H 142641 330326 35.5 27.4 45.4
S40-H 131978 352705 46.4 33.9 52.2

Figure 3

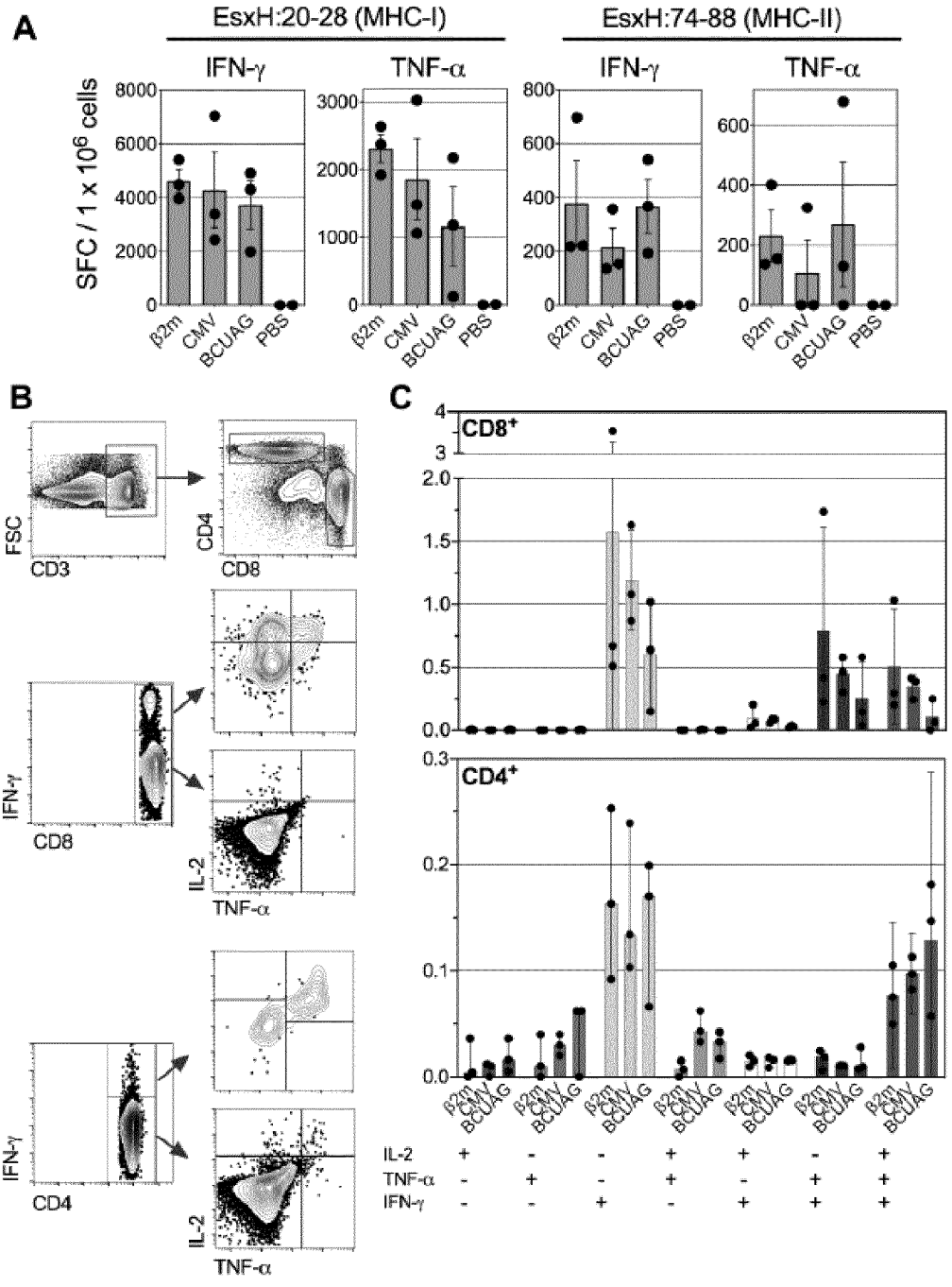

A
EsxH:20-28 (MHC-I)
EsxH:74-88 (MHC-II)
IFN-γ
TNF-α
SFC / 1 x 10^6 cells
β2m
CMV
BCUAG
PBS
B
FSC
CD3
CD4
CD8
IFN-γ
IL-2
TNF-α
C
CD8+
CD4+
IL-2
TNF-α
IFN-γ

Figure 4

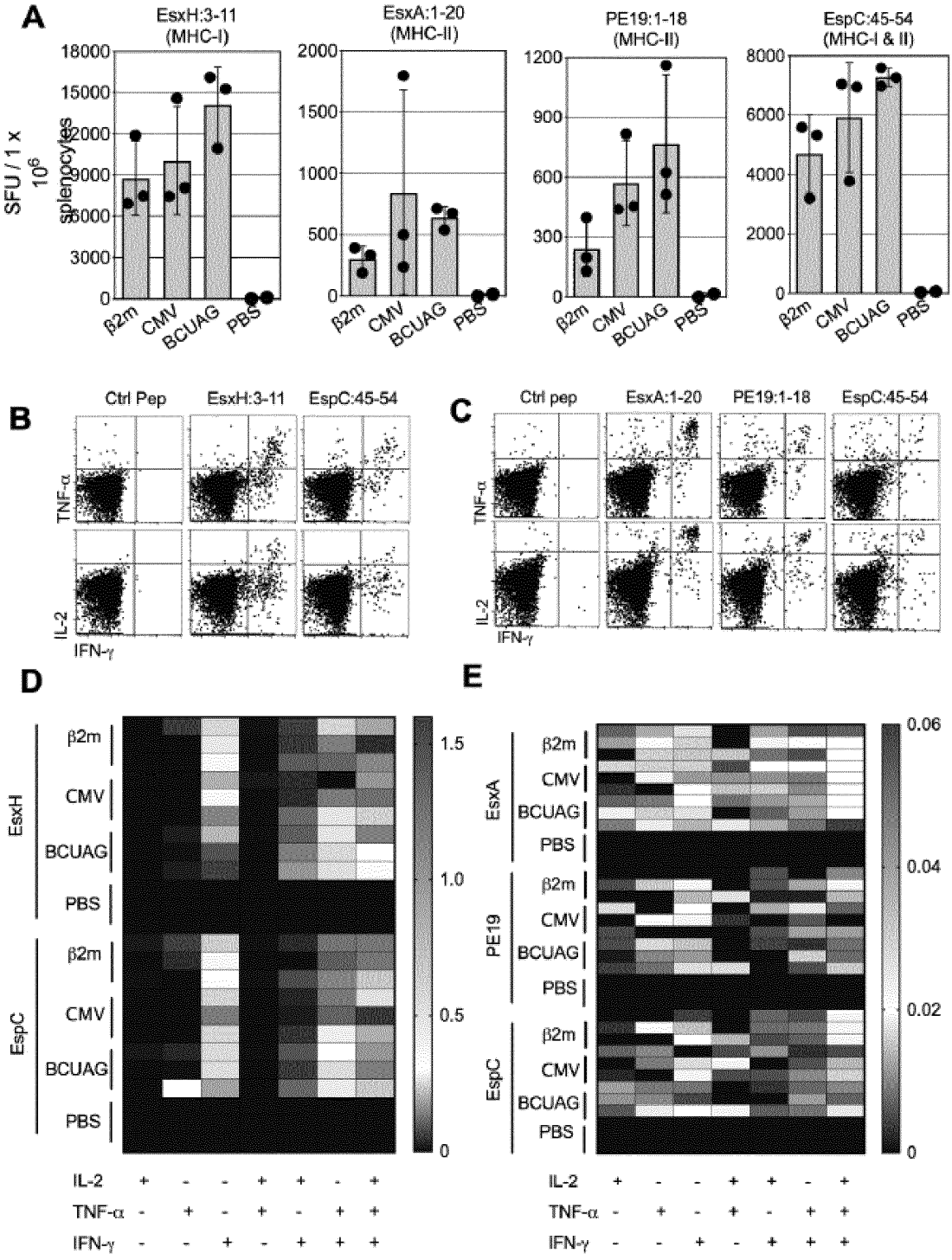

A
EsxH:3-11 (MHC-I)
EsxA:1-20 (MHC-II)
PE19:1-18 (MHC-II)
EspC:45-54 (MHC-I & II)
SFU / 1 x 10^6 splenocytes
β2m
CMV
BCUAG
PBS
B
Ctrl Pep
EsxH:3-11
EspC:45-54
C
Ctrl pep
EsxA:1-20
PE19:1-18
EspC:45-54
TNF-α
IL-2
IFN-γ
D
E
EsxH
EsxA
PE19
EspC
IL-2
TNF-α
IFN-γ

Figure 5

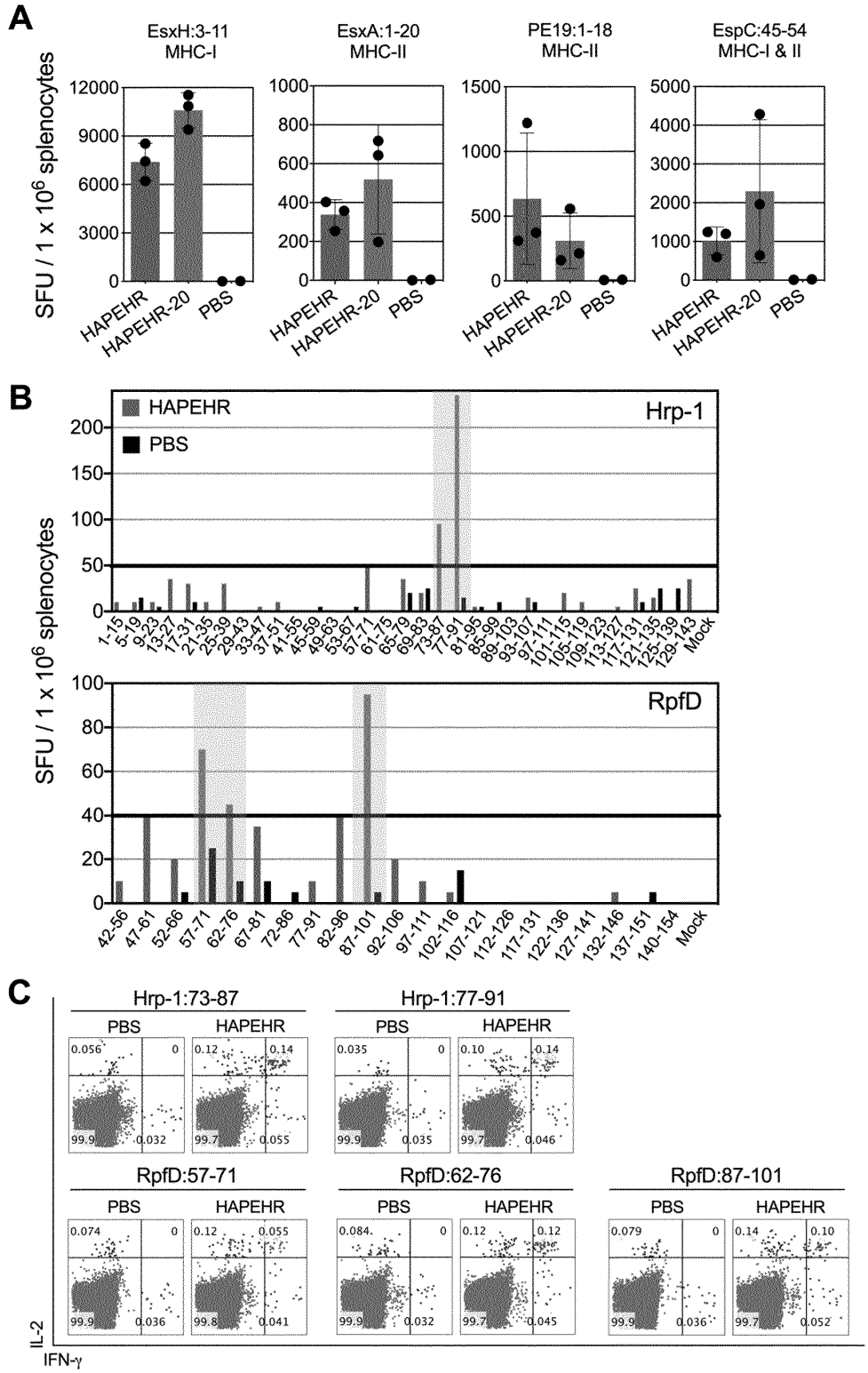

A
EsxH:3-11
MHC-I
EsxA:1-20
MHC-II
PE19:1-18
MHC-II
EspC:45-54
MHC-I & II
SFU / 1 x 10^6 splenocytes
HAPEHR
HAPEHR-20
PBS
B
HAPEHR
PBS
Hrp-1
RpfD
SFU / 1 x 10^6 splenocytes
Mock
C
Hrp-1:73-87
Hrp-1:77-91
RpfD:57-71
RpfD:62-76
RpfD:87-101
PBS
HAPEHR
IL-2
IFN-γ

Figure 6

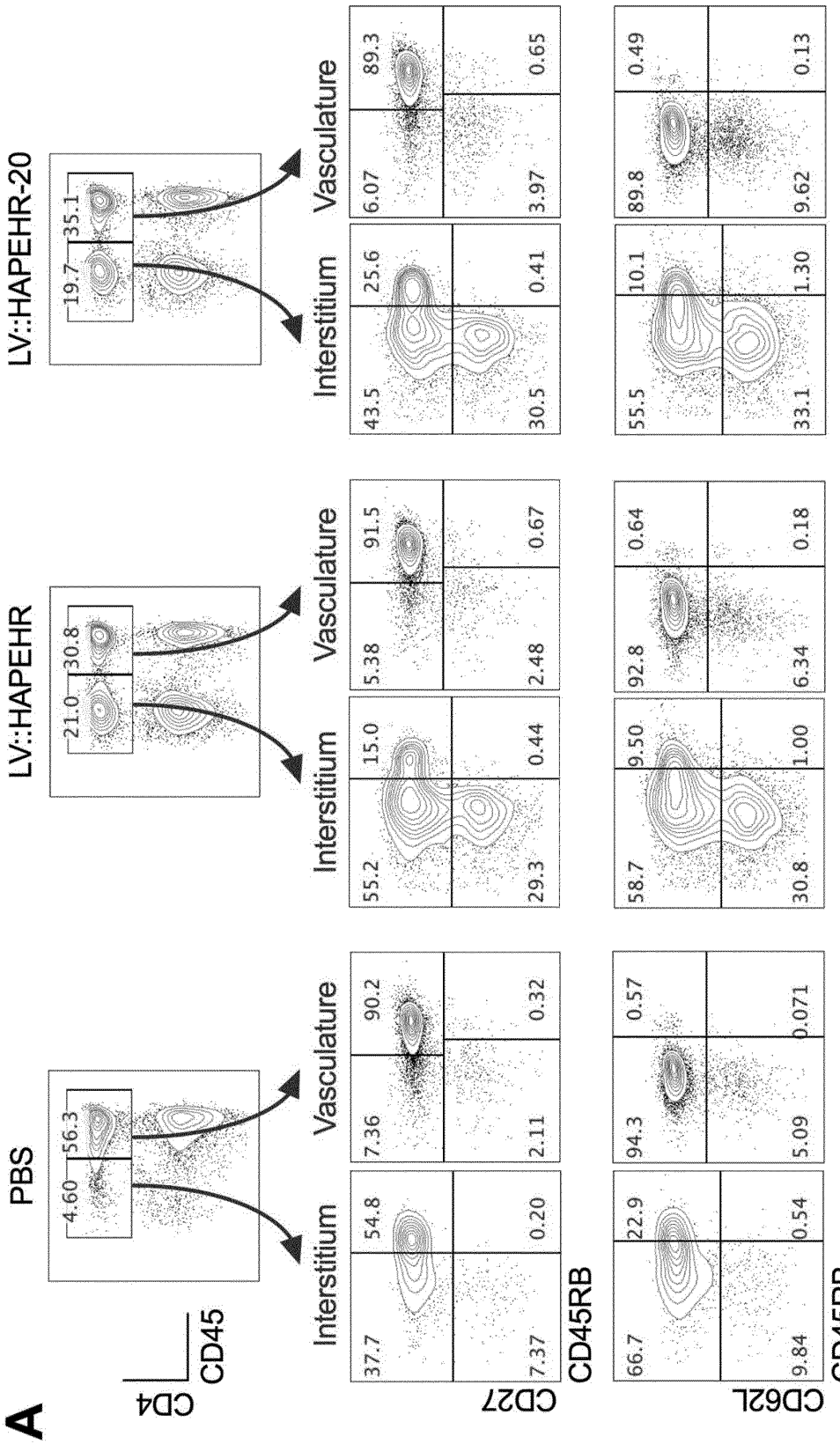

A
PBS
LV::HAPEHR
LV::HAPEHR-20
CD4
CD45
4.60
56.3
21.0
30.8
19.7
35.1
Interstitium
Vasculature
37.7
54.8
7.36
90.2
7.37
0.20
2.11
0.32
55.2
15.0
5.38
91.5
29.3
0.44
2.48
0.67
43.5
25.6
6.07
89.3
30.5
0.41
3.97
0.65
CD27
CD45RB
66.7
22.9
94.3
0.57
9.84
0.54
5.09
0.071
58.7
9.50
92.8
0.64
30.8
1.00
6.34
0.18
55.5
10.1
89.8
0.49
33.1
1.30
9.62
0.13
CD62L
CD45RB

Figure 7

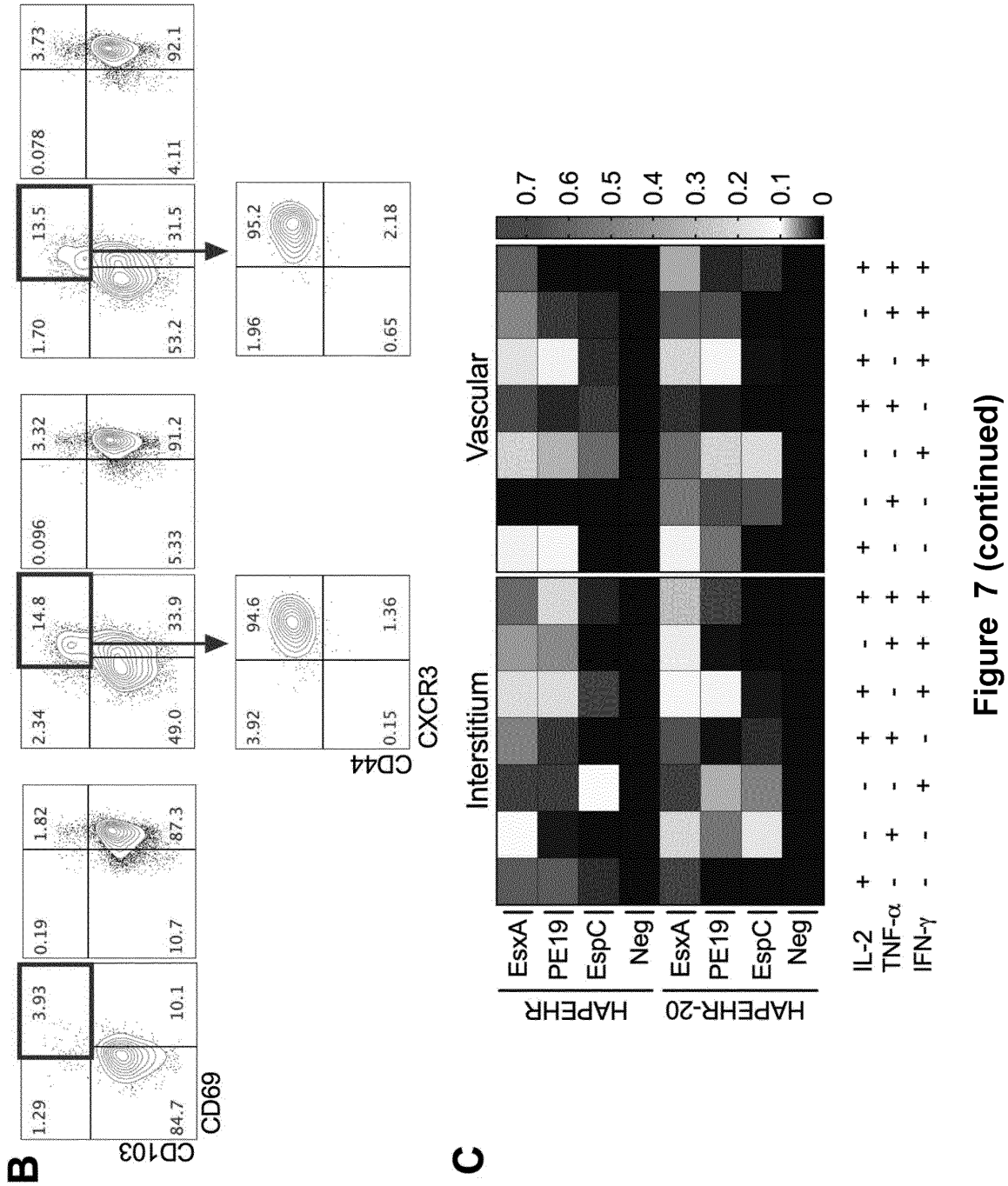


Figure 7 (continued)

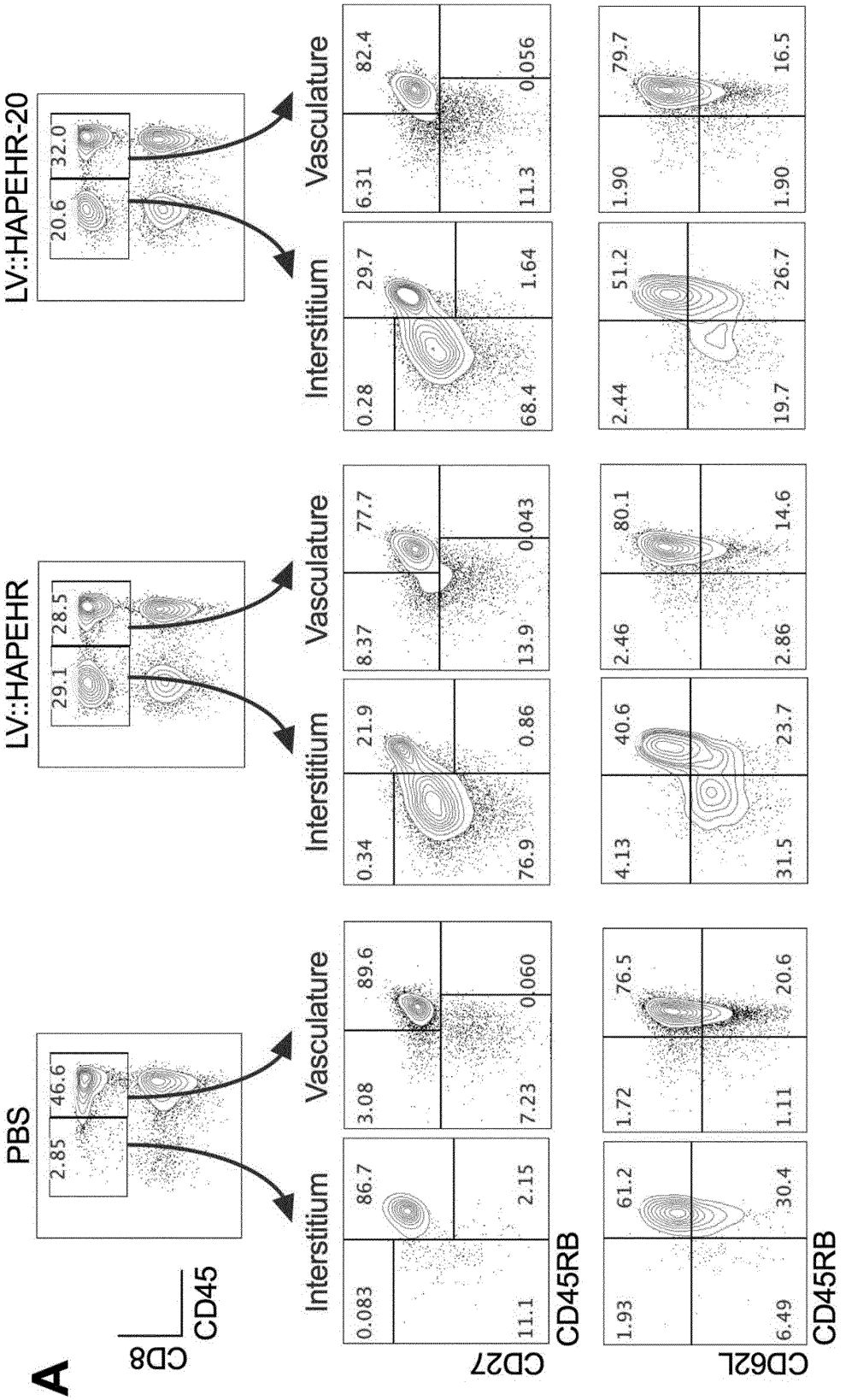

A
PBS
LV::HAPEHR
LV::HAPEHR-20
CD8
CD45
Interstitium
Vasculature
CD27
CD62L
CD45RB
2.85
46.6
29.1
28.5
20.6
32.0
0.083
86.7
11.1
2.15
3.08
89.6
7.23
0.060
0.34
21.9
76.9
0.86
8.37
77.7
13.9
0.043
0.28
29.7
68.4
1.64
6.31
82.4
11.3
0.056
1.93
61.2
6.49
30.4
1.72
76.5
1.11
20.6
4.13
40.6
31.5
23.7
2.46
80.1
2.86
14.6
2.44
51.2
19.7
26.7
1.90
79.7
1.90
16.5

Figure 8

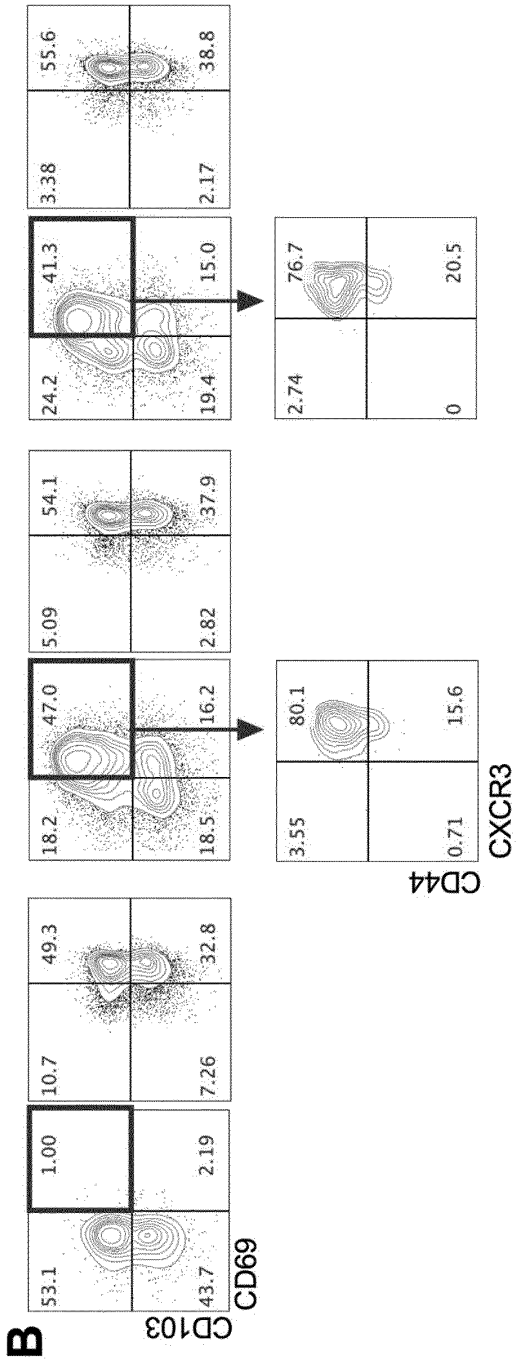


Figure 8 (continued)

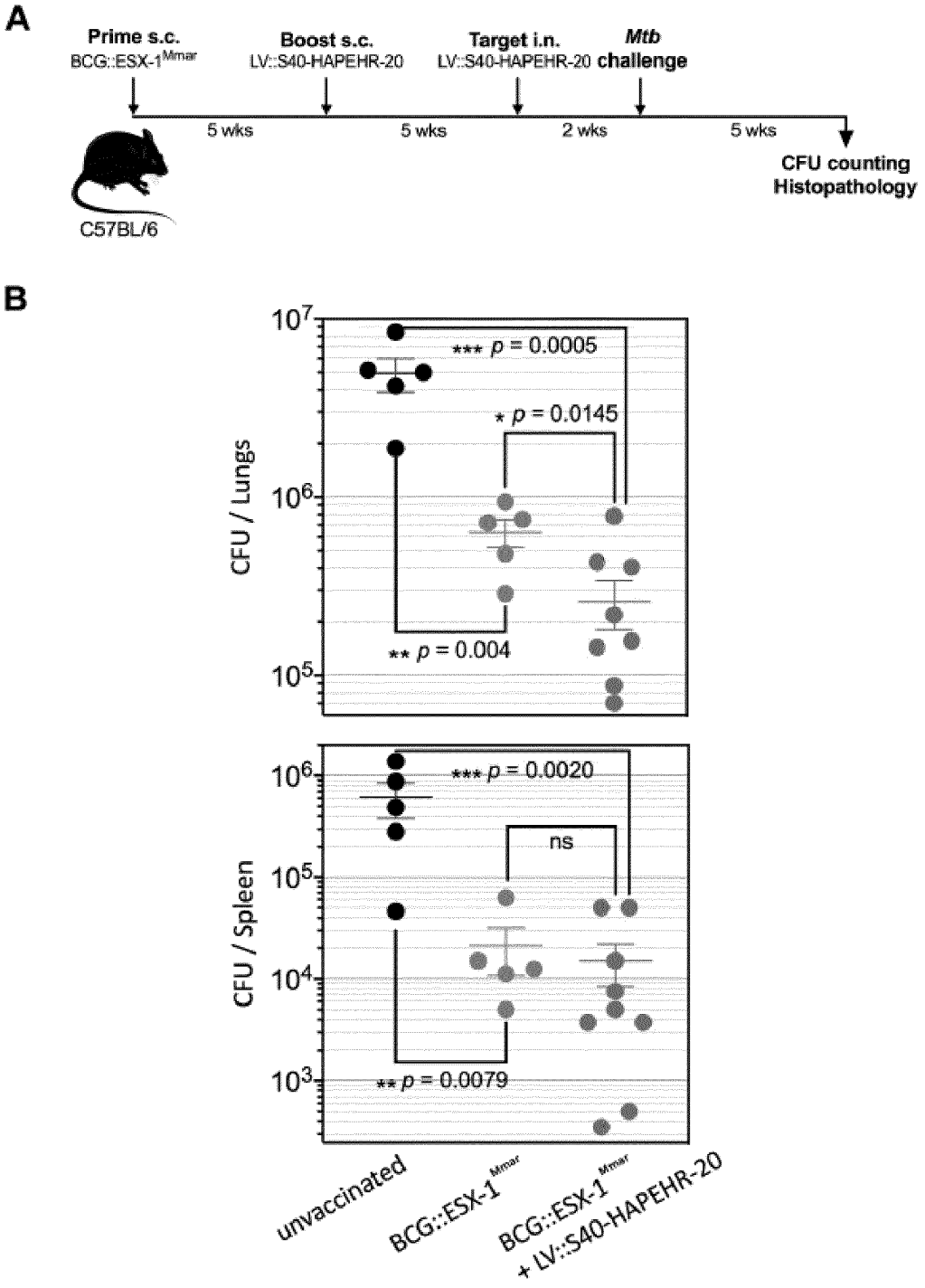

A
Prime s.c.
BCG::ESX-1Mmar
Boost s.c.
LV::S40-HAPEHR-20
Target i.n.
LV::S40-HAPEHR-20
Mtb challenge
5 wks
5 wks
2 wks
5 wks
CFU counting
Histopathology
C57BL/6
B
CFU / Lungs
*** p = 0.0005
* p = 0.0145
** p = 0.004
CFU / Spleen
*** p = 0.0020
ns
** p = 0.0079
unvaccinated
BCG::ESX-1Mmar
BCG::ESX-1Mmar + LV::S40-HAPEHR-20

Figure 9

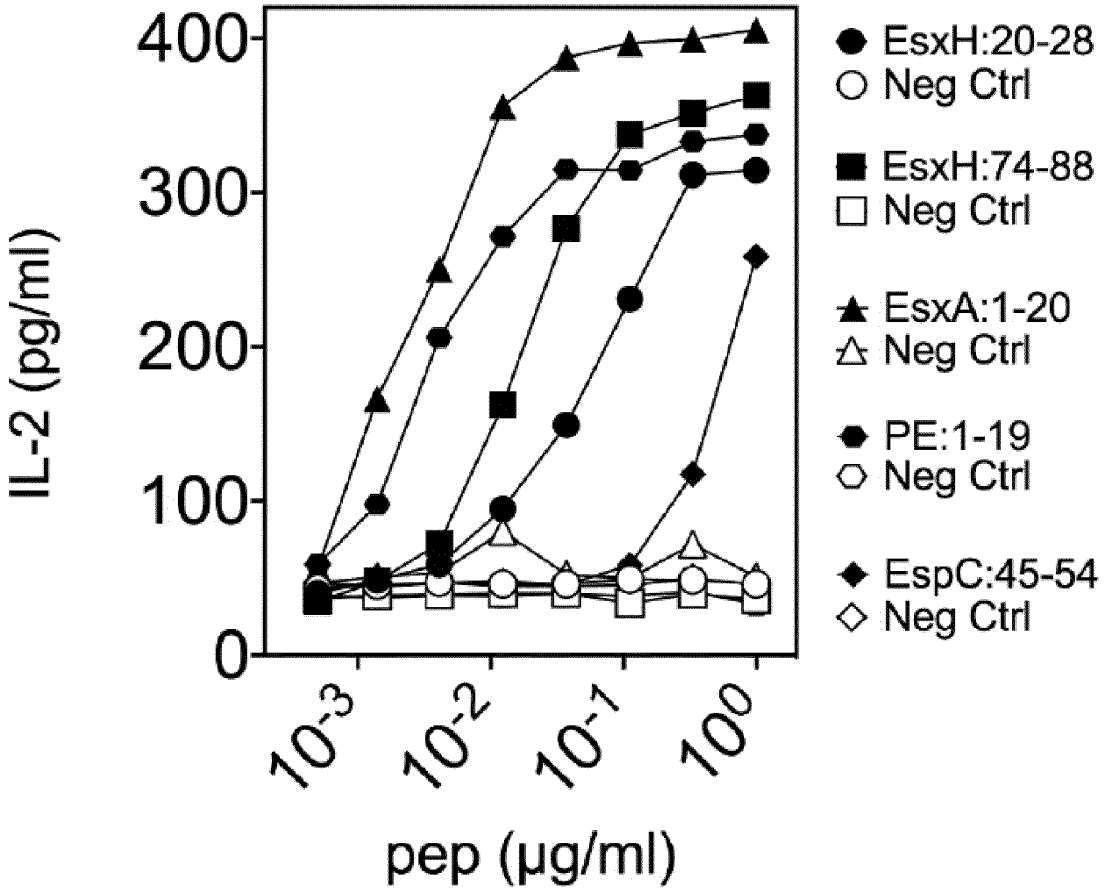

400
300
200
100
0
IL-2 (pg/ml)
10-3
10-2
10-1
100
pep (µg/ml)
EsxH:20-28
Neg Ctrl
EsxH:74-88
Neg Ctrl
EsxA:1-20
Neg Ctrl
PE:1-19
Neg Ctrl
EspC:45-54
Neg Ctrl

Figure 10

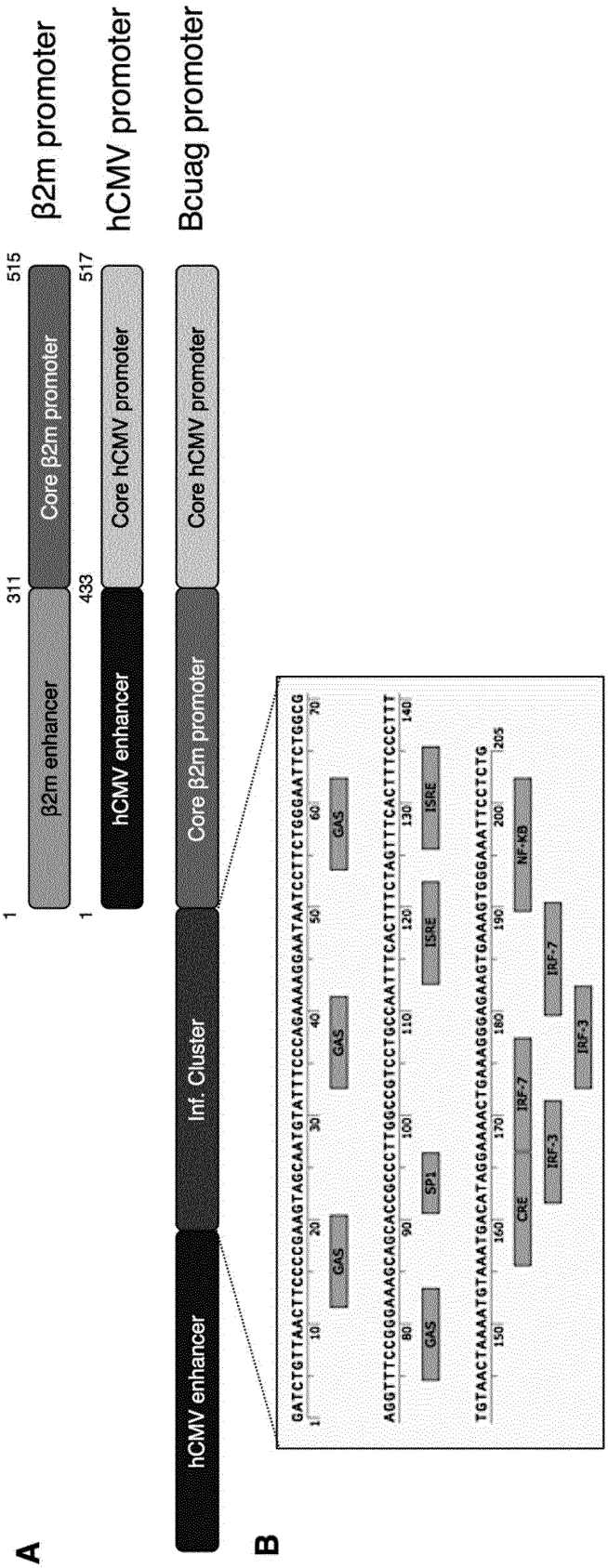

A
B
β2m promoter
hCMV promoter
Bcuag promoter
β2m enhancer
Core β2m promoter
hCMV enhancer
Core hCMV promoter
Inf. Cluster
1
311
515
433
517
GAS
SP1
CRE
IRF-3
IRF-7
ISRE
NF-KB

Figure 11

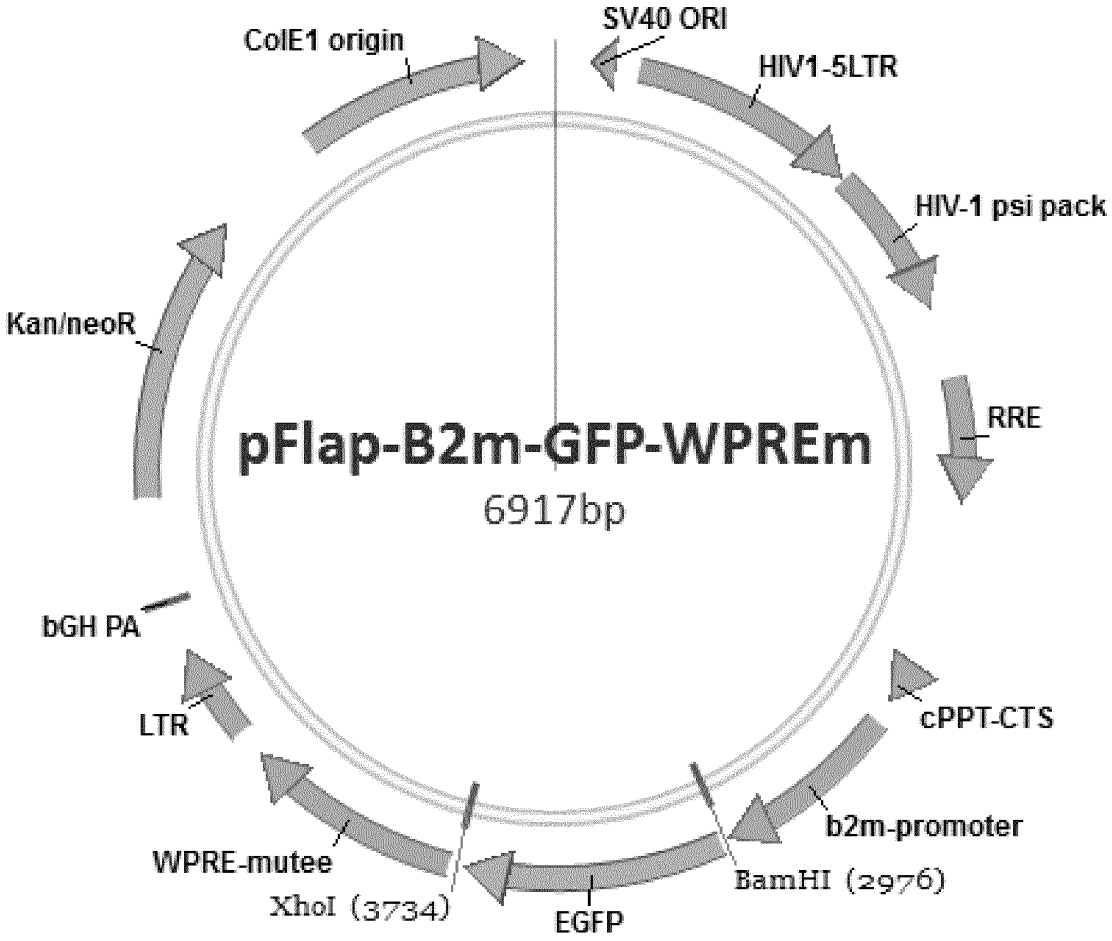

ColE1 origin
SV40 ORI
HIV1-5LTR
HIV-1 psi pack
RRE
cPPT-CTS
b2m-promoter
BamHI (2976)
EGFP
XhoI (3734)
WPRE-mutee
LTR
bGH PA
Kan/neoR
pFlap-B2m-GFP-WPREm
6917bp

Figure 12

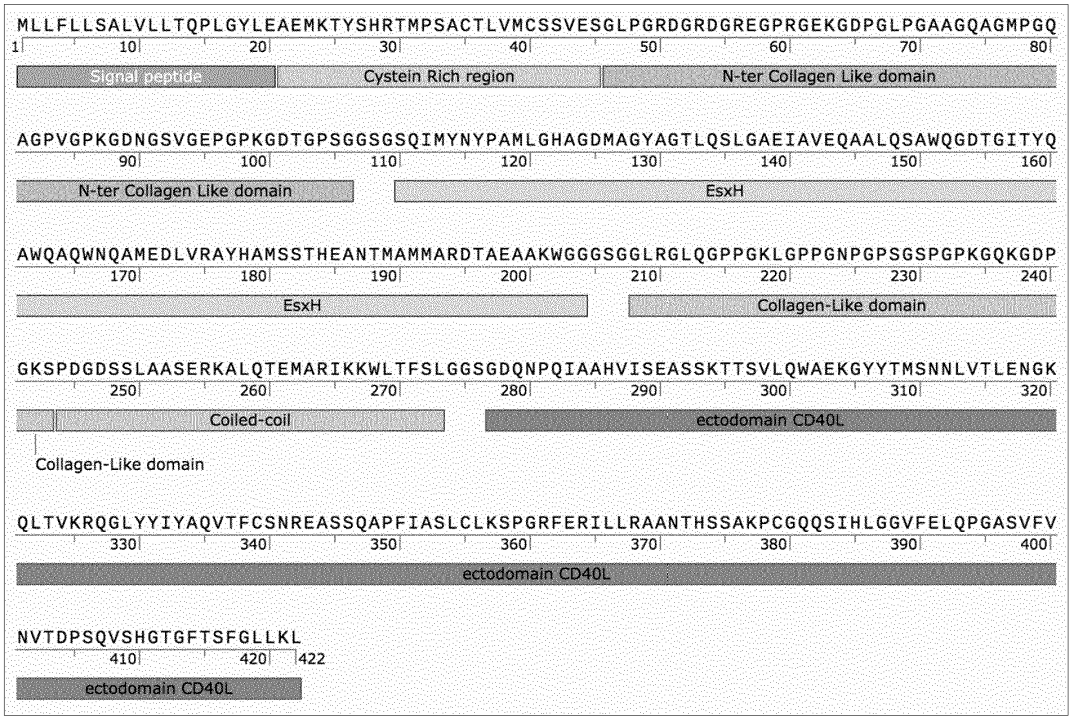

MLLFLLSALVLLTQPLGYLEAEMKTYSHRTMPSACTLVMCSSVESGLPGRDGRDGREGPRGEKGDPGLPGAAGQAGMPGQ
1 10 20 30 40 50 60 70 80
Signal peptide
Cystein Rich region
N-ter Collagen Like domain
AGPVGPKGDNGSVGEPGPKGDTGPSGGSGSQIMYNYPAMLGHAGDMAGYAGTLQSLGAEIAVEQAALQSAWQGDTGITYQ
90 100 110 120 130 140 150 160
N-ter Collagen Like domain
EsxH
AWQAQWNQAMEDLVRAYHAMSSTHEANTMAMMARDTAEAAKWGGGSGGLRGLQGPPGKLGPPGNPGPSGSPGPKGQKGDP
170 180 190 200 210 220 230 240
EsxH
Collagen-Like domain
GKSPDGDSSLAASERKALQTEMARIKKWLTFSLGGSGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGK
250 260 270 280 290 300 310 320
Coiled-coil
ectodomain CD40L
Collagen-Like domain
QLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGASVFV
330 340 350 360 370 380 390 400
ectodomain CD40L
NVTDPSQVSHGTGFTSFGLLKL
410 420 422
ectodomain CD40L

Figure 13