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Supplemental information

Genome-wide fetalization of enhancer architecture in heart disease

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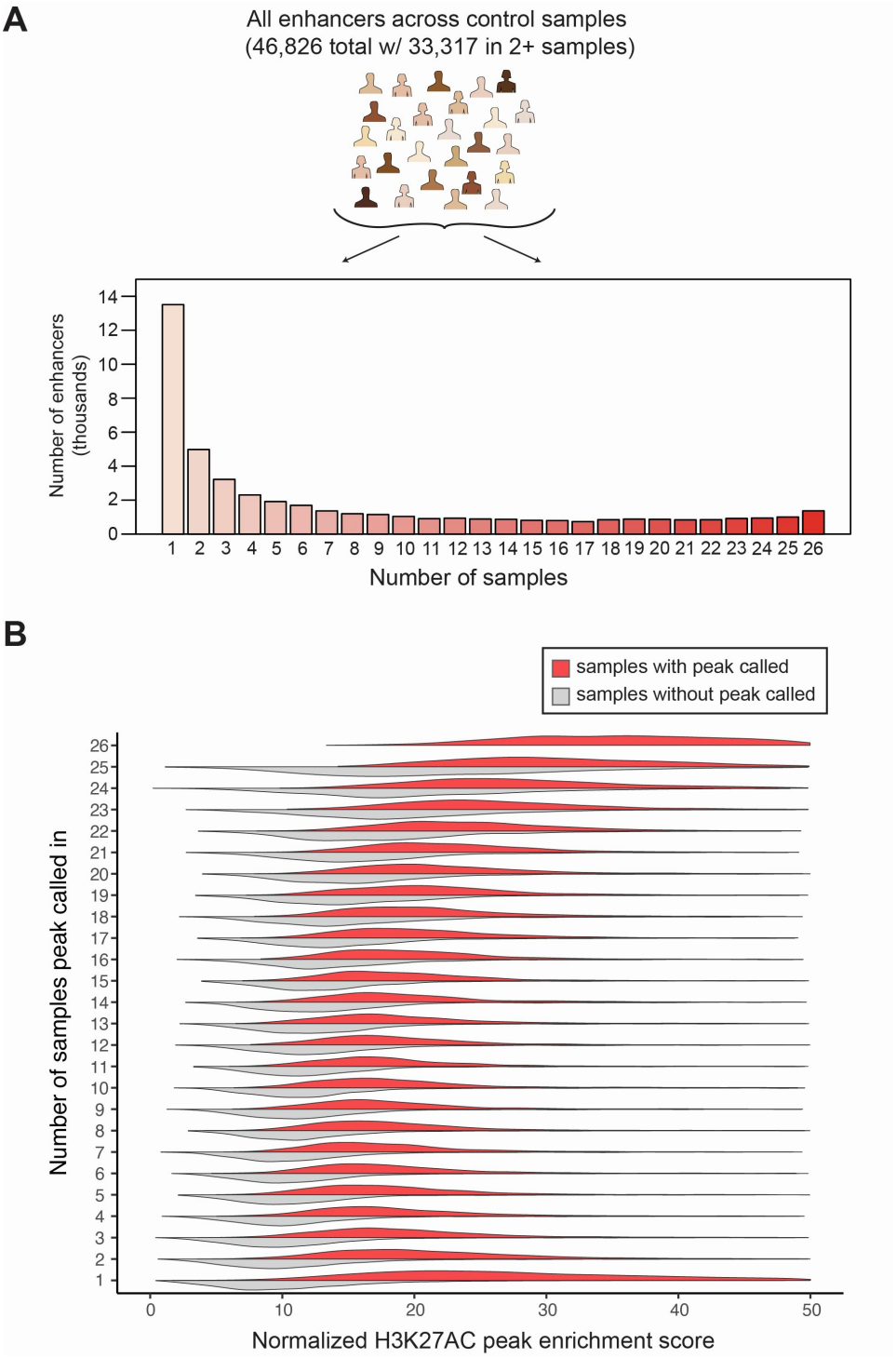
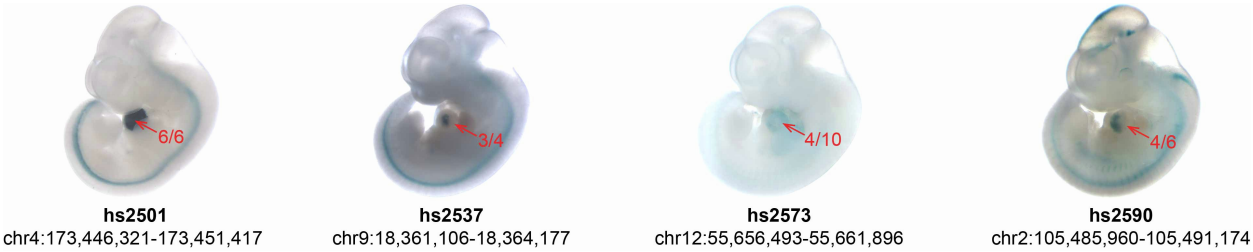
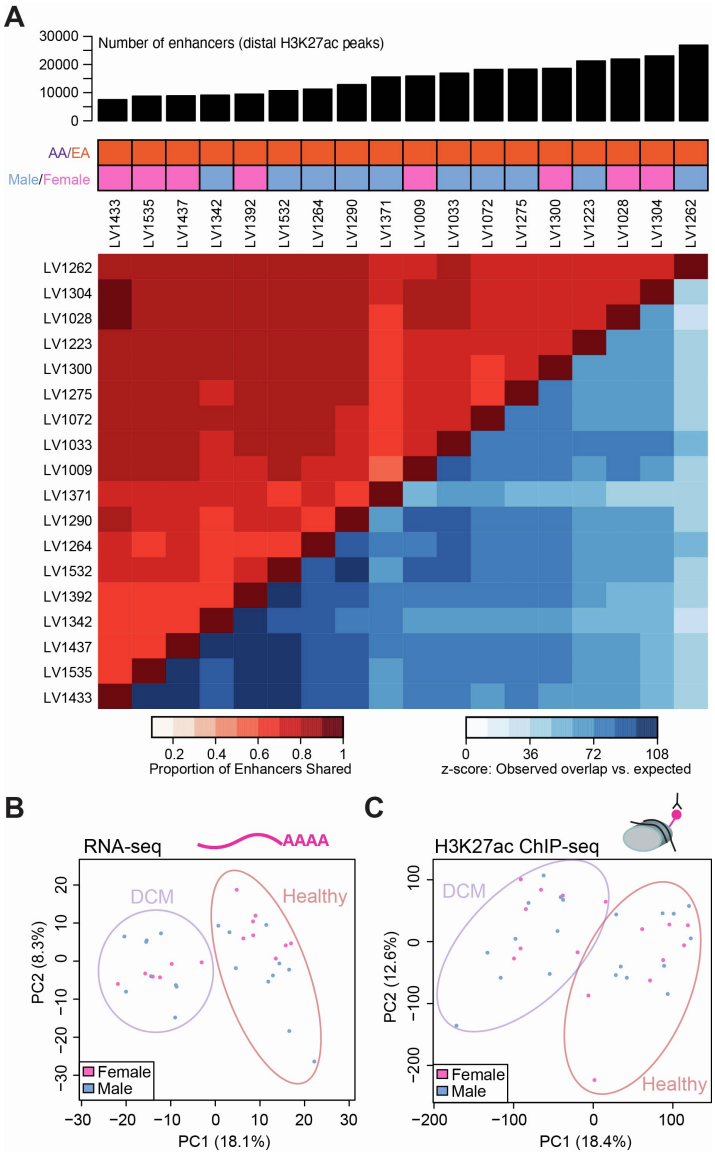


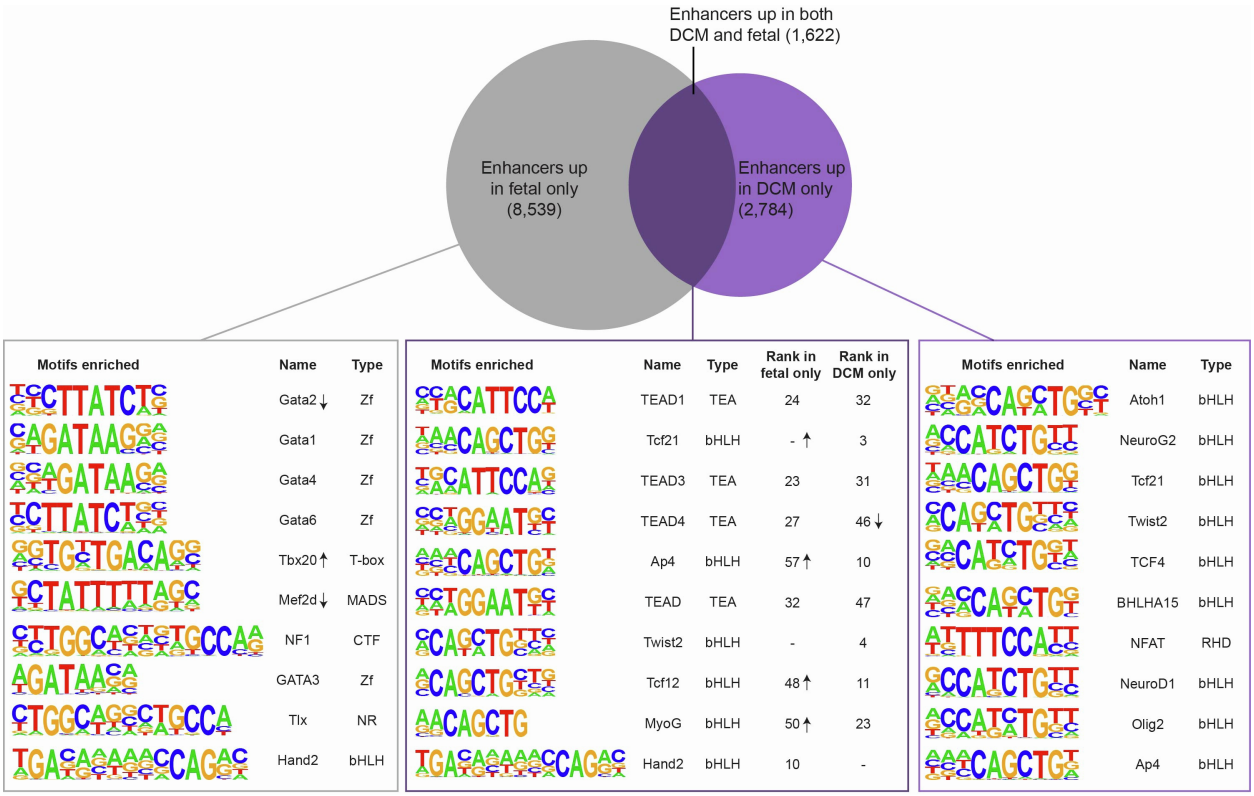
Supplementary Figure 1: Summary of enhancer representation across all healthy left ventricle samples. Related to Figure 2A. (A) Across all 26 healthy samples, we identified 46,826 total predicted enhancers, with 33,317 present in at least two subjects. (B) Split violin plots showing the distribution of average ChIP-seq signal enrichments at enhancer (TSS-distal) regions with a significant H3K27ac enrichment in one or more adult control samples. Distribution of average enrichments at samples either showing or not showing a significant peak are indicated in red and grey, respectively.



Supplementary Figure 2: Transgenic validation of additional predicted human heart enhancers. Related to Figure 2C. Shown are representative transgenic embryonic day 11.5 (E11.5) mouse embryos showing *lacZ* expression (blue staining) driven by one of four different human heart enhancers. Additional examples are shown in Figure 2C. Red numbers indicate reproducibility of the heart expression over the total number of transgene-expressing embryos obtained, hs numbers indicate the unique identifier for the enhancer in the VISTA Enhancer Browser, and the coordinates below are for the tested enhancer in the hg38 assembly of the human genome. Embryos have an average crown-rump length of 6 mm.



Supplementary Figure 3: Chromatin and gene expression profiling of dilated cardiomyopathy. Related to Figure 3. (A) Top: number of predicted enhancers discovered in each DCM left ventricle sample. Middle: demographic information for each subject. Bottom: Heatmap showing the proportion of peaks shared between samples (red tones in top left), along with z-scores indicating how many standard deviations the observed number of shared peaks exceeded random expectation (blue tones in bottom right, see Methods). (B-C) Principal Components (PC1, x-axes; PC2, y-axes) for expressed genes (B) and distal enhancer peaks (C), as in Figure 3A. In this version, samples from males are colored blue and females pink. These data include only autosomal genes and peaks.



Supplementary Figure 4: Transcription factor binding motifs enriched in enhancers upregulated in fetal and DCM samples. Related to Figure 5. Shown are the top ten most significantly enriched transcription factor binding motifs identified by HOMER (see Methods) for enhancers upregulated in fetal samples only (left), enhancers upregulated in both fetal and DCM (middle), and enhancers upregulated in DCM only. The background set used for all comparisons was composed of all enhancers detected in at least two healthy adult, DCM or fetal samples. Arrows next to motif names or ranks indicate the direction of significant change in gene expression (see Methods) of corresponding transcription factor in relevant group compared to healthy adults. Venn diagram not drawn to scale.