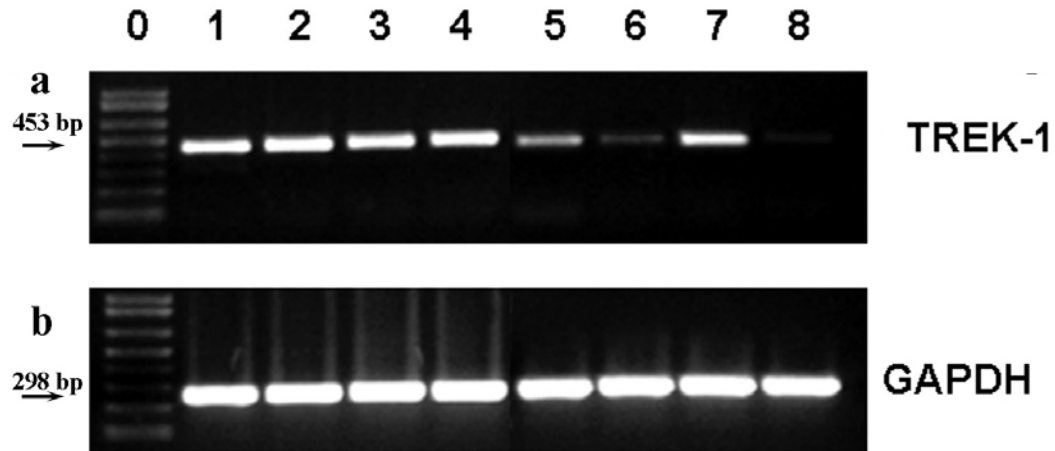


Supplemental Figure 1.



**Sup. Fig. 1.** The developmental expression of TREK-1 studied by one-step RT-PCR. Total RNA was isolated from tubular heart (total) of Hamilton-Hamburger stage 14 (S14), S18, atria and ventricle from ED5, ED8 and ED11 heart by Qiagen RNeasy Kit (Qiagen). 50 ng total RNA was mixed with 1  $\mu$ l Invitrogen SuperScript<sup>TM</sup> one-step RT-PCR Platinum<sup>®</sup> taq, 5  $\mu$ l 10X buffer, 1  $\mu$ l primer mix (final concentration of 0.25  $\mu$ M/L for each) and RNase-free water to a final volume of 50  $\mu$ l. The primers for TREK-1 that span an intron are: sense 5'-TTGCCAAAGTAGAAGATACCTT-3' and antisense 5'-ACTTGTCATAGATCTCCACGCT-3'. One-step RT-PCR cycling conditions were as follows: 30 minutes at 50°C for reverse transcription followed by a 2 minute inactivation step at 94 °C. After reverse transcription a total of 40 cycles for melting, annealing and extension steps (95°C 30s, 55°C 30s and 72°C 45 s) were carried out. 10  $\mu$ L amplification products were verified on 1.5% agarose gels. Lane 0 is 1kb DNA marker (Invitrogen). Lane 1 to 8 represent different chicken developing heart stages as following: S14, S18, ED5A, ED5V, ED8A, ED8V, ED11A and ED11V. TREK-1 is expressed in the early

tubular heart and pre-separated atria and ventricle (ED5). Expression of TREK-1 was restricted to atria (ED8 and later).