Supporting Information

Konno et al. 10.1073/pnas.1012826107

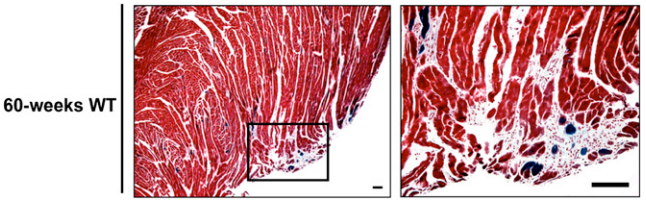

Fig. S1. Colocalization of myocyte enhancer factor-2 (Mef2)-dependent reporter activity with fibrosis in the aging hearts. A representative 60-wk-old WT/Mef2-LacZ (β-galactosidase) mouse heart stained with X-gal followed by Gomori trichrome shows increased Mef2-dependent reporter activity (dark blue) adjacent to regions of fibrosis (light blue). (Scale bars: 100 μm.)

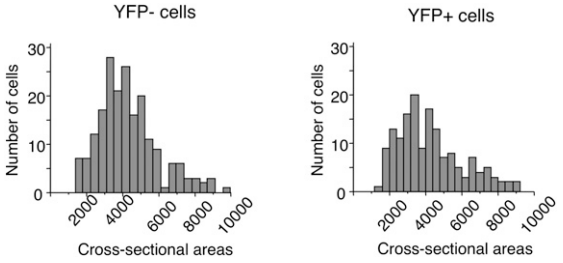

Fig. S2. Distribution of cross-sectional areas of YFP-negative and YFP-positive myocytes in hearts from 30-wk-old MHC$^{403/+}$/Mef2-LacZ/βMHC-YFP mice. Note that the distribution and dispersion of cross-sectional areas was similar between YFP-negative (YFP-) and YFP-positive (YFP+) myocytes.

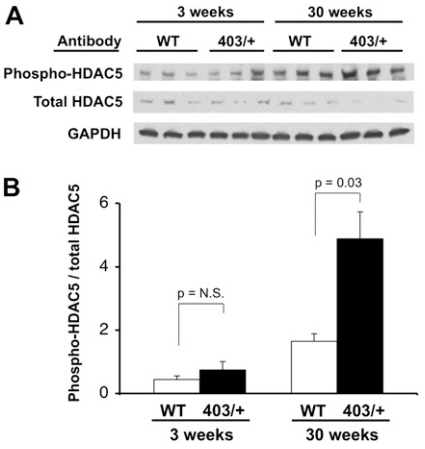

Fig. S3. Western blot analyses of histone deacetylase 5 (HDAC5) phosphorylation levels in MHC$^{403/+}$ mouse hearts. (*A*) Representative Western blots of homogenates from the hearts of 3-wk-old WT, MHC$^{403/+}$ (403/+) mice and 30-wk-old WT, MHC$^{403/+}$ mice reacted with antibodies specific for HDAC5 serine 498, total HDAC5, and GAPDH. (*B*) Phosho-HDAC5 was normalized to total HDAC5 (*n* = three mice per group).

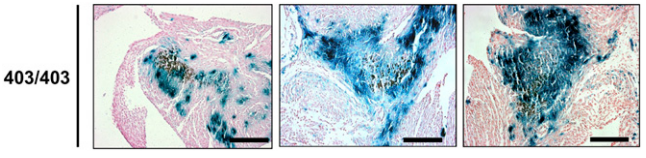

Fig. S4. Mef2-dependent reporter activity associated with necrotic tissue in ventricular walls of 6-d-old homozygous MHC$^{403/403}$ hearts. Additional histological specimens from 6-d-old MHC$^{403/403}$/Mef2-LacZ mouse hearts stained with X-gal (blue) and von Kossa stain (brown). (Scale bars: 100 μm.)

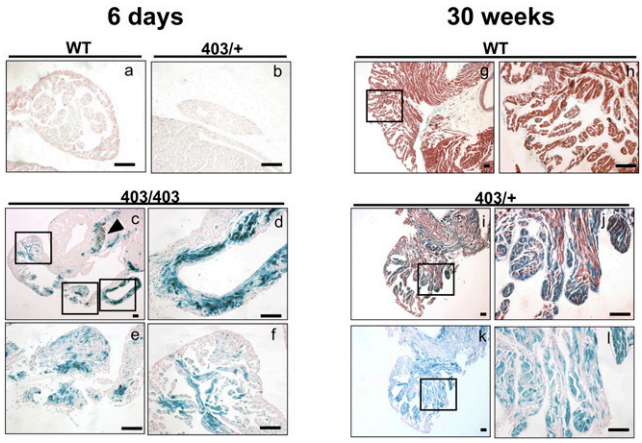

Fig. S5. Mef2-dependent reporter activation without necrosis or fibrosis in atria of MHC$^{403/403}$ and hypertrophic MHC$^{403/+}$ hearts. Atrial sections from 6-d-old WT/Mef2-LacZ (*A*), MHC$^{403/+}$/Mef2-LacZ (*B*), and MHC$^{403/403}$/Mef2-LacZ (*C*–*F*) hearts and 30-wk-old WT/Mef2-LacZ (*G*, *H*) and MHC$^{403/+}$/Mef2-LacZ (*I*–*L*) hearts, stained with X-gal and von Kossa (*A*–*F*, *K*, and *L*) or Gomori trichrome (*G*–*J*). Mef2-dependent reporter was robustly activated in left (*D*, *E*) and right (*F*) atria of MHC$^{403/403}$/Mef2-LacZ hearts. Necrosis was absent from atria but was found in the ventricular septum (*C*, arrowhead) juxtaposed to ventricular myocytes with Mef2-dependent reporter activity. The Mef2-dependent reporter was robustly and heterogeneously activated in the left atria of MHC$^{403/+}$ hearts (*J*–*L*), despite the lack of atrial fibrosis or necrosis. (Scale bars: 100 μm.)

Table S1. Mef2 and TEA domain (TEAD) RNA levels in left ventricular tissues of prehypertrophic and hypertrophic MHC$^{403/+}$ and WT mice measured by deep serial analysis of gene expression (DSAGE)

	Prehypertrophic			Hypertrophic		
Transcript	WT	403/+	*P* value	WT	403/+	*P* value
Mef2a	130	133	N.S.	121	156	3.9×10^{-6}
Mef2b	0	0	N.S.	2	1	N.S.
Mef2c	51	56	N.S.	53	50	N.S.
Mef2d	19	23	N.S.	28	50	3.1×10^{-8}
Tead1	4	7	N.S.	8	10	N.S.
Tead2	4	3	N.S.	3	6	N.S.
Tead3	13	10	N.S.	14	13	N.S.
Tead4	0	1	N.S.	1	2	N.S.

DSAGE, analogous to serial analysis of gene expression (SAGE), measures RNA levels by counting tags derived from RNA molecules (1). RNA was isolated from the left ventricles of prehypertrophic MHC$^{403/+}$ and hypertrophic MHC$^{403/+}$ (403/+) mice and age-matched WT mice. Each tag library had more than 4 million tags that matched to the genome, to RNA transcripts, or both. Tag counts are normalized per million reads of each library; *P* value reflects significance of the difference between prehypertrophic and hypertrophic left ventricle and appropriate control (WT) ventricular RNA. N.S., not significant.

1. Kim JB, et al. (2007) Polony multiplex analysis of gene expression (PMAGE) in mouse hypertrophic cardiomyopathy. *Science* 316:1481–1484.

Table S2. Left ventricular dimensions of prehypertrophic (3-wk-old), hypertrophic (30-wk-old) MHC$^{403/+}$/Mef2-LacZ (403/+), and age-matched WT mice

	Prehypertrophic (3-wk-old) mice		Hypertrophic (30-wk-old) mice	
Genotype	WT	403/+	WT	403/+
Number	4	3	5	8
LVMW (mm)	0.74 ± 0.07	0.75 ± 0.15	0.91 ± 0.02	1.05 ± 0.09*
LVAW (mm)	0.65 ± 0.07	0.64 ± 0.09	0.87 ± 0.05	0.95 ± 0.09
LVPW (mm)	0.70 ± 0.07	0.73 ± 0.17	0.89 ± 0.03	1.01 ± 0.10
LVDD (mm)	3.53 ± 0.31	3.33 ± 0.13	3.61 ± 0.20	3.66 ± 0.38
LVED (mm)	2.02 ± 0.34	2.03 ± 0.17	1.89 ± 0.13	2.18 ± 0.38
LVFS (%)	43 ± 5	39 ± 6	47 ± 4	41 ± 7

LVAW, left ventricular anterior wall thickness; LVDD, left ventricular end-diastolic diameter; LVED, left ventricular end-systolic diameter; LVMW, left ventricular maximal wall thickness; LVPW, left ventricular posterior wall thickness. LVFS, left ventricular fractional shortening.
*Significant difference ($P = 0.02$) compared with the 30-wk-old WT mice.