Supplementary Material: Automated quantification of extranuclear ERα using phosphor-integrated dots for predicting endocrine therapy resistance in HR+/HER2- breast cancer

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Figure S1. Verification of transfection with GFP-ERα or GFP-ERαΔNLS expression plasmids. Relative copy number of ERα mRNA expression level for HeLa cells, GFP-ERα plasmids-transfected HeLa cells, GFP-ERαΔNLS plasmids-transfected HeLa cells.

Figure S2. H-scores for the assessment of ER and PR are highly reproducible. The H-scores of randomly selected 34 cases were assessed with the Aperio image analysis system, which has a strong correlation with manual scoring evaluation by an expert pathologist. Pearson coefficients $r^2=0.67$ (ER, A) and 0.82 (PR, B).
Figure S3. Kaplan-Meier analysis of DFS according to ERα expression. Kaplan-Meier analysis of DFS according to the cut-off values of the ER Allred score (A), ER H-score (B), total ERα PID score (C), extranuclear ERα PID score (D), Ki67 LI (E). P values were determined with the log-rank test.

Figure S4. A comparison of ENR between EDR (estrogen-deprivation-resistant) cell and MCF-7 cell (p = 0.066)
**Supplementary Table S1. Associations between ERα ENR expression and clinicopathological characteristics**

| Characteristics          | No. (%) of Patients |   | P value |
|--------------------------|---------------------|---|---------|
|                          | ERα ENR<0.5 (n=30)  | ERα ENR≥0.5 (n=35) |         |
| Menopausal status        |                     |               |         |
| pre                      | 7 (11)              | 21 (32)       | 0.673   |
| post                     | 11 (17)             | 26 (40)       |         |
| Lymph node status        |                     |               |         |
| N0                       | 12 (18)             | 26 (40)       |         |
| N≥1                      | 6 (9)               | 21 (32)       | 0.406   |
| Stage                    |                     |               |         |
| I                        | 9 (14)              | 20 (31)       |         |
| II                       | 8 (12)              | 21 (32)       |         |
| III                      | 1 (2)               | 6 (9)         | 0.674   |
| Histological grade       |                     |               |         |
| 1                        | 6 (9)               | 14 (22)       |         |
| 2                        | 9 (14)              | 25 (38)       |         |
| 3                        | 3 (5)               | 8 (12)        | 0.961   |
| Ki67 LI (%)              |                     |               |         |
| <14                      | 13 (20)             | 32 (49)       |         |
| ≥14                      | 5 (8)               | 15 (23)       | 0.746   |
| ER H-score               |                     |               |         |
| <110                     | 2 (3)               | 20 (31)       |         |
| ≥110                     | 16 (25)             | 27 (41)       | 0.017   |
| PR H-score               |                     |               |         |
| <100                     | 11 (17)             | 25 (38)       |         |
| ≥100                     | 7 (11)              | 22 (34)       | 0.565   |

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