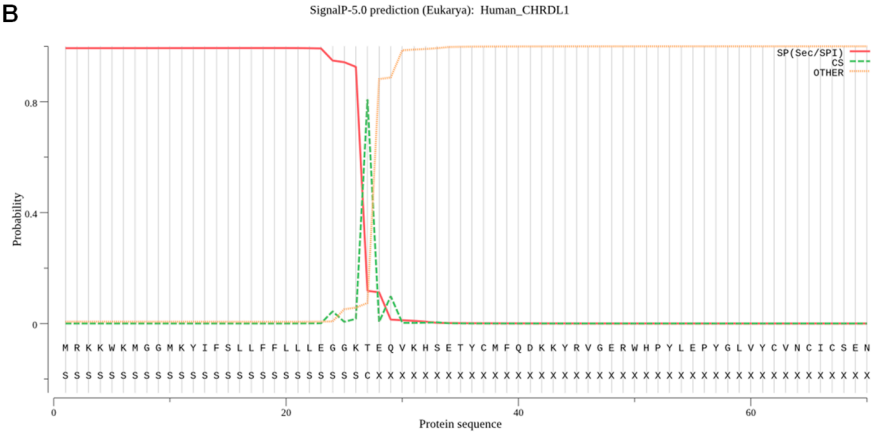
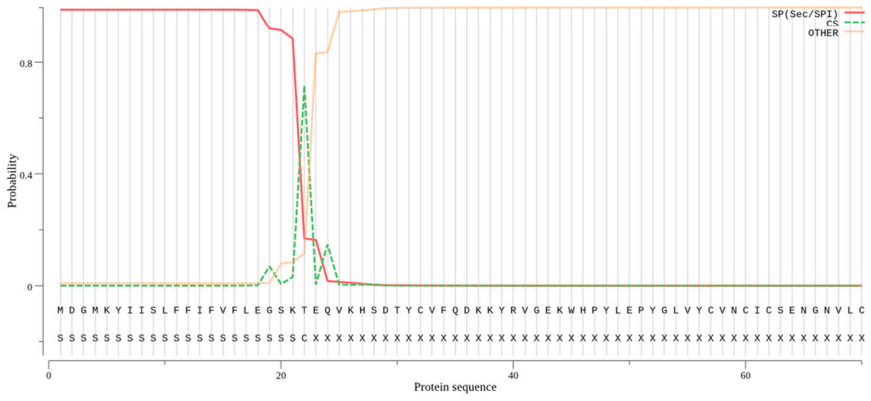
A

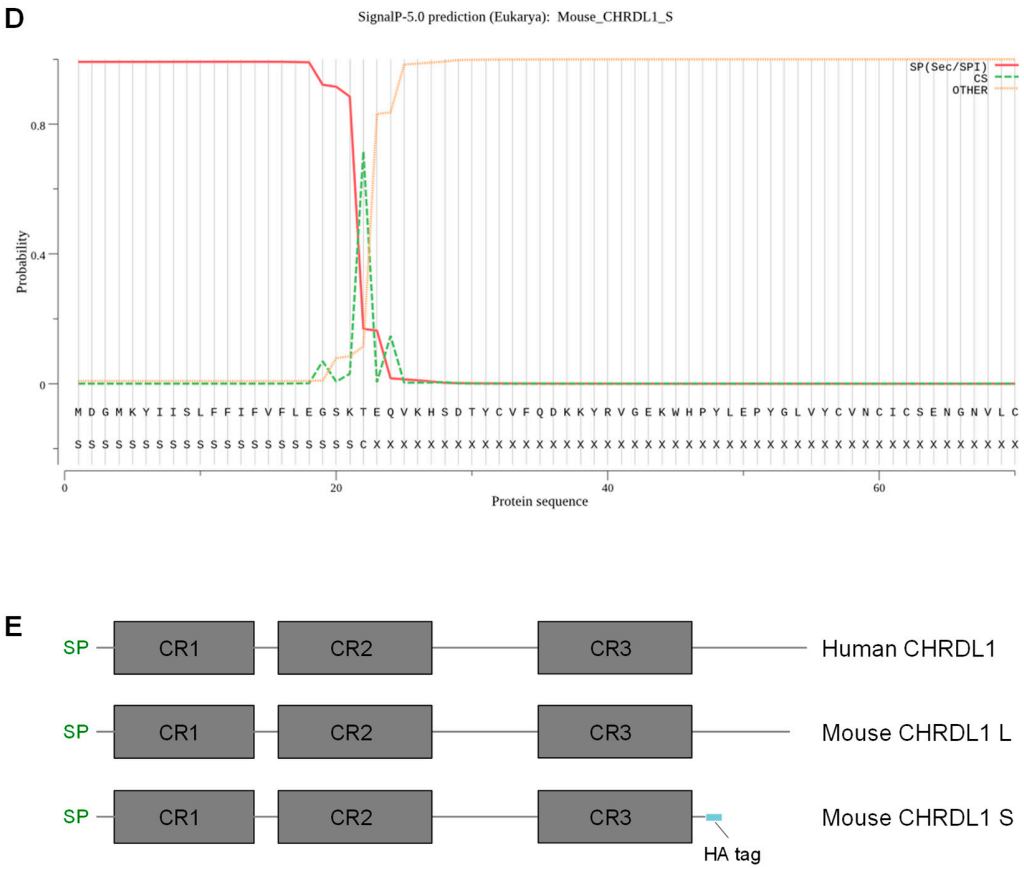
```
                    10        20        30        40        50        60        70        80        90       100
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Human CHRDL1        MRKKWKMGGMKYIFSLLF-FLLLEGGKTEQVKHSETYCMFQDKKYRVGERWHPYLEPYGLVYCVNCICSENGNVLCSRVRCPNVHCLSPVHIPHLCCPRC 99
Mouse CHRDL1 L      ------.D.....I..F.I.VF...S.......D...V..........K.............................SL.................. 94
Mouse CHRDL1 S      ------.D.....I..F.I.VF...S.......D...V..........K.............................SL.................. 94

                   110       120       130       140       150       160       170       180       190       200
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Human CHRDL1        PEDSLPPVNNKVTSKSCEYNGTTYQHGELFVAEGLFQNRQPNQCTQCSCSEGNVYCGLKTCPKLTCAFPVSVPDSCCRVCRGDGELSWEHSDGDIFRQPA 199
Mouse CHRDL1 L      ..............................I.............S.....................................A......A......... 194
Mouse CHRDL1 S      .-.............................I.............S.....................................A......A......... 193

                   210       220       230       240       250       260       270       280       290       300
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Human CHRDL1        NREARHSYHRSHYDPPPSRQAGGLSRFPGARSHRGALMDSQQASGTIVQIVINNKHKHGQVCVSNGKTYSHGESWHPNLRAFGIVECVLCTCNVTKQECK 299
Mouse CHRDL1 L      ........L..P.....N......P....S......VI.....................................................  294
Mouse CHRDL1 S      ........L..P.....N......P....S......VI.....................................................  293

                   310       320       330       340       350       360       370       380       390       400
                    ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Human CHRDL1        KIHCPNRYPCKYPQKIDGKCCKVCPGKKAKEELPGQSFDNKGYFCGEETMPVYESVFMEDGETTRKIALETERPPQVEVHVWTIRKGILQHFHIEKISKR 399
Mouse CHRDL1 L      ........................EEP-----.S.N..S..S.....................V.................Q.............. 389
Mouse CHRDL1 S      ...........................----------KA..ALA.GPAFG---------------------------------------------- 333

                   410       420       430       440       450
                    ....|....|....|....|....|....|....|....|....|....
Human CHRDL1        MFEELPHFKLVTRTTLSQWKIFTEGEAQISQMCSSRVCRTELEDLVKVLYLERSEKGHC 458
Mouse CHRDL1 L      ..G..H..........N...L.......L......Q..........Q....G.P..D.. 448
Mouse CHRDL1 S      ------------------------------------------------------------ 333
```

B



C




D
SignalP-5.0 prediction (Eukarya): Mouse_CHRDL1_S
SP(Sec/SPI)
CS
OTHER
Probability
0.8
0.4
0
M D G M K Y I I S L F F I F V F L E G S K T E Q V K H S D T Y C V F Q D K K Y R V G E K W H P Y L E P Y G L V Y C V N C I C S E N G N V L C
S S S S S S S S S S S S S S S S S S S S C X X X

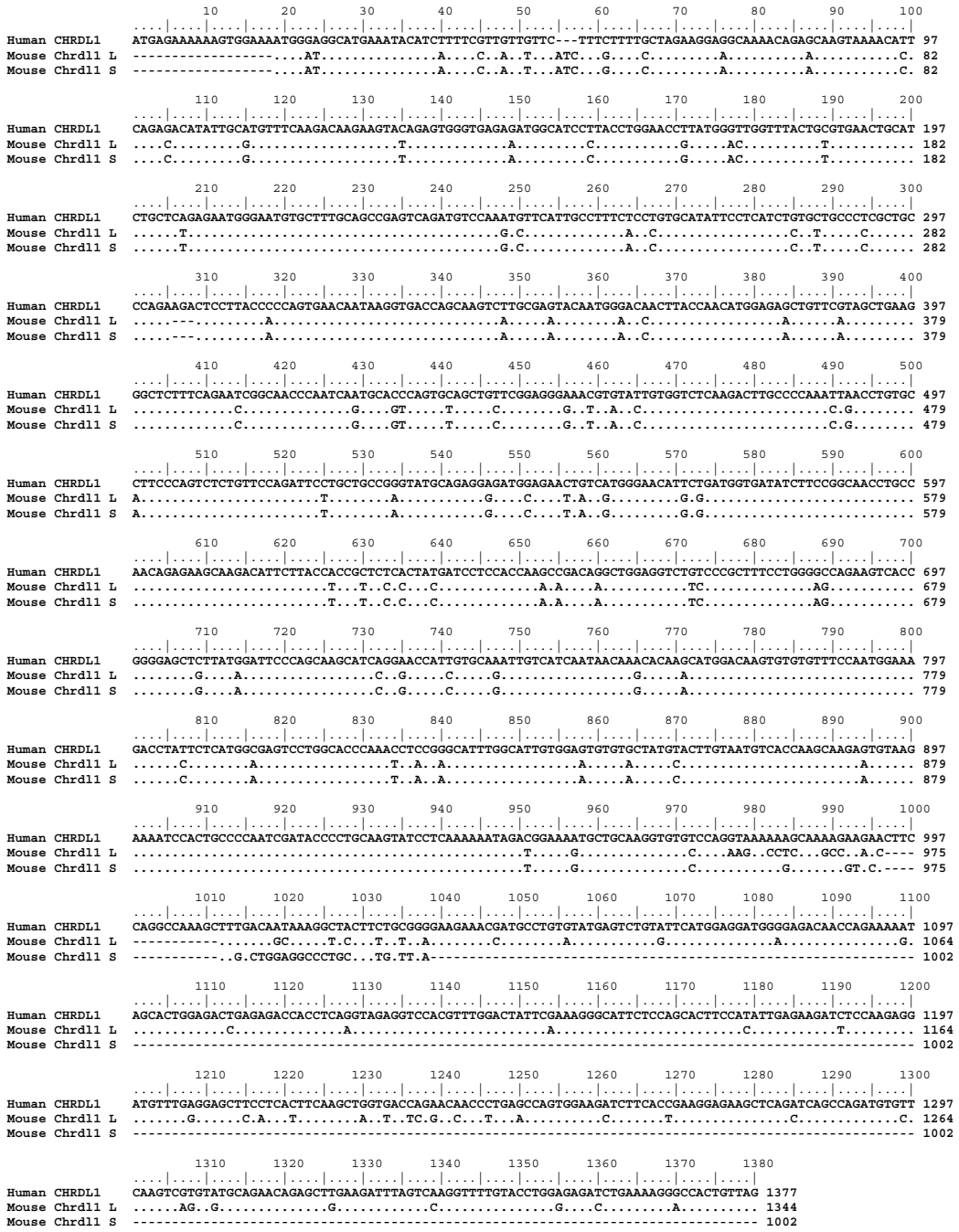
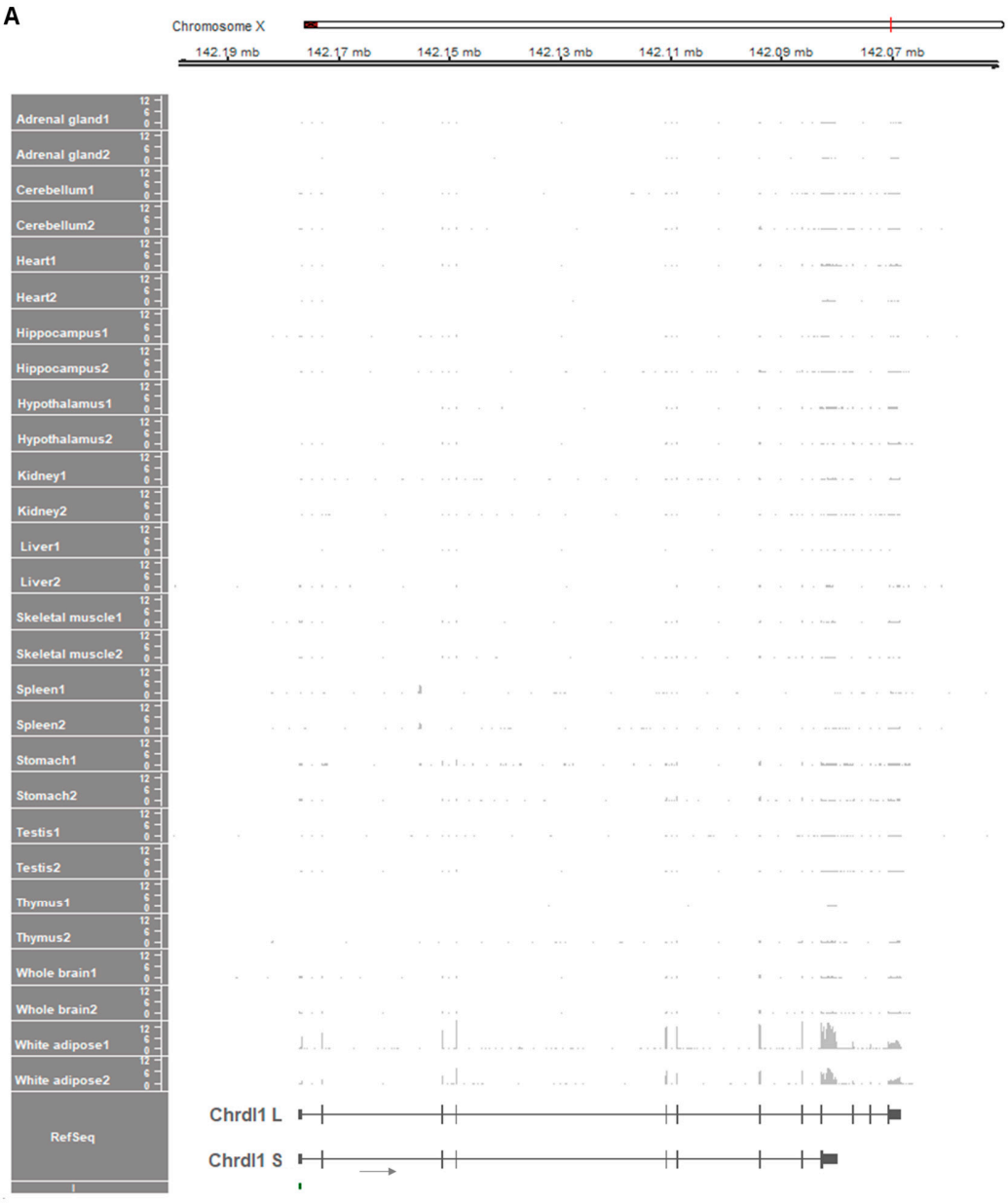


Figure S2. Multiple alignment of chordin-like 1 nucleotide sequences. Human *CHRDL1* and mouse *Chrdl1* protein-coding nucleotide sequences.

A


Chromosome X
142.19 mb
142.17 mb
142.15 mb
142.13 mb
142.11 mb
142.09 mb
142.07 mb
Adrenal gland1
Adrenal gland2
Cerebellum1
Cerebellum2
Heart1
Heart2
Hippocampus1
Hippocampus2
Hypothalamus1
Hypothalamus2
Kidney1
Kidney2
Liver1
Liver2
Skeletal muscle1
Skeletal muscle2
Spleen1
Spleen2
Stomach1
Stomach2
Testis1
Testis2
Thymus1
Thymus2
Whole brain1
Whole brain2
White adipose1
White adipose2
RefSeq
Chrdl1 L
Chrdl1 S

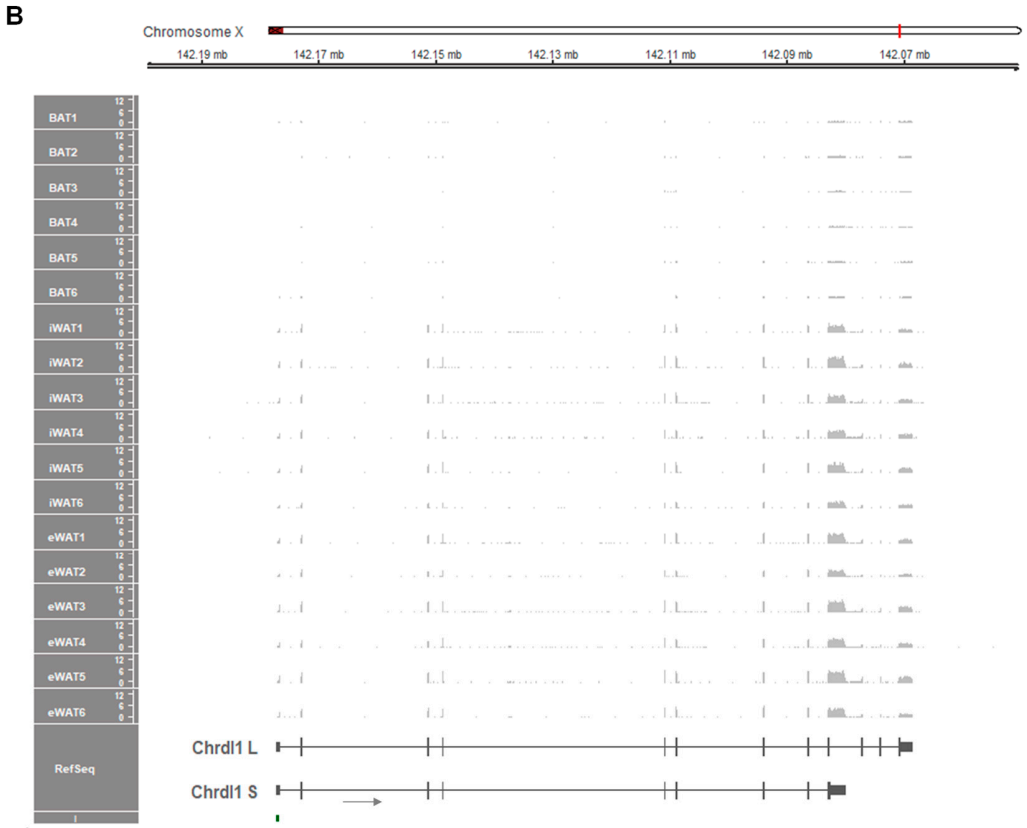


Figure S3. Expression levels of mouse *Chrdl1* mRNA. (A) Mouse *Chrdl1* mRNA expression in various tissues based on RNA-seq data (NCBI SRA study SRP020526). (B) RNA-seq read coverages of mouse *Chrdl1* in BAT, iWAT, and eWAT (GSE131861). TPM values are presented on the y-axis. These read coverages are averaged in Figure 1B. RefSeq, NCBI RefSeq gene; I, GpG island.

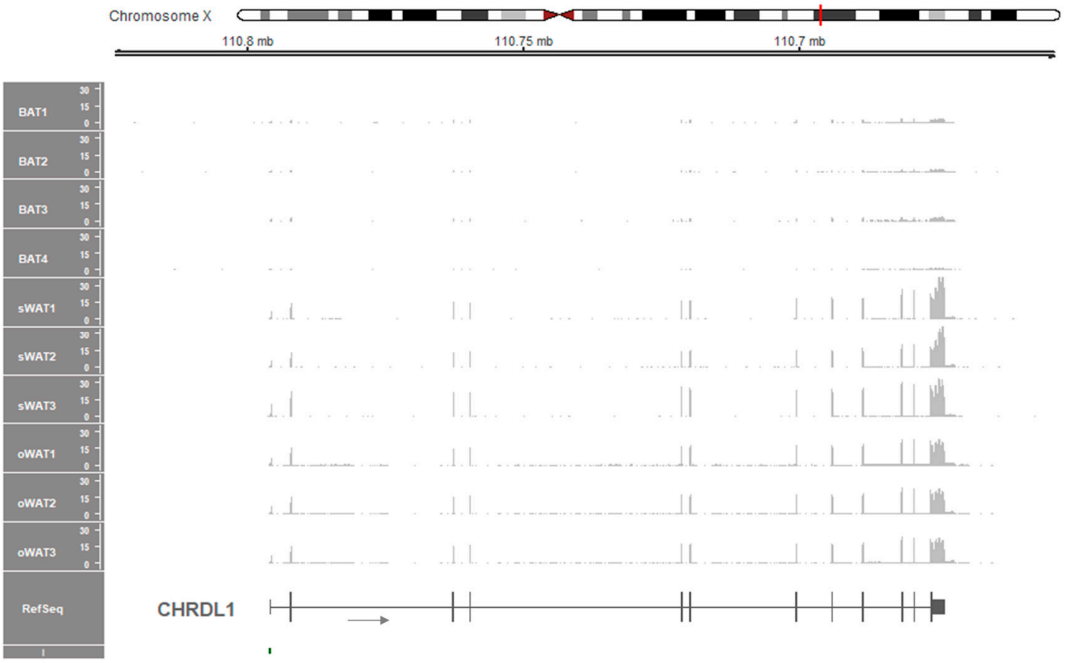


Figure S4. Expression levels of human *CHRDL1* mRNA. RNA-seq read coverages of human *CHRDL1* in BAT, oWAT, and sWAT. The y-axis represents TPM values. These read coverages are averaged in Figure 2B. RefSeq, NCBI RefSeq gene; I, GpG island.

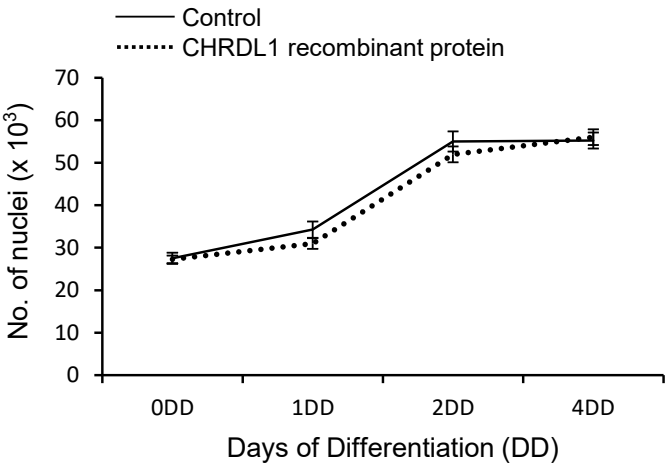


Figure S5. Measurement of cell numbers. Changes in numbers of nuclei in control and CHRDL1 recombinant protein-treated 3T3-L1 cells at 0, 1, 2 and 4 days of differentiation (DD). The Image-based Tool for Counting Nuclei (ITCN) was used for counting after DAPI staining. Values are represented as means ± SEM (n = 4). There was no significant difference between the groups at each time point.