SUPPLEMENTARY MATERIALS

SUPPLEMENTARY METHODS

Immunohistochemistry

We performed immunohistochemistry (IHC) as described in our previous studies [1]. Briefly, sections 4-µm thick were subjected to deparaffinization, antigen retrieval, and blockage of non-specific binding, with the blockage performed by incubation with 10% normal goat serum for 15 min. The sections were incubated with primary antibodies for ORC6 (1:100, Proteintech), CCDC34 (1:100, invitrogen), and SOX4 (1:100, Abcam) at 4°C overnight, and then with a biotinylated secondary antibody. Subsequently, slides were stained with 3,3-diaminobenzidine tetrahydrochloride.

Western blot analysis

Total protein was extracted from tissues using rotor and radio immunoprecipitation assay (RIPA) lysis buffer.

Equal amounts of protein were separated by SDS-PAGE in a 12% gel and transferred to a nitrocellulose membrane. The proteins were detected using an enhanced chemiluminescence system according to the manufacturer's instructions. Membranes were incubated overnight with the following primary antibodies: anti-ORC6 (Proteintech), anti-SOX4 (Abcam), anti-CCDC34 (invitrogen).

REFERENCES

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https://doi.org/10.1111/cas.14438 PMID:32350953