図１－１

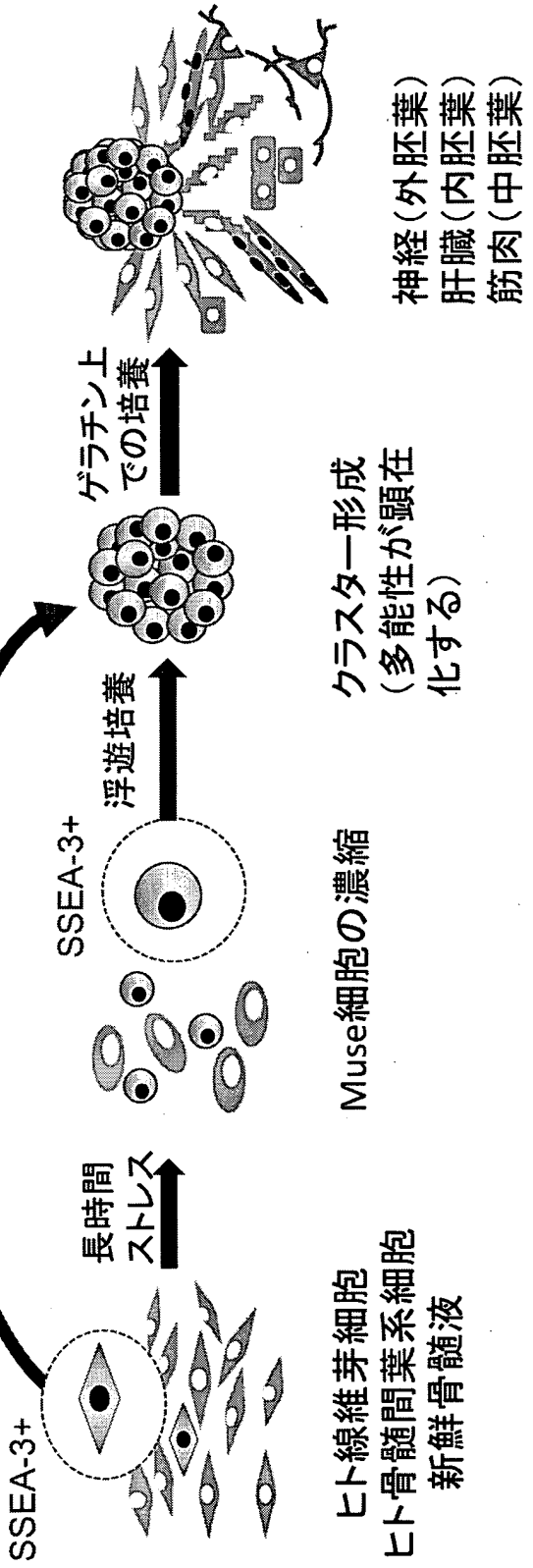

SSEA3陽性細胞を直接分離し、
長時間ストレスなしで浮遊培養することも可能
SSEA-3+
長時間
ストレス
SSEA-3+
浮遊培養
ゲラチン上
での培養
ヒト線維芽細胞
ヒト骨髄間葉系細胞
新鮮骨髄液
Muse細胞の濃縮
クラスター形成
（多能性が顕在
化する）
神経（外胚葉）
肝臓（内胚葉）
筋肉（中胚葉）

図１－２

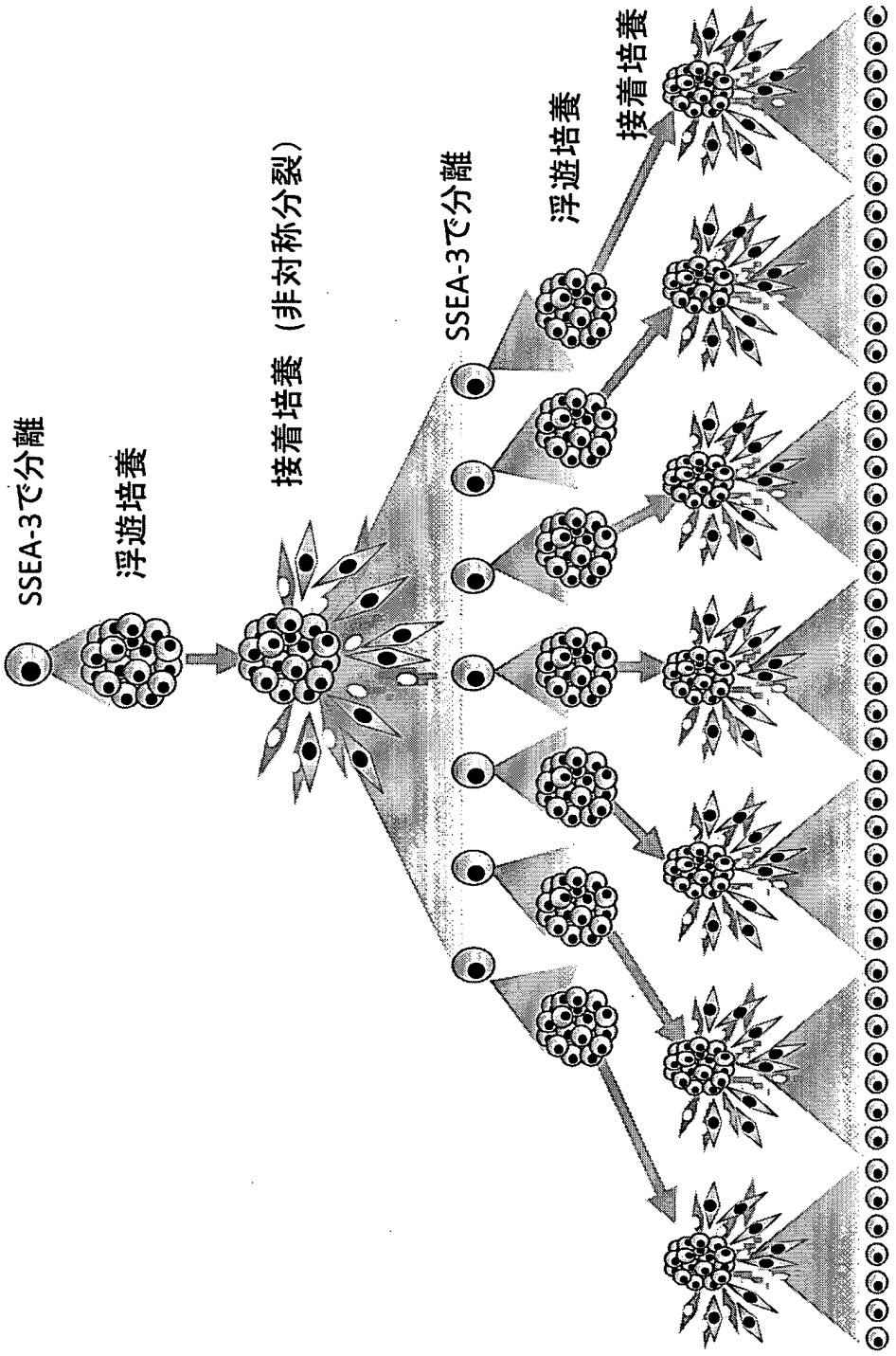


図2

Gene Symbol	Total	D24 EB body/D24 Native	NHDF EB body/NHDF Native	Gene Title
THBD	1267.42467	42.96487494	1224.459795	thrombomodulin
IL1RN	960.4457022	12.0038603	948.4418419	interleukin 1 receptor antagonist
FOS	954.4076469	88.7202031	865.6874438	v-fos FBJ murine osteosarcoma viral oncogene homolog
SLC16A6	849.437506	715.7799818	133.6575242	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)
TYRP1	768.3374259	11.67216252	756.6652634	tyrosinase-related protein 1
CACNA1A	685.5302732	3.084442121	682.445831	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
C16orf81	531.0858738	528.3969782	2.688895627	chromosome 16 open reading frame 81
CHI3L1	489.924397	373.8511267	116.0732704	chitinase 3-like 1 (cartilage glycoprotein-39)
PRSS35	441.7622409	371.6403738	70.12186703	protease, serine, 35
KYNU	420.8532931	182.1885786	238.6647145	kynureninase (L-kynurenine hydrolase)
SLC16A6	407.4159044	315.8163678	91.59953662	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)
APOE	357.4991884	105.5807107	251.9184778	apolipoprotein E
THBD	347.7592226	34.12473279	313.6344898	thrombomodulin
SYTL5	320.0330513	15.80876986	304.2242814	synaptotagmin-like 5
CHI3L1	299.4142542	260.1989748	39.21527948	chitinase 3-like 1 (cartilage glycoprotein-39)
ABCA13	290.3969665	271.9782856	18.41868089	ATP-binding cassette, sub-family A (ABC1), member 13
ANGPTL4	284.3113469	11.53113972	272.7802072	angiopoietin-like 4
PTGS2	273.0706903	201.4865123	71.58417805	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
STC1	255.0878156	29.28287475	225.8049409	stanniocalcin 1
CCDC102B	253.1960755	37.22467468	215.9714008	coiled-coil domain containing 102B

図3

Gene Symbol	Total	D24 EB/hES	NHDF EB/hES	Gene Title
MMP1	36528.90821	6836.563387	29692.34482	matrix metallopeptidase 1 (interstitial collagenase)
EREG	19813.4402	18961.45781	851.9823837	epiregulin
CHI3L1	9641.34444	8842.305483	799.038957	chitinase 3-like 1 (cartilage glycoprotein-39)
---	7940.341316	7298.64809	641.6932268	Transcribed locus
CHI3L1	5714.359227	5385.877946	328.4812811	chitinase 3-like 1 (cartilage glycoprotein-39)
SRGN	4655.28423	4636.821662	18.46256778	serglycin
---	4397.855874	3959.205654	438.6502206	MRNA full length insert cDNA clone EUROIMAGE 1913076
RIN2	3611.379317	859.9132609	2751.466056	Ras and Rab interactor 2
LUM	3396.245648	1797.976375	1598.269272	lumican
CLCA2	3331.259662	747.5657674	2583.693895	CLCA family member 2, chloride channel regulator
IL8	3143.389365	2638.045747	505.3436182	interleukin 8
LOC401097	3012.485859	1860.366679	1152.119181	Similar to LOC166075
DPT	2807.405266	56.39203934	2751.013226	dermatopontin
ELTD1	2658.65006	1751.976082	906.6739784	EGF, latrophilin and seven transmembrane domain containing 1
IGFBP1	2423.035758	2405.299722	17.73603586	insulin-like growth factor binding protein 1
SLC16A4	2305.561138	1826.823338	478.7378001	solute carrier family 16, member 4 (monocarboxylic acid transporter 5)
SRGN	2293.730946	2279.537455	14.19349126	serglycin
GREM2	2053.928699	159.813291	1894.115408	gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)
IGFBP5	1960.755393	1915.425977	45.3294163	insulin-like growth factor binding protein 5
SQRDL	1918.477233	1217.387684	701.0895492	sulfide quinone reductase-like (yeast)

図４

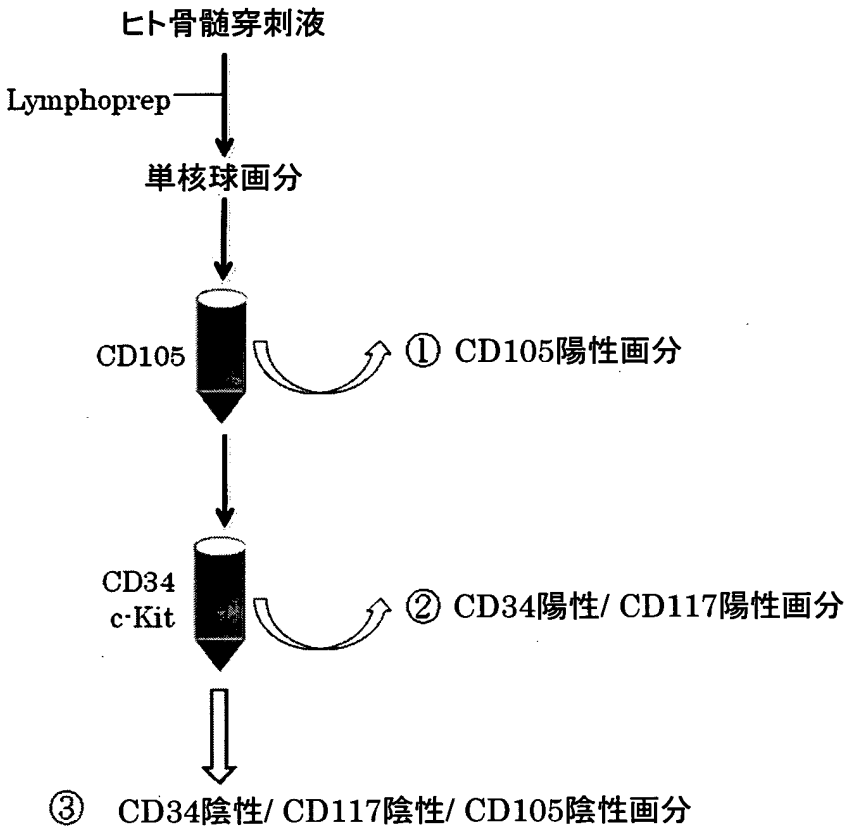

ヒト骨髄穿刺液
Lymphoprep
単核球画分
CD105
① CD105陽性画分
CD34
c-Kit
② CD34陽性/ CD117陽性画分
③ CD34陰性/ CD117陰性/ CD105陰性画分

図5

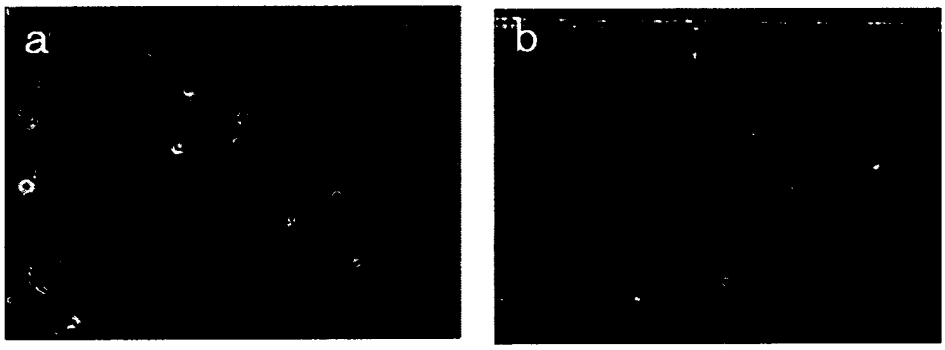


図6

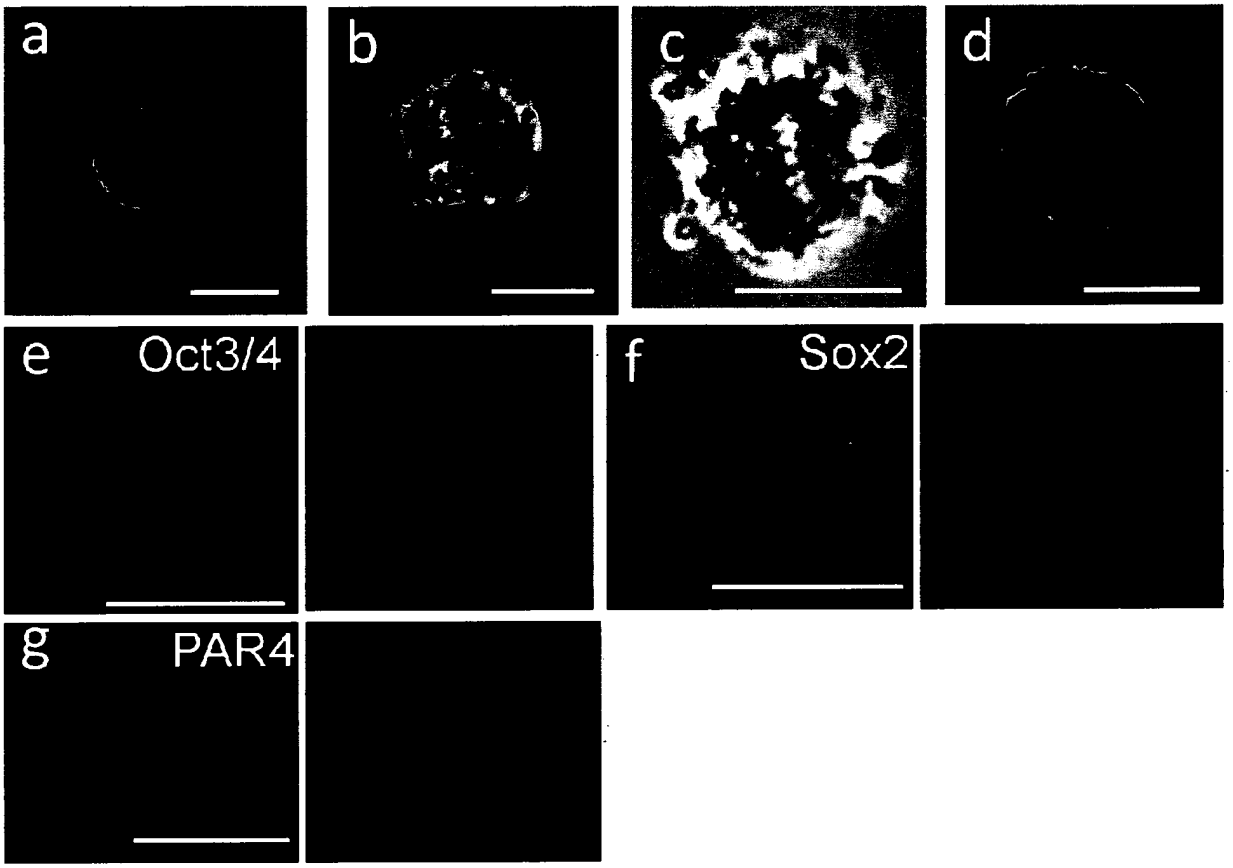


図７－１

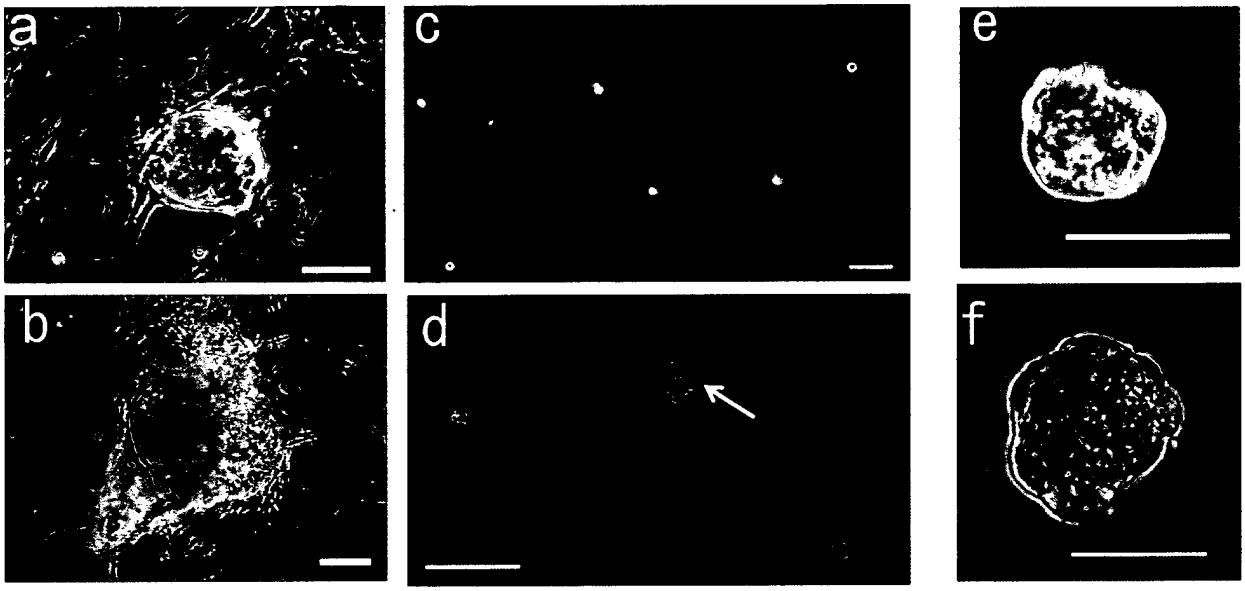


図７－２

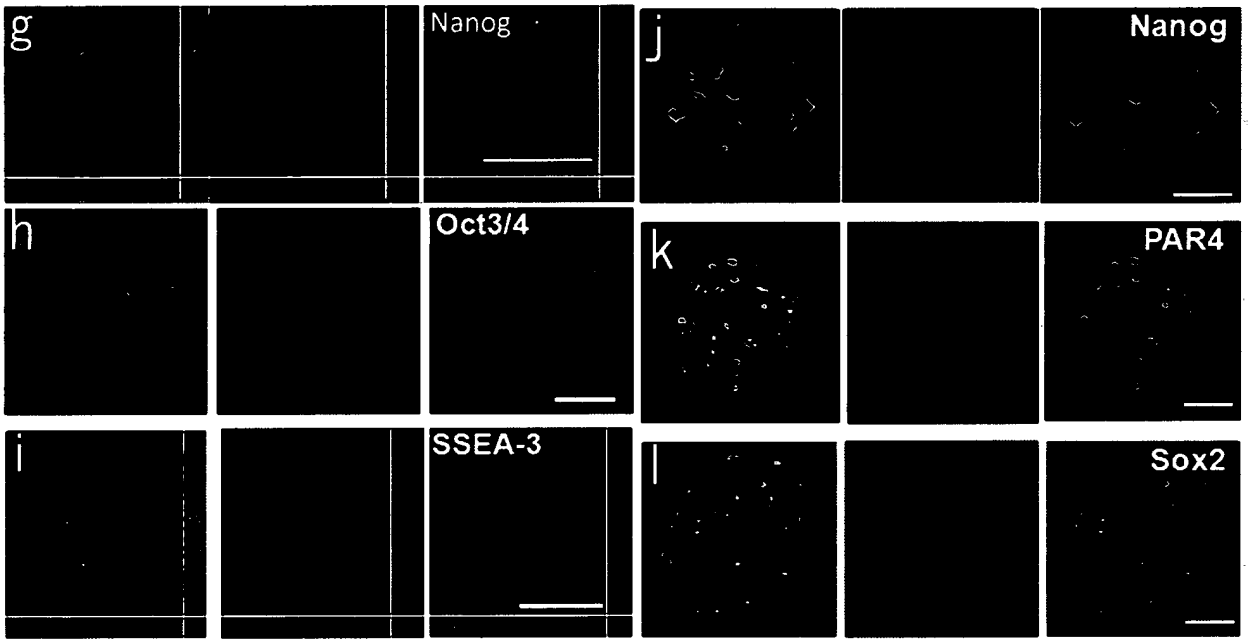


図７－３

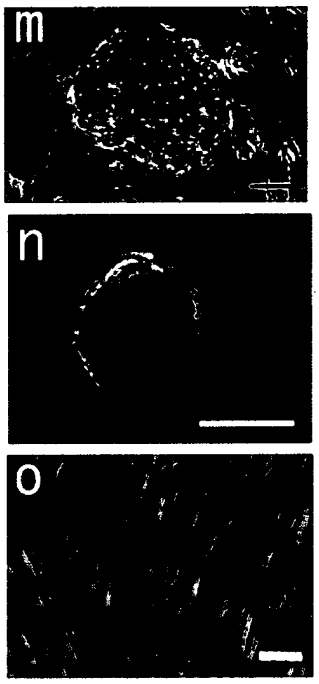


図７－４

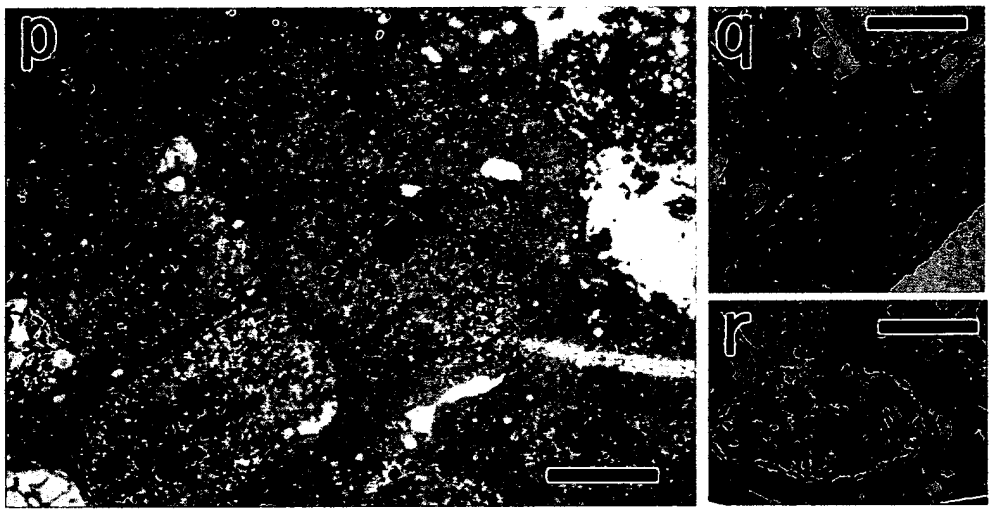


図８－１

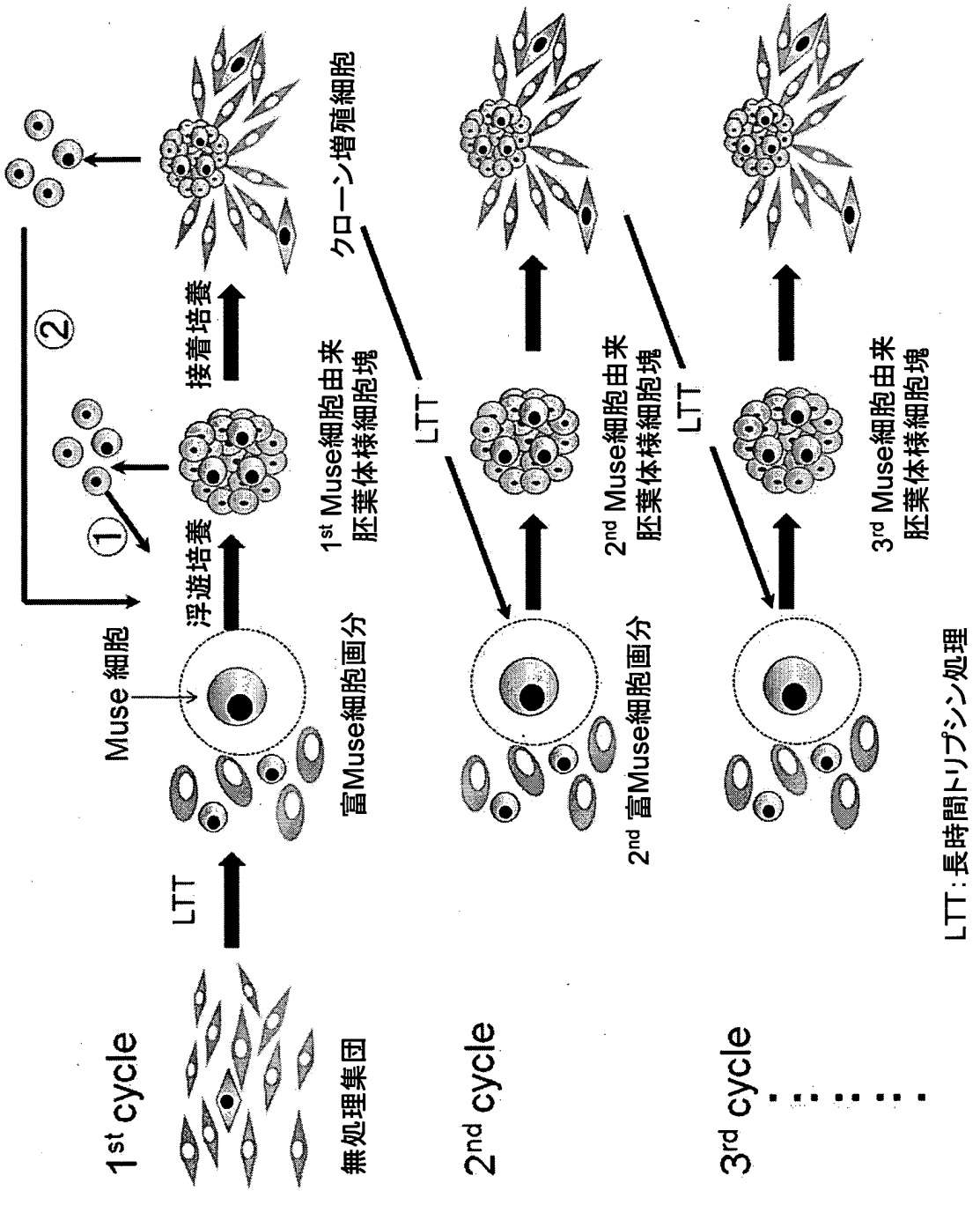

1st cycle
無処理集団
LTT
Muse 細胞
浮遊培養
富Muse細胞画分
1st Muse細胞由来
胚葉体様細胞塊
①
②
接着培養
クローン増殖細胞
LTT
2nd cycle
2nd 富Muse細胞画分
2nd Muse細胞由来
胚葉体様細胞塊
LTT
3rd cycle
3rd Muse細胞由来
胚葉体様細胞塊
LTT:長時間トリプシン処理

図８－２

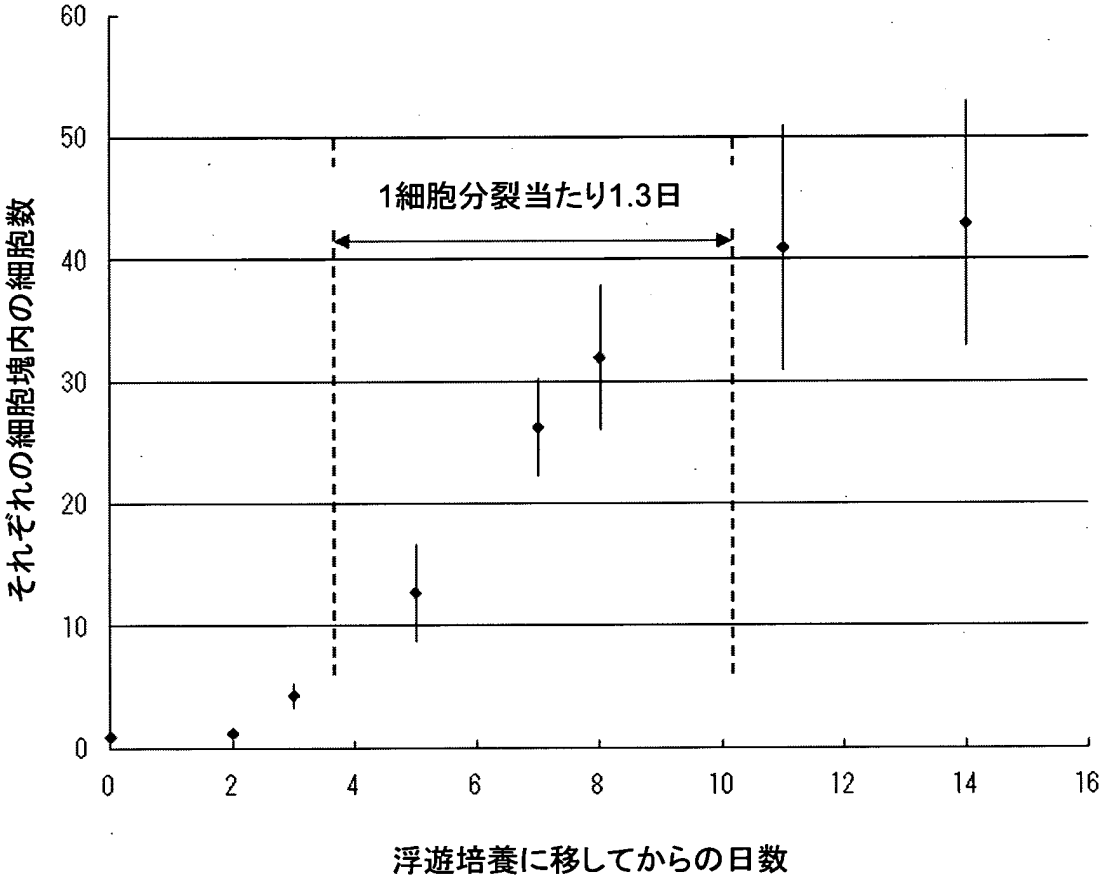

それぞれの細胞塊内の細胞数
60
50
40
30
20
10
0
1細胞分裂当たり1.3日
0
2
4
6
8
10
12
14
16
浮遊培養に移してからの日数

図８－３



図９－１

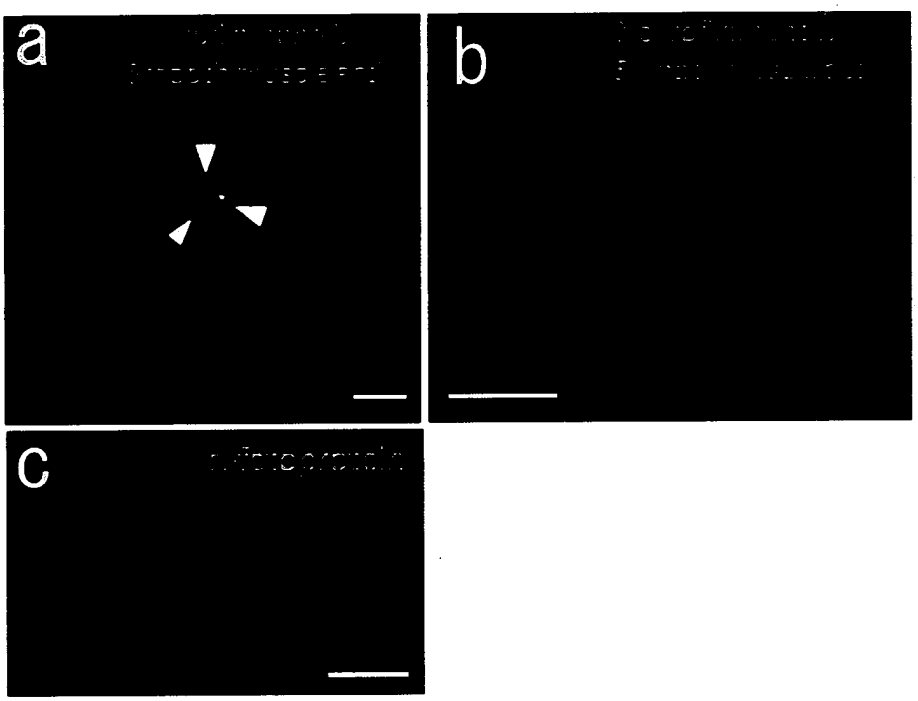


図９－２

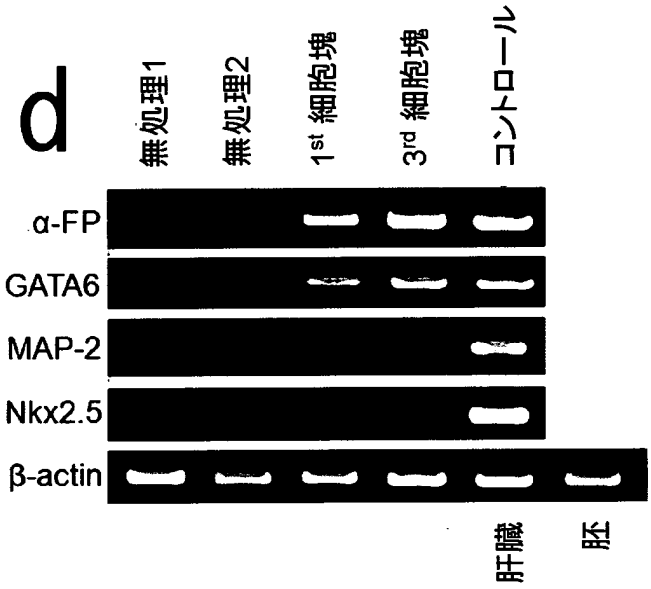

d
無処理1
無処理2
1st 細胞塊
3rd 細胞塊
コントロール
α-FP
GATA6
MAP-2
Nkx2.5
β-actin
肝臓
胚

図９－３

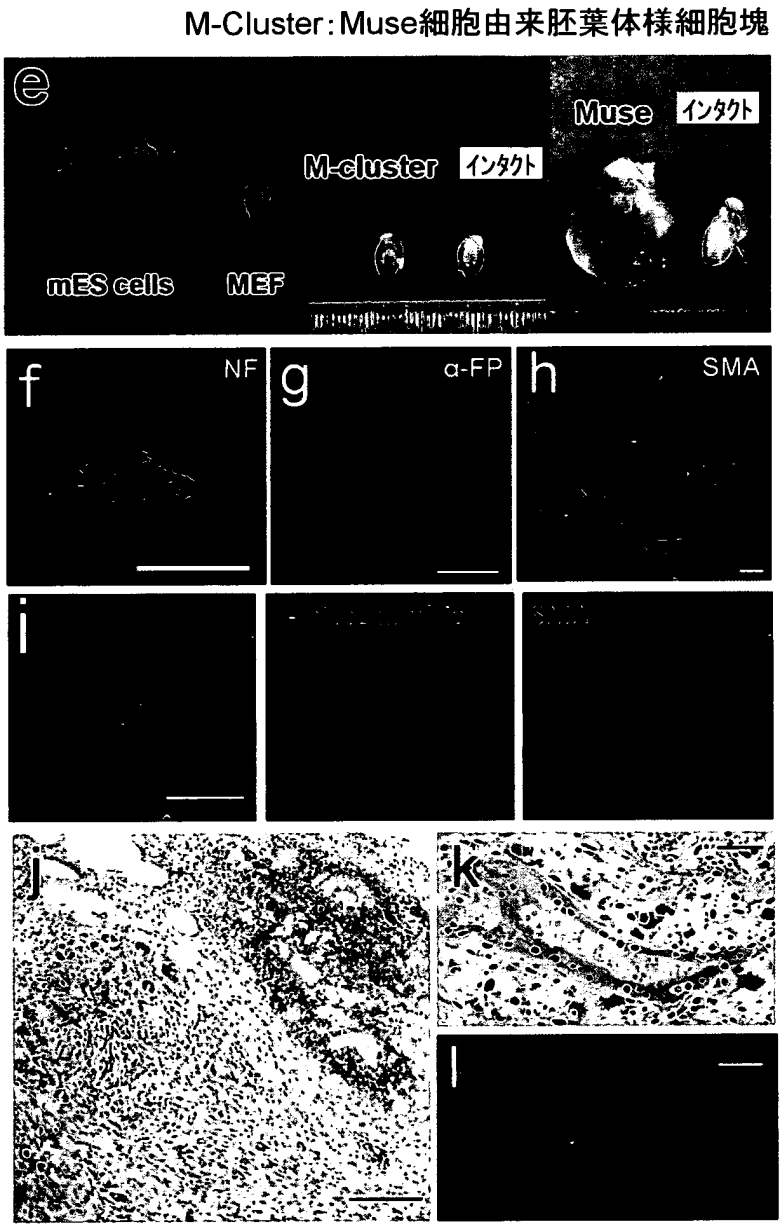

M-Cluster:Muse細胞由来胚葉体様細胞塊
e
Muse
インタクト
M-cluster
インタクト
mES cells
MEF
f
NF
g
α-FP
h
SMA
i
j
k
l

図１０ａ

		MUSE/無処理				細胞塊/無処理			
シンボル	名称	Fibro1	Fibro2	MSC-1	MSC-2	Fibro1	Fibro2	MSC-1	MSC-2
DAZL	Deleted in azoospermia-like			+		+	+		
DPPA2	Developmental pluripotency associated 2 (PESCRG1)	+	+	+	+	+			
DPPA4	Developmental pluripotency associated 4		−			+		+	
ERAS	ES cell expressed Ras	+	+	+	+	+	+	+	
GRB7	Growth factor receptor-bound protein 7	+	+	+		+			
HOXB1	Homeobox B1					+	+		
KLF4	Kruppel-like factor 4 (gut)	+	+	+					−
MYC	c-Myc	+	+	+	+				
PRDM1	PR domain containing 1, with ZNF domain					+	+	+	
SOX2	SRY (sex determining region Y)-box 2	+			+	+			+
SPAG9	Sperm associated antigen 9	+	+	+					
SPRY2	Sprouty homolog 2, antagonist of FGF signaling	+	+	+		+	+	+	+
TDGF1	Teratocarcinoma-derived growth factor 1	+	+	+	+	+	+		
ZFP42	Zinc finger protein 42 homolog (Rex1)		+	+	+				

+ − 変化なし

図１０ｂ

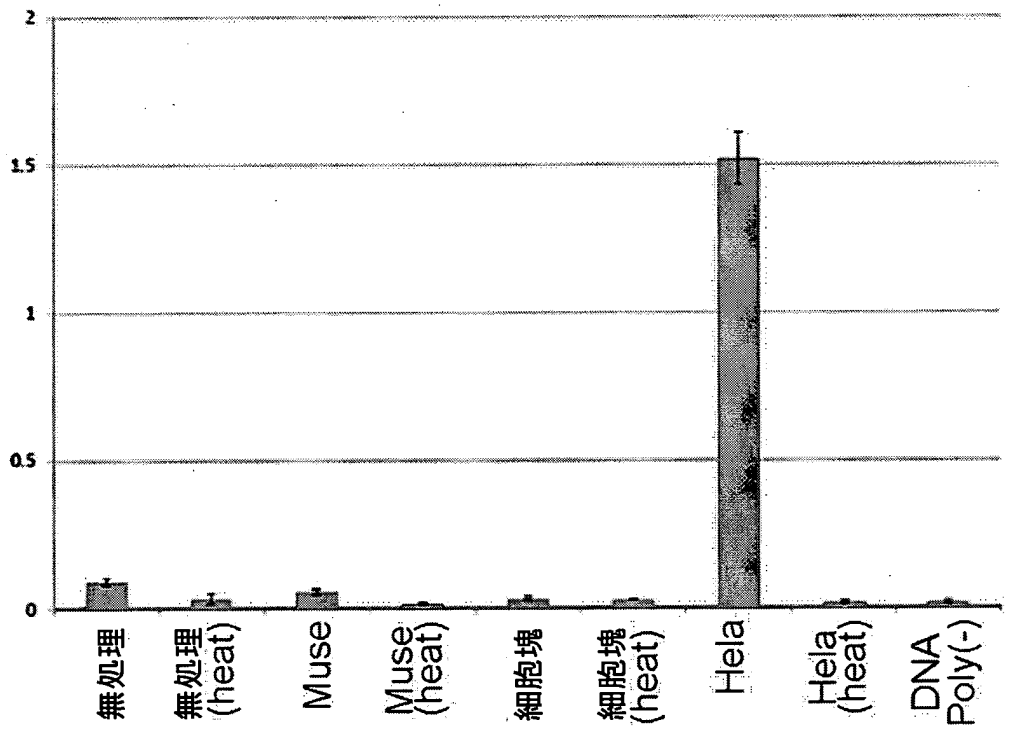

2
1.5
1
0.5
0
無処理
無処理(heat)
Muse
Muse(heat)
細胞塊
細胞塊(heat)
Hela
Hela(heat)
DNA Poly(-)

図１１


0.0
5.09395
8.5507
Mono
MSC-1 無処理
MSC-2 無処理
Fibro-1 無処理
Fibro-2 無処理
MSC-1 Muse
MSC-2 Muse
Fibro-1 Muse
Fibro-2 Muse
MSC-1 EB
MSC-2 EB
Fibro-1 EB
Fibro-2 EB

図１２

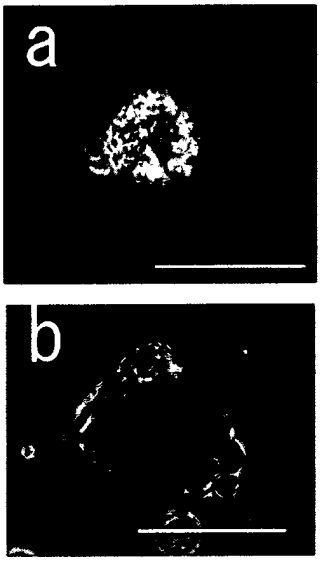


図１３

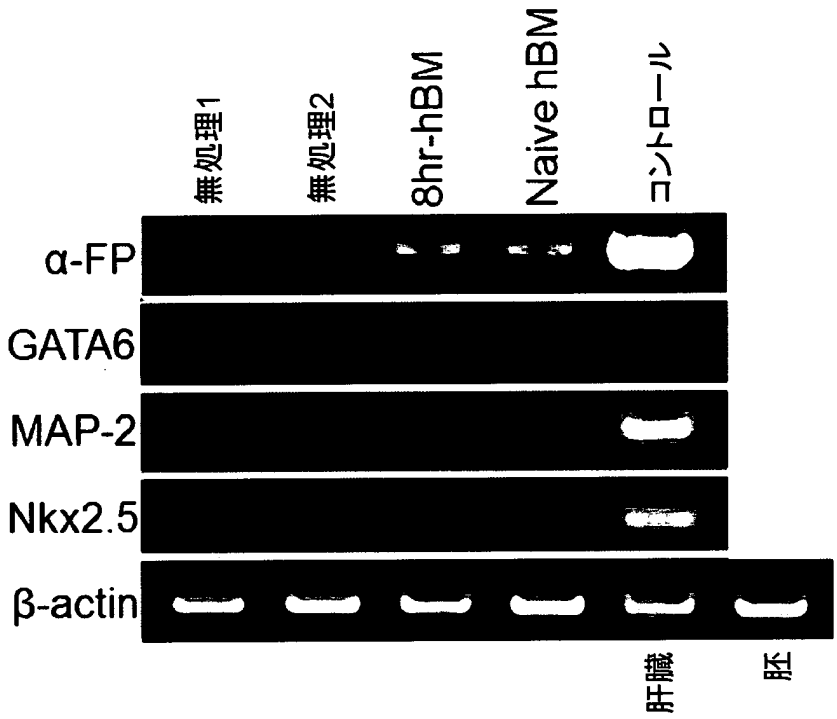

無処理1
無処理2
8hr-hBM
Naive hBM
コントロール
α-FP
GATA6
MAP-2
Nkx2.5
β-actin
肝臓
胚

図１４

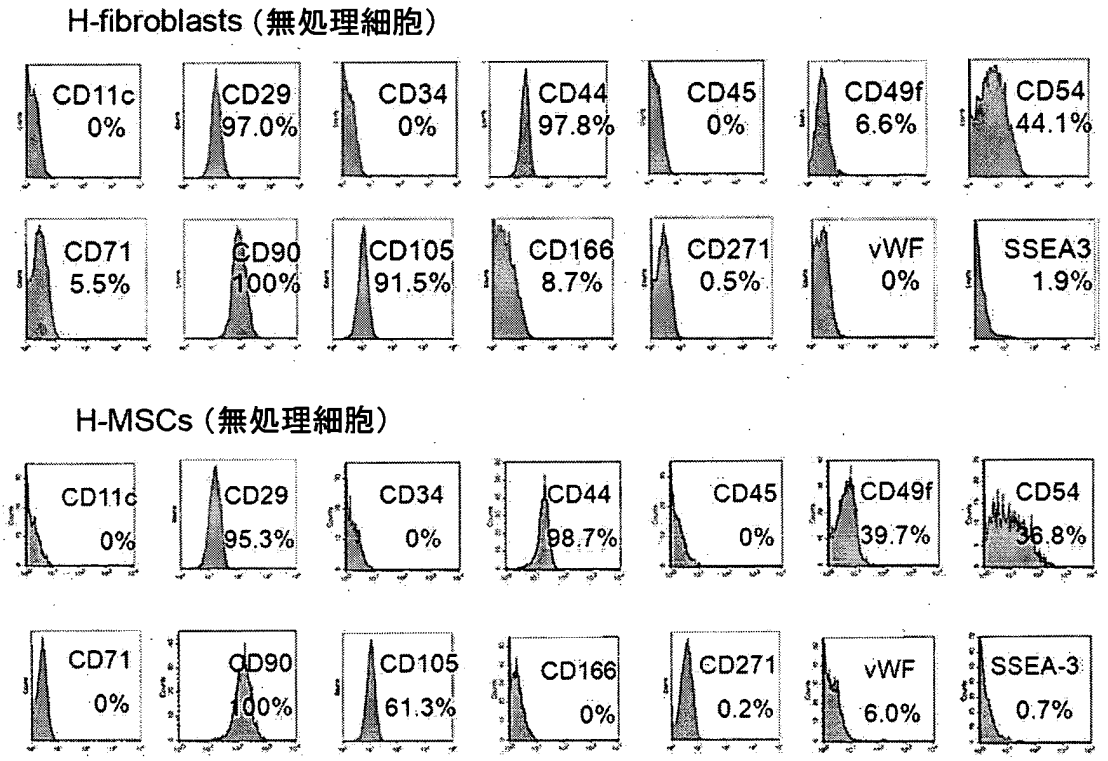

H-fibroblasts（無処理細胞）
CD11c 0%
CD29 97.0%
CD34 0%
CD44 97.8%
CD45 0%
CD49f 6.6%
CD54 44.1%
CD71 5.5%
CD90 100%
CD105 91.5%
CD166 8.7%
CD271 0.5%
vWF 0%
SSEA3 1.9%
H-MSCs（無処理細胞）
CD11c 0%
CD29 95.3%
CD34 0%
CD44 98.7%
CD45 0%
CD49f 39.7%
CD54 36.8%
CD71 0%
CD90 100%
CD105 61.3%
CD166 0%
CD271 0.2%
vWF 6.0%
SSEA-3 0.7%

図１５－１

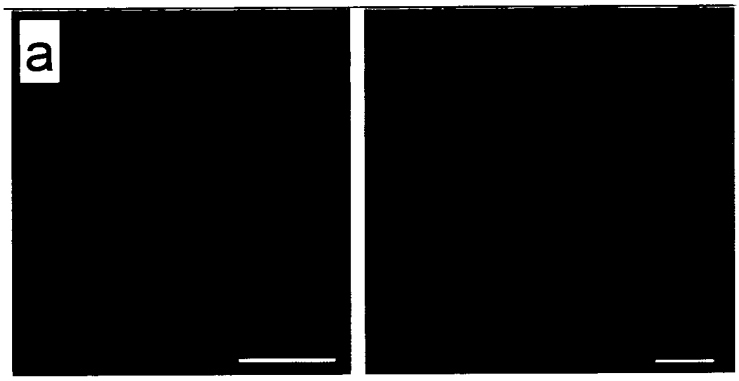


図１５－２

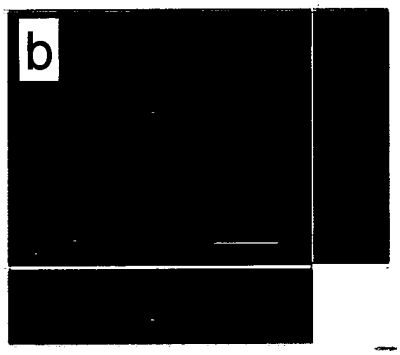


図１５－３

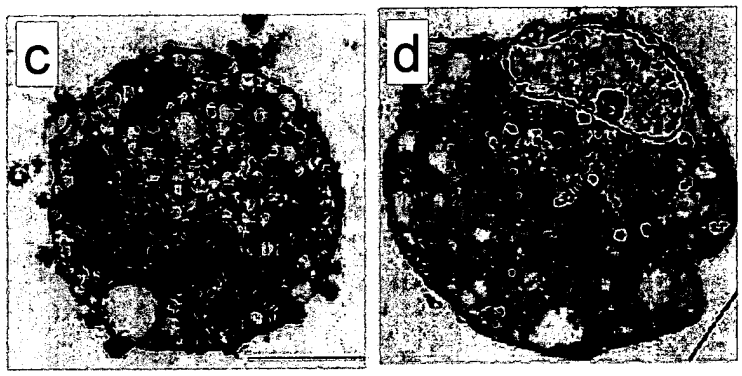


図１５－４

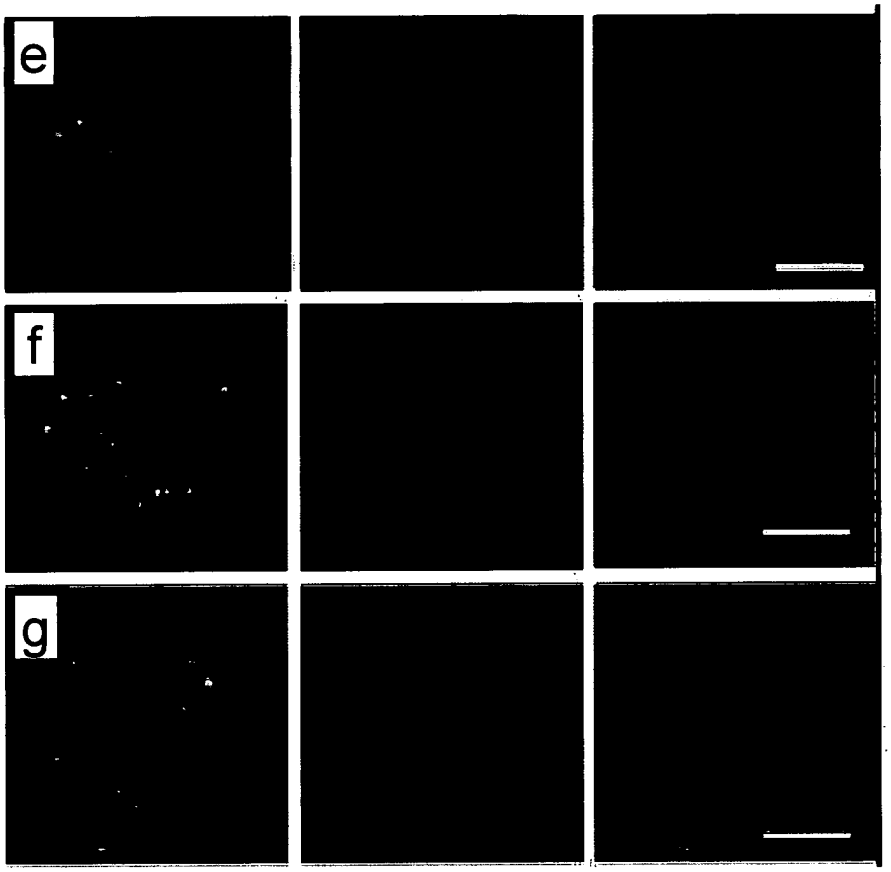


図16－1

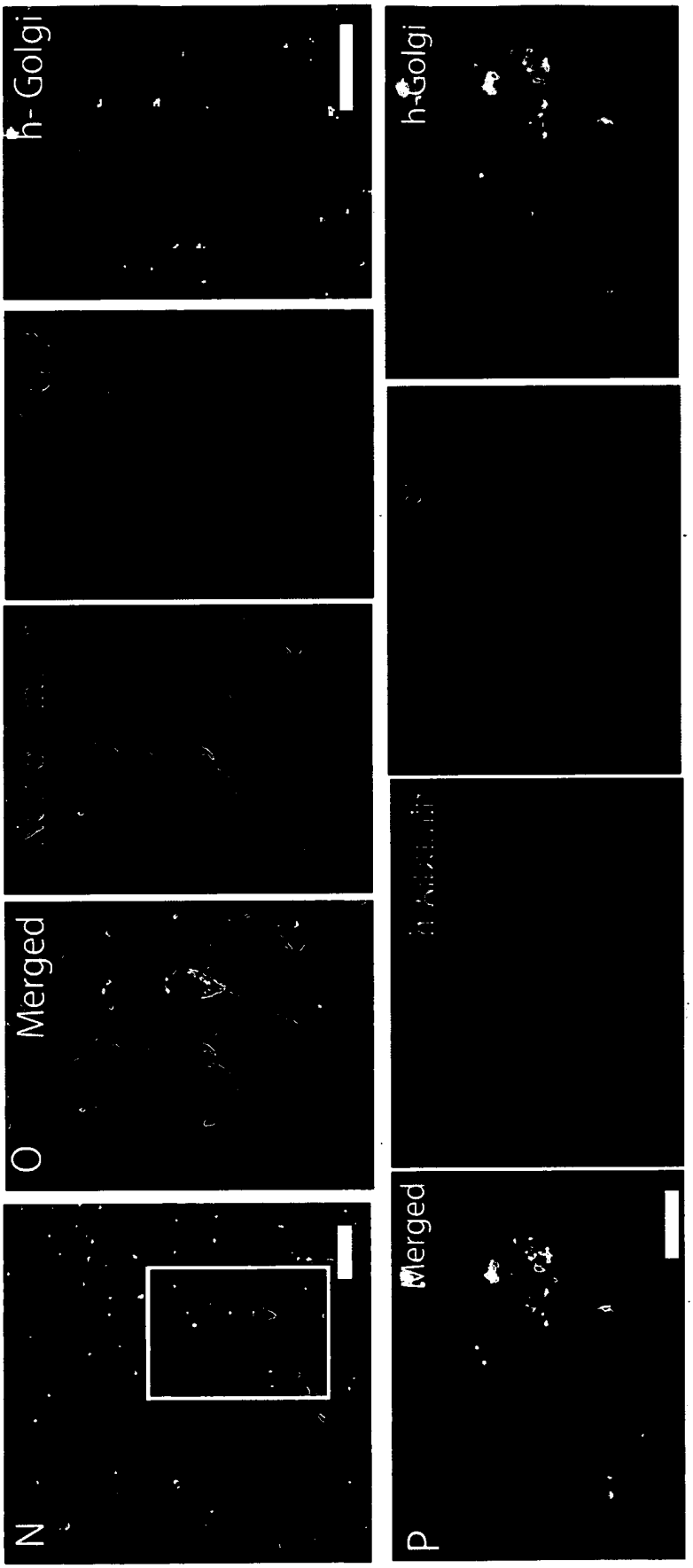


図１６－２

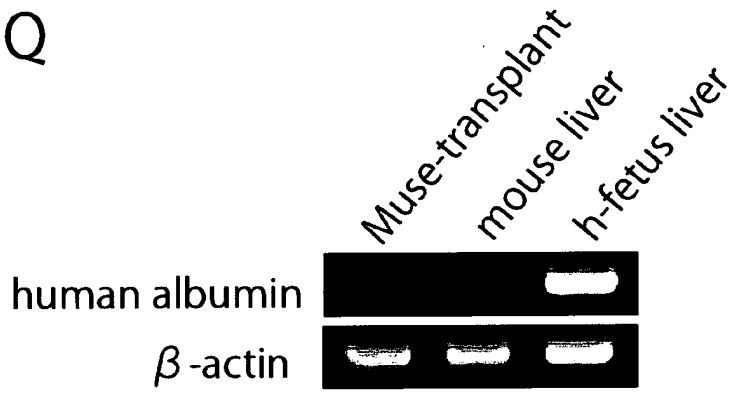

Q
Muse-transplant
mouse liver
h-fetus liver
human albumin
β-actin

図１６－３

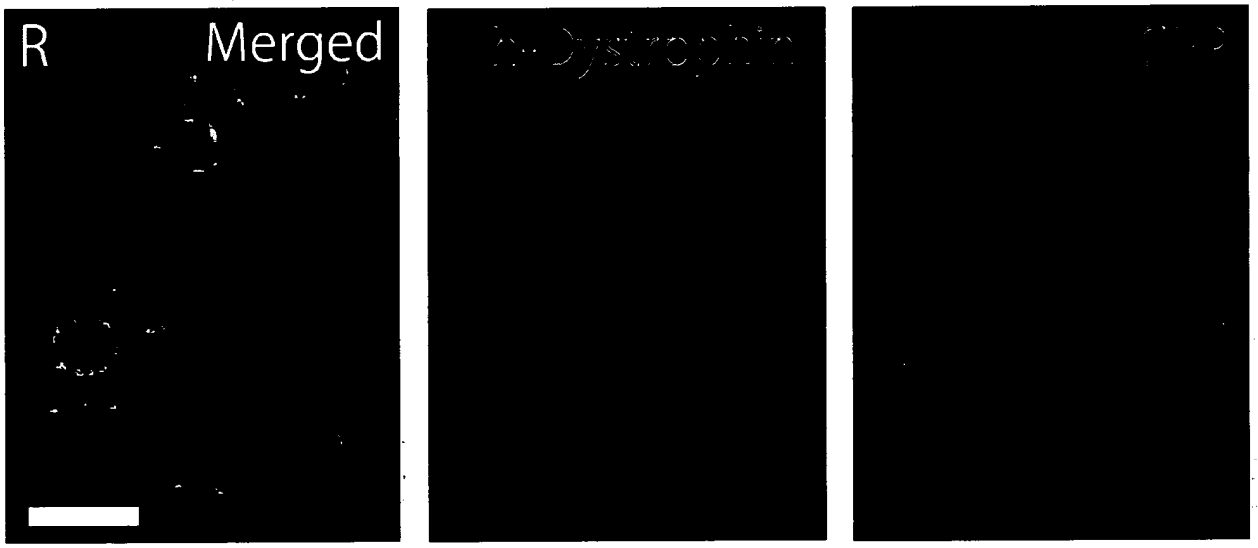


図１７－１

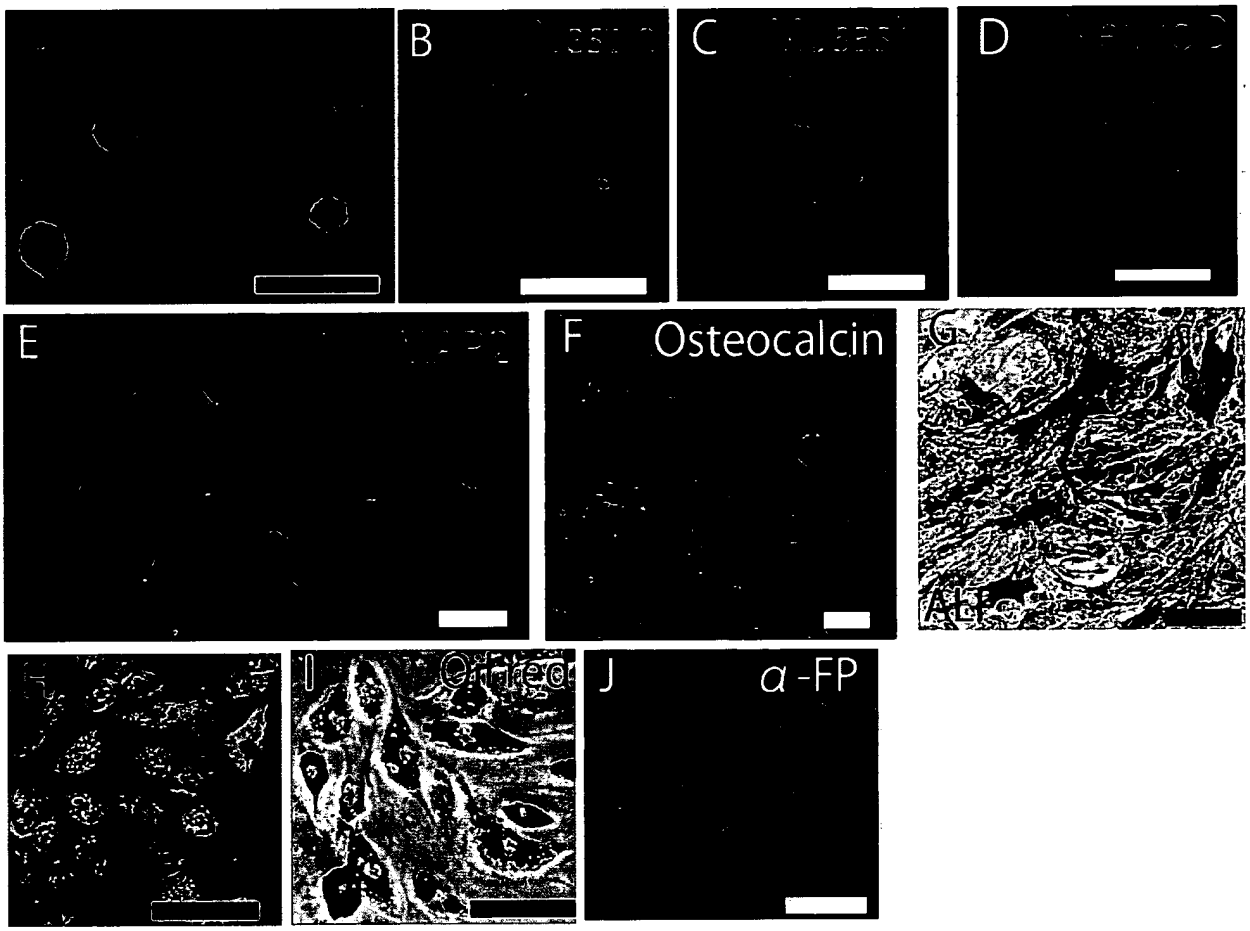


図１７－２

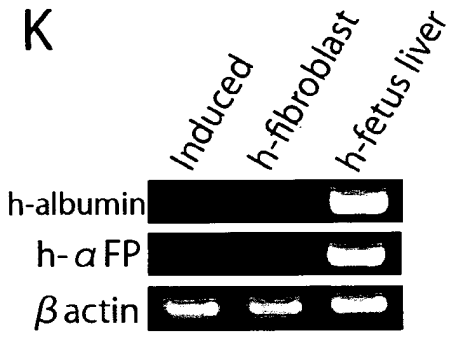


図１８－１

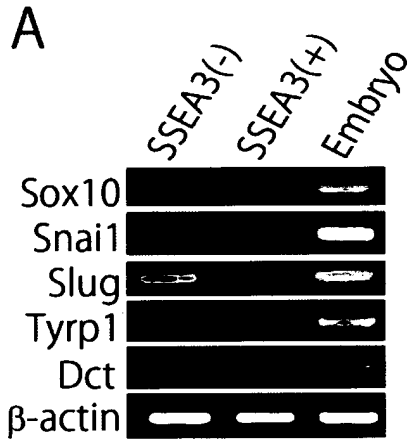


図１８－２

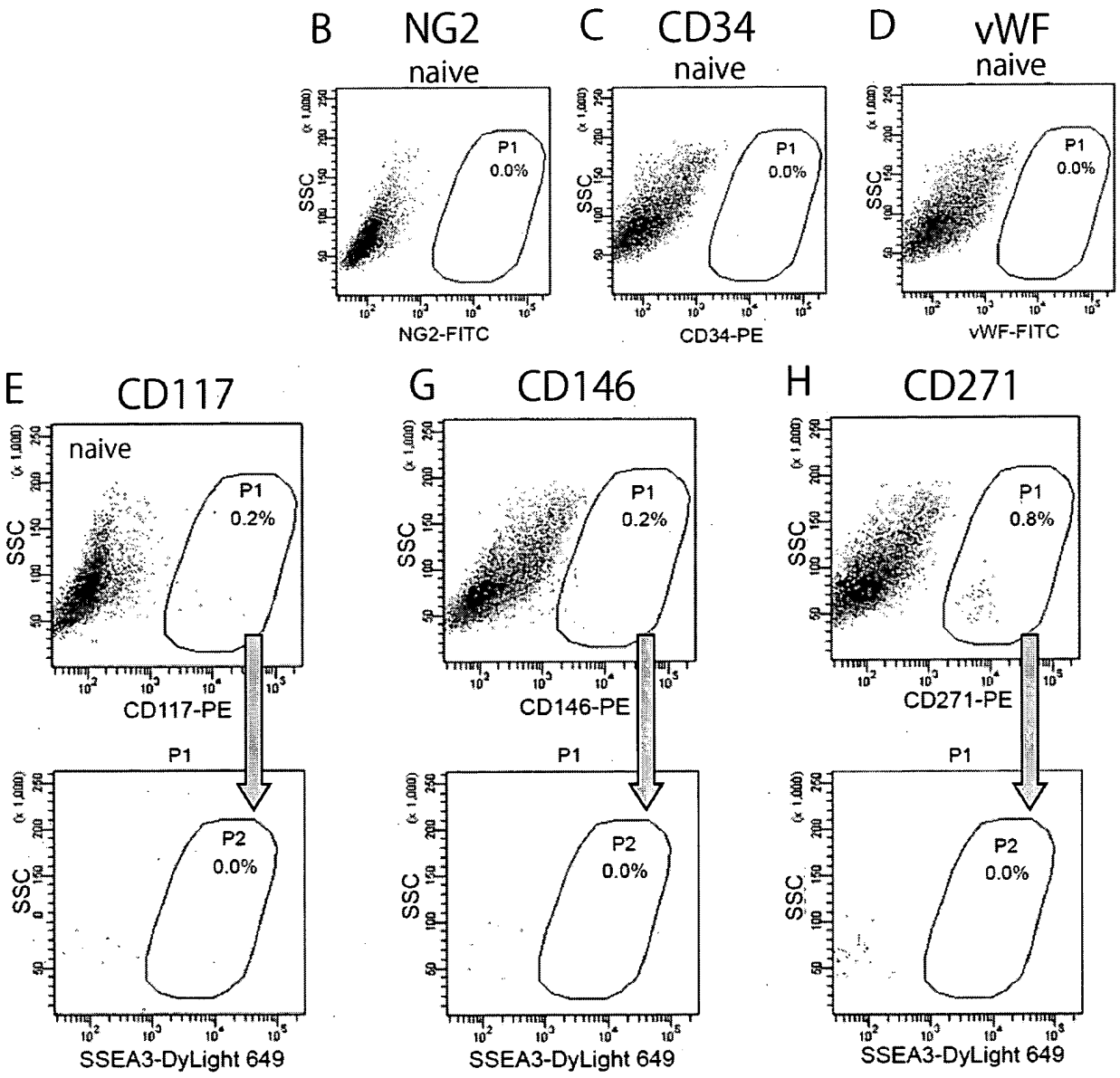

B NG2
naive
P1
0.0%
SSC
NG2-FITC
C CD34
naive
P1
0.0%
SSC
CD34-PE
D vWF
naive
P1
0.0%
SSC
vWF-FITC
E CD117
naive
P1
0.2%
SSC
CD117-PE
P1
P2
0.0%
SSC
SSEA3-DyLight 649
G CD146
P1
0.2%
SSC
CD146-PE
P1
P2
0.0%
SSC
SSEA3-DyLight 649
H CD271
P1
0.8%
SSC
CD271-PE
P1
P2
0.0%
SSC
SSEA3-DyLight 649

図１８－３



図１９

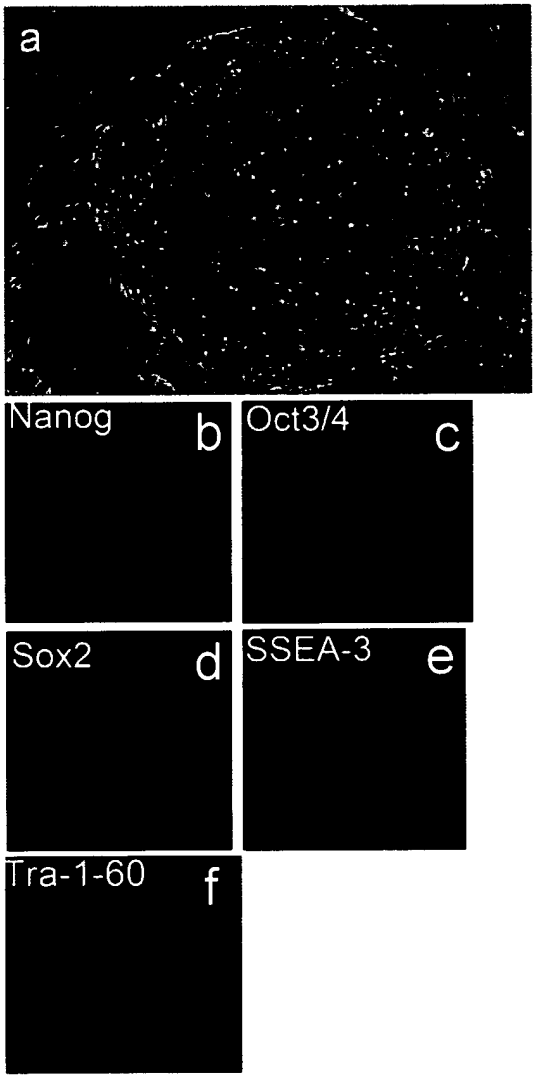


図２０

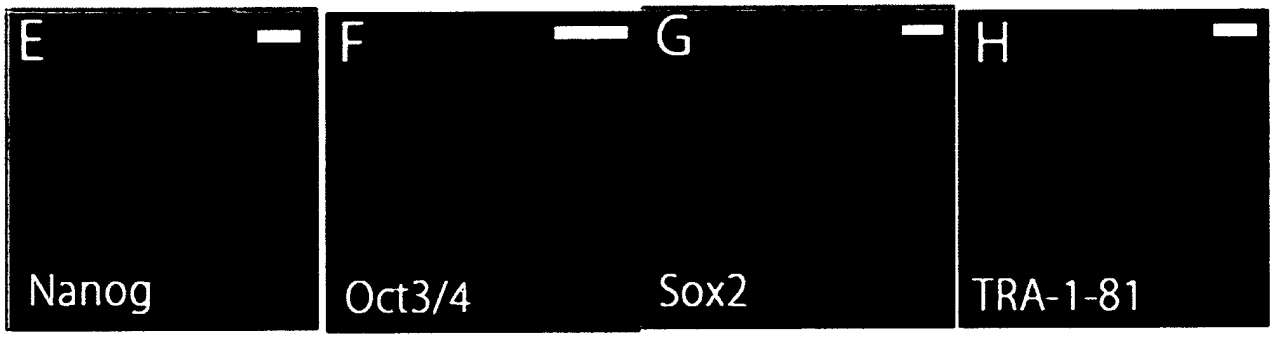


図２１

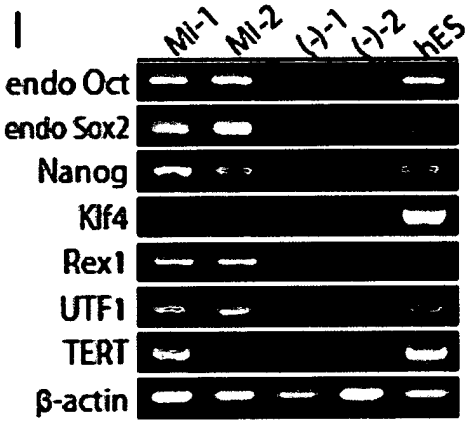

MI-1
MI-2
(-)-1
(-)-2
hES
endo Oct
endo Sox2
Nanog
Klf4
Rex1
UTF1
TERT
β-actin

図２２－１

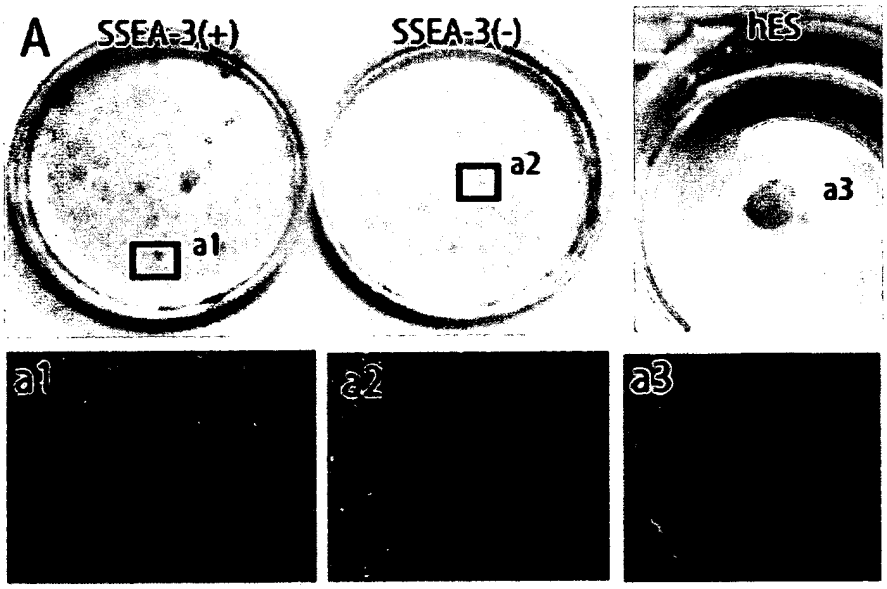


図２２－２

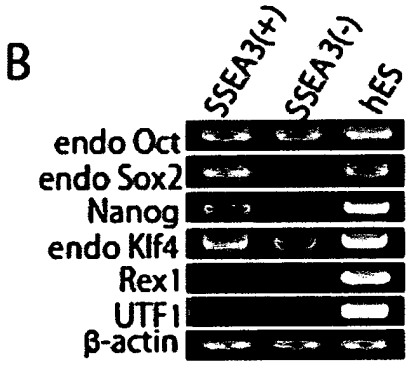

B
SSEA3(+)
SSEA3(-)
hES
endo Oct
endo Sox2
Nanog
endo Klf4
Rex1
UTF1
β-actin

図２２－３

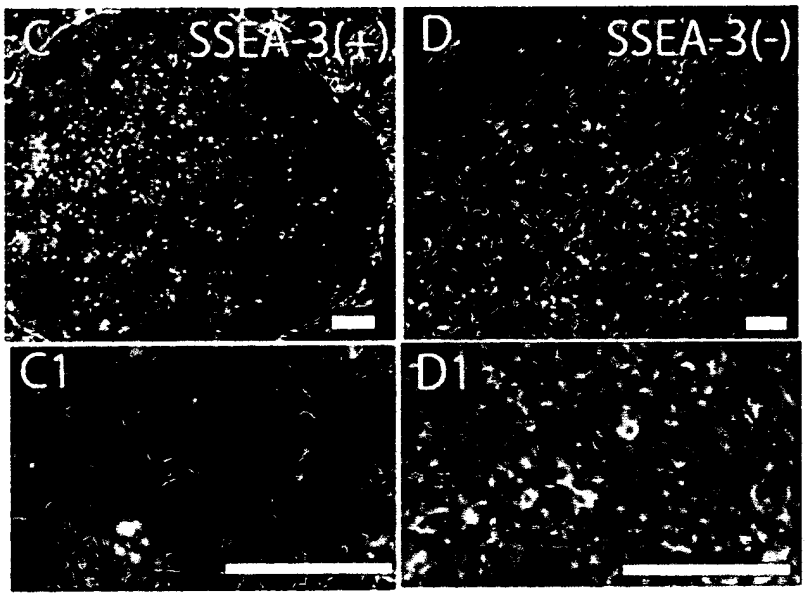


図２３－１

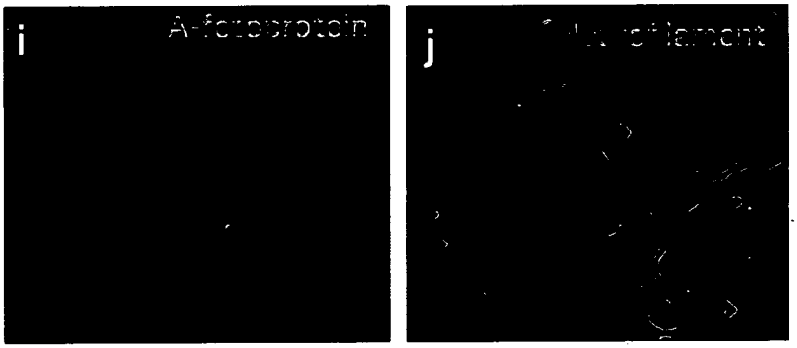


図２３－２

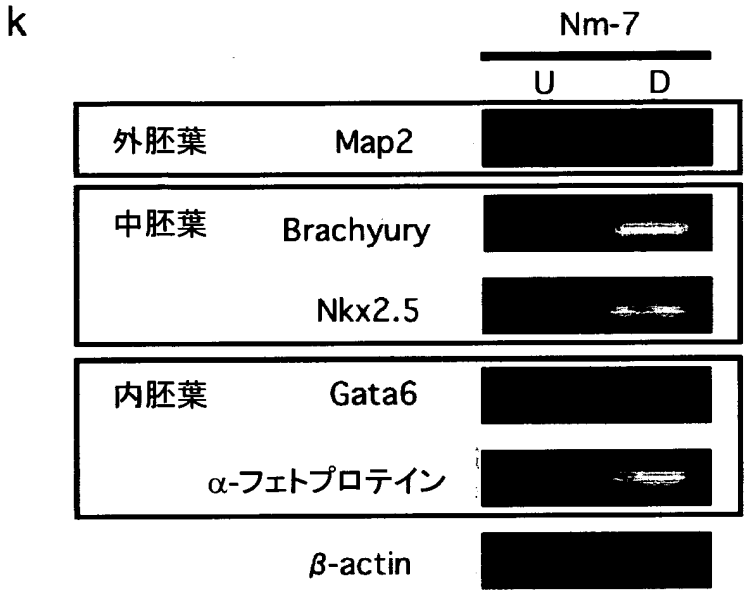

k
Nm-7
U
D
外胚葉
Map2
中胚葉
Brachyury
Nkx2.5
内胚葉
Gata6
α-フェトプロテイン
β-actin

図２３－３

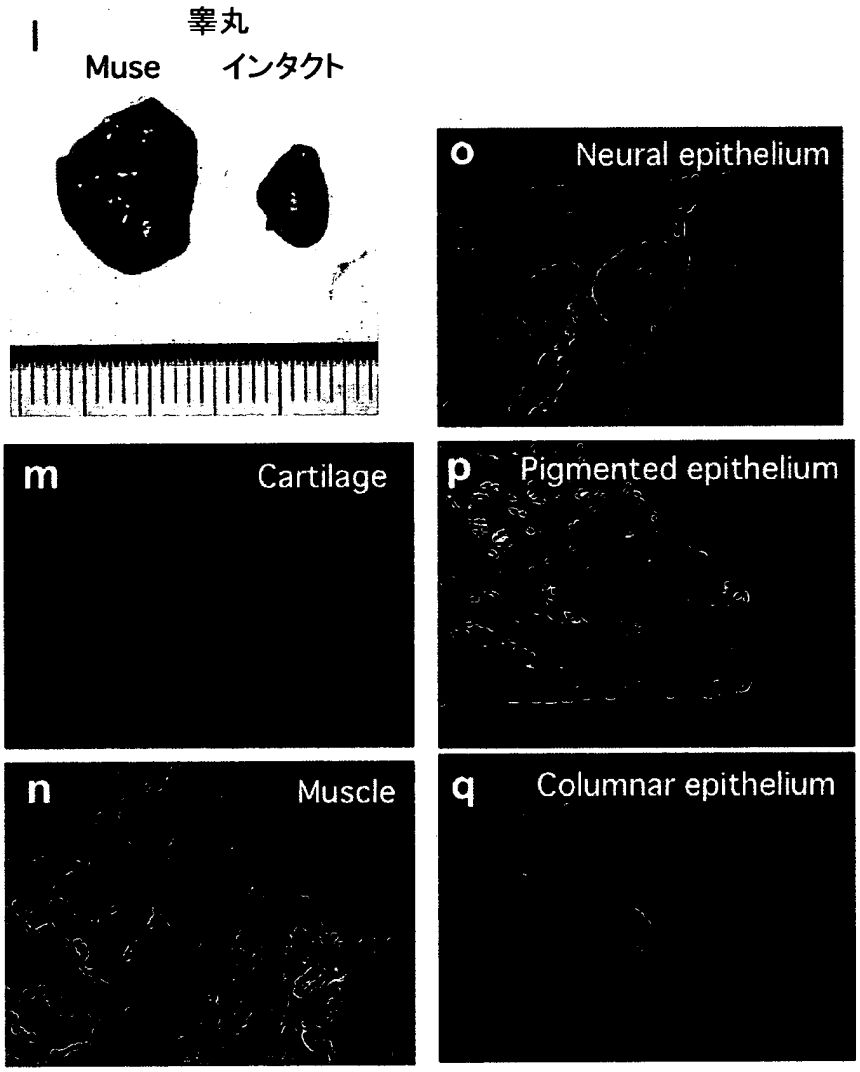

l
睾丸
Muse
インタクト
o
Neural epithelium
m
Cartilage
p
Pigmented epithelium
n
Muscle
q
Columnar epithelium

図２４

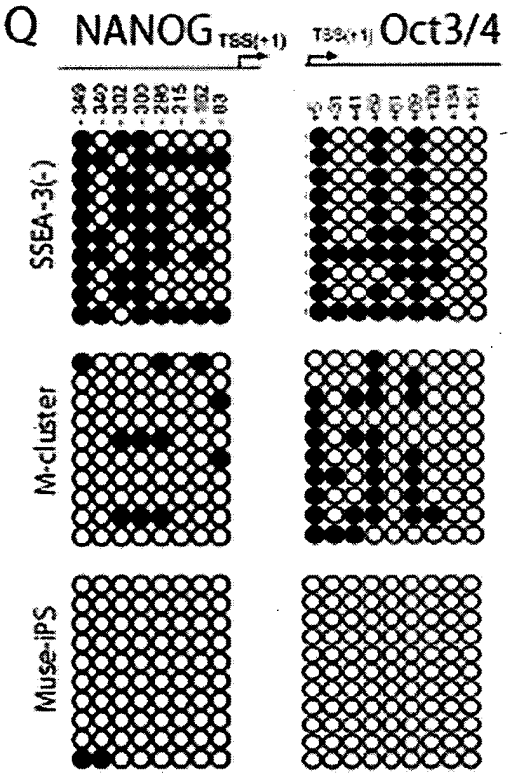


図25

		/Naive		/iPS
Symbol	Description	Cluster	iPS	Cluster
ABL1	C-abl oncogene 1, receptor tyrosine kinase			1.2E+00
ANAPC2	Anaphase promoting complex subunit 2			8.5E-01
ANAPC4	Anaphase promoting complex subunit 4			5.0E-01
ATM	Ataxia telangiectasia mutated			1.7E+00
ATR	Ataxia telangiectasia and Rad3 related			9.5E-01
BRCA1	Breast cancer 1, early onset			1.3E-01*
CCNB1	Cyclin B1			5.9E-02*
CCNB2	Cyclin B2			6.6E-02*
CCNC	Cyclin C			2.2E-01*
CCND1	Cyclin D1			3.7E+00**
CCND2	Cyclin D2			3.7E-02*
CCNE1	Cyclin E1			1.0E-01*
CCNF	Cyclin F			1.1E-01*
CCNG1	Cyclin G1			4.1E-01
CCNG2	Cyclin G2			1.1E+01**
CCNH	Cyclin H			6.6E-01
CCNT1	Cyclin T1			5.8E-01
CCNT2	Cyclin T2			4.0E-01
CDC16	Cell division cycle 16 homolog (S. cerevisiae)			3.3E-01*
CDC2	Cell division cycle 2, G1 to S and G2 to M			6.4E-02*
CDC20	Cell division cycle 20 homolog (S. cerevisiae)			3.3E-02*
CDC34	Cell division cycle 34 homolog (S. cerevisiae)			4.7E-01
CDK2	Cyclin-dependent kinase 2			3.0E-01*
CDK4	Cyclin-dependent kinase 4			6.4E-01
CDK5R1	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)			2.2E-01*
CDK5RAP1	CDK5 regulatory subunit associated protein 1			3.2E-01*
CDK6	Cyclin-dependent kinase 6			5.7E-01
CDK7	Cyclin-dependent kinase 7			3.5E-01
CDK8	Cyclin-dependent kinase 8			4.7E-01
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)			8.3E+01**
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)			8.2E-01
CDKN2A	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)			3.7E+02**
CDKN2B	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)			7.8E+01**
CDKN3	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)			1.3E-01*
CHEK1	CHK1 checkpoint homolog (S. pombe)			1.7E-01*
CHEK2	CHK2 checkpoint homolog (S. pombe)			7.7E-02*
CUL1	Cullin 1			4.2E-01
E2F4	E2F transcription factor 4, p107/p130-binding			4.8E-01
GADD45A	Growth arrest and DNA-damage-inducible, alpha			5.1E+00**
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)			4.4E-02*
MAD2L2	MAD2 mitotic arrest deficient-like 2 (yeast)			8.2E-02*
MCM2	Minichromosome maintenance complex component 2			5.6E-02*
MCM3	Minichromosome maintenance complex component 3			5.7E-02*
MCM4	Minichromosome maintenance complex component 4			2.2E-01*
MCM5	Minichromosome maintenance complex component 5			3.3E-02*
MNAT1	Menage a trois homolog 1, cyclin H assembly factor (Xenopus laevis)			6.8E-01
MRE11A	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)			3.7E-01
PCNA	Proliferating cell nuclear antigen			2.0E-01*
RAD51	RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)			7.0E-02*
RB1	Retinoblastoma 1 (including osteosarcoma)			4.0E+00**
RBBP8	Retinoblastoma binding protein 8			1.8E-01*
RBL1	Retinoblastoma-like 1 (p107)			2.3E-01*
RBL2	Retinoblastoma-like 2 (p130)			5.5E+00**
RPA3	Replication protein A3, 14kDa			1.3E-01*
SKP2	S-phase kinase-associated protein 2 (p45)			1.2E-01*
TERT	Telomerase reverse transcriptase			0.0E+00*
TFDP1	Transcription factor Dp-1			8.4E-01
TFDP2	Transcription factor Dp-2 (E2F dimerization partner 2)			6.6E-01
TP53	Tumor protein p53			3.3E-01*

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		/Naive		/iPS
Symbol	Description	Cluster	iPS	Cluster
ABCA13	ATP-binding cassette, sub-family A (ABC1), member 13			4.1E-01
ALPL	Alkaline phosphatase, liver/bone/kidney			2.9E-03*
ATRX	Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)			9.9E-01
BMP4	Bone morphogenetic protein 4			1.4E-01*
BMPR1A	Bone morphogenetic protein receptor, type IA			6.6E-01
CBX7	Chromobox homolog 7			5.5E+00**
CTR9	Ctr9, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)			6.2E-01
DAZL	Deleted in azoospermia-like			5.8E-02*
DDX4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4			5.0E-01
DNMT1	DNA (cytosine-5-)-methyltransferase 1			2.1E-01*
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta			6.2E-04*
DPPA2	Developmental pluripotency associated 2			1.6E-03*
DPPA3	Developmental pluripotency associated 3			1.1E-02*
DPPA4	Developmental pluripotency associated 4			1.1E-04*
EPC1	Enhancer of polycomb homolog 1 (Drosophila)			8.2E-01
ERAS	ES cell expressed Ras			1.1E-01*
F11R	F11 receptor			2.0E-03*
FGFR1	Fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)			5.8E-01
FOXD3	Forkhead box D3			4.2E-04*
GDF3	Growth differentiation factor 3			0.0E+00*
GRB7	Growth factor receptor-bound protein 7			1.8E-03*
HAND1	Heart and neural crest derivatives expressed 1			1.9E-02*
HES1	Hairy and enhancer of split 1, (Drosophila)			1.4E+00
HEXIM1	Hexamethylene bis-acetamide inducible 1			2.8E+00
HOXB1	Homeobox B1			4.7E-02*
ID1	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein			3.3E+00**
ID3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein			3.0E+00**
IFITM1	Interferon induced transmembrane protein 1 (9-27)			1.7E-01*
KCNK3	Potassium channel, subfamily K, member 3			3.4E+00**
KITLG	KIT ligand			1.2E+01**
KLF4	Kruppel-like factor 4 (gut)			1.0E+00
LIN28	Lin-28 homolog (C. elegans)			3.5E-04*
MSX2	Msh homeobox 2			3.8E-01
MYC	V-myc myelocytomatosis viral oncogene homolog (avian)			7.6E-01
NANOG	Nanog homeobox			4.0E-04*
NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)			9.4E-01
NKX1-2	NK1 homeobox 2			2.3E-03*
NR0B1	Nuclear receptor subfamily 0, group B, member 1			2.4E+01**
OCT3/4	POU class 5 homeobox 1			1.8E-02*
OTX2	Orthodenticle homeobox 2			0.0E+00*
PAN3	PAN3 polyA specific ribonuclease subunit homolog (S. cerevisiae)			4.5E-01
PODXL	Podocalyxin-like			3.8E-02*
PRDM1	PR domain containing 1, with ZNF domain			1.0E+01**
RAG1AP1	Recombination activating gene 1 activating protein 1			8.6E-01
SALL4	Sal-like 4 (Drosophila)			6.4E-04*
SIX4	SIX homeobox 4			9.9E-01
SOX2	SRY (sex determining region Y)-box 2			7.4E-04*
SPAG9	Sperm associated antigen 9			1.3E+00
SPRY1	Sprouty homolog 1, antagonist of FGF signaling (Drosophila)			5.1E-01
SPRY2	Sprouty homolog 2 (Drosophila)			1.9E+00
SSBP2	Single-stranded DNA binding protein 2			1.7E+00
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)			1.6E+00
TDGF1	Teratocarcinoma-derived growth factor 1			3.8E-05*
TFE3	Transcription factor binding to IGHM enhancer 3			1.1E+00
TRDMT1	TRNA aspartic acid methyltransferase 1			7.3E-01
UTF1	Undifferentiated embryonic cell transcription factor 1			0.0E+00*
VIM	Vimentin			1.4E+02**

図２７

動物	リプログラミング因子 [b]	細胞タイプ	時間(週)	効率
マウス	Viral-integrating vectors			
	O, K, S, M	MEFs	2-3	0.01-0.50%
	O, K, S, M	肝細胞	2-3	1-3%
	O, K, S, M	胃上皮細胞	2-3	1-3%
	O, K, S, M	B 細胞	2-3	0.01-0.10%
	O, K, S, M	β 細胞	3-4	0.1-0.2%
	O, K, S, M	神経幹細胞	1-2	3-5%
	O, K, S	MEFs	3-4	0.001-0.010%
	O, K, S	肝細胞	2-3	0.5-1.0%
	O, K, M	神経幹細胞	1-2	0.1-1.0%
	O, K,	神経幹細胞	2-3	0.1-1.0%
	O, M, S	神経幹細胞	3-4	0.1-1.0%
	O, K	神経幹細胞	2-3	0.1-0.2%
	O, M	神経幹細胞	3-4	0.1-0.2%
	Non-integrating viral vectors [c]			
	O, K, S, M	肝細胞	4-5	0.0001 to 0.0010%
	Plasmid-based vedtros [c]			
	O, K, S, M	MEFs	3-4	0.0001 to 0.0010%
ヒト	Viral-integrating vectors			
	O, K, S, M	皮膚線維芽細胞	4-5	0.001-0.002%
	O, K, L, N	皮膚線維芽細胞	2-3	N.D.
	O, K, S, M	骨髄間葉細胞	2-3	N.D.
	O, K, N	皮膚線維芽細胞	2-3	N.D.
	O, K, L	皮膚線維芽細胞	2-3	N.D.
	O, S, K [d]	皮膚線維芽細胞	4	0.1-1.0%
	O, S [d]	皮膚線維芽細胞	4	0.001-0.010%
	O, S, L, N [e]	皮膚線維芽細胞		0.002%
	O, K, S, M, T,LT	皮膚線維芽細胞		0.0417%
	O, K, S, M, p, U	皮膚線維芽細胞		N.D.

MEFs, マウス胚線維芽細胞　　; N.D. , not determined.

[b] O, Oct4; S, Sox2; M, c-Myc; K, Klf4; N, Nanog; L, Lin28; T, hTERT; LT, Large T antigen; p, p53RNAi; U, Utf1
[c] Integration-free clones.
[d] Valpronic acid (VPA) を併用
[e] CHIR99021, PD0325901, A-83-01