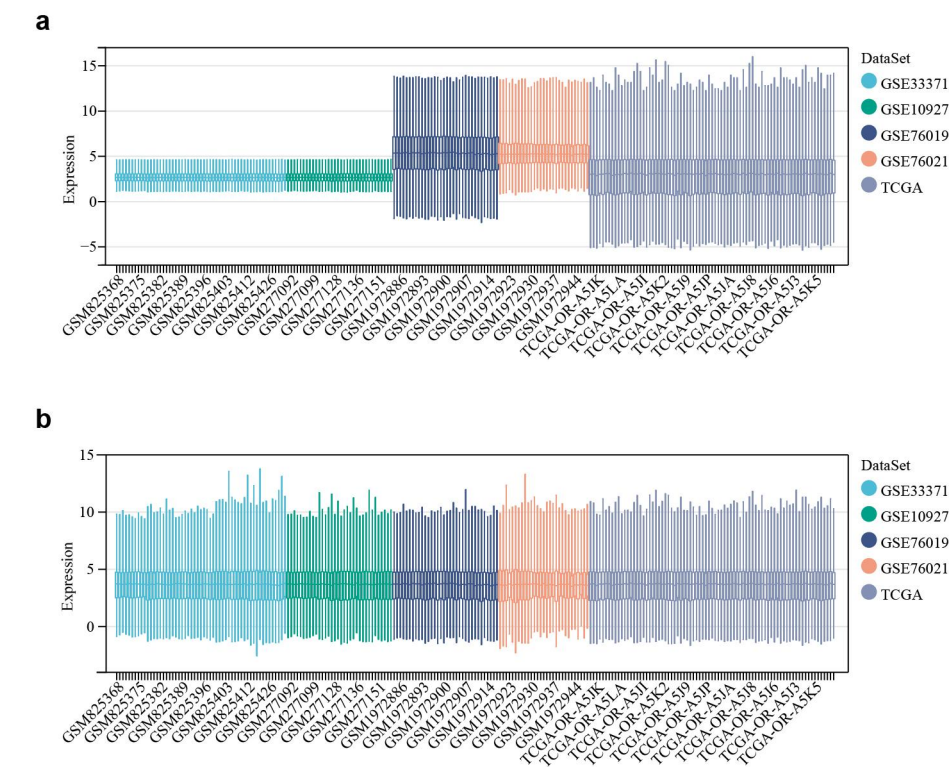


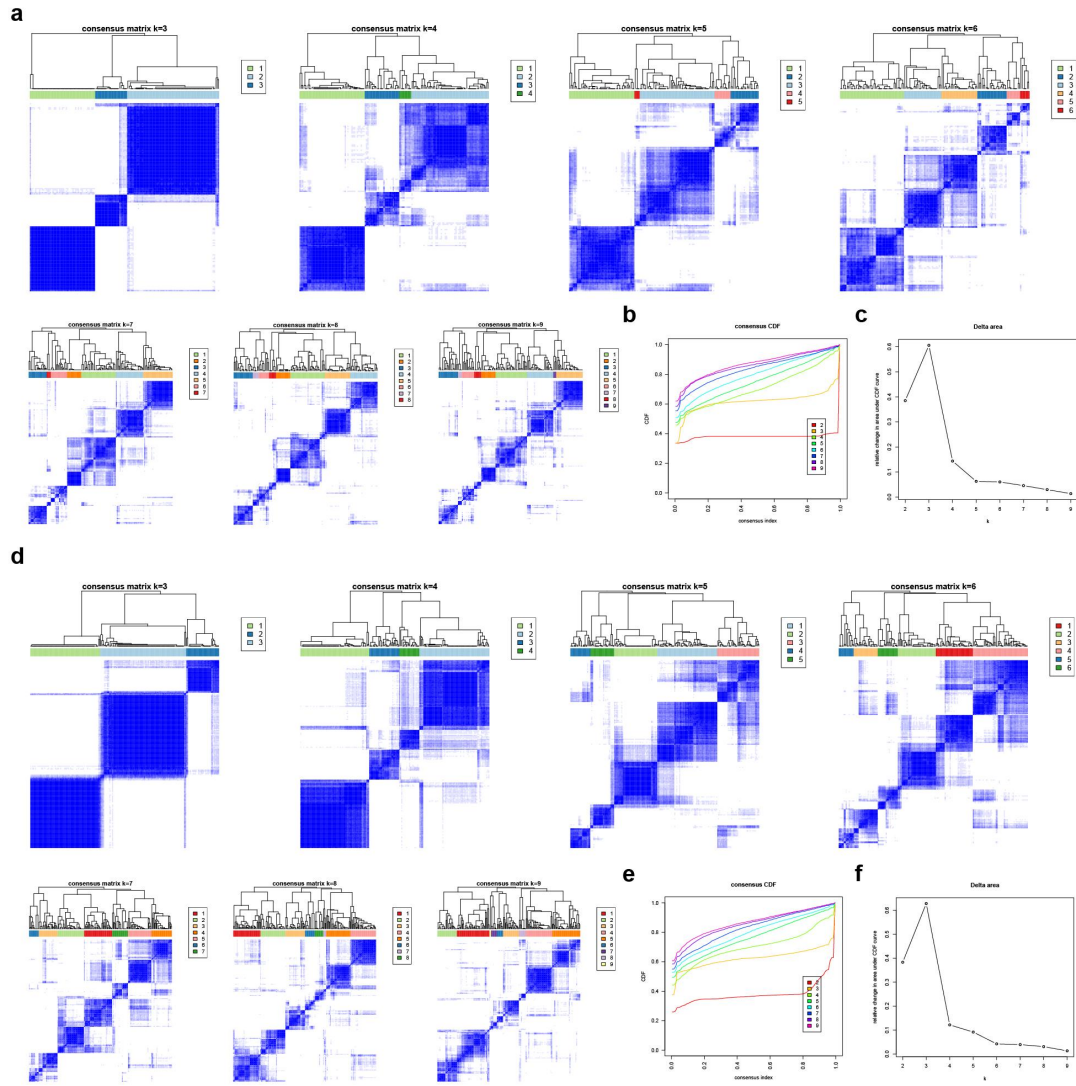
Supplementary Information

Manuscript Title:
Multi-modal characterization of metabolic and immune gene clusters in
adrenocortical carcinoma treatment

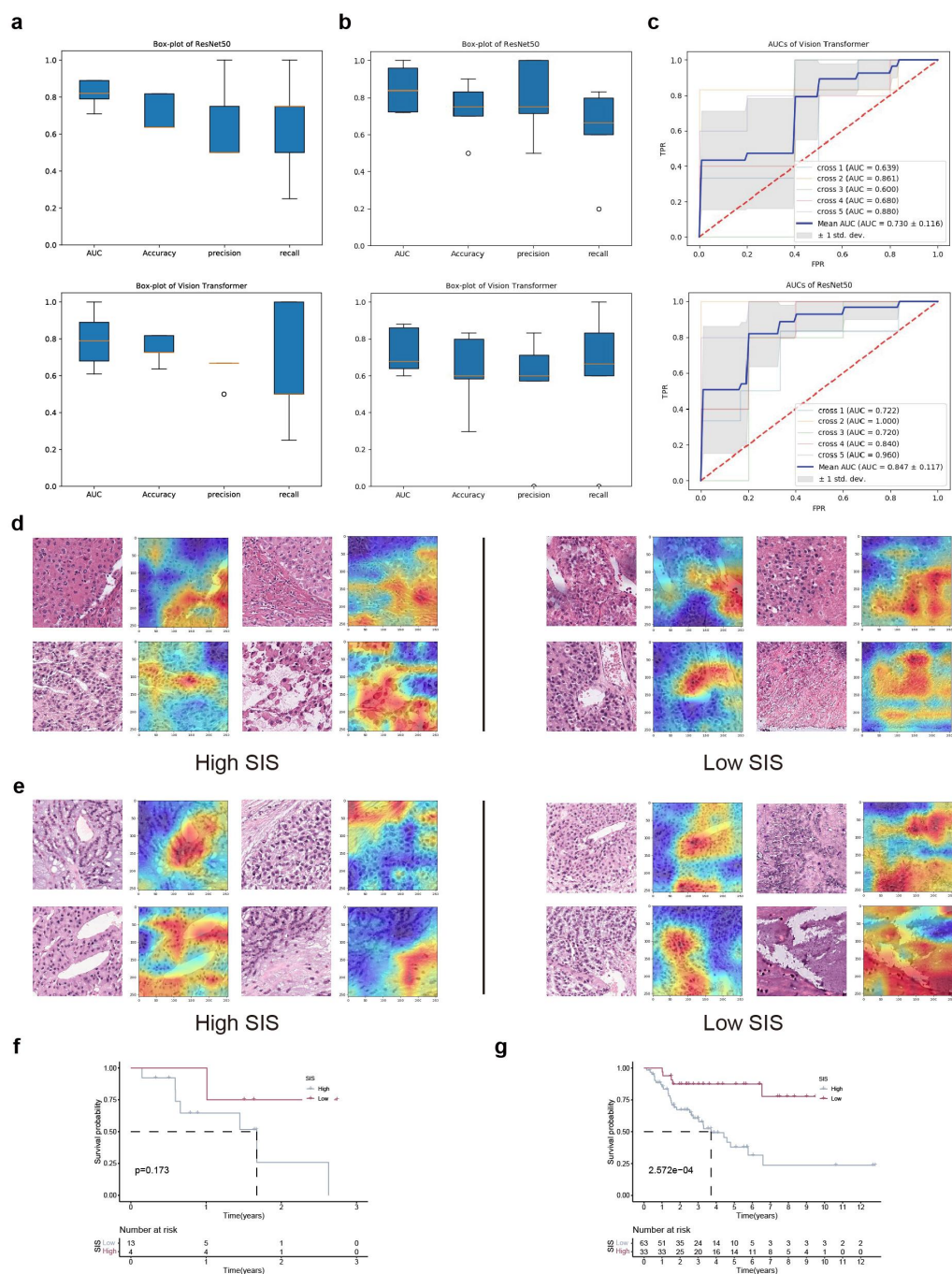
Supplementary Figures



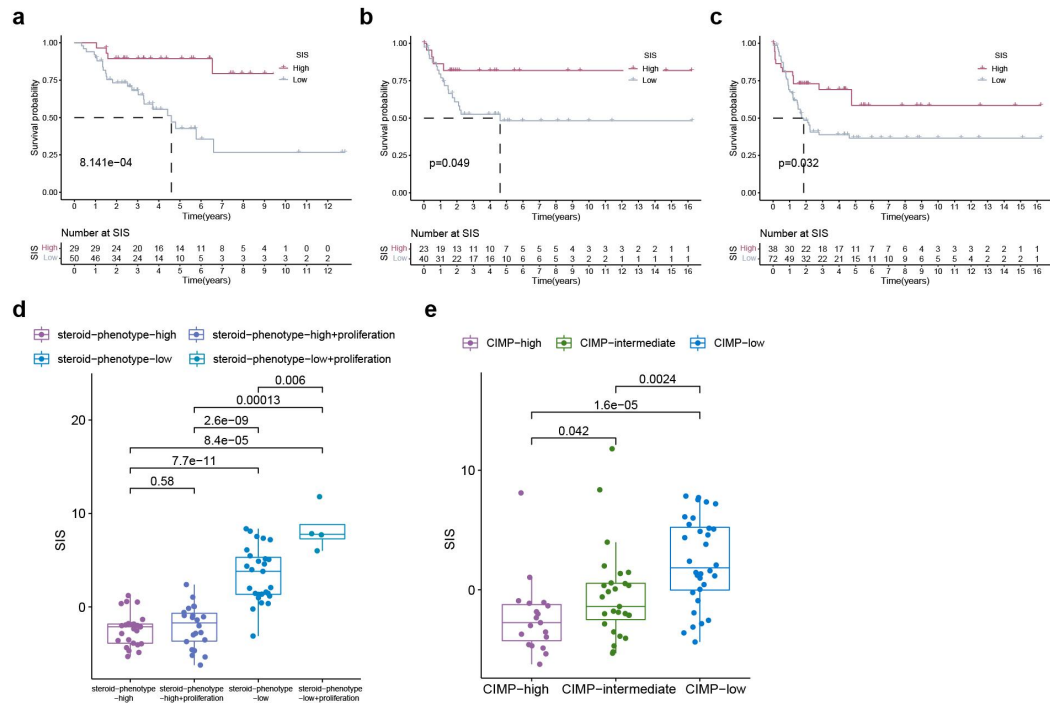
Supplementary Figure 1 | Removal of batch effects in ACC data. a Distribution of data before removing batch effects. **b** Distribution of data after removing batch effects.



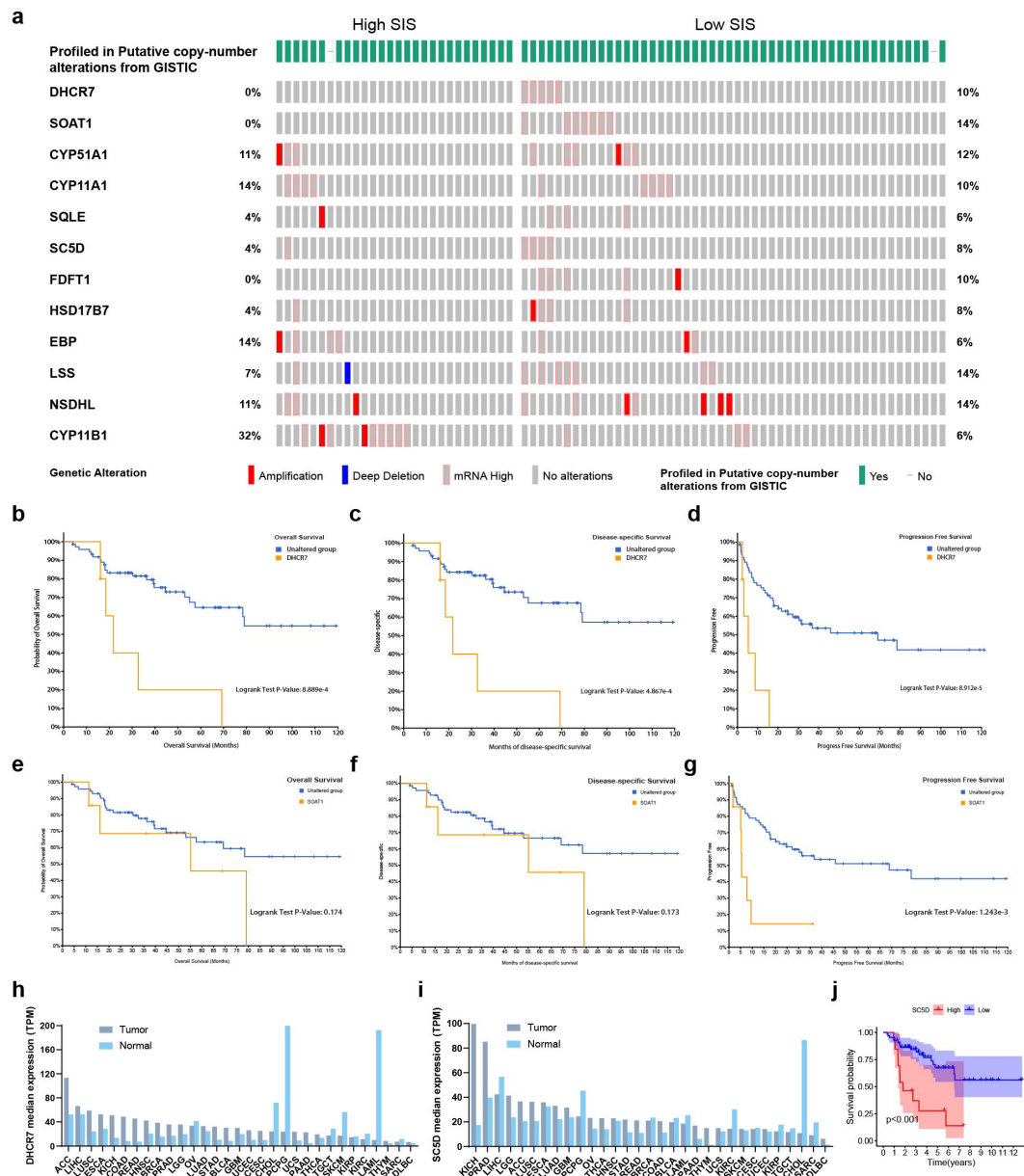
Supplementary Figure 2 | Unsupervised clustering analysis based on DEGs. a Consensus clustering heatmaps with different k values ($k=3, 4, 5, 6, 7, 8, 9$) showing the distribution of ACC patient samples (TCGA, GSE76019, and GSE76021) across different cluster numbers. **b** Consensus cumulative distribution function (CDF) curve for evaluating clustering stability at different k values. **c** Delta area plot to aid in selecting the optimal number of clusters, k . **d** Consensus clustering heatmaps for multiple ACC patient sample sets (TCGA, GSE76019, GSE76021, GSE33371, and GSE10927) with k values of 3, 4, 5, 6, 7, 8, and 9. **e** Consensus CDF curves for these samples, showing the stability of the clustering results. **f** Delta area plot to further confirm the optimal number of clusters, k .



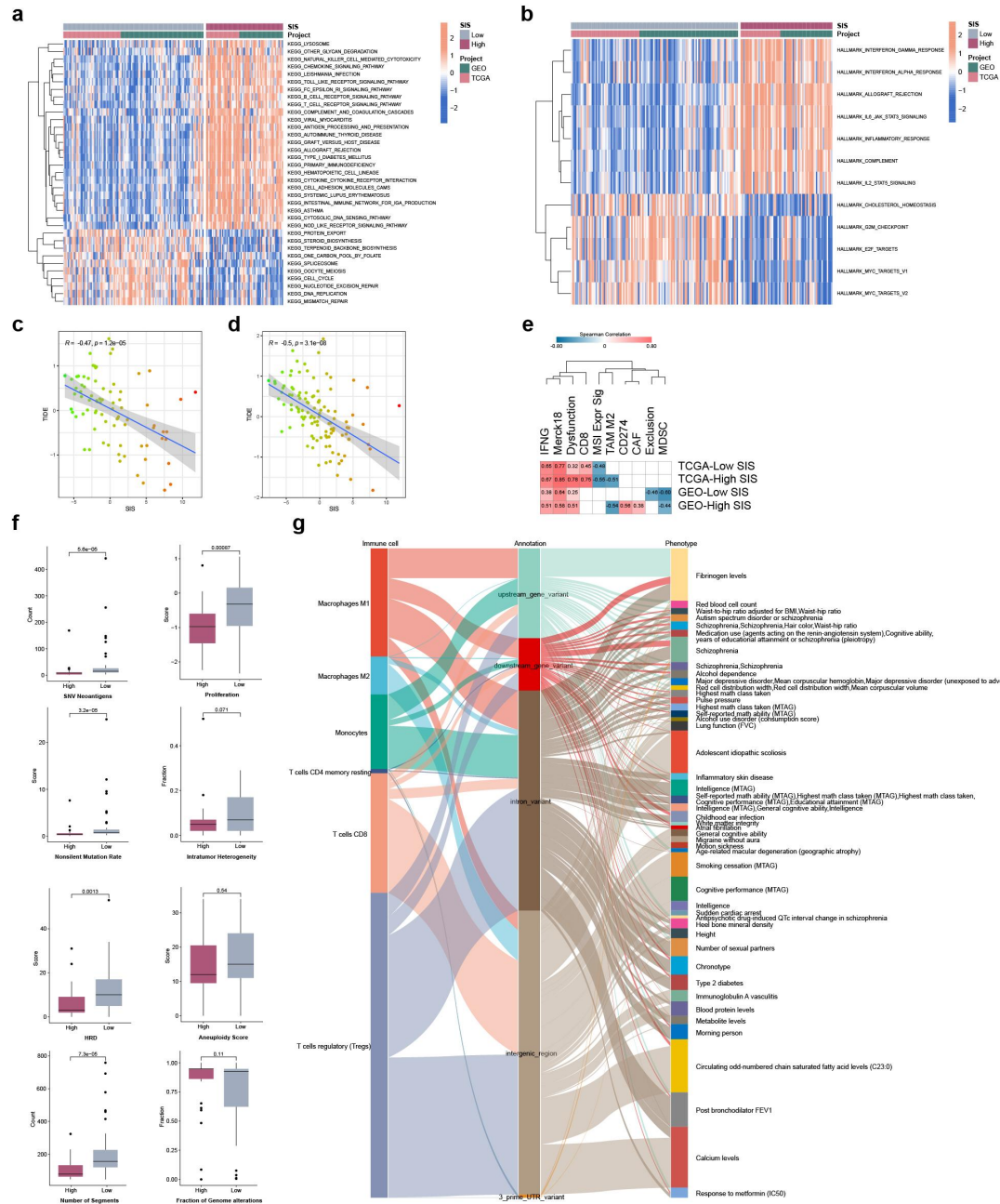
Supplementary Figure 3 | Application and comparison of artificial intelligence in SIS subgroups and C1A/B subgroups. **a** Evaluation of SIS classification performance by ResNet50 and Vision Transformer. **b** Evaluation of C1A/B classification performance by ResNet50 and Vision Transformer. **c** Five-fold cross-validation AUC plots for C1A/B subgroups by ResNet50 and Vision Transformer. **d** Pathological features related to Weiss score identified by CAMs in SIS subgroups. **e** Pathological features related to Weiss score identified by CAMs in the external validation set. **f** Survival prognosis of patients in the validation set. **g** Survival prognosis of the validation set and TCGA patients.



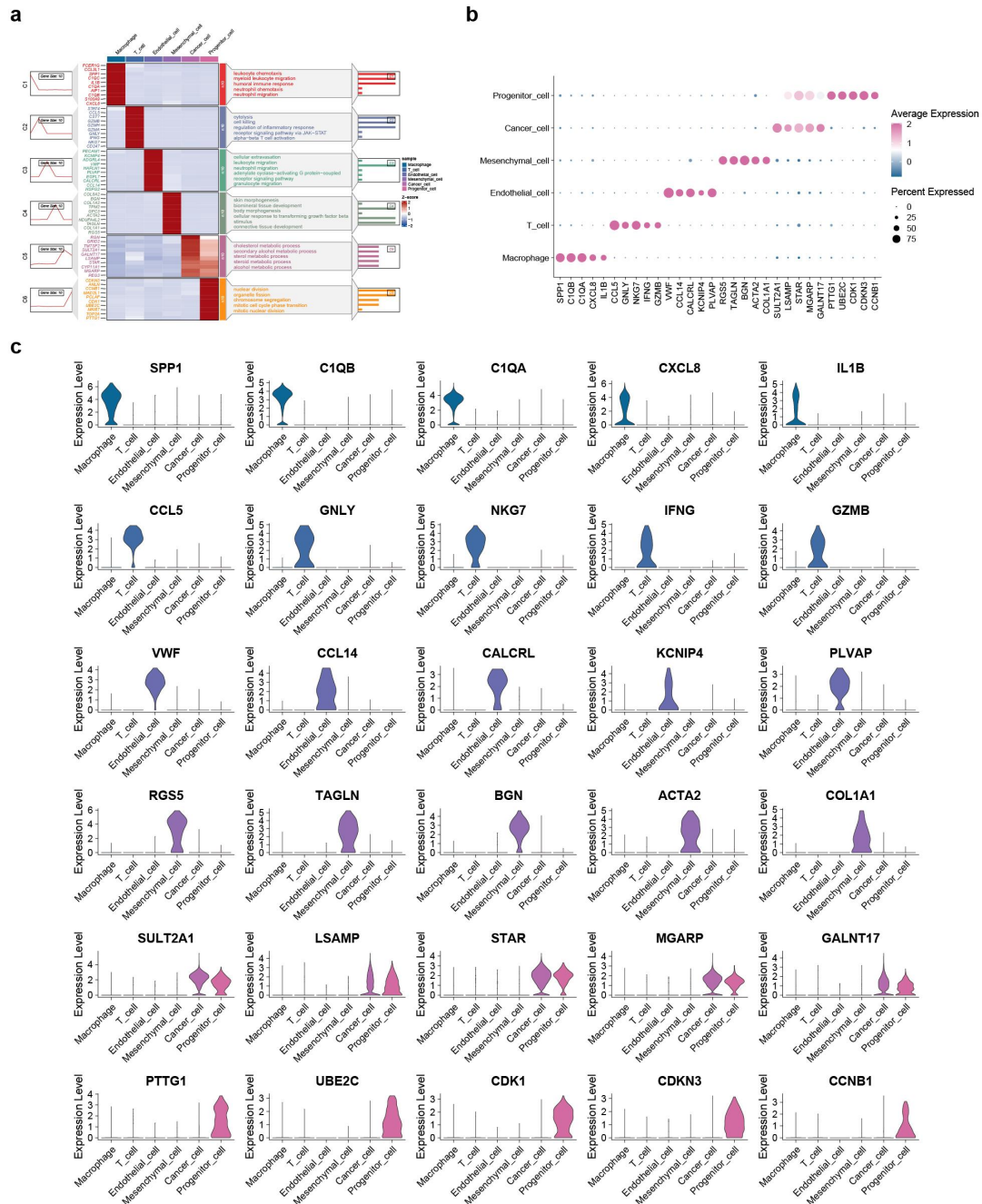
Supplementary Figure 4 | Survival prognosis of SIS subgroups and distribution of SIS in different ACC subtypes. **a** The Kaplan-Meier curve was used to predict the overall survival of the SIS (TCGA) (log-rank test, $P = 8.141 \times 10^{-4}$). **b** The Kaplan-Meier curve was used to predict the overall survival of the SIS (GSE76019+GSE76021) (log-rank test, $P = 0.049$). **c** The Kaplan-Meier curve was used to predict the overall survival of the SIS (GSE76019+GSE76021+GSE33371+GSE10927) (log-rank test, $P = 0.032$). **d** Distribution of SIS in the Expression (K=4) subgroups. **e** Distribution of SIS in methylation subtypes.



Supplementary Figure 5 | Gene alterations and prognosis of metabolism-related genes. **a** Copy number alterations of metabolism-related genes in the SIS subgroups of ACC patients. Kaplan-Meier curve used to predict DHCR7 **b-d** Overall Survival, Disease-specific Survival, and Progression Free Survival in copy number alterations. **e-g** Kaplan-Meier curve used to predict SOAT1 Overall Survival, Disease-specific Survival, and Progression Free Survival in copy number alterations. **h** Expression distribution of DHCR7 in tumor and adjacent non-tumor tissues. **i** Expression distribution of SC5D in tumor and adjacent non-tumor tissues. **j** The Kaplan-Meier curve used to predict the overall survival of SC5D.



Supplementary Figure 6 | Correlation analysis of SIS subgroups with immune characteristics in ACC patients. **a** A heatmap showing differential expression patterns in KEGG pathways for different SIS subgroups, with data from TCGA and GEO projects. **b** A heatmap showing differential expression in HALLMARK pathways for different SIS subgroups. **c** Correlation analysis between SIS (TCGA) and TIDE. **d** Correlation analysis between SIS (GEO) and TIDE. **e** A heatmap showing the correlation of different SIS subgroups with multiple TIDE-related indicators (e.g., Dysfunction, Exclusion). **f** Distribution of DNA damage measures in SIS subgroups. **g** A Sankey plot showing the relationship between ACC-associated survival-immunQTLs and GWAS.



Supplementary Figure 7 | Single-cell RNA sequencing analysis of ACC tumor microenvironment.
a Heatmap of gene expression across different cell types and clusters. **b** Dot plot representing average gene expression and percentage of expression across cell types. **c** Violin plots depicting gene expression distribution across cell types.

Supplementary Tables

Supplementary Table 1 | Principal component scores and SIS values for ACC samples from TCGA and GEO cohorts.

Principal component scores (PC1A and PC1B) and the calculated SIS (Signature Integration Score, $SIS = PC1A + PC1B$) values for each adrenocortical carcinoma (ACC) sample in the TCGA and GEO cohorts.

Sample	PC1A	PC1B	SIS
TCGA_TCGA-OR-A5K0	-2.48038633	-1.23581851	-3.71620484
TCGA_TCGA-OR-A5K9	-2.979462536	-1.606977598	-4.586440135
TCGA_TCGA-OR-A5LD	-3.700151807	0.166560560136605	-3.533591247
TCGA_TCGA-OR-A5JK	4.55989175191839	0.326626023241623	4.88651777516001
TCGA_TCGA-OR-A5K4	-4.02950724	0.910269260645806	-3.11923798
TCGA_TCGA-P6-A50F	-0.641892244	-1.237345423	-1.879237667
TCGA_TCGA-OR-A5JV	6.31944226685478	1.2189036759798	7.53834594283458
TCGA_TCGA-OR-A5J5	-1.941627968	-0.912217875	-2.853845843
TCGA_TCGA-PK-A5H8	1.05067961719157	0.309574318159641	1.36025393535121
TCGA_TCGA-OR-A5JG	-0.281132873	-1.062843812	-1.343976685
TCGA_TCGA-OR-A5LA	5.42337828659977	1.92333463079336	7.34671291739313
TCGA_TCGA-OR-A5JY	0.632601212405326	-1.763785104	-1.131183892
TCGA_TCGA-OR-A5LM	0.322528388284529	0.25305667541898	0.575585063703508
TCGA_TCGA-OR-A5LP	4.85702488717771	1.24137546093377	6.09840034811148
TCGA_TCGA-OR-A5JW	-1.891208943	-1.63312255	-3.524331492
TCGA_TCGA-OR-A5J7	-2.881810558	-1.804800703	-4.686611261
TCGA_TCGA-PK-A5H9	0.676084329087001	-0.900397645	-0.224313316
TCGA_TCGA-OR-A5JI	2.80250390585097	1.17950184672964	3.9820057525806
TCGA_TCGA-OR-A5L6	0.0161488484563322	0.342285423330572	0.358434271786904
TCGA_TCGA-OR-A5KZ	-0.241246113	0.0829061506139408	-0.158339962
TCGA_TCGA-OR-A5KT	7.19491633373782	-1.191897997	6.00301833689948
TCGA_TCGA-OR-A5JB	7.56601627442564	0.269855517600192	7.83587179202583
TCGA_TCGA-OR-A5LR	0.815210171007615	0.773338105785537	1.58854827679315
TCGA_TCGA-OR-A5JL	1.00381381207575	0.209674374955184	1.21348818703094
TCGA_TCGA-OR-A5K2	0.796851914845325	-1.718293993	-0.921442079
TCGA_TCGA-OR-A5JM	-4.853818432	-1.373386391	-6.227204822
TCGA_TCGA-OR-A5L8	-3.557687241	-0.048920804	-3.606608046
TCGA_TCGA-OR-A5L3	0.0717657585005556	-0.984463931	-0.912698172
TCGA_TCGA-OR-A5JQ	3.9461894312694	1.52164286774429	5.46783229901368
TCGA_TCGA-OR-A5K0	-4.965408624	-0.343721202	-5.309129826
TCGA_TCGA-OU-A5PI	-1.971523817	-0.033717493	-2.00524131
TCGA_TCGA-OR-A5J9	-3.131674472	0.786967798742069	-2.344706673
TCGA_TCGA-OR-A5LL	-2.136564344	-0.69569552	-2.832259864
TCGA_TCGA-OR-A5LN	3.89502607905146	0.694541389461953	4.58956746851342

TCGA_TCGA-PK-A5HA	6.42331927718667	1.67824276865032	8.10156204583699
TCGA_TCGA-OR-A5LG	3.19724620161054	-0.809999446	2.38724675520879
TCGA_TCGA-OR-A5L4	0.17486168001558	0.258440063172201	0.433301743187781
TCGA_TCGA-OR-A5L9	4.31419684288969	0.764793386449957	5.07899022933964
TCGA_TCGA-OR-A5JP	-1.587434136	-1.160962182	-2.748396317
TCGA_TCGA-OR-A5L5	4.77829956631022	0.368880129659277	5.1471796959695
TCGA_TCGA-OR-A5J2	-0.474197738	0.511887585199599	0.0376898467328118
TCGA_TCGA-OR-A5LJ	-0.193820222	-1.206400043	-1.400220265
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TCGA_TCGA-OR-A5JF	-2.851855543	-1.032143785	-3.883999328
TCGA_TCGA-OR-A5JA	-1.301036029	-0.535949506	-1.836985535
TCGA_TCGA-OR-A5JR	0.957415515827753	1.11186885491908	2.06928437074683
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TCGA_TCGA-OR-A5K3	1.58213872503159	0.412432636631938	1.99457136166353
TCGA_TCGA-OR-A5JO	8.50436542895915	-0.791131906	7.71323352293572
TCGA_TCGA-OR-A5J8	11.5211647165519	0.272964259479176	11.7941289760311
TCGA_TCGA-OR-A5LS	-1.836900684	-0.29182325	-2.128723934
TCGA_TCGA-OR-A5K8	-1.668553462	-0.120138787	-1.78869225
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GEO_GSM1972933	1.83820611419481	2.27081705230115	4.10902316649596
GEO_GSM1972934	-2.986230399	-1.464520611	-4.45075101
GEO_GSM1972935	4.06115390676286	1.03128404608365	5.0924379528465
GEO_GSM1972936	3.75235354143058	-0.83382109	2.91853245127648
GEO_GSM1972937	-1.320995167	0.17792430257685	-1.143070865
GEO_GSM1972938	0.271833900388906	-0.348785562	-0.076951662
GEO_GSM1972939	-1.996160104	-0.535502577	-2.53166268
GEO_GSM1972940	0.283578116501474	-1.184337591	-0.900759474
GEO_GSM1972941	4.03394358599141	3.12307348804251	7.15701707403393
GEO_GSM1972942	-0.88552452	-0.931268704	-1.816793224
GEO_GSM1972943	2.11620793734862	-1.148725393	0.967482544243372
GEO_GSM1972944	-0.524455975	0.319144032495633	-0.205311942
GEO_GSM1972945	1.30125524403537	-0.235232403	1.06602284103077
GEO_GSM1972946	-1.874544075	-0.535570432	-2.410114507
GEO_GSM1972947	-0.235380069	-0.794825395	-1.030205463

Supplementary Table 2 | Gene weights in PC1A for ACC samples.

These weights were used to calculate the SIS for each sample. The values represent the contribution of each gene to the principal component analysis.

PLEK	0.258427615200504
SIGLEC1	0.257279076197403
AIF1	0.272292435784382
FOLR2	0.253996952100703
MS4A4A	0.274144915324739
HCLS1	0.269470807936666
CD52	0.206663192231167
C1QA	0.27683864853441
FCER1G	0.273915961801974
F13A1	0.239986570086841
C1QB	0.274627734273577

CXCL9	0.18225672472533
COR01A	0.259127563014031
CD163	0.262156568489265
CXCL12	0.215028910046154
CXCL10	0.194611799626555

Supplementary Table 3 | Gene weights in PC1B for ACC samples.

These weights were used to calculate the SIS for each sample. The values represent the contribution of each gene to the principal component analysis.

CYP17A1	-0.707106781
ABAT	0.707106781186549