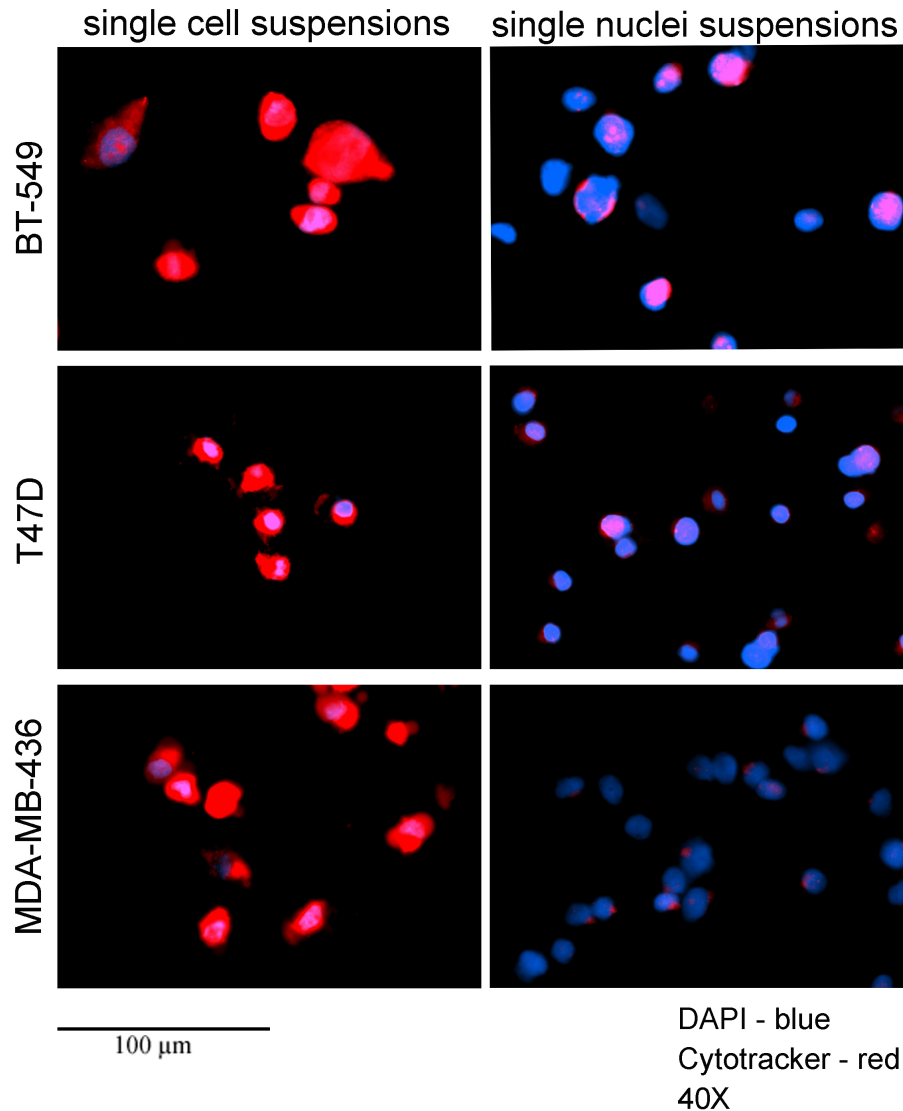


File name: Supplementary Information

Description: Supplementary figures and supplementary tables.

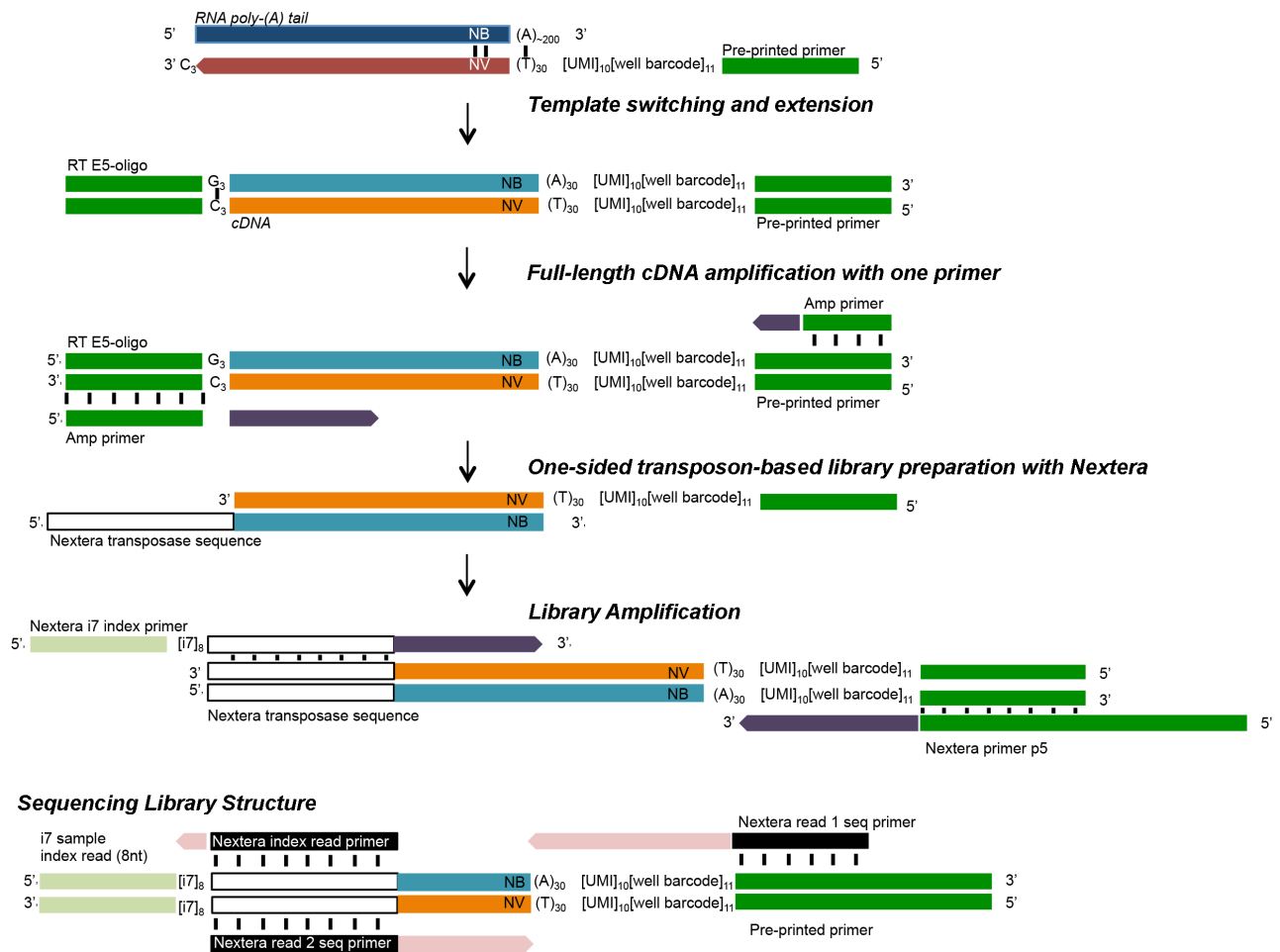
## Supplementary Figures

### Breast Cancer Cell Lines



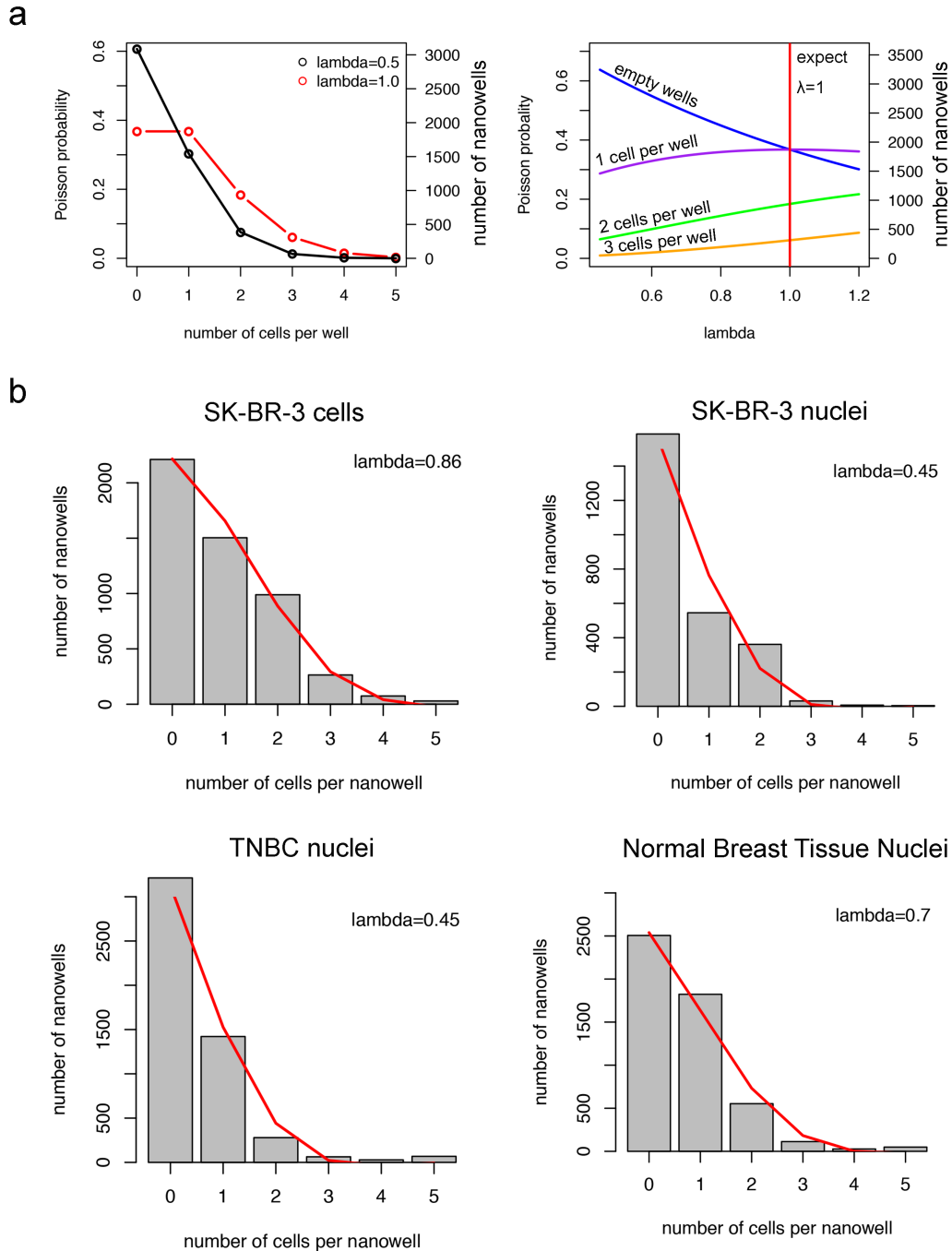
#### Supplementary Figure 1 - Imaging of Single Cell and Nuclei Suspensions.

Purified suspensions of single cells and single nuclei from the MDA-MB436 breast cancer cell line were stained with DAPI (nuclei) or Hoechst (cells) and imaged using fluorescence and brightfield microscopy at 40X magnification. Although the majority of DAPI is excluded by the cell membrane in living cells, there is always a small amount that permeates and shows low-level staining of the nucleus of live cells. However these levels are much lower compared to the DAPI staining in the isolated nuclei (right panels).



## Supplementary Figure 2 – Construction of Nanowell Barcoded Single Cell 3' RNA Sequencing Libraries

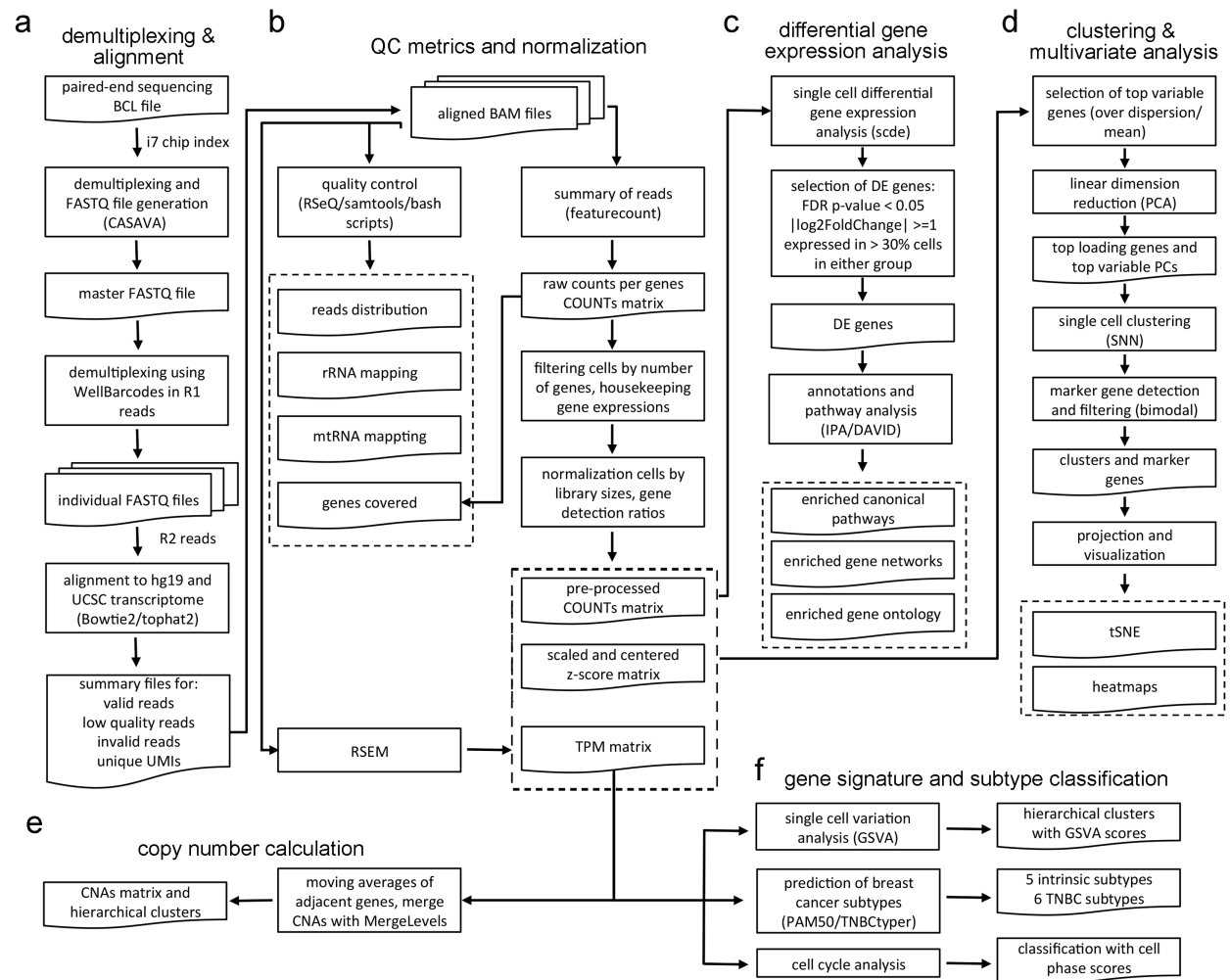
3' Single cell RNA sequencing is performed by hybridization of the polyA mRNA tail to an oligo dT<sub>30</sub> adapter with a 11bp well barcode and 10bp UMI that is pre-printed in each nanowell. Single cells or nuclei undergo reverse-transcription using template switching and extension, followed by full-length cDNA amplification. A one-sided tagmentation reaction is then used to add a Nextera transposase adapter to the 5' end (the other strand is omitted for illustrative purposes). PCR is performed using the nextera P5 primer and i7 index primer to extend the adapter sequences and enrich the library concentrations. The final sequencing reaction involves 3 sequencing primers to read the WBC and UMI (read1), the 3' RNA sequence (read 2) and the i7 nextera sample index (read3). In this figure N represents A, C, G, or T; B represents C, G, or T; and V represents A, C, or G. Dotted lines indicate where the first nucleotide hybridizations occur.



### Supplementary Figure 3 –Poisson Distributions of Cells and Nuclei After Nanodispensing

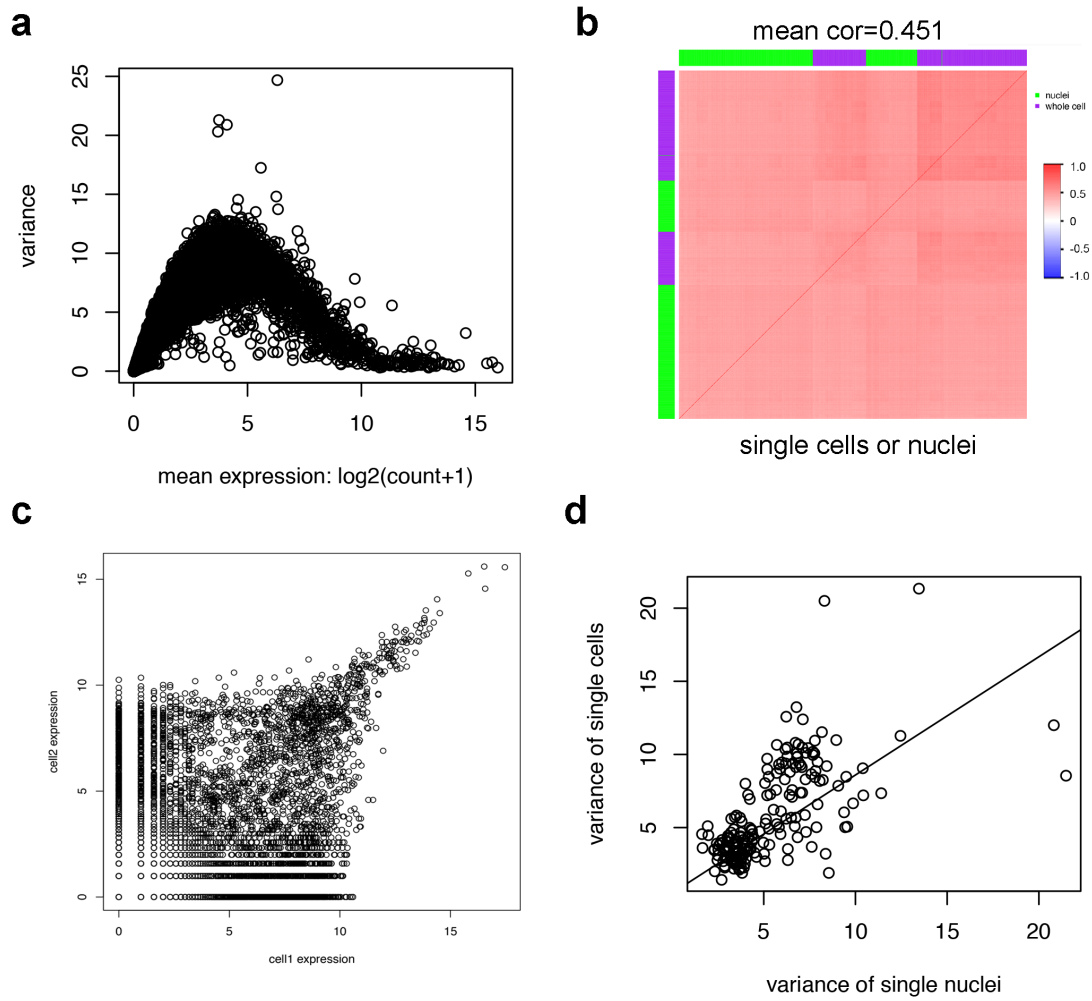
(a) Theoretical Poisson distribution for the number of expected single cells per nanowell (left panel) and probability distribution under series of number of cells per well with expected  $\lambda=1$ , where ~1800 wells with single cells/nuclei are expected to be selected for analysis per chip (right panel). (b) Experimental distributions of cell or nuclei numbers deposited into nanowells from SK-BR-3 cell suspensions, SK-BR-3 nuclear suspensions, TNBC nuclear suspensions and a normal breast tissue nuclei suspension, with observed  $\lambda$  range from 0.45-0.86.





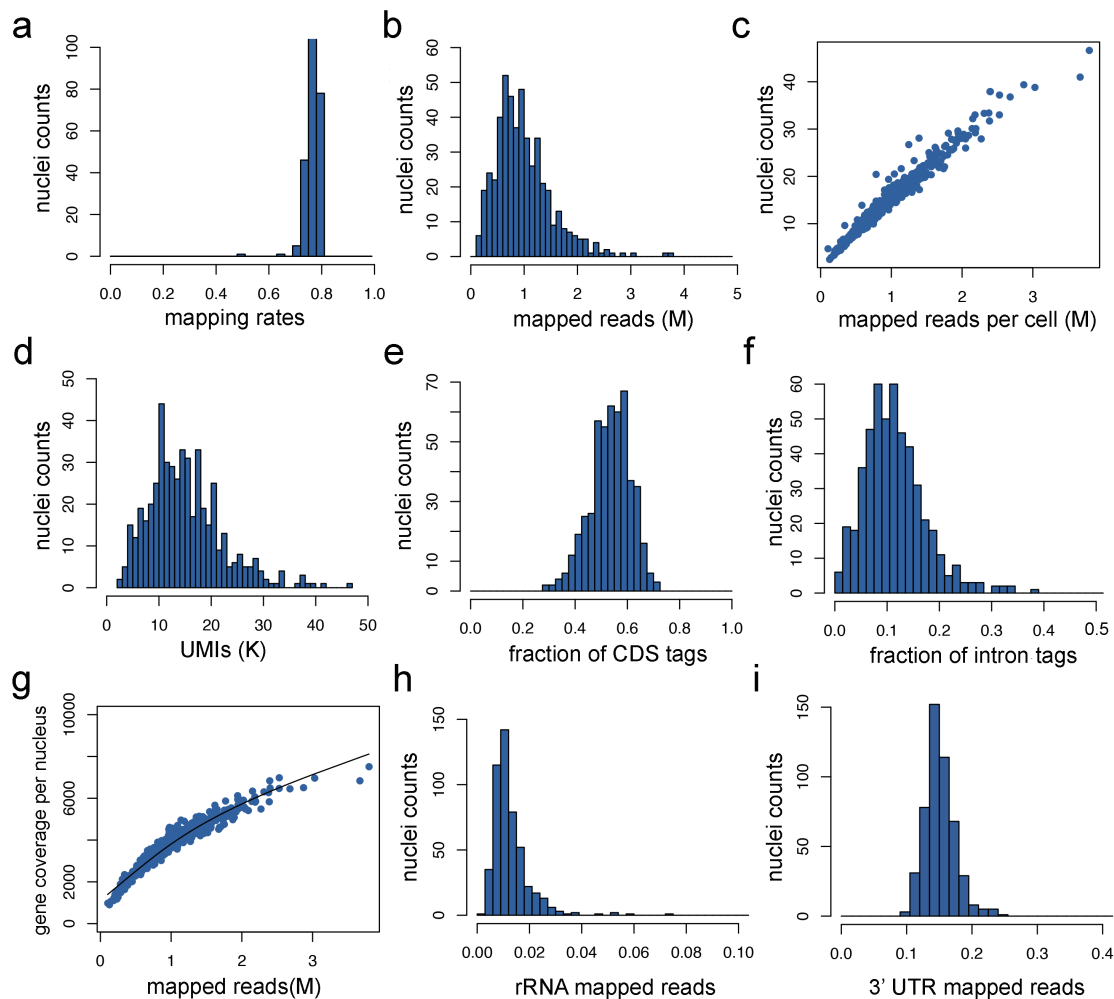
### Supplementary Figure 4 – Single Nuclei and Cell RNA Data Analysis Workflow

Data processing and analysis workflow for single nuclei and single cell RNA data. (a) Sequence reads are demultiplexed by well barcodes (WBC) and aligned to the human genome reference assembly. (b) Quality control metrics are calculated and read counts are normalized by library size and housekeeping genes, transformed to z-scores or TPM using RSEM. (c) Differentially expressed genes are identified using SCDE and annotated for gene ontologies and pathways. (d) Clustering and multivariate analysis is performed to identify groups of cells with similar transcriptional profiles. (e) Copy number profiles are calculated from RNA profiles. (f) Gene signature analysis and classification of breast cancer subtypes is performed.



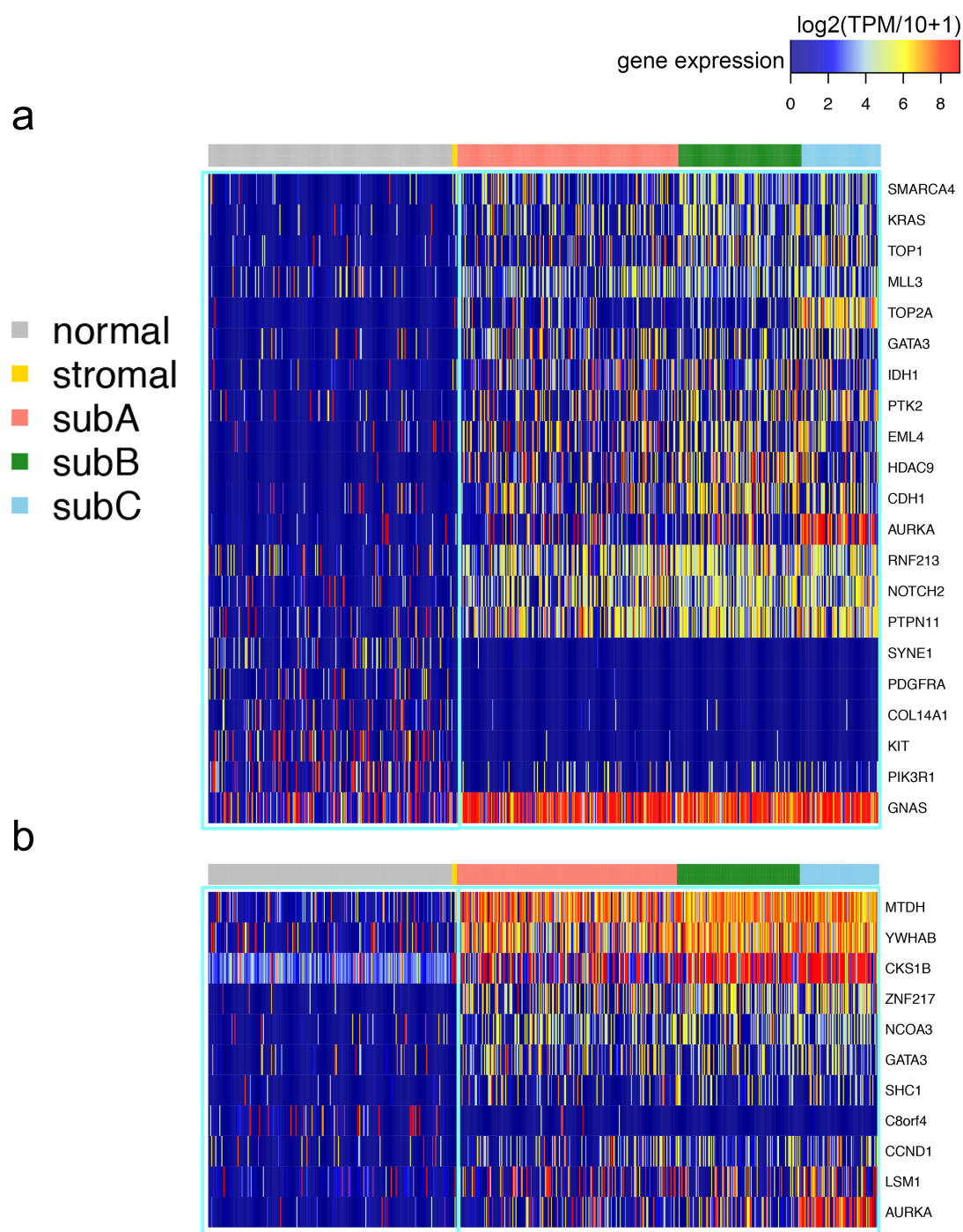
### Supplementary Figure 5 – Variance in Single Cell Gene Expression Data

Calculation of data variation between gene expression levels in single cell and single nuclei data from SK-BR-3. (a) Variance of all detectable genes in SK-BR-3 single cell data as a function of mean expression levels. (b) Pair-wise correlation heatmap of all single nuclei and single cells using 4414 top detected genes. (c) Scatter plot of two representative single cell gene expression levels in  $\log_2(\text{count}+1)$  scale. (d) Scatter plot of single cell and single nuclei within group variations of 196 significant genes detected between both groups by SCDE.



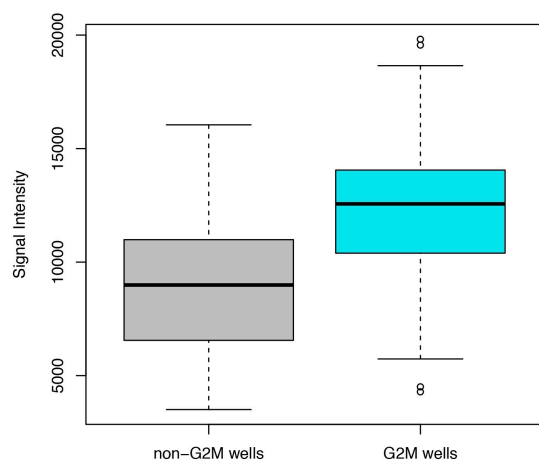
### Supplementary Figure 6 - QC Metrics for the Single Breast Tumor Nuclei

Sequence data metrics were calculated for single nuclei from the triple-negative breast tumor analyzed by nanogrid SNRS, including: (a) Sequence read mapping rates to the human genome reference assembly, (b) number of mapped reads per nucleus, (c) number of mapped reads in millions, (d) number of unique molecular identifiers detected in single nuclei, (e) read tags mapped to coding regions, (f) read tags mapped to intronic regions, (g) number of mapped reads per cell as a function of gene coverage, (h) ribosomal RNA mapped reads, (i) mapped reads to the 3' untranslated region.



**Supplementary Figure 7 –Expression of Cancer Genes in Single Cells from Stromal and Tumor Subpopulations**

Expression of known cancer genes in single cells from normal breast tissues, stroma and tumor tissues using targeted breast cancer gene sets from (a) TCGA and (b) the T200 cancer gene panel. Subpopulations are annotated based on detection of differential gene expression of all genes in Figure 5c and 5d.



### Supplementary Figure 8 – Comparison of G2/M single nuclei sizes from nanogrid imaging data

Boxplots of DAPI-staining intensities of G2/M single nuclei and G1/0/S single nuclei as inferred by RNA-seq data was matched to automated imaging data from the nanogrid system, showing larger nuclear sizes in the G2/M cells. The boxes indicated the 75% IQR and whiskers showed the default values of the boxplot (*graphics*). N= 78 for G2M group and N=338 for the non-G2M group.

## Supplementary Tables

### Supplementary Table 1 - Upregulated Genes in SK-BR-3 Single Nuclei comparing to Cells

This table lists all of the significant upregulated genes in nuclei compared to cells. Columns listed include Gene Identifier (Gene ID), Fold-change, Entrez Gene Name, cellular localization and gene class.

| Gene ID      | FoldChange | Entrez Gene Name                                                       | cellular localization | gene class              |
|--------------|------------|------------------------------------------------------------------------|-----------------------|-------------------------|
| ANKRD20A9P   | 2.0        | ankyrin repeat domain 20 family member A9, pseudogene                  | Other                 | other                   |
| ARHGEF26-AS1 | 2.8        | ARHGEF26 antisense RNA 1                                               | Other                 | other                   |
| ATP6V0A2     | 2.8        | ATPase H+ transporting V0 subunit a2                                   | Cytoplasm             | transporter             |
| BRIP1        | 2.1        | BRCA1 interacting protein C-terminal helicase 1                        | Nucleus               | enzyme                  |
| C12orf65     | 2.2        | chromosome 12 open reading frame 65                                    | Other                 | other                   |
| C21orf62     | 2.8        | chromosome 21 open reading frame 62                                    | Other                 | other                   |
| CASB         | 2.6        | carbonic anhydrase 5B                                                  | Cytoplasm             | enzyme                  |
| CBFA2T2      | 2.7        | CBFA2/RUNX1 translocation partner 2                                    | Nucleus               | transcription regulator |
| CCDC142      | 2.2        | coiled-coil domain containing 142                                      | Other                 | other                   |
| CEACAM22P    | 3.2        | carcinoembryonic antigen related cell adhesion molecule 22, pseudogene | Other                 | other                   |
| CEP104       | 2.1        | centrosomal protein 104                                                | Cytoplasm             | other                   |
| CMBL         | 2.4        | carboxymethylenebutenolidase homolog (Pseudomonas)                     | Cytoplasm             | enzyme                  |
| CNNM3        | 2.6        | cyclin and CBS domain divalent metal cation transport mediator 3       | Other                 | other                   |
| CRX          | 2.1        | cone-rod homeobox                                                      | Nucleus               | transcription regulator |
| CYP4V2       | 2.8        | cytochrome P450 family 4 subfamily V member 2                          | Cytoplasm             | enzyme                  |
| DCAF10       | 2.0        | DDB1 and CUL4 associated factor 10                                     | Other                 | other                   |
| DCUN1D2      | 2.0        | defective in cullin neddylation 1 domain containing 2                  | Other                 | other                   |
| FAM116A      | 2.2        | DENN domain containing 6A                                              | Cytoplasm             | other                   |
| DFFB         | 2.3        | DNA fragmentation factor subunit beta                                  | Nucleus               | enzyme                  |
| DMC1         | 3.2        | DNA meiotic recombinase 1                                              | Nucleus               | enzyme                  |
| ENTPD4       | 2.4        | ectonucleoside triphosphate diphosphohydrolase 4                       | Cytoplasm             | enzyme                  |
| EPHA10       | 3.1        | EPH receptor A10                                                       | Plasma Membrane       | transmembrane receptor  |
| ERVV-1       | 2.8        | endogenous retrovirus group V member 1                                 | Other                 | other                   |
| EXD1         | 2.7        | exonuclease 3'-5' domain containing 1                                  | Other                 | other                   |
| FAM126B      | 2.5        | family with sequence similarity 126 member B                           | Other                 | other                   |
| FAM74A1      | 2.9        | family with sequence similarity 74 member A1                           | Other                 | other                   |
| FBLIM1       | 2.1        | filamin binding LIM protein 1                                          | Plasma Membrane       | other                   |
| FBXO27       | 2.4        | F-box protein 27                                                       | Other                 | other                   |
| FLJ42102     | 2.5        | uncharacterized LOC399923                                              | Other                 | other                   |
| FMN1         | 2.1        | formin 1                                                               | Nucleus               | other                   |
| GFOD2        | 3.0        | glucose-fructose oxidoreductase domain containing 2                    | Extracellular Space   | enzyme                  |
| GGA2         | 2.0        | golgi associated, gamma adaptin ear containing, ARF binding protein 2  | Cytoplasm             | transporter             |
| GNE          | 2.5        | glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase      | Cytoplasm             | kinase                  |
| GREB1        | 2.4        | growth regulation by estrogen in breast cancer 1                       | Cytoplasm             | other                   |
| GRIPAP1      | 2.3        | GRIP1 associated protein 1                                             | Plasma Membrane       | other                   |
| HNRNPA1P10   | 3.1        | heterogeneous nuclear ribonucleoprotein A1 pseudogene 10               | Other                 | other                   |
| IGF2R        | 2.1        | insulin like growth factor 2 receptor                                  | Plasma Membrane       | transmembrane receptor  |
| IL17RD       | 2.4        | interleukin 17 receptor D                                              | Cytoplasm             | other                   |
| IRF1         | 2.5        | interferon regulatory factor 1                                         | Nucleus               | transcription regulator |
| KBTBD12      | 2.9        | kelch repeat and BTB domain containing 12                              | Other                 | other                   |
| KDEL2        | 2.0        | KDEL motif containing 2                                                | Other                 | other                   |
| L1TD1        | 3.3        | LINE-1 type transposase domain containing 1                            | Other                 | other                   |
| LAI1         | 3.0        | leukocyte associated immunoglobulin like receptor 1                    | Plasma Membrane       | transmembrane receptor  |
| LINC00294    | 2.2        | long intergenic non-protein coding RNA 294                             | Other                 | other                   |
| LINC00410    | 5.1        | long intergenic non-protein coding RNA 410                             | Other                 | other                   |
| C14orf23     | 2.6        | long intergenic non-protein coding RNA 1551                            | Other                 | other                   |
| LOC100190986 | 4.0        | uncharacterized LOC100190986                                           | Other                 | other                   |
| LOC643406    | 2.4        | uncharacterized LOC643406                                              | Other                 | other                   |
| LYZ          | 2.6        | lysozyme                                                               | Extracellular Space   | enzyme                  |
| MAB21L3      | 2.9        | mab-21 like 3                                                          | Other                 | other                   |
| MAP1LC3C     | 2.2        | microtubule associated protein 1 light chain 3 gamma                   | Cytoplasm             | other                   |
| MAP7D3       | 2.3        | MAP7 domain containing 3                                               | Cytoplasm             | other                   |
| MBOAT1       | 2.4        | membrane bound O-acyltransferase domain containing 1                   | Cytoplasm             | other                   |
| MEFV         | 3.1        | Mediterranean fever                                                    | Nucleus               | other                   |
| MTFMT        | 2.1        | mitochondrial methionyl-tRNA formyltransferase                         | Cytoplasm             | enzyme                  |
| LOC90834     | 3.9        | NA                                                                     | NA                    | NA                      |
| LOC731424    | 3.4        | NA                                                                     | NA                    | NA                      |
| LOC728558    | 3.0        | NA                                                                     | NA                    | NA                      |
| LOC147670    | 3.0        | NA                                                                     | NA                    | NA                      |
| LOC145663    | 2.5        | NA                                                                     | NA                    | NA                      |
| LOC100190938 | 2.5        | NA                                                                     | NA                    | NA                      |
| LOC642236    | 2.4        | NA                                                                     | NA                    | NA                      |
| NLRP12       | 2.9        | NLR family, pyrin domain containing 12                                 | Cytoplasm             | other                   |
| NPIPL3       | 3.4        | nuclear pore complex interacting protein family member B3              | Other                 | other                   |
| NTSDC3       | 2.5        | 5'-nucleotidase domain containing 3                                    | Other                 | other                   |
| NUAK2        | 2.9        | NUAK family kinase 2                                                   | Other                 | kinase                  |
| NXN          | 2.4        | nucleoredoxin                                                          | Nucleus               | enzyme                  |
| ORA12        | 2.6        | ORA1 calcium release-activated calcium modulator 2                     | Plasma Membrane       | other                   |

|               |     |                                                                      |                     |                            |
|---------------|-----|----------------------------------------------------------------------|---------------------|----------------------------|
| OTUD6A        | 2.1 | OTU deubiquitinase 6A                                                | Other               | peptidase                  |
| P2RX5-TAX1BP3 | 2.4 | P2RX5-TAX1BP3 readthrough (NMD candidate)                            | Other               | other                      |
| PDDC1         | 2.1 | Parkinson disease 7 domain containing 1                              | Cytoplasm           | other                      |
| PDE6A         | 2.4 | phosphodiesterase 6A                                                 | Plasma Membrane     | enzyme                     |
| PGM2L1        | 2.0 | phosphoglucomutase 2-like 1                                          | Cytoplasm           | enzyme                     |
| PGM5P2        | 2.1 | phosphoglucomutase 5 pseudogene 2                                    | Other               | other                      |
| PPP1R3B       | 3.2 | protein phosphatase 1 regulatory subunit 3B                          | Cytoplasm           | other                      |
| PRR11         | 2.2 | proline rich 11                                                      | Other               | other                      |
| PSTPIP2       | 2.2 | proline-serine-threonine phosphatase interacting protein 2           | Cytoplasm           | other                      |
| RBMS2         | 2.2 | RNA binding motif single stranded interacting protein 2              | Nucleus             | other                      |
| RP56KA3       | 2.3 | ribosomal protein S6 kinase A3                                       | Cytoplasm           | kinase                     |
| RRP7B         | 3.0 | ribosomal RNA processing 7 homolog B, pseudogene                     | Other               | other                      |
| KIAA0226      | 2.0 | RUN and cysteine rich domain containing beclin 1 interacting protein | Cytoplasm           | other                      |
| RUNDC1        | 2.2 | RUN domain containing 1                                              | Other               | other                      |
| SCAI          | 2.3 | suppressor of cancer cell invasion                                   | Nucleus             | transcription regulator    |
| SCD5          | 3.4 | stearoyl-CoA desaturase 5                                            | Cytoplasm           | enzyme                     |
| SERPINB9      | 2.3 | serpin family B member 9                                             | Cytoplasm           | other                      |
| SGSM1         | 3.3 | small G protein signaling modulator 1                                | Nucleus             | other                      |
| SKA1          | 2.1 | spindle and kinetochore associated complex subunit 1                 | Nucleus             | other                      |
| MCART1        | 2.3 | solute carrier family 25 member 51                                   | Cytoplasm           | other                      |
| SLC7A5P2      | 2.9 | solute carrier family 7 member 5 pseudogene 2                        | Other               | other                      |
| SPRED1        | 2.5 | sprouty related EVH1 domain containing 1                             | Plasma Membrane     | other                      |
| STAC2         | 3.1 | SH3 and cysteine rich domain 2                                       | Other               | other                      |
| FAM40B        | 2.4 | striatin interacting protein 2                                       | Cytoplasm           | other                      |
| STYX          | 2.1 | serine/threonine/tyrosine interacting protein                        | Cytoplasm           | phosphatase                |
| TACC2         | 3.2 | transforming acidic coiled-coil containing protein 2                 | Nucleus             | other                      |
| TADA2B        | 2.5 | transcriptional adaptor 2B                                           | Nucleus             | other                      |
| TAF8          | 2.0 | TATA-box binding protein associated factor 8                         | Nucleus             | transcription regulator    |
| TBXA2R        | 2.6 | thromboxane A2 receptor                                              | Plasma Membrane     | G-protein coupled receptor |
| TLCD2         | 2.6 | TLC domain containing 2                                              | Other               | other                      |
| TNFAIP8L1     | 2.9 | TNF alpha induced protein 8 like 1                                   | Cytoplasm           | other                      |
| TSIX          | 2.6 | TSIX transcript, XIST antisense RNA                                  | Nucleus             | other                      |
| UCKL1-AS1     | 3.2 | UCKL1 antisense RNA 1                                                | Other               | other                      |
| UTP3          | 2.2 | UTP3, small subunit processome component homolog (S. cerevisiae)     | Nucleus             | other                      |
| VP53          | 2.2 | VP53, GARP complex subunit                                           | Cytoplasm           | other                      |
| VSIG1         | 3.2 | V-set and immunoglobulin domain containing 1                         | Plasma Membrane     | other                      |
| VSTM4         | 3.3 | V-set and transmembrane domain containing 4                          | Other               | other                      |
| WDR45         | 2.2 | WD repeat domain 45                                                  | Other               | other                      |
| WNT7B         | 2.4 | Wnt family member 7B                                                 | Extracellular Space | other                      |
| XIST          | 2.1 | X inactive specific transcript (non-protein coding)                  | Nucleus             | other                      |
| ZC3H12D       | 3.1 | zinc finger CCCH-type containing 12D                                 | Cytoplasm           | other                      |
| ZFYVE19       | 2.6 | zinc finger FYVE-type containing 19                                  | Cytoplasm           | other                      |
| ZKSCAN3       | 2.6 | zinc finger with KRAB and SCAN domains 3                             | Nucleus             | transcription regulator    |
| ZMAT3         | 2.3 | zinc finger matrin-type 3                                            | Nucleus             | other                      |
| ZNF124        | 2.3 | zinc finger protein 124                                              | Nucleus             | other                      |
| ZNF264        | 2.6 | zinc finger protein 264                                              | Nucleus             | other                      |
| ZNF347        | 2.5 | zinc finger protein 347                                              | Nucleus             | other                      |
| ZNF490        | 2.5 | zinc finger protein 490                                              | Nucleus             | other                      |
| ZNF526        | 2.2 | zinc finger protein 526                                              | Other               | other                      |
| ZNF793        | 2.6 | zinc finger protein 793                                              | Other               | other                      |
| ZNF805        | 2.8 | zinc finger protein 805                                              | Other               | other                      |
| ZNF814        | 2.7 | zinc finger protein 814                                              | Other               | other                      |
| ZSCAN22       | 2.7 | zinc finger and SCAN domain containing 22                            | Nucleus             | transcription regulator    |

## Supplementary Table 2 - Downregulated Genes in SK-BR-3 Single Nuclei comparing to Cells

This table lists all of the significant downregulated genes in nuclei compared to cells. Columns listed include Gene Identifier (Gene ID), Fold-change, Entrez Gene Name, cellular localization and gene class.

| Gene ID   | FoldChange | Entrez Gene Name                                        | cellular localization | gene class              |
|-----------|------------|---------------------------------------------------------|-----------------------|-------------------------|
| PTS       | -2.3       | 6-pyruvoyltetrahydropterin synthase                     | Cytoplasm             | enzyme                  |
| AP2S1     | -2.2       | adaptor related protein complex 2 sigma 1 subunit       | Cytoplasm             | transporter             |
| ARF4      | -2.1       | ADP ribosylation factor 4                               | Cytoplasm             | enzyme                  |
| ALG14     | -2.5       | ALG14, UDP-N-acetylglucosaminyltransferase subunit      | Cytoplasm             | enzyme                  |
| ASNS      | -2.2       | asparagine synthetase (glutamine-hydrolyzing)           | Cytoplasm             | enzyme                  |
| BOLA3     | -2.6       | bolA family member 3                                    | Other                 | other                   |
| CD9       | -2.3       | CD9 molecule                                            | Plasma Membrane       | other                   |
| CKS1B     | -2.4       | CDC28 protein kinase regulatory subunit 1B              | Other                 | kinase                  |
| CETN2     | -2.1       | centrin 2                                               | Nucleus               | enzyme                  |
| C11orf73  | -2.0       | chromosome 11 open reading frame 73                     | Cytoplasm             | transporter             |
| COMMD3    | -2.3       | COMM domain containing 3                                | Other                 | other                   |
| COPS4     | -2.0       | COP9 signalosome subunit 4                              | Cytoplasm             | other                   |
| CNIH      | -2.3       | cornichon family AMPA receptor auxiliary protein 1      | Plasma Membrane       | other                   |
| C7orf44   | -2.5       | cytochrome c oxidase assembly factor 1 homolog          | Cytoplasm             | other                   |
| DAZAP2    | -2.0       | DAZ associated protein 2                                | Nucleus               | other                   |
| DRAM2     | -2.5       | DNA damage regulated autophagy modulator 2              | Cytoplasm             | other                   |
| ESD       | -2.3       | esterase D                                              | Cytoplasm             | enzyme                  |
| EIF5A     | -2.0       | eukaryotic translation initiation factor 5A             | Cytoplasm             | translation regulator   |
| FOS       | -2.1       | FBJ murine osteosarcoma viral oncogene homolog          | Nucleus               | transcription regulator |
| GABARAPL2 | -2.5       | GABA type A receptor associated protein like 2          | Cytoplasm             | other                   |
| GTF2IP1   | -5.3       | general transcription factor Ili pseudogene 1           | Other                 | other                   |
| GNPDA1    | -2.2       | glucosamine-6-phosphate deaminase 1                     | Cytoplasm             | enzyme                  |
| GSTM4     | -2.0       | glutathione S-transferase mu 4                          | Cytoplasm             | enzyme                  |
| CGA       | -2.1       | glycoprotein hormones, alpha polypeptide                | Extracellular Space   | other                   |
| H3F3AP4   | -2.7       | H3 histone, family 3A, pseudogene 4                     | Other                 | other                   |
| HIGD1A    | -2.1       | HIG1 hypoxia inducible domain family member 1A          | Cytoplasm             | other                   |
| HAT1      | -2.1       | histone acetyltransferase 1                             | Nucleus               | enzyme                  |
| IGFL1     | -3.6       | IGF like family member 1                                | Extracellular Space   | other                   |
| ID1       | -3.1       | inhibitor of DNA binding 1, HLH protein                 | Nucleus               | transcription regulator |
| ID3       | -2.0       | inhibitor of DNA binding 3, HLH protein                 | Nucleus               | transcription regulator |
| ITGB3BP   | -2.2       | integrin subunit beta 3 binding protein                 | Nucleus               | other                   |
| JKAMP     | -2.9       | JNK1/MAPK8-associated membrane protein                  | Cytoplasm             | other                   |
| LAMTOR1   | -2.0       | late endosomal/lysosomal adaptor, MAPK and MTOR act     | Plasma Membrane       | other                   |
| MAD2L1    | -2.1       | MAD2 mitotic arrest deficient-like 1 (yeast)            | Nucleus               | other                   |
| MEMO1     | -2.3       | mediator of cell motility 1                             | Cytoplasm             | other                   |
| MT1X      | -2.2       | metallothionein 1X                                      | Other                 | other                   |
| MGST1     | -2.0       | microsomal glutathione S-transferase 1                  | Cytoplasm             | enzyme                  |
| BRP44     | -2.6       | mitochondrial pyruvate carrier 2                        | Plasma Membrane       | other                   |
| MRPL39    | -2.1       | mitochondrial ribosomal protein L39                     | Cytoplasm             | other                   |
| MRPS14    | -2.2       | mitochondrial ribosomal protein S14                     | Cytoplasm             | other                   |
| MRPS23    | -2.1       | mitochondrial ribosomal protein S23                     | Cytoplasm             | other                   |
| LOC440354 | -2.1       | NA                                                      | NA                    | NA                      |
| LOC541471 | -2.1       | NA                                                      | NA                    | NA                      |
| LOC550643 | -2.0       | NA                                                      | NA                    | NA                      |
| NDUFA4    | -2.2       | NADH:ubiquinone oxidoreductase complex assembly fac     | Cytoplasm             | other                   |
| NIT2      | -2.2       | nitrilase family member 2                               | Cytoplasm             | enzyme                  |
| NUTF2     | -2.0       | nuclear transport factor 2                              | Nucleus               | transporter             |
| C20orf111 | -2.2       | oxidative stress responsive serine rich 1               | Other                 | enzyme                  |
| PDZD11    | -2.5       | PDZ domain containing 11                                | Extracellular Space   | other                   |
| PRDX3     | -2.3       | peroxiredoxin 3                                         | Cytoplasm             | enzyme                  |
| PIGU      | -2.3       | phosphatidylinositol glycan anchor biosynthesis class U | Cytoplasm             | enzyme                  |
| PHLDA2    | -2.9       | pleckstrin homology like domain family A member 2       | Cytoplasm             | other                   |
| POLR2G    | -2.0       | polymerase (RNA) II subunit G                           | Nucleus               | enzyme                  |
| POLR2K    | -2.5       | polymerase (RNA) II subunit K                           | Nucleus               | enzyme                  |
| PFDN1     | -2.2       | prefoldin subunit 1                                     | Cytoplasm             | transcription regulator |
| PPP6C     | -2.0       | protein phosphatase 6 catalytic subunit                 | Nucleus               | phosphatase             |



|          |      |                                                     |           |                         |
|----------|------|-----------------------------------------------------|-----------|-------------------------|
| PLP2     | -2.5 | proteolipid protein 2 (colonic epithelium-enriched) | Cytoplasm | transporter             |
| RPA3     | -2.0 | replication protein A3                              | Nucleus   | other                   |
| RPL21    | -2.7 | ribosomal protein L21                               | Cytoplasm | other                   |
| SAMM50   | -2.2 | SAMM50 sorting and assembly machinery component     | Cytoplasm | other                   |
| C19orf42 | -2.2 | small integral membrane protein 7                   | Other     | other                   |
| TAX1BP3  | -2.6 | Tax1 binding protein 3                              | Cytoplasm | transcription regulator |
| TXNDC12  | -2.3 | thioredoxin domain containing 12                    | Cytoplasm | enzyme                  |
| TMX2     | -2.2 | thioredoxin related transmembrane protein 2         | Other     | enzyme                  |
| TMEM106C | -2.5 | transmembrane protein 106C                          | Other     | other                   |
| TMEM14A  | -3.6 | transmembrane protein 14A                           | Other     | other                   |
| TMEM18   | -2.3 | transmembrane protein 18                            | Nucleus   | other                   |
| TMEM99   | -2.6 | transmembrane protein 99                            | Other     | other                   |
| TSFM     | -2.5 | Ts translation elongation factor, mitochondrial     | Cytoplasm | translation regulator   |
| UBE2T    | -2.0 | ubiquitin conjugating enzyme E2 T                   | Nucleus   | enzyme                  |
| VPS25    | -2.6 | vacuolar protein sorting 25 homolog                 | Cytoplasm | other                   |
| VDAC3    | -2.0 | voltage dependent anion channel 3                   | Cytoplasm | ion channel             |
| WDR83OS  | -2.1 | WD repeat domain 83 opposite strand                 | Other     | other                   |
| WDYHV1   | -2.5 | WDYHV motif containing 1                            | Cytoplasm | other                   |
| ZNF706   | -2.0 | zinc finger protein 706                             | Other     | other                   |

### Supplementary Table 3 - Differentially Enriched Oncogenic Gene Signatures Between SK-BR-3 Single Nuclei and Cells

This table lists the 5 out of 189 significant differentially enriched oncogenic gene signatures identified by GSVA analysis between single nuclei and single cells. Columns listed include the Gene Signature Identifier, Average difference of GSVA score between nuclei and whole cells (Avg\_diff), p-value (pval) and FDR adjusted p-value (adjPval).

#### Nuclei Enriched oncogenic genesets

| GeneSet Identifier | Avg_diff    | pval     | adjPval  |
|--------------------|-------------|----------|----------|
| CSR LATE UP.V1 UP  | 0.118060921 | 7.22E-38 | 1.14E-36 |
| EIF4E UP           | 0.11607917  | 3.70E-56 | 2.33E-54 |
| RB DN.V1 UP        | 0.102414964 | 1.19E-40 | 2.49E-39 |

#### Whole Cell Enriched oncogenic genesets

| GeneSet Identifier   | Avg_diff    | pval     | adjPval  |
|----------------------|-------------|----------|----------|
| HINATA NFKB IMMU INF | -0.10104583 | 1.38E-13 | 5.23E-13 |
| CSR EARLY UP.V1 DN   | -0.11034172 | 2.12E-73 | 4.01E-71 |

**Supplementary Table 4 – Comparison of Sequencing Metrics of Nanogrid SNRS-seq to Drop-seq.**

This table lists sequencing and QC metrics for single cells that were profiled by nanogrid SNRS and Drop-seq from the SK-BR-3 cell line. To perform a fair comparison the Nanogrid data was downsampled to 90,732 reads per cell before metrics were calculated.

| Platforms                          | Drop-seq | Nanogrid* |
|------------------------------------|----------|-----------|
| median total reads per cell        | 90,732   | 90,732    |
| number of cells sequenced          | 8,128    | 525       |
| median mapping rates per cell      | 29.00%   | 68.72%    |
| median mapped reads per cell       | 26,312   | 62,354    |
| median CDS reads/tags fractions    | 30.60%   | 72.26%    |
| median Intron reads/tags fractions | 6.00%    | 1.33%     |
| median gene covered per cell       | 2,714    | 2,349     |
| median UMIs per cell               | 16,414   | 25,061    |

\*data were downsampled from original sequenced fastq files.