Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Encoded Viral Micropatch for Multiplex Cell-Based Assays through Localized Gene Delivery

Sangkwon Han‡, Hyung Jong Bae‡, Su Deok Kim, Wook Park,* and Sunghoon Kwon*

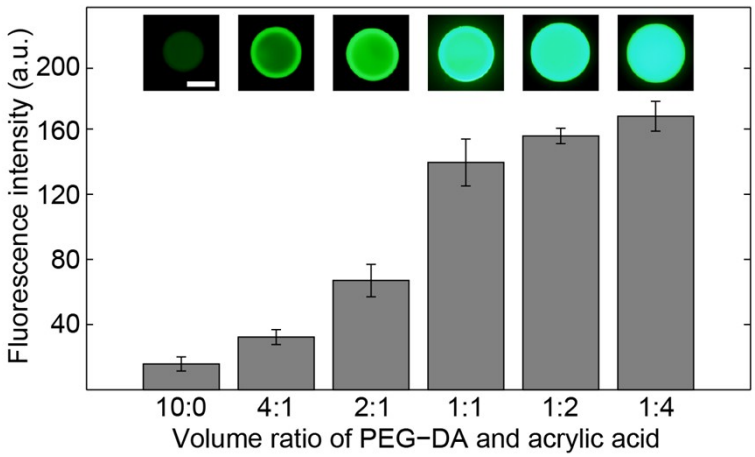

Fig. S1 Examination of carboxyl groups on the microparticles. The carboxylated polymer microparticles were fabricated using a mixture of PEG-DA and acrylic acid with different volume ratios. The particles were reacted with EDC/sulpho-NHS to convert the carboxyl groups to amine-reactive sulpho-NHS esters. The activated particles were then incubated with fluorescein isothiocyanate-conjugated bovine serum albumin (FITC-BSA, Sigma-Aldrich) for 2 h at 4 °C. Data show the mean values ± standard deviation of the fluorescence intensity of FITC-BSA immobilized on the particles (n = 20 for each condition). The insets are fluorescence micrographs of each condition (scale bar: 200 μm). The particles fabricated using higher volume fractions of acrylic acid had more available carboxyl groups.

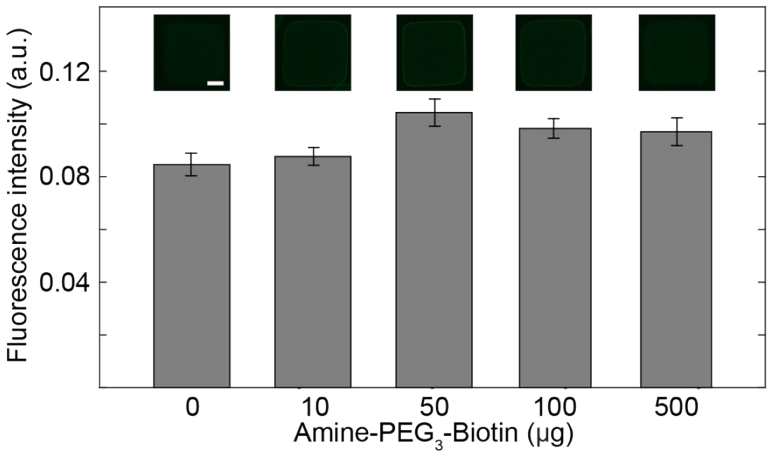

Fig. S2 Examination of the biotinylation of microparticles to maximize the avidin binding. The carboxylated polymer microparticles were biotinylated using different amount of amine-PEG_3-biotin and were examined using FITC-conjugated avidin (Thermo Fisher Scientific). Approximately 600 microparticles were treated with a solution of 2 mg of EDC, 4 mg of sulpho-NHS, and 400 µl of MEST for 30 min at RT. After washing with PBST, the microparticles were biotinylated by reacting with amine-PEG3-biotin in 200 µl of PBST for 2 h at RT. Five different amounts of biotin reagent (0, 10, 50, 100, and 500 µg) were used for microparticle biotinylation. To conjugate FITC-conjugated avidin on the biotinylated surfaces of the microparticles, the microparticles were washed two times with PBST and then vortexed gently in 200 µl of PBS solution containing 1% BSA for 2 h at RT to block non-specific absorption of FITC-avidin. After washing twice with PBST, the biotinylated microparticles were incubated with 0.02 µg of FITC-avidin in 200 µl of PBST for 2 h at RT. To observe the microparticles on a fluorescence microscope, the microparticles were washed two times with PBST. Data show the mean value ± standard deviation of the fluorescence intensity of FITC-conjugated avidin bound to the biotin on the microparticles (n = 10 for each condition). The insets are fluorescence micrographs of each condition (scale bar: 100 µm). The avidin binding on the biotinylated microparticle surface was maximized when 50 µg of amine-PEG_3-biotin was used for 600 microparticles.

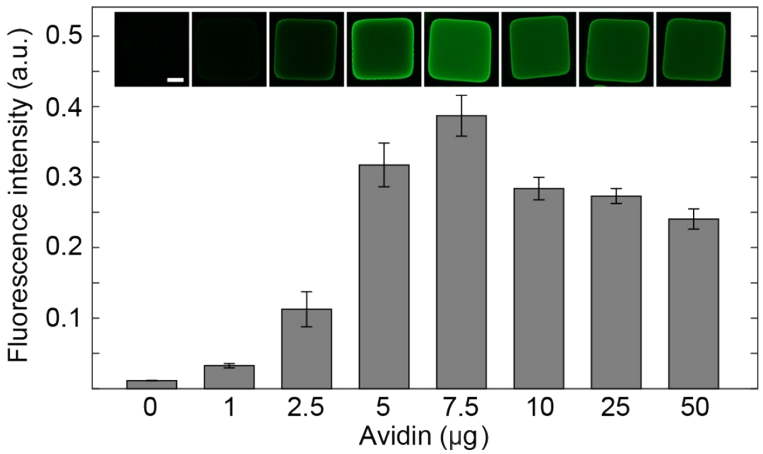

Fig. S3 Examination of the avidinylation of microparticles to maximize the biotin binding. The biotinylated microparticles were avidinylated using different amounts of avidin and were examined using fluorescein-conjugated biotin (Thermo Fisher Scientific). Based on the result in Figure S3, approximately 600 carboxylated microparticles were biotinylated using 50 μg of amine-PEG3-biotin. After washing two times with PBST, the biotinylated microparticles were incubated with avidin in 200 μl of PBST for 2 h at RT. Eight different amounts of avidin (0, 1, 2.5, 5, 7.5, 10, 25, and 50 μg) were used for the avidin conjugation on the biotinylated microparticles. To conjugate fluorescein-conjugated biotin on the avidinylated surface of the microparticles, the microparticles were washed two times with PBST and then vortexed gently in 200 μl of PBS solution containing 1% BSA for 2 h at RT to block non-specific absorption of fluorescein-conjugated biotin. After washing twice with PBST, the avidinylated microparticles were incubated with 200 μl of 0.3 μg/ml fluorescein-conjugated biotin solution for 2 h at RT. Finally, the microparticles were washed two times with PBST. Data show the mean values ± standard deviation of the fluorescence intensity of fluorescein-conjugated avidin bound to the avidin on the microparticles (n = 10 for each condition). The insets are fluorescence micrographs of each condition (scale bar: 100 μm). The biotin binding on the avidinylated microparticle surface was maximized when 7.5 μg of avidin was used for 600 microparticles.

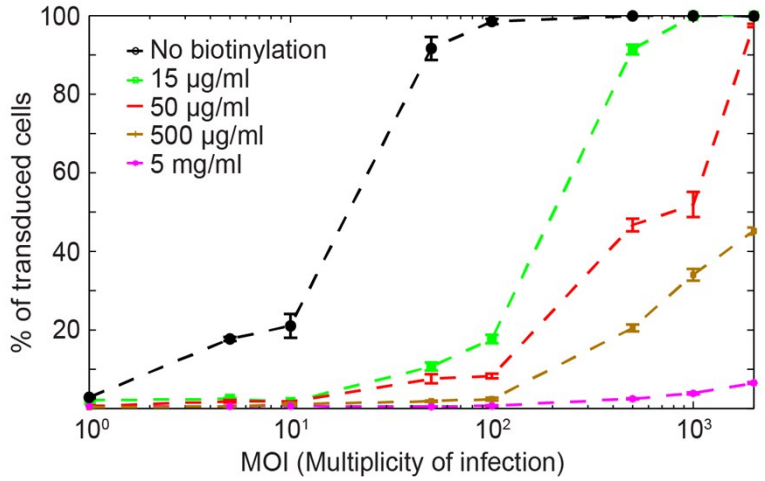

Fig. S4 Infectivity of biotinylated adenoviral vectors. The transduction efficiencies of adenoviral vectors were investigated with respect to their extent of biotinylation. Ad-GFP was biotinylated with five different concentrations of biotin reagent, and the biotinylated vectors with different MOIs were used to transduce U-2 OS cells. The transduction efficiencies were calculated by counting GFP-positive cells versus total cells determined by Hoechst 33342 staining. The transduction efficiency decreased as the extent of biotinylation increased. The error bars represent the standard deviation of the mean of four microscopic views for each data point.

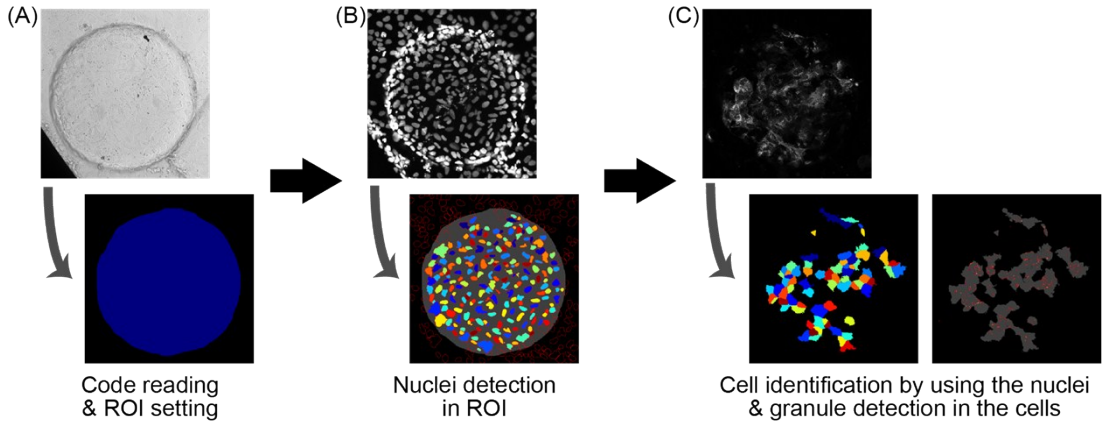

Fig. S5 Image processing for the encoded viral micropatch-based GPCR internalization assay. Images were taken using a confocal laser scanning microscope. (A) The micropatch code was determined based on shape. This shape was used to set a region of interest (ROI) in a transmitted light image. (B) Nuclei in the ROI were detected based on a predefined size and shape in the blue channel. (C) Cells were identified by outward growth from nuclei until reaching the edge of the image threshold or a neighbour in the green channel. Granules, which were identified as accumulations of green fluorescence created by the internalized receptors, were detected using size and intensity thresholds. The fluorescent intensity of cells and granules were measured to calculate receptor internalization ($F_{granule}$).

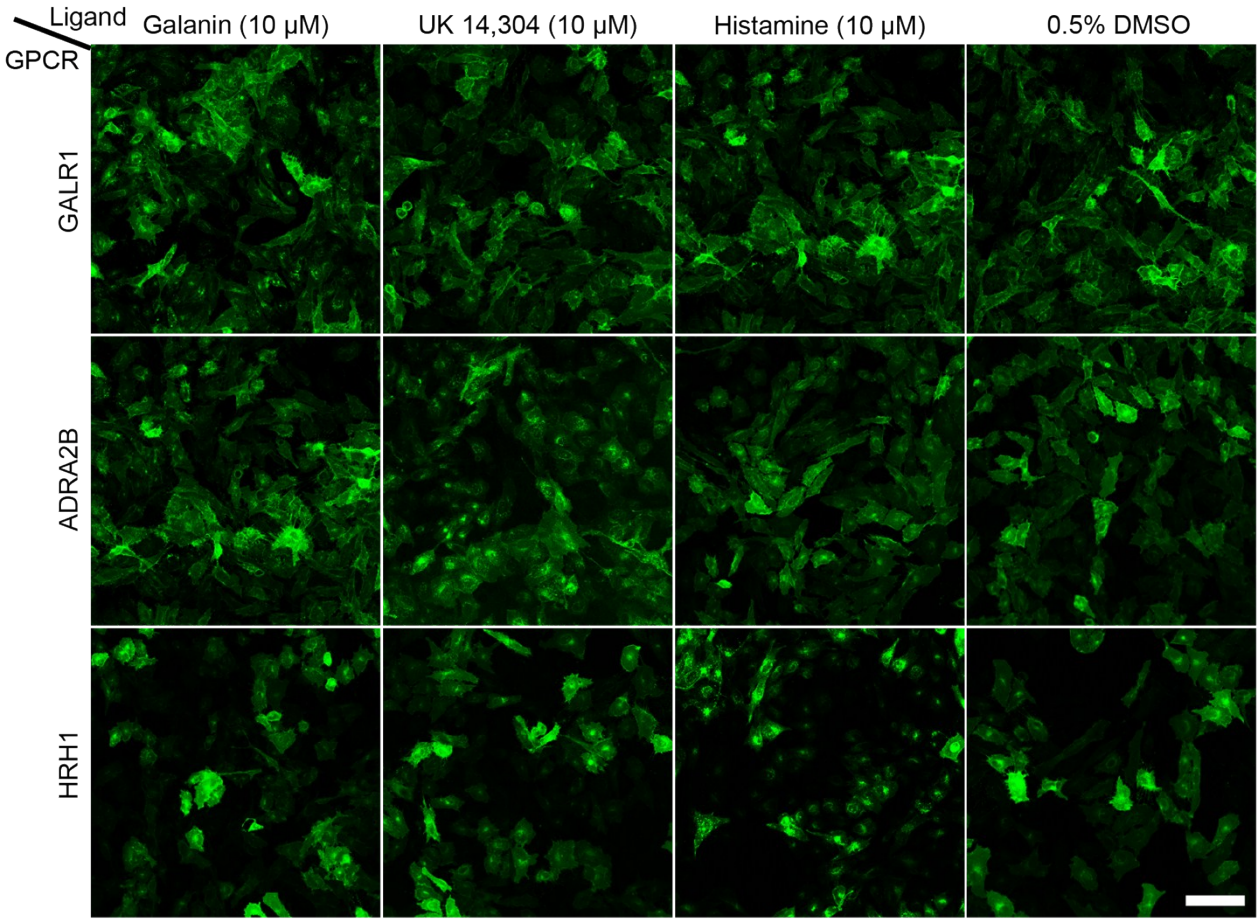

Fig. S6 A conventional singlet GPCR internalization assay using free Ad-GPCR-GFPs. Cells grown in the wells of a 96-well plate were transduced with one adenoviral vector (Ad-GALR1-GFP, Ad-ADRA2B-GFP, or Ad-HRH1-GFP) for GPCR expression. Each well was treated with 10 μM of one of three ligands (galanin, UK 14,304, or histamine) or 0.5% DMSO as a negative control. The confocal laser scanning microscope images show GPCR internalization by ligand activation (scale bar: 100 μm). Greater receptor internalization was observed for properly matched receptor-ligand pairs: galanin for GALR1, UK 14,304 for ADRA2B, and histamine for HRH1.