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Supplementary Materials for

Biological pacemaker created by minimally invasive somatic reprogramming in pigs with complete heart block

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The PDF file includes:

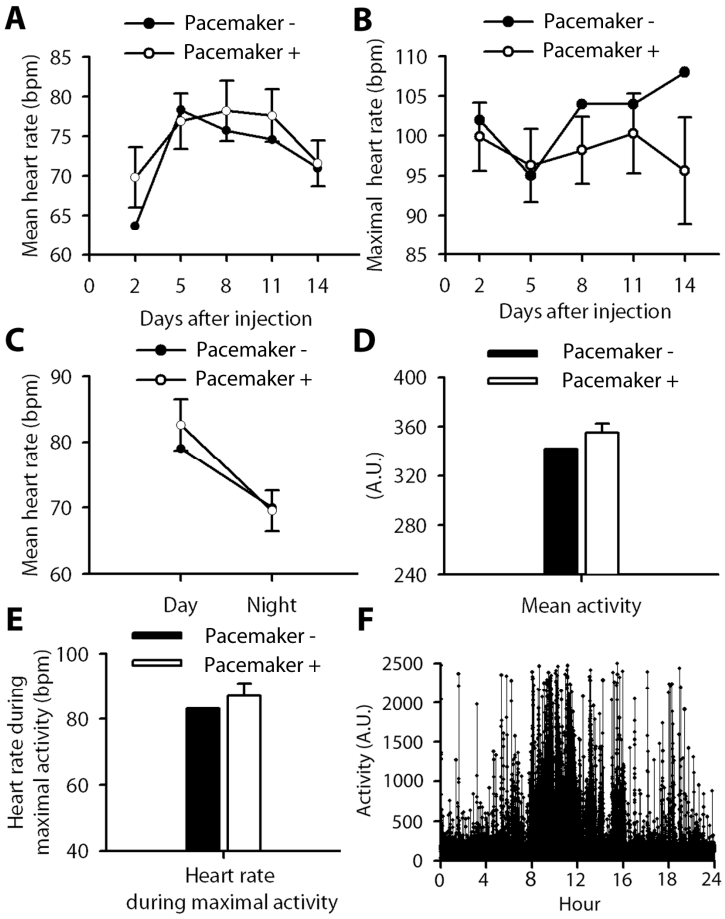

Fig. S1. HR trends, diurnal changes, and physical activity data from a *TBX18*-transduced animal without backup electronic pacing, superimposed on the corresponding pooled data for the routine protocol. *TBX18* vector was transduced in one animal without electronic pacemaker backup. The average heart rate (**A**), maximal heart rate (**B**), diurnal change (**C**), mean activity (**D**), and heart rate during maximal activity (**E**) during the 2-week period after gene transfer in the animal without a back-up electronic pacemaker are superimposed (A and B) or compared (C to E) to the corresponding pooled data from animals with back-up devices. The 24-hour activity pattern in the animal without a backup electronic pacemaker resembles that of animals with backup devices (F). These findings support the notion that the *TBX18* biological pacemaker can support both heart rate and physical activity without the need for a backup electronic pacemaker, at least in the single experiment performed and illustrated here.

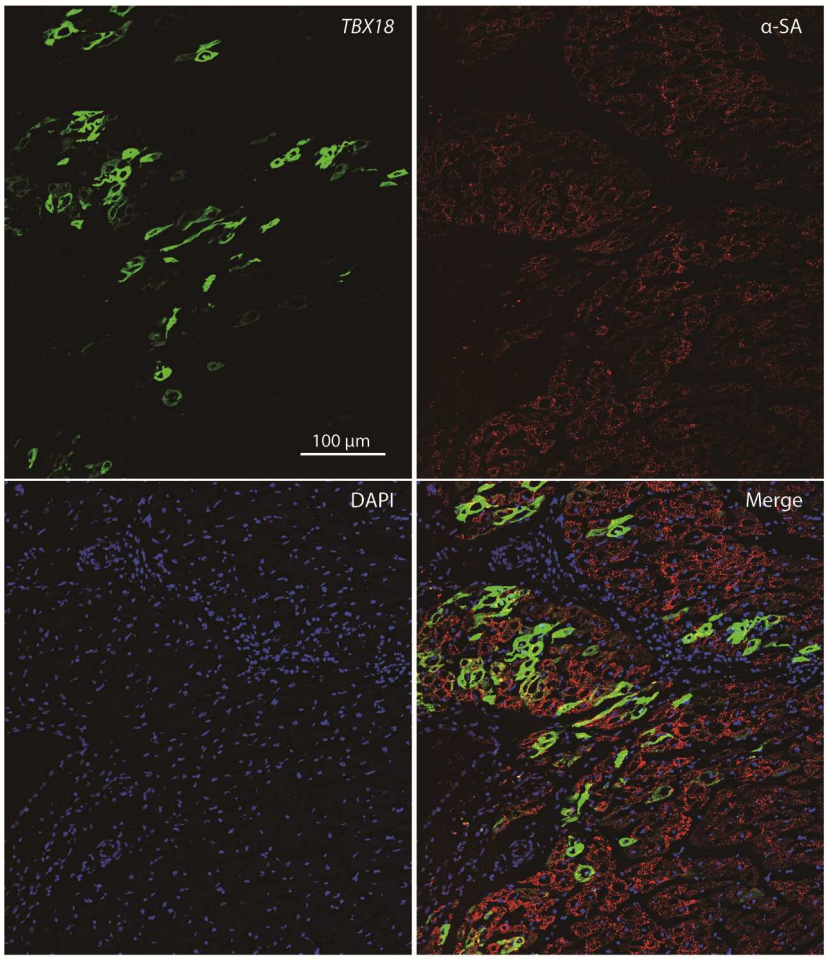

Fig. S2. *TBX18*-transduced cardiomyocytes for quantification. A typical field of *TBX18*-transduced heart proximal to the injection site, with staining for *TBX18* (indexed by coexpressed GFP), α-sarcomeric actin, or DAPI, and a merged image as indicated. Twenty slides derived from two tissue blocks (0.5-cm thickness each), were used to count green cells. Block 1 yielded an average of 246 (range 100-768) green cells per slide, whereas block 2 revealed 342 (182-524) green cells per slide. Each block contained ~25 cell layers, yielding an estimated green cell number of 14,700 per heart. This lower-limit estimate does not count cells remote from the injection site, nor does it account for the loss of GFP expression that may be seen in durably reprogrammed iSAN cells.

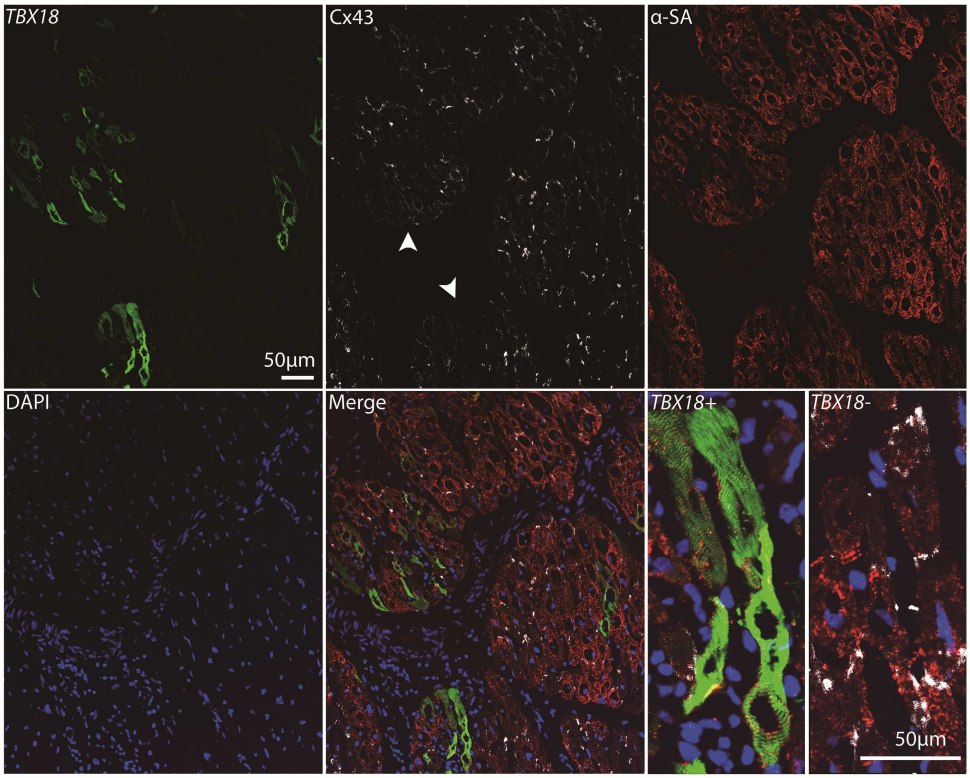

Fig. S3. Cx43 expression in iSAN cells. Myocardial regions (arrowheads) enriched in *TBX18*-transduced cardiomyocytes (GFP+) exhibited a lower density of Cx43 compared with those without *TBX18* transduction. The right lower panel compares enlarged images of cardiomyocytes with or without *TBX18* transduction, showing differences in Cx43 expression (white).

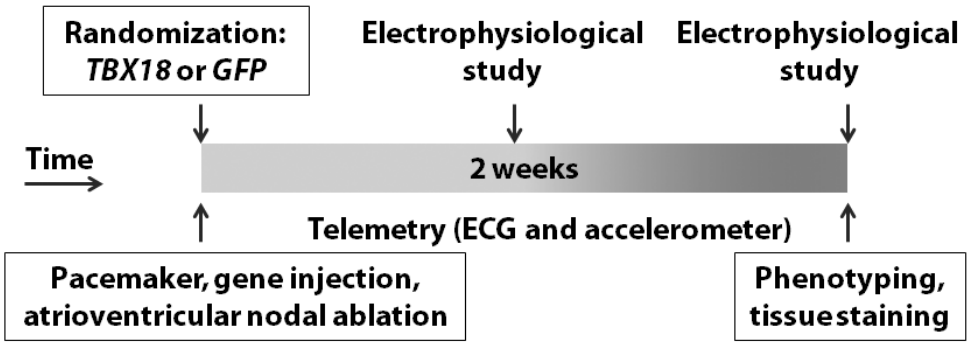

Fig. S4. Schematic of the experimental protocol in the porcine model of complete heart block.

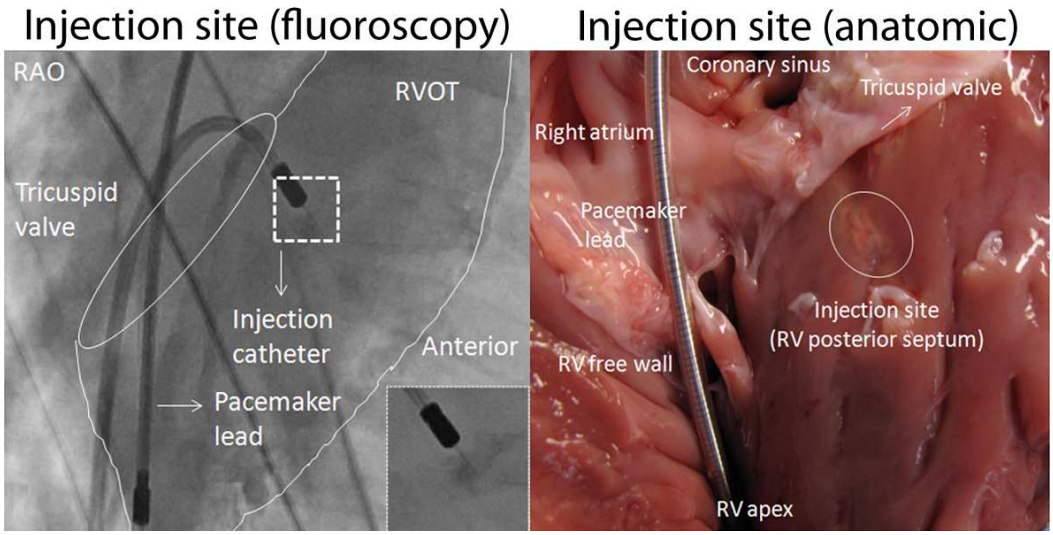

Fig. S5. Injection site images. RAO, right anterior oblique view; RV, right ventricle; RVOT, outflow tract of right ventricle.

SUPPLEMENTARY TABLES

Table S1. Baseline characteristics of *GFP* and *TBX18* groups. Data are expressed as means ± SEM (n = 5 *GFP*, 7 *TBX18*). Averaged APD_{90}: the average of action potential duration at 90% from four locations (anterior, septal, lateral and apical wall); Corrected QT interval: QT interval corrected by Bazett's formula; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CPK: creatine phosphokinase; CKMB, MB isoenzyme of CPK; HGB, hemoglobin; WBC, white blood cell count. *P*-values determined by two-sample *t* test.

	GFP	*TBX18*	*P*-value
Age (months)	3.1±0.2	3.4±0.3	0.10
Body weight (kg)	33.0±2.1	36.5±6.9	0.30
ECG parameters			
Heart rate (ms)	562.2±74.4	586.3±137.7	0.73
P wave duration (ms)	52.1±3.5	56.3±7.4	0.27
PR interval (ms)	102.6±7.8	106.3±12.1	0.57
QRS duration (ms)	42.6±1.8	44.9±3.4	0.21
QT interval (ms)	309.0±28.3	326.0±30.4	0.35
Corrected QT interval (ms)	413.3±13.3	428.8±8.5	0.32
Corrected QT dispersion	42.1±6.3	43.2±3.4	0.87
Average APD_{90} (ms)	207.2±6.9	221.2±10.2	0.32
APD_{90} dispersion (ms)	33.1±4.8	44.1±4.0	0.11
Biochemistry			
Liver function			
AST (IU/l)	25.2±4.9	20.6±1.7	0.33
ALT (IU/l)	22.0±2.6	23.5±3.4	0.66
Total bilirubin (mg/dl)	0.1±0.0	0.1±0.0	0.99
Alk Phosphatase (IU/l)	141.2±16.0	144.3±7.5	0.85
Renal function			
BUN (mg/dl)	10.0±2.1	11.0±0.6	0.61
Creatinine (mg/dl)	1.58±0.07	1.49±0.11	0.52
Pancreas function			
Amylase (IU/l)	1491.4±127.9	1235.7±122.0	0.19
Cardiac enzyme			
CPK (ng/ml)	473.8±52.6	420.7±45.2	0.46
CKMB (%)	1.52±0.75	1.30±0.32	0.77
Troponin I (ng/ml)	0.002±0.002	0.005±0.002	0.24

Hematology			
WBC (10^3/μl)	16.0±1.5	17.1±2.1	0.69
HGB (g/dl)	8.9±0.3	8.3±0.2	0.08
Platelets (10^3/μl)	337.8±49.8	402.6±39.8	0.33
Lymphocytes (10^3/μl)	8.6±0.9	9.5±0.8	0.46
Neutrophils (10^3/μl)	6.6±1.1	7.0±1.4	0.81
Eosinophils (10^3/μl)	0.6±0.2	0.3±0.1	0.18

Table S2. Comparison of HRV between *TBX18* and *GFP* animals. Data are means ± SEM (*n* = 5 *GFP*, 7 *TBX18*). HF, high frequency; LF, low frequency; VLF, very low frequency; norm, normalized; n.u., normalized units. *P*-values determined by two-sample *t* test.

	GFP	***TBX18***	***P*-value**
VLF (ms^2)	0.02±0.01	0.05±0.03	0.40
LF (ms^2)	0.04±0.01	0.04±0.01	0.48
HF (ms^2)	0.05±0.01	0.04±0.01	0.71
Total power (ms^2)	0.11±0.01	0.10±0.02	0.68
LF norm (n.u.)	46.8±6.9	49.5±8.0	0.81
HF norm (n.u.)	53.2±6.9	45.9±3.7	0.34
LF/HF	0.98±0.21	1.91±0.29	0.04

Table S3. Staining conditions for HCN4. Four different antibodies were tested.

	Alomone, APC052	**Abcam, ab32675**	**Novus biological, S114-10**	**Millipore AB5808**
Antibody concentration				
Primary antibody	1:10, 1:20, 1:50, 1:100, 1: 400	1:10, 1:20, 1:50, 1:100, 1: 400	1:10, 1:20, 1:50, 1:100, 1: 400	1:10, 1:50
Secondary antibody	1:400, 1:200	1:400 1:200	1:400 1:200	1:400
Permeabilization				
Triton	+	+	+	+
Saponin	+	+	+	+
Tissue preparation				
Fresh frozen without fixation	+	+	+	
Formalin fixation	+	+	+	+
Antigen retrieval	With and without	With and without	With and without	With and without
Overnight incubation	+	+	+	+

Table 4. Primer sequences for the different genes studied in reprogrammed cells.

	Forward primer	Reverse primer	Probe
HCN4	CAAGCAGGTGGAGCAGTACA	TGCGGCAGTTAAAGTTGATG	CAGCGCATCCACGACTACTA
Cx43	GGATCGTGTGAAGGGAAAGA	GCTCGGCACTGTAATTAGCC	AGCTGGTTACCGGAGACAGA
Cx45	CACCGAGCTCTGGAAGAAAC	ACCTCAAACATGGTCCTTGC	GGACCCCATGATGTATCCAG
Kir2.1	AGTGCCAGGGACTTAGCAGA	TAGAGGTACGCTTGCCTGGT	GTGAAAACGGAGTCCCAGAA
Actinin	CATGCTGCTTTTGGAAGTCA	GGCAGAGGTTTCTTCGACTG	ATTGTTGATGGCAACGTGAA
Nkx2.5	TCGAGCCGATAAGAAAGAGC	AGATCTTGACCTGCGTGGAC	ACAGGTCTACGAGCTGGAGC
Nav1.5	GCTACACCAGCTTCGACTCC	TTCTCCTCCGTCTCAGCAAT	ATGCTTGTCATCTTCCTGGG
Actin	TGTGCTGGACTCTGGAGATG	GTGGTCACGAAGGAGTAGCC	GCCGAGATCTCACCGACTAC