FIGURE LEGENDS FOR SUPPLEMENTARY FIGURES

Fig. S1. Restriction map of wild-type, mutant allele, and targeting vector. (A) Locations of Neo-specific forward and 3' reverse primer are shown by gray arrows. Uncut PCR product=9.2 kb shown by solid line. Location of probe D shown by thick black bar. Restriction products of a partial XbaI digest are shown as dashed and solid lines. Fully digested product=1.6kb, solid line. Incompletely digested products (IC) are shown as dashed lines and correspond to 3.1 and 7.7 kb. (B) Southern analysis of PCR products of ES cell DNA (and controls) with uncut and incomplete XbaI digestion probed with ^{32}P-labeled probe D. ES clone 70, lane 1, shows restriction fragments of expected size with partial digestion. Only one band is by probe 'D' without digestion (lane 8). Positive and negative controls are DNA from the targeting vector and SVJ-ES cells, respectively. Non-recombinant ES clones 72, 105, 141 show no band. (C). DNA harvested from mouse tails was digested with EcoRV (see map in Fig. 1; dashed lines) and probed with 5' probe A using an ECL detection method (CDPStar, GE Healthcare UK, Buckinghamshire, UK). Wild-type band of 9.2kb is identified, as well as a 5.3kb band in mice heterozygous for the mutant allele (lane 2).

Fig. S2. Expression of AKAP13 in adult cardiac tissues. (A) Section through adult heart stained with 1:1000 dilution of 4362. Strong brown staining was present, indicating presence of AKAP13 protein in mature cardiac tissue. Magnification=12X. (B) Section through adult heart reacted with 1:1000 dilution of pre-immune sera (rabbit 4362) as a control. Faint, non-specific light brown staining is noted throughout this control section. (C) Enlarged section of heart stained with 4362 anti-sera showing localization of protein to sarcomeres. (D) Immunoblot of lysates from H9C2 cells stained with 4362 anti-sera directed against AKAP13 (murine BRX). A band migrating with an apparent molecular size of 210-220 kDa was detected (arrow). (F) Immunogold staining of cardiac tissue harvested from a wild-type embryo at E9.5-10.0 and reacted with 4362 antisera. Representative field showind localization of AKAP13 to Z-discs of sarcomeres (bar=0.2μm). Black dots indicate the presence of AKAP13 protein.

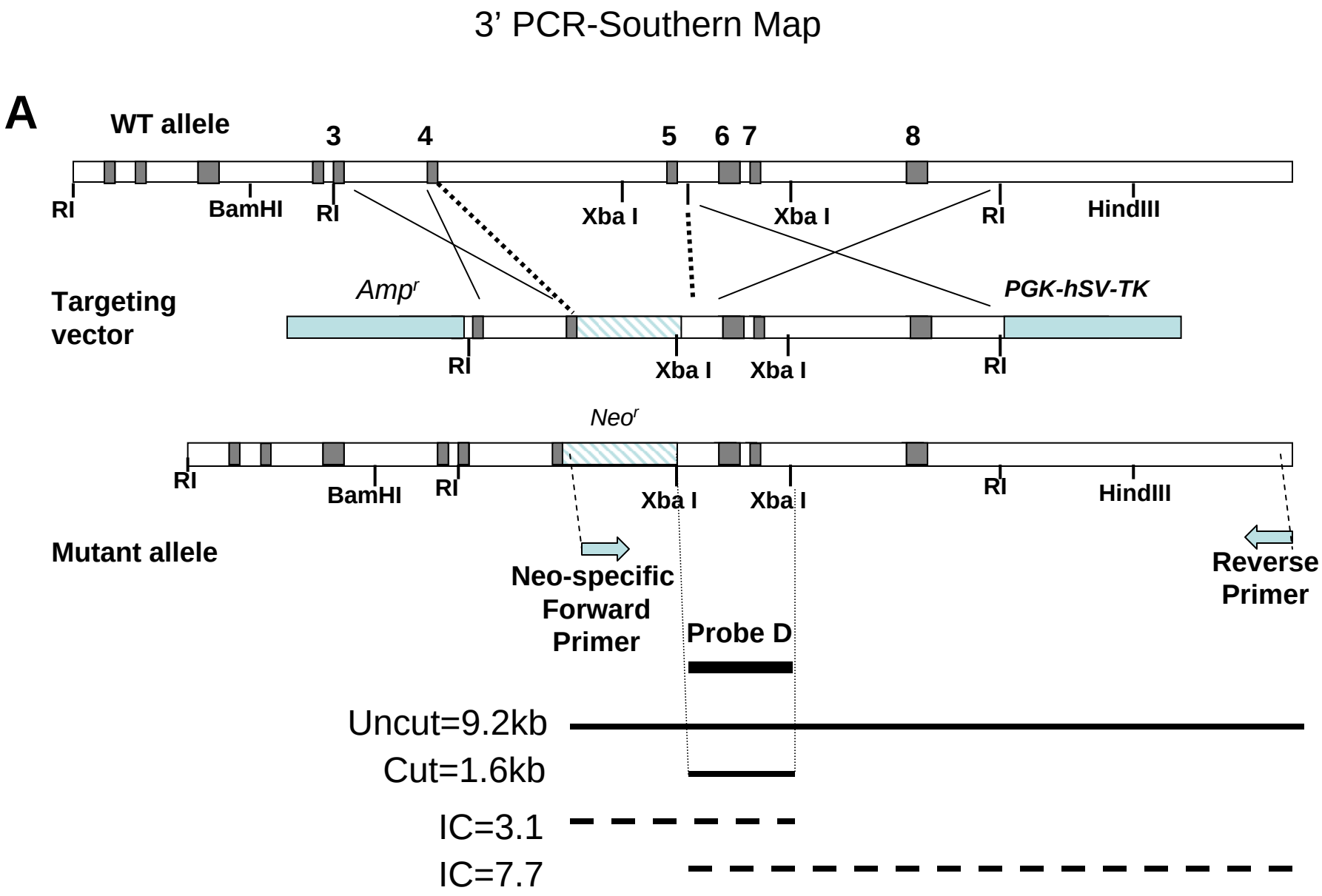
Fig. S3. TUNEL assay of transverse sections of developing embryos containing cardiac tissues at E9.0-9.5. (A) DAPI stained section from wild-type embryo E9.0-9.5. (B) Transverse section from wild-type embryo (shown also in 2M). (C) Tranverse section through AKAP13-null embryo showed no increase in apoptosis (also shown in Fig. 2N). (D) Positive control for assay of wild-type embryo treated with DNASE I (Worthington Biochemicals) for 10 min. at room temperature. (E) Negative control showed no staining. Similar findings were observed in three assays.

Fig. S4. Inactivation of AKAP13 does not reduce *GATA-4* transcripts or protein in the developing heart. (A) Endogenous AKAP13 is not required for optimal GATA-4 expression. H9C2 cells were transfected with control or AKAP-Brx siRNA as indicated. Total RNA was purified and amounts of *AKAP13* or *GATA-4* were determined by real-time RT-PCR. Relative expression levels of mRNA are shown as fold induction over baseline. Results were confirmed in three separate experiments. Error bars=s.e.m. *P* values as indicated. NS=not significant. (B) Immunohistochemical staining of transverse section from wild-type embryo heart at E9.5-10 using antisera directed against GATA-4. Bar=100 microns. (C) Immunohistochemical staining of section from embryo homozygous for the *AKAP13*-null allele reacted with antisera directed against GATA-4. The myocardium is collapsed in this specimen (a common fixation problem due to the extremely thin and fragile myocardium), but staining was not reduced compared to wild-type myocardium (compare open arrows). A pericardial effusion was apparent (black arrowhead). Bar=100 microns.

Fig. S5. AKAP13 expression plasmid did not augment basal activity of the Gal4E1b-luciferase reporter. H9C2 cells grown on 24-well plates to 50% confluence were transfected with 500 ng Gal4E1b-Luc, and 0, 400, or 500 ng AKAP13 expression vector, as indicated. Following overnight incubation, cells were lysed and luciferase assays were performed. Results are from two independent experiments. Error bars=s.e.m.

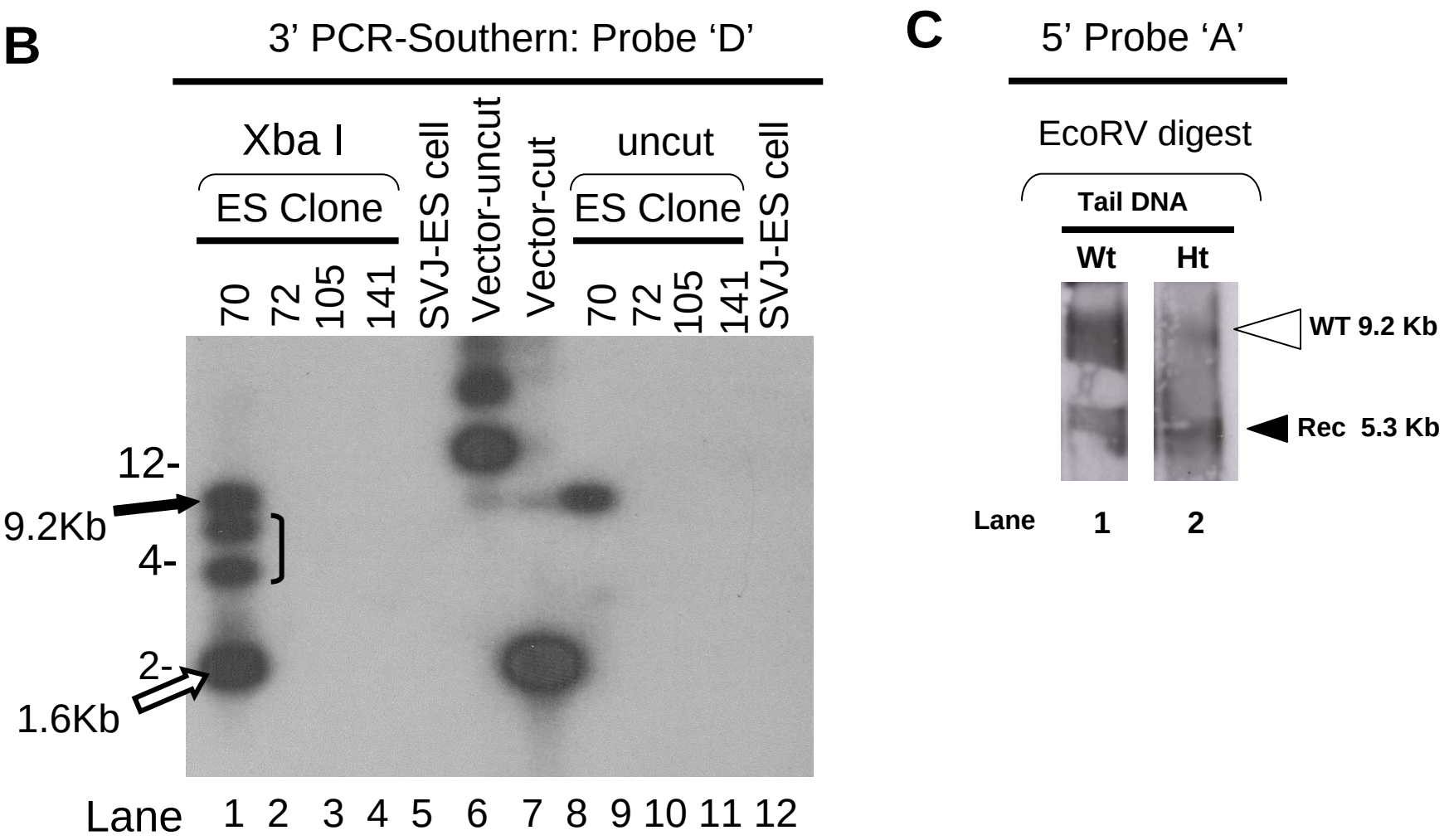
Supplemental Figure S1

Mayers et al_Fig. S1A



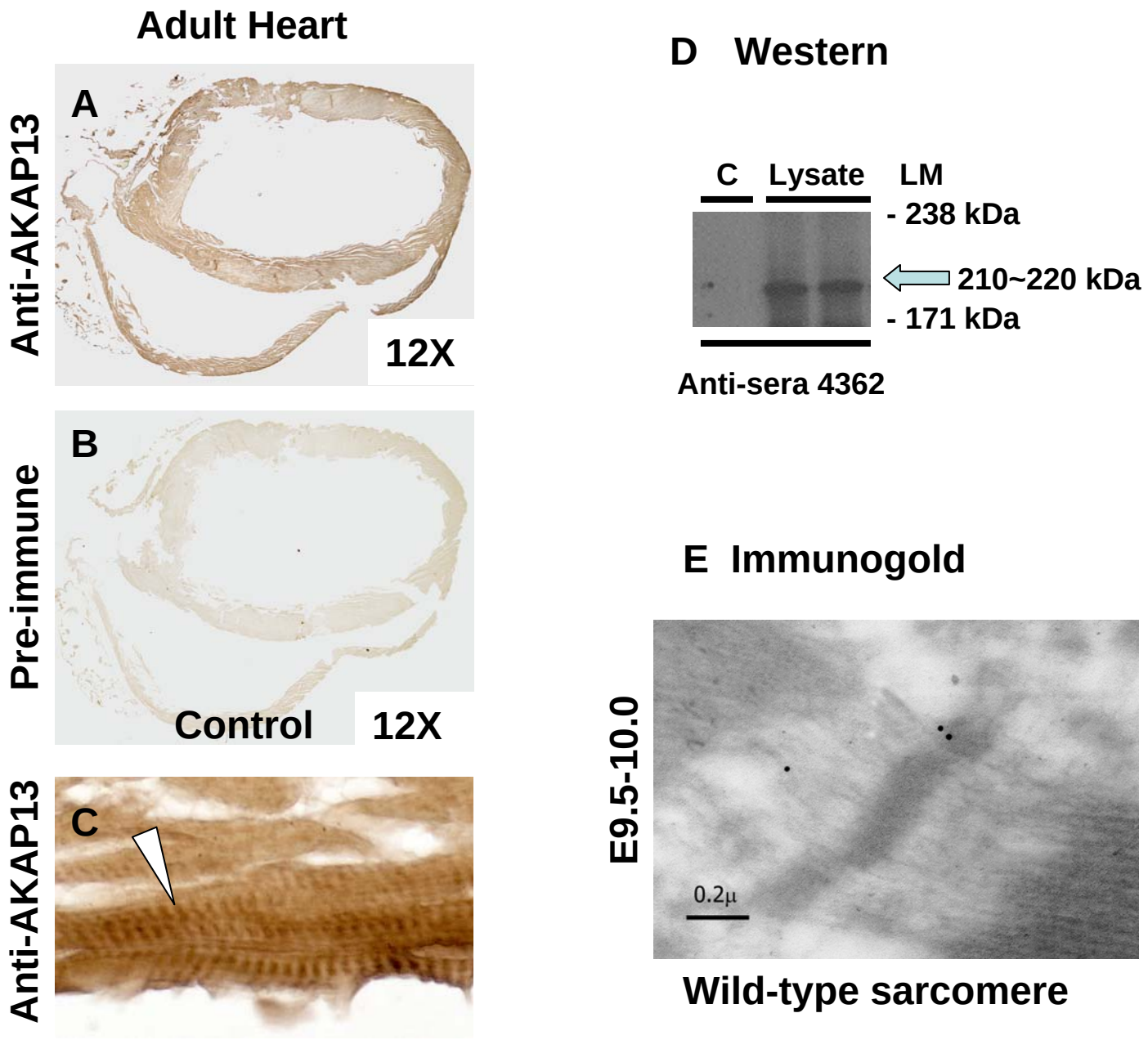
Supplemental Figure S1

Mayers et al_Fig. S1B,C

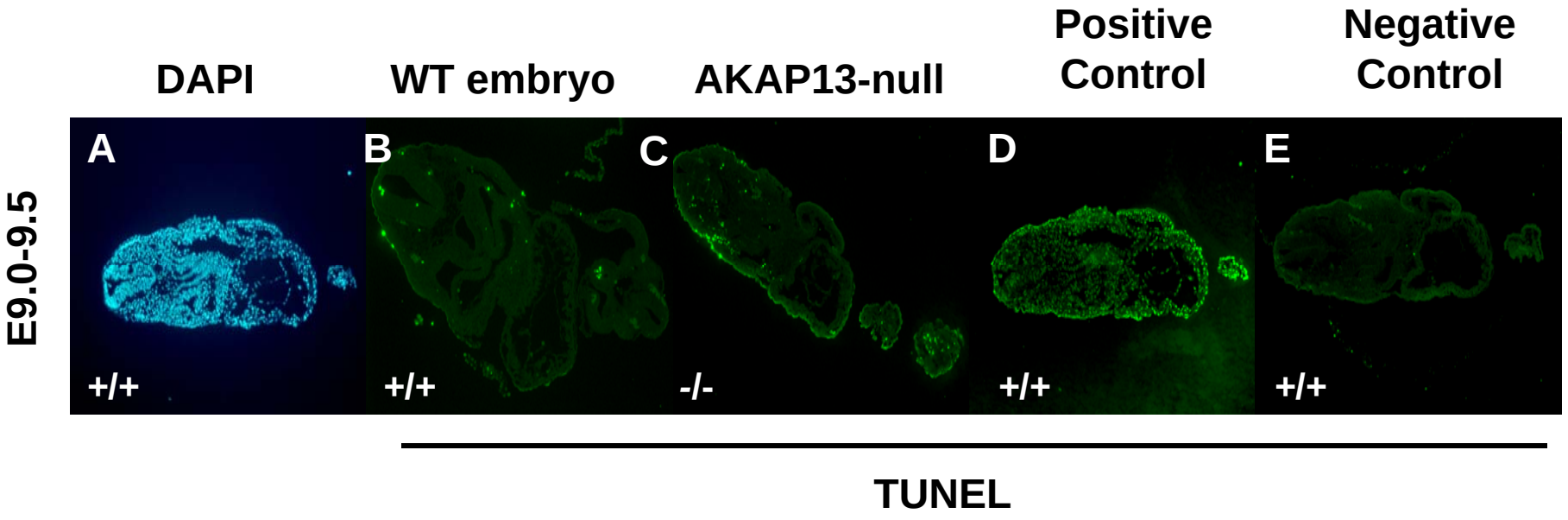


Supplemental Figure S2

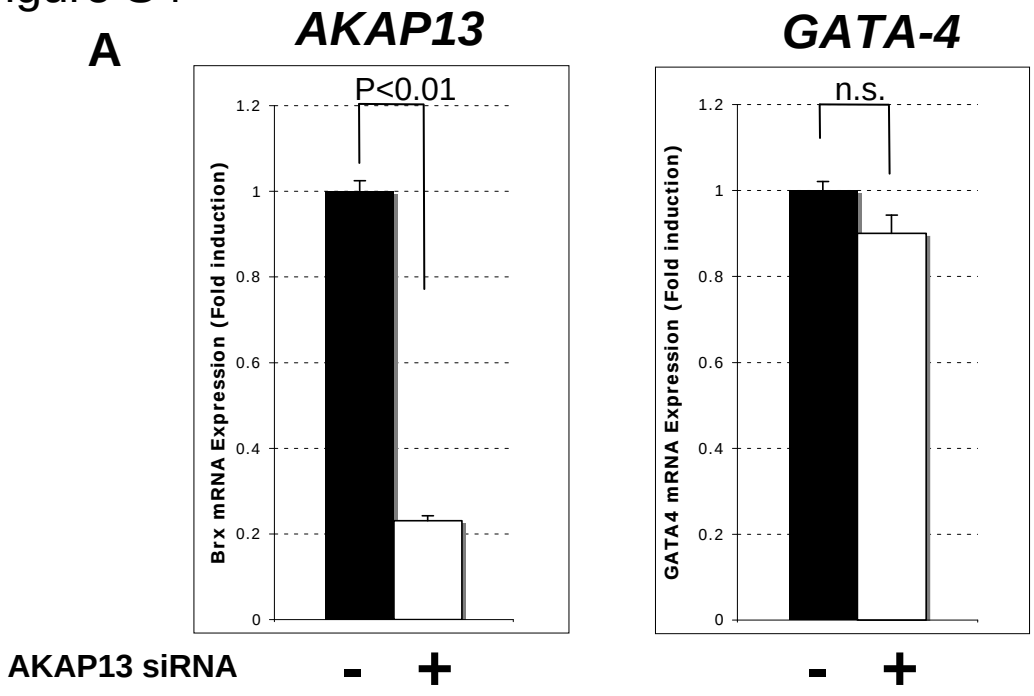
Mayers et al_Fig. S2

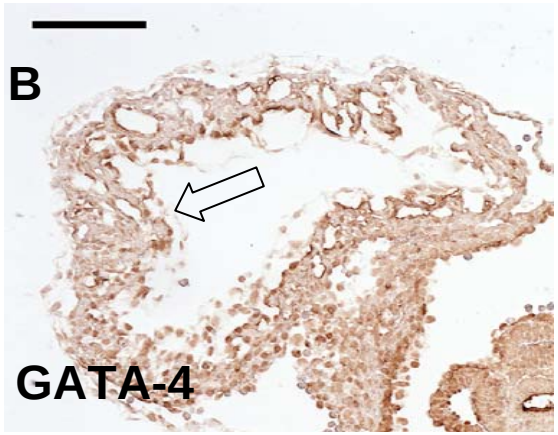


Supplemental Figure S3

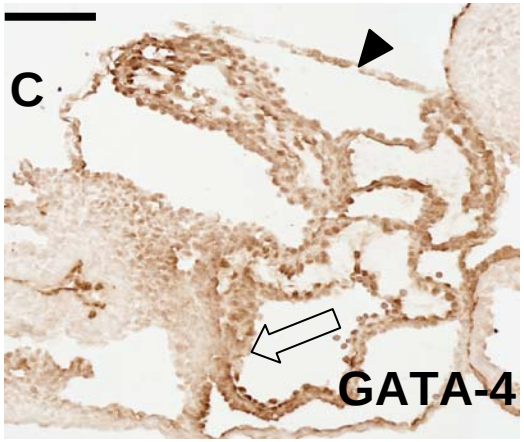


Supplementary Figure S4





Wild-type embryo



AKAP13-null embryo

Supplementary Figure S5