

## Supplementary Material

**Table S1a.** Relationship between tumor markers expression and clinicopathological features.

|                           | AR       |        |          |        | <i>p</i> -value | FOXA1    |        |          |        | <i>p</i> -value | PD-L1    |        |          |         | <i>p</i> -value | BRCA1    |        |          |        | <i>p</i> -value |
|---------------------------|----------|--------|----------|--------|-----------------|----------|--------|----------|--------|-----------------|----------|--------|----------|---------|-----------------|----------|--------|----------|--------|-----------------|
|                           | Negative |        | Positive |        |                 | Negative |        | Positive |        |                 | Negative |        | Positive |         |                 | Negative |        | Positive |        |                 |
| Characteristics           | n        | (%)    | n        | (%)    |                 | n        | (%)    | n        | (%)    |                 | n        | (%)    | n        | (%)     |                 | n        | (%)    | n        | (%)    |                 |
| <i>Patient age</i>        |          |        |          |        |                 |          |        |          |        |                 |          |        |          |         |                 |          |        |          |        |                 |
| ≤ 51 years                | 53       | (57.6) | 8        | (50.0) | 0.571           | 44       | (57.9) | 16       | (43.2) | 0.143           | 45       | (54.1) | 15       | (51.7)  | 0.817           | 23       | (50.0) | 30       | (50.0) | 1.000           |
| > 51 years                | 39       | (42.4) | 8        | (50.0) |                 | 32       | (42.1) | 21       | (56.8) |                 | 38       | (45.8) | 14       | (48.3)  |                 | 23       | (50.0) | 30       | (50.0) |                 |
| <i>Pre/Post</i>           |          |        |          |        |                 |          |        |          |        |                 |          |        |          |         |                 |          |        |          |        |                 |
| Pre                       | 68       | (73.9) | 8        | (50.0) | 0.053           | 55       | (72.4) | 20       | (54.1) | 0.053           | 54       | (65.1) | 21       | (72.4)  | 0.469           | 31       | (67.4) | 38       | (63.3) | 0.664           |
| Post                      | 24       | (26.1) | 8        | (50.0) |                 | 21       | (27.6) | 17       | (45.9) |                 | 29       | (34.9) | 8        | (27.6)  |                 | 15       | (32.6) | 22       | (36.7) |                 |
| <i>Histological type</i>  |          |        |          |        |                 |          |        |          |        |                 |          |        |          |         |                 |          |        |          |        |                 |
| IDC                       | 84       | (91.3) | 15       | (93.8) | 0.836           | 69       | (90.8) | 32       | (86.5) | 0.445           | 79       | (95.2) | 25       | (86.2)  | 0.048           | 42       | (91.3) | 55       | (91.7) | 0.635           |
| ILC                       | 2        | (2.2)  | 0        | (0.0)  |                 | 1        | (1.3)  | 2        | (5.4)  |                 | 2        | (2.4)  | 0        | (0.0)   |                 | 2        | (4.3)  | 1        | (1.7)  |                 |
| Other                     | 6        | (6.5)  | 1        | (6.2)  |                 | 6        | (7.9)  | 3        | (8.1)  |                 | 2        | (2.4)  | 4        | (13.8)  |                 | 2        | (4.3)  | 4        | (6.7)  |                 |
| <i>Tumor size (cm)</i>    |          |        |          |        |                 |          |        |          |        |                 |          |        |          |         |                 |          |        |          |        |                 |
| ≤2 cm                     | 41       | (44.6) | 7        | (43.8) | 0.952           | 34       | (44.7) | 19       | (51.4) | 0.508           | 42       | (51.2) | 10       | (34.5)  | 0.121           | 19       | (41.3) | 30       | (50.8) | 0.331           |
| >2 cm                     | 51       | (55.4) | 9        | (56.2) |                 | 42       | (55.3) | 18       | (48.6) |                 | 40       | (48.8) | 19       | (65.5)  |                 | 27       | (58.7) | 29       | (49.2) |                 |
| <i>Lymph node status</i>  |          |        |          |        |                 |          |        |          |        |                 |          |        |          |         |                 |          |        |          |        |                 |
| Negative                  | 48       | (53.3) | 7        | (46.7) | 0.632           | 40       | (54.1) | 19       | (54.3) | 0.982           | 42       | (53.2) | 15       | (51.7)  | 0.894           | 19       | (42.2) | 33       | (57.9) | 0.116           |
| Positive                  | 42       | (46.7) | 8        | (53.3) |                 | 34       | (45.9) | 16       | (45.7) |                 | 37       | (46.8) | 14       | (48.3)  |                 | 26       | (57.8) | 24       | (42.1) |                 |
| <i>Histological grade</i> |          |        |          |        |                 |          |        |          |        |                 |          |        |          |         |                 |          |        |          |        |                 |
| G1                        | 0        | (0.0)  | 0        | (0.0)  | —               | 0        | (0.0)  | 1        | (2.8)  | 0.016           | 0        | (0.0)  | 0        | (0.0)   | —               | 0        | (0.0)  | 1        | (1.7)  | 0.403           |
| G2                        | 13       | (14.1) | 5        | (31.2) |                 | 9        | (11.8) | 11       | (30.6) |                 | 18       | (21.7) | 3        | (10.3)  |                 | 7        | (15.6) | 15       | (23.3) |                 |
| G3                        | 79       | (85.9) | 11       | (68.8) |                 | 67       | (88.2) | 24       | (66.7) |                 | 65       | (78.3) | 26       | (89.7)  |                 | 38       | (84.4) | 45       | (75.0) |                 |
| <i>Ki67</i>               |          |        |          |        |                 |          |        |          |        |                 |          |        |          |         |                 |          |        |          |        |                 |
| Negative (≤ 20%)          | 6        | (6.7)  | 1        | (6.2)  | 0.951           | 5        | (6.8)  | 6        | (16.2) | 0.116           | 11       | (13.4) | 0        | (0.0)   | 0.041           | 4        | (8.7)  | 6        | (10.3) | 0.777           |
| Positive (> 20%)          | 84       | (93.3) | 15       | (93.8) |                 | 69       | (93.2) | 31       | (83.8) |                 | 71       | (86.6) | 28       | (100.0) |                 | 42       | (91.3) | 52       | (89.7) |                 |

*continued*

IDC: Invasive Ductal Carcinoma; ILC: Invasive Lobular Carcinoma; AR: Androgen Receptor; FOXA1: Forkhead box A1; PD-L1: programmed cell death ligand-1; BRCA1: breast cancer susceptibility protein 1; PARP1: Poly [ADP-Ribose] Polymerase 1; Tumor-infiltrating lymphocytes (TILs); mNHERF1: membranous Na<sup>+</sup>/H<sup>+</sup> Exchanger Regulatory Factor 1; cNHERF1: cytoplasmic NHERF1; nNHERF1: nuclear NHERF1.

**Table S1b.** Relationship between tumor markers expression and clinicopathological features.

| Characteristics    | PARP1    |        |          |         | p-value | mNHERF1  |        |          |        | p-value | cNHERF1  |        |          |        | p-value | nNHERF1  |        |          |        | p-value |
|--------------------|----------|--------|----------|---------|---------|----------|--------|----------|--------|---------|----------|--------|----------|--------|---------|----------|--------|----------|--------|---------|
|                    | Negative |        | Positive |         |         | Negative |        | Positive |        |         | Negative |        | Positive |        |         | Negative |        | Positive |        |         |
|                    | n        | (%)    | n        | (%)     |         | n        | (%)    | n        | (%)    |         | n        | (%)    | n        | (%)    |         | n        | (%)    | n        | (%)    |         |
| Patient age        |          |        |          |         |         |          |        |          |        |         |          |        |          |        |         |          |        |          |        |         |
| ≤51 years          | 43       | (49.4) | 12       | (60.0)  | 0.394   | 40       | (52.6) | 24       | (57.1) | 0.638   | 30       | (52.6) | 34       | (55.7) | 0.735   | 59       | (60.2) | 5        | (25.0) | 0.004   |
| >51 years          | 44       | (50.6) | 8        | (40.0)  |         | 36       | (47.4) | 18       | (42.9) |         | 27       | (47.4) | 27       | (44.3) |         | 39       | (39.8) | 15       | (75.0) |         |
| Pre/Post           |          |        |          |         |         |          |        |          |        |         |          |        |          |        |         |          |        |          |        |         |
| Pre                | 57       | (65.5) | 13       | (65.0)  | 0.965   | 53       | (69.7) | 27       | (64.3) | 0.544   | 39       | (68.4) | 41       | (67.2) | 0.888   | 72       | (73.5) | 8        | (40.0) | 0.004   |
| Post               | 30       | (34.5) | 7        | (35.0)  |         | 23       | (30.3) | 15       | (35.7) |         | 18       | (31.6) | 20       | (32.8) |         | 26       | (26.5) | 12       | (60.0) |         |
| Histological type  |          |        |          |         |         |          |        |          |        |         |          |        |          |        |         |          |        |          |        |         |
| IDC                | 81       | (93.1) | 17       | (85.0)  | 0.097   | 67       | (88.2) | 39       | (92.9) | 0.679   | 52       | (91.2) | 53       | (86.9) | 0.646   | 89       | (90.8) | 17       | (85.0) | 0.303   |
| ILC                | 3        | (3.4)  | 0        | (0.0)   |         | 2        | (2.6)  | 1        | (2.4)  |         | 2        | (3.5)  | 2        | (3.3)  |         | 3        | (3.1)  | 0        | (0.0)  |         |
| Other              | 3        | (3.4)  | 3        | (15.0)  |         | 7        | (9.2)  | 2        | (4.8)  |         | 3        | (5.3)  | 6        | (9.8)  |         | 6        | (6.1)  | 3        | (15.0) |         |
| Tumor size (cm)    |          |        |          |         |         |          |        |          |        |         |          |        |          |        |         |          |        |          |        |         |
| ≤2 cm              | 40       | (46.5) | 10       | (50.0)  | 0.778   | 36       | (48.0) | 19       | (45.2) | 0.774   | 31       | (55.4) | 24       | (39.3) | 0.617   | 46       | (47.4) | 9        | (45.0) | 0.843   |
| >2 cm              | 46       | (53.5) | 10       | (50.0)  |         | 39       | (52.0) | 23       | (54.8) |         | 25       | (44.6) | 37       | (60.7) |         | 51       | (52.6) | 11       | (55.0) |         |
| Lymph node status  |          |        |          |         |         |          |        |          |        |         |          |        |          |        |         |          |        |          |        |         |
| Negative           | 39       | (46.4) | 12       | (63.2)  | 0.188   | 43       | (57.3) | 19       | (47.5) | 0.314   | 31       | (56.4) | 31       | (51.7) | 0.083   | 50       | (52.1) | 12       | (63.2) | 0.376   |
| Positive           | 45       | (53.6) | 7        | (36.8)  |         | 32       | (42.7) | 21       | (52.5) |         | 24       | (43.6) | 29       | (48.3) |         | 46       | (47.9) | 7        | (36.8) |         |
| Histological grade |          |        |          |         |         |          |        |          |        |         |          |        |          |        |         |          |        |          |        |         |
| G1                 | 1        | (1.2)  | 0        | (0.0)   | 0.155   | 0        | (0.0)  | 1        | (2.4)  | 0.701   | 0        | (0.0)  | 1        | (1.7)  | 0.614   | 1        | (1.0)  | 0        | (0.0)  | 0.539   |
| G2                 | 20       | (23.3) | 1        | (5.0)   |         | 14       | (18.4) | 6        | (14.6) |         | 10       | (17.5) | 10       | (16.7) |         | 15       | (15.5) | 5        | (25.0) |         |
| G3                 | 65       | (75.6) | 19       | (95.0)  |         | 62       | (81.6) | 34       | (82.9) |         | 47       | (82.5) | 49       | (81.7) |         | 81       | (83.5) | 15       | (75.0) |         |
| Ki67               |          |        |          |         |         |          |        |          |        |         |          |        |          |        |         |          |        |          |        |         |
| Negative (≤20%)    | 11       | (12.9) | 0        | (0.0)   | 0.089   | 6        | (7.9)  | 4        | (10.0) | 0.701   | 5        | (8.8)  | 5        | (8.5)  | 0.955   | 8        | (8.3)  | 2        | (10.0) | 0.809   |
| Positive (>20%)    | 74       | (87.1) | 20       | (100.0) |         | 70       | (92.1) | 36       | (90.0) |         | 52       | (91.2) | 54       | (91.5) |         | 88       | (91.7) | 184      | (90.0) |         |

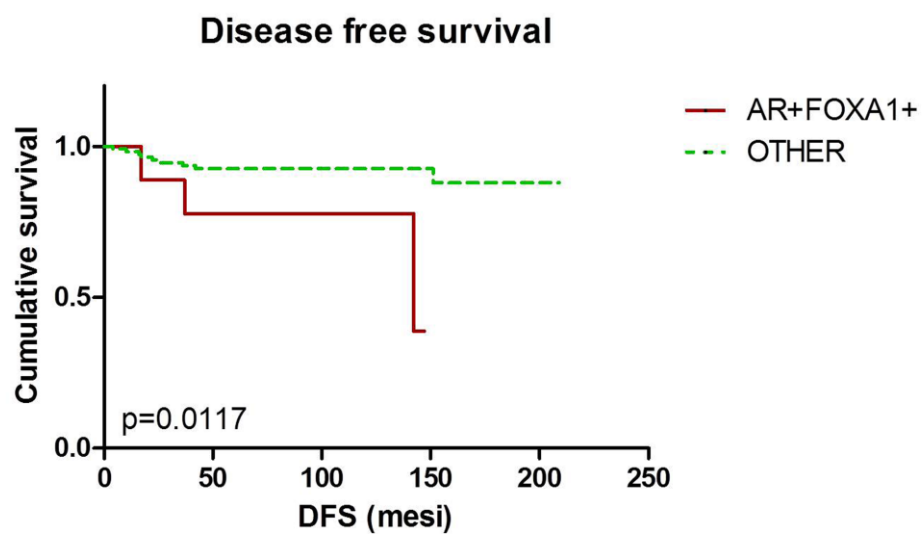
IDC: Invasive Ductal Carcinoma; ILC: Invasive Lobular Carcinoma; AR: Androgen Receptor; FOXA1: Forkhead box A1; PD-L1: programmed cell death ligand-1; BRCA1: breast cancer susceptibility protein 1; PARP1: Poly [ADP-Ribose] Polymerase 1; Tumor-infiltrating lymphocytes (TILs); mNHERF1: membranous Na<sup>+</sup>/H<sup>+</sup> Exchanger Regulatory Factor 1; cNHERF1: cytoplasmic NHERF1; nNHERF1: nuclear NHERF1.

**Table S2.** Dilution, source, staining of antibodies and cut off used.

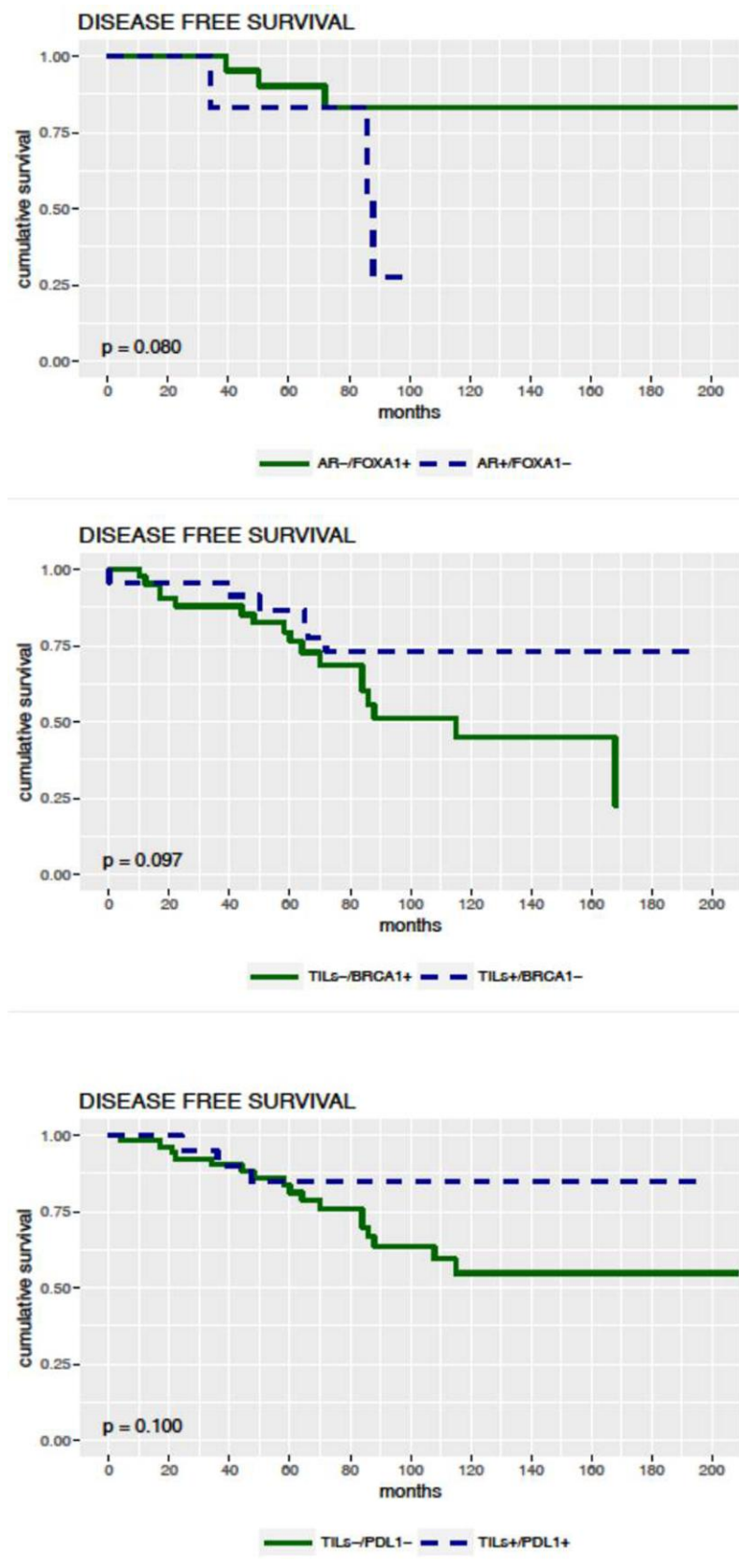
| Biomarkers | Dilution | Source/clone                                           | Staining Localization | Cut off (range) |          |
|------------|----------|--------------------------------------------------------|-----------------------|-----------------|----------|
| NHERF1     | 1:150    | Affinity Bioreagents, rabbit polyclonal EBP50, PA1-090 | membrane              | >0% *           | (0–90%)  |
|            |          |                                                        | cytoplasmic           | ≥70% *          | (0–100%) |
|            |          |                                                        | nuclear               | >0% *           | (0–70%)  |
| BRCA1      | 1:75     | Calbiochem, mouse monoclonal anti-BRCA1, MS110         | nuclear               | ≥3% *           | (0–90%)  |
| PARP1      | 1:100    | Santa Cruz, mouse monoclonal, F-2 nuclear              | nuclear               | ≥10 ** (0–      | (0–18)   |

\* median value; \*\* quick score method. NHERF1: Na<sup>+</sup>/H<sup>+</sup> Exchanger Regulatory Factor 1; BRCA1: breast cancer susceptibility protein 1; PARP1: Poly [ADP-Ribose] Polymerase

1.



**Figure S1.** Survival analysis. DFS curves for patients with simultaneously AR+/FOXA1+ phenotype respect to all other tumors ( $p = 0.0117$ ).



**Figure S2.** Survival analyses. DFS curves for patients with simultaneously AR-/FOXA1+ vs AR+/FOXA1- ( $p = 0.080$ ). TILs-/BRCA1+ vs to TILs+/BRCA1- ( $p = 0.097$ ) and

Table 1. Vs TILs-/PD-L1- ( $p = 0.100$ ) expression.