Additional file 1. IHC-staining protocols used in the current study for demonstrating HER3, NEDD4-1, NRDP1, and Cytokeratin 5/14 protein expression on FFPE breast cancer tissue (LabVision Autostainer platform).

<table>
<thead>
<tr>
<th>HER3 IHC</th>
<th>NEDD4-1 IHC</th>
<th>NRDP1 IHC</th>
<th>Cytokeratin 5/14 IHC</th>
</tr>
</thead>
</table>
| **Pretreatments**: deparaffinization, HIER in Tris-EDTA (TE) buffer pH 9+8°C 15 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 1**: Protein Blocking1 5 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 2**: HER3 primary antibody2 1:100 30 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 3**: Post-antibody blocking3 20 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 4**: HRP-conjugated secondary antibody4 30 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 5**: 3,3'-Diaminobenzidine (DAB)5 5 min  
Rinse with distilled water × 1 and TBS-Tween (0.05%) × 2  
**Step 6**: Mayer’s Hematoxylin 2 min  
Rinse with distilled water × 1 and TBS-Tween (0.05%) × 1 | **Pretreatments**: deparaffinization, HIER in Tris-EDTA (TE) buffer pH 9+8°C 15 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 1**: 3% Hydrogen Peroxide (H₂O₂) 5 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 2**: NEDD4-1 primary antibody4 1:750 30 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 3**: Post antibody blocking2 20 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 4**: HRP-conjugated secondary antibody4 30 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 5**: 3,3'-Diaminobenzidine (DAB)4 5 min  
Rinse with distilled water × 2 and TBS-Tween (0.05%) × 1  
**Step 6**: Mayer’s Hematoxylin 5 min  
Rinse with distilled water × 1 and TBS-Tween (0.05%) × 1 | **Pretreatments**: deparaffinization, HIER in Tris-EDTA (TE) buffer pH 9+8°C 20 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 1**: FLRF/RNF41 primary antibody4 1:3000 30 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 2**: 3% Hydrogen Peroxide (H₂O₂) 5 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 3**: HRP-conjugated secondary antibody2 30 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 4**: 3,3'-Diaminobenzidine (DAB)3 10 min  
Rinse with distilled water × 1 and TBS-Tween (0.05%) × 1  
**Step 5**: 0.5% Copper sulfate (CuSO₄) 30 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 6**: Mayer’s Hematoxylin 1:2  
Rinse with distilled water × 1 and TBS-Tween (0.05%) × 1 | **Pretreatments**: deparaffinization, HIER in Tris-EDTA (TE) buffer pH 9+8°C 15 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 1**: 3% Hydrogen Peroxide (H₂O₂) 5 min  
Rinse with TBS-Tween (0.05%) × 1  
**Step 2**: CK 5/14 primary antibody4 cocktail 1:150 30 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 3**: Post antibody blocking2 20 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 4**: HRP-conjugated secondary antibody4 30 min  
Rinse with TBS-Tween (0.05%) × 2  
**Step 5**: 3,3'-Diaminobenzidine (DAB)4 5 min  
Rinse with distilled water × 2 and TBS-Tween (0.05%) × 1  
**Step 6**: Mayer’s Hematoxylin 2 min  
Rinse with distilled water × 1 and TBS-Tween (0.05%) × 1 |

Additional reagent information:
1) Ultra Vision Protein Block (LabVision),  
2) Mouse monoclonal HER3 antibody DAK-H3-IC (Dako, M7297) diluted in Normal Antibody Diluent (ImmunoLogic, BD09-999),  
3) BrightVision Plus Post-antibody blocking  
4) BrightVision Plus Poly-HRP-Anti Ms/Rb IgG (ImmunoLogic, DPVB110 HRP),  
5) ImmPACT™ DAB Peroxidase Substrate, dilution: 25 µl of Chromogen concentrate added to each 1 ml of DAB Diluent (Vector Laboratories Inc., SK-4105)  
6) Rabbit polyclonal Anti-Nedd4 antibody (Merck KGA, #07-049) diluted in Normal Antibody Diluent (ImmunoLogic, BD09-999),  
7) BrightVision Plus Post-antibody blocking,  
8) Histofine® DAB-2V, dilution: 40 µl Chromogen reagent added to each 1 ml of Chromogen substrate (Nichirei Biosciences Inc., 425312F)  
9) Rabbit polyclonal FLRF/RNF41 antibody (Bethyl Laboratories Inc., A300-049A) diluted in Normal Antibody Diluent (ImmunoLogic, BD09-999),  
10) EnVision™ FLEX High pH HRP,  
11) EnVision™ FLEX DAB+, dilution: 50 µl DAB+ Chromogen added to each 1 ml of DAB+ Substrate Buffer (Dako, K8010)  
12) Mouse monoclonal antibodies for CK5, clone NCL-L-CKS (Leica Biosystems, XM26), and CK14, clone NCL-L-0022 (Leica Biosystems, L0022), diluted in Normal Antibody Diluent (ImmunoLogic, BD09-999),  
13) BrightVision Plus Post-antibody blocking,  
14) ImmPACT™ DAB Peroxidase Substrate, dilution: 25 µl of Chromogen concentrate added to each 1 ml of DAB Diluent (Vector Laboratories Inc., SK-4105)