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Severe disruption and disorganization of dermal collagen fibrils in early striae gravidarum

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Abbreviations: SG, striae gravidarum

Key words: stretch marks, striae gravidarum, collagen fibrils

What's already known about this topic?

- Dermal collagen fibrils are organized as densely packed bundles that provide support.
- Little is known about alterations involving collagen fibrils in striae gravidarum (SG), or stretch marks of pregnancy.

What does this study add?

- In recently developed SG, collagen bundles become markedly separated, and disorganized collagen fibrils emerge and fail to form bundles.

What is the translational message?

- These alterations of collagen fibril organization likely promote atrophy.
- Therapies promoting organization of collagen fibrils into densely packed bundles may improve SG.

(70 words)

ABSTRACT

Background

Striae gravidarum (SG), or stretch marks of pregnancy, begin as erythematous streaks, and mature into hypopigmented atrophic bands.

Objectives

To investigate molecular alterations that may promote atrophy of SG, we investigated dermal type I collagen fibrils, which provide human skin with support.

Methods

We obtained skin samples of recently developed, erythematous abdominal SG from pregnant women. To examine the organization of collagen fibrils, second-harmonic generation imaging was performed using multiphoton microscopy. Immunostaining was used to determine protein expression and localization of type I procollagen, the precursor of type I collagen fibrils. Real-time polymerase chain reaction was used to determine gene expression levels.

Results

In control (hip) and stretched, normal-appearing perilesional abdominal skin, dermal collagen fibrils were organized as tightly packed, interwoven bundles. In SG, collagen bundles appeared markedly separated, especially in the mid-to-deep dermis. In the spaces separating bundles, loosely packed wavy collagen fibrils lacking organization as bundles were present. These disorganized fibrils persisted into the postpartum period and failed to form densely packed bundles. Numerous large fibroblasts displaying type I procollagen expression were in close proximity to the disorganized fibrils, suggesting that the fibrils are newly synthesized. Supporting this possibility, immunostaining and gene expression of type I procollagen were increased throughout the dermis of SG.

Conclusions

Early SG display marked separation of collagen bundles and emergence of disorganized collagen fibrils that fail to form bundles. These alterations may reflect ineffective repair of collagen

bundles disrupted by intense skin stretching. Persistent disruption of the collagenous extracellular matrix likely promotes formation and atrophy of SG.

(250 words)

INTRODUCTION

"Stretch marks," or striae distensae, are associated with obesity, growth spurts, corticosteroid excess, or pregnancy. Stretch marks of pregnancy are known as striae gravidarum (SG) and affect 50-90% of women.[1] Typically, lesions affect the abdomen and breasts, and less frequently, the thighs and buttocks. Initially, lesions appear as erythematous, and sometimes edematous, streaks. Over months to years, they mature into permanent, scar-like, hypopigmented linear bands displaying a crinkled shiny surface, laxity, and atrophy.

Prevention and treatment of SG are difficult, in large part because the molecular pathogenesis of SG remains unclear, with various components of the dermal extracellular matrix (ECM) being implicated.[2-4] The dermal ECM provides human skin with elasticity and strength.[5] Elasticity is provided by elastic fibers, which form a mesh-like network composing 2-4% of the dry weight of the dermis.[6,7] Elastic fibers consist of many proteins. Especially in the mid-to-deep dermis, they consist mainly of elastin, a polymer of cross-linked tropoelastin proteins.[6,7] Strength, resiliency and support of human skin are mainly provided by type I collagen fibrils, which compose 80-90% of the dry weight of the dermis.[8-10] Fragmentation of type I collagen fibrils, as observed in aged or photoaged skin, compromises structural support.[10] Type I collagen fibrils are synthesized in dermal cells (fibroblasts) as a soluble precursor, type I procollagen. After secretion into the extracellular space, type I procollagen is processed to form long type I collagen fibrils.[10,11] Type I collagen fibrils are organized and stabilized by cross-links to form

bundles providing structural support.[9-11] These bundles are interwoven together,[12] and are densely packed in the mid-to-deep (reticular) dermis, but thinner and more loosely packed in the upper (papillary) dermis. Various ECM components, including proteoglycans, glycoproteins, glycosaminoglycans, and other types of collagen, associate with type I collagen fibrils, aiding their organization into bundles.[9,10,13-17]

It is generally believed that pathogenic alterations in striae distensae, including SG, involve collagen fibrils,[18-20] elastic fibers,[4,18,21-23] and/or other dermal ECM components, such as fibrillin.[24] However, how and to what extent these ECM constituents become deranged is not clear. Recently, we analyzed dermal elastic fibers in newly developed, erythematous abdominal SG in pregnant women.[4] We observed severe disruption of the elastic network, accompanied by the emergence of newly synthesized, disorganized tropoelastin-rich fibrils, which persist and fail to form normal-appearing elastic fibers. These changes may compromise skin elasticity, contributing to laxity of mature SG. Here, we expand on those findings, focusing on the structure and metabolism of type I collagen fibrils in SG lesions. Our study includes the use of multiphoton microscopy to perform second-harmonic generation (SHG) imaging, a relatively novel technique for examining fibrillar collagens (including type I collagen) without relying on exogenous labeling. Compared with confocal microscopy, or other traditional methods that require staining of thin tissue sections, SHG imaging is capable of high-resolution imaging of thick skin sections and allows for enhanced, three-dimensional interpretation of collagen organization. Our findings provide insight into the formation of SG and development of atrophy, as observed in mature lesions.

METHODS

Skin samples, immunostaining and gene expression. The study was approved by Institutional Review Board at the University of Michigan, and conducted in accordance to the Declaration of Helsinki principles. Skin biopsies were obtained from 2004 to 2010, and immunostaining, image analysis and gene expression assays were performed, as previously described.[4,25] Antibodies

used for staining are listed in **Table 1**. For real-time polymerase chain reaction, primers and probes for type I procollagen,[26] transforming growth factor-β1 and -β3,[27] connective tissue growth factor,[28] matrix metalloproteinase-1[29] and an internal control (36B4)[28] have been described previously. Sequences of primers and probes for heat shock protein 47, prolyl-4-hydroxylase, and matrix metalloproteinase-3 are provided in **Table 2**.

Second-harmonic generation (SHG) and immunofluorescence imaging using multiphoton microscopy. Multiphoton microscopy was used to perform both SHG and immunofluorescence imaging during separate scans. After fixation in 2% paraformaldehyde for 20 minutes and protein blocking (Biogenex Laboratories) for 30 minutes, skin sections (up to 100 μm) were incubated with primary antibodies (**Table 1**). Following protein blocking for 20 minutes, sections were incubated with anti-rat or anti-mouse secondary antibodies conjugated with Alexa Fluor 488 (2 μg/mL for 45 minutes, Jackson ImmunoResearch). Cell nuclei were stained with SYTOX Orange (1:5000 dilution for 20 minutes, Molecular Probes). Sections were mounted in ProLong Gold (Molecular Probes) for 24 hours. Sections were scanned on a Leica SP8 microscope equipped with a Coherent Chameleon MP laser. Excitation of Alexa Fluor 488 was elicited using two-photon excitation at 775 nm. Fluorescent emissions were detected by a HyD detector through an acousto-optical multi-beam splitter between 490-535 nm (Alexa Fluor 488) and 565-605 nm (SYTOX Orange). SHG emissions were elicited using two-photon excitation at 820 nm and detected through an externally mounted narrow 410/10 bandwidth filter. Forward emissions were detected by an externally mounted forward directed PMT with gain no greater then ~800 mv. For some sections, backward signals were detected using an external Leica HyD detector. Images were captured at 12 bits using 2x max spatial resolution determined by Nyquist sampling parameters per objective. Image stacks were analyzed using ImageJ (v.1.49t, National Institutes of Health), 3-D Gaussian blurred at 1x spatial resolution, and projected using maximum projection or +1 standard deviation projections. Flattened stack images were background subtracted using an average value within an area at least 15 x 15 μm known to contain only background. Paired images were adjusted until an equal percentage of pixels in each image were saturated. To analyze cross-sectional width, 15-30 collagen fibrils were

measured per skin sample. Three-dimensional renders were generated with FluoRender software (v.2.16, University of Utah).[30,31]

Statistics. Group differences were assessed using one-way ANOVA tests with Tukey multiple comparisons adjustment. When appropriate, logarithmic transformations were performed to achieve normality. The Welch-Satterthwaite t-test was used to determine significant group differences in post-partum skin samples. All statistical tests were two-sided using an overall alpha level of 0.05 to determine statistical significance. Data were analyzed using SAS software v.9.3 (SAS Institute Inc.)

RESULTS

Participants

Participants included pregnant women (28 participants; 22 Caucasian and 6 Black or biracial women; mean age 24.9 ± 1.0 years) who had recently developed erythematous abdominal SG (average time of onset 27.4 ± 1.2 weeks of pregnancy; mean duration 6.0 ± 1.0 weeks at the time of skin sampling). Nineteen participants had a family history of SG, 11 had past pregnancies, and six had SG from previous pregnancies. For each participant, we obtained skin samples of: 1) the center of SG lesions; 2) perilesional, normal-appearing, stretched abdominal skin located 2-3 cm from SG lesions; and 3) minimally stretched, normal-appearing skin from the hip (control).[4]

Marked separation of collagen bundles in early striae gravidarum

To investigate the organization of collagen bundles in early SG, we used multiphoton microscopy to perform second harmonic generation (SHG) imaging, which provides high-resolution, three-dimensional imaging of fibrillar collagen based on its inherent optical properties (i.e., exogenous

staining or labeling of collagen is not required).[32] Since type I collagen is the most abundant fibrillar collagen in the dermal ECM of human skin,[8,10] SHG imaging predominantly reflects the structure/organization of this type of collagen.[33]

In control skin from the hip and perilesional abdominal skin, dermal collagen bundles appeared normal, and were organized in a tightly packed, interwoven pattern, especially in the reticular dermis, and to a lesser degree in the papillary dermis (**Fig.1A,B**). In the dermis of SG, there were patchy to broad areas in which collagen bundles appeared abnormally separated (**Fig.1C**). This separation was striking in areas of the reticular dermis, and more modest in the papillary dermis.

Disorganized collagen fibrils lacking organization as bundles in early striae gravidarum

Given the marked separation of collagen bundles in the reticular dermis of SG, we next performed high magnification imaging of dermal areas between collagen bundles. In control (data not shown) and perilesional abdominal skin (**Fig.2A**), SHG imaging showed narrow spaces between collagen bundles in the reticular dermis. Within many of these slender spaces, immunofluorescence staining of tropoelastin revealed the presence of normal elastic fibers, which were mostly horizontally oriented, with respect to the overlying epidermis.

Within the prominent spaces separating collagen bundles in the reticular dermis of SG, high magnification SHG imaging revealed groups or arrays of loosely packed, wavy collagen fibrils that lacked organization as bundles (**Fig.2B**). Mean cross-sectional width of these fibrils was 413±10 nm (n=6 subjects). Similar loosely packed, disorganized collagen fibrils were not observed in control or perilesional skin. Three-dimensional video rendering of images clearly demonstrates that the normal organization of collagen bundles in early SG is severely disrupted, with marked separation of bundles and emergence of disorganized, wavy collagen fibrils (**Video S1**).

Additionally, immunofluorescence staining of tropoelastin revealed a dramatic decrease of normal elastic fibers, and instead, there were numerous disorganized tropoelastin-rich "fibrils" (**Fig.2B**). As previously reported, gene expression of tropoelastin was increased, suggesting that these fibrils are newly synthesized.[4] Many of these tropoelastin-rich fibrils were intermixed with the disorganized collagen fibrils, and some decorated nearby collagen bundles. These observations indicate that disorganized collagen fibrils, as well as disorganized tropoelastin-rich fibrils, are present within the large spaces separating collagen bundles in SG.

Close proximity of procollagen-producing fibroblasts to disorganized collagen fibrils in early striae gravidarum

Next, we examined immunofluorescence staining of type I procollagen, the precursor of type I collagen. In the reticular dermis of control (**Fig.3A**) and perilesional skin (data not shown), type I procollagen staining occurred in fibroblasts that were embedded within the interior of collagen bundles or attached to their exteriors. In the reticular dermis of SG, procollagen staining occurred within numerous large fibroblasts (**Fig.3B**). These fibroblasts were often localized to the exterior of separated collagen bundles, and in close proximity to the disorganized collagen fibrils within the spaces between bundles. Additionally, some fibroblasts were enmeshed within the disorganized collagen fibrils. The spatial relationship of these procollagen-producing fibroblasts to the disorganized collagen fibrils is clearly illustrated in a three-dimensional video rendering of images (**Video S2**).

Increased type I procollagen expression throughout the dermis in early striae gravidarum

Having observed type I procollagen expression in large fibroblasts near disorganized collagen fibrils in SG, we next examined immunostaining of type I procollagen throughout the dermis (from superficial to deep dermis). In control or perilesional skin, procollagen staining of fibroblasts was modest throughout the dermis. In SG, procollagen staining was greater, with

numerous, large, intensely stained fibroblasts (**Fig.4A**). Based on image analysis, the amount of dermal staining was significantly increased 14.3±5.7-fold in SG ($p<0.05$), compared with control skin. Similarly, SG displayed significantly increased expression of heat shock protein (HSP)47, an intracellular chaperone protein required for synthesis of type I procollagen (30.0±8.2-fold, $p<0.05$, **Fig.4B**).

Consistent with these immunostaining results, gene expression of type I procollagen and HSP47 was significantly elevated 7.3±3.4-fold and 2.8±0.7-fold, respectively, in SG lesions ($p<0.05$, **Fig.4C**), compared with control skin. SG also displayed significantly increased gene expression of prolyl-4-hydroxylase, an enzyme required for stable assembly of type I collagen fibrils (3.0±0.8-fold, $p\leq0.05$ compared with control skin, **Fig.4C**), and increased expression of collagen-stimulating growth factors, including transforming growth factor-β1 and -β3 and connective tissue growth factor ($p<0.05$ compared with control skin, **Fig.4C**). Finally, we found no significant changes in gene expression of enzymes that fragment type I collagen fibrils, including matrix metalloproteinase-1 and -3 (data not shown). These data indicate that synthesis, but not enzymatic fragmentation, of type I collagen is increased in early SG.

Prominent vasculature in early striae gravidarum

Since early SG are erythematous and the organization of the collagenous ECM can influence the formation and organization of dermal blood vessels,[34] we investigated vascular endothelial cells by CD31 immunofluorescence staining. In control (**Fig.5A**) and perilesional skin (data not shown), vascular endothelial cells formed normal-appearing, discrete, singular, tubular vessels in the papillary and reticular dermis. In the papillary and reticular dermis of SG, CD31 staining revealed collections of numerous, branching, widened, intertwined vessels, which tended to localize to the prominent spaces formed by separation of collagen bundles (**Fig.5B**). **Video S3** shows this complex, lobule-like organization of vessels in a three-dimensional video rendering. These data reveal that there is abnormally increased prominence and complexity of vasculature in early SG.

Persistence of disorganized collagen fibrils and increased type I procollagen expression in postpartum striae gravidarum

Finally, we investigated collagen organization and production in postpartum women with SG. For each participant, we obtained biopsies of erythematous, abdominal SG and perilesional skin at 4-8 weeks postpartum. These biopsies came from a subset of the same participants that had biopsies of early SG taken during pregnancy (9 participants; 7 Caucasian and 2 Black or biracial women).[4]

In postpartum perilesional skin, we found that the organization of dermal collagen bundles and type I procollagen staining appeared similar to control and perilesional skin during pregnancy (**Fig.6A**). Importantly, postpartum SG continued to display patchy to broad areas with marked separation of collagen bundles, especially the reticular dermis (**Fig.6B**). Between separated bundles, there remained numerous groups of wavy, disorganized collagen fibrils that were similar in appearance and thickness to those observed in SG during pregnancy (mean cross-sectional width 431±9 nm, n=5 subjects). Disorganized tropoelastin-rich fibrils also remained present in these areas (data not shown), consistent with previous findings.[4] In close proximity to the disorganized collagen fibrils were numerous, large type I procollagen-positive fibroblasts (**Fig.6B**). In addition, type I procollagen gene expression remained significantly elevated 9.5±2.6-fold in postpartum SG, compared with postpartum perilesional skin ($p<0.05$, **Fig.6C**). Overall, these data demonstrate that abnormalities in the structure, organization, and production of collagen fibrils persist into the postpartum period.

DISCUSSION

Despite the prevalence of SG, a comprehensive analysis of collagen fibrils in early lesions has been lacking. Here, we observed that early SG displayed severely disrupted organization of dermal collagen fibrils, with marked separation of bundles, especially in patchy to extensive areas of the mid-to-deep dermis.

We considered that enzyme-mediated fragmentation of collagen fibrils may promote these alterations, but did not observe increased expression of matrix metalloproteinase-1 or -3, which are the predominant enzymes that fragment dermal type I collagen fibrils. As previously reported,[4] early SG also did not display increased inflammatory cells, including neutrophils, mast cells, or macrophages, which produce enzymes that fragment collagen and other ECM components. Although we cannot rule out that increased enzymatic or cellular fragmentation of collagen fibrils occurs before lesions become clinically apparent, this possibility is unlikely, since we investigated early SG that were still evolving (in the process of developing and maturing).

Therefore, intense skin stretching that mechanically "pulls apart" the dermal ECM may disrupt the organization of collagen bundles.[4,35] This hypothesis suggests that, in predisposed individuals, the dermal ECM lacks the ability to accommodate to intense skin stretching.[36] Such a hypothesis might explain why SG often occurs on the abdomen, which experiences the greatest stretching of any area of skin during pregnancy. While this hypothesis requires further investigation, a recent study suggests that skin stretching plays a role in SG development, as maternal baseline weight, weight gain during pregnancy, and multiple gestation pregnancy appear to be risk factors.[37] Additional support comes from histological and clinical observations of skin stretching produced by surgical tissue expanders, which can promote separation of dermal collagen bundles[38] and, occasionally, development of striae within 1-3 months.[39]

In the spaces between separated collagen bundles in SG, we found groups of disorganized, wavy collagen fibrils, as well as numerous disorganized tropoelastin-rich fibrils.[4] While some of these collagen fibrils may arise as a consequence of damage to pre-existing collagen bundles, our data suggest that they mainly result from *de novo* synthesis by dermal fibroblasts. Indeed, numerous large type I procollagen-expressing fibroblasts were observed to be in close

proximity to the fibrils. Additionally, staining of procollagen-expressing fibroblasts was increased throughout the dermis of SG. The upregulation of type I procollagen production and synthesis of disorganized collagen fibrils, which apparently last for at least 6-8 weeks postpartum, may represent a reparative response to disruption or "wounding" of normal collagen bundles, a response to intense skin stretching,[8,40] or a combination of both.

Interestingly, disorganized collagen fibrils persisted into the early postpartum period and failed to form normal, densely packed, interwoven collagen bundles. We considered that collagen bundles may form with greater time, but we found that loosely packed, disorganized fibrils remain present even in mature, hypopigmented SG (data not shown). Bundles of collagen fibrils provide strength and support to human skin. Therefore, separation or damage of collagen bundles, coupled with suboptimal repair producing disorganized fibrils, likely translates to decreased dermal structural support. Loss of structural support precedes and, ultimately, may manifest clinically as dermal atrophy in mature SG. These changes occur despite substantial stimulation of the collagen-producing pathway.

The reasons that disorganized collagen fibrils persist in SG are not clear. Elastic fibers are disrupted and tropoelastin-rich fibrils in SG are unable to organize into normal-appearing elastic fibers, possibly because lesions lack ECM components essential for proper elastic fiber assembly.[4] Similarly, ECM components involved in organizing collagen fibrils into bundles may be deficient or dysfunctional in early SG. Such organizational components may include elastic fibers, crosslinking enzymes (e.g., lysyl oxidase), other types of collagen (e.g., type V and XI collagen), proteoglycans (e.g., decorin, biglycan, lumican), glycoproteins (e.g., fibronectin) and glycosaminoglycans (e.g., hyaluronic acid, dermatan sulfate).[9,10,13-17,40,41] As an example, small leucine-rich proteoglycans, such as decorin and biglycan, may play a role in regulating collagen fibril assembly, diameter, length and organization.[16,42] Additionally, increased mechanical tension of skin during pregnancy may play a role in preventing formation of collagen bundles, a situation possibly analogous to impaired healing or "spreading" of surgical wounds under high mechanical tension.[43] Finally, it is possible that hormonal changes during pregnancy may

contribute to persistent disorganization of collagen fibrils in SG.[40,44] Additional research may lend support for these possibilities.

In addition to changes in collagen fibrils, we observed increased prominence of dermal blood vessels in early SG. This change, involving increased number, branching and widening of vessels, likely promotes clinical erythema and explains how the appearance of striae can be improved, in part, by laser therapies targeting dermal vasculature.[45] Interestingly, fragmentation of type I collagen fibrils, and hence decreased density of the collagenous ECM, can induce endothelial cells to form vascular tubes.[34] Similarly, in early SG dispersal of collagen bundles may promote increased prominence or formation of blood vessels. Dispersal of collagen bundles may also promote clinical erythema by allowing easier visualization of color derived from deep dermal vessels. We considered that inflammation may contribute to erythema, but as previously reported,[4] infiltrating immune cells are not substantially present in early SG.

Taken together, our data suggest that strategies aimed at promoting organization of collagen fibrils into densely packed bundles that provide structural support may be effective for preventing or treating SG. In this regard, potential therapies may include topical retinoids, which can improve the clinical appearance of early striae lesions.[46] Some limitations are that topical retinoids are typically avoided during pregnancy, and it remains unclear exactly how these agents impact collagen bundling or whether they can prevent new lesion development.

Finally, it is interesting to note that disruption coupled with suboptimal repair is observed for both elastic fibers[4] and collagen bundles in SG. Furthermore, abnormal collagen fibrils and tropoelastin-rich fibrils localize together in the spaces created by disruption of the normal dense architecture of collagenous dermal ECM. These observations suggest that the organization of collagen bundles and elastic fibers may be interdependent.[40] Investigating the potential role of this interdependence may provide important insights into the pathophysiology of SG, as well as fundamental regulation (or biogenesis) of the human dermal ECM.

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FIGURE LEGENDS

Figure 1. Marked separation of dermal collagen bundles in early striae gravidarum. To examine collagen bundles, second-harmonic generation imaging of skin sections (10 µm thick) was performed using multiphoton microscopy. Collagen bundles appear white. **A)** Minimally stretched, normal-appearing skin from the hip (control) and **B)** perilesional, normal-appearing, stretched skin from the abdomen, with both displaying interwoven, tightly packed collagen bundles, especially in the mid-to-deep dermis. Prominent dark areas correspond to the location of dermal blood vessels. **C)** Striae gravidarum lesions, with collagen bundles appearing separated and loosely packed, particularly in the mid-to-deep dermis. Bottom panels magnify representative areas of the mid-to-deep dermis. Dotted lines indicate the dermal-epidermal junction. Images are representative of 17 participants. Original magnification, x20.

Figure 2. Disorganized collagen fibrils lacking organization as bundles within prominent spaces separating collagen bundles in early striae gravidarum. High magnification second-harmonic generation (SHG) imaging and immunofluorescence staining of skin sections (40 µm thick) were performed. Upper panels display collagen fibrils, which appear white. In the middle panels, red represents immunofluorescence staining of tropoelastin, the main component of elastic fibers. Bottom panels show merged images. **A)** Mid-to-deep dermis of perilesional abdominal skin, displaying collagen fibrils organized as densely packed, interwoven bundles separated by narrow spaces (top panel). Intact elastic fibers were present (middle panel) and mostly localized to the slender spaces between collagen bundles (bottom panel). **B)** Mid-to-deep dermis of striae gravidarum, displaying collagen bundles separated by prominent spaces containing arrays of disorganized, wavy collagen fibrils (top panel). Numerous disorganized tropoelastin-rich fibrils were present in the same location (middle panel), and intermixed with disorganized collagen fibrils (bottom panel). Images are more completely shown in three-dimensional video rendering (Video S1). Images are representative of 13 participants. Original magnification, x40.

Figure 3. Close proximity of procollagen-producing fibroblasts to disorganized collagen fibrils in early striae gravidarum. High magnification second-harmonic generation (SHG) imaging and immunofluorescence staining of skin sections (30 µm thick) were performed. Upper panels display collagen fibrils, which appear white. In the middle panels, blue represents cell nuclei, and red represents immunofluorescence staining of type I procollagen. Bottom panels show merged images. **A)** Mid-to-deep dermis of control skin from the hip, displaying densely packed, interwoven collagen bundles (top panel). Type I procollagen staining appeared within fibroblasts (middle panel) that were mostly embedded within the interior of collagen bundles or attached to their exteriors (bottom panel). **B)** Mid-to-deep dermis of striae gravidarum, displaying collagen bundles separated by prominent spaces containing groups of disorganized, wavy collagen fibrils (top panel). Type I procollagen staining appeared within numerous large fibroblasts (middle panel) that were mostly localized to the exterior of separated collagen bundles, in close proximity to the disorganized collagen fibrils (bottom panel). Images are more completely shown in three-dimensional video rendering (Video S2). Images are representative of 4 participants. Original magnification, x40.

Figure 4. Increased expression of type I procollagen in fibroblasts throughout the dermis in early striae gravidarum. Immunostaining of skin sections (7 µm thick) for **A)** type I procollagen (n=11) and **B)** heat shock protein 47 (n=11), a chaperone protein required for type I procollagen synthesis. Original magnification, x10. Bar graphs show quantification of staining by image analysis. **C)** Using real-time polymerase chain reaction, gene expression of type I procollagen (n=17), heat shock protein 47 (n=12), and prolyl-4-hydroxylase (n=11, an enzyme required for the stable assembly of type I collagen fibrils) was assessed in skin samples. Gene expression of collagen-stimulating growth factors, including transforming growth factor-β1 (n=10) and -β3 (n=11) and connective tissue growth factor (n=11), was also measured. All bar graphs show mean fold change ± SE. *p≤0.05.

Figure 5. Prominent dermal vasculature in early striae gravidarum. High magnification second-harmonic generation (SHG) imaging and immunofluorescence staining of skin sections (60 µm thick) were performed. Upper panels display collagen fibrils, which appear white. In the middle

panels, CD31 immunofluorescence staining of vascular endothelial cells appears green. Bottom panels show merged images. **A**) In the mid-to-deep dermis of control skin, collagen bundles were densely packed (top panel), and CD31 staining localized to thin, tube-like vessels (middle panel), which were situated between collagen bundles (bottom panel). **B**) In the mid-to-deep dermis of striae gravidarum, collagen bundles appeared markedly separated (top panel), and CD31 staining appeared increased, highlighting the presence of multiple, branching, widened, intertwined, tube-like vessels (middle panel), which were localized to the spaces separating collagen bundles (bottom panel). Images are more completely shown in three-dimensional video rendering (Video S3). Images are representative of 4 participants. Original magnification, x40.

Figure 6. Persistence of disorganized collagen fibrils and elevated type I procollagen expression in postpartum striae gravidarum. At 4-8 weeks postpartum, skin samples of recently developed abdominal striae gravidarum and normal-appearing perilesional abdominal skin were obtained from a subset of the same participants who had biopsies taken during pregnancy. High magnification second-harmonic generation (SHG) imaging and immunofluorescence staining of skin sections (100 μm thick) were performed. Upper panels display collagen fibrils, which appear white. In the middle panels, cell nuclei appear blue, and immunofluorescence staining of type I procollagen appears red. Bottom panels show merged images. **A)** Mid-to-deep dermis of perilesional skin, in which collagen bundles continued to appear interwoven and densely packed (top panel). Type I procollagen staining continued to localize to fibroblasts (middle panel) that were embedded within the interior of collagen bundles or attached to their exteriors (bottom panel). **B)** Mid-to-deep dermis of postpartum striae gravidarum, in which collagen bundles continued to appear separated by prominent spaces containing numerous disorganized, wavy collagen fibrils (top panel). Many large fibroblasts staining for type I procollagen remained present (middle panel), and were localized to the exterior of collagen bundles, in close proximity to disorganized collagen fibrils (bottom panel). Images are representative of 5 participants. Original magnification, x40. **C)** Gene expression of type I procollagen (n=7) was assessed in skin samples (mean fold change ± SE). *$p<0.05$.

Table 1. Antibodies used for immunostaining.

Table 2. Primer and probe sequences for real-time polymerase chain reaction.

SUPPLEMENTAL FIGURE LEGENDS

Video S1: Three-dimensional video rendering of images of collagen bundles in early striae gravidarum. Images were obtained by second-harmonic generation imaging. Collagen fibrils appear white. In control skin from the hip, collagen fibrils in the reticular dermis were organized as tightly packed, interwoven bundles. In abdominal striae gravidarum, bundles of collagen fibrils in the reticular dermis appeared loosely packed and markedly separated. Between separated bundles were abnormal arrays of disorganized, wavy collagen fibrils. Each image represents 220 x 220 x 35 μm of tissue. File format mp4.

Video S2: Three-dimensional video rendering of images of synthetically active fibroblasts in early striae gravidarum. Images were obtained by second-harmonic generation imaging and immunofluorescence staining. Collagen fibrils appear white, cell nuclei blue, and type I procollagen staining red. In the mid-to-deep dermis of control skin, type I procollagen staining occurred mostly in fibroblasts embedded within collagen bundles or attached to their exteriors. In the mid-to-deep dermis of striae gravidarum, type I procollagen staining occurred within large fibroblasts in close proximity to disorganized collagen fibrils present within prominent spaces separating collagen bundles. Each image represents 203 X 199 x 25 μm of tissue. File format mp4.

Video S3: Three-dimensional video rendering of images of dermal vasculature in early striae gravidarum. Images were obtained by second-harmonic generation imaging and immunofluorescence staining. Collagen fibrils appear white, and CD31 staining of vascular endothelial cells green. In the mid-to-deep dermis of control skin, CD31 staining highlighted the presence of discrete, tube-like vessels situated between densely packed collagen bundles. In the mid-to-deep dermis of striae gravidarum, CD31 staining was increased and highlighted the presence of multiple, branching, widened, intertwined, tube-like vessels within spaces

separating collagen bundles. Each image represents 310 x 320 x 60 µm of tissue. File format mp4.

Figure 1

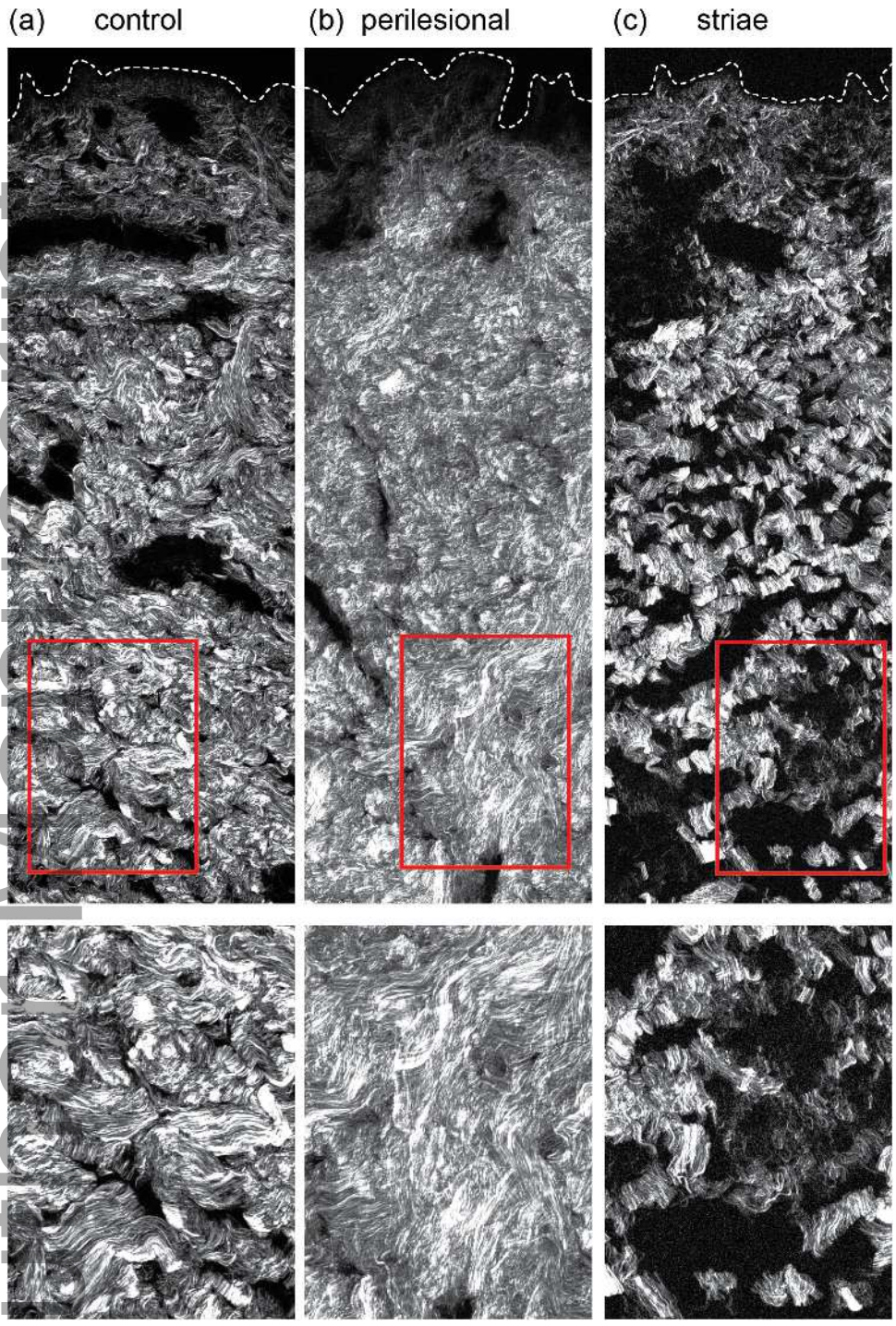

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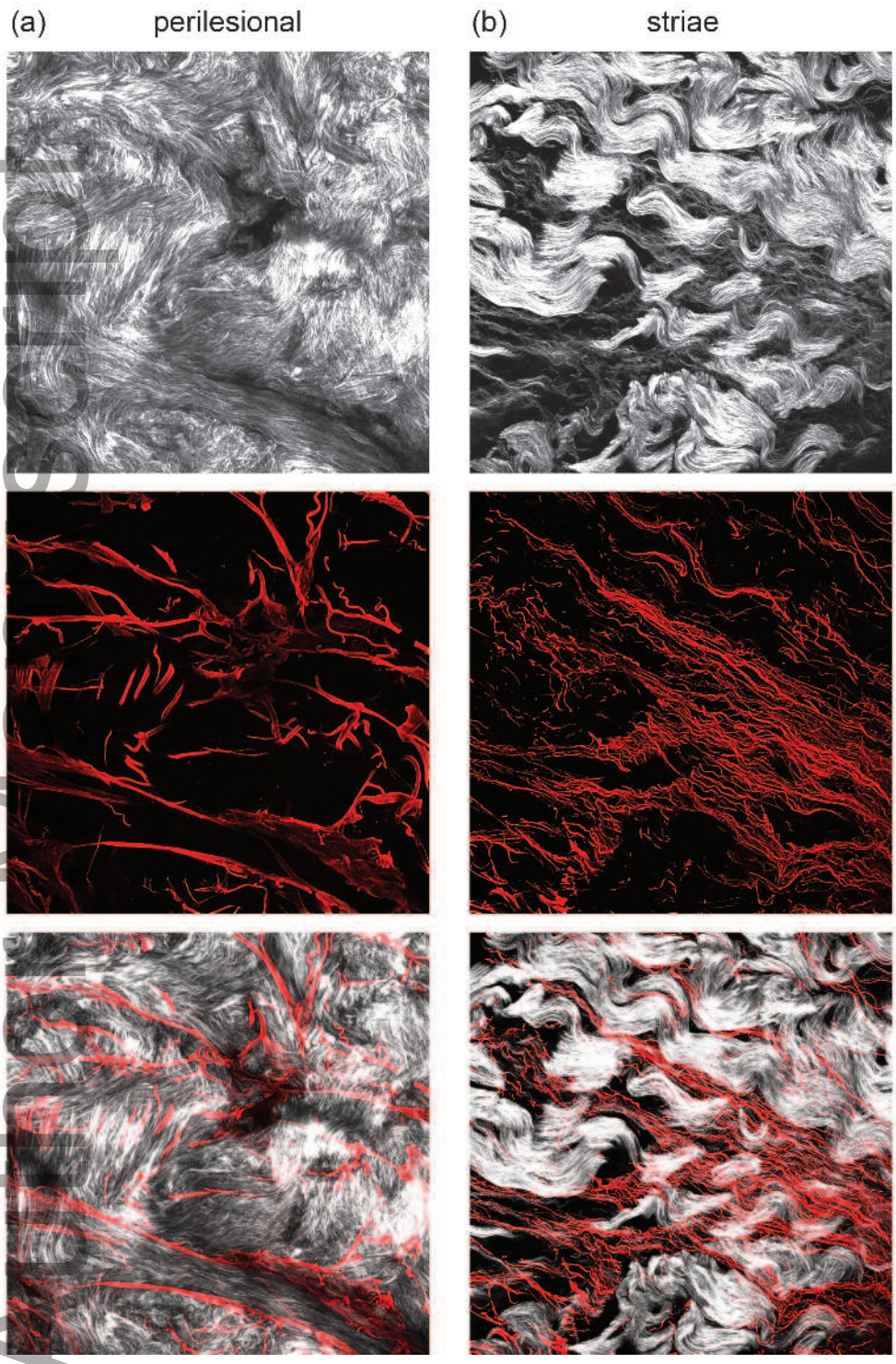
Figure 2



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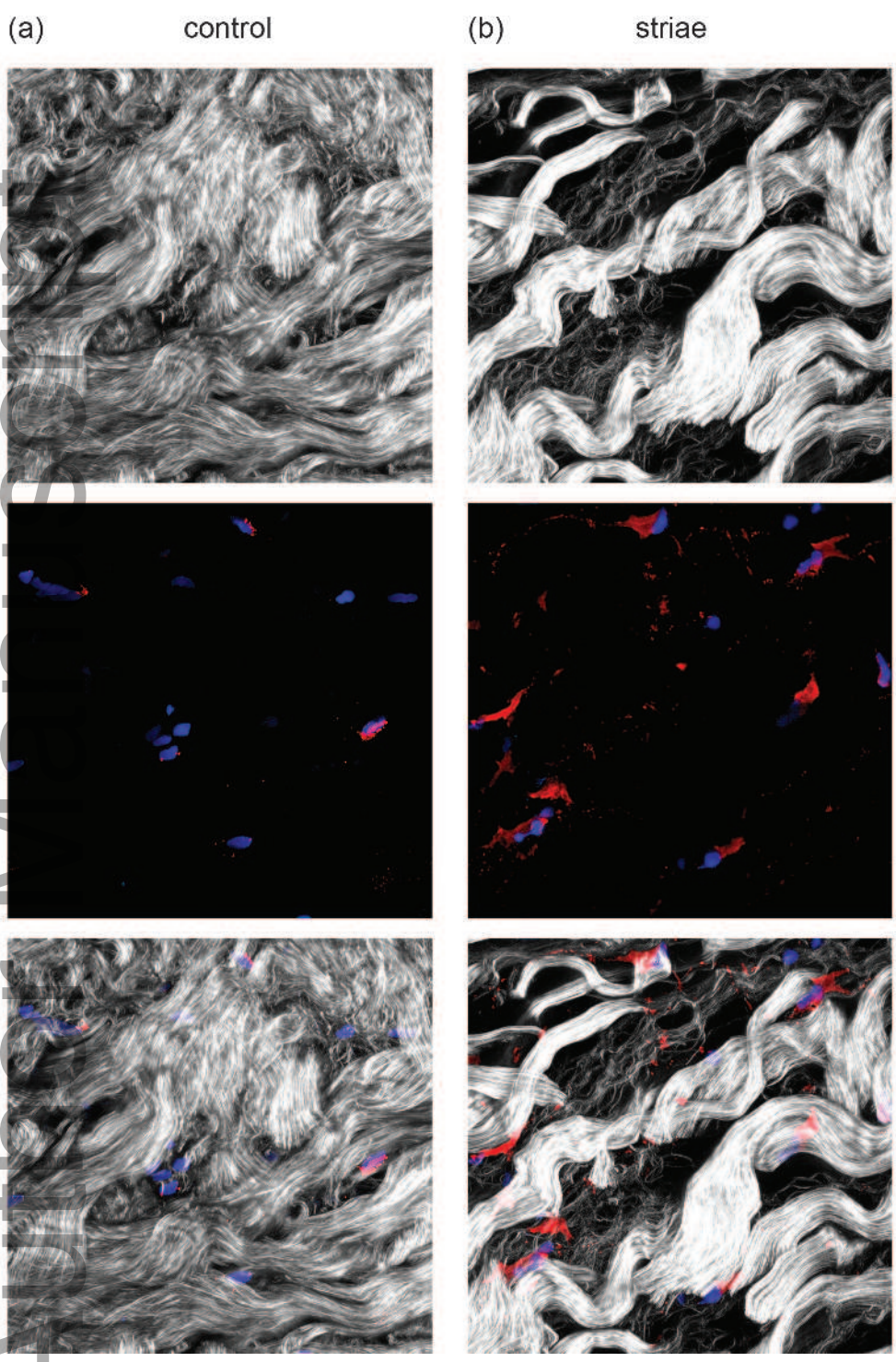

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Figure 4

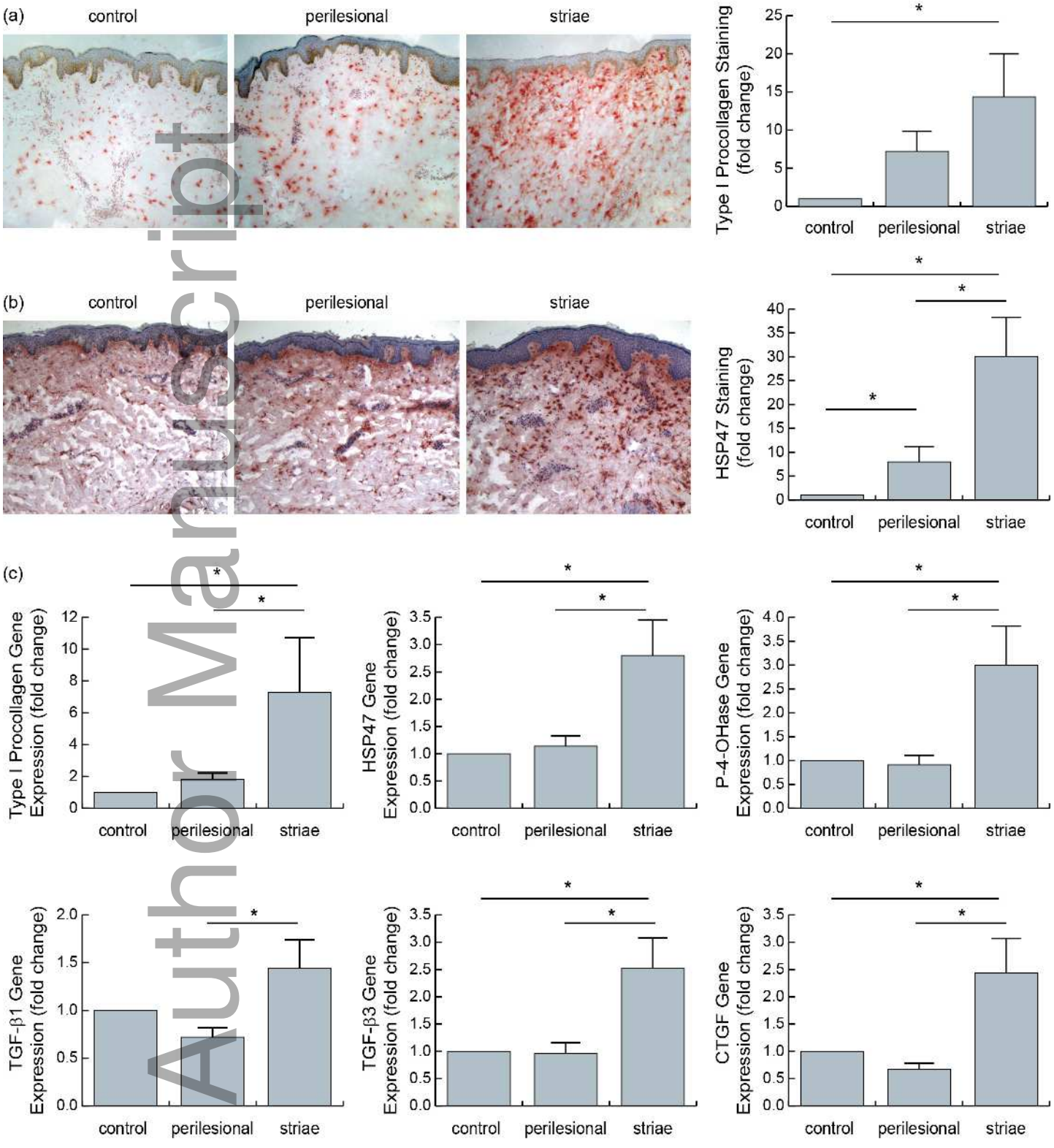

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Figure 5

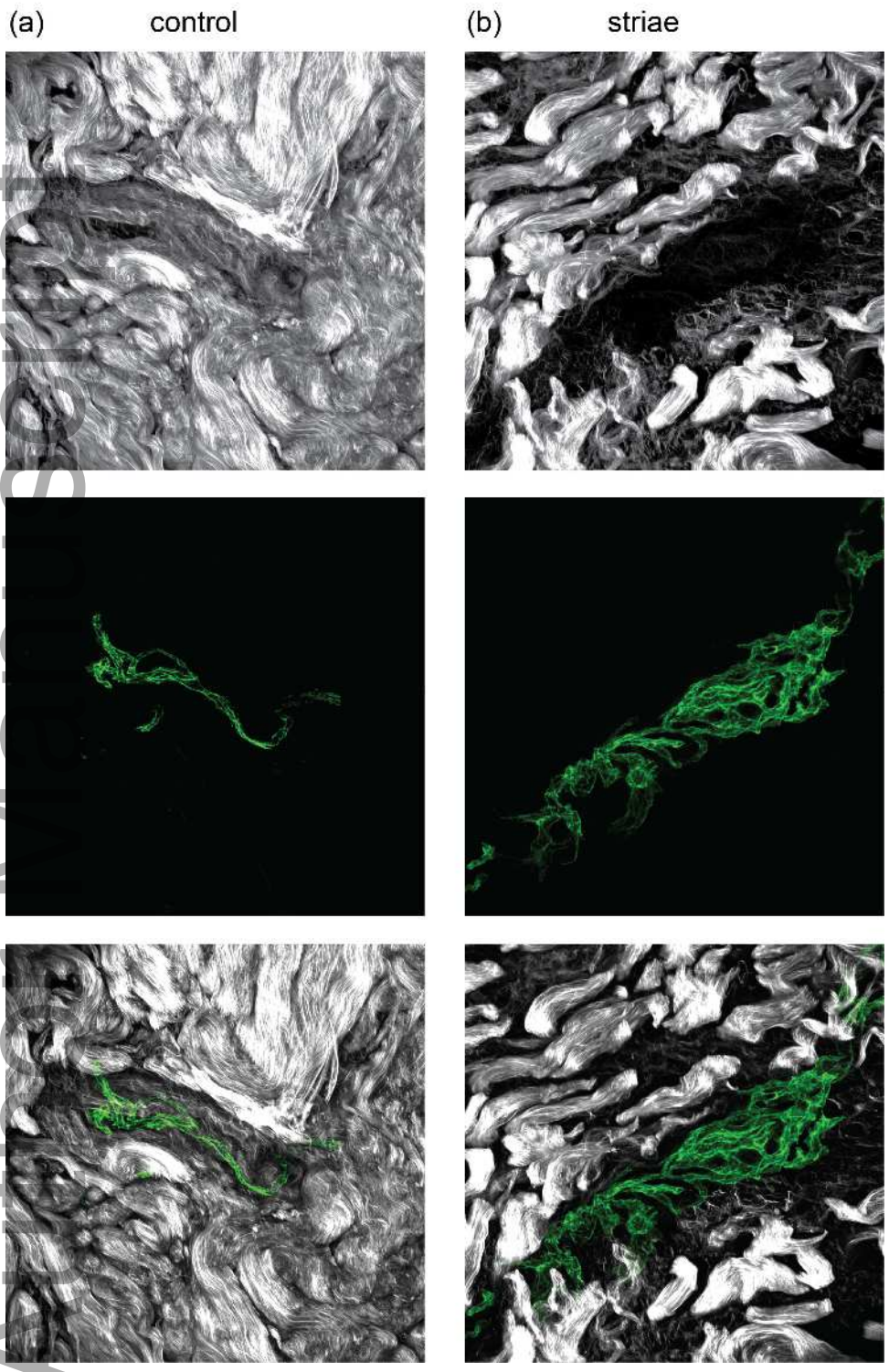

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Figure 6