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# Establishment of a DsRed-Monomer-Harboring ICR Transgenic Mouse Model and Effects of the Transgene on Tissue Development

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## Abstract

**DsRed-monomer is an enhanced red fluorescent protein that may serve as a marker for studies in biotechnology and cell biology. Since the ICR mouse strain is a widely utilized outbred strain for oncology, toxicology, vaccine development and for aging studies, the objective of this study was to produce a DsRed-monomer transgenic mouse by means of pronuclear micro-injection of a vector driven by the cytomegalovirus (CMV) enhancer/chicken beta-actin promoter. Four transgenic mice were successfully produced, one of which expressed the DsRed-monomer protein in every tissue, although at varying levels. High expression levels were observed in the heart, pancreas and muscle. Moreover, amniotic fluid-derived progenitor cells, which also expressed the DsRed-monomer protein, could be collected from the DsRed-monomer-harboring ICR mice. As compared to wild-type mice, a few biochemical and histological dissimilarities were found in the DsRed-monomer transgenic mice, including the presence of intra-cytoplasmic eosinophilic threadlike materials in the acinar cells. Taken together, transgenic mice stably expressing DsRed-monomer can be produced using pronuclear micro-injection; however, expression of the *DsRed-monomer* gene or its insertion position may lead to minor influences.**

**Key Words: DsRed-monomer, ICR, red fluorescence protein, transgenic mice**

## Introduction

The LacZ and alkaline phosphatase reporter genes have been utilized in analyses in gene expression and cell behavior in transgenic animals. For example, the ROSA 26 mouse strain expresses the LacZ reporter gene throughout the body (17, 25, 32). Subsequently, fluorescent proteins have been utilized for studying gene targeting *in vivo* and in biomedical research (5), leading to the identification of the mechanism of embryo development and aetiology (26).

Green fluorescent protein (GFP) has been widely employed in the production of transgenic animals on account of its easy detection in live animals (11, 19, 24, 30). Red fluorescent protein (RFP) is another tracing marker, which was uncovered in coral reefs (*Dicosoma SP*); RFP has the advantage of sharing low homology as compared to GFP without mutual interruption prob-

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lems (18). However, slow maturation of the RFP results in a tetrameric configuration, which is obviously cytotoxic to embryonic stem cells and results in accumulation of the harmful proteins (1, 9, 14), a problem which has hampered the development of RFP transgenic mice (10). RFP has subsequently been modified to permit rapid maturation, a monomeric structure, enhanced red-shifted emission, excitation spectrum and increased tissue penetration capability (3, 16).

The ICR mouse strain is commonly employed for researches (20) in toxicology, pharmacology and in basic biomedical studies (4). Given its applications in many areas of biomedical research, this study sought to generate a DsRed-monomer transgenic mouse with whole-body RFP expression using pronuclear microinjection. This study further analyzes the monomeric RFP expression level in each tissue and organ to investigate whether monomeric RFP influences mouse anatomy, physiology and stem cell differentiation.

## Materials and Methods

*Construction of DsRed-Monomer Transgenic Vectors*

The DsRed-monomer coding sequence (CDS) was excised from the pDsRed-monomer-N1 vector, (Clontech Laboratories Inc., Mountain View, CA, USA) by means of EcoRI enzyme digestion. The DsRed-monomer CDS was inserted with an EcoRI restriction site to substitute for the enhanced GFP (EGFP) CDS of pCX-EGFP (19), resulting in the pCX-DsRed-monomer comprised of the cytomegalovirus (CMV) enhancer, chicken beta-actin promoter, DsRed-monomer cDNA fragment and rabbit beta-globin polyA signal.

*Production of DsRed-Monomer Transgenic Mice*

The ICR mice utilized in the study were acquired from the Laboratory Animal Center of the National Taiwan University College of Medicine (Taipei, Taiwan). DsRed-monomer transgenic mice were generated by pronuclear injection of the pCX-DsRed-monomer DNA into fertilized ICR mouse eggs. This study complied with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Taiwan University (No:NTU-96-EL-20).

*PCR and Southern Blot Analysis*

Genomic DNA was extracted from the mouse tail. After incubation in 500 μL of lysis buffer at 55°C for 12-16 h, the samples were extracted twice with an equal volume of phenol and then twice with chloroform. The aqueous phase was then precipitated with isopropanol and dissolved in TE (10 mM Tris and 0.1 mM EDTA). PCR analysis of the founder genomic DNA was performed by primers specific for the DsRed-monomer (forward, 5'-ATGGACAACACCGAGGAC-GTCATCEGFP-3' and reverse, 5'- TCAGTGGTATTT-GTGAGCCAGGGC-3') that produced a 780-bp product. The PCR reaction was carried out as follows: 94°C for 3 min; 35 cycles at 94°C for 30 sec, 65°C for 30 sec and 72°C for 30 sec; and a final extension at 72°C for 7 min. A total of 10 μg of DNA was digested with 40 units each of XbaI and DraI for Southern blot analysis. After the digess were separated in a 1% agarose gel, DNA fragments were subsequently transferred to a Hybond-N+ membrane (Amersham Pharmacia Biotech, Piscataway, NJ, USA) by capillary diffusion in 0.4 M NaOH after depurination with 0.25 N HCl for 10 min. The blot was probed with a 232-bp PCR fragment (generated using forward primer 5'-CGACATCCCCGACTACATGA -3' and reverse primer 5'- TCCTGGGGGTACAGCTTCTC -3') of the DsRed-monomer, which had been [$^{32}$P]dCTP-labeled using the Rediprime random primed labeling method (Amersham, Little Chalfont, UK). After the last washing step with 2 × SSC/0.1% SDS for 5 min at 65°C, the nylon membrane was sealed and placed in an exposure cassette with an intensifying screen at 4°C for 7 days.

*Hematoxylin and Eosin Staining and Histological Analysis*

Tissues and organs were harvested from the ICR mice and fixed in 10% neutral formaldehyde solution overnight at 4°C before dehydration through a graded ethanol series and embedded in paraffin. Samples were sectioned into 3-μm thickness for histological analysis by standard hematoxylin and eosin staining (H&E).

*Detection of Red Fluorescence*

Isolated organs were fixed in a 10% neutral formaldehyde solution overnight at 4°C. Red fluorescence was detected by a portable light source at excitation 520-560 nm and emission 580-650 nm. Blank slides without staining were also observed under a fluorescent microscope (excitation 510-560 nm and emission 610 nm) equipped with a RFP filter.

*Western Blot Analysis*

Total protein was extracted from tissues and organs in liquid nitrogen using a manual mortar and pestle. After the addition of RIPA lysis buffer (100 mM NaCl, 50 mM Tris-Cl, pH 7.4, 1 mM EDTA, pH 8.0, 0.5% Triton X-100, 0.5% Nonidet P-40) to the tissue, incubation was carried out on ice for 30 min. Following

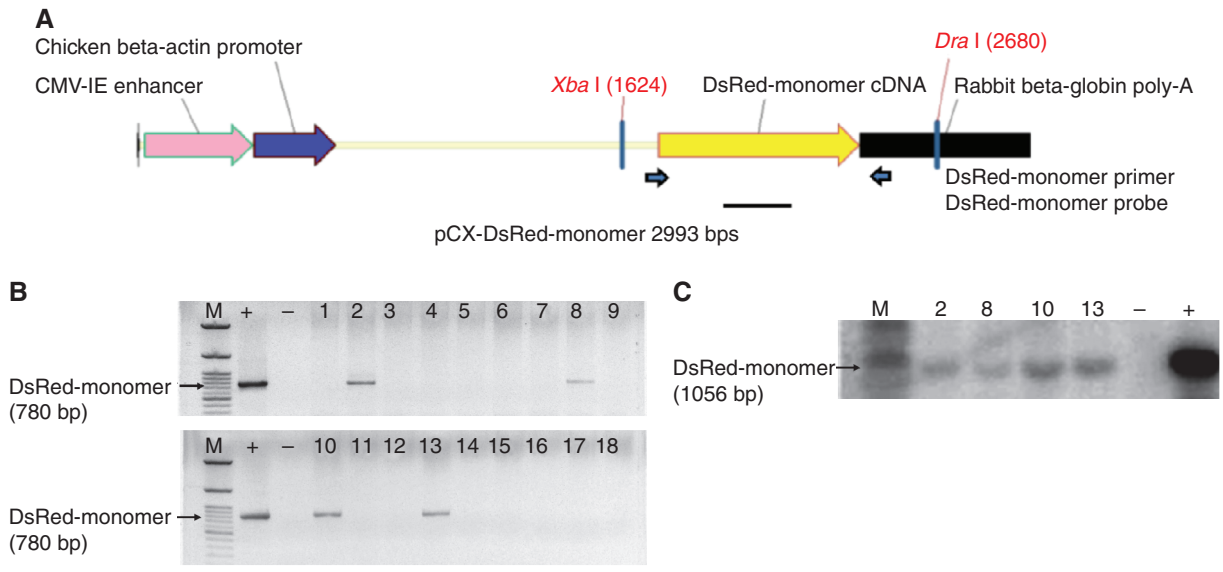


Fig. 1. Generation of the DsRed-monomer transgenic mice. (A) Map of the pCX-DsRed-monomer construct that carries the *DsRed-monomer* gene. Arrows indicate the positions of the DsRed-monomer primers used for PCR. Thick black lines indicate the position of the Southern blot probes. (B and C) Transgenic founder mice carrying the DsRed-monomer were identified by (B) PCR (780 bp product) and (C) Southern blot screening, producing a band of 1.1 kb.

determination of protein concentration, 70 μg protein lysate was separated on a 10% SDS-PAGE gel. After being transferred onto PVDF membranes, the blots were probed with a rabbit anti-DsRed polyclonal antibody (1:1500; BD Clontech, Palo. Alto, CA, USA), detected with a HRP-conjugated goat anti-rabbit antibody (1:15,000; Bio-Rad, Hercules, CA, USA) and visualized using the ECL detection system (Amersham Bioscience, Piscataway, NJ, USA).

*Fluorescence Activated Cell Sorting Analysis*

Cells at passage 5 to 6 were treated with 0.25% trypsin-EDTA for 5 min at 37°C, washed with PBS containing 2% fetal bovine serum, and then dissociated into single cells by gentle pipetting at a density of $1 \times 10^6$ cells/ml. The cells were stained with the following fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse antibodies at 4°C for 30 min: CD29, CD44, CD45, MHCI and ScaI (eBioscience, San Diego, CA, USA). The isotype controls served as the negative control. Fluorescence was measured utilizing a LSR II flow cytometer (Becton, Dickinson and Company Biosciences, San Jose, CA, USA).

*Isolation and Differentiation Mouse Amniotic Fluid Progenitor Cell*

Amniotic fluid progenitor cells (mAFPCs) from DsRed-monomer-harboring pregnant mouse at 11.5 days gestation were obtained. After the pregnant mouse was sacrificed by cervical dislocation, the uterus was harvested, and the collected amniotic fluid was filtered through a 70-μm mesh by centrifugation in 12,000 rpm for 5 min. After the cells were re-suspended, they were cultured on a 10 cm dish (TPP, Trasadingen, Switzerland) at a density of $1 \times 10^5$ cells/cm$^2$. To induce adipogenic differentiation, cells were cultured in StemPro® Adipogenesis Supplement Medium (Gibco BRL, Grand Island, NY, USA) for 21 days. The differentiation medium was changed every 3 days. The cells were stained with 0.5% Oil-Red O (Sigma Aldrich, St. Louis, MO, USA) to verify accumulation of lipid droplets. To investigate osteogenic differentiation potentials, cells were incubated in StemPro® Osteogenesis Supplement Medium (Gibco BRL) for 7 days. Bone calcium deposition was analyzed by Alizarin red S (Sigma Aldrich). For chondrogenic differentiation, a pellet cell was cultured in StemPro® Chondrogenesis Supplement Medium (Gibco BRL) for 10 days. The production of proteoglycan was assessed by staining with toluidine blue (Sigma Aldrich).

## Results

The transgene construct map with restriction enzyme digestion sites is shown in Fig. 1A. The construct includes the CMV enhancer/chicken β-actin (CAG) promoter and the *DsRed-monomer* gene. After injection of 304 pronuclear embryos with the *DsRed-monomer* transgene, 164 embryos were transplanted to five surrogate mice, from which 31 pups were born. PCR and Southern blot analyses for transgene integration revealed that lineages 2, 8, 10 and

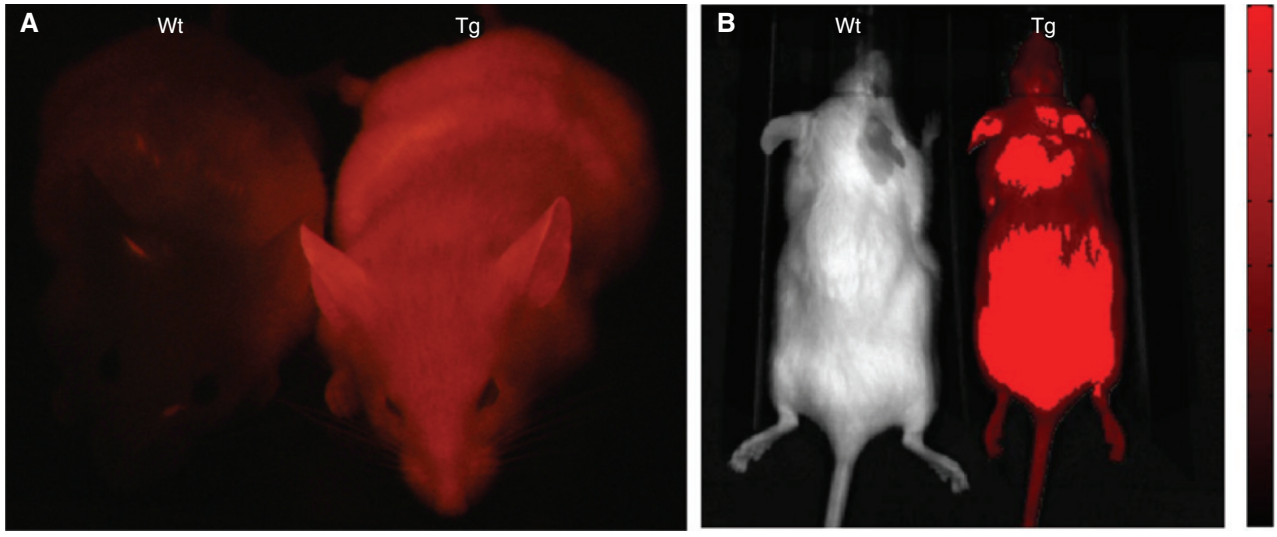


Fig. 2. Whole-body fluorescence of the transgenic mice produced by pronuclear microinjection with the *DsRed-monomer* gene. (A) Wild-type (Wt) and transgenic (Tg) mice with systemic red fluorescence expression using a portable fluorescence source. (B) Wt and Tg mice imaged in the IVIS imaging system under anaesthesia with 2.5% isoflurane.

**Table 1. Generation of DsRed-monomer transgenic mice**

| | Number |
|---|---|
| Donor number | 11 |
| Pronuclear eggs | 397 |
| Microinjected pronuclear eggs | 304 |
| Embryos transferred to recipients | 164 |
| Recipients | 5 |
| Pregnant recipients (%) | 3 (60.0%) |
| Birth/embryos transferred (%) | 31 (19.3%) |
| Transgenic pups that survived (%) | 4 (13.3%) |
| Germline transmission rate (%) | 58/110 (52.72%) |

13 carried the DsRed-monomer gene (Fig. 1, B and C) with a germ-line transmission rate of approximately 52.72 % (Table 1).

By means of a portable fluorescence imaging and an *in vivo* image system (IVIS), DsRed-monomer expression in the transgenic mice was observed (Fig. 2). Specifically, fluorescence microscopy revealed that all the examined organs and tissues in the transgenic mice expressed high levels of DsRed-monomer protein in the brain, heart, lung, liver, pancreas, stomach, spleen, intestine, kidney, testis, muscle, ovary and eye (Fig. 3). Analysis of the pathological sections revealed varying DsRed-monomer protein expression in different cells (Fig. 4). The DsRed-monomer protein of different tissues was extracted and quantified. DsRed-monomer protein expression levels in the heart, pancreas and muscle were found to be considerably higher than in other organs (Fig. 5).

H&E staining revealed that the histological structures of the hippocampus, cortex, cerebellum, eye, heart, lung, liver, stomach, spleen, muscle, kidney, intestine and testis of the wild type mice were normal as compared to the transgenic mice (Fig. 6). However, the pancreas of the transgenic mice showed additional intra-cytoplasmic eosinophilic threadlike materials in the acinar cells as compared with the wild-type mice (Fig. 7).

Blood samples from six wild-type and six DsRed-monomer transgenic mice were collected to determine the haematological and serum parameters. The levels of lymphocytes, alanine transaminase (ALT) and aspartate transaminase (AST) were significantly higher in the transgenic mice than in the wild-type controls (Table 2; $P < 0.05$). Conversely, the levels of monocytes were significantly lower in the transgenic mice (Table 2; $P < 0.05$).

Due to the histopathological abnormality observed in the pancreatic acinar cells, we suspected that the DsRed-monomer transgenic mice might be suffering from pancreatitis. Thus, the pancreatitis markers, amylase and lipase, were analyzed. To determine whether β cells were also affected, glucose and insulin levels were detected as well. No differences in glucose, insulin, amylase and lipase levels were detected between the wild-type and transgenic mice (Table 3).

mAFPCs were collected from DsRed-monomer transgenic mice to determine expression of CD29, CD44 and ScaI (stem cell markers) (Fig. 8). The proportions of mAFPCs that were CD29-, CD44- and ScaI-positive were 64.5%, 5.3% and 16.5%, respectively. Further, the ability of mAFPCs isolated from

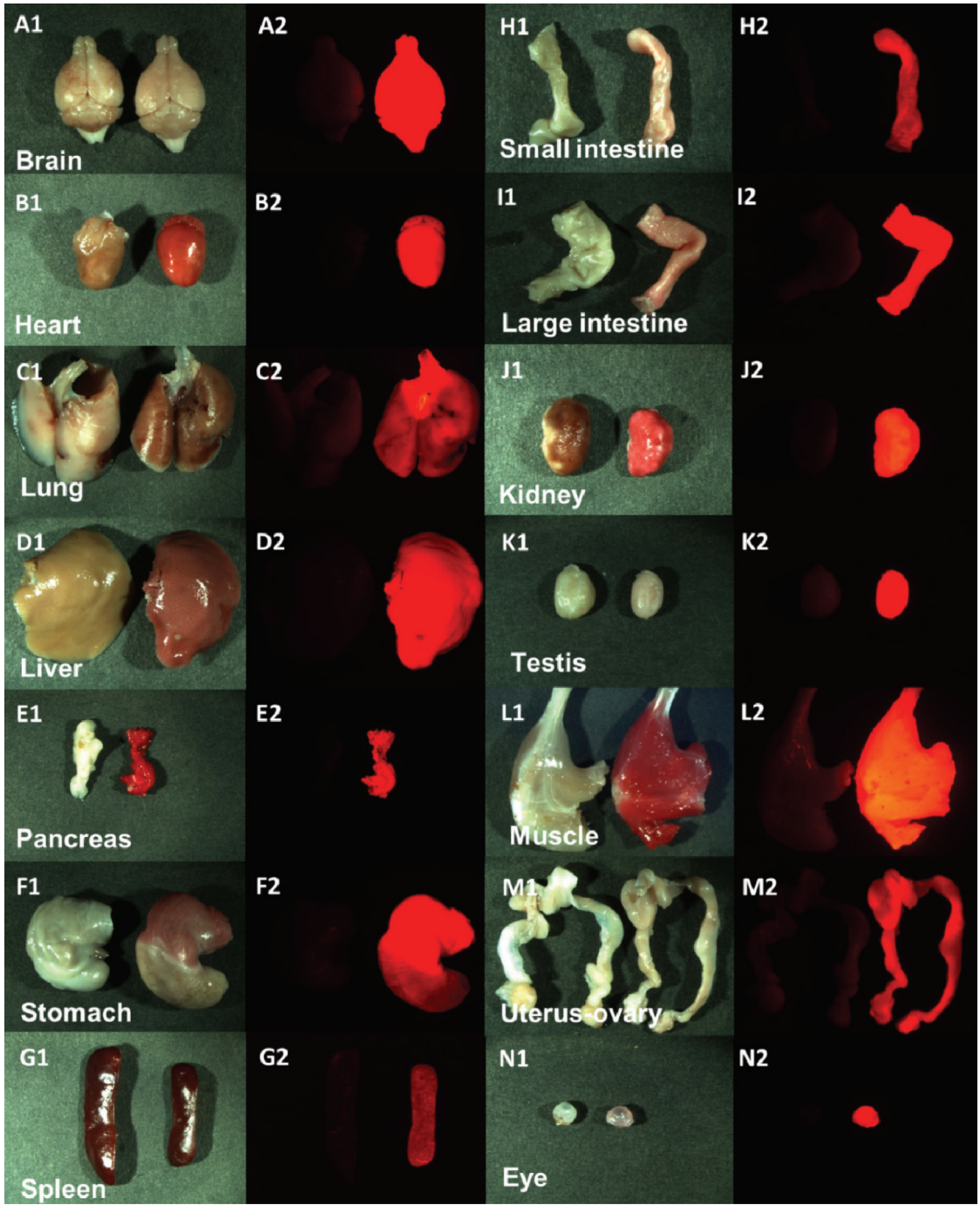


Fig. 3. Systemic expression of DsRed-monomer in the transgenic mice. Bright-field images (A1-N1) of organs from wild-type (left) and transgenic (right) mice. Fluorescence images (A2-N2) show organs from the transgenic mice (right), but not the wild-type controls (left) exhibiting distinctive fluorescence. The tissues and organs examined were: Brain (A1, A2); Heart (B1, B2); Lung (C1, C2); Liver (D1, D2); Pancreas (E1, E2); Stomach (F1, F2); Spleen (G1, G2); Small Intestine (H1, H2); Large Intestine (I1, I2); Kidney (J1, J2); Testis (K1, K2); Muscle (L1, L2); Uterus-Ovary (M1, M2); and Eye (N1, N2).

the DsRed-monomer transgenic mice to differentiate into adipocytes, osteoblasts and chondrocytes was confirmed by Oil Red O staining for oil droplets, Alizarin red S staining for mineralized foci and toluidine blue staining for glycosaminoglycans within the extracellular matrix, respectively (Fig. 9).

## Discussion

With advancements in biotechnology and biomedicine, another fluorescent marker in addition to GFP is needed for research (15). The outbred ICR mouse strain shares genetic similarity to humans and

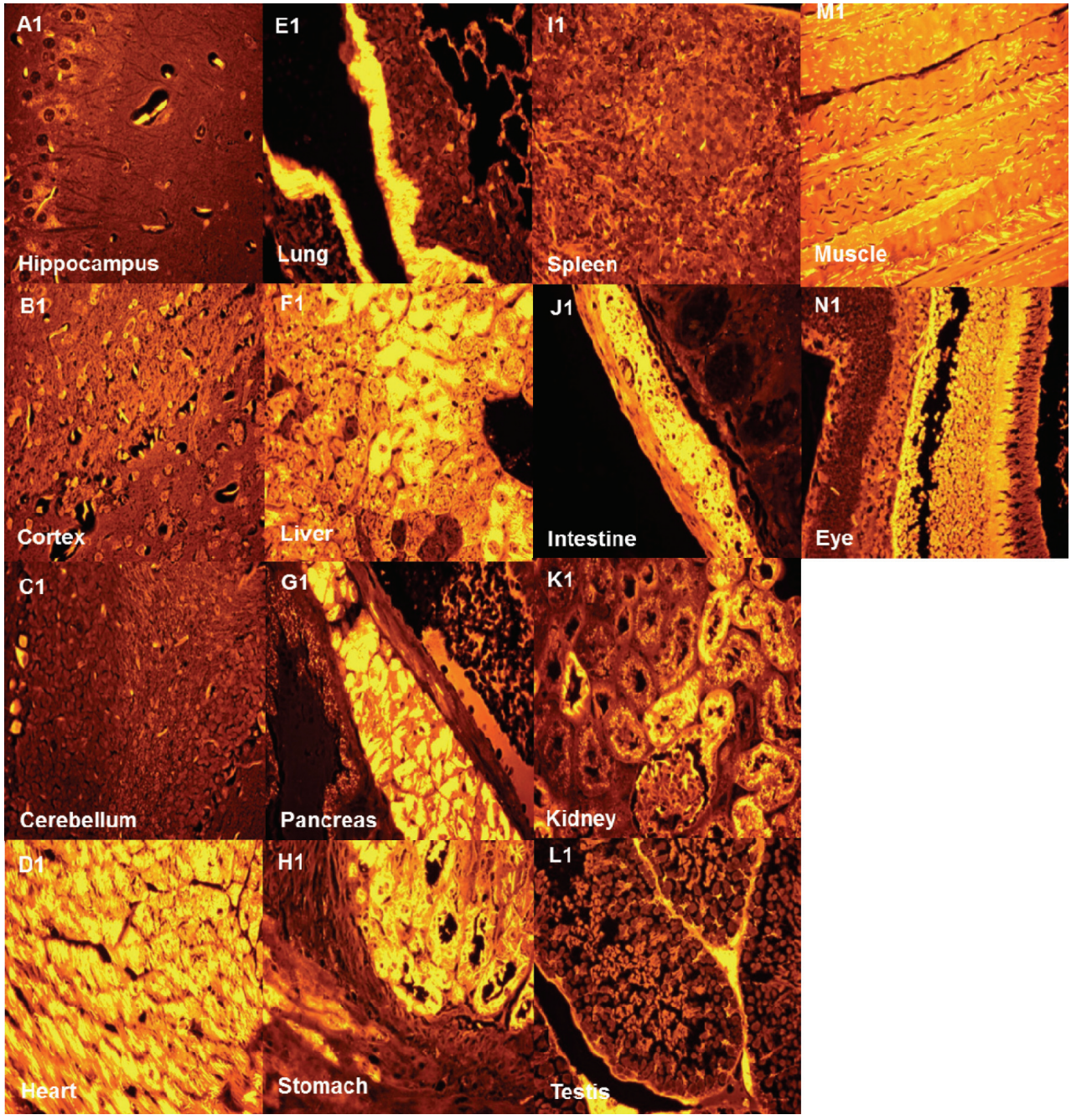


Fig. 4. Histological analysis of tissues from the DsRed-monomer transgenic mouse with systemic red fluorescence expression using fluorescence microscopy. Cerebellum (A1); Cortex (B1); Hippocampus (C1); Heart (D1); Lung (E1); Liver (F1); Pancreas (G1); Stomach (H1); Spleen (I1); Intestine (J1); Kidney (K1); Testis (L11); Muscle (K1); Eye (N1). Magnification was 400×.

has been demonstrated to be an ideal animal model for studies in various biomedical fields, such as vaccine testing, aging, oncology, toxicology and biology (4). Thus, we aimed to produce a DsRed-monomer transgenic ICR mouse and analyzed the effects of expression of the RFP monomer protein *in vivo*. At present, there are two major methods for establishing transgenic mice, including DNA microinjection and embryonic stem cell-mediated gene transfer (13, 21). In this study, we chose to produce the DsRed-monomer transgenic ICR mouse by pronuclear microinjection. *DsRed-monomer* transgene-harboring ICR mice with whole body expression were generated, which were confirmed by the presence of red fluorescence protein in all the organs analyzed, as reported in previous studies using different strains and different production methods (8, 16, 31, 33).

Analyses demonstrated that four transgenic

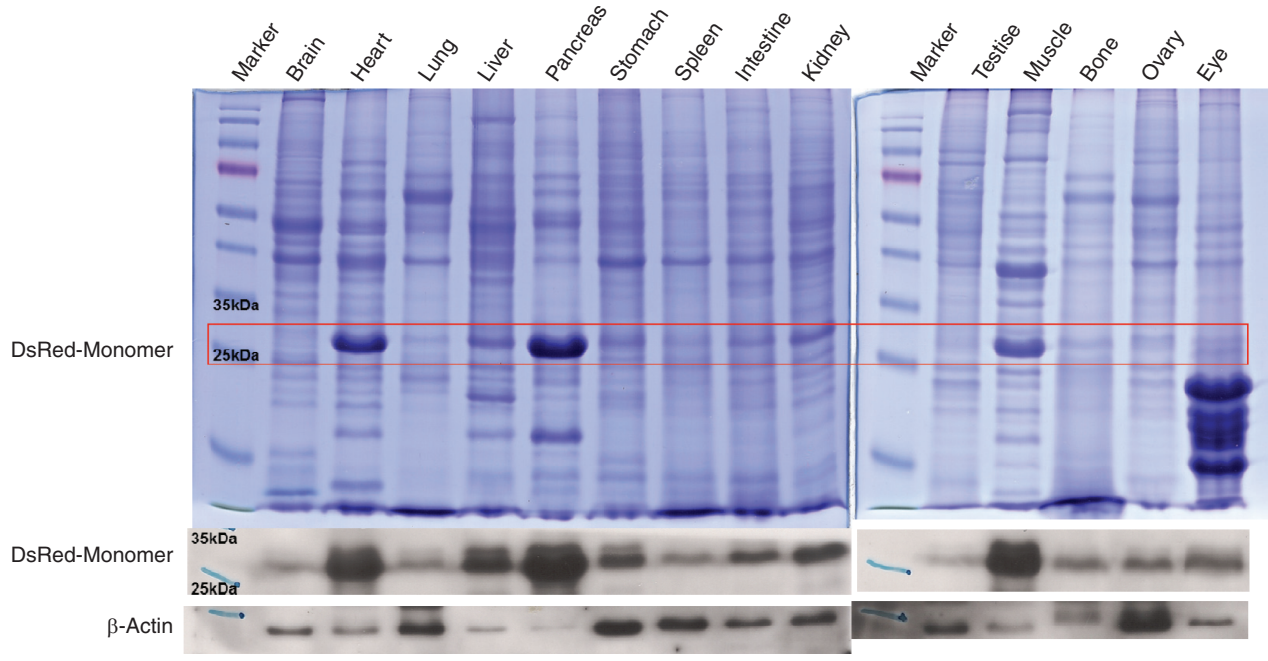


Fig. 5. SDS-PAGE and western blot analysis of DsRed-monomer transgenic mouse tissue samples. Tissues were isolated from the transgenic DsRed-monomer mice, and proteins were separated by SDS-PAGE. Protein extracts (70 μg per well) were incubated with a Ds-Red-specific antibody. The levels of the 28-kDa DsRed-monomer protein are indicated. Actin served as the internal control.

founder mice carried the *DsRed-monomer* gene, yet only one line (number 10) expressed the DsRed-monomer protein in the whole body. This outcome is consistent with the previous report of mRFP transgenic mice produced by embryonic stem cell-mediated gene transfer (8, 16). Sawicki *et al.* (22) previously demonstrated that the CMV enhancer/chicken beta-actin promoter was active in most cell types, which was confirmed in line 10 reported here that showed ubiquitous expression of the red fluorescent protein.

The other three lines only expressed the DsRed-monomer protein in selected tissues and organs probably as a consequence of gene insertion positions, resulting in gene silencing in many tissues and organs (2, 6, 29). In line 10, several tissues, such as heart, pancreas and muscle, strongly expressed the DsRed-monomer protein, which is consistent with the same microinjection approach in a former report (33). However, as reported for the embryonic stem cell-mediated gene transfer method, there is no expression differences in previous papers (8, 16). It is possible that tissue-specific transcription factors in these tissues may have enhanced the *DsRed-monomer* gene expression level.

The *DsRed-monomer* gene was also evaluated for possible influence in normal physiological functions and homeostasis in the transgenic mice (23, 28). Histopathologic evaluation of the wild-type and transgenic mouse tissue sections revealed fine tissue penetration without the phenomena of auto-fluorescence, which is concordant with a previous report (3). However, distinct histological discrepancies were observed in the pancreatic tissues of the transgenic mice in that additional intra-cytoplasmic eosinophilic threadlike materials in the acinar cells were detected. However, this abnormality did not affect β cell conformation or normal insulin secretion. Nevertheless, the cause of the abnormal structure of the acinar cells, including the location of the gene insertion or cell toxicity caused by DsRed protein (1), requires further investigation. This phenomenon has not been mentioned by previous reports (8, 16, 33).

Parameters in hematology, serology and plasma biochemitry are important for assessing the physical conditions and health of an animal. Statistically significant differences in lymphocyte, monocyte, ALT and AST levels were found between the wild-type and transgenic mice. Some red fluorescent proteins have been reported to result in the death of some transgenic species, such as the red fluorescent transgenic dog that died of chronic bronchopneumonia (12). However, the DsRed-monomer transgenic mice reported here survived and transmitted the transgene to their offspring despite the presence of certain some biochemical changes, an observation not previously reported (8). AFPCs hold the promise to give rise to multiple cellular lineages (7). mAFPCs derived from the DsRed-monomer transgenic mice showed a spindle

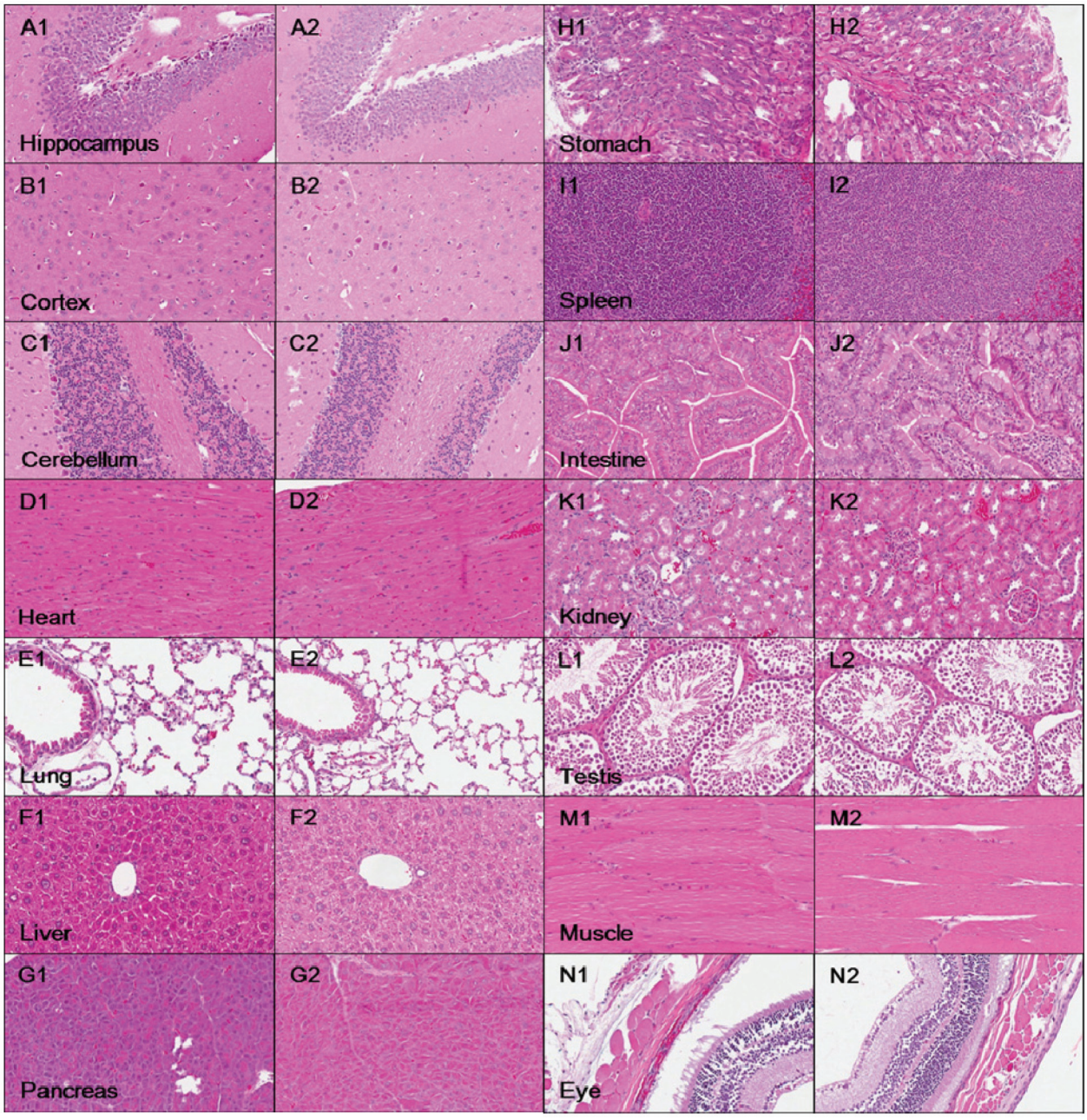


Fig. 6. Histological analysis of tissues of the DsRed-monomer transgenic mice. Displayed are photomicrographs of representative H&E-stained slides showing various tissues from wild-type (A1-N1) and transgenic mice (A2-N2). Hippocampus (A1, A2); Cortex (B1, B2); Cerebellum (C1, C2); Heart (D1, D2); Lung (E1, E2); Liver (F1, F2); Pancreas (G1, G2); Stomach (H1, H2); Spleen (I1, I2); Intestine (J1, J2); Kidney (K1, K2); Testis (L1, L2); Muscle (M1, M2); Eye (N1, N2). Magnification was 200×.

shape and expressed stem cell-specific markers. The transgenic mouse-derived mAFPCs also differentiated to osteocytes, adipocytes and chondrocytes and consistently expressed the DsRed-monomer protein after differentiation and after several passages. Given that DsRed is a potential tracing marker after transplantation into a recipient (31), the continued expression of the DsRed-monomer protein in these cells will permit researchers to trace the fate of the cells even after differentiation into specific cell types. Moreover, due to the fetal origin of mADPCs, DsRed-monomer-expression mAFPCs could potentially be applied to disease modeling relevant to all three germ layers. Therefore, these DsRed-monomer-labeled cells may be an ideal cell source for future transplantation research and for studying mechanisms underlying cell

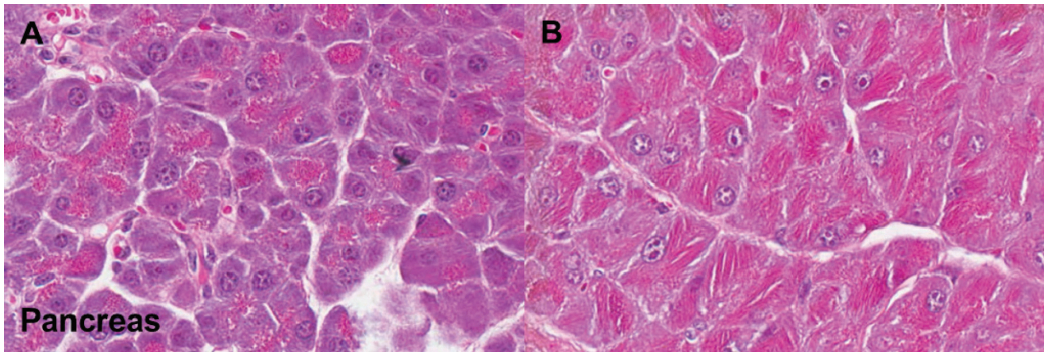


Fig. 7. H&E-stained sections of pancreatic tissues and islets of wild-type and transgenic mice. Pancreatic tissues were isolated from a wild-type mouse (A), which showed normal morphology. Transgenic mouse (B) pancreas showed pathological changes. Magnification was 200×.

**Table 2. Biochemical analysis of wild-type and transgenic mice**

| | Wild-type n = 6 (♂) | Transgenic n = 6 (♂) | *P*-value |
|---|---|---|---|
| *Blood Routine* | | | |
| Hemoglobin (g/dl) | 13.48 ± 1.55 | 13.98 ± 0.72 | 0.490 |
| Red blood cells (× $10^3$/$mm^3$) | 8.66 ± 0.75 | 9.22 ± 0.69 | 0.212 |
| Mean corpuscular volume (fl) | 46.62 ± 2.70 | 46.12 ± 1.15 | 0.685 |
| Mean corpuscular hemoglobin (pg) | 15.53 ± 1.05 | 15.2 ± 0.58 | 0.515 |
| Mean corpuscular hemoglobin concentration (%) | 33.43 ± 2.08 | 32.95 ± 1.83 | 0.678 |
| White blood cells (cells/$mm^3$) | 4616.67 ± 1205.68 | 5133.33 ± 1623.17 | 0.545 |
| Segment (%) | 36.33 ± 7.58 | 31.00 ± 12.76 | 0.399 |
| Eosinophils (%) | 6.00 ± 4.00 | 2.50 ± 1.29 | 0.153 |
| Lymphocytes (%) | 46.50 ± 6.83 | 60.33 ± 13.08 | 0.045* |
| Monocytes (%) | 14.17 ± 4.58 | 7.00 ± 3.23 | 0.011* |
| Platelets (× $10^3$/$mm^3$) | 896.8 ± 186.0 | 966.5 ± 355.1 | 0.714 |
| *Nutritional Index* | | | |
| Total protein (g/dl) | 5.43 ± 0.46 | 5.42 ± 0.17 | 0.936 |
| Albumin (g/dl) | 2.30 ± 0.13 | 2.37 ± 0.05 | 0.260 |
| *Renal Function Index* | | | |
| Blood urea nitrogen (mg/dl) | 22.50 ± 3.45 | 21.83 ± 2.71 | 0.718 |
| Creatinine (mg/dl) | 0.30 ± 0.09 | 0.33 ± 0.10 | 0.563 |
| *Liver Function Index* | | | |
| Alanine aminotransferase (U/dl) | 54.33 ± 10.82 | 75.00 ± 15.17 | 0.022* |
| Aspartate aminotransferase (U/dl) | 131.67 ± 33.86 | 188.17 ± 46.27 | 0.036* |
| Alkaline phosphatase (U/dl) | 57.83 ± 18.42 | 68.50 ± 14.75 | 0.294 |
| *Metabolic Index* | | | |
| Glucose (mg/dl) | 120.67 ± 27.65 | 139.00 ± 19.44 | 0.214 |
| Cholesterol (mg/dl) | 74.17 ± 8.16 | 72.83 ± 18.61 | 0.876 |
| Triglyceride (mg/dl) | 68.33 ± 40.19 | 57.17 ± 33.48 | 0.612 |

Comparisons between wild-type and transgenic mice were undertaken using an unpaired *t*-test. *$P < 0.05$

**Table 3. Insulin, amylase and lipase levels in wild-type and transgenic mice**

| | Wild type n = 6 (♂) | Transgenic n = 6 (♂) | *P*-value |
|---|---|---|---|
| Insulin (mU/l) | < 0.5 | < 0.5 | N/A |
| Amylase (mU/l) | 2389 ± 341 | 2093 ± 135 | 0.071 |
| Lipase (mU/l) | 137 ± 25 | 141 ± 41 | 0.382 |

Comparisons between wild-type and transgenic mice were undertaken using an unpaired *t*-test. N/A: not available.

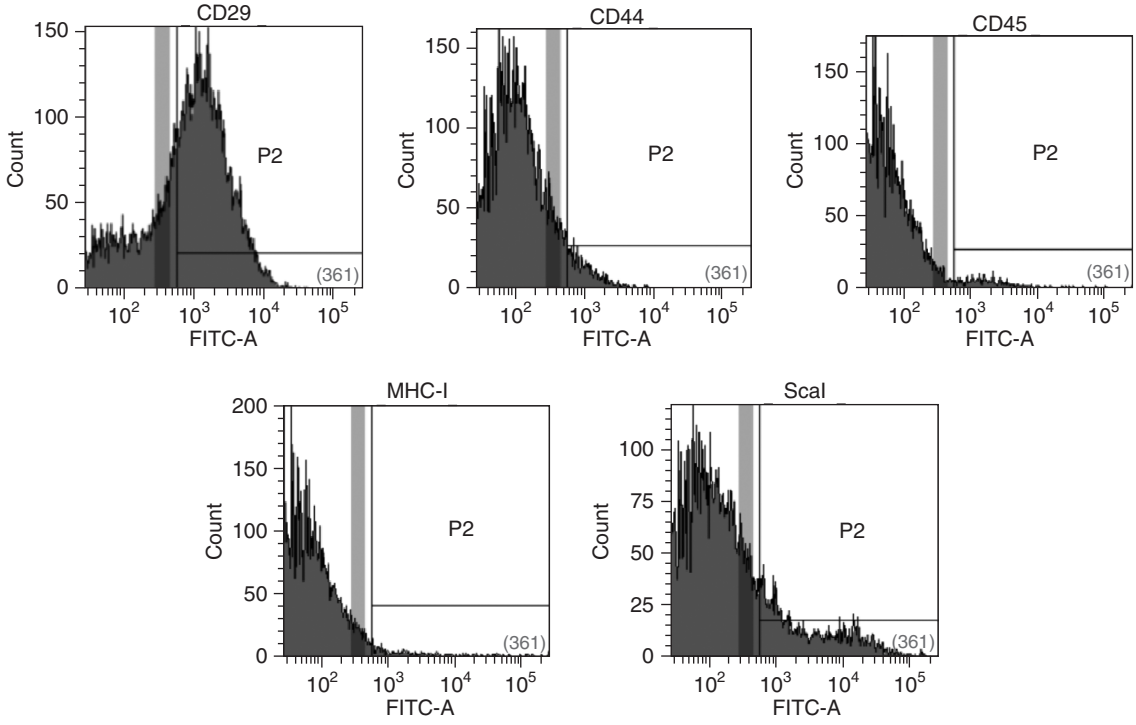


Fig. 8. Cytometric analyses of expression of CD29, CD44, CD45, MHC-I and ScaI transgenic mAFPCs. Surface marker expression of culture-expanded mAFPCs was analyzed by FACS analysis.

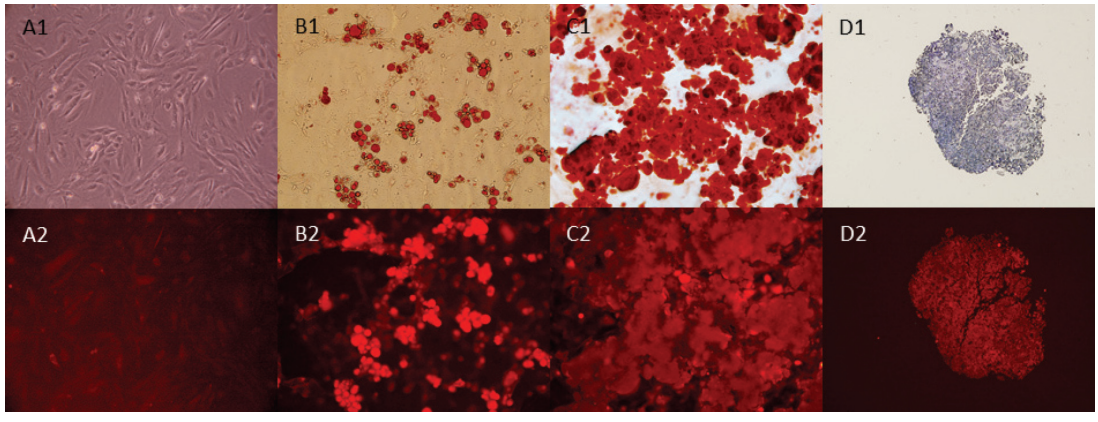


Fig. 9. Differentiation potential of mAFPCs isolated from DsRed-monomer transgenic mice. DsRed-monomer mAFPCs were analyzed using bright field (A1-D1) or fluorescence microscopy (A2-D2). Displayed are illustrations of DsRed-monomer mAFPCs with spindle-shaped morphology (A1, A2). Lipid droplets are illustrated in the DsRed-monomer mAFPCs after adipogenic differentiation at day 21 using Oil Red O staining (B1). Calcium accumulation is shown in the Ds-red mAFPCs after osteogenic differentiation at day 7 by Alizarin Red staining (C1). Collagen was revealed in the Ds-red mAFPCs after chondrogenic differentiation at day 10 using Toluidine Blue staining (D1). Fluorescence microscopy revealed expression of the Ds-red fluorescent protein after osteogenesis (B2), adipogenesis (C2) and chondrogenesis (D2) differentiation.

engraftment, differentiation, paracrine secretion or cell fusion.

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