Supplementary Figure 1

Supplementary figure 1. The DCISion questionnaire

DCISion QUESTIONNAIRE

Please tick the most appropriate box, or fill in your answer in the available grey text box. In case of multiple choice questions, only one answer is allowed.

1. How many years are you in practice as a pathologist (excluding the years in training)?

2. Do you work in an academic or non-academic laboratory?
   - ☐ Academic laboratory (i.e. a laboratory associated with a university hospital).
   - ☐ Non-academic laboratory
   - ☐ Both academic and non-academic: I work in more than one laboratory.

3. Do you use conventional light microscopy or digital pathology in daily clinical routine?
   - ☐ Conventional light microscopy
   - ☐ Digital pathology
   - ☐ Both

4. Based on a fulltime (5 days per week = 100%) working schedule, how much of your time do you spend on breast pathology?
   - ☐ Less than one day (<20%)
   - ☐ At least one day but less than two days (≥ 20% and < 40%)
   - ☐ At least two days but less than three days (≥ 40% and < 60%)
   - ☐ At least three days but less than four days (≥ 60% and < 80%)
   - ☐ At least four days (≥ 80%)

5. What do you do when you encounter heterogeneous DCIS in daily clinical practice? Do you mention the highest nuclear grade or the predominant nuclear grade?
   - ☐ Highest grade
   - ☐ Predominant grade
   - ☐ I do not know

6. Which grading system do you use in daily clinical routine for grading DCIS?
   - ☐ ASCO/CAP guidelines
   - ☐ Holland classification
   - ☐ Lagios system
   - ☐ Pinder pathological classification
   - ☐ Van Nuys histological classification
   - ☐ WHO grading
   - ☐ Other; please specify:

7. Do you routinely mention the extent of stromal inflammation or tumour-infiltrating lymphocytes in the histopathological report when you encounter pure DCIS in a biopsy or resection specimen in daily clinical practice?
   - ☐ Yes, always
   - ☐ Yes, sometimes
   - ☐ No, never

8. Do you routinely mention the presence or absence of lobular cancerisation when you encounter pure DCIS in a biopsy or resection specimen in daily clinical practice?
   - ☐ Yes, always
   - ☐ Yes, sometimes
   - ☐ No, never

9. Do you routinely mention the presence or absence of intraductal calcifications when you encounter pure DCIS in a biopsy or resection specimen in daily clinical practice?
   - ☐ Yes, always
   - ☐ Yes, sometimes
   - ☐ No, never

10. Do you routinely mention the presence or absence of (comedo)necrosis when you encounter pure DCIS in a biopsy or resection specimen in daily clinical practice?
    - ☐ Yes, always
    - ☐ Yes, sometimes
    - ☐ No, never

11. Do you routinely mention the type of growth pattern (DCIS architecture) when you encounter pure DCIS in a biopsy or resection specimen in daily clinical practice?
    - ☐ Yes, always
    - ☐ Yes, sometimes
    - ☐ No, never

Supplementary Figure 2

Supplementary figure 2. All participants were provided with written definitions for each histopathological characteristic.

SUPPLEMENTARY FIGURE 2

DEFINITIONS OF HISTOPATHOLOGICAL FEATURES

1. Nuclear grade

Many ways for assessment of nuclear grade in DCIS have been described. In this study, the method proposed by the ASCO/CAP was preferred.[1] This method was adapted according to the previously determined binary cut-off [2]. Necrosis is not taken into account.

Non-high	High
➢ Limited to moderate pleomorphism ➢ Size is 1.5x to 2.5x the size of a normal red blood cell or a normal duct epithelial cell nucleus ➢ Usually diffuse finely dispersed chromatin distribution; vesicular nuclei are rare ➢ Prominent nucleoli are rare. If present, nucleoli cannot be observed at 40x magnification. ➢ Only occasional mitoses ➢ Usually (some areas with) polarized cells toward the luminal space.	➢ Markedly pleomorphic ➢ Size: >2.5x the size of a normal red blood cell or a normal duct epithelial cell nucleus ➢ Nuclei are usually vesicular with irregular chromatin distribution ➢ Nucleoli are prominent and often multiple, and can be observed at 40x magnification ➢ Mitoses are usually easily observed and can be frequent ➢ Usually not polarized toward the luminal space.

STUDY CODES NUCLEAR GRADE:

- 0 = non-high grade
- 1 = high grade

2. DCIS architecture

Architectural types include solid, cribriform, papillary and micropapillary growth (regardless of the presence of comedonecrosis)[4]. In this study, the presence (or absence) of ≥50% solid growth will be assessed (as described by Pinder et al.).[3]

STUDY CODES DCIS ARCHITECTURE:

- 0 = < 50% of ducts affected by DCIS show a solid growth pattern.
- 1 = ≥50% of ducts affected by DCIS show a solid growth pattern.

3. Necrosis

Necrosis is classified into two categories: no or single cell necrosis versus any amount of comedonecrosis. Comedonecrosis is defined by areas of confluent dirty necrosis, i.e. confluent eosinophilic material, often containing ghost cells and karyorrhectic debris, which is easily detected at low magnification. Necrosis is assessed without taking nuclear grade into account.

STUDY CODES NECROSIS :

- 0 = no necrosis or single-cell necrosis.
- 1 = any amount of comedonecrosis.

4. Intraductal calcifications

Intraductal calcifications within the DCIS lesion are scored either as present or absent (regardless the size and regardless the number of ducts with calcifications).

STUDY CODES INTRADUCTAL CALCIFICATIONS :

- 0 = absent
- 1 = present

5. Periductal stromal changes

The architecture of the periductal stroma is recorded as either sclerotic or myxoid. Myxoid stroma is defined as loosely arranged collagen fibers, often interspersed with an amorphous, slightly basophilic substance (illustrated in the DCISion poster and in the photographs in Van Bockstal et al.)[2, 4]. Stromal architecture is divided into 2 categories (< 33% or ≥ 33% of ducts surrounded by myxoid stroma), and preferentially assessed at low magnification.[2, 4]

CAVEAT: try not to assess periductal stromal changes near the site of a previous biopsy.

STUDY CODES PERIDUCTAL STROMAL ARCHITECTURE:

- 0 = SCLEROTIC = no or limited amount of myxoid stroma i.e. < 33% (1/3) of ducts shows myxoid changes in the periductal stroma.
- 1 = MYXOID = extensive myxoid stroma i.e. ≥33% (1/3) of ducts show myxoid changes in the periductal stroma.

6. Stromal inflammation

The presence and degree of a chronic inflammatory infiltrate in the periductal stroma (regardless of its architecture) was recorded in a semi-quantitative manner as described by Pinder et al.,[3] and preferentially assessed at low magnification. Definitions for the current study are based on previous findings.[2]

CAVEAT: try not to assess stromal inflammation near the site of a previous biopsy.

STUDY CODES STROMAL INFLAMMATION:

- 0 = LOW = absent or mild stromal inflammation. The periductal stroma is not infiltrated by lymphocytes, or the periductal stroma surrounding the affected ducts is infiltrated by few loosely arranged lymphocytes with apparent intervening stroma. Dense lymphocytic aggregates are absent. The nature of the stromal architecture is easy to perceive.
- 1 = HIGH = moderate to extensive inflammation. The periductal stroma contains a chronic inflammatory infiltrate that consists of at least one lymphoid aggregate (i.e. any infiltrate that is more than just loosely arranged lymphocytes; this means lymphocytes stick together without intervening collagenous stroma). Lymphoid follicle formation can be present but is not a prerequisite. Assessment of the stromal architecture can be hampered by the density of the inflammatory infiltrate.

7. Tumor-infiltrating lymphocytes (TILs)

The stromal inflammatory infiltrate mainly consists of lymphocytes. The DCISion study would like to compare semi-quantitative assessment of this infiltrate (as mentioned under 6.) with assessment of the percentage of TILs. Therefore, TILs will also be assessed according to the standardized method as proposed by the International Immuno-oncology Biomarkers Working Group [5]. The number of TILs should be noted in two different ways. First, assess the TILs as a percentage, as has been described by Hendry et al. and Pruneri et al. [5,6] Next, you note this TILS percentage as a dichotomous feature with the following cut-off: <50% versus ≥50%.

STUDY CODES TILs (for dichotomous assessment):

- 0 = LOW = <50% of TILs in the surrounding stroma, according to the method described by Hendry et al. [5] (see DCISion poster)
- 1 = HIGH = ≥50% of TILs in the surrounding stroma, according to the method described by Hendry et al. [5] (see DCISion poster)

8. Lobular cancerization

Lobular cancerization is defined as the presence of DCIS tumor cells within breast lobules, with preservation of the normal lobular architecture. Lobular cancerization is assessed as either absent of present in the digital slide, regardless its extent.

STUDY CODES LOBULAR CANCERIZATION :

- 0 = absent
- 1 = present

REFERENCES

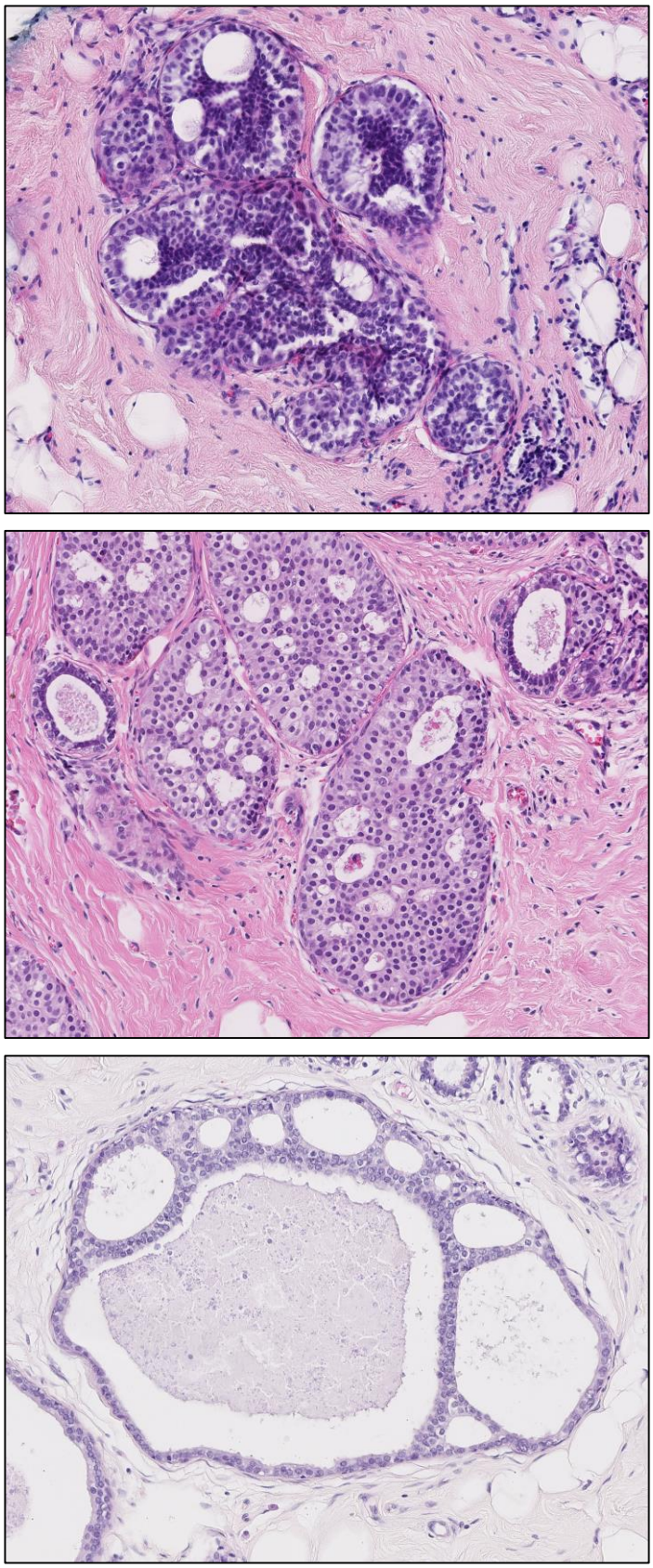
1. Lester SC, Bose S, Chen YY, Connolly JL, de Baca ME, Fitzgibbons PL, Hayes DF et al. Protocol for the examination of specimens from patients with ductal carcinoma in situ of the breast. Arch Pathol Lab Med 2009 (133): 15-25.
2. Van Bockstal M, Baldewijns M, Colpaert C, Dano Hélène, Floris G, Galant C, Lambein K, Peeters D, Van Renterghem S, Van Rompuy AS, Verbeke S, Verschuere S, Van Dorpe J. Dichotomous histopathological assessment of ductal carcinoma in situ of the breast results in substantial inter-observer concordance. Histopathology 2018 (73): 923-932.
3. Pinder S, Duggan C, Ellis IO, Forbes JF, Bishop H, Fentiman IS, George WD. A new pathological system for grading DCIS with improved prediction of local recurrence: results from the UKCCC/ANZ DCIS trial. British Journal of Cancer 2010 (103): 94-100.
4. Van Bockstal M, Libbrecht L, Floris G, Lambein K. The Baader-Meinhof Phenomenon in Ductal Carcinoma in Situ of the Breast. Histopathology 2016 (69): 522-528.
5. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B et al. Assessing Tumor-infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method From the International Immunooncology Biomarkers Working Group: Part 1: Assessing the Host Immune Response, TILs in Invasive Breast Carcinoma and Ductal Carcinoma In Situ, Metastatic Tumor Deposits and Areas for Further Research. Adv Anat Pathol 2017(24): 235-251.
6. Pruneri G, Lazzeroni M, Bagnardi V, Tiburzio GB, Rotmensz N, DeCensi A, Guerrieri-Gonzaga A et al. The prevalence and clinical relevance of tumor-infiltrating lymphocytes (TILs) in ductal carcinoma in situ of the breast. Ann Oncol 2017(28): 321-328.

Supplementary Figure 3

Supplementary figure 3. The DCISion poster, with a series of images for each histopathological feature.

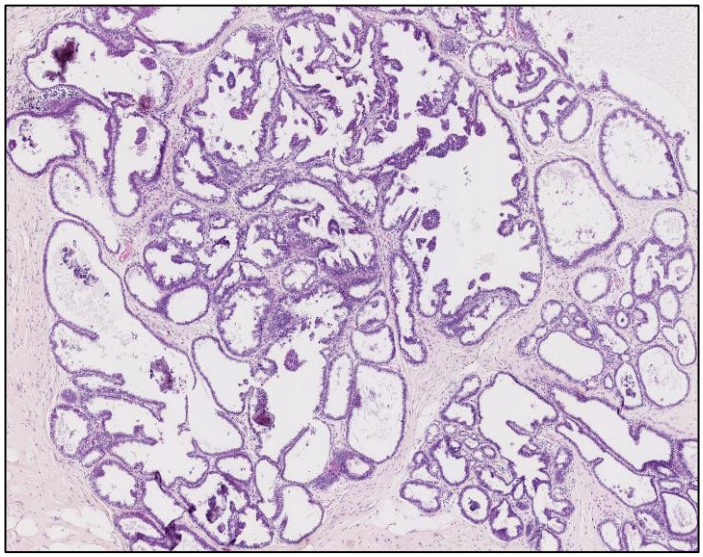
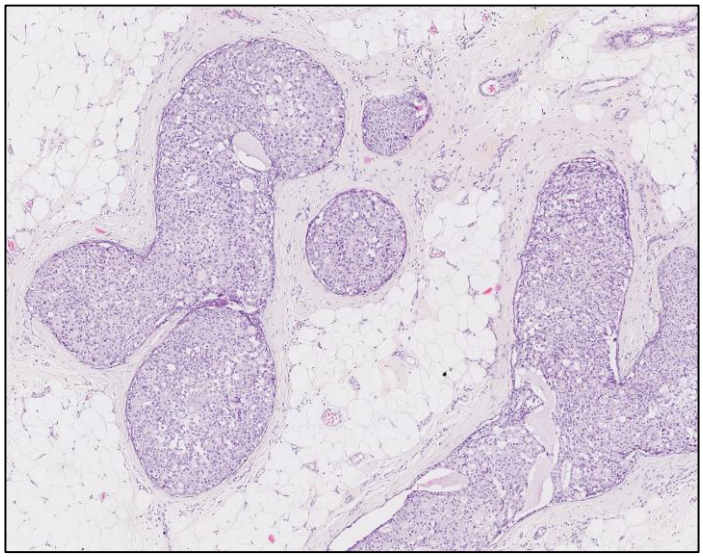
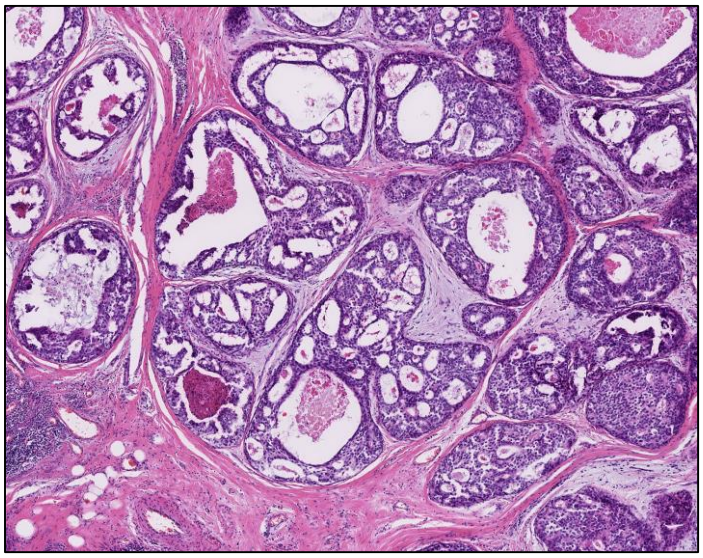
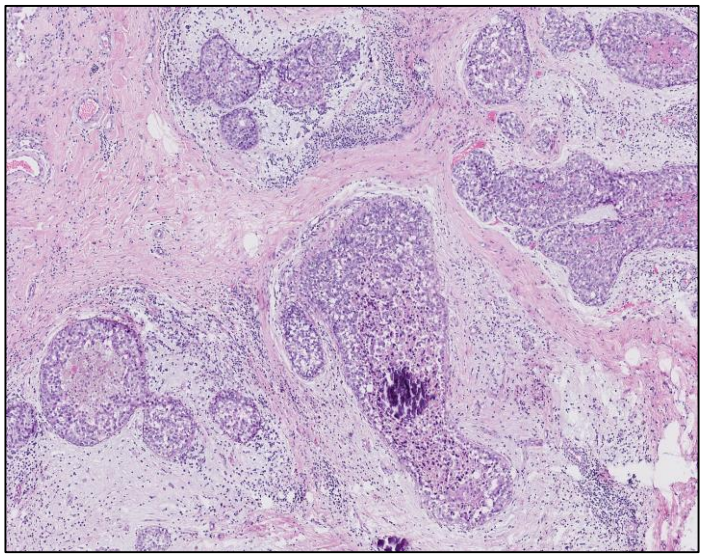
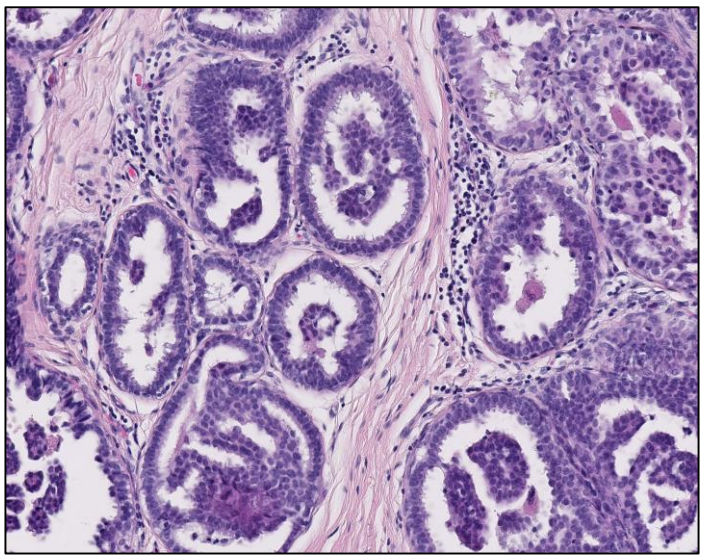
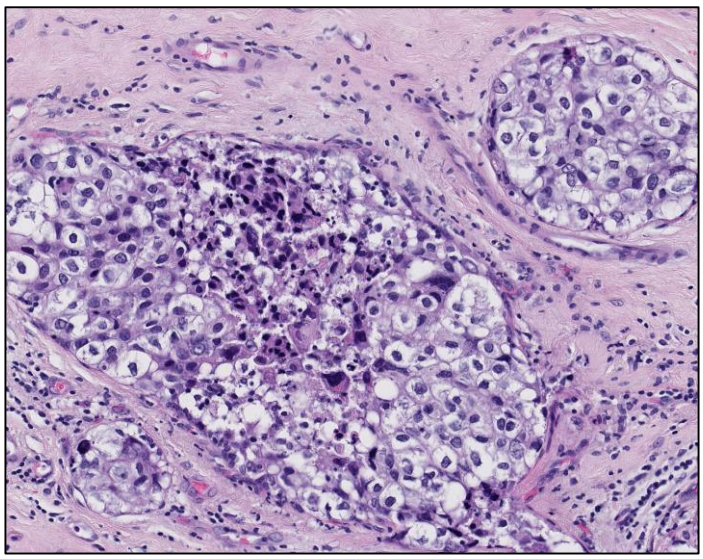
Nuclear grade

Non-high grade	High grade



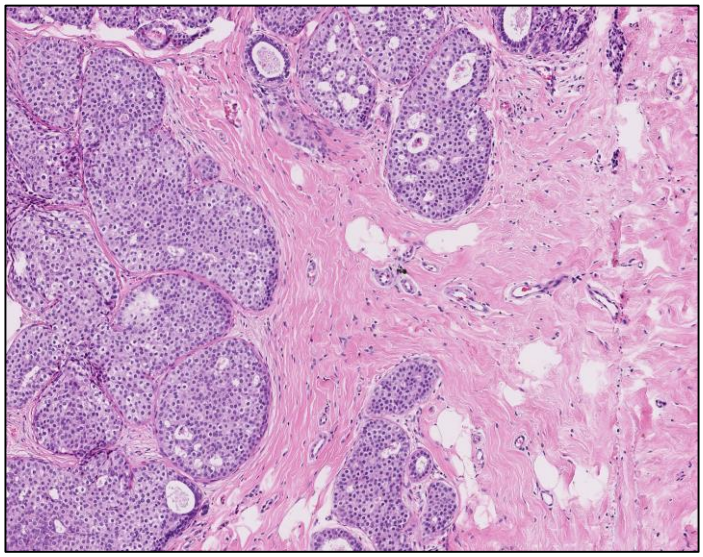
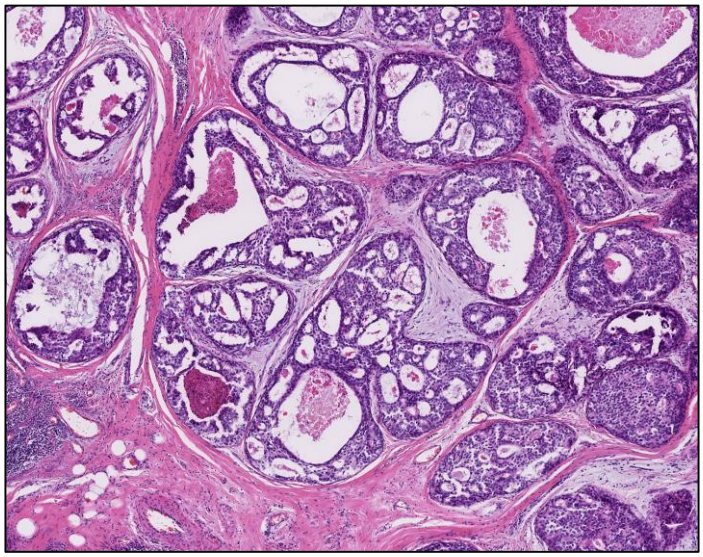
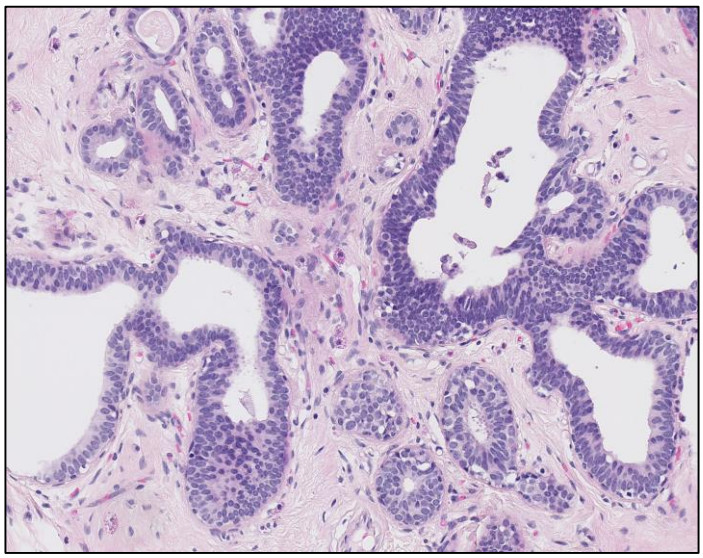
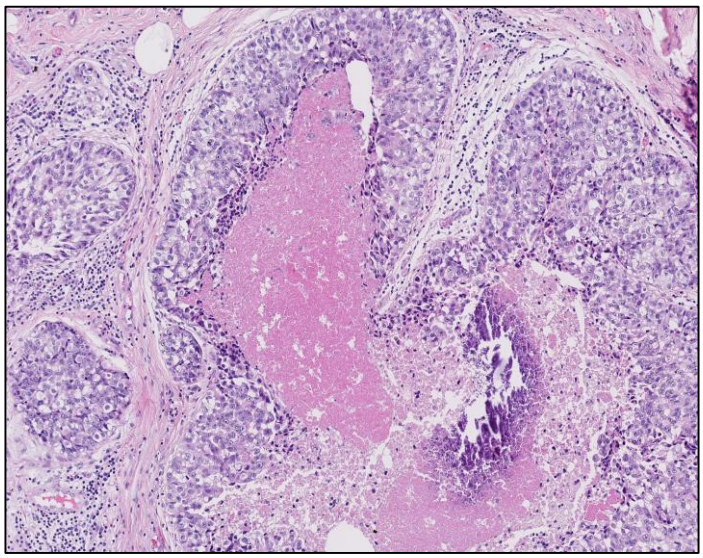
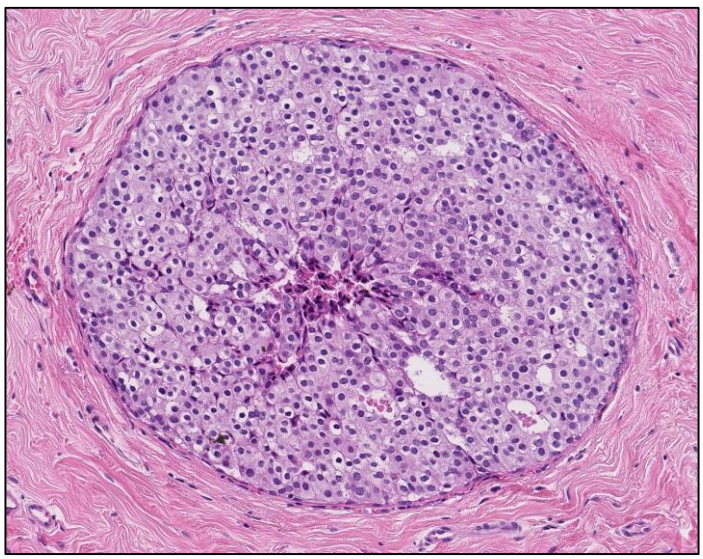
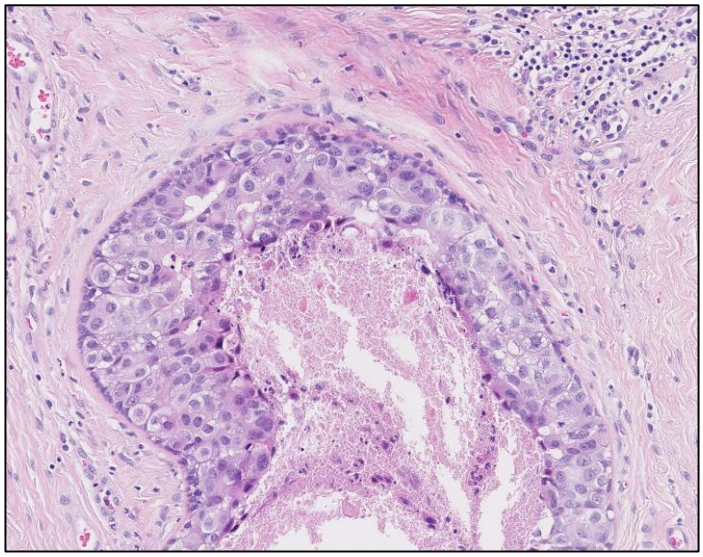
DCIS architecture : growth pattern

Predominantly non-solid	Predominantly solid

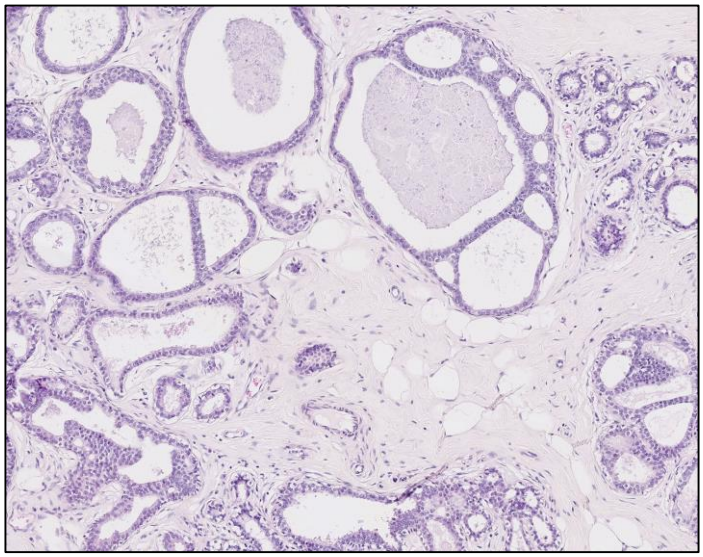
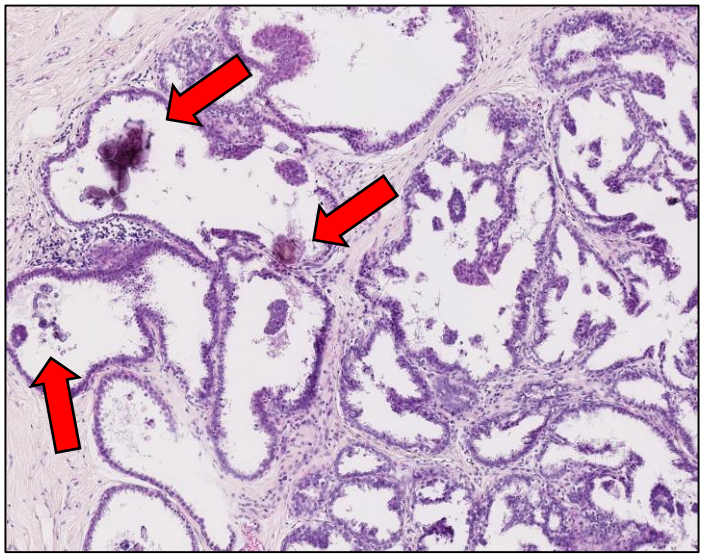
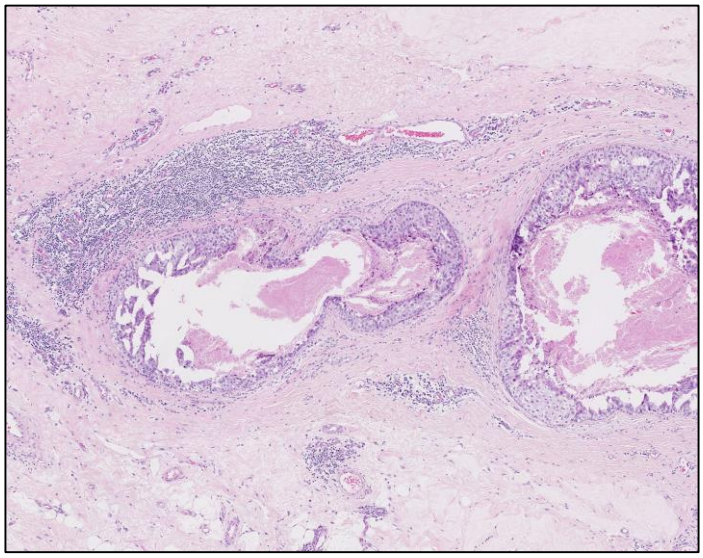
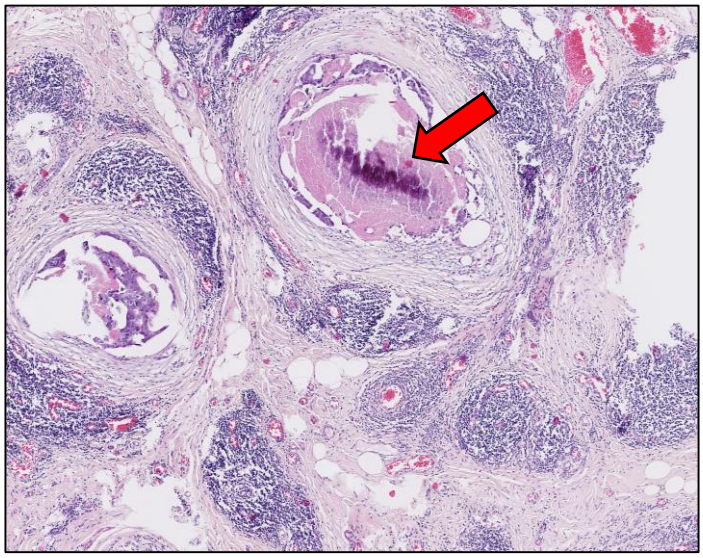
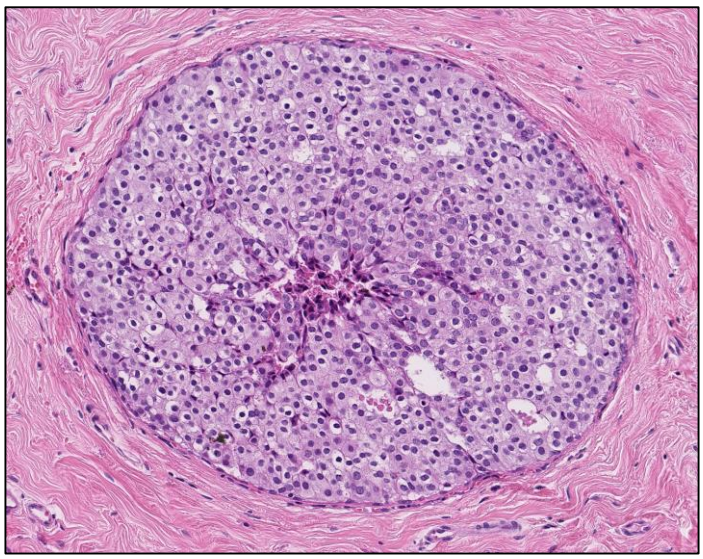
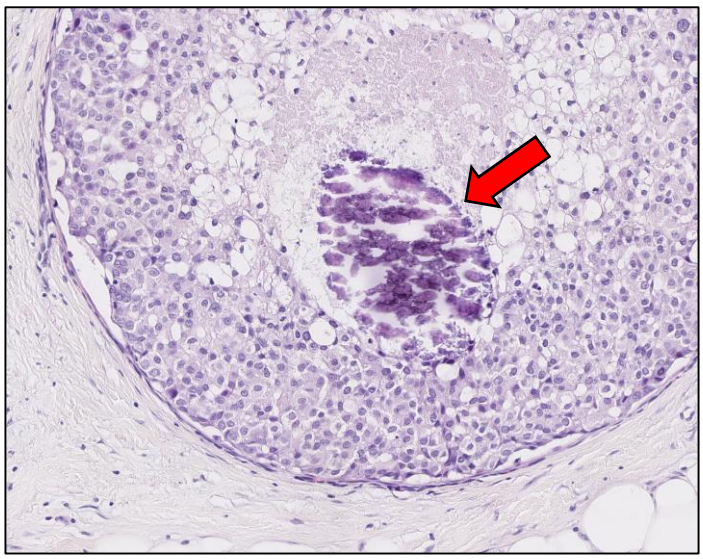







Comedonecrosis

No necrosis or single cell necrosis	Any amount of comedonecrosis

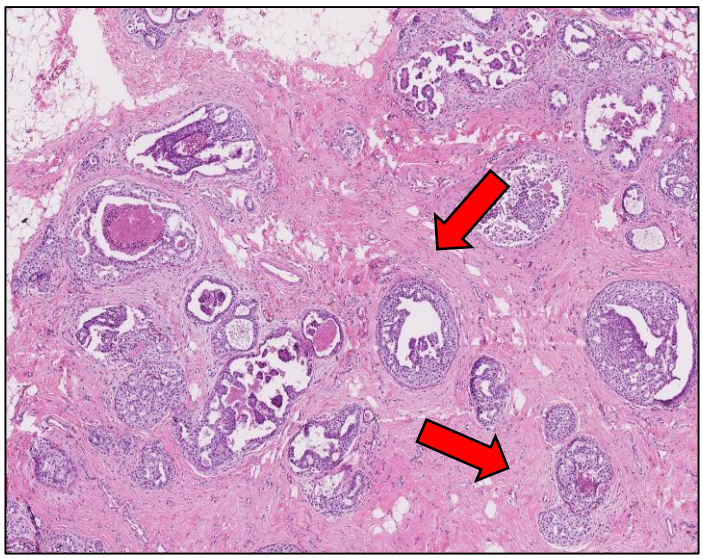







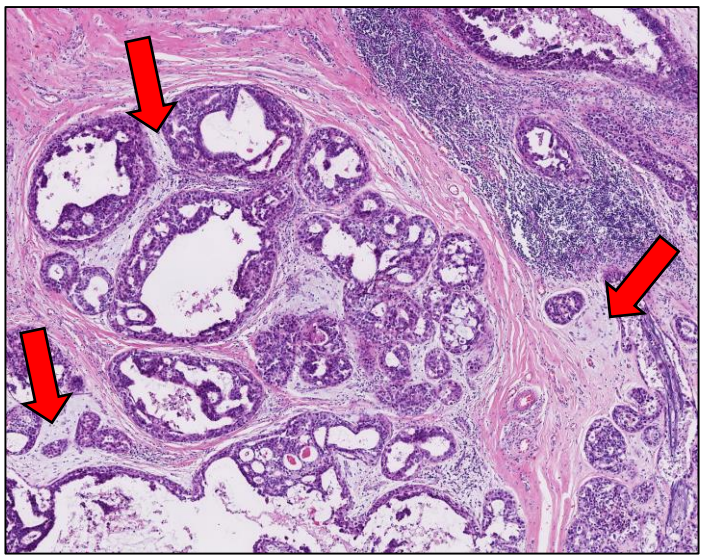
Calcifications

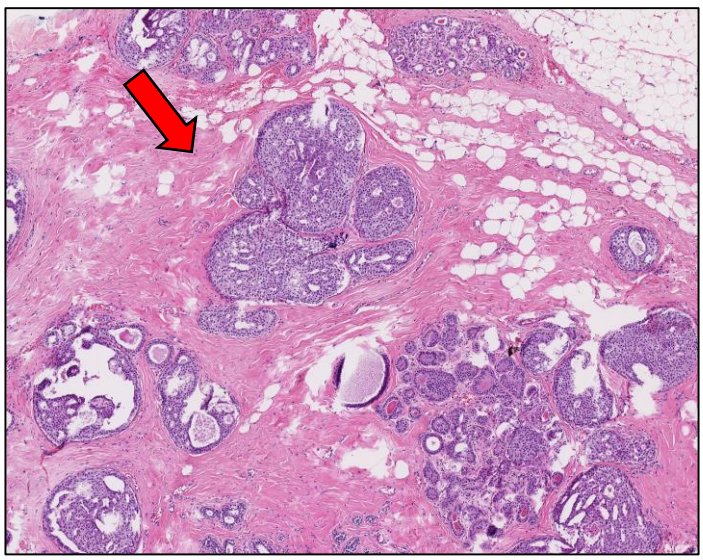
Absent	Present
	
	
	

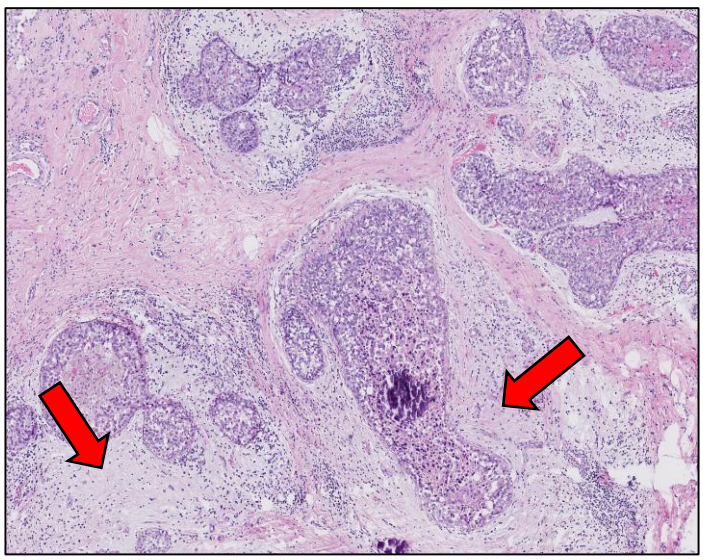
Stromal architecture : periductal stromal changes

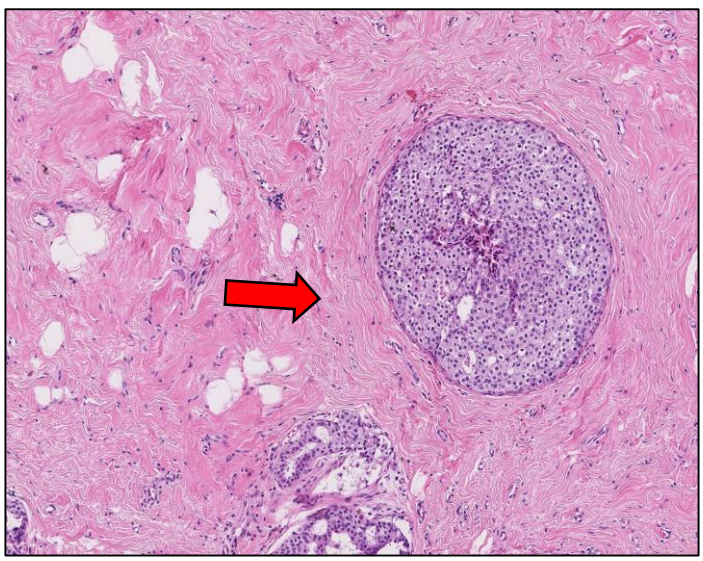
Sclerotic	Myxoid

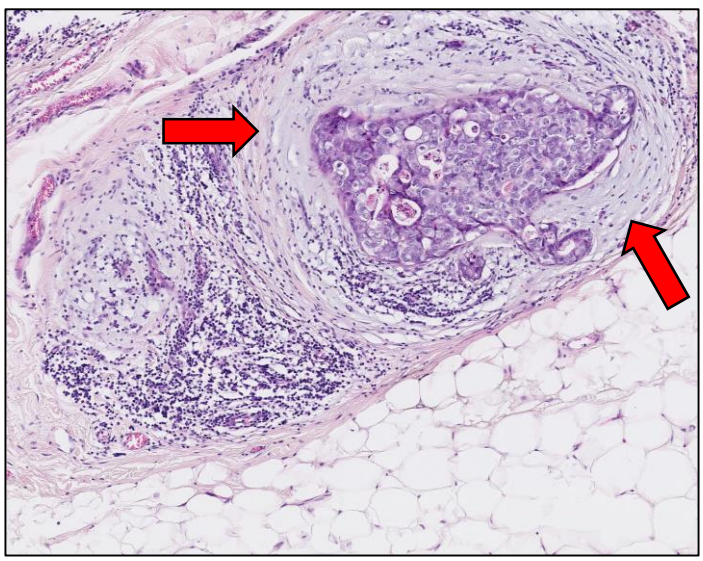




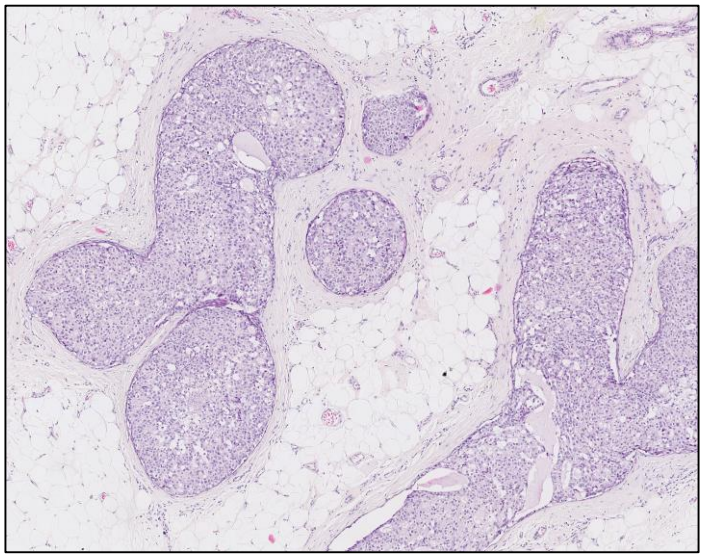
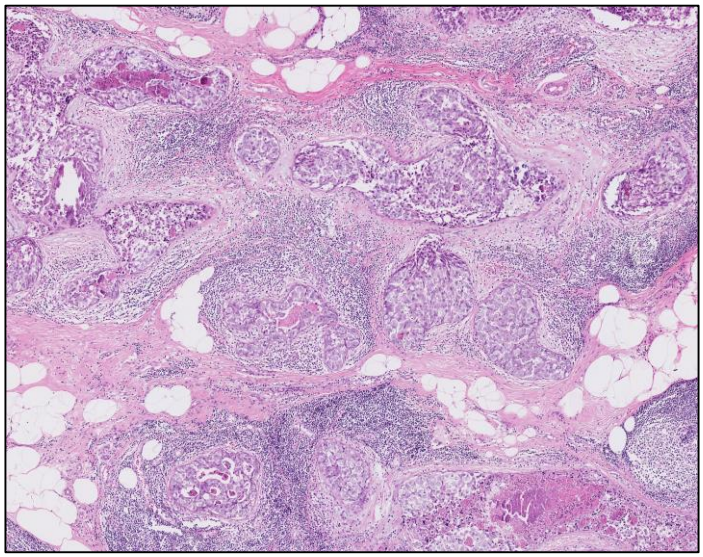
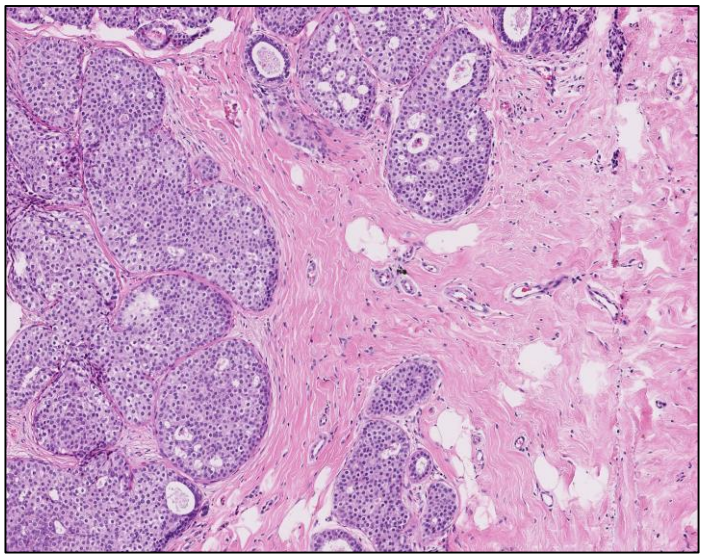
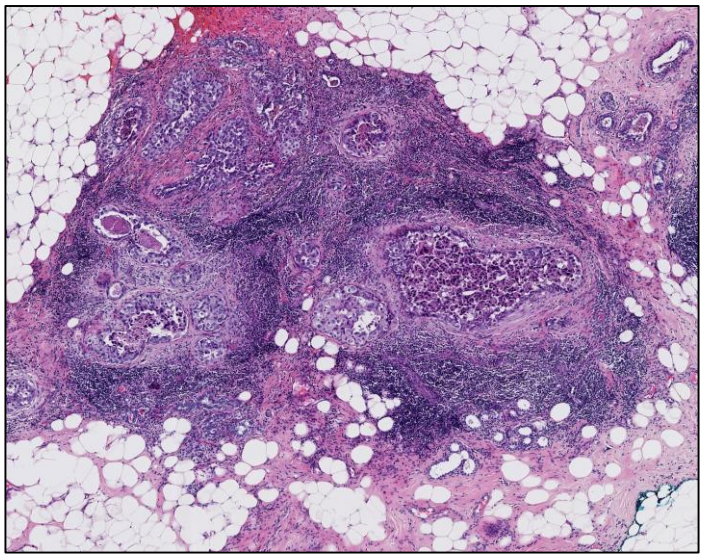
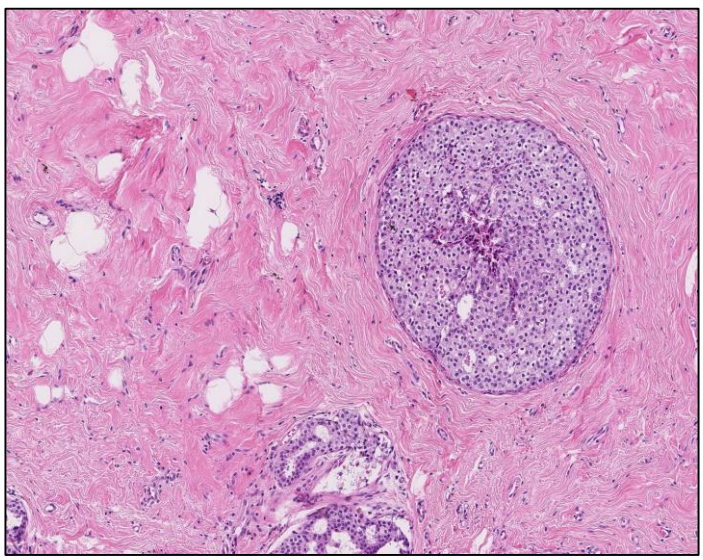
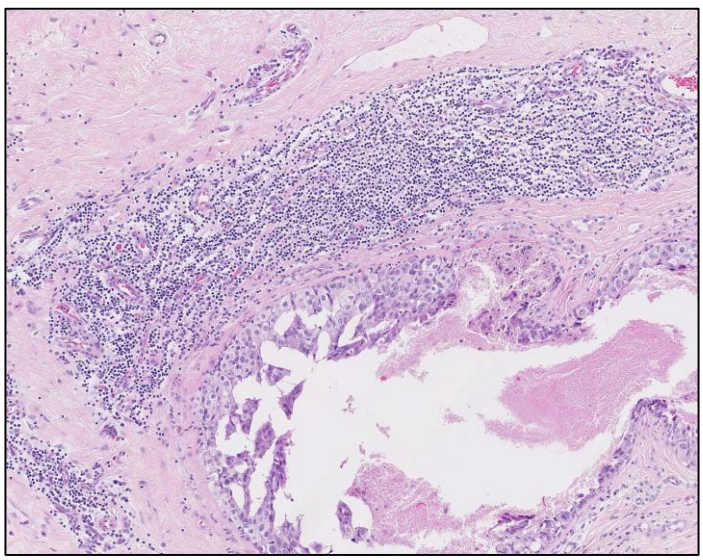






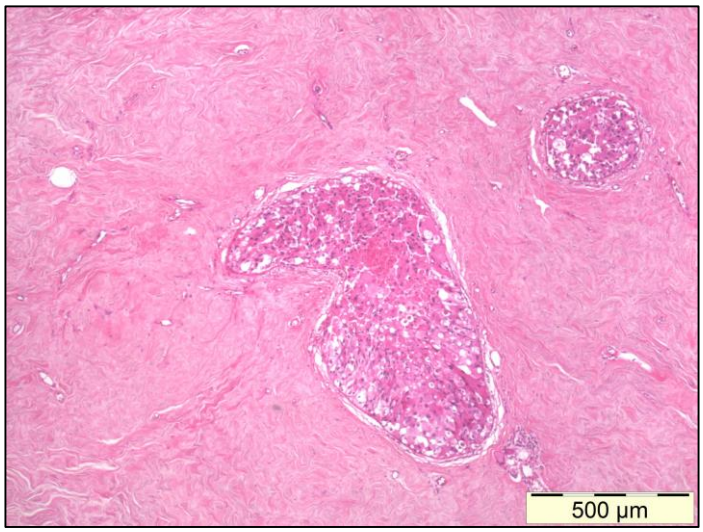


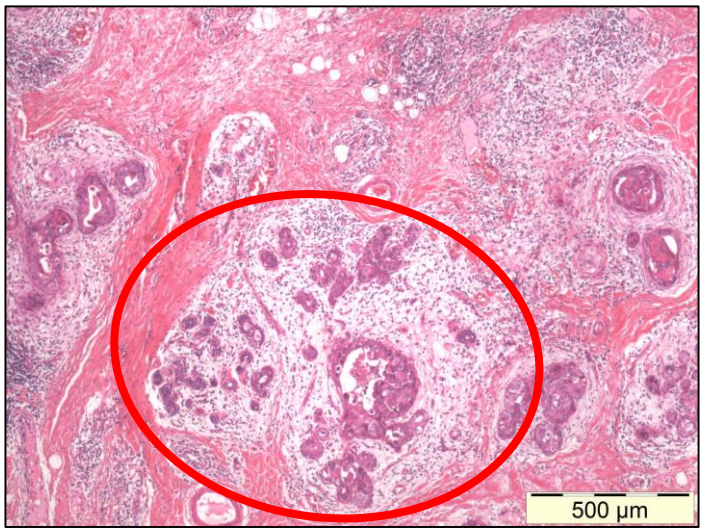
Stromal inflammation

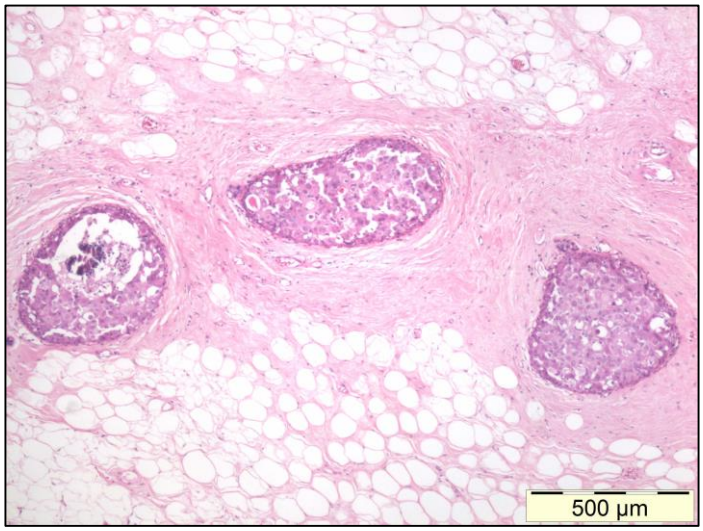
Low	High
	
	
	

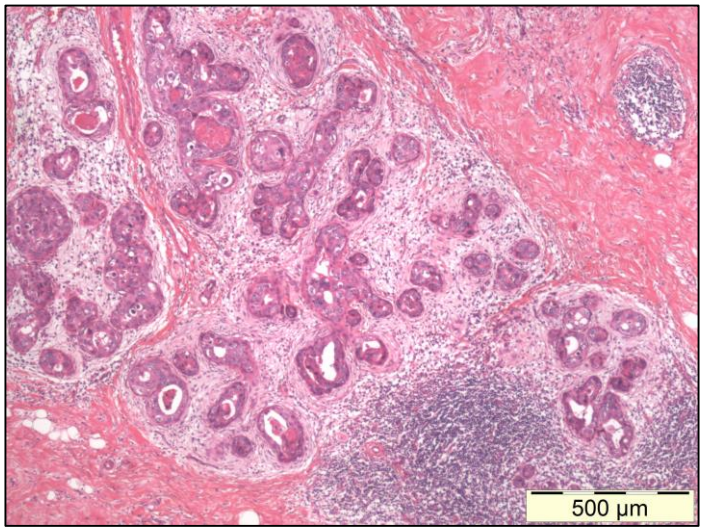
Lobular cancerisation

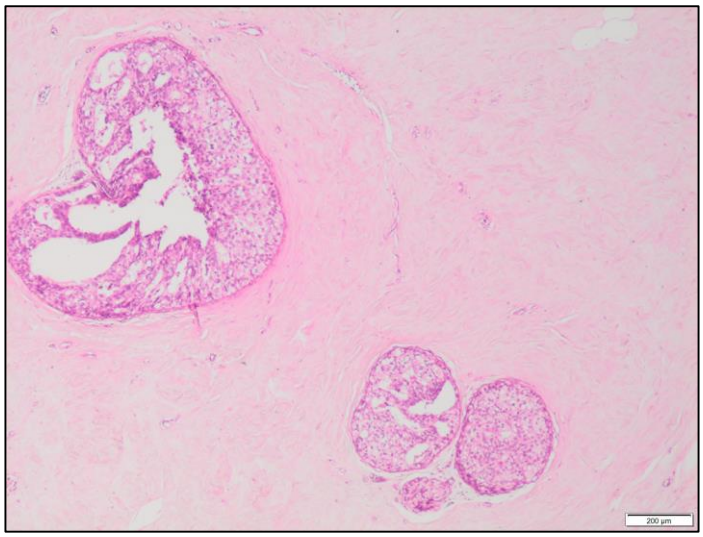
Absent	Present

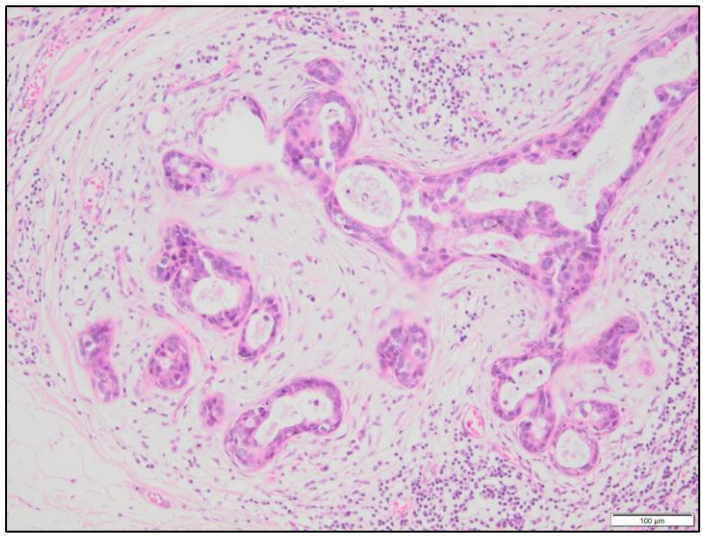










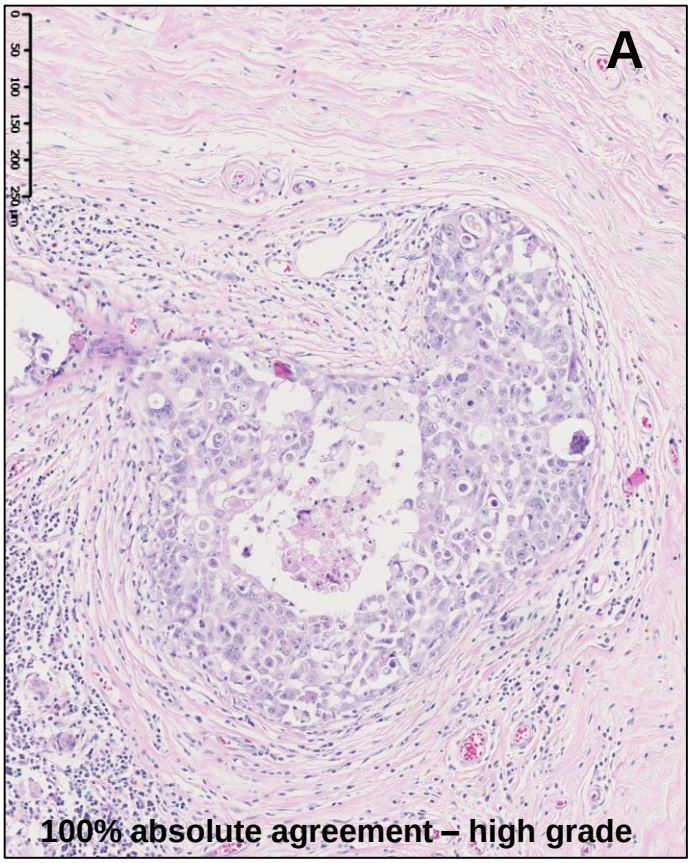


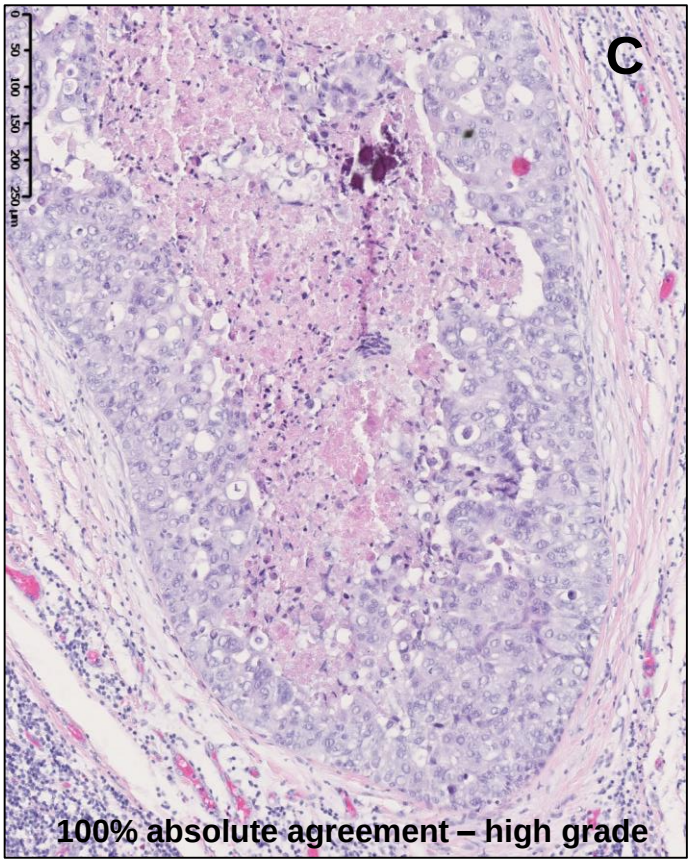
Supplementary Figure 4

Supplementary figure 4. A series of images as part of the DCISion study with 100% absolute agreement regarding nuclear grade (A,C; original magnification 100x), stromal inflammation (E, G; original magnification 40x) and semi-quantitatively assessed TILs with a cut-off of 50% (I, K; original magnification 25x), and a series of images with lowest absolute agreement regarding nuclear grade (B, D; original magnification 100x), stromal inflammation (F, H; original magnification 50x) and semi-quantitatively assessed TILs with a cut-off of 50% (J, L; original magnification 25x).

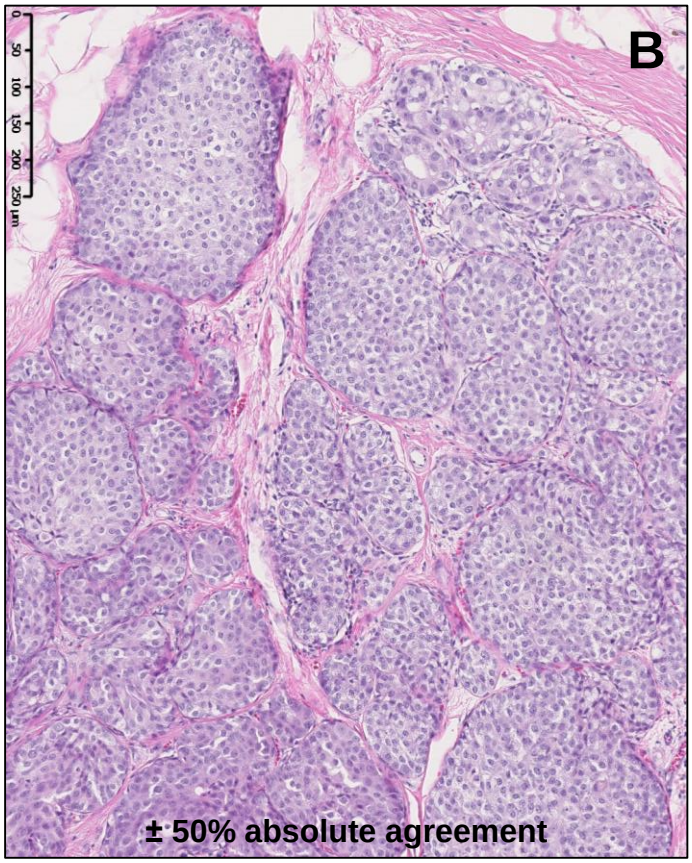
Nuclear grade

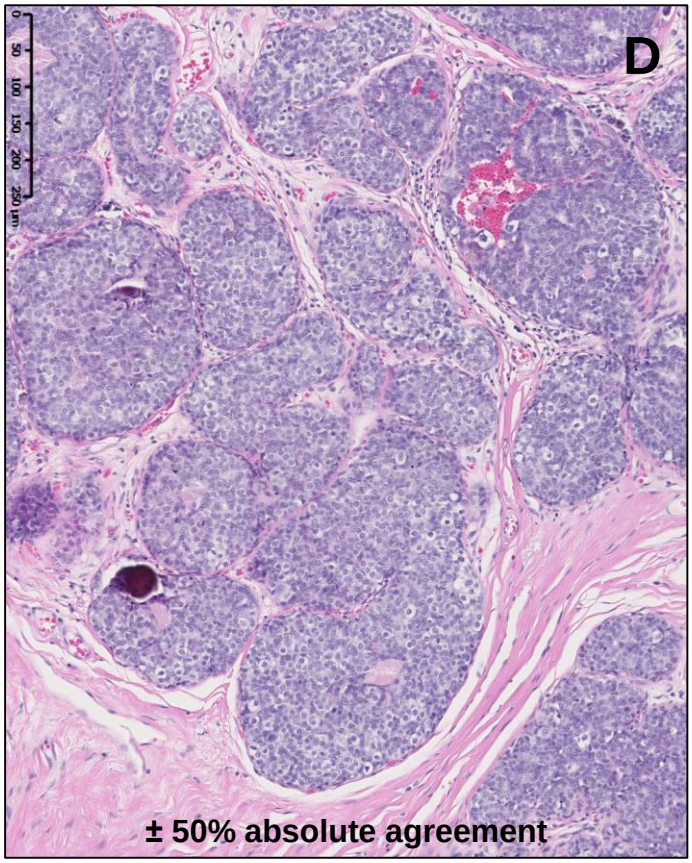
High concordance





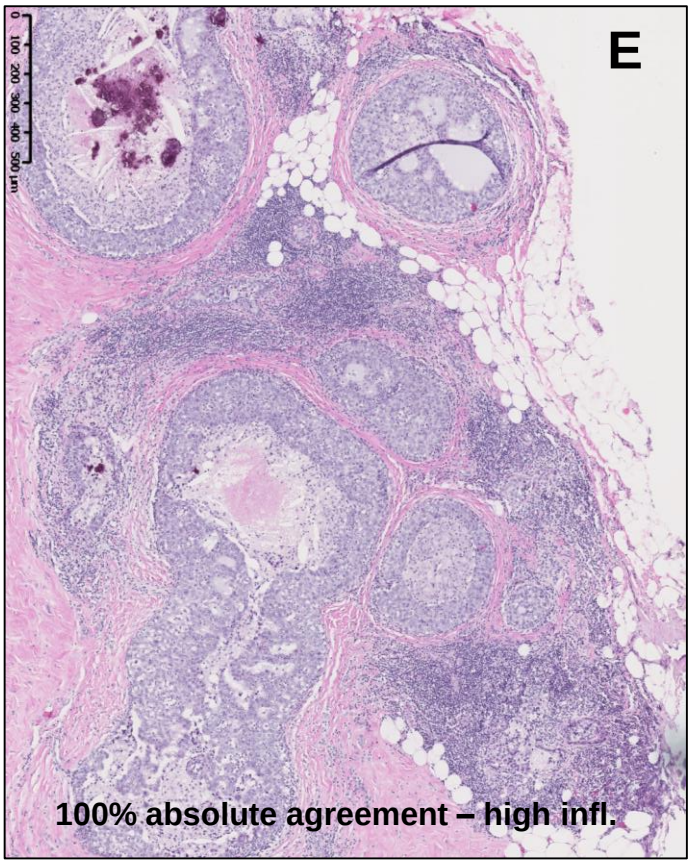
Low concordance

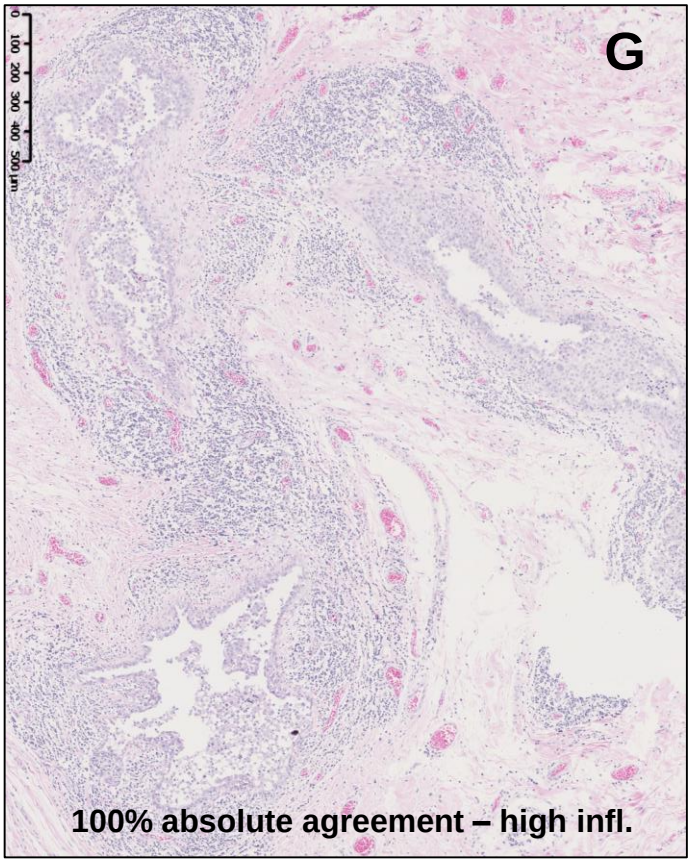




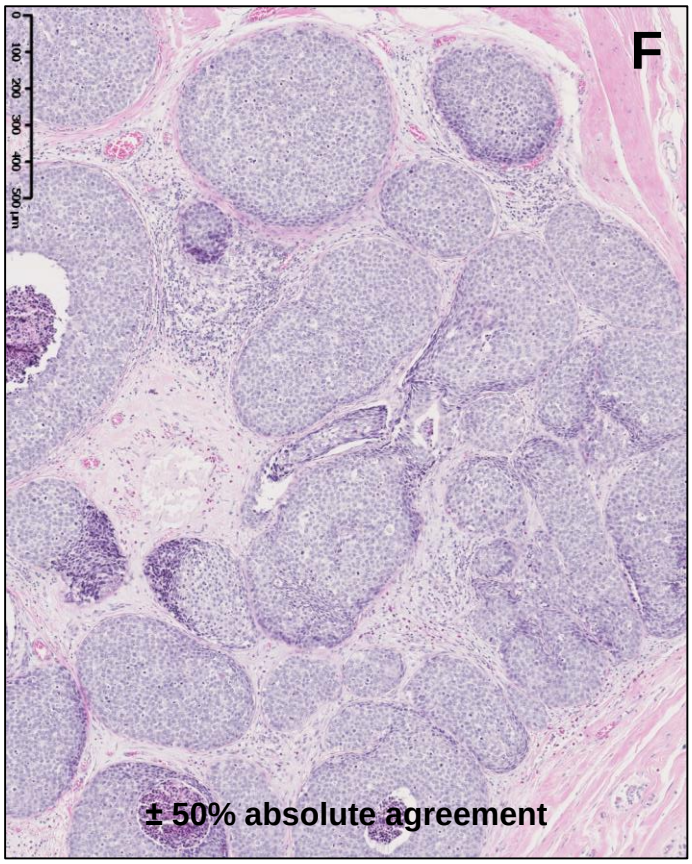
Stromal inflammation

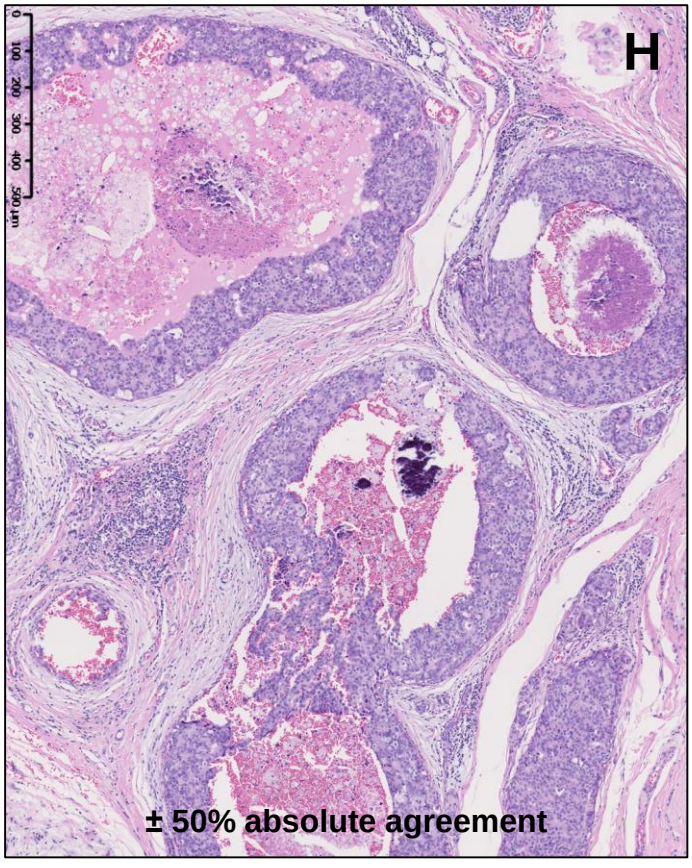
High concordance





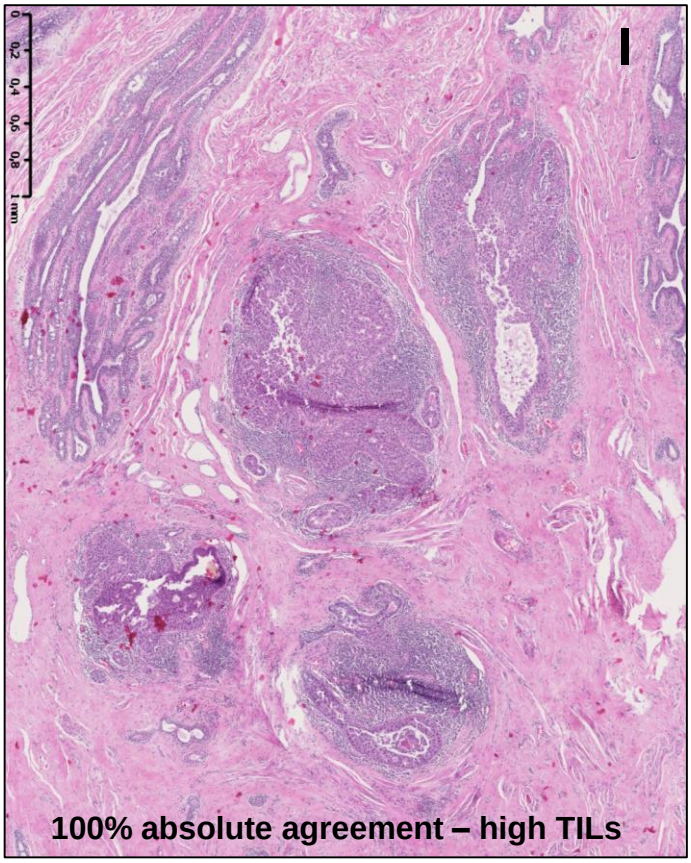
Low concordance

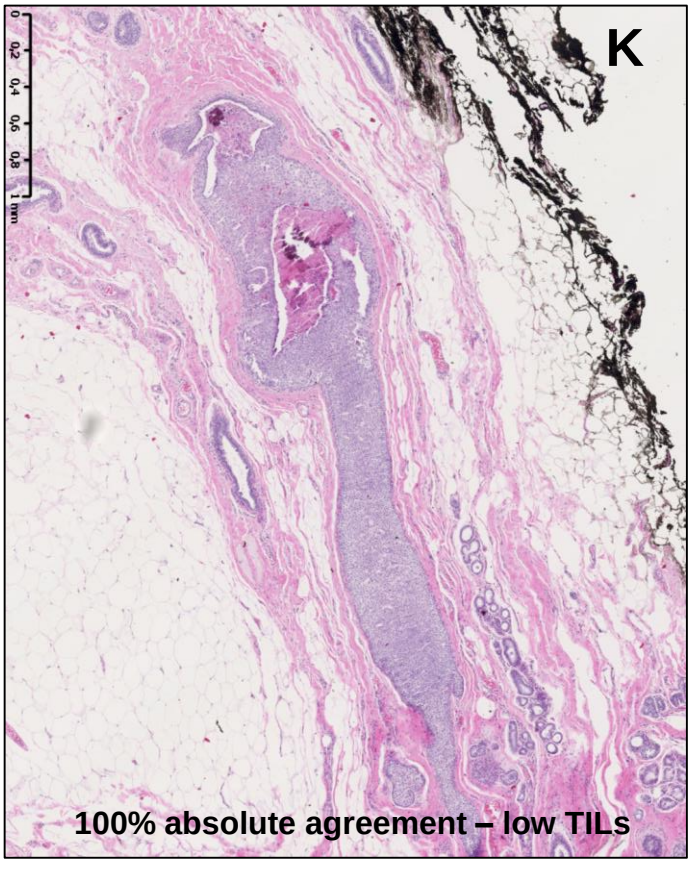




Tumour-infiltrating lymphocytes (TILs)

High concordance





Low concordance