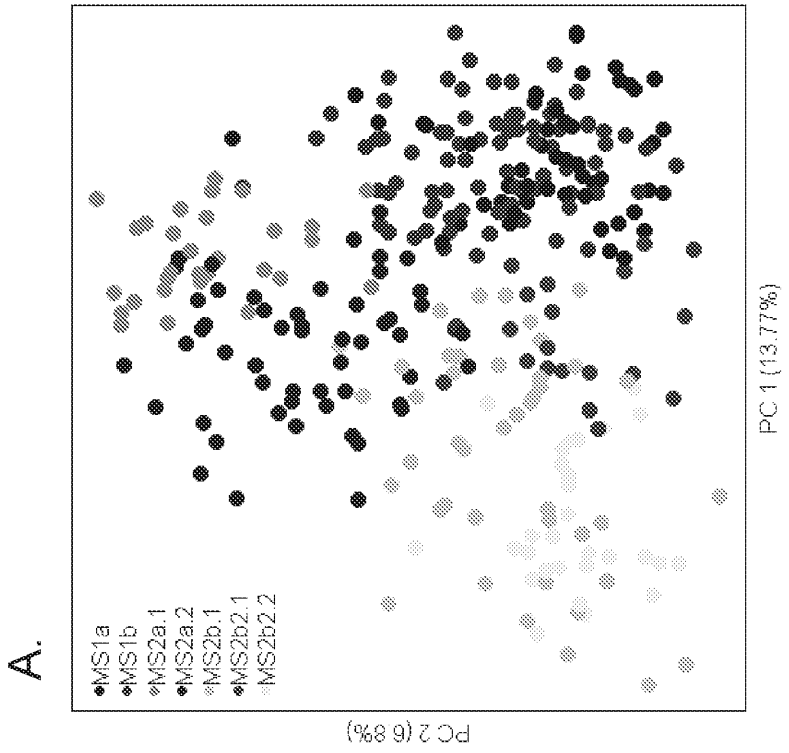
Figure 1

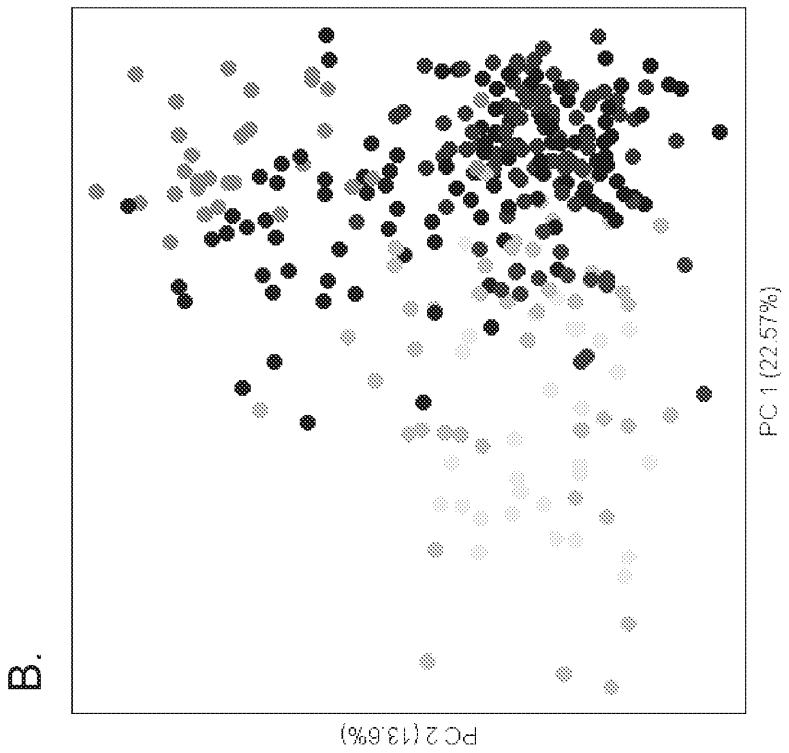
Total Samples = 204	Number	Percentage
AGE at diagnosis		
Median	65	
Range	35 - 91	
Standard Deviation	11.51	
GENDER		
Male	164	79%
Female	43	21%
TISSUE SPECIMENS		
Primary Bladder Cancer (T0-T4)	154	75.5%
Non-Muscle-Invasive (T0, T1, Tis)	93	45.5%
Muscle-Invasive (T2, T3)	61	30.0%
Metastatic Bladder Cancer (T4)	53	26.0%
Lymph Nodes	45	22.0%
Distal Sites	8	4.0%

	Number	Censored (%)	Median (Years)
DISEASE FREE SURVIVAL			
All Patients	142	50	4.6
Primary Bladder Cancer	102	66	NR*
Metastatic Bladder Cancer	40	10	0.9
OVERALL SURVIVAL			
All Patients	142	78	NR*
Primary Bladder Cancer	102	100	NR*
Metastatic Bladder Cancer	40	23	1.3

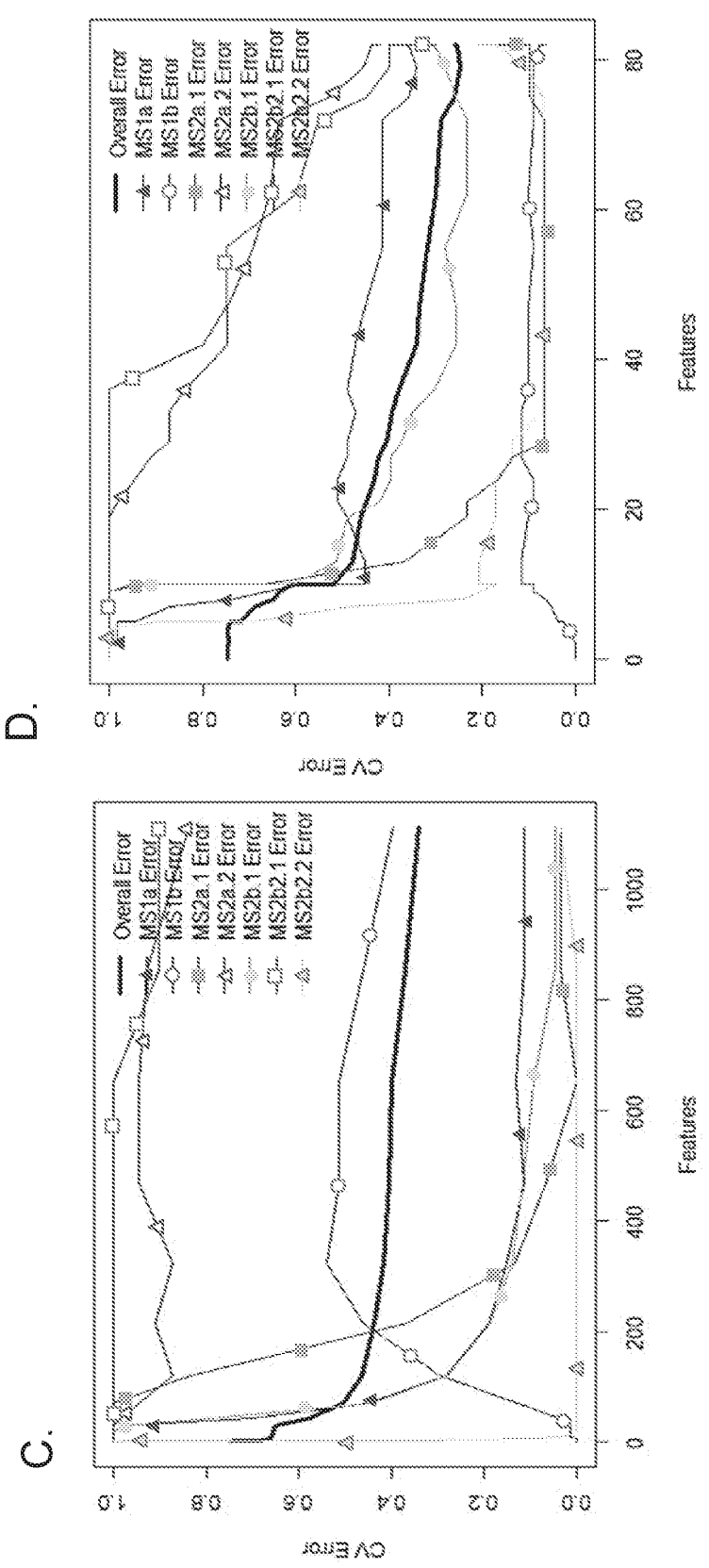
*NR: Not reached

Figures 2A-2B

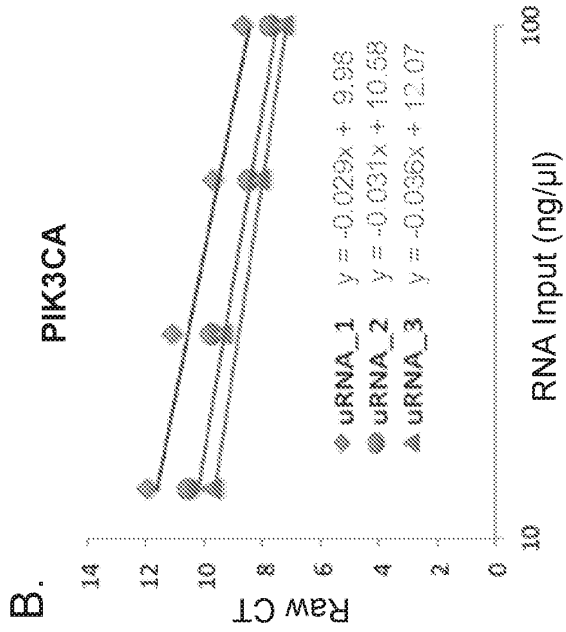


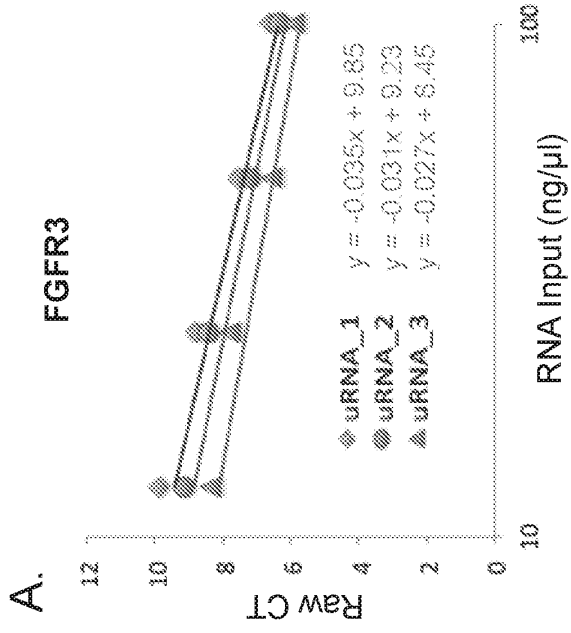


Figures 2C-2D

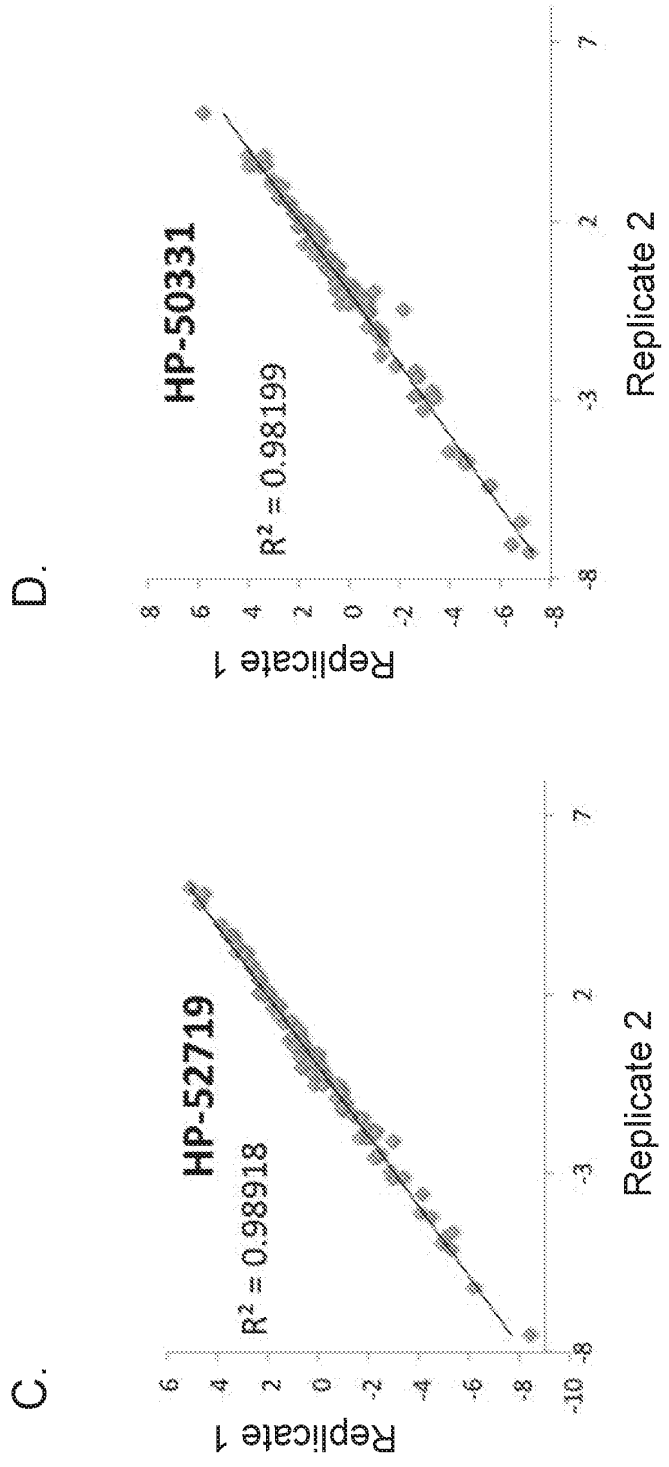


Figures 3A-3B





Figures 3C-3D



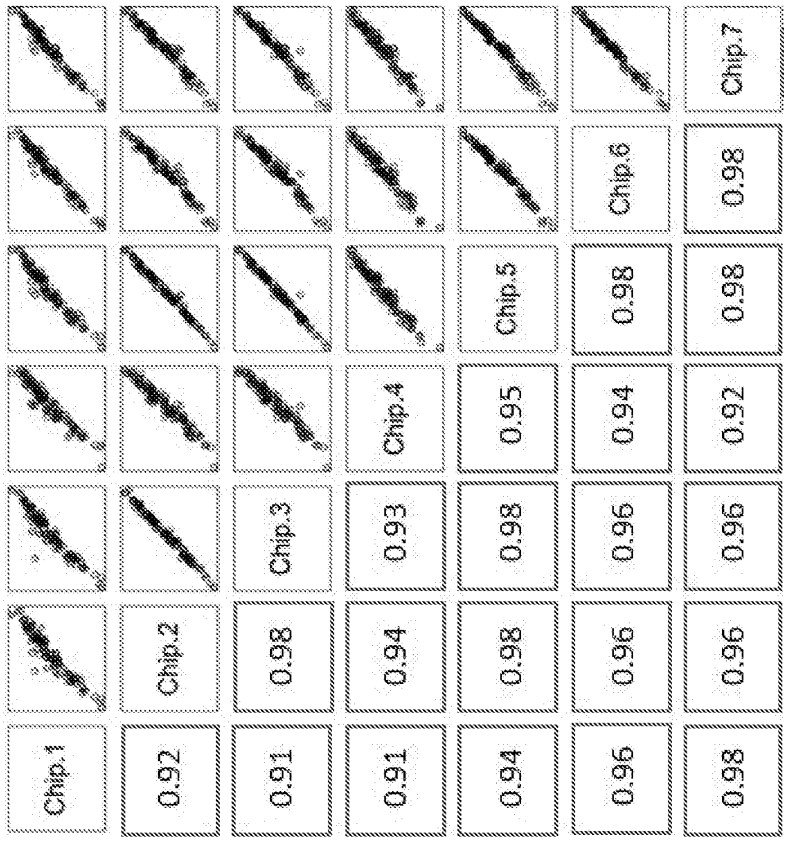
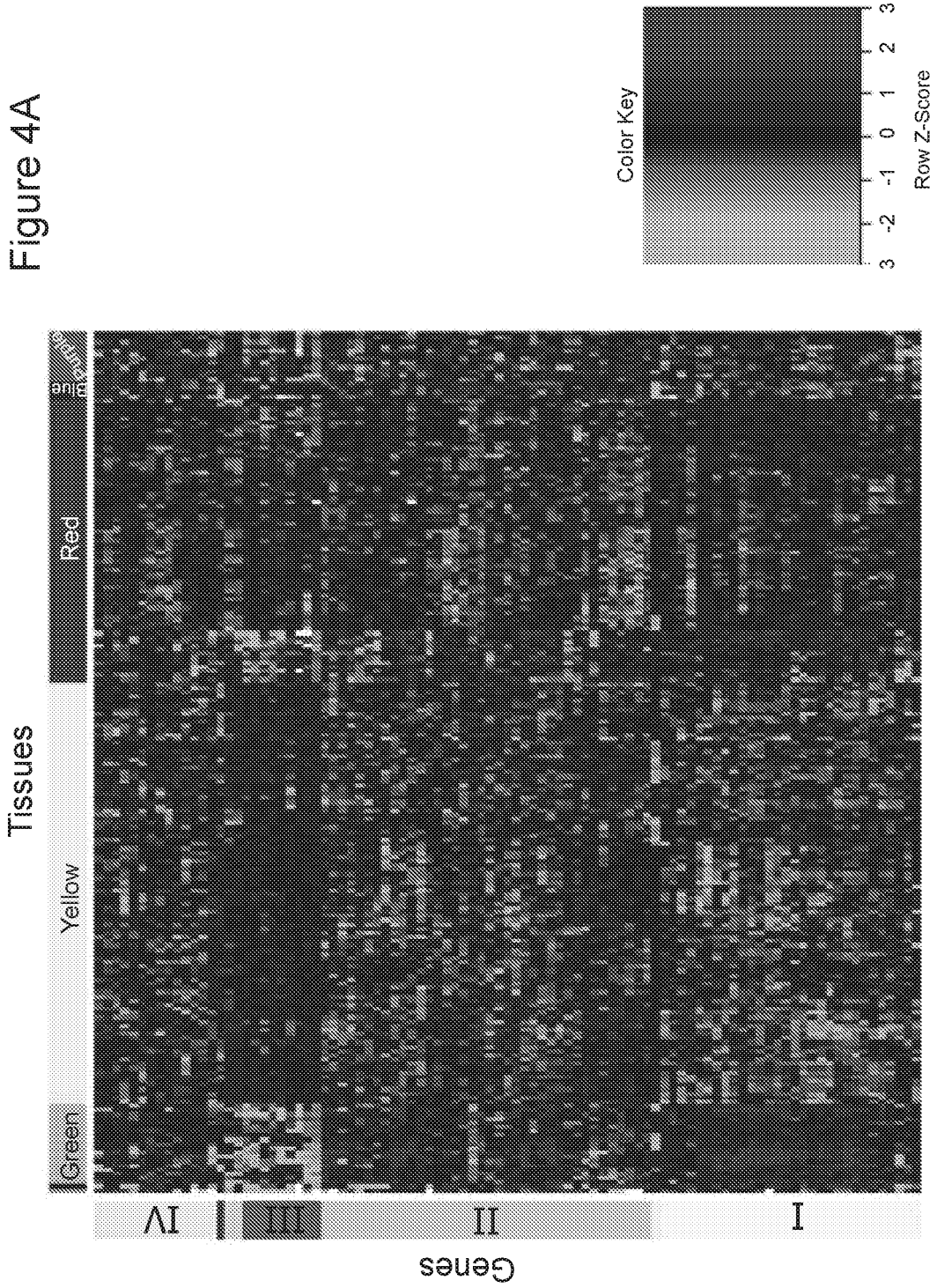

Chip.1
Chip.2
Chip.3
Chip.4
Chip.5
Chip.6
Chip.7
0.92
0.91
0.91
0.94
0.96
0.98
0.98
0.94
0.98
0.93
0.98
0.94
0.96
0.96
0.96
0.98
0.95
0.96
0.92
0.98
0.98
0.98

Figure 3E

Figure 4A


Color Key
3 -2 -1 0 1 2 3
Row Z-Score
Tissues
Green
Yellow
Red
Genes
IV
III
II
I

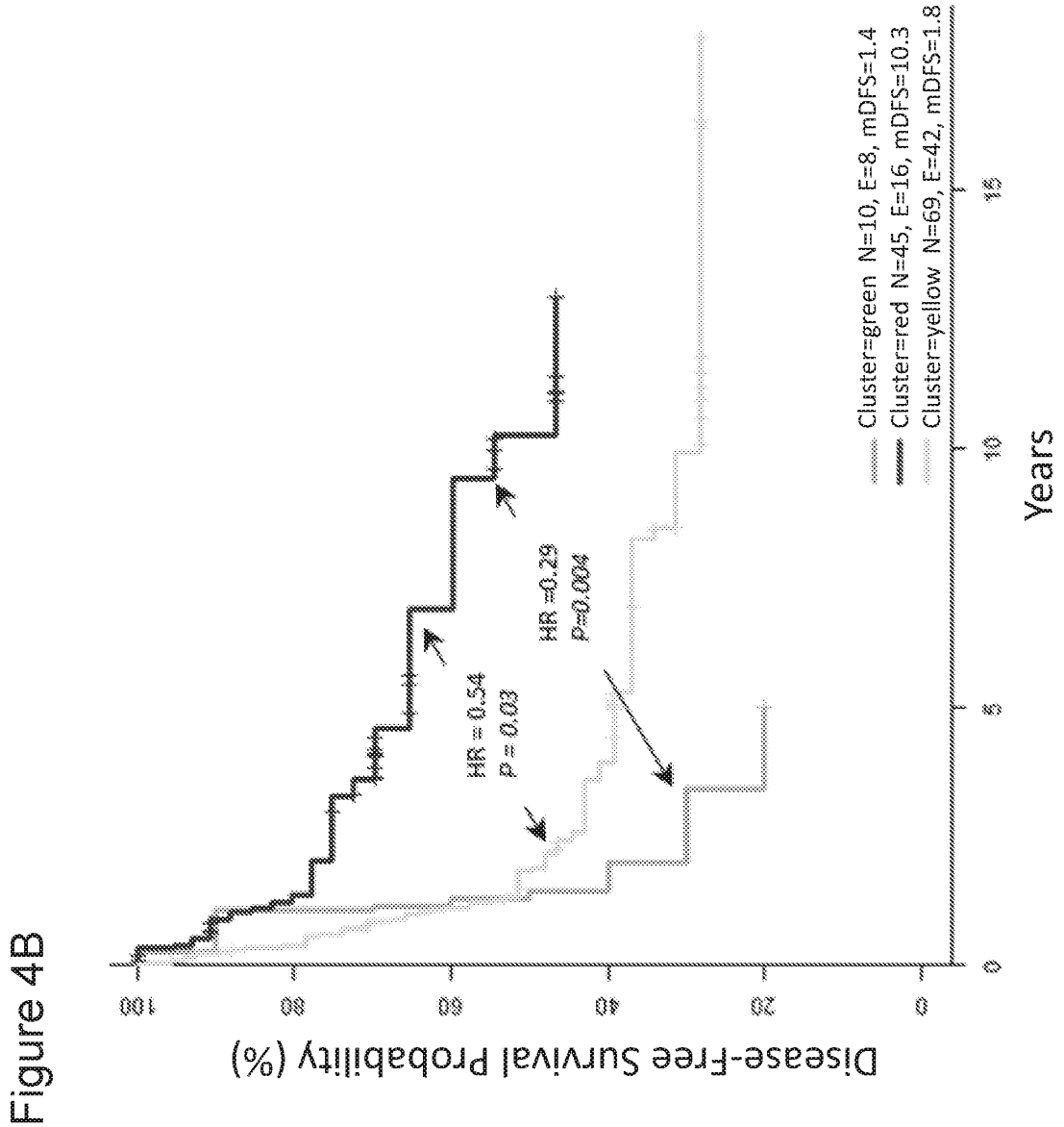
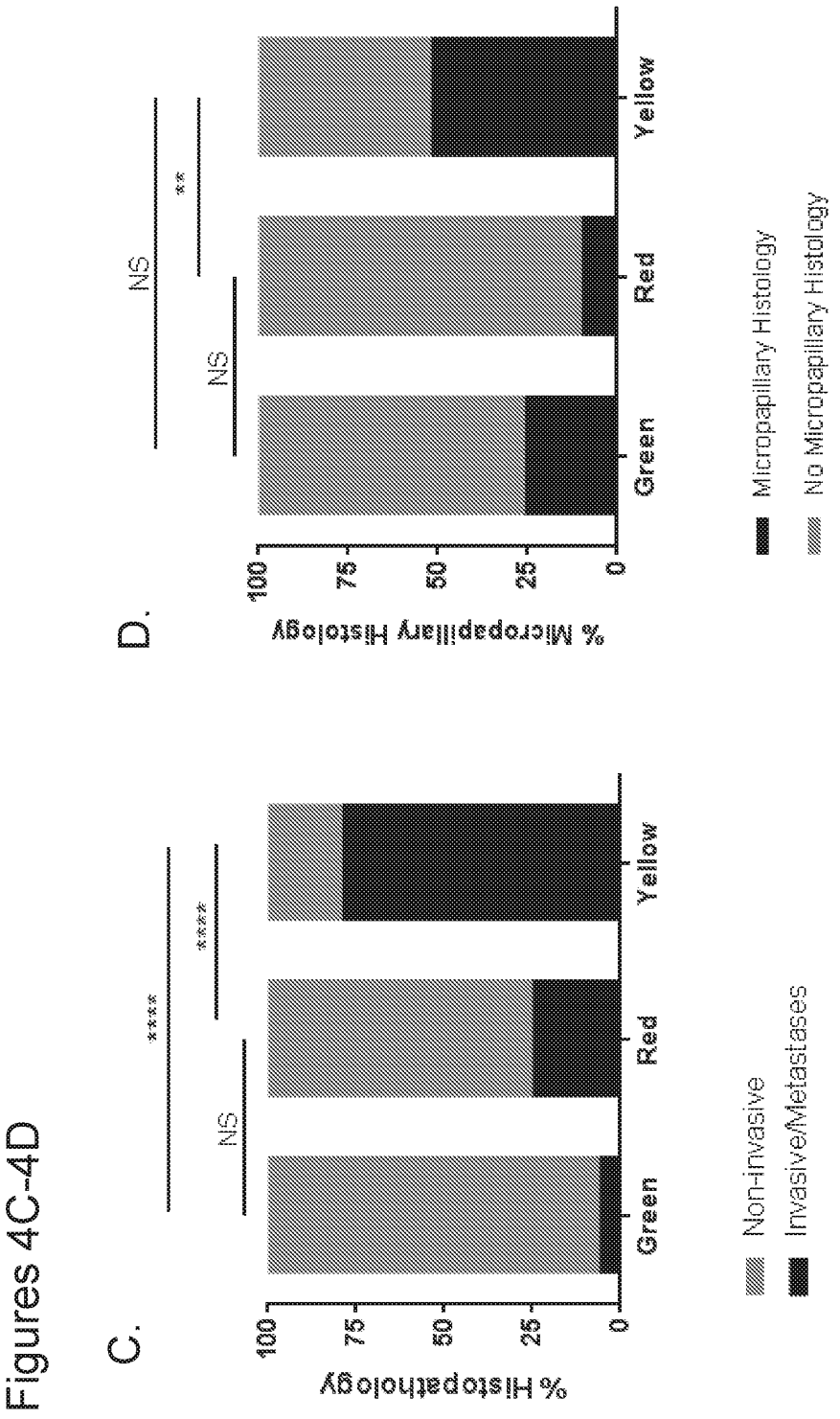
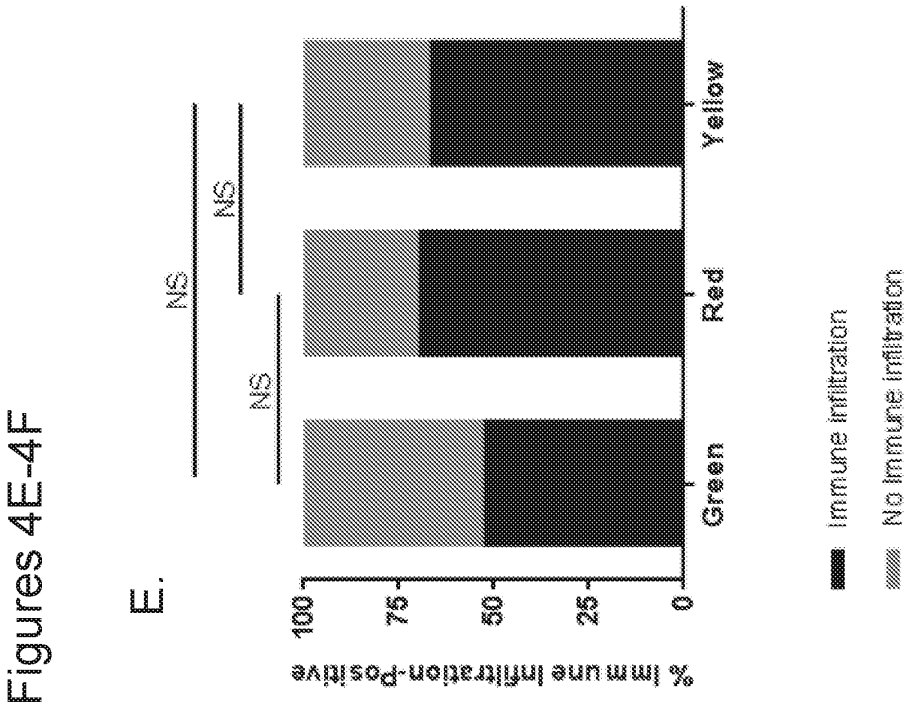

Disease-free Survival Probability (%)
100
80
60
40
20
0
0
5
10
15
Years
Cluster=green N=10, E=8, mDFS=1.4
Cluster=red N=45, E=16, mDFS=10.3
Cluster=yellow N=69, E=42, mDFS=1.8
HR = 0.54
P = 0.03
HR =0.29
P=0.004

Figure 4B

Figures 4C-4D



Figures 4E-4F



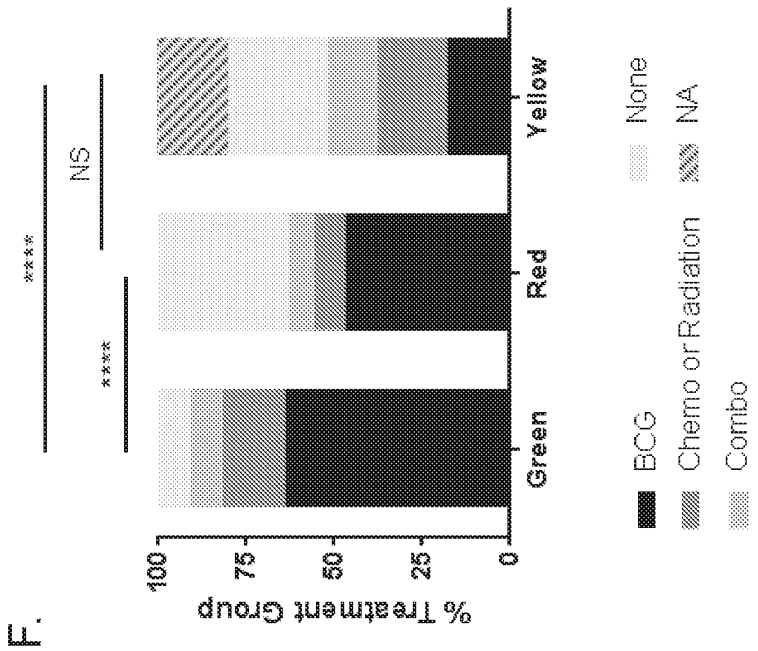


Figure 5A

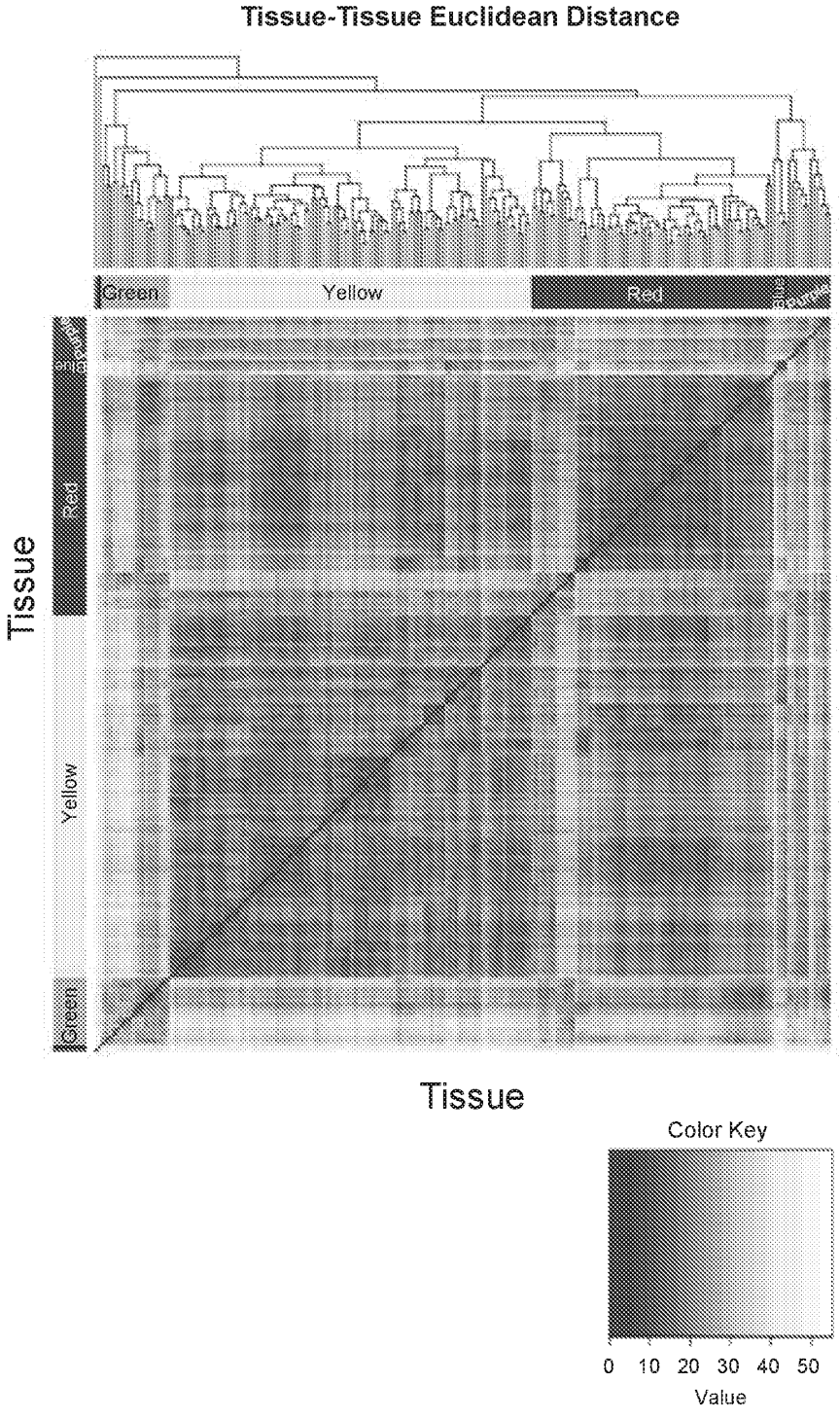
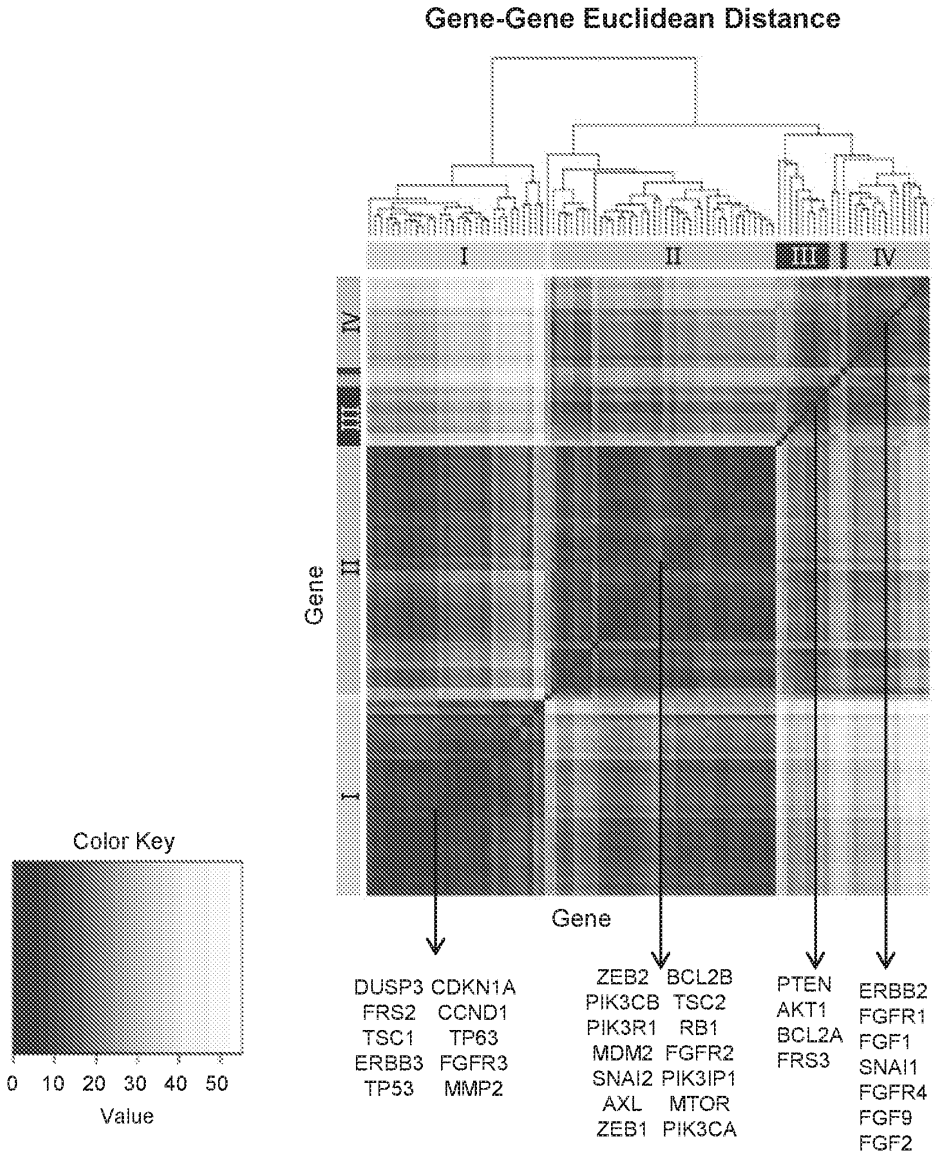

Tissue-Tissue Euclidean Distance
Green
Yellow
Red
Red
Yellow
Green
Tissue
Tissue
Color Key
0 10 20 30 40 50
Value

Figure 5B


Gene-Gene Euclidean Distance
I
II
III
IV
Gene
Gene
Color Key
0 10 20 30 40 50
Value
DUSP3 CDKN1A
FRS2 CCND1
TSC1 TP63
ERBB3 FGFR3
TP53 MMP2
ZEB2 BCL2B
PIK3CB TSC2
PIK3R1 RB1
MDM2 FGFR2
SNAI2 PIK3IP1
AXL MTOR
ZEB1 PIK3CA
PTEN
AKT1
BCL2A
FRS3
ERBB2
FGFR1
FGF1
SNAI1
FGFR4
FGF9
FGF2

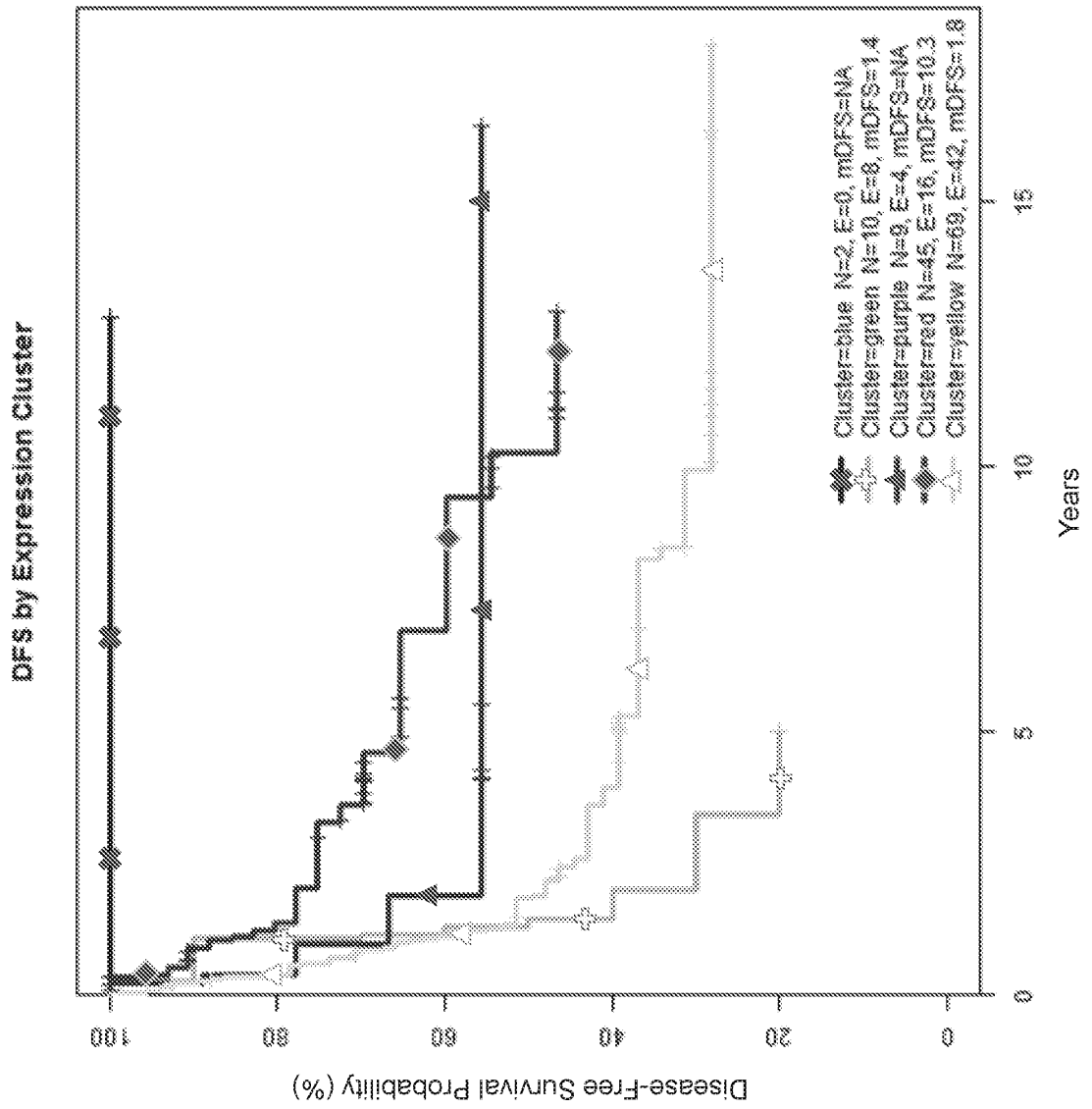
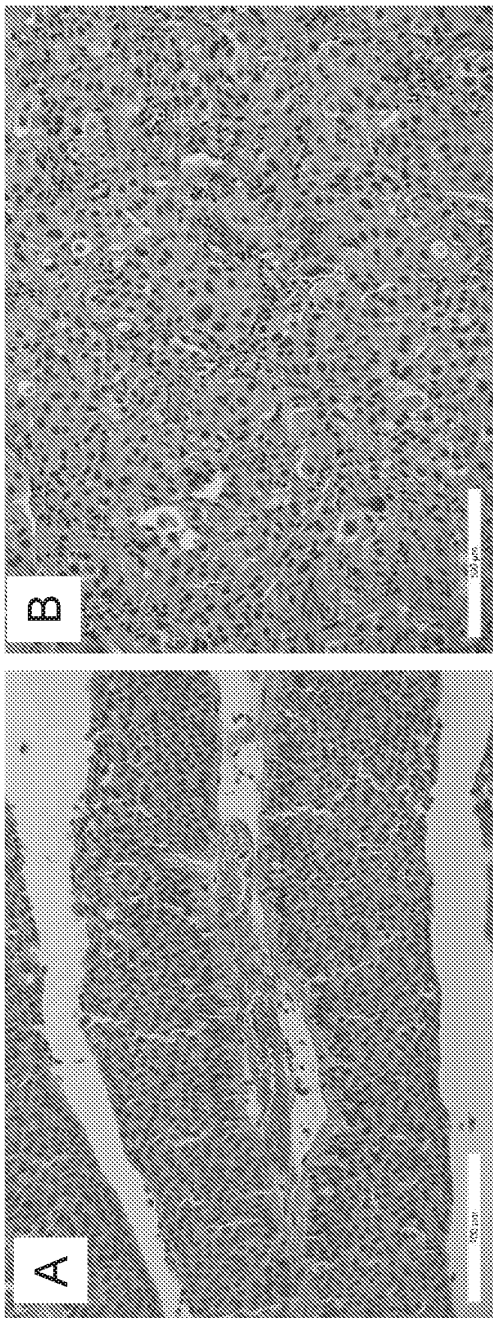
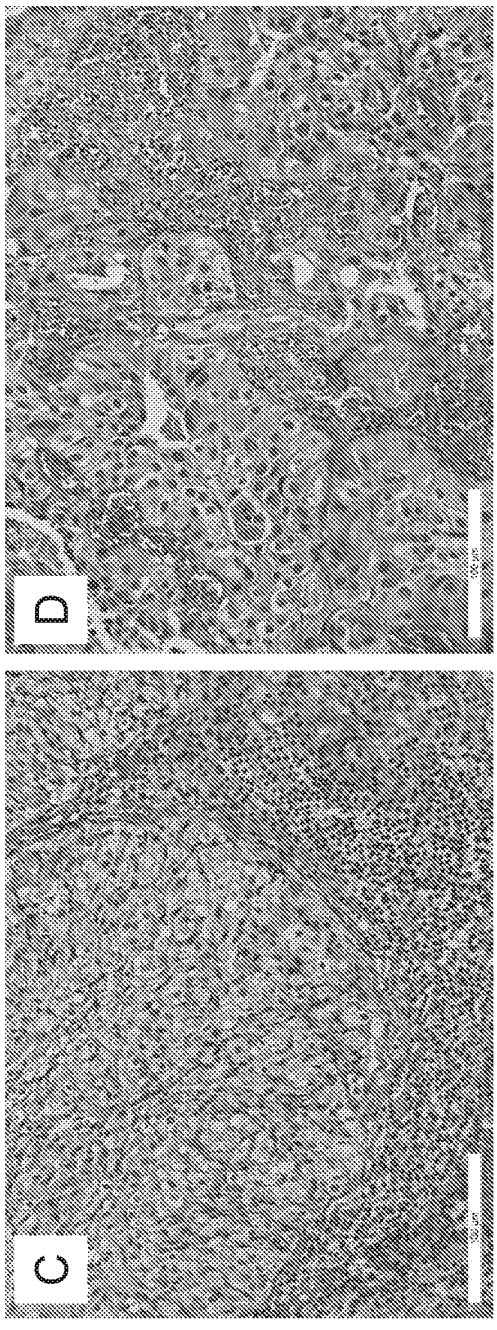

DFS by Expression Cluster
Disease-Free Survival Probability (%)
0
20
40
60
80
100
0
5
10
15
Years
Cluster=blue N=2, E=0, mDFS=NA
Cluster=green N=10, E=8, mDFS=1.4
Cluster=purple N=9, E=4, mDFS=NA
Cluster=red N=45, E=16, mDFS=10.3
Cluster=yellow N=69, E=42, mDFS=1.8

Figure 5C

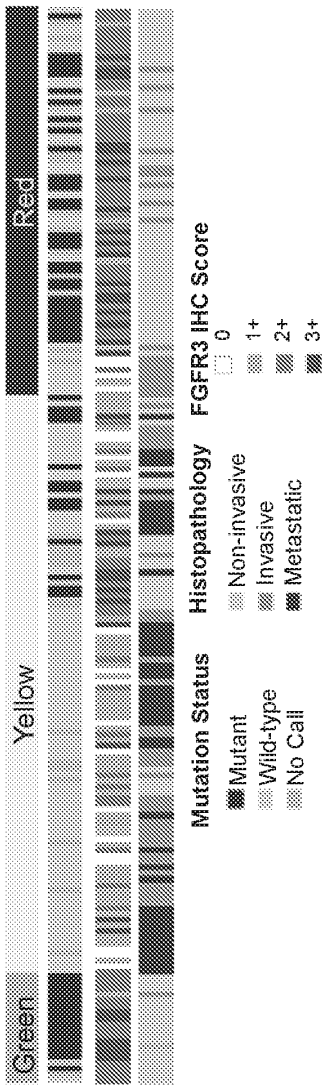


Figures 6A-6B

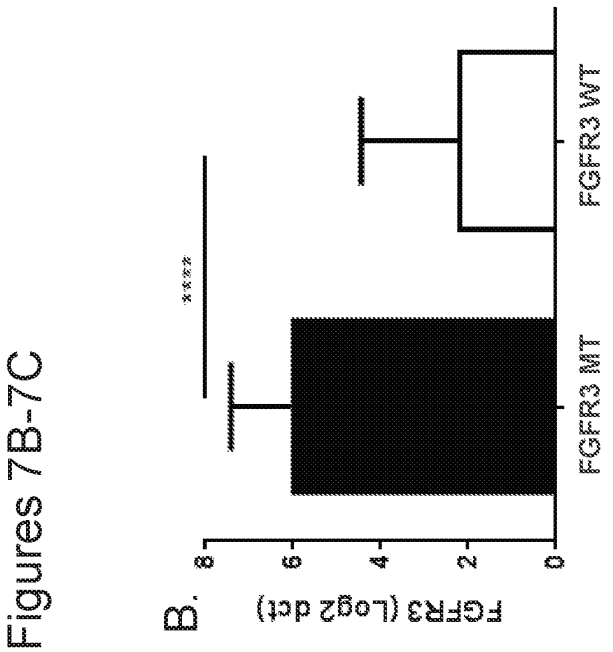


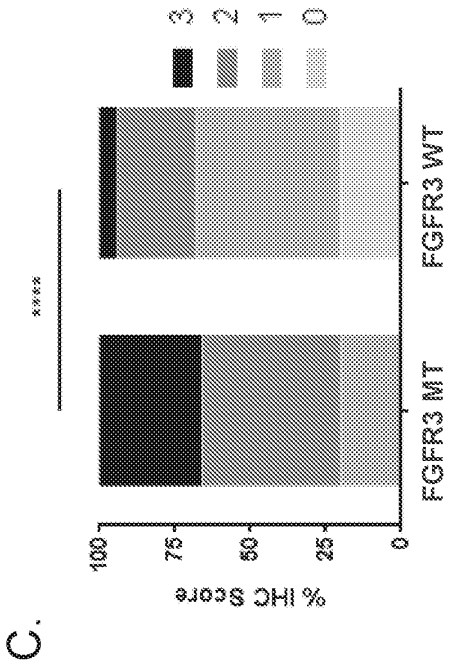
Figures 6C-6D

Figure 7A


Tissue Clusters
Green
Yellow
Red
FGFR3 Mutation
FGFR3 IHC
Histopathology
Mutation Status
Mutant
Wild-type
No Call
Histopathology
Non-invasive
Invasive
Metastatic
FGFR3 IHC Score
0
1+
2+
3+

Figures 7B-7C





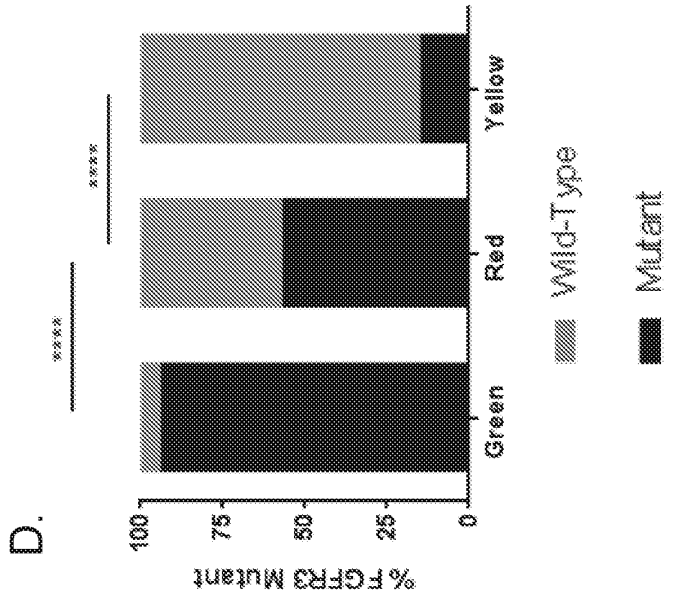
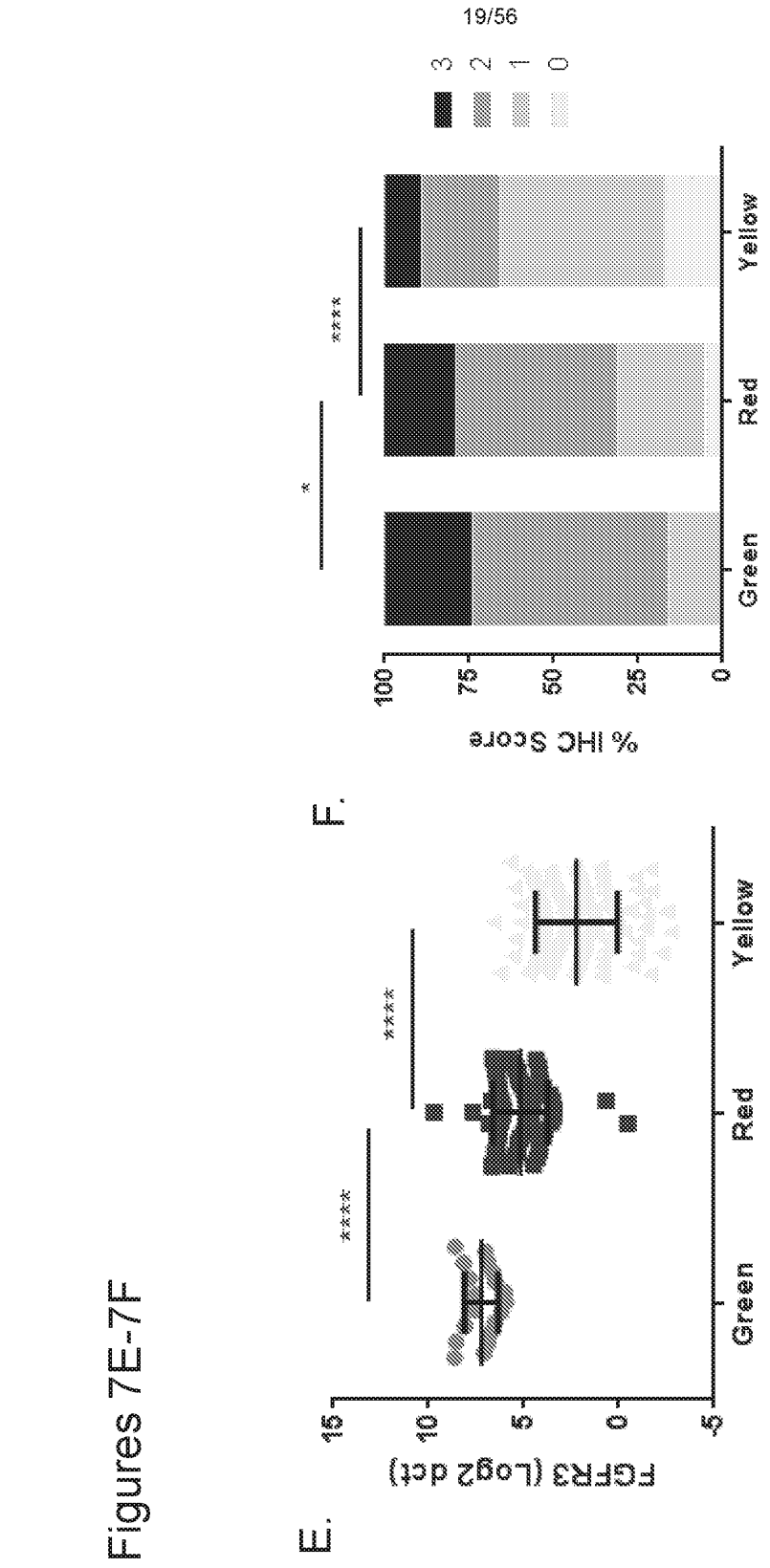
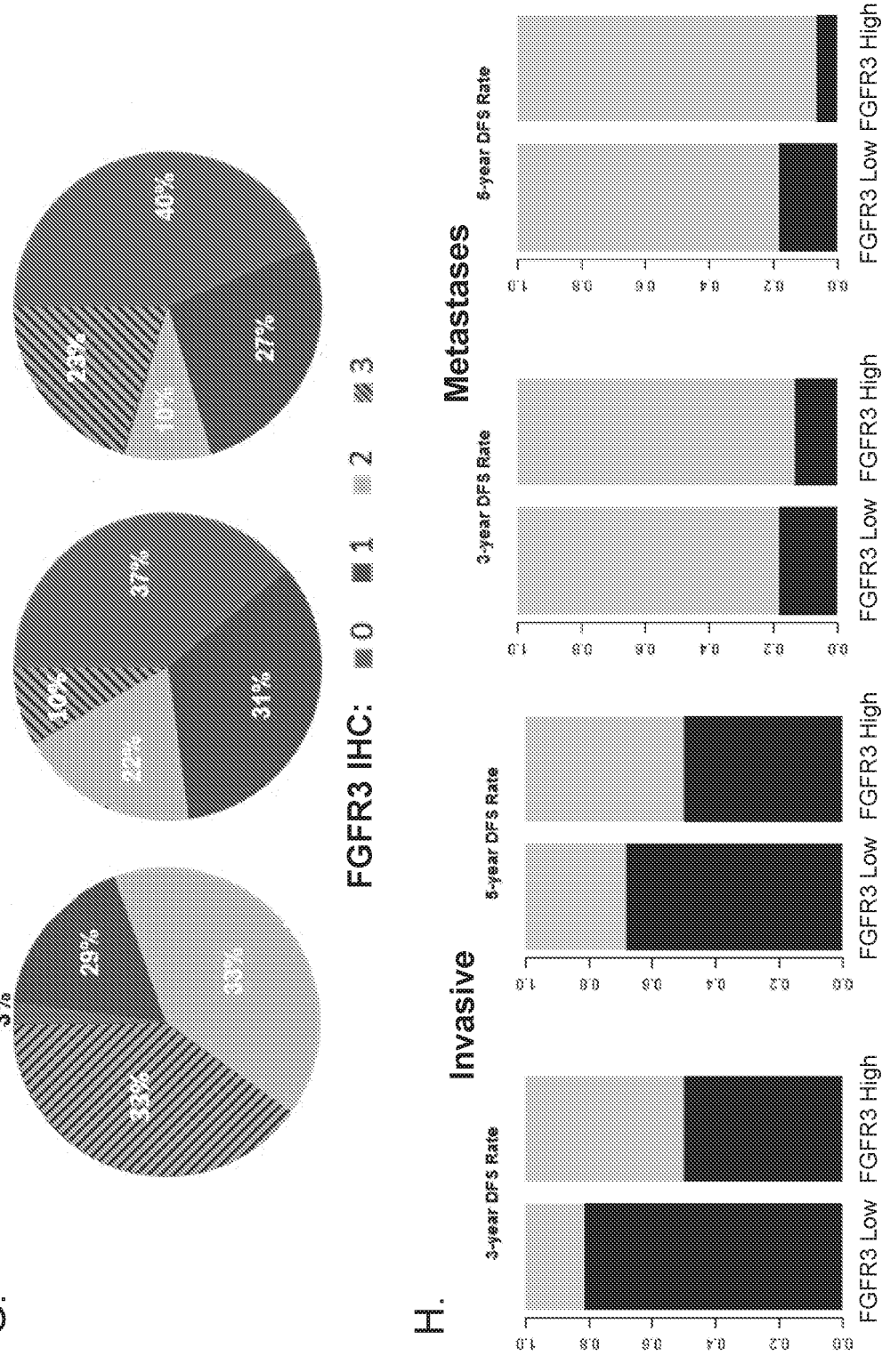

D.
% FGFR3 Mutant
100
75
50
25
0
Green
Red
Yellow
****
****
Wild-Type
Mutant

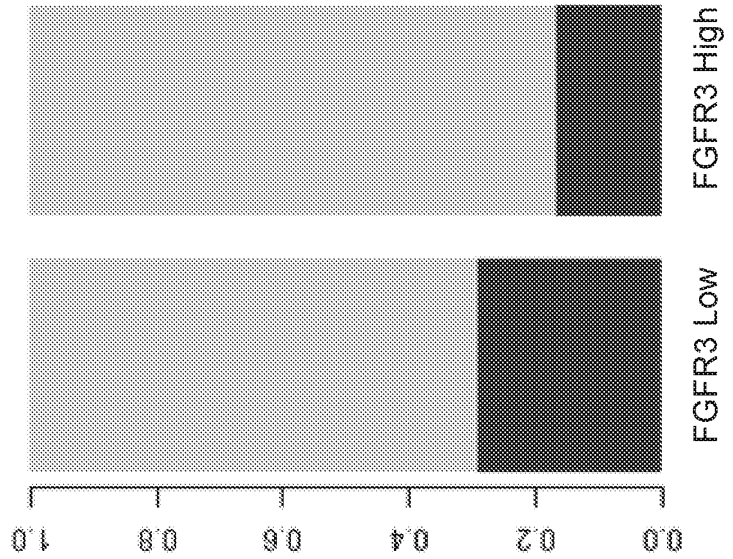
Figure 7D

Figures 7E-7F



Figures 7G-7H




5-year OS Rate
FGFR3 High
FGFR3 Low
0.0
0.2
0.4
0.6
0.8
1.0

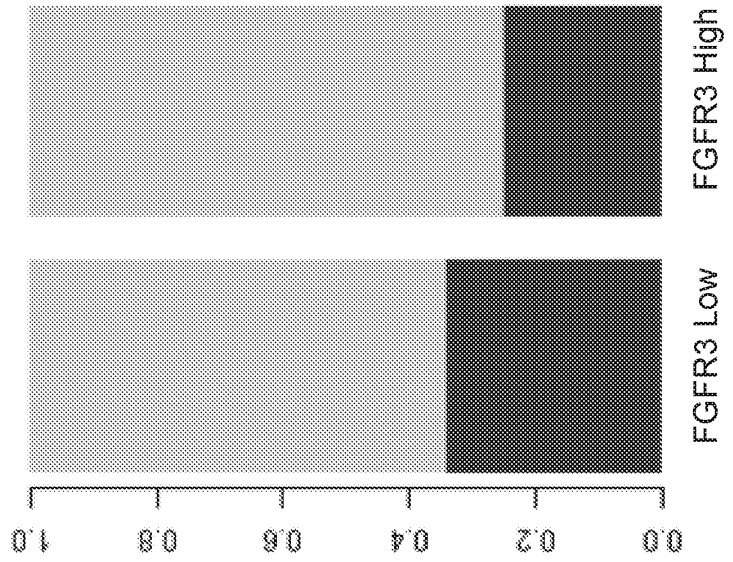

3-year OS Rate
FGFR3 High
FGFR3 Low
0.0
0.2
0.4
0.6
0.8
1.0

Figure 7I

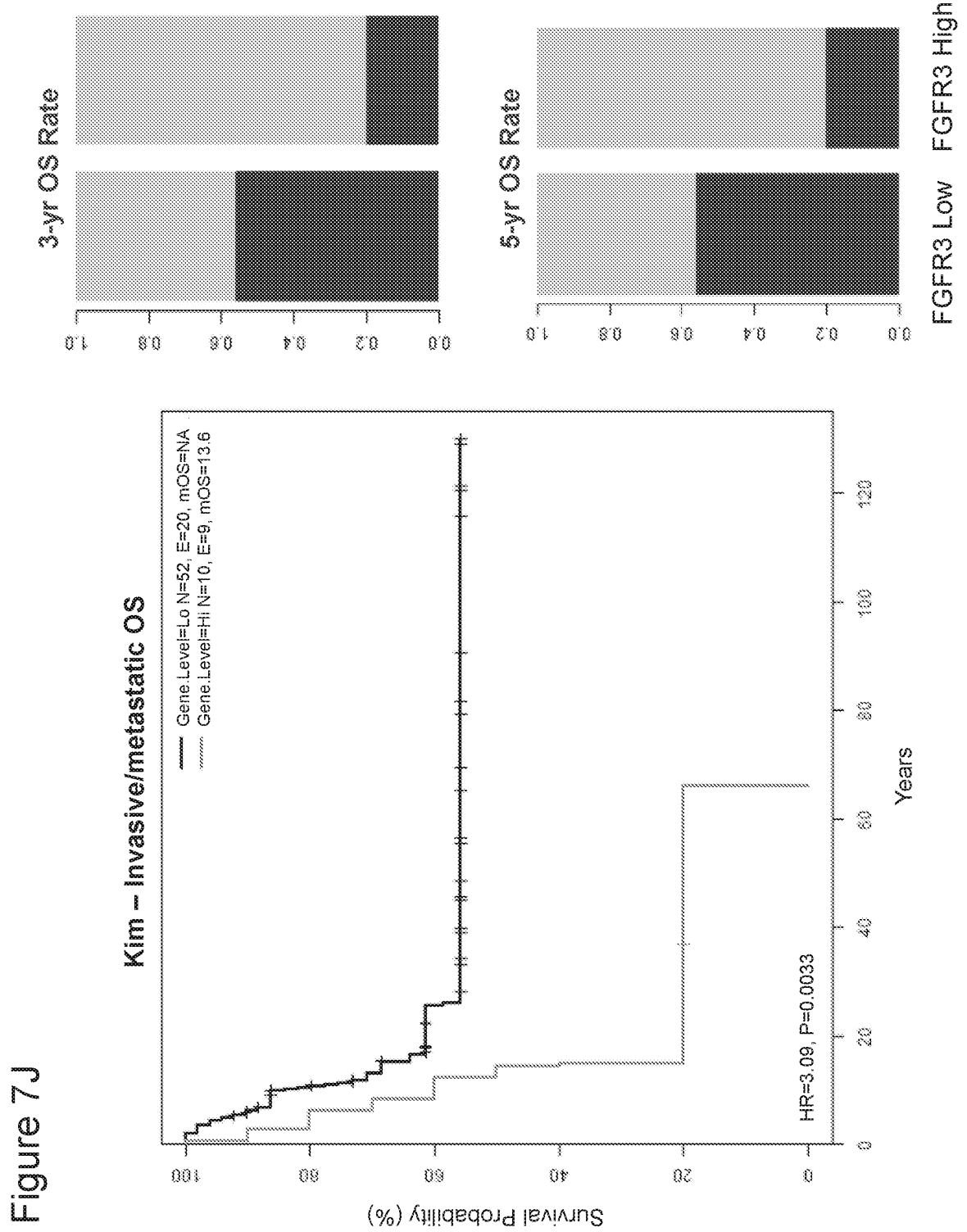

3-yr OS Rate
5-yr OS Rate
FGFR3 Low
FGFR3 High
Kim – Invasive/metastatic OS
Gene.Level=Lo N=52, E=20, mOS=NA
Gene.Level=Hi N=10, E=9, mOS=13.6
HR=3.09, P=0.0033
Survival Probability (%)
Years

Figure 7J

Figure 8

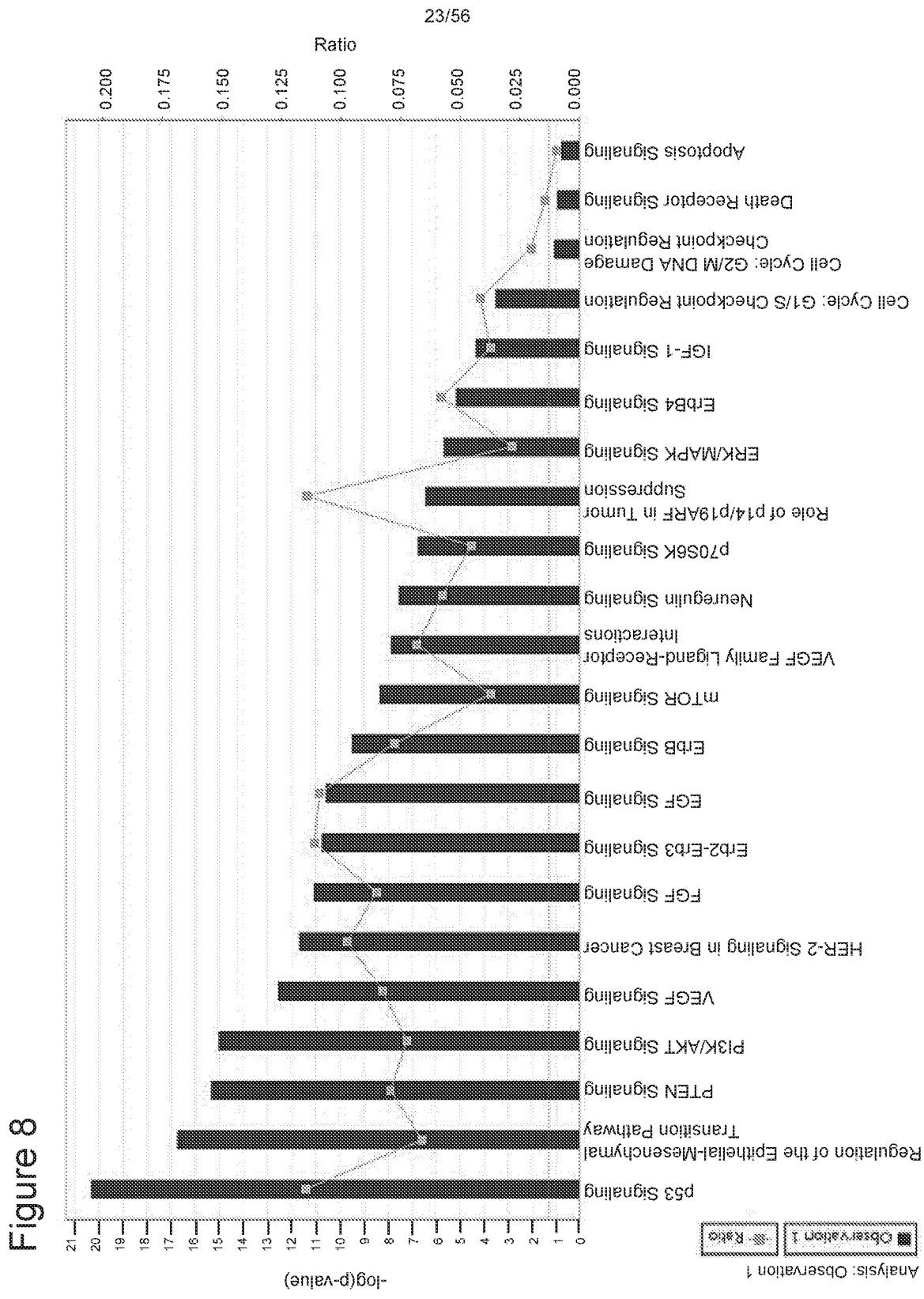
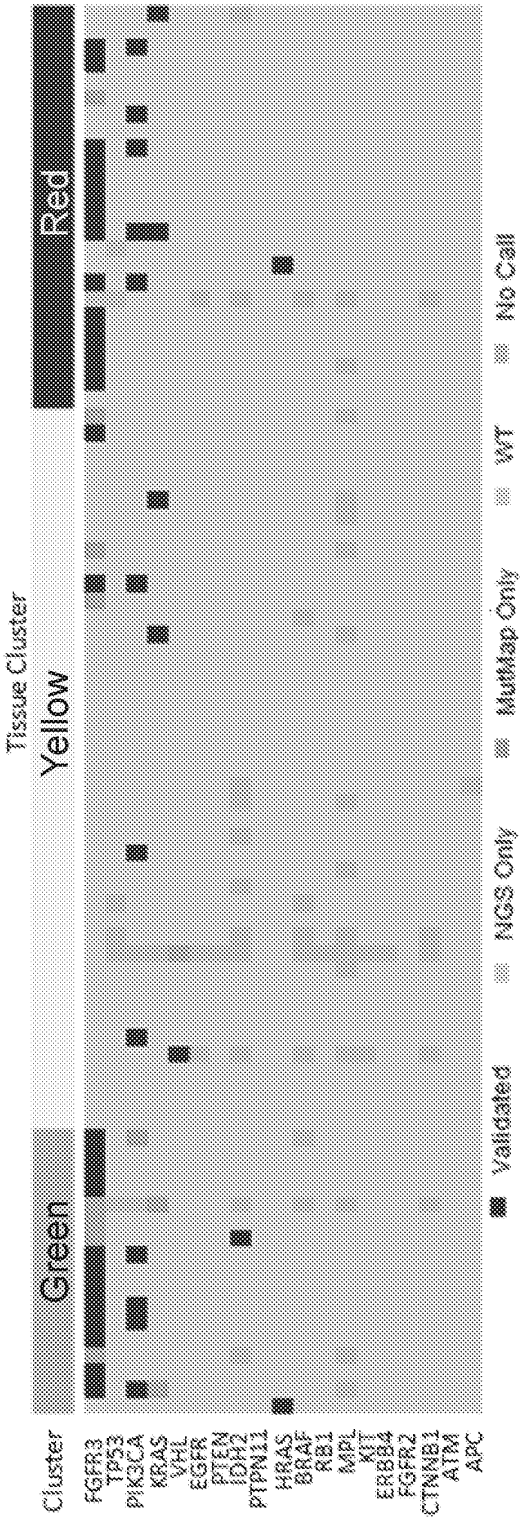

Ratio
0.200
0.175
0.150
0.125
0.100
0.075
0.050
0.025
0.000
Apoptosis Signaling
Death Receptor Signaling
Cell Cycle: G2/M DNA Damage Checkpoint Regulation
Cell Cycle: G1/S Checkpoint Regulation
IGF-1 Signaling
ErbB4 Signaling
ERK/MAPK Signaling
Role of p14/p19ARF in Tumor Suppression
p70S6K Signaling
Neuregulin Signaling
VEGF Family Ligand-Receptor Interactions
mTOR Signaling
ErbB Signaling
EGF Signaling
ErbB2-ErbB3 Signaling
FGF Signaling
HER-2 Signaling in Breast Cancer
VEGF Signaling
PI3K/AKT Signaling
PTEN Signaling
Regulation of the Epithelial-Mesenchymal Transition Pathway
p53 Signaling
21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
-log(p-value)
Analysis: Observation 1
Observation 1
Ratio

Figure 9A


Tissue Cluster
Green
Yellow
Red
Cluster
FGFR3
TP53
PIK3CA
KRAS
VHL
EGFR
PTEN
IDH2
PTPN11
HRAS
BRAF
RB1
MPL
KIT
ERBB4
FGFR2
CTNNB1
ATM
APC
Validated
NGS Only
MutMap Only
WT
No Call

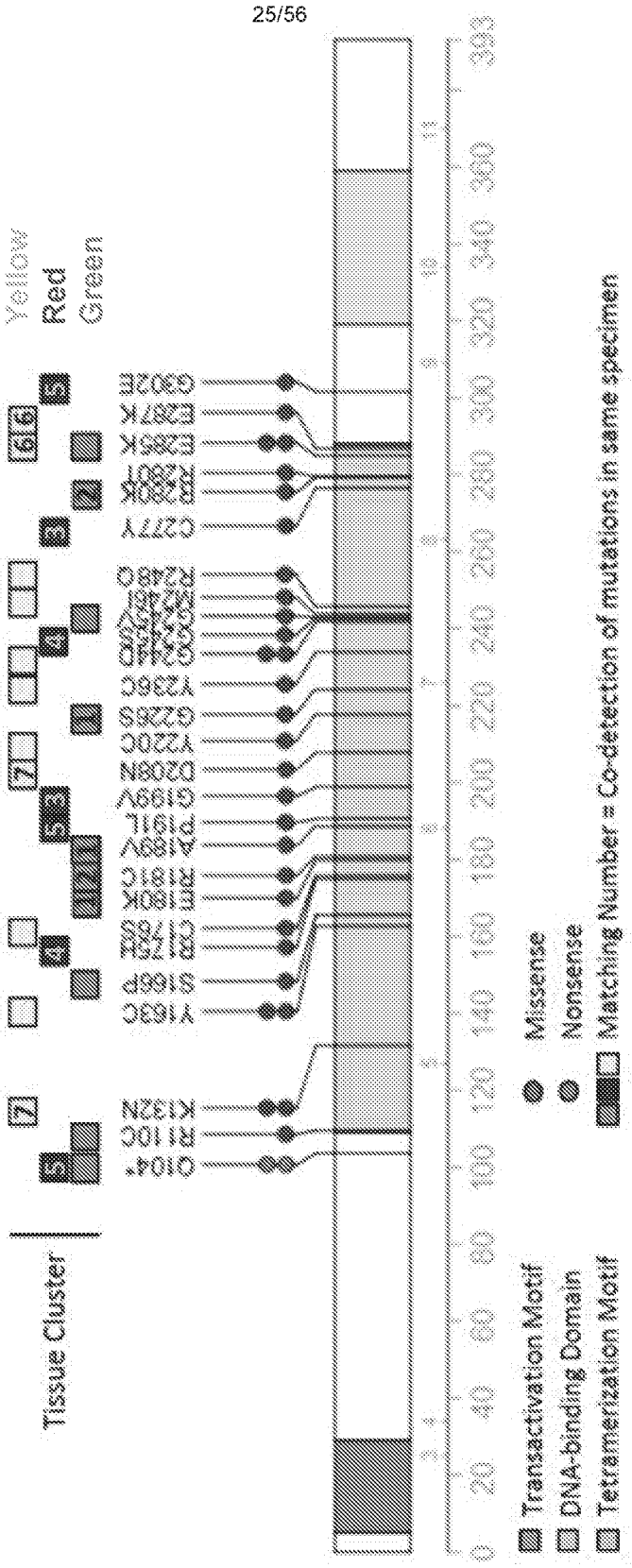
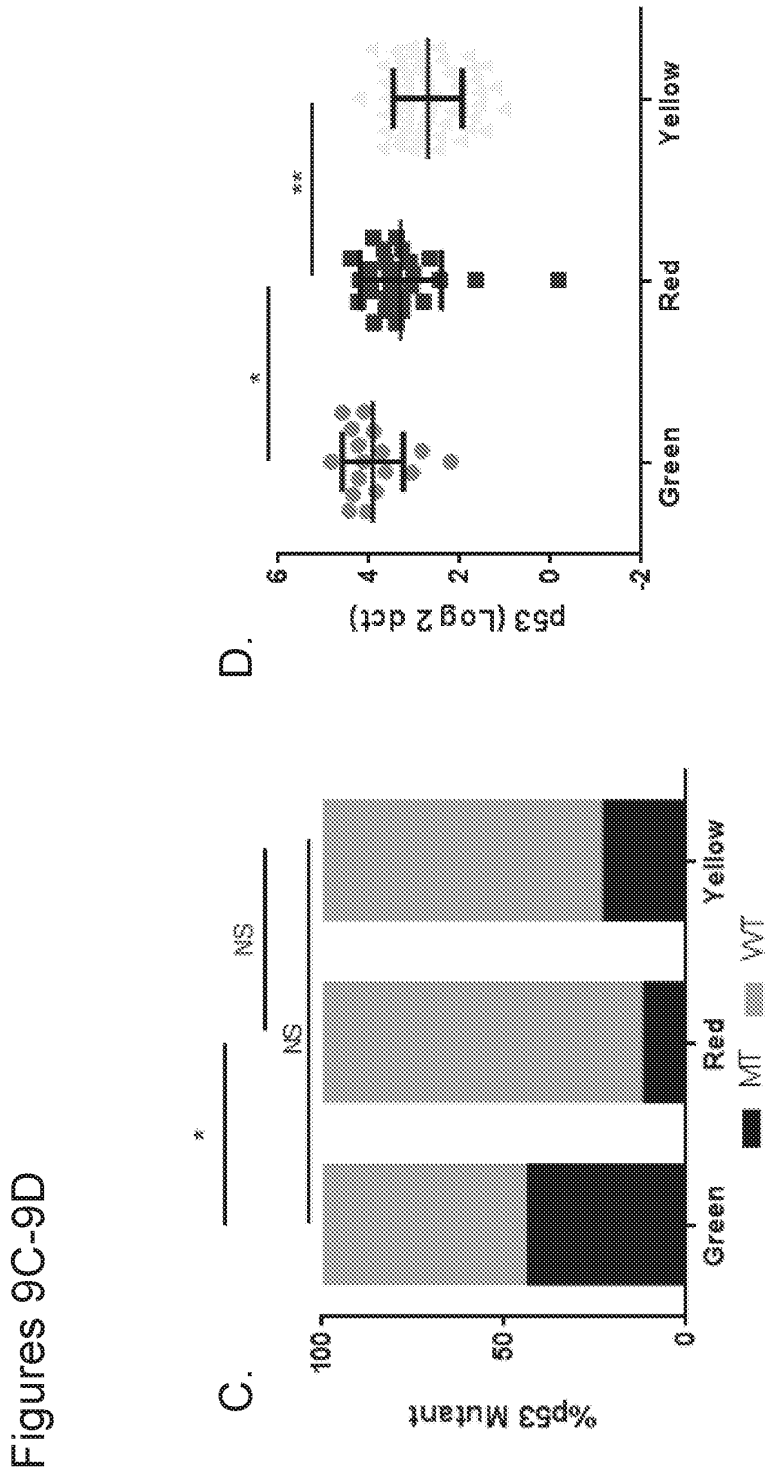
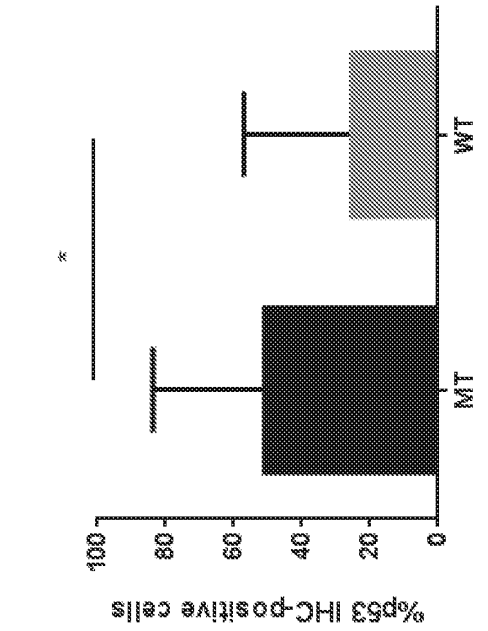
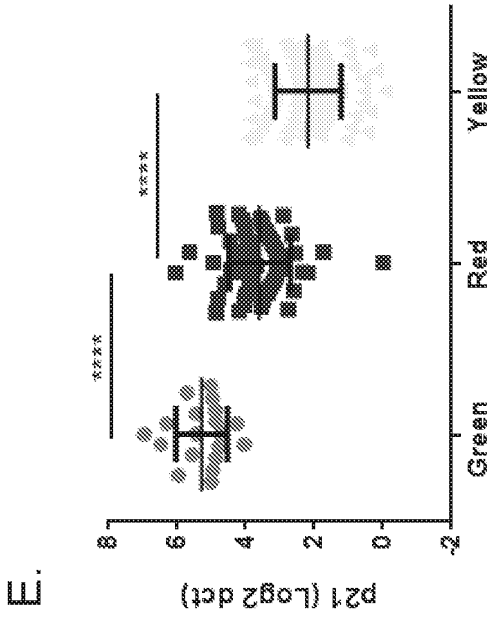

Yellow
Red
Green
Tissue Cluster
Q104*
R110C
K132N
Y163C
S166P
C176S
R181C
A189V
Y191L
G199V
Q208N
Y220C
G228S
Y236C
S241P
R248Q
C277Y
R280K
R280T
E286K
E287K
G302E
Missense
Nonsense
Matching Number = Co-detection of mutations in same specimen
Transactivation Motif
DNA-binding Domain
Tetramerization Motif
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
393

Figure 9B

Figures 9C-9D







Figures 9E-9F

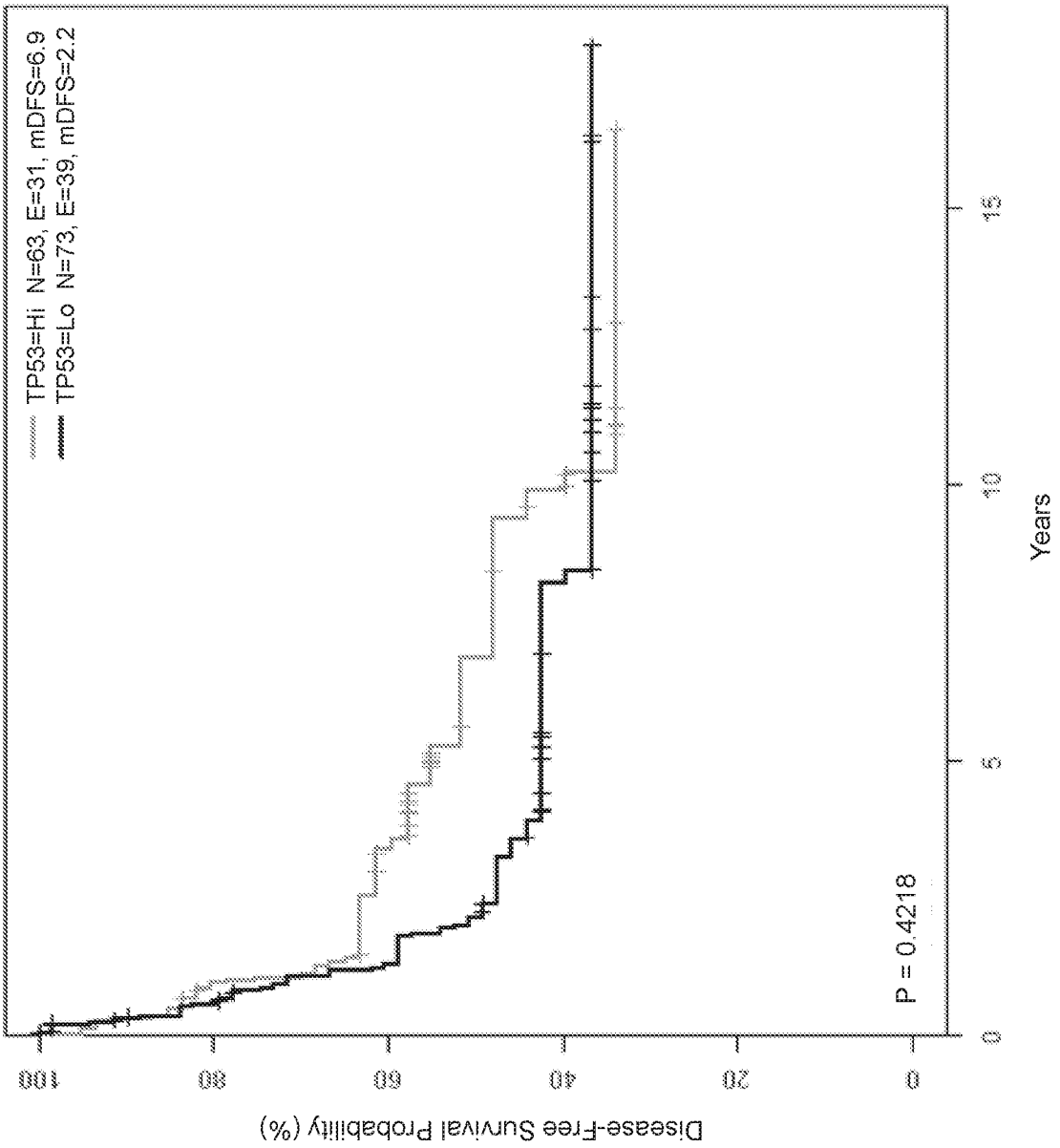

TP53=Hi N=63, E=31, mDFS=6.9
TP53=Lo N=73, E=39, mDFS=2.2
P = 0.4218
Disease-Free Survival Probability (%)
0
20
40
60
80
100
0
5
10
15
Years

Figure 9G

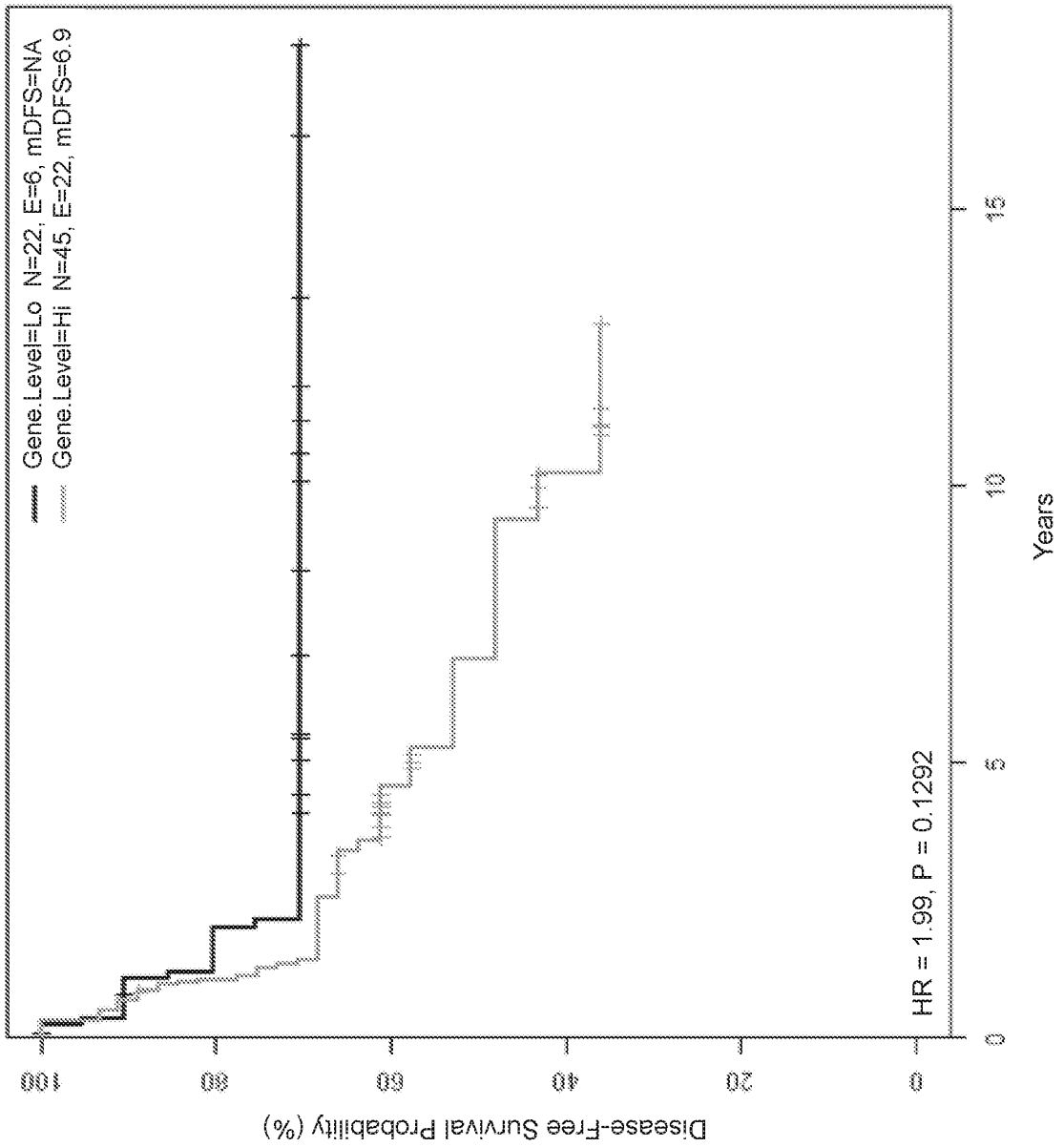

Gene.Level=Lo N=22, E=6, mDFS=NA
Gene.Level=Hi N=45, E=22, mDFS=6.9
HR = 1.99, P = 0.1292
Disease-Free Survival Probability (%)
0
20
40
60
80
100
0
5
10
15
Years

Figure 9H

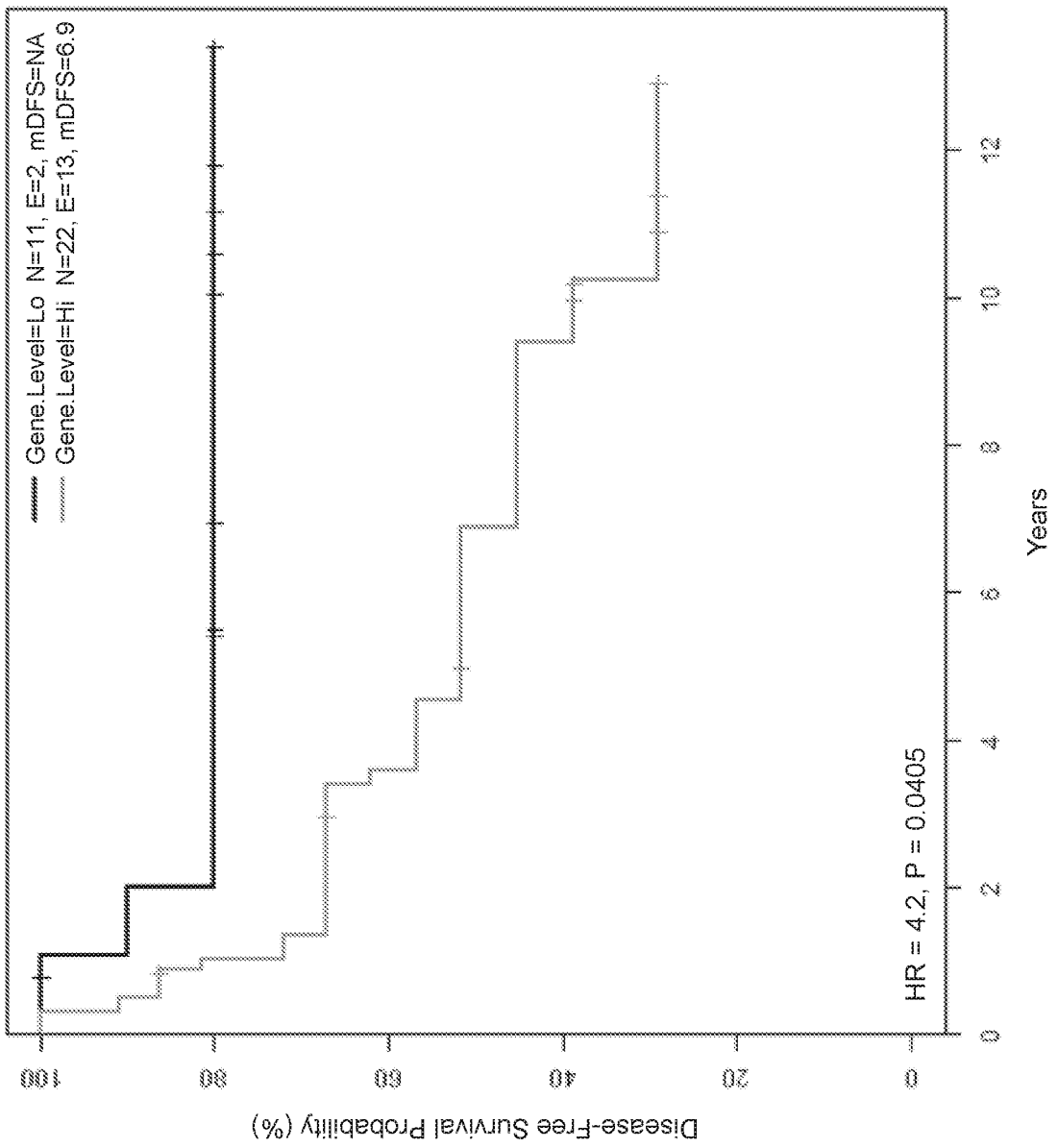

Gene.Level=Lo N=11, E=2, mDFS=NA
Gene.Level=Hi N=22, E=13, mDFS=6.9
HR = 4.2, P = 0.0405
Disease-Free Survival Probability (%)
0
20
40
60
80
100
0
2
4
6
8
10
12
Years

Figure 9I

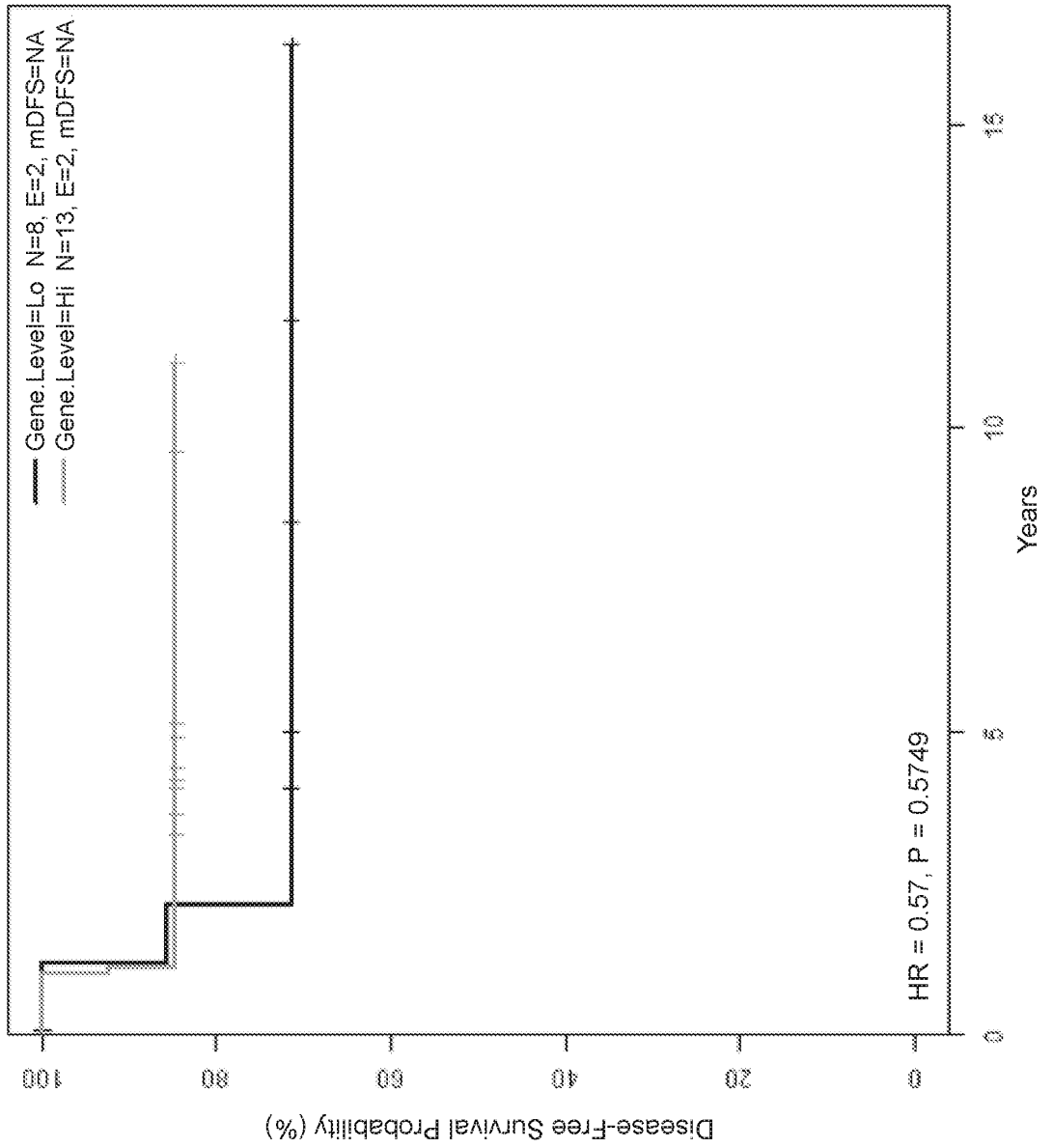

Gene.Level=Lo N=8, E=2, mDFS=NA
Gene.Level=Hi N=13, E=2, mDFS=NA
HR = 0.57, P = 0.5749
Disease-Free Survival Probability (%)
0
20
40
60
80
100
0
5
10
15
Years

Figure 9J

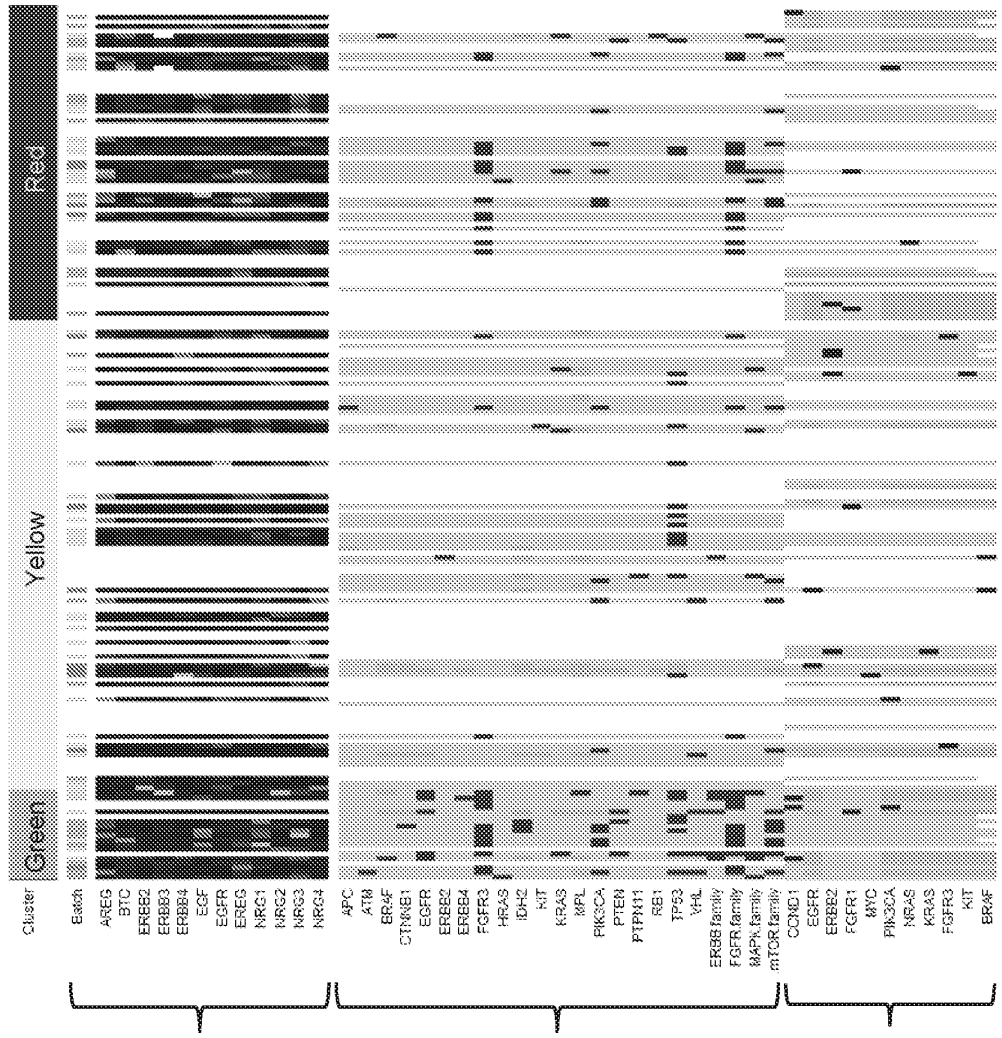

Cluster
Green
Yellow
Red
Batch
AREG
BTC
ERBB2
ERBB3
ERBB4
EGF
EGFR
EREG
NRG1
NRG2
NRG3
NRG4
APC
ATM
BRAF
CTNNB1
EGFR
ERBB2
ERBB4
FGFR3
HRAS
IDH2
KIT
KRAS
MPL
PIK3CA
PTEN
PTPN11
RB1
TP53
VHL
ERBB family
FGFR family
MAPK family
mTOR family
CCND1
EGFR
ERBB2
FGFR1
MYC
PIK3CA
NRAS
KRAS
FGFR3
KIT
BRAF
Fluidigm Expression Analysis
Fluidigm Mutation Analysis
Fluidigm DNA Copy Number Analysis

Figure 10

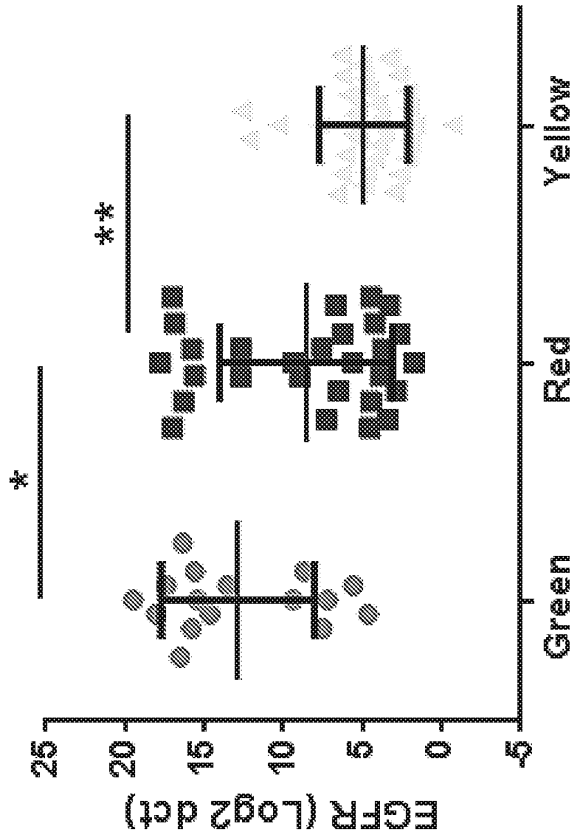


Figure 11A

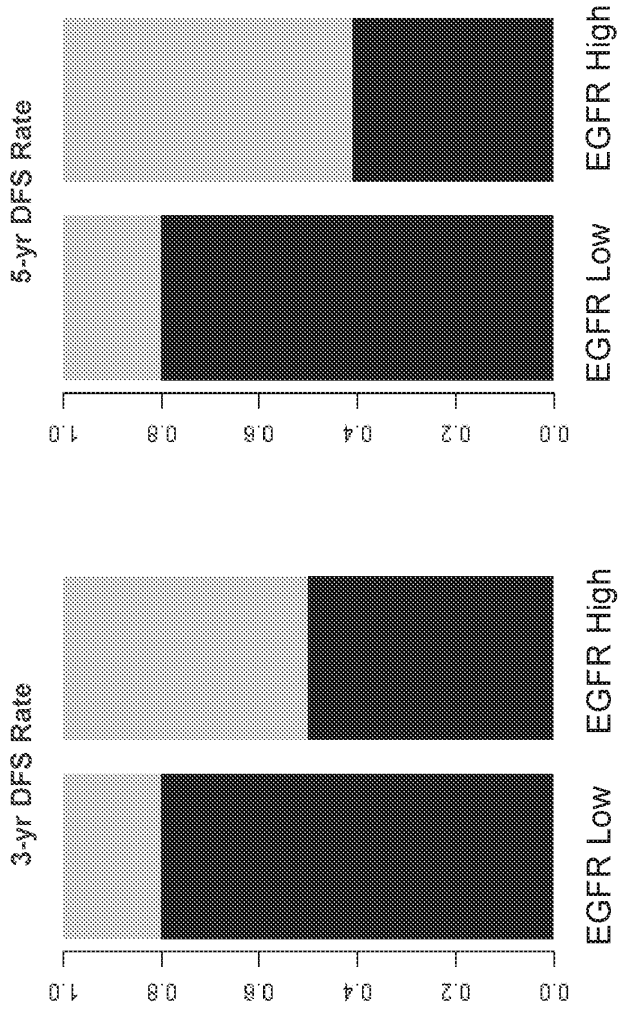
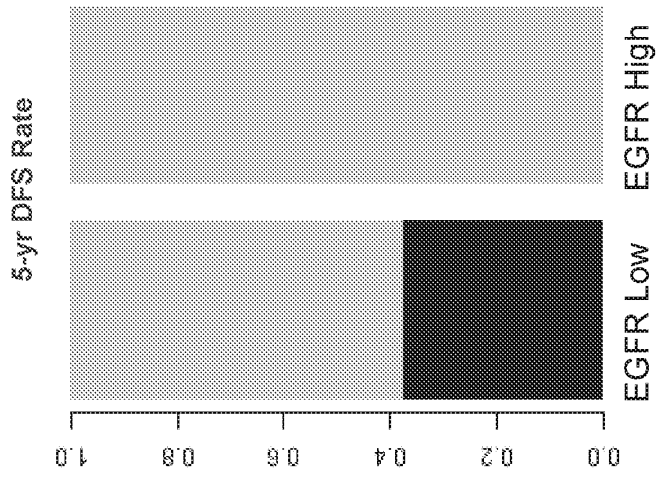


Figure 11B


5-yr DFS Rate
EGFR High
EGFR Low
0.0
0.2
0.4
0.6
0.8
1.0

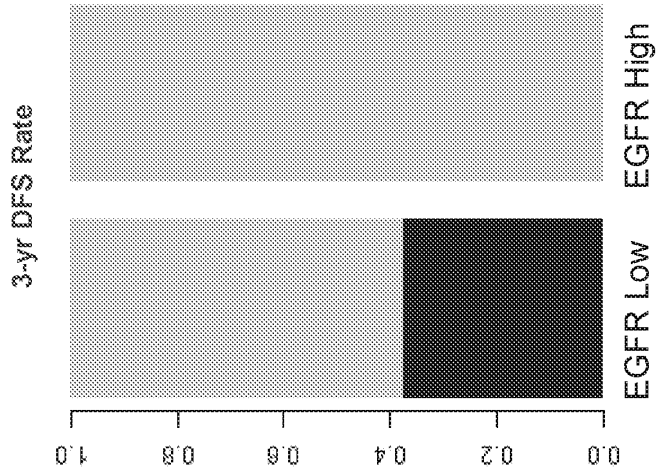

3-yr DFS Rate
EGFR High
EGFR Low
0.0
0.2
0.4
0.6
0.8
1.0

Figure 11C

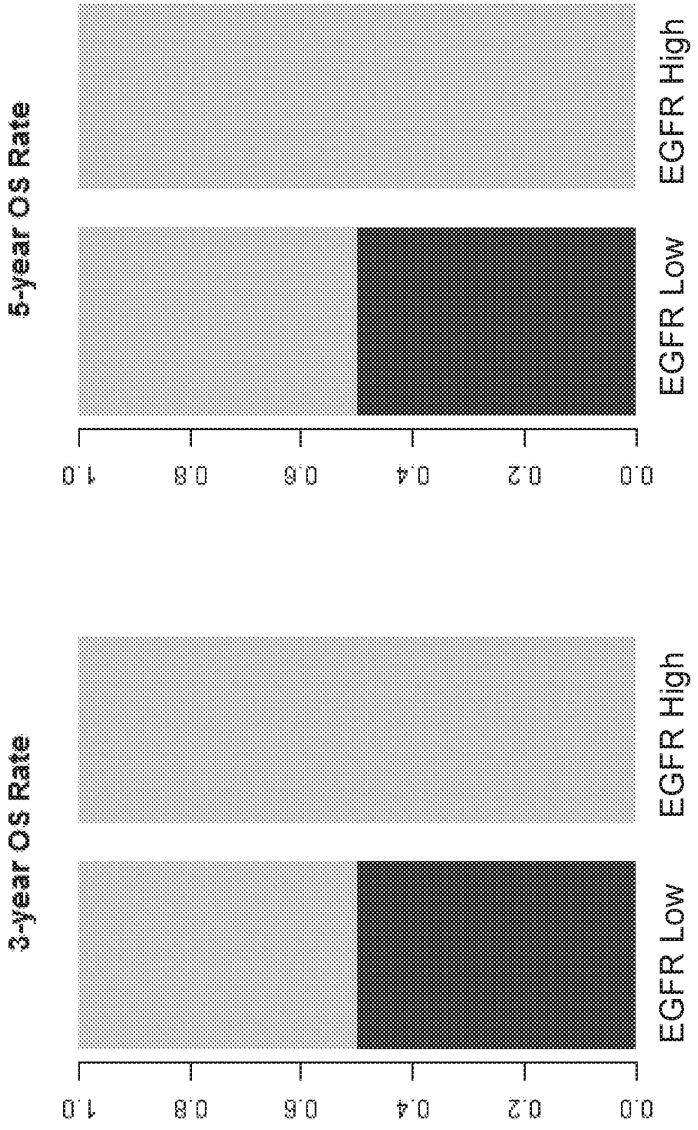

5-year OS Rate
EGFR High
EGFR Low
0.0
0.2
0.4
0.6
0.8
1.0
3-year OS Rate
EGFR High
EGFR Low
0.0
0.2
0.4
0.6
0.8
1.0

Figure 11D

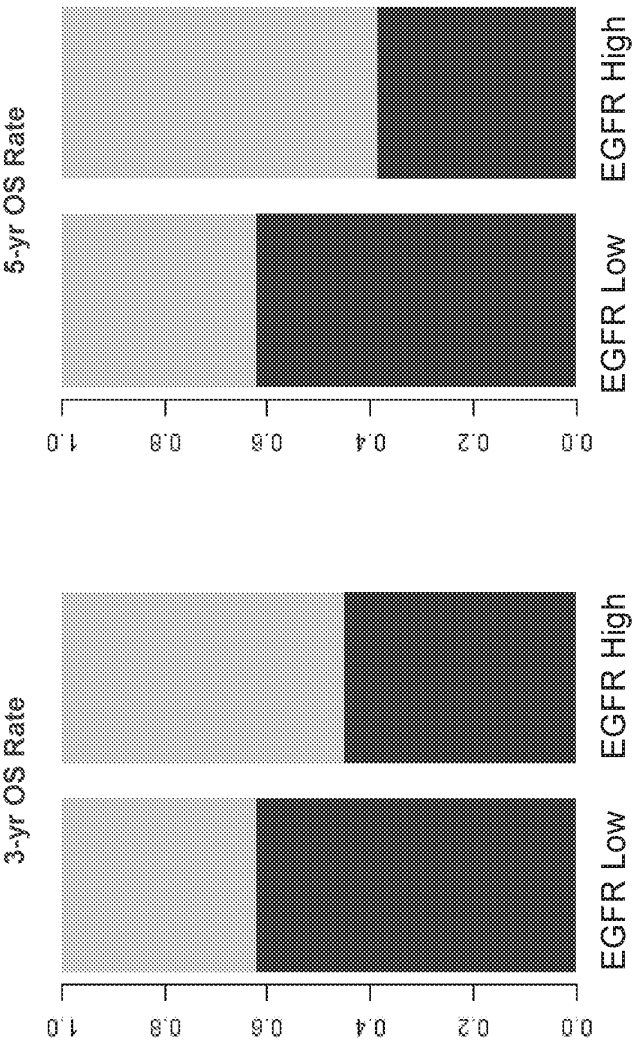

3-yr OS Rate
EGFR Low
EGFR High
0.0
0.2
0.4
0.6
0.8
1.0
5-yr OS Rate
EGFR Low
EGFR High
0.0
0.2
0.4
0.6
0.8
1.0

Figure 11E

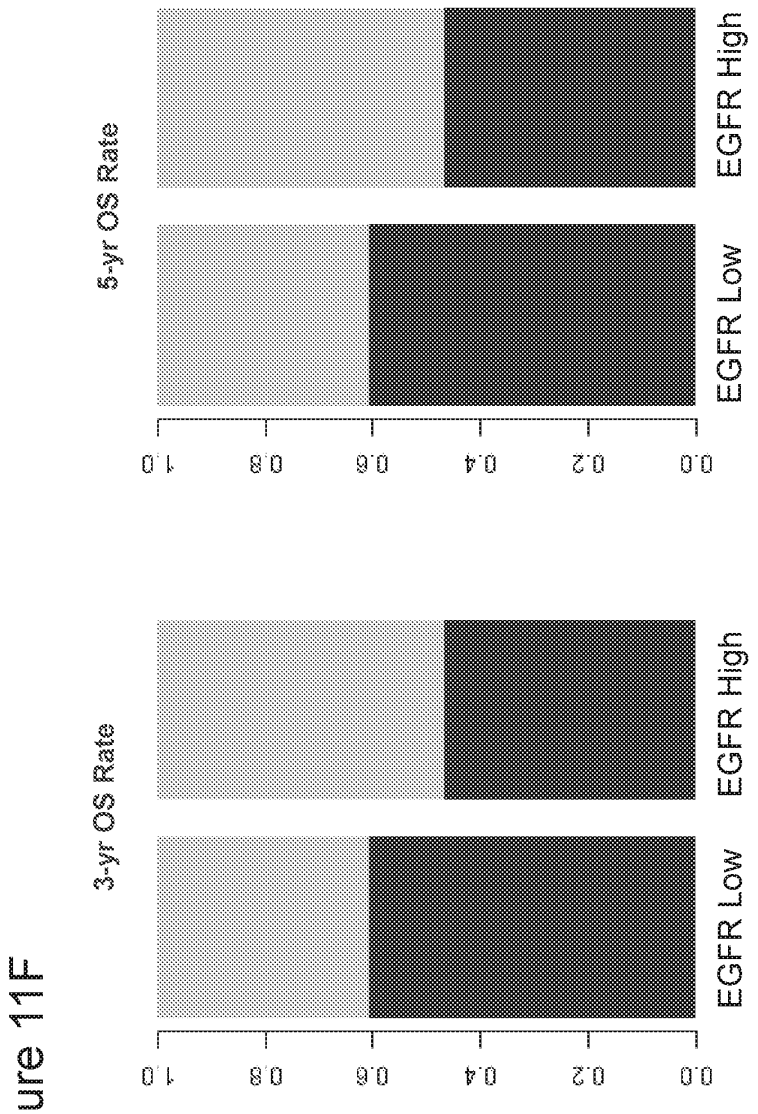
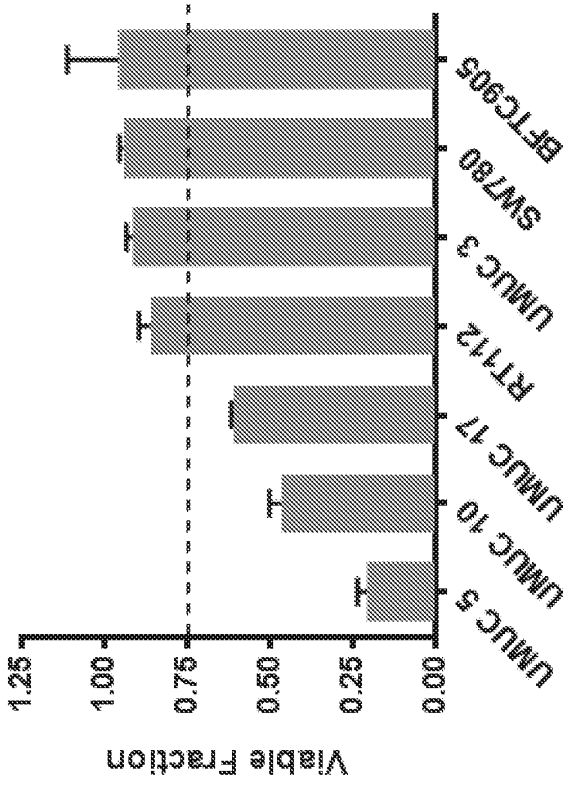

5-yr OS Rate
EGFR High
EGFR Low
0.0
0.2
0.4
0.6
0.8
1.0
3-yr OS Rate
EGFR High
EGFR Low
0.0
0.2
0.4
0.6
0.8
1.0

Figure 11F

Figures 11G-11H

G.



H.

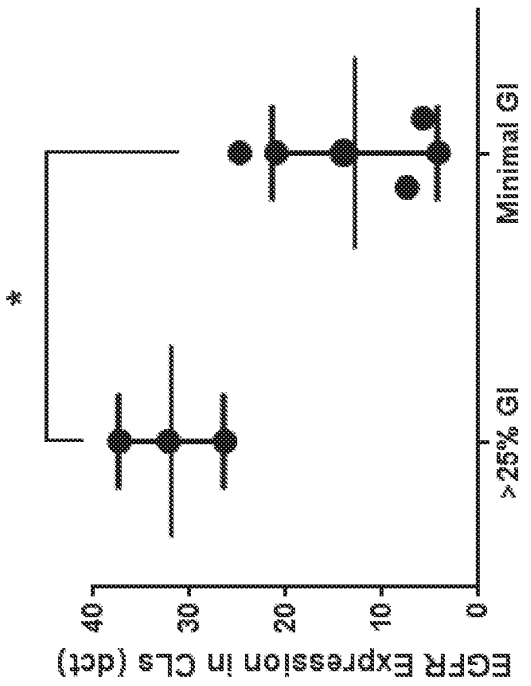
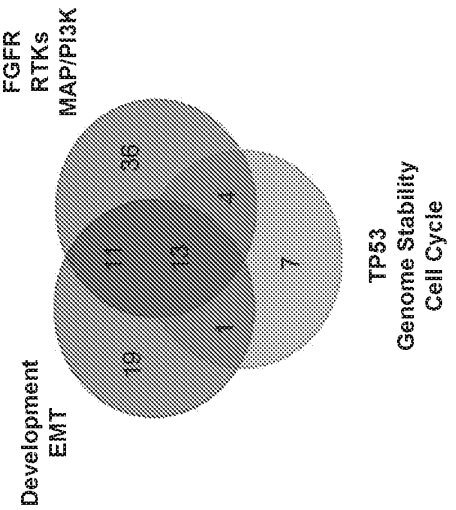


Figure 12A


FGFR
RTKs
MAP/PI3K
TP53
Genome Stability
Cell Cycle
Development
EMT
36
4
11
13
7
19
1

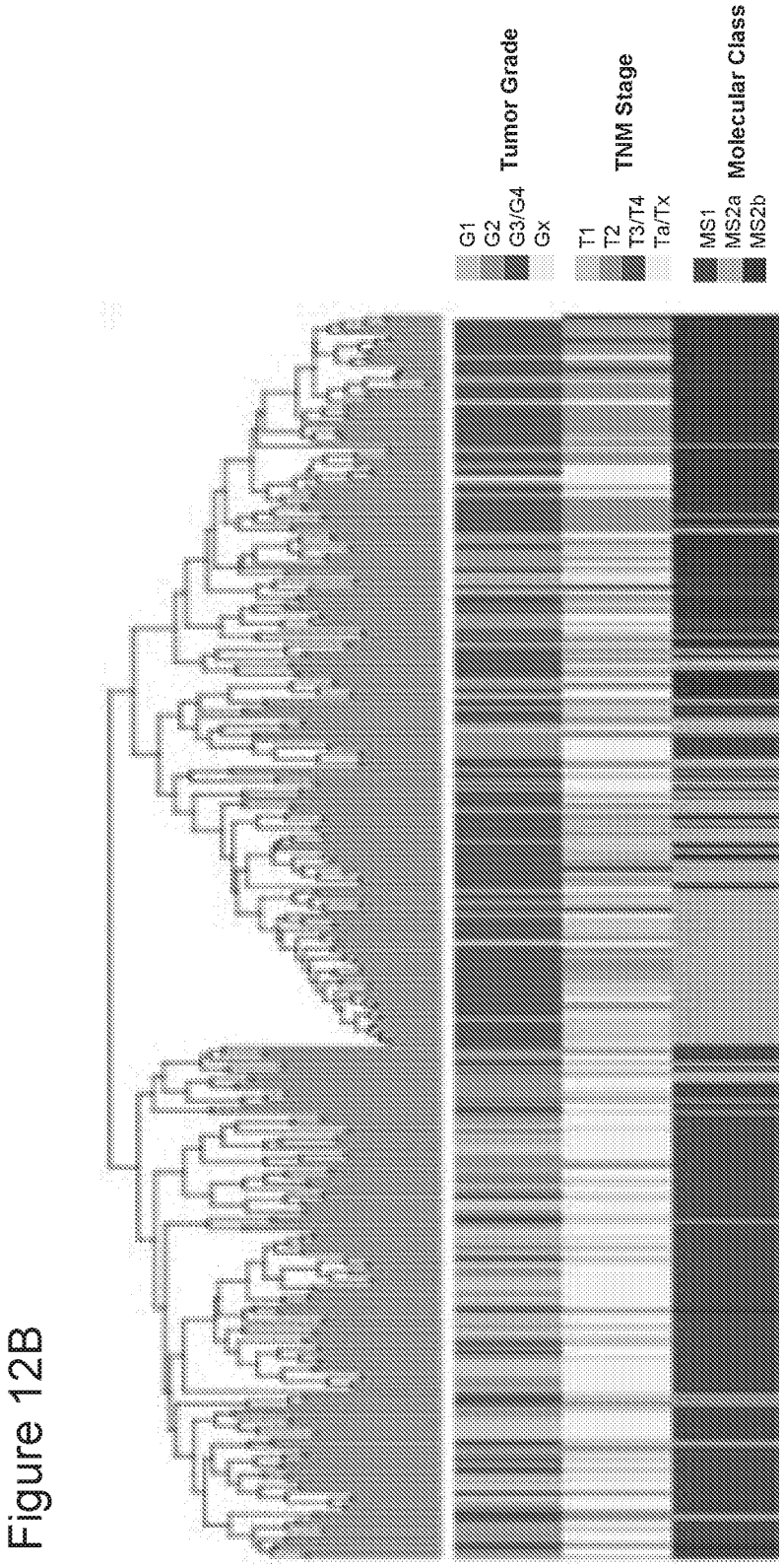
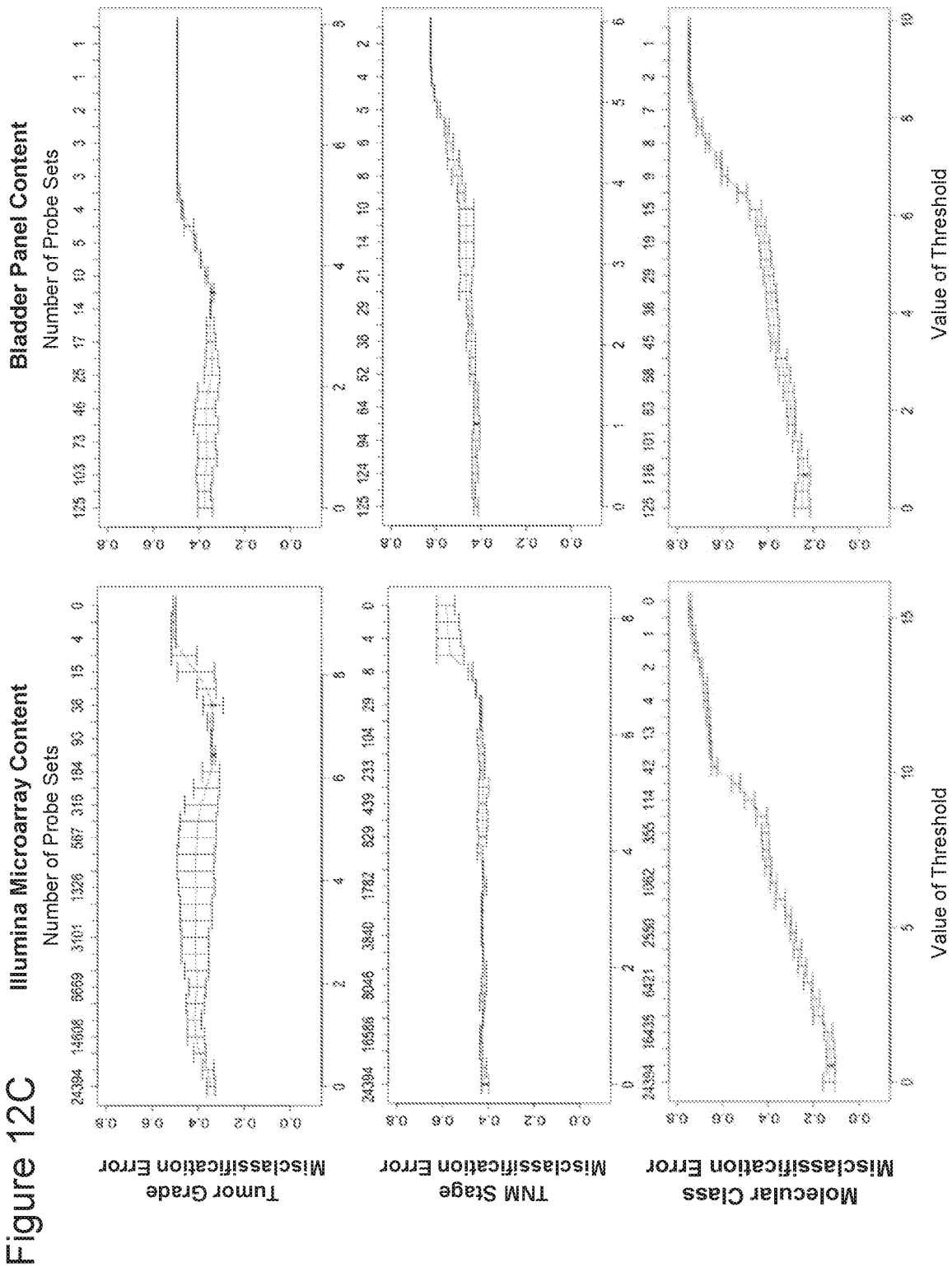

Tumor Grade
G1
G2
G3/G4
Gx
TNM Stage
T1
T2
T3/T4
Ta/Tx
Molecular Class
MS1
MS2a
MS2b

Figure 12B

Figure 12C


Illumina Microarray Content
Bladder Panel Content
Number of Probe Sets
Number of Probe Sets
Molecular Class Misclassification Error
TNM Stage Misclassification Error
Tumor Grade Misclassification Error
Value of Threshold
Value of Threshold

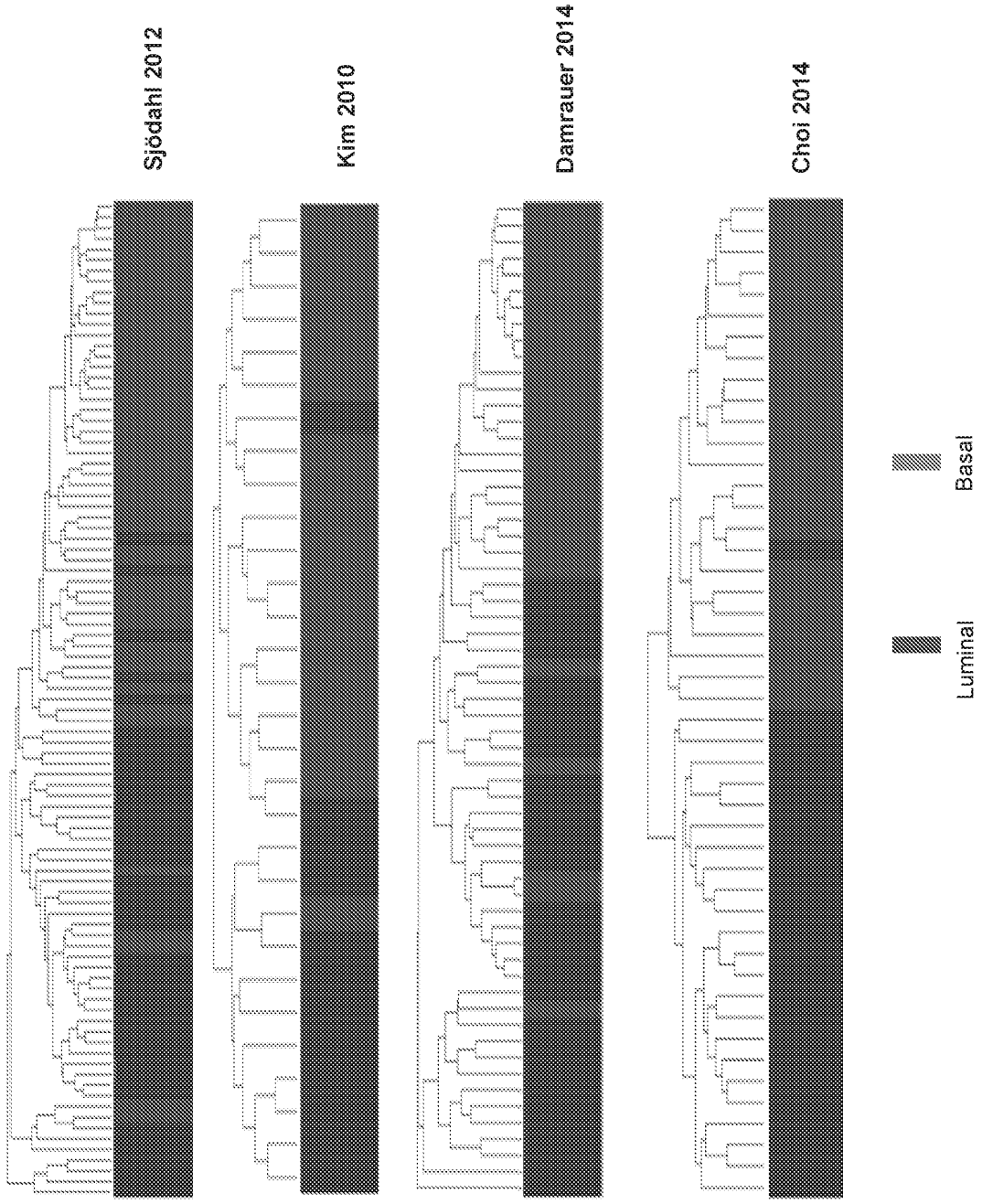

Sjödahl 2012
Kim 2010
Damrauer 2014
Choi 2014
Basal
Luminal

Figure 12D

Figure 13A

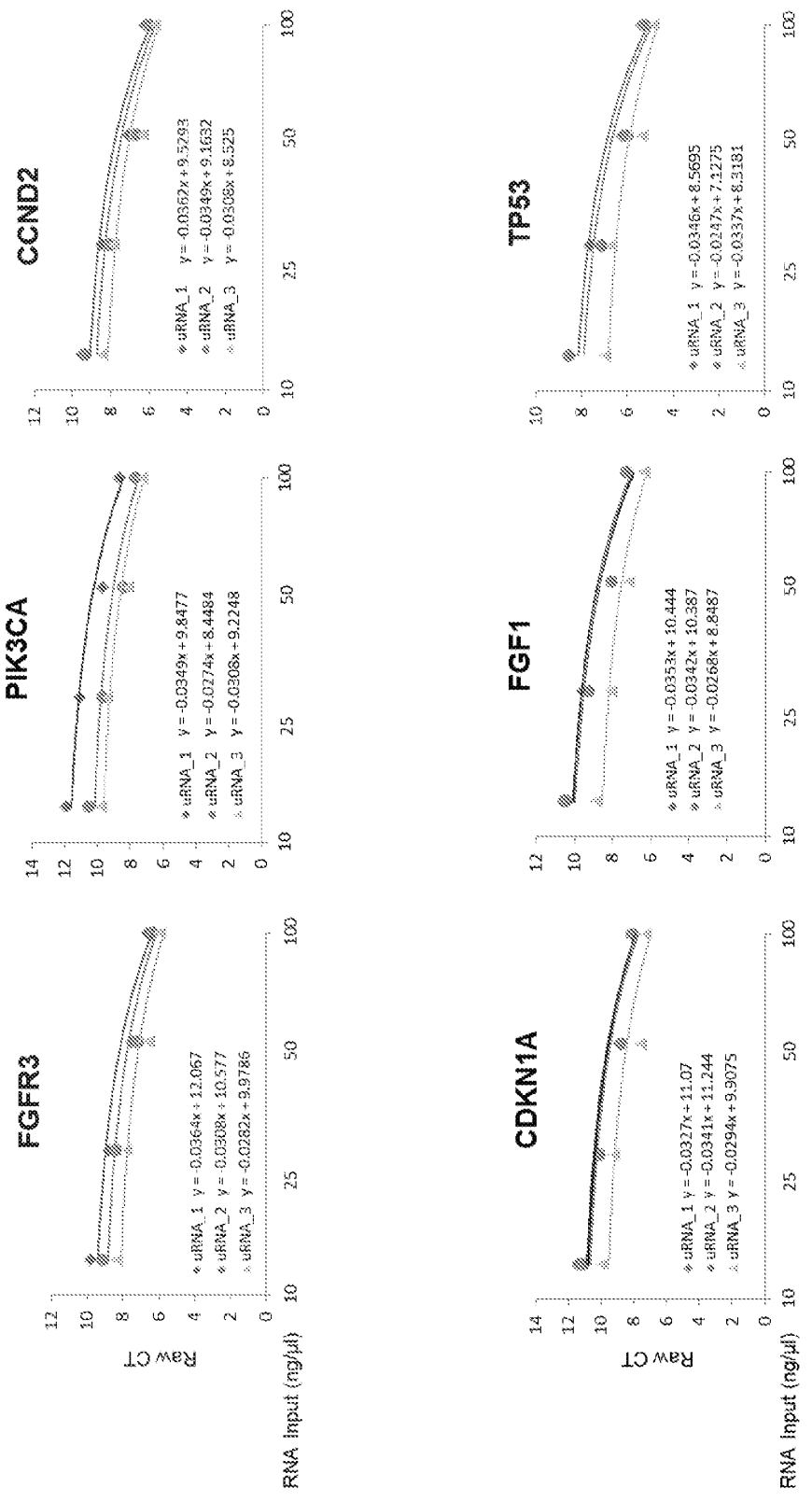
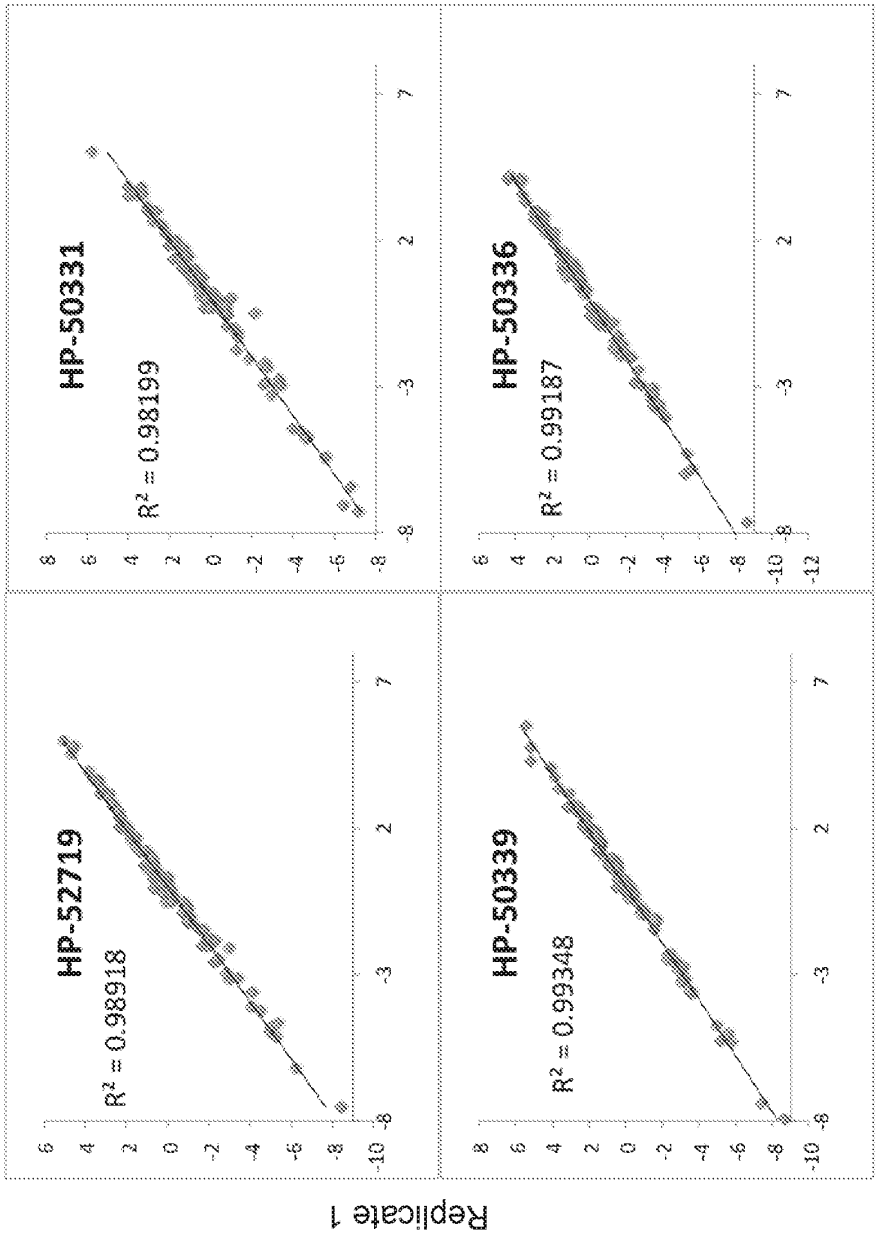

FGFR3
PIK3CA
CCND2
CDKN1A
FGF1
TP53
Raw CT
RNA input (ng/µl)
uRNA_1 y = -0.0364x + 12.067
uRNA_2 y = -0.0308x + 10.577
uRNA_3 y = -0.0282x + 9.9786
uRNA_1 y = -0.0349x + 9.8477
uRNA_2 y = -0.0274x + 8.4484
uRNA_3 y = -0.0308x + 9.2248
uRNA_1 y = -0.0362x + 9.5293
uRNA_2 y = -0.0349x + 9.1632
uRNA_3 y = -0.0306x + 8.525
uRNA_1 y = -0.0327x + 11.07
uRNA_2 y = -0.0341x + 11.244
uRNA_3 y = -0.0294x + 9.9075
uRNA_1 y = -0.0353x + 10.444
uRNA_2 y = -0.0342x + 10.387
uRNA_3 y = -0.0268x + 8.8487
uRNA_1 y = -0.0346x + 8.5695
uRNA_2 y = -0.0347x + 7.1275
uRNA_3 y = -0.0337x + 8.3181

Figure 13B


HP-52719
R² = 0.98918
HP-50331
R² = 0.98199
HP-50339
R² = 0.99348
HP-50336
R² = 0.99187
Replicate 1
Replicate 2

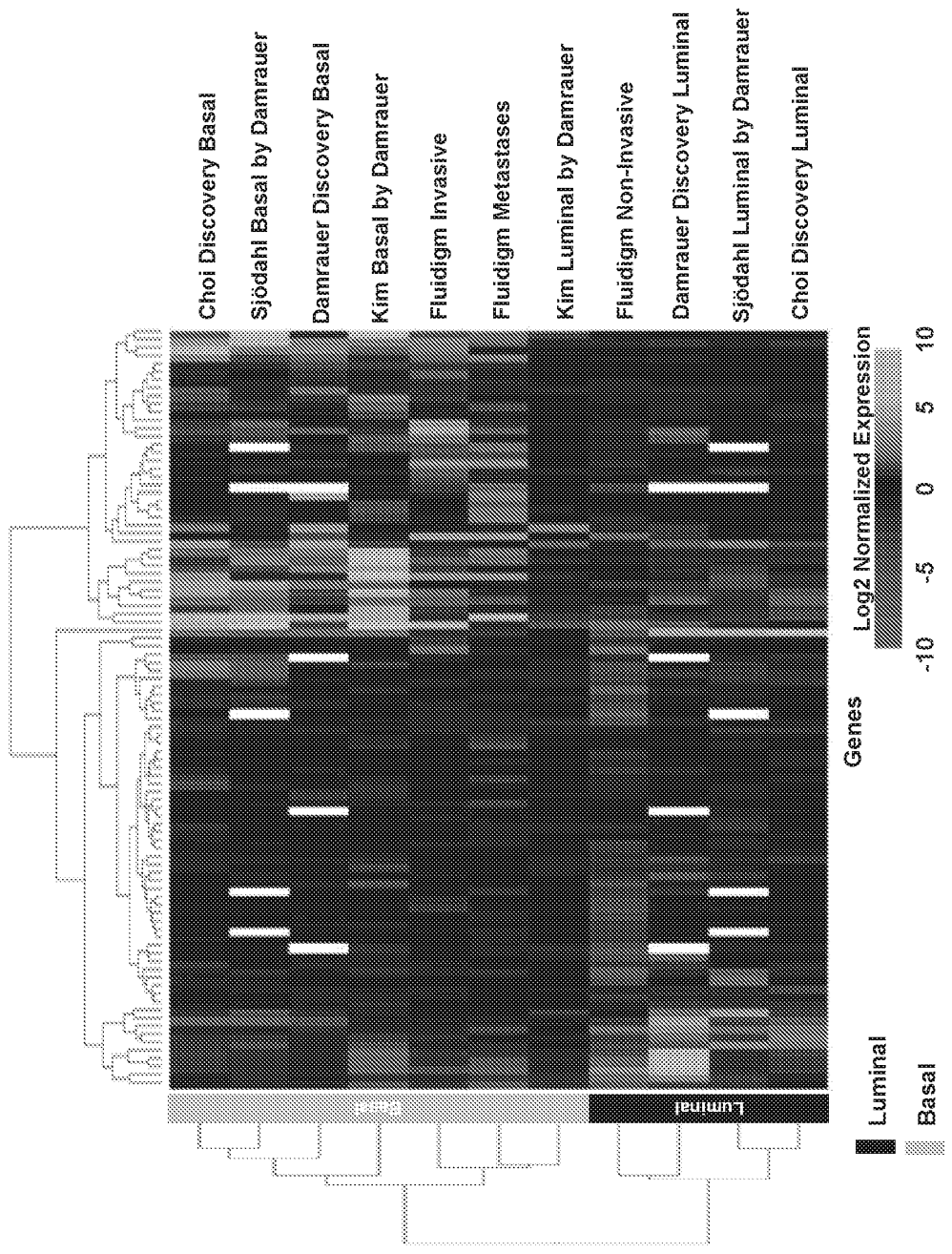

Choi Discovery Basal
Sjödahl Basal by Damrauer
Damrauer Discovery Basal
Kim Basal by Damrauer
Fluidigm Invasive
Fluidigm Metastases
Kim Luminal by Damrauer
Fluidigm Non-invasive
Damrauer Discovery Luminal
Sjödahl Luminal by Damrauer
Choi Discovery Luminal
Log2 Normalized Expression
10
5
0
-5
-10
Genes
Luminal
Basal

Figure 14A

Figure 14B

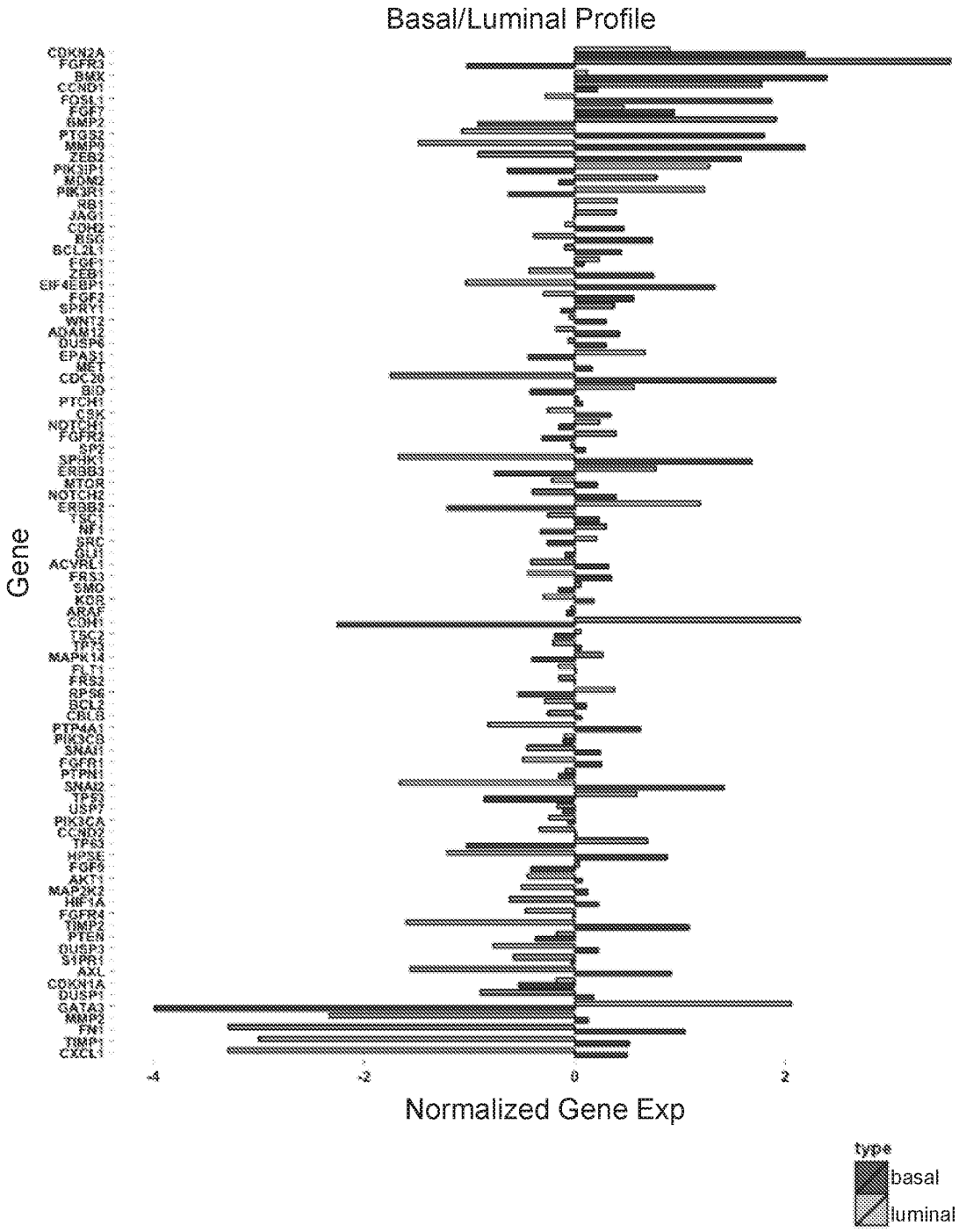
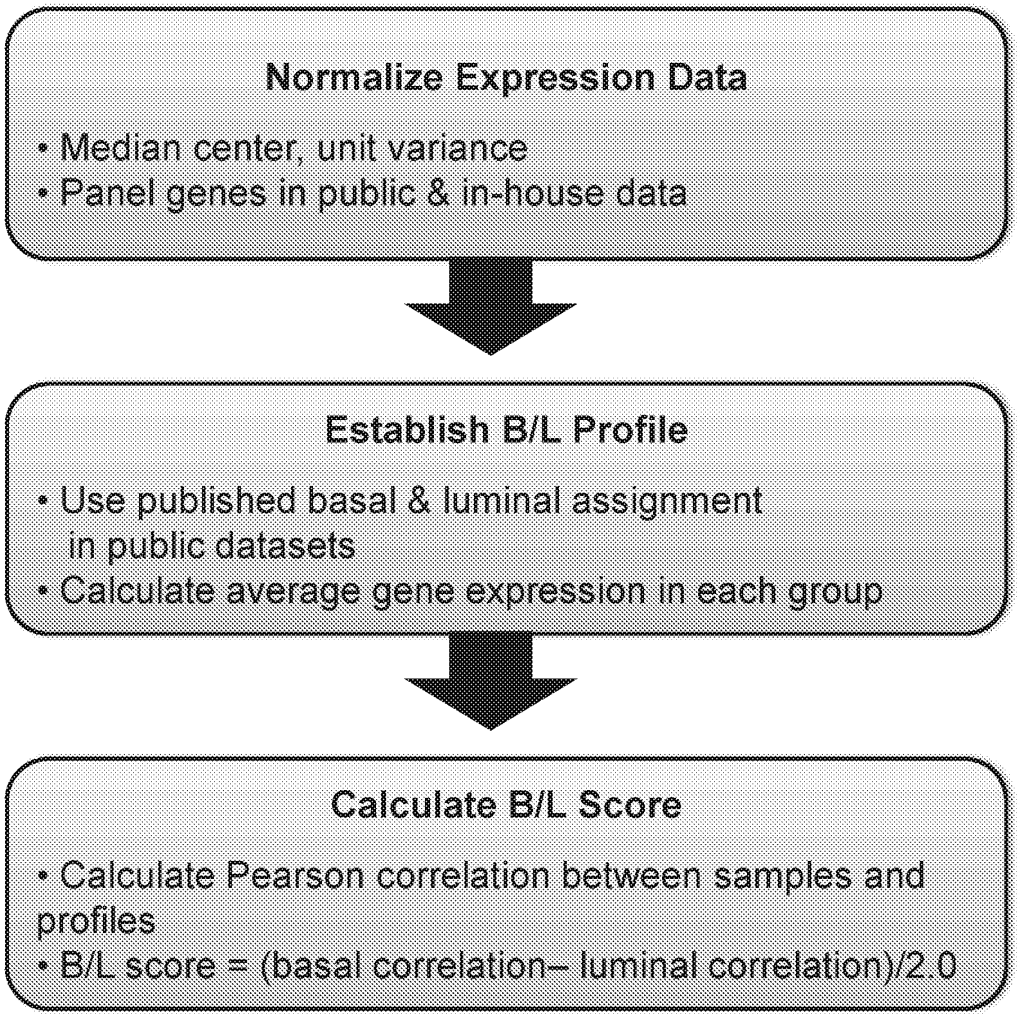

Basal/Luminal Profile
Gene
CDKN2A
FGFR3
BMX
CCND1
FOSL1
FGF7
GMPR2
PTGS2
MMP9
ZEB2
PKNOX1
MDM2
PIK3R1
RB1
JAG1
CDH2
RSC0
BCL2L1
FGF1
ZEB1
EIF4EBP1
FGF2
SPRY1
WNT3
ADAM12
DUSP6
EPAS1
MET
CDC20
BID
PTCH1
CSK
NOTCH1
FGFR2
SP2
SPOK1
ERBB3
MTOR
NOTCH2
ERBB2
TSC1
NF1
SRC1
GLI1
ACVRL1
FRS3
SMO
KGS
ARAF
CDH1
TSC2
TP73
MAPK1A
FLT1
FRS2
RPS6
BCL2
CBLB
PTP4A1
PIK3CB
SNAI1
FGFR1
PTPN1
SNAI2
TP53
USP7
PIK3CA
CCND2
TP63
HPSE
FGF5
AKT1
MAP2K2
HIF1A
FGFR4
TIMP2
PTEN
USP3
S1PR1
AXL
CDKN1A
DUSP1
GATA3
MMP2
FN1
TIMP1
CXCL1
-4
-2
0
2
Normalized Gene Exp
type
basal
luminal

Figure 14C


Normalize Expression Data
• Median center, unit variance
• Panel genes in public & in-house data
Establish B/L Profile
• Use published basal & luminal assignment in public datasets
• Calculate average gene expression in each group
Calculate B/L Score
• Calculate Pearson correlation between samples and profiles
• B/L score = (basal correlation– luminal correlation)/2.0

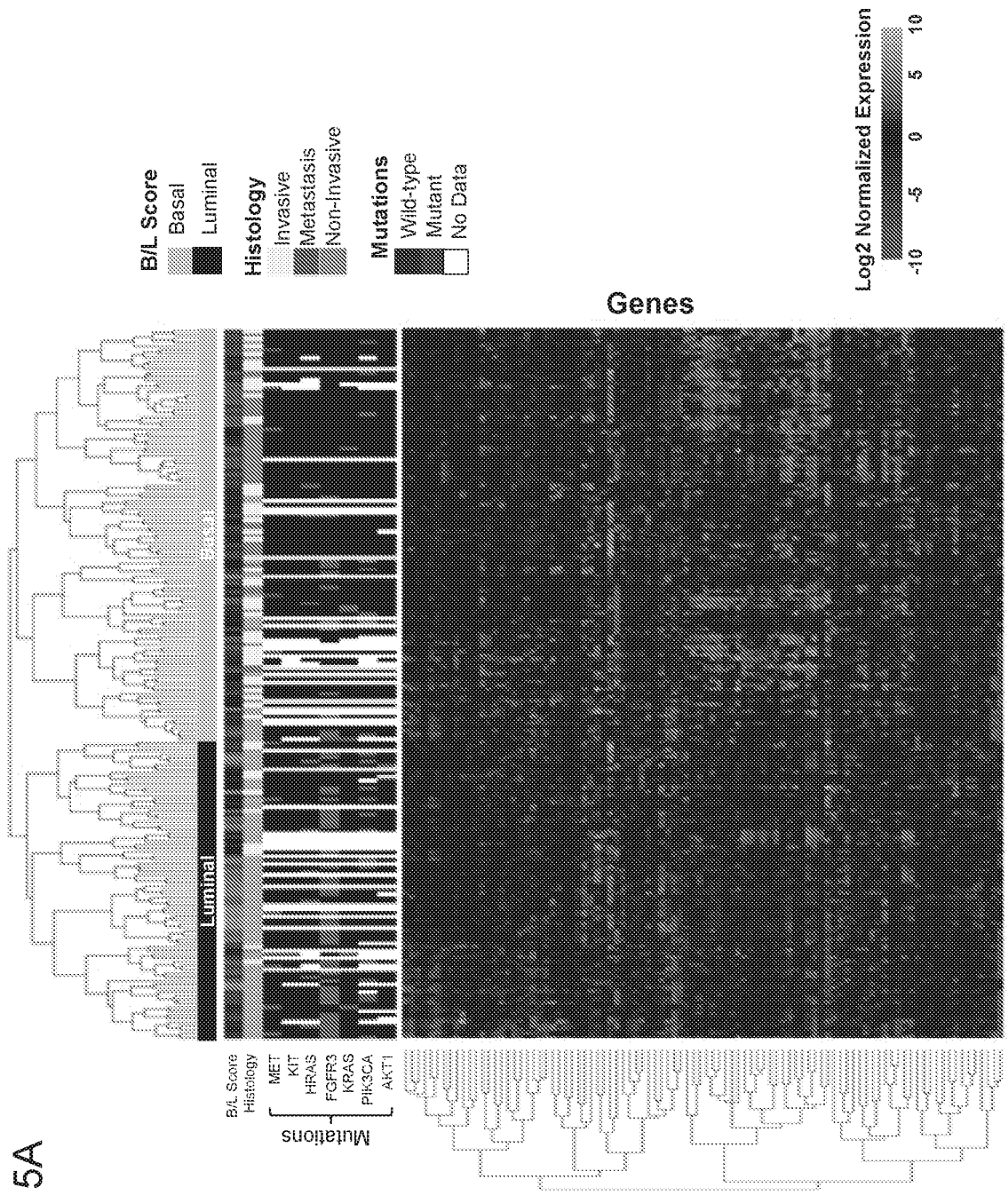
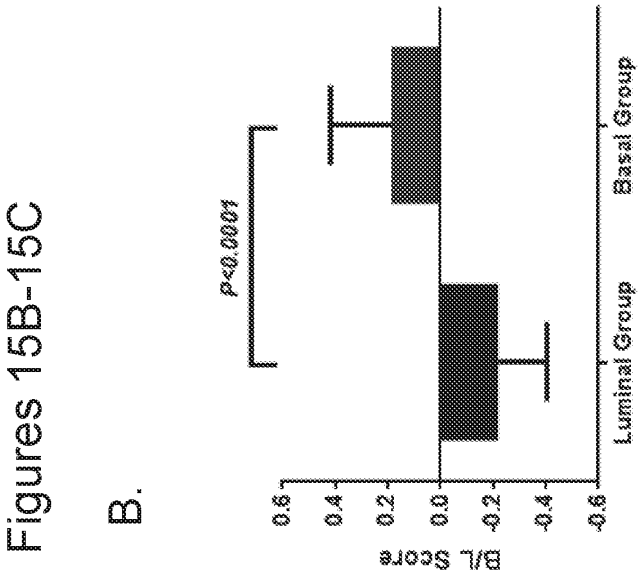
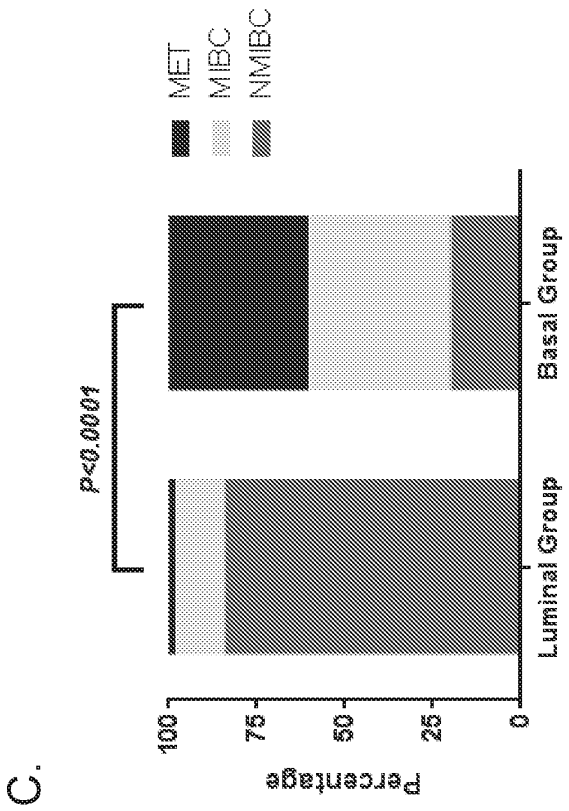

B/L Score
Basal
Luminal
Histology
Invasive
Metastasis
Non-Invasive
Mutations
Wild-type
Mutant
No Data
Log2 Normalized Expression
-10
-5
0
5
10
Genes
Luminal
B/L Score
Histology
Mutations
MET
KIT
HRAS
FGFR3
KRAS
PIK3CA
AKT1

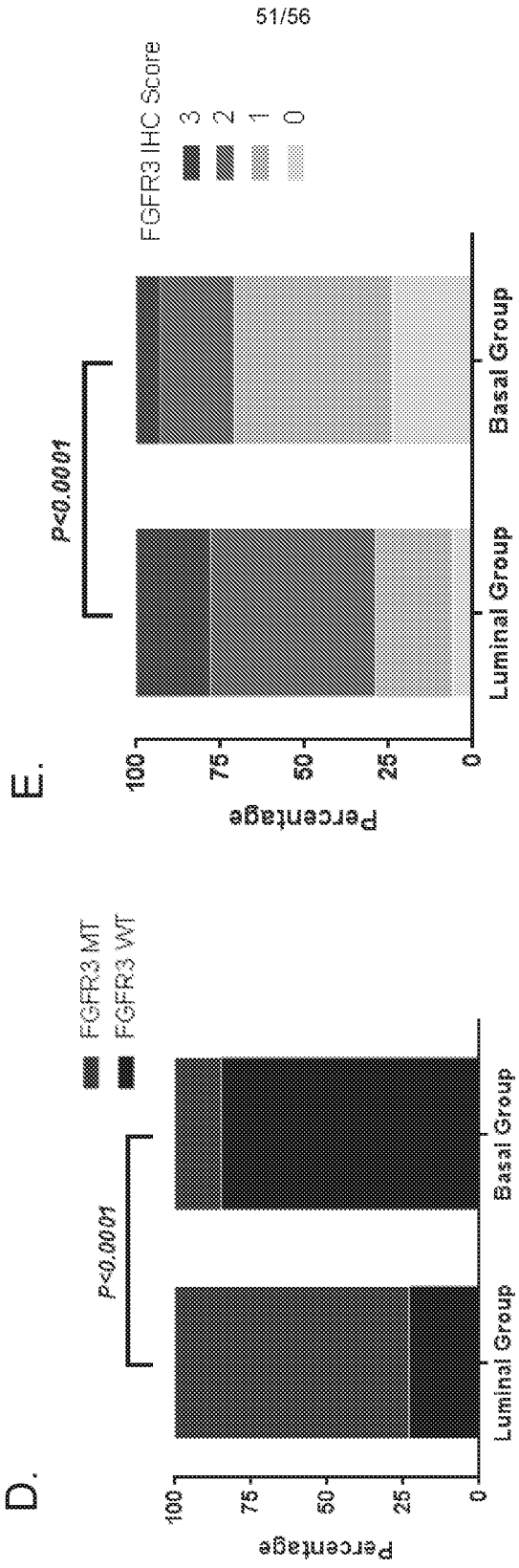
Figure 15A

Figures 15B-15C





Figures 15D-15E



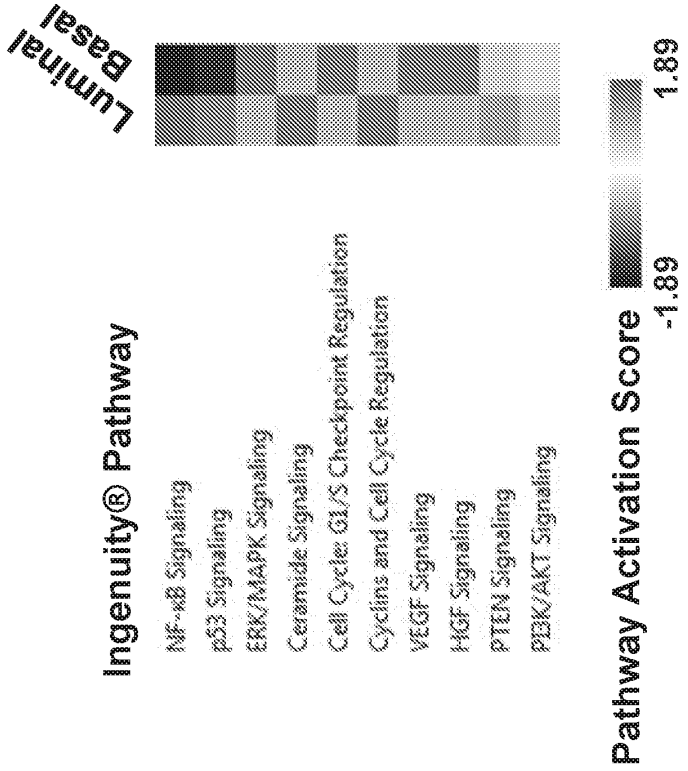

Luminal
Basal
Ingenuity® Pathway
NF-κB Signaling
p53 Signaling
ERK/MAPK Signaling
Ceramide Signaling
Cell Cycle: G1/S Checkpoint Regulation
Cyclins and Cell Cycle Regulation
VEGF Signaling
HGF Signaling
PTEN Signaling
PI3K/AKT Signaling
Pathway Activation Score
-1.89
1.89

Figure 15F

Figure 16A

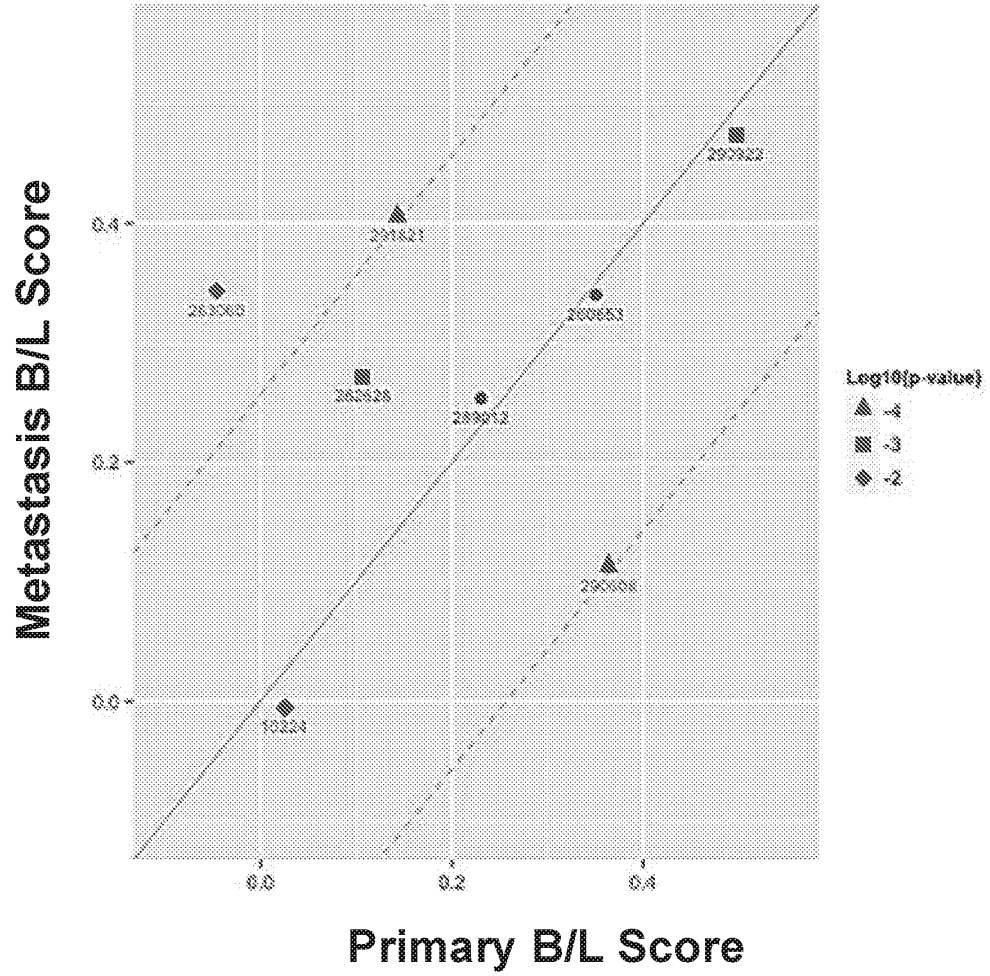

Metastasis B/L Score
Primary B/L Score
0.0
0.2
0.4
Log10(p-value)
-4
-3
-2

Figure 16B

B/L Status in Matched Primary (Pri) tumors vs. matched metastases (met)

Patient ID	Pri B/L Score	Met B/L Score	Pri-Met B/L change p-value
10224	0.024	-0.005	*0.813*
10239			*0.270*
260653	0.350	0.340	*0.934*
262628	0.107	0.172	*0.194*
263060	-0.046	0.335	***0.005***
289012	0.230	0.253	*0.851*
290608	0.364	0.112	*0.054*
290922			*0.852*
291821	0.144	0.436	***0.046***

Luminal Basal

Figures 17A-17B

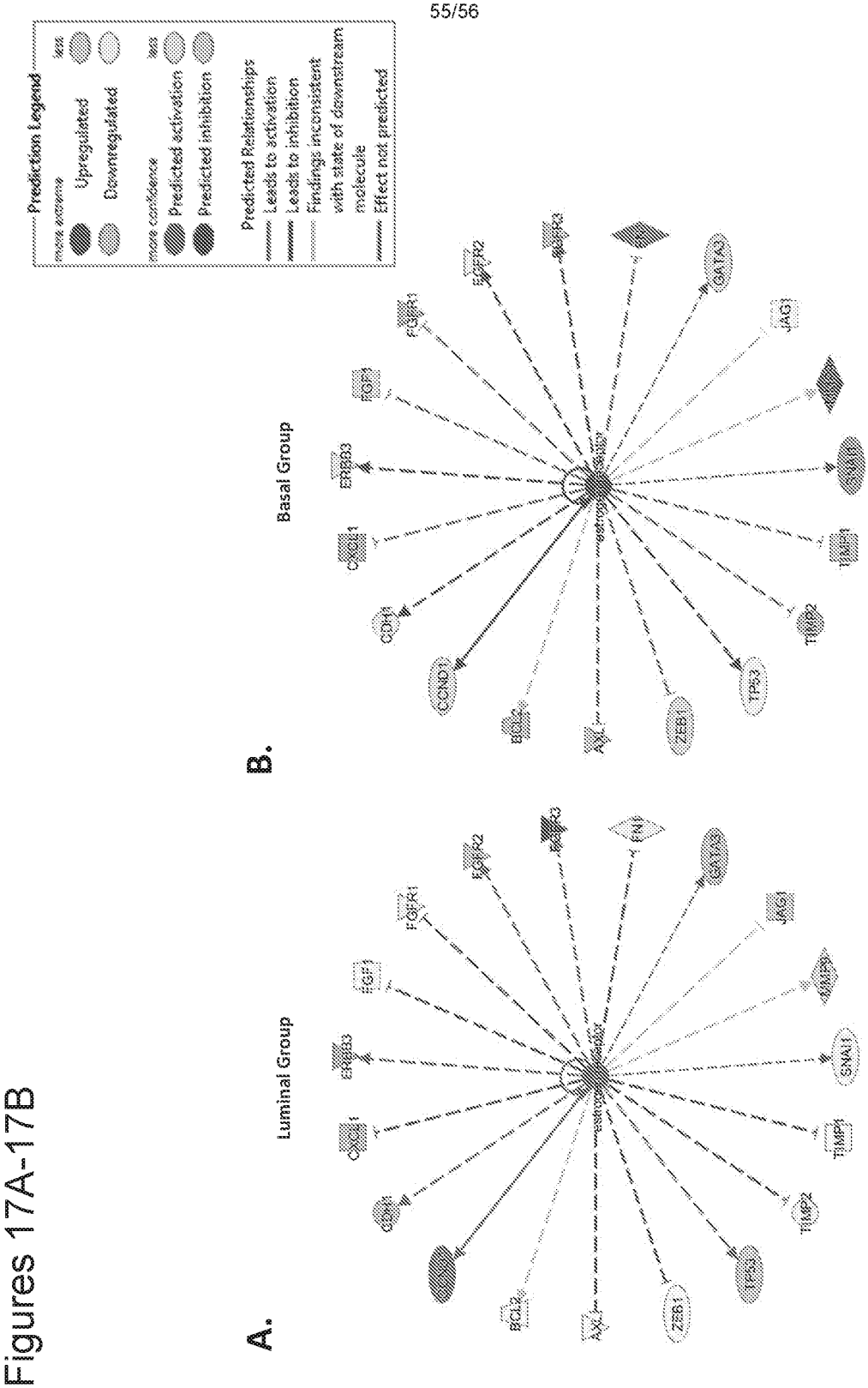
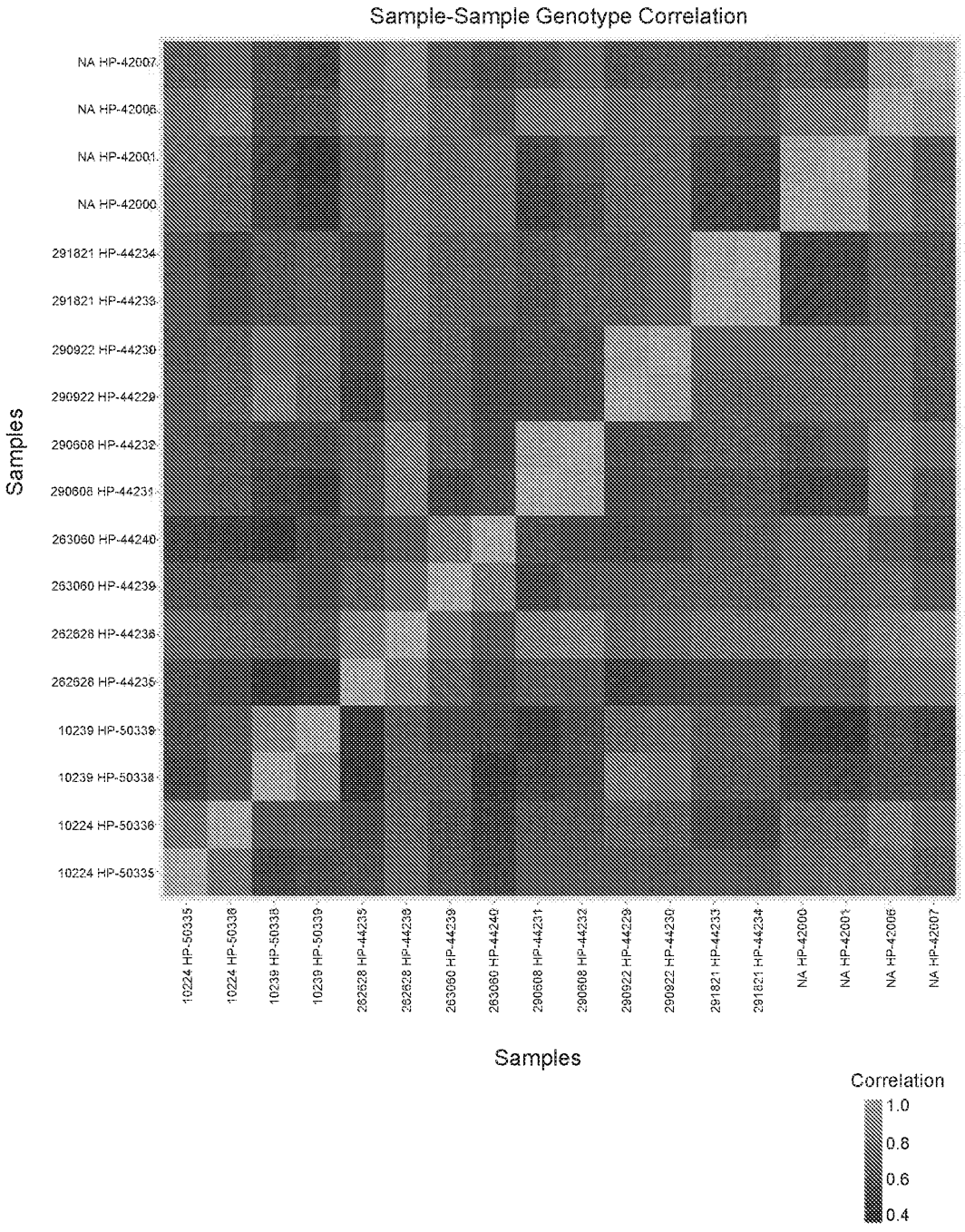


Figure 18


Sample-Sample Genotype Correlation
Samples
NA HP-42007
NA HP-42006
NA HP-42001
NA HP-42000
291821 HP-44234
291821 HP-44233
290922 HP-44230
290922 HP-44229
290608 HP-44232
290608 HP-44231
263060 HP-44240
263060 HP-44239
262628 HP-44238
262628 HP-44235
10239 HP-50339
10239 HP-50338
10224 HP-50336
10224 HP-50335
10224 HP-50335
10224 HP-50338
10239 HP-50338
10239 HP-50339
262628 HP-44235
262628 HP-44238
263060 HP-44239
263060 HP-44240
290608 HP-44231
290608 HP-44232
290922 HP-44229
290922 HP-44230
291821 HP-44233
291821 HP-44234
NA HP-42000
NA HP-42001
NA HP-42006
NA HP-42007
Samples
Correlation
1.0
0.8
0.6
0.4