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A C E F

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A B A C

CD132 -2.
CD132 (CD132), D A CD132 ,
CD132 ,
-2- . F CD132
CD132
2, 84E94 95 96 97 98 -
96
E A, . A
- C -
CD132.
CD132 -2.

1. Introduction

γ (γ , CD132),
, -2, -4, -7, -9, -15 -21 1
3 . CD132
4 . γ
- , 5 .
A γ
-2, -4, -7
. -2
(-2), βγ -2 100-
-2 β 6 , -2 αβγ
10- αβ 1 . A
3 5-
-4 7 -7 8 .
C
- . -2 A (6
30), A′ (33 40), B (53 C58), B′ (59 A73), C (82 97), D (114 130)
9 . , γ ,
β- .
( )
(C ,)
/ 10 . (32 125) C
(129 226) ~120°,
(126 127 128) 3_{10} γ
11 . A -2 -2
. -2/γ
γ
-2 A D 11,12 . A
γ , -4 α/γ / -4, 13 .
-2 E15, 19, 126,
130 -4 8, 12, 121, 125
A D ,
γ
) F 2 (206 221) .
-2 -4 , -
. ,
γ
γ - ,
13 .
-D 14 -2 (-
2) , -2

Abbreviations: γ , ; CD132, γ ;
CD132, γ ; FA, ; C , ;
C,

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15 18 . , -2
(-2) , -
2 α -2 γ 19,20 .
γ (CD132)
-2.
CD132 -
A .

2. Materials and methods

2.1. Cells

(E) (B , D) 10%
(C). (C)
19 .

2.2. RNA isolation, RT-PCR and DNA sequencing

C 6-
1×10^7 /. 10 μ /. C A 1640
10% C 39 °C (
41 °C) 24 . C A-
C 5 2500 /.
4 °C. A
(,C , CA), D A
( )18 . CD132
C (
5′-A C CCCC C-3′
5′- CAC C CCCAC A C -3′) -2 γ D A
(B . AJ419898). C
30
95 °C 1 , 60 °C 45 D A
72 °C 1 . ,
D18- (,).
CD132
(:// . / / ED -
) CD132 (CD132)
(B . 000197) D A A 5.0
(D A A ., ,).

2.3. Construction of model of tertiary structure of chCD132

A CD132
C 2.0 (:// / /
C /). C 2.0
/ ,
CD132
- (DB D
2E C),
(C) 11 .

2.4. Bacterial expression of recombinant chCD132 fusion protein

CD132
C CD132
: 5′-A AA C CA CCCC-
CA CCCCAAA -3′ E
5′-C C AC CAC A CCA AACC-3′ .
C E
E 28
(, ,).
E 28 - CD132. CD132
(CD132)
E 28 - CD132 *Escherichia coli*
B 21 (DE3) . - CD132
(., , CA). CD132
D - A E
- A (A , A).

2.5. Production and purification of mAb to chCD132

F F BA B/
50 μ CD132 F ' ( )
F ' ( ).
100 μ CD132.
2/0
.
CD132 . F
A CD132
E A
17 *E. coli* B 21 (DE3)

-8 (C - 8®, D , , J) . C -2 1640 , . : = (D – D)/(D – D) × 100%.

2.7. Kinetics analysis of chCD132 expression on Con A-stimulated SMCs

C 6- 1×10^7 / 1640 10% C 10 μ / C A 41 °C . - C . B C 5 2500 / . D . F . (2×10^6 /) 30 5% C 1640 4 °C. A 1 4000 / , 100 μ A CD132 60 μ / 4 °C 1 . C B 30 100 μ F C- - - (1:400, B A ., B , A). C B 500 μ (1% , 0.02% $_3$, 2% B). C C FC 500 (B C , ., F , CA) . C 1 .

2.8. Epitope mapping using a phage display peptide library

.D.-12 (EB, ,) - . B , 96- ( ) A CD132 100 μ / 0.1 / C $_3$ (8.6). A - 0.5% B A 0.5% B . F , 2×10^{11} F 1 . F , 500 μ / CD132.

E. coli E 2738. A D A . F , A CD132 E A . C / . CD132 .

2.9. Competitive ELISA to identify the native epitope in chCD132

A CD132 , $^{82\ 101}$ (E E) - (^{96}A) $^{82\ 101}$. (E E- A) - - (., A). 90% C . B A - - - CC (, A). $^{82\ 101}$. A C10 CD132 E A 21 . B , 0.17 μ / CD132 0.1 / C $_3$ (8.6) 4 °C . D A C10 6.7 / 5% 8 μ / - 1 37 °C. , CD132- 100 μ / , 37 °C 1 . A , - - , B. E . B A CD132 (8 μ /) , .

3. Results

3.1. Sequence and structure of the chCD132 ectodomain

D A (B . D 852357) CD132 20 . CD132 214 . - , CD132 C 2.0 . C C -4- -4 - (DB D 3B C) 13 . CD132 (F . 1), CD132 , (C). E β- (A, B, C, C′, E, F,). ($^{95\ 96\ 97}$) 3_{10} CD132.

3.2. Expression of rchCD132 protein and generation of mAbs

E 28 - CD132 *E. coli* B 21 (DE3) , CD132 . CD132 . CD132 28 D . BA B/ CD132 - CD132 ( ). A (1) CD132

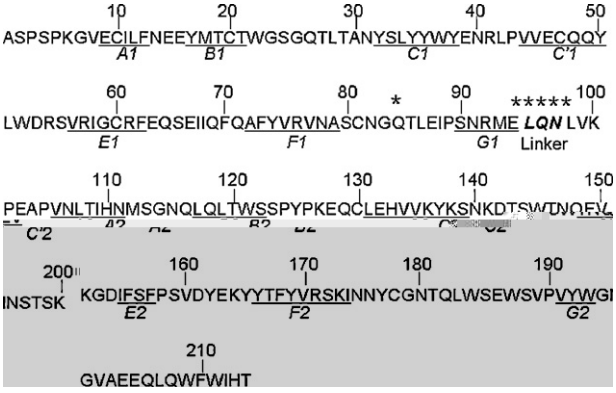

Fig. 1. CD132 . , β- . 20 ; *underlined* *A1* *G1* *A2* *G2* C . . A -2.

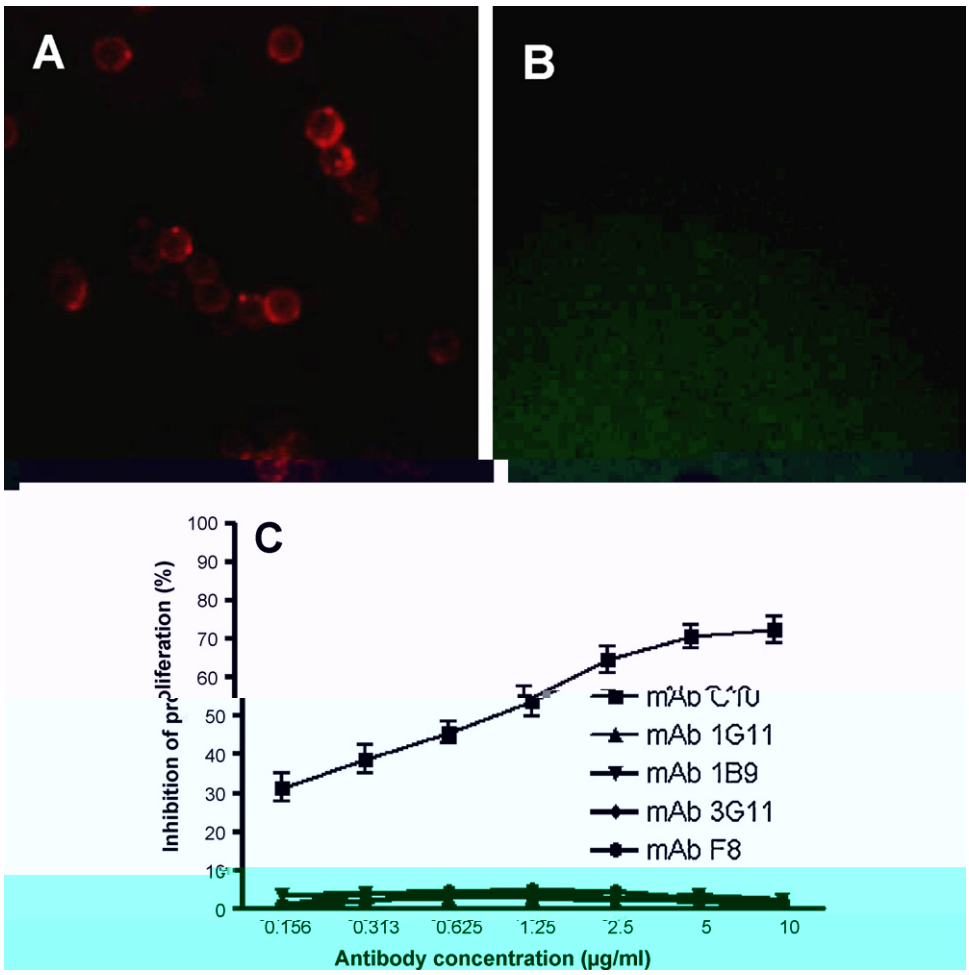

Fig. 2. B - CD132 A C CD132 C . (A) A - CD132 A C10 CD132 C A- C FA (200×). (B) A - CD132 A F8 FA. (C) -2- C . C 2. ± D.

E. coli. , , , A (1B9, 1 11, 3 11, C10 F8) CD132 ( ). A C10 CD132 C A- C (F . 2A), (A F8) CD132 C A- C (F . 2B). F , A -2- - . A F . 2C, -2 A C10 - . - CD132 A C10 CD132 .

3.3. Kinetics of chCD132 expression on Con A-activated SMCs

C C C A 0, 4, 8, 16, 24, 32, 40 48 , CD132 . A F . 3, CD132 C A (0), 3.04% (%) CD132 . C A , C CD132 3.12% 48 . , CD132 C - C A . 18.25% 40 18.13% 48 , 40 48 . - CD132 - C C A .

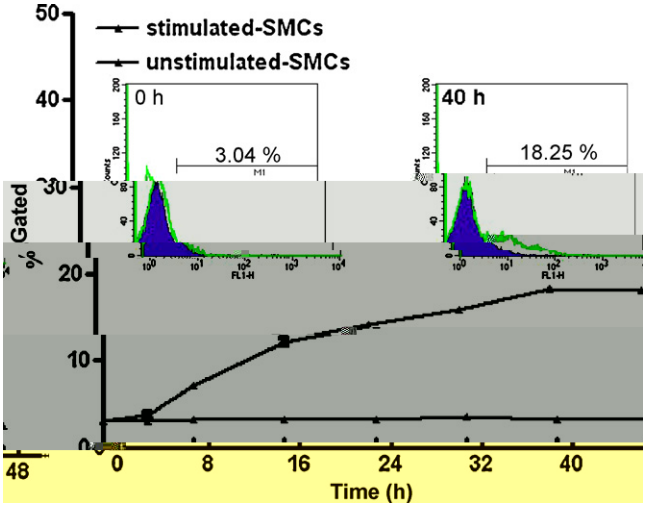

Fig. 3. CD132 C A- C . C A- C - CD132 A C10 F C- - . C 1 .

Table 1
A C10.

	F	D450	
		A	B A
C10-1	QD QNL	1/8	0.873	0.054
C10-2	D DHQNL A	1/8	0.834	0.052
C10-3	EHQNL A	1/8	0.835	0.059
C10-4	QE QN W A	1/8	0.882	0.058
C10-5	E WQEHQNF	1/8	0.901	0.059
C10-6	WD QE QN	1/8	0.743	0.066
C10-7	AQEHQNL W	1/8	0.505	0.087
C10-8	D Q WQNF	1/8	0.868	0.068
C	QEHQNL			

A - C W
A.
B A B A E A.

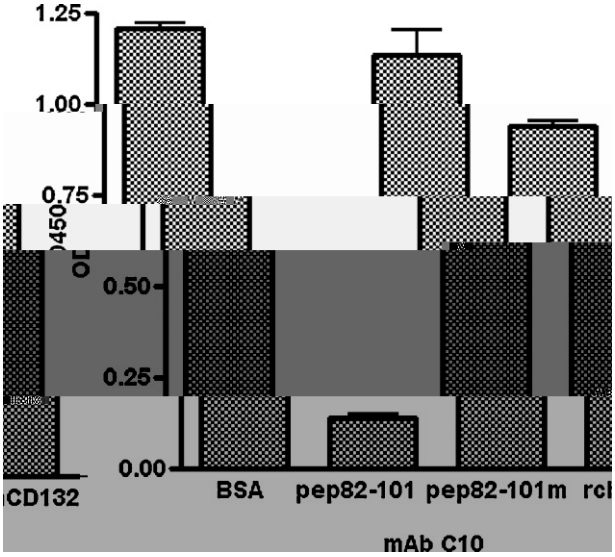

Fig. 4. B A CD132 .
A E A 2.9.
82 101 82 101. 8 μ /
A C10 CD132. B A CD132
8 μ /

3.4. Mimotope motif of chCD132 selected by a phage display peptide library

CD132 -

A C10.
1.
E C W. A CD132,
CD132 84E94 95 96 97 98
82 101 (E E).
C E A
A
- (F . 4). A C10
CD132 82 101
82 101
CD132 84E94 95 96 97 98
CD132, 96 127 CD132.
127 CD132
96
-2 12 .
CD132 -2, 82 101.
96A F . 4
82 101
96 CD132
-2.
84E94 95 96 97 98
CD132,
E115 125 126 127 128 129 CD132, 96 CD132
-2.

3.5. Location of the functional domain in the tertiary structure of chCD132

CD132
CD132, 84E94 95 96 97 98 -
C
84 F 1 (80 89)
95 96 97 310
- C
(F . 1 5A). E115 125 126 127 128 129
CD132 C
(F . 5B).

4. Discussion

CD132 *E. coli*, A
CD132. , A , A C10
CD132
C (F . 2A), - CD132. A
CD132
C , A C10 C
-2 (F . 2C).

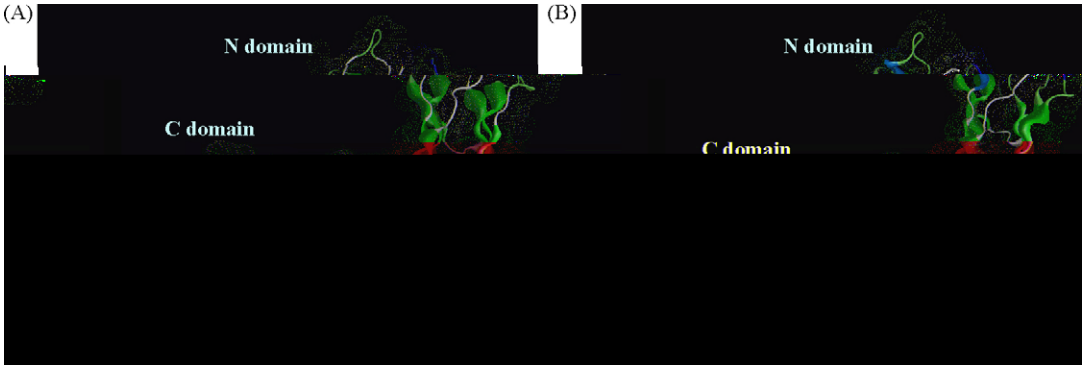

Fig. 5. - CD132 . (A) C CD132 ; (B) C CD132
. B . 84 E94 95 97 98 CD132 CD132
96 . C 2.0

Acknowledgements

F C (. 30625030 30771589), A -

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