

**Supplementary Table 1. IHC antibody information**

| <b>Antibody</b> | <b>Supplier</b>  | <b>Catalogue number</b> | <b>Dilution</b> | <b>Incubation</b> | <b>HIER buffer</b> |
|-----------------|------------------|-------------------------|-----------------|-------------------|--------------------|
| ER $\alpha$     | DAKO             | M7047                   | 1:50            | 30 min            | pH 9               |
| PR              | DAKO             | M3569                   | 1:150           | 30 min            | pH 9               |
| p53             | DAKO             | M7001                   | 1:1000          | 30 min            | pH 9               |
| EpCAM           | Cell signaling   | D9S3P                   | 1:200           | 60 min            | pH 6               |
| L1CAM           | BioLegend        | SIG-3911                | 1:100           | 60 min            | pH 9               |
| PTEN            | Cell Signaling   | 9188                    | 1:100           | 4°C overnight     | pH 6               |
| ARID1A          | Abcam            | ab182560                | 1:2000          | 60 min            | pH 6               |
| MSH6            | Leica Biosystems | MSH6-L-CE               | 1:25            | 60 min            | pH 9               |
| MSH2            | Leica Biosystems | MSH2-L-CE               | 1:50            | 30 min            | pH 6               |
| PMS2            | Leica Biosystems | PMS2-L-CE               | 1:25            | 60 min            | pH 9               |
| MLH1            | Leica Biosystems | MLH1-L-CE               | 1:100           | 30 min            | pH 9               |
| Ki67            | Abcam            | Ab16667                 | 1:100           | 60 min            | pH 6               |

**Supplementary Table 2. IMC antibody information**

| <b>Mass isotope</b> | <b>Target</b>    | <b>Clone</b> | <b>Staining Conc<sup>a</sup></b> | <b>Supplier</b> | <b>Catalogue no</b> |
|---------------------|------------------|--------------|----------------------------------|-----------------|---------------------|
| <b>141 Pr</b>       | $\alpha$ -SMA    | 1A4          | 2.5                              | Fluidigm        | 3141017D            |
| <b>143 Nd</b>       | Vimentin         | D21H3        | 5.0                              | Fluidigm        | 3143027D            |
| <b>144 Nd</b>       | EpCAM            | 9C4          | 6.7                              | Fluidigm        | 3144026D            |
| <b>146 Nd</b>       | CD16             | EPR16784     | 5.0                              | Fluidigm        | 3146020D            |
| <b>148 Nd</b>       | Pan-cytokeratin  | AE1/AE3      | 10.0                             | Fluidigm        | 3148022D            |
| <b>150 Nd</b>       | PD-L1            | E1L3N        | 5.0                              | Fluidigm        | 3150031D            |
| <b>151 Eu</b>       | CD31             | EPR3094      | 5.0                              | Fluidigm        | 3151025D            |
| <b>152 Sm</b>       | CD45             | CD45-2B11    | 6.7                              | Fluidigm        | 3152016D            |
| <b>154 Sm</b>       | ER               | SP1          | 5.0                              | Abcam           | ab187260            |
| <b>156 Gd</b>       | CD4              | EPR6115      | 5.0                              | Fluidigm        | 3156033D            |
| <b>158 Gd</b>       | E-Cadherin       | 24E10        | 5.0                              | Fluidigm        | 3158029D            |
| <b>159 Tb</b>       | CD68             | KP1          | 5.0                              | Fluidigm        | 3159035D            |
| <b>161 Dy</b>       | CD20             | H1           | 1.3                              | Fluidigm        | 3161029D            |
| <b>162 Dy</b>       | CD8a             | D8A8Y        | 5.0                              | Fluidigm        | 3162035D            |
| <b>163 Dy</b>       | VEGF             | G153-694     | 5.0                              | Fluidigm        | 3163028D            |
| <b>164 Dy</b>       | PDGFR $\alpha$   | EPR22059-270 | 10.0                             | Abcam           | ab234965            |
| <b>165 Ho</b>       | $\beta$ -catenin | D13A1        | 2.5                              | Fluidigm        | 3165032D            |
| <b>167 Er</b>       | Podoplanin       | D2-40        | 5.0                              | Biolegend       | 916606              |
| <b>168 Er</b>       | Ki-67            | B56          | 5.0                              | Fluidigm        | 3168022D            |
| <b>169 Tm</b>       | Collagen type I  | Polyclonal   | 5.0                              | Fluidigm        | 3169023D            |
| <b>170 Er</b>       | CD3              | C-terminal   | 10.0                             | Fluidigm        | 3170019D            |
| <b>171 Yb</b>       | pERK1/2          | D13.14.E4    | 10.0                             | Fluidigm        | 3171021D            |
| <b>173 Yb</b>       | PR               | SP2          | 6.7                              | Abcam           | ab239793            |
| <b>175 Lu</b>       | pS6              | N7-548       | 6.7                              | Fluidigm        | 3175031D            |
| <b>176 Yb</b>       | HH3              | D1H2         | 1.3                              | Fluidigm        | 3176023D            |
| <b>191/193 Ir</b>   | Iridium          | -            | 1:300                            | Fluidigm        | 201192A             |

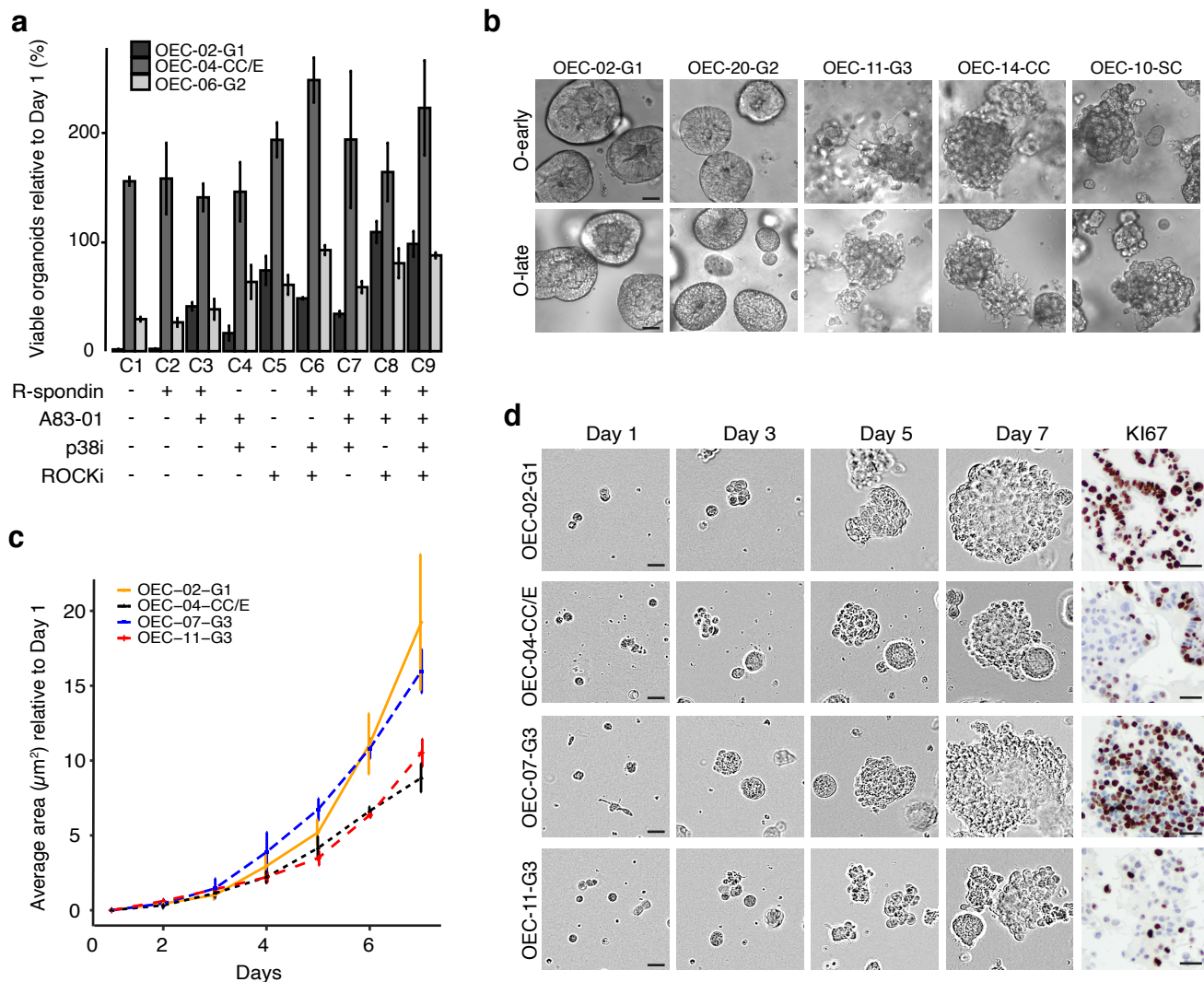
<sup>a</sup>  $\mu$ g/ml

**Supplementary Table 3. PCR primers for sequencing of POLE exon 9, 11, 13 and 14.**

| <b>Exon</b> | <b>Forward PCR primer (5`-3`)*</b>               | <b>Reverse PCR primer (5`-3`)*</b>                       | <b>PCR size</b> |
|-------------|--------------------------------------------------|----------------------------------------------------------|-----------------|
| 9           | <b>TGTAAAACGACGGCCAGT</b> GCCTAATGGGGAGTTTAGAGC  | <b>CAGGAAACAGCTATGACCT</b> ACTTCCCAGAAGCCACCTG           | 219             |
| 11          | <b>TGTAAAACGACGGCCAGT</b> GAGAAAGAGCAGACCTCTGAC  | <b>CAGGAAACAGCTATGACCC</b> CAGTTACTCATAGAGAAGACAC<br>AGA | 193             |
| 13          | <b>TGTAAAACGACGGCCAGT</b> TCTGTTCTCATTCTCCTTCCAG | <b>CAGGAAACAGCTATGACCC</b> GGGATGTGGATTACGTG             | 210             |
| 14          | <b>TGTAAAACGACGGCCAGT</b> TCTGGCGTTCTCTCCTCAG    | <b>CAGGAAACAGCTATGACCC</b> GACAGGACAGATAATGCTCA          | 191             |

\* Universal primers (bold) are incorporated into forward and reverse primers for subsequent Sanger sequencing.

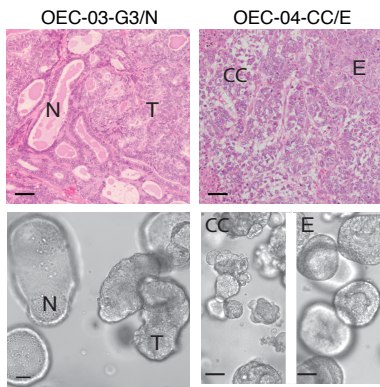




**Supplementary Figure 1. Modified medium allows long-term expansion of EC organoids.**

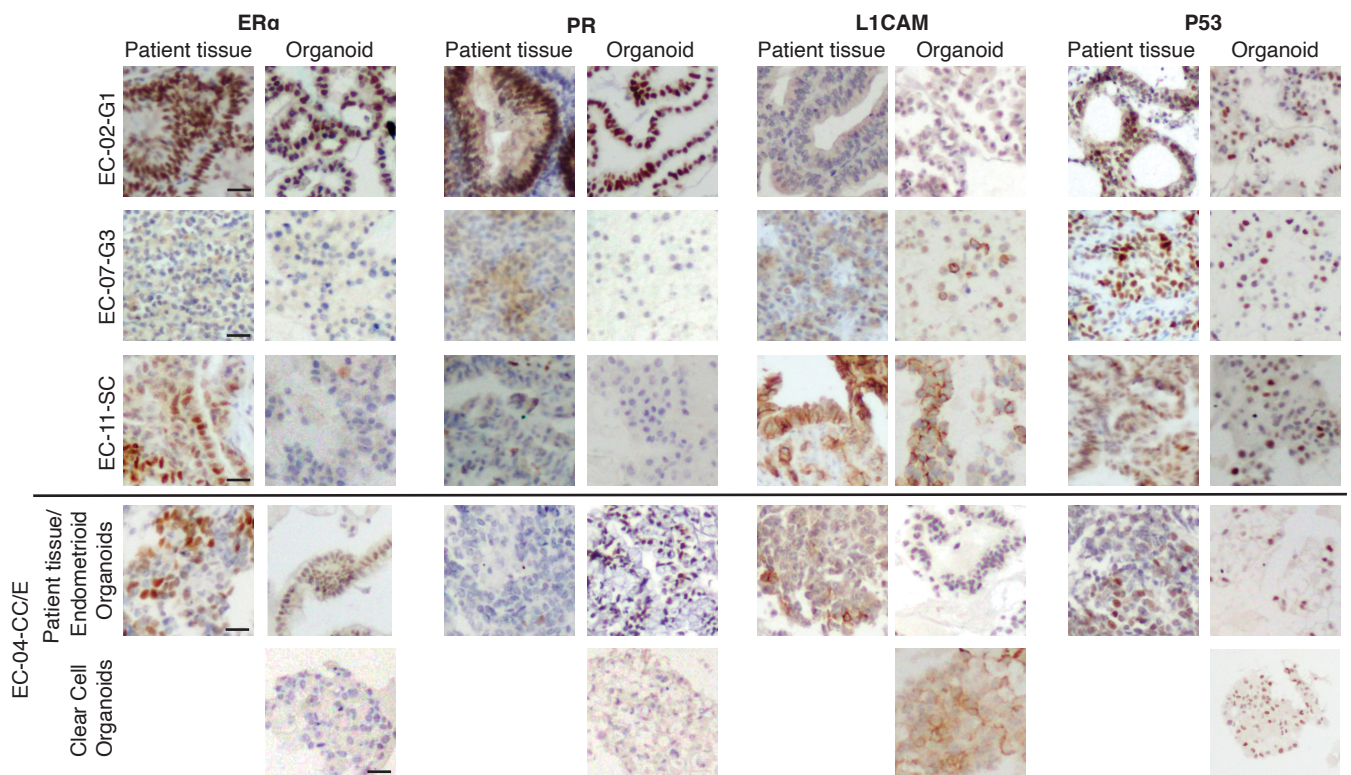
**Growth rates differ between the cultures.**

**a** Medium withdrawal experiment showing viable organoids in three different organoid cultures after eight days of culture with nine different medium conditions (C1-C9). Organoids were seeded as small cell aggregates and number of viable organoids ( $\geq 2$  cells in cluster) at Day 8 was normalized to counts at Day 1. Error bars represent SD of three technical replicates. **b** Representative brightfield images of early- and late-passaged organoids derived from different subtypes of endometrial tumors. **c** Average phase object area ( $\text{mm}^2$ ) of selected organoid cultures at day 2-7 normalized to Day 1. Phase object area for each organoid culture was calculated using the IncuCyte ZOOM software. Error bars represent SD of two technical replicates. **d** Representative brightfield images of organoid cultures at 1, 3, 5 and 7 days after seeding. Organoids were seeded as small aggregates of cells in Matrigel coated wells in EC-ExM/5% Matrigel. Right panel depicts Ki67 immunohistochemical staining of corresponding organoids. Scale bars = 20  $\mu\text{m}$ .



### Supplementary Figure 2. Mixed tumor histology is retained in the organoid cultures.

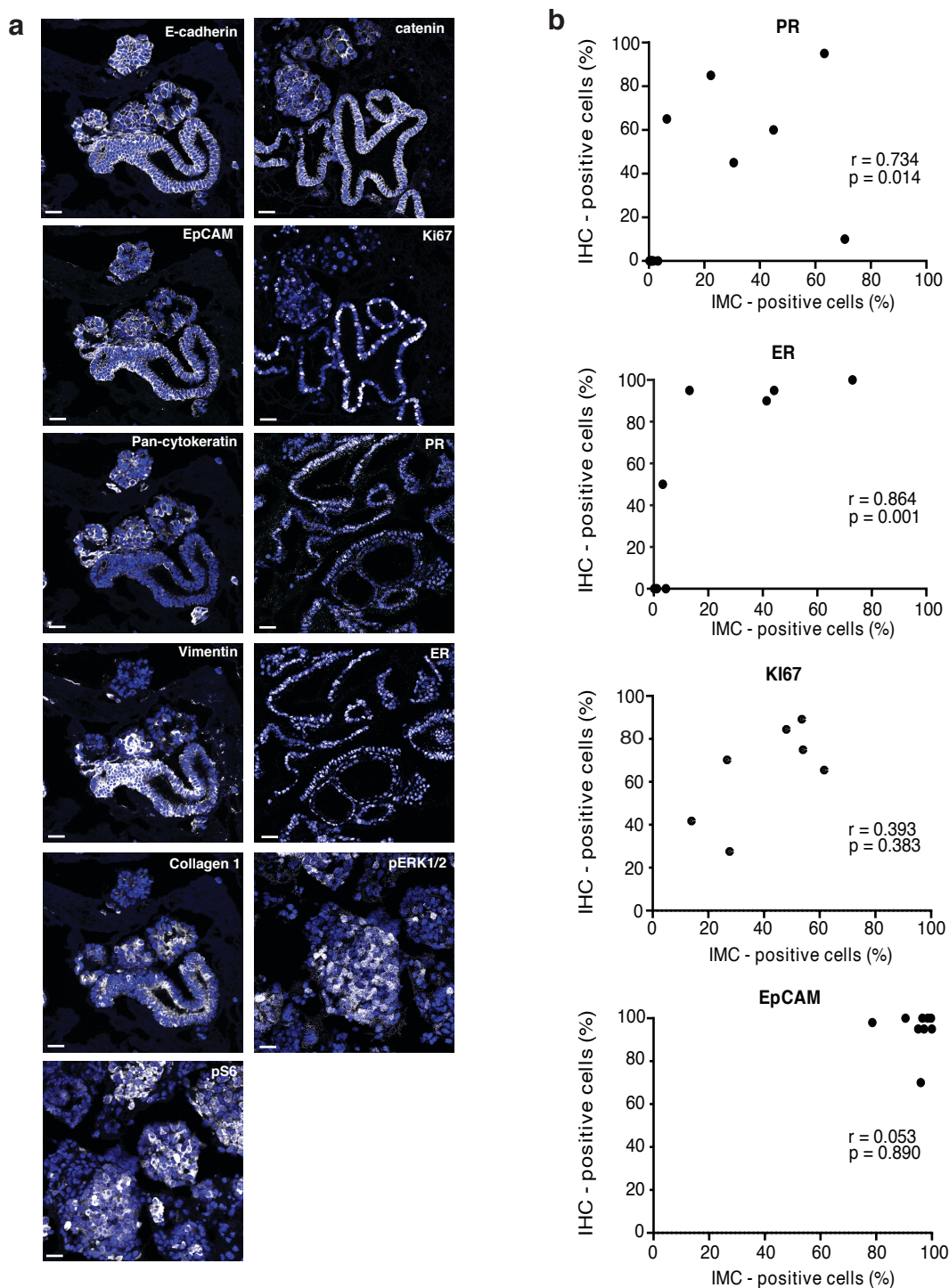
Representative H&E stained sections of patient tissue and brightfield images of corresponding mixed-morphology organoid cultures. Subclones are indicated with T=tumor, N=normal, CC=clear cell, E=endometrioid. Scale bar = 50  $\mu$ m.



### Supplementary Figure 3. Patient tissue biomarker expression is retained in the organoids.

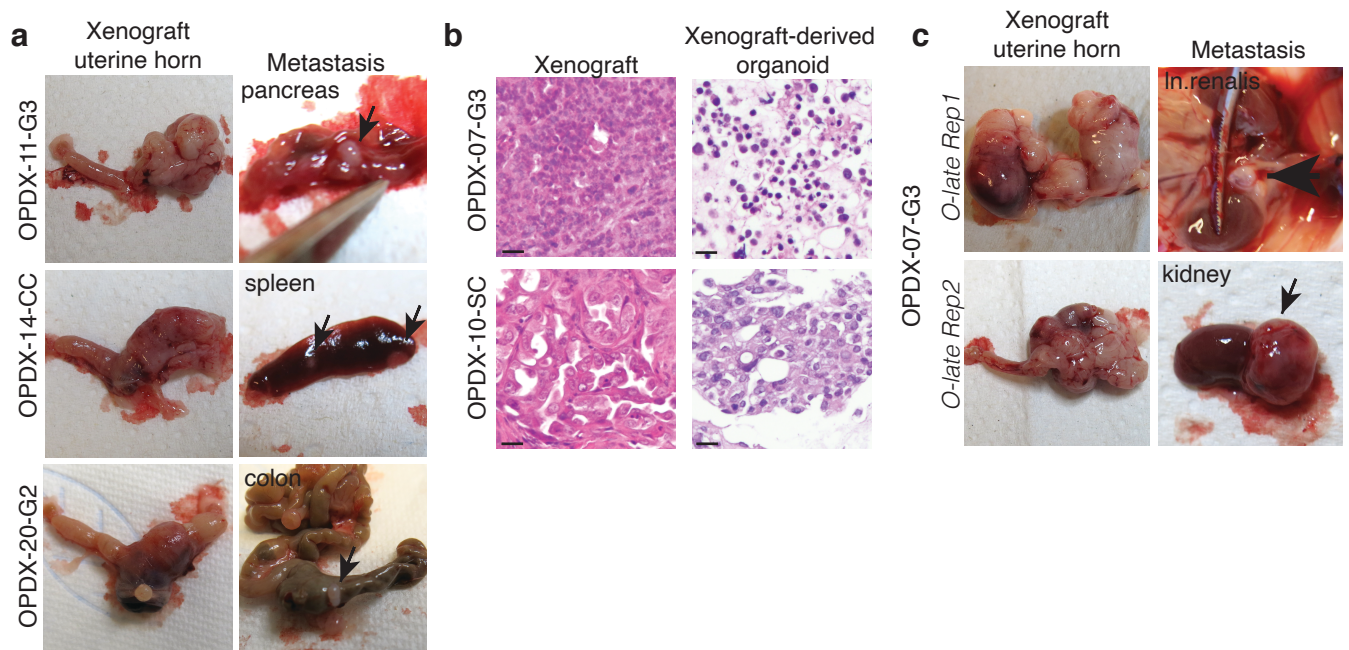
Representative images of selected primary tumor-organoid pairs showing immunohistochemical staining of ER, PR, L1CAM and p53. In the lower panel, stained sections of the clear cell component of EC-04-CC/E patient tissue are depicted as well as the subcloned pure endometrioid and clear cell organoid cultures. Scale bars = 20  $\mu$ m.





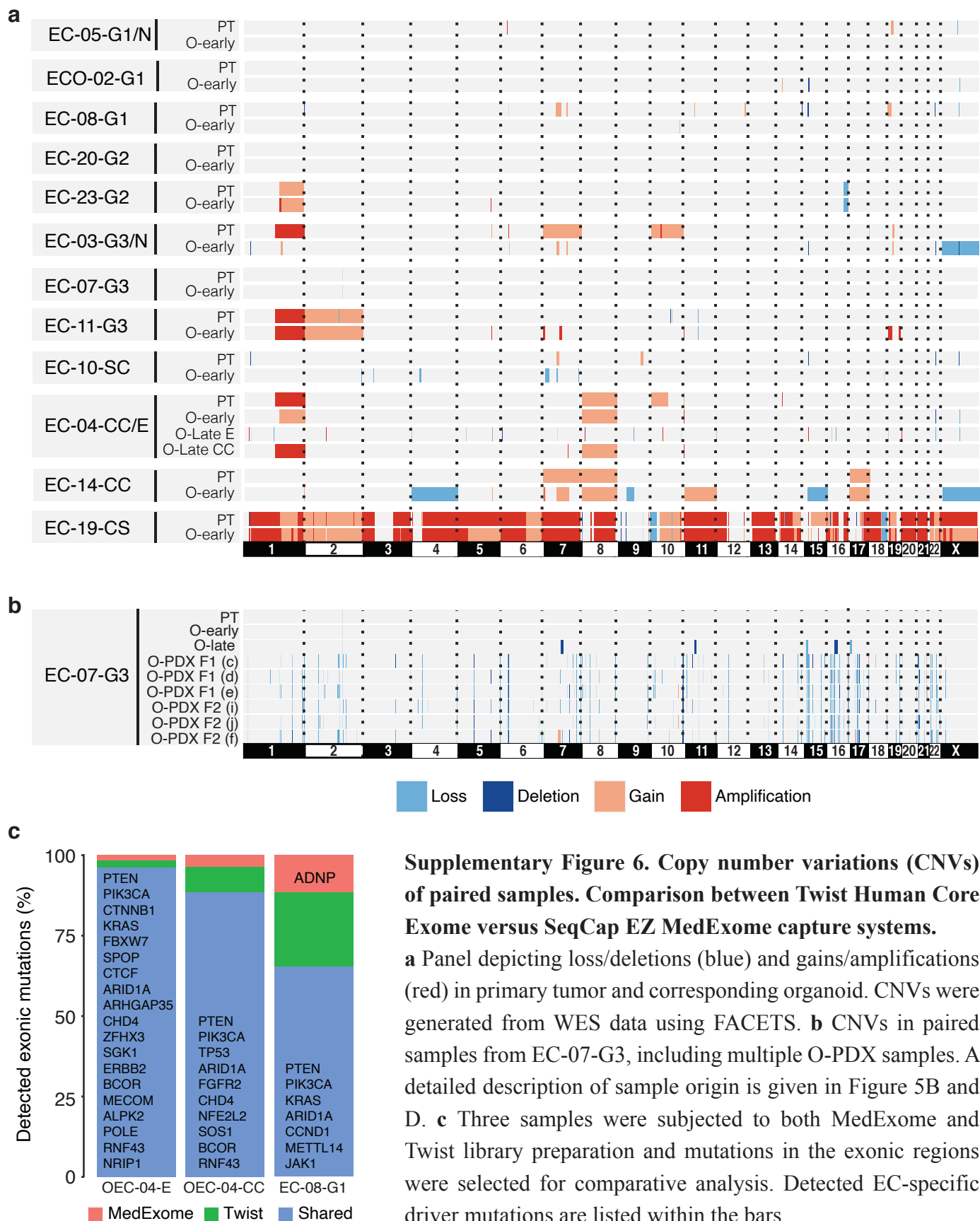
**Supplementary Figure 4. Marker expression measured by imaging mass cytometry (IMC) and immunohistochemistry (IHC) is similar.**

**a** Example images from IMC depicting positive staining of the tumor cell markers Vimentin, Pan-cytokeratin, EpCAM, Collagen I, PR, ER,  $\beta$ -catenin, Ki67, pERK1/2, pS6 and E-cadherin. Scale bar = 20  $\mu$  m. **b** For IMC, single cells positive for a marker of interest were quantified by manually gating single cells in HistoCAT. Percent positive cells identified by IMC was plotted against percent positive cells determined by manually scoring of IHC stained sections. The concordance between the two methods were calculated using Spearman correlation.



**Supplementary Figure 5. Orthotopically implanted organoids metastasize to lymph nodes and distant organs.**

**a** Representative O-PDX models with large tumor mass in left uterine horn and metastases to distant organs. **b** Representative H&E stained sections of uterine xenografts and corresponding xenograft-derived organoids. **c** O-PDX model generated by implanting late-passaged organoids. Mice developed large uterine tumors with metastases to either renal lymph nodes or kidney. Scale bar = 20  $\mu$ m.



**Supplementary Table 4. Differentially expressed genes in cluster 2 versus cluster 1**

| <b>Transcript</b> | <b>Log2 fold change</b> | <b>FDR (q-value)</b> |
|-------------------|-------------------------|----------------------|
| S100A9            | 7.80                    | <0.001               |
| SORCS2            | 7.03                    | <0.001               |
| POU3F3            | 7.24                    | <0.001               |
| COL4A2            | 6.97                    | <0.001               |
| COL4A1            | 6.55                    | <0.001               |
| RNF182            | 5.96                    | <0.001               |
| PRTG              | 7.78                    | <0.001               |
| SIRPA             | 6.70                    | <0.001               |
| SAMD5*            | 7.47                    | <0.001               |
| MMP26             | -7.87                   | <0.001               |
| SCGB2A1           | -8.09                   | <0.001               |
| SCGB1D2           | -7.38                   | <0.001               |
| PGR               | -8.88                   | <0.001               |
| KIAA1324          | -6.87                   | <0.001               |
| CST1              | -7.71                   | <0.001               |
| SCGB2A2           | -7.90                   | <0.001               |
| PKHD1L1           | -6.82                   | <0.001               |
| ESR1              | -8.48                   | <0.001               |
| SERPINA6          | -6.42                   | <0.001               |
| CAPN6             | -6.62                   | <0.001               |
| IHH*              | -8.09                   | <0.001               |
| RP11-6E9.4*       | -7.30                   | <0.001               |
| RP11-474D1.3*     | -6.48                   | <0.001               |

\*Genes excluded from 19-gene signature (see Methods section)

**Supplementary Table 5. Signature score associates with aggressive features of endometrial cancer.**

|                                               | <b>Low risk sign.</b> | <b>High risk sign.</b> | P-value <sup>d</sup> |
|-----------------------------------------------|-----------------------|------------------------|----------------------|
|                                               | n (%)                 | n(%)                   |                      |
| <b>Number of patients</b>                     | 148                   | 108                    |                      |
| <b>Age, median</b>                            |                       |                        | <b>0.002</b>         |
| <67                                           | 90 (66.7)             | 45 (33.3)              |                      |
| ≥67                                           | 58 (47.9)             | 63 (52.1)              |                      |
| <b>Histologic type and grade <sup>a</sup></b> |                       |                        | <b>&lt;0.001</b>     |
| Endometrioid grade 1-2                        | 120 (81.1)            | 28 (18.9)              |                      |
| Endometrioid grade 3                          | 18 (36.0)             | 32 (64.0)              |                      |
| Non-endometrioid                              | 5 (9.4)               | 48 (90.6)              |                      |
| <b>FIGO stage</b>                             |                       |                        | <b>&lt;0.001</b>     |
| I                                             | 122 (66.3)            | 62 (33.7)              |                      |
| II                                            | 11 (55.0)             | 9 (45.0)               |                      |
| III                                           | 13 (36.1)             | 23 (63.9)              |                      |
| IV                                            | 2 (12.5)              | 14 (87.5)              |                      |
| <b>Metastatic lymph nodes<sup>b</sup></b>     |                       |                        | <b>&lt;0.001</b>     |
| Yes                                           | 104 (61.9)            | 64 (38.1)              |                      |
| No                                            | 9 (27.3)              | 24 (72.7)              |                      |
| <b>Myometrial infiltration<sup>c</sup></b>    |                       |                        | <b>0.012</b>         |
| <50%                                          | 85 (65.9)             | 44 (34.1)              |                      |
| >50%                                          | 63 (50.4)             | 62 (49.6)              |                      |

Abbreviations: FIGO: International Federation of Gynaecology and Obstetrics.

<sup>a</sup>Data missing for 5 patients, <sup>b</sup>Data missing for 55 patients, <sup>c</sup>Data missing for 2 patients,

<sup>d</sup>Pearson chi-square test.

**Supplementary Table 6. Signature score in relation to clinicopathological factors in the L1000 and TCGA transcriptomic EC datasets.**

|                                  | <b>L1000</b>          |                        | P-value <sup>e</sup> | <b>TCGA</b>           |                        | P-value <sup>c</sup> |
|----------------------------------|-----------------------|------------------------|----------------------|-----------------------|------------------------|----------------------|
|                                  | <b>Low risk sign.</b> | <b>High risk sign.</b> |                      | <b>Low risk sign.</b> | <b>High risk sign.</b> |                      |
| <b>Number of patients</b>        | 230                   | 150                    |                      | 293                   | 213                    |                      |
| <b>Age, median<sup>a</sup></b>   |                       |                        | <b>0.001</b>         |                       |                        | <b>0.001</b>         |
| <64                              | 131 (68.6)            | 60 (31.4)              |                      | 177 (64.8)            | 96 (35.2)              |                      |
| ≥64                              | 99 (52.4)             | 90 (47.6)              |                      | 114 (49.6)            | 116 (50.4)             |                      |
| <b>Histologic subtype</b>        |                       |                        | <b>&lt;0.001</b>     |                       |                        | <b>&lt;0.001</b>     |
| Endometrioid grade 1-2           | 203 (82.2)            | 44 (17.8)              |                      | 179 (84.0)            | 34 (16.0)              |                      |
| Endometrioid grade 3             | 18 (33.3)             | 36 (66.7)              |                      | 100 (54.3)            | 84 (45.7)              |                      |
| Non-endometrioid                 | 6 (7.9)               | 70 (92.1)              |                      | 14 (12.8)             | 95 (87.2)              |                      |
| <b>FIGO stage</b>                |                       |                        | <b>0.001</b>         |                       |                        |                      |
| I                                | 192 (64.6)            | 105 (35.4)             |                      |                       |                        |                      |
| II                               | 18 (56.3)             | 14 (43.8)              |                      |                       |                        |                      |
| III                              | 18 (47.4)             | 20 (52.6)              |                      |                       |                        |                      |
| IV                               | 2 (15.4)              | 11 (84.6)              |                      |                       |                        |                      |
| <b>Metastatic LN<sup>b</sup></b> |                       |                        | <b>0.004</b>         |                       |                        |                      |
| Yes                              | 175 (62.3)            | 106 (37.7)             |                      |                       |                        |                      |
| No                               | 13 (37.1)             | 22 (62.9)              |                      |                       |                        |                      |
| <b>Myometrial infiltration</b>   |                       |                        | 0.079                |                       |                        |                      |
| <50%                             | 145 (64.2)            | 81 (35.8)              |                      |                       |                        |                      |
| ≥50%                             | 85 (55.2)             | 69 (44.8)              |                      |                       |                        |                      |

Abbreviations: FIGO: International Federation of Gynaecology and Obstetrics.

<sup>a</sup>Data missing for 3 patients in the TCGA cohort, <sup>b</sup>LN: lymph nodes: Data missing for 64 patients in the L1000 cohort, <sup>c</sup>Pearson chi-square test.