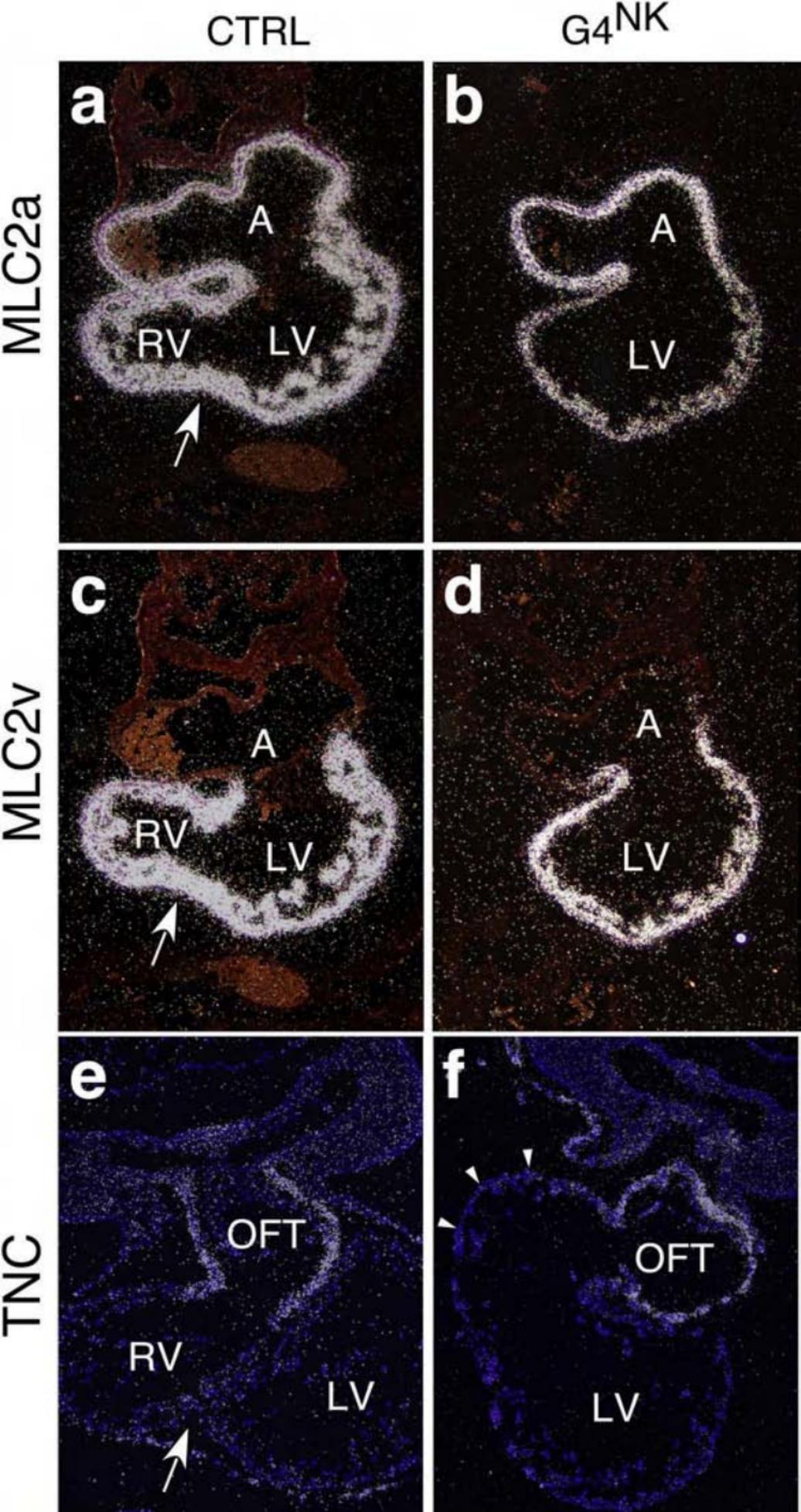
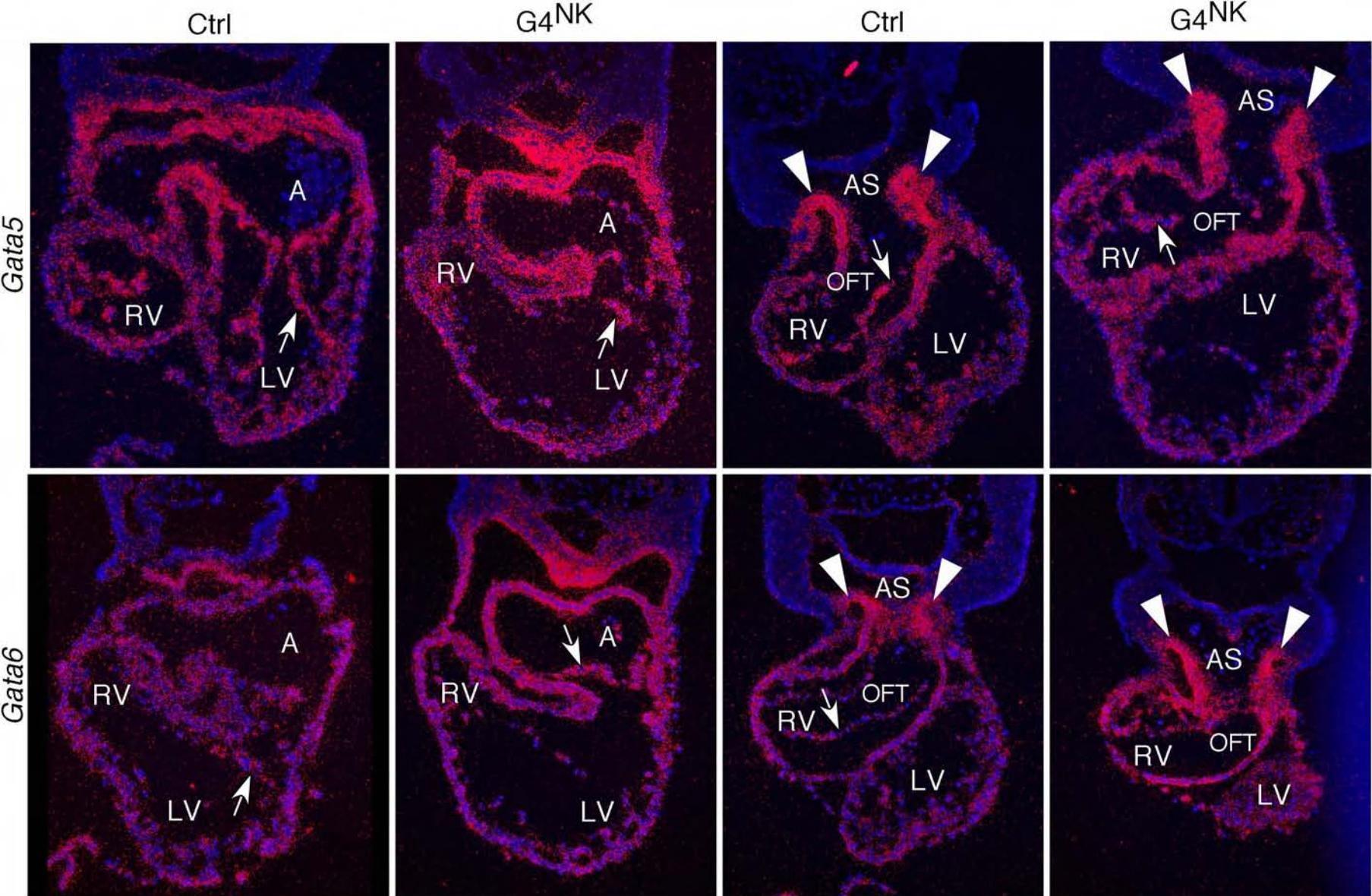


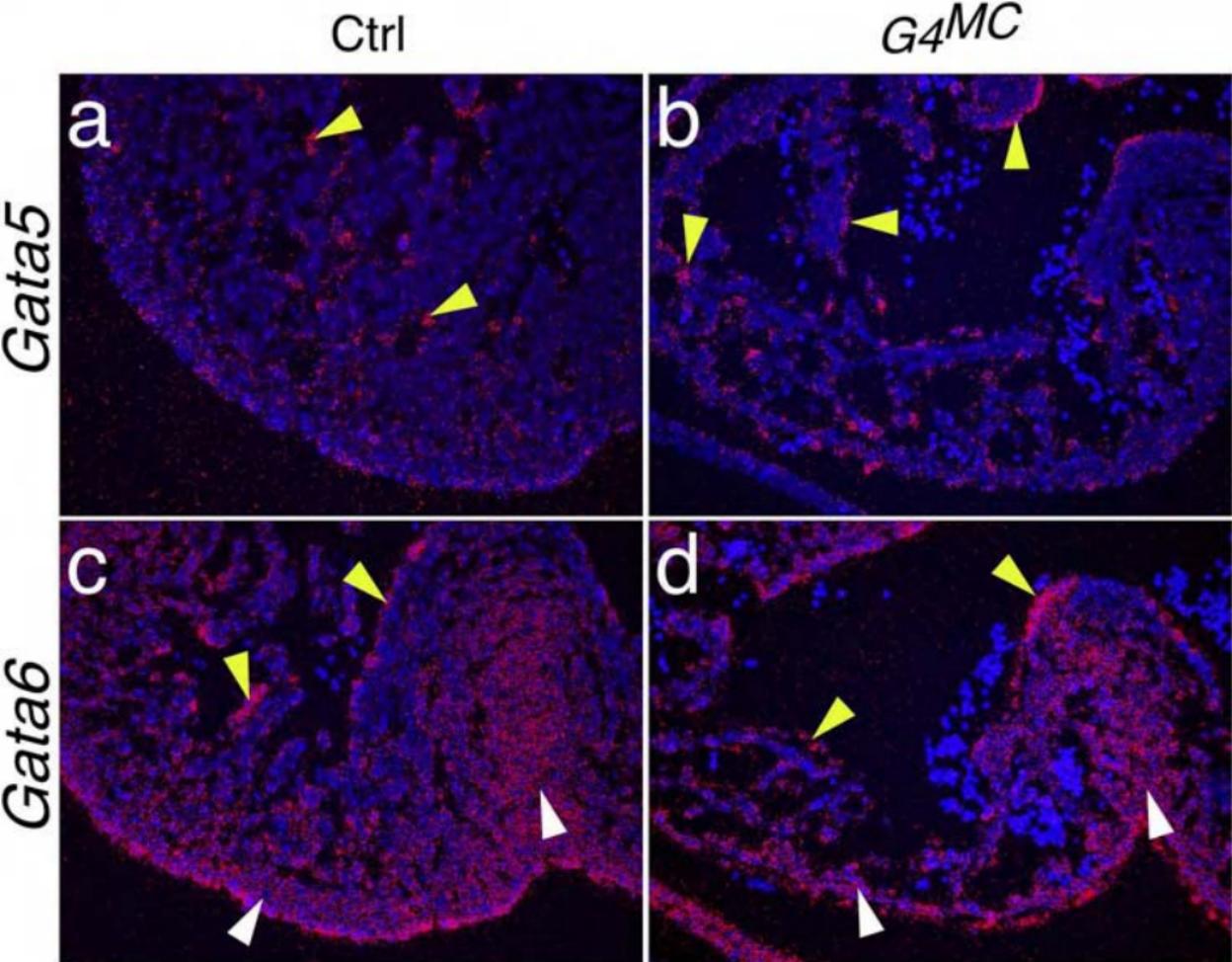
Supplementary Figure 1



Supplementary Figure 2.



Supplementary Figure 3.



Supplementary Figure 4.

Supplementary Figure 1

Intact endocardial specification in $G4^{NK}$ mutant embryos. Immunofluorescent detection of Nfatc1 (red) in the endocardium of $G4^{NK}$ and control embryos at E10.0. Note the abundant mesenchymal cells within the control endocardial cushion (white arrowheads), which are absent in mutant endocardial cushion (asterisk). Green, desmin; blue, nuclei. A, atrium; V, ventricle.

Supplementary Figure 2

Expression of Chamber Specific Markers in Control and $G4^{NK}$ Hearts. a-d. Expression of *MLC2a* and *MLC2v* were detected in adjacent sections of control and mutant E9.75 embryos by *in situ* hybridization (signal displayed in white). Arrow indicates early interventricular groove. e-f. Expression of tenascin C (*TNC*, white), a marker of outflow tract myocardium, in E9.75 embryos. In control embryos, *TNC* was expressed in outflow tract myocardium from the confluence of the aortic sac to its junction with the RV. The RV was located to the right of the LV, and an interventricular groove was well defined (arrow). In mutant embryos, a region of myocardium rostral to the LV with a tubular structure was *TNC* negative (arrowheads) and likely represented the residual, hypoplastic RV. Labels as in figure 1.

Supplementary Figure 3

Expression of *Gata5* and *Gata6* in control and $G4^{NK}$ embryos. *Gata5* and *Gata6* expression was measured by *in situ* hybridization (red) on sections of E9.5 embryos (all 22 somites). Nuclei were detected by DAPI staining (blue). Both genes are expressed in myocardium, endocardium (arrows), and splanchnic mesoderm (arrowheads). Myocardial *Gata4* inactivation did not alter expression of *Gata5* or *Gata6*. AS, aortic sac, OFT, outflow tract. Other labels as in figure 1.

Supplementary Figure 4

Expression of *Gata5* and *Gata6* in control and $G4^{MC}$ E12.5 embryos. *Gata5* (a,b) and *Gata6* (c,d) expression were measured by *in situ* hybridization (displayed as red pseudocolor). At this stage, cardiac *Gata5* was limited to the endocardium (yellow arrowheads), while *Gata6* was expressed in the myocardium (white arrowheads), and endocardium. Myocardial *Gata4* deletion by MHC α Cre did not alter the pattern of *Gata5* (b) or *Gata6* (d) expression. Blue, DAPI.

Supplemental Table 1

	Forward	Reverse	Probe
cyclin D1	TCCGCAAGCATGCACAGA	GGTGGGTTGGAAATGAACTTCA	CTTTGTGGCCCTCTGTGCCACAGA
cyclin D2	GCTCTGTGCGCTACCGACTT	CACGCTTCCAGTTGCAATCA	AAGTTTGCCATGTACCCGCCATCG
cyclin D3	TGATTGCGCACGACTTCCT	CAAAGCCTGCCGGTCACT	TGATTCTGCACCCGCTGTCTCTGC
GATA6	agatgaatggcctcagcagg	caagccgctgatgaa	ctcatcaagccacagaagcgcgtg
GATA4	ccctggaagacacccaat	tggacatgccccacaat	tcgatatgtttgatgacttctcagaaggcagag
MEF2C	tcaagtacaccgagtacaacgagc	tctttctgttcaatgcctcca	cacgagagccggacaaactcagacatt
Nkx2-5	tgaccagccaaagaccct	ccatccgtctcggtttgt	aaagagctgtgctgctgcaga
ANF	ggccatattggagcaaatcctgtg	catgacctcatcttaccggcat	agtgcggtgtccaacacagatctgat
HOP	ggtggagatcctggagtacaactt	caggcgtgcttaaccatttctg	aacaaggtaacaagcaccggacc
CARP	ctcaatgccaaggacagagaagga	caccgaaggtcatcaagagtcgaa	atgatgctgtgaggctgaaccgctat
BMP10	cctcctgggtatgaagcc	attcttgaggtgaccaa	tggcggagcactcacac
18S	from Applied Biosystems		
BNP	tgggaagtccatgaccagtctc	ctgtctctgggccatttctc	sybr green
MHCa	ccactgtggtgcctcgctt	ggtccgtcattctgtcactc	sybr green
MHCb	aagggcctgaatgaggagtagatc	tgcaaaggctccaggctctga	sybr green
GAPDH	tgaccaccaactgcttagc	ggcatggactgtggtcatgag	sybr green