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).

HER3 IHC	NEDD4-1 IHC	NRDP1 IHC	Cytokeratin 5/14 IHC
Pretreatments: deparaffinization, HIER in Tris-EDTA (TE) buffer pH9 +98°C 15 min Rinse with TBS-Tween (0.05%) × 1 **Step 1**: *Protein Blocking*[1] 5 min Rinse with TBS-Tween (0.05%) × 1 **Step 2**: *HER3 primary antibody*[2] 1:100 30 min Rinse with TBS-Tween (0.05%) × 2 **Step 3**: *Post-antibody blocking*[3] 20 min Rinse with TBS-Tween (0.05%) × 2 **Step 4**: *HRP-conjugated secondary antibody*[4] 30 min Rinse with TBS-Tween (0.05%) × 2 **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 pH9 +98°C 15 min Rinse with TBS-Tween (0.05%) × 1 **Step 1**: *3% Hydrogen Peroxide (H_2O_2)* 5 min Rinse with TBS-Tween (0.05%) × 1 **Step 2**: *NEDD4-1 primary antibody*[1] 1:750 30 min Rinse with TBS-Tween (0.05%) × 2 **Step 3**: *Post antibody blocking*[2] 20 min Rinse with TBS-Tween (0.05%) × 2 **Step 4:** *HRP-conjugated secondary antibody*[3] 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**: *0.5% Copper sulfate ($CuSO_4$)* added as enhancement in *Mayer's Hematoxylin* 5 min Rinse with distilled water × 1 and TBS-Tween (0.05%) × 1	**Pretreatments**: deparaffinization, HIER in Tris-EDTA (TE) buffer pH9 +98°C 20 min Rinse with TBS-Tween (0.05%) × 1 **Step 1**: *FLRF/RNF41 primary antibody*[1] 1:3000 30 min Rinse with TBS-Tween (0.05%) × 2 **Step 2**: *3% Hydrogen Peroxide (H_2O_2)* 5 min Rinse with TBS-Tween (0.05%) × 2 **Step 3**: *HRP-conjugated secondary antibody*[2] 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 ($CuSO_4$)* enhancement 5 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 pH9 +98°C 15 min Rinse with TBS-Tween (0.05%) × 1 **Step 1**: *3% Hydrogen Peroxide (H_2O_2)* 5 min Rinse with TBS-Tween (0.05%) × 1 **Step 2**: *CK 5/14 primary antibody*[1] *cocktail* 1:150 30 min Rinse with TBS-Tween (0.05%) × 2 **Step 3**: *Post antibody blocking*[2] 20 min Rinse with TBS-Tween (0.05%) × 2 **Step 4:** *HRP-conjugated secondary antibody*[3] 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)	*Additional reagent information*: [1)] Rabbit polyclonal Anti-Nedd4 antibody (Merck KGaA, #07-049) diluted in Normal Antibody Diluent (ImmunoLogic, BD09-999), [2)] BrightVision Plus Post-antibody blocking, [3)] BrightVision Plus Poly-HRP-Anti Ms/Rb IgG (ImmunoLogic, DPVB110 HRP), [4)] Histofine® DAB-2V, dilution: 40 µl Chromogen reagent added to each 1 ml of Chromogen substrate (Nichirei Biosciences Inc., 425312F)	*Additional reagent information*: [1)] Rabbit polyclonal FLRF/RNF41 antibody (Bethyl Laboratories Inc., A300-049A) diluted in Normal Antibody Diluent (ImmunoLogic, BD09-999), [2)] EnVision™ FLEX High pH HRP, [3)] EnVision™ FLEX DAB+, dilution: 50 µl DAB+ Chromogen added to each 1 ml of DAB+ Substrate Buffer (Dako, K8010)	*Additional reagent information*: [1)] Mouse monoclonal antibodies for CK5, clone NCL-L-CK5 (Leica Biosystems, XM26), and CK14, clone NCL-L-LL0022 (Leica Biosystems, LL0022), diluted in Normal Antibody Diluent (ImmunoLogic, BD09-999), [2)] BrightVision Plus Post-antibody blocking, [3)] BrightVision Plus Poly-HRP-Anti Ms/Rb IgG (ImmunoLogic, DPVB110 HRP), [4)] ImmPACT™ DAB Peroxidase Substrate, dilution: 25 µl of Chromogen concentrate added to each 1 ml of DAB Diluent (Vector Laboratories Inc., SK-4105)