**Early enrichment of ESR1 mutations and the impact on gene expression in primary breast cancer treated with aromatase inhibitors in the pre-surgical setting**

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**Supplementary methods**

## E2F activation metagene

The E2F activation metagene was generated using the expression values of 24-gene E2F signature devoid of cell cycle-associated genes previously described by Miller et al (1): *ARHGAP11A*, *ATAD2*, *C10ORF119*, *CASP8AP2*, *CLSPN*, *DCK*, *DNAJC9*, *FANCD2*, *FBXO5*, *FKBP5*, *H2AFZ*, *KIAA0101*, *KPNB1*, *NUP62*, *RANBP1*, *RET*, *SFRS1*, *SFRS10*, *SFRS7*, *SNRPD1*, *STMN1*, *TMPO*, *TREX2* and *ZNF367*. The activation z-score was calculated as previously described by G (2). Firstly, values across all genes for each tumor were added to generate an un-scaled E2F score. The un-scaled E2F score was then standardized to z-score by subtracting from each patient's score the mean score in the cohort, and dividing it by the scores' SD.

## Fluorescence activated cell sorting

Tumours with VAF <1% were validated by ddPCR after fluorescence activated cell sorting (FACS) to enrich the number neoplastic cells evaluated. This approach was also used to confirm the lack of mutation in pre-NAI samples.

FACS sorting enabled separation of stromal cells from cancer cells in disintegrated FFPE tumours. 50-µm section scroll was cut from each FFPE block and was placed into 2-ml eppendorf tube. Section scrolls were then deparaffinized in xylene and rehydrated in a descending alcohol series. Rehydrated tissue sections were incubated in 10 mM sodium citrate buffer to reverse the protein cross-linking. Tissue sections were then disintegrated using enzymatic digestion with collagenase 1A (Merck) and dispase (Merck), as previously described by (3), followed by gentle pipetting. Undigested tissue fragments and large cell clusters were removed by 30-µm filters. Single cell suspensions were labelled simultaneously for cytokeratin (AlexaFluor 488; primary antibody: MNF116, Agilent; AE1/AE3, Merk Millipore), vimentin (AlexaFluor 647; primary antibody: Vim3B4, Agilent), and DNA (DAPI; Merk), and then analyzed using BD FACSAria III flow cytometer. Cytokeratin positive (cancer) cells were separated from vimentin positive (stromal) cells by gating on a dot plot showing green (AlexaFluor 488) versus far red (AlexaFluor 647) fluorescence. Stromal and cancer cell populations were lysed immediately after sorting during overnight incubation. DNA was subsequently extracted using QIAamp DNA FFPE Tissue kit (Qiagen) and 5 ng was used for each ddPCR analysis.

**References**

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