ACCSCIENCE PUBLISHING

Microbes & Immunity

COMMUNICATION

Establishment of a highly sensitive and specific anti-EphB2 monoclonal antibody (Eb_2Mab-12) for flow cytometry

Supplementary File

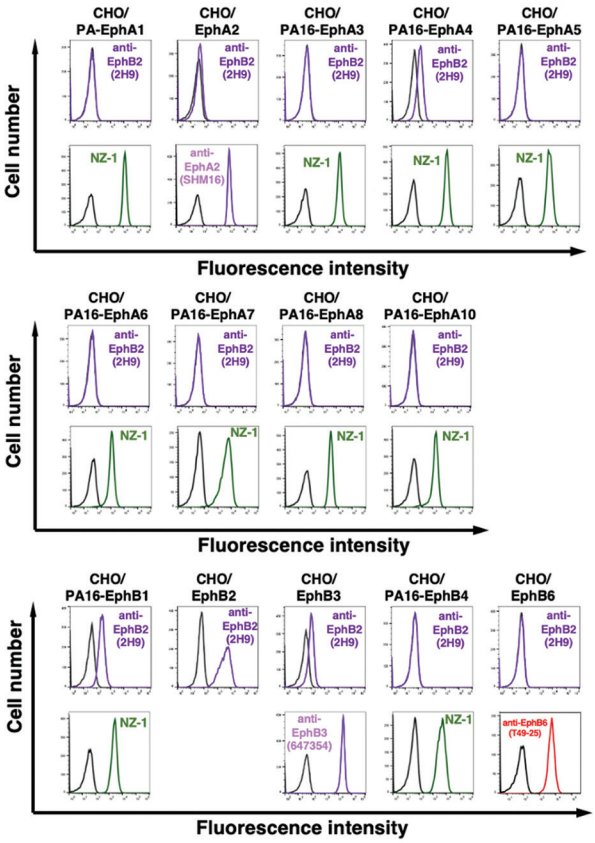

Figure S1. Flow cytometry of an anti-EphB2 mAb (clone 2H9) in Eph receptor-expressed CHO-K1 cells. The 14 Eph receptor-expressed CHO-K1 cells were treated with 1 μg/mL of 2H9 conjugated with RB545 (purple line) or control blocking buffer (black line). The cells were treated with corresponding mAbs, including an anti-EphA2 mAb (clone SHM16, 5 μg/mL), an anti-EphB3 mAb (clone 647354, 1 μg/mL), an anti-EphB6 mAb (clone T49-25, 0.5 μg/mL), or an anti-PA tag mAb (clone NZ-1, 1 μg/mL). Then, cells were treated with corresponding secondary Ab conjugated with Alexa Fluor 488. Fluorescence data were collected using the SA3800 Cell Analyzer.
Abbreviations: Ab: Antibody; mAbs: Monoclonal antibodies.

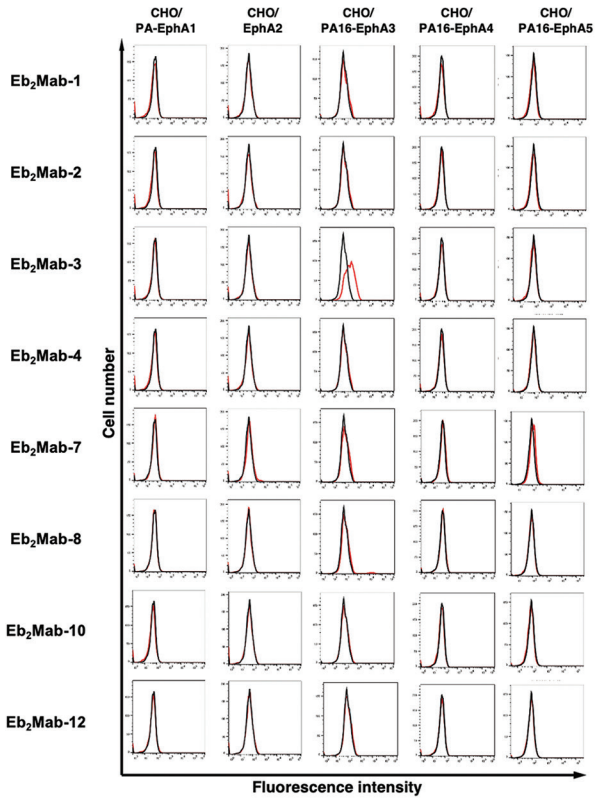

Figure S2. Flow cytometry of Eb_2Mabs (10 μg/mL) in EphA1, EphA2, EphA3, EphA4, and EphA5-expressed CHO-K1 cells. The cells were treated with anti-mouse IgG conjugated with Alexa Fluor 488. Fluorescence data were collected using the SA3800 Cell Analyzer.
Abbreviations: Ab: Antibody; CHO: Chinese hamster ovary; mAbs: Monoclonal antibodies.

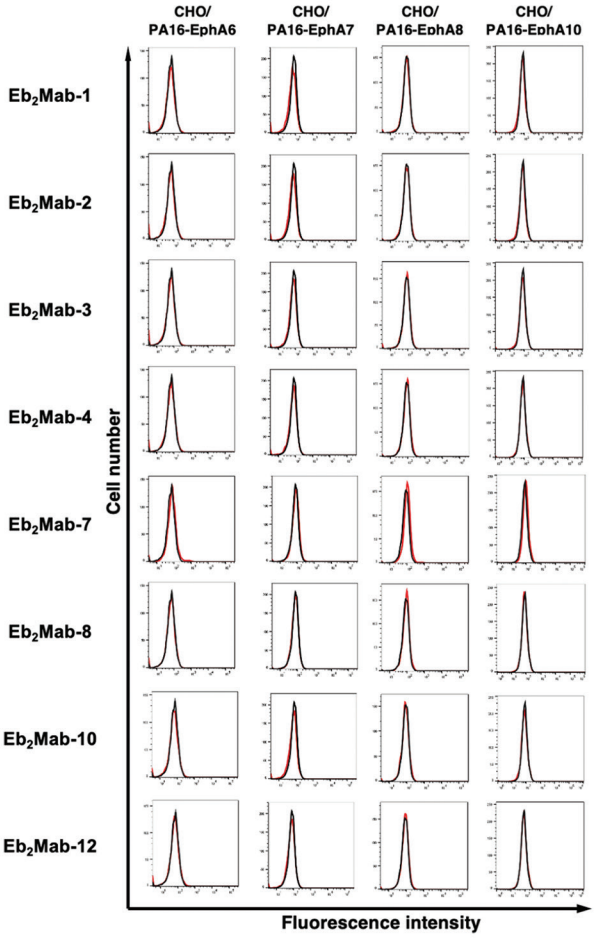

Figure S3. Flow cytometry of Eb_2Mabs (10 μg/mL) in EphA6, EphA7, EphA8, and EphA10-expressed CHO-K1 cells. The cells were treated with anti-mouse IgG conjugated with Alexa Fluor 488. Fluorescence data were collected using the SA3800 Cell Analyzer.
Abbreviations: Ab: Antibody; CHO: Chinese hamster ovary; mAbs: Monoclonal antibodies.

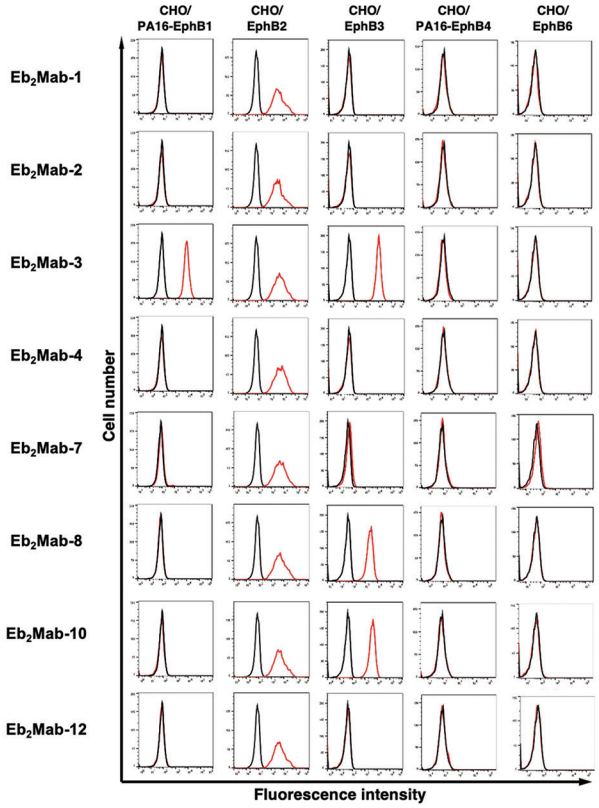

Figure S4. Flow cytometry of of Eb_2Mabs (10 μg/mL) in EphB1, EphB2, EphB3, EphB4, and EphB6-expressed CHO-K1 cells. The cells were treated with anti-mouse IgG conjugated with Alexa Fluor 488. Fluorescence data were collected using the SA3800 Cell Analyzer.
Abbreviations: Ab: Antibody; CHO: Chinese hamster ovary; mAbs: Monoclonal antibodies.

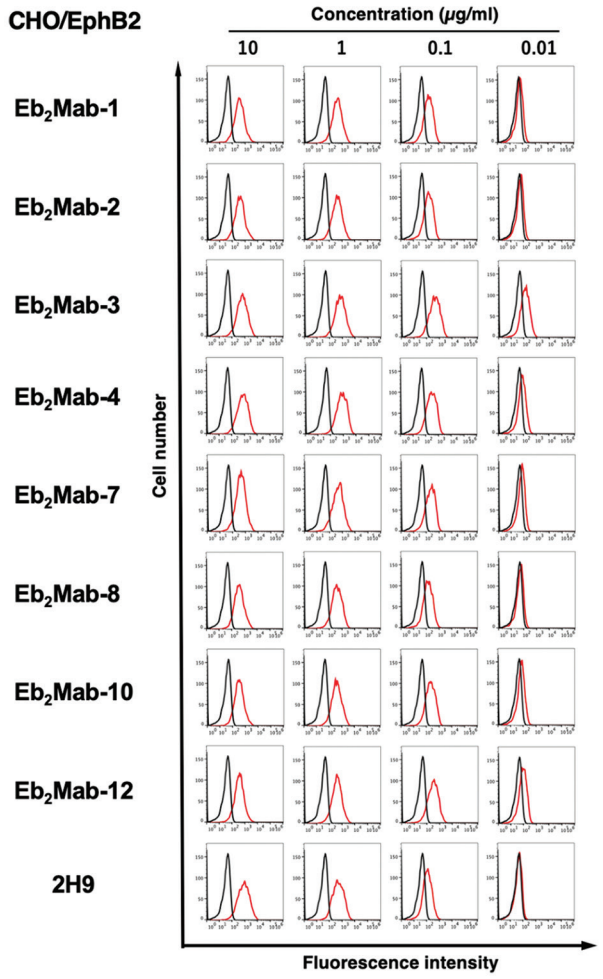

Figure S5. Flow cytometry of EphB2-expressed CHO-K1 cells using Eb_2Mabs and 2H9. CHO/EphB2 cells were treated with 0.01–10 μg/mL of Eb_2Mabs or 2H9 conjugated with RB545 (red line). The Eb_2Mabs-treated cells were incubated with anti-mouse IgG conjugated with Alexa Fluor 488. The fluorescence data were subsequently collected using the SA3800 Cell Analyzer. The black line represents the negative control (blocking buffer).

Abbreviations: CHO/EphB2: EphB2-overexpressed Chinese hamster ovary-K1 cells; CHO: Chinese hamster ovary; IgG: Immunoglobulin G; mAbs: Monoclonal antibodies.

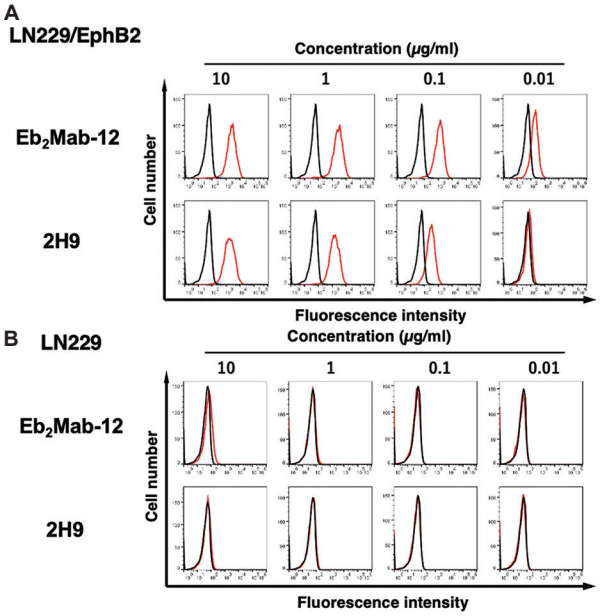

Figure S6. Flow cytometry of EphB2-expressed LN229 cells using Eb_2Mab-12 and 2H9. LN229/EphB2 (A) and LN229 (B) cells were treated with 0.01–10 μg/mL of Eb_2Mab-12 or 2H9 conjugated with RB545 (red line). The Eb_2Mab-12-treated cells were further incubated with anti-mouse IgG conjugated with Alexa Fluor 488. The fluorescence data were subsequently collected using the SA3800 Cell Analyzer. The black line represents the negative control (blocking buffer).

Abbreviations: IgG: Immunoglobulin G; mAbs: Monoclonal antibodies.

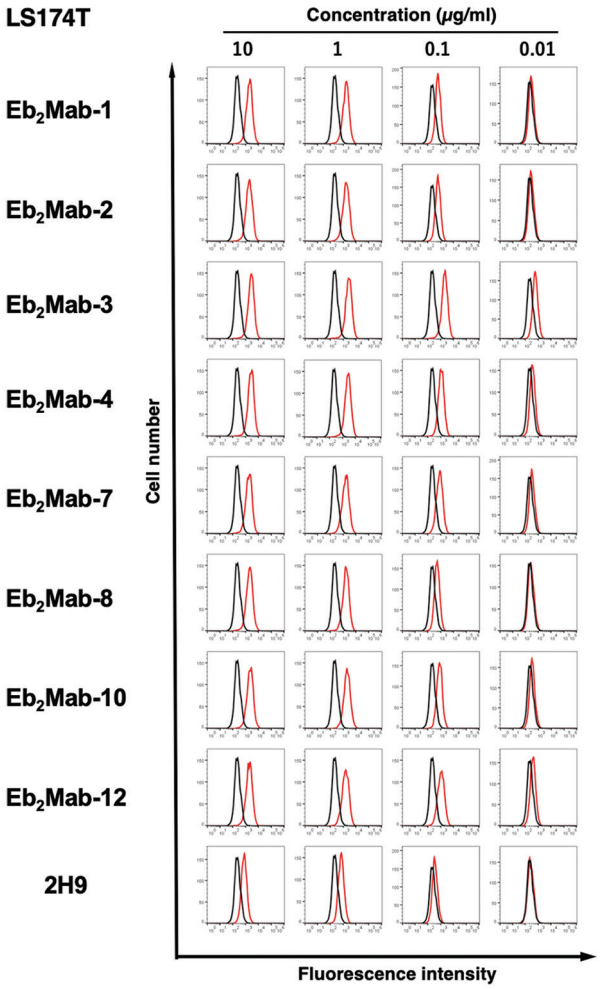

Figure S7. Flow cytometry of endogenous EphB2-expressing cells using Eb_2Mabs and 2H9. LS174T cells were treated with 0.01–10 μg/mL of Eb_2Mabs, or 2H9 conjugated with RB545 (red line). The Eb_2Mabs-treated cells were incubated with anti-mouse IgG conjugated with Alexa Fluor 488. The fluorescence data were subsequently collected using the SA3800 Cell Analyzer. The black line represents the negative control (blocking buffer).
Abbreviations: IgG: Immunoglobulin G; mAbs: Monoclonal antibodies.