

# Chemical Landscape of Adipocytes Derived from 3T3-L1 Cells Investigated by Fourier Transform Infrared and Raman Spectroscopies

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**Table S1.** RS band positions and their assignments to vibrational modes of biomolecules [1–8].

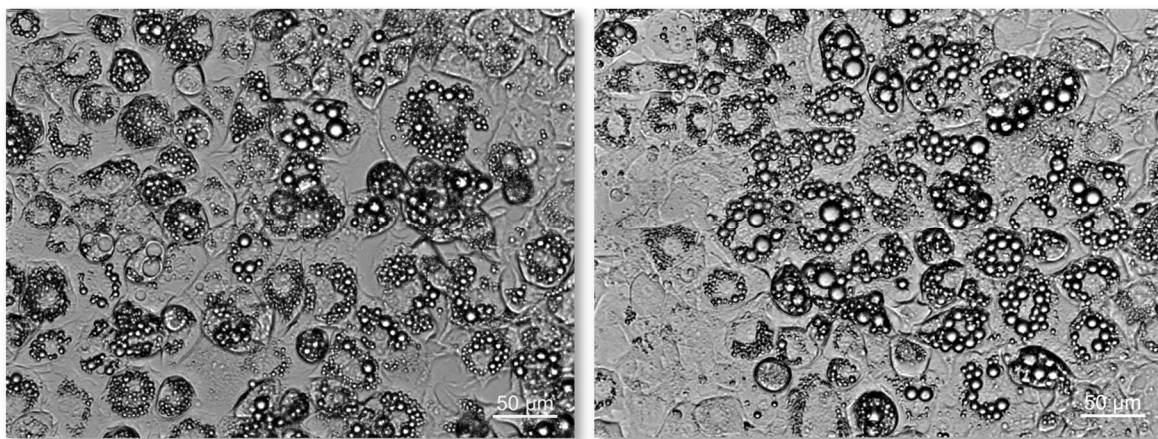
<i>Band position [cm<sup>-1</sup>]</i>	<i>Assignment to biomolecules and vibrational modes</i>
<b>605</b>	Cholesterol, cholesterol esters; $\nu$ (steroid ring)
<b>643</b>	Tyr (proteins); $\delta$ (C-C)
<b>750</b>	Cyt. c and c <sub>1</sub> ; $\nu$ (porphyrin ring)
<b>790</b>	Nucleic acids; $\nu_s$ (PO <sub>2</sub> )
<b>896</b>	Proteins; $\delta$ (CH <sub>2</sub> )
<b>1007</b>	Phe (proteins); ring breathing
<b>1040</b>	Cross-linked Phe (proteins)
<b>1070</b>	Fatty acids; $\nu$ (C-C)
<b>1099</b>	Phosphate-containing molecules; $\nu_s$ (PO <sub>2</sub> )
<b>1129</b>	Phospholipids; $\nu$ (C-C) *Cyt. c and c <sub>1</sub> ; $\nu$ (porphyrin ring)
<b>1240</b>	Phosphate-containing molecules; $\nu_{as}$ (PO <sub>2</sub> )
<b>1257</b>	Proteins (amide III); $\nu$ (C-N), $\delta$ (N-H), $\nu$ (CH <sub>3</sub> -C)
<b>1270</b>	Unsaturated lipids; $\delta$ (=CH)
<b>1306</b>	Unsaturated fatty acids; $\tau$ (CH <sub>2</sub> /CH <sub>3</sub> )
<b>1316</b>	G (nucleic acids); ring breathing, $\delta$ (C-H) *Cyt. c and c <sub>1</sub> ; $\delta$ (C-H)
<b>1340</b>	Nucleic acids, proteins; ring breathing; $\delta$ (C-H) Lipids; $\tau/\delta$ (CH <sub>2</sub> /CH <sub>3</sub> )
<b>1375</b>	A (nucleic acids); $\delta$ (CH <sub>3</sub> )
<b>1451</b>	Proteins, lipids; $\delta$ (CH <sub>2</sub> , CH <sub>3</sub> )
<b>1585</b>	A, G (nucleic acids); ring breathing *Reduced cyt. c, c <sub>1</sub> and b; $\nu$ (methine bridges – C <sub>a</sub> C <sub>m</sub> , C <sub>a</sub> C <sub>m</sub> H bonds)
<b>1660</b>	Unsaturated fatty acids; $\nu$ (C=C)
<b>1743</b>	Triacylglycerols; $\nu_{ester}$ (C=O)
<b>2855</b>	Long chain fatty acids; $\nu_s$ (CH <sub>2</sub> )
<b>3010</b>	Unsaturated fatty acids; $\nu$ (=C-H)

$\nu$  – stretching mode, as – asymmetric, s – symmetric;  $\delta$  – in-plane deformations; ;  $\tau$  – twisting; cyt – cytochromes; A – adenine; G – guanine; Tyr – tyrosine; Phe – phenylalanine. \*Bands at 1130, 1311, and 1587 cm<sup>-1</sup> are assigned to cytochromes only if the ~750 cm<sup>-1</sup> band is present.

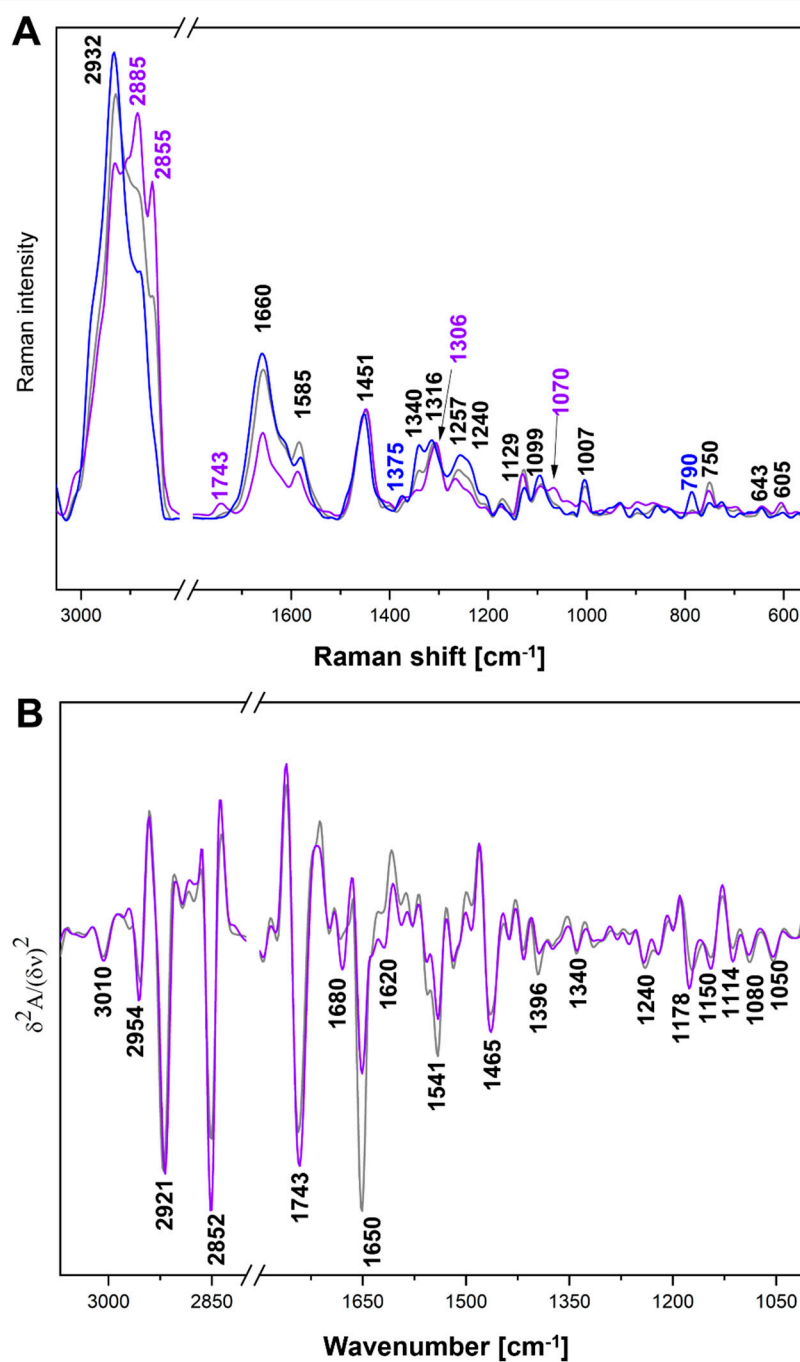
**Table S2.** FTIR band positions and their assignments to vibrational modes of biomolecules [9–14].

<i>Band position [cm<sup>-1</sup>]</i>	<i>Assignment to biomolecules and vibrational modes</i>
1050	Poly/sugars; $\nu(\text{CC-OC})$
1080	Nucleic acids; $\nu_s(\text{PO}_2^-)$ Phospholipids; $\nu_s(\text{PO}_2^-)$
1114	Ribose (RNA); $\nu(\text{C-O})$ Poly/sugars; $\nu(\text{CC-OC})$
1150	Poly/sugars; $\nu(\text{CC-OC})$
1178	Cholesterol esters; $\nu_{\text{as}}(\text{CO-O-C})$
1240	Phospholipids; $\nu_{\text{as}}(\text{PO}_2^-)$
1340	Phospholipids, fatty acids, triacylglycerols; $\nu(\text{CH}_2)$
1396	Free amino acids; $\nu_s(\text{COO}^-)$
1465	Fatty acids; $\delta(\text{CH}_2, \text{CH}_3)$
1620	Intercellular aggregations of cellular proteins
1650	$\alpha$ -helices in proteins (amide I); $\nu(\text{C=O})$ and $\delta(\text{N-H})$
1680	$\beta$ -turns in proteins (amide I); $\nu(\text{C=O})$ and $\delta(\text{N-H})$
1743	Triacylglycerols; $\nu_{\text{ester}}(\text{C=O})$
2852	Long chain FAs; $\nu_s(\text{CH}_2)$
2954	Lipids and proteins; $\nu_{\text{as}}(\text{CH}_3)$
3010	Unsaturated lipids; $\nu_s(\text{=CH})$

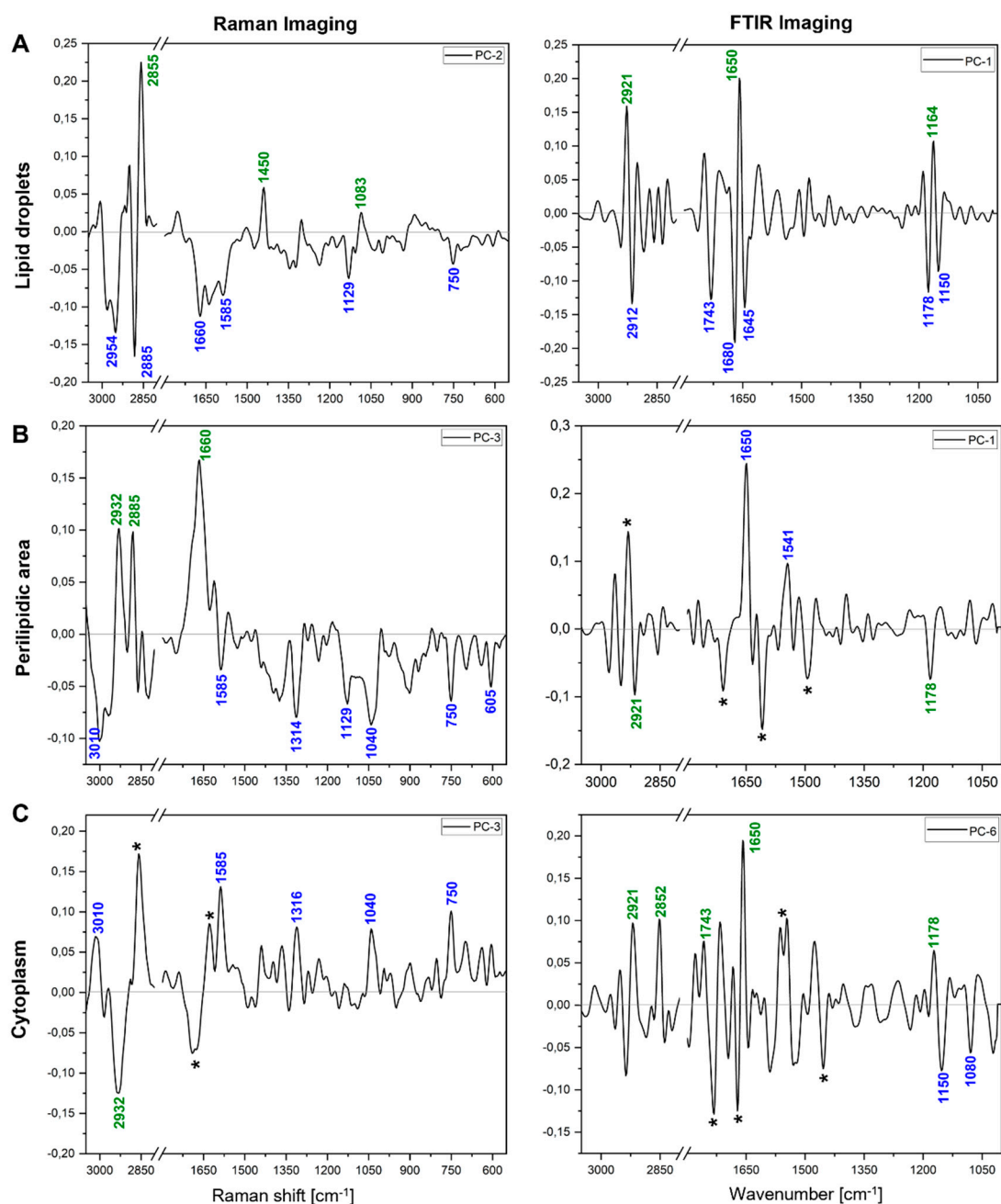
$\nu$  – stretching mode, as – asymmetric, s – symmetric;  $\delta$  – in-plane deformations.



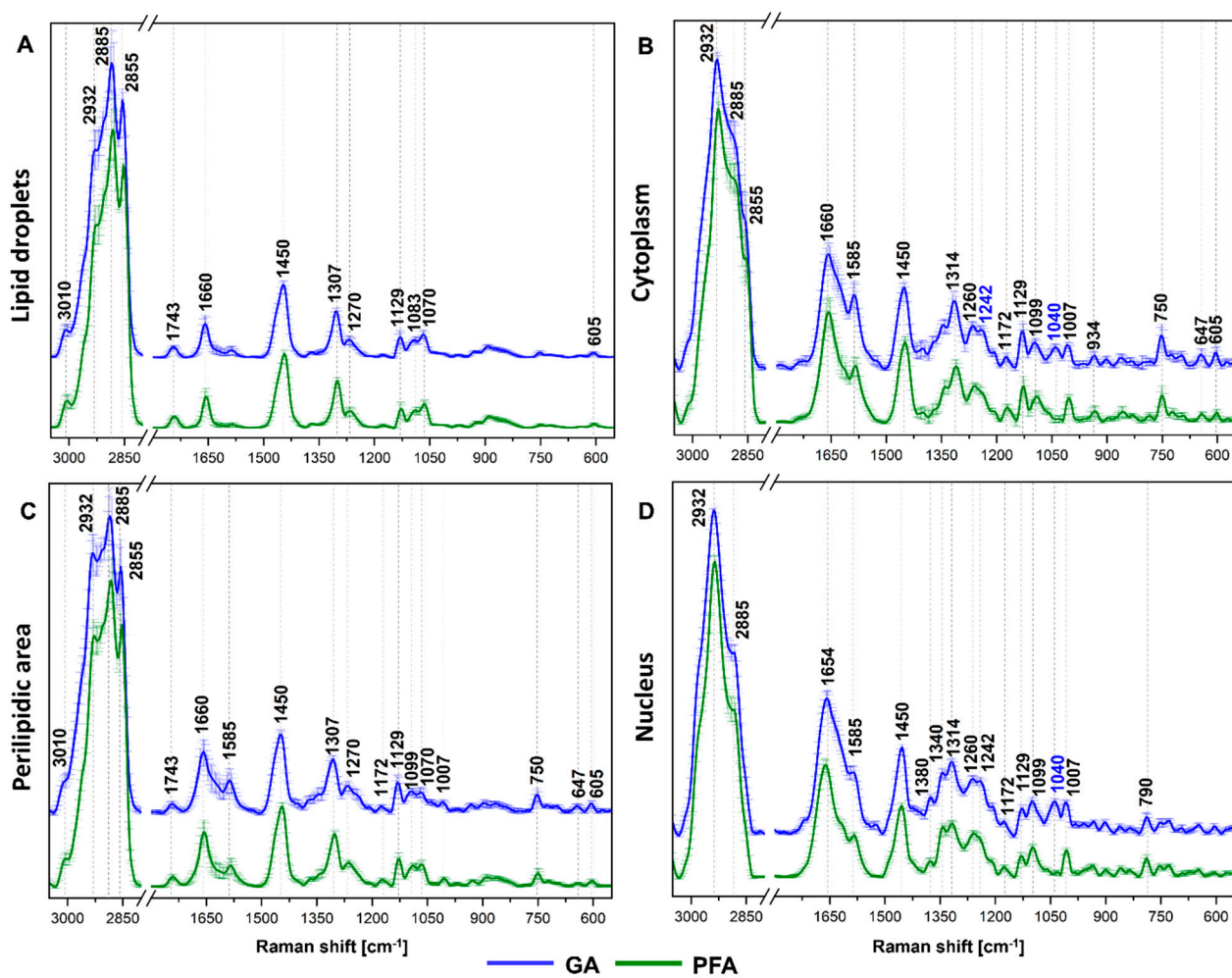
**Figure S1.** Exemplary bright-field optical images of 3T3-L1 derived adipocytes.



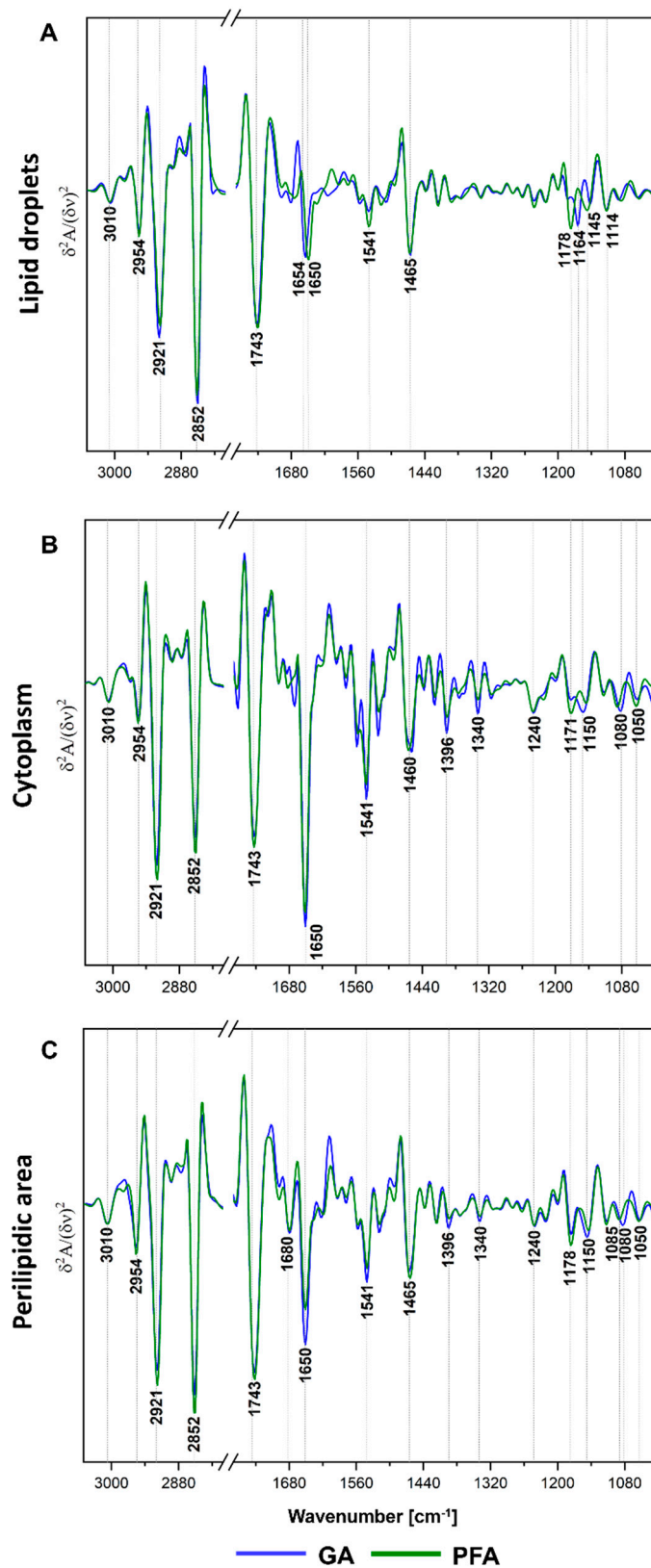
**Figure S2.** (A) The averaged Raman (N = 100) and (B) FTIR (N = 30) spectra of perilipidic area (purple), cytoplasm (gray), and nucleus (blue) from PFA-fixed adipocytes in the spectral regions of 3050-550 cm<sup>-1</sup> and 3070-1005 cm<sup>-1</sup>, respectively.



**Figure S3.** The PCA loading graphs of RS and FTIR datasets in the spectral regions of 3050-550  $\text{cm}^{-1}$  and 3070-1005  $\text{cm}^{-1}$ , respectively. **(A)** lipid droplets, **(B)** perilipidic area, and **(C)** cytoplasm from GA- (in blue) and PFA-fixed (in green) adipocytes. Artifacts marked with asterisks.

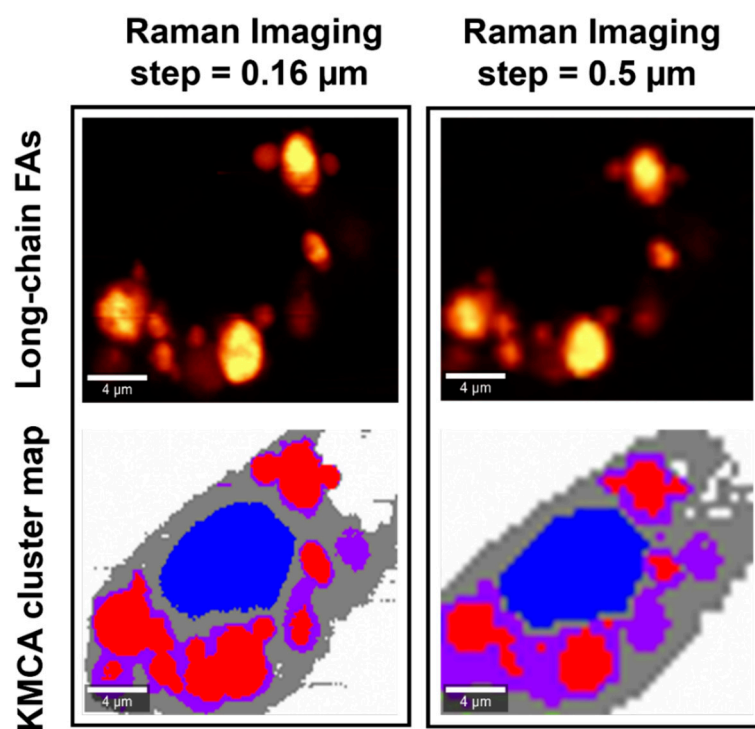


**Figure S4.** Comparison of averaged Raman spectra of (A) lipid droplets, (B) cytoplasm, (C) perilipidic area, and (D) nucleus, obtained from the differentiated 3T3-L1 cells fixed with glutaraldehyde (blue) and paraformaldehyde (green). Shade represents the  $\pm$  standard deviation.



**Figure S5.** Comparison of averaged FTIR spectra of (A) lipid droplets, (B) cytoplasm, and (C) perilipid area, obtained from the differentiated 3T3-L1 cells fixed with glutaraldehyde (blue) and paraformaldehyde (green).





**Figure S6.** Distribution maps of long-chain fatty acids constructed from Raman images acquired with  $0.16\ \mu\text{m}$  and  $0.5\ \mu\text{m}$  step sizes, respectively. KMCA segregated the cell interior into the same compartments with acceptable accuracy.

1. Bik, E.; Dorosz, A.; Mateuszuk, L.; Baranska, M.; Majzner, K. Fixed versus Live Endothelial Cells: The Effect of Glutaraldehyde Fixation Manifested by Characteristic Bands on the Raman Spectra of Cells. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **2020**, *240*, 118460, doi:10.1016/j.saa.2020.118460.
2. Majzner, K.; Chlopicki, S.; Baranska, M. Lipid Droplets Formation in Human Endothelial Cells in Response to Polyunsaturated Fatty Acids and 1-Methyl-Nicotinamide (MNA); Confocal Raman Imaging and Fluorescence Microscopy Studies. *J. Biophotonics* **2016**, *9*, 396–405, doi:10.1002/jbio.201500134.
3. Czamara, K.; Majzner, K.; Pacia, M.Z.; Kochan, K.; Kaczor, A.; Baranska, M. Raman Spectroscopy of Lipids: A Review. *J. Raman Spectrosc.* **2015**, *46*, 4–20, doi:10.1002/jrs.4607.
4. Prescott, B.; Steinmetz, W.; Thomas, G.J. Characterization of DNA Structures by Laser Raman Spectroscopy. *Biopolymers* **1984**, *23*, 235–256, doi:10.1002/bip.360230206.
5. Wu, M.; Pu, K.; Jiang, T.; Zhai, Q.; Ma, Z.; Ma, H.; Xu, F.; Zhang, Z.; Wang, Q. Early Label-Free Analysis of Mitochondrial Redox States by Raman Spectroscopy Predicts Septic Outcomes. *J. Adv. Res.* **2021**, *28*, 209–219, doi:10.1016/j.jare.2020.06.027.
6. Bik, E.; Mielniczek, N.; Jarosz, M.; Denbigh, J.; Budzynska, R.; Baranska, M.; Majzner, K. Tunicamycin Induced Endoplasmic Reticulum Changes in Endothelial Cells Investigated: In Vitro by Confocal Raman Imaging. *Analyst* **2019**, *144*, 6561–6569, doi:10.1039/c9an01456j.
7. Matthäus, C.; Chernenko, T.; Newmark, J.A.; Warner, C.M.; Diem, M. Label-Free Detection of Mitochondrial Distribution in Cells by Nonresonant Raman Microspectroscopy. *Biophys. J.* **2007**, *93*, 668–673, doi:10.1529/biophysj.106.102061.
8. Meade, A.D.; Clarke, C.; Draux, F.; Sockalingum, G.D.; Manfait, M.; Lyng, F.M.; Byrne, H.J. Studies of Chemical Fixation Effects in Human Cell Lines Using Raman Microspectroscopy. *Anal. Bioanal. Chem.* **2010**, *396*, 1781–1791, doi:10.1007/s00216-009-3411-7.
9. Sahu, R.K.; Argov, S.; Salman, A.; Huleihel, M.; Grossman, N.; Hammody, Z.; Kapelushnik, J.; Mordechai, S. Characteristic Absorbance of Nucleic Acids in the Mid-IR Region as Possible Common Biomarkers for Diagnosis of Malignancy. *Technol. Cancer Res. Treat.* **2004**, *3*, 629–638, doi:10.1177/153303460400300613.
10. Szafraniec, E.; Wiercigroch, E.; Czamara, K.; Majzner, K.; Staniszevska-Slezak, E.; Marzec, K.M.; Malek, K.; Kaczor, A.; Baranska, M. Diversity among Endothelial Cell Lines Revealed by Raman and Fourier-Transform Infrared Spectroscopic Imaging. *Analyst* **2018**, *143*, 4323–4334, doi:10.1039/c8an00239h.
11. Perez-Guaita, D.; Kochan, K.; Martin, M.; Andrew, D.W.; Heraud, P.; Richards, J.S.; Wood, B.R. Multimodal Vibrational Imaging of Cells. *Vib. Spectrosc.* **2017**, *91*, 46–58, doi:10.1016/j.vibspec.2016.07.017.
12. Wiercigroch, E.; Staniszevska-Slezak, E.; Szkaradek, K.; Wojcik, T.; Ozaki, Y.; Baranska, M.; Malek, K. FT-IR Spectroscopic Imaging of Endothelial Cells Response to Tumor Necrosis Factor- $\alpha$ : To Follow Markers of Inflammation Using Standard and High-Magnification Resolution. *Anal. Chem.* **2018**, *90*, 3727–3736, doi:10.1021/acs.analchem.7b03089.
13. Banyay, M.; Sarkar, M.; Gräslund, A. A Library of IR Bands of Nucleic Acids in Solution. *Biophys. Chem.* **2003**, *104*, 477–488, doi:10.1016/S0301-4622(03)00035-8.
14. Staniszevska, E.; Malek, K.; Baranska, M. Rapid Approach to Analyze Biochemical Variation in Rat Organs by ATR FTIR Spectroscopy. *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.* **2014**, *118*, 981–986, doi:10.1016/j.saa.2013.09.131.