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Supplemental information

Exocyst controls exosome biogenesis via Rab11a

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Figure S1.

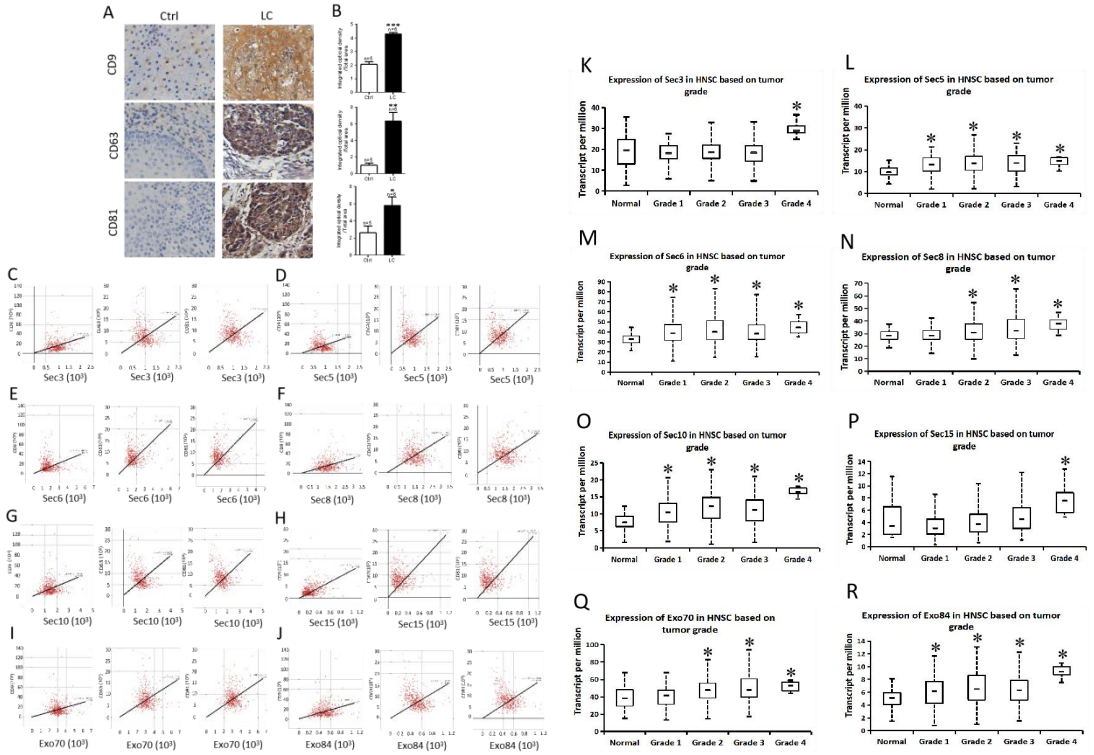

Figure S1. Correlation between exosome and exocyst subunits in HNC. (A and B) The expression of CD9, CD63 and CD81 in LC tissue compared with Ctrl. (A) Representative images of immunohistochemistry staining. (B) Data summary of immunohistochemistry staining. LC: laryngocarcinoma tissue, Ctrl: adjacent normal tissues. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ by Student's t test. (C-J) Correlation analysis about exocyst and exosomal markers. (K-R) The expression of exocyst subunits in HNC based on tumor grade. $^{*}P<0.05$ compared with normal.

Figure S2.

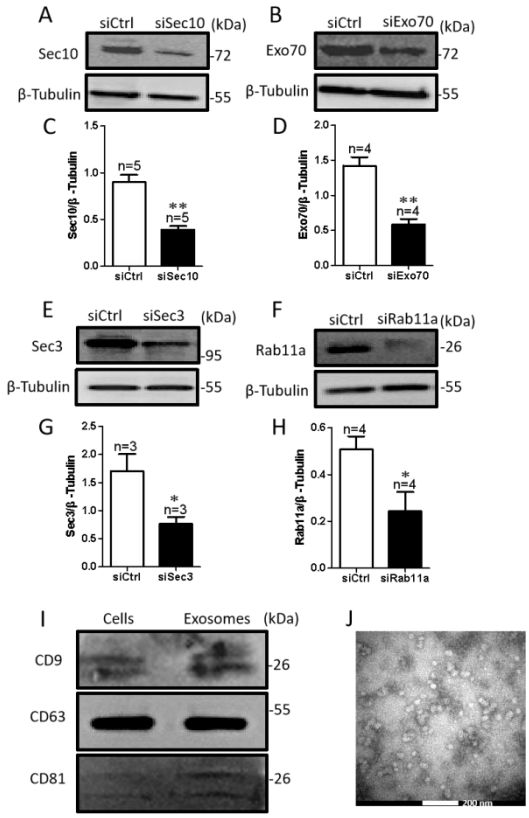

Figure S2. Transfection efficiency of siRNA and exosomes identification. (A and C) The expression of Sec10 after transfection with siSec10 in HN4 cells. (A) Representative western blot images about the expression of Sec10. (C) Summary data showing the expression of Sec10. $^{**}P<0.01$ by Student's t test. (B and D) The expression of Exo70 after transfection with siExo70 in HN4 cells. (E and G) Representative western blot images and summary data about the expression of Sec3 after transfection with siSec3 in HN4 cells. (F and H) Representative western blot images and summary data about the expression of Rab11a after transfection with siRab11a in HN4 cells. $^{*}P<0.05$ by Student's t test. (I and J) Identification of purified exosomes. (I) The typical proteins CD9, CD63 and CD81 for exosome identification by western blot. (J) Representative TEM photograph of exosomes, scale bar, 200 nm.

Figure S3.

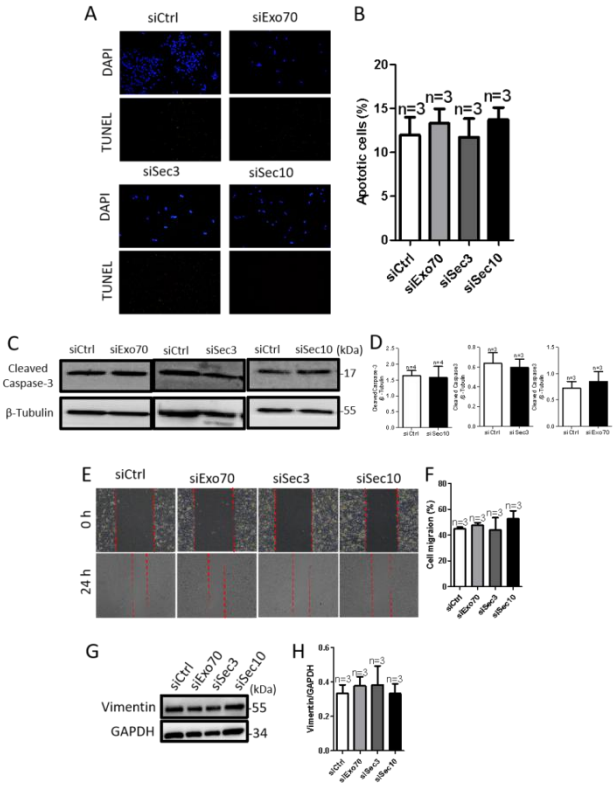

Figure S3. No effect of decreased exocyst on cell apoptosis and migration. (A and B) The apoptosis of HN4 cells after knock down the expression Exo70, Sec3 or Sec10 by TUNEL detection. (A) Representative images showing HN4 cell nuclei (blue, DAPI) and apoptotic (green, TUNEL) HN4 cells. (B) Summary data showing the percentage of apoptotic cells. (C and D) Representative western blot images and summary data about the apoptosis of HN4 cells after knock down the expression Exo70, Sec3 or Sec10 by western blot. (E and H) The migration of HN4 cells after knock down the expression Exo70, Sec3 or Sec10 by scratch-wound assay and western blot. (E) Representative images showing migration of HN4 cell after 24 h. (F) Summary data showing the percentage of cell migration compared with 0 h. (G) Representative western blot images of Vimentin. (H) Summary data showing the

expression level of Vimentin in HN4 cells after exosome complex component knockdown or transfection with siCtrl.

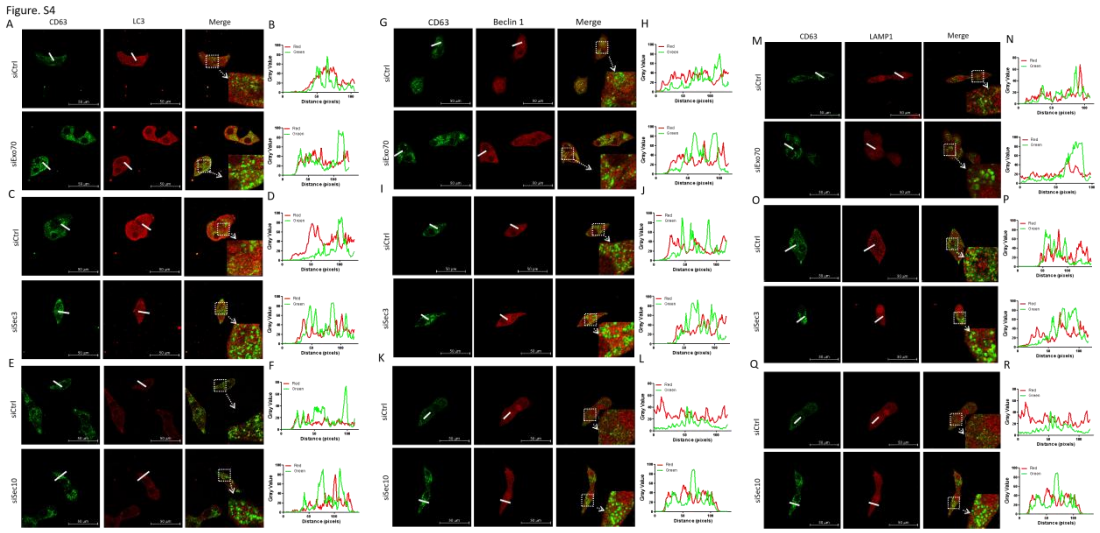

Figure S4. Co-localization analysis of MVBs and autophagosomes and lysosomes after knockdown of exocyst subunits. (A-B) Immunofluorescence labeling to assess the localization and intensity (at the white line) for CD63 (green) and LC3 (red) in HN4 cells after knock down the expression of Exo70 (A and B), Sec3 (C and D) and Sec10 (E and F). (G-L) Immunofluorescence labeling to assess the localization and intensity (at the white line) for CD63 (green) and Beclin1 (red) in HN4 cells after knock down the expression of Exo70 (G and H), Sec3 (I and J) and Sec10 (K and L). (M-R) Immunofluorescence labeling to assess the localization and intensity (at the white line) for CD63 (green) and LAMP1 (red) in HN4 cells after knock down the expression of Exo70 (M and N), Sec3 (O and P) and Sec10 (Q and R).