

## **Supplemental Information Inventory**

**Figure S1 (related to Figure 1).** Analysis of the genomic regions enriched in HAND2 chromatin complexes and associated genes.

**Figure S2 (related to Figure 1).** Activities of the VISTA cardiac enhancers that overlap genomic regions enriched by HAND2 ChIP-Seq (E10.5).

**Figure S3 (related to Figure 1).** HAND2 target genes encoding ligands for signaling pathways that function in OFT and/or right ventricle morphogenesis.

**Figure S4 (related to Figure 4).** Transcriptome analysis identifies the HAND2 target genes with significantly altered expression in *Hand2*-deficient AVCs.

**Figure S5 (related to Figures 5 and 6).** Analysis of HAND2 target genes in the developing AVC.

**Figure S6 (related to Figure 6).** The *Snai1* transcript distribution overlaps the CRM+57kb activity in craniofacial structures, branchial arches and early limb buds.

**Table S1 (related to Figure 1).** List of the statistically validated HAND2 ChIP-Seq peaks in the dataset from mouse embryonic hearts (E10.5) and associated genes. This table is included as a **separate Excel file**.

**Table S2 (related to Figure 1).** HAND2-enriched genomic regions associated to genes whose altered expression in *Hand2*-deficient mouse embryonic hearts have been published.

**Table S3 (related to Figure 1 and S2).** HAND2 ChIP-Seq peaks overlapping VISTA enhancers active in mouse embryonic hearts.

**Table S4 (related to Figures 1 and S3).** HAND2 target genes annotated to GO terms relevant for outflow tract and right ventricle development.

**Table S5 (related to Figure 4 and S4).** Comparative transcriptome analysis of the genes expressed in wild-type and *Hand2*-deficient AVCs (E9.25-E9.5). This table is included as a **separate Excel file**.

**Table S6 (related to Figures 4D, 5, 6 and S5).** Difference in expression levels of HAND2 target genes with functions in EMT and/or AVC cardiac cushion development.

**Supplemental Experimental Procedures.** Detailed description of the ChIP-Seq peak-calling and data analysis performed, such as e.g. de novo motif discovery and CHIP-qPCR analysis. A detailed description of the AVC transcriptome analysis and the functional annotation of differentially expressed genes is included. Details on various other methods used such as AVC explant cultures, in situ hybridization, immunofluorescence and the list of all antibodies used are given. This section also lists all relevant primers for qPCR analysis and the genomic coordinates for generating LacZ reporter constructs.

**Supplemental References**