Figure S1. Flow cytometry using 5-mG_{2a} and C_{44}Mab-46-mG_{2a}. CHO-K1 cells were treated with 10-0.01 μg/ml of 5-mG_{2a} and C_{44}Mab-46-mG_{2a} (red) or buffer control (black), followed by Alexa Fluor 488-conjugated anti-mouse IgG. Fluorescence data were analyzed using the SA3800 Cell Analyzer. mAb, monoclonal antibody.

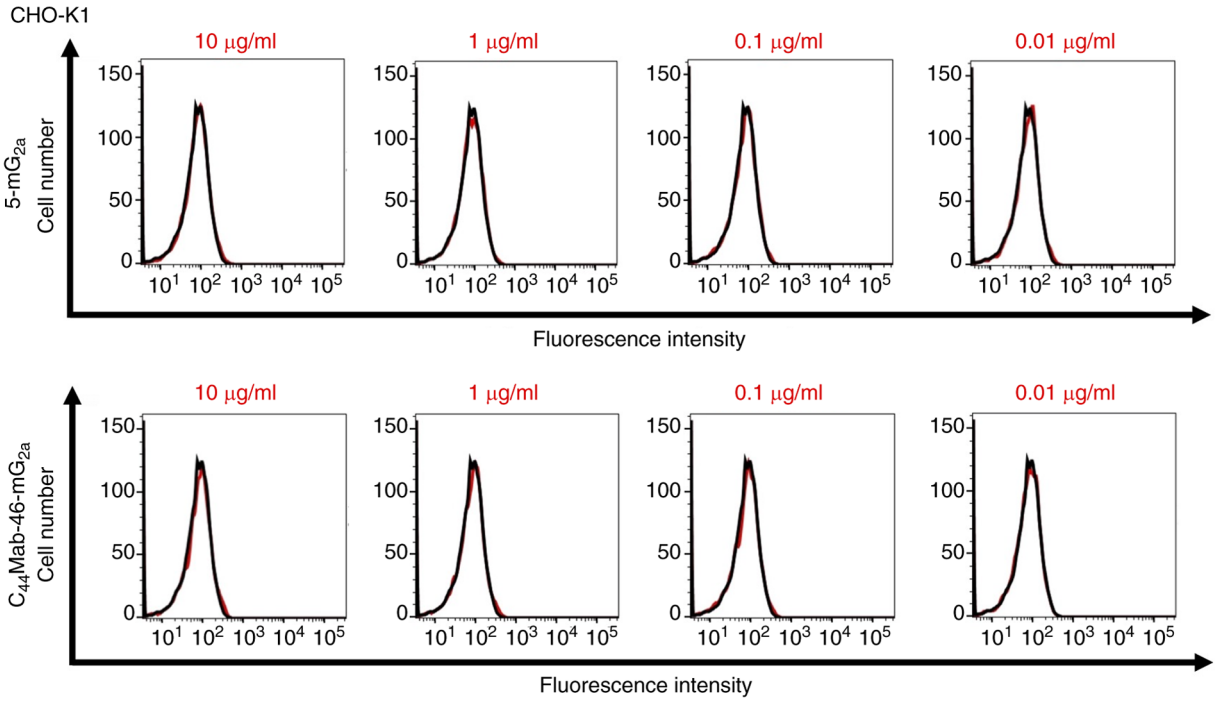

Figure S2. Flow cytometry using anti-CD44v mAbs. KYSE770 cells were treated with 10 μg/ml of C_{44}Mab-46, C_{44}Mab-6 (an anti-CD44v3 mAb), C44Mab-3 (an anti-CD44v5 mAb), C_{44}Mab-9 (an anti-CD44v6 mAb), C_{44}Mab-18 (an anti-CD44v10 mAb) (red), or buffer control (black), followed by Alexa Fluor 488-conjugated anti-mouse IgG. Fluorescence data were analyzed using the SA3800 Cell Analyzer. mAb, monoclonal antibody.

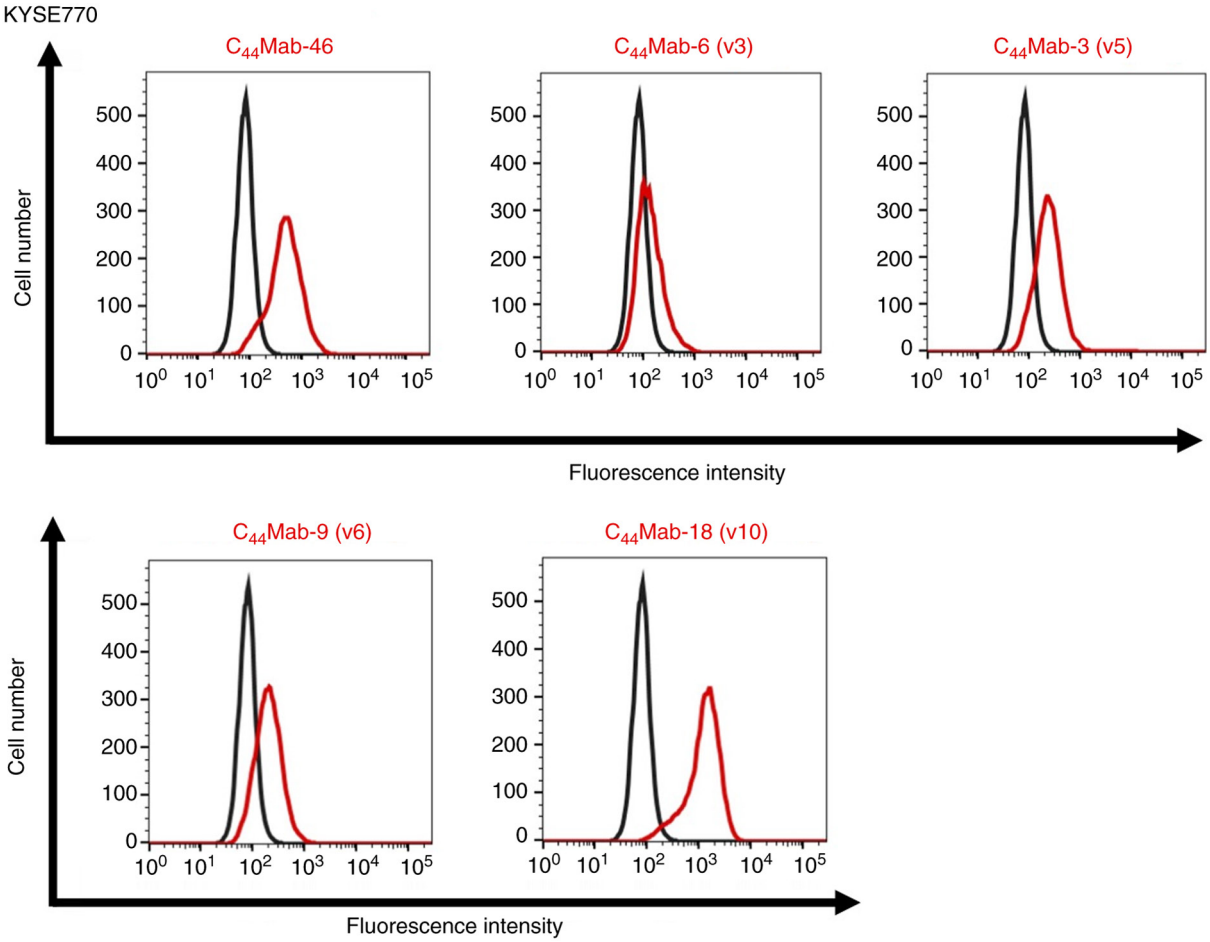

Figure S3. Histological analyses of 5-mG_{2a}, C_{44}Mab-46-mG_{2a}, or control mouse IgG_{2a} (281-mG_{2a})-treated CHO/CD44s xenograft tumors. (A) The sections were performed with H&E. (B-D) Immunohistochemistry using (B) C_{44}Mab-46, (C) mPMab-1 (an anti-mouse podoplanin mAb) and (D) no 1st Ab control (no Ab), followed by treatment with the Envision+ kit. The chromogenic reaction was performed using DAB, and the sections were counterstained with hematoxylin. Scale bar, 100 μm. Arrow, lymphatic vessels; Arrowheads, podoplanin-positive macrophages. mAb, monoclonal antibody.

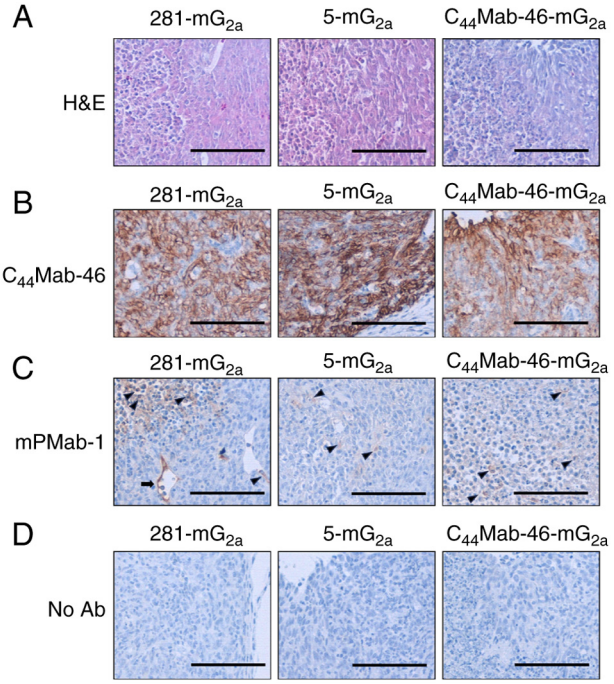

Figure S4. Antitumor activity of 5-mG_{2a} and C_{44}Mab-46-mG_{2a} against KYSE770 xenograft (low dosage). (A) Measurement of tumor volume in KYSE770 xenograft. KYSE770 cells ($5x10^6$ cells) were injected into mice subcutaneously. On day 7, 100 μg of 5-mG_{2a} and C_{44}Mab-46-mG_{2a} or control mouse IgG_{2a} (281-mG_{2a}) were injected into mice intraperitoneally. On days 14 and 21, additional antibodies were injected. The tumor volume was measured on days 7, 9, 14, 17, 21 and 23 following the inoculation. Values are presented as the mean ± SEM. There was no significant difference; two-way ANOVA and Sidak's multiple comparisons test. (B) The weight (left) and appearance (right) of the excised KYSE770 xenografts. The weight was measured on day 23. Values are presented as the mean ± SEM. Scale bar, 1 cm. There was no significant difference; one-way ANOVA with Tukey's multiple comparisons test. (C) The body weight (left) and appearance (right) of control mouse IgG_{2a}, 5-mG_{2a} and C_{44}Mab-46-mG_{2a}-treated mice. mAb, monoclonal antibody.

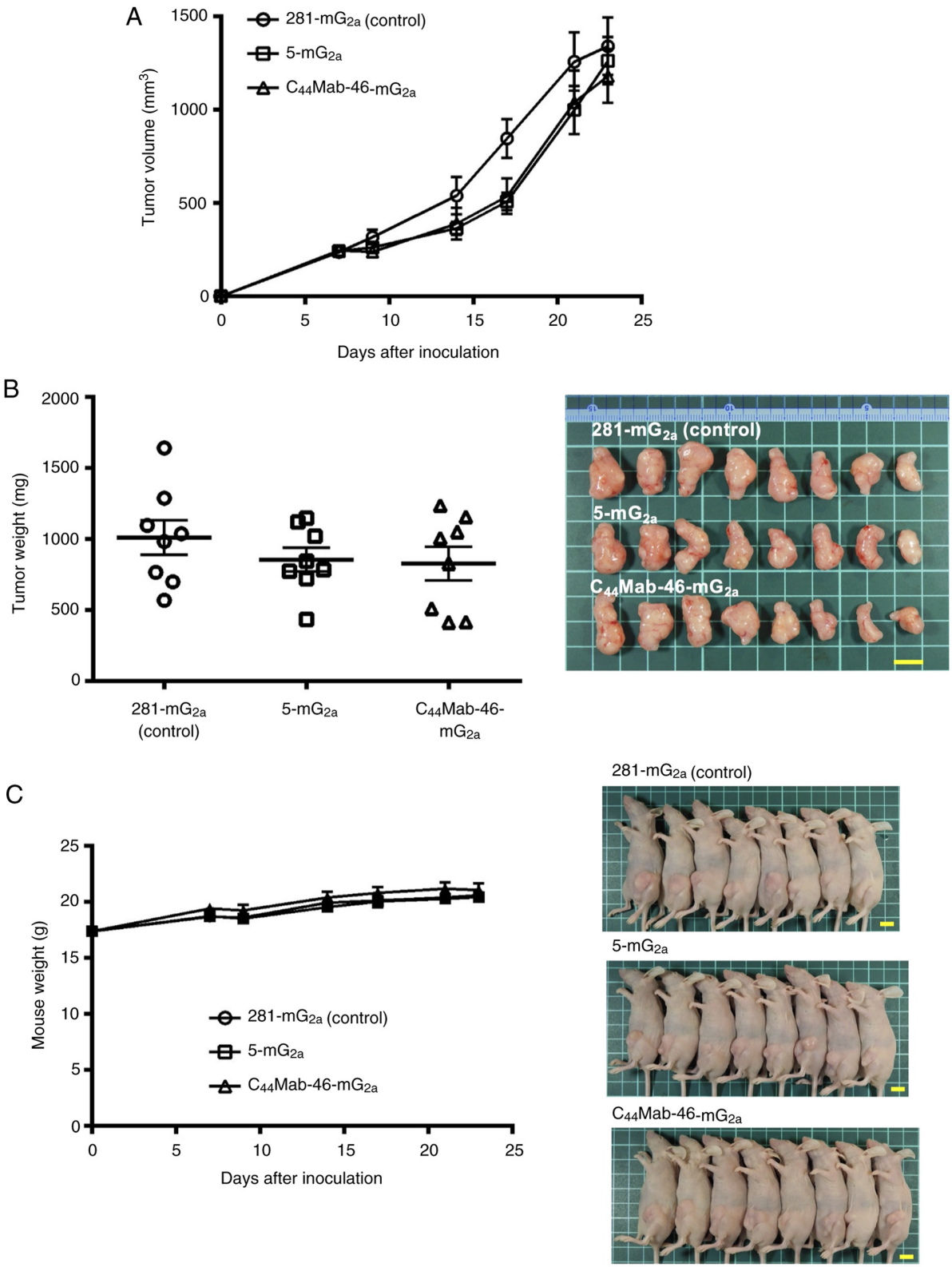

Figure S5. Histological analyses of 5-mG_{2a}, C_{44}Mab-46-mG_{2a}, or control mouse IgG_{2a} (281-mG_{2a})-treated KYSE770 xenograft tumors. (A) The sections were performed with H&E staining. (B-D) Immunohistochemistry using (B) C44Mab-46, (C) mPMab-1 (an anti-mouse podoplanin mAb) and (D) no 1st Ab control (no Ab), followed by treatment with the Envision+ kit. The chromogenic reaction was performed using DAB, and the sections were counterstained with hematoxylin. Scale bar, 100 μm. Arrowheads, podoplanin-positive macrophages. mAb, monoclonal antibody.