

Figure S1: Representative images of heart defect assessment of E17.5 embryos using OPT imaging and 3D reconstruction.

(A) 3D reconstruction of a normal E17.5 mouse embryonic heart. (B-C) Representative images of cardiac transverse sections of a normal tricuspid aortic valve (B, arrowhead) and a bicuspid aortic valve (C, BAV, arrowhead). (D-G) Representative images of cardiac coronal sections of a normal heart (D) and heart displaying a ventricular septal defect (E, VSD, arrowhead), an overriding aorta (F, OA, arrowhead) and a double outlet right ventricle (G, DORV, arrowhead). RA: right atrium, LA: left atrium, RV: right ventricle, LV: left ventricle, Ao: Aorta, Pa: pulmonary artery, Th: thymus. The scale bar represents 0.5 mm

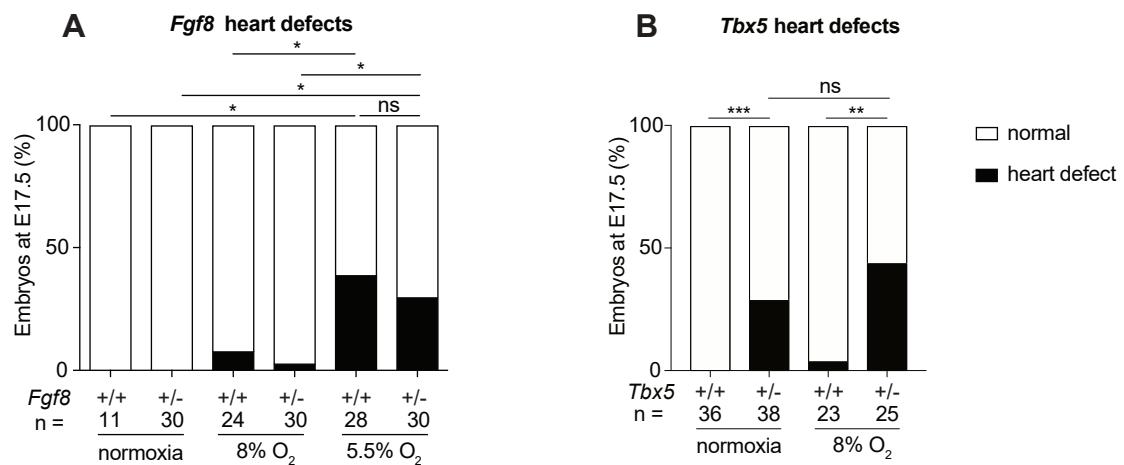


Figure S2: Incidence of heart defects in embryos with mutated cardiac development genes following maternal exposure to low oxygen. Histograms showing the percentage of heart defects present in E17.5 embryos following maternal exposure to the indicated level of hypoxia at E9.5. (A) *Fgf8* and (B) *Tbx5*. Data were tested for statistical significance by two-tailed Fisher's exact test. All relevant pairs were statistically tested and only the significant and/or relevant ones are shown. ns not significant, * P<0.05, **P<0.01, *** P<0.001.

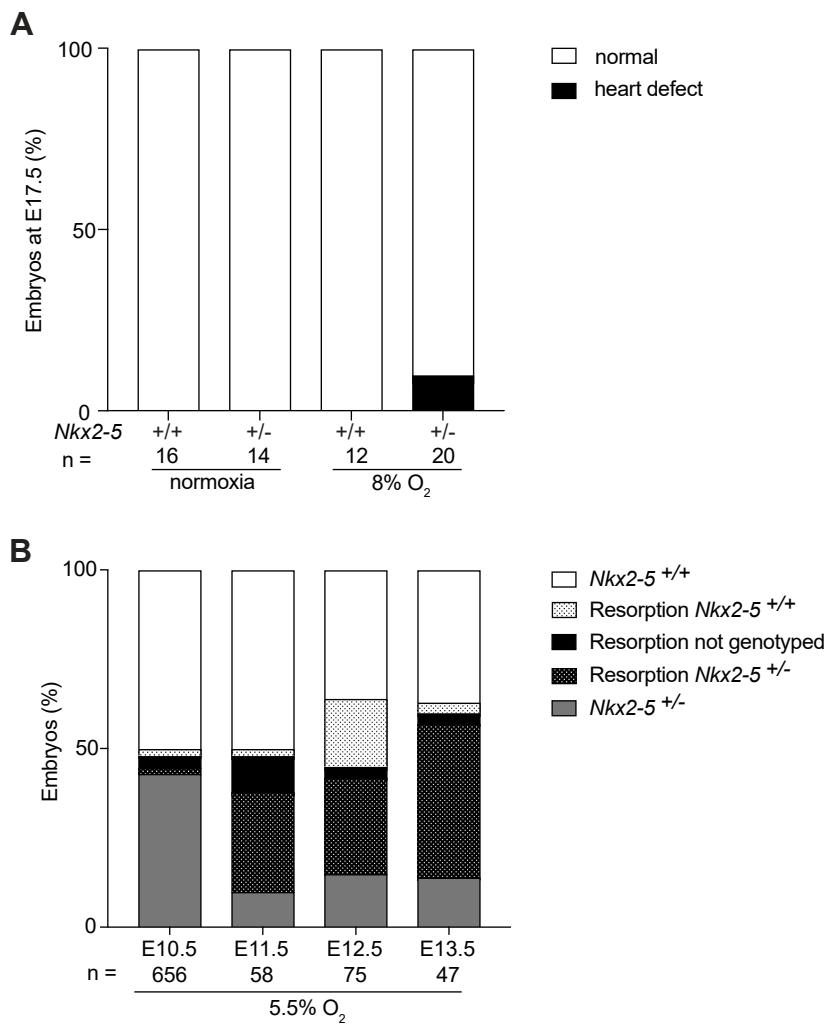


Figure S3: Embryonic survival and resorption genotypes following maternal exposure to low oxygen. (A) Histograms showing the percentage of heart defects present in E17.5 *Nkx2-5* embryos following maternal exposure to 8% Oxygen at E9.5. (B) Histogram showing the percentage of *Nkx2-5*^{+/+} embryos alive (white), *Nkx2-5*^{+/+} resorbed (white with dots), *Nkx2-5*^{+/-} alive (grey), *Nkx2-5*^{+/-} resorbed (black with dots) and the resorptions that could not be genotyped (black) exposed to 5.5% Oxygen at the indicated developmental stages.

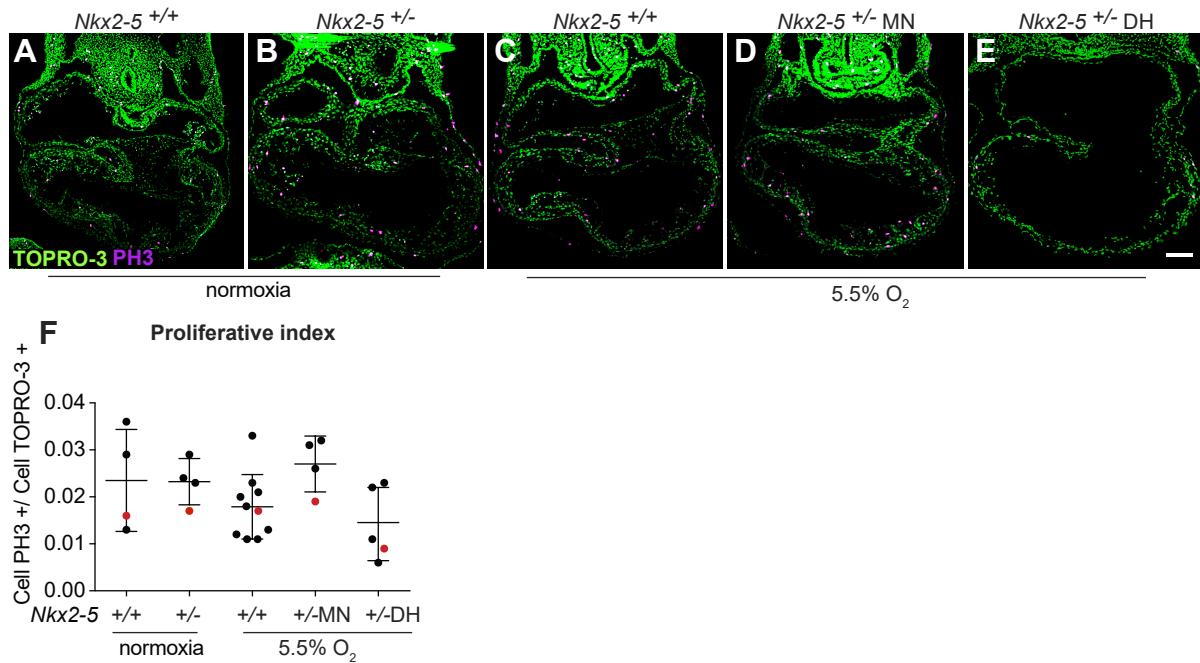


Figure S4: Proliferative index of *Nkx2-5* embryos following maternal exposure to low oxygen. (A-E) Comparison of Phospho-HISTONE H3 expression (magenta) in *Nkx2-5*^{+/+} controls (A, n=4), *Nkx2-5*^{-/-} controls (B, n=4), and exposed to 5.5% oxygen *Nkx2-5*^{+/+} (C, n=10), *Nkx2-5*^{-/-} with a morphologically normal heart (D, n=4) and *Nkx2-5*^{-/-} with a dilated heart (E, n=5). Nuclei are stained with TOPRO-3 (green). (F) Quantification of phospho-histone H3-positive nuclei. Red dots indicate quantification of the images shown in A-E. Data were tested for statistical significance by one-way ANOVA with Tukey's *post hoc* test. Error bars show standard deviations. DH: dilated heart, MN: morphologically normal heart. The scale bar represents 100 μ m.

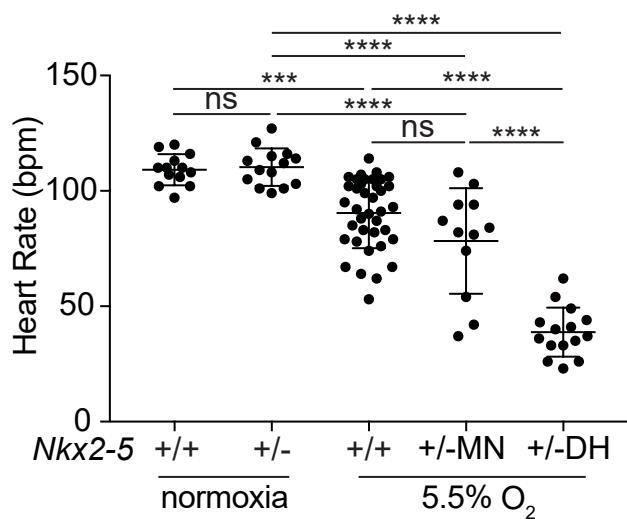
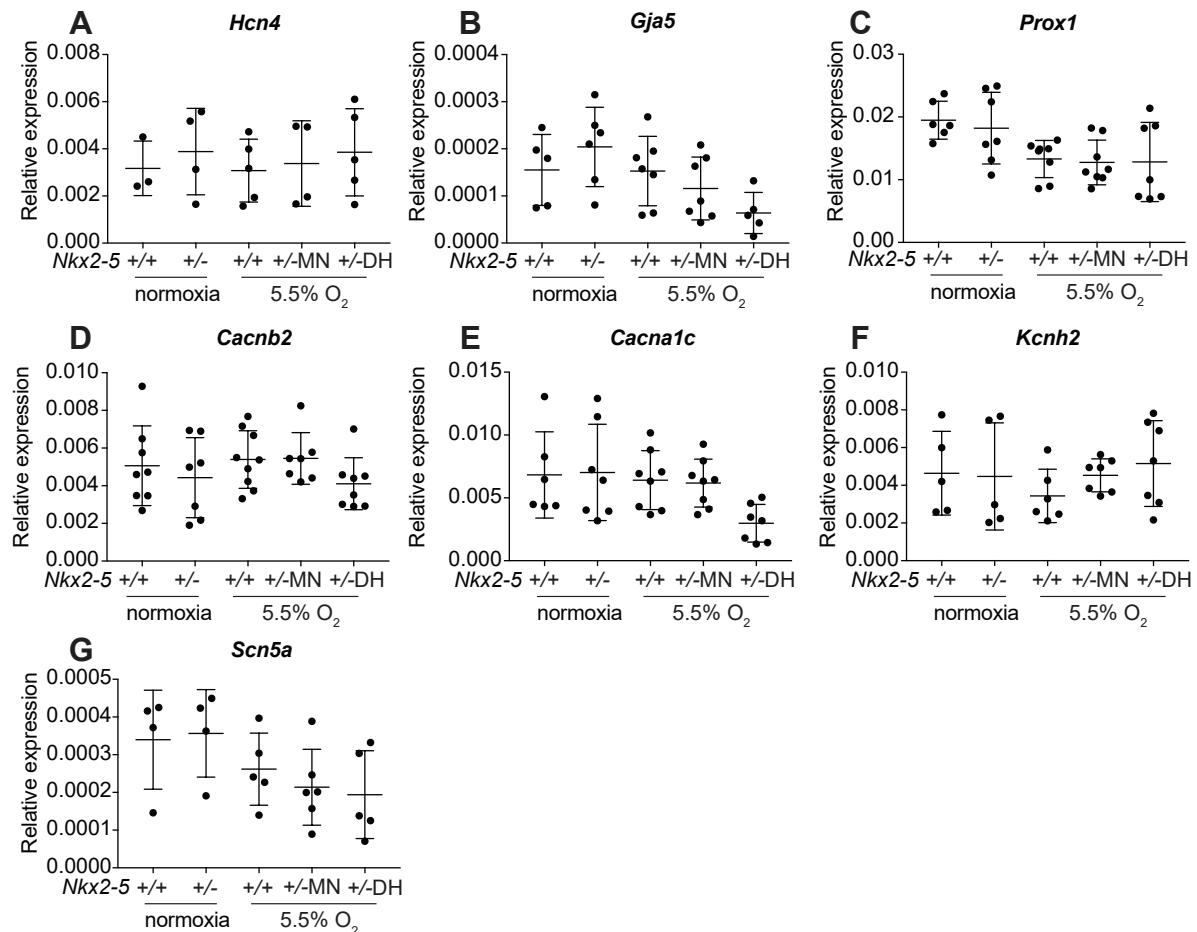
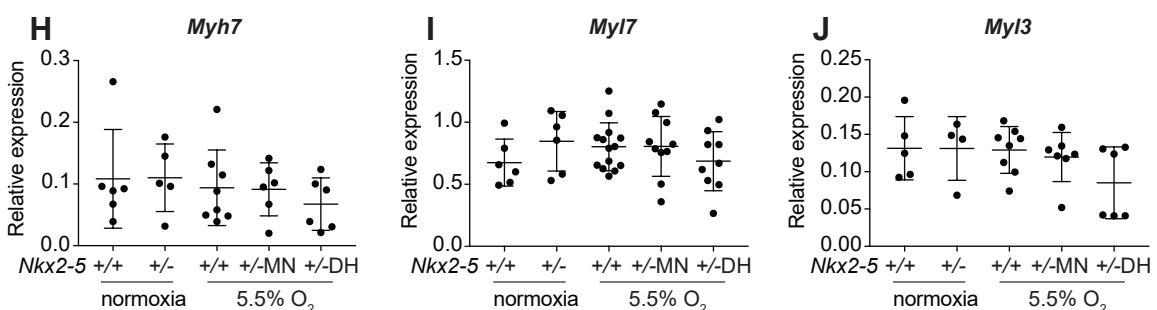


Figure S5: *In utero* embryonic cardiac chronotropy assessment. Quantification of the heart rate of each embryo in the given genotypes and conditions at E10.5. Data were tested for statistical significance by one-way ANOVA with Tukey's *post hoc* test. Error bars show standard deviations. ns not significant, *** P<0.001, **** P<0.0001. DH: dilated heart, MN: morphologically normal heart, bpm: beats per minute.

CONDUCTION SYSTEM



CONTRACTILITY



OTHER

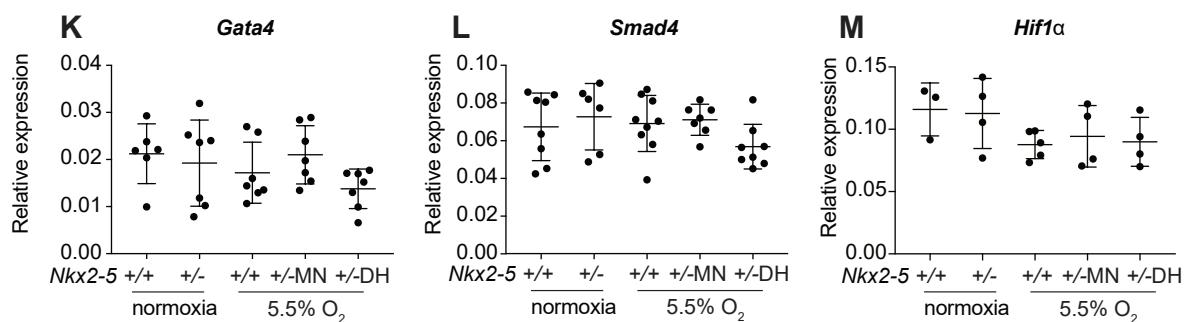
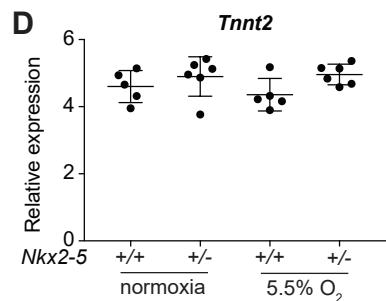
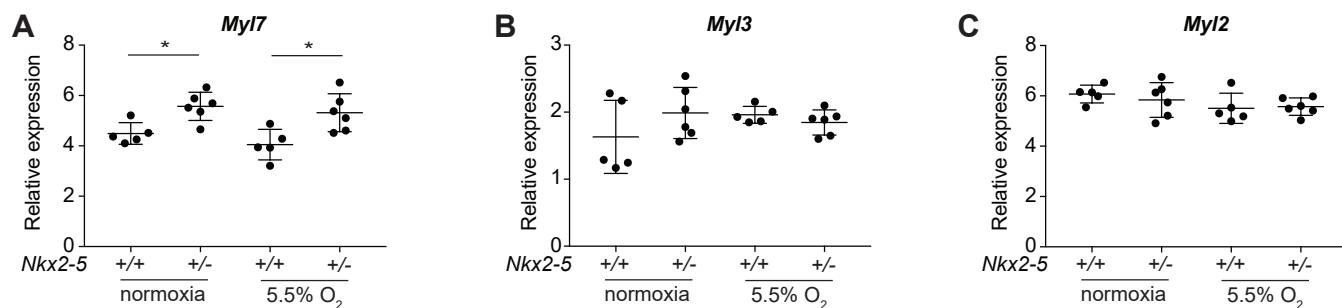


Figure S6: Effects of maternal exposure to low oxygen on embryonic levels of cardiac gene transcripts at E10.5. (A-J) Quantification of transcript levels of (A) *Hcn4*, (B) *Gja5*, (C) *Prox1*, (D) *Cacnb2*, (E) *Cacna1c*, (F) *Kcnh2*, (G) *Scn5a*, (H) *Myh7*, (I) *Myl7*, (J) *Myl3*, (K) *Gata4*, (L) *Smad4* and (M) *Hif1α* at E10.5 by qPCR relative to *Gapdh* and *Eef1e1*. Data were tested for statistical significance by one-way ANOVA with Tukey's post hoc test. Error bars show standard deviations. DH: dilated heart, MN: morphologically normal heart.

CONTRACTILITY



CONDUCTION SYSTEM

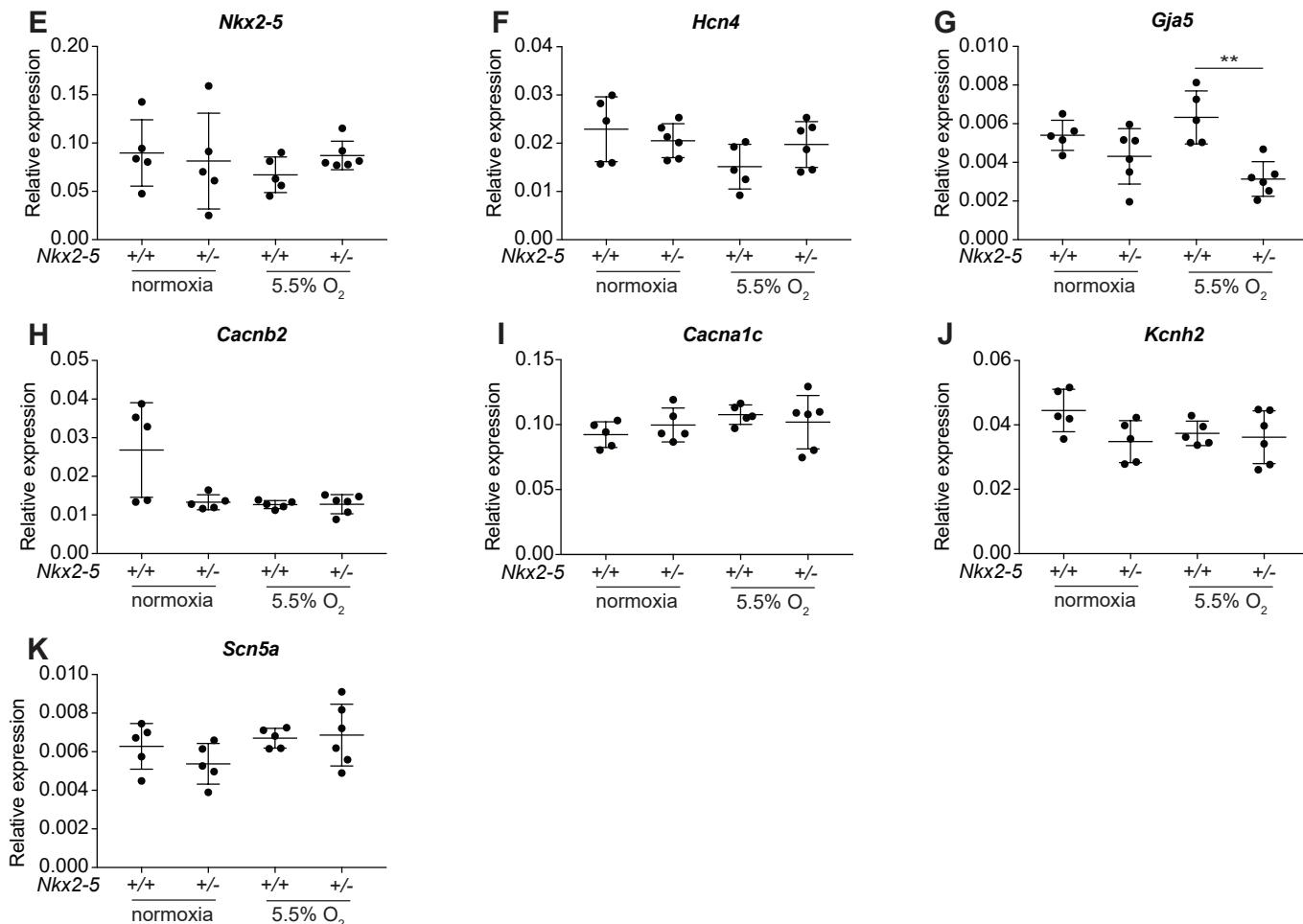


Figure S7: Effects of maternal exposure to low oxygen on embryonic levels of cardiac gene transcripts at E12.5. (A-K) Quantification of transcript levels of (A) *Myl7*, (B) *Myl3*, (C) *Myl2*, (D) *Tnnt2*, (E) *Nkx2-5*, (F) *Hcn4*, (G) *Gja5*, (H) *Cacnb2*, (I) *Cacna1c*, (J) *Kcnh2*, and (K) *Scn5a* at E12.5 by qPCR relative to *Gapdh* and *Eef1e1*. Data were tested for statistical significance by one-way ANOVA with Tukey's *post hoc* test. Error bars show standard deviations. DH: dilated heart, MN: morphologically normal heart. * P<0.05, ** P<0.01.

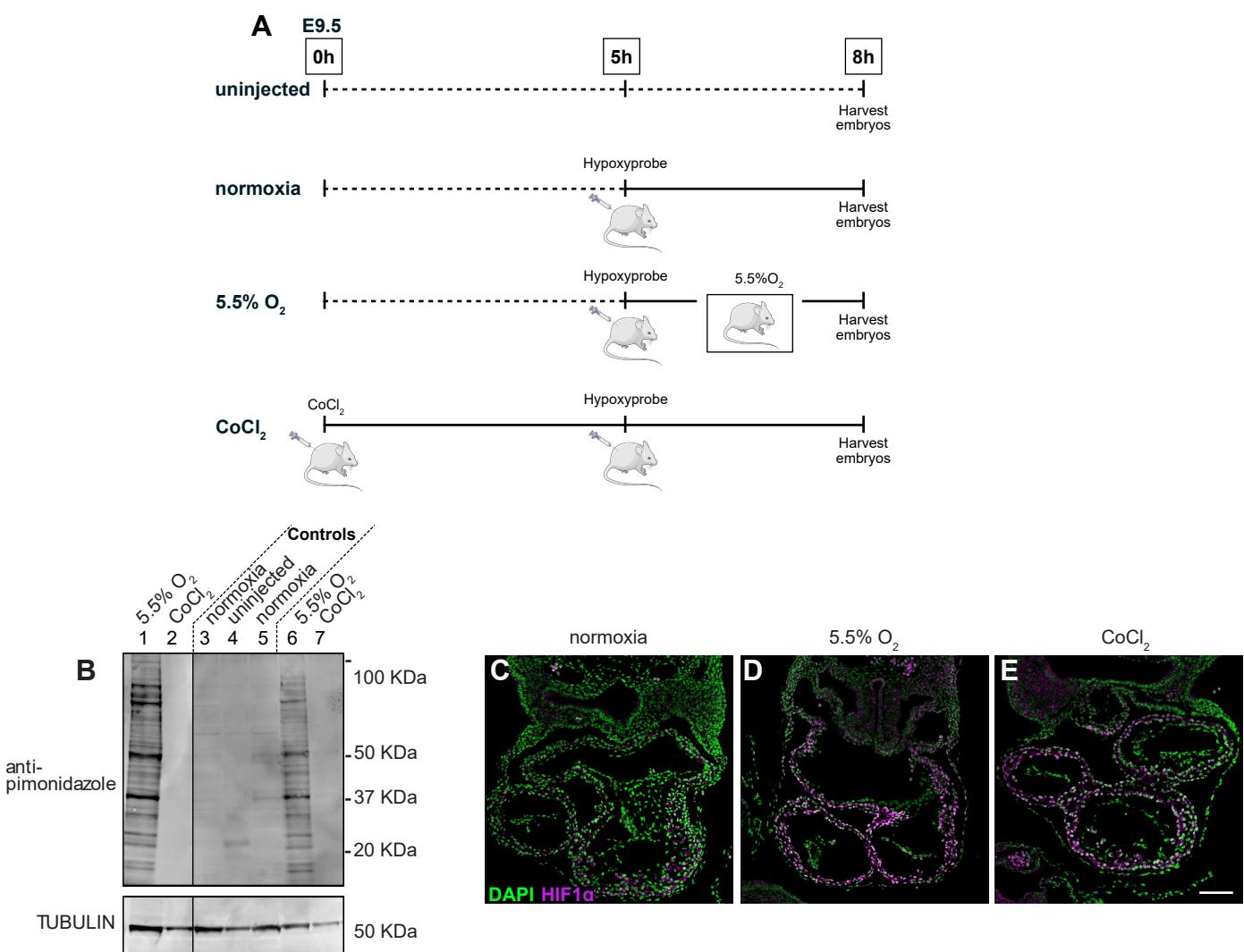


Figure S8: Induction of hypoxia and accumulation of HIF1 α in embryonic heart following maternal cobalt chloride injection in wildtype E9.5 embryos. (A) Schematic diagram illustrating the experimental design. (B) Western Blot showing quantification of Hypoxyprobe™ adducts in E9.5 embryo. Embryos were uninjected (n=1, lane 4), normoxic (n=2, lane 3 and 5), exposed to 5.5% O_2 for 3 hours (n=2, lanes 1 and 6), and 8 hours after injection of 40mg/kg cobalt chloride (n=2, lanes 2 and 7). β -TUBULIN was used as the loading control. (C-E) Comparison of expression levels of HIF1 α (magenta) in control embryos (C, n=2), embryos after 3 hours maternal exposure to low oxygen (D, n=2), and embryos 8 hours after maternal cobalt chloride injection (E, n=2). The scale bar represents 100 μm in C-E.

O ₂ %	Embryo genotype	Embryos examined	Normal	Abnormal	Type of heart defect observed												
					VSD	Musc VSD	OA	DORV	BAV	ASD	TGA	SOTV	PTA	EC	HRH		
21%	<i>Fgf8</i>	+/-	11	11	0	0	0	0	0	0	0	0	0	0	0	0	
		+/-	30	30	0	0	0	0	0	0	0	0	0	0	0	0	
		+/-	24	22	2	0	2	0	0	0	0	0	0	0	0	0	
		+/-	30	29	1	0	1	0	0	0	0	0	0	0	0	0	
		+/-	28	17	11	10	0	6	1	1	0	0	0	0	1	0	
		+/-	30	21	9	8	0	2	1	1	0	2	1	0	0	0	
21%	<i>Fgfr1</i>	+/-	+/-	20	20	0	0	0	0	0	0	0	0	0	0	0	
		+/-	+/-	21	21	0	0	0	0	0	0	0	0	0	0	0	
		+/-	+/-	20	20	0	0	0	0	0	0	0	0	0	0	0	
		+/-	+/-	22	22	0	0	0	0	0	0	0	0	0	0	0	
		+/-	+/-	52	48	4	2	1	1	1	0	0	0	0	0	0	0
		+/-	+/-	37	33	4	3	1	1	0	1	0	0	0	0	0	0
		+/-	+/-	42	39	3	3	1	1	1	0	0	0	0	0	0	0
		+/-	+/-	34	25	9	7	1	1	0	1	0	0	0	0	0	1

Table S1: Pregnant mice were exposed at the indicated oxygen concentration for 8 hours at E9.5 days of gestation. Mice were returned to normoxia, and embryos allowed to develop until E17.5, when heart morphology was analysed using OPT in 3D. VSD: membranous ventricular septal defect; muscVSD: muscular ventricular septal defect; OA: overriding aorta; DORV: double-outlet right ventricle; TGA: transposition of the great arteries; ASD: atrial septal defect; PTA: persistent truncus arteriosus; SOTV: straddling overriding tricuspid valve; BAV: bicuspid aortic valve; EC: ectopia cordis; HRH: hypoplastic right heart. Note that a particular embryo may have more than one type of defect. The heart defects in bold are more severe than the ones in regular font. Also see Figure S1 for representative images of heart defects.

O ₂ %	Embryo genotype	Embryos examined	Normal	Abnormal	Type of heart defect observed									
					VSD	Musc VSD	OA	DORV	BAV	ASD	TGA	SOTV	PTA	HLH
21%	<i>Tbx5</i>	+/-	36	36	0	0	0	0	0	0	0	0	0	0
		+/-	38	27	11	5	0	3	1	0	3	0	3	1
		+/-	23	22	1	1	0	1	0	0	0	0	0	0
		+/-	25	14	11	4	3	5	2	0	1	0	1	1
21%	<i>Tbx1</i>	+/-	11	11	0	0	0	0	0	0	0	0	0	0
		+/-	20	19	1	1	0	0	0	0	0	0	0	0
		+/-	32	31	1	1	0	0	0	0	0	0	0	0
		+/-	37	35	2	0	2	0	0	0	0	0	0	0
5.5%	<i>Nkx2-5</i>	+/-	43	27	16	4	3	5	1	0	0	1	0	1
		+/-	31	8	23	8	3	8	3	0	0	1	0	0
		+/-	16	16	0	0	0	0	0	0	0	0	0	0
		+/-	14	14	0	0	0	0	0	0	0	0	0	0
8%	<i>Nkx2-5</i>	+/-	12	12	0	0	0	0	0	0	0	0	0	0
		+/-	20	18	2	1	0	0	0	0	1	0	0	0

Table S2: Pregnant mice were exposed at the indicated oxygen concentration for 8 hours at E9.5 days of gestation. Mice were returned to normoxia, and embryos allowed to develop until E17.5, when heart morphology was assessed using OPT in 3D. VSD: membranous ventricular septal defect; muscVSD: muscular ventricular septal defect; OA: overriding aorta; DORV: double-outlet right ventricle; BAV: bicuspid aortic valve; ASD: atrial septal defect; TGA: transposition of the great arteries; SOTV: straddling overriding tricuspid valve; PTA: persistent truncus arteriosus; HLH: hypoplastic left heart. The heart defects in bold are more severe than the ones in regular font.

Embryonic stage after exposure at E9.5 to 5.5% oxygen for 8 hours														
	E9.5 0h after 5.5%O ₂		E9.5 – E10 5h after 5.5%O ₂			E10.5			E11.5		E12.5		E17.5	
	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
<i>Nkx2-5</i>	=	=	↓	↑↑										
HIF1 α	(F6)	(F6)	(F6)	(F6)										
Live embryos (%)	100 (F6)	100 (F6)	100 (F6)	100 (F6)	96 (F2, FS3)	96 (F2, FS3)	96 (F2, FS3)	27 (F2, FS3)	66 (F2, FS3)	25 (F2, FS3)	94 (F1)	18 (F1)		
Heart morphology (%)	normal 100 (F6)	normal 100	normal 100	normal 55 (F6)	DH 45	normal 100 (F2)	normal 50 (F2)	DH 50	normal 100 (F2)	normal 100 (F2)	normal 100 (F2)	normal 100 (FS3)	normal 100	normal 100
Nkx2-5 expression						=	↓	↓↓						
Proliferation/apoptosis						=	=	=						
Heart rate						↓	↓↓	↓↓↓						
Conduction						=	=	=			=	=		
Contractility						=	↓	↓↓			=	=		

Table S3: Summary of molecular and morphological cardiac phenotype of *Nkx2-5* embryos following maternal low oxygen exposure. Embryonic day (E), figure (F), supplementary figure (FS), equal levels (=), elevated levels (↑), reduced levels (↓), wildtype (+/+), heterozygous null (+/-), dilated heart (DH).

Candidate	RefSeq	Primer Sequence	Reference
<i>Nkx2-5</i>	NM_008700.2	AAGTGCTCTCCTGCTTCCC GCGCGCACAGCTTTTT	This study
<i>Myh7 (Beta-MHC)</i>	NM_001361607.1 NM_080728.3	CCTTACTTGCTACCCTCAGGTG GGCTGAGCCTTGGATTCTCAAA	This study
<i>Myl7 (MLC2a)</i>	NM_022879.2	GGCACAAACGTGGCTTCTAA TGCAGATGATCCCATCCCTGT	(Koren et al., 2013)
<i>Myl3 (MLC1v)</i>	NM_001364484.1 NM_010859.3	GGAAGCCGAGTTGATGCC GCCTCCTTGAACTCTTCATCT	This study
<i>Actc1</i>	NM_009608.4	GAACCACAGGCATTGTTCTGG CGCATGATGGCATGGGTAA	This study
<i>Myl2 (MLC2v)</i>	NM_010861.4	ATCGACAAGAATGACCTAACGGGA ATTTTACGTTCACTCGCCT	(Koren et al., 2013)
<i>Tnnt2 (cTNT)</i>	NM_001130174.2 NM_001130175.2 NM_001130176.1 NM_001130177.2 NM_001130178.2 NM_001130179.2 NM_001130180.2 NM_001130181.2 NM_011619.3	ATGATGCACTTGGAGGGTACA GGCCTTCTCTCTCAGTTGGT	This study
<i>Hcn4</i>	NM_001081192.1	CAGCGTCAGAGCGGATACTT CTTCTTGCCATGCGGTCCA	This study
<i>Gja5 (Cx40)</i>	NM_001271628.1 NM_008121.3	AGCTCTAACGTGGAAGGCTC CCGATGACTGTGGAGTGCTT	This study
<i>Prox1</i>	NM_001360827.1 NM_008937.3	AAAGTCAAATGTACTCCGCAAGC CTGGGAAATTATGGTTGCTCCT	(Zhou et al., 2013)
<i>Cacnb2</i>	NM_001309519.1 NM_001252533.1 NM_023116.4	TCTCGAGGGAAATCTCAAGCA ATT CCTGCGGAGGACATTGG	This study
<i>Cacna1c</i>	NM_001159533.2 NM_001159534.2 NM_001159535.2 NM_001255997.2 NM_001255998.2 NM_001255999.2 NM_001256000.2 NM_001256001.2 NM_001256002.2 NM_001290335.1 NM_009781.4	CATGAAGCTCAACTCAACTGTTTC CGTGGGCTCCCATAAGTTG	(Furtado et al., 2017)
<i>Kcnh2</i>	NM_001294162.1 NM_013569.2	GATCGCCTTCTACCGGAAA CATTCTTCACGGGTACCACCA	(Furtado et al., 2017)
<i>Scn5a</i>	NM_001253860.1 NM_021544.4	GATGAGGAGAACAGCCTTGG CACAACTTGGATTCTGCT	(Furtado et al., 2016)
<i>Gata4</i>	NM_001310610.1 NM_008092.4	GCTCCATGTCCCAGACATTC ATGCATAGCCTTGTGGGAC	This study
<i>Smad4</i>	NM_008540.2	CCAAGCGCGTATATAAAGGTCT GAGCTATTCCACCCACGGAC	This study
<i>Hif1α</i>	NM_001313919.1 NM_001313920.1 NM_010431.2	TCATCAGTTGCCACTTCCCC CTGTCTAGACCACCAGGCATC	This study

Table S4: Primer sequences used for qPCR

Target	Name	Catalogue number	Species and type	Supplier	Dilution for IHC	Dilution for IB
Primary Antibodies						
α-Smooth Muscle-FITC	clone 1A4	F3777	Mouse monoclonal	Sigma-Aldrich	1:1,000	na
Phospho-histone H3	(Ser 10)- R	sc-8656	Rabbit polyclonal	Santa Cruz Biotechnology	1:200	na
NKX2-5		13921-1-AP	Rabbit polyclonal	Proteintech	na	1:1,000
β-Tubulin	Clone TUB 2.1	T5201	Mouse monoclonal	Sigma-Aldrich	na	1:5,000
HIF1α		NB100-449	Rabbit polyclonal	Novus Biologicals	1:400	na
Hypoxyprobe TM	4.3.11.3		Mouse monoclonal	Hypoxyprobe, Inc.	1:500	na
Secondary Antibodies						
Donkey anti-rabbit Cy™3		711-165-152		Jackson ImmunoResearch	1:500	na
Donkey anti-mouse AlexaFluor ® 488		715-545-151		Jackson ImmunoResearch	1:500	na
Goat anti-mouse IRDye® 800CW		926-32210		LI-COR	na	1:10,000
Donkey anti-rabbit biotinylated		711-065-152		Jackson ImmunoResearch	1:500	na
Tertiary reagents						
Streptavidin Cy™3		GTX8590 2		GeneTex	1:1,000	na
Nuclear stain						
TO-PRO®-3 Iodide		T3605		Life Technologies	1:10,000	na
DAPI		1023627 6001		Sigma-Aldrich	1:1,000	na

Table S5: Antibodies used for immunodetection