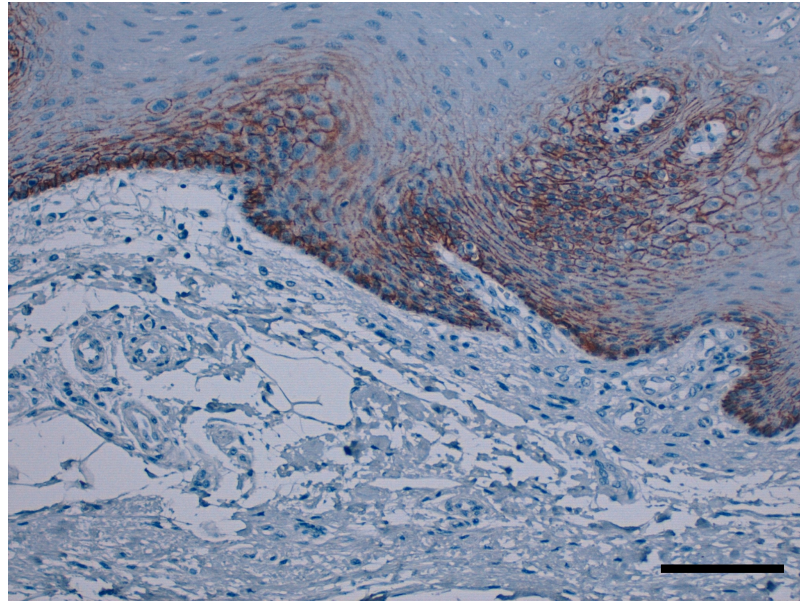


Figure S1 Conformation of the recognition of CHO/CD44s and CHO/CD44v3-10 by C₄₄Mab-46 by flow cytometry. CHO/CD44v3-10 (A), CHO/CD44s (B), and CHO-K1 (C) were treated with 0.01-10 $\mu\text{g/mL}$ of C₄₄Mab-46, followed by treatment with Alexa Fluor 488-conjugated anti-mouse IgG (Red line). The black line represents the negative control (blocking buffer).

C₄₄Mab-1



C₄₄Mab-46

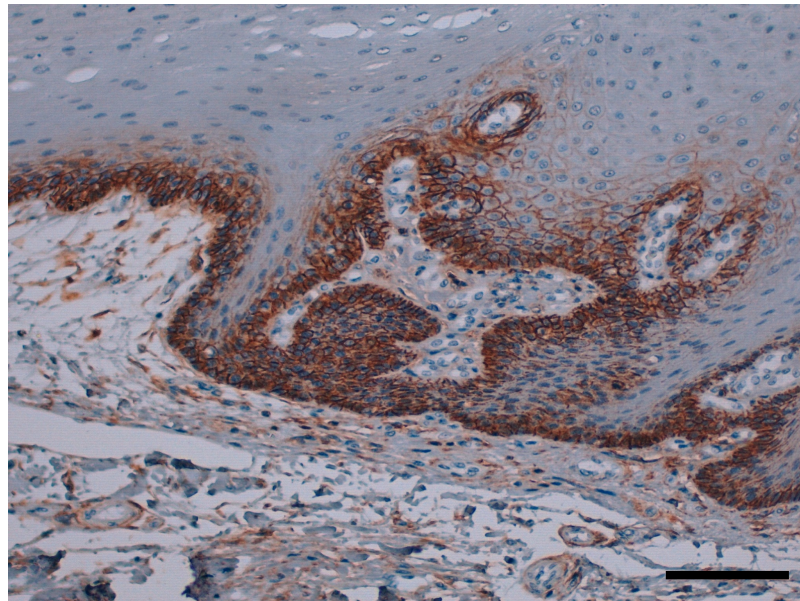


Figure S2 Immunohistochemical analysis using C₄₄Mab-1 and C₄₄Mab-46 against oral squamous cell carcinoma tissues. After antigen retrieval, the sections were incubated with 1 μ g/mL of C₄₄Mab-1 (A) and 1 μ g/mL of C₄₄Mab-46 (B), followed by treatment with the Envision+ kit. The color was developed using 3,3'-diaminobenzidine tetrahydrochloride (DAB), and the sections were counterstained with hematoxylin. Scale bar = 100 μ m.

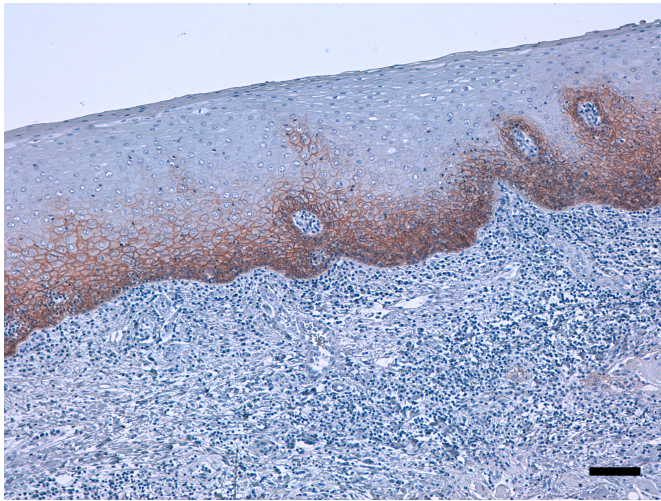
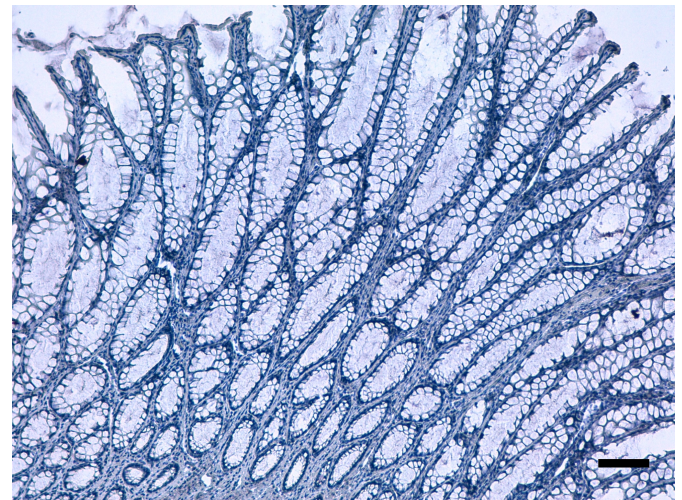
A**C₄₄Mab-1****oral SCC****C₄₄Mab-1 + p471-490 peptide****oral SCC****B****PMab-44 (Isotype control)****oral SCC****C****C₄₄Mab-1****cat rectum
(negative tissue control)**

Figure S3 The blocking assay by an epitope peptide, isotype control, and negative tissue control in immunohistochemistry. (A) Blocking of the C₄₄Mab-1 reactivity to oral SCC tissue (positive tissue control) by the CD44 peptide (aa 471–490) containing the C₄₄Mab-1 epitope. After antigen retrieval, sections were incubated with C₄₄Mab-1 (1 µg/mL) or C₄₄Mab-1 (1 µg/mL) plus human CD44 peptide (aa 471–490, 10 µg/mL). (B) The oral SCC tissue section was incubated with an isotype control mAb, PMab-44 (1 µg/mL). (C) A negative control tissue section (cat rectum) was incubated with C₄₄Mab-1 (1 µg/mL). The tissues were further treated with the Envision+ kit. The color was developed using DAB, and sections were counterstained with hematoxylin. Scale bar = 100 µm.