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Supplemental information

Combined tumor and immune signals from genomes or transcriptomes predict outcomes of checkpoint inhibition in melanoma

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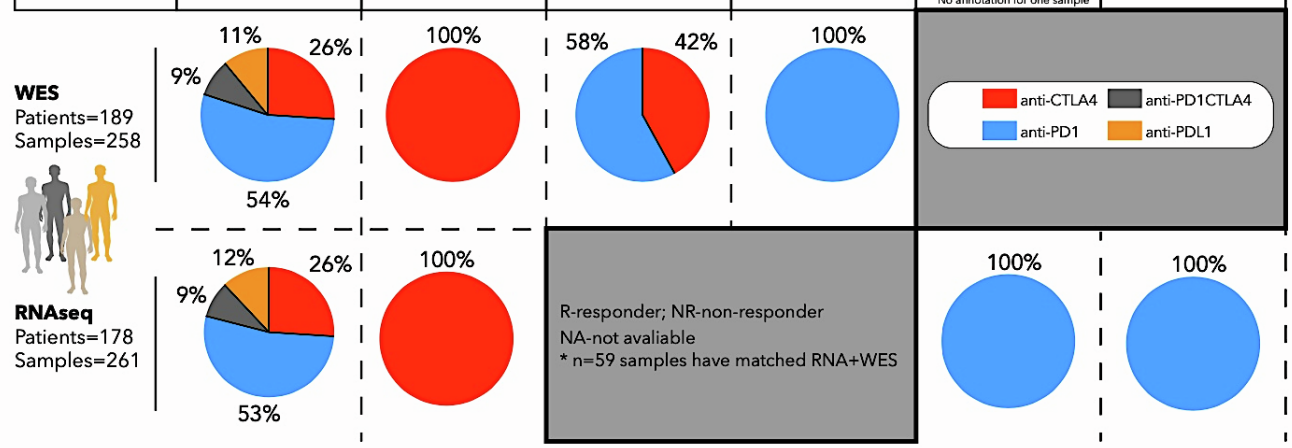
Supplementary Figures

List of Supplementary Figures:

A

Primary Cohort

Cohort	MGH	Van Allen et al. *Science* 2015	Roh et al. *Science Translational Medicine* 2015	Zaretsky et al. *NEJM* 2016	Riaz et al. *Cell* 2017	Hugo et al. *Cell* 2016
DNA* (WES)	Patients*=56 Samples=109 * 37 patients with pre/post samples	Patients*=98 *only baseline	Patients*=31 Samples=43 * 12 patients with pre/post samples	Patients*=4 Samples=8 * 4 patients with pre/post samples	NA	NA
RNA (bulk)	Patients*=48 Samples=88 *26 patients with pre/post samples	Patients*=39 *only baseline	NA	NA	Patients*=65 Samples=108 * 43 patients with pre/post samples	Patients*=26 *only baseline
Clinical response (sample)	WES: R=23, NR=86 RNA: R=27, NR=61	WES: R=24, NR=74 RNA: R=13, NR=26	WES: R=9, NR=34	WES: R=4	RNA*: R=23, NR=84 * No annotation for one sample	RNA: R=14, NR=12



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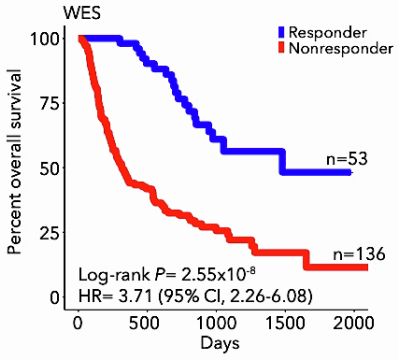
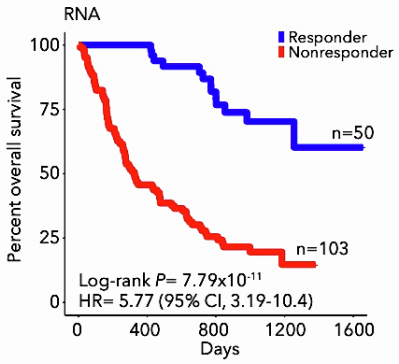
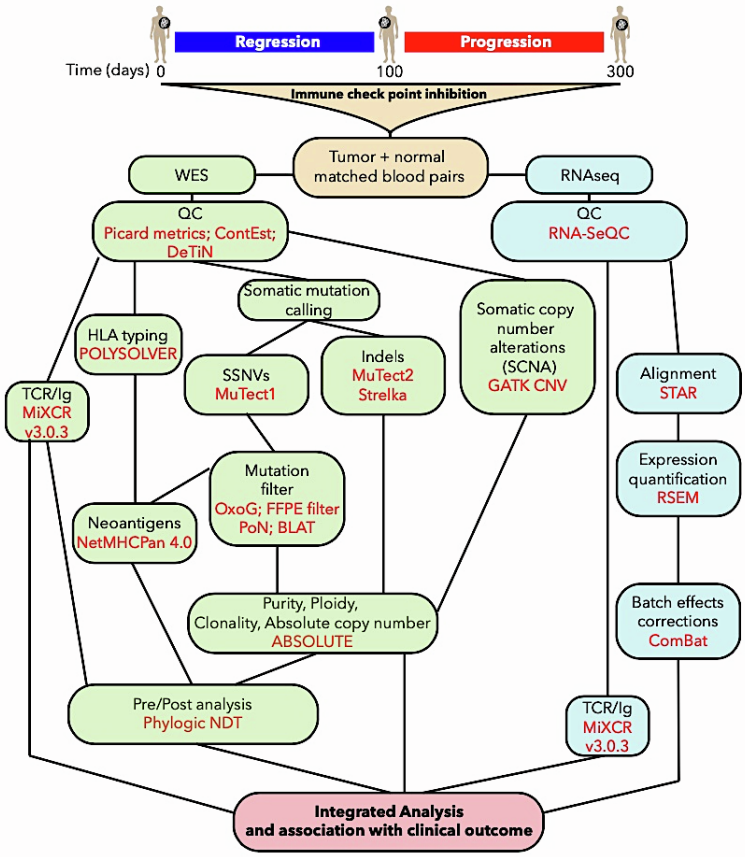


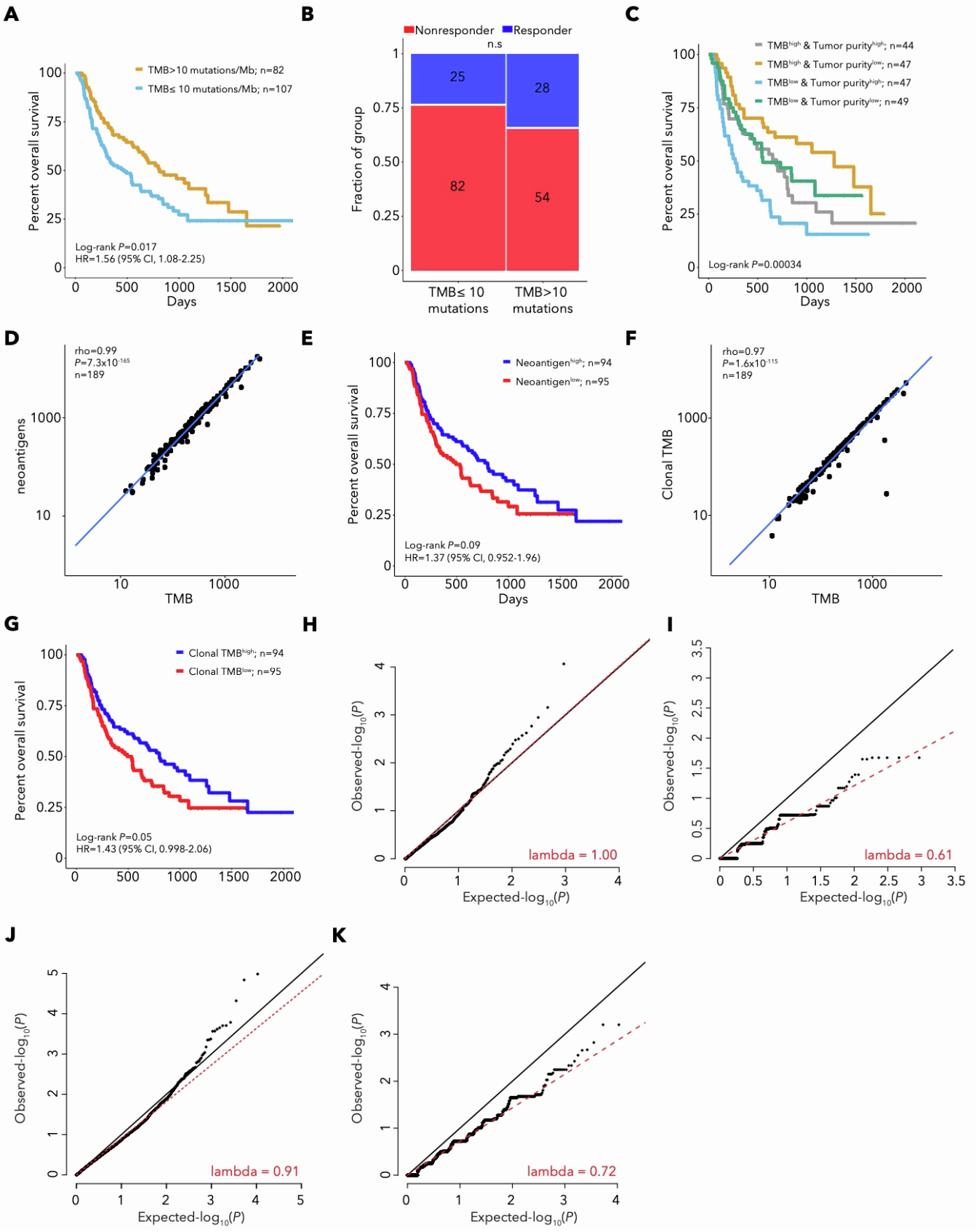
C



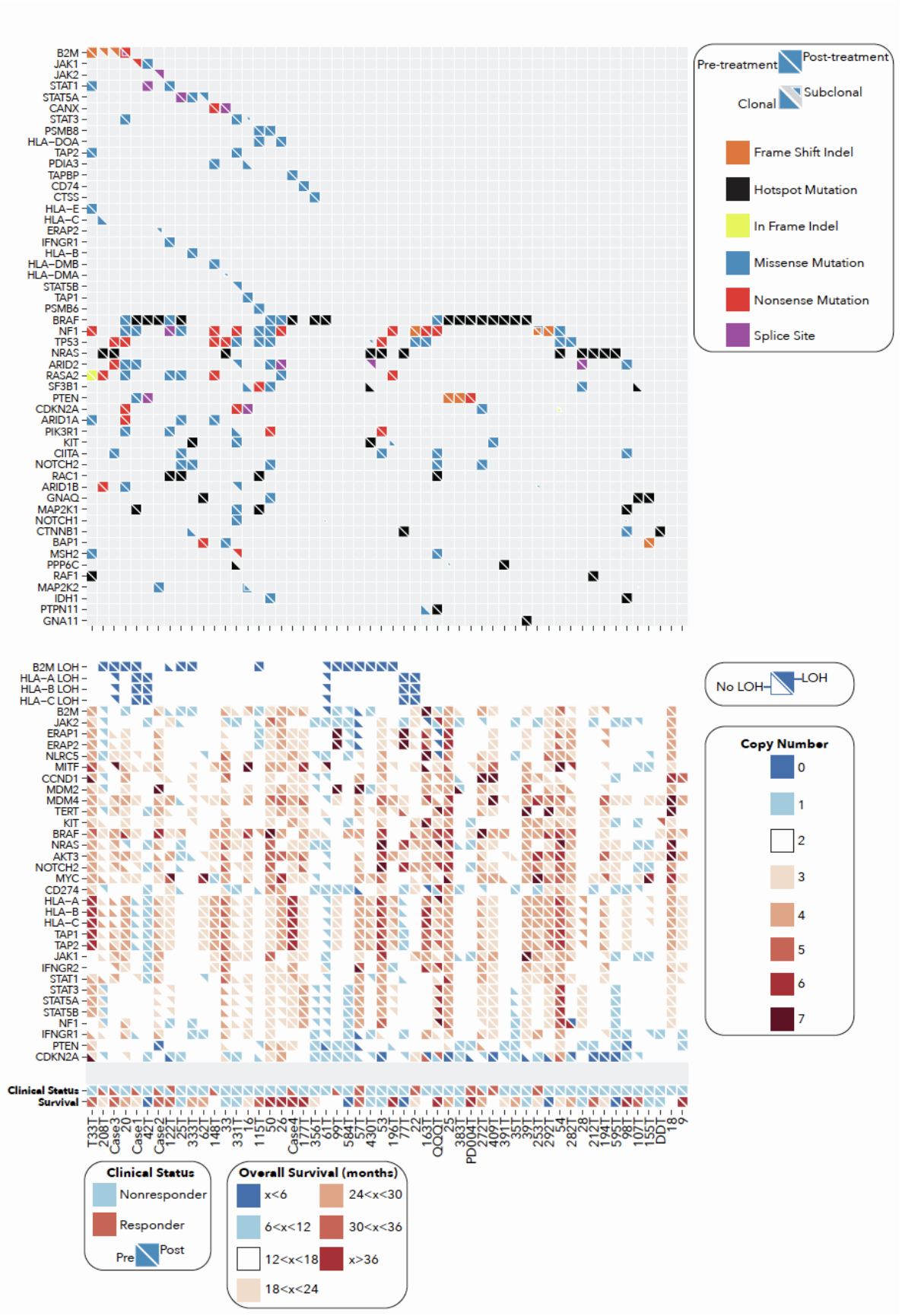
D



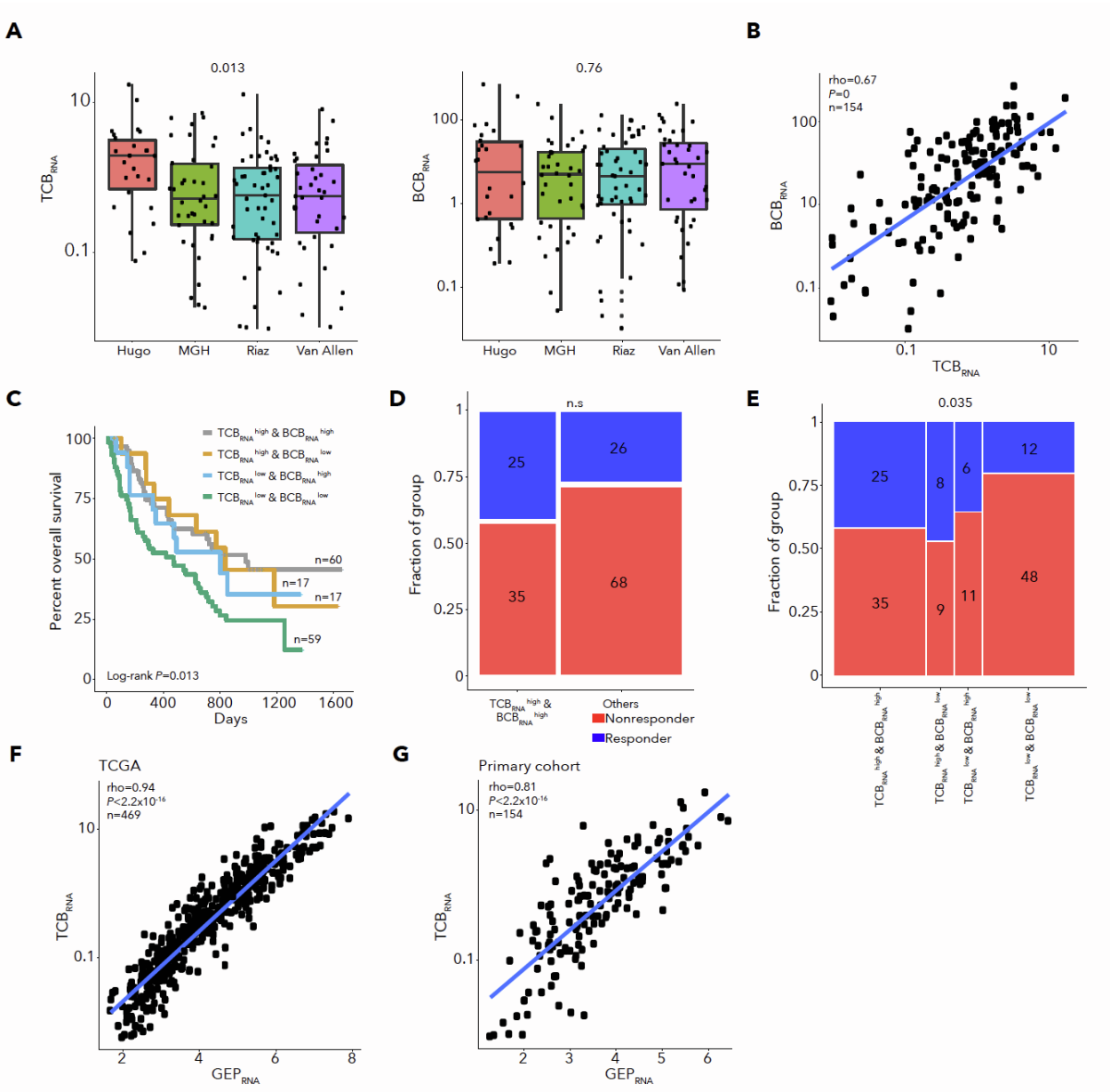
Supplementary Figure 1. **Primary cohort composition in meta-analysis and analysis workflow, Related to Figure 1 and STAR methods quantification and statistical analysis. A.** Six cohorts were included in the primary cohort meta-analysis. Table indicated cohorts for which DNA and/or RNA was available. The clinical response and numbers of pre-treatment and post-treatment samples are indicated for each cohort. Treatment for patients in each cohort is indicated in the pie charts. **B-C.** Kaplan-Meier survival curve for responders and nonresponders in the primary cohort for DNA (WES) (**B**) and RNA (**C**) samples is shown. One patient in the Hugo cohort had response data but no survival data. **D.** Flow chart of analysis pipeline used to process DNA (WES) and RNA-seq data in this study.


A
TMB>10 mutations/Mb; n=82
TMB≤ 10 mutations/Mb; n=107
Percent overall survival
Log-rank P=0.017
HR=1.56 (95% CI, 1.08-2.25)
Days
B
Nonresponder
Responder
n.s
25
28
82
54
Fraction of group
TMB≤ 10 mutations
TMB>10 mutations
C
TMB^high & Tumor purity^high; n=44
TMB^high & Tumor purity^low; n=47
TMB^low & Tumor purity^high; n=47
TMB^low & Tumor purity^low; n=49
Percent overall survival
Log-rank P=0.00034
Days
D
rho=0.99
P=7.3x10^-165
n=189
neoantigens
TMB
E
Neoantigen^high; n=94
Neoantigen^low; n=95
Percent overall survival
Log-rank P=0.09
HR=1.37 (95% CI, 0.952-1.96)
Days
F
rho=0.97
P=1.6x10^-115
n=189
Clonal TMB
TMB
G
Clonal TMB^high; n=94
Clonal TMB^low; n=95
Percent overall survival
Log-rank P=0.05
HR=1.43 (95% CI, 0.998-2.06)
H
lambda = 1.00
Observed-log10(P)
Expected-log10(P)
I
lambda = 0.61
Observed-log10(P)
Expected-log10(P)
J
lambda = 0.91
Observed-log10(P)
Expected-log10(P)
K
lambda = 0.72
Observed-log10(P)
Expected-log10(P)

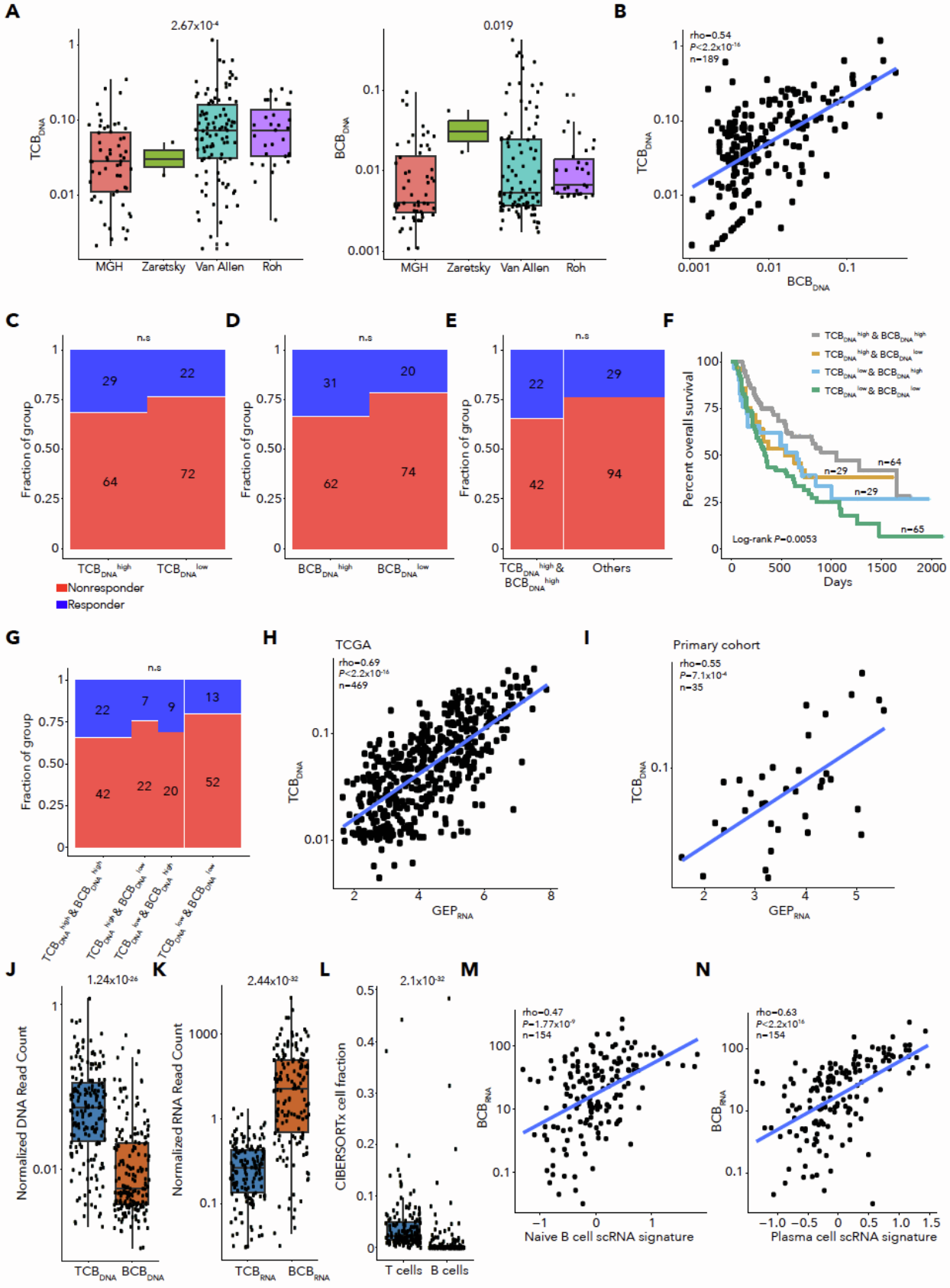
Supplementary Figure 2. **TMB, neoantigens, tumor purity and single gene mutation models, Related to Figure 1.** **A.** Kaplan-Meier survival curve for TMB high and low subgroups using TMB=10 mutations/Mb as a threshold (rather than the median). **B.** Response for patients with TMB over or under 10 mutations/Mb. **C.** Kaplan-Meier survival curve for all four subgroups of TMB (using the median threshold) and tumor purity. **D.** Spearman correlation of TMB with the number of neoantigens. **E.** Kaplan-Meier survival curve for patients with neoantigen burden above or below median. **F.** Correlation between clonal non-silent mutation burden (Clonal TMB) and TMB. **G.** Kaplan-Meier survival curve for patients with clonal TMB above or below median. **H.** qq plot for p values of the addition of each gene's mutation status to TMB in a Cox model for survival using loss-of-function mutation status for genes with 3 or more loss-of-function mutations. **I.** qq plot for p values of the addition of each gene's mutation status to TMB in a logistic regression model for response status using loss-of-function mutation status for genes with 3 or more loss-of-function mutations. **J.** qq plot for p values of the addition of each gene's mutation status to TMB in a Cox model for survival using non-synonymous mutation status for genes with 3 or more non-synonymous mutations. **K.** qq plot for p values of the addition of each gene's mutation status to TMB in a logistic regression model for response status using non-synonymous mutation status for genes with 3 or more non-synonymous mutations.


B2M
JAK1
JAK2
STAT1
STAT5A
CANX
STAT3
PSMB8
HLA−DOA
TAP2
PDIA3
TAPBP
CD74
CTSS
HLA−E
HLA−C
ERAP2
IFNGR1
HLA−B
HLA−DMB
HLA−DMA
STAT5B
TAP1
PSMB6
BRAF
NF1
TP53
NRAS
ARID2
RASA2
SF3B1
PTEN
CDKN2A
ARID1A
PIK3R1
KIT
CIITA
NOTCH2
RAC1
ARID1B
GNAQ
MAP2K1
NOTCH1
CTNNB1
BAP1
MSH2
PPP6C
RAF1
MAP2K2
IDH1
PTPN11
GNA11
Pre-treatment
Post-treatment
Clonal
Subclonal
Frame Shift Indel
Hotspot Mutation
In Frame Indel
Missense Mutation
Nonsense Mutation
Splice Site
B2M LOH
HLA−A LOH
HLA−B LOH
HLA−C LOH
B2M
JAK2
ERAP1
ERAP2
NLRC5
MITF
CCND1
MDM2
MDM4
TERT
KIT
BRAF
NRAS
AKT3
NOTCH2
MYC
CD274
HLA−A
HLA−B
HLA−C
TAP1
TAP2
JAK1
IFNGR2
STAT1
STAT3
STAT5A
STAT5B
NF1
IFNGR1
PTEN
CDKN2A
Clinical Status
Survival
T33T
208T
Case3
20
Case1
42T
Case2
125T
23T
333T
62T
148T
33
331T
16
115T
50
26
Case4
177T
356T
61T
99T
584T
57T
430T
53
192T
77T
72
163T
QQQT
25
383T
PD0041T
272T
409T
391T
35T
39T
253T
292T
54
282T
28
212T
194T
595T
98T
107T
155T
DDT
18
9
No LOH
LOH
Copy Number
0
1
2
3
4
5
6
7
Clinical Status
Nonresponder
Responder
Pre
Post
Overall Survival (months)
x<6
6<x<12
12<x<18
18<x<24
24<x<30
30<x<36
x>36

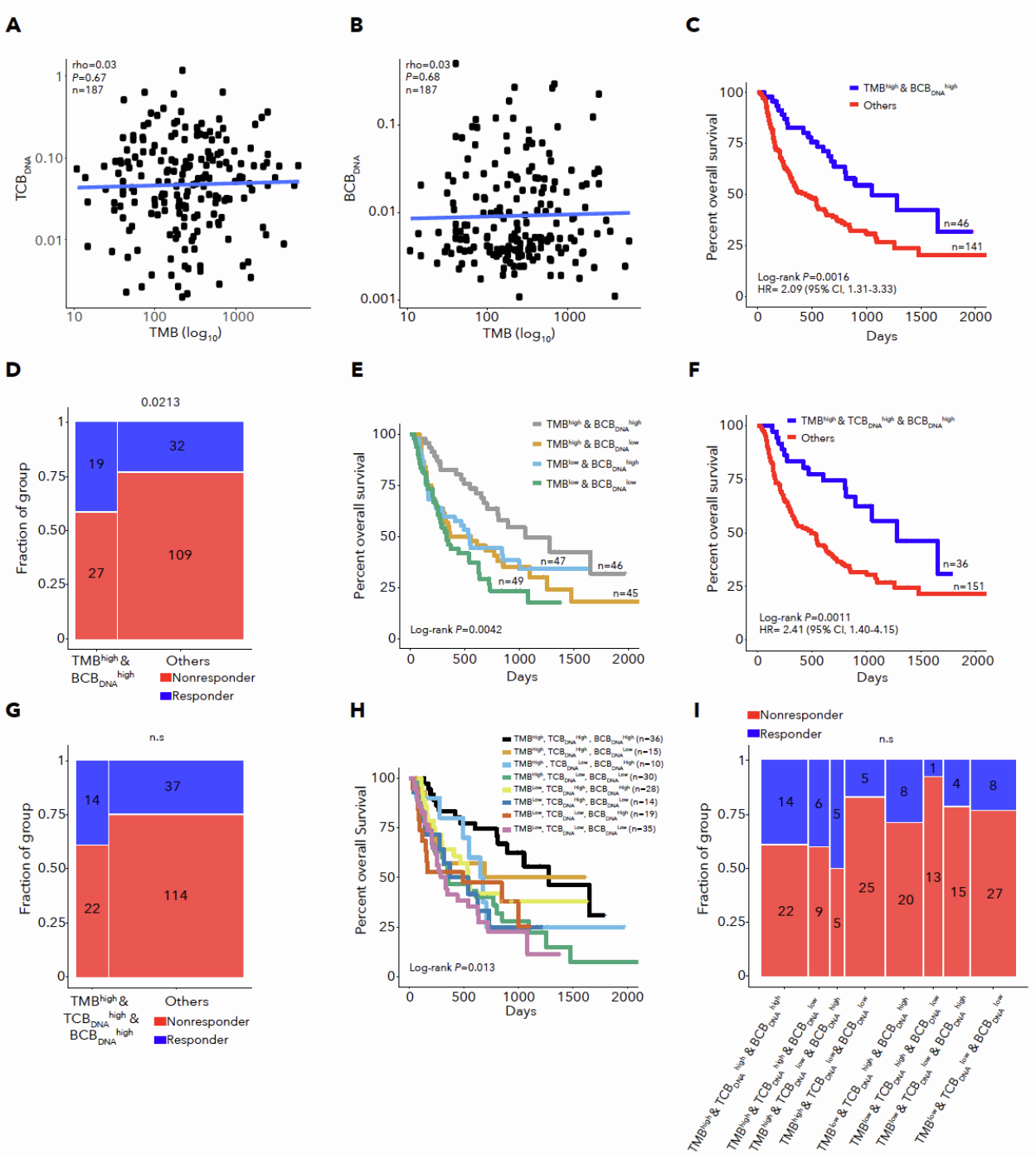
Supplementary Figure 3. **Comparison between paired pre-treatment and post-treatment biopsies, Related to Figure 1.** Upper panel shows a plot of mutations for selected genes in matched pre-treatments and post-treatment biopsies. The lower panel shows the integer copy number and LOH status for selected genes. Mutation clonality is represented by the area of each triangle in the upper panel. Lower triangles indicate mutations or copy number pre-treatment and upper triangles indicate mutations or copy number post-treatment. In the lower panel, white indicates absence of LOH in the first four rows and a copy number of 2 in the remaining rows. Patient response characteristics and survival are shown in the bottom two rows.



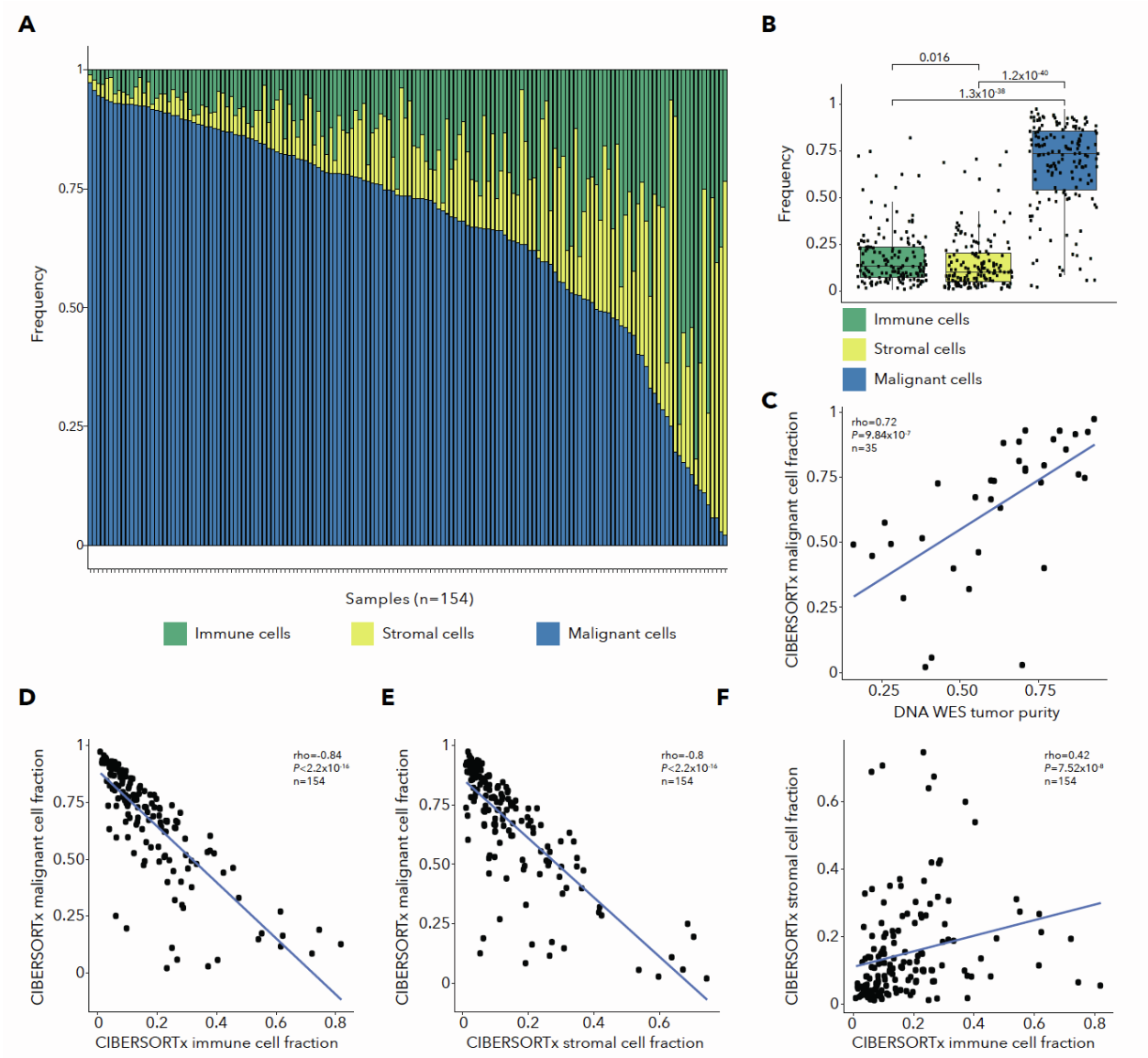
Supplementary Figure 4. Performance of TCB_{RNA} and BCB_{RNA} models, Related to Figure 2. A. TCB_{RNA} (left) and BCB_{RNA} (right) for each cohort with Kruskal-Wallis test p values. **B.** Correlation between TCB_{RNA} and BCB_{RNA} for primary cohort samples. **C.** Kaplan-Meier survival curve for all TCB_{RNA} and BCB_{RNA} high and low subgroups. **D.** Response for TCB_{RNA} high, BCB_{RNA} high subgroup vs. others. **E.** Response for all TCB_{RNA} and BCB_{RNA} high and low subgroups. **F.** Correlation between TCB_{RNA} and GEP[32] in TCGA melanoma samples. **G.** Correlation between TCB_{RNA} and GEP in primary cohort samples.


A
2.67x10-4
0.019
TCBDNA
BCBDNA
MGH
Zaretsky
Van Allen
Roh
B
rho=0.54
P<2.2x10-16
n=189
C
D
E
F
n.s
Fraction of group
Nonresponder
Responder
Percent overall survival
Log-rank P=0.0053
Days
G
H
TCGA
rho=0.69
P<2.2x10-16
n=469
I
Primary cohort
rho=0.55
P=7.1x10-4
n=35
J
1.24x10-26
Normalized DNA Read Count
K
2.44x10-32
Normalized RNA Read Count
L
2.1x10-32
CIBERSORTx cell fraction
T cells
B cells
M
rho=0.47
P=1.77x10-9
n=154
Naive B cell scRNA signature
N
rho=0.63
P<2.2x10-16
n=154
Plasma cell scRNA signature

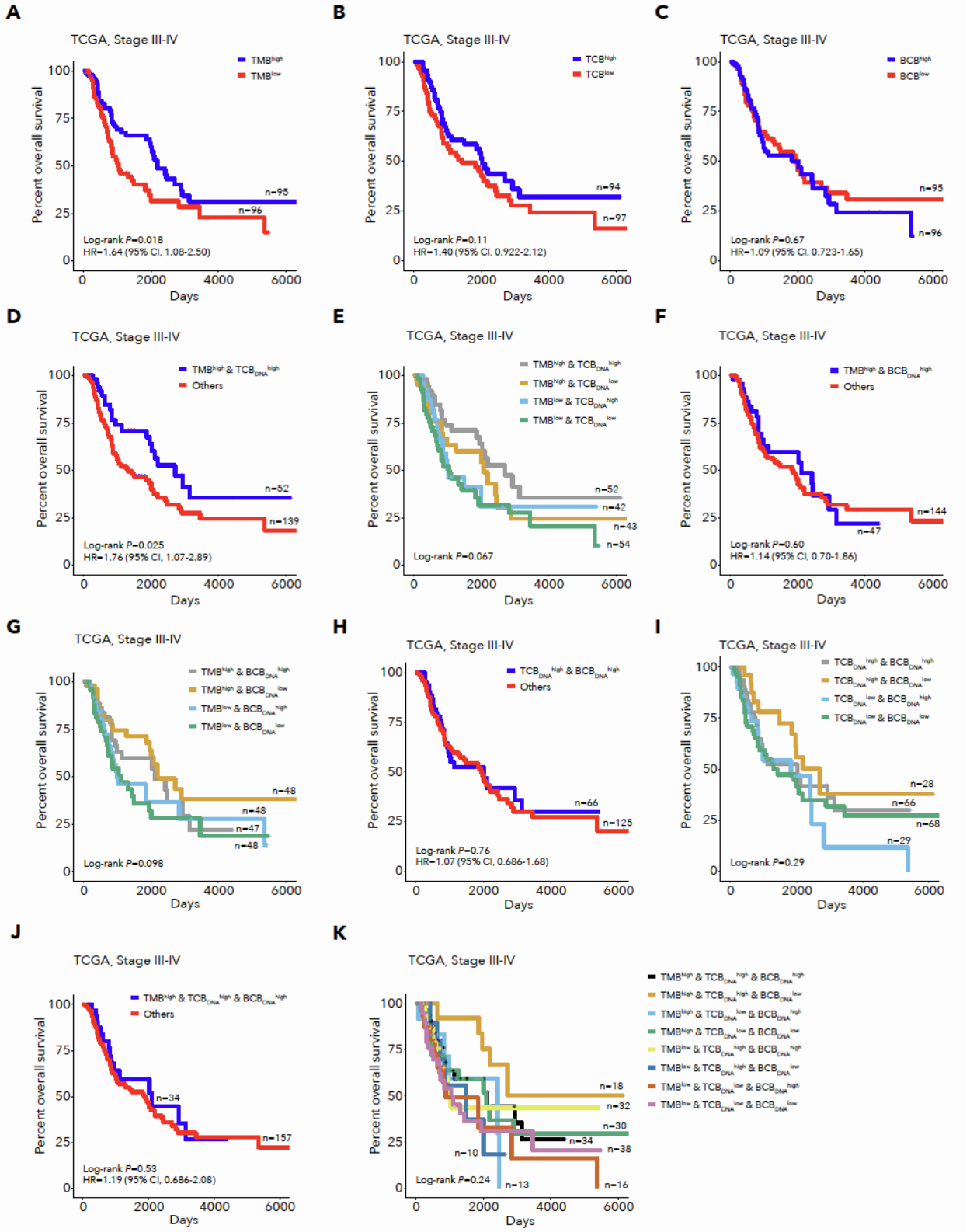
Supplementary Figure 5. **Performance of TCB_{DNA} and BCB_{DNA} models, Related to Figure 2. A.** TCB_{DNA} (left) and BCB_{DNA} (right) for each cohort with Kruskal-Wallis test p values. Due to cohort differences, we classified samples as above or below median TCB_{DNA} or BCB_{DNA} within each cohort. **B.** Correlation between TCB_{DNA} and BCB_{DNA} for primary cohort samples. **C.** Response for TCB_{DNA} high and low subgroups. **D.** Response for BCB_{DNA} high and low subgroups. **E.** Response for TCB_{DNA} high, BCB_{DNA} high subgroup vs. others. **F.** Kaplan-Meier survival curve for all TCB_{DNA} and BCB_{DNA} high and low subgroups. **G.** Response for all TCB_{DNA} and BCB_{DNA} high and low subgroups. **H.** Correlation between TCB_{DNA} and GEP_{RNA}[32] in TCGA melanoma samples. **I.** Correlation between TCB_{DNA} and GEP_{RNA} in primary cohort samples, for samples with DNA and RNA extracted from the same location in the tumor. **J.** Normalized DNA read counts for TCB_{DNA} vs. BCB_{DNA} comparison, with Wilcoxon test p value. **K.** Normalized RNA read counts for TCB_{RNA} vs. BCB_{RNA} comparison, with Wilcoxon test p value. **M.** CIBERSORTx cell fraction for T cells and B cells, with Wilcoxon test p value. **M.** Correlation between BCB_{RNA} and naive B cell scRNA signature for primary cohort samples. **N.** Correlation between BCB_{RNA} and plasma B cell scRNA signature for primary cohort samples.



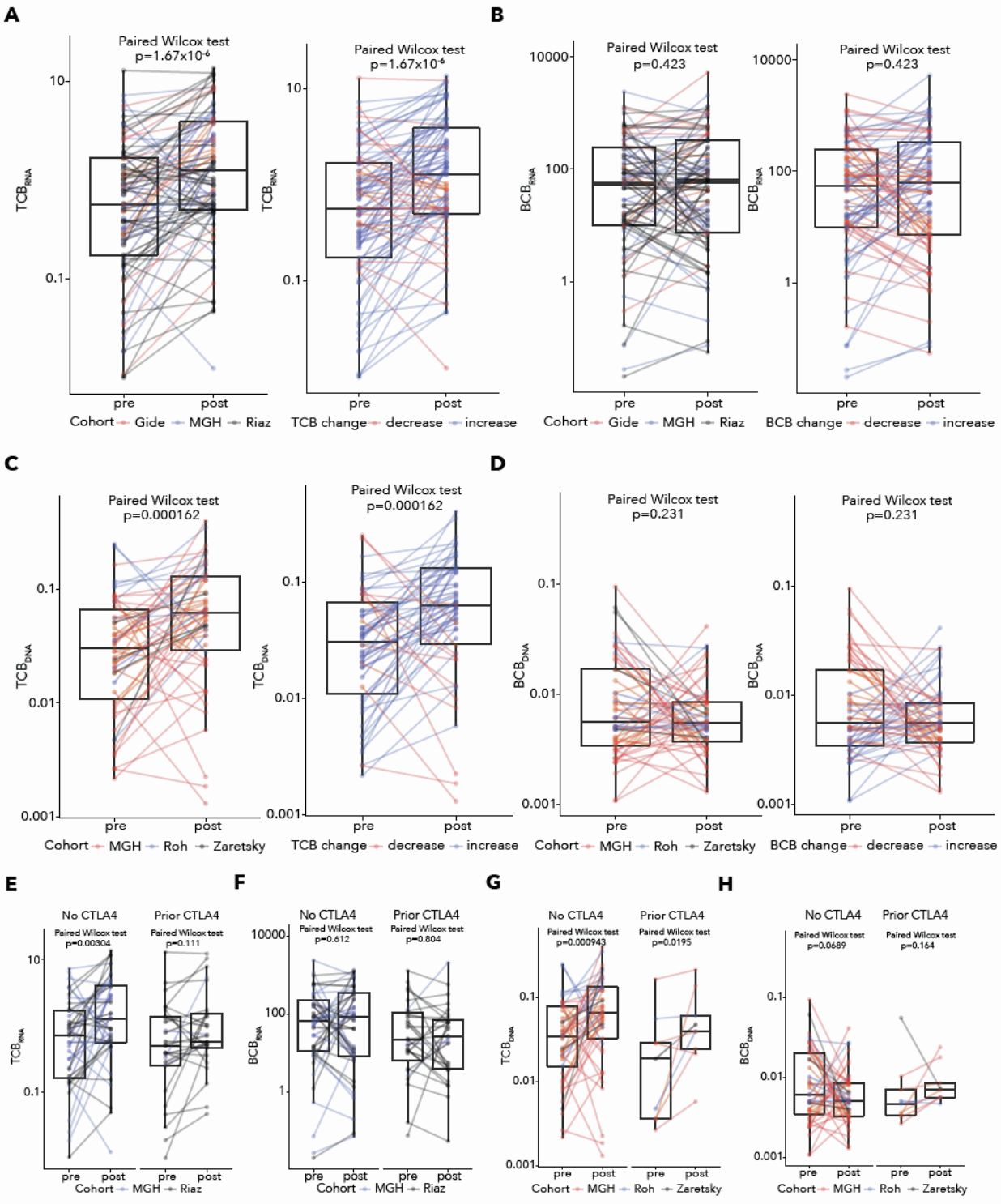
Supplementary Figure 6. Performance of integrative DNA-based models for survival and response prediction, Related to Figure 2. A. Correlation between TMB and TCB_{DNA}. **B.** Correlation between TMB and BCB_{DNA}. **C.** Kaplan-Meier survival curve for TMB high, BCB_{DNA} high subgroup vs. other patients. **D.** Response for TMB high, BCB_{DNA} high subgroup vs. other patients. **E.** Kaplan-Meier survival curve for all combined TMB and BCB_{DNA} subgroups. **F.** Kaplan-Meier survival curve for TMB high, TCB_{DNA} high, BCB_{DNA} high subgroup vs. other patients. **G.** Response for TMB high, TCB_{DNA} high, BCB_{DNA} high subgroup vs. other patients. **H.** Kaplan-Meier survival curve for all combined TMB, TCB_{DNA}, and BCB_{DNA} subgroups. **I.** Response for all combined TMB, TCB_{DNA}, and BCB_{DNA} subgroups.



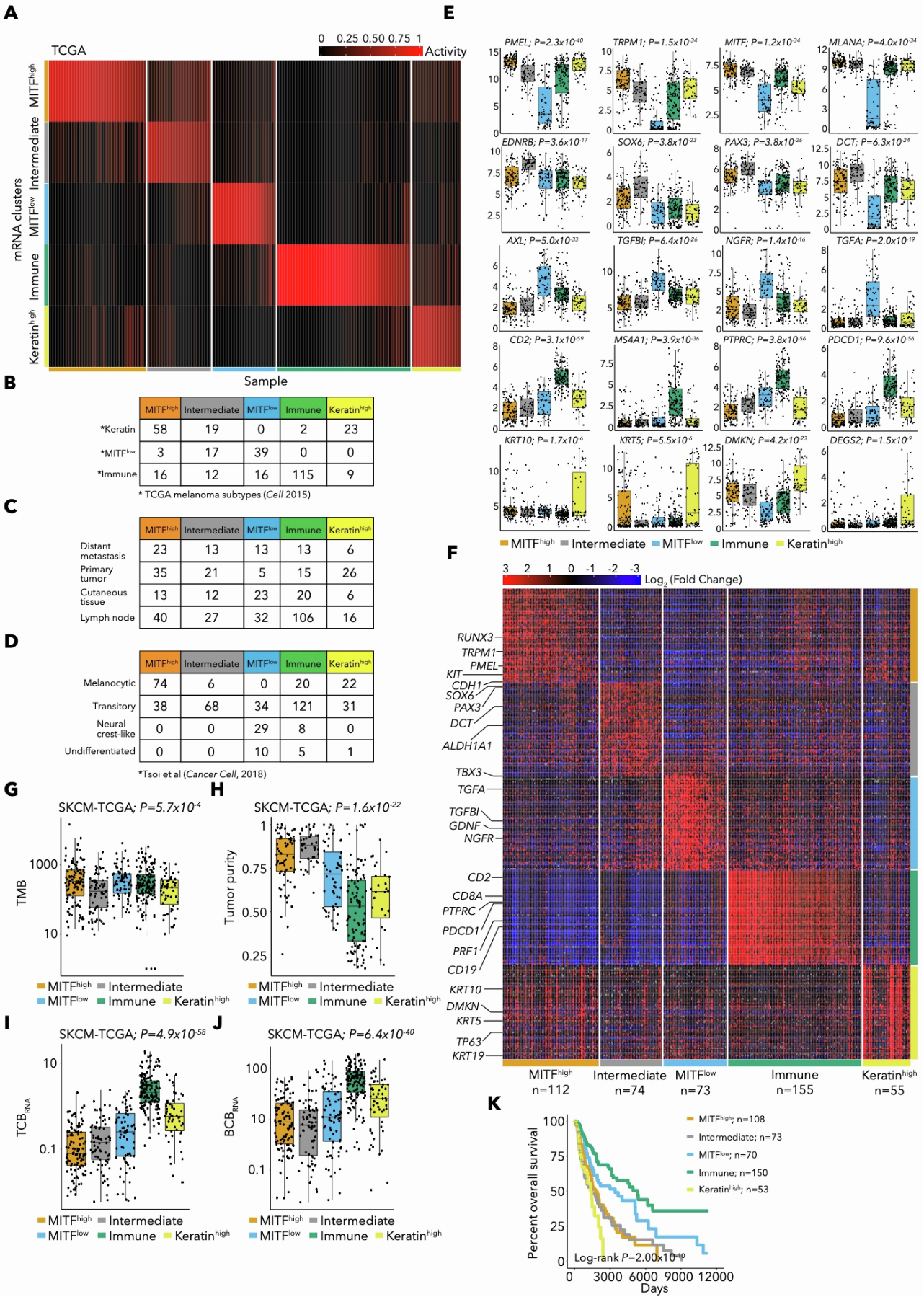
Supplementary Figure 7. **Quantification of immune and stromal cell fractions using CIBERSORTx, Related to Figure 2. A.** CIBERSORTx immune, stromal and malignant cell fractions for each patient in primary cohort RNA samples (n=154). **B.** Cell fraction estimation for immune, stromal and malignant cells using CIBERSORTx, with Wilcox test p value. **C.** Correlation between CIBERSORTx malignant cell fraction from RNA-Seq and DNA WES tumor purity for samples with matched DNA and RNA from the same tumor location in primary cohort (n=35). **D.** Correlation between CIBERSORTx immune and malignant cell fractions for primary cohort samples. **E.** Correlation between CIBERSORTx stromal and malignant cell fractions for primary cohort samples. **F.** Correlation between CIBERSORTx immune and stromal cell fractions for primary cohort samples.


A
TCGA, Stage III-IV
TMB^high
TMB^low
Percent overall survival
n=95
n=96
Log-rank P=0.018
HR=1.64 (95% CI, 1.08-2.50)
Days
B
TCGA, Stage III-IV
TCB^high
TCB^low
n=94
n=97
Log-rank P=0.11
HR=1.40 (95% CI, 0.922-2.12)
C
TCGA, Stage III-IV
BCB^high
BCB^low
n=95
n=96
Log-rank P=0.67
HR=1.09 (95% CI, 0.723-1.65)
D
TCGA, Stage III-IV
TMB^high & TCB_DNA^high
Others
n=52
n=139
Log-rank P=0.025
HR=1.76 (95% CI, 1.07-2.89)
E
TCGA, Stage III-IV
TMB^high & TCB_DNA^high
TMB^high & TCB_DNA^low
TMB^low & TCB_DNA^high
TMB^low & TCB_DNA^low
n=52
n=42
n=43
n=54
Log-rank P=0.067
F
TCGA, Stage III-IV
TMB^high & BCB_DNA^high
Others
n=144
n=47
Log-rank P=0.60
HR=1.14 (95% CI, 0.70-1.86)
G
TCGA, Stage III-IV
TMB^high & BCB_DNA^high
TMB^high & BCB_DNA^low
TMB^low & BCB_DNA^high
TMB^low & BCB_DNA^low
n=48
n=48
n=47
n=48
Log-rank P=0.098
H
TCGA, Stage III-IV
TCB_DNA^high & BCB_DNA^high
Others
n=66
n=125
Log-rank P=0.76
HR=1.07 (95% CI, 0.686-1.68)
I
TCGA, Stage III-IV
TCB_DNA^high & BCB_DNA^high
TCB_DNA^high & BCB_DNA^low
TCB_DNA^low & BCB_DNA^high
TCB_DNA^low & BCB_DNA^low
n=28
n=66
n=68
n=29
Log-rank P=0.29
J
TCGA, Stage III-IV
TMB^high & TCB_DNA^high & BCB_DNA^high
Others
n=34
n=157
Log-rank P=0.53
HR=1.19 (95% CI, 0.686-2.08)
K
TCGA, Stage III-IV
n=18
n=32
n=30
n=34
n=38
n=10
n=13
n=16
Log-rank P=0.24
TMB^high & TCB_DNA^high & BCB_DNA^high
TMB^high & TCB_DNA^high & BCB_DNA^low
TMB^high & TCB_DNA^low & BCB_DNA^high
TMB^high & TCB_DNA^low & BCB_DNA^low
TMB^low & TCB_DNA^high & BCB_DNA^high
TMB^low & TCB_DNA^high & BCB_DNA^low
TMB^low & TCB_DNA^low & BCB_DNA^high
TMB^low & TCB_DNA^low & BCB_DNA^low
Percent overall survival
Days
0
25
50
75
100
2000
4000
6000

Supplementary Figure 8. **Performance of integrative DNA-based models for survival and response prediction for TCGA melanoma stage III-IV cases, Related to Figure 2. A.** Kaplan-Meier survival curve for TMB high and low subgroups. **B.** Kaplan-Meier survival curve for TCB_{DNA} high vs. other patients. **C.** Kaplan-Meier survival curve for BCB_{DNA} high vs. other patients. **D.** Kaplan-Meier survival curve for TMB high, TCB_{DNA} high subgroup vs. other patients. **E.** Kaplan-Meier survival curve for all combined TMB and TCB_{DNA} subgroups. **F.** Kaplan-Meier survival curve for TMB high, BCB_{DNA} high subgroup vs. other patients. **G.** Kaplan-Meier survival curve for all combined TMB and BCB_{DNA} subgroups. **H.** Kaplan-Meier survival curve for TCB_{DNA} high, BCB_{DNA} high subgroup vs. other patients. **I.** Kaplan-Meier survival curve for all combined TCB_{DNA} and BCB_{DNA} subgroups. **J.** Kaplan-Meier survival curve for TMB high, TCB_{DNA} high, BCB_{DNA} high subgroup vs. other patients. **K.** Kaplan-Meier survival curve for all combined TMB, TCB_{DNA} and BCB_{DNA} subgroups.


A
Paired Wilcox test
p=1.67x10⁻⁶
TCB_RNA
10
0.1
pre
post
Cohort Gide MGH Riaz
Paired Wilcox test
p=1.67x10⁻⁶
TCB_RNA
10
0.1
pre
post
TCB change decrease increase
B
Paired Wilcox test
p=0.423
BCB_RNA
10000
100
1
pre
post
Cohort Gide MGH Riaz
Paired Wilcox test
p=0.423
BCB_RNA
10000
100
1
pre
post
BCB change decrease increase
C
Paired Wilcox test
p=0.000162
TCB_DNA
0.1
0.01
0.001
pre
post
Cohort MGH Roh Zaretsky
Paired Wilcox test
p=0.000162
TCB_DNA
0.1
0.01
0.001
pre
post
TCB change decrease increase
D
Paired Wilcox test
p=0.231
BCB_DNA
0.1
0.01
0.001
pre
post
Cohort MGH Roh Zaretsky
Paired Wilcox test
p=0.231
BCB_DNA
0.1
0.01
0.001
pre
post
BCB change decrease increase
E
No CTLA4
Paired Wilcox test
p=0.00304
Prior CTLA4
Paired Wilcox test
p=0.111
TCB_RNA
10
0.1
pre post pre post
Cohort MGH Riaz
F
No CTLA4
Paired Wilcox test
p=0.612
Prior CTLA4
Paired Wilcox test
p=0.804
BCB_RNA
10000
100
1
pre post pre post
Cohort MGH Riaz
G
No CTLA4
Paired Wilcox test
p=0.000943
Prior CTLA4
Paired Wilcox test
p=0.0195
TCB_DNA
0.1
0.01
0.001
pre post pre post
Cohort MGH Roh Zaretsky
H
No CTLA4
Paired Wilcox test
p=0.0689
Prior CTLA4
Paired Wilcox test
p=0.164
BCB_DNA
0.1
0.01
0.001
pre post pre post
Cohort MGH Roh Zaretsky

Supplementary Figure 9. **Dynamics of DNA and RNA-based TCB and BCB abundance between paired pre-treatment and post-treatment biopsies, Related to Figure 2. A.** Changes in TCB_{RNA} between matched pre-treatment and post-treatment samples, colored by cohort (left) or decrease/increase (right). **B.** Changes in BCB_{RNA} between matched pre-treatment and post-treatment samples, colored by cohort (left) or decrease/increase (right). **C.** Changes in TCB_{DNA} between matched pre-treatment and post-treatment samples, colored by cohort (left) and decrease/increase (right). **D.** Changes in BCB_{DNA} between matched pre-treatment and post-treatment samples, colored by cohort (left) and decrease/increase (right). **E.** Changes in TCB_{RNA} between matched pre-treatment and post-treatment samples, with no prior CTLA-4 therapy (left) or prior CTLA-4 therapy (right). **F.** Changes in BCB_{RNA} between matched pre-treatment and post-treatment samples, with no prior CTLA-4 therapy (left) or prior CTLA-4 therapy (right). **G.** Changes in TCB_{DNA} between matched pre-treatment and post-treatment samples, with no prior CTLA-4 therapy (left) or prior CTLA-4 therapy (right). **H.** Changes in BCB_{DNA} between matched pre-treatment and post-treatment samples, with no prior CTLA-4 therapy (left) or prior CTLA-4 therapy (right).



B

	MITF^{high}	Intermediate	MITF^{low}	Immune	Keratin^{high}
*Keratin	58	19	0	2	23
*MITF^{low}	3	17	39	0	0
*Immune	16	12	16	115	9

* TCGA melanoma subtypes (*Cell* 2015)

C

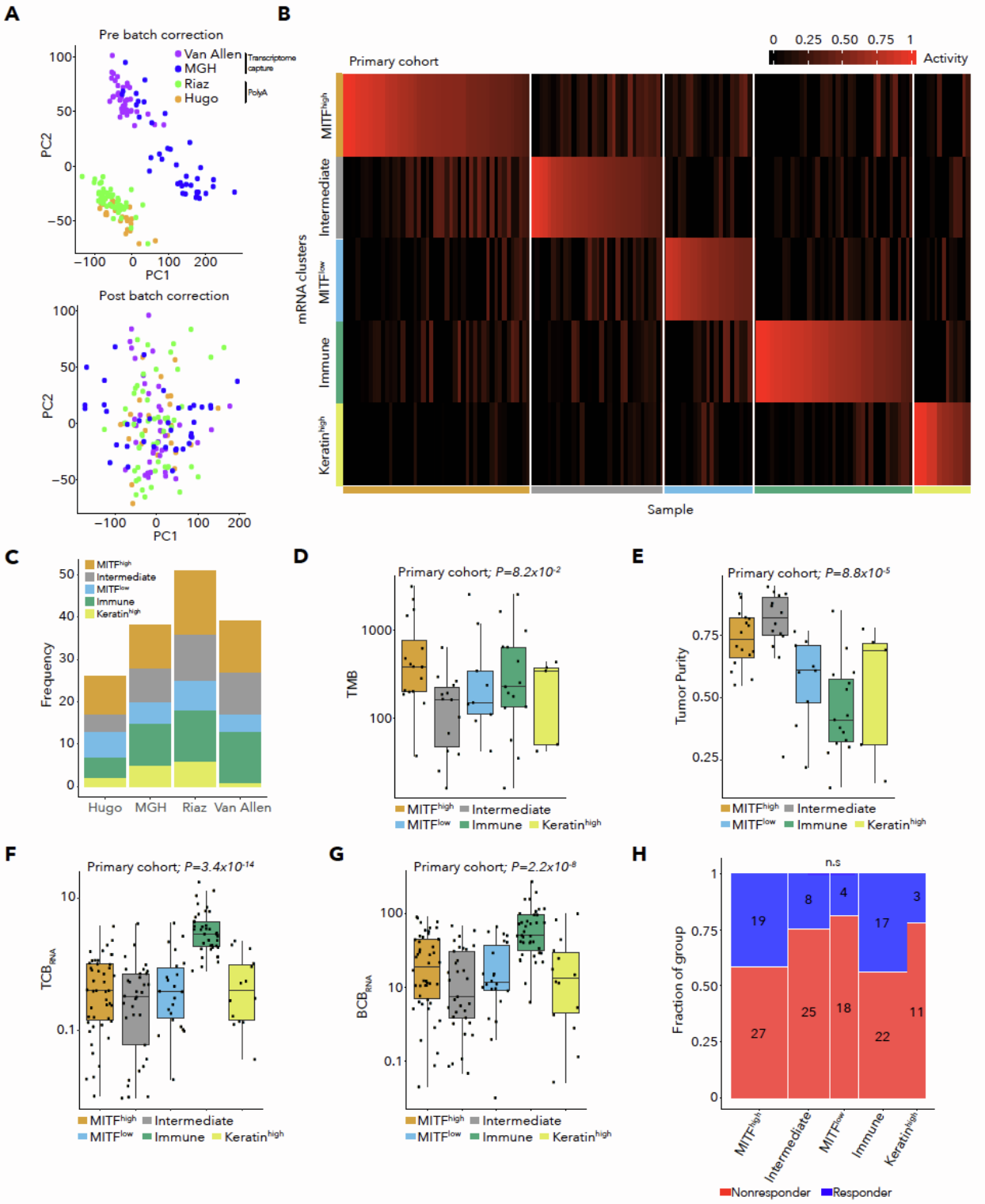
	MITF^{high}	Intermediate	MITF^{low}	Immune	Keratin^{high}
Distant metastasis	23	13	13	13	6
Primary tumor	35	21	5	15	26
Cutaneous tissue	13	12	23	20	6
Lymph node	40	27	32	106	16

D

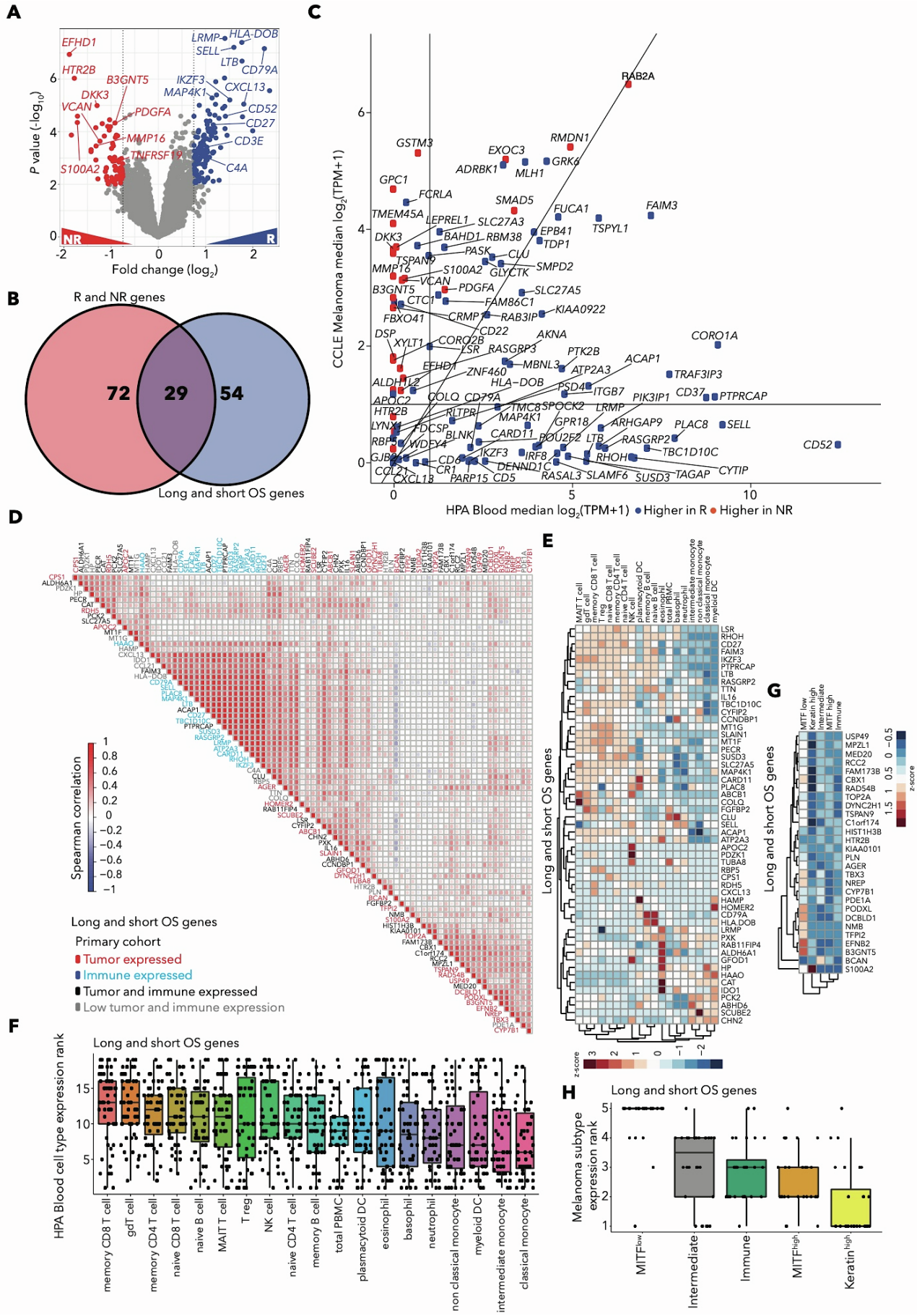
	MITF^{high}	Intermediate	MITF^{low}	Immune	Keratin^{high}
Melanocytic	74	6	0	20	22
Transitory	38	68	34	121	31
Neural crest-like	0	0	29	8	0
Undifferentiated	0	0	10	5	1

*Tsoi et al (*Cancer Cell*, 2018)

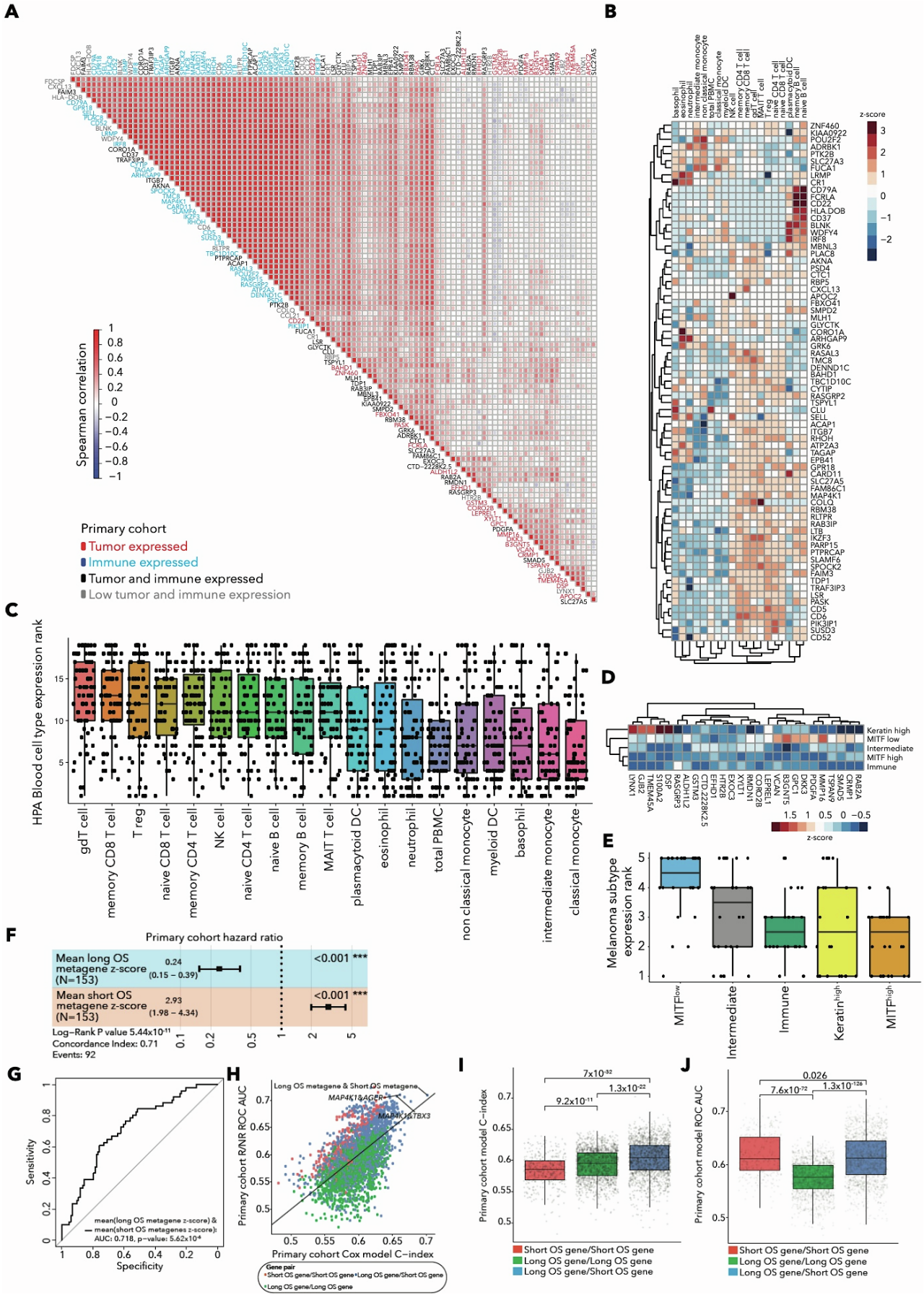
Supplementary Figure 10. **Subtypes identified using NMF clustering of TCGA melanoma RNA-seq and their tumor related features, Related to Figure 3. A.** NMF H matrix from TCGA melanoma NMF clustering (n=469) identified 5 subtypes. Activity values indicate the probability that a sample is associated with a cluster. Samples are sorted by activity value within subtypes. **B.** Comparison of subtype membership to previously identified TCGA subtypes[38]. **C.** TCGA biopsy locations and subtype membership for all samples. The Immune subtype is enriched for lymph node biopsy samples, but all other subtypes contain lymph node samples as well. **D.** Comparison of subtype membership to melanoma differentiation subtypes[48]. **E.** Boxplots of gene expression for selected TCGA melanoma NMF cluster marker genes. All genes were identified through automated marker selection and were included in the heatmap except *MITF*, *MLANA* and *AXL*. **F.** Heatmap of marker genes identified for each NMF subtype in TCGA melanoma data. Initial $\log_2$(TPM+1) values were median centered to obtain $\log_2$(Fold change) values. We selected marker genes which were overexpressed in each cluster relative to all other samples. **G-J.** Kruskal-wallis p values for association of gene expression with subtype are displayed above plots, (**G**) TMB ($\log_{10}$ scale), (**H**) tumor purity, (**I**) TCB_{RNA} and (**J**) BCB_{RNA}, for TCGA samples by RNA-seq subtype. **K.** Kaplan-Meier survival curve for TCGA samples by RNA-seq subtype.


A
Pre batch correction
Van Allen
MGH
Riaz
Hugo
Transcriptome capture
PolyA
PC2
PC1
Post batch correction
B
Primary cohort
0 0.25 0.50 0.75 1
Activity
mRNA clusters
MITF^high
Intermediate
MITF^low
Immune
Keratin^high
Sample
C
Frequency
Hugo MGH Riaz Van Allen
D
Primary cohort; P=8.2x10^-2
TMB
E
Primary cohort; P=8.8x10^-5
Tumor Purity
F
Primary cohort; P=3.4x10^-14
TCB_RNA
G
Primary cohort; P=2.2x10^-8
BCB_RNA
H
n.s
Fraction of group
19
27
8
25
4
18
17
22
3
11
Nonresponder
Responder

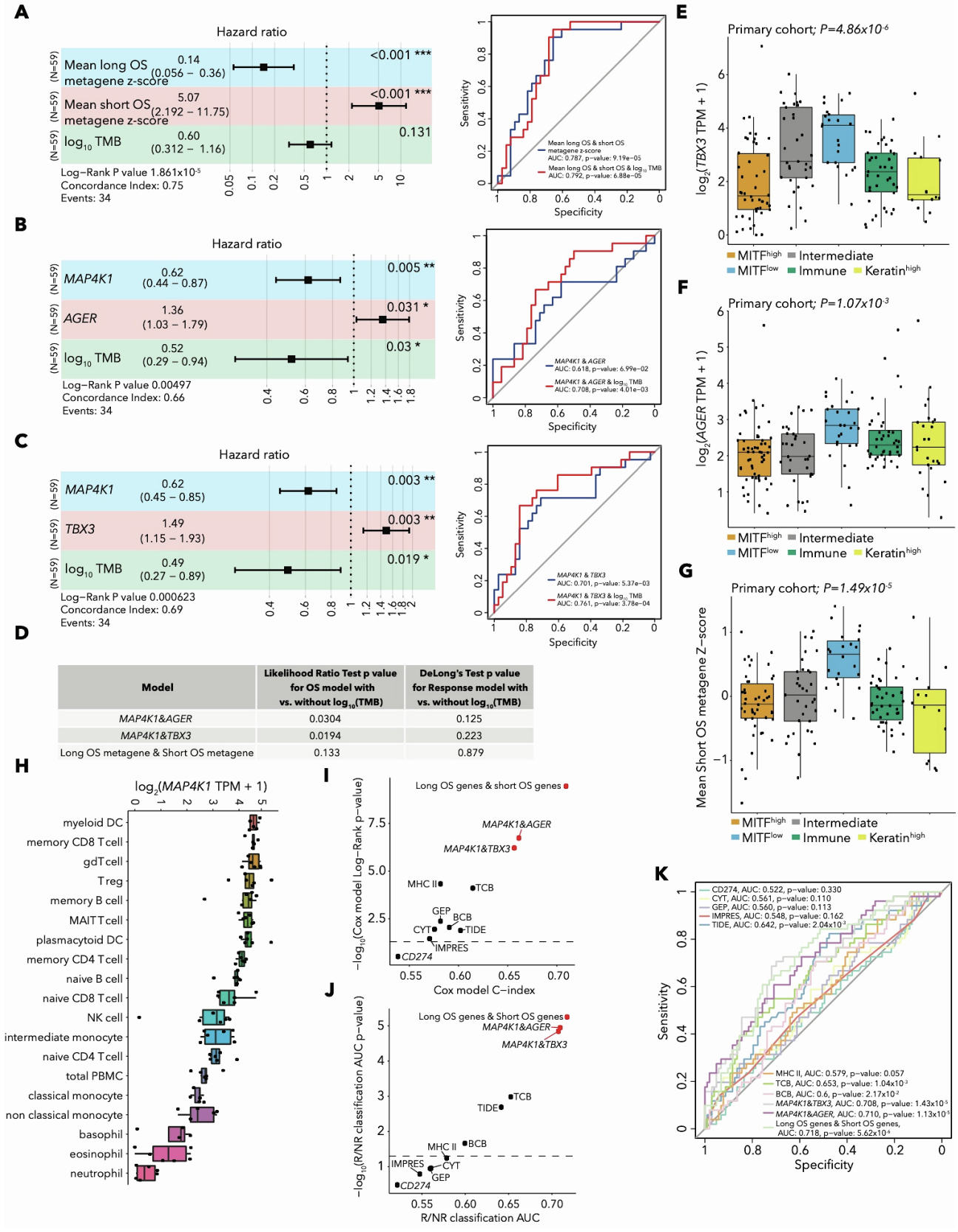
Supplementary Figure 11. **Subtype classification for primary cohort RNA-seq samples, Related to Figure 3. A.** PCA of protein coding gene $\log_2$(TPM+1) values before (upper plot) and after batch effects correction with ComBat[83] (lower plot). Before batch effects correction, samples cluster by cohort and library preparation method (polyA selection vs. transcriptome capture). **B.** NMF H matrix for primary cohort sample subtyping using subtypes and marker genes identified in TCGA melanoma samples. **C.** Subtypes by RNA-seq sample for each sample in the primary cohort. **D-G.** Kruskal-wallis p values for association of gene expression with subtype are displayed above plots, (**D**) TMB ($\log_{10}$ scale), (**E**) tumor purity for RNA-seq samples with matched WES data, (**F**) TCB_{RNA} and (**G**) BCB_{RNA}. **H.** Number of responders and non-responders by subtype.


A
P value (-log10)
Fold change (log2)
NR
R
EFHD1
HTR2B
B3GNT5
DKK3
VCAN
PDGFA
MMP16
TNFRSF19
S100A2
LRMP
HLA-DOB
SELL
LTB
CD79A
IKZF3
MAP4K1
CXCL13
CD52
CD27
CD3E
C4A
B
R and NR genes
72
29
54
Long and short OS genes
C
CCLE Melanoma median log2(TPM+1)
HPA Blood median log2(TPM+1)
Higher in R
Higher in NR
RAB2A
GSTM3
EXOC3
RMDN1
ADRBK1
GRK6
MLH1
GPC1
FCRLA
SMAD5
FAIM3
FUCA1
TMEM45A
LEPREL1
SLC27A3
TSPYL1
DKK3
BAHD1
RBM38
EPB41
TDP1
PASK
CLU
TSPAN9
GLYCTK
SMPD2
MMP16
S100A2
VCAN
B3GNT5
PDGFA
SLC27A5
CTC1
FAM86C1
KIAA0922
FBXO41
CRMP1
RAB3IP
DSP
CD22
AKNA
CORO1A
XYLT1
CORO2B
LSR
RASGRP3
PTK2B
EFHD1
MBNL3
ACAP1
ZNF460
ATP2A3
TRAF3IP3
ALDH1L2
HLA-DOB
PSD4
ITGB7
APOC2
COLQ
CD79A
PIK3IP1
CD37
PTPRCAP
HTR2B
TMC8
SPOCK2
LRMP
RLTPR
MAP4K1
GPR18
ARHGAP9
PLAC8
SELL
LYN
FCGR3B
BLNK
CARD11
POU2F2
LTB
RASGRP2
RBP5
WDFY4
TBC1D10C
CD52
GJB2
IKZF3
IRF8
RHOH
CD6
CR1
DENND1C
CYTIP
CCL21
CXCL13
PARP15
CD5
RASAL3
SLAMF6
SUSD3
TAGAP
D
Spearman correlation
Long and short OS genes
Primary cohort
Tumor expressed
Immune expressed
Tumor and immune expressed
Low tumor and immune expression
E
Long and short OS genes
z-score
F
Long and short OS genes
HPA Blood cell type expression rank
memory CD8 T cell
gdT cell
memory CD4 T cell
naive CD8 T cell
naive B cell
MAIT T cell
T reg
NK cell
naive CD4 T cell
memory B cell
total PBMC
plasmacytoid DC
eosinophil
basophil
neutrophil
non classical monocyte
myeloid DC
intermediate monocyte
classical monocyte
G
MITF low
Keratin high
Intermediate
MITF high
Immune
Long and short OS genes
z-score
H
Long and short OS genes
Melanoma subtype expression rank
MITFlow
Intermediate
Immune
MITFhigh
Keratinhigh

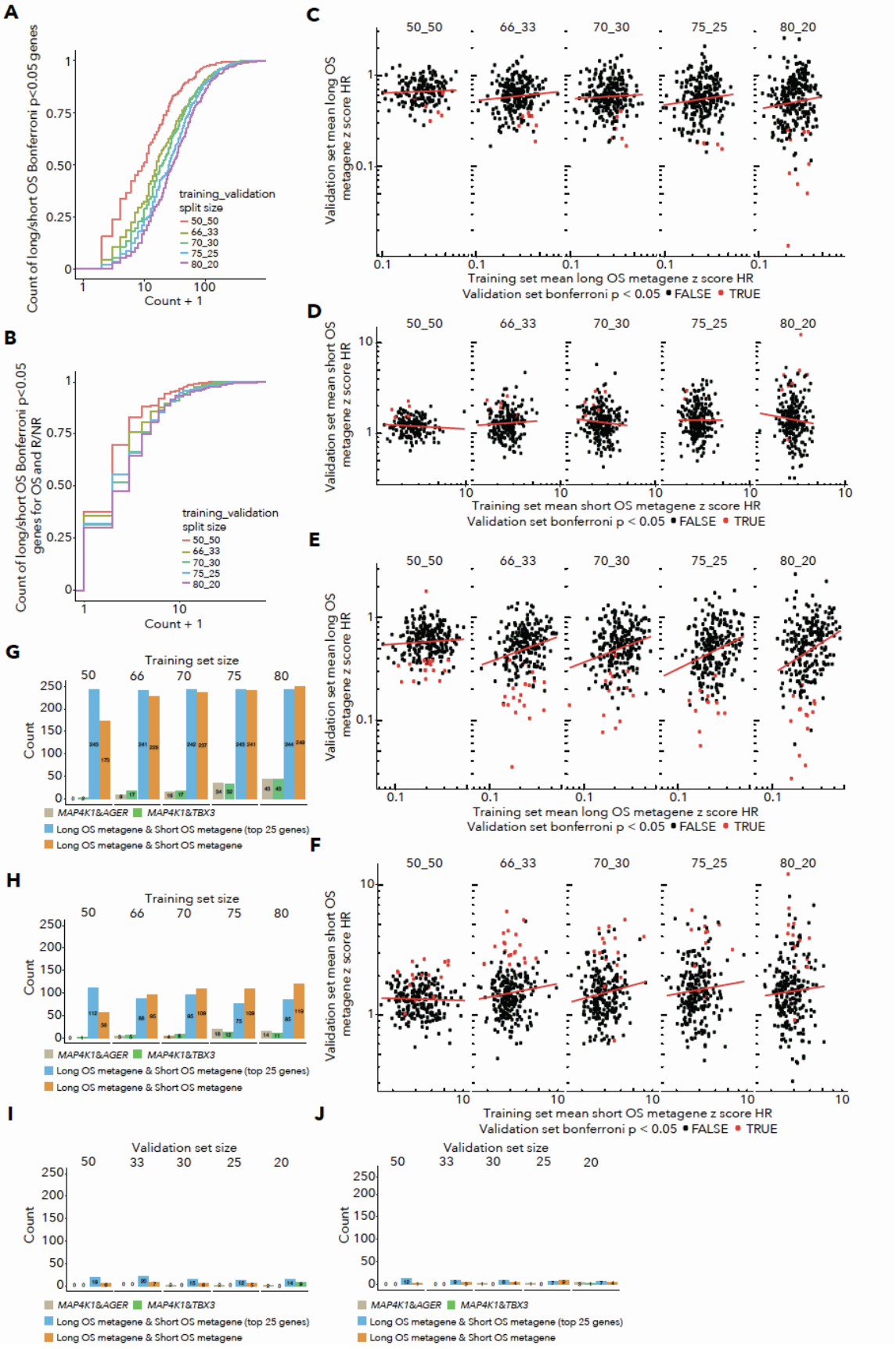
Supplementary Figure 12. **Genes associated with response in the primary cohort and expression patterns for long and short OS differentially expressed genes in the primary cohort, Related to Figure 3. A.** Differential expression between responders (R) and non-responders (NR) in the primary cohort. **B.** Venn diagram for differentially expressed genes in the high vs. low OS and responder vs. non-responder comparisons. In total, 29 genes were differentially expressed in both analyses. **C.** Expression of responder and non-responder differentially expressed genes in melanoma CCLE cell lines and Human Protein Atlas blood cell types. **D.** Co-expression for long and short OS differentially expressed genes in the primary cohort. **E.** Expression of genes overexpressed in long OS patients in Human Protein Atlas (HPA) blood cell types. **F.** Ranks of HPA cell type expression for genes overexpressed in long OS patients. **G.** Expression of genes overexpressed in short OS patients in primary cohort samples grouped by melanoma subtype. **H.** Ranks of melanoma subtype expression for genes overexpressed in short OS patients.


A
Spearman correlation
Primary cohort
Tumor expressed
Immune expressed
Tumor and immune expressed
Low tumor and immune expression
B
z-score
C
HPA Blood cell type expression rank
gdT cell
memory CD8 T cell
T reg
naive CD8 T cell
memory CD4 T cell
NK cell
naive CD4 T cell
naive B cell
memory B cell
MAIT T cell
plasmacytoid DC
eosinophil
neutrophil
total PBMC
non classical monocyte
myeloid DC
basophil
intermediate monocyte
classical monocyte
D
Keratin high
MITF low
Intermediate
MITF high
Immune
z-score
E
Melanoma subtype expression rank
MITF low
Intermediate
Immune
Keratin high
MITF high
F
Primary cohort hazard ratio
Mean long OS metagene z-score (N=153)
0.24 (0.15 – 0.39)
<0.001 ***
Mean short OS metagene z-score (N=153)
2.93 (1.98 – 4.34)
<0.001 ***
Log−Rank P value 5.44x10-11
Concordance Index: 0.71
Events: 92
G
Sensitivity
Specificity
mean(long OS metagene z-score) & mean(short OS metagenes z-score):
AUC: 0.718, p−value: 5.62x10-8
H
Long OS metagene & Short OS metagene
MAP4K1&AGER
MAP4K1&TBX3
Primary cohort R/NR ROC AUC
Primary cohort Cox model C−index
Gene pair
Short OS gene/Short OS gene
Long OS gene/Short OS gene
Long OS gene/Long OS gene
I
7x10-32
1.3x10-22
9.2x10-11
Primary cohort model C−index
Short OS gene/Short OS gene
Long OS gene/Long OS gene
Long OS gene/Short OS gene
J
0.026
7.6x10-72
1.3x10-126
Primary cohort model ROC AUC
Short OS gene/Short OS gene
Long OS gene/Long OS gene
Long OS gene/Short OS gene

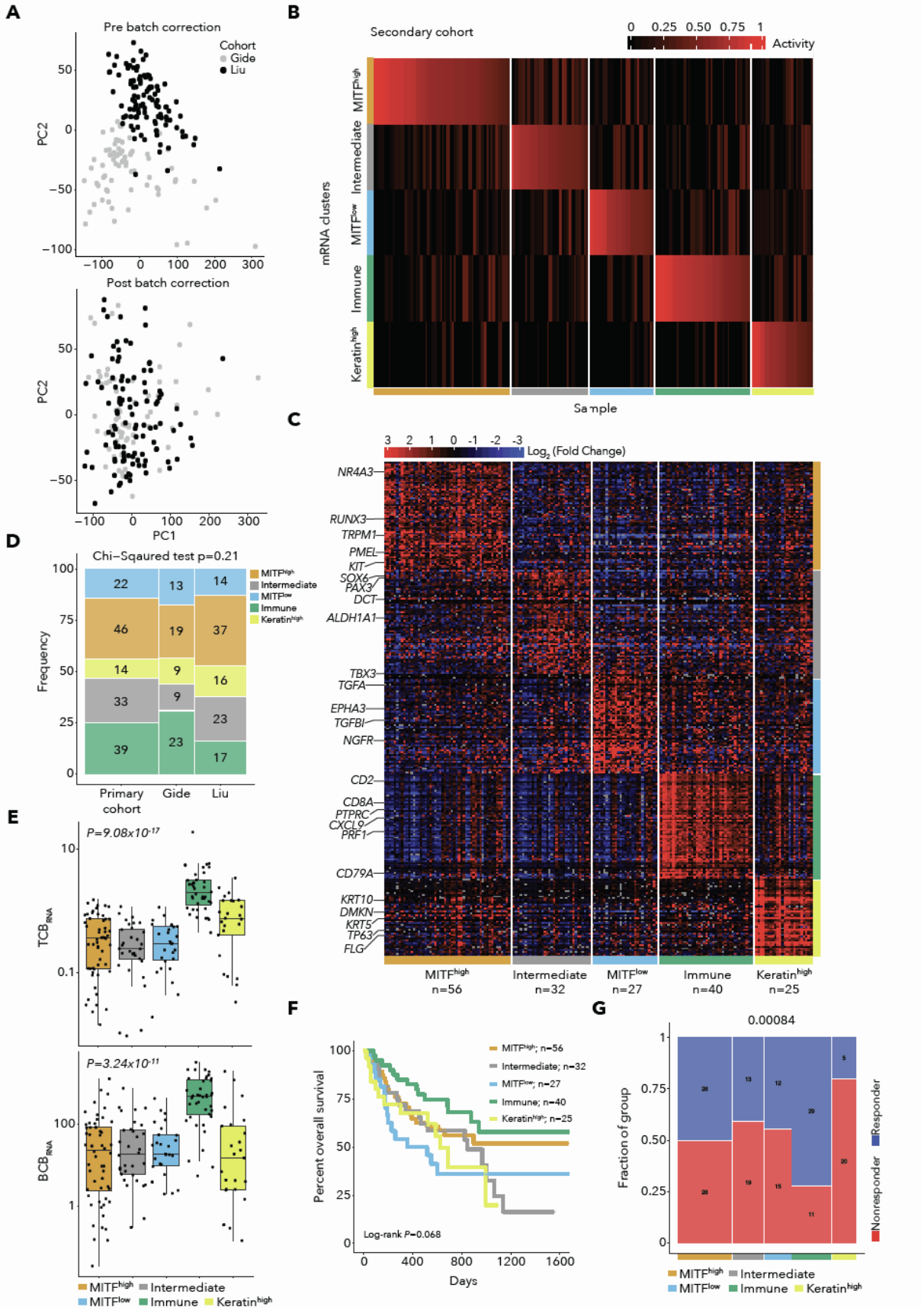
Supplementary Figure 13. **Expression patterns for responder and non-responder differentially expressed genes and performance of gene-pair models in the primary cohort in the primary cohort, Related to Figure 3 and Figure 4. A.** Co-expression for responder and non-responder differentially expressed genes in the primary cohort. **B.** Expression of genes overexpressed in responders in Human Protein Atlas (HPA) blood cell types. **C.** Ranks of HPA cell type expression for genes overexpressed in responders. **D.** Expression of genes overexpressed in non-responders in primary cohort samples grouped by melanoma subtype. **E.** Ranks of melanoma subtype expression for genes overexpressed in non-responders. **F.** Forest plot for the long OS and short OS metagene pair model in the primary cohort. Error bars represent 95% confidence intervals for Cox model hazard ratio estimates. **G.** ROC curve for the long OS and short OS metagene pair model in the primary cohort. **H.** Performance for all gene pair models using genes derived from long OS vs. short OS differential expression in terms of survival and response predictions in the primary cohort based on survival C-index and response AUC. Each point represents one gene pair model, and points are colored by the gene pair type (Short OS/Short OS gene pair- red, Long OS/Long OS gene pair- green or Short OS/Long OS gene pair- blue). **I.** Survival C-index for gene pair models using genes derived from long OS vs. short OS differential expression by gene pair type. **J.** Response AUC for gene pair models using genes derived from long OS vs. short OS differential expression by gene pair type.


A
Hazard ratio
Mean long OS metagene z-score
0.14 (0.056 – 0.36)
<0.001 ***
Mean short OS metagene z-score
5.07 (2.192 – 11.75)
<0.001 ***
log10 TMB
0.60 (0.312 – 1.16)
0.131
Log−Rank P value 1.861x10-5
Concordance Index: 0.75
Events: 34
Sensitivity
Specificity
Mean long OS & short OS metagene z-score AUC: 0.787, p−value: 9.19e−05
Mean long OS & short OS & log10 TMB AUC: 0.792, p−value: 6.88e−05
B
Hazard ratio
MAP4K1
0.62 (0.44 – 0.87)
0.005 **
AGER
1.36 (1.03 – 1.79)
0.031 *
log10 TMB
0.52 (0.29 – 0.94)
0.03 *
Log−Rank P value 0.00497
Concordance Index: 0.66
Events: 34
MAP4K1 & AGER AUC: 0.618, p−value: 6.99e−02
MAP4K1 & AGER & log10 TMB AUC: 0.708, p−value: 4.01e−03
C
Hazard ratio
MAP4K1
0.62 (0.45 – 0.85)
0.003 **
TBX3
1.49 (1.15 – 1.93)
0.003 **
log10 TMB
0.49 (0.27 – 0.89)
0.019 *
Log−Rank P value 0.000623
Concordance Index: 0.69
Events: 34
MAP4K1 & TBX3 AUC: 0.701, p−value: 5.37e−03
MAP4K1 & TBX3 & log10 TMB AUC: 0.761, p−value: 3.78e−04
D
Model
Likelihood Ratio Test p value for OS model with vs. without log10(TMB)
DeLong's Test p value for Response model with vs. without log10(TMB)
MAP4K1&AGER 0.0304 0.125
MAP4K1&TBX3 0.0194 0.223
Long OS metagene & Short OS metagene 0.133 0.879
E
Primary cohort; P=4.86x10-6
log2(TBX3 TPM + 1)
MITFhigh Intermediate
MITFlow Immune Keratinhigh
F
Primary cohort; P=1.07x10-3
log2(AGER TPM + 1)
MITFhigh Intermediate
MITFlow Immune Keratinhigh
G
Primary cohort; P=1.49x10-5
Mean Short OS metagene Z−score
MITFhigh Intermediate
MITFlow Immune Keratinhigh
H
log2(MAP4K1 TPM + 1)
myeloid DC
memory CD8 T cell
gdT cell
Treg
memory B cell
MAIT T cell
plasmacytoid DC
memory CD4 T cell
naive B cell
naive CD8 T cell
NK cell
intermediate monocyte
naive CD4 T cell
total PBMC
classical monocyte
non classical monocyte
basophil
eosinophil
neutrophil
I
Long OS genes & short OS genes
MAP4K1&AGER
MAP4K1&TBX3
MHC II
TCB
GEP
BCB
CYT
TIDE
IMPRES
CD274
−log10(Cox model Log−Rank p−value)
Cox model C−index
J
Long OS genes & Short OS genes
MAP4K1&AGER
MAP4K1&TBX3
TCB
TIDE
MHC II
BCB
IMPRES
CYT
GEP
CD274
−log10(R/NR classification AUC p−value)
R/NR classification AUC
K
CD274, AUC: 0.522, p−value: 0.330
CYT, AUC: 0.561, p−value: 0.110
GEP, AUC: 0.560, p−value: 0.113
IMPRES, AUC: 0.548, p−value: 0.162
TIDE, AUC: 0.642, p−value: 2.04x10-3
MHC II, AUC: 0.579, p−value: 0.057
TCB, AUC: 0.653, p−value: 1.04x10-3
BCB, AUC: 0.6, p−value: 2.17x10-2
MAP4K1&TBX3, AUC: 0.708, p−value: 1.43x10-5
MAP4K1&AGER, AUC: 0.710, p−value: 1.13x10-5
Long OS genes & Short OS genes, AUC: 0.718, p−value: 5.62x10-6
Sensitivity
Specificity

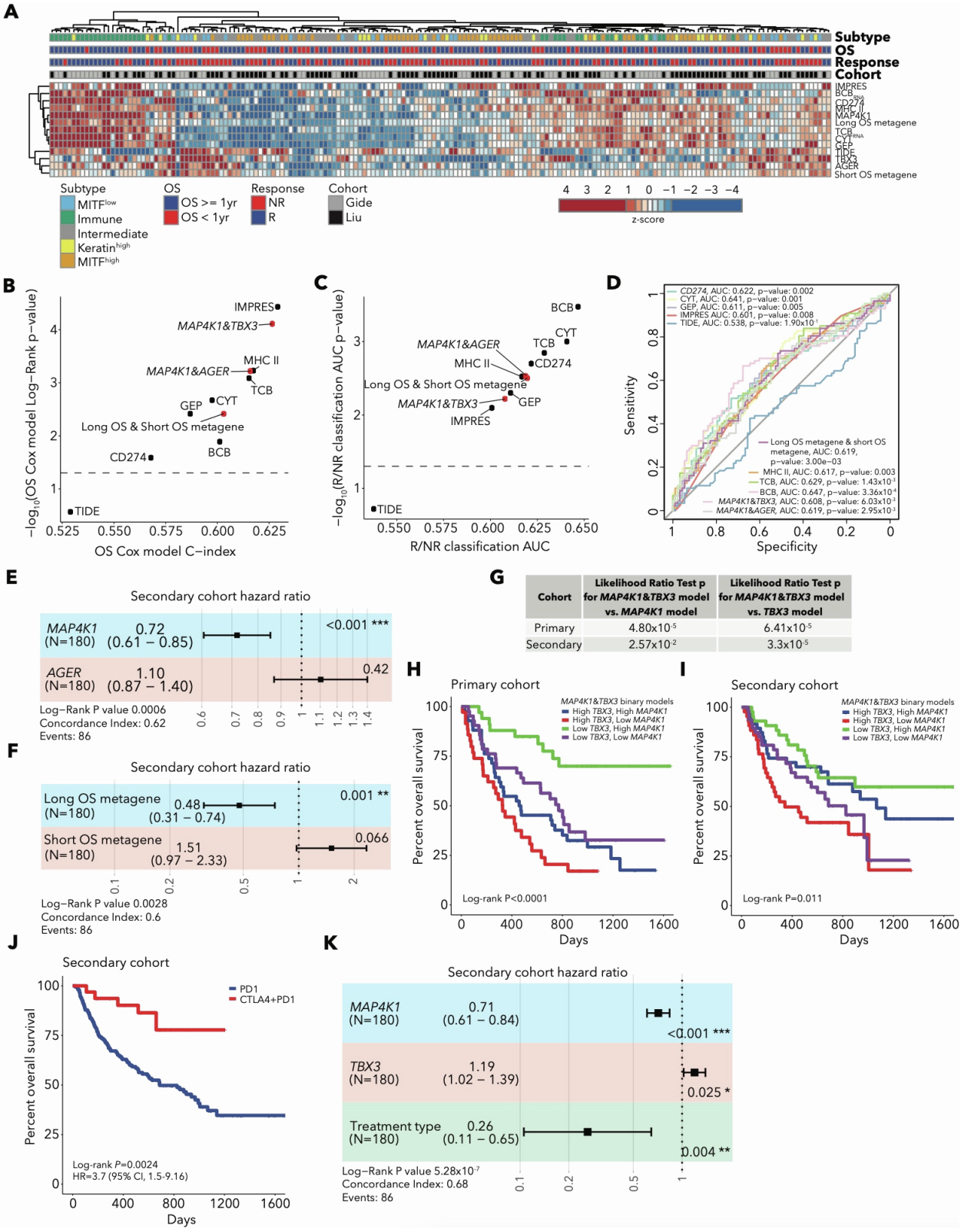
Supplementary Figure 14. **Performance of the three RNA gene-pair models with TMB in the primary cohort, expression of genes from top gene pair models and gene pair model performance, Related to Figure 4. A.** Forest plot (upper) for Cox survival model and ROC curves for response classification (lower) incorporating long OS metagene, short OS metagene and TMB in the primary cohort. Error bars represent 95% confidence intervals for Cox model hazard ratio estimates. **B.** Forest plot (upper) for Cox survival model and ROC curves for response classification (lower) incorporating *MAP4K1* expression, *AGER* expression and TMB in the primary cohort. Error bars represent 95% confidence intervals for Cox model hazard ratio estimates. **C.** Forest plot (upper) for Cox survival model and ROC curves for response classification (lower) incorporating *MAP4K1* expression, *TBX3* expression and TMB in the primary cohort. Error bars represent 95% confidence intervals for Cox model hazard ratio estimates. **D.** Likelihood ratio test and DeLong's test results comparing Cox survival models or response model ROC curves, respectively for the *MAP4K1*&*AGER*, *MAP4K1*&*TBX3* or long OS metagene & short OS metagene pair with or without TMB in the primary cohort. **E.** Expression of *TBX3* in the primary cohort by melanoma subtype with Kruskal-Wallis test P-value. **F.** Expression of *AGER* in the primary cohort by melanoma subtype with Kruskal-Wallis test P-value. **G.** Expression of the short OS metagene in the primary cohort by melanoma subtype with Kruskal-Wallis test P-value. **H.** Expression of *MAP4K1* in Human Protein Atlas (HPA) cell types. **I.** Performance of melanoma immunotherapy survival models in the primary cohort in terms of C-index and Cox model log-rank P-value. **J.** Performance of melanoma immunotherapy response models in the primary cohort in terms of AUC and AUC P-value. **K.** ROC curves for response classification for all models in the primary cohort.


A
Count of long/short OS Bonferroni p<0.05 genes
Count + 1
training_validation split size
50_50
66_33
70_30
75_25
80_20
B
Count of long/short OS Bonferroni p<0.05 genes for OS and R/NR
Count + 1
C
Validation set mean long OS metagene z score HR
Training set mean long OS metagene z score HR
Validation set bonferroni p < 0.05 FALSE TRUE
D
Validation set mean short OS metagene z score HR
Training set mean short OS metagene z score HR
E
Validation set mean long OS metagene z score HR
Training set mean long OS metagene z score HR
F
Validation set mean short OS metagene z score HR
Training set mean short OS metagene z score HR
G
Training set size
Count
MAP4K1&AGER
MAP4K1&TBX3
Long OS metagene & Short OS metagene (top 25 genes)
Long OS metagene & Short OS metagene
H
I
Validation set size
J

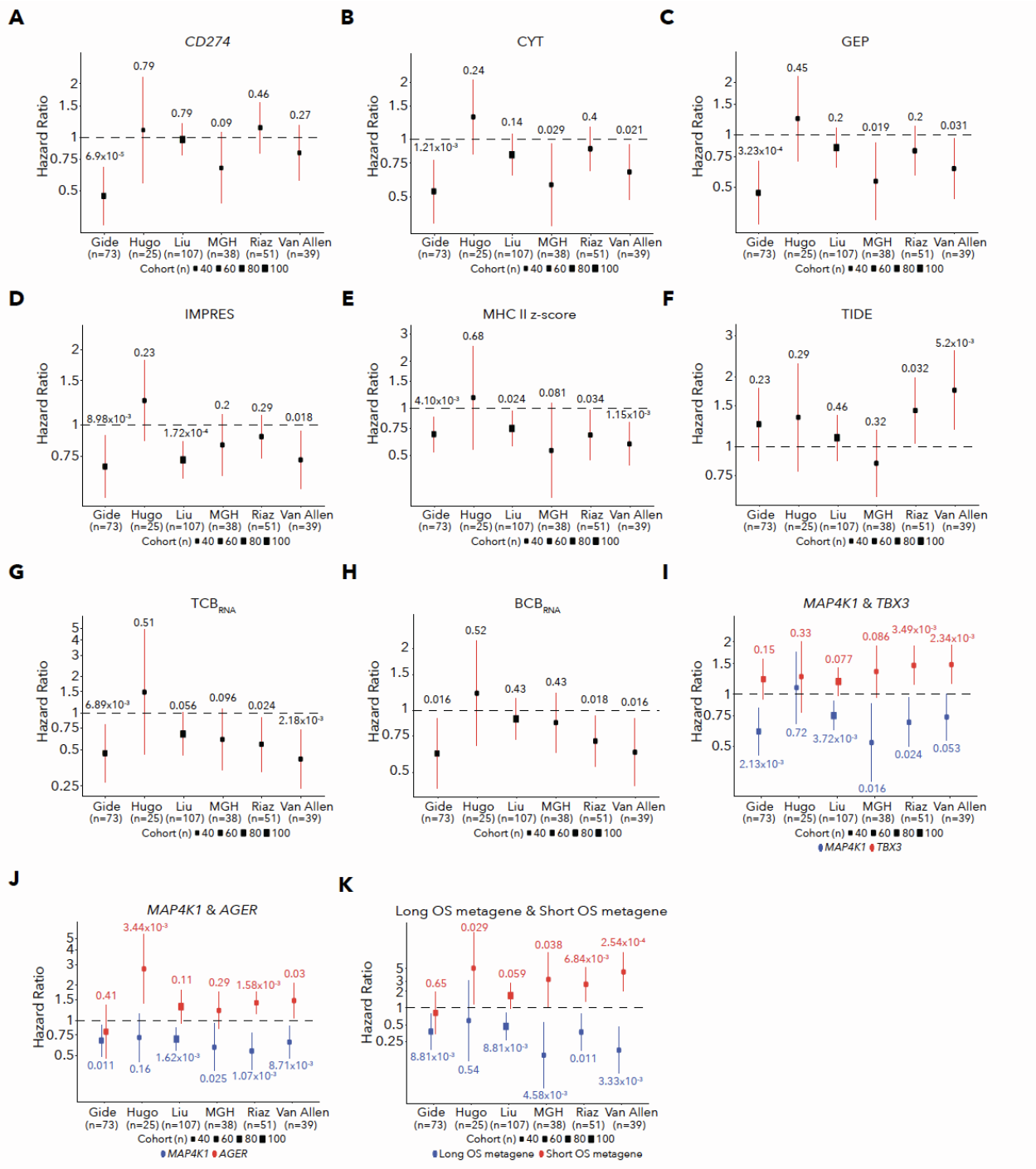
Supplementary Figure 15. **Cross-validation of gene pair model discovery and validation in the primary cohort, Related to Figure 4. A.** Empirical cumulative distribution of the number of gene pairs discovered with Bonferroni p<0.05 for association with survival in training sets in the cross validation using genes differentially expressed between patients with long and short OS (DESeq q<0.05) within the training set. Each line represents a different training and validation set split size, with 250 cross validation training/validation splits per split size. **B.** Empirical cumulative distribution of the number of gene pairs discovered with Bonferroni p<0.05 for association with survival and response in training sets in the cross validation using genes differentially expressed between patients with long and short OS (DESeq q<0.05) within the training set. Each line represents a different training and validation set split size, with 250 cross validation training/validation splits per split size. **C-D.** Performance of the long OS metagene & short OS metagene model in cross-validation training and validation sets. Each point represents the hazard ratio (HR) of the long OS metagene (**C**) and short OS metagene (**D**) discovered in that training/test set split. In training/validation splits, the metagenes are composed of the genes that were differentially expressed between long and short OS patients with DESeq q<0.05 in the training set samples. Panels represent different split sizes and red lines are linear regressions. Points are colored by whether the metagene had Bonferroni p<0.05 for survival association in the validation set. **E-F.** Performance of the the long OS metagene & short OS metagene model in cross-validation training and validation sets where metagenes were defined using top 25 long OS or short OS genes ranked by DESeq p value. **G-H.** Frequency of selected gene pairs with Bonferroni p<0.05 for association with survival (**G**) or Bonferroni p<0.05 for association with survival and response (**H**) in training sets. Training set sizes are listed above with 250 training/validation splits for each split size. Metagene pair models using the top 25 genes ranked by DESeq p value or the genes with DESeq q<0.05 in the training set are shown separately. **I-J.** Frequency of selected gene pairs with Bonferroni p<0.05 for association with survival (**I**) or Bonferroni p<0.05 for association with survival and response (**J**) in validation sets. Validation set sizes are listed above with 250 training/validation splits for each split size. Metagene pair models using the top 25 genes ranked by DESeq p value or the genes with DESeq q<0.05 in the training set are shown separately.


A
Pre batch correction
Cohort
Gide
Liu
PC2
Post batch correction
PC1
B
Secondary cohort
Activity
mRNA clusters
Sample
C
Log2 (Fold Change)
NR4A3
RUNX3
TRPM1
PMEL
KIT
SOX6
PAX3
DCT
ALDH1A1
TBX3
TGFA
EPHA3
TGFBI
NGFR
CD2
CD8A
PTPRC
CXCL9
PRF1
CD79A
KRT10
DMKN
KRT5
TP63
FLG
MITFhigh n=56
Intermediate n=32
MITFlow n=27
Immune n=40
Keratinhigh n=25
D
Chi−Sqaured test p=0.21
Frequency
Primary cohort
Gide
Liu
E
P=9.08x10-17
TCBRNA
P=3.24x10-11
BCBRNA
F
Percent overall survival
Log-rank P=0.068
Days
G
0.00084
Fraction of group
Responder
Nonresponder

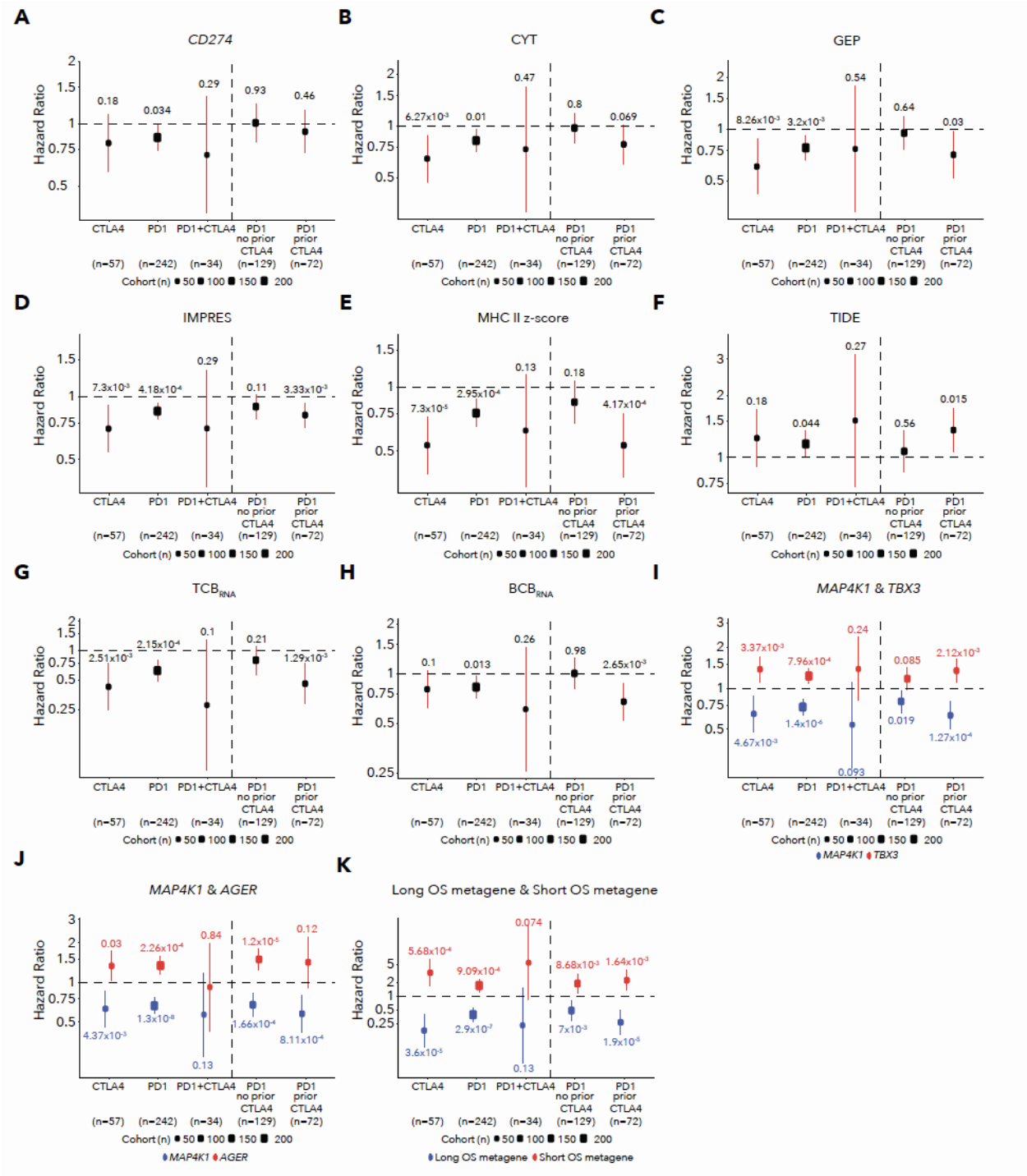
Supplementary Figure 16. **Batch effects correction and melanoma subtyping for the secondary cohort, Related to Figure 4. A.** PCA of secondary cohort (Gide and Liu cohorts) protein coding gene $\log_2$(TPM+1) values before (upper plot) and after (lower plot) batch-effects correction with ComBat[83]. Before batch effects correction, samples cluster by cohort. **B.** NMF H matrix for secondary cohort sample subtyping using subtypes and marker genes identified in TCGA melanoma samples. **C.** Heatmap of marker gene expression for samples in the secondary cohort with samples grouped by subtype. **D.** Comparison of frequency of subtypes in the primary cohort and the Gide and Liu cohorts. **E.** TCB_{RNA} and BCB_{RNA} by subtype for secondary cohort samples with Kruskal-Wallis P values for associations with subtype. **F.** Kaplan-Meier survival curve by subtype for patients in the secondary cohort. **G.** Number of responder and non-responders by subtype in the secondary cohort with Fisher's exact test P value.


A
Subtype
OS
Response
Cohort
IMPRES
BCB RNA
CD274
MHC II
MAP4K1
Long OS metagene
TCB
CYT RNA
GEP
TIDE
TBX3
AGER
Short OS metagene
Subtype
MITF low
Immune
Intermediate
Keratin high
MITF high
OS
OS >= 1yr
OS < 1yr
Response
NR
R
Cohort
Gide
Liu
4 3 2 1 0 −1 −2 −3 −4
z-score
B
−log10(OS Cox model Log−Rank p−value)
OS Cox model C−index
IMPRES
MAP4K1&TBX3
MHC II
MAP4K1&AGER
TCB
GEP
CYT
Long OS & Short OS metagene
BCB
CD274
TIDE
C
−log10(R/NR classification AUC p−value)
R/NR classification AUC
BCB
CYT
MAP4K1&AGER
TCB
MHC II
CD274
Long OS & Short OS metagene
MAP4K1&TBX3
GEP
IMPRES
TIDE
D
CD274, AUC: 0.622, p−value: 0.002
CYT, AUC: 0.641, p−value: 0.001
GEP, AUC: 0.611, p−value: 0.005
IMPRES AUC: 0.601, p−value: 0.008
TIDE, AUC: 0.538, p−value: 1.90x10−1
Long OS metagene & short OS metagene, AUC: 0.619, p−value: 3.00e−03
MHC II, AUC: 0.617, p−value: 0.003
TCB, AUC: 0.629, p−value: 1.43x10−3
BCB, AUC: 0.647, p−value: 3.36x10−4
MAP4K1&TBX3, AUC: 0.608, p−value: 6.03x10−2
MAP4K1&AGER, AUC: 0.619, p−value: 2.95x10−3
Sensitivity
Specificity
E
Secondary cohort hazard ratio
MAP4K1 (N=180) 0.72 (0.61 – 0.85) <0.001 ***
AGER (N=180) 1.10 (0.87 – 1.40) 0.42
Log−Rank P value 0.0006
Concordance Index: 0.62
Events: 86
F
Secondary cohort hazard ratio
Long OS metagene (N=180) 0.48 (0.31 – 0.74) 0.001 **
Short OS metagene (N=180) 1.51 (0.97 – 2.33) 0.066
Log−Rank P value 0.0028
Concordance Index: 0.6
Events: 86
G
Cohort | Likelihood Ratio Test p for MAP4K1&TBX3 model vs. MAP4K1 model | Likelihood Ratio Test p for MAP4K1&TBX3 model vs. TBX3 model
Primary | 4.80x10-5 | 6.41x10-5
Secondary | 2.57x10-2 | 3.3x10-5
H
Primary cohort
MAP4K1&TBX3 binary models
High TBX3, High MAP4K1
High TBX3, Low MAP4K1
Low TBX3, High MAP4K1
Low TBX3, Low MAP4K1
Percent overall survival
Log-rank P<0.0001
Days
I
Secondary cohort
MAP4K1&TBX3 binary models
High TBX3, High MAP4K1
High TBX3, Low MAP4K1
Low TBX3, High MAP4K1
Low TBX3, Low MAP4K1
Percent overall survival
Log-rank P=0.011
Days
J
Secondary cohort
PD1
CTLA4+PD1
Percent overall survival
Log-rank P=0.0024
HR=3.7 (95% CI, 1.5-9.16)
Days
K
Secondary cohort hazard ratio
MAP4K1 (N=180) 0.71 (0.61 – 0.84) <0.001 ***
TBX3 (N=180) 1.19 (1.02 – 1.39) 0.025 *
Treatment type (N=180) 0.26 (0.11 – 0.65) 0.004 **
Log−Rank P value 5.28x10-7
Concordance Index: 0.68
Events: 86

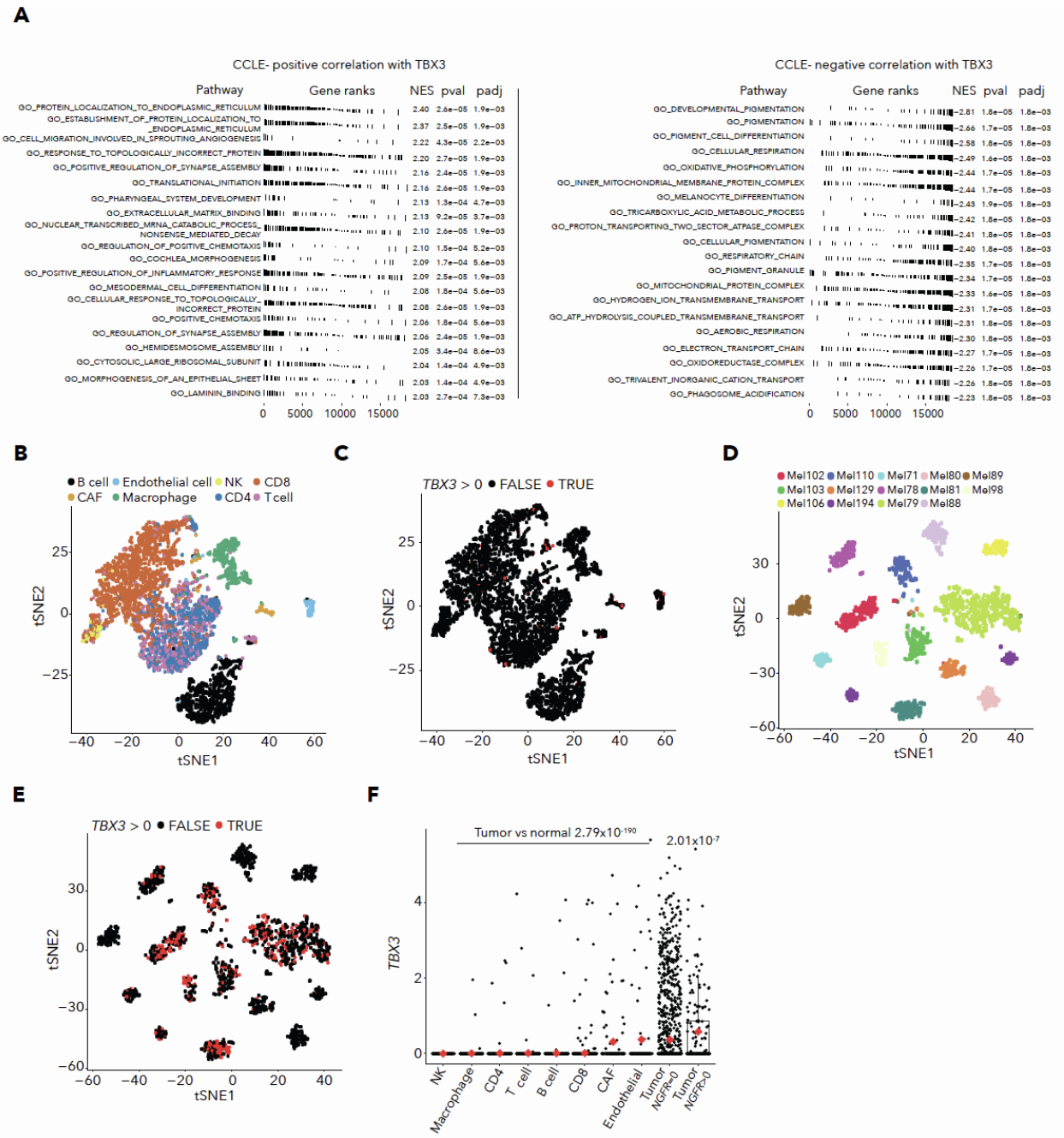
Supplementary Figure 17. **Performance of top gene pair models in the secondary cohort, Related to Figure 4. A.** Heatmap of z-scored values for immunotherapy predictive models and top gene pairs in the secondary cohort. **B.** Performance of pairwise gene models in comparison to previous immunotherapy predictive models in significance and effect size of predictions of survival in the secondary cohort. **C.** Performance of pairwise gene models in comparison to previous immunotherapy predictive models in significance and effect size of predictions of response in the secondary cohort. **D.** ROC curve of pairwise gene models and previous immunotherapy models in classification of response in the secondary cohort. **E.** Forest plot for *MAP4K1*&*AGER* gene pair Cox survival model in the secondary cohort. Error bars represent 95% confidence intervals for Cox model hazard ratio estimates. **F.** Forest plot for the metagene pair Cox survival model in the secondary cohort. Error bars represent 95% confidence intervals for Cox model hazard ratio estimates. **G.** Likelihood ratio test results comparing Cox survival models with *MAP4K1*&*TBX3* to Cox survival models with *MAP4K1* or *TBX3* alone in the primary and secondary cohorts. In both cohorts, the gene pair models outperformed both single gene models. **H.** Kaplan-Meier survival curve for groups based on binary *MAP4K1* and *TBX3* expression (above or below median) in the primary cohort. **I.** Kaplan-Meier survival curve for groups based on binary *MAP4K1* and *TBX3* expression (above or below median) in the secondary cohort. **J.** Kaplan-Meier survival curve for groups based on treatment (PD-1 alone or combination CTLA-4/PD-1 therapy) in the secondary cohort. **K.** Forest plot for Cox survival model incorporating *MAP4K1* expression, *TBX3* expression and treatment (PD-1 alone or combination CTLA-4/PD-1) in the secondary cohort. After including treatment in the model, *MAP4K1* and *TBX3* expression both remain significant. Error bars represent 95% confidence intervals for Cox model hazard ratio estimates.



Supplementary Figure 18. **Performance of all models within each cohort separately, Related to Figure 4. A-G.** Performance of univariate Cox models within each cohort separately. Hazard ratios and error bars representing 95% confidence intervals of Hazard ratio estimates are plotted and Wald test P values are indicated. (**A**) *CD274* (**B**) CYT (**C**) GEP (**D**) IMPRES (**E**) MHC II (**F**) TIDE (**G**) TCB_{RNA} (**H**) BCB_{RNA}. **I-K**. Performance of Cox models using gene pairs within each cohort. Hazard ratios and confidence intervals are colored by the gene or metagene and the Wald test P values are indicated. (**I**) *MAP4K1*&*TBX3* (**J**) *MAP4K1*&*AGER* (**K**) metagene pair model.



Supplementary Figure 19. **Performance of all models in patients treated with different checkpoint blockade therapies, Related to Figure 4. A-G.** Performance of univariate Cox models for different checkpoint blockade therapies. Hazard ratios and error bars representing 95% confidence intervals of Hazard ratio estimates are plotted and Wald test P values are indicated. (**A**) *CD274* (**B**) CYT (**C**) GEP (**D**) IMPRES (**E**) MHC II (**F**) TIDE (**G**) TCB_{RNA} (**H**) BCB_{RNA}. **I-K**. Performance of Cox models for patients treated with different therapies using gene pairs. Hazard ratios and confidence intervals are colored by the gene or metagene and the Wald test P values are indicated. (**I**) *MAP4K1*&*TBX3* (**J**) *MAP4K1*&*AGER* (**K**) metagene pair model.



Supplementary Figure 20. **Analysis of melanoma *TBX3* expression, Related to Figure 4. A.** GSEA for genes ordered by spearman correlation of gene expression with *TBX3* gene expression in CCLE melanoma cell lines using GO terms. Top GSEA results for genes positively correlated with *TBX3* (left), and top GSEA results for genes negatively correlated with *TBX3* (right). GO terms associated with genes negatively correlated with *TBX3* include pigmentation and melanocyte gene sets. **B-C**. tSNE plot of scRNA data[59] from immune cells with cells labelled by lineage (**B**) or *TBX3* expression status (**C**). *TBX3* is rarely expressed in any immune cell type. **D-E**. tSNE plot of scRNA data[59] from melanoma tumor cells with cells labelled by patient (**D**) or *TBX3* expression status (**E**). *TBX3* is expressed in tumor cells from some patients. **F.** *TBX3* expression in scRNA data[59] by immune cell type or tumor cell type. Melanoma tumor cells are split between cells with no *NGFR* expression or cells with non-zero *NGFR* expression. Mean expression by group is indicated by red dots. P values for Wilcoxon tests of *TBX3* expression in tumor single cells vs. normal single cells and tumor *NGFR*>0 cells vs. tumor *NGFR*=0 cells are indicated above.