SUPPLEMENTARY TEXT

Differential roles of endocardial and myocardial Brg1 in heart development

We previously reported that *Tie2cre;Brg1*$^{F/F}$ embryos that lacked endocardial *Brg1* had defects in the patterning of trabeculi[1]. In contrast, *Sm22αCre;Brg1*$^{F/F}$ embryos that lacked myocardial *Brg1* had normal trabecular patterning at E10.5[1] and E11.5 (Supplementary Fig. 3a-f). By alcian blue staining[1], these embryos did not have changes of extracellular matrix as observed in *Tie2cre;Brg1*$^{F/F}$ mice (Supplementary Fig. 4b). Therefore, the thin compact myocardium phenotypes of *Sm22αCre;Brg1*$^{F/F}$ embryos are distinct from the trabecular patterning defects of *Tie2cre;Brg1*$^{F/F}$ embryos. Furthermore, the molecular pathways controlled by myocardial Brg1 (current studies) are different from that (Adamts1) controlled by endocardial Brg1[1]. Thus, our observations indicate that the trabecular patterning and compact myocardium formation in early embryos are two different developmental processes regulated separately by endocardial and myocardial Brg1.

The J1 anti-Brg1 antibody immunoprecipitates Brg1 on *MHC* promoters

Although the J1 anti-Brg1 antibody was reported to have some cross-reactivity with the closely related Brahma ATPase (Brm) when Brm was overexpressed[2], we did not observe such cross-reactivity in the immunostaining of paraffin sections of embryonic hearts. J1 specifically recognized Brg1 in embryonic hearts despite the presence of Brm proteins there

(Supplementary Fig. 2b,c; data not shown). To conclude that Brg1 binds to the *MHC* promoters, we first performed J1-ChIP analysis using mice that lack *Brm*[3]. In the hearts of *Brm*-null mice that underwent TAC 2 weeks before, we found that the J1 antibody immunoprecipitated the BAF complex on both the α-*MHC* and β-*MHC* promoters (Supplementary Fig. 10f) in a pattern similar to that of the TAC'ed control mice (Fig. 4f). Since *Brm*-null mice have only minimal amount of truncated Brm proteins[2], these J1-ChIP results suggest that Brg1, indeed, binds to the *a-MHC* and β-*MHC* promoters. Conversely, we used J1 to perform ChIP analysis of normal non-stressed adult hearts that predominantly express Brm with undetectable Brg1 proteins in cardiomyocytes (Fig. 4c; data not shown). This J1-ChIP analysis showed no binding of proteins to either α-*MHC* or β-*MHC* promoters (Supplementary Fig. 10e), indicating J1 does not immunoprecipitate Brm proteins on the *MHC* promoters. These results are consistent with the reporter assays that showed Brg1 restoration alone in SW13 cells (which lack both Brg1 and Brm) was sufficient to repress α-*MHC* and activates β-*MHC* promoters (Fig. 2f,i). Together, these observations indicate that the J1 antibody detects Brg1 on the *MHC* promoters.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Dynamic changes of *MHC* gene expression under different pathophysiological conditions. Embryonic cardiomyocytes are highly proliferative and

express primarily *β-MHC*. Adult cardiomyocytes are post-mitotic and mainly express *α-MHC*. Under stress conditions, adult cardiomyocytes undergo hypertrophy and a shift from adult α-MHC to embryonic β-MHC isoforms.

Supplementary Figure 2. Embryos lacking myocardial *Brg1* die at E11.5-E12.5.

a, Whole mount β-galactosidase staining of *Sm22αCre;R26R* embryos.

b, **c**, Immunostaining of Brg1 (red, J1 antibody) and Troponin T (green) of E9.5 control and *Sm22αCre;Brg1*$^{F/F}$ embryos, showing specific deletion of Brg1 in myocardial cells. Nuclei: blue by DAPI staining. Short arrows: endocardial cells. Long arrows: myocardial cells.

d, **e**, Control and mutant (*Sm22αCre;Brg1*$^{F/F}$) embryos whole mount at E11.5. The left upper limb was removed to expose the heart.

f, The frequency of recovering mutant embryos at different gestational dates.

Supplementary Figure 3. *Sm22αCre;Brg1*$^{F/F}$ embryonic myocardium fails to proliferate.

a, **b**, H&E sections of E10.5 embryos. Arrows: interventricular septum. Asterisks: endocardial cushion.

c, Quantitation of compact myocardial thickness. p-value: Student-t test. Error bars: standard deviation.

d, **e**, H&E sections of E11.5 embryos. Arrows: interventricular septum. Asterisks: endocardial

cushion.

f, Quantitation of trabecular myocardium. p-value: Student-t test. Error bars: standard deviation.

g-j, TUNEL staining of E10.5 and E11.5 wildtype and *Sm22αCre;Brg1*$^{F/F}$ embryos.

k, **l**, Activated caspase 3 immunostaining E11.5 embryos. Green: auto fluorescence from red blood cells.

m, **n**, BrdU immunostaining E10.5 embryos in the septal primordia.

o, Quantification of BrdU incorporation of endocardium, endocardial cushion, and epicardium of control (black bar) and *Sm22αCre;Brg1*$^{F/F}$ (gray bar) embryonic hearts.

Supplementary Figure 4. *Sm22αCre;Brg1*$^{F/F}$ embryos have normal cardiac jelly and vascular pattern at E11.5.

a, b, Alcian blue staining[1] of control (**a**) and *Sm22αCre;Brg1*$^{F/F}$ (**b**) embryos at E11.5. arrow: extracellular matrix in cardiac jelly.

c, d, Whole mount Pecam1 immunostaining (brown) of cranial vessels (arrows) in control (**c**) and *Sm22αCre;Brg1*$^{F/F}$ (**d**) embryos at E11.5.

e, f, Whole mount Pecam1 immunostaining (brown) of intersomitic vessels (arrows) in control (**e**) and *Sm22αCre;Brg1*$^{F/F}$ (**f**) embryos at E11.5.

**Supplementary Figure 5. *Sm22αCre;Brg1*$^{F/F}$ embryos have normal expression of many

genes.

RNA *in-situ* hybridization of E11.5 control and *Sm22αCre;Brg1*$^{F/F}$ hearts. Expression of these transcripts are comparable in amount and localization between control and mutants.

Supplementary Figure 6. *Sm22αCre;Brg1*$^{F/F}$ mutants have ectopic expression *of* p57^{kip2}.

a, Quantitation of wildtype BrdU incorporation and p57^{kip2} expression, which show inverse correlation with each other.

b, p57^{kip2} immunostaining of E10.5 control and *Sm22αCre;Brg1*$^{F/F}$ hearts. Asterisks: septal primordium.

c, Gross morphology of cultured embryos.

Supplementary Figure 7. Regulation of BMP10, p57^{kip2} and myocardial proliferation by Brg1 in the myocardium.

a, **b**, Brg1 immunostaining of E10.5 wildtype and *Mef2cCre;Brg1*$^{F/F}$ hearts at E10.5. Asterisks: endocardium. Arrows: right ventricular myocardium. Arrowheads: left ventricular myocardium. RV: right ventricle. LV: left ventricle.

c, **d**, RNA *in situ* hybridization of *BMP10* in E10.5 control and *Mef2cCre;Brg1*$^{F/F}$ embryos. Arrows: right ventricular myocardium. Arrowheads: left ventricular myocardium.

e, **f**, p57^{kip2} immunostaining of E10.5 embryos. Asterisks: endocardium. Arrows: right

ventricular myocardium. Arrowheads: left ventricular myocardium.

g, **h**, BrdU immunostaining of E10.5 embryos. Asterisks: endocardium. Arrows: right ventricular myocardium. Arrowheads: left ventricular myocardium.

i, j, H&E sections of control (**i**) and *Mef2cCre;Brg1*$^{F/F}$ (**j**) hearts at E10.5. The cardiac outflow tract (OFT) of *Mef2cCre;Brg1*$^{F/F}$ embryos is hypoplastic.

Supplementary Figure 8. *BMP10* and *MHC* promoter analysis.

a, Sequence alignment of the *BMP10* locus from mouse, human, and rat. Height of individual peaks indicates degree of sequence homology. Black boxes (p1-p4) are regions of high sequence homology and further analyzed by ChIP. Red: promoter elements. Blue: exons. Green: transposons and repeat elements. Yellow: untranslated regions.

b, c, PCR analysis of Brg1-immunoprecipitated chromatin from E11.5 hearts (**b**) and adult hearts 2 weeks after the TAC procedure (**c**). α-HRP: anti-horse radish peroxidase antibody for negative control.

d, Deletional analyses of the α-*MHC* promoter in luciferase reporter assays in SW13 cells. p-value: Student t test. Error bar: standard deviation.

e, Deletional analyses of the β-*MHC* promoter in luciferase reporter assays in SW13 cells. p-value: Student t test. Error bar: standard deviation.

Supplementary Figure 9. Brg1 commands parallel pathways to regulate myocardial proliferation and differentiation.

a, **b**, α-actinin immunostaining (green) of E9.5 control (**a**) and *Sm22αCre;Brg1*$^{F/F}$ (**b**) embryos. Nuclei: blue (Dapi staining). Arrowheads point to dotted α-actinin proteins in (**a**). Arrows points to α-actinin in a thread-like, striated pattern (**b**).

c, **d**, Gross morphology of cultured embryos.

d, **f**, BrdU immunostaining of cultured embryos treated with DMSO and TSA.

g, BrdU incorporation quantitation in myocardial cells of cultured embryos. p-value: Student-t test.

h, **i**, Quantitative RT-PCR analysis of ventricular α-*MHC* and β-*MHC* mRNA (**h**) and α-*MHC*/β-*MHC* ratio (**i**) in cultured embryos treated with BSA or BMP10. Ctrl: control embryos. Mut: *Sm22αCre;Brg1*$^{F/F}$ embryos.

Supplementary Figure 10. Brg1 is required for cardiac hypertrophy, fibrosis, and *MHC* switch in adult mice.

a, Section of a whole mount β-galactosidase stained (blue) heart of *Tnnt2-rtTA;Tre-Cre;Rosa26LacZ* mice treated with doxycycline for 5 days.

b, Cardiac ventricular weight/body weight ratio. p-value: Student-t test.

c, **d**, Trichrome staining of doxycycline-treated control and *Tnnt2-rtTA;Tre-Cre;Brg1*$^{F/F}$ mice

after the TAC procedure. Black: nuclei, red: cardiomyocytes, blue: fibrotic areas.

e, PCR analysis of Brg1-immunoprecipitated chromatin from wildtype adult hearts in mice that received sham operation. a1-a7, b1-b5: primers targeting conserved regions of the α-*MHC* and β-*MHC* promoter, respectively.

f, PCR analysis of J1-immunoprecipitated chromatin from *Brm*-null adult hearts in mice that received TAC operation 2 weeks before. a1-a7, b1-b5: primers targeting conserved regions of the α-*MHC* and β-*MHC* promoter, respectively.

Supplementary Figure 11. ChIP analysis, expression and co-immunoprecipitation of PARP1 and HDAC.

a, PCR analysis of PARP1-immunoprecipitated chromatin from adult hearts 2 weeks after TAC. Numbers (p1-p4) indicate conserved regions of the *BMP10* promoter illustrated in Supplementary Fig. 8a.

b, Immunostaining and quantitation of myocardial BrdU incorporation in embryos cultured with BrdU from E9.5 to E10.5 in the presence or absence of PJ34.

c, PCR analysis of PARP1-immunoprecipitated chromatin from E11.5 hearts.

d, PARP1 (Green) immunostaining of E11.5 embryonic heart. Asterisks: myocardial cells.

e, Co-immunoprecipiation of PARP1 and HDAC2 from adult myocardium.

f, PCR analysis of HDAC9-immunoprecipitated chromatin from adult hearts two weeks after

TAC. IgG: negative control antibody. Numbers (a1-a7) and (b1-b5) indicate conserved regions of *α-MHC* and *β-MHC* promoter.

Supplementary Figure 12. Characteristics of human hypertrophic cardiomyopathy.

a, Demographic data of control subjects and patients with hypertrophic cardiomyopathy (HCM). IVSd is measured by cardiac magnetic resonance imaging.

b, Cardiac magnetic resonance imaging (MRI) of a normal subject and a HCM patient listed in (**a**). The arrows denote the thickness of interventricular septum measured during diastole (IVSd). LV, RV: left, right ventricle. LA, RA: left, right atrium.

c, Myocardial thickness (IVSd) in normal and HCM subjects. p-value: Student-t test. Error bar: standard deviation.

d, Mathematical derivation of the inflection point of the regression curve described in Fig. 5b.

e, Mathematical derivation of the inflection point of the regression curve described in Fig. 5c.

References:

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