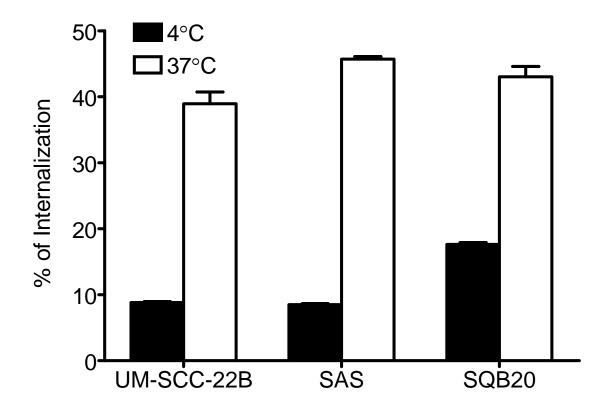


SUPPLEMENTAL FIGURE 1. Schematics of panitumumab localization in HNSCC tumors. In SQB20 tumor (A), low vascular density and permeability caused hindrance for extravasculation of panitumumab. High EGFR expression on tumor cells depleted antibodies and further weakened the antibody penetration. To the contrary, in UM-SCC-22B tumor (B), high vascular density and permeability facilitated antibody diffusion from circulation. Relatively low EGFR expression on tumor cells induced more homogeneous distribution of antibodies.



SUPPLEMENTAL FIGURE 2. Panitumumab internalization in HNSCC cells. HNSCC cells were harvested and washed with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA). Upon blockade by 2% BSA in PBS, the cells were incubated with panitumumab (10 µg/mL in PBS containing 2% BSA). FITC-conjugated donkey anti-human IgG (1:200) was then added and allowed to incubate for 120 min at 4°C or 37°C. Then the cells were washed twice with cold 0.2 M glycine buffer containing 0.15 M NaCl (pH 3.0), followed by 3 times cold PBS wash. The cells were analyzed using an LSR flow cytometer (Beckman Coulter). The FITC signal intensity was analyzed using the Cell-Quest software (version 3.3, Becton-Dickinson). The percentage of internalization was calculated from dividing mean fluorescence intensity of acid washed cells by that of cells without acid wash.