## Supplemental Table 1. Statistical analysis between WT and Mef2d mutant mice

Echocardiogram		Fetal gene expression	
Parameters	p values	Genes	p values
LVIDd <sup>A</sup>	$< 0.7^{\mathrm{B}}$	$ANP^{C}$	<1.0 <sup>D</sup>
LVIDs	< 0.005	BNP	< 0.08
FS	< 0.005	Myh6	<1.0
HR	< 0.3	Myh7	<0.4
AWd	< 0.6	Actal	< 0.09
PWd	< 0.2	Ctgf	<0.5
		Col1a2	< 0.9
		Col3a1	< 0.5
		Mef2a	<0.7

<sup>A</sup>LVIDd, left ventricular end-diastolic diameter; LVIDs, left ventricular end-systolic diameter; FS, fractional shortening; HR, heart rate; AWd, anterior wall thickness in diastole; PWd, posterior wall thickness in diastole.

<sup>C</sup>ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; Myh6, myosin, heavy polypeptide 6, cardiac muscle, alpha; Myh7, myosin, heavy polypeptide 7, cardiac muscle, beta; Acta1, actin, alpha 1, skeletal muscle; Ctgf, connective tissue growth factor; Col1a2, procollagen, type I, alpha 2; Col3a1, procollagen, type III, alpha 1; Mef2a, myocyte enhancer factor-2a.

<sup>&</sup>lt;sup>B</sup>These p values relate to Table 1 and Figure 3.

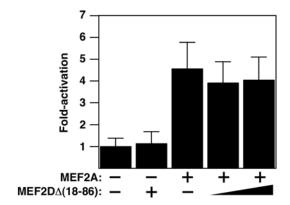
<sup>&</sup>lt;sup>D</sup>These p values relate to Figure 4.

## **Supplemental Methods**

## Tissue culture and transfection

COS-7 cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum. Fugene 6 (Roche) was used for transient transfection assay according to the manufacturer's instructions. A luciferase reporter plasmid containing three copies of a high-affinity MEF2 binding site and a mammalian expression construct of MEF2A have been previously described (39). A coding sequence of MEF2DΔ(18-86) was generated by PCR and ligated into pcDNA 3.1 (Invitrogen) to generate the expression construct. Plasmids were co-transfected with 10 ng of a CMV-β-galactosidase reporter plasmid to control for transfection efficiency.

## **Supplemental Figure**



**Figure S1.** COS-7 cells were cotransfected with a MEF2-dependent luciferase reporter and expression vectors of MEF2A and MEF2D $\Delta$ (18-86) to demonstrate that the presence of mutant MEF2D protein has a minimal effect on MEF2 activity. Error bars indicate one standard deviation.