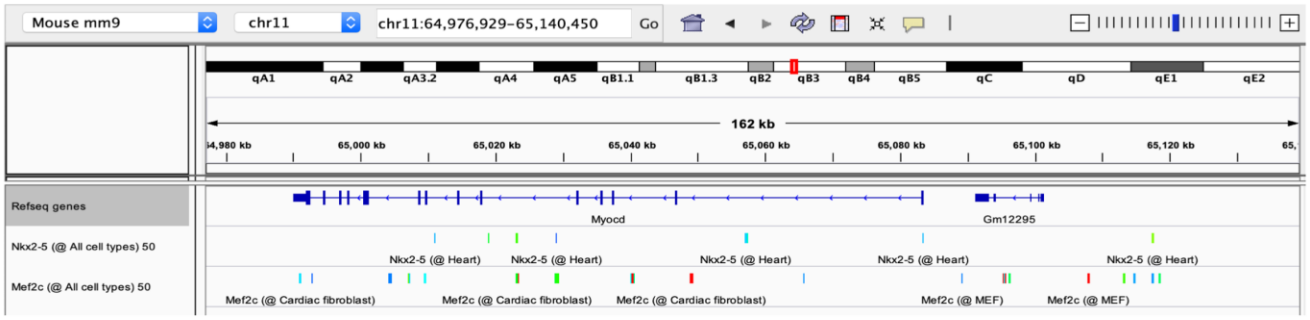
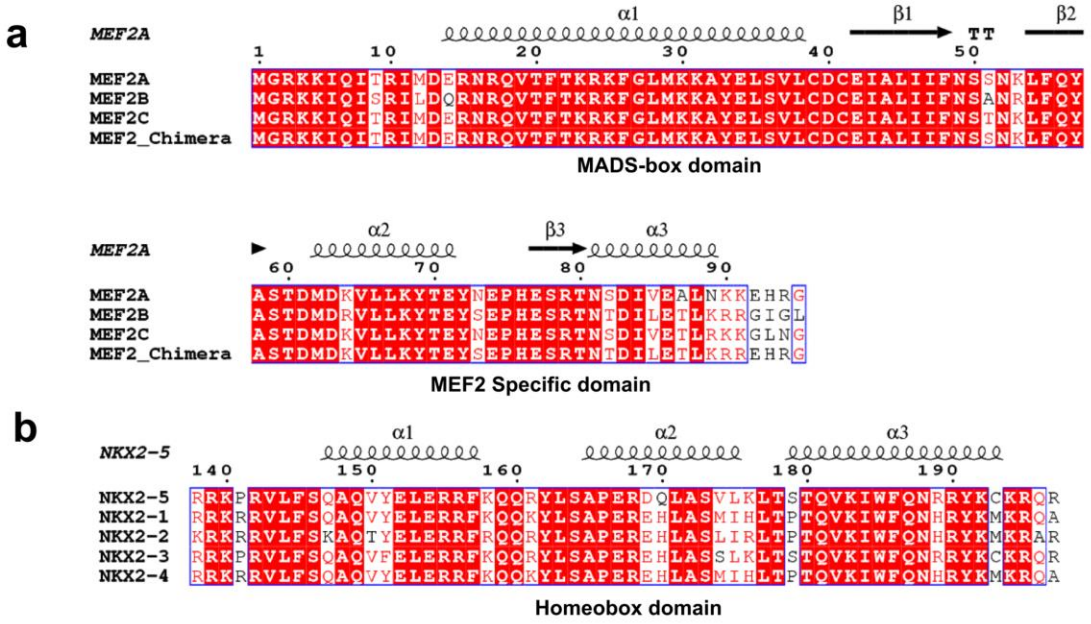
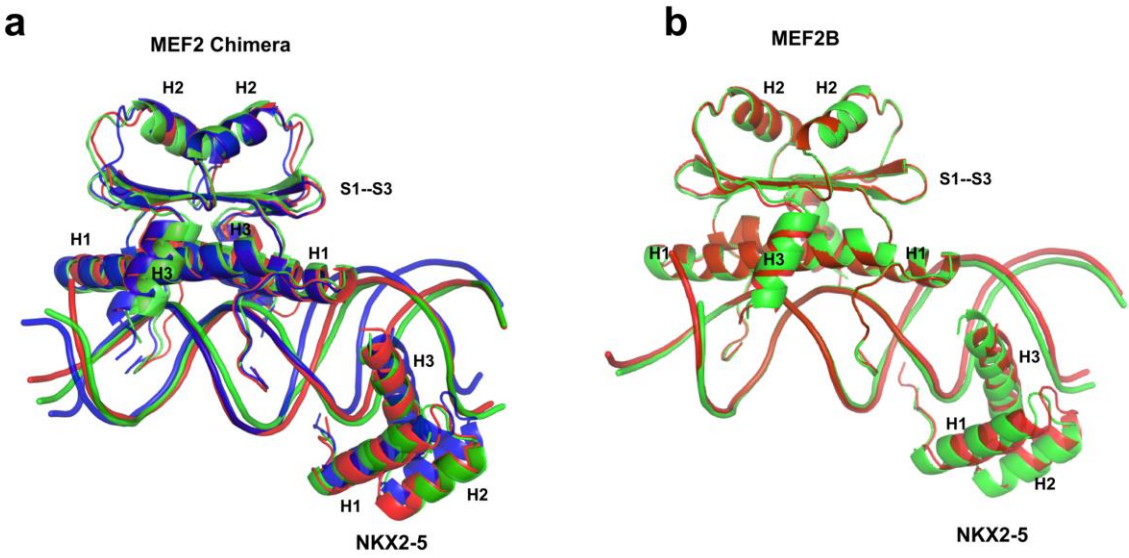
a



Supplemental Figure 1. MEF2C and NKX2-5 Chip-seq peaks in myocardin regulatory elements. (a) Chip-seq peaks of mouse MEF2C and NKX2-5 in myocardin regulatory elements. Myocd: myocardin gene. Mouse myocardin gene is in chromosome 11 (chr11). Peaks data were retrieved from ChIP-Atlas database and were visualized in IGV genome browser.

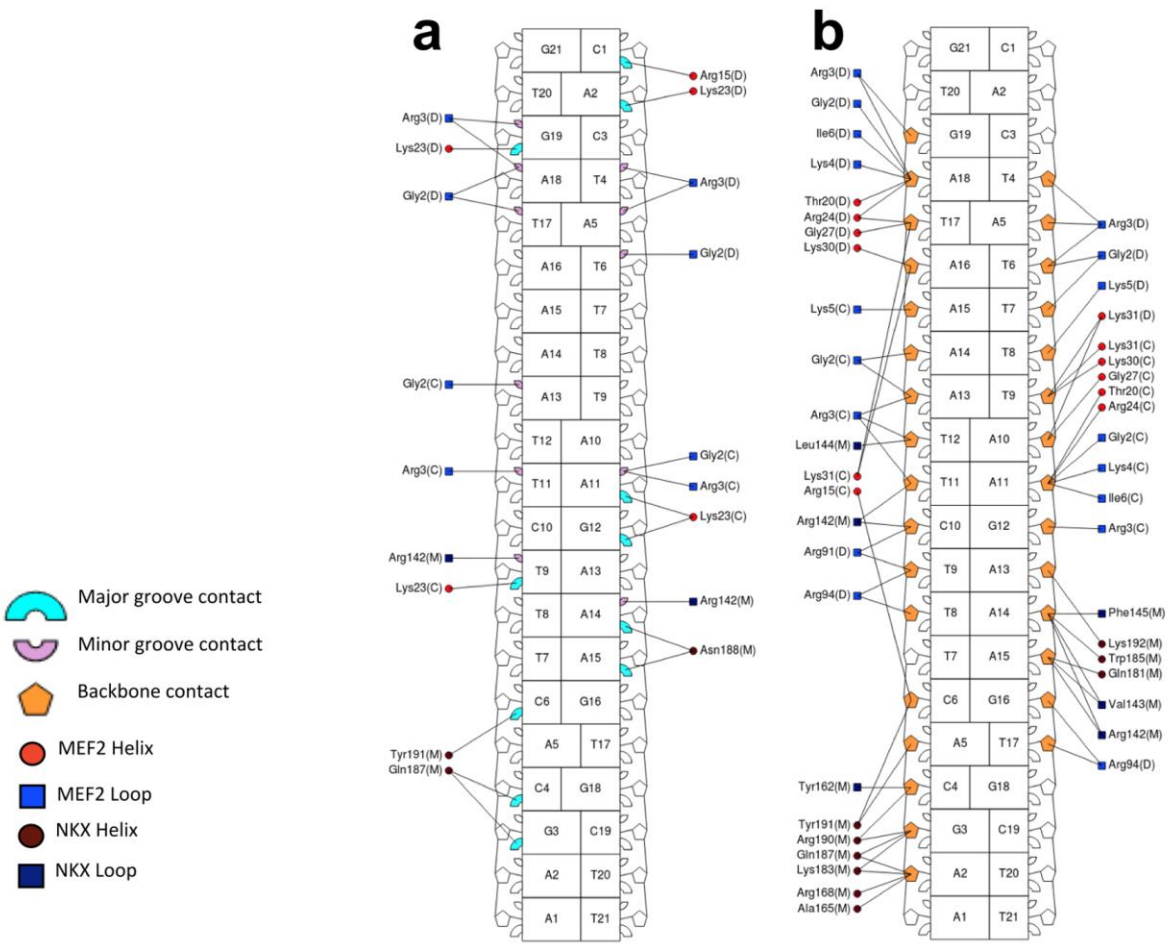


Supplemental Figure 2. Sequence alignment of protein constructs used in this study. (a) Sequence alignment of MEF2_Chimera constructs to MEF2A, MEF2B, and MEF2C. TT: β turns. (b) Sequence alignment of NKX2-5 to NKX2-1, NKX2-2, NKX2-3, and NKX2-4. The NKX2-5 construct in crystallization contains C194S mutation. Sequence alignment was performed with Clustal Omega and visualized with ESPript 3.0.



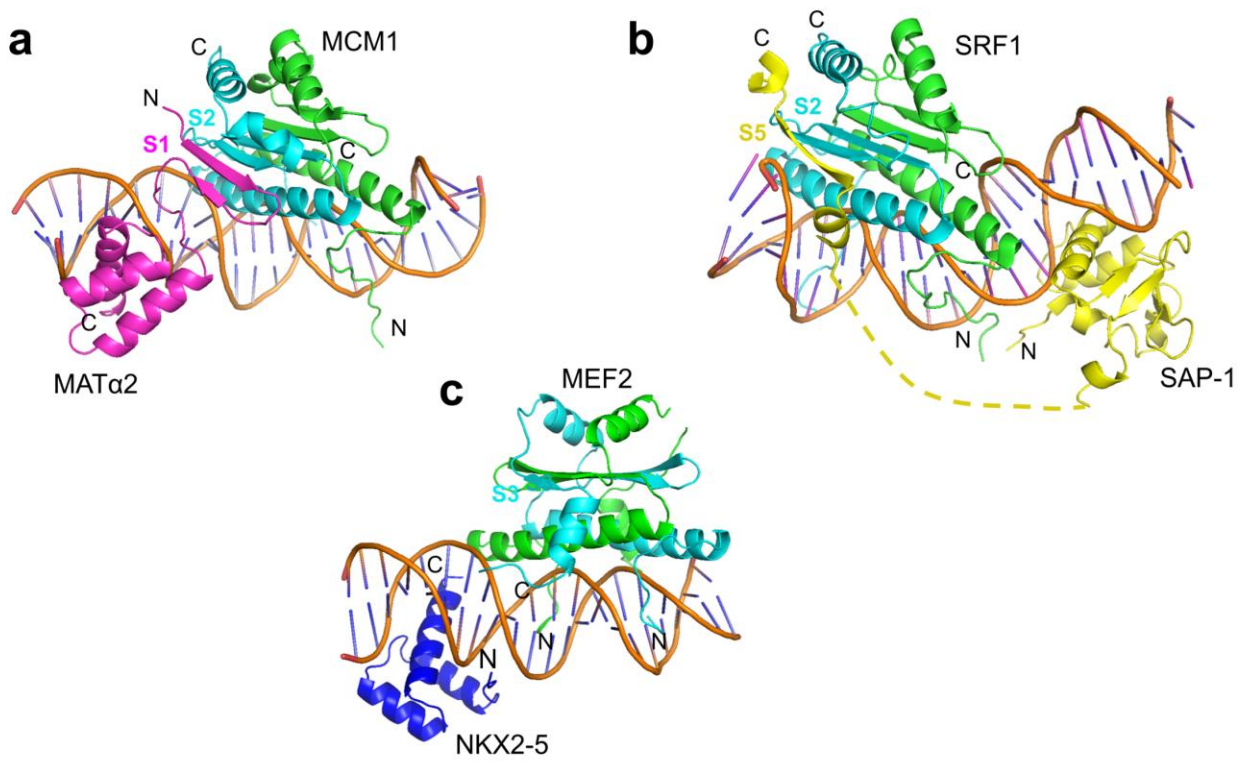
Supplemental Figure 3. Structural comparison of MEF2/NKX2-5/DNA ternary complexes in an asymmetric unit

(a) Superposition of MEF2 chimera/NKX2-5/DNA complexes in the asymmetric unit (ASU), each complex is colored in red, green and blue respectively. (b) Superposition of MEF2B/NKX2-5/DNA complexes in the asymmetric unit (ASU), each complex is colored in red and green respectively. Secondary structural elements are labelled: H: alpha helix, S: beta strand.

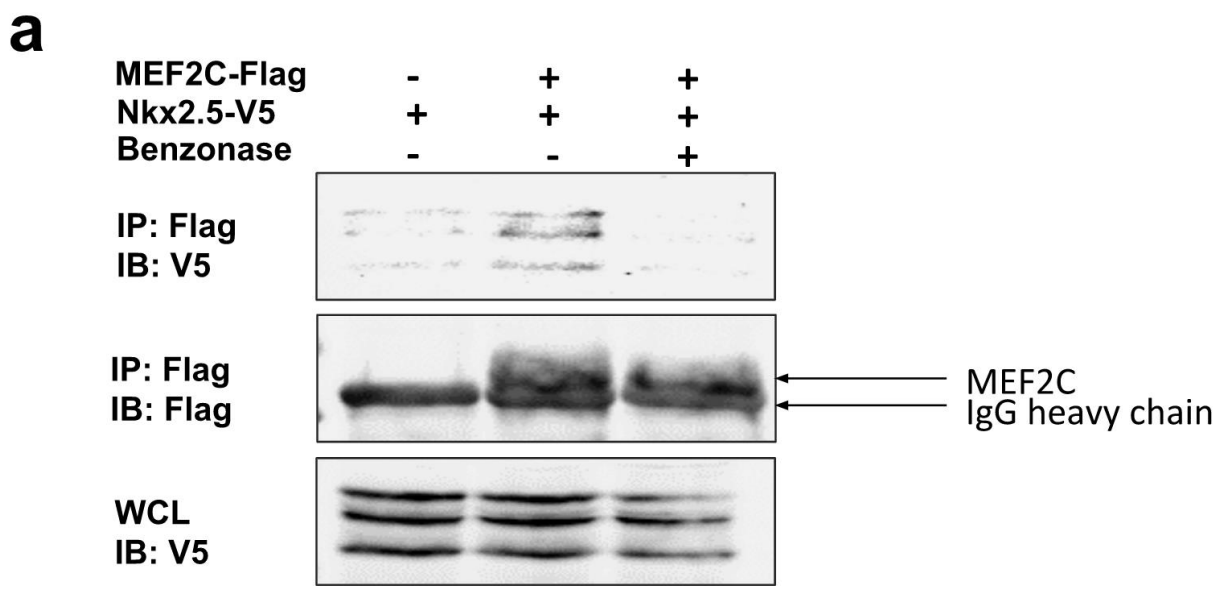


Supplemental Figure 4. Protein and DNA contacts diagram of MEF2 Chimera/NKX2-5/DNA crystal structure

(a) Schematic diagrams show interactions between protein and DNA bases in major and minor grooves. (b) Schematic diagrams show interactions between protein and DNA backbones. Plots were generated with DNAproDB. Letters in parenthesis indicate protein chains in structure (MEF2 chain C and chain D, NKX2-5 chain M).



Supplemental Figure 5. Cocrystals structures of MADS-box transcription factors with their transcription partners in the literature show protein-protein interactions involving secondary structures, whereas no protein-protein interaction involving secondary structures was observed in the cis-mode of MEF2/NKX2-5/DNA interaction.
(a) Structure of the yeast MADS-box protein MCM1/Matα2/DNA complex (PDB code: 1MNM). MCM1 dimer is colored in green and cyan, MATα2 is colored in magenta. Strands involved in protein-protein interactions are labeled as S1 and S2. (b) Structure of the human MADS-box protein SRF1/SAP-1/DNA complex (PDB code: 1HBX). SRF dimer is shown in green and cyan, SAP-1 is colored in yellow, yellow dash line indicates disordered region. Strands involved in protein-protein interactions are labeled as S2 and S5 (c) structure of MADS box protein MEF2/NKX2-5/DNA in this study, MEF2 dimer is colored in green and cyan, NKX2-5 is colored in blue.



Supplemental Figure 6. NKX2-5 and MEF2C do not interact with each other without DNA. (a) HEK293T cells were transfected with plasmids containing FLAG tagged wild-type MEF2C and V5 tagged Nkx2-5. Whole cell lysates were extracted after 48 hours and treated with or without Benzonase for 1 hour at 37℃. MEF2C was then immunoprecipitated with mouse anti-FLAG antibody and NKX2-5 was analyzed by immunoblotting with rabbit anti-V5 antibody.