Lack of both androgen receptor and forkhead box A1 (FOXA1) expression is a poor prognostic factor in estrogen receptor-positive breast cancers

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: Genomic expression on the eBioPortal website. (A) Genomic alteration is presented by the OncoPrint using the METABRIC dataset. Associations of mRNA expression are shown with correlation coefficients (B) between ESR1 and AR, (C) between ESR1 and FOXA1, and (D) between AR and FOXA1.

Supplementary Figure 2: Survival curves according to AR and FOXA1 status in all patients of the TMA study. (A) Disease-free survival and (B) Overall survival curves are shown according to immunohistochemical AR and FOXA1 status.
Supplementary Figure 3: Survival curves according to AR and FOXA1 status in all patients of the METABRIC dataset. (A) Disease-free survival and (B) Overall survival curves are presented according to AR and FOXA1 mRNA levels of the METABRIC dataset from the cBioPortal for Cancer Genomics (http://www.cbioportal.org).

Supplementary Figure 4: Relapse-free survival curves stratified by the intrinsic subtype in the KM Plotter. Plots are generated according to AR and FOXA1 levels in each (A) luminal A, (B) luminal B, (C) HER2-enriched, and (D) basal subtype from the KM Plotter.
Supplementary Table 1: Clinicopathological characteristics according to AR and FOXA1 expression in the whole population of the TMA study

| Factor                              | AR(+)/ FOXA1(+) (n = 384, %) | AR(+)/ FOXA1(-) (n = 99, %) | AR(-)/ FOXA1(+) (n = 240, %) | AR(-)/ FOXA1(-) (n = 143, %) | p-value |
|-------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------|
| Age (yrs)                           |                             |                             |                             |                             |         |
| ≤ 50                                | 241 (62.8)                  | 53 (53.5)                   | 162 (67.5)                  | 91 (63.6)                   | 0.115   |
| > 50                                | 143 (37.2)                  | 46 (46.5)                   | 78 (32.5)                   | 52 (36.4)                   |         |
| Type                                |                             |                             |                             |                             |         |
| Ductal                              | 347 (90.4)                  | 93 (93.9)                   | 207 (86.2)                  | 129 (90.2)                  | 0.158   |
| Lobular/ special                    | 37 (9.6)                    | 6 (6.1)                     | 33 (13.8)                   | 14 (9.8)                    |         |
| Tumor stage                         |                             |                             |                             |                             |         |
| pT1                                 | 207 (53.9)                  | 41 (41.4)                   | 88 (36.7)                   | 50 (35.0)                   | < 0.001 |
| pT2-4                               | 177 (46.1)                  | 58 (58.6)                   | 152 (63.3)                  | 93 (65.0)                   |         |
| Node stage                          |                             |                             |                             |                             |         |
| pN0                                 | 204 (53.1)                  | 46 (46.5)                   | 120 (50.0)                  | 66 (46.2)                   | 0.424   |
| pN1-3                               | 180 (46.9)                  | 53 (53.5)                   | 120 (50.0)                  | 77 (53.8)                   |         |
| Histologic grade                    |                             |                             |                             |                             |         |
| I/II                                | 325 (84.6)                  | 77 (77.8)                   | 145 (60.4)                  | 82 (57.3)                   | < 0.001 |
| III                                 | 59 (15.4)                   | 22 (22.2)                   | 95 (39.6)                   | 61 (42.7)                   |         |
| ER                                  |                             |                             |                             |                             |         |
| Positive                            | 356 (92.7)                  | 68 (68.7)                   | 141 (58.8)                  | 60 (42.0)                   | < 0.001 |
| Negative                            | 28 (7.3)                    | 31 (31.3)                   | 99 (41.2)                   | 83 (58.0)                   |         |
| PR                                  |                             |                             |                             |                             |         |
| Positive                            | 307 (79.9)                  | 61 (61.6)                   | 122 (50.8)                  | 49 (34.3)                   | < 0.001 |
| Negative                            | 77 (20.1)                   | 38 (38.4)                   | 118 (49.2)                  | 94 (65.7)                   |         |
| HER2                                |                             |                             |                             |                             |         |
| Negative                            | 293 (76.3)                  | 64 (64.6)                   | 180 (75.0)                  | 117 (81.8)                  | 0.023   |
| Positive                            | 91 (23.7)                   | 35 (35.4)                   | 60 (25.0)                   | 26 (18.2)                   |         |
| Ki-67 (n = 864)                     |                             |                             |                             |                             |         |
| < 15%                               | 345 (90.1)                  | 89 (90.8)                   | 156 (65.0)                  | 73 (51.0)                   | < 0.001 |
| ≥ 15%                               | 38 (9.9)                    | 9 (9.2)                     | 84 (35.0)                   | 70 (49.0)                   |         |
| Breast cancer subtypes (n = 864)    |                             |                             |                             |                             |         |
| Luminal A                           | 267 (69.7)                  | 52 (53.1)                   | 98 (40.8)                   | 42 (29.4)                   | < 0.001 |
| Luminal B                           | 89 (23.2)                   | 17 (17.3)                   | 53 (22.1)                   | 26 (18.2)                   |         |
| HER2-positive                       | 18 (4.7)                    | 20 (20.4)                   | 21 (8.8)                    | 16 (11.2)                   |         |
| TNBC                                | 9 (2.3)                     | 9 (9.2)                     | 68 (28.3)                   | 59 (41.3)                   |         |
| Type of surgery                     |                             |                             |                             |                             |         |
| BCS                                 | 116 (30.2)                  | 28 (28.3)                   | 68 (28.3)                   | 37 (25.9)                   | 0.800   |
| TM                                  | 268 (69.8)                  | 71 (71.7)                   | 172 (71.7)                  | 106 (74.1)                  |         |
| Radiation therapy                   |                             |                             |                             |                             |         |
| Not done                            | 201 (52.3)                  | 49 (49.5)                   | 125 (52.1)                  | 75 (52.4)                   | 0.964   |
| Done                                | 183 (47.7)                  | 50 (50.5)                   | 115 (47.9)                  | 68 (47.6)                   |         |
| Chemotherapy                        |                             |                             |                             |                             |         |
| Not done                            | 58 (15.1)                   | 16 (16.2)                   | 25 (10.4)                   | 15 (10.5)                   | 0.210   |
| Done                                | 326 (84.9)                  | 83 (83.8)                   | 215 (89.6)                  | 128 (89.5)                  |         |
| Endocrine therapy                   |                             |                             |                             |                             |         |
| Not done                            | 58 (15.1)                   | 37 (37.4)                   | 110 (45.8)                  | 87 (60.8)                   | < 0.001 |
| Done                                | 326 (84.9)                  | 62 (62.6)                   | 130 (54.2)                  | 56 (39.2)                   |         |

AR: androgen receptor, ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor 2, TNBC: triple-negative breast cancer, BCS: breast conservation surgery, TM: total mastectomy.
**Supplementary Table 2: Clinicopathological characteristics according to AR and FOXA1 mRNA status in the whole population of the METABRIC dataset**

| Factor                          | AR-high/ FOXA1-high (n, %) | AR-high/ FOXA1-low (n, %) | AR-low/ FOXA1-high (n, %) | AR-low/ FOXA1-low (n, %) | p-value |
|--------------------------------|-----------------------------|---------------------------|---------------------------|---------------------------|---------|
| Age (yrs)                      |                             |                           |                           |                           |         |
|  
| ≤ 50                           | 223 (17.1)                  | 38 (22.9)                 | 40 (23.8)                 | 119 (37.5)                | < 0.001 |
|  > 50                          | 1,080 (82.9)                | 131 (77.1)                | 128 (76.2)                | 198 (62.5)                |         |
| TNM stage                      |                             |                           |                           |                           |         |
|  
| Stage I                        | 403 (31.1)                  | 45 (26.9)                 | 67 (40.1)                 | 84 (27.0)                 | 0.004   |
| Stage II                       | 757 (58.5)                  | 92 (55.1)                 | 87 (52.1)                 | 186 (59.8)                |         |
| Stage III                      | 134 (10.4)                  | 30 (18.0)                 | 13 (7.8)                  | 41 (13.2)                 |         |
| Histologic grade               |                             |                           |                           |                           |         |
| I/II                           | 748 (60.1)                  | 49 (30.2)                 | 84 (50.9)                 | 52 (16.8)                 | < 0.001 |
| III                            | 496 (39.9)                  | 113 (69.8)                | 81 (49.1)                 | 257 (83.2)                |         |
| ER*                            |                             |                           |                           |                           |         |
| Positive                       | 1,168 (91.1)                | 97 (58.1)                 | 148 (89.2)                | 72 (23.4)                 | < 0.001 |
| Negative                       | 114 (8.9)                   | 70 (41.9)                 | 18 (10.8)                 | 236 (76.6)                |         |
| PR†                            |                             |                           |                           |                           |         |
| Positive                       | 865 (66.4)                  | 54 (31.8)                 | 86 (51.2)                 | 26 (8.2)                  | < 0.001 |
| Negative                       | 438 (33.6)                  | 116 (68.2)                | 82 (48.8)                 | 291 (91.8)                |         |
| HER2†                          |                             |                           |                           |                           |         |
| Positive                       | 1,148 (88.1)                | 125 (73.5)                | 146 (86.9)                | 297 (93.7)                | < 0.001 |
| Negative                       | 155 (11.9)                  | 45 (26.5)                 | 22 (13.1)                 | 20 (6.3)                  |         |
| PAM50 subtype                  |                             |                           |                           |                           |         |
| Luminal A                      | 632 (48.7)                  | 19 (11.3)                 | 60 (35.7)                 | 4 (1.3)                   | < 0.001 |
| Luminal B                      | 388 (29.9)                  | 20 (11.9)                 | 66 (39.5)                 | 6 (1.9)                   |         |
| HER2                           | 146 (11.2)                  | 45 (26.8)                 | 25 (14.9)                 | 22 (6.9)                  |         |
| Basal                          | 23 (1.8)                    | 45 (26.8)                 | 7 (4.2)                   | 250 (78.9)                |         |
| Normal                         | 110 (8.5)                   | 39 (23.2)                 | 10 (6.0)                  | 35 (11.0)                 |         |
| Type of surgery                |                             |                           |                           |                           |         |
| BCS                            | 499 (38.6)                  | 63 (38.2)                 | 74 (44.3)                 | 143 (46.3)                | 0.054   |
| TM                             | 795 (61.4)                  | 102 (61.8)                | 93 (55.7)                 | 166 (53.7)                |         |
| Radiotherapy                   |                             |                           |                           |                           |         |
| Not done                       | 576 (44.2)                  | 53 (31.2)                 | 68 (40.5)                 | 96 (30.3)                 | < 0.001 |
| Done                           | 727 (55.8)                  | 117 (68.8)                | 100 (59.5)                | 221 (69.7)                |         |
| Chemotherapy                   |                             |                           |                           |                           |         |
| Not done                       | 1,134 (87.0)                | 120 (70.6)                | 140 (83.3)                | 155 (48.9)                | < 0.001 |
| Done                           | 169 (13.0)                  | 50 (29.4)                 | 28 (16.7)                 | 162 (51.1)                |         |
| Hormone therapy                |                             |                           |                           |                           |         |
| Not done                       | 409 (31.4)                  | 80 (47.1)                 | 53 (31.5)                 | 208 (65.6)                | < 0.001 |
| Done                           | 894 (68.6)                  | 90 (52.9)                 | 115 (68.5)                | 109 (34.4)                |         |

*Positive criteria of ER was measured by immunohistochemistry in the METABRIC dataset.
†Positive criteria of PR and HER2 were defined as expression status in the METABRIC dataset.
Supplementary Table 3: Primers used for real-time reverse transcription-polymerase chain reaction (RT-PCR)

| Gene symbol | GenBank Accession No. | Sequence               |
|-------------|-----------------------|------------------------|
| ESR1        | NM_000125             | FW: ATGACTATGCTTCAGGCTACCATT  |
|             |                       | RV: GTGGCTGGACACATATAGTCGTTA  |
| AR          | NM_000044             | FW: CGACCAGATGGCTGTCATTTC  |
|             |                       | RV: TGTGCATGCGGTACTCATTG  |
| FOXA1       | NM_004496             | FW: ACTCCTTCAACCACCCGTTC  |
|             |                       | RV: GCGAGTATTGCGTCCTGT  |