

# An Immunohistochemical Tool to Visualize Hydroxyproline in FFPE Tissue

## INTRODUCTION

Hydroxyproline is a major component of and is expressed almost exclusively in collagen. Measurement of hydroxyproline content in tissues is a commonly used means of quantifying collagen in the study of fibrosis. Classical assays used to quantify hydroxyproline in tissue require tedious sample processing steps that utilize hazardous, toxic and highly reactive chemicals that require special handling and disposal<sup>1,2</sup>.

Here we describe a means of visualizing hydroxyproline in paraffin-embedded tissues using a routine immunohistochemical assay that is easily implemented by any laboratory. Using our hydroxyproline antibody in the IHC assay, we can detect global collagen in tissue, providing contextual information that is lost in traditional hydroxyproline assays. Additionally, the hydroxyproline antibody can be combined with other antibodies to enable detection of other markers of interest, something that is not easily achievable with the Sirius Red stain, a conventional means of global collagen detection<sup>3</sup>.

## METHODS

**Single Stain:** FFPE tissues were deparaffinized and rehydrated, subjected to antigen retrieval, then incubated overnight at 4°C with Hydroxyproline Antibody #73812 or COL1A1 (E8F4L) Rabbit mAb diluted in SignalStain® Antibody Diluent #8112. Detection was performed using SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114 and SignalStain® DAB Substrate Kit #8059.

**Dual Stain:** Deparaffinized and rehydrated sections were subjected to antigen retrieval, then incubated 1 hour at room temperature with E-Cadherin (4A2) Mouse mAb #14472 and Hydroxyproline Antibody #73812 diluted in SignalStain® Antibody Diluent #8112. Detection was performed using SignalStain® Boost IHC Detection Reagent (HRP, Mouse) #8125 and DAB Substrate Kit #8059, followed by SignalStain® Boost IHC Detection Reagent (AP, Rabbit) #18653 and SignalStain® Vibrant Red Alkaline Phosphatase Substrate Kit #76713.

C57BL/6NTac mice (Taconic Biosciences) were fed a diet with 5% fat (control mice) or Amylin liver NASH (AMLN) diet (Diet # D09100310i) for 28 weeks to induce NASH.

## CONCLUSIONS

- Hydroxyproline Antibody enables visualization of global collagen in FFPE tissue samples by a routine IHC assay.

- Hydroxyproline Antibody allows for species-independent detection of global collagen in FFPE tissues.

- Hydroxyproline Antibody can be combined with other antibodies to enable visualization of collagen along with other markers of interest.

## REFERENCES

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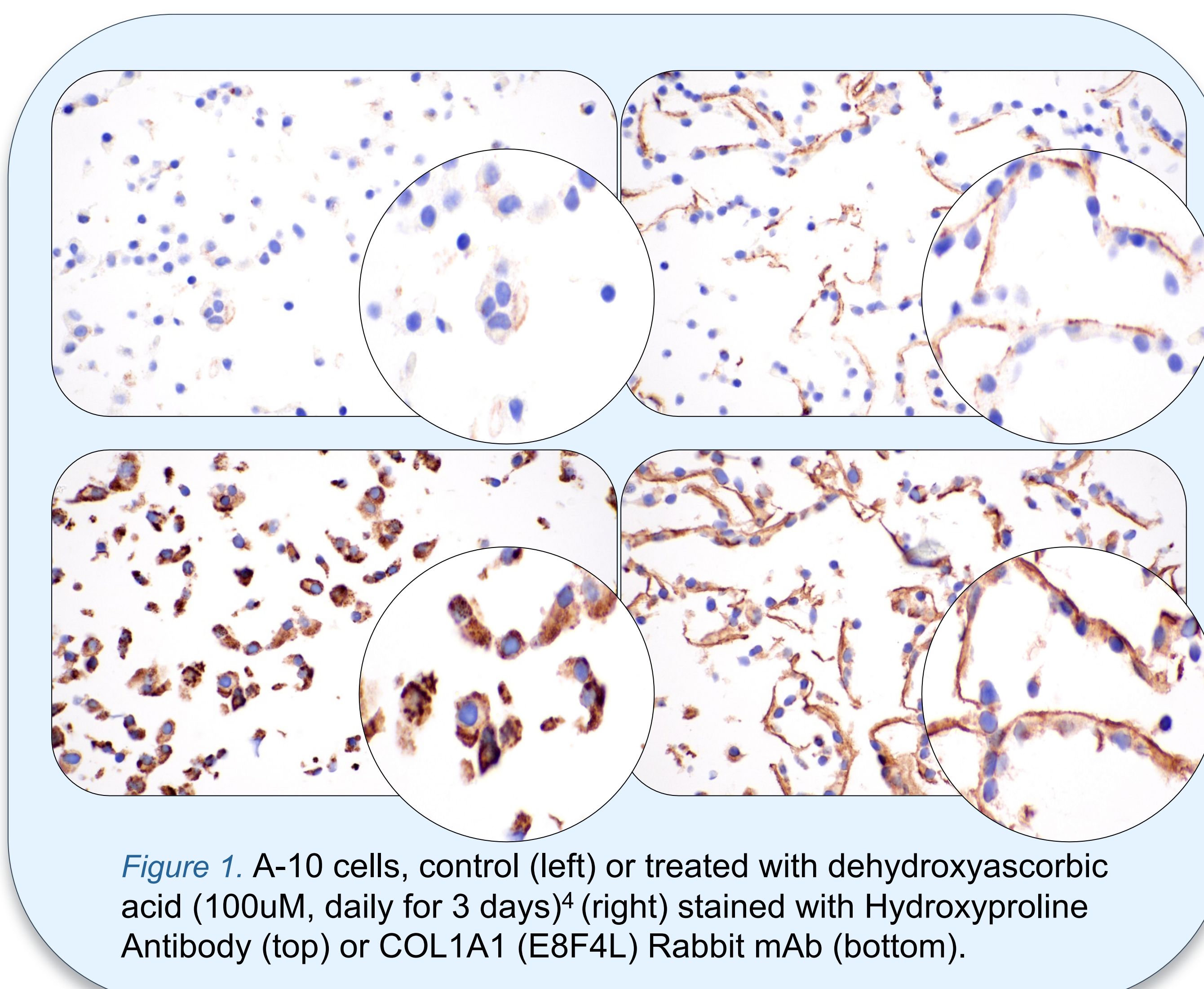


Figure 1. A-10 cells, control (left) or treated with dehydroxyascorbic acid (100uM, daily for 3 days)<sup>4</sup> (right) stained with Hydroxyproline Antibody (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

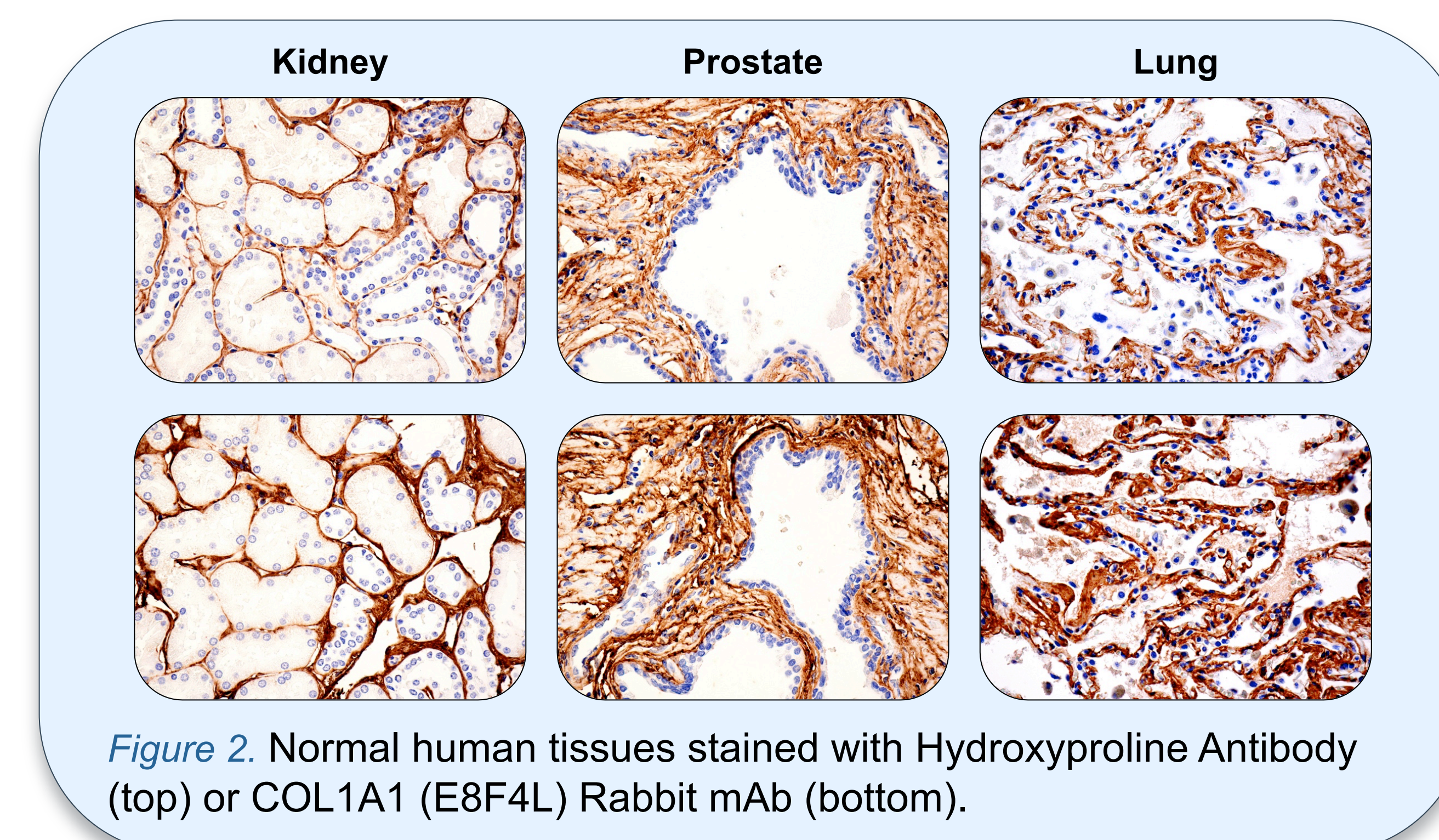


Figure 2. Normal human tissues stained with Hydroxyproline Antibody (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

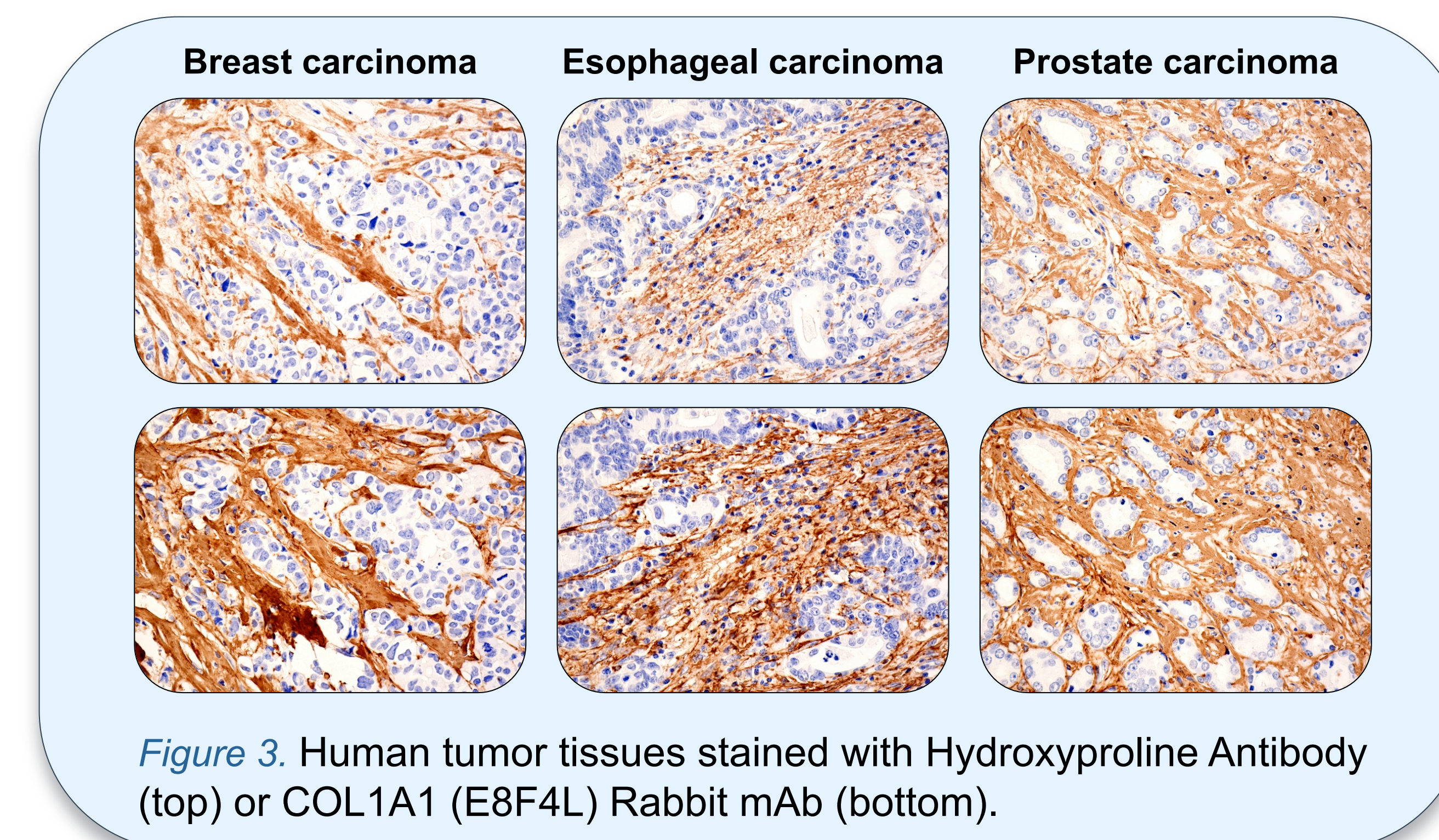


Figure 3. Human tumor tissues stained with Hydroxyproline Antibody (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

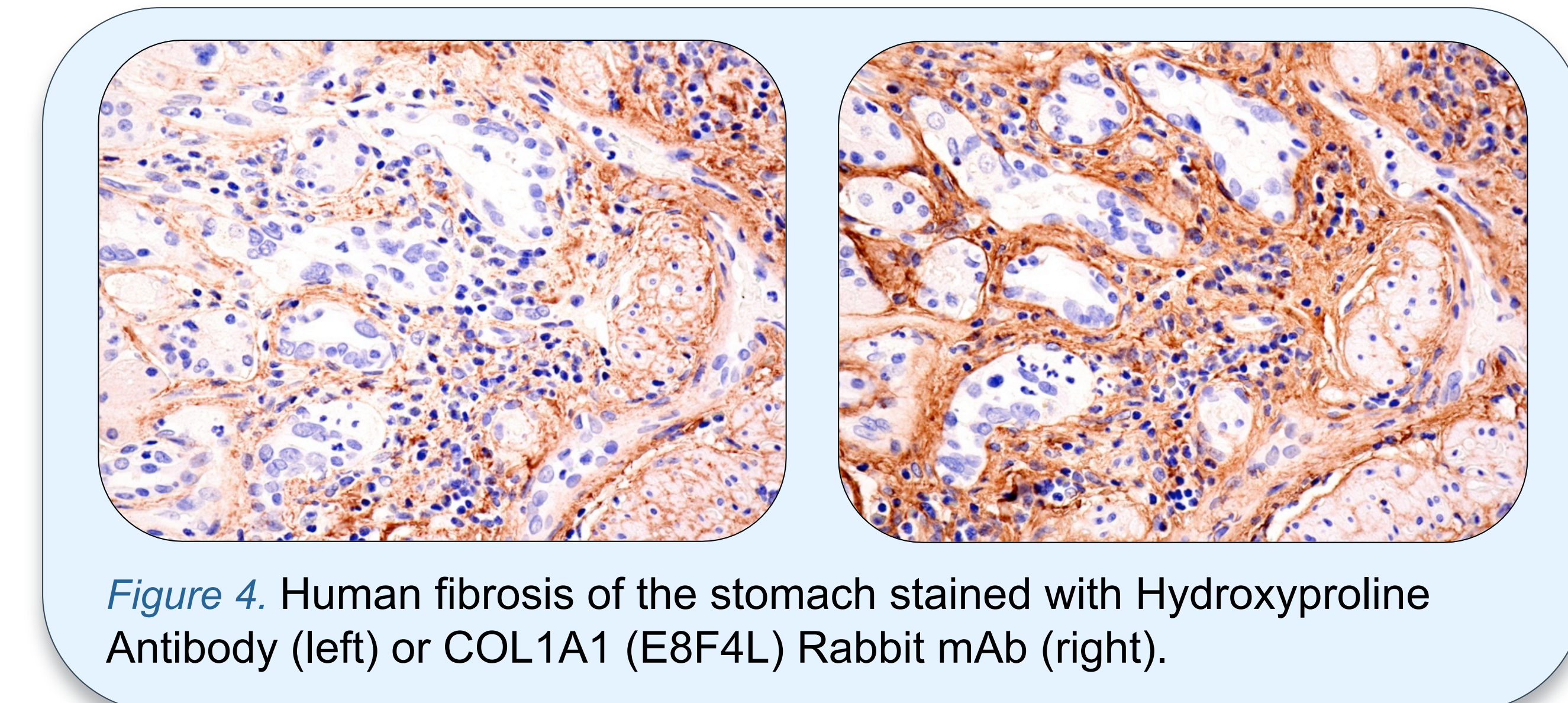


Figure 4. Human fibrosis of the stomach stained with Hydroxyproline Antibody (left) or COL1A1 (E8F4L) Rabbit mAb (right).

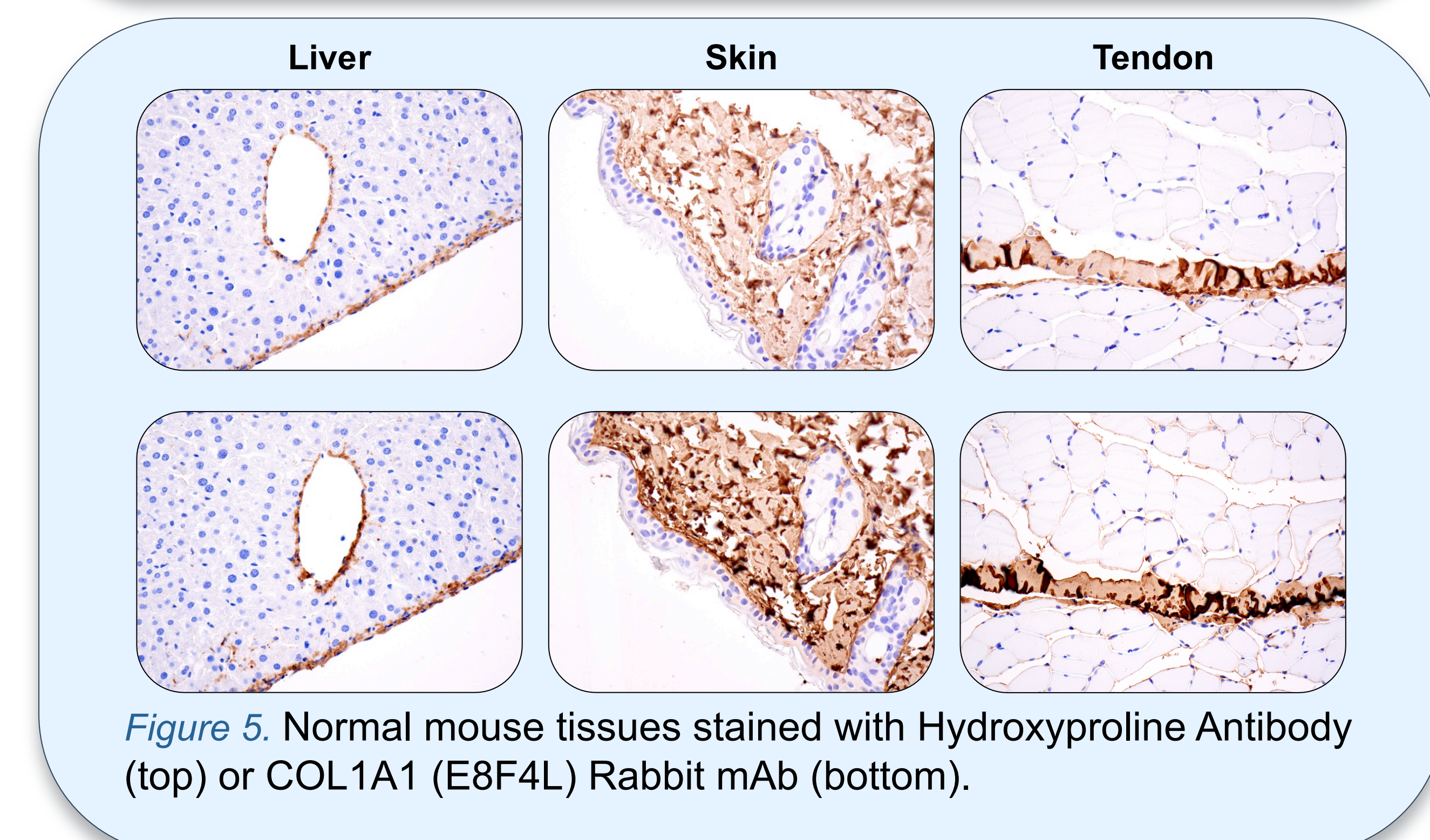


Figure 5. Normal mouse tissues stained with Hydroxyproline Antibody (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

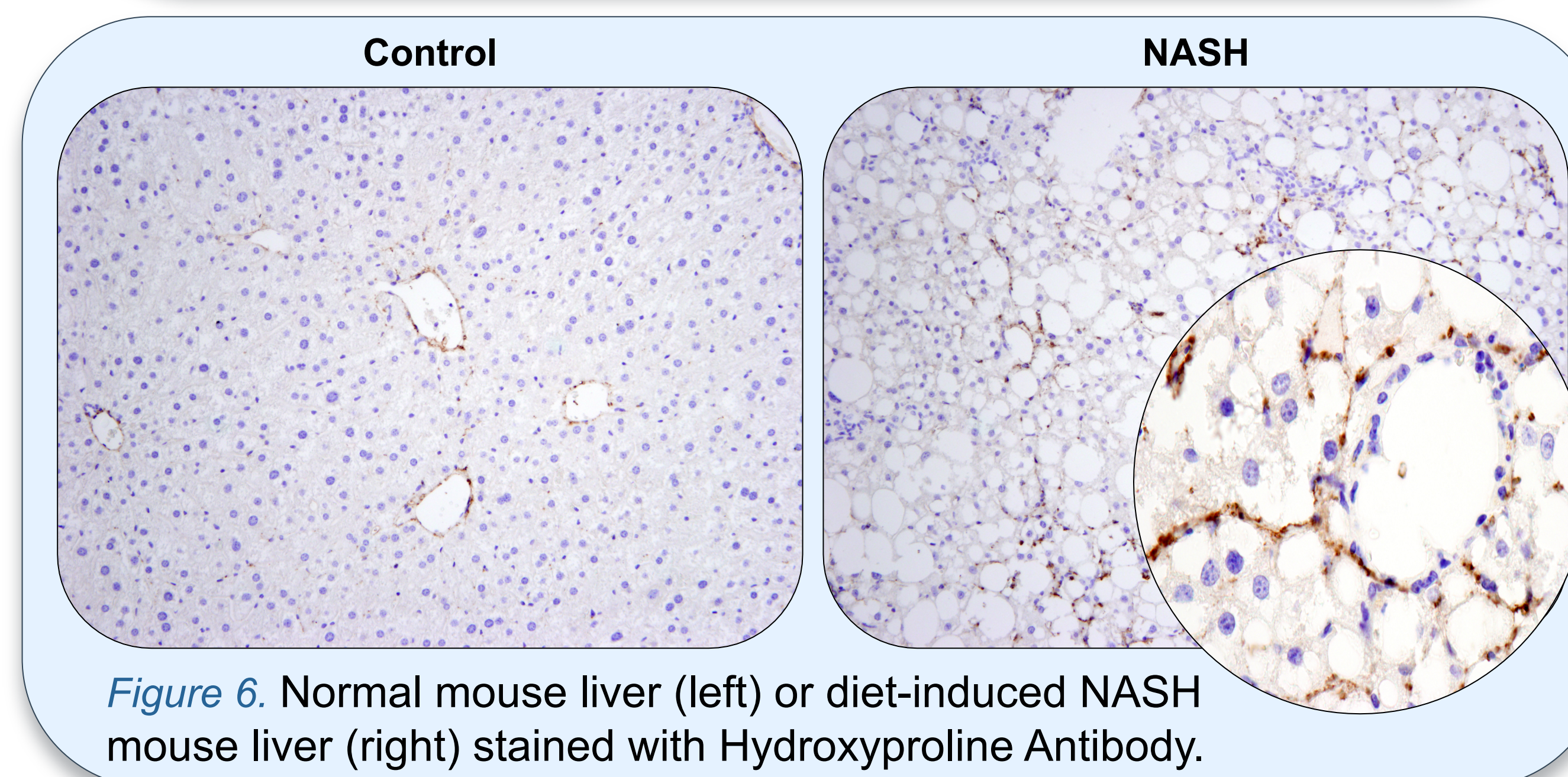


Figure 6. Normal mouse liver (left) or diet-induced NASH mouse liver (right) stained with Hydroxyproline Antibody.

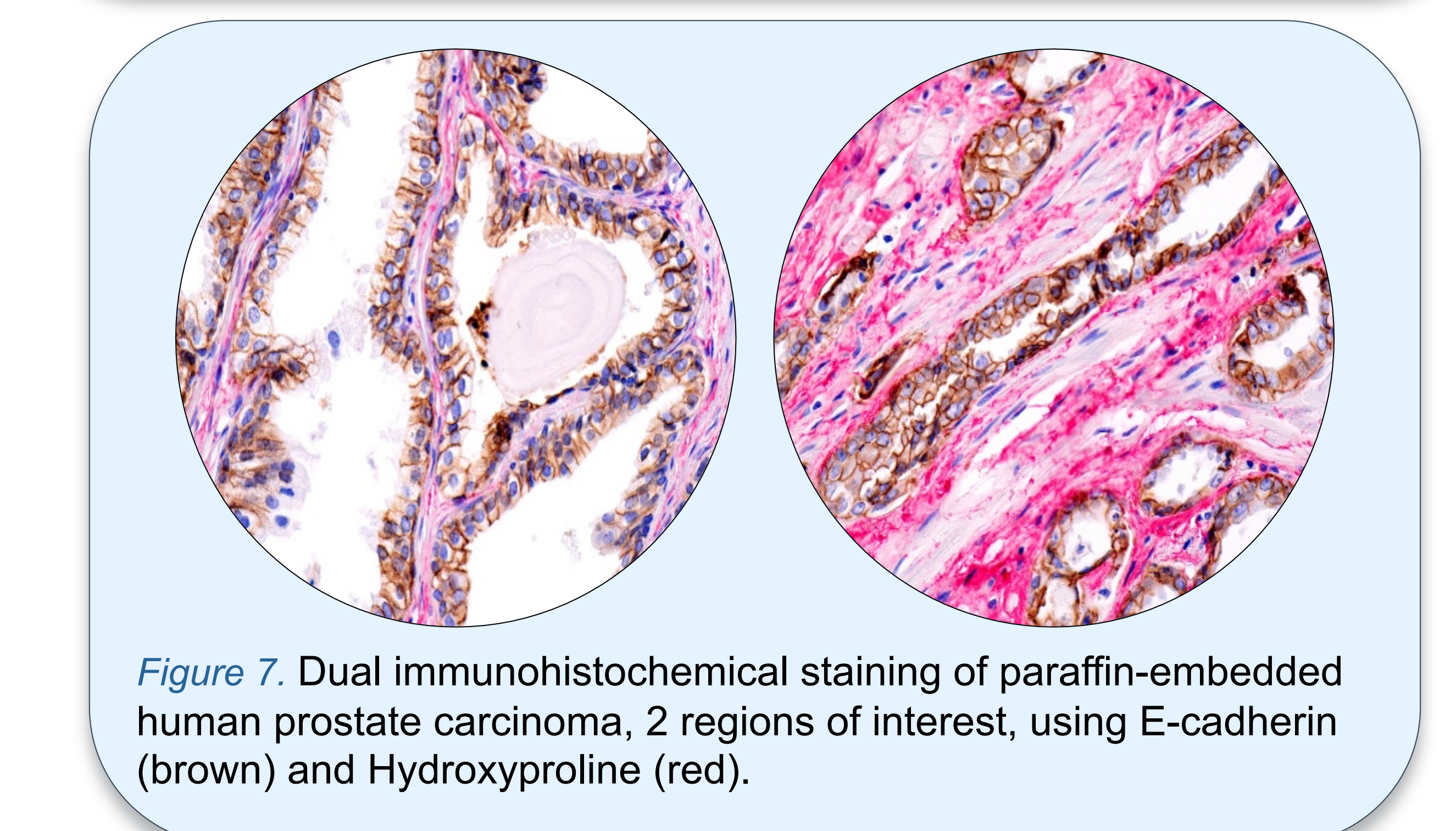


Figure 7. Dual immunohistochemical staining of paraffin-embedded human prostate carcinoma, 2 regions of interest, using E-cadherin (brown) and Hydroxyproline (red).

