SUPPLEMENTAL FIGURE LEGENDS

Supplementary Figure 1. Generation and validation of A420P and CBM mutant iPSCs through CRISPR/CAS9 gene editing.

The single-stranded oligodeoxynucleotides (ssODN), 150bp DNA sequence was used for homologous recombination and aligned with the DNA sequence of human *ATP1A1* gene (Panel A). *Tse*I restriction site was introduced for clone selection. Genotyping PCR amplified the DNA sequence flanking the target region from the genomic DNA of wild type human iPSCs and three A420P mutant iPSCs clones (Panel B), further validated by DNA sequencing. Panel C shows the DNA sequences in the reverse direction where both wild type A420 and the mutant P420 reverse sequences are highlighted in blue-lined boxes). An absence of off-target effect was verified through genotyping PCR and DNA sequencing. DNA sequences flanking the CBM region of *ATP1A2*, *ATP1A3* and *ATP1A4* genes (panel D, E and F), top-matched regions for gRNAs used in generation of mCBM (panel G and H), and A420P (panel I, J and K) mutations through CRISPR gene editing were PCR-amplified from the genomic DNA of iPSCs. The amplicons were gel-purified and sequenced. All sequences were 100% matched with NCBI human genomic DNA database (wild type); no abnormal mutations were found. *FAM* short for the matched sequence in the gene *FAM120AOS* Ensembl accession code ENSG00000188938; LY86 for *LY86-AS1*, Ensembl accession code ENSG00000216863; CAD12, Ensembl accession code ENSG00000154162; CBF for the gene *CBFA2T2,* Ensembl accession code ENSG00000079699; NOD1, Ensembl accession code ENSG00000106109.

Supplementary Figure 2. Sequential induction of marker genes' expression during iSkm differentiation from wild type human iPSCs.

Panel A illustrates the consecutive steps of skeletal muscle cells (Skm) differentiation from wild type human iPSCs. Marker genes' expression indicates specific differentiation stage. The first step induced in SKM01 for one week gives rise to myogenic precursor cells, including the early induction of mesoderm (characterized by *T*, *MIXL1* and *MSGN1*), presomitic mesoderm (marked by *PAX3*, panel B), as well as decreased expression of pluripotency markers *NANOG* and *OCT4*. The second induction with SKM02 for one week led to myoblast cells, marked by myogenic transcription factors *MYOD* (panel C) and *MYOG* (panel D). The final step with SKM03 for one week induces the formation of myotube cells, actively expressing genes encoding proteins forming cell structure, *MYH8* (E), *CAV3* (F) and *TNNT1* (G). mRNA Induction for each gene was calculated and compared to the parental/un-induced iPSC control by one-way ANOVA. RT-qPCR results were shown as mean±SE. N=3 for each gene group, **p<0.01, ***p<0.001.

Supplemental Figure 3. Verification of defective Skm differentiation in an alternate clone of iPSC-mCBM (clone mCBM#2).

mRNA induction for the myogenic marker genes was compared to that in wild type iPSCs. As observed for the representative clone shown in Figure 2, CBM mutation dramatically inhibited induction of myogenic marker genes *MYOD*, *MYOG*, *TNNT1*, *MYH8*, *MYH3* and *CAV3* (panel A-F). Moreover, immunostaining for β-catenin (panel G) in this clone also mimics the results in Figure 6. Data are expressed as mean±SE and analyzed with Student's t-test. N=3 for WT and mCBM, ***p<0.001.

Supplemental Figure 4. Adipocyte differentiation from human iPSCs.

Schematic for *in vitro* differentiation of adipocytes from human iPSCs (panel A). As their wild type counterparts, mutant iPSCs (iPSCs-mCBM) readily differentiated into adipocytes, activating mRNA expression for the marker genes to levels equivalent to (*PPARG* and *FASN*), or even higher than (*ADIPOQ* and *FABP4*) wild type iPSCs-derived adipocytes (Adi-WT). Of note, mRNA expression for these markers was not detected (short for n/d) in MSC (panel B-E, real-time RT-qPCR, n=3-6, unpaired t-test). Adi-mCBM developed a weaker oil red O staining than Adi-WT (panel F, representative images from three independent repeats and quantitation of lipid droplets per cell, n=8, scale bar = 500 μm. Data are shown as mean ± SE. **, $P < 0.01$; ***, $P < 0.001$. MSC stands for mesenchymal stem cell; Adi stands for Adipocytes differentiated from iPSCs-WT (Adi-WT) or iPSC-mCBM (Adi-mCBM).

Supplementary Figure 5. Characterization of iSkm-A420P with immunostaining and gene expression.

Comparison of fold induction for the myogenic marker genes between iSkm-WT and iSkm-A420P (panel A). Data were analyzed with t-test and shown as mean ± SE. N=4 for each group, **, $P < 0.01$; ***, $P < 0.001$. iSkm-A420P were positively stained with skeletal muscle antibodies, anti-sarcomeric alpha actinin antibody and anti-alpha skeletal muscle actin. Red signals indicate the positive staining of differentiated muscle fibers while blue signals show the nuclei (panel B). C2C12 myoblast cells were used to evaluate these antibodies. The positive staining for Actinin (panel C) and Actin (panel D) was observed in the differentiated C2C12 cells (five days in the differentiation medium, C2C12 5d-Diff), but absent in the undifferentiated iPSCs-WT or iPSC-mCBM.

Supplementary Figure 6. Rescue iPSCs-mCBM with transgene *Myod1*.

Comparison of *MYOD* and *MYF5* induction in the myoblast (SKM02) and myotube (SKM03) differentiated from wild type iPSCs indicates *MYOD* as a major player in the Skm differentiation system used. Induction fold of each gene was calculated in the induced cells (SKM02 or SKM03) normalized against their parental iPSCs (panel A). Mouse *Myod1* cDNA was transfected into iPSCs-mCBM cells through lentivirus. Compared to the parental mCBM cells, the mouse *Myod1* (mMyod1) mRNA is highly increased in transfected Myod-mCBM cells (panel B). Muscle fusion in iSkm was quantified (panel C). Fusion index was calculated as the ratio of number of nuclei per myocyte with the positive staining of myosin heavy chain antibody (shown in Figure 3), to the number of total nuclei. Data reported as mean ± SE. N=3 for each gene group. ***p<0.001, N.S.: not significant by student's t-test).

Supplementary Figure 7. Heatmap of mCBM regulated genes associated to pluripotency and Wnt signaling.

RNA-seq data analyses for iPSCs-mCBM v.s. iPSCs-WT (panel A for pluripotency markers, B and D for Wnt and TGF-β signaling, respectively), SKM01 induced myogenic precursor cells from iPSCs-mCBM and iPSCs-WT (panel C and E for Wnt and TGF-β signaling, respectively) were analyzed and plotted through Cytoscape. Colors on the heatmap present the average gene Log2Fold of Change in reads of mCBM vs. WT triplicate samples.

Supplementary Figure 8. Protein expression and distribution in myogenic precursor cells.

Western blots show protein alteration in myogenic precursor cells, SKM01-induced human iPSCs. Blot intensities in panel A are quantified in panel B. Data are analyzed with t-test and shown as mean ± SE. N=4 for each group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Immunofluorescence staining in myogenic precursor cells shows the localization of β-catenin is shifted away from cell membrane by CBM mutation (panel C), reminiscent of the parental iPSCs in Figure 6.

Supplementary Figure 9. Premature activation of Wnt signaling by CBM mutation.

Compared to wild type iPSCs (iPSCs-WT), CBM mutant iPSCs (iPSCs-mCBM) increased *PAX3* (panel A) and *MSGN1* (panel B) mRNA expression. However, this untimely activation desensitized iPSCs-mCBM of further induction in *PAX3* mRNA by Wnt ligands, CHIR (Figure 5), or SKM01 medium (panel A). Skm01-WT and Skm01-mCBM stand for the SKM01-induced wild type or CBM mutant iPSCs, i.e. myogenic precursor cells. Data was analyzed with one-way ANOVA. N=3 for each gene group, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

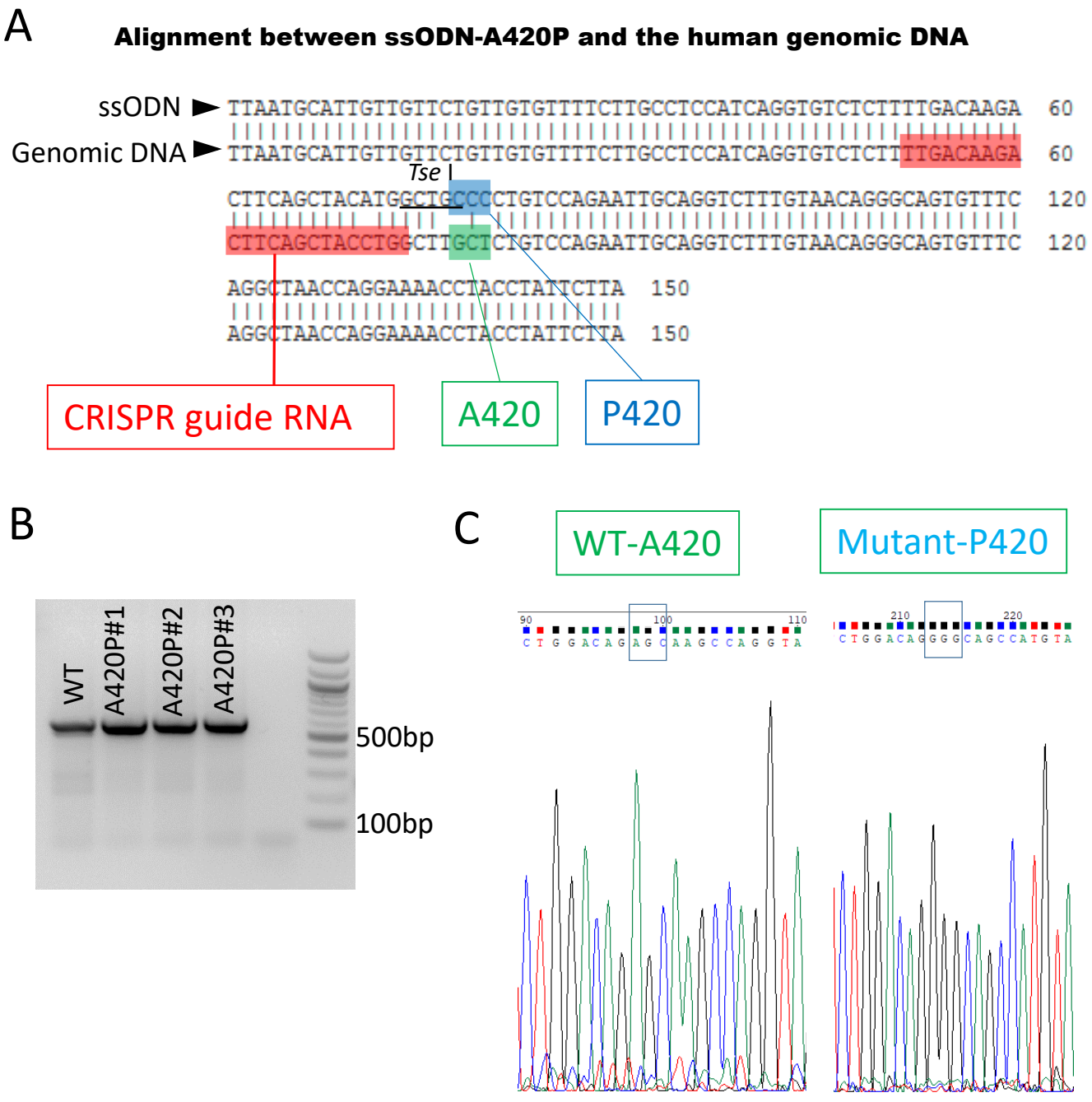
Supplementary Table 1. RNA-seq profiling gene expression between wild type iPSCs (iPSCs-WT) and CBM mutant iPSCs (iPSCs-mCBM).

Supplementary Table 2. RNA-seq profiling gene expression between SKM01-WT and SKM01-mCBM, SKM01-induced myogenic precursor cells from wild type or CBM mutant iPSCs.

Supplementary Video 1. Movement recording for the induced skeletal muscle cells from wild type iPSCs (iSkm-WT). File A is AVI format for PC while file B is MOV format for Mac.

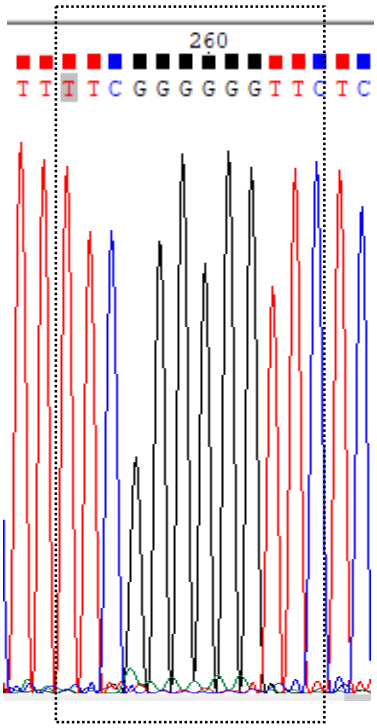
Supplementary Video 2. Movement recording for the induced skeletal muscle cells from A420P mutant iPSCs (iSkm-A420P). File A is AVI format for PC while file B is MOV format for Mac.

Suppl. Fig. 1

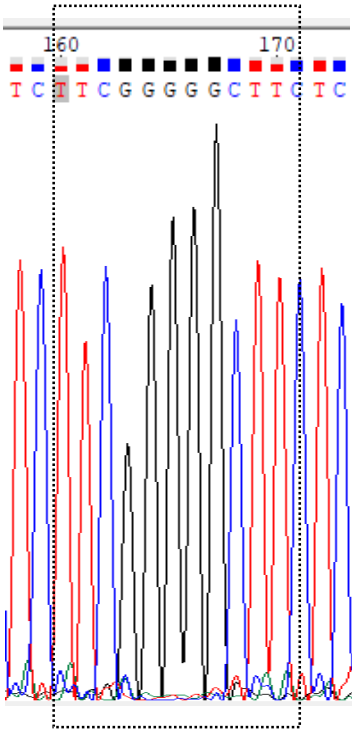

A
Alignment between ssODN-A420P and the human genomic DNA
ssODN
Genomic DNA
TTAATGCATTGTTGTTCTGTTGTGTTTTCTTGCCTCCATCAGGTGTCTCTTTTGACAAGA 60
TTAATGCATTGTTGTTCTGTTGTGTTTTCTTGCCTCCATCAGGTGTCTCTTTTGACAAGA 60
Tse I
CTTCAGCTACATGGCTGCCCTGTCCAGAATTGCAGGTCTTTGTAACAGGGCAGTGTTTC 120
CTTCAGCTACCTGGCTTGCTCTGTCCAGAATTGCAGGTCTTTGTAACAGGGCAGTGTTTC 120
AGGCTAACCAGGAAAACCTACCTATTCTTA 150
AGGCTAACCAGGAAAACCTACCTATTCTTA 150
CRISPR guide RNA
A420
P420
B
WT
A420P#1
A420P#2
A420P#3
500bp
100bp
C
WT-A420
Mutant-P420
90
100
110
C T G G A C A G A G C A A G C C A G G T A
210
220
C T G G A C A G G G G C A G C C A T G T A

Suppl. Fig. 1 continued D-G

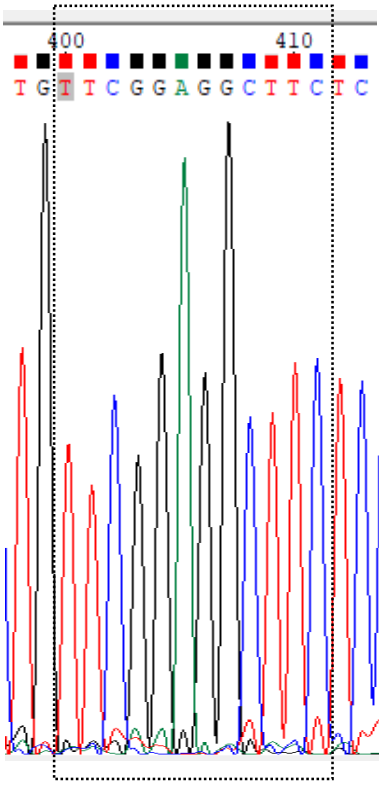
D *ATP1A2* CBM sequence



E *ATP1A3* CBM sequence



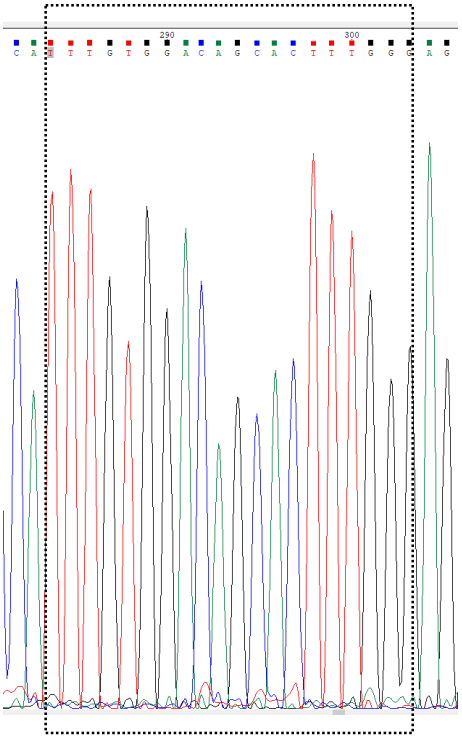
F *ATP1A4* CBM sequence



G

gRNA:TTTGTCGGCAGCTCTTTGGG

FAM: TTTGTGGACAGCACTTTGGG

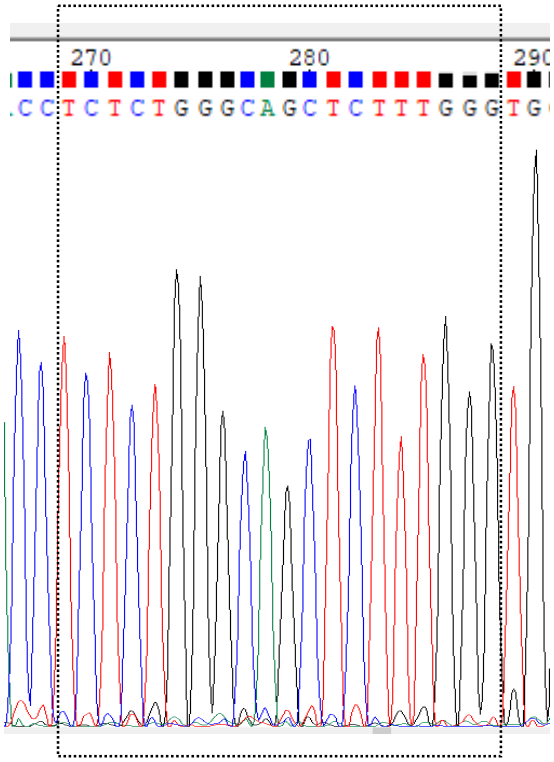


Suppl. Fig. 1 continued H-K

H

gRNA: TTTGTCGGCAGCTCTTTGGG

LY86: TCTCTGGGCAGCTCTTTGGG

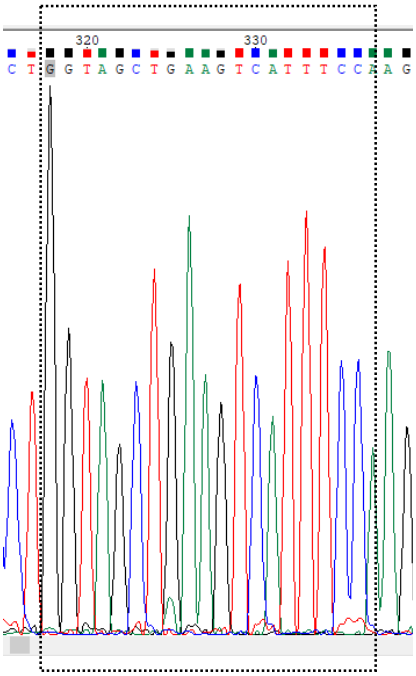


I

gRNA: TTGACAAGACTTCAGCTACC

Reverse: GGTAGCTGAAGTCTTGTCAA

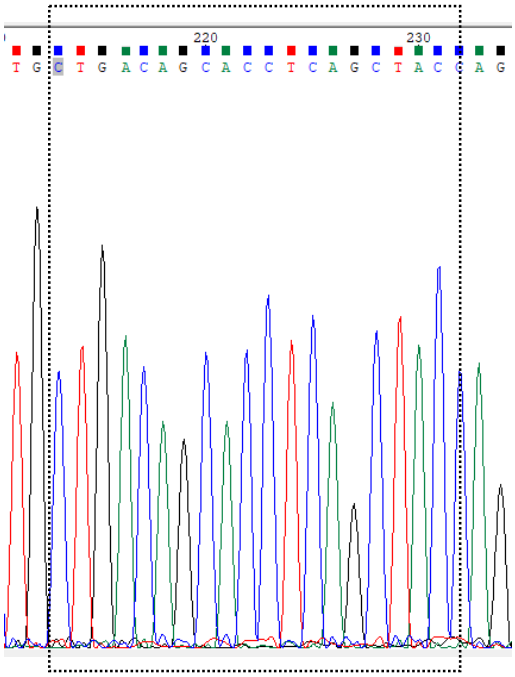
CAD12: GGTAGCTGAAGTCATTTCCA



J

gRNA: TTGACAAGACTTCAGCTACC

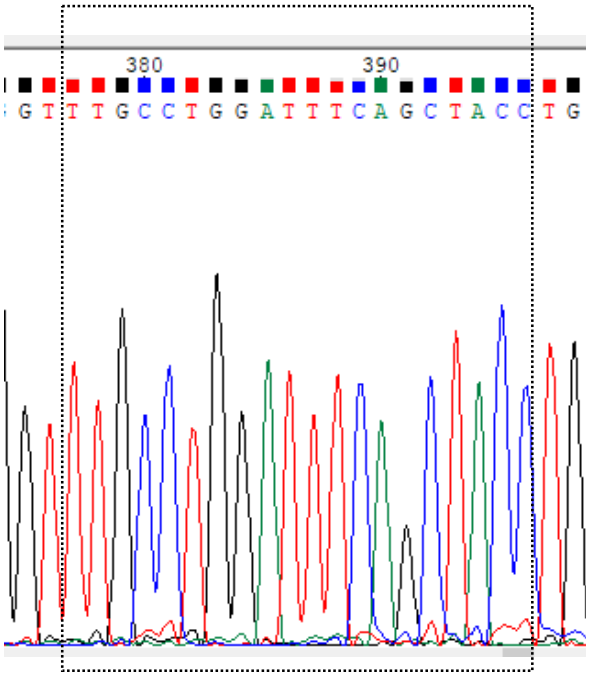
CBF: CTGACAGCACCTCAGCTACC



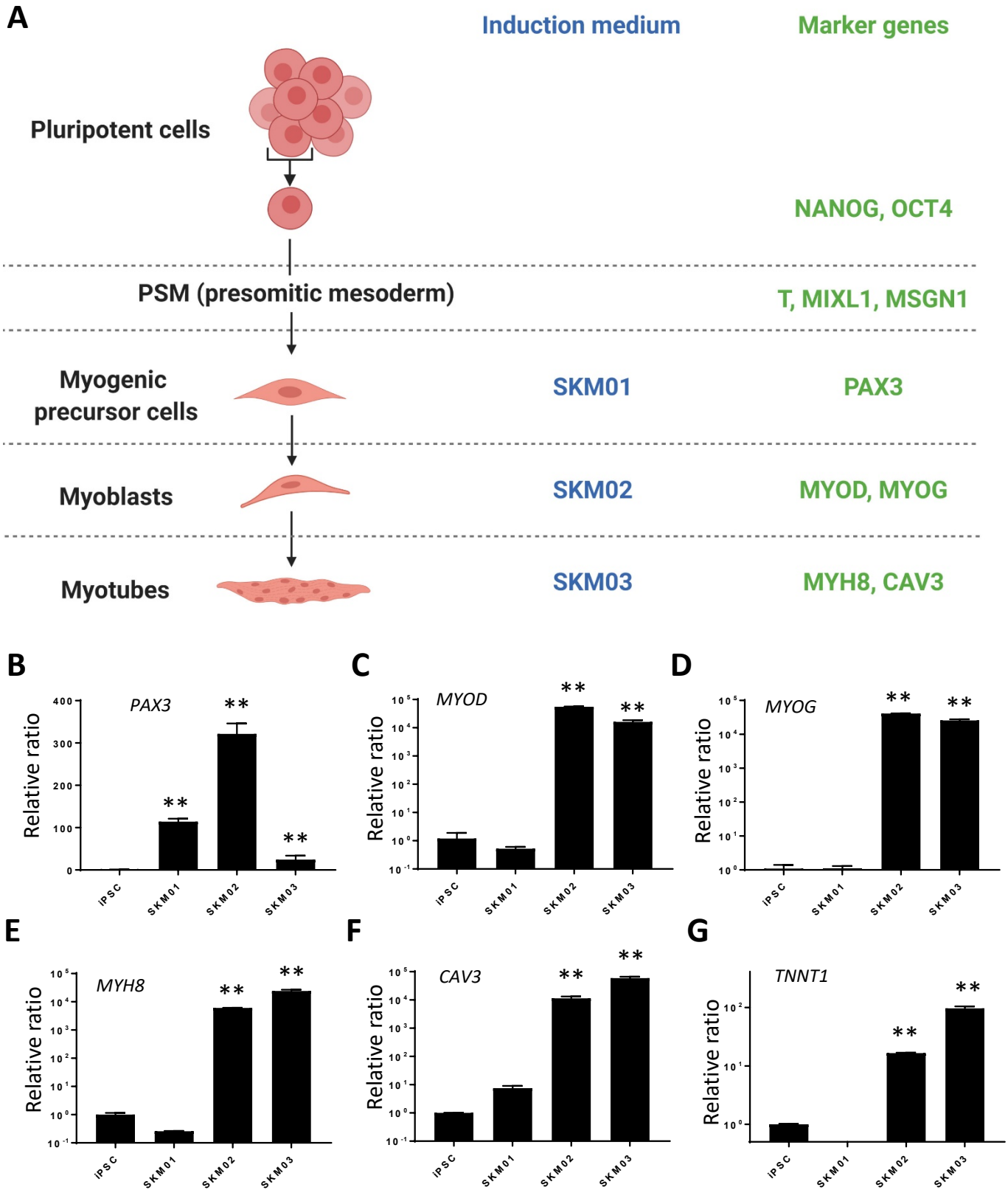
K

gRNA: TTGACAAGACTTCAGCTACC

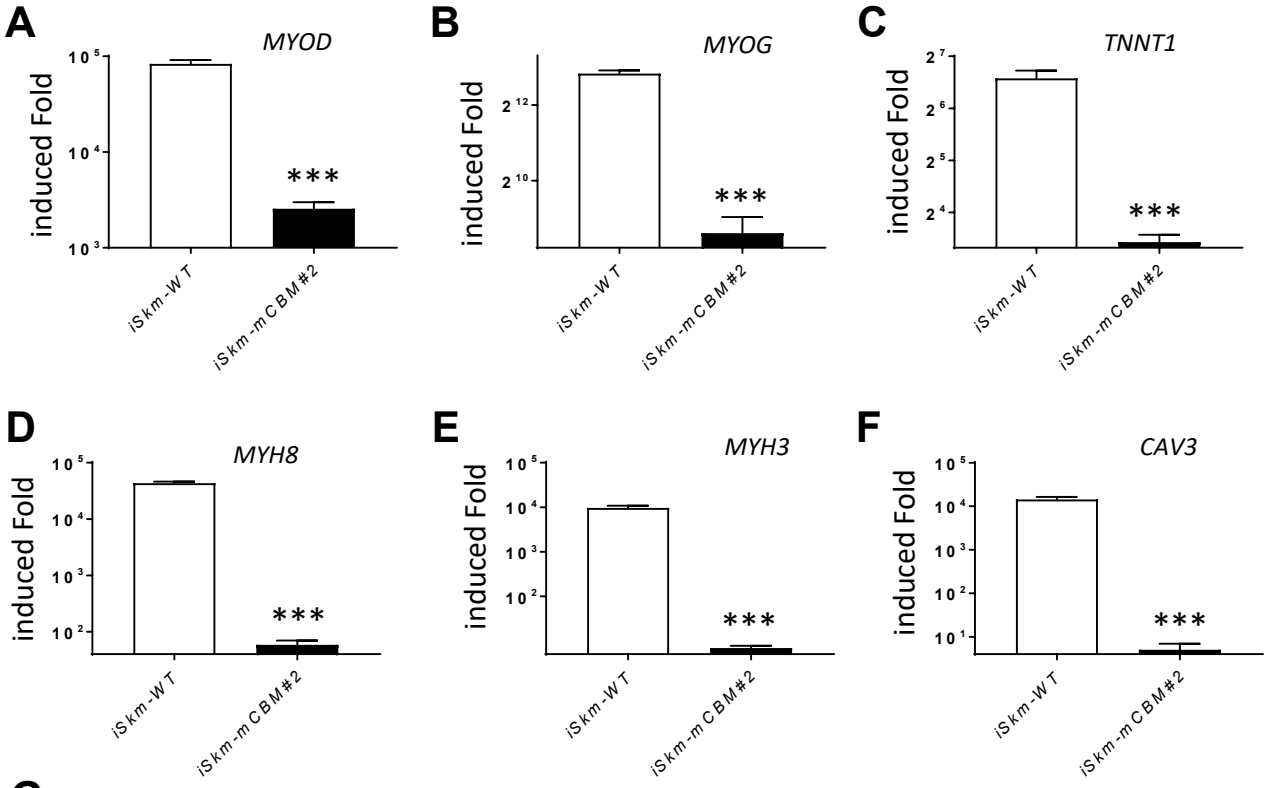
NOD: TTGCCTGGATTTCAGCTACC

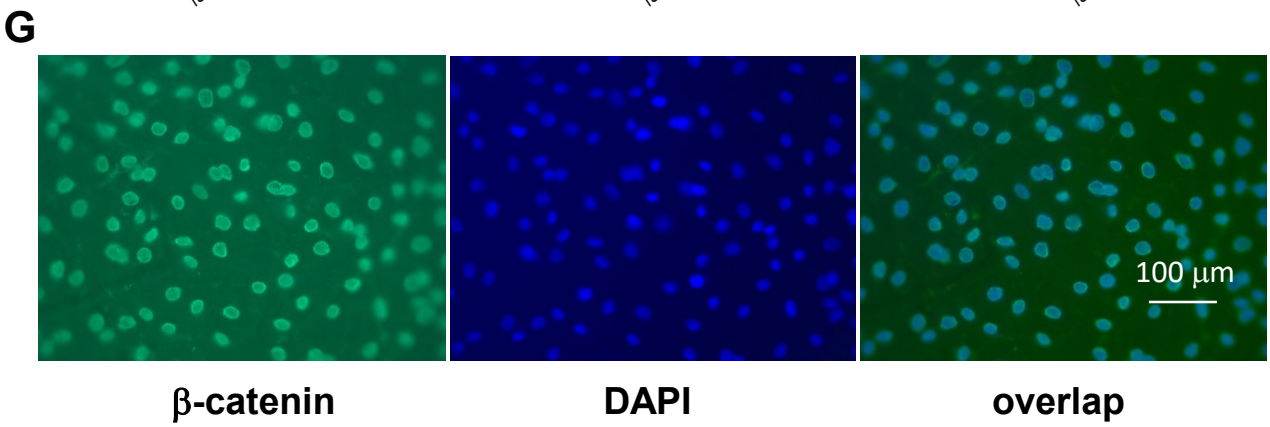


Suppl. Fig. 2

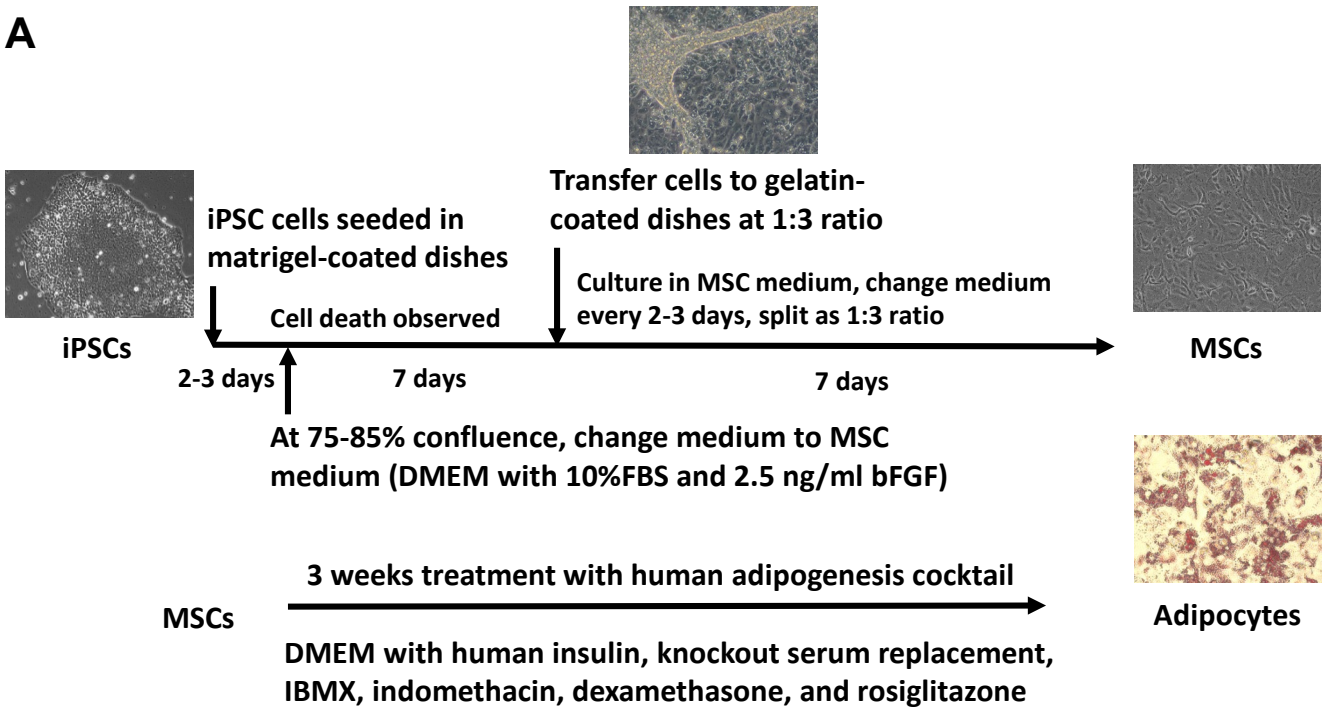

A
Induction medium
Marker genes
Pluripotent cells
NANOG, OCT4
PSM (presomitic mesoderm)
T, MIXL1, MSGN1
Myogenic precursor cells
SKM01
PAX3
Myoblasts
SKM02
MYOD, MYOG
Myotubes
SKM03
MYH8, CAV3
B
PAX3
C
MYOD
D
MYOG
E
MYH8
F
CAV3
G
TNNT1
Relative ratio
iPSC
SKM01
SKM02
SKM03
**

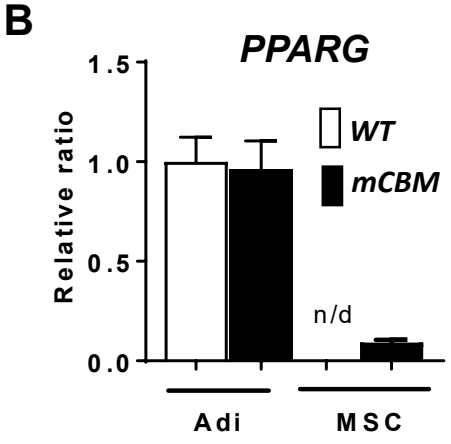
Suppl. Fig. 3

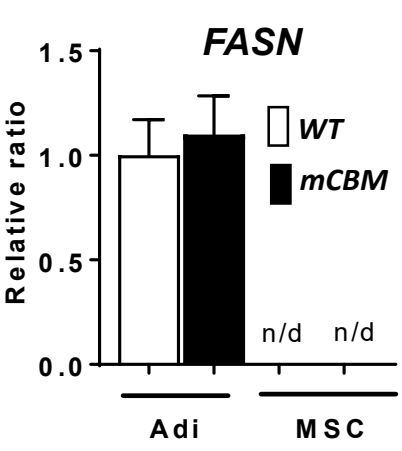

A
MYOD
induced Fold
***
iSkm-WT
iSkm-mCBM#2
B
MYOG
induced Fold
***
iSkm-WT
iSkm-mCBM#2
C
TNNT1
induced Fold
***
iSkm-WT
iSkm-mCBM#2
D
MYH8
induced Fold
***
iSkm-WT
iSkm-mCBM#2
E
MYH3
induced Fold
***
iSkm-WT
iSkm-mCBM#2
F
CAV3
induced Fold
***
iSkm-WT
iSkm-mCBM#2

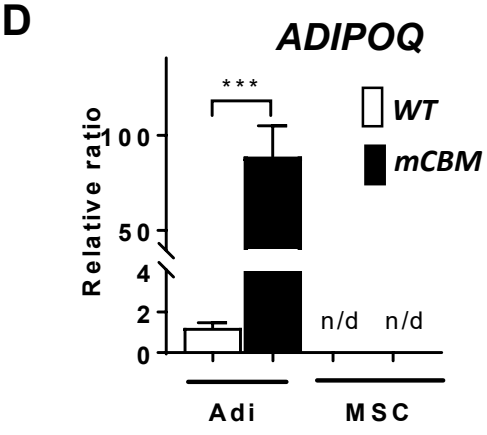

G
100 µm
β-catenin
DAPI
overlap

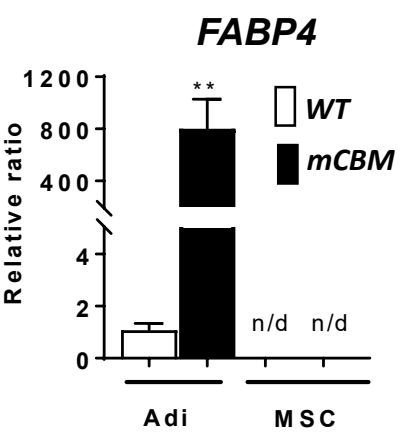
Suppl. Fig. 4


A
iPSCs
iPSC cells seeded in matrigel-coated dishes
Cell death observed
2-3 days
7 days
Transfer cells to gelatin-coated dishes at 1:3 ratio
Culture in MSC medium, change medium every 2-3 days, split as 1:3 ratio
7 days
MSCs
At 75-85% confluence, change medium to MSC medium (DMEM with 10%FBS and 2.5 ng/ml bFGF)
MSCs
3 weeks treatment with human adipogenesis cocktail
DMEM with human insulin, knockout serum replacement, IBMX, indomethacin, dexamethasone, and rosiglitazone
Adipocytes


B
PPARG
Relative ratio
1.5
1.0
0.5
0.0
WT
mCBM
n/d
Adi
MSC

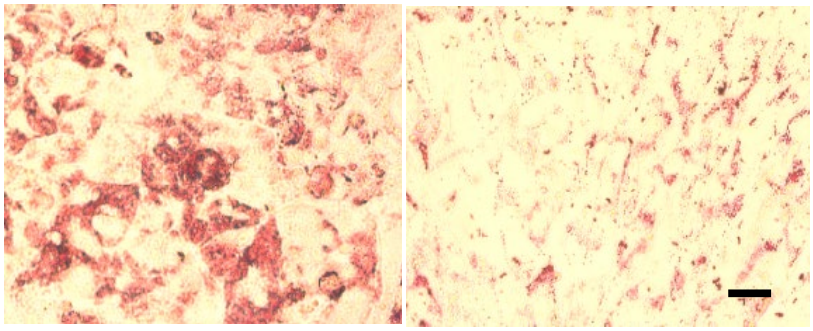

C
FASN
Relative ratio
1.5
1.0
0.5
0.0
WT
mCBM
n/d
n/d
Adi
MSC


D
ADIPOQ
Relative ratio
***
100
50
4
2
0
WT
mCBM
n/d
n/d
Adi
MSC

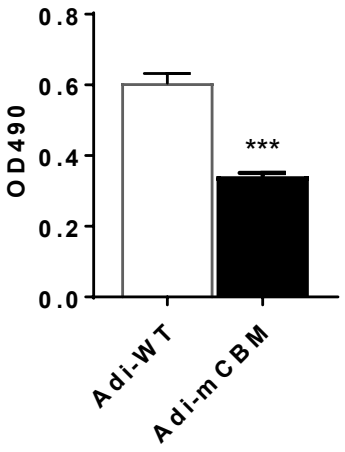

E
FABP4
Relative ratio
**
1200
800
400
4
2
0
WT
mCBM
n/d
n/d
Adi
MSC

Suppl. Fig. 4 continued

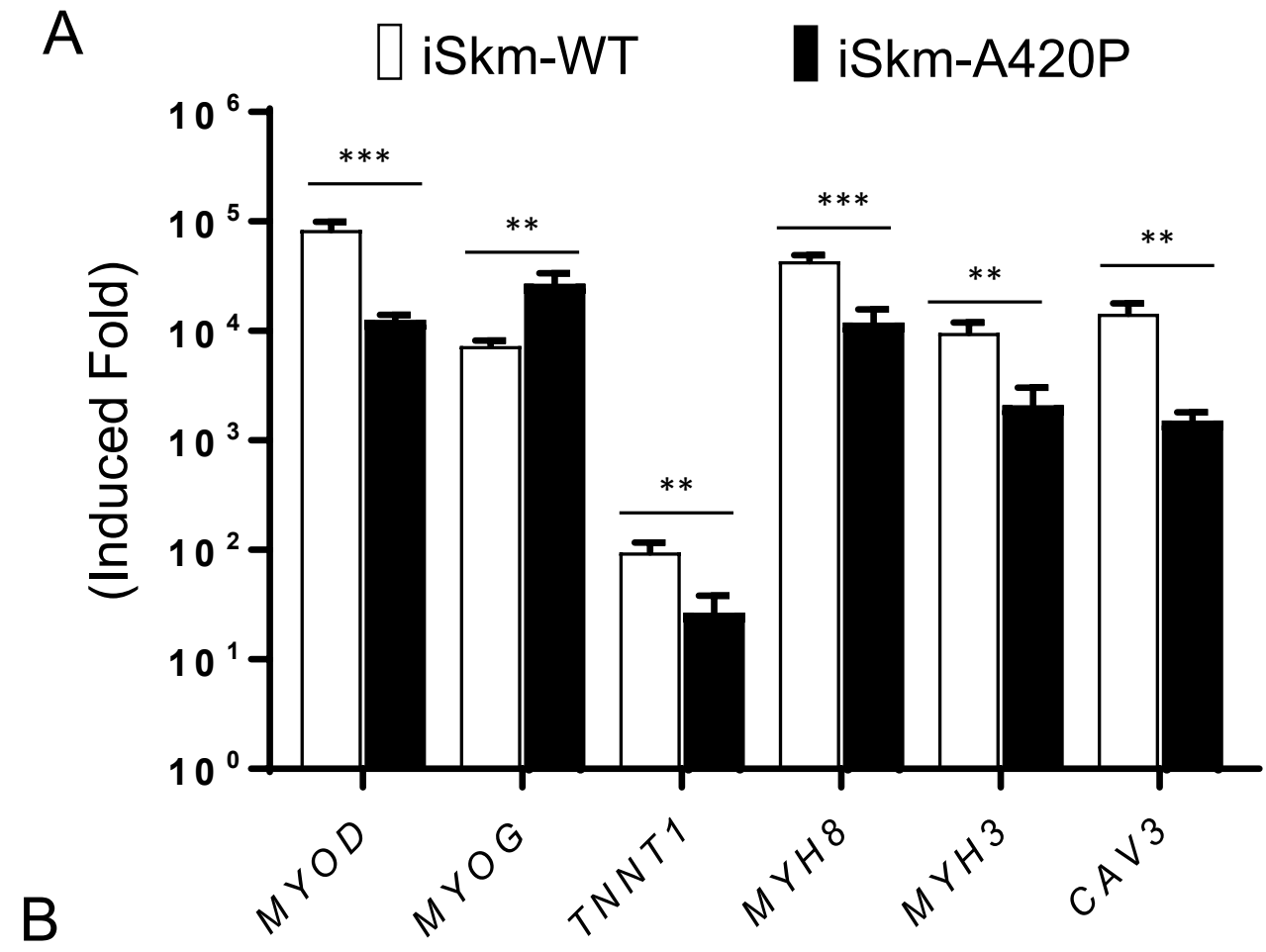
F

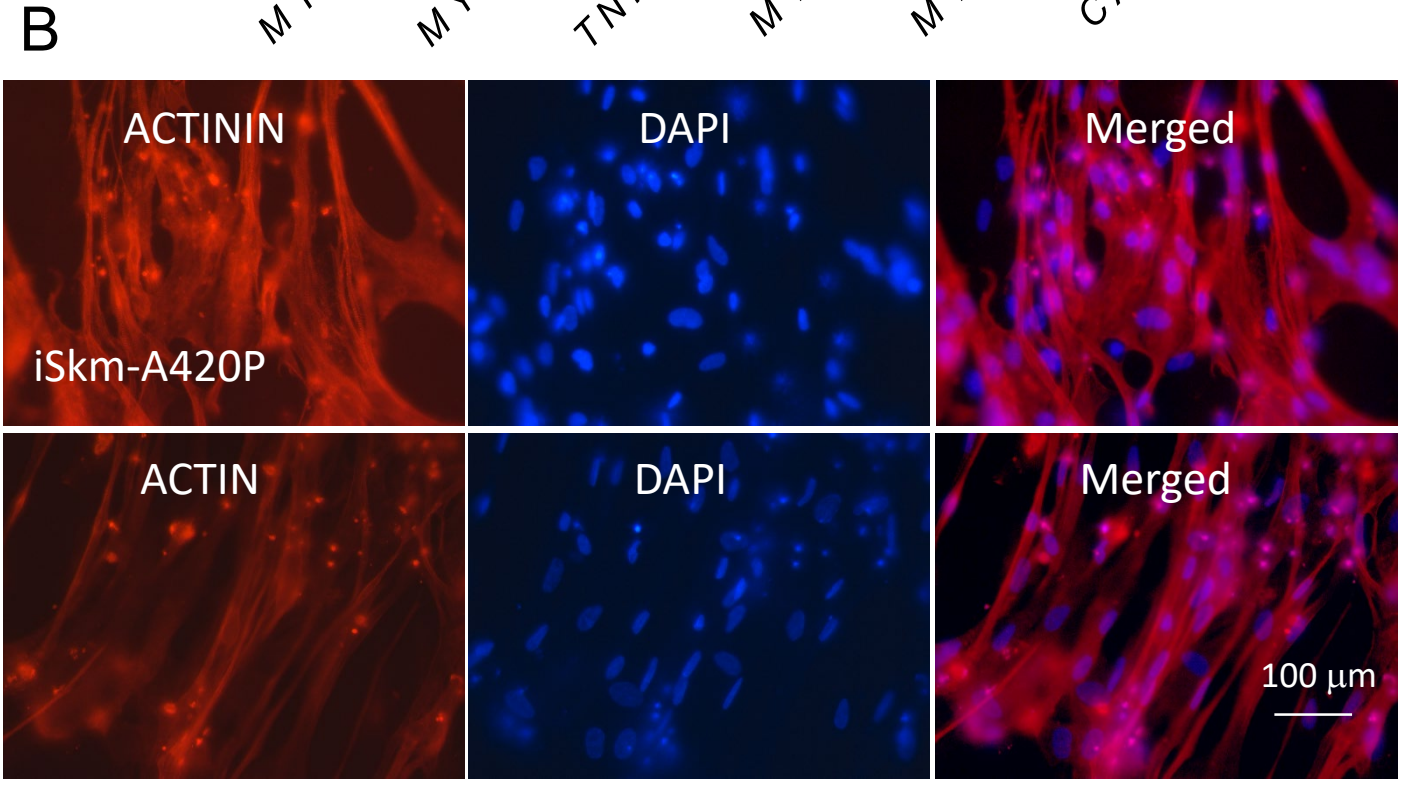


Adi-WT Adi-mCBM

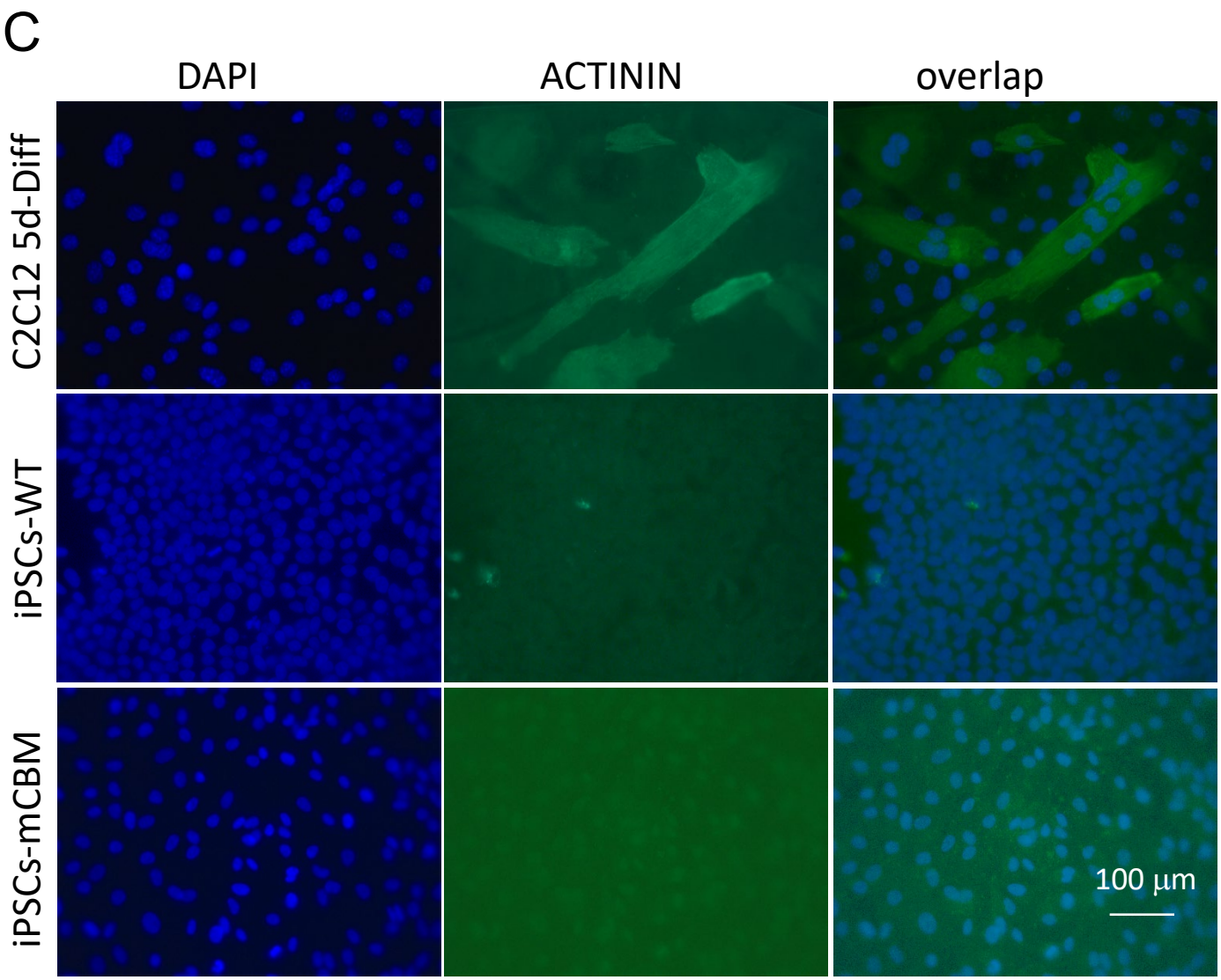


Suppl. Fig. 5

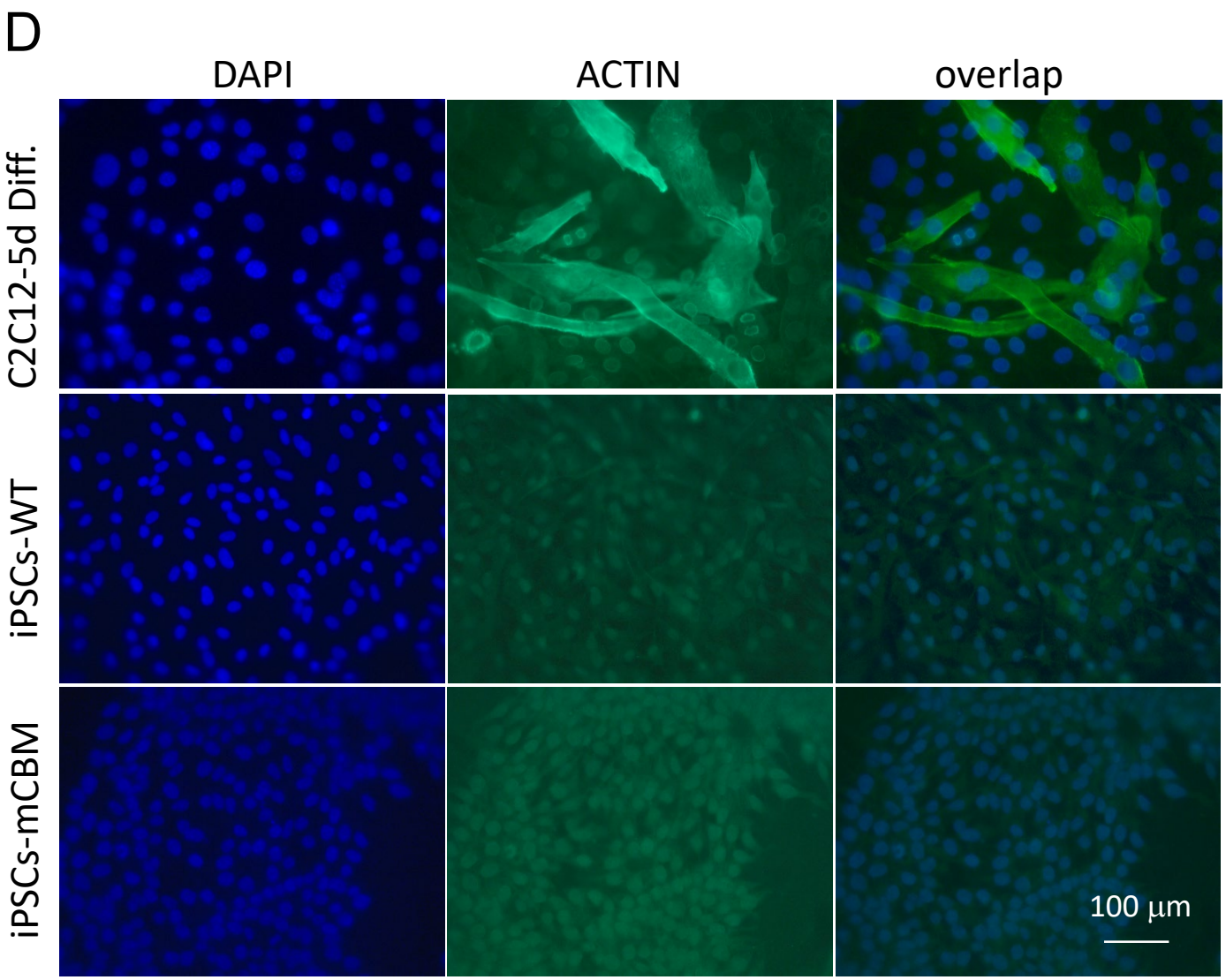

A
iSkm-WT
iSkm-A420P
(Induced Fold)
10 6
10 5
10 4
10 3
10 2
10 1
10 0
***
**
**
***
**
**
MYOD
MYOG
TNNT1
MYH8
MYH3
CAV3


B
ACTININ
DAPI
Merged
iSkm-A420P
ACTIN
DAPI
Merged
100 µm

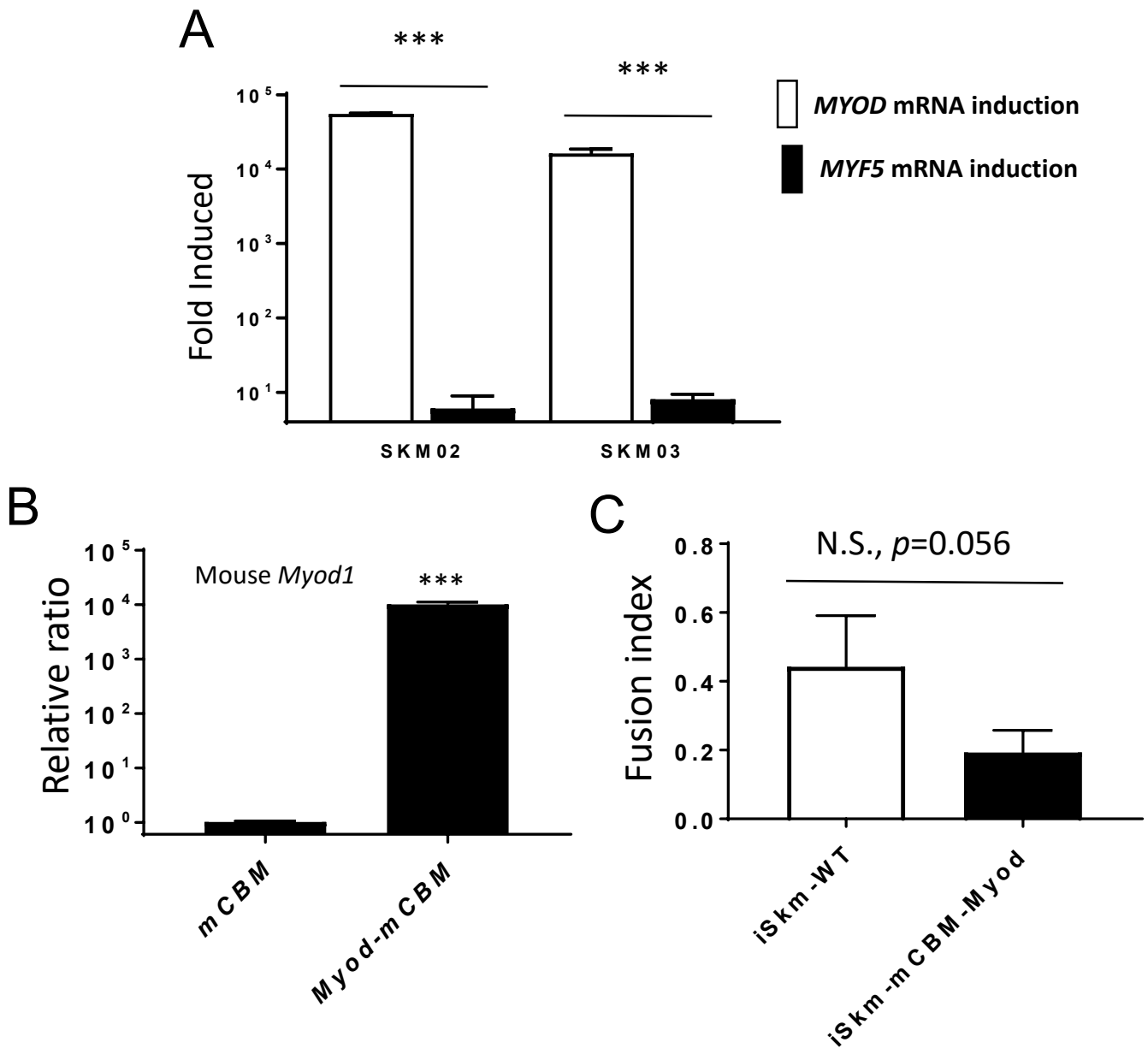
Suppl. Fig. 5 continued C



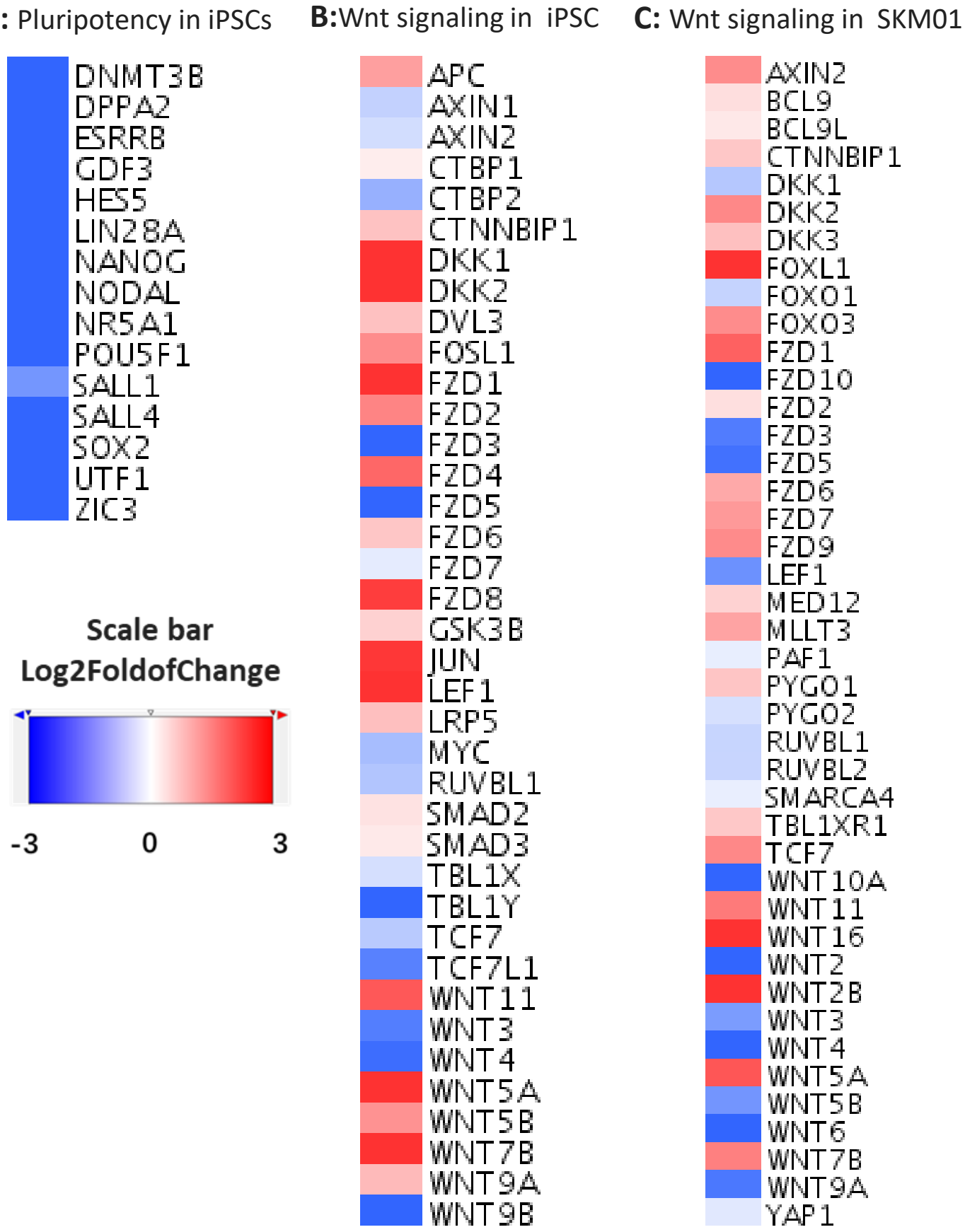
Suppl. Fig. 5 continued D



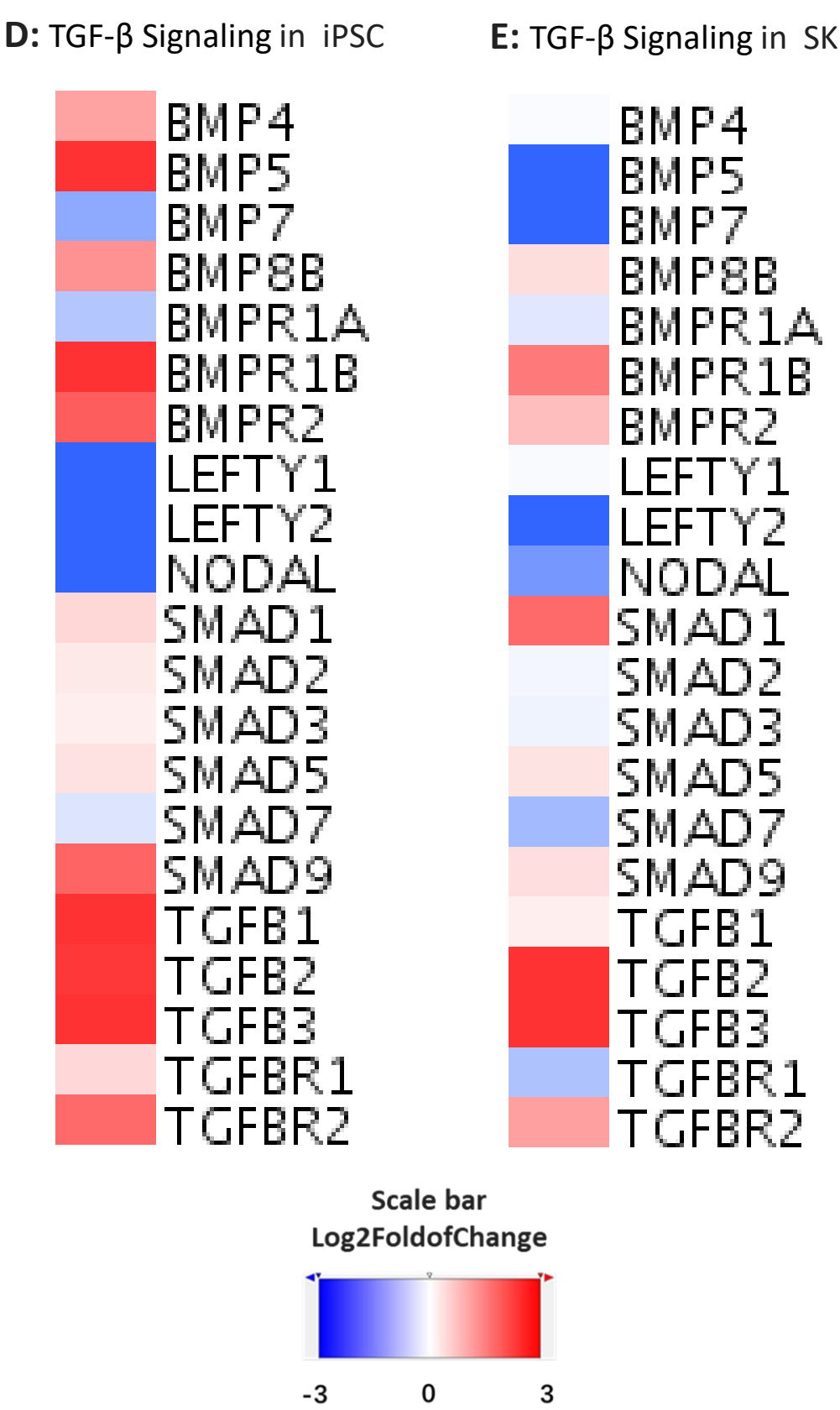
Suppl. Fig. 6


A
***
***
Fold Induced
10 5
10 4
10 3
10 2
10 1
MYOD mRNA induction
MYF5 mRNA induction
SKM02
SKM03
B
Relative ratio
Mouse Myod1
***
10 5
10 4
10 3
10 2
10 1
10 0
mCBM
Myod-mCBM
C
N.S., p=0.056
Fusion index
0.8
0.6
0.4
0.2
0.0
iSkm-WT
iSkm-mCBM-Myod

Suppl. Fig.7

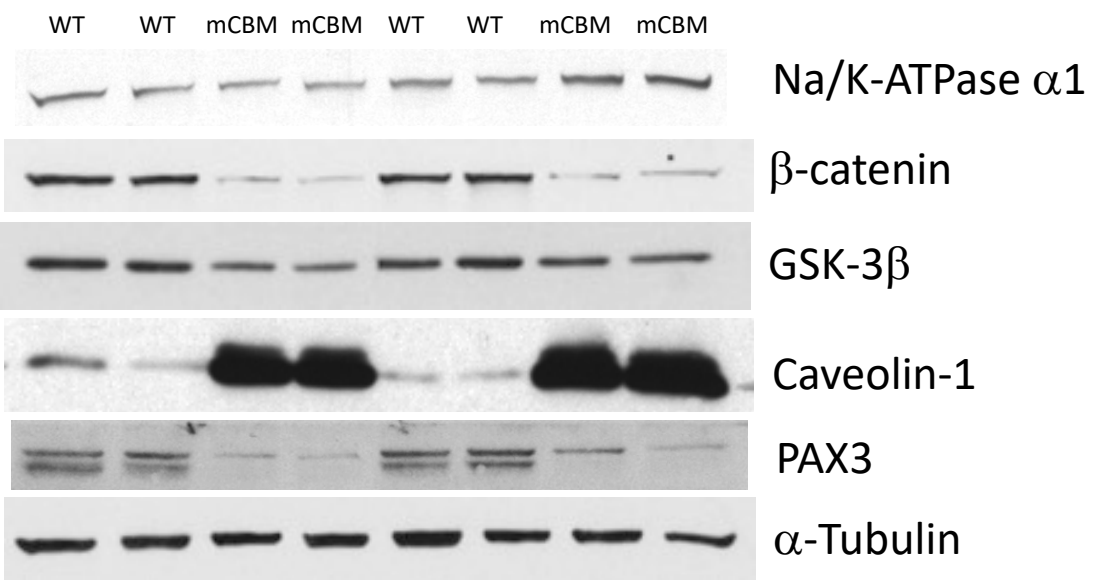

A: Pluripotency in iPSCs
DNMT3B
DPPA2
ESRRB
GDF3
HES5
LIN28A
NANOG
NODAL
NR5A1
POU5F1
SALL1
SALL4
SOX2
UTF1
ZIC3
Scale bar
Log2FoldofChange
-3
0
3
B:Wnt signaling in iPSC
APC
AXIN1
AXIN2
CTBP1
CTBP2
CTNNBIP1
DKK1
DKK2
DVL3
FOSL1
FZD1
FZD2
FZD3
FZD4
FZD5
FZD6
FZD7
FZD8
GSK3B
JUN
LEF1
LRP5
MYC
RUVBL1
SMAD2
SMAD3
TBL1X
TBL1Y
TCF7
TCF7L1
WNT11
WNT3
WNT4
WNT5A
WNT5B
WNT7B
WNT9A
WNT9B
C: Wnt signaling in SKM01
AXIN2
BCL9
BCL9L
CTNNBIP1
DKK1
DKK2
DKK3
FOXL1
FOXO1
FOXO3
FZD1
FZD10
FZD2
FZD3
FZD5
FZD6
FZD7
FZD9
LEF1
MED12
MLLT3
PAF1
PYGO1
PYGO2
RUVBL1
RUVBL2
SMARCA4
TBL1XR1
TCF7
WNT10A
WNT11
WNT16
WNT2
WNT2B
WNT3
WNT4
WNT5A
WNT5B
WNT6
WNT7B
WNT9A
YAP1

Suppl. Fig. 7 continued

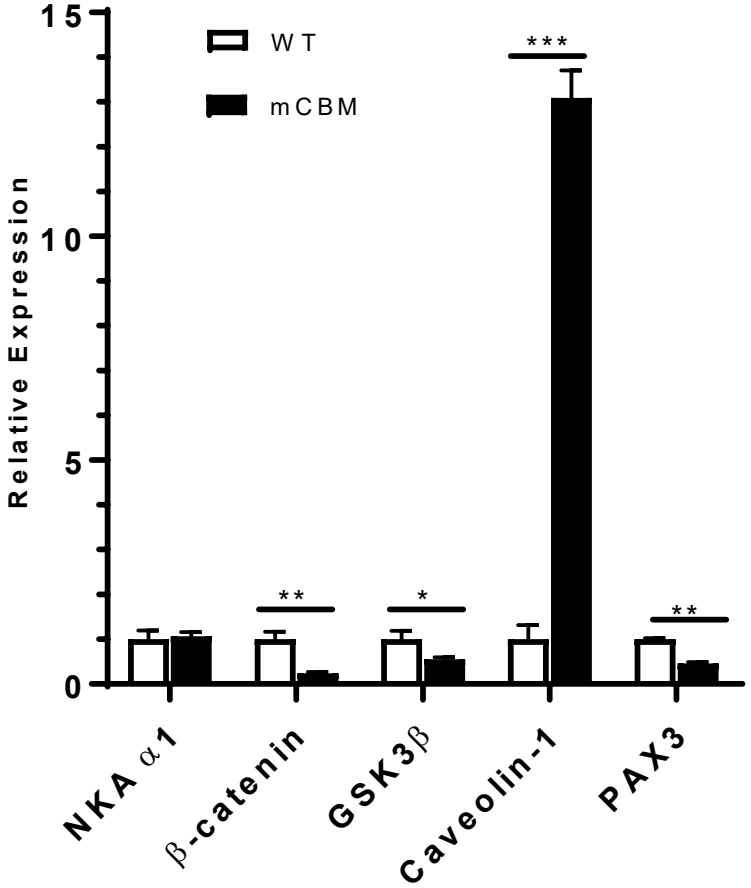


Suppl. Fig. 8

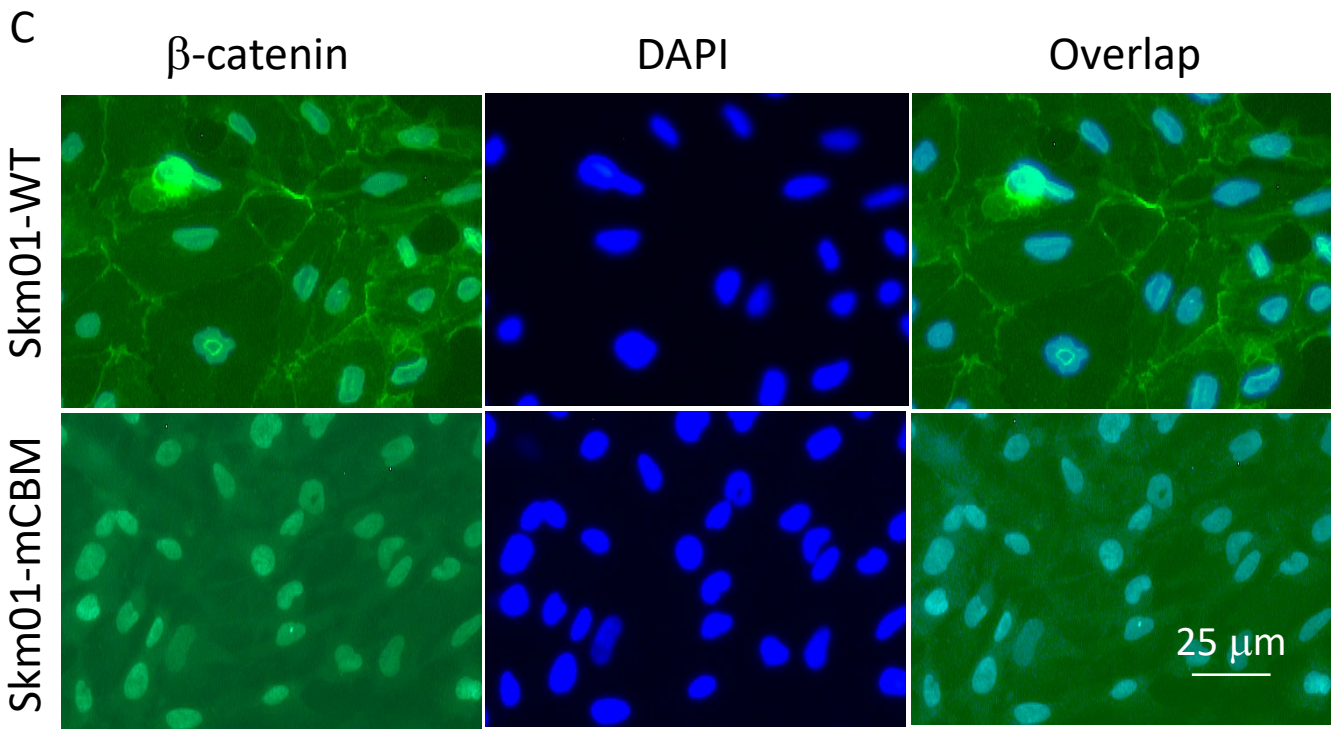
A



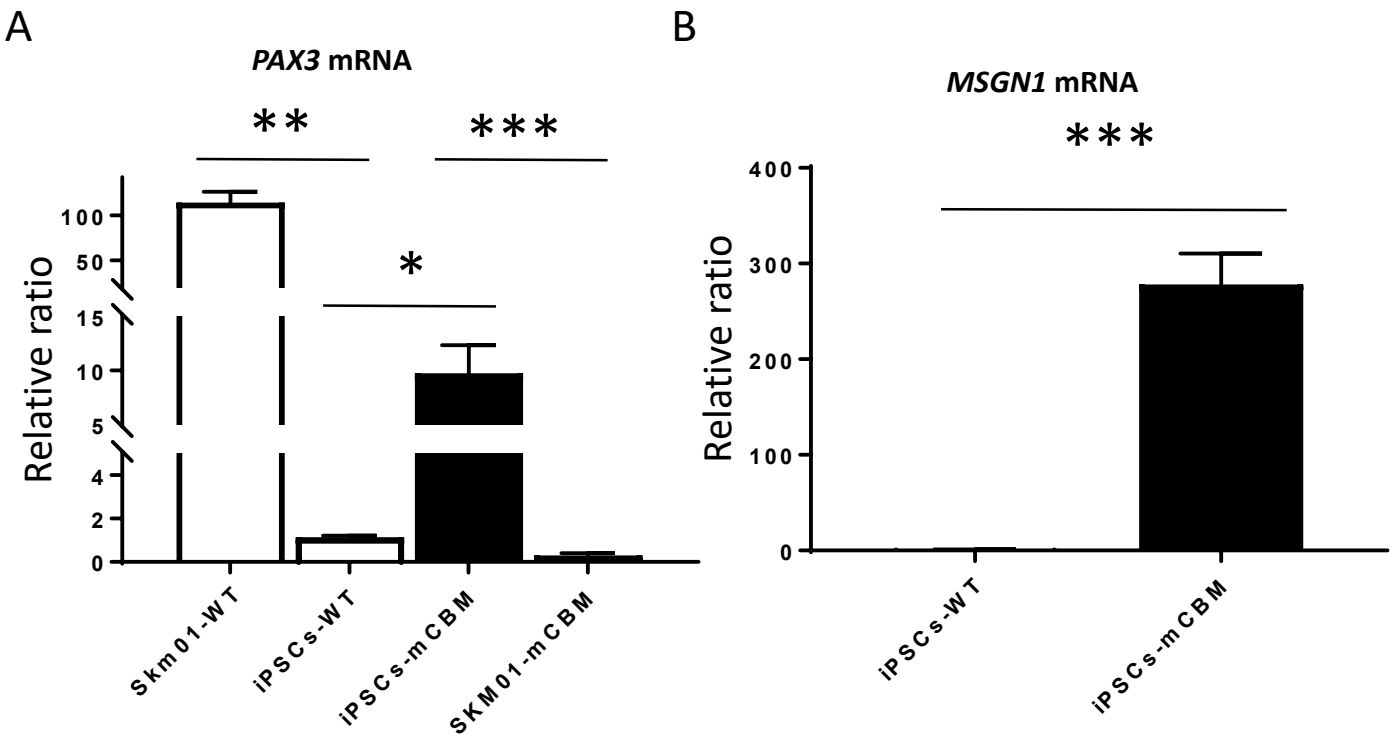
B



Suppl Fig. 8 continued



Suppl. Fig. 9


A
PAX3 mRNA
**
***
*
Relative ratio
100
50
15
10
5
4
2
0
Skm01-WT
iPSCs-WT
iPSCs-mCBM
SKM01-mCBM
B
MSGN1 mRNA
***
Relative ratio
400
300
200
100
0
iPSCs-WT
iPSCs-mCBM