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P Nizet, V Untereiner, Gd Sockalin, I Proult, C Terryn, et al.. Assessment of Ovarian Tumor Growth in WildType and Lumican-Deficient Mice: Insights Using Infrared Spectral Imaging, Histopathology, and Immunohistochemistry. Federation of European Biochemical Societies, FEBS, May 2022, HERAKLION, Greece. hal-03926295

HAL Id: hal-03926295

<https://hal.univ-reims.fr/hal-03926295>

Submitted on 6 Jan 2023

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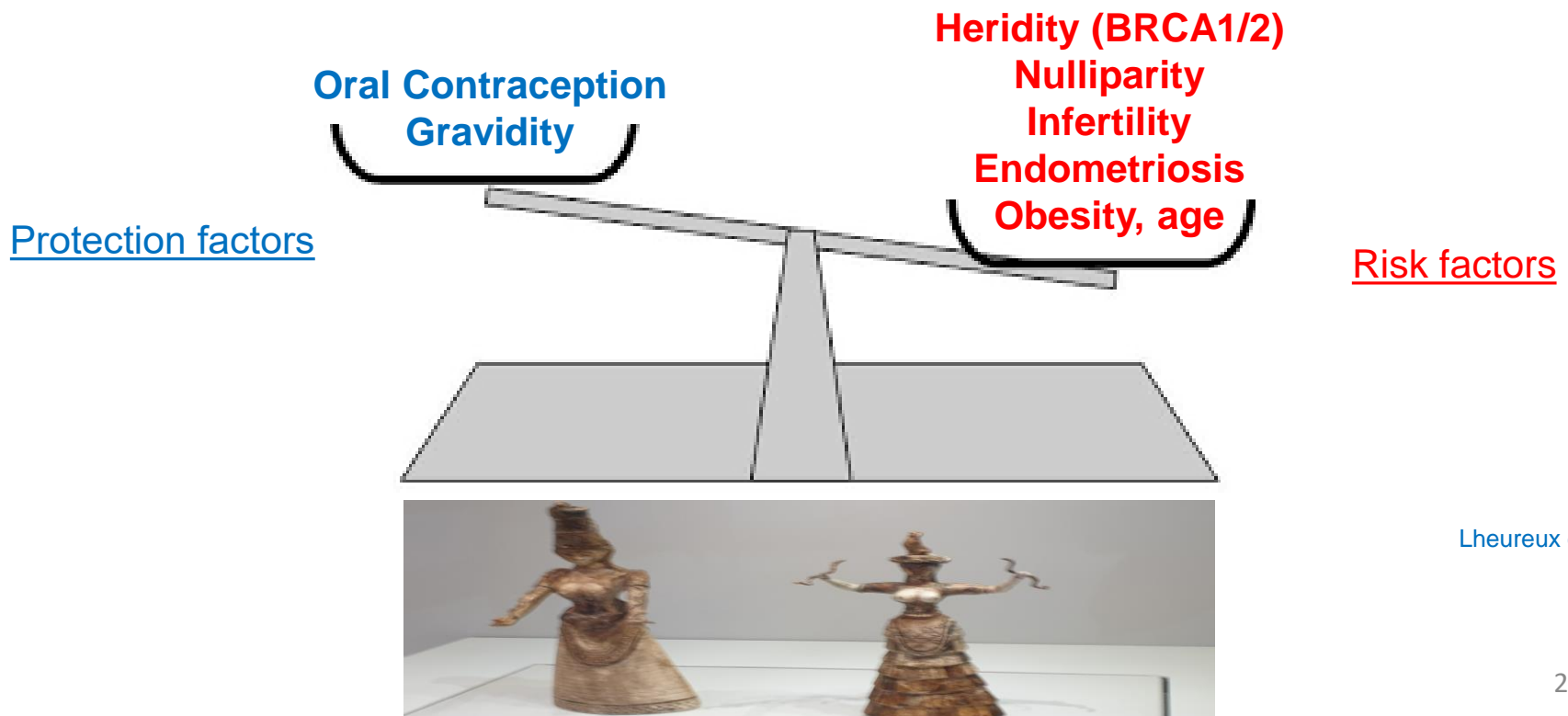
Assessment of Ovarian Tumor Growth in WildType and Lumican-Deficient Mice: Insights Using Infrared Spectral Imaging, Histopathology, and Immunohistochemistry

Nizet P, Untereiner V, Sockalingum GD, Proult I, Terryn C, Jeanne A, Nannan L, Boulagnon-Rombi C, Sellier C, Rivet R, Ramont L, Brézillon S.

Extracellular Matrix and Cell Dynamics (MEDyC), CNRS UMR/URCA N° 7369,
Faculté de Médecine, Reims, France
Head: Pr L. Martiny

Ovarian Cancer (OVC)

- **7th cancer worldwide**
 - 3.6% of cancers
 - 230.000 new cases per year
- **Second cause of mortality by gynecological cancer in the world**
 - 150.000 deaths per year
 - 46% survival 5 years after the diagnosis.



Ovarian Cancer (OVC)

- Diagnosis

- Frequently very late (asymptomatic disease)

About 75% of patients are diagnosed at an advanced stage because of the asymptomatic nature of EOC.

Late stage presentation has a 5-year relative survival rate of 29%, by contrast with 92% for early-stage disease.

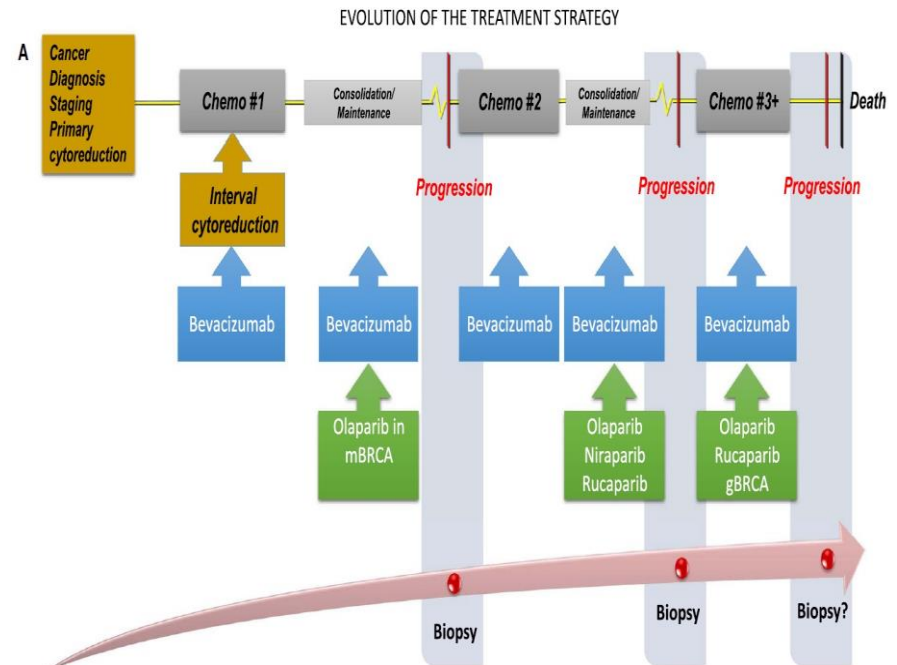
- Clinical imaging
- Dosage of CA-125 or HE4

- Treatment

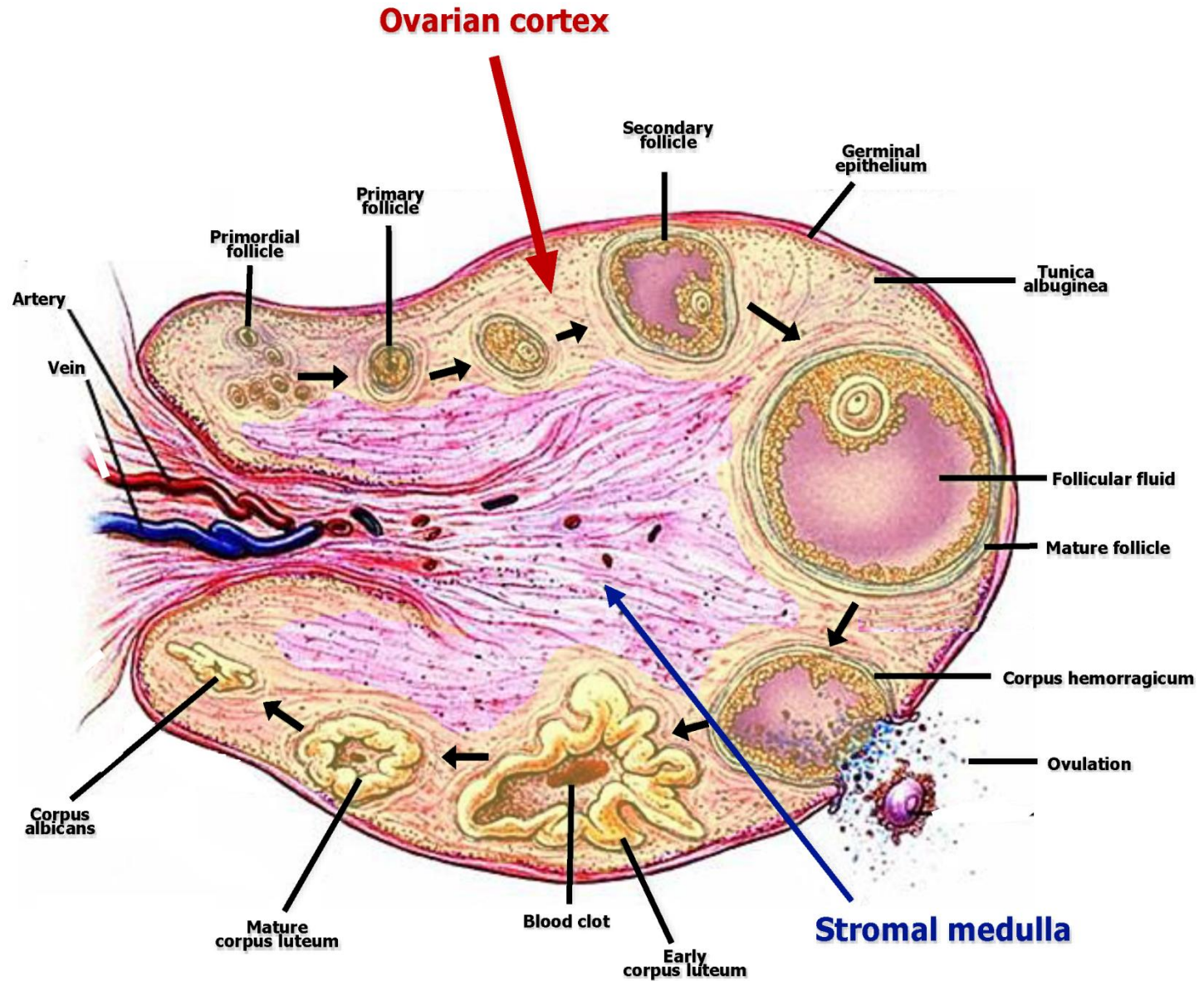
- Surgery
- Chemotherapy (Carboplatin + Paclitaxel)
- anti-angiogenic treatment (Bevacizumab)
- Inhibitors of PARP (Olaparib, niraparib, rucaparib)

- But still low efficiency due to

- Strong heterogeneity of the tumors
- High relapse rate



Ovary structure

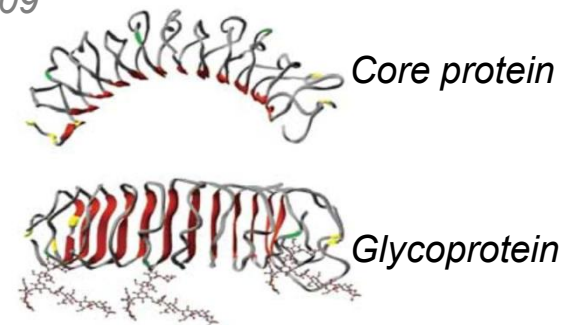
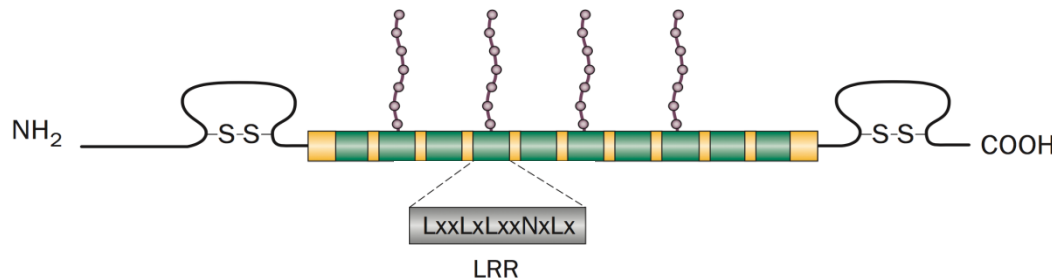


Lumican

- ✓ small leucine-rich proteoglycan (SLRP)
- ✓ abundant within tumor reactive stroma

In melanoma:

- ↘ lumican expression = more infiltrative disease *Brézillon et al., Clin Exp Dermatol 2007*
- promotes cell adhesion and inhibits cell migration
*D'Onofrio et al., Biochem Biophys Res Commun 2008 ;
Brézillon et al., Cancer Lett 2009 ; Zeltz et al., Exp Cell Res 2010 ; Stasiak et al., PLoS One 2016;
Jeanne et al., Scientific reports, 2017;
Brézillon et al., Frontiers in Cell and developmental Biology, 2020;*
- angiostatic properties *Brézillon et al., J Physiol Pharmacol 2009*



Kao et al, 2006

LUMICAN = KEY REGULATOR OF COLLAGEN FIBRILLOGENESIS

OVC & lumican

- Glycoproteomic Analysis of Malignant **Ovarian Cancer Ascites** Fluid Identifies Unusual Glycopeptides.

Miyamoto S *et al.* J Proteome Res. 2016 Sep 2;15(9):3358-76.

- O-Linked glycome and proteome of high-molecular-mass proteins in human **ovarian cancer ascites**: Identification of sulfation, disialic acid and O-linked fucose.

Karlsson NG, McGuckin MA. Glycobiology. 2012 Jul;22(7):918-29.

- The significance of **lumican** expression in **ovarian cancer** drug-resistant cell lines.

Klejewski A *et al.* Oncotarget. 2017 Aug 10;8(43):74466-74478.

- HMGA2 overexpression-induced **ovarian** surface epithelial transformation is mediated through regulation of EMT genes.

Wu J *et al.* Cancer Res. 2011 Jan 15;71(2):349-59.

Addressed Questions:

1) May lumican expression within an OVC microenvironment influence tumor matrix assembly as well as microvascular density?

- *In vivo* ovarian cancer allograft model
- Histological analyses
- IHC analyses of isolated tumors (Lumican, α v Integrin subunits, Cyclin D1, CD31)

2) Does lumican control tumor collagen molecular assembly?

- Second Harmonic Generation (SHG) imaging
- Polarized light microscopy (Picrosirius red staining)
- Fourier Transform infrared (FTIR) tumor spectral images

ID8 murine cell line
Ovarian Surface Epithelium
Epithelial phenotype
Agressiveness +++

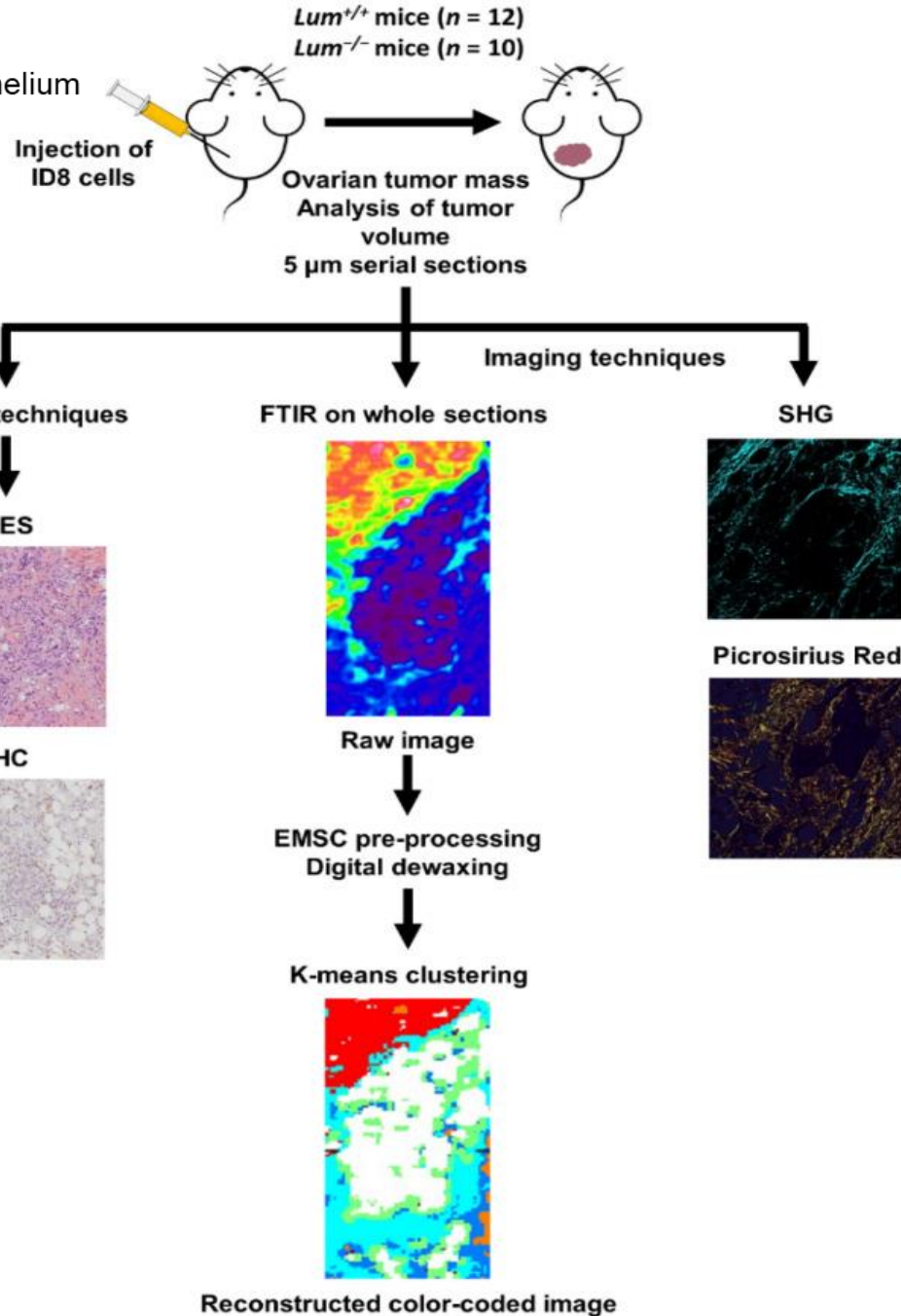


Figure 1: Workflow showing the histology, the immunohistochemistry of formalin-fixed paraffin-embedded ID8 ovarian tumor⁸ sections, SHG imaging, Picrosirius red staining (polarized light), and analysis of FTIR images using common K-means clustering

Results

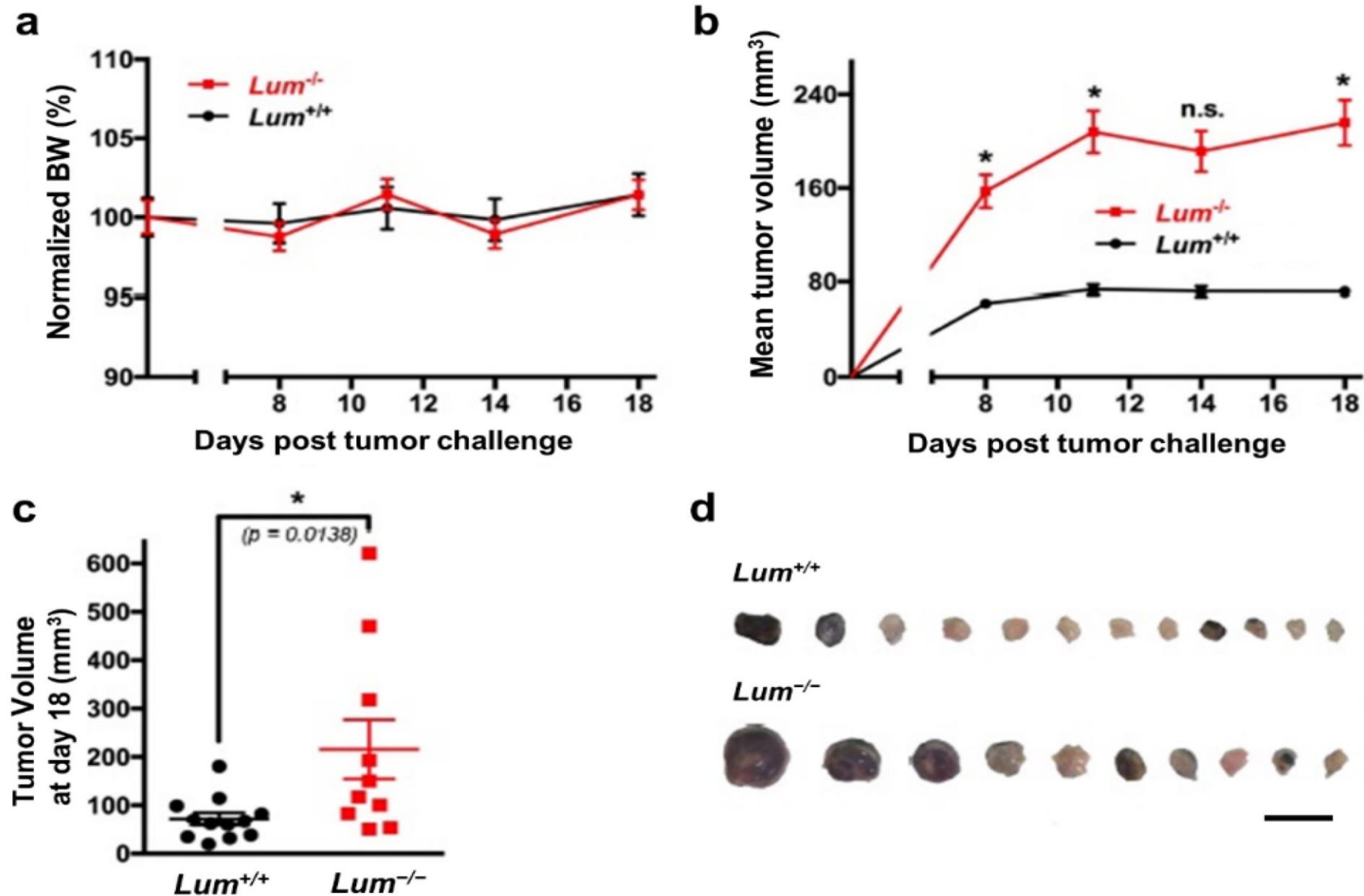


Figure 2: Evaluation of endogenous lumican impact on tumor growth in an ovarian allograft model.

(a–d) ID8 ovarian tumor cells (2.5×10^5) were s.c. inoculated in wild-type ($Lum^{+/+}$) or lumican-deficient ($Lum^{-/-}$) syngeneic C57BL/6J mice.

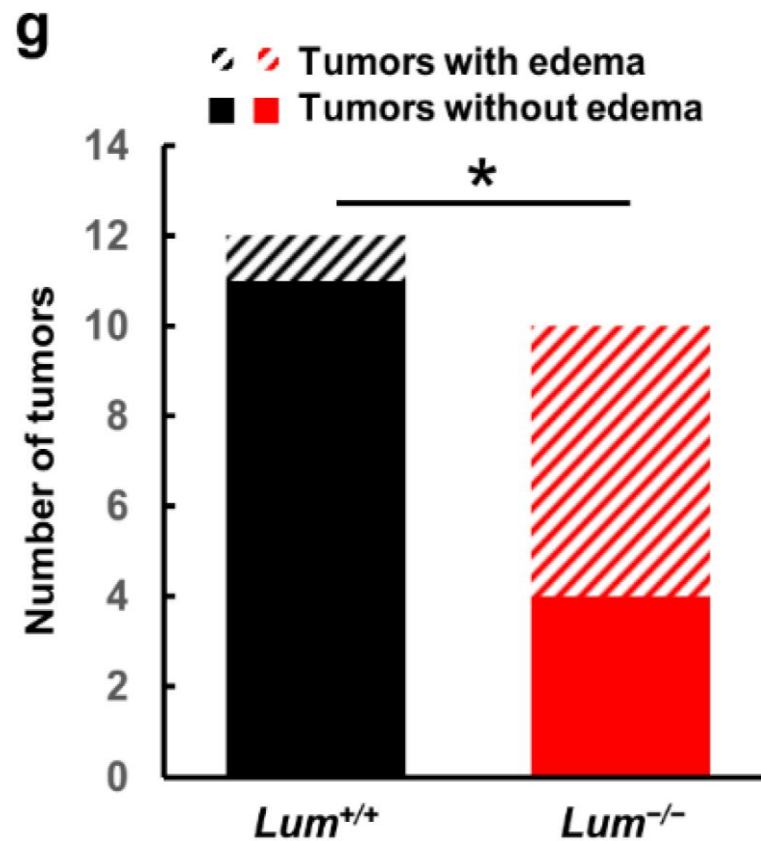
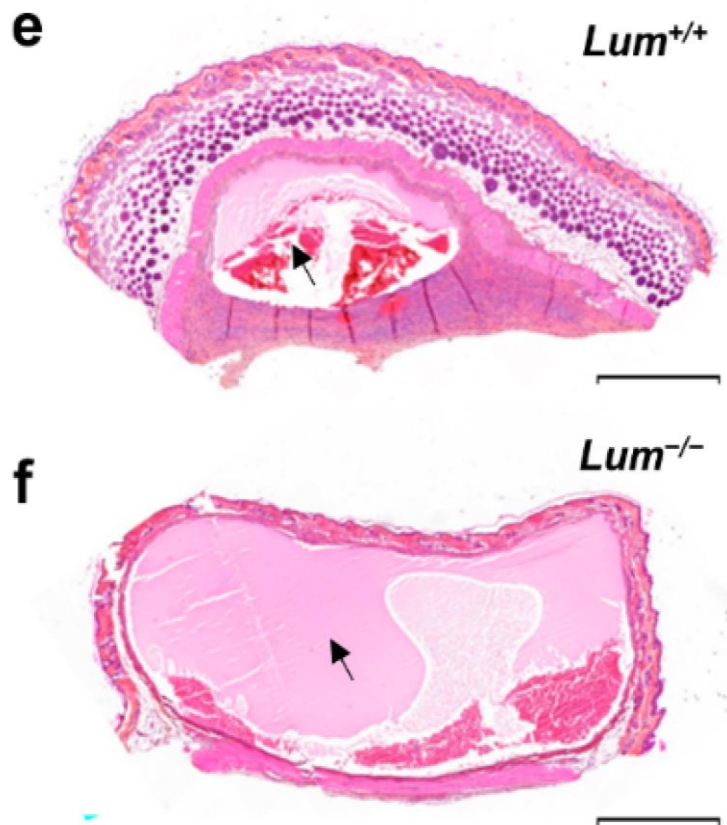


Figure 2 (continued):

Representative images of edemas observed in HES staining of *Lum*^{+/+} (**e**) and *Lum*^{-/-} (**f**) tumor sections are shown (scale bar, 500 μ m);

(**g**) Quantification of the number of edemas observed in ovarian tumor sections of *Lum*^{+/+} or *Lum*^{-/-} syngeneic C57BL/6J mice (* $p < 0.05$).

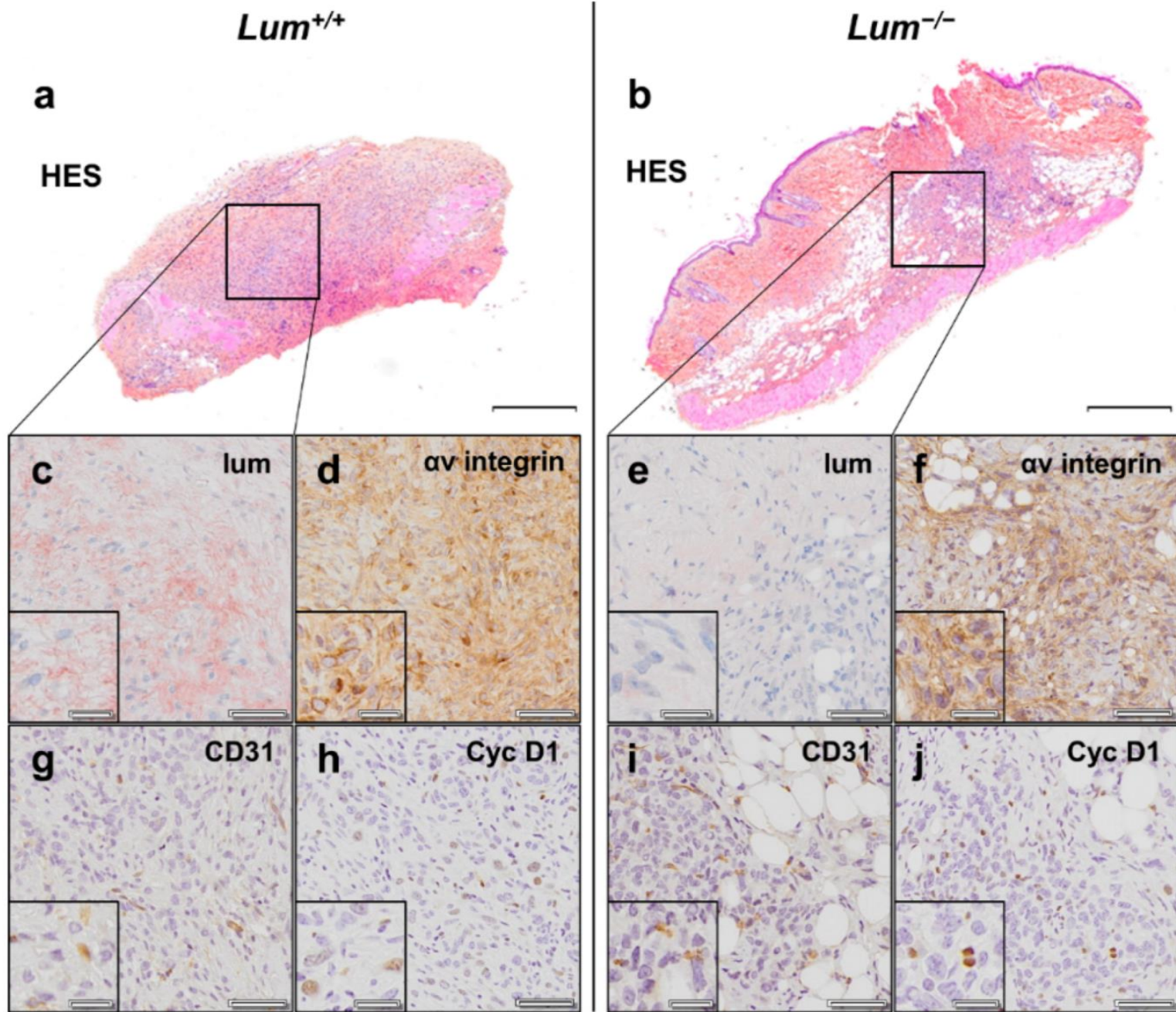
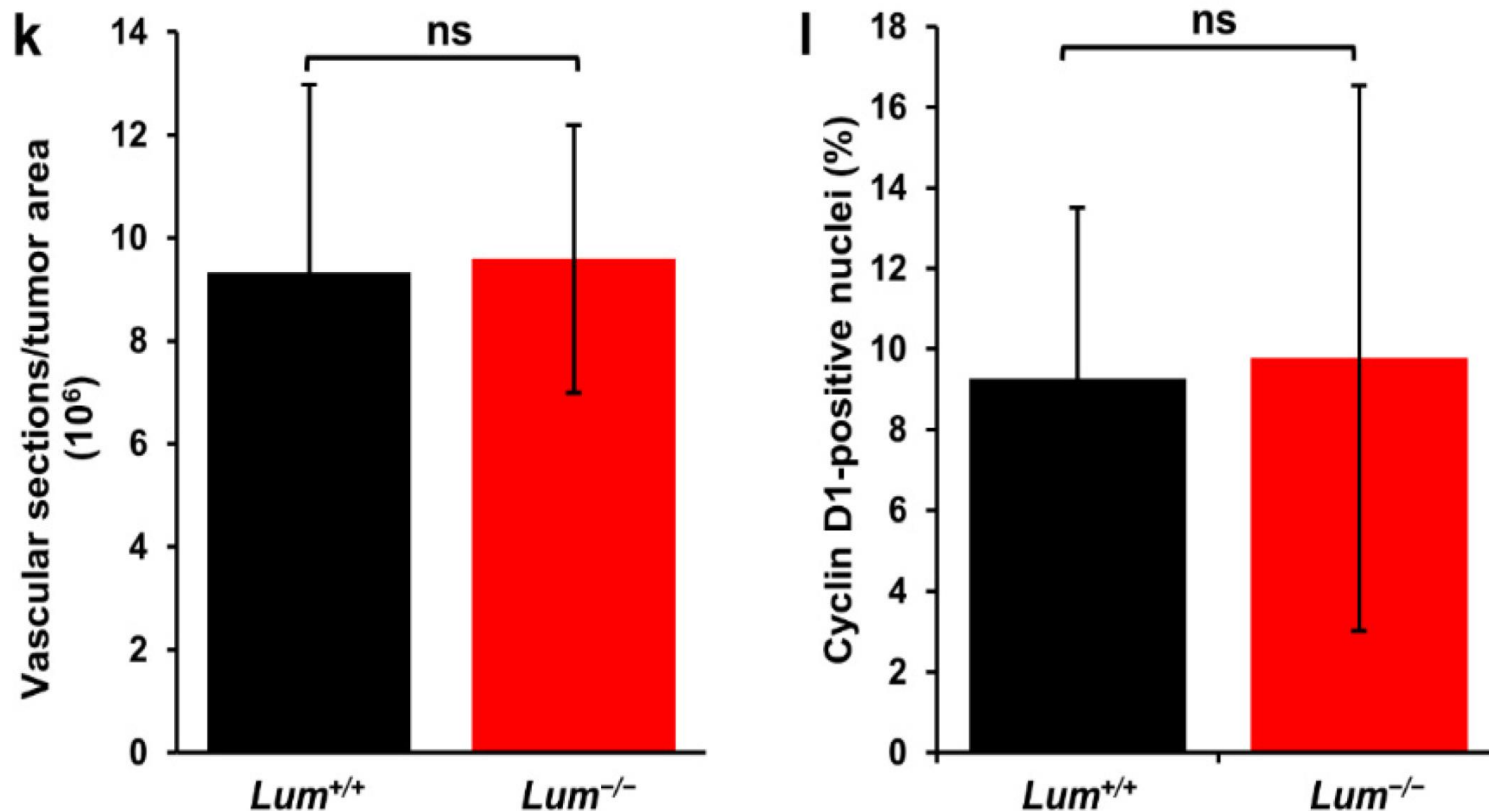


Figure 3: Histological and immunohistochemical analysis of ovarian tumor sections.



[Figure 3](#) (continued):

(k) Quantification of percentage of **CD31**-positive blood vessels. The quantification of the MicroVascular Density (MVD) was based on a manual counting of full vascular sections formed by CD31-positive endothelial cells. All acquisitions were performed with a 20× magnification.

(i) cyclin D1-positive areas (number of positive cyclin D1 tumor cell nuclei normalized to the total number of tumor cell nuclei) **(l)** (mean ± SD, ns: not significant).

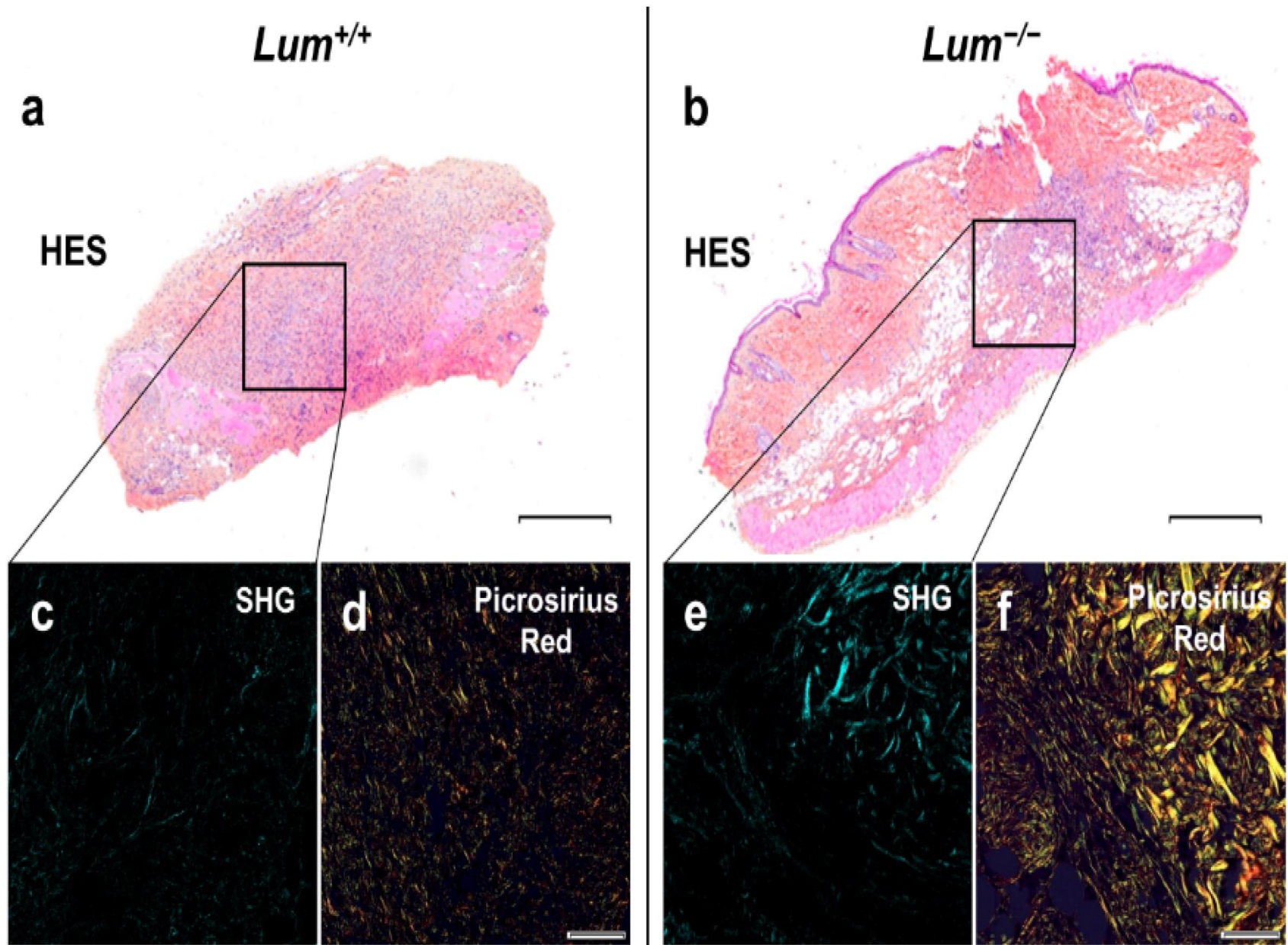


Figure 4: Analysis of collagen organization in ovarian tumor sections of wild-type and lumican-deficient mice.

Birefringence of collagen fibers allows distinction between type I (red) and type III (green) collagens.

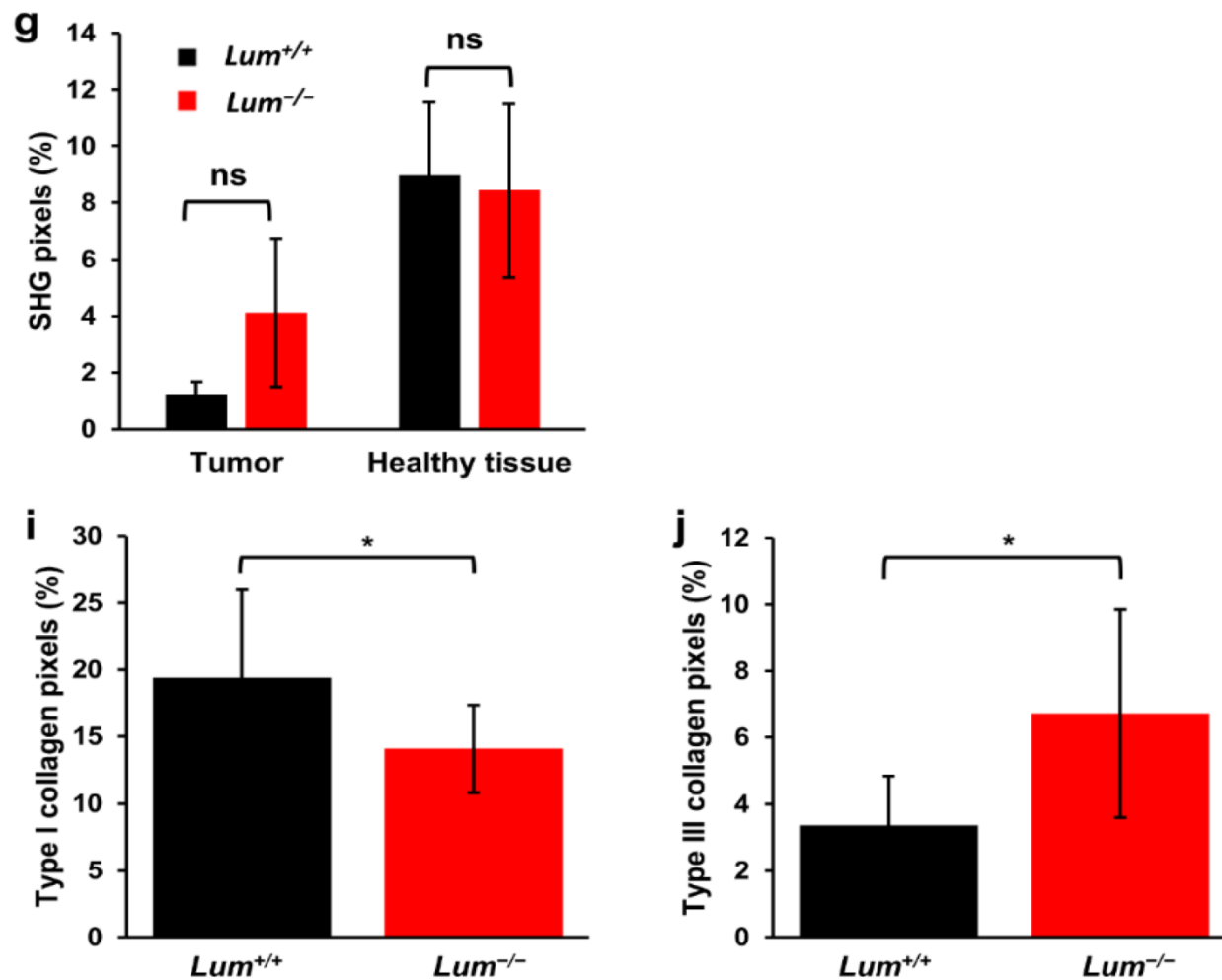


Figure 4 (continued) :

(g) Analysis of **collagen fibers intensity by SHG** in tumors and healthy tissues present in each section (mean \pm SD, ns: not significant);

(i,j) Quantification on Picrosirius red stained sections of the relative distribution of red pixels (corresponding to **type I collagen**) and of green pixels (corresponding to **type III collagen**) within tumors of *Lum*^{+/+} and *Lum*^{-/-} sections (mean \pm SD, * $p < 0.05$).

✓ **Significant decrease of Type I/III collagen pixels ratio within tumors of *Lum*^{-/-} sections**

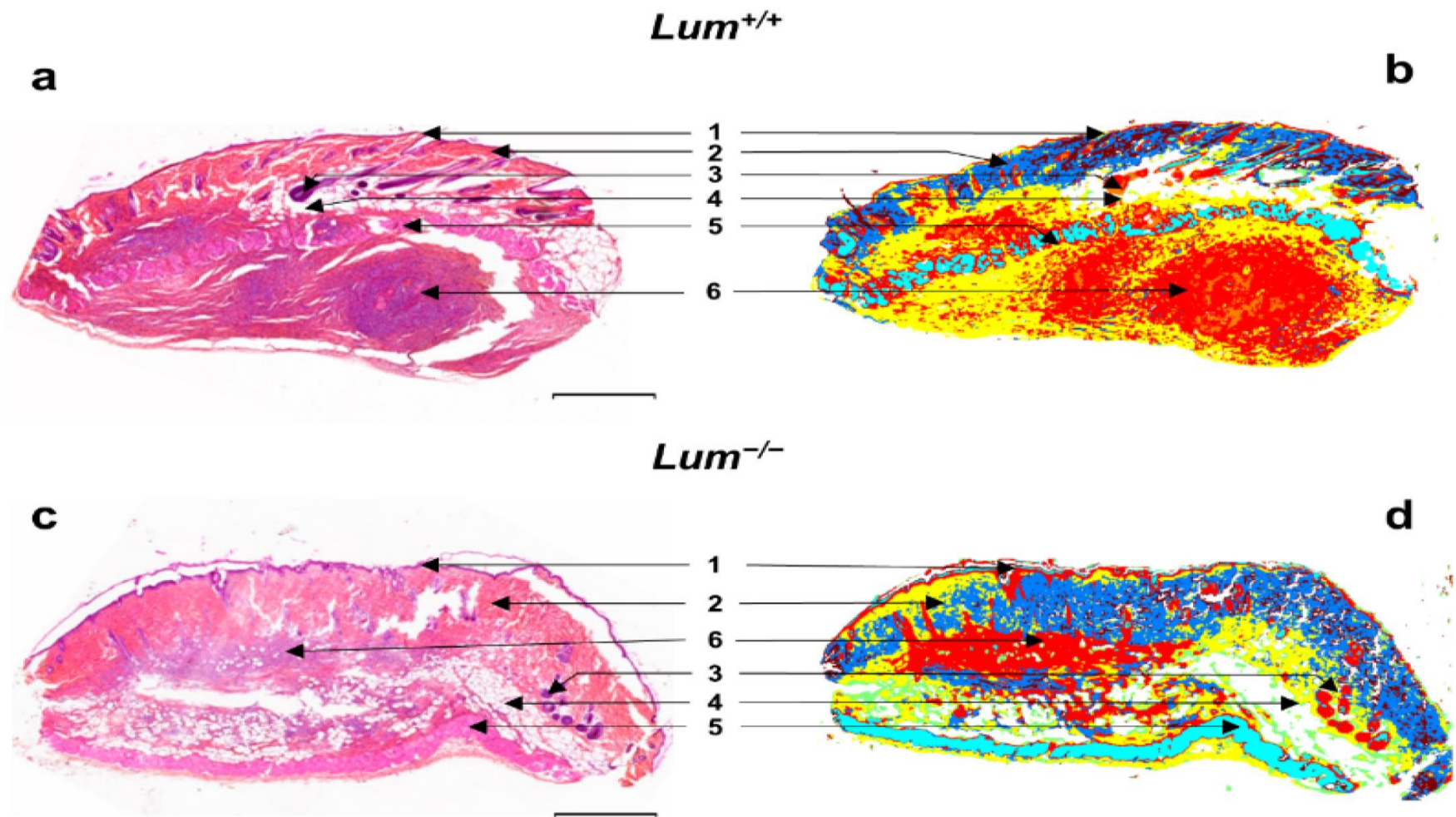
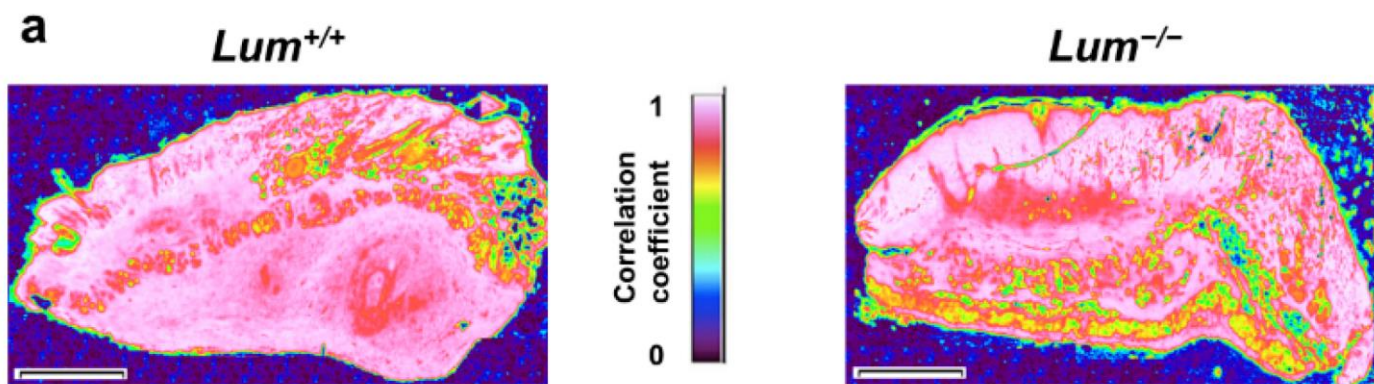


Figure 5: K-means clustering of FTIR spectral images of ovarian tumor sections in wild-type and lumican-deficient mice.

(a,c) Example of s.c. allograft whole sections stained with **HES** (original magnification 20× scale bar 500 μm) in *Lum^{+/+}* (a) and *Lum^{-/-}* mice (c); (b,d) Representative color-coded K-means (7 classes) clustered images of tumor sections in *Lum^{+/+}* (b) and *Lum^{-/-}* mice (d)

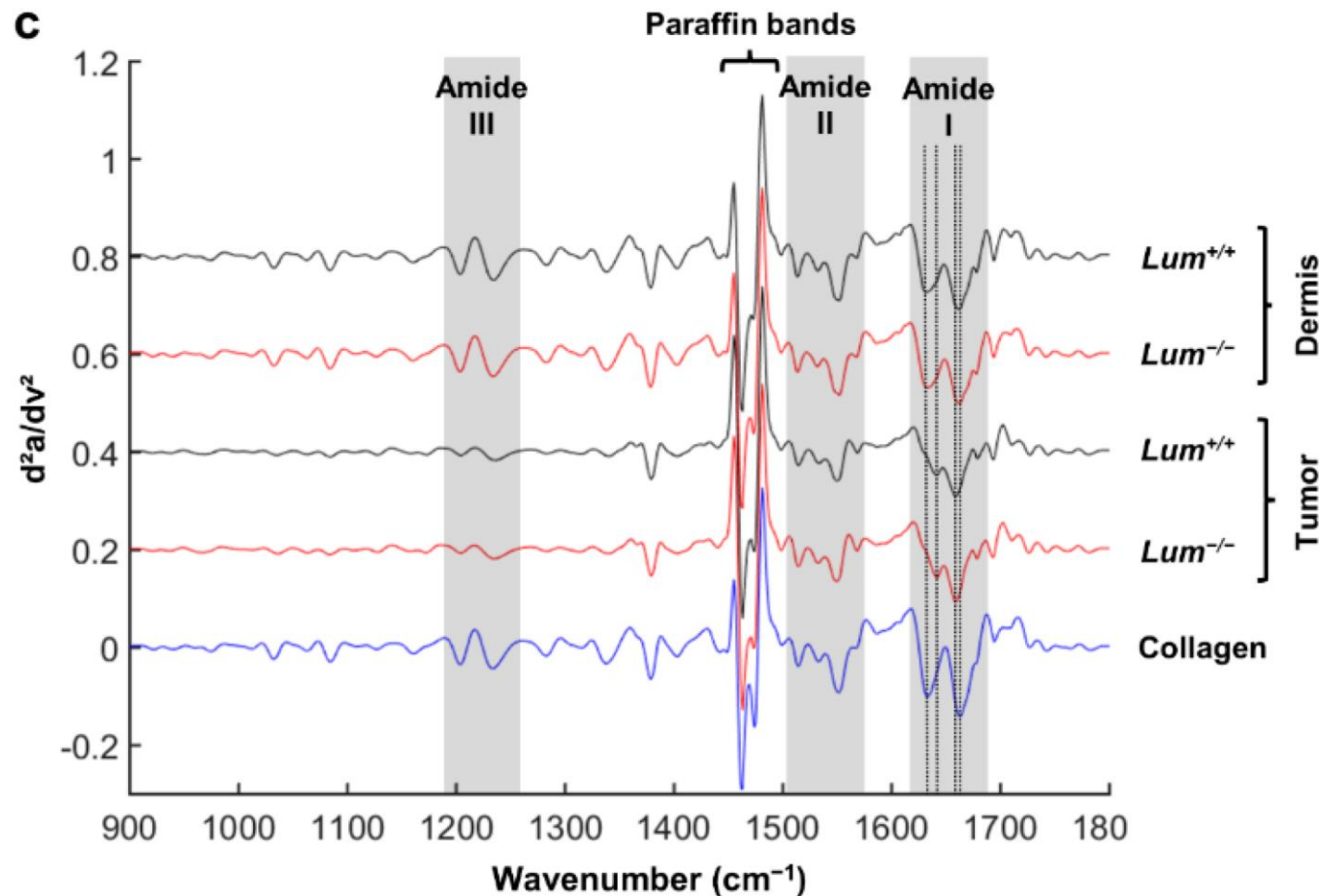
(1: epidermis, 2: dermis, 3: hair bulb, 4: hypodermis, 5: smooth muscle, 6: tumor)

- ✓Capacity of infrared spectral histology to discriminate tissue structure;
- ✓Loss of ECM integrity in *Lum^{-/-}* mice



b [Figure 6:](#) Correlation maps using type I collagen reference spectrum.

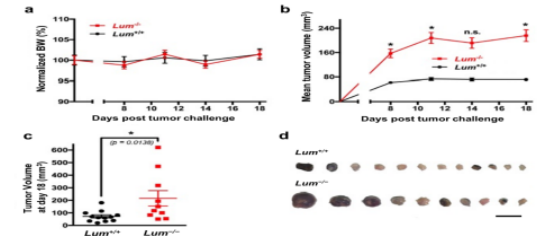
(a,b) Type I collagen spectral correlation images of *Lum*^{+/+} (a) and *Lum*^{-/-} (b) tissue sections (scale bar 500 μm). Original images were each correlated with a pure type I collagen spectrum. Provided scale indicates the degree of correlation from 0 (black, not correlated) to 1 (white, completely correlated).



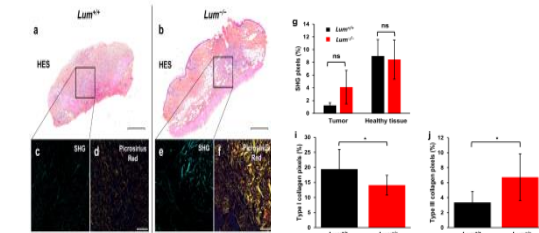
(c) Comparison between type I collagen second derivative spectrum (blue line) with second derivative spectra taken randomly from the dermis and tumors of *Lum*^{+/+} (black lines) and *Lum*^{-/-} (red lines) mice skin tissues.

Key findings

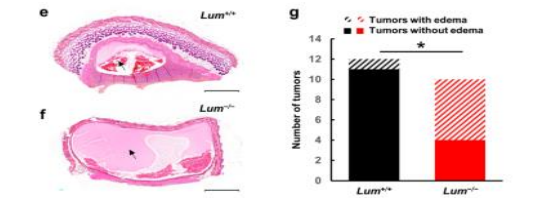
- ✓ Major role of lumican in the **maintenance of the extracellular matrix integrity** in the context of **ovarian cancer**, showing its **inhibitory role** in **primary ovarian tumor allografts growth**.



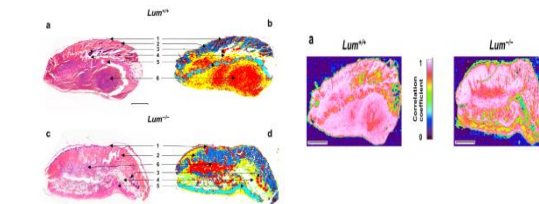
- ✓ Using Multimodal approach, combining **histopathology**, **immunohistochemistry**, and three optical imaging techniques (polarized light (Picrosirius red staining), SHG and FTIR-imaging), the **alteration of collagen organization** could be demonstrated in tumors from lumican-deficient mice.



- ✓ This **collagen disorganization** was **associated with a significant increase in tumor growth** and **edema** formation within the tumors.



- ✓ Non-invasive methods such as **FTIR imaging** represent **potential diagnostic techniques** for **detection of ovarian tumors** at **early stages**.



- > These **techniques** are **promising in evaluating ECM integrity**, leading to a **more appropriate treatment to target cancer cells** while **preserving ECM structure**

ACKNOWLEDGEMENTS

Extracellular Matrix and Cell Dynamic, CNRS/URCA UMR N°7369

Head: Pr. L. Martiny

Team 1

- Extracellular matrix, Cancer and therapeutic targets
- PI: Pr. S. Dedieu and Dr. S. Brézillon



Proteoglycan group:

S. Brézillon, L. Ramont, R. Rivet, P. Nizet,
C. Colin-Pierre (FEBS ALC Selected Talk 11)
C. Sellier, I. Proult, L. Huber

Team 2

- Matrix aging and vascular remodeling

Team 3

- Modeling and multiscale imaging

Vibrational Spectroscopy

G.D. Sockalingum

Imaging Platform (PICT)

C. Terryn

V. Untereiner



[Assessment of Ovarian Tumor Growth in Wild-Type and Lumican-Deficient Mice: Insights Using Infrared Spectral Imaging, Histopathology, and Immunohistochemistry](#). Nizet P *et al.*, *Cancers*. 2021;13(23):5950.