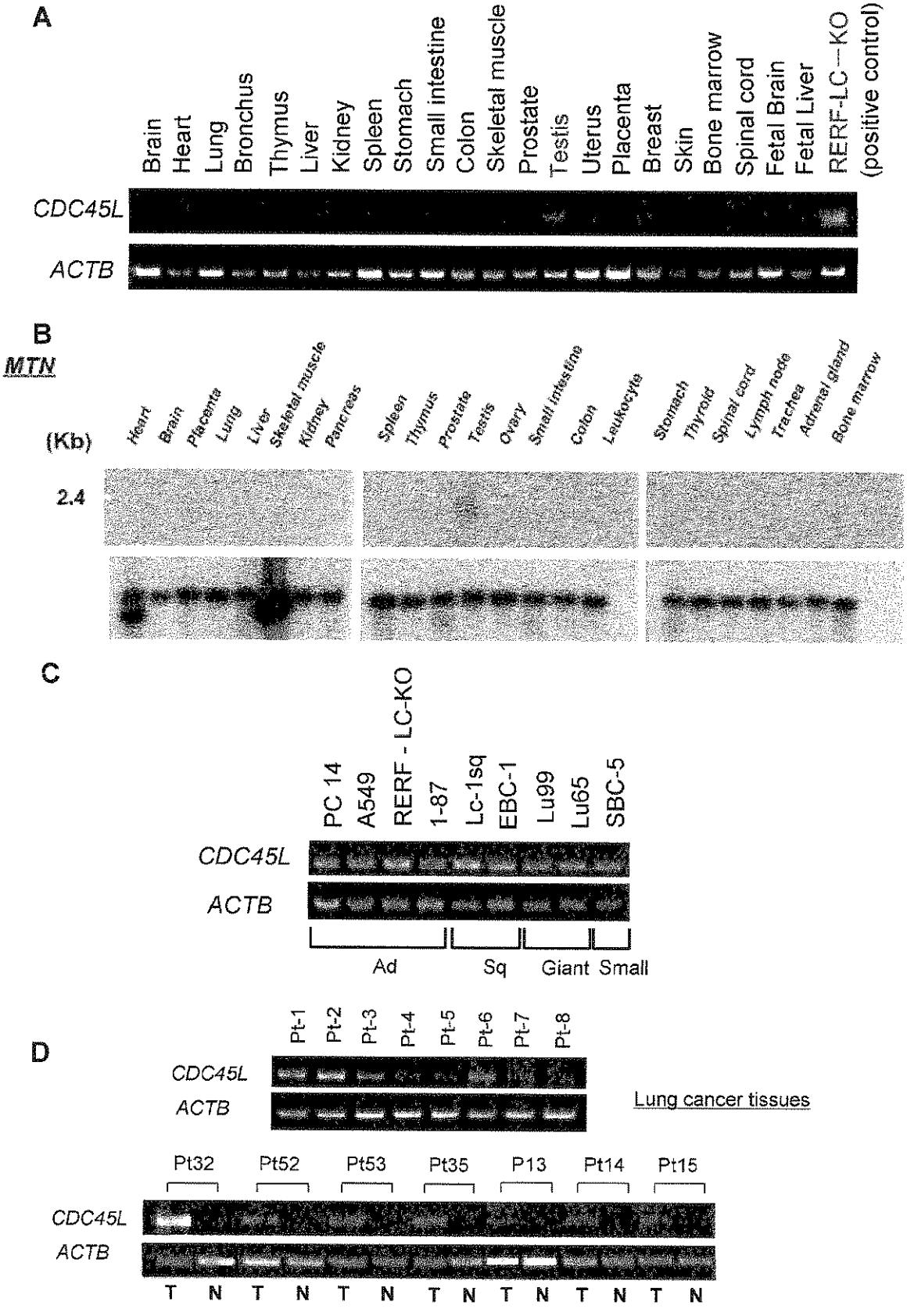
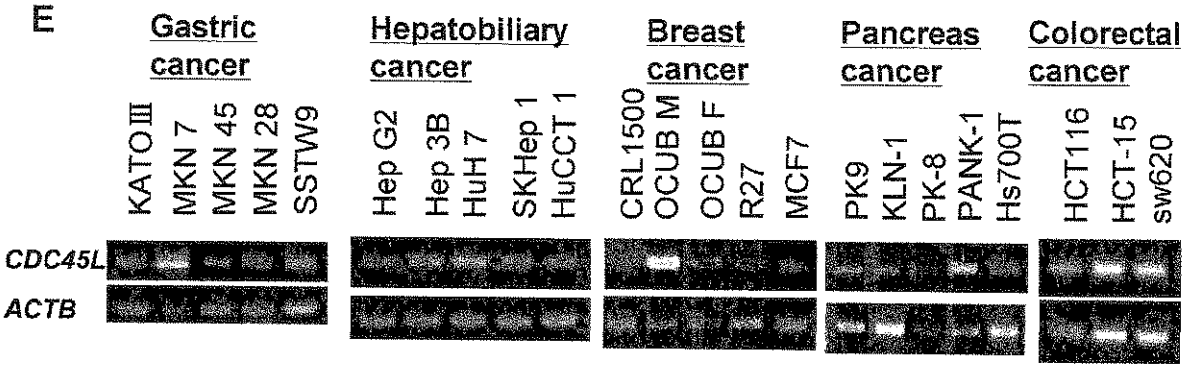
[Fig. 1a-d]

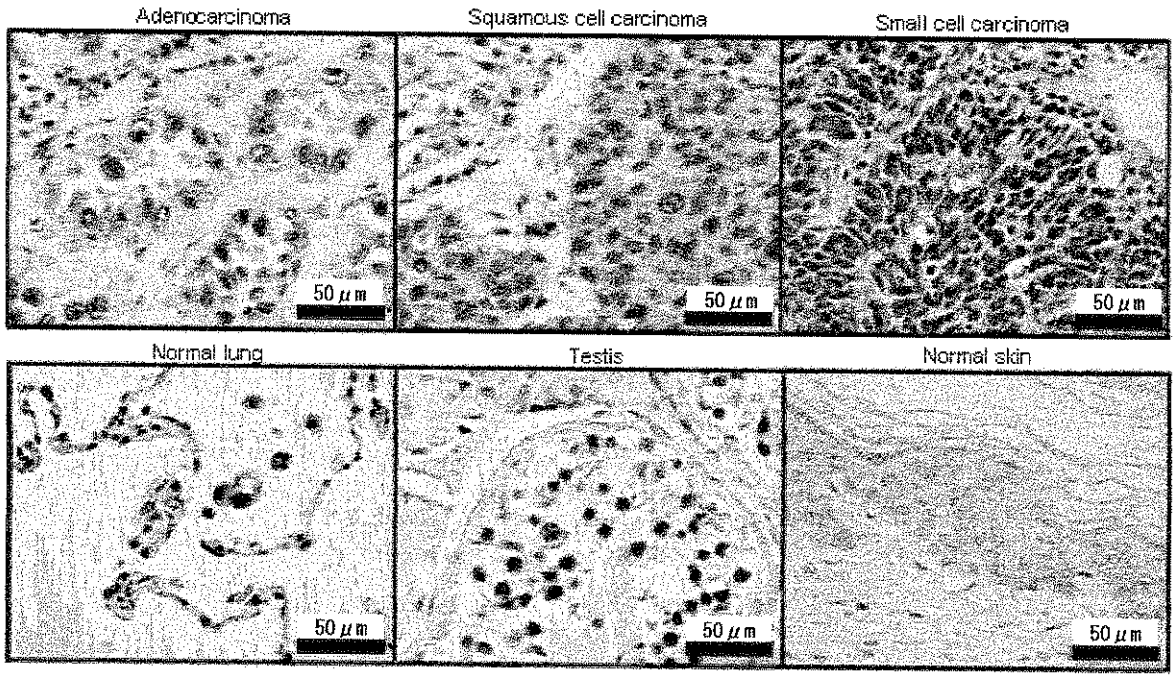

A
Brain
Heart
Lung
Bronchus
Thymus
Liver
Kidney
Spleen
Stomach
Small intestine
Colon
Skeletal muscle
Prostate
Testis
Uterus
Placenta
Breast
Skin
Bone marrow
Spinal cord
Fetal Brain
Fetal Liver
RERF-LC-KO (positive control)
CDC45L
ACTB
B
MTN
(Kb)
2.4
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas
Spleen
Thymus
Prostate
Testis
Ovary
Small intestine
Colon
Leukocyte
Stomach
Thyroid
Spinal cord
Lymph node
Trachea
Adrenal gland
Bone marrow
C
PC 14
A549
RERF - LC-KO
1-87
Lc-1sq
EBC-1
Lu99
Lu65
SBC-5
CDC45L
ACTB
Ad
Sq
Giant
Small
D
Pt-1
Pt-2
Pt-3
Pt-4
Pt-5
Pt-6
Pt-7
Pt-8
CDC45L
ACTB
Lung cancer tissues
Pt32
Pt52
Pt53
Pt35
P13
Pt14
Pt15
CDC45L
ACTB
T N T N T N T N T N T N T N

[Fig. 1e-f]

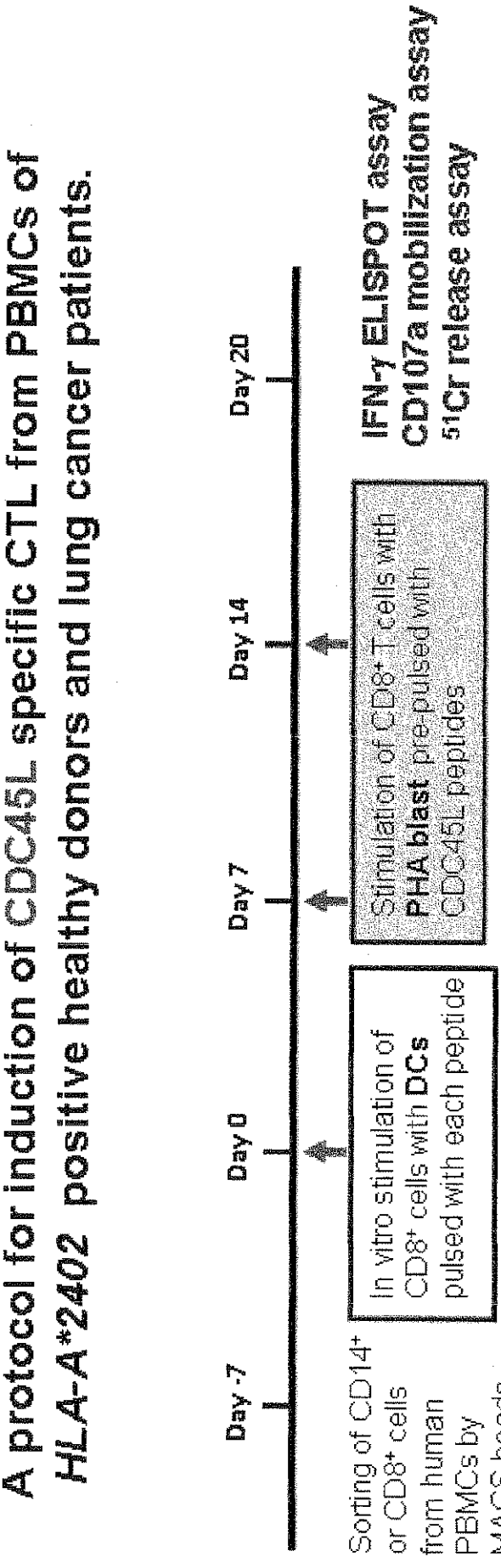
E


Gastric cancer
Hepatobiliary cancer
Breast cancer
Pancreas cancer
Colorectal cancer
KATO III
MKN 7
MKN 45
MKN 28
SSTW9
Hep G2
Hep 3B
HuH 7
SKHep 1
HuCCT 1
CRL1500
OCUB M
OCUB F
R27
MCF7
PK9
KLN-1
PK-8
PANK-1
Hs700T
HCT116
HCT-15
sw620
CDC45L
ACTB

F

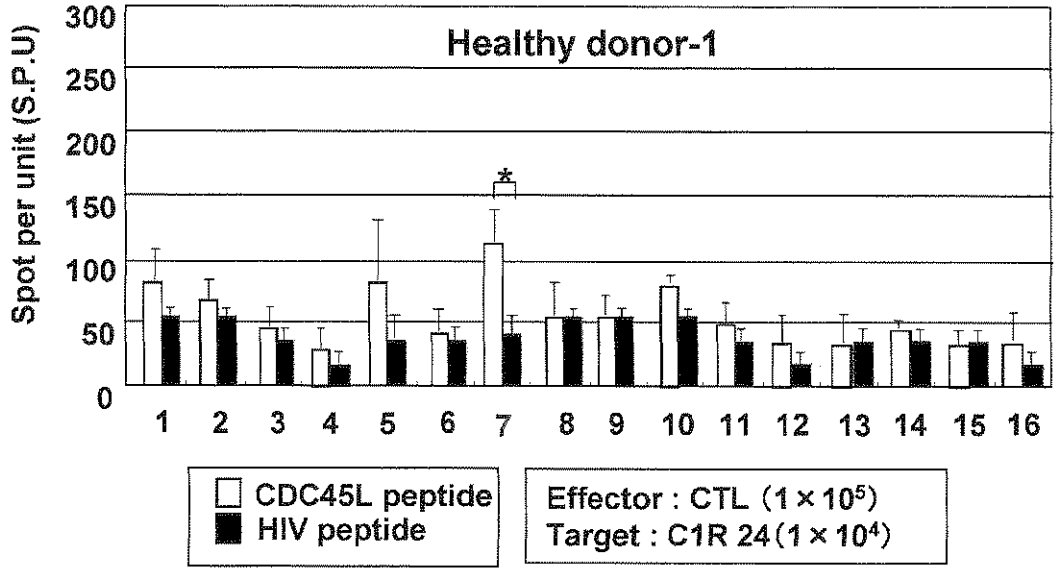

Adenocarcinoma
Squamous cell carcinoma
Small cell carcinoma
Normal lung
Testis
Normal skin
50 μm

[Fig. 2]

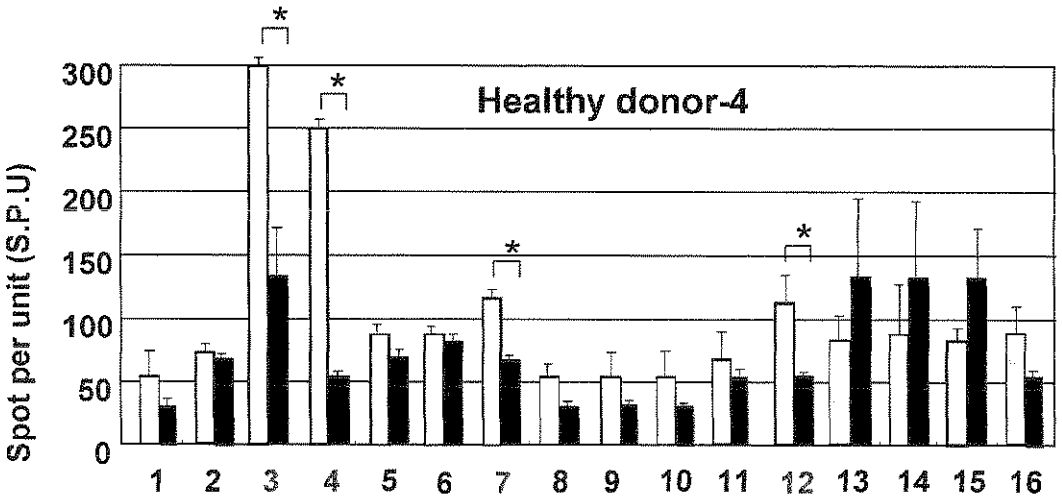

A protocol for induction of CDC45L specific CTL from PBMCs of HLA-A*2402 positive healthy donors and lung cancer patients.
Day -7
Day 0
Day 7
Day 14
Day 20
Sorting of CD14+ or CD8+ cells from human PBMCs by MACS beads
In vitro stimulation of CD8+ cells with DCs pulsed with each peptide
Stimulation of CD8+ T cells with PHA blast pre-pulsed with CDC45L peptides
IFN-γ ELISPOT assay
CD107a mobilization assay
51Cr release assay

[Fig. 3]

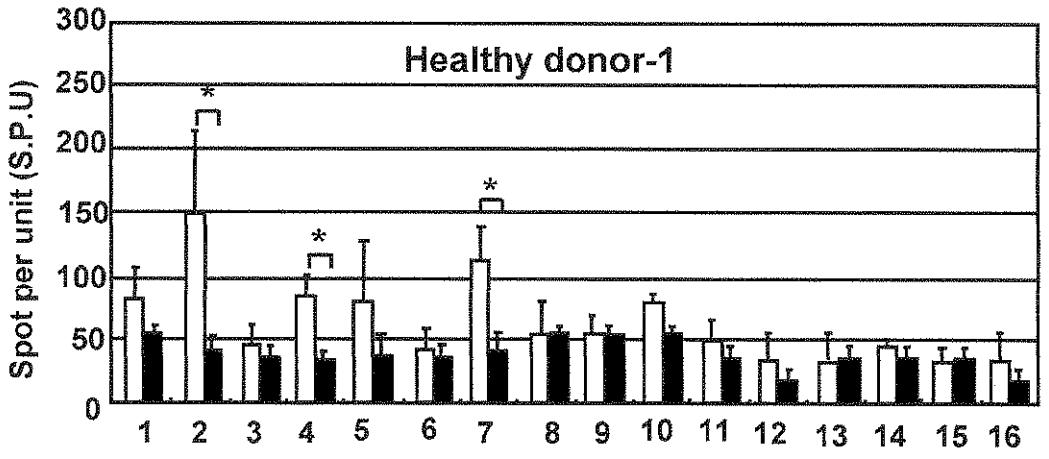
A


Healthy donor-1
Spot per unit (S.P.U)
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
*
CDC45L peptide
HIV peptide
Effector : CTL (1×10^5)
Target : C1R 24(1×10^4)

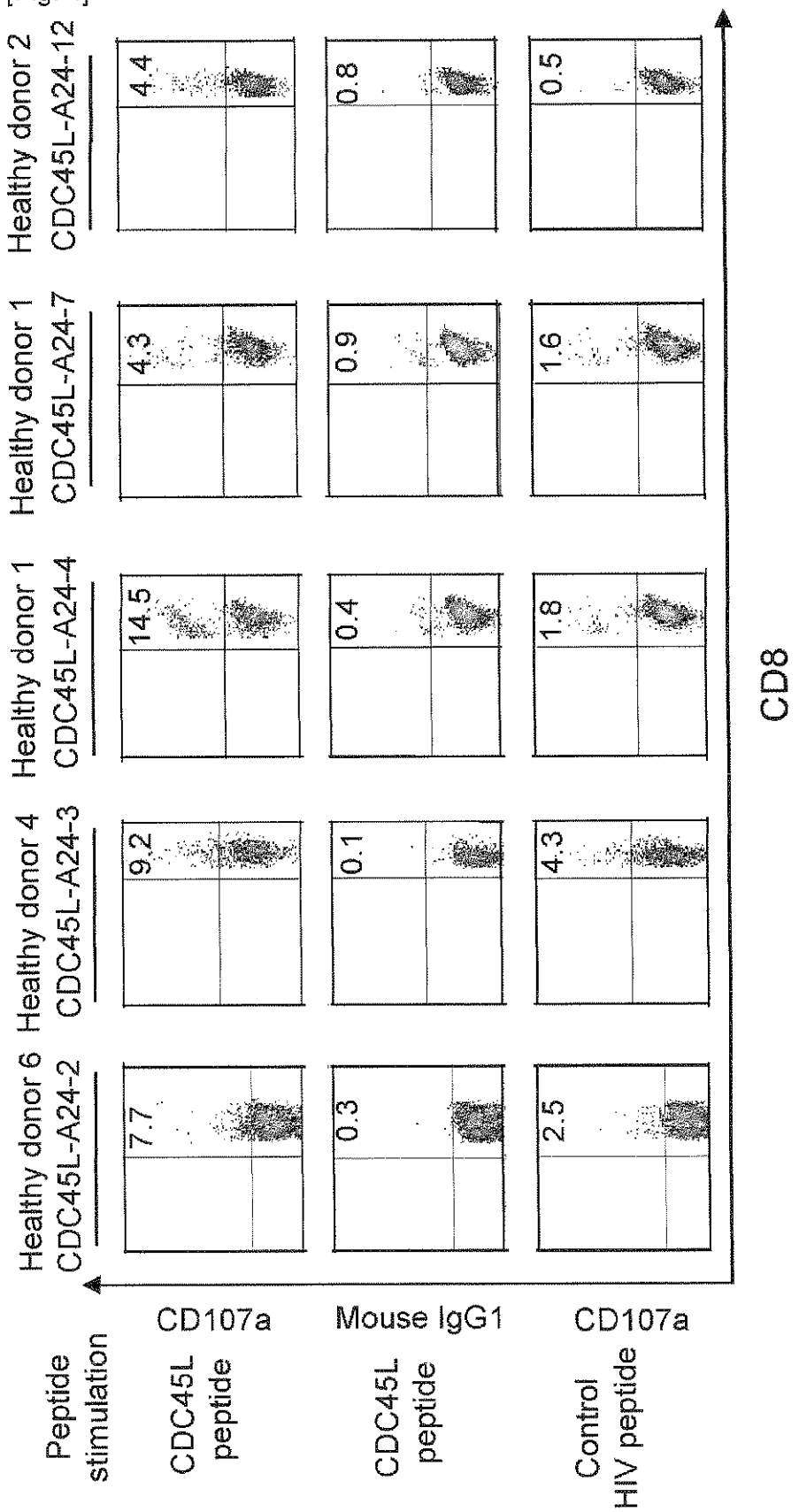
B


Healthy donor-4
Spot per unit (S.P.U)
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
*

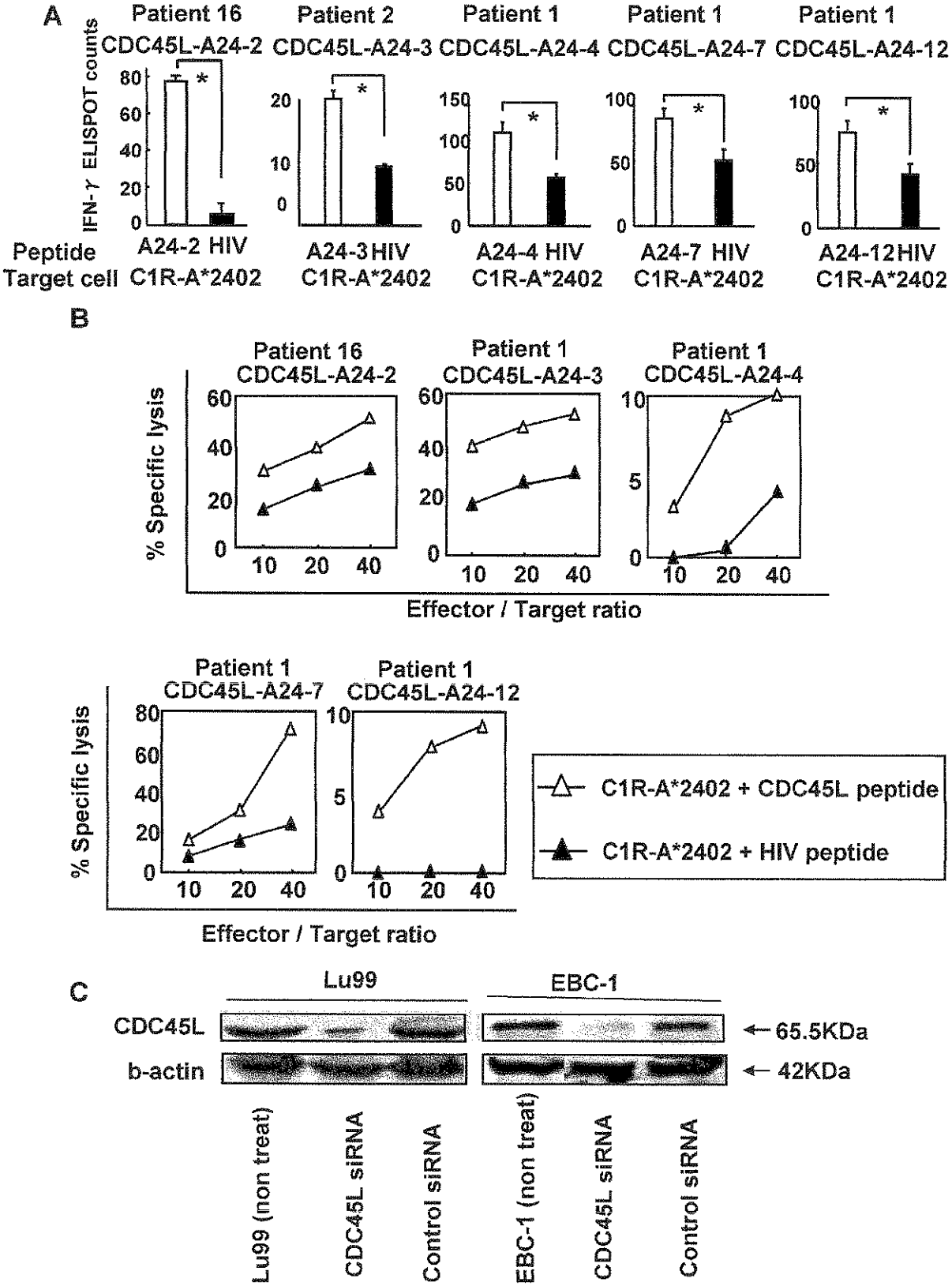
C


Healthy donor-1
Spot per unit (S.P.U)
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
*

[Fig. 4]

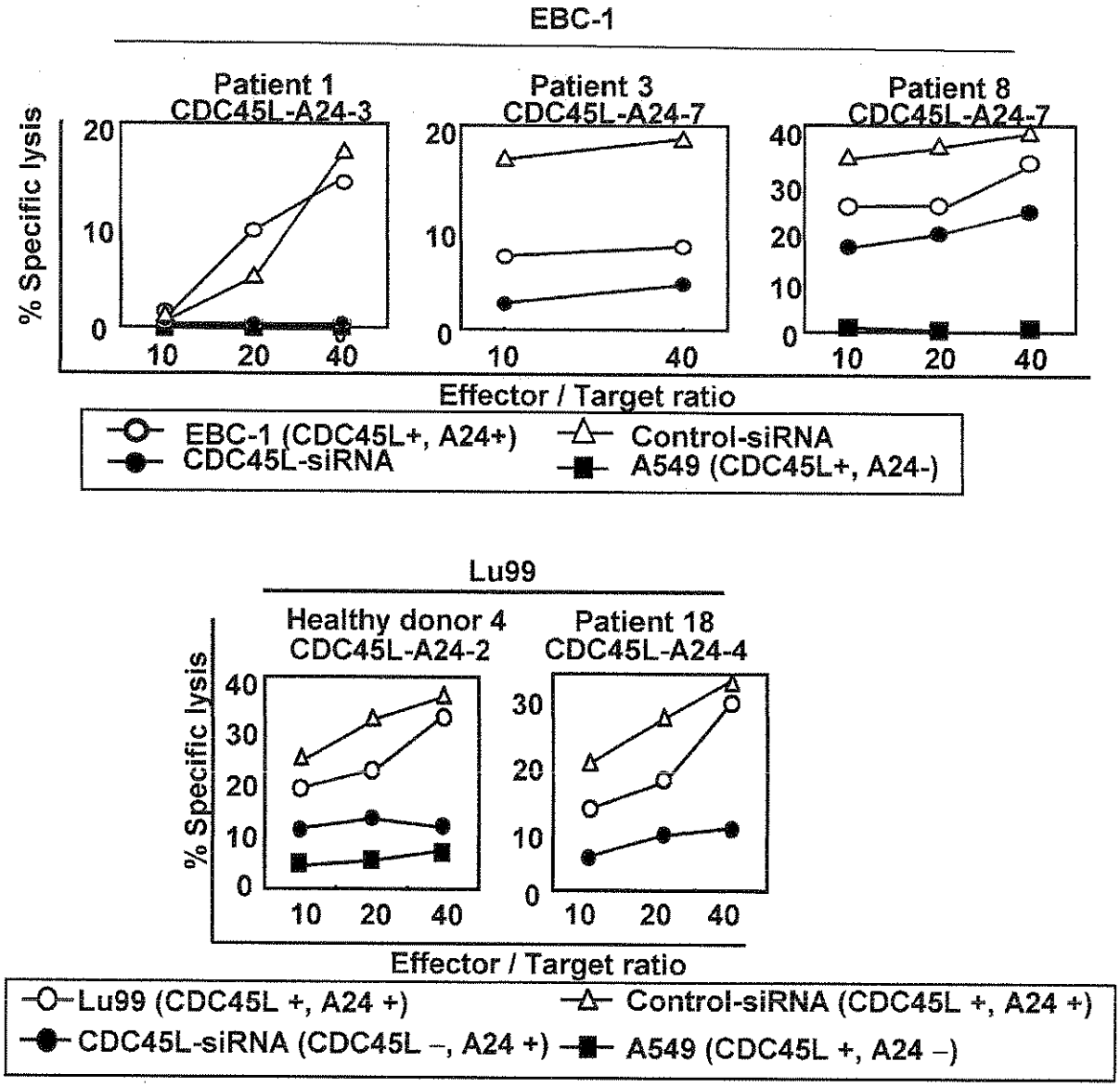

Healthy donor 6 CDC45L-A24-2
Healthy donor 4 CDC45L-A24-3
Healthy donor 1 CDC45L-A24-4
Healthy donor 1 CDC45L-A24-7
Healthy donor 2 CDC45L-A24-12
7.7
9.2
14.5
4.3
4.4
0.3
0.1
0.4
0.9
0.8
2.5
4.3
1.8
1.6
0.5
CD8
CD107a
Mouse IgG1
CD107a
Peptide stimulation
CDC45L peptide
CDC45L peptide
Control HIV peptide

[Fig. 5a-c]

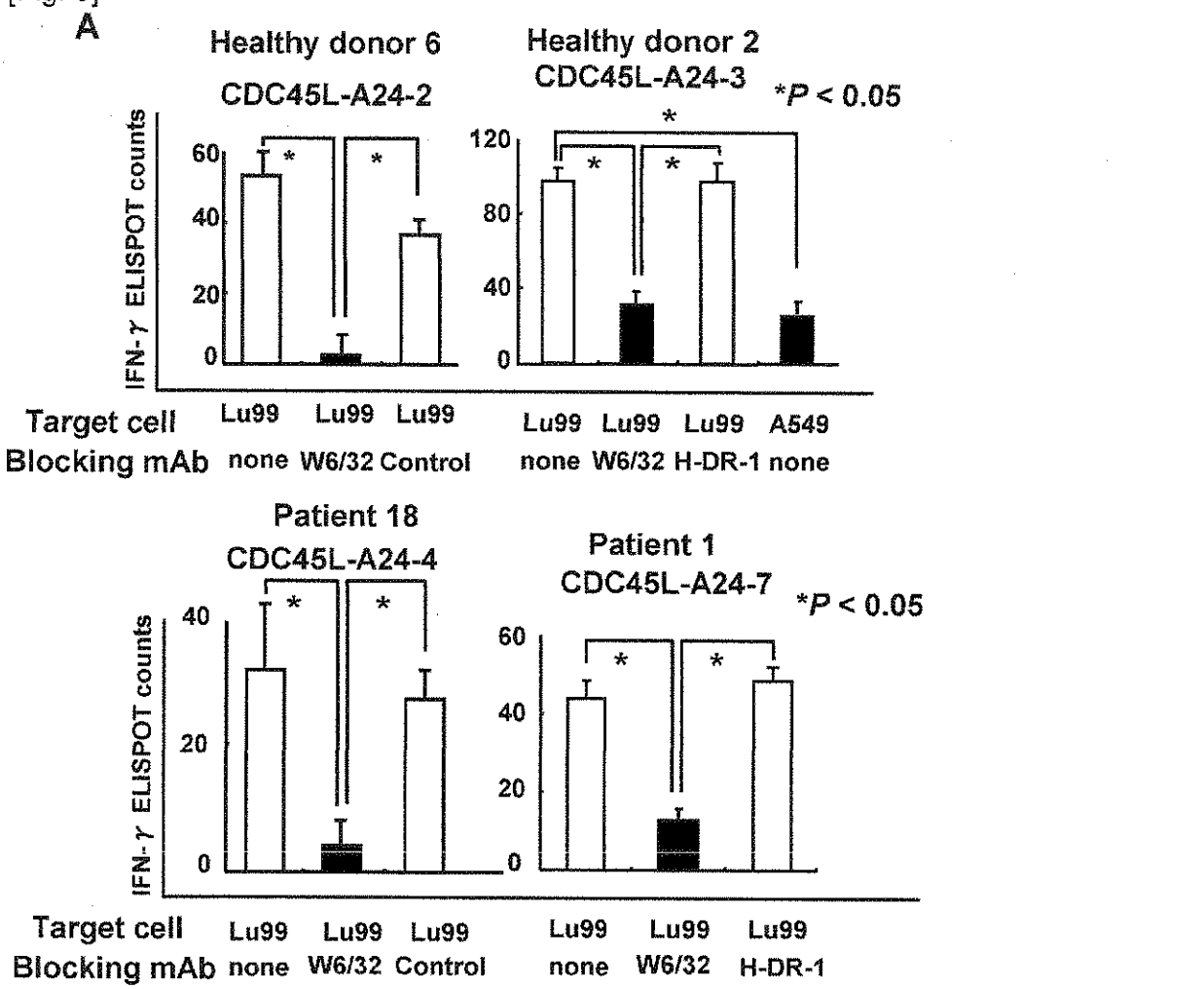

A
Patient 16
CDC45L-A24-2
Patient 2
CDC45L-A24-3
Patient 1
CDC45L-A24-4
Patient 1
CDC45L-A24-7
Patient 1
CDC45L-A24-12
IFN-γ ELISPOT counts
Peptide
A24-2 HIV
A24-3 HIV
A24-4 HIV
A24-7 HIV
A24-12 HIV
Target cell
C1R-A*2402
B
Patient 16
CDC45L-A24-2
Patient 1
CDC45L-A24-3
Patient 1
CDC45L-A24-4
% Specific lysis
Effector / Target ratio
Patient 1
CDC45L-A24-7
Patient 1
CDC45L-A24-12
C1R-A*2402 + CDC45L peptide
C1R-A*2402 + HIV peptide
C
Lu99
EBC-1
CDC45L
b-actin
65.5KDa
42KDa
Lu99 (non treat)
CDC45L siRNA
Control siRNA
EBC-1 (non treat)
CDC45L siRNA
Control siRNA

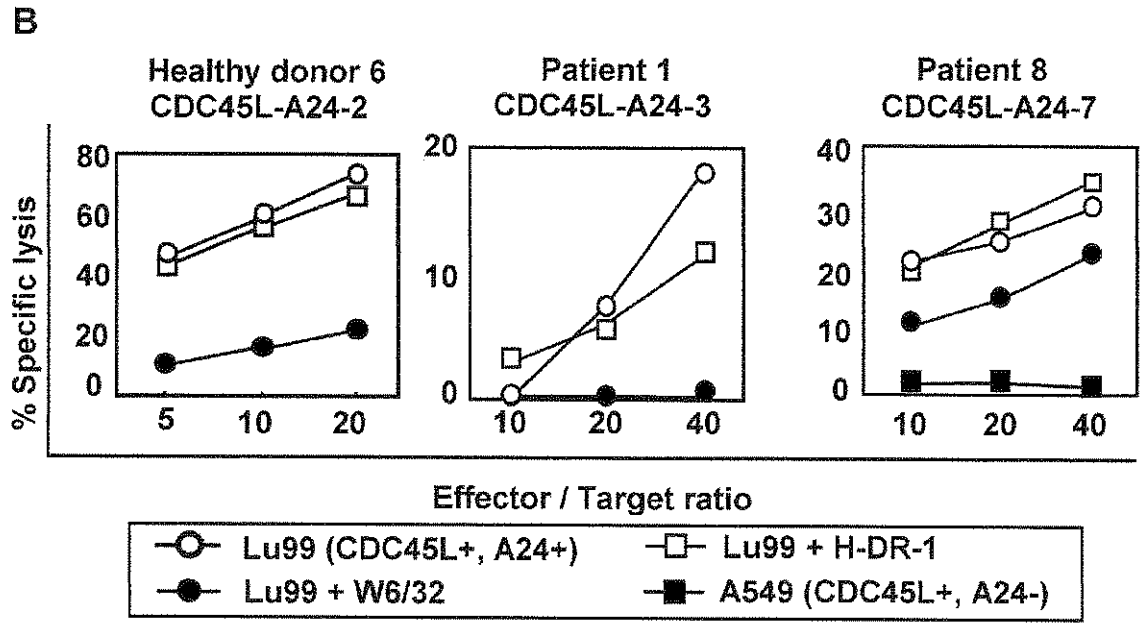
[Fig. 5d]

D

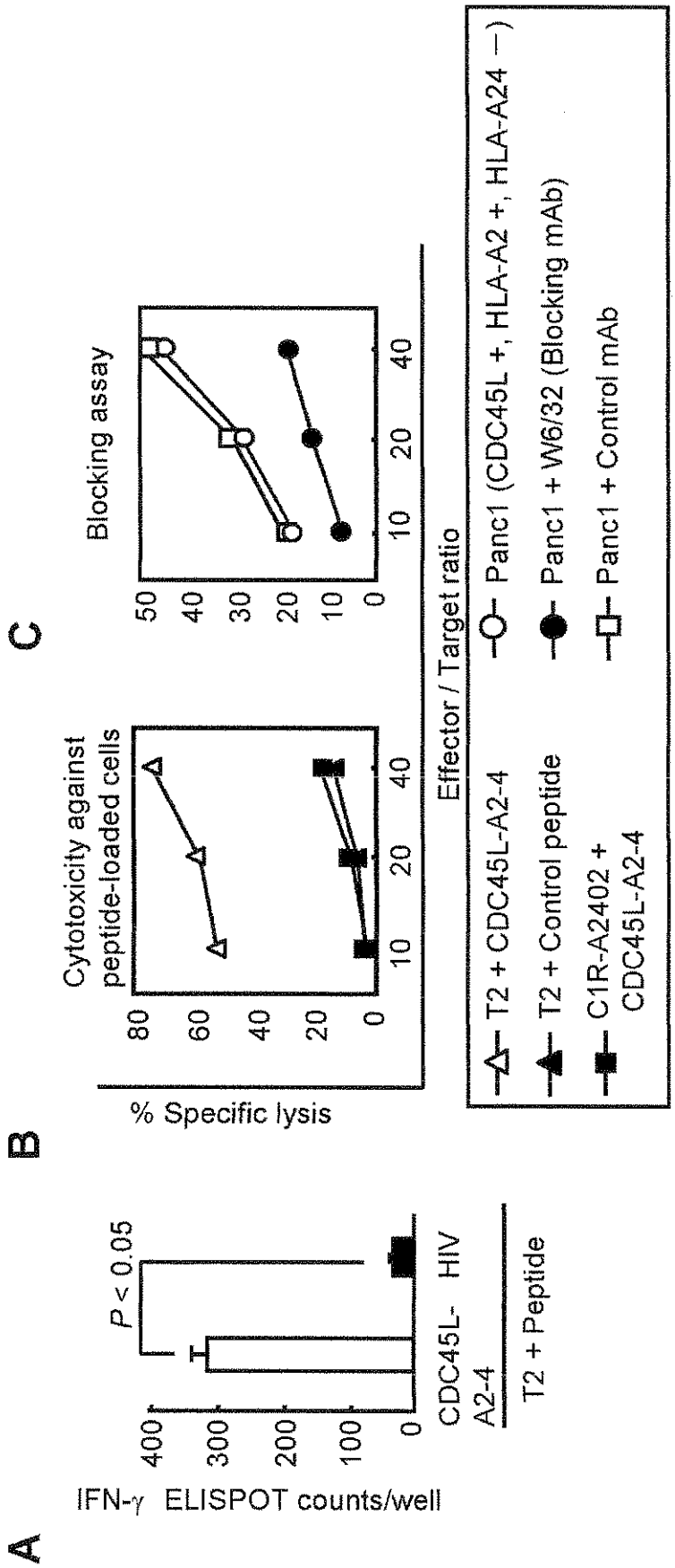

EBC-1
Patient 1
CDC45L-A24-3
Patient 3
CDC45L-A24-7
Patient 8
CDC45L-A24-7
% Specific lysis
Effector / Target ratio
EBC-1 (CDC45L+, A24+)
CDC45L-siRNA
Control-siRNA
A549 (CDC45L+, A24-)
Lu99
Healthy donor 4
CDC45L-A24-2
Patient 18
CDC45L-A24-4
% Specific lysis
Effector / Target ratio
Lu99 (CDC45L +, A24 +)
CDC45L-siRNA (CDC45L –, A24 +)
Control-siRNA (CDC45L +, A24 +)
A549 (CDC45L +, A24 –)

[Fig. 6]

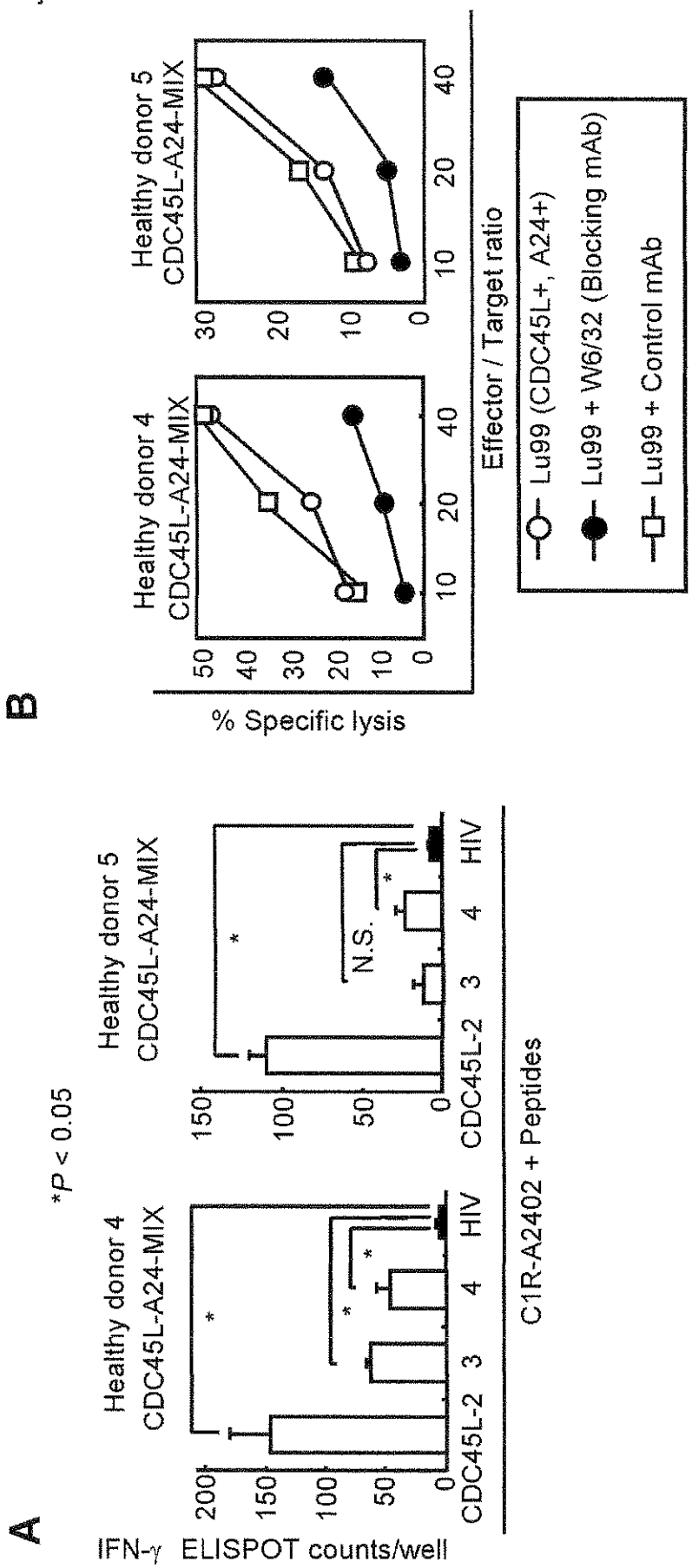

A
Healthy donor 6
CDC45L-A24-2
Healthy donor 2
CDC45L-A24-3
*P < 0.05
IFN-γ ELISPOT counts
Target cell
Blocking mAb
Lu99 Lu99 Lu99 Lu99 Lu99 Lu99 A549
none W6/32 Control none W6/32 H-DR-1 none
Patient 18
CDC45L-A24-4
Patient 1
CDC45L-A24-7
*P < 0.05
IFN-γ ELISPOT counts
Target cell
Blocking mAb
Lu99 Lu99 Lu99 Lu99 Lu99 Lu99
none W6/32 Control none W6/32 H-DR-1


B
Healthy donor 6
CDC45L-A24-2
Patient 1
CDC45L-A24-3
Patient 8
CDC45L-A24-7
% Specific lysis
Effector / Target ratio
Lu99 (CDC45L+, A24+)
Lu99 + H-DR-1
Lu99 + W6/32
A549 (CDC45L+, A24-)

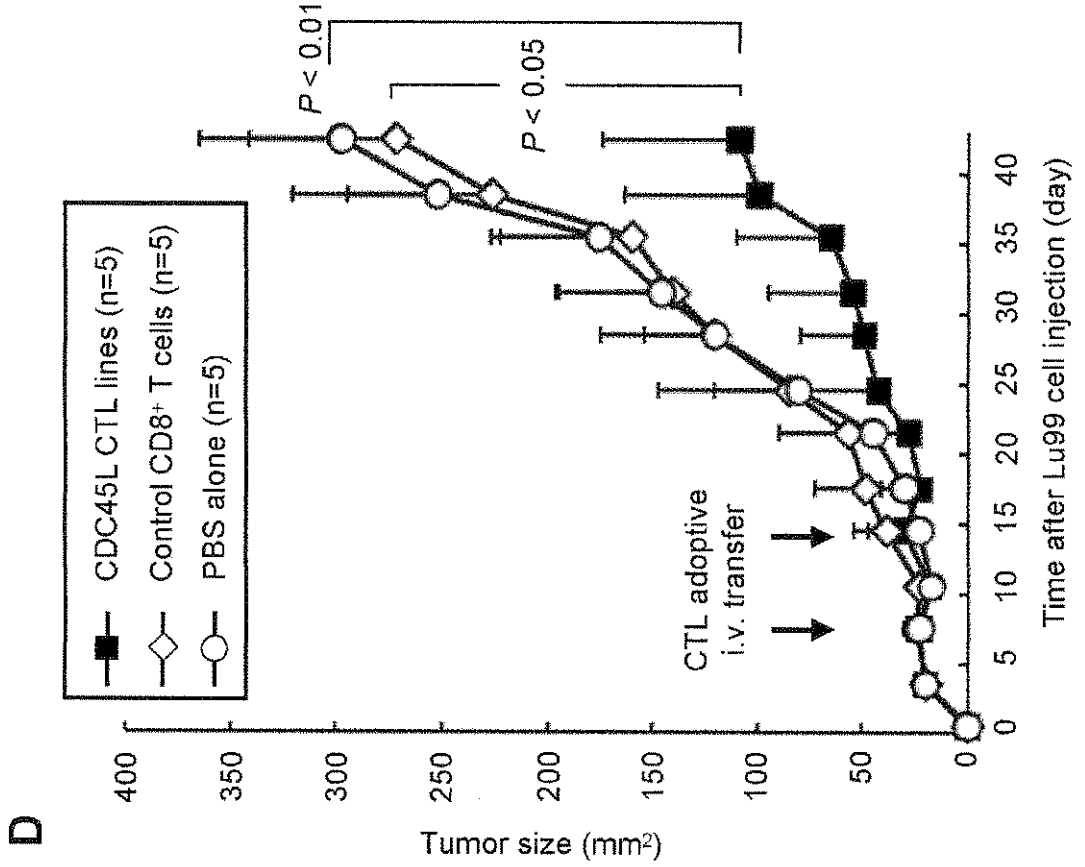
[Fig. 7]

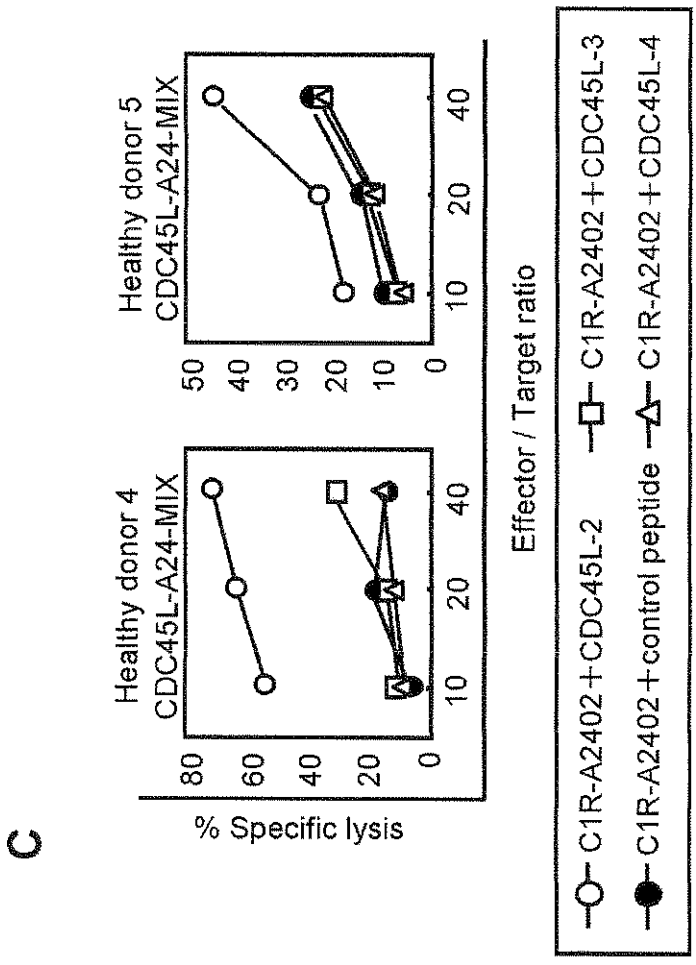

A
IFN-γ ELISPOT counts/well
P < 0.05
400
300
200
100
0
CDC45L-A2-4
HIV
T2 + Peptide
B
% Specific lysis
Cytotoxicity against peptide-loaded cells
80
60
40
20
0
10
20
40
C
Blocking assay
50
40
30
20
10
0
10
20
40
Effector / Target ratio
T2 + CDC45L-A2-4
T2 + Control peptide
C1R-A2402 + CDC45L-A2-4
Panc1 (CDC45L +, HLA-A2 +, HLA-A24 −)
Panc1 + W6/32 (Blocking mAb)
Panc1 + Control mAb

[Fig. 8a-b]


A
B
Healthy donor 4
CDC45L-A24-MIX
Healthy donor 5
CDC45L-A24-MIX
IFN-γ ELISPOT counts/well
*P < 0.05
CDC45L-2
HIV
C1R-A2402 + Peptides
N.S.
% Specific lysis
Effector / Target ratio
Lu99 (CDC45L+, A24+)
Lu99 + W6/32 (Blocking mAb)
Lu99 + Control mAb

[Fig. 8c-d]


D
CDC45L CTL lines (n=5)
Control CD8+ T cells (n=5)
PBS alone (n=5)
P < 0.01
P < 0.05
CTL adoptive i.v. transfer
Tumor size (mm2)
Time after Lu99 cell injection (day)


C
Healthy donor 4 CDC45L-A24-MIX
Healthy donor 5 CDC45L-A24-MIX
% Specific lysis
Effector / Target ratio
C1R-A2402+CDC45L-2
C1R-A2402+CDC45L-3
C1R-A2402+control peptide
C1R-A2402+CDC45L-4