Inter-observer agreement for the histological diagnosis of invasive lobular breast cancer

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Figure S2. CDH1 mutations. Lollipop plot of CDH1 mutations detected in the study collection. Blue circles correspond to frameshift mutations, red circles correspond to nonsense mutations, green circles correspond to missense mutations and black circles correspond to mutations affecting splicing sites. Assignment of the corresponding BCs to either ILC or NST/non-lobular BC by participants and the reference is shown on the top. The E-cadherin protein expression status of the corresponding specimens is also shown on the top.
Figure S3. Alternative visualization of BC subtype calls. Cases from set A and set B were re-ordered from left to right, according to increasing participant calls for ILC. Next, percent participant calls for ILC (red), mixed BC NST/ILC (gray), or NST/non-lobular BC was plotted on the y-axis and linked with a connecting line. Please note that in set A just one specimen received 100% participant calls for ILC (upper right corner in the first plot).
Figure S4. E-cadherin status calls. (A) Thirty-five experienced pathologists independently classified the E-cadherin IHC status in n=62 BC specimens based on centrally stained sections (set B). Tumor characteristics, as defined by the reference standard are shown in the top panel. A two-dimensional presentation of participant calls is shown in the lower panel. (B) Inter-observer agreement (left), agreement with the reference standard for a positive, aberrant or negative E-cadherin status (middle), and agreement with the reference standard for positive or aberrant/negative E-cadherin status (negative and aberrant grouped together) expressed as accuracy [%]. Data are presented as traditional Tukey plots. Horizontal lines indicate the median, boxes indicate the interquartile range (IQR), whiskers indicate the 1.5-fold interquartile distance, or the minimal/maximal values, whichever is shorter.
Figure S5. Lobular carcinoma in situ (LCIS) in cases B056 and B062. Representative photomicrographs of HE- and IHC-stained sections at x200 magnification. Scale bars correspond to 200 µm. B056 represents an example of LCIS with pagetoid extension in a mammary duct. LCIS cells (E-cadherin-negative) undermine the luminal epithelial cell layer (E-cadherin-positive). B062 showed the unusual constellation of an E-cadherin-negative LCIS and an E-cadherin-positive ILC with dissociated and single file growth pattern (for details see text). The photomicrographs of the IHC stainings for beta-catenin and p120-catenin were taken from a different LCIS focus, because the lobule depicted in the E-cadherin staining was not represented on the sections of the beta-catenin and p120-catenin staining.
**Figure S6.** Variable growth pattern in case B056. Representative photomicrographs of HE-stained sections at x5 (center) and x200 (periphery) magnification. Areas with classic growth pattern (single files, dissociated growth) accounted for approximately 80-85%. Areas with tubular elements accounted for approximately 15-20% of the invasive carcinoma. The region with pagetoid LCIS (green, R7) is shown in detail in Supplemental Data Figure 5.
Figure S7. Histology of ILCs classified as NST in set A. (A) Pie charts showing proportional subtype calls for cases A040, A011, and A036. Case IDs and BC subtypes according to the reference standard are given on top. (B) Representative photomicrographs of HE-stained sections and E-cadherin IHC at x200 magnification. Scale bars correspond to 200 µm. Insets fitted over E-cadherin IHC stainings indicate the CDH1 mutation status. (C and D) Participant calls for ILC in BCs that were classified as ILC with predominantly dissociated growth pattern (diss.), single file growth pattern (s.files), or trabecular growth pattern (trab.) by the reference (ref.). Data are presented as traditional Tukey plots. Horizontal lines indicate the median, boxes indicate the interquartile range (IQR). Statistical significance was determined with the Kruskal-Wallis test.
Table S1. Antibodies and IHC scoring methods

| antigen      | antibody     | source                      | dilution          | antigenic retrieval | detection system          | scoring               | cutoff               |
|--------------|--------------|-----------------------------|-------------------|---------------------|---------------------------|-----------------------|----------------------|
| ER           | clone SP1    | Ventana                     | undiluted, ready-to-use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semi quantitative, 0-100 | 0-5, neg.; 10-100, pos. |
| PR           | clone 1E2    | Ventana                     | undiluted, ready-to-use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semi quantitative, 0-100 | 0-5, neg.; 10-100, pos. |
| HER2         | clone 4B     | Ventana                     | undiluted, ready-to-use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | 0, 1+, 2+, 3+          | 0-1, neg.; 2+, equivocal; 3+, positive |
| Ki67         | clone 30-9   | Ventana                     | undiluted, ready-to-use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semi quantitative, 0-100 | n.a.                 |
| CKS/14       | XM26+LL002   | Diagnostic BioSystems       | 1:200             | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | i) completely neg.; ii) focally pos. (< approx. 25% cells); iii) positive (> approx. 25% cells) | n.a.                 |
| E-cadherin   | clone ECH-6  | Zytomed                     | 1:100             | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | Remmele IRS           | 0-1; negative 2-12, positive |
| beta-catenin | clone 14     | BD Transduction Laboratories | 1:75              | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | i) completely neg.; ii) focally pos. (< approx. 75% cells); iii) positive (> approx. 75% cells) | n.a.                 |
| p120-catenin (expression) | clone 98 | BD Transduction Laboratories | 1:250             | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | Remmele IRS           | 0-2, neg.; 3-12, pos. |
| p120-catenin (misloc.) | clone 98 | BD Transduction Laboratories | 1:250             | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | i) normal (membranous); ii) aberrant (nuclear or cytosolic); iii) non-informative (low/neg. expression) | n.a.                 |
| P-cadherin   | clone 56     | BD Transduction Laboratories | 1:100             | CC1 mild protocol (Ventana) | CC1 mild protocol (Ventana) | i) completely neg.; ii) focally pos. (< approx. 75% cells); iii) positive (> approx. 75% cells) | n.a.                 |
Table S2. Tumor characteristics of NST/non-lobular BC, as defined by the reference standard, are balanced between set A and B.

|        | reference       | all cases | set A  | set B   | test | P value |
|--------|----------------|-----------|--------|---------|------|---------|
|        |                 | n    | %      | n    | %      | n    | %      |
| all NST/nonlobular BC cases | 81   | 100  | 40    | 49    | 41   | 51     |
| grade  |                 |       |       |       |      |        |
|        | G1  | 3    | 100   | 1    | 33    | 2    | 67     | CSTT 0.459 |
|        | G2  | 47   | 100   | 26   | 55    | 21   | 45     |
|        | G3  | 31   | 100   | 13   | 42    | 18   | 58     |
| mBSR: architecture | 1   | 0    | 0     | 0    | 0     | 0    | 0      | CSTT 0.286 |
|        | 2   | 22   | 100   | 13   | 59    | 9    | 41     |
|        | 3   | 59   | 100   | 13   | 42    | 18   | 58     |
| mBSR: nuc. grade | 1   | 0    | 0     | 0    | 0     | 0    | 0      | CSTT 0.904 |
|        | 2   | 44   | 100   | 22   | 50    | 22   | 50     |
|        | 3   | 37   | 100   | 18   | 49    | 19   | 51     |
| mBSR: proliferation | 1   | 12   | 100   | 8    | 67    | 4    | 33     | CSTT 0.357 |
|        | 2   | 52   | 100   | 24   | 46    | 28   | 54     |
|        | 3   | 17   | 100   | 8    | 47    | 9    | 53     |
| ER     | neg. | 1    | 100   | 1    | 100   | 0    | 0      | FET 0.488 |
|        | pos. | 79   | 100   | 38   | 48    | 41   | 52     |
|        | n.a. | 1    | 100   | 1    | 100   | 0    | 0      |
| PR     | neg. | 7    | 100   | 3    | 43    | 4    | 57     | FET 1.000 |
|        | pos. | 74   | 100   | 37   | 50    | 37   | 50     |
| HER2   | 0/1+ | 62   | 100   | 29   | 47    | 33   | 53     | CSTT 0.576 |
|        | 2+/-neg. | 16   | 100   | 10   | 63    | 6    | 37     |
|        | 2+/F. n.a. | 2    | 100   | 1    | 50    | 1    | 50     |
|        | 3+, 2+/F.-pos. | 1    | 100   | 0    | 0     | 1    | 100    |
| Ki67   | <10% | 4    | 100   | 3    | 75    | 1    | 25     | CSTT 0.725 |
|        | 10-19% | 19   | 100   | 8    | 42    | 11   | 58     |
|        | 20-34% | 49   | 100   | 25   | 51    | 24   | 49     |
|        | 35-100% | 9    | 100   | 4    | 44    | 5    | 56     |
| E-cadherin | neg. | 1    | 100   | 0    | 0     | 1    | 100    | FET 1.000 |
|        | aberrant | 0    | 0     | 0    | 0     | 0    | 0      |
|        | pos.   | 80   | 100   | 40   | 50    | 40   | 50     |
| CDH1 status | wild-type | 80   | 100   | 40   | 50    | 40   | 50     | FET 1.000 |
|        | mutant | 1    | 100   | 0    | 0     | 1    | 100    |
| beta-catenin | neg. | 0    | 0     | 0    | 0     | 0    | 0      | CSTT 0.320 |
|        | focally pos. | 1    | 100   | 0    | 0     | 1    | 100    |
|        | pos.   | 80   | 100   | 40   | 50    | 40   | 50     |
| p120-catenin | low.neg. | 4    | 100   | 1    | 25    | 3    | 75     | FET 0.616 |
|        | pos.   | 77   | 100   | 39   | 51    | 38   | 49     |
| p120-catenin mislocation | membranous | 75   | 100   | 39   | 52    | 36   | 48     | FET 0.240 |
|        | mislocated | 2    | 100   | 0    | 0     | 2    | 100    |
|        | not informative | 4    | 100   | 1    | 25    | 3    | 75     |
| P-cadherin | neg. | 67   | 100   | 34   | 51    | 33   | 49     | FET 0.770 |
|        | focally pos. | 14   | 100   | 6    | 43    | 8    | 57     |
|        | pos.   | 0    | 0     | 0    | 0     | 0    | 0      |

FET; Fisher’s exact test (set A versus set B), CSTT; Chi square test for trends (set A versus set B), pos.; positive, neg.; negative.
Table S3. Tumor characteristics of ILCs, as defined by the reference standard, are balanced between set A and B

|                  | reference | all cases | set A | set B | test | P value |
|------------------|-----------|-----------|-------|-------|------|---------|
|                  |           | n   | %    | n   | %    | n   | %    |       |       |
| all ILC cases    |           | 42  | 100  | 21  | 50   | 21  | 50   | CSTT  | 0.740 |
| grade            | G1        | 1   | 100  | 0   | 0    | 1   | 100  | CSTT  | 0.781 |
|                  | G2        | 31  | 100  | 17  | 55   | 14  | 45   | CSTT  | 0.464 |
|                  | G3        | 10  | 100  | 4   | 40   | 6   | 60   | CSTT  | 0.464 |
| mBSR: architecture | 1        | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
|                  | 2        | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
|                  | 3        | 42  | 100  | 21  | 50   | 21  | 50   | CSTT  | 0.781 |
| mBSR: nuc. grade | 1        | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
|                  | 2        | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
|                  | 3        | 6   | 100  | 2   | 33   | 4   | 67   | CSTT  | 0.781 |
| mBSR: proliferation | 1       | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
|                  | 2        | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
|                  | 3        | 3   | 100  | 2   | 33   | 4   | 67   | CSTT  | 0.781 |
| ER               | neg.     | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
|                  | pos.     | 42  | 100  | 21  | 50   | 21  | 50   | CSTT  | 0.781 |
|                  | n.a.     | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.781 |
| PR               | neg.     | 3   | 100  | 2   | 67   | 1   | 33   | FET   | 1.000 |
|                  | pos.     | 39  | 100  | 19  | 49   | 20  | 51   | FET   | 1.000 |
| HER2             | 0/1+     | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.293 |
|                  | 2+/F.-neg. | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.293 |
|                  | 2+/F. n.a. | 0   | 0    | 0   | 0    | 0   | 0    | CSTT  | 0.293 |
| Ki67             | <10%     | 2   | 100  | 0   | 0    | 2   | 100  | CSTT  | 0.460 |
|                  | 10-19%   | 18  | 100  | 9   | 50   | 9   | 50   | CSTT  | 0.460 |
|                  | 20-34%   | 21  | 100  | 12  | 57   | 9   | 43   | CSTT  | 0.460 |
|                  | 35-100%  | 1   | 100  | 0   | 0    | 1   | 100  | CSTT  | 0.460 |
| E-cadherin       | neg.     | 40  | 100  | 20  | 50   | 20  | 50   | FET   | 1.000 |
|                  | aberrant | 0   | 0    | 0   | 0    | 0   | 0    | FET   | 1.000 |
|                  | pos.     | 2   | 100  | 1   | 50   | 1   | 50   | FET   | 1.000 |
| CDH1 status      | wild-type | 10  | 100  | 5   | 50   | 5   | 50   | FET   | 1.000 |
|                  | mutant   | 32  | 100  | 16  | 50   | 16  | 50   | FET   | 1.000 |
| beta-catenin     | neg.     | 37  | 100  | 18  | 49   | 19  | 51   | CSTT  | 0.750 |
|                  | focally pos. | 3  | 100  | 2   | 67   | 1   | 33   | CSTT  | 0.750 |
|                  | pos.     | 2   | 100  | 1   | 50   | 1   | 50   | CSTT  | 0.750 |
| p120-catenin     | low/neg. | 8   | 100  | 6   | 75   | 2   | 25   | FET   | 0.238 |
|                  | pos.     | 34  | 100  | 15  | 44   | 19  | 56   | FET   | 0.238 |
| p120-catenin mislocation | membranous | 1   | 100  | 1   | 100  | 0   | 0    | FET   | 0.441 |
|                  | mislocated | 33  | 100  | 14  | 42   | 19  | 58   | FET   | 0.441 |
|                  | not informative | 8  | 100  | 6   | 75   | 2   | 25   | FET   | 0.441 |
| P-cadherin       | neg.     | 35  | 100  | 19  | 54   | 16  | 46   | FET   | 0.410 |
|                  | focally pos. | 7  | 100  | 2   | 29   | 5   | 71   | FET   | 0.410 |
|                  | pos.     | 0   | 0    | 0   | 0    | 0   | 0    | FET   | 0.410 |
| variant/growth pattern | classical/dissociated | 17  | 100  | 9   | 53   | 8   | 47   | FET   | 1.000 |
|                  | classical/single files | 16 | 100  | 8   | 50   | 8   | 50   | FET   | 1.000 |
|                  | trabecular | 8   | 100  | 4   | 50   | 4   | 50   | FET   | 1.000 |
|                  | solid     | 1   | 100  | 0   | 0    | 1   | 100  | FET   | 1.000 |
|                  | pleo., alv., others | 0  | 0    | 0   | 0    | 0   | 0    | FET   | 1.000 |

FET; Fisher's exact test (set A versus set B), CSTT; Chi square test for trends (set A versus set B), pos.; positive, neg.; negative. Pleo.; pleomorphic, alv.; alveolar, others refers to histiocytoid ILC and other rare variants. *Classical versus non-classical. 1Refers to the predominant growth pattern.
Table S4. ILCs, as defined by the reference standard, are associated with loss of E-cadherin expression and CDH1 mutation

| reference | all cases (n=123) | set A (n=61) | set B (n=62) |
|-----------|------------------|-------------|-------------|
|           | NST       | ILC       | NST       | ILC       | NST       | ILC       |
|           | n  | %       | n  | %       | n  | %       |
| grade     |     |         |     |         |     |         |
| G1        | 3  | 4        | 2  | 0.188   | 1  | 2        | 0  | 0.393   | 2  | 5        | 1  | 5        | 0.317 CSTT |
| G2        | 47 | 38       | 31 | 74      | 26 | 65       | 17| 81      | 21| 51       | 14| 67       |
| G3        | 31 | 38       | 10 | 24      | 13 | 33       | 4 | 19      | 18| 44       | 6 | 28       |
| mBSR: architecture |     |         |     |         |     |         |
| 1         | 0  | 0        | 0  | 0       | 0  | 0        |     |         |     |         |     |         |
| 2         | 22 | 27       | 0  | 0       | 13| 32       | 0  | 0       | 9  | 22       | 0  | 0       |
| 3         | 59 | 73       | 42 | 100     | 27| 68       | 21| 100     | 32| 78       | 21| 100     |
| mBSR: nucl. grade |     |         |     |         |     |         |
| 1         | 0  | 0        | 7  | 17      | 0  | 0        | 3 | 14      | 0  | 0        | 4 | 19       | 0.003 CSTT |
| 2         | 44 | 54       | 29 | 69      | 22| 55       | 16| 76      | 22| 54       | 13| 62       |
| 3         | 37 | 46       | 6  | 14      | 18| 45       | 2 | 10      | 19| 46       | 4 | 19       |
| mBSR: proliferation |     |         |     |         |     |         |
| 1         | 12| 15       | 10 | 24      | 0  | 0        | 24| 5       | 28| 68       | 11| 52       |
| 2         | 52| 64       | 25 | 59      | 24| 60       | 14| 67      | 29| 68       | 11| 52       |
| 3         | 17| 21       | 7  | 17      | 8  | 20       | 2 | 9       | 9  | 22       | 5 | 24       |
| ER        |     |         |     |         |     |         |
| neg.      | 1  | 1        | 0  | 0       | 1  | 1        | 0  | 0       |     |         |     |         |
| pos.      | 79| 98       | 42 | 100     | 38| 95       | 21| 100     | 41| 100      | 21| 100     |
| PR        |     |         |     |         |     |         |
| neg.      | 7  | 9        | 3  | 7       | 3  | 7        | 2 | 10      | 1  | 0        | 0  | 0       |
| pos.      | 74| 91       | 39 | 93      | 37| 93       | 19| 90      | 37| 90       | 20| 95       |
| HER2      |     |         |     |         |     |         |
| 0/1+      | 62| 77       | 38 | 90      | 29| 73       | 18| 86      | 33| 81       | 20| 95       | 0.156 CSTT |
| 2+/F. -neg.| 16| 20       | 4  | 10      | 10| 25       | 3 | 14      | 6  | 15       | 1 | 5        |
| 2+/F. n.a. | 2  | 2        | 0  | 0       | 1  | 2        | 0 | 0       | 1  | 2        | 0 | 0        |
| 3+/2+/F. -pos.| 1  | 1        | 0  | 0       | 0  | 0        | 0 | 0       | 1  | 2        | 0 | 0        |
| K67       |     |         |     |         |     |         |
| <10%      | 4  | 5        | 2  | 5       | 3  | 7        | 0 | 0       | 0  | 0        | 0  | 0       |
| 10-19%    | 19| 23       | 18 | 43      | 8  | 20       | 9 | 43      | 11| 27       | 9 | 43       | 0.049 CSTT |
| 20-34%    | 49| 61       | 21 | 50      | 25| 63       | 12| 57      | 24| 59       | 9 | 43       |
| 35-100%   | 9  | 11       | 1  | 2       | 4  | 10       | 0 | 0       | 5  | 12       | 1 | 5        |
| E-cadherin|     |         |     |         |     |         |
| neg.      | 1  | 1        | 40| 95      | 0  | 0        | 20| 95      | 1  | 2        | 20| 95      | P<0.001 FET |
| aberrant  | 0  | 0        | 0  | 0       | 0  | 0        | 0 | 0       | 0  | 0        | 0  | 0       |
| pos.      | 80| 99       | 2  | 5       | 40| 100      | 1 | 5       | 40| 98       | 1 | 5        |
| CDH1 status |     |         |     |         |     |         |
| wild-type mutant | 80 | 99    | 10 | 24     | 40| 100     | 5 | 24     | 40| 98       | 5 | 24       |
| mutant    | 1  | 1        | 32| 76      | 0  | 0        | 16| 76      | 1  | 2        | 16| 76      |
| beta-catenin |     |         |     |         |     |         |
| neg.      | 0  | 0        | 37| 88      | 0  | 0        | 18| 86      | 0  | 0        | 19| 90       |
| focally pos. | 1  | 1        | 3  | 7       | 0  | 0        | 2 | 9       | 1  | 2        | 1 | 5        |
| pos.      | 80| 99       | 2  | 5       | 40| 100      | 1 | 5       | 40| 98       | 1 | 5        |
| p120-catenin |     |         |     |         |     |         |
| neg.      | 4  | 5        | 8  | 19      | 1  | 3        | 6 | 29      | 3  | 7        | 2 | 10       | 1.000 FET |
| pos.      | 77| 95       | 34 | 81      | 39| 98       | 15| 71      | 38| 93       | 19| 90       |
| p120-catenin mislocation |     |         |     |         |     |         |
| membranous | 75 | 93     | 1  | 2       | 39| 98       | 1 | 5       | 36| 88       | 0 | 0        |
| not informative | 2  | 2        | 33| 79      | 0  | 0        | 14| 67      | 2  | 5        | 19| 90       |
| P-cadherin |     |         |     |         |     |         |
| neg.      | 67| 83       | 35 | 83      | 34| 85       | 19| 90      | 33| 80       | 16| 76       | 0.748 FET |
| focally pos. | 14 | 17       | 7  | 17      | 6 | 15       | 2 | 10      | 8 | 20       | 5 | 24       |
| pos.      | 0  | 0        | 0  | 0       | 0  | 0        | 0 | 0       | 0  | 0        | 0  | 0       |

FET; Fisher’s exact test, CSTT; Chi square test for trends; pos.; positive, neg.; negative.
### Table S5. Proportions of category II cases in set A and set B

| calls for ILC | explanation                                      | category | all cases (n=123) | set A (n=61) | set B (n=62) |
|--------------|--------------------------------------------------|----------|------------------|--------------|--------------|
|              |                                                  |          | n    | %    | n    | %    | n    | %    |
| 0/35         | 0 complete consensus against ILC                 | cat. I   | 55   | 45   | 24   | 39   | 31   | 50   |
| 1/35         | 3 one dissenting call for ILC                     |          | 18/35 | 51   | 30   | 49   | 15   | 24   |
| 2/35         | 6 two dissenting calls for ILC                    |          |       |      |      |      |      |      |
| 3/35         | 9 three dissenting calls for ILC                  |          |       |      |      |      |      |      |
| 32/35        | 91 balanced calls for/against ILC                 | cat. II  | 45   | 36   | 30   | 49   | 15   | 24   |
| 33/35        | 94 two dissenting calls against ILC               |          |       |      |      |      |      |      |
| 34/35        | 97 one dissenting call against ILC                |          |       |      |      |      |      |      |
| 35/35        | 100 complete consensus for ILC                    | cat. III | 23   | 19   | 7    | 12   | 16   | 26   |
|              |                                                  | test     | FET   | P value | FET (cat. I+III vs cat. II) | P=0.005 |

FET, Fisher’s exact test.
Table S6. Participants' written comments for case B056.

| participant | comment |
|-------------|---------|
| p01_i01     | LIN present. ILC-like but tubules. |
| p02_i01     | Possibly tubulo-lobular? |
| p04_i01     | ILC but tubular too. |
| p07_i03     | Heterogeneous. |
| p08_i04     | E-cadherin seems reliably negative. |
| p09_i01     | Difficult. I'm undecided. |
| p11_i06     | Partially tubules. |
| p14_i09     | Not easy. Impression of a lobular component. |
| p14_i09     | Is E-cad. False-negative in the ductal component? |
| p18_i01     | LIN present. Partially lobular and ductal pattern. What are the molecular features? |
| p21_i15     | Some areas show a delicate and faint E-cad.-positivity. Surprisingly low E-cad. staining in well differentiated glands. I'm in favor of a mixed NST+lobular or tubulo-lobular BC. |
| p22_i16     | Considered as ILC (although there are some tubules). |
| p23_i17     | Tubular-lobular growth. E-cad.: NST area with very weak expression. ILC area shows a complete loss. |
| p24_i18     | Some cells are possibly very weakly E-cadherin-positive? |
| p27_i20     | I'm not sure: In some areas it is unequivocally lobular. In other areas the growth pattern would correspond to NST. E-cad. didn't help me to decide. |
| p32_i25     | I don't want to believe E-cad., but it looks real. I would sign out as carcinoma with mixed features. |
| p33_i26     | Very weak focal expression of E-cad. in tubular structures? |
### Table S7. Features of BCs with variable subtype calls (category II specimens)

| Reference | cat. I | cat. II | cat. III | P-value | set A (n=61) | cat. I | cat. II | cat. III | P-value | set B (n=62) | cat. I | cat. II | cat. III | P-value |
|-----------|--------|---------|----------|---------|-------------|--------|---------|----------|---------|-------------|--------|---------|----------|---------|
| All cases | 55/100 | 45/100 | 23/100   | 0.336   | 0.797/1    | 2/7    | 0/0    | 0/0     | 0.451 CST |
| Grade     | 0.16   | 6/0    | 0/0     | 0.001   | 0.001/0.01 | 0/0    | 0/0    | 0/0     | 0.019 CST |
| mBSR: architecture | 0/0 | 0/0 | 0/0 | <0.001 | 0.001/0.001 | 0/0 | 0/0 | 0/0 | 0.019 CST |
| mBSR: nucleo. grade | 29/53 | 29/64 | 15/65 | 14/58 | 19/63 | 5/71 | 15/48 | 10/67 | 10/63 |
| mBSR: proliferation | 10/42 | 9/30 | 1/14 | 0.12   | 4/13 | 2/13 | 3/19 | 0.937 CST |
| ER        | 0/0 | 1/2 | 0/0 | 0.474 | 0.711 | 0/0 | 0/0 | 0/0 | 0.999 CST |
| PR        | 4/7 | 2/4 | 3/13 | 0.436 | 0.091 | 2/6 | 1/7 | 1/6 | 0.999 CST |
| HER2      | 43/78 | 37/82 | 21/95 | 0.702 | 1.000 | 24/78 | 15/93 | 19/94 | 0.709 CST |
| K67       | 4/7 | 2/4 | 0/0 | 0.445 | 0.154 | 1/3 | 2/10 | 0.357 CST |
| E-cadherin | 0/0 | 13/40 | 7/100 | 0.001 | 0.001 | 0/0 | 5/3 | 3/16 | 0.001 CST |
| CDH2 status | 55/100 | 30/67 | 5/22 | <0.001 | 0.001 | 0/0 | 0/0 | 0/0 | 0.001 CST |
| Beta-catenin | 0/0 | 11/36 | 7/100 | 0.001 | 0.001 | 0/0 | 3/20 | 16/100 | 0.001 CST |
| p120-catenin | 0/0 | 10/33 | 4/57 | 0.017 | 0.017 | 0/0 | 3/10 | 0/0 | 0.017 CST |
| p120-catenin misloc. | 23/96 | 17/57 | 0/0 | <0.001 | <0.001 | 27/87 | 9/60 | 0/0 | <0.001 CST |
| P-cadherin | 49/89 | 34/76 | 19/83 | 0.201 | 0.991 | 28/90 | 8/53 | 13/81 | 0.015 CST |
| E / P-cadherin | 0/0 | 13/40 | 6/86 | <0.001 | <0.001 | 0/0 | 2/13 | 13/82 | 0.011 CST |

CST: Chi square test for trends. Pos.: positive, neg.: negative.

*Cutoffs to separate category I, II, and III were set at <3/35 (<9%) and >32/35 (>91%) participant calls for ILC.*
Table S8. Educational BC subtype assessment*

|                      | inter-observer agreement (pairwise) | agreement with the reference standard | accuracy for detection of a lobular component |
|----------------------|-------------------------------------|--------------------------------------|-----------------------------------------------|
|                      | set A  | set B  | set A  | set B  | set A  | set B  |
| median [kappa]       | 0.36   | 0.35   | 0.38   | 0.55   | 75%    | 77%    |
| IQR                  | 0.26-0.44 | 0.22-0.47 | 0.30-0.51 | 0.25-0.69 | 67-80% | 62-90% |
| range                | 0.06-0.67 | -0.01-0.87 | 0.10-0.70 | 0.10-0.90 | 51-85% | 52-95% |
| P value              | n.s.   | P=0.015 |        |        |        |        |

*Participants included 15 medical students, 1 pathologist assistant educated in histopathology, 1 early resident in clinical pathology, and 1 biomedical scientists. All medical students had attended a course in basic histopathology at the MHH (40 hours lectures, 40 hours practical training in microscopy using 122 different tissue specimens, including 1 representative ductal carcinoma in situ, 1 representative invasive BC of NST, and 1 representative ILC) before participation.