

An optimised patient-derived explant platform for breast cancer reflects clinical responses to chemotherapy and antibody-directed therapy

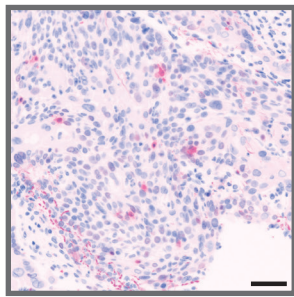
Constantinos Demetriou¹, Naila Abid¹, Michael Butterworth¹, Larissa Lezina¹, Pavandeep Sandhu¹, Lynne Howells¹, Ian R Powley¹, James Howard Pringle¹, Zahirah Sidat², Omar Qassid^{1,3}, Dave Purnell³, Monika Kaushik⁴, Kaitlin Duckworth⁴, Helen Hartshorn⁴, Anne Thomas¹, Jacqui A Shaw¹, Marion MacFarlane^{5,6*}, Catrin Pritchard^{1*}, Gareth J Miles^{1*}

Additional File 4. Optimisation of multiplexed Immuno-Fluorescence

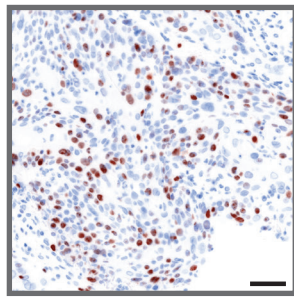
Whole slide mIF-stained FFPE sections of BC-PDEs were digitised using a Vectra Polaris and analysed in InForm. **A**, Captured AF, Ki67, CK and cPARP staining was used to generate pseudo-H&E and -DAB images. InForm was then trained to segment tissue into tumour (yellow), stroma (green), necrosis (red) and background/glass (blue). Next, cells were segmented and phenotyped into Ki67 (green), cPARP (Red) and DAPI (blue). **B**, Representative examples of vehicle- and FET-treated BC-PDEs showing high Ki67 staining in vehicle samples and low Ki67 staining in FET-treated samples as well as high cPARP staining in FET-treated samples, with low staining in vehicle samples. Scale bars = 50 μm . **C**, Representative examples of necrosis shown in mIF, with CK+ve cPARP+ve DAPI-ve, and as a H&E. Scale bars = 20 μm .

Additional File 4

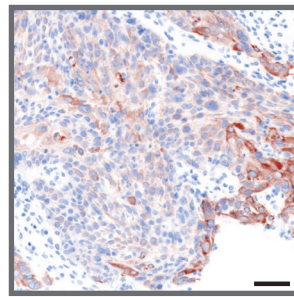
A



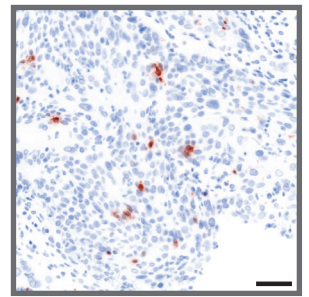
Pseudo-H&E



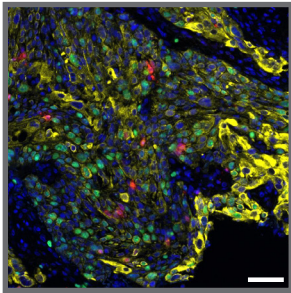
Pseudo-Ki67



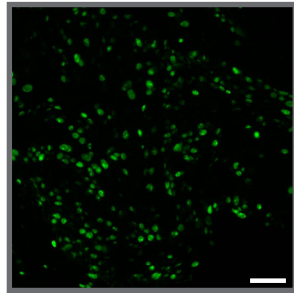
Pseudo-Cytokeratin



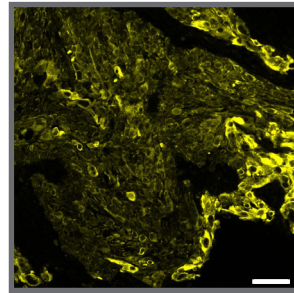
Pseudo-cPARP



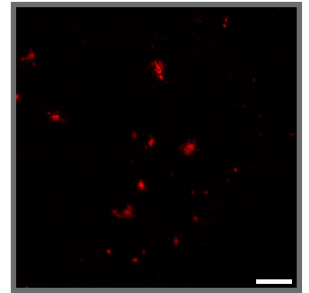
Merge



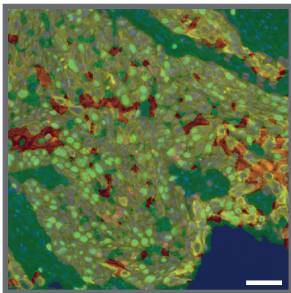
Ki67



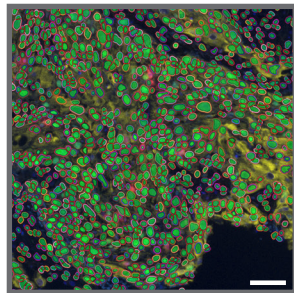
Cytokeratin



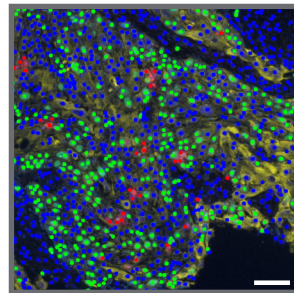
cPARP



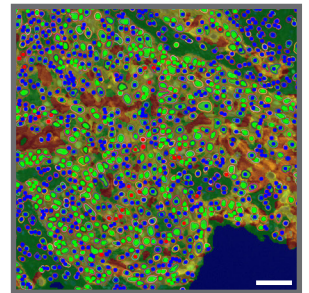
Tissue Segmentation



Cell Segmentation



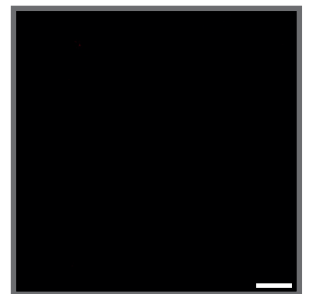
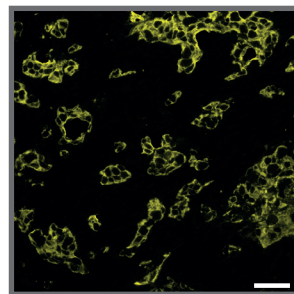
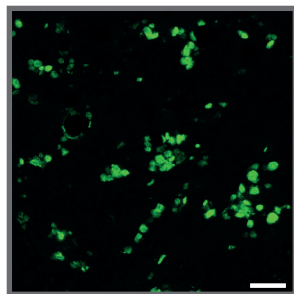
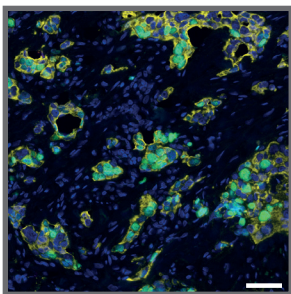
Phenotyping



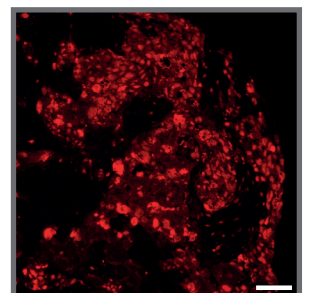
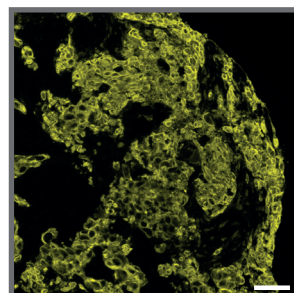
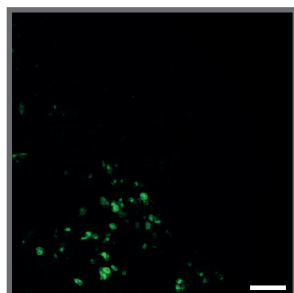
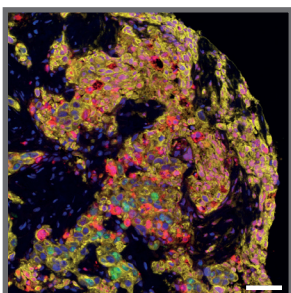
All Processing

B

Control



FET



Merge

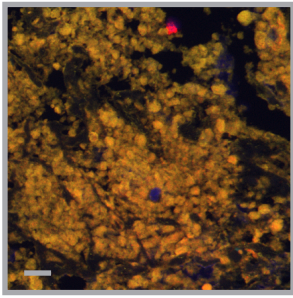
Ki67

Cytokeratin

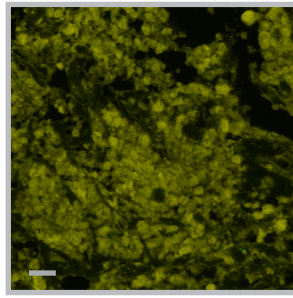
cPARP

Additional File 4

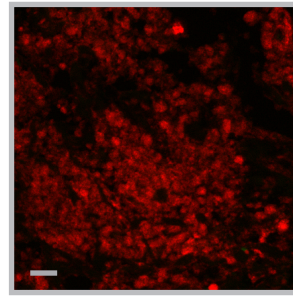
C



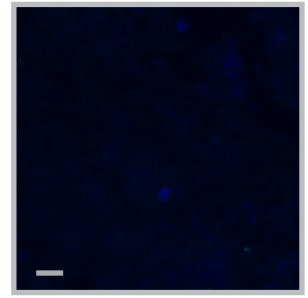
Merge



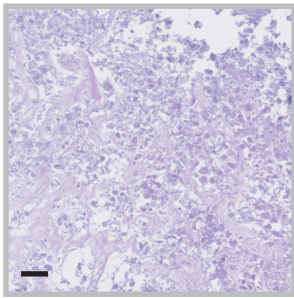
Cytokeratin



cPARP



DAPI



H&E