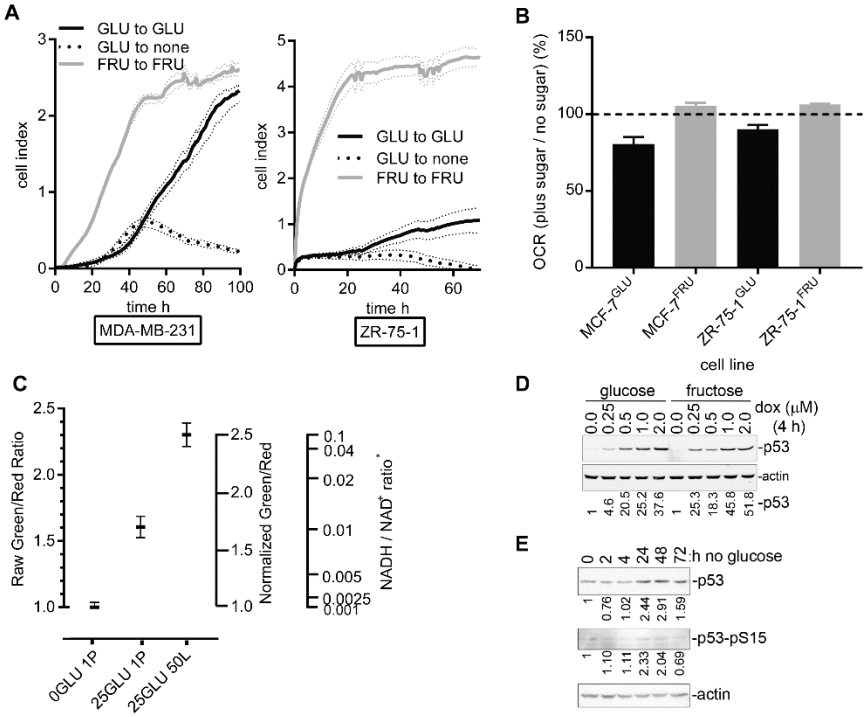

Fig. S1. Effects of altered media sugar composition on the proliferation, metabolism and p53 expression in breast cancer cells . (**A**) Glucose- and fructose-adapted MDA-MB-231 (left) and ZR-75-1 (right) cells had the medium replaced with one containing the indicated sugar, and the cells were then analyzed for proliferation rate using xCELLigence. Data are means ± range of at least two experiments. Equal numbers of cells were plated per well. (**B**) Effect of sugar on OCR measured in conjunction with the ECAR readings shown in Fig. 1. (**C**) Calibration of the NADH/NAD^+ biosensor (Peredox) in MCF-7 cells. MCF-7 cells growing in standard DMEM culture medium including 25 mM glucose and 1 mM pyruvate were transferred to modified assay medium [serum- and sodium bicarbonate–free DMEM (Sigma #D5030), 2 mM Hepes (pH 7.4), 2 mM glutamine] with (i) 0 mM glucose and 1 mM pyruvate, (ii) 25 mM glucose and 1 mM pyruvate, or (iii) 25 mM glucose and 50 mM lactate. After 30 min (i) or 10 min (ii and iii), the cells were imaged by fluorescence microscopy. For (i), an additional medium change was made 10 min before imaging to remove any lactate produced from residual glucose in the cells. The fluorescence intensity of each individual cell was calculated with Image J software and, after background subtraction, raw green/red ratios were calculated and baselined to 1 (0GLU 1P). This resulted in a maximum range for the raw ratios of 1 (0GLU 1P) to 2.3 (25GLU 50L). As described by Hung *et al*. (*11*), these data were normalized onto a 1 to 2.5 range (right-hand axis; this scale was then used in the main manuscript). The asterisk indicates NADH/NAD^+ ratios determined by Hung *et al*. (*11*) that correspond to the full range of the Peredox sensor are shown for reference. (**D**) $MCF\text{-}7^{GLU}$ and $MCF\text{-}7^{FRU}$ cells were exposed to the indicated concentrations of doxorubicin for 4 hours and the effect on p53 protein abundance was determined by Western blotting analysis. (**E**) $MCF\text{-}7^{GLU}$ cells were transferred to medium lacking added sugar (0 mM glucose) and were analyzed by Western blotting to determine relative p53 protein abundance and phosphorylation at Ser^{15}. Blots in (D) and (E) are representative of two experiments.

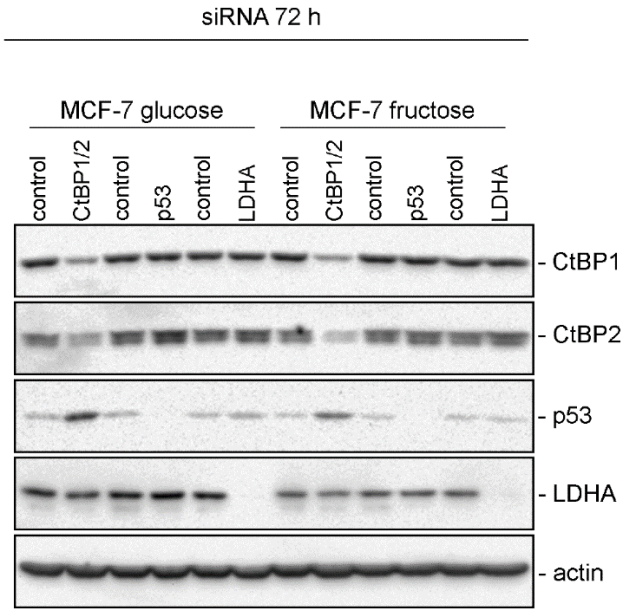

Fig. S2. Validation of siRNA efficacy in MCF-7 cells. MCF-7^{GLU} cells and MCF-7^{FRU} cells were transfected with the indicated siRNAs. Seventy-two hours later, the cells were analyzed by Western blotting with antibodies against the indicated proteins. Blots are representative of at least two experiments.

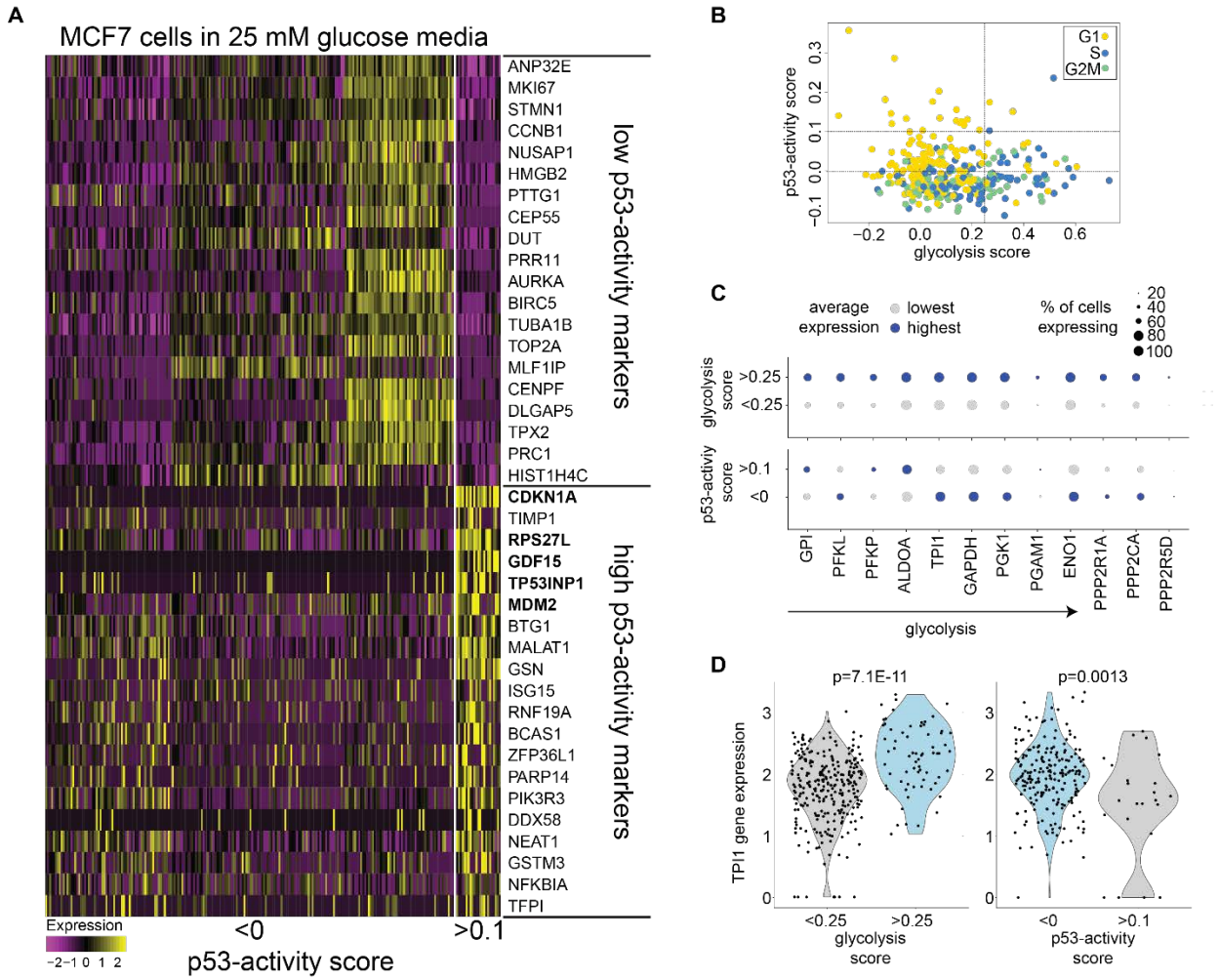

Fig. S3. Extended analysis of single cell mRNA sequencing of MCF7 cells . (**A**) Heatmap of genes differentially expressed between MCF-7^{GLU} cells with a p53-activity score >0.1 compared to MCF-7^{GLU} cells with a p53-activity score <0. The 20 genes with the greatest average fold change in expression are shown. Genes in bold are present in the validated set of 116 p53-activated target genes used to generate the p53-activity score. (**B**) GenePlot of glycolysis score versus p53-activity score in MCF-7^{GLU} cells. Colors indicate the assigned cell cycle phases for each cell. (**C**) Dot plots showing genes from the glycolysis gene set that were revealed as statistically significant markers of MCF-7^{GLU} cells with a glycolysis score >0.25 versus those with a score <0.25. Top: The expression of cells identified by glycolysis score. Bottom: The expression of genes by p53-activity score. Genes encoding glycolytic enzymes are arranged as per their order in the glycolytic pathway (PPP2 is involved in the positive regulation of PFK enzymes). (**D**) Violin plot showing *TPI1* expression in MCF-7^{GLU} cells identified by their glycolysis score (left) or p53 activity score (right). Statistical analysis using the Wilcoxon rank sum test was performed with Seurat v2.3.1 (R). A-D show combined data from three independent experiments.

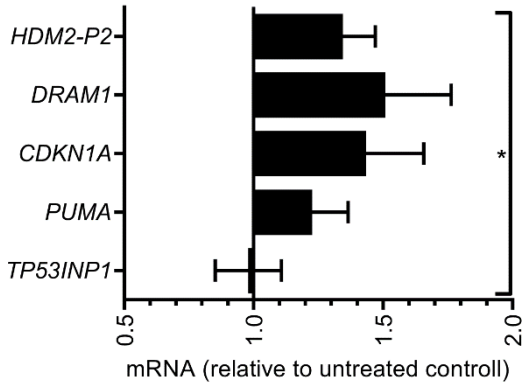

Fig. S4. The effect of lactate on the induction of p53-responsive genes. MCF-7 cells were cultured as described in Fig. 3A for 20 hours in the absence or presence of 50 mM lactate before being subjected to RT-qPCR analysis of the expression of the indicated genes. Data are means ± SEM of three experiments. Genes were selected for analysis on the basis of their being either classically defined as p53-response genes in response to multiple stimuli (*HDM2-P2*, *CDKN1A*, and *PUMA*) or those that showed a strong response to sodium oxamate (*DRAM1* and *TP53INP1*). The statistical significance of lactate on the combined gene set was tested by one-sample *t* test $*P < 0.05$.

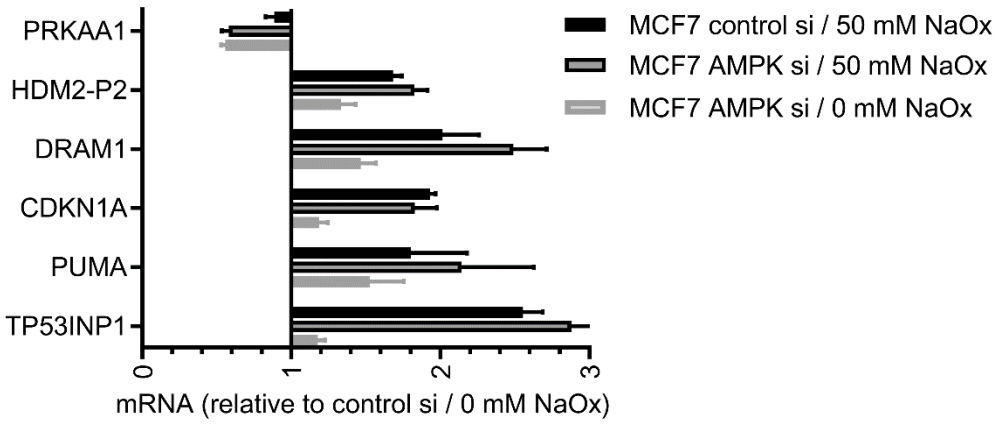

Fig. S5. The effect of AMPKα siRNA on the induction of p53-responsive genes by oxamate . MCF-7 cells were transfected with an siRNA pool targeting *PRKAA1* and *PRKAA2* mRNAs, which encode AMPKα (Santa Cruz Biotechnology, sc-45312). Seventy-two hours later, cells were treated with vehicle or 50 mM sodium oxamate for 6 hours before being subjected to lysis, mRNA extraction, and RT-qPCR analysis. Data are means ± SEM of three experiments. The efficacy of knockdown was validated by RT-qPCR analysis of *PRKAA1* expression

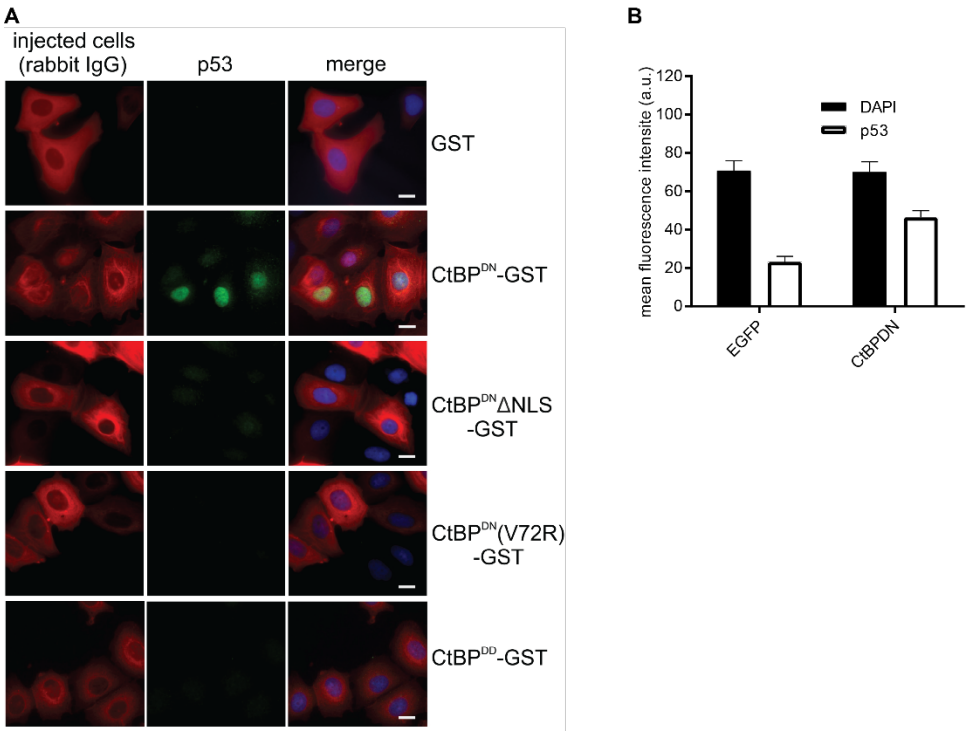

Fig. S6. Effect of dominant negative CtBP fragments on p53 expression in MCF-7 cells. (**A**) Immunohistochemical analysis of the MCF-7 cells microinjected with the indicated GST miniproteins as depicted in Fig. 4D. The injected cells are stained red, p53 is shown in green, and DNA is shown in blue (DAPI). (**B**) Relative p53 abundance in the nuclei of synchronized MCF-7 cells transfected with the EGFP-N1 and CtBPDN-EGFP constructs 6 hours before being released from serum starvation. Cells were fixed 20 hours after release, and quantitative immunofluorescence analysis of p53 abundance was performed with ImageJ software. Bars show means ± SEM of 22 cells from a representative of two experiments.

Time	Quantification: CtBP siRNA / control siRNA			
	p53	pSer15-p53	pSer392-p53	ac382-p53
24 h	12.08	3.79	4.08	0.60
48 h	84.16	19.44	13.07	5.24
72 h	6.28	6.45	5.17	1.98

Fig. S7 Quantification of the effect of CtBP siRNA on p53 post translational modifications. MCF-7 cells were transfected with the indicated siRNAs at the point of release from 48 hours of serum-starvation. Samples were prepared for Western blotting at the indicated times after release. Quantification shows background-subtracted band intensities, normalized to actin and expressed relative to the control siRNA.

Table S1. The qPCR primers and probes used in this study.

Roche universal probe library system			
Gene	**Left primer**	**Right primer**	**Probe**
DDB2	tcaaggacaaacccaccttc	aaacttcagcccagtgatgc	#78
DRAM-1	tgtctgtgcttcactaatttcca	tcacagatcgcactcactacg	#78
RRM1	aagcaccctgactatgctatcc	gttatagaggtcttccatcacatcac	#71
EDA2R	ccagcccttaactctaatgagg	tgctgctgttgtggtatagga	#61
RPS27L	tctatcccggaagttgatgc	caagctcagccctaccagac	#23
Applied Biosystems "assays on demand"			
Gene	**Assay**		
CDKN1A	Hs00355782_m1		
PUMA	Hs00248075_m1		
TP53INP1	Hs01003820_m1		
SESN1	Hs00902787_m1		
TIGAR	Hs00608644_m1		
Custom qPCR			
Gene	**Left primer**	**Right primer**	**Probe**
HDM2-P2	cggacgcacgccactt	cagtaggtacagacatgttggtattgc	(5' FAM, 3' TAMRA) ttctctgctgatccaggcaaatg

Glycolysis Gene Annotation list for Dropseq analysis

Downloaded 27-11-18 from:

http://software.broadinstitute.org/gsea/msigdb/geneset_page.jsp?geneSetName=REACTOME_GLYCOLYSIS&keywords=glycolysis

ALDOA; ALDOB; ALDOC; ENO1; ENO2; ENO3; GAPDH; GAPDHS; GPI; LOC642969; PFKFB1; PFKFB2; PFKFB3; PFKFB4; PFKL; PFKM; PFKP; PGAM1; PGAM2; PGK1; PKLR; PKM2; PPP2CA; PPP2CB; PPP2R1A; PPP2R1B; PPP2R5D; TPI1; TPI1P1

R Script for Dropseq analysis

```
### Written for Seurat v 2.3.1

library(Seurat)
library(dplyr)
library(Matrix)

### can move straight to reloading pre-analysed datasets at this point

### ensure the working directory is set
### read in raw DGE dat
MCF7FRU.data=read.table("MCF7_2D_FRUCTOSE.dge.txt",sep="\t",header=TRUE,row.n
ames=1)
MCF7GLU.data=read.table("MCF7_2D_GLUCOSE.dge.txt",sep="\t",header=TRUE,row.na
mes=1)
### change working directory if required

### initialize the Seurat object with the raw (non-normalized data).
### keep all genes expressed in >= 3 cells. Keep all cells with at least 2000
detected genes.
MCF7FRU <- CreateSeuratObject(raw.data = MCF7FRU.data, min.cells = 3, min.genes
= 2000, project = "BLAYDES", names.field = 1, names.delim = "_", do.scale =
FALSE, do.center = FALSE)
MCF7GLU <- CreateSeuratObject(raw.data = MCF7GLU.data, min.cells = 3, min.genes
= 2000, project = "BLAYDES", names.field = 1, names.delim = "_", do.scale =
FALSE, do.center = FALSE)
MCF7GLUFRU <- MergeSeurat(MCF7GLU,MCF7FRU,min.cells = 0, min.genes = 0, project
= "BLAYDES", names.field = 1, names.delim = "_", do.scale = FALSE, do.center =
FALSE, do.normalize = FALSE)

### normalise the data
MCF7GLUFRU <- NormalizeData(object = MCF7GLUFRU, normalization.method =
"LogNormalize", scale.factor = 10000)

MCF7GLU <- NormalizeData(object = MCF7GLU, normalization.method =
"LogNormalize", scale.factor = 10000)

MCF7FRU <- NormalizeData(object = MCF7FRU, normalization.method =
"LogNormalize", scale.factor = 10000)

### calculate mitochondrial content for each cell
mito.genes <- grep("^MT-", rownames(MCF7GLUFRU@data), value = T)
percent.mito                                                          <-
colSums(expm1(MCF7GLUFRU@data[mito.genes, ]))/colSums(expm1(MCF7GLUFRU@data))
MCF7GLUFRU <- AddMetaData(MCF7GLUFRU, percent.mito, "percent.mito")

mito.genes <- grep("^MT-", rownames(MCF7GLU@data), value = T)
percent.mito                                                          <-
colSums(expm1(MCF7GLU@data[mito.genes, ]))/colSums(expm1(MCF7GLU@data))
MCF7GLU <- AddMetaData(MCF7GLU, percent.mito, "percent.mito")

mito.genes <- grep("^MT-", rownames(MCF7FRU@data), value = T)
percent.mito                                                          <-
colSums(expm1(MCF7FRU@data[mito.genes, ]))/colSums(expm1(MCF7FRU@data))
MCF7FRU <- AddMetaData(MCF7FRU, percent.mito, "percent.mito")
```

```
### plots of confounding variables (end of script) used to identify settings
with which to subset data
### subset data
MCF7GLUFRU <- SubsetData(MCF7GLUFRU, subset.name = "nUMI", accept.high = 15000)
MCF7GLUFRU <- SubsetData(MCF7GLUFRU, subset.name = "percent.mito", accept.high
= 0.1)

MCF7GLU <- SubsetData(MCF7GLU, subset.name = "nUMI", accept.high = 15000)
MCF7GLU <- SubsetData(MCF7GLU, subset.name = "percent.mito", accept.high = 0.1)

MCF7FRU <- SubsetData(MCF7FRU, subset.name = "nUMI", accept.high = 15000)
MCF7FRU <- SubsetData(MCF7FRU, subset.name = "percent.mito", accept.high = 0.1)

### FindVariableGenes not used here for UMI datasets where technical variables
are regressed out
### define all the genes not expressed from mitochondrial DNA
GLUFRUnonmito.genes <- grep("^MT-", rownames(MCF7GLUFRU@data), value = T,
invert = TRUE)
writeLines(GLUFRUnonmito.genes, con = "GLUFRUnonmito.genes.txt")

GLUnonmito.genes <- grep("^MT-", rownames(MCF7GLU@data), value = T, invert =
TRUE)
writeLines(GLUnonmito.genes, con = "GLUnonmito.genes.txt")

FRUnonmito.genes <- grep("^MT-", rownames(MCF7FRU@data), value = T, invert =
TRUE)
writeLines(FRUnonmito.genes, con = "FRUnonmito.genes.txt")

### perform negative-binomial regression on the nonmito genes, this sets their
value in XXXXX@scale.data, which is used for PCA/clustering
### to use all genes: genes.use = rownames(MCF7GLUFRU@data)
MCF7GLUFRU <- ScaleData(MCF7GLUFRU, vars.to.regress = c("nUMI", "percent.mito"),
genes.use = GLUFRUnonmito.genes, model.use = "negbinom", do.scale = T, do.center
= T)

MCF7GLU <- ScaleData(MCF7GLU, vars.to.regress = c("nUMI", "percent.mito"),
genes.use = GLUnonmito.genes, model.use = "negbinom", do.scale = T, do.center
= T)

MCF7FRU <- ScaleData(MCF7FRU, vars.to.regress = c("nUMI", "percent.mito"),
genes.use = FRUnonmito.genes, model.use = "negbinom", do.scale = T, do.center
= T)

save(MCF7GLUFRU, file="MCF7GLUFRU.robj")
save(MCF7GLU, file="MCF7GLU.robj")
save(MCF7FRU, file="MCF7FRU.robj")
save(MCF7FRU.data, file="MCF7FRU.RDATA")
save(MCF7GLU.data, file="MCF7GLU.RDATA")

### _____________________________
### ANALYSIS of combined GLUFRU dataset
### _____________________________

load(file="MCF7GLUFRU.robj")
load(file="MCF7FRU.RDATA")
load(file="MCF7GLU.RDATA")
```

```
nonmito.genes <- readLines(con="GLUFRUnonmito.genes.txt")

write.csv(MCF7GLUFRU@cell.names,file="cellnames.csv",quote=F,row.names = F)

###
_____________
###             Cell             Cycle             Phase             Annotation
(http://satijalab.org/seurat/cell_cycle_vignette.html)
###
_____________

cc.genes      <-      readLines(con      =      "C:/Program      Files/R/R-
3.4.1/seurat_resources/regev_lab_cell_cycle_genes.txt")
s.genes <- cc.genes[1:43]
g2m.genes <- cc.genes[44:98]
MCF7GLUFRU  <-  CellCycleScoring(object  =  MCF7GLUFRU,  s.genes  =  s.genes,
g2m.genes = g2m.genes, set.ident = TRUE)
head(x = MCF7GLUFRU@meta.data)
JoyPlot(object  =  MCF7GLUFRU,  features.plot  =  c("PCNA",  "TOP2A",  "MCM6",
"MKI67"), nCol = 2)

###_____________________________________
### p53 transcriptional signature annotation: Table 1 in Fischer et al 2017
(PMID 28288132)
###_____________________________________

p53.genes      <-      readLines(con      =      "C:/Program      Files/R/R-
3.4.1/seurat_resources/Fischer_116_p53_induced_gene_list.txt")
P53.GENES <- list(p53.genes)
MCF7GLUFRU <- AddModuleScore(
  object = MCF7GLUFRU,
  genes.list = P53.GENES,
  ctrl.size = 100,
  enrich.name = "P53_GENES"
)
head(x = MCF7GLUFRU@meta.data)

VlnPlot(MCF7GLUFRU,   "P53_GENES1",   group.by   =   "orig.ident",   cols.use   =
c("lightgrey", "lightgrey"), point.size.use = 3)
VlnPlot(MCF7GLUFRU,   "P53_GENES1",   group.by   =   "orig.ident",   cols.use   =
c("orange", "lightblue"), point.size.use = 3)

MCF7GLUFRUp53 <- SubsetData(MCF7GLUFRU, subset.name = "P53_GENES1", accept.low
= 0.1)
write.csv(MCF7GLUFRUp53@cell.names,file="cellnamesp53.csv",quote=F,row.names =
F)

### _____________________________________________
### Glycolysis Gene Annotation :    Downloaded          271118          from
      http://software.broadinstitute.org/gsea/msigdb/geneset_page.jsp?geneSet
Name=REACTOME_GLYCOLYSIS&keywords=glycolysis
### _____________________________________________________

glycol.genes      <-      readLines(con      =      "C:/Program      Files/R/R-
3.4.1/seurat_resources/glycolysis_gene_set.txt")
```

```
GLYCOL.GENES <- list(glycol.genes)
MCF7GLUFRU <- AddModuleScore(
  object = MCF7GLUFRU,
  genes.list = GLYCOL.GENES,
  ctrl.size = 100,
  enrich.name = "GLYCOL_GENES"
)
head(x = MCF7GLUFRU@meta.data)

VlnPlot(MCF7GLUFRU, "GLYCOL_GENES1", group.by = "orig.ident", cols.use = c("lightgrey", "lightgrey"), point.size.use = 3)
VlnPlot(MCF7GLUFRU, "GLYCOL_GENES1", group.by = "orig.ident", cols.use = c("orange", "lightblue"), point.size.use = 3)


MCF7GLUFRUglycol <- SubsetData(MCF7GLUFRU, subset.name = "GLYCOL_GENES1", accept.low = 0.35)
write.csv(MCF7GLUFRUglycol@cell.names,file="cellnamesglycol.csv",quote=F,row.names = F)

VlnPlot(MCF7GLUFRU, c("percent.mito"), nCol=1, group.by = "orig.ident", cols.use = c("lightgrey", "lightgrey"), point.size.use = 3)
VlnPlot(MCF7GLUFRU, c("percent.mito"), nCol=1, group.by = "orig.ident", cols.use = c("orange", "lightblue"), point.size.use = 3)


### Further analysis of glycolytic status
MCF7GLUFRUnotglycol <- SubsetData(MCF7GLUFRU, subset.name = "GLYCOL_GENES1", accept.high = 0.25)

MCF7GLUFRUglycol@meta.data$cell.type <- "GLUFRU_glycol"
MCF7GLUFRUnotglycol@meta.data$cell.type <- "GLUFRU_notglycol"
MCF7GLUFRU3 <- MergeSeurat(MCF7GLUFRUglycol,MCF7GLUFRUnotglycol,min.cells = 0, min.genes = 0, project = "BLAYDES", names.field = 1, names.delim = "_", do.scale = FALSE, do.center = FALSE, do.normalize = FALSE)
MCF7GLUFRU3 <- NormalizeData(object = MCF7GLUFRU3, normalization.method = "LogNormalize", scale.factor = 10000)
MCF7GLUFRU3 <- ScaleData(MCF7GLUFRU3, genes.use = nonmito.genes, model.use = "negbinom", do.scale = T, do.center = T)

MCF7GLUFRU3 <- RunPCA(MCF7GLUFRU3, pc.genes = nonmito.genes, pcs.compute = 20, pcs.print = 20)
PCHeatmap(MCF7GLUFRU3,pc.use = 1:12,100)
PCHeatmap(MCF7GLUFRU3,pc.use = 13:20,100)
PCElbowPlot(MCF7GLUFRU3, num.pc = 20)

### select PCAs for downstream analysis, run TSNE and find clusters. PC1 and PC2 are commonly cell cycle
PCA <-c(1:5)
MCF7GLUFRU3 <- RunTSNE(MCF7GLUFRU3, dims.use = PCA , do.fast = T)

### save.SNN means that you can easily re-run with different resolution values.
MCF7GLUFRU3 <- FindClusters(MCF7GLUFRU3, reduction.type = "pca", dims.use = PCA, resolution = seq(0.5,1.25,0.25), force.recalc = TRUE, save.SNN = T, print.output = FALSE)
```

```
### outputs based on glycolytic status
MCF7GLUFRU3 <- SetAllIdent(MCF7GLUFRU3, id = "cell.type")
TSNEPlot(MCF7GLUFRU3,do.label = F, pt.size=2.5, colors.use = c("darkorange","blue4"))
MCF7GLUFRU3.origidentmarkers <- FindAllMarkers(MCF7GLUFRU3, genes.use = nonmito.genes, only.pos = TRUE, min.pct = 0.25, thresh.use = 0.25, min.diff.pct = -Inf, test.use = "bimod")
MCF7GLUFRU3.origidentmarkers %>% group_by(cluster) %>% top_n(2, avg_logFC)
write.csv (MCF7GLUFRU3.origidentmarkers, "origidentmarkers.csv")
MCF7GLUFRU3.origidentmarkers %>% group_by(cluster) %>% top_n(20, avg_logFC) -> top20
DoHeatmap(MCF7GLUFRU3, genes.use = top20$gene, slim.col.label = TRUE, remove.key = FALSE, rotate.key = TRUE, cex.row = 15, title = "Original Cell Identity")

### _____
### ANALYSIS of GLU dataset
### _____

load(file="MCF7GLU.robj")
load(file="MCF7GLU.RDATA")
nonmito.genes <- readLines(con="GLUnonmito.genes.txt")

write.csv(MCF7GLU@cell.names,file="cellnames.csv",quote=F,row.names = F)

###
_____
_____
### Cell Cycle Modified PCA preparation (http://satijalab.org/seurat/cell_cycle_vignette.html)
###
_____
_____

cc.genes <- readLines(con = "C:/Program Files/R/R-3.4.1/seurat_resources/regev_lab_cell_cycle_genes.txt")
s.genes <- cc.genes[1:43]
g2m.genes <- cc.genes[44:98]
MCF7GLU <- CellCycleScoring(object = MCF7GLU, s.genes = s.genes, g2m.genes = g2m.genes, set.ident = TRUE)
head(x = MCF7GLU@meta.data)
JoyPlot(object = MCF7GLU, features.plot = c("PCNA", "TOP2A", "MCM6", "MKI67"), nCol = 2)
### FeaturePlot(MCF7GLU, "S.Score", cols.use = c("gold", "black"))
### FeaturePlot(MCF7GLU, "G2M.Score", cols.use = c("gold", "black"))

###_____
### p53 transcriptional signature annotation: Table 1 in Fischer et al 2017 (PMID 28288132)
###_____
p53.genes <- readLines(con = "C:/Program Files/R/R-3.4.1/seurat_resources/Fischer_116_p53_induced_gene_list.txt")

P53.GENES <- list(p53.genes)
MCF7GLU <- AddModuleScore(
  object = MCF7GLU,
  genes.list = P53.GENES,
```

```
  ctrl.size = 100,
  enrich.name = "P53_GENES"
)
head(x = MCF7GLU@meta.data)

VlnPlot(MCF7GLU, "P53_GENES1", group.by = "Phase", cols.use = c("gold",
"seagreen1", "dodgerblue2"), point.size.use = 2)
write.csv(MCF7GLU@meta.data,file="metadataGLU.csv",quote=F,row.names = F)

### ____________________________________________________
### Glycolysis Gene Annotation :    Downloaded          271118          from
http://software.broadinstitute.org/gsea/msigdb/geneset_page.jsp?geneSetName=R
EACTOME_GLYCOLYSIS&keywords=glycolysis

### ____________________________________________________________

glycol.genes      <-      readLines(con      =      "C:/Program      Files/R/R-
3.4.1/seurat_resources/glycolysis_gene_set.txt")

GLYCOL.GENES <- list(glycol.genes)
MCF7GLU <- AddModuleScore(
  object = MCF7GLU,
  genes.list = GLYCOL.GENES,
  ctrl.size = 100,
  enrich.name = "GLYCOL_GENES"
)
head(x = MCF7GLU@meta.data)

VlnPlot(MCF7GLU, "GLYCOL_GENES1", group.by = "Phase", cols.use = c("gold",
"seagreen1", "dodgerblue2"), point.size.use = 2)
GenePlot(MCF7GLU,   "GLYCOL_GENES1",   "P53_GENES1",   col.use   =   c("gold",
"seagreen1", "dodgerblue2"), cex.use = 3)

MCF7GLUglycol <- SubsetData(MCF7GLU, subset.name = "GLYCOL_GENES1", accept.low
= 0.25)
write.csv(MCF7GLUglycol@meta.data,file="metadataGLUglycol.csv",quote=F,row.na
mes = F)

MCF7GLUp53 <- SubsetData(MCF7GLU, subset.name = "P53_GENES1", accept.low = 0.1)
MCF7GLUnotp53 <- SubsetData(MCF7GLU, subset.name = "P53_GENES1", accept.high =
0)

write.csv(MCF7GLUp53@cell.names,file="cellnamesp53.csv",quote=F,row.names = F)
VlnPlot(MCF7GLU, c("P53_GENES1"), nCol = 1, group.by = "orig.ident")
write.csv(MCF7GLUp53@meta.data,file="metadataGLUp53.csv",quote=F,row.names = F)

MCF7GLUp53@meta.data$cell.type <- "GLU_P53"
MCF7GLUnotp53@meta.data$cell.type <- "GLU_notP53"
MCF7GLU2 <- MergeSeurat(MCF7GLUp53,MCF7GLUnotp53,min.cells = 0, min.genes = 0,
project = "BLAYDES", names.field = 1, names.delim = "_", do.scale = FALSE,
do.center = FALSE, do.normalize = FALSE)
MCF7GLU2   <-   NormalizeData(object   =   MCF7GLU2,   normalization.method   =
"LogNormalize", scale.factor = 10000)
MCF7GLU2  <-  ScaleData(MCF7GLU2,  genes.use  =  nonmito.genes,  model.use  =
"negbinom", do.scale = T, do.center = T)
```

```
MCF7GLU2 <- RunPCA(MCF7GLU2, pc.genes = nonmito.genes, pcs.compute = 20, pcs.print = 20)
PCHeatmap(MCF7GLU2,pc.use = 1:12,100)
PCHeatmap(MCF7GLU2,pc.use = 13:20,100)
PCElbowPlot(MCF7GLU2, num.pc = 20)

### select PCAs for downstream analysis, run TSNE and find clusters. PC1 and PC2 are commonly cell cycle
PCA <-c(1:5)
MCF7GLU2 <- RunTSNE(MCF7GLU2, dims.use = PCA , do.fast = T)

### save.SNN means that you can easily re-run with different resolution values.
MCF7GLU2 <- FindClusters(MCF7GLU2, reduction.type = "pca", dims.use = PCA, resolution = seq(0.5,1.25,0.25), force.recalc = TRUE, save.SNN = T, print.output = FALSE)

### outputs based on p53 status
MCF7GLU2 <- SetAllIdent(MCF7GLU2, id = "cell.type")
TSNEPlot(MCF7GLU2,do.label = F, pt.size=2.5, colors.use = c("darkorange","blue4"))
MCF7GLU2.origidentmarkers <- FindAllMarkers(MCF7GLU2, genes.use = nonmito.genes, only.pos = TRUE, min.pct = 0.25, thresh.use = 0.25, min.diff.pct = -Inf, test.use = "bimod")
MCF7GLU2.origidentmarkers %>% group_by(cluster) %>% top_n(2, avg_logFC)
write.csv (MCF7GLU2.origidentmarkers, "origidentmarkers.csv")
MCF7GLU2.origidentmarkers %>% group_by(cluster) %>% top_n(20, avg_logFC) -> top20
DoHeatmap(MCF7GLU2, genes.use = top20$gene, slim.col.label = TRUE, remove.key = FALSE, rotate.key = TRUE, cex.row = 15, title = "Original Cell Identity")

### Further analysis of glycolytic status
MCF7GLUnotglycol <- SubsetData(MCF7GLU, subset.name = "GLYCOL_GENES1", accept.high = 0.25)

MCF7GLUglycol@meta.data$cell.type <- "GLU_glycol"
MCF7GLUnotglycol@meta.data$cell.type <- "GLU_notglycol"
MCF7GLU3 <- MergeSeurat(MCF7GLUglycol,MCF7GLUnotglycol,min.cells = 0, min.genes = 0, project = "BLAYDES", names.field = 1, names.delim = "_", do.scale = FALSE, do.center = FALSE, do.normalize = FALSE)
MCF7GLU3 <- NormalizeData(object = MCF7GLU3, normalization.method = "LogNormalize", scale.factor = 10000)
MCF7GLU3 <- ScaleData(MCF7GLU3, genes.use = nonmito.genes, model.use = "negbinom", do.scale = T, do.center = T)

MCF7GLU3 <- RunPCA(MCF7GLU3, pc.genes = nonmito.genes, pcs.compute = 20, pcs.print = 20)
PCHeatmap(MCF7GLU3,pc.use = 1:12,100)
PCHeatmap(MCF7GLU3,pc.use = 13:20,100)
PCElbowPlot(MCF7GLU3, num.pc = 20)

### select PCAs for downstream analysis, run TSNE and find clusters. PC1 and PC2 are commonly cell cycle
PCA <-c(1:5)
MCF7GLU3 <- RunTSNE(MCF7GLU3, dims.use = PCA , do.fast = T)

### save.SNN means that you can easily re-run with different resolution values.
```

```
MCF7GLU3 <- FindClusters(MCF7GLU3,  reduction.type = "pca", dims.use = PCA, resolution = seq(0.5,1.25,0.25), force.recalc = TRUE, save.SNN = T, print.output = FALSE)

### outputs based on glycolytic status
MCF7GLU3 <- SetAllIdent(MCF7GLU3, id = "cell.type")
TSNEPlot(MCF7GLU3,do.label     =     F,     pt.size=2.5,     colors.use     = c("darkorange","blue4"))
MCF7GLU3.origidentmarkers <- FindAllMarkers(MCF7GLU3, genes.use = nonmito.genes, only.pos = TRUE, min.pct = 0.25, thresh.use = 0.25, min.diff.pct = -Inf, test.use = "bimod")
MCF7GLU3.origidentmarkers %>% group_by(cluster) %>% top_n(2, avg_logFC)
write.csv (MCF7GLU3.origidentmarkers, "origidentmarkers.csv")
MCF7GLU3.origidentmarkers %>% group_by(cluster) %>% top_n(20, avg_logFC) -> top20
DoHeatmap(MCF7GLU3, genes.use = top20$gene, slim.col.label = TRUE, remove.key = FALSE, rotate.key = TRUE, cex.row = 15, title = "Original Cell Identity")
```