Supplementary information, Fig. S9



Low density (sEV) High density (NV) Low density (sEV) High density (NV) Low density (sEV) High density (NV)

Supplementary information, Fig. S9. EGFR phosphorylates RAB31 to drive EGFR into exosomes and the exosomes promoted by RAB31 mediate resistance to erlotinib. a Western blotting analyses of whole-cell lysates (WCL) from the indicated stable NCI-H1975 cells under serum starvation (SS). b Western blotting analyses of WCL from the indicated stable HeLa cells under SS. c Western blotting analyses of WCL and immunoprecipitates (IP) from the indicated stable NCI-H1975 cells treated with DMSO, afatinib or erlotinib under SS. d Immunofluorescence of endogenous EGFR (green) and CD63 (red) in NCI-H1975 cells treated with afatinib or erlotinib under SS. e Immunofluorescence of endogenous RAB31 (green) and CD63 (red) in NCI-H1975 cells treated with afatinib or erlotinib under SS. f Western blotting analyses of WCL from the indicated stable NCI-H1975 cells under SS. g Western blotting analyses of WCL and IP from the indicated stable NCI-H1975 cells under SS. h Western blotting analyses of the concentrated conditional media from the indicated stable NCI-H1975 cells used in g. i Representative clone images of PC9-GFP cells co-cultured with the indicated stable NCI-H1975 cells without or with erlotinib. j, Quantification of the cell numbers of 20 colones in each group in i. Data are means \pm S.D. of cell numbers of 20 clones in each group. Unpaired *t*-test was used to analyze the difference between the two groups. ****P < 0.0001, NS, no statistical significance. k Density gradient fractionation of small EVs isolated from the concentrated conditional media derived from stable NCI-H1975 cells under SS. After flotation of sample in high-resolution iodixanol gradients, equal volumes of each fraction were loaded on SDS-PAGE gels, and membranes were blotted with the indicated antibodies. NV, non-vesicular; sEV, small EV. Scale bars, 10 µm (d and e), 200 µm (i).