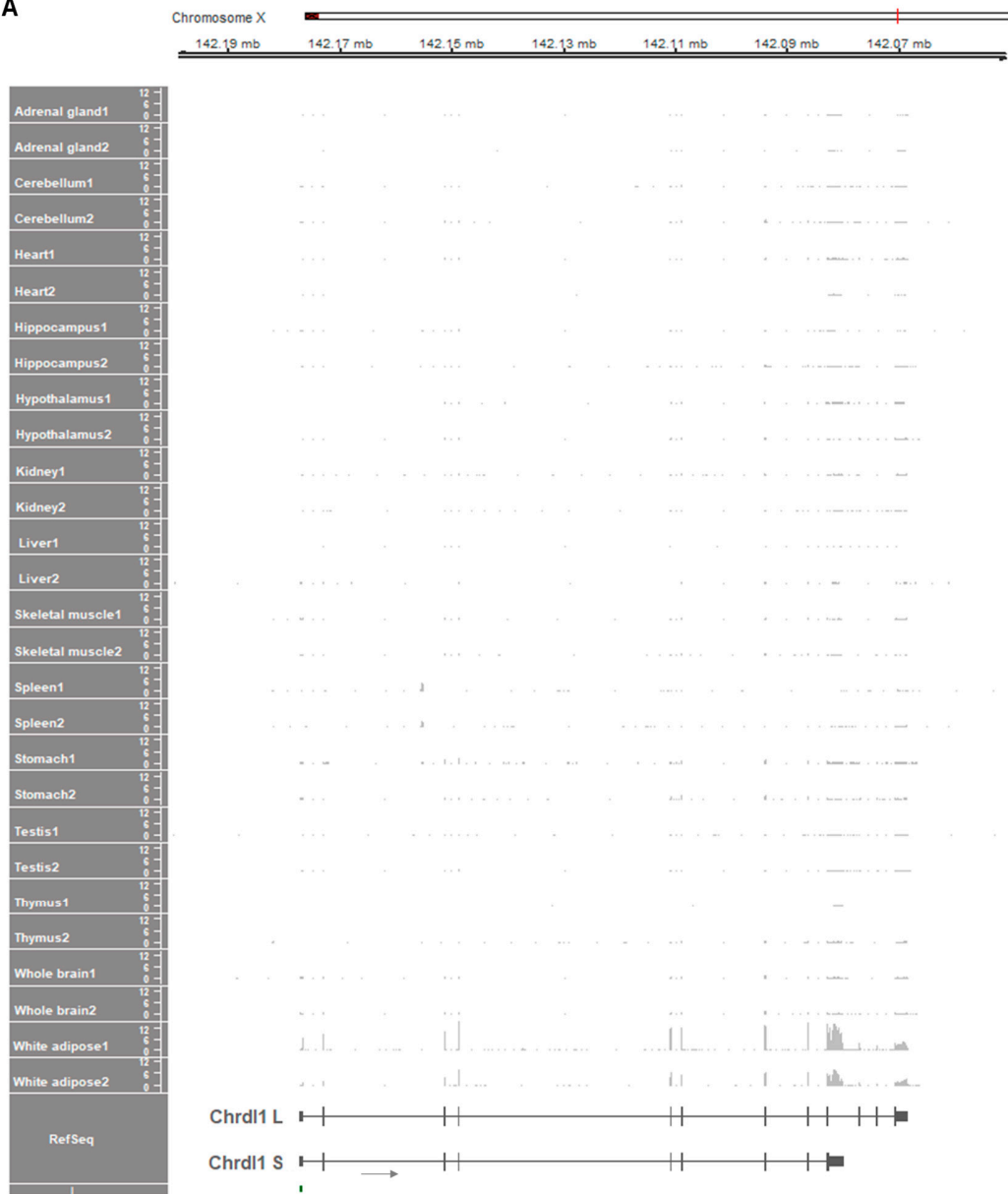


		10	20	30	40	50	60	70	80	90	100	
Human	CHRD1L	ATGAGAAAAAGTGGAAATGGGAGGCATGAAATACATCTTTTCGTGTTGTTC	---	TTTCTTTTGTCTAGAGGAGGCAAAACAGAGCAAGTAAACATT	97							
Mouse	Chrd11 LAT.....A.....C..A..T..ATC..G...C.....A.....A.....CA.....C.....C.....A.....C.....C.....A.....C.....C	82								
Mouse	Chrd11 SAT.....A.....C..A..T..ATC..G...C.....A.....A.....CA.....C.....C.....A.....C.....C.....A.....C.....C	82								
		110	120	130	140	150	160	170	180	190	200	
Human	CHRD1L	CAGAGACATATTGCATGTTTCAAGACAAGAAGTACAGAGTGGGTGAGAGATGGCATCCTTACTCGAACTTATGGGTGGTTTACTGCGTGAACATGCAT	197									
Mouse	Chrd11 LC.....G.....T.....A.....C.....C.....G.....AC.....T.....T.....	182									
Mouse	Chrd11 SC.....G.....T.....A.....C.....C.....G.....AC.....T.....T.....	182									
		210	220	230	240	250	260	270	280	290	300	
Human	CHRD1L	CTGCTCAGAGAAATGGAAATGTGCTTTGCAGCCGAGTCAGATGTCCAAATGTTCAATTGCCCTTCTCCTGTGCATATTCTCATCTGTGCTGCCCTCGCTGC	297									
Mouse	Chrd11 LT.....C.....G.....T.....G..C..C.....A..C.....C.....T.....C.....	282									
Mouse	Chrd11 ST.....C.....G.....T.....G..C..C.....A..C.....C.....T.....C.....	282									
		310	320	330	340	350	360	370	380	390	400	
Human	CHRD1L	CCAGAGACTCCTTACCCCCAGTGAACAAATGAAGGTGACCAGCAAGTCTTGCGAGTACAATGGGCAAACTTACCAACATGGAGAGCTGTTCTGAGCTGAAG	397									
Mouse	Chrd11 LA.....A.....A.....A.....A.....A.....C.....A.....A.....A.....	379									
Mouse	Chrd11 SA.....A.....A.....A.....A.....A.....C.....A.....A.....A.....	379									
		410	420	430	440	450	460	470	480	490	500	
Human	CHRD1L	GGCTCTTTTCAGAAATCGGCAACCAATCAATGCACCCAGTGCAGCTGTTTGGAGGGAAACGTGTATTGGTCTCAAGACTTGCCCCAAATTAACCTGTGC	497									
Mouse	Chrd11 LC.....G.....GT.....T.....C.....G.....T.....A..C.....C.....G.....	479									
Mouse	Chrd11 SC.....G.....GT.....T.....C.....G.....T.....A..C.....C.....G.....	479									
		510	520	530	540	550	560	570	580	590	600	
Human	CHRD1L	CTTCCAGTCTCTGTTTCCAGATTCTGCTGCGGGTATGCAGAGGAGATGGAGAACTGTCAATGGGAACATTCTGATGGTGATATCTTCCGGCAACCTGCC	597									
Mouse	Chrd11 L	A.....T.....A.....G.....C.....T.....A..G.....G.....G.....	579									
Mouse	Chrd11 S	A.....T.....A.....G.....C.....T.....A..G.....G.....G.....	579									
		610	620	630	640	650	660	670	680	690	700	
Human	CHRD1L	AACAGAGAAGCAAGACATCTTACCACCGCTCTCACTATGATCTCCACCAAGCCGACAGGCTGGAGGTCTGTCCCGCTTCTCTGGGGCCAGAAATCACC	697									
Mouse	Chrd11 LT...T..C.C.C...C.....A.A...A.....TC.....AG.....	679									
Mouse	Chrd11 ST...T..C.C.C...C.....A.A...A.....TC.....AG.....	679									
		710	720	730	740	750	760	770	780	790	800	
Human	CHRD1L	GGGAGCTCTTATGGATTCCAGCAAGCATCAGGAACCATTTGTCAAATTTGTCATCAATAACAACACAGCATGGACAAGTGTGTGTTTCCAATGGAAA	797									
Mouse	Chrd11 LG.....A.....C.....G.....C.....G.....G.....A.....	779									
Mouse	Chrd11 SG.....A.....C.....G.....C.....G.....G.....A.....	779									
		810	820	830	840	850	860	870	880	890	900	
Human	CHRD1L	GACCTATTCTCATGGCCAGTCTCGGACCCAAACCTCCGGGCATTTGGCATTGTGGAAGTGTGTGCTATGTAATGTGTAATGTACCAAGCAAGAGTGTAAAG	897									
Mouse	Chrd11 LC.....A.....T.....A.....A.....A.....A.....C.....A.....A.....	879									
Mouse	Chrd11 SC.....A.....T.....A.....A.....A.....A.....C.....A.....A.....	879									
		910	920	930	940	950	960	970	980	990	1000	
Human	CHRD1L	AAAAATCCACTGCCCAATCGATACCCCTGCAAGTATCCTCAAAAAATAGACGGAAAAATGCTGCAAGGTGTGTCCAGGTAAAAAGCAAAAGAAAGAACTTC	997									
Mouse	Chrd11 LT.....G.....C.....AAG...CCTC...GCC...A.C----	975									
Mouse	Chrd11 ST.....G.....C.....AAG...CCTC...GCC...A.C----	975									
		1010	1020	1030	1040	1050	1060	1070	1080	1090	1100	
Human	CHRD1L	CAGGCCAAAGCTTTGACAAATAAAGGCTACTTCTCGGGGAAGAAACGATGCCCTGTGTATGAGTCTGTATTCTATGAGGATGGGGAGACAAACAGAAAAAT	1097									
Mouse	Chrd11 LGC.....T.C...T..T..A.....C.....A.....G.....A.....G.....	1064									
Mouse	Chrd11 SG.CTGGAGGCCCTGC...TG.TT.A-----	1002									
		1110	1120	1130	1140	1150	1160	1170	1180	1190	1200	
Human	CHRD1L	AGCACTGGAGACTGAGAGACCACCTCAGGTAGAGGTCCACGTTTGGACTATTTCGAAAGGGCATTCTCCAGCACTTCCATATTGAGAAGATCTCCAAGAGG	1197									
Mouse	Chrd11 LC.....A.....A.....A.....A.....A.....C.....C.....T.....	1164									
Mouse	Chrd11 SC.....A.....A.....A.....A.....A.....C.....C.....T.....	1002									
		1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	
Human	CHRD1L	ATGTTTGAGGAGCTTCTCTCAAGCTGGTGACCAACAACCTGAGCCAGTGGAAAGATCTTACCGAAGGAGAAGCTCAGATCAGCCAGATGTGTT	1297									
Mouse	Chrd11 LG.....C.A...T.....A..T..TC.G..C...T...A.....C.....T.....C.....	1264									
Mouse	Chrd11 SG.....C.A...T.....A..T..TC.G..C...T...A.....C.....T.....C.....	1002									
		1310	1320	1330	1340	1350	1360	1370	1380			
Human	CHRD1L	CAAGTCGTGTATGCAGAACAGAGCTTGAAGATTAGTCAAGGTTTGTACTGGAGAGATCTGAAAAGGGCCACTGTTAG	1377									
Mouse	Chrd11 LAG..G.....G.....C.....C.....G.....C.....A.....	1344									
Mouse	Chrd11 SAG..G.....G.....C.....C.....G.....C.....A.....	1002									

Figure S2. Multiple alignment of chordin-like 1 nucleotide sequences. Human *CHRD1* and mouse *Chrd11* protein-coding nucleotide sequences.

A



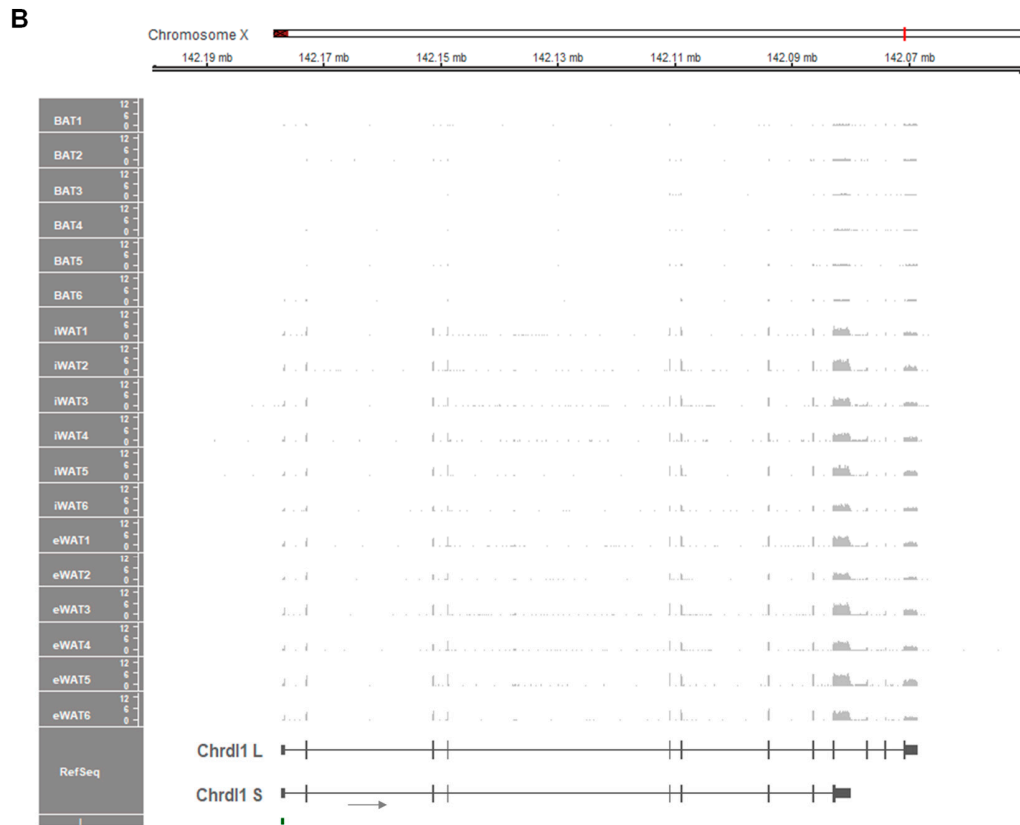


Figure S3. Expression levels of mouse *Chrdl1* mRNA. (A) Mouse *Chrdl1* mRNA expression in various tissues based on RNA-seq data (NCBI SRA study SRP020526). (B) RNA-seq read coverages of mouse *Chrdl1* in BAT, iWAT, and eWAT (GSE131861). TPM values are presented on the y-axis. These read coverages are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

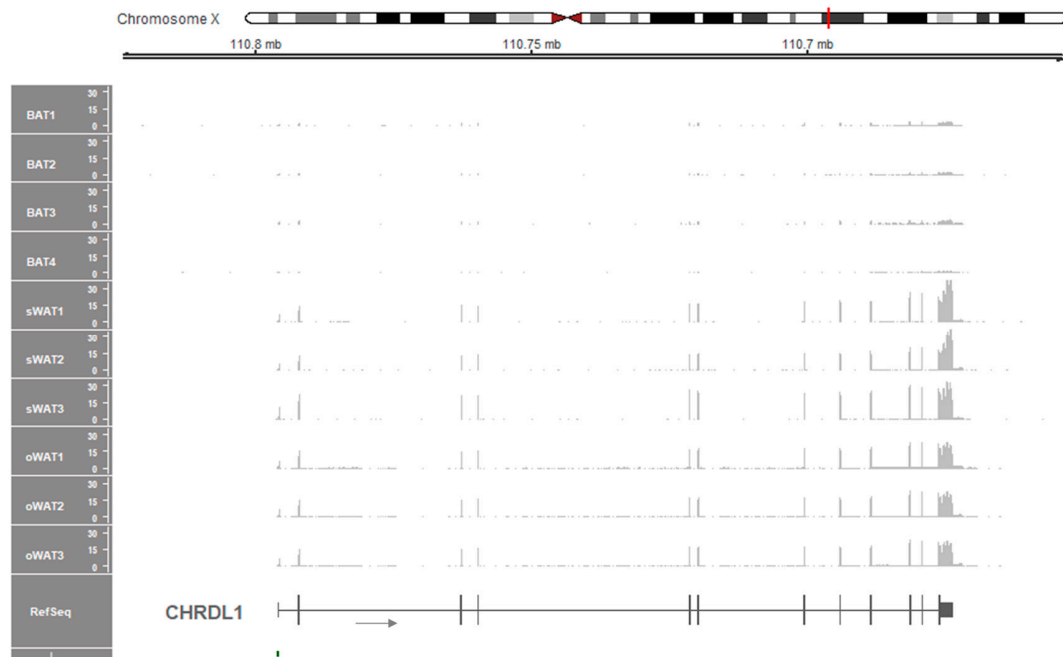


Figure S4. Expression levels of human *CHRD1* mRNA. RNA-seq read coverages of human *CHRD1* in BAT, oWAT, and sWAT. The y-axis represents TPM values. These read coverages are averaged in Figure 2B. RefSeq, NCBI RefSeq gene; I, GpG island.

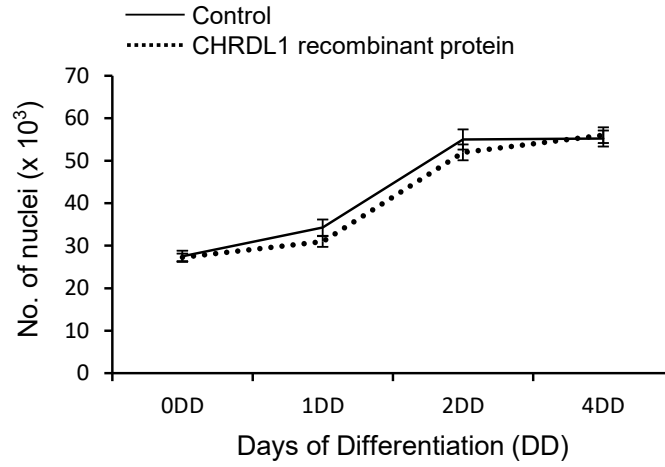


Figure S5. Measurement of cell numbers. Changes in numbers of nuclei in control and CHRDL1 recombinant protein-treated 3T3-L1 cells at 0, 1, 2 and 4 days of differentiation (DD). The Image-based Tool for Counting Nuclei (ITCN) was used for counting after DAPI staining. Values are represented as means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