Supporting Information

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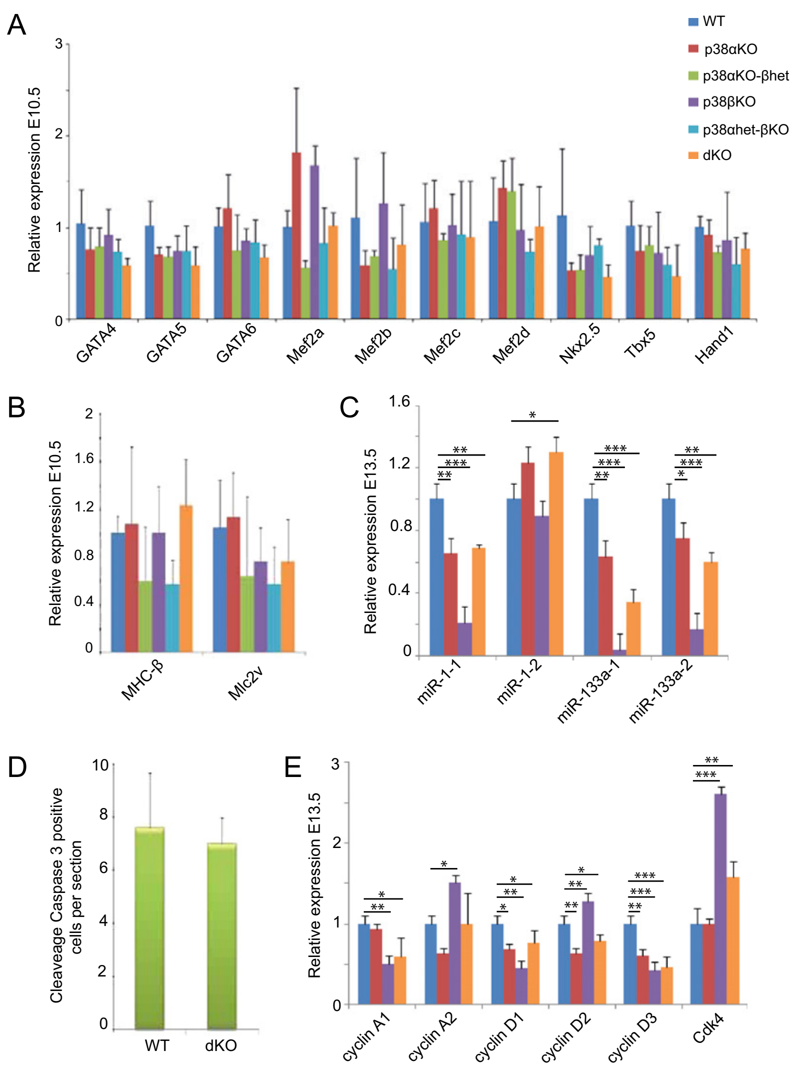

Fig. S1. Modulation of cardiac gene expression and apoptosis. Expression of the indicated genes was analyzed by quantitative RT-PCR in biological triplicates of the following genotypes: wild-type (WT), *p38*$\alpha^{(\Delta/\Delta)}$ *Sox2-Cre* (p38αKO), *p38*$\alpha^{(\Delta/\Delta)}$ *p38*$\beta^{(+/-)}$ *Sox2-Cre* (p38αKO-βhet), *p38*$\beta^{(-/-)}$ (p38βKO), *p38*$\alpha^{(\Delta/+)}$ *p38*$\beta^{(-/-)}$ *Sox2-Cre* (p38αhet-βKO) or *p38*$\alpha^{(\Delta/\Delta)}$*p38*$\beta^{(-/-)}$ *Sox2-Cre* (dKO). RNA was isolated from three individual hearts in E10.5 embryos, and from three pools of three hearts each (nine hearts total) in E13.5 embryos. (*A*) No significant variation was detected in the expression of various cardiac transcription factors in E10.5 hearts. (*B*) Expression of the contractile proteins MHC-β and Mlc2v was not altered in E10.5 hearts. (*C*) The microRNAs miR-1-1, miR-133a-l, and miR-133a-2 were down-regulated in E13.5 p38βKO hearts. (*D*) No differences in apoptosis were detected in E13.5 dKO hearts by cleavage Caspase3 staining. Positive cells were counted in three sections for each genotype. (*E*) Cell-cycle regulators Cyclin A2, Cyclin D2, and Cdk4 were up-regulated in E13.5 p38βKO hearts, whereas dKO hearts showed a general down-regulation of cyclins A and D. MHC-β, myosin heavy chain-β; Mlc2V, myosin light Chain2V; Error bars indicate SD. Statistical significance ($n = 3$) was determined by using one-way ANOVA-Tukey's test. Changes are referred to the expression levels in WT hearts (given the value of 1 for each gene) and indicated as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

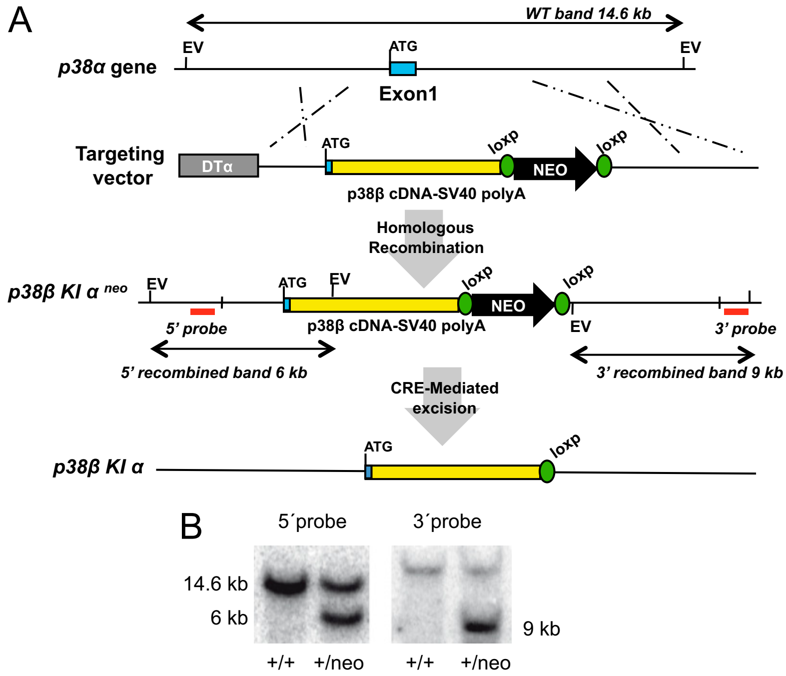

Fig. S2. Generation of the *p38βKIα* allele. (*A*) Strategy for targeting the *p38α* locus. The genomic structure, targeting vector, and targeted allele for *p38βKIα* are shown. The construct consisted of an in-frame-cloned *p38β* cDNA fused to a SV40 polyA sequence followed by a *loxP*-flanked neomycin resistance cassette, a 3.3-kb 5′ homology arm, and a 7.9-kb 3′ homology arm. The A subunit of diphtheria toxin was used as a counter selection marker. Heterozygous *p38α*$^{(\beta KI/+)}$ mice were intercrossed with the CMV-Cre transgenic line to remove the neomycin resistance cassette. (*B*) ES cells were electroporated, and correct recombination events were verified by Southern blot analysis of genomic DNA digested with EcoRV (EV) using external probes. The wild-type band migrates at 14.6 kb, and the mutant band migrates at 6 kb (5′ probe) or 9 kb (3′probe).

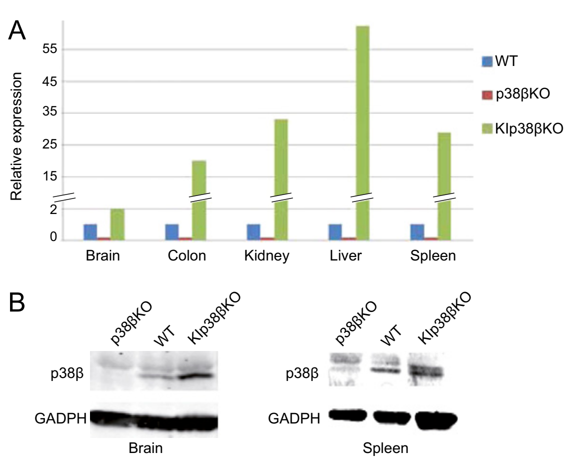

Fig. S3. Expression of p38β under control of the endogenous p38α promoter. (*A*) Quantitative RT-PCR analysis of *p38β* mRNA expression in the indicated adult tissues of mice wild-type (WT), *p38β*$^{(-/-)}$ (p38βKO), and *p38α*$^{(\beta KI/+)}$*p38β*$^{(-/-)}$ (KIp38βKO). (*B*) Immunoblot analysis of p38β protein expression in brain and spleen obtained from the indicated adult mice.

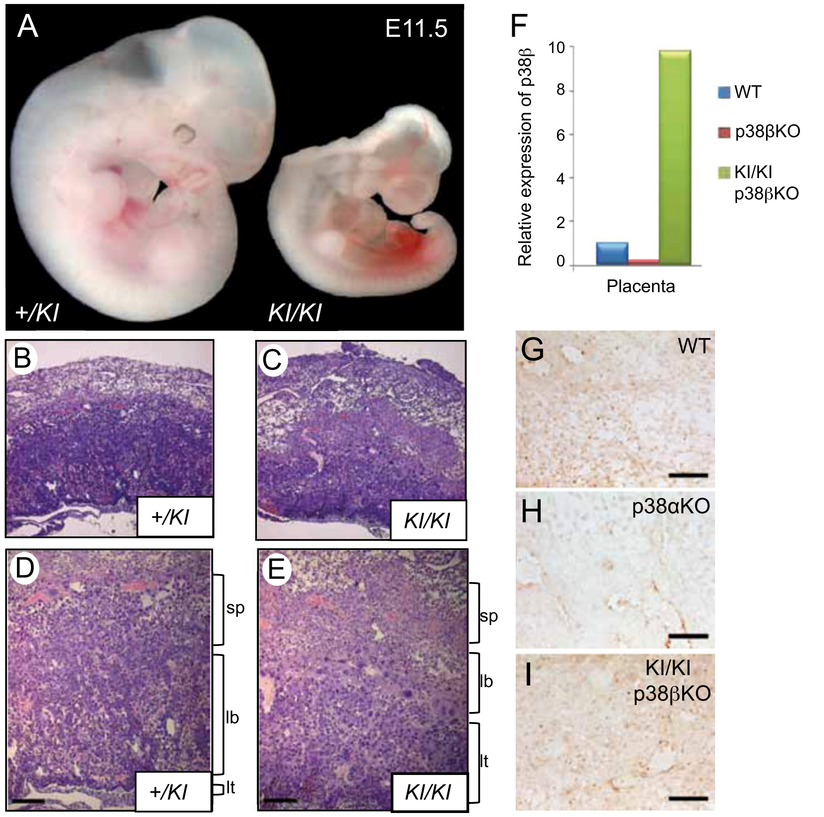

Fig. S4. Phenotypic analysis of $p38\alpha^{(\beta KI/\beta KI)}$ mutant embryos and placenta at E11.5. (*A*) Severe growth retardation in $p38\alpha^{(\beta KI/\beta KI)}$ embryos compared with $p38\alpha^{(\beta KI/+)}$ littermate embryos. (*B–E*) Hematoxylin and eosin staining of placental sections. (*B* and *C*) Abnormal layering in $p38\alpha^{(\beta KI/\beta KI)}$ placentas compared with $p38\alpha^{(\beta KI/+)}$ placentas. (*D* and *E*) Magnification showing the reduced size of the labyrinthine layer and the thickening of the labyrinthine trophoblast in $p38\alpha^{(\beta KI/\beta KI)}$ placentas. Abbreviations: *+/KI*, $p38\alpha^{(\beta KI/+)}$; *KI/KI*, $p38\alpha^{(\beta KI/\beta KI)}$; lt, labyrinthine trophoblast; lb, labyrinthine layer; sp, spongiotrophoblast layers. (Scale bars: 50 μm.) (*F*) Quantitative RT-PCR analysis showing placental expression of *p38*β mRNA under control of the endogenous *p38*α promoter in *p38*β null background ($p38\alpha^{(\beta KI/\beta KI)}p38\beta^{(-/-)}$). (*G*) Immunohistochemistry analysis showing similar levels of phospho-p38 MAPK in placentas $p38\alpha^{(\beta KI/\beta KI)}p38\beta^{(-/-)}$ (KI/KI p38βKO) and wild-type (WT). Placentas $p38\alpha^{(-/-)}$ (p38αKO) were analyzed as control.

Table S1. Expected and observed frequencies of genotypes and phenotypes from breeding $p38\alpha^{(lox/lox)}p38\beta^{(+/-)}$ and $p38\alpha^{(\Delta/+)}p38\beta^{(\Delta/+)}$Sox2-Cre mice

	E13.5								
		Phenotypes				E16.5		E18.5	
Genotype*	Expected	Observed	SB	E	VSD	Expected	Observed	Expected	Observed
Wild-type	7/112	6/112	0/8	0/8	0/3	3/48	4/48	3/48	3/48
$p38\alpha^{het}$ $p38\beta^{wt}$	14/112	15/112	0/14	0/14	0/3	6/48	5/48	6/48	7/48
$p38\alpha^{KO}$ $p38\beta^{wt}$	7/112	6/112	0/6	0/6	0/3	3/48	3/48	3/48	4/48
$p38\alpha^{KO}$ $p38\beta^{het}$	14/112	13/112	0/13	0/13	0/3	6/48	5/48	6/48	5/48
$p38\alpha^{wt}$ $p38\beta^{het}$	14/112	16/112	0/15	0/15	0/3	6/48	6/48	6/48	7/48
$p38\alpha^{wt}$ $p38\beta^{KO}$	7/112	8/112	0/7	0/7	0/3	3/48	4/48	3/48	3/48
$p38\alpha^{het}$ $p38\beta^{KO}$	14/112	16/112	0/14	0/14	0/3	7/48	8/48	7/48	6/48
$p38\alpha^{het}$ $p38\beta^{het}$	28/112	26/112	0/13	0/13	0/3	12/48	11/48	12/48	12/48
$p38\alpha^{KO}$ $p38\beta^{KO}$	7/112	6/112	7/7	2/7	4/4	3/48	2/48	3/48	$0^{\dagger}$/48

*$p38\alpha^{het}$ $p38\beta^{wt}$ include $p38\alpha^{(\Delta/lox)}p38\beta^{(+/+)}$ and $p38\alpha^{(\Delta/+)}p38\beta^{(+/+)}$Sox2-Cre embryos; $p38\alpha^{het}$ $p38\beta^{KO}$ include $p38\alpha^{(\Delta/lox)}p38\beta^{(-/-)}$ and $p38\alpha^{(\Delta/+)}p38\beta^{(-/-)}$Sox2-Cre embryos; and $p38\alpha^{het}$ $p38\beta^{het}$ include $p38\alpha^{(\Delta/lox)}p38\beta^{(+/-)}$ and $p38\alpha^{(\Delta/+)}p38\beta^{(+/-)}$Sox2-Cre embryos.

†One embryo was reabsorbed.

SB, spina bifida; E, exencephaly; VSD, ventricular septal defects.

Table S2. Expected and observed frequencies of phenotypes in embryos that express p38β under control of the endogenous p38α promoter

			Observed phenotype		
Stage	Expected	Observed	Spina bifida	Exencephaly	Heart defects
E13.5	12/48	13/48	1/13	0/13	7/9
E18.5	14/58	12/58	0/12	0/12	N.A.
P20	8/34	0/34	N.A.	N.A.	N.A.

$p38\alpha^{(\beta KI/\Delta)}p38\beta^{(-/-)}$Sox2-Cre embryos were obtained from breeding *$p38\alpha^{(\beta KI/+)}p38\beta^{(+/-)}$ Sox2-Cre* males with *$p38\alpha^{(lox/lox)}p38\beta^{(+/-)}$* females. Note that the *p38βKIα* allele rescued the early lethality phenotype, but no *$p38\alpha^{(\beta KI/\Delta)}p38\beta^{(-/-)}$Sox2-Cre* mice were found at weaning. Spina bifida and Exencephaly were also rescued whereas heart defects largely remained in *$p38\alpha^{(\beta KI/\Delta)}p38\beta^{(-/-)}$Sox2-Cre* embryos. N.A., not analyzed.

Table S3. Primers used for quantitative RT-PCR

Gene	5′-Forward primer-3′	5′-Reverse primer-3′	Anneal temperature, °C
ANF	gccctgagtgagcagactg	cggaagctgttgcagccta	60
BNP	ccggatcggatccgtcagtcgt	gttgtggcaagtttgtgctccaaga	60
Cdk4	ttgtacggctgatggatg	cggtcccattacttgtcac	60
CRT	aggctcctggaggatgatt	tcccactctccatccatctc	60
CyclinA1	gcggctggaaagaaagtgctctct	ctgaaccaaaatccgttgcttcctc	60
CyclinA2	gcagttttgaatcaccacatgc	tggctgcctcttcatgtaacc	60
CyclinD1	ctgcaaatggaactgcttctggtga	agcaggagaggaagttgttggggct	60
CyclinD2	ggaactggtagtgttgggtaa	tgctgctcttgacggaactgc	60
CyclinD3	ttgcgcacgacttcctggcctt	cagacatagagcaggcgcctaggc	60
GADPH	cttcacaccatggaggaggc	ggcatggactgtggtcatgag	60
GATA4	ggttcccaggcctcttgcaatgcgg	agtggcattgctggagttaccgctg	60
GATA5	gtcaaccgaccgctagtgaggc	cattgccagtggccttggcac	60
GATA6	gccaactgtcacaccacaac	tgttaccggagcaagctttt	60
Hand1	ggatgcacaagcaggtgac	cactggtttagctccagcg	60
Hand2	ccgacaccaaactctccaag	tcttgtcgttgctgctcact	60
Mef2a	gtagcggagactcggaattg	atcttctttcgccccatttt	60
Mef2b	ctggagagaagctgctgagg	caaggtggcttggagagaag	60
Mef2c	tgccagttaccatcccagtgtccag	cgtggatccttccaacaccttgtga	60
Mef2d	cagcagccagcactacagag	acttggcagggatgactttg	60
MHC-α	tggtcaccaacaacccatacgact	tgtcagcttgtagacaccagcctt	60
MHC-β	gccaacaccaacctgtccaagttc	tgcaaaggctccaggtctgagggc	60
Mlc2v	tgttcctcacgatgtttggg	ctcagtccttctcttctccg	60
miR-1–1	cctgcttgggacacatacttc	cagtctggcgagagagttcc	60
miR-1–2	cattccatagcacgaatgttcata	ggctgcttcatgttttcaca	60
miR-133a-1	cattgaagaggcgatttggt	gagctgcaagaacagcagtg	60
miR-133a-2	agccaaatgctttgctgaag	tgcggcgtgatcaatg	60
p38α	gattctggattttgggctggctcg	atcttctccagtaggtcgacagcc	60
p38β	atccatcgaggatttcagcg	cctccatgattcgcttcagc	60