Table S1. Sequences of siRNAs used to KD gene/protein expression.

	Supplier	**Sequence**
Rat		
Allstars Negative Control siRNA	Qiagen, Venlo, Netherlands	Not provided
siNova1#1 Stealth Select siRNAi Duplex Oligoribonucleotides	Invitrogen, Pasley, UK	5´-CCGCAGGGCUAUUAGGACAUGCUAA-3´
		5´-UUAGCAUGUCCUAAUAGCCCUGCGG-3
siNova1#2 Stealth Select siRNAi Duplex Oligoribonucleotides	Invitrogen, Pasley, UK	5´-TAGCCAGCTATGGATATAATCTCAA-3´
		5´-UUGAGAUUAUAUCCAUAGCUGGCUA-3´
siFoxO3A#1 Stealth Select siRNAi Duplex Oligoribonucleotides	Invitrogen, Pasley, UK	5´-ACAGCCGUUUCAUGCGGGGUUCAGAA-3´
		5´-UUCUGAACCCGCAUGAAACGGCUGU-3
siFoxO3A#2 Stealth Select siRNAi Duplex Oligoribonucleotides	Invitrogen, Pasley, UK	5´-GGCAAGAGCUCUUGGUGGAUCAUCA-3´
		5´-UGAUGAUCCACCAAGAGCUCUUGCC-3´
siBim Stealth Select siRNAi Duplex Oligoribonucleotides	Invitrogen, Pasley, UK	5´-GAGUUCAAUGAGACUUACACGAGGA-3´
		5´-CGAGGAGGGCGUUUGCAAACGAUUA-3´
siSNAP25 b Stealth Select siRNAi Duplex Oligoribonucleotides	Invitrogen, Pasley, UK	5´-GAGGAAGGGAUGGACCAAAUCAAUA-3´
		5´-UAUUGAUUUGGUCCAUCCCUUCCUC-3´
Human		
siNova1 Stealth Select siRNAi Duplex Oligoribonucleotides	Invitrogen, Pasley, UK	5´-UUUGCAACUGAACAAUUGUCUGUCC-3´
		5´-GGACAGACAAUUGUUCAGUUGCAAA-3´

Table S2. Primers used for real time and splicing analyses.

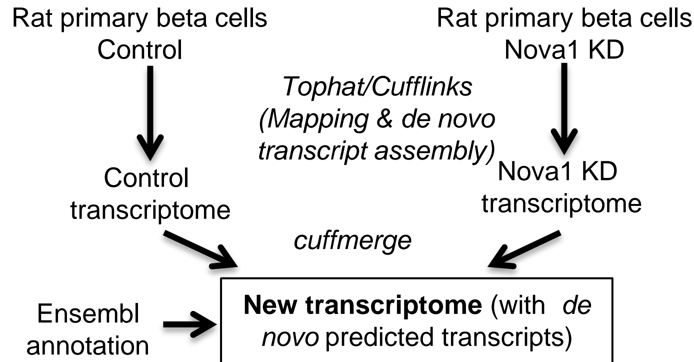
Primer used for real time analyses			
Name	**Forward (5´-3´)**	**Reverse (5´-3´)**	**Lenght**
RAT			
GAPDH ST	ATGACTCTACCCACGGCAAG	TGTGAGGGAGATGCTCAGTG	930 bp
GAPDH RT	AGTTCAACGGCACAGTCAAG	TACTCAGCACCAGCATCACC	136 bp
NOVA1 ST	TCTGACCCCATGACCACCTCCA	CTGCTGGGAAGGCCGCAACA	421 bp
NOVA1 RT	GGAGCAGTCAGGGGCTTGGG	TGAGACAGCTGCCACTCTGTGGA	161 bp
FOXO1ST	AGGCTAGGAGTTAGTGAGCAGGCAA	AACCTGTGGGTGCTGGGCAA	200 bp
FOXO1RT	GGCTAGGAGTTAGTGAGCAGGCAAC	GGGTGAAGGGCATCTTTGGACTGC	91 bp
SNAP25ART	CAACTCGATCGTGTCGAAGA	ATTATTGCCCCAGGCTTTTT	159 bp
SNAP25BRT	GAAAATTCTGCGGGCTTTGT	ATTATTGCCCCAGGCTTTTT	80 bp
HUMAN			
ACTB ST	AAATCTGGCACCACACCTTC	CCGATCCACACGGAGTACTT	805 bp
ACTB RT	CTGTACGCCAACACAGTGCT	GCTCAGGAGGAGCAATGATC	127 bp
NOVA1 ST	TGCAGCTGCTCCTCAGCCCT	ACTGGCCGTCTTCGCCCGTA	363 bp
NOVA1 RT	CCGGTAGCAGCGGCAGGAAC	AGCGGCCTTTTCCGCGAGTC	116 bp
Primers used for splicing analysis			
Name	**Forward (5´-3´)**	**Reverse (5´-3´)**	
RAT			
GABRG2	AGCCCGGAAGTCTCTGCCCA	CCCGTGTCTCCAGGCTCCTGT	
NOVAEX4	TGCCCCAAAATGTTGCCAAGACA	AGCCTTCACAGTAGCACCTCCCT	
NEUREXIN	CACGAGCAGACAGGCTGGCC	CTGGCCCTGCTCTTTCCCGC	
PAX6EX11	TCACATCCCTATCAGCAGCA	TGGGCTGACTGTTCATGTGT	
SNAP25-A	AACAACTCGATCGTGTCGAAG	CATCTGCTCCCGTTCATCC	
SNAP25-B	TGACGGACCTAGGAAAATTCTG	CATCTGCTCCCGTTCATCC	
INSR	TGCACAACGTGGTTTTTGTT	TCTCTTCTGGGGAGTCCTGA	
MAPK9	TGAGTGACAGTAAAAGCGATGG	GTTTGGTTCTGAAAAGGACGAC	
ANK3	TGCCATCACAGGGGACACTGAC	GACCTATCCGAACTGAAGGAGCG	
CACNA1C1	AGTGATCCCTGGAATGTTTTTG	AGGACTTGATGAAGGTCCACAG	
CACNA1C2	GATATAGCAATCACCGAGGTACAC	AGGACTTGATGAAGGTCCACAG	
ARHGEF12	CCGAGAGTCACCAACAGATAAG	TTGTACGAAGACTGGATTGTCC	
CYB5R4	CTGCCTCCAAGTACTCACCTTC	GACTGGACATGTGAGACAAAGC	
SFRS9	GGACCTCGAGGACTTGTTCTAC	GGAAATCTGACCGTCTTGTAGG	
MAP3K9	AGCTCAGTTGTTCCAAAAGAGG	TATCTCCATAAGGCTGGTGAAC	
CLASP1	TGACCTCACCCACCAACTGTTCC	TGGCTTCTGTAACTCCACGCAAAG	
NUMB	GGGATTTCCTGCTCTTAGCC	GAGAGGCAGCACCAGAAGAC	
KCNMA1	TCACACCTCCTGGAATGGACAG	GGTCATCATCATCGTCTTGGTC	

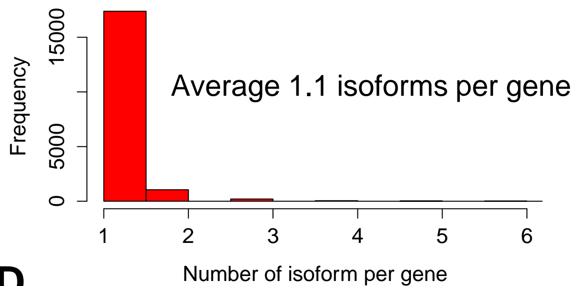
ST denotes standard PCR, RT denotes real time qPCR.

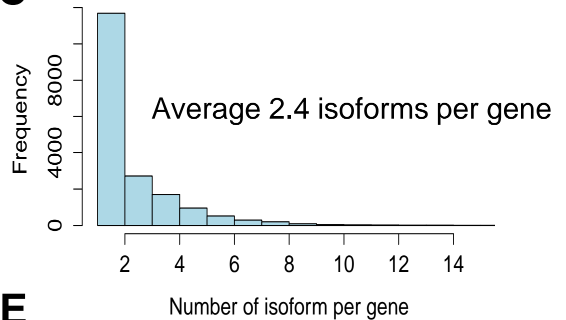
Table S3. Reads count and mapping statistics after RNA-seq.

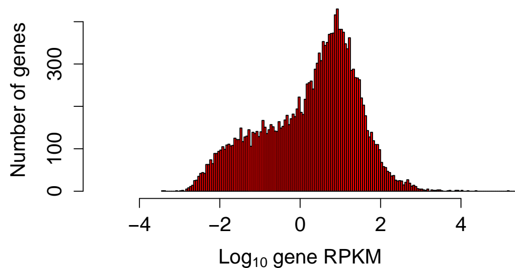
Sample	Condition	Reads 1	Reads 2	Total reads 1+2	Mapped reads	Mapped total	Properly paired	% mapped
1	CTL 1	153.174.762	153.174.762	306.349.524	260.657.490	355.836.052	302.888.416	85%
2	Nova1 KD 1	68.685.321	68.685.321	137.370.642	116.197.690	159.271.991	135.962.398	85%
3	CTL 2	67.568.538	67.568.538	135.137.076	106.831.580	162.088.157	125.538.492	79%
4	Nova1 KD 2	53.447.414	53.447.414	106.894.828	81.799.358	126.311.494	95.700.154	77%
5	CTL 3	109.127.969	109.127.969	218.255.938	179.430.848	260.732.788	209.394.344	82%
6	Nova1 KD 3	79.355.128	79.355.128	158.710.256	130.637.368	185.478.101	149.657.890	82%

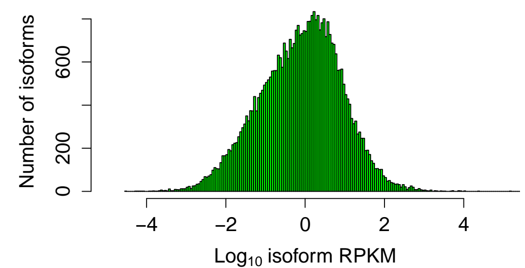
SUPPLEMENTARY FIGURE 1

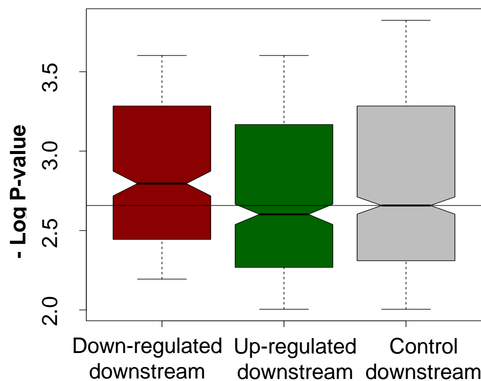


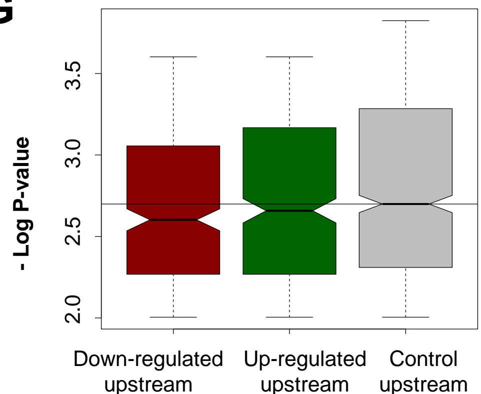












SUPPLEMENTARY FIGURE 2

A

-log(p-value)

0,0 0,5 1,0 1,5 2,0 2,5 3,0 3,5 4,0 4,5 5,0 5,5 6,0

Threshold

CELL SIGNALLING

MOLECULAR TRANSPORT

VITAMIN AND MINERAL METABOLISM

CELL MORPHOLOGY

CELLULAR ASSEMBLY AND ORGANIZATION

CELLULAR DEVELOPMENT

CELL FUNCTION AND MAINTENANCE

CELL-TO-CELL SIGNALLING AND INTERACTION

NERVOUS SYSTEM DEVELOPMENT AND FUNCTION

TISSUE DEVELOPMENT

CELL DEATH AND SURVIVAL

POST-TRANSLATIONAL MODIFICATION

B

-log(p-value)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Threshold

CELLULAR ASSEMBLY AND ORGANIZATION

CELL FUNCTION AND MAINTENANCE

CELL MORPHOLOGY

CELLULAR DEVELOPMENT

CELL-TO-CELL SIGNALLING AND INTERACTION

CELLULAR COMPROMISE

CELLULAR MOVEMENT

CELL SIGNALLING

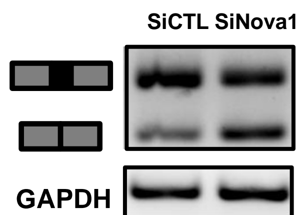
MOLECULAR TRANSPORT

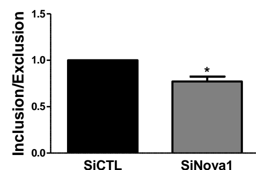
VITAMIN AND MINERAL METABOLISM

POST-TRANSLATIONAL MODIFICATION

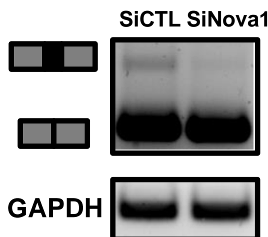
SUPPLEMENTARY FIGURE 3

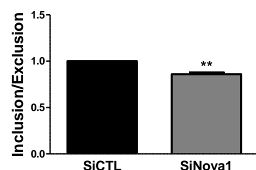
ARHGEF12



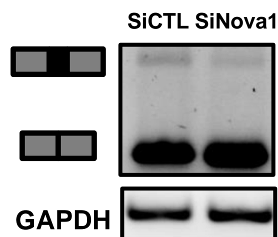


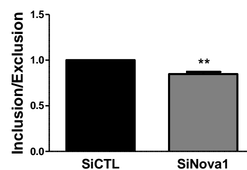
KCNMA1



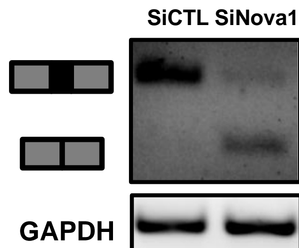


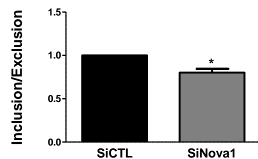
MAPK9



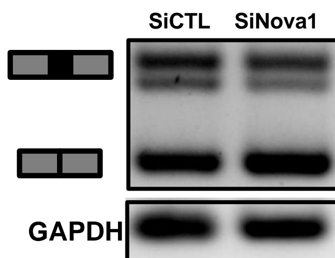


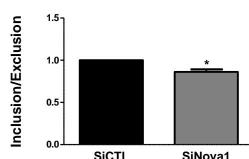
SFRS9



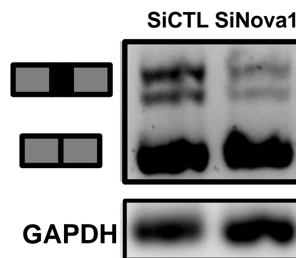


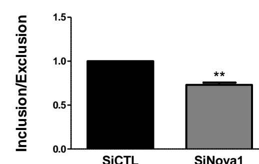
MAP3K9



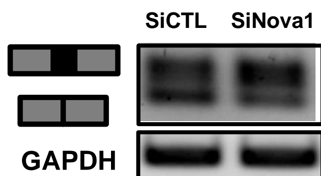


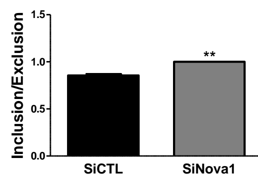
NUMB



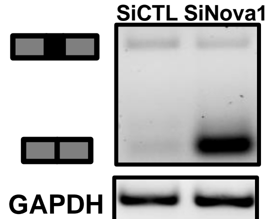


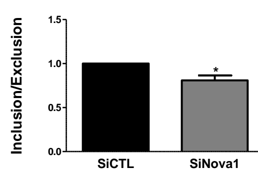
ANK3



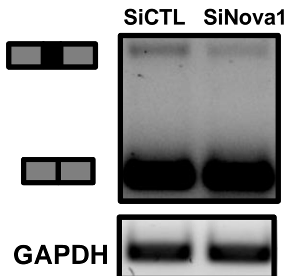


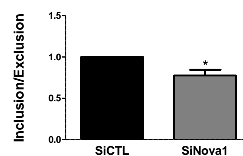
CYB5R4





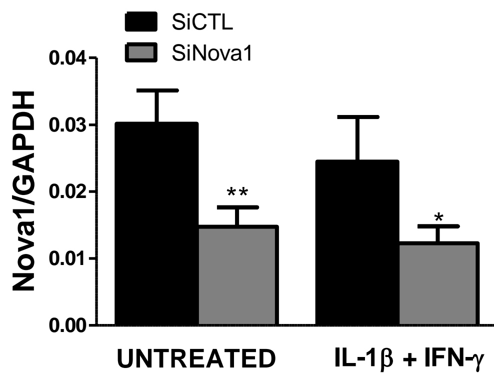
CLASP1



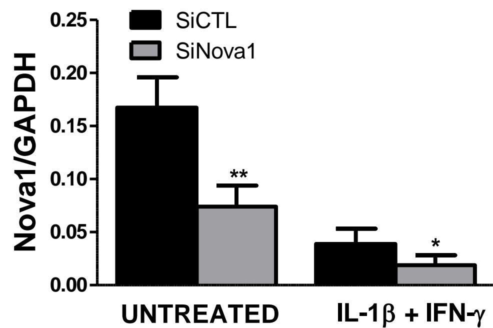


SUPPLEMENTARY FIGURE 4

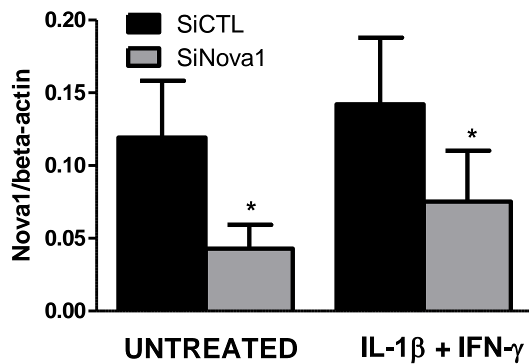
A



B

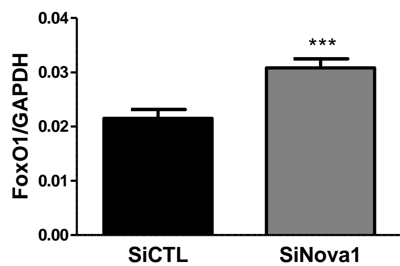


C

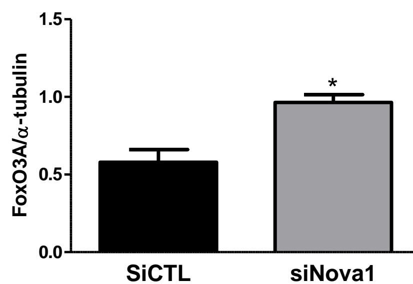


SUPPLEMENTARY FIGURE 5

A



B



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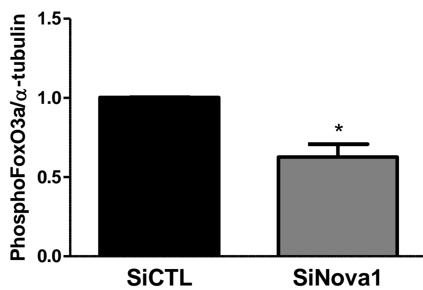

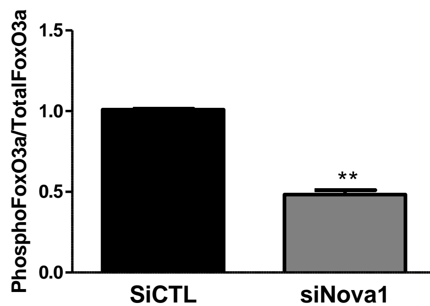

D

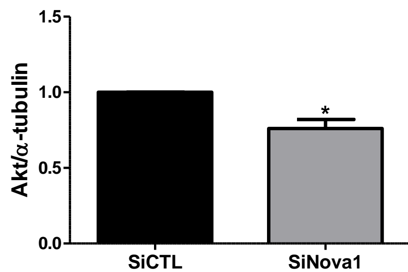

Table S3. Reads count and mapping statistics after RNA-seq. Summary of the read counts after sequencing and mapping of three FACS-purified primary rat beta cell preparations cultured under control condition (CTL 1,2,3) or after *Nova1* KD (*Nova1* KD 1,2,3). An average of 177 million total reads per sample was obtained and 82% of them were properly mapped using Tophat. The mappings were subsequently analyzed with Cufflinks for the discovery of unknown expressed isoforms. The numbers in the column "mapped total" is greater than that in "mapped reads" column since some reads were mapped to more than one alternative location.

Figure S1. *De novo* transcript assembly of the RNA-seq data and analysis of YCAY clusters. A. Description of the strategy used for the *de novo* isoforms assembly using the RNA-seq samples and a Tophat/Cufflinks pipeline. B-C. Distribution of isoforms per gene as annotated in Ensembl version 70 (B) and after *de novo* assembly with discovery of unknown isoforms (C). *De novo* discovery of expressed but unknown isoforms increased the number of identified isoforms per genes (2.4 versus 1.1 with Ensembl annotation only). D-E. Histograms of RPKM levels of the rat genes in beta cell (D) and the *de novo* assembled isoforms (E). F-G. Distributions of the P-values of predicted clusters of Nova1 YCAY motifs in downstream (F) and upstream (G) regions. Compared to random control, downstream regions (where Nova acts as activator) of down-regulated exons after Nova1 KD showed more significant YCAY motifs clusters (F, red box, Wilcoxon-Mann-Wittney P-value: 0.002). In contrast, the downstream junctions regions of up-regulated exons after Nova1 KD showed less significant YCAY clusters (F, green box, Wilcoxon-Mann-Wittney P-value: 0.036) as the upstream regions of down-regulated genes showed (G, red box, Wilcoxon-Mann-Wittney P-value: 0.027). There were no differences in significant YCAY motifs clusters in the upstream regions of up-regulated exons compared to random control (G, green box).

Figure S2. Ingenuity Pathway Analysis of *Nova1* KD- differentially spliced transcripts. The length of the blue bars indicates the significance of the association between the set of genes and the keyword, and is expressed as minus the logarithm of the probability that a random set of genes from the human genome would be associated with the same keyword. The straight orange line indicates a threshold of 0.05 (corresponding to a -log(B-H p-value) of 1.3). A. IPA analysis of all transcripts modified by *Nova1* in pancreatic beta cells. B. IPA analysis of transcripts modified by *Nova1* and shared by brain and pancreatic beta cells.

Figure S3. RT-PCR validation of Nova1 target exons in INS-1E cells. Each panel shows a predicted Nova1 target alternative splicing event. The alternative exon(s) with Nova1-regulated inclusion or exclusion are highlighted in black or white at the left side of the figures. The result of RT-PCR analysis comparing siCTL and siNova1 is shown at the right side. Each agarose gel is representative of three independent experiments. The respective quantification of inclusion/exclusion of each exon (done as described in Figure 1) is shown in the figure below the picture of the gel. * $P<0.05$ or ** $P<0.01$ *vs*. siCTL by paired *t*-test.

Figure S4. Confirmation of *Nova1* KD. INS-1E cells, primary rat beta cells and human islet cells were transfected with siCTL and siNova1 and then exposed or not to cytokines. After 24 h or 48h cells were used for qRT-PCR analyses and apoptosis was measured in parallel (data shown in Figure 4). Confirmation of *Nova1* KD in INS-1E cells (A), rat primary beta cells (B) and human islet cells (C). Results are mean ± SEM (n=4) * $P<0.05$ or ** $P<0.01$ *vs*. siCTL untreated or after cytokine treatment by paired *t*-test.

Figure S5. *Nova1* regulates the expression of the *FoxO* family of transcription factors and the *Akt* kinase. INS-1E cells were transfected with siCTL or siNova1 and collected after 48h for mRNA analysis. A. Expression of the transcription factor *FoxO1* after *Nova1* KD measured by

qRT-PCR. Results are mean ± SEM (n=4). *** $P<0.001$ *vs*. siCTL. B. and C. Densitometry analysis of the Western blots shown in Figure 7 for B. FoxO3A and C. PhosphoFoxO3A using respectively α-tubulin or total FoxO3A to correct for protein loading. Results are mean ± SEM (n=5). * $P<0.05$ or ** $P<0.01$ *vs*. siCTL by paired *t*-test. D. Densitometry analysis of the Western blot shown in Figure 7E for Akt. Results are mean ± SEM (n=4). * $P<0.05$ *vs*. siCTL by paired *t*-test