Supplementary Figures

Supplementary Figure 1: PMSC Isolation from 15 weeks preterm placenta. (A) The dissected villous tissue was digested enzymatically and cells were separated using a discontinuous Percoll gradient. Five cell fractions were typically obtained corresponding to five different densities and cells were isolated from layers 3, 4, and 5. **(B)** Phase contrast images of the isolated PMSCs, from all three layers, grown in culture after 4 weeks. **(C)** PMSCs from passage 4 of all three layers were positive for CD73 and CD105 (>98%), and were negative for CD117 (<1%) (measured by flow cytometry). Flow cytometry histograms are representative of all 3 layers from 3 placental tissue as they showed the same results.

Supplementary Figure 2: IGFBP-6 levels in response to IGFBP-6 supplementation in PMSCs under skeletal muscle differentiation conditions. (A) PMSCs cultured under muscle differentiation conditions showed increased IGFBP-6 protein levels, using western blots, in response to different doses of recombinant human IGFBP-6 protein supplementation with 375 ng/mL and 450 ng/mL having the highest band intensity. **(B)** IGFBP-6 secretion into the media was increased with the supplementation of recombinant human IGFBP-6 protein (375 ng/mL) that reduced by time and was lower compared to control at day 3. Data is presented as the mean ± SEM of 3 independent experiments. Two-way ANOVA with Bonferroni's multiple comparison test was performed to determine ***P<0.001.

Supplementary Figure 3: PMSCs cultured under muscle differentiation conditions showed the formation of multi-nucleated fibers and lower cell count compared to control. (A) At 14

days post-differentiation, PMSCs are immunoreactive for MHC (Red-Alexa 568, λ-568 nm) with cell alignment and multi-nucleated fiber formation (5X). Nuclei, were stained with Hoechst dye (blue, λ=340 nm). **(B)** PMSCs grown in muscle differentiation media showed multi-nucleated skeletal muscle fiber formation (40X). Black arrows indicate the multi-nucleated muscle. **(C)** PMSCs under muscle differentiation conditions showed lower cell count per field compared to control. Data is presented as the mean ± SEM of 15 different fields from 3 independent experiments. One-way ANOVA followed by a Student's t-test, **P<0.01.

Supplementary Figure 4: PMSCs cultured under skeletal muscle differentiation conditions showed a decreased frequency of cells with high ALDH-activity. Representative flow cytometry dot plots showing the frequency of PMSC with high ALDH-activity with Aldefluor and an inhibitor of ALDH (DEAB) or with ALDH alone when cultured under control (10% FBS) or muscle differentiation conditions at **(A)** day 1, **(B)** day 3, **(C)** day 7, **(D)** and day 14.

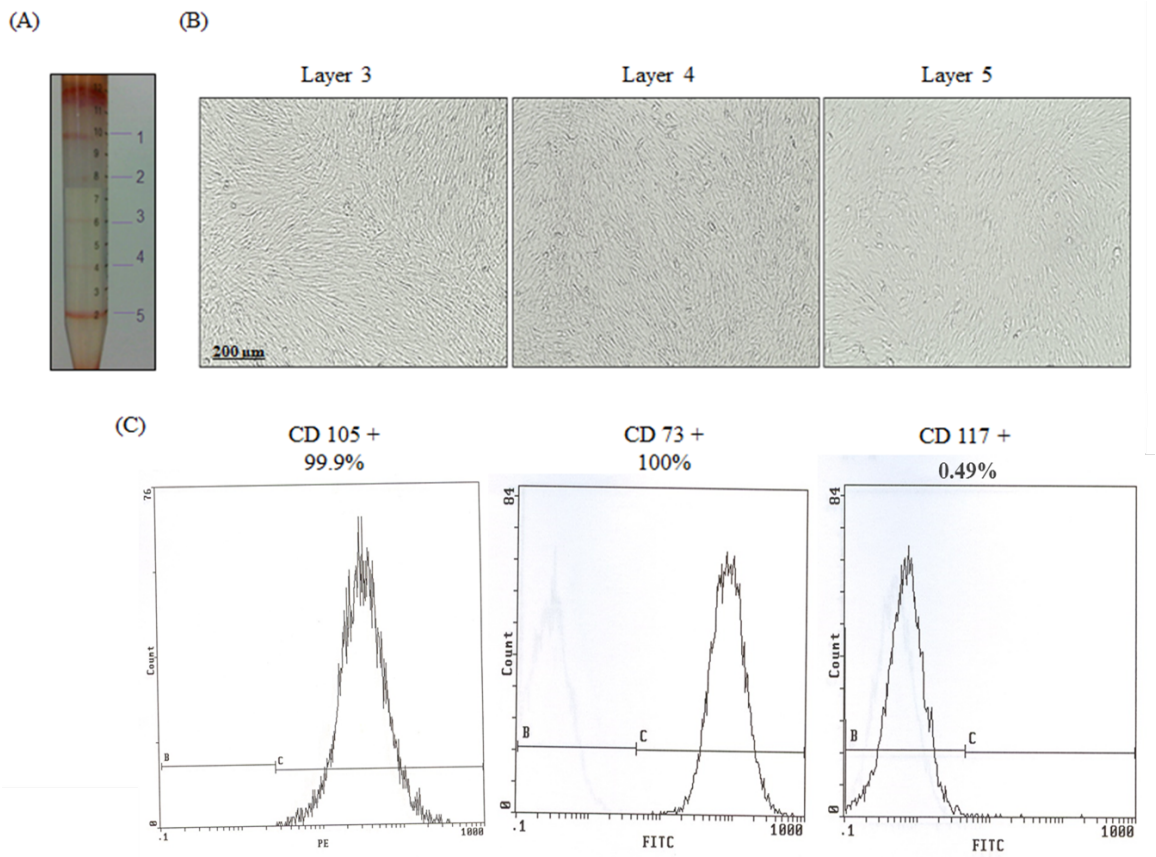
Supplementary Figure 5: IGFBP-6 treatment increased the frequency of PMSCs with high ALDH-activity. Representative flow cytometry dot plots with Aldefluor and an inhibitor (DEAB) or with ALDH alone in PMSCs cultured under muscle differentiation conditions with or without IGFBP-6 addition at **(A)** day 1, **(B)** day 3, **(C)** day 7, **(D)** and day 14.

Supplementary Figure 6: IGFBP-6 siRNA in PMSCs cultured under muscle differentiation conditions decreased the frequency of cells with high ALDH-activity. Representative flow cytometry dot plots with Aldefluor and an inhibitor of ALDH (DEAB) or with ALDH alone of

PMSCs treated with IGFBP-6 siRNA at **(A)** day 1, **(B)** day 3, and **(C)** day 7 under muscle differentiation conditions.

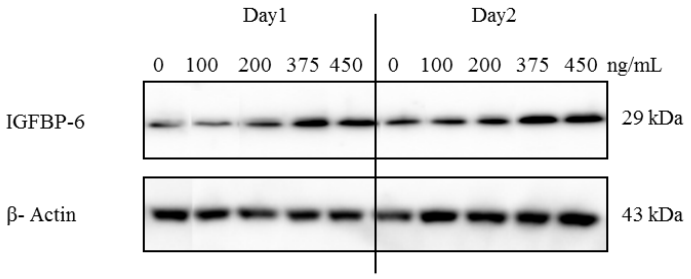
Supplementary Figure 7: IGF-2 secretion in PMSCs treated with IGFBP-6 or IGFBP-6 siRNA under muscle differentiation conditions. (A) IGF-2 levels secreted into the media were significantly decreased at each time point after IGFBP-6 addition compared the control. **(B)** After treatment with siRNA against IGFBP-6 compared to controls (scrambled siRNA), IGF-2 levels increased at the first 48 hours with siRNA treatment applied every 3 days. Data is presented as the mean ± SEM of 3 independent experiments. Two-way ANOVA with Bonferroni's multiple comparison test was performed to determine *P<0.05, **P<0.001.

Supplementary Figure 1.

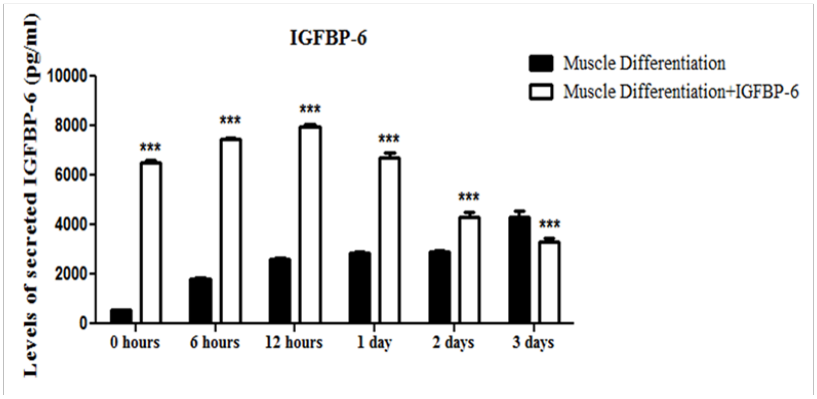


Supplementary Figure 2.

(A)

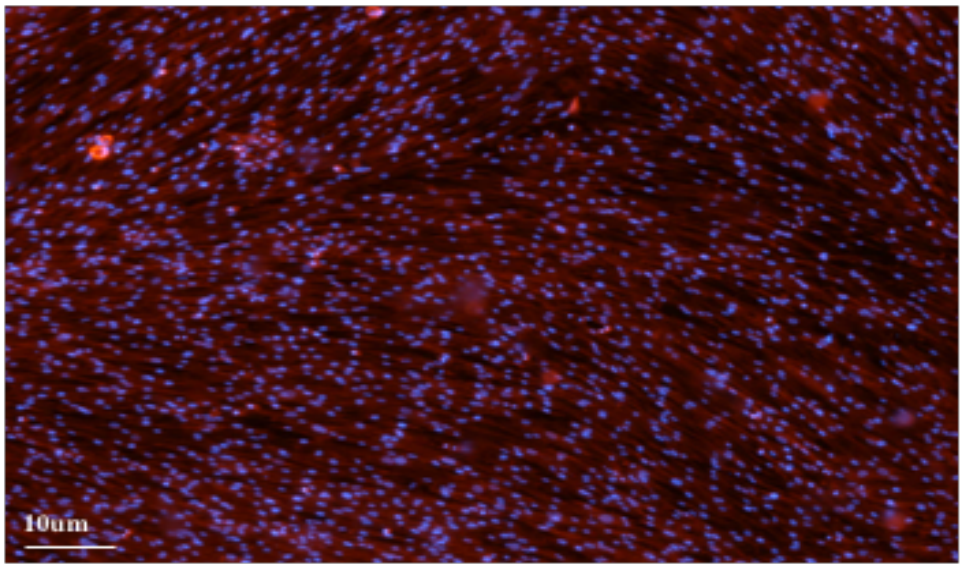


(B)

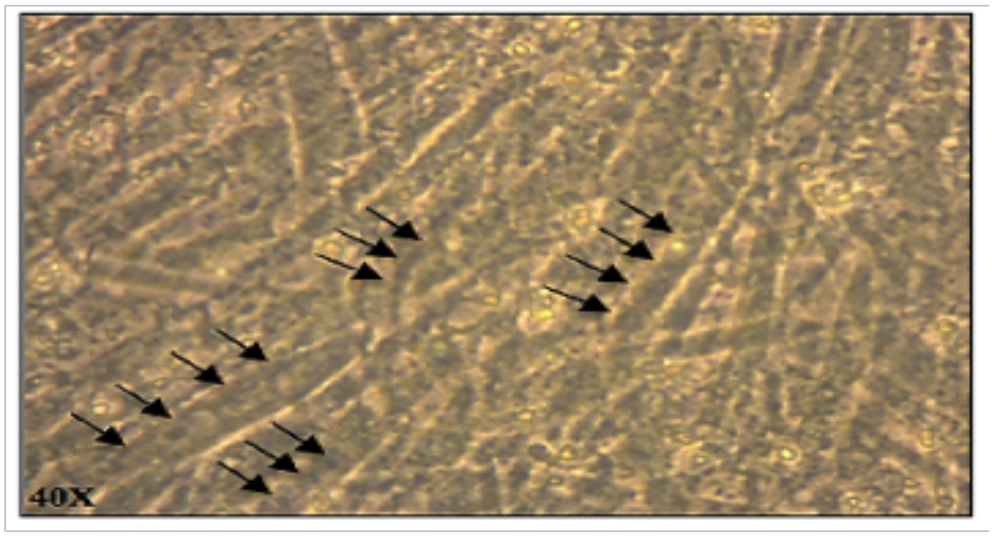


Supplementary Figure 3.

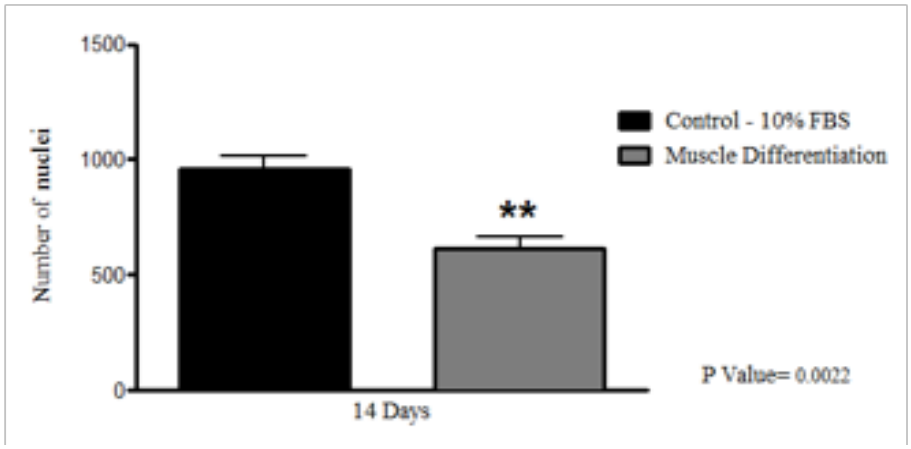
(A)



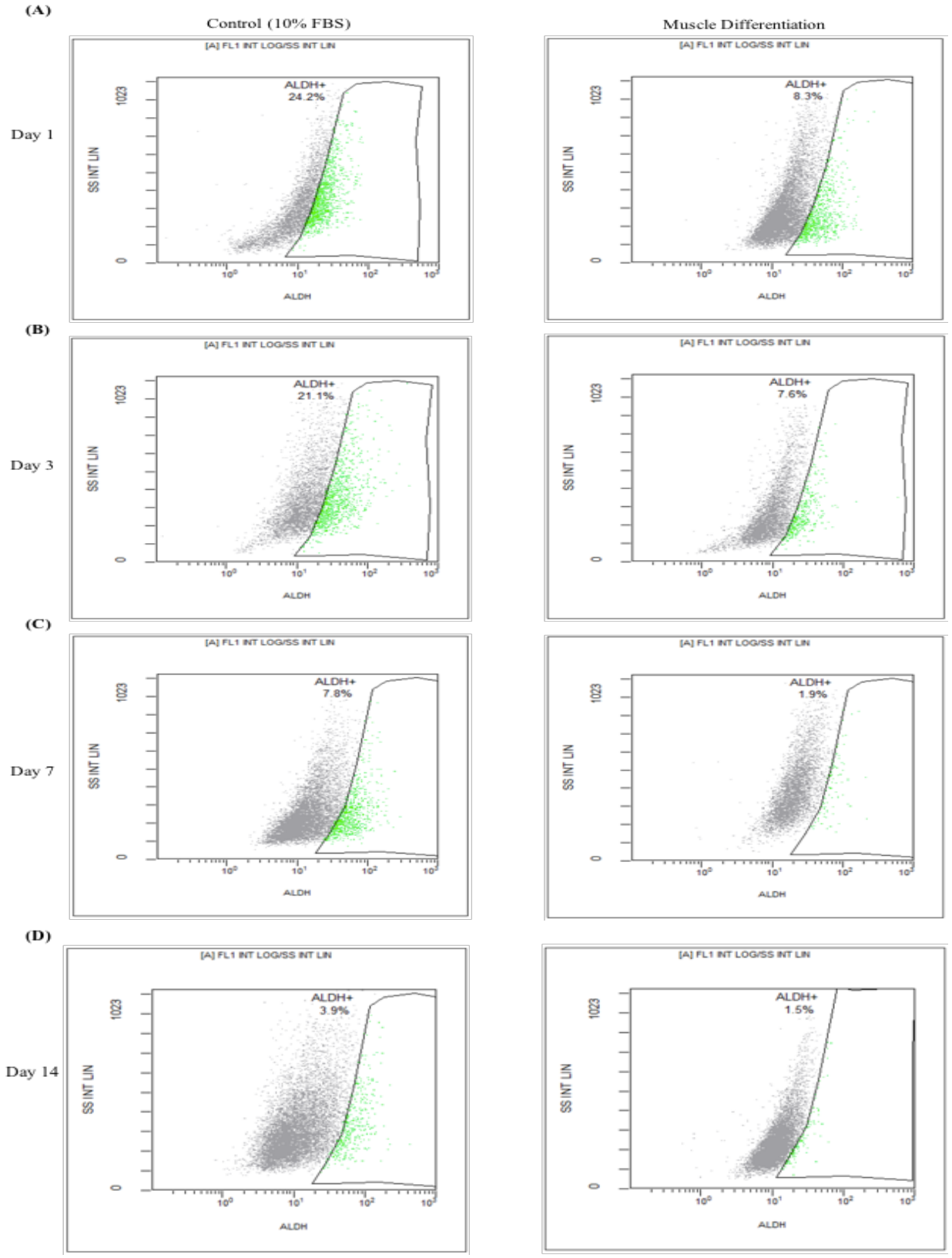
(B)



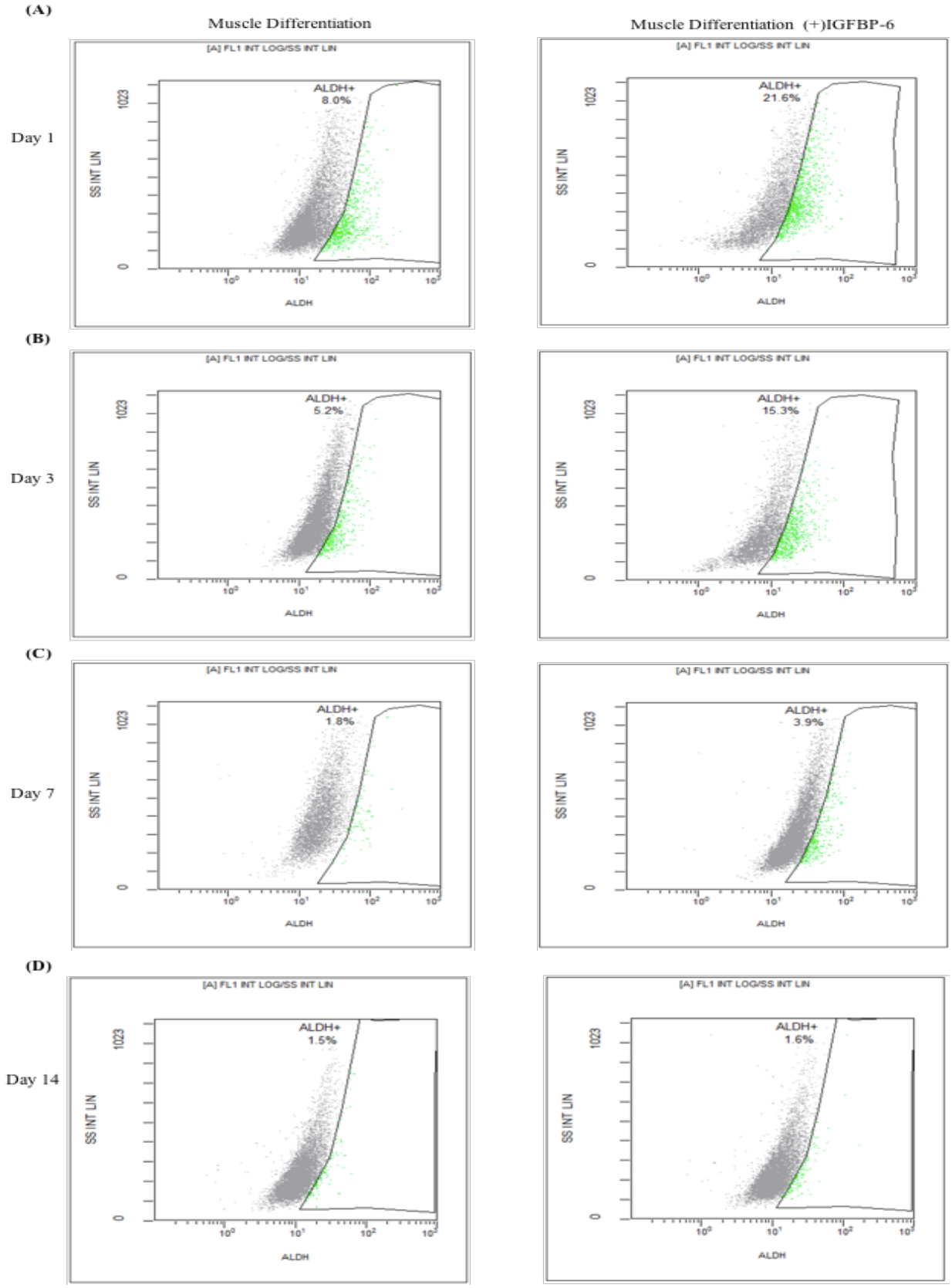
(C)



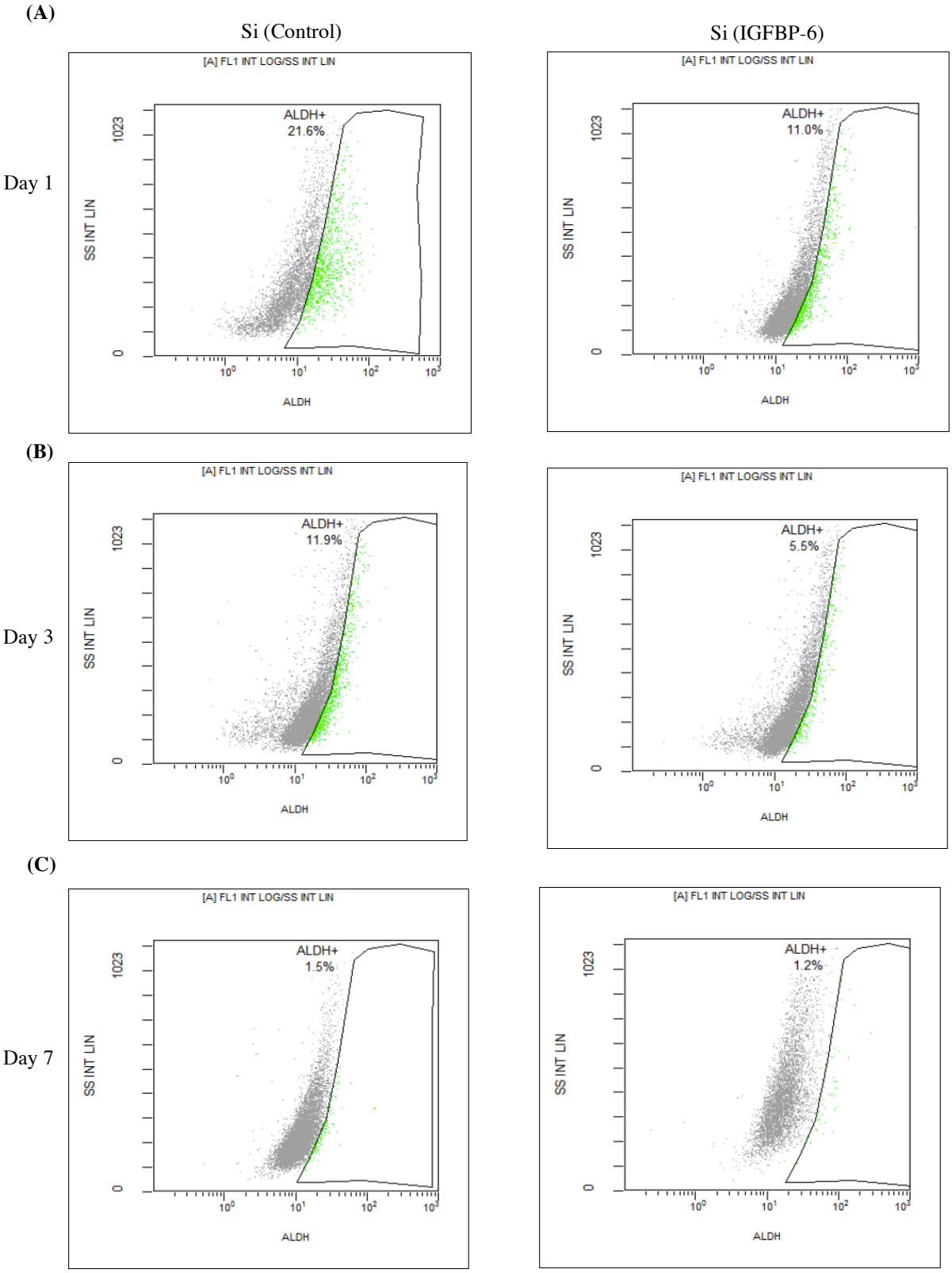
Supplementary Figure 4.



Supplementary Figure 5.

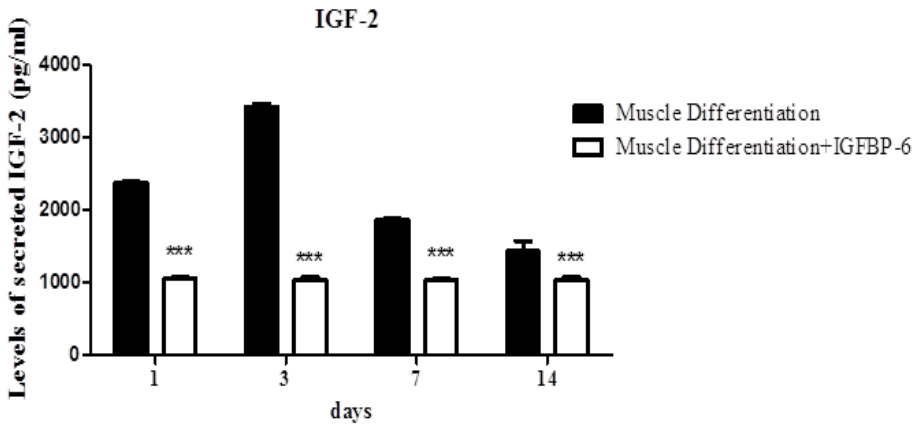


Supplementary Figure 6.



Supplementary Figure 7.

(A)



(B)