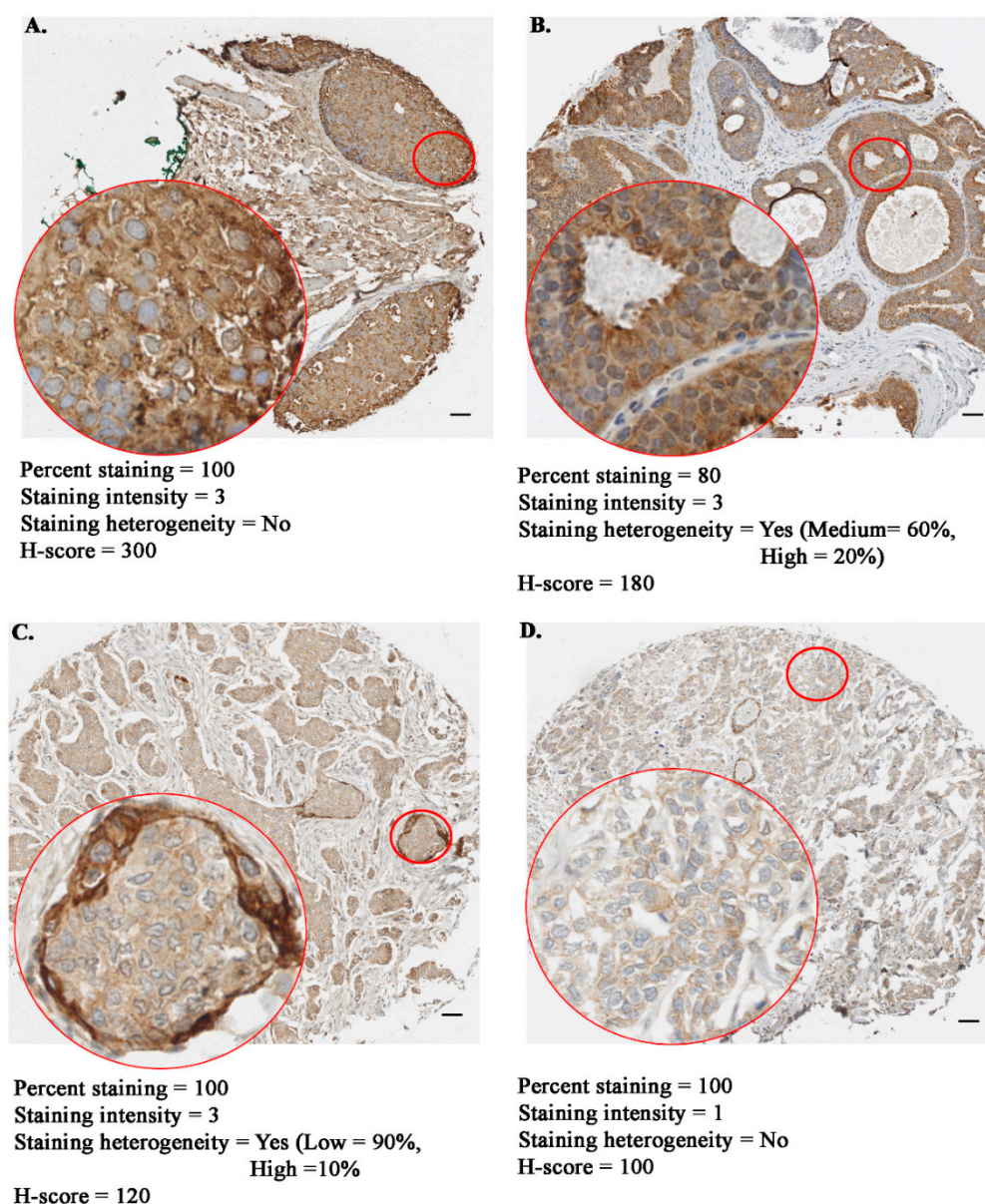
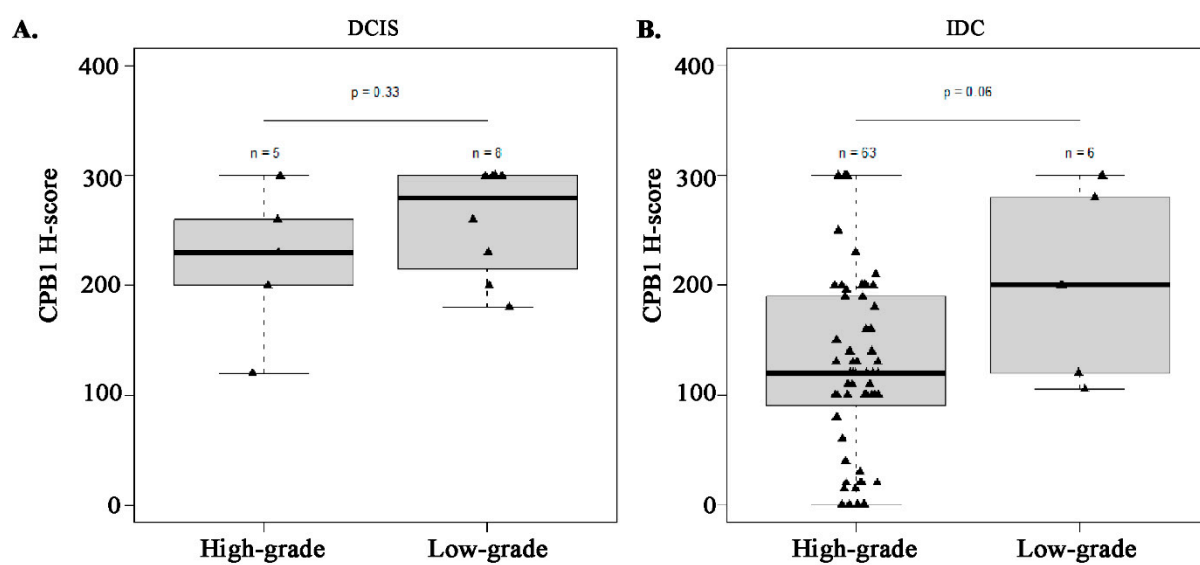


# Supplementary Materials: Is Carboxypeptidase B1 a Prognostic Marker for Ductal Carcinoma In Situ?

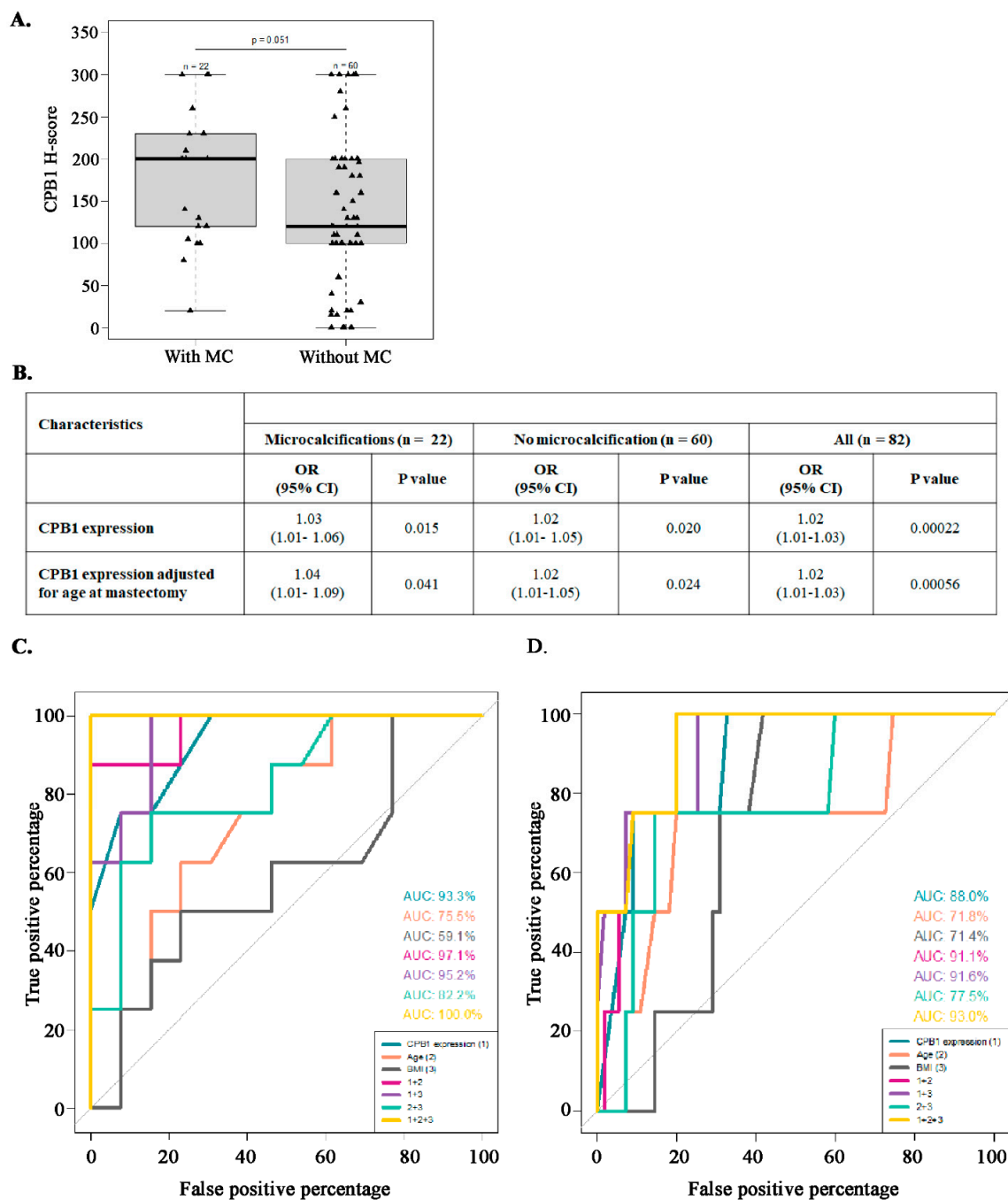
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**Figure S1.** Representative images depicting the scoring method used for quantitation of immunohistochemistry (IHC) staining. H-score = (% of cells stained with low-intensity \* 1) + (% of cells stained with medium-intensity \* 2) + (% of cells stained with high-intensity \* 3). carcinoma in situ (DCIS) (A,B), invasive ductal carcinoma (IDC) (C,D). Scale bar 100  $\mu$ m.



**Figure S2.** Carboxypeptidase 1 (CPB1) expression based on H-score in our cohort stratified according to grade in DCIS (A) and IDC (B).



**Figure S3.** Association of CPB1 with microcalcification. Boxplot depicting H-score of CPB1 in patients with and without microcalcifications (**A**). Association of CPB1 expression in our BC cohort stratified by microcalcifications (**B**). Receiver operating characteristic (ROC) curve depicting the possibility of identification of DCIS based on expression of CPB1 in BC patients with microcalcifications (DCIS = 8; IDC = 3) (**C**) and without microcalcifications (DCIS = 4; IDC = 55) (**D**).

**Table S1.** Primers used.

| Gene Symbol | Description                                                                                        | Size (bp) | Primer Sequence 5'→3'<br>Forward/Reverse                       |
|-------------|----------------------------------------------------------------------------------------------------|-----------|----------------------------------------------------------------|
| CPB1        | <i>Homo sapiens</i> carboxypeptidase B1 (CPB1)                                                     | 92        | TGCTGGTTGGTGTGAA<br>ATTGGAG/<br>GGGCCTTGGTCTCCTTT<br>TCAG      |
| SFRP1       | <i>Homo sapiens</i> secreted frizzled-related protein 1 (SFRP1)                                    | 111       | TCTACCCGTGTCGCTGG<br>CTCT/<br>CCTCGGGGAACCTGTGTC<br>ACACTTAA   |
| FN1         | <i>Homo sapiens</i> fibronectin 1 (FN1), 7 transcripts                                             | 104       | GCTCAAGTGGTCTGTGTC<br>GAAGTAT/<br>GTACTTGGAATGTGA<br>GATGGCTGT |
| SPP1        | <i>Homo sapiens</i> secreted phosphoprotein 1 (SPP1), 5 transcripts                                | 108       | AAGAAGTTTCGCAGAC<br>CTGACATC/<br>AACGGGGATGGCCTTG<br>TATGC     |
| OS9         | <i>Homo sapiens</i> OS9 endoplasmic reticulum lectin (OS9), 7 transcripts                          | 128       | CCTTCCTACAGCCTGA<br>GGAGTA/CCAGACTTGG<br>TCTCACTCCACAC         |
| HIF-1α      | <i>Homo sapiens</i> hypoxia-inducible factor 1 subunit alpha (HIF1A), 3 transcripts                | 144       | TATGAGCCAGAAGAAC<br>TTTTAGGCC/CCACCTC<br>TTTTGGCAAGCATCCTG     |
| TUBB3       | <i>Homo sapiens</i> tubulin beta 3 class III (TUBB3), 2 transcripts                                | 119       | TCAGCGTCTACTACAA<br>CGAGGCC/GCCTGAAG<br>AGATGTCCAAAGGC         |
| ATP5O       | <i>Homo sapiens</i> ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, O subunit | 103       | ATTGAAGGTCGCTATG<br>CCACAG/<br>CCTTCAGGATTTGTGCT<br>ACTCTCA    |
| HPRT1       | <i>Homo sapiens</i> hypoxanthine phosphoribosyltransferase 1                                       | 86        | CGTCGTGATTAGTGAT<br>GATGAACCA/<br>ACACCCTTTCCAAATC<br>CTCAGC   |
| GAPDH       | <i>Homo sapiens</i> glyceraldehyde-3-phosphate dehydrogenase                                       | 93        | CGGGGCTCTCCAGAAC<br>ATCAT/<br>ATGCCAGTGAGCTTCC<br>CGTTC        |

**Table S2.** Clinicopathological information of patients with DCIS.

|                  | Age | Histological Type | Stage | Tumor Size (mm) | Grade | Lymph Nodes | BMI  | Menopause Status | Microcalcifications            | CPB1 H-Score | Relapse *           |
|------------------|-----|-------------------|-------|-----------------|-------|-------------|------|------------------|--------------------------------|--------------|---------------------|
| DCIS without IDC | 34  | Non-invasive      | 0     | 56              | 2     | NA          | 21.5 | No               | In cancer cells under analysis | 300          | Yes                 |
|                  | 45  | Non-invasive      | 0     | 13              | 2     | Negative    | 22.3 | No               | No microcalcification          | 300          | No                  |
|                  | 38  | Non-invasive      | 0     | 50              | 2     | Negative    | 23.5 | No               | No microcalcification          | 260          | No                  |
|                  | 42  | Non-invasive      | 0     | 50              | 2     | NA          | 24.2 | No               | No microcalcification          | 180          | Yes (contralateral) |

|               |    |              |   |     |   |          |      |     |                                                           |     |                         |
|---------------|----|--------------|---|-----|---|----------|------|-----|-----------------------------------------------------------|-----|-------------------------|
| DCIS with IDC | 43 | Non-invasive | 0 | 45  | 3 | Negative | 31.5 | No  | In cancer cells under analysis                            | 260 | NA                      |
|               | 41 | Non-invasive | 0 | 60  | 3 | NA       | NA   | No  | In cancer cells under analysis and in benign/normal cells | 120 | No                      |
|               | 30 | Invasive     | 1 | 13  | 1 | Negative | 22.2 | No  | In cancer cells under analysis                            | 200 | No                      |
|               | 59 | Invasive     | 1 | 0.5 | 2 | Negative | 20.8 | Yes | In biopsy                                                 | 230 | Yes (DCIS, same breast) |
|               | 50 | Invasive     | 1 | 6   | 2 | Negative | 19.4 | No  | In cancer cells under analysis                            | 300 | No                      |
|               | 57 | Invasive     | 1 | 6   | 3 | Negative | 25.5 | Yes | In benign/normal cells                                    | 200 | No                      |
|               | 43 | Invasive     | 2 | 22  | 2 | Negative | 32   | No  | In cancer cells under analysis                            | 300 | No                      |
|               | 68 | Invasive     | 2 | 18  | 3 | Positive | 25.5 | Yes | NA                                                        | 300 | No                      |
|               | 54 | Invasive     | 3 | 40  | 3 | Positive | 30.8 | Yes | In biopsy                                                 | 230 | Yes (IDC contralateral) |

\* After resection surgery.