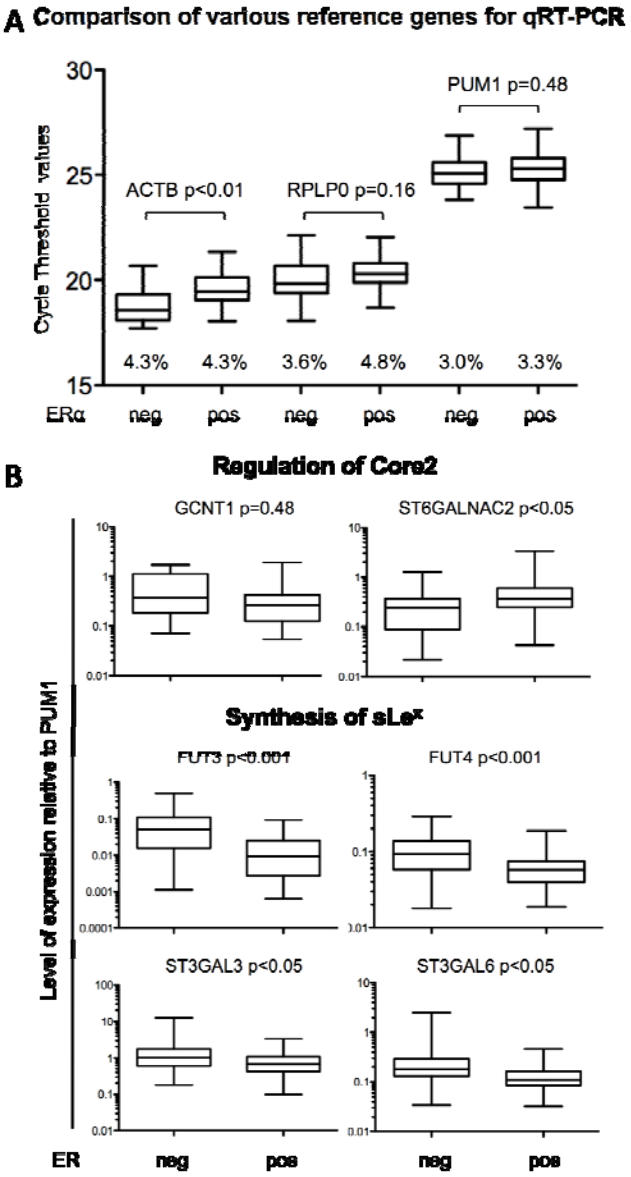
Supplementary Figure 2



Supplementary Figure 2: qRT-PCR analysis of glycosyltransferases expression in breast primary tumours. A: we first assessed the stability of different genes to be used for normalisation: *ACTB, RPLP0* and *PUM1*. In accordance with previous study (1), PUM1 was found to be similarly expressed in ER-negative compared with ER-positive samples with the lowest coefficient of variation for both groups (3% and 3.3% respectively). B: we compared the expression of 6 glyco-genes (*GCNT1, ST6GALNAC2 FUT3, FUT4, ST3GAL4* and *ST3GAL6*) in ER-negative and ER-positive tumours. GCNT1 was more expressed in ER-negative tumours, albeit not significantly, while *ST6GALNAC2* was significantly less expressed, as observed in microarray analyses. Similarly, *FUT3, FUT4, ST3GAL3* and *ST3GAL6* were all found more expressed in ER-negative tumours, in agreement with the microarray data. All PCR reaction were performed in triplicate and repeated twice for each tumour sample. ER negative: n=24, ER positive: n=49, *PUM1:* Pumilio homolog 1. Statistical significance was tested using the Mann-Whitney test on Prism 5 (Graph-Pad Software). All p values were two-tailed and $p<0.05$ was considered as statistically significant**.**

Material and method

RNA extraction, reverse transcription and quantitative real-time-PCR (qRT-PCR) performed on human samples:

Frozen tissue (125mm3) from 73 primary breast tumours were crushed to powder using a mikrodismembrator II (Sartorius, Epsom, UK) and lysed in Qiazol (Qiagen, Crawley, UK). Total RNA was then extracted from lysates using the RNEasy Lipid tissue kit (Qiagen) according to manufacturer's instructions. Reverse transcription and qRT-PCR was performed as indicated in the main text.

1. Lyng MB, Laenkholm AV, Pallisgaard N, Ditzel HJ Identification of genes for normalization of real-time RT-PCR data in breast carcinomas. BMC Cancer 2008;8:20.