Original Article

Pancreatic cancer differential methylation atlas in blood, peri-carcinomatous and diseased tissue

Huan Wang[1#], Fan Yin[2#], Fang Yuan[1#], Yuehua Men[3], Muhong Deng[1], Yang Liu[4], Qingfang Li[1]

[1]Cancer Center, General Hospital of PLA, Beijing 100086, China; [2]Department of Oncology, The Second Medical Centre & National Clinical Research Center of Geriatric Disease, Chinese PLA General Hospital, Beijing 100086, China; [3]Department of Dermatology, Peking University Third Hospital, Beijing 100191, China; [4]Endocrine Department, Chinese PLA 309 Hospital, Beijing 100193, China

Contributions: (I) Conception and design: H Wang, Q Li; (II) Administrative support: H Wang; (III) Provision of study materials or patients: F Yuan, M Deng; (IV) Collection and assembly of data: Y Men, Y Liu; (V) Data analysis and interpretation: H Wang, F Yin, Q Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Dr. Qingfang Li. Cancer Center, General Hospital of PLA, No. 28, Fuxing Road, Beijing 100853, China. Email: liqfluck@126.com.

Background: Pancreatic cancer is common in elderly persons, and less than 20% of patients present with localized, potentially curable tumors.

Methods: We compared the methylated sites and genes in pericarcinous tissues compared to cancer tissue, and blood compared to pericarcinous tissues in order to harvest methylation markers for putative diagnostic and therapy monitoring purposes.

Results: Of 15,397 CpG sites detected in 7,440 genes, 5,605 (36.4%, 5,605 of 15,397) CpG sites were hypomethylated and 5,870 (38.12%, 5,870 of 15,397) CpG sites were hypermethylated. We then performed Gene Ontology (GO) and KEGG analysis to systematically characterize the ten significantly differentially methylated genes: *PTPRN2*, *MAD1L1*, *TNXB*, *PRDM16*, *GNAS*, *KCNQ1*, *TSNARE1*, *HDAC4*, *TBCD*, and *DIP2C*. Meanwhile, function analysis of genes with differentially methylated sites located in promoter regions of overlap group was also performed. According to previous studies, we further screened 22 pancreatic cancer related key genes. The results suggested that these key genes can influence methylation. GO and KEGG analysis indicated that these genes are involved in a wide range of functions.

Conclusions: The identification of differentially methylated genes in this study provides valuable information for liquid biopsy methylation markers in pancreatic cancer.

Keywords: Pancreatic cancer; blood; DNA methylation; CpG sites; KEGG

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Introduction

Pancreatic cancer is more common in elderly persons than in younger persons, and less than 20% of patients present with localized, potentially curable tumors (1). The estimated incidence of pancreatic cancer in the United States was 37,700 cases, and an estimated 34,300 patients died from the disease in 2008. The overall 5-year survival rate among patients with pancreatic cancer is <5% (2). Several environmental factors have been implicated, but evidence of a causative role exists only for tobacco use. The risk of pancreatic cancer in smokers is 2.5 to 3.6 times that in nonsmokers (3) Some studies have shown an increased incidence of pancreatic cancer among patients with a history of diabetes or chronic pancreatitis, and there is also evidence that chronic cirrhosis, a high-fat, high-cholesterol diet, and previous cholecystectomy are associated with an increased incidence (4,5).

Presently, there is no valid diagnostic marker for pancreatic cancer. Carbohydrate antigen 19-9 (CA 19-9) levels are elevated in pancreatic cancer but frequently only

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in advanced disease. It can also be elevated in other cancers, chronic pancreatitis, and autoimmune diseases such as rheumatoid arthritis. Approximately 10% of the population lacks expression of Lewis antigen, which is required to produce CA 19-9. Furthermore, CA 19-9 is used in a clinical setting based on response to treatment (6,7). Up to now, a combination of complex and advanced imaging modalities, such as positron emission tomography scanning, 3-phase computed tomography scanning, endoscopic ultrasound, laparoscopic ultrasound, endoscopic retrograde cholangiopancreatography, and trans-abdominal ultrasound, are necessary for the diagnosis of pancreatic cancer. However, several of these methods are invasive and thus risk complications. Consequently, a minimally or noninvasive marker for pancreatic cancer is urgently needed.

Epigenetics is defined as the study of mitotically or meiotically heritable variations in gene function that cannot be explained by changes in DNA sequence (8). Epigenetic modifications, such as DNA promoter hypermethylation, are known to be aspects of early carcinogenesis and have shown significant potential in the development of a useful diagnostic marker (9,10). Recently, attention to its role in pancreatic cancer has recently increased. DNA methylation has gained much recent interest for its role in cancer biology. Aberrant patterns of DNA methylation can be associated with carcinogenesis and affect the regulation of genome stability and gene transcription (11). Genome wide studies of CpG islands have uncovered thousands of loci where differential methylation can segregate pancreatic tumor tissue from normal tissue (12).

Cancer-linked global genomic hypomethylation in tumor tissue is a common characteristic in a wide variety of malignancies, ranging from solid tumors, such as breast, colon, oral, and lung cancers, to cancers of the blood (13,14). In this study, in order to identify candidate liquid biopsy methylation markers in pancreatic cancer, we have employed a global methylation profiling platform to comprehensively survey a large scale of CpG sites between blood and cancer tissues versus pericarcinous tissues. We compared pericarcinous tissues *vs.* cancer tissue and blood *vs.* pericarcinous tissues in order to harvest methylation markers for diagnostic purposes. These genes could be the most likely candidate methylation markers for future liquid biopsies in pancreatic cancer.

Methods

Subjects

Six patients with pancreatic cancer (2 males and 4 females, mean age: 58.83±14.95 y), without radiation, chemotherapy and immunotherapy treatment, were recruited from the Chinese General Hospital of PLA in China (*Table S1*). The diagnosis of pancreatic cancer was made by at least two experienced oncologists. Sample collection was carried out accorded to the following criteria: (I) the minimum diameter of tumor was greater than 2 cm. Meanwhile, pancreatic cancer was identified by Hematoxylin and Eosin (H&E) staining and the ratio of cancer cells in the whole cells section was over 80%. (II) Tissue adjacent to cancer was collected as far as possible from the cancer tissue in order to avoid the mistake sampling. (III) Blood samples were collected before surgery. Pancreatic cancer tissue and tissue adjacent to cancer of each patient were collected and stored in liquid nitrogen immediately for DNA extraction. All specimens were subjected to autolysis for 4 to 8 h and then snap-frozen at –80 ℃ until use in analysis. DNA was extracted from 25 mg samples of the tissue specimens using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. gDNA of Blood samples were extracted by FitAmp™ Plasma/Serum DNA Isolation Kit (Epigentek, USA) according to the manufacturer's instructions. The DNA yield and purity were determined spectrophotometrically (NanoDrop® ND1000; Thermo Fisher Scientific Inc., Waltham, MA, USA) and by gel electrophoresis, respectively. DNA of sample was stored at –20 ℃ for further study. This study was approved by the Ethics Committee of Chinese General Hospital of PLA (No. S2018-013-02). All patients provided signed informed consent.

DNA methylation methods

Bisulfite conversion of 500 ng genomic DNA was performed using the EZ DNA methylation kit (Zymo Research). DNA methylation level was assessed according to the manufacturer's instructions using Infinium-HumanMethylation450 Beadchips (Illumina Inc.). The technical schemes, the accuracy, and the high reproducibility of this array have been described previously (15). Quantitative measurements of DNA methylation were determined for 485,577 CpG dinucleotides, which covered 99% of the RefSeq genes and were distributed across the whole gene regions, including promoter, gene body, and 30-untranslated regions (UTRs). They also covered 96% of CGIs from the UCSC database with additional coverage in CGI shores (0–2 kb from CGI) and CGI shelves (2–4 kb from CGI). Detailed information on the contents of the array is available in the Infinium HumanMethylation450 User Guide and Human-Methylation 450 manifest (www.

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Table 1 Basic information of six patients in this study

Patient	Age	A (blood)	B (pericarcinous tissue)	C (pancreatic cancer tissue)
Patient 1 (F)	74	A1[&]	B1[&]	C1[&]
Patient 2 (M)	36	A2[&]	B2*	C2[&]
Patient 3 (M)	66	A3[&]	B3[&]	C3[&]
Patient 4 (F)	60	A4[&]	B4*	C4[&]
Patient 5 (F)	46	A5[&]	B5[&]	C5[&]
Patient 6 (F)	71	A6[&]	B6[&]	C6[&]

[&], represents qualified sample; *, represents unqualified samples. F, female; M, male.

illumina.com) and in recent papers (16). DNA methylation data were analyzed with the methylation analysis module within the BeadStudio software (Illumina Inc.). DNA methylation status of the CpG sites was calculated as the ratio of the signal from a methylated probe relative to the sum of both methylated and unmethylated probes. This value, known as b, ranges from 0 (completely unmethylated) to 1 (fully methylated). Given the batch effects normally associated with this platform and especially for small sample sizes as in the current study, we performed batch effect correction as described previously (17). For intra-chip normalization of probe intensities, colored balance and background corrections in every set of ten samples from the same chip were performed using internal control probes. X chromosome CpG sites in the CGIs in the AR gene in this array as well as the internal control probes were checked to validate the DNA methylation measurements.

Bioinformatics

GO enrichment analysis was performed using GOEAST (http://omicslab.genetics.ac.cn/GOEAST/index.php). Hypergeometric distribution was used to calculate the P value of GOID enrichment, and P<1E–4 cut-off value was applied (18). The graph size was reduced by condensing non-significant nodes to points. The smaller the P value is, the more significant the GO term is enriched in the dataset. And the graph size was reduced by condensing non-significant nodes to points. KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-through put experimental technologies (http://www.genome.jp/kegg/). We used KOBAS software to test the statistical enrichment of differentially methylated genes in KEGG pathways.

Results

Overlap of differential DNA methylation sites between pericarcinous tissues vs. cancer tissue and blood vs. pericarcinous tissues

DNA methylation levels were compared between four pericarcinous tissues (B) *vs.* six pancreatic cancer tissues (C) and six blood samples (A) *vs.* four pericarcinous tissues (B) using Infinium HumanMethylation450 Bead Chips (*Table 1*). Sites simultaneously present in B versus C and A versus B group comparisons were group defined as hypermethylation sites. Same was done to define hypomethylation sites. Meanwhile, hypomethylation sits simultaneously existed in B *vs.* C group and A *vs.* B group was defined as hypomethylation sits. Of 485,577 CpG sites, significant diagnostic differences in DNA methylation were observed at 15,397 CpG sites representing 7,440 genes at FDR 5 % correction (*Figure 1* and http://fp.amegroups.cn/cms/9614487675fcbfcb574c6af25b586775/tcr.2019.11.26-1.pdf). Of these sites, 5,605 (36.4%, 5,605 of 15,397) CpG sites were hypomethylated and 5,870 (38.12%, 5,870 of 15,397) CpG sites were hypermethylated. Functional distribution of 5,870 hypermethylated CpG sites suggested that 47.4% of these sites were located in promoter regions, 38.86% of these sites were located in gene bodies, 12.42% of these sites were located in intergenic regions and 6.01% of these sites were located in the 3'-untranslated regions (UTRs). Furthermore, sublocation analysis of 2,659 CpG sites in promoter region with hypermethylated indicated that 31.74% of these sites were located in regions from –200 to –1,500 nt upstream of the transcription start site (TSS1500),

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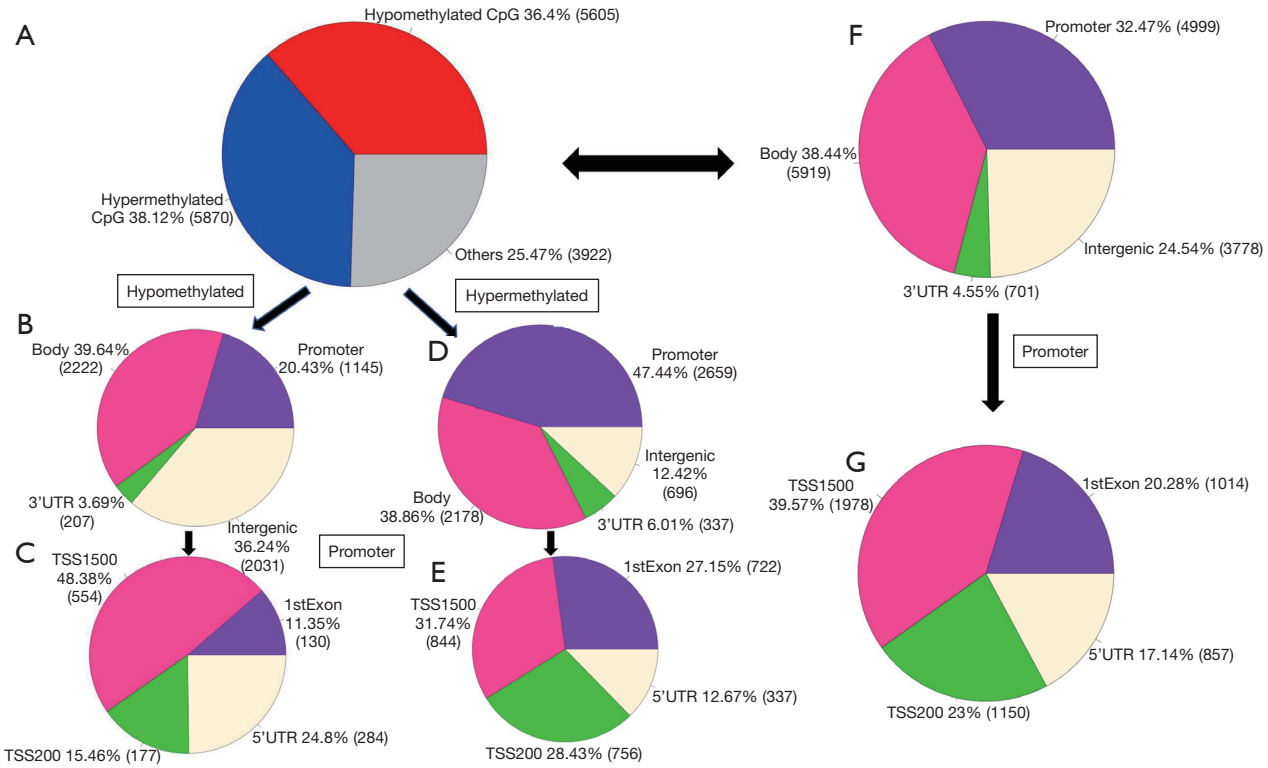

Figure 1 Graphic illustration of functional distribution and differentially methylated CpG sites identified in this study.

28.43% of these sites were located in regions from −200 nt upstream to the TSS itself (TSS200), 27.15% of these sites were located in 1st Exon regions and 12.67% of these sites were located in the 5'-untranslated regions (UTRs). These hypermethylated CpG sites were mostly located in gene bodies and promoter regions. Meanwhile, Functional distribution of 5,605 hypomethylated CpG sites suggested that 20.43% of these sites were located in promoter regions, 39.64% of these sites were located in gene bodies, 36.24% of these sites were located in intergenic regions and 3.69% of these sites were located in 3'UTR regions. Furthermore, sublocation analysis of 5,605 hypomethylated CpG sites in promoter regions indicated that 48.38% of these sites were located in TSS1500 regions, 15.46% of these sites were located in TSS200 regions, 11.35% of these sites were located in 1st Exon regions and 24.8% of these sites were located in 5'UTR regions. These hypomethylated CpG sites were mostly located in gene bodies, promoter regions and intergenic regions. The results above seem to be in apparent contradiction to widely held belief that promoter hypomethylation is correlated to increased transcription and vice versa. This also indicates the possibility that transcription factors are modified which dictate their regulation of anomalous transcription in the cancer cells.

Because the 15,397 methylated CpG sites corresponded to 7,440 genes, some of the methylated genes must contain more than one methylated site. Further analysis showed that among the 7,440 methylated genes, 4,962 (67%) possessed only one methylated site, 1,590 (21%) contained two methylated sites, and 888 (12%) contained three or more methylated sites (*Figure 2* and http://fp.amegroups.cn/cms/3adcaa480666f581911c4ab936783571/tcr.2019.11.26-2.pdf). In particular, one methylated gene (PTPRN2) possessed 40 methylated sites in overlap. Meanwhile, the MAD1 mitotic arrest deficient-like 1 (yeast) (MAD1L1, ENSG00000002822) possessed over 25 methylated sites (*Figure 3*). Of note, number of methylation sites can be correlated to gene length and mere presence of more methylation sites does not mean increased methylation-based regulation. Instead, methylation sites normalized over gene length is a better indicator of propensity to regulation by methylation.

Gene Ontology (GO) and KEGG pathway analysis of differentially methylated genes in overlap group

In order to improve the credibility of this research, the

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genes with counts of methylation sites were equal or greater than 15 were selected to perform intensive study. After such screening, 10 genes with more than three counts of differentially methylated CpG sites were harvested. GO terms were further assigned to Homo sapiens differentially methylated genes based on their sequence similarities to known proteins in the UniProt database annotated with GO terms as well as InterPro and Pfam domains they contain. GO annotation and enrichment analysis of ten significantly differentially methylated genes was implemented by GOEAST software (http://omicslab.genetics.ac.cn/GOEAST/index.php), in which gene length bias was corrected. GO terms with corrected P value less than 10^{-4} were considered significantly enriched (*Figure 4*). Biological processes, cellular components, and molecular functions are shown in *Figure 4* and *Table S2*. From the perspective of biological processes, there are 75 GO terms were assigned under this catalogues. Among these terms, spindle checkpoint (GO: 0031577, P value: 8.98E–21), mitotic spindle assembly checkpoint (GO: 0007094, P value: 3.5E–21) and negative regulation of mitotic sister chromatid segregation (GO: 0033048, P value: 3.5E–21) were the top three significantly enriched terms. From the cellular component perspective, there are 3 GO terms were assigned under this catalogues. Among these terms, A band (GO: 0031672, P value: 1.1E–05) was the top significantly enriched terms. From the molecular function perspective, there are 4 GO terms were assigned under this catalogues. Among these terms, G-protein beta/gamma-subunit complex binding (GO: 0031683, P value: 2.4E–11) was the top significantly over-represented terms.

In vivo, various biological functions were implemented by cooperation of different genes. Pathways enrichment analysis can give some clues to the biochemical and signal transduction pathways that differentially expressed genes may participate in. KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-through put experimental technologies (http://www.genome.jp/kegg/). We used KOBAS software to test the statistical enrichment of differentially methylated genes in KEGG pathways (19). In this study, ten significantly differentially methylated genes involve 52 pathways (*Table S3*). It was worthy noticed that 43 pathways owned the same corrected P value (0.31). *Table S3* shows the results of pathways enrichment, it clearly displays that vibrio cholerae infection were the top enriched

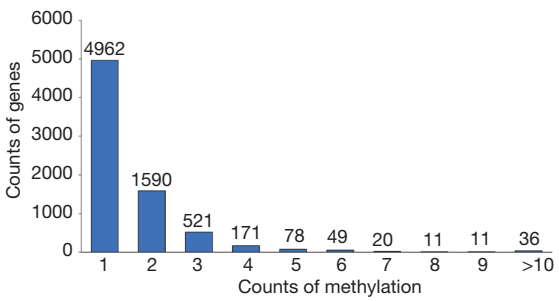

Figure 2 Analysis of the identified methylated CpG sites. Distribution of the methylated CpG sites in the methylated genes.

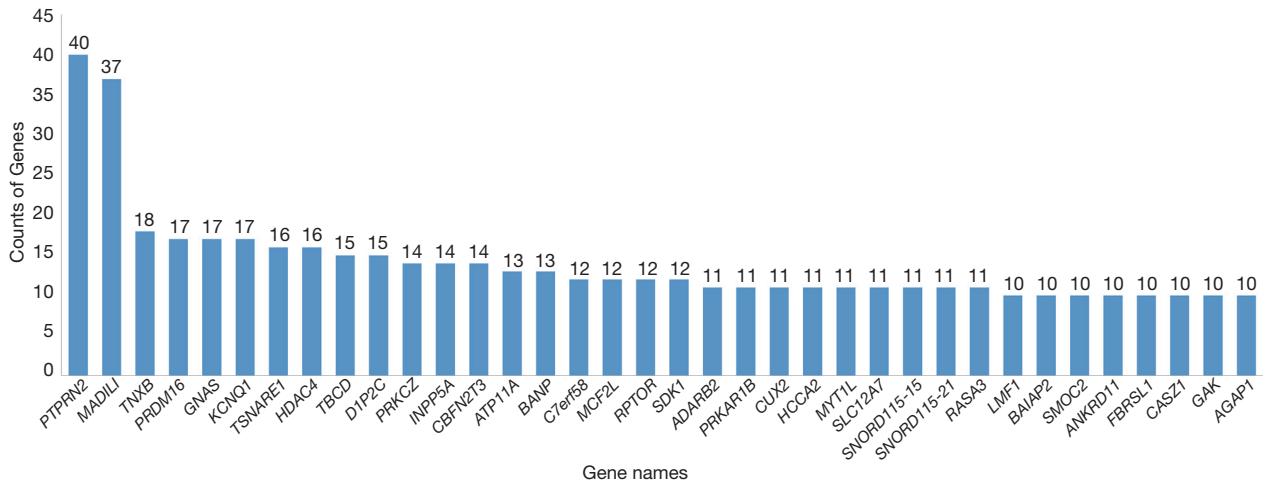

Figure 3 Methylated genes with over ten methylated CpG sites.

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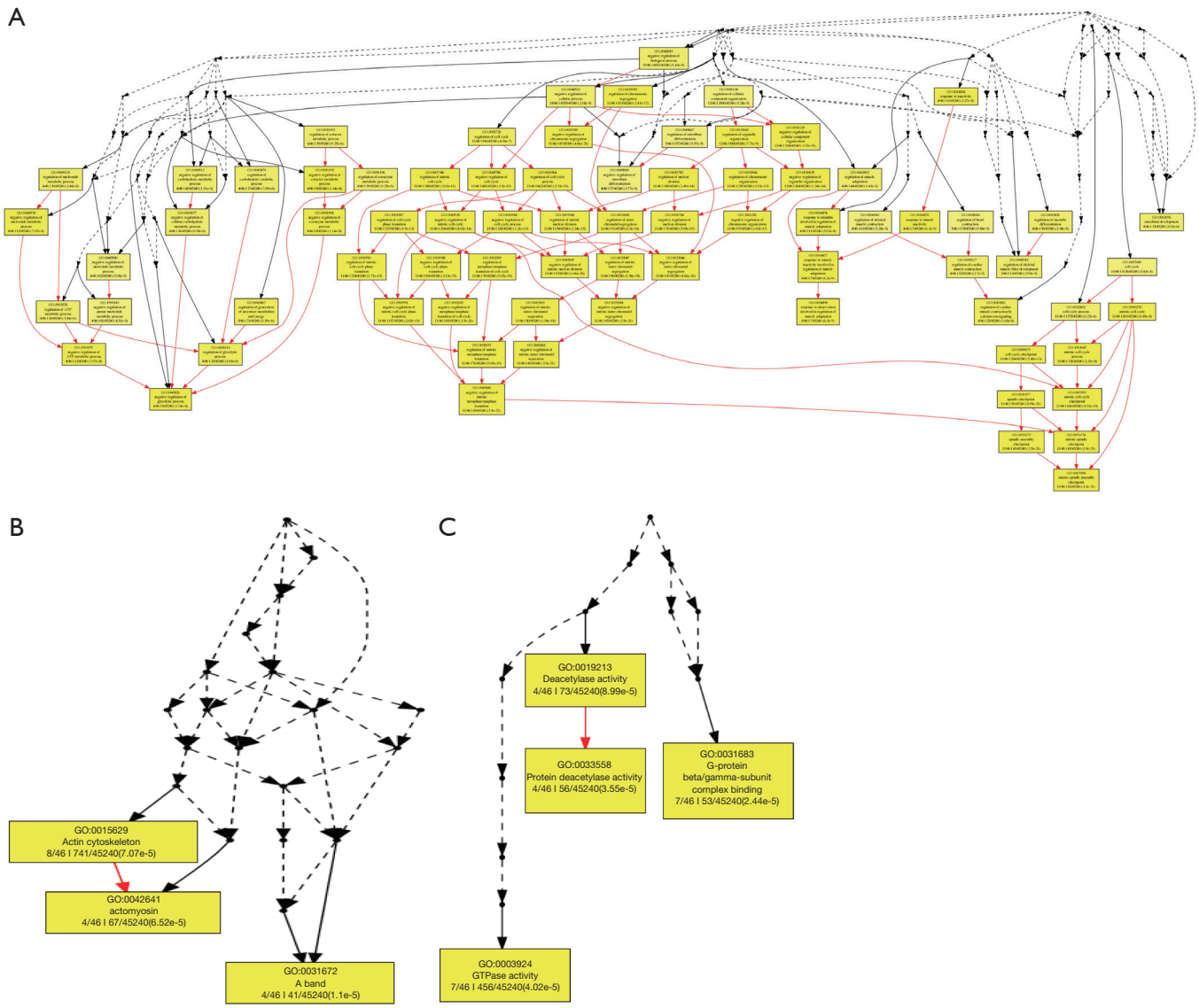

Figure 4 GO enrichment analysis of ten significant differentially methylated genes (≥15 methylated CpG sites). The figure is composed of three parts: "biological processes (BP, *Figure 4A*)", "molecular functions (MF, *Figure 4B*)", and "cellular components (CC, *Figure 4C*)". Hypergeometric statistical test methods were used for analysis, and the significance level of enrichment was set at P value <10–4. Black solid lines symbolize the connections between enriched terms. The boxes contain GO functional positioning that is equivalent to the significant GO terms. GO, Gene Ontology.

term. Two differentially methylated genes that identified in our study participate in this pathway. Moreover, it is worth noting that pancreatic secretion, type I diabetes mellitus, Insulin secretion and Adrenergic signaling in cardiomyocytes were also significant enriched in this study. The pathways mentioned above were adopted with the function that pancreas played.

GO and KEGG pathway analysis of differentially methylated sites located in promoter regions of genes in overlap group

The promoter contains specific DNA sequences that are recognized by proteins known as transcription factors. These factors bind to the promoter sequences, recruiting RNA polymerase, the enzyme that synthesizes the RNA

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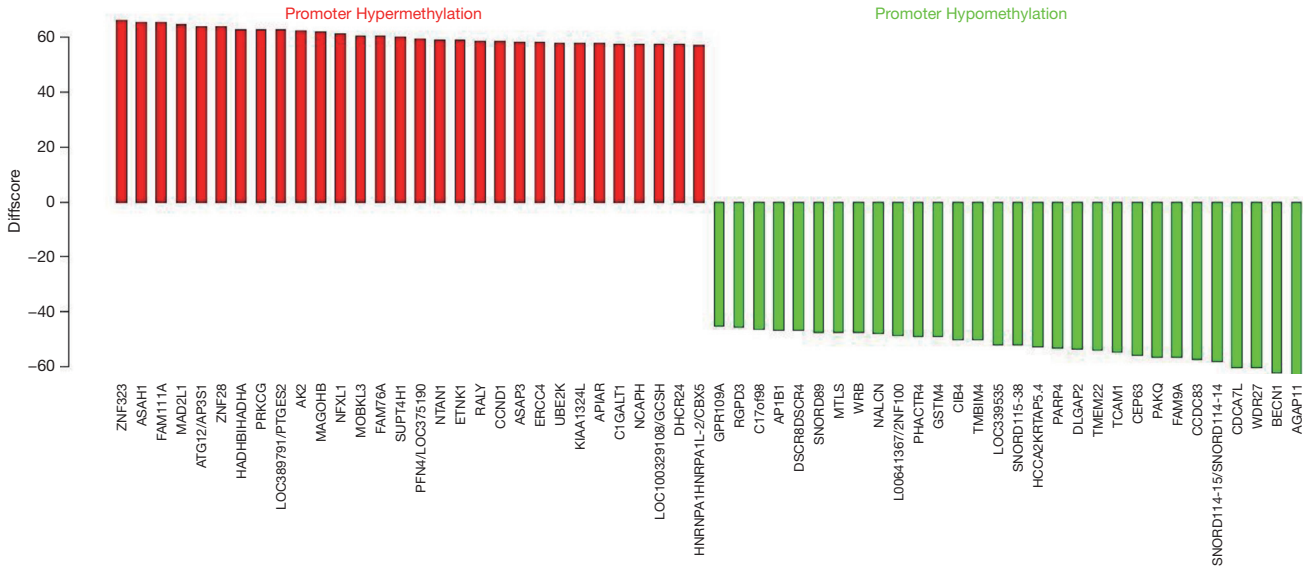

Figure 5 Sixty candidate genes with hypermethylation and hypomethylation status.

from the coding region of the gene. Eukaryotic promoters are extremely diverse and are difficult to characterize. They typically lie upstream of the gene and can have regulatory elements several kilobases away from the transcriptional start site. In eukaryotes, the transcriptional complex can cause the DNA to bend back on itself, which allows for placement of regulatory sequences far from the actual site of transcription. Many eukaryotic promoters, contain a TATA box (sequence TATAAA), which in turn binds a TATA binding protein which assists in the formation of the RNA polymerase transcriptional complex. Of this study, we identified 4,999 differentially methylated sites located in promoter regions in overlap group (http://fp.amegroups.cn/cms/24bc751fdb7f41b7ce54e74bde803221/tcr.2019.11.26-3.pdf). Moreover, we picked out 30 genes with significantly hypermethylation and 30 genes with significantly hypomethylation in the overlap group (*Figure 5* and *Table S4*). GO and KEGG analysis were performed with these 60 aberrant methylation genes. Of the GO analysis (*Figure 6* and *Table S5*), GO terms with corrected P value less than 10^{-4} were considered significantly enriched. From the perspective of biological processes, there are three GO terms were assigned under this catalogues. Among these terms, autophagosome assembly (GO: 0000045, P value: 5.8E−05), autophagy (GO: 0006914, P value: 3.8E−10) and autophagosome organization (GO: 1905037, P value: 5.8E−05) were the top three significantly enriched terms. From the cellular component perspective, there are three GO terms were assigned under this catalogues. Among these terms, mitochondrial fatty acid beta-oxidation multienzyme complex (GO: 0016507, P value: 6.4E−07), fatty acid beta-oxidation multienzyme complex (GO: 0036125, P value: 6.4E−07) and glycine cleavage complex (GO: 0005960, P value: 6.4E−07) were the top three significantly enriched terms. From the molecular function perspective, there are five GO terms were assigned under this catalogues. Among these terms, long-chain-3-hydroxyacyl-CoA dehydrogenase activity (GO: 0016509, P value: 6.4E−07) was the top significantly over-represented terms. Of the KEGG analysis (*Table S6*), it clearly displays that Regulation of autophagy were the top enriched term. Two differentially methylated genes that identified in our study participate in this pathway. Moreover, it is worth noting that Non-small cell lung cancer, Glioma, ErbB signaling pathway and Fc gamma R-mediated phagocytosis were also significant enriched in this study.

Methylation status of key genes related to pancreatic cancer

To pinpoint the methylation status of pancreatic cancer related genes (*Table 2*). We check out 22 pancreatic cancer related genes, including *ERBB2*, *AKT1*, *CDC42*, *KRAS*, *RAC1*, *RALB*, *RALA*, *PIK3R3*, *PIK3R2*, *AKT2*, *PLD1*, *RALBP1*, *SMAD4*, *RAF1*, *SMAD3*, *SMAD2*, *RB1*, *MAPK10*, *BAD*, *CDK4*, *STAT3* and *CCND1*, which has been reported before. The results indicated that *ERBB2*, *KRAS*, *PIK3R3*, *PLD1*, *RALBP1*, *RB1* and *MAPK10* all

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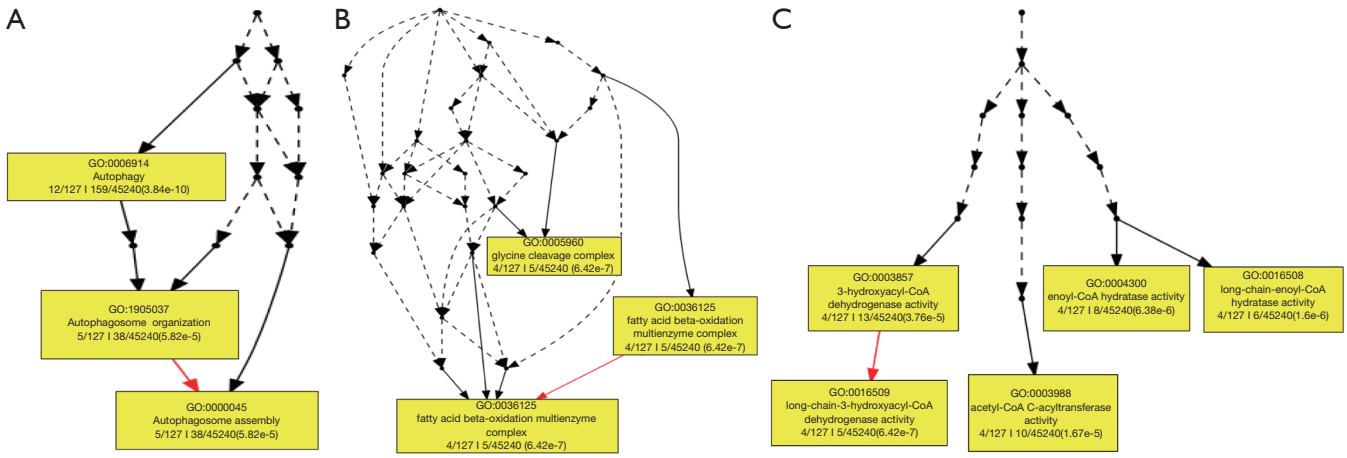

Figure 6 GO enrichment analysis of 60 candidate genes with hypermethylation and hypomethylation status. The figure is composed of three parts: "biological processes (BP, *Figure 6A*)", "molecular functions (MF, *Figure 6B*)", and "cellular components (CC, *Figure 6C*)". Hypergeometric statistical test methods were used for analysis, and the significance level of enrichment was set at P value <10–4. Black solid lines symbolize the connections between enriched terms. The boxes contain GO functional positioning that is equivalent to the significant GO terms. GO, Gene Ontology.

showed hypomethylation status. On the contrary, the other genes all showed hypermethylation status. Of note, effect size estimation was not calculated in this case.

Discussion

It is now evident that epigenetic abnormalities are extremely common in cancers, and these abnormalities provide an alternative mechanism of transcriptional silencing. Epigenetic abnormalities in cancer predominantly encompass methylation of CG dinucleotides (CpG islands) in the 5' regulatory region of tumor suppressor genes, which abrogates RNA polymerase from binding and initiating transcription. In cancers, there is preferential methylation of the gene promoter, but not in the corresponding normal cells within the tissue of origin. Methylome sequencing, without a priori bias to known CpG islands, yielded novel highly discriminant methylation markers for pancreatic cancer. Importantly, these findings were confirmed using an independent sample set of tumor and control tissues, showing that the method used in this study successfully identify pancreatic cancer markers with low background levels. Many of the markers with the strongest association to pancreatic cancer also showed greater than 10-fold increases in the median copies per sample compared with controls; this observation is critical to the application of these markers in diagnostic test development where assays must detect tumor signal against the background biologic milieu. Novel candidates identified by this method were clinically piloted by assay from pancreatic juice, demonstrating utility for the detection of pancreatic cancer in blinded comparisons, even to diseased controls with chronic pancreatitis.

In this study, genome-wide DNA methylation profiling was conducted between four pericarcinous tissues *vs.* six pancreatic cancer tissues and six blood samples *vs.* four pericarcinous tissues using Infinium HumanMethylation450 Beadchips. Sampling from pancreatic cancer tissues, pericarcinous tissues and blood of one patient is a useful method for investigating DNA methylation biomarkers without the influence of genetic discordance. Actually, the approach used in this study has identified vagarious epigenetic differences, including non-small cell lung cancer (20), colorectal carcinoma (21) and hepatocellular carcinoma (22), etc. Of this study, a total of 15,397 differentially methylated CpG sites (3.2%, of 485,577 CpG sites,) corresponding 7,440 genes that were identified in overlap. Of these 15,397 CpG sites with significant diagnostic differences in DNA methylation, 5,605 (36.4%, 5,605 of 15,397) CpG sites were hypomethylated and 5,870 (38.12%, 5,870 of 15,397) CpG sites were hypermethylated. Functional distribution of 5,870 hypermethylated CpG sites suggested that 47.4% of these sites were located in promoter regions, 38.86% of these sites were located in gene bodies, 12.42% of these

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Table 2 Methylation status of key genes related to pancreatic cancer

Series number	Gene	Methylation status	
		Hypermethylation	Hypomethylation
1	*ERBB2*	N/A	Yes
2	*AKT1*	Yes	N/A
3	*CDC42*	Yes	N/A
4	*KRAS*	N/A	Yes
5	*RAC1*	Yes	N/A
6	*RALB*	Yes	N/A
7	*RALA*	Yes	N/A
8	*PIK3R3*	N/A	Yes
9	*AKT2*	Yes	N/A
10	*PIK3R2*	Yes	N/A
11	*PLD1*	N/A	Yes
12	*RALBP1*	N/A	Yes
13	*SMAD4*	Yes	N/A
14	*RAF1*	Yes	N/A
15	*SMAD3*	Yes	N/A
16	*SMAD2*	Yes	N/A
17	*RB1*	N/A	Yes
18	*MAPK10*	N/A	Yes
19	*BAD*	Yes	N/A
20	*CDK4*	Yes	N/A
21	*STAT3*	Yes	N/A
22	*CCND1*	Yes	N/A

sites were located in intergenic regions and 6.01% of these sites were located in the 3'-untranslated regions (UTRs). Furthermore, sublocation analysis of 2,659 CpG sites in promoter region with hypermethylated indicated that 31.74% of these sites were located in regions from –200 to –1,500 nt upstream of the transcription start site (TSS1500), 28.43% of these sites were located in regions from –200 nt upstream to the TSS itself (TSS200), 27.15% of these sites were located in 1st Exon regions and 12.67% of these sites were located in the 5'-untranslated regions (UTRs). These hypermethylated CpG sites were mostly located in gene bodies and promoter regions. Meanwhile, Functional distribution of 5,605 hypomethylated CpG sites suggested that 20.43% of these sites were located in promoter regions, 39.64% of these sites were located in gene bodies, 36.24% of these sites were located in intergenic regions and 3.69% of these sites were located in 3'UTR regions. Furthermore, sublocation analysis of 5,605 hypomethylated CpG sites in promoter regions indicated that 48.38% of these sites were located in TSS1500 regions, 15.46% of these sites were located in TSS200 regions, 11.35% of these sites were located in 1st Exon regions and 24.8% of these sites were located in 5'UTR regions. This seems to be consistent with previous findings that methylation of these regions inhibits transcription. For example, Irizarry *et al.* demonstrated that altered DNA methylation in cancer occurred in CGI shores rather than in the CGIs, and DNA methylation changes in CGI shores were strongly related to gene expression (23). In addition, we had noticed that numerous differential CpG sites were located in gene bodies. Recently, it became apparent that CGIs in gene bodies act as alternative promoters (24,25) and that tissue-specific or cell type-specific CGI methylation is prevalent in gene bodies (26). GO analysis of these significantly differentially methylated genes revealed that spindle checkpoint, mitotic spindle assembly checkpoint and negative regulation of mitotic sister chromatid segregation were the top three significantly enriched terms from perspective of biological processes. Meanwhile, from the cellular component perspective, there are 3 GO terms were assigned under this catalogues. Among these terms, A band was the top significantly enriched terms. In addition, from the molecular function perspective, there are 4 GO terms were assigned under this catalogues. Among these terms, G-protein beta/gamma-subunit complex binding was the top significantly over-represented terms. KEGG analysis showed that vibrio cholerae infection was the top enriched term. Moreover, pancreatic secretion, Type I diabetes mellitus, Insulin secretion and Adrenergic signaling in cardiomyocytes were also significant enriched in this study. Furthermore, GO analysis of differentially methylated sites located in promoter regions of genes showed that autophagosome assembly, autophagy and autophagosome organization were the top three significantly enriched terms from the perspective of biological processes. From the cellular component perspective, there are three GO terms were assigned under this catalogues. Among these terms, mitochondrial fatty acid beta-oxidation multienzyme complex, fatty acid beta-oxidation multienzyme complex and glycine cleavage complex were the top three significantly enriched terms. From the molecular function perspective, long-chain-3-hydroxyacyl-CoA dehydrogenase activity was the top significantly over-represented terms. Of

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the KEGG analysis, it clearly displays that Regulation of autophagy were the top enriched term. It is worth noting that Non-small cell lung cancer, Glioma, ErbB signaling pathway and Fc gamma R-mediated phagocytosis were also significant enriched in this study. Meanwhile, we have invested methylation status of 22 pancreatic cancer related key genes, and revealed the aberrant methylation status. For example, Cyclin D1 (CCND1) has been showed to be over-expressed in human pancreatic cancer (27). Here, CCND1 was identified as hypermethylated candidate gene that is inconsistent with a previous study (28), which suggested that over-expression of cyclin D1 in pancreatic cancer is associated with the loss of methylation.

There are several limitations to the present study. First, the sample size was not large. Further validation in studies encompassing more samples is warranted in the future. Second, the analyzed CpG sites were limited in number, although the 450 K microarray is one of the most powerful and cost-effective tools currently available for assessing methylation changes. Third, it is not possible to differentiate methylation from 5-hydroxymethylation of cytosine, which also plays a critical role in gene regulation (29). In summary, aberrant DNA methylation in pancreatic cancer tissues was identified at numerous CpG sites across the whole genome in using two independent sets of samples. Of the differently methylated CpG sites in the CGIs, most of them were located in the promoter regions. These findings support the hypothesis that altered DNA methylation could be involved in the pathophysiology of pancreatic cancer. Although the number of analyzed individuals was limited, the analysis was sufficient to provide DNA methylation distribution patterns across different genomic regions that were largely in agreement with patterns previously observed. The methylome data alone was sufficient for correctly distinguishing between all the ten tissues studied, collectively demonstrating that tissues are characterized by distinctive methylation patterns that reflect their tissue-specific functions. Our study provoked the question, of how differentially methylated CpG sites mechanistically contribute to the gene functions, especially for the numerous methylation regions that were found in gene body areas. In addition, it remains unclear, however, how the gene body differentially methylated CpG sites may function as regulators of gene expression, and this question should be addressed in the future epigenetic studies.

In conclusion, previous studies have demonstrated that DNA methylation play important roles in the regulation of developmental processes of several cancers. The identification of differentially methylated genes in this study provides information valuable to the in-depth study of pancreatic cancer. Moreover, the results of this study will not only improve our understanding of the differentially methylated genes but will also help to enhance methylome studies of pancreatic cancer.

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Footnote

Conflicts of Interest: The authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.11.26). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Chinese General Hospital of PLA. All patients provided signed informed consent.

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Supplementary

Table S1 Clinicopathological details of patients

Patient No.	Age	Gender	Histology
1	F	74	Highly differentiated ductal adenocarcinoma
2	M	36	Poorly differentiated adenocarcinoma
3	M	66	Moderately differentiated adenocarcinoma
4	F	60	Moderately differentiated ductal adenocarcinoma
5	F	46	Moderately differentiated ductal adenocarcinoma
6	F	71	Moderately-poorly differentiated adenocarcinoma

F, female; M, male.

Table S2 Gene ontology annotation of the 10 genes with significant methylation frequency of the overlaps group (≥15 counts)

GOID	Ontology	Term	Level	q	m	t	k	Gene IDs	Symbols	Log odds ratio	P
GO: 0048519	Biological process	Negative regulation of biological process	2	19	4,961	45,240	46	Q92932, B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9, Q9HAZ2, P51787, C9J0X4, F5GX36, F5H0B1, P56524, Q9BTW9	PTPRN2, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, PRDM16, KCNQ1, HDAC4, HDAC4, HDAC4, HDAC4, TBCD	1.913262	1.42711E−05
GO: 0000075	Biological process	Cell cycle checkpoint	3	11	264	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	5.356787	5.86265E−13
GO: 0000278	Biological process	Mitotic cell cycle	2	11	810	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	3.739403	6.84714E−08
GO: 0007049	Biological process	Cell cycle	2	11	1,636	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	2.725224	5.43394E−05
GO: 0007088	Biological process	Regulation of mitotic nuclear division	4	11	154	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	6.134394	2.24109E−15
GO: 0007093	Biological process	Mitotic cell cycle checkpoint	7	11	166	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	6.026142	4.53154E−15
GO: 0007094	Biological process	Mitotic spindle assembly checkpoint	13	11	42	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	8.008864	3.49706E−21
GO: 0007346	Biological process	Regulation of mitotic cell cycle	2	11	388	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	4.801268	3.6196E−11
GO: 0010564	Biological process	Regulation of cell cycle process	2	11	462	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	4.549432	2.32842E−10
GO: 0010639	Biological process	Negative regulation of organelle organization	5	12	264	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9, Q9BTW9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, TBCD	5.482318	1.24116E−14
GO: 0010948	Biological process	Negative regulation of cell cycle process	3	11	226	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	5.581002	1.20706E−13
GO: 0010965	Biological process	Regulation of mitotic sister chromatid separation	5	11	80	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.079253	1.5405E−18
GO: 0022402	Biological process	Cell cycle process	3	11	1,278	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	3.081509	6.23166E−06
GO: 0030071	Biological process	Regulation of mitotic metaphase/anaphase transition	7	11	76	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.153253	9.65074E−19
GO: 0031577	Biological process	Spindle checkpoint	3	11	50	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.757325	8.97641E−21
GO: 0033043	Biological process	Regulation of organelle organization	2	12	854	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9, Q9BTW9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, TBCD	3.78862	7.71321E−09
GO: 0033044	Biological process	Regulation of chromosome organization	2	11	239	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	5.500314	2.01867E−13
GO: 0033045	Biological process	Regulation of sister chromatid segregation	4	11	91	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	6.893386	6.4978E−18
GO: 0033046	Biological process	Negative regulation of sister chromatid segregation	7	11	47	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.846592	4.66045E−21
GO: 0033047	Biological process	Regulation of mitotic sister chromatid segregation	5	11	80	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.079253	1.5405E−18
GO: 0033048	Biological process	Negative regulation of mitotic sister chromatid segregation	8	11	45	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.909328	3.49706E−21
GO: 0045786	Biological process	Negative regulation of cell cycle	3	11	481	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	4.491288	3.49572E−10
GO: 0045839	Biological process	Negative regulation of mitotic nuclear division	7	11	53	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.673261	1.66166E−20
GO: 0045841	Biological process	Negative regulation of mitotic metaphase/anaphase transition	9	11	45	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.909328	3.49706E−21
GO: 0045930	Biological process	Negative regulation of mitotic cell cycle	3	11	204	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	5.728756	4.01676E−14
GO: 0048523	Biological process	Negative regulation of cellular process	3	18	4,555	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9, Q9HAZ2, P51787, C9J0X4, F5GX36, F5H0B1, P56524, Q9BTW9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, PRDM16, KCNQ1, HDAC4, HDAC4, HDAC4, HDAC4, TBCD	1.958439	2.17828E−05
GO: 0051128	Biological process	Regulation of cellular component organization	2	12	2,006	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9, Q9BTW9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, TBCD	2.556606	5.28453E−05
GO: 0051129	Biological process	Negative regulation of cellular component organization	4	12	524	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9, Q9BTW9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, TBCD	4.493289	3.19493E−11
GO: 0051726	Biological process	Regulation of cell cycle	2	11	966	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	3.485302	4.03765E−07
GO: 0051783	Biological process	Regulation of nuclear division	2	11	186	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	5.862022	1.49082E−14
GO: 0051784	Biological process	Negative regulation of nuclear division	5	11	71	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.251434	5.05177E−19
GO: 0051983	Biological process	Regulation of chromosome segregation	2	11	103	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	6.71468	2.42944E−17
GO: 0051985	Biological process	Negative regulation of chromosome segregation	4	11	47	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.846592	4.66045E−21
GO: 0071173	Biological process	Spindle assembly checkpoint	3	11	45	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.909328	3.49706E−21
GO: 0071174	Biological process	Mitotic spindle checkpoint	11	11	45	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.909328	3.49706E−21
GO:1901987	Biological process	Regulation of cell cycle phase transition	2	11	237	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	5.512438	1.9028E−13
GO:1901988	Biological process	Negative regulation of cell cycle phase transition	3	11	160	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	6.079253	3.13253E−15
GO:1901990	Biological process	Regulation of mitotic cell cycle phase transition	3	11	234	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	5.530816	1.71147E−13
GO:1901991	Biological process	Negative regulation of mitotic cell cycle phase transition	5	11	157	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	6.10656	2.65226E−15
GO:1902099	Biological process	Regulation of metaphase/anaphase transition of cell cycle	4	11	76	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.153253	9.65074E−19
GO:1902100	Biological process	Negative regulation of metaphase/anaphase transition of cell cycle	4	11	45	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.909328	3.49706E−21
GO:1903047	Biological process	Mitotic cell cycle process	4	11	726	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	3.897355	2.31135E−08
GO:2000816	Biological process	Negative regulation of mitotic sister chromatid separation	8	11	45	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	7.909328	3.49706E−21
GO:2001251	Biological process	Negative regulation of chromosome organization	5	11	99	45,240	46	B3KR41, C9J9H5, C9JIR0, C9JJ38, C9JKI7, C9JP81, C9JPS1, C9JTA2, C9JX80, C9K086, Q9Y6D9	MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1, MAD1L1	6.771824	1.62527E−17
GO: 0015629	Cellular component	Actin cytoskeleton	4	8	741	45,240	46	B3KR41, C9JKI7, C9JP81, Q9Y6D9, C9J0X4, F5GX36, F5H0B1, P56524	MAD1L1, MAD1L1, MAD1L1, MAD1L1, HDAC4, HDAC4, HDAC4, HDAC4	3.40842	7.07259E−05
GO: 0043467	Biological process	Regulation of generation of precursor metabolites and energy	3	5	71	45,240	46	Q9HAZ2, C9J0X4, F5GX36, F5H0B1, P56524	PRDM16, HDAC4, HDAC4, HDAC4, HDAC4	6.11393	1.58738E−06
GO: 0003924	Molecular function	Gtpase activity	1	7	456	45,240	46	A2A2R6, H0Y7E8, H0Y7F4, P63092, Q5JWD1, Q5JWE9, Q5JWF2	GNAS, GNAS, GNAS, GNAS, GNAS, GNAS, GNAS	3.916214	4.02427E−05
GO: 0031683	Molecular function	G-protein beta/gamma-subunit complex binding	2	7	53	45,240	46	A2A2R6, H0Y7E8, H0Y7F4, P63092, Q5JWD1, Q5JWE9, Q5JWF2	GNAS, GNAS, GNAS, GNAS, GNAS, GNAS, GNAS	7.021184	2.43723E−11
GO: 0045667	Biological process	Regulation of osteoblast differentiation	3	5	157	45,240	46	Q5JWF2, C9J0X4, F5GX36, F5H0B1, P56524	GNAS, HDAC4, HDAC4, HDAC4, HDAC4	4.969057	5.56829E−05
GO: 0008016	Biological process	Regulation of heart contraction	2	5	178	45,240	46	P51787, C9J0X4, F5GX36, F5H0B1, P56524	KCNQ1, HDAC4, HDAC4, HDAC4, HDAC4	4.787944	9.84102E−05
GO: 0002076	Biological process	Osteoblast development	6	4	33	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.897355	5.03412E−06
GO: 0006942	Biological process	Regulation of striated muscle contraction	2	4	63	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	5.964469	5.28453E−05
GO: 0010882	Biological process	Regulation of cardiac muscle contraction by calcium ion signaling	5	4	25	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	7.297893	1.65414E−06
GO: 0055117	Biological process	Regulation of cardiac muscle contraction	3	4	52	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.24131	2.70438E−05
GO: 0014854	Biological process	Response to inactivity	1	4	10	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	8.619821	3.27042E−08
GO: 0014870	Biological process	Response to muscle inactivity	1	4	7	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	9.134394	6.30331E−09
GO: 0014874	Biological process	Response to stimulus involved in regulation of muscle adaptation	3	4	11	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	8.482318	5.02378E−08
GO: 0014877	Biological process	Response to muscle inactivity involved in regulation of muscle adaptation	5	4	7	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	9.134394	6.30331E−09
GO: 0014894	Biological process	Response to denervation involved in regulation of muscle adaptation	5	4	7	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	9.134394	6.30331E−09
GO: 0043502	Biological process	Regulation of muscle adaptation	3	4	44	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.482318	1.42711E−05
GO: 0031672	Cellular component	A band	7	4	41	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.584197	1.10087E−05
GO: 0019213	Molecular function	Deacetylase activity	1	4	73	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	5.751925	8.98652E−05
GO: 0033558	Molecular function	Protein deacetylase activity	1	4	56	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.134394	3.5536E−05
GO: 0042641	Cellular component	Actomyosin	6	4	67	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	5.87566	6.51755E−05
GO: 0045668	Biological process	Negative regulation of osteoblast differentiation	5	4	57	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.108859	3.76518E−05
GO: 0006110	Biological process	Regulation of glycolytic process	7	4	25	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	7.297893	1.65414E−06
GO: 0009118	Biological process	Regulation of nucleoside metabolic process	4	4	30	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	7.034859	3.44042E−06
GO: 0010677	Biological process	Negative regulation of cellular carbohydrate metabolic process	6	4	36	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.771824	6.98124E−06
GO: 0043470	Biological process	Regulation of carbohydrate catabolic process	3	4	37	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.732296	7.68907E−06
GO: 0045820	Biological process	Negative regulation of glycolytic process	12	4	8	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	8.941749	1.14254E−08
GO: 0045912	Biological process	Negative regulation of carbohydrate metabolic process	4	4	40	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.619821	1.01024E−05
GO: 0045978	Biological process	Negative regulation of nucleoside metabolic process	7	4	12	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	8.356787	7.06904E−08
GO: 0045980	Biological process	Negative regulation of nucleotide metabolic process	8	4	62	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	5.987553	5.08282E−05
GO: 0051193	Biological process	Regulation of cofactor metabolic process	3	4	39	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.656347	9.24809E−06
GO: 0051195	Biological process	Negative regulation of cofactor metabolic process	6	4	8	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	8.941749	1.14254E−08
GO: 0051196	Biological process	Regulation of coenzyme metabolic process	3	4	39	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.656347	9.24809E−06
GO: 0051198	Biological process	Negative regulation of coenzyme metabolic process	7	4	8	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	8.941749	1.14254E−08
GO:1900543	Biological process	Negative regulation of purine nucleotide metabolic process	9	4	60	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.034859	4.51066E−05
GO:1903578	Biological process	Regulation of ATP metabolic process	5	4	30	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	7.034859	3.44042E−06
GO:1903579	Biological process	Negative regulation of ATP metabolic process	11	4	12	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	8.356787	7.06904E−08
GO: 0010830	Biological process	Regulation of myotube differentiation	3	4	50	45,240	46	C9J0X4, F5GX36, F5H0B1, P56524	HDAC4, HDAC4, HDAC4, HDAC4	6.297893	2.33836E−05
GO: 0048742	Biological process	Regulation of skeletal muscle fiber development	5	3	13	45,240	46	C9J0X4, F5GX36, F5H0B1	HDAC4, HDAC4, HDAC4	7.826272	3.04774E−05

Statistical test method: hypergeometric test/Fisher's exact test. FDR correction method: Benjamini and Hochberg.

Table S3 KEGG analysis of the 10 genes with significant methylation frequence of the overlap group (≥15 counts)

#Term	Database	ID	Input number	Background number	P value	Corrected P value		Input	Hyperlink
Vibrio cholerae infection	KEGG PATHWAY	hsa05110	2	50	0.006229	0.308577	0.510637	ENSG00000087460, ENSG00000053918	http://www.genome.jp/kegg-bin/show_pathway?hsa05110/hsa:2778%09red/hsa:3784%09red
Gastric acid secretion	KEGG PATHWAY	hsa04971	2	74	0.012932	0.308577	0.510637	ENSG00000087460, ENSG00000053918	http://www.genome.jp/kegg-bin/show_pathway?hsa04971/hsa:2778%09red/hsa:3784%09red
Pancreatic secretion	KEGG PATHWAY	hsa04972	2	96	0.020893	0.308577	0.510637	ENSG00000087460, ENSG00000053918	http://www.genome.jp/kegg-bin/show_pathway?hsa04972/hsa:2778%09red/hsa:3784%09red
Adrenergic signaling in cardiomyocytes	KEGG PATHWAY	hsa04261	2	151	0.047242	0.308577	0.510637	ENSG00000087460, ENSG00000053918	http://www.genome.jp/kegg-bin/show_pathway?hsa04261/hsa:2778%09red/hsa:3784%09red
Alcoholism	KEGG PATHWAY	hsa05034	2	180	0.064213	0.308577	0.510637	ENSG00000087460, ENSG00000068024	http://www.genome.jp/kegg-bin/show_pathway?hsa05034/hsa:9759%09red/hsa:2778%09red
Viral carcinogenesis	KEGG PATHWAY	hsa05203	2	207	0.08156	0.308577	0.510637	ENSG00000002822, ENSG00000068024	http://www.genome.jp/kegg-bin/show_pathway?hsa05203/hsa:9759%09red/hsa:8379%09red
Type I diabetes mellitus	KEGG PATHWAY	hsa04940	1	42	0.093054	0.308577	0.510637	ENSG00000155093	http://www.genome.jp/kegg-bin/show_pathway?hsa04940/hsa:5799%09red
Vasopressin-regulated water reabsorption	KEGG PATHWAY	hsa04962	1	45	0.099219	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04962/hsa:2778%09red
Endocrine and other factor-regulated calcium reabsorption	KEGG PATHWAY	hsa04961	1	47	0.103306	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04961/hsa:2778%09red
Cocaine addiction	KEGG PATHWAY	hsa05030	1	49	0.107375	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa05030/hsa:2778%09red
Ovarian steroidogenesis	KEGG PATHWAY	hsa04913	1	52	0.113444	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04913/hsa:2778%09red
Regulation of lipolysis in adipocytes	KEGG PATHWAY	hsa04923	1	58	0.12546	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04923/hsa:2778%09red
MicroRNAs in cancer	KEGG PATHWAY	hsa05206	2	273	0.128856	0.308577	0.510637	ENSG00000068024, ENSG00000168477	http://www.genome.jp/kegg-bin/show_pathway?hsa05206/hsa:9759%09red/hsa:7148%09red
Long-term depression	KEGG PATHWAY	hsa04730	1	61	0.131408	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04730/hsa:2778%09red
Renin secretion	KEGG PATHWAY	hsa04924	1	64	0.137317	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04924/hsa:2778%09red
Amphetamine addiction	KEGG PATHWAY	hsa05031	1	67	0.143185	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa05031/hsa:2778%09red
Bile secretion	KEGG PATHWAY	hsa04976	1	71	0.150949	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04976/hsa:2778%09red
Thyroid hormone synthesis	KEGG PATHWAY	hsa04918	1	71	0.150949	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04918/hsa:2778%09red
Aldosterone synthesis and secretion	KEGG PATHWAY	hsa04925	1	80	0.168167	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04925/hsa:2778%09red
ECM-receptor interaction	KEGG PATHWAY	hsa04512	1	83	0.173829	0.308577	0.510637	ENSG00000168477	http://www.genome.jp/kegg-bin/show_pathway?hsa04512/hsa:7148%09red
Insulin secretion	KEGG PATHWAY	hsa04911	1	87	0.18132	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04911/hsa:2778%09red
Gap junction	KEGG PATHWAY	hsa04540	1	88	0.183182	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04540/hsa:2778%09red
Salivary secretion	KEGG PATHWAY	hsa04970	1	90	0.186894	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04970/hsa:2778%09red
Dilated cardiomyopathy	KEGG PATHWAY	hsa05414	1	90	0.186894	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa05414/hsa:2778%09red
Protein digestion and absorption	KEGG PATHWAY	hsa04974	1	90	0.186894	0.308577	0.510637	ENSG00000053918	http://www.genome.jp/kegg-bin/show_pathway?hsa04974/hsa:3784%09red
Morphine addiction	KEGG PATHWAY	hsa05032	1	91	0.188744	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa05032/hsa:2778%09red
GnRH signaling pathway	KEGG PATHWAY	hsa04912	1	92	0.19059	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04912/hsa:2778%09red
Circadian entrainment	KEGG PATHWAY	hsa04713	1	95	0.196102	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04713/hsa:2778%09red
ProgesteronE–mediated oocyte maturation	KEGG PATHWAY	hsa04914	1	97	0.199757	0.308577	0.510637	ENSG00000002822	http://www.genome.jp/kegg-bin/show_pathway?hsa04914/hsa:8379%09red
Endocrine resistance	KEGG PATHWAY	hsa01522	1	99	0.203395	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa01522/hsa:2778%09red
Melanogenesis	KEGG PATHWAY	hsa04916	1	100	0.205207	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04916/hsa:2778%09red
Amoebiasis	KEGG PATHWAY	hsa05146	1	100	0.205207	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa05146/hsa:2778%09red
Inflammatory mediator regulation of TRP channels	KEGG PATHWAY	hsa04750	1	101	0.207016	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04750/hsa:2778%09red
Estrogen signaling pathway	KEGG PATHWAY	hsa04915	1	101	0.207016	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04915/hsa:2778%09red
Glucagon signaling pathway	KEGG PATHWAY	hsa04922	1	102	0.208821	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04922/hsa:2778%09red
Chagas disease (American trypanosomiasis)	KEGG PATHWAY	hsa05142	1	106	0.216	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa05142/hsa:2778%09red
Cholinergic synapse	KEGG PATHWAY	hsa04725	1	113	0.228409	0.308577	0.510637	ENSG00000053918	http://www.genome.jp/kegg-bin/show_pathway?hsa04725/hsa:3784%09red
Serotonergic synapse	KEGG PATHWAY	hsa04726	1	113	0.228409	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04726/hsa:2778%09red
Glutamatergic synapse	KEGG PATHWAY	hsa04724	1	115	0.231919	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04724/hsa:2778%09red
Vascular smooth muscle contraction	KEGG PATHWAY	hsa04270	1	123	0.245803	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04270/hsa:2778%09red
Cell cycle	KEGG PATHWAY	hsa04110	1	124	0.247521	0.308577	0.510637	ENSG00000002822	http://www.genome.jp/kegg-bin/show_pathway?hsa04110/hsa:8379%09red
Platelet activation	KEGG PATHWAY	hsa04611	1	125	0.249235	0.308577	0.510637	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04611/hsa:2778%09red
Dopaminergic synapse	KEGG PATHWAY	hsa04728	1	129	0.256053	0.309646	0.509135	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04728/hsa:2778%09red
Phospholipase D signaling pathway	KEGG PATHWAY	hsa04072	1	146	0.284358	0.336059	0.473584	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04072/hsa:2778%09red
Oxytocin signaling pathway	KEGG PATHWAY	hsa04921	1	160	0.306871	0.354606	0.450254	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04921/hsa:2778%09red
Calcium signaling pathway	KEGG PATHWAY	hsa04020	1	179	0.336314	0.380181	0.42001	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04020/hsa:2778%09red
cAMP signaling pathway	KEGG PATHWAY	hsa04024	1	201	0.368872	0.399073	0.398948	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04024/hsa:2778%09red
Epstein-Barr virus infection	KEGG PATHWAY	hsa05169	1	204	0.373188	0.399073	0.398948	ENSG00000068024	http://www.genome.jp/kegg-bin/show_pathway?hsa05169/hsa:9759%09red
Focal adhesion	KEGG PATHWAY	hsa04510	1	206	0.376049	0.399073	0.398948	ENSG00000168477	http://www.genome.jp/kegg-bin/show_pathway?hsa04510/hsa:7148%09red
Rap1 signaling pathway	KEGG PATHWAY	hsa04015	1	216	0.390165	0.405771	0.391719	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa04015/hsa:2778%09red
PI3K-Akt signaling pathway	KEGG PATHWAY	hsa04151	1	343	0.544312	0.554985	0.255719	ENSG00000168477	http://www.genome.jp/kegg-bin/show_pathway?hsa04151/hsa:7148%09red
Pathways in cancer	KEGG PATHWAY	hsa05200	1	399	0.599439	0.599439	0.222255	ENSG00000087460	http://www.genome.jp/kegg-bin/show_pathway?hsa05200/hsa:2778%09red

Statistical test method: hypergeometric test/Fisher's exact test. FDR correction method: Benjamini and Hochberg.

Table S4 60 genes with significant hypermethylation and hypomethylation of overlap genes between B (pericarcinous tissues) *vs.* C (pancreatic cancer tissue) and A (blood) *vs.* B (pericarcinous tissues)

Genes	Diff score	Chromosome	Position	Methylation status	P value (B *vs.* C)	P value (A *vs.* B)
ZNF323	131.89468	6	28431998	High-GpG	4.16001E–07	1.55395E–07
ASAH1	130.81353	8	17986262	High-GpG	2.01169E–07	4.1218E–07
FAM111A	130.45708	11	58666572	High-GpG	1.21744E–05	7.39343E–09
MAD2L1	129.33632	4	121207392	High-GpG	5.45286E–07	2.1367E–07
ATG12	127.27827	5	115205360	High-GpG	8.27127E–07	2.26256E–07
ZNF28	127.26429	19	58016010	High-GpG	1.55587E–06	1.2067E–07
HADHB	125.49116	2	26320955	High-GpG	4.79904E–06	5.88478E–08
PRKCG	125.39503	19	59077027	High-GpG	2.97625E–05	9.70126E–09
PTGES2	124.99208	9	129930459	High-GpG	8.51945E–07	3.7186E–07
AK2	124.18006	1	33275020	High-GpG	1.48229E–07	2.57668E–06
MAGOHB	123.44346	12	10657370	High-GpG	8.11057E–05	5.57958E–09
NFXL1	122.4017	4	47611255	High-GpG	1.88006E–06	3.05955E–07
MOBKL3	120.93052	2	198088826	High-GpG	2.86446E–06	2.81777E–07
FAM76A	120.92863	1	27925162	High-GpG	1.06657E–06	7.57092E–07
SUPT4H1	119.87514	17	53784566	High-GpG	1.36289E–05	7.55137E–08
PFN4	118.79988	2	24199741	High-GpG	5.71282E–06	2.30761E–07
NTAN1	117.88967	16	15057701	High-GpG	1.57434E–06	1.03261E–06
ETNK1	117.59242	12	22669361	High-GpG	3.17717E–06	5.47921E–07
RALY	117.18969	20	32046086	High-GpG	9.3884E–06	2.03442E–07
CCND1	116.8124	11	69164711	High-GpG	1.57231E–06	1.32502E–06
ASAP3	116.03716	1	23683861	High-GpG	7.38714E–05	3.37139E–08
ERCC4	115.92696	16	13921604	High-GpG	2.69769E–07	9.46918E–06
UBE2K	115.73845	4	39375775	High-GpG	0.000128084	2.08286E–08
KIAA1324L	115.34069	7	86526859	High-GpG	3.39017E–06	8.62402E–07
AP1AR	115.30214	4	113372285	High-GpG	4.0348E–06	7.31079E–07
C1GALT1	114.78336	7	7188867	High-GpG	4.95185E–06	6.71268E–07
NCAPH	114.77977	2	96365157	High-GpG	6.31399E–05	5.26889E–08
GCSH	114.74176	16	79687498	High-GpG	4.30184E–07	7.80136E–06
DHCR24	114.43711	1	55125751	High-GpG	1.87238E–05	1.92263E–07
HNRNPA1	114.07533	12	52960808	High-GpG	0.000139304	2.80869E–08
GPR109A	–90.42935	12	121755189	Low-CpG	7.59402E–06	0.000119287
RGPD3	–91.08637	2	106451234	Low-CpG	4.72388E–05	1.64841E–05
C17orf98	–92.50327	17	34251672	Low-CpG	0.00010681	5.2609E–06
AP1B1	–93.62296	22	28115355	Low-CpG	2.05317E–05	2.11484E–05
DSCR8	–93.67115	21	38415359	Low-CpG	0.002349546	1.82768E–07
SNORD89	–94.64155	2	101256138	Low-CpG	2.65111E–05	1.29544E–05
MTL5	–95.11474	11	68275537	Low-CpG	8.07261E–06	3.81515E–05
WRB	–95.16274	21	39672973	Low-CpG	1.04278E–05	2.92101E–05
NALCN	–95.55199	13	100866990	Low-CpG	0.000157631	1.76669E–06
ZNF100	–97.47202	19	21725295	Low-CpG	1.15163E–05	1.55412E–05
PHACTR4	–97.70953	1	28567843	Low-CpG	7.41887E–06	2.28407E–05
GSTM4	–97.92513	1	109998812	Low-CpG	9.94493E–06	1.62138E–05
CIB4	–99.87754	2	26718375	Low-CpG	0.000282136	3.64575E–07
TMBIM4	–100.22459	12	64851491	Low-CpG	3.88671E–06	2.4432E–05
LOC339535	–103.632	1	236716068	Low-CpG	5.41422E–06	8.0032E–06
SNORD115-38	–103.94379	15	23034641	Low-CpG	3.08714E–06	1.30636E–05
HCCA2	–105.64957	11	1601150	Low-CpG	1.64093E–05	1.65941E–06
PARP4	–105.90702	13	23979063	Low-CpG	2.30641E–05	1.11266E–06
DLGAP2	–106.78054	8	1442761	Low-CpG	1.34894E–06	1.5558E–05
TMEM22	–108.01178	3	138039519	Low-CpG	8.62848E–06	1.83184E–06
TCAM1	–109.0501	17	59288076	Low-CpG	3.68175E–06	3.38015E–06
CEP63	–111.64598	3	135690134	Low-CpG	0.000347835	1.96801E–08
PAK2	–112.77409	3	197954174	Low-CpG	5.13802E–06	1.02753E–06
FAM9A	–112.86534	X	8729344	Low-CpG	2.79992E–06	1.84638E–06
CCDC83	–114.29396	11	85246352	Low-CpG	5.92682E–06	6.27745E–07
SNORD114-15	–115.87882	14	100508183	Low-CpG	9.26565E–06	2.78767E–07
CDCA7L	–120.37022	7	21930929	Low-CpG	2.20559E–06	4.16346E–07
WDR27	–120.65469	6	169839513	Low-CpG	5.12794E–06	1.67721E–07
BECN1	–124.31615	17	38230497	Low-CpG	6.43061E–06	5.75617E–08
AGAP11	–138.8236	10	88746560	Low-CpG	8.04749E–08	1.62922E–07

Table S5 Gene ontology annotation of 60 genes with significantly hypermethylation and hypomethylation of overlap genes between B (pericarcinous tissues) *vs.* C (pancreatic cancer tissue) and a (blood) *vs.* B (pericarcinous tissues)

GOID	Ontology	Term	Level	q	m	t	k	Gene IDs	Symbols	Log odds ratio	P
GO: 0000045	Biological process	Autophagosome assembly	4	5	38	45,240	127	C1IDX9, O94817, K7EPZ0, K7EQQ7, Q14457	ATG12, ATG12, BECN1, BECN1, BECN1	5.550627	5.82E–05
GO: 0006914	Biological process	Autophagy	1	12	159	45,240	127	C1IDX9, O94817, E7EV84, K7ELY9, K7EMA2, K7EN35, K7EPZ0, K7EQQ7, K7ER46, K7ERY0, K7ESG3, Q14457	ATG12, ATG12, BECN1, BECN1, BECN1, BECN1, BECN1, BECN1, BECN1, BECN1, BECN1, BECN1	4.748706	3.84E–10
GO:1905037	Biological process	Autophagosome organization	2	5	38	45,240	127	C1IDX9, O94817, K7EPZ0, K7EQQ7, Q14457	ATG12, ATG12, BECN1, BECN1, BECN1	5.550627	5.82E–05
GO: 0003857	Molecular function	3-hydroxyacyl-CoA dehydrogenase activity	1	4	13	45,240	127	C9JE81, C9JEY0, C9K0M0, P55084	HADHB, HADHB, HADHB, HADHB	6.776187	3.76E–05
GO: 0003988	Molecular function	Acetyl-CoA C-acyltransferase activity	1	4	10	45,240	127	C9JE81, C9JEY0, C9K0M0, P55084	HADHB, HADHB, HADHB, HADHB	7.154699	1.67E–05
GO: 0004300	Molecular function	Enoyl-CoA hydratase activity	1	4	8	45,240	127	C9JE81, C9JEY0, C9K0M0, P55084	HADHB, HADHB, HADHB, HADHB	7.476627	6.38E–06
GO: 0016507	Cellular component	Mitochondrial fatty acid beta-oxidation multienzyme complex	8	4	5	45,240	127	C9JE81, C9JEY0, C9K0M0, P55084	HADHB, HADHB, HADHB, HADHB	8.154699	6.42E–07
GO: 0036125	Cellular component	Fatty acid beta-oxidation multienzyme complex	1	4	5	45,240	127	C9JE81, C9JEY0, C9K0M0, P55084	HADHB, HADHB, HADHB, HADHB	8.154699	6.42E–07
GO: 0016508	Molecular function	Long-chain-enoyl-CoA hydratase activity	1	4	6	45,240	127	C9JE81, C9JEY0, C9K0M0, P55084	HADHB, HADHB, HADHB, HADHB	7.891664	1.6E–06
GO: 0016509	Molecular function	Long-chain-3-hydroxyacyl-CoA dehydrogenase activity	1	4	5	45,240	127	C9JE81, C9JEY0, C9K0M0, P55084	HADHB, HADHB, HADHB, HADHB	8.154699	6.42E–07
GO: 0005960	Cellular component	Glycine cleavage complex	5	4	5	45,240	127	H3BNV1, H3BQ30, H3BUG8, P23434	GCSH, GCSH, GCSH, GCSH	8.154699	6.42E–07

Statistical test method: hypergeometric test/Fisher's exact test. FDR correction method: Benjamini and Hochberg.

Table S6 KEGG analysis of 60 genes with significantly hypermethylation and hypomethylation of overlap genes between B (pericarcinous tissues) vs. C (pancreatic cancer tissue) and A (blood) vs. B (pericarcinous tissues)

#Term	Database	ID	Input number	Background number	P value	Corrected P value	Input	Hyperlink
Regulation of autophagy	KEGG PATHWAY	hsa04140	2	40	0.023689	0.583858	ENSG00000126581, ENSG00000145782	http://www.genome.jp/kegg-bin/show_pathway?hsa04140/hsa:9140%09red/hsa:86789%09red
Non-small cell lung cancer	KEGG PATHWAY	hsa05223	2	58	0.045599	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05223/hsa:5595%09red/hsa:5582%09red
Glioma	KEGG PATHWAY	hsa05214	2	67	0.058495	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05214/hsa:5595%09red/hsa:5582%09red
ErbB signaling pathway	KEGG PATHWAY	hsa04012	2	90	0.090676	0.583858	ENSG00000126583, ENSG00000180370	http://www.genome.jp/kegg-bin/show_pathway?hsa04012/hsa:5062%09red/hsa:5582%09red
Fc gamma R-mediated phagocytosis	KEGG PATHWAY	hsa04666	2	96	0.106773	0.583858	ENSG00000088280, ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04666/hsa:55616%09red/hsa:5582%09red
Steroid biosynthesis	KEGG PATHWAY	hsa00100	1	20	0.112548	0.583858	ENSG00000116133	http://www.genome.jp/kegg-bin/show_pathway?hsa00100/hsa:1718%09red
Focal adhesion	KEGG PATHWAY	hsa04510	3	206	0.11596	0.583858	ENSG00000126583, ENSG00000180370, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04510/hsa:5595%09red/hsa:5062%09red/hsa:5582%09red
Fatty acid elongation	KEGG PATHWAY	hsa00062	1	25	0.137434	0.583858	ENSG00000138029	http://www.genome.jp/kegg-bin/show_pathway?hsa00062/hsa:3032%09red
Glycolysate and dicarboxylate metabolism	KEGG PATHWAY	hsa00630	1	28	0.152032	0.583858	ENSG00000140905	http://www.genome.jp/kegg-bin/show_pathway?hsa00630/hsa:2653%09red
Thyroid hormone signaling pathway	KEGG PATHWAY	hsa04919	2	121	0.154242	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04919/hsa:5595%09red/hsa:5582%09red
Thyroid cancer	KEGG PATHWAY	hsa05216	1	29	0.156843	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05216/hsa:5595%09red
Sphingolipid signaling pathway	KEGG PATHWAY	hsa04071	2	123	0.1582	0.583858	ENSG00000126583, ENSG00000104763	http://www.genome.jp/kegg-bin/show_pathway?hsa04071/hsa:4279%09red/hsa:5582%09red
Cell cycle	KEGG PATHWAY	hsa04110	2	124	0.160187	0.583858	ENSG00000110092, ENSG00000164109	http://www.genome.jp/kegg-bin/show_pathway?hsa04110/hsa:5595%09red/hsa:4085%09red
Lysosome	KEGG PATHWAY	hsa04142	2	124	0.160187	0.583858	ENSG00000104763, ENSG00000100280	http://www.genome.jp/kegg-bin/show_pathway?hsa04142/hsa:1629%09red/hsa:4279%09red
Mucin type O-Glycan biosynthesis	KEGG PATHWAY	hsa00512	1	31	0.166384	0.583858	ENSG00000106392	http://www.genome.jp/kegg-bin/show_pathway?hsa00512/hsa:56913%09red
Base excision repair	KEGG PATHWAY	hsa03410	1	33	0.175818	0.583858	ENSG00000102699	http://www.genome.jp/kegg-bin/show_pathway?hsa03410/hsa:1439%09red
Apoptosis–multiple species	KEGG PATHWAY	hsa04215	1	33	0.175818	0.583858	ENSG00000126581	http://www.genome.jp/kegg-bin/show_pathway?hsa04215/hsa:8678%09red
African trypanosomiasis	KEGG PATHWAY	hsa05143	1	34	0.180495	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa05143/hsa:5582%09red
FoxO signaling pathway	KEGG PATHWAY	hsa04068	2	135	0.18232	0.583858	ENSG00000110092, ENSG00000145782	http://www.genome.jp/kegg-bin/show_pathway?hsa04068/hsa:9140%09red/hsa:5595%09red
Spliceosome	KEGG PATHWAY	hsa03040	2	136	0.184355	0.583858	ENSG00000111196, ENSG00000135486	http://www.genome.jp/kegg-bin/show_pathway?hsa03040/hsa:55110%09red/hsa:317%09red
Wnt signaling pathway	KEGG PATHWAY	hsa04310	2	142	0.196631	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04310/hsa:5595%09red/hsa:5582%09red
Glycine, serine and threonine metabolism	KEGG PATHWAY	hsa00260	1	40	0.20801	0.583858	ENSG00000140905	http://www.genome.jp/kegg-bin/show_pathway?hsa00260/hsa:2653%09red
Hepatitis B	KEGG PATHWAY	hsa05161	2	148	0.209005	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05161/hsa:5595%09red/hsa:5582%09red
Bladder cancer	KEGG PATHWAY	hsa05219	1	41	0.212505	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05219/hsa:5595%09red
Aldosterone[–regulated sodium reabsorption	KEGG PATHWAY	hsa04960	1	41	0.212505	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04960/hsa:5582%09red
Fatty acid degradation	KEGG PATHWAY	hsa00071	1	45	0.230236	0.583858	ENSG00000138029	http://www.genome.jp/kegg-bin/show_pathway?hsa00071/hsa:3032%09red
Oxytocin signaling pathway	KEGG PATHWAY	hsa04921	2	160	0.233969	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04921/hsa:5595%09red/hsa:5582%09red
Hedgehog signaling pathway	KEGG PATHWAY	hsa04340	1	46	0.234606	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04340/hsa:5595%09red
Nucleotide excision repair	KEGG PATHWAY	hsa03420	1	46	0.234606	0.583858	ENSG00000171595	http://www.genome.jp/kegg-bin/show_pathway?hsa03420/hsa:2072%09red
Endocrine and other factor-regulated calcium reabsorption	KEGG PATHWAY	hsa04961	1	47	0.238952	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04961/hsa:5582%09red
Sphingolipid metabolism	KEGG PATHWAY	hsa00600	1	47	0.238952	0.583858	ENSG00000104763	http://www.genome.jp/kegg-bin/show_pathway?hsa00600/hsa:427%09red
Valine, leucine and isoleucine degradation	KEGG PATHWAY	hsa00280	1	48	0.243273	0.583858	ENSG00000138029	http://www.genome.jp/kegg-bin/show_pathway?hsa00280/hsa:3032%09red
Fatty acid metabolism	KEGG PATHWAY	hsa01212	1	49	0.24757	0.583858	ENSG00000138029	http://www.genome.jp/kegg-bin/show_pathway?hsa01212/hsa:3032%09red
Glutathione metabolism	KEGG PATHWAY	hsa00480	1	51	0.256091	0.583858	ENSG00000168765	http://www.genome.jp/kegg-bin/show_pathway?hsa00480/hsa:29480%09red/hsa:5553%09red
RNA transport	KEGG PATHWAY	hsa03013	2	171	0.257003	0.583858	ENSG00000153165, ENSG00000111196	http://www.genome.jp/kegg-bin/show_pathway?hsa03013/hsa:65348%09red/hsa:55110%09red
Endometrial cancer	KEGG PATHWAY	hsa05213	1	54	0.268693	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05213/hsa:5595%09red
Fanconi anemia pathway	KEGG PATHWAY	hsa03460	1	56	0.276976	0.583858	ENSG00000175595	http://www.genome.jp/kegg-bin/show_pathway?hsa03460/hsa:2072%09red
Viral myocarditis	KEGG PATHWAY	hsa05416	1	57	0.281083	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05416/hsa:5595%09red
Acute myeloid leukemia	KEGG PATHWAY	hsa05221	1	59	0.289227	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05221/hsa:5595%09red
Long-term depression	KEGG PATHWAY	hsa04730	1	61	0.297279	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04730/hsa:5582%09red
VEGF signaling pathway	KEGG PATHWAY	hsa04370	1	64	0.309188	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04370/hsa:5582%09red
Arachidonic acid metabolism	KEGG PATHWAY	hsa00590	1	64	0.309188	0.583858	ENSG00000148334	http://www.genome.jp/kegg-bin/show_pathway?hsa00590/hsa:4spl%09red
Colorectal cancer	KEGG PATHWAY	hsa05210	1	64	0.309188	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05210/hsa:5595%09red
Shigellosis	KEGG PATHWAY	hsa05131	1	66	0.317016	0.583858	ENSG00000167232	http://www.genome.jp/kegg-bin/show_pathway?hsa05131/hsa:3751891%09red
Long-term potentiation	KEGG PATHWAY	hsa04720	1	66	0.317016	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04720/hsa:5582%09red
Amphetamine addiction	KEGG PATHWAY	hsa05031	1	67	0.320896	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa05031/hsa:5582%09red
Pancreatic cancer	KEGG PATHWAY	hsa05212	1	68	0.324755	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05212/hsa:5595%09red
Drug metabolism-cytochrome P450	KEGG PATHWAY	hsa00982	1	68	0.324755	0.583858	ENSG00000168765	http://www.genome.jp/kegg-bin/show_pathway?hsa00982/hsa:1576%09red
Renal cell carcinoma	KEGG PATHWAY	hsa05211	1	69	0.328592	0.583858	ENSG00000180370	http://www.genome.jp/kegg-bin/show_pathway?hsa05211/hsa:5062%09red
p53 signaling pathway	KEGG PATHWAY	hsa04115	1	69	0.328592	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04115/hsa:5595%09red
RIG-I-like receptor signaling pathway	KEGG PATHWAY	hsa04622	1	70	0.332408	0.583858	ENSG00000145782	http://www.genome.jp/kegg-bin/show_pathway?hsa04622/hsa:9140%09red
Proteoglycans in cancer	KEGG PATHWAY	hsa05205	2	208	0.33428	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05205/hsa:5595%09red/hsa:5582%09red
Thyroid hormone synthesis	KEGG PATHWAY	hsa04918	1	71	0.336201	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04918/hsa:5582%09red
Metabolism of xenobiotics by cytochrome P450	KEGG PATHWAY	hsa00980	1	72	0.339974	0.583858	ENSG00000168765	http://www.genome.jp/kegg-bin/show_pathway?hsa00980/hsa:29480%09red
Melanoma	KEGG PATHWAY	hsa05218	1	73	0.343725	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05218/hsa:5595%09red
Gastric acid secretion	KEGG PATHWAY	hsa04971	1	74	0.347455	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04971/hsa:5582%09red
Prolactin signaling pathway	KEGG PATHWAY	hsa04917	1	74	0.347455	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04917/hsa:5595%09red
Rap1 signaling pathway	KEGG PATHWAY	hsa04015	2	216	0.350752	0.583858	ENSG00000167232, ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04015/hsa:3751891%09red/hsa:5582%09red
Chronic myeloid leukemia	KEGG PATHWAY	hsa05220	1	75	0.351164	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05220/hsa:5595%09red
Platinum drug resistance	KEGG PATHWAY	hsa01524	1	76	0.354852	0.583858	ENSG00000168765	http://www.genome.jp/kegg-bin/show_pathway?hsa01524/hsa:29480%09red
Regulation of actin cytoskeleton	KEGG PATHWAY	hsa04810	2	219	0.356894	0.583858	ENSG00000167232, ENSG00000180370	http://www.genome.jp/kegg-bin/show_pathway?hsa04810/hsa:3751891%09red/hsa:5062%09red
Aldosterone synthesis and secretion	KEGG PATHWAY	hsa04925	1	80	0.369397	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04925/hsa:5582%09red
Chemical carcinogenesis	KEGG PATHWAY	hsa05204	1	82	0.376547	0.583858	ENSG00000168765	http://www.genome.jp/kegg-bin/show_pathway?hsa05204/hsa:29480%09red
EGFR tyrosine kinase inhibitor resistance	KEGG PATHWAY	hsa01521	1	83	0.380091	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa01521/hsa:5582%09red
Ras signaling pathway	KEGG PATHWAY	hsa04014	2	231	0.381248	0.583858	ENSG00000126583, ENSG00000180370	http://www.genome.jp/kegg-bin/show_pathway?hsa04014/hsa:5062%09red/hsa:5582%09red
Salmonella infection	KEGG PATHWAY	hsa05132	1	86	0.390606	0.583858	ENSG00000167232	http://www.genome.jp/kegg-bin/show_pathway?hsa05132/hsa:3751891%09red
Insulin secretion	KEGG PATHWAY	hsa04911	1	87	0.394071	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04911/hsa:5582%09red
GABAergic synapse	KEGG PATHWAY	hsa04727	1	88	0.397517	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04727/hsa:5582%09red
Small cell lung cancer	KEGG PATHWAY	hsa05222	1	88	0.397517	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05222/hsa:5595%09red
Gap junction	KEGG PATHWAY	hsa04540	1	88	0.397517	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04540/hsa:5582%09red
Salivary secretion	KEGG PATHWAY	hsa04970	1	90	0.40435	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04970/hsa:5582%09red
Prostate cancer	KEGG PATHWAY	hsa05215	1	91	0.407737	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05215/hsa:5595%09red
Morphine addiction	KEGG PATHWAY	hsa05032	1	91	0.407737	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa05032/hsa:5582%09red
mRNA surveillance pathway	KEGG PATHWAY	hsa03015	1	91	0.407737	0.583858	ENSG00000111196	http://www.genome.jp/kegg-bin/show_pathway?hsa03015/hsa:55110%09red
Metabolic pathways	KEGG PATHWAY	hsa01100	8	1240	0.412411	0.583858	ENSG00000139163, ENSG00000140905, ENSG00000148334, ENSG00000116133, ENSG00000106392, ENSG00000104763, ENSG00000138029, ENSG00000140905	http://www.genome.jp/kegg-bin/show_pathway?hsa01100/hsa:2653%09red/hsa:427%09red/hsa:56913%09red/hsa:55500%09red/hsa:3032%09red/hsa:80142%09red/hsa:1718%09red/hsa:2645%09red
Circadian entrainment	KEGG PATHWAY	hsa04713	1	95	0.421098	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04713/hsa:5582%09red
Pancreatic secretion	KEGG PATHWAY	hsa04972	1	96	0.424391	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04972/hsa:5582%09red
Glycerophospholipid metabolism	KEGG PATHWAY	hsa00564	1	96	0.424391	0.583858	ENSG00000139163	http://www.genome.jp/kegg-bin/show_pathway?hsa00564/hsa:55500%09red
Progesterone-mediated oocyte maturation	KEGG PATHWAY	hsa04914	1	97	0.427665	0.583858	ENSG00000164109	http://www.genome.jp/kegg-bin/show_pathway?hsa04914/hsa:4085%09red
MAPK signaling pathway	KEGG PATHWAY	hsa04010	2	257	0.432595	0.583858	ENSG00000126583, ENSG00000180370	http://www.genome.jp/kegg-bin/show_pathway?hsa04010/hsa:5062%09red/hsa:5582%09red
Endocrine resistance	KEGG PATHWAY	hsa01522	1	99	0.434158	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa01522/hsa:5595%09red
HTLV-I infection	KEGG PATHWAY	hsa05166	2	259	0.436454	0.583858	ENSG00000110092, ENSG00000164109	http://www.genome.jp/kegg-bin/show_pathway?hsa05166/hsa:5595%09red/hsa:4085%09red
Amoebiasis	KEGG PATHWAY	hsa05146	1	100	0.437378	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa05146/hsa:5582%09red
Melanogenesis	KEGG PATHWAY	hsa04916	1	100	0.437378	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04916/hsa:5582%09red
Inflammatory mediator regulation of TRP channels	KEGG PATHWAY	hsa04750	1	101	0.440579	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04750/hsa:5582%09red
Retrograde endocannabinoid signaling	KEGG PATHWAY	hsa04723	1	101	0.440579	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04723/hsa:5582%09red
Phosphatidylinositol signaling system	KEGG PATHWAY	hsa04070	1	101	0.440579	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04070/hsa:5582%09red
AGE–RAGE signaling pathway in diabetic complications	KEGG PATHWAY	hsa04933	1	103	0.446926	0.583858	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04933/hsa:5595%09red
Choline metabolism in cancer	KEGG PATHWAY	hsa05231	1	104	0.450074	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa05231/hsa:5582%09red
HIF-1 signaling pathway	KEGG PATHWAY	hsa04066	1	105	0.453203	0.583858	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04066/hsa:5582%09red
T cell receptor signaling pathway	KEGG PATHWAY	hsa04660	1	107	0.459408	0.583858	ENSG00000180370	http://www.genome.jp/kegg-bin/show_pathway?hsa04660/hsa:5062%09red
MicroRNAs in cancer	KEGG PATHWAY	hsa05206	2	273	0.46306	0.583858	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05206/hsa:5595%09red/hsa:5582%09red
Serotonergic synapse	KEGG PATHWAY	hsa04726	1	113	0.477608	0.589389	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04726/hsa:5582%09red
Cholinergic synapse	KEGG PATHWAY	hsa04725	1	113	0.477608	0.589389	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04725/hsa:5582%09red
Glutamatergic synapse	KEGG PATHWAY	hsa04724	1	115	0.485339	0.590426	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04724/hsa:5582%09red
Oocyte meiosis	KEGG PATHWAY	hsa04114	1	120	0.498074	0.595635	ENSG00000164109	http://www.genome.jp/kegg-bin/show_pathway?hsa04114/hsa:4085%09red
Leukocyte transendothelial migration	KEGG PATHWAY	hsa04670	1	120	0.498074	0.595635	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04670/hsa:5582%09red
Vascular smooth muscle contraction	KEGG PATHWAY	hsa04270	1	123	0.506599	0.599648	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04270/hsa:5582%09red
AMPK signaling pathway	KEGG PATHWAY	hsa04152	1	125	0.512203	0.600157	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04152/hsa:5595%09red
Dopaminergic synapse	KEGG PATHWAY	hsa04728	1	129	0.523221	0.604046	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04728/hsa:5582%09red
Natural killer cell mediated cytotoxicity	KEGG PATHWAY	hsa04650	1	130	0.525936	0.604046	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04650/hsa:5582%09red
Ubiquitin mediated proteolysis	KEGG PATHWAY	hsa04120	1	137	0.54452	0.613122	ENSG00000078140	http://www.genome.jp/kegg-bin/show_pathway?hsa04120/hsa:3093%09red
Measles	KEGG PATHWAY	hsa05162	1	138	0.547116	0.613122	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05162/hsa:5595%09red
Tight junction	KEGG PATHWAY	hsa04530	1	139	0.549696	0.613122	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04530/hsa:5582%09red
Apoptosis	KEGG PATHWAY	hsa04210	1	142	0.55735	0.615739	ENSG00000102699	http://www.genome.jp/kegg-bin/show_pathway?hsa04210/hsa:1439%09red
Hippo signaling pathway	KEGG PATHWAY	hsa04390	1	153	0.584325	0.638598	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04390/hsa:5595%09red
mTOR signaling pathway	KEGG PATHWAY	hsa04150	1	155	0.589052	0.638598	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04150/hsa:5582%09red
Jak-STAT signaling pathway	KEGG PATHWAY	hsa04630	1	160	0.600635	0.645126	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04630/hsa:5595%09red
Purine metabolism	KEGG PATHWAY	hsa00230	1	177	0.637645	0.670677	ENSG00000004455	http://www.genome.jp/kegg-bin/show_pathway?hsa00230/hsa:2049%09red
Axon guidance	KEGG PATHWAY	hsa04360	1	178	0.639713	0.670677	ENSG00000180370	http://www.genome.jp/kegg-bin/show_pathway?hsa04360/hsa:5062%09red
Calcium signaling pathway	KEGG PATHWAY	hsa04020	1	179	0.641769	0.670677	ENSG00000126583	http://www.genome.jp/kegg-bin/show_pathway?hsa04020/hsa:5582%09red
Pathways in cancer	KEGG PATHWAY	hsa05200	2	399	0.665937	0.689721	ENSG00000126583, ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05200/hsa:5595%09red/hsa:5582%09red
cAMP signaling pathway	KEGG PATHWAY	hsa04024	1	201	0.684166	0.702329	ENSG00000182782	http://www.genome.jp/kegg-bin/show_pathway?hsa04024/hsa:3384421%09red
Viral carcinogenesis	KEGG PATHWAY	hsa05203	1	207	0.694837	0.707027	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa05203/hsa:5595%09red
Endocytosis	KEGG PATHWAY	hsa04144	1	264	0.779936	0.786718	ENSG00000088280	http://www.genome.jp/kegg-bin/show_pathway?hsa04144/hsa:9516%09red
PI3K-Akt signaling pathway	KEGG PATHWAY	hsa04151	1	343	0.860284	0.860284	ENSG00000110092	http://www.genome.jp/kegg-bin/show_pathway?hsa04151/hsa:5595%09red

Statistical test method: hypergeometric test/Fisher's exact test. FDR correction method: Benjamini and Hochberg.