

Supplementary information, Fig. S10. RAB31 sequesters EGFR in CD63-positive MVEs. a Up-panels Immunofluorescence of EGFR-HA (green) with CD63 (red), EGFR-HA (green) with EEA1 (red) or EGFR-HA (red) with GFP-RAB5 (green) in the indicated stable HeLa cells transiently expressing EGFR-HA treated with 100 ng/mL of EGF at the indicated times. Low-panel left, the ratio of co-localization of EGFR-HA with EEA1-positive vesicle in Vector (n = 9 fields) and RAB31^{WT} (n = 8fields) at 8 min of EGF treatment. Low-panel right, the ratio of co-localization of EGFR-HA with GFP-RAB5-positive vesicle in Vector (n = 8 fields) and RAB31^{WT} (n= 8 fields) at 8 min of EGF treatment. Data are means \pm S.D. Unpaired *t*-test was used to analyze the difference between the two groups. NS, no statistical significance. b Immunofluorescence of EGFR-HA (red) with LAMP1 (green) in the indicated stable HeLa cells transiently expressing EGFR-HA treated with 100 ng/mL of EGF at the indicated times. c Western blotting analyses of whole-cell lysates (WCL) and immunoprecipitates (IP) from HEK-293T cells co-expressing EGFR-HA and Flag-RAB31. d Western blotting analyses of WCL and IP from the indicated stable HeLa cells. e Western blotting analyses of WCL from various cancer cell lines stably expressing Flag-RAB31. Scale bars, 10 µm.