Supplementary Materials

1.1 Main Materials for the Experiment

1.1.1 Main Instruments

No.	Equipment	Brand	Model
1	Benchtop High-Speed Freezing Centrifuge	cence	TGL-16
2	Ultra-Micro Spectrophotometer	Bio-DL	Micro Drop
3	Gene Amplifier	Bio-rad	MyCycler
4	Fluorescent Quantitative PCR Instrument	ABI	ViiA7

1.1.2 Main Reagents

No.	Reagent	Brand	Catalog No.
1	RNAiso Plus	TAKARA	9109
2	PrimeScript™ RT reagent Kit	TAKARA	RR037A
3	PerfectStart Universal Green qPCR SuperMix	TransGen Biotech	AQ631
4	Primers	Tsingke	——

1.2 Experimental Procedures

1.2.1Total RNA Extraction

1.2.1.1 Sample Pretreatment

Add 1 mL of RNAiso Plus to the EP tube containing the cell sample. Use a pipette to gently agitate the cells until all cells are lysed into the RNAiso Plus solution. Incubate at room temperature for approximately 5 minutes.

1.2.1.2 Phase Separation

Add 0.2 mL of chloroform to each 1 mL of RNAiso Plus. Vortex for 15 seconds, then let it sit at room temperature for about 3 minutes. Centrifuge at 4°C, 12,000 rpm for 15 minutes.

1.2.1.3 Precipitation

Transfer the aqueous phase to a new 1.5 mL centrifuge tube. Add 0.5 mL of isopropanol per 1 mL of RNAiso Plus, mix thoroughly, and let it sit at room temperature for 10 minutes. Centrifuge at 4°C, 12,000 rpm for 10 minutes.

1.2.1.4 Wash

Discard the supernatant. Add 1 mL of 75% ethanol per 1 mL of RNAiso Plus, mix thoroughly, and centrifuge at 4°C, 7,500 rpm for 5 minutes.

1.2.1.5 Dissolution

Discard the supernatant, air-dry the RNA precipitate for approximately 5 minutes (note: do not completely

dry; only dry until the precipitate turns white), then add an appropriate amount of DEPC-treated water to dissolve the RNA precipitate.

1.2.1.6 Determination of Concentration and Purity

Measure the RNA concentration and purity using a spectrophotometer and record the data.

1.2.2 Reverse transcription reaction

1.2.2.1 Prepare the RT reaction solution according to the following groups

To ensure the accuracy of the reaction solution preparation and reduce errors caused by aliquoting, prepare the reaction solution in a volume slightly larger than the actual amount used, and finally add the RNA sample.

mRNA primer reverse transcription system:

Reagent Component	Volume	Final Concentration
5×PrimeScript Buffer（for Real Time）	2uL	1×
PrimeScript RT Enzyme Mix I	0.5uL	
Oligo dT Primer（50 μM）*1	0.5uL	25 pmol
Random 6 mers（100 μM）*1	0.5uL	50 pmol
Total RNA	600ng	
RNase Free dH2O	Make up to 10 μL	

1.2.2.2 Perform reverse transcription according to the following procedure:

37°C, 15 min; 85°C, 5 sec.

Store at -20°C for future use.

1.2.3 Quantitative PCR Detection

1.2.3.1 Dissolve the mix at 4°C, gently invert to mix, and centrifuge briefly.

1.2.3.2 Prepare the reaction solution in the table below on ice.

Component	Volume	Final Concentration
2xPerfectStart Universal Green qPCR SuperMix	5μL	1×
Forward Primer (10μM)	0.2μL	0.2 μM
Reverse Primer (10μM)	0.2μL	0.2 μM
cDNA（diluted 1:10）	2μL	
RNase-free Water	to 10μL	

1.2.3.3 Centrifuge the reaction tube briefly to ensure that all reaction liquid is at the bottom of the

reaction well.

1.2.3.4 Perform the reaction using a three-step procedure:

Cycle number	Step	Temperature	Time	Fluorescence collection
1	Pre-denaturation	95℃	1min	No
40	Denaturation	95℃	5s	No
	Annealing	60℃	30s	Yes
After the above reaction is complete, perform melting curve analysis during the process from 60°C to 95°C.				

1.2.4. Principles and Methods of Relative Quantitative △△Ct Analysis

1.2.4.1. Theoretical Equation for Fluorescence Signal

$R_n = R_B + X_0 (1 + E)^n R_s$

The fluorescence signal intensity (R_n) at the nth PCR cycle equals the background signal intensity (R_B) plus the product of the fluorescence intensity per molecule (i.e., unit fluorescence intensity R_S) and the number of molecules.

Or total signal = background signal + molecular quantity × unit signal intensity

Where R_n is the total signal at the nth cycle, R_B is the background signal, R_S is the unit signal intensity, X_0 is the initial template quantity, and E is the PCR efficiency.

1.2.4.2. Formula calculation

When the number of cycles n = Ct value, assuming E = 100%:

$R_{Ct} = R_B + X_0 \times 2^{Ct} R_s$

$X_0 \times 2^{Ct} = (R_{Ct} - R_B) / R_s = b$

$X_0 = b / 2^{Ct} = b \times 2^{-Ct}$

1.2.4.3. Internal reference gene normalization of sample differences

Each sample is tested for the target gene and internal reference gene. The internal reference gene is used for normalization to correct differences in nucleic acid quantity among samples. The correction value = target gene/internal reference gene, i.e., $X1/X2 = 2^{-(Ct1 - Ct2)} = 2^{-\Delta Ct}$.

$2^{-\Delta Ct}$ represents the normalized molecular quantity of the target gene in each sample.

1.2.4.4. Comparison of treated samples and control samples

To compare the expression levels of a specific gene between different samples, the relative value is calculated as treated sample / control sample.

The fold change is calculated as $2^{-\triangle Ct1} / 2^{-\triangle Ct2} = 2^{-\triangle\triangle Ct}$.

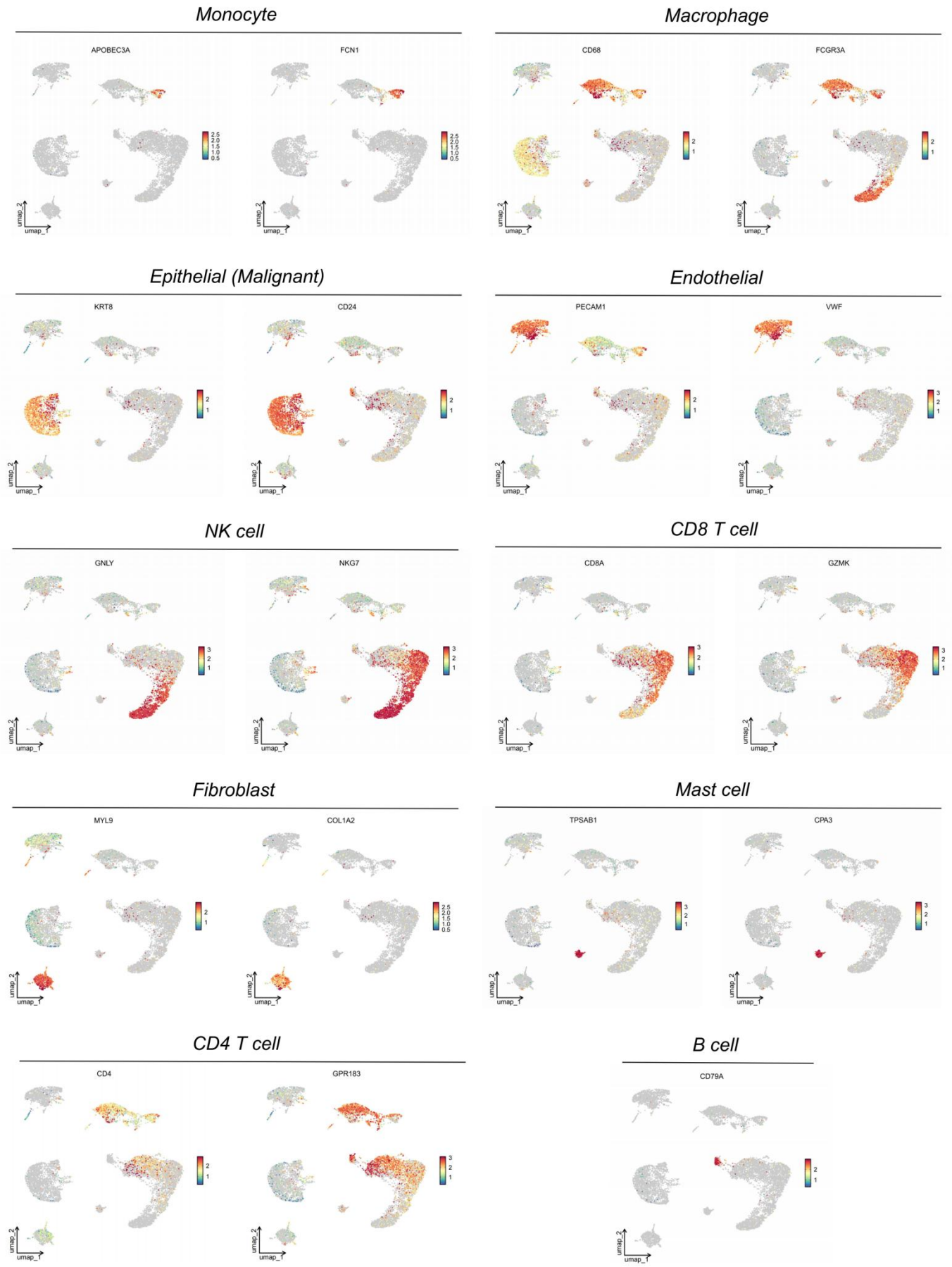

Fig S1. Cell markers (all types)

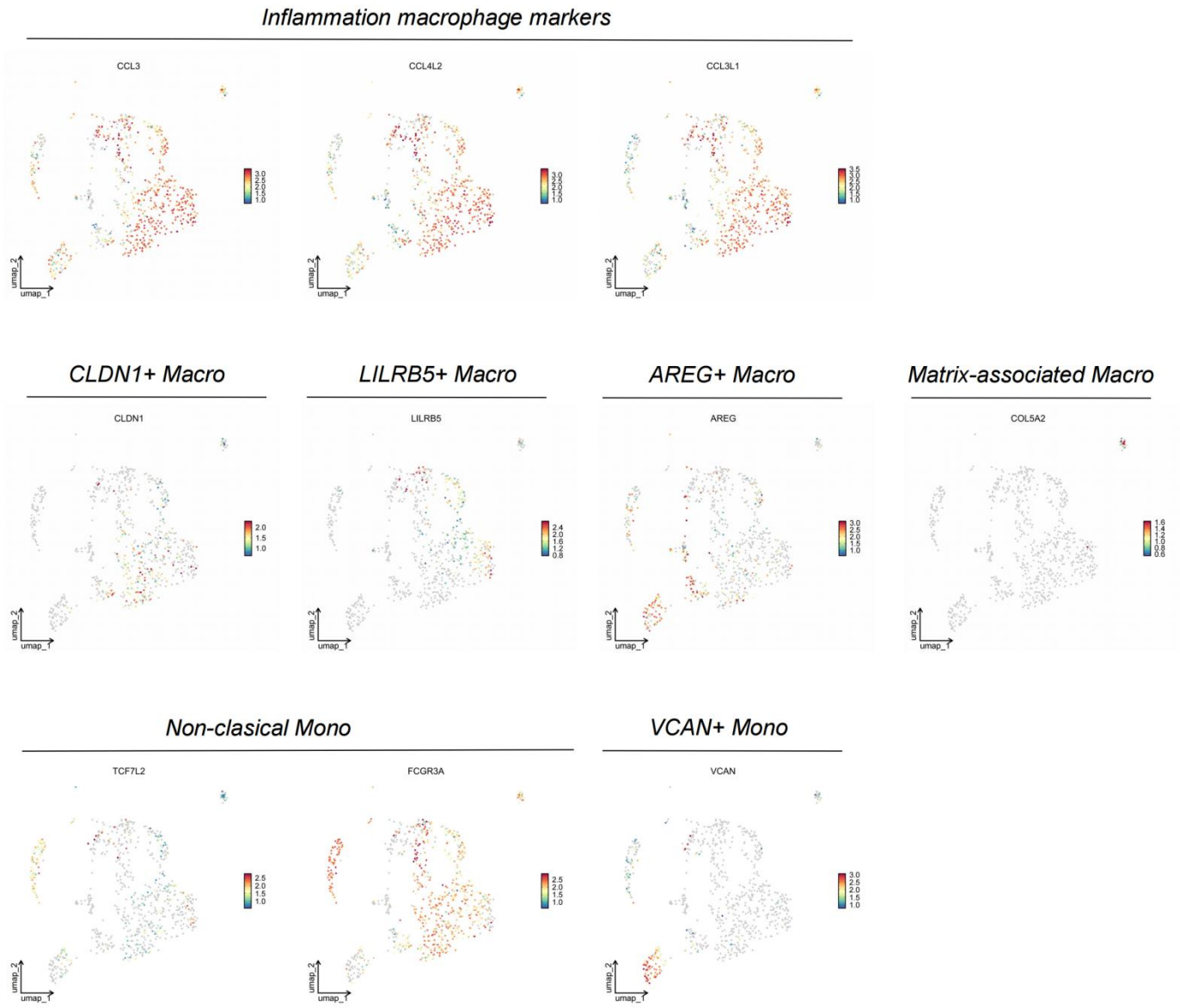

Fig S2. Cell markers (Monocyte and Macrophage)

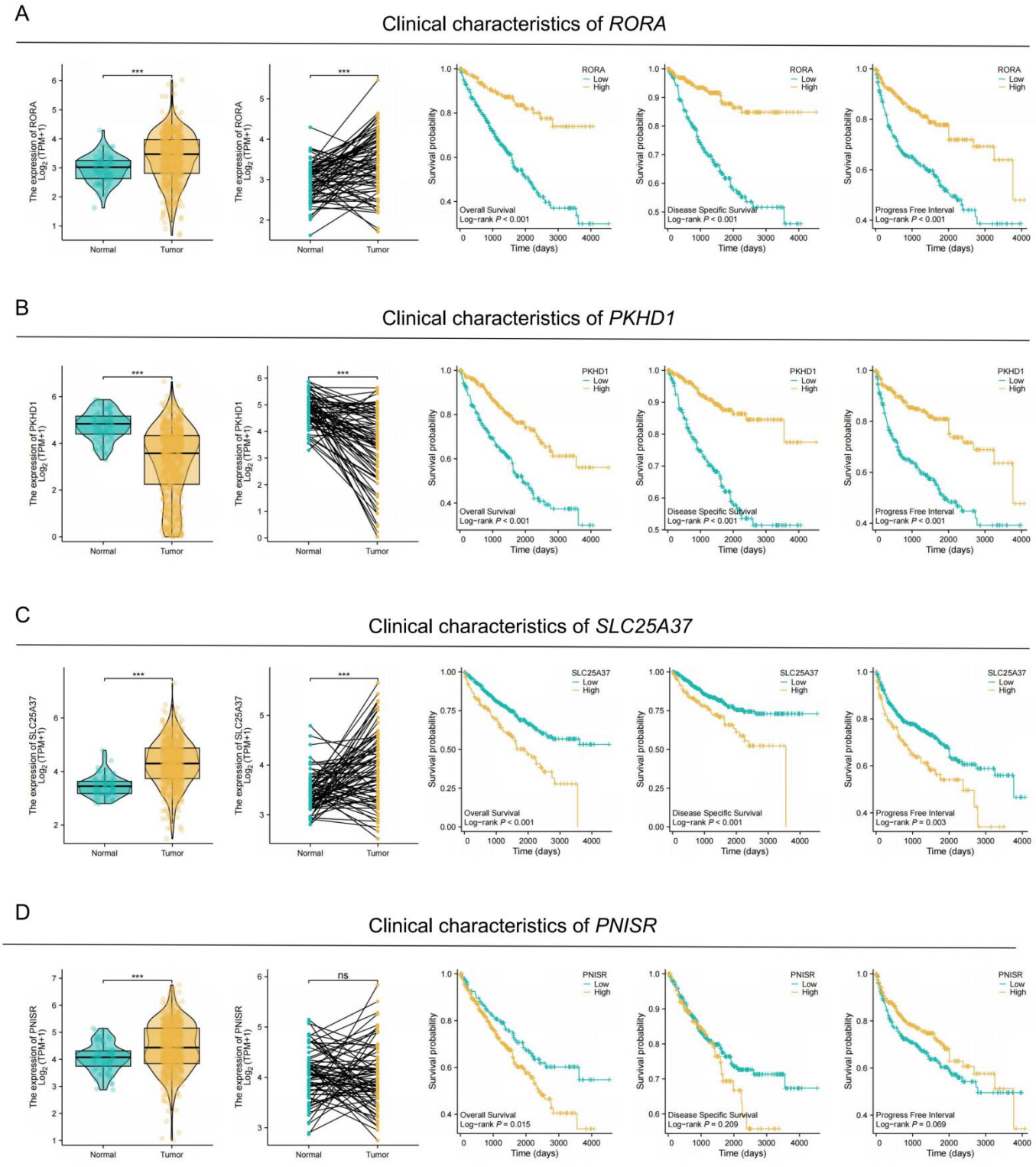

Fig S3. Clinical characteristics of model genes