Generating Hypoimmunogenic Human Embryonic Stem Cells by the Disruption of Beta 2-Microglobulin

[15]. HLA a a
, a a a
[7]. C a a HLA a I -
a a a a a
HLA a I (HLA-A,
HLA-B a HLA-C) a a a β2 . I a
β2 , a I HLA a a a a
a [16,
17]. I , a ESC
β2 a
HLA a ESC .

Materials and Methods

C C

I a a CF1 Ma - a
a a a 30,000 / 2 2 a
ESC a a . T
D , Ea , (DMEM)
(I) 10 % a
(FBS; H). T a a
ESC ESC a a . T ESC
a a ESC , a DMEM/F12
(I) 20 % -
a (I), 4 / L a a a -
(FGF; I), 2 /L L a (I),
1 % a a a (I) a 0.1 /L β-
a a (S a-A). T ESC a a
a a a . C a a IV a
a a . T ESC
a ESC
a a a a a a
a . T a a
ESC 20 % a
FBS (G), 70 % ESC a 10 % -
(DMSO). 293 T a a DMEM
(I) 10 % FBS (H).

TALEN E D

T TALEN Fa TALE TALEN A -
K (S a a). T TALEN
a 293 T . T DNA
293 T a a 3 a a a . N , PCR
a a
TALEN .

D a HLA C a I-D ESC L

H1 X1 a a HLA a I-
ESC . F , TALEN a
ESC F GENE HD a a
(P a) - . T a
a T LE (I)
5 a - a a CF1- a a a
a 500 / 2. T a a a ,
a
CF1- a , a a a , a PCR
(F140, L T) a a .

R T a -P a C a R a a R a -T P a C a R a

T a RNA a a a RN a (Q a),
a a a a a - a
a a (RT-PCR). RT-PCR a a
E Ma a a - PCR
SYBR G - a PCR Ma (TOYOBO).
S a a a a
a a (G3PD). T a
S a Ta 1.

W B

C a , a a
: a - a 2- (1:1000, Sa a C B -
), a -HLA-A/B/C (LY5.1, 1:1000, Sa a C
B) a a -GAPDH (1:5000, A a).

FACS

ESC a T LE, a 5 10^5
a 30 a 37 C 100 μL 0.5 % FBS PBS
a a a a (PE)
PE-CY7- a a . P a a
a a 2- (BD B) a HLA -A,
-B a -C (BD B). T a a a
a FACSCa , a
a a a F J a (T S a ,
A a , OR).

I a a C T a a a

T a a a -O 4 (1:100, Sa a
C B), a -S 2 (1:1000, M), a -
Na (1:150, Sa a C B), a -T a-1-81
(1:150, C), a -T a-1-60 (1:150, C), a -
CDH1 (1:100, BD), a -SSEA1 (A , 1:500, D -
a S H a Ba), a -SSEA3 (A ,
1:400, D a S H a Ba) a a -
SSEA4 (A , 1:400, D a S H a
Ba) ESC a a a -S 17 (1:100, R&D),
a -M a (1:100, D a S H a
Ba) a a -T 1 (1:100, Sa a C B)

(EB) a . 1 10^6 ESC a a 100-µL . T a a 6–8- Ba / . T a 48 - a a a , 4 % a a a a a a 20 % a 30 % a 4 C . T a a 20 µ a a, a a 10 a . T a a a a a a a CD3 (1: 400, A a) a KLRA1 (1:200, A a). F a a - a a a , a a a ESC . T CD3- KLRA1- a a a .

T a a F a

C a a - a / - (NOD/SCID) (a - a 5 10^6). A a a 2 , a - a . A a a a G Ca a U A a R a P a a Z a U A a Ca C .

E A a

F a , a - a a (PBMC) a a a a a . E a a 5 10^5 C- a ESC a a 5 10^5 a PBMC a a , (Ma) IFN- a a a a . T a a a a a E a a (CTL) a a a a .

S a a A a

A a a a a SEM. S a a a a a a a S , *t*- , a a $p<0.05$.

Results

D B a 2-M ESC TALEN T β2 a a a a a - a (TALEN) [18–20] a 2 (F . 1a). S a TALEN (F . S1A)

Fig. 1 D a 2- ESC TALEN . a Ta 2 a β2 a a a TALEN . b A a TALEN a . T (*dashes*) (*red*) a ( ) a a . c E a β2 - ESC TALEN

, a a 293 T (F . S1B). T TALEN a (L86&R102) a β2 H1 [1] a X1 [21] ESC (F . 1a). B (β2 $^{+/-}$) a (β2 $^{-/-}$) ESC a a (F . 1). I a a , 44 %–87 % a (F . 1).

I a HLA C a I E β2 N ESC

T a β2 a HLA a I - (β2 $^{+/-}$) a (β2 $^{-/-}$) ESC , RT-PCR, a FACS a a . β2 RNA , a a a (β2 $^{+/-}$) a (β2 $^{-/-}$) ESC a ESC (F . 2a), a β2 a a . I a a (IFN-γ), a a a a , a β2 a HLA-A, -B a -C [22]. M , β2 RNA - a a IFN-γ a , β2 $^{+/-}$ a β2 $^{-/-}$ ESC . H , β2 (F . 2) a β2 (F . 2) β2 $^{-/-}$ ESC , a IFN-γ a , β2 β2 $^{+/-}$ a ESC a a a a (F . 2). T a a , β2 $^{+/-}$ a β2 $^{-/-}$ a HLA-A, -B a -C RNA (F . 2 , a) (F . 2), IFN-γ a . H , a HLA a I a β2 $^{-/-}$ ESC (F . 2), a IFN-γ a . H ESC HLA a I a a ESC . I a - , HLA-A, -B a -C a a β2 $^{+/-}$ ESC a a a IFN-γ (F . 2). T , a HLA a I- ESC β2 .

T P HLA C a I-D ESC

N , a a a a a HLA a I- ESC . β2 $^{-/-}$ ESC , a O 4, Na , S 2, D a2, D a4, N a , D 3 a R 1, - a ESC (F . 3a). F , a

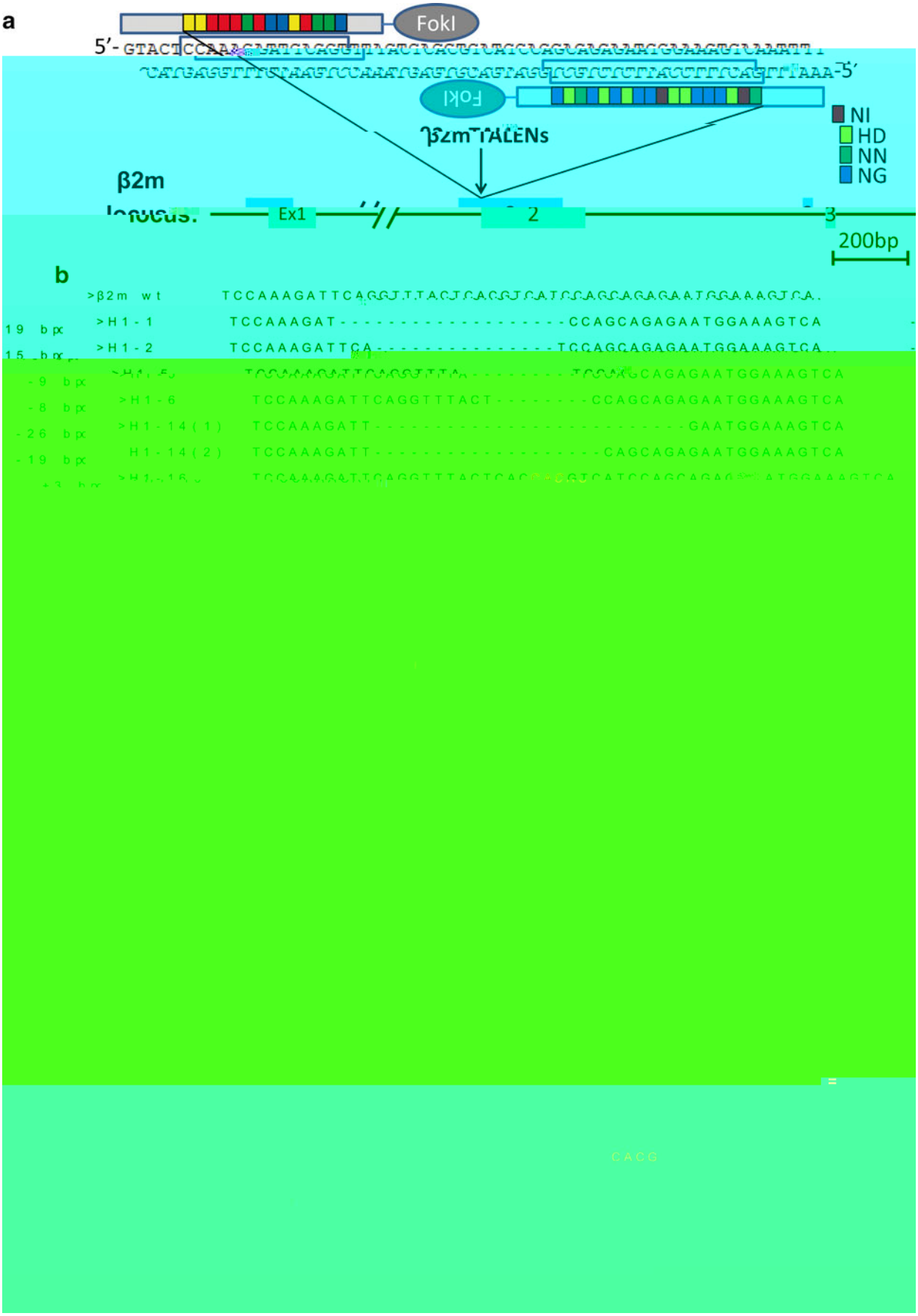
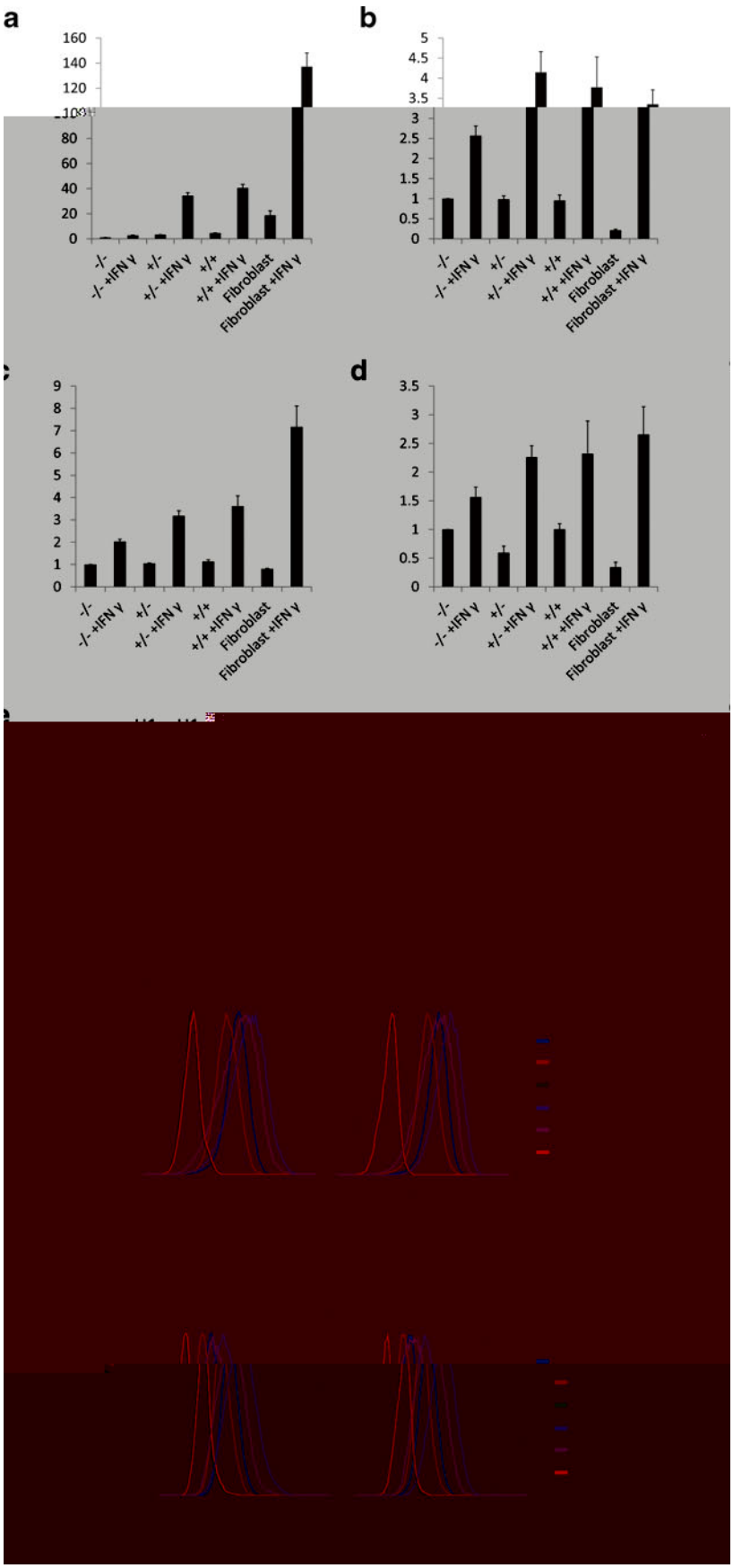

a
FokI
5′- GTACTCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTT
CATGAGGTTTCTAAGTCCAAATGAGTGCAGTAGGTCCGTCTCTTACCTTTCAGTTTAAA-5′
FokI
β2mTALENs
NI
HD
NN
NG
β2m
locus
Ex1
2
3
200bp
b
>β2m wt TCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCA
19 bp >H1-1 TCCAAAGAT----------CCAGCAGAGAATGGAAAGTCA
15 bp >H1-2 TCCAAAGATTCA----------TCCAGCAGAGAATGGAAAGTCA
-9 bp TCCAAAGATTCAGGTTTA---------TCCAGCAGAGAATGGAAAGTCA
-8 bp >H1-6 TCCAAAGATTCAGGTTTACT--------CCAGCAGAGAATGGAAAGTCA
-26 bp >H1-14(1) TCCAAAGATT----------GAATGGAAAGTCA
-19 bp H1-14(2) TCCAAAGATT----------CAGCAGAGAATGGAAAGTCA
+3 bp >H1-16 TCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCA
CACG

Fig. 2 I g a HLA a I β2 ESC . a–d R a RNA β2 g (a) a HLA-A (b), -B (c) a -C (d). e W g a a β2 a HLA-A, -B a -C β2 - ESC a a IFN-γ (500 U/) a 24 . f–g FACS a a β2 (f) a HLA-A, -B a -C (g) a β2 - ESC a IFN-γ (500 U/) a 24



a β2 −/− ESC O 4, S 2, Na , T a-1-60, T a-1-81, CDH1, SSEA3 a SSEA4 SSEA1 (F . 3). W a a a a β2 −/− ESC a . T β2 −/− ESC a a a (F . 3 a). W β2 −/− ESC NOD/SCID , a a a 2 , a a a a a - (HE) a (F . 3). W a a a (F . 3) a (F . 3) ESC .

T I HLA C a I-D ESC T a β2 - ESC , a - (ELISPOT) a a a IFN-γ a a a (PBMC) -

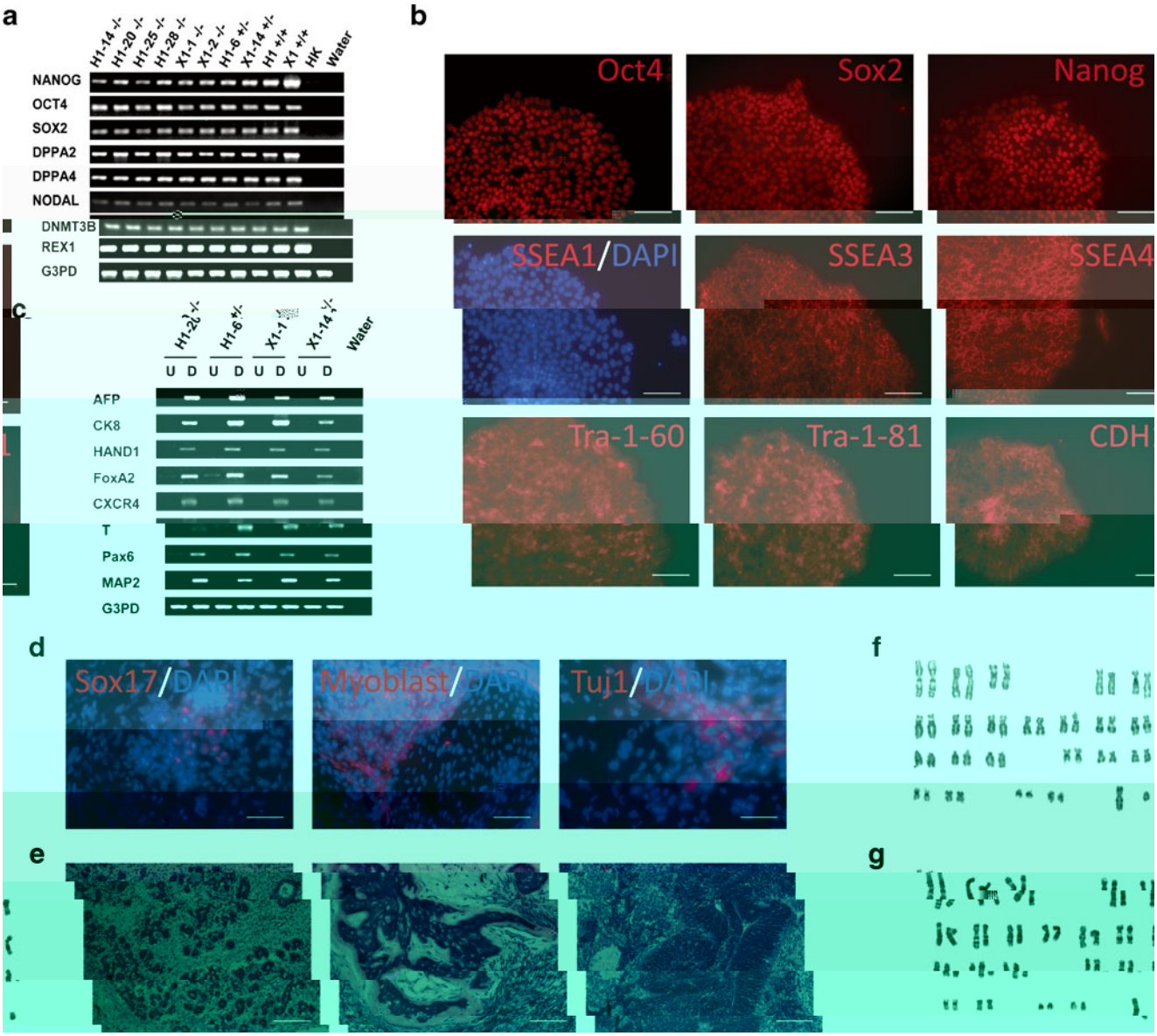


Fig. 3 T HLA a I- ESC . a RT-PCR a a a β2 - ESC . b I a a β2 −/− ESC . S a a , 100 µ . c RT-PCR a a a β2 - ESC- . U, a ; D, a . d I a a a e HE a a ((*left*), (*middle*) a (*right*)) a a β2 −/− ESC . f–g Ka a a β2 (f) a - (g) ESC

β2 - ESC . W ESC a a a a PBMC IFN-γ, β2 $^{-/-}$ ESC a a a IFN-γ (F . 4a). T a a (β2 $^{-/-}$) ESC a -a .

T a β2 - ESC , , β2 $^{+/-}$ a β2 $^{-/-}$ ESC a a a a Ba / . T a a a 2 a - a a a . I a , a a T (F . 4) a a a (NK) (F . 4) a (β2 $^{+/-}$) a (β2 $^{-/-}$) ESC a ESC . T β2 - ESC - , - a a a a Ba / .

Discussion

T a a a . F , HLA a I- ESC a a a a a a a . U a a a a “ - - ” ESC .

S , a a a HLA a I- ESC a PSC . T a - a TALEN [23, 24], a a a a a [11, 12]. T , HLA a I- ESC a a a PSC . I HLA a I- ESC , a a a ESC . H , a a a a PSC - , a a a a - [25, 26]. A a , a PSC a a PSC ESC a , a a a a a a a . I , a a ESC , a a a a a . T , HLA C a I- ESC- a a , - a a a , a a a a . I a , a a a , a a . W - a ESC- - a - a , a a .

F , HLA a I T (CTL) a HLA a I [27]. A HLA a I a a a NK , a CTL, HLA a I NK a NK- a [28]. T HLA a I- ESC a a a a CTL a a a NK . U T [29], a a NK - a - a a a a a [30, 31]. T , a a a . T , β2 - ESC . O a a [32]. I a a a

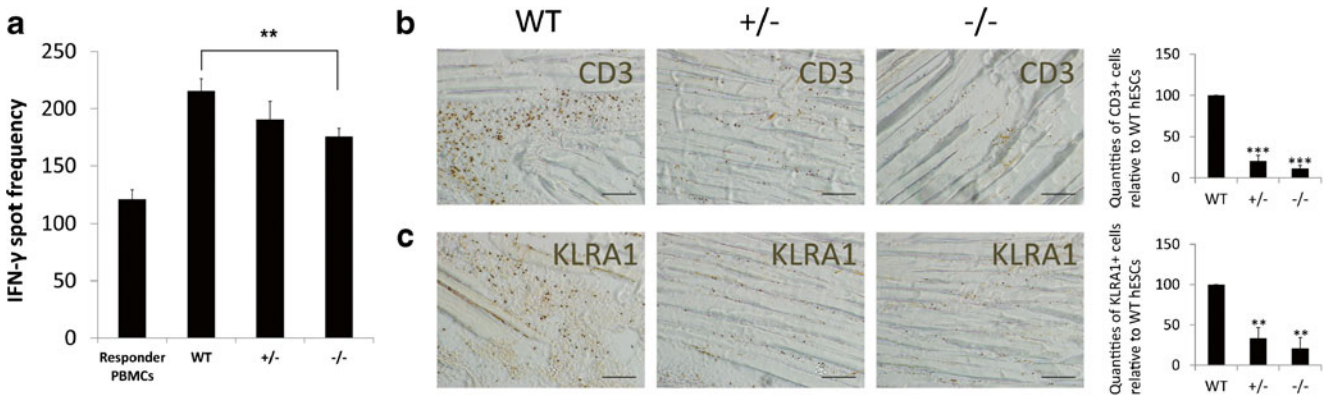


Fig. 4 T HLA a I- ESC . a IFN-γ a a a a (PBMC) β2 - a ESC . *N*>20. ** p<0.01. T a a a a SEM. b–c B β2 - a ESC a a Ba / . T a - a a a , a a -330( )18(a).4( )15..59. 215.6(a)-10 . -33 .

a a , a
β2 - ESC a a a a NK
. M , HLA a I- ESC a
a a a a a
.
F a , ESC a
a , a
a ESC a a a .

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Na a Na a S F a C a (31271577), M
S a T C a (2012AA020503, 2012CB966601,
a 2011AA020108), F a a R a F C a
U .

Submission Statement T a a a a
.

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