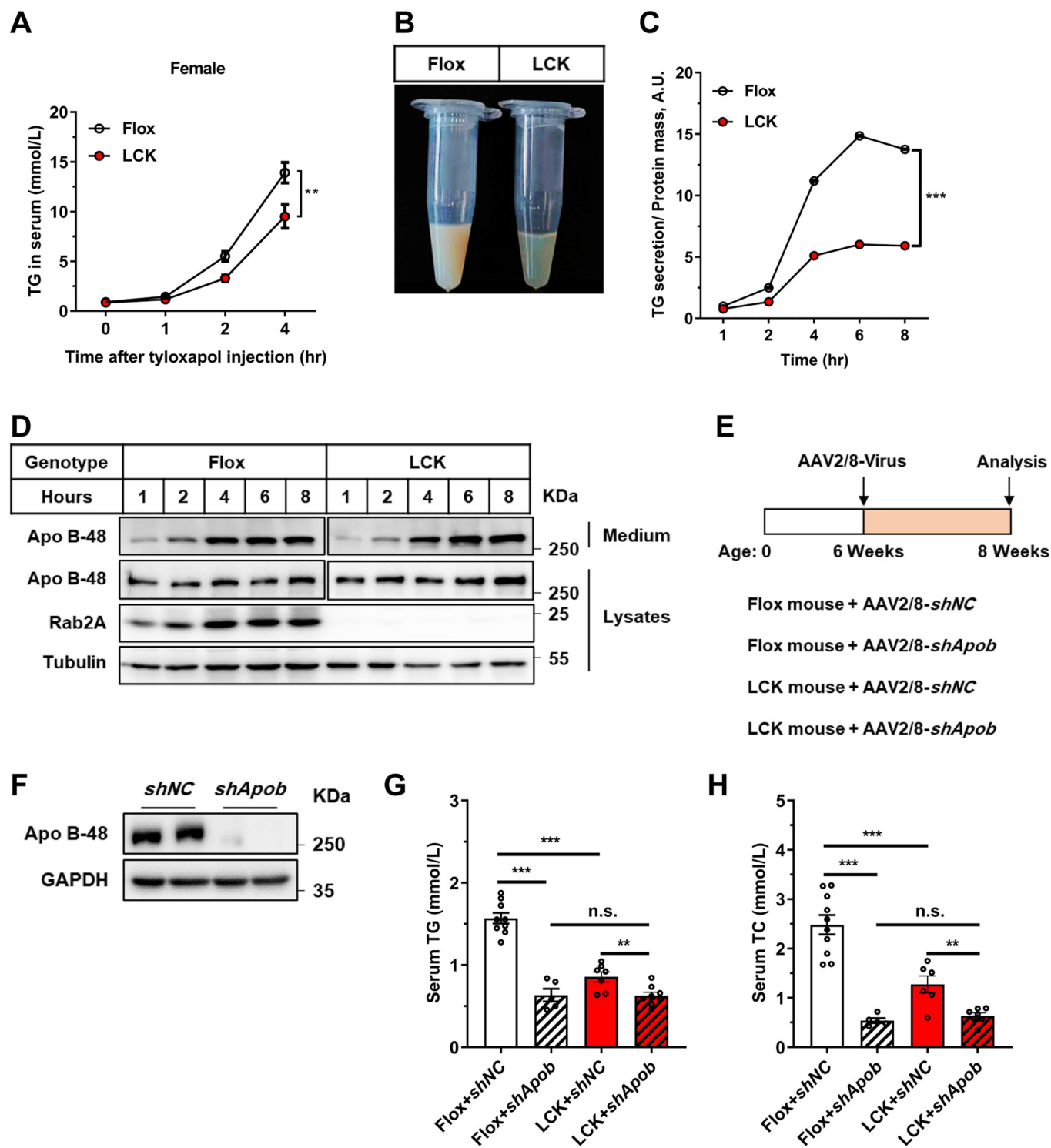


Expanded View Figures

Figure EV1. Evaluating the rates of lipid secretion in Flox and LCK mice.

(A) Triglyceride (TG) secretion in female mice were assessed through a tyloxapol injection assay ($n = 7$ vs. 6 mice) ($P = 0.0021$). (B) A visual examination of blood transparency in Flox and LCK mice was conducted four hours post-tyloxapol injection. (C, D) The evaluation of the TG secretion in primary hepatocytes involved quantifying the secretion level of TG (C) ($P < 0.0001$) and Apo B-48 (D), with normalization of Flox samples' secretion level at 1-hour to 1. (E-H) The schematic representation outlines the timeline for the *ApoB* knockdown assay (E), followed by the validation of Apo B-48 proteins levels (F) and assessment of serum TG (G) (Flox (*shNC* vs. *shApoB*), $P < 0.0001$; *shNC* (Flox vs. LCK), $P < 0.0001$; LCK (*shNC* vs. *shApoB*), $P = 0.0079$) and TC (H) (Flox (*shNC* vs. *shApoB*), $P < 0.0001$; *shNC* (Flox vs. LCK), $p = 0.0009$; LCK (*shNC* vs. *shApoB*), $P = 0.0034$) levels under "Fasted" condition (Male, $n = 10$ vs. 5 vs. 7 vs. 8 mice). Data information: Data in (A, C, G, H) are presented as mean $\pm$ SEM. Circles in (G, H) correspond to individual mice. P values in (A, C) were determined using two-way ANOVA. P values in (G, H) were determined using unpaired two-tailed Student's t -test. n.s. indicates no significant difference ($P > 0.05$), ** indicates $P < 0.01$; *** indicates $P < 0.001$.



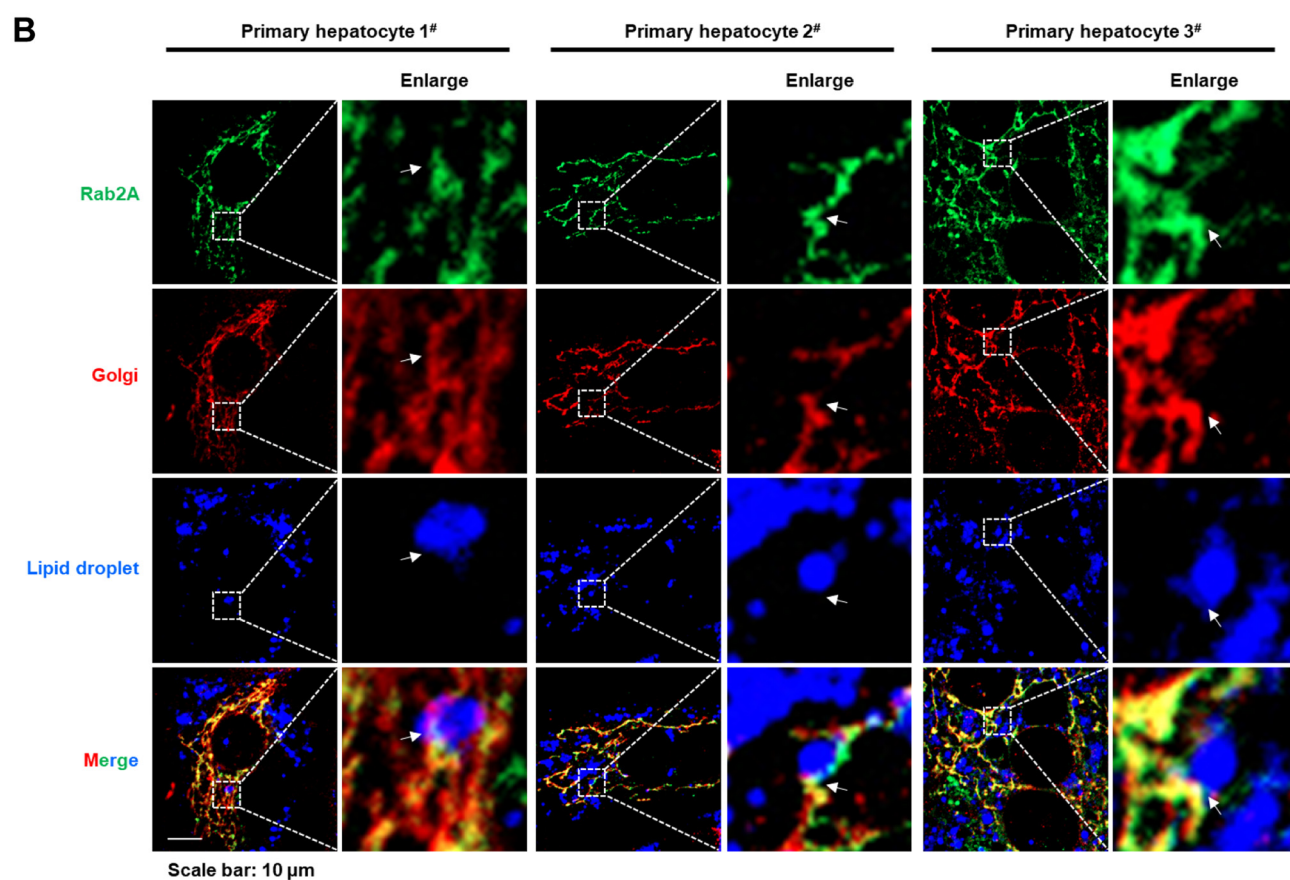
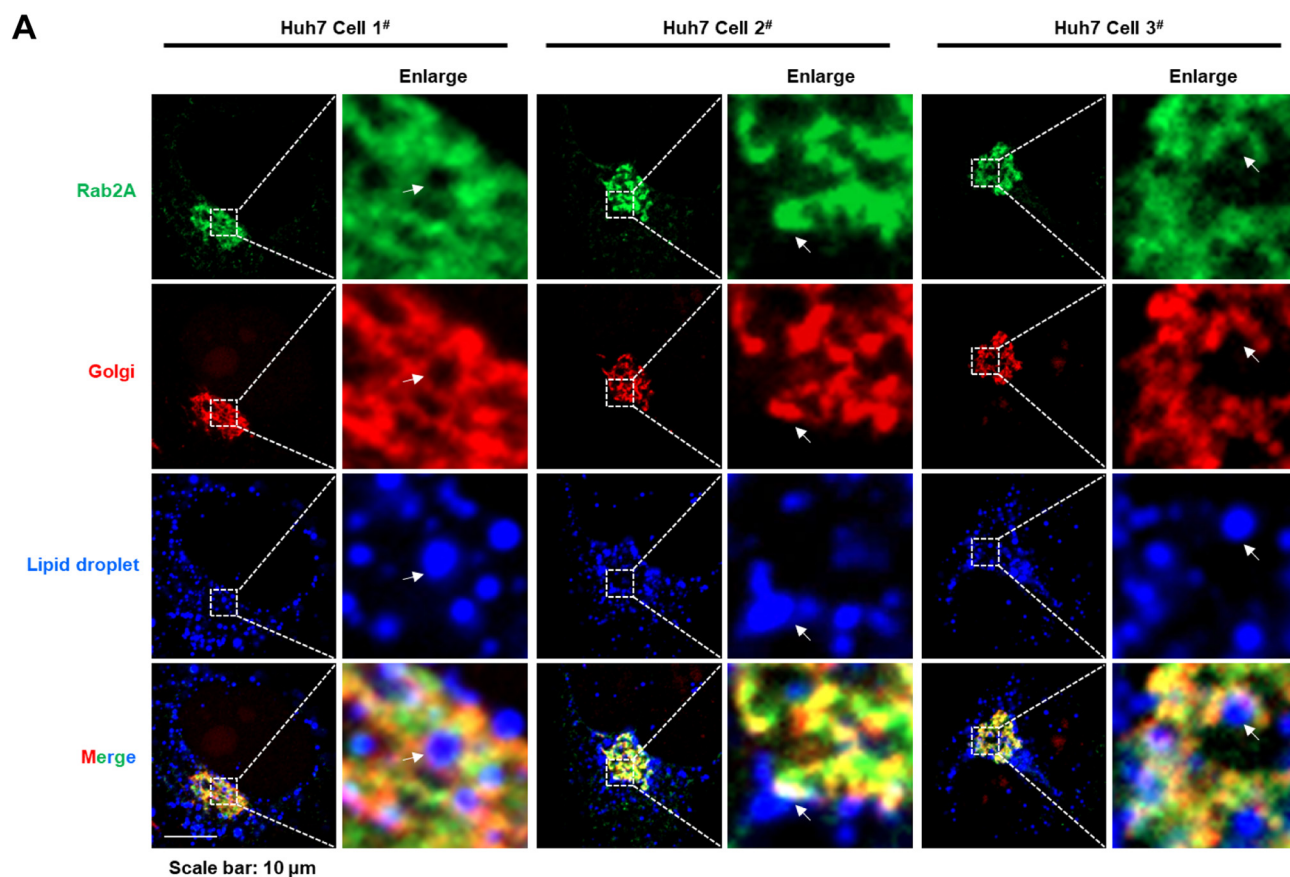


Figure EV2. Golgi-localized Ras-related protein Rab-2A (Rab2A) contacts with LDs.

(A, B) Immunofluorescence analysis elucidated Rab2A-mediated organelle contacts between Golgi and LDs in Huh7 cells (A) and primary hepatocytes (B) with overexpression of EGFP-Rab2A plasmids. Golgi was labeled using a primary antibody targeting GM130, while LDs were stained with Bodipy (The white arrow indicates different types of contact points).

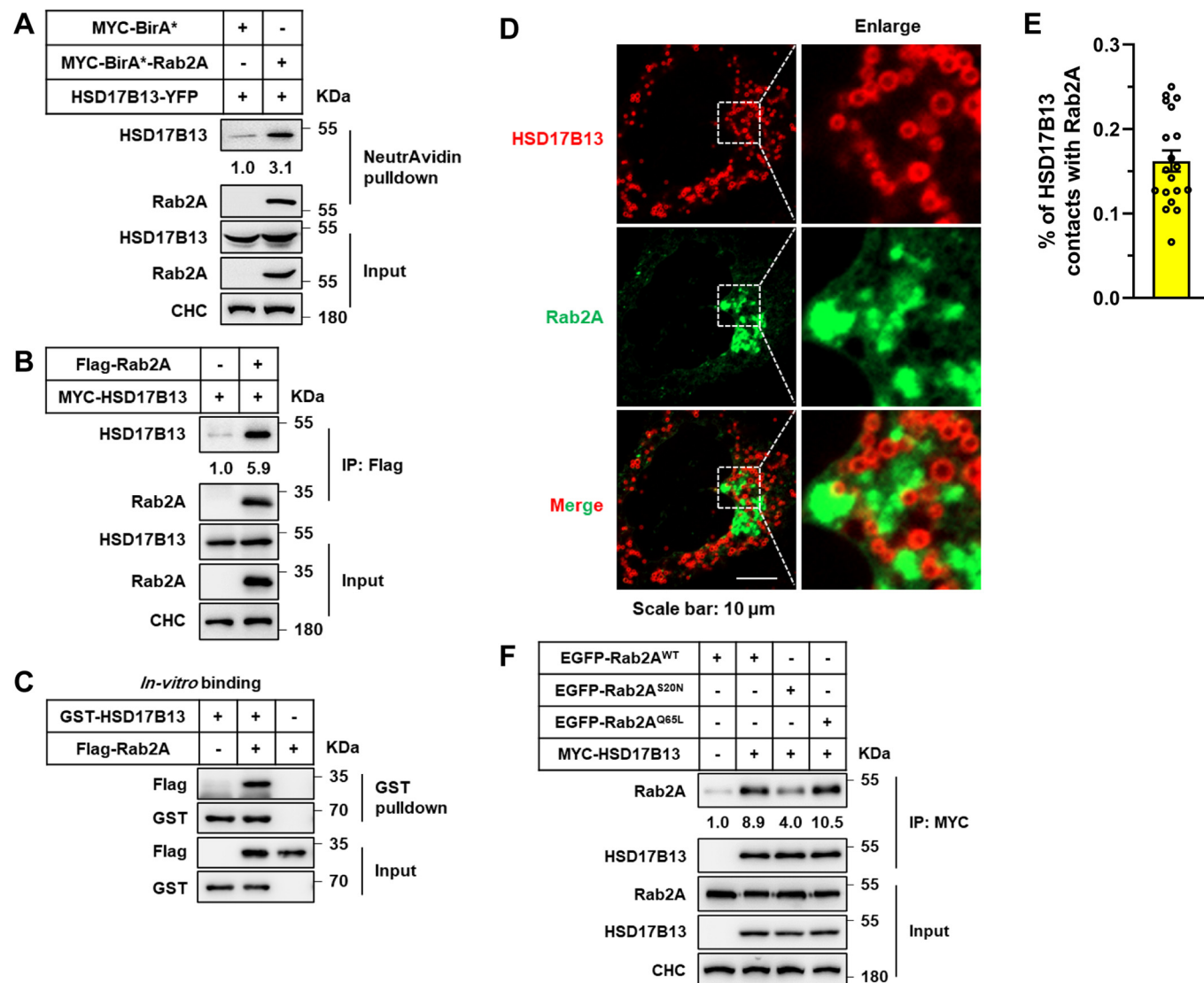
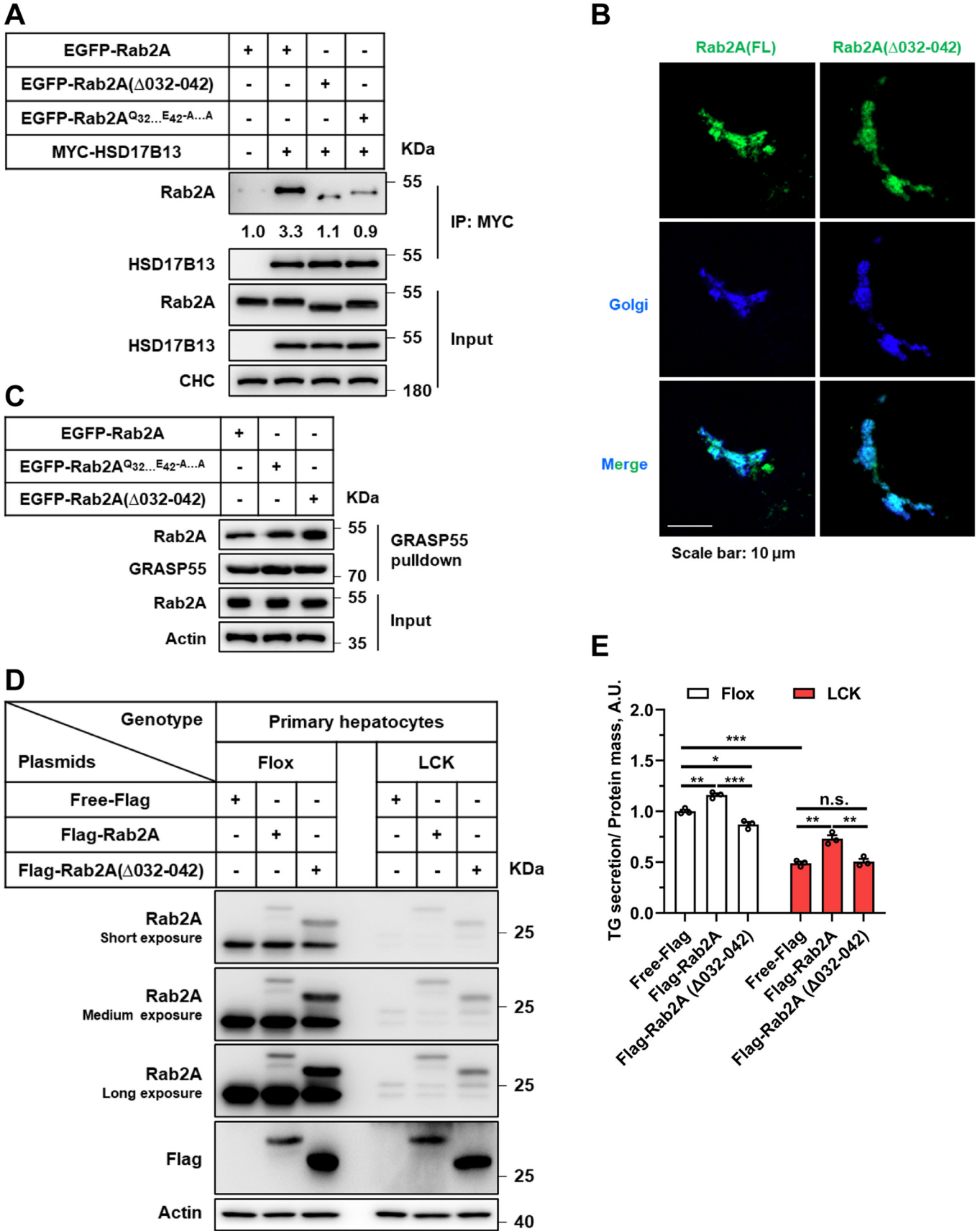


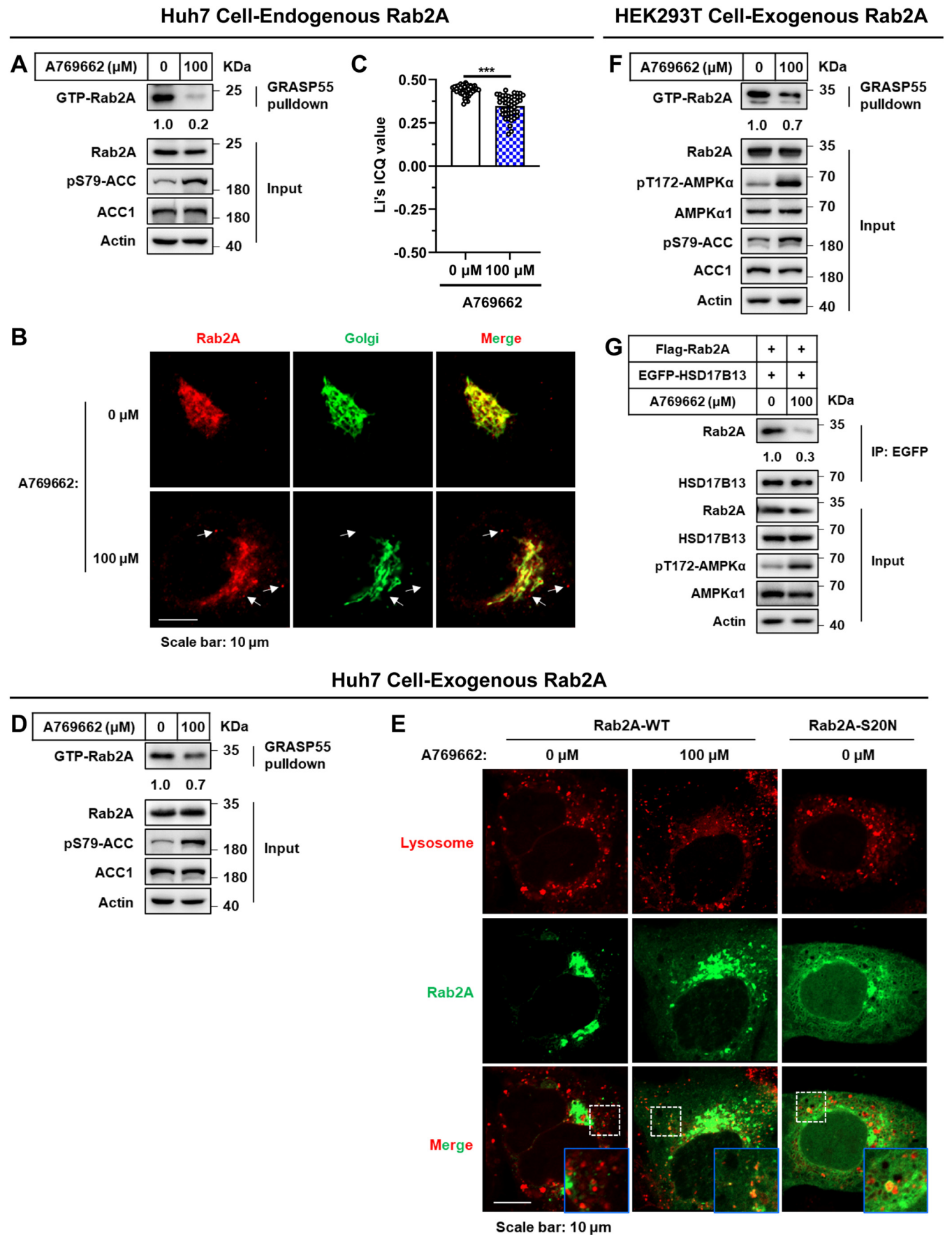
Figure EV3. Confirming the binding efficiency between Rab2A and 17-beta-hydroxysteroid dehydrogenase 13 (HSD17B13) using exogenously expressed Rab2A and HSD17B13.

(A) HEK293T cells were transfected with the BirA*-Rab2A and HSD17B13 plasmids. Rab2A-HSD17B13 binding was evaluated via NeutrAvidin beads pulldown, followed by visualization using Western blotting. (B) HEK293T cells were transfected with Flag-Rab2A and MYC-HSD17B13 plasmids, followed by a Flag-affinity beads pulldown assay to confirm Rab2A and HSD17B13 binding. (C) *In vitro* binding assay was conducted using purified GST-HSD17B13 and Flag-Rab2A proteins. (D, E) Huh7 cells underwent transfection with HSD17B13-mCherry and EGFP-Rab2A plasmids. Immunofluorescence was employed to observe the binding efficiency between Rab2A and HSD17B13. Representative images are shown (D), and the percentage of HSD17B13 contacting with Rab2A was quantified (E) ($n = 19$ cells). (F) The regulatory effect of Rab2A activity on the binding between Rab2A and HSD17B13 was examined in HEK293T cells by transfecting with different types of Rab2A plasmids, followed by MYC-affinity beads pulldown assay. Data information: Data in (E) are presented as mean $\pm$ SEM. Circles in (E) correspond to individual Huh7 cell. Source data are available online for this figure.



◀ **Figure EV4. Blocking the binding between Rab2A and HSD17B13 using a Rab2A mutant protein suppresses very-low-density lipoprotein (VLDL) secretion in primary hepatocytes.**

(A–E) Examination of the effects of Rab2A and HSD17B13 binding on TG secretion involving multiple steps. First, vital amino acids for complex binding in Rab2A were identified via Western blotting in HEK293T cells after transfection with relative plasmids (A). Second, the localization of mutant Rab2A protein was assessed using immunofluorescence in Huh7 cells following transfection with EGFP-Rab2A plasmids, with Golgi labeling accomplished using RCAS1-BFP (B). Third, the activity of both wild-type and mutant Rab2A proteins was detected using a GST-GRASP55 pulldown assay to assess influence of specific mutations on Rab2A's activity (C). Finally, the impact of deficient complex binding on TG secretion was evaluated in Flox and LCK primary hepatocytes after protein overexpression with the AAV-virus system (D). The medium was collected for testing, with values in Flox samples with Free-Flag overexpression normalized to 1 (E) (Flox hepatocytes (Free-Flag vs. Flag-Rab2A), $P = 0.0025$; Flox hepatocytes (Free-Flag vs. Flag-Rab2A ($\Delta 032-042$)), $P = 0.0101$; Flox hepatocytes (Flag-Rab2A vs. Flag-Rab2A ($\Delta 032-042$)), $P = 0.0005$; Free-Flag (Flox vs. LCK), $P < 0.0001$; LCK hepatocytes (Free-Flag vs. Flag-Rab2A), $P = 0.0030$; LCK hepatocytes (Flag-Rab2A vs. Flag-Rab2A ($\Delta 032-042$)), $P = 0.0063$). Data information: Data in (E) are presented as mean $\pm$ SEM. Circles in (E) correspond to individual test. P values in (E) were determined using unpaired two-tailed Student's t -test. n.s. indicates no significant difference ($P > 0.05$), * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$.



◀ **Figure EV5. A769662 stimulation attenuates Rab2A activity, Golgi-localization and Rab2A-HSD17B13 binding.**

(A–C) Huh7 cells were stimulated with A769662 (100 μ M), an AMPK agonist, for 4 h in DMEM medium without fetal bovine serum (FBS). Rab2A activity was evaluated using the GRASP55 pulldown assay (A). Its subcellular localization was assessed with immunofluorescence (B). Endogenous Rab2A was stained with a primary antibody, and the Golgi apparatus was labeled with a primary antibody against GM130. The dispersion of Rab2A signaling was quantified via Li's ICQ value (Li's colocalization value) in Image J ($n = 44$ vs. 44 cells), with white arrows indicating potential signaling away from the Golgi (C) ($P < 0.0001$). (D, E) Huh7 cells, transfected with exogenously expressing Rab2A, were stimulated with A769662 (100 μ M) for 4 h in DMEM medium without FBS. The activity of Rab2A was assessed using a GRASP55 pulldown assay and analyzed by Western blotting (D). The subcellular localization of Rab2A was evaluated with immunofluorescence, with lysosomes stained using LysoTracker (E). (F, G) HEK293T cells were transfected with exogenously expressing Rab2A plasmids, with or without co-transfection of HSD17B13 plasmids, and subsequently stimulated with A769662 (100 μ M) for 4 h in DMEM medium without FBS. Rab2A activity was evaluated using the GRASP55 pulldown assay (F). The binding efficiency of Rab2A-HSD17B13 complex was assessed by an EGFP-affinity beads pulldown assay (G). Data information: Data in (C) are presented as mean $\pm$ SEM. Circles in (C) correspond to individual cell. P value in (C) was determined using unpaired two-tailed Student's t -test. *** indicates $P < 0.001$.