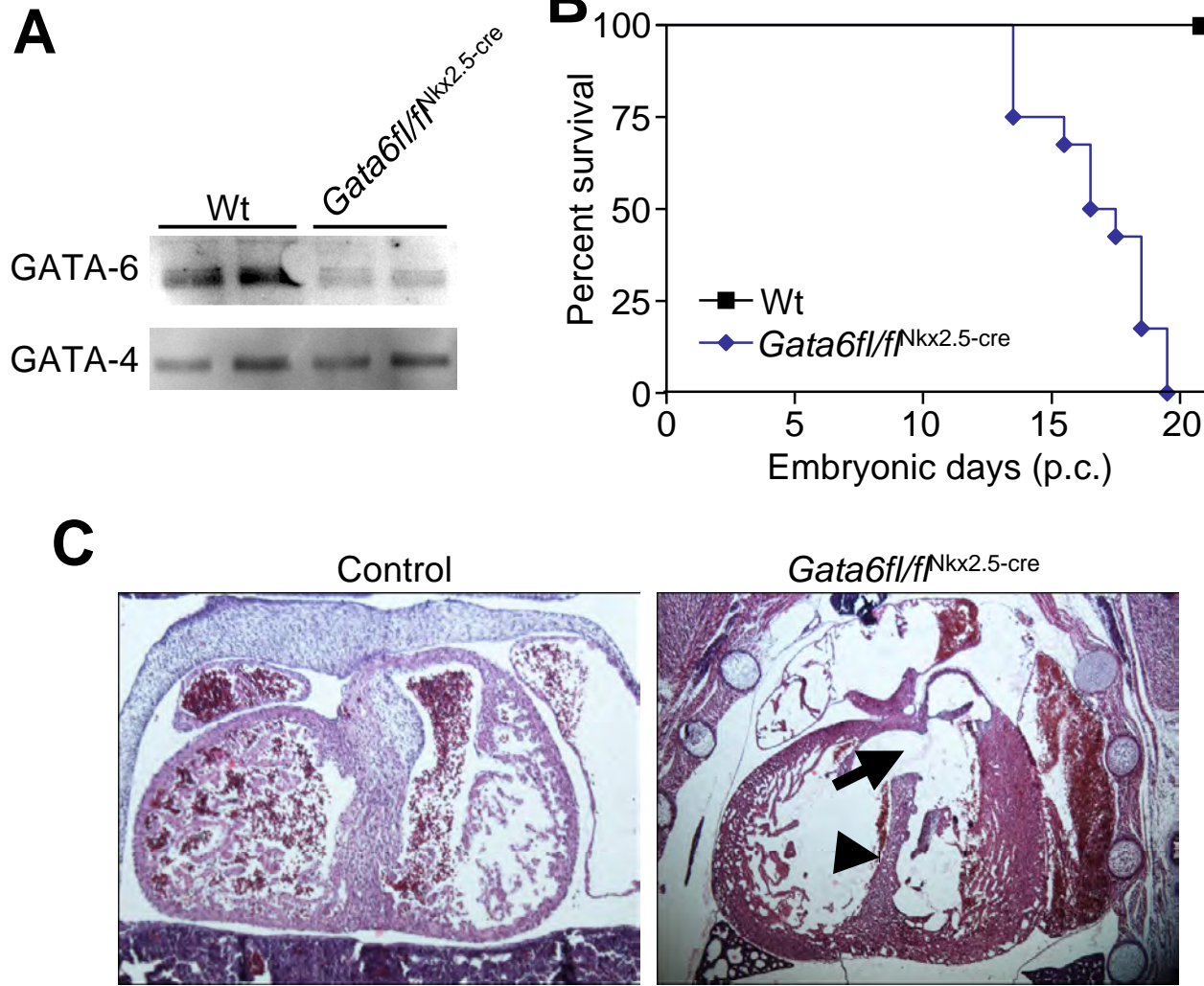
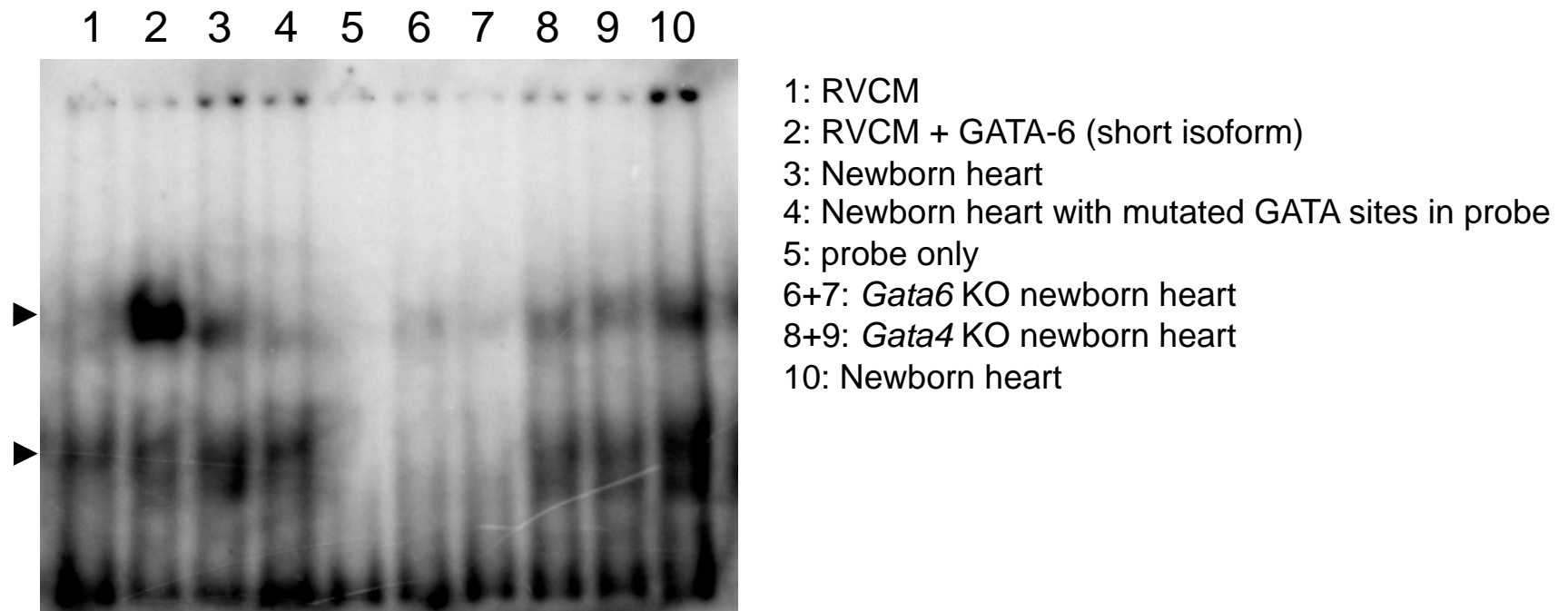


## Supplemental Figure I



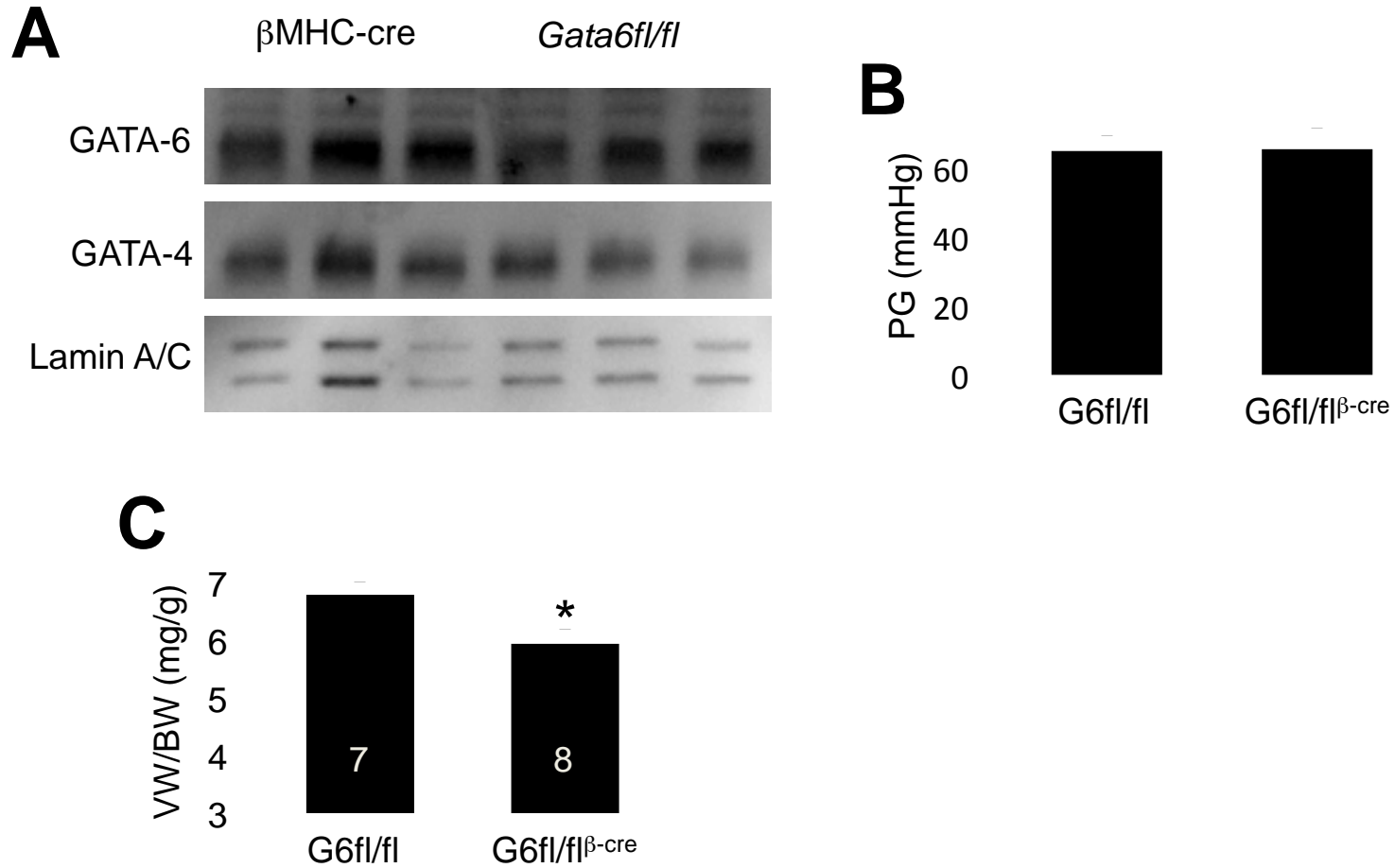
**Supplemental Figure I.** Developmental phenotype of *Gata6fl/fl<sup>Nkx2.5-cre</sup>* mice. A. Western blot for GATA-6 and GATA-4 from cardiac protein lysates from embryos at day 14.5. B. Survival curve of wildtype and *Gata6*-deleted embryos. Time is indicated in embryonic developmental day after conception. C. Immunohistochemistry of control and *Gata6fl/fl<sup>Nkx2.5-cre</sup>* embryos at E16.5 showing ventricular septum defect (arrow) and thinned (arrowhead) septum in *Gata6*-deleted embryos.

## Supplemental Figure II



**Supplemental Figure II.** Gel shift for total GATA DNA binding activity in the adult heart of *Gata6* and *Gata4* heart-specific KO mice. The data show that loss of GATA-6 from the heart reduces total GATA DNA binding activity better than loss of GATA-4. Control samples are from newborn hearts and rat ventricular myocytes with and without infection with AdGATA-6 adenovirus as a migration marker of GATA-6 on the gel shift. A probe only negative control is also shown where the GATA binding sites are mutated. Arrows indicate specific GATA binding activity.

### Supplemental Figure III



**Supplemental Figure III.** A, Western blot for GATA-6 and GATA-4 of nuclear extracts from  $\beta$ MHC-Cre and *Gata6fl/fl* shows equal GATA-6 expression (i.e. the loxP allele is not hypomorphic). B, Doppler echocardiography 1 day after TAC surgery to measure pressure gradients across the constriction shows equal gradients between *Gata6fl/fl* and *Gata6fl/fl* $\beta$ <sup>MHC-Cre</sup>. C, 1 week after induction of pressure overload in the same mice shown in B, *Gata6fl/fl* mice again show significantly higher ventricular/body weight ratio's than *Gata6fl/fl* $\beta$ <sup>MHC-Cre</sup> mice (\* p<0.05).

## Supplemental Tables

**Supplemental table I.** Assessment of cardiac dimensions and function of 1 year-old mice of indicated genotypes, measured by M-mode echocardiography.

Genotype	Number of mice	IVS (mm)	LVPW (mm)	LVED (mm)	LVES (mm)	FS (%)
<b>β-Cre</b>	13	0.94±0.03	1.00±0.02	4.08±0.13	2.76±0.15	32.7±1.7
<b>G6fl/fl</b>	17	0.95±0.03	1.01±0.02	3.93±0.09	2.63±0.08	33.3±0.8
<b>G6fl/flβ-Cre</b>	8	0.93±0.05	0.96±0.03	4.21±0.12	2.86±0.13	32.2±1.5

Abbreviations: IVS, interventricular septum; LVPW, left ventricular posterior wall; LVED, left ventricular end diastolic dimension; LVES, left ventricular end systolic dimension; FS, fractional shortening.

**Supplemental table II.** Assessment of cardiac dimensions and function up to 6 weeks after TAC, measured by M-Mode echocardiography, \*p<0.05 G6fl/flβ-Cre vs β-Cre at the same time point, †p<0.05 G6fl/flβ-Cre vs G6fl/fl, ‡p<0.05 G6fl/fl vs β-Cre.

Genotype	Week	IVS (mm)	LVPW (mm)	LVED (mm)	LVES (mm)	FS (%)
<b>β-Cre</b> (N=16)	0	0.79±0.02	0.92±0.02	4.02±0.08	2.62±0.07	33.1±0.7
	2	1.14±0.04	1.28±0.04	3.41±0.10	2.26±0.08	33.9±0.9
	4	1.15±0.05	1.22±0.04	3.78±0.08	2.67±0.08	29.4±1.3
	6	1.18±0.05	1.25±0.04	3.81±0.10	2.72±0.08	28.5±1.0
<b>G6fl/fl</b> (N=9)	0	0.83±0.04	0.96±0.05	3.55±0.08‡	2.25±0.07	37.3±1.0
	2	1.01±0.06	1.12±0.06‡	3.64±0.13	2.49±0.08	31.8±1.7
	4	0.98±0.05‡	1.19±0.06	3.86±0.13	2.78±0.14	28.2±1.2
	6	1.01±0.07‡	1.21±0.06	3.90±0.14	2.87±0.11	26.3±1.5
<b>G6fl/flβ-Cre</b> (N=13)	0	0.73±0.02	0.97±0.03	4.04±0.08†	2.68±0.08	33.5±0.8
	2	0.86±0.03*	1.07±0.04*	3.86±0.13*	2.91±0.16*	25.3±1.7*†
	4	0.87±0.04*	1.01±0.04*†	4.26±0.16*	3.20±0.31*	19.8±1.1*†
	6	0.85±0.05*	1.04±0.04*†	4.37±0.18*	3.54±0.19*†	19.4±1.4*†

Abbreviations: IVS, intraventricular septum; LVPW, left ventricular posterior wall; LVED, left ventricular end diastolic dimension; LVES, left ventricular end systolic dimension; FS, fractional shortening.

**Supplemental table III.** Assessment of cardiac dimensions and function of control and GATA-6 overexpressing mice after Sham and TAC surgery, measured by M-mode echocardiography.

<b>Genotype</b>	<b>treatment</b>	<b>Number of mice</b>	<b>IVS (mm)</b>	<b>LVPW (mm)</b>	<b>LVED (mm)</b>	<b>LVES (mm)</b>	<b>FS (%)</b>
<b>TTA</b>	Sham	11	0.85±0.03	0.92±0.03	3.63±0.09	2.30±0.10	36.9±1.5
	TAC	14	1.06±0.03	1.07±0.04	3.50±0.05	2.22±0.06	36.6±1.1
<b>Low</b>	Sham	8	0.89±0.02	0.97±0.03	3.69±0.07	1.97±0.23	40.0±0.9
	TAC	9	1.13±0.04	1.07±0.03	3.45±0.07	2.13±0.08	38.4±1.2
<b>Medium</b>	Sham	6	0.90±0.04	0.97±0.05	3.53±0.22	2.24±0.12	36.5±2.1
	TAC	13	1.05±0.04	1.15±0.04	3.38±0.12	2.16±0.11	36.7±1.4
<b>High</b>	Sham	7	0.92±0.04	1.07±0.05	3.46±0.12	1.92±0.07	44.6±0.8
	TAC	12	1.10±0.04	1.15±0.04	3.57±0.13	2.18±0.13	39.0±1.7

Abbreviations: IVS, intraventricular septum; LVPW, left ventricular posterior wall; LVED, left ventricular end diastolic dimension; LVES, left ventricular end systolic dimension; FS, fractional shortening.