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

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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.**

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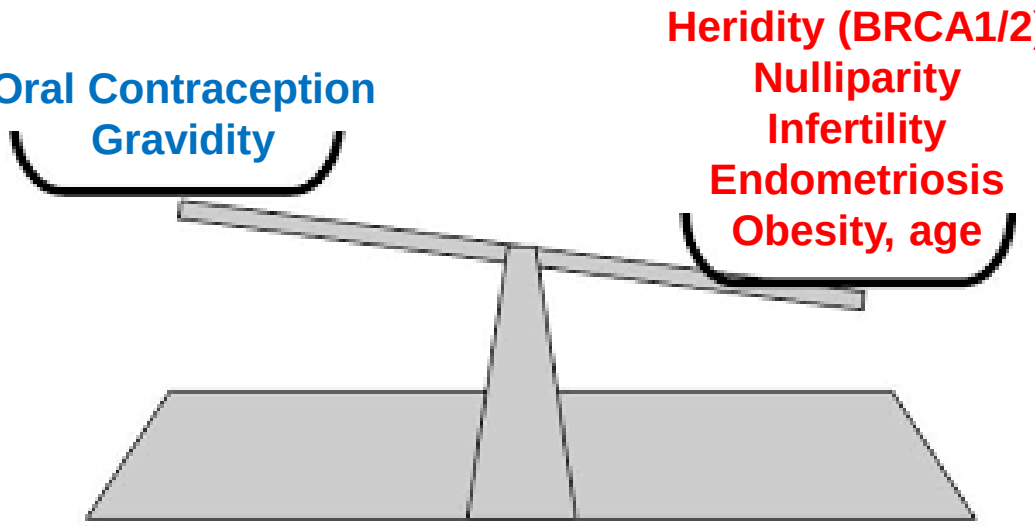
Ovarian Cancer (OVC)

- **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.



Protection factors

Risk factors

Lheureux et al. 2019

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.

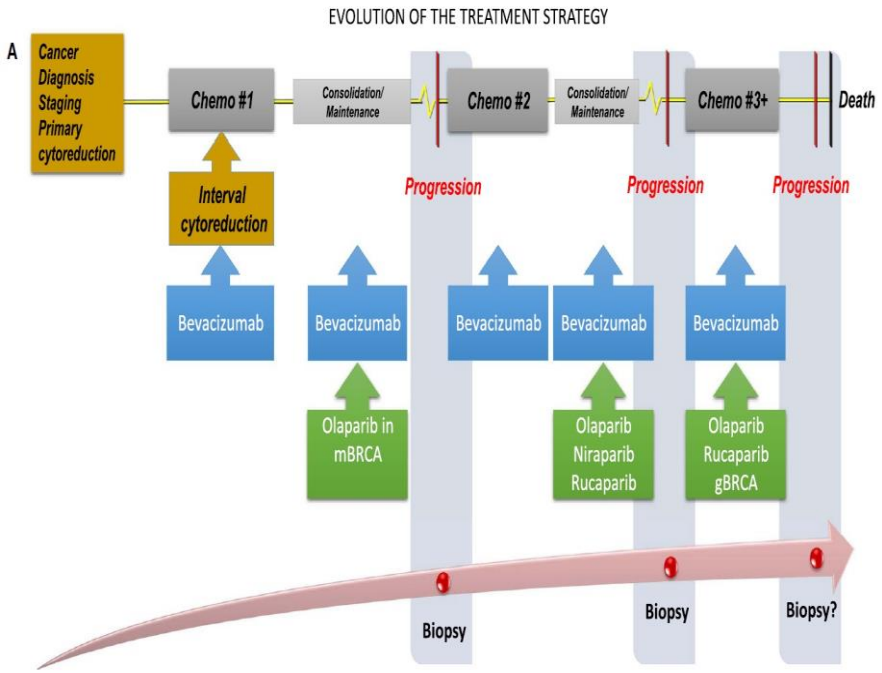
  - Clinical imaging
  - Dosage of CA-125 or HE4

- **Treatment**

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

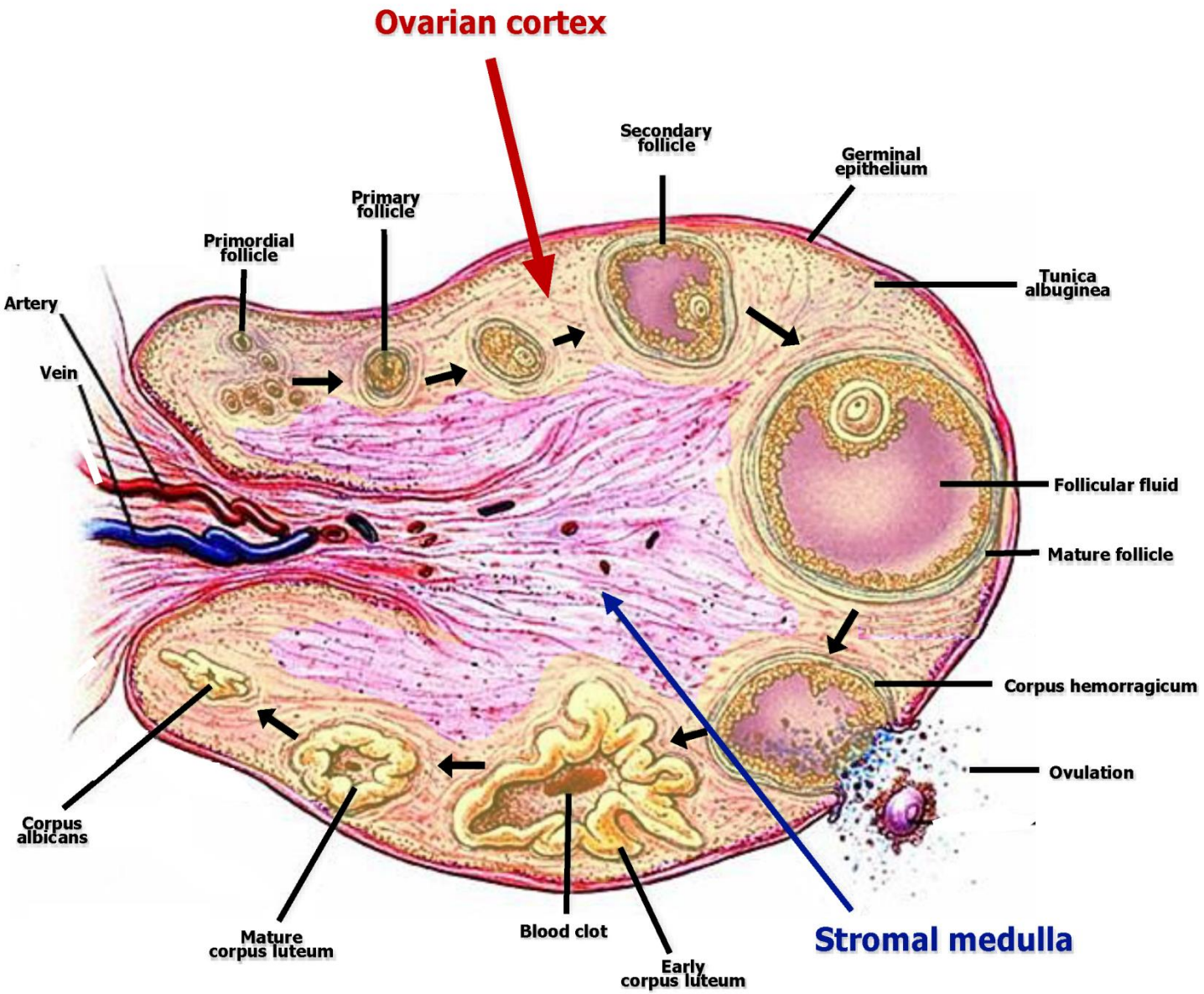
- **But still low efficiency due to**

  - Strong heterogeneity of the tumors
  - High relapse rate



Lheureux *et al.* 2019

Ovary structure



Silvestris et al, Int. J. Mol. Sci. **2020**, *21*(9), 3245;

Lumican

✓ **small leucine-rich proteoglycan (SLRP)**

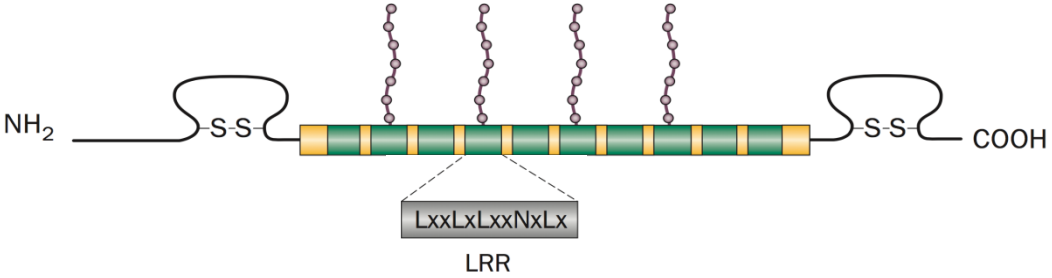
✓ **abundant within tumor reactive stroma**

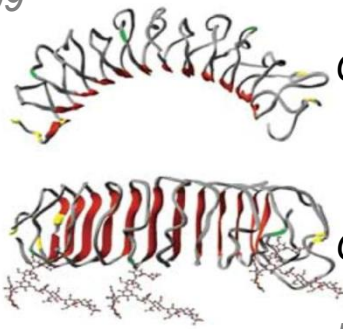
In melanoma:

- ↘ 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 at 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.

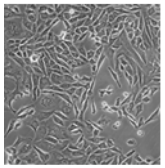
Addressed Questions:

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

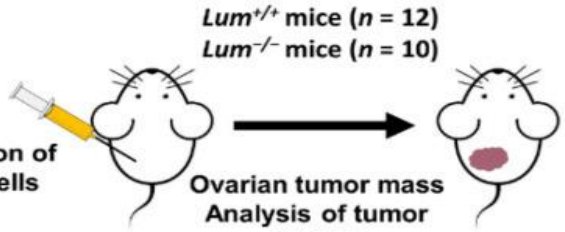
- ***In vivo* ovarian cancer allograft model**
- **Histological analyses**
- **IHC analyses of isolated tumors (Lumican, αv Integrin subunits, Cyclin D1, CD31)**

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 +++



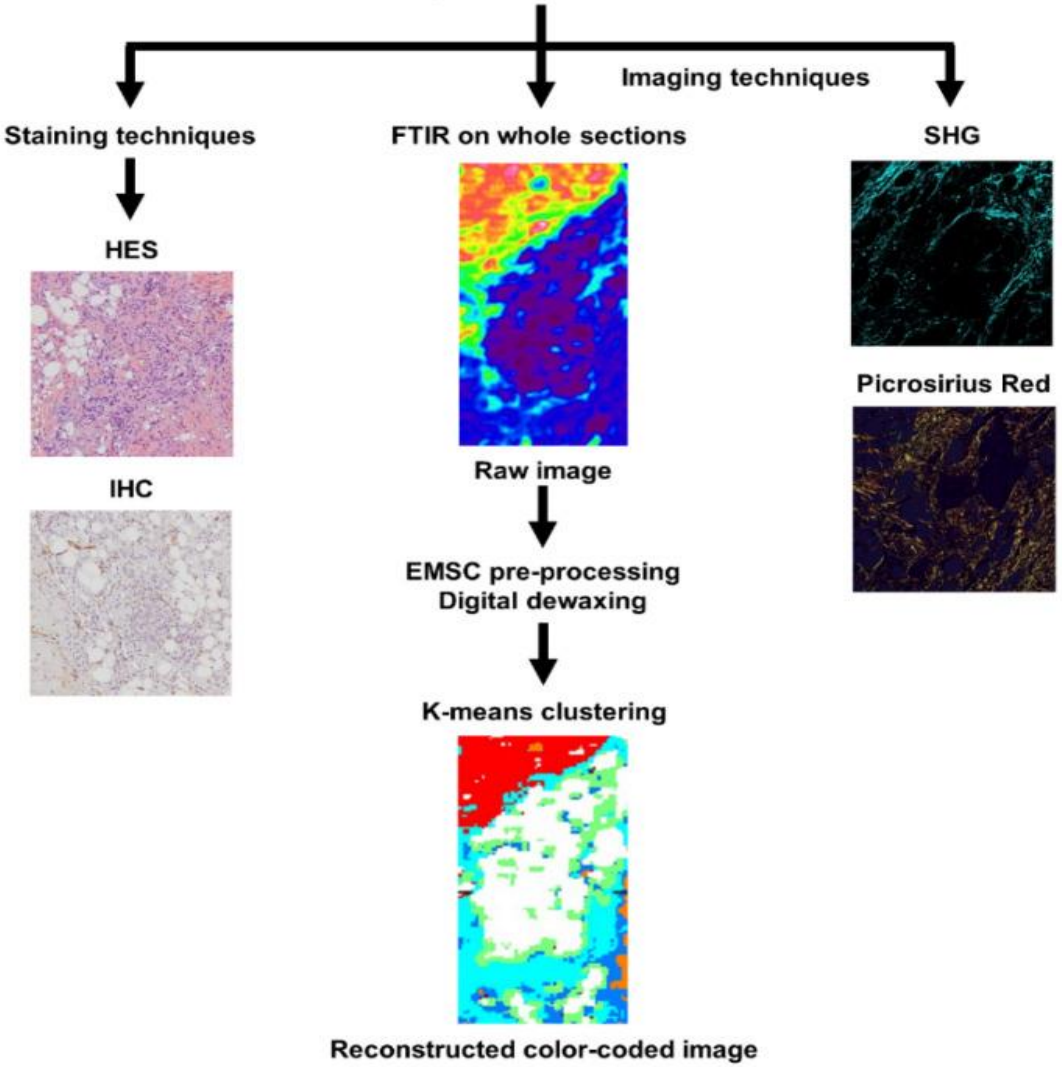


Figure 1: **Workflow** showing the histology, the immunohistochemistry of formalin-fixed paraffin-embedded ID8 ovarian tumor sections, SHG imaging, Picrosirius red staining (polarized light), and analysis of FTIR images using common K-means clustering

Results

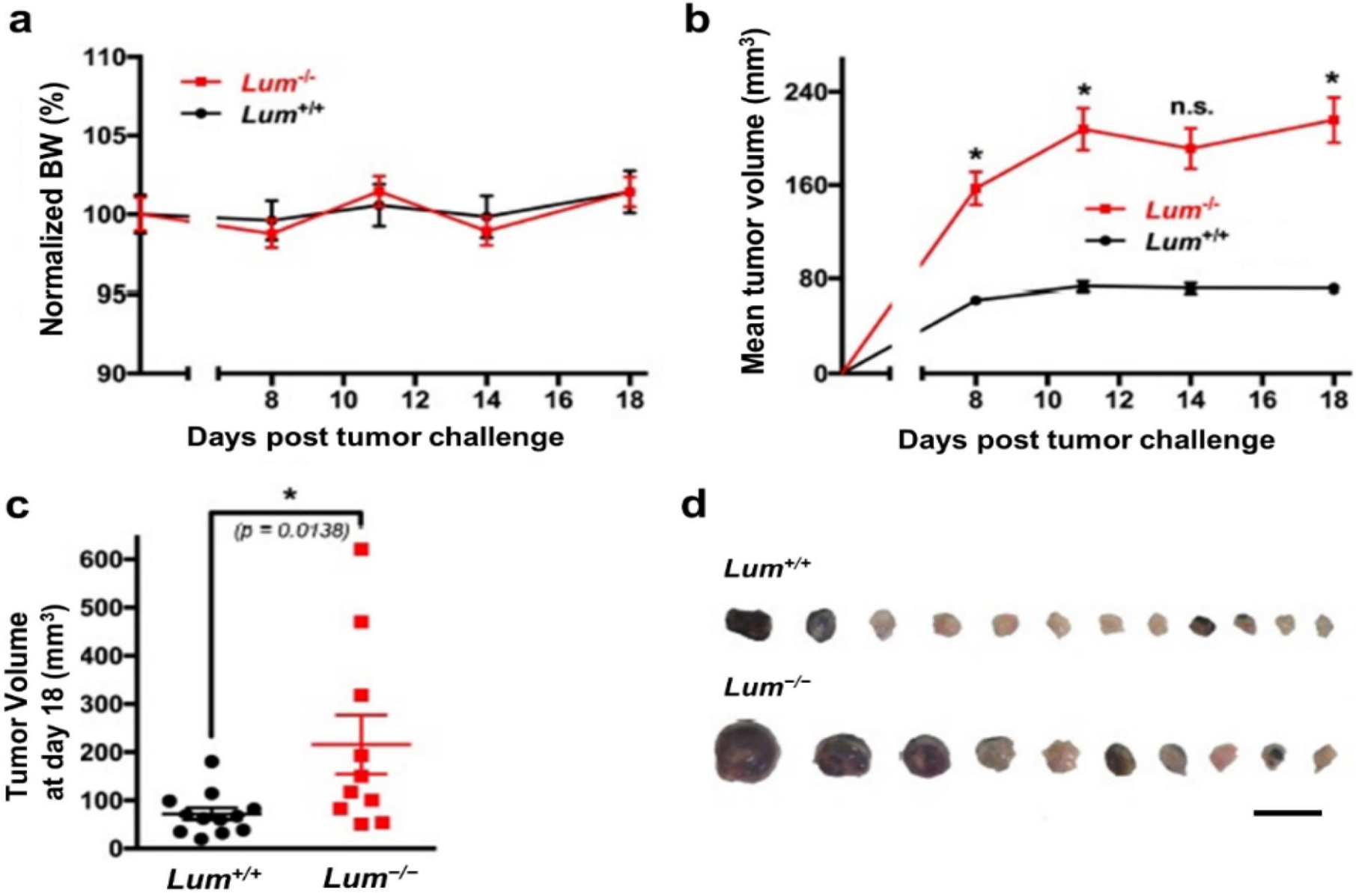


Figure 2: **Evaluation of endogenous lumican impact on tumor growth in an ovarian allograft model.**
(**a**–**d**) ID8 ovarian tumor cells (2.5 × 10^5) were s.c. inoculated in wild-type (*Lum*$^{+/+}$) or lumican-deficient (*Lum*$^{-/-}$) syngeneic C57BL/6J mice.

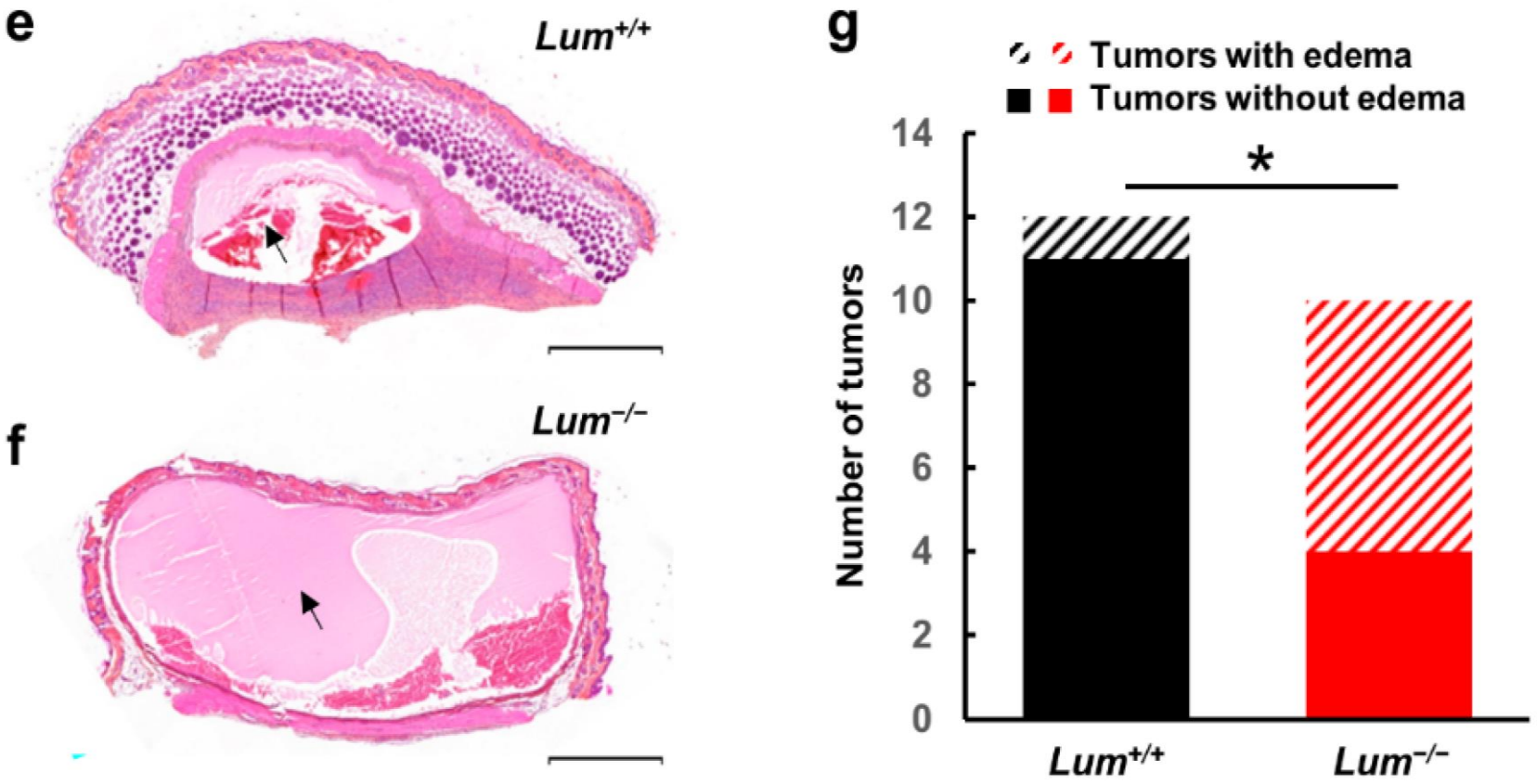


Figure 2 (continued):
Representative images of edemas observed in HES staining of *Lum*$^{+/+}$ (**e**) and *Lum*$^{-/-}$ (**f**) tumor sections are shown (scale bar, 500 µm);

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

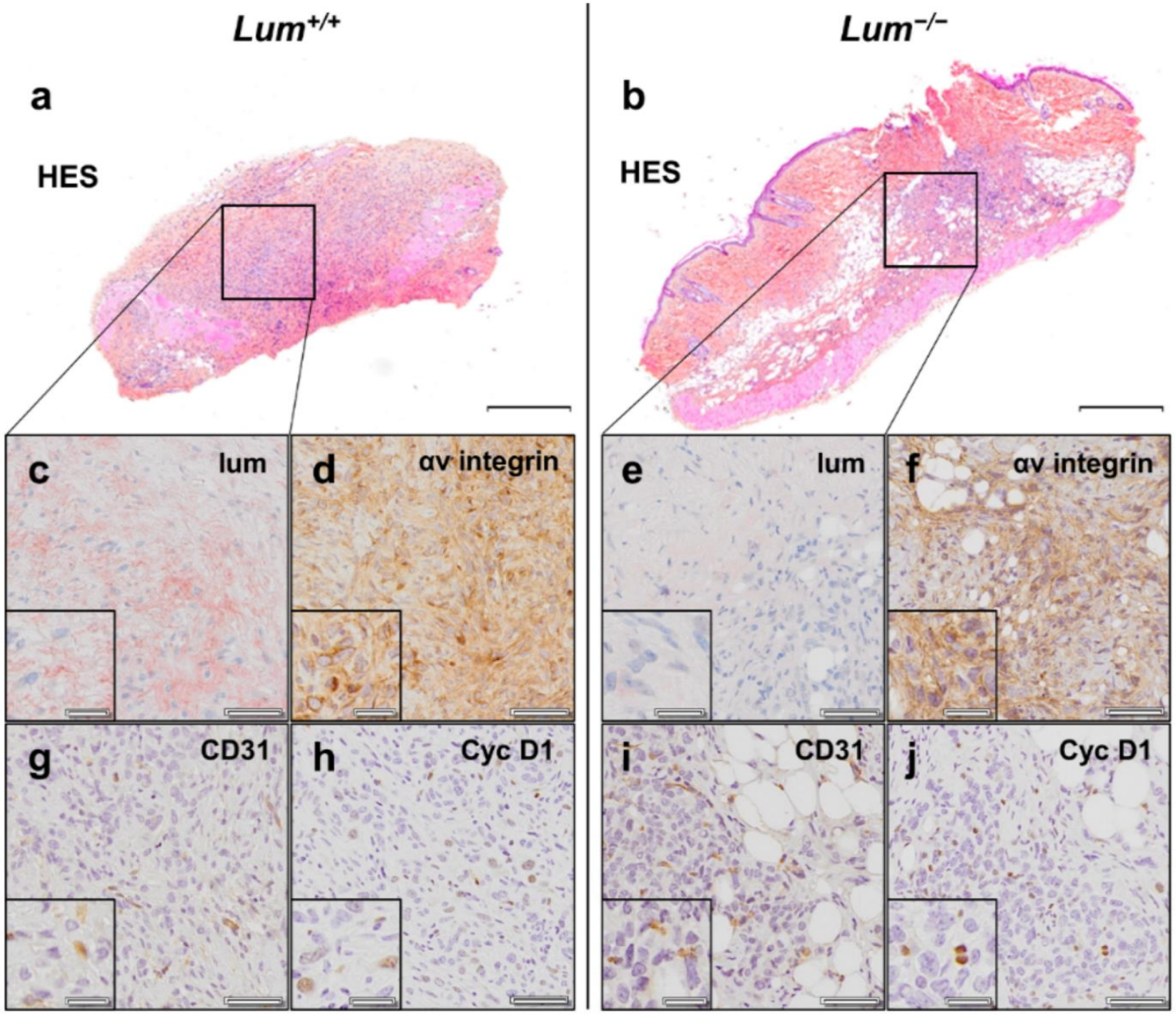


Figure 3: **Histological and immunohistochemical analysis of ovarian tumor sections.**

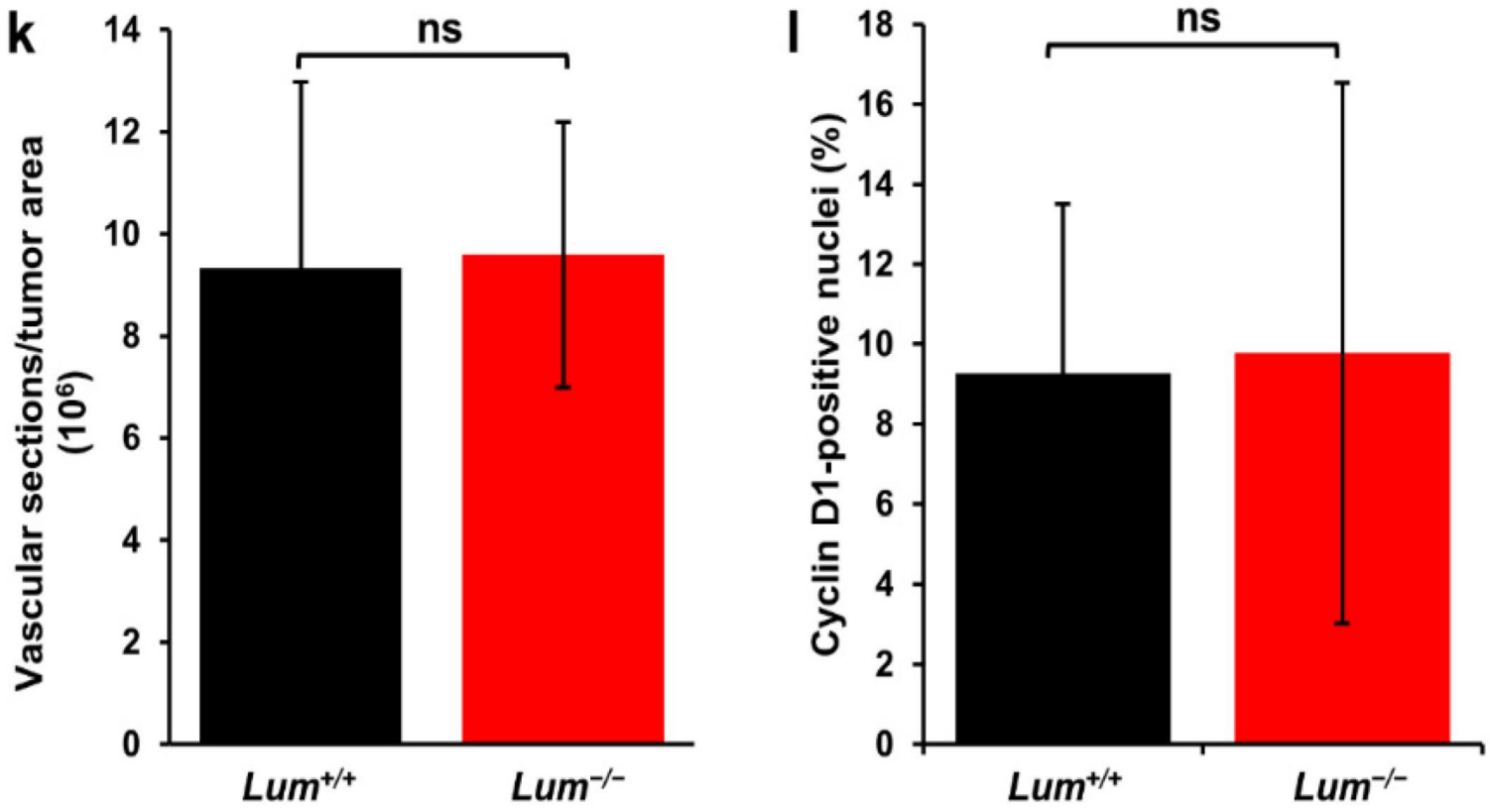


Figure 3 (continued):

(**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).

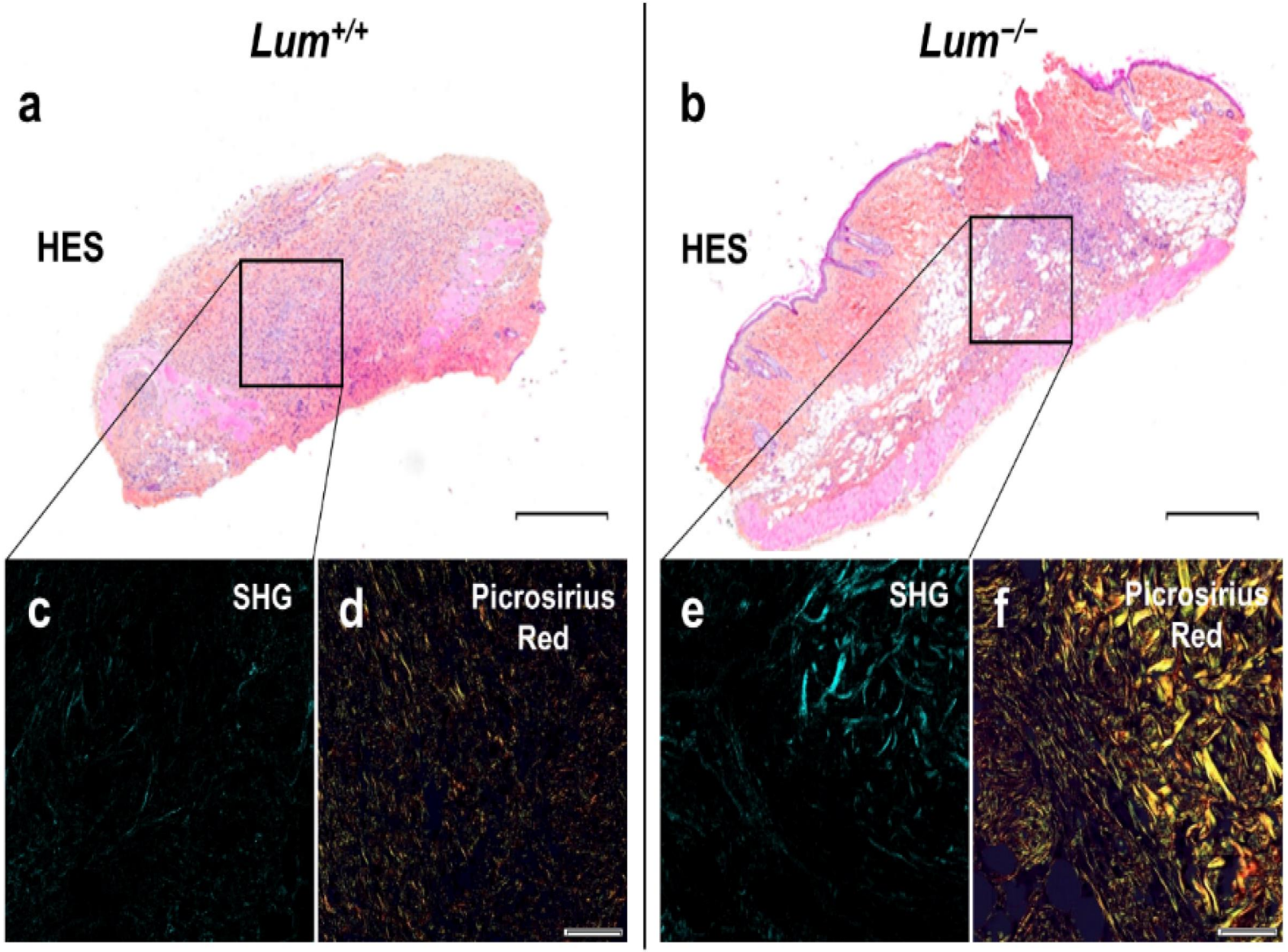


Figure 4: **Analysis of collagen organization in ovarian tumor sections of wild-type and lumican-deficient mice.**
Birefringence of collagen fibers allows distinction between type I (red) and type III (green) collagens.

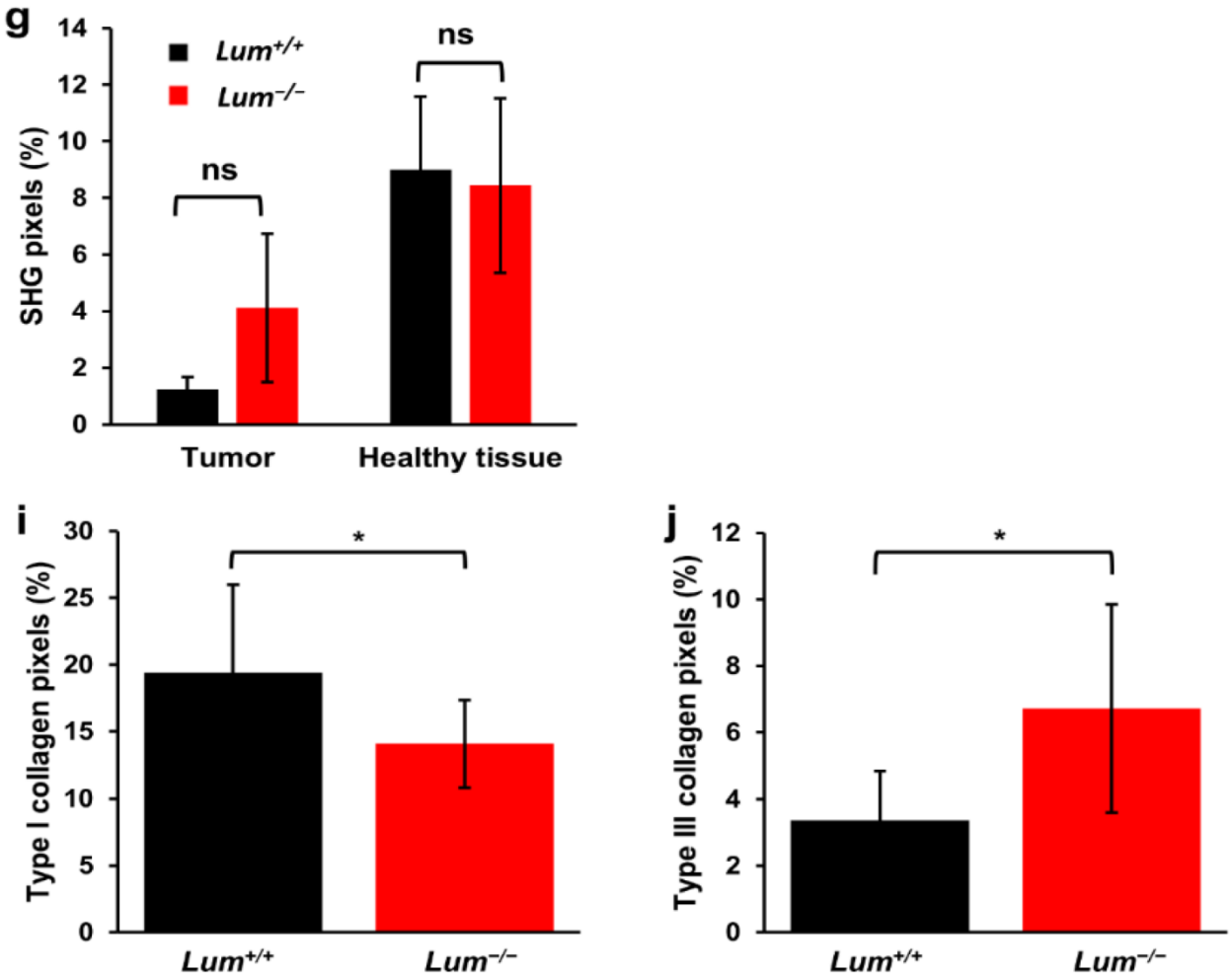


Figure 4 (continued) :

(**g**) Analysis of **collagen fibers intensity by SHG** in tumors and healthy tissues present in each section (mean ± 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 ± SD, * $p < 0.05$).

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

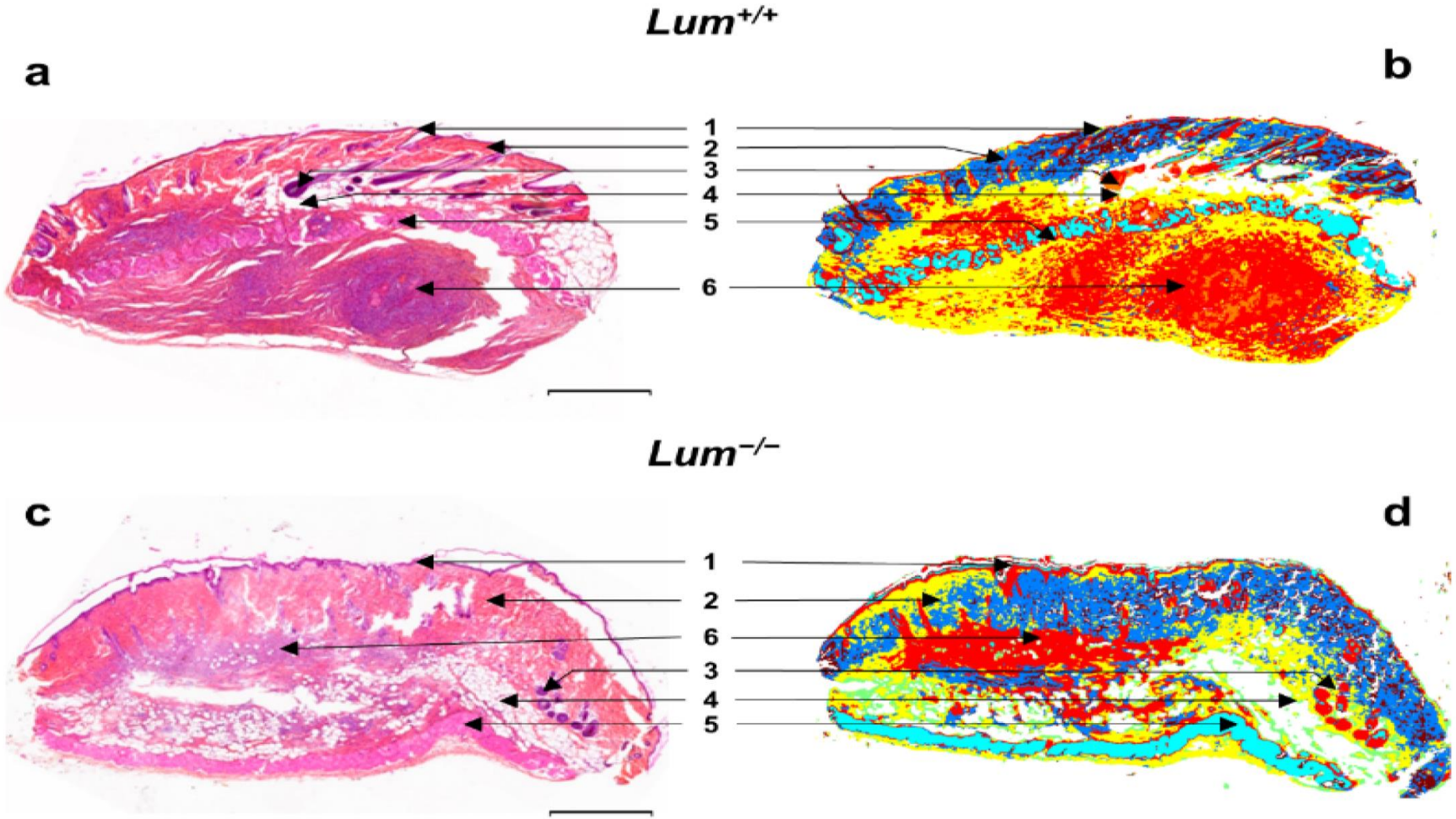


Figure 5: **K-means clustering of FTIR spectral images of ovarian tumor sections in wild-type and lumican-deficient mice.**

(**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**

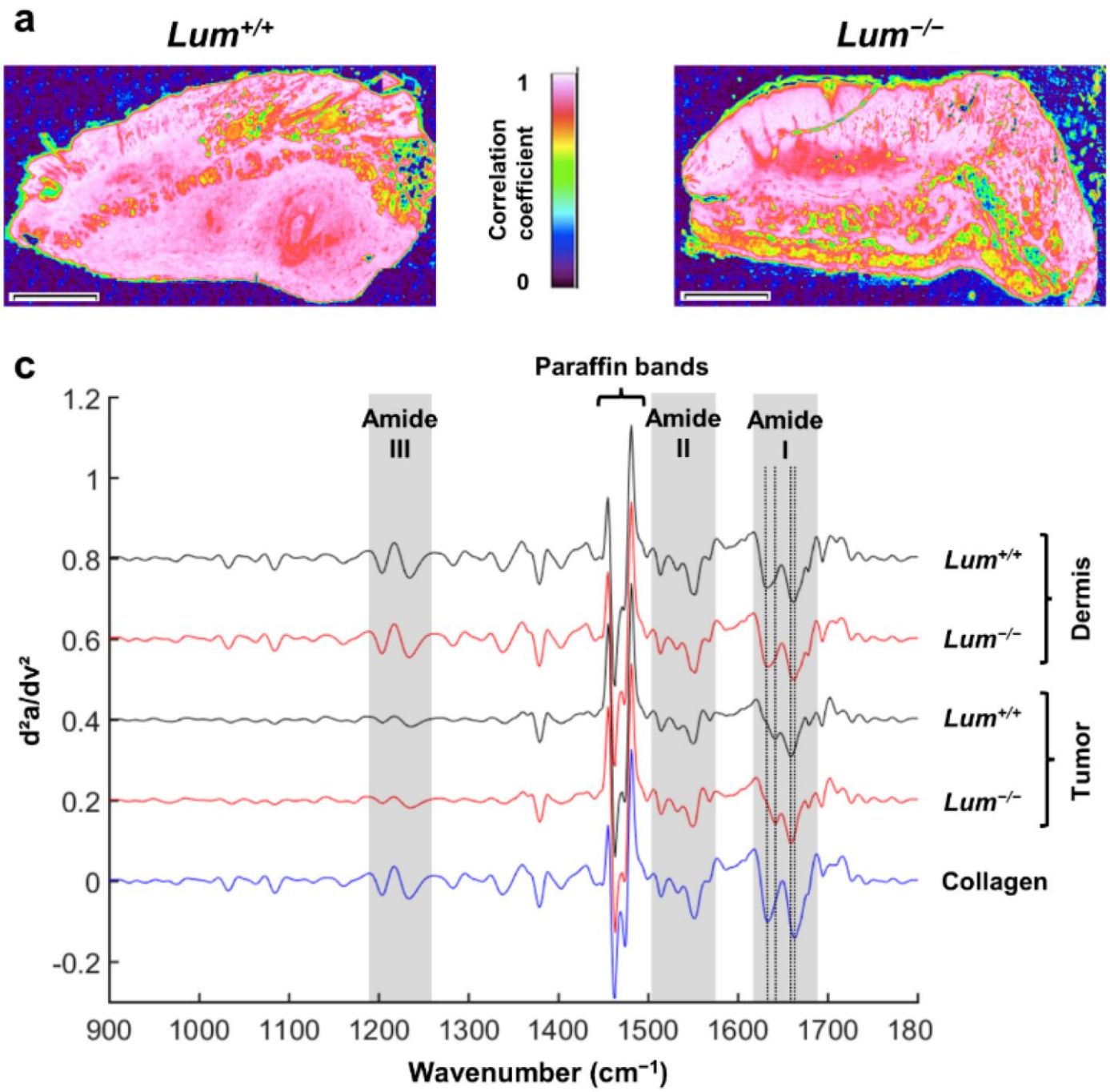


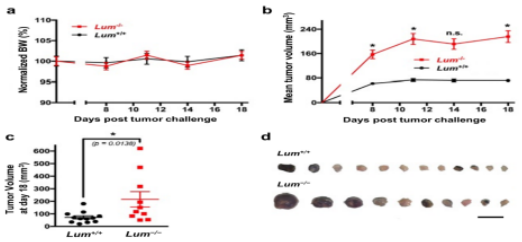
Figure 6: Correlation maps using type I collagen reference spectrum.

(**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).

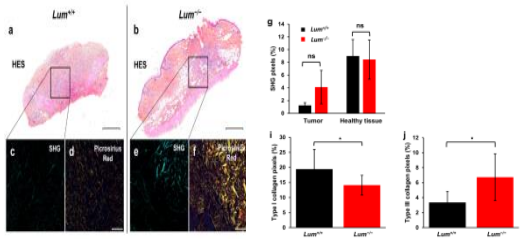
(**c**) Comparison between type I collagen second derivative spectrum (blue line) with second derivative spectra taken randomly from the dermis and tumors of *Lum*$^{+/+}$ (black lines) and *Lum*$^{-/-}$ (red lines) mice skin tissues.

Key findings

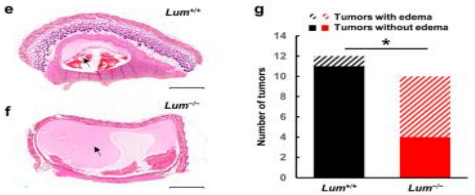
- ✓ Major role of lumican in the **maintenance of the extracellular matrix integrity** in the context of **ovarian cancer**, showing its **inhibitory role in primary ovarian tumor allografts growth**.



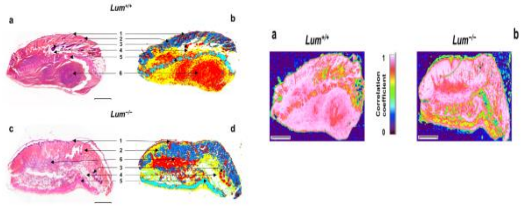
- ✓ Using Multimodal approach, combining **histopathology, immunohistochemistry, and three optical imaging techniques (polarized light (Picrosirius red staining), SHG and FTIR-imaging),** the **alteration of collagen organization** could be demonstrated **in tumors from lumican-deficient mice**.



- ✓ 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**.



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

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C. Terryn
V. Untereiner

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

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.