**Christgen *et al.* 2020**

**E-cadherin to P-cadherin switching in lobular breast cancer with tubular elements**

# Supplementary Material

**1. List of Supplemental data**

## 2. Supplemental Data Table 1

Supplemental Data Table 1
Antibodies used for immunohistochemical analyses

*A) ILBC with tubular elements*

| antigen | study | antibody | species | source | dilution | antigenic retrieval | detection system | scoring | cutoff |
|---|---|---|---|---|---|---|---|---|---|
| ER | ILBC-TE | clone SP1 | rabbit | Ventana | undiluted, ready-to use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semiquantitative, 0-100 | 10 |
| PR | ILBC-TE | clone 1E2 | rabbit | Ventana | undiluted, ready-to use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semiquantitative, 0-100 | 10 |
| HER2 | ILBC-TE | clone 4B5 | mouse | Ventana | undiluted, ready-to use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | 0, 1+, 2+, 3+ | 2+, 3+ |
| Ki67 | ILBC-TE | clone 30-9 | rabbit | Ventana | undiluted, ready-to use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semiquantitative, 0-100 | n.a. |
| E-cadherin | ILBC-TE | clone ECH-6 | mouse | Zytomed | 1:100 | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | Remmele IRS | 0 |
| beta-catenin | ILBC-TE | clone 14 | mouse | BD Transduction Laboratories | 1:75 | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | Remmele IRS | 0 |
| p120-catenin | ILBC-TE | clone 98 | mouse | BD Transduction Laboratories | 1:250 | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | Remmele IRS | >/=3, localization |
| P-cadherin | ILBC-TE | clone 56 | mouse | BD Transduction Laboratories | 1:100 | CC1 mild protocol (Ventana) | CC1 mild protocol (Ventana) | Remmele IRS | >/=3 |
| R-cadherin | ILBC-TE | clone D9 | mouse | Santa Cruz | 1:50 | CC1 mild protocol (Ventana) | CC1 mild protocol (Ventana) | Remmele IRS | >/=3 |
| N-cadherin | ILBC-TE | clone 3B9 | mouse | Thermo Fisher Scientific | 1:100 | CC1 mild protocol (Ventana) | CC1 mild protocol (Ventana) | Remmele IRS | >/=3 |

*B) TMA reference cohort*

| antigen | study | antibody | species | source | dilution | antigenic retrieval | detection system | scoring | cutoff |
|---|---|---|---|---|---|---|---|---|---|
| ER | TMA | clone SP1 | rabbit | Neomarkers, MEDAC | 1:100 | citric acid, pH 6.0, pressure cooker, 125 °C | ZytoChem-Plus HRP Kit (Zytomed) | Remmele IRS | 3 |
| PR | TMA | clone PgR636 | mouse | Dako | 1:100 | citric acid, pH 6.0, pressure cooker, 125 °C | ZytoChem-Plus HRP Kit (Zytomed) | Remmele IRS | 3 |
| AR | TMA | clone AR441 | mouse | Dako | 1:40 | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | Remmele IRS | 3 |
| BCL2 | TMA | clone 124 | mouse | Dako | 1:100 | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | Remmele IRS | 3 |
| HER2 | TMA | clone 4B5 | mouse | Ventana | undiluted, ready-to use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | 0, 1+, 2+, 3+ | 2+, 3+ |
| CK5/14 | TMA | clones XM26 and LL002 | mouse | Zytomed | 1:200 | citric acid, pH 6.0, pressure cooker, 125 °C | ZytoChem-Plus HRP Kit (Zytomed) | Remmele IRS | 3 |
| EGFR | TMA | clone 2.1E1 | mouse | Zytomed | 1:600 | Fast Enzyme Solution (Zytomed) | ZytoChem-Plus HRP Kit (Zytomed) | Remmele IRS | 3 |
| p53 | TMA | clone DO-7 | mouse | Novocastra | 1:100 | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semiquantitative, 0-100 | >95 |
| E-cadherin | TMA | clone 4A2C7 | mouse | Invitrogen | 1:50 | citric acid, pH 6.0, pressure cooker, 125 °C | ZytoChem-Plus HRP Kit (Zytomed) | semiquantitative 0-100 | 0 |
| beta-catenin | TMA | clone #14 | mouse | BD Transduction Laboratories | 1:100 | citric acid, pH 6.0, pressure cooker, 125 °C | ZytoChem-Plus HRP Kit (Zytomed) | Remmele IRS | 3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ki67 | TMA | clone 30-9 | rabbit | Ventana | undiluted, ready-to use solution | CC1 mild protocol (Ventana) | ultraView DAB Kit (Ventana) | semiquantitative, 0-100 | 10, 25, 35 |
| P-cadherin | TMA | clone 56 | mouse | BD Transduction Laboratories | 1:100 | CC1 mild protocol (Ventana) | CC1 mild protocol (Ventana) | Remmele IRS | >/=3 |

## 3. Supplemental Data Table 2

Supplemental Data Table 2
NGS details

| case | format | histology | pre-preparation | reads | coverage | gene | mut. status | freq. |
|---|---|---|---|---|---|---|---|---|
| *A) primary tumors: customized CDH1 panel* | | | | | | | | |
| 1 | RS | ILBC with tubular elements | microdissected | 222775 | 4790 | *CDH1* | p.H97I_fs*20 | 0,61 |
| 2 | RS | ILBC with tubular elements | bulk | 1891618 | 57331 | *CDH1* | p.F86S_fs12 | 0,42 |
| 3 | RS | ILBC with tubular elements, ovarian met. | microdissected region with only tubular elements | 804829 | 25954 | *CDH1* | p.S9* | 0,75 |
| 4 | NB | ILBC with tubular elements | bulk | 692796 | 22824 | *CDH1* | p.E841* | 0,37 |
| 5 | RS | ILBC with tubular elements | microdissected | 707935 | 22699 | *CDH1* | p.Q23* | 0,38 |
| 6 | NB | ILBC with tubular elements | bulk | 936629 | 23332 | *CDH1* | wild-type | ina |
| 7 | NB | ILBC with tubular elements | bulk | 725175 | 23535 | *CDH1* | p.Q177* | 0,47 |
| 8 | NB | ILBC with tubular elements | bulk | 35952 | 857 | *CDH1* | p.E445* | 0,61 |
| 9 | RS | ILBC with tubular elements | microdissected | 251137 | 8181 | *CDH1* | splicing | 0,28 |
| 10 | RS | ILBC with tubular elements | bulk | 47660 | 1155 | *CDH1* | p.T295I | 0,04 |
| 11 | RS | ILBC with tubular elements | bulk | 38333 | 1060 | *CDH1* | p.P537R_fs*20 | 0,31 |
| 12 | NB | ILBC with tubular elements | bulk | 101856 | 3141 | *CDH1* | p.Q23* | 0,43 |
| 13 | RS | ILBC with tubular elements | bulk | 524266 | 18456 | *CDH1* | wild-type | ina |
| *B) additional tumors/lesions: customized CDH1 panel* | | | | | | | | |
| 1 | RS | 2nd tumor, ipsilateral, ILBC with classic growth pattern | microdissected | 122103 | 3287 | *CDH1* | p.H97I_fs*20 | 0,27 |
| 1 | RS | 3nd tumor, ipsilateral, ILBC with solid growth pattern | microdissected | 198147 | 3462 | *CDH1* | p.H97I_fs*20 | 0,58 |
| 1 | RS | adjacent LCIS | microdissected | 273633 | 6170 | *CDH1* | p.H97I_fs*20 | 0,22 |
| 3 | RS | ILBC with conventional growth pattern, ovarian met. | microdissected region with only conventional growth pattern | 796760 | 25914 | *CDH1* | p.S9* | 0,70 |
| 4 | NB | 2nd tumor, ipsilateral, ILBC with classic growth pattern | bulk | 821730 | 27473 | *CDH1* | p.E841* | 0,10 |
| 4 | NB | 3rd tumor, contralateral, ILBC with classic growth pattern | bulk | 798321 | 26415 | *CDH1* | p.Y523* | 0,36 |
| 5 | RS | 2nd tumor, ipsilateral, ILBC with trabecular growth pattern | microdissected | 635777 | 20215 | *CDH1* | p.Q23* | 0,12 |
| 9 | RS | 2nd tumor, ipsilateral, ILBC with classic growth pattern | bulk | 243778 | 7545 | *CDH1* | splicing | 0,28 |
| *C)* case 3: additional Oncomine comprehensive assay | | | | | | | | |
| 3 | RS | ILBC with tubular elements, ovarian met. | microdissected region with only tubular elements | 5562786 | 1587 | *TSC1*<br>*RNF43* | p.G568*<br>p.A193Pfs*10 | 0,80<br>0,33 |
| 3 | RS | ILBC with conventional growth pattern, ovarian met. | microdissected region with only conventional growth pattern | 9907247 | 2856 | *TSC1*<br>*RNF43*<br>*PTEN* | p.G568*<br>p.A193Pfs*10<br>p.V271L | 0,60<br>0,39<br>0,05 |

## 4. Supplemental Data Table 3

Supplemental Data Table 3

Association of P-Cadherin with clinicopathological characteristics, TMA cohort

| | | all cases | | P-cadherin-pos. | | P-cadherin-neg. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | number | percent | number | percent | number | percent | P value | test | note |
| all cases | | 268 | 100 | 36 | 13 | 232 | 87 | | | |
| age | | | | | | | | 0.368 | FET | |
| | <60 | 120 | 45 | 19 | 16 | 101 | 84 | | | |
| | >/=60 | 148 | 55 | 17 | 11 | 131 | 88 | | | |
| lesion type | | | | | | | | 0.051 | FET | PT vs DOM |
| | PT | 182 | 68 | 26 | 14 | 156 | 86 | | | |
| | LRT | 36 | 13 | 8 | 22 | 28 | 78 | | | |
| | DOM | 50 | 19 | 2 | 4 | 48 | 96 | | | |
| pT stage | | | | | | | | 0.805 | FET | pT1/2 vs pT3/4 |
| | pT1/2 | 157 | 59 | 23 | 15 | 134 | 85 | | | |
| | pT3/4 | 41 | 15 | 5 | 12 | 36 | 88 | | | |
| | n.a. | 20 | 7 | 3 | 15 | 17 | 85 | | | |
| | ina. | 50 | 19 | 5 | 10 | 45 | 90 | | | |
| pN stage | | | | | | | | 0.278 | FET | pN0 vs pN1+ |
| | pN0 | 98 | 37 | 12 | 12 | 86 | 88 | | | |
| | pN1+ | 70 | 26 | 13 | 19 | 57 | 81 | | | |
| | n.a. | 50 | 19 | 9 | 18 | 41 | 82 | | | |
| | ina. | 50 | 19 | 2 | 4 | 48 | 96 | | | |
| histology | | | | | | | | 0.082 | FET | NST vs lobular |
| | NST | 175 | 65 | 29 | 17 | 146 | 83 | | | |
| | lobular | 84 | 31 | 7 | 8 | 77 | 92 | | | |
| | mucinous | 8 | 3 | 0 | 0 | 8 | 100 | | | |
| | tubular | 1 | 1 | 0 | 0 | 1 | 100 | | | |
| grade | | | | | | | | <0.001 | * CSTT | |
| | G1 | 9 | 3 | 0 | 0 | 9 | 100 | | | |
| | G2 | 148 | 55 | 10 | 7 | 138 | 93 | | | |
| | G3 | 98 | 37 | 25 | 25 | 73 | 75 | | | |
| | n.a. | 13 | 5 | 1 | 8 | 12 | 92 | | | |
| ER status | | | | | | | | <0.001 | * FET | |
| | pos. | 203 | 76 | 11 | 5 | 192 | 95 | | | |
| | neg. | 65 | 24 | 25 | 38 | 40 | 62 | | | |
| PR status | | | | | | | | <0.001 | * FET | |
| | pos. | 126 | 47 | 7 | 6 | 119 | 94 | | | |
| | neg. | 142 | 53 | 29 | 20 | 113 | 80 | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| AR status | | | | | | | | | | |
| | pos. | 62 | 23 | 6 | 10 | 56 | 90 | 0.211 | | FET |
| | neg. | 160 | 60 | 27 | 17 | 133 | 83 | | | |
| | n.a. | 46 | 17 | 3 | 6 | 43 | 94 | | | |
| BCL2 status | | | | | | | | <0.001 | * | FET |
| | pos. | 118 | 44 | 4 | 3 | 114 | 97 | | | |
| | neg. | 142 | 53 | 30 | 21 | 112 | 79 | | | |
| | n.a. | 8 | 3 | 2 | 25 | 6 | 75 | | | |
| HER2 status | | | | | | | | 0.323 | | FET |
| | 0/1+ | 241 | 90 | 32 | 13 | 209 | 87 | | | |
| | 2+/F- | 7 | 3 | 0 | 0 | 7 | 100 | | | |
| | 2+/F+ | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | 3+ | 20 | 7 | 4 | 20 | 16 | 80 | | | |
| CK5/14 status | | | | | | | | <0.001 | * | FET |
| | pos. | 22 | 8 | 12 | 54 | 10 | 46 | | | |
| | neg. | 243 | 91 | 23 | 9 | 220 | 91 | | | |
| | n.a. | 3 | 1 | 1 | 33 | 2 | 67 | | | |
| EGFR status | | | | | | | | 0.046 | * | FET |
| | pos. | 3 | 1 | 2 | 67 | 1 | 33 | | | |
| | neg. | 263 | 98 | 33 | 12 | 230 | 88 | | | |
| | n.a. | 2 | 1 | 1 | 50 | 1 | 50 | | | |
| P53 nuc. accumulation | | | | | | | | 0.002 | * | FET |
| | pos. | 35 | 13 | 11 | 31 | 24 | 69 | | | |
| | neg. | 227 | 85 | 23 | 10 | 204 | 90 | | | |
| | n.a. | 6 | 2 | 2 | 33 | 4 | 67 | | | |
| E-cadherin status | | | | | | | | 0.083 | | FET |
| | pos. | 170 | 63 | 26 | 15 | 144 | 85 | | | |
| | neg. | 91 | 34 | 7 | 8 | 84 | 92 | | | |
| | n.a. | 7 | 3 | 3 | 43 | 4 | 57 | | | |
| Beta-catenin status | | | | | | | | 0.051 | | FET |
| | pos. | 180 | 67 | 29 | 16 | 151 | 84 | | | |
| | neg. | 85 | 32 | 6 | 7 | 79 | 93 | | | |
| | n.a. | 3 | 1 | 1 | 33 | 2 | 67 | | | |
| Ki67 index | | | | | | | | <0.001 | * | CSTT |
| | 0-10 | 84 | 31 | 6 | 7 | 78 | 93 | | | |
| | 11-25 | 103 | 38 | 10 | 10 | 93 | 90 | | | |
| | 26-35 | 26 | 10 | 3 | 11 | 23 | 89 | | | |
| | 36-100 | 55 | 21 | 17 | 31 | 38 | 69 | | | |
| molecular subtype | | | | | | | | <0.001 | * | CSTI |
| | luminal A/B | 195 | 73 | 11 | 6 | 184 | 94 | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| luminal / HER2-pos. | 9 | 3 | 0 | 0 | 9 | 100 | | | | |
| HER2-pos. | 11 | 4 | 4 | 36 | 7 | 64 | | | | |
| basal | 22 | 8 | 12 | 54 | 10 | 46 | | | | |
| undefined | 31 | 12 | 9 | 29 | 22 | 71 | | | | |
| | | | | | | | <0.001 | * | CSTI | |
| triple-neg. | 53 | 20 | 21 | 40 | 32 | 60 | 0.127 | | FET | luminal NST vs ILBC |
| luminal, and NST | 113 | 42 | 4 | 3 | 109 | 97 | | | | |
| luminal, and ILBC | 78 | 29 | 7 | 9 | 71 | 91 | | | | |

## 5. Supplemental Data Table 4

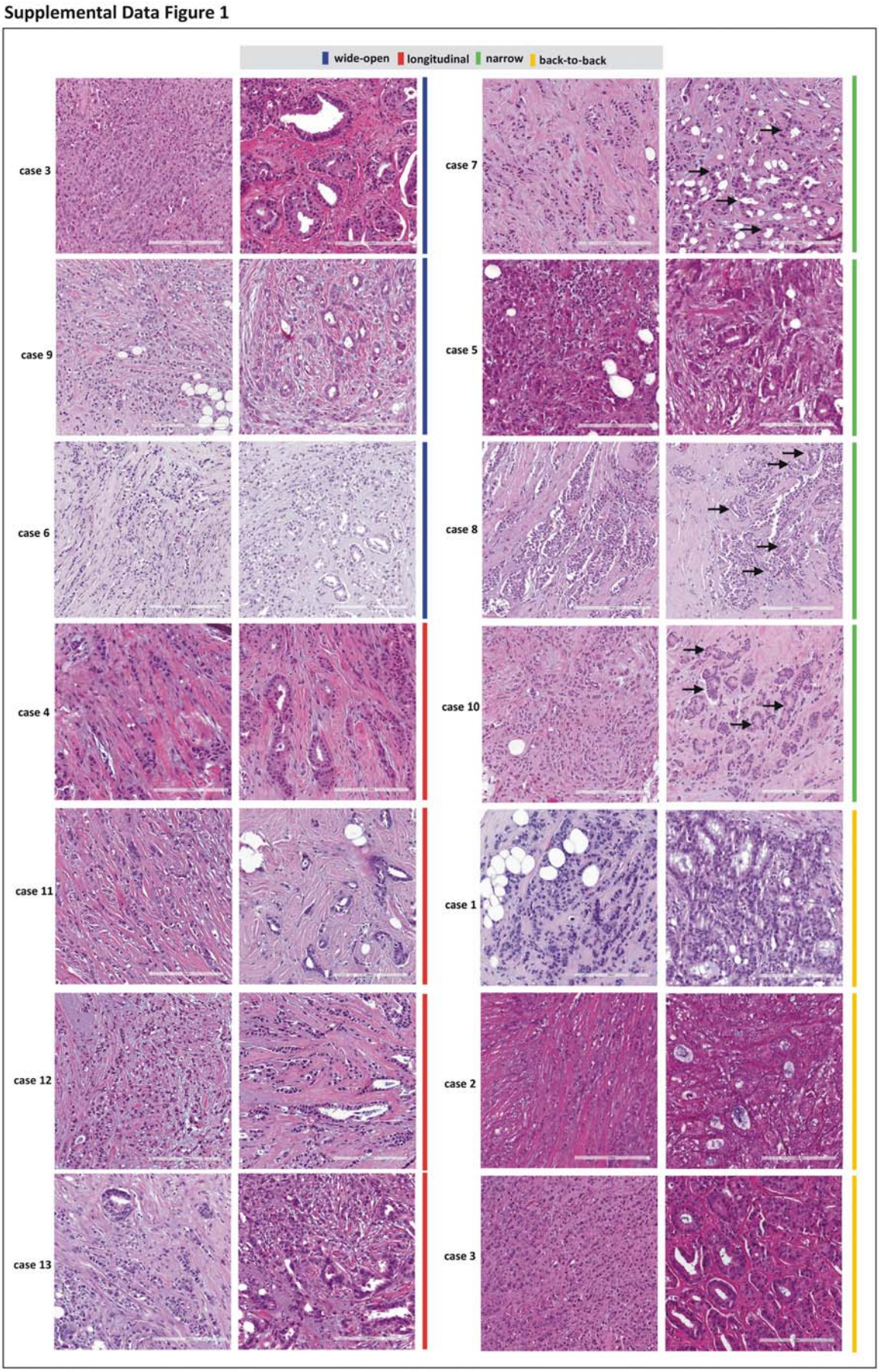
Supplemental Data Table 4

P-cadherin and E-cadherin expression in TNBC

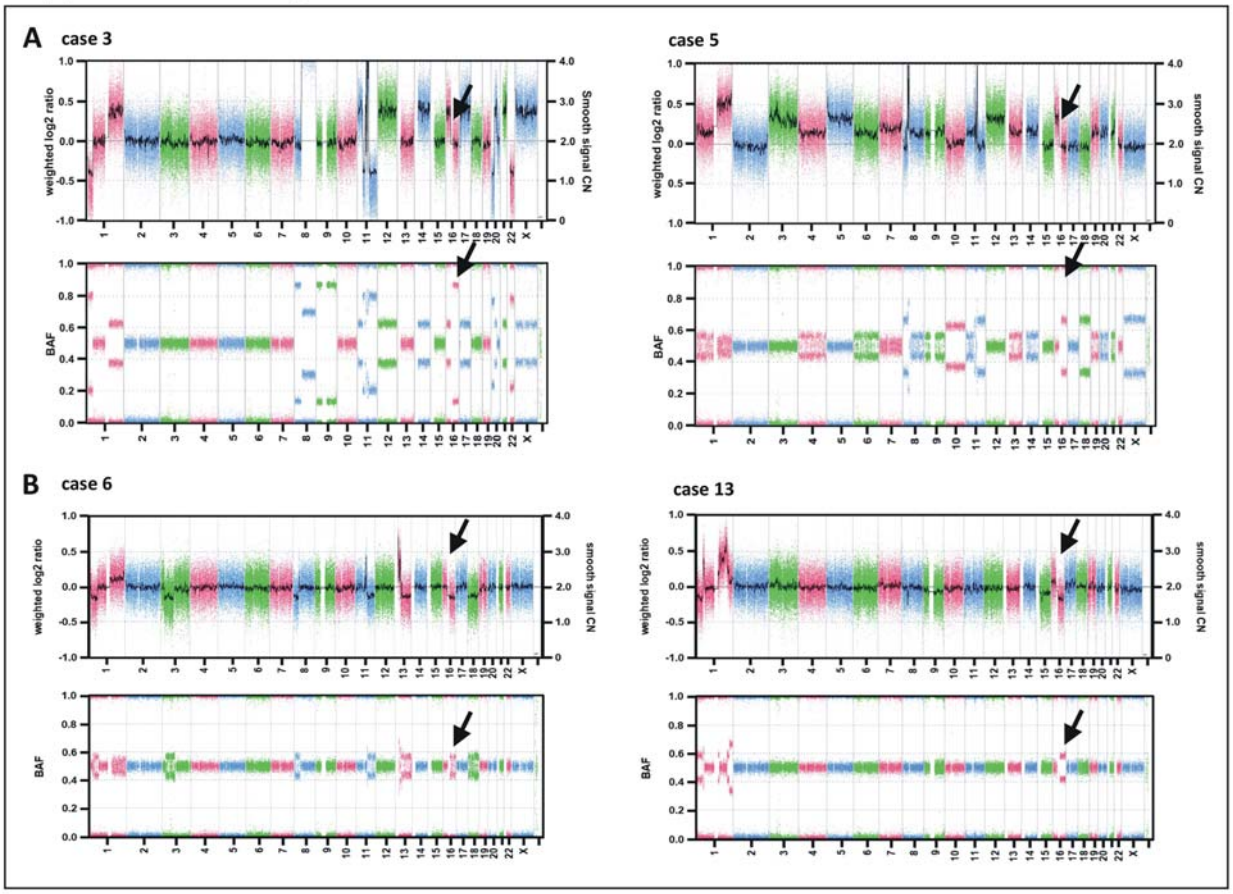
| | | P-cad. | | |
|---|---|---|---|---|
| | | pos | neg | P value |
| | pos | 18 (34%) | 22 (41%) | |
| E-cad. | neg | 0 (0%) | 10 (19%) | 0.009* |
| | na | 3 (6%) | 0 (0%) | |

* Fisher's exact test

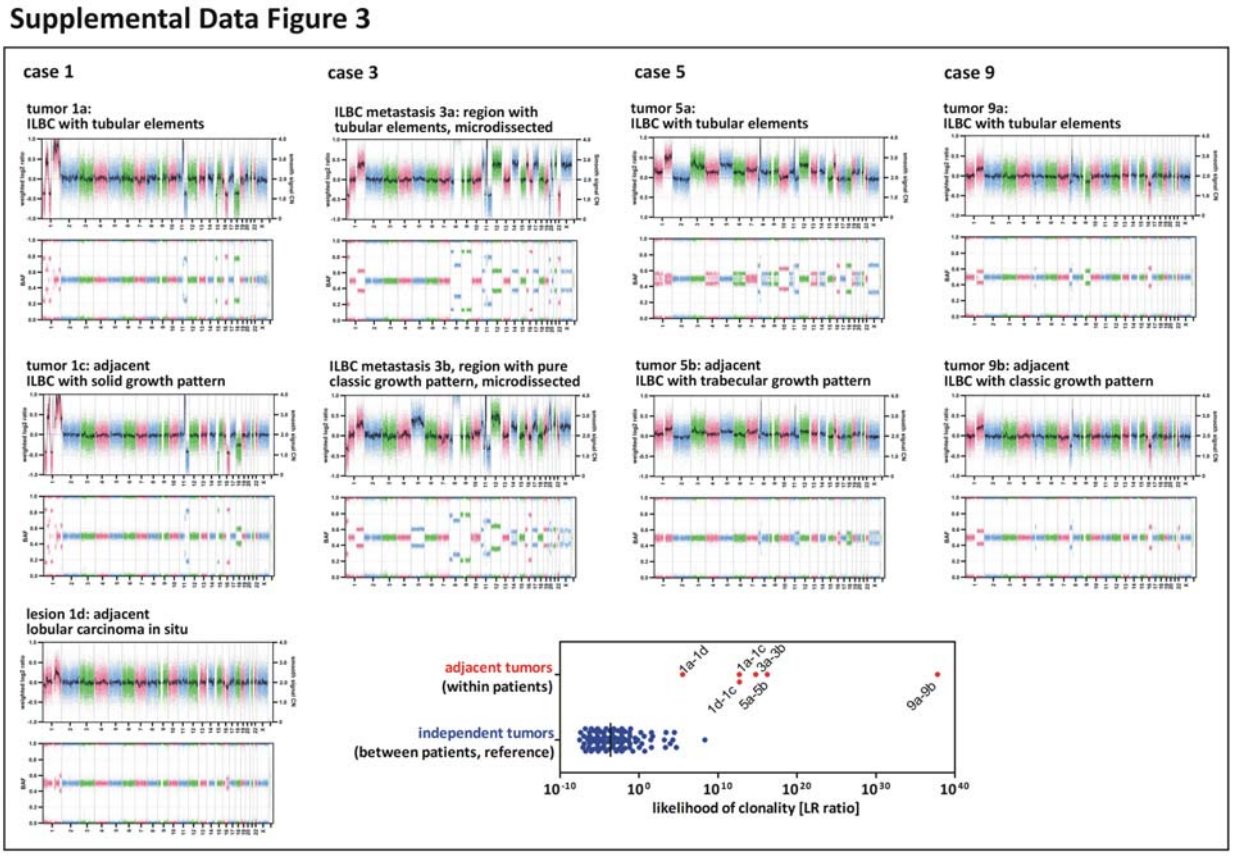
## 6. Supplemental Data Figure 1



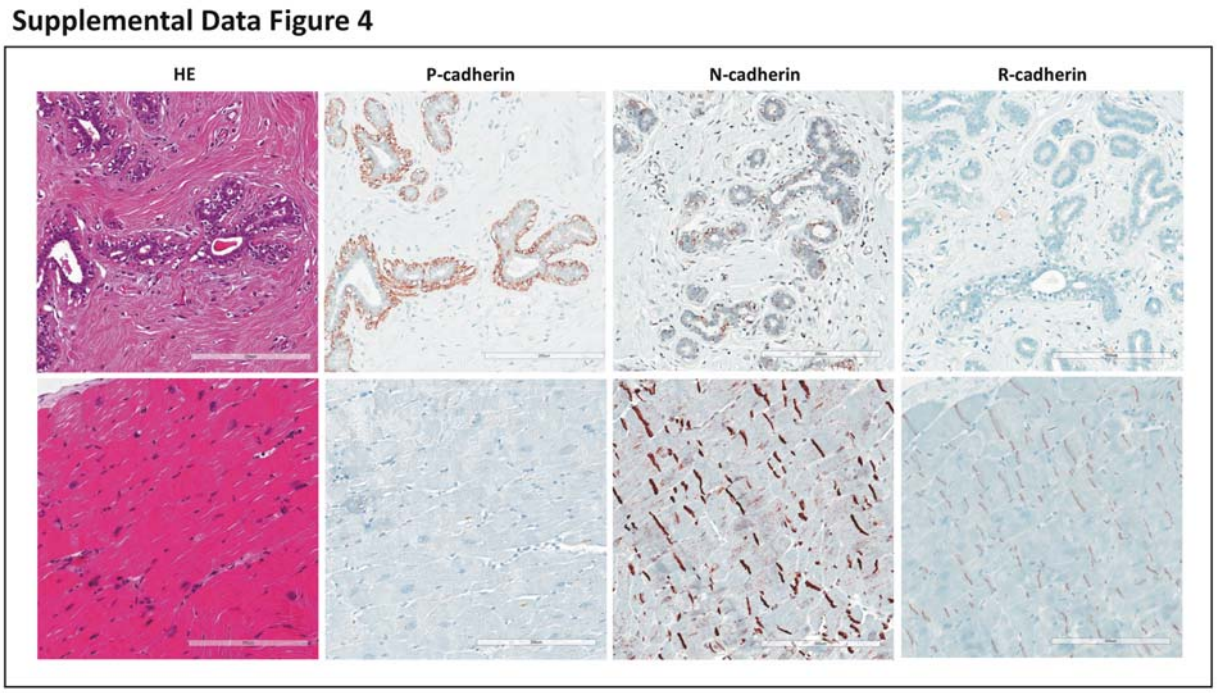
## 7. Supplemental Data Figure 2



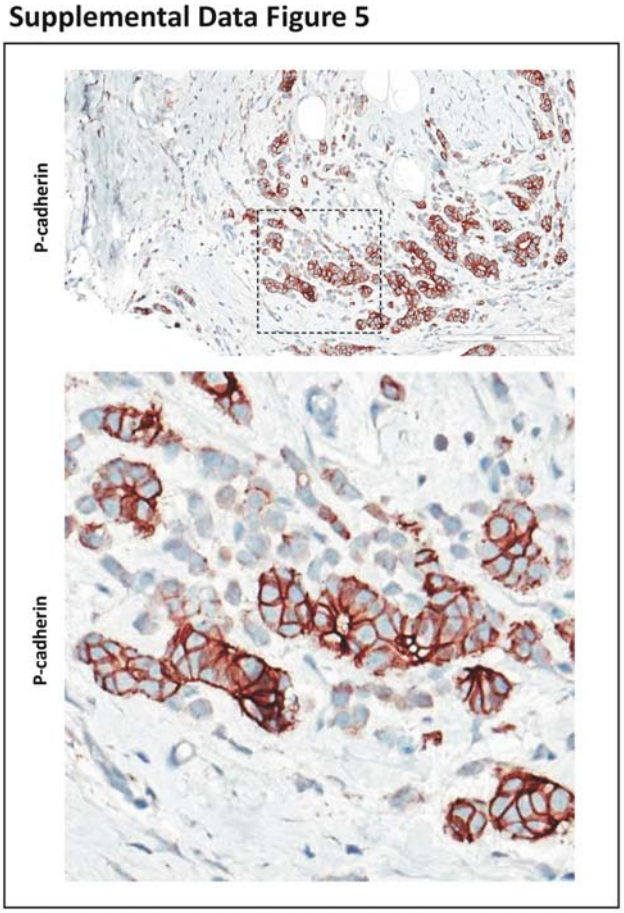
## 8. Supplemental Data Figure 3



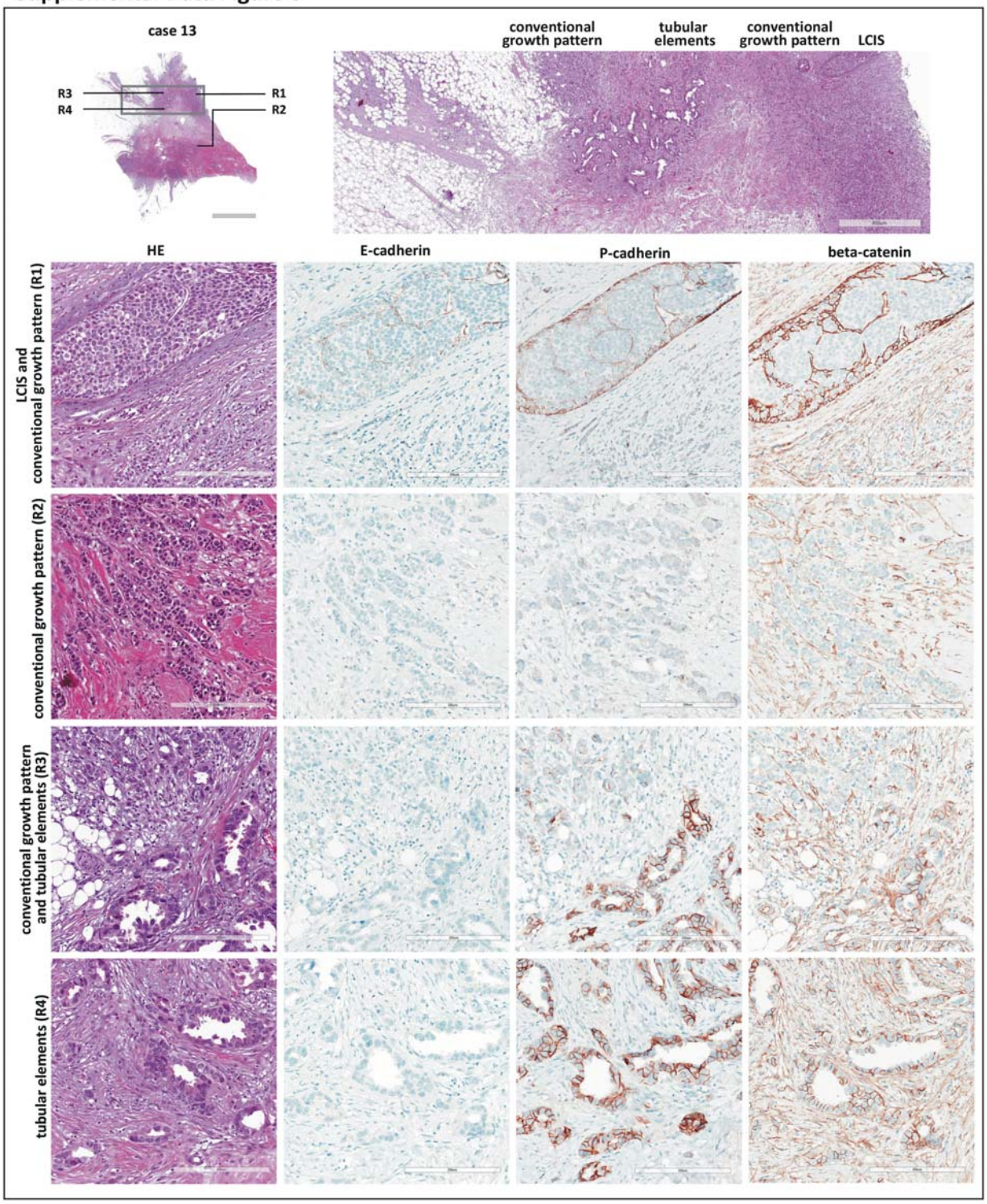
**9. Supplemental Data Figure 4**



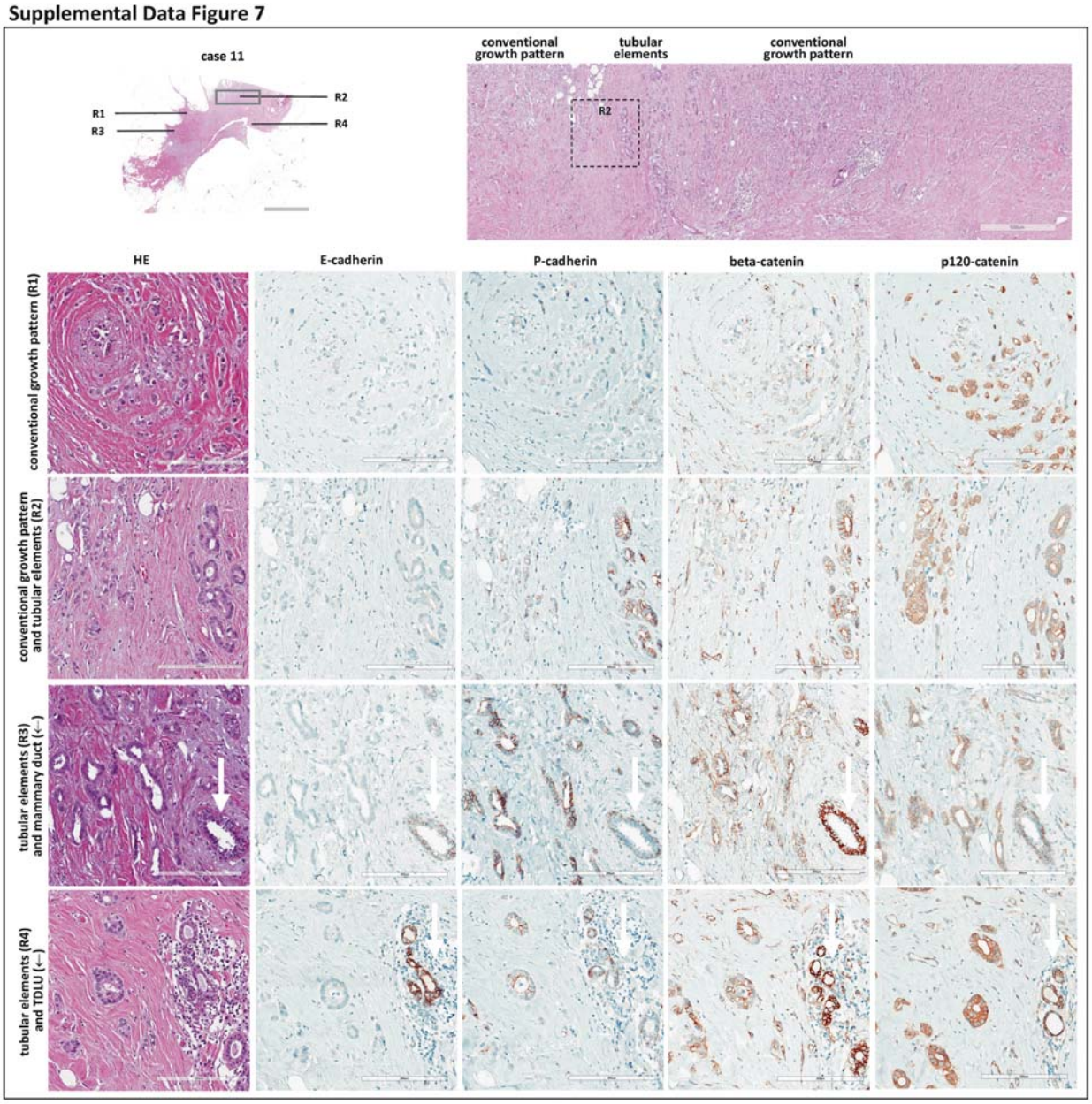
**10. Supplemental Data Figure 5**



## 11. Supplemental Data Figure 6



## 12. Supplemental Data Figure 7



## 13. Supplemental Data Figure 8