Supporting Information

Plasmon-enhanced biosensing for multiplexed profiling of extracellular vesicles

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Supporting experimental section

Western blotting
Cells and EVs were lysed in RIPA buffer (Cell Signaling Technology) supplemented with protease inhibitor cocktail (Roche). Western blotting analysis was conducted as previously described (Cell Report 2 8, 3105). After transfer onto nitrocellulose membrane, blots were probed with anti-EGFR (1:1000 dilution, Cell Signaling Technology), anti-GAPDH (1:2000 dilution, Cell Signaling Technology), anti-CD9 (1:500 dilution, BD Biosciences), anti-CD63 (1:500 dilution, Ancell), and anti-CD81 (1:500 dilution, Santa Cruz Biotechnology).

Supporting Figures
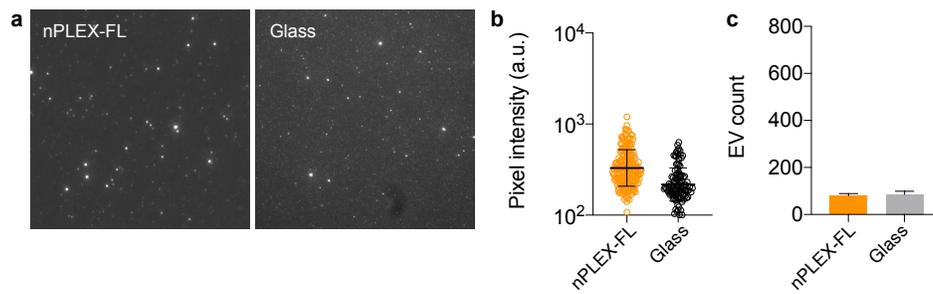
Figure S1. a) Biotinylated EVs were captured on glass and nPLEX-FL substrates coated with bioadhesive. The captured EVs were labeled with AF488-conjugated streptavidin, and then imaged. b) Comparison of the mean fluorescence intensities and the number of detected EVs on the glass and nPLEX-FL substrates.
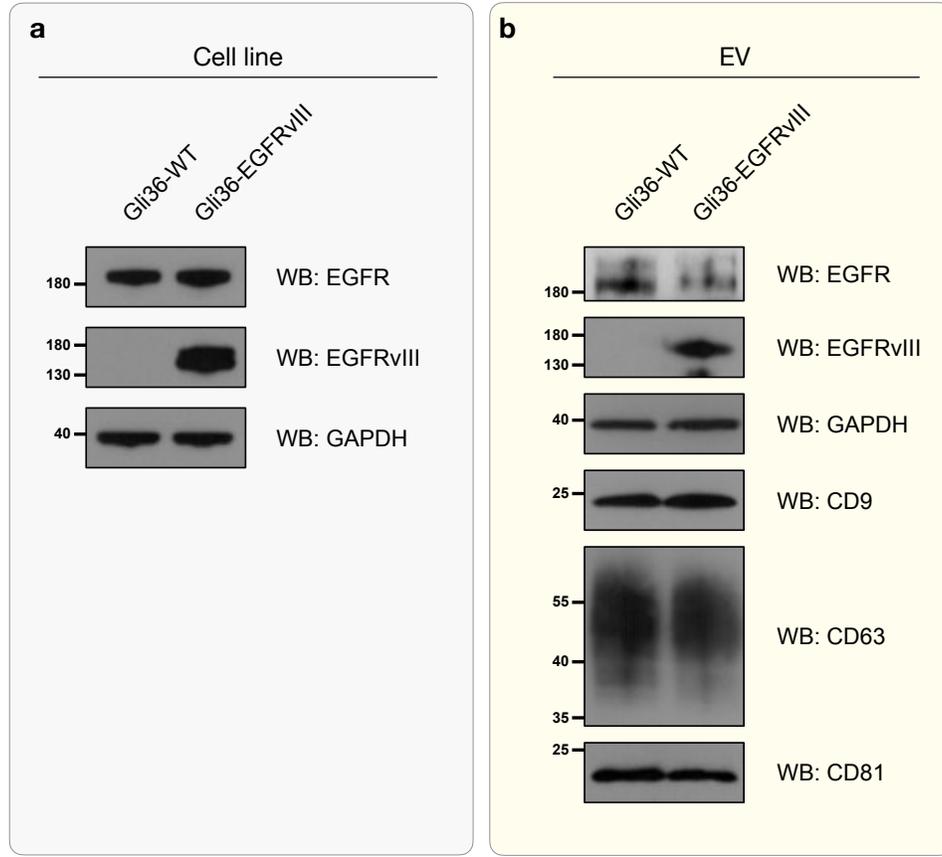
Figure S2. a-b) Western blot analysis of various marker expression in the Gli36-WT and Gli36-EGFRvIII cells (a) and EVs (b). Comparable EGFR and EGFRvIII expression was observed in both cells and EVs. Strong tetraspanin expression (CD9, CD63, CD81) was observed in EVs derived from the Gli36-WT and Gli36-EGFRvIII cell lines. Blotting antibody against GAPDH was used for loading control.
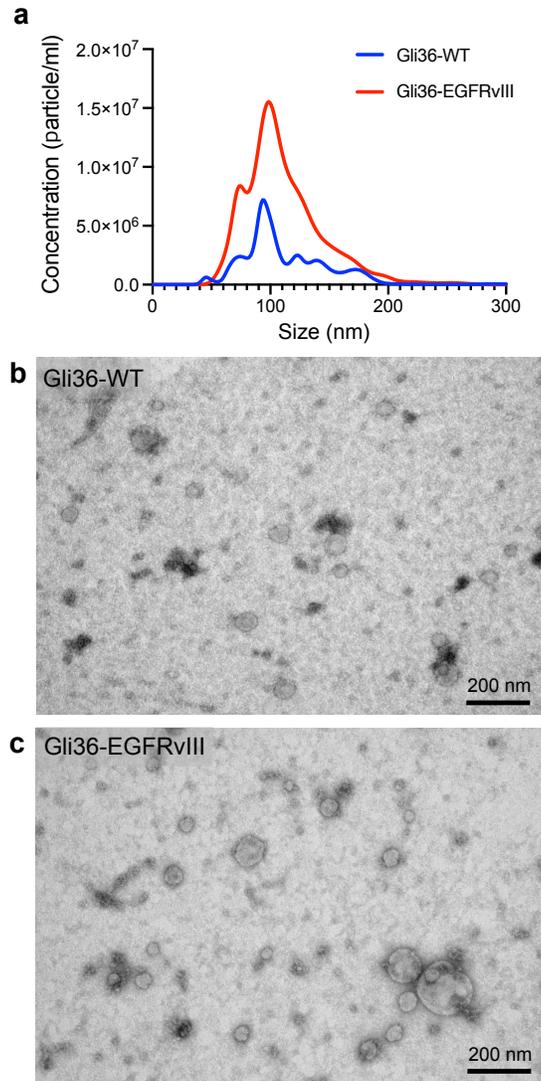
Figure S3. Characterization of EVs isolated from Gli36-WT and Gli36-EGFRvIII. a) Size distribution of EVs from Gli36-WT and Gli36-EGFRvIII cell lines obtained by nanoparticle tracking analysis (NTA). b-c) Transmission electron micrographs of Gli36-WT (b) and Gli36-EGFRvIII EVs (c).
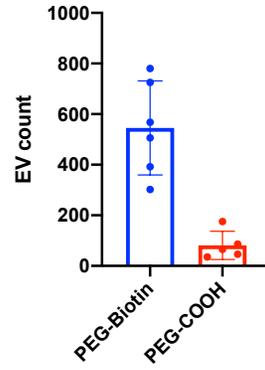
Figure S4. Capture efficiency of biotinylated EVs were tested on PEG-biotin and PEG-COOH modified nPLEX-FL chip. Note that Neutravidin was coated on PEG-biotin modified surface. Biotinylated and non-biotinylated EVs were labeled with streptavidin-AF488.
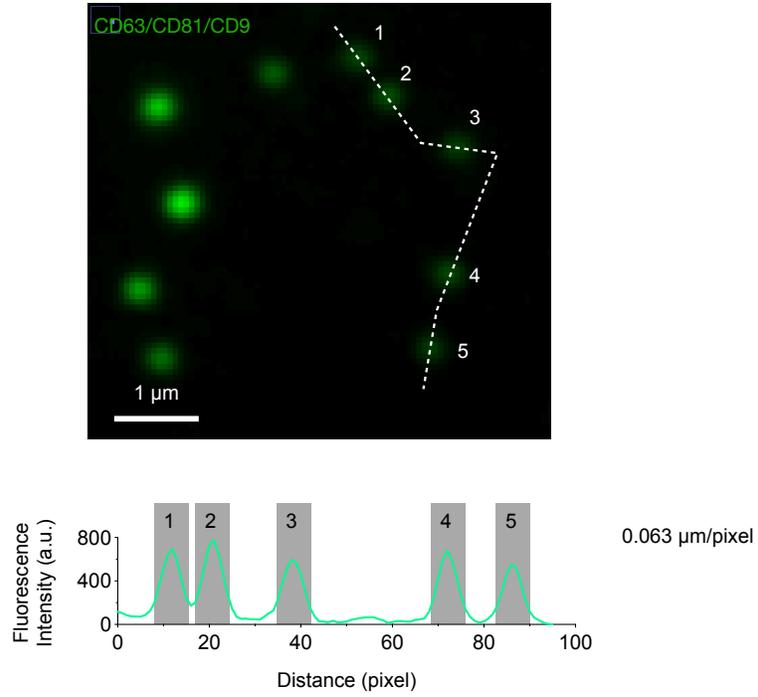
Figure S5. (Top) EVs labeled with fluorescent antibodies against transmembrane EV markers (CD9/CD63/CD81). (Bottom) Line scan showing size of for the chosen vesicles in the image. Gray shading highlights EV positions. Pixel size: 63 nm.
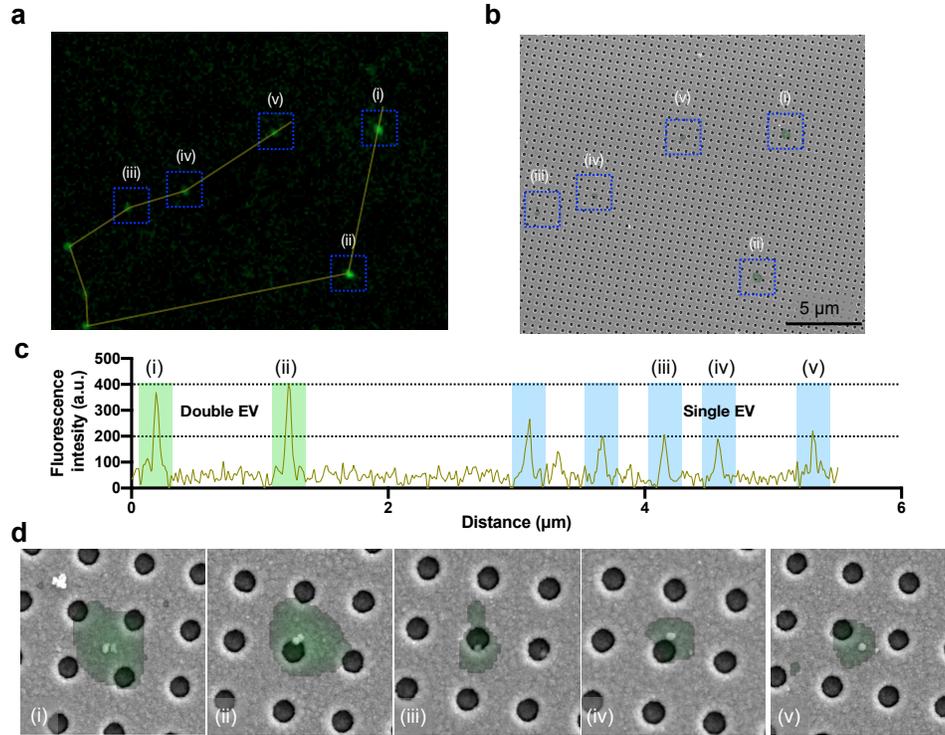
Figure S6. Correlative analysis of single EVs with scanning electron micrography (SEM) and fluorescence images. a-b) a fluorescence image (a) overlaid with a SEM (b). A sample used in nPLEX-FL measurements was imaged by SEM. c) Line profile of fluorescence intensity of five EVs detected. Doublet EVs showed higher fluorescence intensity. d) Zoomed-in images of five EVs marked by blue dashed boxes in (a).
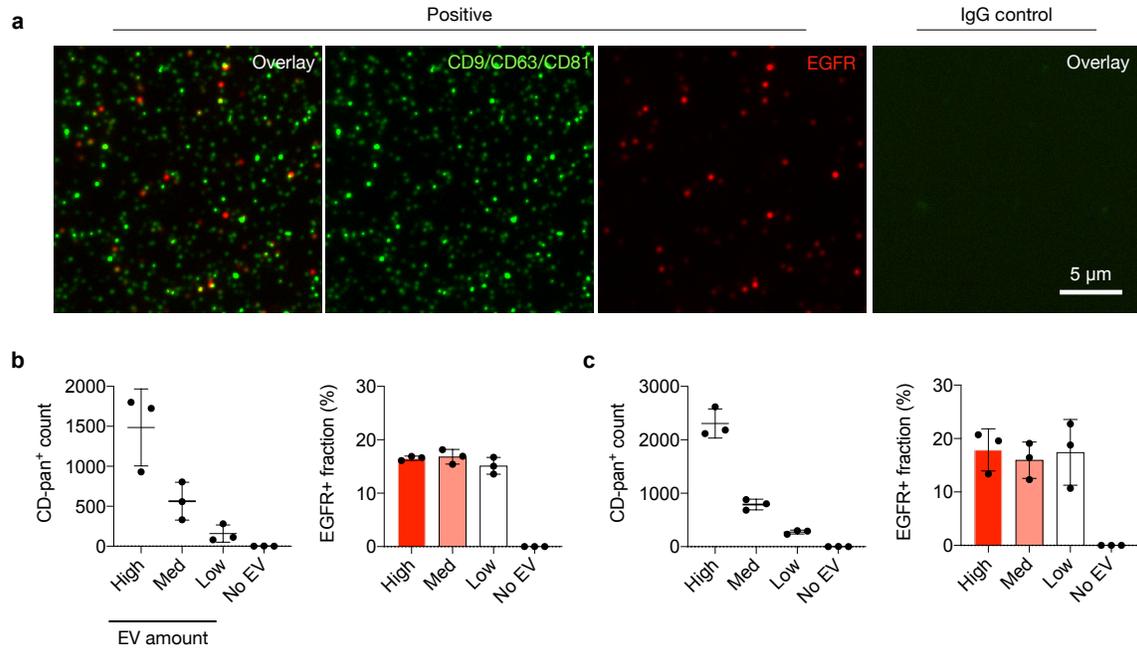
Figure S7. (a) EVs from the GBM cell line were biotinylated and captured on the device. EVs were labeled against the EV tetraspanin marker—combination of CD9, CD63, and CD81—(AF488) and EGFR (Cy5). EVs were artificially color-coded for visual aid. (b-c) We varied EV concentrations (high, med, low represent 1-, 4-, 10-fold diluted) and analyzed for EVs from Gli36-WT (b) and Gli36-EGFRvIII (c) cell lines. Regardless of the EV concentrations, roughly 15-20% the CD-pan+ EVs expressed EGFR.
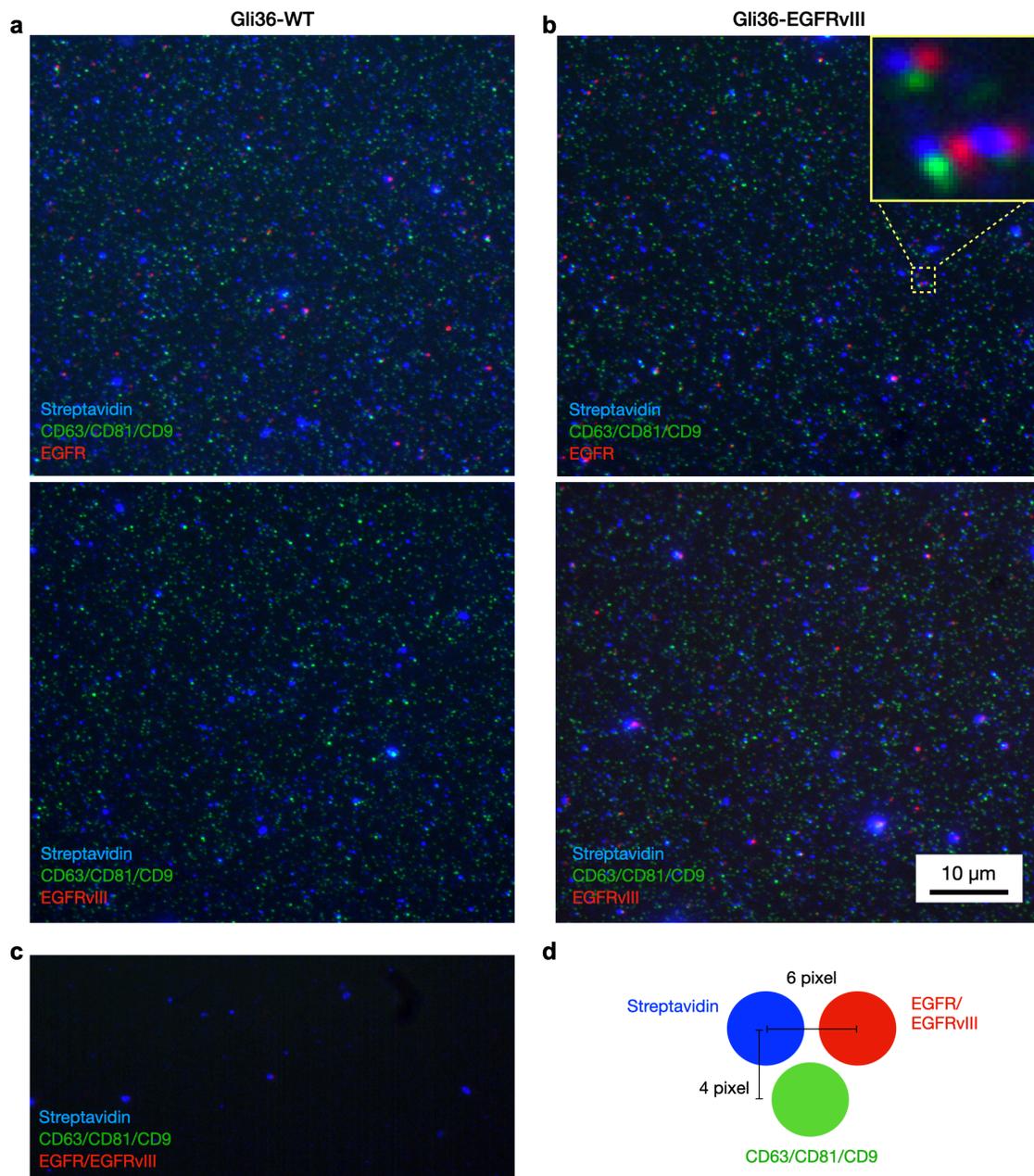
Figure S8. Representative images with a large field of view (120 μm × 100 μm). a-b) Biotinylated EVs from the Gil36-WT (a) and Gil36-EGFRvIII (b) cell lines were captured on the device and labeled against streptavidin-Cy3 (blue), CD-pan markers (CD9/CD63/CD81, green) and target cancer makers (EGFR or EGFRvIII, red): EGFR in the top row and EGFRvIII in the bottom row. c) The negative control was prepared with the same procedure with no EV incubation. d) The three channel images were overlaid with pixel shifts for better visualization: green channel shifted by 3 pixels in the *x*-direction and 4 pixels in the *y*-direction; red channel shifted by 6 pixels in the *x*-direction.

a

b

c

d
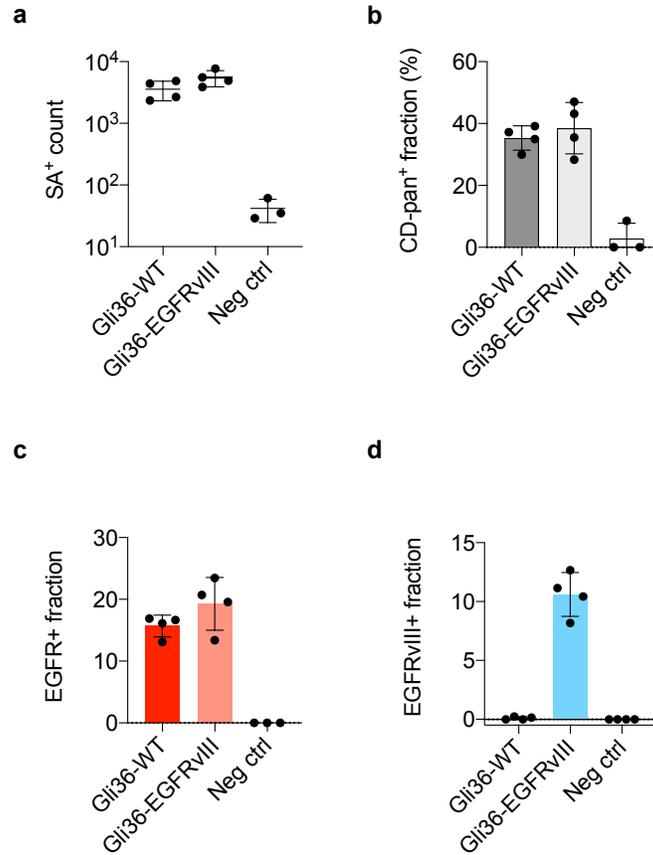
Figure S9. EV detection and marker profiling. EVs from two different cell lines (Gil36-WT, Gli36-EGFRvIII) in PBS solutions were analyzed by the analytic algorithm described in Figure 4A. The negative control was prepared with the same procedure with no EV incubation.
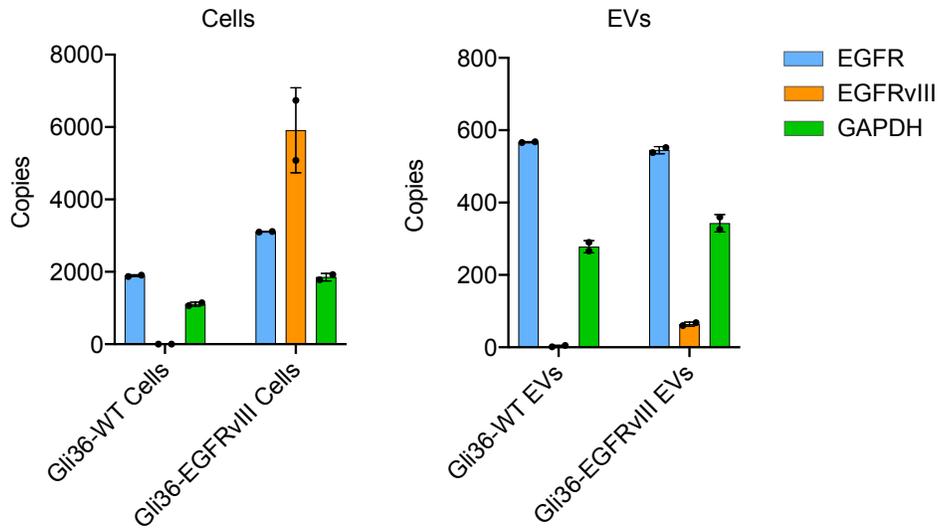
Figure S10. Droplet digital PCR measurements for cellular and EV RNA measurements. RNAs from Gli36-WT and Gli36-EGFRvIII cells and their EVs were extracted with RNeasy and exoRNease kits (QIAGEN), respectively. 10 ng input was used for each sample. Measurements were done in duplicate.

Table S1: Antibodies list and dilution factors

Antibody	Vendor	Cat No.	Dilution factor for nPLEX-FL	Dilution factor for WB
Primary antibodies				
CD9 (mouse)	BD Biosciences	312102	1:200	1:500
CD63 (mouse)	Ancell	215-820	1:200	1:500
CD81 (mouse)	Santa Cruz	sc-166029	1:100	1:500
EGFR (rabbit)	CST	54359S	1:50	1:1000
EGFRvIII (rabbit)	CST	64952S	1:1600	1:1000
GAPDH (rabbit)	CST	2118S	1:100	1:2000
Secondary Antibody				
Alexa 488 Goat anti-mouse IgG	CST	4408S	1:1000	
Alexa 555 Goat anti-mouse IgG	CST	4409S	1:1000	
Alexa 647 Goat anti-rabbit IgG	CST	4414S	1:1000	
HRP Goat anti-rabbit IgG Antibody	CST	7074S		1:3000
HRP Goat anti-mouse IgG Antibody	CST	7076S		1:3000
Streptavidin (SA)				
SA Dy405	Thermofisher	21831	1:400-1000	
SA Alexa 488	BioLegend	405235	1:400-1000	
SA cy3	BioLegend	405215	1:400-1000	
SA cy5	BioLegend	405209	1:400-1000	
SA cy5.5	Rockland	S000-13	1:400-1000	