Increased gene expression variability in *BRCA1*-associated and basal-like breast tumours

Wiggins, George A R¹, Black, Michael A², Dunbier, Anita², Morley-Bunker, Arthur E¹, kConFab Investigators³,⁴, Pearson, John F¹,⁵#, Walker, Logan C¹#*

¹Department of Pathology and Biomedical Science, University of Otago Christchurch, NZ.
²Department of Biochemistry, University of Otago Dunedin, NZ.
³Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, VIC, Australia
⁴Research Department, Peter MacCallum Cancer Center, Melbourne, VIC, Australia
⁵Biostatistics and Computational Biology Unit, University of Otago Christchurch, NZ.
#Contributed equally
*Corresponding author: Logan C. Walker
Tel: +64 3 364 0544
Email: logan.walker@otago.ac.nz
**Supplementary Table 1.** RNAscope scoring criteria

| Score | Criteria |
|-------|----------|
| 0     | No punctuated signal |
| 0.5   | 0-30% of cells with 1-3 punctuate signals/cell |
| 1     | >30% of cells with 1-3 punctuate signals/cell |
| 2     | 4-9 punctuate signals/cell with no clustering |
| 3     | 10+ punctuate signals/cell with <10% of signals clustering |
| 4     | 10+ punctuate signals/cell with >10% of signals clustering |
**Supplementary Table 2.** Clinicopathological data for breast tumours used for RNAscope analysis

|                | TMA8 | TMA9 | TMA10 | TMA11 | TMA12 | TMA16 | TMA17 |
|----------------|------|------|-------|-------|-------|-------|-------|
| N              | 60   | 60   | 60    | 57    | 24    | 121   | 121   |
| BRCA status    |      |      |       |       |       |       |       |
| BRCA1          | 60   | -    | 60    | -     | 24    | 4     | 3     |
| BRCA2          | -    | 60   | -     | 57    | -     | 3     | 4     |
| ER status      |      |      |       |       |       |       |       |
| Positive       | 12   | 30   | 7     | 25    | 1     | 60    | 51    |
| Negative       | 34   | 7    | 34    | 11    | 10    | 19    | 23    |
| Unknown        | 14   | 23   | 19    | 21    | 13    | 42    | 47    |
| PR Status      |      |      |       |       |       |       |       |
| Positive       | 11   | 25   | 8     | 23    | 2     | 56    | 43    |
| Negative       | 34   | 23   | 30    | 10    | 4     | 18    | 20    |
| Unknown        | 15   | 12   | 22    | 24    | 18    | 47    | 58    |
| HER2           |      |      |       |       |       |       |       |
| Positive       | 4    | 5    | 3     | 4     | 1     | 7     | 13    |
| Negative       | 19   | 12   | 14    | 3     | 1     | 14    | 20    |
| Unknown        | 37   | 43   | 43    | 50    | 22    | 100   | 88    |
| CK5            |      |      |       |       |       |       |       |
| Positive       | 31   | 8    | 27    | 5     | 13    | 10    | 8     |
| Negative       | 25   | 48   | 26    | 41    | 9     | 94    | 88    |
| Unknown        | 4    | 4    | 7     | 11    | 2     | 17    | 25    |
Supplementary Fig 1. Transcriptome-wide gene expression variability in breast tumours as measured by gene-specific CV and MAD. *BRCA1*-associated and basal-like breast tumours each show greater gene-specific CV and MAD values compared to BRCAx and non-basal tumour, respectively. A model of equity (red line) was compared to the linear model (blue dashed line) and polynomial regression (sky blue line).
Supplementary Fig 2. Transcriptome-wide gene expression variability in breast tumours as measured by gene-specific SD, CV and MAD. BRCA2-associated gene expression variability is inconsistent compared to BRCAx across the three microarray datasets. In contrast, global gene-specific means between tumour groups are comparable. A model of equity (red line) was compared to the linear model (blue dashed line) and polynomial regression (sky blue line).
Supplementary Fig 3. Correlation of EN1 copy number with EN1 gene expression (left) and EN1 variability (right). Variability is described as the absolute deviation from the median within each copy number status. The linear model (blue) describes the strength of correlation.