Cell Reports, Volume *25*

Supplemental Information

Promotion of Myoblast Differentiation by Fkbp5 via Cdk4 Isomerization

Mercedes Ruiz-Estevez, James Staats, Ellen Paatela, Dane Munson, Nobuko Katoku-Kikyo, Ce Yuan, Yoko Asakura, Reilly Hostager, Hiroshi Kobayashi, Atsushi Asakura, and Nobuaki Kikyo

Fig S1

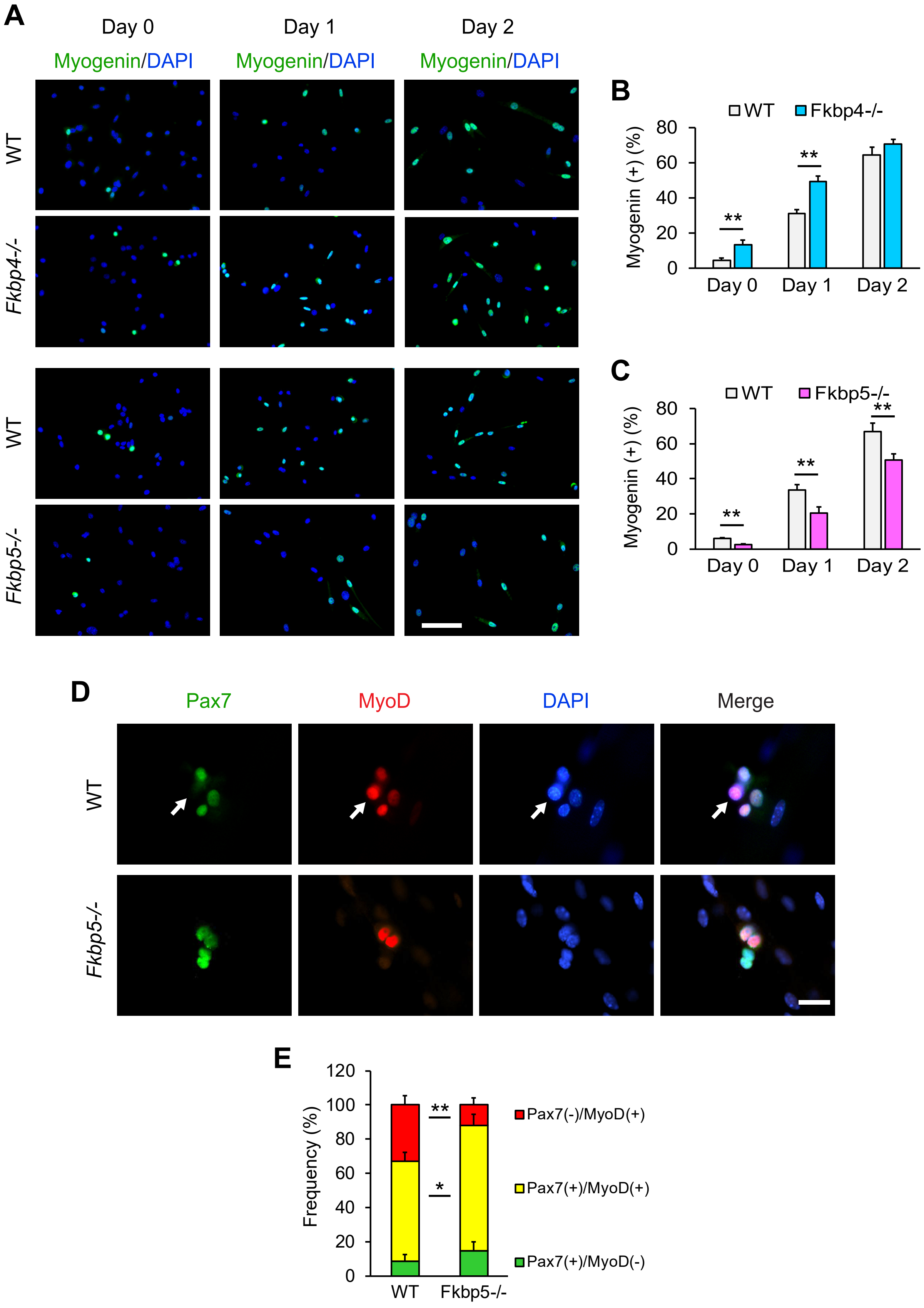

A
Day 0
Day 1
Day 2
Myogenin/DAPI
Myogenin/DAPI
Myogenin/DAPI
WT
Fkbp4-/-
WT
Fkbp5-/-
B
WT
Fkbp4-/-
Myogenin (+) (%)
80
60
40
20
0
**
**
Day 0
Day 1
Day 2
C
WT
Fkbp5-/-
Myogenin (+) (%)
80
60
40
20
0
**
**
**
Day 0
Day 1
Day 2
D
Pax7
MyoD
DAPI
Merge
WT
Fkbp5-/-
E
Frequency (%)
120
100
80
60
40
20
0
**
*
WT
Fkbp5-/-
Pax7(-)/MyoD(+)
Pax7(+)/MyoD(+)
Pax7(+)/MyoD(-)

Figure S1. Differentiation of primary myoblasts and myofibers prepared from *Fkbp* KO mice, Related to Figure 1.

(A) Temporal profile of myogenin expression in primary myoblasts prepared from WT and KO mice. DNA was counter stained with DAPI. Bar, 100 µm.
(B) Frequency of myogenin (+) nuclei representing differentiating myoblasts prepared from WT and *Fkbp4*$^{-/-}$ mice.
(C) Frequency of myogenin (+) nuclei representing differentiating myoblasts prepared from WT and *Fkbp5*$^{-/-}$ mice.
(D) Immunofluorescence staining of single myofibers with antibodies against Pax7 (green) and MyoD (red) after culture for 72 hr. A Pax7(-)/MyoD(+) differentiating myoblast is indicated by arrows. Bars, 50 µm.
(E) Frequency of Pax7(+)/MyoD(-), Pax7(+)/MyoD(+), and Pax7(-)/MyoD(+) cells per cluster in myofibers after culture for 72 hr. More than ten myofibers were counted for each genotype.
* $< p < 0.05$ and ** $p < 0.01$ with Student's t-test in (B), (C), and (E). Data are presented as mean ± SD.

Fig S2

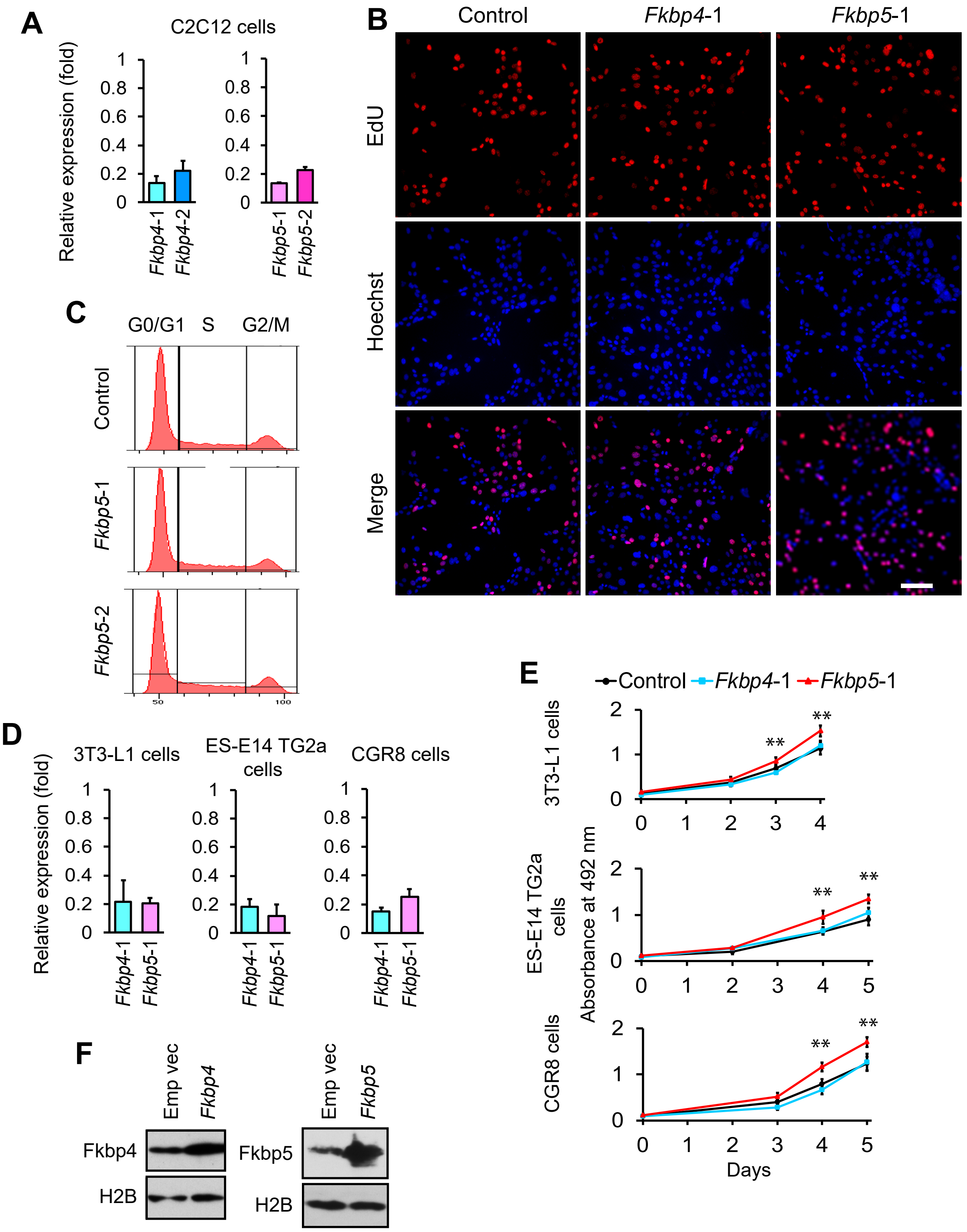
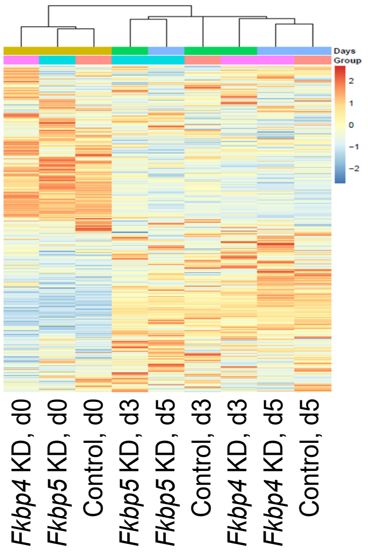

A
C2C12 cells
Relative expression (fold)
1
0.8
0.6
0.4
0.2
0
Fkbp4-1
Fkbp4-2
Fkbp5-1
Fkbp5-2
B
Control
Fkbp4-1
Fkbp5-1
EdU
Hoechst
Merge
C
G0/G1
S
G2/M
Control
Fkbp5-1
Fkbp5-2
50
100
D
3T3-L1 cells
ES-E14 TG2a cells
CGR8 cells
Relative expression (fold)
Fkbp4-1
Fkbp5-1
E
Control
Fkbp4-1
Fkbp5-1
3T3-L1 cells
ES-E14 TG2a cells
CGR8 cells
Absorbance at 492 nm
**
Days
0
1
2
3
4
5
F
Emp vec
Fkbp4
Fkbp5
Fkbp4
Fkbp5
H2B

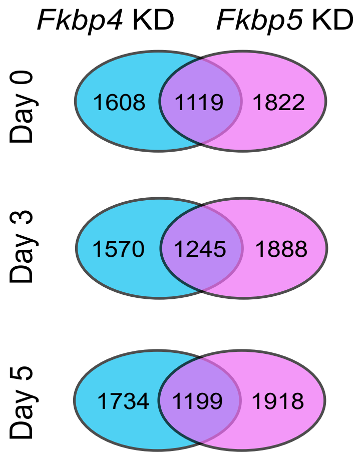
Figure S2. Proliferation of various cell lines with *Fkbp4* and *Fkbp5* KD and overexpression, Related to Figure 2.

(A) Relative expression levels of *Fkbp4* and *Fkbp5* mRNAs in C2C12 cells after KD with two shRNA clones. The value obtained with the scrambled control shRNA was defined as 1.0 for each gene.
(B) EdU uptake in C2C12 cells with *Fkbp4* and *Fkbp5* KD. shRNA clone #1 was used for each. Bar, 100 µm.
(C) Flow cytometry analysis of the cell cycle phases in C2C12 cells with *Fkbp5* KD.
(D) Relative expression levels of *Fkbp4* and *Fkbp5* mRNAs in the indicated three cell lines after KD with shRNA clone #1 for each gene.
(E) MTS assay for the proliferation of undifferentiated cells after KD.
(F) Western blotting of Fkbp4 and Fkbp5 comparing empty vector (Emp vec) and overexpressed cells. Histone H2B was detected as the loading control.
** $p < 0.01$ with Student's t-test in comparison to the control cells. Data are presented as mean + or ± SD of technical triplicates.

A



B



C

Day	GO Biological Process	GO Term	Q-value
Upregulated by Fkbp4 KD but unchanged by Fkbp5 KD			
Day 0	sensory perception of chemical stimulus	GO:0007606	3.99E-05
	muscle organ development	GO:0007517	5.58E-05
	sensory perception of smell	GO:0007608	7.21E-05
	muscle structure development	GO:0061061	0.000447
	muscle system process	GO:0003012	0.000858
	skeletal muscle organ development	GO:0060538	0.00123
	striated muscle contraction	GO:0006941	0.00149
	striated muscle tissue development	GO:0014706	0.00178
	skeletal muscle tissue development	GO:0007519	0.0023
	muscle tissue development	GO:0060537	0.00272
Day 3	N/A		
Day 5	sensory perception of chemical stimulus	GO:0007606	6.29E-05
	sensory perception of smell	GO:0007608	6.75E-05
	DNA repair	GO:0006281	0.0168
	DNA conformation change	GO:0071103	0.0188
	DNA packaging	GO:0006323	0.019
	chromosome condensation	GO:0030261	0.0199
	sensory perception	GO:0007600	0.0201
	nuclear chromosome segregation	GO:0098813	0.0241
	chromosome segregation	GO:0007059	0.0387
	central nervous system development	GO:0007417	0.0466
Downregulated by Fkbp4 KD but unchanged by Fkbp5 KD			
Day 0	sensory perception of chemical stimulus	GO:0007606	5.67E-05
	sensory perception of smell	GO:0007608	0.00331
Day 3	sensory perception of chemical stimulus	GO:0007606	0.000232
	sensory perception of smell	GO:0007608	0.000358
	sensory perception	GO:0007600	0.0195
	mitotic cell cycle	GO:0000278	0.0214
	G-protein coupled receptor signaling pathway	GO:0007186	0.0243
	regulation of apoptotic process	GO:0042981	0.0446
	vasculature development	GO:0001944	0.0494
	mitotic cell cycle process	GO:1903047	0.0549
	nervous system process	GO:0050877	0.0556
Day 5	sensory perception	GO:0007600	6.55E-06
	sensory perception of chemical stimulus	GO:0007606	1.29E-05
	nervous system process	GO:0050877	1.35E-05
	sensory perception of smell	GO:0007608	0.000055
	system process	GO:0003008	0.000142
	G-protein coupled receptor signaling pathway	GO:0007186	0.0141

D

Gene	Fkbp4 KD	Fkbp5 KD	Control
Acta1	64.3789	21.9912	26.3394
Actc1	16.1701	5.27573	5.30062
Asb2	1.01968	0.119246	0.10109
Atp2a1	5.22118	1.59093	2.18857
Casq2	7.64579	3.91062	3.42909
Gm7325	5.52258	3.30605	2.6316
Hira	26.2598	19.9358	13.7143
Klhl40	1.14976	0.550772	0.373528
Klhl41	4.1346	1.59973	1.62738
Lmod3	1.93046	0.739803	1.04527
Mef2c	5.93802	5.19829	2.9072
Mybph	15.038	3.48579	4.89398
Myh3	7.31454	1.62762	2.23672
Myl1	162.849	116.985	76.0385
Myl6b	13.9434	8.44615	6.64869
Mylpf	528.335	248.413	173.238
Myog	22.1951	8.25881	10.7462
Nf1	6.56455	3.29237	3.46269
Nog	1.36385	0.498907	0.422942
Pbrm1	30.2152	16.0045	16.4193
Pde5a	22.0994	15.9786	11.5854
Ryr1	1.53666	0.898607	0.847074
Tbx18	10.1294	4.3859	5.46377
Tmem8c	26.5212	7.78822	10.7065
Tnnc1	96.1855	41.7279	44.3501
Tnnc2	33.4149	14.4973	10.5947
Tnnt1	30.7782	20.0999	15.9788
Tnnt3	26.4005	8.46402	10.5598
Ttn	1.10678	0.350495	0.543402
Zfp609	7.08215	4.00654	3.9139
Zfp689	2.77448	1.49771	1.08703

Figure S3. RNA-seq analysis of *Fkbp4* and *Fkbp5* KD cells, Related to Figure 3.

(A) Heat map comparing the transcriptome of KD cells.
(B) Venn diagrams displaying the number of genes whose expression levels were >180% or <56% of those of the control cells. shRNA clone #1 was used for *Fkbp4* and *Fkbp5* KD.
(C and D) Enriched gene ontology (GO) terms in each comparison of the cell types. The genes belonging to the highlighted GO terms in yellow in (C) are listed in (D). The FPKM (Fragments per Kilobase of Exon per Million Fragments Mapped) was compared between *Fkbp4* KD, *Fkbp5* KD, and the control cells.

Fig S4

A

Day	GO Biological Process	GO Term	Q-value
Upregulated by Fkbp5 KD but unchanged by Fkbp4 KD			
Day 0	sensory perception of smell	GO:0007608	0.0001
	sensory perception of chemical stimulus	GO:0007606	0.000138
	sensory perception	GO:0007600	0.0355
Day 3	sensory perception of chemical stimulus	GO:0007606	6.8E-09
	sensory perception of smell	GO:0007608	1.24E-07
	sensory perception	GO:0007600	3.34E-05
	G-protein coupled receptor signaling pathway	GO:0007186	0.0205
	negative regulation of cellular process	GO:0048523	0.0286
	cell projection organization	GO:0030030	0.0304
	protein phosphorylation	GO:0006468	0.0309
	nervous system process	GO:0050877	0.0376
	phosphorylation	GO:0016310	0.0457
Day 5	sensory perception of chemical stimulus	GO:0007606	8.66E-08
	sensory perception of smell	GO:0007608	3.25E-06
	sensory perception	GO:0007600	0.00172
	G-protein coupled receptor signaling pathway	GO:0007186	0.0063
	regulation of cellular component movement	GO:0051270	0.0326
Downregulated by Fkbp5 KD but unchanged by Fkbp4 KD			
Day 0	sensory perception of chemical stimulus	GO:0007606	1.68E-06
	negative regulation of cellular process	GO:0048523	4.21E-05
	negative regulation of biological process	GO:0048519	6.49E-05
	negative regulation of cellular metabolic process	GO:0031324	9.23E-05
	sensory perception of smell	GO:0007608	0.000112
	negative regulation of metabolic process	GO:0009892	0.000615
	G-protein coupled receptor signaling pathway	GO:0007186	0.000647
	negative regulation of nitrogen compound metabolic	GO:0051172	0.000793
	cellular metabolic process	GO:0044237	0.00148
	macromolecule metabolic process	GO:0043170	0.0016
Day 3	sensory perception of chemical stimulus	GO:0007606	1.26E-08
	sensory perception of smell	GO:0007608	3.03E-06
	muscle system process	GO:0003012	1.27E-05
	muscle contraction	GO:0006936	2.00E-05
	sensory perception	GO:0007600	7.36E-05
	striated muscle contraction	GO:0006941	3.28E-04
	myofibril assembly	GO:0030239	1.77E-03
	sarcomere organization	GO:0045214	4.55E-03
	muscle structure development	GO:0061061	1.37E-02
	actomyosin structure organization	GO:0031032	1.49E-02
Day 5	sensory perception of chemical stimulus	GO:0007606	2.80E-08
	metabolic process	GO:0008152	1.71E-07
	muscle contraction	GO:0006936	9.63E-06
	cellular metabolic process	GO:0044237	9.64E-06
	macromolecule metabolic process	GO:0043170	0.000011
	muscle structure development	GO:0061061	1.37E-05
	muscle system process	GO:0003012	2.26E-05
	regulation of nitrogen compound metabolic process	GO:0051171	3.05E-05
	muscle cell differentiation	GO:0042692	3.31E-05
	striated muscle contraction	GO:0006941	3.38E-05

B

Gene	Fkbp4 KD	Fkbp5 KD	Control
Actc1	926.468	245.7	697.408
Actn2	8.79924	4.099	7.89083
Adora1	3.55611	1.99214	4.46089
Angpt1	5.65446	4.8588	9.91276
Ankrd2	22.7712	6.92621	20.6512
Casq1	2.33726	2.0505	3.84586
Casq2	258.243	121.178	235.089
Cav3	16.6712	3.1437	16.5747
Chrnd	121.751	38.5849	91.3575
Gm7325	161.08	69.269	201.406
Grip2	0.769205	0.457966	1.12782
Hmcn2	1.24435	0.934076	2.09645
Igf2	62.5932	37.025	82.4576
Kcnh2	2.34116	1.02116	3.28211
Kcnj2	3.73417	1.02099	3.78962
Klf5	24.033	9.73356	23.9487
Ldb3	38.6925	21.2559	39.6167
Limch1	1.56533	0.909299	2.17372
Lmod2	19.442	12.5597	26.9969
Mapk14	32.7387	14.0297	29.0461
Murc	114.807	55.2924	118.682
Mybph	460.696	269.843	495.758
Myc	16.9573	10.821	24.2648
Myh4	2.46112	0.331384	1.47547
Myh8	1.88479	0.79193	1.59269
Myl4	540.681	333.925	727.592
Myog	236.673	110.397	263.407
Nog	3.36497	1.00468	1.96747
Pou4f1	1.47128	0.71782	1.43465
Prkar1a	382.861	197.468	360.034
Rgs2	1.70049	0.382702	1.53391
Smarca2	3.89398	2.27508	5.32207
Smpx	81.4153	25.0869	89.7319
Sox11	9.26246	6.28244	12.0245
Tcap	2.92216	1.43814	2.72965
Tnnc2	1056.72	660.142	1263.69
Tnni1	1029.76	630.594	1165.65
Tnnt3	686.332	359.046	696.913
Trim63	3.84917	2.59227	4.78362
Unc45b	30.0017	9.98042	25.9177

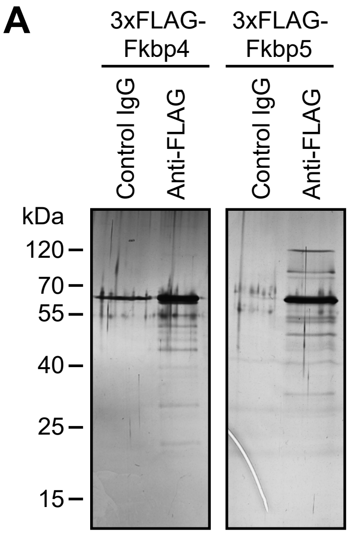
C

Gene	Fkbp4 KD	Fkbp5 KD	Control
Actn2	55.82	13.0564	35.5083
Atp2a1	403.873	100.48	346.637
Atp2a2	225.592	122.373	224.952
Cacna1s	61.9828	26.8631	49.6371
Cav3	43.6789	15.5797	42.3502
Chd2	20.7953	6.07509	24.5802
Chrne	3.31282	0.913014	4.62856
Clcn1	1.42818	0	1.04377
Egr2	2.42194	1.32013	3.07006
Eid2	1.70921	0.694328	1.54422
Fer1l5	1.20298	0.542698	1.3587
Fhod3	4.29211	2.02096	3.74561
Foxn2	3.90664	3.29544	6.23112
Gpd1l	18.2464	8.77815	21.069
Homer1	15.2308	7.20533	13.3751
Kcnj2	5.92892	1.18063	3.63235
Klhl41	149.169	75.657	154.556
Kmt5b	15.2283	9.23787	16.8488
Mapk14	42.4241	13.7468	30.1726
Megf10	23.2779	13.4282	25.4735
Mybpc1	40.4816	6.00567	24.3031
Myh1	56.7749	13.7139	45.3377
Myh2	2.20026	0.549313	2.2822
Myh3	629.797	290.569	530.028
Myh4	61.1483	3.2321	37.8226
Myh8	14.1357	2.22	12.04
Mylk	5.97113	1.59709	4.47072
Mylk2	11.0139	6.23079	11.6872
Myo18b	5.79329	2.057	5.93707
Myom1	43.5553	18.0599	33.6233
Myoz1	2.04917	1.13513	2.73498
Mypn	4.02729	1.43149	4.73011
Neb	47.54	14.8121	38.3272
Nfatc4	1.12756	0.813389	1.57317
Nog	2.17899	0.968149	2.30702
Obsl1	128.244	55.5888	117.483
Pgam2	207.203	117.049	214.532
Popdc2	3.48599	1.48254	3.62219
Scn5a	5.33043	1.1992	4.798
Sik1	30.9275	12.1614	23.4803
Six4	5.65298	2.78037	5.27422
Smpx	172.341	63.5886	198.735
Sox11	13.0495	8.4705	16.5194
Tanc1	28.0129	13.5502	26.2681
Tbx18	11.6084	7.16329	14.1116
Tcf7l2	4.57542	2.0448	5.70948
Tgfbr3	1.3814	1.16771	2.35669
Tmtc3	11.3647	4.84463	12.1146
Tnnc2	2197.18	998.407	2116.24
Unc45b	50.4629	15.0574	39.1639

Figure S4. RNA-seq analysis of *Fkbp4* and *Fkbp5* KD cells, Related to Figure 3.

(A, B, and C) Enriched GO terms in each comparison of the cell types. The genes belonging to the highlighted GO terms in orange and blue in (A) are listed in (B) and (C), respectively. The FPKM was compared between *Fkbp4* KD, *Fkbp5* KD, and the control cells.

Fig S5



B

3xFLAG-Fkbp4-binding proteins

Accession	Coverage (%)	#Peptides	#Unique	Description
gi|6753882	69	43	43	peptidyl-prolyl cis-trans isomerase Fkbp4
gi|40556608	24	17	8	heat shock protein HSP 90-beta
gi|6754254	19	14	5	heat shock protein HSP 90-alpha
gi|1351907	22	13	13	Serum albumin precursor cRAP
gi|269914154	11	9	1	uncharacterized protein LOC239673
gi|31981690	8	5	5	heat shock cognate 71 kDa protein
gi|47059013	9	7	1	keratin type II cytoskeletal 73
gi|6753380	11	3	3	cyclin-dependent kinase 4
gi|162461907	5	3	3	stress-70 protein mitochondrial
gi|190194418	1	4	4	desmoplakin
gi|28395018	4	3	3	junction plakoglobin
gi|30061403	17	2	2	histone H4
gi|306482623	17	2	2	histone H4
gi|30061351	17	2	2	histone H4
gi|46430493	17	2	2	histone H4
gi|30061355	17	2	2	histone H4
gi|30061349	17	2	2	histone H4
gi|28316746	17	2	2	histone H4
gi|21361209	17	2	2	histone H4
gi|23943922	17	2	2	histone H4
gi|30089708	17	2	2	histone H4
gi|30089714	17	2	2	histone H4
gi|30061359	17	2	2	histone H4
gi|30061405	17	2	2	histone H4
gi|6753884	4	2	2	peptidyl-prolyl cis-trans isomerase Fkbp5
gi|6755632	11	1	1	small proline-rich protein 2D
gi|115654	12	3	3	Alpha-S2-casein precursor cRAP
gi|258679542	8	2	2	uncharacterized protein LOC66533

C

3xFLAG-Fkbp5-binding proteins

Accession	Coverage (%)	#Peptides	#Unique	Description
gi|6753884	75	56	56	peptidyl-prolyl cis-trans isomerase Fkbp5
gi|40556608	36	30	21	heat shock protein HSP 90-beta
gi|6754254	35	25	16	heat shock protein HSP 90-alpha
gi|6753380	34	9	9	cyclin-dependent kinase 4
gi|31981690	21	11	9	heat shock cognate 71 kDa protein
gi|1351907	21	12	12	Serum albumin precursor cRAP
gi|7949018	20	7	7	hsp90 co-chaperone Cdc37
gi|31982755	17	7	6	vimentin
gi|28395018	10	7	7	junction plakoglobin
gi|269914154	9	6	1	uncharacterized protein LOC239673
gi|6755372	27	6	6	40S ribosomal protein S3
gi|224994260	10	2	2	40S ribosomal protein SA
gi|309264022	10	2	2	PREDICTED: 40S ribosomal protein SA-like
gi|13385652	25	3	3	40S ribosomal protein S20
gi|162461907	7	4	4	stress-70 protein mitochondrial
gi|190194418	1	3	3	desmoplakin
gi|27545181	3	2	2	serine/threonine-protein kinase A-Raf isof 1
gi|254675270	17	3	3	40S ribosomal protein S5
gi|6671569	7	2	2	60S acidic ribosomal protein P0
gi|30061381	18	3	3	histone H2B type 1-F/J/L
gi|30061389	18	3	3	histone H2B type 1-F/J/L
gi|30061395	18	3	3	histone H2B type 1-F/J/L
gi|68131547	18	3	3	histone H2B type 1-C/E/G
gi|294997245	18	3	3	histone H2B type 1-C/E/G
gi|30061385	18	3	3	histone H2B type 1-M
gi|30061383	18	3	3	histone H2B type 1-F/J/L
gi|30089704	18	3	3	histone H2B type 1-K
gi|30089706	18	3	3	histone H2B type 1-C/E/G
gi|30061387	18	3	3	histone H2B type 1-H
gi|68226433	18	3	3	histone H2B type 2-B
gi|28316760	18	3	3	histone H2B type 1-B
gi|30061377	18	3	3	histone H2B type 1-C/E/G
gi|28316750	18	3	3	histone H2B type 1-A
gi|160420310	17	3	3	H2b histone family member A
gi|160420312	17	3	3	histone cluster 1 H2br
gi|160420308	17	3	3	histone H2B type 1-P
gi|6996913	3	1	1	annexin A2
gi|23956082	8	2	2	60S ribosomal protein L5
gi|309267832	7	2	2	PREDICTED: 60S ribosomal protein L5-like

Figure S5. Immunoprecipitation of Fkbp4- and Fkbp5-binding proteins from C2C12 cell extracts, Related to Figure 4.

(A) Silver staining of SDS-PAGE gels loaded with immunoprecipitated proteins from 3xFLAG-Fkbp4- and 3xFLAG-Fkbp5-expressing undifferentiated cells using anti-FLAG antibody and control IgG.

(B and C) Lists of co-precipitated proteins with 3xFLAG-Fkbp4 (B) and 3xFLAG-Fkbp5 (C). Proteins in which more than one peptide sequences were detected are listed. Known Fkbp4- or Fkbp5-binding proteins are highlighted in red. We adjusted peptide false discovery rate (FDR) to 0.5% and protein FDR to 1.0%.

Fig S6

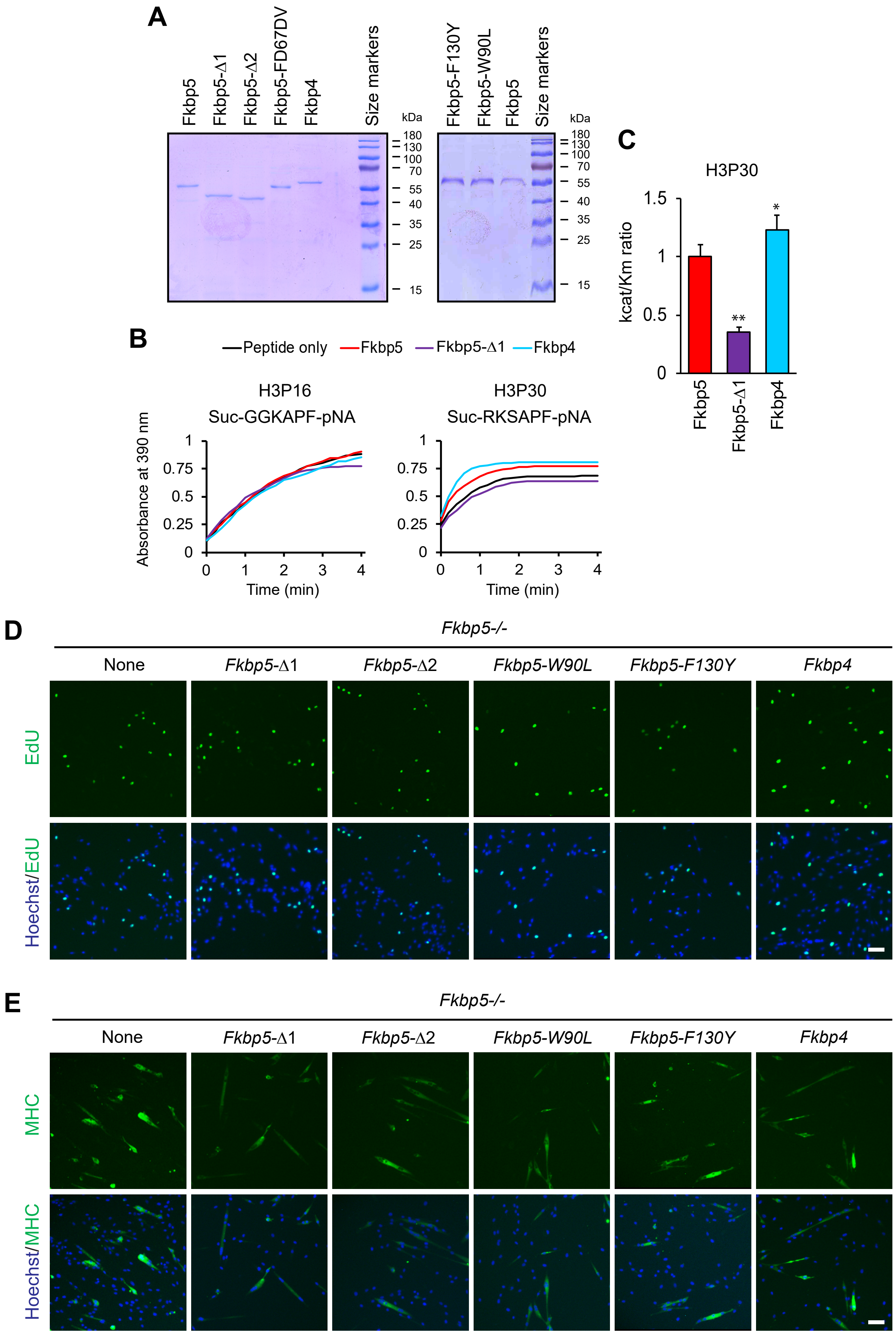

A
Fkbp5
Fkbp5-Δ1
Fkbp5-Δ2
Fkbp5-FD67DV
Fkbp4
Size markers
kDa
180
130
100
70
55
40
35
25
15
Fkbp5-F130Y
Fkbp5-W90L
Fkbp5
Size markers
kDa
180
130
100
70
55
40
35
25
15
B
Peptide only
Fkbp5
Fkbp5-Δ1
Fkbp4
H3P16
Suc-GGKAPF-pNA
H3P30
Suc-RKSAPF-pNA
Absorbance at 390 nm
1
0.75
0.5
0.25
0
0
1
2
3
4
Time (min)
1
0.75
0.5
0.25
0
0
1
2
3
4
Time (min)
C
H3P30
kcat/Km ratio
1.5
1
0.5
0
**
*
Fkbp5
Fkbp5-Δ1
Fkbp4
D
Fkbp5-/-
None
Fkbp5-Δ1
Fkbp5-Δ2
Fkbp5-W90L
Fkbp5-F130Y
Fkbp4
EdU
Hoechst/EdU
E
Fkbp5-/-
None
Fkbp5-Δ1
Fkbp5-Δ2
Fkbp5-W90L
Fkbp5-F130Y
Fkbp4
MHC
Hoechst/MHC

Figure S6. PPIase activity and myogenic activity of Fkbp proteins, Related to Figures 5 and 6.

(A) Coomassie brilliant blue staining of an SDS-PAGE gel loaded with recombinant Fkbp4 and Fkbp5 proteins.
(B) PPIase assays with indicated proteins and histone H3 peptides. Average of technical triplicates is shown.
(C) Catalytic efficiency k_{cat}/K_M with Fkbp proteins and the H3P30 peptide. * $p < 0.05$ and ** $p < 0.01$ with Student's t-test in comparison to Fkbp5. Data are presented as mean + SD of technical triplicates.
(D) Uptake of EdU in *Fkbp5*$^{-/-}$ myoblasts transduced with Fkbp proteins on differentiation day 1. Bar, 100 μm.
(E) Immunostaining of MHC in *Fkbp5*$^{-/-}$ myoblasts transduced with Fkbp proteins on differentiation day 1.

Fig S7

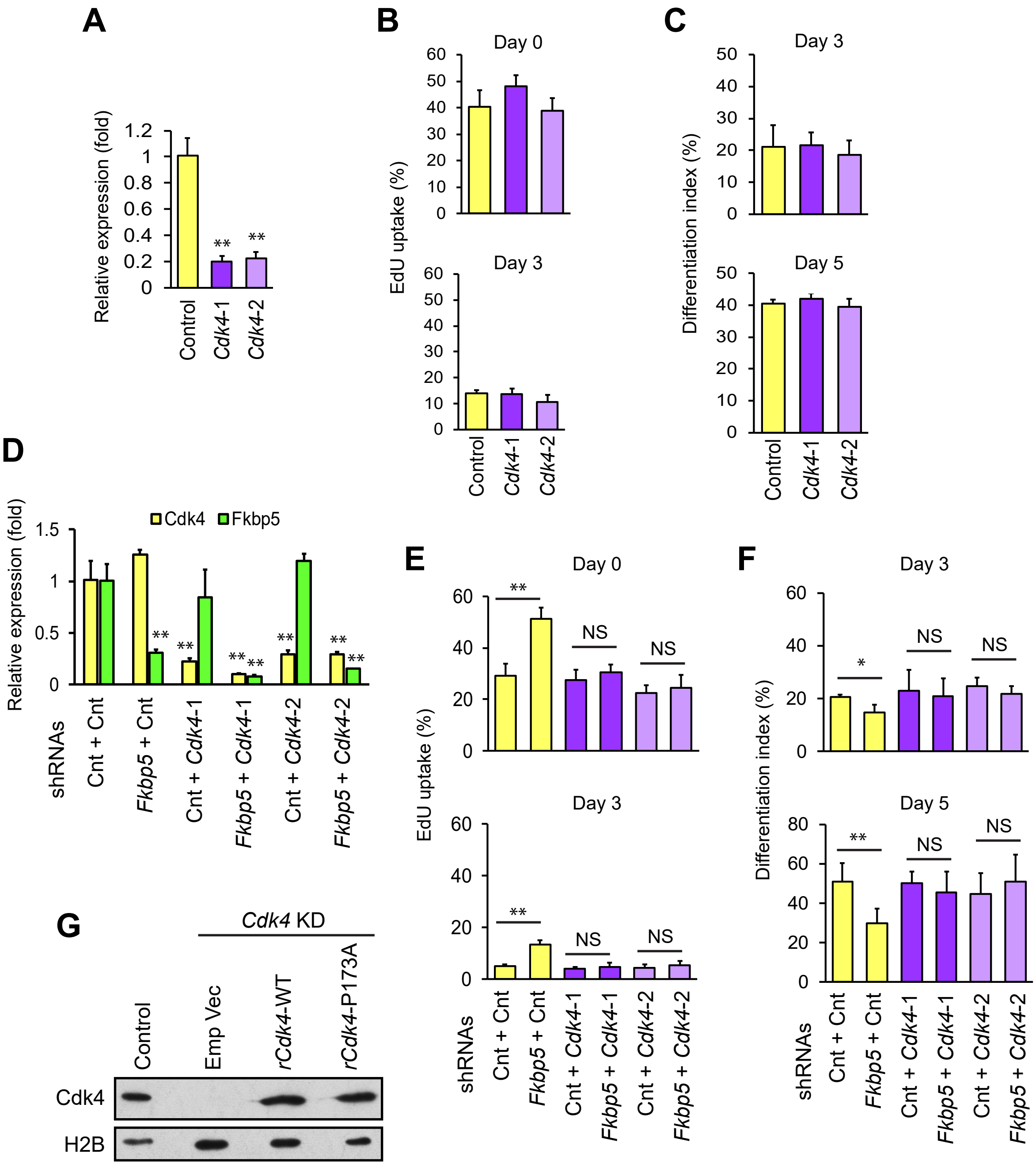

A
Relative expression (fold)
1.2
1
0.8
0.6
0.4
0.2
0
**
**
Control
Cdk4-1
Cdk4-2
B
Day 0
EdU uptake (%)
60
50
40
30
20
10
0
Day 3
Control
Cdk4-1
Cdk4-2
C
Day 3
Differentiation index (%)
50
40
30
20
10
0
Day 5
Control
Cdk4-1
Cdk4-2
D
Relative expression (fold)
Cdk4
Fkbp5
1.5
1
0.5
0
**
**
**
**
**
**
**
**
shRNAs
Cnt + Cnt
Fkbp5 + Cnt
Cnt + Cdk4-1
Fkbp5 + Cdk4-1
Cnt + Cdk4-2
Fkbp5 + Cdk4-2
E
Day 0
EdU uptake (%)
60
40
20
0
**
NS
NS
Day 3
**
NS
NS
shRNAs
Cnt + Cnt
Fkbp5 + Cnt
Cnt + Cdk4-1
Fkbp5 + Cdk4-1
Cnt + Cdk4-2
Fkbp5 + Cdk4-2
F
Day 3
Differentiation index (%)
60
40
20
0
*
NS
NS
Day 5
80
60
40
20
0
**
NS
NS
shRNAs
Cnt + Cnt
Fkbp5 + Cnt
Cnt + Cdk4-1
Fkbp5 + Cdk4-1
Cnt + Cdk4-2
Fkbp5 + Cdk4-2
G
Cdk4 KD
Control
Emp Vec
rCdk4-WT
rCdk4-P173A
Cdk4
H2B

Figure S7. Requirement of Cdk4 P173 for the inhibition of C2C12 cell proliferation by Fkbp5, Related to Figure 7.

(A) Relative expression levels of *Cdk4* mRNA in KD C2C12 cells with two shRNAs against *Cdk4*. The value obtained with the control shRNA was defined as 1.0.
(B and C) EdU uptake (B) and the differentiation index (C) in the *Cdk4* KD cells.
(D) Relative expression levels of *Cdk4* and *Fkbp5* mRNAs in double KD C2C12 cells. Cnt indicates the control shRNA.
(E and F) EdU uptake (E) and the differentiation index (F) in the double KD cells.
(G) Western blotting of overexpressed r*Cdk4*-WT and r*Cdk4*-P173A in the double KD cells.
* $p < 0.05$ and ** $p < 0.01$ with Student's t-test in comparison to the control cells (D, Cnt + Cnt) or between the two conditions indicated by the bar. NS indicates that the difference was statistically not significant. Data are presented as mean + SD of technical triplicates.

Table S1. shRNA clones. Related to Figures 2, 3, 4, 7, S2, S3, S4, and S7.

Gene	Manufacturer	Catalog #
Control	Sigma-Aldrich	SHC016-1EA
Cdk4-1	GE Life Sciences	TRCN0000023176
Cdk4-2	GE Life Sciences	TRCN0000000364
Fkbp4-1	GE Life Sciences	TRCN0000111861
Fkbp4-2	GE Life Sciences	TRCN0000111862
Fkbp5-1	GE Life Sciences	TRCN0000111799
Fkbp5-2	GE Life Sciences	TRCN0000111796

Table S2. Sequences of qPCR primers. Related to Figures 3, 7, S2, and S7.

Gene	Forward	Reverse
MyoD	TGAGCAAAGTGAATGAGGCCTTCG	TGCAGACCTTCGATGTAGCGGAT
Myogenin (*Myog*)	CCCTATTTCTACCAGGAGCCCCAC	GCGCAGGATCTCCACTTTAGGCAG
Myomaker (*Mymk*)	ATCGCTACCAAGAGGCGTT	CACAGCACAGACAAACCAGG
MHC3 (*Myh3*)	CACCTGGAGAGGATGAAGAAGAA	AAGACTTGACTTTCACTTGGAGTTTATC
Ckm	CTCAGCAAGCACAACAATCAC	GATGACATCGTCCAGAGTGAAG
Gapdh	TGCACCACCAACTGCTTAG	GATGCAGGGATGATGTTC
Cdk4	CTGATGGATGTCTGTGCTACTT	AGGTGCTTTGTCCAGGTATG
Fkbp4	TTCCATCGTGTACCTCAAACC	TCTTCAGCCGCACTTCATAC
Fkbp5	GAAAGGCGAGGGATACTCAAA	CCACATCTCGGCAATCAAATG
*Hsp90*b	CCTCAAAGAAGACCAGACAGAG	CTCCCGTTCCTTCTCCAAATAG