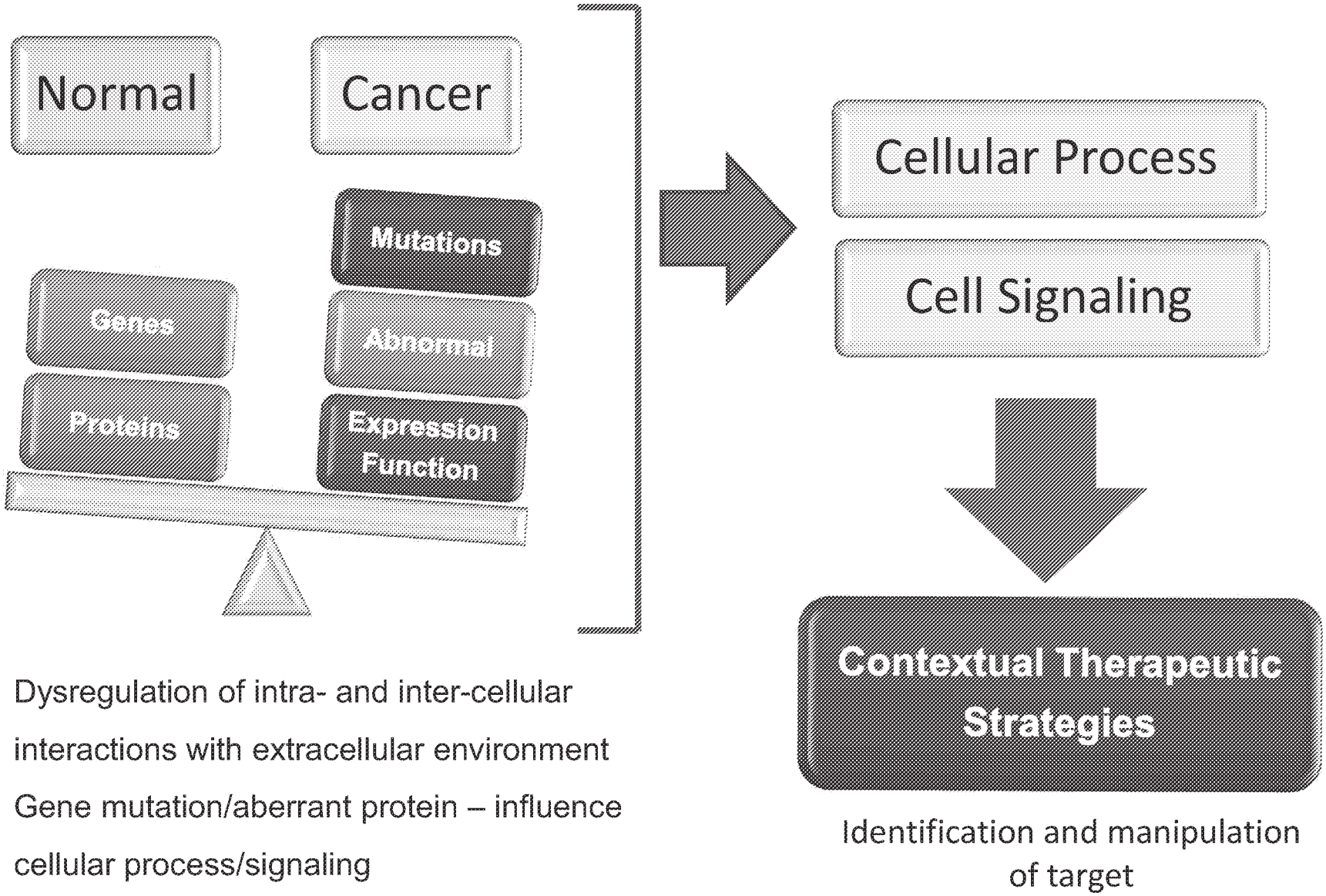


- Dysregulation of intra- and inter-cellular interactions with extracellular environment
- Gene mutation/aberrant protein – influence cellular process/signaling

Figure 1

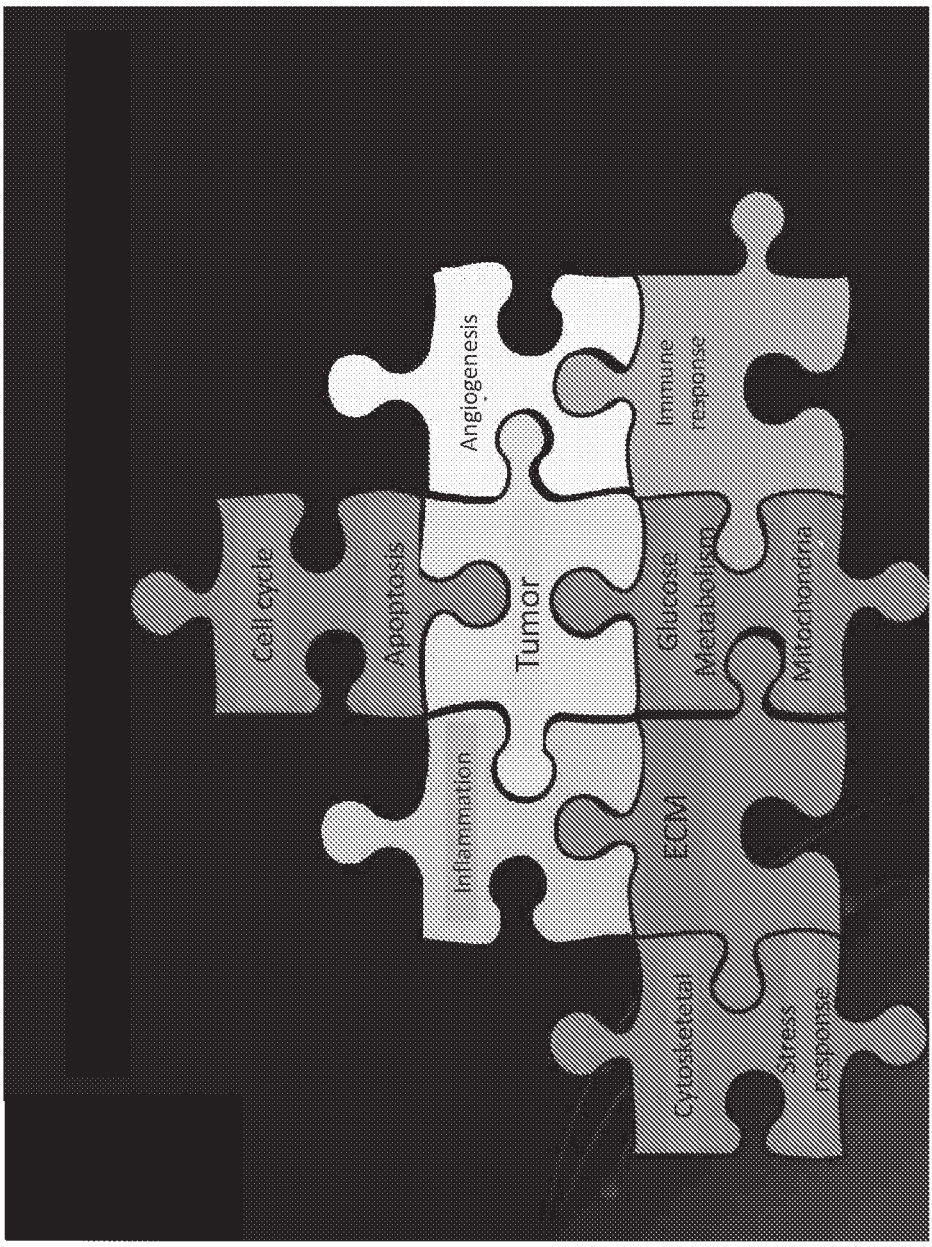

Cell cycle
Apoptosis
Angiogenesis
Immune response
Inflammation
Tumor
Glucose Metabolism
Mitochondria
Cytoskeletal
ECM
Stress response

Figure 2

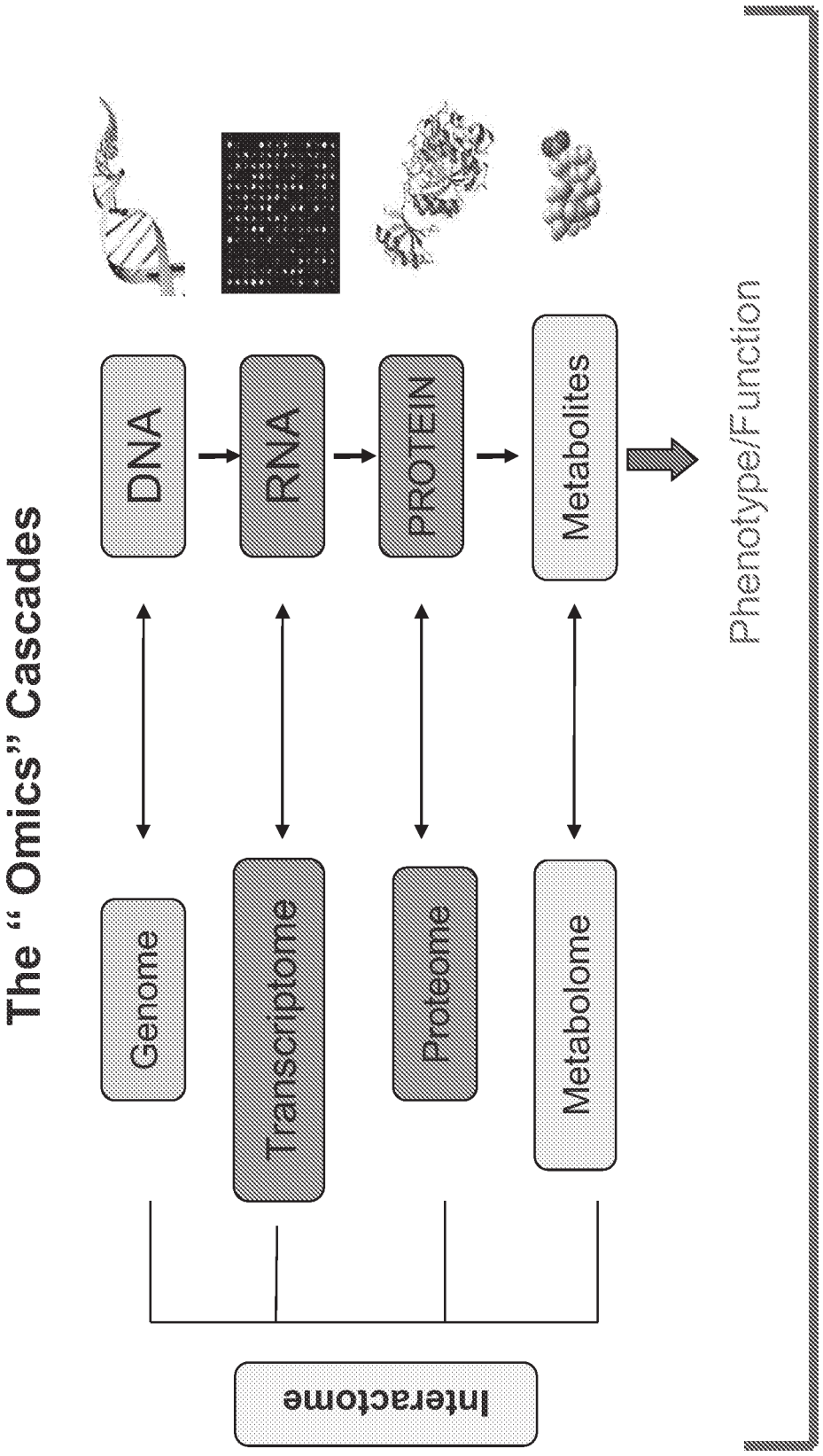

The " Omics" Cascades
DNA
RNA
PROTEIN
Metabolites
Phenotype/Function
Genome
Transcriptome
Proteome
Metabolome
Interactome

Figure 3

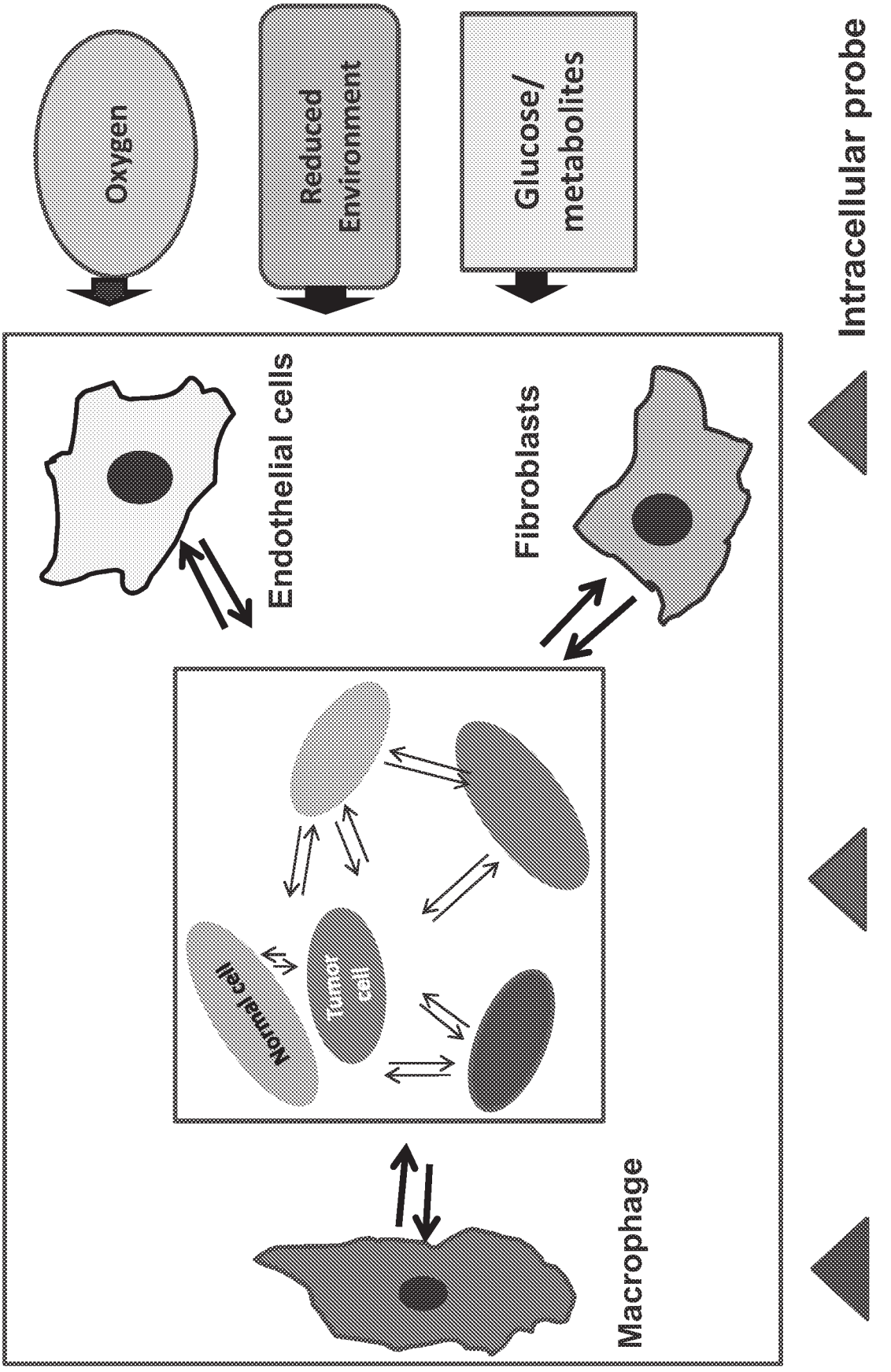

Oxygen
Reduced Enviornment
Glucose/ metabolites
Intracellular probe
Endothelial cells
Fibroblasts
Normal cell
Tumor cell
Macrophage

Figure 4

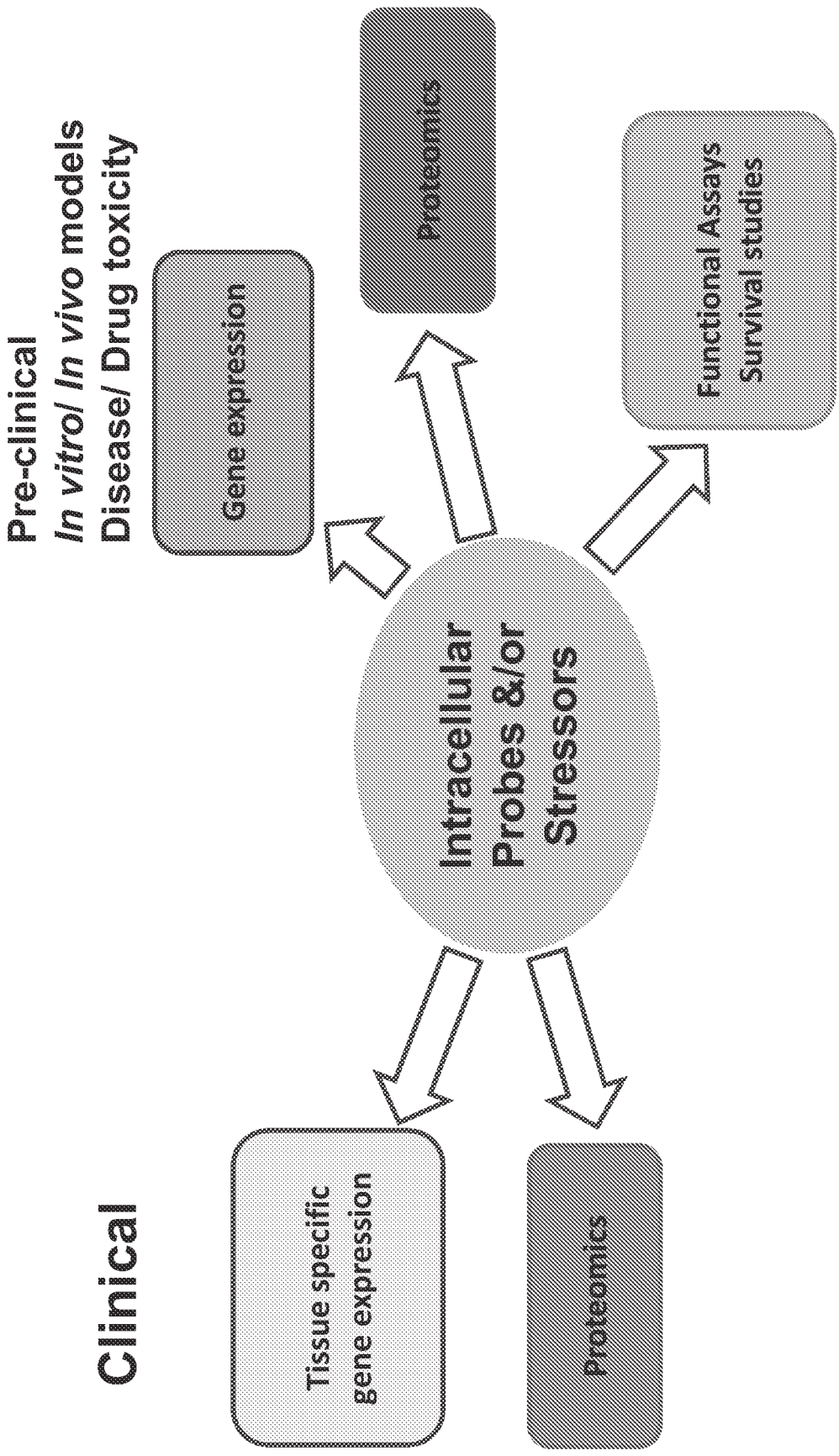

Pre-clinical
In vitro/ In vivo models
Disease/ Drug toxicity
Gene expression
Proteomics
Functional Assays
Survival studies
Intracellular
Probes &/or
Stressors
Clinical
Tissue specific
gene expression
Proteomics

Figure 5

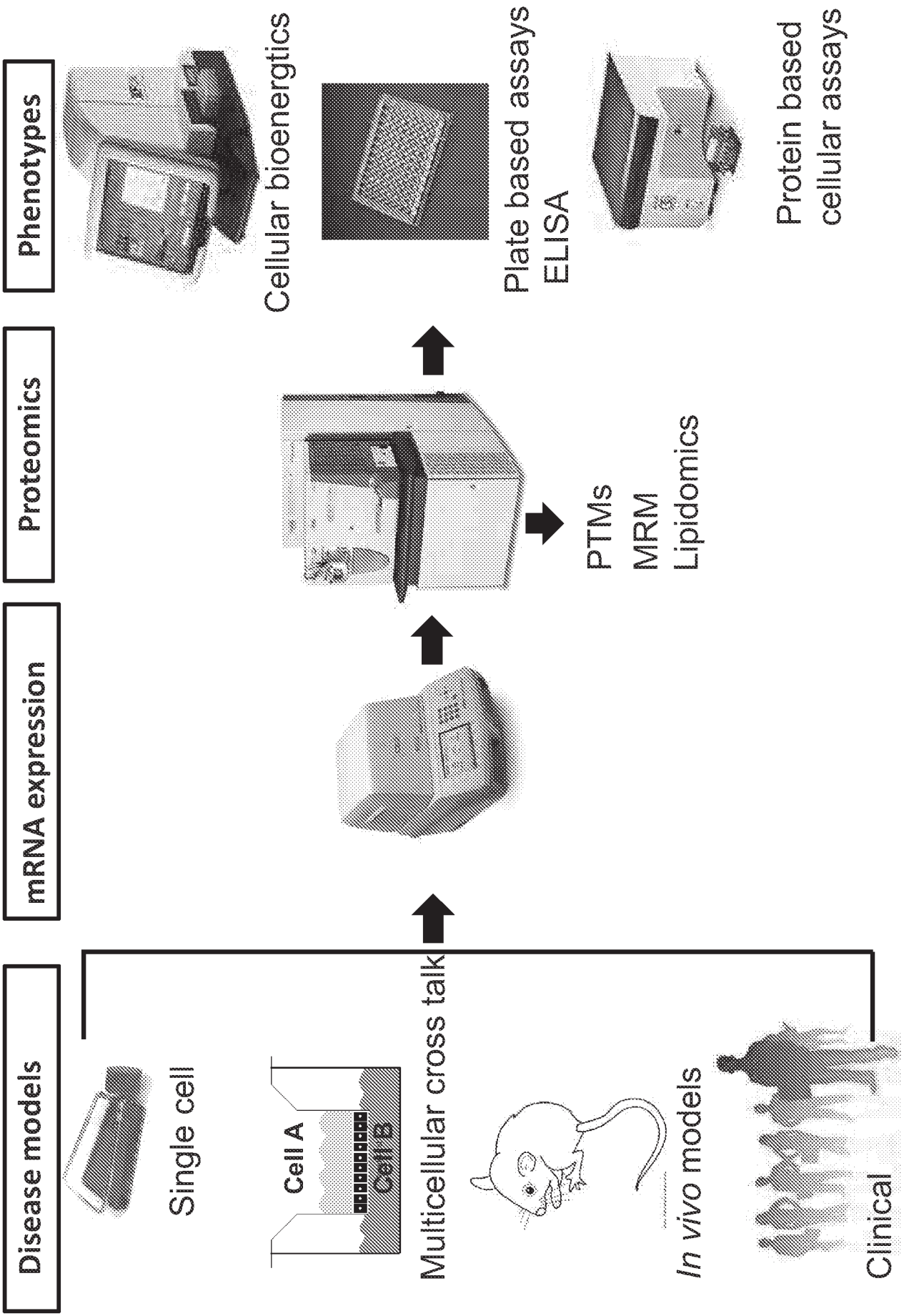

Disease models
mRNA expression
Proteomics
Phenotypes
Single cell
Cell A
Cell B
Multicellular cross talk
In vivo models
Clinical
Cellular bioenergtics
Plate based assays
ELISA
Protein based
cellular assays
PTMs
MRM
Lipidomics

Figure 6

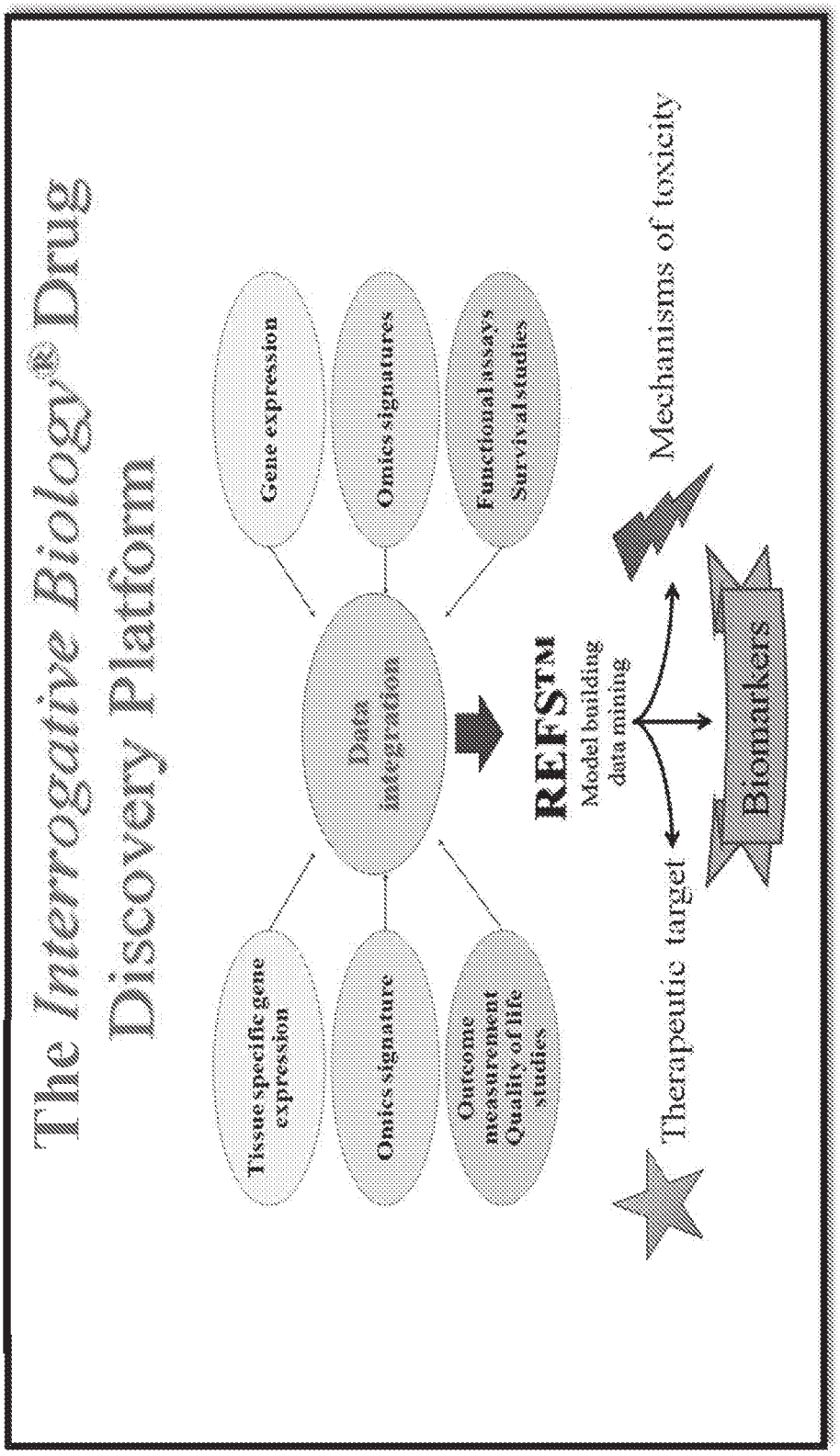

The Interrogative Biology® Drug Discovery Platform
Gene expression
Omics signatures
Functional assays Survival studies
Tissue specific gene expression
Omics signature
Outcome measurement Quality of life studies
Data integration
REFS™
Model building data mining
Mechanisms of toxicity
Therapeutic target
Biomarkers

Figure 7

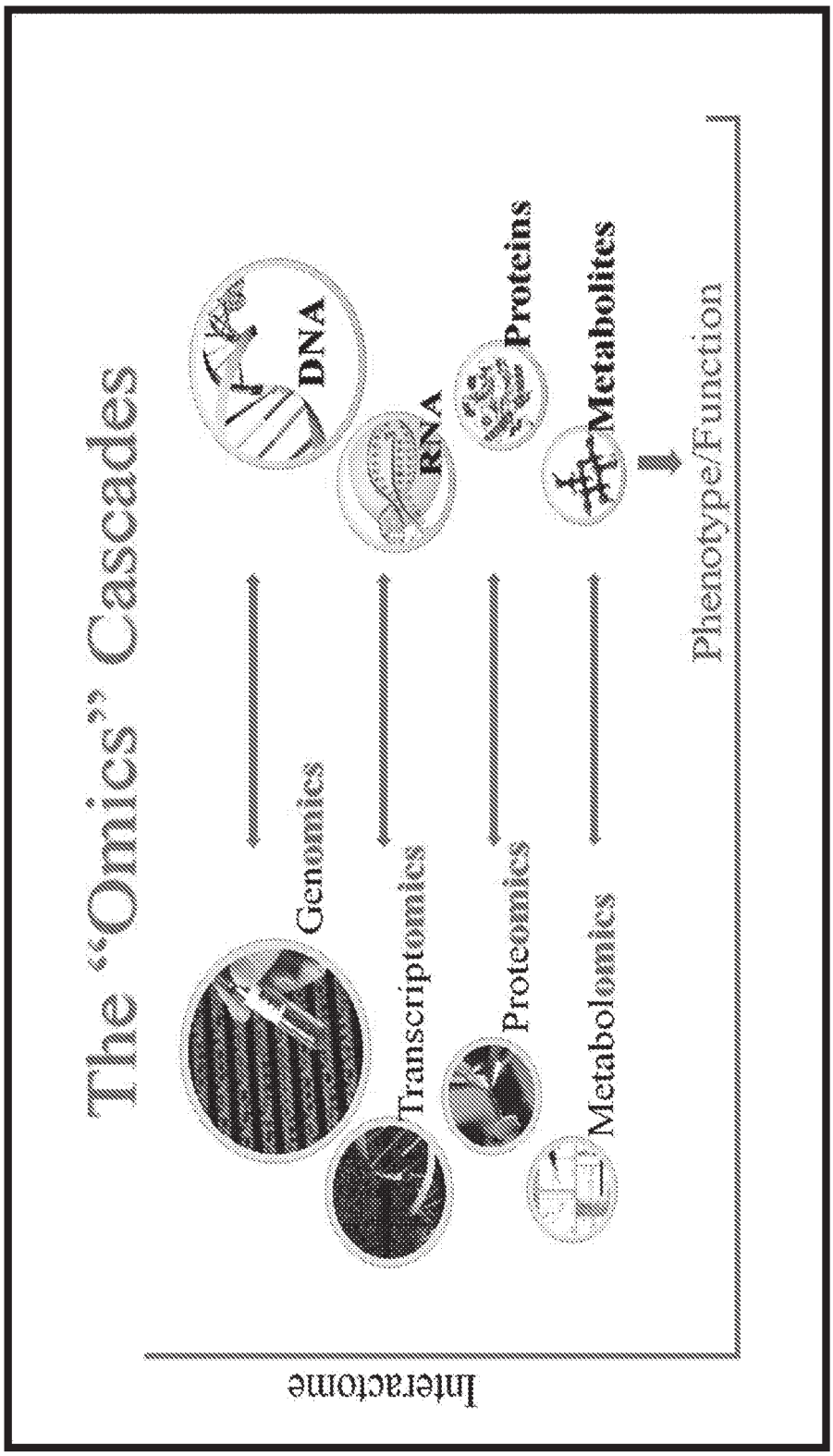

The "Omics" Cascades
Genomics
Transcriptomics
Proteomics
Metabolomics
DNA
RNA
Proteins
Metabolites
Phenotype/Function
Interactome

Figure 8

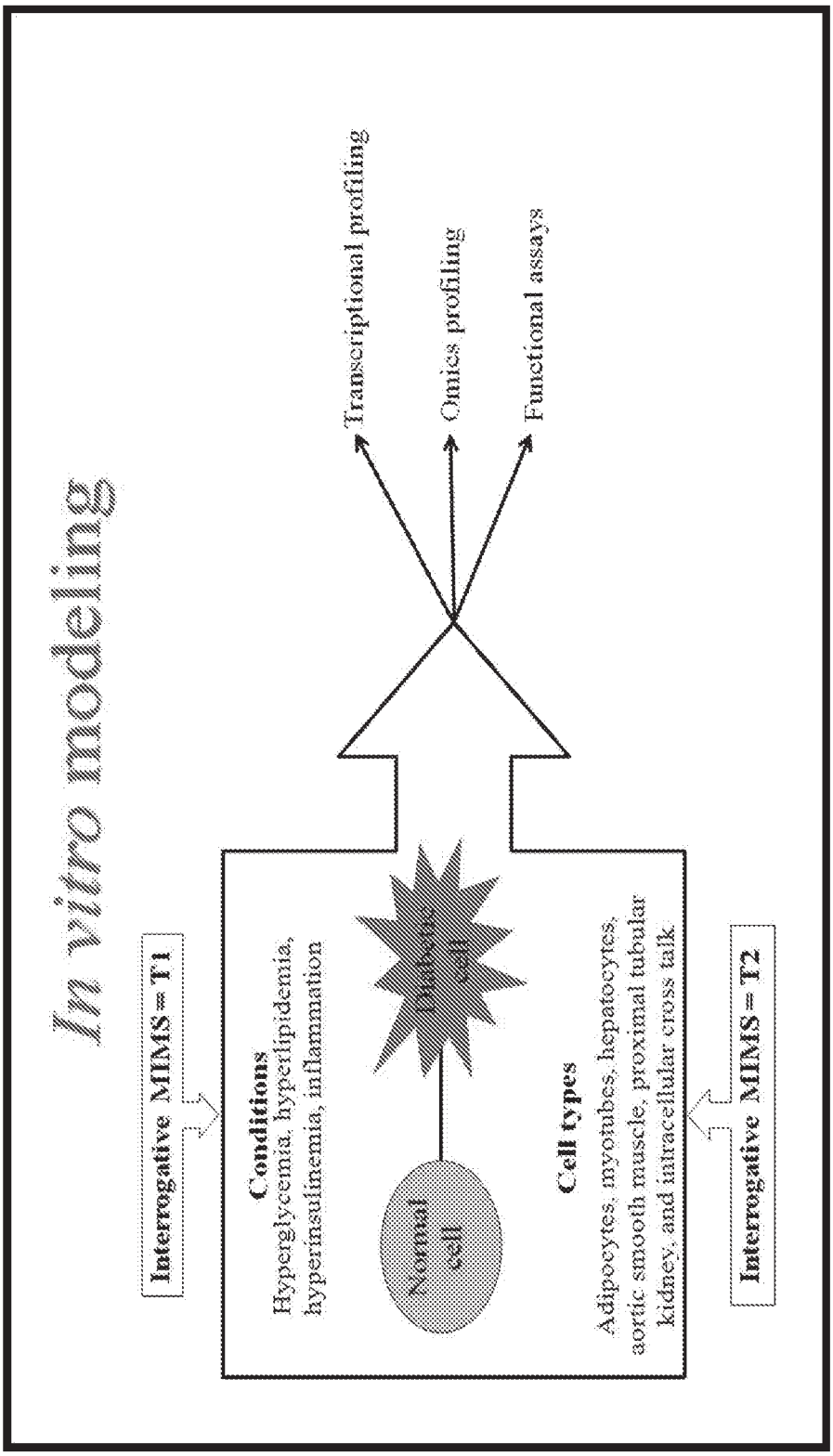

In vitro modeling
Interrogative MIMS = T1
Conditions
Hyperglycemia, hyperlipidemia, hyperinsulinemia, inflammation
Diabetic cell
Normal cell
Cell types
Adipocytes, myotubes, hepatocytes, aortic smooth muscle, proximal tubular kidney, and intracellular cross talk
Interrogative MIMS = T2
Transcriptional profiling
Omics profiling
Functional assays

Figure 9

Building Blocks
Perturbation to Network Component Network Fragments
Component to Component Network Fragments
Component to Outcome Network Fragments
Outcome to Component Network Fragments
Incorporates multiple data types – continuous, discrete, boolean from unlimited number of data modalities (clinical, 'omic, etc.)
Ensembles of Models
REFS™
REFS™
Requires massively parallel IBM Blue Gene machines of 30,000+ processors
Model Queries
Prediction
Control
Clinical Phenotype
Enables high-throughput *in silico* testing of interventions (single and in combination)
Assigns rank order and confidence metrics to predictions
Provides verifiable hypotheses

Figure 10

(Normal vs. Diabetic) Vs. (Diabetic T1 vs. Diabetic)
Normal
Diabetic
Diabetic T1
Diabetic
Compare the unique edges of Normal in the normal vs. disease delta network with the unique edges of Disease T1 in the disease vs. disease T1 delta network. Edges in the intersection of normal and disease T1 are disease edges that were restored to normal when treated with T1.

Figure 11

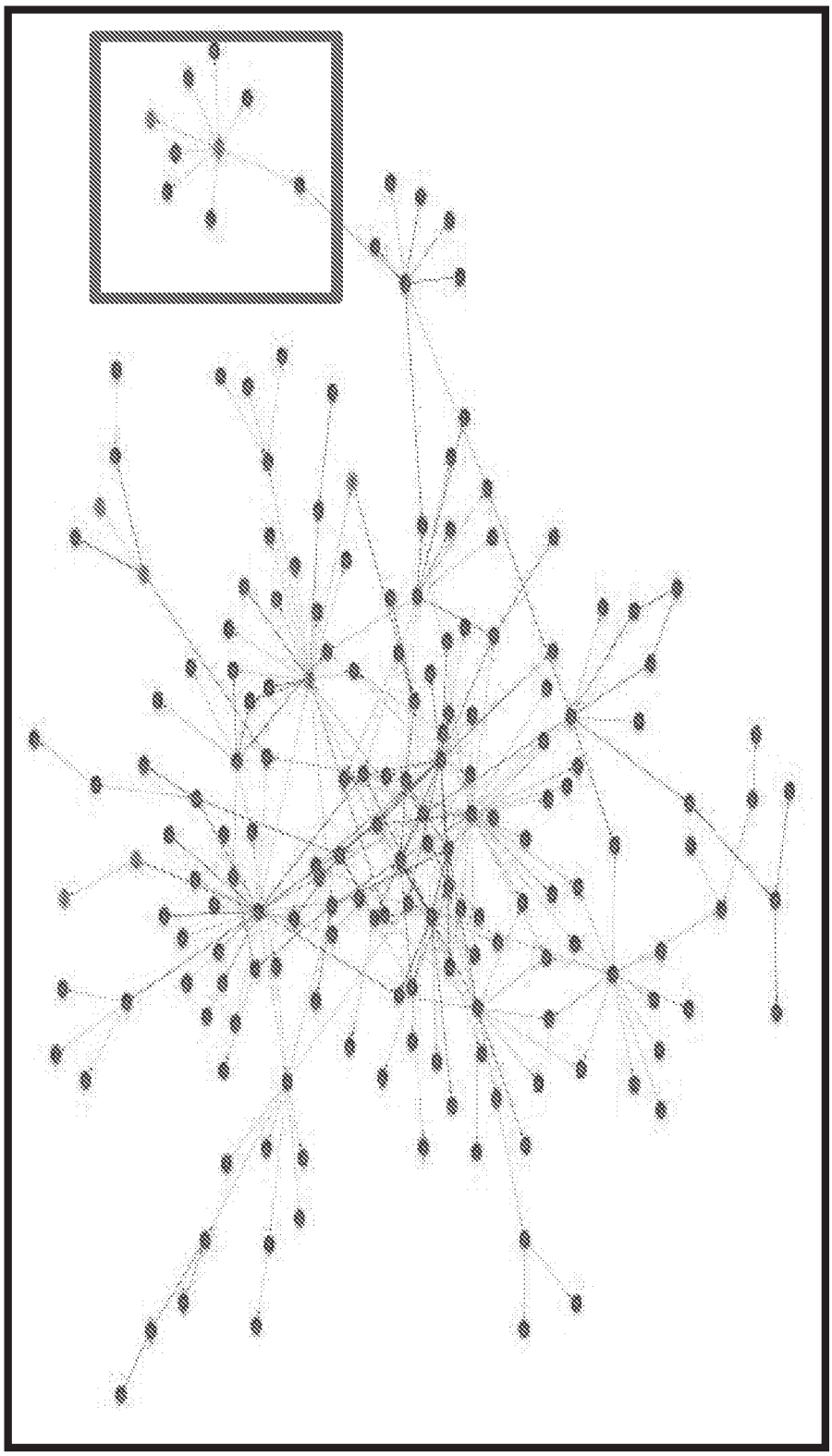


Figure 12

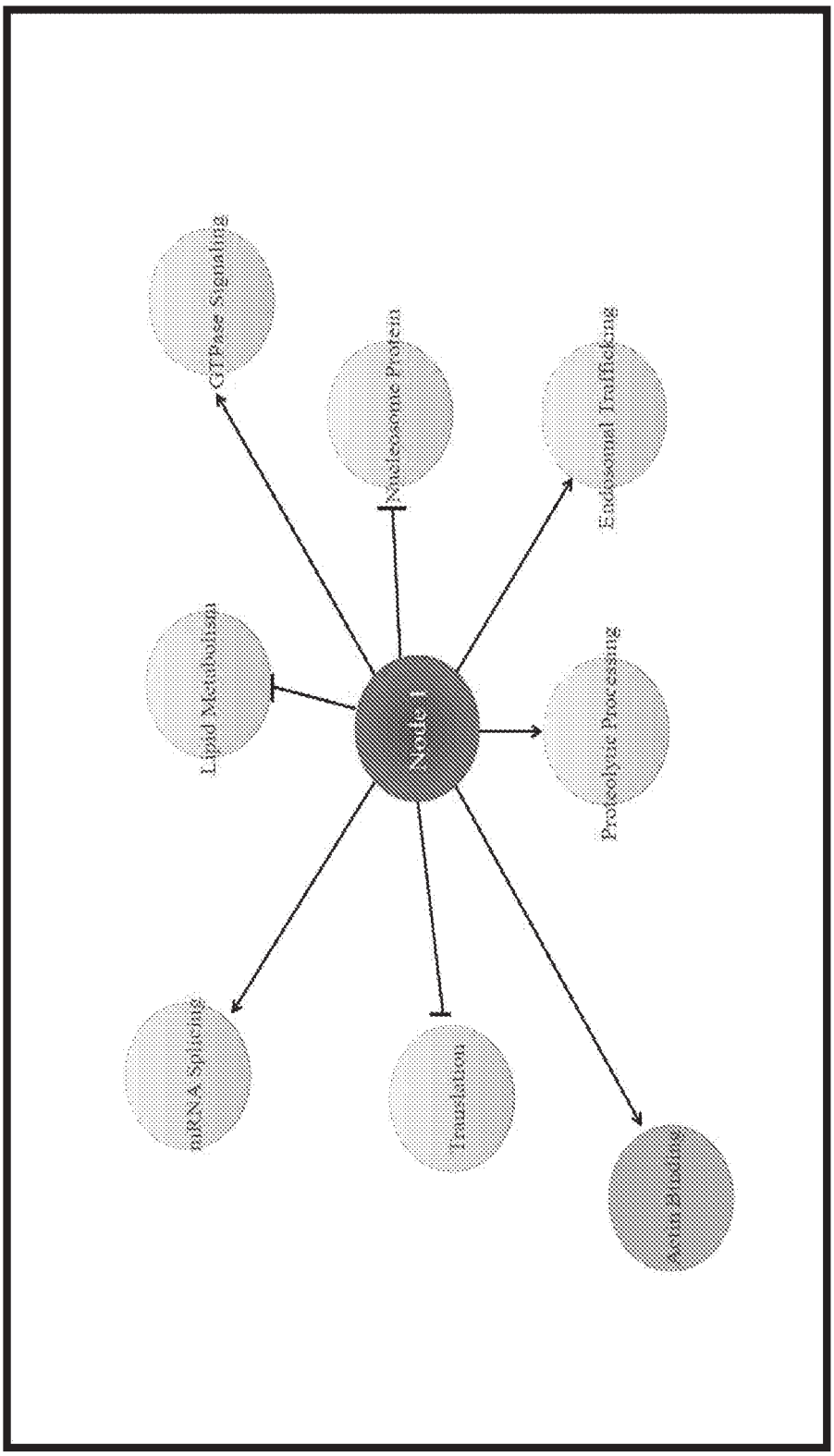

GTPase Signaling
Proteasome Protein
Endosomal Trafficking
Lipid Metabolism
Node 1
Proteolytic Processing
mRNA Splicing
Translation
Actin Binding

Figure 13

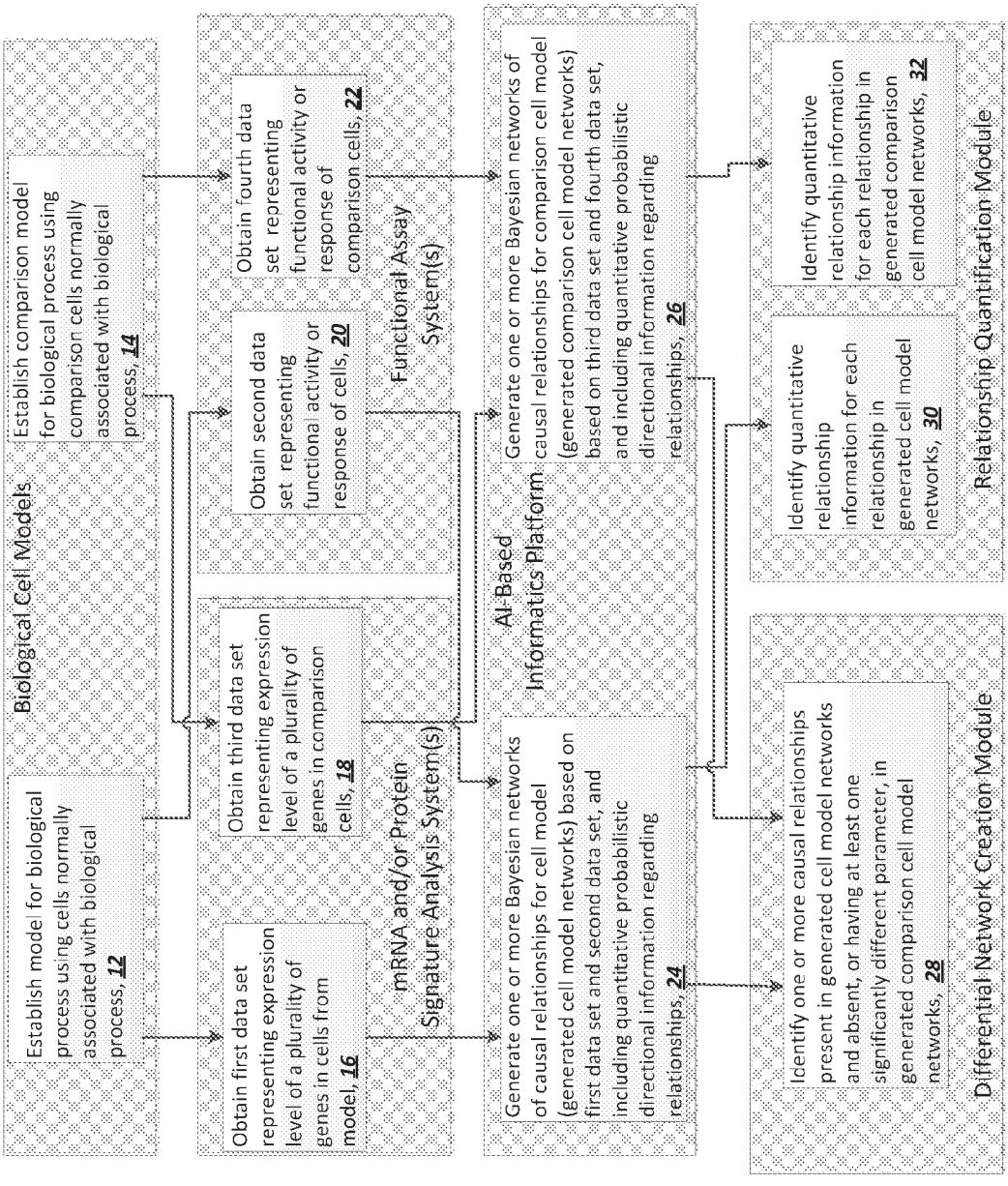

Biological Cell Models
Establish model for biological process using cells normally associated with biological process, 12
Establish comparison model for biological process using comparison cells normally associated with biological process, 14
mRNA and/or Protein Signature Analysis System(s)
Obtain first data set representing expression level of a plurality of genes in cells from model, 16
Obtain third data set representing expression level of a plurality of genes in comparison cells, 18
Functional Assay System(s)
Obtain second data set representing functional activity or response of cells, 20
Obtain fourth data set representing functional activity or response of comparison cells, 22
AI-Based Informatics Platform
Generate one or more Bayesian networks of causal relationships for cell model (generated cell model networks) based on first data set and second data set, and including quantitative probabilistic directional information regarding relationships, 24
Generate one or more Bayesian networks of causal relationships for comparison cell model (generated comparison cell model networks) based on third data set and fourth data set, and including quantitative probabilistic directional information regarding relationships, 26
Differential Network Creation Module
Identify one or more causal relationships present in generated cell model networks and absent, or having at least one significantly different parameter, in generated comparison cell model networks, 28
Relationship Quantification Module
Identify quantitative relationship information for each relationship in generated cell model networks, 30
Identify quantitative relationship information for each relationship in generated comparison cell model networks, 32

Figure 14

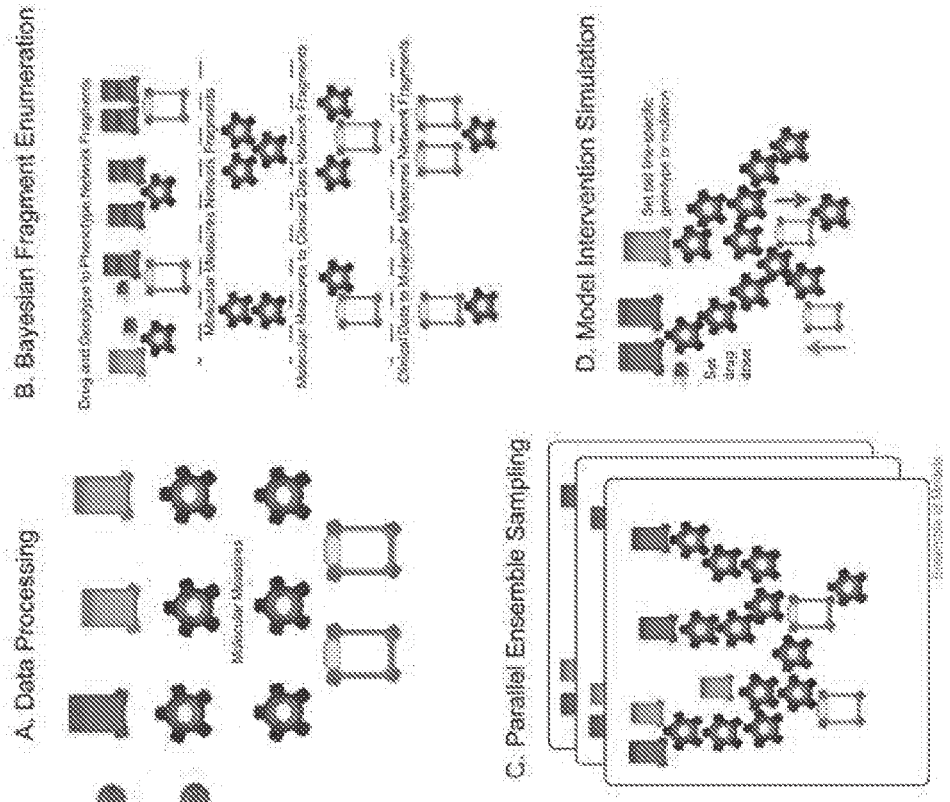
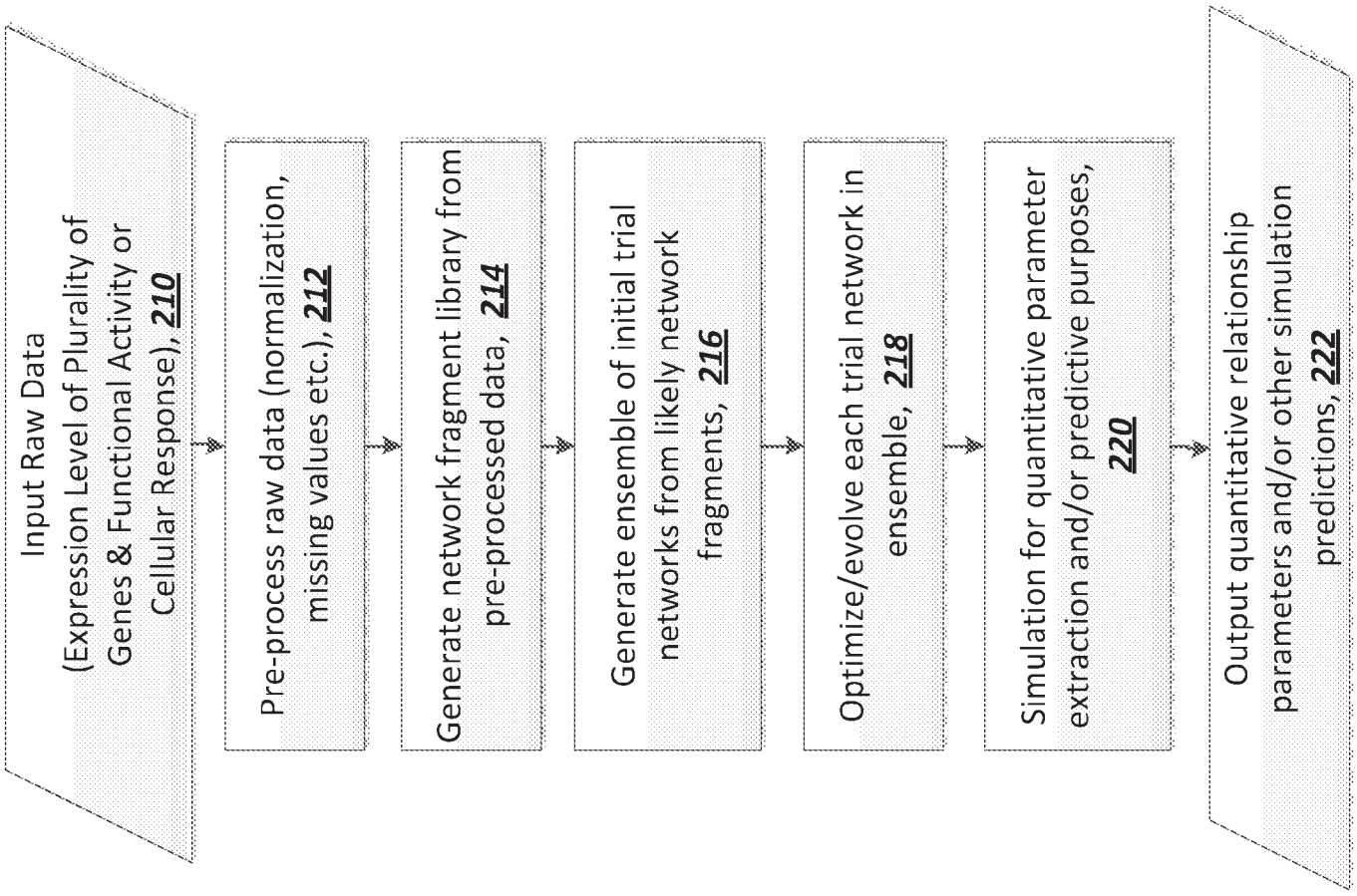

A. Data Processing
B. Bayesian Fragment Enumeration
C. Parallel Ensemble Sampling
D. Model Intervention Simulation

Figure 15

Figure 16


Input Raw Data (Expression Level of Plurality of Genes & Functional Activity or Cellular Response), 210
Pre-process raw data (normalization, missing values etc.), 212
Generate network fragment library from pre-processed data, 214
Generate ensemble of initial trial networks from likely network fragments, 216
Optimize/evolve each trial network in ensemble, 218
Simulation for quantitative parameter extraction and/or predictive purposes, 220
Output quantitative relationship parameters and/or other simulation predictions, 222

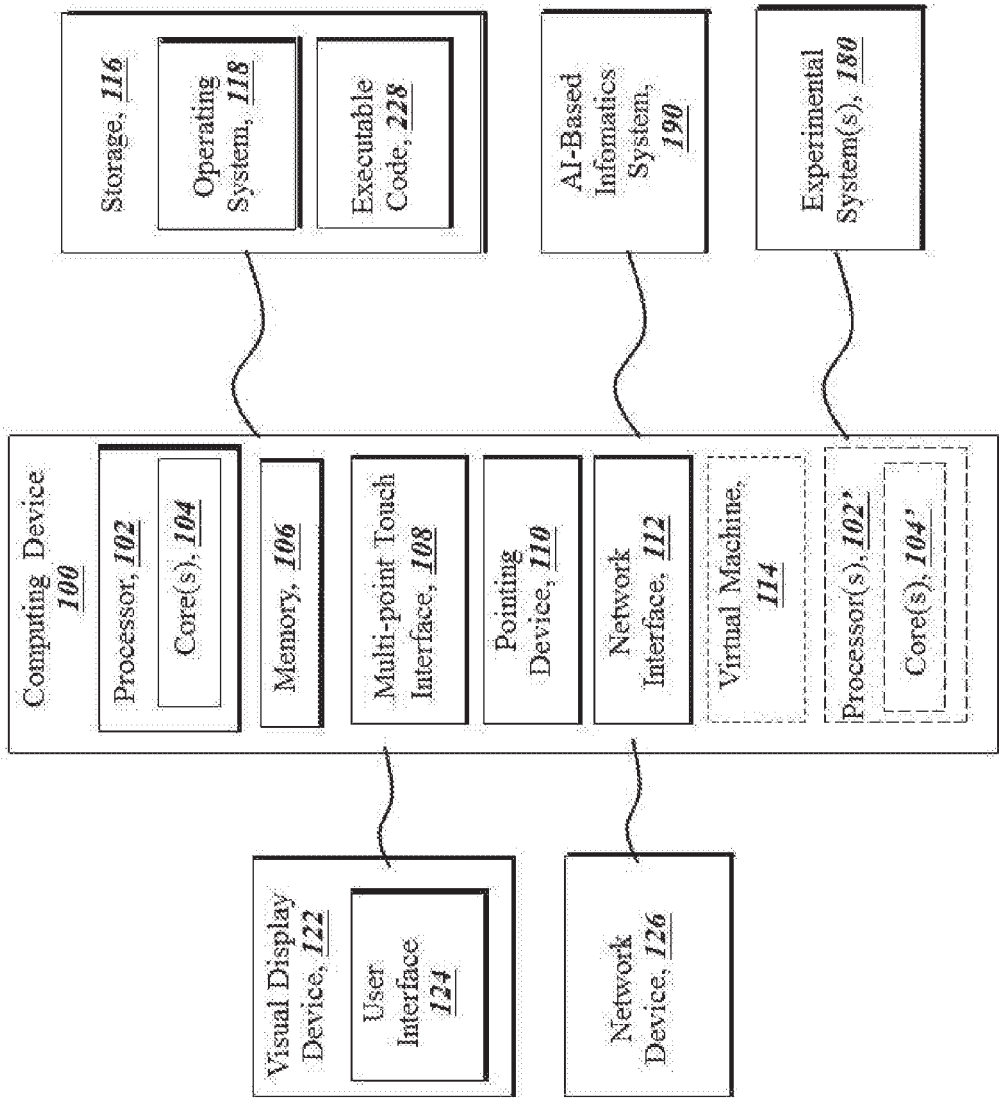

Computing Device 100
Processor, 102
Core(s), 104
Memory, 106
Multi-point Touch Interface, 108
Pointing Device, 110
Network Interface, 112
Virtual Machine, 114
Processor(s), 102'
Core(s), 104'
Storage, 116
Operating System, 118
Executable Code, 228
AI-Based Informatics System, 190
Experimental System(s), 180
Visual Display Device, 122
User Interface 124
Network Device, 126

Figure 17

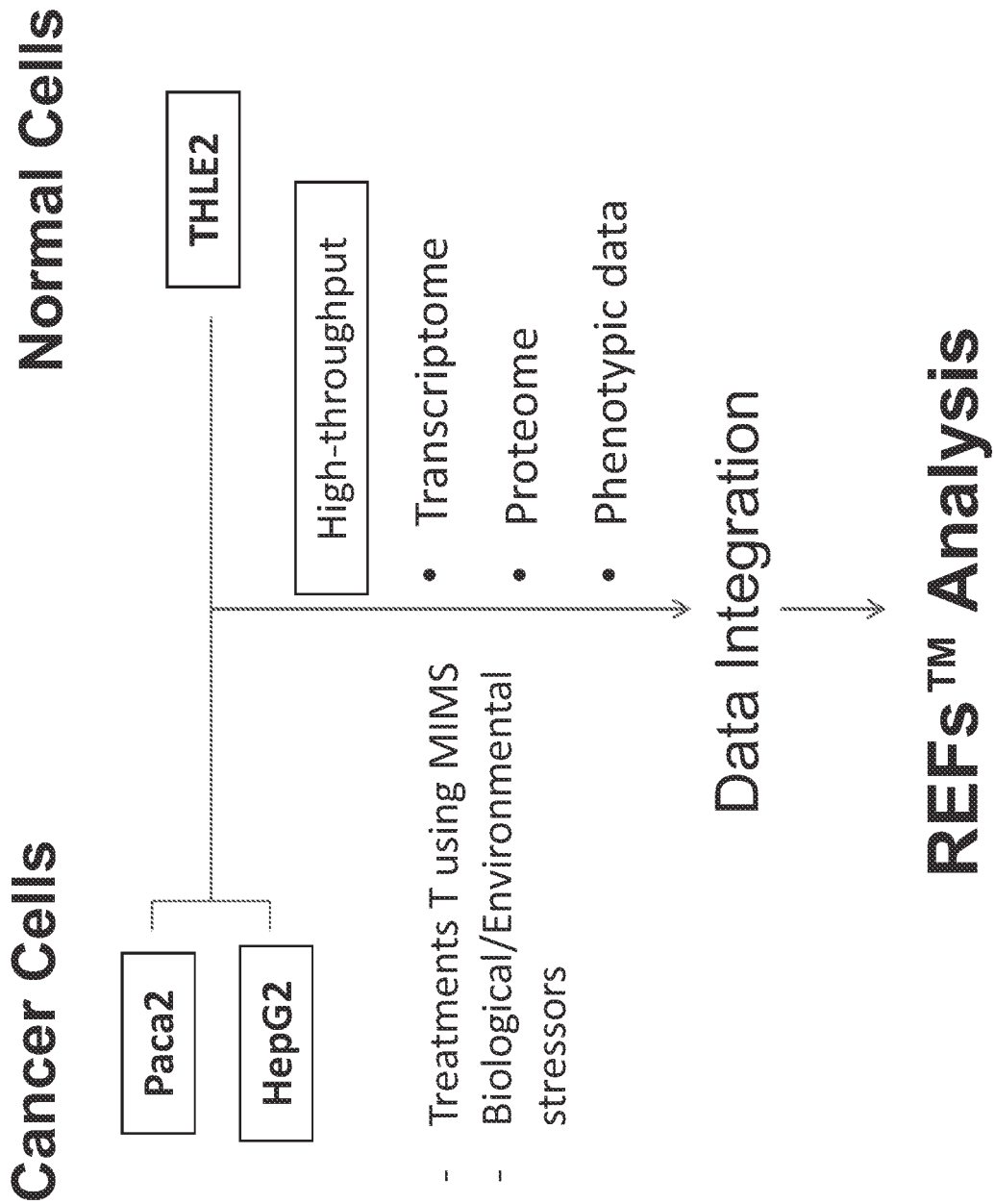

Cancer Cells
Normal Cells
Paca2
HepG2
THLE2
High-throughput
- Treatments T using MIMS
- Biological/Environmental stressors
• Transcriptome
• Proteome
• Phenotypic data
Data Integration
REFs™ Analysis

Figure 18

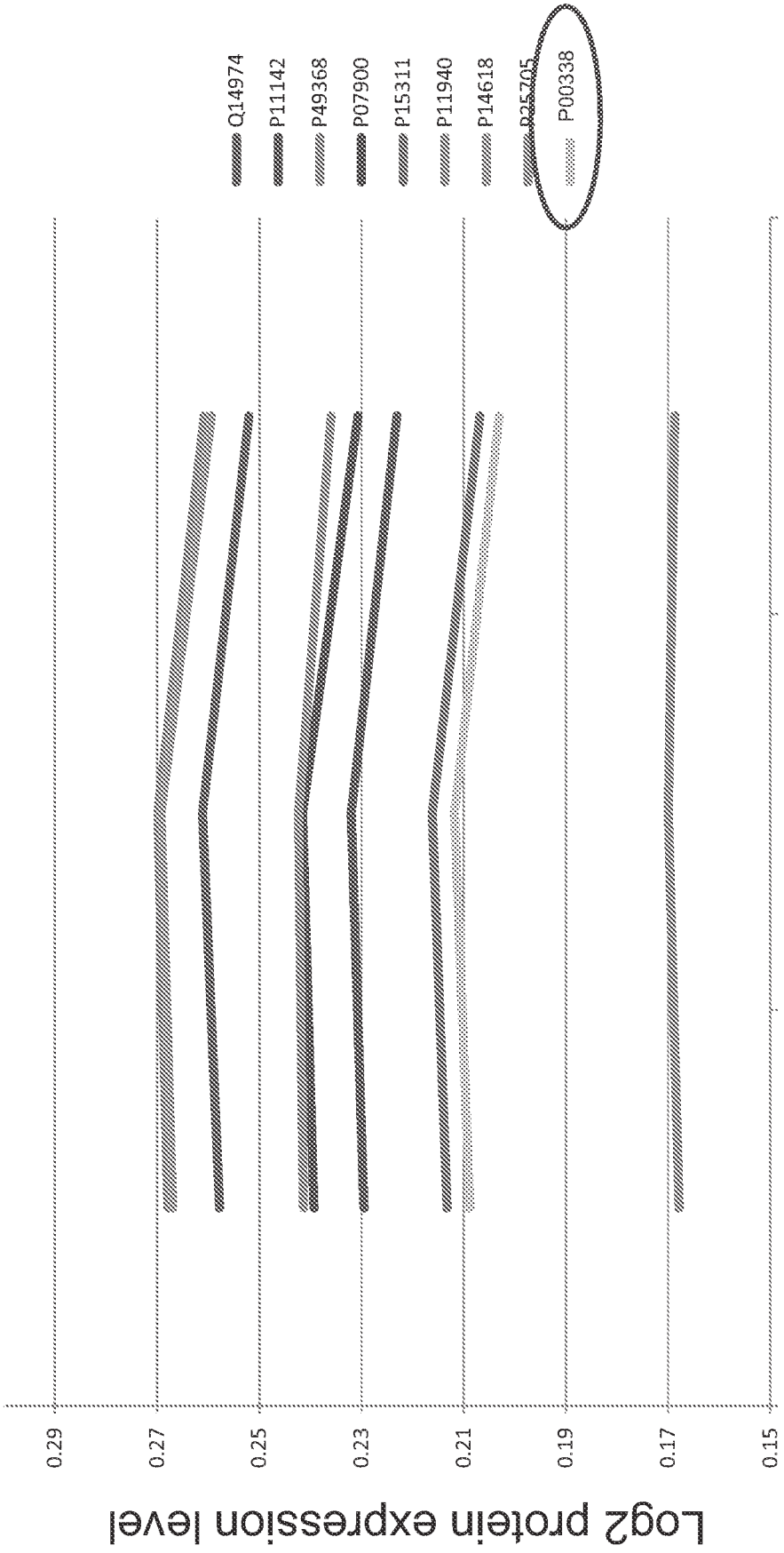

Log2 protein expression level
0.29
0.27
0.25
0.23
0.21
0.19
0.17
0.15
None
50 uM
100 uM
CoQ10 Treatment
Q14974
P11142
P49368
P07900
P15311
P11940
P14618
P25705
P00338

Figure 19

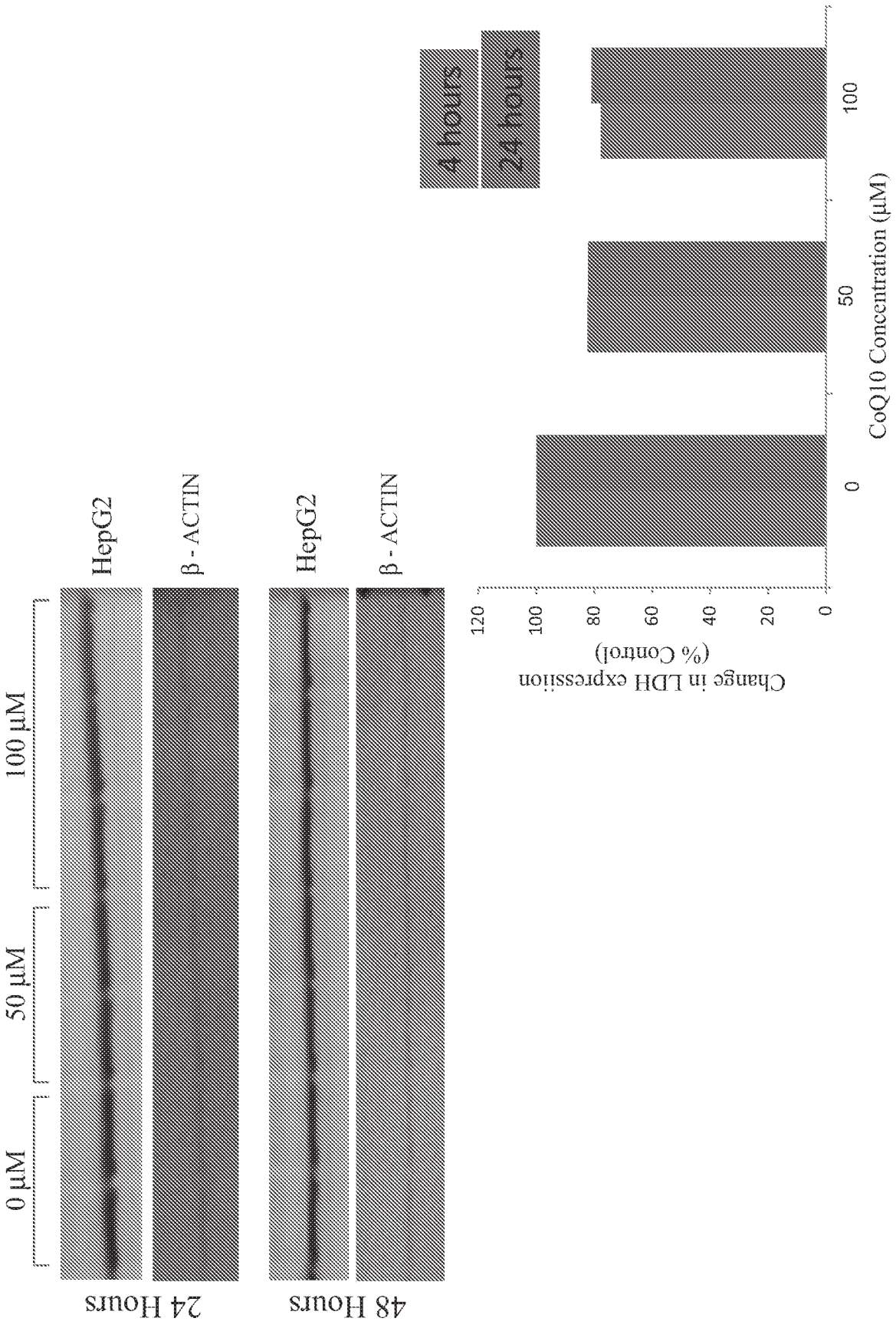

0 µM
50 µM
100 µM
HepG2
β - ACTIN
HepG2
β - ACTIN
24 Hours
48 Hours
4 hours
24 hours
Change in LDH expression
(% Control)
120
100
80
60
40
20
0
0
50
100
CoQ10 Concentration (µM)

Figure 20

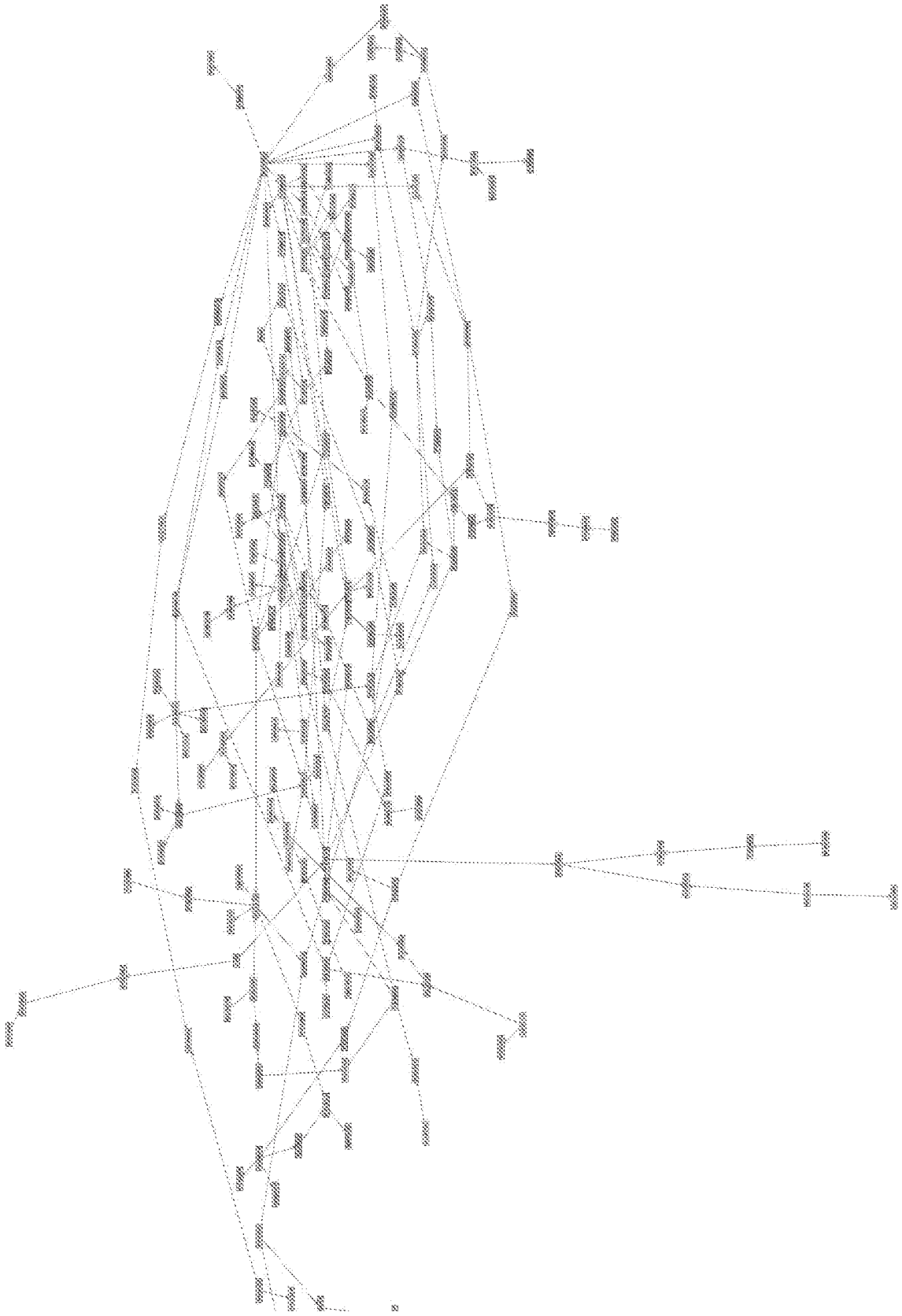


Figure 21

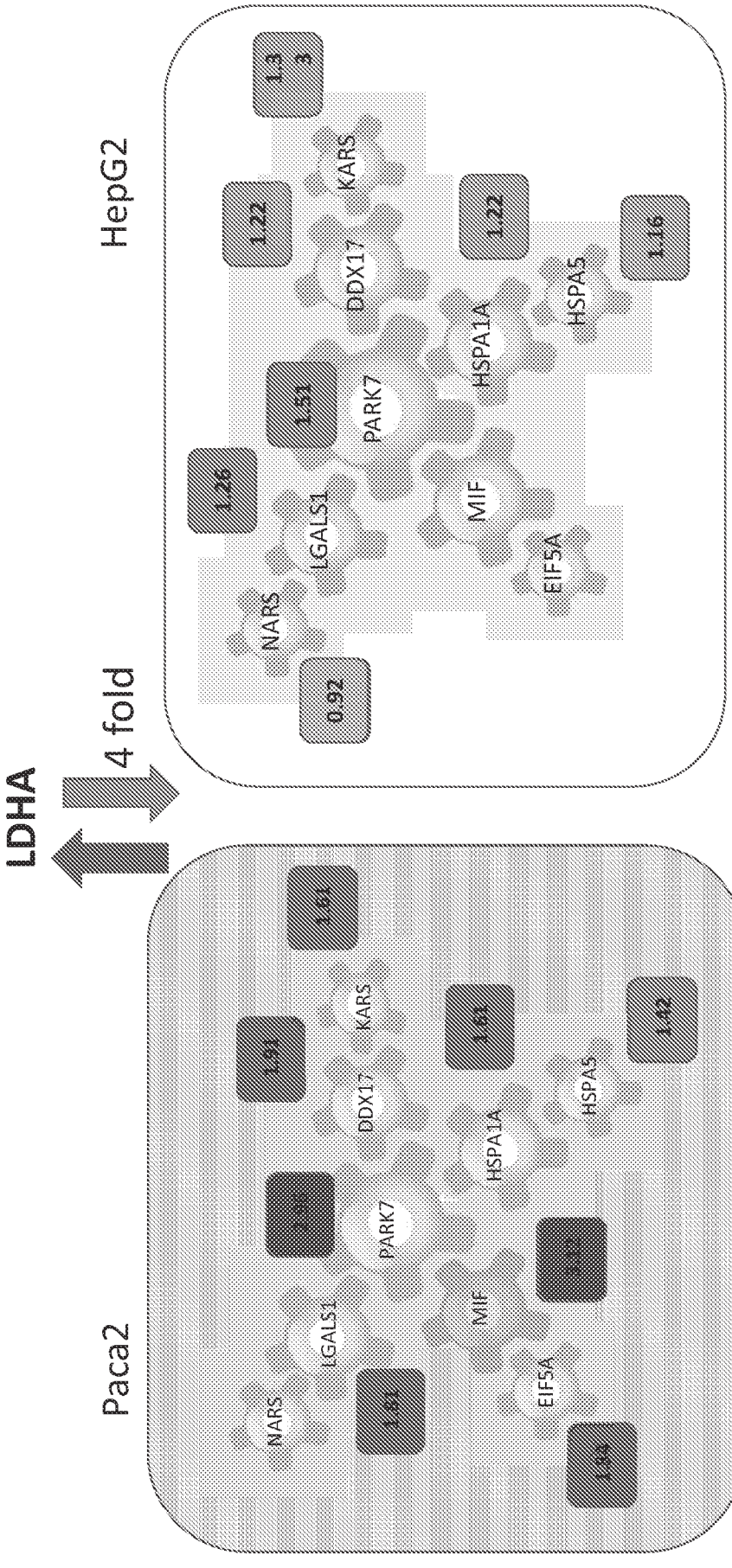

LDHA
4 fold
HepG2
Paca2
NARS
LGALS1
PARK7
DDX17
KARS
MIF
HSPA1A
EIF5A
HSPA5
0.92
1.26
1.51
1.22
1.22
1.16
1.3
3
1.61
1.61
1.61
1.61
1.42
1.94

Figure 22

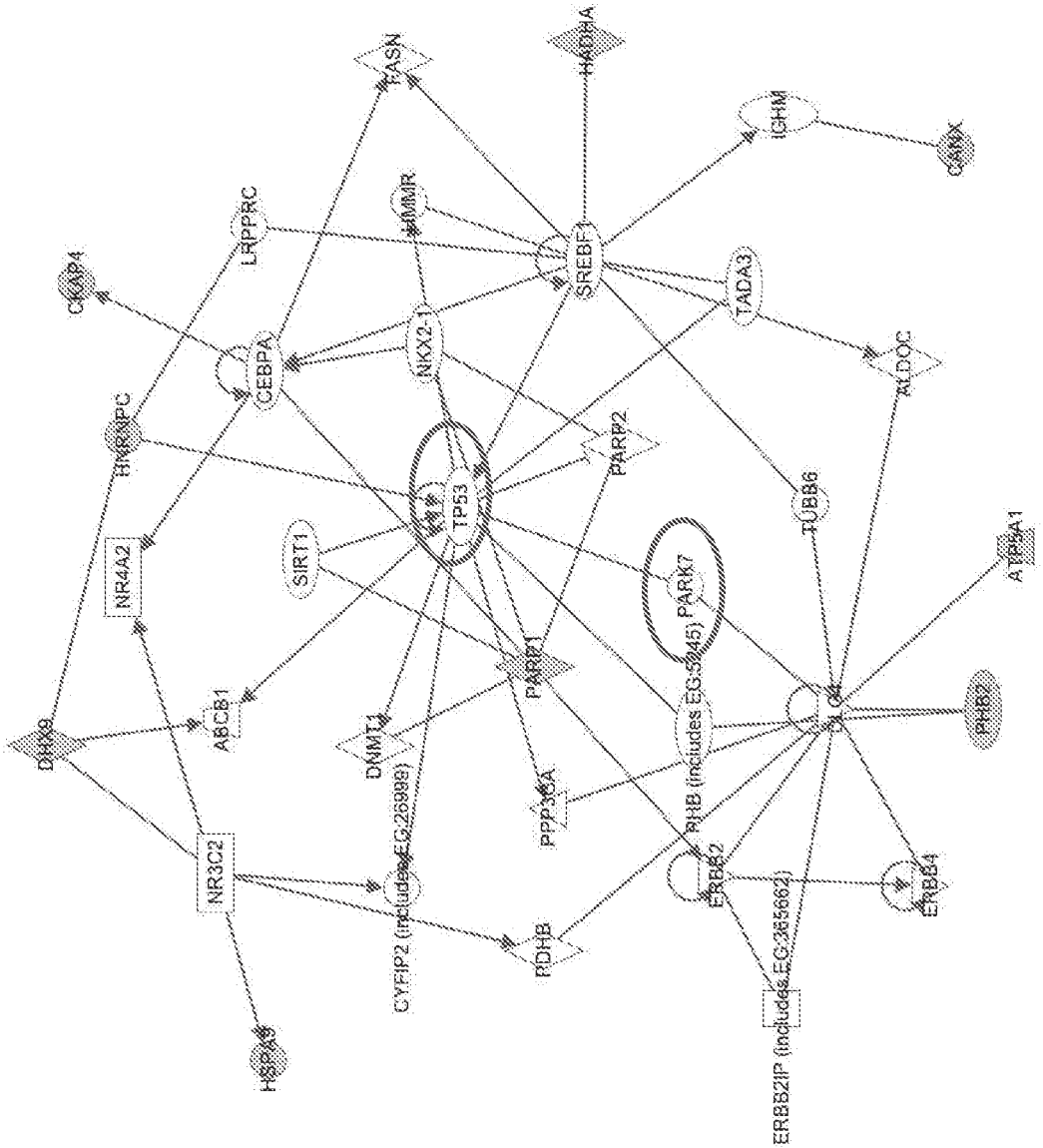

FASN
HADHA
IGHM
CA9X
LRPPRC
HMMR
SREBF1
CCAR4
CEBPA
NKX2-1
TADA3
HNRNPC
PARP2
ALDOC
TP53
NR4A2
SIRT1
TUBB6
PARK7
ATP5A1
PARP1
ABCB1
DHX9
DNMT1
PPP1CA
PHB (includes EG:5245)
PHB2
NR3C2
CYFIP2 (includes EG:26999)
RDHB
ERBB2
ERBB4
ERBB2IP (includes EG:285662)
HSPA8

Figure 23

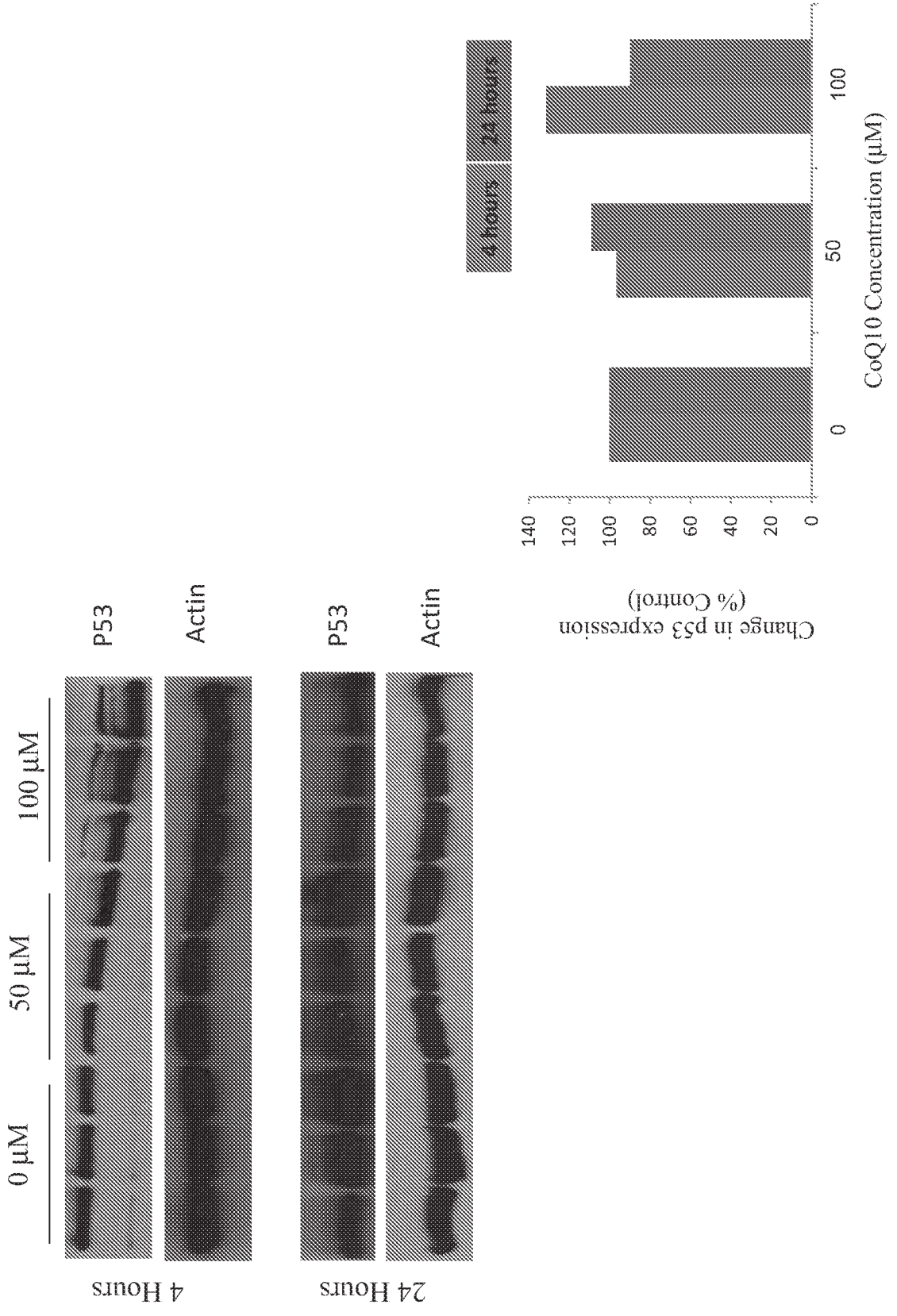

0 µM
50 µM
100 µM
P53
Actin
4 Hours
24 Hours
Change in p53 expression
(% Control)
140
120
100
80
60
40
20
0
0
50
100
CoQ10 Concentration (µM)
4 hours
24 hours

Figure 24

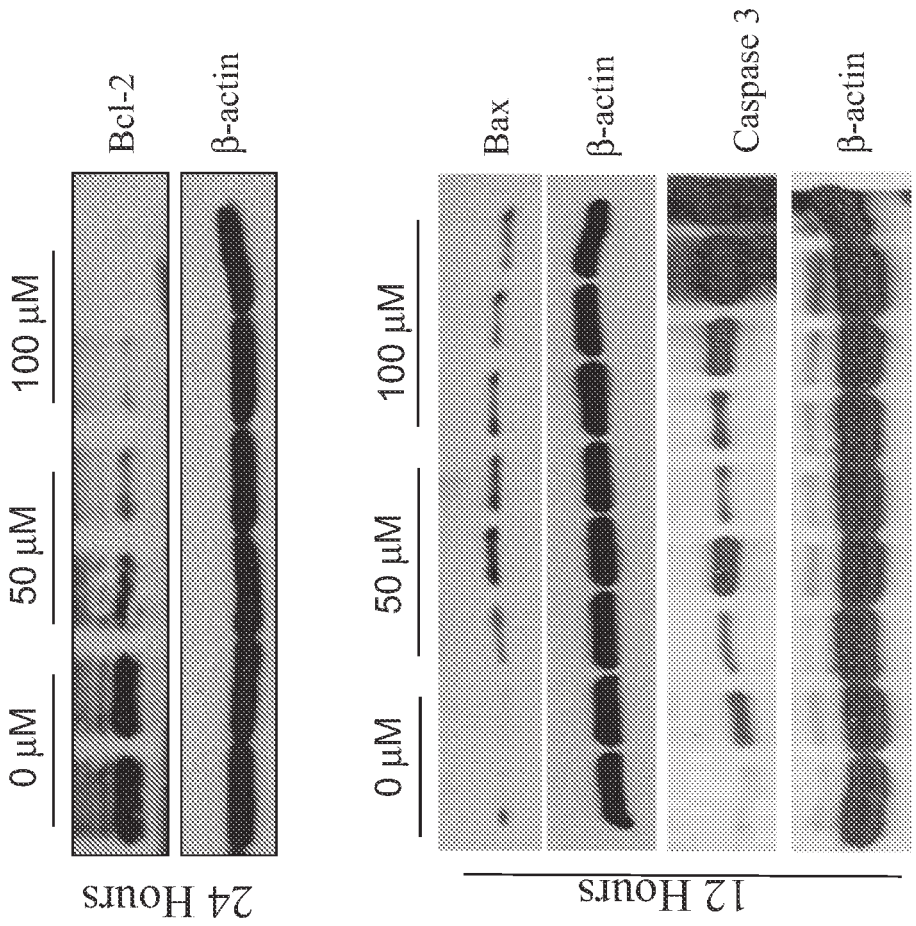

24 Hours
0 µM
50 µM
100 µM
Bcl-2
β-actin
12 Hours
0 µM
50 µM
100 µM
Bax
β-actin
Caspase 3
β-actin

Figure 25

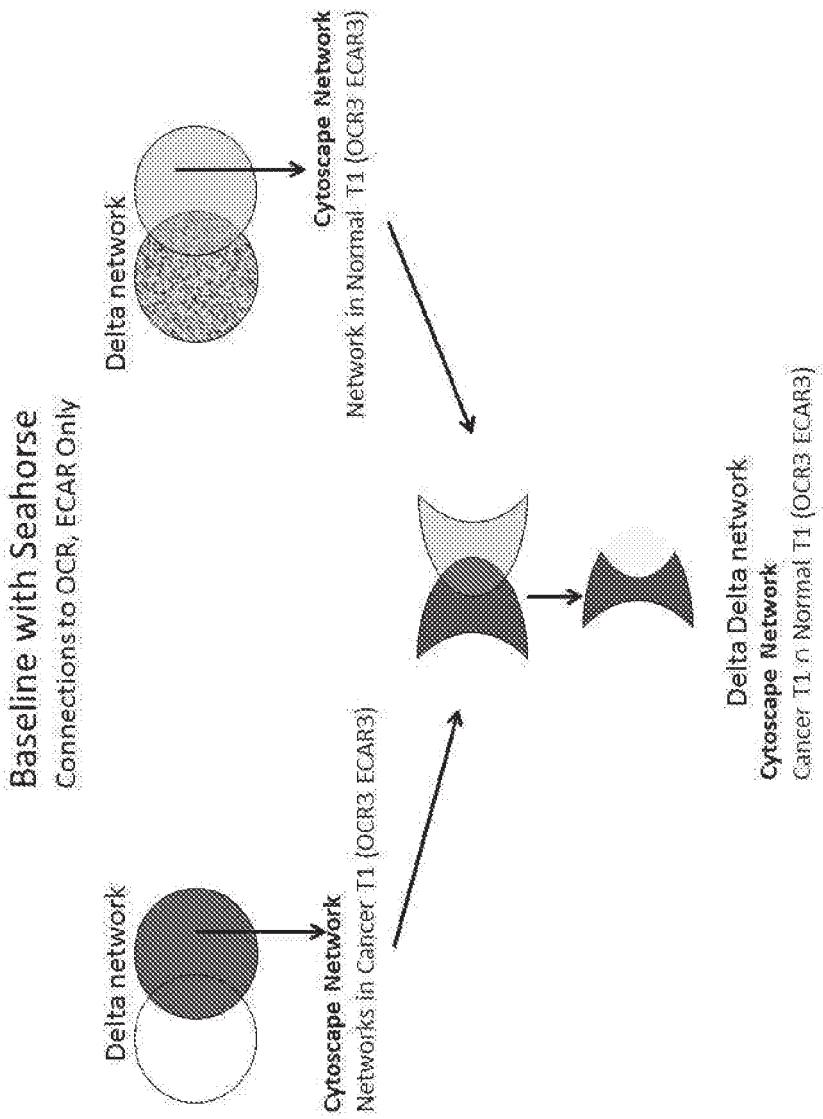

Baseline with Seahorse
Connections to OCR, ECAR Only
Delta network
Cytoscape Network
Networks in Cancer T1 (OCR3 ECAR3)
Delta network
Cytoscape Network
Network in Normal T1 (OCR3 ECAR3)
Delta Delta network
Cytoscape Network
Cancer T1 n Normal T1 (OCR3 ECAR3)

Figure 26

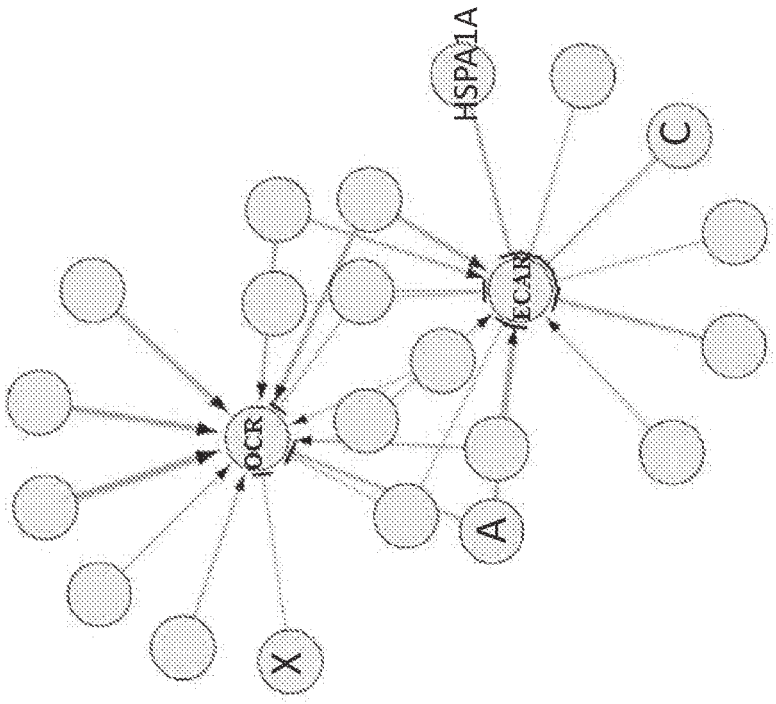


Figure 27

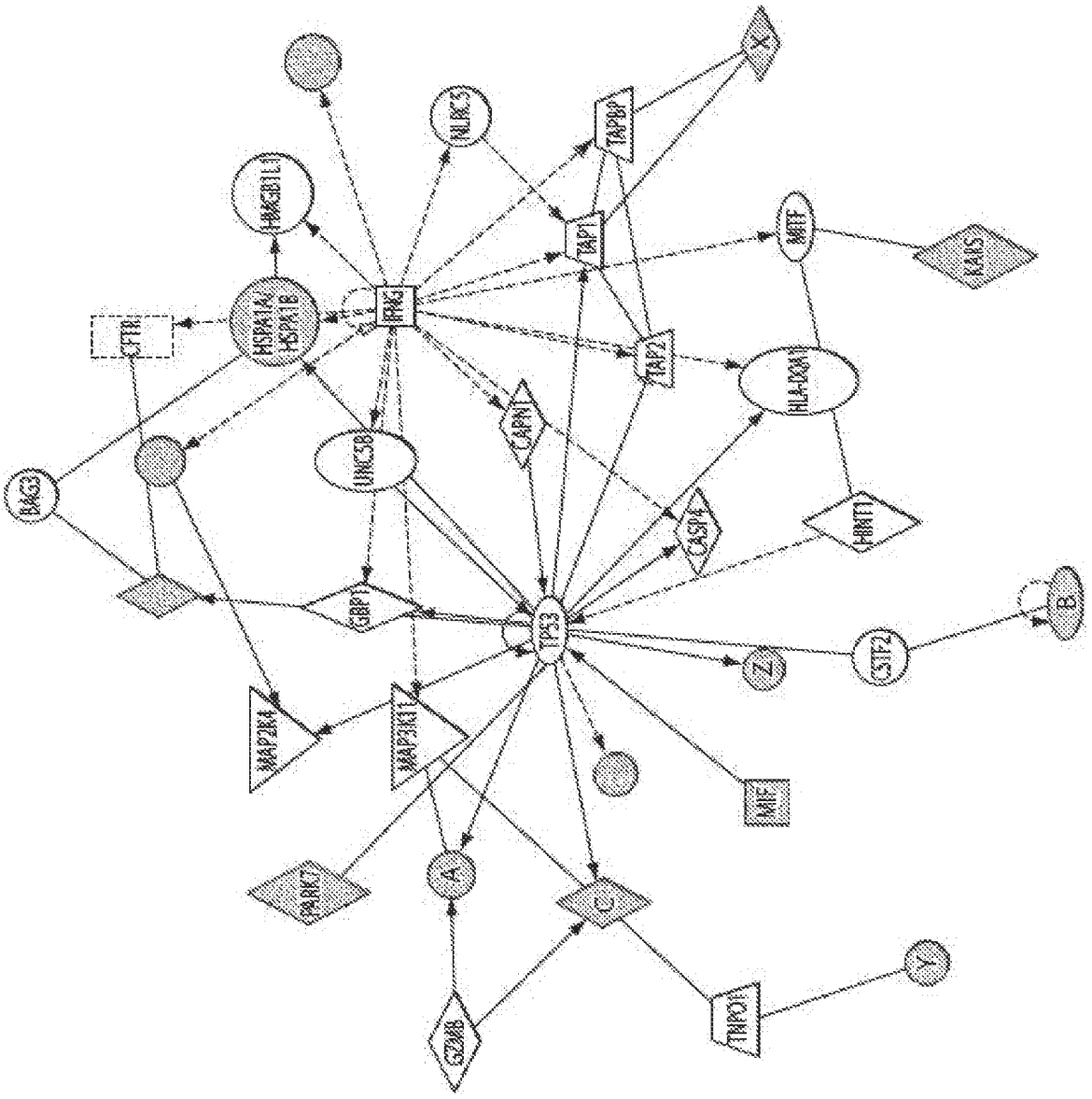

X
NLRC3
TAPBP
HMGB1L1
TAP1
MHF
KAAS
HSPA1A, HSPA1B
IFNG
CFTR
TAP2
HLA-DQA1
UNC93B
CAPN1
BAG3
CASP4
HBMT1
GBP1
TP53
B
Z
STF2
MAP2K4
MAP3K11
MIF
PRKCT
A
C
GZMB
TNFSF13
Y

Figure 28

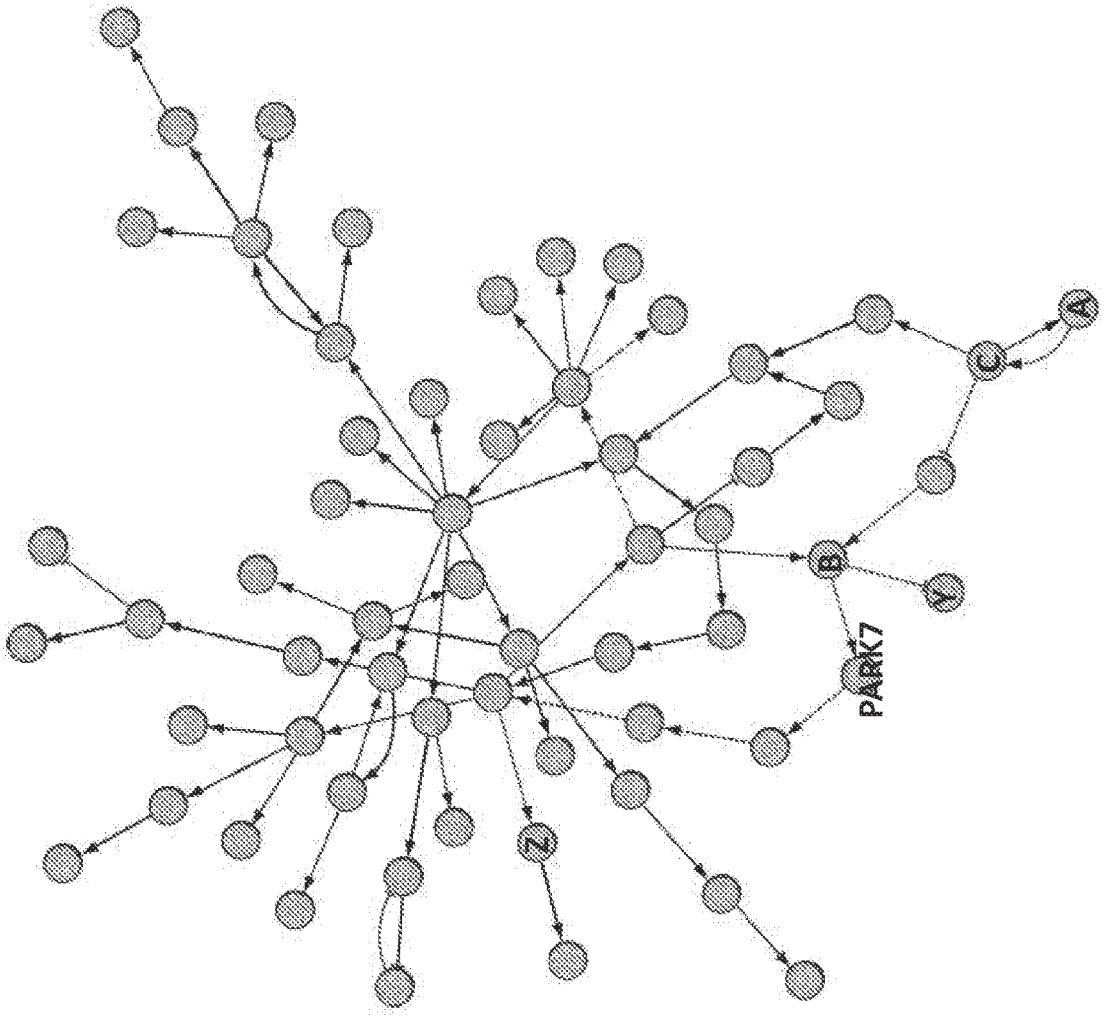


Figure 29

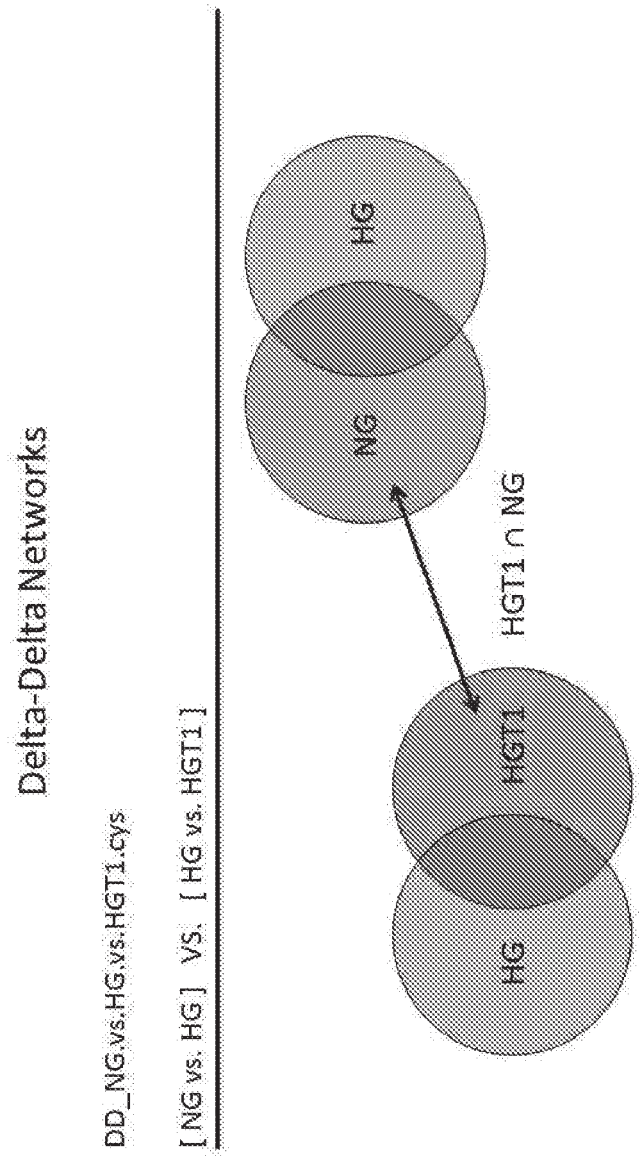

Delta-Delta Networks
DD_NG.vs.HG.vs.HGT1.cys
[NG vs. HG] VS. [HG vs. HGT1]
HG
NG
HGT1 ∩ NG
HGT1
HG

Figure 30

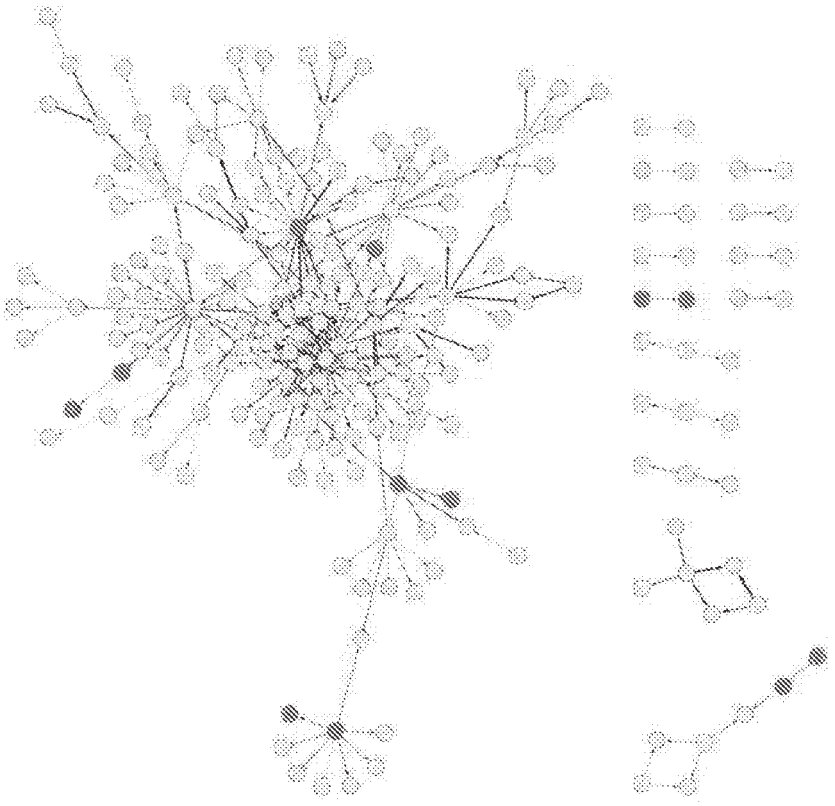


Figure 31

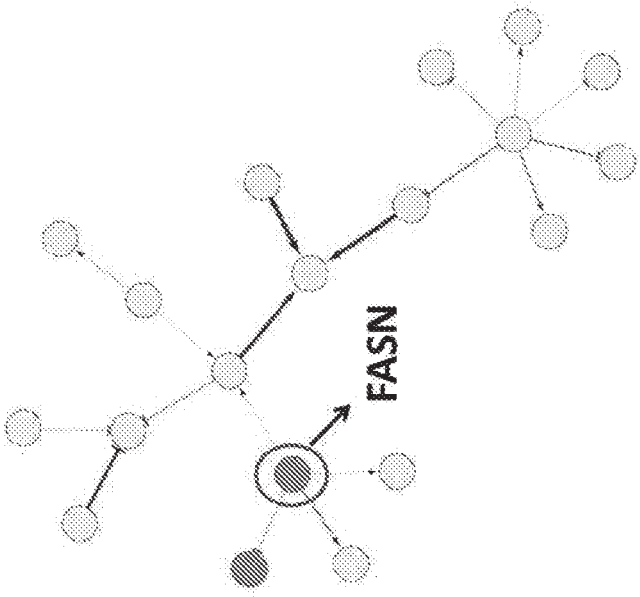


Figure 32

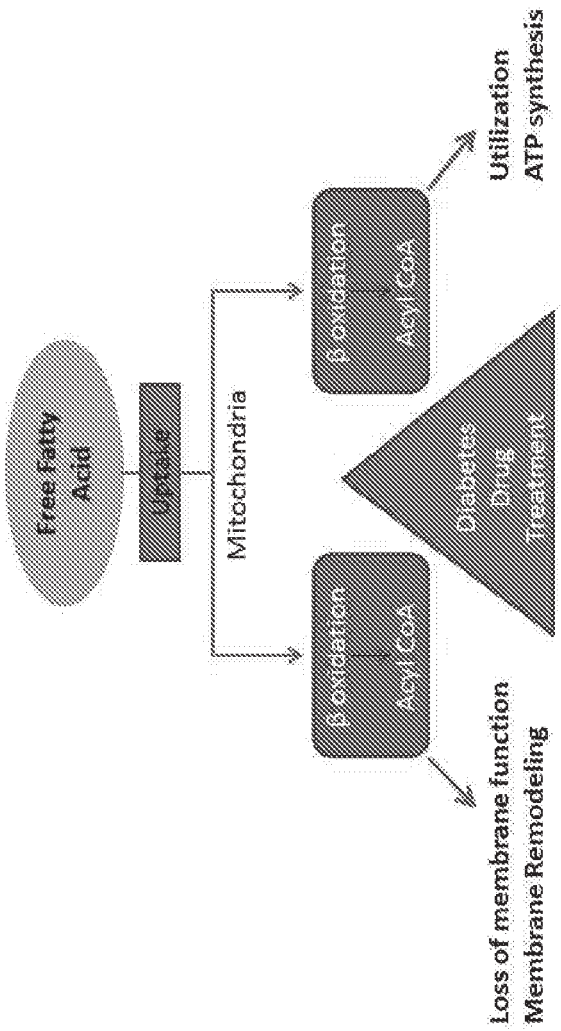

Free Fatty Acid
Uptake
Mitochondria
β oxidation
Acyl CoA
β oxidation
Acyl CoA
Diabetes Drug Treatment
Utilization
ATP synthesis
Loss of membrane function
Membrane Remodeling

Figure 33

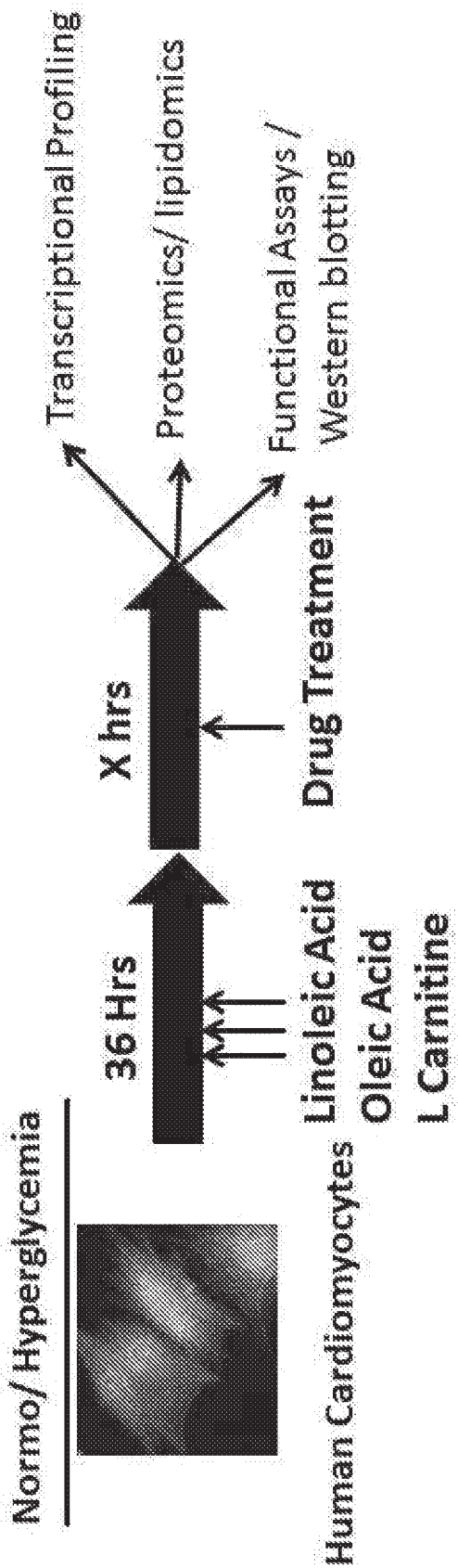

Normo/ Hyperglycemia
Human Cardiomyocytes
36 Hrs
Linoleic Acid
Oleic Acid
L Carnitine
X hrs
Drug Treatment
Transcriptional Profiling
Proteomics/ lipidomics
Functional Assays / Western blotting

Figure 34

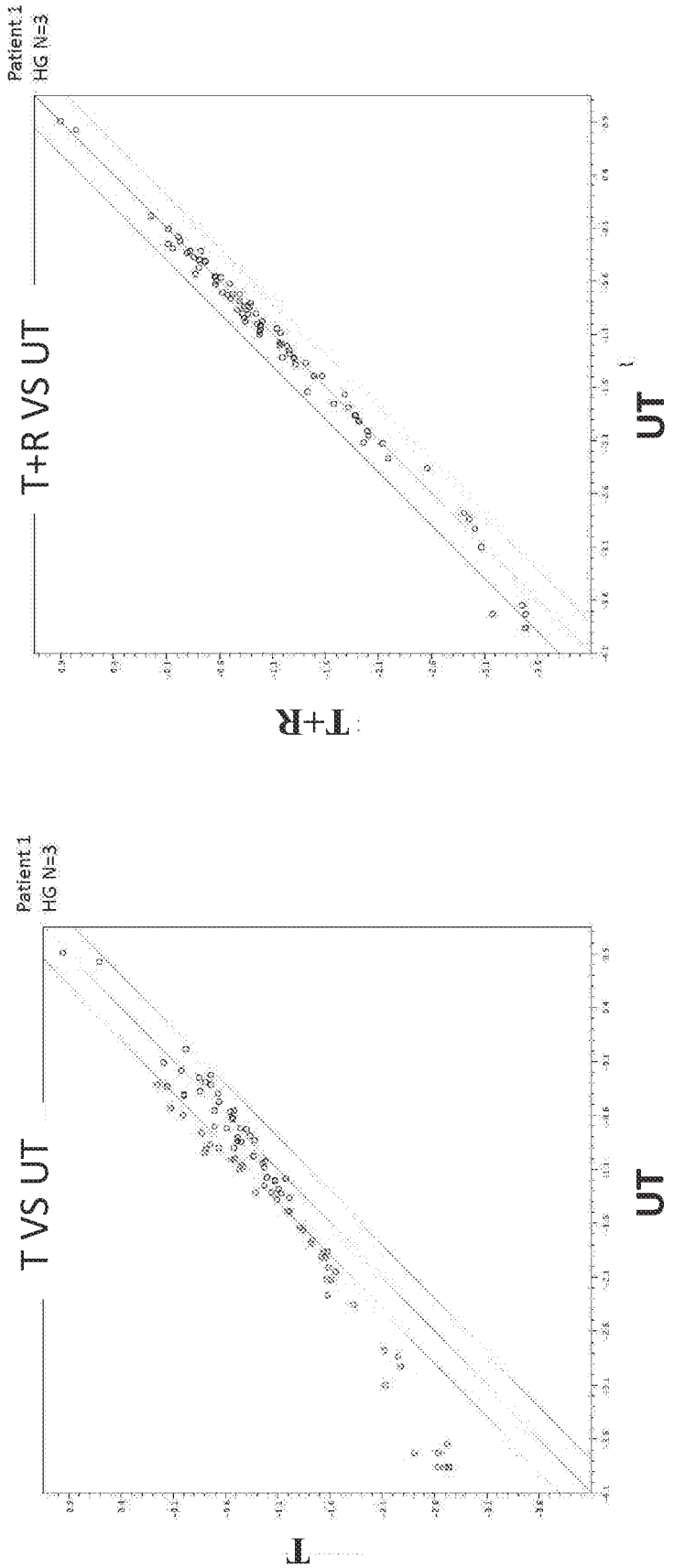


Figure 35

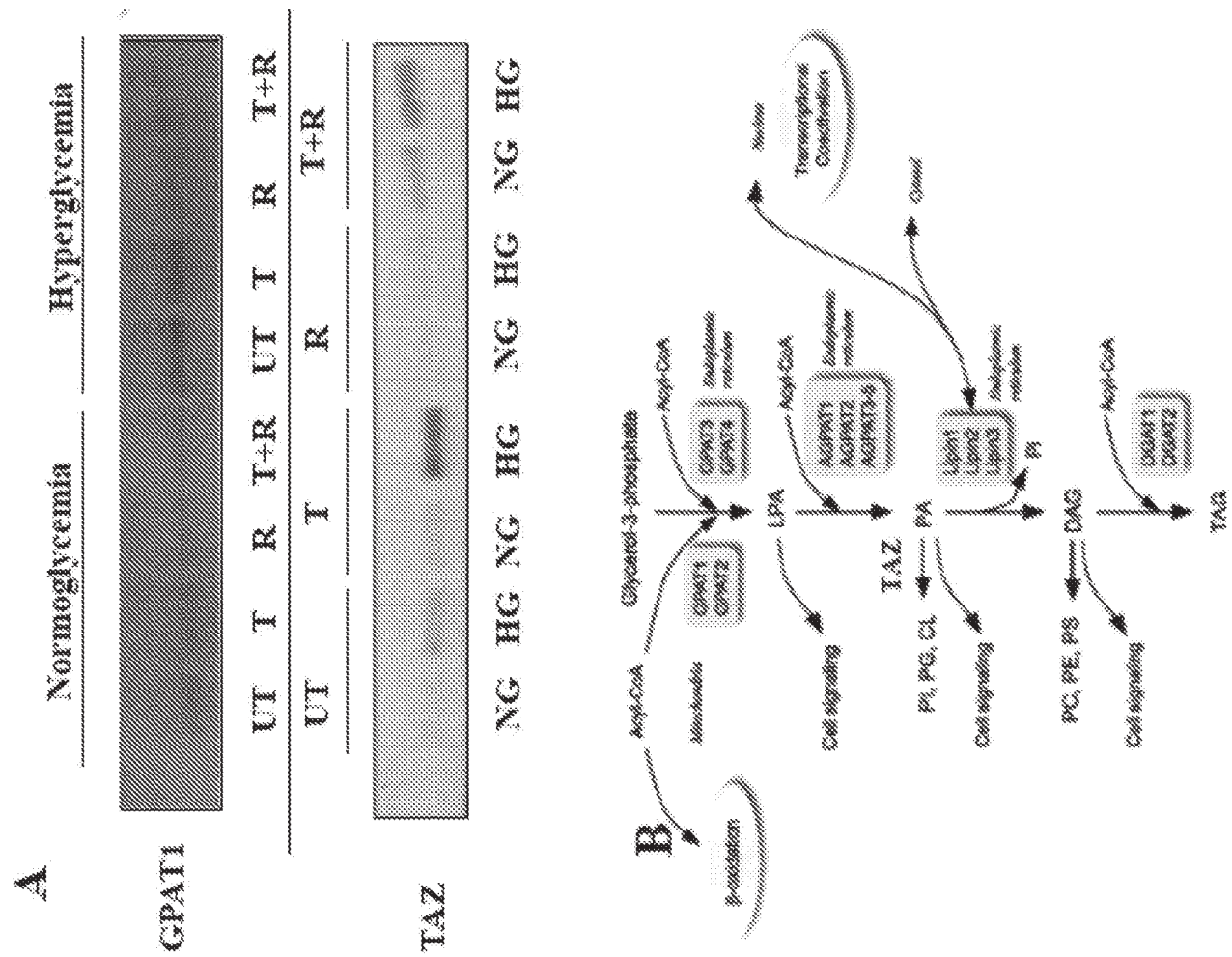
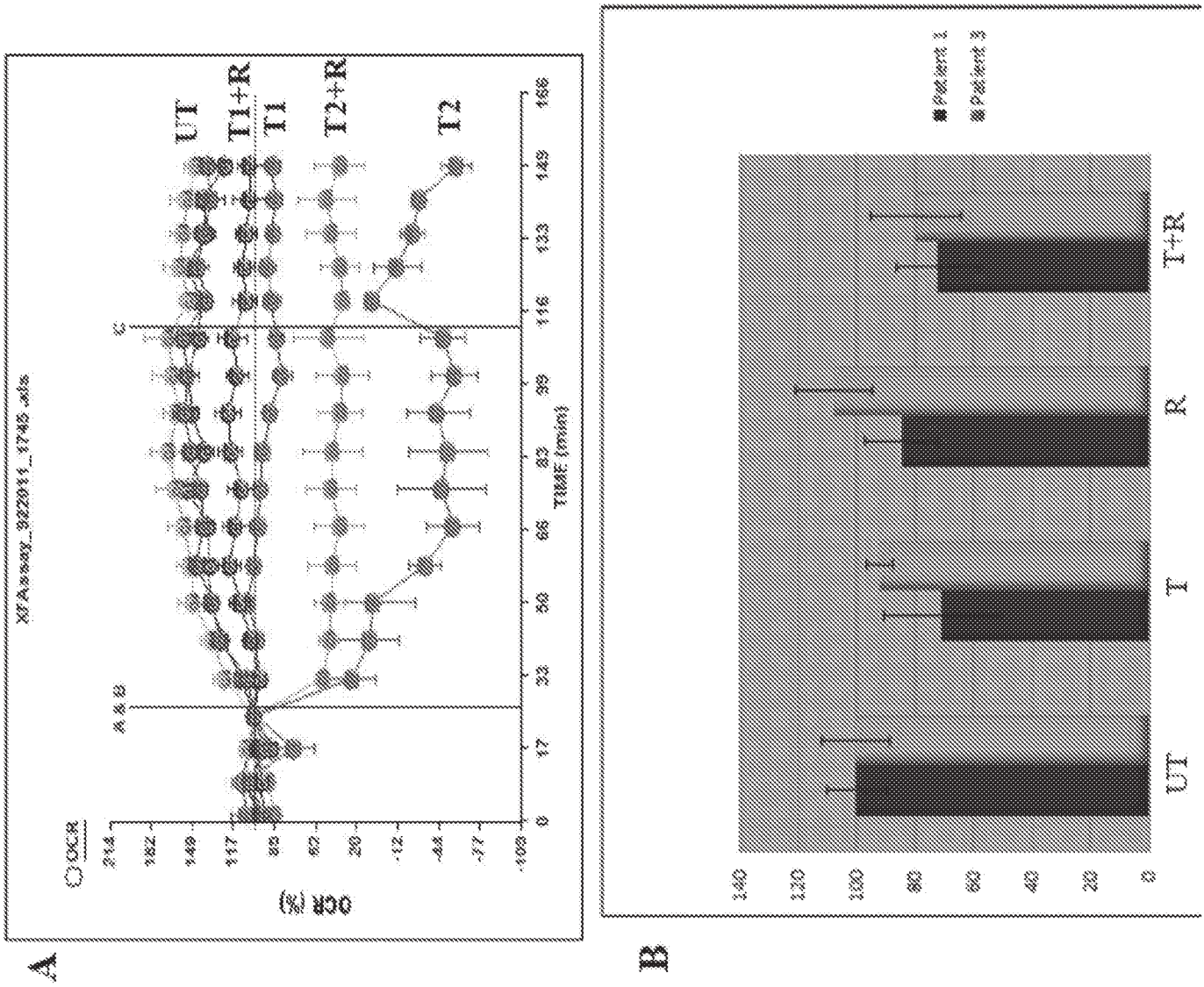

A
Normoglycemia
Hyperglycemia
GPAT1
UT T R T+R UT T R T+R
UT T T+R
TAZ
NG HG NG HG NG HG NG HG
B
Acyl-CoA
Glycerol-3-phosphate
Ambient
Activation
GPAT3
GPAT4
GPAT1
GPAT2
LPA
Cell signaling
Acyl-CoA
AGPAT1
AGPAT2
AGPAT3/4
TAZ
PA
PA, PG, CL
Cell signaling
Lipin1
Lipin2
Lipin3
PI
DAG
PC, PE, PS
Cell signaling
Acyl-CoA
DGAT1
DGAT2
TAG
Nuclear
Transcriptional Coactivation
Cytosol

Figure 36

Figure 37


A
XFAssay_3222011_1745.xls
OCR
OCR (%)
A
B
C
UT
T1+R
T1
T2+R
T2
TIME (min)
B
Patient 1
Patient 3
UT
T
R
T+R

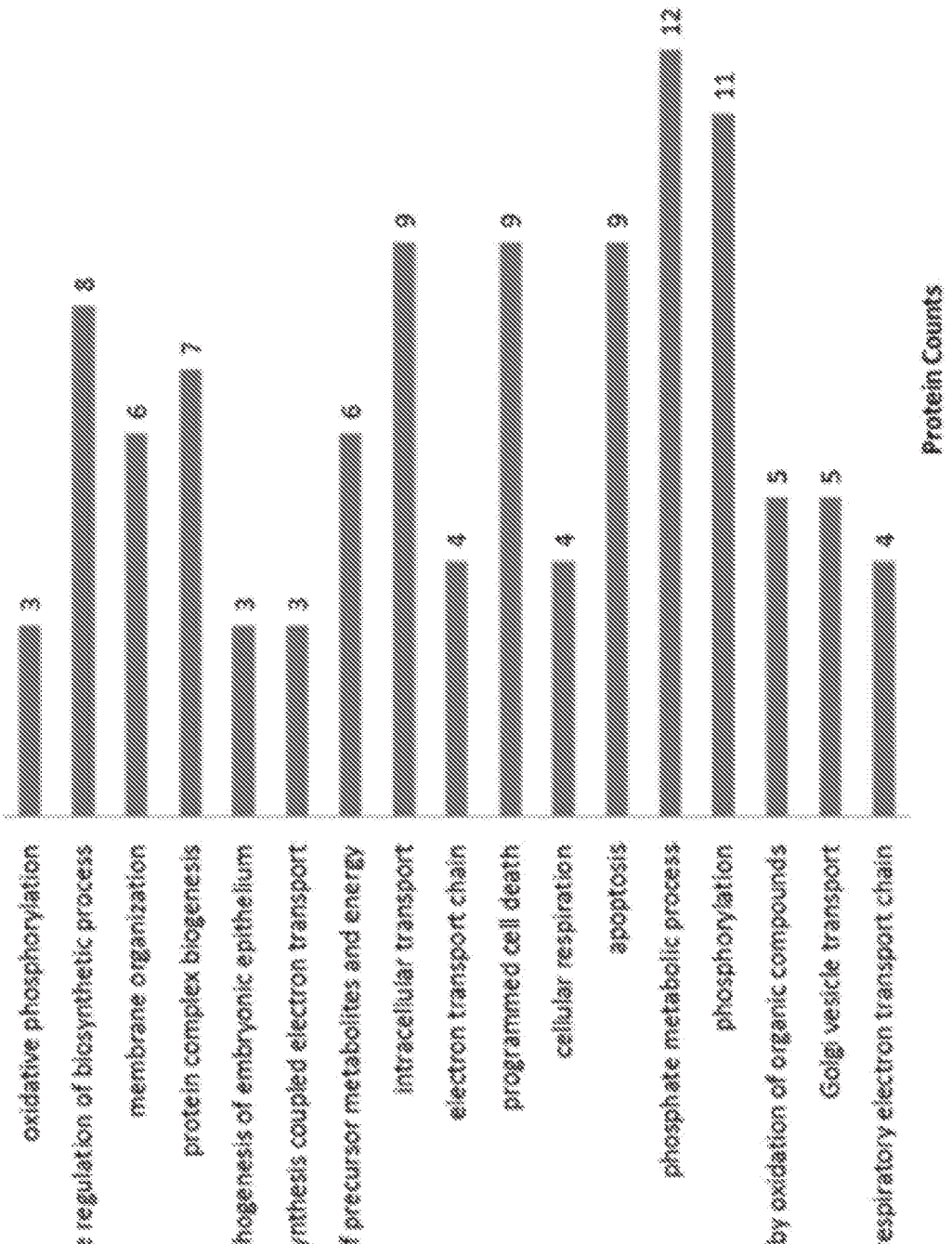

oxidative phosphorylation
positive regulation of biosynthetic process
membrane organization
protein complex biogenesis
morphogenesis of embryonic epithelium
mitochondrial ATP synthesis coupled electron transport
generation of precursor metabolites and energy
intracellular transport
electron transport chain
programmed cell death
cellular respiration
apoptosis
phosphate metabolic process
phosphorylation
energy derivation by oxidation of organic compounds
Golgi vesicle transport
respiratory electron transport chain
3
8
6
7
3
3
6
9
4
9
4
9
12
11
5
5
4
Protein Counts

Figure 38

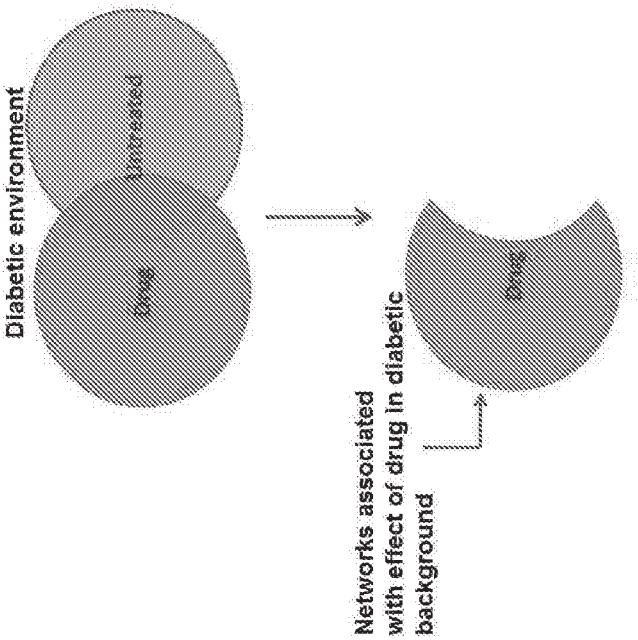

Diabetic environment
Untreated
Drug
Networks associated
with effect of drug in diabetic
background
Drug

Figure 39

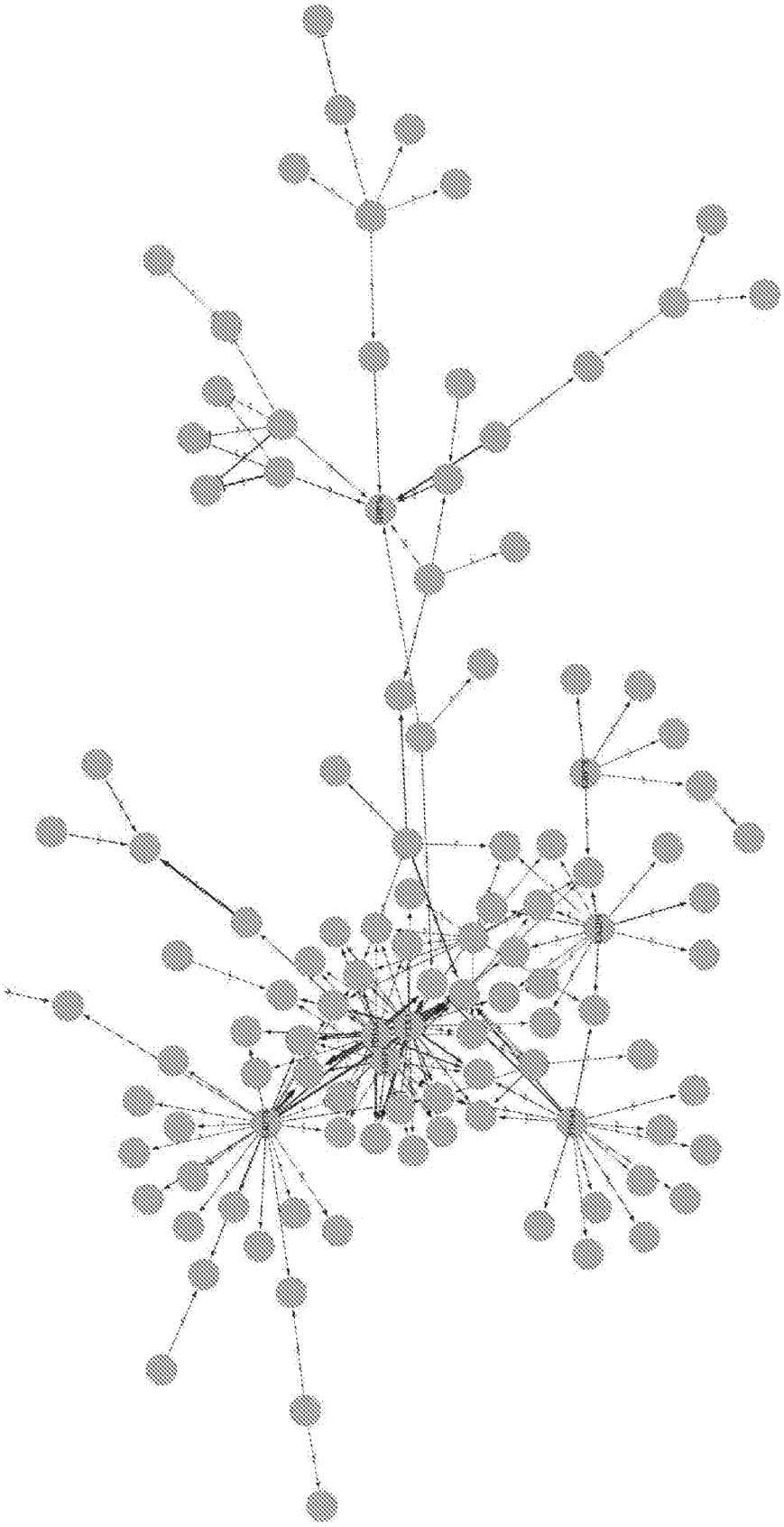


Figure 40