

SUPPLEMENTAL MATERIAL

Supplemental Table 1: Summary of surface ECG analysis 4 weeks of age with 1 or 3% isoflurane anesthesia.

genotype	Isoflu- rane	SCL (ms)	HR (bpm)	PR (ms)	QRS (ms)	Qtc (ms)
W/W (n=5)	1%	143 ± 9	423 ± 22	33.2 ± 0.8	9.1 ± 0.4	23.9 ± 1.4
W/ R52G (n=6)	1%	141 ± 4	428 ± 12	36.5 ± 0.6*	11.8 ± 0.4**	26.0 ± 0.6
W/W (n=5)	3%	139 ± 7	434 ± 23	41.5 ± 1.5	10.3 ± 0.3	26.0 ± 1.0
W/R52G (n=6)	3%	146 ± 8	416 ± 23	54.2 ± 2.2**	12.2 ± 0.4**	29.0 ± 1.2

SCL, sinus cycle length; HR, heart rate; bpm. beats per minute.

Mean ± SE. *P*, *P* value between two different mouse genotypes. **P* < 0.05, ***P* < 0.01

Figure S1. Profound effects of higher dose of isoflurane (3%) anesthesia on prolongation of PR interval and QRS duration in *Nkx2-5^{+R52G}* mice. Representative surface ECG recordings obtained from 4 weeks old control *Nkx2-5^{+/+}* (top) and *Nkx2-5^{+R52G}* (bottom) mice either with 1% or 3% isoflurane anesthesia. Signal-averaged ECG waves were utilized for analysis shown in Supplemental Table S1. HR, heart rate.

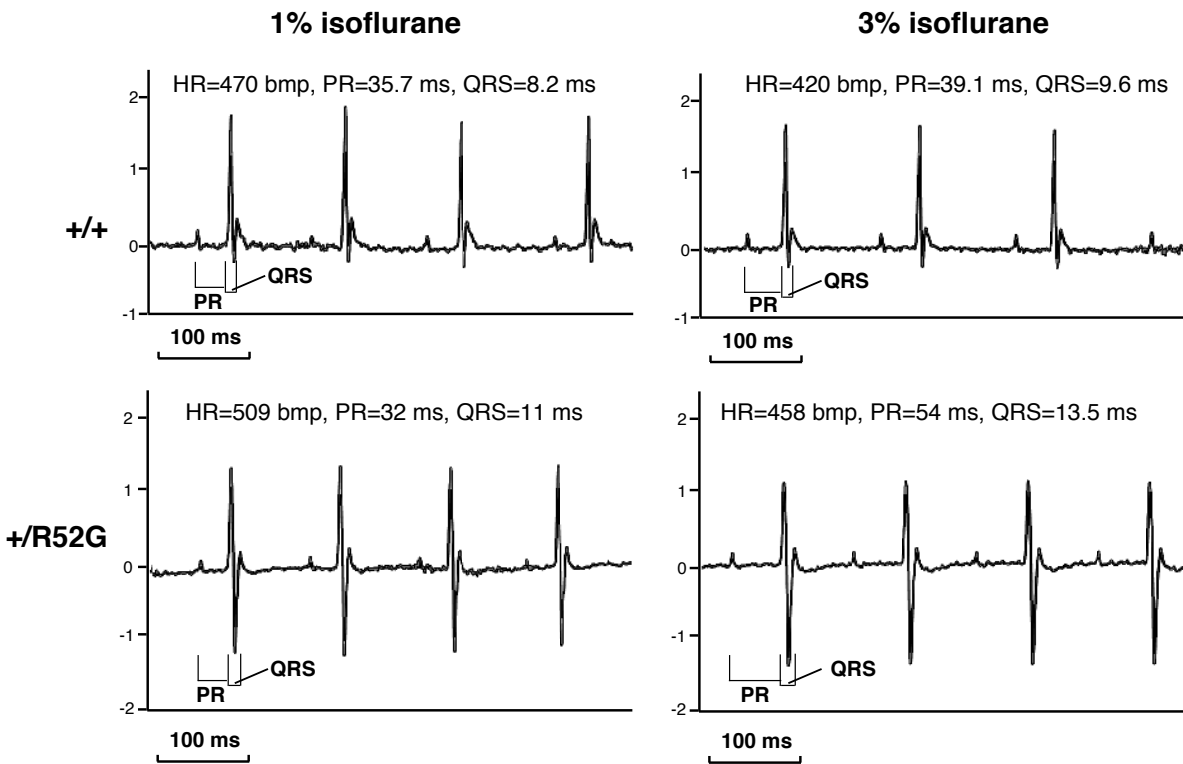


Figure S2: Representative images of serial tissue sections positively (+) or negatively (-) stained with HCN4, connexin 40, connexin 43, and Na_v1.5 (green) in AV node, penetrating AV bundle (traced by white dots) and left bundle branch (arrowheads) from 4 week-old mice. Anti-actinin antibody was used for co-immunostaining (red). Of note, connexin40 staining was not evident in penetrating AV bundle and left bundle branch differing from P1 hearts demonstrated in Figure 6E.

