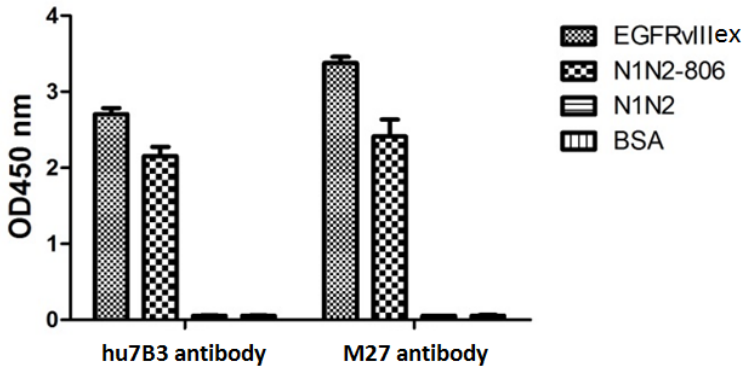
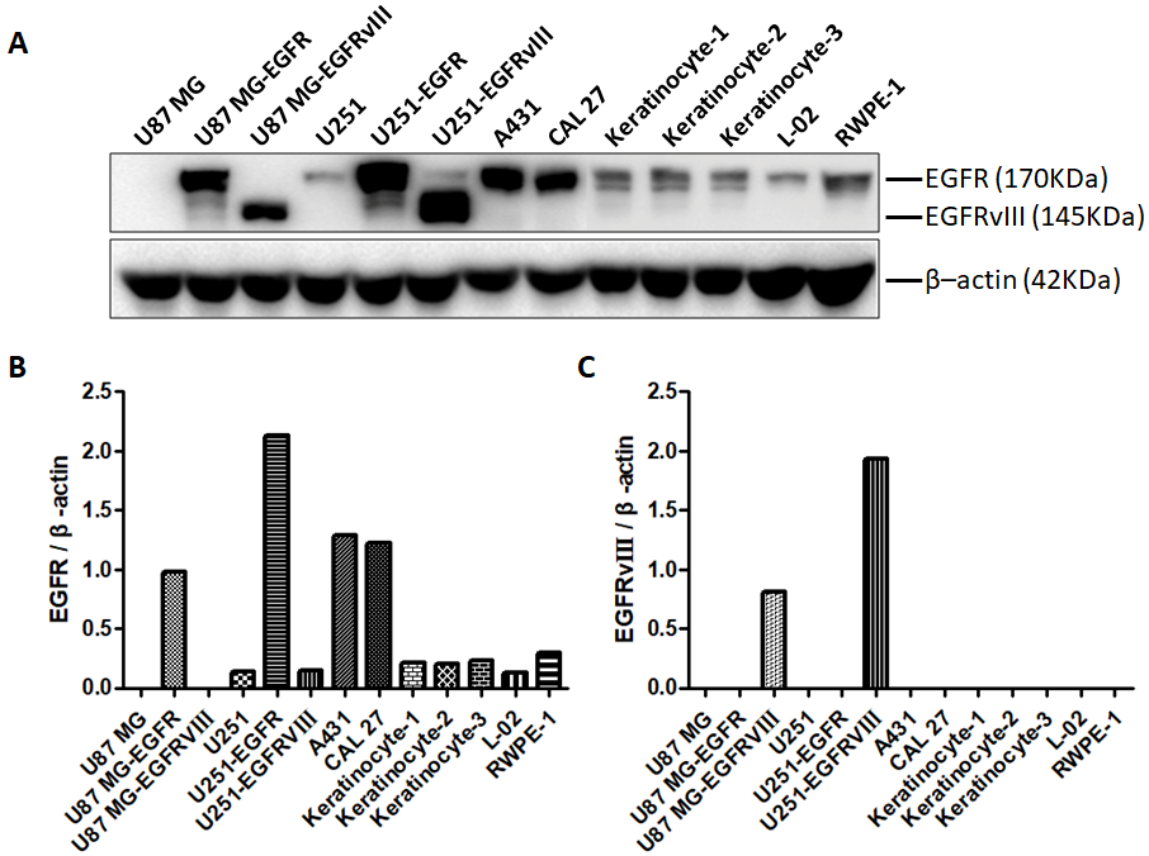
Supplementary Figures and legends

Supplementary Figure S1



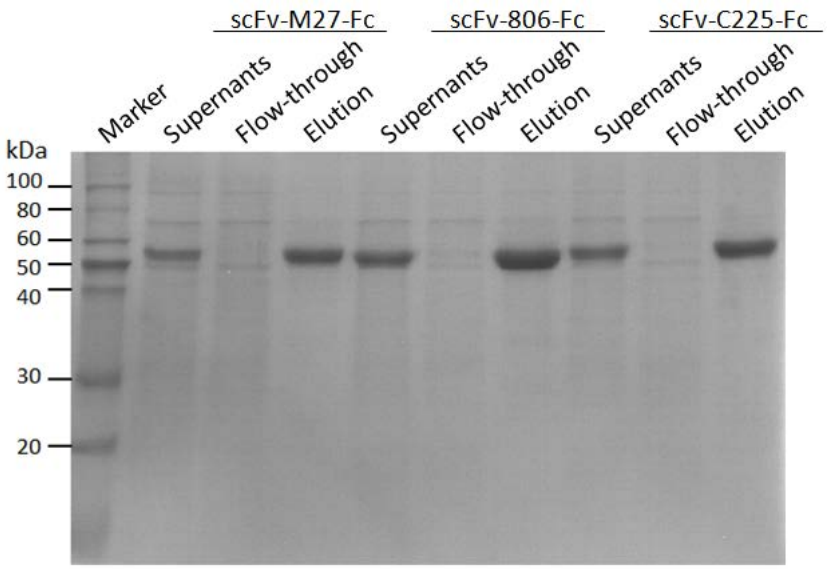
Supplementary Figure S1. Determination the binding epitope of hu7B3 and M27 antibodies by monoclonal phage ELISA. EGFRvIIIex was a recombinant extracellular portion of EGFRvIII which was expressed in *E.Coli*. N1N2-806 was a fusion protein in which $EGFR^{287-302}$ epitope was fused to the N1N2 domain of pIII protein from phage. BSA (bovine serum albumin) and N1N2 were set as a negative antigen control. All recombinant proteins were purified by an NTA-Ni affinity column (GE Healthcare Bio-Sciences). EGFR-derived recombinant proteins were coated on an ELISA plate. hu7B3 and M27 antibodies were used as primary antibody.

Supplementary Figure S2



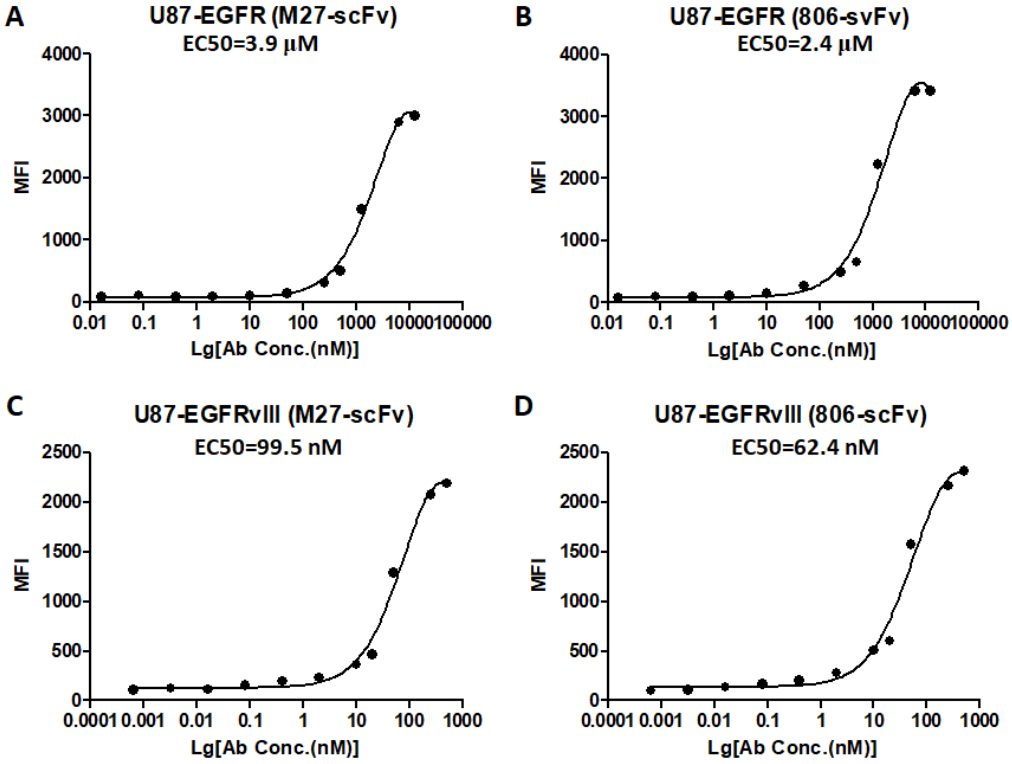
Supplementary Figure S2. The Western blot analysis of EGFR or EGFRvIII expression in different cell lines. (A) EGFR or EGFRvIII expression in indicated cells was detected by Western blot analysis with an antibody against EGFR (sc-03, Santa Cruz, CA) detected full-length EGFR (approximately 170 kDa) and the truncated EGFRvIII (approximately 145 kDa). β-actin served as loading control. (B) EGFR expression was quantified and normalized to β-actin. (C) EGFRvIII expression was quantified and normalized to β-actin.

Supplementary Figure S3



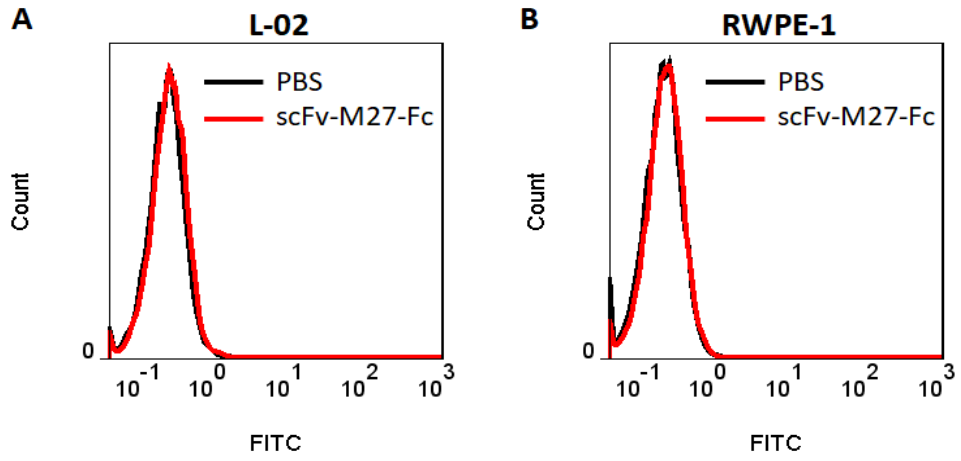
Supplementary Figure S3. Expression and purification of scFv-M27-Fc, scFv-806-Fc and scFv-C225-Fc. ScFv-Fc proteins were transiently expressed in FreeStyle™ 293F cells. After the cells were cultured for 6-7 days, proteins in cell culture supernatant were purified by protein A affinity chromatography. The purified proteins were analyzed by SDS-PAGE.

Supplementary Figure S4



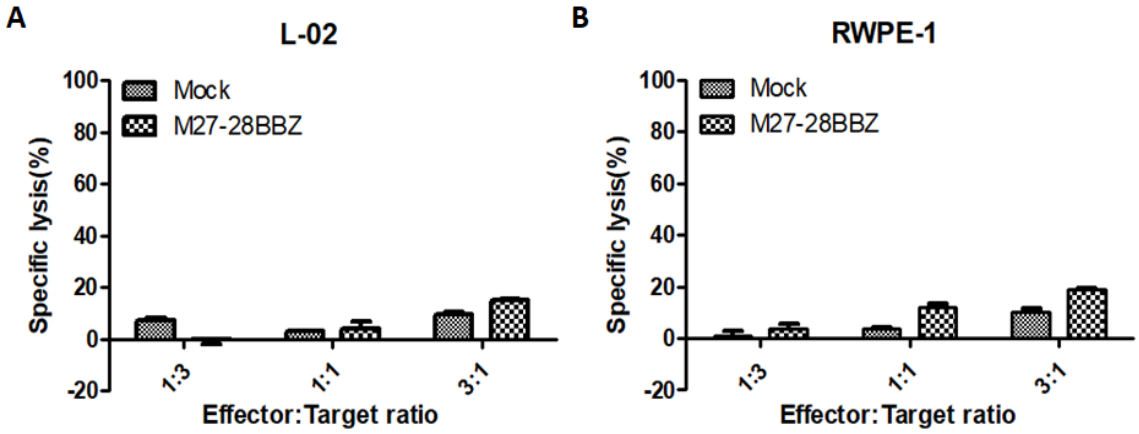
Supplementary Figure S4. EC50 test of M27 and 806 scFv binding to human tumor cell lines. (A and B) The binding of M27 and 806 scFv to U87MG-EGFR was assessed by FACS analysis. (C and D) The binding of M27 and 806 scFv to U87MG-EGFRvIII was tested by FACS analysis. The cells were incubated with series dilution of both antibodies. The EC50 were calculated by the GraphPad Prism 5.0 software.

Supplementary Figure S5



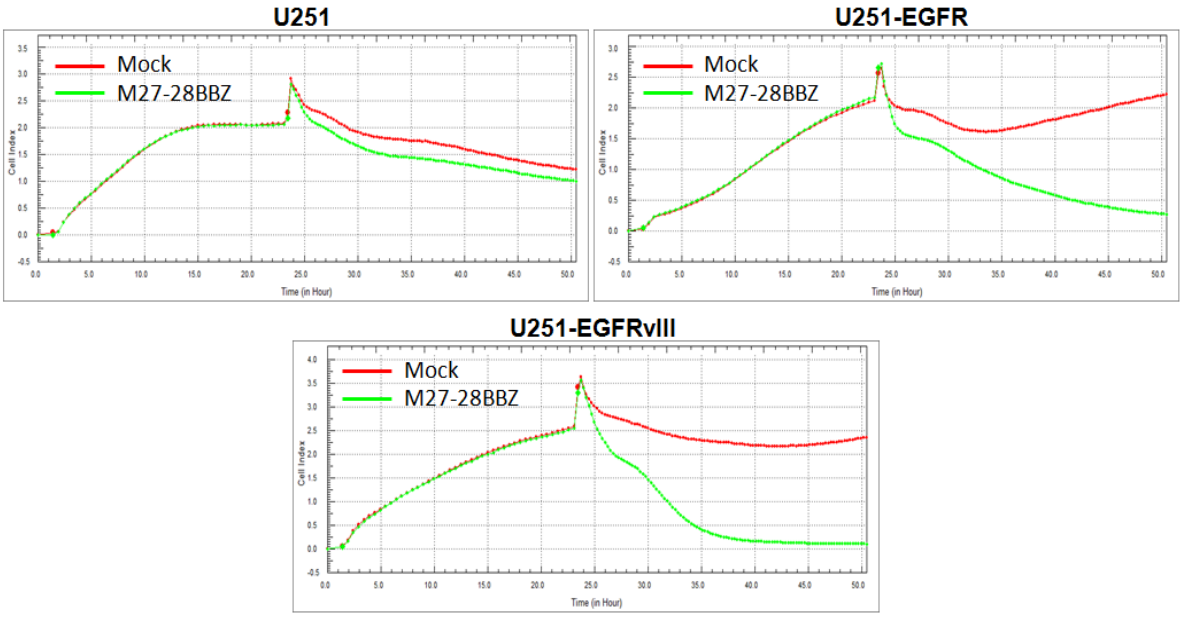
Supplementary Figure S5. FACS analysis of scFv-M27-Fc binding to the indicated target cells. To test the binding of scFv-M27-Fc to human hepatocyte cell L-02 (A) and human prostate epithelial cell RWPE-1 (B), 1×10^{6} cells were incubated with scFv-M27-Fc proteins for 45 min at 4°C. After wash with FACS buffer, the cells were stained with an FITC-conjugated goat anti-human antibody for 45 min at 4°C. Fluorescence was determined by using a BD FACSCelesta flow cytometer.

Supplementary Figure S6



Supplementary Figure S6. *In vitro* cytotoxic activities of M27-28BBZ CAR-T in the presence of L-02 and RWPE-1 cells. Primary human T-cells transduced with the indicated lentiviral vectors were incubated with L-02 (A) or RWPE-1 (B) cell lines at the varying effector:target ratios for 18 hours, respectively. Cell lysis was determined by using a standard nonradioactive cytotoxic assay. Each data point reflects the mean ± SEM of triplicates.

Supplementary Figure S7



Supplementary Figure S7. Dynamic monitoring of M27-28BBZ CAR-T-cells mediated cytotoxicity. Continuous graphical output of cell index values up to the 72h from different target cancer cells during incubation with M27-28BBZ CAR-T-cells by using the xCELLigence impedance system. Target cancer cells were seeded in electrodecoated 16-well plates (e-plates) in triplicates. After 24-hour target cell recovery, T cells were added at 1:1 ratio of effector:target. Electrical impedance was recorded continuously as an indicator of U251 density.