

Supplementary Material (ESI) for Molecular BioSystems
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Supporting information for the manuscript

Fluorescent labeling of membrane proteins on the surface of living cells by a self-catalytic glutathione S-transferase omega 1 tag

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Supplemental Data

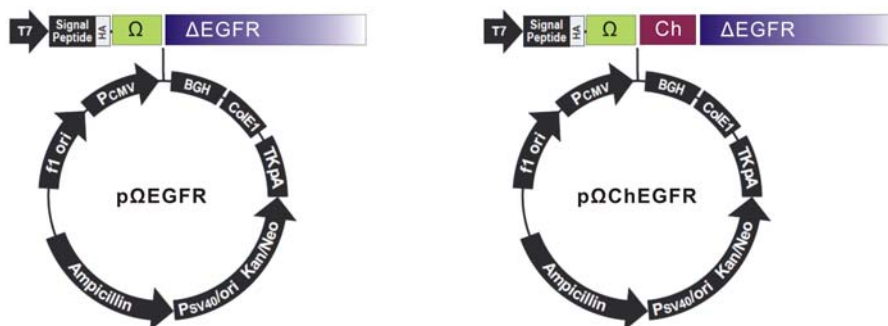


Fig. S1. Illustration for the Ω tagged recombinant EGFRs. Amino acids 1 to 24 of EGFR are its signal peptide that directs the membrane transport of EGFR. We amplified a truncated cDNA fragment of EGFR (amino acids 25 to 1210: Δ EGFR) excluding the signal peptide and the resulting PCR product was subcloned into the Sall/NotI sites of pDisplay- Ω or pDisplay- Ω Ch. Ω means GSTO1 and Ch means the monomeric Cherry protein. The Sall/NotI digestion on those constructs results in the deletion out of the DNA fragment encoding the PDGFR transmembrane domain. Therefore, the resulting plasmids encode chimeric EGFRs which have the signal peptide of IgK light chain and Ω tag or Ω Ch tag in frame with the Δ EGFR. Those chimeric EGFRs are displayed on the cell membrane by the transmembrane domain of EGFR itself.

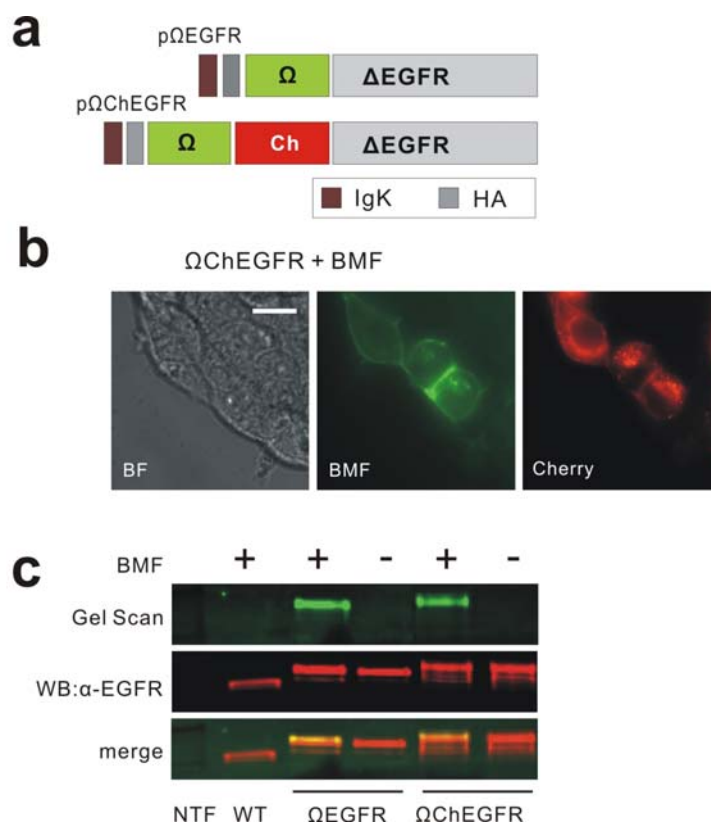


Fig. S2. Fluorescence labeling of EGFR by the Ω tagging method (a) Chimeric EGFRs fused to the Ω tag (b) HEK293 cells were used as they are EGFR-negative cells. HEK 293 cells transiently expressing Ω ChEGFR were stained with BMF (0.5 μ M, in serum free medium, 37°C for 20 min) in live cell condition. After washing normal growth medium one time, cells were supplemented with fresh growth medium (10% FBS containing DMEM (Dulbeccos modified eagle's medium)) and images were taken in live cell condition by a fluorescence microscope (Eclipse Ti-E, Nikon, with 100x oil immersion lens). Scale bar is 10 μ m (c) Analysis of BMF labeling to Ω -tagged EGFRs in SDS-PAGE. HEK293 cells were transiently transfected with p Ω EGFR (Ω) or p Ω ChEGFR (Ω Ch), and wild type EGFR (WT) expressing vector. Cells were stained with BMF and the total protein lysates was resolved in SDS-PAGE and scanned. Western blotting against EGFR confirmed the expression of exogenous EGFRs (both wild-type and chimeric EGFRs) and gel scanning revealed the BMF labeling on the Ω tagged EGFRs.

NTF : non-transfected cell lysate as a negative control, BF: bright field, WB: western blotting, IgK: signal peptide of the immunoglobulin kappa chain, HA: hemagglutinin tag, a small peptide tag encoded by the pDisplay vector.