Figure S1. Brief overview of CAIA for nuclear area in a specimen stained with Feulgen (case #41). (A) Automatically detected area by CAIA and (B) manually selected nuclei for data acquisition. Red line surrounding tumor parenchyma in (A) is the annotated area. Red lines surrounding each nucleus in (B) are manually selected as nuclei for data acquisition. Black arrows in (A and B) indicate aggregated nuclei detected as a single, large area in (A), which was eliminated from analysis in (B). (C) Examples of individual data images of automatically detected nuclear area in (A). Arrow indicates the same area shown by arrows in (A and B). (D) Individual data images after the manual elimination of aggregated nuclear area, which are shown in black arrows in (A and C), from analysis data. (E) Other examples of eliminated nuclei.



TIFF files saved from Virtual slide data

Slide converter

| Acquired information from the saved files              | Hight 1088 pixel<br>Width 1848 pixel<br>=2010624 pixel<br>Exact area: 0.101mm <sup>2</sup>  | Micrometer/pixel<br>X-axis: 0.504<br>Y-axis: 0.504 |  |  |
|--|---|--|--|--|
| Interpretation by us                                   | Converted area: 0.510731 mm <sup>2</sup> for 0.101mm <sup>2</sup><br>=5.056739267mm <sup>2</sup> for 1mm <sup>2</sup><br>For length evaluation, we took the square root<br>=2.248719473mm for 1mm |  |  |  |
| Analyzed results<br>shown in HistoQuant<br>Module data | Example data (1 nuclei in case #1)<br>Nuclear area: 405.92μm²<br>Nuclear perimeter: 118.02μm  |  |  |  |
|  | To get exact area and perimeter<br>Area data was divided by 5.0567<br>Perimeter data was divided by 2   | 739267.<br>2.248719473.                            |  |  |
| Calculated nuclear area and perimeter                  | Example data (1 nuclei in case #1)<br>Nuclear area: 80.27μm²<br>Nuclear perimeter: 52.48μm  |  |  |  |

|                      | Protocol number <sup>a</sup> |       |       |       |  |  |
|----------------------|------------------------------|-------|-------|-------|--|--|
| Application          | 1                            | 2     | 3     | 4     |  |  |
| Color range          |                              |       |       |       |  |  |
| Red                  | 0-244                        | 0-241 | 0-245 | 0-232 |  |  |
| Green                | 0-228                        | 0-222 | 0-220 | 0-211 |  |  |
| Blue                 | 0-255                        | 0-255 | 0-245 | 0-236 |  |  |
| Noise reduction      |                              |       |       |       |  |  |
| Туре                 |                              | Ga    | uss   |       |  |  |
| Size, 0-32           |                              | 2     | 4     |       |  |  |
| Separation           |                              |       |       |       |  |  |
| Steps, 1-10          |                              |       | 1     |       |  |  |
| Fill holes           |                              | Y     | es    |       |  |  |
| Size filter, 0-∞     | 150-1400                     |       |       |       |  |  |
| Shape filter, 0-1.00 | 0-1.00                       |       |       |       |  |  |
| Number of cases      | 18                           | 49    | 65    | 8     |  |  |

Table SI. Protocols for the image analysis of Feulgen-stained specimens.

<sup>a</sup>Depending on the darkness of nuclei, the color range (Red, Green and Blue) was adjusted individually

| Table SII. | Comparison | of nuclear factors | between FIGO | stages I+II | and III+IV. |
|------------|------------|--------------------|--------------|-------------|-------------|
|            | 1          |                    |              | 0           |             |

|                                |                              | Nuclear factors            |         |                          |         |                         |         |
|--------------------------------|------------------------------|----------------------------|---------|--------------------------|---------|-------------------------|---------|
| Subtype                        | FIGO<br>stage                | Nuclear area, $\mu m^2$    | P-value | Nuclear<br>perimeter, µm | P-value | Nuclear<br>shape factor | P-value |
| High-grade<br>serous carcinoma | I+II (n=10)<br>III+IV (n=28) | 51.50±7.96<br>56.08±11.11  | 0.1761  | 30.36±3.03<br>32.24±3.83 | 0.1323  | 0.70±0.07<br>0.68±0.06  | 0.3116  |
| Clear cell<br>carcinoma        | I+II (n=55)<br>III+IV (n=8)  | 60.30±12.62<br>57.64±11.06 | 0.5479  | 33.05±4.12<br>32.75±5.00 | 0.8788  | 0.69±0.05<br>0.68±0.08  | 0.7679  |
| Endometrioid carcinoma         | I+II (n=24)<br>III+IV (n=1)  | 49.41±6.40<br>51.95        | NE      | 30.54±2.74<br>31.16      | NE      | 0.67±0.06<br>0.67       | NE      |
| Mucinous<br>carcinoma          | I+II (n=13)<br>III+IV (n=1)  | 51.93±11.62<br>66.42       | NE      | 30.69±3.02<br>32.84      | NE      | 0.69±0.04<br>0.75       | NE      |

 $NE, not \ evaluated \ due \ to \ the \ small \ number \ (n=1) \ of \ patients \ in \ FIGO \ stage \ III + IV. \ FIGO, \ International \ Federation \ of \ Gynecology \ and \ Obstetrics.$ 

| Table SIII. | Comparison | of Lamin A ar | nd emerin | between FIGO | stages I+II | and III+IV. |
|-------------|------------|---------------|-----------|--------------|-------------|-------------|
|             | 1          |               |           |              | 0           |             |

|                             |                              | Expression                 |         |                            |         |  |
|-----------------------------|------------------------------|----------------------------|---------|----------------------------|---------|--|
| Subtype                     | FIGO stage                   | Lamin A-positive rate, %   | P-value | Emerin-positive rate, %    | P-value |  |
| High-grade serous carcinoma | I+II (n=10)<br>III+IV (n=28) | 67.15±24.74<br>55.41±26.16 | 0.2217  | 57.53±18.44<br>53.79±30.02 | 0.6496  |  |
| Clear cell carcinoma        | I+II (n=55)<br>III+IV (n=8)  | 48.12±23.18<br>55.71±25.87 | 0.4534  | 27.54±24.55<br>29.70±30.61 | 0.8533  |  |
| Endometrioid carcinoma      | I+II (n=24)<br>III+IV (n=1)  | 56.23±23.35<br>82.61       | NE      | 29.14±27.62<br>42.83       | NE      |  |
| Mucinous carcinoma          | I+II (n=13)<br>III+IV (n=1)  | 87.67±11.87<br>94.63       | NE      | 57.34±26.43<br>35.82       | NE      |  |

 $NE, not \ evaluated \ due \ to \ the \ small \ number \ (n=1) \ of \ patients \ in \ FIGO \ stage \ III + IV. \ FIGO, \ International \ Federation \ of \ Gynecology \ and \ Obstetrics.$