Idiopathic Pulmonary Fibrosis: Registration of extracellular matrix components comprising the fibroblastic focus

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Supplemental Online Material

Supplemental Figure Legends:

Supplemental Table I: List of antibodies used for immunohistochemistry. Antibodies and the titers used are listed here as described in the materials and methods.

Supplemental Figure 1. 3-D reconstruction of collagen I, αSMA, and pro-collagen I immunostains of the IPF fibroblastic focus. (**A**) 9 serial sections (5 µm each) of one IPF specimen were immunostained in sequence for collagen I (left panels), αSMA (middle panels), and pro-collagen I (right panels) spanning 45 µm. (**B**) Each immunostain in (A) was color-separated and anatomically aligned for collagen I (left panel), αSMA (middle panel), and pro-collagen I (right panel). (**C**) The aligned images in (B) were pseudo-colored [collagen I (red), αSMA (green), and pro-collagen I (blue)] and combined into a flattened image. Scale bars represents 100 µm.

Supplemental Figure 2. 3-D reconstruction of collagen I, collagen V, and hyaluronan immunostains of the IPF fibroblastic focus. (**A**) 9 serial sections (5 µm each) of one IPF specimen were immunostained in sequence for collagen I (left panels), collagen V (middle panels), and hyaluronan (right panels) spanning 45 µm. (**B**) Each immunostain in (A) was color-separated and anatomically aligned for collagen I (left panel), collagen V (middle panel), and hyaluronan (right panel). (**C**) The aligned images in (B) were pseudo-colored [collagen I (red), collagen V (green), and hyaluronan (blue)] and combined into a flattened image. Scale bars represents 100 µm.

Supplemental Figure 3. 3-D reconstruction of collagen I, fibronectin, and collagen VI immunostains of the IPF fibroblastic focus. (**A**) 9 serial sections (5 µm each) of one IPF specimen were immunostained in sequence for collagen I (left panels), fibronectin (middle panels), and collagen VI (right panels) spanning 45 µm. (**B**) Each immunostain in (**A**) was color-separated and anatomically aligned for collagen I (left panel), fibronectin (middle panel), and collagen VI (right panel). (**C**) The aligned images in (**B**) were pseudo-colored [collagen I (red), fibronectin (green), and collagen VI (blue)] and combined into a flattened image. Scale bars represent 100 µm.

Supplemental Figure 4. Two patterns of collagen IV immunoreactivity in the IPF fibroblastic focus. FFPE serial sections of 8 IPF specimens were immunostained for collagen I (left panels) and collagen IV (middle panels) with high magnification insets of collagen IV (right panels). IPF 1 through 4 show characteristic patchy/layering of collagen IV as previously described [26] where immunoreactivity is stronger towards the airspace. IPF 5 through 8 show collagen IV immunoreactivity throughout the myofibroblast core as previously described [25]. As an internal control, collagen IV immunoreacts with blood vessels (shown in red asterisks). Scale bars represent 100 µm.

Supplemental Figure 5. 3-D reconstruction of collagen I, collagen IV, and versican immunostains of the IPF fibroblastic focus. (**A**) 9 serial sections (5 µm each) of one IPF specimen were immunostained in sequence for collagen I (left panels), collagen IV

(middle panels), and versican (right panels) spanning 45 µm. (**B**) Each immunostain in (**A**) was color-separated and anatomically aligned for collagen I (left panel), collagen IV (middle panel), and versican (right panel). (**C**) The aligned images in (**B**) were pseudo-colored [collagen I (red), collagen IV (green), and versican (blue)] and combined into a flattened image. Scale bars represents 100 µm.

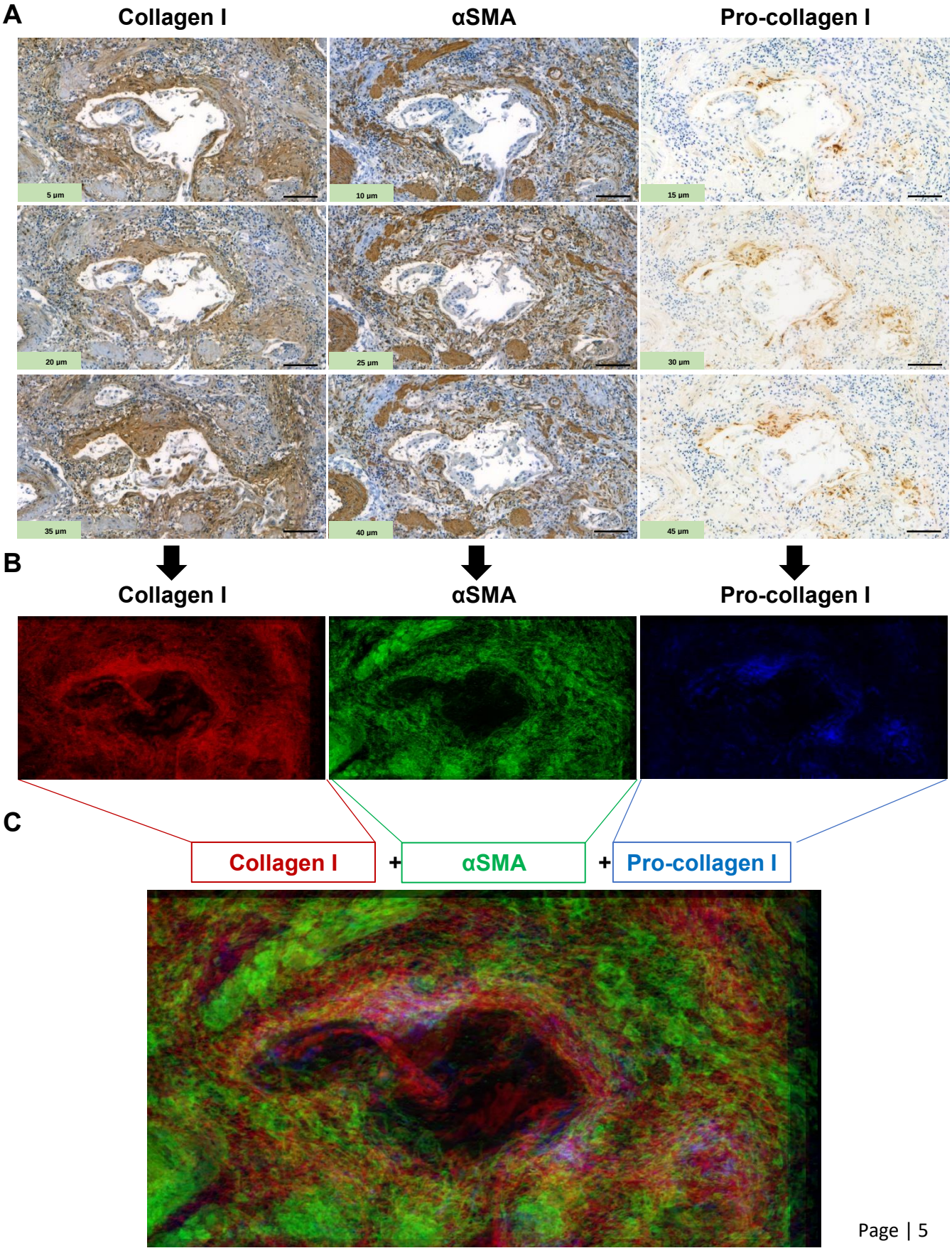
Supplemental Figure 6. 3-D reconstruction of collagen I, collagen III, and fibrinogen immunostains of the IPF fibroblastic focus. (**A**) 9 serial sections (5 µm each) of one IPF specimen were immunostained in sequence for collagen I (left panels), collagen III (middle panels), and fibrinogen (right panels) spanning 45 µm. (**B**) Each immunostain in (**A**) was color-separated and anatomically aligned for collagen I (left panel), collagen III (middle panel), and fibrinogen (right panel). (**C**) The aligned images in (**B**) were pseudo-colored [collagen I (red), collagen III (green), and fibrinogen (blue)] and combined into a flattened image. Scale bars represents 100 µm.

Supplemental Table I

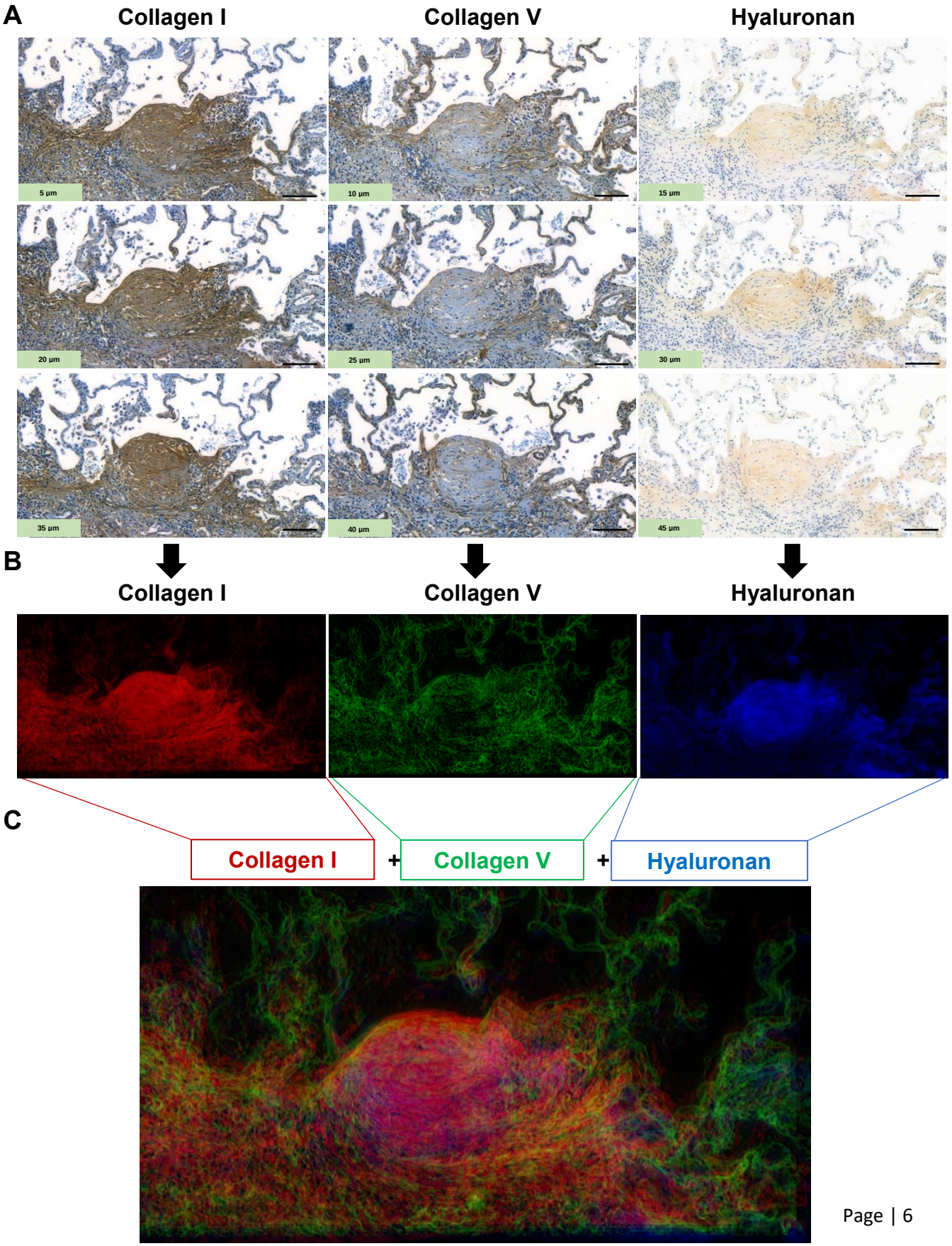
Antigen	Company	Catalogue	Titer
alpha-smooth muscle actin	Cell Signal	19245	1:500
collagen I	Abcam	ab138492	1:30,000
collagen III	Abcam	ab7778	1:12,000
collagen IV	Abcam	ab6586	1:12,000
collagen V	Abcam	ab134800	1:2,000
collagen VI	Abcam	ab182744	1:80,000
fibrinogen	Abcam	ab58207	1:14,000
fibronectin	Abcam	ab2413	1:2,500
versican	Abcam	ab177480	1:10,000
human procollagen I	Abcam	ab64409	1:800

Antibodies used for immunohistochemistry

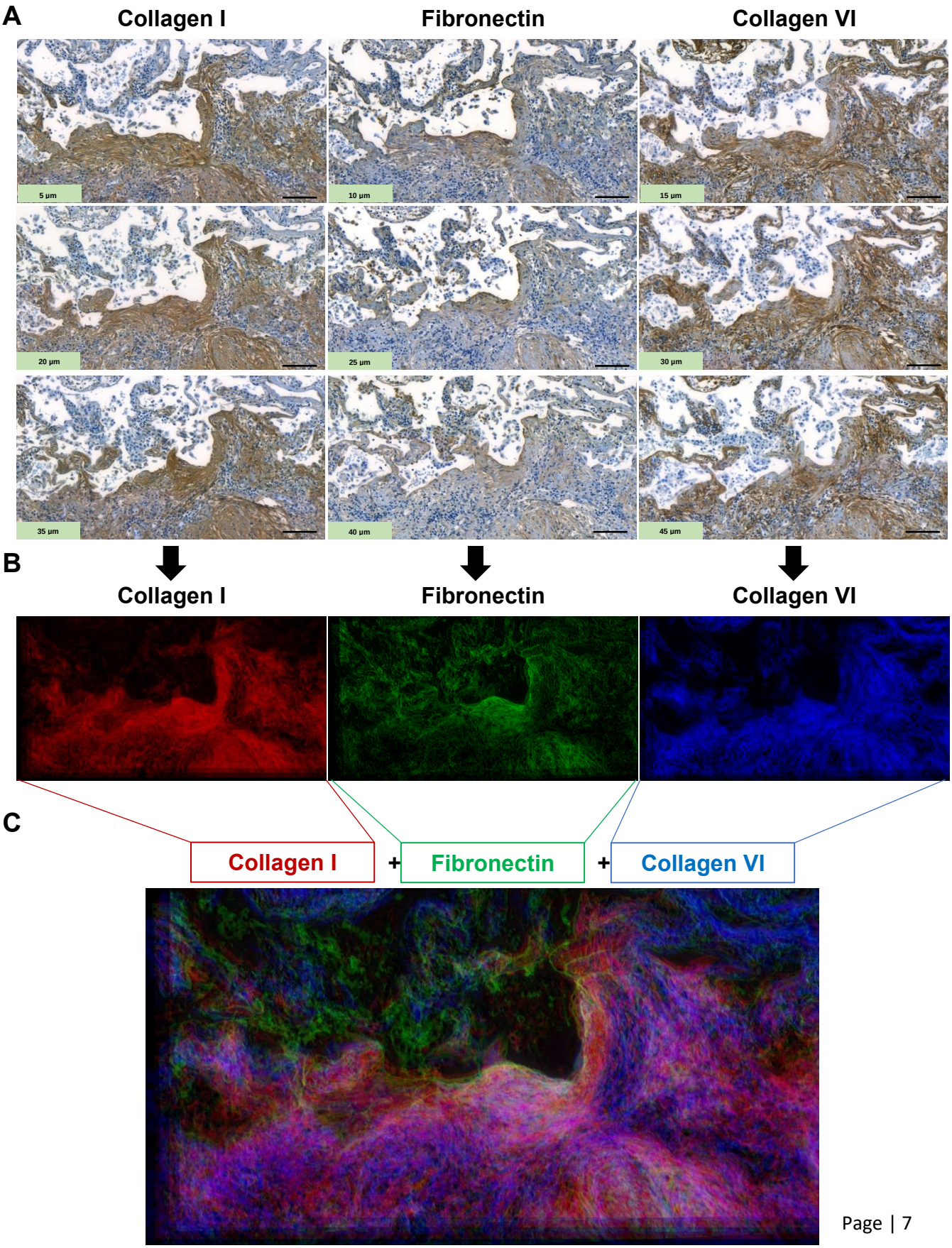
Supplemental Figure 1



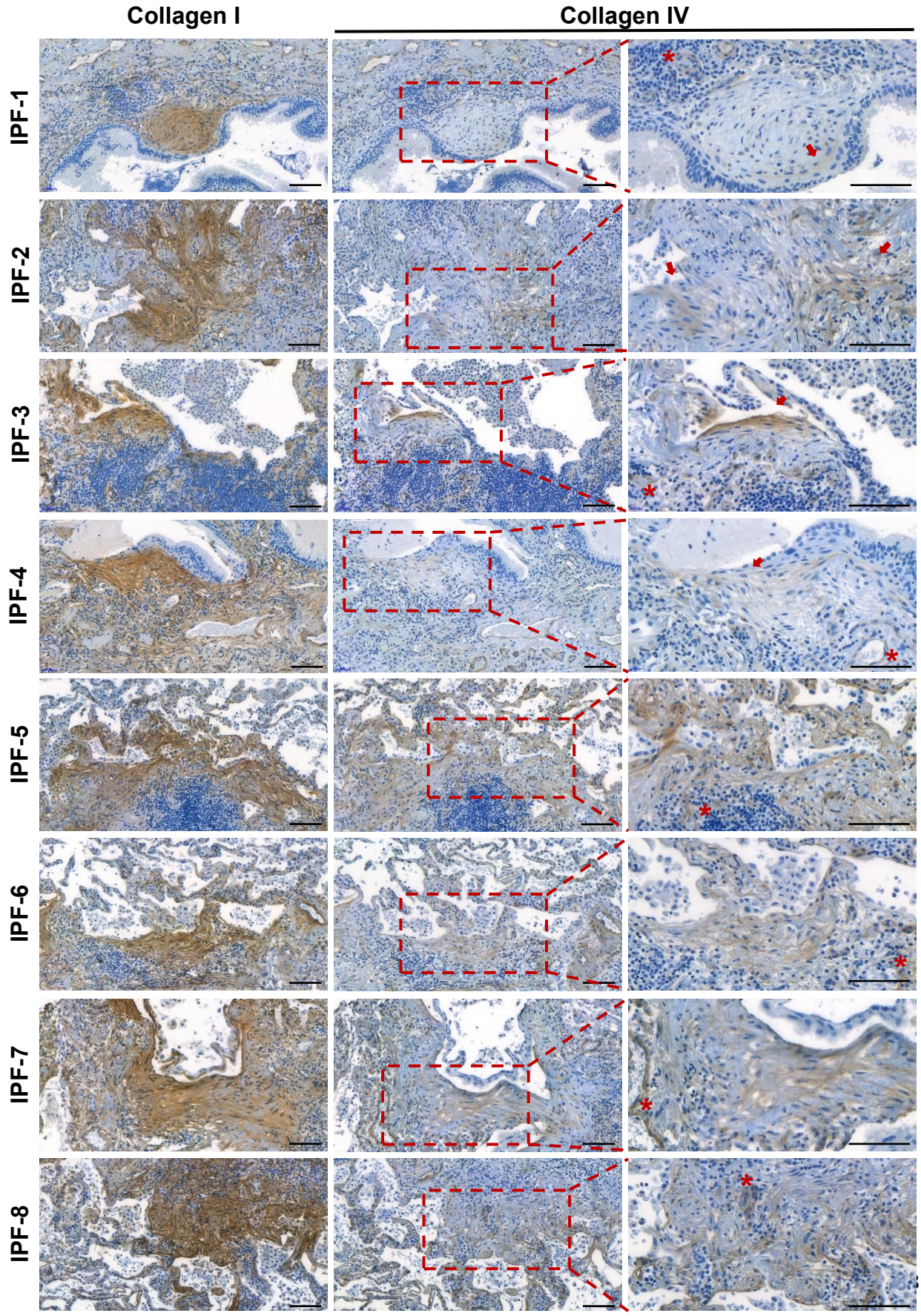
Supplemental Figure 2



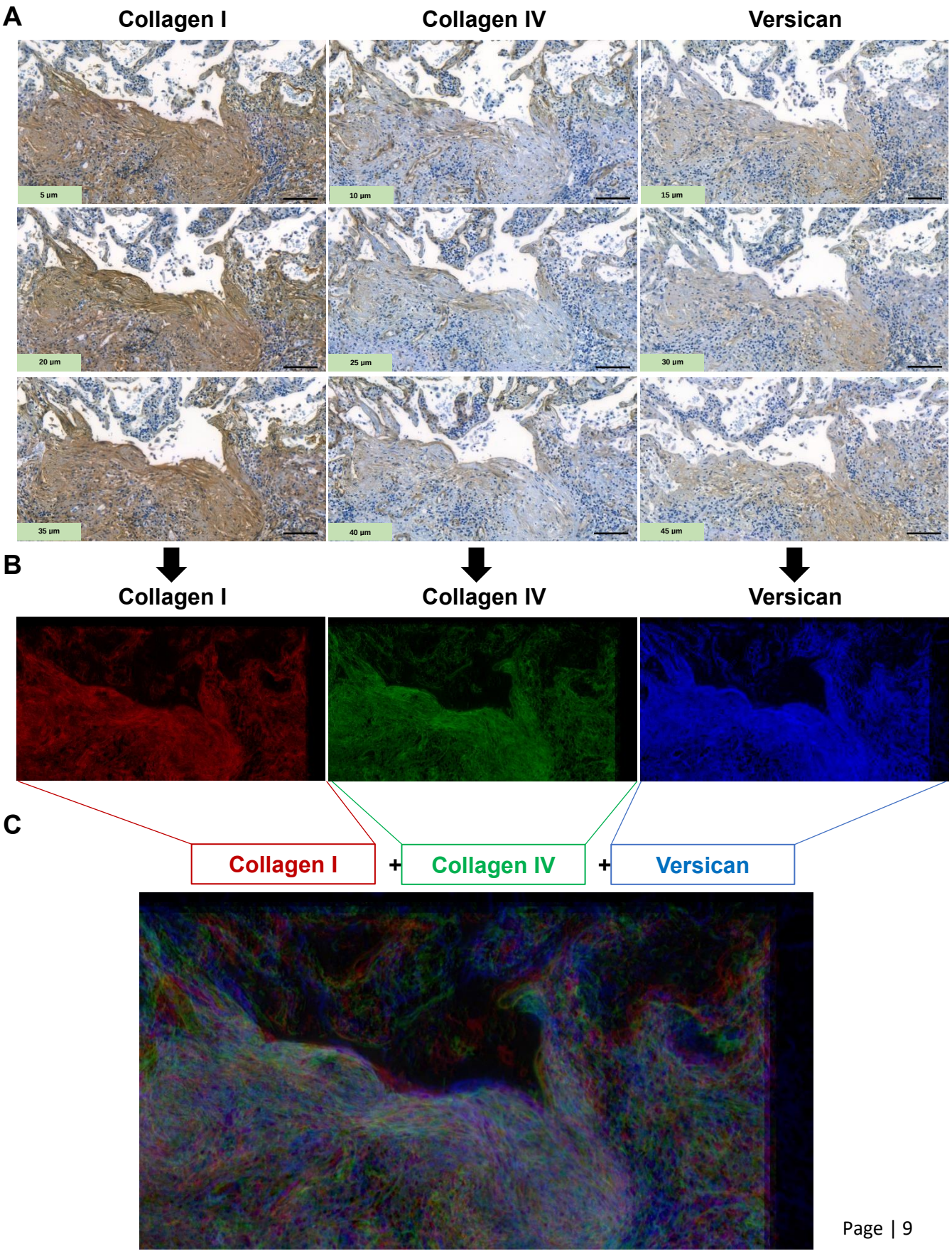
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6