Tumor analysis: freeze–thawing cycle of triple-negative breast cancer cells alters tumor CD24/CD44 profiles and the percentage of tumor-infiltrating immune cells

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Abstract

Objective: The use of novel methods to characterize living tumor cells relies on well-conceived biobanks. Herein, we raised the question of whether the composition of fresh and freeze/thawed dissociated tumor samples is comparable in terms of quantitative and qualitative profiling.

Results: Breast cancer is a heterogeneous disease, encompassing luminal A and B, basal/triple-negative breast cancer (TNBC), and ERBB2-like tumors. We examined living cells dissociated from TNBC and found that a classical freeze/thaw protocol leads to a marked reduction in the number of CD45−CD44LowCD24Low tumor cells. This, in turn, changed the percentage of tumor cells with certain CD44/CD24 expression patterns and changed the percentage of tumor-infiltrating immune cells. These cryopreservation-driven alterations in cellular phenotype make it impossible to compare fresh and frozen samples from the same patient directly. Moreover, the freeze/thaw process changed the transcriptomic signatures of triple-negative cancer stem cells in such a manner that hierarchical clustering no longer ranked them according to expected inter-individual differences. Overall, this study suggests that all analyses of living tumor cells should be conducted only using freshly dissociated tumors if we are to generate a robust scoring system for prognostic/predictive markers.

Keywords: Triple negative breast cancer, Immune infiltrate, Living biobank, Frozen

Introduction

Personalized medicine necessitates identification of biomarkers that are accurate, sensitive, and disease-specific. This is particularly true for cancer, which is a disease characterized by marked tumor heterogeneity. Although thousands of biomarkers have been described, few have translated successfully to the clinic. Biomarkers are crucial not only for tumor diagnosis and prognosis, but also for better stratification of patients, which reduces concerns related to over-treatment of indolent cancers and under-treatment of aggressive cancers.

Among women, breast cancer is the most common cause of cancer, and the second leading cause of cancer death after lung cancer [1, 2]. Triple-negative breast cancer (TNBC) represents 15–20% of all breast cancer cases. It comprises a heterogeneous tumor subset that lacks expression of estrogen and progesterone receptors and does not overexpress HER2. As a group, TNBCs are aggressive and prognoses and clinical outcomes are poor [1]. The low percentage of tumor-infiltrating lymphocytes (TILs) [3] and accumulation of cancer stem cells (CSCs) [4] mean that TNBCs often show drug resistance, recurrence, and metastasis [5, 6]. Therefore, exhaustive characterization of tumor-infiltrating immune cells and
tumor cell heterogeneity is crucial if we are to identify new prognostic and predictive biomarkers and therapeutic targets.

Most, if not all, genomic and proteomic studies are performed using flash-frozen tumor tissues; hopefully, such tissues will yield transcriptomic and/or protein signatures that can be used to develop a personalized medicine approach. However, the complexity and heterogeneity of TNBC tumors mean that clinicians have yet to achieve this goal. We hypothesized that the use of novel methods to exhaustively characterize dissociated and living tumor cells may move us a step closer. For instance, multiparameter flow cytometry (i.e., cyTOF, single cell sequencing) can reveal detailed signatures that are unique to cells inside a tumor (e.g., immune, stromal, and tumor cells) and, by so-doing, identify new markers associated with relapse, and/or targets for a new generation of therapeutic drugs. Accordingly, laboratories will come to rely on well-conceived biobanks to develop dissociated cancer tissues.

Main text
Methods and patients
Patients
Patients (n=15) were diagnosed and treated at the Centre Eugène Marquis between 2017 and 2018. None showed evidence of relapse at the time of diagnosis and none received chemotherapy, endocrine therapy, or radiation therapy prior to surgery. Treatment decisions and follow-up processes were based solely on international recommendations.

Tumor samples
Triple-negative breast tumors were collected by a pathologist after resection by a surgeon and immediately placed in RPMI medium. The dissociation process initiated within 2 h after surgical resection. Tumors were dissociated using the tumor dissociation kit (Human) (Miltenyi Biotec GmbH), which is optimized to deliver a high yield of tumor cells and TILs while preserving important cell surface epitopes. TNBC pieces were weighed and cut into small pieces (<2 mm³), which were then treated with dissociation kit (Human) in a gentleMACS Dissociator, according to the manufacturer’s recommendations. Briefly, tumors were mechanically dissociated in the gentleMACS dissociator for 36 s and then incubated at 37 °C for 30 min under continuous rotation. Next, a cycle of mechanical–chemical–mechanical dissociation was performed and dissociated cells were resuspended in RPMI. Macroscopic pieces were removed using a Corning® cell strainer (70 μm). Tumor cells were then washed twice in RPMI (20 ml) and counted using a hemocytometer.

Flow cytometry
Tumor cells (50,000 cells) were suspended in PBS supplemented with 2% BSA, 2% FCS, and FcR block (Miltenyi Biotec GmbH) at 4 °C for 20 min. Cells were then stained for 30 min at 4 °C with anti-CD24 PE (clone ML5, BD Biosciences), anti-CD44 APC (clone G44-26, BD Biosciences), and anti-CD45 PE-Vio770 (clone 5B1, Miltenyi Biotec GmbH) antibodies. Isotypic antibodies were used as a control for each fluorochrome (obtained from the same manufacturer). Cells were then washed twice in PBS supplemented with 2% BSA and 2% FCS and resuspended in PBS. To assess cell viability, cells were incubated with 7-AAD (BD Biosciences) for 10 min prior to cytometry analysis. Data were acquired using a Novo- cytocyte cytometer (ACEA Biosciences) and analyzed using FlowJo or Novoexpress software.

Cryopreservation and storage
The freezing process was carried out using standardized freezing procedures, following the guidelines issued by the “Haute Autorité de Santé”, the government agency regulating the French healthcare system, for human tissue and cell samples biobanking. Freshly dissociated tumor cells were frozen in 1 ml of human serum albumin (HSA) Vialebex® (LFB BIOMEDICAMENTS, Les Ullis, France) supplemented with 10% DMSO (Sigma Aldrich). Each vial contained 2–5×10⁶ cells (depending on the tumor dissociation yield). Briefly, freshly dissociated cell pellets were resuspended in 500 µl of pre-cooled HSA. Then, 500 µl of pre-cooled HSA containing 20% DMSO solution was added drop by drop to the cell suspension. The suspension was then homogenized and transferred to a cryotube. To ensure a standardized and controlled rate of freezing (−1 °C/min), cryotubes were first placed in a CoolCell® LX Cell Freezing Container (BioCision) at −80 °C. After 24 h, cells were transferred to a freezer set at −150 °C. All freezers were monitored; no critical temperature variations were recorded during storage.

For thawing, cells were placed in a water bath at 37 °C and then transferred to RPMI (40 ml) at RT to allow complete thawing. After a second wash in 20 ml of RPMI, the cells were counted using a hemocytometer. Cell viability was assessed using Trypan Blue.

Generation of mammospheres
Matched freshly dissociated and thawed tumor cells from the same patient were treated in the same way. Cells (1.5×10⁶) were seeded in 2 ml of Mammocult medium (StemCell Technologies) supplemented with heparin (4 µg/ml; Stem Cell Technologies), hydrocortisone (480 ng/ml; Stem Cell Technologies), penicillin (100 units/ml), and streptomycin (100 µg/ml) (Gibco) in
ultra-low binding 6-well plates (Corning). After 15 days at 37 °C/5% CO₂, mammospheres were collected and passed through a cell strainer (40 μm) to separate suspended cells from mammospheres. Next, mammospheres were dissociated with trypsin/EDTA (0.05% trypsin; Gibco) for 5 min. Dissociated cells were washed twice in PBS and stained as described above. RNA extracted from mammospheres using the NucleoSpin RNA XS extraction kit (Macherey–Nagel), according to the manufacturer’s recommendations.

Microarray analysis
RNA quality was assessed using an RNA6000 nano chip (Agilent). For each condition (fresh or freeze/thawed), 9 ng of RNA was reverse transcribed using the Ovation PicoSL WTA System V2 (Nugen, Leek, The Netherlands). Fragmented cDNAs were hybridized to GeneChip Human Gene 2.0 ST microarrays (Affymetrix), which were scanned by a GeneChip Scanner 3000 7G (Affymetrix). Raw data and quality-control metrics were generated using Expression Console software (Affymetrix). Probes were mapped using Brainarray V23 CDF files (http://brainarray.mbni.med.umich.edu/) and normalized by robust multi-array averaging with R software. Statistical analyses were performed using the limma R package; genes showing a twofold change in expression and a P value of 0.05 were considered significant. Gene Ontology terms enrichment analyses were performed using the ToppGene Suite (Chen J, Bardes EE, Aronow BJ, Jegga AG 2009. ToppGene Suite for gene list enrichment and candidate gene prioritization. Nucleic Acids Research 10.1093/nar/gkp427). Hierarchical clustering was performed using Morpheus Matrix visualization and analysis software (https://software.broadinstitute.org/morpheus/).

Accession numbers
Raw and normalized data have been deposited to the GEO database accession ID GSE114359.

Results
We raised the question of whether the composition of fresh and freeze/thawed samples from the same patients was sufficiently similar in terms of quantitative and qualitative profiling and asked whether both could be used to investigate TILs (CD45-positive cells) and the phenotype (CD45negCD24LowCD44High) of CSCs. To address this, we used multiparameter flow cytometry to compare the phenotypes of 15 freshly dissociated TNBCs and their matched cryopreserved counterparts. Briefly, to minimize mortality due to sample processing time, the pathologist collected samples in RPMI medium and the dissociation process was started within a maximum of 120 min after surgical resection. Fresh TNBCs were dissociated using the Miltenyi Tumor dissociation kit (Human); 0.8 million cells were analyzed. In parallel, we cultured 1.5 × 10⁶ cells in serum-free medium in low-attachment plates to isolate spheroids, which are enriched in CSCs (see “Methods and patients” section). The remaining cells were frozen using a standardized procedure following the French “Haute Autorité de Santé” guidelines to biobanks and kept for 2 weeks in a freezer at −150 °C. The cells were then thawed and analyzed immediately by flow cytometry; spheroids were also generated. Total RNA was isolated from fresh and thawed spheroids and the transcriptomic signatures compared (see Additional file 1: Figure S1).

Cell viability was assessed by 7-AAD staining. The results showed that the freezing/thawing procedure for dissociated TNBC cells yielded 25.4% dead cells, with five dissociated tumor samples showing a drop in absolute numbers of 10% or less, and four tumors showing more than 50% dead cells (Fig. 1a) after thawing. The reasons for this were unknown since all samples were treated in a similar manner. Strikingly, most of the cells that died following the freezing/thawing process originated from the CD45− population (Fig. 1b), which in turn impacted the percentage of tumor-infiltrating leukocytes (i.e., CD45+ cells; Fig. 1b). Of note, the percentage of tumor cells with a CD45−CD24LowCD44Low phenotype fell markedly after thawing (Fig. 1c, d). Two hypotheses may explain these losses: either these cells are highly sensitive to freezing/thawing, or they are reprogrammed to exhibit a different CD24/CD44 phenotype. The percentage of dead cells observed in Fig. 1a and the short delay between thawing and immunophenotyping are not compatible with the latter hypothesis, strongly suggesting that CD45−CD24LowCD44Low cells do not tolerate the freeze/thaw process well. Loss of this population resulted mainly in artificial enrichment of the CD24High/CD44Low cell population and, albeit to a lesser extent, the CD24LowCD44High and CD24HighCD44High populations (Fig. 1d). Because the CD24LowCD44High lineage was enriched after the freeze/thaw cycle, and this cell subset seems to correspond to stem/progenitor cancer cells [5, 6], we next wondered whether the transcriptomic signature of these enriched CSCs varied after the freezing/thawing process. To compare the transcriptome of CSCs derived from fresh and frozen samples, we used stringent cell culture procedures to culture spheroid cells. Compared with the bulk of dissociated tumor cells, spheroids were enriched for CD24LowCD44High cells (Fig. 2a). Next, we examined freshly dissociated TNBC cells from three different TNBC patients, along with the cells from the same patients frozen immediately after dissociation and cryopreserved for 15 days before thawing. Both sets of
cells were cultured for 15 days in serum-free medium in low-adherence plates to select CSCs (CD44^{High}CD24^{Low}) and the transcriptomic signatures were compared (Fig. 2b). Unexpectedly, unsupervised hierarchical clustering of these tumor cells ranked tumors according to whether they had been subjected to freeze/thaw treatment and not according to their inter-individual differences; this shows that freeze/thawing the cells had a deleterious effect in their transcriptomic signatures. Gene Ontology terms enrichment analysis revealed that the freeze/thaw process drove deregulation of 274 genes (-fold change > 2, P < 0.05); most of these were associated to epithelial to mesenchymal transition (EMT), tissues morphogenesis and cell junction and adhesion (Fig. 2c). Of these, 224 genes (82%) in freeze/thaw spheroids were significantly down-regulated when compared with those in fresh spheroids (Table 1). Because these genes play a role in organization of cell junctions and are lost during freeze/thaw of CSCs, we suggest that cryopreservation either spares breast cancer stem cells showing the most dedifferentiated mesenchymal profiles or favors EMT reprogramming (Fig. 2c and Additional file 2: Table S1).

Discussion

Although one study did examine the impact of cryopreservation on gametes and embryos, this study appears to be the first to examine phenotypic changes caused by freeze/thawing living cells isolated from cancer patients [7].

Although molecular oncology has opened new avenues to classifying human cancers from a molecular standpoint, a number of issues associated with heterogeneous genomic platforms limit their ability to identifying signatures capable of predicting biological events related to the treatment and outcome of tumors.
behavior and/or identifying new molecular targets for more effective and less toxic therapeutic interventions [8]. We identified a novel issue using data meta-analysis based on profiling of fresh and frozen tumor samples; our findings suggest that it will be difficult to identify robust signatures transposable to patients if freeze/thawed dissociated cancer tissues are used.

We strongly discourage the use of frozen dissociated tumor sample for accurate cell population characterization, as thawing creates a disequilibrium in the proportion of immune and tumor cell subpopulations. In addition, because the transcriptomic signatures of mammospheres derived from frozen/thawed samples are not representative of those of initial CSCs, this study rules out their use for identification of new prognostic and/or therapeutic targets. We strongly suggest that future studies involving dissociated tumor cells should be conducted using fresh tumors only; in parallel, tumor biobanks should develop validate methods of freezing living cells isolated from resected tumors that preserves tumor heterogeneity. In this way, we may be able to generate robust scoring systems for prognostic, predictive, and therapeutic markers. Such a system is an unmet clinical need with respect to patients with TNBC.

**Limitations**

Although, the freeze–thaw shock seems mainly to affect immune cells, a more detailed analysis would be required to further investigate whether some minor immune subsets could be affected by this stress and thereby, could also alter the conclusions drawn from analyses performed using freeze/thaw living cells.

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**Fig. 2** Transcriptomic signatures of fresh and freeze/thawed CSCs. **a** Flow cytometry analysis of freshly dissociated tumor cells and spheroids derived from the tumor after 15 days of culture in low serum/low adherence conditions. The dot plot is representative of data obtained from all 15 tumors. **b** Heatmap showing RNA expression by spheroids derived from fresh or frozen tumor cells from three different patients. Hierarchical clustering was performed on Robust Multi-Array Average (rma)-normalized expression data using the One minus Pearson’s correlation method. **c** Overall, 274 genes showed differential expression in spheroids isolated from fresh and frozen tumors. Network representation of GO terms (biological process) significantly enriched in the 224 genes downregulated in freeze/thawed mammospheres as compared to fresh ones. Similar go terms were spatially clustered and colors represent corrected p-values.
## Table 1 Transcriptomic comparison between fresh TNBC cells and their freeze/thawed counterparts

| ProbeID  | Gene name   | logFC | FC  | t   | P value | Adj. P value | B DESC | ENTREZID | ENSEMBL | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|----------|-------------|-------|-----|-----|---------|--------------|--------|----------|---------|-------------|-------------|-------------|--------------|--------------|--------------|
| 102716_at| PRG4        | 2.97  | 7.81| 2.57| 0.04    | 0.85         | Proteoglycan 4 | 10216.00 | ENSG00000116690 | 5.18 | 3.84 | 5.88 | 8.73 | 5.41 | 9.64 |
| 664613_at| MIR544A     | 2.46  | 5.48| 4.28| 0.00    | 0.85         | MicroRNA 544a | 66461.00 | ENSG00000207587 | 4.13 | 2.41 | 2.94 | 4.70 | 6.14 | 6.01 |
| 767568_at| SNORD113-8  | 2.42  | 5.35| 2.56| 0.04    | 0.85         | Small nucleolar RNA, C/D box 113-8 | 767568.00 | ENSG00000201967 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| 407000_at| MR218-1     | 2.31  | 4.95| 2.74| 0.03    | 0.85         | MicroRNA 218-1 | 407000.00 | ENSG00000201967 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| 102723604_at| LOC102723604 | 2.14  | 4.42| 3.04| 0.02    | 0.85         | Uncharacterized LOC102723604 | 102723604.00 | ENSG00000207732 | 4.05 | 3.47 | 3.05 | 4.37 | 6.98 | 7.63 |
| 105369519_at| LOC105369519 | 2.08  | 4.22| 2.52| 0.04    | 0.85         | Uncharacterized LOC105369519 | 105369519.00 | ENSG00000207732 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| 2633_at  | GBP1        | 2.06  | 4.17| 2.62| 0.04    | 0.85         | Guanylate binding protein 1 | 2633.00 | ENSG00000117228 | 5.85 | 3.16 | 3.90 | 6.79 | 6.98 | 5.32 |
| 26822_at | SNORD14A    | 2.05  | 4.13| 3.54| 0.01    | 0.85         | Small nucleolar RNA, C/D box 14A | 26822.00 | ENSG00000207732 | 4.05 | 3.47 | 3.05 | 4.37 | 6.98 | 7.63 |
| 767579_at| SNORD114-3  | 1.99  | 3.97| 2.87| 0.03    | 0.85         | Small nucleolar RNA, C/D box 114-3 | 767579.00 | ENSG00000201967 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| 342908_at| ZNF404      | 1.78  | 3.44| 4.01| 0.01    | 0.85         | Zinc finger protein 404 | 342908.00 | ENSG00000176228 | 7.15 | 5.29 | 4.22 | 7.18 | 6.18 | 7.07 |
| 107983995_at| LOC107983995 | 1.65  | 3.14| 3.44| 0.01    | 0.85         | Small nucleolar RNA, C/D box 114-36 | 107983995.00 | ENSG00000207732 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| 767606_at| SNORD114-26 | 1.64  | 3.11| 3.43| 0.01    | 0.85         | Uncharacterized LOC101366014 | 767606.00 | ENSG00000207732 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| 644612_at| MIR539      | 1.57  | 2.98| 3.38| 0.01    | 0.85         | MicroRNA 539 | 64461.00 | ENSG00000207732 | 7.15 | 5.29 | 4.22 | 7.18 | 6.18 | 7.07 |
| 54518_at | APBB1IP     | 1.57  | 2.97| 2.52| 0.04    | 0.85         | Amyloid beta precursor protein binding family member 7 interacting protein | 54518.00 | ENSG00000116690 | 5.85 | 3.16 | 3.90 | 6.79 | 6.98 | 5.32 |
| 100033820_at| LOC100033820 | 1.53  | 2.88| 2.46| 0.05    | 0.85         | Small nucleolar RNA, C/D box 116-28 | 100033820.00 | ENSG00000207732 | 4.05 | 3.47 | 3.05 | 4.37 | 6.98 | 7.63 |
| 8404_at  | SPARQL1     | 1.48  | 2.78| 2.65| 0.04    | 0.85         | SPARC like 1 | 8404.00 | ENSG00000116690 | 5.85 | 3.16 | 3.90 | 6.79 | 6.98 | 5.32 |
| 100289635_at| ZNF605     | 1.45  | 2.73| 3.65| 0.01    | 0.85         | Zinc finger protein 605 | 100289635.00 | ENSG00000176228 | 7.15 | 5.29 | 4.22 | 7.18 | 6.18 | 7.07 |
| 105376805_at| LOC105376805 | 1.41  | 2.66| 3.09| 0.02    | 0.85         | Uncharacterized LOC105376805 | 105376805.00 | ENSG00000207732 | 4.47 | 3.47 | 3.05 | 4.37 | 6.98 | 7.63 |
| 100128002_at| LOC100128002 | 1.41  | 2.66| 2.52| 0.04    | 0.85         | Uncharacterized LOC100128002 | 100128002.00 | ENSG00000176228 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| 158131_at| OR1Q1       | 1.36  | 2.57| 2.49| 0.05    | 0.85         | Olfactory receptor family 1 subfamily Q member 1 | 158131.00 | ENSG00000176228 | 7.15 | 5.29 | 4.22 | 7.18 | 6.18 | 7.07 |
| 100616251_at| MIR1587  | 1.36  | 2.57| 2.60| 0.04    | 0.85         | MicroRNA 1587 | 100616251.00 | ENSG00000207732 | 4.55 | 4.60 | 4.25 | 5.02 | 5.44 | 7.03 |
| 1006481206_at| RNU6-94P | 1.31  | 2.47| 2.59| 0.04    | 0.85         | RNA, U6 small nuclear 94, pseudogene | 1006481206.00 | ENSG00000207732 | 3.96 | 3.09 | 3.01 | 4.14 | 5.27 | 7.91 |
| ProbeId       | Gene name      | logFC | FC  | t       | P      | Adj. P  | DESC                                                | ENTREZID | ENSEMBL                      | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|--------------|----------------|-------|-----|---------|--------|---------|-----------------------------------------------------|----------|------------------------------|-------------|-------------|-------------|--------------|--------------|--------------|
| 57562_at     | CEP126         | 1.30  | 2.46| 4.04    | 0.01   | 0.85    | −9.30 Centrosomal protein 126                        | 57562.00 | ENSG00000110318 | 4.80 | 3.93 | 4.71 | 6.19 | 5.70 | 5.45 |
| 5170_at      | PDPK1          | 1.29  | 2.44| 2.47    | 0.05   | 0.85    | −4.42 3-Phosphoinositide dependent protein kinase 1  | 5170.00  | ENSG00000140992 | 4.17 | 3.49 | 3.67 | 3.69 | 5.04 | 4.07 |
| 26787_at     | SNORD61        | 1.28  | 2.43| 3.60    | 0.01   | 0.85    | −4.33 Small nucleolar RNA, C/D box 61                | 26787.00 | NA              | 267.00 | NA | 5.33 | 5.01 | 6.87 | 5.94 | 5.55 |
| 26770_at     | SNORD79        | 1.23  | 2.35| 2.91    | 0.03   | 0.85    | −4.38 Small nucleolar RNA, C/D box 79               | 26770.00 | NA              | 267.00 | NA | 5.33 | 5.01 | 6.87 | 5.94 | 5.55 |
| 90499_at     | FAM95A         | 1.22  | 2.32| 2.49    | 0.05   | 0.85    | −4.42 Family with sequence similarity 95 mem-ber A  | 90499.00 | NA              | 90499.00 | NA | 3.93 | 3.53 | 3.65 | 4.23 | 4.49 | 6.04 |
| 100126355_at | MIR365A        | 1.20  | 2.30| 2.60    | 0.04   | 0.85    | −4.41 MicroRNA 365a                                 | 100126355.00 | ENSG00000199130 | 5.26 | 3.96 | 4.05 | 6.12 | 4.97 | 5.78 |
| 441728_at    | LOC441728      | 1.20  | 2.29| 3.29    | 0.02   | 0.85    | −4.35 Golgin-like pseudogene                        | 441728.00 | NA              | 441728.00 | NA | 3.29 | 3.39 | 3.15 | 4.38 | 3.82 | 5.21 |
| 5228_at      | PGF            | 1.19  | 2.28| 2.78    | 0.03   | 0.85    | −4.39 Placental growth factor                        | 5228.00  | ENSG00000119630 | 3.93 | 3.80 | 5.04 | 4.97 | 5.99 | 5.36 |
| 619564_at    | SNORD72        | 1.17  | 2.24| 3.08    | 0.02   | 0.85    | −4.36 Small nucleolar RNA, C/D box 72               | 619564.00 | ENSG00000212296 | 2.29 | 1.80 | 1.89 | 2.94 | 3.95 | 2.60 |
| 168667_at    | BMPER          | 1.15  | 2.21| 5.79    | 0.00   | 0.85    | −4.24 BMP binding endothelial regulator             | 168667.00 | ENSG00000164619 | 4.90 | 4.78 | 4.60 | 6.05 | 5.64 | 6.02 |
| 64853_at     | AIDA           | 1.15  | 2.21| 2.51    | 0.05   | 0.85    | −4.42 Axin interactor, dorni-tilization associated    | 64853.00  | ENSG00000166063 | 4.41 | 2.58 | 3.68 | 4.84 | 4.63 | 4.64 |
| 100462981_at | MTRNR2L2       | 1.15  | 2.21| 2.64    | 0.04   | 0.85    | −4.40 MT-RNR2-like 2                                | 100462981.00 | ENSG00000269028 | 2.95 | 2.61 | 3.15 | 3.89 | 3.39 | 4.95 |
| 100379296_at | RN4P13         | 1.14  | 2.20| 3.05    | 0.02   | 0.85    | −4.37 RNA, Ro-associated Y4 pseudogene 13           | 100379296.00 | NA              | 100379296.00 | NA | 3.42 | 3.02 | 3.47 | 4.06 | 4.02 | 5.24 |
| 100873920_at | NHS-A51        | 1.12  | 2.17| 4.52    | 0.00   | 0.85    | −4.28 NHS antisense RNA 1                           | 100873920.00 | ENSG00000230020 | 3.29 | 2.94 | 3.06 | 3.81 | 4.57 | 4.27 |
| 340527_at    | NHSL2          | 1.11  | 2.16| 3.27    | 0.02   | 0.85    | −4.35 NHS like 2                                    | 340527.00  | ENSG00000204131 | 4.91 | 4.01 | 4.04 | 5.86 | 5.05 | 5.38 |
| 7857_at      | SCC2           | 1.10  | 2.15| 3.01    | 0.02   | 0.85    | −4.37 Secretogranin II                              | 7857.00   | ENSG00000171951 | 4.11 | 3.41 | 4.48 | 5.64 | 4.83 | 4.84 |
| 503582_at    | ARGFX          | 1.10  | 2.14| 2.84    | 0.03   | 0.85    | −4.39 Arginine-fifty home-box                       | 503582.00 | ENSG00000186103 | 2.98 | 2.69 | 2.91 | 3.90 | 3.24 | 4.74 |
| 574494_at    | MIR521-1       | 1.10  | 2.14| 2.91    | 0.03   | 0.85    | −4.38 MicroRNA 521-1                                | 574494.00 | ENSG00000207634 | 3.23 | 3.03 | 3.58 | 3.92 | 4.03 | 5.17 |
| 26022_at     | TMEM98         | 1.08  | 2.12| 4.33    | 0.00   | 0.85    | −4.29 Transmembrane protein 98                      | 26022.00  | ENSG00000060942 | 4.10 | 3.42 | 3.55 | 4.73 | 4.57 | 5.03 |
| 343521_at    | TCTEX1D4       | 1.08  | 2.11| 5.33    | 0.00   | 0.85    | −4.25 Tctex1 domain containing 4                    | 343521.00 | ENSG00000188969 | 4.98 | 4.86 | 4.69 | 5.83 | 6.19 | 5.73 |
| ProbeId   | Gene name            | logFC | FC  | t   | P     | Adj. P value | B   | DESC                                                                 | ENTREZID | ENSEMBL | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|-----------|----------------------|-------|-----|-----|-------|---------------|-----|-----------------------------------------------------------------------|----------|----------|-------------|-------------|-------------|---------------|-------------|--------------|
| 4919_at   | ROR1                 | 1.07  | 2.10| 2.78| 0.03  | 0.85          |     | Receptor tyrosine kinase like orphan receptor 1                      | 4919.00  | ENSG0000185483 | 4.27 | 4.19 | 5.12 | 6.24 | 5.25 | 5.30 |
| 440910_at | LOC4040910           | 1.07  | 2.09| 5.58| 0.00  | 0.85          |     | Uncharacterized LOC4040910                                          | 440910.00| ENSG0000231431 | 4.11 | 3.73 | 3.77 | 5.05 | 4.78 | 4.81 |
| 105373893_at | LOC105373893         | 1.06  | 2.09| 3.87| 0.01  | 0.85          |     | Uncharacterized LOC105373893                                      | 105373893.00 | NA      | 2.54 | 3.11 | 3.03 | 3.60 | 4.36 | 3.91 |
| 105375249_at | LOC105375249         | 1.06  | 2.05| 2.92| 0.03  | 0.85          |     | Uncharacterized LOC105375249                                      | 105375249.00 | NA      | 3.06 | 2.60 | 2.85 | 3.12 | 4.33 | 4.23 |
| 84973_at  | SNHG7                | 1.03  | 2.05| 2.97| 0.02  | 0.85          |     | Small nuclear RNA host gene 7                                       | 84973.00 | ENSG000023016 | 4.84 | 4.29 | 4.02 | 5.62 | 4.82 | 5.81 |
| 400728_at | FAM878               | 1.03  | 2.04| 3.33| 0.02  | 0.85          |     | Family with sequence similarity 87 member 8                         | 400728.00| ENSG0000177757 | 4.65 | 4.11 | 4.01 | 5.48 | 4.76 | 5.63 |
| 283685_at | GOLGA6L2             | 1.03  | 2.04| 2.49| 0.05  | 0.85          |     | Golgin A6 family like 2                                             | 283685.00| ENSG0000174450 | 4.22 | 4.06 | 4.06 | 4.52 | 4.84 | 6.07 |
| 9376_at   | SLC22A8              | 1.01  | 2.02| 3.32| 0.02  | 0.85          |     | Solute carrier family 22 member 8                                   | 9376.00 | ENSG000019452  | 3.30 | 3.43 | 3.29 | 3.09 | 3.97 | 5.00 |
| 9787_at   | DLGAP5               | −1.00 | −2.00| 2.66| 0.04  | 0.85          |     | DLG associated protein 5                                            | 9787.00 | ENSG0000126787 | 5.91 | 5.43 | 4.60 | 4.59 | 4.40 | 3.95 |
| 5709_at   | PSMD3                | −1.00 | −2.00| 3.12| 0.02  | 0.85          |     | Proteasome 26S subunit, non-ATPase 3                               | 5709.00 | ENSG0000108344 | 6.29 | 6.90 | 7.03 | 5.88 | 6.05 | 5.23 |
| 5154_at   | PDGFA                | −1.00 | −2.00| 3.42| 0.01  | 0.85          |     | Platelet derived growth factor subunit A                             | 5154.00 | ENSG0000197461 | 5.01 | 6.02 | 5.81 | 4.54 | 4.60 | 4.69 |
| 161050_at | ZNF564               | −1.00 | −2.00| 3.74| 0.01  | 0.85          |     | Zinc finger protein 564                                             | 161050.00| ENSG0000249709 | 5.69 | 6.30 | 6.20 | 4.67 | 5.30 | 5.22 |
| 7405_at   | UVRAG                | −1.00 | −2.01| 2.59| 0.04  | 0.85          |     | UV radiation resistance associated                                  | 7405.00 | ENSG0000198382 | 6.49 | 7.01 | 7.35 | 6.44 | 6.17 | 5.23 |
| 283624_at | UNC00641             | −1.01 | −2.01| 3.15| 0.02  | 0.85          |     | Long intergenic non-protein coding RNA 641                          | 283624.00| ENSG0000258441 | 5.99 | 6.25 | 6.36 | 5.41 | 5.63 | 4.55 |
| 3931_at   | LCAT                 | −1.01 | −2.01| 5.27| 0.00  | 0.85          |     | Lecithin-cholesterol acyltransferase                                | 3931.00 | ENSG0000213398 | 5.49 | 5.73 | 5.80 | 4.83 | 4.71 | 4.45 |
| 1318_at   | SLC31A2              | −1.01 | −2.01| 2.96| 0.02  | 0.85          |     | Solute carrier family 31 member 2                                   | 1318.00 | ENSG0000136867 | 5.63 | 6.51 | 6.83 | 5.56 | 5.17 | 5.21 |
| 4771_at   | NF2                  | −1.01 | −2.02| 2.70| 0.03  | 0.85          |     | Neurofibrin 2                                                       | 4771.00 | ENSG0000186575 | 5.54 | 6.07 | 5.83 | 5.22 | 5.18 | 4.00 |
| 54947_at  | LPCAT2               | −1.01 | −2.02| 2.51| 0.05  | 0.85          |     | Lysophosphatidylcholine acyltransferase 2                           | 54947.00 | ENSG00001872253 | 5.56 | 6.63 | 7.04 | 5.19 | 5.34 | 5.67 |
| 1978_at   | Eif4EBP1             | −1.01 | −2.02| 2.98| 0.02  | 0.85          |     | Eukaryotic translation initiation factor 4E binding protein 1       | 1978.00 | ENSG0000187840 | 5.94 | 6.91 | 6.67 | 5.68 | 5.79 | 5.01 |
| 407043_at | MIR7-1               | −1.02 | −2.02| 3.21| 0.02  | 0.85          |     | MicroRNA-7-1                                                       | 407043.00| ENSG0000284179 | 4.77 | 5.46 | 5.67 | 4.54 | 3.86 | 4.46 |
| ProbeID | Gene name                | logFC | FC  | t   | P value | Adj. P val | DESC                                         | ENTREZID | ENSEMBL | BT133_fresh | BT133_thawed | BT134_fresh | BT134_thawed | BT138_fresh | BT138_thawed | BT138_thawed |
|---------|--------------------------|-------|-----|-----|---------|------------|---------------------------------------------|----------|---------|--------------|---------------|--------------|---------------|--------------|---------------|---------------|
| 8893_at | EIF2B5                   | −0.92 | −2.03 | −2.44 | 0.05    | 0.85       | −4.42 Eukaryotic translation initiation factor 2B subunit epsilon | 8893.00  | ENSG00000145191 | 6.73 | 7.46 | 6.76 | 6.48 | 6.28 | 5.13 |
| 205564_at | SENP5                  | −0.92 | −2.03 | −2.45 | 0.05    | 0.85       | −4.42 SUMO1/retin specific peptidase 5       | 205564.00 | ENSG00000119231 | 6.72 | 6.75 | 7.15 | 6.53 | 6.06 | 4.98 |
| 2305_at | FOXM1                    | −0.92 | −2.03 | −2.84 | 0.03    | 0.85       | −4.39 Forkhead box M1                        | 2305.00  | ENSG00000112106 | 5.61 | 5.60 | 5.15 | 4.64 | 4.96 | 3.71 |
| 113263_at | GLCCI1                  | −0.92 | −2.03 | −2.65 | 0.04    | 0.85       | −4.40 Glucocorticoid induced 1               | 113263.00 | ENSG00000106115 | 6.15 | 6.57 | 6.53 | 6.11 | 5.41 | 4.66 |
| 2030_at | SLC29A1                  | −0.92 | −2.03 | −2.60 | 0.04    | 0.85       | −4.41 Solute carrier family 29 member 1 (Augustine blood group) | 2030.00  | ENSG00000117279 | 5.22 | 6.50 | 6.64 | 5.02 | 5.06 | 5.20 |
| 6660_at | SOX5                     | −0.92 | −2.03 | −4.88 | 0.00    | 0.85       | −4.27 SUMO1/sentrin specific peptidase 5    | 6660.00  | ENSG00000134532 | 4.32 | 3.94 | 4.54 | 3.13 | 3.33 | 3.24 |
| 144100_at | PLEKHA7                 | −0.92 | −2.03 | −2.57 | 0.04    | 0.85       | −4.41 Pleckstrin homology domain containing A7 | 144100.00 | ENSG0000016689 | 3.85 | 5.38 | 4.81 | 3.67 | 3.85 | 3.44 |
| 23248_at | RPRD2                   | −0.92 | −2.03 | −2.45 | 0.05    | 0.85       | −4.42 Regulation of nuclear pre-mRNA domain containing 2 | 23248.00 | ENSG00000163125 | 6.94 | 7.36 | 7.59 | 6.80 | 6.62 | 5.38 |
| 26273_at | FBXO3                    | −0.92 | −2.03 | −3.22 | 0.02    | 0.85       | −4.35 F-box protein 3                        | 26273.00 | ENSG00000110429 | 5.28 | 6.10 | 6.40 | 5.06 | 4.69 | 4.94 |
| 57037_at | ANXWY2                   | −0.92 | −2.03 | −2.84 | 0.03    | 0.85       | −4.39 Ankyrin repeat and MYND domain containing 2 | 57037.00 | ENSG00000106524 | 5.45 | 6.12 | 5.33 | 5.19 | 4.55 | 4.06 |
| 9701_at  | PPP6R2                   | −0.92 | −2.03 | −2.88 | 0.03    | 0.85       | −4.38 Protein phosphatase 6 regulatory subunit 2 | 9701.00  | ENSG0000010229 | 5.03 | 5.51 | 5.22 | 4.64 | 4.56 | 3.46 |
| 10564_at | ARFGEF2                  | −0.92 | −2.03 | −2.46 | 0.05    | 0.85       | −4.42 ADP ribosylation factor guanine nucleotide exchange factor 2 | 10564.00 | ENSG00000124198 | 7.10 | 7.70 | 7.10 | 6.75 | 6.66 | 5.37 |
| 79005_at | SCN1M1                   | −0.92 | −2.06 | −3.37 | 0.01    | 0.85       | −4.34 Sodium channel modifier 1              | 79005.00 | ENSG00000163156 | 4.53 | 4.80 | 5.40 | 4.27 | 3.81 | 3.54 |
| 6558_at  | SLC12A2                  | −0.92 | −2.06 | −2.88 | 0.03    | 0.85       | −4.38 Solute carrier family 12 member 2      | 6558.00  | ENSG00000164651 | 7.47 | 8.24 | 7.42 | 7.15 | 6.77 | 6.09 |
| 377677_at | CA13                     | −0.92 | −2.06 | −3.02 | 0.02    | 0.85       | −4.37 Carboxid anhydrase 13                   | 377677.00 | ENSG00000185015 | 3.82 | 4.88 | 4.77 | 3.82 | 3.32 | 3.19 |
| 10678_at | B3GNT2                   | −0.92 | −2.06 | −2.52 | 0.00    | 0.85       | −4.35 UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2 | 10678.00 | ENSG00000170340 | 6.95 | 7.17 | 7.39 | 6.29 | 5.94 | 6.15 |
| 6641_at  | SNTB1                    | −0.92 | −2.07 | −2.91 | 0.03    | 0.85       | −4.38 Synthrophin beta 1                    | 6641.00  | ENSG00000172164 | 6.23 | 7.02 | 7.18 | 5.99 | 6.09 | 5.19 |
| 51050_at | PI15                     | −0.92 | −2.08 | −3.28 | 0.02    | 0.85       | −4.35 Peptidase inhibitor 15                | 51050.00 | ENSG00000137558 | 4.70 | 3.74 | 3.81 | 2.77 | 2.96 | 3.35 |
| 222068_at | TMED4                   | −0.92 | −2.08 | −2.62 | 0.04    | 0.85       | −4.41 Transmembrane p24 trafficking protein 4 | 222068.00 | ENSG0000158604 | 6.22 | 6.92 | 6.84 | 6.17 | 5.82 | 4.81 |
| ProbeId | Gene name                  | logFC  | FC    | t   | P value | Adj. P value | B     | DESC                                      | ENTREZID   | ENSEMBL       | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|---------|---------------------------|--------|-------|-----|---------|--------------|-------|------------------------------------------|------------|---------------|-------------|-------------|-------------|---------------|---------------|---------------|
| 1719_at | DHFR                      | −1.06  | −2.08 | −2.82 | 0.03    | 0.85         | −4.39| Dihydrofolate reductase                  | 1719.00    | ENSG00000228716 | 4.72        | 5.34        | 5.88        | 4.73          | 4.22          | 3.81          |
| 51203_at| NUSAP1                    | −1.06  | −2.09 | −2.70 | 0.03    | 0.85         | −4.40| Nucleolar and spindle-associated protein 1| 51203.00   | ENSG00000137804 | 6.15        | 5.52        | 5.25        | 4.53          | 5.23          | 3.97          |
| 6217_at | RPS16                     | −1.06  | −2.09 | −2.75 | 0.03    | 0.85         | −4.39| Ribosomal protein S16                   | 6217.00    | ENSG00000105193 | 8.53        | 9.00        | 9.28        | 8.50          | 7.94          | 7.17          |
| 289600_at| DHCP5                    | −1.07  | −2.10 | −2.51 | 0.05    | 0.85         | −4.42| Decapping enzyme, scavenger             | 28960.00   | ENSG00000110063 | 5.10        | 5.68        | 5.59        | 4.75          | 5.06          | 4.25          |
| 9939_at | JOSD1                     | −1.07  | −2.10 | −2.52 | 0.04    | 0.85         | −4.42| Josephin domain containing 1            | 9929.00    | ENSG00000100221 | 6.81        | 7.41        | 7.05        | 6.93          | 5.64          | 5.48          |
| 54354_at| MRPL50                    | −1.07  | −2.10 | −2.90 | 0.03    | 0.85         | −4.38| Mitochondrial ribosomalprotein L50     | 54354.00   | ENSG00000136897 | 5.55        | 6.48        | 6.67        | 5.08          | 5.61          | 4.80          |
| 51706_at| CYB5R1                    | −1.08  | −2.11 | −2.67 | 0.04    | 0.85         | −4.40| Cytochrome b5 reductase                 | 51706.00   | ENSG00000159348 | 5.42        | 5.82        | 6.36        | 4.62          | 5.51          | 4.24          |
| 6615_at | SNAI1                     | −1.08  | −2.11 | −4.82 | 0.00    | 0.85         | −4.27| Snail family transcriptional repressor 1| 6615.00    | ENSG00000124216 | 5.55        | 5.85        | 5.80        | 4.36          | 4.62          | 4.99          |
| 1063_at | CENPF                     | −1.08  | −2.12 | −3.03 | 0.02    | 0.85         | −4.37| Centromere protein F                    | 1063.00    | ENSG00000117724 | 7.15        | 6.27        | 6.93        | 5.82          | 6.15          | 5.12          |
| 5832_at | ALDH18A1                  | −1.08  | −2.12 | −2.73 | 0.03    | 0.85         | −4.40| Aldehyde dehydrogenase 18 family member A1| 5832.00    | ENSG00000159573 | 6.94        | 7.26        | 7.20        | 6.51          | 6.48          | 5.16          |
| 116092_at| DNTTIP1                  | −1.09  | −2.12 | −2.57 | 0.04    | 0.85         | −4.41| Deoxynucleotidyltransferase terminal interacting protein 1| 116092.00 | ENSG00000101457 | 5.89        | 6.68        | 6.59        | 5.61          | 5.82          | 4.47          |
| 6241_at | RRMI2                     | −1.10  | −2.14 | −2.83 | 0.03    | 0.85         | −4.39| Ribonucleoside reductase regulatory subunit M2| 6241.00    | ENSG00000171848 | 7.05        | 6.78        | 5.62        | 5.47          | 5.24          | 5.44          |
| 54764_at| ZRANB1                    | −1.10  | −2.14 | −3.01 | 0.02    | 0.85         | −4.37| Zinc finger RANBP2-type containing 1    | 54764.00   | ENSG00000199995 | 7.41        | 8.66        | 7.86        | 7.18          | 6.91          | 6.54          |
| 50_at   | ACO2                      | −1.10  | −2.14 | −2.43 | 0.05    | 0.85         | −4.43| Aconitase 2                              | 50.00      | ENSG00000100412  | 4.95        | 6.14        | 5.36        | 4.01          | 5.18          | 3.97          |
| 84546_at| SNORD358                  | −1.10  | −2.15 | −2.56 | 0.04    | 0.85         | −4.41| Small nucleolar RNA, C/D box 35B       | 84546.00   | ENSG0000020530  | 5.69        | 5.74        | 6.57        | 4.42          | 5.71          | 4.55          |
| 677845_at| SNORA79                  | −1.11  | −2.15 | −2.50 | 0.05    | 0.85         | −4.42| Small nucleolar RNA, H/ACA box 79      | 677845.00  | ENSG00000221303 | 4.99        | 6.70        | 5.51        | 4.76          | 4.70          | 4.42          |
| 54700_at| RNR3                      | −1.11  | −2.16 | −2.52 | 0.04    | 0.85         | −4.42| RRN3 homolog, RNA polymerase I transcribing factor | 54700.00   | ENSG00000085721  | 5.79        | 5.48        | 5.91        | 5.61          | 4.19          | 4.05          |
| 56907_at| SPRE1                     | −1.11  | −2.17 | −3.21 | 0.02    | 0.85         | −4.35| Spire type actin nucleation factor 1    | 56907.00   | ENSG00000134278  | 6.98        | 7.96        | 7.50        | 6.63          | 6.63          | 5.83          |
| 1739_at | DLG1                      | −1.11  | −2.17 | −3.42 | 0.01    | 0.85         | −4.34| Discs large MAGUK scaffold protein 1    | 1739.00    | ENSG00000075711  | 6.80        | 7.23        | 7.18        | 6.51          | 6.01          | 5.36          |
| 9991_at | PTBP3                     | −1.12  | −2.17 | −2.96 | 0.02    | 0.85         | −4.38| Polyaminidine tract binding protein 3   | 9991.00    | ENSG00000119314  | 8.29        | 9.03        | 8.69        | 7.97          | 7.88          | 6.81          |
| ProbeId  | Gene name       | logFC | FC   | t     | P value | Adj. P value | DESC                                | ENTREZID | ENSEMBL       | BT133_fresh | BT134_fresh | BT133_thawed | BT134_thawed | BT138_fresh | BT133_thawed | BT134_thawed |
|----------|-----------------|-------|------|-------|---------|--------------|-------------------------------------|----------|----------------|-------------|-------------|--------------|--------------|-------------|--------------|--------------|
| 54512_at | DIO2C4          | −1.12 | 2.17 | 2.99  | 0.03    | 0.85         | −4.38 Exosome component 4           | 54512.00 | ENSG0000017896 | 6.25        | 9.44        | 6.57         | 5.37         | 6.31        | 5.35         |              |
| 56910_at | STARD7          | −1.12 | 2.17 | 2.79  | 0.03    | 0.85         | −4.39 STAR related lipid transfer domain containing 7 | 56910.00 | ENSG0000084090 | 8.17        | 9.00        | 7.59         | 7.98         | 6.66        |              |
| 5894_at  | RAF1            | −1.12 | 2.17 | 2.62  | 0.04    | 0.85         | −4.41 Raf-1 proto-oncogene, serine/threonine kinase | 5894.00  | ENSG00000132155 | 7.08        | 7.18        | 7.55         | 6.44         | 6.79        | 5.22         |              |
| 3099_at  | HK2             | −1.12 | 2.18 | 2.98  | 0.03    | 0.85         | −4.39 Hexokinase 2                  | 3099.00  | ENSG00000159399 | 6.52        | 7.18        | 6.28         | 6.31         | 5.72        |              |
| 1017_at  | CDK2            | −1.13 | 2.18 | 2.60  | 0.04    | 0.85         | −4.41 Cyclin dependent kinase 2     | 1017.00  | ENSG00000123374 | 6.47        | 7.67        | 7.78         | 5.95         | 6.73        | 5.86         |              |
| 9388_at  | LIPG            | −1.13 | 2.19 | 2.82  | 0.03    | 0.85         | −4.39 Lipase G, endothelial type     | 9388.00  | ENSG00000101670 | 4.66        | 6.09        | 5.53         | 4.62         | 4.32        | 3.94         |              |
| 100128998_at  | LOC100128998 | −1.14 | 2.20 | 2.82  | 0.03    | 0.85         | −4.39 Uncharacterized LOC100128998  | 100128998.00 | ENSG00000176593 | 4.70        | 4.73        | 5.99         | 4.23         | 4.13        | 3.65         |              |
| 8714_at | ABCC3           | −1.14 | 2.21 | 2.45  | 0.05    | 0.85         | −4.42 ATP binding cassette subfamily C member 3 | 8714.00  | ENSG00000108846 | 6.34        | 7.36        | 7.00         | 6.03         | 6.39        | 4.86         |              |
| 64866_at | CDCP1           | −1.15 | 2.22 | 2.46  | 0.05    | 0.85         | −4.42 CUB domain containing protein 1 | 64866.00 | ENSG00000163814 | 7.31        | 8.23        | 7.10         | 7.00         | 6.62        | 5.57         |              |
| 2673_at  | GFPT1           | −1.15 | 2.23 | 2.86  | 0.03    | 0.85         | −4.38 Glutamine/fructose-6-phosphate transaminase 1 | 2673.00  | ENSG00000198380 | 7.65        | 8.48        | 7.99         | 7.48         | 7.05        | 6.13         |              |
| 6376_at  | CX3CL1          | −1.16 | 2.23 | 2.48  | 0.05    | 0.85         | −4.42 C-X3-C motif chemokine ligand 1 | 6376.00  | ENSG0000006210  | 5.36        | 6.55        | 6.82         | 4.74         | 5.71        | 4.81         |              |
| 116113_at | FOXP4           | −1.16 | 2.24 | 3.51  | 0.01    | 0.85         | −4.33 Forkhead box P4                | 116113.00 | ENSG00000137166 | 5.23        | 6.44        | 5.95         | 4.53         | 4.84        | 4.78         |              |
| 203413_at | CTB3            | −1.16 | 2.24 | 2.49  | 0.05    | 0.85         | −4.42 Cancer/testis antigen 83      | 203413.00 | ENSG00000204019 | 4.95        | 6.72        | 5.25         | 4.50         | 4.48        | 4.45         |              |
| 8503_at  | RIK3R3          | −1.17 | 2.25 | 2.52  | 0.04    | 0.85         | −4.42 Phosphoinositide 3-kinase regulatory subunit 3 | 8503.00  | ENSG00000117461 | 6.13        | 7.36        | 5.60         | 5.48         | 5.17        | 4.93         |              |
| 22992_at | KDM2B           | −1.17 | 2.25 | 2.51  | 0.04    | 0.85         | −4.42 Lysine demethylase 2A           | 22992.00 | ENSG00000173120 | 8.13        | 8.42        | 8.27         | 7.76         | 7.52        | 6.03         |              |
| 55707_at | NERCA2          | −1.18 | 2.26 | 2.77  | 0.03    | 0.85         | −4.39 NERCA endonuclease associated 2 | 55707.00 | ENSG00000157191 | 5.93        | 6.96        | 7.07         | 5.21         | 6.14        | 5.08         |              |
| 55227_at | LRRC1           | −1.18 | 2.26 | 2.74  | 0.03    | 0.85         | −4.39 Leucine rich repeat containing 1 | 55227.00 | ENSG00000137269 | 3.60        | 5.28        | 4.36         | 3.44         | 3.21        | 3.05         |              |
| 8694_at  | DGAT1           | −1.19 | 2.28 | 2.63  | 0.04    | 0.85         | −4.40 Diacylglycerol O-acyltransferase 1 | 8694.00  | ENSG00000185000 | 5.46        | 6.59        | 7.05         | 5.26         | 5.54        | 4.74         |              |
| 64225_at | AT1L2           | −1.19 | 2.28 | 3.39  | 0.01    | 0.85         | −4.34 Aflavidin GTPase 2             | 64225.00 | ENSG00000119787 | 5.73        | 6.75        | 6.41         | 5.31         | 5.42        | 4.61         |              |
| 6301_at  | SARS            | −1.19 | 2.28 | 2.62  | 0.04    | 0.85         | −4.41 Seryl-RNA synthetase            | 6301.00  | ENSG00000031698 | 6.58        | 6.90        | 7.30         | 6.15         | 6.30        | 4.76         |              |
| ProbeId | Gene name      | logFC | FC  | t   | P value | Adj. P value | DESC                                                                 | ENTREZID   | ENSEMBL | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|---------|----------------|-------|-----|-----|---------|--------------|-----------------------------------------------------------------------|------------|---------|-------------|-------------|-------------|--------------|--------------|-------------|
| 6536_at | SLC6A9         | 1.19  | 2.28| 3.92| 0.01    | 0.85         | Member 9, solute carrier family 6                                      | 6536.00    | ENSG0000196517 | 6.25 | 6.41 | 7.04 | 5.30 | 5.04 | 5.79 |
| 4494_at | MT1F           | 1.19  | 2.29| 2.61| 0.04    | 0.85         | Metallothionein 1F                                                     | 4494.00    | ENSG0000198417 | 7.00 | 7.65 | 7.47 | 6.32 | 6.97 | 5.25 |
| 54069_at| M5S18A         | 1.20  | 2.29| 3.11| 0.02    | 0.85         | MS 18 kinocore protein A                                               | 54069.00   | ENSG0000159055 | 6.46 | 7.26 | 6.78 | 6.34 | 5.50 | 5.06 |
| 139231_at| FAM199X        | 1.20  | 2.29| 4.94| 0.00    | 0.85         | Family with sequence similarity 199, X-linked                         | 139231.00  | ENSG0000123575 | 6.30 | 6.70 | 6.83 | 5.73 | 5.35 | 5.14 |
| 81502_at| M13            | 1.20  | 2.30| 2.64| 0.04    | 0.85         | Histocompatibility minor 13                                            | 81502.00   | ENSG0000101294 | 6.54 | 6.81 | 7.20 | 6.04 | 6.24 | 4.66 |
| 81875_at| ISG20L2        | 1.20  | 2.30| 2.49| 0.05    | 0.85         | Interferon stimulated exonuclease gene 20 like 2                      | 81875.00   | ENSG0000143319 | 6.63 | 6.70 | 7.71 | 6.03 | 6.46 | 4.94 |
| 10040_at| TOMIL1         | 1.22  | 2.32| 2.98| 0.02    | 0.85         | Target of myb1 like 1 membrane trafficking protein                     | 10040.00   | ENSG0000141198 | 6.21 | 7.28 | 5.96 | 5.74 | 5.08 | 4.99 |
| 440138_at| ALG11          | 1.22  | 2.33| 2.47| 0.05    | 0.85         | ALG11, alpha-1,2-mannosyltransferase                                   | 440138.00  | ENSG0000253710 | 6.85 | 6.96 | 7.14 | 6.24 | 6.43 | 4.62 |
| 57669_at| EPB41L5        | 1.22  | 2.33| 3.05| 0.02    | 0.85         | Erythrocyte membrane protein band 4.1 like 5                           | 57669.00   | ENSG0000115109 | 5.94 | 6.75 | 6.66 | 5.73 | 5.48 | 4.48 |
| 2120_at | ETV6           | 1.23  | 2.34| 2.75| 0.03    | 0.85         | ETS variant 6                                                          | 2120.00    | ENSG0000139083 | 6.38 | 7.66 | 7.03 | 6.26 | 6.06 | 5.07 |
| 85461_at| TANC1          | 1.24  | 2.36| 2.81| 0.03    | 0.85         | Tetraoctapeptide repeat, ankyrin repeat and coiled-coil containing 1   | 85461.00   | ENSG0000115183 | 6.36 | 7.37 | 6.29 | 5.86 | 5.76 | 4.69 |
| 127544_at| RNF198         | 1.24  | 2.36| 3.13| 0.02    | 0.85         | Ring finger protein 198                                               | 127544.00  | ENSG0000116514 | 5.77 | 6.76 | 6.27 | 5.32 | 5.44 | 4.33 |
| 8351_at | HIST1H3D       | 1.24  | 2.36| 2.71| 0.03    | 0.85         | Histone cluster 1 H3 family member d                                   | 8351.00    | ENSG0000197409 | 6.54 | 6.61 | 7.43 | 6.39 | 4.83 | 5.65 |
| 2762_at | GMDS           | 1.25  | 2.38| 2.97| 0.02    | 0.85         | GDP-mannose 4-beta-dehydrogenase                                       | 2762.00    | ENSG0000112699 | 5.64 | 6.19 | 6.75 | 5.17 | 5.43 | 4.22 |
| 55298_at| RNF121         | 1.25  | 2.39| 2.52| 0.04    | 0.85         | Ring finger protein 121                                               | 55298.00   | ENSG0000137522 | 4.68 | 5.17 | 5.44 | 4.28 | 4.49 | 2.76 |
| 6809_at | STX3           | 1.26  | 2.39| 3.25| 0.02    | 0.85         | Syntaxin 3                                                             | 6809.00    | ENSG0000166900 | 6.03 | 6.89 | 7.18 | 5.46 | 5.92 | 4.94 |
| 51514_at| DTL            | 1.26  | 2.39| 3.24| 0.02    | 0.85         | Denticless E3 ubiquitin protein ligase homolog                          | 51514.00   | ENSG0000143476 | 5.78 | 6.08 | 6.58 | 4.27 | 4.83 | 4.22 |
| 1903_at | S1PR3          | 1.26  | 2.40| 3.11| 0.02    | 0.85         | Sphingosine-1-phosphate receptor 3                                     | 1903.00    | ENSG0000213694 | 5.47 | 5.96 | 6.54 | 5.20 | 4.95 | 4.04 |
| 100288069_at| LOC100288069 | 1.27  | 2.40| 3.58| 0.01    | 0.85         | Uncharacterized LOC100288069                                          | 100288069  | NA      | 5.03 | 5.98 | 5.56 | 4.54 | 3.69 | 4.55 |
| ProbeId   | Gene name               | logFC  | FC   | t    | P value | Adj. P value | DESC                                                                 | ENTREZID      | ENSEMBL          | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|----------|-------------------------|--------|------|------|---------|--------------|----------------------------------------------------------------------|---------------|-----------------|-------------|-------------|-------------|---------------|---------------|---------------|
| 221079_at| ARL5B                   | −1.27  | −2.41| −2.86| 0.03    | 0.85         | ADP ribosylation factor like GTPase 5B                              | 221079.00     | ENSG00000165997 | 6.61        | 8.07        | 7.89        | 6.64          | 6.53          | 5.78          |
| 72_at    | ACTG2                   | −1.27  | −2.41| −3.35| 0.01    | 0.85         | Actin, gamma 2, smooth muscle, enteic                               | 72.00         | ENSG00000163017 | 3.76        | 4.44        | 3.10        | 2.36          | 2.87          | 2.25          |
| 23636_at | NUP62                   | −1.27  | −2.41| −2.97| 0.02    | 0.85         | Nucleoporin 62                                                      | 23636.00      | ENSG00000213024 | 5.99        | 6.14        | 7.01        | 5.40          | 5.58          | 4.35          |
| 57154_at | SMURF1                  | −1.28  | −2.43| −2.97| 0.02    | 0.85         | SMAD specific E3 ubiquitin protein ligase 1                         | 57154.00      | ENSG0000019742  | 5.85        | 6.76        | 5.71        | 5.32          | 5.08          | 4.08          |
| 9055_at  | PRC1                    | −1.28  | −2.43| −4.05| 0.01    | 0.85         | Protein regulator of cytokinesis 1                                  | 9055.00       | ENSG0000019801  | 7.49        | 7.99        | 7.11        | 6.27          | 6.64          | 5.83          |
| 201475_at| RAB12                   | −1.28  | −2.43| −2.88| 0.03    | 0.85         | RAB12, member RAS oncogene family 3                                 | 201475.00     | ENSG00000206148 | 7.79        | 8.27        | 7.74        | 6.98          | 7.29          | 5.68          |
| 11123_at | RCAN3                   | −1.30  | −2.46| −2.97| 0.02    | 0.85         | RCAN family member 3                                               | 11123.00      | ENSG00000117602 | 5.67        | 7.23        | 6.70        | 5.67          | 5.10          | 4.94          |
| 10276_at | NET1                    | −1.30  | −2.46| −3.19| 0.02    | 0.85         | Neuroepithelial cell transforming 1                                 | 10276.00      | ENSG00000173848 | 6.53        | 7.72        | 6.78        | 5.85          | 6.14          | 5.14          |
| 79065_at | ATG9A                   | −1.30  | −2.47| −3.05| 0.02    | 0.85         | Autophagy related 9A cyclin A2                                      | 79065.00      | ENSG00000198925 | 6.34        | 7.11        | 7.46        | 5.94          | 6.12          | 4.93          |
| 890_at   | CCNA2                   | −1.31  | −2.47| −2.50| 0.05    | 0.85         | Occludin                                                           | 890.00        | ENSG00000145386 | 6.91        | 6.72        | 5.52        | 5.40          | 5.63          | 4.20          |
| 100506658_at| OCLN                  | −1.31  | −2.48| −3.25| 0.02    | 0.85         | Occludin                                                           | 100506658.00  | ENSG00000197822 | 3.76        | 5.05        | 5.21        | 3.44          | 3.42          | 3.24          |
| 5209_at  | PKFB3                   | −1.31  | −2.49| −2.46| 0.05    | 0.84         | 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3              | 5209.00       | ENSG00000170525 | 4.95        | 5.48        | 6.21        | 4.44          | 5.01          | 3.25          |
| 51361_at | HOOK1                   | −1.31  | −2.49| −2.65| 0.04    | 0.85         | Hook microtubule tethering protein 1                                | 51361.00      | ENSG00000134709 | 3.25        | 4.78        | 5.06        | 2.77          | 3.04          | 3.34          |
| 701_at   | BUB1B                   | −1.32  | −2.50| −5.79| 0.00    | 0.85         | BUB1 mitotic checkpoint serine/threonine kinase B                   | 701.00        | ENSG00000156970 | 6.46        | 6.40        | 5.91        | 5.01          | 4.70          | 5.10          |
| 85406_at | DNAJC14                 | −1.33  | −2.52| −3.14| 0.02    | 0.85         | DnaJ heat shock protein family (Hsp40) member C14                  | 85406.00      | ENSG00000135392 | 5.91        | 6.24        | 6.27        | 5.19          | 5.38          | 3.85          |
| 6907_at  | TBL1X                   | −1.34  | −2.53| −3.73| 0.01    | 0.85         | Transducin beta like 1 X-linked                                     | 6907.00       | ENSG00000101849 | 4.83        | 5.79        | 4.97        | 4.30          | 3.90          | 3.38          |
| 3728_at  | JUP                     | −1.35  | −2.54| −2.69| 0.04    | 0.85         | Junction plakoglobin                                                | 3728.00       | ENSG00000173801 | 5.39        | 7.24        | 6.88        | 4.82          | 5.41          | 5.25          |
| 7153_at  | TOP2A                   | −1.35  | −2.55| −2.47| 0.05    | 0.85         | DNA topoisomerase II alpha                                         | 7153.00       | ENSG00000131747 | 8.13        | 6.98        | 7.44        | 6.45          | 6.96          | 5.08          |
| 56888_at | KCNFM                   | −1.35  | −2.56| −2.57| 0.04    | 0.85         | Potassium channel modulatory factor 1                               | 56888.00      | ENSG00000176407 | 7.45        | 8.10        | 8.39        | 7.40          | 6.94          | 5.54          |
Table 1 (continued)

| ProbeID | Gene name | logFC | FC  | t    | P    | Adj. P | DESC | ENTREZID | ENSEMBL | BT133       | BT134       | BT138     | BT133     | BT134     | BT138     |
|---------|-----------|-------|-----|------|------|--------|------|----------|---------|------------|------------|----------|-----------|------------|----------|
| 112616_at | CMTM7 | −1.35 | −2.56 | −2.58 | 0.04 | 0.85   | −4.41 | CCK-like MARVEL transmembrane domain containing 7 | 112616.00 | ENSG0000015355 | 5.31 | 7.09 | 6.28   | 4.46 | 5.58 | 4.57 |
| 4318_at   | MMP9    | −1.36 | −2.57 | −2.86 | 0.03 | 0.85   | −4.38 | Matrix metallopeptidase 9 | 4318.00 | ENSG00000100985 | 5.69 | 6.61 | 7.44   | 5.56 | 5.32 | 4.77 |
| 57355_at  | KIA1324 | −1.36 | −2.57 | −3.51 | 0.01 | 0.85   | −4.33 | KIA1324 | 57355.00 | ENSG00000116299 | 5.17 | 6.65 | 5.85   | 4.34 | 4.75 | 4.49 |
| 57162_at  | PEL1    | −1.38 | −2.60 | −2.82 | 0.03 | 0.85   | −4.39 | Pellino 3 ubiquitin protein ligase 1 | 57162.00 | ENSG00000197329 | 5.32 | 7.19 | 6.58   | 5.08 | 5.22 | 4.64 |
| 9654_at   | MMP9    | −1.36 | −2.57 | −2.86 | 0.05 | 0.85   | −4.42 | Tubulin tyrosine ligase like 4 | 9654.00 | ENSG00000135912 | 6.28 | 7.67 | 8.29   | 6.19 | 6.45 | 5.46 |
| 493856_at | CISD2   | −1.39 | −2.62 | −4.51 | 0.00 | 0.85   | −4.28 | CDGSH iron sulfur domain 2 | 493856.00 | ENSG00000145354 | 6.53 | 7.52 | 7.37   | 6.00 | 5.65 | 5.61 |
| 1857_at   | DVL3    | −1.39 | −2.62 | −2.90 | 0.03 | 0.85   | −4.38 | Dishevelled segment polarity protein 3 | 1857.00 | ENSG00000161202 | 7.03 | 8.07 | 7.79   | 6.77 | 6.66 | 5.30 |
| 102723739_at | LOC102723739 | −1.39 | −2.63 | −2.66 | 0.04 | 0.85   | −4.40 | Uncharacterized | 102723739.00 | NA | 3.14 | 4.09 | 5.29 | 2.79 | 2.80 | 2.75 |
| 91452_at  | ACBD5   | −1.40 | −2.63 | −2.44 | 0.05 | 0.85   | −4.42 | Acyl-CoA binding domain 5 | 91452.00 | ENSG00000107897 | 5.59 | 7.02 | 5.87   | 5.24 | 5.35 | 3.71 |
| 79622_at  | SNRNP25 | −1.41 | −2.66 | −3.19 | 0.02 | 0.85   | −4.36 | Small nuclear ribonucleoprotein U1/U12 subunit 25 | 79622.00 | ENSG00000161981 | 5.44 | 6.73 | 6.20   | 4.54 | 5.39 | 4.20 |
| 200634_at | KRTCAP3 | −1.41 | −2.66 | −2.79 | 0.03 | 0.85   | −4.39 | Keratinocyte associated protein 3 | 200634.00 | ENSG00000157992 | 5.96 | 7.90 | 6.93   | 5.99 | 5.84 | 5.12 |
| 6533_at   | SLC6A6  | −1.41 | −2.66 | −2.61 | 0.04 | 0.85   | −4.41 | Solute carrier family 6 member 6 | 6533.00 | ENSG00000131389 | 6.44 | 7.15 | 8.29   | 5.87 | 6.50 | 5.28 |
| 10809_at  | STARD10 | −1.42 | −2.68 | −2.82 | 0.03 | 0.85   | −4.39 | StAR related lipid transfer domain containing 10 | 10809.00 | ENSG00000214530 | 5.74 | 7.20 | 6.04   | 4.20 | 4.94 | 5.57 |
| 440278_at | CATSPER2P1 | −1.43 | −2.70 | −3.43 | 0.01 | 0.85   | −4.34 | Cation channel sperm associated 2 pseudogene 1 | 440278.00 | ENSG00000215771 | 4.41 | 5.58 | 4.78   | 4.17 | 3.16 | 3.15 |
| 8985_at   | PLOD3   | −1.46 | −2.74 | −3.09 | 0.02 | 0.85   | −4.36 | Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3 | 8985.00 | ENSG00000106397 | 7.17 | 8.09 | 8.06   | 6.92 | 6.63 | 5.41 |
| ProbeId | Gene name | logFC | FC  | t   | P    | Adj. P val | DESC                                                                 | ENTREZID | ENSEMBL | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|---------|-----------|-------|-----|-----|------|------------|----------------------------------------------------------------------|----------|----------|-------------|-------------|-------------|--------------|--------------|--------------|
| 7978_at | MTERF1    | −1.46 | −2.74 | −3.13 | 0.02 | 0.85       | −4.36 Mitochondrial transcription termination factor 1 | 7978.00  | ENSG0000012798 | 5.75        | 5.78        | 5.99        | 4.98         | 3.31         | 4.87         |
| 146223_at | CMTM4   | −1.47 | −2.78 | −4.22 | 0.01 | 0.85       | −4.29 C/EBP-like Mar/ER transmembrane domain containing 4 | 146223.00 | ENSG00000183723 | 6.79        | 7.29        | 6.90        | 5.74         | 6.00         | 4.82         |
| 64426_at | SUDS3    | −1.48 | −2.78 | −3.91 | 0.01 | 0.85       | −4.31 SDS3 homolog, SIN3A corepressor complex component | 64426.00 | ENSG00000111707 | 5.36        | 5.85        | 6.04        | 4.52         | 4.77         | 3.53         |
| 57530_at | CGN      | −1.48 | −2.79 | −2.47 | 0.05 | 0.85       | −4.42 Cingulin | 57530.00 | ENSG00000143375 | 3.80        | 5.94        | 5.36        | 2.96         | 4.09         | 3.61         |
| 375035_at | SFT2D2 | −1.49 | −2.81 | −2.72 | 0.03 | 0.85       | −4.40 SFT2 domain containing 2 | 375035.00 | ENSG0000023064 | 8.02        | 8.63        | 9.94        | 7.04         | 8.03         | 7.05         |
| 158586_at | ZXDB    | −1.50 | −2.82 | −2.98 | 0.02 | 0.85       | −4.37 Zinc finger, X-linked, duplicated B | 158586.00 | ENSG00000198455 | 6.61        | 7.35        | 6.90        | 6.50         | 5.24         | 4.62         |
| 9368_at  | SLC9A3R1 | −1.50 | −2.82 | −3.06 | 0.02 | 0.85       | −4.37 SLC9A3 regulator 1 | 9368.00  | ENSG00000141574 | 5.80        | 6.88        | 8.25        | 5.56         | 5.25         | 5.61         |
| 1717_at  | DHCR7    | −1.51 | −2.84 | −2.91 | 0.03 | 0.85       | −4.38 7-Dehydrocholesterol reductase | 1717.00  | ENSG00000172893 | 6.16        | 8.01        | 7.10        | 6.15         | 5.50         | 5.10         |
| 7097_at  | TLR2     | −1.51 | −2.84 | −3.05 | 0.02 | 0.85       | −4.37 Toll like receptor 2 | 7097.00  | ENSG00000137462 | 6.01        | 6.65        | 6.31        | 3.74         | 5.13         | 4.89         |
| 22974_at | TPX2     | −1.54 | −2.91 | −3.85 | 0.01 | 0.85       | −4.31 TPX2, microtubule nucleation factor | 22974.00 | ENSG00000188325 | 6.77        | 6.86        | 6.44        | 5.59         | 5.59         | 4.27         |
| 1838_at  | DTNB     | −1.55 | −2.92 | −3.70 | 0.01 | 0.85       | −4.32 Dystrobrevin beta | 1838.00  | ENSG00000138101 | 4.93        | 5.76        | 6.59        | 4.31         | 4.17         | 4.17         |
| 51176_at | LEF1     | −1.55 | −2.92 | −3.59 | 0.01 | 0.85       | −4.33 Lymphoid enhancer binding factor 1 | 51176.00 | ENSG00000138795 | 4.60        | 4.50        | 5.54        | 4.01         | 2.71         | 3.28         |
| 10207_at | PATJ     | −1.55 | −2.94 | −3.54 | 0.01 | 0.85       | −4.33 PATJ, crumbs cell polarity complex component | 10207.00 | ENSG0000012849 | 5.44        | 7.03        | 6.29        | 5.14         | 4.59         | 4.38         |
| 29028_at | ATAD2    | −1.56 | −2.95 | −3.11 | 0.02 | 0.85       | −4.36 ATPase family, AAA domain containing 2 | 29028.00 | ENSG00000156802 | 6.59        | 6.84        | 6.37        | 5.91         | 5.26         | 3.94         |
| 4301_at  | AFDN     | −1.58 | −2.99 | −3.08 | 0.02 | 0.85       | −4.37 Afadin, adherens junction formation factor | 4301.00  | ENSG00000130396 | 7.28        | 8.54        | 7.96        | 6.92         | 6.74         | 5.38         |
| 3838_at  | KPNA2    | −1.58 | −2.99 | −2.62 | 0.04 | 0.85       | −4.41 Karyopherin subunit alpha 2 | 3838.00  | ENSG00000182481 | 7.53        | 7.88        | 7.85        | 7.19         | 6.54         | 4.78         |
| ProbeId     | Gene name            | logFC | FC   | t    | P  | Adj. P  | B      | DESC                                                                 | ENTREZID  | ENSEMBL                  | BT133 fresh | BT134 fresh | BT138 fresh | BT133 thawed | BT134 thawed | BT138 thawed |
|-------------|----------------------|-------|------|------|----|---------|--------|----------------------------------------------------------------------|-----------|---------------------------|-------------|-------------|-------------|---------------|---------------|---------------|
| 79837_at    | PIP4K2C              | −1.58 | −2.99| −2.43| 0.05| 0.85    | −4.42 | Phosphatidylinositol-5-phosphate 4-kinase type 2 gamma               | 79837.00  | ENSG00000166908           | 5.00        | 7.63        | 6.73        | 4.54          | 5.18          | 4.91          |
| 3691_at     | ITGB4                | −1.59 | −3.01| −3.59| 0.01| 0.85    | −4.33 | Integrin subunit beta 4                                             | 3691.00   | ENSG00000132470           | 4.89        | 6.44        | 6.02        | 3.86          | 4.64          | 4.08          |
| 8342_at     | HIST1H2BM            | −1.59 | −3.02| −7.74| 0.00| 0.85    | −4.21 | Histone cluster H2B family member m                                  | 8342.00   | ENSG00000273703           | 6.08        | 6.15        | 6.49        | 4.88          | 4.55          | 4.50          |
| 1317_at     | SLC31A1              | −1.60 | −3.03| −2.62| 0.04| 0.85    | −4.41 | Solute carrier family 31 member 1                                    | 1317.00   | ENSG00000136868           | 7.61        | 8.97        | 8.40        | 7.02          | 7.62          | 5.54          |
| 360019_at   | KRT18P10             | −1.61 | −3.05| −3.19| 0.02| 0.85    | −4.36 | Keratin 18 pseudogene 10                                             | 360019.00 | NA                        | 3.49        | 5.09        | 3.33        | 2.02          | 2.32          | 2.73          |
| 388564_at   | TMEM238              | −1.62 | −3.07| −2.91| 0.03| 0.85    | −4.38 | Transmembrane protein 238                                           | 388564.00 | ENSG0000023493           | 5.09        | 6.52        | 7.12        | 5.18          | 4.36          | 4.34          |
| 4316_at     | MMP7                 | −1.62 | −3.07| −2.79| 0.03| 0.85    | −4.39 | Matrix metallopeptidase 7                                            | 4316.00   | ENSG00000137673           | 6.90        | 8.39        | 6.73        | 6.19          | 6.15          | 4.82          |
| 27350_at    | APOBEC3C             | −1.62 | −3.08| −3.59| 0.01| 0.85    | −4.33 | Apolipoprotein B mRNA editing enzyme catalytic subunit 3C            | 27350.00  | ENSG00000244509          | 6.78        | 6.83        | 7.46        | 6.07          | 5.66          | 4.47          |
| 26578_at    | OSTF1                | −1.62 | −3.08| −2.59| 0.04| 0.85    | −4.41 | Osteoclast stimulating factor 1                                      | 26578.00  | ENSG0000014996           | 6.81        | 7.32        | 7.15        | 6.36          | 6.06          | 4.00          |
| 8424_at     | BBOX1                | −1.62 | −3.08| −2.43| 0.05| 0.85    | −4.43 | Gamma Butyrobetaine hydroxylase 1                                   | 8424.00   | ENSG00000129151           | 3.19        | 4.73        | 5.98        | 2.95          | 3.10          | 2.97          |
| 56990_at    | CDC42SE2             | −1.63 | −3.10| −2.83| 0.03| 0.85    | −4.39 | CDC42 small effector 2                                               | 56990.00  | ENSG00000158985           | 6.36        | 6.43        | 6.68        | 5.77          | 5.28          | 3.52          |
| 27095_at    | TRAPP1C              | −1.64 | −3.11| −2.68| 0.04| 0.85    | −4.40 | Trafficking protein particle complex 3                               | 27095.00  | ENSG0000054116            | 7.43        | 8.62        | 9.34        | 7.39          | 7.21          | 5.87          |
| 1672_at     | DEFB1                | −1.64 | −3.12| −2.76| 0.03| 0.85    | −4.39 | Defensin beta 1                                                      | 1672.00   | ENSG00000164825           | 6.43        | 8.53        | 7.29        | 5.20          | 6.46          | 5.67          |
| 7045_at     | TGFBI                | −1.65 | −3.13| −2.51| 0.04| 0.85    | −4.42 | Transforming growth factor beta induced                              | 7045.00   | ENSG00000120708           | 8.86        | 7.38        | 7.93        | 6.92          | 7.22          | 5.10          |
| 105374310_at| LOC105374310         | −1.65 | −3.15| −3.11| 0.02| 0.85    | −4.36 | Uncharacterized LOC105374310                                         | 105374310.00 | NA               | 4.90        | 6.39        | 6.91        | 4.27          | 4.80          | 4.16          |
| 1836_at     | SLC26A2              | −1.67 | −3.17| −2.90| 0.03| 0.85    | −4.38 | Solute carrier family 26 member 2                                    | 1836.00   | ENSG00000155850           | 6.09        | 7.64        | 6.78        | 5.93          | 5.41          | 4.17          |
| 196_at      | AHR                  | −1.68 | −3.21| −3.62| 0.01| 0.85    | −4.33 | Aryl hydrocarbon receptor                                           | 196.00    | ENSG00000106546           | 8.48        | 9.69        | 8.80        | 7.66          | 7.75          | 6.77          |
| 4233_at     | MET                  | −1.70 | −3.24| −2.82| 0.03| 0.85    | −4.39 | MET proto-oncogene, receptor tyrosine kinase                         | 4233.00   | ENSG00000105976           | 6.25        | 6.90        | 5.63        | 5.72          | 4.39          | 3.58          |
| 10652_at    | YKT6                 | −1.70 | −3.24| −3.35| 0.01| 0.85    | −4.34 | YKT6 v-SNARE homolog                                                | 10652.00  | ENSG00000106636           | 6.29        | 7.03        | 7.09        | 5.77          | 5.51          | 4.03          |
| ProbeID | Gene name | logFC | FC | t | P value | Adj. P val | DESC | ENTREZID | ENSEMBL | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|---------|-----------|-------|----|---|---------|------------|------|----------|----------|------------|------------|------------|--------------|--------------|--------------|
| 5768_at | QSOX1     | −1.70 | 3.25 | 2.53 | 0.04    | 0.85       | −4.41 | Quercin sulfhydryl oxidase 1 | 5768.00  | ENSG00000116260 | 5.27       | 7.66       | 7.06         | 5.27         | 5.40         | 4.22         |
| 93474_at| ZNF670    | −1.72 | 3.30 | 0.97 | 0.00    | 0.85       | −4.24 | Zinc finger protein 670 | 93474.00 | ENSG000000277462 | 4.16       | 4.70       | 4.86         | 3.29         | 2.65         | 2.61         |
| 7020_at | TFAP2A    | −1.74 | 3.34 | 2.58 | 0.04    | 0.85       | −4.41 | Transcription factor AP-2 alpha | 7020.00  | ENSG00000137203 | 5.64       | 6.83       | 8.41         | 5.11         | 5.46         | 5.09         |
| 11221_at| DUSP10    | −1.74 | 3.34 | 3.52 | 0.01    | 0.85       | −4.33 | Dual specificity phosphatase 10 | 11221.00 | ENSG00000143507 | 7.27       | 7.36       | 8.15         | 6.18         | 6.53         | 4.85         |
| 56900_at| TMEM167B  | −1.75 | 3.36 | 2.51 | 0.04    | 0.85       | −4.42 | Transmembrane protein 167B | 56900.00 | ENSG00000215717 | 6.05       | 7.34       | 7.33         | 5.78         | 5.98         | 3.71         |
| 5557_at | PRIM1     | −1.77 | 3.42 | 3.72 | 0.01    | 0.85       | −4.32 | DNA primase subunit 1 | 5557.00  | ENSG00000019056 | 5.69       | 6.34       | 5.89         | 5.24         | 3.85         | 3.52         |
| 5795_at | PTPRU     | −1.78 | 3.45 | 2.69 | 0.04    | 0.85       | −4.40 | Protein tyrosine phosphatase, receptor type J | 5795.00  | ENSG000000149177 | 5.39       | 8.03       | 6.45         | 4.69         | 5.31         | 4.51         |
| 79022_at| TMEM106C  | −1.79 | 3.46 | 2.53 | 0.04    | 0.85       | −4.41 | Transmembrane protein 106C | 79022.00 | ENSG00000134291 | 5.41       | 7.66       | 6.77         | 5.50         | 5.25         | 3.73         |
| 481_at  | ATP1B1    | −1.80 | 3.47 | 2.47 | 0.05    | 0.85       | −4.42 | ATPase Na+/K+ transporting subunit beta 1 | 481.00   | ENSG000000143153 | 7.64       | 10.31      | 8.18         | 6.69         | 7.58         | 6.48         |
| 105376171_at | LOC105376171 | −1.81 | 3.51 | 3.93 | 0.01    | 0.85       | −4.31 | Uncharacterized LOC105376171 | 105376171.00 | ENSG00000231521 | 5.53       | 6.06       | 6.77         | 5.09         | 4.15         | 3.71         |
| 283987_at | HD1      | −1.84 | 3.59 | 3.15 | 0.02    | 0.85       | −4.36 | HD1 domain containing | 283987.00 | ENSG000000167861 | 5.14       | 7.50       | 6.41         | 4.34         | 4.84         | 4.34         |
| 1942_at | EFNA1     | −1.85 | 3.59 | 3.36 | 0.01    | 0.85       | −4.34 | Ephrin A1 | 1942.00  | ENSG000000169242 | 3.73       | 5.03       | 5.96         | 2.89         | 3.02         | 3.27         |
| 84844_at | PHF5A     | −1.85 | 3.60 | 2.79 | 0.03    | 0.85       | −4.39 | PHD finger protein 5A | 84844.00 | ENSG00000109410 | 4.91       | 6.55       | 7.50         | 4.70         | 3.97         | 4.75         |
| 10653_at | SPINT2    | −1.86 | 3.63 | 2.56 | 0.04    | 0.85       | −4.41 | Serine peptidase inhibitor, Kunitz type 2 | 10653.00 | ENSG00000167842 | 5.07       | 7.84       | 6.95         | 4.08         | 5.09         | 5.10         |
| 100313770_at | MIR548K | −1.87 | 3.66 | 2.88 | 0.03    | 0.85       | −4.38 | MicroRNA 548k | 100313770.00 | ENSG00000221333 | 3.67       | 4.79       | 6.36         | 3.00         | 3.16         | 3.05         |
| 83481_at | EPPK1     | −1.90 | 3.73 | 2.96 | 0.02    | 0.85       | −4.38 | Epipalakin 1 | 83481.00 | ENSG0000001261150 | 3.92       | 5.27       | 6.60         | 3.38         | 3.37         | 3.34         |
| 55971_at | BAIAP2L1  | −1.90 | 3.74 | 2.68 | 0.04    | 0.85       | −4.40 | BAI1 associated protein 2 like 1 | 55971.00 | ENSG0000006453 | 5.12       | 7.77       | 6.23         | 4.14         | 5.25         | 4.03         |
| 3675_at | ITGA3     | −1.91 | 3.75 | 2.69 | 0.03    | 0.85       | −4.40 | Integrin subunit alpha 3 | 3675.00  | ENSG0000005884 | 6.84       | 7.81       | 7.05         | 6.01         | 6.25         | 3.72         |
| 1545_at | CYP1B1    | −1.95 | 3.86 | 2.71 | 0.03    | 0.85       | −4.40 | Cytochrome P450 family 1 subfamily B member 1 | 1545.00  | ENSG00000138061 | 7.76       | 8.41       | 9.92         | 7.13         | 7.54         | 5.58         |
| 57133_at | SLC4A2    | −1.96 | 3.88 | 2.62 | 0.04    | 0.85       | −4.41 | Solute carrier family 44 member 2 | 57133.00 | ENSG00000129353 | 6.65       | 8.62       | 8.63         | 6.29         | 6.93         | 4.82         |
| 11009_at | IL24      | −1.96 | 3.90 | 2.95 | 0.02    | 0.85       | −4.38 | Interleukin 24 | 11009.00 | ENSG00000162892 | 6.61       | 7.42       | 7.13         | 4.36         | 6.63         | 4.28         |
| ProbeId | Gene name | logFC | FC | t  | P  | Adj. P | DESC | ENTREZID | ENSEMBL | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|--------|------------|------|----|----|----|-------|------|----------|---------|------------|-------------|-------------|--------------|--------------|--------------|--------------|
| 6272_at | SORT1      | -1.99 | -3.97 | 2.78 | 0.03 | 0.85 | -4.39 | Sortilin 1 | 6272.00  | ENSG00000134243 | 5.51 | 7.42 | 7.93 | 5.07 | 5.70 | 4.12 |
| 6692_at | SPINT1     | -2.00 | -4.01 | 3.35 | 0.01 | 0.85 | -4.35 | Serine peptidase inhibitor, Kunitz type 1 | 6692.00  | ENSG00000166145 | 5.61 | 8.07 | 6.52 | 4.84 | 4.67 | 4.68 |
| 129642_at | MBOAT2 | -2.01 | -4.03 | 3.80 | 0.01 | 0.85 | -4.32 | Membrane bound O-acyltransferase domain containing 2 | 129642.00 | ENSG00000143797 | 6.45 | 7.14 | 6.80 | 5.33 | 5.43 | 3.59 |
| 1829_at | DSG2       | -2.01 | -4.04 | 3.39 | 0.01 | 0.85 | -4.33 | Desmoglein 2 | 1829.00  | ENSG00000046604 | 6.13 | 8.00 | 6.21 | 4.67 | 5.29 | 4.34 |
| 92421_at | CHMP4C     | -2.07 | -4.19 | 2.76 | 0.03 | 0.85 | -4.39 | Charged multivesicular body protein 4C | 92421.00 | ENSG00000164695 | 4.82 | 7.95 | 6.36 | 4.17 | 4.22 | 4.54 |
| 219907_at | GJA1TL2 | -2.07 | -4.19 | 5.74 | 0.00 | 0.85 | -4.24 | Glycine-N-acyltransferase like 2 | 219907.00 | ENSG00000156689 | 6.74 | 6.55 | 6.57 | 5.30 | 4.44 | 3.92 |
| 1366_at | CLDN7      | -2.10 | -4.30 | 3.20 | 0.02 | 0.85 | -4.36 | Claudin 7 | 1366.00  | ENSG00000181885 | 5.76 | 8.28 | 7.54 | 4.96 | 5.60 | 4.71 |
| 55041_at | PLEKH8B    | -2.12 | -4.34 | 2.52 | 0.04 | 0.85 | -4.42 | Pleckstrin homology domain containing B2 | 55041.00 | ENSG00000115762 | 5.98 | 7.53 | 7.36 | 5.85 | 5.63 | 3.04 |
| 337875_at | HIST2H28A | -2.16 | -4.47 | 3.17 | 0.02 | 0.85 | -4.36 | Histone cluster 2 H2B family member a (pseudogene) | 337875.00 | NA | 5.74 | 6.89 | 7.36 | 5.17 | 5.17 | 3.17 |
| 6768_at | ST14       | -2.18 | -4.53 | 2.65 | 0.04 | 0.85 | -4.40 | Suppression of tumorigenicity 14 | 6768.00  | ENSG00000149418 | 4.10 | 7.39 | 6.61 | 3.59 | 4.00 | 3.98 |
| 8140_at | SLC7A5     | -2.20 | -4.58 | 2.99 | 0.02 | 0.85 | -4.37 | Solute carrier family 7 member 5 | 8140.00  | ENSG00000130257 | 6.75 | 9.18 | 8.08 | 5.84 | 6.74 | 4.84 |
| 1824_at | DSC2       | -2.20 | -4.61 | 3.51 | 0.01 | 0.85 | -4.33 | Desmocollin 2 | 1824.00  | ENSG00000134755 | 7.20 | 9.00 | 8.68 | 6.43 | 6.77 | 5.07 |
| 200508_at | FLJ23867 | -2.20 | -4.61 | 3.11 | 0.02 | 0.85 | -4.36 | Uncharacterized protein FLJ23867 | 200508.00 | NA | 6.24 | 9.21 | 8.00 | 5.63 | 5.62 | 5.39 |
| 51330_at | TNFRSF12A  | -2.28 | -4.86 | 2.71 | 0.03 | 0.85 | -4.40 | TNF receptor superfamily member 12A | 51330.00 | ENSG00000006327 | 6.98 | 8.53 | 10.21 | 5.90 | 7.16 | 5.82 |
| 2517_at | FUC1A      | -2.31 | -4.97 | 2.64 | 0.04 | 0.85 | -4.40 | alpha-L-Fucosidase 1 | 2517.00  | ENSG00000179163 | 5.26 | 7.76 | 8.62 | 5.33 | 5.19 | 4.17 |
| 1832_at | DSP        | -2.34 | -5.06 | 6.25 | 0.00 | 0.85 | -4.23 | Desmoplakin | 1832.00  | ENSG00000196696 | 7.04 | 8.21 | 8.17 | 5.74 | 5.56 | 5.11 |
| 1500_at | CTNNBD1    | -2.38 | -5.22 | 2.78 | 0.03 | 0.85 | -4.39 | Catenin delta 1 | 1500.00  | ENSG00000198561 | 6.60 | 9.08 | 8.82 | 5.74 | 7.01 | 4.60 |
| 55959_at | SULF2      | -2.39 | -5.24 | 3.21 | 0.02 | 0.85 | -4.36 | Sulfatase 2 | 55959.00 | ENSG00000196562 | 6.43 | 8.46 | 6.99 | 4.71 | 6.15 | 3.84 |
| 1022723517_at | LOC1022723517 | -2.42 | -5.34 | 2.87 | 0.03 | 0.85 | -4.38 | Uncharacterized LOC1022723517 | 1022723517.00 | ENSG00000234899 | 7.56 | 9.83 | 10.72 | 6.94 | 7.68 | 6.25 |
| 79679_at | VTCN1      | -2.42 | -5.36 | 2.75 | 0.03 | 0.85 | -4.39 | V-set domain containing T-cell activation inhibitor 1 | 79679.00  | ENSG00000134258 | 4.11 | 6.81 | 7.68 | 3.76 | 3.66 | 3.92 |
| 50848_at | F11R       | -2.45 | -5.45 | 2.56 | 0.04 | 0.85 | -4.41 | F11 receptor | 50848.00 | ENSG00000158769 | 5.11 | 8.90 | 7.36 | 4.60 | 5.42 | 4.00 |
| 4071_at | TM4SF1     | -2.46 | -5.50 | 2.70 | 0.03 | 0.85 | -4.40 | Transmembrane 4 L six family member 1 | 4071.00  | ENSG00000169908 | 5.87 | 9.24 | 7.98 | 5.71 | 5.82 | 4.19 |
| 999_at | CDH1       | -2.46 | -5.50 | 3.13 | 0.02 | 0.85 | -4.36 | Cadherin 1 | 999.00 | ENSG00000239068 | 6.21 | 9.33 | 6.90 | 4.97 | 5.32 | 4.78 |
| ProbeId       | Gene name                                | logFC | FC  | t    | P  | Adj. P val | DESC                                      | ENTREZID  | ENSEMBL  | BT133_fresh | BT134_fresh | BT138_fresh | BT133_thawed | BT134_thawed | BT138_thawed |
|--------------|------------------------------------------|-------|-----|------|----|------------|-------------------------------------------|-----------|-----------|-------------|-------------|-------------|--------------|--------------|--------------|
| 2568_at      | GABRP                                    | −2.51 | −5.70 | −292 | 0.03 | 0.85       | gamma-Aminobutyric acid type A receptor pi subunit | 2568.00   | ENSG0000094755 | 5.83 | 8.82 | 8.48 | 4.59 | 6.09 | 4.90 |
| 54845_at     | ESRP1                                    | −2.56 | −5.91 | −258 | 0.04 | 0.85       | Epithelial splicing regulatory protein 1 | 54845.00  | ENSG0000010413 | 3.97 | 8.06 | 6.65 | 3.27 | 4.06 | 3.66 |
| 3655_at      | ITGA6                                    | −2.58 | −5.97 | −273 | 0.03 | 0.85       | Integrin subunit alpha 6 | 3655.00   | ENSG0000019409 | 5.87 | 9.56 | 8.76 | 4.93 | 5.84 | 5.69 |
| 3854_at      | KRT68                                    | −2.61 | −6.11 | −262 | 0.04 | 0.85       | Keratin 68 | 3854.00  | ENSG0000018579 | 9.02 | 9.60 | 9.42 | 8.23 | 7.64 | 4.34 |
| 3904_at      | ITGB6                                    | −2.63 | −6.18 | −573 | 0.00 | 0.85       | Integrin subunit beta 6 | 3904.00   | ENSG0000015221 | 7.21 | 8.24 | 7.04 | 4.28 | 5.59 | 4.73 |
| 27075_at     | TSPAN13                                   | −2.75 | −6.73 | −270 | 0.03 | 0.85       | Tetraspanin 13 | 27075.00 | ENSG00000106537 | 5.26 | 9.38 | 8.10 | 4.39 | 4.75 | 5.35 |
| 654319_at    | SNORA5A                                   | −2.76 | −6.79 | −319 | 0.02 | 0.85       | Small nuclear RNA, H/ACA box 5A | 654319.00 | ENSG00000206838 | 3.56 | 6.90 | 6.28 | 3.32 | 2.69 | 2.45 |
| 8842_at      | PROM1                                     | −2.76 | −6.79 | −256 | 0.04 | 0.85       | Prominin 1 | 8842.00 | ENSG0000007062 | 5.98 | 9.88 | 7.29 | 4.82 | 6.17 | 4.86 |
| 102723505_at | LINC02095                                 | −2.86 | −7.25 | −267 | 0.04 | 0.85       | Long intergenic non-protein coding RNA 2095 | 102723505.00 | ENSG00000228639 | 5.17 | 8.97 | 8.94 | 4.90 | 5.43 | 4.17 |
| 114569_at    | MAL2                                      | −3.05 | −8.28 | −335 | 0.01 | 0.85       | mal T-cell differentiation protein 2 (gene/pseudogene) | 114569.00 | ENSG00000147676 | 7.95 | 10.23 | 9.52 | 6.32 | 7.65 | 4.58 |
| 938_at       | CD2AP4                                    | −3.29 | −9.81 | −313 | 0.02 | 0.85       | CD24 molecule pseudogene 4 | 938.00 | NA | 6.48 | 7.89 | 7.12 | 3.49 | 6.16 | 1.96 |
| 105376425_at | LOC105376425                              | −3.46 | −11.03 | −340 | 0.01 | 0.85       | Uncharacterized LOC105376425 | 105376425.00 | NA | 6.44 | 9.61 | 6.90 | 5.20 | 4.68 | 2.68 |
| 8364_at      | HIST1H4C                                  | −3.63 | −12.39 | −325 | 0.02 | 0.85       | Histone cluster 1 H1 family member c | 8364.00  | ENSG00000197061 | 6.78 | 5.03 | 9.38 | 4.50 | 2.75 | 3.05 |
| 260436_at    | FDSCR                                    | −4.42 | −21.46 | −281 | 0.03 | 0.85       | Follicular dendritic cell secreted protein | 260436.00 | ENSG00000181617 | 8.08 | 11.32 | 11.19 | 4.13 | 9.03 | 4.16 |
| 6279_at      | S100A8                                    | −4.79 | −27.66 | −393 | 0.01 | 0.85       | S100 calcium binding protein AII | 6279.00  | ENSG00000143546 | 5.66 | 9.82 | 10.42 | 3.96 | 3.56 | 4.01 |

BT133, BT134 and BT138 corresponds to three different TNBC patients
Additional files

Additional file 1: Figure S1. Flowchart showing the TNBC dissociation and spheroid protocols.
Additional file 2: Table S1. Gene ontology enrichment associated with the freeze/thaw process in TNBCs.

Authors’ contributions
MLG, TMR and AP conducted the experiments and analyzed data. VL, PT, JL and FG provided tumor samples and analyzed data. MLG, TMR, VL, JL, FG and PL wrote the paper. PL designed the experiments and supervised the project. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The study was conducted with human samples and clinical data of Rennes Biobank Breast Cancer Collection (BRIF Number: BB-0033-00056) certified NF S96900 for receipt preparation preservation and provision of biological resources. Raw and normalized transcriptomic data have been deposited to the GEO database accession ID GSE114359. All data generated or analyzed during this study are included in this published article.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The breast Cancer Collection was declared and approved by the French Ethics Committee for the Protection of Research Participants (CCPPRB). Rennes CCPPRB approved the use of tumor tissues for this study (6 May 2013: No. 357/2013). Collection of tumors was approved by French Minister of higher education and research (No. AC-2008-141). The study did not need additional ethical approval.

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