

Research Article

Endothelial TLR4 Activation by Endogenous Ligands Contributes to Small Blood Vessels Formation in Angiolipoma

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Abstract

Angiolipomas are benign subcutaneous nodules characterized by the presence of mature adipocytes intermingled with cluster of small blood vessels, often displaying intraluminal fibrin microthrombi. It is known that fibrinogen and/or fibrin intraluminal and extracellular deposition during immune responses affect Endothelial Cell (EC) functioning and leukocyte trafficking. Also, it is known that Toll-Like Receptors (TLRs) are not only expressed in Immune Cells (ICs) but also in ECs and that excessive endothelial activation through TLR interactions with endogenous ligands such as fibrinogen, Heparan Sulfate Proteoglycans (HSPGs), Fibronectin (FN), Tenascin-C (TN-C), hyaluronan and galectin-3, contributes to EC dysfunction promoting endothelial proliferation, migration, apoptosis and tube-like structures formation. Nevertheless, studies involving the endothelial TLR4 activation by specific ligands and their contribution to the small blood vessels formation in angiolipoma has not been considered.

Herein, we show that in angiolipoma TLR4 and some of their ligands such as fibrinogen, FN, HSPGs including agrin, perlecan and Synd-1 and galectin-3 as well as some glycoconjugates associated to these ligands including VE-cadherin, VCAM-1, ICAM-1, PECAM-1, endoglin and CD44 were immunolocalized in the ECs from the small vessels and some ICs. We propose that in angiolipoma tissues galectin-3 oligomerization upon binding to these TLR4 endogenous ligands and glycoproteins associated can lead to the formation of gal-glycan lattices on the endothelial surface that might be facilitating not only the activation of TLR4, but also contributing to the vasculature formation regulated by signaling pathways mediated by certain cytokines, chemokines and growth factors.

Keywords: Angiolipoma; Fibrin Microthrombi; Endothelial Cell; Toll-Like Receptor 4; Galectin-3; Gal-Glycan Lattice

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Introduction

Angiolipomas are benign subcutaneous nodules characterized by the presence of mature adipocytes intermingled with clusters of small blood vessels generally situated at the periphery of the lesion. The clusters are composed in part of well formed, dilated small vessels containing Red Blood Cells (RBCs). Other blood vessels appear tortuous and have poorly formed lumina. Nearly all angiolipomas display intraluminal fibrin microthrombi formed from fibrinogen, being the presence of fibrin microthrombi considered as a diagnostic hallmark of angiolipoma and associated with its painful nature [1-6]. The multidomain glycoprotein fibrinogen, together with fibrin, plays overlapping roles in blood clotting, fibrinolysis, cellular and Extracellular Matrix (ECM) interactions, inflammatory responses, wound healing, angiogenesis and tumorigenesis [7-16]. It is known that fibrinogen and/or fibrin intraluminal and extraluminal deposition during immune responses affect Endothelial Cell (EC) functioning and leukocyte trafficking. Both provoked by inflammatory mediators that include cytokines, chemokines and growth factors [5,8-10,14,16].

Of note, interaction of fibrinogen/fibrin with EC via endothelial integrin receptors including $\alpha 5\beta 1$ and $\alpha v\beta 3$ and non-integrin receptors such as Vascular Endothelial-cadherin (VE-cadherin), Neural-cadherin (N-cadherin) and the Intercellular Cell Adhesion Molecule-1 (ICAM-1) inducing the formation of capillary-like structures by human vascular ECs is well documented [7-9,11,14,17-21]. However, despite the presence of intraluminal fibrinogen/fibrin microthrombi in the small blood vessels in angiolipoma is well known, the interaction between fibrinogen/fibrin and ECs has not been clarified.

It has further reported that Toll-Like Receptors (TLRs), a family of innate immune receptors, are not only expressed in immune cells (ICs) but also in ECs [22-32].

TLRs are type I integral membrane glycoproteins that possess a N-terminal extracellular domain containing multiple Leucine-rich repeat (LRR) motifs required for ligand binding, a transmembrane fragment and an intracellular C-terminal binding domain of Toll / Interleukin-1 (IL-1) Receptor (TIR) homology domain [37-42]. In humans, TLRs comprise ten functional members that are expressed on the cell surface (TLR1, 2, 4, 5, 6 and 10) or in the intracellular compartments (TLR3, 7, 8, 9, occasionally TLR4), the TLR4 being one of most studied [37-42]. Notably, TLR4 activation by endogenous components such as fibrinogen, Heparan Sulfate Proteoglycans (HSPGs), Fibronectin (FN), Tenascin-C (TN-C) and hyaluronan, has been suggested [43-50]. In ECs, accumulating evidence suggest that inappropriate or excessive endothelial activation through TLR interactions with endogenous ligands generated upon cellular injury, cell death or stressed tissues (Damage-Associated Molecular Patterns, DAMPs) contributes to endothelial dysfunction promoting endothelial proliferation, migration, apoptosis and tube formation by secretion of cytokines, chemokines and growth factors by both ECs and ICs as well as upregulating the expression of endothelial adhesion molecules and activation of coagulation and NFkB pathways [22-28,31,32,51,52]. Another important endogenous ligand and activator of TLR4 that is expressed and released during the neurodegenerative disorders and other inflammatory diseases is the galectin-3 [53-57]. This chimeric protein that has a great affinity for the β -galactoside residues present in the TLR4 and TLR2 ectodomains through its Carbohydrate Recognition Domain (CRD) and its N-terminal, form dimers and oligomeric structures, respectively [53-62]. The latter structure gives way to galectin-3 molecules with multivalent CRDs that form lattice on the cell surface facilitating cell adhesion and activation [59-62]. Of note, dimeric and oligomeric structures regulate innate and adaptive responses and promote angiogenesis [54,59,63-69]. Nevertheless, to the best of our knowledge, studies involving the endothelial TLR4 activation by specific ligands and their contribution to the small blood vessels (vasculature) formation in angiolipoma, has not been considered.

In this context, the goal of the current study was to examine the presence and localization in angiolipoma tissues of endothelial TLR4 and some of their endogenous ligands including fibrinogen/fibrin, HSPGs, Fibronectin (FN) and galectin-3, as well as of some glycoconjugates associated to these ligands such as Vascular-Endothelial cadherin (VE-cadherin), Intercellular Cell Adhesion Molecule-1 (ICAM-1) (CD54), Vascular Cell-Adhesion Molecule-1 (VCAM-1) (CD106), Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1 (CD31), endoglin (CD105) and CD44 and whose upregulation has been related with TLR4 activation. We also discuss the contribution of endothelial TLR4 and their endogenous ligands to the vasculature formation in the angiolipoma.

Material and Methods

Seven biopsies were obtained from patients clinically and histopathologically (H&E staining) diagnosed with angiolipoma. Data regarding gender, age and site of lesion appear listed in Table 1. This study was approved by the Ethics Committee of the ASIB and performed in line with the principles of the Declaration of Helsinki.

Indirect Immunofluorescence Staining of Tissue Sections

For immunohistochemistry paraffin-embedded skin tissue blocks were cut into sections of 4 μ m of thick each. Paraffin sections were then set onto silanised glass slides, dewaxed in xylene, dehydrated through graded alcohol series (from 100 to 70%) and rehydrated in distilled water and equilibrated in Phosphate-Buffered Saline (PBS) for 10 min. Prior to incubation with the primary antibodies, sections were blocked for 1 hour in a humid chamber with Phosphate-Buffered Saline (PBS) containing 3% BSA and 0.1% Tween 20, in order to prevent non-specific staining. Sections were then incubated with the primary antibodies diluted in PBS, 3% BSA, 0.1% Tween 20 (Table 2) for two hours at RT followed by incubation with anti-rabbit IgG Cruz Fluor 594 (Santa Cruz Biotechnology Inc. TX, USA) diluted with PBS containing 3% BSA and 0.1% Tween20 for 30 min in a humid chamber in the dark. Finally, the sections were washed in PBS and coverslipped with mounting medium containing DAPI (4', 6-

Diamidino-2-phenylindole) (Santa Cruz Biotechnology).

For autofluorescence, selected sections of 4µm dewaxed and without staining were examined with the CLSM. Autofluorescence was determined from the ratio between the emission fluorescence in the wavelength range between 603 and 646nm and the excitation light with a wavelength of between 491 and 592 nm. To validate antibody binding specificity, some sections were incubated with only primary antibody or secondary antibody. All images were captured using a 1X81 Olympus inverted microscope with the Fluo View Confocal Laser Scanning configuration (CLSM) (Olympus America) equipped with software program FV10.ASW.

Results

Histopathological analysis of the angiolipoma tissue sections showed the presence of dilated and tortuous small blood vessels intermingled with mature adipocytes that were situated in the periphery of the lesion (Fig. 1). Intraluminal microthrombi consisting of fibrinogen/fibrin strands and Red Blood Cells (RBCs) were also frequently observed in numerous vessels (Fig. 1). These microthrombi generally appeared delimited by Endothelial Cells (ECs) and some ICs (Fig. 2). Yellow dotted lines enclosed dilated and tortuous blood vessels.

In-vivo PECAM-1 (CD31) Immunostaining

Because PECAM-1 is a transmembrane glycoprotein with pro-inflammatory and pro-angiogenic activities highly expressed on the surface of activated Endothelial Cells (ECs), we examined the presence and distribution of this glycoprotein in the small blood vessels of angiolipoma [70,71]. Immunofluorescence analysis by confocal microscopy showed that PECAM-1 was localized in the ECs of the tortuous and dilated small blood vessels (Fig. 3). In addition, moderate PECAM-1 immunoreactivity was observed in some ICs (Fig. 3). Exacerbated immunopositivity in RBCs, ICs and microthrombi provoked by autofluorescence at the red wavelength was also observed (Supplemental Fig. 1).

In-vivo Perilipin 1 (PLIN1) Immunostaining

As PLIN1 is a lipid droplet-associated protein that is considered specific biomarker of adipocytes, we examined the presence and localization of this protein in the angiolipoma tissue sections [72]. Immunofluorescence showed mature adipocytes containing PLIN1 (Fig. 4).

In-vivo VE-cadherin, ICAM-1 (CD54) and VCAM-1 (CD106) Immunostaining

Since VE-cadherin, ICAM-1 (CD54) and VCAM-1 (CD106) are glycoproteins that mediate the interaction of fibrinogen/fibrin with ECs modulating fibrin-dependent inflammation and angiogenesis and given that exaggerated endothelial TLR4 activation can induce the expression of ICAM-1 and VCAM-1, we examined the presence and localization of these glycoproteins in the small blood vessels of angiolipoma tissue sections [7,9,16,19,20,24,27,31,32]. Immunofluorescence showed that ICAM-1 and VCAM-1 were localized in the ECs of the dilated and tortuous small blood vessels (Fig. 5).

In-vivo Toll-Like Receptor 4 (TLR4) Immunostaining

Considering that exaggerated activation of TLR4 contributes to endothelial dysfunction, mediating immune response and angiogenesis and can induce the expression of ICAM-1 and VCAM-1, we tested the presence and distribution of this receptor in angiolipoma tissue sections [22-32]. Strong TLR4 staining was observed in the ECs of the dilated and tortuous small blood vessels, as well as in some adipocytes (Fig. 6) and some immunopositive CD68 ICs (Fig. 6). No immunoreactivity was detected when the primary antibody was omitted or replaced by PBS (not shown).

In-vivo Fibronectin (FN), Syndecan-1 (Synd-1), Agrin, Perlecan and CD44 Immunostaining

We next examined the presence and localization of the glycoprotein FN, a glycoprotein that interacts with fibrinogen/fibrin, HSPGs agrin, perlecan and Synd-1 (CD138) and the CSPG CD44 [73,74]. Proteoglycans involved in the angiogenic and proliferative responses in ECs and considered endogenous activators of TLR4 [27,33-36,42-50]. We found that FN, agrin, perlecan, Synd-1 and CD44 were present in the small blood vessels, in some ICs, as well as in the extracellular space (Fig 7).

In-vivo Galectin-3 Immunostaining

Because galectin-3, a glycan-binding protein, has been identified as another important endogenous ligand and activator of TLR4,

we also examined the localization and distribution of this galectin in the small blood vessels of angiolipoma tissue sections [53-58]. Immunofluorescence evidenced immunoreactivity for galectin-3 in the ECs of small blood vessels (Fig. 8). Likewise, galectin-3 was observed in some adipocytes and ICs (Fig. 8).

In-vivo Endoglin (CD105) Immunostaining

As endoglin (CD105) is a homodimeric glycoprotein that plays an essential role during angiogenesis and innate and adaptive immunity and that its expression can be upregulated by activated TLR4, we examined the presence and localization of this glycoprotein in the small vessels of angiolipoma tissue sections [75-77]. Immunofluorescence demonstrated that endoglin was localized in the ECs of the small vessels and in some ICs (Fig. 9,10).

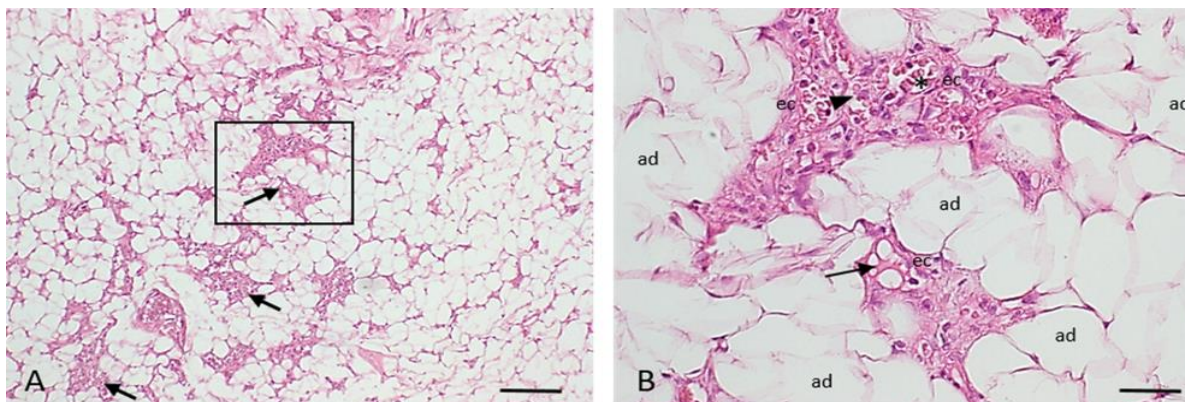


Figure 1: (A,B) Hematoxylin (H&E) staining from a biopsy of angiolipoma tissue located on thigh, showing the presence of dilated and tortuous small blood vessels containing intraluminal microthrombi (arrows), ICs (arrowhead) and RBCs (asterisk), intermingled with mature adipocytes (ad). ec, endothelial cell. Scale bars: 375 μ m (A); 75 μ m (B).

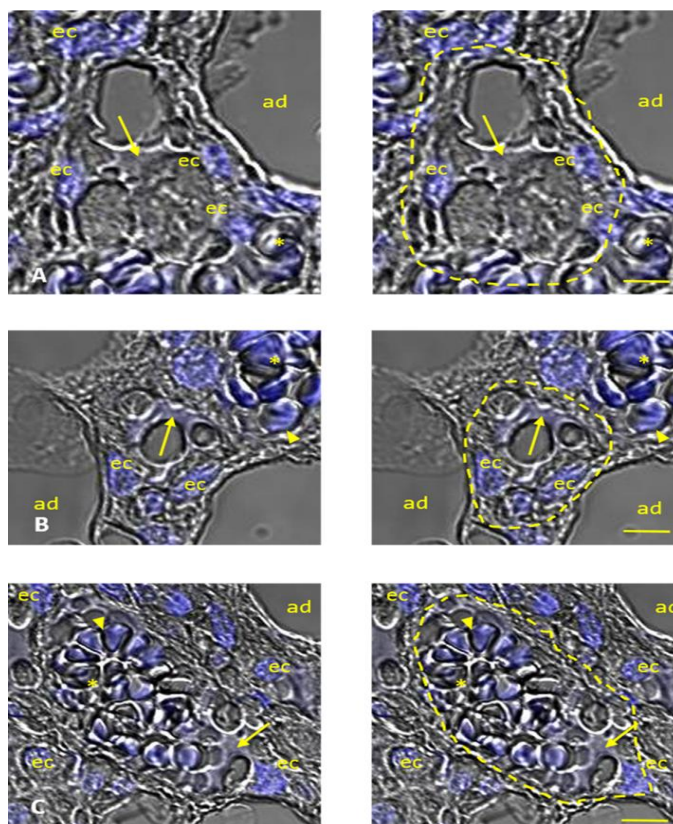


Figure 2: Differential interference contrast (DIC) images from two biopsies of angiolipoma tissues (A, arm; B,C, thigh). Note the presence of intraluminal microthrombi (arrows) delimited by ECs (ec), some ICs (arrowhead) and RBCs (asterisks). Yellow dotted lines enclose the small blood vessels. Blue, nuclear DAPI staining. Scale bars: 15 μ m (A); 25 μ m (B,C).

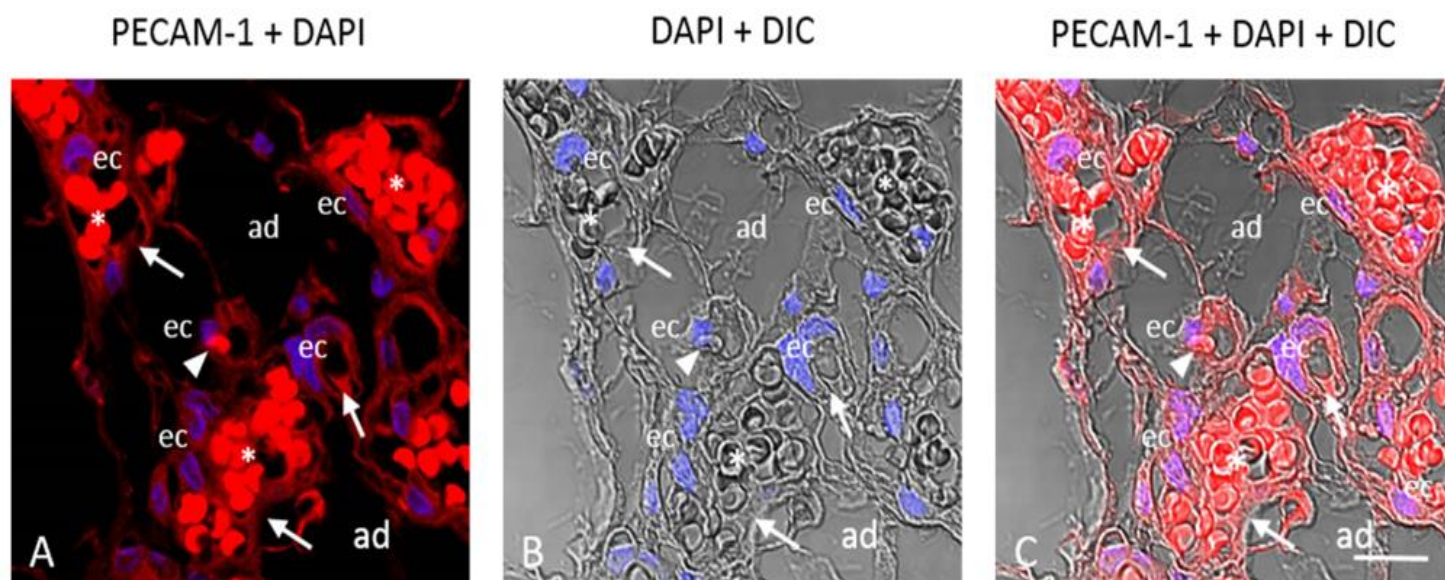


Figure 3: CLSM images of PECAM-1 (CD31) in the angioliipoma tissue located on thigh. (A,C) PECAM-1 (CD31) immunolocalization in the ECs (ec) of the tortuous and dilated small blood vessels containing intraluminal microthrombi (arrows), as well as in some ICs (arrowhead). Blue, nuclear DAPI staining. (B,C) DIC, differential interference contrast images. (C) Merge of images. Scale bar: 75 μ m. ad, mature adipocytes; RBCs (asterisks).

Perilipin

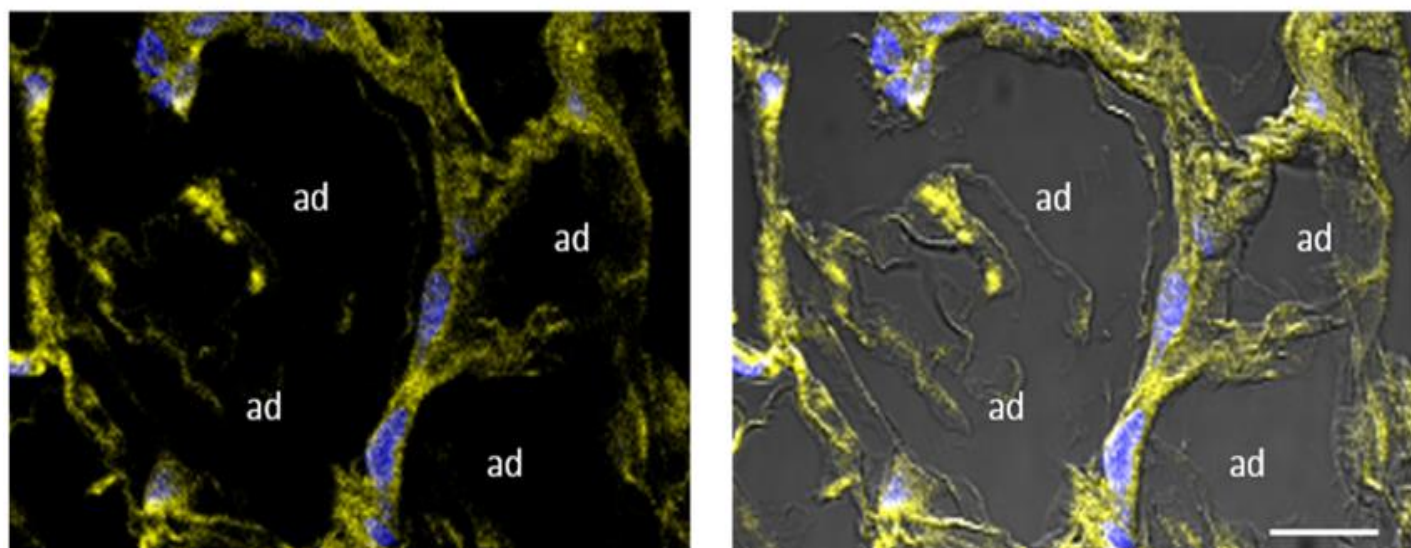


Figure 4: CLSM fluorescence image of perilipin in the same biopsy. Perilipin is immunolocalized in the mature adipocytes (ad). Blue, nuclear DAPI staining. Scale bar: 75 μ m. The corresponding overlay of fluorescence and DIC images is shown.

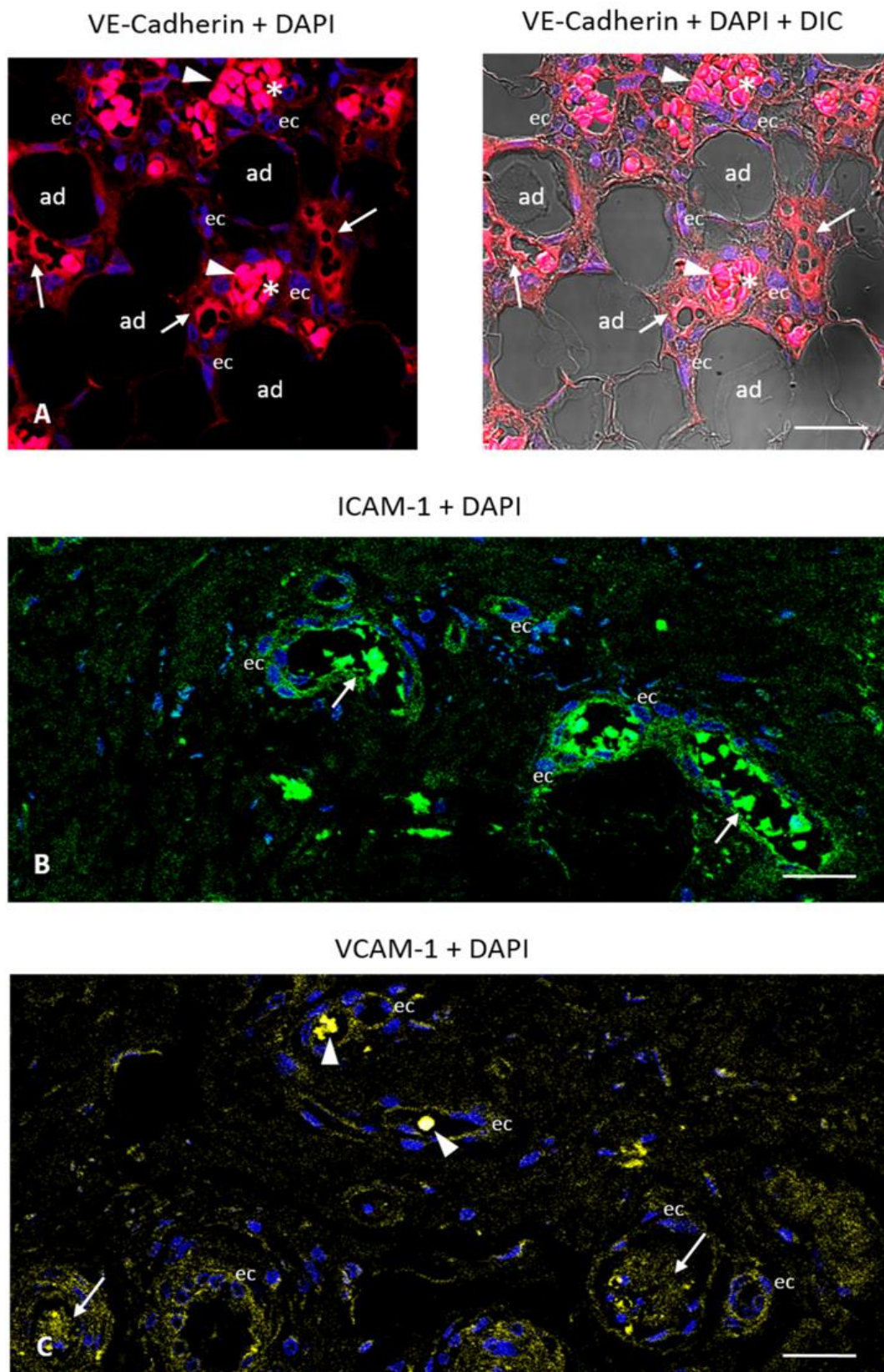


Figure 5: CLSM fluorescence image of VE-cadherin in the biopsy of angioliopoma located on thigh and ICAM-1 (CD54) and VCAM-1(CD106) in the biopsy of angioliopoma located on arm. VE-cadherin, ICAM-1 (CD54) and VCAM-1 (CD106) were immunolocalized in the ECs (ec) of the tortuous and dilated small blood vessels containing intraluminal microthrombi (arrows), as well as in some ICs (arrowhead). Blue, nuclear DAPI staining. DIC, differential interference contrast image. Scale Bar: 75µm. ad, mature adipocytes; RBCs (asterisks).

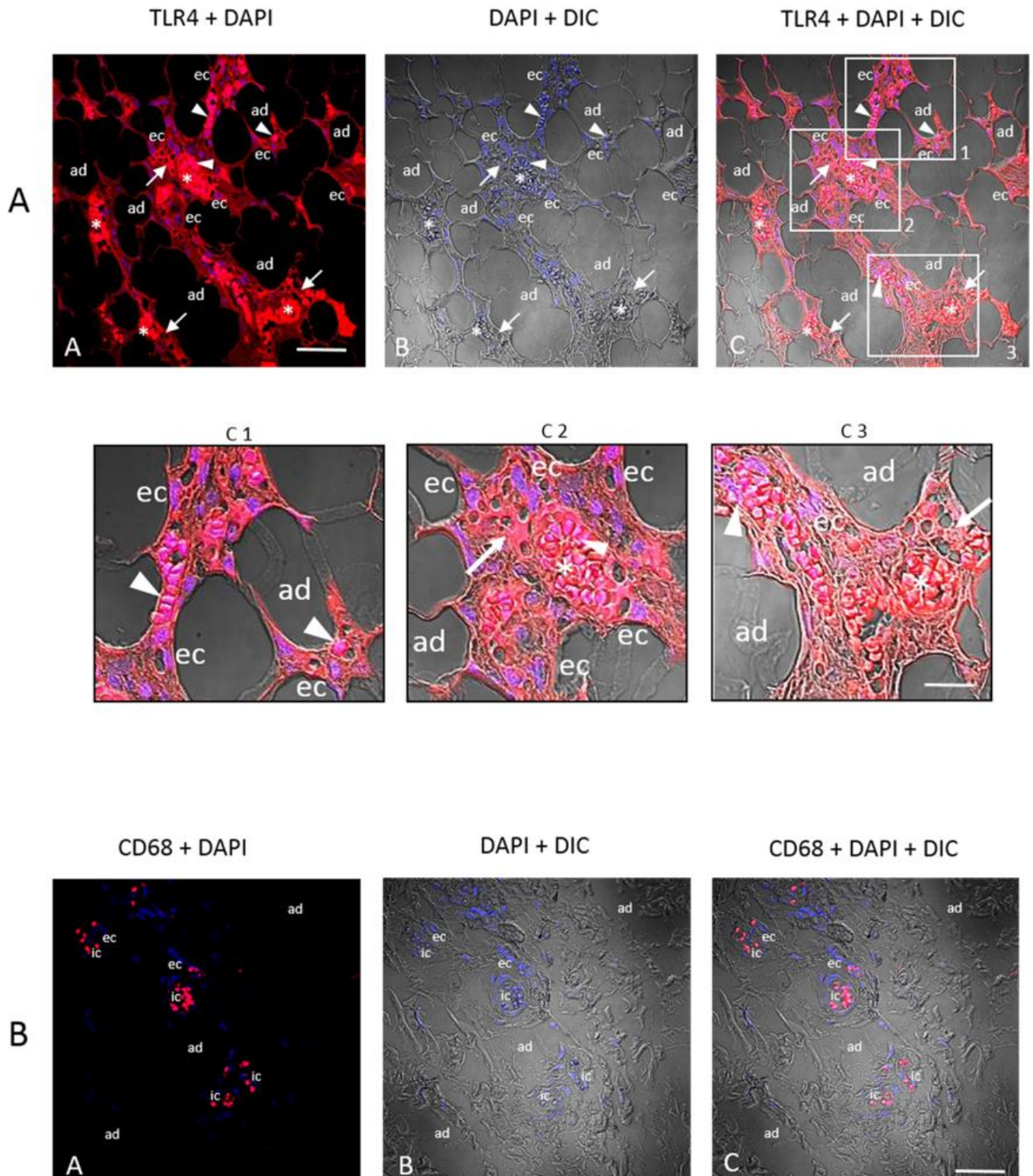


Figure 6: (A) CLSM images of TLR4 in the biopsy of angiolipoma located on thigh. (A,C) TLR4 immunolocalization in the ECs (ec) of the tortuous and dilated small blood vessels containing intraluminal microthrombi (arrows), as well as in some ICs (arrowhead) and some mature adipocytes (ad). Blue, nuclear DAPI staining. (B,C) DIC, differential interference contrast images. (C) Merge of images. Scale bar: 90 μ m. RBCs (asterisks). Enlargements (C1,C2,C3) show the immunoreactivity in the ECs (ec), some ICs (arrowheads) and mature adipocytes (ad). Blue, nuclear DAPI staining. Scale bar: 45 μ m. (B) CLSM images of CD68 in the same biopsy. (A,C) CD68 immunolocalization in ICs. Blue, nuclear staining. (B,C) DIC, differential interference contrast images. (C) Merge of images. Scale bar: 90 μ m.

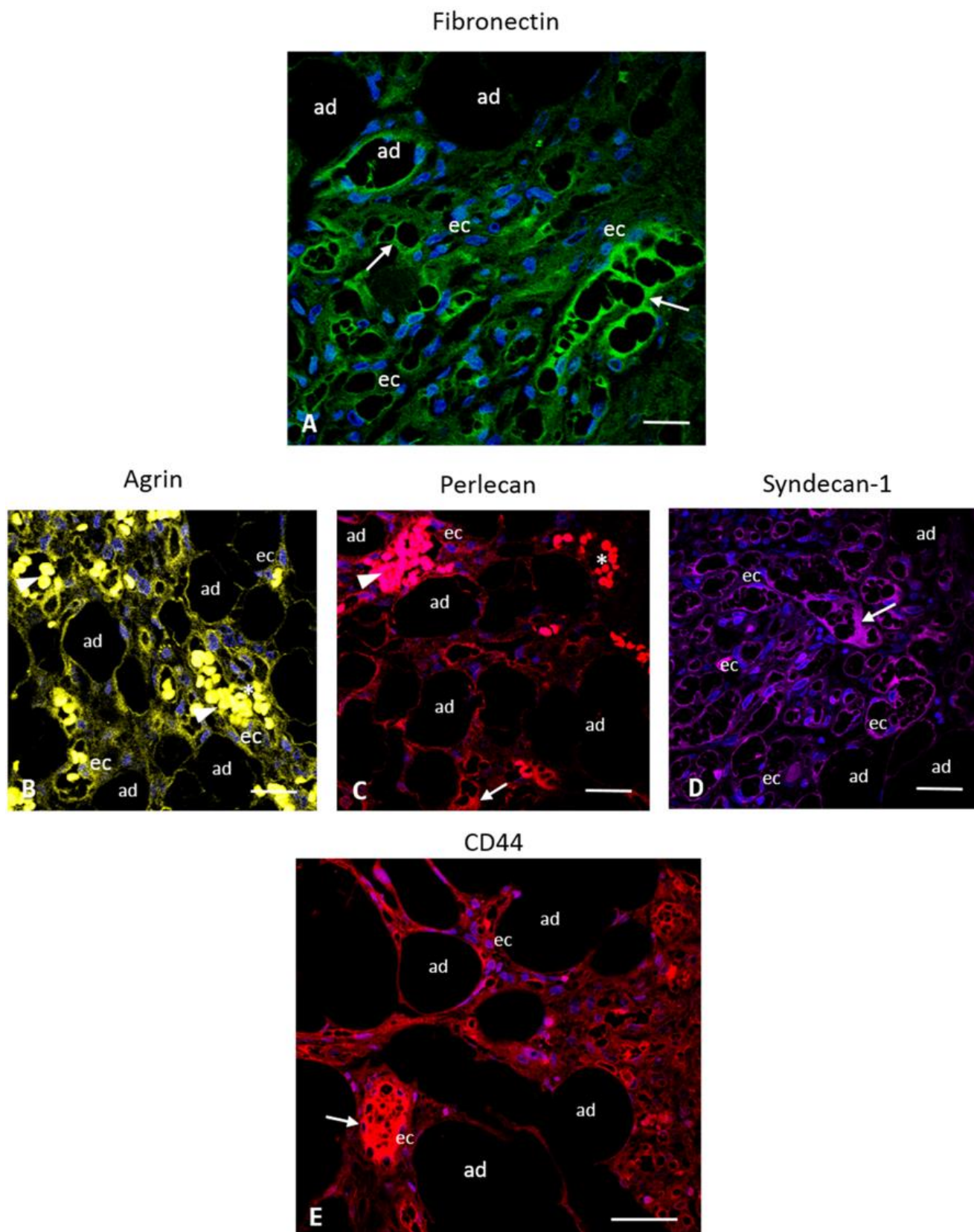


Figure 7: A series of representative CLSM fluorescence images of FN, agrin, perlecan, syndecan-1 and CD44 in a biopsy of angiolipoma located on arm. Immunoreactivities are observed in the small blood vessels, in some ICs (arrowheads), as well as in the extracellular space. Blue, nuclear DAPI staining. Scale bars: 50 μ m; 75 μ m. ec, endothelial cell; intraluminal microthrombi (arrows); ad, mature adipocyte. RBCs (asterisk).

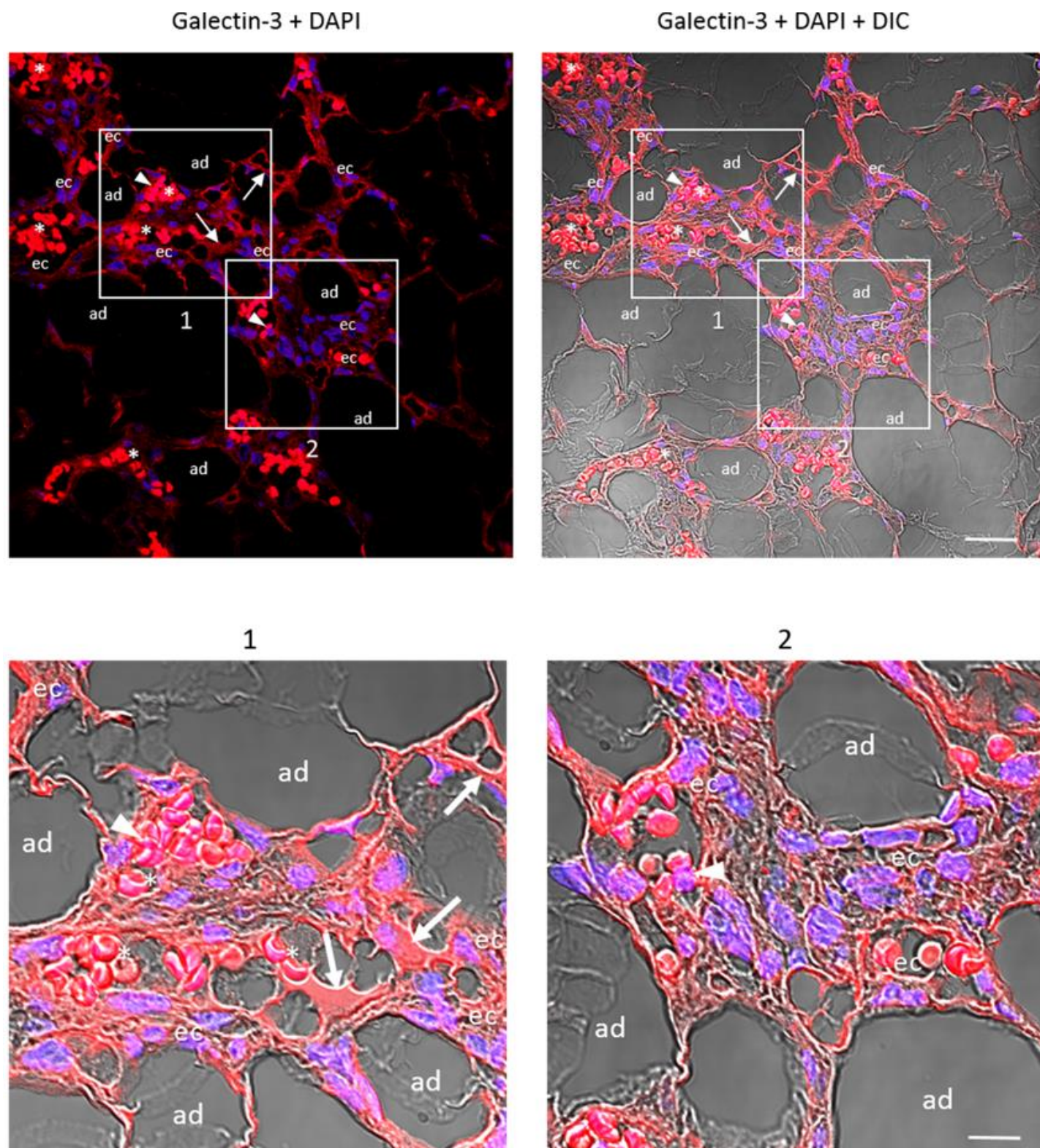


Figure 8: Immunolocalization of galectin-3 in the biopsy of angioliipoma located on thigh. Galectin-3 immunoreactivity is observed in the ECs (ec) of small blood vessels containing intraluminal microthrombi (arrows) as well as in some ICs (arrowheads) and mature adipocytes (ad). Blue, nuclear DAPI staining. DIC, differential interference contrast. Enlargements (1,2) show the galectin-3 immunoreactivity in the ECs (ec), ICs (arrowheads) and mature adipocytes (ad). Scale bars: 75 μ m; 20 μ m.

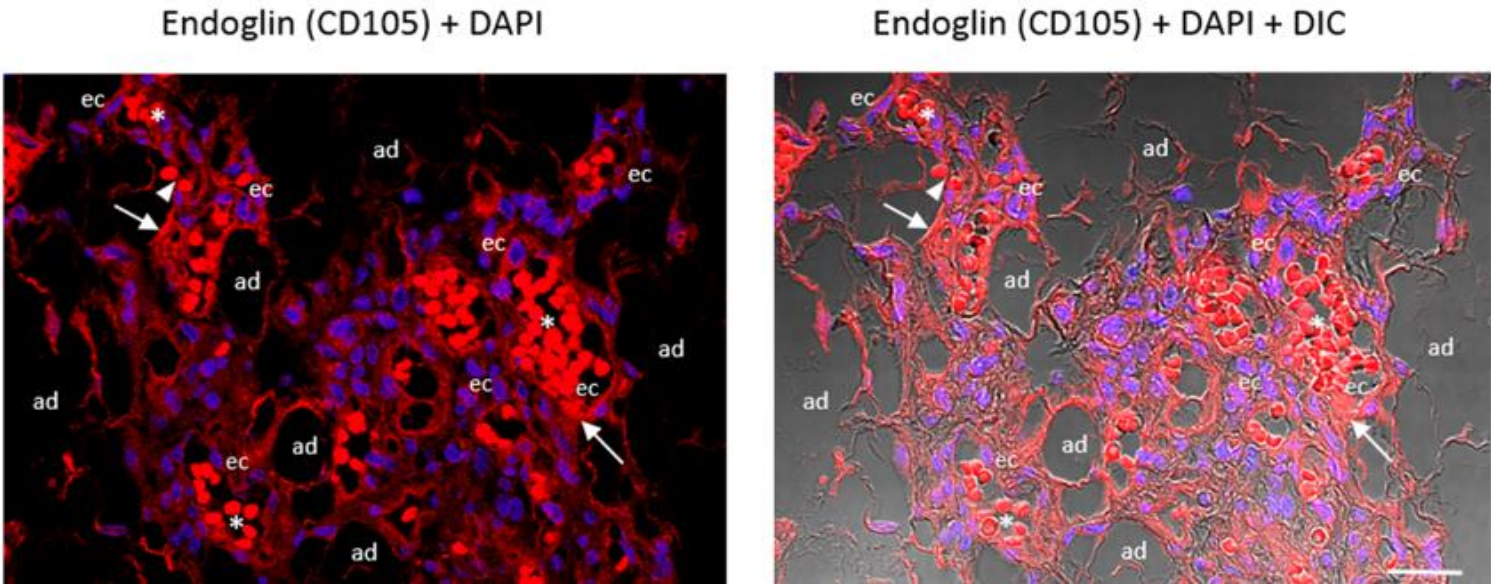


Figure 9: CLSM fluorescence image of endoglin (CD105) in the same biopsy. Endoglin is immunolocalized in the ECs (ec) of the tortuous and dilated small blood vessels containing intraluminal microthrombi (arrows), as well as in some ICs (arrowhead). Blue, nuclear DAPI staining. DIC, differential interference contrast image. Scale Bar: 40µm. ad, mature adipocytes; RBCs (asterisks). The corresponding overlay of fluorescence and DIC images is shown.

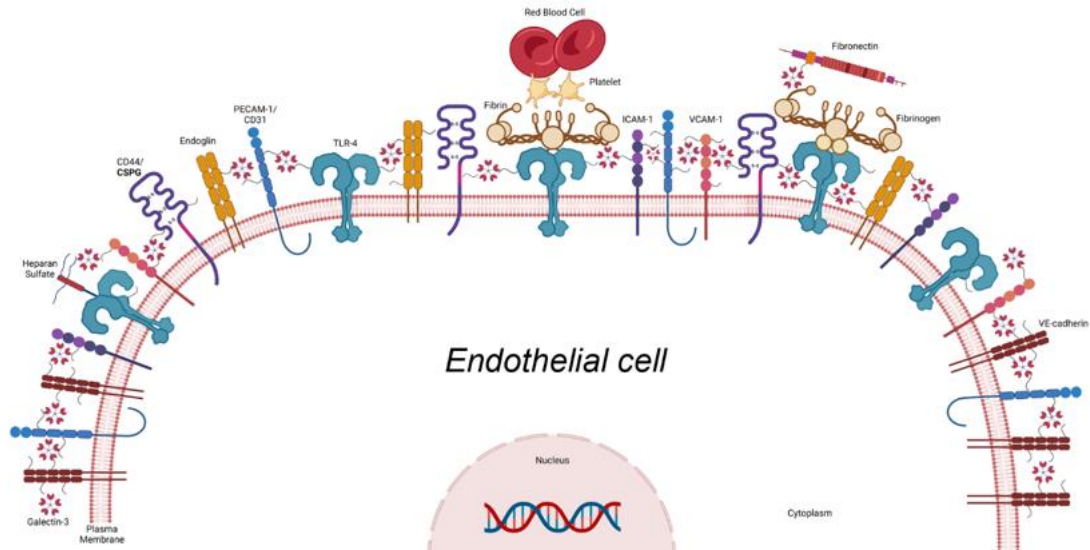


Figure 10: Schematic representation of galectin-3 forming a gal-glycan lattice on the endothelial surface with activated TLR4 and some of its endogenous ligands that include fibrinogen/fibrin, HSPGs and FN, as well as with some glycoconjugates associated to these ligands such as VE-cadherin, PECAM-1 (CD31), ICAM-1 (CD54), VCAM-1 (CD106), endoglin (CD105) and CD44.

Patient	Gender	Age	Site of Lesion
1	Female	41 years	Thigh
2	Male	62 years	Thigh
3	Female	48 years	Arm
4	Male	24 years	Arm
5	Male	38 years	Wrist
6	Female	48 years	Scapular region
7	Male	28 years	Arm

Table 1: Patients diagnosed with angiolipoma included in the study.

Primary Antibody	Host	Producer
Agrin	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
CD44	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
CD68	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Endoglin (CD105)	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Fibronectin	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Galectin-3	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
ICAM-1 (CD54)	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
PECAM-1(CD31)	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Perilipin (PLIN1)	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Perlecan	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Syndecan-1 (CD138)	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
TLR4	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
VCAM-1 (CD106)	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
VE-cadherin	Mouse monoclonal	Santa Cruz Biotechnology, Inc., Dallas, TX, USA

Table 2: Antibodies.

Discussion

In this study, we showed the presence of intraluminal fibrinogen/fibrin microthrombi delimited by ECs that exhibited immunoreactivities to VE-cadherin, ICAM-1 (CD54), VCAM-1 (CD106), PECAM-1 (CD31) and endoglin (CD105) and by some immunopositive CD68 ICs that also displayed ICAM-1, PECAM-1 and endoglin immunoreactivities in the small blood vessels of angioliopoma (arterioles, capillary-like structures and venules). In this regard, several reports in vascular ECs have established that fibrinogen/fibrin directly interacts with ECs through VE-cadherin, ICAM-1 and VCAM-1, operating as bridging molecule and facilitating the formation of capillary-like structures and promoting angiogenesis [7-10,16-20]. The same studies suggest that fibrinogen endothelial binding also increases the expression of growth factors such as FGF-2 and VEGF and inflammatory cytokines including Interleukin- β 1 (IL- β 1) and Tumor Necrosis Factor- α (TNF- α) stimulating inflammatory response and angiogenesis [8-10,16]. Similarly, fibrinogen binding to ICAM-1 has been shown to enhance IC-endothelium interaction with the participation of VE-cadherin, promoting leukocyte transmigration and inflammation [12,16,78]. Thus, it is likely that fibrinogen and fibrin serves a scaffold of glycoproteins that could be facilitating the EC-EC and IC-EC interactions through VE-cadherin, ICAM-1, VCAM-1, PECAM-1 and in turn, contributing to the vasculature formation in angioliopoma [7,11,13,21].

Of relevance, binding of fibrinogen/fibrin to TLR4 triggering the TLR4 activation and promoting inflammatory response and angiogenesis has been suggested [12,25,27,78]. Moreover, fibrinogen/fibrin effects have been associated with TLR4 signaling. In fact, there is evidence that fibrinogen stimulates monocyte/macrophage chemokine secretion at sites of inflammation through TLR4 activation [25,27,79]. Noteworthy, studies have highlighted that inappropriate endothelial activation through TLR4

contributes not only to initial microthrombus formation, but also to endothelial dysfunction [26-28,32,51]. The same studies propose that TLR4 raised the levels of Tissue Factor (TF), a blood clotting protein that contributes to thrombus formation [26-28,32,51]. In this context, other studies report that exaggerated activation of TLR4 can induce the expression of endothelial adhesion ICAM-1 and VCAM-1 [24,26,27,31,32,36]. In line with these reports, elevated mRNA levels expression of ICAM-1, VCAM-1 and E-selectin by microvascular ECs in response to TLR4 has been demonstrated [23,24,28,36]. Furthermore, inappropriate activation of endothelial TLR4 and up-regulation of endoglin expression promoting endothelial dysfunction and contributing to inflammation and angiogenesis has been proposed [77]. However, despite it has been suggested that inappropriate activation of ECs through TLR4 could contribute to tissue damage and defective angiogenesis, the mechanisms underlying the interaction of ECs with TLR4 are still being explored [26,28,29,32].

In this study, immunofluorescence staining revealed that TLR4 and some of their endogenous ligands such as FN, HSPGs including agrin, perlecan and Synd-1, as well as the glycoconjugates associated to these ligands such as VE-cadherin, VCAM-1, ICAM-1, PECAM-1, endoglin and CD44 were localized in the ECs from the small vessels and some ICs. Incidentally, studies on ECs and TLR4 activation have proposed that excessive TLR4 activation by endogenous ligands including ECM components such as fibrinogen, FN, HSPGs, TN-C and hyaluronan, contribute to endothelial dysfunction promoting endothelial proliferation, migration, apoptosis and tube-like structures formation, involving upregulation of ICAM-1 and VCAM-1 expression and secretion of cytokines, chemokines and growth factors [24,25,27,32]. Similarly, studies on inflammation, angiogenesis and TLRs have suggested that endothelial dysfunction via TLRs interaction with endogenous ligands generated upon cellular injury, cell death or stressed tissues contributes to inflammation and angiogenesis [23,26-28,31]. Therefore, it is likely that the endothelial TLR4 endogenous ligands contribute not only to the activation of TLR4 but also to the vasculature formation in angiolipoma. With respect to Gal-3, another important endogenous ligand and activator of TLR4, immunofluorescence also evidenced immunoreactivity for galectin-3 in the ECs of small vessels as well as in some ICs. Consistent with this, studies on galectin-3 have suggested that this glycan-binding protein has a great affinity for the β -galactoside residues present in the TLR4 and TLR2 ectodomains regulating innate and adaptive responses and promoting inflammatory response and angiogenesis [54,58]. Remarkably, studies have point to the involvement of galectin-3 in the leukocyte recruitment *in-vivo* and angiogenesis [54,59,63-69]. Furthermore, galectin-3 oligomerization upon binding to glycoconjugates containing β -galactoside residues on EC surface such as Synd-1, CD44, ICAM-1, VCAM-1, VE-cadherin, N-cadherin, PECAM-1 and endoglin has been proposed, suggesting a critical role for galectin-3 in endothelial morphogenesis and angiogenesis [59,65,68,80]. This is interesting if we consider that published evidence indicates that galectin-3 oligomerization can generate galectin-lattices on the endothelial surface and ICS which facilitate endothelial activation, migration, proliferation and tubule-like structures formation [68,69,80-82]. What is more, the glycoconjugates above mentioned, including fibrinogen, in addition to being assumed as endogenous ligands and activators of endothelial TLR4 are also considered as potential binding partners for galectin-3, as they contain N- and O-linked glycan residues in their ectodomains [24,25,27,32,80,82].

Conclusion

Therefore, it is possible that in angiolipoma tissues galectin-3 oligomerization can lead to the formation of gal-glycan lattices on the endothelial surface that might be facilitating not only the activation of endothelial TLR4, but also contributing to the vasculature formation regulated by signaling pathways mediated by certain cytokines, chemokines and growth factors (Fig.10).

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Author Contributions

E.A. analyzed the data and wrote the paper; H.R., A.D., A.S. and R.R. analyzed the data and constructed the images; A.D. constructs the scheme; J.P. and R.C. examined the biopsies. All authors read and approved the final manuscript.

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Supplementary File

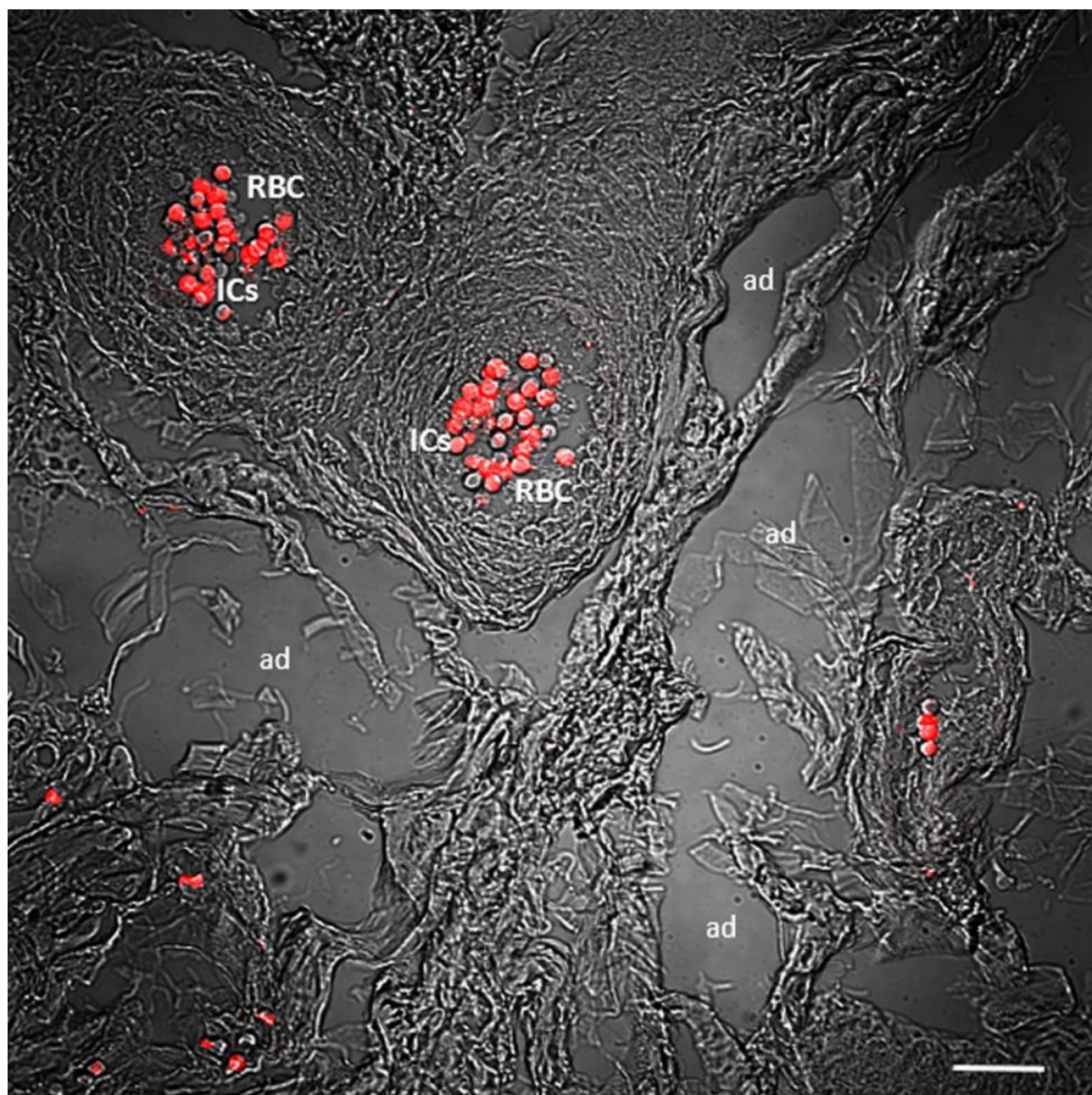


Figure S1: Autofluorescence at the red wavelength.

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