

Expanded View Figures

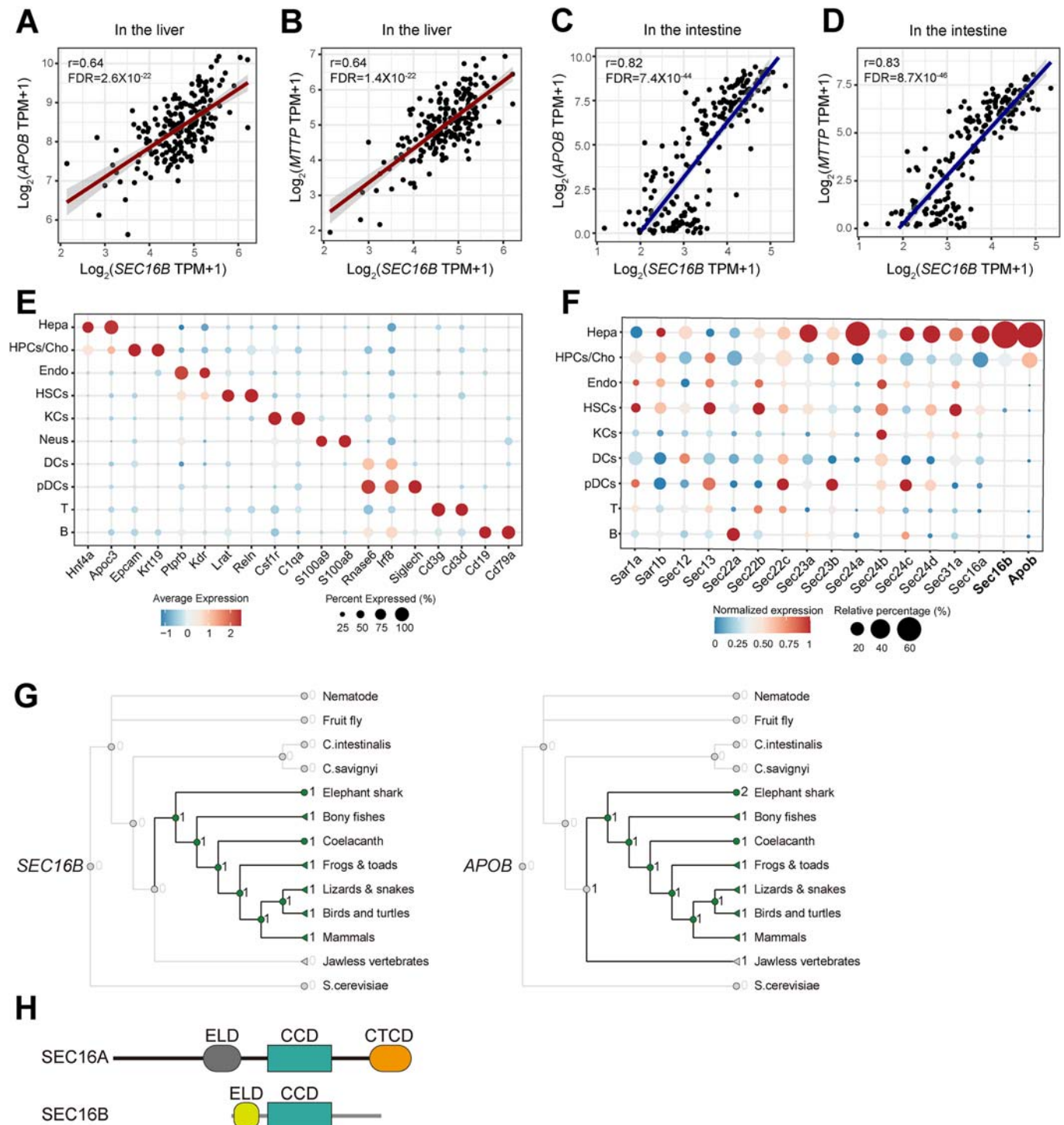


Figure EV1. Identification of a tissue-selective *SEC* gene *SEC16B*.

(A, B) Correlation analysis of *SEC16B* with *APOB* expression (A) and *MTTP* expression (B) in human liver. (C, D) Correlation analysis of *SEC16B* with *APOB* expression (C) and *MTTP* expression (D) in human intestine. (E) Bubble heatmap of marker gene expression for each identified cell cluster in Fig. 1F. Hepa hepatocytes, hPCS/Cho hepatic progenitor cells/cholangiocyte, Endo endothelial cells, HSCs hepatic stellate cells, KCs kupffer cells, Neus neutrophils, DCs dendritic cells, pDCs plasmacytoid dcs, T T cells, B B cells. (F) Bubble heatmap of *Sec* gene expression patterns across cell types from Fig. 1F. Sizes represent the percentage of cells expressing the indicated gene within each cluster. Color represents the normalized expression levels in each cell cluster. The expression of *Sec31b* was not detected. Neutrophils were excluded from the analysis due to low cell counts ($n < 10$) to avoid potential statistical bias. (G) Gene gain and loss events of *SEC16B* and *APOB* by using the Ensembl browser. (H) Domain schematics of *SEC16A* and *SEC16B*. ELD ER localizing domain, CCD central conserved domain, CTCD C-terminal conserved domain.

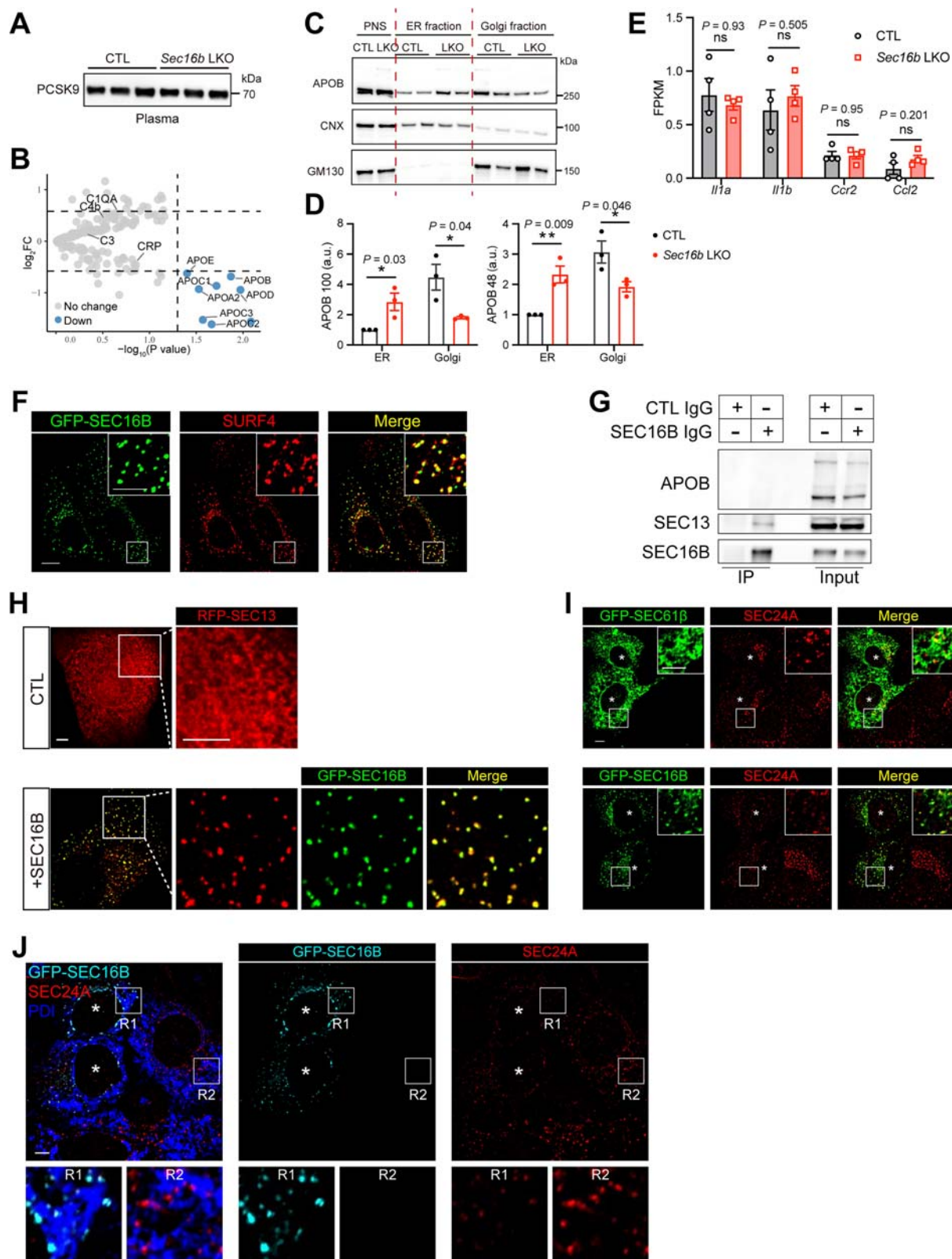



Figure EV2. SEC16B selectively regulates hepatic lipid secretion.

(A) IB analysis of PCSK9 in plasma samples from CTL and *Sec16b* LKO mice. Representative of three independent experiments is shown. (B) Volcano plot of quantitative proteomics in plasma samples from CTL and *Sec16b* LKO mice. Significantly altered proteins with $|FC| > 1.5$ and adjusted P value < 0.05 are colored in blue. $n = 3$ for each genotype. (C) IB analysis of ER-enriched fractions and Golgi-enriched fractions isolated from CTL and *Sec16b*-KO mouse livers. Representative of three independent experiments is shown. (D) Quantification of IB signals in (C). $n = 3$ biological independent replicates for each group. Data are shown as mean $\pm$ SEM. $*P < 0.05$; $**P < 0.01$ (two-tailed Student's t test). (E) Analysis of inflammatory gene expression using hepatic RNA-seq data from Fig. 3G with CTL and *Sec16b* LKO mice under fasting conditions. $n = 4$ mice for each group. Statistic was analyzed using the Wald test in DESeq2 with adjustment by Benjamini-Hochberg. Data are shown as mean $\pm$ SEM. ns, no significance. (F) Co-localization of SEC16B with SURF4. Huh7 cells transfected with GFP-SEC16B were fixed and stained with an anti-SURF4 (red) antibody, prior to confocal microscopy. Scale bars = 5 μ m. (G) Co-IP of endogenous SEC16B and SEC13 in mouse liver samples. Liver lysates were subjected to anti-SEC16B or CTL IgG IP, followed by SDS-PAGE and IB with the indicated antibodies. (H) SEC16B promotes membrane localization of outer coat proteins. Huh7 cells transfected with HA-SEC31A and RFP-SEC13, plus empty vector (upper row) or GFP-SEC16B WT (lower row) were analyzed by confocal microscopy. Scale bars = 5 μ m. (I) SEC61 β overexpression exhibits little effect on COPII coalescence compared to neighboring cells. Huh7 cells transfected with GFP-SEC61 β (upper) or GFP-SEC16B (lower) were fixed and stained with an anti-SEC24A (red) antibody, prior to confocal microscopy. Scale bars = 5 μ m. Asterisks, positive cells with GFP-SEC61 β or GFP-SEC16B expression. (J) Similar localization on the ER surface of SEC24A between SEC16B-expressing and neighboring control cells. Huh7 cells transfected with GFP-SEC16B were fixed and co-stained with anti-SEC24A (red) and anti-PDI (blue) antibodies, prior to confocal microscopy. Scale bars = 5 μ m. Asterisks, positive cells with GFP-SEC16B expression. Source data are available online for this figure.

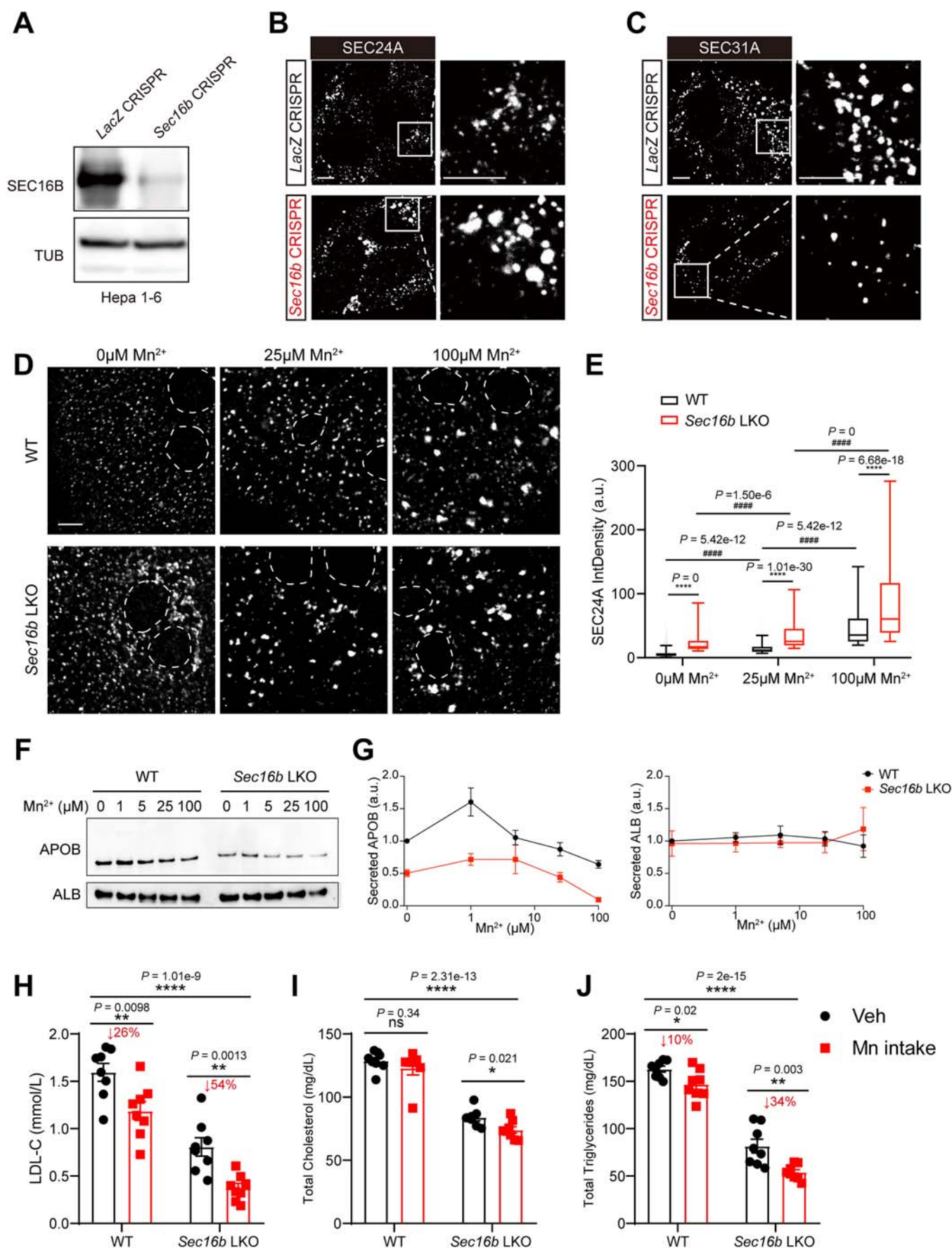


Figure EV3. A cooperative effect between SEC16B loss and Mn^{2+} signals on COPII coalescence and lipid secretion.

(A) IB analysis of SEC16B protein depletion in Hepa 1-6 with CRISPR-mediated gene editing. (B) Loss of SEC16B enhances SEC24A coalescence in Hepa 1-6. CTL (*LacZ* CRISPR) or *Sec16b* KO (*Sec16b* CRISPR) Hepa 1-6 cells were stained with the anti-SEC24A antibody prior to confocal microscopy. Scale bars = 5 μ m. (C) Loss of SEC16B decreases SEC31A signals in puncta. The same set of cells as in (B) were stained with the anti-SEC31A antibody prior to confocal microscopy. Scale bars = 5 μ m. (D) SEC16B loss enhances the promoting effect of Mn^{2+} signals on COPII coalescence. Primary hepatocytes isolated from control (WT) or *Sec16b* KO mice were treated with $MnCl_2$ prior to SEC24A staining. Dashed lines: nucleus. Scale bars = 5 μ m. (E) Quantification of the integrated fluorescence signal density of SEC24A puncta in (D). Data are shown as a box plot with 5-95% distribution, with the line inside the box and box edges representing median and the interquartile ranges, respectively. $n = 2615, 747, 896, 512, 425,$ and 271 puncta for WT, *Sec16b* KO, WT with 25 μ M $MnCl_2$ treatment, *Sec16b* KO with 25 μ M $MnCl_2$ treatment, WT with 100 μ M $MnCl_2$ treatment, and *Sec16b* KO with 100 μ M $MnCl_2$ treatment, respectively. Data were pooled from 10 cells per condition. Statistical analysis between WT and *Sec16b* KO under the same $MnCl_2$ treatment was determined by two-tailed Student's t test. **** $P < 0.0001$. Statistical analysis among different $MnCl_2$ treatments within the same genotype was determined by ANOVA with the Tukey post hoc test. **** $P < 0.0001$. a.u. arbitrary units. (F) Cooperative regulation of SEC16B loss and Mn^{2+} signals in APOB secretion. Primary hepatocytes isolated from WT or *Sec16b* LKO mice were treated with TPEN for 30 min, followed by $MnCl_2$ treatment for 8 h with indicated doses. APOB and albumin in the medium were analyzed by IB. Representative of three independent experiments is shown. (G) Quantification of IB signals in (F). The IB signals were normalized to the secretion level of WT at 0 μ M $MnCl_2$ treatment. $n = 3$ biologically independent replicates for each genotype at each treatment. Data are shown as mean $\pm$ SEM. a.u. arbitrary units. (H-J) Cooperative effects between SEC16B loss and Mn^{2+} signals in lowering plasma lipids. Total LDL-C (H), total cholesterol (I) and triglyceride (J) levels in plasma of WT and *Sec16b* LKO mice with vehicle or $MnCl_2$ intake were measured. $n = 8$ for each group. Data are shown as mean $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$. ns no significance (two-tailed Student's t test). Source data are available online for this figure.

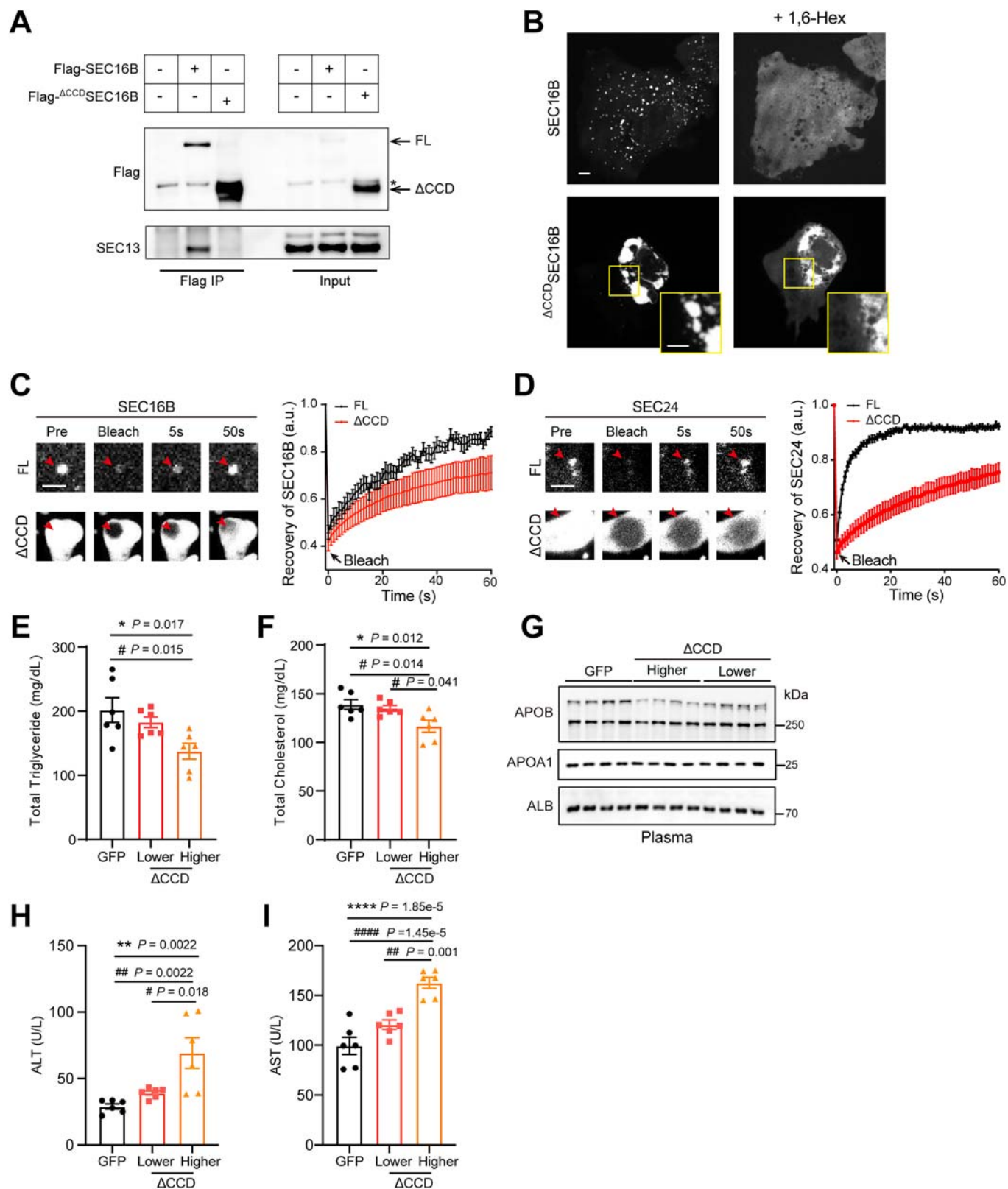
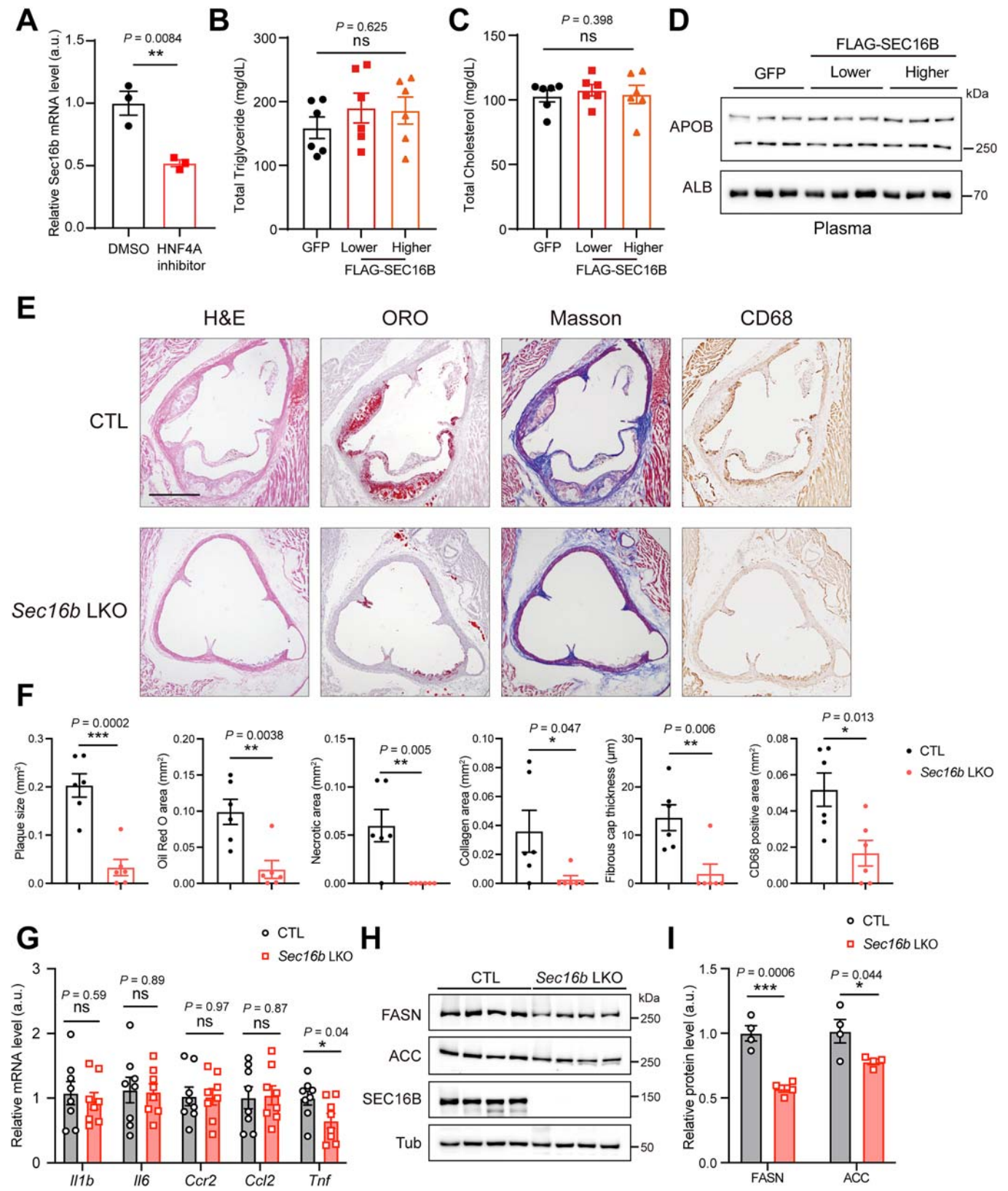



Figure EV4. Abnormal COPII assembly impairs secretion.

(A) SEC16B lacking CCD domain (Δ^{CCD} SEC16B) fails to interact with SEC13. Mouse livers expressing Flag-SEC16B or Flag- Δ^{CCD} SEC16B were lysed and subjected to anti-Flag Co-IP, followed by SDS-PAGE and IB with the indicated antibodies. (B) Confocal image of Huh7 cells transfected with GFP-SEC16B or GFP- Δ^{CCD} SEC16B before or after 1,6-Hex treatments for 30 s. Scale bars = 5 μ m. (C) FRAP comparison of GFP-SEC16B coalescence and Δ^{CCD} SEC16B over-aggregates in Huh7 cells. Arrowheads: selected foci for photobleaching in each condition. Scale bars = 2.5 μ m. Right, quantification analysis, shown as mean $\pm$ SEM. $n = 9$ for GFP-SEC16B coalescence and 7 for GFP- Δ^{CCD} SEC16B aggregates, respectively. (D) FRAP comparison of RFP-SEC24D colocalized with GFP-SEC16B coalescence or Δ^{CCD} SEC16B over-aggregates in Huh7 cells. Arrowheads: selected foci for photobleaching in each condition. Scale bars = 2.5 μ m. Right, quantification analysis, shown as mean $\pm$ SEM. $n = 11$ and 9 for RFP-SEC24D colocalized with GFP-SEC16B coalescence or Δ^{CCD} SEC16B aggregates, respectively. (E) Plasma triglyceride levels of mice receiving AAV-TBG-GFP or AAV-TBG- Δ^{CCD} SEC16B. Data are shown as mean $\pm$ SEM. $n = 6$ mice for each group. * $P < 0.05$ determined by ANOVA; # $P < 0.05$ determined by the Tukey post hoc test. (F) Plasma total cholesterol levels of mice in (E). Data are shown as mean $\pm$ SEM. * $P < 0.05$ determined by ANOVA; # $P < 0.05$ determined by the Tukey post hoc test. (G) IB analysis of plasma samples from mice in (E). Representative of three independent experiments is shown. (H) Plasma ALT levels of mice in (E). Data are shown as mean $\pm$ SEM. ** $P < 0.01$ determined by ANOVA; #, $P < 0.05$; ## $P < 0.01$ determined by the Tukey post hoc test. (I) Plasma AST levels of mice in (E). Data are shown as mean $\pm$ SEM. **** $P < 0.0001$ determined by ANOVA; ## $P < 0.01$; #### $P < 0.0001$ determined by the Tukey post hoc test. Source data are available online for this figure.



◀ **Figure EV5. Therapeutic potentials of SEC16B targeting in dyslipidemia and ASCVD.**

(A) Relative *Sec16b* mRNA levels in murine primary hepatocytes treated with DMSO control, or BI6015, a HNF4A inhibitor, as measured by reverse transcription-quantitative PCR (RT-qPCR). Data are shown as mean $\pm$ SEM. $n = 3$ biological replicates. P values were determined by two-tailed Student's t test. (B) Plasma triglyceride levels of mice receiving AAV-TBG-GFP or AAV-TBG-SEC16B. $n = 6$ mice for each group. Data are shown as mean $\pm$ SEM. ns, no significance determined by ANOVA test. (C) Plasma total cholesterol levels of mice in (B). Data are shown as mean $\pm$ SEM. $n = 6$ mice for each group. ns, no significance determined by the ANOVA test. (D) IB analysis of plasma samples from mice in (B). Representative of three independent experiments is shown. (E) Representative of H&E, ORO, Masson and CD68 IHC at aortic root cross-sections from mice in Fig. 7A. Scale bars = 100 μ m. (F) Quantification of plaque size, ORO positive area, necrotic area, collagen area, fibrous cap and CD68 positive are using aortic root sections in (E). Data are shown as mean $\pm$ SEM. $n = 6$ mice for each group. $^*P < 0.05$; $****P < 0.0001$ (two-tailed Student's t test). (G) Relative expression levels of hepatic inflammatory genes in mice from Fig. 7A. Hepatic mRNA from the indicated mouse liver samples were subjected to RT-qPCR using primers as listed in Dataset EV1. $n = 8$ mice for each group. P values were determined by two-tailed Student's t test. (H) IB analysis of liver samples from mice in Fig. 7A. (I) Quantification of IB signals in (H). Data are shown as mean $\pm$ SEM. $n = 4$ mice for each group. $^*P < 0.05$; $^{**}P < 0.01$ (two-tailed Student's t test). Source data are available online for this figure.