## SUPPLEMENTARY INFORMATION

## Angiogenesis inhibitor or aggressiveness marker? The function of endostatin in cancer through electrochemical biosensing

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**Table S1.**  $t_{exp}$  values obtained in the comparison between the slope values of the calibration plots constructed with the bioplatform for endostatin prepared in buffer solution and in the indicated amount/dilution of each biological matrix.

|                    |                 | Intercent nA              | Slope, nA mL    | t <sub>evn**</sub> | ftab(05% 2 2 tails)**  |  |
|--------------------|-----------------|---------------------------|-----------------|--------------------|------------------------|--|
|                    | Intercept, in s |                           | $pg^{-1}$       | cexp               | (au)(3570, 2, 2 tails) |  |
| Buffered solutions |                 | $73 \pm 2$ $0.09 \pm 0.0$ |                 |                    |                        |  |
| Tissues (0.05 µg)  | NT5*            | $93.7\pm0.7$              | $0.07\pm0.02$   | 4.809              |                        |  |
|                    | T5 (III)*       | $107 \pm 2$               | $0.07\pm0.02$   | 5.231              |                        |  |
| Plasma (1/150)     | 2*              | $82 \pm 2$                | $0.105\pm0.005$ | 1.028              |                        |  |
|                    | 16*             | $108 \pm 2$               | $0.106\pm0.005$ | 0.814              | 4.303                  |  |
| Cell extracts      | SW480           | $85 \pm 4$                | $0.10 \pm 0.01$ | 0.279              |                        |  |
| (0.1 µg)           | KM12SM          | $118\pm9$                 | $0.10\pm0.03$   | 0.232              |                        |  |
| Cell secretomes    | SW480           | $85\pm 6$                 | $0.10\pm0.02$   | 0.238              |                        |  |
| (1/75)             | KM12SM          | $108\pm10$                | $0.09\pm0.03$   | 0.301              |                        |  |

\*These codes correspond to those of **Table 3** in the manuscript. \*\*Estimated as described in [1] by comparing the slope values.



**Fig. S1.** ROC curves of the bioplatform diagnostic value to discriminate the metastatic capabilities of cancer cells and between healthy subjects and CRC patients through the determination of endostatin in cell extracts or exosomes, or in tissue and plasma samples, respectively.

**Table S2.** Potential of the bioplatform to assist in CRC staging through the determination of endostatin in plasma and tissue samples and estimated cut-off values.

|                       | Plasma samples                    |     |             |             |                               | Tissue samples                    |     |             |             |                               |
|-----------------------|-----------------------------------|-----|-------------|-------------|-------------------------------|-----------------------------------|-----|-------------|-------------|-------------------------------|
| Comparison            | Cut-off<br>(ng mL <sup>-1</sup> ) | AUC | Specificity | Sensitivity | p-value<br>(Mann-<br>Whitney) | Cut-off<br>(pg µg <sup>-1</sup> ) | AUC | Specificity | Sensitivity | p-value<br>(Mann-<br>Whitney) |
| CT_Stage I            | 67.8                              | 100 | 100         | 100         | 0.00020794                    | 191.59                            | 100 | 100         | 100         | 3.37E-06                      |
| CT_Stage II           | 77.635                            | 100 | 100         | 100         | 0.00020794                    | 195.81                            | 100 | 100         | 100         | 3.37E-06                      |
| CT_Stage III          | 81.96                             | 100 | 100         | 100         | 0.00020794                    | 226.405                           | 100 | 100         | 100         | 3.37E-06                      |
| CT_Stage IV           | 89.735                            | 100 | 100         | 100         | 0.00020794                    | 235.38                            | 100 | 100         | 100         | 3.37E-06                      |
| Stage I_Stage II      | 99.545                            | 100 | 100         | 100         | 0.0021645                     | 212.86                            | 100 | 100         | 100         | 0.0021645                     |
| Stage I_Stage III     | 103.87                            | 100 | 100         | 100         | 0.0021645                     | 243.455                           | 100 | 100         | 100         | 0.0021645                     |
| Stage I_Stage IV      | 111.645                           | 100 | 100         | 100         | 0.0021645                     | 252.43                            | 100 | 100         | 100         | 0.0021645                     |
| Stage II_Stage III    | 113.92                            | 100 | 100         | 100         | 0.0021645                     | 251.03                            | 100 | 100         | 100         | 0.0021645                     |
| Stage II_Stage IV     | 121.695                           | 100 | 100         | 100         | 0.0021645                     | 260.005                           | 100 | 100         | 100         | 0.0021645                     |
| Stage III_Stage<br>IV | 126.805                           | 100 | 100         | 100         | 0.0021645                     | 288.975                           | 100 | 100         | 100         | 0.0021645                     |

## Reference

[1] J.M. Andrade, M.G. Estévez-Pérez, Statistical comparison of the slopes of two regression lines: A tutorial, Anal. Chim. Acta 838 (2014) 1–12, <u>https://doi.org/10.1016/j.aca.2014.04.057</u>.