SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure 1. Absence of cardiac developmental abnormalities in mice deleted for GSK-3α. H and E-stained sections of P0 neonatal hearts from *gsk*-3$\alpha^{(-/-)}$ and wild-type neonates demonstrating normal heart development.

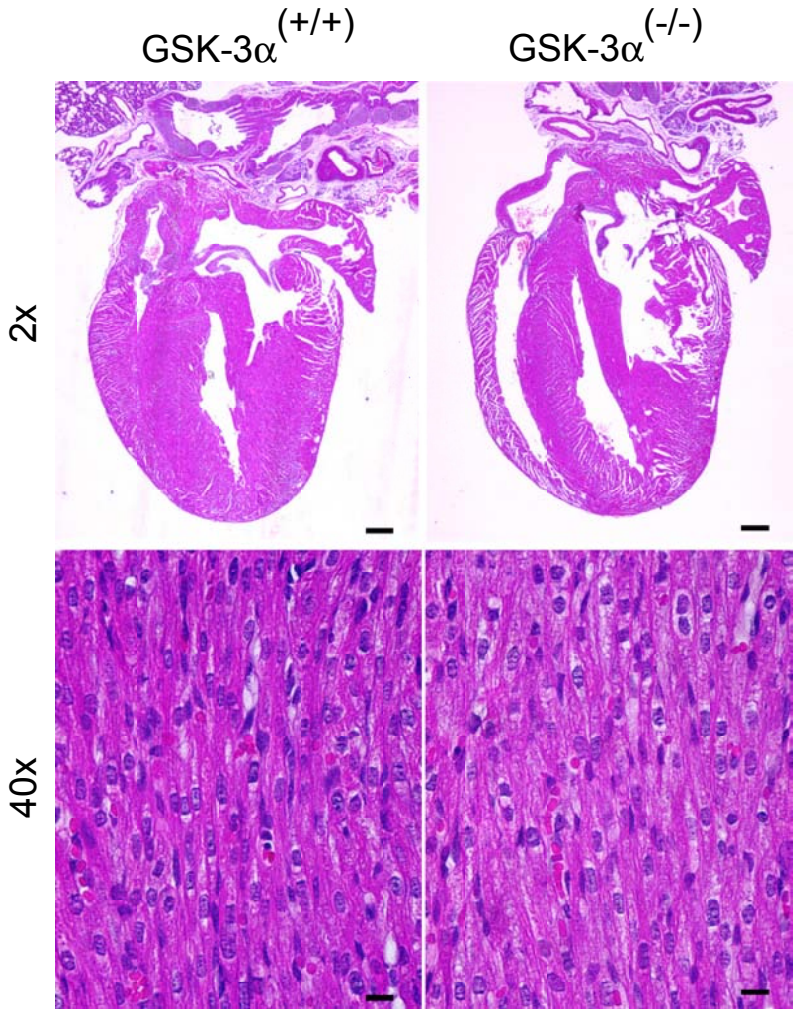
Supplemental Figure 2. Hematoxylin and eosin (HE) stained frontal sections through the developing aortic arch arteries (top two rows) and heart (bottom two rows) of wild type (WT) and *gsk*-3$\beta^{(-/-)}$ (KO) embryos are shown. Adjacent sections are stained for alpha smooth muscle actin (α-SMA) by immunohistochemistry (green) and for Sema3C and PlexinA2 by in situ hybridization. SMA staining is normal in KO embryos, indicating appropriate differentiation of neural crest into vascular smooth muscle surrounding the aortic arch arteries. Sema3C and PlexinA2 expression is also normal, indicating appropriate patterning of cardiac neural crest. a, atrium. v, ventricle. Asterisk (*) indicates the outflow tract of the heart

Supplemental Figure 3. Cardiomyoycte size in GSK-3β-deficient heart. (*A*) Sections of E17.5 wild-type and *gsk*-3$\beta^{(-/-)}$ hearts were stained with FITC-conjugated wheat germ agglutinin in order to quantify cardiomyocyte cross-sectional area. (*B*) Analysis of cardiomyocyte size in GSK-3β-deficient vs. wild-type hearts. Shown is the composite mean ± SE of cardiomyocyte cross-sectional area measured in n = 3 *gsk*-3$\beta^{(-/-)}$ and 4 *gsk*-3$\beta^{(+/+)}$ E15.5 embryos, with at least 10 sections per heart quantified. ** $p<0.01$ vs wild-type. (*C*) Analysis of glycogen storage in hearts of GSK-3β-deficient vs wild type mice. Sequential sections were stained with PAS with (+) or without (-) prior glycase treatment, which digests glycogen. Tissue staining following glycogen digestion is shown (left panels). Selective magenta colored cellular deposits in non-digested samples (right panels) is consistent with glycogen. PAS staining in the hearts was comparable between GSK-3β-deficient and wild type mice.

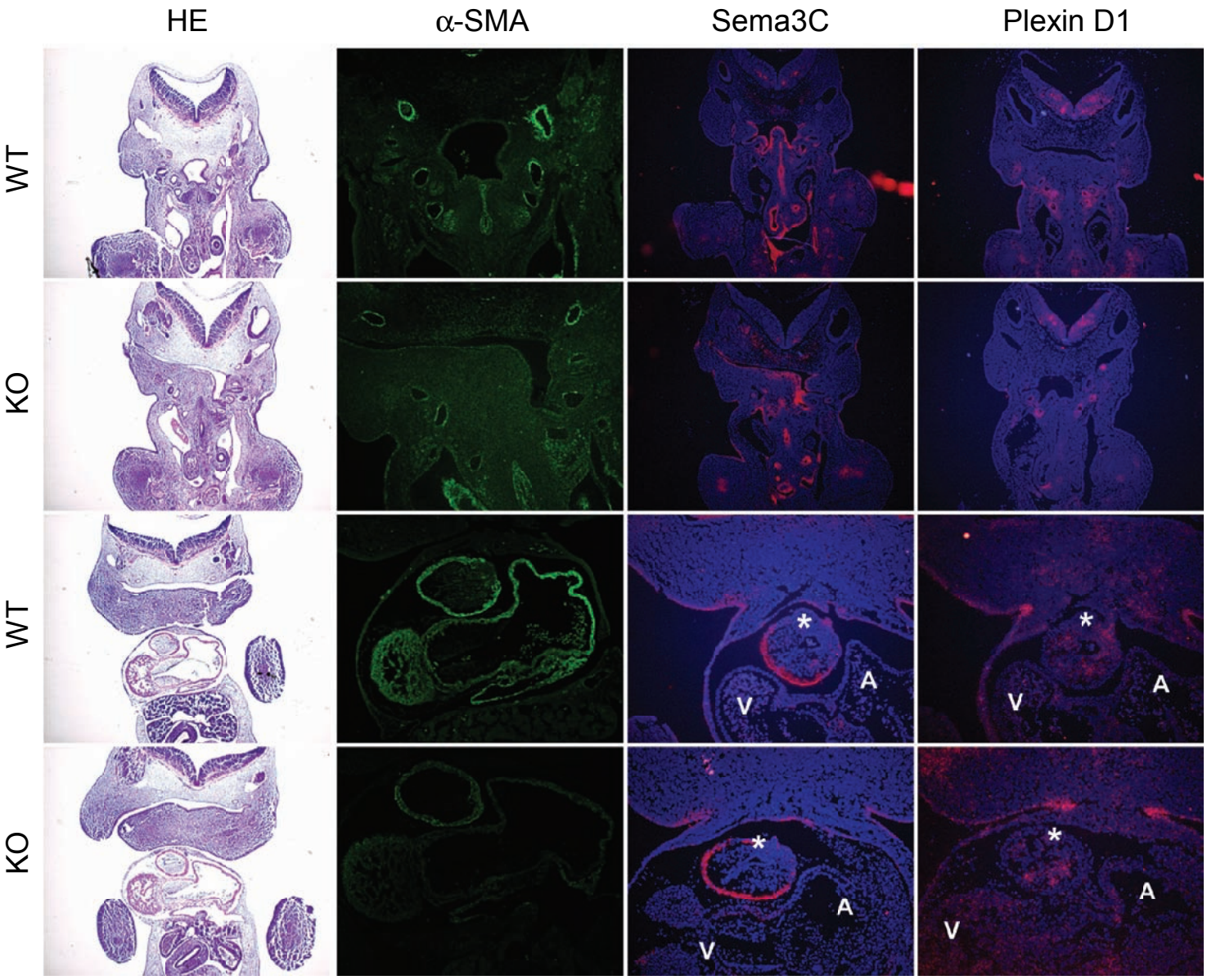
Supplemental Figure 4. Apoptosis in the myocardium. Quantification of TUNEL staining in the left ventricles of wild-type (WT, n=4) and *gsk*-3$\beta^{(-/-)}$ (KO, n=5) mice at E17.5 demonstrating no differences in rates of apoptosis . TUNEL positive cells are expressed as a percent of total cells, based on staining with DAPI.

Supplemental Figure 5. β-catenin expression in the myocardium (A) and heart valve-forming regions (B) in E15.5 mice. In (A), note the intense staining for β-catenin in the cardiomyocyte membrane in both *gsk*-3$\beta^{(+/+)}$ and *gsk*-3$\beta^{(-/-)}$ with no differences between the genotypes. In (B), there is significant staining in the pulmonic valve-forming regions but, again, there are no apparent differences between wild-type and *gsk*-3$\beta^{(-/-)}$ in β-catenin distribution or intensity of staining.

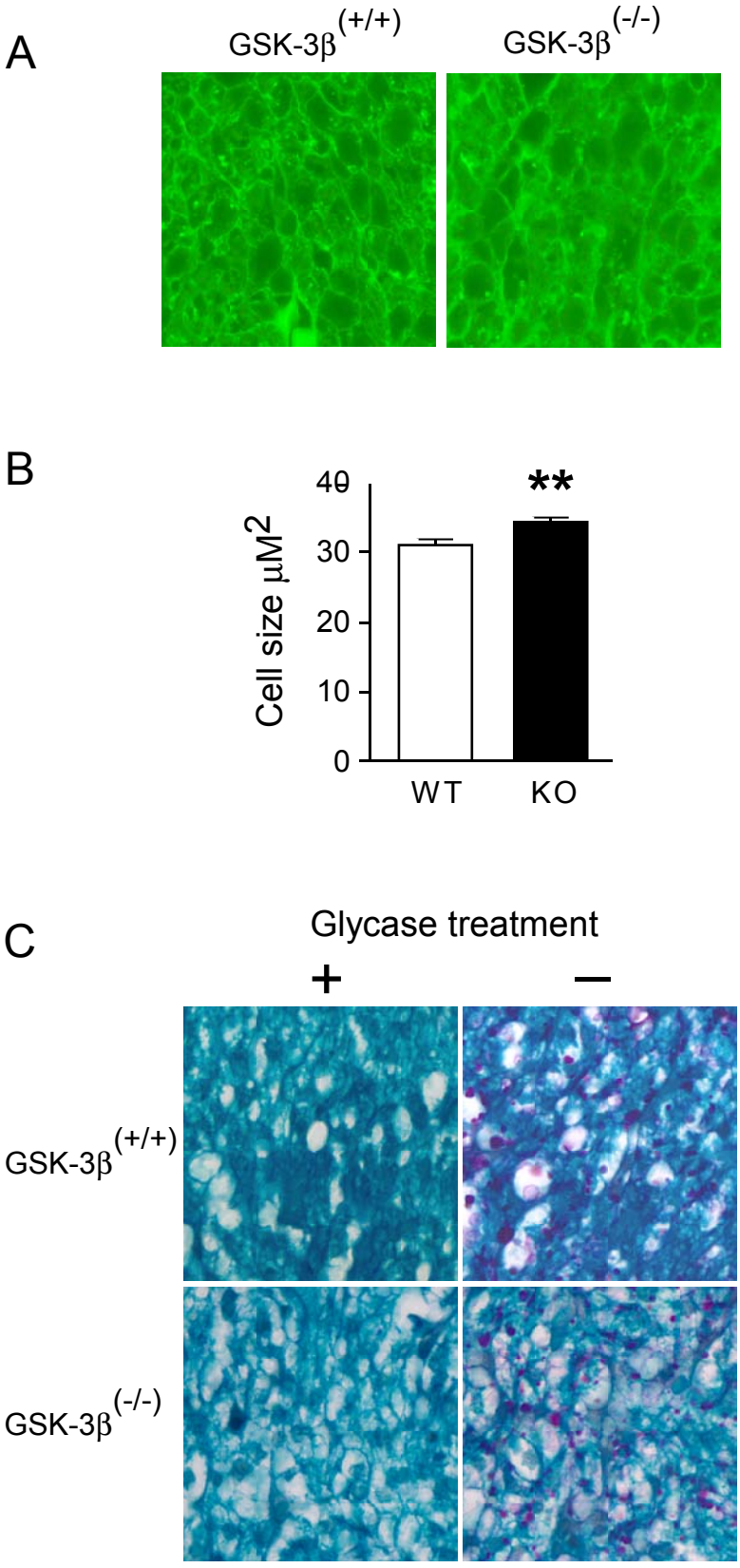
Supplemental Figure 1



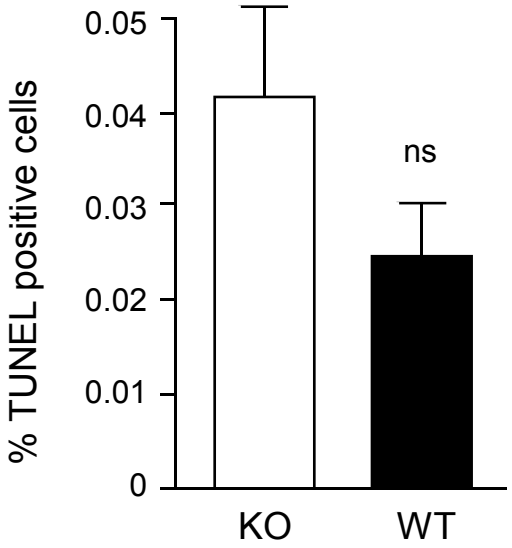
Supplemental Figure 2



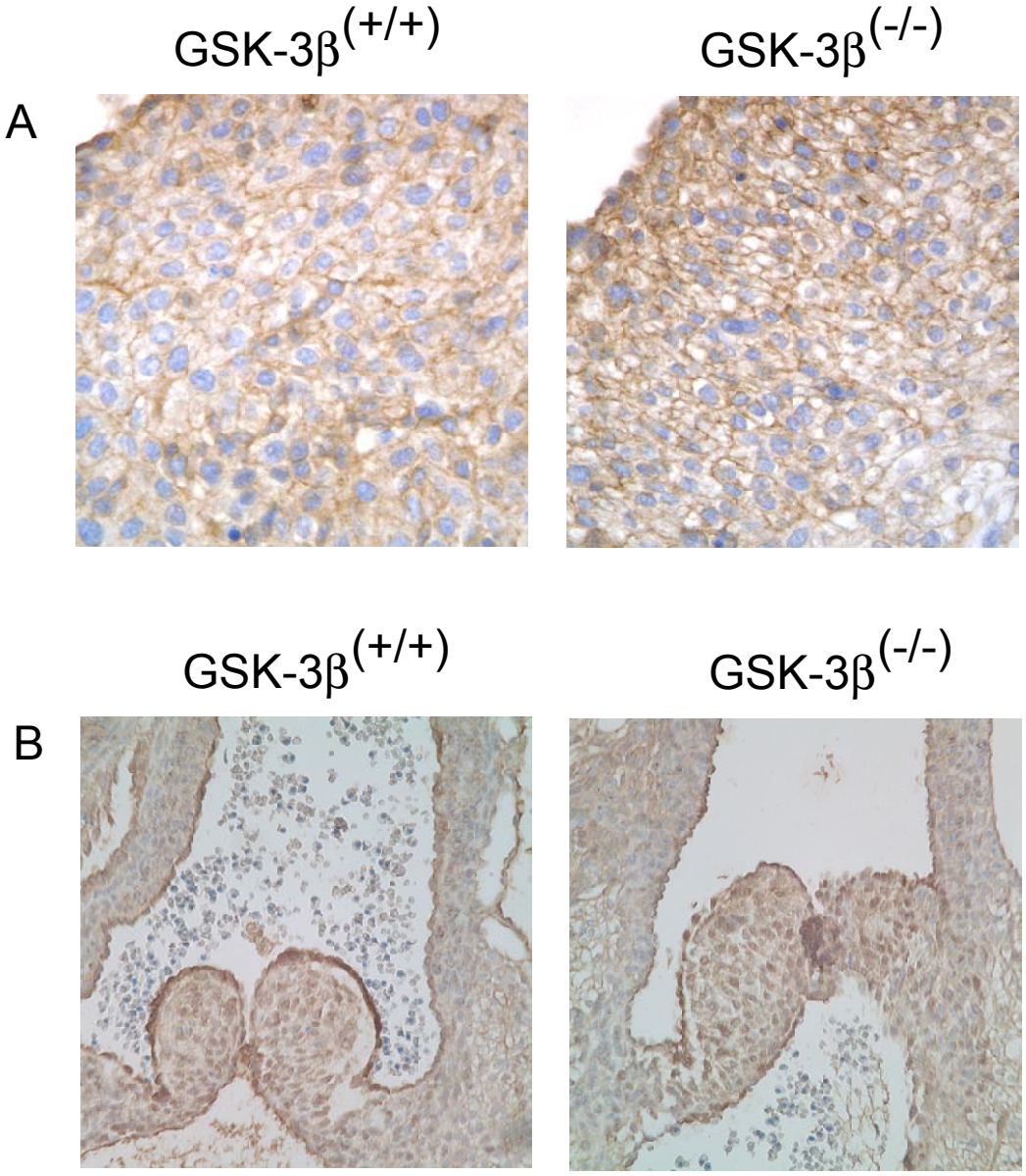
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Methods

TUNEL Staining. For visualization of apoptotic cells by terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL), we used a kit from Chemicon according to the manufacturers instructions. Images were viewed with a Nikon Eclipse 80i microscope. Spot Imaging software was used to record immunofluorescence images.

PAS/Glycase staining methods. Staining for glycogen content of the heart was performed using a kit from Poly Scientific (Bay Shore, NY) according to the manufacturer's instructions. Briefly, sections were deparaffinized in xylenes, hydrated through an alcohol series and washed with water. For glycase treatment, 0.5% diastase in water was added onto the sections for 20 minutes. Following this, the periodic acid Schiff reaction was performed according to the manufacturer's instructions, nuclei were stained with Weigert's iron hematoxylin and fast green solution was used for detection. Finally, the slides were dehydrated, cleared in xylenes, and mounted with Permount (Biomeda, Foster City, CA).

Studies of the neural crest. Embryos for radioactive in situ hybridization were harvested and fixed for 48 hours in 4% paraformaldehyde (PFA) in PBS. Embryos were dehydrated through a graded ethanol series and stored in 100% ethanol at -20°C, then paraffin embedded and sectioned at 10 mm. PlexinA2 probes encompass nucleotides 2121-4330 of the GenBank mouse *PlexinA2* sequence D86949. 35S-labeled sense and antisense riboprobes were synthesized with SP6, T7 or T3 RNA polymerase and 35S-UTP as previously described (1, 2). Hybridization was carried out at 55°C overnight. Successful hybridzation was assessed by overnight exposure of slides to Kodak X-OMAT film. Slides were dipped in Kodak NTB-2 emulsion, exposed for 5-7 days a 4°C, developed and fixed in Kodak Dektol developer and fixer. Cell nuclei were counterstained with Hoechst 33258 (Sigma, St. Louis, MO) and mounted in Canada balsam/methyl salicylate. Sections were digitally photographed on a Zeiss Axioplan 2 microscope.

qRT-PCR Primers

	Forward	Reverse	Reference
Nanog	cctcagcctccagcagatgc	ccgcttgcacttcacccttg	(3)
Brachury	catcggaacagctctccaacctat	gtgggctggcgttatgactca	(3)
GATA-4	aagcccaagaacctgaataaatct	gtctgagtgacaggagatgcatag	
Nkx2.5	caaagaccctcgggcggataaaaag	gacggttctggaaccagatcttgac	
α-MHC	ggacaagctacagttgaaggtgaa	tttattgtgtattggccacagc	
β-MHC	ctggagaagatccgaaagca	gtgtccttcagcaaactctgg	
BNP	agtcctagccagtctccagagcaa	caacttcagtgcgttacagcccaa	
SERCA2	catagagatgtgtaatgccctcaa	aggagattttcagcccatcag	
GAPDH	caactcactcaagattgtcagcaa	ggcatggactgtggtcatga	(4)

References

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3. Willems, E. 2006. RTPrimerDB entry.
4. Rube, C.E., Wilfert, F., Uthe, D., Schmid, K.W., Knoop, R., Willich, N., Schuck, A., and Rube, C. 2002. Modulation of radiation-induced tumour necrosis factor alpha (TNF-alpha) expression in the lung tissue by pentoxifylline. *Radiother Oncol* 64:177-187.