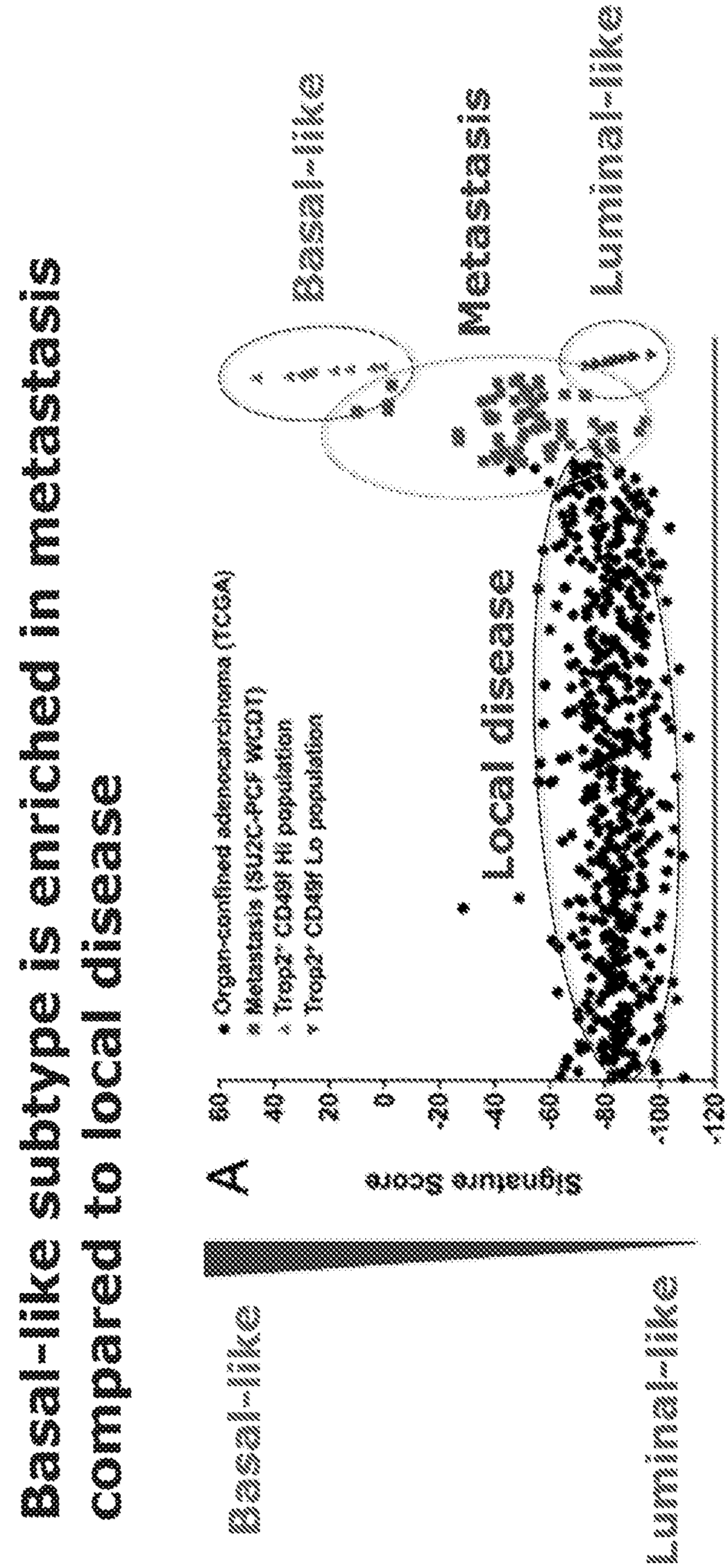


Modified from Smith et al., PNAS (2013)

FIG. 1A

Function Differences Between Luminal and Basal Subtypes in Prostate

Category	Description
1	Neural and neuronal development
2	Cell adhesion, extracellular matrix (ECM), cytoskeleton (CSK) and cell motility
3	Development and stem cells
4	Transcription/nuclear factors, and epigenetic regulators
5	Small nucleolar RNAs (snoRNAs)
6	RNA metabolism, ribosome biogenesis, and protein translation
7	Signal transduction
8	Cytokines, immunity and inflammation
9	Non-coding RNAs (pseudogenes, asRNAs, miRNAs)
10	Intermediate metabolism and transporters
11	Others
12	Androgen-responsive gene (ARGs) and AR regulated gene (ARRGs)
13	Prostate functions (steroidogenesis, prostaglandin and phospholipid synthesis, intermediate metabolism, secretion, transport, reproduction)

Basal DEG (%)	
1	11.14
2	15.59
3	9.26
4	6.80
5	4.45
6	10.08
7	14.77
8	4.57
9	1.76
10	6.57
11	15.01

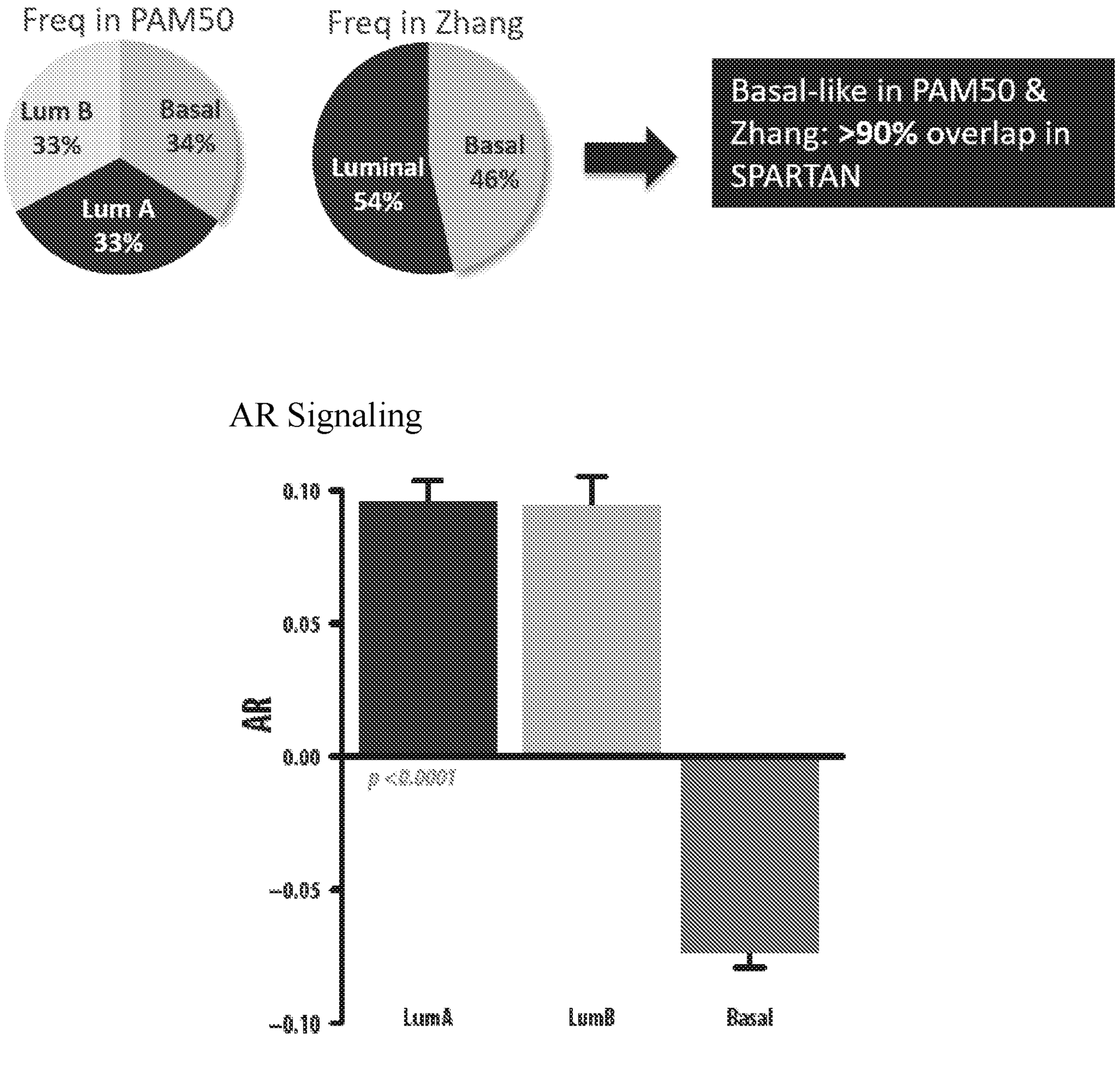
Luminal DEG (%)	
1	7.55
2	8.72
3	3.30
4	4.04
12	10.64
13	24.57
7	16.91
8	8.30
9	1.38
11	14.60

4 + 5 + 6: Transcription/RNA biogenesis
12 + 13: Prostate/androgen function

Adapted from Zhang et al., Nat Commun 2016

FIG. 1B

Molecular Subtypes of Prostate Tumors: PAM50 vs. Zhang



Adapted from Zhao et al., JAMA Oncol. 2017

FIG. 2

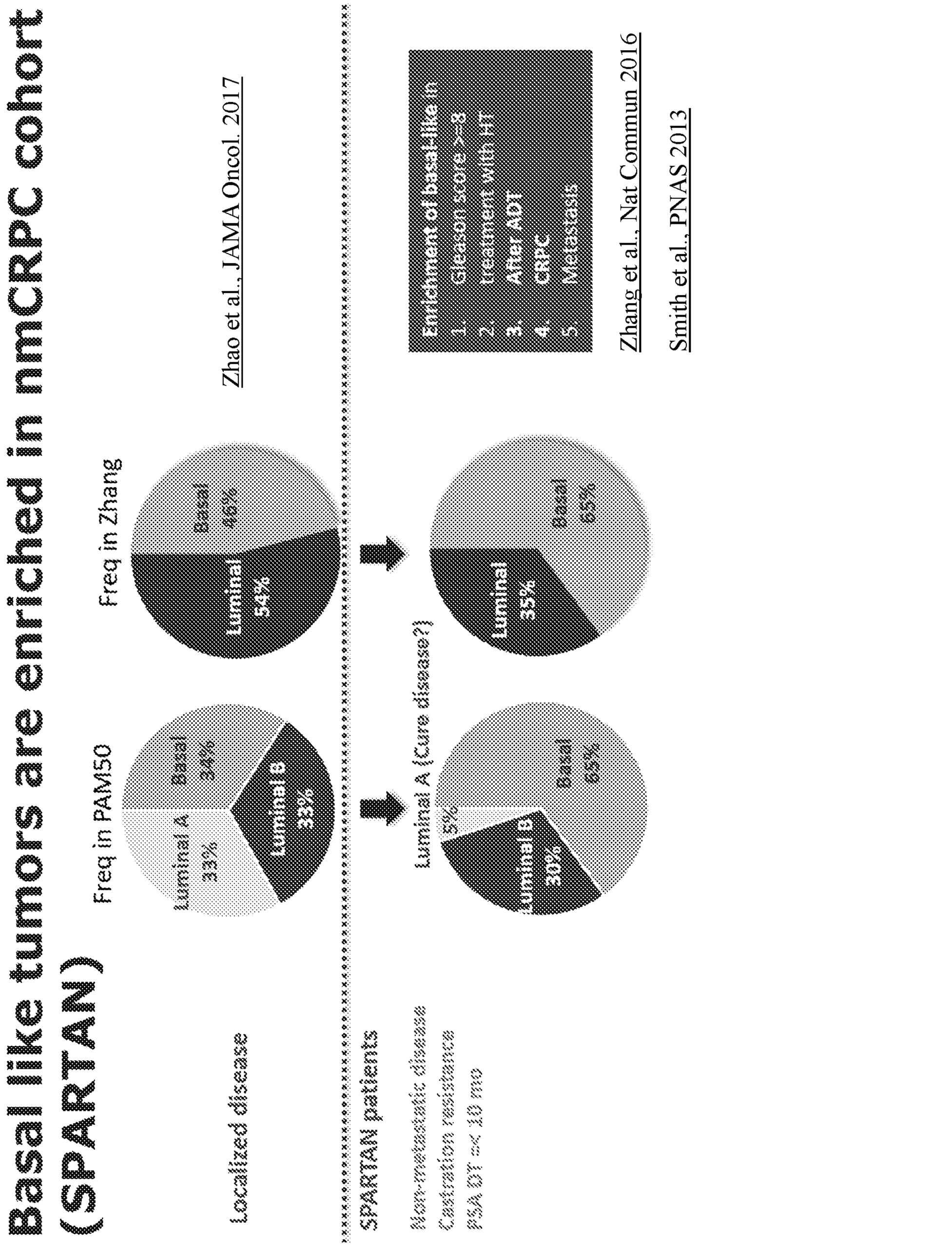
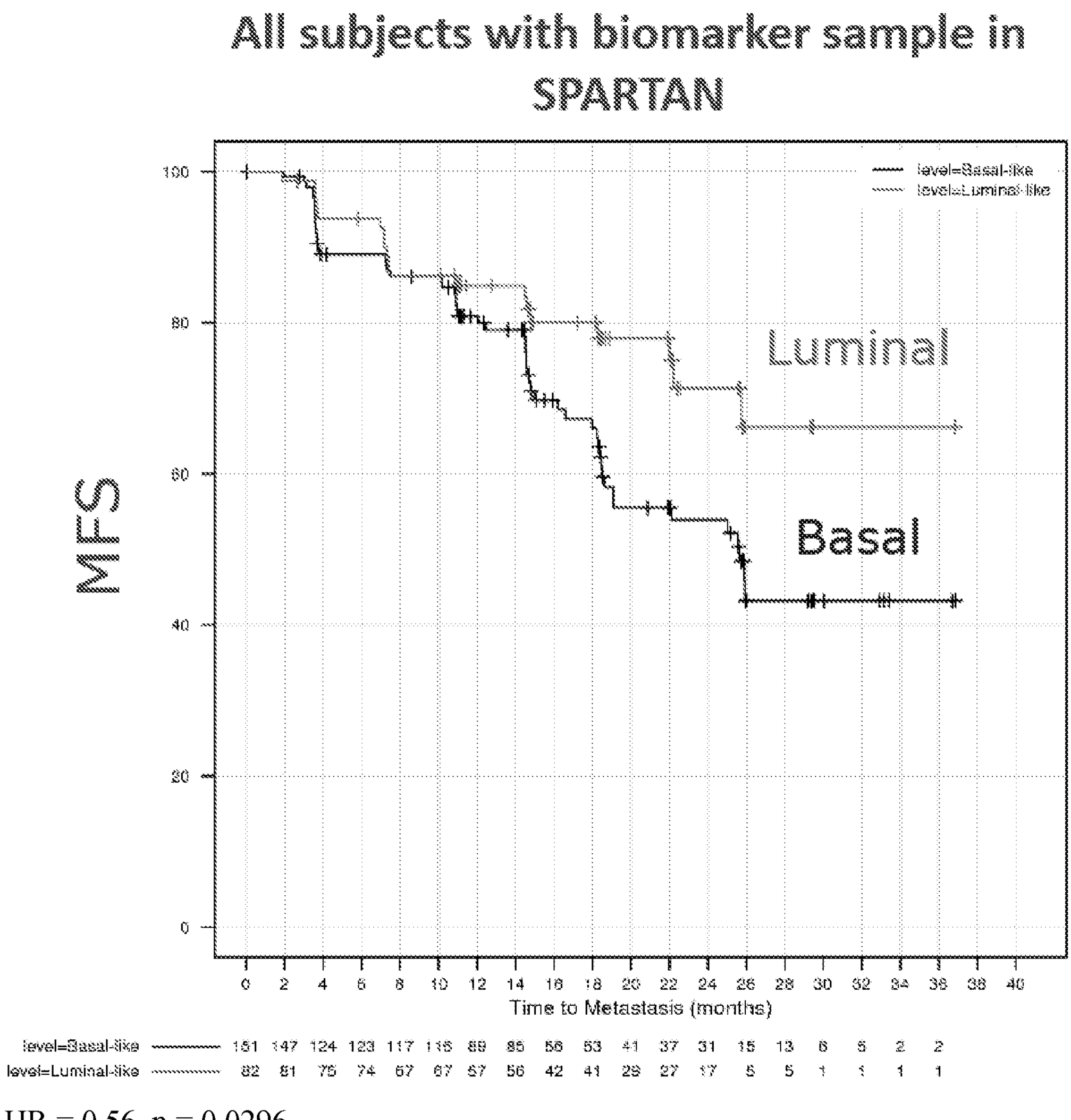

Basal like tumors are enriched in mCRPC cohort
(SPARTAN)
Freq in PAM50
Luminal A 33%
Basal 34%
Luminal B 33%
Freq in Zhang
Luminal 54%
Basal 46%
Zhao et al., JAMA Oncol. 2017
Luminal A (Cure disease?)
5%
Luminal B 30%
Basal 65%
Luminal 35%
Basal 65%
Zhang et al., Nat Commun 2016
Enrichment of basal like in
1. Gleason score ≥8
2. treatment with HT
3. After ADT
4. CRPC
5. Metastasis
Smith et al., PNAS 2013
Localized disease
SPARTAN patients
Non-metastatic disease
Castration resistance
PSA DT =< 10 mo

FIG. 3



HR = 0.56, p = 0.0296
Median MFS of 25.6 m (Basal) vs. unreached (Luminal)

FIG. 4

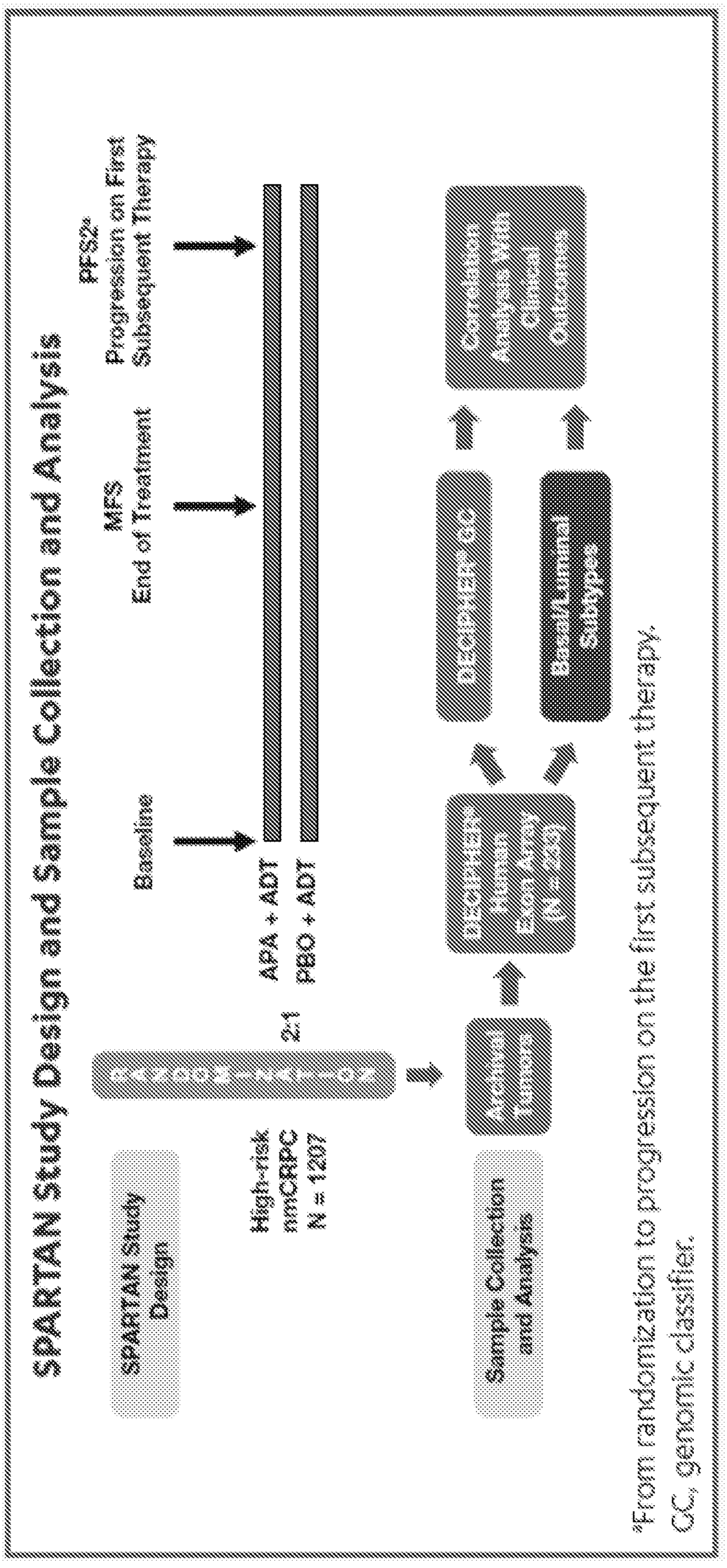

SPARTAN Study Design and Sample Collection and Analysis
SPARTAN Study Design
High-risk nmCRPC N = 1207
Baseline
2:1
APA + ADT
PBO + ADT
End of Treatment
MFS
PFS2*
Progression on First Subsequent Therapy
Sample Collection and Analysis
Archival Tumor
Baseline Blood
Enzalutamide
Apalutamide
Darolutamide
Cabazitaxel
Docetaxel
Other
Subsequent therapy
*From randomization to progression on the first subsequent therapy
GC, genomic classifier.

FIG. 5

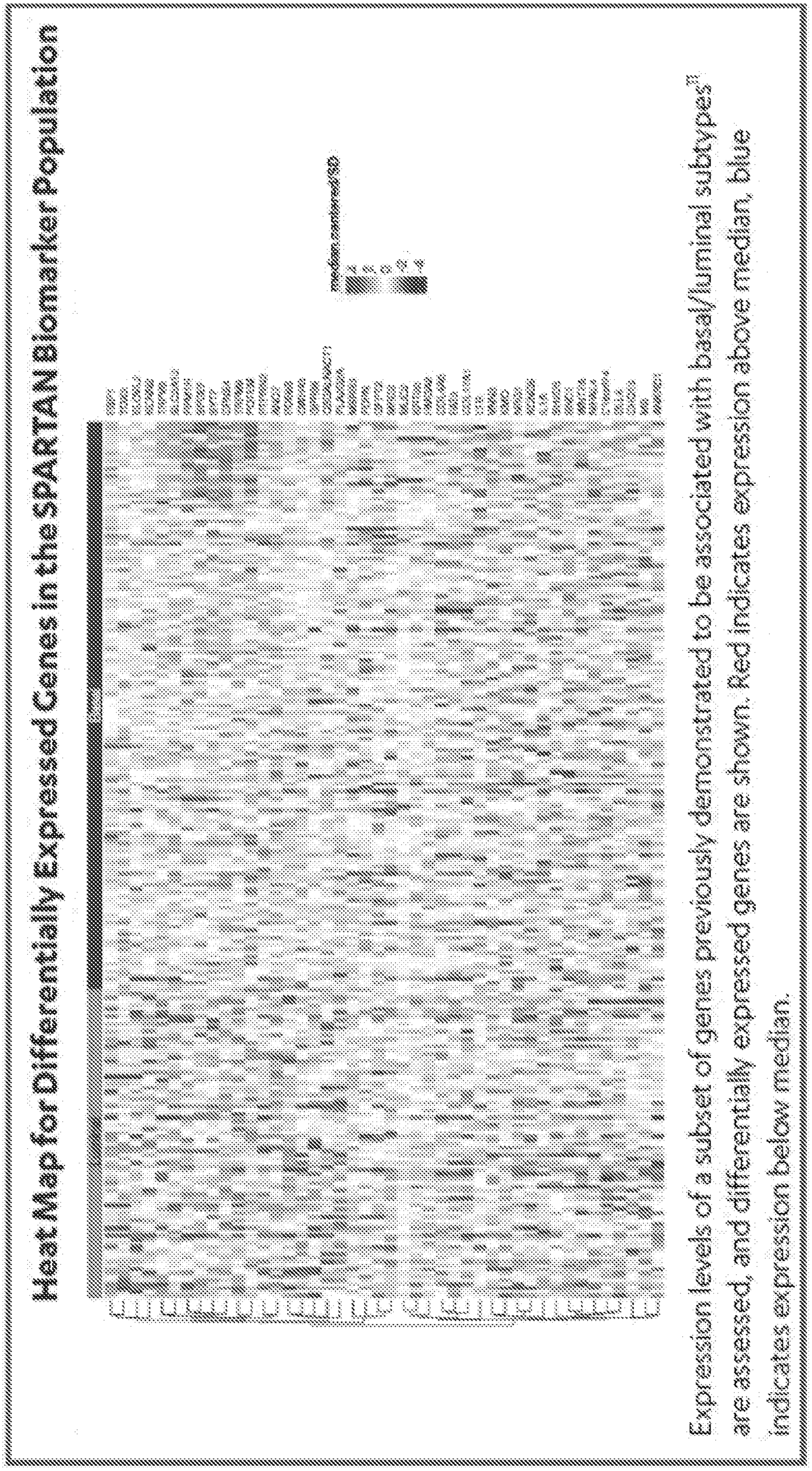

Heat Map for Differentially Expressed Genes in the SPARTAN Biomarker Population
Expression levels of a subset of genes previously demonstrated to be associated with basal/luminal subtypes are assessed, and differentially expressed genes are shown. Red indicates expression above median, blue indicates expression below median.

FIG. 6

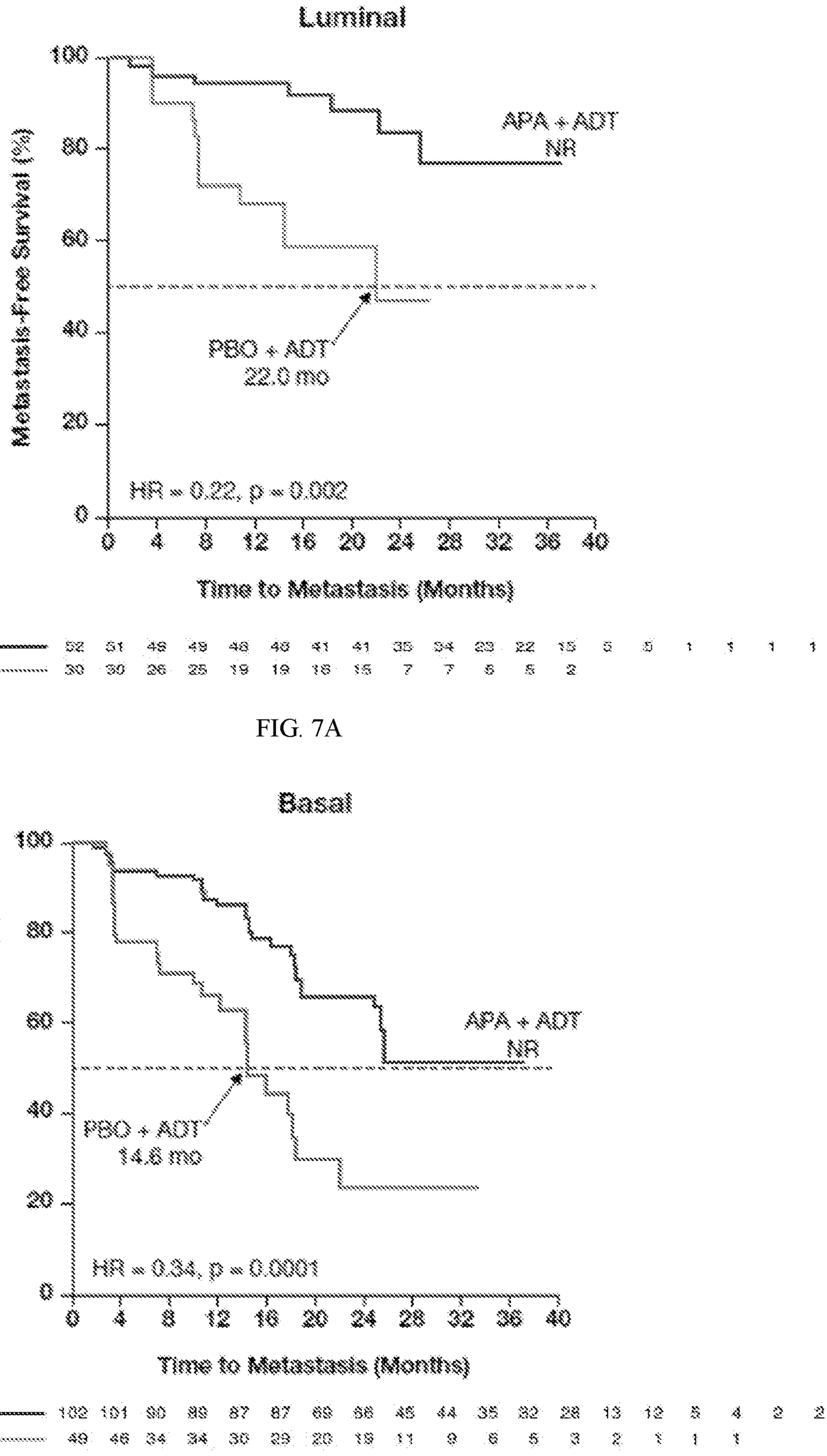

Luminal
Metastasis-Free Survival (%)
100
80
60
40
20
0
APA + ADT
NR
PBO + ADT
22.0 mo
HR = 0.22, p = 0.002
0 4 8 12 16 20 24 28 32 36 40
Time to Metastasis (Months)
Apalutamide 52 51 49 49 48 40 41 41 35 34 23 22 15 5 5 1 1 1 1
Placebo 30 30 26 25 19 19 16 15 7 7 5 5 2

FIG. 7A

Basal
Metastasis-Free Survival (%)
100
80
60
40
20
0
APA + ADT
NR
PBO + ADT
14.6 mo
HR = 0.34, p = 0.0001
0 4 8 12 16 20 24 28 32 36 40
Time to Metastasis (Months)
Apalutamide 102 101 90 89 87 87 69 66 45 44 35 32 28 13 12 5 4 2 2
Placebo 49 46 34 34 30 29 20 18 11 9 6 5 3 2 1 1 1

FIG. 7B

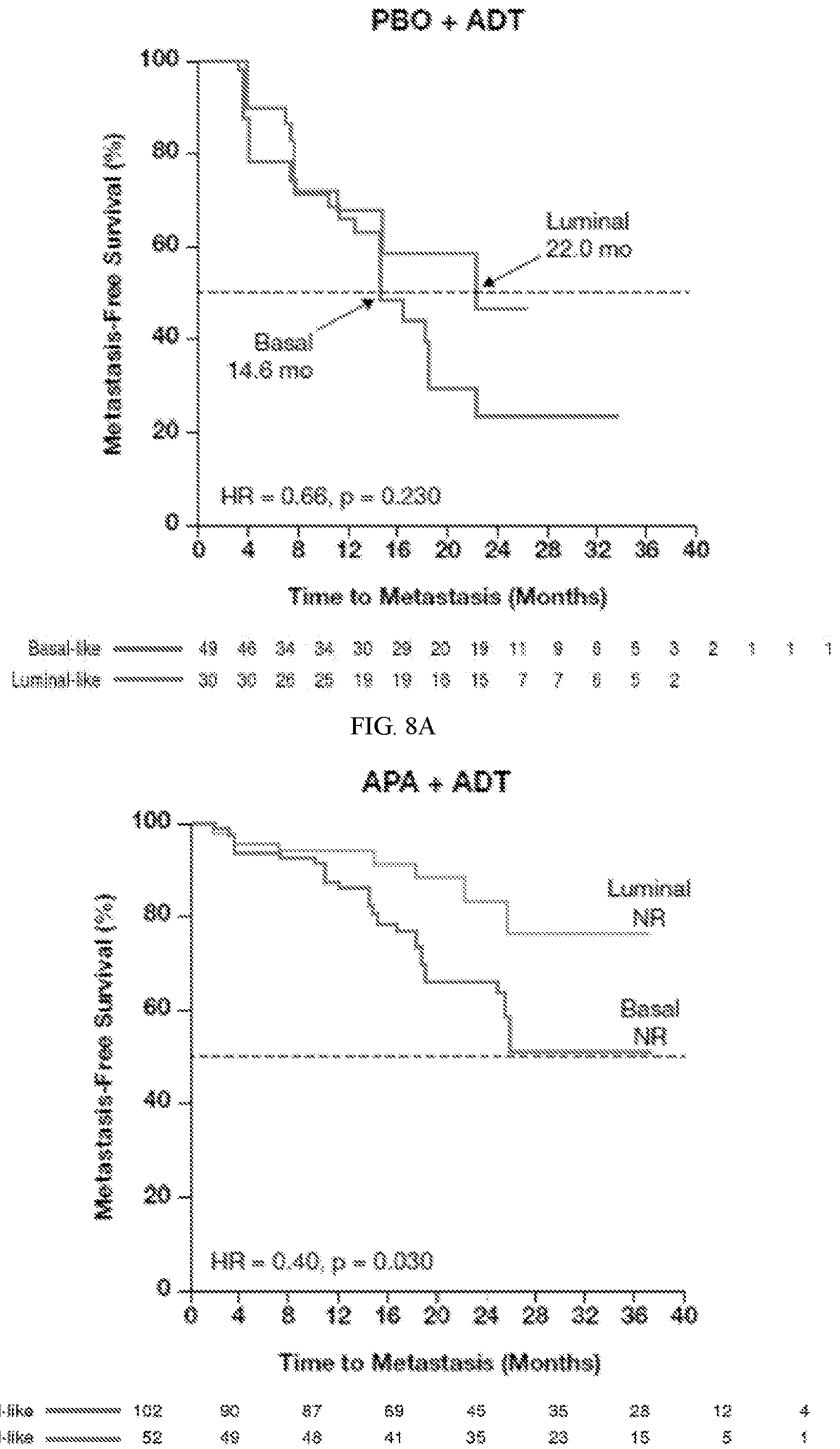

PBO + ADT
Metastasis-Free Survival (%)
100
80
60
40
20
0
Luminal
22.0 mo
Basal
14.6 mo
HR = 0.66, p = 0.230
0 4 8 12 16 20 24 28 32 36 40
Time to Metastasis (Months)
Basal-like 49 46 34 34 30 29 20 19 11 9 6 6 3 2 1 1 1
Luminal-like 30 30 26 25 19 19 16 15 7 7 6 5 2
APA + ADT
Metastasis-Free Survival (%)
100
80
60
40
20
0
Luminal
NR
Basal
NR
HR = 0.40, p = 0.030
0 4 8 12 16 20 24 28 32 36 40
Time to Metastasis (Months)
Basal-like 102 90 67 69 45 35 28 12 4 2
Luminal-like 52 49 48 41 35 23 15 5 1 1

FIG. 8A

FIG. 8B

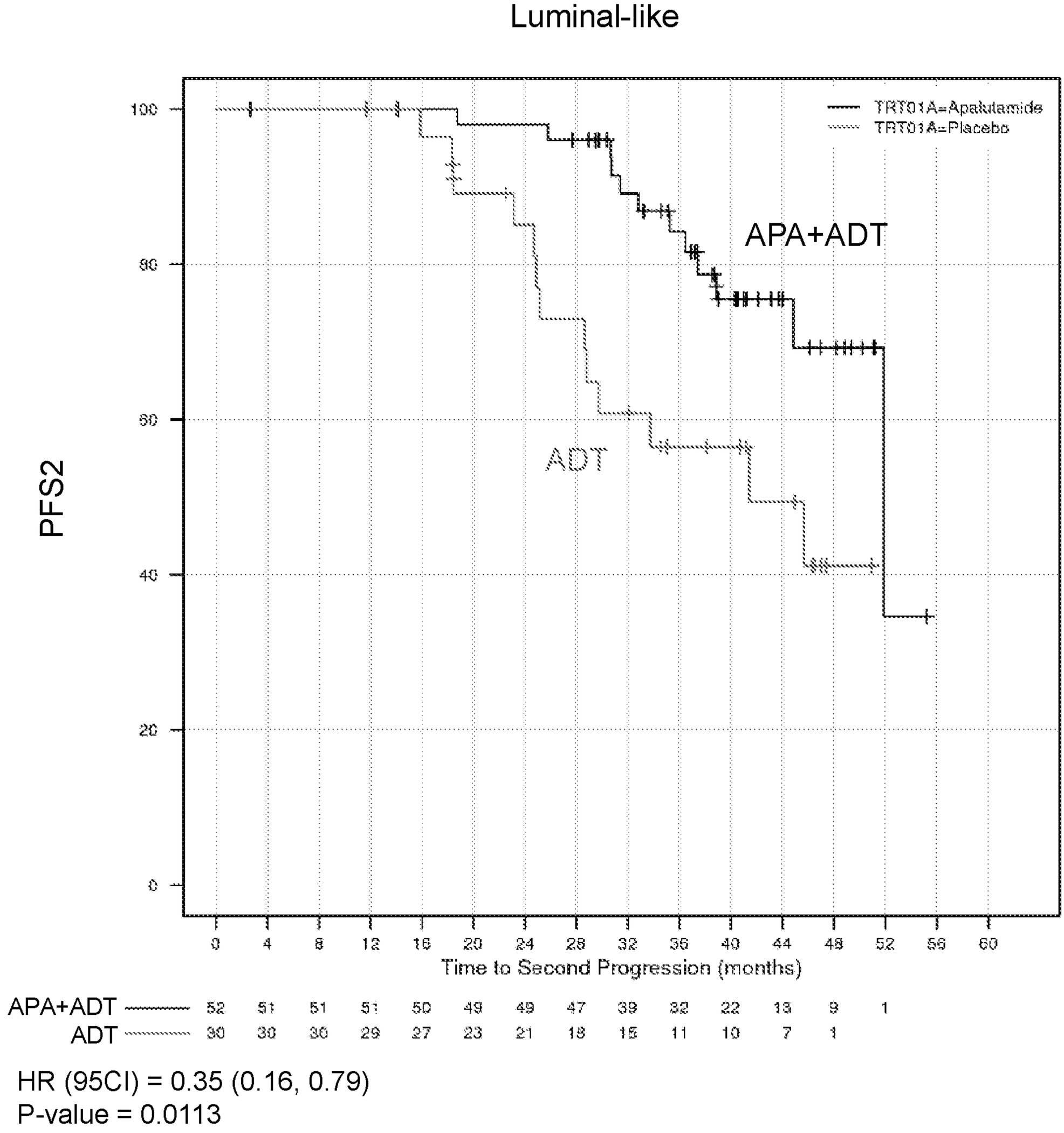

Luminal-like
PFS2
100
80
60
40
20
0
TRT01A=Apalutamide
TRT01A=Placebo
APA+ADT
ADT
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
Time to Second Progression (months)
APA+ADT 52 51 51 51 50 49 49 47 39 32 22 13 9 1
ADT 30 30 30 29 27 23 21 18 15 11 10 7 1
HR (95CI) = 0.35 (0.16, 0.79)
P-value = 0.0113

FIG. 9A

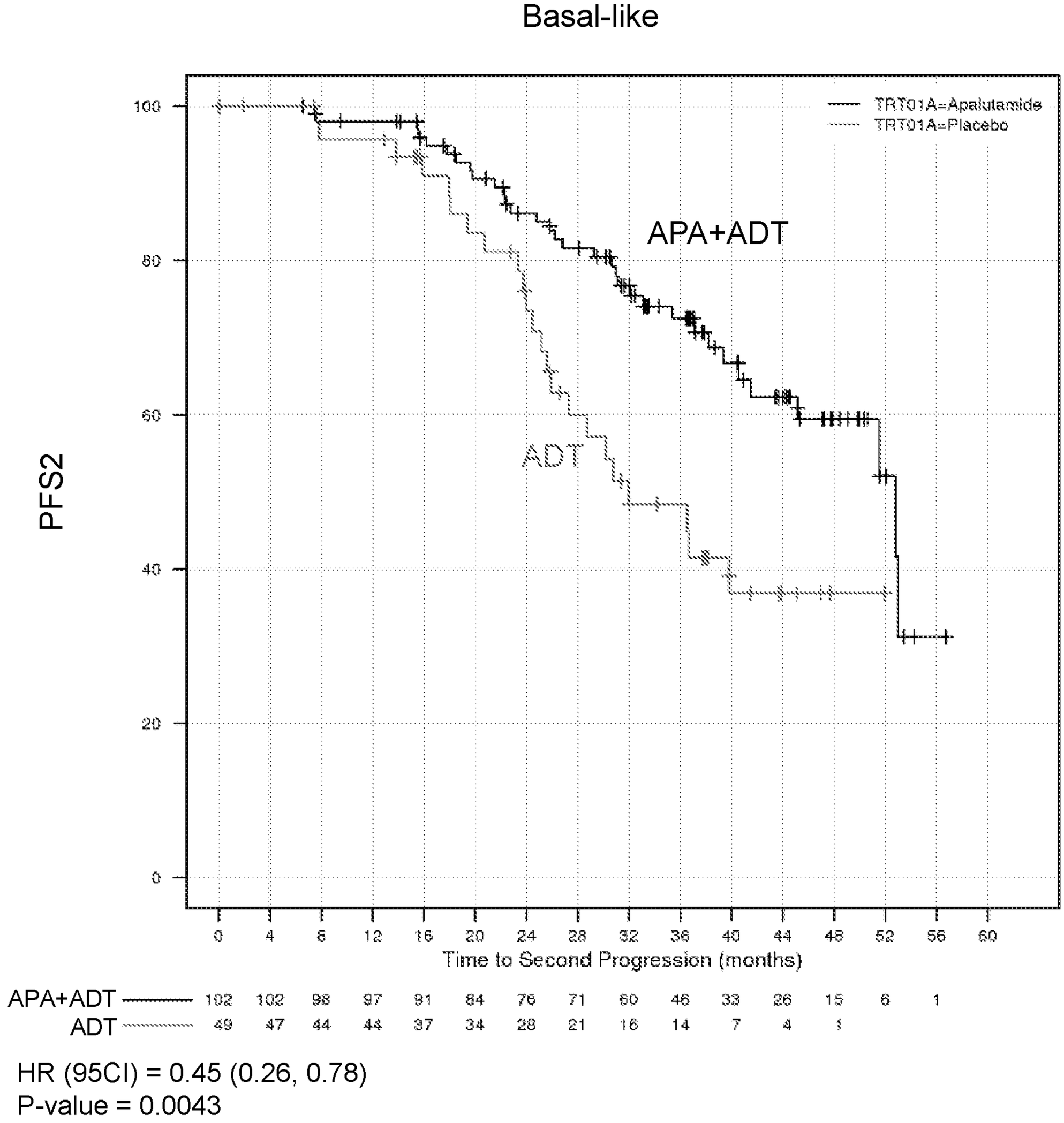

Basal-like
TRT01A=Apalutamide
TRT01A=Placebo
APA+ADT
ADT
PFS2
100
80
60
40
20
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
Time to Second Progression (months)
APA+ADT 102 102 98 97 91 84 76 71 60 46 33 26 15 6 1
ADT 49 47 44 44 37 34 28 21 16 14 7 4 1
HR (95CI) = 0.45 (0.26, 0.78)
P-value = 0.0043

FIG. 9B

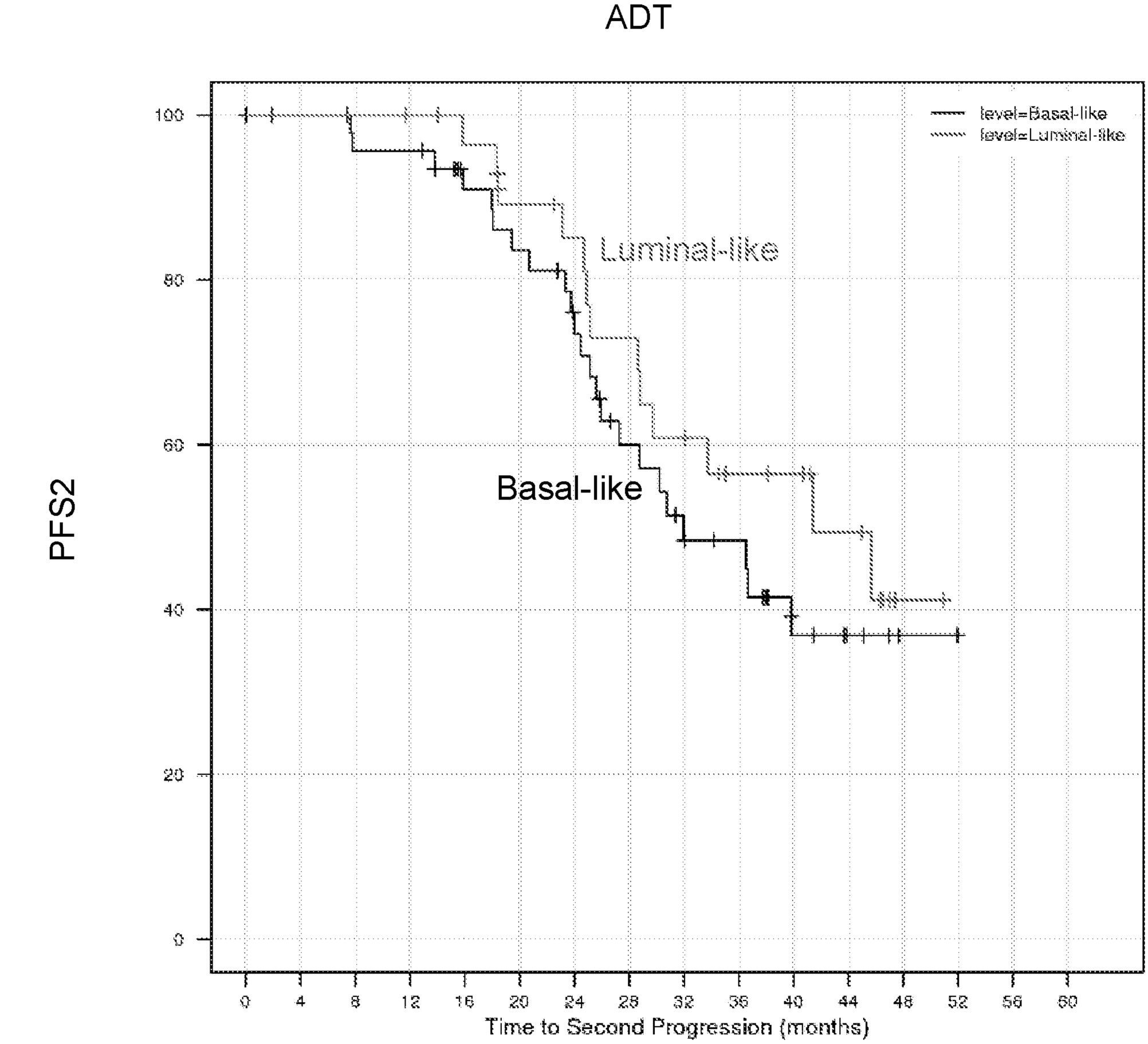

ADT
level=Basal-like
level=Luminal-like
Luminal-like
Basal-like
PFS2
100
80
60
40
20
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
Time to Second Progression (months)
Basal-like 49 47 44 44 37 34 28 21 16 14 7 4 1
Luminal-like 30 30 30 29 27 23 21 18 15 11 10 7 1
HR (95CI) = 0.72 (0.36, 1.42)
P-value = 0.3415

FIG. 9C

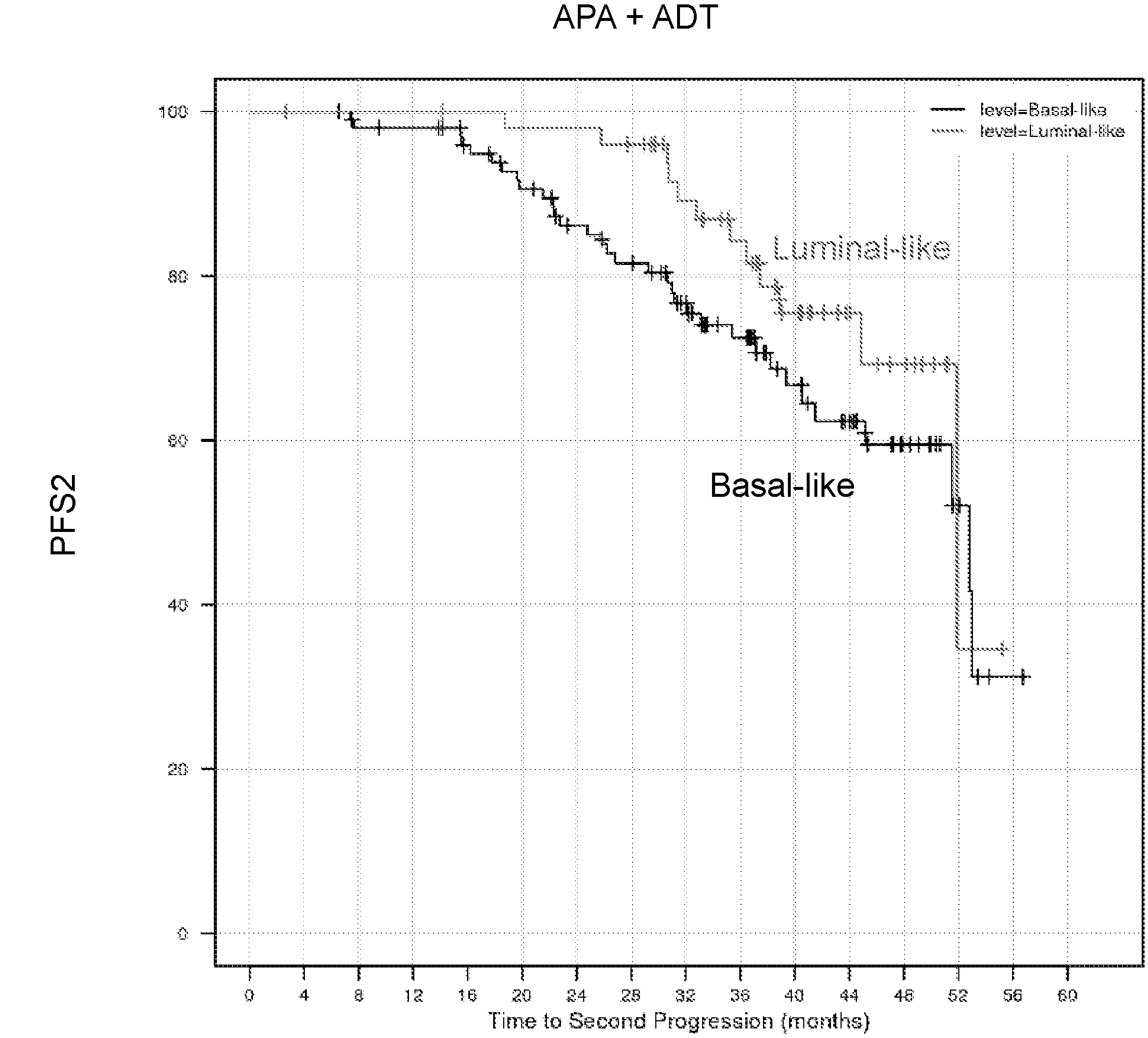
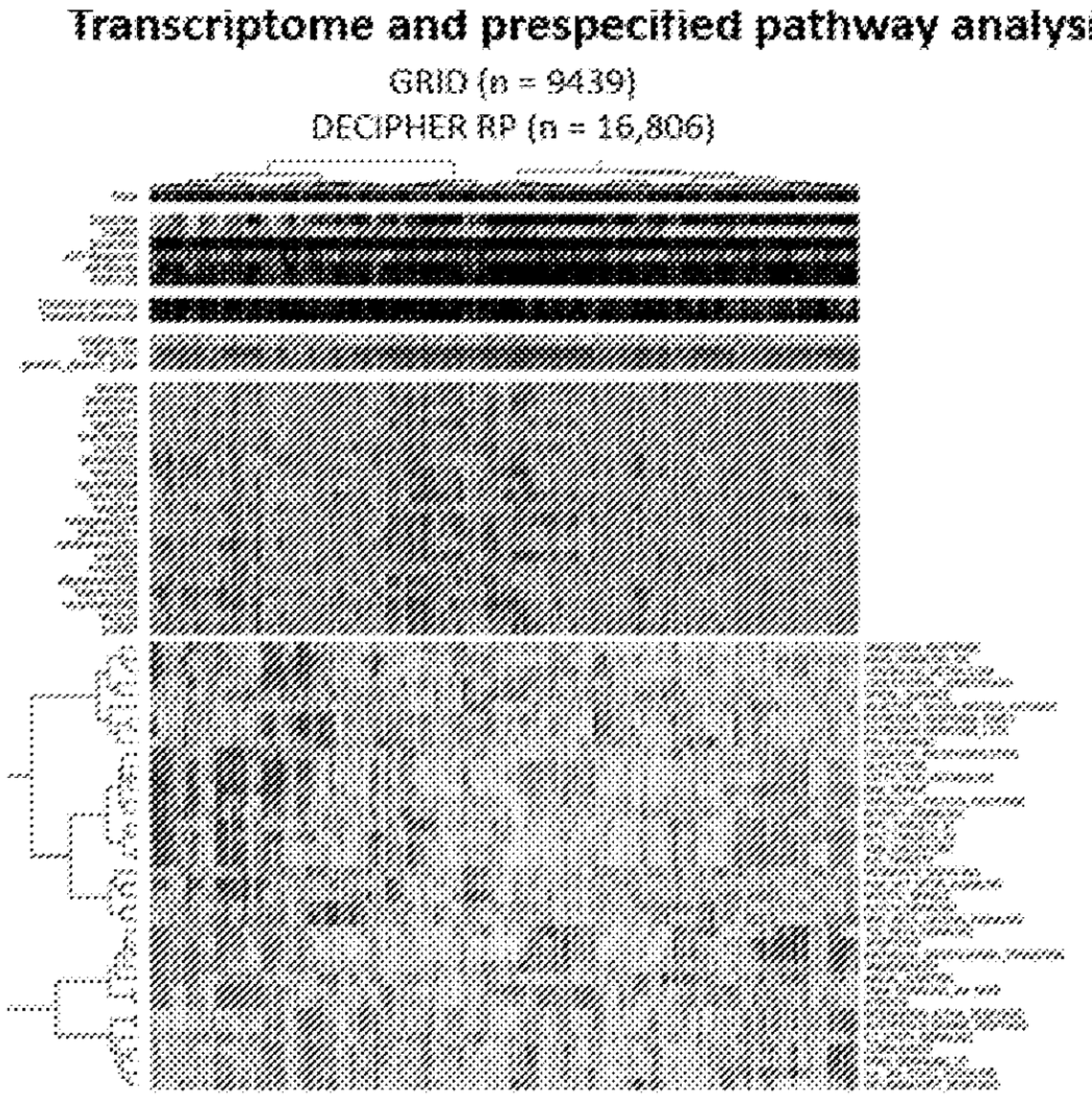

APA + ADT
level=Basal-like
level=Luminal-like
Luminal-like
Basal-like
PFS2
100
80
60
40
20
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
Time to Second Progression (months)
Basal-like 102 102 98 97 91 84 76 71 60 46 33 26 15 6 1
Luminal-like 52 51 51 51 50 49 49 47 39 32 22 13 9 1
HR (95CI) = 0.62 (0.32, 1.21)
P-value = 0.1601

FIG. 9D



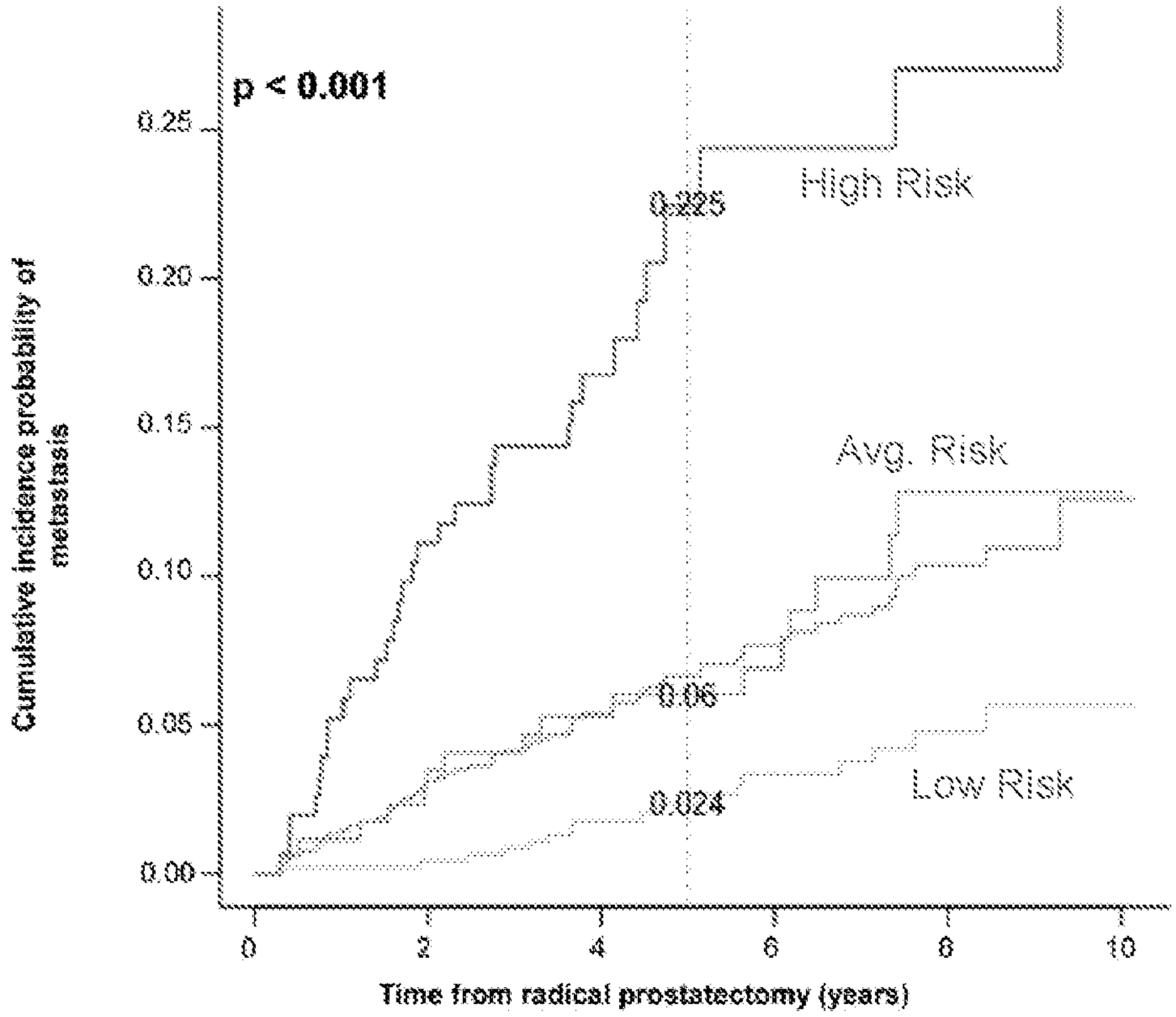
Multivariate analysis

Pathways Associated With Basal Subtype	
Decipher high risk	Genomic risk classifier
Androgen receptor activity low	Low sensitivity to ADT
Small-cell prostate cancer (NEPC)	**Neuroendocrine differentiation**
pRB Loss	
Kegg_Homologous_Recombination	DNA repair
PORTOS-Higher RT response	Radiation sensitivity
Hypoxia signature	Angiogenesis
Angiogenesis_Signature	
EMT Signature (Wnt-B-catenin)	**EMT/metastasis**
TGF beta	
Inflammatory_response	**Immune response**
IFN_alpha_response	
IFN_gamma_response	

INF, interferon. TGF, tumor growth factor.

FIG. 10

DECIPHER® GC Is Associated with Metastasis



Karnes et al., J Urol. 2013

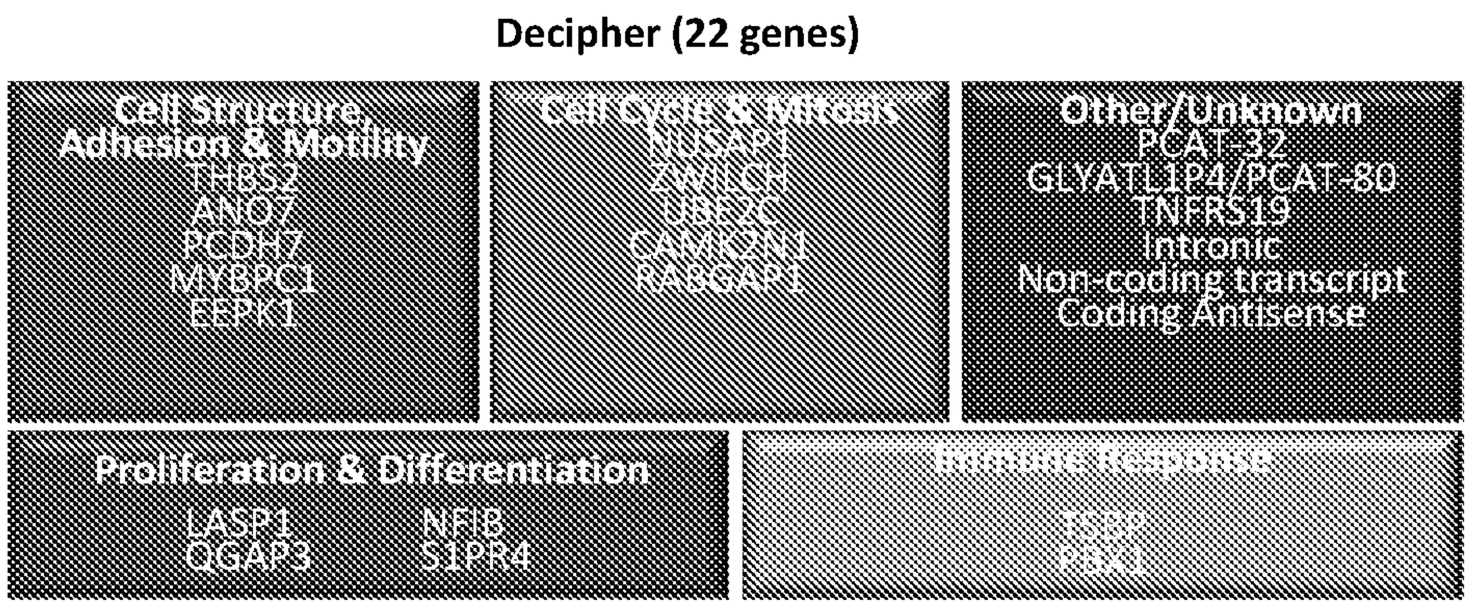


FIG. 11

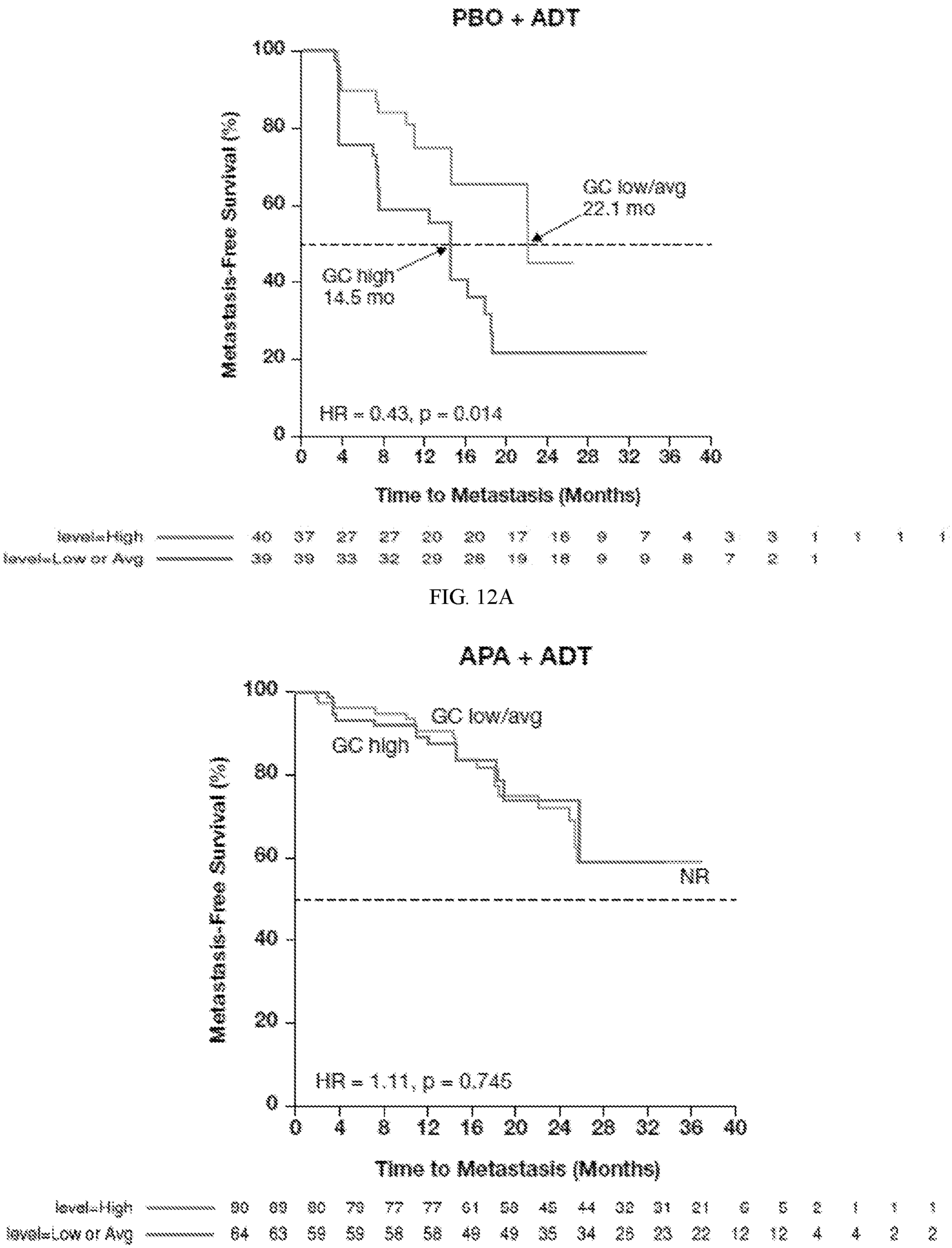


FIG. 12A

FIG. 12B

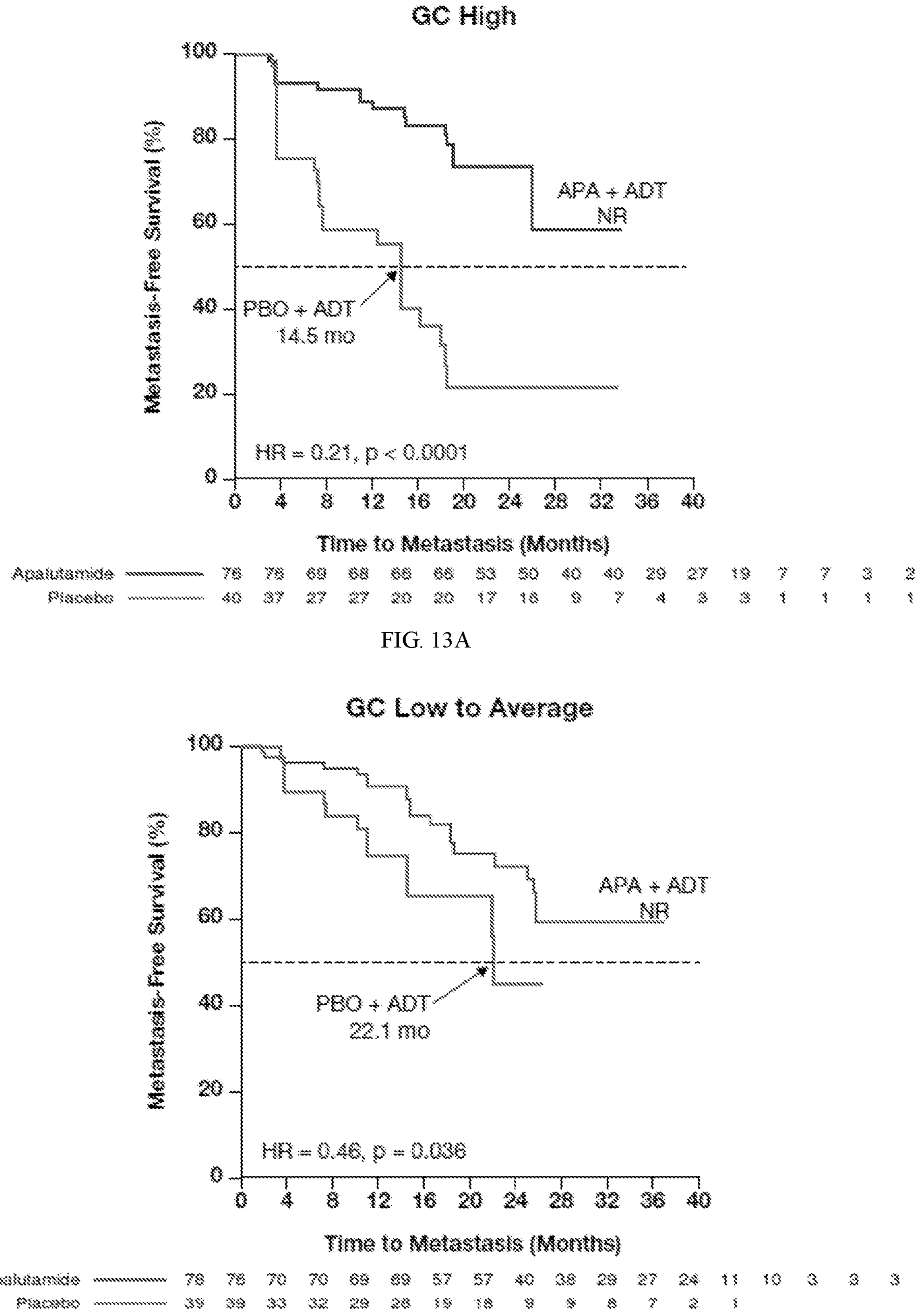


FIG. 13A

FIG. 13B

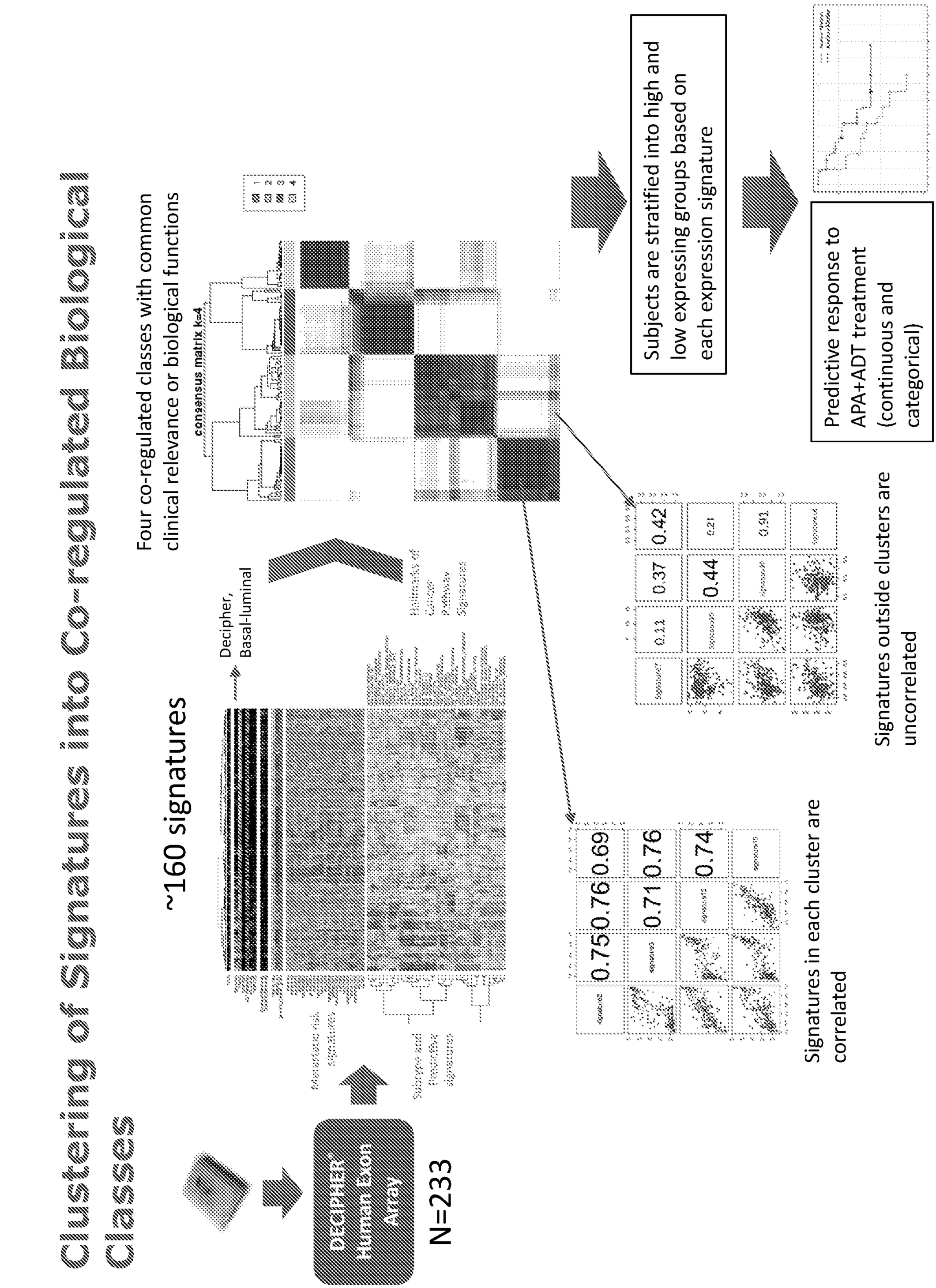
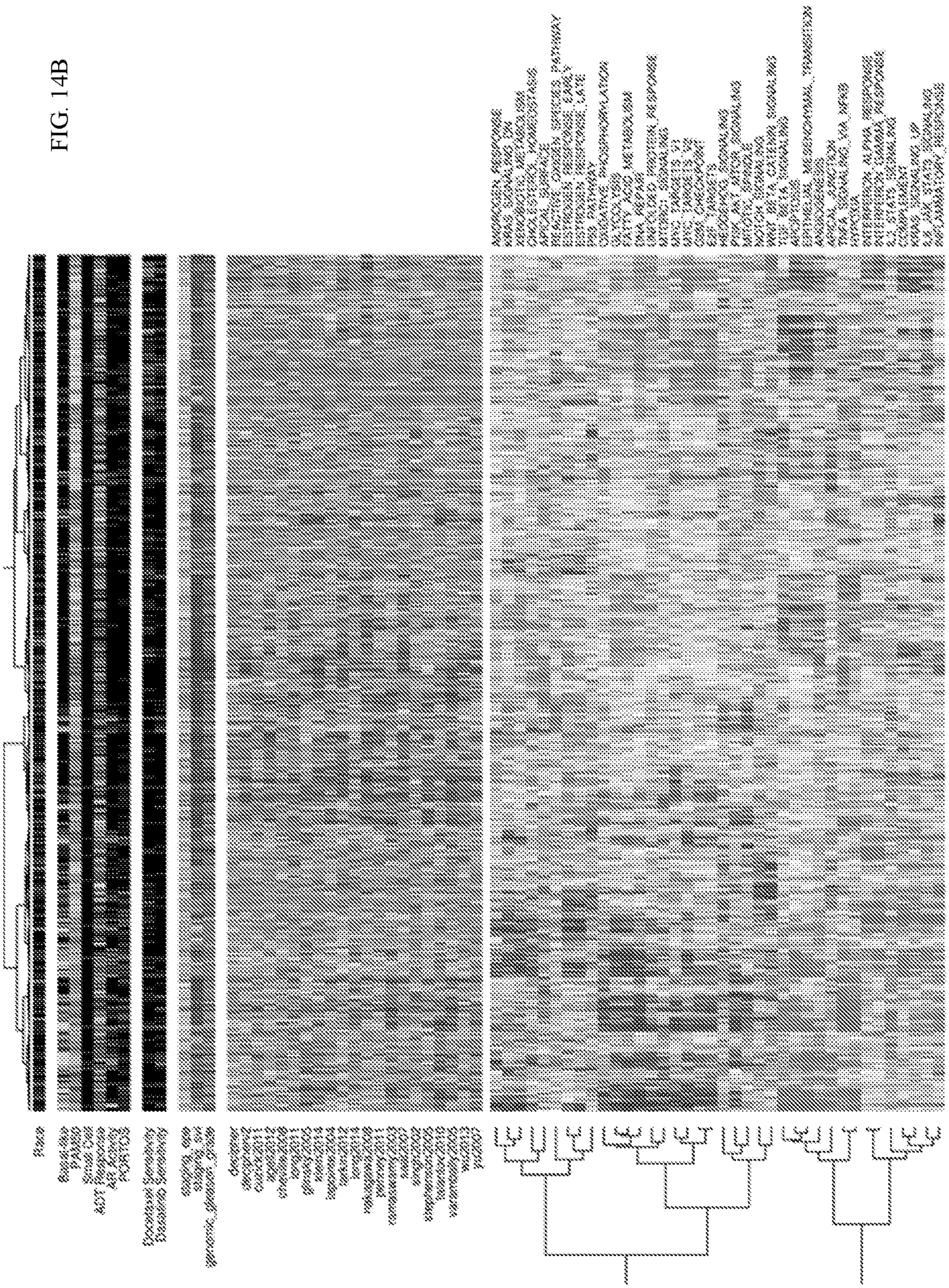

Clustering of Signatures into Co-regulated Biological Classes
Four co-regulated classes with common clinical relevance or biological functions
consensus matrix k=4
Decipher, Basal-luminal
~160 signatures
Signatures in each cluster are correlated
Signatures outside clusters are uncorrelated
Subjects are stratified into high and low expressing groups based on each expression signature
Predictive response to APA+ADT treatment (continuous and categorical)
Clustering Classes
DECIPHER® Human Exon Array
N=233

FIG. 14A

FIG. 14B



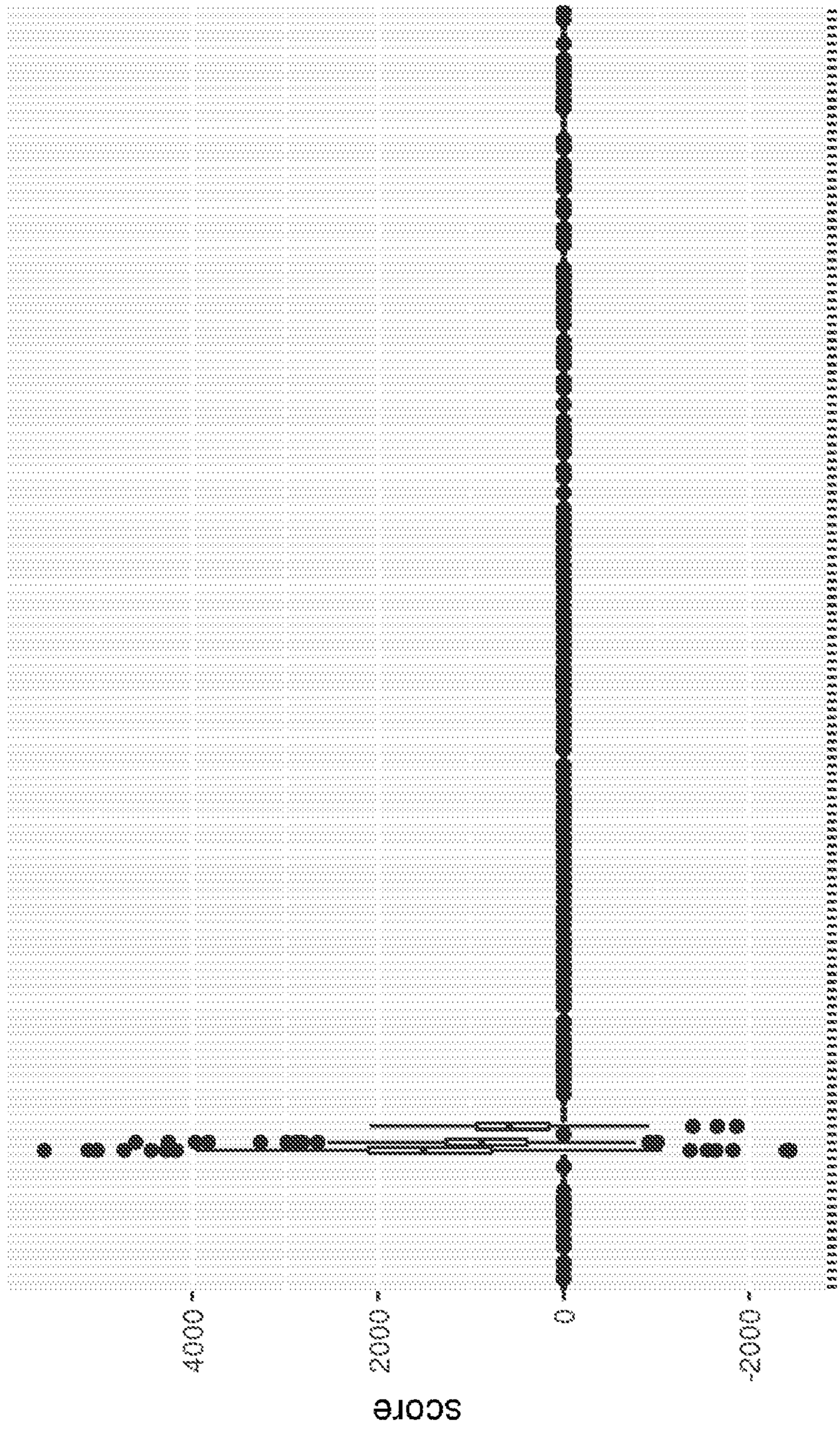

SPARTAN signature raw score
4000
2000
0
-2000
score
signature

FIG. 14C

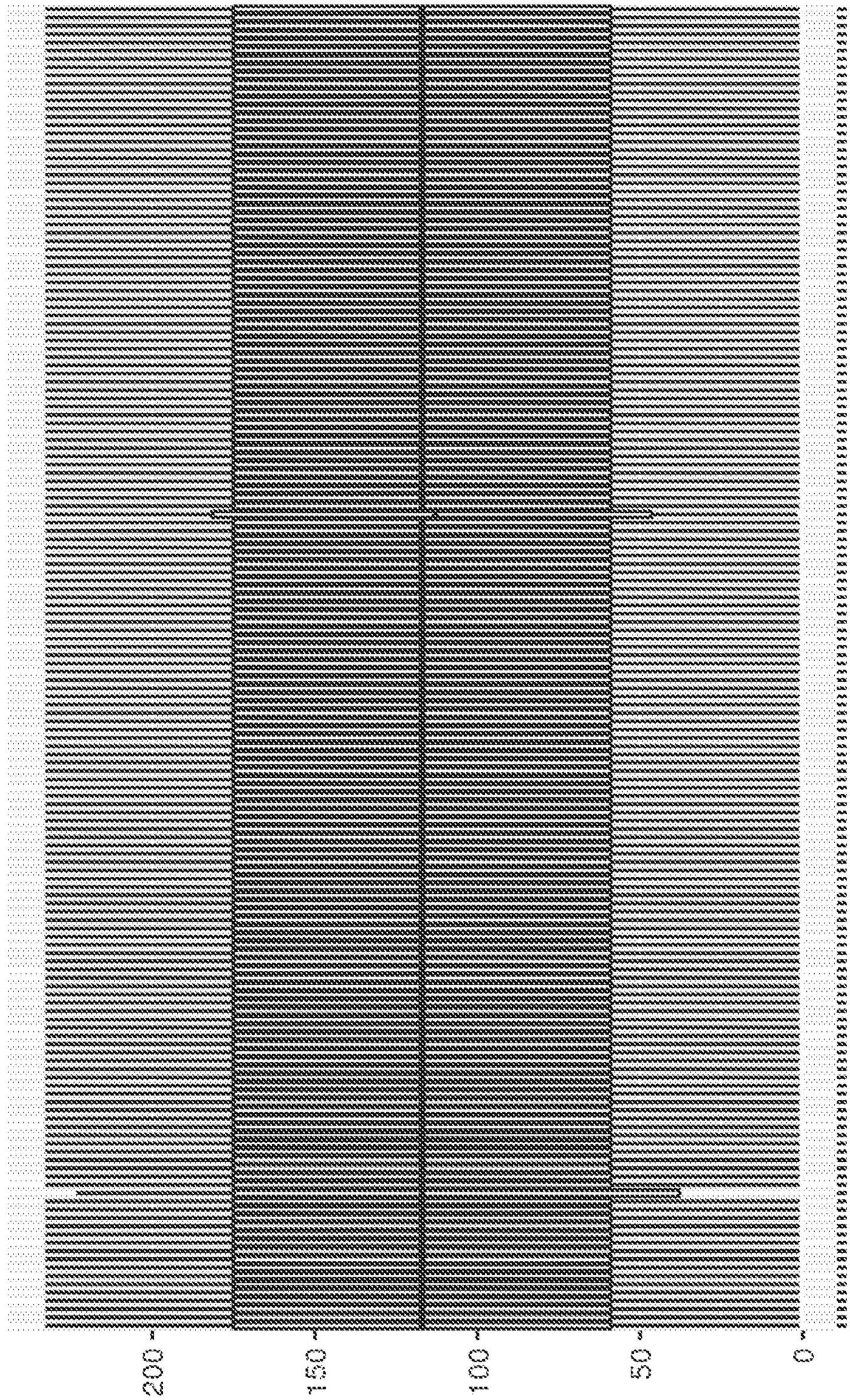


FIG. 14D

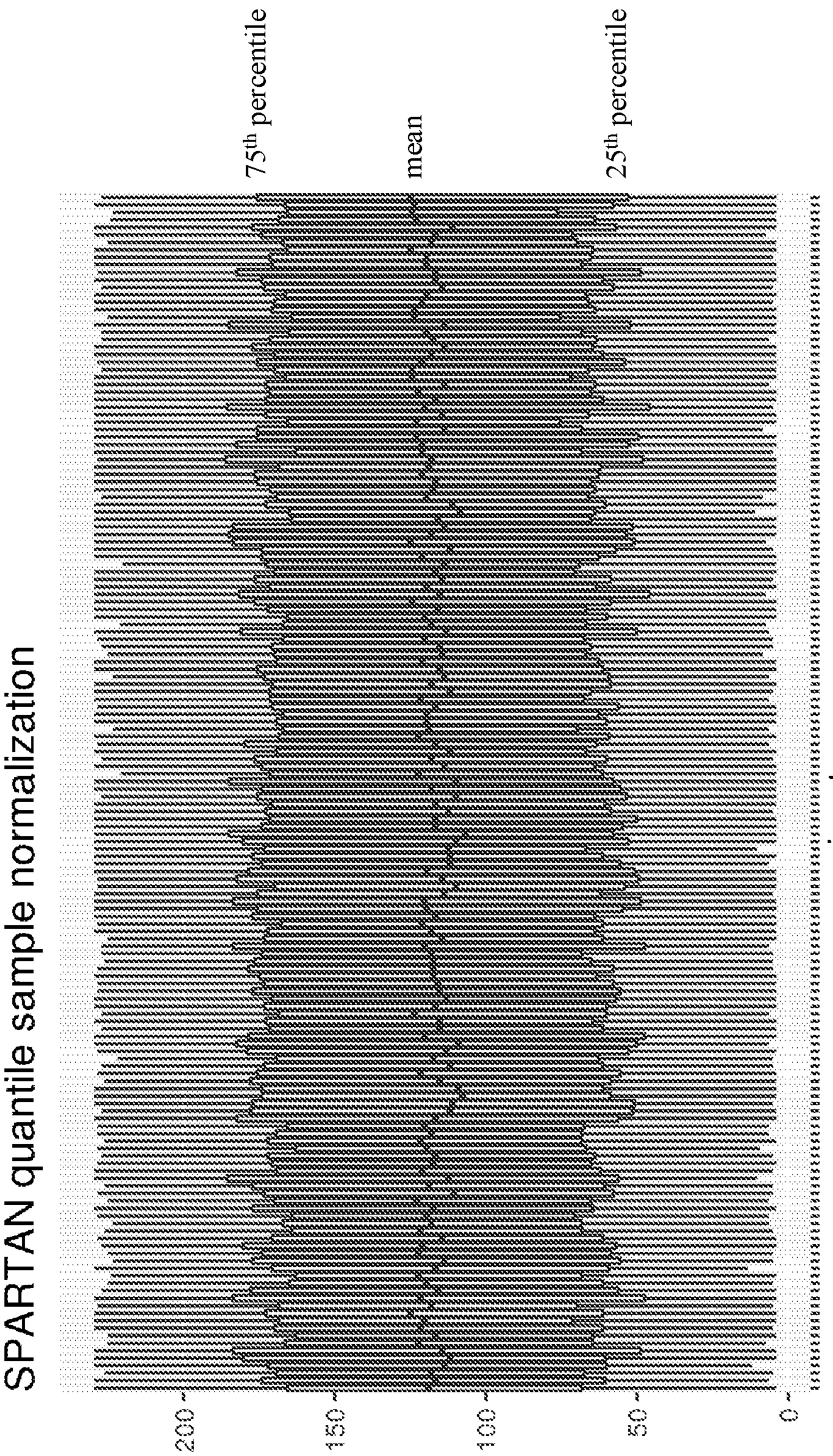

75th percentile
mean
25th percentile
normalization
signature
SPARTAN quantile sample n
value
200
150
100
50
0

FIG. 14E

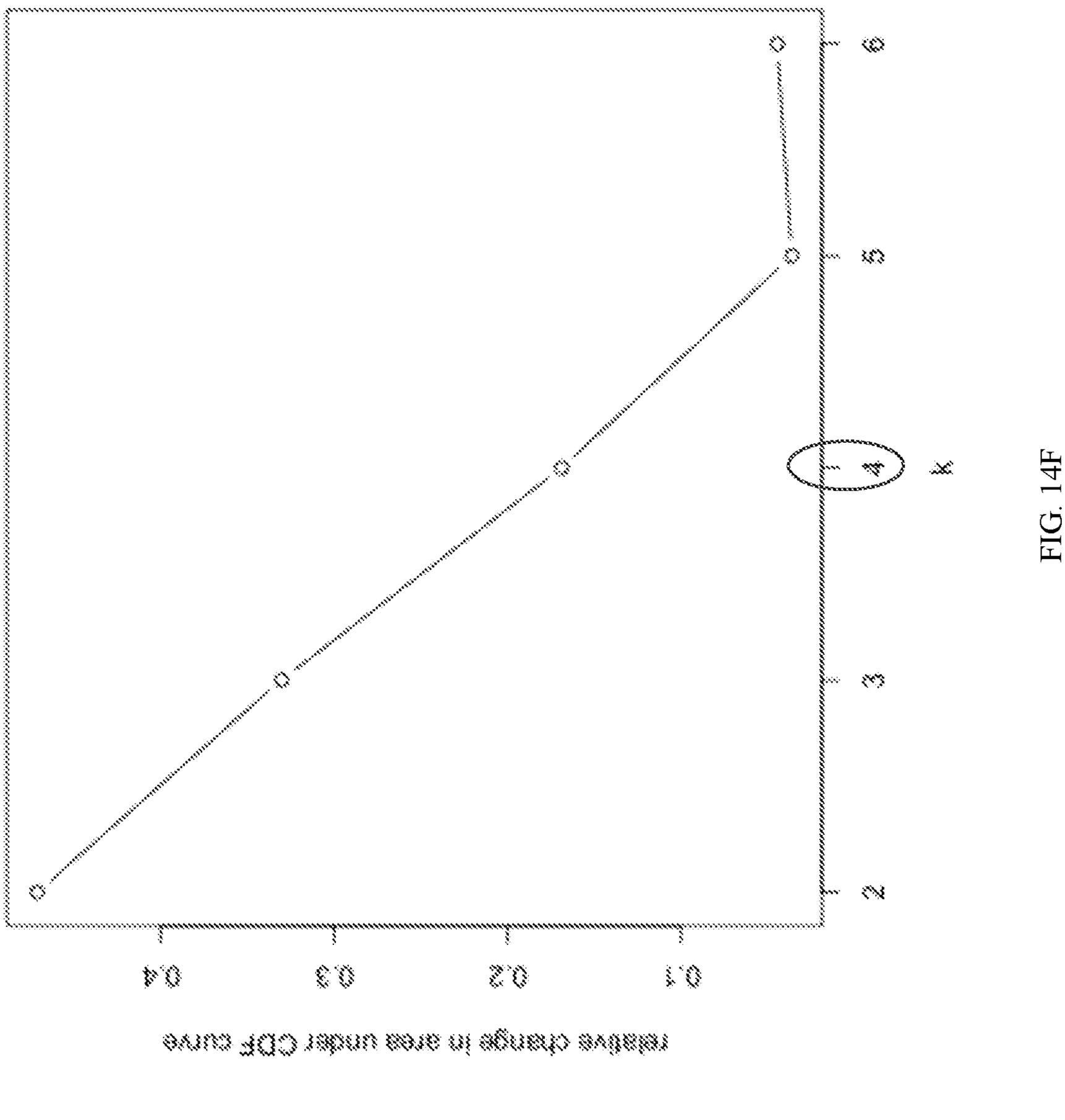

Delta area
relative change in area under CDF curve
0.1
0.2
0.3
0.4
2
3
4
5
6
x

FIG. 14F

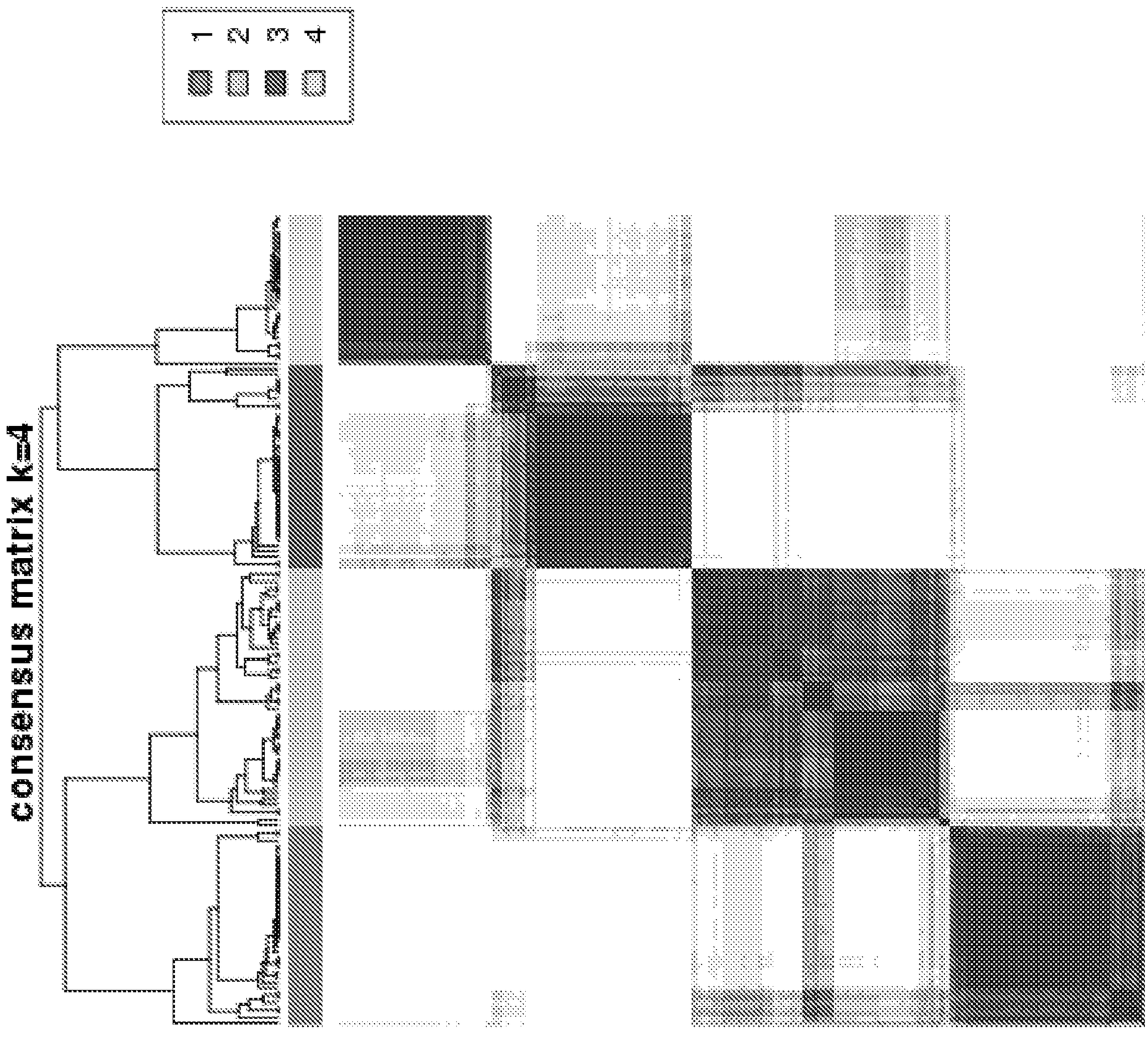


FIG. 14G

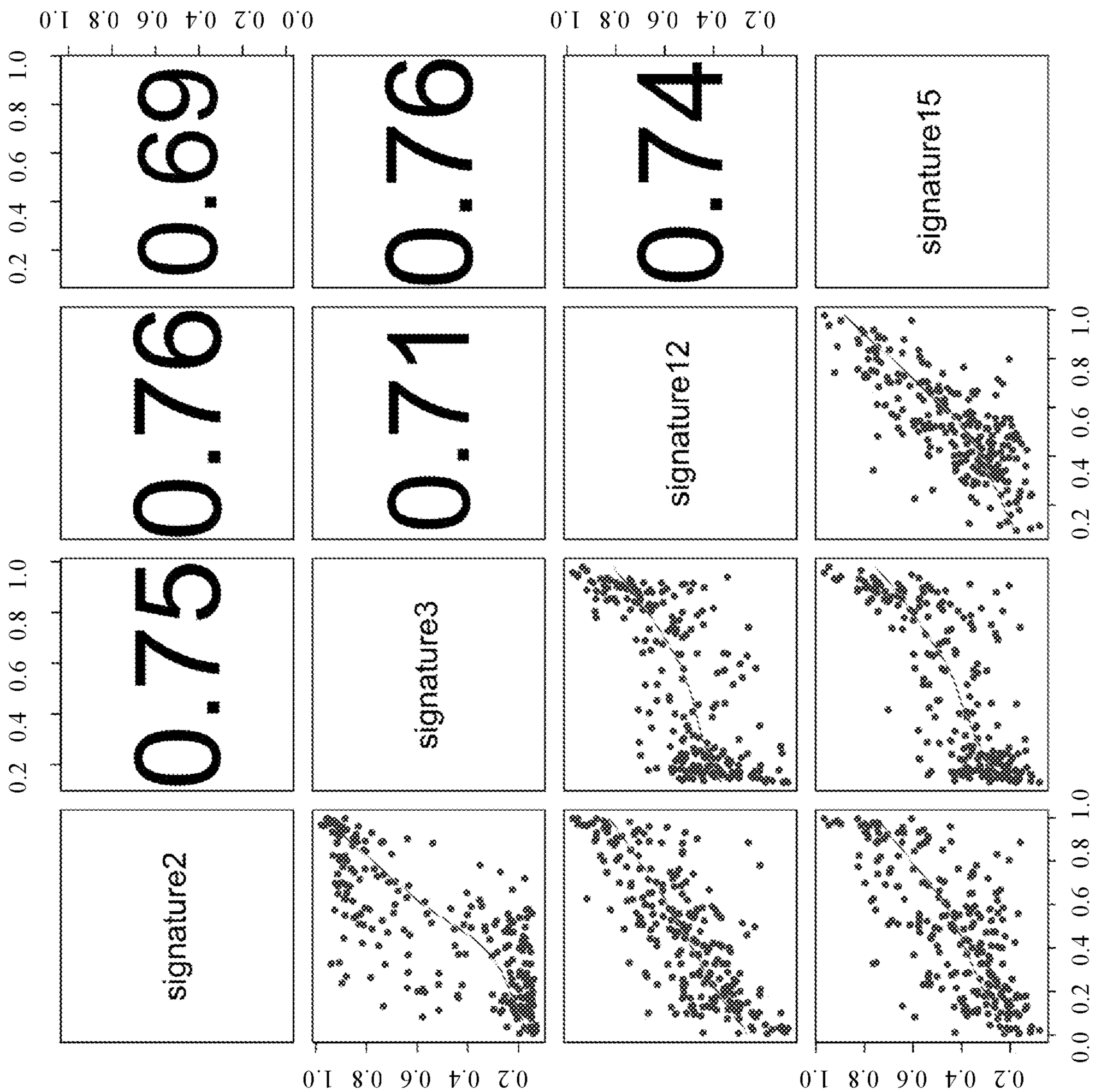

0.76
0.69
0.71
0.76
0.74
0.75
signature12
signature15
signature2
signature3
0.0 0.2 0.4 0.6 0.8 1.0

FIG. 14H

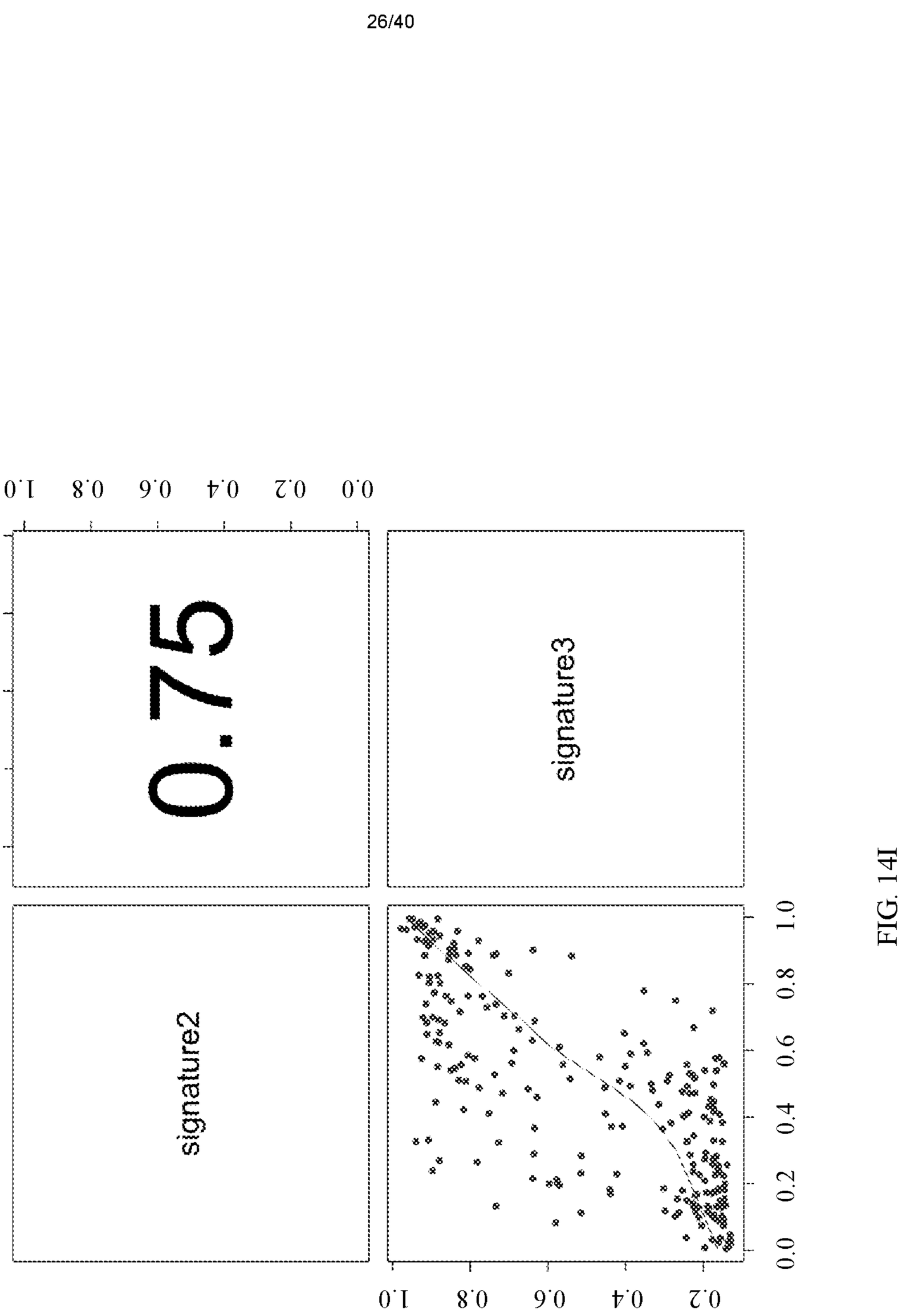


FIG. 14I

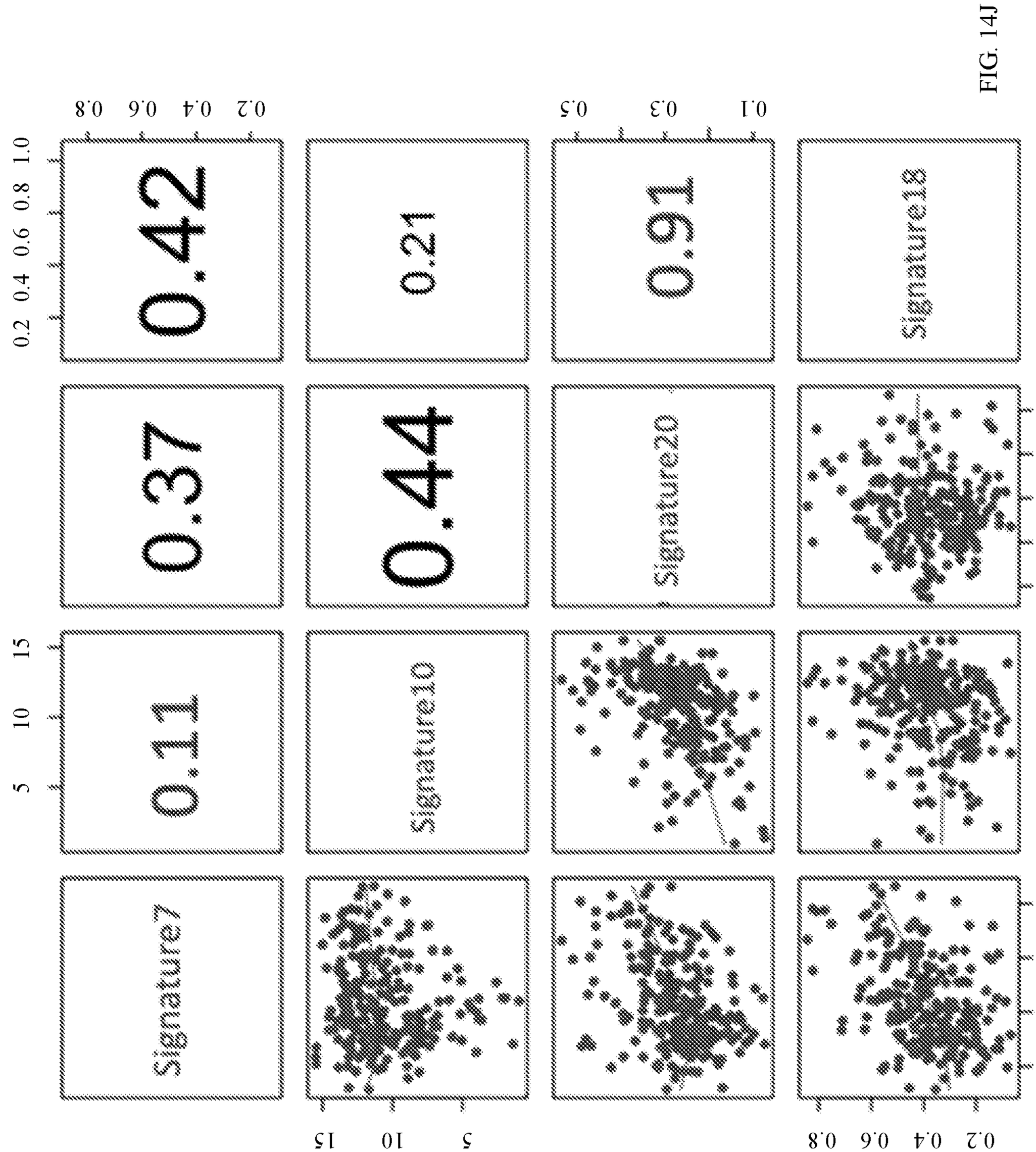

0.42
0.21
0.91
Signature19
0.37
0.44
Signature20
0.11
Signature10
Signature7
0.2 0.4 0.6 0.8 1.0
0.1 0.3 0.5
5 10 15
0.2 0.4 0.6 0.8

FIG. 14J

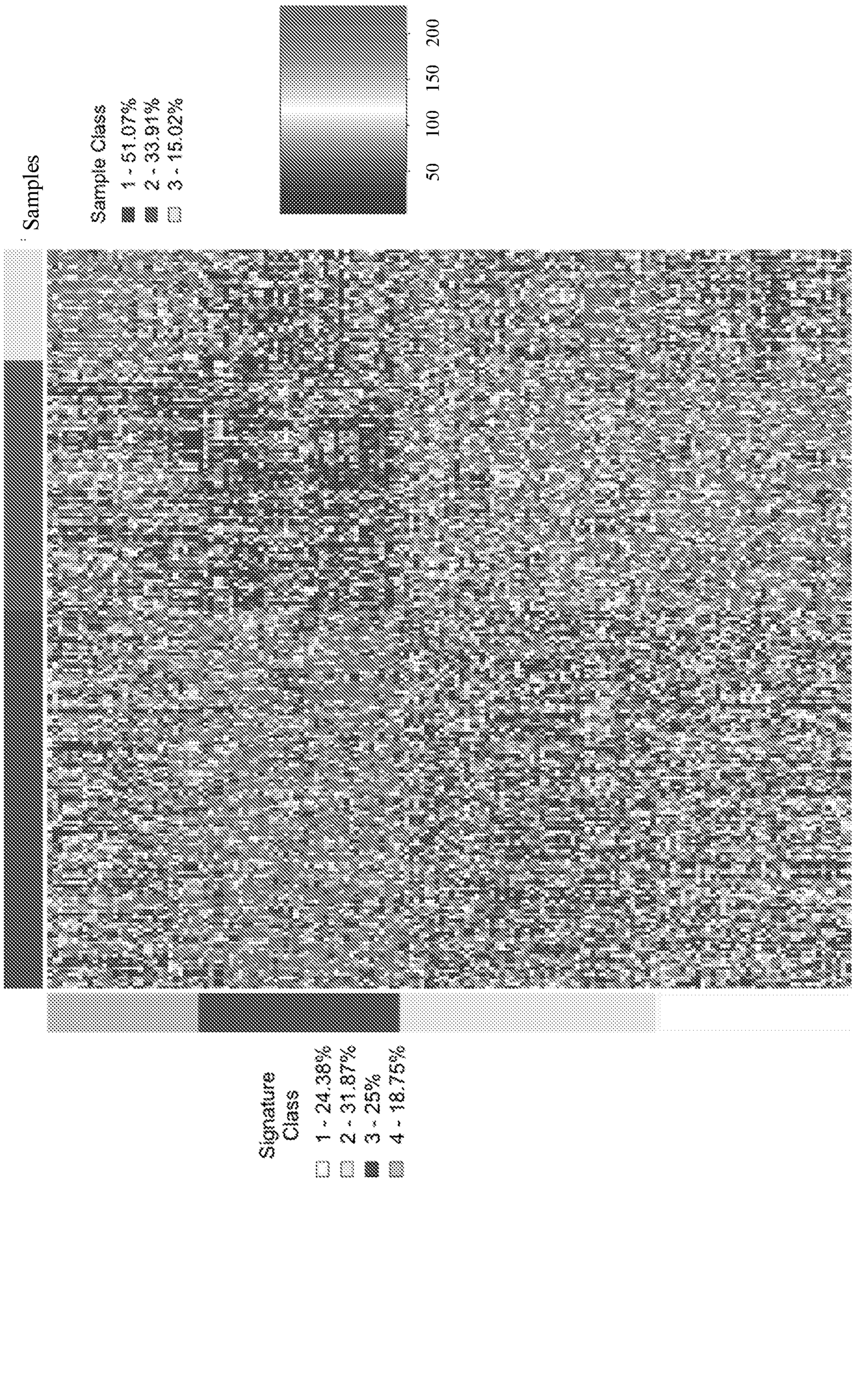

Signatures
50 100 150 200
Sample Class
1 - 51.07%
2 - 33.91%
3 - 15.02%
Signature Class
1 - 24.38%
2 - 31.87%
3 - 25%
4 - 18.75%

FIG. 14K

Class 1: Risk
Representative signature (RS): Genomic Gleason Grade 2

ADT

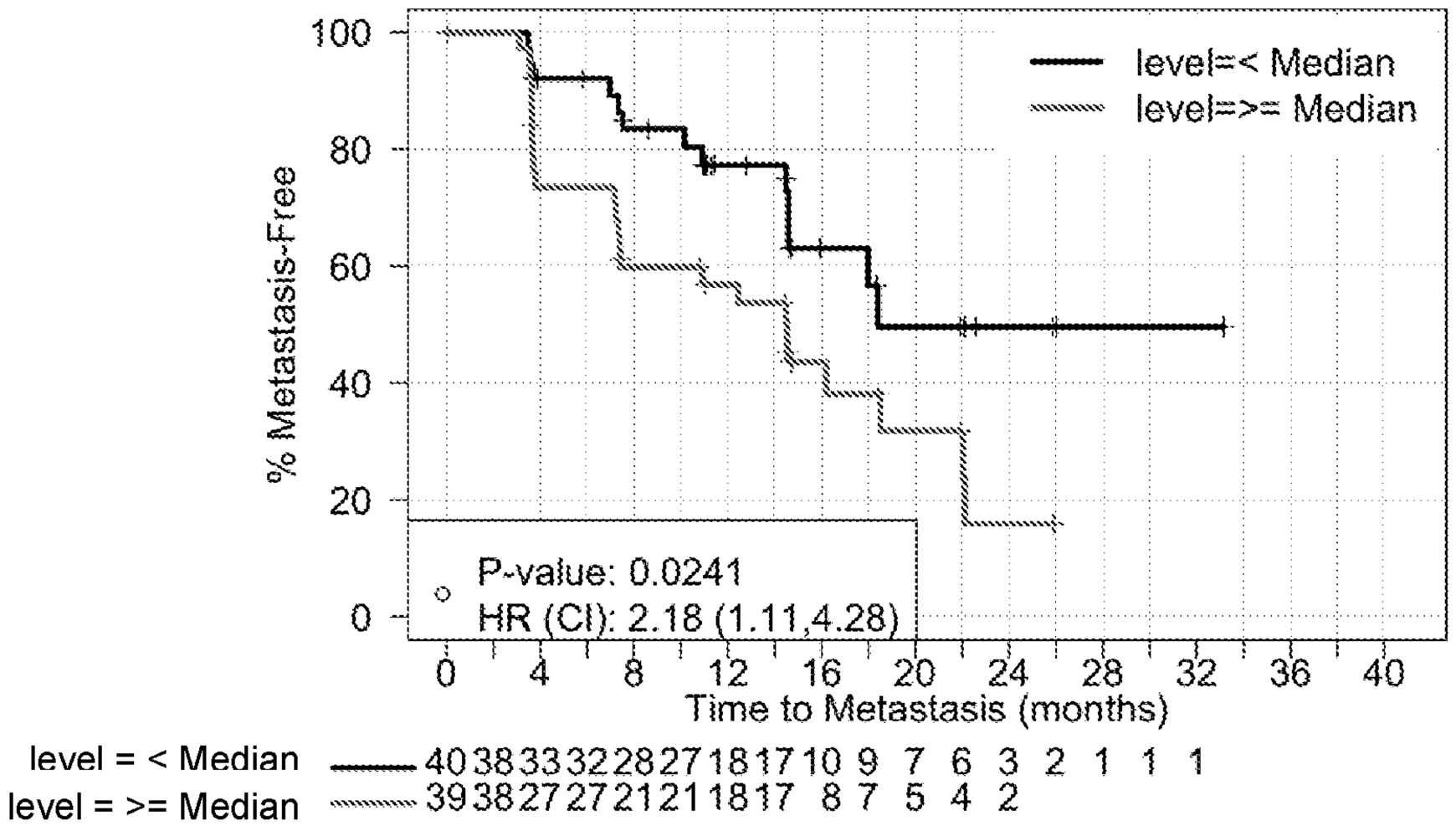


FIG. 15A

APA + ADT

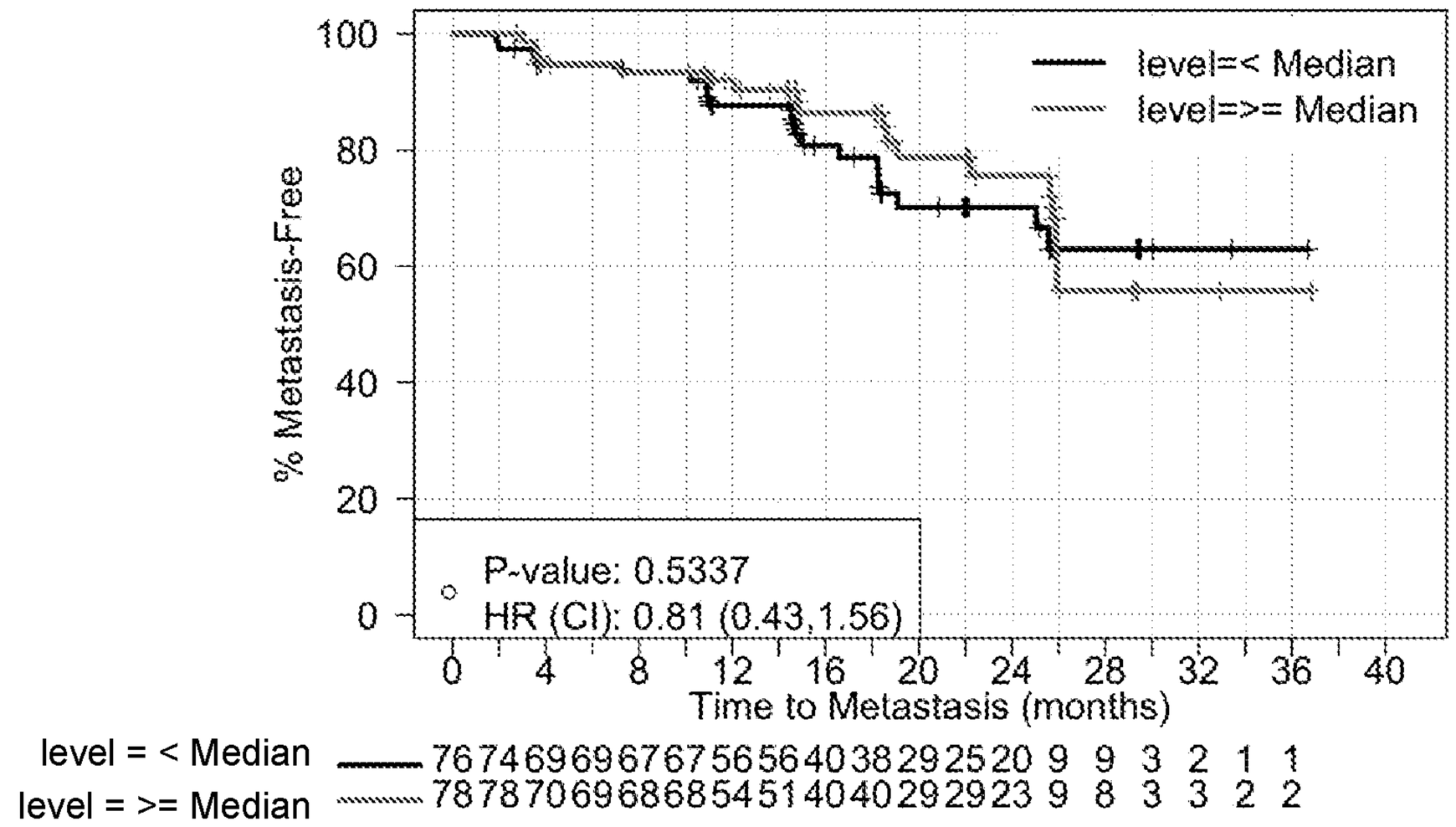


FIG. 15B

Class 1: High-risk
Representative signature (RS): Genomic Gleason Grade 2

High Expression

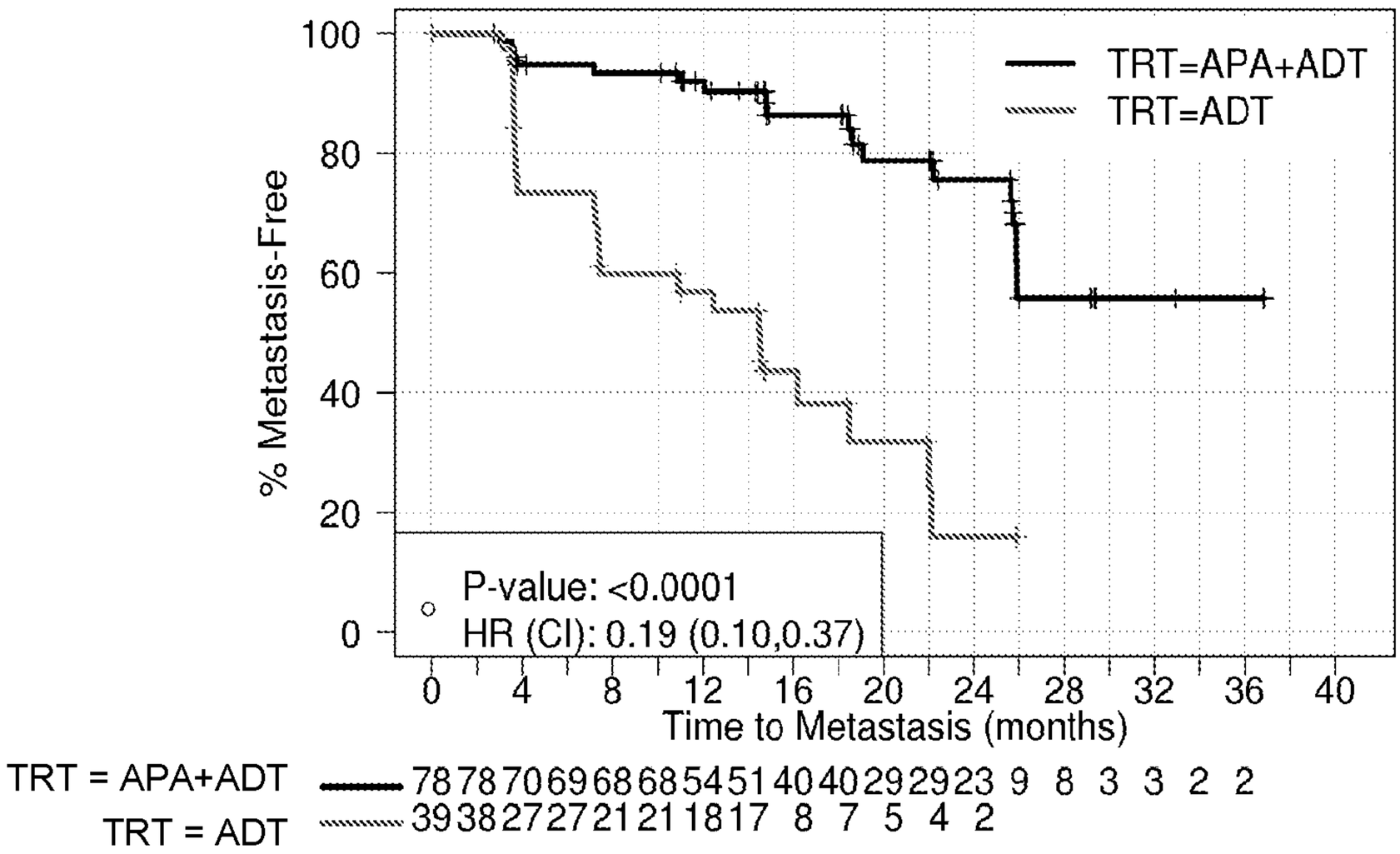


FIG. 15C

Low Expression

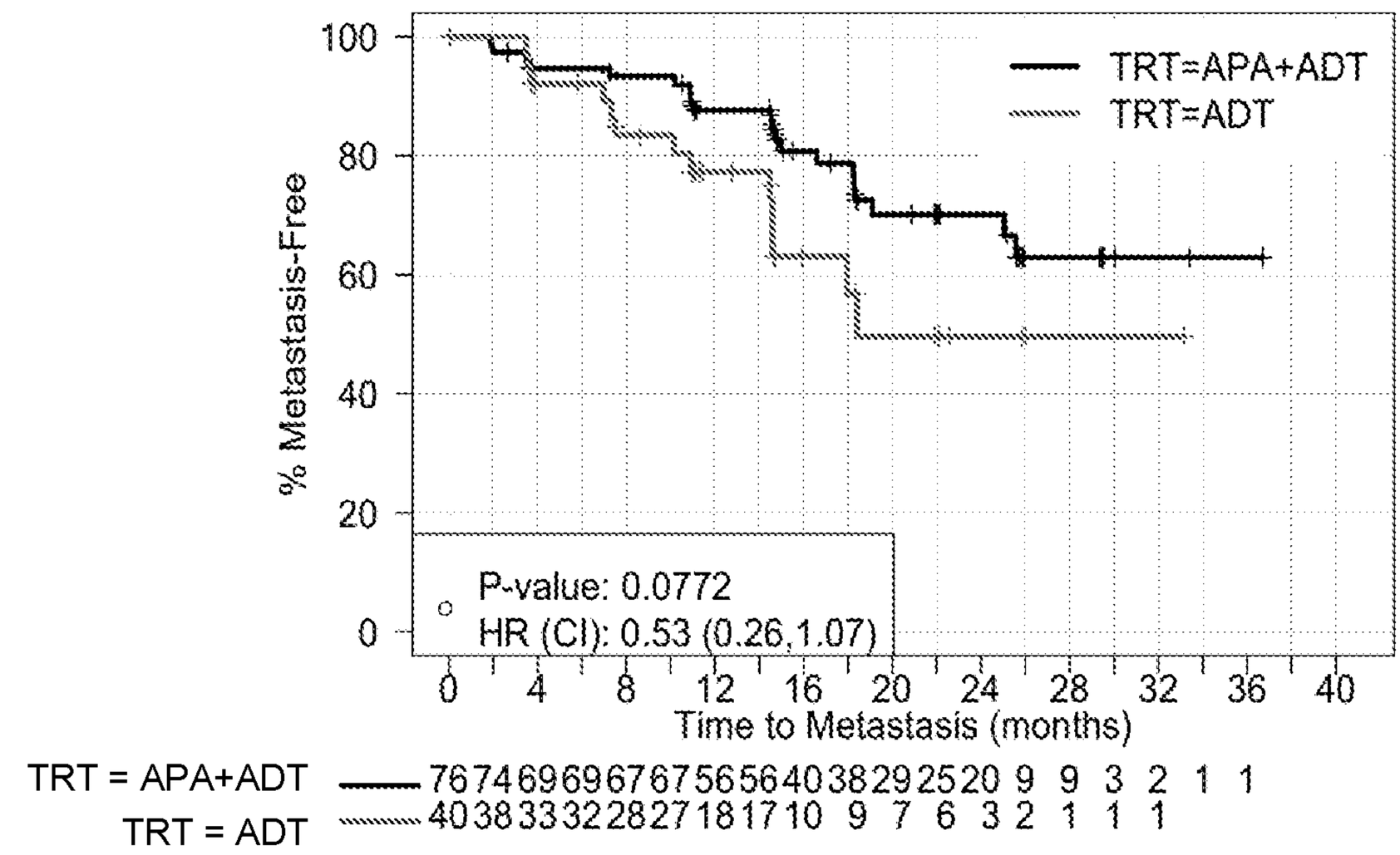


FIG. 15D

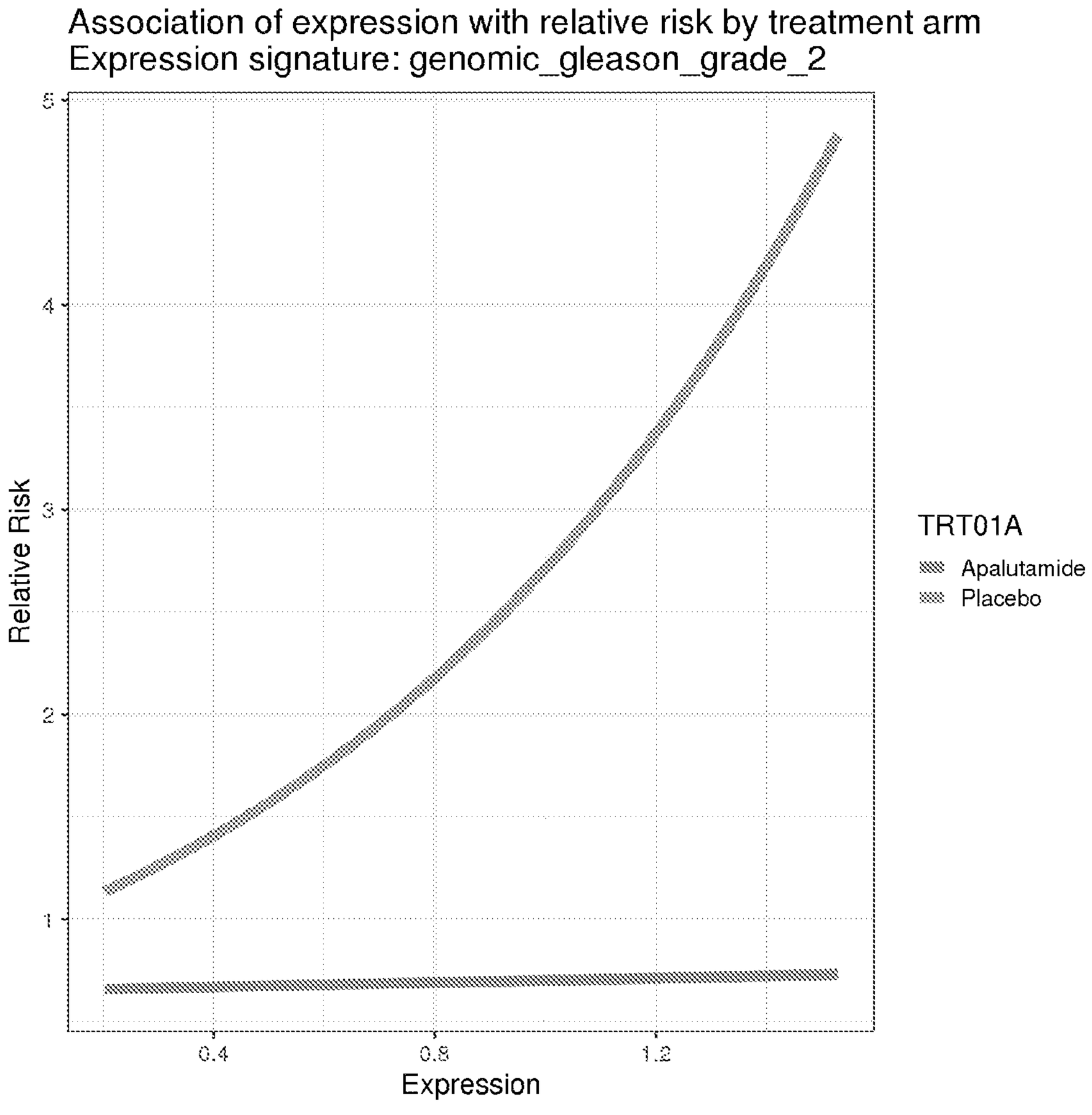

Association of expression with relative risk by treatment arm
Expression signature: genomic_gleason_grade_2
Relative Risk
1
2
3
4
5
0.4
0.8
1.2
Expression
TRT01A
Apalutamide
Placebo

FIG. 15E

Class 2: Steroid Homeostasis
Representative signature (RS): Hallmark Cholesterol Homeostasis

ADT

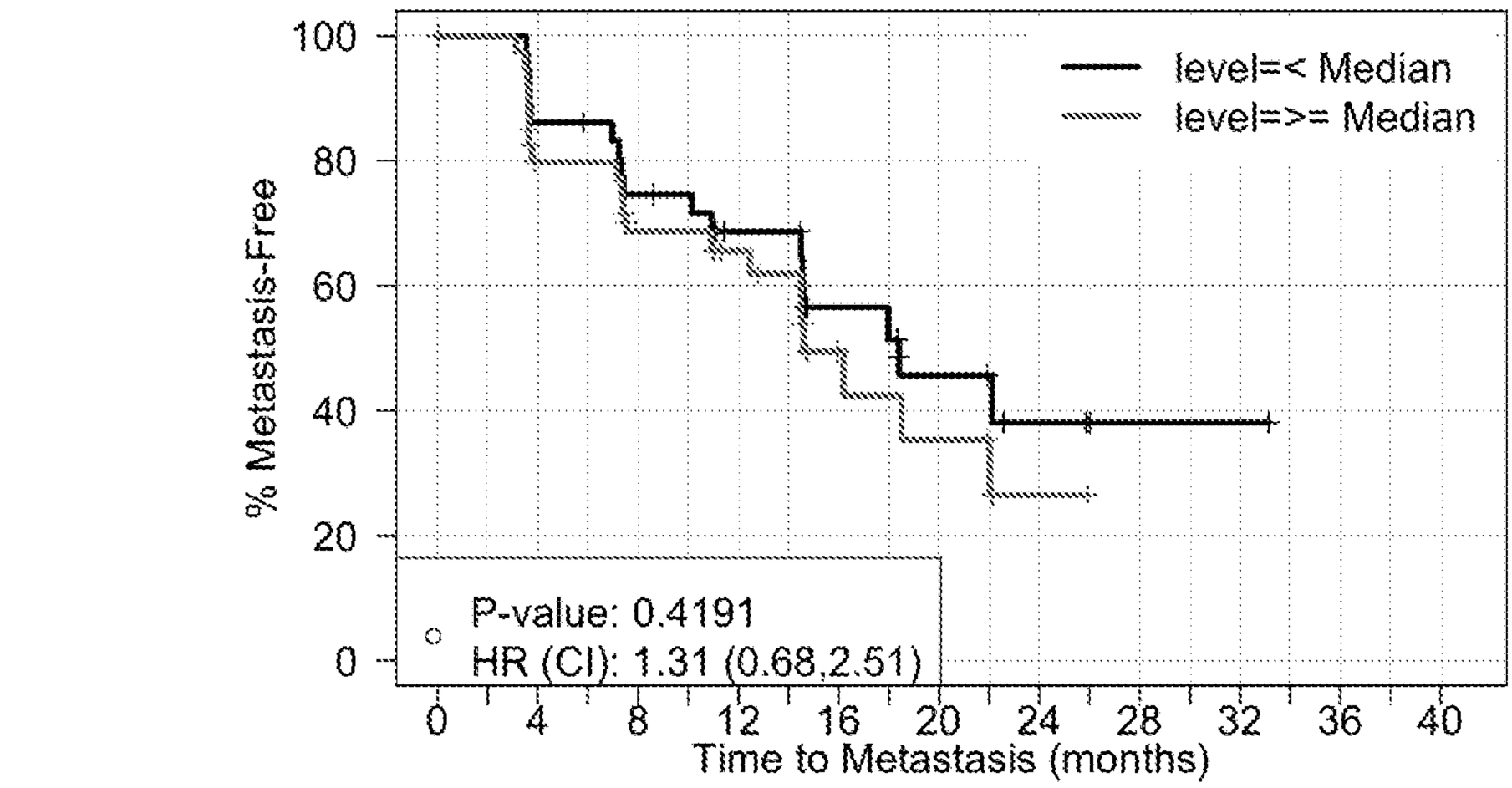


FIG. 16A

APA + ADT

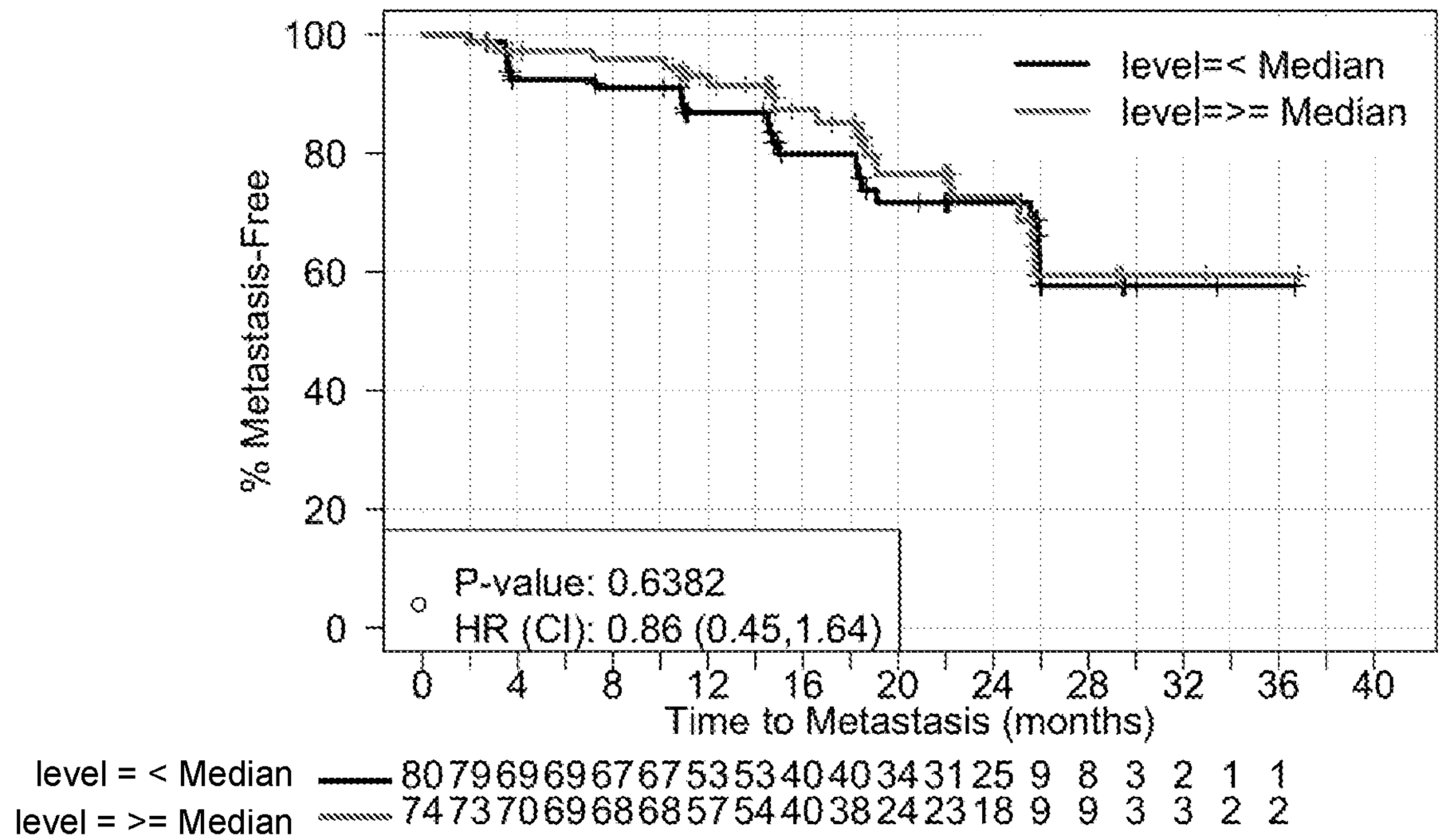


FIG. 16B

Class 2: Steroid Homeostasis
Representative signature (RS): Hallmark Cholesterol Homeostasis

High Expression

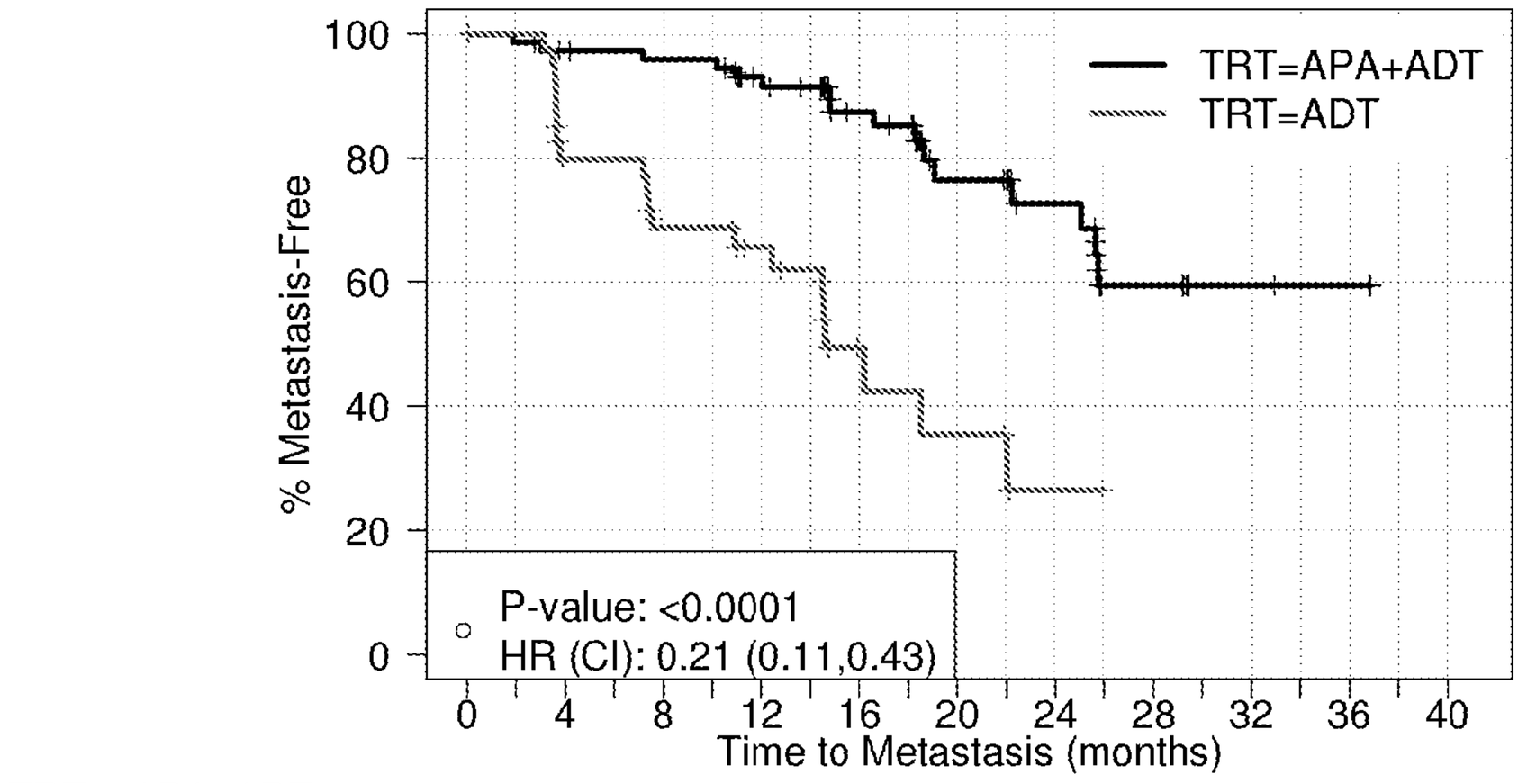


FIG. 16C

Low Expression

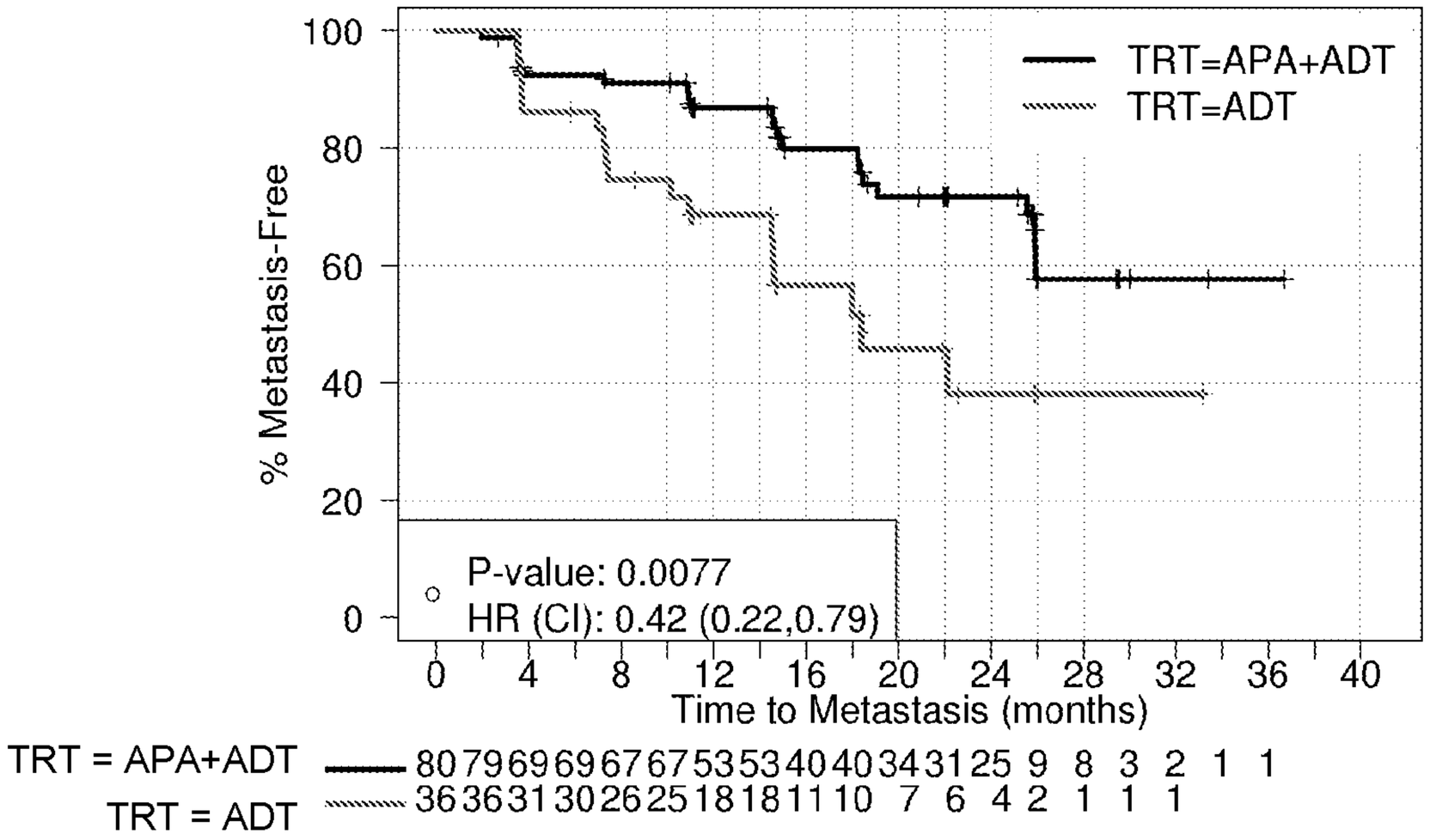


FIG. 16D

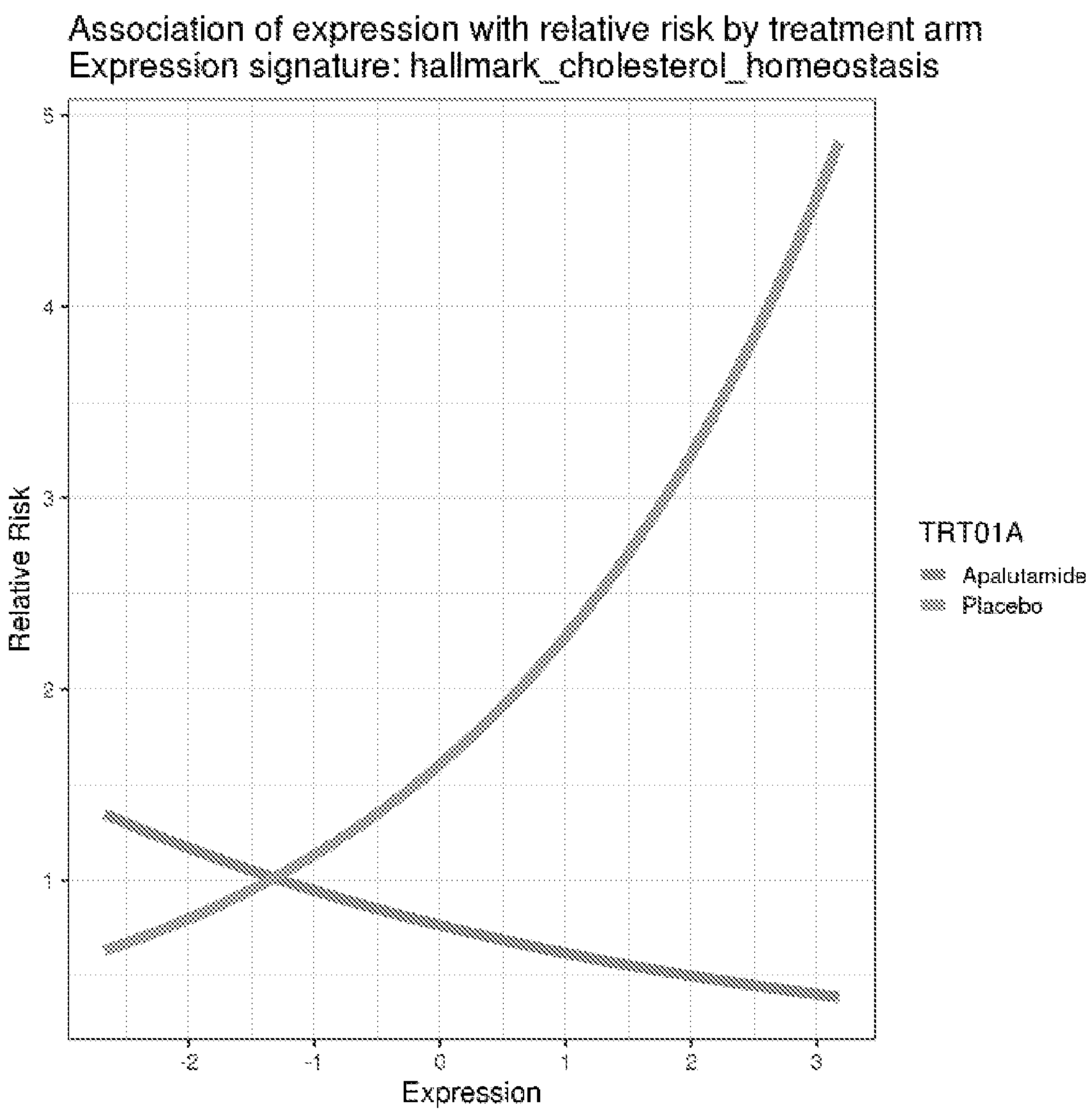

Association of expression with relative risk by treatment arm
Expression signature: hallmark_cholesterol_homeostasis
Relative Risk
Expression
TRT01A
Apalutamide
Placebo

FIG. 16E

Class 3: Adeno with NE Like Features
Representative signature (RS): Beltran Neuroendocrine

ADT

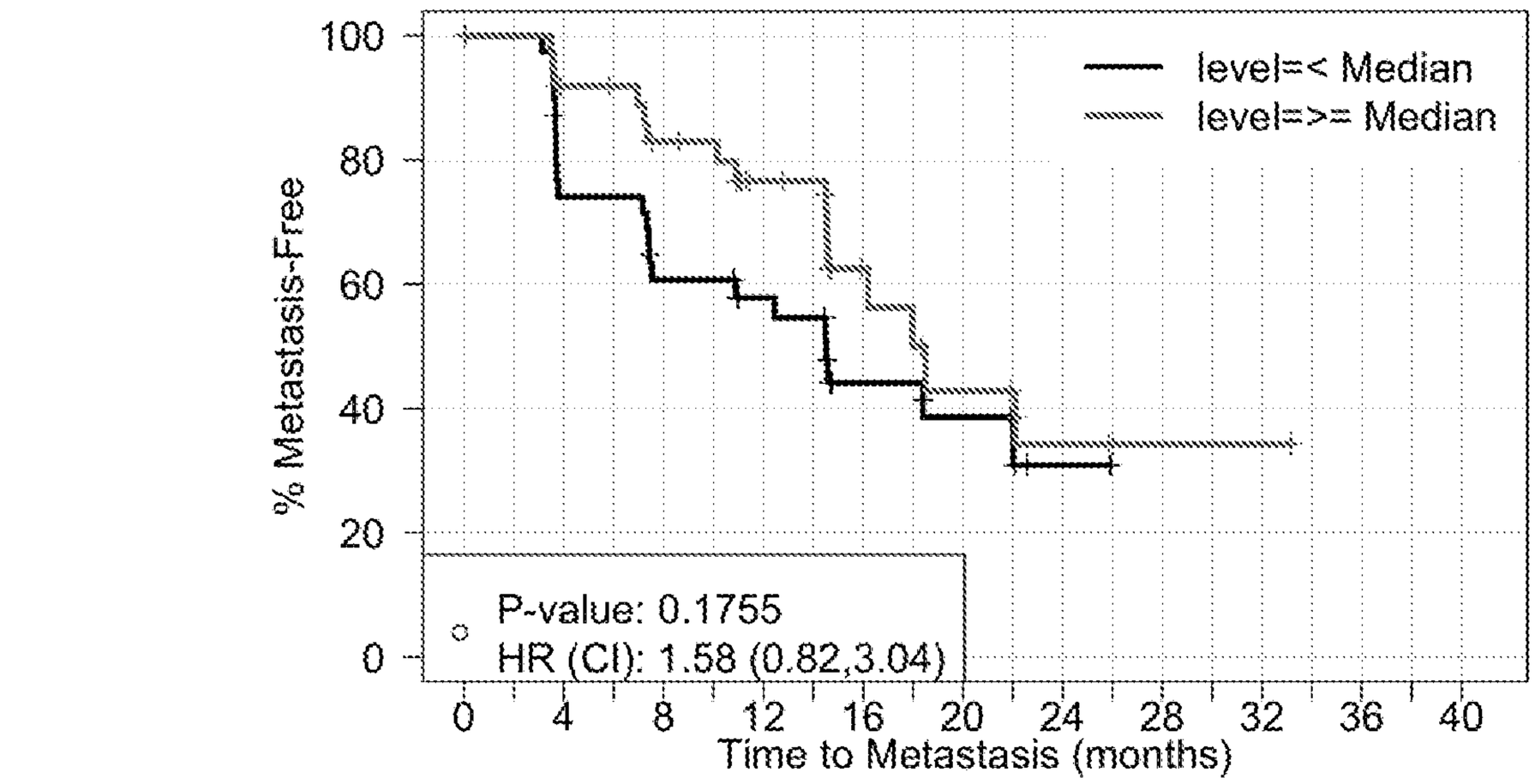


FIG. 17A

APA + ADT

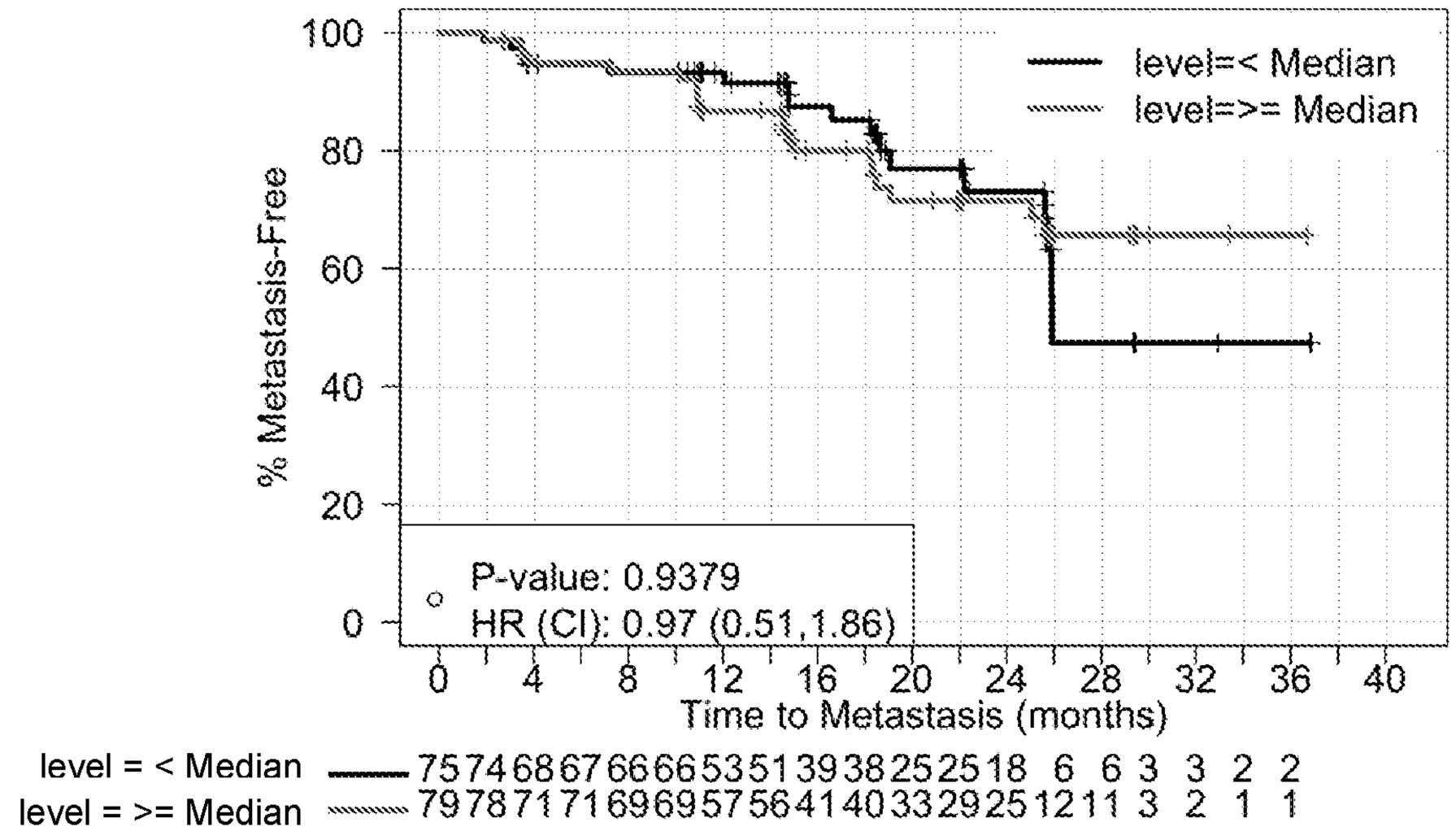


FIG. 17B

Class 3: Adeno with NE Like Features
Representative signature (RS): Beltran Neuroendocrine

High Expression

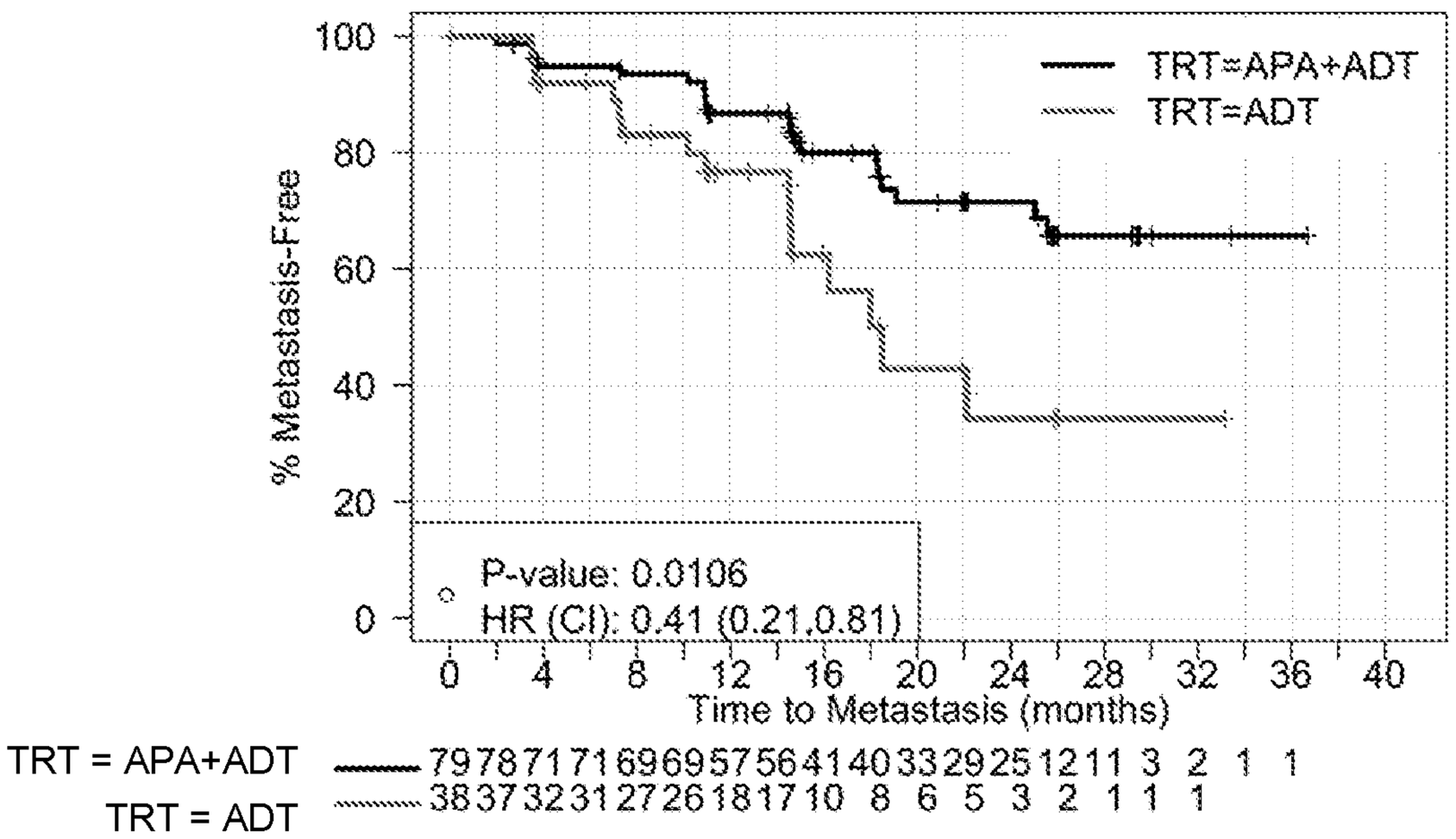


FIG. 17C

Low Expression

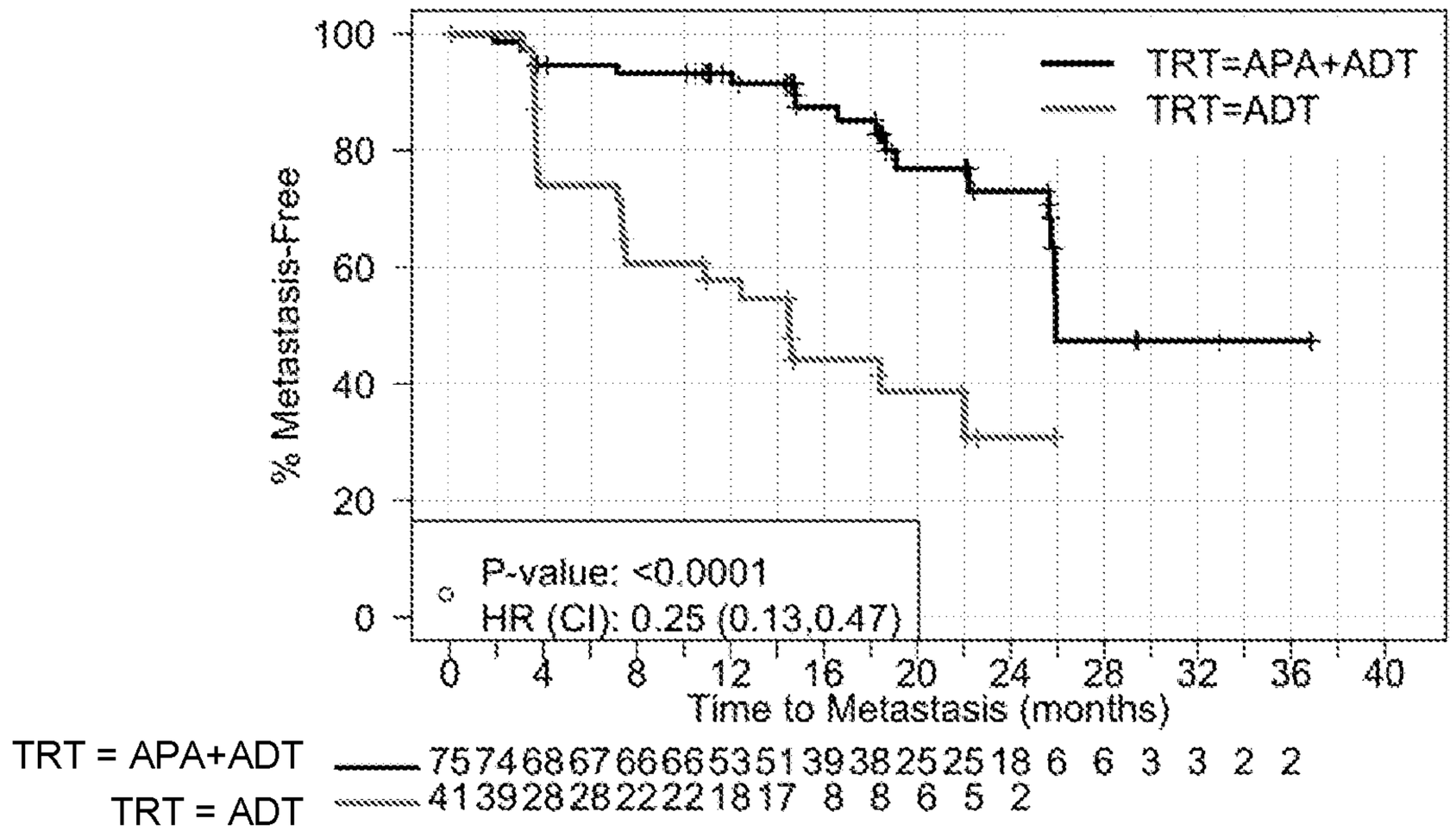


FIG. 17D

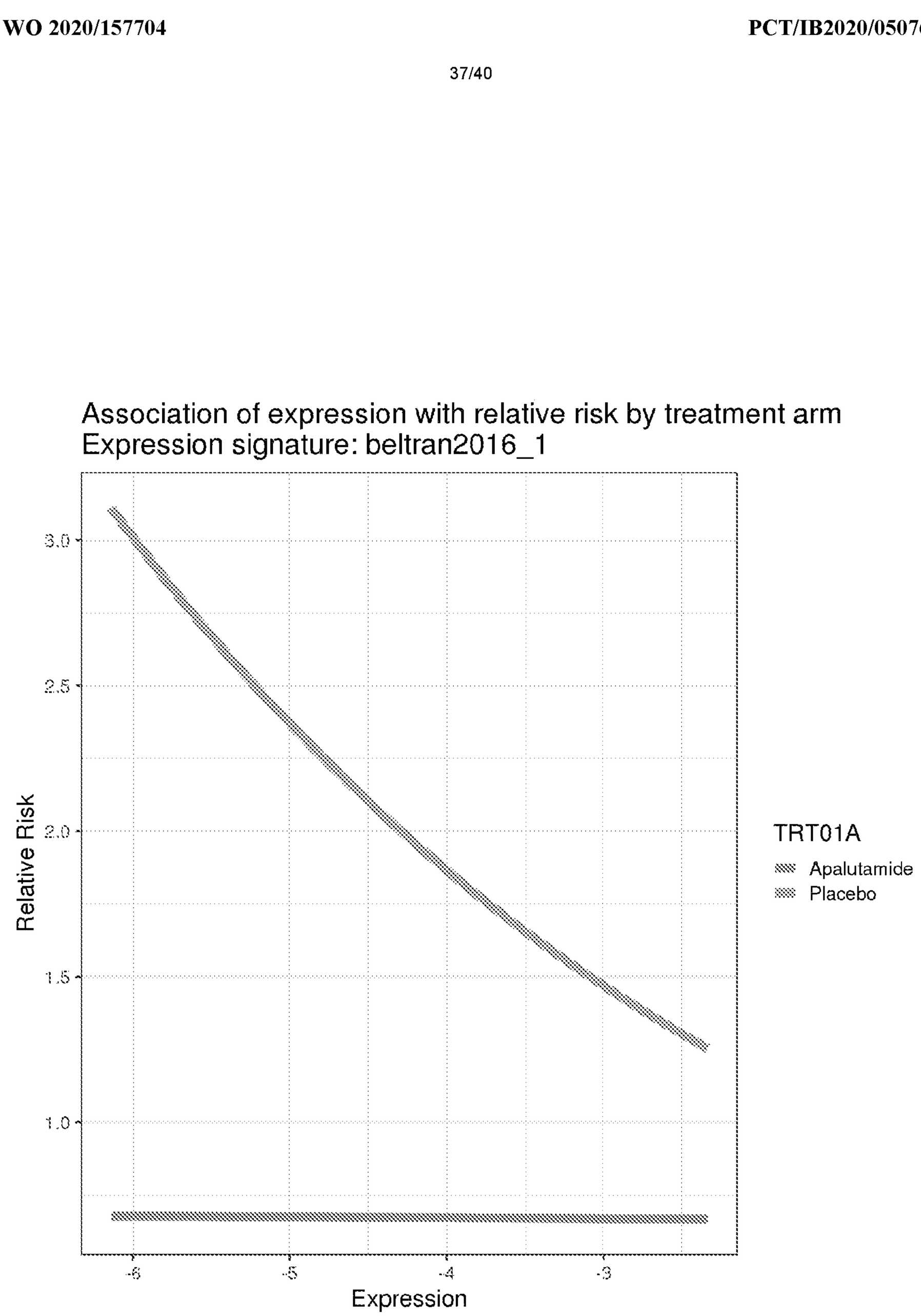

Association of expression with relative risk by treatment arm
Expression signature: beltran2016_1
Relative Risk
3.0
2.5
2.0
1.5
1.0
-6
-5
-4
-3
Expression
TRT01A
Apalutamide
Placebo

FIG. 17E

Class 4: Immune/Stromal
Representative signature (RS): Hallmark IL2 JAK STAT5 Signaling

ADT

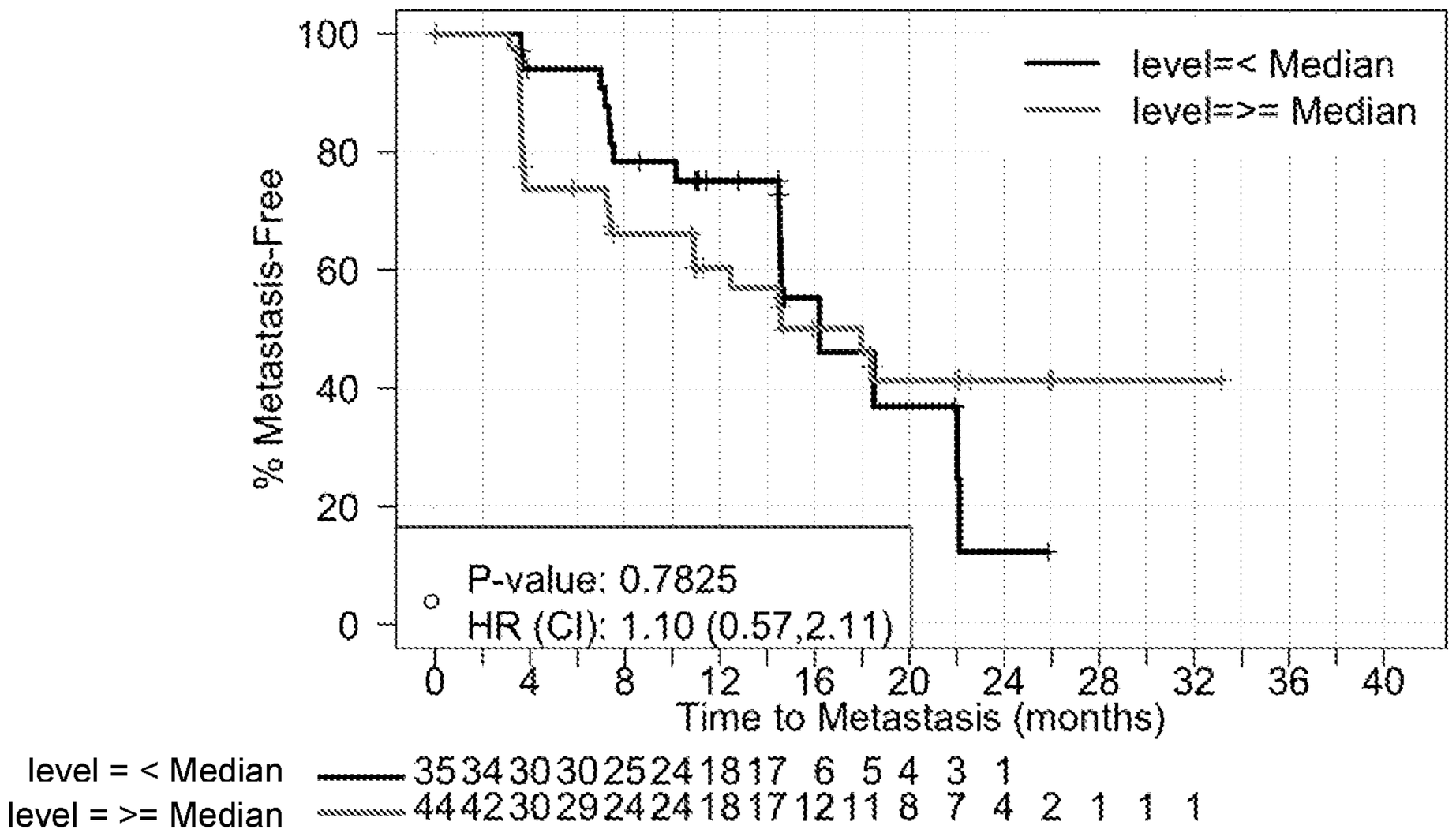


FIG. 18A

APA + ADT

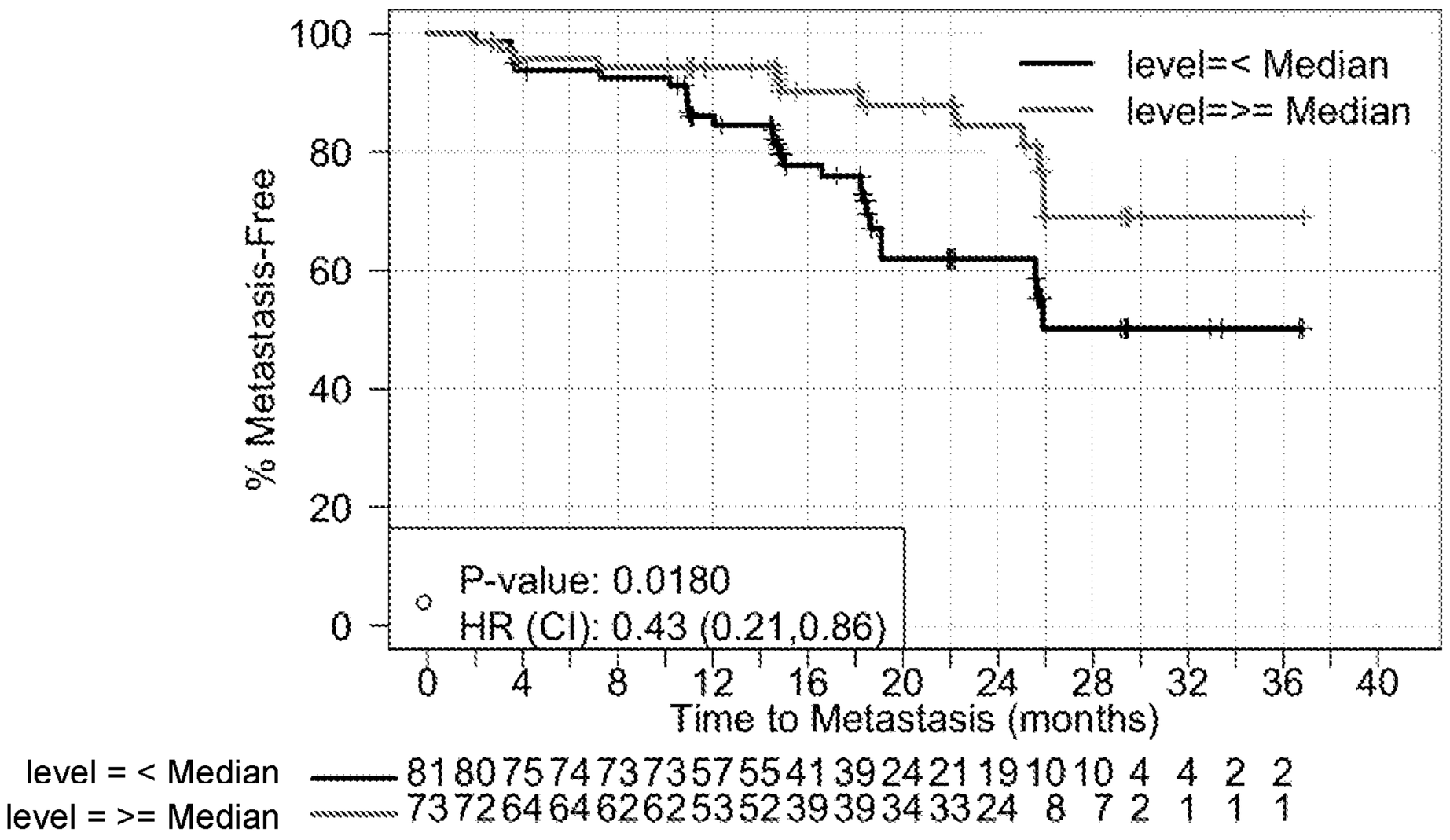


FIG. 18B

Class 4: Immune/Stromal
Representative signature (RS): Hallmark IL2 JAK STAT5 Signaling

High Expression

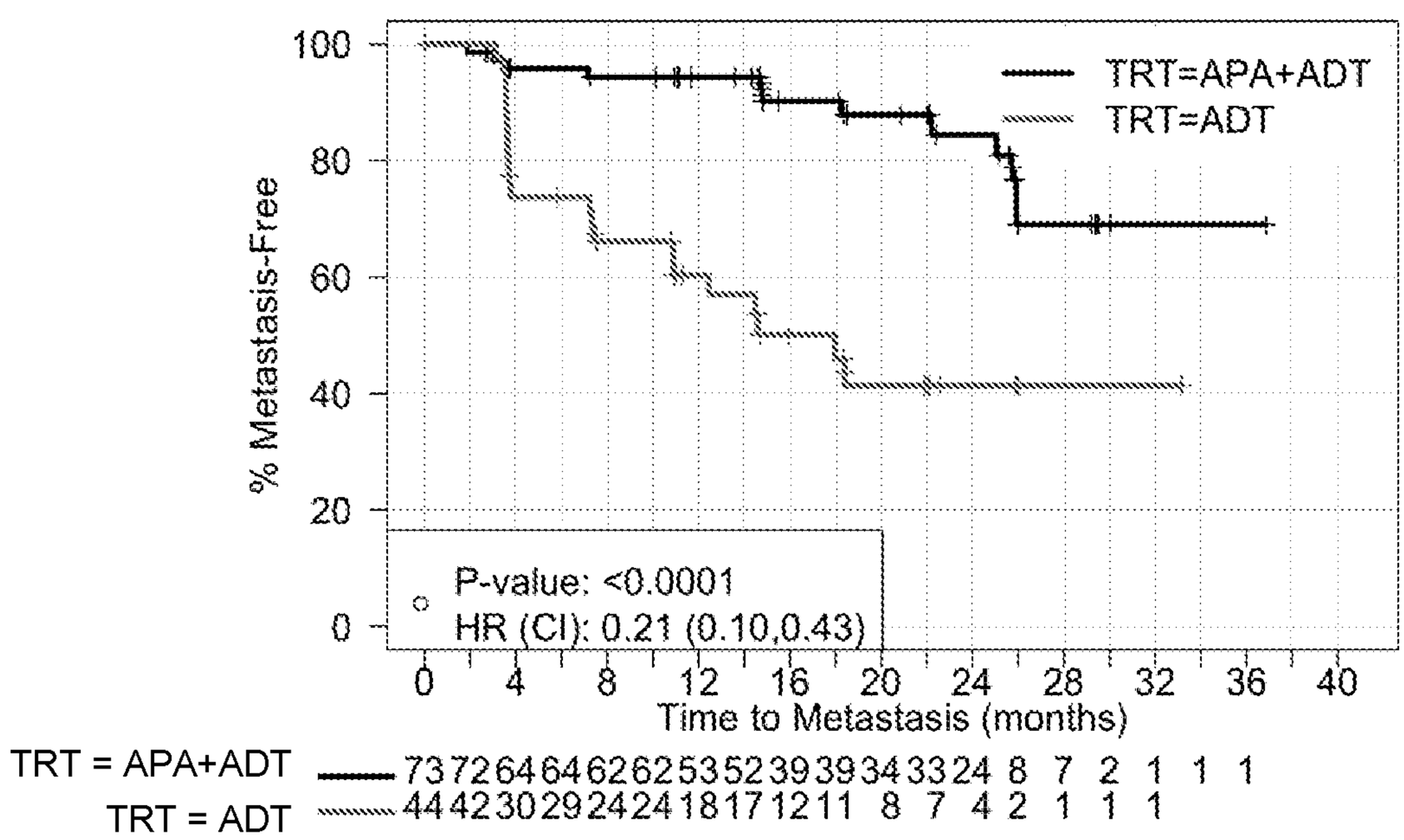


FIG. 18C

Low Expression

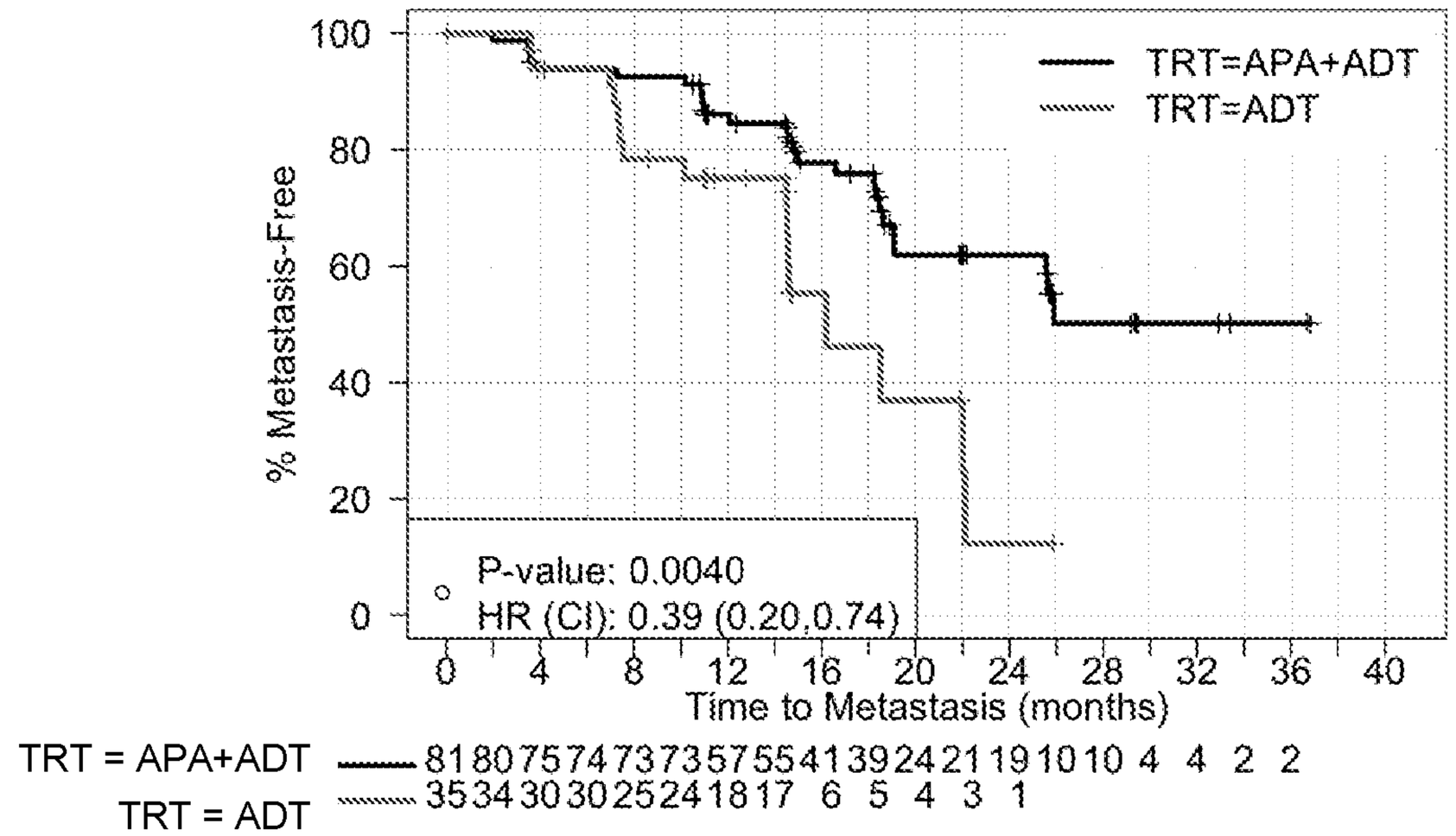


FIG. 18D

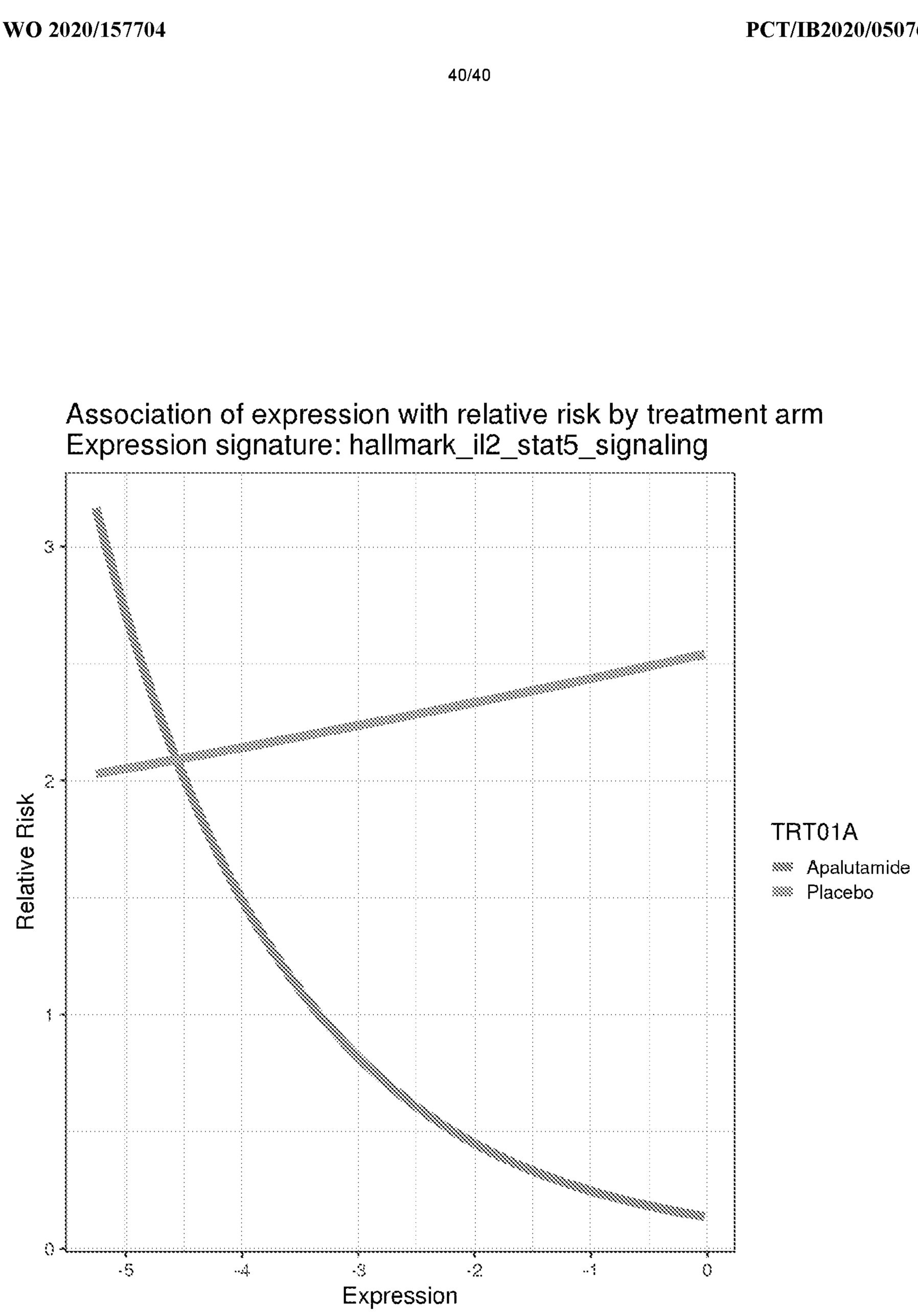

Association of expression with relative risk by treatment arm
Expression signature: hallmark_il2_stat5_signaling
Relative Risk
0
1
2
3
-5
-4
-3
-2
-1
0
Expression
TRT01A
Apalutamide
Placebo

FIG. 18E