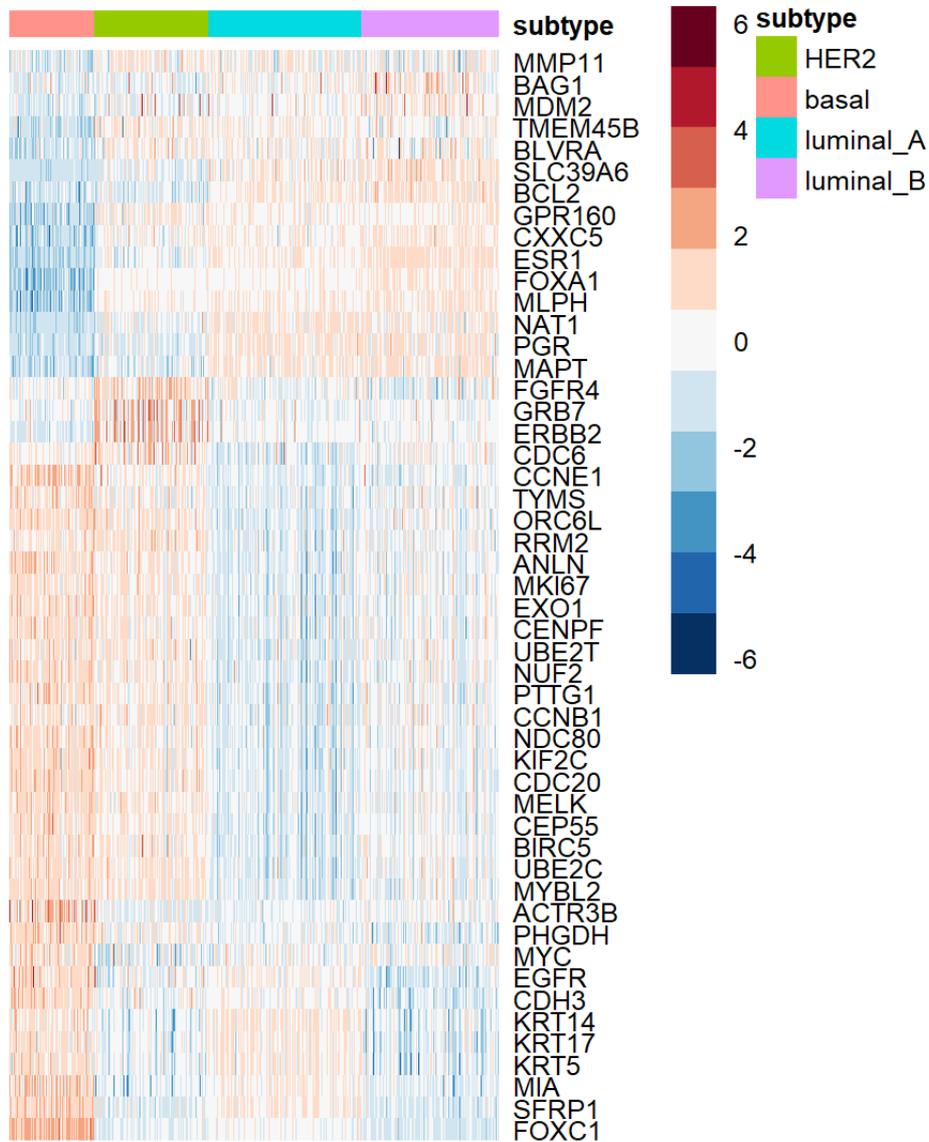
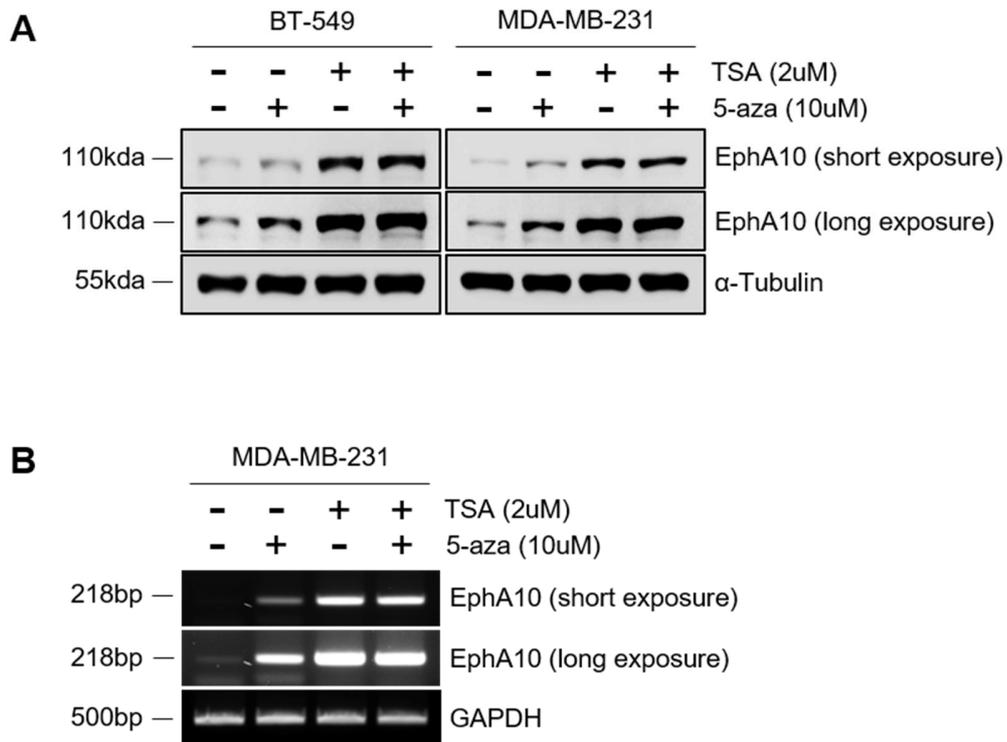


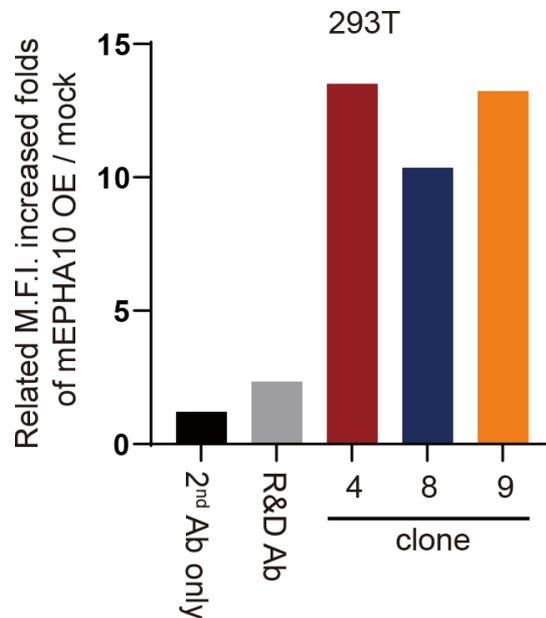
TCGA BRCA subtype by PAM50



Supplementary Figure 1. Characteristics of the TCGA BRCA subtype. The TCGA BRCA tumor tissues used in Figure 1A were subtyped according to the expression of several markers. The “basal” group is the PAM 50 definition and is well-correlated to TNBC.

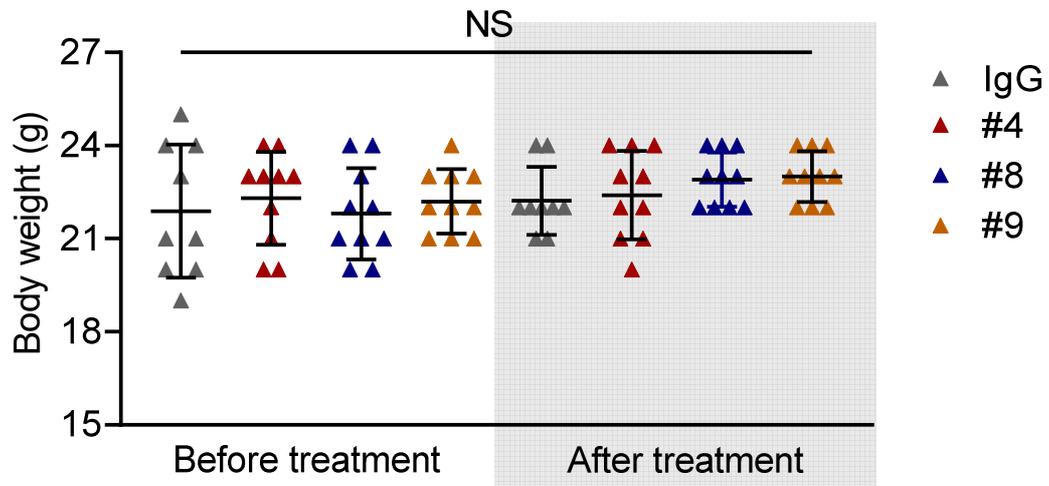


Supplementary Figure 2. EphA10 expression could be regulated epigenetically. A, EphA10 protein and **B**, mRNA levels are highly increased by treatment with epigenetic drugs such as DNA methyltransferase inhibitors 5-azacytidine (5-aza) and HDAC inhibitor TSA.

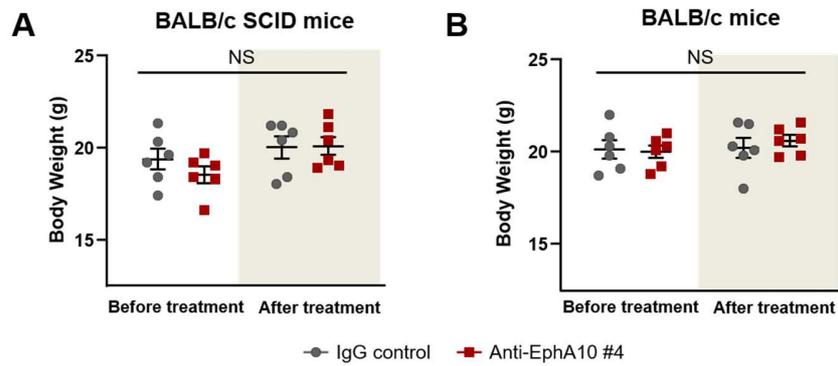


Supplementary Figure 3. Anti-EphA10 Abs of clone 4, 8, and 9 can binds with mouse EphA10.

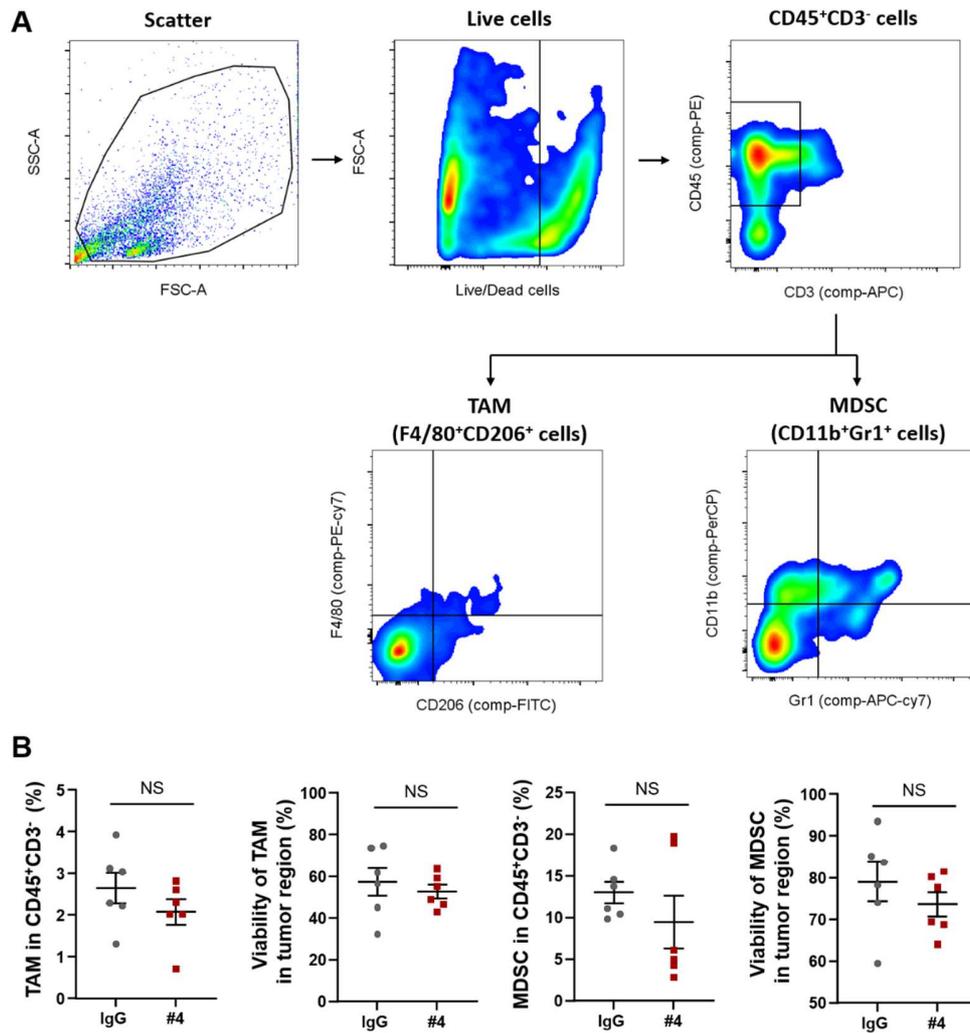
Quantification of flow cytometry analysis measuring fluorescence intensity of the indicated antibodies in 293T cells expressing mouse EphA10 or mock control. 2nd Ab serves as a negative control and commercial anti-EphA10 ab (R&D system, MAB5188) serves as a positive control. MFI, mean fluorescence intensity.



Supplementary Figure 4. Body weight of the mice did not change significantly during the course of treatment. Measurement of body weight from Figure 3A, before and after treatment with the indicated antibodies. Gray box indicates the body weight at the end point at day 21. Error bar, mean \pm SD. One-way ANOVA. NS, not significant.

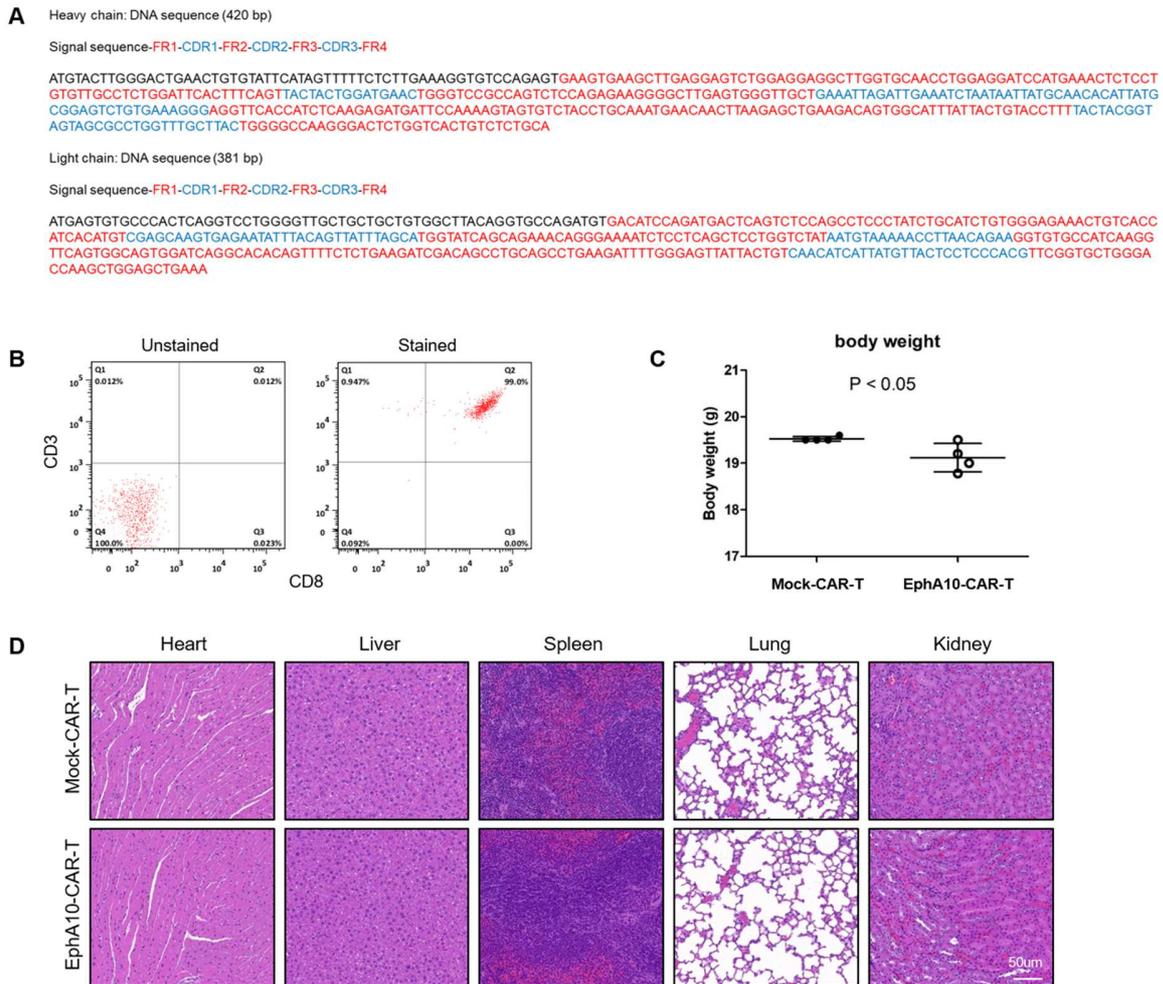


Supplementary Figure 5. Body weight of BALB/c-SCID (A) and BALB/c (B) mice bearing orthotopic injected 4T1 tumor in Fig. 4 A and B. Mice (n = 6 per group) were treated with anti-EphA10 mAb (clone #4) or IgG control. Measurement of body weight before and after treatment with the indicated antibodies. Gray box indicates the body weight at the end point. Error bar represent mean \pm SEM. Data were analyzed by two-way ANOVA analysis. NS, not significant, and ***p < 0.001.

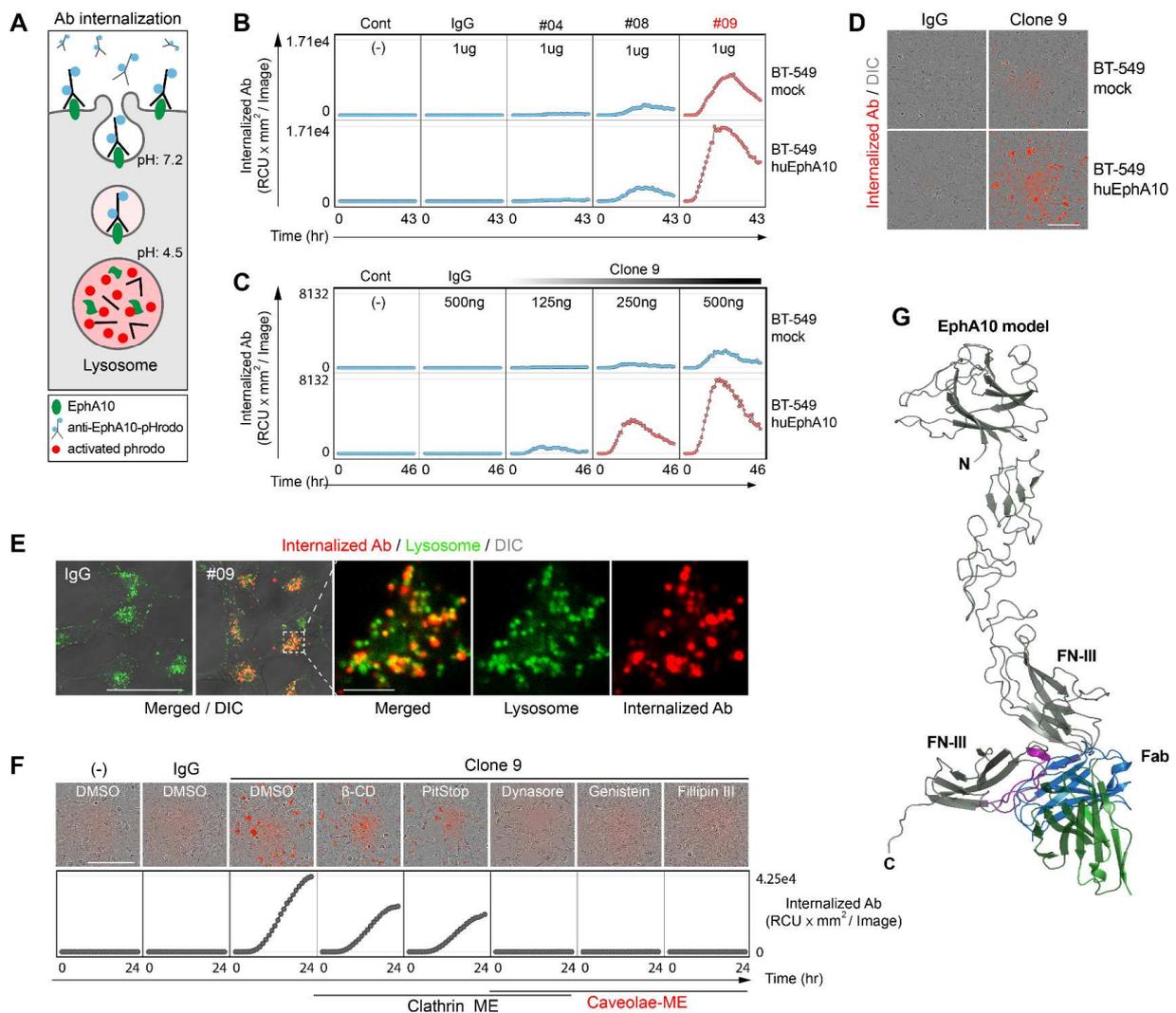


Supplementary Figure 6. Analysis of TAM and MDSC in 4T1 syngeneic mouse model.

(A) The gating strategy used for flow cytometric analysis of (C). Cells were analyzed by forward scatter (FSC)/side scatter (SSC) parameters and Zombie Violet⁺ staining to determine total live events, in which various cell populations were identified on the basis of marker expression: CD45⁺ (immune cell population), CD3⁻/F4/80⁺/CD206⁺ (TAM cells), and CD3⁻/CD11b⁺/Gr1⁺ (MDSC cells). (B) Flow cytometry analysis of the percentage of F4/80⁺/CD206⁺ TAM in CD45⁺CD3⁻ cells, the viability of F4/80⁺/CD206⁺ TAM, the percentage of CD11b⁺/Gr1⁺ MDSC in CD45⁺CD3⁻ cells and the viability of CD11b⁺/Gr1⁺ MDSC in 4T1 tumor region under IgG control or anti-EphA10 #4 antibody treatment (300 μg/mouse). n = 6 mice per group.



Supplementary Figure 7. Additional results of xenograft mouse model for EphA10-specific CAR-T cells and the DNA sequence of clone #4 scFv. A, The DNA sequence of clone #4 scFv. **B,** Flow cytometry analysis of CD8⁺ T cell population after positive selection from healthy volunteers. **C,** The end-point body weights of the mice treated with mock-transduced T cells or EphA10 CAR-T cells. The body weights of mice which treated with mock transduced T cells were 19.53 ± 0.05 gram, whereas the body weights of mice which treated with EphA10 CAR-T cells were 19.12 ± 0.30 gram. **D,** Pathological analysis of important organs of the mice by hematoxylin and eosin (HE) stain. The organs were resected and preserved in 4% paraformaldehyde at 4°C, and then paraffin embedded sections of tissue were stained.



Supplementary Figure 8. Anti-EphA10 antibody clone #9 induces EphA10 internalization.

A, Schematic of antibody internalization assay. Anti-EphA10 antibodies were labeled with a pH-sensitive fluorophore pHrodo Red for monitoring a dynamic internalization of antibodies into the lysosome. **B**, antibody internalization assay of each anti-EphA10 clones in BT-549 cells expressing huEphA10 or mock control at each concentration and time point. **C**, antibody internalization assay of anti-EphA10 clone #9 at serial concentrations. **D**, representative phase and red fluorescent merged images at 500 ng and 24 h are shown. Scale bar, 200 μ m. **E**, Internalization of anti-EphA10 clone #9 and its co-localization in the lysosome. Antibodies were labeled with pHrodo Red and

then added to huEphA10-expressing BT-549 cells (500 ng, 24 h) with LysoTracker Green (75 nM, 16 h). The yellow merged signals indicate the localization of anti-EphA10 clone #9 in the lysosome after internalization. Scale bar, 50 μm (left), 5 μm (right). **F**, Antibody internalization assay of anti-EphA10 clone #9 in huEphA10-expressing BT-549 cells treated with various inhibitors of clathrin-mediated endocytosis (clathrin-ME) or caveolae-mediated endocytosis (caveolae-ME) as indicated, including β -cyclodextrin (Sigma, #C4555), PitStop (Sigma, #SML1169), Dynasore (Sigma, #D7693), Genistain (Sigma, #G6649), and Filipin III (Sigma, #F4767). Scale bar, 200 μm . **G**, Computational prediction of EphA10 antigen regions recognized by anti-EphA10 clone #9. Figure was prepared by using PyMOL, an open source molecular visualization system. The magenta color highlights where the epitope may be located based on the top1 docking result. Blue- and green-colored areas are the variable regions of the heavy and light chain, respectively, of anti-EphA10 clone #9. FN-III, fibronectin III domains.

Supplementary Table 1. Characterization and potential applications of anti-EphA10 monoclonal antibodies.

	Specificity	Internalization	IHC ^a	Therapeutic Ab	CAR-T ^a	ADC ^{a,b}
#4	O	X	X	++	O	X
#8	O	X	O	+	O	X
#9	O	O	X	+	O	O

^aAbbreviations: IHC, immunohistochemistry; CAR-T, chimeric antigen receptor T cells; ADC, antibody-drug conjugates. ^bA potential application in this study.