

# Supplementary information

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## 1. Cell preparation

### 1.1. Cell culture

For the cell transfection experiments Colon carcinoma cells, RKO, were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA). Then they were supplemented with 10% fetal bovine serum and 100 units/mL penicillin, 100 g/mL streptomycin and 0.25 g/mL amphotericin B (all HyClone, Logan, UT, USA). Prior to the experiments they were placed in a Nunc™ six-well plate and allowed to grow overnight in an incubator with a temperature of 37 °C and 5 % CO<sub>2</sub> to let the cells attach to the surface of individual wells. With this procedure we obtained 80-90% confluency per well.

### 1.2. Molecular delivery

Two fluorescent molecules were used to investigate the cell membrane poration: Calcein (623 Da, Stokes radius 0.6-0.7 nm,  $\lambda_{exc}$  = 490 nm,  $\lambda_{em}$  = 520 nm, Sigma-Aldrich, St. Louis, USA) and FITC-Dextran (10.000 Da, Stokes radius 2.3 nm,  $\lambda_{exc}$  = 490 nm,  $\lambda_{em}$  = 520 nm, Sigma-Aldrich, St. Louis, USA), henceforth referred to as Calcein and FITC-Dextran respectively. Both solutions were then dissolved in 99.9% anhydrous Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA) and DI water, respectively, to obtain stock solutions of 100 mg/mL. Before the experiments were conducted, the stock solutions were further diluted with culture medium to final concentrations of 0.3 mg/mL for Calcein and 2.0 mg/mL for FITC-Dextran. The cells in the wells were then washed once with 1x phosphate buffered saline (PBS) (Gibco, Grand Island, NY, USA) and 200  $\mu$ L of the working solution containing Calcein or FITC-Dextran was added to the cells with droplets to achieve homogeneous spreading of the working solution over the cells.

### 1.3. Molecular uptake evaluation

We evaluate the molecular uptake of Calcein and FITC-Dextran by the cells with an inverted microscope (Olympus, IX71) with a 4x or 10x microscope objective. Fluorescence was excited with a mercury lamp (U-RFL-T, Olympus) and observed with an appropriate filter block (U-FBN, Olympus, band pass excitation filter 470 nm <  $\lambda$  < 490 nm, long band pass emission filter  $\lambda$  > 510 nm). Still images were recorded using a monochrome digital 12-bit CCD camera (Sensicam QE). The exposure time of the images captured for calcein and FITC-Dextran were set to  $t_e = 0.105$  s and  $t_e = 2.175$  s, respectively.