Microarray experiments:

The Whole Human Genome Oligo Microarray Kit 4x44K v2 (G4845A, design ID 026652, Agilent Technologies) was utilized in this study. Microarrays of this design type contain 44495 probes, covering roughly 26000 transcripts.

200ng of total RNA were used to prepare aminoallyl-UTP-modified (aaUTP) cRNA (Amino Allyl MessageAmp™ II Kit; #AM1753; Life Technologies) as directed by the company (applying one-round of amplification). Prior to the reverse transcription reaction, 1µl of a 1:5000 dilution of Agilent's 'One-Color spike-in Kit stock solution' (#5188-5282, Agilent Technologies) was added to each single sample. The labeling of aaUTP-cRNA was performed by use of Alexa Fluor 555 Reactive Dye (#A32756; LifeTechnologies).

cRNA fragmentation, hybridization and washing steps were carried-out as recommended in the 'One-Color Microarray-Based Gene Expression Analysis Protocol V5.7', except that 500ng of each labeled cRNA population were used for hybridization.

Slides were scanned on the Agilent Micro Array Scanner G2565CA (pixel resolution 5 µm, bit depth 20). Data extraction was performed with the 'Feature Extraction Software V10.7.3.1' using the extraction protocol file 'GE1_107_Sep09.xml'.

Processed intensity values of the green channel ('gProcessedSignal' or 'gPS') were normalized by global linear scaling: All gPS values of one sample were multiplied by an array-specific scaling factor. This scaling factor was calculated by dividing a 'reference 75th Percentile value' (set as 1500 for the whole series) by the 75th Percentile value of the particular Microarray ('Array I' in the formula shown below). Accordingly, normalized gPS values for all samples (microarray data sets) were calculated by the following formula:

$$normalized\ gPS_{Array\ i} = gPS_{Array\ i}\ x\ (1500 / 75^{th}\ Percentile_{Array\ i})$$

Normalized gPS values of on-chip replicate probes were averaged arithmetically. Measurements that were i) manually flagged, ii) identified as outliers by the Feature Extraction Software, or, iii) lie outside the interval of '1.42 x interquartile range' regarding the normalized gPS distribution of the respective on-chip replicate population, were excluded from averaging.

Finally, a lower intensity threshold (surrogate value) was fixed at 1% of the reference 75th Percentile value (= 15). All of those normalized gPS values that fell below this intensity border, were substituted by the respective surrogate value of 15.