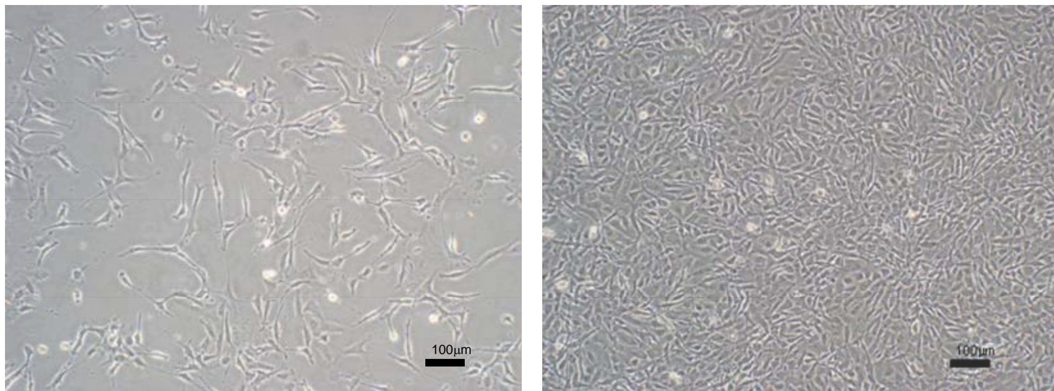
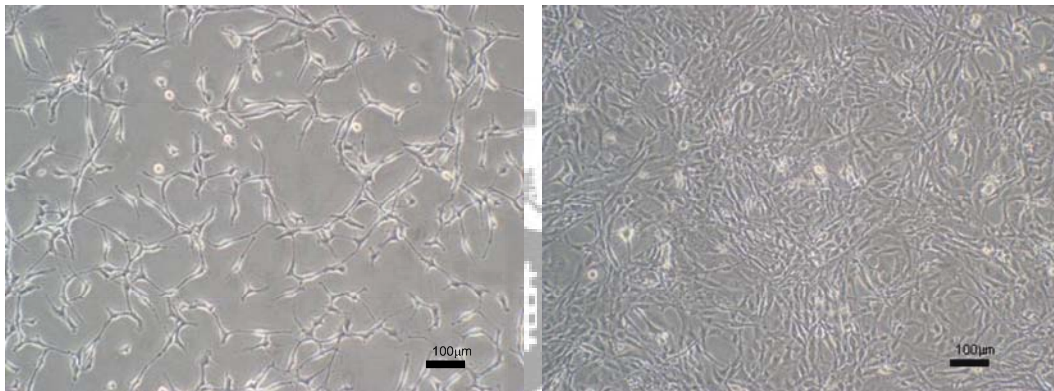
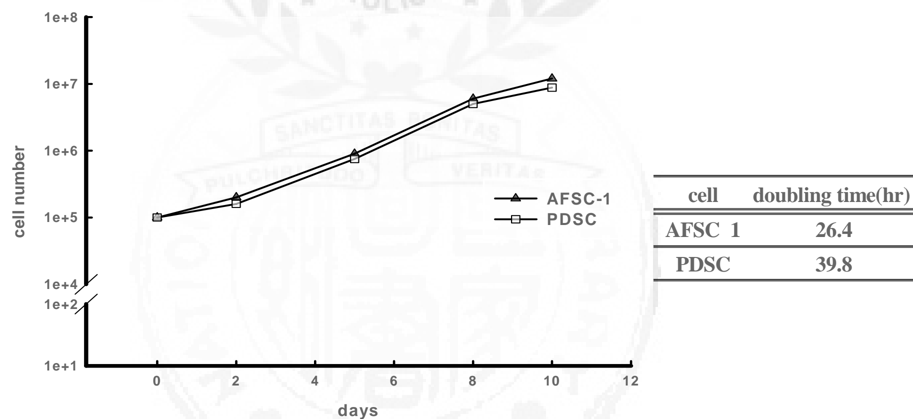
(A-1) AFSC-1



(A-2) PDSC



(B)



cell	doubling time(hr)
AFSC 1	26.4
PDSC	39.8

Figure 1. Morphology and growth kinetic of amniotic fluid and placenta-derived stromal cells. Appearance of fibroblast-look and epitheloid-look cells at passage 12 of amniotic fluid derived stromal cells (AFSC) and placenta-derived stromal cells (PDSC) on day 1 and day 4 culture, respectively. Magnification of phase contrast as scale bar = 100 μ m. **(B).** The initiated number of 1 x 10^5 AFSC (▲) or PDSC (□) cells were seeded in 6-well culture plates. Adherent cells in cultures were harvested and the cell numbers were counted every 2-3 day for 10 days in duplicated. The cell doubling time were calculated and indicated as table.

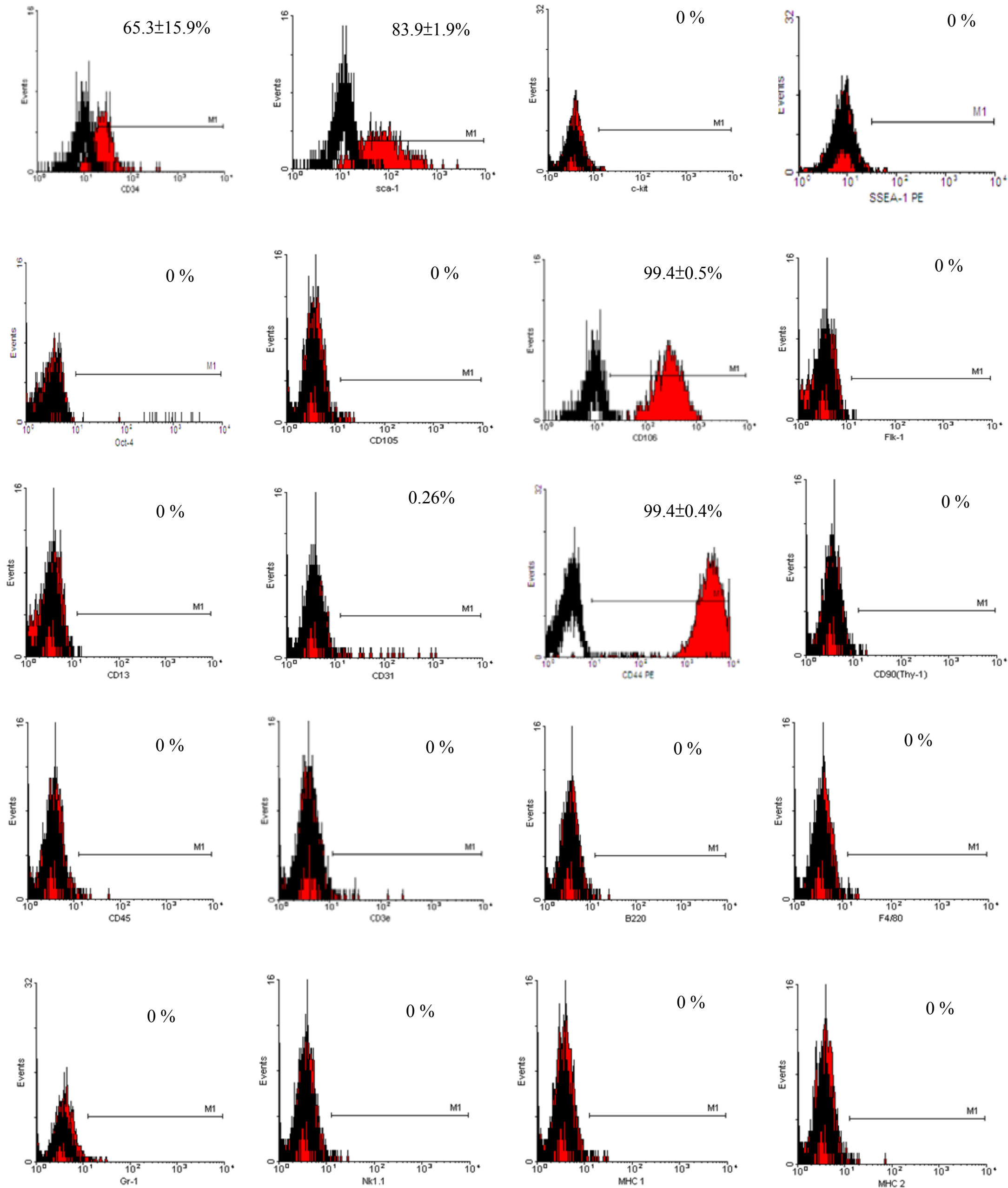


Figure 2. Phenotypic characteristics of AFSC cells. Amniotic fluid-derived stromal cells were collected and stained with FITC- or PE-conjugated anti-mouse moAbs including stem cell, hematopoietic, endothelial, lineage panel or adhesion receptor related markers. The expressive level of specific molecules was examined by flow cytometry. Mean of results is shown as percentage of positive cells (± SD, n = 3). Histogram results are shown in the picture, black line display as isotype control and red solid area as positive staining cells.

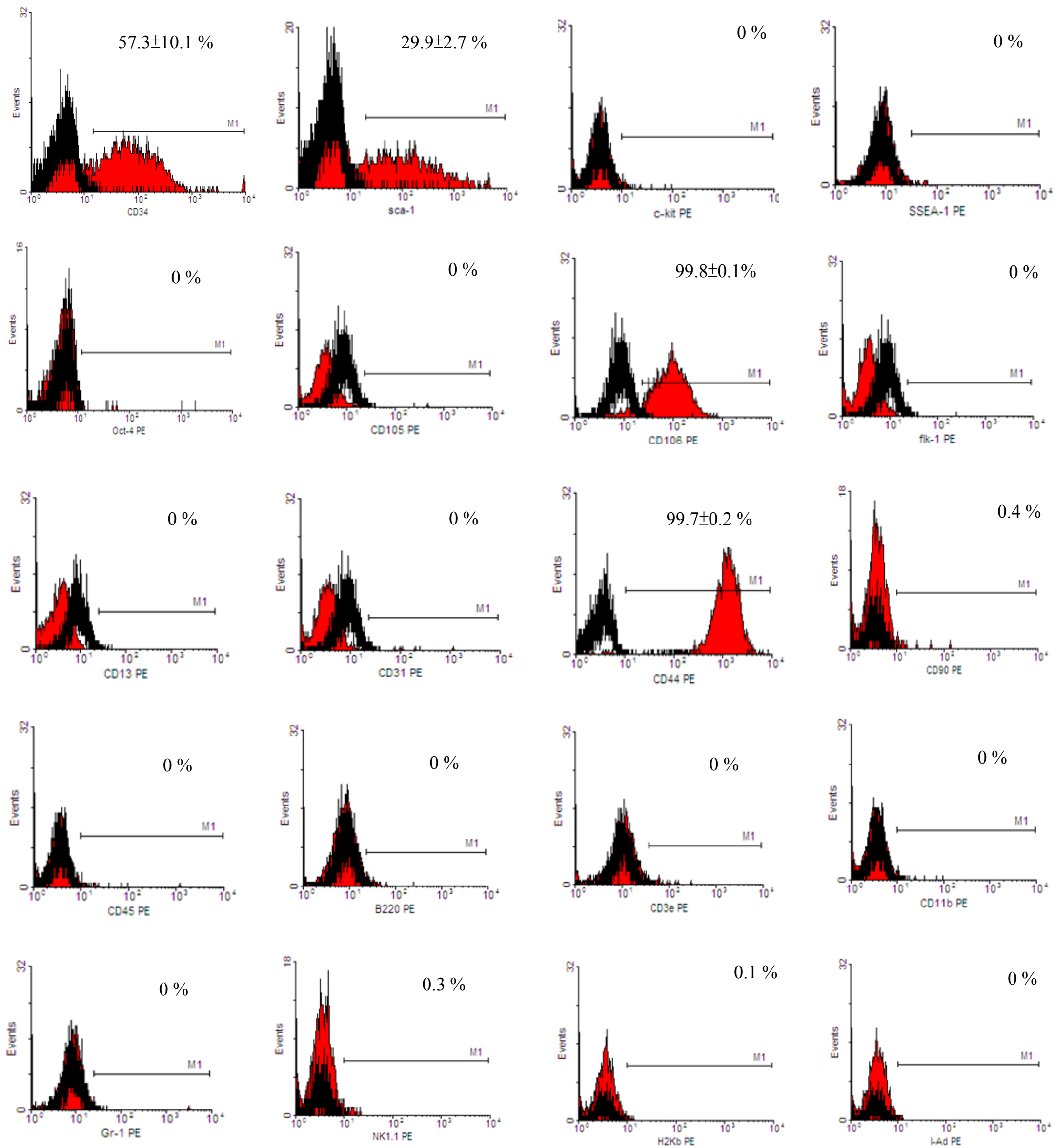
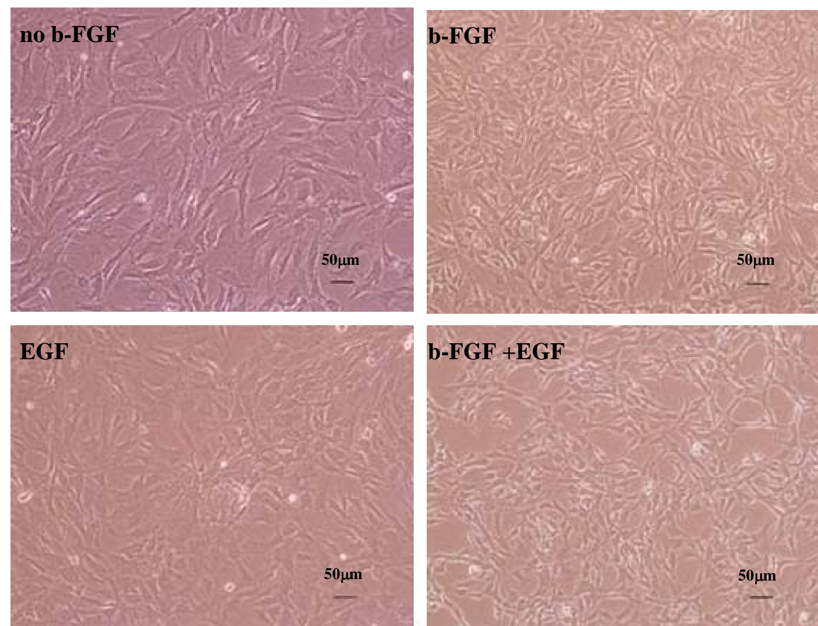


Figure 3. Phenotypic characteristics of PDSC cells. Placenta-derived progenitor cells were collected and stained with FITC- or PE-conjugated anti-mouse moAbs including stem cell, hematopoietic, endothelial, lineage panel or adhesion receptor related markers. The expressive level of specific molecules was examined by flow cytometry. Mean of results is shown as percentage of positive cells (± SD, n = 3). Histogram results are shown in the picture, black line display as isotype control and red solid area as positive staining cells.

(A)



(B)

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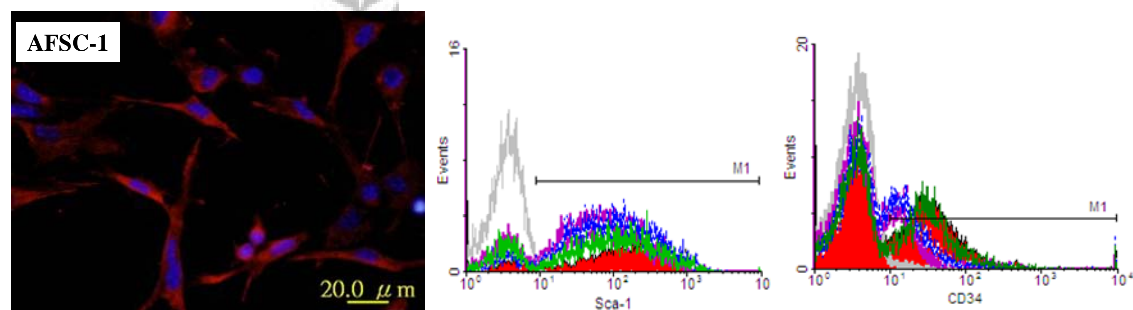
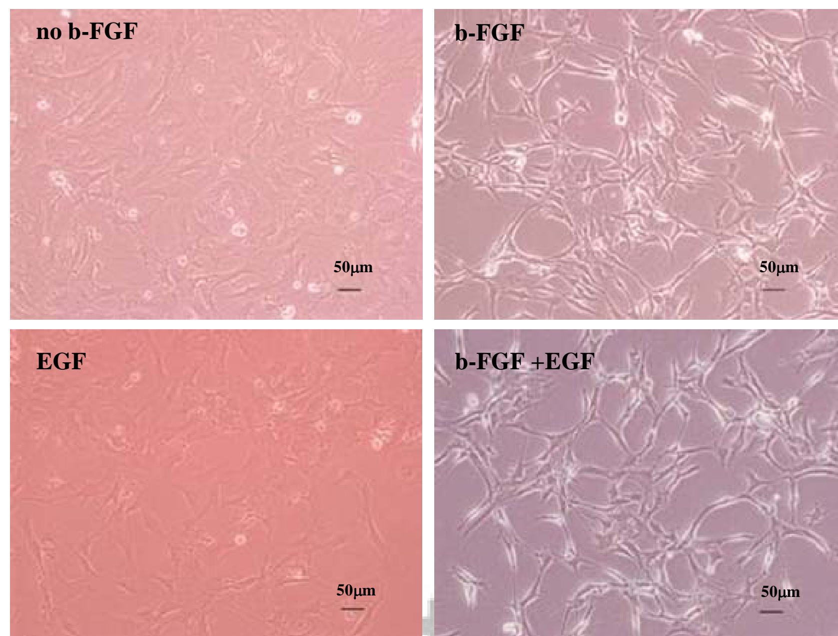


Figure 4. Morphologic and phenotypic characteristics of AFSC cells in cultures with or without b-FGF or/and EGF. (A) Phase-contrast microscopic images of cultured AFSC cells with or without or included both b-FGF (10ng/ml) and EGF (10 ng/ml) for 2 days. Magnification of phase contrast as scale bar as 50 µm. (B) Flow Cytometry of the status of AFSC cells were collected and stained with PE-conjugated anti-mouse moAbs Abs of sca-1 or CD34 (red). Nuclei were counterstained with DAPI (blue). The expressive level of specific molecules was examined by flow cytometry. Histogram results are shown in the picture, gray line display as isotype control, purple line display as no growth factor, blue line display as EGF condition, green line display as b-FGF+EGF conditon and red solid area as b-FGF condition. As well as, an immunofluorscent staining for sca-1 was visualized under a fluorscence microscope. Magnification scale bar = 20 µm.

(A)



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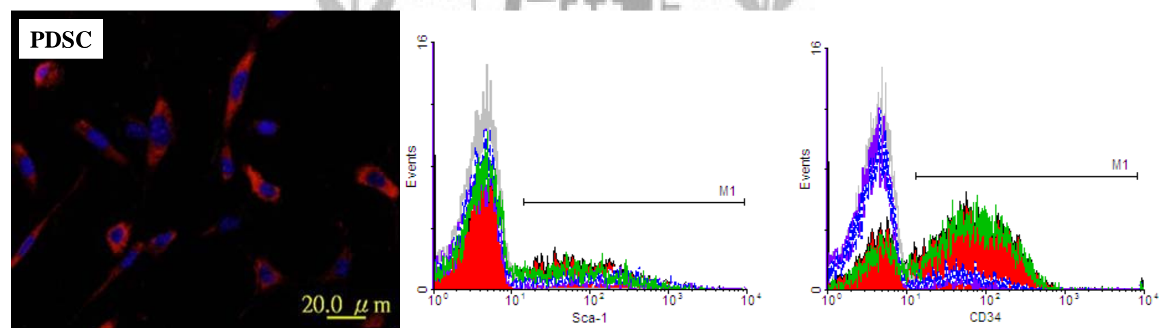


Figure 5. Morphologic and phenotypic characteristics of PDSC cells in cultures with or without b-FGF or/and EGF. (A) Phase-contrast microscopic images of cultured PDSC cells with or without or included both b-FGF (10 ng/ml) and EGF (10 ng/ml) for 2 days. Magnification of phase contrast as scale bar = 50 μm. (B) Flow Cytometry of the status of PDSC cells were collected and stained with PE-conjugated anti-mouse moAbs Abs of sca-1 or CD34 (red). Nuclei were counterstained with DAPI (blue). The expressive level of specific molecules was examined by flow cytometry. Histogram results are shown in the picture, gray line display as isotype control, purple line display as no growth factor, blue line display as EGF condition, green line display as b-FGF+EGF conditon and red solid area as b-FGF condition.As well as, an immunofluorscent staining for sca-1 was visualized under a fluorscence microscope. Magnification scale bar = 200 μm.

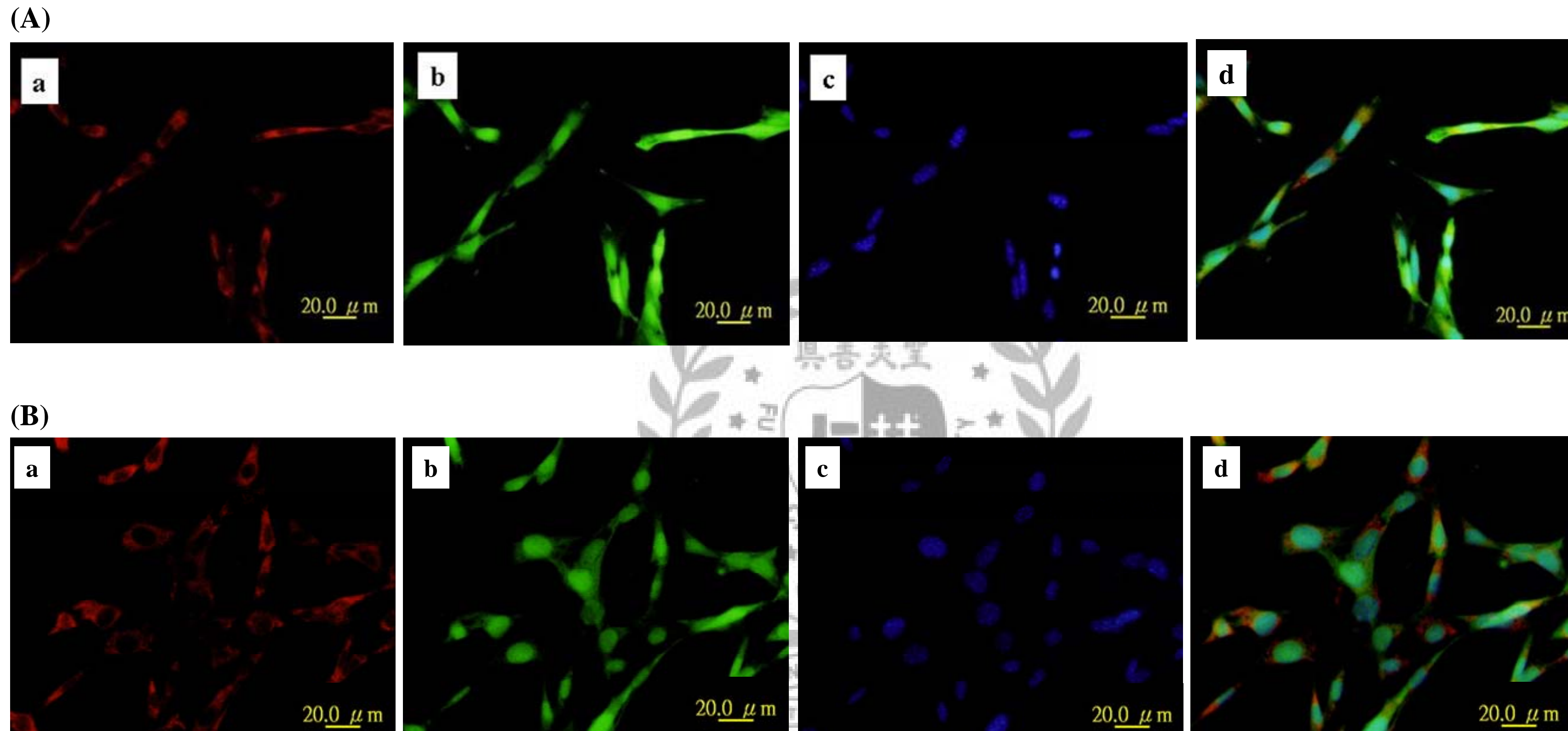
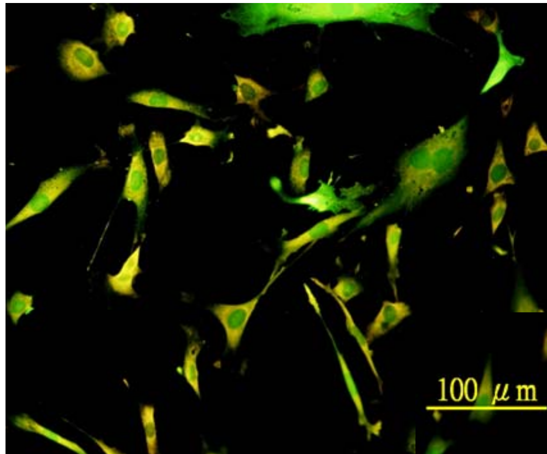


Figure 6. Immunocytochemical detection of CXCR-4 molecules in cultured AFSC-1 and PDSC cells. The cells were seeded on coverslips in culture medium with b-FGF presence for 24hr, then, GFP-origin AFSC-1 (A) or PDSC (B) cells were fixed with 4% paraformaldehyde solution (b: green) and stained with PE-conjugated anti-mouse moAbs Abs of CXCR 4 (a: red). Nuclei were counterstained with DAPI (c: blue). Single color(a, b, c) and merge results(d) of stain were visualized under a fluorscence microscope and image were analysized by by DPController & DPManager.Magnification scale bar = 20μm.

(A) AFSC-1 **(B) PDSC**



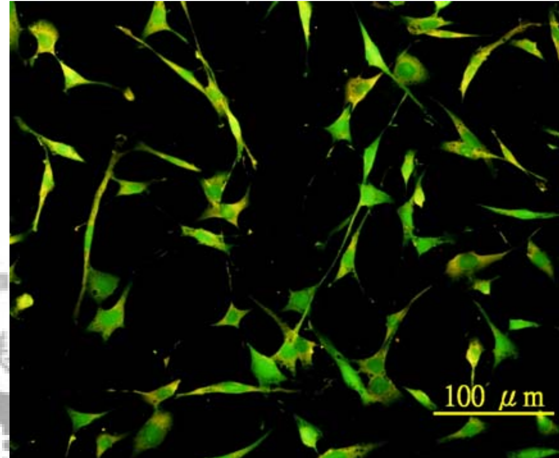


Figure 7. Immunocytochemical detection of cytokeratin in cutured AFSC and PDSC cells. The cells were seeded on coverslips in culture medium with b-FGF presence for 24hr, then, GFP-origin AFSC (A) or PDSC (B) cells were fixed with 4% paraformaldehyde solution and stained with PE-conjugated anti-mouse moAbs Abs of pan-cytokeratin. The merge results of Stain were visualized under a fluorscence microscope and image were analysized by DPController & DPManager. Magnification scale bar = 100μm.

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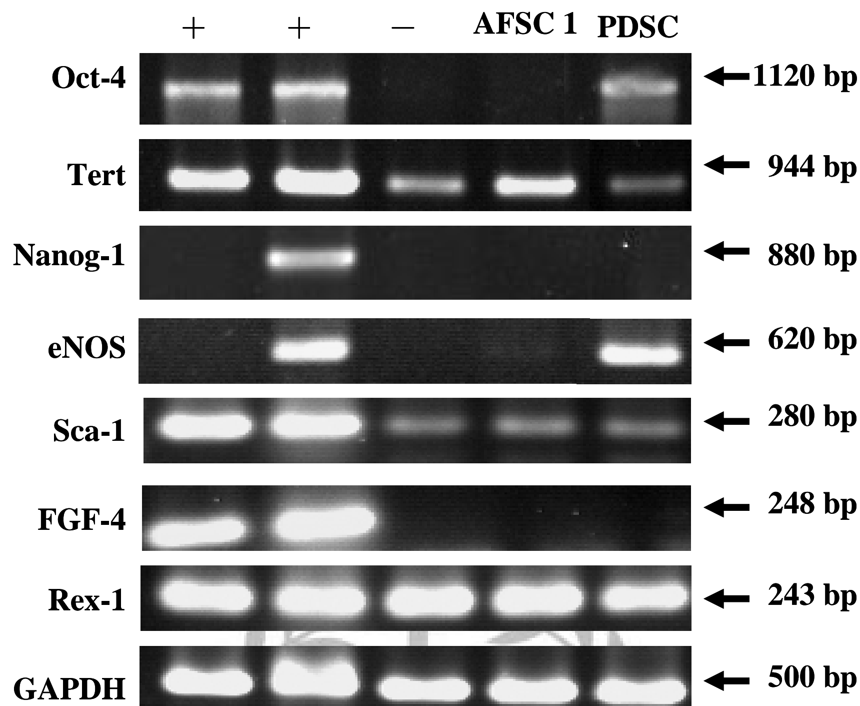
(A)



(B)

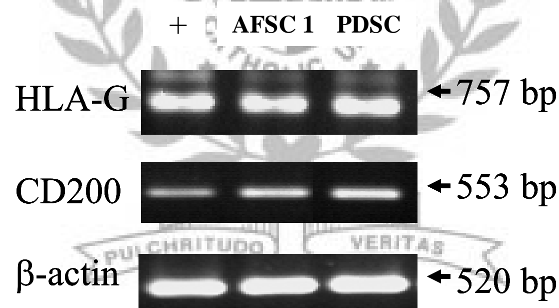
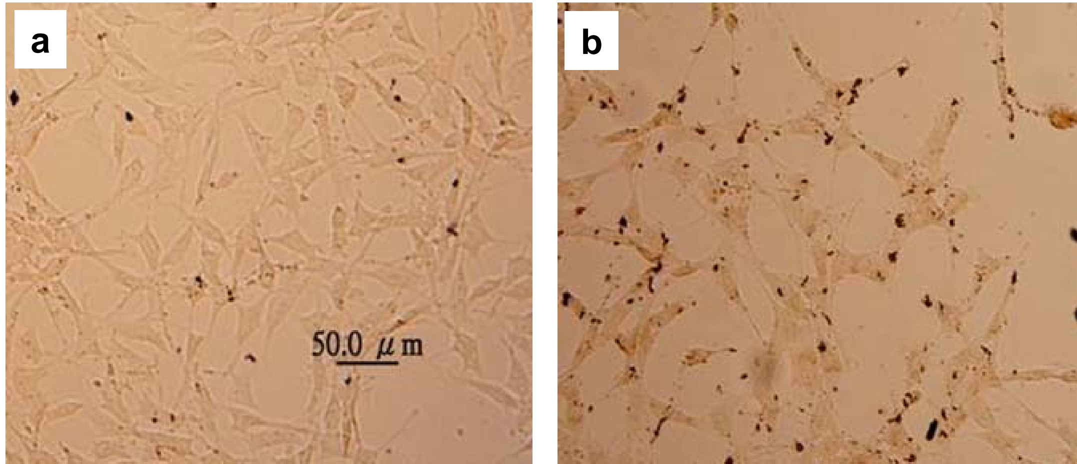


Figure 8. The mRNA expression of stem cell related and immuno-tolerance related genes in AFSC and PDSC. Total RNA from cell at passage 12 were extracted by RNAZolB RNA extraction kit, and cDNAs were obtained by reverse transcription and PCR was performed after. Stem cell related gene primers and tolerance related gene primers were used. Results of stem cell related genes are showed in gel images **(A).** ES-J1 and ES-R1 were used as positive control. MEF, a fetal fibroblast primary cell line, was used as negative control. Results of tolerance related genes are showed in gel images (B). The fetal liver primary cells were used as positive control.

(A) AFSC-1



(B) PDSC

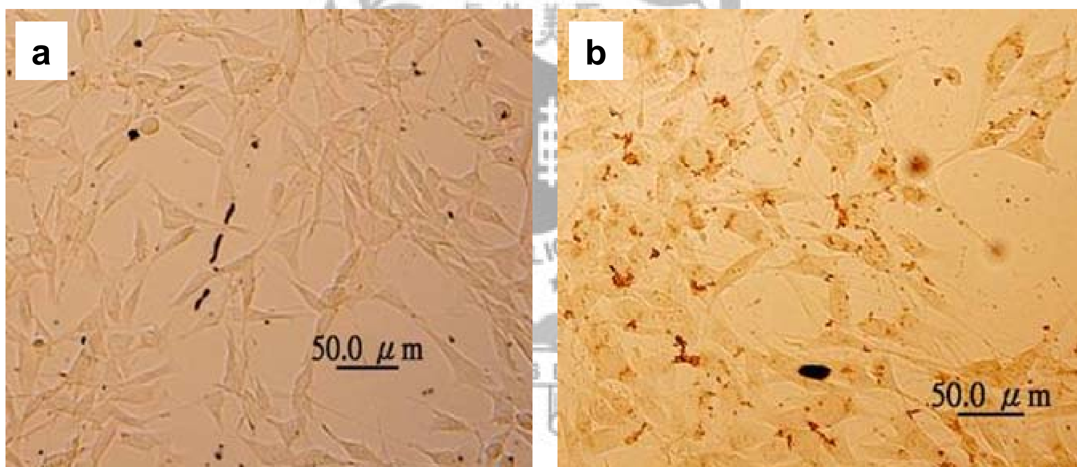


Figure 9. Phosphatase activities of AFSC and PDSC cells. Both cells at passage 12 were seeded on coverslips in culture with b-FGF, then cells were fixed by 3.7% formaldehyde, respectively. Specific enzyme substrate were used to detect alkalin or acid phosphatase activity of cells. The results of staining on alkalin phosphatase (a) and acid phosphatase (b) were visualized under a light microscope. Magnification scale bar = 200 μm.

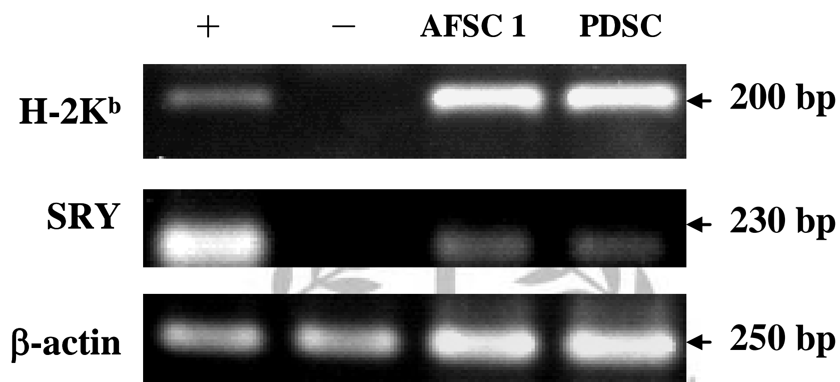


Figure 10. AFSC and PDSC cells are fetal origin. Total DNAs from cell at passage 12 were extracted by DNA extraction kit. H-2K^b and Y antigen–specific gene, SRY, primers were used. The male C57BL/6 leukocytes were used as positive control. The female BALB/c leukocytes were used as Negative control. Results are shown in gel image.

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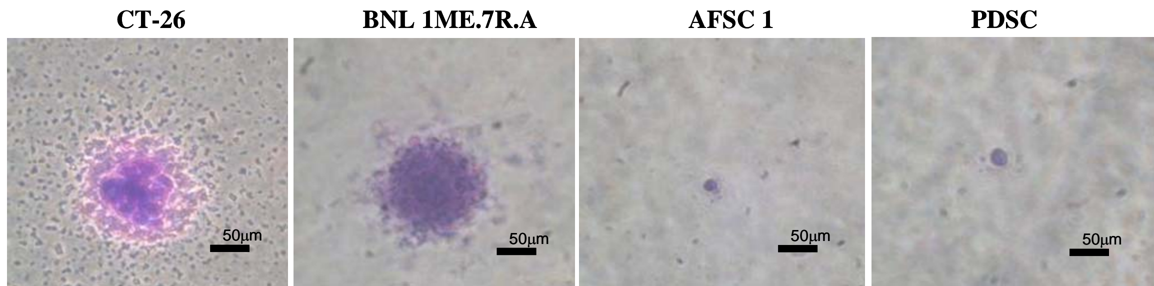
(A)



(B)

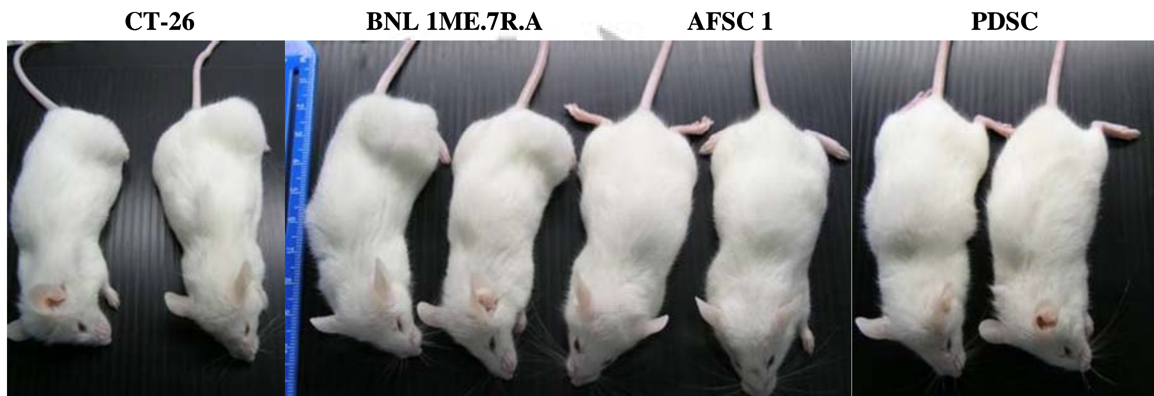
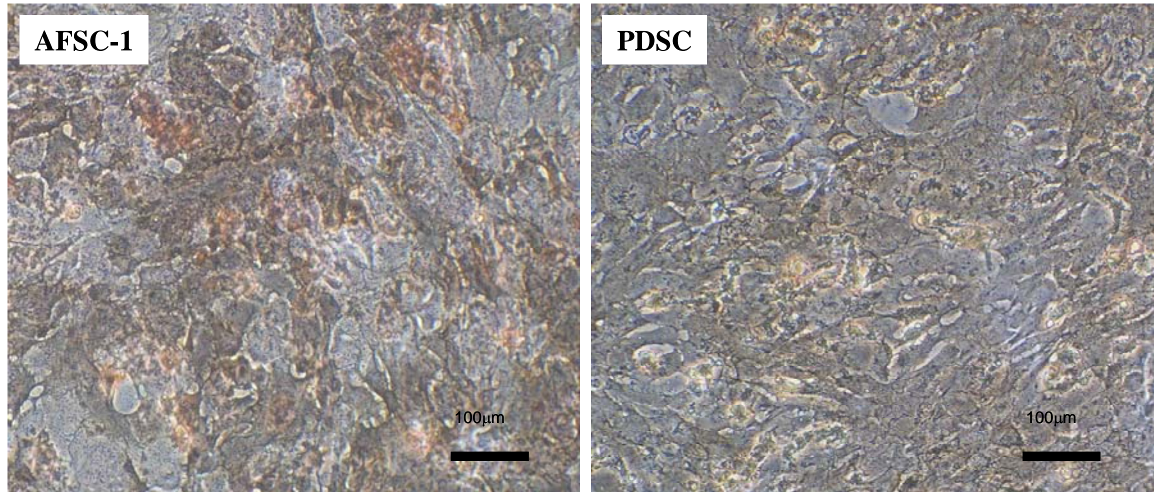


Figure 11. Tumorigenicity of AFSC and PDSC cells. The tumorigenicity of cells were evaluated by in vitro colony forming assay (A) and transplanted mice models (B). In colony assay, control tumor cell lines (CT-26 and BNL 1ME.7R.A) and tested cells (AFSC-1 and PDSC) were cultured in soft agar to incubate assay at 37℃in humidified incubator for 2-4 weeks. Plates were stained with 0.5ml of 0.005% Crystal Violet (in methanol), then plates were destained with ddH_2O, and colonies from each plate were counted by used a dissecting microscope. Magnification scale bar = 50 µm. In transplanted mice model, cells were examined for tumorigenicity by injection 1 x 10^4 of tumor or 1 x 10^7 of tested cells per site into the rear leg muscle (n = 2) of SCID mice in 100µl of PBS. Animals after transplanted were observed for up to 3 months with no evidence of tumor formation, whereas the tumor cell line formed tumors in 4-7 weeks.

(A) Osteogenesis



(B) Adipogenesis

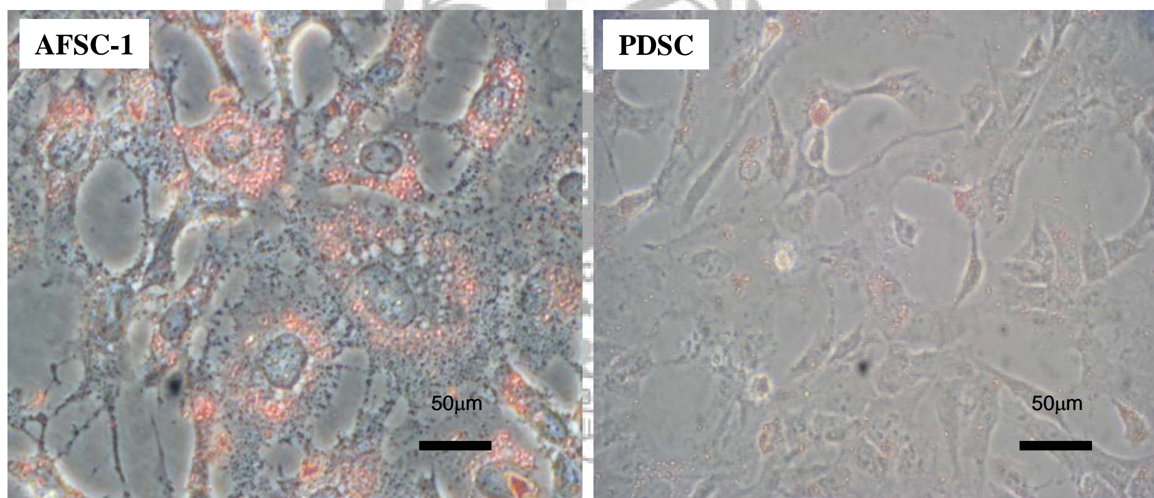


Figure 12. Osteogenic and adipogenic differentiations of AFSC and PDSC cells. (A). Osteogenic differentiation evidenced by the formation of mineralized matrix as shown by von Kossa staining. Magnification scale bar = 100 μm. **(B):** Adipogeneic differentiation was indicated by the presence of neutral lipid vacuoles that stained with Oil-red –O. Magnification scale bar = 50 μm. (C)

(A)

(B)

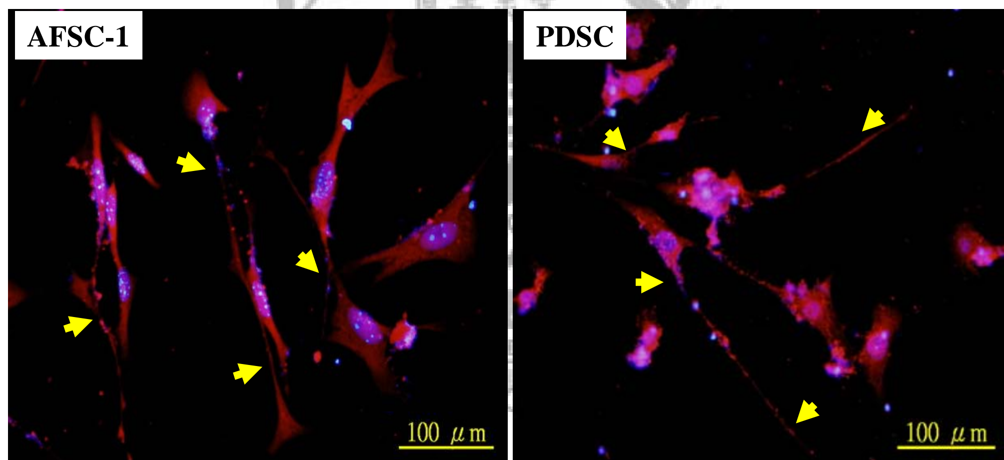


Figure 13. Neurogenic differentiation of AFSC and PDSC cells. (A) Both cells were showed neuron-like morphology after neurogenic induction. Phase contrast magnification scale bar = 50 μm. (B).Immunofluorsecent staining for nuron protein. The cells were fixed by 3% paraformaldehyde, then incubated with primary moAbs against the PGP9.5 and with rhodamine-conjucated secondary Abs. Stained results were visualized under a fluorscence microscope. Magnification scale bar = 100 μm.

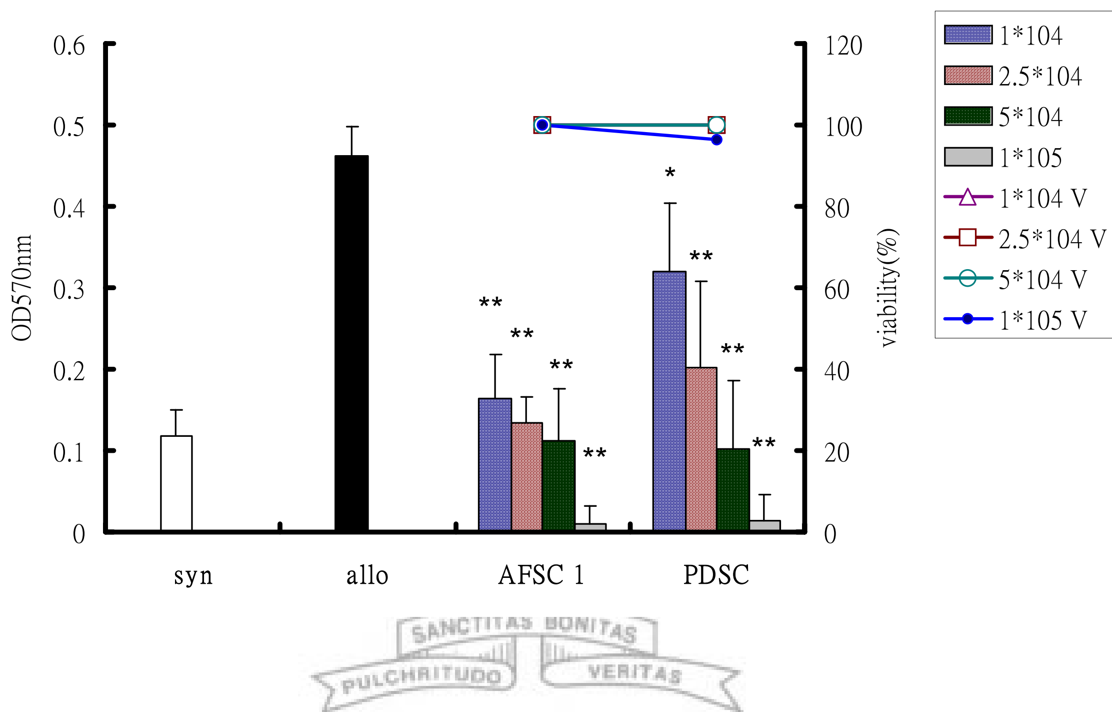
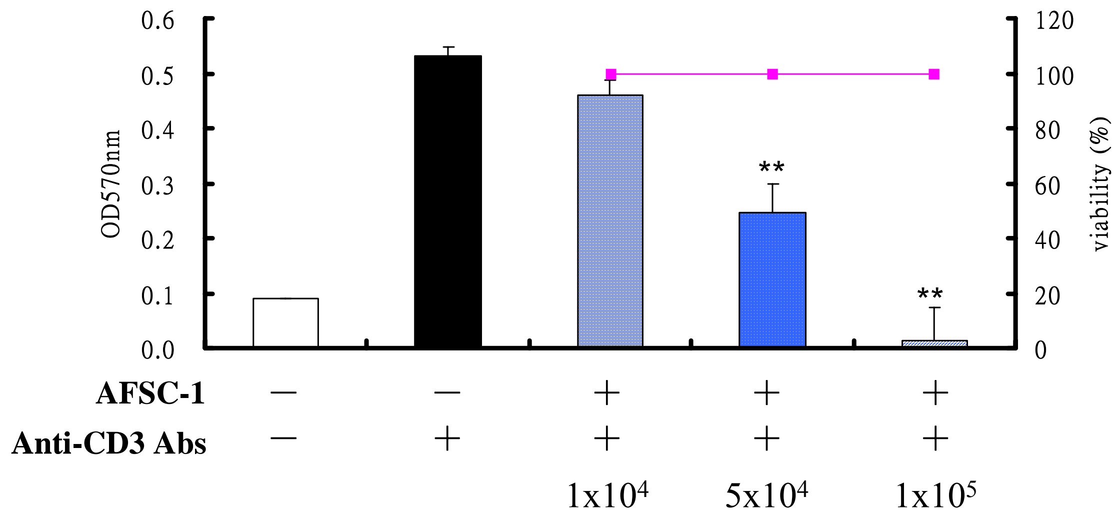


Figure 14. AFSC and PDSC cells inhibit allogeneic lymphocyte responses in a dose dependent manner. The immunogenicity of AFSCs and PDSCs were tested by using either health donor T cells (4 x 10^5), AFSCs or PDSCs (1, 2.5, 5, 10 x 10^4) as stimulators in one-way allogenic MLRs. Stimulators were treated with mitomycin C (0.5 mg/ml) and then co-cultured with responder T cells (4 x 10^5) for 3 days. T cell proliferation was measured by MTT assay. The groups of syngenic and allogenic were acted as negative and positive controls. The data are expressed as mean of OD_{570} ± SE of triplicates , n = 3; * indicated as $p \leqq .05$ and ** indicated as $p \leqq .01$.

(A) anti-CD3 Abs stimulation



(B) Con A stimulation

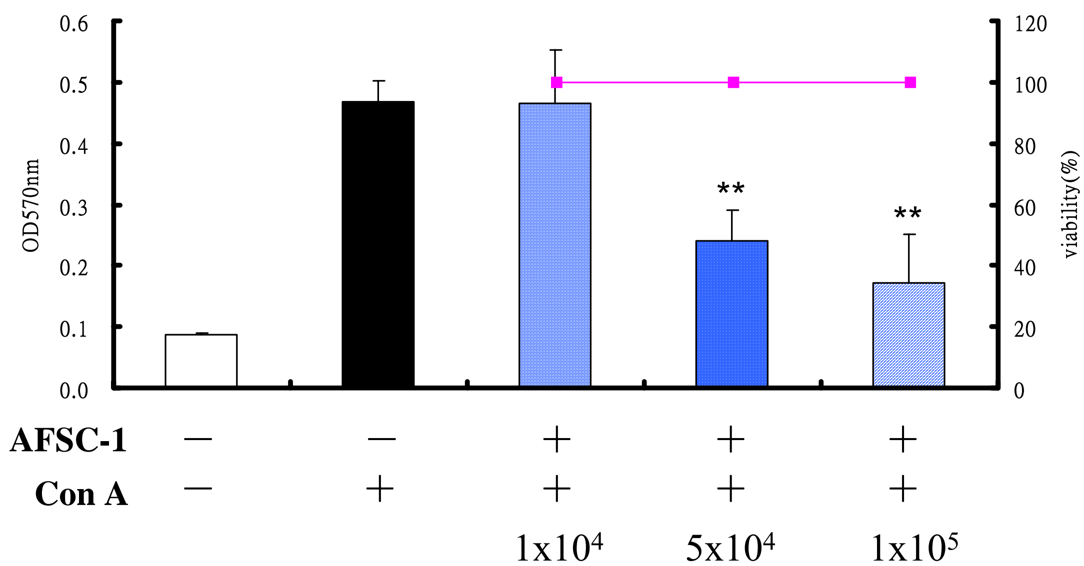
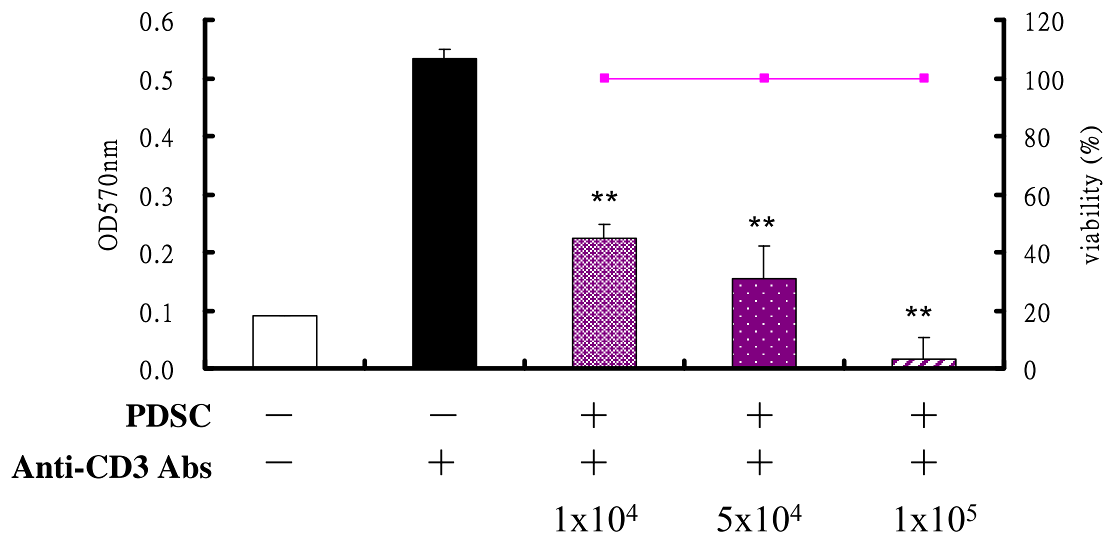


Figure 15. Proliferation of T cells responses stimulated by Con A and anti-CD3 antibodies were suppressed in presence of AFSCs culture. Three-day co-cultures were performed with (black bar) or without (blue bar) AFSCs. AFSCs stimulators (1, 5, or 10 x 10^4) were treated with mitomycin C (0.5 mg/ml) before co-cultured with responder T cells (2 x 10^5) in presence of CD3 antibodies (2 μg/ml, panel A) or Con A(2.5 μg/ml, panel B). T cell proliferation was measured by MTT assay. The groups of without AFSC and CD3 Abs were acted as negative controls. The groups of with CD3 antibody only were acted as positive controls. The data are expressed as mean of OD_{570} ± SE of triplicates , n = 3; * indicated as $p \leqq .05$ and ** indicated as $p \leqq .01$.

(A) anti-CD3 Abs stimulation



(B) Con A stimulation

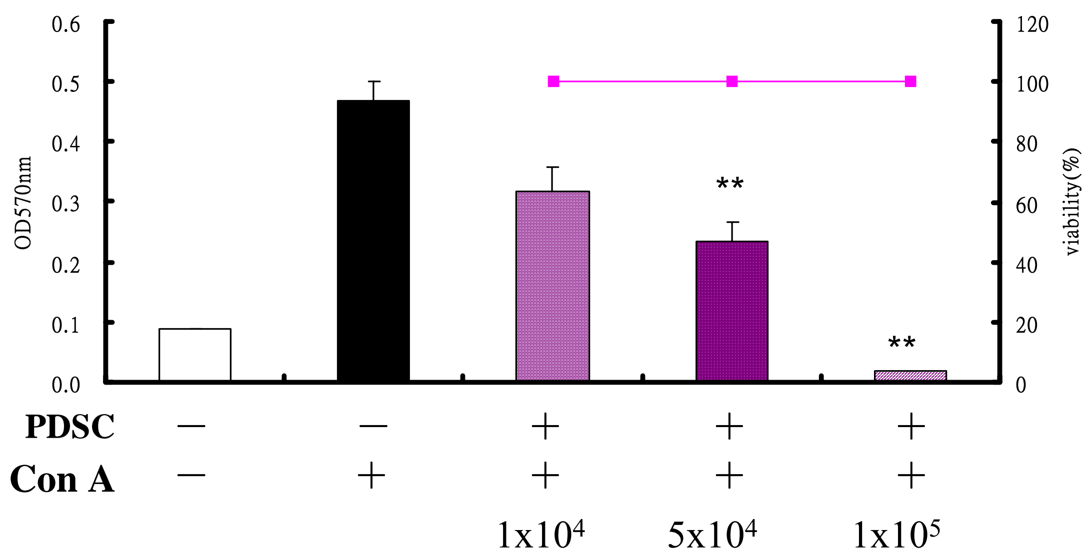


Figure 16. Proliferation of T cells responses stimulated by Con A and anti-CD3 antibodies were suppressed in presence of PDSCs culture. Three-day co-cultures were performed with (black bar) or without (purple bar) PDSCs. PDSCs stimulators (1, 5, or 10 x 10^4) were treated with mitomycin C (0.5 mg/ml) before co-cultured with responder T cells (2 x 10^5) in presence of CD3 antibodies (2 μg/ml, panel A) or Con A (2.5 μg/ml, panel B). T cell proliferation was measured by MTT assay. The groups of without PDSC and CD3 Abs were acted as negative controls. The groups of with CD3 antibody only were acted as positive controls. The data are expressed as mean of OD_{570} ± SE of triplicates , n = 3; * indicated as p ≦.05 and ** indicated as *p* ≦.01.