

## Supplementary Appendix

### Integrated Bioinformatics Analysis Reveals the Aberrantly Methylated Differentially Expressed Genes in Dilated Cardiomyopathy

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### Supplementary Materials and Methods

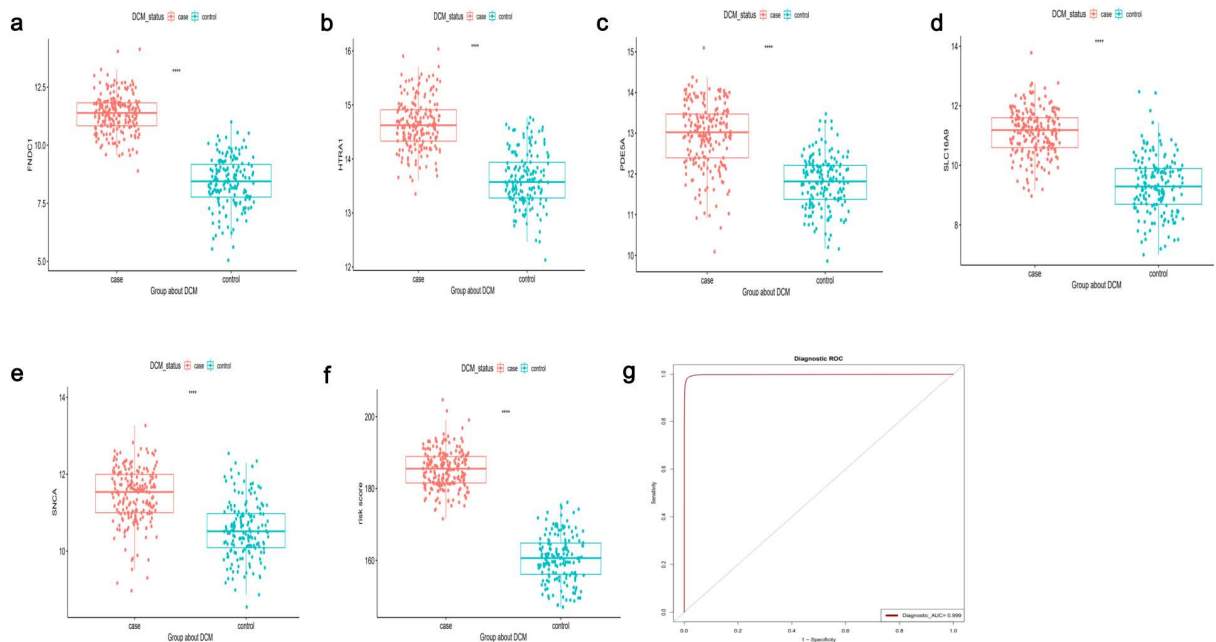
#### DNA Methylation Array and Gene Expression Data Acquisition

Seven datasets were searched and filtered using the keyword "Dilated cardiomyopathy" from the Gene Expression Omnibus database (<https://www.ncbi.nlm.nih.gov/geo/>), including the DNA methylation dataset GSE81337 and the original gene expression datasets GSE42955 [1], GSE79962 [2], GSE57338 [3], GSE84796 [4], GSE111544, and GSE141910. The screening criterion was a sample size of >10. In the GSE81337 dataset, methylation data were available for 27 samples, including 18 DCM left and nine DCM right ventricular samples on the dataset platform GPL13534 (HumanMethylation45015017482). The GSE42955 dataset contained 12 dilated cardiomyopathy (DCM) cardiac and five control cardiac tissue samples on the platform GPL6244 (Affymetrix Human Gene 1.0 ST Array). The platform for the GSE79962 dataset was also GPL6244, which comprised nine DCM cardiac and 11 control cardiac tissue samples. The GSE57338 dataset had 218 collected samples, including 82 DCM cardiac and 136 normal cardiac tissue samples, and the dataset platform was GPL11532 (Affymetrix Human Gene 1.1 ST Array). The platform for the GSE84796 and GSE111544 datasets was GPL14550, and because the GSE111544 dataset contained only DCM cardiac tissue sample data, the GSE111544 and GSE84796 datasets were combined for analysis. Twenty-four DCM cardiac and seven normal cardiac tissue samples were collected. The GSE141910 dataset contained 200 DCM cardiac and 166 normal cardiac tissue samples with the platform GPL16791 (Illumina HiSeq 2500; Illumina Inc., San Diego, CA, USA).

38 **Supplementary Figures**

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40 **Fig. S1** Box diagrams before and after standardization of the GSE42955, GSE79962, GSE57338, and GSE84796  
41 + GSE111544 datasets  
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44 **Fig. S2** Receiver operating characteristic (ROC) curves of positive genes from logistic regression of the  
45 GSE141910 dataset  
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 48 **Fig. S3** Results of logistic regression prediction model for the GSE141910 dataset. (a) Significant differences in  
 49 risk scores were observed between the dilated cardiomyopathy (DCM) and non-DCM samples. (b–f) Expression  
 50 values of five aberrantly methylated differentially expressed genes (DEGs) (*SLC16A9*, *SNCA*, *PDE5A*, *FND1*,  
 51 and *HTRA1*) were significantly different between DCM and non-DCM samples. (g) Receiver operating  
 52 characteristic (ROC) curve of the logistic regression prediction model. \*\*\* $P < 0.0001$   
 53

54 **Table S1.**

Table S1. Primer sequences used in RT-qPCR

Primer name	Sequence (5'→3')
GAPDH-F	CCATCTTCCAGGAGCGAGACCC
GAPDH-R	CCACCCTGTTGCTGTAGCCGTA
RGS9BP-F	ACAAGACCACAGCGTGCTACCAT
RGS9BP-R	CCATGTCGTCAATCATCTCGCCCACC
FND1-F	GCCGGAAGATGAATTATGTCCCAC
FND1-R	TCCACTTGCCATCTCGTTCACC
PDE5A-F	AAAGGAACAAATGCCACTAACC
PDE5A-R	TCAAAGATTAAGCTGGCAAGGTCA
SLC16A9-F	CCTCTCATCTCCATTTTCGGCAT
SLC16A9-R	TGTCAATCCCCACAGTCTTCGT
CHDH-F	CCACCAGCAGGAAGCTTACCAG
CHDH-R	CCCTGAGGTTTTCTACGCCGAT
HTRA1-F	AAGATCCCAACAGTTTGCGTCA
HTRA1-R	TGTTAATCCCAATCACCTCGCCAT
NPTX2-F	GCGAGGCAACAGTGCATTCAAGTCACC
NPTX2-R	AACGCTTCCCACATGCCGTCT
SNCA-F	TGAGAAAACCAAGCAGGGTGT
SNCA-R	GCTCATAGTCTTGGTAGCCTT

56 *GAPDH*: glyceraldehyde 3-phosphate dehydrogenase; *RGS9BP*: regulator of G protein signaling 9 binding  
 57 protein; *FND1*: fibronectin type III domain containing 1; *PDE5A*: phosphodiesterase 5A; *SLC16A9*: solute

58 carrier family 16 member 9; *CHDH*: choline dehydrogenase; *HTRAI*: HtrA serine peptidase 1; *NPTX2*: neuronal  
59 pentraxin 2; *SNCA*: synuclein alpha

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62 **References**

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