Supplemental Results

K-means Clustering of a subset of five datasets that were identified as TNBC by IHC. Since TNBCs are not identified by GE in the clinical setting, we performed a similar GE analysis on a dataset from TNBCs identified using IHC (n=183) complied from five datasets: GSE7904 (n= 22), GSE19615 (n= 34), GSE20194 (n= 67), E-TABM-158 (n= 30) and GSE22513, GSE-XXX (n= 30). This compilation included 136 tumors (74.3%) that were identified by our bimodal filtering analysis (Table S4). K-means clustering performed on the most differentially expressed genes (SD> 0.8) resulted in the identification of 5 TNBC subtypes (Figure S4). One subtype contained only three tumors and analysis of GE showed these tumors to have high levels of ER and PR (Figure S4B). These tumors were considered to be false negatives by IHC and were removed from Comparison of these IHC-identified TNBC samples using further analysis. genes differentially expressed from the six TNBC subtypes revealed similar patterns of gene enrichment (Figure S5).

Supplemental Methods

Immunostaining. Both formalin fixed paraffin embedded (FFPE) and frozen tissue were used for immunohistochemical studies. When frozen tissue was used for immunohistochemistry, staining was performed on sections taken from the same tissue block from which RNA was isolated for microarray. AR, Ki67, EGFR and CK 5/6 expression were evaluated in frozen tissue using the DAKO (Carpinteria, CA) antibodies: AR (clone AR411) at a 1:50 dilution, EGFR at 1:200, CK5/6 at 1:50 and Ki67 antibody (MIB1 clone) at a 1:200 dilution for 1 h at room temperature. FFPE tissue was subject to antigen retrieval with high pH buffer (pH 8.0) followed by overnight incubation with an AR (1:30) or Ki67 (1:75) antibody dilution overnight. AR expression was scored as both the percentage of tumor cells with nuclear staining as well as the intensity of staining (scored as 0-3+). An AR intensity score was calculated as follows: (AR intensity x 100) + % AR positive cells. For Ki67 the percentage of cells demonstrating nuclear staining at any intensity was recorded.

Immunoblotting. Cells were trypsinized, lysed and relative protein expression was determined by Western blot as previously described with the following antibodies; HSP90 monoclonal antibody, clone F-18 (Santa Cruz Biotechnology, Santa Cruz, CA) and the AR polyclonal antibody, SC-N20 (Santa Cruz Biotechnology).

Colony Formation. MFM-223, MDA-MB-453 and CAL-148 cells (3000 cells/well) were reverse-transfected with 1.25 pmole of siRNAs to AR [ON-TARGET plus SMARTpool, cat# L-003400-00 (Dharmacon, Lafayette, CO)] or non-targeting control (ON-TARGETplus Non-targeting Pool cat# D-001810-10-05) with 0.25 μL Dharmafect #3 (MFM-223), or 0.25 μL Dharmafect #1 (MDA-MB-453 and CAL-148). Colonies were stained and quantified 14 d following transfection using Cell Profiler 2.0 (Broad Institute, Cambridge, MA). Experiments were preformed in triplicate and error bars reflect standard deviation.

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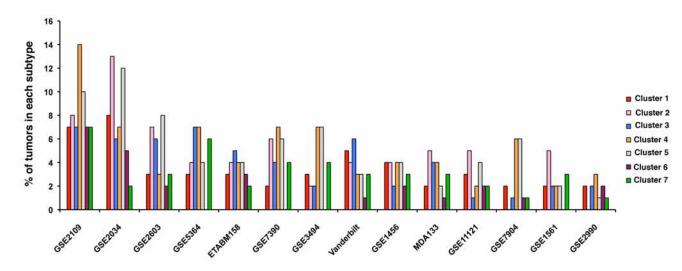


Figure S1. Random distribution of TNBC subtypes found within datasets. (A) The percent of tumors in each cluster is displayed across 14 studies that comprise the training dataset.

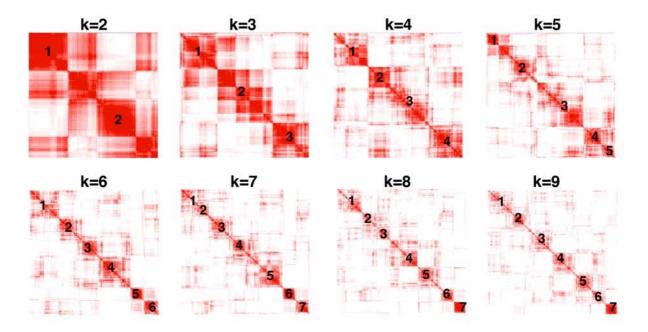


Figure S2. Consensus clustering of training and validation TNBC datasets (n= 587). Heat map displays consensus clustering results depicting the robustness of sample classification. Red areas indicate samples that frequently cluster with each other over multiple iterations (1000) of k-means clustering (k= 2 to k= 9).

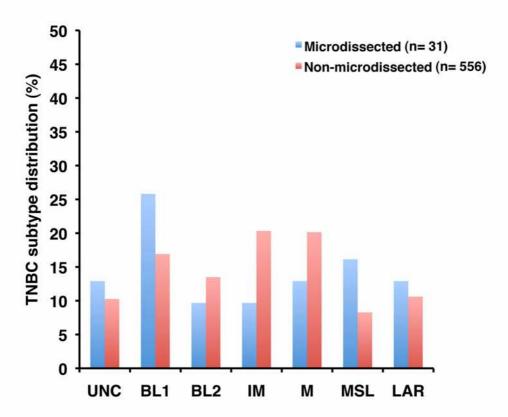
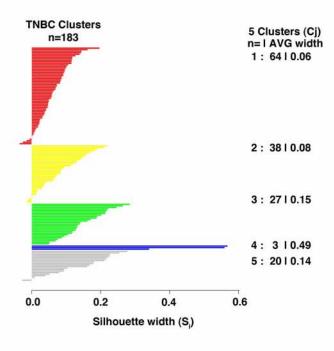


Figure S3. Gene expression profiles derived from tumors that were laser-capture microdissected represent all TNBC subtypes. Bar graph depicts the percentage of tumors that were microdissected (blue bars) present in the GSE584 (n=17) and Vanderbilt (n=14) datasets relative to non-microdissected tumors in the combined dataset (red bars).

Figure S4





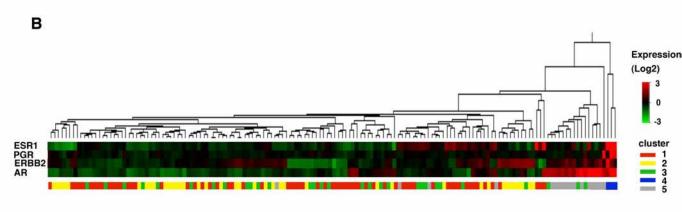


Figure S4. TNBC subtypes identified by IHC. (A) Silhouette plot showing the composition (n= number of tumors) and stability (AVG width) of k-means clustering on the TNBC training set. Clusters with a silhouette width, s(i)>0 were considered stable. (B) Heatmap displays hierarchical clustering of ERBB2, PGR, ESR1 and AR expression in the tumors identified by IHC. Color bar identifies the cluster associated with each tumor.

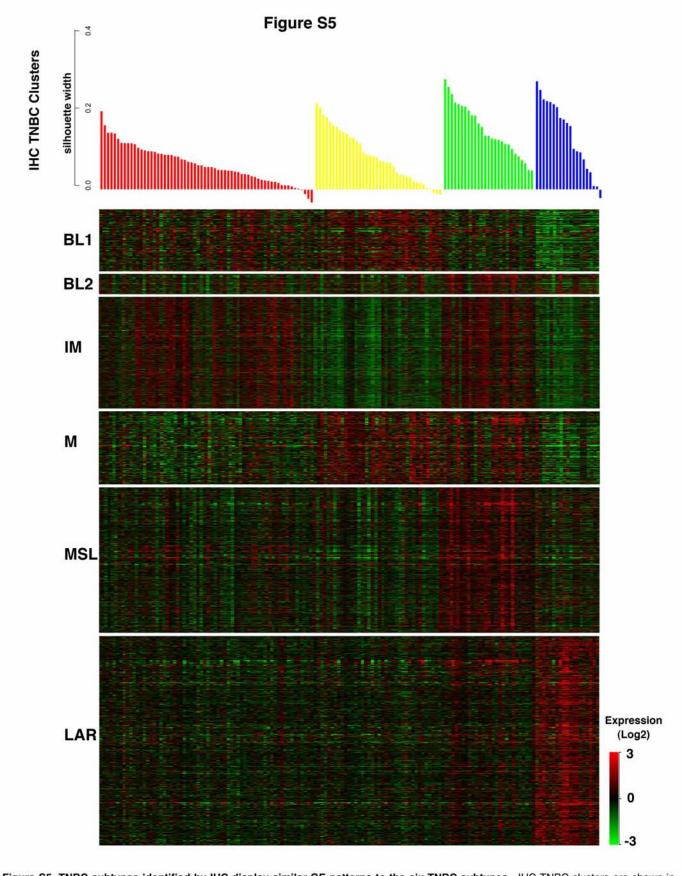


Figure S5. TNBC subtypes identified by IHC display similar GE patterns to the six TNBC subtypes. IHC TNBC clusters are shown in silhouette plot with relative GE for genes unique to the six TNBC subtypes shown below.

Figure S6

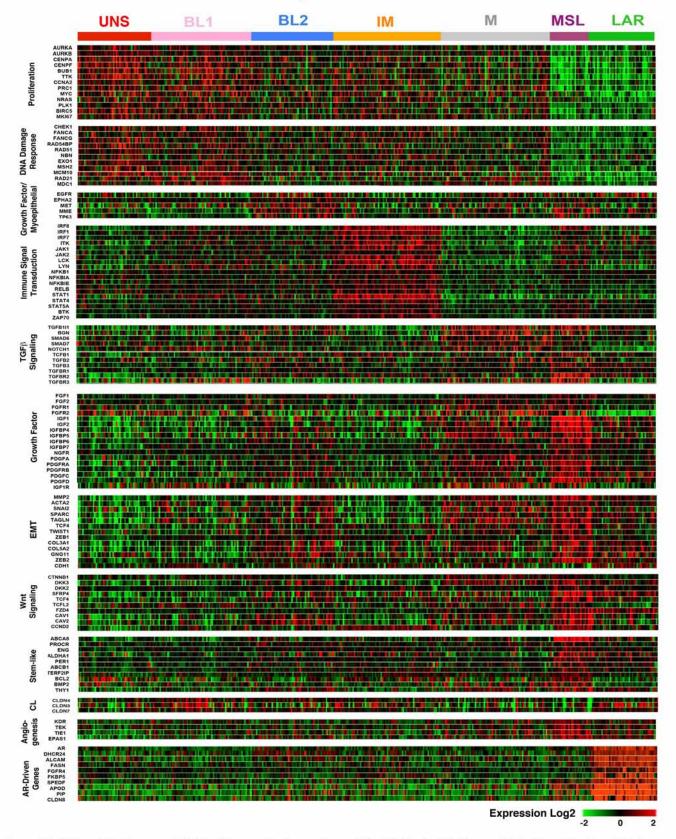
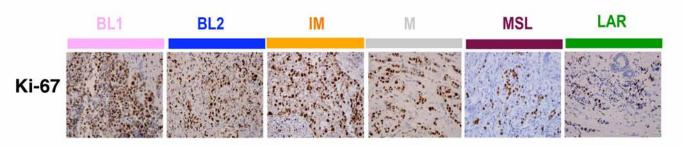


Figure S6. Differential GE across TNBC subtypes. Heatmaps show relative GE (log2, -2 to 2) associated with proliferation, DNA damage response, myoepithelial genes, immune signal transduction, TGFβ signaling, growth factor receptors, EMT, WNT signaling, stem-like, claudin (CL), angiogenesis, AR and of AR target genes across TNBC subtypes (as in Figure 3).

A



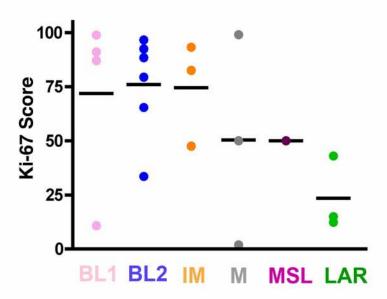


Figure S7. TNBC tumor subtypes differentially stain for the proliferation marker Ki-67. (A) Representative micrographs from 20 tumors showing IHC staining of the proliferation marker, Ki-67, in tumors from different TNBC subtypes. (B) Dot plot showing the mean and distribution of Ki-67 staining within TNBC subtypes as scored by study pathologist. Subtypes: BL1= basal-like 1, BL2= basal-like 2, IM= immunomodulatory, M= mesenchymal-like, MSL= mesenchymal stem-like and LAR= luminal AR.

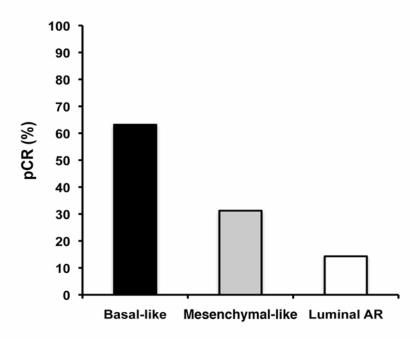


Figure S8. Response rates differ across TNBC subtypes in taxane-treated patients. Percent of patients achieving pathologic complete response (pCR) after taxane-based treatment was significantly (P = 0.042, chi-squared analysis) different for patients whose tumors correlated to basal-like (n = 19), mesenchymal-like (n = 16) and luminal AR (n = 7) subtypes, from studies in which response data were available.

Figure S9

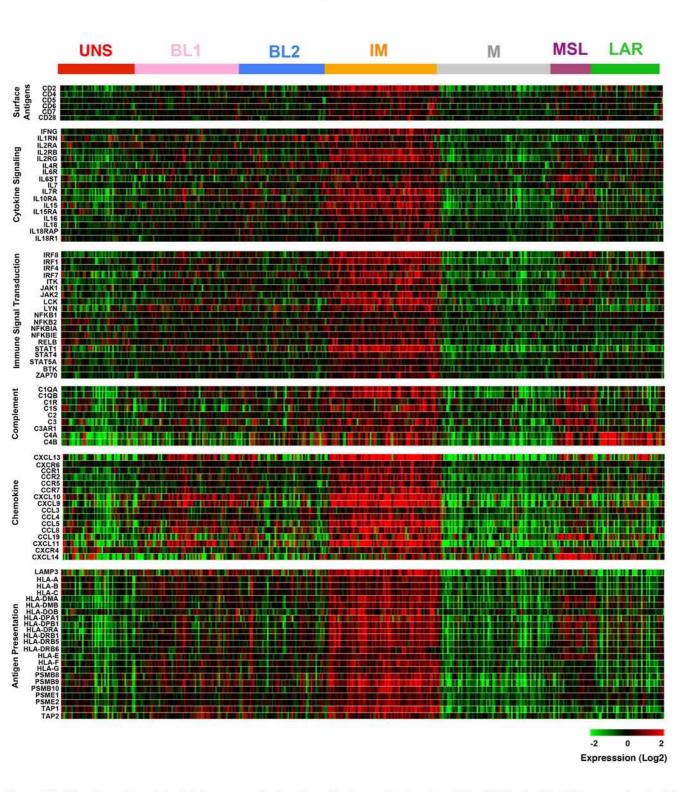


Figure S9. IM subtype is enriched in immune cell signaling. Heatmaps showing the relative GE (log2, -2 to 2) for genes involved in immune cell surface antigens, cytokines, immune cell signal transduction, complement, chemokine, and antigen presentation across TNBC subtypes (as in Figure 3).

Figure S10

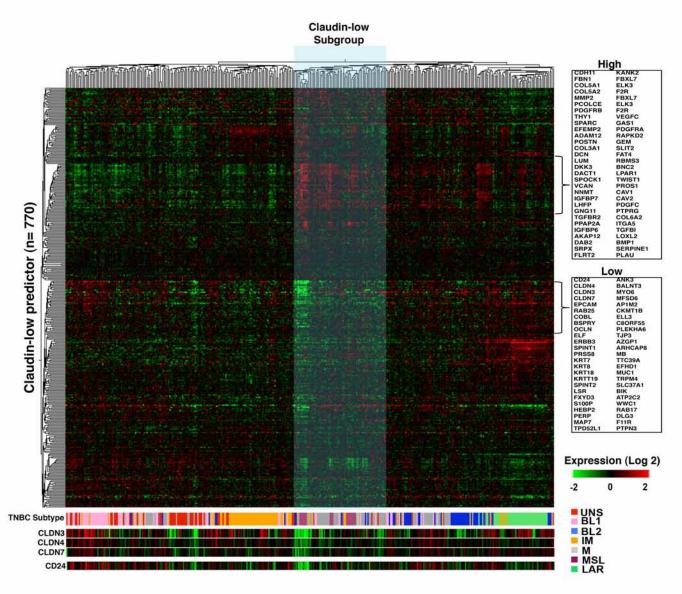
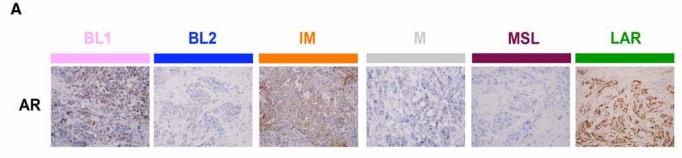


Figure S10. The claudin-low predictor gene set identifies a sub-population of MSL tumors. Unsupervised hierarchical clustering was performed on the training TNBC tumors using genes (n= 770) unique to the claudin-low subgroup [26]. Displayed to the right of the heatmap are the genes that are most differentially expressed (either high or low) in the claudin-low tumor set. Colorbar displays the TNBC subtype and heatmaps show relative levels (Log 2) of claudins (3, 4 and 7) and CD24, markers of this subgroup.

Figure S11



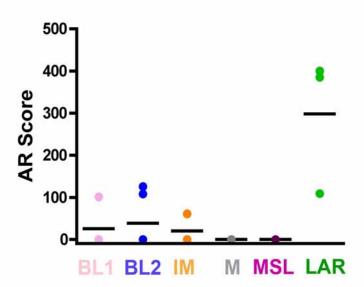
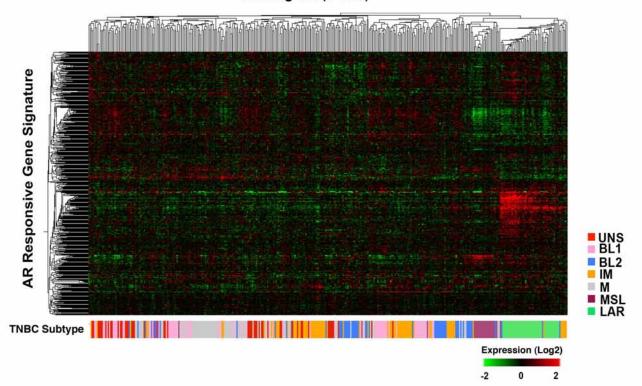
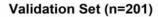


Figure S11. TNBC tumor subtypes differentially stain for AR by IHC. (A) IHC staining for AR from 20 tumors with representative samples from each TNBC subtype shown. (B) Dot plot showing the quantification of nuclear AR staining based on intensity and the percent of nuclei staining positive for AR in 20 tumors; Note, in some cases one dot represents overlapping dots from multiple tumors.

Figure S12

Training Set (n=386)





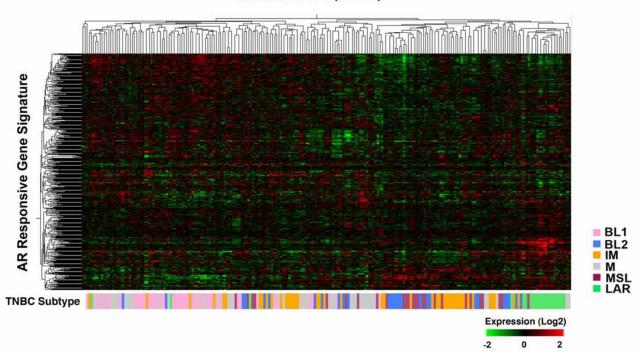
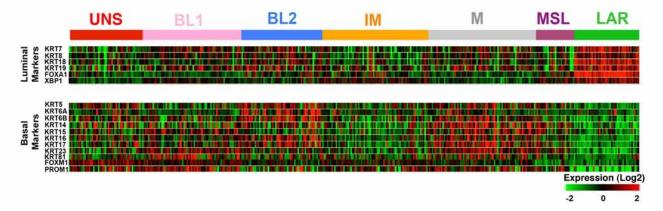


Figure S12. An androgen-inducible gene signature segregates LAR tumors. Hierarchical clustering was performed on both the (A) training and (B) validation TNBC tumor set using a 559 androgen-inducible gene signature [27].





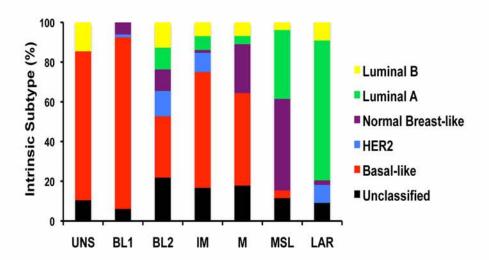


Figure S13. TNBC subtypes differentially correlate with the intrinsic molecular subtypes. (A) Heatmaps show relative GE (log2, -2 to 2) of luminal and basal markers of breast cancer across all TNBC subtypes. (B) Bar graph shows the distribution of intrinsic molecular subtypes of breast cancer (luminal A and B, normal breast-like, HER2, basal-like or unclassified) within each TNBC subtype (UNS = unstable, BL1= basal-like1, BL2 = basal-like 2, IM = immunomodulatory, M = mesenchymal-like, MSL = mesenchymal stem-like and LAR = luminal AR) as determined by best-fit Spearman correlation to the intrinsic centroids.

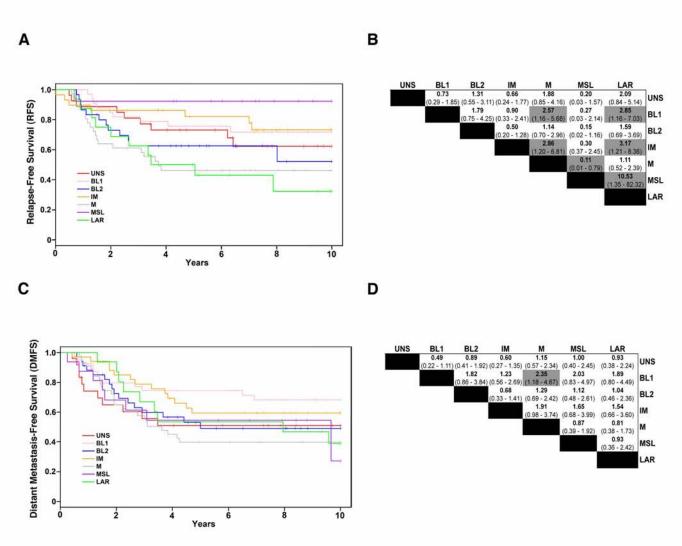
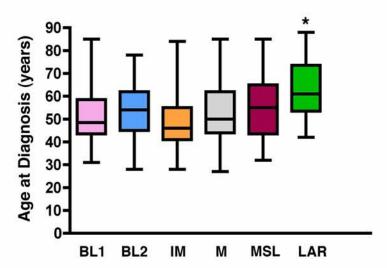
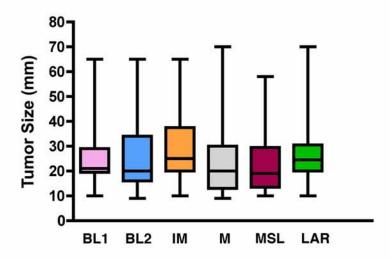


Figure S14. TNBC subtypes differ in relapse-free survival and distant metastasis-free survival. Kaplan-Meier plot showing (A) 10-year RFS or (C) DMFS in TNBC subtypes. RFS (B) and DMFS (D) hazard ratios (bold) and 95% CI (parentheses) for patients from TNBC subtypes. Shaded boxes indicate significant (P < 0.05) comparisons.

Α



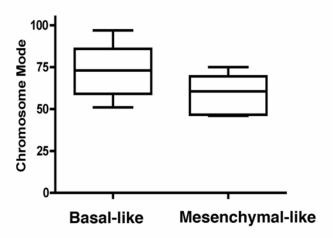
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(rectangle) and SD (error bars) of (A) age at diagnosis and (B) tumor size (mm) between TNBC subtypes (as in Figure 3). * P = 9.0e-6

Figure S15. TNBC subtypes differ in age, but are similar size upon diagnosis. Box plots show the median (horizontal line), range

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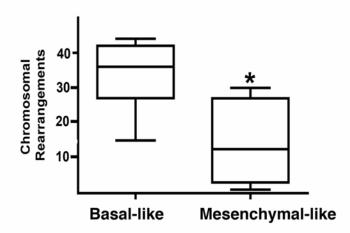


Figure S16. Chromosomal Aberrations in TNBC cell lines. (A) Box plots depicting the average number of chromosomes (mode) in breast cancer cell lines correlating to basal-like (n=8) vs. mesenchymal-like (n=6) subtypes. (B) Box plots depicting the average number of chromosomal rearrangements (translocations, inversions, and deletions) in basal-like vs. mesenchymal-like subtypes. Chromosome mode and rearrangements were obtained from the Departments of Pathology and Oncology, University of Cambridge (http://www.path.cam.ac.uk/~pawefish/cell%20line%20catalogues/breast-cell-lines.htm). *P <0.01 by unpaired t-test

Figure S17

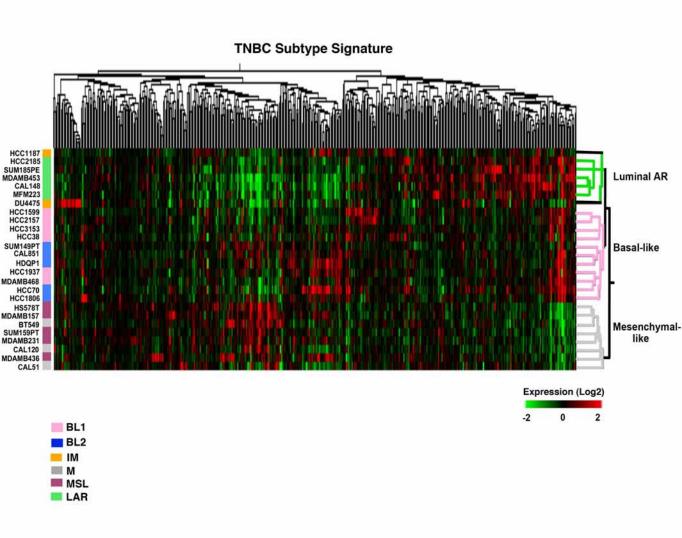


Figure S17. TNBC cell lines cluster in three major groups: luminal AR, basal-like and mesenchymal-like. Unsupervised hierarchical clustering of TNBC cell lines performed on genes unique to TNBC subtypes (n= 2188). Colorbar shows the best correlation of cell lines to the TNBC subtypes.

Figure S18

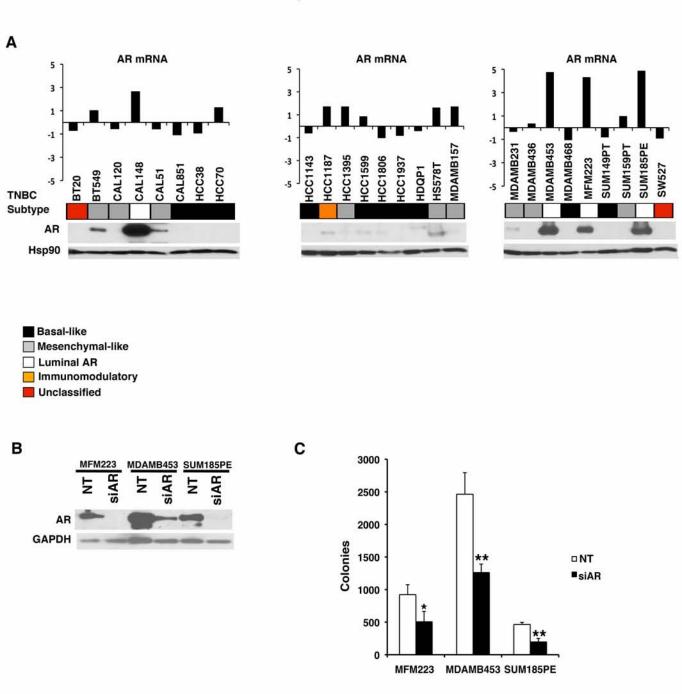


Figure S18. LAR cell lines depend on AR expression for colony formation. (A) Top panel depicts relative AR mRNA levels obtained from GE microarrays performed on TNBC cell lines (log 2, centered on 0). Color bar identifies TNBC subtype classification for each cell line (immunomodulatory shown in orange and unclassified shown in red). Immunoblots showing relative expression AR protein in TNBC cell lines, Hsp90 serves as a loading control. (B) AR expression 72h following transfection with siRNA pools of non-targeting (NT) or targeting AR in MFM-223, MDA-MB-453 and SUM185PE cells. GAPDH expression serves as a loading control. (C) Colony formation of MFM-223, MDA-MB-453 and SUM185PE cells 14d following siRNA transfection of AR or non-targeting control (NT).

Table S7. Top gene ontologies for TNBC subtypes in the training and validation datasets

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		EPOPATHWAY	ERK1 ERK2 MAPK PATHWAY
SPRYPATHWAY GLUCONEOGENESIS			

TRKA RECEPTOR 1 AND 2 METHYLNAPHTHALENE DEGRADATION PHOSPHOINOSITIDE 3 KINASE PATHWAY **VEGFPATHWAY PROTEASOME ETSPATHWAY P38 MAPK PATHWAY** PROSTAGLANDIN SYNTHESIS REGULATION PATHOGENIC ESCHERICHIA COLI INFECTION EPEC BENZOATE DEGRADATION VIA COA LIGATION **NFKBPATHWAY** CHOLERA INFECTION **TIDPATHWAY** HEPARAN SULFATE BIOSYNTHESIS **ECMPATHWAY PDGFPATHWAY** ARGININE AND PROLINE METABOLISM **GLYCOLYSIS PROTEASOMEPATHWAY NTHIPATHWAY** EPITHELIAL CELL SIGNALING IN HELICOBACTER PYLORI INFECTION **NGFPATHWAY ETSPATHWAY ERK1 ERK2 MAPK PATHWAY GALACTOSE METABOLISM** GAP JUNCTION **ERYTHPATHWAY GLYCOLYSIS AND GLUCONEOGENESIS** MYOCYTE AD PATHWAY CTLA4PATHWAY **NO2IL12PATHWAY** IM **NO2IL12PATHWAY CTLA4PATHWAY** TH1TH2PATHWAY **IL12PATHWAY CSKPATHWAY TYPE I DIABETES MELLITUS NKTPATHWAY NKCELLSPATHWAY COMPPATHWAY** TH1TH2PATHWAY **TNFR2PATHWAY IL7PATHWAY PROTEASOME CSKPATHWAY IL7PATHWAY NKCELLSPATHWAY TYPE I DIABETES MELLITUS AMIPATHWAY** ANTIGEN PROCESSING AND PRESENTATION **AMIPATHWAY** ANTIGEN PROCESSING AND PRESENTATION **NFKBPATHWAY** TUMOR NECROSIS FACTOR PATHWAY **IL12PATHWAY LAIRPATHWAY PROTEASOME TNFR2PATHWAY NKTPATHWAY DCPATHWAY** T CELL SIGNAL TRANSDUCTION **TIDPATHWAY DEATHPATHWAY APOPTOSIS GENMAPP APOPTOSIS GENMAPP NFKBPATHWAY** DCPATHWAY **PROTEASOMEPATHWAY** RELAPATHWAY T CELL SIGNAL TRANSDUCTION **TOB1PATHWAY TUMOR NECROSIS FACTOR PATHWAY APOPTOSIS CASPASEPATHWAY** APOPTOSIS KEGG T CELL RECEPTOR SIGNALING PATHWAY **APOPTOSIS** NATURAL KILLER CELL MEDIATED CYTOTOXICITY **CYTOKINEPATHWAY TOB1PATHWAY** CASPASEPATHWAY **B CELL RECEPTOR SIGNALING PATHWAY TIDPATHWAY** T CELL RECEPTOR SIGNALING PATHWAY **IL2PATHWAY CYTOKINEPATHWAY** NATURAL KILLER CELL MEDIATED CYTOTOXICITY **CELL ADHESION MOLECULES** CASPASE CASCADE **DEATHPATHWAY LAIRPATHWAY APOPTOSIS KEGG HEMATOPOIETIC CELL LINEAGE TOLL LIKE RECEPTOR SIGNALING PATHWAY IL3PATHWAY PROTEASOME COMPPATHWAY HEMATOPOIETIC CELL LINEAGE TCRPATHWAY B CELL RECEPTOR SIGNALING PATHWAY RELAPATHWAY IL2PATHWAY STEMPATHWAY MITOCHONDRIAPATHWAY MITOCHONDRIAPATHWAY CELL ADHESION MOLECULES TOLLPATHWAY TOLLPATHWAY B CELL ANTIGEN RECEPTOR INFLAMPATHWAY STRESSPATHWAY IL2RBPATHWAY IL2RBPATHWAY IL3PATHWAY INFLAMPATHWAY STEMPATHWAY** CYTOKINE CYTOKINE RECEPTOR INTERACTION **BCR SIGNALING PATHWAY** PIP3 SIGNALING IN B LYMPHOCYTES **TOLL LIKE RECEPTOR SIGNALING PATHWAY HIVNEFPATHWAY TCRPATHWAY** 41BBPATHWAY

CASPASE CASCADE B CELL ANTIGEN RECEPTOR PIP3 SIGNALING IN B LYMPHOCYTES BCR SIGNALING PATHWAY NTHIPATHWAY PROTEASOME CERAMIDEPATHWAY 41BBPATHWAY **CCR5PATHWAY APOPTOSIS** CYTOKINE CYTOKINE RECEPTOR INTERACTION **JAK STAT SIGNALING PATHWAY INTERLEUKIN 4 PATHWAY EPOPATHWAY STRESSPATHWAY NTHIPATHWAY IL1RPATHWAY CD40PATHWAYMAP APOPTOSIS CALCINEURIN NF AT SIGNALING EICOSANOID SYNTHESIS HIVNEFPATHWAY UBIQUITIN MEDIATED PROTEOLYSIS EICOSANOID SYNTHESIS CELLCYCLEPATHWAY BCRPATHWAY CALCINEURIN NF AT SIGNALING** FC EPSILON RI SIGNALING PATHWAY FC EPSILON RI SIGNALING PATHWAY **KERATINOCYTEPATHWAY AMINOACYL TRNA BIOSYNTHESIS** AMINOACYL TRNA BIOSYNTHESIS **JAK STAT SIGNALING PATHWAY CELLCYCLEPATHWAY FASPATHWAY B CELL RECEPTOR COMPLEXES IL6PATHWAY PTEN PATHWAY B CELL RECEPTOR COMPLEXES PROTEASOMEPATHWAY** DNA REPLICATION REACTOME **CCR5PATHWAY FOLATE BIOSYNTHESIS ATRBRCAPATHWAY ATRBRCAPATHWAY GHPATHWAY IL6PATHWAY FOLATE BIOSYNTHESIS** LEUKOCYTE TRANSENDOTHELIAL MIGRATION PEPTIDE GPCRS **GLEEVECPATHWAY GLEEVECPATHWAY TNFR1PATHWAY FASPATHWAY** COMPLEMENT AND COAGULATION CASCADES ABC TRANSPORTERS GENERAL **BCRPATHWAY INTERLEUKIN 4 PATHWAY EPOPATHWAY IL4RECEPTOR IN B LYPHOCYTES GHPATHWAY** ADIPOCYTOKINE SIGNALING PATHWAY **TPOPATHWAY** PROSTAGLANDIN AND LEUKOTRIENE METABOLISM AMINOACYL TRNA BIOSYNTHESIS **CDMACPATHWAY** PEPTIDE GPCRS **TPOPATHWAY** N GLYCAN BIOSYNTHESIS HSP27PATHWAY ALZHEIMERS DISEASE **TNFR1PATHWAY ACUTE MYELOID LEUKEMIA** INOSITOL PHOSPHATE METABOLISM **ACUTE MYELOID LEUKEMIA GAQ PATHWAY CD40PATHWAYMAP** LEUKOCYTE TRANSENDOTHELIAL MIGRATION METHIONINE METABOLISM **IL1RPATHWAY** GPCRDB CLASS B SECRETIN LIKE **DENTATORUBROPALLIDOLUYSIAN ATROPHY IL4RECEPTOR IN B LYPHOCYTES OVARIAN INFERTILITY GENES CERAMIDEPATHWAY PTEN PATHWAY** FCER1PATHWAY G1 TO S CELL CYCLE REACTOME PARKINSONS DISEASE **CHEMICALPATHWAY CELL CYCLE KEGG** SNARE INTERACTIONS IN VESICULAR TRANSPORT **ATMPATHWAY** NICOTINATE AND NICOTINAMIDE METABOLISM **KERATINOCYTEPATHWAY** HYPERTROPHY MODEL ADIPOCYTOKINE SIGNALING PATHWAY **DENTATORUBROPALLIDOLUYSIAN ATROPHY FMLPPATHWAY** DICTYOSTELIUM DISCOIDEUM CAMP CHEMOTAXIS **PATHWAY BIOPEPTIDESPATHWAY** PANCREATIC CANCER **FAS SIGNALING PATHWAY RACCYCDPATHWAY** AMINOSUGARS METABOLISM **CELL COMMUNICATION PTENPATHWAY** М HEPARAN SULFATE BIOSYNTHESIS DNA REPLICATION REACTOME ECM RECEPTOR INTERACTION **RIBOSOME** TRANSLATION FACTORS **ALKPATHWAY UCALPAINPATHWAY** RNA POLYMERASE STRIATED MUSCLE CONTRACTION **DNA POLYMERASE BASAL CELL CARCINOMA** BASAL TRANSCRIPTION FACTORS

REGULATION OF THE ACTIN CYTOSKELETON BY

RHO GTPASES

TGF BETA SIGNALING PATHWAY

HDACPATHWAY

HEDGEHOG SIGNALING PATHWAY INOSITOL PHOSPHATE METABOLISM

IGF1PATHWAY
ECMPATHWAY
ERK5PATHWAY
FOCAL ADHESION
EDG1PATHWAY
CARM ERPATHWAY

INSULINPATHWAY ETHER LIPID METABOLISM

TELPATHWAY

NOTCH SIGNALING PATHWAY WNT BETA CATENIN PATHWAY

MEF2DPATHWAY
INTEGRINPATHWAY
MELANOGENESIS
VEGFPATHWAY
WNTPATHWAY
ETSPATHWAY

DORSO VENTRAL AXIS FORMATION

IGF1MTORPATHWAY

RNA TRANSCRIPTION REACTOME CHOLESTEROL BIOSYNTHESIS

GLYCOSYLPHOSPHATIDYLINOSITOL ANCHOR BIOSYNTHESIS

ECMPATHWAY UCALPAINPATHWAY

EIF4PATHWAY RIBOSOMAL PROTEINS CELL CYCLE KEGG

MTORPATHWAY

REGULATION OF THE ACTIN CYTOSKELETON BY RHO

GTPASES

G1 TO S CELL CYCLE REACTOME MRNA PROCESSING REACTOME UBIQUITIN MEDIATED PROTEOLYSIS

WNTPATHWAY
ALKPATHWAY
G2PATHWAY
TELPATHWAY
CELL CYCLE

ATRBRCAPATHWAY

MSL

PROSTAGLANDIN SYNTHESIS REGULATION

BADPATHWAY LAIRPATHWAY IL7PATHWAY COMPPATHWAY

RENIN ANGIOTENSIN SYSTEM

AMIPATHWAY CSKPATHWAY ERYTHPATHWAY

ECM RECEPTOR INTERACTION

TOB1PATHWAY

COMPLEMENT AND COAGULATION CASCADES

CARDIACEGFPATHWAY
HISTIDINE METABOLISM
GATA3PATHWAY
NDKDYNAMINPATHWAY
CCR5PATHWAY
FOCAL ADHESION

BETA ALANINE METABOLISM

INTRINSICPATHWAY PAR1PATHWAY ALKPATHWAY

CALCINEURINPATHWAY NTHIPATHWAY

CTLA4PATHWAY
RETA ALANINE ME

BETA ALANINE METABOLISM

GCRPATHWAY

HEMATOPOIETIC CELL LINEAGE

NO1PATHWAY VIPPATHWAY UCALPAINPATHWAY EDG1PATHWAY IL3PATHWAY IGF1PATHWAY

TCRPATHWAY EICOSANOID SYNTHESIS

NKCELLSPATHWAY

VALINE LEUCINE AND ISOLEUCINE DEGRADATION

PPARAPATHWAY SPRYPATHWAY HDACPATHWAY

STATIN PATHWAY PHARMGKB GLYCAN STRUCTURES DEGRADATION SMOOTH MUSCLE CONTRACTION

TH1TH2PATHWAY

ECMPATHWAY

NO2IL12PATHWAY

CSKPATHWAY LAIRPATHWAY TOB1PATHWAY CTLA4PATHWAY AMIPATHWAY

TH1TH2PATHWAY

RENIN ANGIOTENSIN SYSTEM

PAR1PATHWAY
NKTPATHWAY
IL7PATHWAY
NKCELLSPATHWAY
DCPATHWAY
ERYTHPATHWAY

HEMATOPOIETIC CELL LINEAGE
TYPE I DIABETES MELLITUS

IL3PATHWAY

GLYCOSAMINOGLYCAN DEGRADATION

IL12PATHWAY IL2PATHWAY

PROSTAGLANDIN SYNTHESIS REGULATION

EICOSANOID SYNTHESIS COMPPATHWAY

CCR5PATHWAY

GLYCAN STRUCTURES DEGRADATION

CALCINEURINPATHWAY

COMPLEMENT AND COAGULATION CASCADES

ECM RECEPTOR INTERACTION

TCRPATHWAY
TNFR2PATHWAY
NO1PATHWAY
INTRINSICPATHWAY

STATIN PATHWAY PHARMGKB ALKALOID BIOSYNTHESIS II

LEUKOCYTE TRANSENDOTHELIAL MIGRATION HSA04514 CELL ADHESION MOLECULES

INFLAMPATHWAY MEF2DPATHWAY IL2RBPATHWAY

WNT BETA CATENIN PATHWAY HSA04510 FOCAL ADHESION

NTHIPATHWAY BCRPATHWAY

BCR SIGNALING PATHWAY

GCRPATHWAY

GLYCEROLIPID METABOLISM

GPCRPATHWAY

PDGFPATHWAY

INTEGRIN MEDIATED CELL ADHESION KEGG

FATTY ACID METABOLISM

NKTPATHWAY

CXCR4PATHWAY

TOLLPATHWAY

TNFR2PATHWAY

LEUKOCYTE TRANSENDOTHELIAL MIGRATION

PPAR SIGNALING PATHWAY

AMYOTROPHIC LATERAL SCLEROSIS

GLYCEROLIPID METABOLISM

GLYCOSPHINGOLIPID METABOLISM

MTORPATHWAY

NICOTINATE AND NICOTINAMIDE METABOLISM

PGC1APATHWAY **METPATHWAY**

WNT BETA CATENIN PATHWAY

TPOPATHWAY

BUTANOATE METABOLISM

TGF BETA SIGNALING PATHWAY

GSK3PATHWAY

GLYCOSAMINOGLYCAN DEGRADATION

PROPANOATE METABOLISM

CELL COMMUNICATION

OVARIAN INFERTILITY GENES

VALINE LEUCINE AND ISOLEUCINE DEGRADATION

BILE ACID BIOSYNTHESIS

IL6PATHWAY

BCR SIGNALING PATHWAY

CELL ADHESION MOLECULES

B CELL RECEPTOR SIGNALING PATHWAY

BCRPATHWAY

PTENPATHWAY

MCALPAINPATHWAY

1 AND 2 METHYLNAPHTHALENE DEGRADATION

GPCRDB CLASS B SECRETIN LIKE

IL2PATHWAY

PROPANOATE METABOLISM

MELANOMA

INTEGRINPATHWAY

ALKALOID BIOSYNTHESIS II

ETHER LIPID METABOLISM

NUCLEAR RECEPTORS

CK1PATHWAY

TYPE I DIABETES MELLITUS

NO2IL12PATHWAY

RHOPATHWAY

CYTOKINE CYTOKINE RECEPTOR INTERACTION

BLOOD CLOTTING CASCADE

INFLAMPATHWAY

DICTYOSTELIUM DISCOIDEUM CAMP CHEMOTAXIS

PATHWAY

DIFFERENTIATION PATHWAY IN PC12 CELLS

PENTOSE AND GLUCURONATE INTERCONVERSIONS

GLUTATHIONE METABOLISM

BIOSYNTHESIS OF STEROIDS

ANDROGEN AND ESTROGEN METABOLISM

GLYCOSPHINGOLIPID METABOLISM

TYPE III SECRETION SYSTEM

GAMMA HEXACHLOROCYCLOHEXANE DEGRADATION

FLAGELLAR ASSEMBLY

CITRATE CYCLE TCA CYCLE

PHENYLALANINE METABOLISM

ATP SYNTHESIS

PHOTOSYNTHESIS

INOSITOL PHOSPHATE METABOLISM **BADPATHWAY**

CYTOKINE CYTOKINE RECEPTOR INTERACTION

PIP3 SIGNALING IN B LYMPHOCYTES

GATA3PATHWAY

PEPTIDE GPCRS

MONOAMINE GPCRS

HISTIDINE METABOLISM

NFKBPATHWAY

ECMPATHWAY

1 AND 2 METHYLNAPHTHALENE DEGRADATION

B CELL RECEPTOR SIGNALING PATHWAY

VIPPATHWAY

CARDIACEGFPATHWAY

ALKPATHWAY

IL4RECEPTOR IN B LYPHOCYTES

GHPATHWAY

NATURAL KILLER CELL MEDIATED CYTOTOXICITY

RAC1PATHWAY

FDG1PATHWAY

BETA ALANINE METABOLISM

UCALPAINPATHWAY

GPCRPATHWAY

ERK1 ERK2 MAPK PATHWAY

INTEGRIN MEDIATED CELL ADHESION KEGG

ARACHIDONIC ACID METABOLISM

ABC TRANSPORTERS GENERAL

RHOPATHWAY

JAK STAT SIGNALING PATHWAY

HDACPATHWAY

BILE ACID BIOSYNTHESIS

TOLLPATHWAY

SPPAPATHWAY

NDKDYNAMINPATHWAY

SMOOTH MUSCLE CONTRACTION CALCIUM SIGNALING PATHWAY

GLYCEROLIPID METABOLISM

ANTIGEN PROCESSING AND PRESENTATION ADIPOCYTOKINE SIGNALING PATHWAY

LAR

CHOLESTEROL BIOSYNTHESIS PENTOSE AND GLUCURONATE

INTERCONVERSIONS

BIOSYNTHESIS OF STEROIDS

TYROSINE METABOLISM

GAMMA HEXACHLOROCYCLOHEXANE

DEGRADATION

PORPHYRIN AND CHLOROPHYLL METABOLISM

PHENYLALANINE METABOLISM

GAMMA HEXACHLOROCYCLOHEXANE

DEGRADATION

CHREBPPATHWAY

GLUTATHIONE METABOLISM

1 AND 2 METHYLNAPHTHALENE DEGRADATION

GLYCOSPHINGOLIPID METABOLISM

PORPHYRIN AND CHLOROPHYLL METABOLISM

GLUTATHIONE METABOLISM

CHOLESTEROL BIOSYNTHESIS

TYROSINE METABOLISM

PORPHYRIN AND CHLOROPHYLL METABOLISM

ANDROGEN AND ESTROGEN METABOLISM

PHENYLALANINE METABOLISM CITRATE CYCLE TCA CYCLE

TYROSINE METABOLISM

METABOLISM OF XENOBIOTICS BY CYTOCHROME

P450

EICOSANOID SYNTHESIS

VALINE LEUCINE AND ISOLEUCINE DEGRADATION

GLYCAN STRUCTURES DEGRADATION

GLYCOSYLPHOSPHATIDYLINOSITOL ANCHOR **BIOSYNTHESIS**

BUTANOATE METABOLISM

ARGININE AND PROLINE METABOLISM

GLUTATHIONE METABOLISM

CK1PATHWAY CITRATE CYCLE

PANTOTHENATE AND COA BIOSYNTHESIS

PROPANOATE METABOLISM **FATTY ACID METABOLISM**

ANDROGEN AND ESTROGEN METABOLISM

GLUTAMATE METABOLISM N GLYCAN BIOSYNTHESIS HISTIDINE METABOLISM BILE ACID BIOSYNTHESIS PROPANOATE METABOLISM **HCMVPATHWAY**

TRYPTOPHAN METABOLISM

ALANINE AND ASPARTATE METABOLISM

VALINE LEUCINE AND ISOLEUCINE DEGRADATION

PPAR SIGNALING PATHWAY

CREBPATHWAY ATP SYNTHESIS

FLAGELLAR ASSEMBLY

TYPE III SECRETION SYSTEM SPRYPATHWAY

ERYTHPATHWAY PHOTOSYNTHESIS HISTIDINE METABOLISM MCALPAINPATHWAY

FRUCTOSE AND MANNOSE METABOLISM

GLYCOSAMINOGLYCAN DEGRADATION

SPHINGOLIPID METABOLISM **GLUTAMATE METABOLISM** LINOLEIC ACID METABOLISM GLYCEROLIPID METABOLISM

ARGININE AND PROLINE METABOLISM

SNARE INTERACTIONS IN VESICULAR TRANSPORT SELENOAMINO ACID METABOLISM

PENTOSE PHOSPHATE PATHWAY

FRUCTOSE AND MANNOSE METABOLISM

NO1PATHWAY

BILE ACID BIOSYNTHESIS

STARCH AND SUCROSE METABOLISM

CYSTEINE METABOLISM

GATA3PATHWAY

ALANINE AND ASPARTATE METABOLISM

LIMONENE AND PINENE DEGRADATION

ABC TRANSPORTERS GENERAL

UREA CYCLE AND METABOLISM OF AMINO

GROUPS

OXIDATIVE PHOSPHORYLATION N GLYCAN BIOSYNTHESIS

GLYCEROLIPID METABOLISM

KREBS TCA CYCLE

AMINOSUGARS METABOLISM

ARACHIDONIC ACID METABOLISM

TRYPTOPHAN METABOLISM

PENTOSE PHOSPHATE PATHWAY

NAPHTHALENE AND ANTHRACENE DEGRADATION

OXIDATIVE PHOSPHORYLATION

STARCH AND SUCROSE METABOLISM

PORPHYRIN AND CHLOROPHYLL METABOLISM

ARGININE AND PROLINE METABOLISM

GAMMA HEXACHLOROCYCLOHEXANE DEGRADATION METABOLISM OF XENOBIOTICS BY CYTOCHROME P450

FRUCTOSE AND MANNOSE METABOLISM

CARBON FIXATION

GLYCAN STRUCTURES DEGRADATION

CITRATE CYCLE

COMPPATHWAY

PARKINSONS DISEASE

ANDROGEN AND ESTROGEN METABOLISM

PANTOTHENATE AND COA BIOSYNTHESIS FATTY ACID METABOLISM

RENIN ANGIOTENSIN SYSTEM

TYROSINE METABOLISM

GLUTAMATE METABOLISM ALANINE AND ASPARTATE METABOLISM

CARBON FIXATION

EICOSANOID SYNTHESIS

GLYCOSAMINOGLYCAN DEGRADATION

PHENYLALANINE METABOLISM

CHREBPPATHWAY

CK1PATHWAY

MONOAMINE GPCRS

SPHINGOLIPID METABOLISM

P53HYPOXIAPATHWAY TRYPTOPHAN METABOLISM

NICOTINATE AND NICOTINAMIDE METABOLISM

GLUTAMATE METABOLISM

UCALPAINPATHWAY

FRUCTOSE AND MANNOSE METABOLISM

KREBS TCA CYCLE

OXIDATIVE PHOSPHORYLATION

HISTIDINE METABOLISM

GALACTOSE METABOLISM

PENTOSE PHOSPHATE PATHWAY

OXIDATIVE PHOSPHORYLATION

N GLYCAN BIOSYNTHESIS LINOLEIC ACID METABOLISM

BLOOD CLOTTING CASCADE

ARACHIDONIC ACID METABOLISM

MCALPAINPATHWAY

ERYTHPATHWAY

ABC TRANSPORTERS GENERAL

BUTANOATE METABOLISM

O GLYCAN BIOSYNTHESIS

PPAR SIGNALING PATHWAY

ALANINE AND ASPARTATE METABOLISM

VALINE LEUCINE AND ISOLEUCINE DEGRADATION

NO1PATHWAY

STARCH AND SUCROSE METABOLISM **ARGININE AND PROLINE METABOLISM**

COMPLEMENT AND COAGULATION CASCADES

GALACTOSE METABOLISM

PENTOSE PHOSPHATE PATHWAY

UNC	DNA REPLICATION REACTOME	
UNC	ATRBRCAPATHWAY	
	DNA POLYMERASE	
	CELL CYCLE KEGG	
	CELLCYCLEPATHWAY	
	G1 TO S CELL CYCLE REACTOME	
	CELL CYCLE	
	G1PATHWAY	
	G1 AND S PHASES	
	BASAL TRANSCRIPTION FACTORS	
	RNA TRANSCRIPTION REACTOME	
	AMINOACYL TRNA BIOSYNTHESIS	
	RNA POLYMERASE	
	PYRIMIDINE METABOLISM	
	CARM ERPATHWAY	
	PYRIMIDINE METABOLISM	
	ALANINE AND ASPARTATE METABOLISM	
	ALANINE AND ASPARTATE METABOLISM	
	AMINOACYL TRNA BIOSYNTHESIS	
	G2PATHWAY	
	P53PATHWAY	
	SELENOAMINO ACID METABOLISM	
	GLUTAMATE METABOLISM	
	PENTOSE PHOSPHATE PATHWAY	
	MRNA PROCESSING REACTOME	
	PENTOSE PHOSPHATE PATHWAY	
<u> </u>		

Bold- shared between training and validation set