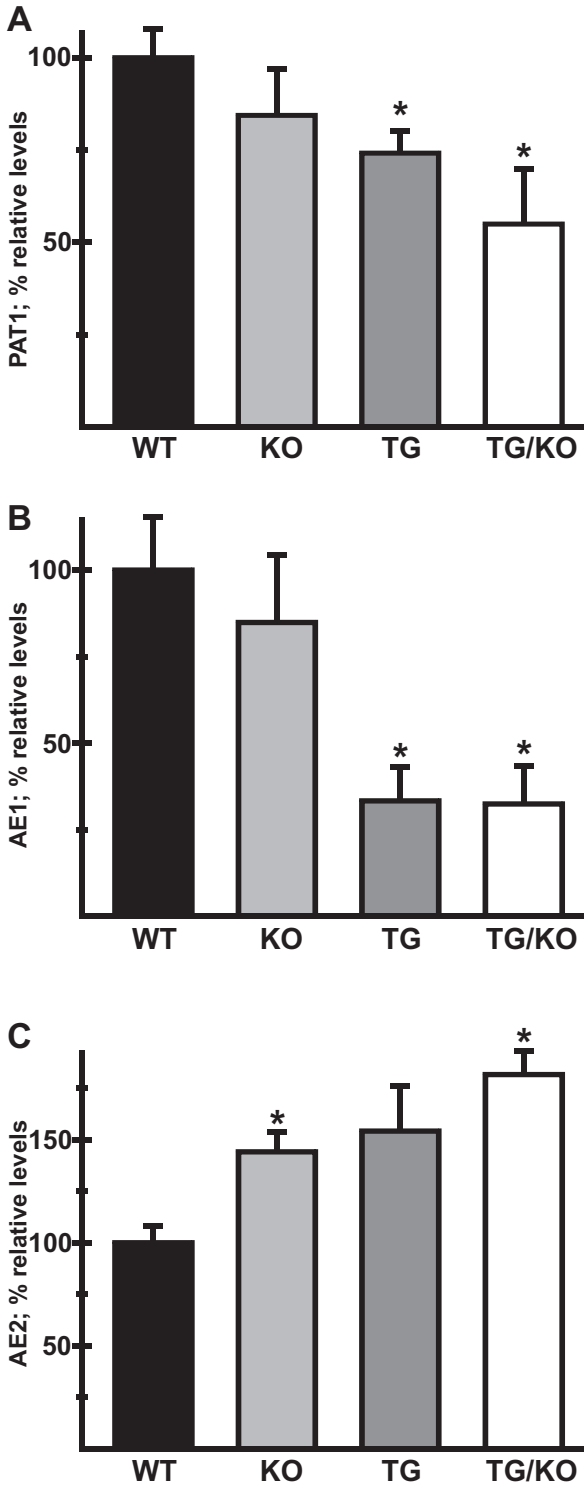
Supplementary Figures
Figure S1



Methods for Supplementary Figure

Real-time PCR analyses

Total RNA was isolated using Tri-reagent (Molecular Research Center; TR118). RNA concentration was quantified by UV spectrophotometry. RNA samples were stored at -80ºC until further use. cDNA was synthesized using oligo dT primers (for GAPDH and PAT-1) or random hexamers (for AE1 and AE2) in the SuperScript III first strand synthesis system (Invitrogen; 18080-051). Real-time PCR analysis was carried out using the iQ SYBR Green system (Bio-Rad Laboratories; 170-8882) and the Opticon2 DNA Engine (MJ Research Inc.). Levels of GAPDH, PAT1, and AE2 were determined using primers from PrimerBank [1,2] (GAPDH: PrimerBank ID 6679937a3; PAT1: Primer Bank ID 31981655a1; AE2: 10798998a1). AE1 levels were determined using the following primers: 5' CCTGCTTGTGCTAGGCTTCT 3' and 5' CTGGGTGTATCGGGA GATGT 3' to generate a 190 bp amplicon. This primer pair was designed, using the Primer3 (Ver. 4.0) and the NCBI Primer-BLAST programs, to identify all three known transcripts for AE1 [3,4]. Results obtained using this primer pair were validated by results obtained using primers from PrimerBank (PrimerBank ID 6755560a1), which identifies the full-length (erythroid) isoform of AE1. All reactions were run in triplicate. Results were analyzed using the Opticon Monitor Analysis Software (Ver. 2.02) and levels of PAT-1, AE1 and AE2 were normalized to GAPDH levels.

References for Supplementary Figure

1] Spandidos A, Wang X, Wang H, Seed B. PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification. Nucl Acids Res 2010; 38:D792-9.

2] Wang X and Seed B. A PCR primer bank for quantitative gene expression analysis. Nucl Acids Res 2003;31:1-8.

3] Kudrycki KE, Shull GE. Primary structure of the rat kidney band 3 anion exchange protein deduced from a cDNA. J Biol Chem 1989;264:8185-92.

4] Richards SM, Jaconi ME, Vassort G, Puceat M. A spliced variant of AE1 gene encodes a truncated form of Band 3 in heart: the predominant anion exchanger in ventricular myocytes. J Cell Sci 1999;112:1519-28.

Legend for Supplementary Figure

Fig. S1. Expression levels of PAT1 (*Slc26a6*), AE1, and AE2 in wild-type, AE3-null, TM180 and TM180/AE3 double mutant hearts. Real-time PCR analyses of total RNA isolated from wild-type (WT), AE3-null (KO), TM180 (TG) and TM180/AE3 (TG/KO) hearts was carried out as described in the supplementary methods section. Results, presented as percent levels, show expression-levels of PAT1 (A), AE1 (B), and AE2 (C) normalized to GAPDH, in wild-type and mutant hearts. n = 4-6 for each genotype, except for determination of AE1 levels in wild-type hearts (n = 3). $^{*}p < 0.05$ vs WT. The increase in AE2 levels in TG hearts did not achieve statistical significance (p = 0.059).