図44　E-boxクラスター領域のｈTERT転写活性化へのc-mycの関与について

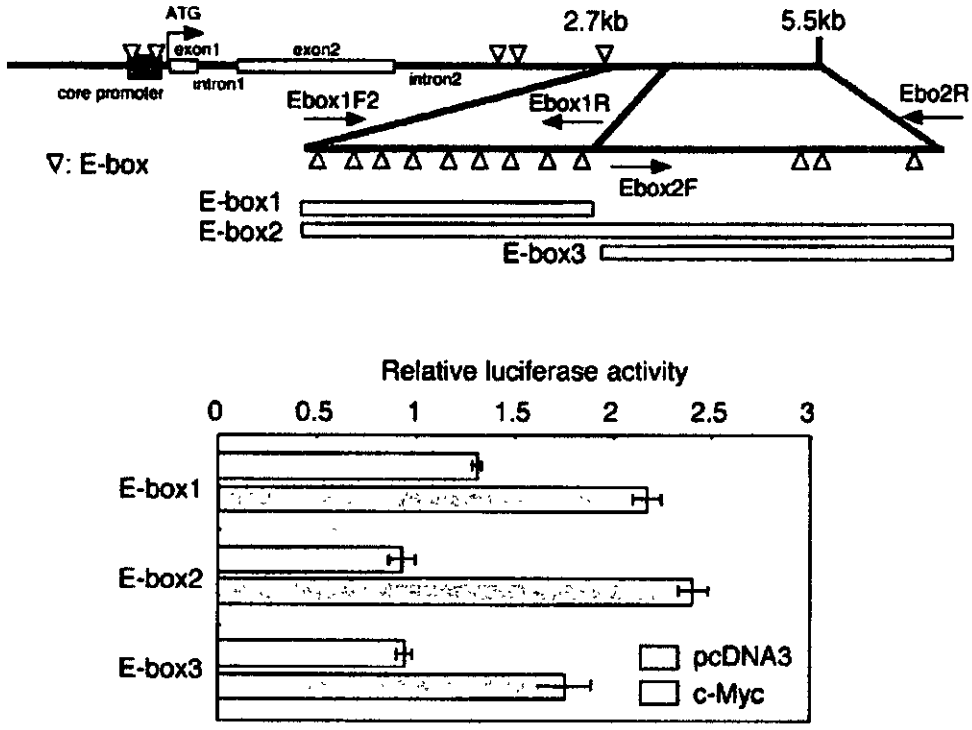


Figure　Transcriptional activation of the *hTERT* promoter containing E-box cluster by *c-myc*. *Upper panel* shows the schematic representation of the *hTERT* promoter region. The genomic fragments of E-box cluster were amplified using the indicated primer sets (*arrows*) as described in "Materials and Methods". Then, the genomic fragments of E-box cluster (E-box1, 2, 3) were inserted into CMV promoter driven-luciferase construct (pGL3-Promoter), and used for analysis of transcriptional activation by *c-myc*. The c-Myc binding sites (E-box) are indicated by *triangles* (Wu et al., 1999), and *bent arrow* indicates traslation start site (ATG). *Lower panel* shows the transcriptional activation of *hTERT* by *c-myc*. A *c-myc* expression vector (pcDNA3 /HA-c-myc) or control vector (pcDNA3) were co-transfected into A549 cells with different E-box cluster-reporter plasmids by LipofectAMINE PLUS reagent. For each transfection, the firefly luciferase activity of hTERT E-box cluster-reporter constructs shown in the *upper panel* was normalized with *Renilla reniformis* luciferase activity driven from the co-transfected pRL-SV40. The means from the three independent experiments are shown. *Bars* indicate standard deviations.

図45 MyoDによるhTERTコアプロモーターの転写制御について

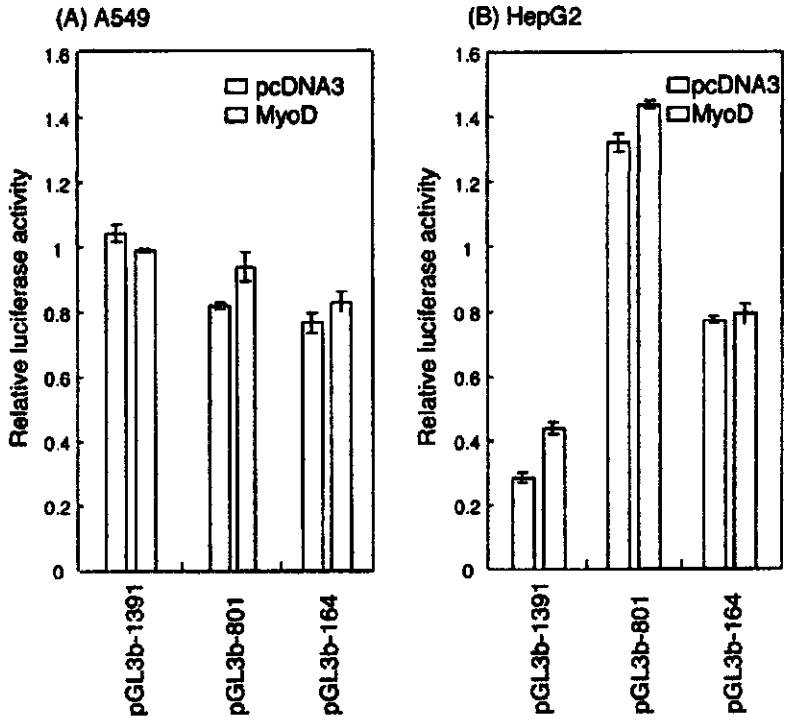


Figure Transcriptional repression of the *hTERT* promoter by MyoD. A human *MyoD* cDNA expression plasmid (pcDNA3-MyoD) or vector alone (pcDNA3) was co-transfected with the firefly luciferase construct (pGL3b-1391, pGL3b-801 or pGL3b-286) and the pRL-TK into the A549 cells (A) or HepG2 (B). The means from the three independent experiments are shown. *Bars* indicate standard deviations.

図46　IRF-1によるhTERTコアプロモーターの転写制御について

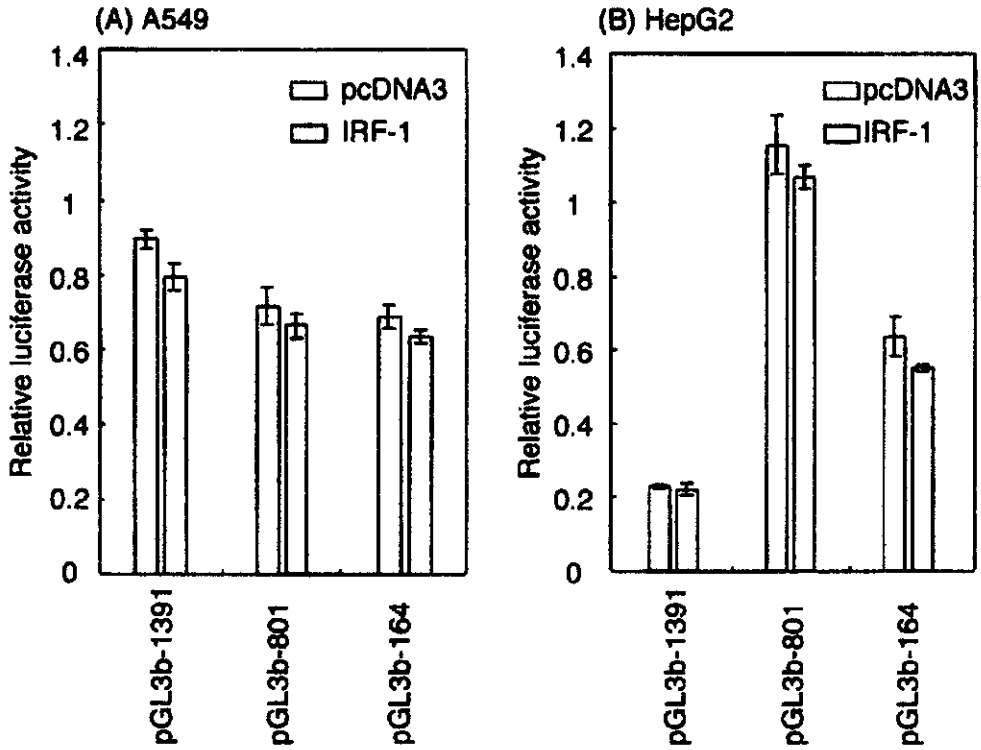


Figure Transcriptional repression of the *hTERT* promoter by IRF-1. A human *IRF*-1 cDNA expression plasmid (pcDNA3-IRF-1) or vector alone (pcDNA3) was co-transfected with the firefly luciferase construct (pGL3b-1391, pGL3b-801 or pGL3b-286) and the pRL-TK into the A549 cells (A) or HepG2 (B). The means from the three independent experiments are shown. *Bars* indicate standard deviations.

表15．分離された形質転換巣の形質転換能確認のための
トランスフォーメーション試験

parental or transformant cells	cloning efficiency (% of control)	No. of foci/plate*
A3111	100	0.3 ±0.48
A5	106.3	2.3±1.23
A6	110.8	47.0±6.28
L11	144.8	114.1±13.32
L21	126.2	84.5±5.10
A3111+3-MC**	2.2	30.0±5.15

*average with SD of 15 plates
**A3111 cells were treated with 0.5 μg/ml of 3-MC (positive control)

表16．発現が親株の5倍以上亢進した遺伝子

Code	Gene
A08e	integrin beta 7
A14l	insulin-like growth factor binding protein 10
B02a	c-fos proto-oncogene
B02b	FBJ osteosarcoma oncogene B
B02l	Jun oncogene
B02m	junB proto-oncogene
B06g	HSP27, HSP25,HSPB1,growth-related 25-kDa protein
B07e	N-oxide forming dimethylaniline monooxygenase 1 hepatic flavin-containing monooxygenase 1 dimethylaniline oxidase 1
C01k	retinoic acid receptor beta,nuclear receptor subfamily 1 group Bmember 2
C03m	caspase-activated DNase,DNase inhibited by DNA fragmentation factor
D05b	insulin-like growth factor II precursor, multiplication-stimulating polypeptide
D05e	leukemia inhibitory factor,cholinergic differentiation factor
D06d	proliferin
E07k	non-receptor type 16 protein tyrosine phosphatase

表17　発現が親株の5倍以上抑制された遺伝子

Code	Gene
A06f	Cdk 6 inhibitor, Cdk 4 inhibitor C, Cdk inhibitor 2C
A08c	fat tumor suppressor homolog (Drosophila)
A10n	thrombospondin 1
A11c	vascular cell adhesion molecule 1 precursor
A12a	cysteine rich intestinal protein
A12e	delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG
B01l	EB1 APC-binding protein
B14d	HSP60, HSP65, HSPD1, mitochondrial matrix protein P1 precursor, 60-kDa chaperonin, GroEL protein
C01c	apoptosis inhibitor 1
C03j	clusterin precursor, clustrin, apolipoprotein J, sulfated glycoprotein 2
C07m	platelet-derived growth factor receptor alpha precursor
C12n	Hek2 murine homolog, Mdk5 mouse developmental kinase, Eph-related tyrosine-protein kinase receptor
D06b	pleiotrophin
D06l	small inducible cytokine A9
D14g	avian sarcoma virus CT10 (v-crk) oncogene homolog
E02n	non-receptor type 11 protein tyrosine phosphatase, phosphotyrosine phosphatase
E04f	Cdk7, CDC2-related kinase 4, Cdk-activating kinase, 39-kDa protein kinase, MO15, MPK7
E08f	serum-inducible kinase
E13k	histidine triad nucelotide-binding protein, protein kinase C iota
E13n	menage a trois 1
F01i	a disintegrin-like and metalloprotease with thrombospondin type 1 motif protein 1
F02g	matrix metalloproteinase 9
F03a	large multifunctional protease 7
F09h	developmentally d neural precursor cell expressed
F10j	tubulin cofactor a

図47　いずれかの形質転換巣で、その発現が５倍以上亢進していた遺伝子

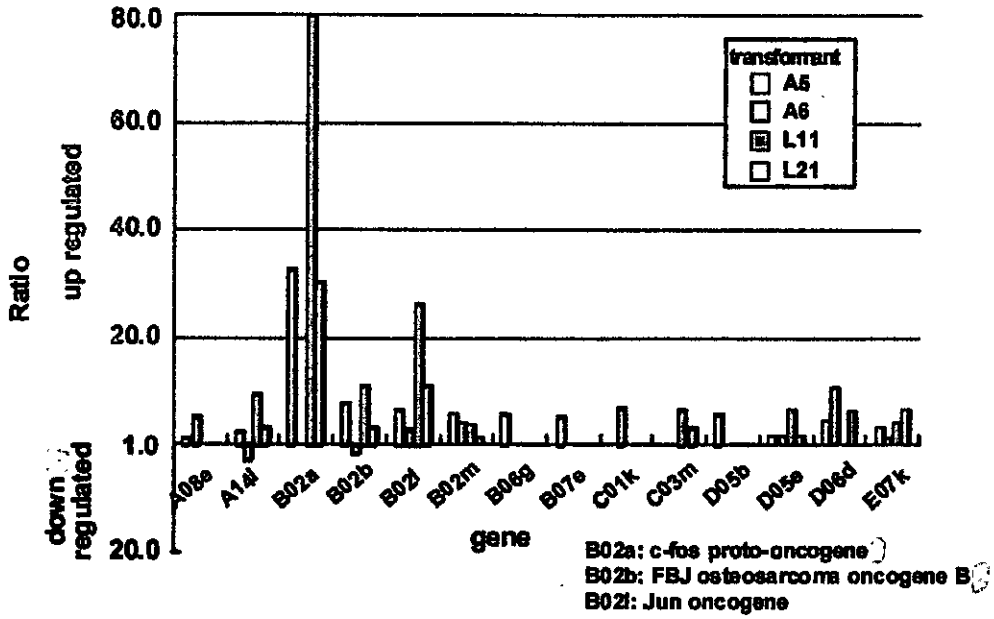


図48. いずれかの形質転換巣で、その発現が５倍以上低下していた遺伝子

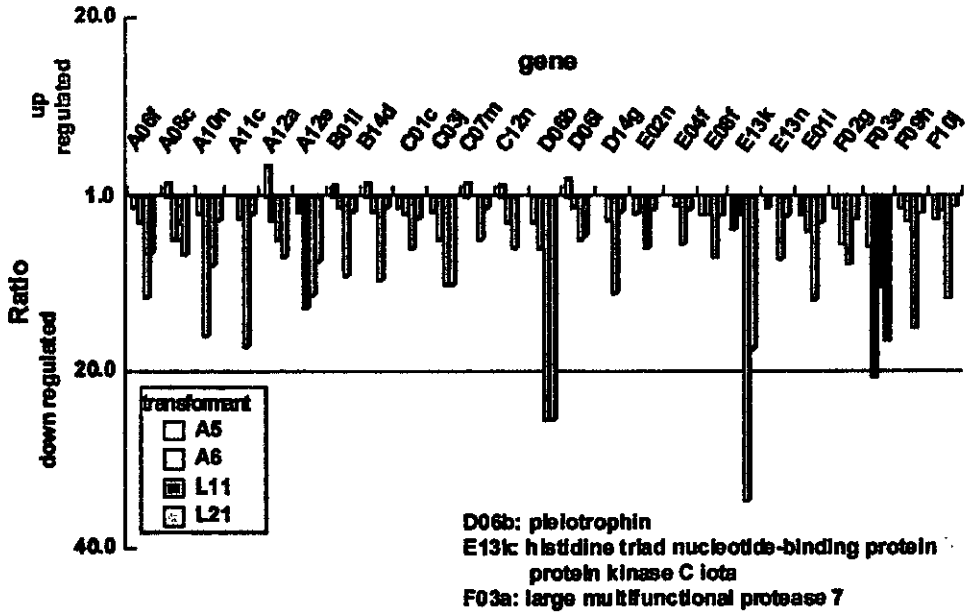


表18　発現が親株の5倍以上変化した遺伝子

transformant	Genes upregulated	Genes downregulated
A5	c-fos proto-oncogene (33.0) FBJ osteosarcoma oncogene B Jun oncogene junB proto-oncogene HSP27, HSP25, HSPB1, growth-related 25-kDa protein N-oxide forming dimethylaniline monooxygenase 1 hepatic flavin-containing monooxygenase 1 dimethylaniline oxidase 1 insulin-like growth factor II precursor, multiplication-stimulating polypeptide	large multifunctional protease 7
A6	integrin beta 7 retinoic acid receptor beta, nuclear receptor subfamily 1 group B member 2 proliferin (10.7)	fat tumor suppressor homolog (Drosophila) thrombospondin 1 (15 8) delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG (12.7) clusterin precursor, clustrin, apolipoprotein J, sulfated glycoprotein 2 pleiotrophin matrix metalloproteinase 9 large multifunctional protease 7 (20.7)
L11	insulin-like growth factor binding protein 10 c-fos proto-oncogene (79.8) FBJ osteosarcoma oncogene B (10.9) Jun oncogene (26.3) caspase-activated DNase, DNase inhibited by DNA fragmentation factor leukemia inhibitory factor, cholinergic differentiation factor	Cdk 6 inhibitor, Cdk 4 inhibitor C, Cdk inhibitor 2C (11 5) fat tumor suppressor homolog (Drosophila) thrombospondin 1 vascular cell adhesion molecule 1 precursor (17.0) delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG (11.2) EB1 APC-binding protein HSP60, HSP65, HSPD1, mitochondrial matrix protein P1 precursor, 60-kDa chaperonin, GroEL protein apoptosis inhibitor 1 clusterin precursor, clustrin, apolipoprotein J, sulfated glycoprotein 2 (10.0) platelet-derived growth factor receptor alpha precursor Hek2 murine homolog, Mdk5 mouse developmental kinase, Eph-related tyrosine-protein kinase receptor pleiotrophin (25.2) small inducible cytokine A9 avian sarcoma virus CT10 (v-crk) oncogene homolog (11.0) non-receptor type 11 protein tyrosine phosphatase, phosphotyrosine phosphatase Cdk7, CDC2-related kinase 4, Cdk-activating kinase, 39-kDa protein kinase, MO15, MPK7 serum-inducible kinase histidine triad nucelotide-binding protein, protein kinase C iota (34.4) menage a trois 1 a disintegrin-like and metalloprotease with thrombospondin type 1 motif protein 1 (11.8) matrix metalloproteinase 9 large multifunctional protease 7 (10.4) developmentally d neural precursor cell expressed (15.0) tubulin cofactor a (11.8)
L21	c-fos proto-oncogene (30.3) Jun oncogene (11.1) proliferin non-receptor type 16 protein tyrosine phosphatase	Cdk 6 inhibitor, Cdk 4 inhibitor C, Cdk inhibitor 2C fat tumor suppressor homolog (Drosophila) cysteine rich intestinal protein delta-like homolog 1, preadipocyte factor 1, SCP 1, FA1, ZOG clusterin precursor, clustrin, apolipoprotein J, sulfated glycoprotein 2 (10.0) pleiotrophin (25.2) histidine triad nucelotide-binding protein, protein kinase C iota (17.2) large multifunctional protease 7 (16.6)

Genes in black and red were up- or down-regulated more than 5-fold to less than 10-fold and more than 10-fold, respectively.
Figures in parentheses indicate fold-increase or -decrease in gene expression compared to parental cells.

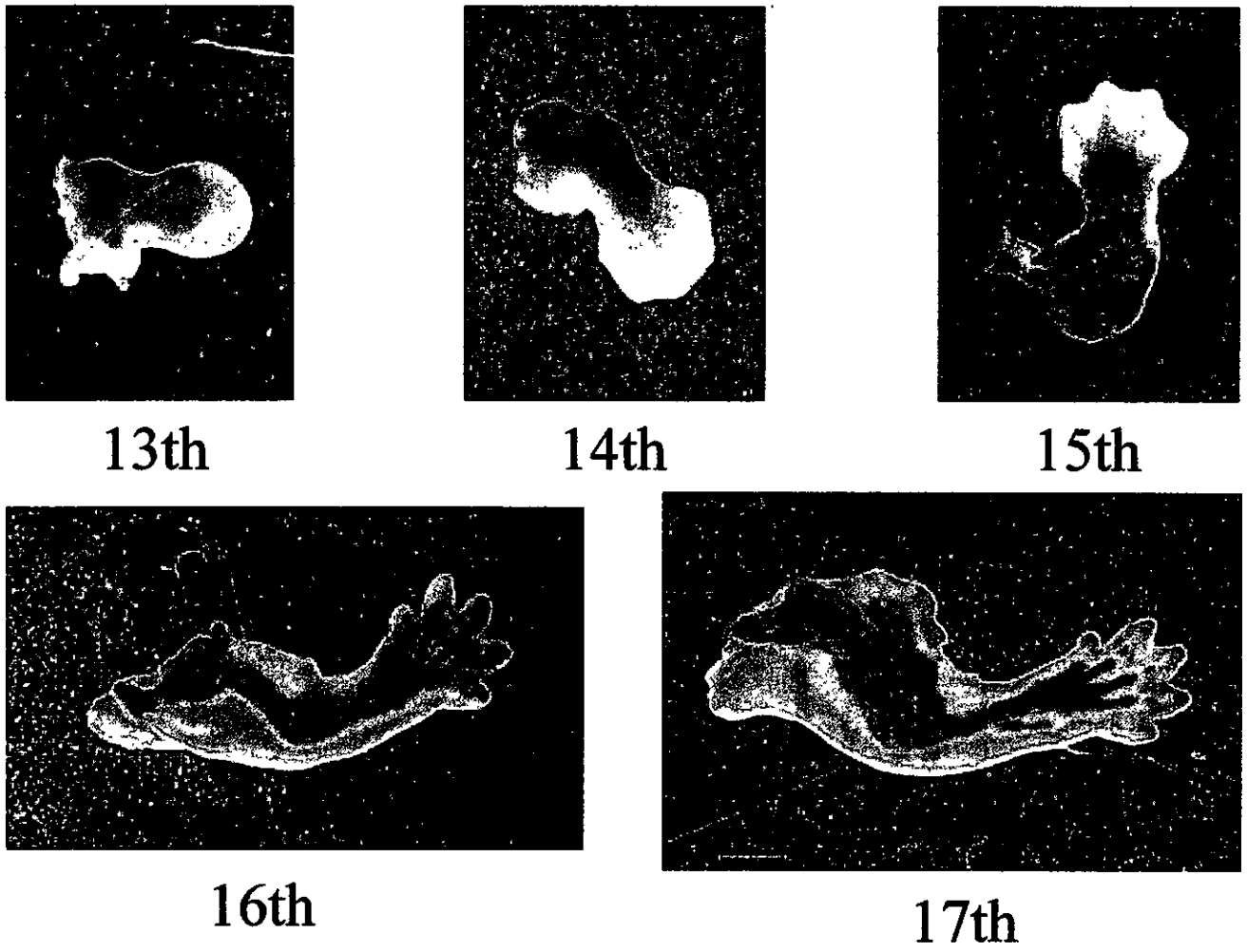


図49　ラット胎児の肢器官のアルシアンブルー染色標本

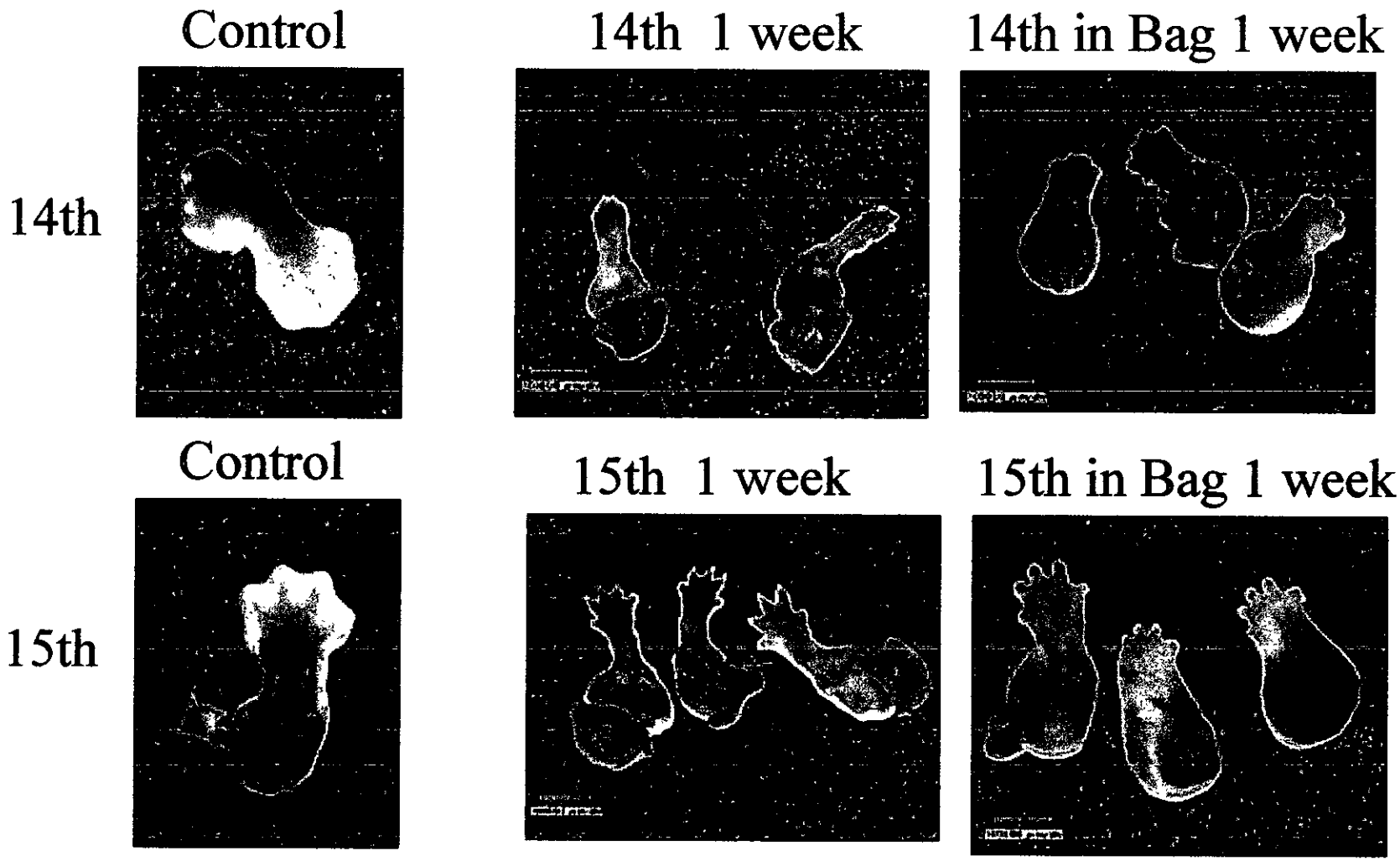


図50 肢芽に動的ストレスを与えて培養した際の
形態変化と軟骨分化能

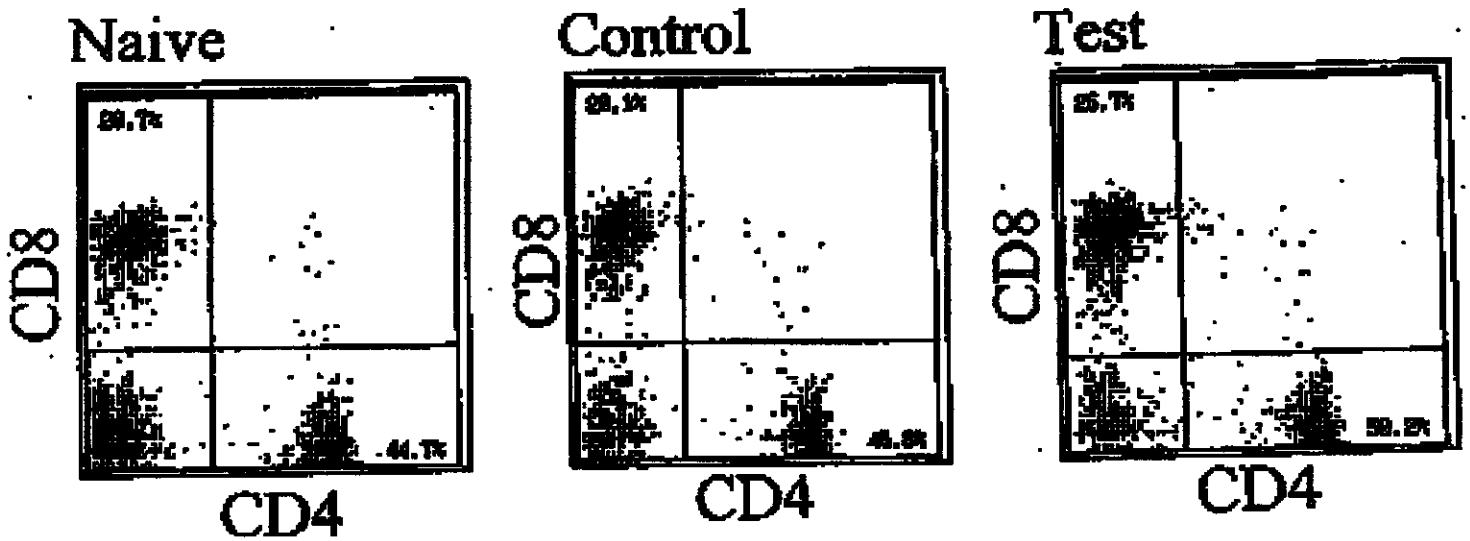
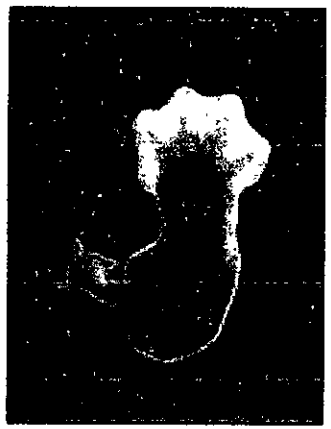
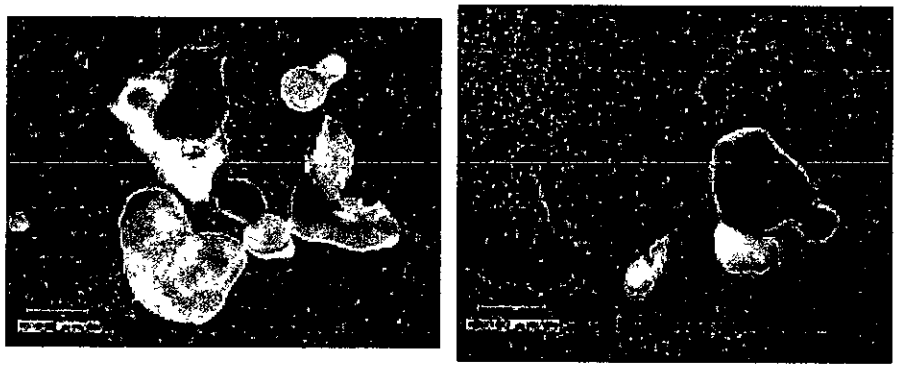


図51　肢芽埋入群とコントロール群の末梢血Tリンパ球
のサブセットの割合の比較

14th



15th



14th Limb buds in bags for 1 week

図52 マイクロメンブレン・バッグに入れ1週間腹腔内培養した肢芽と、胎齢14日及び15日目の肢芽との比較

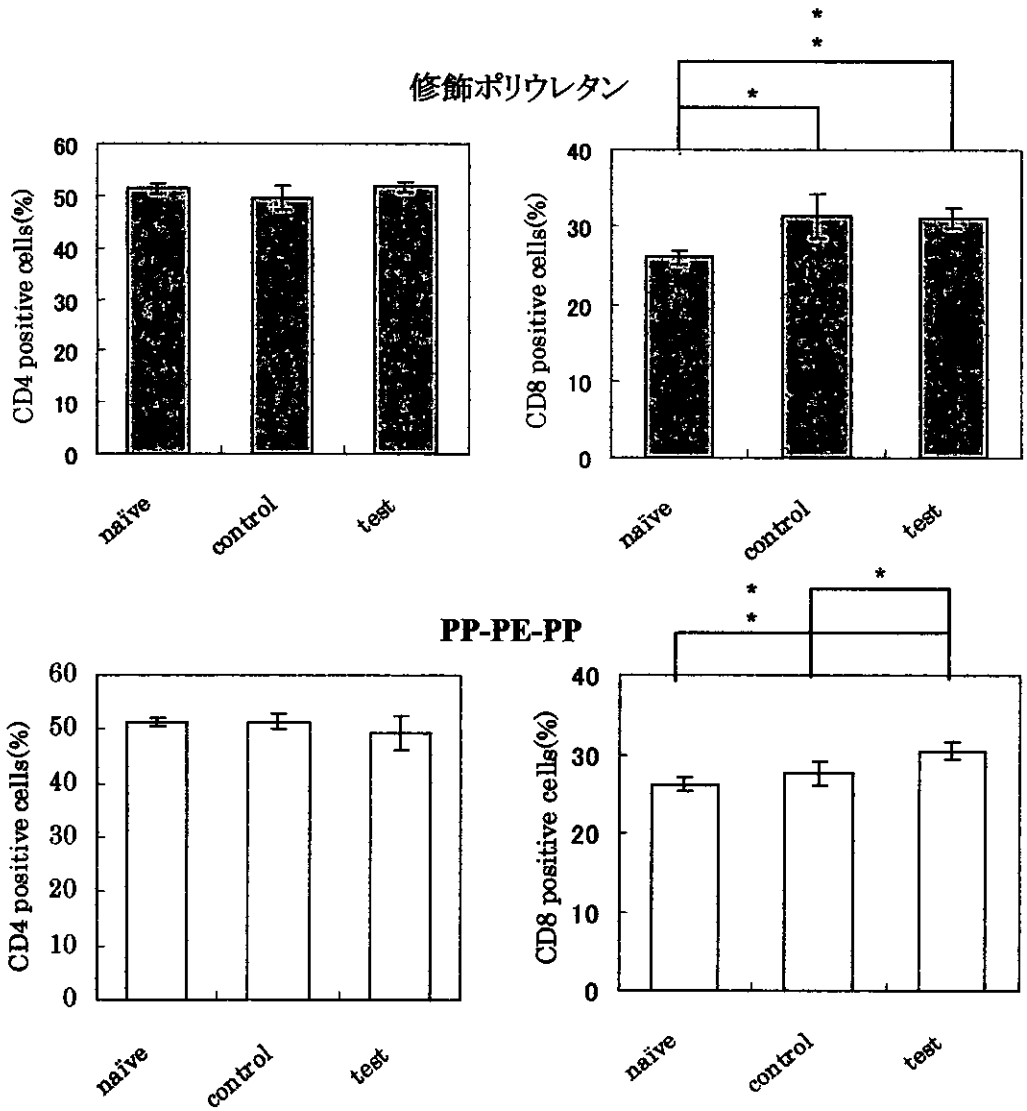
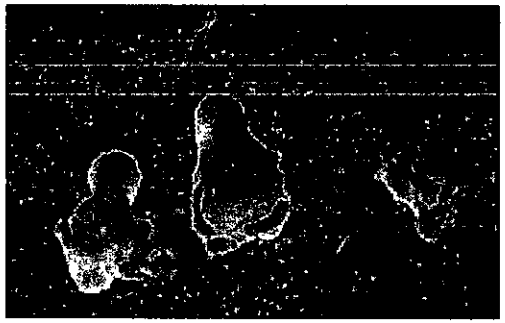


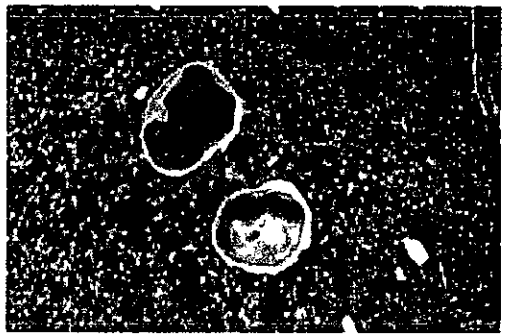
図53　異系ラット肢芽埋入群とコントロール群の末梢血Tリンパ球サブセットの割合の比較

14th
Limb bud
BN rat

修飾ポリウレタン



PP-PE-PP



14th Limb buds in bags for 1 week
BN rat

図54 異系ラット肢芽をバッグに入れ1週間腹腔内培養した場合の肢芽と、胎齢14日目の肢芽との比較

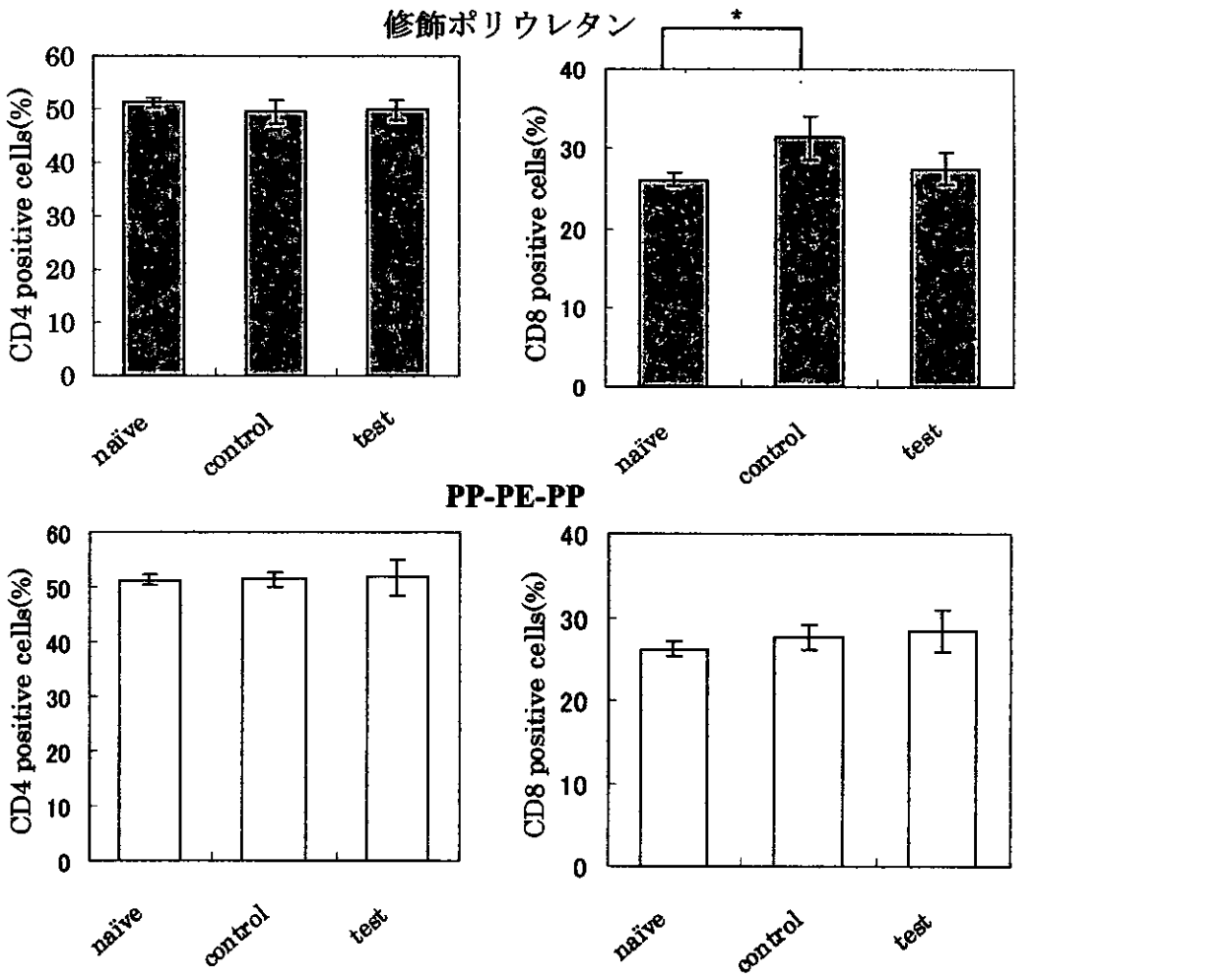


図55　異種であるICRマウスの肢芽埋入群とコントロール群の末梢血Tリンパ球サブセットの割合の比較

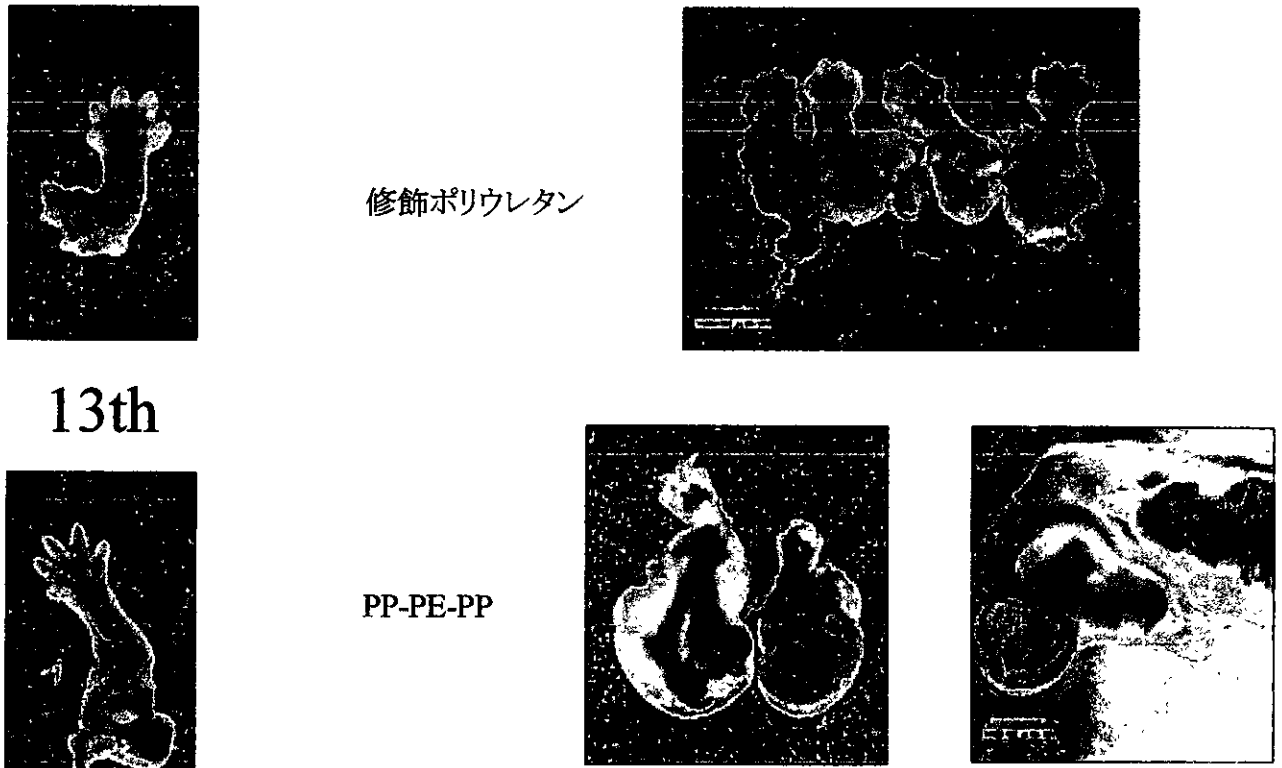


図56 異種であるマウスの肢芽をバッグに入れ1週間腹腔内培養した場合の肢芽と、胎齢13日目の肢芽との比較

図57　レシピエントラットの末梢血 Tリンパ球サブセットの割合の比較
（12週間埋植群）

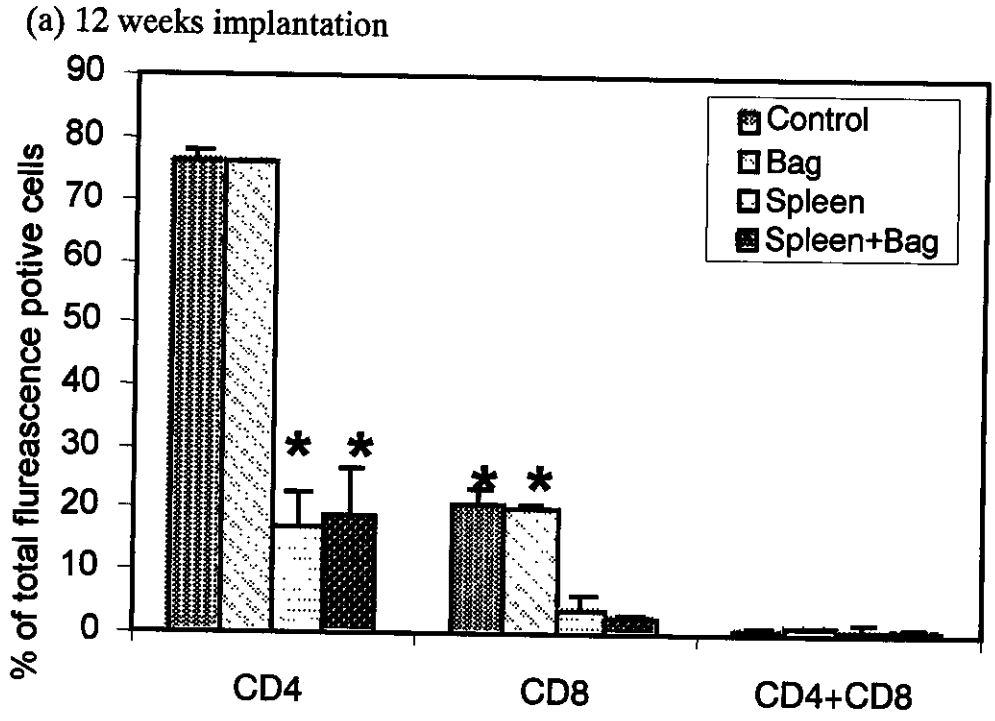


表19　レシピアントラットの末梢血 Tリンパ球によるサイトカイン産生
（12および24週間埋植群）

	12 weeks				24 weeks			
	C	B	S	BS	C	B	S	BS
TNF-α	-	↑↑	↑↑↑	↑	-	±	±	±
IL-4	-	-	-	↑	-	-	-	-
IL-13	-	-	-	-		ND		
IFN-γ	-	↓	↓	-		ND		

C, control; B, bag; S, spleen; BS, spleen inside bag.
N.D., no determination; ↑, increase; ↓, decrease; -, no increase than control; ±, no or almost no increase than control.

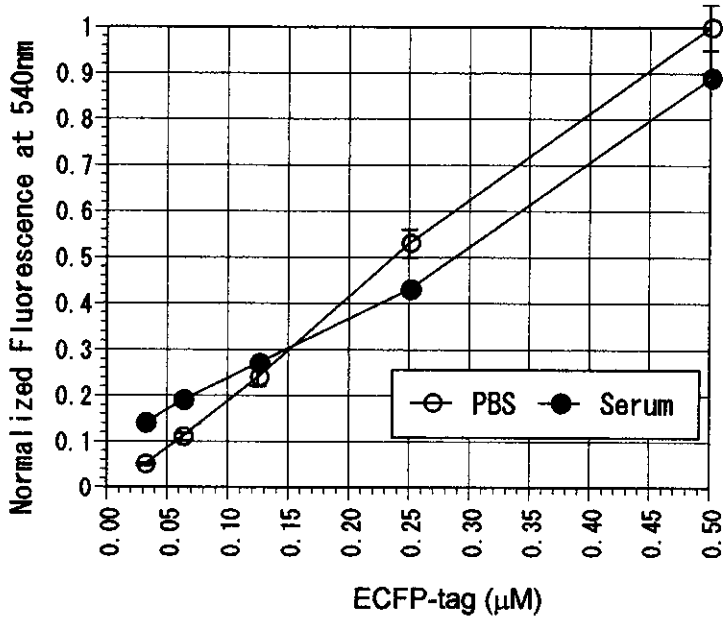


図 58　タグペプチドへの FlAsH の結合量におけるタグペプチド量に対する直線性の検討

研究方法、研究結果を参照。実験は４～５回行い（血清はそれぞれ異なった動物から得た）、結果は平均±標準偏差で示した。

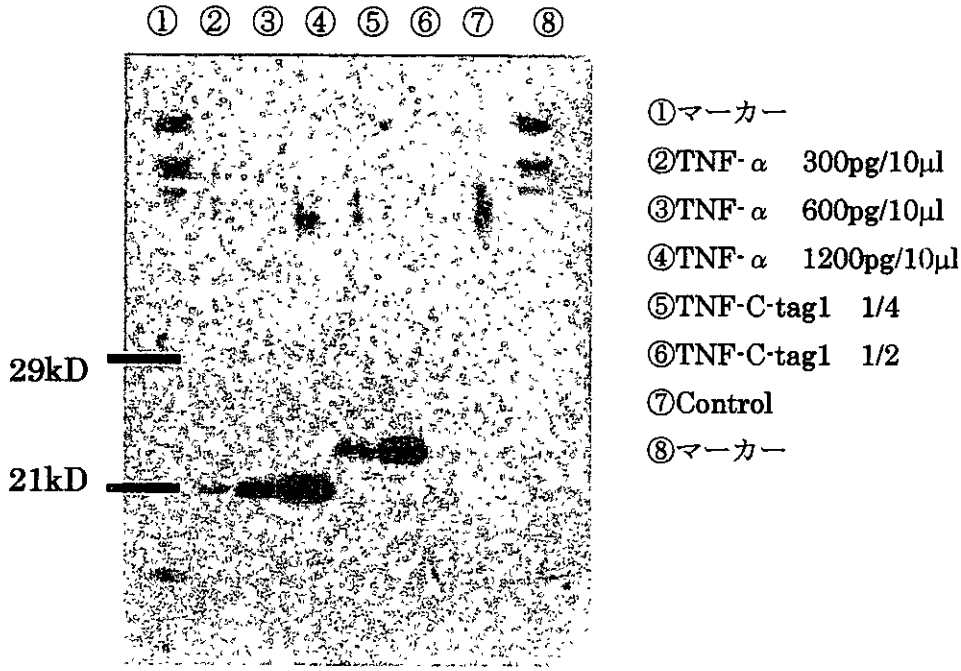


図 59　C-末端にα-ヘリックス型タグを付加した TNF-α の発現および分泌

TNF-α抗体を用いた培養上清のウェスタンブロット結果である。Control は Shuttle ベクターを処置した HeLa 細胞の培養上清。TNF-C-tag1 の濃度は培養上清の希釈倍率であらわしている

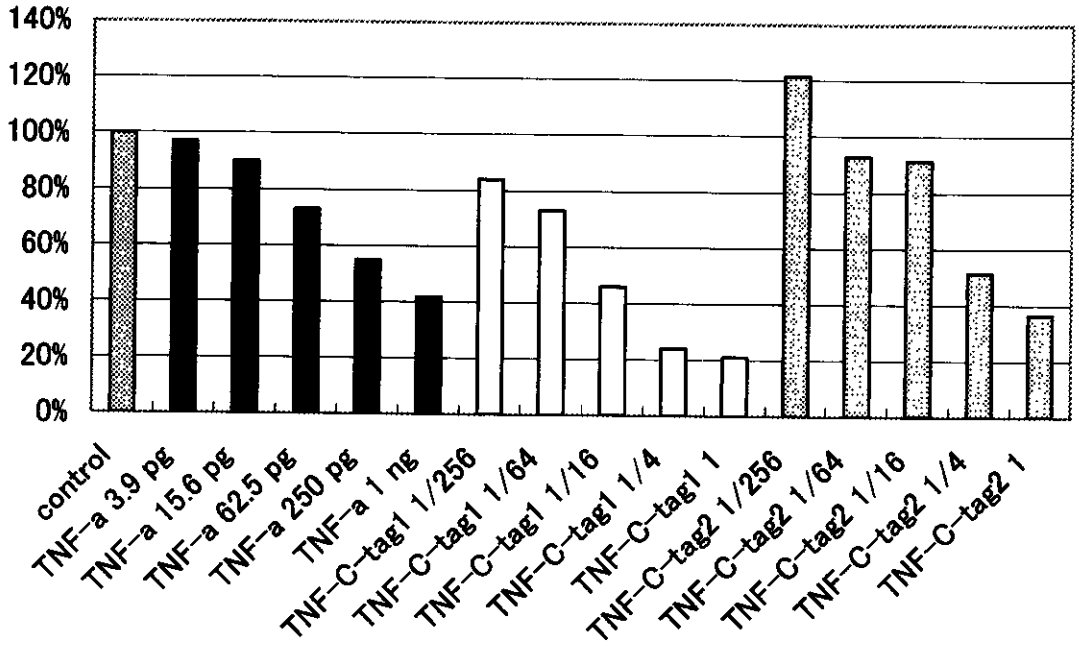


図 60　C-末端にヘアピン型あるいはαヘリックス型タグを付加した TNF-αの細胞障害活性

Control は Shuttle ベクターを処置した HeLa 細胞の培養上清を表す。結果は Control を 100%として、その百分率で表す。TNF-αは試料 50μl 中に添加した量を表す。その他は培養上清の希釈倍率を表す。

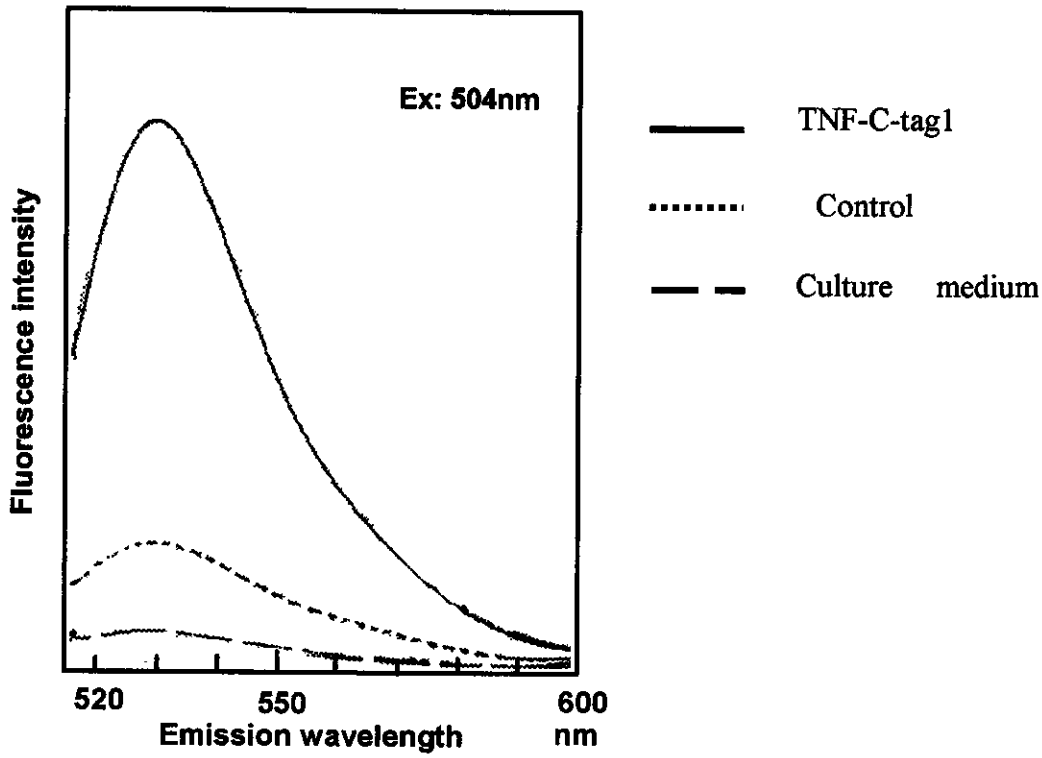
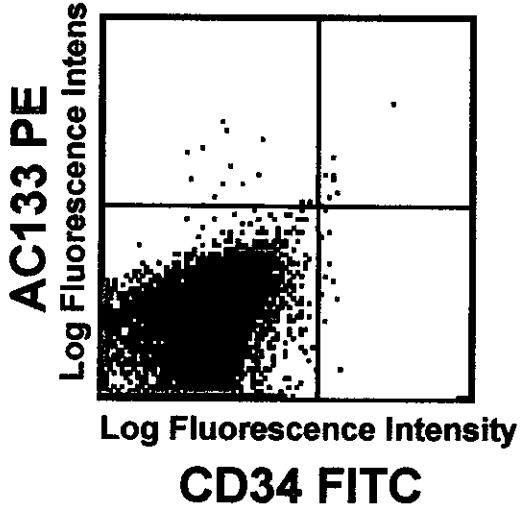


図 61　標識条件の検討後の C-末端にタグを付加した TNF-α (TNF-C-tag1) の FlAsH による蛍光標識　　TNF-C-tag1 はヒスチジンタグを利用して精製したものである。

(a)



	Mean ± SD (Percent)
$AC133^{+}$ $CD34^{-}$	0.21 ±0.035
$AC133^{-}$ $CD34^{+}$	0.20 ± 0.085
$AC133^{+}$ $CD34^{+}$	0.08 ± 0.032

(b)

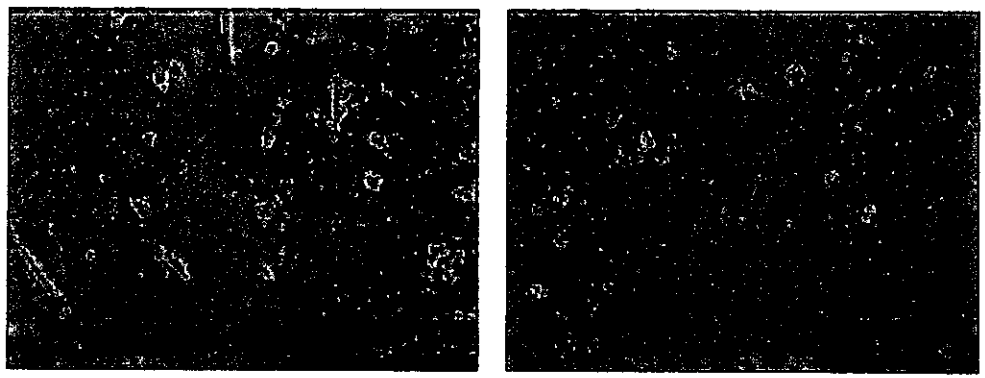


図62. AC133及びCD34陽性細胞からの接着細胞の誘導

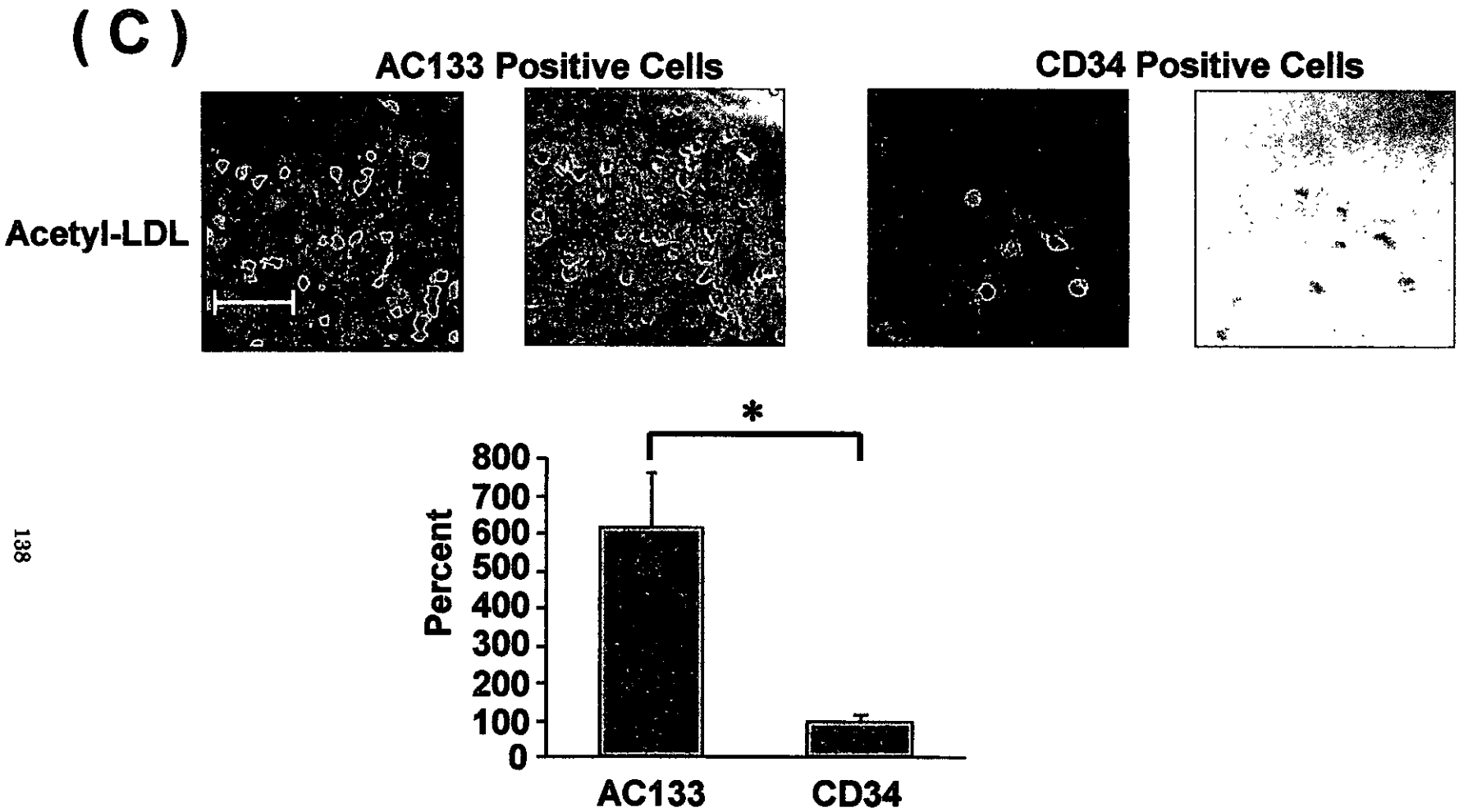


図63. 末梢血AC133陽性細胞及びCD34陽性細胞由来接着細胞のアセチル-LDLの取り込み能