Supplementary Materials: Integrative Analysis Identifies Multi-Omics Signatures That Drive Molecular Classification of Uveal Melanoma

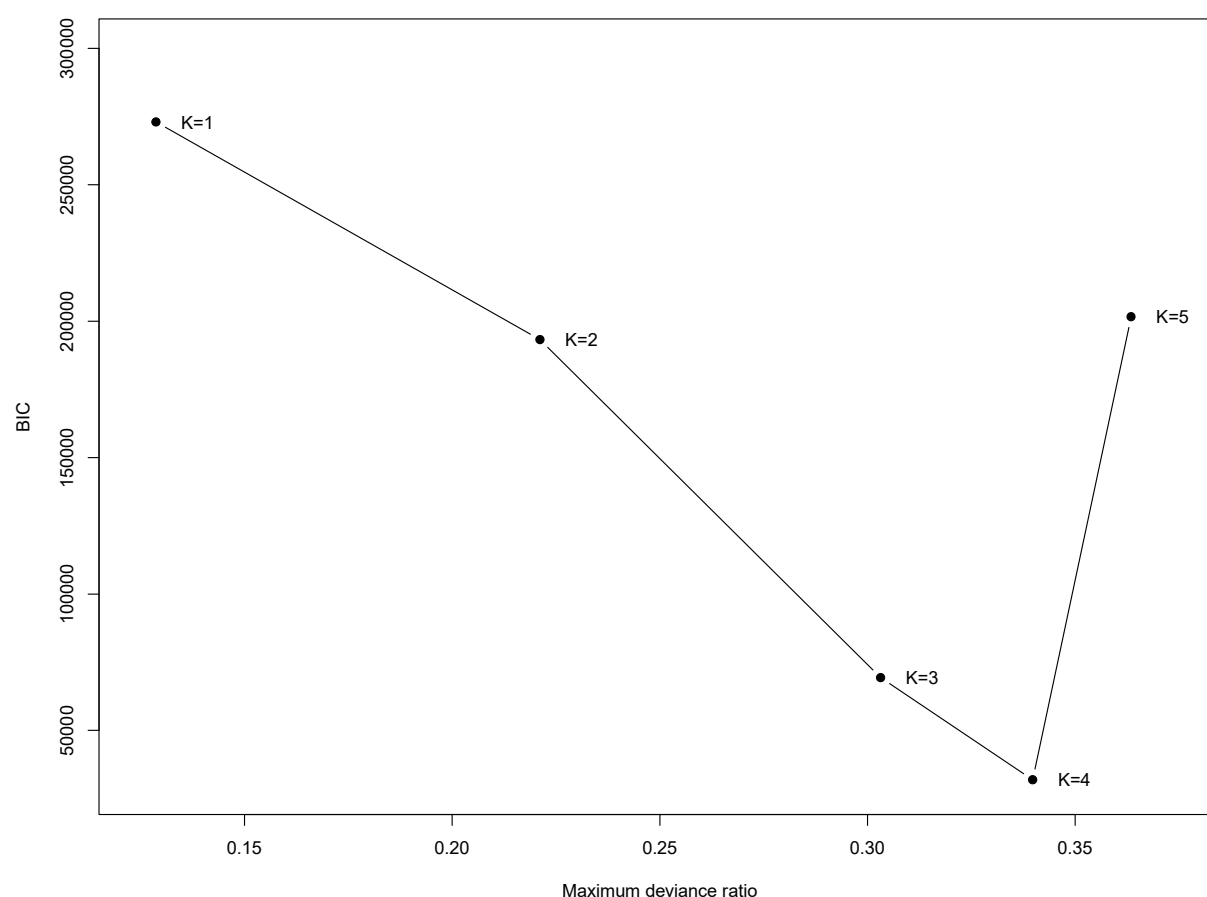**Qianxing Mo, Lixin Wan, Michael J. Schell, Heather Jim, Shelley S. Tworoger and Guang Peng**

Figure S1. Model fitting parameters of iClusterPlus. Bayesian information criterion (BIC) and the maximum deviance ratio for the cluster parameter K=1,2,3,4,5 are shown. For a given k, the samples can be divided into K+1 clusters. Based on the BIC and maximum deviance ratio, K=3 (4 clusters) and K=4 (5 clusters) are two optimal solutions. .
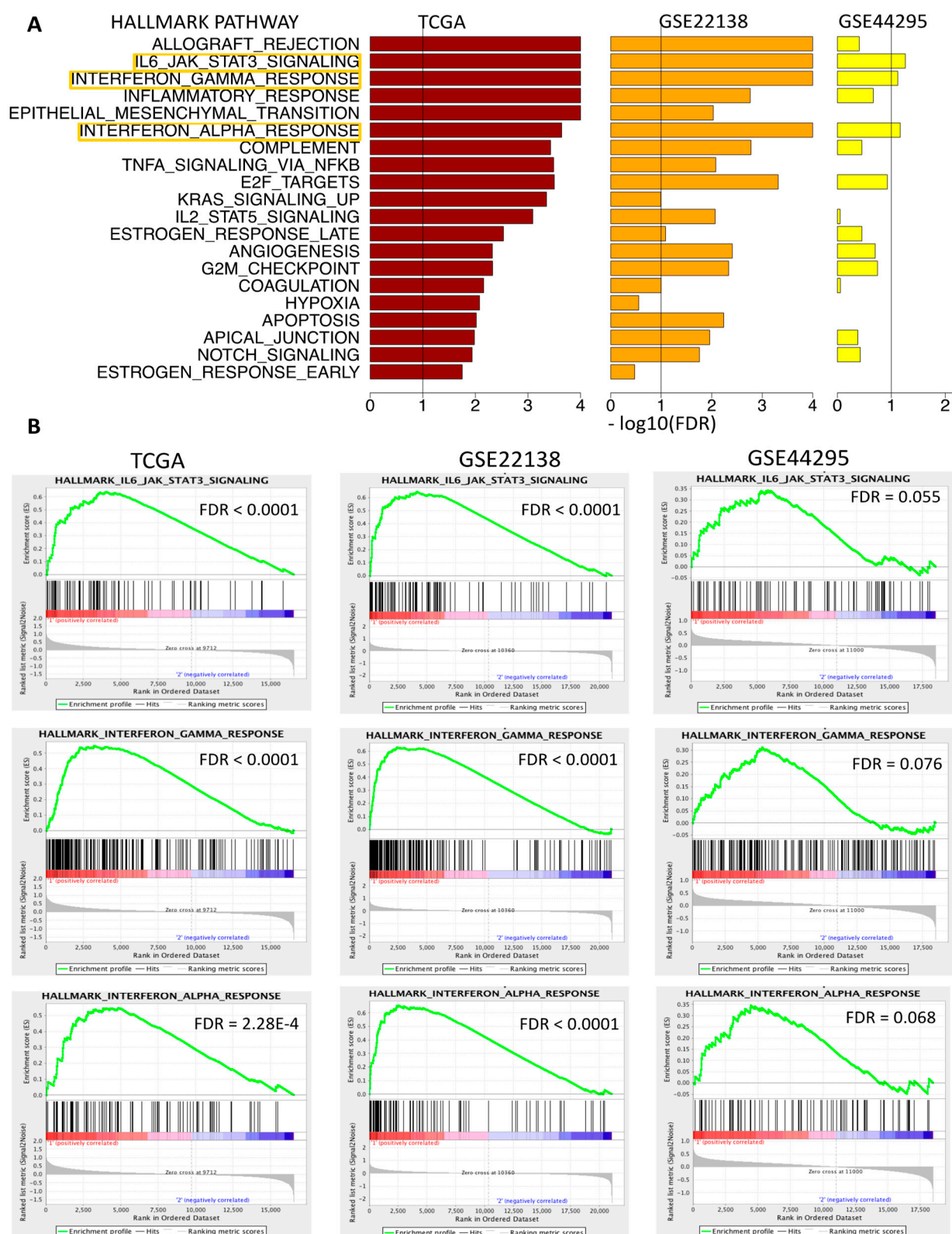
Figure S2. Top up-regulated pathways in the M3 subtype of the TCGA, GSE22138 and GSE44295 cohorts. **(A)** Comparison of the significance levels of the top up-regulated pathways. **(B)** Enrichment plots of the most significantly up-regulated pathways.
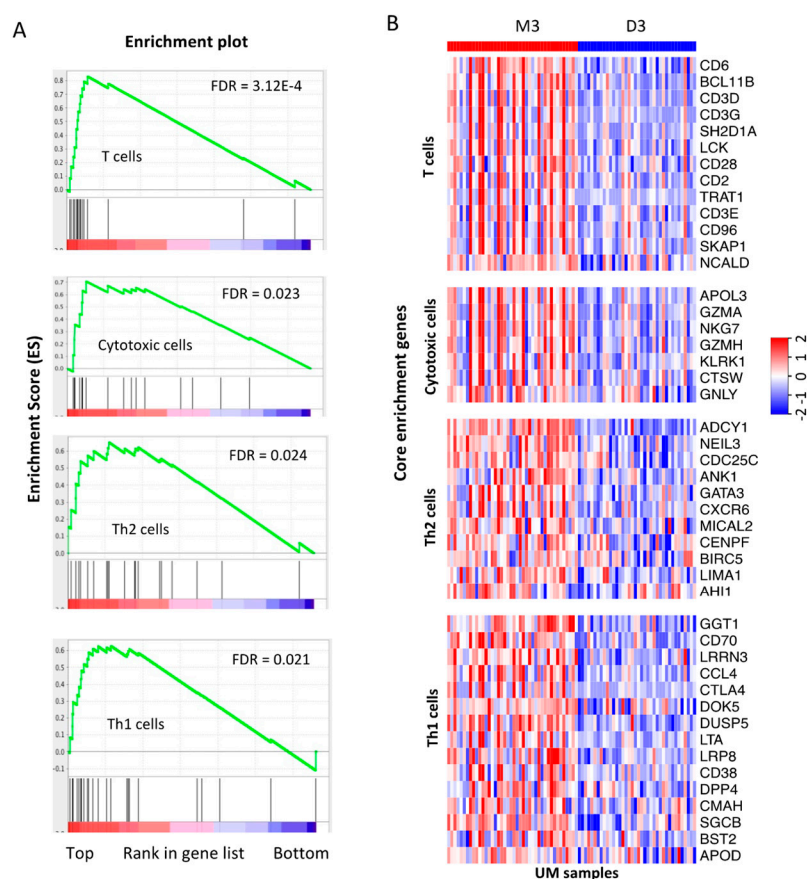
Figure S3. Up-regulated immune gene sets in the M3 iSubtype of the TCGA UM samples.　**(A)** Enrichment plot of the enriched immune gene sets.　Gene-based permutation test was used to calculate the FDR.　**(B)** Heatmaps of the core enrichment genes of the up-regulated immune gene sets in the M3 iSubtype.　On the heatmap, red and blue represent high and low expression, respectively.
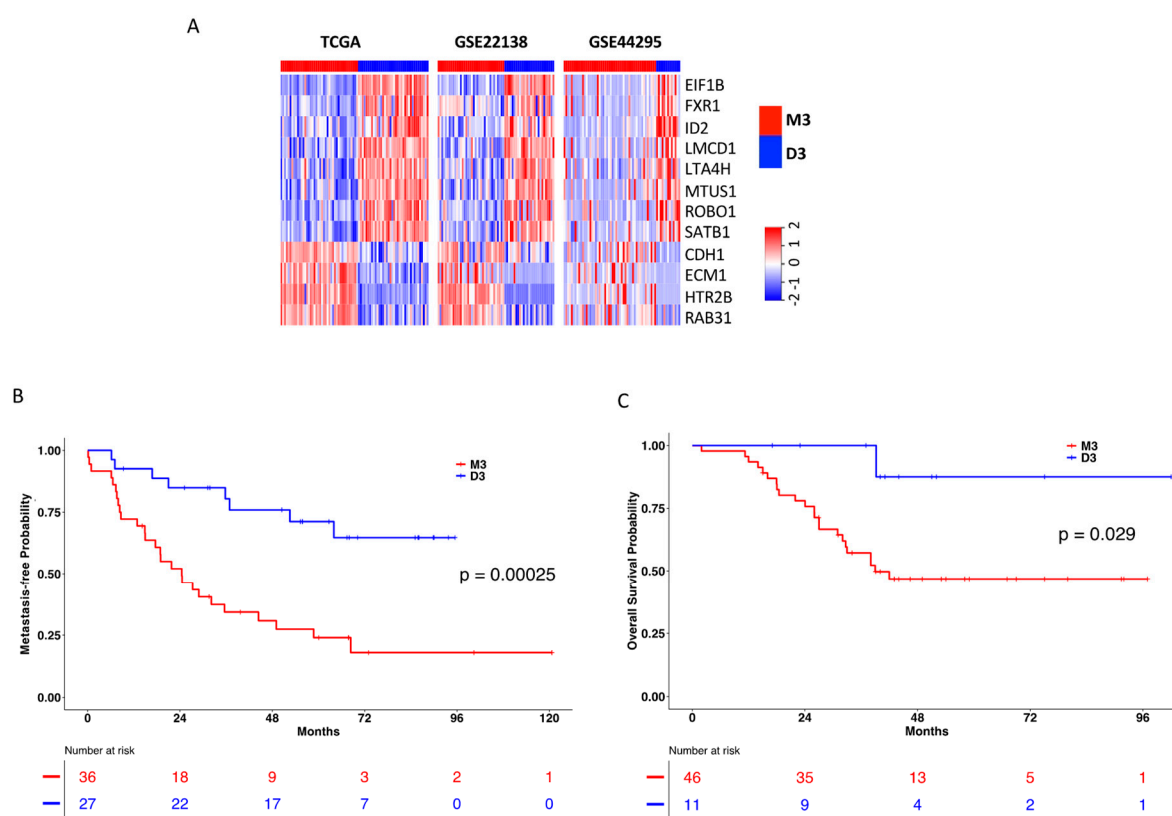
Figure S4. Prognostic power of the 12 gene expression signatures. **(A)** Heatmaps of the 12 gene expression of the training and testing data sets. **(B)** Patient metastasis-free survival stratified by the M3 and D3 subtypes defined by the 12 genes in the GSE22138 cohort. **(C)** Patient overall survival stratified by the M3 and D3 subtypes defined by the 12 genes in the GSE44295 cohort.
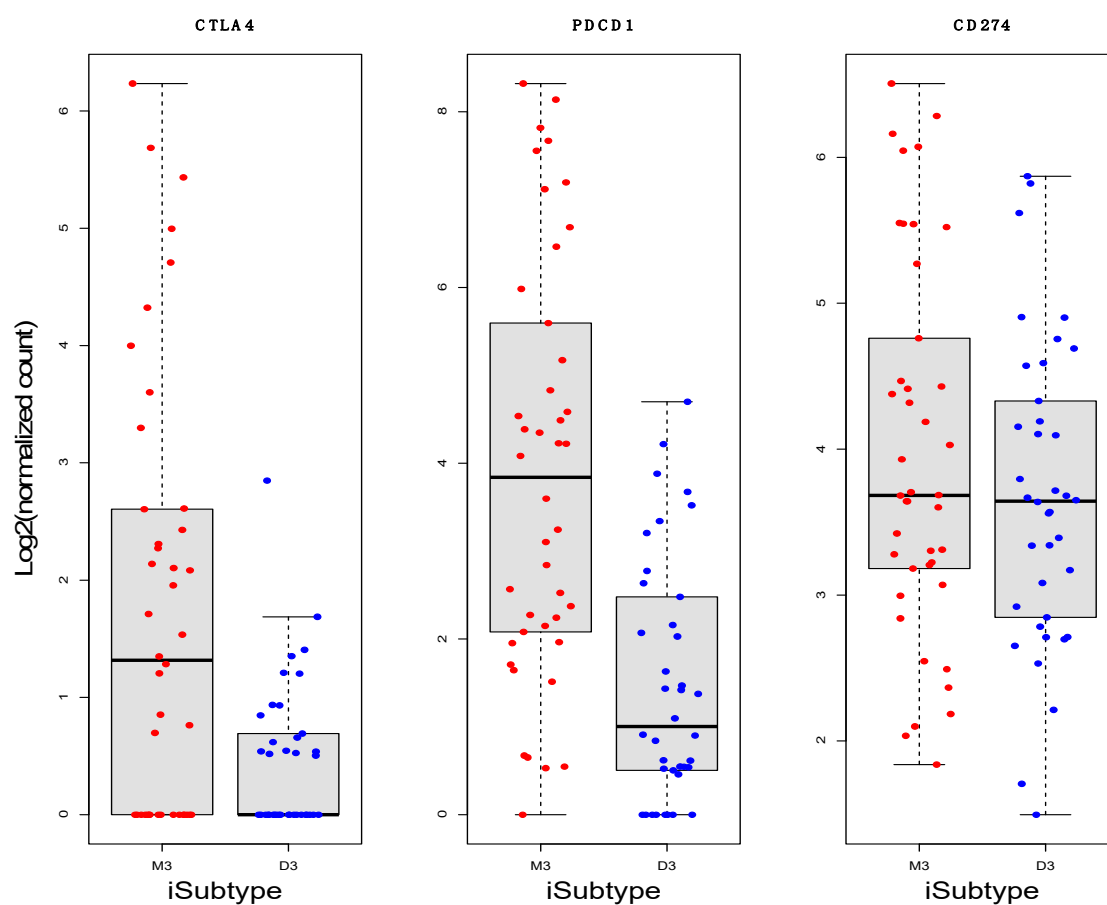
Figure S5. CTLA4, PDCD1 (PD1), and CD274(PDL1) expression in the M3 and D3 iSubtypes of the TCGA cohort.

Table S1. Comparison of 4-cluster solution (K = 3) and 5-cluster solution (K = 4).

	5-cluster solution				
4-cluster solution	**M.1**	**M.2**	**D.1**	**D.2**	**D.3**
M.1	**29**	0	0	0	0
M.2	0	**13**	0	0	0
D.1	0	0	**9**	**1**	0
D.2	0	0	0	**23**	**5**

The values in the table are the numbers of common samples shared by any pair of subtypes in the 4- and 5-cluster solutions.

Table S2. Top up-regulated BIOCARTA pathways in the M3 iSubtype of the TCGA cohort.

BIOCARTA Pathway Name	Description	SIZE	ES	NES	p-value	FDR
CTLA4	The Co-Stimulatory Signal During T-cell Activation	19	0.79	2.01	0.0000	0.0021
CSK	Activation of Csk by cAMP-dependent Protein Kinase Inhibits Signaling through the T Cell Receptor	19	0.79	2.00	0.0000	0.0012
SPPA	Aspirin Blocks Signaling Pathway Involved in Platelet Activation	15	0.75	1.81	0.0005	0.0313
BAD	Regulation of BAD phosphorylation	23	0.67	1.76	0.0014	0.0490
TCR	T Cell Receptor Signaling Pathway	44	0.57	1.75	0.0004	0.0494
INTRINSIC	Intrinsic Prothrombin Activation Pathway	16	0.70	1.72	0.0041	0.0601
EDG1	Phospholipids as signalling intermediaries	22	0.65	1.71	0.0036	0.0538
PAR1	Thrombin signaling and protease-activated receptors	18	0.68	1.71	0.0047	0.0517

INFLAM	Cytokines and Inflammatory Response	15	0.71	1.69	0.0038	0.0620
TOB1	Role of Tob in T-cell activation	16	0.68	1.66	0.0086	0.0798
CHREBP	ChREBP regulation by carbohydrates and cAMP	16	0.66	1.61	0.0140	0.1261
IL2RB	IL-2 Receptor Beta Chain in T cell Activation	37	0.54	1.59	0.0078	0.1381
NKT	Selective expression of chemokine receptors during T-cell polarization	20	0.62	1.59	0.0192	0.1329
IL12	IL12 and Stat4 Dependent Signaling Pathway in Th1 Development	19	0.61	1.54	0.0244	0.1975
NKCELLS	Ras-Independent pathway in NK cell-mediated cytotoxicity	16	0.63	1.54	0.0288	0.1856
MPR	How Progesterone Initiates the Oocyte Maturation	20	0.59	1.52	0.0318	0.2091
NO2IL12	NO2-dependent IL 12 Pathway in NK cells	15	0.63	1.51	0.0360	0.2114
CCR5	Pertussis toxin-insensitive CCR5 Signaling in Macrophage	17	0.60	1.50	0.0366	0.2091
EICOSANOID	Eicosanoid Metabolism	22	0.57	1.50	0.0333	0.2000
ATM	ATM Signaling Pathway	19	0.59	1.50	0.0361	0.1927

SIZE: number of genes in the pathway; ES: enrichment score of GSEA; NES: normalized enrichment score; p-value: raw p-value; FDR: adjusted p-value for multiple comparison.

Table S3. Classification results of the GSE22138 cohort using the expression signatures of the DEG368, DMEG, and the 12 classical genes. DEG368: differentially expressed genes on chr3, 6, 8 with FDR < 0.05 and absolute fold change of M3/D3 > 2; DMEG: 378 differentially methylated and expressed iSubtype-driving genes.

	Subtypes predicted by					
Chr3 status	**DEG368**		**DMEG**		**The 12 genes**	
	M3	**D3**	**M3**	**D3**	**M3**	**D3**
disomy	1	17	2	16	1	17
monosomy	30	2	30	2	29	3
NA	7	1	7	1	6	2
Partial monosomy	1	4	0	5	0	5