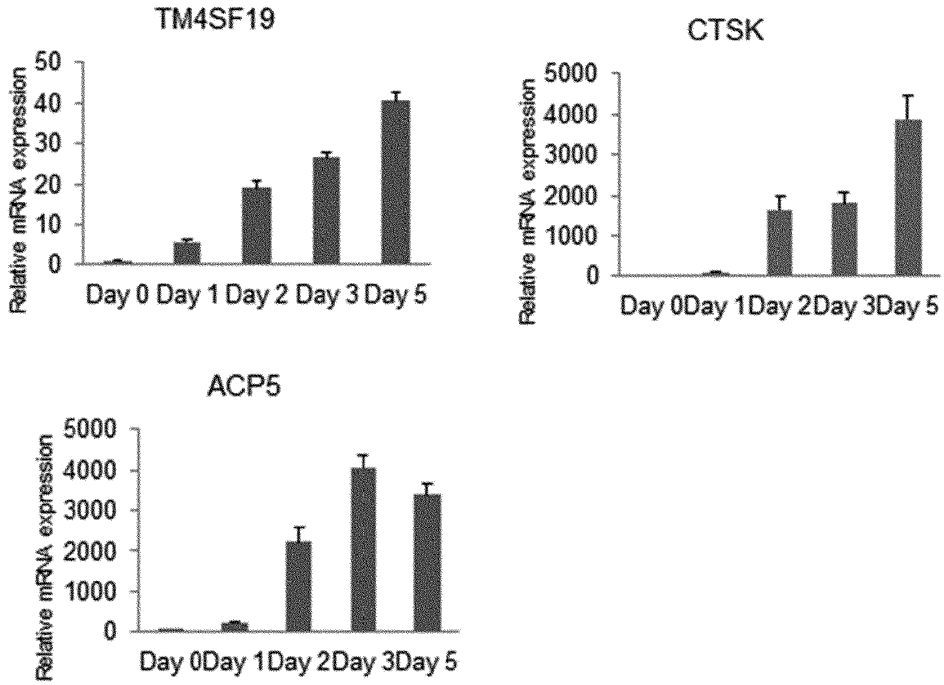
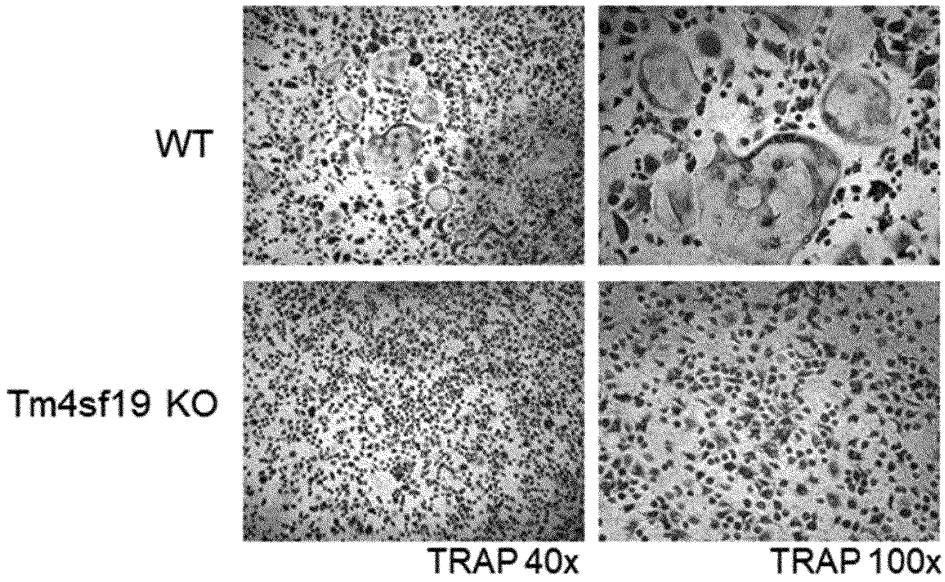
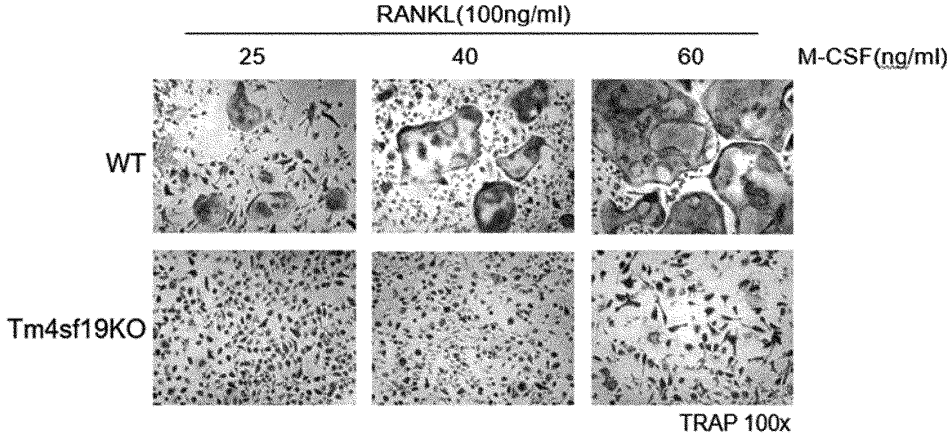
[도1]


TM4SF19
Relative mRNA expression
50
40
30
20
10
0
Day 0 Day 1 Day 2 Day 3 Day 5
CTSK
Relative mRNA expression
5000
4000
3000
2000
1000
0
Day 0Day 1Day 2Day 3Day 5
ACP5
Relative mRNA expression
5000
4000
3000
2000
1000
0
Day 0Day 1Day 2Day 3Day 5

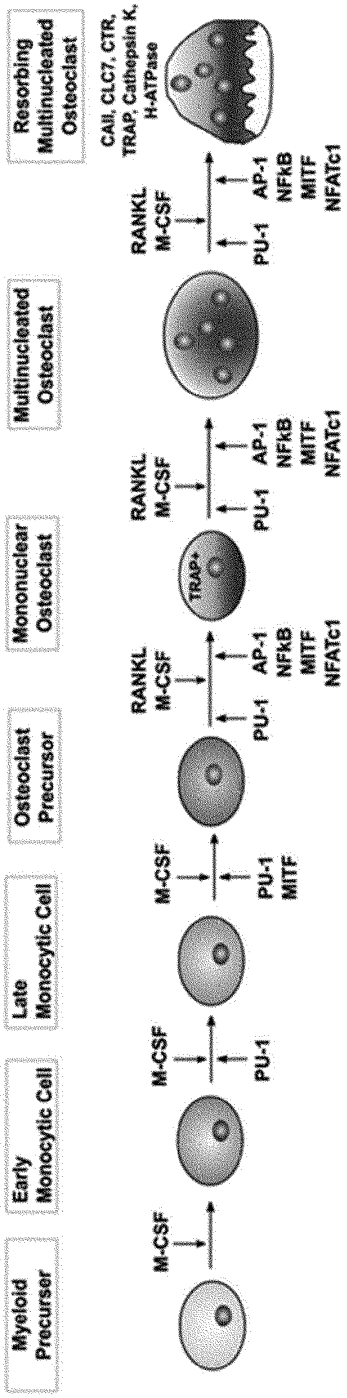
[도2a]


WT
Tm4sf19 KO
TRAP 40x
TRAP 100x

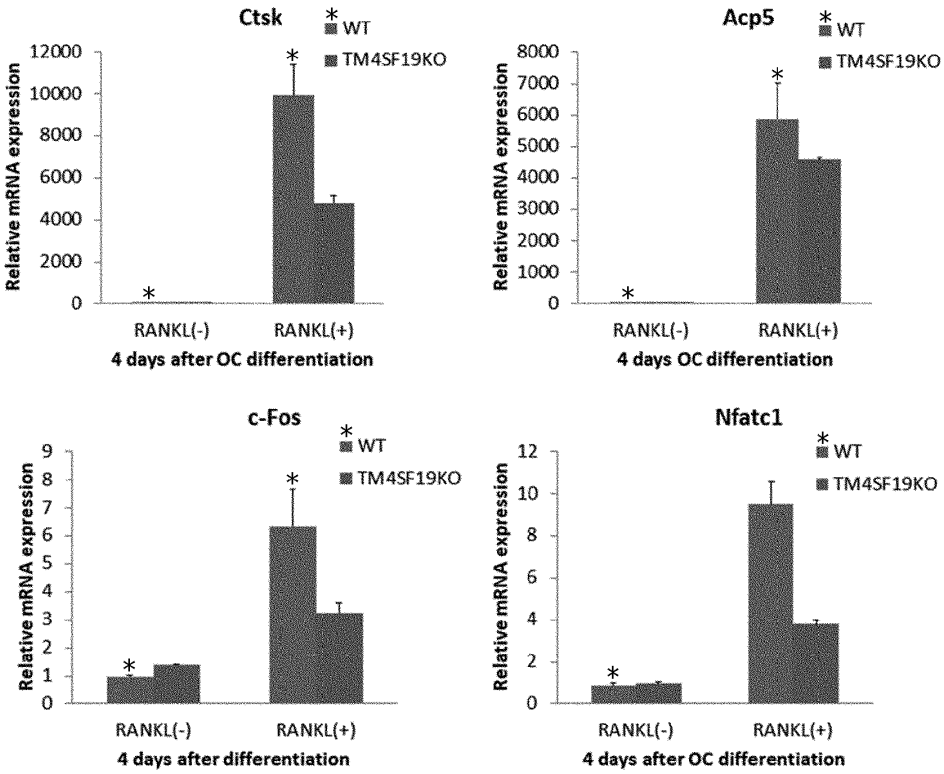
[도2b]



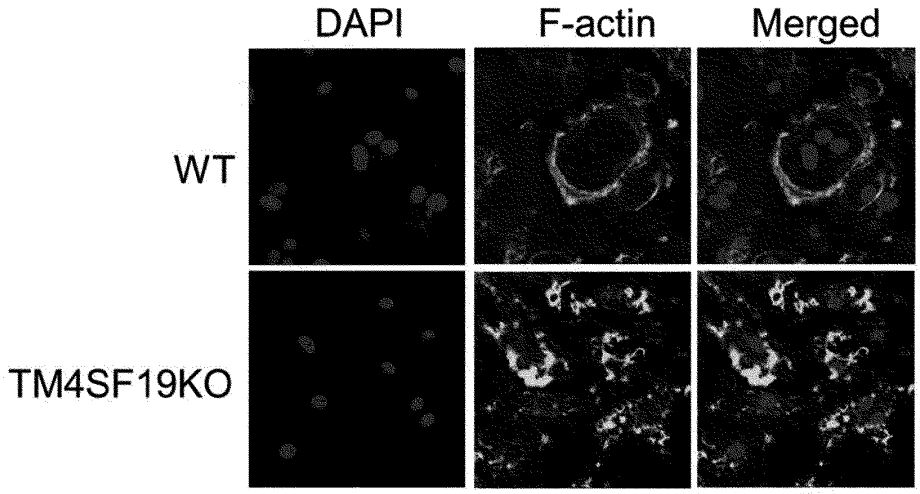
[도2c]


Myeloid Precurser
Early Monocytic Cell
Late Monocytic Cell
Osteoclast Precursor
Mononuclear Osteoclast
Multinucleated Osteoclast
Resorbing Multinucleated Osteoclast
M-CSF
PU-1
M-CSF
PU-1
M-CSF
PU-1
MITF
RANKL
M-CSF
PU-1
AP-1
NFkB
MITF
NFATc1
TRAP+
RANKL
M-CSF
PU-1
AP-1
NFkB
MITF
NFATc1
RANKL
M-CSF
PU-1
AP-1
NFkB
MITF
NFATc1
CAII, CLC7, CTR, TRAP, Cathepsin K, H-ATPase

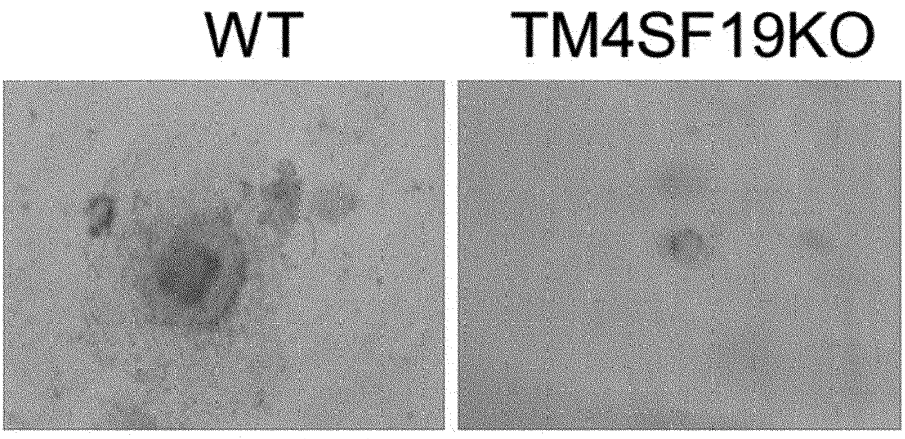
[도3]


Ctsk
WT
TM4SF19KO
Relative mRNA expression
12000
10000
8000
6000
4000
2000
0
RANKL(-)
RANKL(+)
4 days after OC differentiation
Acp5
WT
TM4SF19KO
Relative mRNA expression
8000
7000
6000
5000
4000
3000
2000
1000
0
RANKL(-)
RANKL(+)
4 days OC differentiation
c-Fos
WT
TM4SF19KO
Relative mRNA expression
9
8
7
6
5
4
3
2
1
0
RANKL(-)
RANKL(+)
4 days after differentiation
Nfatc1
WT
TM4SF19KO
Relative mRNA expression
12
10
8
6
4
2
0
RANKL(-)
RANKL(+)
4 days after OC differentiation

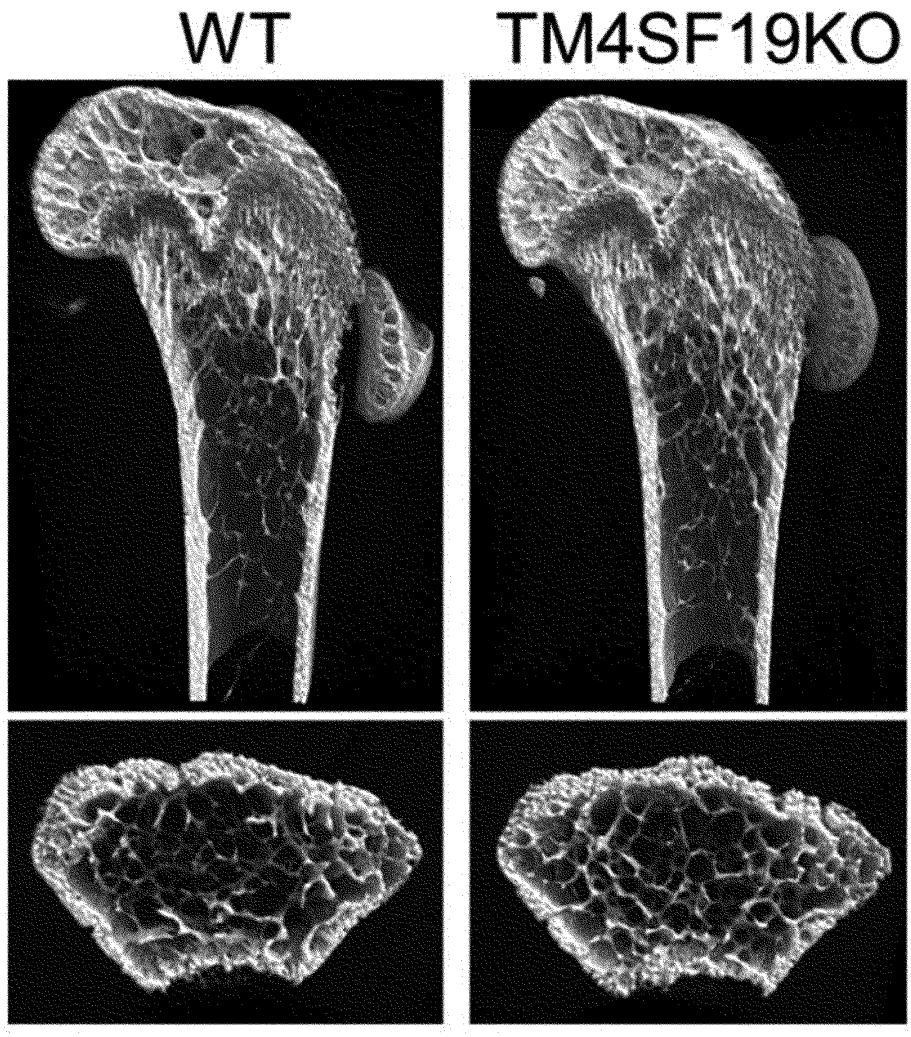
[도4a]


DAPI
F-actin
Merged
WT
TM4SF19KO

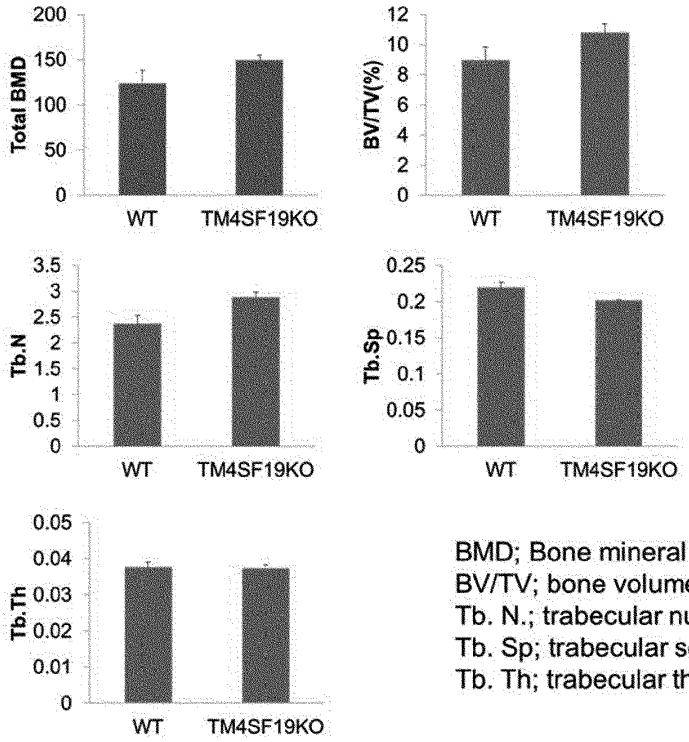
[도4b]



[도5a]

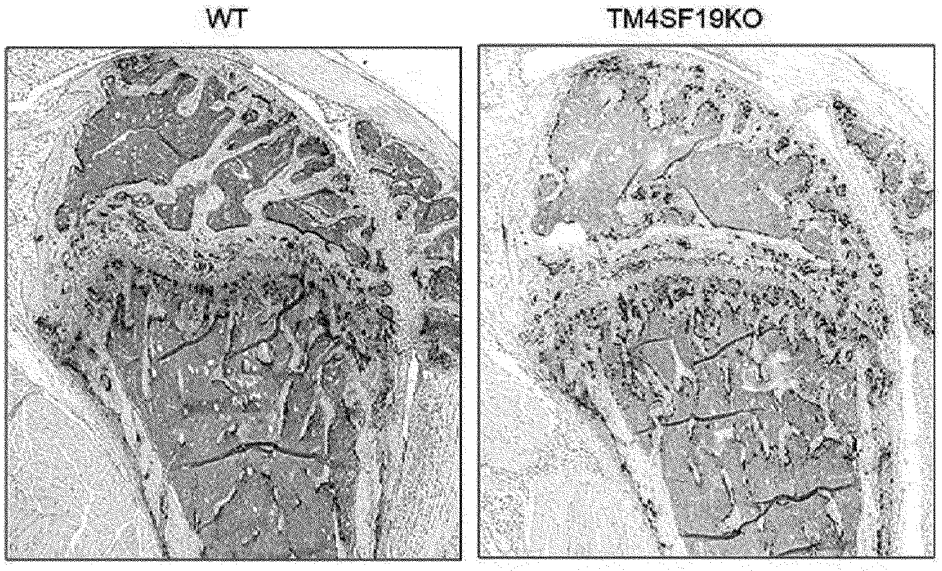


[도5b]



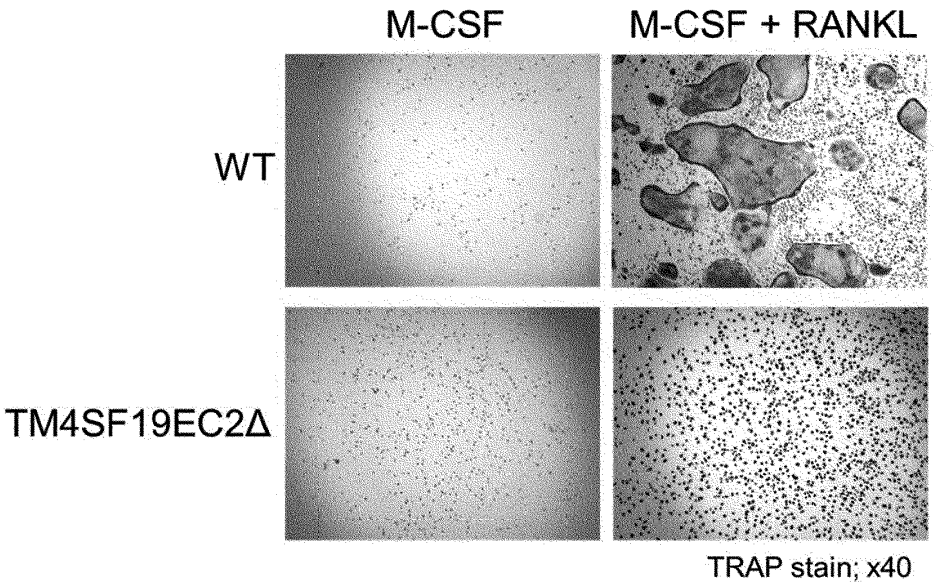
BMD; Bone mineral density
BV/TV; bone volume per tissue volume
Tb. N.; trabecular number
Tb. Sp; trabecular separation
Tb. Th; trabecular thickness

[도6]

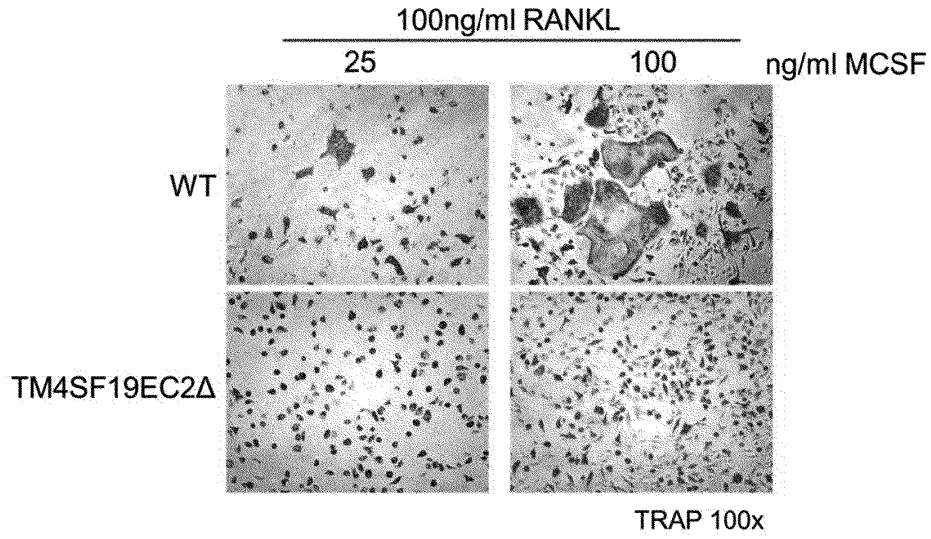


TRAP staining, 8 weeks female femur

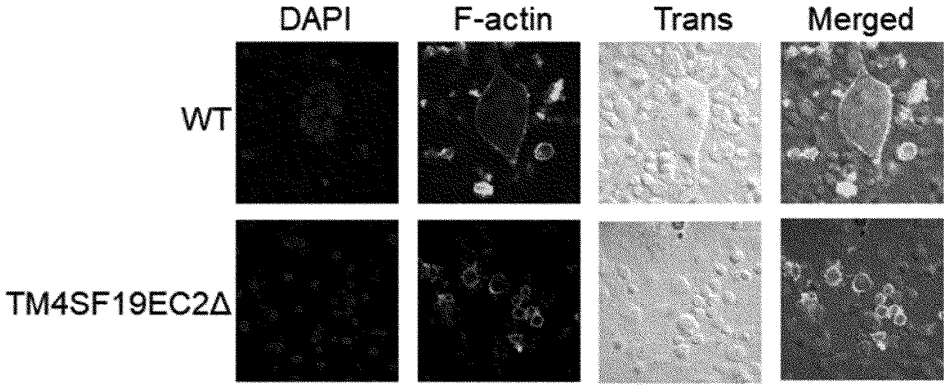
[도7a]


M-CSF
M-CSF + RANKL
WT
TM4SF19EC2Δ
TRAP stain; x40

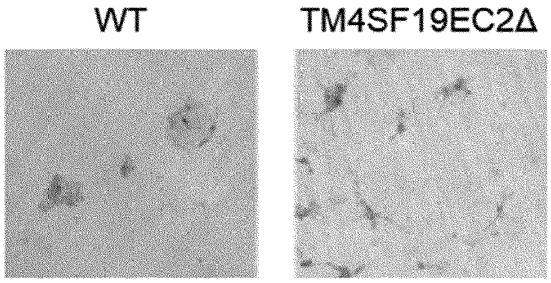
[도7b]


100ng/ml RANKL
25
100
ng/ml MCSF
WT
TM4SF19EC2Δ
TRAP 100x

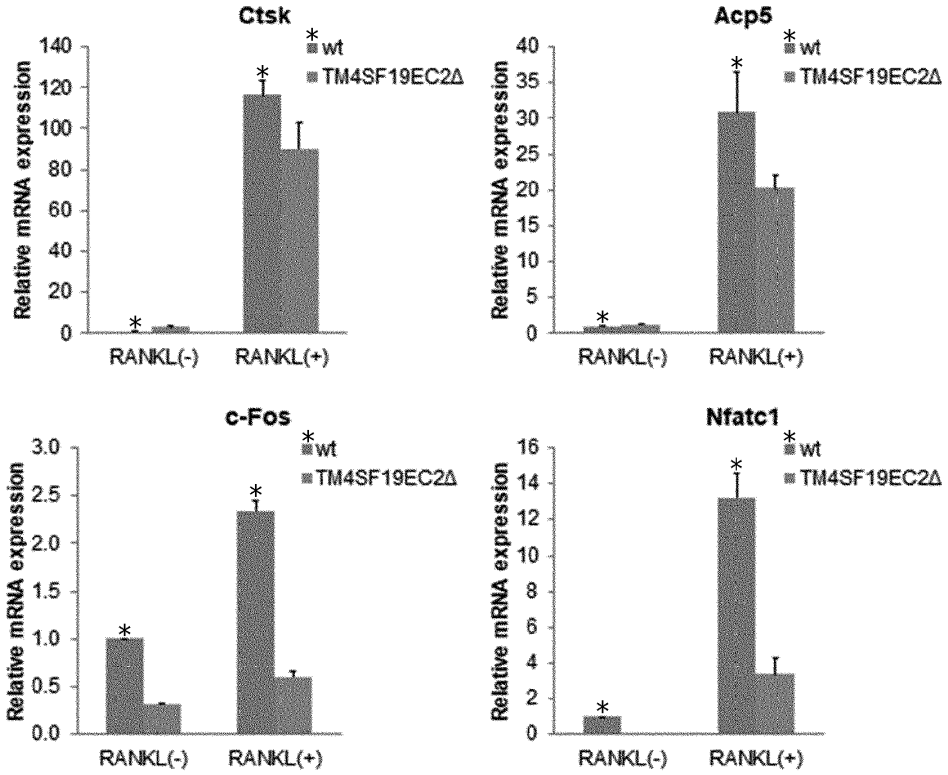
[도7c]


DAPI
F-actin
Trans
Merged
WT
TM4SF19EC2Δ

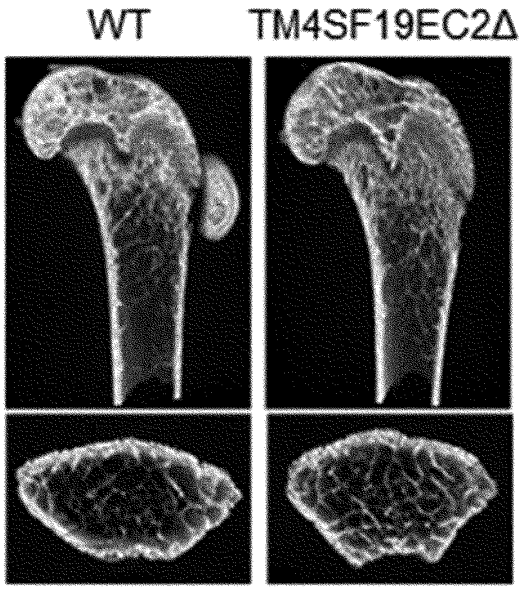
[도7d]


WT
TM4SF19EC2Δ

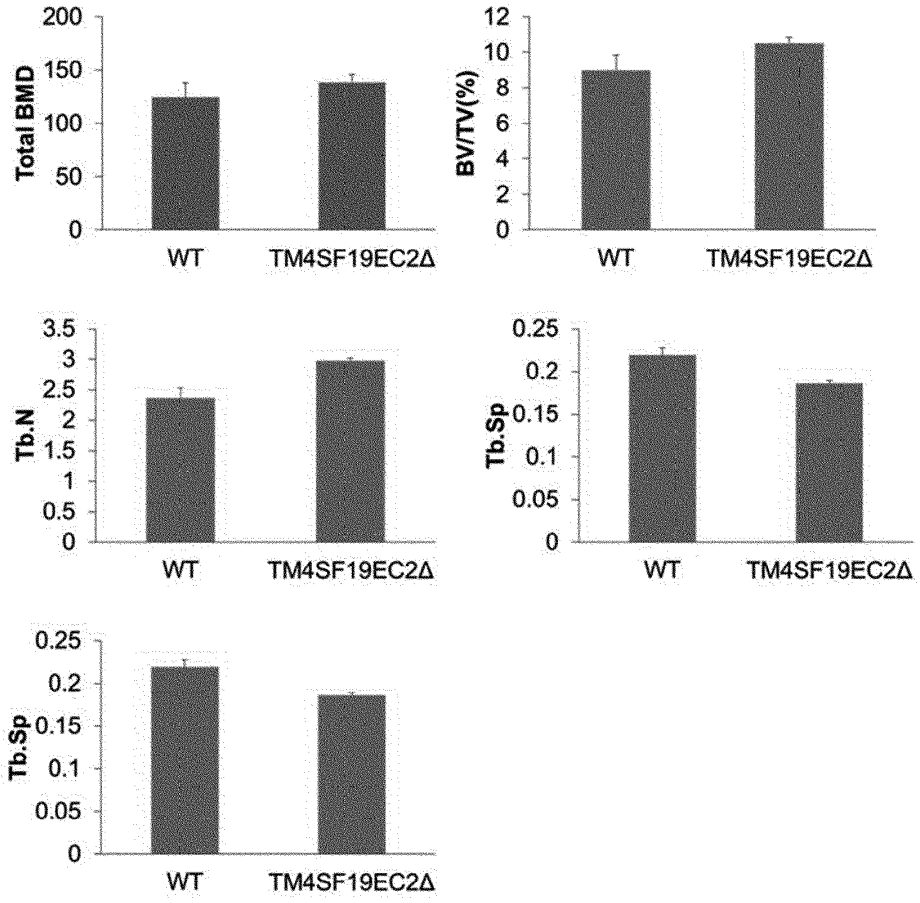
[도8]


Ctsk
Acp5
c-Fos
Nfatc1
wt
TM4SF19EC2Δ
Relative mRNA expression
RANKL(-)
RANKL(+)

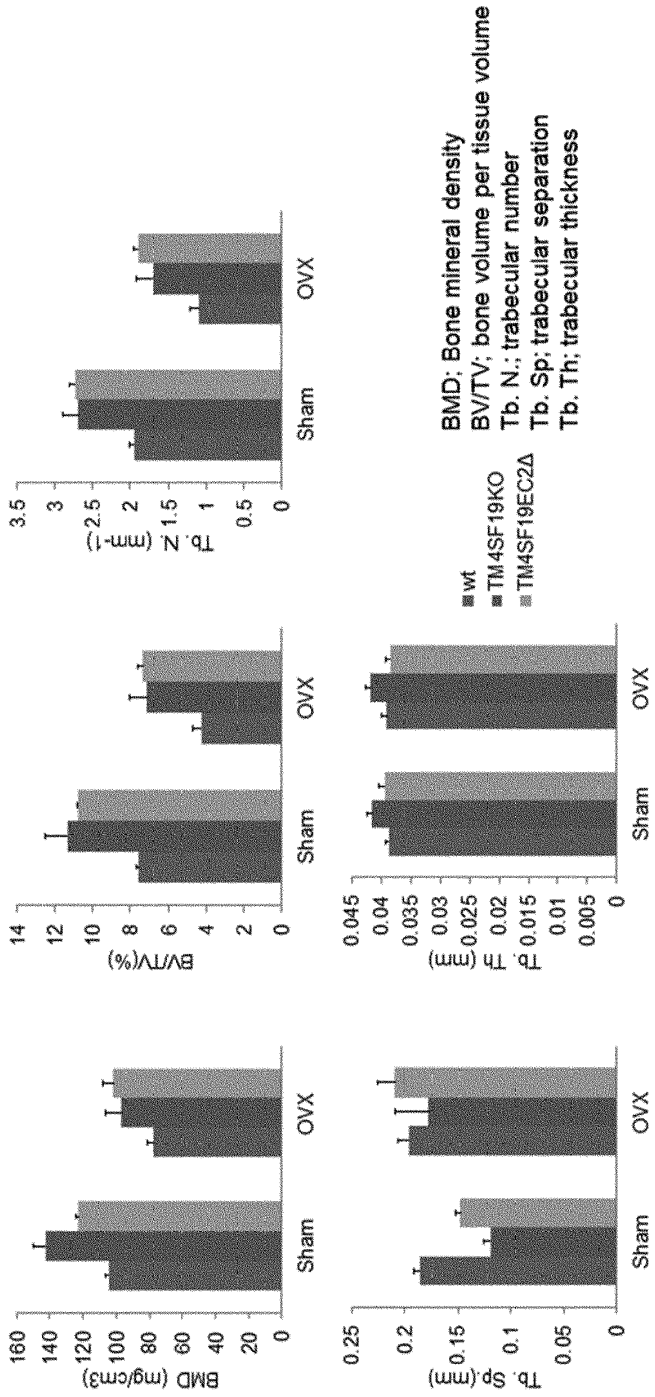
[도9a]


WT
TM4SF19EC2Δ

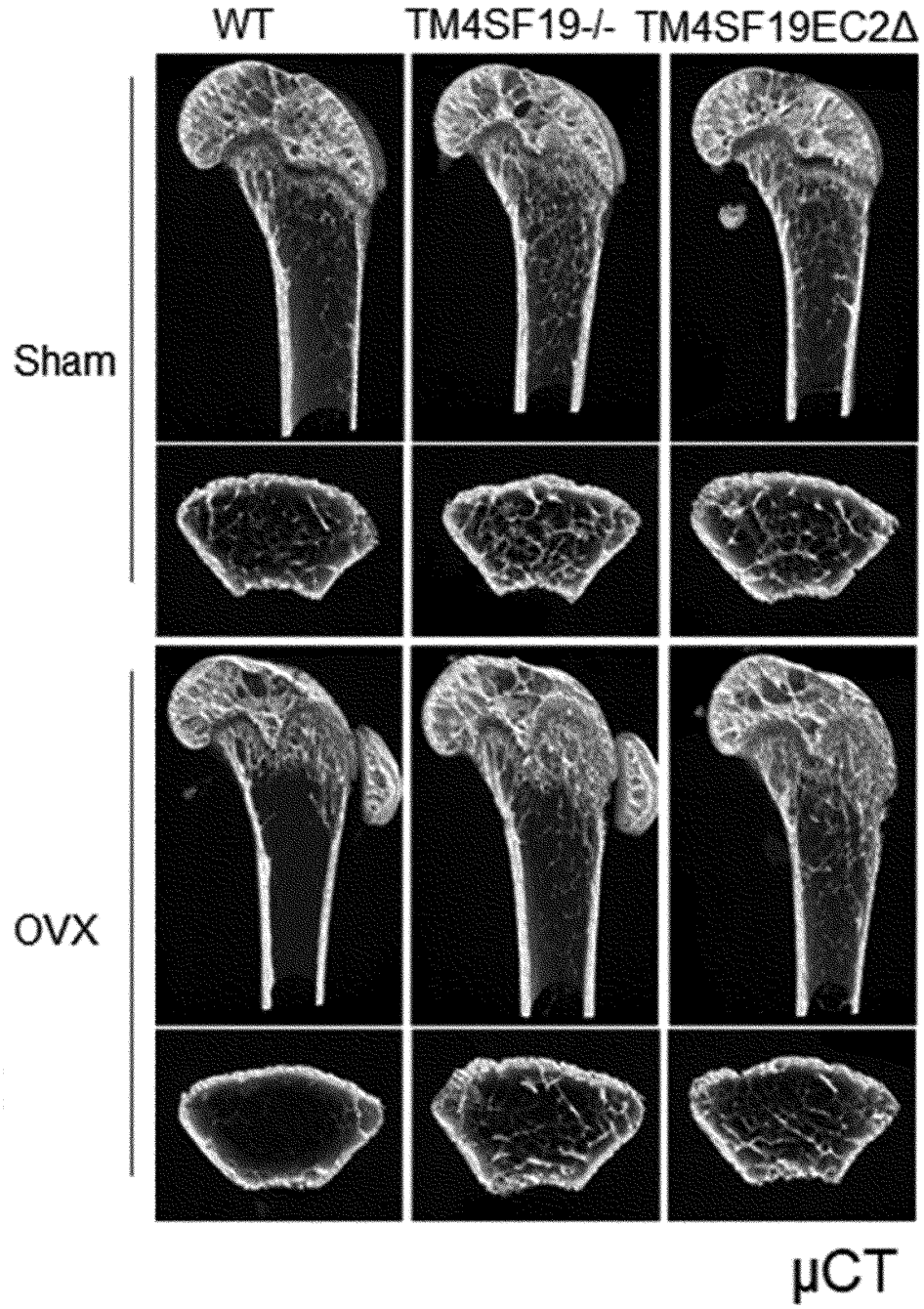
[도9b]


Total BMD
200
150
100
50
0
WT
TM4SF19EC2Δ
BV/TV(%)
12
10
8
6
4
2
0
WT
TM4SF19EC2Δ
Tb.N
3.5
3
2.5
2
1.5
1
0.5
0
WT
TM4SF19EC2Δ
Tb.Sp
0.25
0.2
0.15
0.1
0.05
0
WT
TM4SF19EC2Δ
Tb.Sp
0.25
0.2
0.15
0.1
0.05
0
WT
TM4SF19EC2Δ

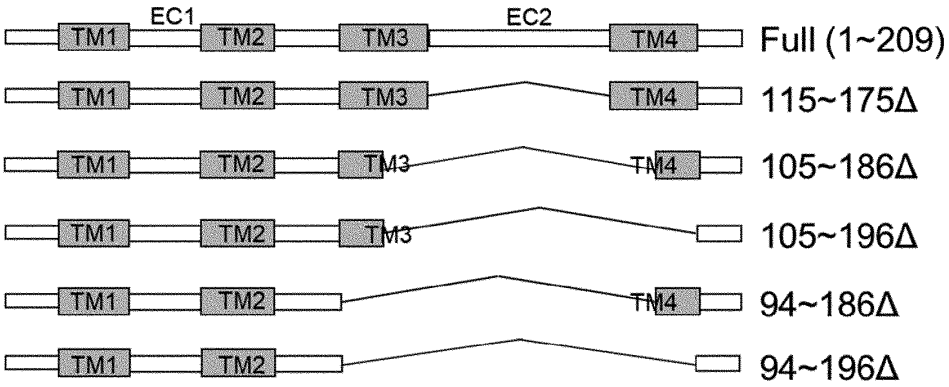
[도10]


BMD; Bone mineral density
BV/TV; bone volume per tissue volume
Tb. N.; trabecular number
Tb. Sp; trabecular separation
Tb. Th; trabecular thickness
wt
TM4SF19KO
TM4SF19EC2Δ
Sham
OVX
BMD (mg/cm3)
BV/TV(%)
Tb. N. (mm-1)
Tb. Th (mm)
Tb. Sp.(mm)

[도11]

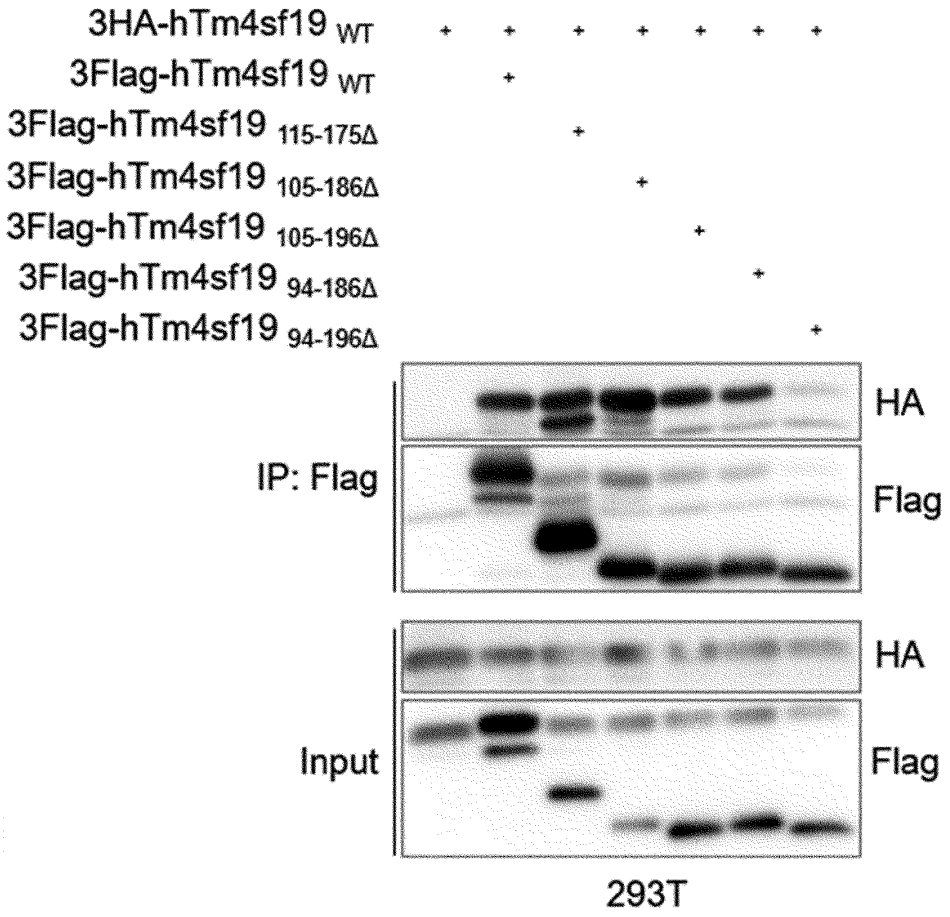


[도12a]

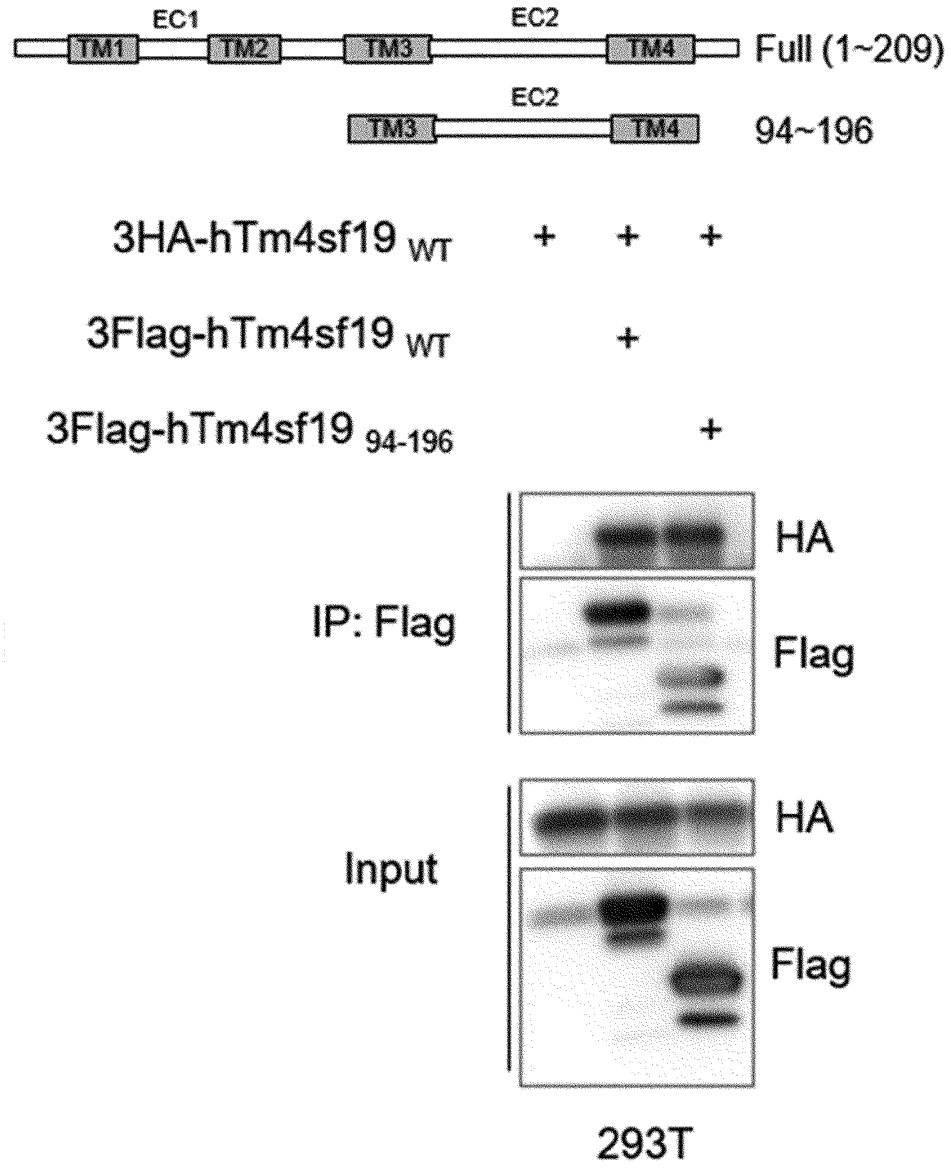


- EC1: Extracellular region 1
- EC2: Extracellular region 2
- TM: Transmembrane

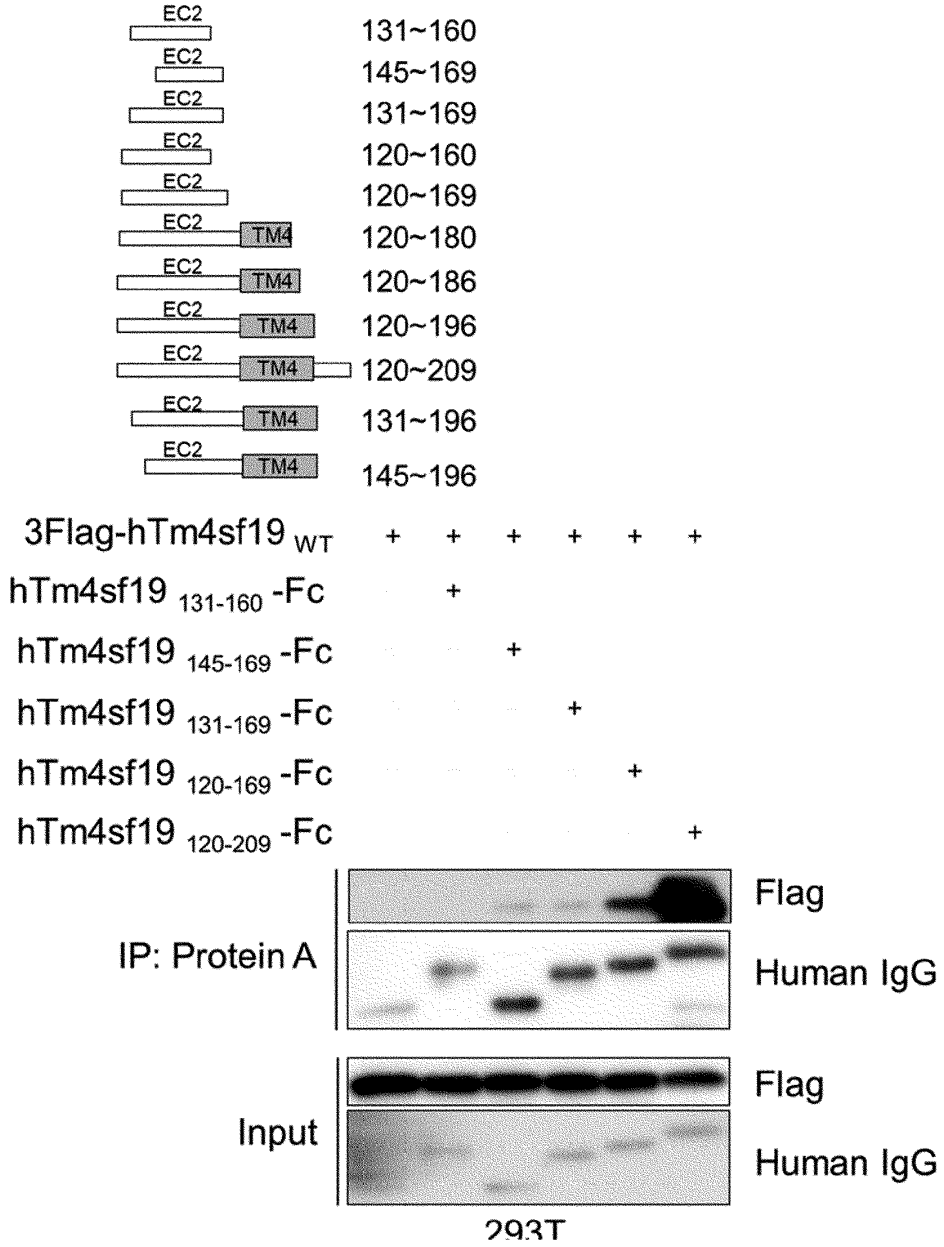
[도12b]



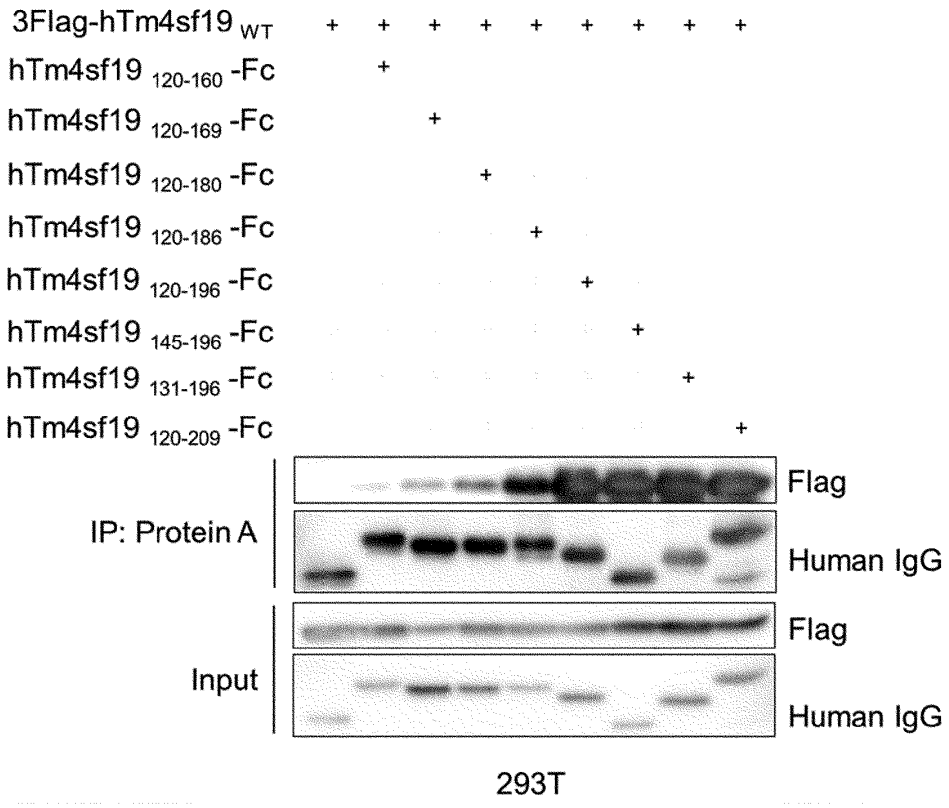
[도12c]


EC1
EC2
TM1
TM2
TM3
TM4
Full (1~209)
EC2
TM3
TM4
94~196
3HA-hTm4sf19 WT + + +
3Flag-hTm4sf19 WT +
3Flag-hTm4sf19 94-196 +
IP: Flag
HA
Flag
Input
HA
Flag
293T

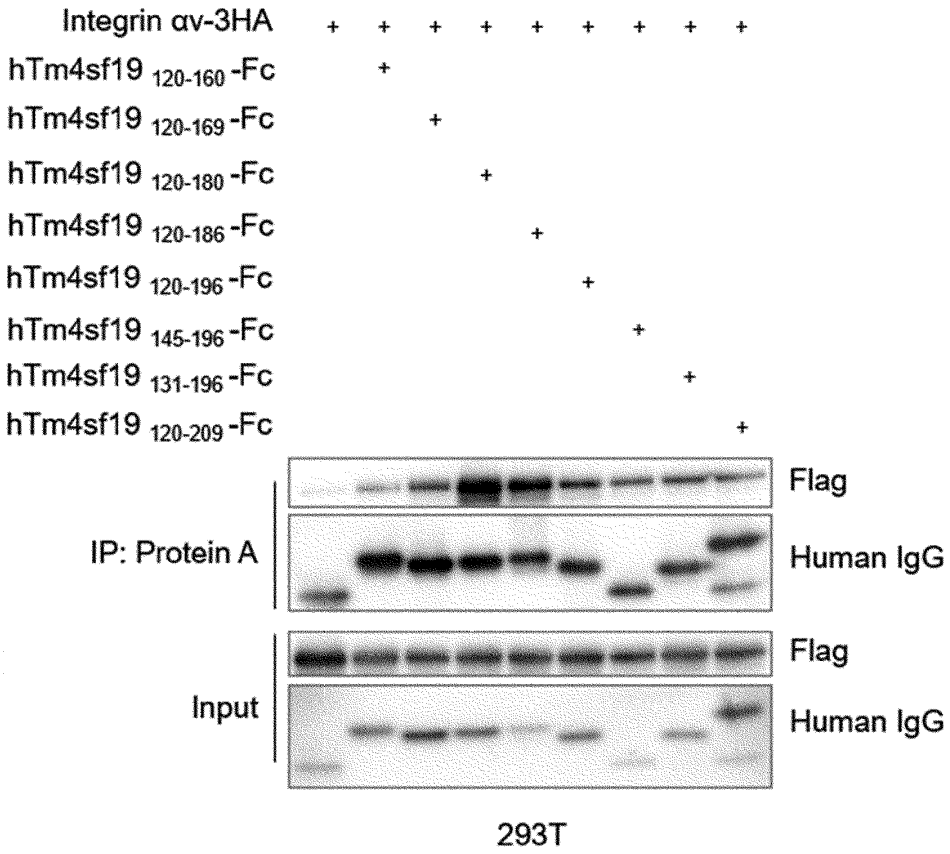
[도12d]


EC2
TM4
131~160
145~169
131~169
120~160
120~169
120~180
120~186
120~196
120~209
131~196
145~196
3Flag-hTm4sf19 WT
hTm4sf19 131-160 -Fc
hTm4sf19 145-169 -Fc
hTm4sf19 131-169 -Fc
hTm4sf19 120-169 -Fc
hTm4sf19 120-209 -Fc
IP: Protein A
Flag
Human IgG
Input
Flag
Human IgG
293T

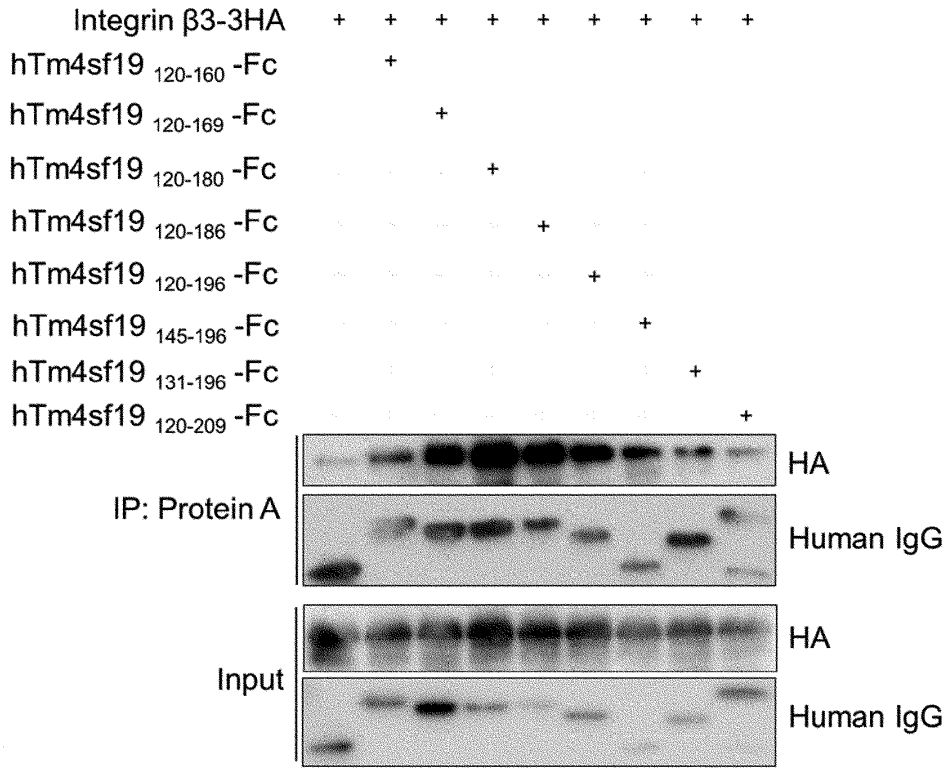
[도12e]


3Flag-hTm4sf19 WT
hTm4sf19 120-160 -Fc
hTm4sf19 120-169 -Fc
hTm4sf19 120-180 -Fc
hTm4sf19 120-186 -Fc
hTm4sf19 120-196 -Fc
hTm4sf19 145-196 -Fc
hTm4sf19 131-196 -Fc
hTm4sf19 120-209 -Fc
IP: Protein A
Flag
Human IgG
Input
Flag
Human IgG
293T

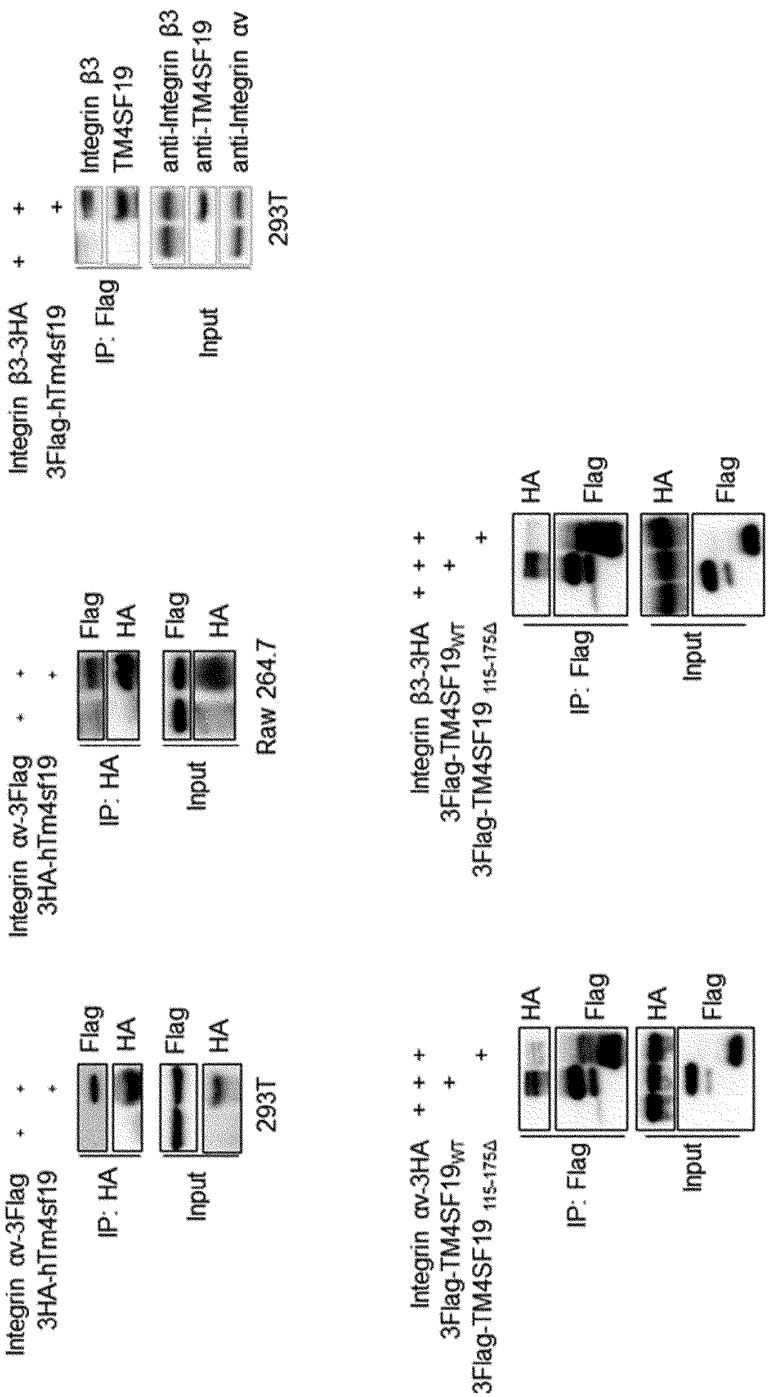
[도12f]


Integrin αv-3HA
hTm4sf19 120-160 -Fc
hTm4sf19 120-169 -Fc
hTm4sf19 120-180 -Fc
hTm4sf19 120-186 -Fc
hTm4sf19 120-196 -Fc
hTm4sf19 145-196 -Fc
hTm4sf19 131-196 -Fc
hTm4sf19 120-209 -Fc
IP: Protein A
Flag
Human IgG
Input
Flag
Human IgG
293T

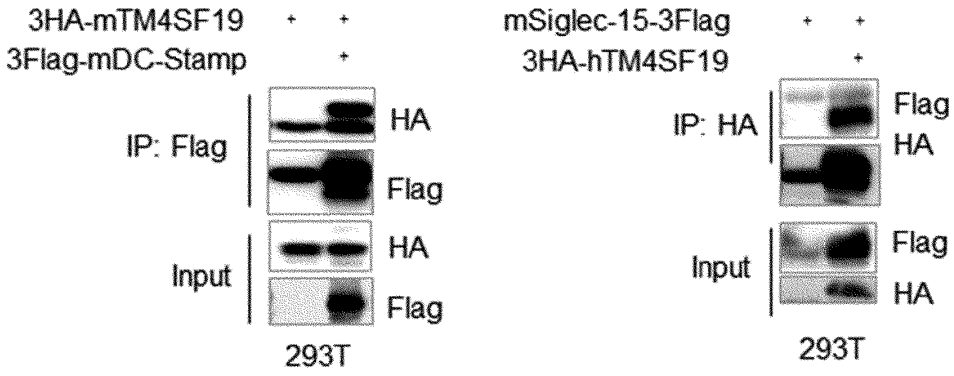
[도12g]


Integrin β3-3HA
hTm4sf19 120-160 -Fc
hTm4sf19 120-169 -Fc
hTm4sf19 120-180 -Fc
hTm4sf19 120-186 -Fc
hTm4sf19 120-196 -Fc
hTm4sf19 145-196 -Fc
hTm4sf19 131-196 -Fc
hTm4sf19 120-209 -Fc
IP: Protein A
HA
Human IgG
Input
HA
Human IgG

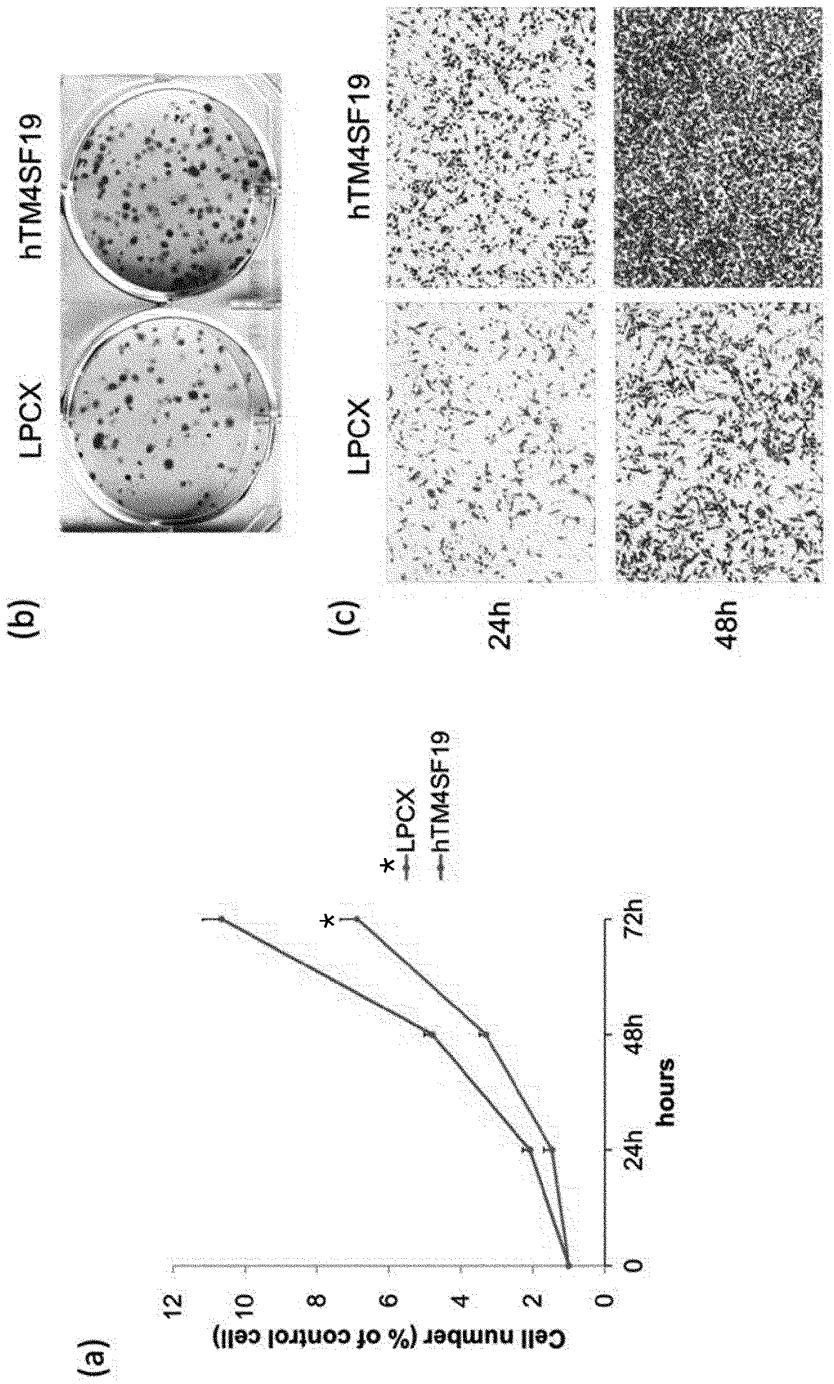
[도12h]


Integrin αv-3Flag + + +
3HA-hTm4sf19 +
IP: HA
Flag
HA
Input
Flag
HA
293T
Integrin αv-3Flag + + +
3HA-hTm4sf19 +
IP: HA
Flag
HA
Input
Flag
HA
Raw 264.7
Integrin β3-3HA + + +
3Flag-hTm4sf19 +
IP: Flag
Integrin β3
TM4SF19
Input
anti-Integrin β3
anti-TM4SF19
anti-Integrin αv
293T
Integrin αv-3HA + + +
3Flag-TM4SF19WT +
3Flag-TM4SF19 115-175Δ +
IP: Flag
HA
Flag
Input
HA
Flag
Integrin β3-3HA + + +
3Flag-TM4SF19WT +
3Flag-TM4SF19 115-175Δ +
IP: Flag
HA
Flag
Input
HA
Flag

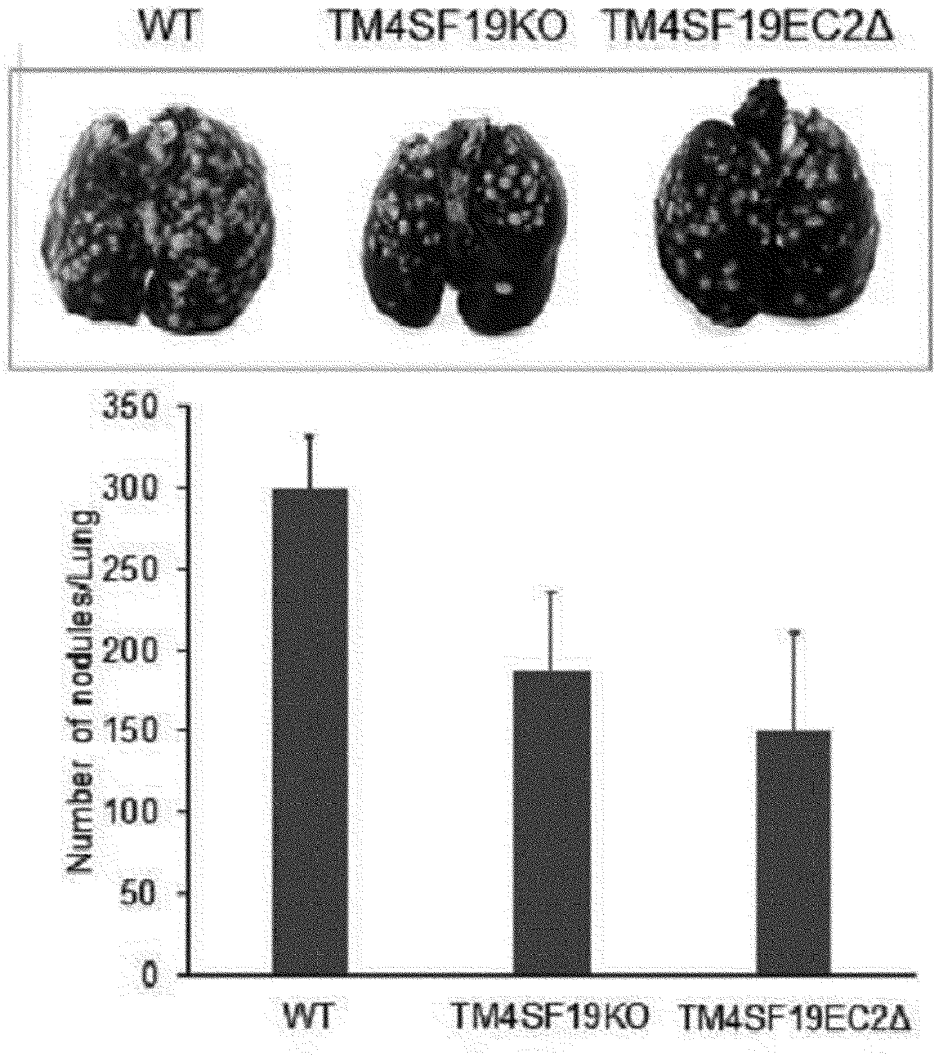
[도12i]


3HA-mTM4SF19 + +
3Flag-mDC-Stamp +
IP: Flag
HA
Flag
Input
HA
Flag
293T
mSiglec-15-3Flag + +
3HA-hTM4SF19 +
IP: HA
Flag
HA
Input
Flag
HA
293T

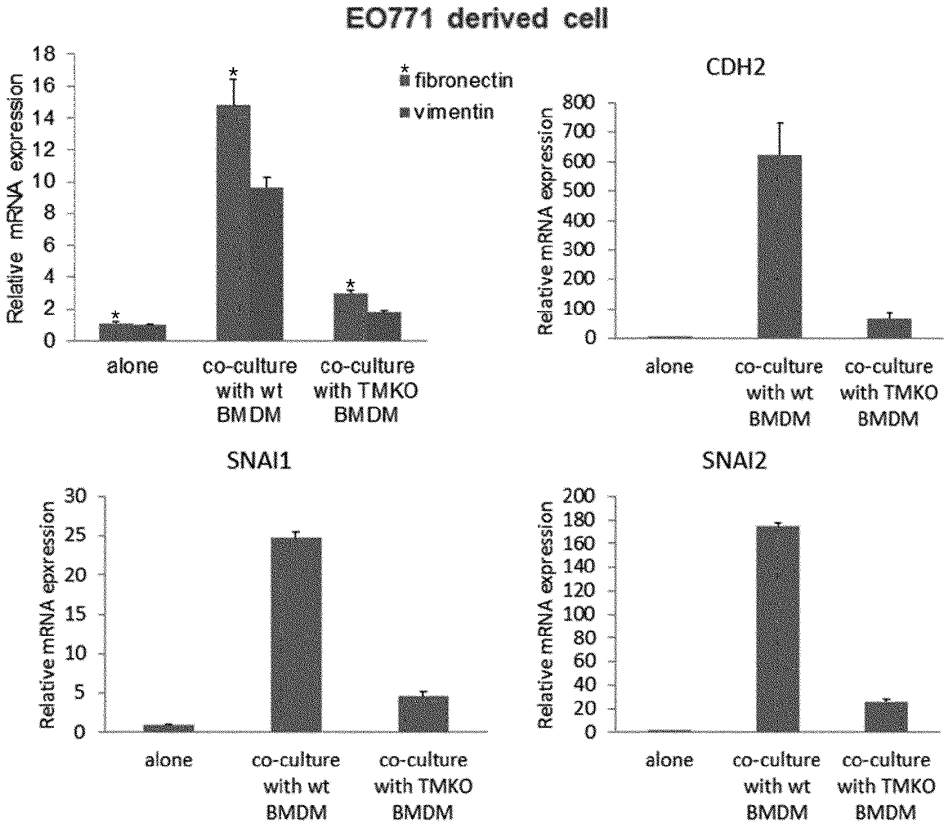
[도13]


(a)
Cell number (% of control cell)
0
2
4
6
8
10
12
0
24h
48h
72h
hours
*
*LPCX
hTM4SF19
(b)
LPCX
hTM4SF19
(c)
LPCX
hTM4SF19
24h
48h

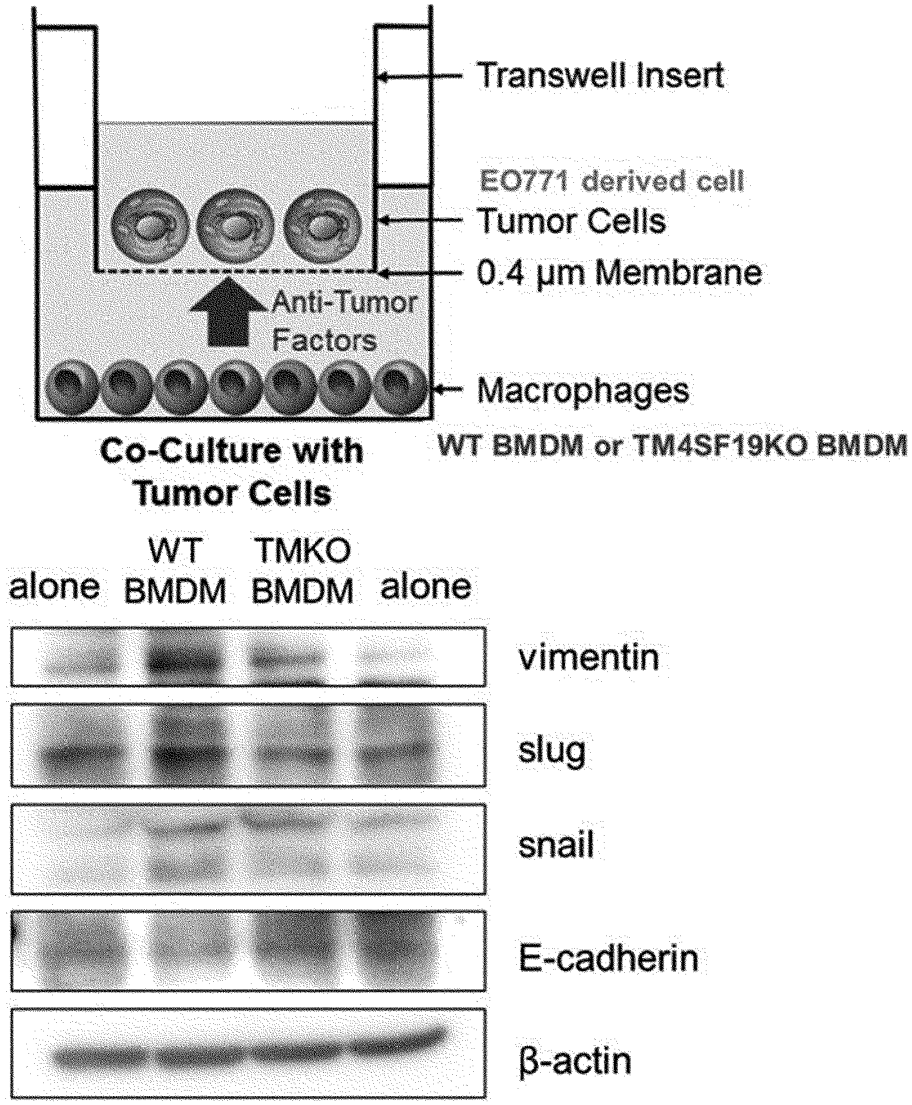
[도14]


WT
TM4SF19KO
TM4SF19EC2Δ
Number of nodules/Lung
350
300
250
200
150
100
50
0
WT
TM4SF19KO
TM4SF19EC2Δ

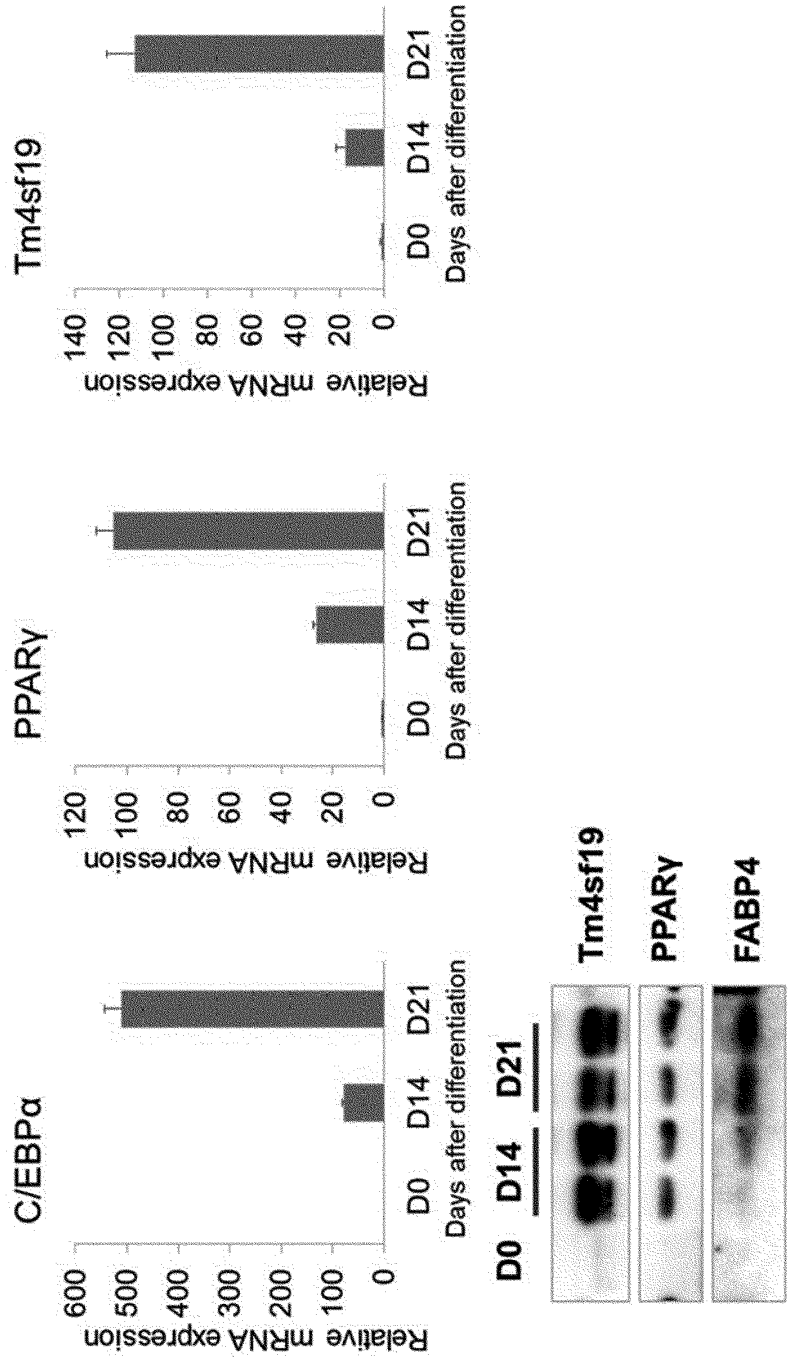
[도15a]


EO771 derived cell
Relative mRNA expression
fibronectin
vimentin
alone
co-culture with wt BMDM
co-culture with TMKO BMDM
CDH2
SNAI1
SNAI2

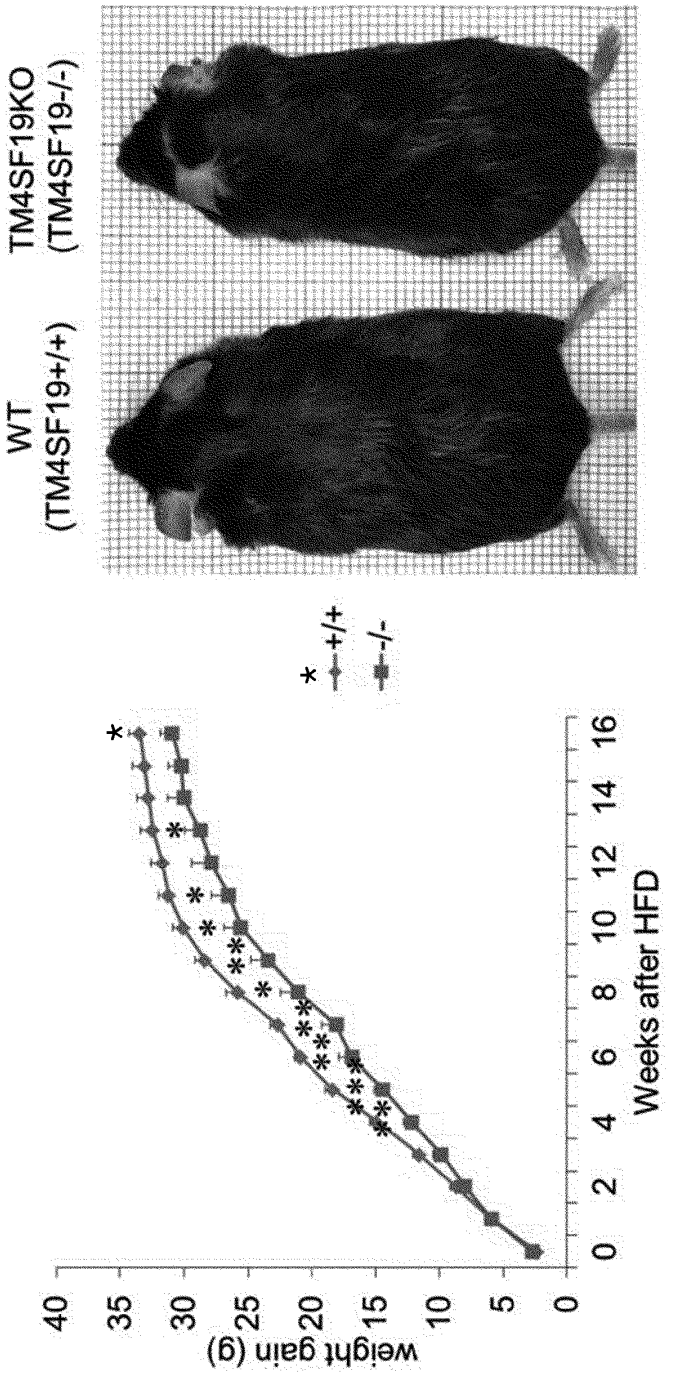
[도15b]


Transwell Insert
EO771 derived cell
Tumor Cells
0.4 μm Membrane
Anti-Tumor Factors
Macrophages
WT BMDM or TM4SF19KO BMDM
Co-Culture with Tumor Cells
alone
WT BMDM
TMKO BMDM
alone
vimentin
slug
snail
E-cadherin
β-actin

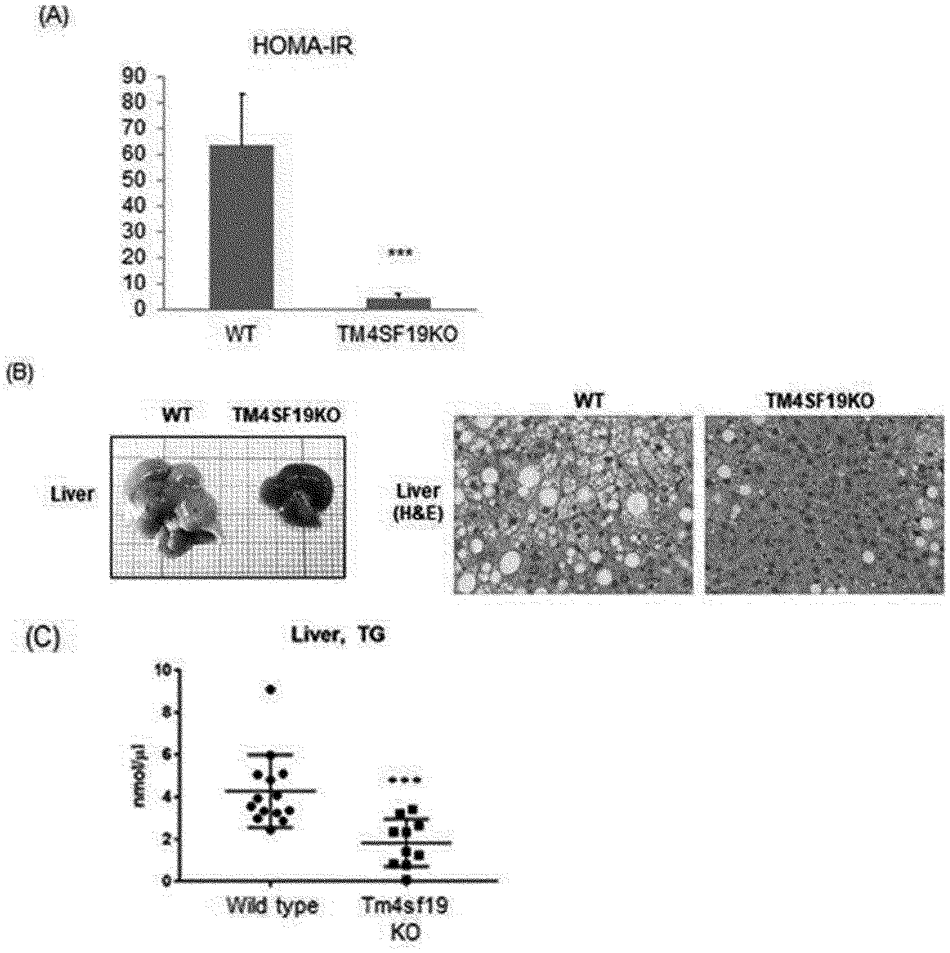
[도16]


C/EBPα
PPARγ
Tm4sf19
Relative mRNA expression
Days after differentiation
D0
D14
D21
D0 D14 D21
Tm4sf19
PPARγ
FABP4

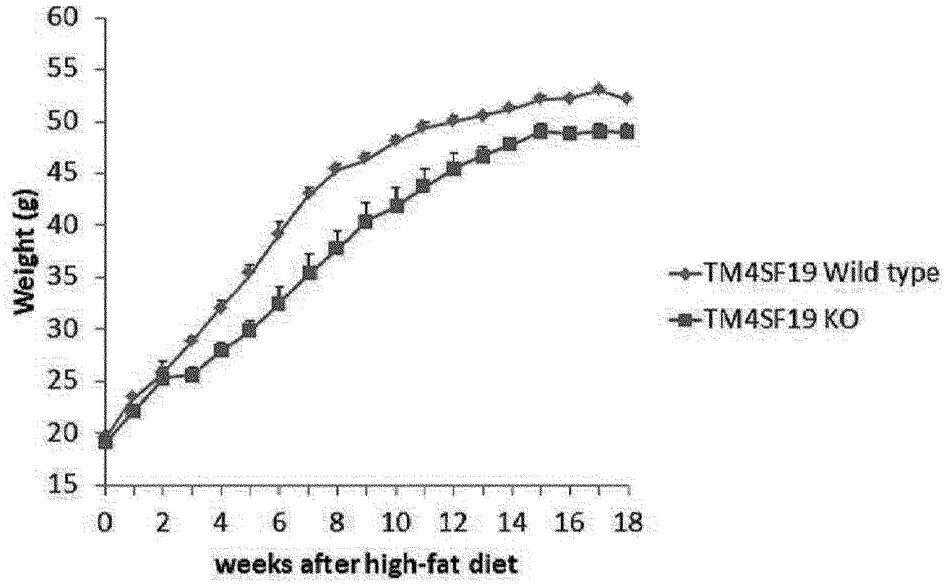
[도17]


WT
(TM4SF19+/+)
TM4SF19KO
(TM4SF19-/-)
* +/+
-/-
weight gain (g)
Weeks after HFD
0
5
10
15
20
25
30
35
40
0
2
4
6
8
10
12
14
16

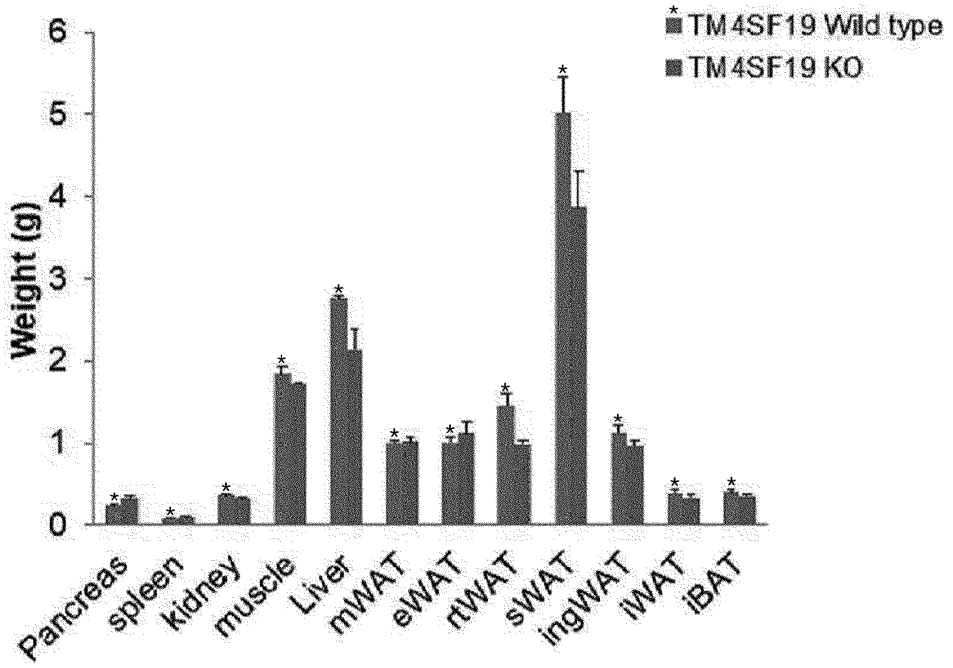
[도18]


(A)
HOMA-IR
90
80
70
60
50
40
30
20
10
0
***
WT
TM4SF19KO
(B)
WT
TM4SF19KO
Liver
Liver
(H&E)
WT
TM4SF19KO
(C)
Liver, TG
nmol/μl
10
8
6
4
2
0
***
Wild type
Tm4sf19
KO

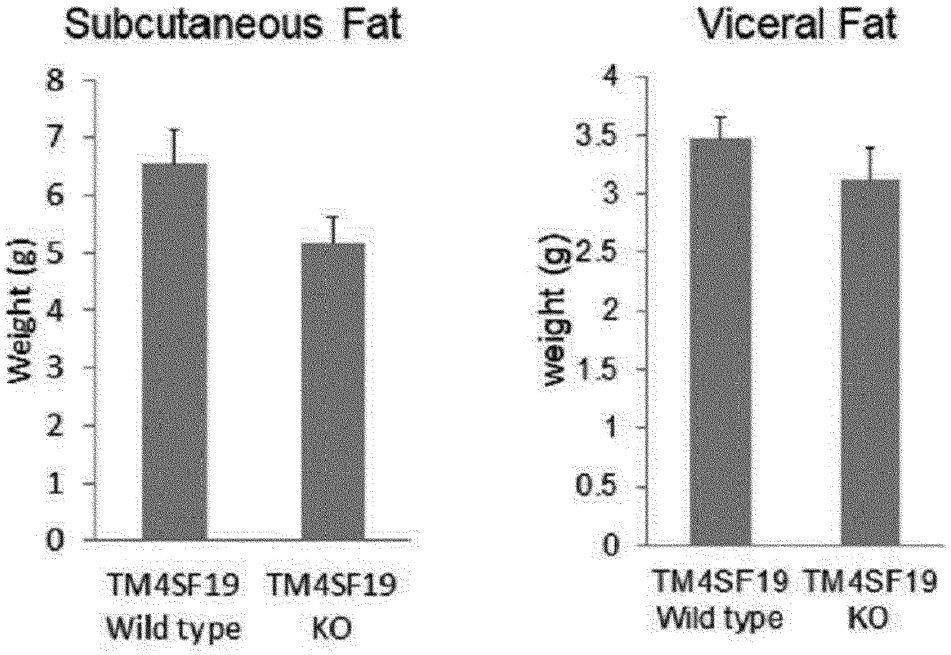
[도19a]


Weight (g)
60
55
50
45
40
35
30
25
20
15
0
2
4
6
8
10
12
14
16
18
weeks after high-fat diet
TM4SF19 Wild type
TM4SF19 KO

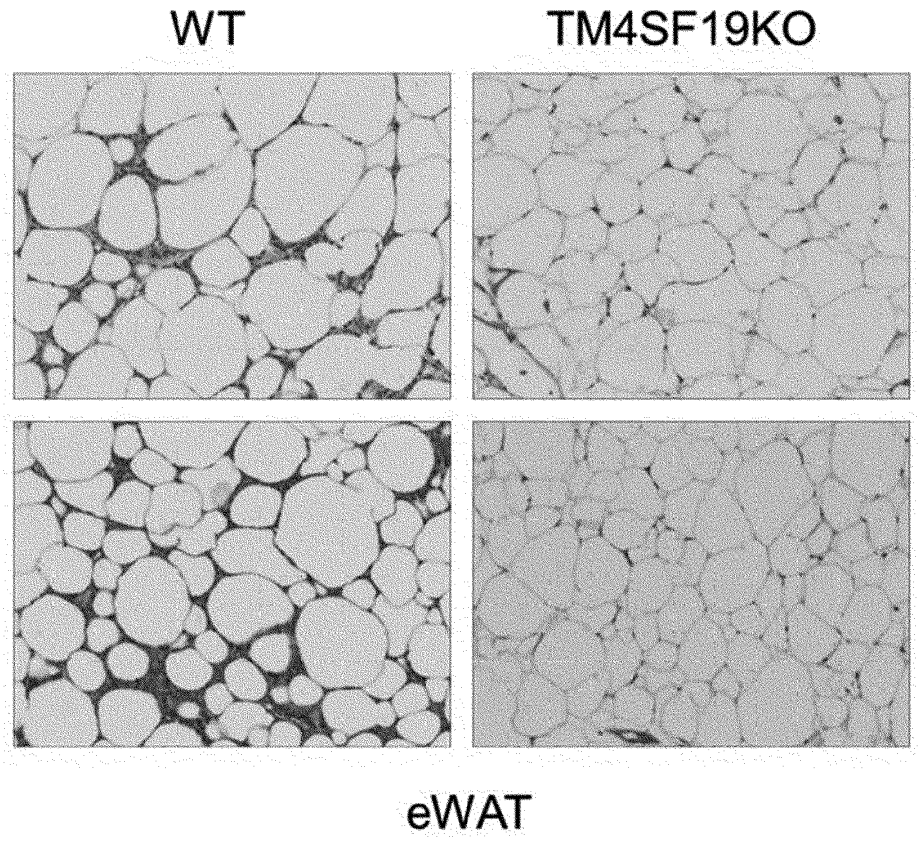
[도19b]


TM4SF19 Wild type
TM4SF19 KO
Weight (g)
0
1
2
3
4
5
6
Pancreas
spleen
kidney
muscle
Liver
mWAT
eWAT
rtWAT
sWAT
ingWAT
iWAT
iBAT

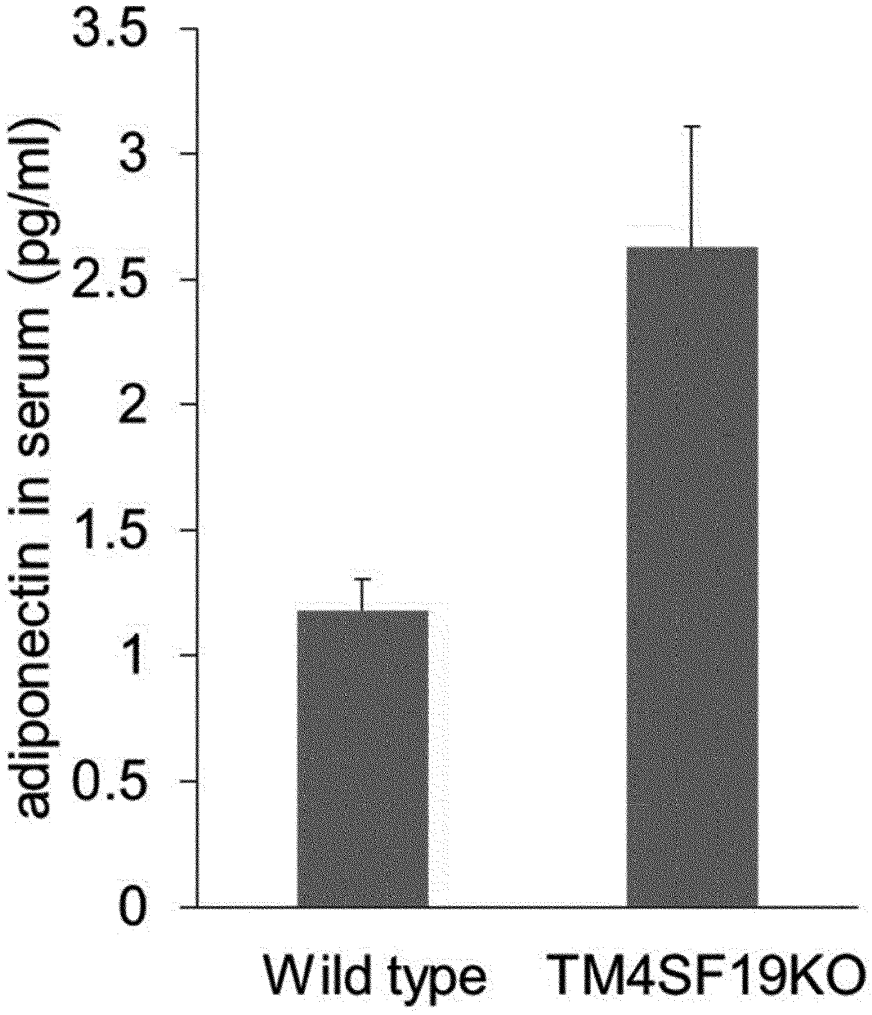
[도19c]


Subcutaneous Fat
Weight (g)
0
1
2
3
4
5
6
7
8
TM4SF19 Wild type
TM4SF19 KO
Viceral Fat
weight (g)
0
0.5
1
1.5
2
2.5
3
3.5
4
TM4SF19 Wild type
TM4SF19 KO

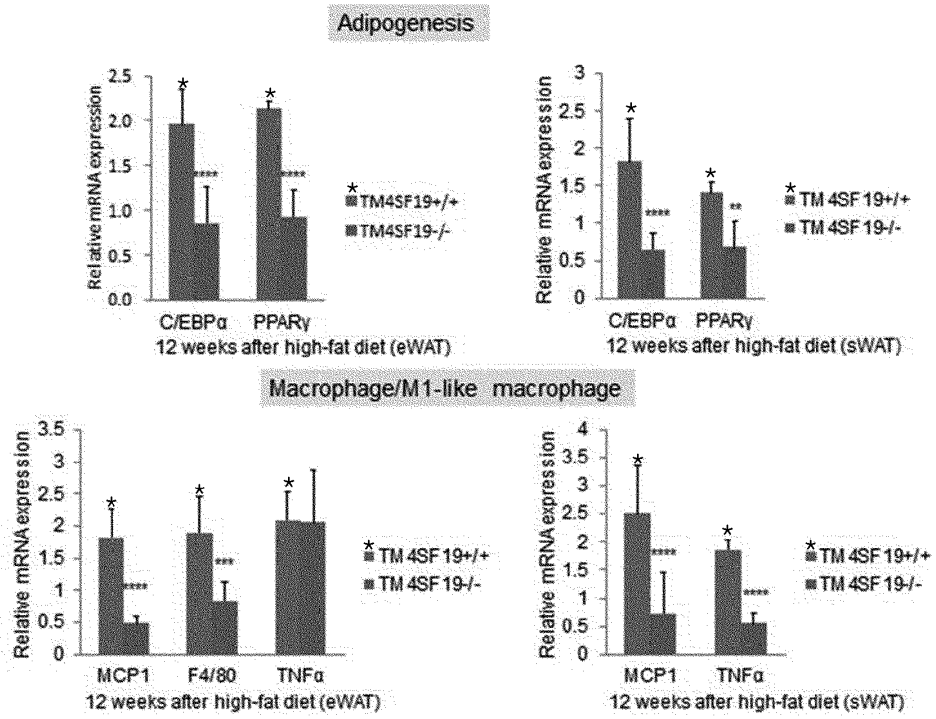
[도19d]



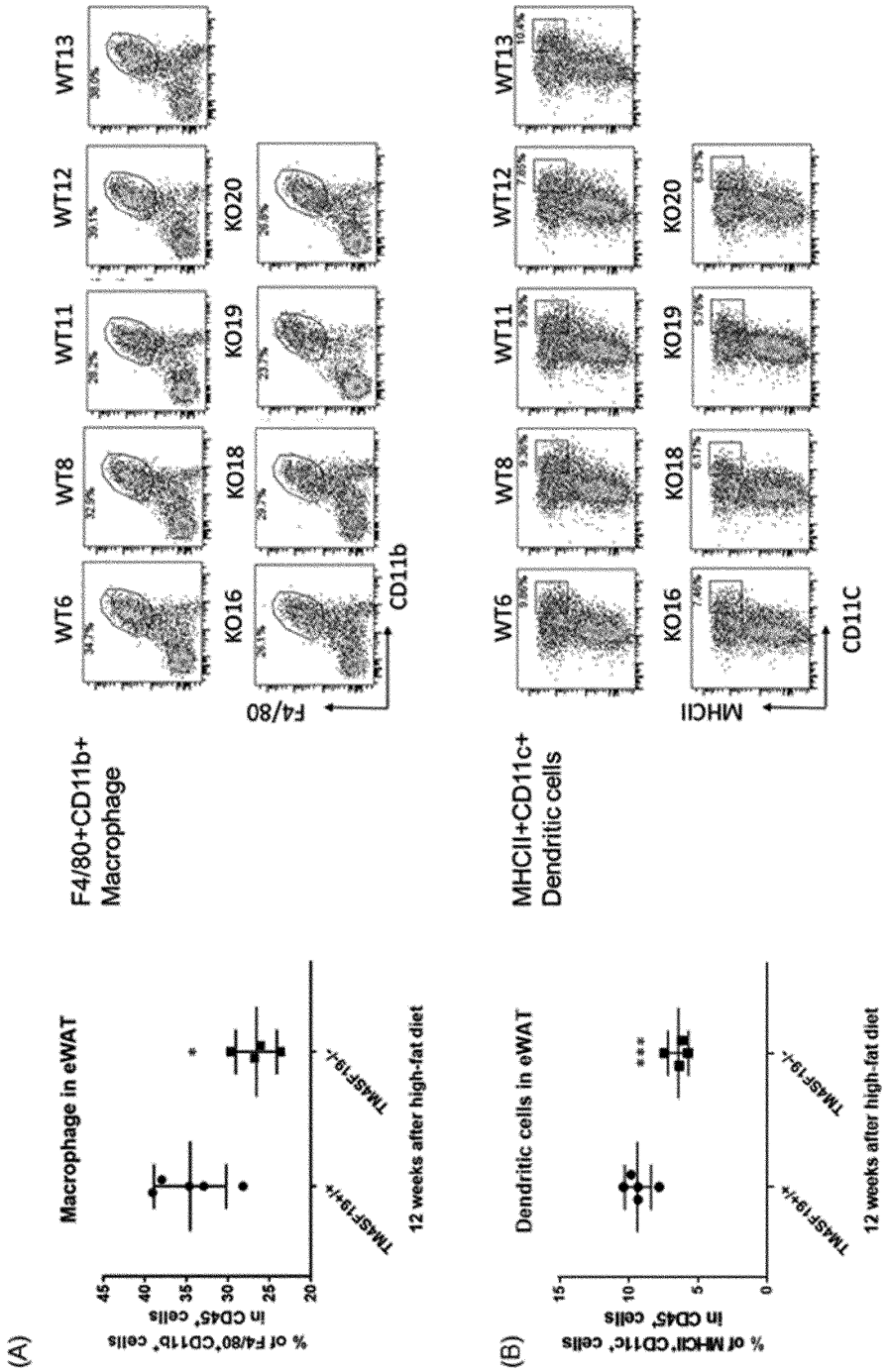
[도19e]


adiponectin in serum (pg/ml)
3.5
3
2.5
2
1.5
1
0.5
0
Wild type
TM4SF19KO

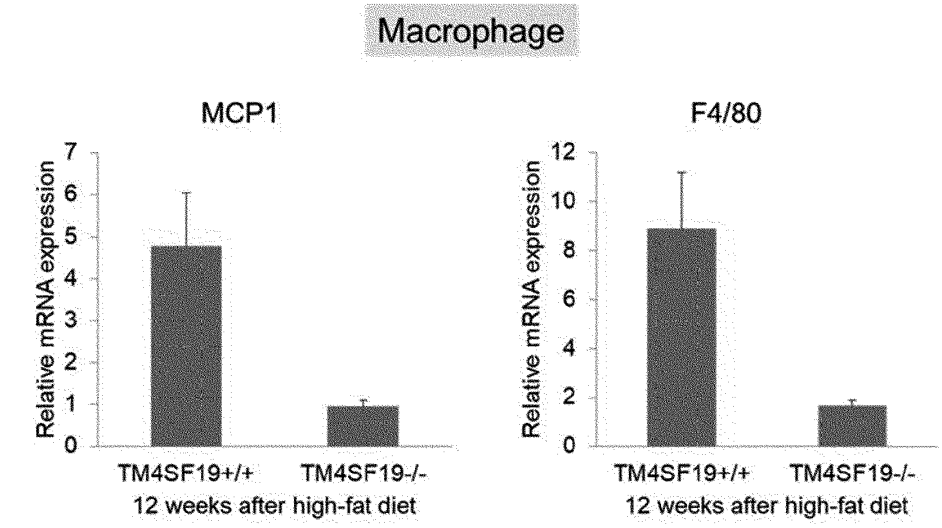
[도20]


Adipogenesis
Relative mRNA expression
C/EBPα
PPARγ
12 weeks after high-fat diet (eWAT)
TM4SF19+/+
TM4SF19-/-
12 weeks after high-fat diet (sWAT)
Macrophage/M1-like macrophage
MCP1
F4/80
TNFα

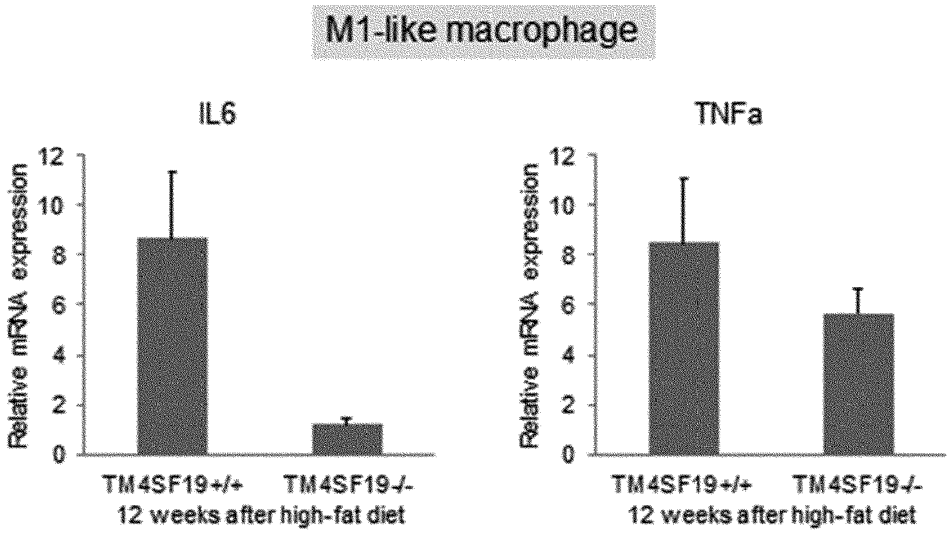
[도21]


(A)
F4/80+CD11b+
Macrophage
WT6
WT8
WT11
WT12
WT13
KO16
KO18
KO19
KO20
F4/80
CD11b
Macrophage in eWAT
% of F4/80+CD11b+ cells
in CD45+ cells
TMASF19+/+
TMASF19+/-
12 weeks after high-fat diet
(B)
MHCII+CD11c+
Dendritic cells
MHCII
CD11C
Dendritic cells in eWAT
% of MHCII+CD11c+ cells
in CD45+ cells
***

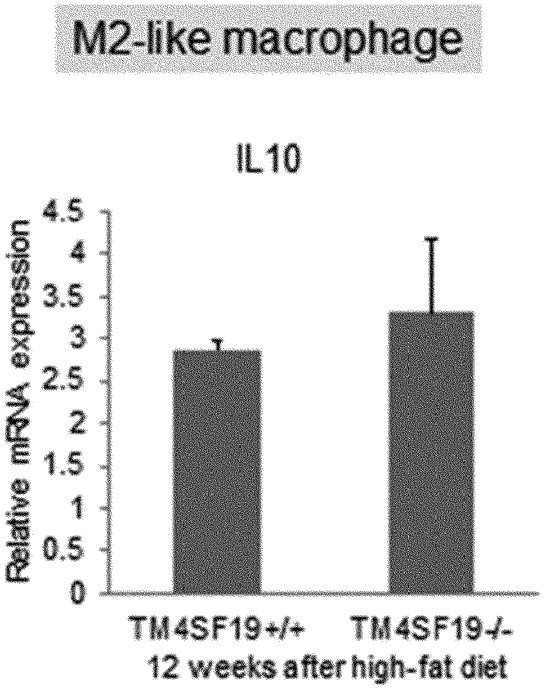
[도22a]


Macrophage
MCP1
Relative mRNA expression
0
1
2
3
4
5
6
7
TM4SF19+/+
TM4SF19-/-
12 weeks after high-fat diet
F4/80
Relative mRNA expression
0
2
4
6
8
10
12
TM4SF19+/+
TM4SF19-/-
12 weeks after high-fat diet

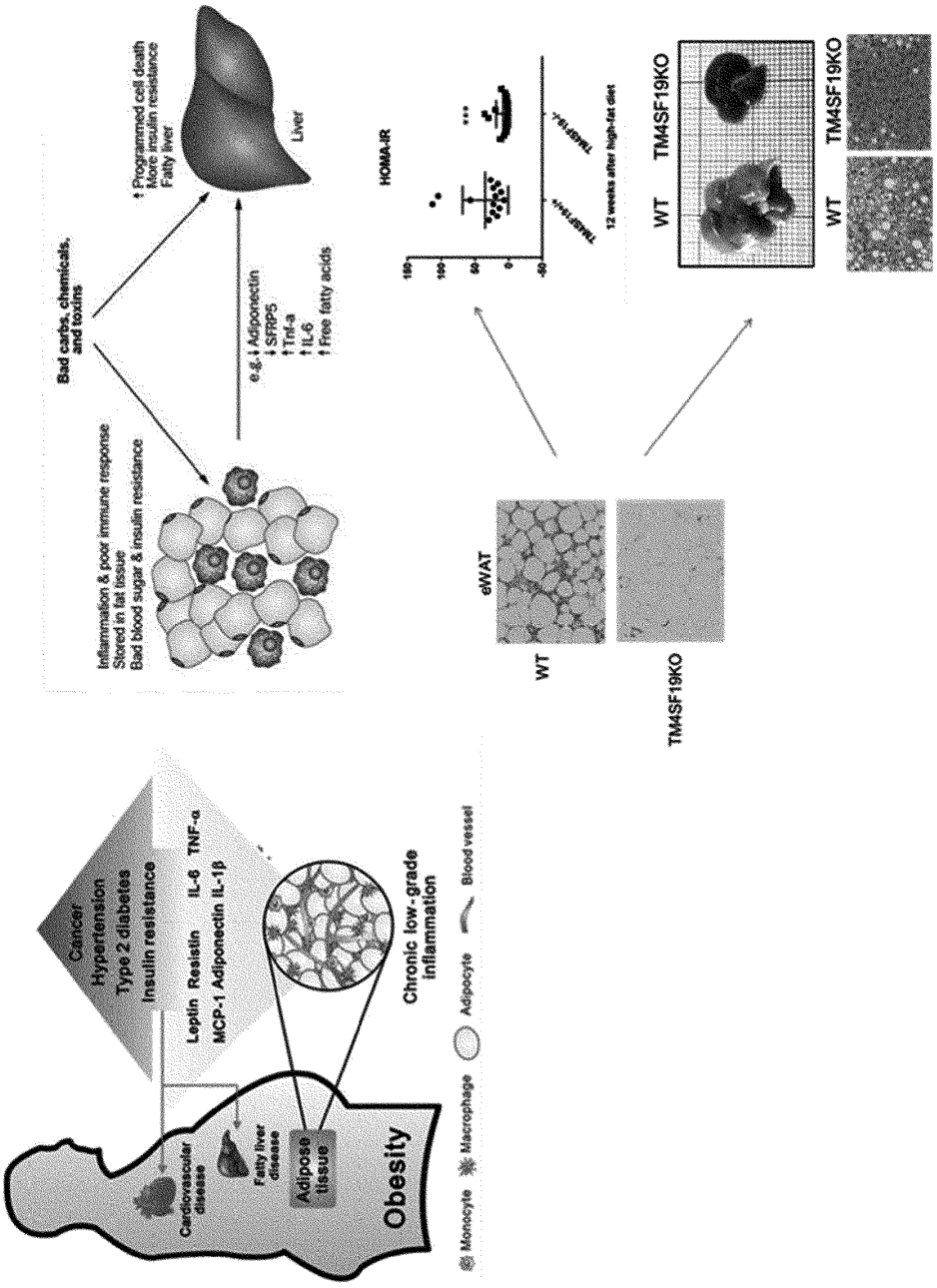
[도22b]


M1-like macrophage
IL6
Relative mRNA expression
0
2
4
6
8
10
12
TM4SF19+/+
TM4SF19-/-
12 weeks after high-fat diet
TNFa
Relative mRNA expression
0
2
4
6
8
10
12
TM4SF19+/+
TM4SF19-/-
12 weeks after high-fat diet

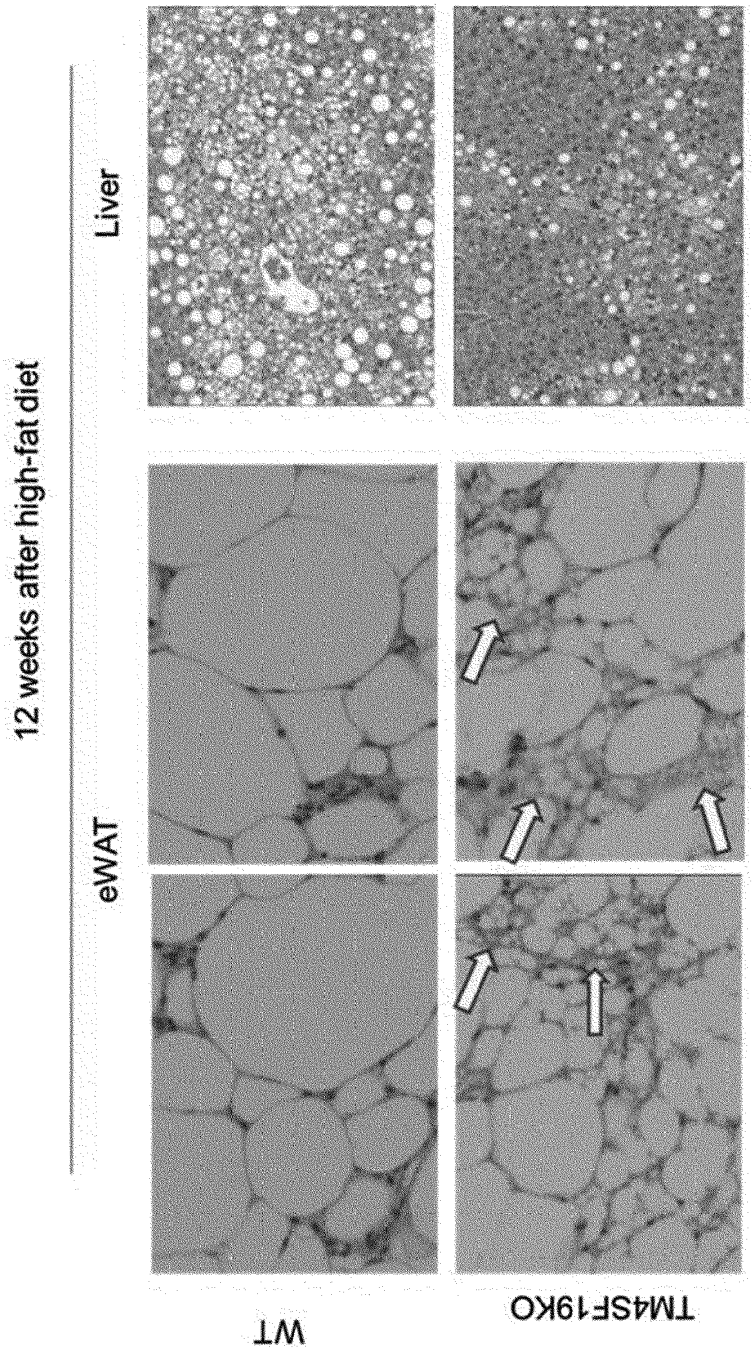
[도22c]


M2-like macrophage
IL10
Relative mRNA expression
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
TM4SF19+/+
TM4SF19-/-
12 weeks after high-fat diet

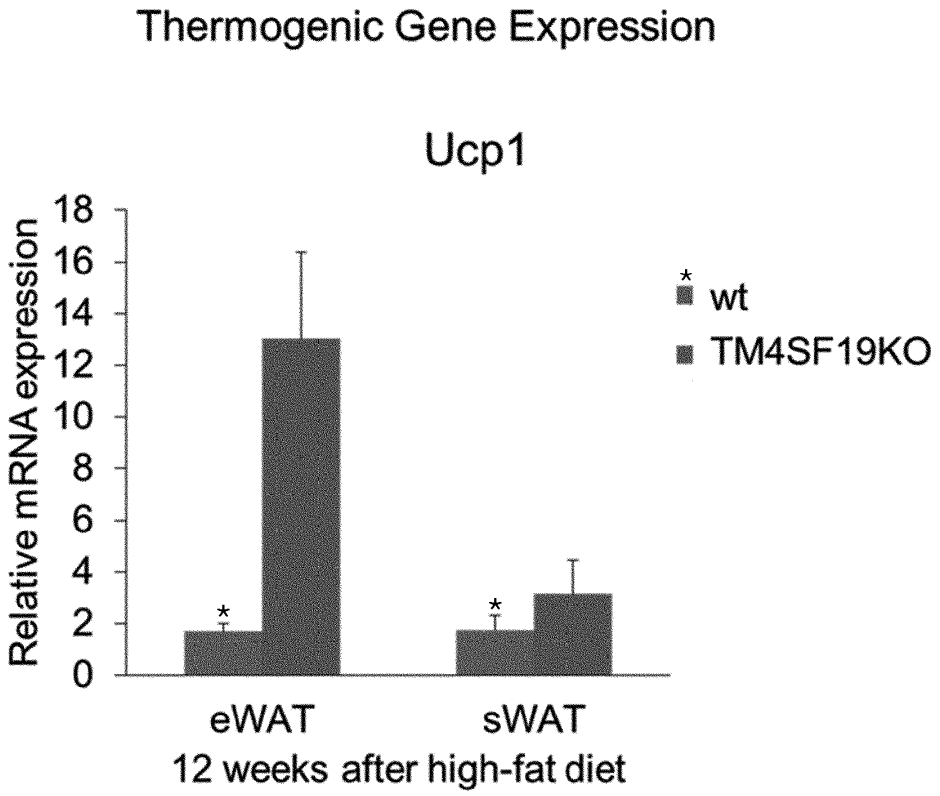
[도23]


Bad carbs, chemicals, and toxins
Inflammation & poor immune response
Stored in fat tissue
Bad blood sugar & insulin resistance
e.g.↓ Adiponectin
↓ SFRP5
↑ Tnf-a
↑ IL-6
↑ Free fatty acids
↑ Programmed cell death
More insulin resistance
Fatty liver
Liver
HOMA-IR
12 weeks after high-fat diet
eWAT
WT
TM4SF19KO
Cancer
Hypertension
Type 2 diabetes
Insulin resistance
Leptin Resistin IL-6 TNF-α
MCP-1 Adiponectin IL-1β
Chronic low-grade inflammation
Cardiovascular disease
Fatty liver disease
Adipose tissue
Obesity
Monocyte
Macrophage
Adipocyte
Blood vessel

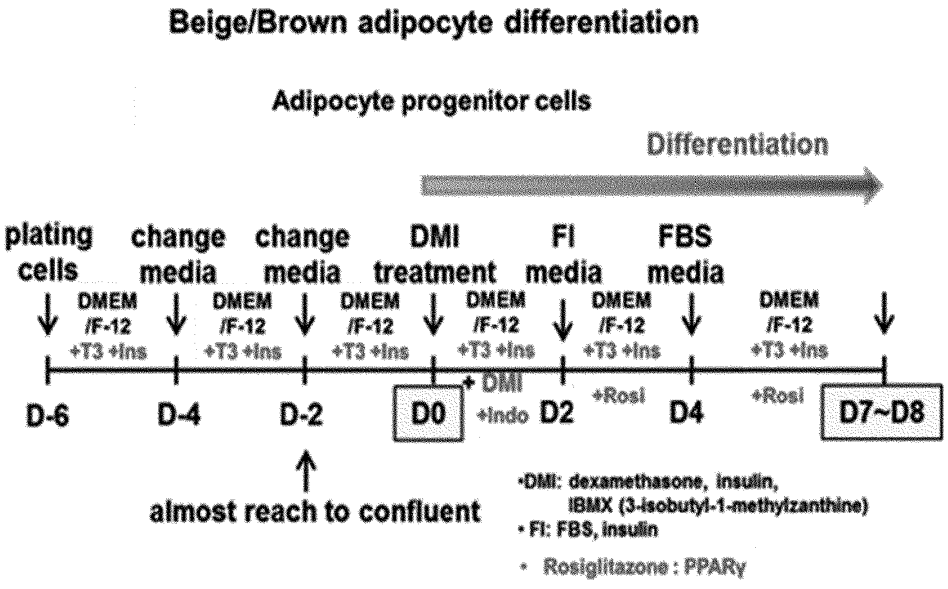
[도24a]



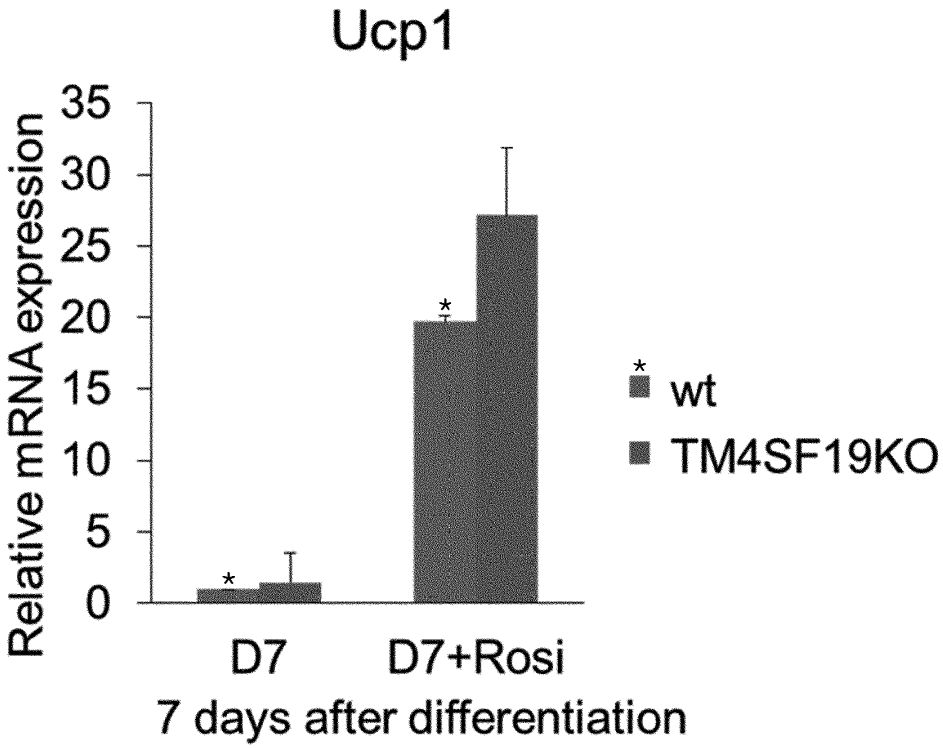
[도24b]


Thermogenic Gene Expression
Ucp1
Relative mRNA expression
18
16
14
12
10
8
6
4
2
0
*
*
wt
TM4SF19KO
eWAT
sWAT
12 weeks after high-fat diet

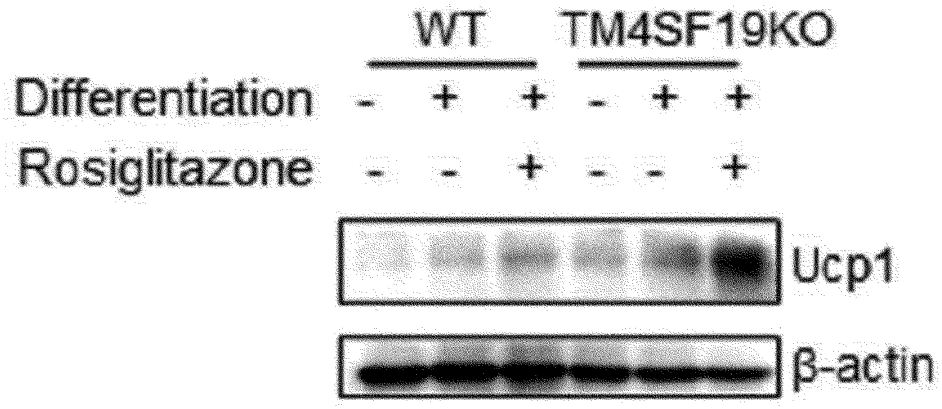
[도24c]


Beige/Brown adipocyte differentiation
Adipocyte progenitor cells
Differentiation
plating cells
change media
change media
DMI treatment
FI media
FBS media
DMEM /F-12 +T3 +Ins
+ DMI +Indo
+Rosi
+Rosi
D-6
D-4
D-2
D0
D2
D4
D7~D8
almost reach to confluent
•DMI: dexamethasone, insulin, IBMX (3-isobutyl-1-methylzanthine)
• FI: FBS, insulin
• Rosiglitazone : PPARγ

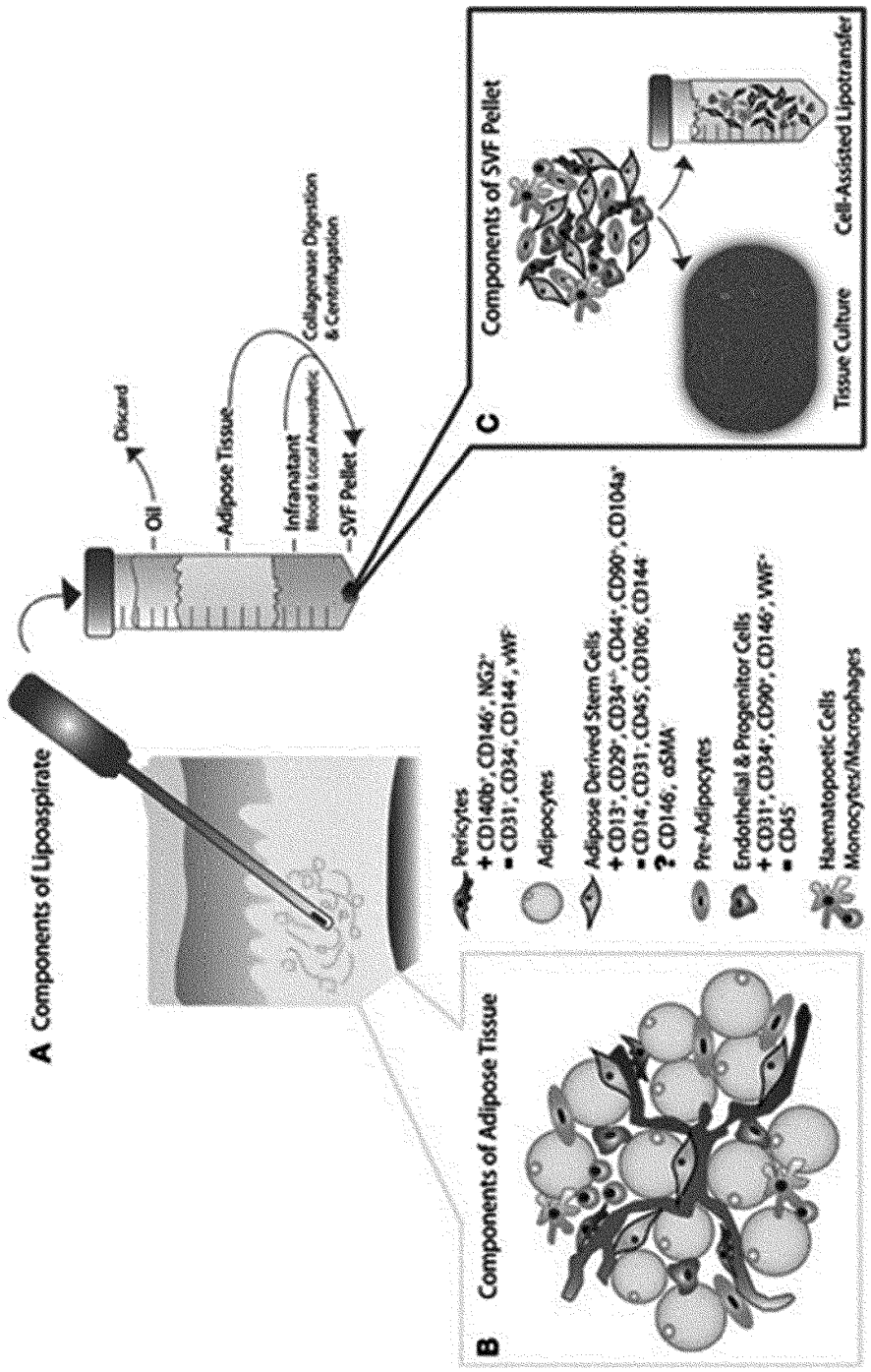
[도24d]


Ucp1
Relative mRNA expression
35
30
25
20
15
10
5
0
*
*
wt
TM4SF19KO
D7
D7+Rosi
7 days after differentiation

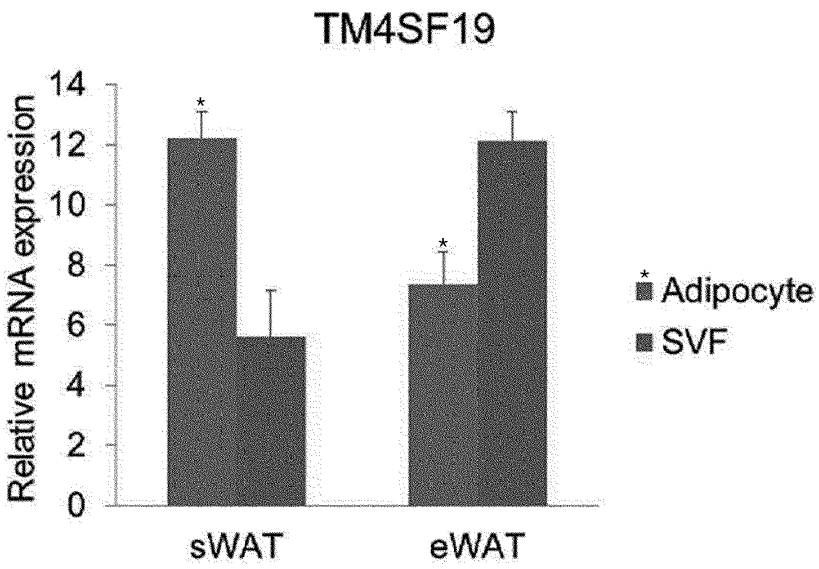
[도24e]


WT
TM4SF19KO
Differentiation - + + - + +
Rosiglitazone - - + - - +
Ucp1
β-actin

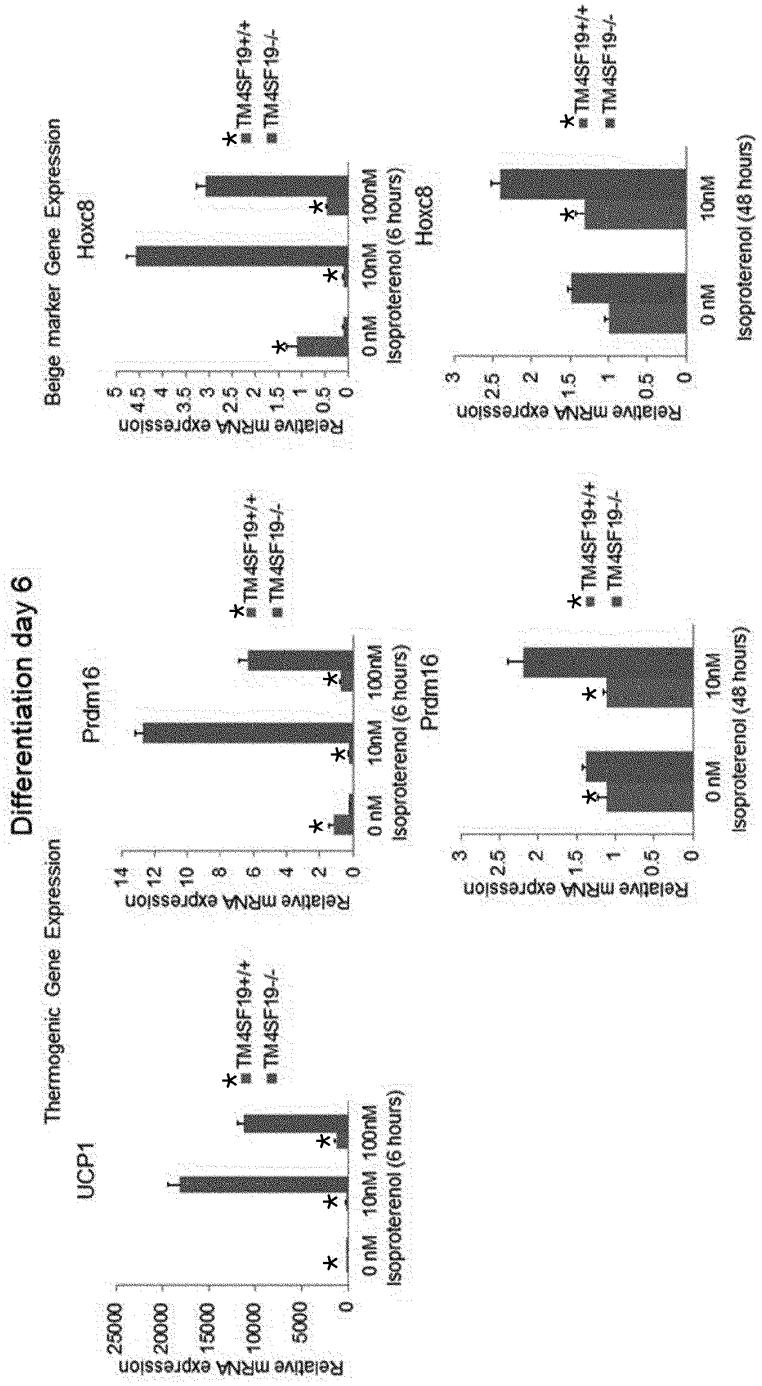
[도25a]


A Components of Lipoaspirate
Oil
Discard
Adipose Tissue
Infranatant
Blood & Local Anaesthetic
Collagenase Digestion
& Centrifugation
SVF Pellet
B Components of Adipose Tissue
Pericytes
+ CD140b+, CD146+, NG2+
- CD31-, CD34-, CD144-, vWF-
Adipocytes
Adipose Derived Stem Cells
+ CD13+, CD29+, CD34+/-, CD44+, CD90+, CD105+
- CD14-, CD31-, CD45-, CD106-, CD144-
? CD146-, αSMA+
Pre-Adipocytes
Endothelial & Progenitor Cells
+ CD31+, CD34+, CD90+, CD146+, VWF+
- CD45-
Haematopoetic Cells
Monocytes/Macrophages
C Components of SVF Pellet
Tissue Culture
Cell-Assisted Lipotransfer

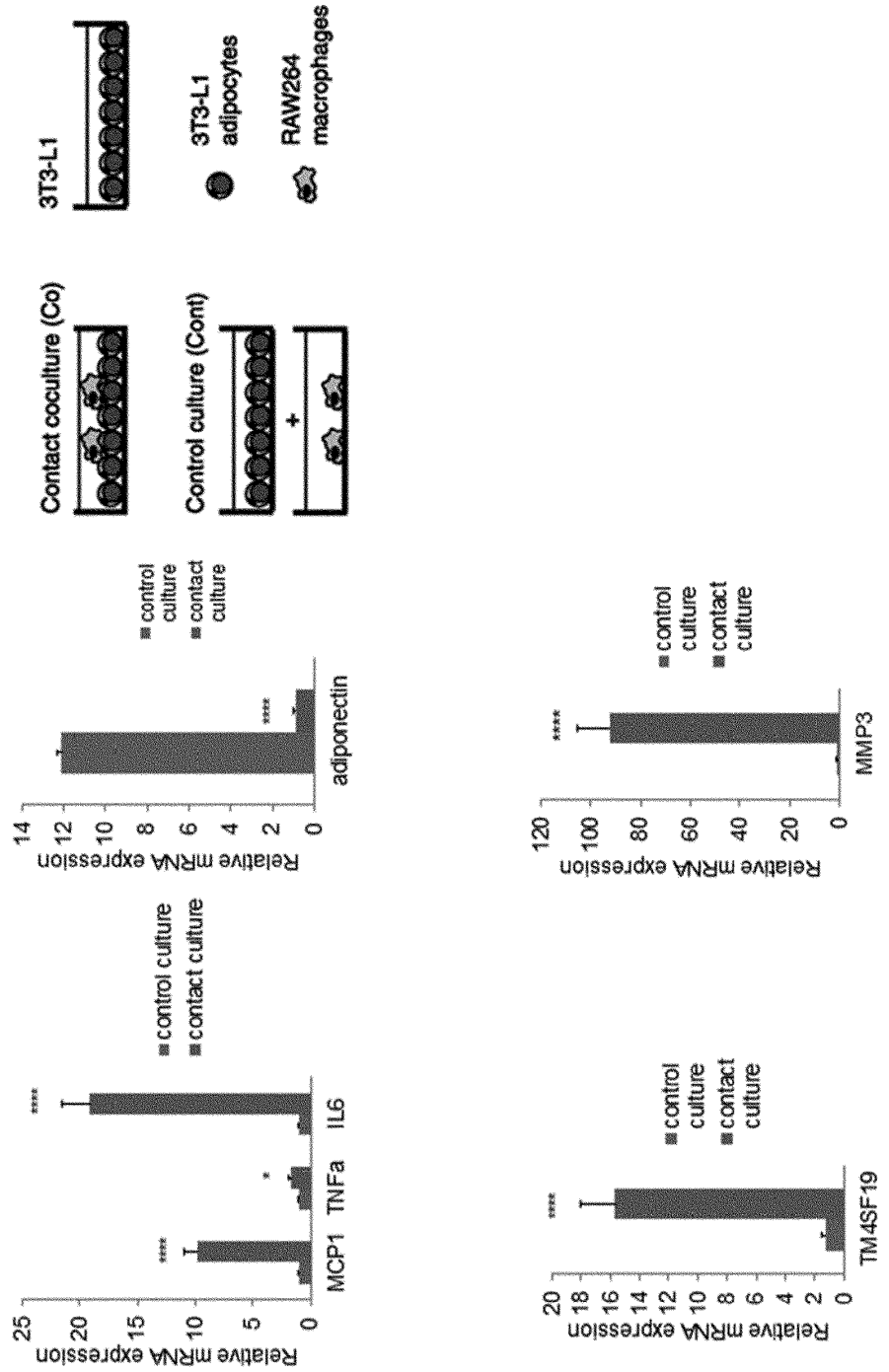
[도25b]


TM4SF19
Relative mRNA expression
14
12
10
8
6
4
2
0
*
*
sWAT
eWAT
* Adipocyte
SVF

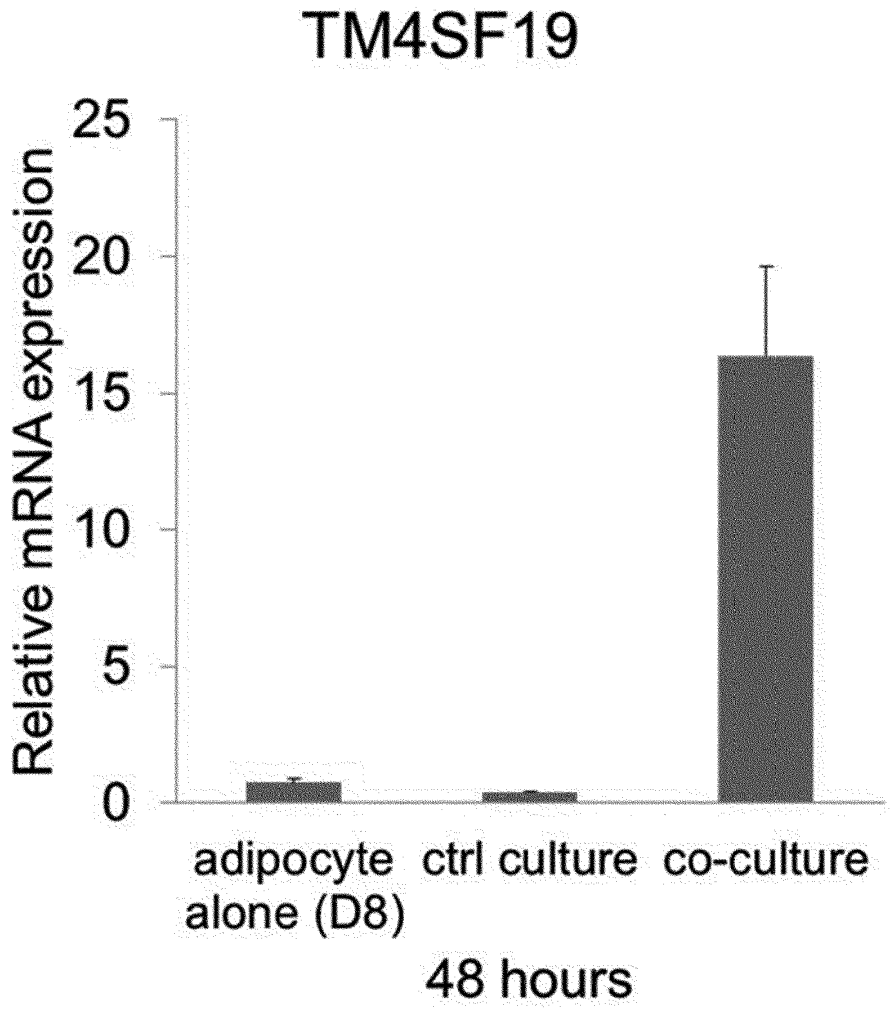
[도26]


Differentiation day 6
Thermogenic Gene Expression
UCP1
Prdm16
Beige marker Gene Expression
Hoxc8
Relative mRNA expression
TM4SF19+/+
TM4SF19-/-
0 nM
10nM
100nM
Isoproterenol (6 hours)
Isoproterenol (48 hours)

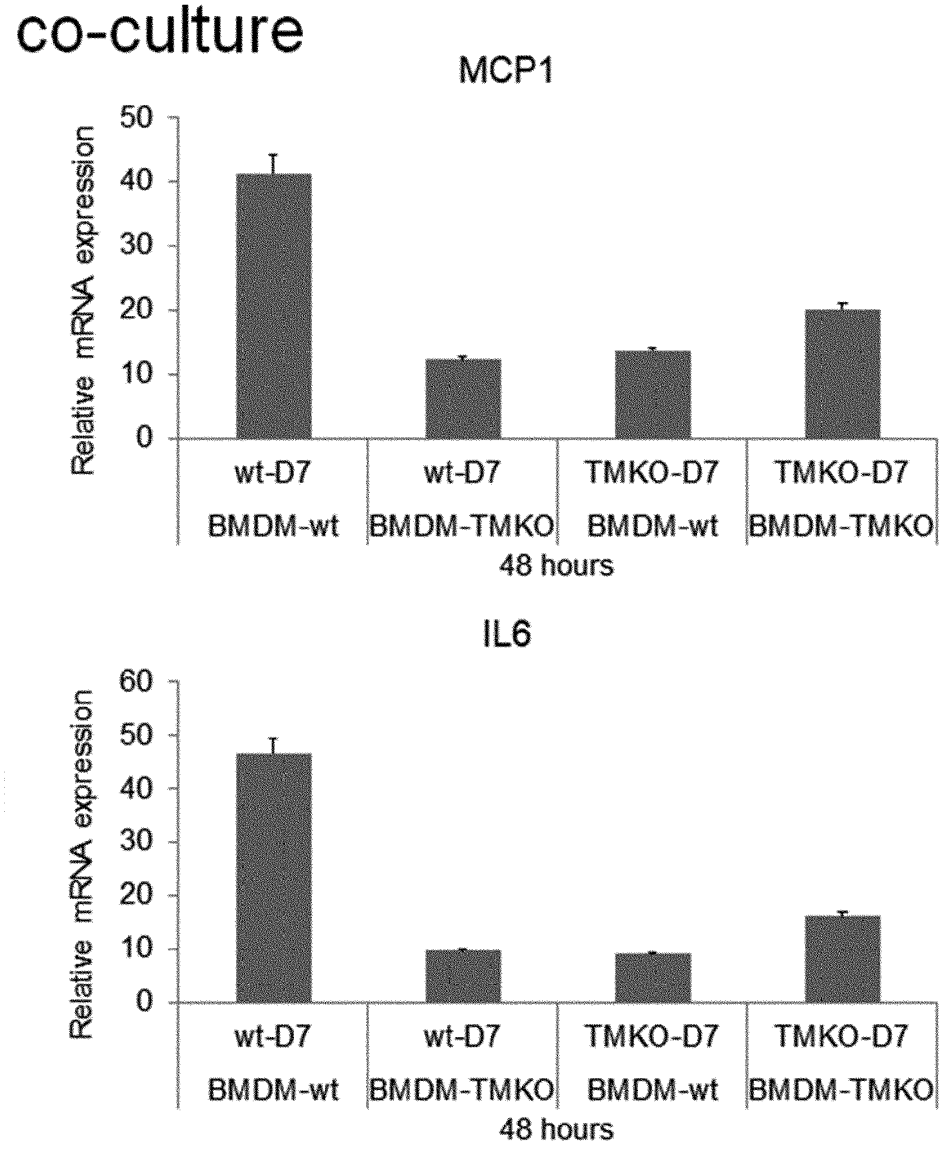
[도27]


3T3-L1
3T3-L1 adipocytes
RAW264 macrophages
Contact coculture (Co)
Control culture (Cont)
control culture
contact culture
Relative mRNA expression
MCP1
TNFa
IL6
adiponectin
MMP3
TM4SF19

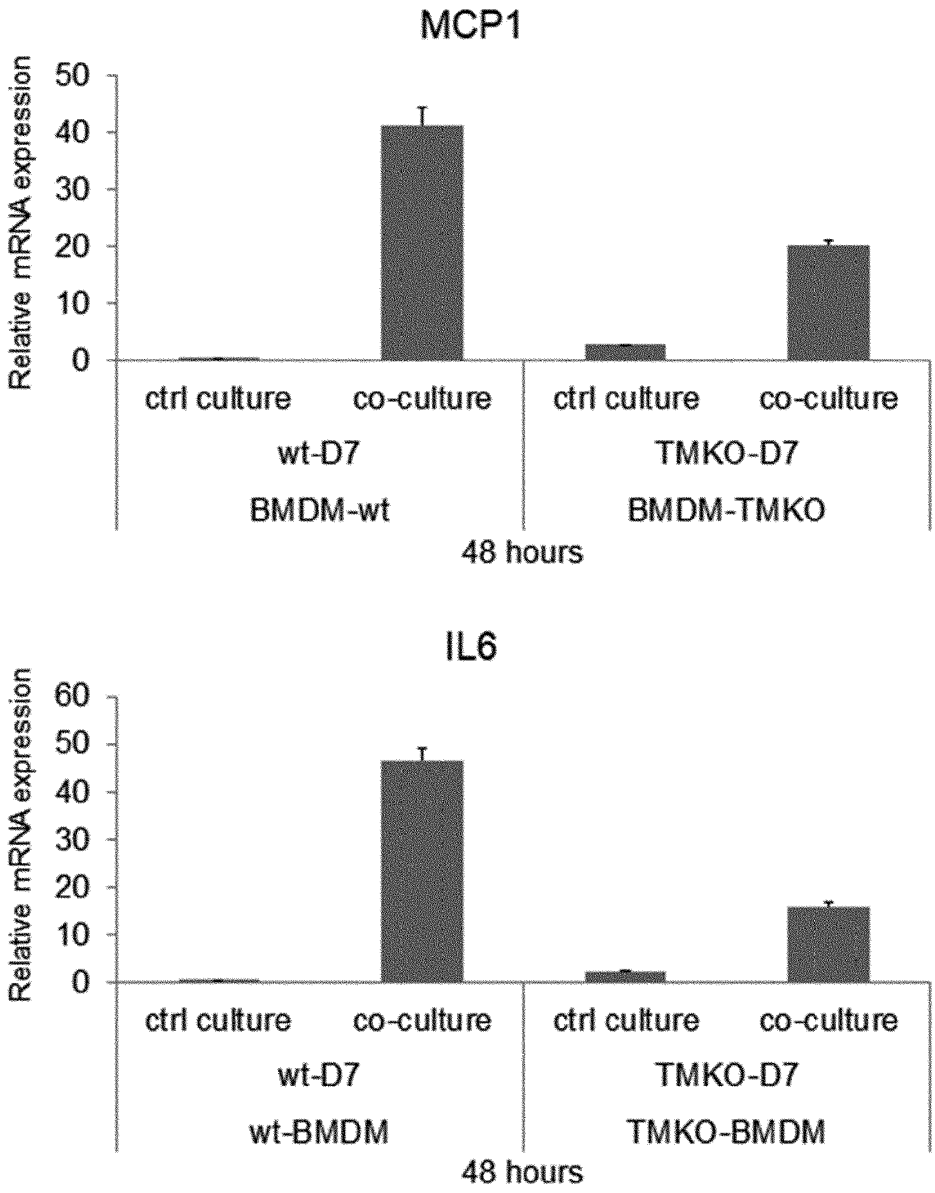
[도28]


TM4SF19
Relative mRNA expression
25
20
15
10
5
0
adipocyte alone (D8)
ctrl culture
co-culture
48 hours

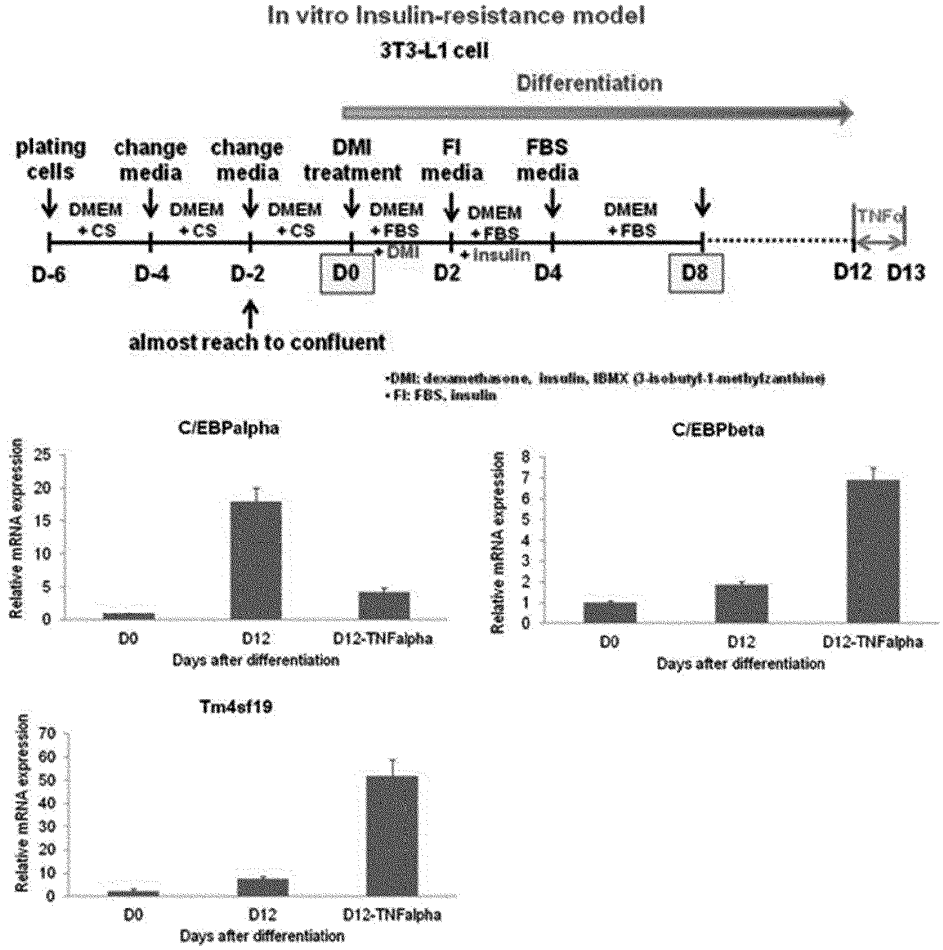
[도29]


co-culture
MCP1
Relative mRNA expression
50
40
30
20
10
0
wt-D7
BMDM-wt
wt-D7
BMDM-TMKO
TMKO-D7
BMDM-wt
TMKO-D7
BMDM-TMKO
48 hours
IL6
Relative mRNA expression
60
50
40
30
20
10
0
wt-D7
BMDM-wt
wt-D7
BMDM-TMKO
TMKO-D7
BMDM-wt
TMKO-D7
BMDM-TMKO
48 hours

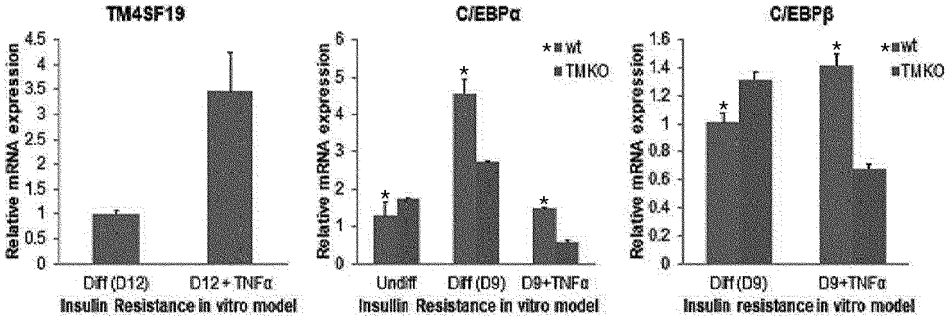
[도30]


MCP1
Relative mRNA expression
50
40
30
20
10
0
ctrl culture
co-culture
ctrl culture
co-culture
wt-D7
TMKO-D7
BMDM-wt
BMDM-TMKO
48 hours
IL6
Relative mRNA expression
60
50
40
30
20
10
0
ctrl culture
co-culture
ctrl culture
co-culture
wt-D7
TMKO-D7
wt-BMDM
TMKO-BMDM
48 hours

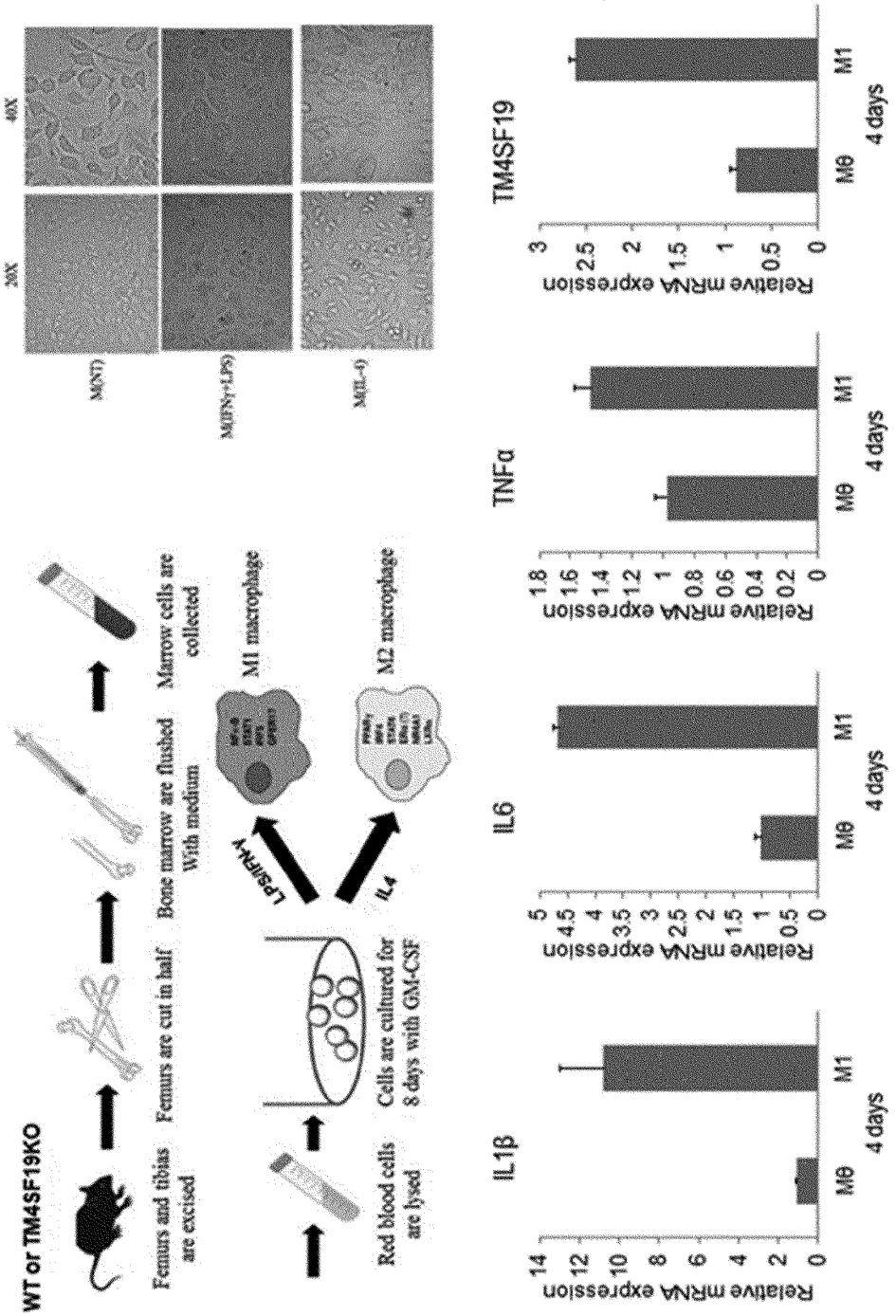
[도31]


In vitro Insulin-resistance model
3T3-L1 cell
Differentiation
plating cells
change media
change media
DMI treatment
FI media
FBS media
DMEM +CS
DMEM +FBS +DMI
DMEM +FBS +Insulin
DMEM +FBS
TNFα
D-6
D-4
D-2
D0
D2
D4
D8
D12
D13
almost reach to confluent
•DMI: dexamethasone, insulin, IBMX (3-isobutyl-1-methylxanthine)
• FI: FBS, insulin
C/EBPalpha
C/EBPbeta
Tm4sf19
Relative mRNA expression
D0
D12
D12-TNFalpha
Days after differentiation

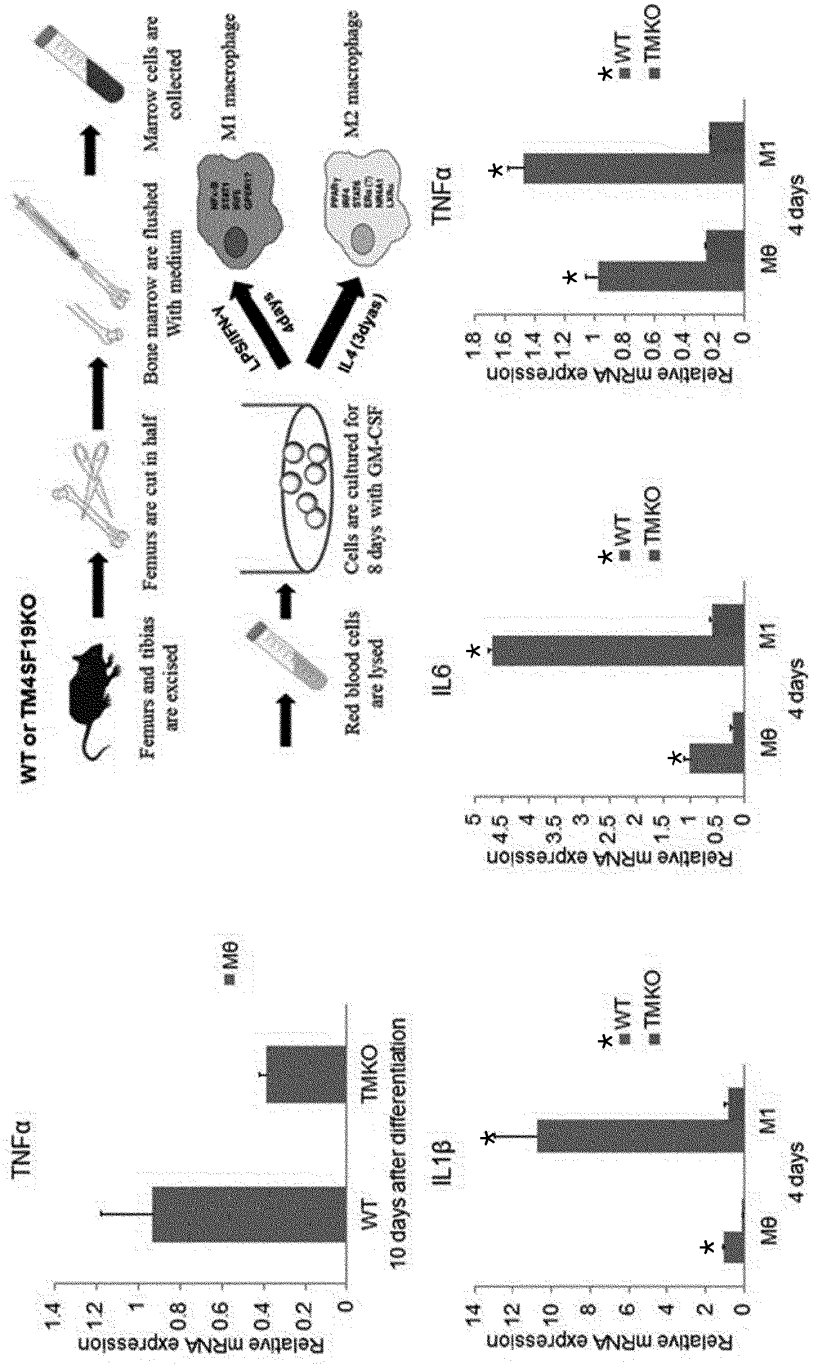
[도32]


TM4SF19
C/EBPα
C/EBPβ
wt
TMKO
Relative mRNA expression
Diff (D12)
D12 + TNFα
Undiff
Diff (D9)
D9+TNFα
Insulin Resistance in vitro model
Insulin resistance in vitro model

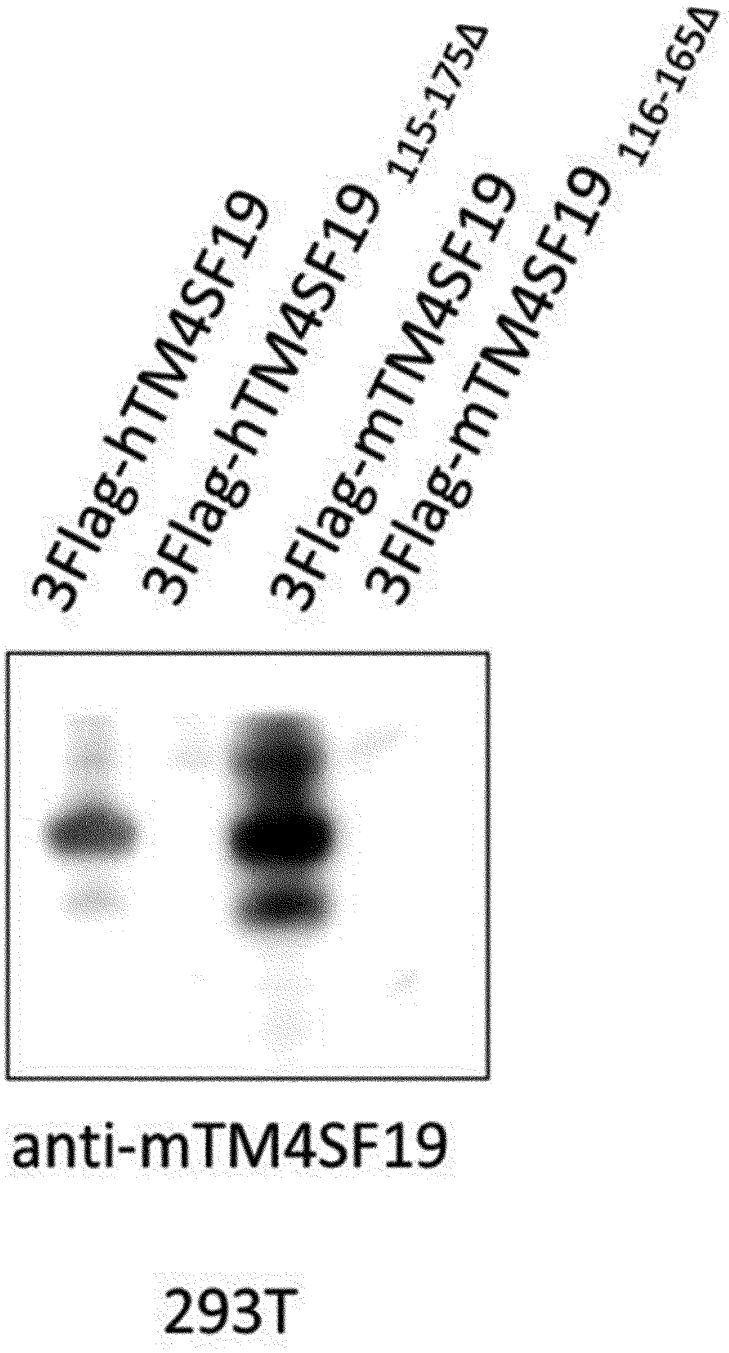
[도33]


WT or TM4SF19KO
Femurs and tibias are excised
Femurs are cut in half
Bone marrow are flushed With medium
Marrow cells are collected
Red blood cells are lysed
Cells are cultured for 8 days with GM-CSF
LPS/IFN-γ
IL4
M1 macrophage
M2 macrophage
M(NT)
M(IFN-γ+LPS)
M(IL-4)
20X
40X
IL1β
IL6
TNFα
TM4SF19
Relative mRNA expression
Mθ
M1
4 days

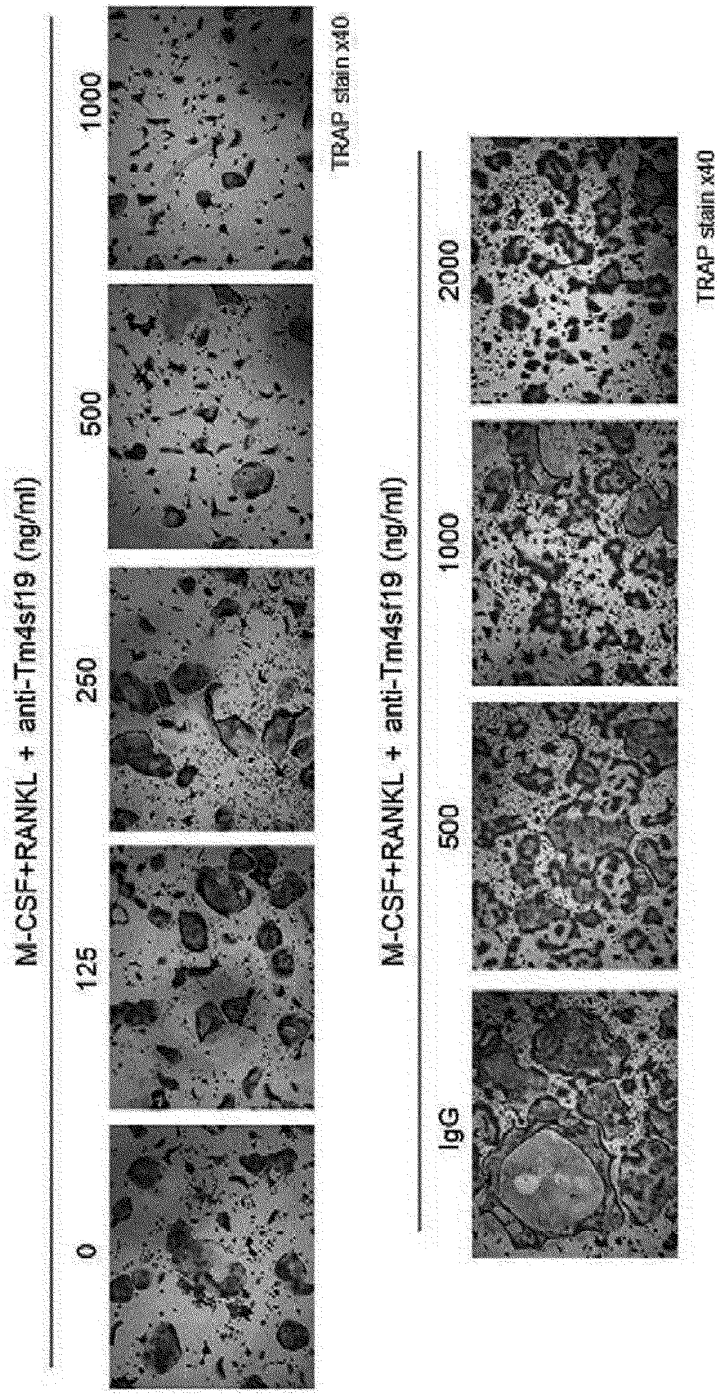
[도34]


WT or TM4SF19KO
Femurs and tibias are excised
Femurs are cut in half
Bone marrow are flushed With medium
Marrow cells are collected
Red blood cells are lysed
Cells are cultured for 8 days with GM-CSF
M1 macrophage
M2 macrophage
LPS/IFN-γ 4days
IL4 (3days)
TNFα
IL6
IL1β
Relative mRNA expression
WT
TMKO
Mθ
M1
4 days
10 days after differentiation

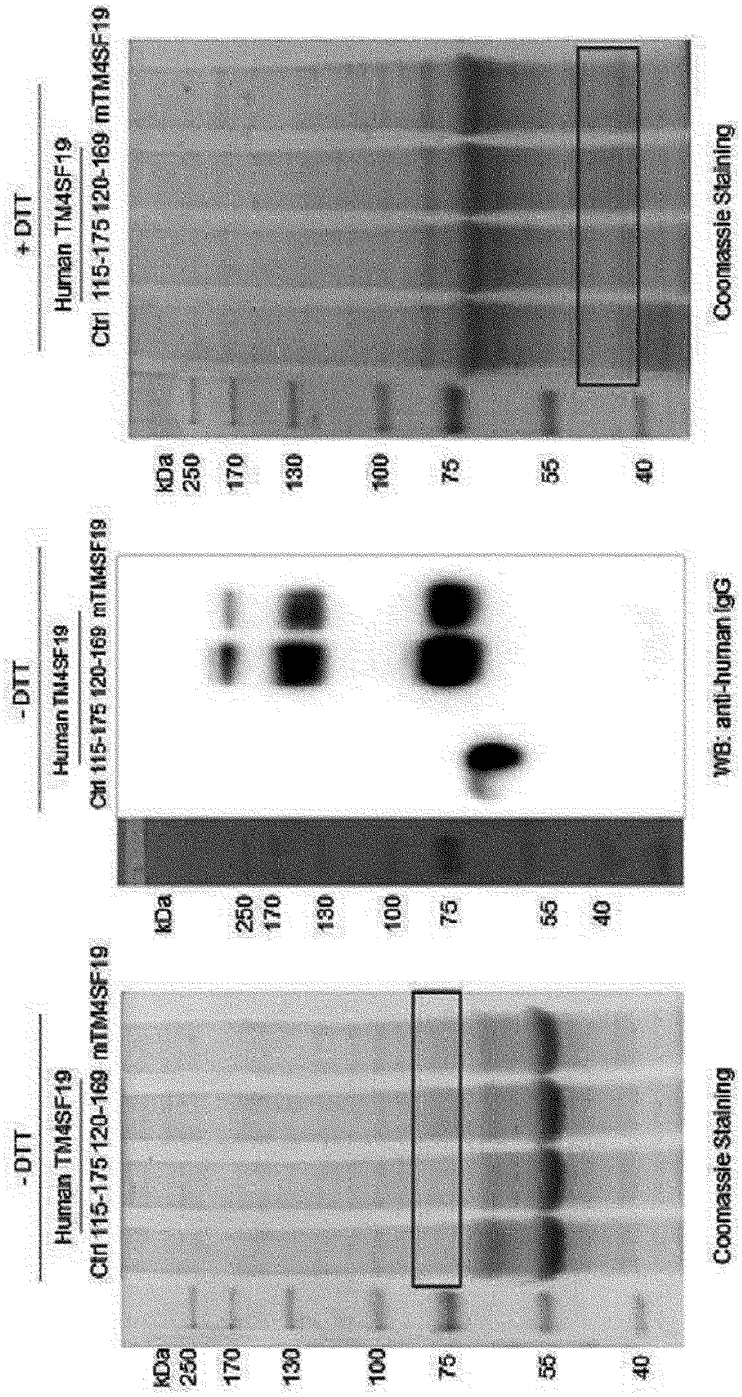
[도35]


3Flag-hTM4SF19
3Flag-hTM4SF19 115-175Δ
3Flag-mTM4SF19
3Flag-mTM4SF19 116-165Δ
anti-mTM4SF19
293T

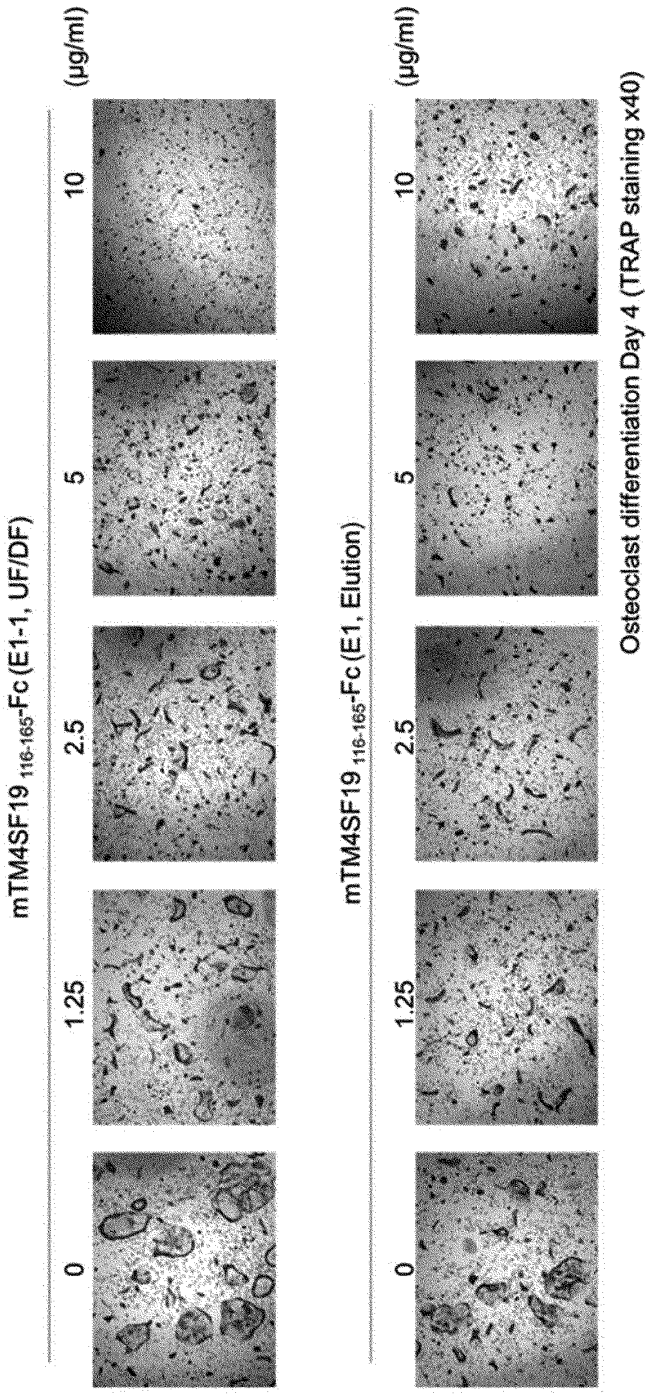
[도36]


M-CSF+RANKL + anti-Tm4sf19 (ng/ml)
0
125
250
500
1000
TRAP stain x40
M-CSF+RANKL + anti-Tm4sf19 (ng/ml)
IgG
500
1000
2000
TRAP stain x40

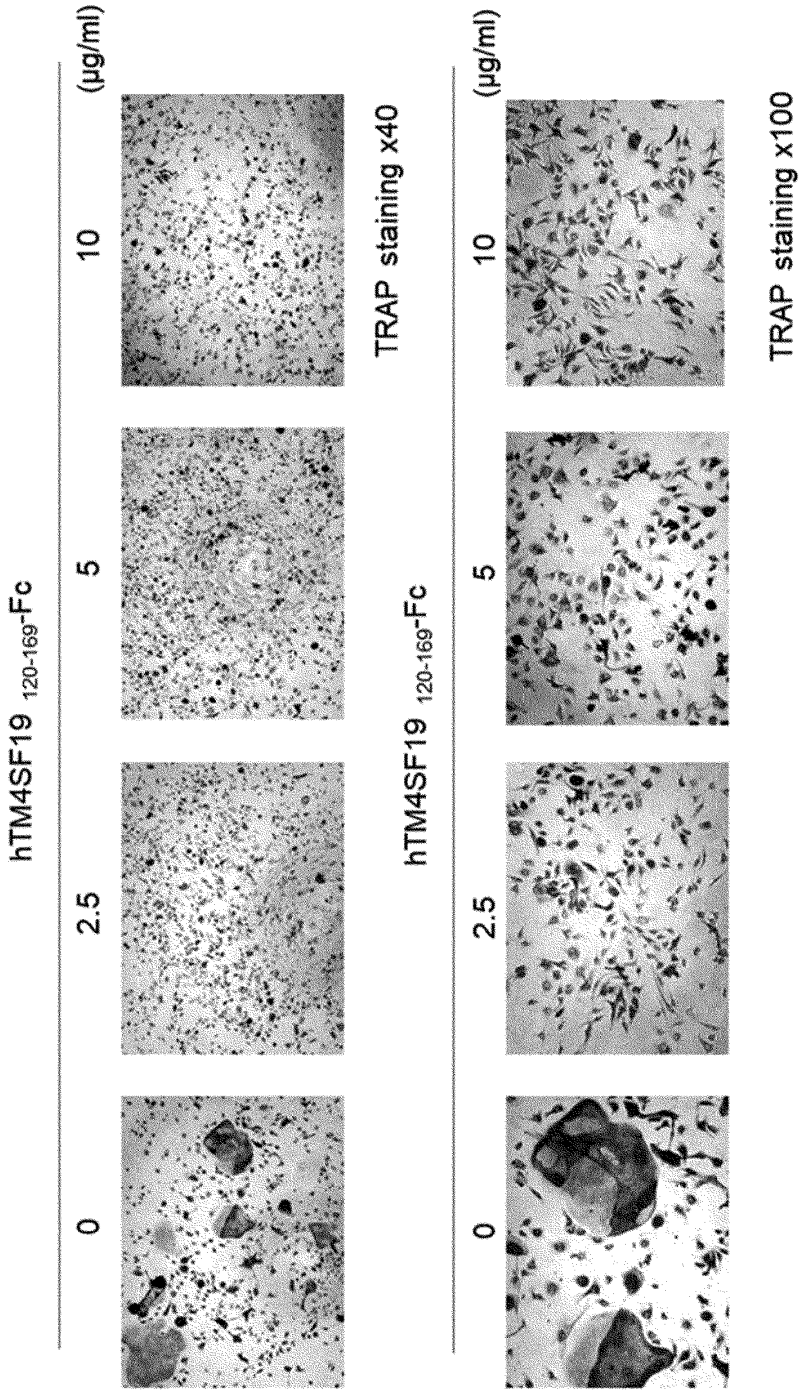
[도37]


- DTT
Human TM4SF19
Ctrl 115-175 120-169 mTM4SF19
kDa
250
170
130
100
75
55
40
Coomassie Staining
- DTT
Human TM4SF19
Ctrl 115-175 120-169 mTM4SF19
WB: anti-human IgG
+ DTT
Human TM4SF19
Ctrl 115-175 120-169 mTM4SF19
Coomassie Staining

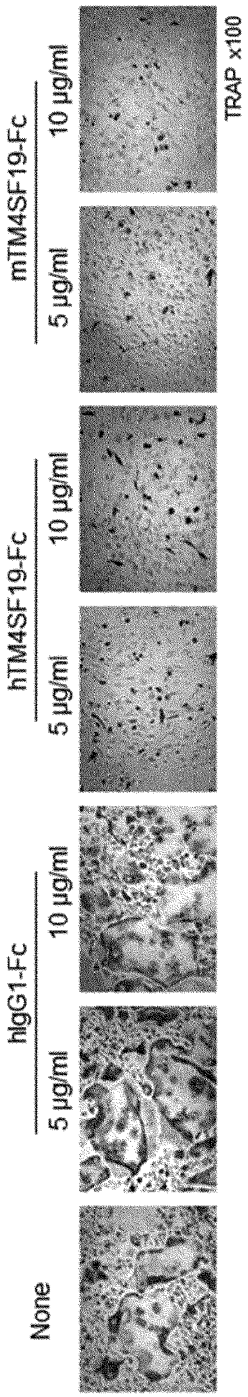
[도38a]


mTM4SF19 116-165-Fc (E1-1, UF/DF)
(μg/ml)
0
1.25
2.5
5
10
mTM4SF19 116-165-Fc (E1, Elution)
(μg/ml)
0
1.25
2.5
5
10
Osteoclast differentiation Day 4 (TRAP staining x40)

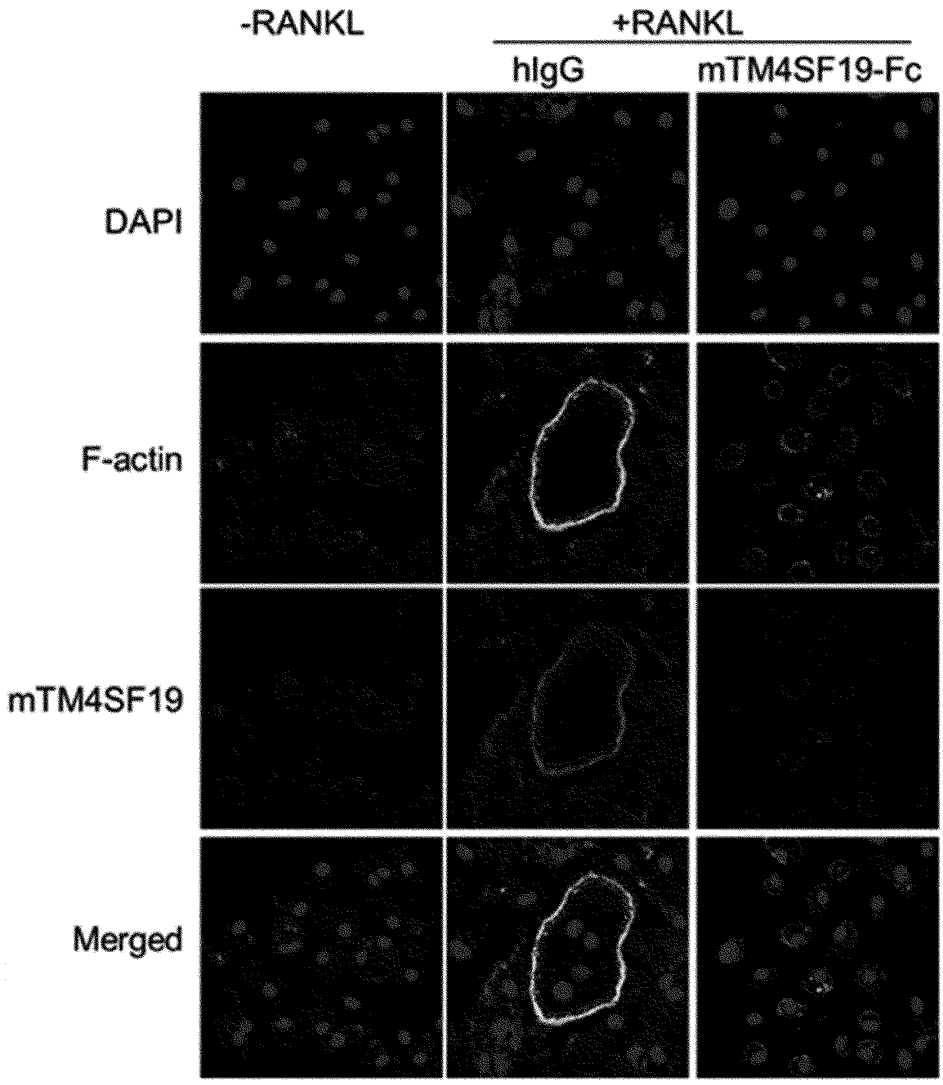
[도38b]


hTM4SF19 120-169-Fc
0
2.5
5
10
(μg/ml)
TRAP staining x40
hTM4SF19 120-169-Fc
0
2.5
5
10
(μg/ml)
TRAP staining x100

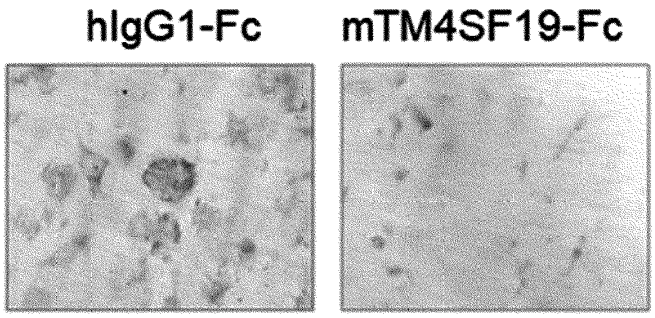
[도38c]


TRAP x100
mTM4SF19-Fc
10 μg/ml
5 μg/ml
hTM4SF19-Fc
10 μg/ml
5 μg/ml
hIgG1-Fc
10 μg/ml
5 μg/ml
None

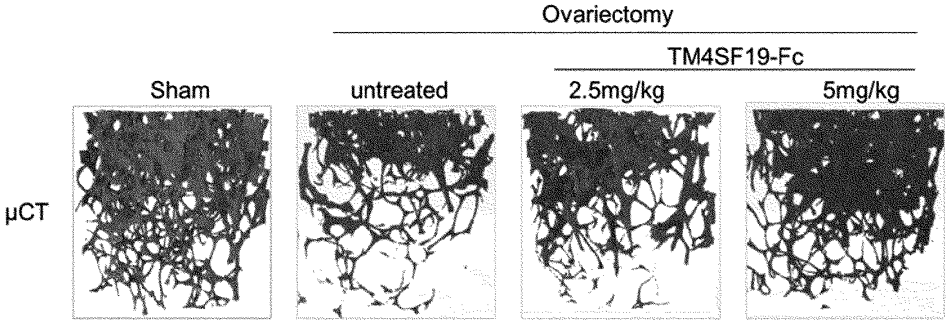
[도38d]


-RANKL
+RANKL
hIgG
mTM4SF19-Fc
DAPI
F-actin
mTM4SF19
Merged

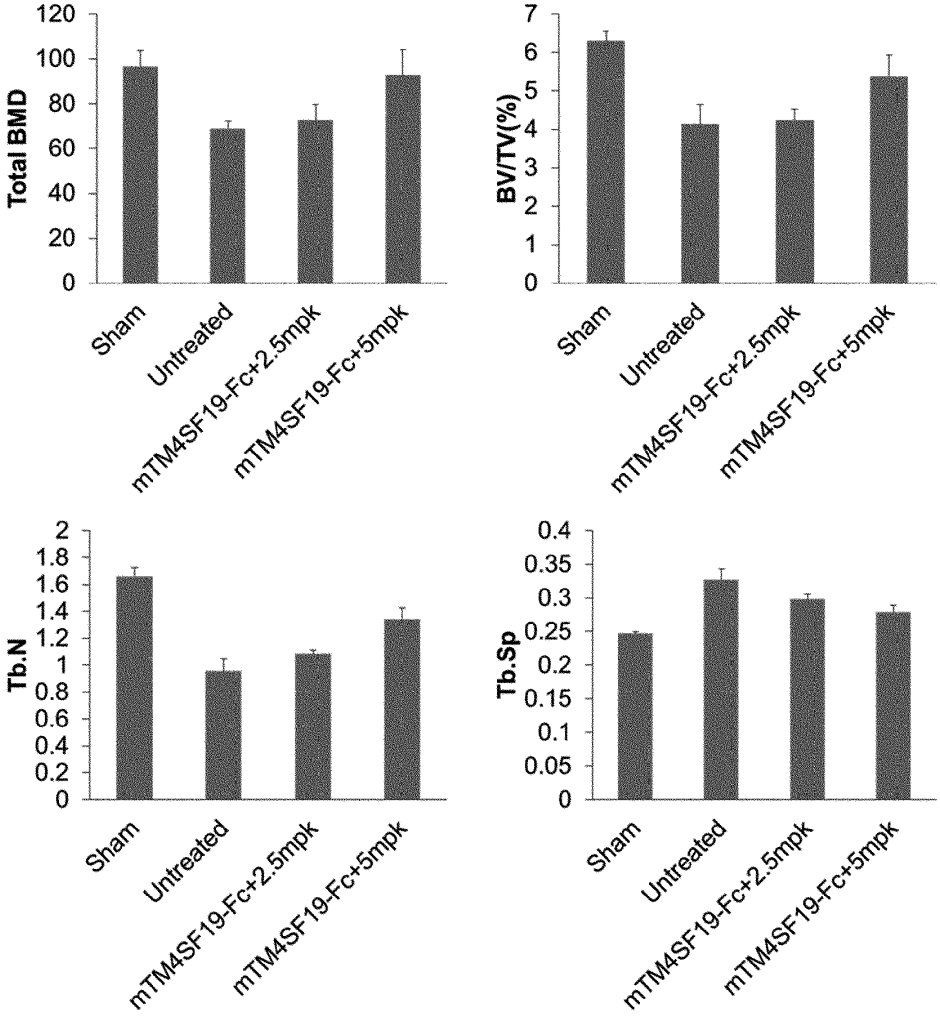
[도38e]


hIgG1-Fc
mTM4SF19-Fc

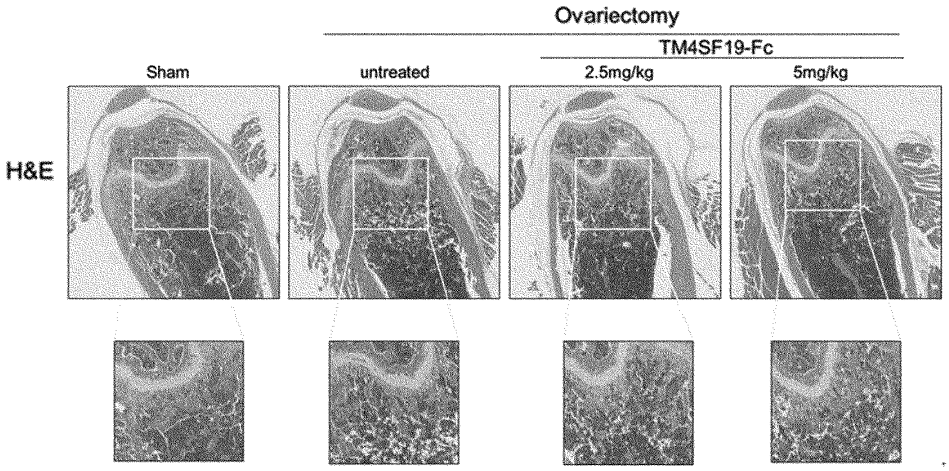
[도39a]


Ovariectomy
TM4SF19-Fc
Sham
untreated
2.5mg/kg
5mg/kg
μCT

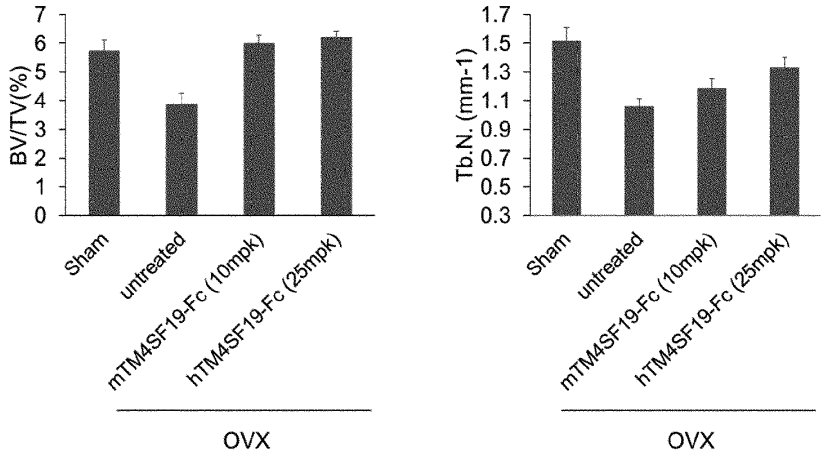
[도39b]


Total BMD
BV/TV(%)
Tb.N
Tb.Sp
Sham
Untreated
mTM4SF19-Fc+2.5mpk
mTM4SF19-Fc+5mpk

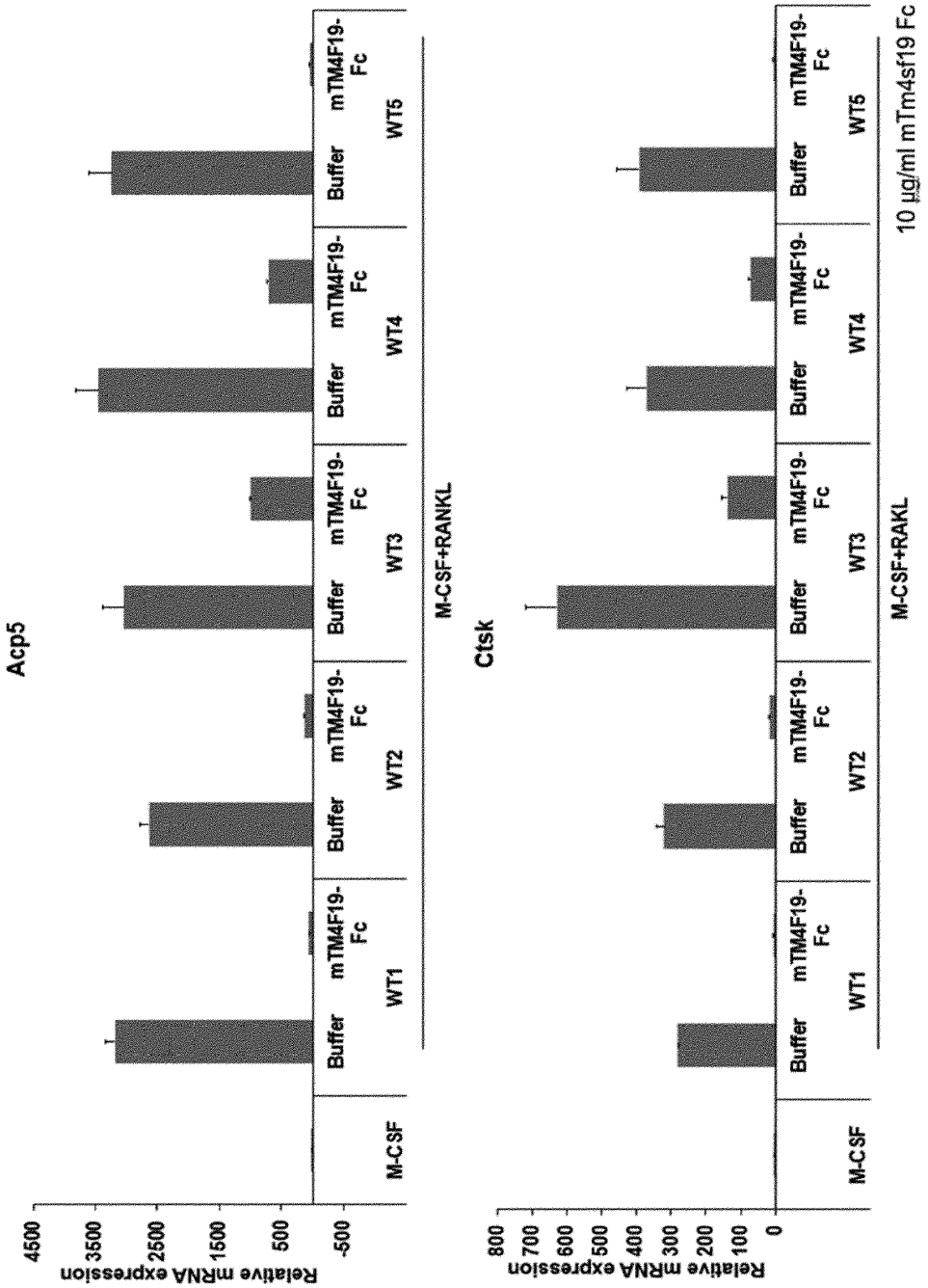
[도39c]


Ovariectomy
TM4SF19-Fc
Sham
untreated
2.5mg/kg
5mg/kg
H&E

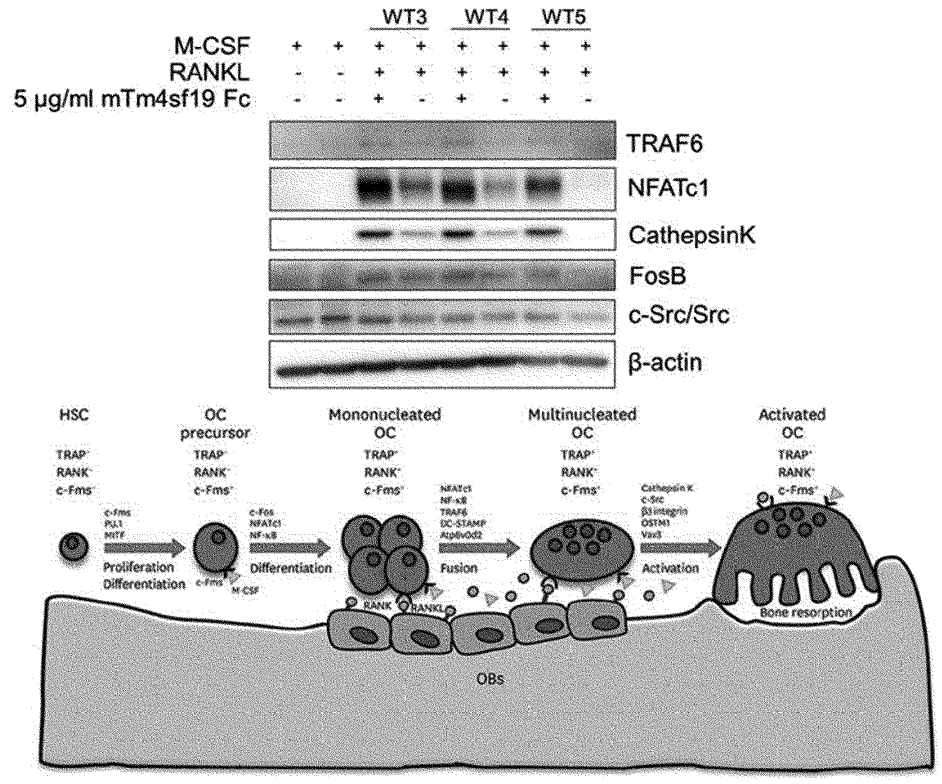
[도39d]


BV/TV(%)
7
6
5
4
3
2
1
0
Sham
untreated
mTM4SF19-Fc (10mpk)
hTM4SF19-Fc (25mpk)
OVX
Tb.N. (mm-1)
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
Sham
untreated
mTM4SF19-Fc (10mpk)
hTM4SF19-Fc (25mpk)
OVX

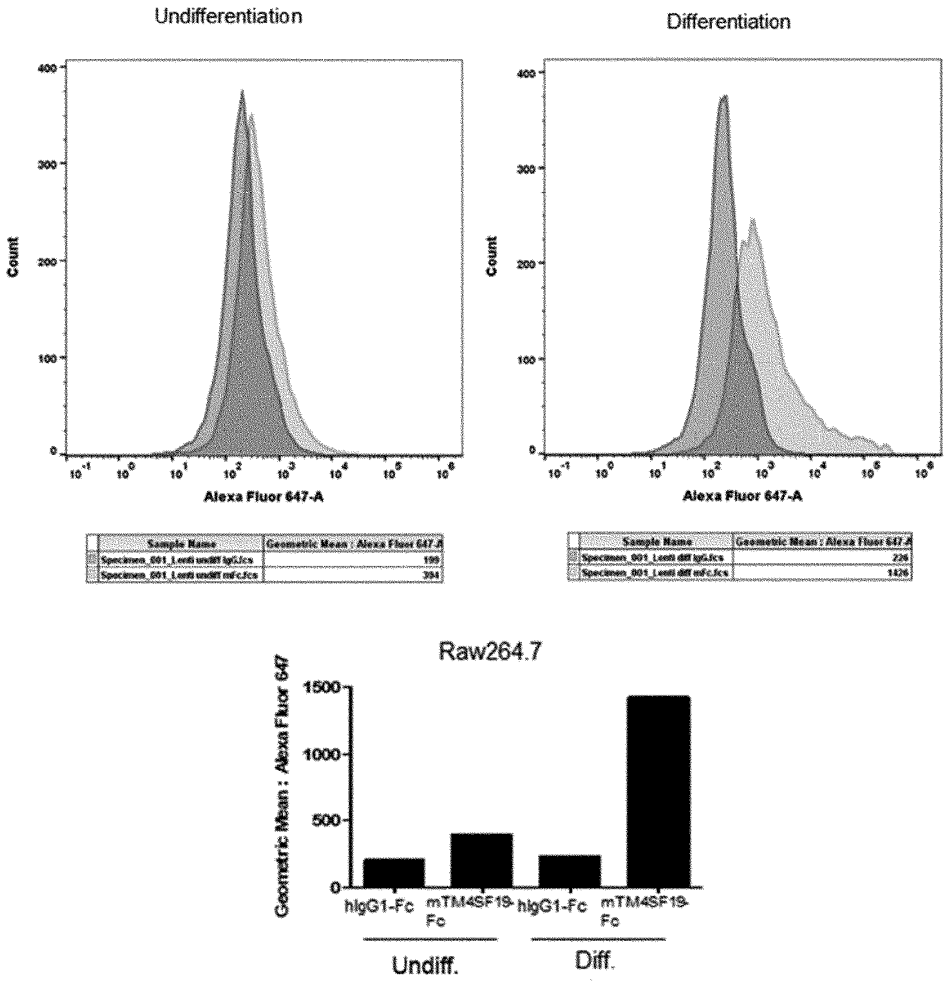
[도40a]


Acp5
Relative mRNA expression
4500
3500
2500
1500
500
-500
M-CSF
Buffer
mTM4F19-Fc
WT1
WT2
WT3
WT4
WT5
M-CSF+RANKL
Ctsk
800
700
600
500
400
300
200
100
0
M-CSF+RAKL
10 μg/ml mTm4sf19 Fc

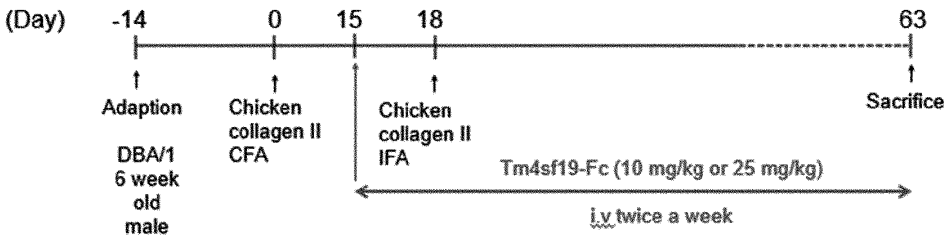
[도40b]


WT3
WT4
WT5
M-CSF
RANKL
5 μg/ml mTm4sf19 Fc
TRAF6
NFATc1
CathepsinK
FosB
c-Src/Src
β-actin
HSC
OC precursor
Mononucleated OC
Multinucleated OC
Activated OC
TRAP-
RANK-
c-Fms-
TRAP+
RANK+
c-Fms+
c-Fms
PU.1
MITF
Proliferation
Differentiation
c-Fos
NFATc1
NF-κB
Differentiation
M-CSF
NFATc1
NF-κB
TRAF6
DC-STAMP
Atp6v0d2
Fusion
RANK
RANKL
Cathepsin K
c-Src
β3 integrin
OSTM1
Vav3
Activation
Bone resorption
OBs

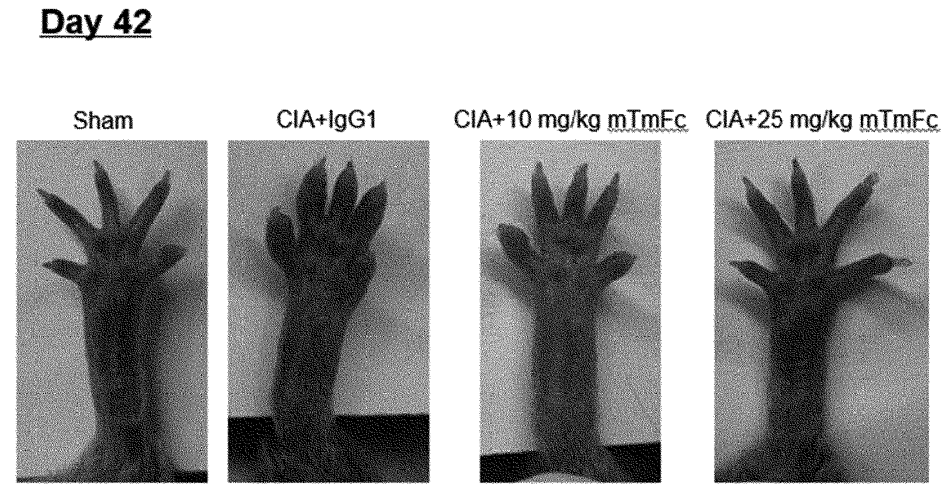
[도41]


Undifferentiation
Differentiation
Count
Alexa Fluor 647-A
Sample Name
Geometric Mean : Alexa Fluor 647-A
Raw264.7
Geometric Mean : Alexa Fluor 647
hIgG1-Fc
mTM4SF19-Fc
Undiff.
Diff.

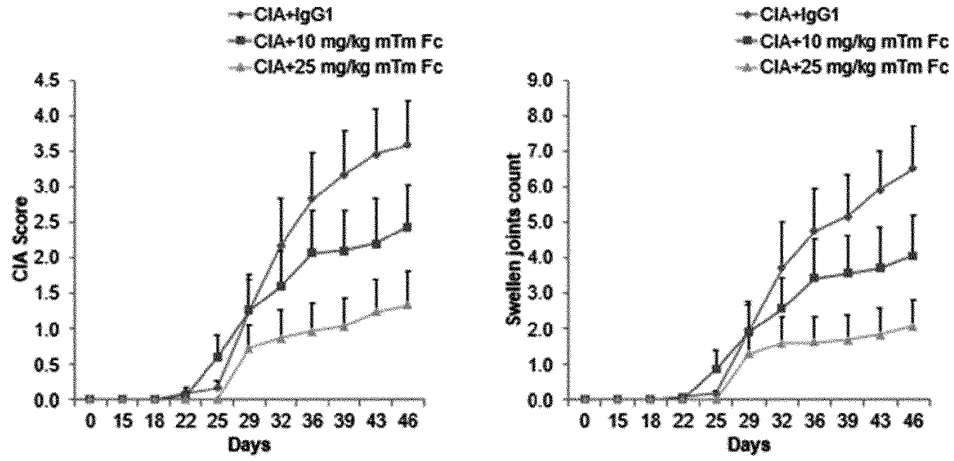
[도42a]


(Day)
-14
0
15
18
63
Adaption
DBA/1
6 week
old
male
Chicken
collagen II
CFA
Chicken
collagen II
IFA
Sacrifice
Tm4sf19-Fc (10 mg/kg or 25 mg/kg)
i.v twice a week

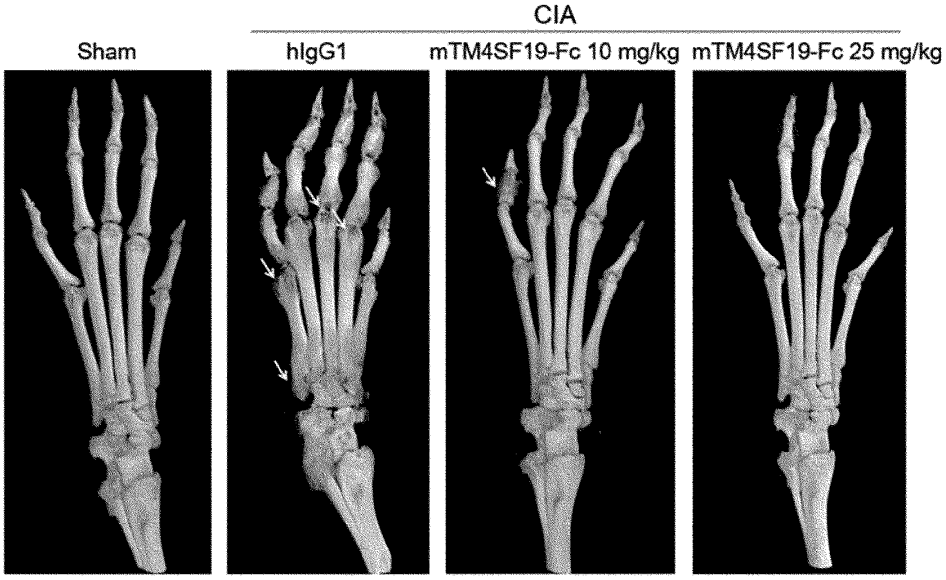
[도42b]



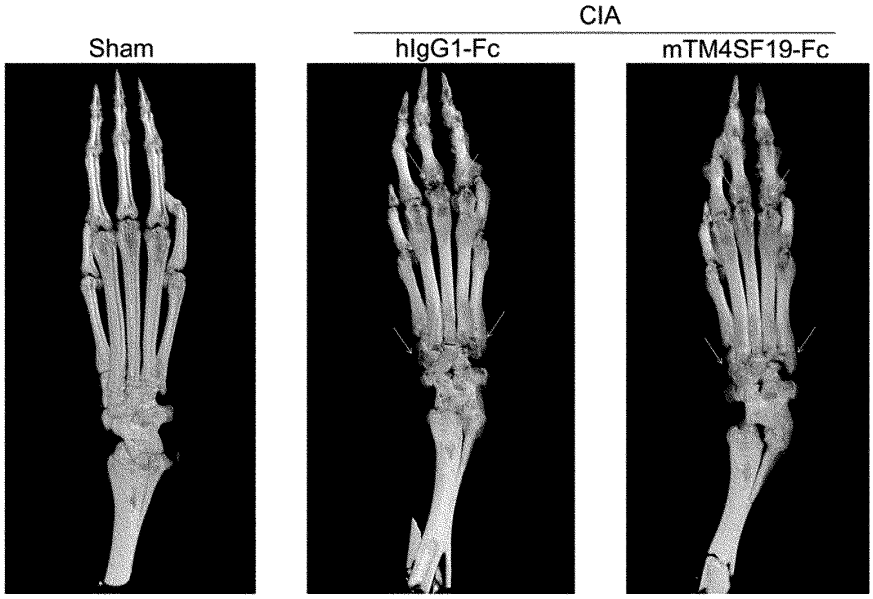
[도42c]



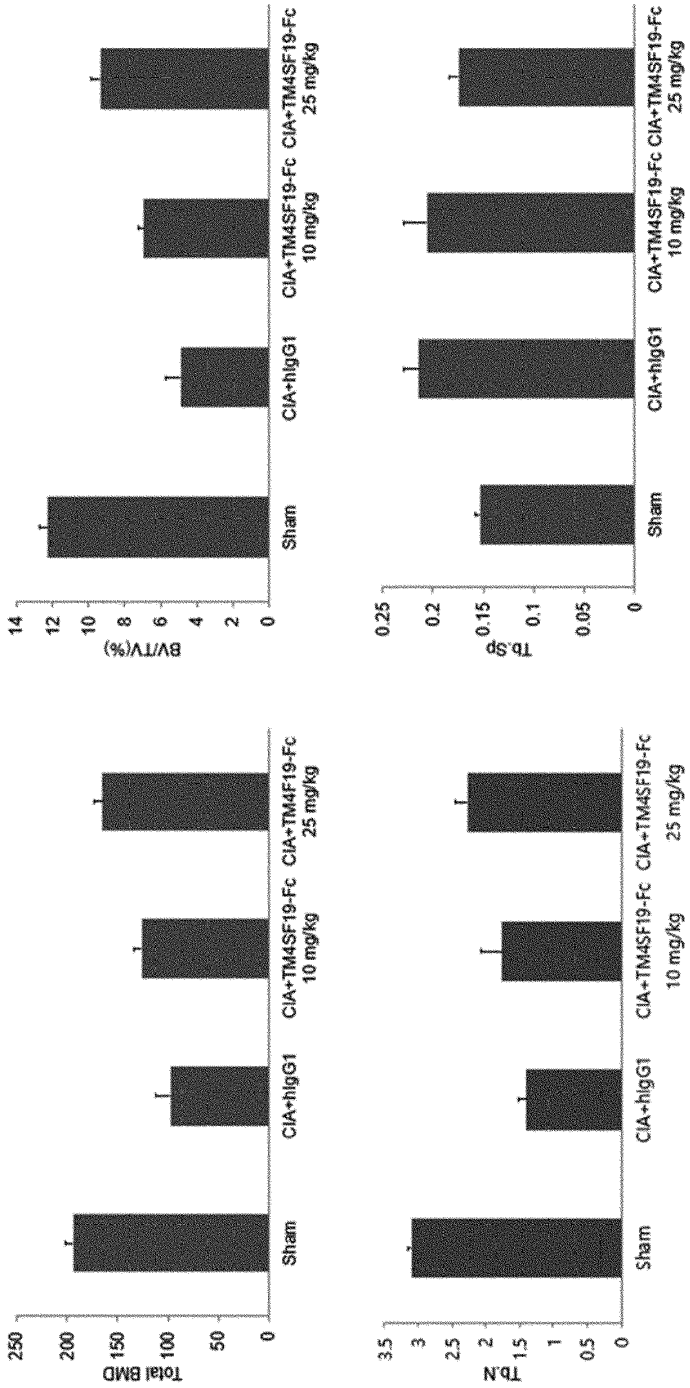
[도42d]



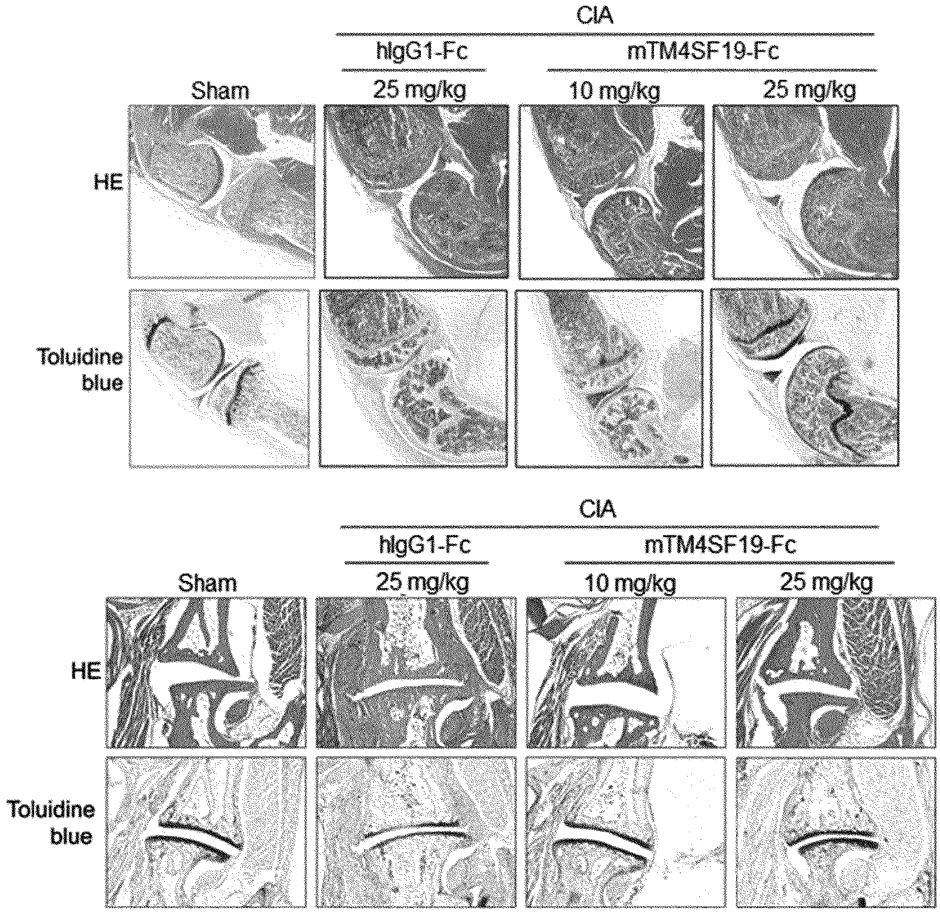
[도42e]



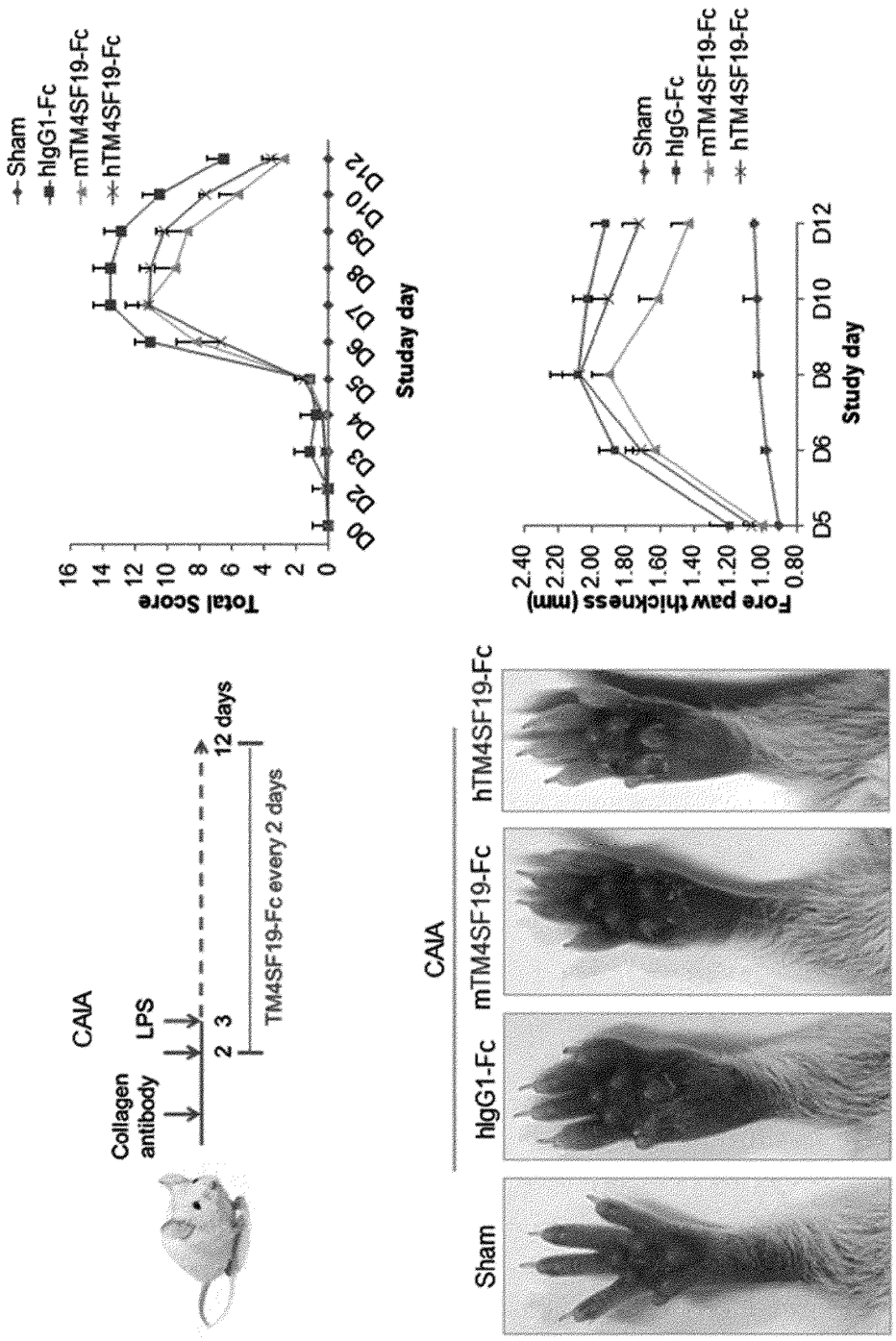
[도42f]


BV/TV(%)
14
12
10
8
6
4
2
0
Tb.Sp
0.25
0.2
0.15
0.1
0.05
0
Total BMD
250
200
150
100
50
0
Tb.N
3.5
3
2.5
2
1.5
1
0.5
0
Sham
CIA+hIgG1
CIA+TM4SF19-Fc 10 mg/kg
CIA+TM4SF19-Fc 25 mg/kg

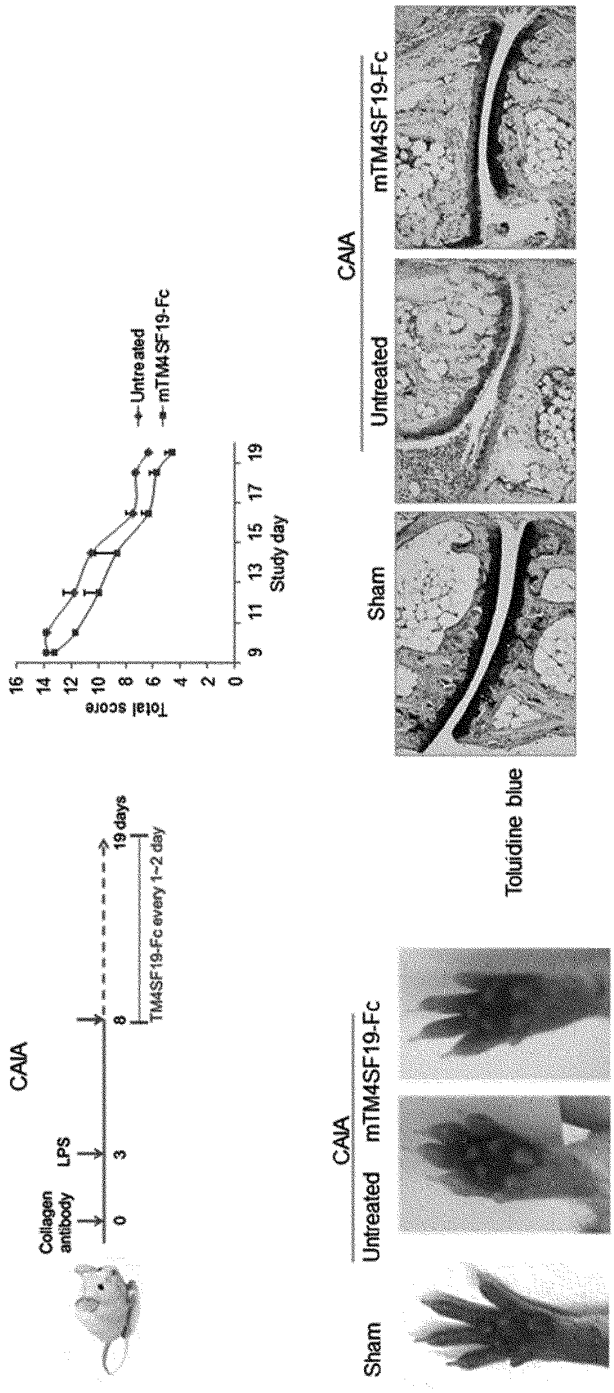
[도42g]


CIA
hIgG1-Fc
mTM4SF19-Fc
Sham
25 mg/kg
10 mg/kg
25 mg/kg
HE
Toluidine blue
CIA
hIgG1-Fc
mTM4SF19-Fc
Sham
25 mg/kg
10 mg/kg
25 mg/kg
HE
Toluidine blue

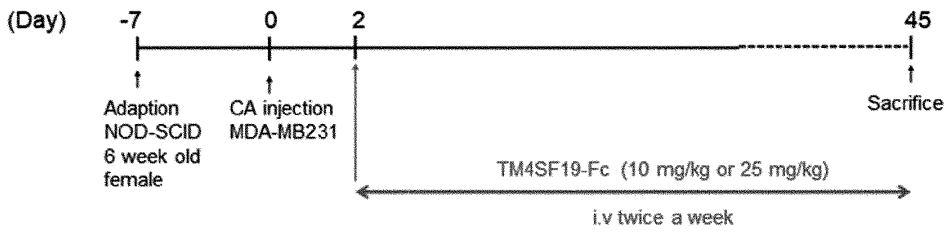
[도42h]


Sham
hIgG1-Fc
mTM4SF19-Fc
hTM4SF19-Fc
Total Score
Studay day
D0 D2 D3 D4 D5 D6 D7 D8 D9 D10 D12
Fore paw thickness (mm)
Study day
D5 D6 D8 D10 D12
CAIA
Collagen antibody
LPS
2 3
12 days
TM4SF19-Fc every 2 days
Sham
hIgG1-Fc
mTM4SF19-Fc
hTM4SF19-Fc

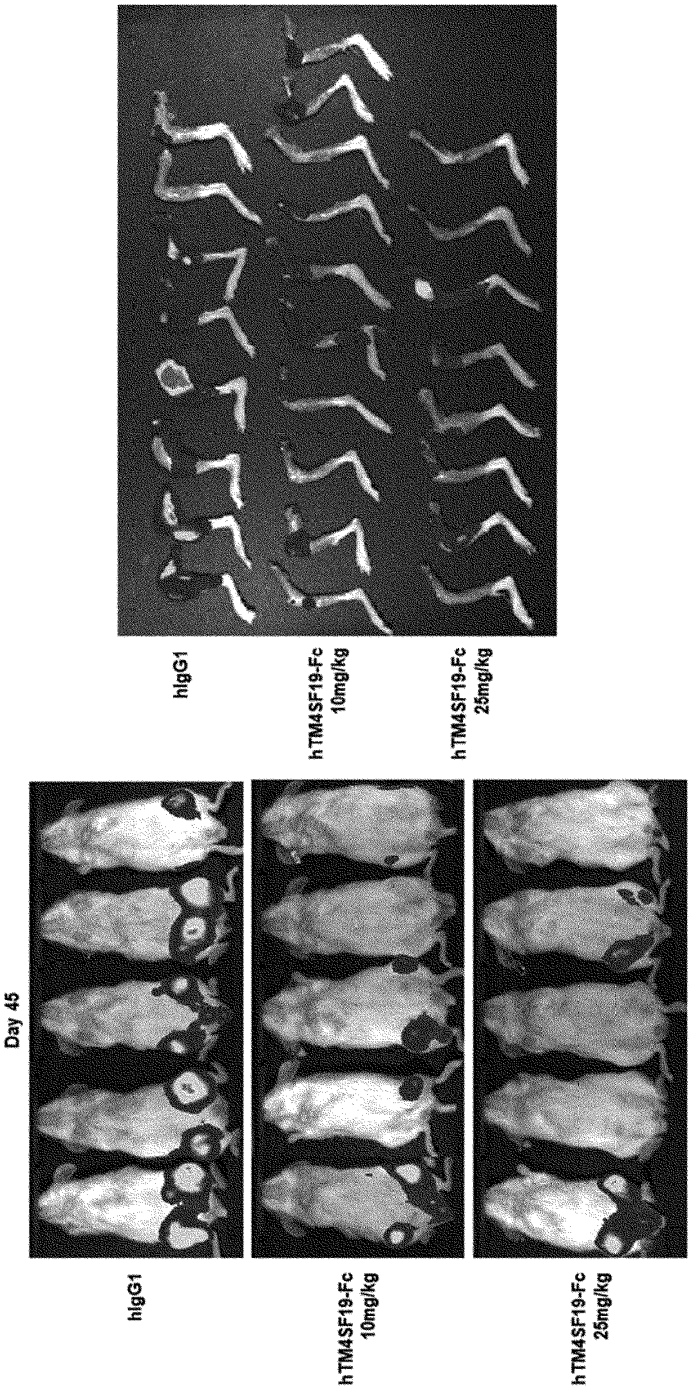
[도42i]


Untreated
mTM4SF19-Fc
Total score
Study day
CAIA
Sham
Untreated
mTM4SF19-Fc
Toluidine blue
Collagen antibody
LPS
CAIA
19 days
TM4SF19-Fc every 1~2 day

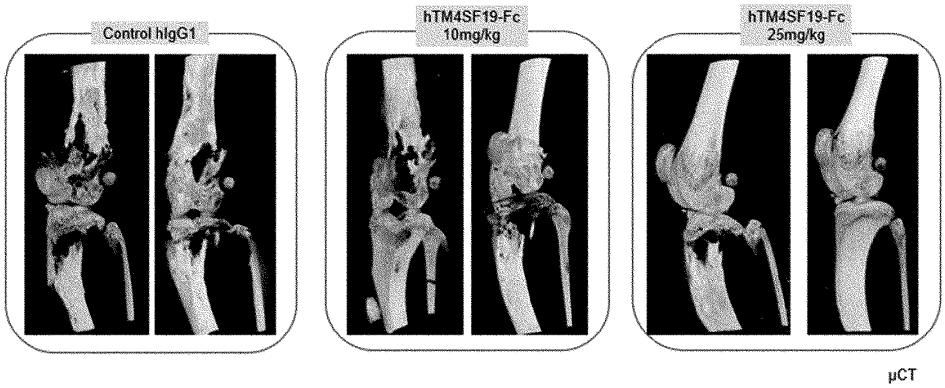
[도43a]


(Day)
-7
0
2
45
Adaption
NOD-SCID
6 week old
female
CA injection
MDA-MB231
Sacrifice
TM4SF19-Fc (10 mg/kg or 25 mg/kg)
i.v twice a week

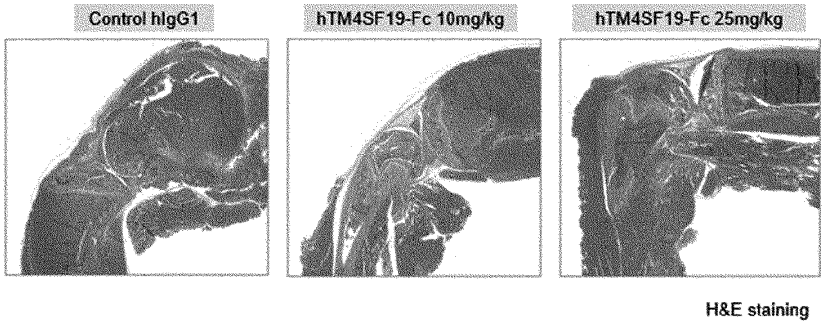
[도43b]


hIgG1
hTM4SF19-Fc 10mg/kg
hTM4SF19-Fc 25mg/kg
Day 45
hIgG1
hTM4SF19-Fc 10mg/kg
hTM4SF19-Fc 25mg/kg

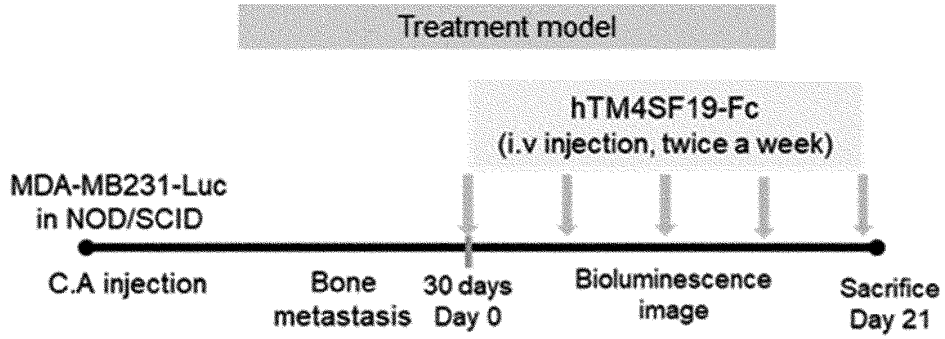
[도43c]



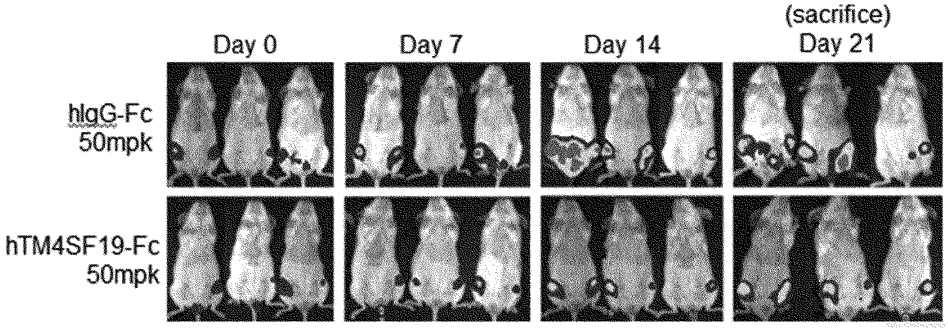
[도43d]



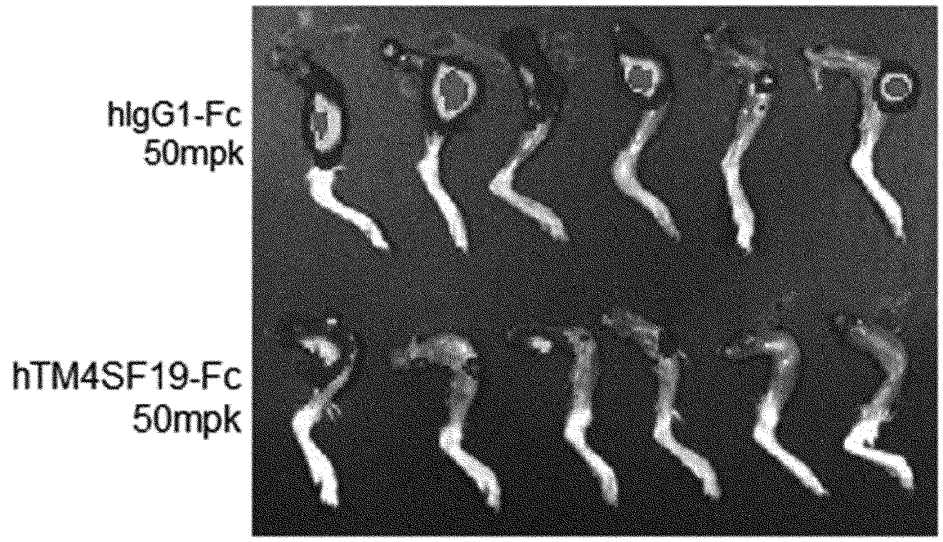
[도44a]



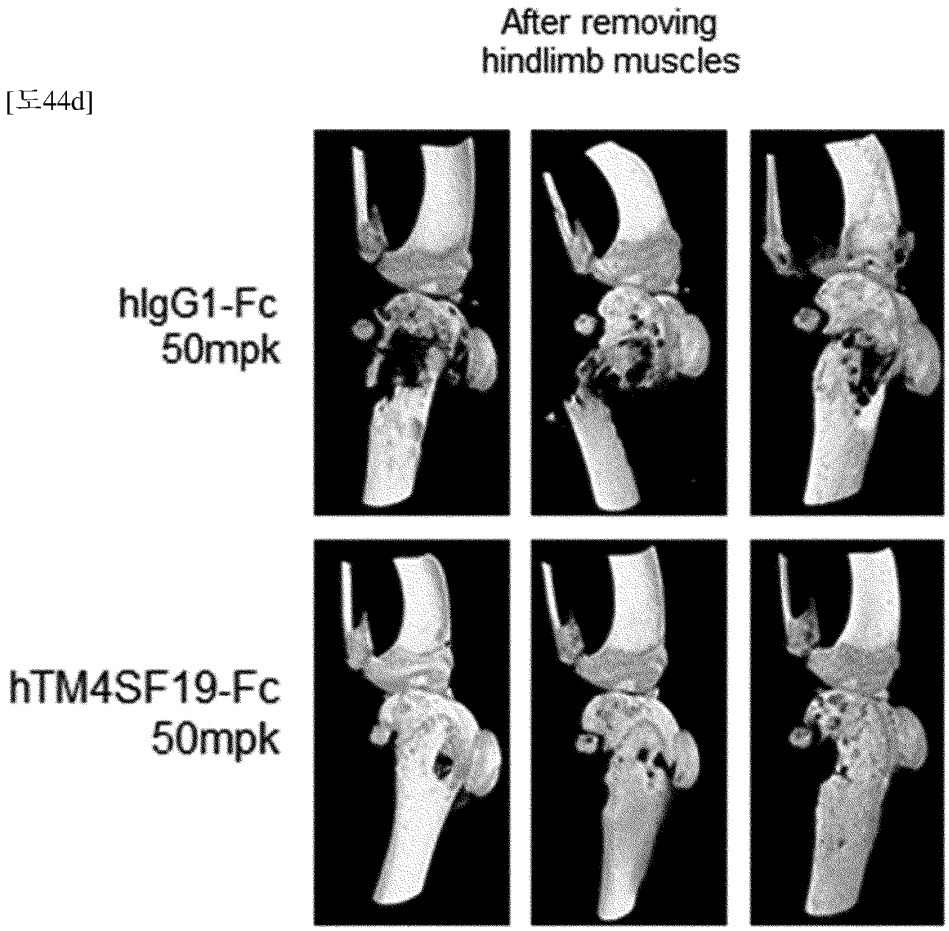
[도44b]



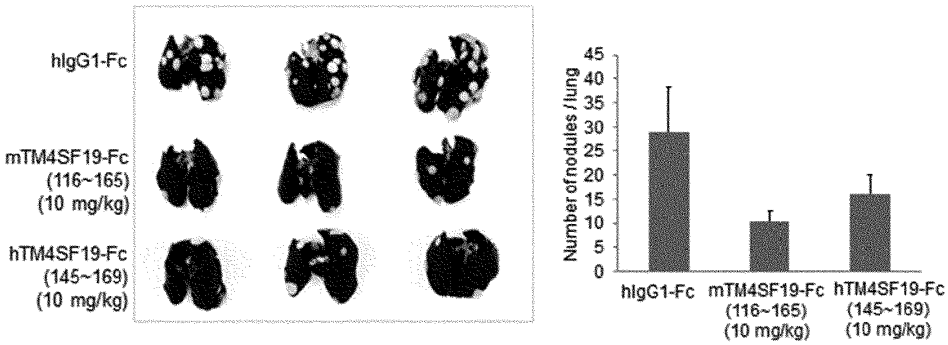
[도44c]


hIgG1-Fc
50mpk
hTM4SF19-Fc
50mpk

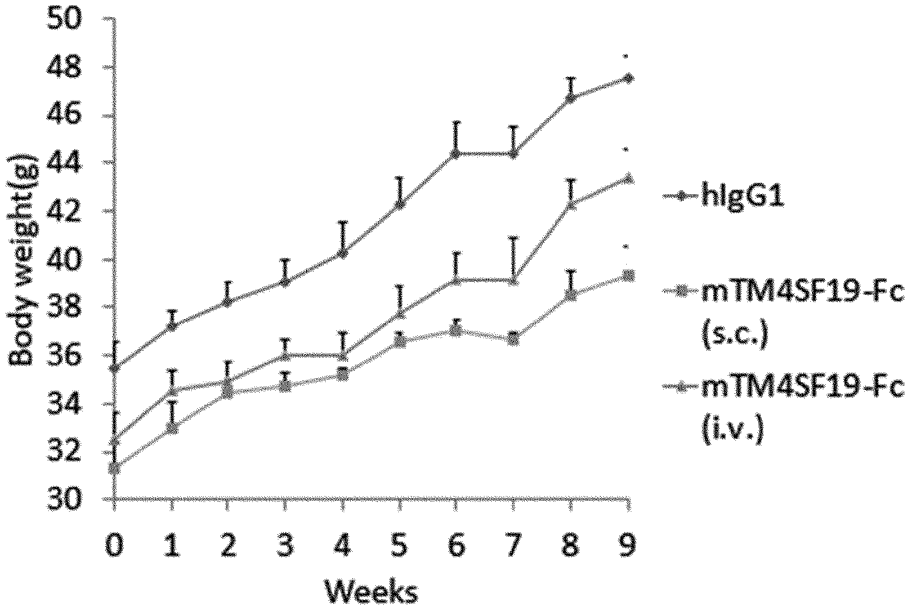
[도44d]

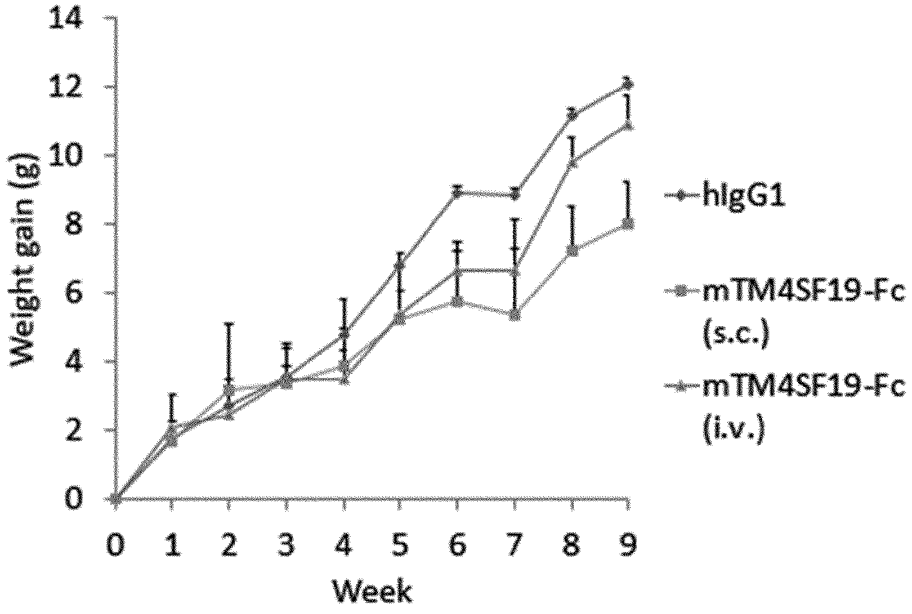

After removing
hindlimb muscles
hIgG1-Fc
50mpk
hTM4SF19-Fc
50mpk

[도45]

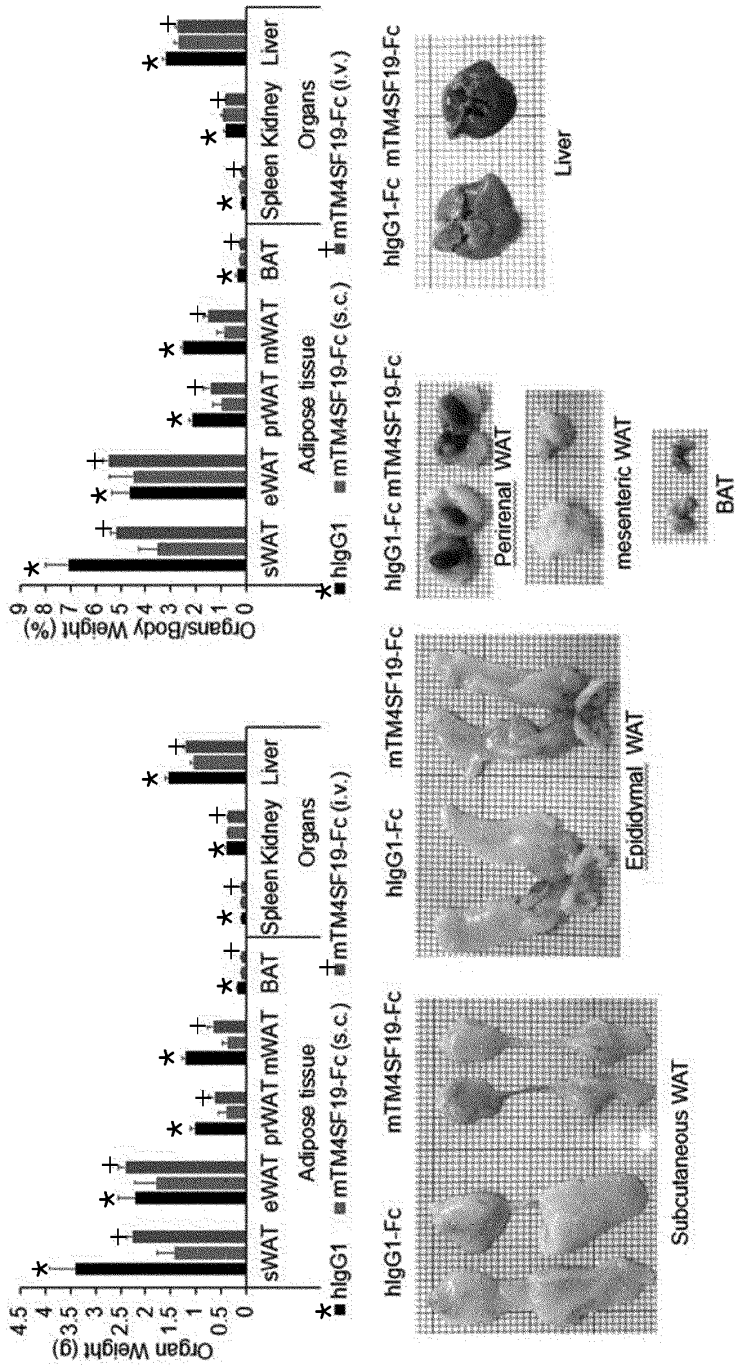

hIgG1-Fc
mTM4SF19-Fc (116~165) (10 mg/kg)
hTM4SF19-Fc (145~169) (10 mg/kg)
Number of nodules / lung
45
40
35
30
25
20
15
10
5
0
hIgG1-Fc
mTM4SF19-Fc (116~165) (10 mg/kg)
hTM4SF19-Fc (145~169) (10 mg/kg)

[도46a]

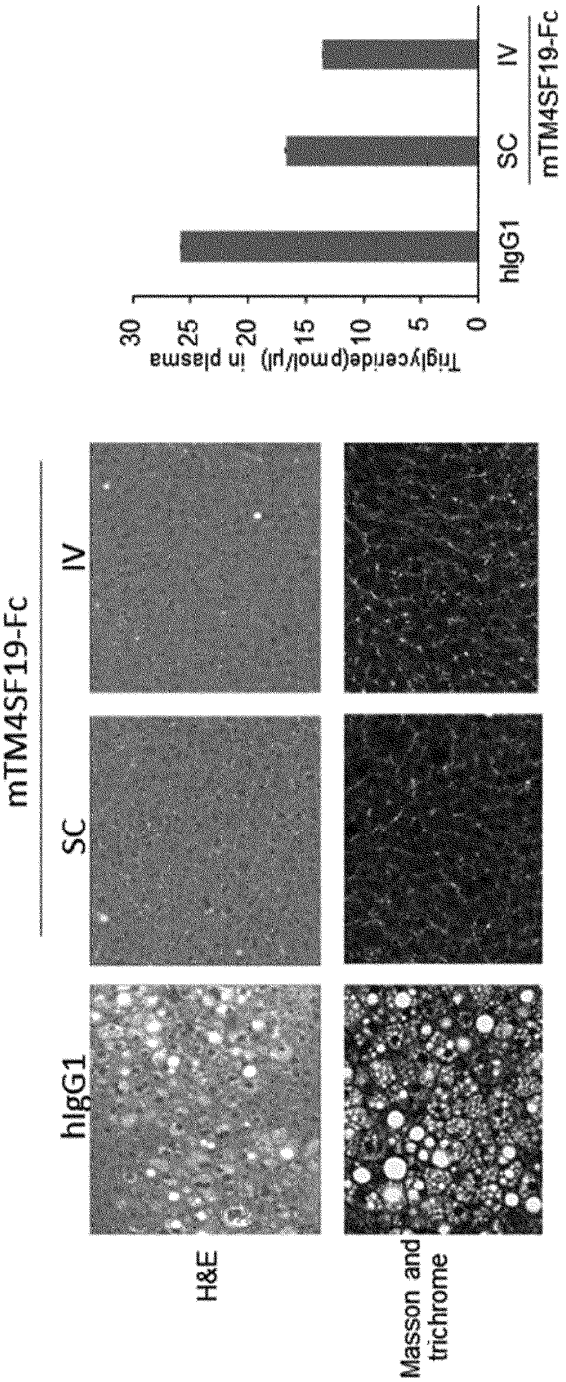

Body weight(g)
50
48
46
44
42
40
38
36
34
32
30
0 1 2 3 4 5 6 7 8 9
Weeks
hIgG1
mTM4SF19-Fc (s.c.)
mTM4SF19-Fc (i.v.)


Weight gain (g)
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9
Week
hIgG1
mTM4SF19-Fc (s.c.)
mTM4SF19-Fc (i.v.)

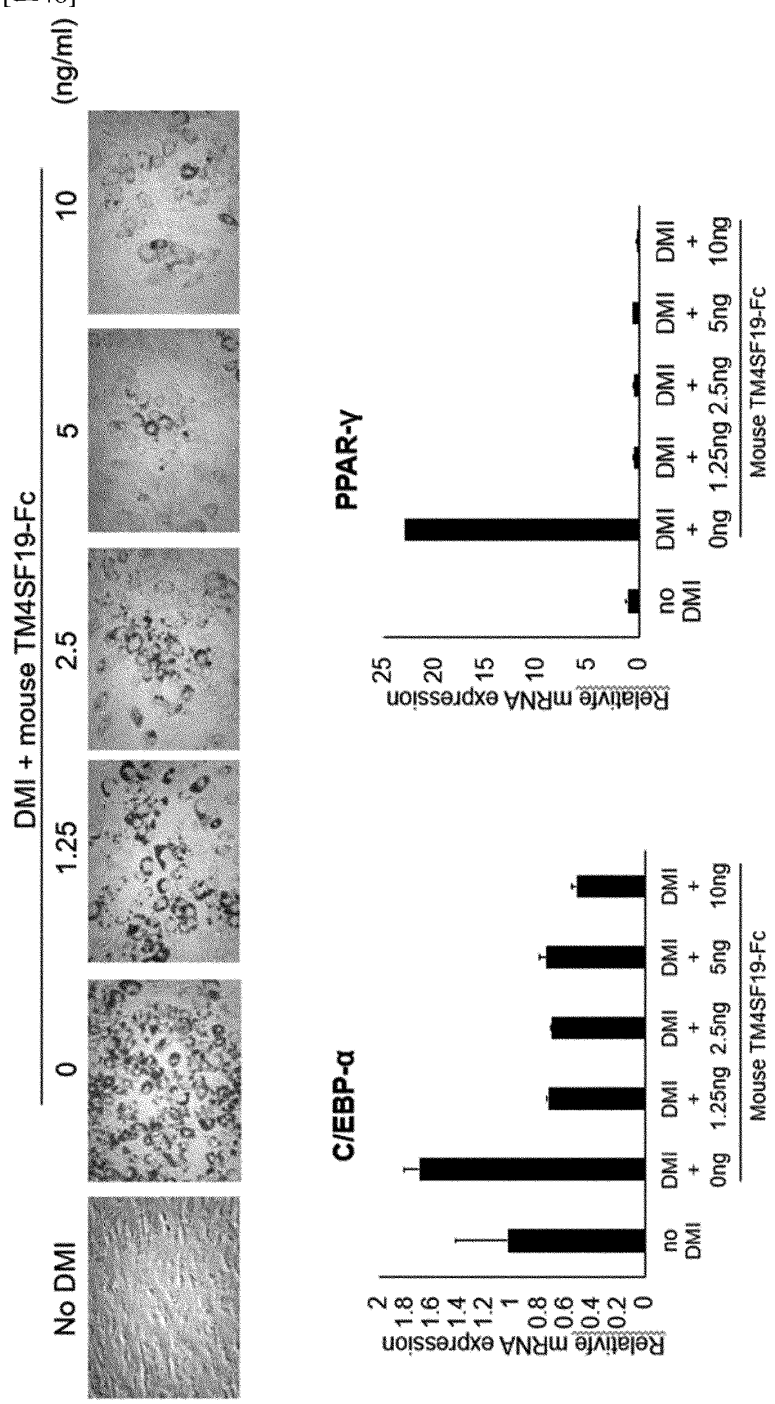
[도46b]


Organs/Body Weight (%)
Organ Weight (g)
sWAT eWAT prWAT mWAT BAT Spleen Kidney Liver
Adipose tissue
Organs
hIgG1
mTM4SF19-Fc (s.c.)
mTM4SF19-Fc (i.v.)
hIgG1-Fc
mTM4SF19-Fc
Subcutaneous WAT
Epididymal WAT
Perirenal WAT
mesenteric WAT
BAT
Liver

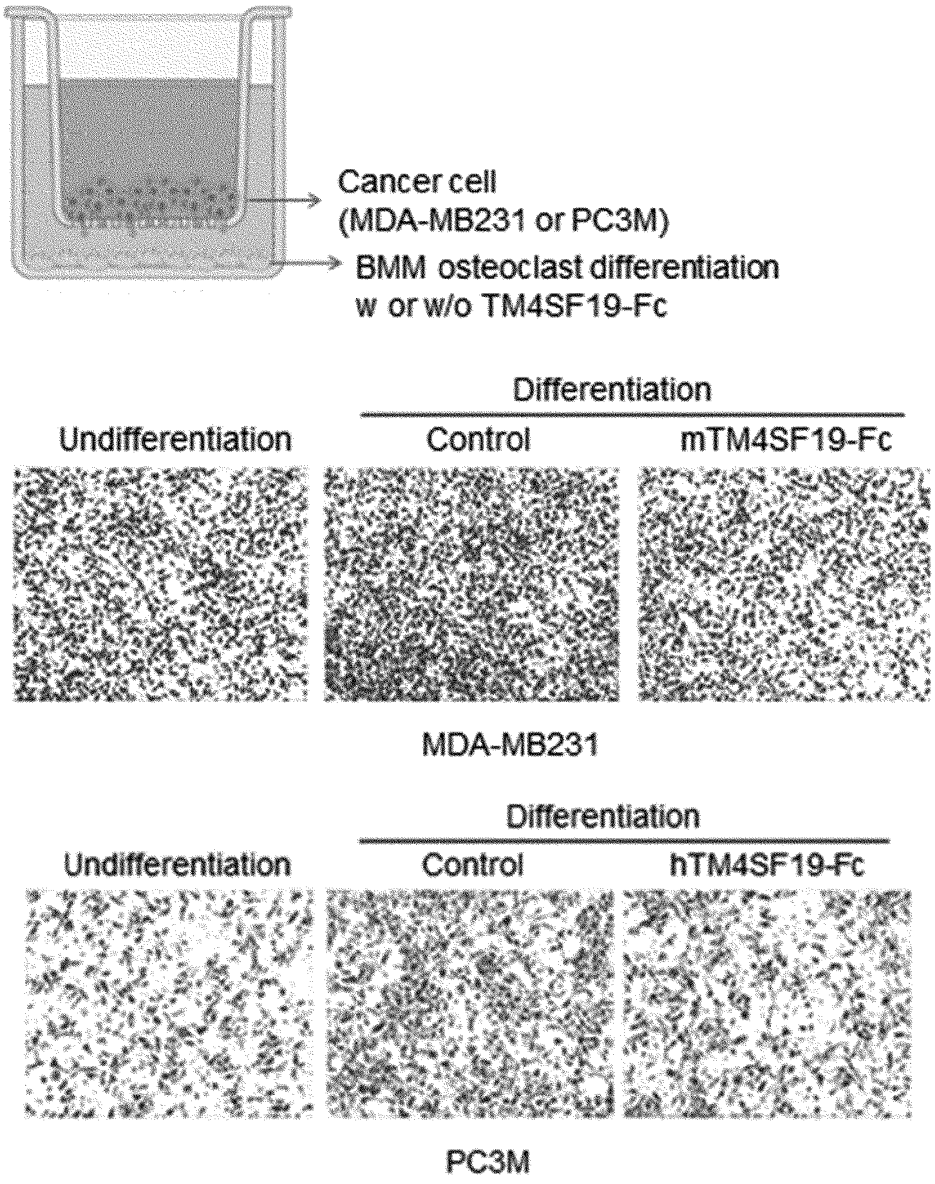
[도47]


Triglyceride(pmol/μl) in plasma
0
5
10
15
20
25
30
hIgG1
SC
IV
mTM4SF19-Fc
mTM4SF19-Fc
hIgG1
SC
IV
H&E
Masson and trichrome

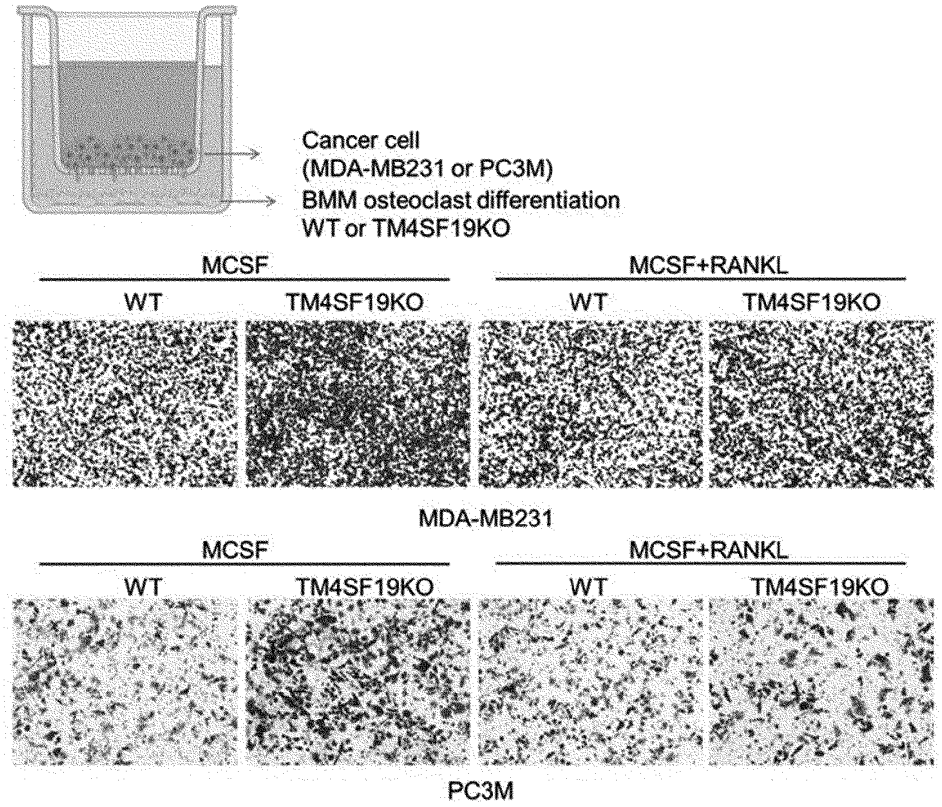
[도48]


No DMI
DMI + mouse TM4SF19-Fc (ng/ml)
0
1.25
2.5
5
10
C/EBP-α
Relative mRNA expression
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
no DMI
DMI + 0ng
DMI + 1.25ng
DMI + 2.5ng
DMI + 5ng
DMI + 10ng
Mouse TM4SF19-Fc
PPAR-γ
Relative mRNA expression
25
20
15
10
5
0
no DMI
DMI + 0ng
DMI + 1.25ng
DMI + 2.5ng
DMI + 5ng
DMI + 10ng
Mouse TM4SF19-Fc

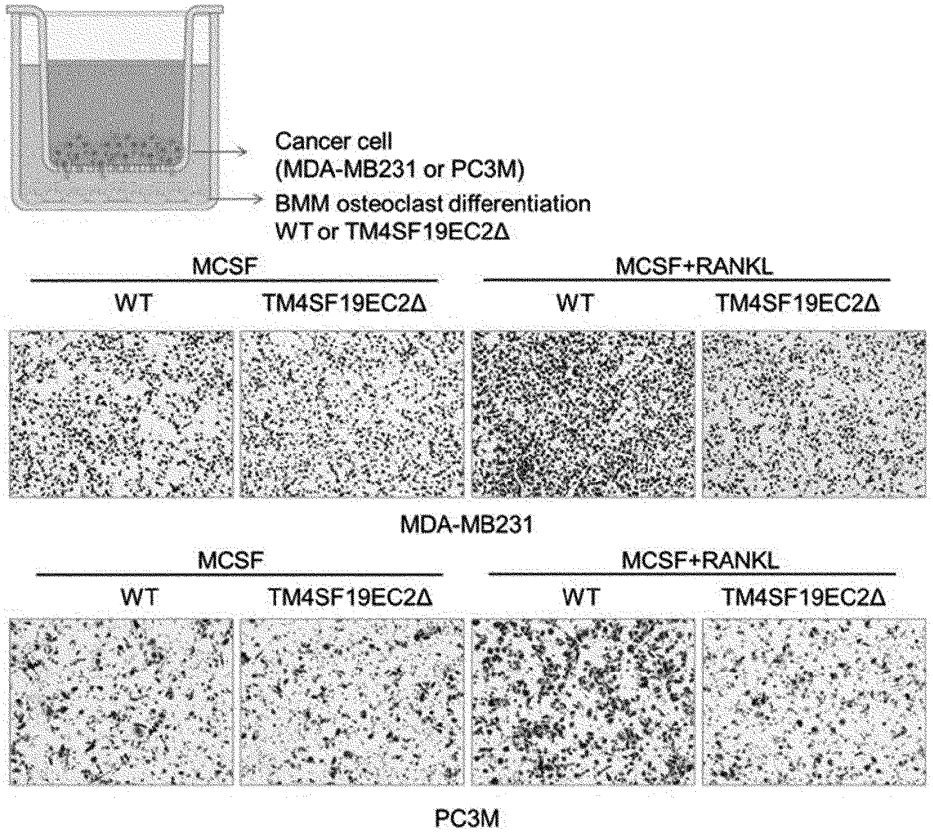
[도49]


Cancer cell
(MDA-MB231 or PC3M)
BMM osteoclast differentiation
w or w/o TM4SF19-Fc
Differentiation
Undifferentiation
Control
mTM4SF19-Fc
MDA-MB231
Differentiation
Undifferentiation
Control
hTM4SF19-Fc
PC3M

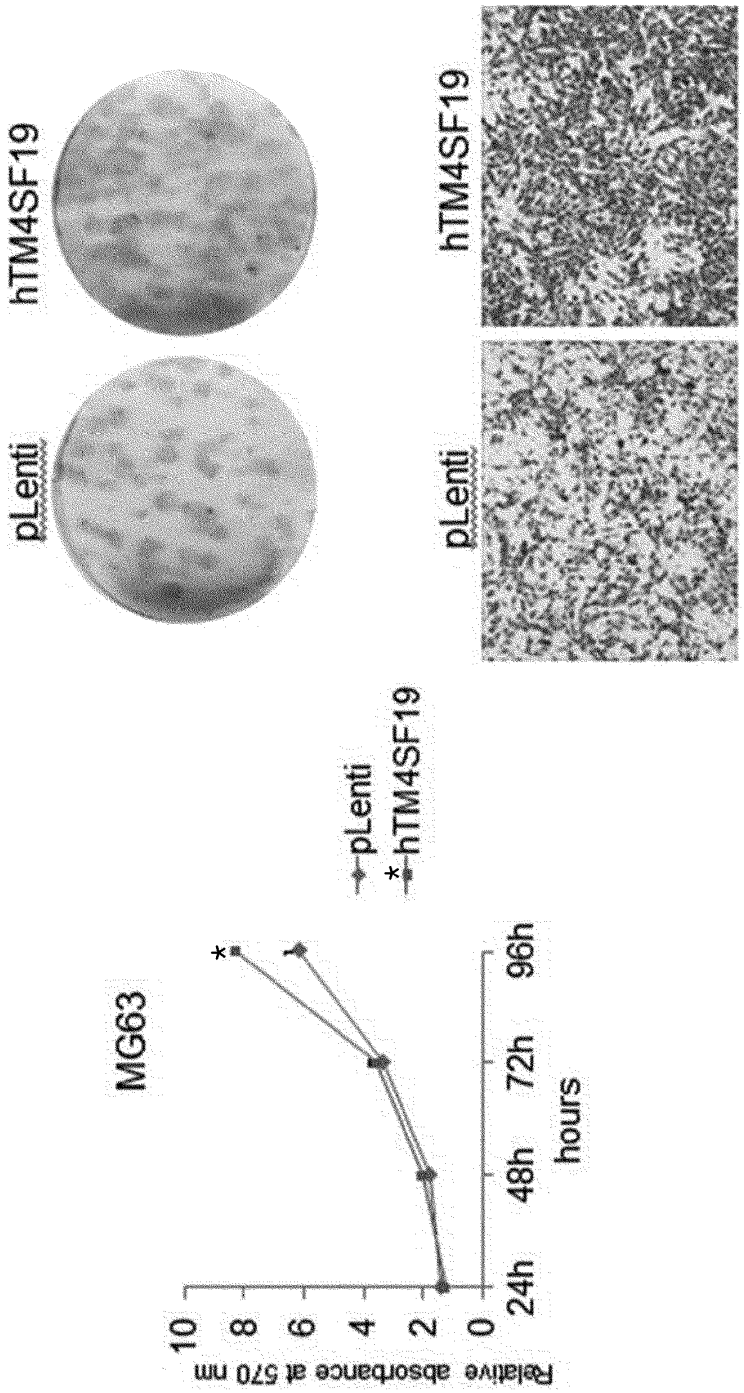
[도50a]


Cancer cell
(MDA-MB231 or PC3M)
BMM osteoclast differentiation
WT or TM4SF19KO
MCSF
MCSF+RANKL
WT
TM4SF19KO
WT
TM4SF19KO
MDA-MB231
MCSF
MCSF+RANKL
WT
TM4SF19KO
WT
TM4SF19KO
PC3M

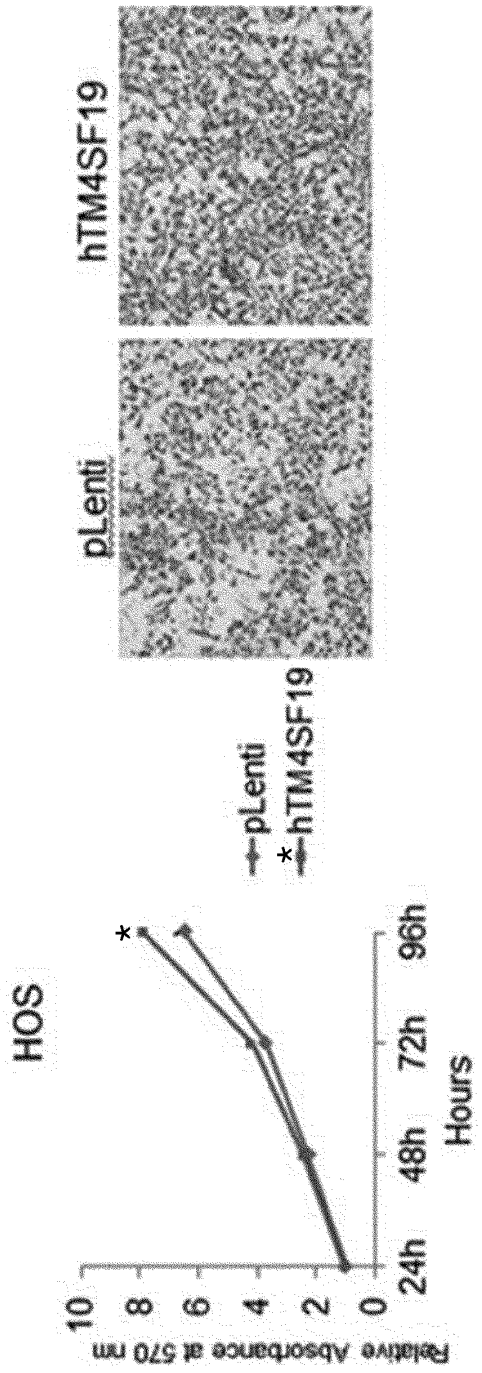
[도50b]


Cancer cell
(MDA-MB231 or PC3M)
BMM osteoclast differentiation
WT or TM4SF19EC2Δ
MCSF
MCSF+RANKL
WT
TM4SF19EC2Δ
WT
TM4SF19EC2Δ
MDA-MB231
MCSF
MCSF+RANKL
WT
TM4SF19EC2Δ
WT
TM4SF19EC2Δ
PC3M

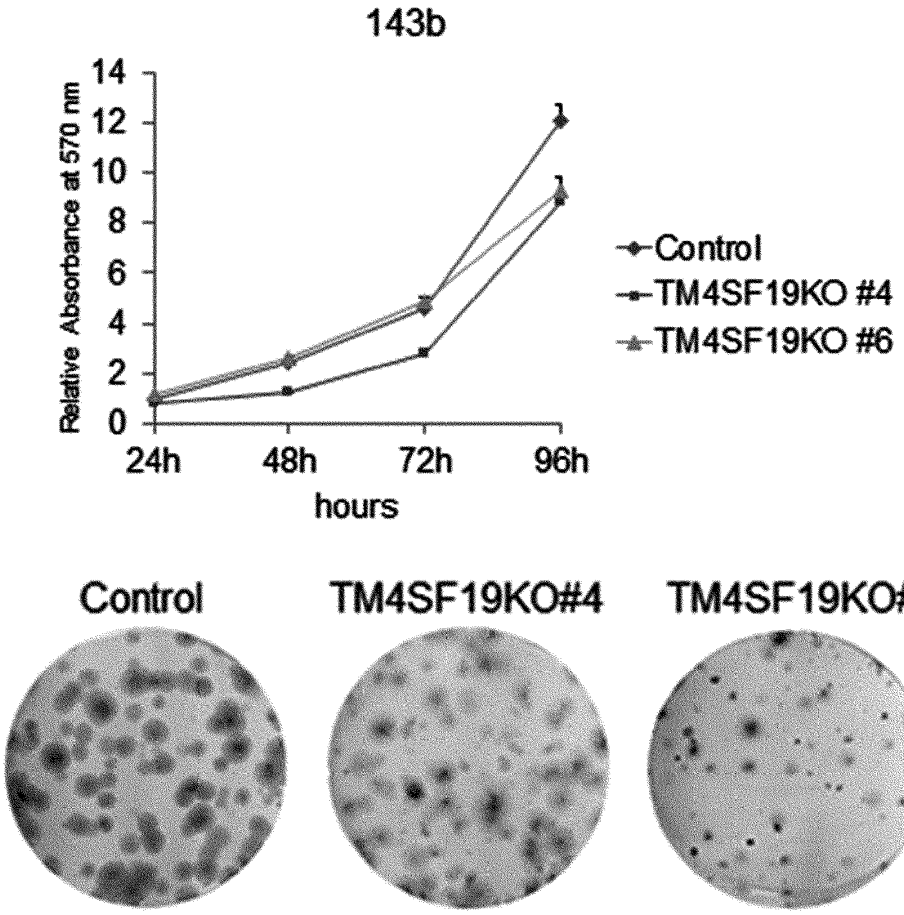
[도51]


hTM4SF19
pLenti
hTM4SF19
pLenti
MG63
pLenti
*hTM4SF19
Relative absorbance at 570 nm
10
8
6
4
2
0
24h
48h
72h
96h
hours
*

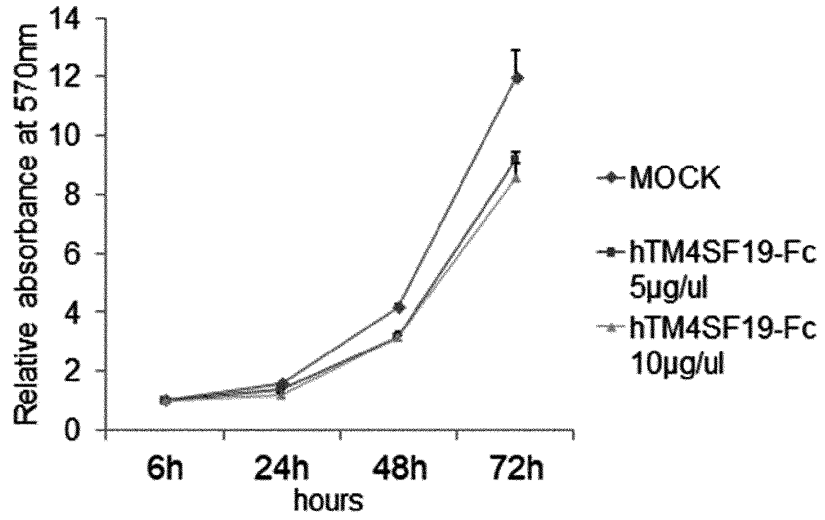
[도52]


hTM4SF19
pLenti
pLenti
*hTM4SF19
HOS
*
24h
48h
72h
96h
Hours
0
2
4
6
8
10
Relative Absorbance at 570 nm

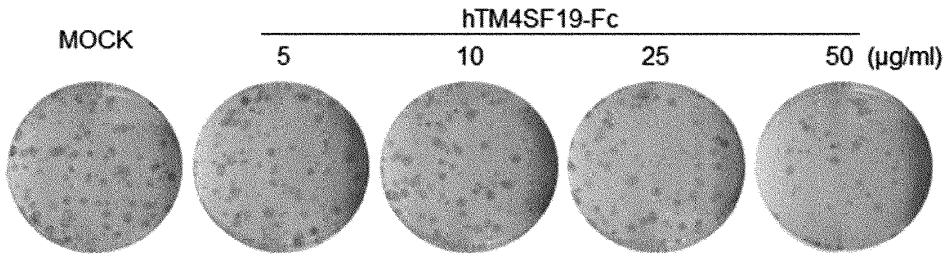
[도53]


143b
Relative Absorbance at 570 nm
14
12
10
8
6
4
2
0
24h
48h
72h
96h
hours
Control
TM4SF19KO #4
TM4SF19KO #6
Control
TM4SF19KO#4
TM4SF19KO#6

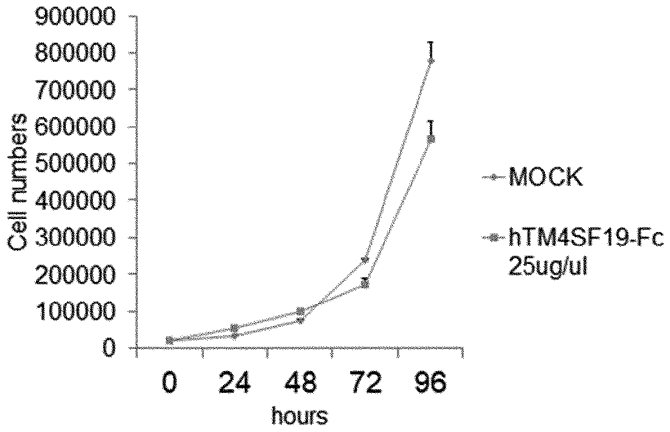
[도54a]


Relative absorbance at 570nm
14
12
10
8
6
4
2
0
6h
24h
48h
72h
hours
MOCK
hTM4SF19-Fc 5μg/ul
hTM4SF19-Fc 10μg/ul

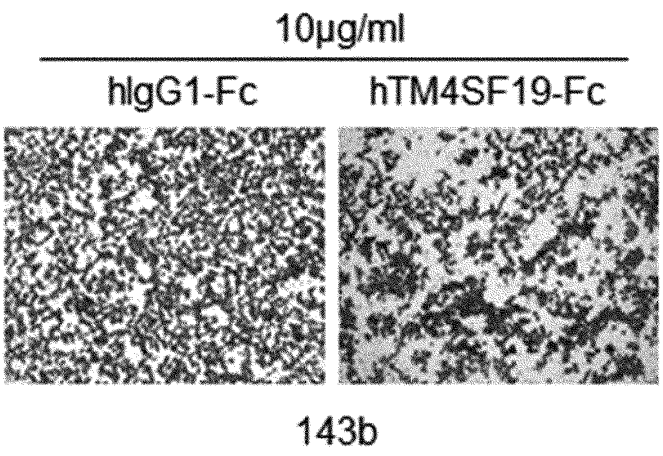
[도54b]



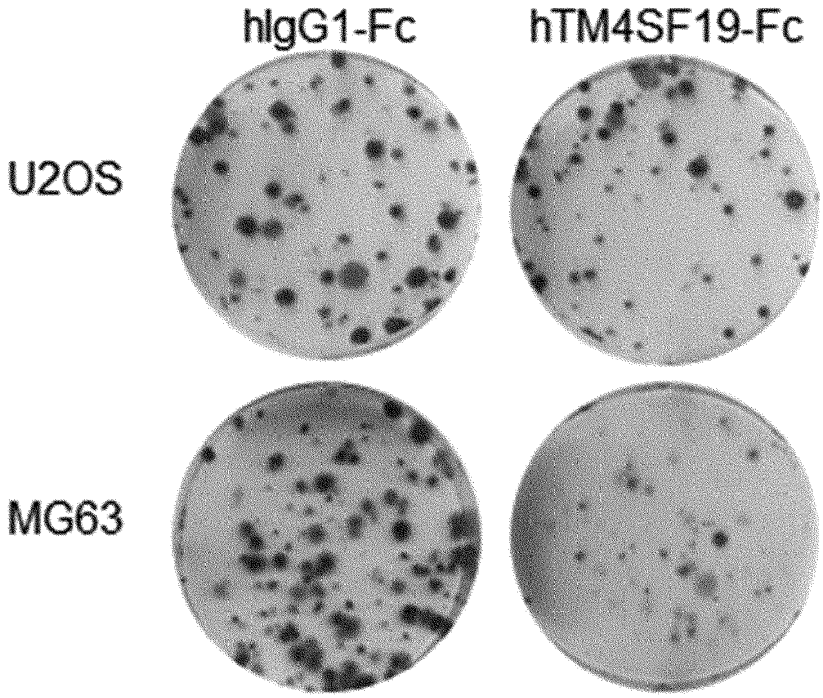
[도54c]



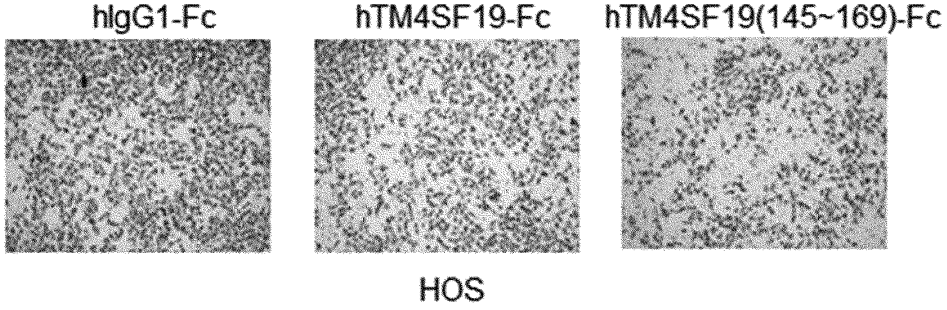
[도54d]



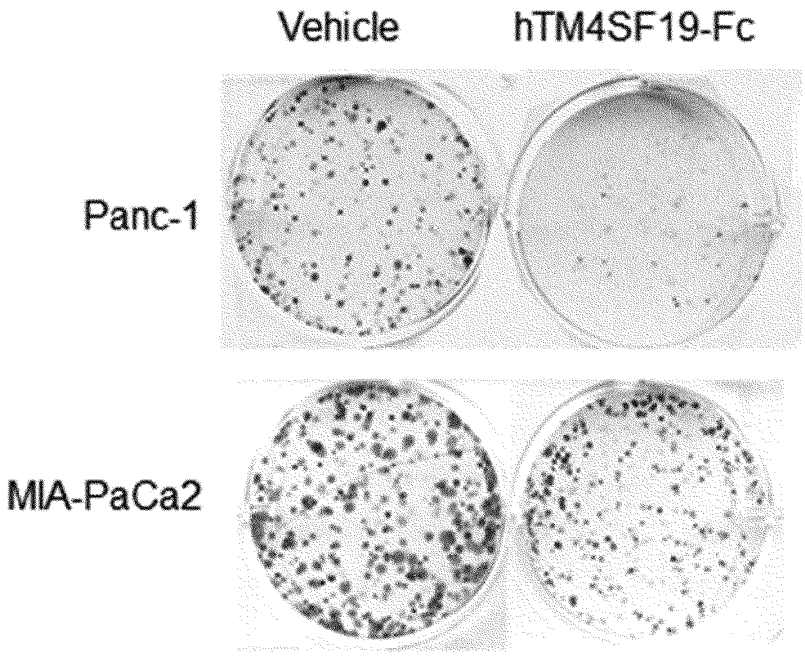
[도55]


hIgG1-Fc
hTM4SF19-Fc
U2OS
MG63

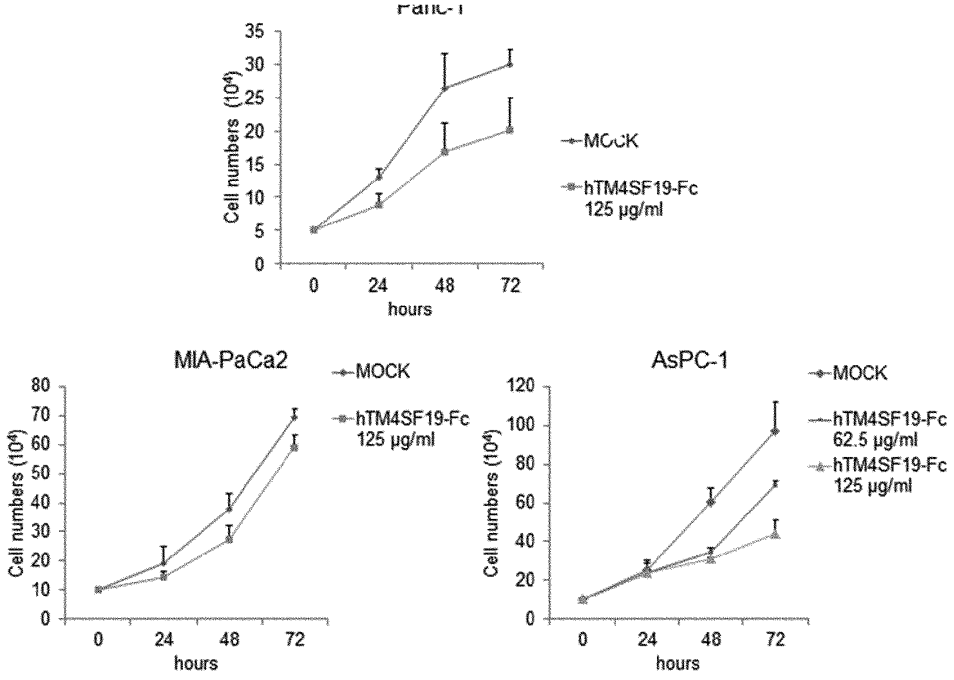
[도56]


hIgG1-Fc
hTM4SF19-Fc
hTM4SF19(145~169)-Fc
HOS

[도57a]


Vehicle
hTM4SF19-Fc
Panc-1
MIA-PaCa2

[도57b]


Panc-1
Cell numbers (10^4)
hours
MOCK
hTM4SF19-Fc 125 μg/ml
MIA-PaCa2
AsPC-1
hTM4SF19-Fc 62.5 μg/ml

[도58a]

Mouse TM4SF19$_{116-165}$-Fc : SEQ ID NO: 10

Target Sequence: SEQ ID NO: 8

Enzyme Site

hIgG1-Fc : SEQ ID NO: 9

Mouse 116-165 amino acid seq

KDGPFCMFDVSSFNQTQAWKFGYPFKDLHNRNYLYDRSLWTSVCLEPSKATSEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

Nucleotides encoding Mouse TM4SF19$_{116-165}$-Fc : SEQ ID NO: 11

**AAAGATGGTCCCTTTTGCATGTTTGATGTCTCATCCTTCAATCAGACACAAGCTTGGAAATTCGG
CTATCCCTTTAAAGATCTACACAACAGGAATTATCTGTATGACCGTTCACTTTGGACCTCCGTTT
GCCTGGAGCCCTCTAAGGCT**ACTAGTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGT
GCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC
TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGG
TCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG
CAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAA
GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAA
GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGA
GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
GA

[도58b]

Human TM4SF19$_{120-169}$-Fc : SEQ ID NO: 13

Target Sequence : SEQ ID NO: 12

Enzyme Site

hIgG1-Fc : SEQ ID NO: 9

KDGPFCMFDVSSFNQTQAWKYGYPFKDLHSRNYLYDRSLWNSVCLEPSAATSEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

Nucleotides encoding Human TM4SF19$_{120-169}$-Fc : SEQ ID NO: 14

**AAAGATGGTCCTTTTTGCATGTTTGATGTTTCATCCTTCAATCAGACACAAGCTTGGAAATATGG
TTACCCATTCAAAGACCTGCATAGTAGGAATTATCTGTATGACCGTTCGCTCTGGAACTCCGTCT
GCCTGGAGCCCTCTGCAGCT**ACTAGTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGT
GCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC
TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGG
TCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG
CAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAA
GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAA
GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGA
GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
GA

[도58c]

Human TM4SF19$_{145-169}$-Fc : SEQ ID NO: 16

Target Sequence : SEQ ID NO: 15

Enzyme Site

hIgG1-Fc: SEQ ID NO: 9

KDLHSRNYLYDRSLWNSVCLEPSAATSEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Nucleotides encoding Human Human TM4SF19$_{145-169}$-Fc : SEQ ID NO: 17

AAAGACCTGCATAGTAGGAATTATCTGTATGACCGTTCGCTCTGGAACTCCGTCTGCCTGGAGC
CCTCTGCAGCTACTAGTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCAC
CTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTC
CCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCA
ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC
AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGT
CAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG
GCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[도58d]

Human TM4SF19$_{131-169}$-Fc : SEQ ID NO: 19

Target Sequence : SEQ ID NO: 18

Enzyme Site

hIgG1-Fc : SEQ ID NO: 9

SFNQTQAWKYGYPFKDLHSRNYLYDRSLWNSVCLEPSAATSEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Nucleotides encoding Human TM4SF19$_{131-169}$-Fc SEQ ID NO: 20

TCCTTCAATCAGACACAAGCTTGGAAATATGGTTACCCATTCAAAGACCTGCATAGTAGGAATT
ATCTGTATGACCGTTCGCTCTGGAACTCCGTCTGCCTGGAGCCCTCTGCAGCTACTAGTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCA
GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG
TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGT
CCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT
ACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGA
GCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACA
CGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

Human TM4SF19 extracellular loop 2, 115~175, 60 aa: SEQ ID NO: 21

SGVALKDGPFCMFDVSSFNQTQAWKYGYPFKDLHSRNYLYDRSLWNSVCLEPSAAVVWHVS