

Supplemental figure legends

Fig.S1. Differentially expressed protein-coding genes between HCM patients and healthy donors.

Dark goldenrod dots were up-regulated DEGs with $FDR < 0.05$ and $\log_2(\text{Fold Change}) > 0.5$; cyan dots were down-regulated DEGs with $FDR < 0.05$ and $\log_2(\text{Fold Change}) < -0.5$. Squares meant that these genes met the criteria: $FDR < 10^{-30}$ or $FDR < 10^{-10}$ and absolute value of $\log_2(\text{Fold Change})$ was greater than 4.

Fig.S2. The comparison of HCM patients and healthy controls at endogenous DNA methylation levels.

- (A) Line plot showing the average endogenous DNA methylation levels in the gene body and 2 kb upstream and downstream regions of gene body in HCM patient ind110. Different colors indicated different technology replications.
- (B) PCA plot showing the endogenous DNA methylation pattern of the whole genome in HCM patients and healthy controls. PC1 and PC2 had variance values of 4.3% and 3.8%, respectively.
- (C) Bar plots showing the numbers of hypermethylated and hypomethylated DMRs located in different genome elements.
- (D) Bar plot showing the average endogenous DNA methylation levels in different genome elements. ns: P value > 0.05 .
- (E) PCA plot showing the endogenous DNA methylation pattern of gene-bodies in HCM patients and controls. PC1 and PC2 had variance values of 10.1% and 4.6%, respectively.
- (F) PCA plot showing the endogenous DNA methylation pattern of CpG Islands in HCM patients and controls. PC1 and PC2 had variance values of 9.4% and 6.7%, respectively.
- (G) Heatmap showing endogenous DNA methylation levels in the gene body ± 10 kb regions of *ACTA1* in all HCM patients and healthy controls. The gene expression levels of *ACTA1* were showed in the boxplot on the right, and the gene expression levels were quantified by $\log_2(\text{RPKM} + 1)$. The color bars in the heatmap represented the regions of gene body and promoter (from 1 kb upstream of the TSS to 0.5 kb downstream of the TSS), and the direction of the arrow indicated the direction of transcription. ns: P value > 0.05 .
- (H) IGV image showing the endogenous DNA methylation levels of *ACTA1* and adjacent regions in 4 HCM patients and 4 controls. Each vertical line represented a site, and the height of the vertical line represented the endogenous DNA methylation level. DMR_NC and DMR_NHCM represent the hypomethylated DMRs and hypermethylated DMRs identified in HCM patients, respectively. ENCODE_cCRE_Hg38ToHg19 showed the position of the enhancers. Statistical significance was performed by two-tailed Student t test.

Fig.S3. The changes of chromatin accessibility between HCM patients and controls.

- (A) Line plot showing the average chromatin accessibility levels in the 2 kb upstream and downstream regions of TSS in HCM patient ind110. Different colors indicated different technology replications.
- (B) Boxplot showing the average chromatin accessibility levels in the proximal and distal NDRs in HCM patients and healthy adult controls. ns: P value > 0.05 , ***: P value < 0.001 .
- (C) PCA plot showing the chromatin accessibility pattern of proximal NDRs in HCM patients and

- controls. PC1, PC2 and PC3 had variance values of 9.7%, 4.3% and 3.9%, respectively.
- (D) PCA plot showing the chromatin accessibility pattern of distal NDRs in HCM patients and controls. PC1, PC2 and PC3 had variance values of 5.9%, 3.7% and 3.5%, respectively.
 - (E) PCA plot showing the chromatin accessibility pattern of promoters in HCM patients and healthy donors. PC1 and PC2 had variance values of 18.5% and 6.2%, respectively.
 - (F) PCA plot showing the chromatin accessibility pattern of gene-bodies in HCM patients and healthy donors. PC1 and PC2 had variance values of 24.9% and 5.1%, respectively.
 - (G) PCA plot showing the chromatin accessibility pattern of enhancers in HCM patients and healthy donors. PC1 and PC2 had variance values of 6.6% and 3.5%, respectively. Statistical significance was performed by two-tailed Student *t* test.

Fig.S4. Correlations among transcriptome, DNA methylome, and chromatin accessibility in HCM patients and healthy controls.

- (A) Line plots showing the average endogenous DNA methylation level in the gene body ± 2 kb regions of protein-coding genes in controls and HCM patients. Different colors represented 4 gene groups classified by gene expression levels. Silenced genes: $RPKM \leq 0.1$; low-expression genes: $0.1 < RPKM \leq 1$; intermediate-expression genes: $1 < RPKM \leq 10$; high-expression genes: $RPKM > 10$.
- (B) Line plots showing the average chromatin accessibility level in the TSS ± 2 kb regions of protein-coding genes in controls and HCM patients. Different colors represented 4 gene groups classified by gene expression levels.
- (C) Line plots showing the average endogenous DNA methylation levels (green) and the average chromatin accessibility levels (blue) in the promoters of corresponding genes. Genes were ranked by expression levels (red), and the x-axis from left to right indicated genes with increased expression levels. R1 indicated spearman correlation coefficients between endogenous DNA methylation levels and gene expression levels and R2 indicated spearman correlation coefficients between chromatin accessibility levels and gene expression levels.
- (D) Line plots showing the average endogenous DNA methylation levels of the gene bodies in each bin. Genes ($RPKM > 0$) were ranked by expression levels and grouped into 100 bins, and the x-axis from left to right indicated genes with increased expression levels. Each point indicated a bin. The shadow represented the 0.95 confidence interval around smooth.
- (E) Line plots showing the average chromatin accessibility level of the gene bodies in each bin.

Fig.S5. Multi-omics analysis of the adult controls, HCM patients and fetuses.

- (A) Heatmap showing row z-score scaled gene expression levels of co-upregulated and co-downregulated DEGs (protein-coding genes) in both HCM patients and fetuses compared with adult controls.
- (B) GO terms of corresponding co-regulated DEGs.
- (C) Violin plot showing the genome-wide endogenous DNA methylation levels (left) and line plot showing the average endogenous DNA methylation levels in the gene body and 2 kb upstream and downstream regions of gene body in the adult controls, HCM patients and fetuses (right). ns: $P > 0.05$.
- (D) PCA plot showing the DNA methylation pattern of promoters in healthy donors, HCM patients and fetuses. PC1 and PC2 had variance values of 8.4% and 5.7%, respectively.

- (E) PCA plot showing the DNA methylation pattern of gene-bodies in three groups. PC1 and PC2 had variance values of 11.3% and 7.8%, respectively.
- (F) PCA plot showing the DNA methylation pattern of enhancers in three groups. PC1 and PC2 had variance values of 12.7% and 3.7%, respectively.
- (G) Violin plot showing the total accessibility levels of whole genome (left) and line plot showing the average chromatin accessibility levels in the 2 kb upstream and downstream regions of TSS in the adult controls, HCM patients and fetuses (right). ns: $P > 0.05$.
- (H) PCA plot showing the chromatin accessibility pattern of distal NDRs in three groups. PC1 and PC2 had variance values of 5.6% and 3.2%, respectively.
- (I) Unsupervised hierarchical clustering analysis of chromatin accessibility of distal NDRs in the adult controls, the HCM patients and the fetuses. Statistical significance was performed by two-tailed Student t test.

Fig.S6. The expression of SP1 and EGR1 in the adult controls, patients with HCM and fetus.

- (A) Boxplot showing the expression levels of *SP1* in healthy controls, HCM patients and fetuses. Gene expression levels were quantified with $\log_2(\text{RPKM} + 1)$.
- (B) Boxplot showing the expression levels of *EGR1* in healthy controls, HCM patients and fetuses.
- (C) Bar plot showing the relative expression of *SP1* in controls and HCM patients by RT-qPCR. Values were normalized to *GAPDH* expression (n = 6 people in controls and n = 15 people in HCM patients).
- (D) Bar plot showing the relative expression of *EGR1* in controls and HCM patients by RT-qPCR. Values were normalized to *GAPDH* expression (n = 6 people in controls and n = 15 people in HCM patients).
- (E) Western blot (left) and bar plots (right) showing SP1 and EGR1 protein levels in controls and HCM patients (n = 4 people per group).
- (F) The schematic diagram of breeding strategy for HCM mice carrying *Myh6* (c.1360 C>T) and *Tnnt2* (c.379 A>T) mutations. Mice with *Myh6*^{R454C/+} or *Tnnt2*^{R127W/+} mutation were constructed, and the double-mutant HCM mice model (*Myh6*^{R454C/+}*Tnnt2*^{R127W/+}) was generated by cross-breeding between the two genotypes. Data in (C, D, E) were expressed as mean \pm SEM, and statistical significance was performed by two-tailed Student t test. ns: P value > 0.05 , *: P value ≤ 0.05 , **: P value ≤ 0.01 , ***: P value ≤ 0.001 .

Fig S1

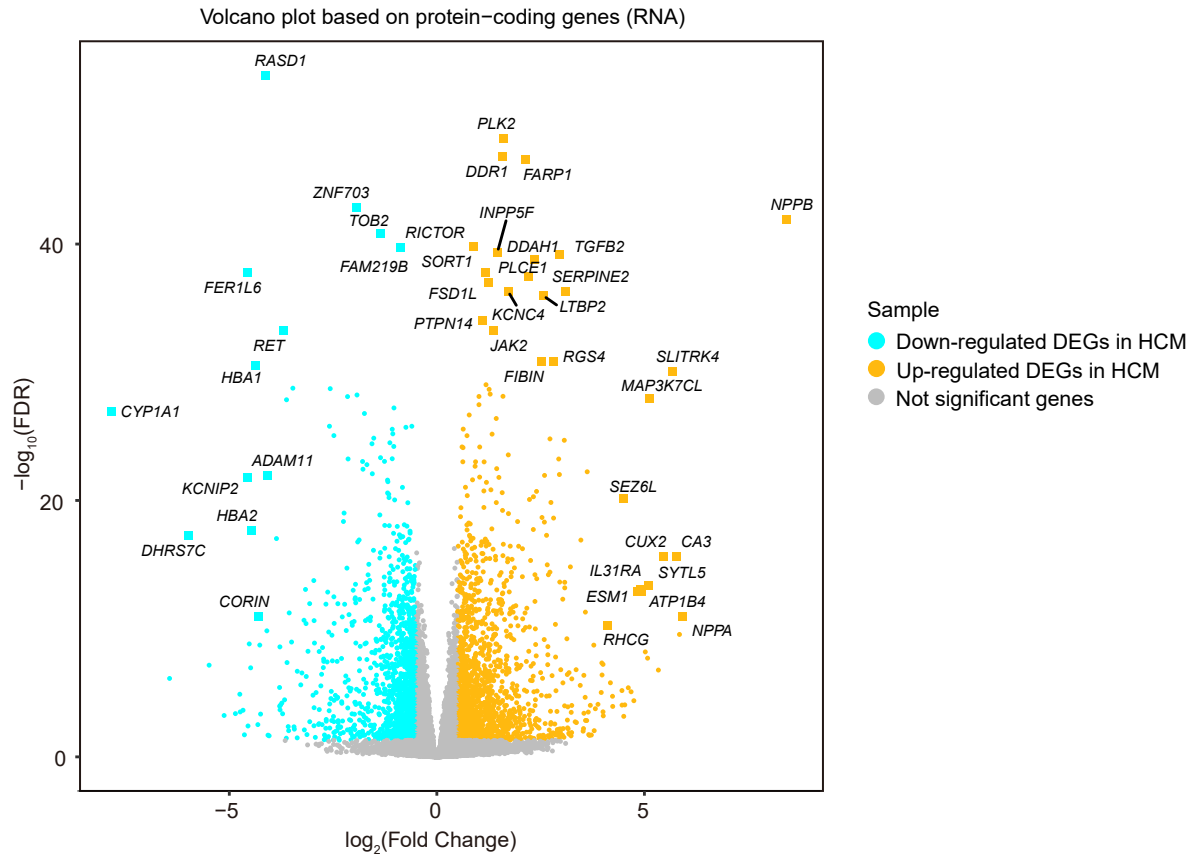


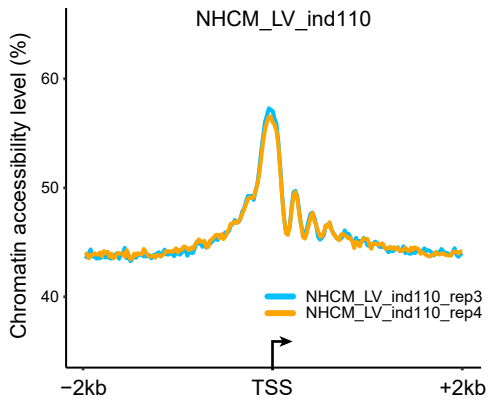
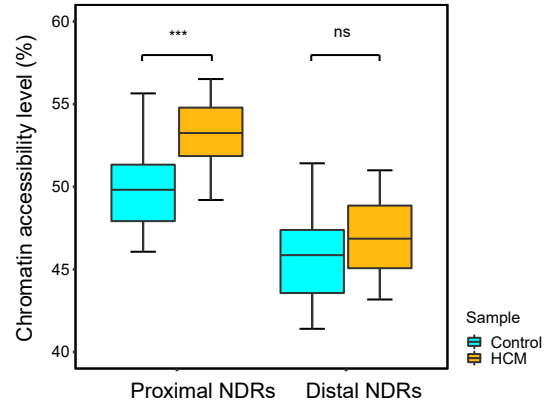
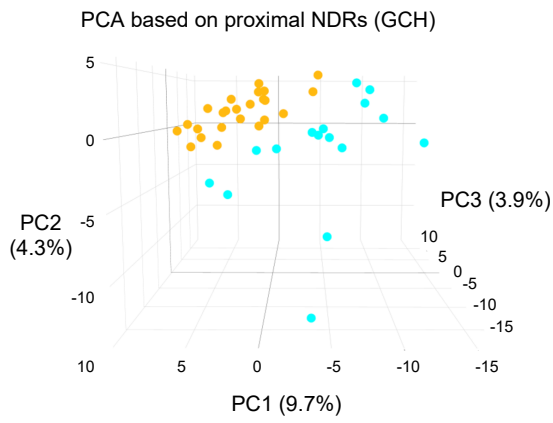
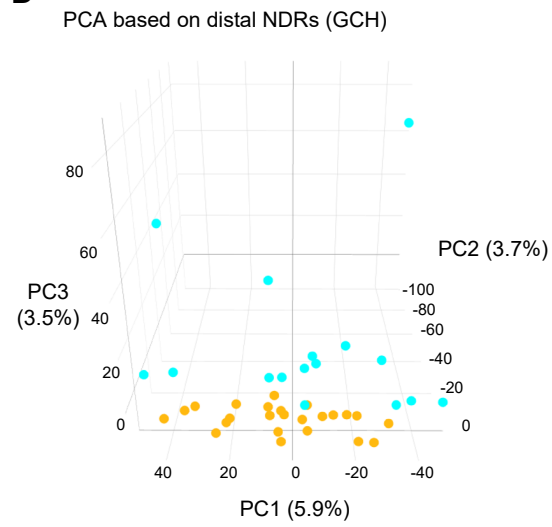
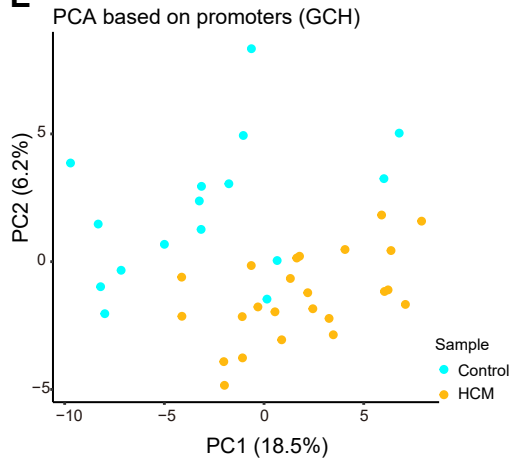
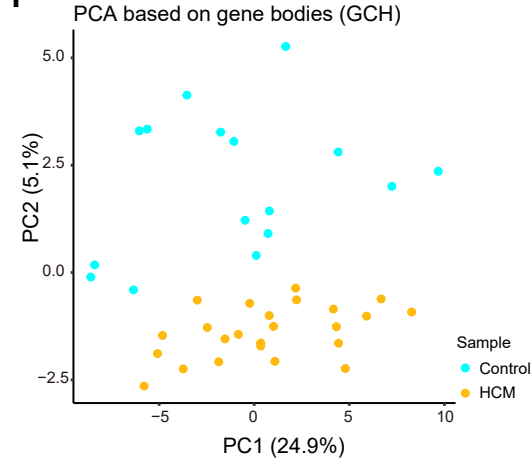
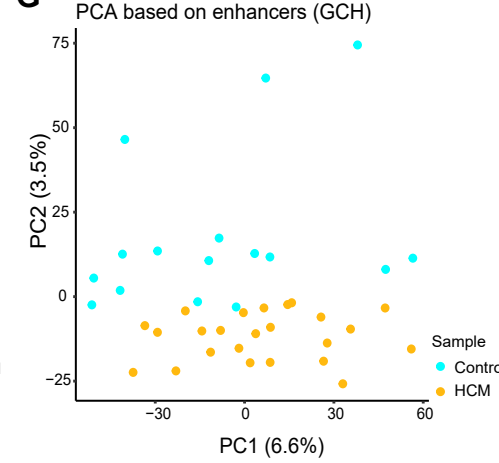
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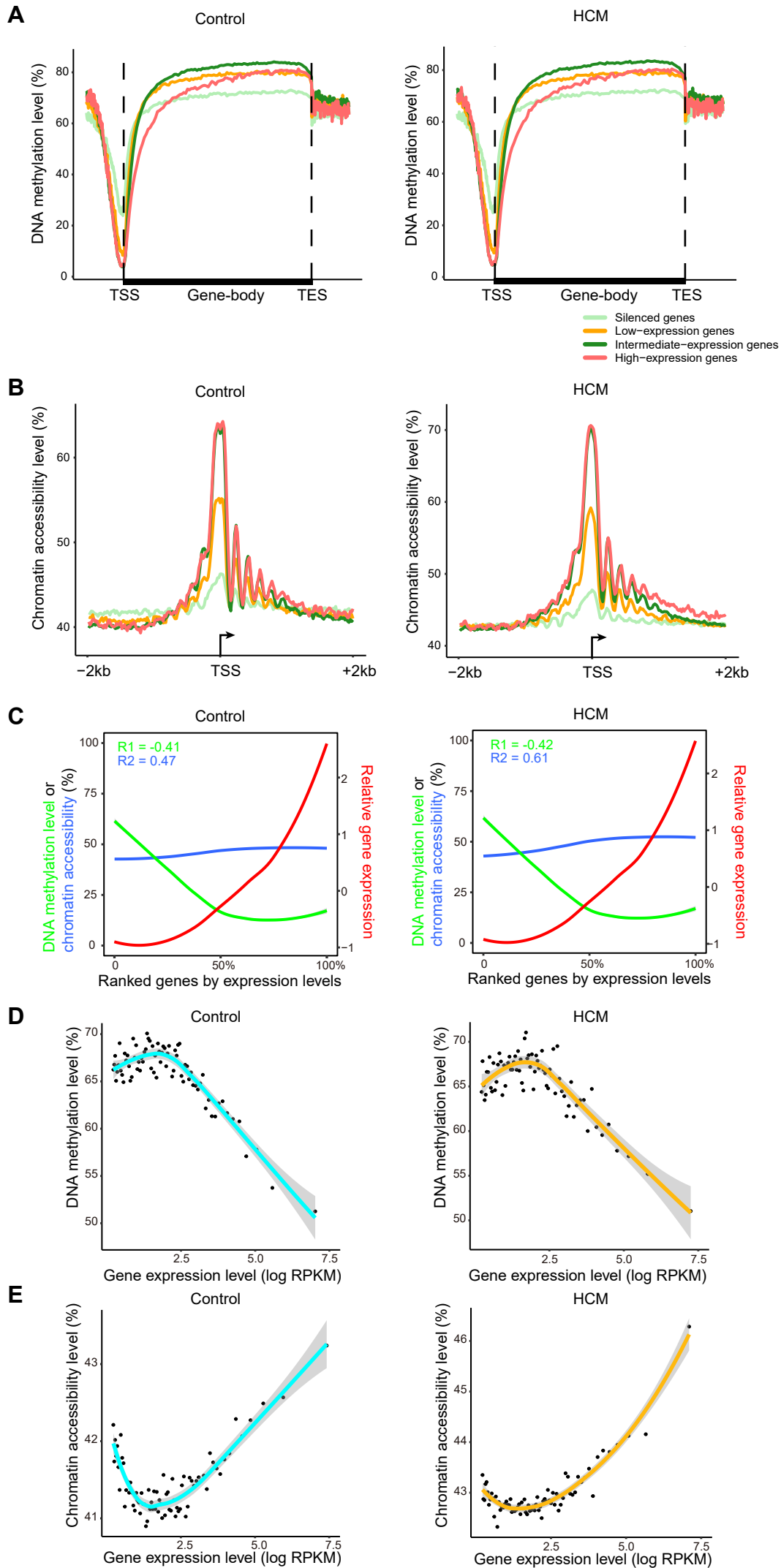
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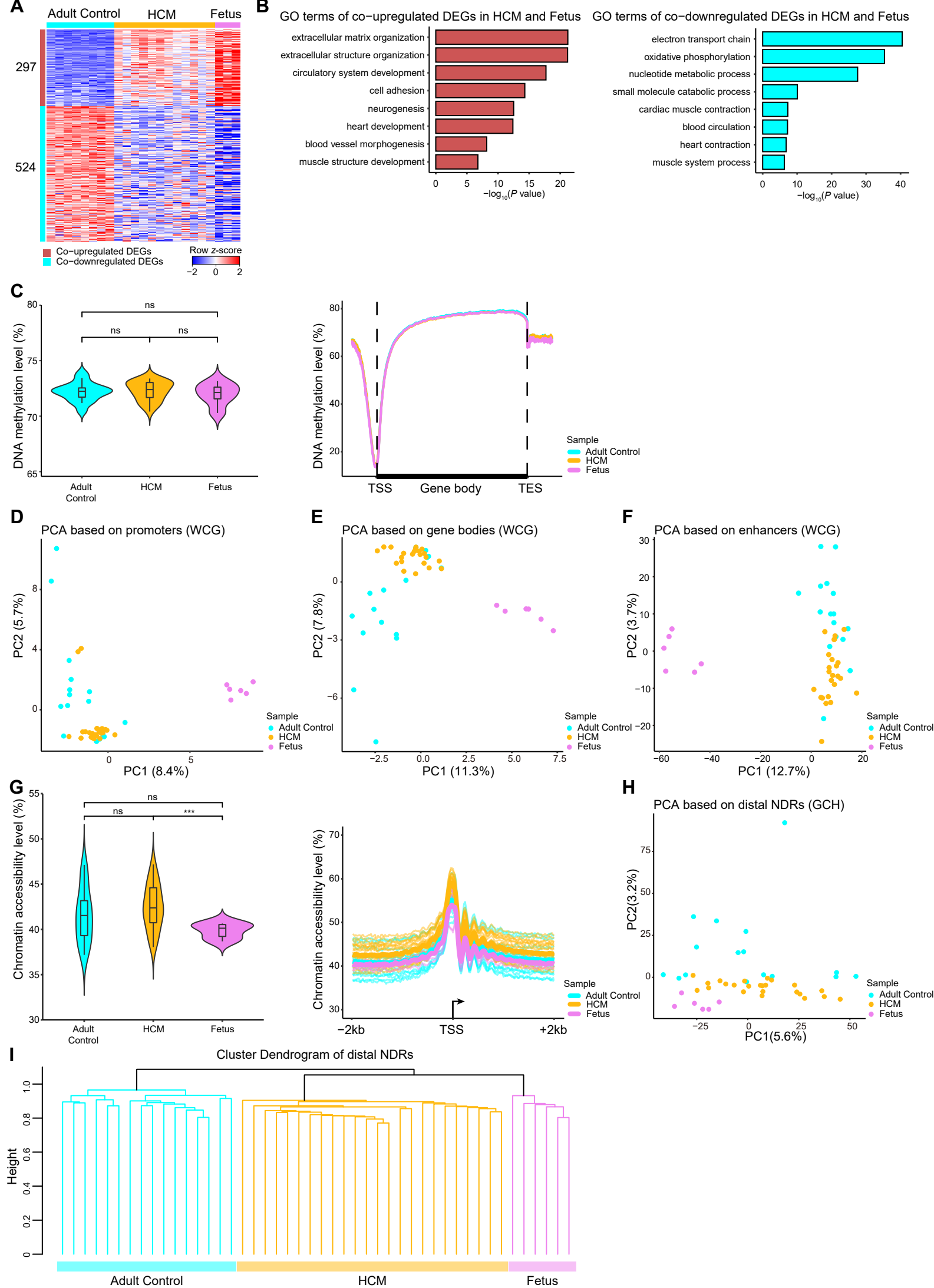
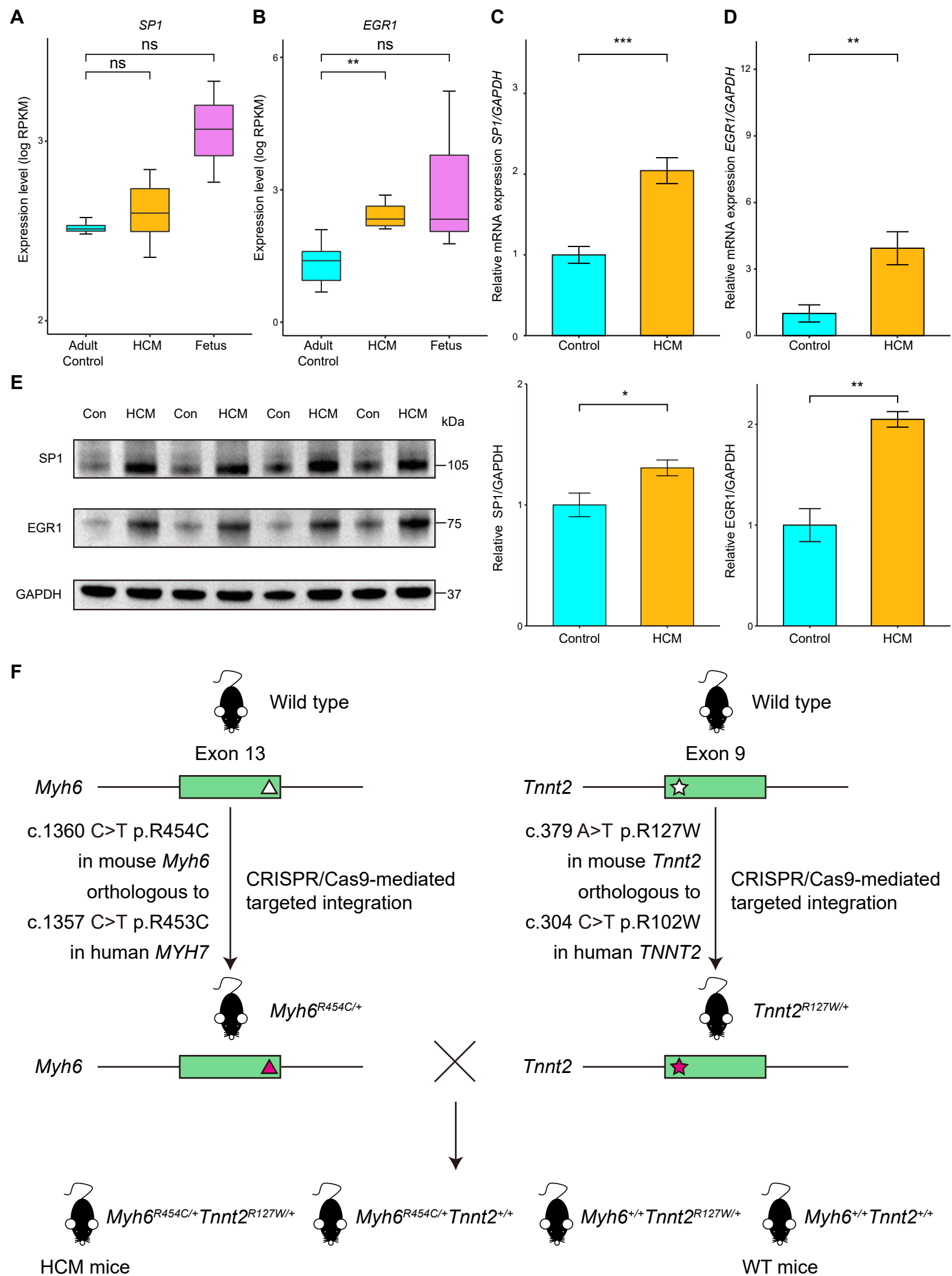
Fig S5

Fig S6



Supplemental tables

Table S1. The clinical parameters of HCM patients.

Table S2. The sequence information of NOME-seq and RNA-seq.

Table S3. DEGs (protein-coding genes and lncRNAs) between the adult controls and HCM patients.

Table S4. DMRs between the adult controls and HCM patients.

Table S5. Co-regulated DEGs in both fetuses and HCM patients compared with adult controls.

Table S6. Motifs enriched in distal and proximal NDRs in the adult controls, HCM patients and fetuses.

Table S7. DEGs between HCM mice and WT mice.

Table S8. DEGs between HCM mice and HCM mice treated with plicamycin or ML264.

Table S9. Co-regulated DEGs in fetal mice and HCM mice compared with WT mice.