Supporting Information

Dual imaging and photoactivatable nanoprobe for controlled cell tracking

Sarit S. Agasti,[1] Rainer H. Kohler,[1] Monty Liong,[1] Vanessa M. Peterson,[1] Hakho Lee,[1] and Ralph Weissleder[1,2]*

[1]Center for Systems Biology, Massachusetts General Hospital/Harvard Medical School, 185 Cambridge St., Boston, MA 02114 (USA)
[2]Prof. R. Weissleder, Department of Systems Biology, Harvard Medical School, 200 Longwood Ave., Alpert 536, Boston, MA 02115 (USA)

*E-mail: rweissleder@mgh.harvard.edu

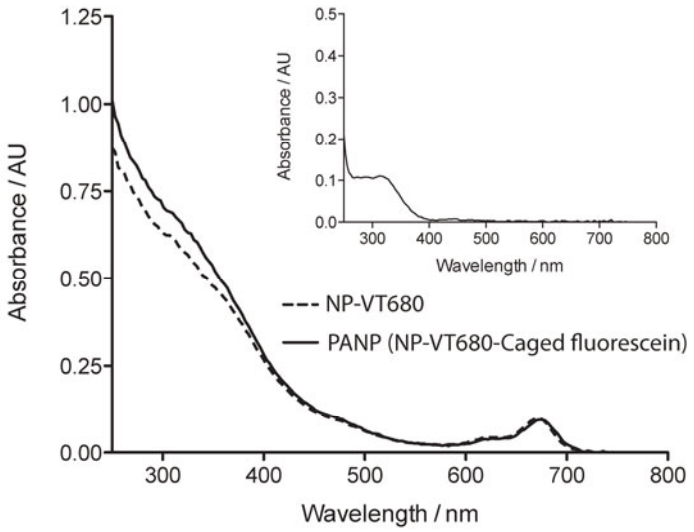

Figure S1. Absorption spectra of the nanoprobes (0.15 mg Fe/ml solution in PBS). Absorption peak at 670 nm indicated successful conjugation of the VT680 fluorophore. After conjugation of the caged fluorescein to the VT680 functionalized nanoprobe (NP-VT680) an increase in absorbance below 400 nm was observed, thus indicating attachment of the caged fluorescein to the PANP. The inset shows the absorption spectrum of the caged fluorescein (80 μM solution in PBS). Absorption spectra were recorded on a NanoDrop spectrophotometer.

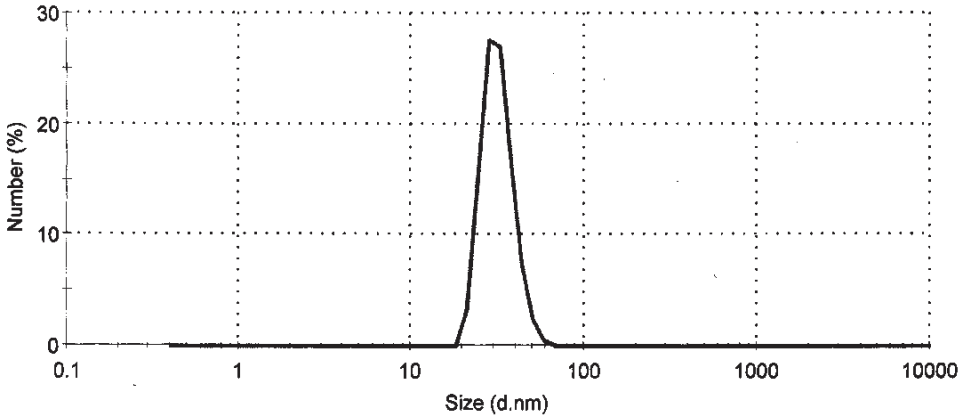

Figure S2. Dynamic light scattering (DLS) measurement showing the hydrodynamic size (diameter, d) distribution of PANP. PANP solution (0.1 mg of Fe/ml) in PBS was used for DLS measurement.

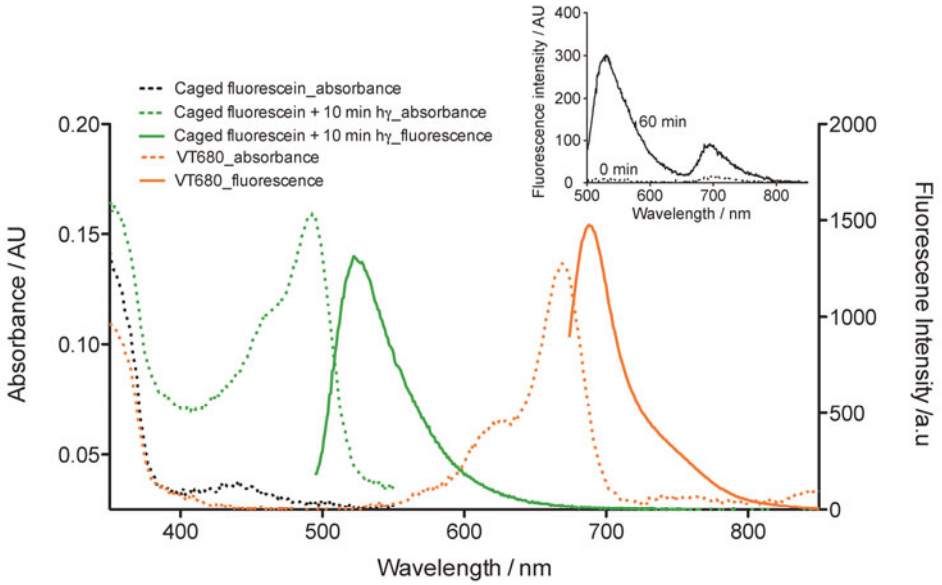

Figure S3. Absorption and fluorescence spectra of the free dyes. Spectra were recorded using PBS solution of 1 μM caged fluorescein and 0.1 μM VT680. Due to a small overlap between the fluorescence spectrum of the fluorescein and the absorption spectrum of the VT680, we have observed FRET from the PANP. Inset shows the fluorescence spectra before and after photoactivation (excitation at 470 nm) of the PANP, indicating increase in VT680 fluorescence after photoactivation due to FRET.

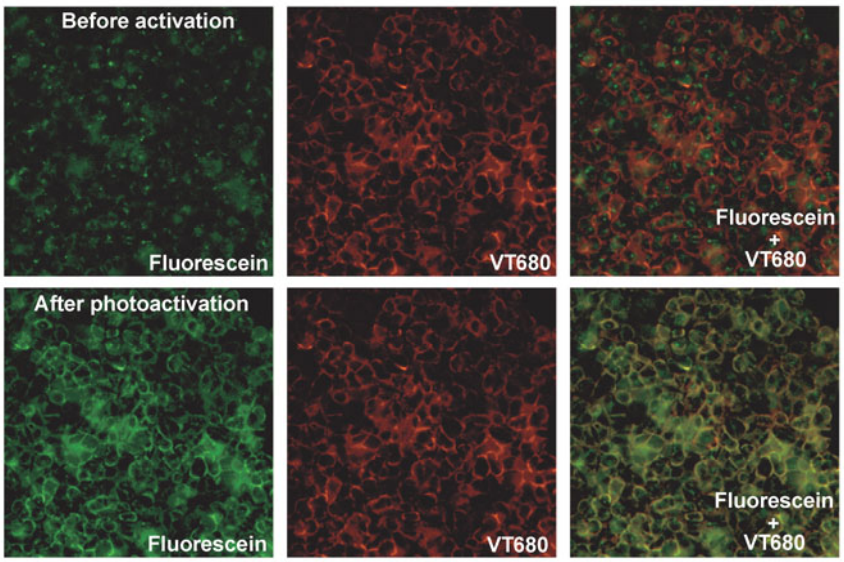

Figure S4. Spatiotemporally controlled dual-color labeling of the SK-BR-3 cells. TCO modified HER2/*neu* antibodies were targeted to SK-BR-3 cells and then used as scaffolds for bioorthogonal coupling of Tz-PANP in live cells. A similar protocol, described for the labeling of A431 cells, was used for labeling of SK-BR-3 cells. A Nikon 80i epifluorescence microscope was used for photoactivation and imaging of SK-BR-3 cells. SK-BR-3 cells were photoactivated using the microscope light passing through a DAPI filter set. Fluorescein (pseudo-colored green) and VT680 (pseudo-colored red) were imaged using FITC filter set and Cy5.5 filter set, respectively.

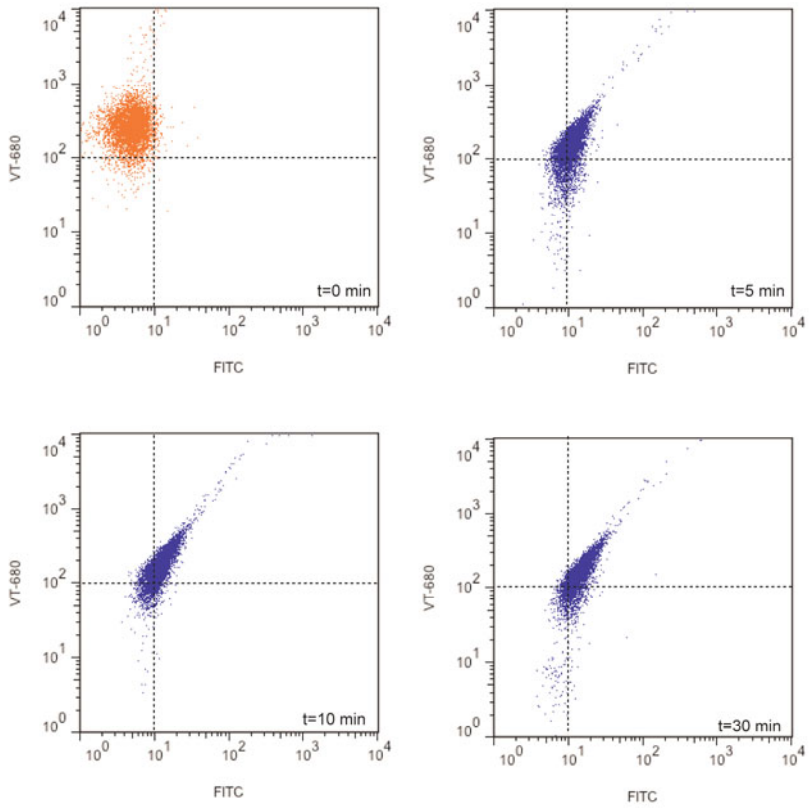

Figure S5. Flow cytometry analysis showing the formation of dual-labelled cells following photoactivation. For this experiment, live A431 cells were first coupled with PANP using the bioorthogonal method. Briefly, 10^6 cells suspended in 400 µl of PBS (containing 1%BSA and 2% FBS) were first incubated with TCO-anti-EGFR (10 µg/mL) for 15 minutes at room temperature. Following aspiration and washing with PBS (3 times), the cells were mixed with 400 µl of Tz-PANP (25 µg of Fe/mL) in PBS (containing 1%BSA and 2% FBS) at room temperature. After 15 min, excess Tz-PANP solution was removed and the cells were washed 3 times with PBS. Live cells were then suspended in PBS (containing 1%BSA and 2% FBS) and irradiated with a long wavelengh (>365 nm) hand held UV lamp. At different time points, fractions of the cells were removed and flow cytometry analysis was performed. Increase in fluorescence fluorescence signal after photoactivation indicated the formation of dual labelled cells.

Determination of the number of caged and uncaged fluorochromes attached to a single PANP: The degree of conjugation of VT680 moieties was quantified using the absorption spectrum of the PANP. Briefly, the absorbance was determined at 673 nm from a suspension of PANP. The concentration of VT680 was calculated using an extinction coefficient of 230,000 $M^{-1}cm^{-1}$. For the caged fluorescein, the PANP was first photoactivated and then the absorbance was determined at 494 nm. The concentration of fluorescein was calculated using an extinction coefficient of 73,000 $M^{-1}cm^{-1}$to get the degree of conjugation for caged fluorescein.

Caption for Movie 1: Photoactivated dual-labelled cells imaged over time showing the internalization of the PANP into the endosomes and the movement of the endosomes inside the cells. After photoactivation, fluorescent images were acquired from both fluorescein channel (pseudo-colored green) and VT680 channel (pseudo-colored red). Images were taken at 1 min intervals. Video shows the merged images.

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