Supplementary Figure 1

a

LVDP-p16:

5' LTR | DsRed ◄ hPGK | cHS4 | Tactb | SPA | ZsG | p16 | 3' LTR

b

NANOG:

5' LTR | TRE ► NANOG | UbC ► rtTA | IRES | Puro | 3' LTR

c

NANOG — 40KDa
β-tubulin — 50KDa

YM   SM   SMN

d

Key
YM (green)
SM (red)
SMN (blue)

Cell Count vs ZsGreen-A

e

% of p16(+) cells

** ****

YM   SM   SMN

f

YM   SM   SMN

100 µm

g

SA-β-gal⁺ Cells (%)

**** ****

YM   SM   SMN

h

YM   SM   SMN

100 µm

i

Glucogen stain (PAS absorption cell⁻¹)

*** **

YM   SM   SMN

j

IGG   YM+ins

PE-Texas Red-A vs FSC-A

0   0.95   0   74.9
0   99.1   0   25.1
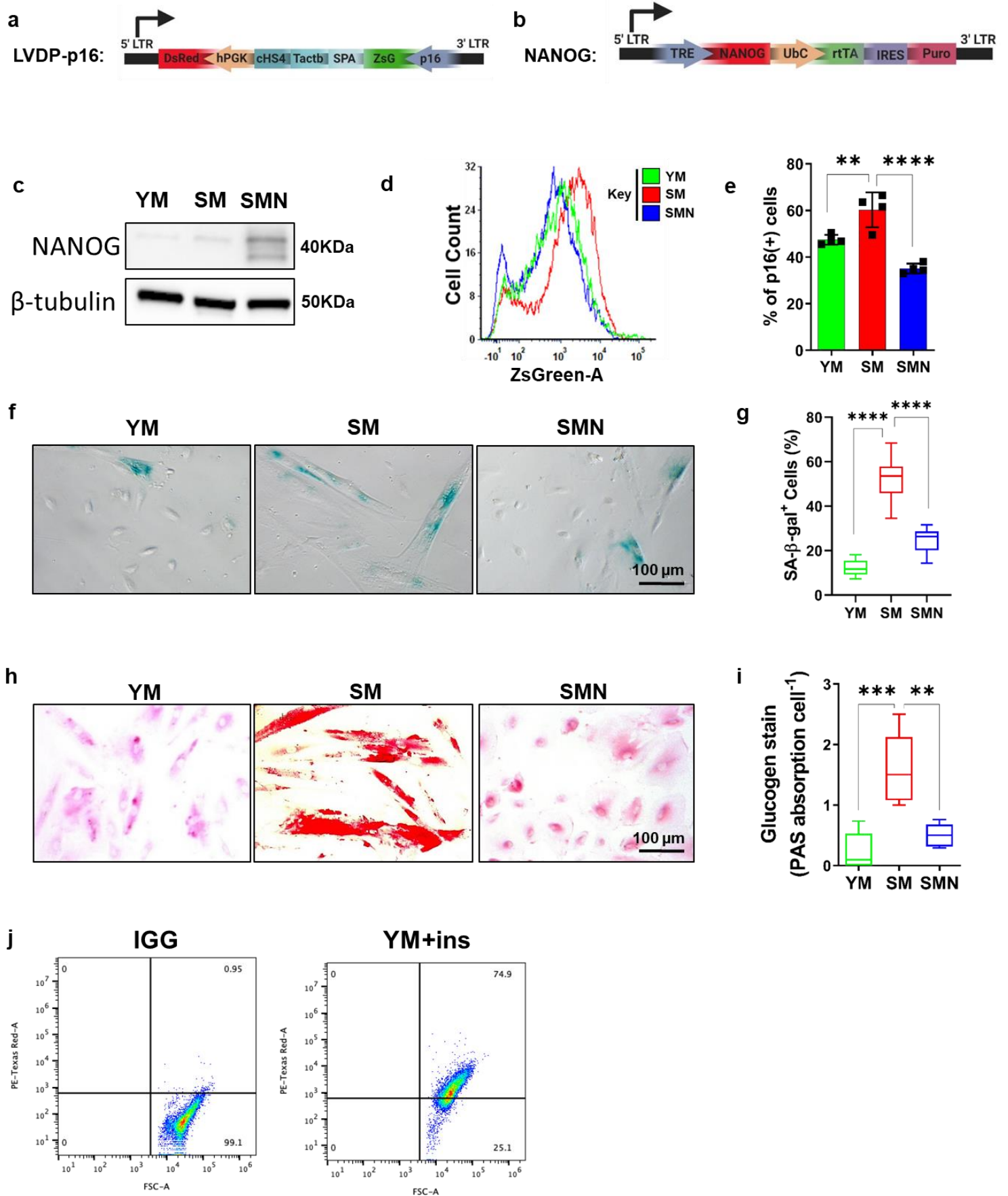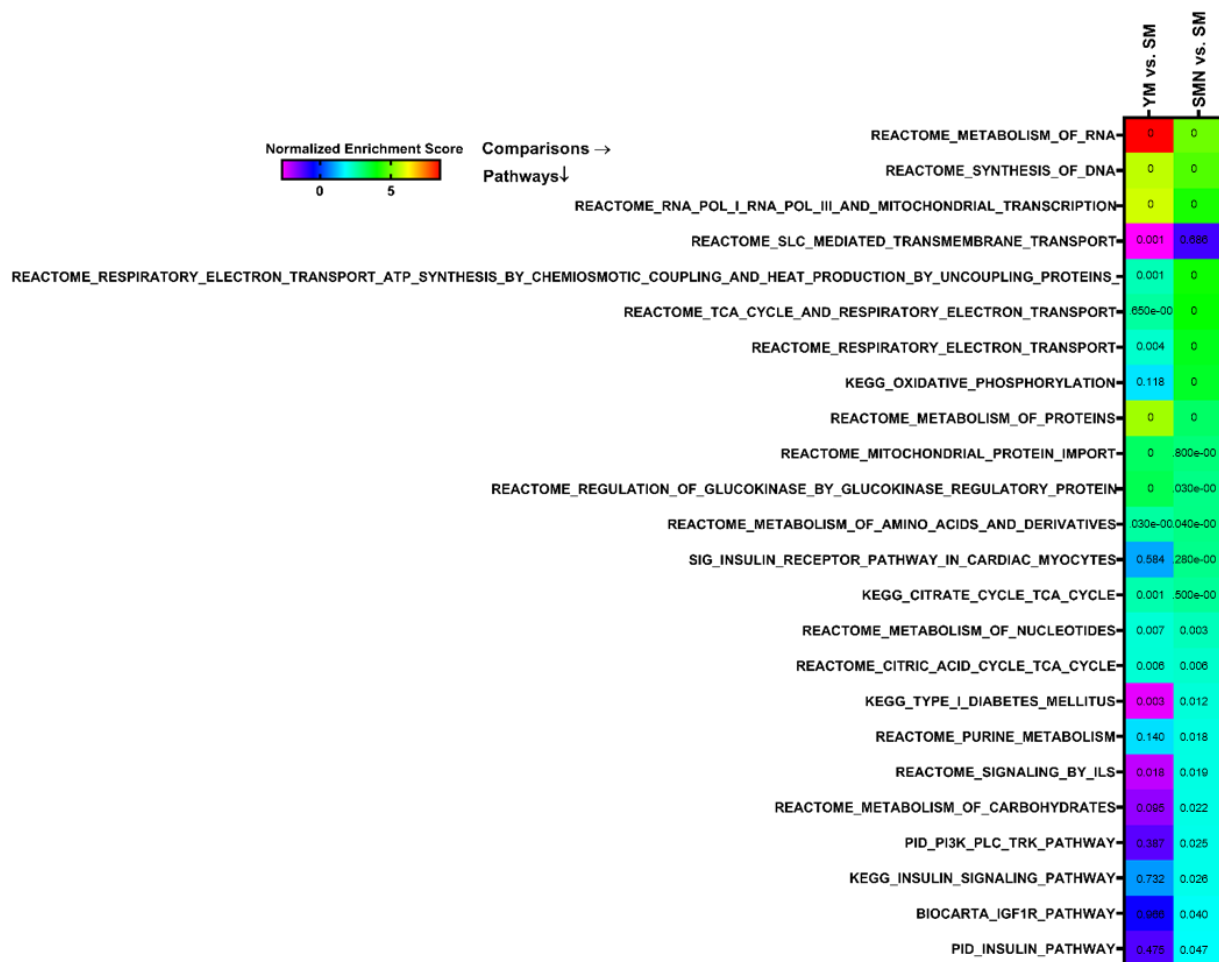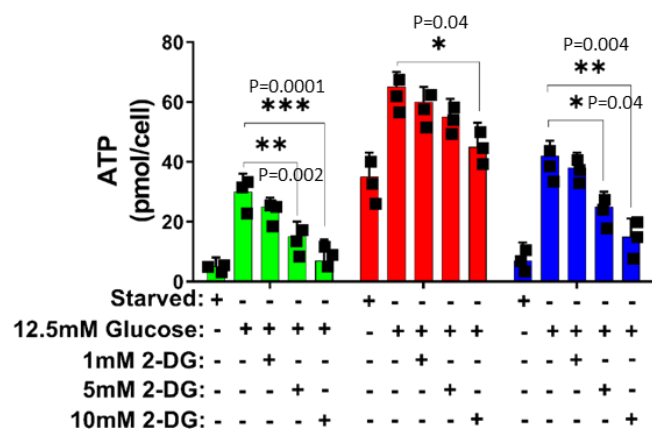
Supplementary Figure 1. a Schematic of the vector containing two expression cassettes in trans of the lentiviral backbone: one encodes for ZsGreen under the p16 promoter; the other encodes for DsRed under the constitutive hPGK promoter. **b** Schematic of the lentivirus encoding for NANOG under the tetracycline-regulatable promoter (TRE); the reverse tetracycline trans-activator, rtTA is expressed constitutively under the ubiquitin (UbC) promoter, which also drives puromycin phosphotransferase downstream of IRES. **c** Western blots for NANOG in human myoblast cells. β-tubulin served as loading control. **d,e** Flow cytometry for p16 and percentage p16+ cells transduced with LVDP-p16 vector. Unstained cell which incubated with flurochrome control were used to gate immunopositive shift. **f,g** Staining for SA-β-Gal in YM, SM and SMN cells and quantification of the percentage of SA-β-Gal-positive cells (n = 250 cells) (scale bar = 100 μm). Statistical significance using one-way ANOVA with Tukey's multiple comparisons test (*** $p = 0.009$, and **** $p < 0.0001$). **h,i** PAS stain for glycogen accumulation YM, SM, and SMN and quantification for Schiff absorbance per cell (n=250 cells) (scale bar=100μm). Statistical significance using one-way ANOVA with Tukey's multiple comparisons test (** $p < 0.01$, and *** $p < 0.001$). **j** For flow cytometry gating, the corresponding IgG conjugated antibody was used to establish proper gating of positive/negative cell populations. Young myoblast: YM, Senescent myoblast: SM, and Senescent NANOG myoblast: SMN. YM: green bars or green lines, SM: red bars or red lines, and SMN: blue bars or blue lines. n=3 human donors/group. Data in bar graphs are presented as mean ± SD. For the boxplots, the top and bottom lines of each box represent the 75th and 25th percentiles of the samples, respectively. The line inside each box represents the median of the samples. The upper and lower lines above and below the boxes are the whiskers.
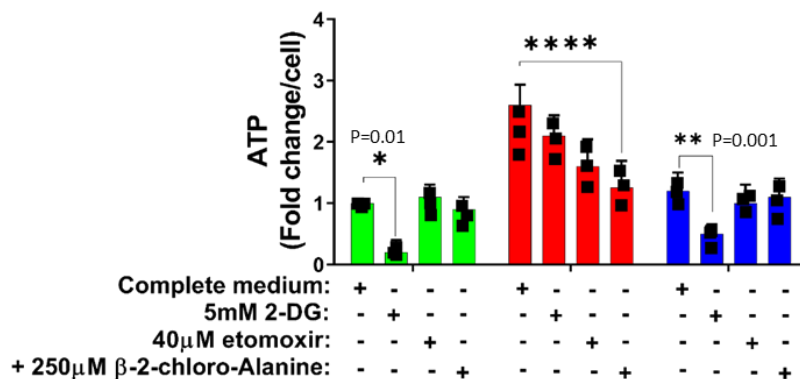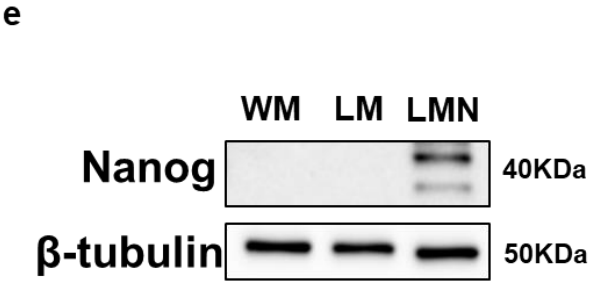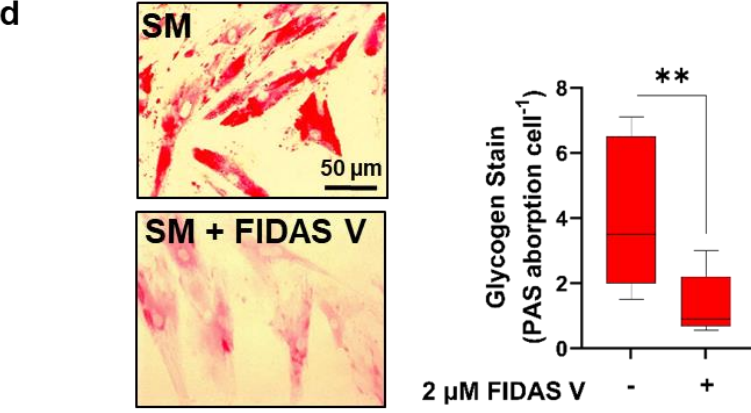
Supplementary Figure 2
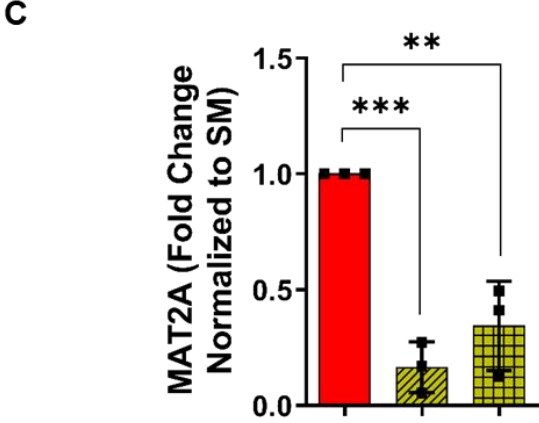
a

Normalized Enrichment Score

Comparisons →

Pathways↓

Pathways	YM vs. SM	SMN vs. SM
REACTOME_METABOLISM_OF_RNA	0	0
REACTOME_SYNTHESIS_OF_DNA	0	0
REACTOME_RNA_POL_I_RNA_POL_III_AND_MITOCHONDRIAL_TRANSCRIPTION	0	0
REACTOME_SLC_MEDIATED_TRANSMEMBRANE_TRANSPORT	0.001	0.686
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS	0.001	0
REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT	650e-00	0
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT	0.004	0
KEGG_OXIDATIVE_PHOSPHORYLATION	0.118	0
REACTOME_METABOLISM_OF_PROTEINS	0	0
REACTOME_MITOCHONDRIAL_PROTEIN_IMPORT	0	800e-00
REACTOME_REGULATION_OF_GLUCOKINASE_BY_GLUCOKINASE_REGULATORY_PROTEIN	0	030e-00
REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES	030e-00	040e-00
SIG_INSULIN_RECEPTOR_PATHWAY_IN_CARDIAC_MYOCYTES	0.584	280e-00
KEGG_CITRATE_CYCLE_TCA_CYCLE	0.001	500e-00
REACTOME_METABOLISM_OF_NUCLEOTIDES	0.007	0.003
REACTOME_CITRIC_ACID_CYCLE_TCA_CYCLE	0.006	0.006
KEGG_TYPE_I_DIABETES_MELLITUS	0.003	0.012
REACTOME_PURINE_METABOLISM	0.140	0.018
REACTOME_SIGNALING_BY_ILS	0.018	0.019
REACTOME_METABOLISM_OF_CARBOHYDRATES	0.095	0.022
PID_PI3K_PLC_TRK_PATHWAY	0.387	0.025
KEGG_INSULIN_SIGNALING_PATHWAY	0.732	0.026
BIOCARTA_IGF1R_PATHWAY	0.938	0.040
PID_INSULIN_PATHWAY	0.475	0.047

0   5

b

ATP (pmol/cell)

P=0.0001 ***
P=0.002
**
P=0.04 *
P=0.004 **
* P=0.04

Starved:	+	-	-	-	-
12.5mM Glucose:	-	+	+	+	+
1mM 2-DG:	-	-	+	-	-
5mM 2-DG:	-	-	-	+	-
10mM 2-DG:	-	-	-	-	+

c

ATP (Fold change/cell)

P=0.01 *
****
** P=0.001

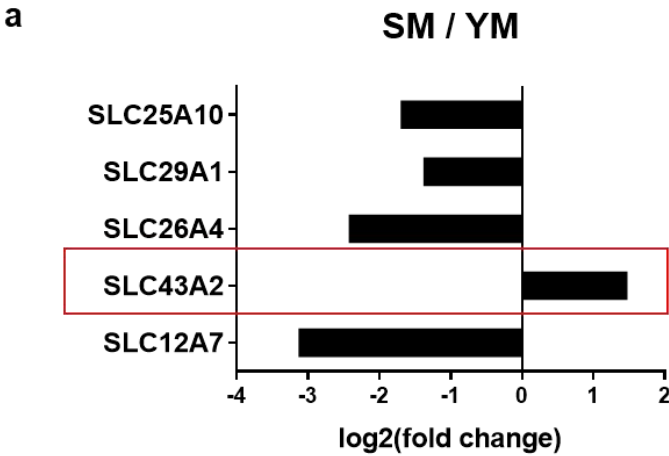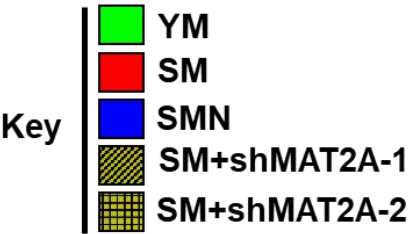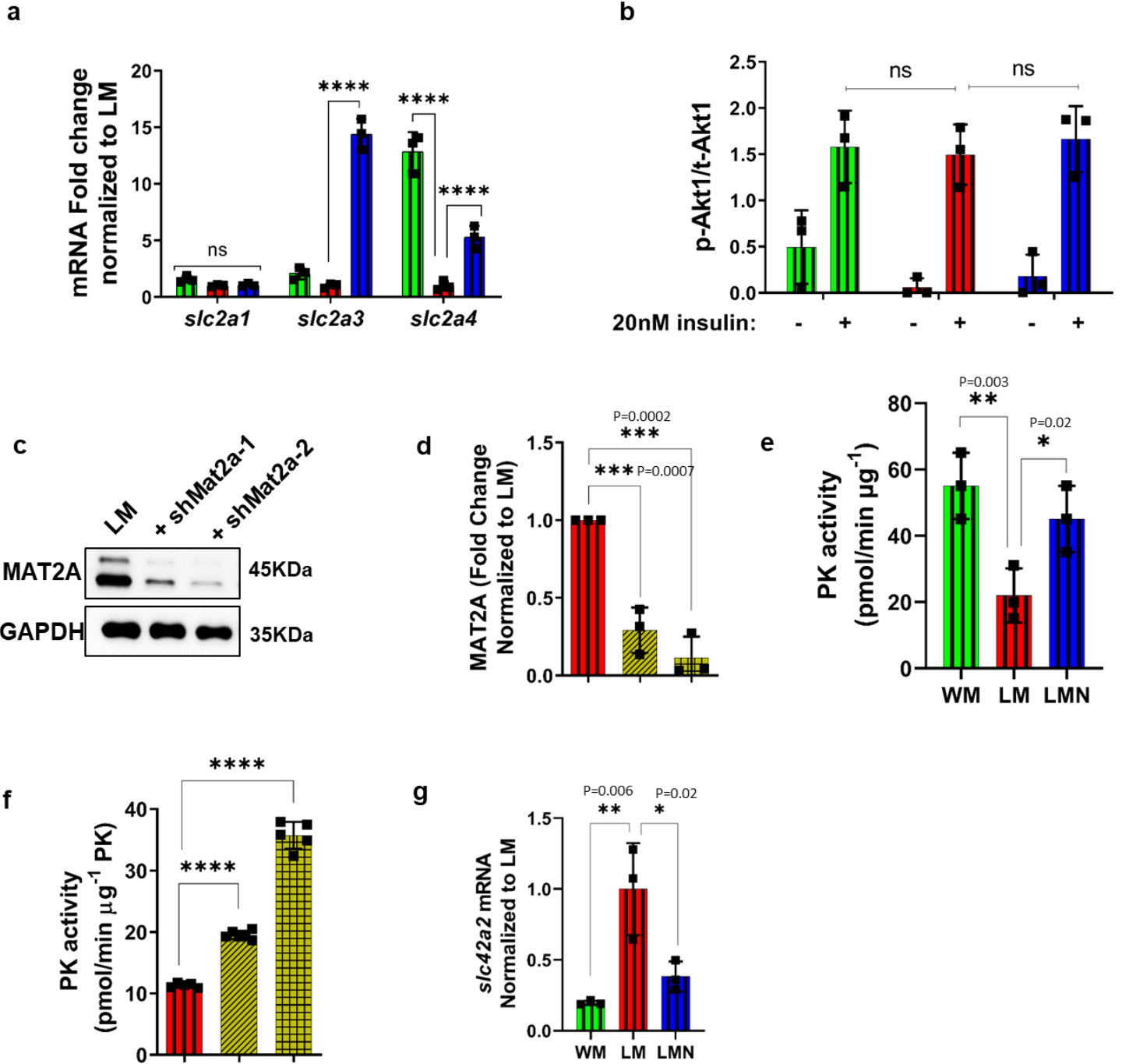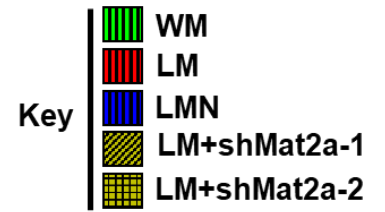|				
Complete medium:	+	-	-	-
5mM 2-DG:	-	+	-	-
40μM etomoxir:	-	-	+	-
+ 250μM β-2-chloro-Alanine:	-	-	-	+

Supplementary Figure 2. a A heat map of representative metabolic pathways that are significantly enriched after 12 days of NANOG expression (FDR is written inside the cells, and when FDR<0.001 is depicted as 0). **b** The levels of ATP level measured in the presence of the indicated concentrations of 2-DG (hexokinase inhibitor, IC_{50}=5mM) in 12.5mM glucose containing medium. **c** ATP levels of YM, SM, and SMN in the presence of 2-DG, Etomoxir or β-2-chloro-alanine inhibitors. Statistical significance using two-way ANOVA with Tukey's multiple comparisons test. YM: green bars, SM: red bars, and SMN: blue bars. n=3 human donors/group. Data in bar graphs are presented as mean ± SD.

Supplementary Figure 3

Key
- YM
- SM
- SMN
- SM+shMAT2A-1
- SM+shMAT2A-2

a

SM / YM

b

MAT2A — 45KDa

GAPDH — 35KDa

C

d

SM

SM + FIDAS V

50 µm

2 µM FIDAS V   −   +

e

WM LM LMN

Nanog — 40KDa

β-tubulin — 50KDa
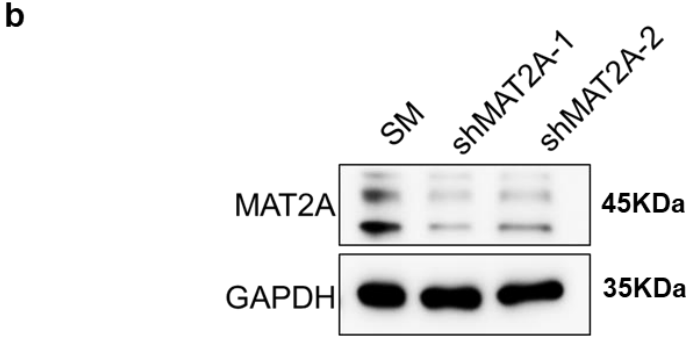
Supplementary Figure 3. a Changes in gene expression of representative solute transporters (SLC) in SM/YM as measured by RNA-seq. **b** Western blot and band quantification of MAT2A knockdown in SM cells using two distinct shRNAs. GAPDH served as loading control. Statistical significance using one-way ANOVA with Tukey's multiple comparisons test. (** $p = 0.001$, and *** $p = 0.005$). **d** PAS stain for glycogen accumulation SM and SM+FIDAS V and quantification for Schiff absorbance per cell (n=250 cells) (scale bar=50μm). (**$p < 0.001$ using unpaired t-test). n=3 human donors/group. **e** Western blots for NANOG in mouse myoblast cells. β-tubulin served as loading control. n=3 human donors/group. SM: red bars, and SM+shMAT2A-1,2: yellow bars. Data in bar graphs are presented as mean ± SD. For the boxplots, the top and bottom lines of each box represent the 75th and 25th percentiles of the samples, respectively. The line inside each box represents the median of the samples. The upper and lower lines above and below the boxes are the whiskers.
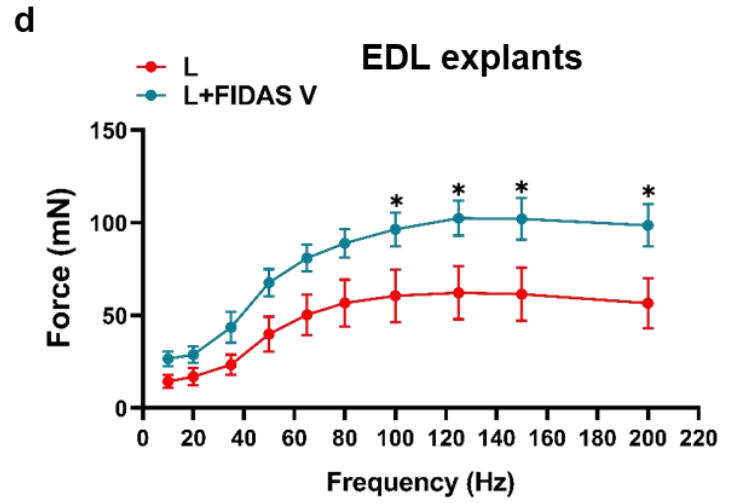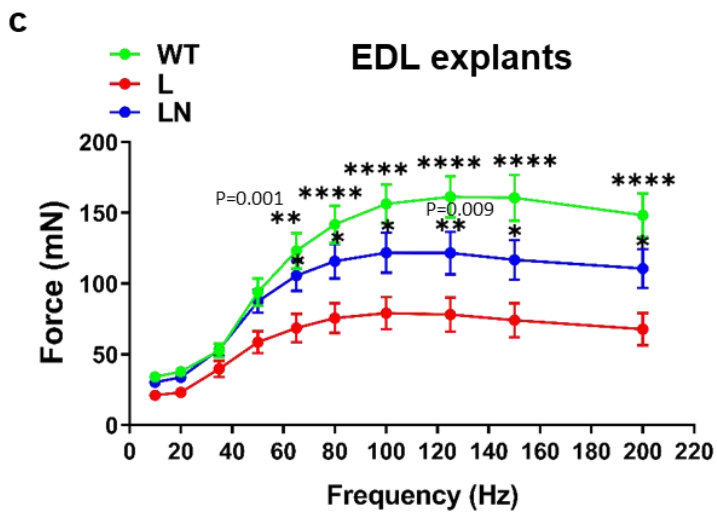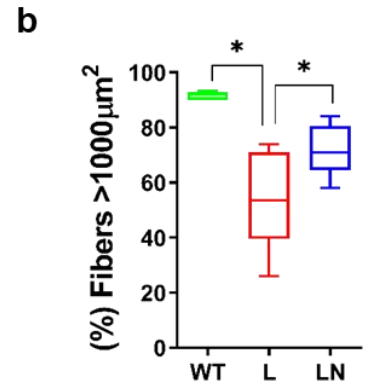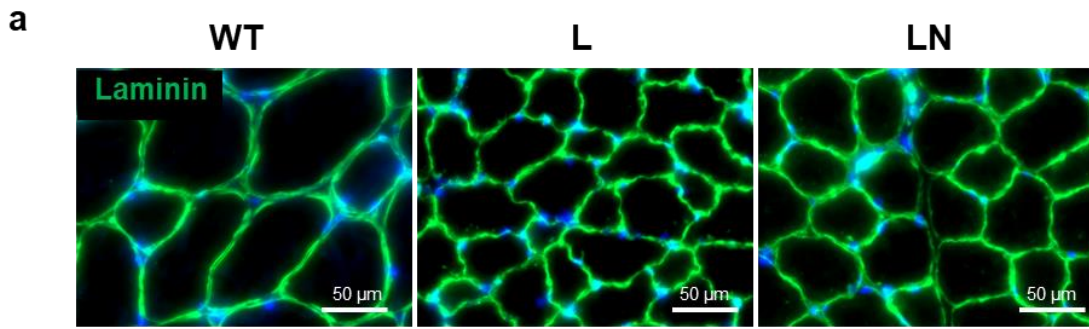
Supplementary Figure 4. a Effect of NANOG on mouse *slc2a1*, *slc2a3* and *slc2a4* transcripts. Statistical significance using two-way ANOVA with Tukey's multiple comparisons test. (**** $p < 0.0001$, and ns represents $p > 0.6$). **b** Band quantification of western blot for the effect of insulin on mouse Akt1 and p-Akt1; GAPDH served as loading control. (ns represents $p > 0.3$) statistical significance using two-way ANOVA with Sidak's multiple comparisons test. **c,d** Western blot and band quantification of MAT2A knockdown in LM cells using two distinct shRNAs. GAPDH served as loading control. **e** Effect of NANOG on mouse myoblast PK activity. **f** Effect of MAT2A inhibition on LM PK activity. **g** Effect of NANOG on mouse *slc42a2* transcripts. Statistical significance using one-way ANOVA with Tukey's multiple comparisons test. (**** $p < 0.0001$). WM: green bars, LM: red bars, LMN: blue bars, and LM+shMat2a-1,2: yellow bars. n=5 mice donors/group. Data in bar graphs are presented as mean $\pm$ SD.

Supplementary Figure 5

WM **LM** **LMN**
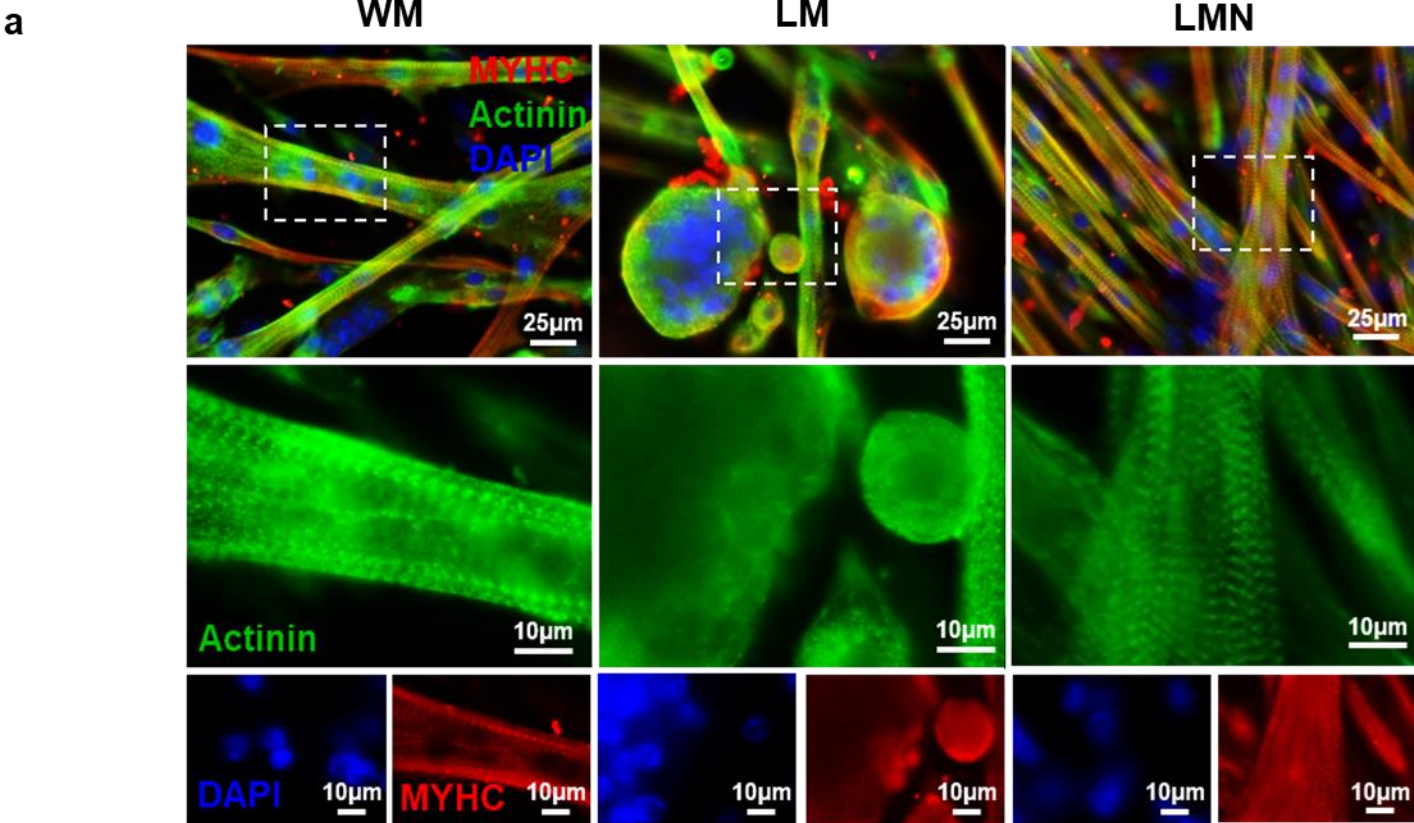
Supplementary Figure 5. a Immunostaining for myosin heavy chain (MyHC) (red) and actinin (green) showing skeletal muscle differentiation (scale bar= 25µm). **b** Videos showing contraction of differentiated mouse myofibers. n=5 mice donors/group.

a

WT   L   LN

Laminin

50 µm   50 µm   50 µm

b

(%) Fibers >1000µm²

WT   L   LN

c

EDL explants

Force (mN)

WT
L
LN

P=0.001
P=0.009
**
*
****
****
****
*
****
**
*
****
*

Frequency (Hz)

d

EDL explants

Force (mN)

L
L+FIDAS V

*
*
*
*

Frequency (Hz)

Supplementary Figure 6. a,b Immunostaining of laminin and quantification of the percentage of fibers with area $> 1,000 \ \mu m^2$ (scale bar= 50μm). n=6 mice/group. Statistical significance using one-way ANOVA with Tukey's multiple comparisons test. (* $p = 0.01$). **c** Effect of NANOG on isometric contraction performed *ex vivo* on EDL harvested from WT, L, and LN mice. n=6 mice/group. **d** Effect of FIDAS V on isometric contraction performed *ex vivo* on EDL harvested from LAKI animals. L: LAKI control; L + FIDAS V: muscles from LAKI mice that received FIDAS V. n=4 mice/group. Statistical significance using two-way ANOVA with Tukey's multiple comparisons test. (* $p = 0.01$, **** $p < 0.0001$). WT are green boxes or green lines, L are red boxes or red lines, LN are blue boxes or blue lines, and L+FIDAS V are light blue bars or light blue lines. n=4-6 animals per cohort. Data in lines are presented as mean ± SEM. For the boxplots, the top and bottom lines of each box represent the 75th and 25th percentiles of the samples, respectively. The line inside each box represents the median of the samples. The upper and lower lines above and below the boxes are the whiskers.

Supplementary Table 1

Target gene	Sequence 5'→ 3'
Nanog primers	CCCTCCATGTGTGACCAAGG-common
	GCACAGCATTGCGGACATGC-wt
	GCAGAAGCGCGGCCGTCTGG-tg
rtTA primers	AAAGTCGCTCTGAGTTGTTAT-common
	GCGAAGAGTTTGTCCTCAACC-mut F
	GGAGCGGGAGAAATGGATATG-mut R
lmna primers	GGTTCCCACTGCAGCGGCTC- mm-lmna forward exon 1
	GGACCCCACTCCCTTGGGCT-mm-lmna reverse (intron)

Supplementary Table 2

Target gene	Sequence 5'→ 3'
p16-F	CGGATCGATTACAGGTGATTTCGATTTCTCGGTGGGG
p16-R	ATTACCGGTGGCACCAGCCGGAAGCAGCC

Supplementary Table 3

Target gene	Sequence 5'→ 3'
MAT2A-1	GCAACAGTCACCAGATATTGC
MAT2A-2	GGGATGCCCTAAAGGAGAAAG
Mat2a-1	GGTCACCCAGATAAGATTTGT
Mat2a-2	GCCATAAAGCACATTGGATAT

Supplementary Table 4

Target gene	Forward 5'→ 3'	Reverse 5'→ 3'
MAT2A-human	ATGAACGGACAGCTCAACGG	CCAGCAAGAAGGATCATTCCAG
RPL-32-human	GCCCAAGATCGTCAAAAAGAGA	TCCGCCAGTTACGCTTAATTT
slc2a1-mouse	GCAGTTCGGCTATAACACTGG	GCGGTGGTTCCATGTTTGATTG
slc2a3-mouse	ATGGGGACAACGAAGGTGAC	CAGGTGCATTGATGACTCCAG
slc2a4-mouse	ACACTGGTCCTAGCTGTATTCT	CCAGCCACGTTGCATTGTA
slc42a2-mouse	TGCTATCTTTGTCCGGTACAAC	AGCTTGGGTAGCGAAAGTAAAAC
Rp-11β-mouse	GGTCAGAAGGGAACTTGTGGTAT	GCATCATTAAATGGAGTAGCGTC

Supplementary Table 5

Antibody(Clone#)	Catalog#	Dilution	Company
RHCG(5A4)	WH0051458M6	WB: 1:1000	Millipore
Akt1(9Q7)	AHO1112	WB: 1:1000	Invitrogen
pAkt1- Ser473(14-6)	44-621G	WB: 1:1000	Invitrogen
Akt2 (4H7)	MA1-034	WB: 1:1000	Invitrogen
pAkt2-Ser474	PA5-104870	WB: 1:1000	Invitrogen
InsR (CT-3)	MA5-13783	WB: 1:250	Invitrogen
MAT2A	NB110-94158	WB: 1:1000, IF: 1:200	Novus Biological
GAPDH	5174	WB: 1:10000	Cell signaling
Pax7	AB-528428	IF:1:10	Developmental Studies Hybridoma Bank
Laminin	L9393	IF: 1:200	Sigma-Aldrich
Sarcomeric Alpha Actinin	ab68167	IF: 1:200	Abcam
MyHC(clone A4.1025)	05-716	IF: 1:200	Millipore
eMyHC	Sc-53091	IF:1:100	Santa Cruz Biotechnology
NANOG	Ab109250	WB:1:500, IF: 1:250	Abcam