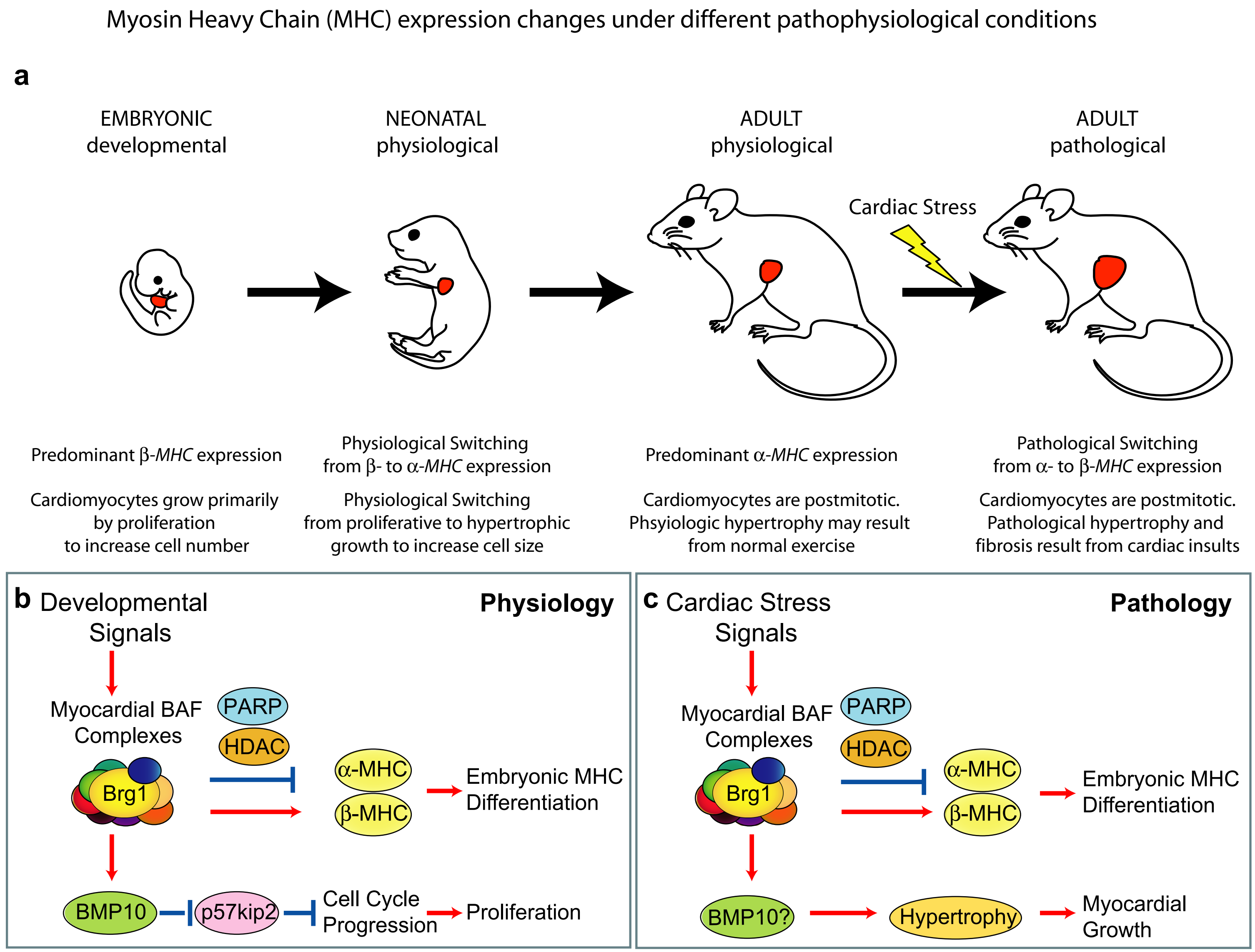
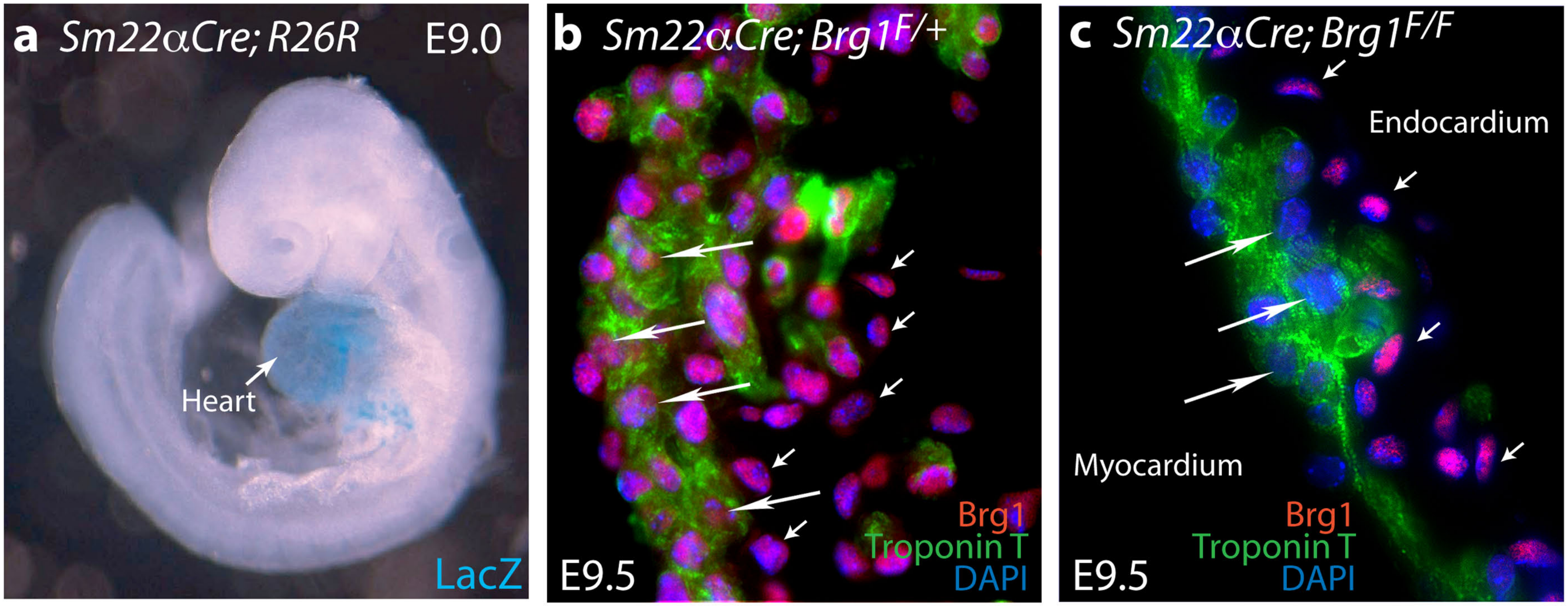
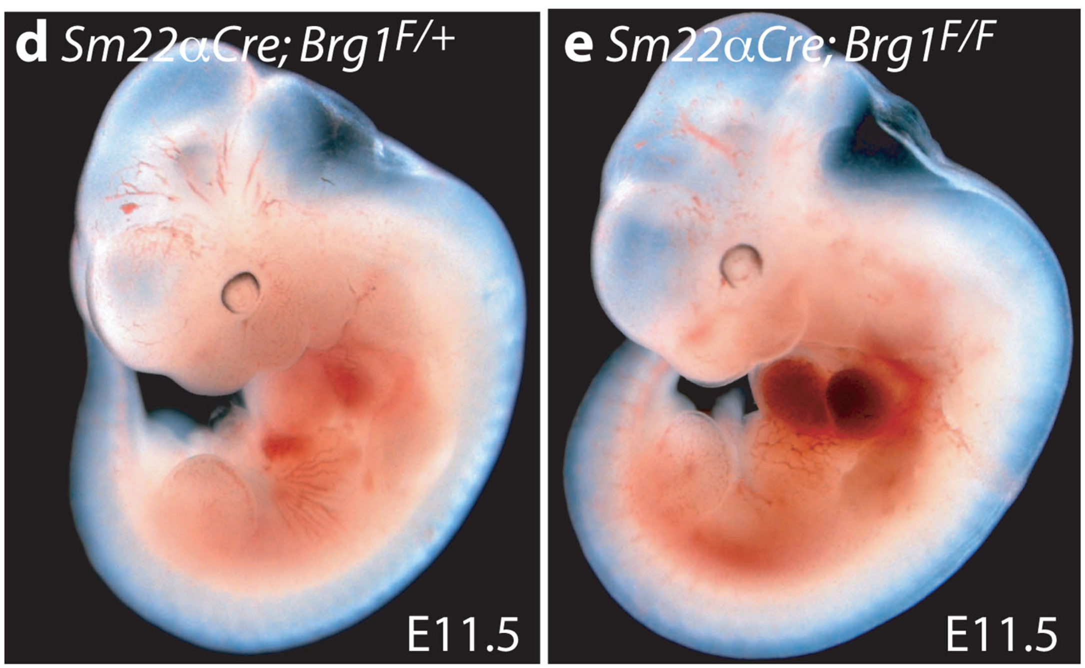


Myosin Heavy Chain (MHC) expression changes under different pathophysiological conditions
a
EMBRYONIC developmental
NEONATAL physiological
ADULT physiological
ADULT pathological
Cardiac Stress
Predominant β-MHC expression
Cardiomyocytes grow primarily by proliferation to increase cell number
Physiological Switching from β- to α-MHC expression
Physiological Switching from proliferative to hypertrophic growth to increase cell size
Predominant α-MHC expression
Cardiomyocytes are postmitotic. Phsyiologic hypertrophy may result from normal exercise
Pathological Switching from α- to β-MHC expression
Cardiomyocytes are postmitotic. Pathological hypertrophy and fibrosis result from cardiac insults
b Developmental Signals
Physiology
Myocardial BAF Complexes
PARP
HDAC
Brg1
α-MHC
β-MHC
Embryonic MHC Differentiation
BMP10
p57kip2
Cell Cycle Progression
Proliferation
c Cardiac Stress Signals
Pathology
Myocardial BAF Complexes
PARP
HDAC
Brg1
α-MHC
β-MHC
Embryonic MHC Differentiation
BMP10?
Hypertrophy
Myocardial Growth

Embryos lacking myocardial Brg1 die at E11.5-E12.5

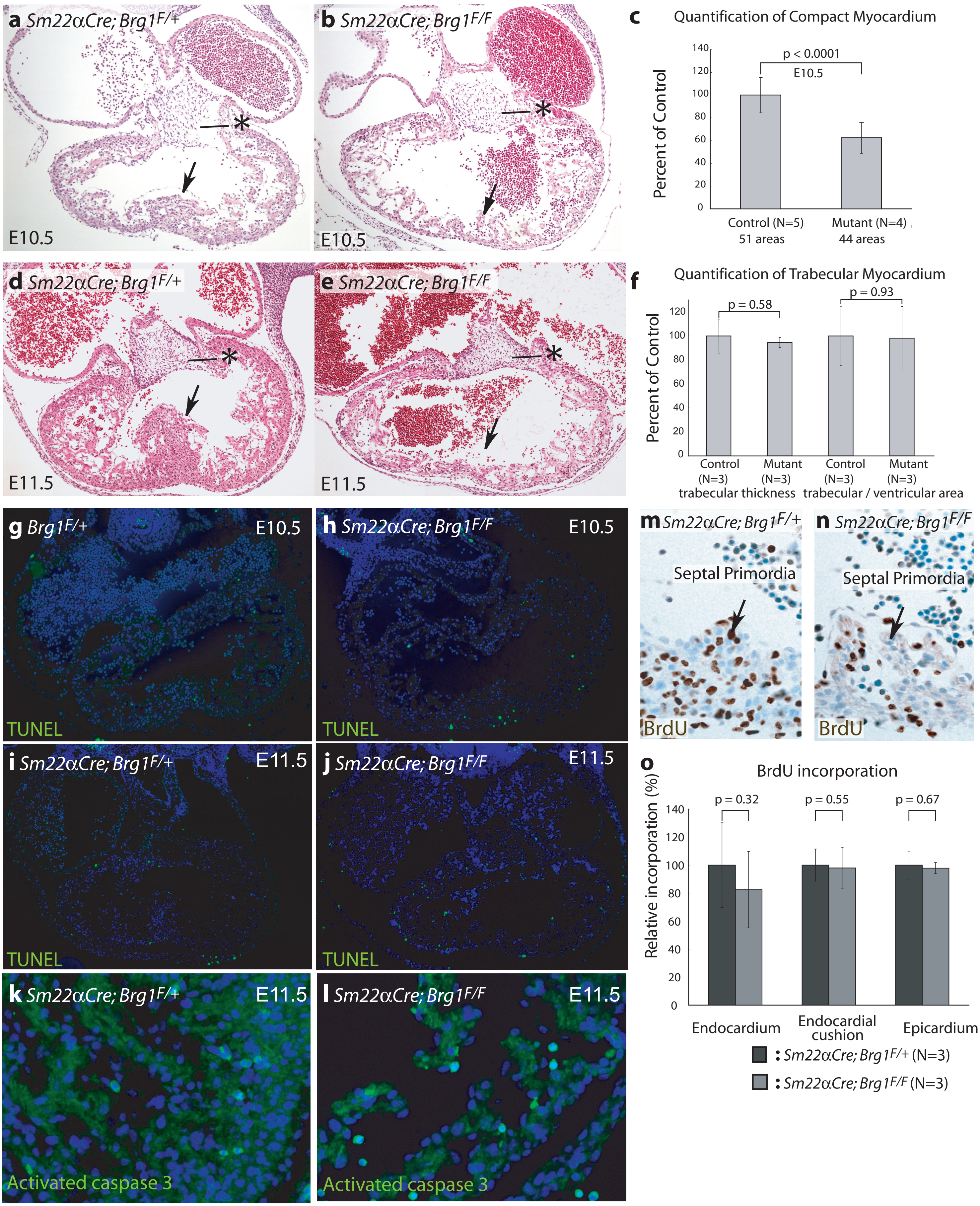




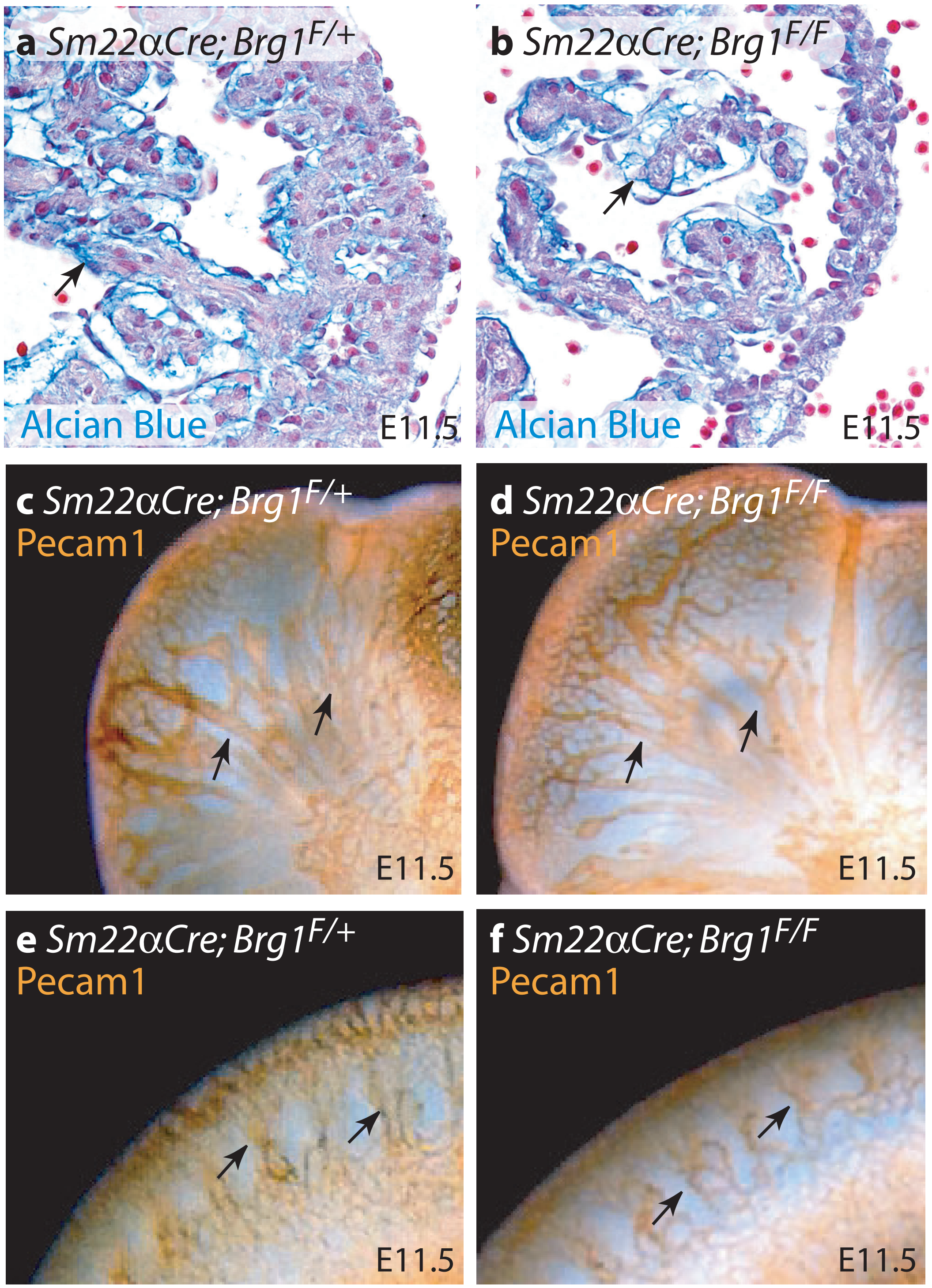
f Frequency of *Sm22αCre; Brg1*$^{F/F}$
(from *Sm22αCre;Brg1*$^{F/+}$ x *Brg1* $^{F/F}$)

Age	Expected	Observed
E8.5	0.25	0.29 (N= 7)
E9.5	0.25	0.24 (N= 25)
E10.5	0.25	0.23 (N=105)
E11.5	0.25	0.24 (N=230)
E12.5	0.25	0.15 (N=106)
>E13.5	0.25	0.00 (N= 21)
P0	0.25	0.00 (N= 85)

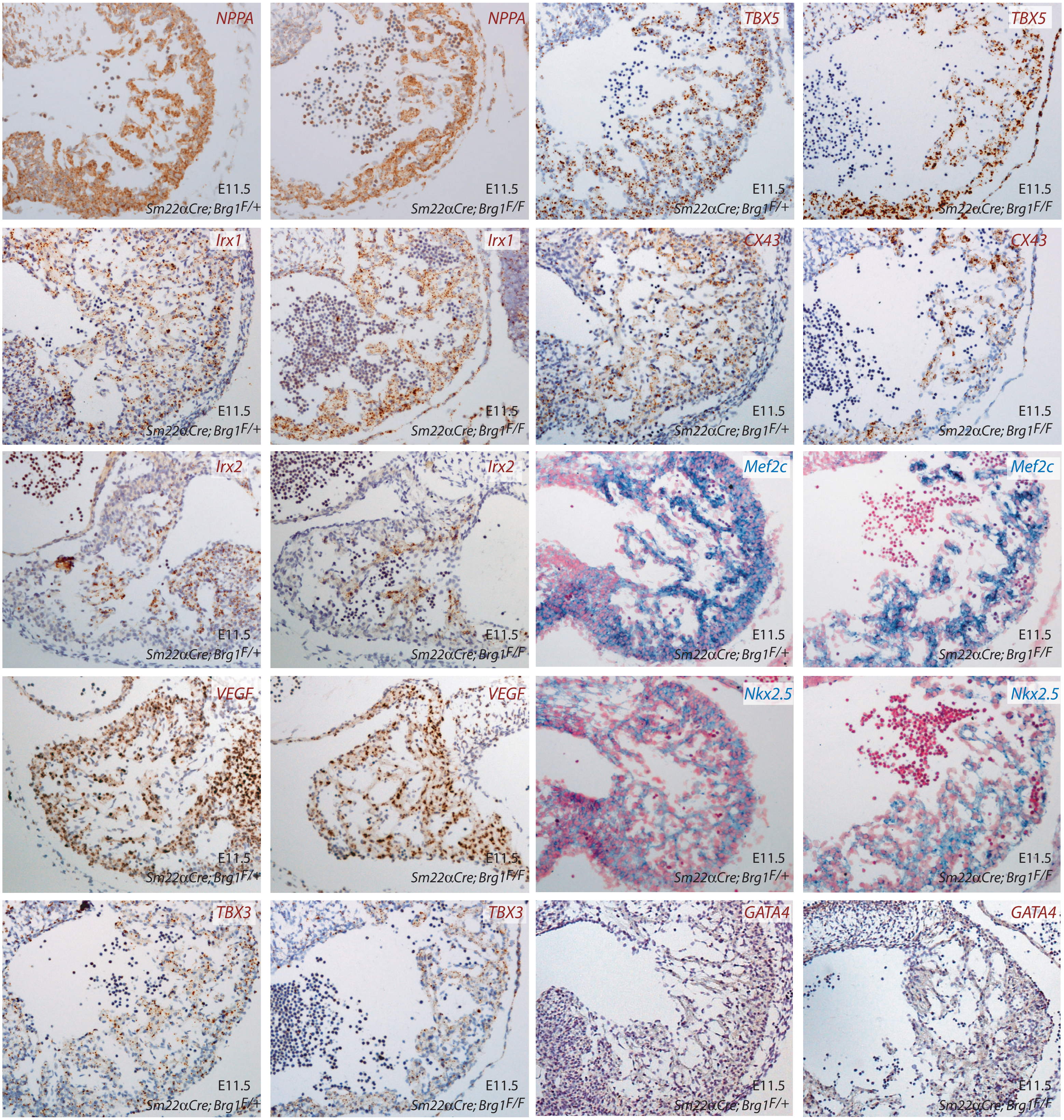
*Sm22αCre; Brg1^F/F^* embryonic myocardium fails to proliferate



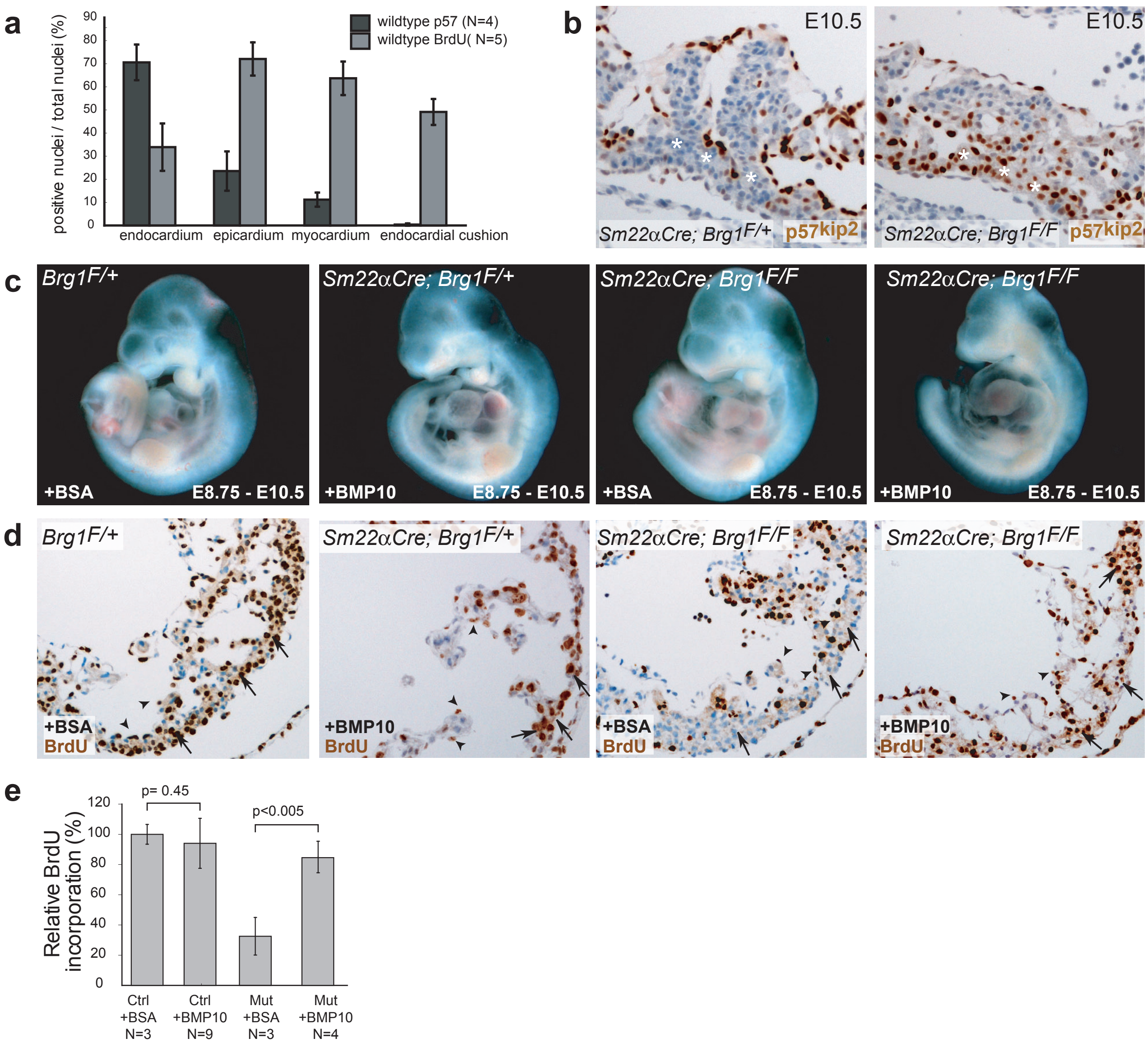
Sm22αCre; Brg1$^{F/F}$ embryos have normal cardiac jelly and vascular pattern at E11.5



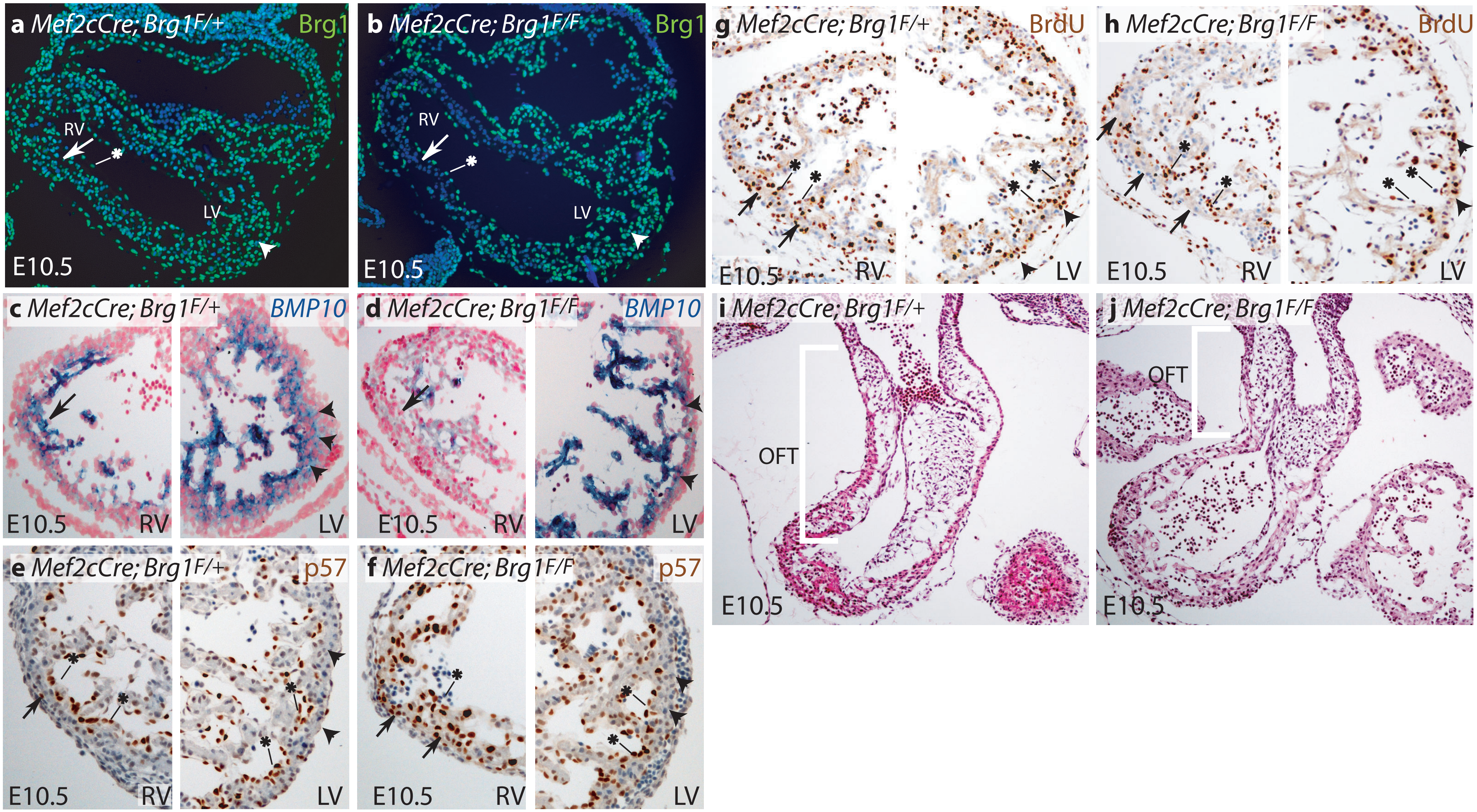
Sm22αCre; Brg1$^{F/F}$ embryos have normal expression of many cardiac genes

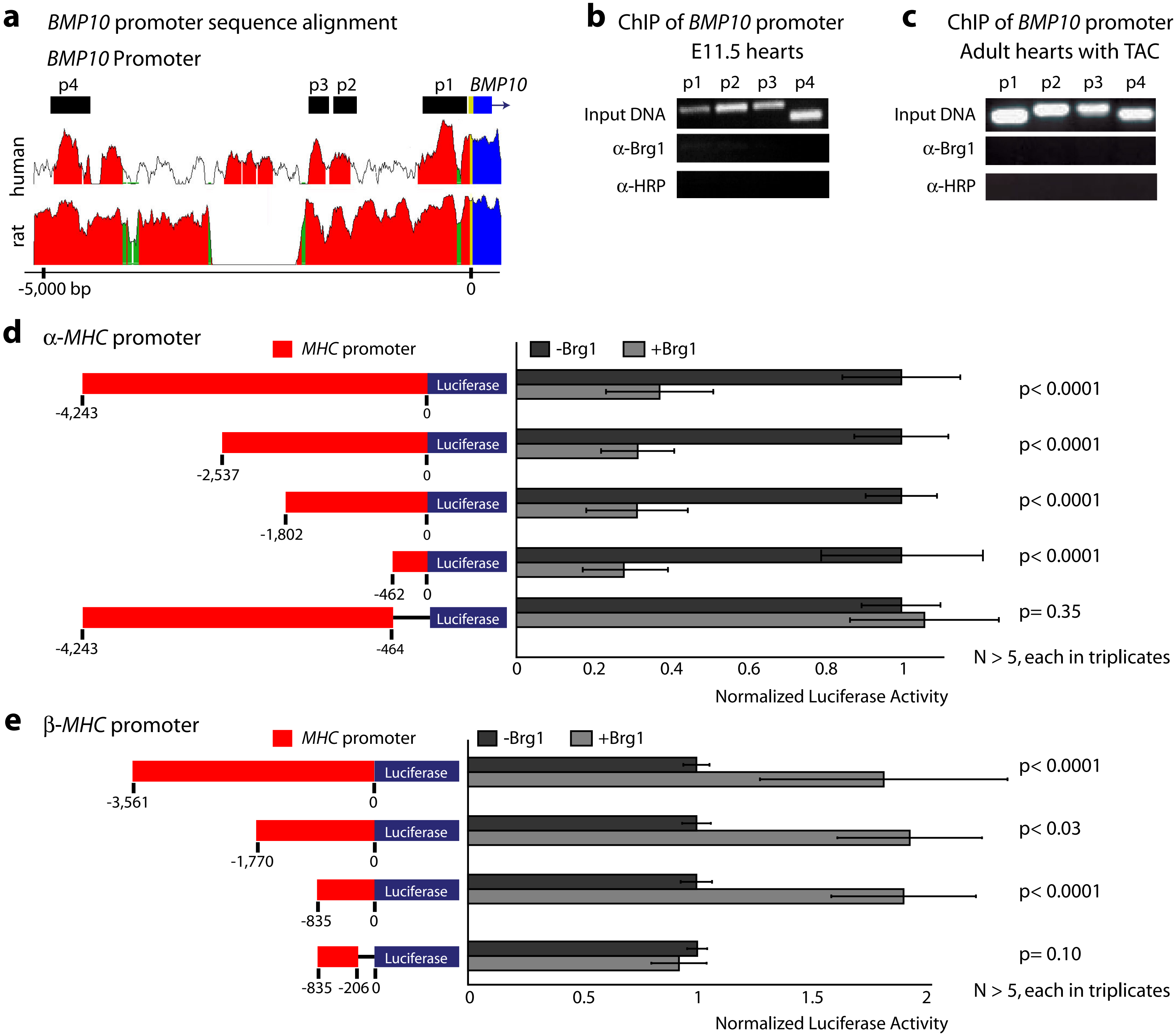


Sm22αCre; Brg1$^{F/F}$ mutants have ectopic expression of p57^{kip2}

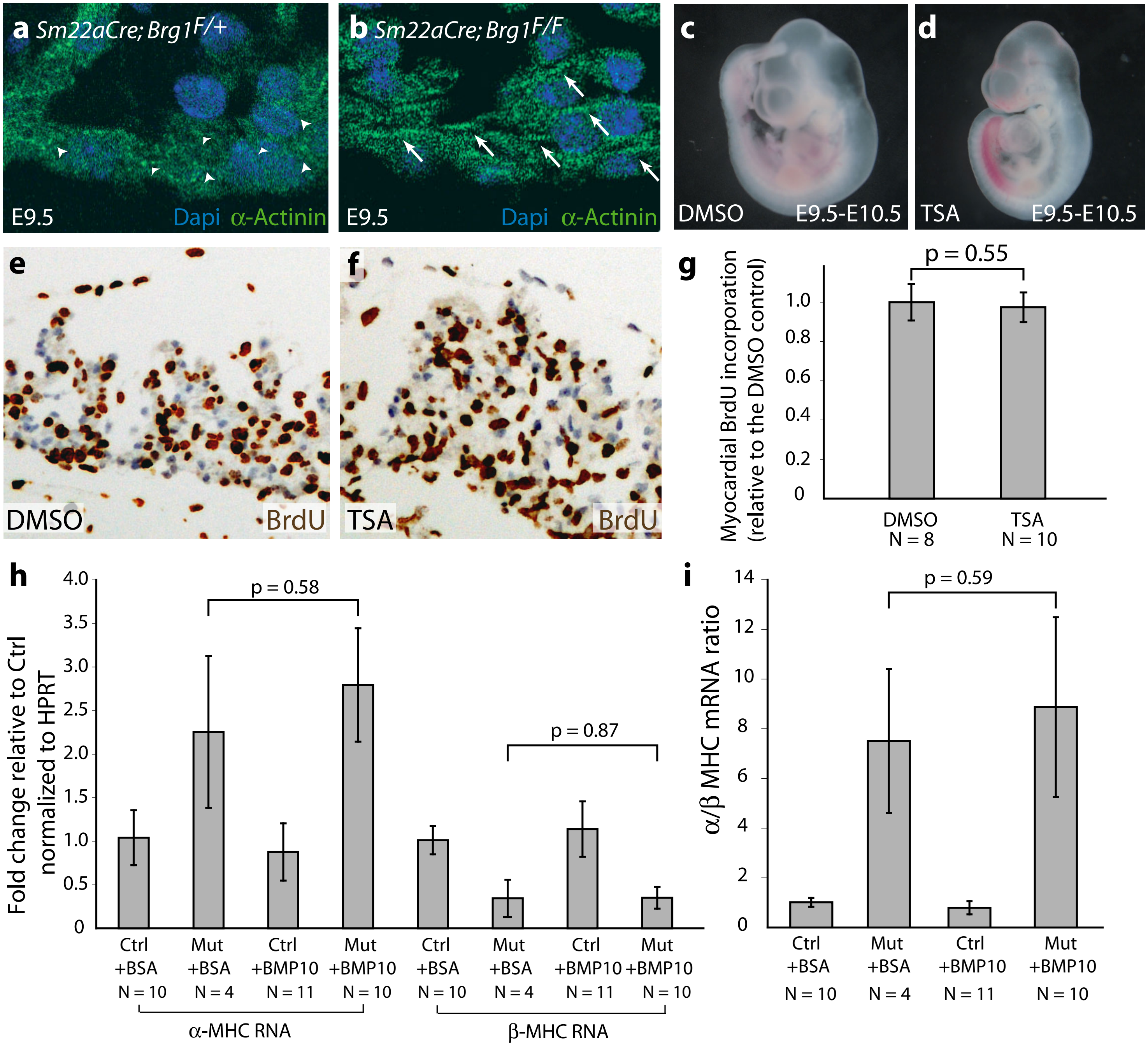


Regulation of BMP10, p57kip2 and cell proliferation by Brg1 in the myocardium

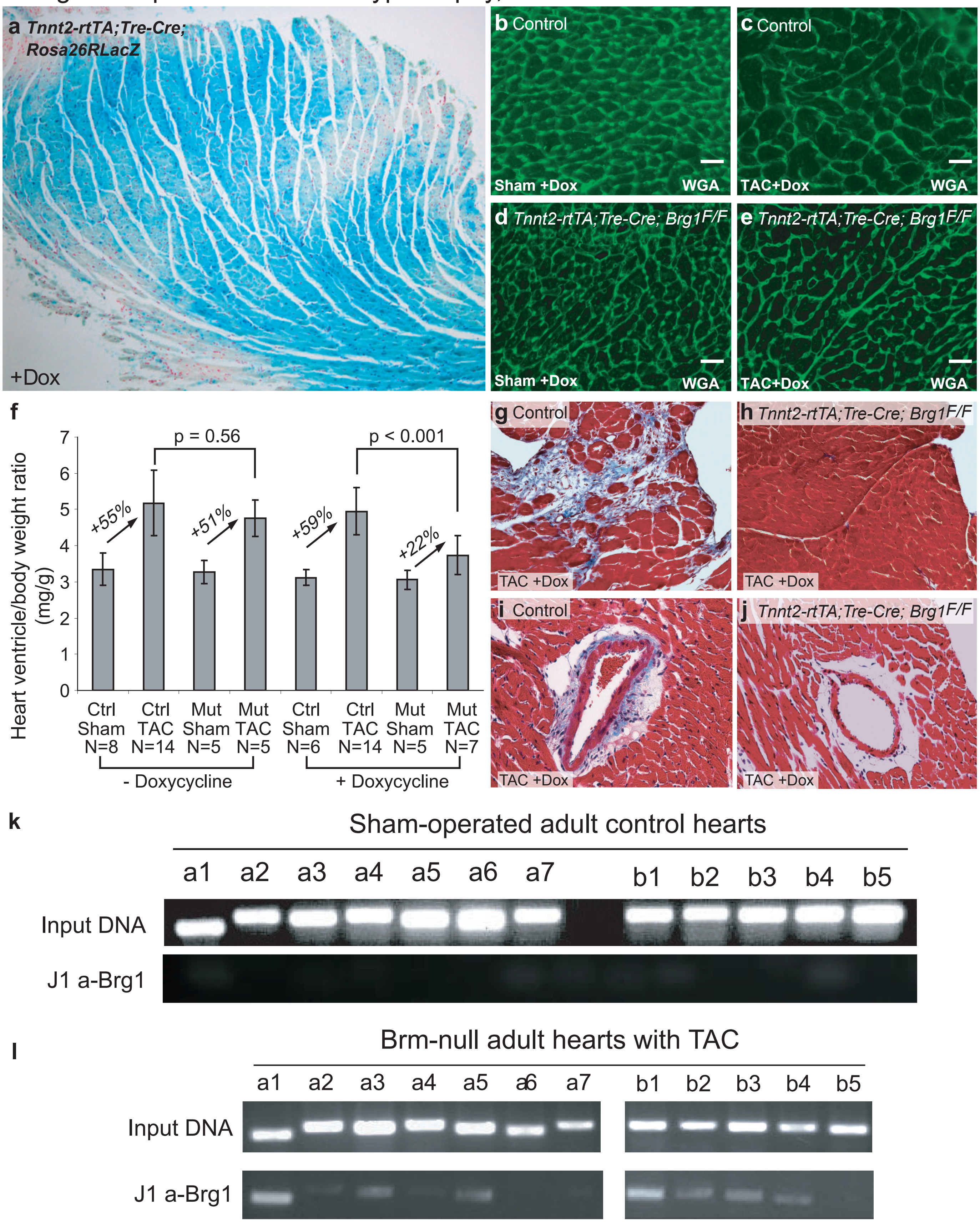



a
BMP10 promoter sequence alignment
BMP10 Promoter
p4
p3 p2
p1 BMP10
human
rat
-5,000 bp
0
b
ChIP of BMP10 promoter
E11.5 hearts
p1 p2 p3 p4
Input DNA
α-Brg1
α-HRP
c
ChIP of BMP10 promoter
Adult hearts with TAC
p1 p2 p3 p4
Input DNA
α-Brg1
α-HRP
d
α-MHC promoter
MHC promoter
-Brg1 +Brg1
Luciferase
-4,243
0
-2,537
-1,802
-462
-464
p< 0.0001
p< 0.0001
p< 0.0001
p< 0.0001
p= 0.35
N > 5, each in triplicates
0 0.2 0.4 0.6 0.8 1
Normalized Luciferase Activity
e
β-MHC promoter
MHC promoter
-Brg1 +Brg1
Luciferase
-3,561
0
-1,770
-835
-206
p< 0.0001
p< 0.03
p< 0.0001
p= 0.10
N > 5, each in triplicates
0 0.5 1 1.5 2
Normalized Luciferase Activity

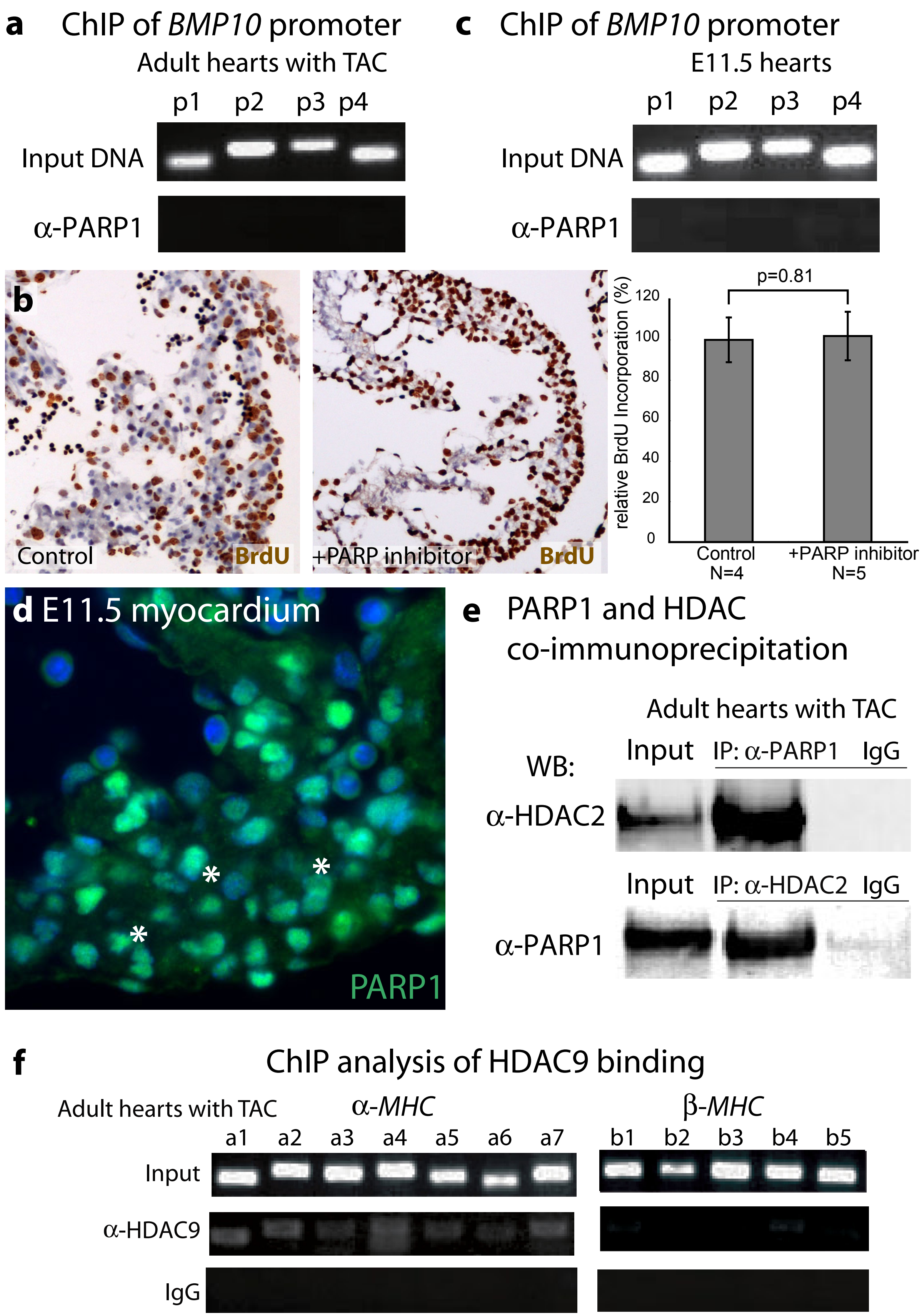
Brg1 commands parallel pathways to regulate myocardial proliferation and differentiation



Brg1 is required for cardiac hypertrophy, fibrosis and MHC switches in adult mice



PARP1 ChIP analysis, expression and co-IP with HDAC



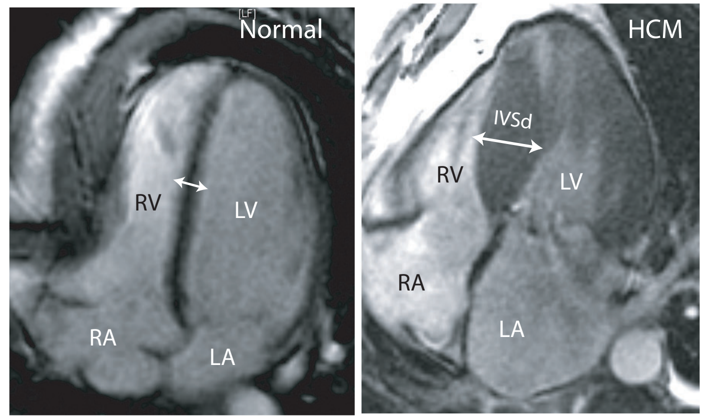
a Control individuals with normal hearts

Age	Gender	Diagnosis	IVSd (cm)
38	M	Normal relative of a HCM patient	0.96
42	F	Normal relative of a HCM patient	0.88
44	M	Normal relative of a HCM patient	0.83
49	M	Normal relative of a HCM patient	1.1
20	M	Normal athlete	1.0
31	M	Heart transplant donor	1.1

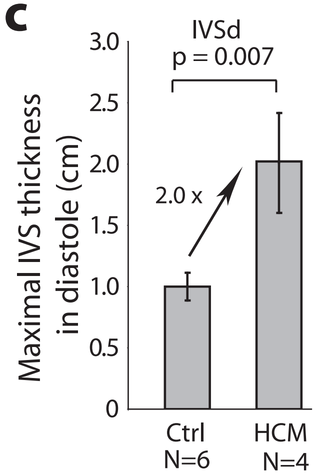
Patients with hypertrophic cardiomyopathy (HCM)

Age	Gender	Diagnosis	Tissue Source	IVSd (cm)
38	F	HCM	Surgical myectomy	2.5
48	M	HCM	Surgical myectomy	2.1
39	M	HCM	Heart transplantation	1.7
35	M	HCM	Surgical myectomy	1.6

b Cardiac MRI



c



d Derivation of the inflection point for Fig. 5b

$y = a + b*[1+e^{(c-dx)}]^{-1}$, $a = 0.94, b=1.2, c=30, d=20$

$$\frac{dy}{dx} = bd*[e^{(c-dx)}]*[1+e^{(c-dx)}]^{-2}$$

$$\frac{d}{dx}\left(\frac{dy}{dx}\right) = (bd^2)*[e^{(c-dx)}]*\{[1+e^{(c-dx)}]^{-2}\}*\{2*[e^{(c-dx)}]*[1+e^{(c-dx)}]^{-1}-1\}$$

$\because$ Inflection point (x, y) occurs when $\frac{d}{dx}\left(\frac{dy}{dx}\right) = 0$

$\therefore bd^2*[e^{(c-dx)}]*\{[1+e^{(c-dx)}]^{-2}\}*\{2*[e^{(c-dx)}]*[1+e^{(c-dx)}]^{-1}-1\} = 0$ for inflection at (x, y)

Since $e^{(c-dx)}$, $[1+e^{(c-dx)}] > 0$,

$\therefore 2*[e^{(c-dx)}]*[1+e^{(c-dx)}]^{-1}-1=0$

$\therefore e^{(c-dx)}=1 \quad \therefore c-dx=0$

$\therefore x = c/d = 30/20 = 1.50$, $y = a + b*(1+e^0)^{-1} = 0.94+1.2/2=1.54$

$\therefore$ Inflection point (x, y) = (1.50, 1.54)

e Derivation of the inflection point for Fig. 5c

$y = a*e^{-e^{(b-cx)}}$, $a=150, b=160, c=110$

$$\frac{dy}{dx} = ac*\left[e^{-e^{(b-cx)}}\right]*\left[e^{(b-cx)}\right]$$

$$\frac{d}{dx}\left(\frac{dy}{dx}\right) = ac^2*\left[e^{-e^{(b-cx)}}\right]*\left[e^{(b-cx)}\right]*\left[1-e^{(b-cx)}\right]$$

$\because$ Inflection point (x, y) occurs when $\frac{d}{dx}\left(\frac{dy}{dx}\right) = 0$

$\therefore ac^2*\left[e^{-e^{(b-cx)}}\right]*\left[e^{(b-cx)}\right]*\left[1-e^{(b-cx)}\right] = 0$ for inflection point (x, y)

Since $e^{-e^{(b-cx)}}>0$ and $e^{(b-cx)}>0$,

$\therefore 1-e^{(b-cx)} = 0$

$\therefore x = b/c = 160/110 = 1.45$

$\therefore y = a*e^{-e^0} = 150*e^{-1} = 55.2$

$\therefore$ Inflection point (x, y) = (1.45, 55.2)