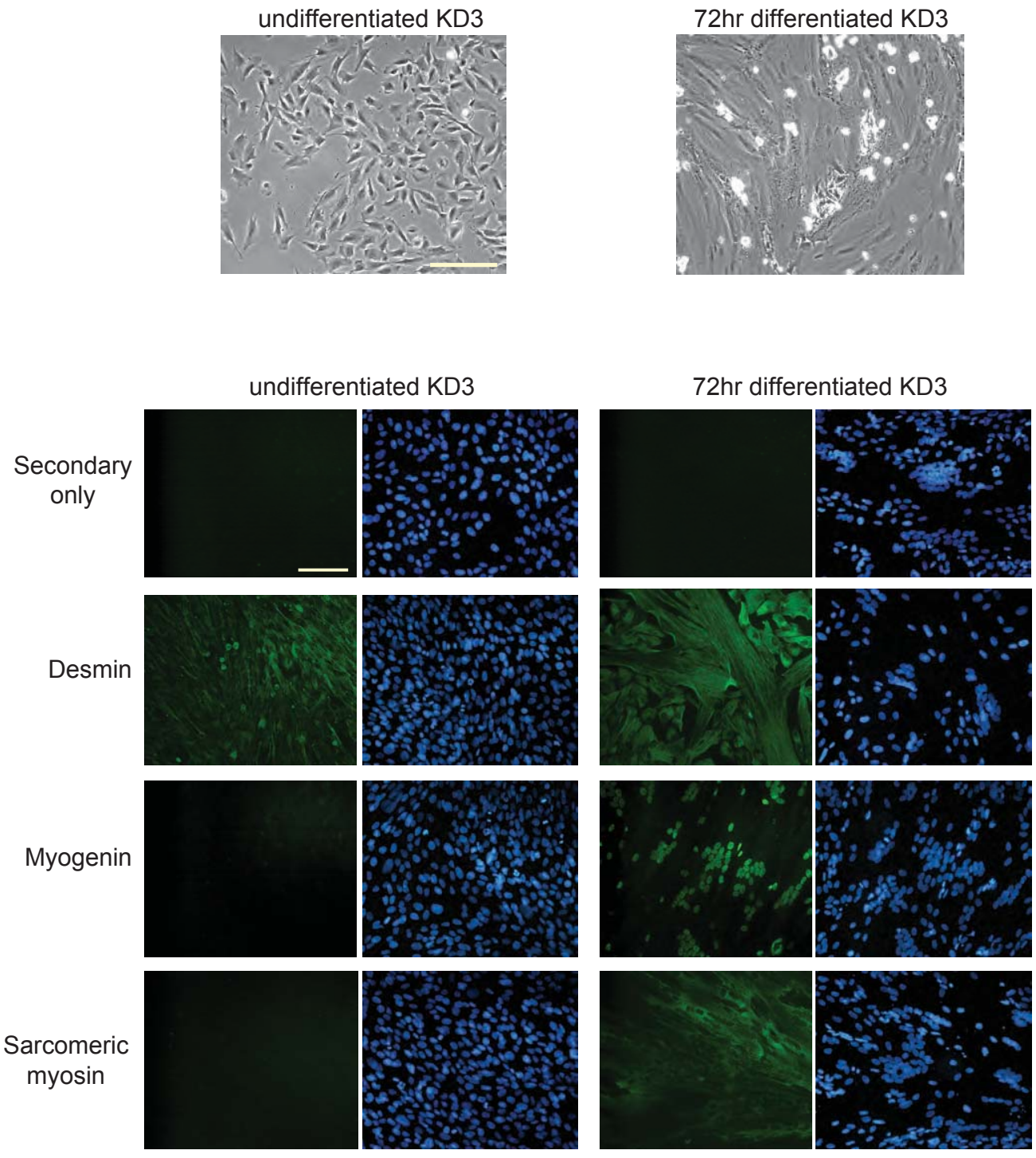
Fig S1



Scale bar is 100μm.

FigS2

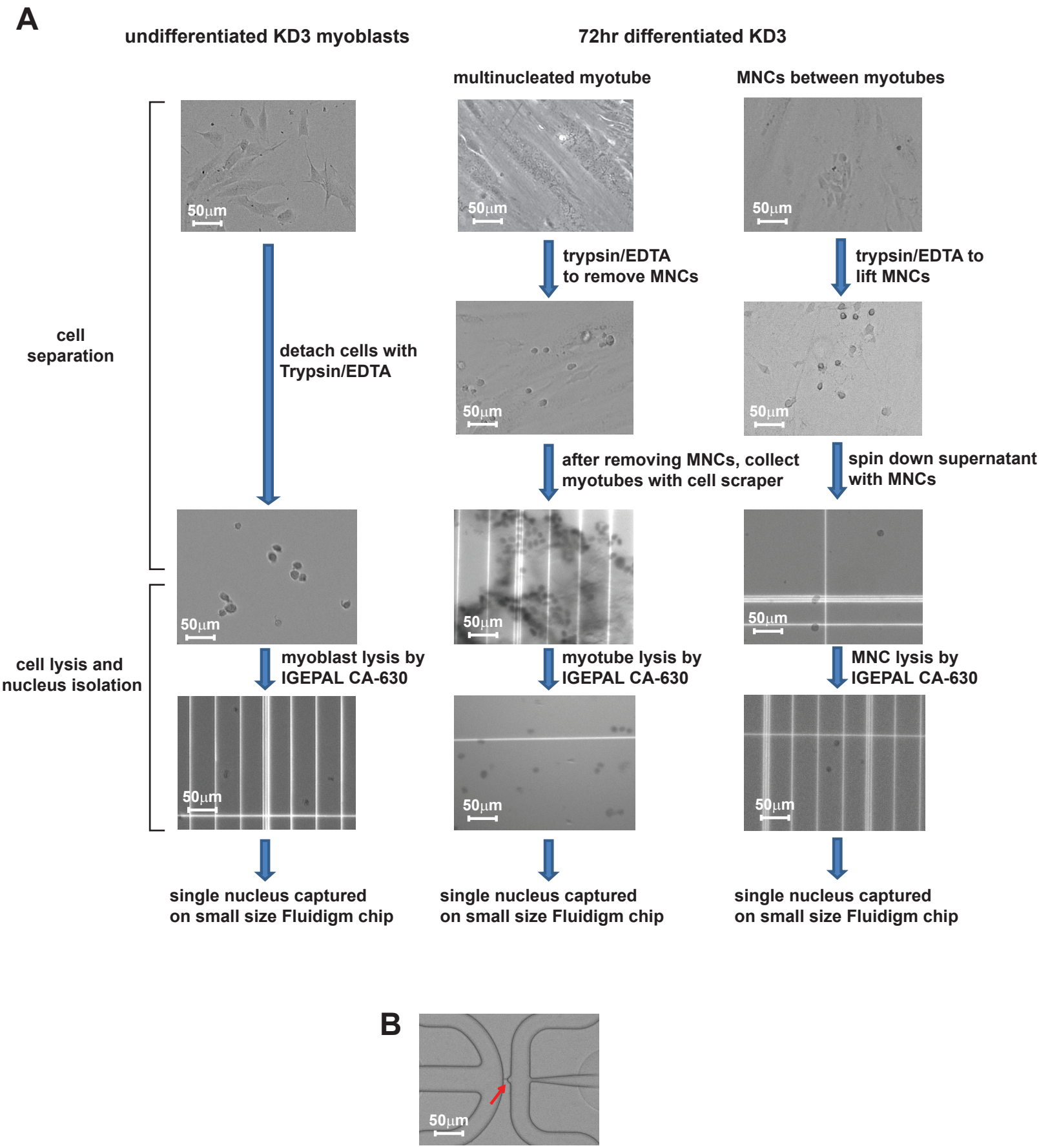

A
undifferentiated KD3 myoblasts
72hr differentiated KD3
multinucleated myotube
MNCs between myotubes
50μm
trypsin/EDTA
to remove MNCs
trypsin/EDTA to
lift MNCs
cell
separation
detach cells with
Trypsin/EDTA
after removing MNCs, collect
myotubes with cell scraper
spin down supernatant
with MNCs
cell lysis and
nucleus isolation
myoblast lysis by
IGEPAL CA-630
myotube lysis by
IGEPAL CA-630
MNC lysis by
IGEPAL CA-630
single nucleus captured
on small size Fluidigm chip
single nucleus captured
on small size Fluidigm chip
single nucleus captured
on small size Fluidigm chip
B
50μm

Fig S3

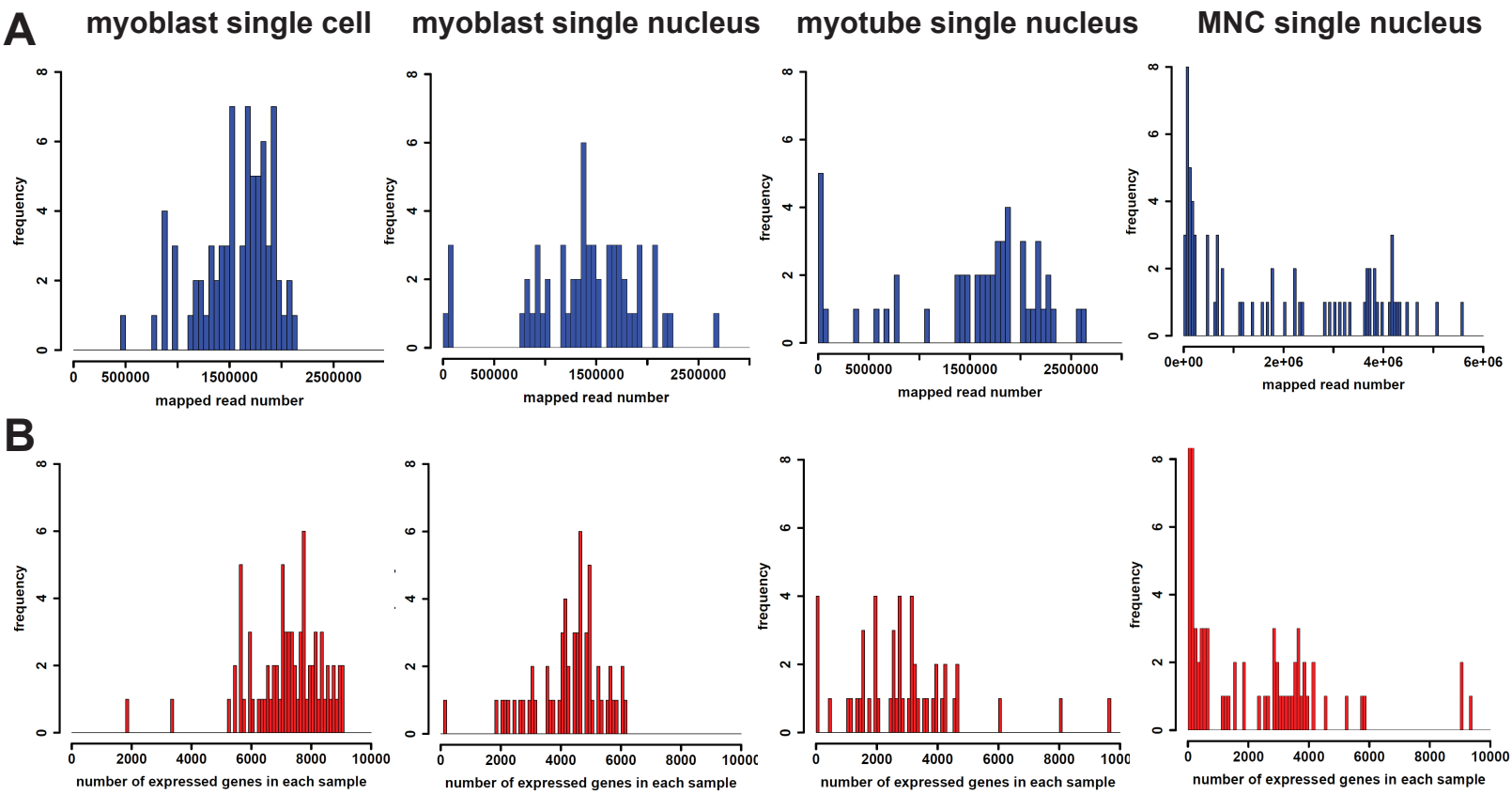
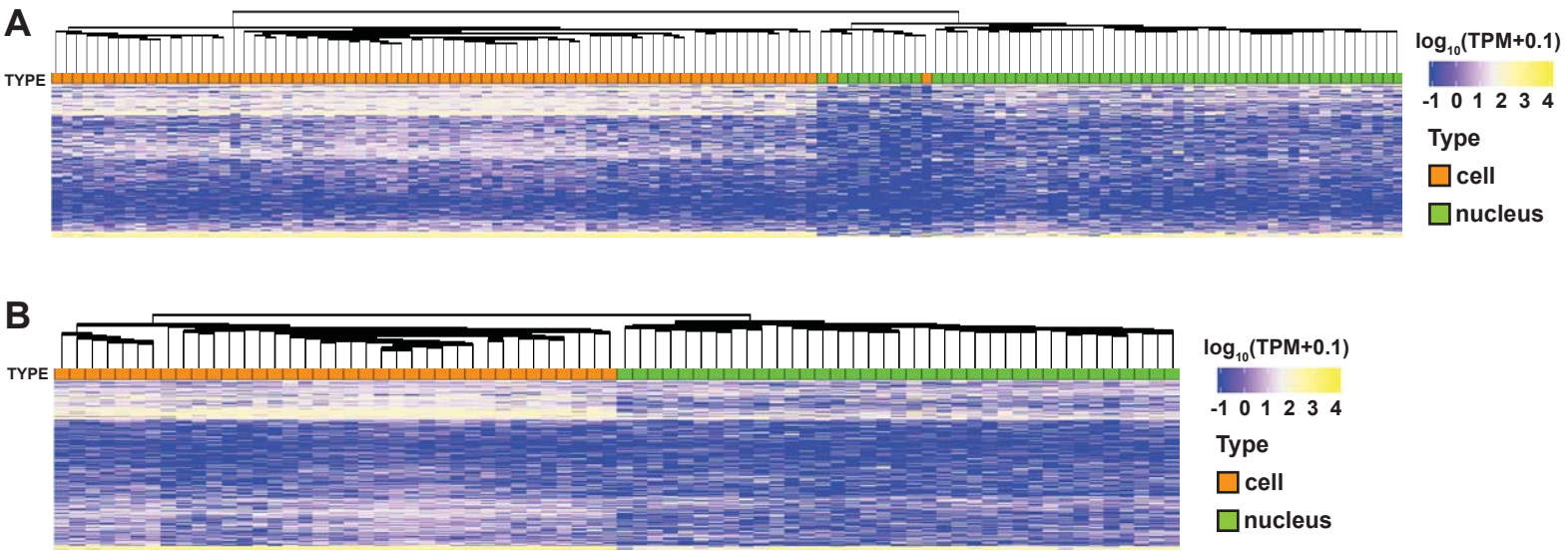

A
myoblast single cell
myoblast single nucleus
myotube single nucleus
MNC single nucleus
frequency
mapped read number
0
500000
1500000
2500000
0e+00
2e+06
4e+06
6e+06
B
number of expressed genes in each sample
0
2000
4000
6000
8000
10000

Fig S4


A
TYPE
log10(TPM+0.1)
-1 0 1 2 3 4
Type
cell
nucleus

B
TYPE
log10(TPM+0.1)
-1 0 1 2 3 4
Type
cell
nucleus

Fig S5

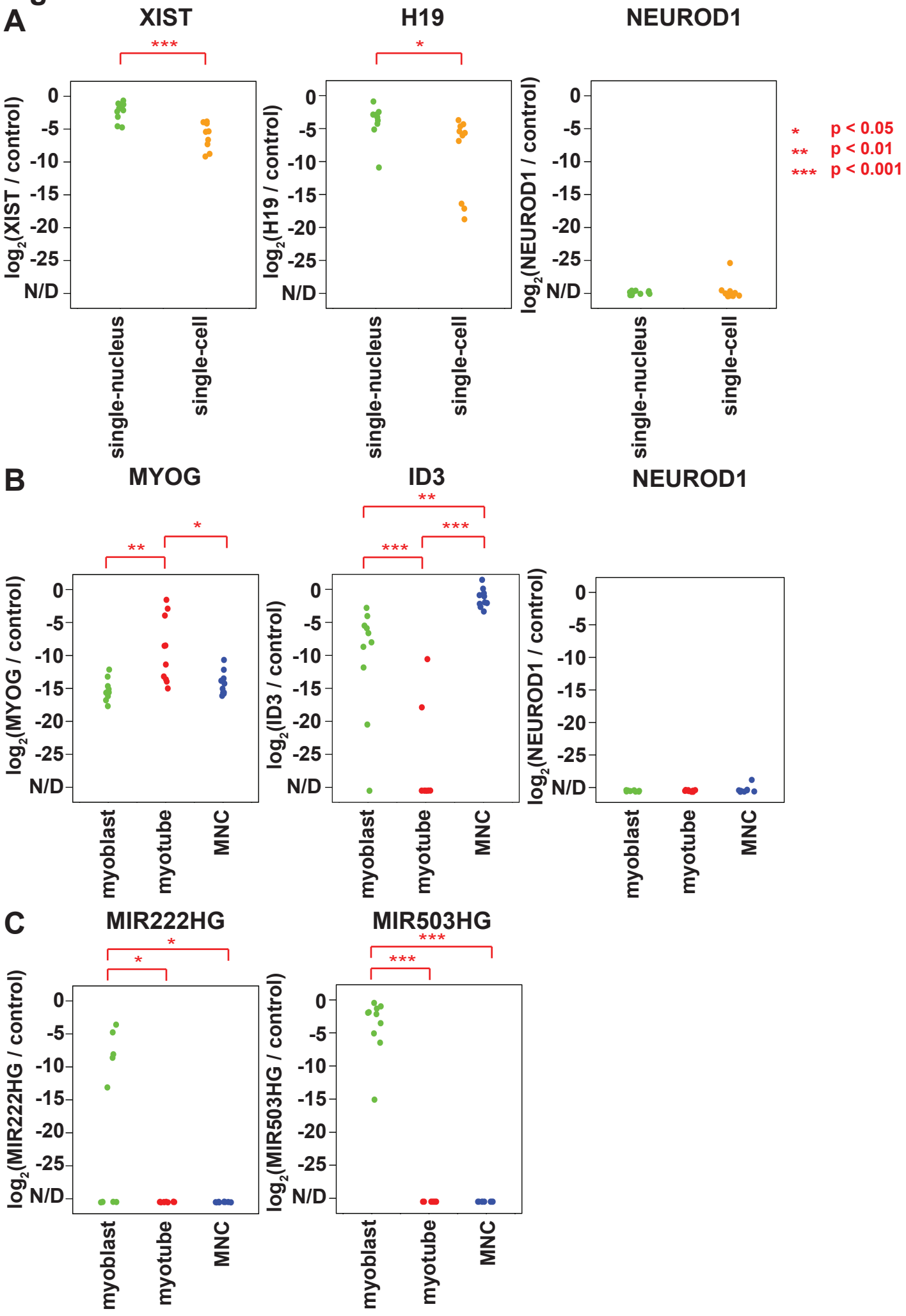

A
XIST
H19
NEUROD1
***
*
log2(XIST / control)
log2(H19 / control)
log2(NEUROD1 / control)
0
-5
-10
-15
-20
-25
N/D
single-nucleus
single-cell
* p < 0.05
** p < 0.01
*** p < 0.001
B
MYOG
ID3
NEUROD1
**
*
***
log2(MYOG / control)
log2(ID3 / control)
log2(NEUROD1 / control)
myoblast
myotube
MNC
C
MIR222HG
MIR503HG
log2(MIR222HG / control)
log2(MIR503HG / control)

Fig S6

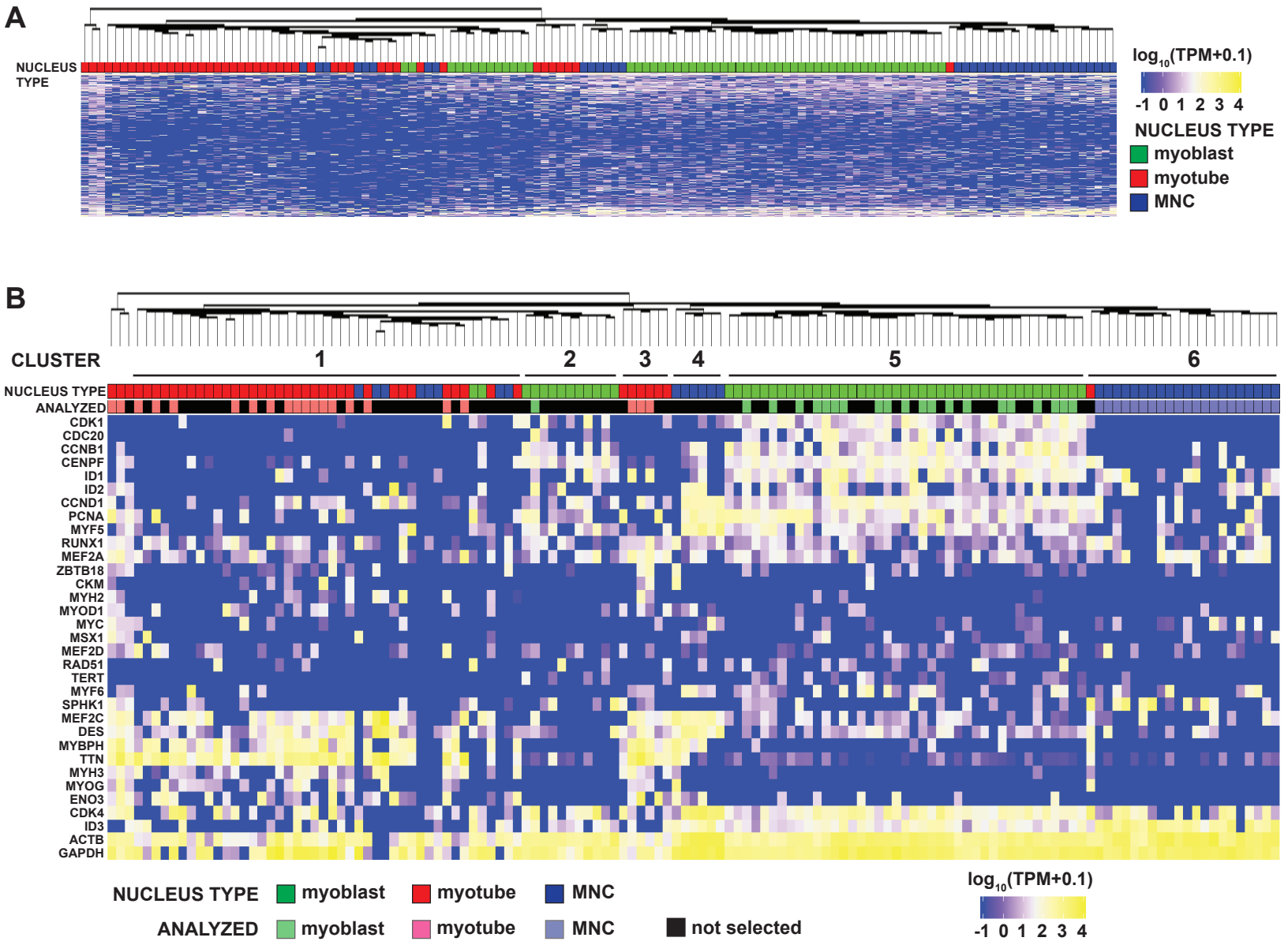

A
NUCLEUS TYPE
log10(TPM+0.1)
-1 0 1 2 3 4
NUCLEUS TYPE
myoblast
myotube
MNC
B
CLUSTER
1
2
3
4
5
6
NUCLEUS TYPE
ANALYZED
CDK1
CDC20
CCNB1
CENPF
ID1
ID2
CCND1
PCNA
MYF5
RUNX1
MEF2A
ZBTB18
CKM
MYH2
MYOD1
MYC
MSX1
MEF2D
RAD51
TERT
MYF6
SPHK1
MEF2C
DES
MYBPH
TTN
MYH3
MYOG
ENO3
CDK4
ID3
ACTB
GAPDH
NUCLEUS TYPE myoblast myotube MNC
ANALYZED myoblast myotube MNC not selected
log10(TPM+0.1)
-1 0 1 2 3 4

Fig S7

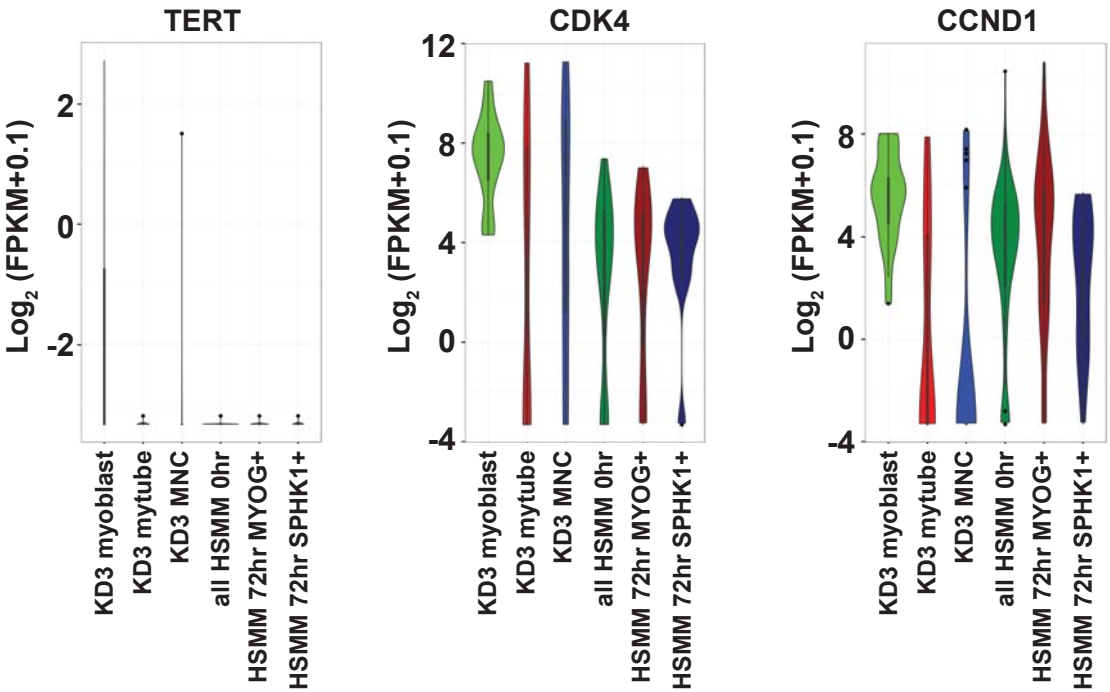

TERT
CDK4
CCND1
Log2 (FPKM+0.1)
2
0
-2
12
8
4
0
-4
KD3 myoblast
KD3 mytube
KD3 MNC
all HSMM 0hr
HSMM 72hr MYOG+
HSMM 72hr SPHK1+

Fig S8

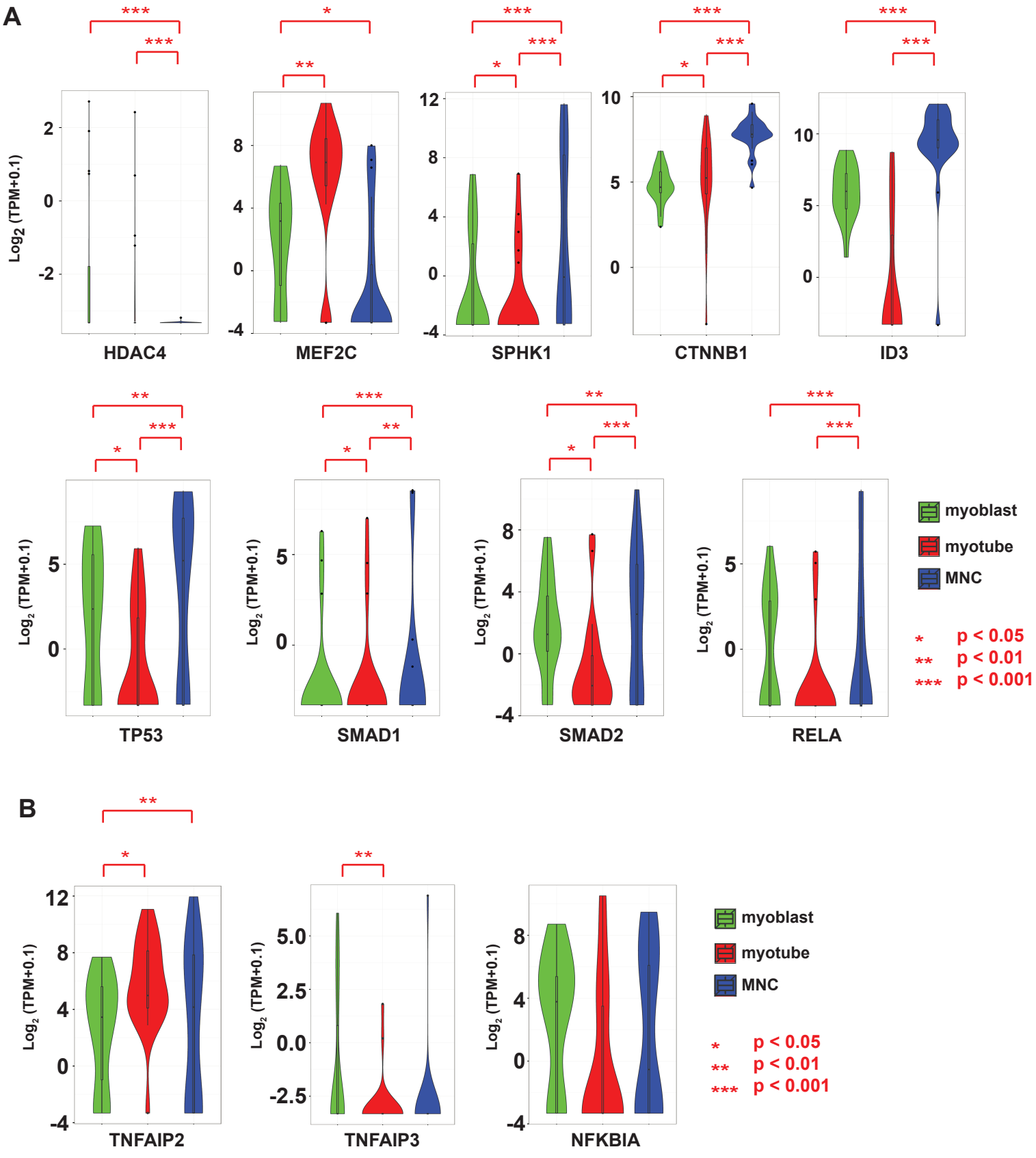
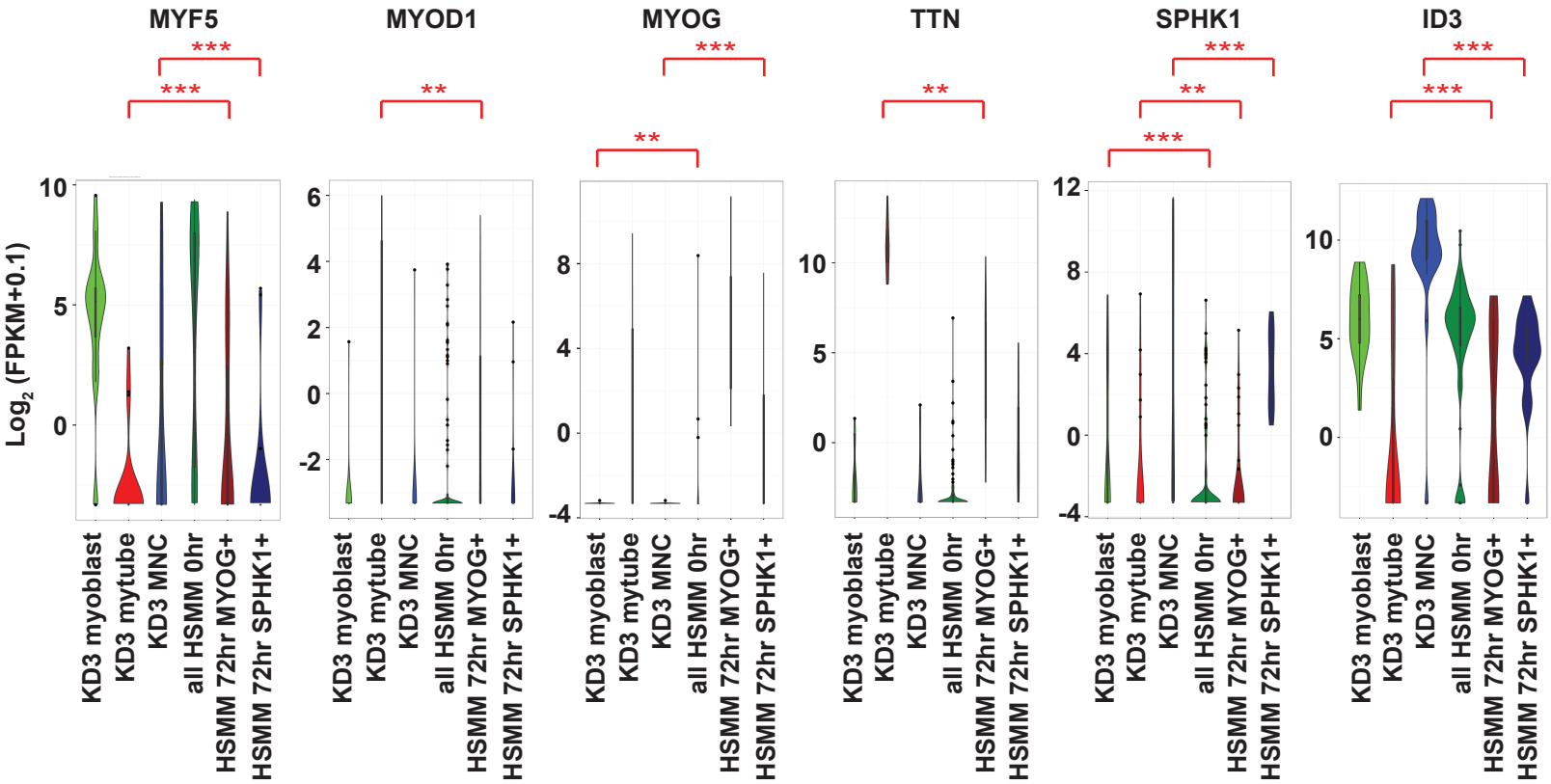

A
HDAC4
MEF2C
SPHK1
CTNNB1
ID3
TP53
SMAD1
SMAD2
RELA
Log2 (TPM+0.1)
myoblast
myotube
MNC
* p < 0.05
** p < 0.01
*** p < 0.001
B
TNFAIP2
TNFAIP3
NFKBIA

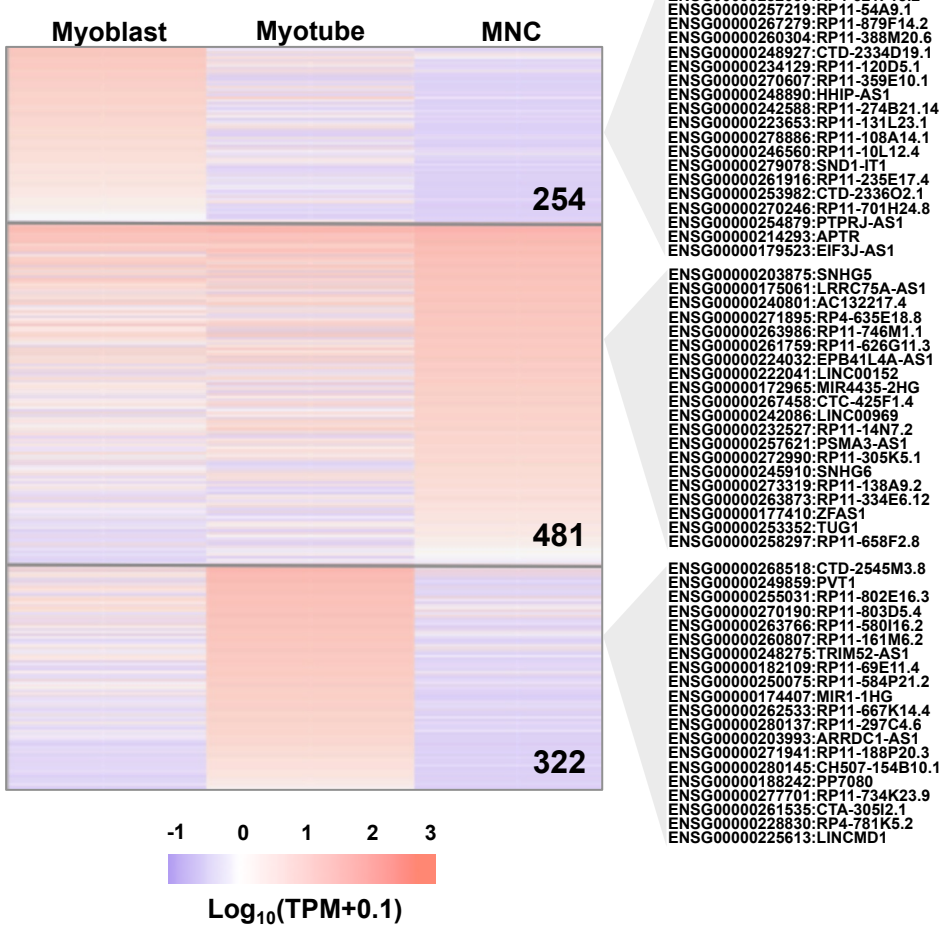
Fig S9



* p < 0.05
** p < 0.01
*** p < 0.001

Fig S10

A


Myoblast
Myotube
MNC
254
481
322
-1 0 1 2 3
Log10(TPM+0.1)
ENSG00000281881:SPRY4-IT1
ENSG00000282057:RP4-621F18.2
ENSG00000257219:RP11-54A9.1
ENSG00000267279:RP11-879F14.2
ENSG00000260304:RP11-388M20.6
ENSG00000248927:CTD-2334D19.1
ENSG00000234129:RP11-120D5.1
ENSG00000270607:RP11-359E10.1
ENSG00000248890:HHIP-AS1
ENSG00000242588:RP11-274B21.14
ENSG00000223653:RP11-131L23.1
ENSG00000278886:RP11-108A14.1
ENSG00000246560:RP11-10L12.4
ENSG00000279078:SND1-IT1
ENSG00000261916:RP11-235E17.4
ENSG00000253982:CTD-2336O2.1
ENSG00000270246:RP11-701H24.8
ENSG00000254879:PTPRJ-AS1
ENSG00000214293:APTR
ENSG00000179523:EIF3J-AS1
ENSG00000203875:SNHG5
ENSG00000175061:LRRC75A-AS1
ENSG00000240801:AC132217.4
ENSG00000271895:RP4-635E18.8
ENSG00000263986:RP11-746M1.1
ENSG00000261759:RP11-626G11.3
ENSG00000224032:EPB41L4A-AS1
ENSG00000222041:LINC00152
ENSG00000172965:MIR4435-2HG
ENSG00000267458:CTC-425F1.4
ENSG00000242086:LINC00969
ENSG00000232527:RP11-14N7.2
ENSG00000257621:PSMA3-AS1
ENSG00000272990:RP11-305K5.1
ENSG00000245910:SNHG6
ENSG00000273319:RP11-138A9.2
ENSG00000263873:RP11-334E6.12
ENSG00000177410:ZFAS1
ENSG00000253352:TUG1
ENSG00000258297:RP11-658F2.8
ENSG00000268518:CTD-2545M3.8
ENSG00000249859:PVT1
ENSG00000255031:RP11-802E16.3
ENSG00000270190:RP11-803D5.4
ENSG00000263766:RP11-580I16.2
ENSG00000260807:RP11-161M6.2
ENSG00000248275:TRIM52-AS1
ENSG00000182109:RP11-69E11.4
ENSG00000250075:RP11-584P21.2
ENSG00000174407:MIR1-1HG
ENSG00000262533:RP11-667K14.4
ENSG00000280137:RP11-297C4.6
ENSG00000203993:ARRDC1-AS1
ENSG00000271941:RP11-188P20.3
ENSG00000280145:CH507-154B10.1
ENSG00000188242:PP7080
ENSG00000277701:RP11-734K23.9
ENSG00000261535:CTA-305I2.1
ENSG00000228830:RP4-781K5.2
ENSG00000225613:LINCMD1

B

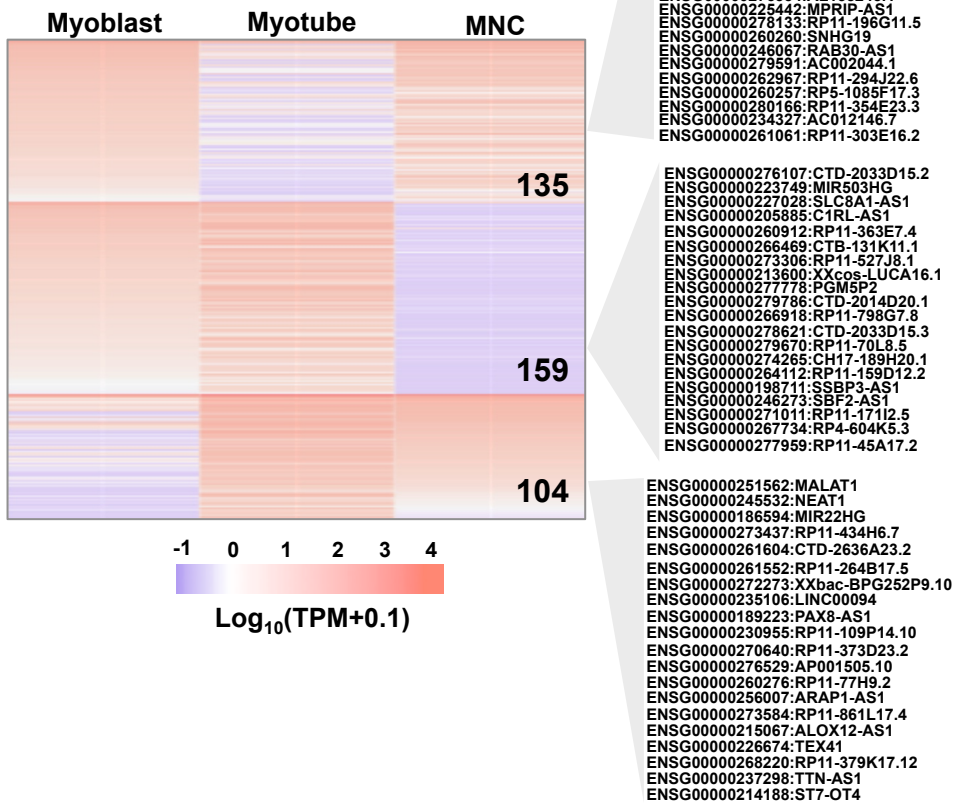
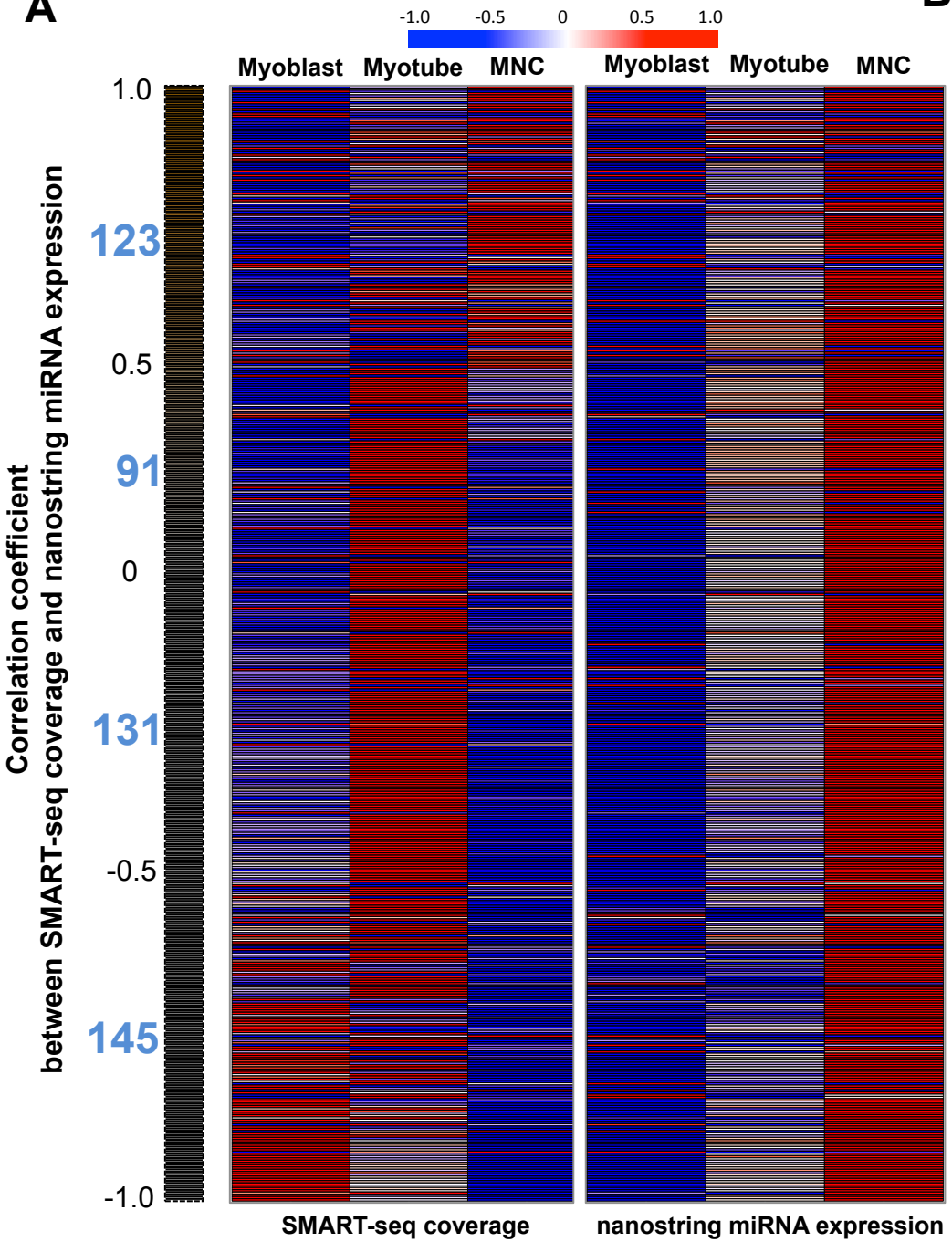

Myoblast
Myotube
MNC
135
159
104
-1 0 1 2 3 4
Log10(TPM+0.1)
ENSG00000258017:RP11-386G11.10
ENSG00000258232:RP11-161H23.5
ENSG00000234741:GAS5
ENSG00000179818:PCBP1-AS1
ENSG00000280120:RP11-546D6.3
ENSG00000279632:RP11-286N22.6
ENSG00000267058:RP11-15A1.3
ENSG00000242125:SNHG3
ENSG00000255135:RP11-111M22.3
ENSG00000276334:AL133243.1
ENSG00000225442:MPRIP-AS1
ENSG00000278133:RP11-196G11.5
ENSG00000260260:SNHG19
ENSG00000246067:RAB30-AS1
ENSG00000279591:AC002044.1
ENSG00000262967:RP11-294J22.6
ENSG00000260257:RP5-1085F17.3
ENSG00000280166:RP11-354E23.3
ENSG00000234327:AC012146.7
ENSG00000261061:RP11-303E16.2
ENSG00000276107:CTD-2033D15.2
ENSG00000223749:MIR503HG
ENSG00000227028:SLC8A1-AS1
ENSG00000205885:C1RL-AS1
ENSG00000260912:RP11-363E7.4
ENSG00000266469:CTB-131K11.1
ENSG00000273306:RP11-527J8.1
ENSG00000213600:XXcos-LUCA16.1
ENSG00000277778:PGM5P2
ENSG00000279786:CTD-2014D20.1
ENSG00000266918:RP11-798G7.8
ENSG00000278621:CTD-2033D15.3
ENSG00000279670:RP11-70L8.5
ENSG00000274265:CH17-189H20.1
ENSG00000264112:RP11-159D12.2
ENSG00000198711:SSBP3-AS1
ENSG00000246273:SBF2-AS1
ENSG00000271011:RP11-171I2.5
ENSG00000267734:RP4-604K5.3
ENSG00000277959:RP11-45A17.2
ENSG00000251562:MALAT1
ENSG00000245532:NEAT1
ENSG00000186594:MIR22HG
ENSG00000273437:RP11-434H6.7
ENSG00000261604:CTD-2636A23.2
ENSG00000261552:RP11-264B17.5
ENSG00000272273:XXbac-BPG252P9.10
ENSG00000235106:LINC00094
ENSG00000189223:PAX8-AS1
ENSG00000230955:RP11-109P14.10
ENSG00000270640:RP11-373D23.2
ENSG00000276529:AP001505.10
ENSG00000260276:RP11-77H9.2
ENSG00000256007:ARAP1-AS1
ENSG00000273584:RP11-861L17.4
ENSG00000215067:ALOX12-AS1
ENSG00000226674:TEX41
ENSG00000268220:RP11-379K17.12
ENSG00000237298:TTN-AS1
ENSG00000214188:ST7-OT4

Fig S11

A



B

miRNA	lncRNA	r
MIR421	FTX	0.999971921
MIR421	RP3-368A4.5	0.999971921
MIR221	MIR222HG	0.999777411
MIR628	DYX1C1-CCPG1	0.999254049
MIR29A	AC058791.1	0.998871016
MIR148B	RP11-968A15.8	0.997806686
MIR222	MIR222HG	0.993906409
MIR210	MIR210HG	0.992865427
MIR141	U47924.27	0.991674063
MIR141	U47924.29	0.991674063
MIR2113	RP11-436D23.1	0.989367849
MIR30B	AC083843.1	0.986931656
MIR675	H19	0.986901119
MIR223	RP6-159A1.4	0.985055911
MIR770	MEG3	0.984900118
MIR133A2	MIR1-1HG	0.982896359
MIR133B	LINCMD1	0.979690547
MIR92A1	MIR17HG	0.973743515
MIR24-2	CTD-3252C9.4	0.963019249
MIR23A	CTD-3252C9.4	0.961190177
MIR568	RP11-553L6.5	0.959749143
MIR503	MIR503HG	0.958898365
MIR655	MIR381HG	0.952452199
MIRLET7D	RP11-2B6.3	0.930720304
MIRLET7D	RP11-2B6.2	0.930720304
MIR206	LINCMD1	0.930719587
MIR181A1	MIR181A1HG	0.910454043
MIR381	MIR381HG	0.893540499
MIR487A	MIR381HG	0.843149071
MIR1305	RP11-402C9.1	0.81880738
MIR487B	MIR381HG	0.814219188
MIRLET7A1	RP11-2B6.2	0.785889497
MIRLET7A1	RP11-2B6.3	0.785889497
MIR20A	MIR17HG	0.784714255
MIR133A1	MIR133A1HG	0.78166434
MIRLET7F1	RP11-2B6.3	0.755822968
MIRLET7F1	RP11-2B6.2	0.755822968
MIR544A	MIR381HG	0.740151196
MIR181B1	MIR181A1HG	0.731594025
MIR155	MIR155HG	0.729875964
MIR195	RP11-589P10.7	0.717107837
MIR195	MIR497HG	0.717107837
MIR889	MIR381HG	0.705686504
MIR570	LINC00969	0.680459534
MIR1539	MIR1539	0.642441322
MIR134	MIR381HG	0.616418919
MIRLET7B	MIRLET7BHG	0.613282134
MIR1-1	MIR1-1HG	0.605615978
MIR125B1	RP11-166D19.1	0.563329507
MIRLET7I	RP11-631N16.2	0.525892573
MIRLET7I	RP11-631N16.4	0.525892573
MIR370	MEG8	0.510520736