# Supplementary Materials

# HPV infection significantly accelerates glycogen metabolism in cervical cells with large nuclei: Raman microscopic study with subcellular resolution

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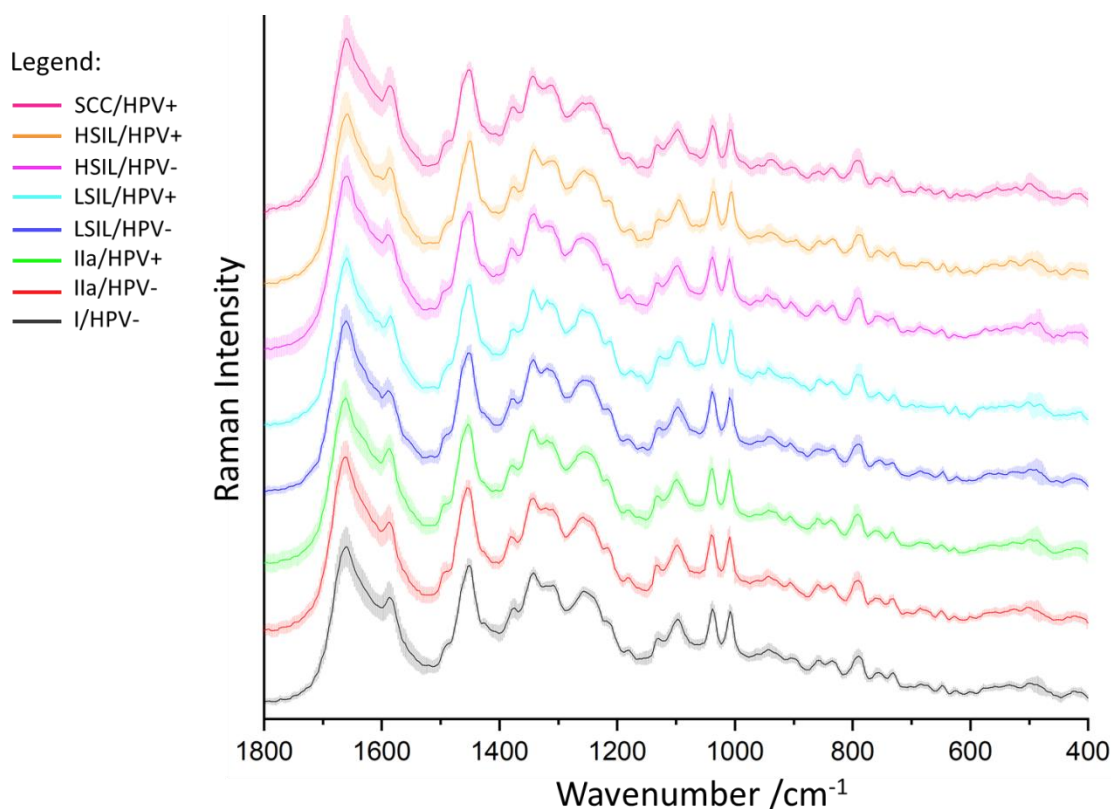
**Figure S1. Raman average spectra of nuclei in cervical cells in studied groups of females.** Presented Raman spectra were averaged over all nuclei in the studied group and the nuclei were separated based on Cluster Analysis (K-means, Manhattan distance).
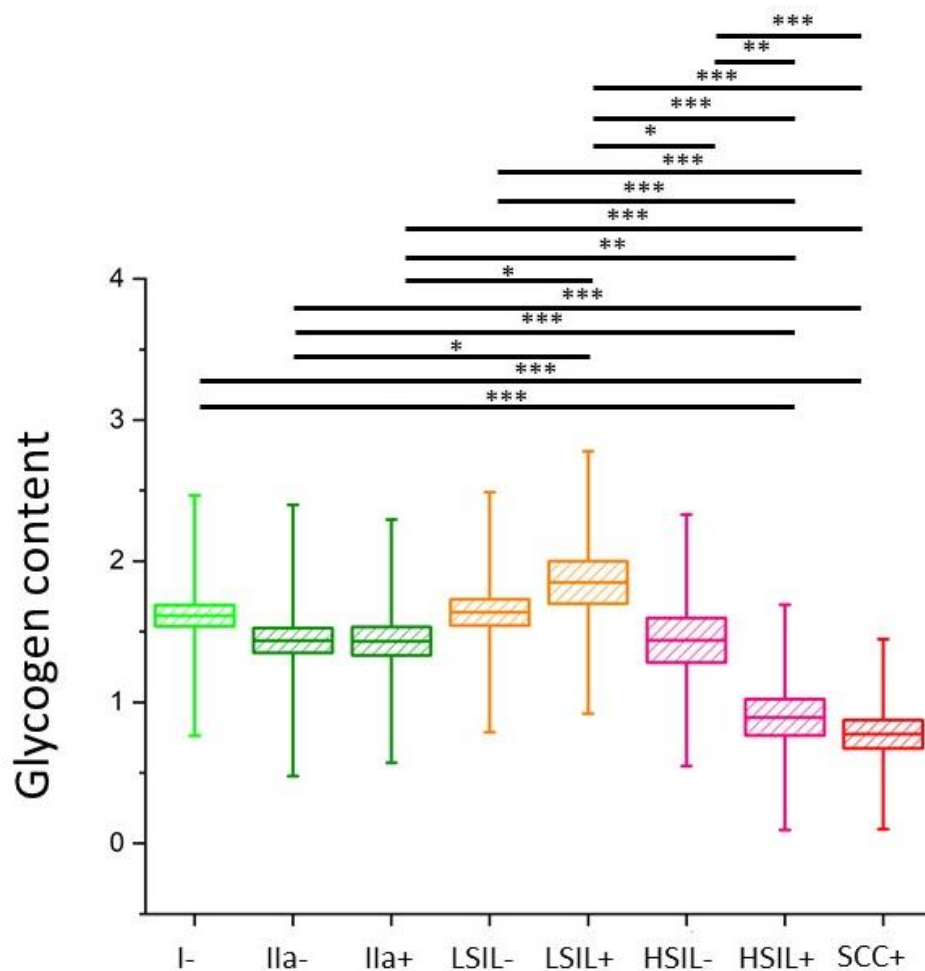
**Figure S2. Glycogen content in the cytoplasm of cervical epithelial cells.** The comparison of the glycogen content in the cytoplasm of cervical epithelial cells in studied groups: I/HPV- (bright green), IIa/HPV- (dark green), IIa/HPV + (dark green), LSIL/HPV- (orange), LSIL/HPV+ (orange), HSIL/HPV- (pink), HSIL/HPV+ (pink), SCC/HPV+ (red) obtained by calculations of the integral intensity of the glycogen marker band at 486 cm-1 in studied cells (in total 560). Mean values ± SEM are given as box plots: mean (horizontal line), SEM (box), minimal and maximal values (whiskers). * p < 0.05 ** p < 0.01 *** p < 0.001.
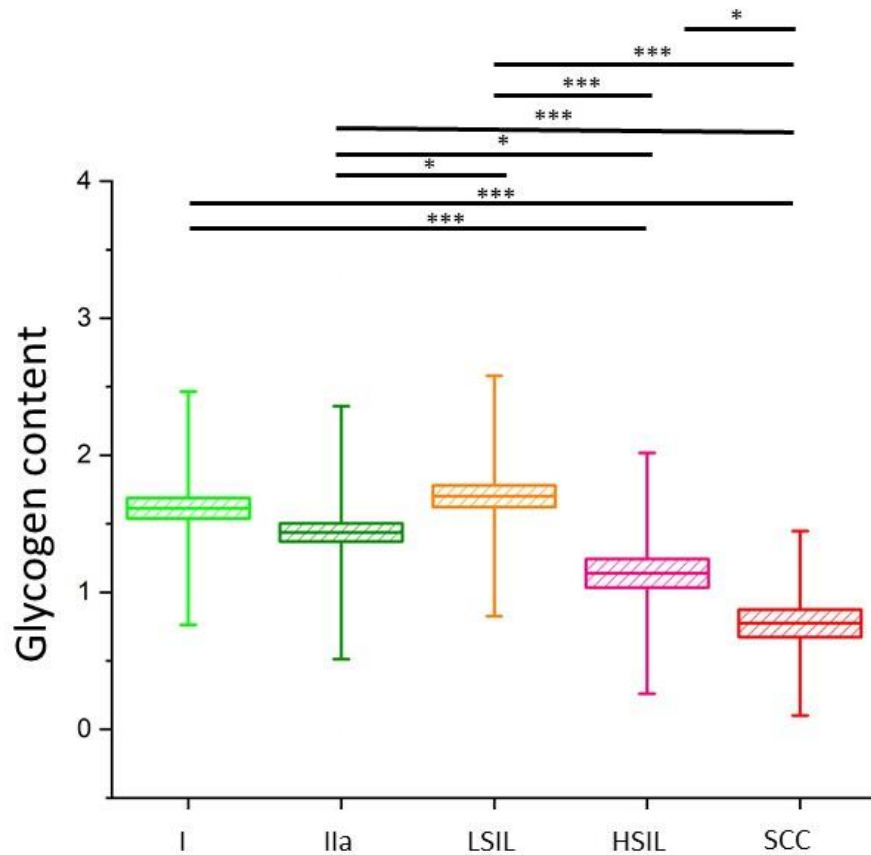
**Figure S3. Glycogen content in the cytoplasm of cervical epithelial cells without HPV-infection based differentiation.** The comparison of the glycogen content in the cytoplasm of cervical epithelial cells in studied groups: I (bright green), IIa (dark green), LSIL (orange), HSIL (pink), SCC (red) obtained by calculations of the integral intensity of the glycogen marker band at 486 cm-1 in studied cells (in total 560). Mean values ± SEM are given as box plots: mean (horizontal line), SEM (box), minimal and maximal values (whiskers). * $p < 0.05$ *** $p < 0.001$.
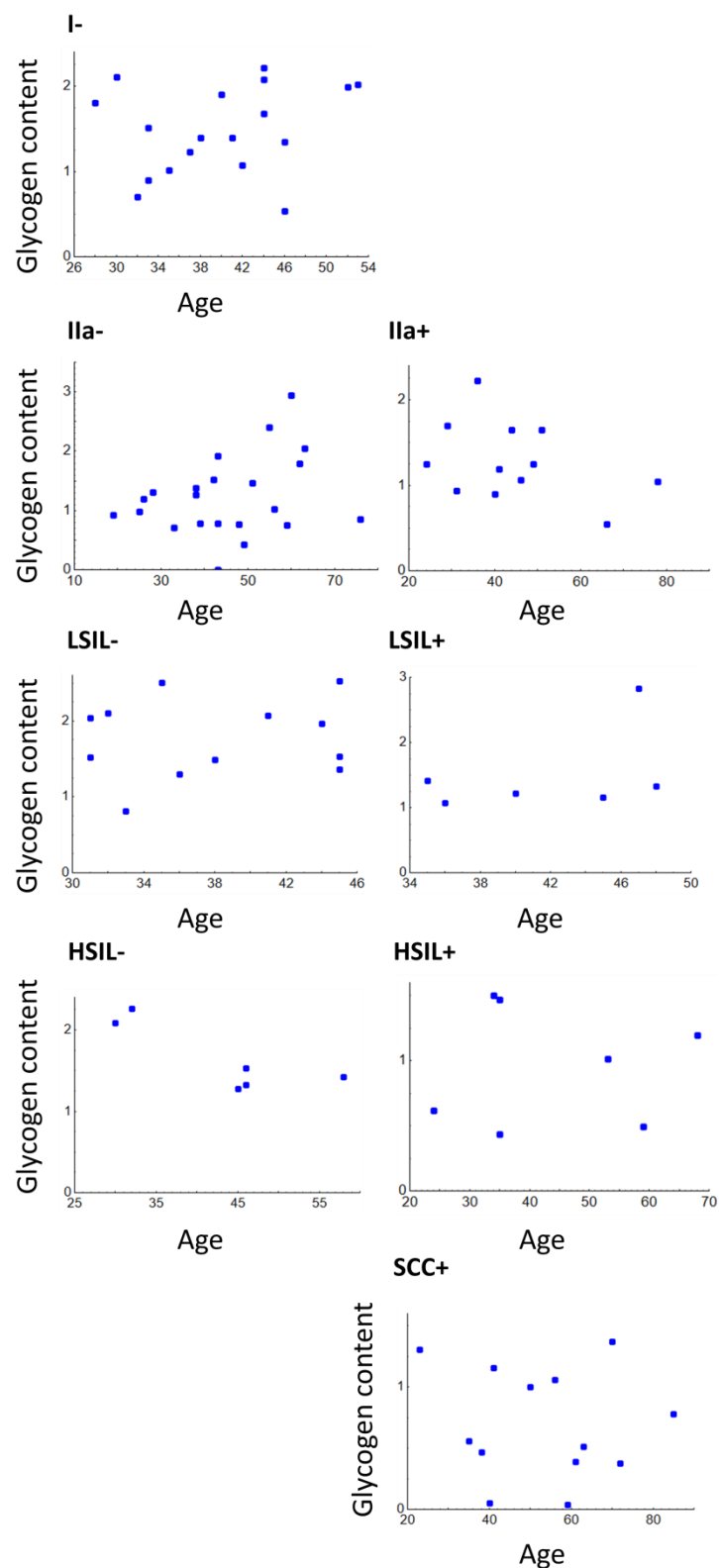
**Figure S4. Glycogen content in the cytoplasm of cervical epithelial cells as a function of age.** Analysis of the correlation between the average amount of glycogen (obtained by calculations of the integral intensity of the glycogen marker band at 941 cm$^{-1}$) in the cytoplasm of cells and the age of females in individual groups. Each blue circle represents average data for one patient. Graphs present individual groups of patients: I/HPV- (A), IIa/HPV- (B), IIa/HPV+ (C), LSIL/HPV- (D), LSIL/HPV+ (E), HSIL/HPV- (F), HSIL/HPV+ (G), SCC/HPV+ (H).
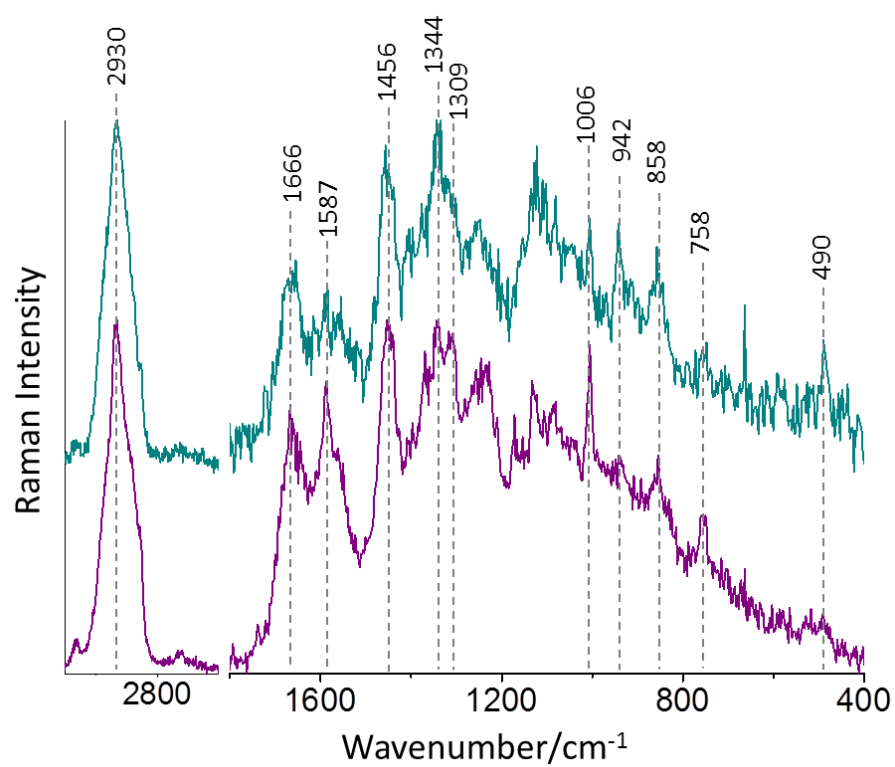
**Figure S5. Single Raman spectra of dry pellets of cervical epithelial cells of two patients differing in the glycogen level obtained using fiber optic Raman spectroscopy.** Spectra were acquired with a novel portable Raman fiber optic probe (WITec, Germany) using the 532 nm laser excitation. Each spectrum was accumulated with the 4 s integration time and 10 accumulations.

**Table S1. The number of patients in studied groups.**

| Analyzed group | HPV status | Number of patients |
|---|---|---|
| I | HPV-<br>HPV+ | 18<br>0 |
| IIa | HPV-<br>HPV+ | 22<br>12 |
| IIID-1=LSIL[a] | HPV-<br>HPV+ | 12<br>6 |
| IIID-2=HSIL[b] | HPV-<br>HPV+ | 6<br>7 |
| SCC[c] | HPV-<br>HPV+ | 0<br>13 |

[a] low-grade squamous intraepithelial lesion

[b] high-grade squamous intraepithelial lesion

[c] histopathologically confirmed squamous cell carcinoma

**Table S2. Glycogen content in studied epithelial cervical cells.** Glycogen content calculated based on the relative intensity of the Raman band at 486 $cm^{-1}$.

| Classification | Number of cells | Relative glycogen content | Statistical significance |
|---|---|---|---|
| **Cells with small-diameter nuclei** | | | |
| **I/HPV-** | 83 | Mean: 1.75<br>SD: 0.84<br>SE: 0.09 | LSIL/HPV+***<br>HSIL/HPV+*<br>SCC/HPV+*** |
| **IIa/HPV-** | 65 | Mean: 1.61<br>SD: 1.00<br>SE: 0.12 | LSIL/HPV+***<br>SCC/HPV+*** |
| **IIa/HPV+** | 55 | Mean: 1.69<br>SD: 0.81<br>SE: 0.11 | LSIL/HPV+***<br>HSIL/HPV+*<br>SCC/HPV+*** |
| **LSIL/HPV-** | 68 | Mean: 1.65<br>SD: 0.85<br>SE: 0.10 | LSIL/HPV+***<br>HSIL/HPV+*<br>SCC/HPV+*** |
| **LSIL/HPV+** | 24 | Mean: 2.38<br>SD: 0.69<br>SE: 0.14 | I/HPV-***<br>IIa/HPV-***<br>IIa/HPV+***<br>LSIL/HPV-***<br>HSIL/HPV-**<br>HSIL/HPV+***<br>SCC/HPV+*** |

| | | | |
|---|---|---|---|
| **HSIL/HPV-** | 18 | Mean: 1.70<br>SD: 0.73<br>SE: 0.17 | LSIL/HPV+**<br>HSIL/HPV+*<br>SCC/HPV+*** |
| **HSIL/HPV+** | 17 | Mean: 1.16<br>SD: 0.92<br>SE: 0.22 | I/HPV-*<br>IIa/HPV+*<br>LSIL/HPV-*<br>LSIL/HPV+***<br>HSIL/HPV-*<br>SCC/HPV+* |
| **SCC/HPV+** | 21 | Mean: 0.56<br>SD: 0.55<br>SE: 0.12 | I/HPV-***<br>IIa/HPV-***<br>IIa/HPV+***<br>LSIL/HPV-***<br>LSIL/HPV+***<br>HSIL/HPV-***<br>HSIL/HPV+* |
| <span style="color:red">**Cells with large-diameter nuclei**</span> | | | |
| **I/HPV-** | 43 | Mean: 1.35<br>SD: 0.82<br>SE: 0.12 | IIa/HPV+**<br>HSIL/HPV+***<br>SCC/HPV+ * |
| **IIa/HPV-** | 56 | Mean: 1.23<br>SD: 0.88<br>SE: 0.12 | IIa/HPV+*<br>HSIL/HPV+ ** |
| **IIa/HPV+** | 19 | Mean: 0.68<br>SD: 0.49<br>SE: 0.11 | I/HPV-**<br>IIa/HPV-*<br>LSIL/HPV-** |
| **LSIL/HPV-** | 17 | Mean: 1.57<br>SD: 0.87<br>SE: 0.21 | IIa/HPV+ **<br>LSIL/HPV+*<br>HSIL/HPV+**<br>SCC/HPV+* |
| **LSIL/HPV+** | 14 | Mean: 0.95<br>SD: 0.48<br>SE: 0.13 | LSIL/HPV-*<br>HSIL/HPV+* |
| **HSIL/HPV-** | 14 | Mean: 1.10<br>SD: 0.99<br>SE: 0.26 | - |
| **HSIL/HPV+** | 22 | Mean: 0.69<br>SD: 0.63<br>SE: 0.13 | I/HPV-***<br>IIa/HPV-**<br>LSIL/HPV-**<br>LSIL/HPV+* |
| **SCC/HPV+** | 24 | Mean: 0.97<br>SD: 0.72<br>SE: 0.15 | I/HPV-*<br>LSIL/HPV-* |

*p<0.05**p<0.01***p<0.001

**Table S3. Glycogen content in studied epithelial cervical cells.** Glycogen content calculated based on the percentage of cells stained with the PAS method.

| Classification | Number of samples | Relative glycogen content | Statistical significance |
|---|---|---|---|
| I | 10 | Mean: 70.1<br>SD: 8.7<br>SE: 2.9 | HSIL**<br>SCC* |
| IIa | 13 | Mean: 64.2<br>SD: 8.6<br>SE: 2.4 | HSIL**<br>SCC* |
| LSIL | 13 | Mean: 62.2<br>SD: 15.1<br>SE: 4.2 | HSIL**<br>SCC* |
| HSIL | 5 | Mean: 24.6<br>SD: 22.8<br>SE: 10.2 | I**<br>IIa**<br>LSIL** |
| SCC | 3 | Mean: 38.3<br>SD: 11.7<br>SE: 6.2 | I*<br>IIa*<br>LSIL* |

*$p<0.05$**$p<0.01$