

Appendix A

Appendix

HeLa and A431 cells were cultured in Dulbeccos Modified Eagles medium (DMEM) with 10To tag EGFRs in the plasma membranes of live cells, anti-EGFR antibody (Thermo Fisher Scientific, Waltham, MA, USA) was first biotinylated. The resulting biotinylated anti-EGFRs were conjugated with quantum dots (Qdot585-streptavidin, Thermo Fisher Scientific). Cells were incubated in a cell culture containing 10 nM of anti-EGFR-Qdot585 for 15 min and then washed three times with phosphate buffered saline (PBS). Fluorescent EGF (EGF-Qdot585) was synthesized by conjugating biotin-EGF from Invitrogen to Qdot585-streptavidin in PBS. To activate EGFRs, cells were incubated in a cell culture containing 40 ng/mL of EGF-Qdot585. Fluorescent anti-CD59 antibody was synthesized by biotinylating anti-CD59 antibody (Sigma-Aldrich, St. Louis, MO, USA) and then conjugated with Qdot585-streptavidin in PBS. To label CD59, we followed the same protocol as described above for labeling EGFR.

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