## Development of a sequential workflow based on LC-PRM for the verification of endometrial cancer protein biomarkers in uterine aspirate samples

## **Supplementary Materials**





Supplementary Figure S1: Biomarker candidates rejected from the study due to their high abundance in blood. Concentration profile of the peptides coming from 32 proteins showing increasing expression profiles when uterine aspirate samples were diluted with increasing amount of full blood (0, 10, 20, 40% (v/v)).



**Supplementary Figure S2: TIC of each injected sample to control the total amount of protein injected.** Area of the total ion current chromatogram (TIC) of the samples. Samples 199T to 175T and 300N to 337N were acquired in a different batch. T, endometrial cancer patients; N, control women.







CONTROL

TUMOR

-0.5







































Supplementary Figure S3: Scattering plots of the expression of 31 peptides coming from the 16 differentially expressed biomarkers with AUC between 0.75-0.89. Scattering plots depicting the light/heavy (L/H) ratios across the 20 EC patients and 18 controls of the 16 differentially expressed biomarkers not shown in Figure 4 (*p*-value<0.05 and fold change higher than 3; AUC below 0.9).

**Supplementary Table S1: List of proteins and related peptides detected by DDA analysis in uterine aspirates samples.** In bold, the 158 proteins identified out of the initial list of 506 biomarker candidates. See Supplementary\_File\_S1

Supplementary Table S2: List of precursors and related fragment ions extracted from PRM acquisition. See Supplementary\_File\_S2

Supplementary Table S3: Raw integration data extracted from Skyline (area ratio L/H). See Supplementary\_File\_S3

Supplementary Table S4: Cleansed integration data, mean of duplicates (area ratio L/H). See Supplementary File S4

**Supplementary Table S5: Clinico-pathological characteristics of the 42 women enrolled in the study.** DDA, data-dependent acquisition; PRM, parallel-reaction monitoring. See Supplementary File S5