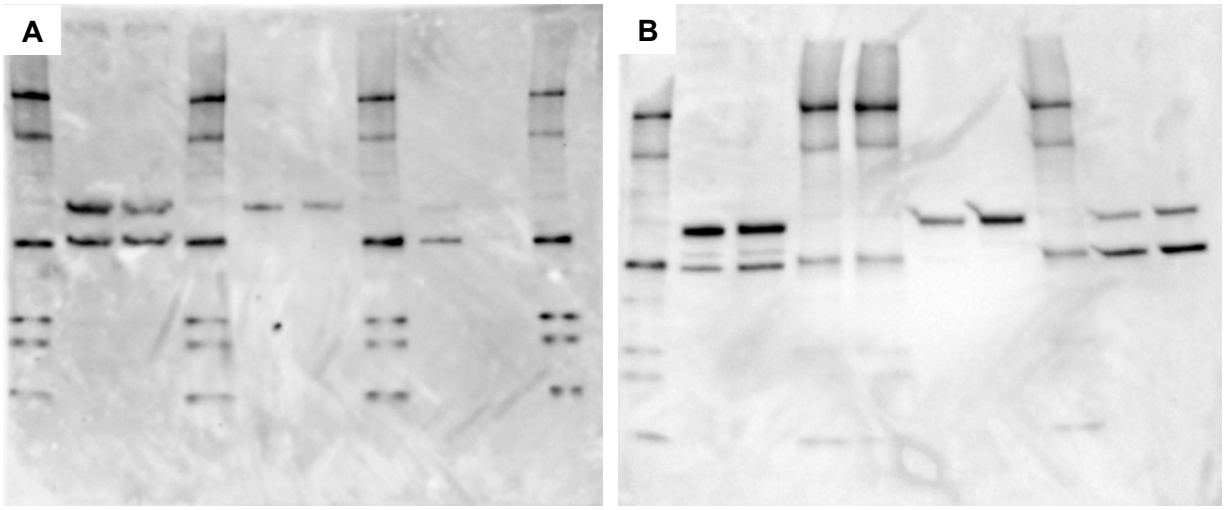

Fig. S1. Primary CA-IV antibody validation using 4-day control and hypoxia heart tissue. Two CA-IV antibodies were tested at 1:1,000 concentration: (A) red drum CA4-1 (B) red drum CA4-2. Red drum CA4-2 was used for the current study. Tissues were processed with RIPA buffer or Mem-PER Plus membrane protein extraction kit. For (A), protein loaded in lanes 2 and 3 were processed using RIPA buffer, protein loaded in lanes 5 and 6 were isolated from the cytoplasmic fraction of the Mem-PER Plus isolation, and protein loaded in lanes 8 and 9 were isolated from the membrane fraction of the Mem-PER Plus isolation. For (B), protein loaded in lanes 2 and 3 were processed using RIPA buffer, protein loaded in lanes 6 and 7 were isolated from the cytoplasmic fraction of the Mem-PER Plus isolation, and lanes 9 and 10 were isolated from the membrane fraction of the Mem-PER Plus isolation. 10 µg of protein was loaded for each sample, and each set of tissues consisted of one control and one hypoxia tissue (e.g., lanes 2 and 3 are control and hypoxia 4-day heart samples, respectively). Precision Plus Protein™ Unstained protein standards (Bio-Rad) were used for the ladders.

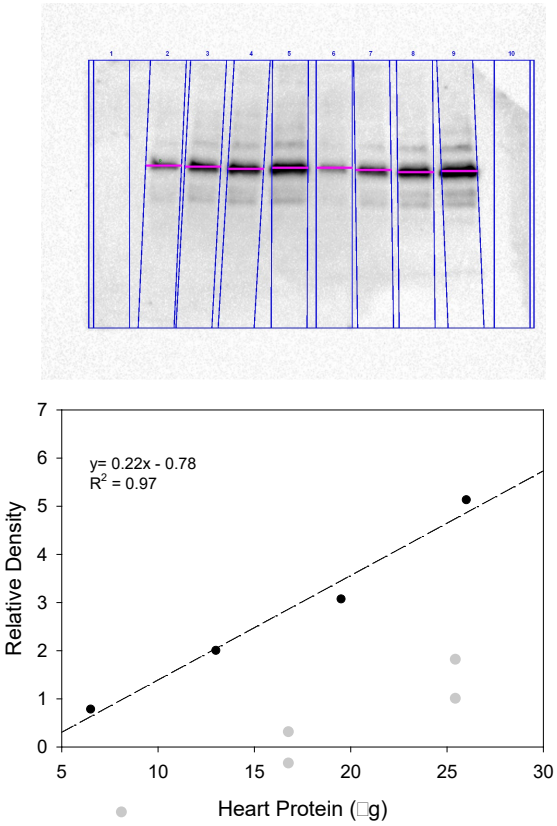

Fig. S2. CA-IV antibody linear response validation using control heart tissue. (A) Western blot demonstrated the linearity of the CA-IV antibody response across a range of heart protein concentrations. Note that in this non-experimental sample the 56 kDa band was dominant relative to the 43 kDa band, and as such the linearity was calculated only relative to this band. (B) The calculated linear function and R^2 based on the relative densities across the loaded total heart protein demonstrated linearity of the CA-IV antibody response in relation to quantity of protein. Black dots represent the mean at each total protein concentration, and gray dots represent the individual data points (n=2 per protein concentration). All bands are calculated relative to the band in lane 2.

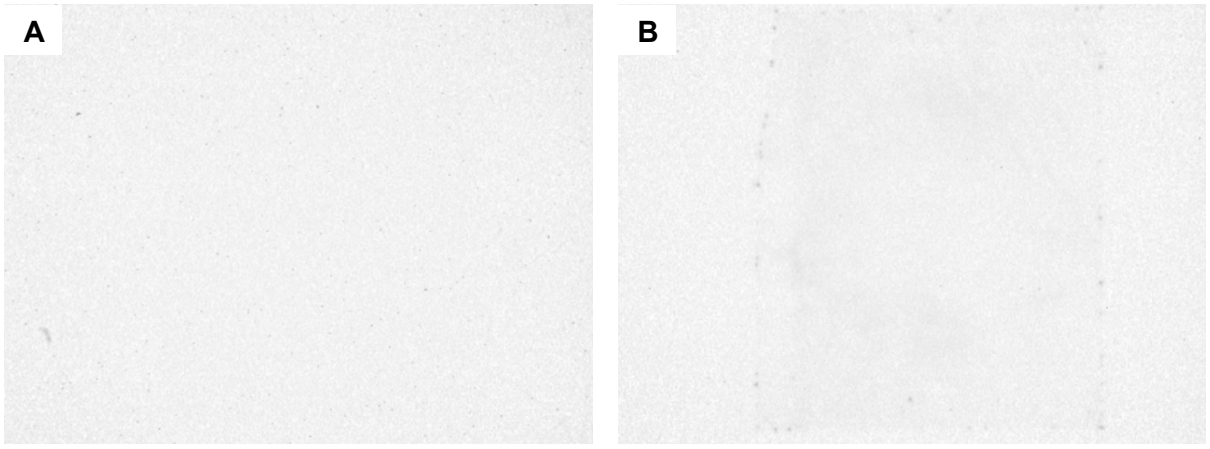

Fig. S3. Primary CA-IV antibody validation using (A) antigen excess and (B) no primary controls. 10 µg of heart protein was loaded for each sample, and each set of tissues consisted of one control and one hypoxia tissue. (A) Antigen excess control consisted of incubating antibody with 10x antigen provided from the manufacturers (GenScript) overnight at 4°C. Western blot protocol was performed as described in Materials and Methods section. No bands were present for either control demonstrating that the primary CA-IV antibody is specific to CA-IV protein and responsible for the bands present in Figure S1.

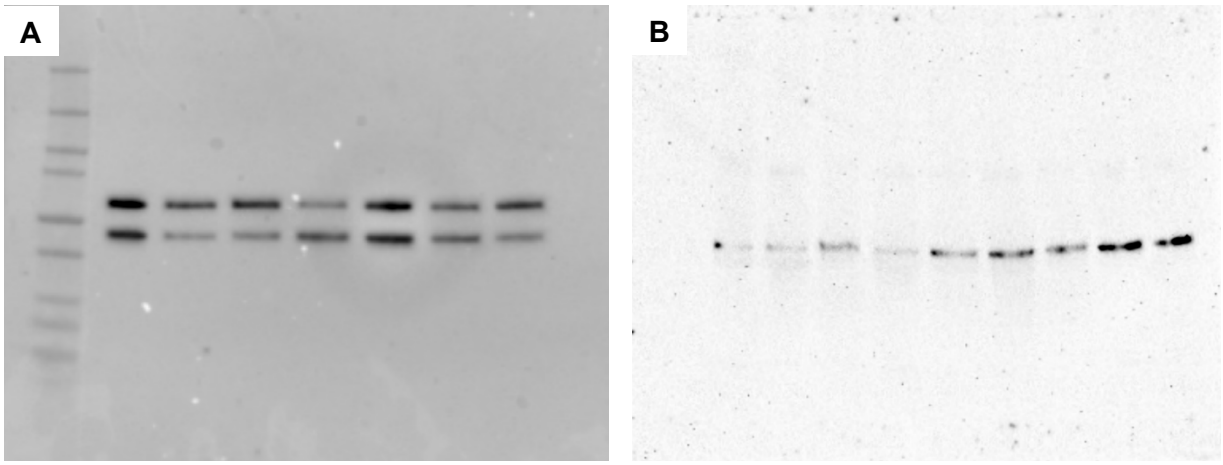

Fig. S4. Representative Western blot images for CA-IV protein at 8 days in the (A) heart and (B) red muscle. (A) A composite image of the Precision Plus Protein™ Dual Color ladder (Bio-Rad) under UV illumination and chemiluminescent image of CA-IV protein demonstrates the weight of each protein band (composite image not used for protein quantification).

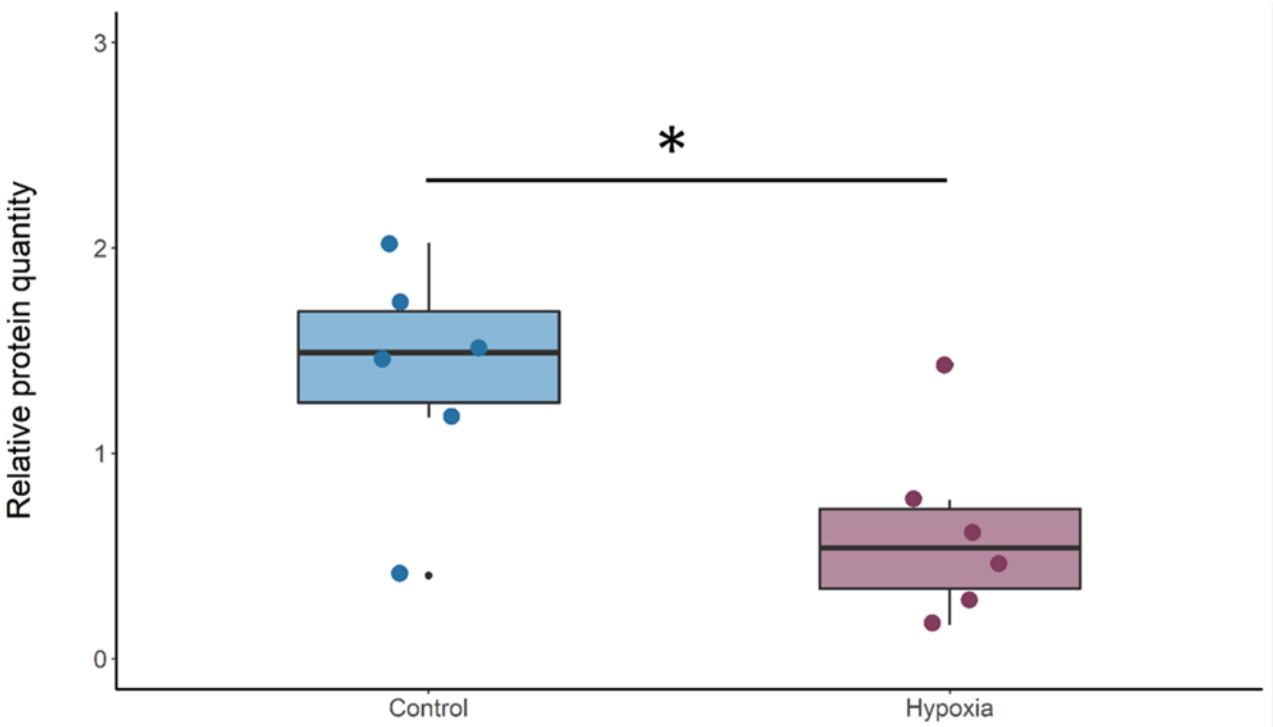

Fig. S5. Comparison of immature CA-IV protein quantity in the heart of red drum acclimated to control seawater or hypoxia for 8 days. There were significant differences in the immature CA-IV protein between treatments for heart (student's t-test, P=0.03). Quantities were calculated relative to a control 8-day (C1A8D) heart sample. Relative quantities were calculated for both for CA-IV protein content and total protein content of each sample. Relative CA-IV protein content was normalized to relative total protein content for each sample to account for any overall reduction in protein due to hypoxia exposure. Therefore, the relative protein quantity of C1A8D is 1. Relative protein quantities were assessed for the 56 kDa band, which we consider the immature form of CA-IV protein. All data are represented as box plots, where the minimum, first quartile, median, third quartile and maximum are presented for each treatment (control presented in blue, hypoxia presented in pink). Asterisk denotes significant difference between treatments. Individual data are overlayed (n=6 per treatment).