Supplementary methods

H&E staining of paraffin sections

1. Preparation of paraffin sections

Sampling. Fresh tissue was fixed with fixative solution for >24 h. The tissue was then removed from the fixative and the area of interest was trimmed smoothly using a scalpel in a fume hood. The trimmed tissue and its corresponding label were placed into a dehydration cassette.

Dehydration and wax infiltration. The dehydration cassettes were placed into the dehydrator for dehydration through a graded series of alcohols: 75% alcohol for 4 h, 85% alcohol for 2 h, 90% alcohol for 2 h, 95% alcohol for 1 h, absolute ethanol I for 30 min, absolute ethanol II for 30 min, alcohol-xylene for 5-10 min, xylene I for 5-10 min, xylene II for 5-10 min, followed by infiltration in melted paraffin wax at 65˚C: Paraffin I for 1 h, Paraffin II for 1 h, Paraffin III for 1 h.

Embedding. The wax-infiltrated tissues were embedded using an embedding machine. Molten wax was poured into an embedding mold, and before the wax solidified, the tissue was taken out from the dehydration cassette and placed into the mold according to the orientation requirements for the embedding surface, followed by attaching the corresponding label. The mold was cooled on a -20˚C freezing stage. After the wax solidified, the wax block was removed from the mold and trimmed.

Sectioning. The trimmed wax blocks were cooled on the -20˚C freezing stage. The cooled blocks were then sectioned at 4 μm thickness using the paraffin microtome. The sections were floated on a 40˚C warm water bath in the spreading machine to flatten the tissue. The flattened sections were picked up onto glass slides, which were then baked in a 60˚C oven until the water evaporated and the wax melted. The slides were stored at room temperature for future use.

2. H&E staining

Deparaffinization and hydration. Slides were sequentially placed in xylene I for 20 min -> xylene II for 20 min -> absolute ethanol I for 5 min -> absolute ethanol II for 5 min -> 75% alcohol for 5 min, followed by rinsing with tap water.

Hematoxylin staining. Slides were stained in hematoxylin staining solution for 3-5 min, rinsed with tap water, differentiated in differentiation solution, rinsed with tap water, blued in bluing solution and rinsed under running water.

Eosin staining. Slides were sequentially dehydrated in 85 and 95% graded alcohols for 5 min each, and then stained in eosin staining solution for 5 min.

Dehydration and mounting. Slides were sequentially placed in absolute ethanol I for 5 min -> absolute ethanol II for 5 min -> absolute ethanol III for 5 min -> xylene I for 5 min -> xylene II for 5 min for clearing, and finally mounted with Neutral balsam.

Microscopic examination and image acquisition and analysis. Nuclei appear blue and cytoplasm appears red.

3. Main experimental reagents

Name	Manufacturer	Product code
Absolute ethyl alcohol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Xylene	Sinopharm Chemical Reagent Co., Ltd.	10023418
HE Staining Kit	Servicebio	G1003
Neutral gum	Sinopharm Chemical Reagent Co., Ltd.	10004160

Immunohistochemistry of paraffin sections

Dewaxing of paraffin sections to water. Put the sections into environmentally friendly dewaxing solution I for 10 min, then environmentally friendly dewaxing solution II for 10 min, followed by environmentally friendly dewaxing solution III for 10 min, anhydrous ethanol I for 5 min, anhydrous ethanol II for 5 min, anhydrous ethanol III for 5 min and finally rinse with distilled water.

Antigen retrieval. Refer to the above table for retrieval. During this process, excessive evaporation of the buffer should be prevented, and the sections must not be dried out. After natural cooling, place the glass slides in PBS (pH 7.4) and wash with shaking on a decolorizing shaker 3 times, 5 min each time (the retrieval solution and retrieval conditions are determined according to the tissue).

Blocking endogenous peroxidase. Put the sections into 3% hydrogen peroxide solution, incubate at room temperature in the dark for 25 min. Then place the glass slides in PBS (pH 7.4) and wash with shaking on a decolorizing shaker 3 times, 5 min each time.

Serum blocking. Add 3% BSA dropwise within the immunohistochemical circle to cover the tissue evenly, and block at room temperature for 30 min (if the primary antibody is derived from goats, rabbit serum is used for blocking; for primary antibodies from other sources, BSA is used for blocking).

Adding primary antibody. Gently shake off the blocking solution, add the primary antibody prepared with PBS at a certain ratio dropwise onto the sections. Place the sections flat in a wet box and incubate at 4˚C overnight.

Adding secondary antibody. Place the glass slides in PBS (pH 7.4) and wash with shaking on a decolorizing shaker 3 times, 5 min each time. After slightly drying the sections by shaking, add the secondary antibody (HRP-labeled) corresponding to the species of the primary antibody dropwise within the circle to cover the tissue and incubate at room temperature for 50 min.

DAB chromogenic reaction. Place the glass slides in PBS (pH 7.4) and wash with shaking on a decolorizing shaker 3 times, 5 min each time. After slightly drying the sections by shaking, add freshly prepared DAB chromogenic solution dropwise within the circle. Control the chromogenic time under a microscope; positive results show as a brownish-yellow color. Rinse the sections with tap water to terminate the chromogenic reaction.

Counterstaining of cell nuclei. Counterstain with hematoxylin for ~3 min, rinse with tap water, differentiate with hematoxylin differentiation solution for a few seconds, rinse with tap water, blue with hematoxylin bluing solution and rinse with running water.

Dehydration and mounting. Put the sections into 75% ethanol for 5 min, then 85% ethanol for 5 min, anhydrous ethanol I for 5 min, anhydrous ethanol II for 5 min, n-butanol for 5 min and xylene I for 5 min for dehydration and clearing. Take the sections out of xylene, air-dry slightly and mount with mounting medium.

Microscopic examination. Place the sections under a bright-field microscope for result interpretation. Cell nuclei stained with hematoxylin are blue and positive expression shown by DAB is brownish-yellow.

Table SI. Antibody information and retrieval conditions.

Antigen retrieval condition	Primary antibody name	Primary antibody product no.	Primary antibody manufacturer	Primary antibody species	Primary antibody dilution ratio	Corresponding secondary antibody name
EDTA high pressure, 5 min	CD21	GB14031	Servicebio	Mouse	1:200	HRP-labeled goat anti-mouse IgG
Heat retrieval, 15 min (100˚C)	CD23	GB14032	Servicebio	Mouse	1:200	HRP-labeled goat anti-mouse IgG
Heat retrieval, 15 min (100˚C)	CD35	GB112007	Servicebio	Rabbit	1:650	HRP-labeled goat anti-rabbit IgG

Table SII. Secondary antibodies.

Corresponding secondary antibody name	Corresponding secondary product no.	Corresponding secondary manufacturer	Corresponding secondary dilution ratio
HRP-labeled goat anti-mouse IgG	GB23301	Servicebio	1:200
HRP-labeled goat anti-rabbit IgG	GB23303	Servicebio	1:200