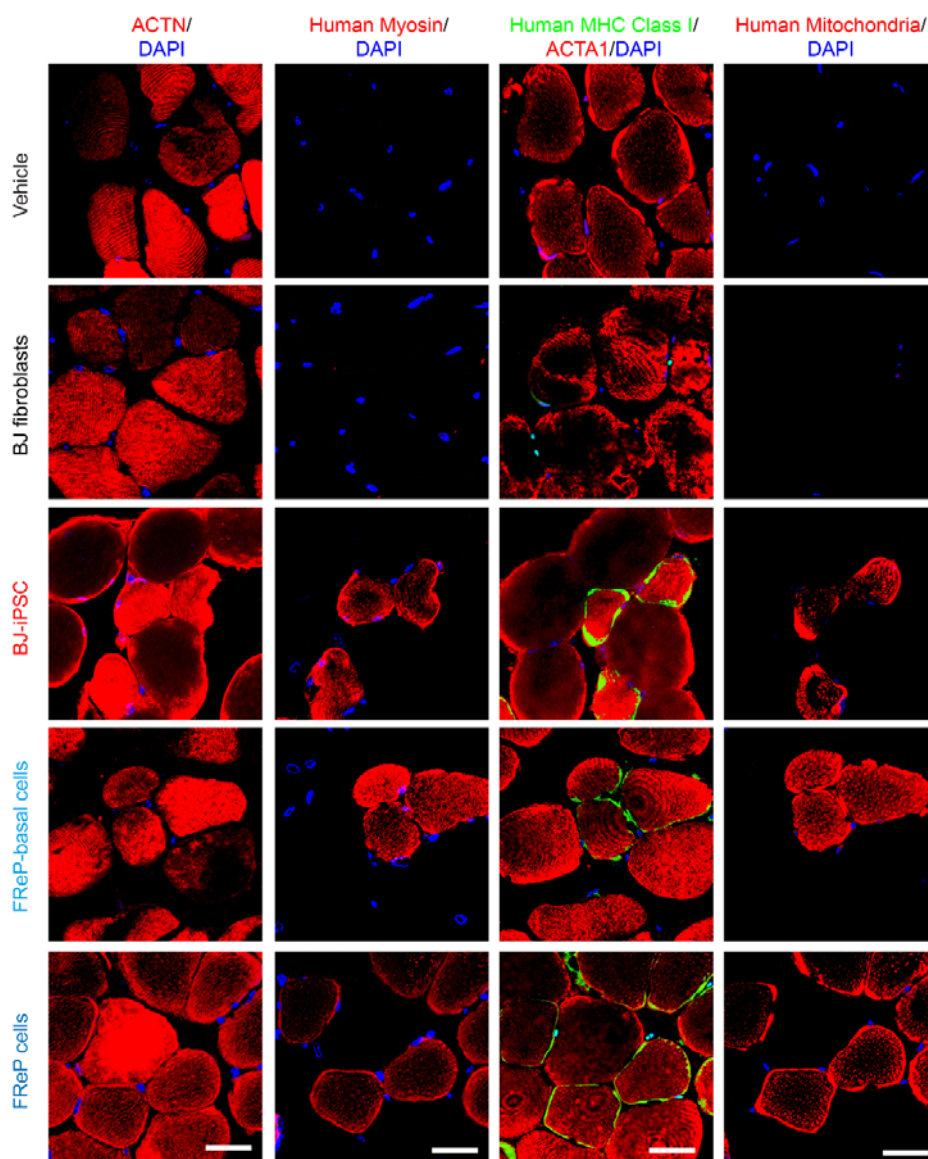


## Supplemental Information

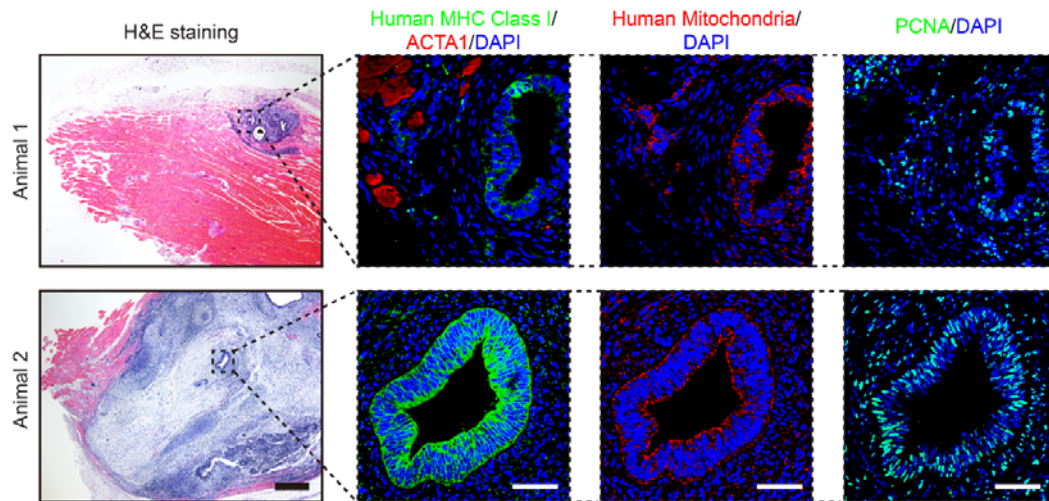
### Supplemental Figures



**Supplemental Figure 1: Persistence, engraftment, and myogenesis of FReP cells in SCID mice TA muscles.**

Confocal microscopy images at transverse section view of SCID mice TA muscles at 6-weeks post-implantation are shown. Cellular myogenesis was documented by ACTN, myosin, and ACTA1 staining, while cell persistence and engraftment were indicated by the staining of

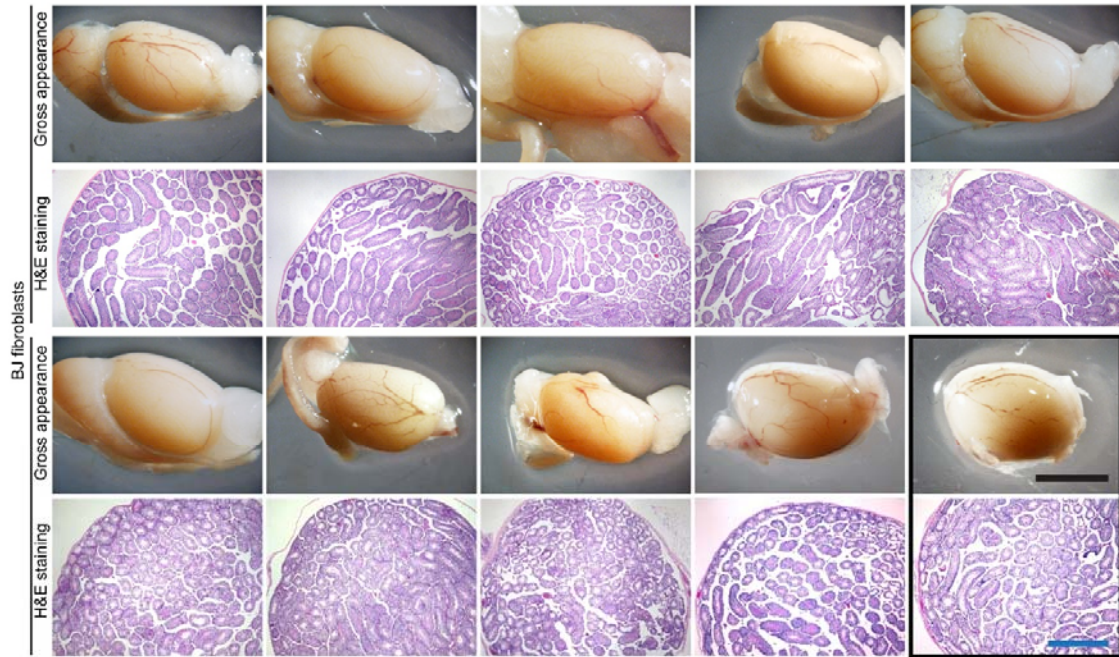
human myosin (using an anti-myosin antibody that recognized human antigen, but not mouse antigen), human MHC Class I, and human mitochondria. Scale bar = 25  $\mu\text{m}$ .



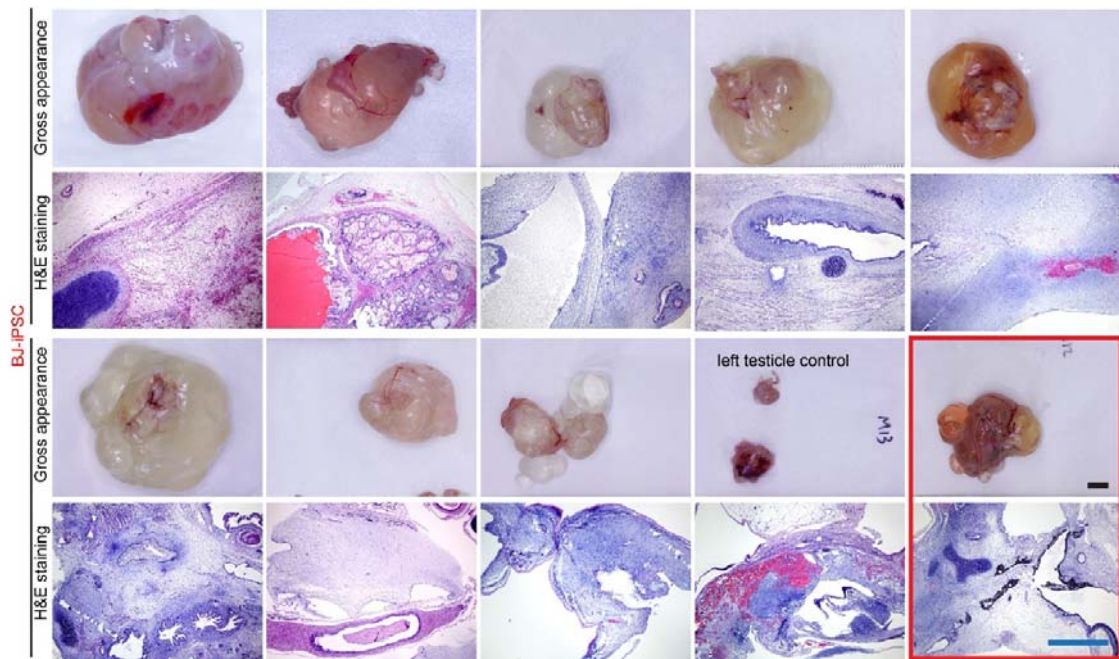
**Supplemental Figure 2: Intramuscular implantation of iPSC bears a high risk of tumorigenesis in vivo.**

6 weeks after implantation of retrovirus-mediated BJ-iPSC in the left TA muscles of SCID mice, 2 of the 8 animals (25%; the dashed outlines highlighted animals in **Figure 2**) experienced tumor formation instead of muscle generation. Proliferating cell nuclear antigen (PCNA) was used to identify the proliferating cells. Scale bar = 500 μm (black) or 50 μm (white).

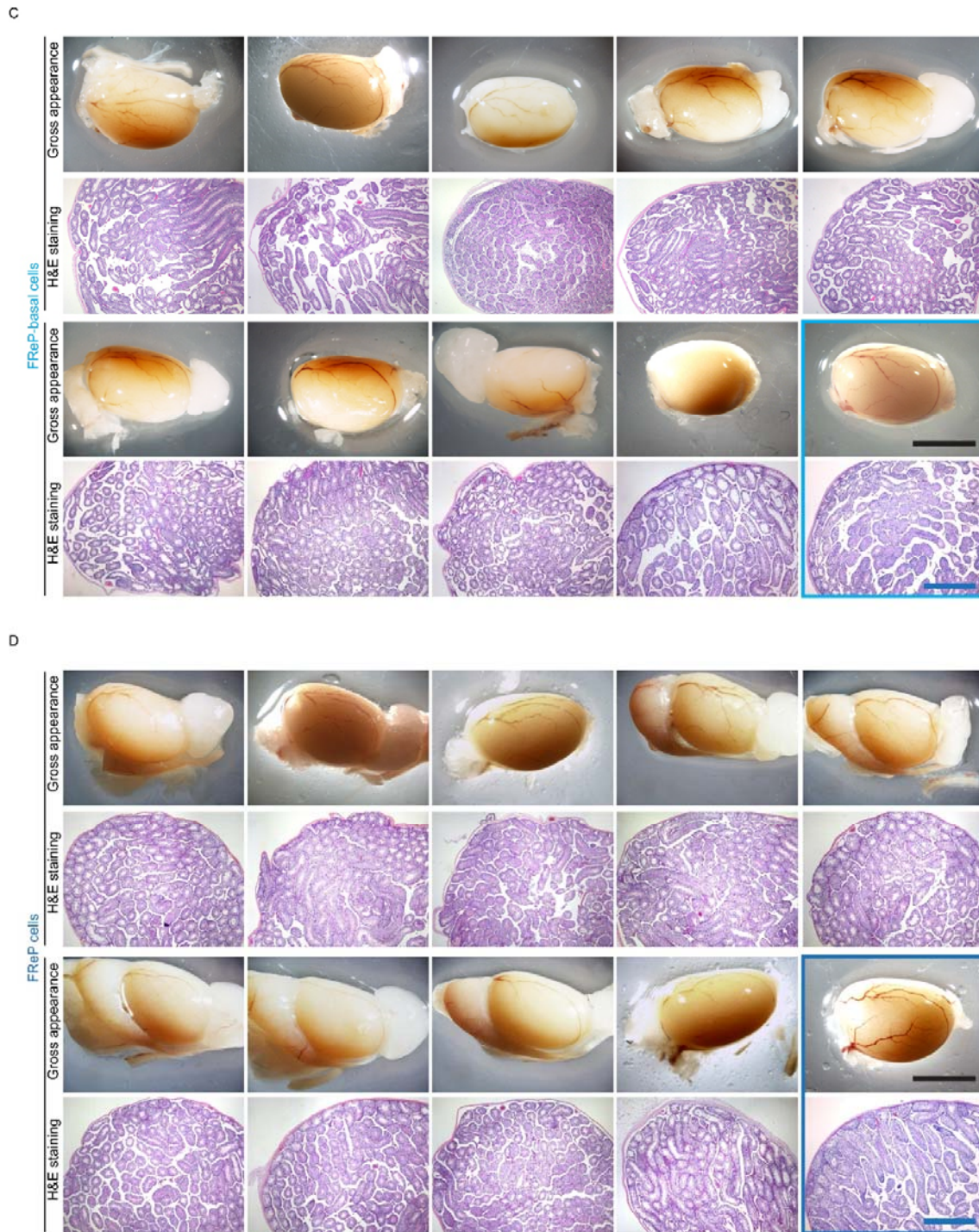
A



B



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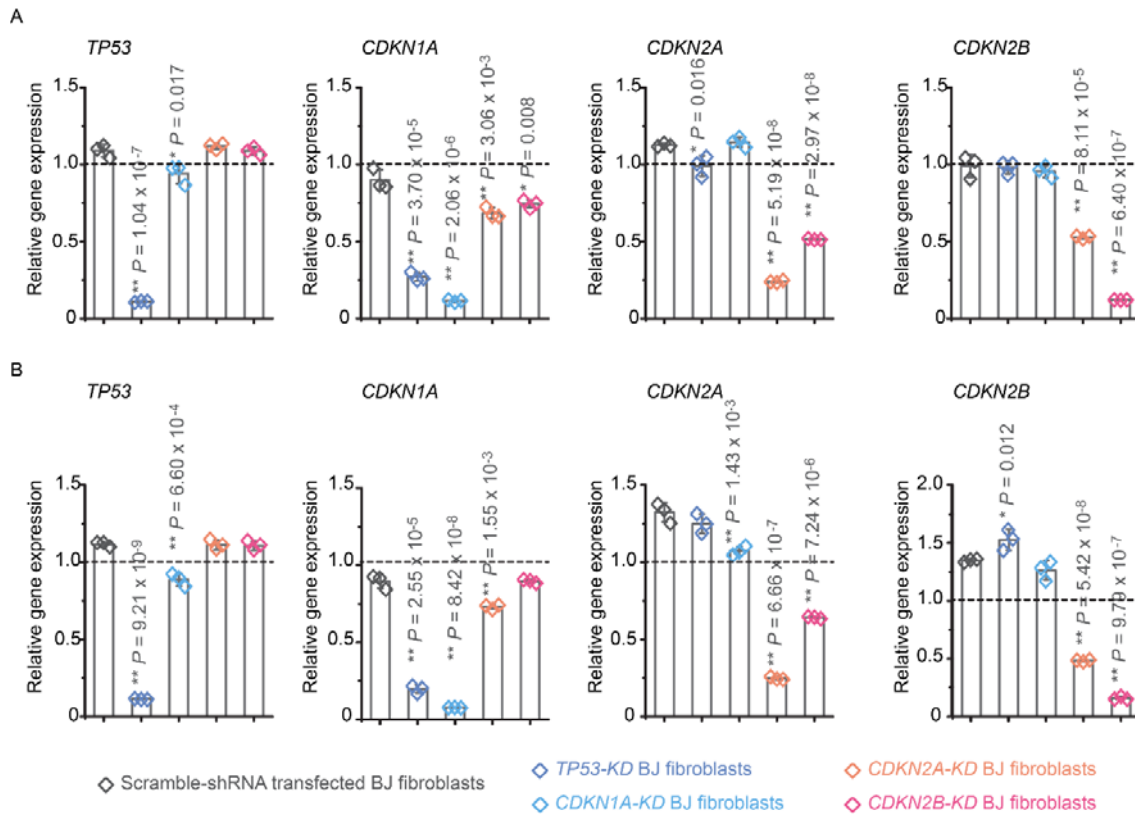
**Supplemental Figure 3: No tumor formation was observed in Fox Chase SCID-Beige mice that underwent intratesticular implantation of FReP cells.**

Gross appearance and histological evaluation (H&E staining) of adult Fox Chase SCID-Beige mice testes implanted with  $1 \times 10^6$  (A) BJ fibroblasts [The representative gross appearance and H&E staining images shown in Figure 4A (leftmost panels in the first and second rows)]

are reshowed and outlined by the Black box here], (B) retrovirus-mediated BJ-iPSC [The representative gross appearance and H&E staining images shown in Figure 4A (second from left panels in the first and second rows) are reshowed and outlined by the Red box here], (C) FReP basal cells [The representative gross appearance and H&E staining images shown in Figure 4A (second from right panels in the first and second rows) are reshowed and outlined by the Cyan box here], and (D) FReP cells [The representative gross appearance and H&E staining images shown in Figure 4A (rightmost panels in the first and second rows) are reshowed and outlined by the Denim box here] in 30  $\mu$ l Matrigel™ hESC-qualified Matrix evaluated 4 months post-implantation. Scale bar = 5 mm (black) or 1 mm (blue), respectively.

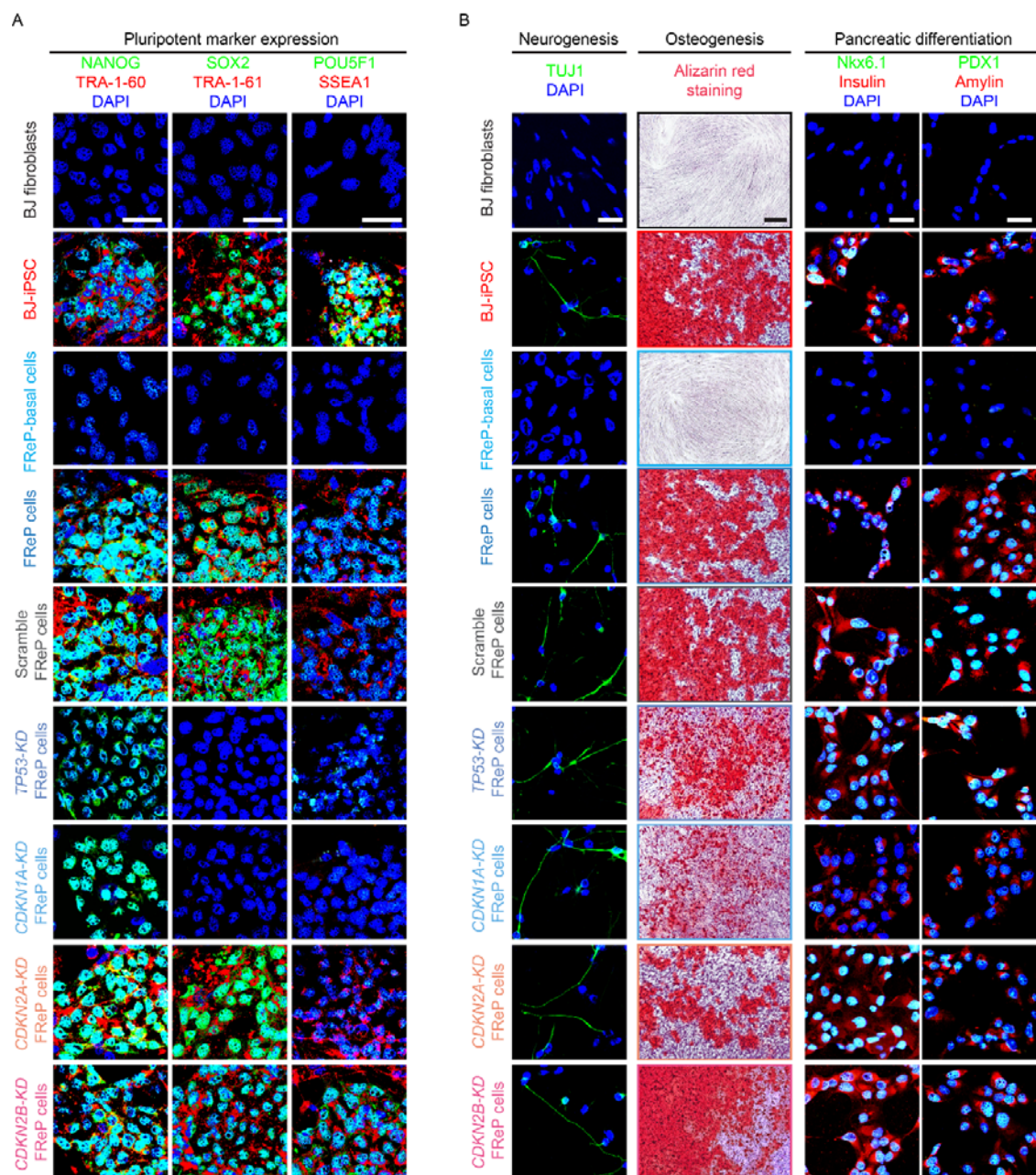


human proto-oncogenes and (C) tumor suppressor genes listed in the UniProt database. (D) Tumor suppressor genes with high levels of expression (faint blue balloons) in FReP cells were further analyzed by the STRING database to retrieve the relative genes predicted by known protein-protein interactions (dark blue diamonds). The size of the balloons indicates fold difference of gene expression levels in FReP cells vs. BJ-derived iPSC generated without genome integration (e.g., larger balloons indicate higher levels of gene expression in FReP cells than the expression levels in BJ-derived iPSC generated without genome integration).



**Supplemental Figure 5: Multiple stable knockdown BJ cells were established by shRNA transduction.**

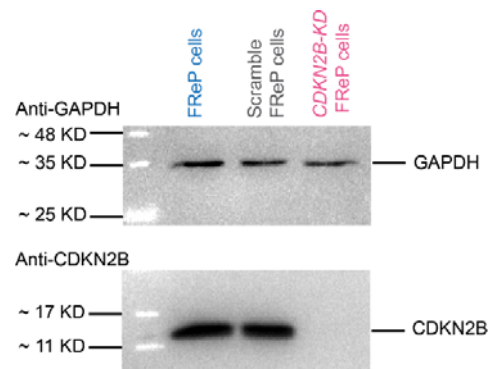
(A) Expression levels of *TP53* and *CDKN* genes were tracked in the screened knockdown (KD) BJ cells to confirm the efficacy of RNAi and reveal interactions between these genes. (B) After undergoing extensive passage for 4 weeks, gene expression of *TP53*- and *CDKN*-KD BJ cells were re-examined to ensure that the duration of the RNAi effects would extend longer than the 3-week FMOD reprogramming process. Data are normalized to BJ fibroblasts without any shRNA transfection (in brief, BJ fibroblasts), presented as mean  $\pm$  the standard deviation, and analyzed by one-way ANOVA and one-tailed two-sample *t*-tests. \*,  $P < 0.05$ ; \*\*,  $P < 0.005$ ;  $n = 3$  independent experiments performed in duplicate. Dashed lines indicate the gene expression levels of BJ fibroblasts; stars indicate significance in comparison with scramble-shRNA transfected BJ fibroblasts.



**Supplemental Figure 6: *TP53*- and *CDKN*-KD altered the multipotency of FReP cells.**

(A) Expression of pluripotent markers was examined by immunofluorescent staining. Compared with scramble FReP cells and *CDKN2B-KD* FReP cells, *TP53*- and *CDKN1A-KD* FReP cells had reduced expressions of all the pluripotent markers, except for NANOG. (B) In vitro multipotent differentiation capability was assessed by: Ectoderm – neuron-like morphology and neuron-specific  $\beta_{III}$ -tubulin (TUJ1) staining; Mesoderm – Alizarin red staining for osteogenesis; Endoderm – pancreatic differentiation with Nkx6.1/insulin and pancreatic

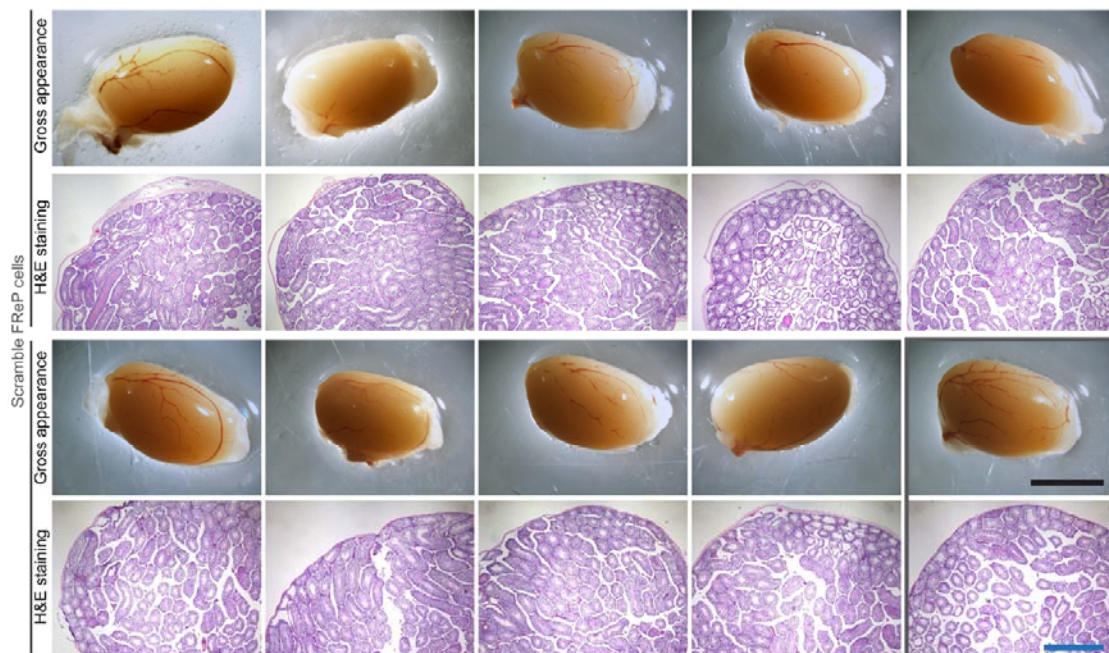
and duodenal homeobox 1 (PDX1)/Amylin staining. *TP53*-, *CDKN1A*-, and *CDKN2A-KD* FReP cells also exhibited reduced osteogenic differentiation/mineralization ability, and *CDKN1A-KD* FReP cells expressed fewer pancreatic transcription factors. However, knockdown *TP53* or *CDKNs* did not appear to halt ectodermic differentiation in the yielded FReP cells. Scale bar = 50  $\mu\text{m}$  (white) or 200  $\mu\text{m}$  (black), respectively.



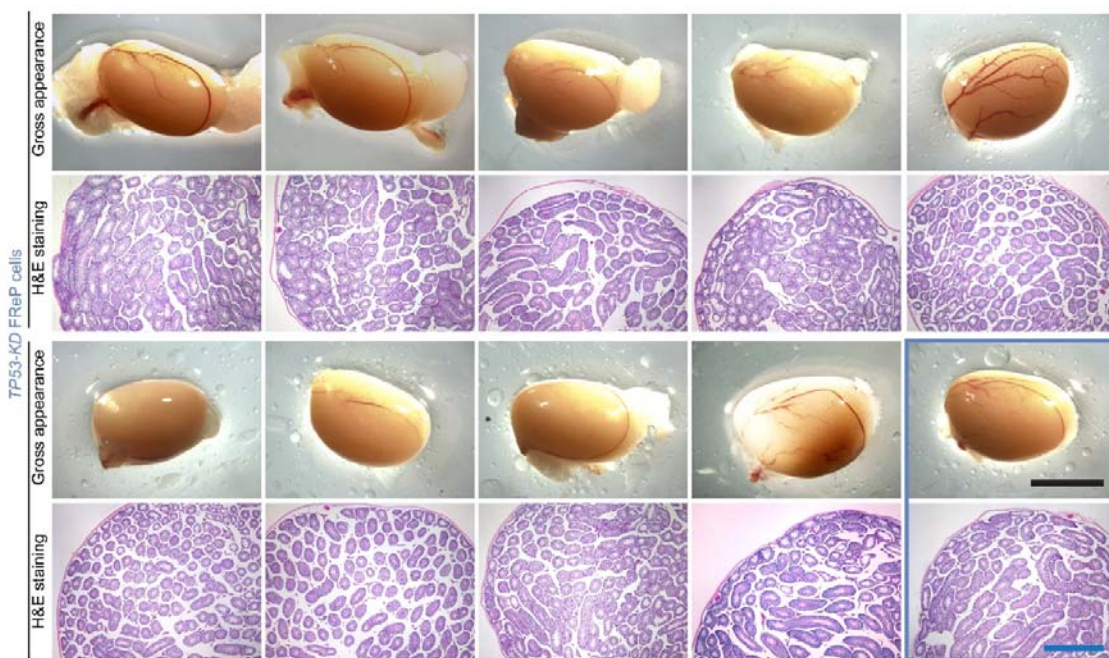
**Supplemental Figure 7: Western blotting verified the efficacy of *CDKN2B*-KD at the protein level.**

Under undifferentiated conditions, CDKN2B protein levels of *CDKN2B*-KD FReP cells were significantly reduced in comparison with those of FReP cells (generated from non-transfected BJ fibroblasts) or scramble FReP cells.

A

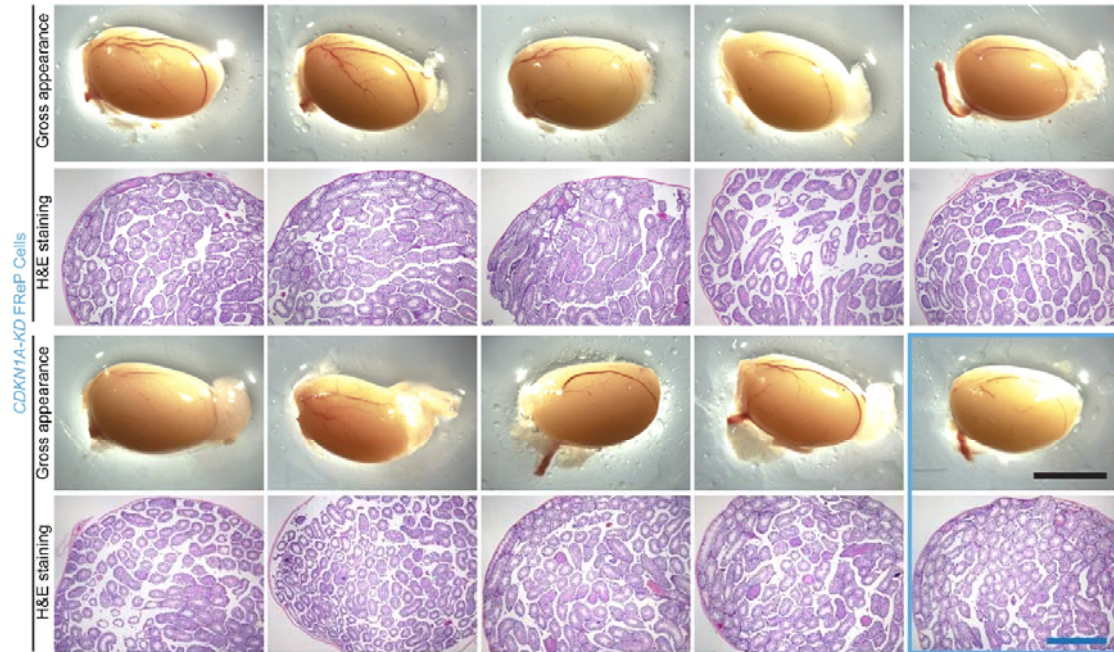


B

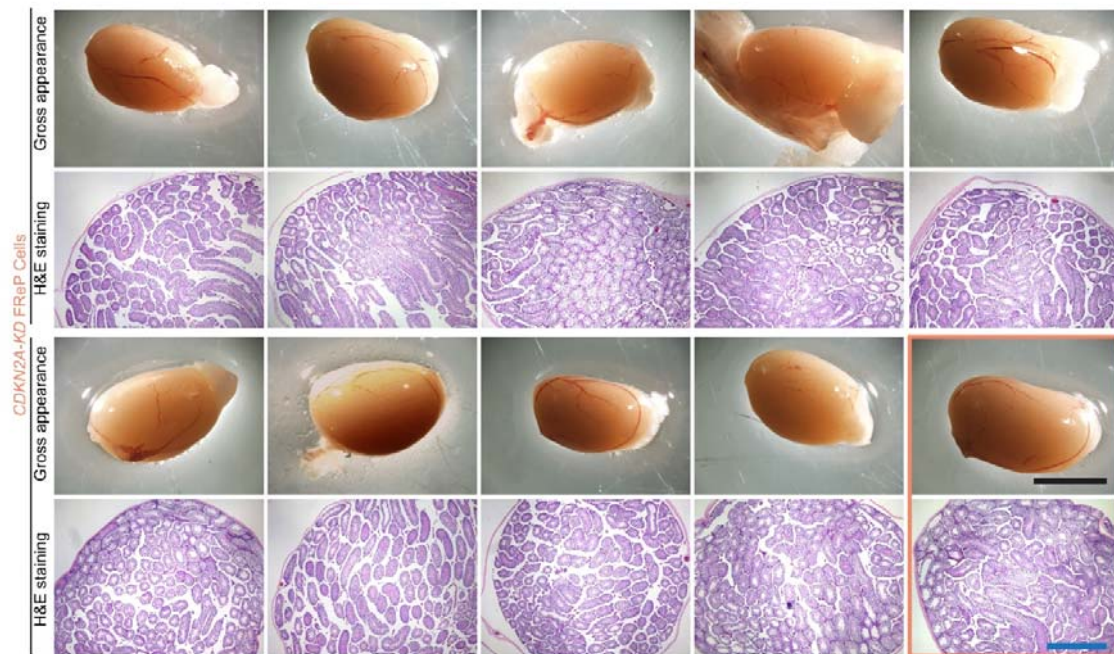


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C

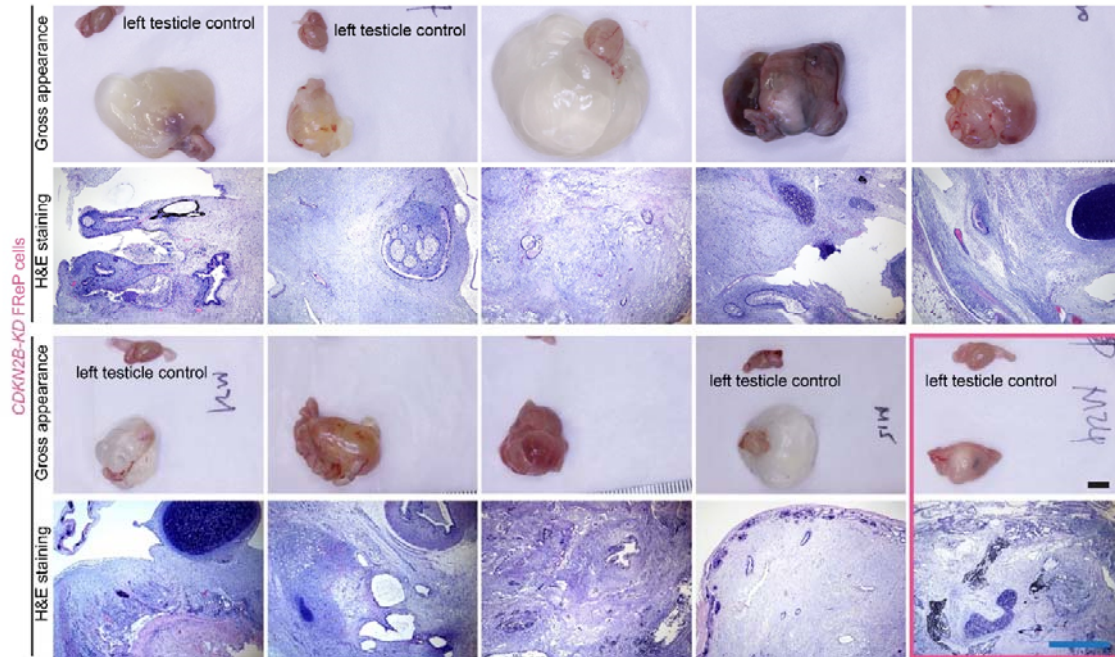


D



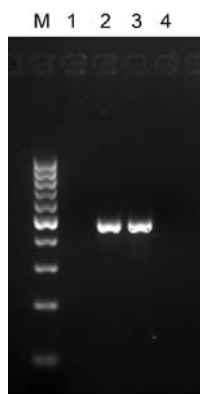
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E



**Supplemental Figure 8: Tumor formation was observed in Fox Chase SCID-Beige mice with intratesticular implantation of *CDKN2B-KD* FReP cells.**

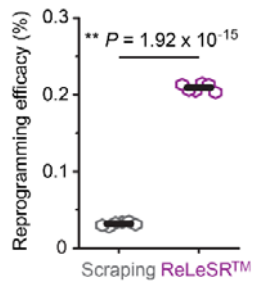
Gross appearance and histological evaluation (H&E staining) of adult Fox Chase SCID-Beige mice testes implanted with  $1 \times 10^6$  (A) scramble FReP cells [The representative gross appearance and H&E staining images shown in Figure 8A (leftmost panels) are reshown and outlined by the Bright Gray box here ], (B) *TP53-KD* FReP cells [The representative gross appearance and H&E staining images shown in Figure 8A (second from left panels) are reshown and outlined by the Danube box here], (C) *CDKN1A-KD* FReP cells [The representative gross appearance and H&E staining images shown in Figure 8A (third from right panels) are reshown and outlined by the Malibu box here], (D) *CDKN2A-KD* FReP cells [The representative gross appearance and H&E staining images shown in Figure 8A (second from right panels) are reshown and outlined by the Salmon box here], and (E) *CDKN2B-KD* FReP cells [The representative gross appearance and H&E staining images shown in Figure 8A (rightmost panels) are reshown and outlined by the French Rose box here] in 30  $\mu$ l Matrigel™ hESC-qualified Matrix evaluated 4 months post-implantation. Scale bar = 5 mm (black) or 1 mm (blue), respectively.



**Supplemental Figure 9: No mycoplasma contamination was found in the BJ fibroblasts used in this study.**

Mycoplasma contamination was tested using the Universal Mycoplasma Detection Kit (ATCC).

M: 100 bp DNA ladder with a highlighted band at 500 bp; lane 1, BJ fibroblasts; lane 2, positive control + BJ fibroblasts; lane 3, positive control; and lane 4, negative control.



**Supplemental Figure 10: ReLeSR™-based technology significantly increased the efficacy of FMOD reprogramming.**

Data are presented with mean value. \*\*,  $P < 0.005$  (analyzed by one-way ANOVA and one-tailed two-sample  $t$ -tests);  $n = 6$  independent experiments.

**Supplemental Table 1: Gene expression profile of FReP cells during myogenic differentiation, as determined by Human Skeletal Muscle Myogenesis & Myopathy RT<sup>2</sup> PCR Array: C<sub>T</sub> values.**

Symbol	Description	Myogenic differentiation time							
		Week 0		Week 1		Week 2		Week3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>ACTA1</i>	Skeletal muscle $\alpha$ -actin	33.73	0.26	32.19	0.40	31.15	0.01	30.55	0.37
<i>ACTN3</i>	$\alpha$ -Actinin skeletal muscle isoform 3	31.62	0.12	30.42	0.17	29.25	0.13	28.63	0.11
<i>ACVR2B</i>	Activin A receptor, type IIB	26.96	0.05	28.58	0.30	27.23	0.10	26.57	0.18
<i>ADIPOQ</i>	Adiponectin, C1Q and collagen domain containing	36.03	0.05	34.45	0.21	32.95	0.05	33.20	0.39
<i>ADRB2</i>	Adrenergic, $\beta$ 2 receptor, surface	28.40	0.24	27.94	0.13	27.40	0.27	26.87	0.15
<i>AGRN</i>	Agrin	24.49	0.20	24.25	0.13	23.60	0.25	22.70	0.22
<i>AKT1</i>	V-akt murine thymoma viral oncogene homolog 1	22.95	0.06	22.85	0.12	21.1	0.14	19.17	0.17
<i>AKT2</i>	V-akt murine thymoma viral oncogene homolog 2	22.18	0.08	22.40	0.22	21.11	0.14	19.76	0.16
<i>ATP2A1</i>	ATPase, Ca <sup>2+</sup> transporting, cardiac muscle, fast twitch 1	30.86	0.14	29.3	0.34	28.64	0.38	28.07	0.07
<i>BCL2</i>	B-cell CLL/lymphoma 2	28.67	0.09	29.97	0.19	28.24	0.11	27.75	0.24
<i>BMP4</i>	Bone morphogenetic protein 4	27.14	0.05	23.55	0.14	21.42	0.11	20.49	0.19

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Symbol	Description	Myogenic differentiation time							
		Week 0		Week 1		Week 2		Week3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>CAMK2</i>	Calcium/calmodulin-dependent protein kinase II	24.50	0.11	23.95	0.12	23.48	0.07	22.79	0.20
<i>CAPN2</i>	Calpain 2	20.65	0.09	19.85	0.17	19.35	0.15	18.75	0.28
<i>CAPN3</i>	Calpain 3	26.65	0.15	26.78	0.29	24.32	0.23	22.97	0.20
<i>CASP3</i>	Caspase 3	25.24	0.08	24.12	0.14	22.91	0.15	22.61	0.24
<i>CAST</i>	Calpastatin	22.14	0.05	20.87	0.11	19.06	0.09	17.27	0.28
<i>CAV1</i>	Caveolin 1	19.60	0.05	18.08	0.17	16.42	0.04	15.87	0.23
<i>CAV3</i>	Caveolin 3	34.54	0.23	32.06	0.17	32.27	0.03	32.08	0.48
<i>CRYAB</i>	$\alpha\beta$ -Crystallin	21.88	0.06	22.80	0.15	21.76	0.21	20.62	0.16
<i>CS</i>	Citrate synthase	21.93	0.06	22.00	0.18	21.38	0.06	20.84	0.26
<i>CTNNB1</i>	Catenin (cadherin-associated protein) $\beta$ 1	23.82	0.05	22.97	0.23	22.22	0.11	21.39	0.33
<i>DAG1</i>	Dystroglycan 1	22.35	0.08	22.03	0.13	21.13	0.07	20.04	0.15
<i>DES</i>	Desmin	27.12	0.04	26.91	0.13	25.99	0.26	23.56	0.26
<i>DMD</i>	Dystrophin	27.45	0.05	27.18	0.26	25.89	0.23	24.78	0.26
<i>DMPK</i>	Dystrophia myotonica-protein kinase	25.31	0.14	23.73	0.22	22.94	0.26	21.90	0.13
<i>DYSF</i>	Dysferlin	32.47	0.26	30.18	0.22	28.50	0.11	26.92	0.24
<i>FBXO32</i>	F-box protein 32	26.79	0.05	25.49	0.20	24.87	0.32	25.19	0.36
<i>FGF2</i>	Fibroblast growth factor 2	22.58	0.09	21.53	0.20	20.63	0.25	19.86	0.11
<i>FOXO1</i>	Forkhead box O1	24.23	0.07	24.21	0.60	22.91	0.12	23.58	0.28
<i>FOXO3</i>	Forkhead box O3	22.93	0.05	22.99	0.23	22.06	0.13	21.36	0.23

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Symbol	Description	Myogenic differentiation time							
		Week 0		Week 1		Week 2		Week3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>HDAC5</i>	Histone deacetylase 5	23.09	0.07	23.05	0.10	22.19	0.16	21.47	0.15
<i>HK2</i>	Hexokinase 2	26.55	0.17	25.03	0.23	24.72	0.32	24.20	0.39
<i>IGF1</i>	Insulin-like growth factor 1	28.13	0.05	24.30	0.17	24.07	0.16	23.94	0.31
<i>IGF2</i>	Insulin-like growth factor 2	23.28	0.12	24.72	0.27	21.62	0.10	19.82	0.10
<i>IGFBP3</i>	Insulin-like growth factor binding protein 3	18.77	0.08	19.97	0.19	17.80	0.10	16.96	0.20
<i>IGFBP5</i>	Insulin-like growth factor binding protein 5	19.72	0.15	19.22	0.18	18.62	0.06	17.60	0.24
<i>IKBKB</i>	Inhibitor of $\kappa$ light polypeptide gene enhancer in B-cells, kinase $\beta$	25.33	0.09	25.18	0.29	24.14	0.16	23.75	0.18
<i>IL1B</i>	Interleukin 1 $\beta$	29.81	0.23	25.58	0.33	24.15	0.16	24.37	0.20
<i>IL6</i>	Interleukin 6	26.91	0.11	24.17	0.16	23.55	0.54	23.06	0.37
<i>LEP</i>	Leptin	low	-	30.53	0.16	30.43	0.04	30.34	0.18
<i>LMNA</i>	Lamin A/C	21.12	0.07	20.86	0.10	20.32	0.14	19.59	0.13
<i>MAPK1</i>	Mitogen-activated protein kinase 1	21.94	0.06	22.05	0.15	21.31	0.09	20.66	0.22
<i>MAPK14</i>	Mitogen-activated protein kinase 14	23.85	0.06	23.96	0.15	22.99	0.10	22.27	0.23
<i>MAPK3</i>	Mitogen-activated protein kinase 3	23.90	0.05	23.36	0.19	22.72	0.22	21.95	0.13

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Symbol	Description	Myogenic differentiation time							
		Week 0		Week 1		Week 2		Week3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>MAPK8</i>	Mitogen-activated protein kinase 8	25.84	0.05	24.83	0.28	24.17	0.13	23.42	0.32
<i>MB</i>	Myoglobin	39.86	0.07	36.47	0.15	35.52	0.14	34.76	0.27
<i>MEF2C</i>	Myocyte enhancer factor 2C	25.00	0.12	26.87	0.10	25.37	0.37	25.63	0.23
<i>MMP9</i>	Matrix metalloproteinase 9	31.74	0.07	31.48	0.32	32.63	0.35	33.24	0.41
<i>MSTN</i>	Myostatin	34.57	0.08	34.47	0.47	34.63	0.17	34.54	0.23
<i>MUSK</i>	Muscle, skeletal, receptor tyrosine kinase	34.53	0.30	31.26	0.15	29.92	0.34	29.87	0.20
<i>MYF5</i>	Myogenic factor 5	36.43	0.15	32.56	0.19	31.25	0.22	30.12	0.31
<i>MYF6</i>	Myogenic factor 6	34.75	0.07	31.63	0.31	30.19	0.21	30.47	0.08
<i>MYH1</i>	Skeletal muscle myosin, heavy chain 1	27.85	0.10	29.10	0.15	25.88	0.29	24.07	0.14
<i>MYH2</i>	Skeletal muscle myosin, heavy chain 2	39.00	0.05	33.40	0.48	31.23	0.04	29.31	0.19
<i>MYOD1</i>	Myogenic differentiation 1	38.63	0.34	34.82	0.21	31.72	0.25	33.31	0.33
<i>MYOG</i>	Myogenin	38.93	0.15	36.12	0.15	31.79	0.14	32.42	0.25
<i>MYOT</i>	Myotilin	30.22	0.20	31.26	0.40	29.48	0.36	28.80	0.10
<i>NEB</i>	Nebulin	36.67	0.27	36.12	0.44	34.87	0.13	33.79	0.06
<i>NFKB1</i>	Nuclear factor of $\kappa$ light polypeptide gene enhancer in B-cells 1	24.62	0.12	24.65	0.13	23.65	0.07	22.95	0.25
<i>NOS2</i>	Nitric oxide synthase 2	30.45	0.19	33.98	0.39	32.32	0.19	31.87	0.18

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Symbol	Description	Myogenic differentiation time							
		Week 0		Week 1		Week 2		Week3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>PAX3</i>	Paired box 3	26.66	0.07	23.40	0.19	24.45	0.22	24.81	0.08
<i>PAX7</i>	Paired box 7	41.58	0.22	33.45	0.34	33.15	0.10	32.74	0.17
<i>PDK4</i>	Pyruvate dehydrogenase kinase, isozyme 4	28.54	0.24	26.88	0.20	25.00	0.17	25.09	0.19
<i>PPARG</i>	Peroxisome proliferator- activated receptor $\gamma$	26.67	0.17	25.43	0.07	23.74	0.16	23.25	0.23
<i>PPARGC1A</i>	Peroxisome proliferator- activated receptor G coactivator 1 $\alpha$	28.12	0.10	26.97	0.34	25.07	0.27	23.71	0.02
<i>PPARGC1B</i>	Peroxisome proliferator- activated receptor $\gamma$ coactivator 1 $\beta$	25.95	0.14	32.57	0.38	32.63	0.12	31.85	0.14
<i>PPP3CA</i>	Protein phosphatase 3, catalytic subunit, $\alpha$ isozyme	22.23	0.06	24.03	0.13	23.35	0.21	22.18	0.24
<i>PRKAA1</i>	Protein kinase, AMP- activated, $\alpha$ 1 catalytic subunit	23.23	0.06	22.96	0.19	22.14	0.09	21.62	0.26
<i>PRKAB2</i>	Protein kinase, AMP- activated, $\beta$ 2 non-catalytic subunit	24.89	0.15	24.95	0.05	24.45	0.20	23.71	0.23

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Symbol	Description	Myogenic differentiation time							
		Week 0		Week 1		Week 2		Week3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>PRKAG1</i>	Protein kinase, AMP-activated, $\gamma$ 1 non-catalytic subunit	22.96	0.03	23.17	0.13	22.42	0.08	21.77	0.18
<i>PRKAG3</i>	Protein kinase, AMP-activated, $\gamma$ 3 non-catalytic subunit	34.20	0.24	32.79	0.42	32.55	0.29	31.59	0.15
<i>RHOA</i>	Ras homolog gene family, member A	20.40	0.08	19.75	0.22	19.33	0.10	18.62	0.38
<i>RPS6KB1</i>	Ribosomal protein S6 kinase, polypeptide 1	23.89	0.15	24.37	0.26	23.48	0.07	22.87	0.22
<i>SGCA</i>	Sarcoglycan $\alpha$	31.53	0.59	30.57	0.50	29.23	0.13	29.99	0.52
<i>SLC2A4</i>	Solute carrier family 2, member 4	31.54	0.09	31.29	0.39	31.61	0.16	31.55	0.20
<i>TGFB1</i>	Transforming growth factor $\beta$ 1	19.77	0.08	22.38	0.13	21.65	0.13	21.03	0.15
<i>TNF</i>	Tumor necrosis factor	38.45	0.06	33.43	0.41	31.62	0.23	31.75	0.38
<i>TNNC1</i>	Troponin C type 1	31.56	0.61	28.32	0.41	27.20	0.23	26.00	0.29
<i>TNNI2</i>	Troponin I type 2	33.49	0.30	30.30	0.37	29.42	0.15	29.27	0.32
<i>TNNT1</i>	Troponin T type 1	27.67	0.22	25.72	0.16	25.41	0.10	24.20	0.34
<i>TNNT3</i>	Troponin T type 3	36.85	0.29	34.76	0.29	32.98	0.30	32.17	0.25
<i>TRIM63</i>	Tripartite motif containing 63	32.91	0.19	34.17	0.51	33.08	0.16	32.09	0.10
<i>TTN</i>	Titin	39.76	0.06	34.34	0.37	34.60	0.15	34.61	0.04

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Symbol	Description	Myogenic differentiation time							
		Week 0		Week 1		Week 2		Week3	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>UTRN</i>	Utrophin	24.11	0.13	24.56	0.12	22.39	0.10	21.46	0.40
<i>ACTB</i>	$\beta$ -actin*	15.57	0.11	16.13	0.10	16.01	0.17	15.16	0.32
<i>B2M</i>	$\beta$ -2-microglobulin*	17.18	0.13	17.79	0.18	16.65	0.03	15.87	0.37
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase*	17.48	0.05	17.55	0.22	17.51	0.14	17.18	0.17
<i>HPRT1</i>	Hypoxanthine phosphoribosyltransferase 1*	23.58	0.21	24.40	0.23	23.96	0.13	23.69	0.22
<i>RPLP0</i>	Ribosomal protein, large, P0*	15.54	0.09	16.73	0.18	16.19	0.18	15.66	0.46

\* housekeeping genes.

N = 3 independent experiments.