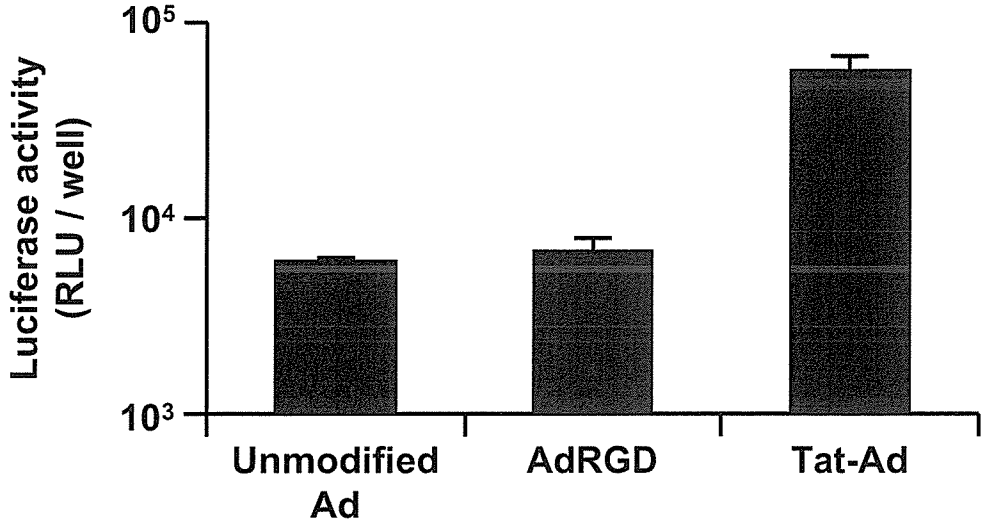


Fig. 129 Transduction efficiency of Tat-Ad in KG-1a cells. KG-1a cells were transduced with unmodified Ad-Luc, AdRGD-Luc, or Tat-Ad-Luc at 10000 VP/cell. After 24 h-cultivation, luciferase activity was measured using the kit according to the manufacture's instructions. Data represent the mean ± SD of results from triplicate culture.

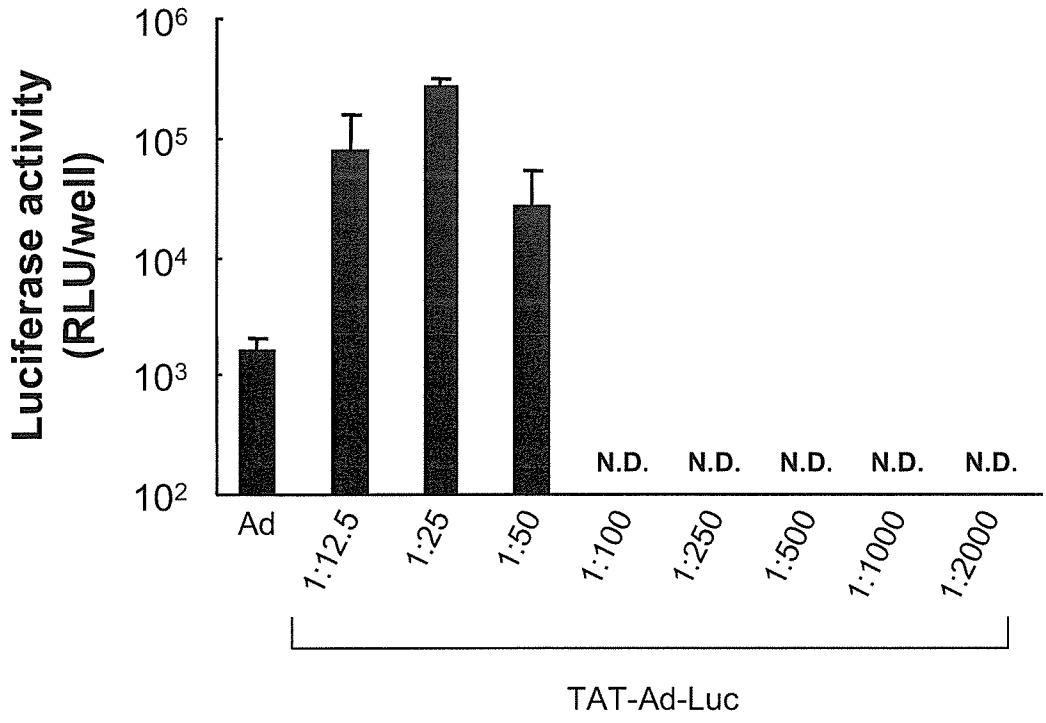


Fig. 130 Transduction efficiency of Tat-Ad with various modification ratio. B16BL6 cells (1×10^4 cells) were transduced with 10,000 particles/cell of Ad or each Tat-Ads encoding the luciferase gene. Luciferase expression was measured after 24 hr. Each bar represents the mean ± S.D. (n = 4). (Tat-Ad, virus lysine residue:Tat peptide= 1:12.5 (mole:mole), 1:25, 1:50, 1:100, 1:250, 1:500, 1:1000, 1:2000).

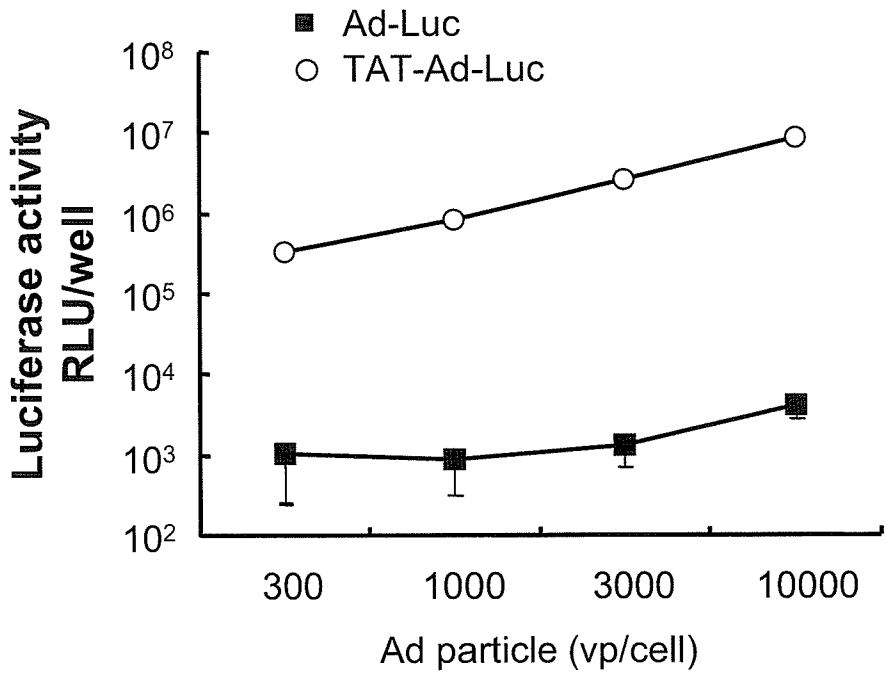


Fig. 131 Transduction efficiency of Tat-Ad into B16BL6 cells. B16BL6 cells (1×10^4 cells) were transduced with 300, 1000, 3,000 or 10,000 particles/cell of Ad/CMV-Luc or each Tat-Ad-Luc encoding the luciferase gene. Luciferase expression was measured after 24 hr. Each bar represents the mean ± S.D. (n = 4).

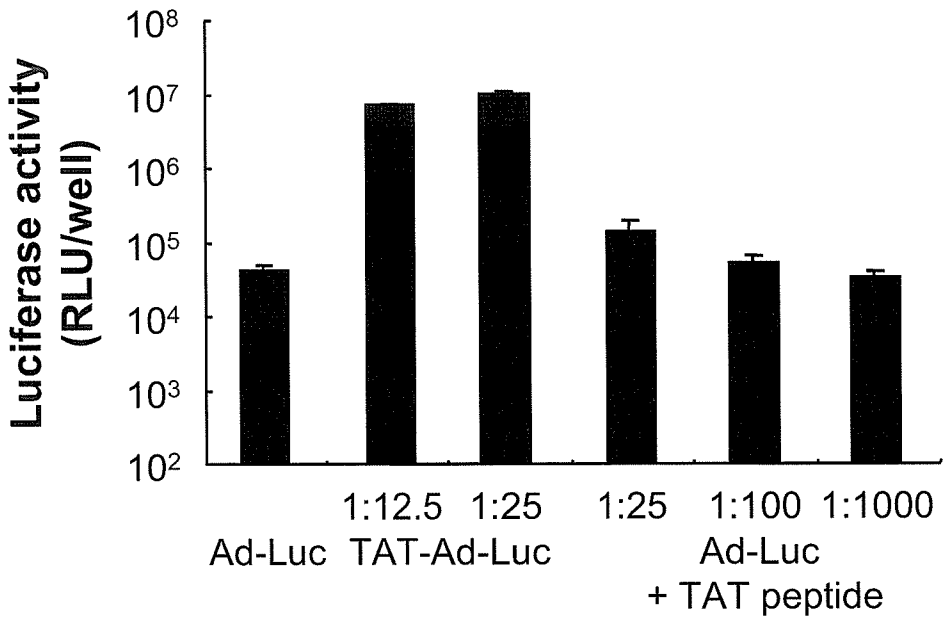


Fig. 132 Transduction efficiency of Ad, TAT-Ad or Ad with TAT peptide. B16BL6 cells (1×10^4 cells) were transduced with 10,000 particles/cell of Ad-Luc, TAT-Ad-Luc or Ad-Luc with TAT peptide. Luciferase expression was measured after 24 hr. Each point represents the mean ± S.D. (n = 4). (TAT-Ad; virus lysine residue:TAT peptide= 1:12.5 (mole:mole), 1:25), (TAT peptide; virus lysine residue:TAT peptide= 1:25, 1:100, 1:1000).

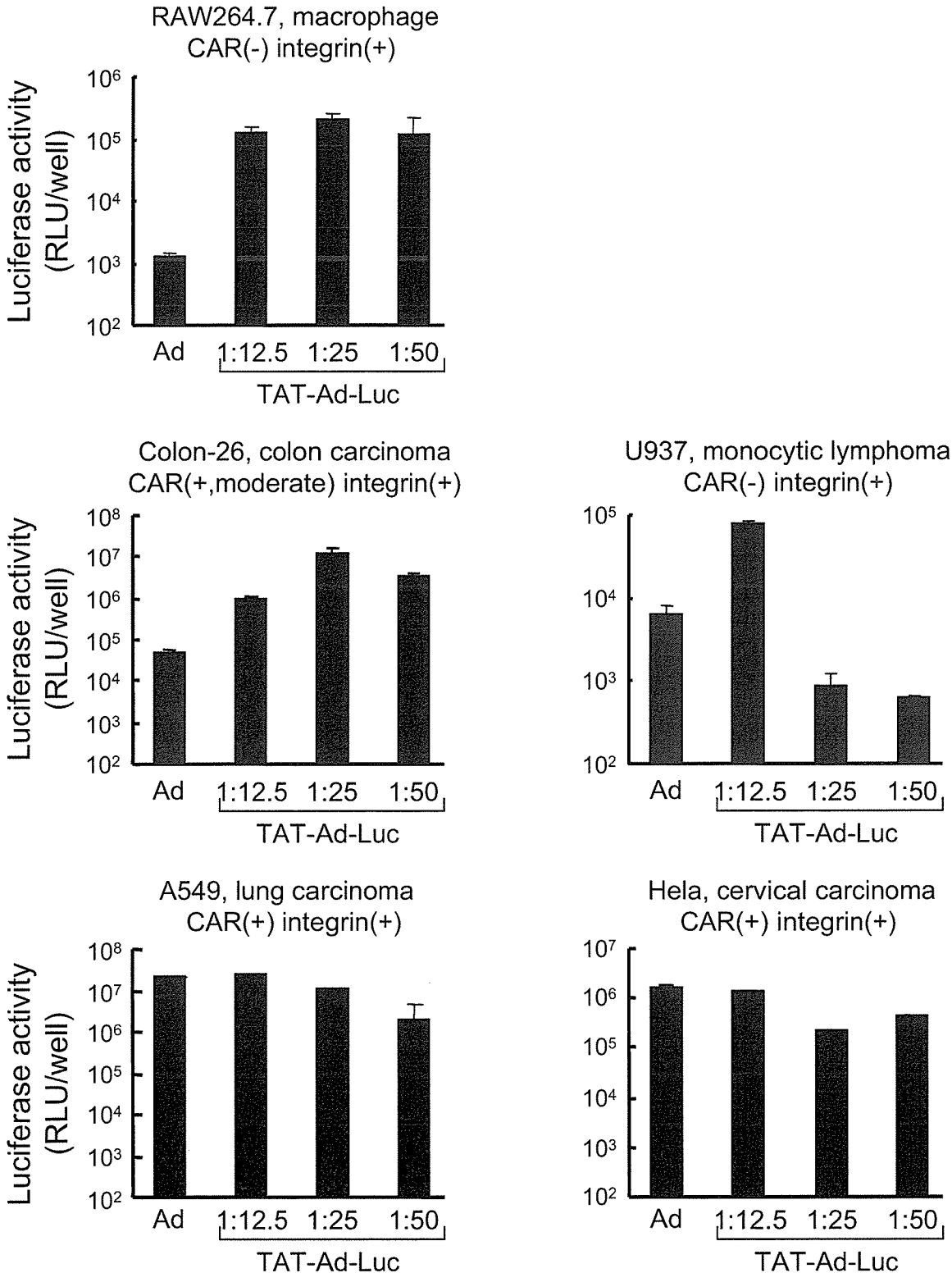


Fig. 133 Transduction efficiency of Tat-Ad into various cells. RAW264.7 cells, CT26 cells, U937 cells, A549 cells and Hela cells (1×10^4 cells) were transduced with 10,000 particles/cell of Ad-Luc or each Tat-Ad-Luc encoding the luciferase gene. Luciferase expression was measured after 24 hr. Each bar represents the mean ± S.D. (n = 4).

Table 14 Adenovirus vectors used in this study

Name	Fiber type	Gene of interest
AdCALacZ	Type 5 fiber	CA promoter + LacZ
AdRGD-CALacZ	RGD peptide in the HI-loop of the fiber knob	CA promoter + LacZ
AdK7-CALacZ	Polylysine peptide in the C-terminal of the fiber knob	CA promoter + LacZ
AdF35-CALacZ	Chimeric type 5 and type 35 fiber	CA promoter + LacZ
AdK7-H1	Polylysine peptide in the C-terminal of the fiber knob	H1 promoter
AdK7-H1-PPARγ	Polylysine peptide in the C-terminal of the fiber knob	H1 promoter + shRNA for PPARγ
AdK7-H1-Scramble	Polylysine peptide in the C-terminal of the fiber knob	H1 promoter + shRNA for Scramble
AdK7-Null	Polylysine peptide in the C-terminal of the fiber knob	None

CA promoter: β-actin promoter/CMV enhancer with β-actin intron.

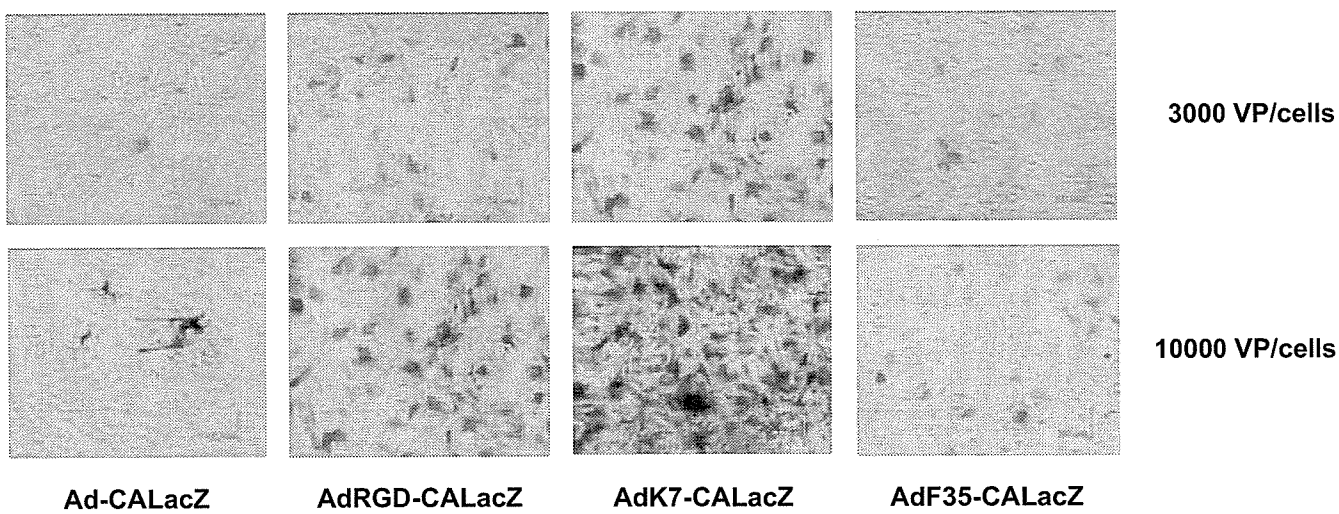


Fig. 134 Comparison of the transduction efficiency of various types of fiber-modified Ad vector into 3T3-L1 preadipocytes.
3T3-L1 preadipocytes were transduced with Ad-CALacZ, AdRGD-CALacZ, AdK7-CALacZ or AdF35-CALacZ (3000 or 10000 VP/cells) for 1.5 h. After 48 h in culture, LacZ expression was determined by X-gal staining.

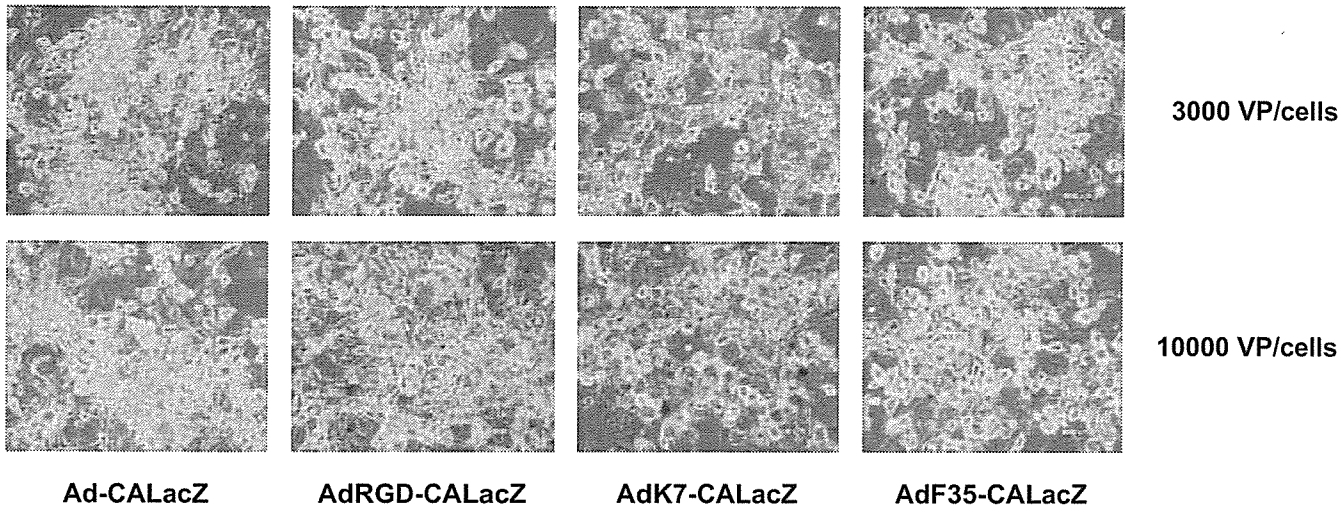


Fig. 135 Comparison of the transduction efficiency of various types of fiber-modified Ad vector into 3T3-L1 adipocytes. 3T3-L1 adipocytes, which were cultured in differentiation medium containing pioglitazone, insulin, dexamethasone and 3-isobutyl-1-methylxanthine for 6 days, were transduced with Ad-CALacZ, AdRGD-CALacZ, AdK7-CALacZ or AdF35-CALacZ (3000 or 10000 VP/cells) for 1.5 h. After 48 h in culture, LacZ expression was determined by X-gal staining.

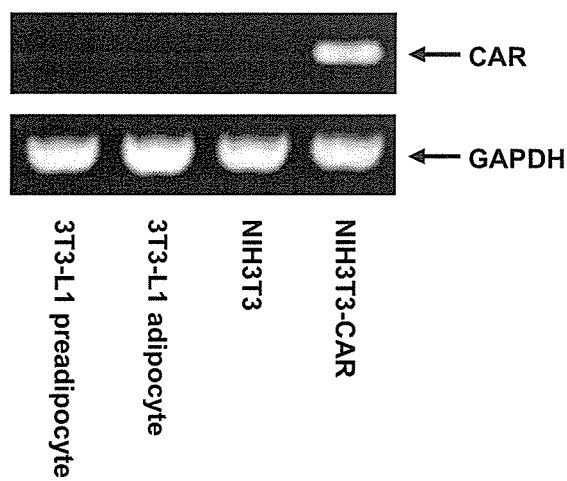


Fig. 136 RT-PCR analysis of CAR mRNA expression in 3T3-L1 preadipocytes and adipocytes.
Total RNA was isolated from 3T3-L1 preadipocytes and adipocytes differentiated for 6 days, and RT-PCR analysis was performed as described in Materials and Methods section. NIH3T3 and NIH3T3-CAR cells were also analyzed as a negative and positive control of CAR mRNA expression, respectively.

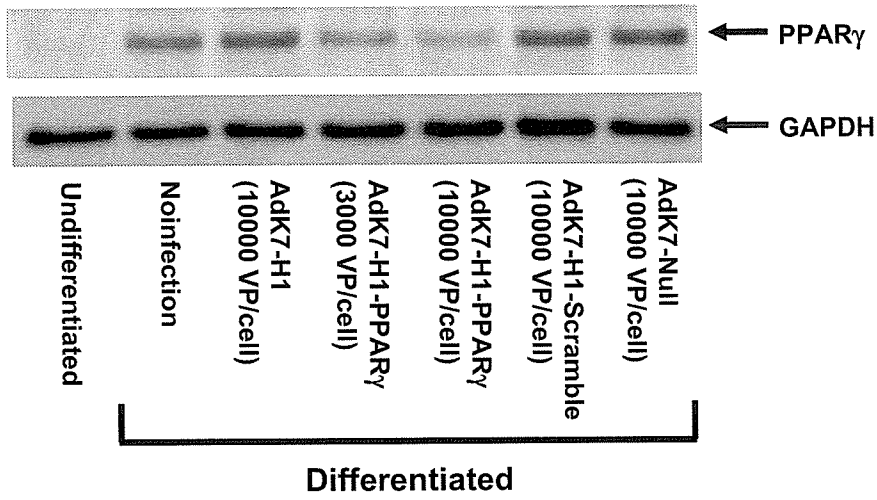


Fig. 137 Suppression of PPARγ expression in 3T3-L1 cells transduced with AdK7-H1-PPARγ. 3T3-L1 preadipocytes were transduced with each Ad vector for 1.5 h. On the following day, the cells reached confluence. From three days after Ad treatment, the cells were cultured with differentiation medium containing pioglitazone, insulin, dexamethasone and 3-isobutyl-1-methylxanthine for 4 days. Proteins were then extracted from the cells, and the levels of PPARγ expression were examined by western blotting. The GAPDH bands served as an internal control for equal total protein loading.

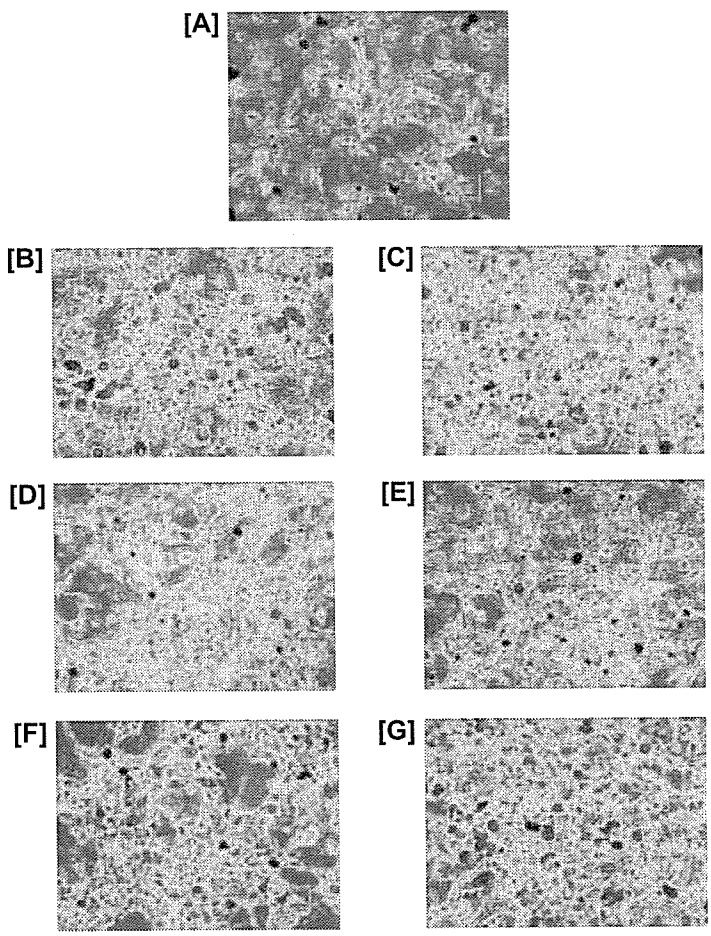


Fig. 138 Suppression of preadipocyte-to-adipocyte differentiation by transduction with AdK7-H1-PPARγ. 3T3-L1 preadipocytes were transduced with each Ad vector for 1.5 h. On the following day, the cells reached confluence. From three days after Ad treatment, the cells were cultured with differentiation medium containing pioglitazone, insulin, dexamethasone and 3-isobutyl-1-methylxanthine for 9 days. Then, the intracellular lipid accumulation, which was used as the marker of preadipocyte-to-adipocyte differentiation, was determined by Oil red O staining. A, 3T3-L1 preadipocytes (3T3-L1 cells cultured with normal medium); B, 3T3-L1 adipocytes (3T3-L1 cells cultured with differentiation medium without Ad treatment); C, 3T3-L1 cells cultured with differentiation medium with AdK7-H1 (10000 VP/cell) treatment; D, 3T3-L1 cells cultured with differentiation medium with AdK7-H1-PPARγ (3000 VP/cell) treatment; E, 3T3-L1 cells cultured with differentiation medium with AdK7-H1-PPARγ (10000 VP/cell) treatment; F, 3T3-L1 cells cultured with differentiation medium with AdK7-H1-Scramble (10000 VP/cell) treatment; G, 3T3-L1 cells cultured with differentiation medium with AdK7-Null (10000 VP/cell) treatment.

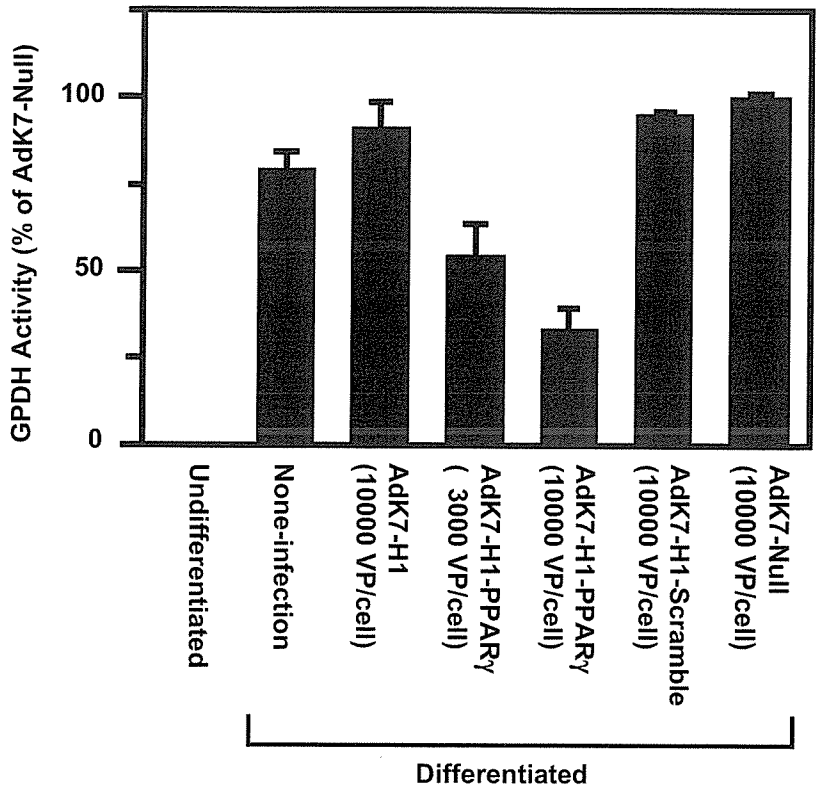


Fig. 139 Suppression of the fatty synthesis on 3T3-L1 cells transduced with AdK7-H1-PPARγ.
3T3-L1 preadipocytes were transduced with each Ad vector for 1.5 h. On the following day, the cells reached confluence. From three days after Ad treatment, the cells were cultured with differentiation medium containing pioglitazone, insulin, dexamethasone and 3-isobutyl-1-methylxanthine for 9 days. The fatty synthesis was determined by the measurement of GPDH activity in 3T3-L1 cells. Data were expressed as percentage of the GPDH activity of 3T3-L1 cells cultured with differentiation medium with AdK7-Null (10000 VP/cell) treatment.

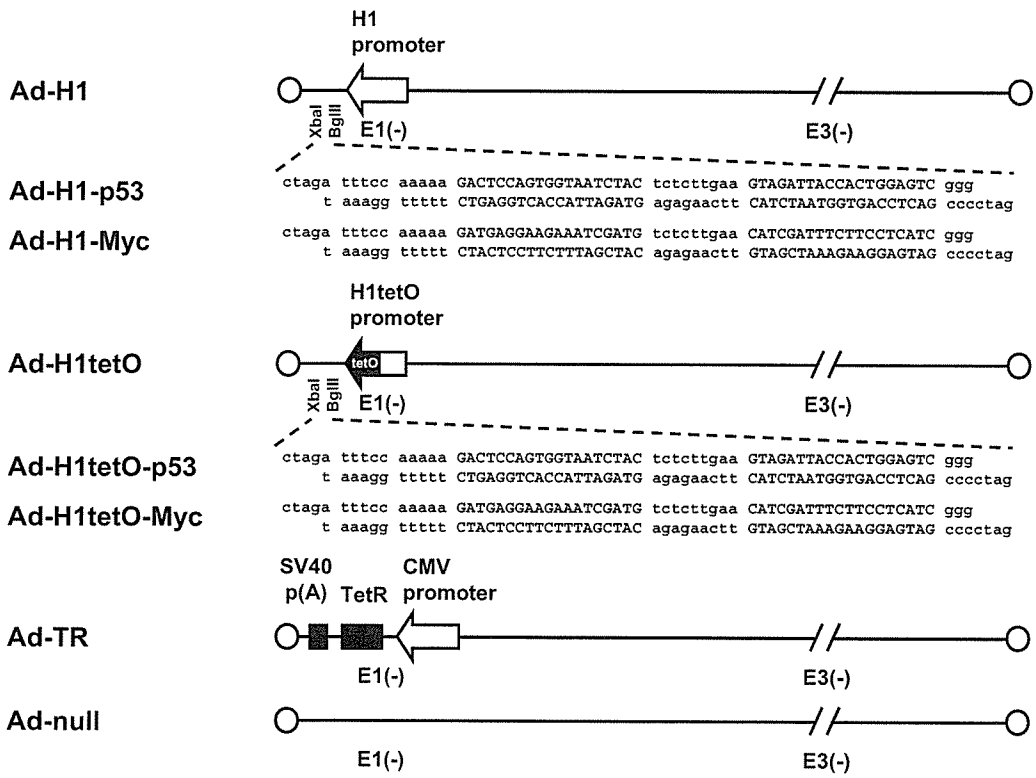


Fig. 140 The structure of Ad vectors used in the present study.
The H1 promoter-based siRNA expression cassette was inserted into the E1 deletion region of Ad genome. For the inducible siRNA expression, a tetracycline operator (tetO) sequence was introduced in the downstream of the TATA box in the H1 promoter, as described in Materials and Methods section. The target sequences against the p53 and c-myc gene were shown in a large letter. Ad-TR is the Ad vector containing a tetracycline repressor sequence under the control of the CMV promoter/enhancer. Ad-null is the Ad vector without foreign genes in the E1 deletion region.

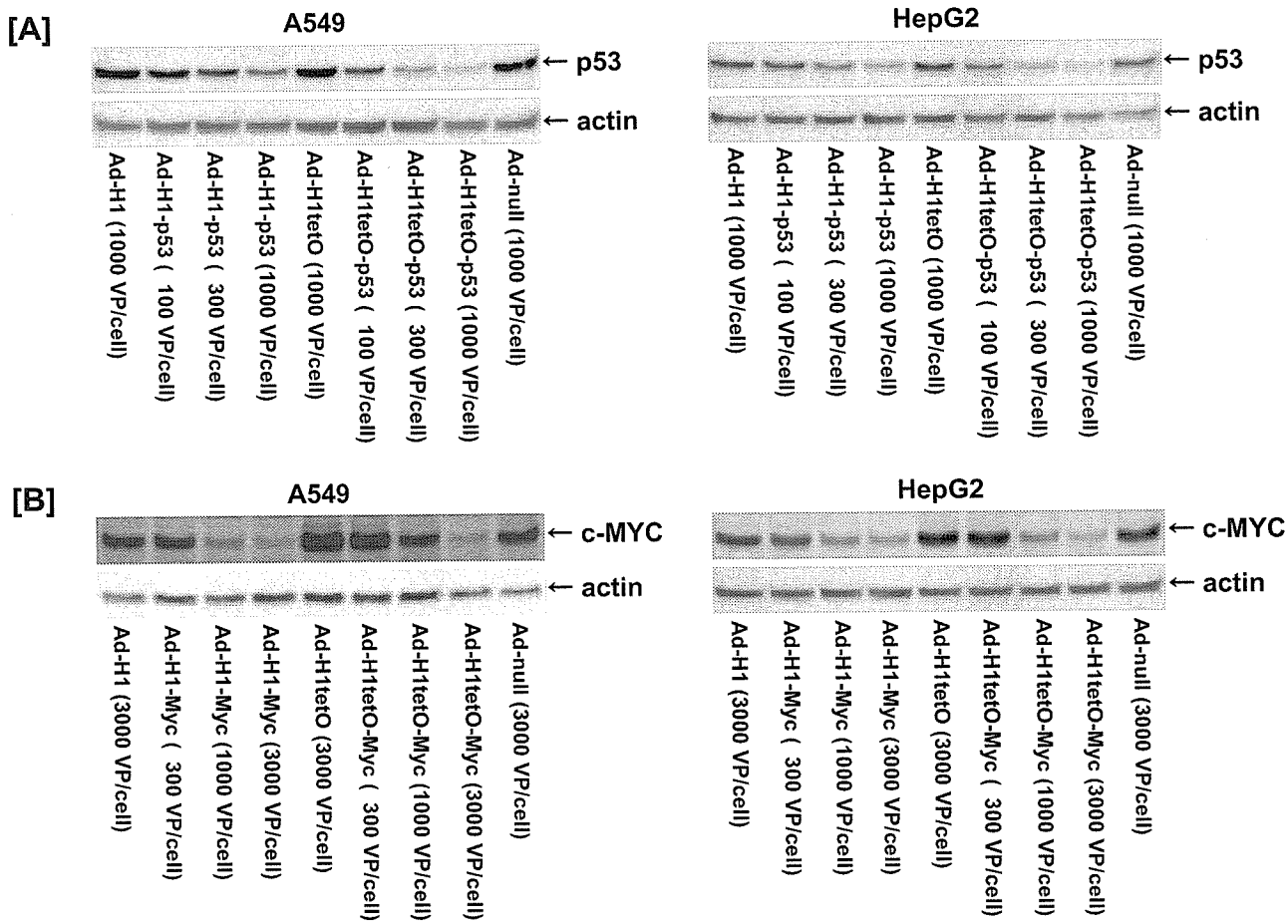


Fig. 141 The dose-dependent suppression of the p53 and c-MYC expression by Ad vector-delivered siRNA. A549 and HepG2 cells were infected with each Ad vector for 1.5 hr, and then cultured for 3 days. The proteins were then extracted from the cells, and the levels of p53 (panel A) and c-MYC (panel B) expression were examined by Western blotting. The actin bands served as an internal control for equal total protein loading.

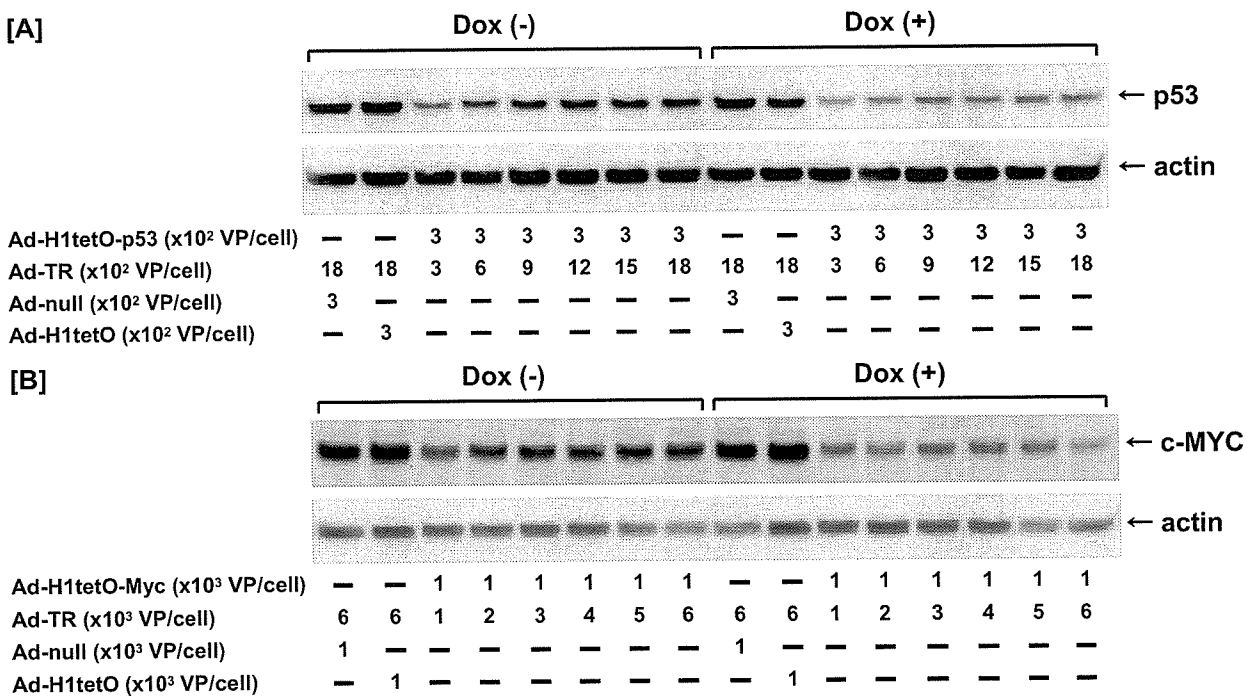


Fig. 142 Regulated suppression of the p53 and c-MYC expression by the co-infection of Ad-H1tetO-p53 or Ad-H1tetO-Myc plus Ad-TR.
A549 cells were infected with the indicated amounts of Ad-H1tetO-p53 or Ad-H1tetO-Myc plus Ad-TR for 1.5 hr, and then cultured with or without Dox (10 μg/ml) for 3 days. The cells were also infected with Ad-null or Ad-H1tetO plus Ad-TR. The proteins were then extracted from the cells, and the levels of p53 (panel A) and c-MYC (panel B) expression were examined by Western blotting. The actin bands served as an internal control for equal total protein loading.

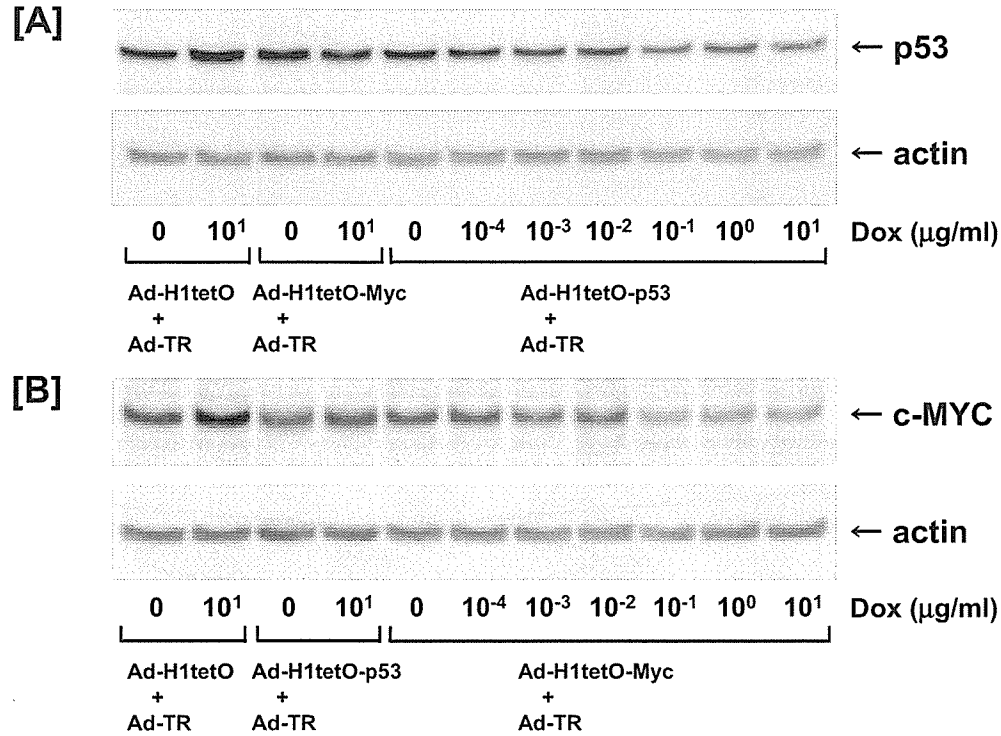


Fig. 143 Dox dose-dependent suppression of the p53 and c-MYC expression by the co-infection of Ad-H1tetO-p53 or Ad-H1tetO-Myc plus Ad-TR.

A549 cells were infected with Ad-H1tetO-p53 (300 VP/cell) plus Ad-TR (1800 VP/cell) or Ad-H1tetO-Myc (1000 VP/cell) plus Ad-TR (6000 VP/cell) for 1.5 hr, and then cultured with various concentrations of Dox for 3 days. The cells were also infected with Ad-H1tetO plus Ad-TR. The proteins were then extracted from the cells, and the levels of p53 (panel A) and c-MYC (panel B) expression were examined by Western blotting. The actin bands served as an internal control for equal total protein loading.

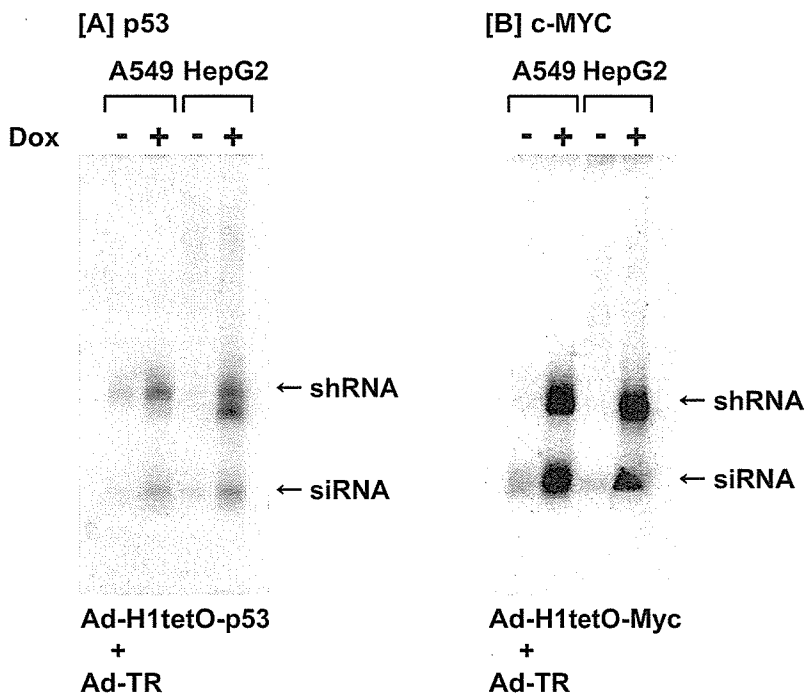


Fig. 144 Dox inducible p53 or c-Myc siRNAs expression by the co-infection of Ad-H1tetO-p53 or Ad-H1tetO-Myc plus Ad-TR.

A549 and HepG2 cells were infected with Ad-H1tetO-p53 (300 VP/cell) plus Ad-TR (1800 VP/cell) or Ad-H1tetO-Myc (1000 VP/cell) plus Ad-TR (6000 VP/cell) for 1.5 hr, and then cultured with or without Dox (1 mg/ml) for 3 days. The total RNAs were then extracted from the cells, and the levels of p53 and c-myc siRNAs expression were examined by Northern blotting.

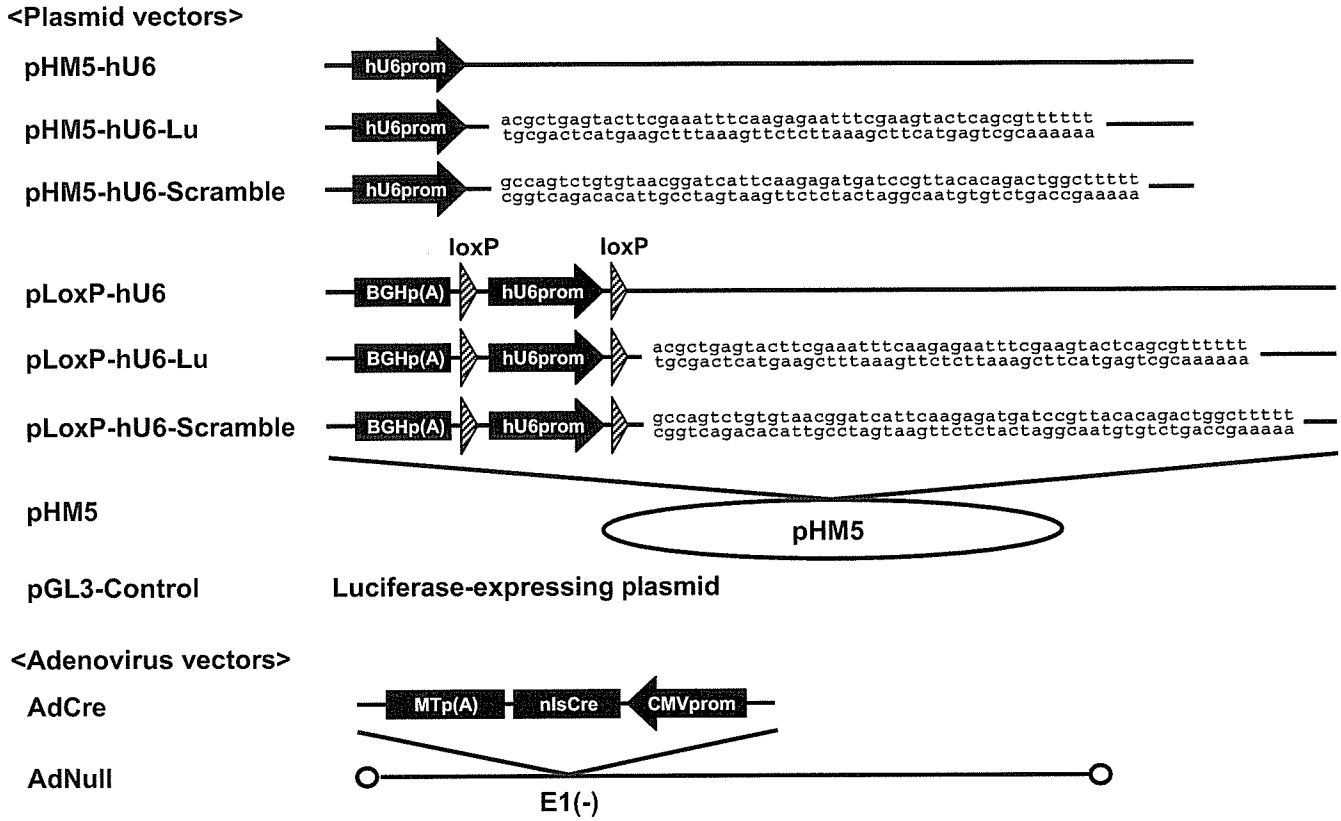


Fig. 145 The structure of plasmid vectors and Ad vectors used in the present study.

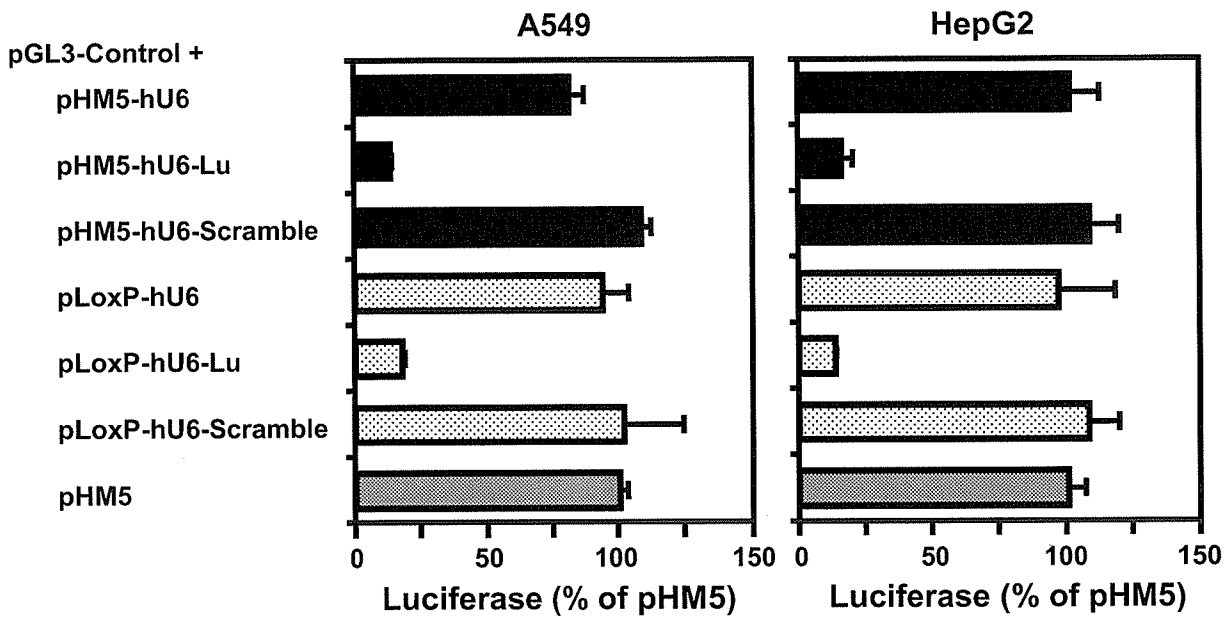


Fig. 146 The effects of insertion of loxP in the plasmid vector expressed siRNA on the suppression of the expression of luciferase.

A549 and HepG2 cells were transfected with each plasmid vector for 2.5 hr, and then cultured for 2 days. The luciferase activity was measured in Materials and Methods.

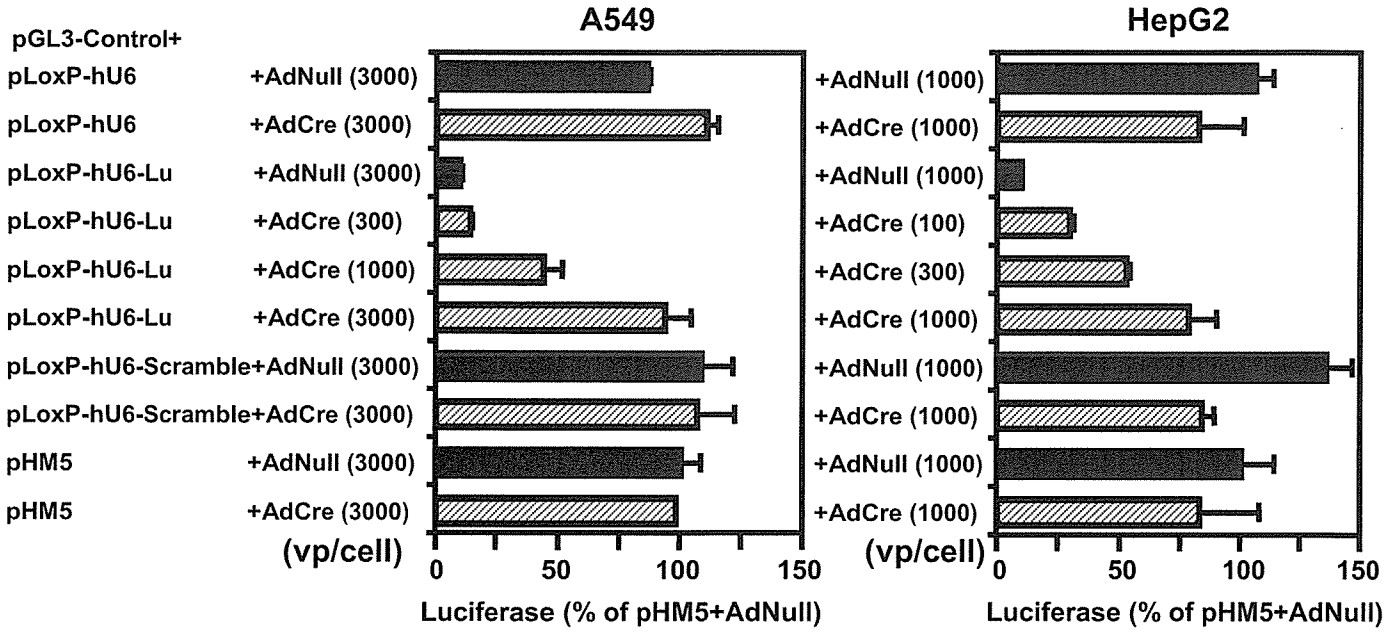


Fig.147 AdCre vector dose-dependent release of the suppressed expression of luciferase by the transfection of pLoxP-hU6-Lu.
A549 and HepG2 cells were infected with AdCre vector for 1.5 hr. The next day, cells were transfected with each plasmid vector for 2.5 hr, and then cultured for 2 days. The luciferase activity was measured in Materials and Methods.

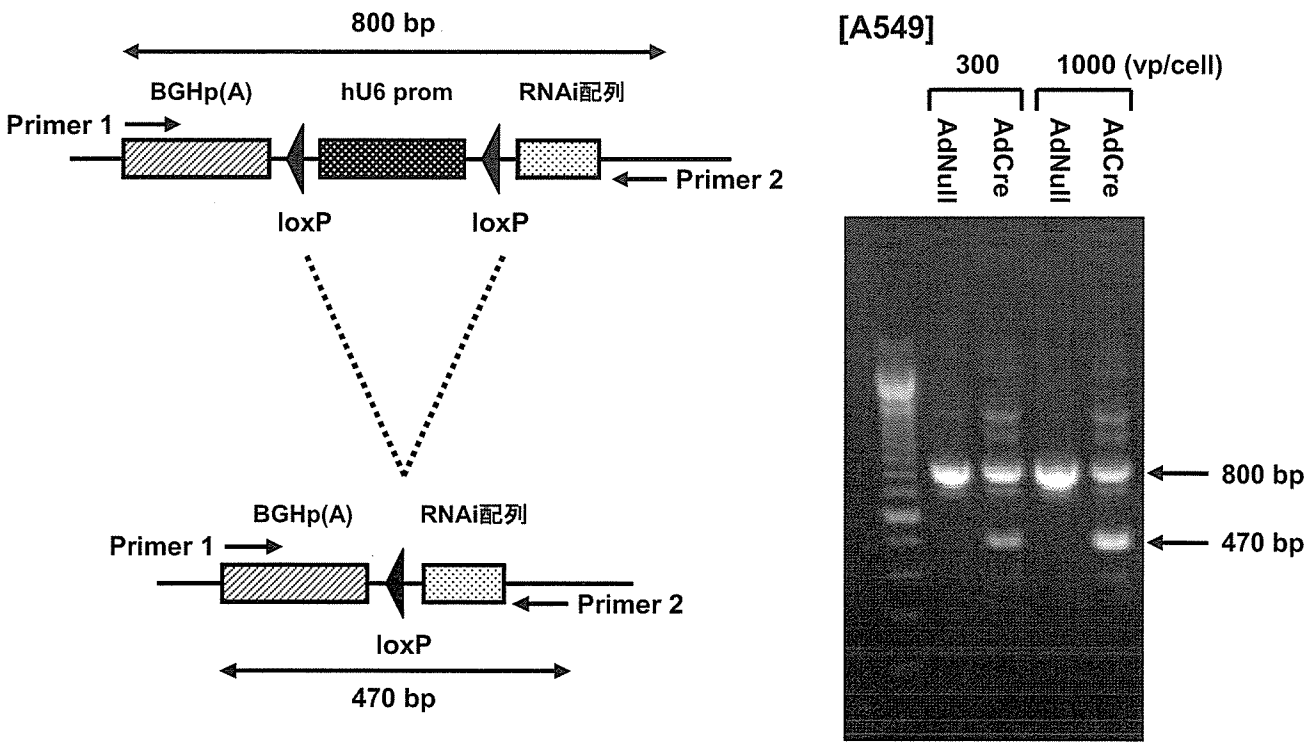


Fig. 148 The removes of hU6 promoter from pLoxP-hU6-Lu treated with AdCre.
A549 and HepG2 cells were infected with AdCre vector for 1.5 hr. The next day, cells were transfected with each plasmid vector for 2.5 hr, and then cultured for 2 days. The nuclear DNA was prepared in Materials and Methods.

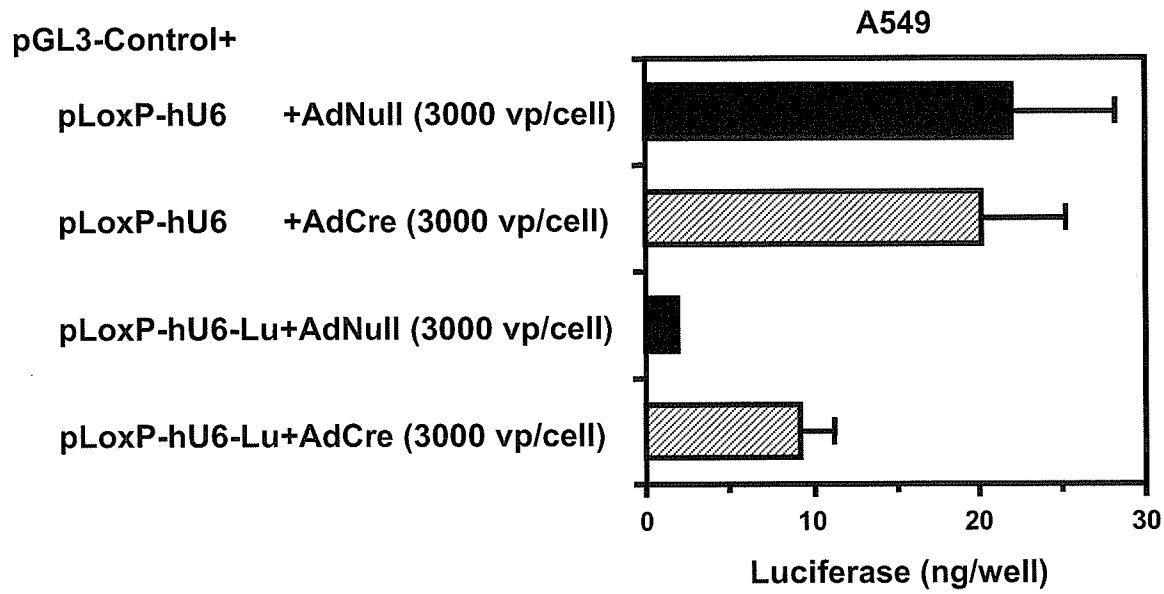


Fig. 149 The recovery by the infected AdCre of the suppressed expression of luciferase by the transfection of pLoxP-hU6-Lu.
A549 and HepG2 cells were transfected with each plasmid vector for 2.5 hr. The next day, cells were infected with AdCre vector for 1.5 hr, and then cultured for 4 days. The luciferase activity was measured in Materials and Methods.

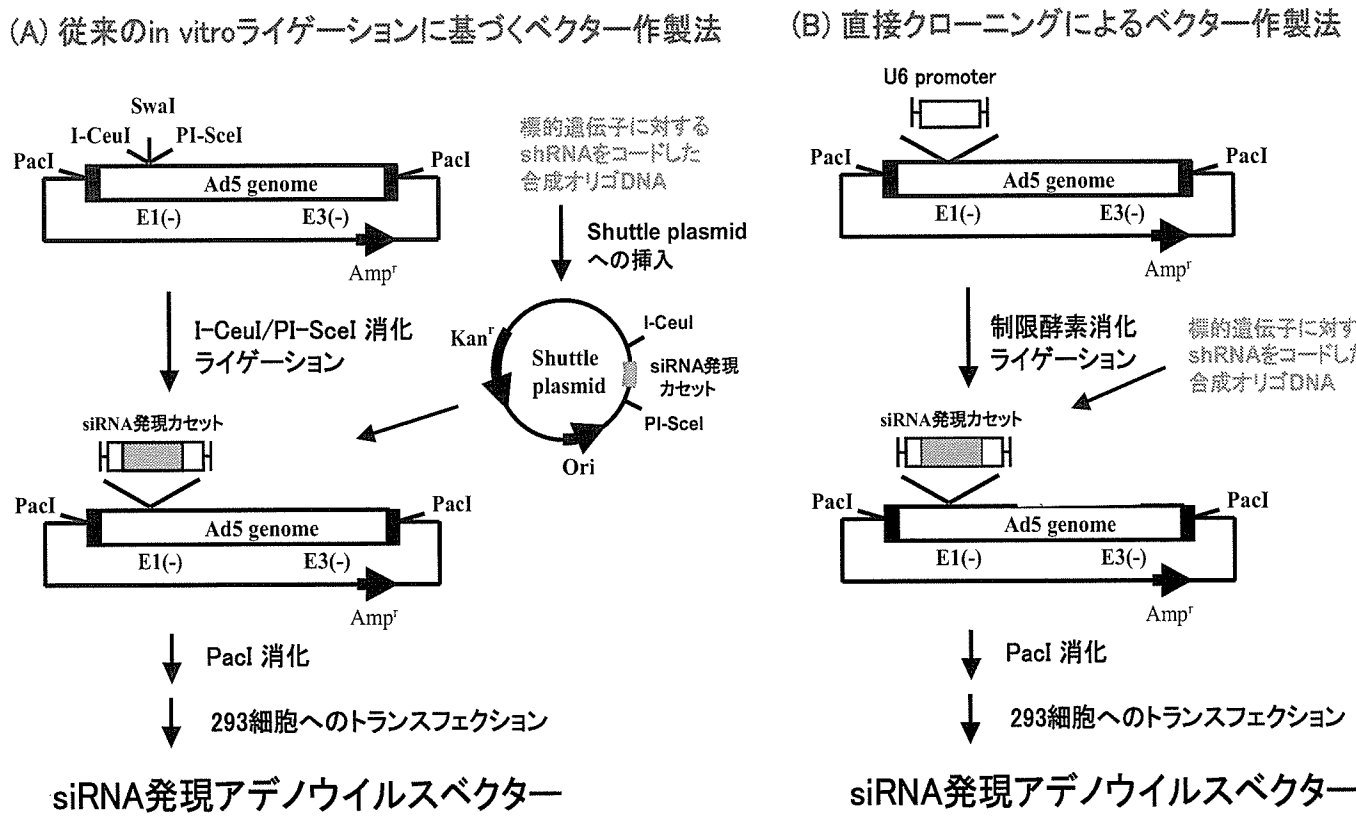


Fig. 150 ベクタープラスミドへの直接クローニングによる迅速なsiRNA発現アデノウイルスベクター作製法
(A) 従来のin vitroライゲーションに基づくベクター作製法、(B)直接クローニングによるベクター作製法

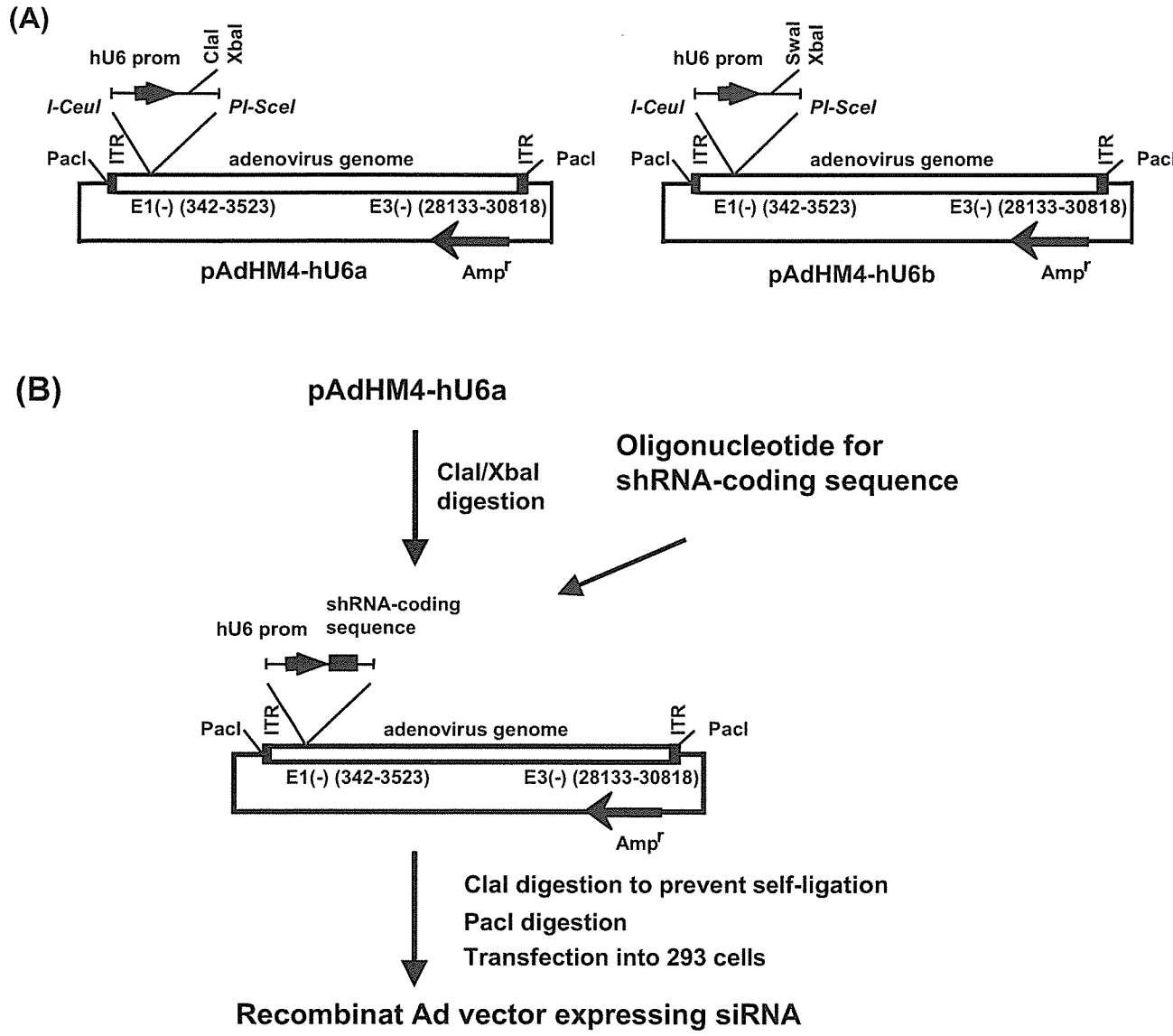


Fig. 151 The vector plasmids and the construction strategy for the Ad vectors expressing siRNA.
(A) The vector plasmids, pAdHM4-hU6a and –hU6b. pAdHM4-hU6a contains a unique ClaI site at the transcription start site of the hU6 promoter sequence and an XbaI site downstream from the promoter sequence. pAdHM4-hU6b contains a unique the SwaI site at the transcription start site of the hU6 promoter sequence and an XbaI site downstream from the promoter sequence.
(B) The construction strategy for the Ad vector expressing siRNA. pAdHM4-hU6a was digested with ClaI/XbaI and ligated with oligonucleotides for the shRNA-coding sequence. Ligation products were then digested with ClaI to prevent the generation of non-recombinant parental plasmid. The resulting plasmid was linearized by digestion with PacI and transfected into 293 cells, generating recombinant Ad vectors expressing siRNA. pAdHM4-hU6b are similarly used.

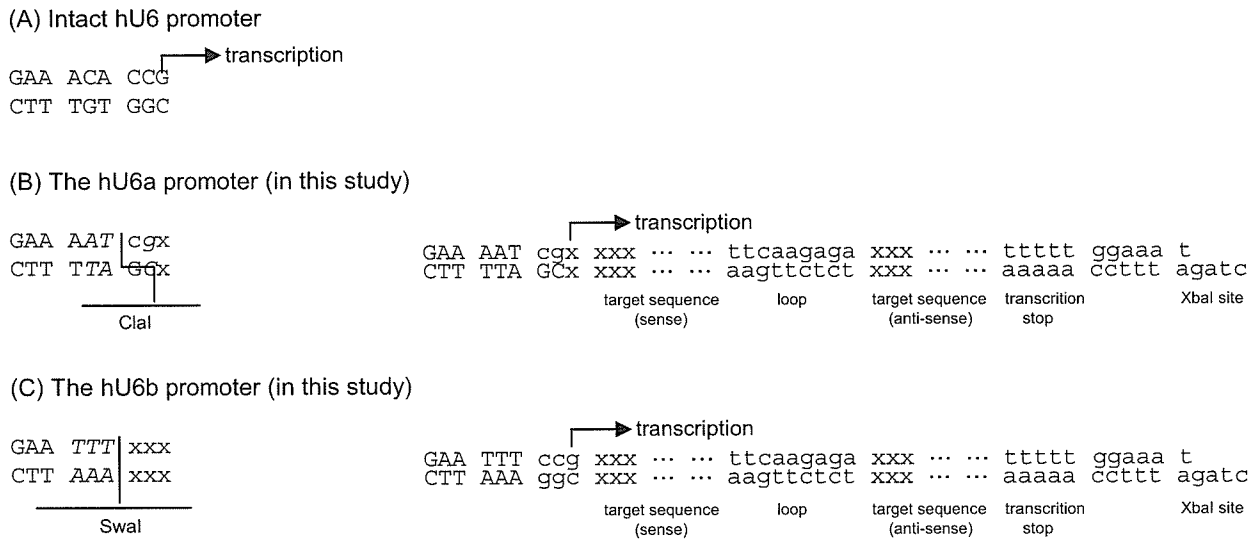


Fig. 152 Sequences at the transcription start site of new hU6 promoter.
(A) Intact hU6 promoter sequence. (B) The hU6a promoter sequence. In this promoter, the ClaI site is placed at the transcription start site. (C) The hU6b promoter sequence. In this promoter, a SwaI site is placed at the transcription start site. ShRNA-coding oligonucleotides to be synthesized for each promoter are shown in small letters in the right side.

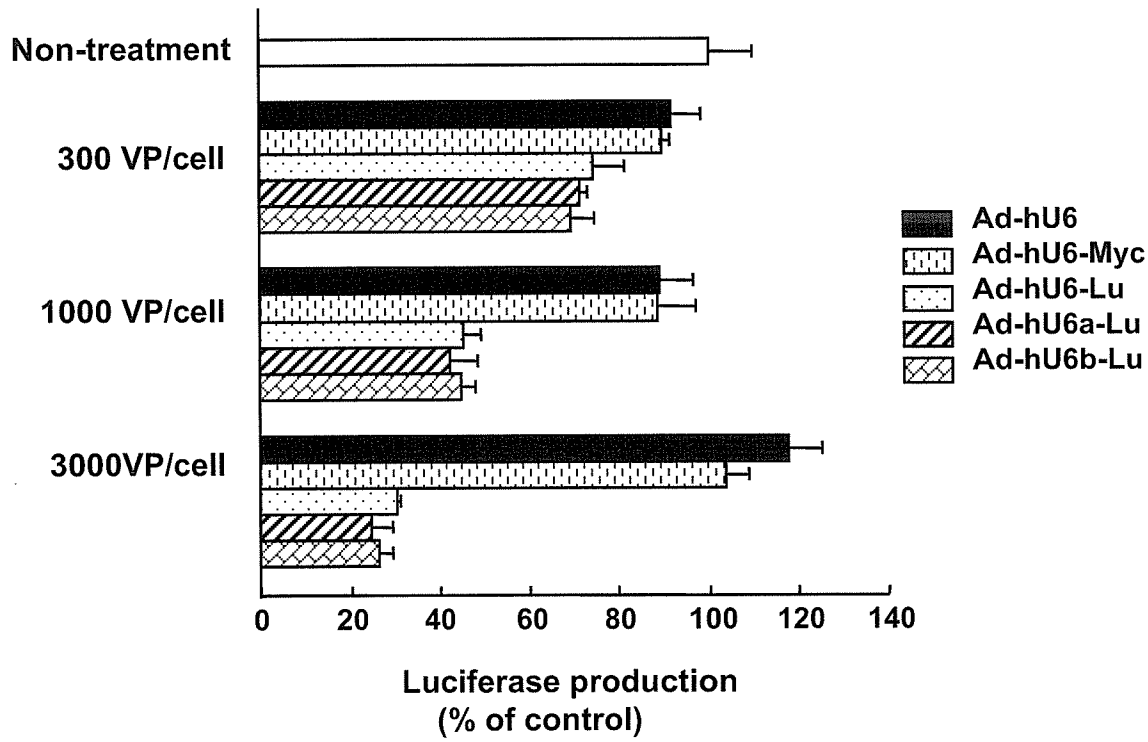


Fig. 153 Suppression of luciferase expression by the Ad vector expressing siRNA.
A549-Luc cells, which stably express luciferase, were transduced with 300, 1000, or 3000 VP/cell of Ad-hU6, Ad-hU6-p53, Ad-hU6-Lu, Ad-hU6a-Lu, or Ad-hU6b-Lu for 1.5 hr. After culturing for 72 hours, luciferase production in the cells was measured by luminescent assay. The data are expressed as means ± S.D. (n=4).

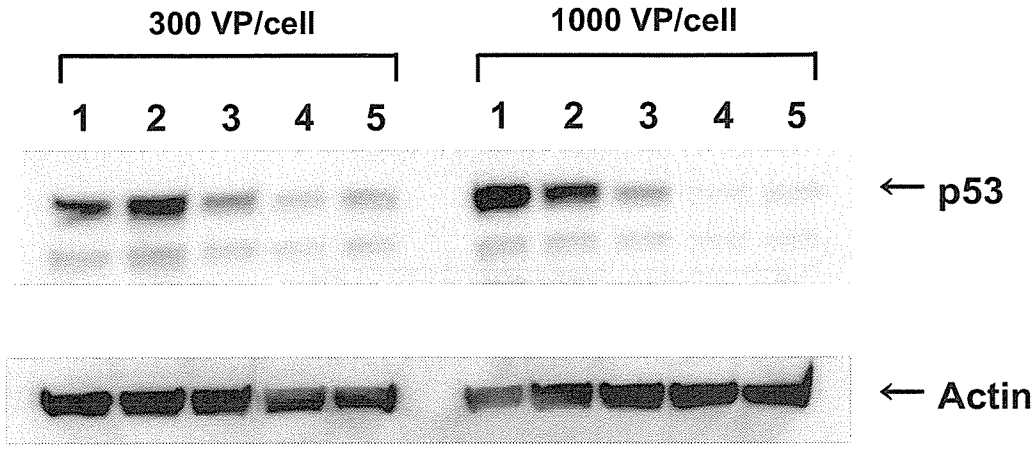


Fig. 154 Suppression of human p53 expression by the Ad vector expressing siRNA.
A549 cells were transduced with 300 or 1000 VP/cell of Ad-hU6 (lane 1), Ad-hU6-Lu (lane 2), Ad-hU6-p53 (lane 3), Ad-hU6a-p53 (lane 4), or Ad-hU6b-p53 (lane 5) for 1.5 hr, and then cultured for 3 days. The proteins were then extracted from the cells, and the levels of p53 expression were examined by Western blotting. The actin bands served as an internal control for equal total protein loading. The extra (lower) bands of p53 are non-specific.

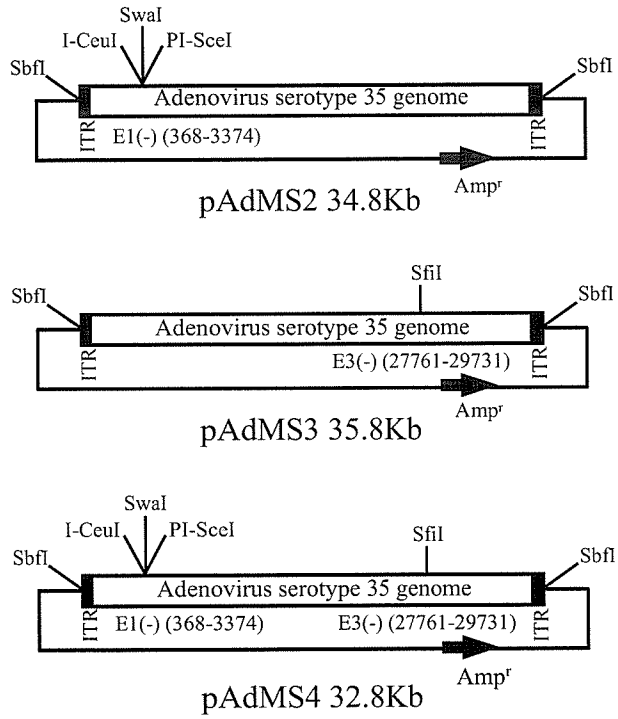


Fig. 155 The structure of the vector plasmids pAdMS2, -3, and -4 for construction of Ad35 vectors by the improved in vitro ligation method.

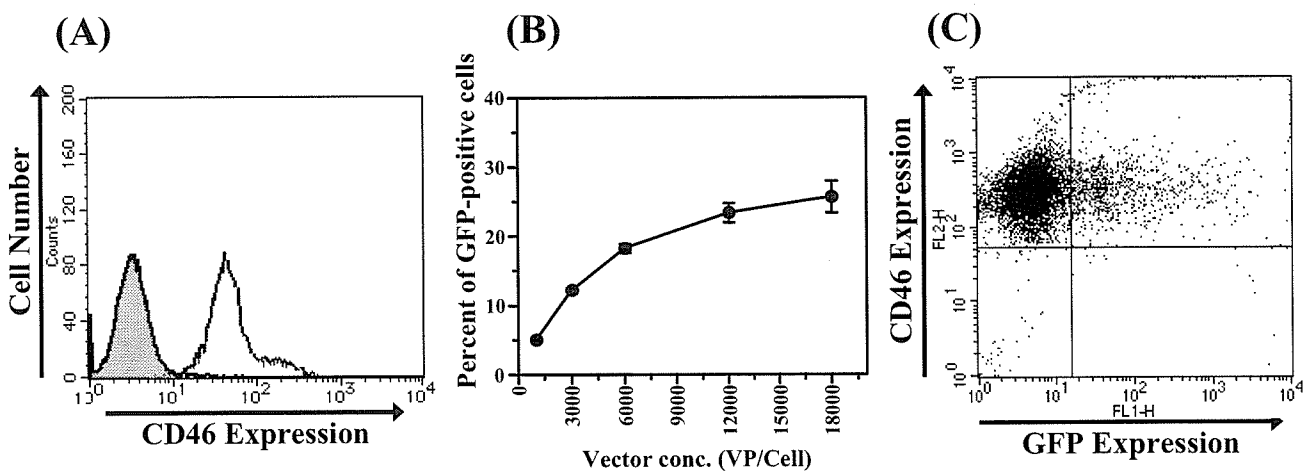


Fig. 156 (A) CD46 expression in human bone marrow CD34$^+$ cells. The cells were incubated with FITC-conjugated anti-CD46 antibody. As a negative control, the cells were incubated with an irrelevant antibody (shaded histogram). Similar levels of CD46 were found in the cells from three different donors. (B) Dose response of the percentage of GFP-positive cells following transduction with Ad35 vector containing a CMV promoter-driven GFP expression cassette. Human bone marrow CD34$^+$ cells were transduced with the Ad35 vector at the indicated vector concentrations for 6 hrs, washed, and resuspended in medium. Forty-eight hours later, GFP expression was measured by flow cytometry. All data represent the means ± S.D. of three experiments. (C) The relationship between the CD46 expression level on human bone marrow CD34$^+$ cells and GFP expression levels following Ad35 vector transduction. The cells were transduced with the Ad35 vector containing the CMV promoter at 6000 VP/cell for 6 hrs, washed and resuspended in the medium. After 48 hr of incubation and washing, the transduced cells were incubated with an anti-CD46 antibody. The cells were then washed, resuspended, and incubated with PE-conjugated second antibody. Data shown are from one representative experiment of the three performed.

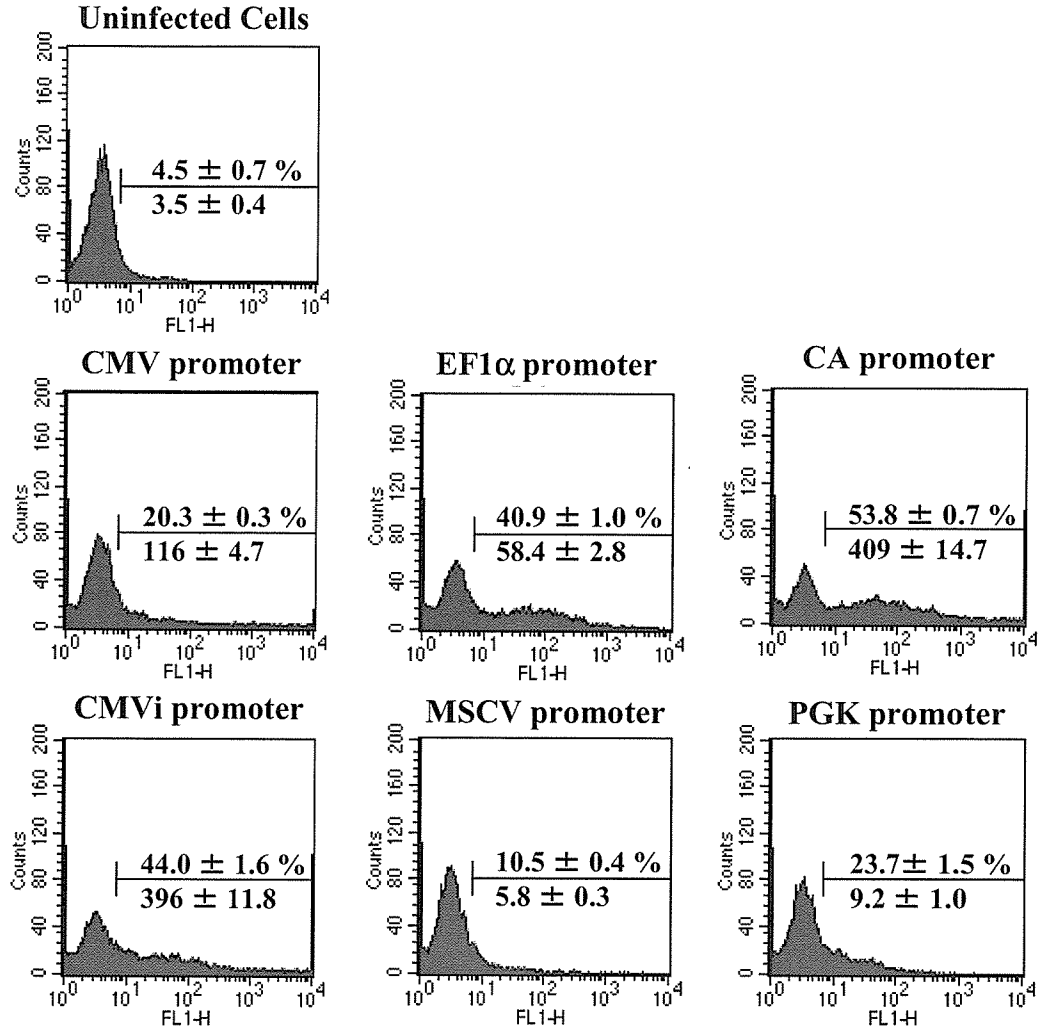


Fig. 157 Comparison of promoter activities in human bone marrow CD34⁺ cells transduced with Ad35 vectors. The results are shown as a percentage of GFP-positive cells (upper) and the meanfluorescence intensity (MFI) (lower) in the panel. The CD34⁺ cells were transduced with Ad35 vectors at 6000 VP/cell for 6 hrs, washed, and resuspended in medium. Forty-eight hours later, GFP expression was measured by flow cytometry. All data represent the means ± S.D. of three experiments.

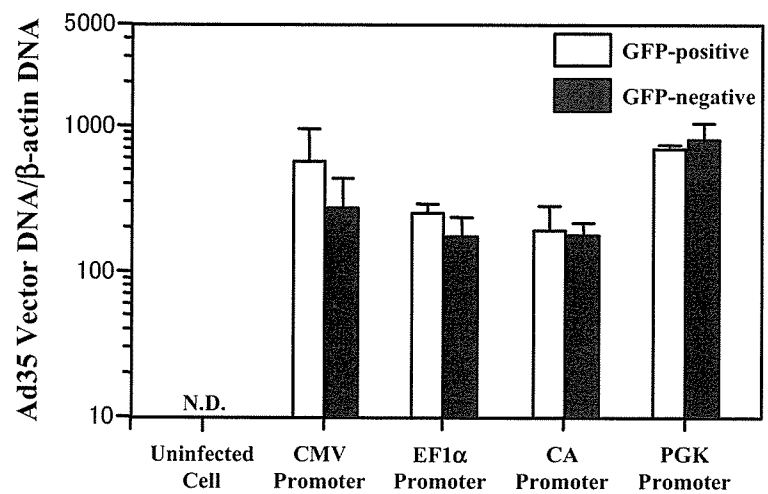


Fig. 158 Ad35 vector copy numbers in GFP-positive and GFP-negative cells following Ad35 vector transduction into huma bone marrow CD34⁺ cells. The CD34⁺ cells were transduced with Ad35 vectors at 6000 VP/cell for 6 hrs, washed, and resuspended in medium. Forty-eight hours later, GFP-positive and GFP-negative cells were sorted and the total DNA was extracted from the cells. The copy numbers of Ad35 vectors and b-actin were analyzed by Taqman PCR. All data represent the means ± S.D. of two independent experiments. N.D., not detected (under the limit of detection).

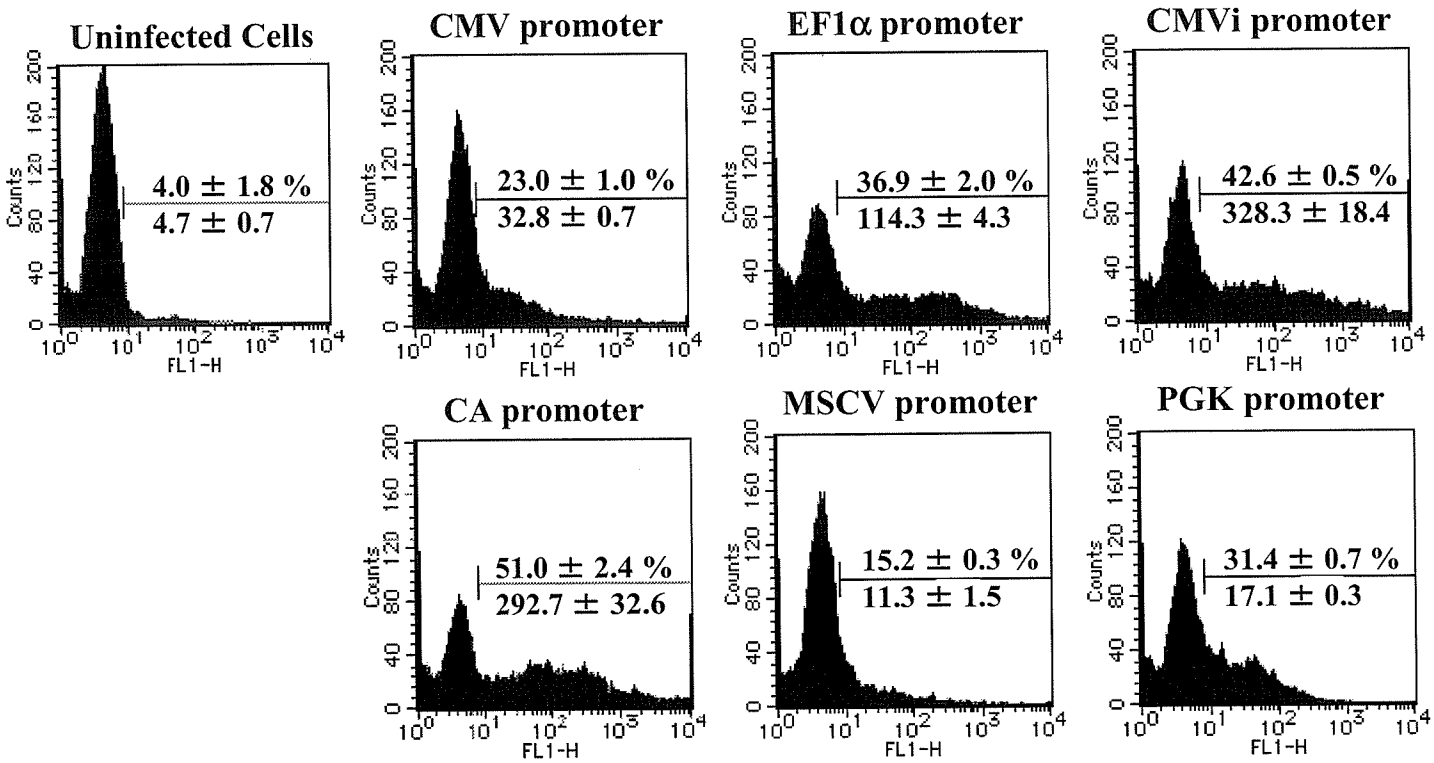


Fig. 159 Comparison of promoter activities in the CD34$^+$CD38$^{low/-}$ subsets transduced with Ad35 vectors. Results are shown as the percentage of GFP-positive cells (upper) and the mean fluorescence intensity (MFI) (lower) in the panel. The CD34$^+$CD38$^{low/-}$ subsets were transduced at 6000 VP/cell for 6 hrs, washed, and resuspended in medium. Forty-eight hours later, GFP expression was measured by flow cytometry. All datarepresent the means ± S.D. of two experiments.

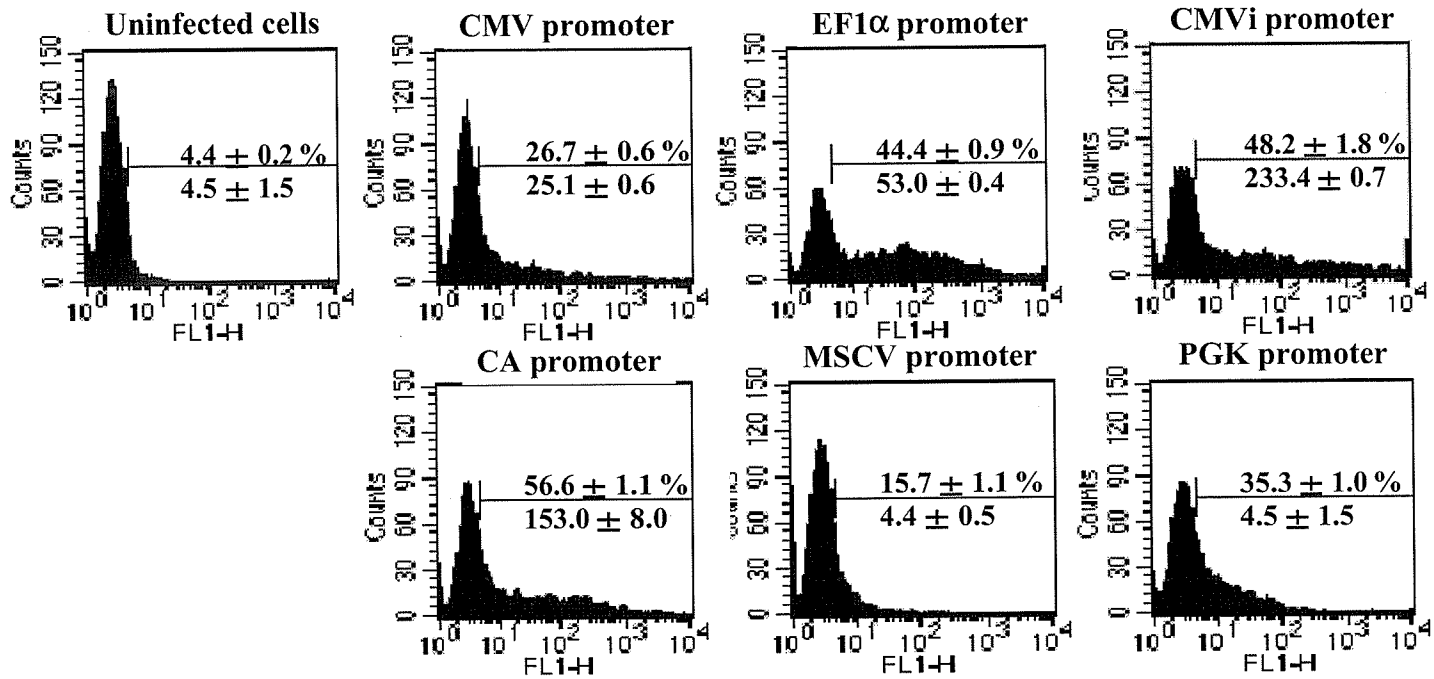


Fig. 160 Comparison of promoter activities in human bone marrow CD34$^+$AC133$^+$ subsets transduced with Ad35 vectors. Results are shown as the percentage of GFP-positive cells (upper) and the mean fluorescence intensity (MFI) (lower) in the panel. The CD34$^+$AC133$^+$ subsets were transduced at 6000 VP/cell for 6 hrs, washed, and resuspended in medium. Forty-eight hours later, GFP expression was measured by flow cytometry. All data represent the means ± S.D. of two experiments.

Table. 15 Numbers of colonies derived from GFP-positive and GFP-negative cells following transduction with the Ad35 vector containing the CA promoter in human CD34$^+$ cells.

CD34$^+$ cells		Total	BFU-E	CFU-GM	CFU-Mix
Sample 1	uninfected cells	222.8 ± 25.5	72 ± 11.8	150.3 ± 14.2	0.5 ± 0.6
	GFP-positive	193.5 ± 29 (86.9 %)	61 ± 9.9 (84.7 %)	131.5 ± 17.7 (87.5 %)	1 ± 1.4
	GFP-negative	180.5 ± 13.4 (81 %)	24.5 ± 0.7 (34 %)	155.5 ± 13.4 (103.5 %)	0.5 ± 0.7
Sample 2	uninfected cells	124.8 ± 13.5	44.5 ± 6.1	78.8 ± 9.5	1.5 ± 0.6
	GFP-positive	115 ± 11.3 (92 %)	29 ± 5.7 (65.2 %)	85.5 ± 6.4 (108.5 %)	0.5 ± 0.7
	GFP-negative	158 ± 19.8 (127 %)	26 ± 0 (58.4 %)	130.5 ± 20.5 (165.6 %)	1.5 ± 0.7

The data represent the mean number of colonies ± S.D. from duplicate cultures and the percentage of number of colonies/uninfected cells.

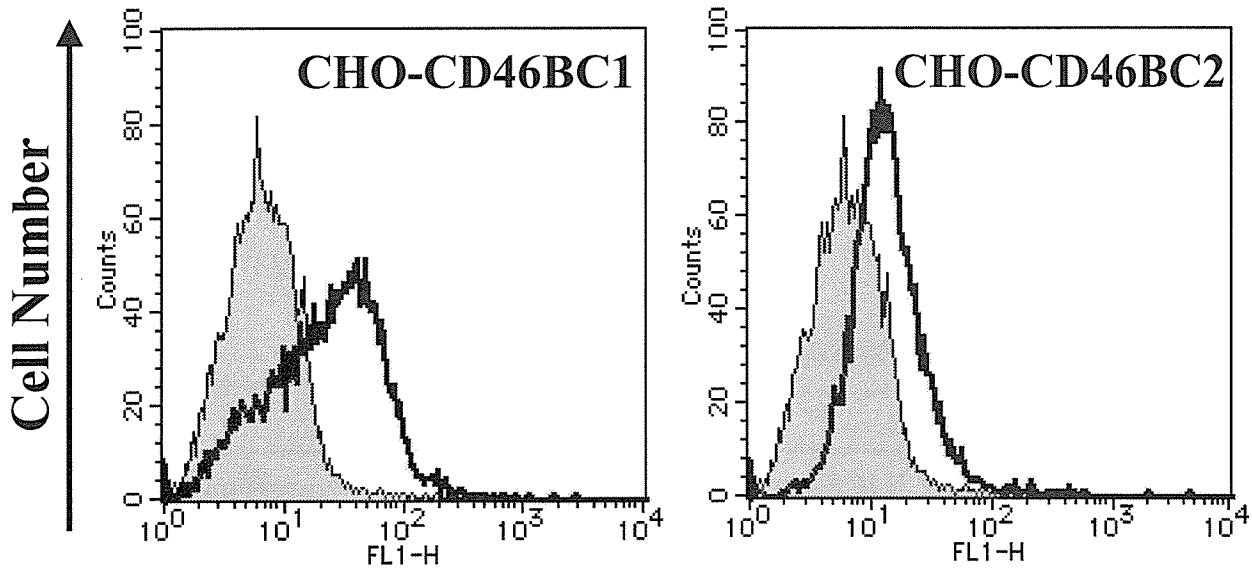


Fig. 161 Human CD46 expression levels in CHO cell transfectants. The expression of CD46 on CHO cells transfected with cDNAs encoding the BC1 and BC2 idoforms of CD46 was investigated by using flow cytometry with the monoclonal anti-CD46 antibody. CD46 expression level on wild type CHO cell were shown as shaded histogram.

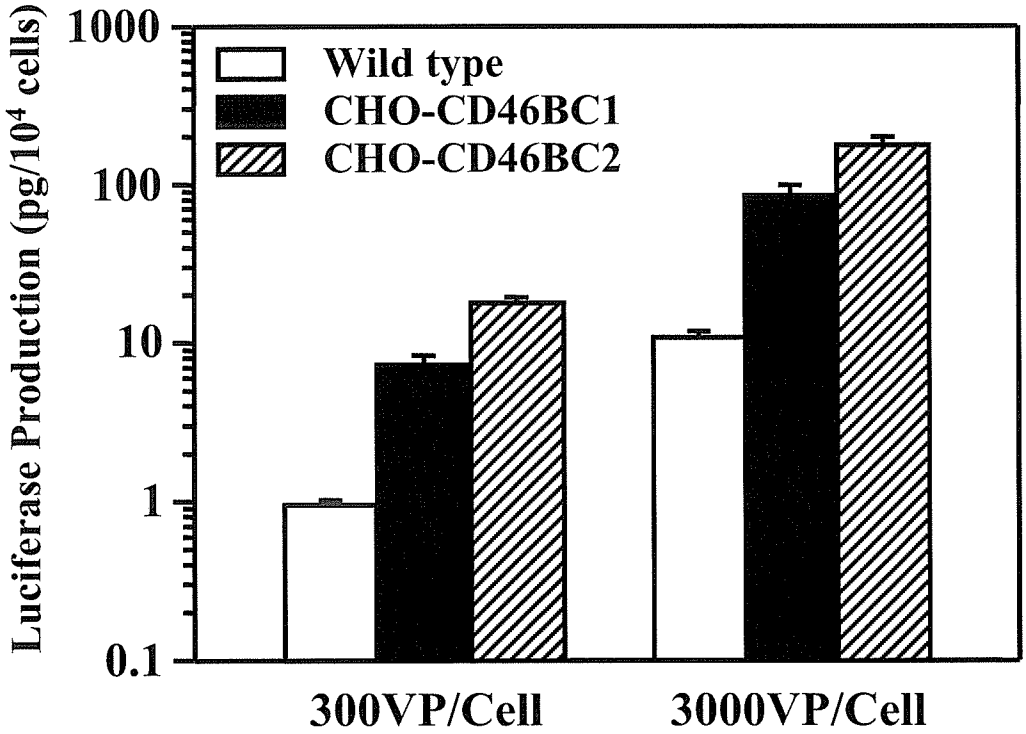
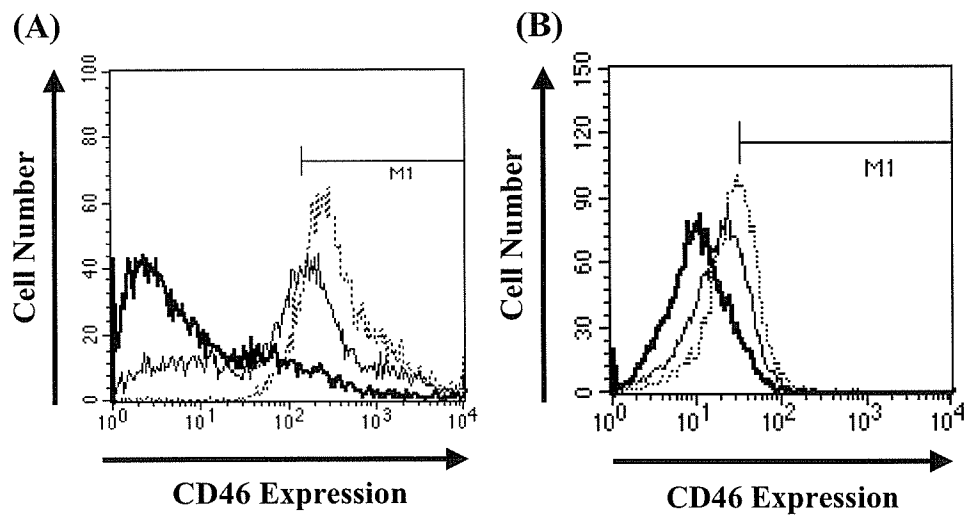


Fig. 162 Ad35 vector-mediated Transduction into CHO Cells Expressing Human CD46. CHO cell and CHO transfectants we transduced with 300 and 3000 VP/cell of luciferase-expressing Ad35 vector for 1.5h. Forty-eight hours later, luciferase production was measured by luminescent assay. All data represent the mean ± S.D. of four experiments.



	% of M1-gated
Wild-type	8.2 %
Hemi TG	46.6 %
Homo TG	86.6 %

	% of M1-gated
Wild-type	5.0 %
Hemi TG	19.8 %
Homo TG	40.5 %

Fig 163 CD46 expression in (A) mBM-DC and (B) peritoneal macrophages from wild-type mice and CD46TG mice. Thick lines, thin lines, and dotted lines represent cells from wild-type mice (C57Bl6), and hemizygous (Hemi TG), and homozygous (Homo TG) CD46TG mice, respectively. mBM-DC and peritoneal macrophages were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-human CD46 antibody after incubation with anti-FcgRII/III monoclonal antibody to block nonspecific binding of the anti-human CD46 antibody. After being washed thoroughly, 10^4 stained cells were analyzed using flow cytometory.