Figure S1. Process of screening the RPL9 protein. (A) The statistical graph depicts the differential protein expression among the three groups. (B) The differentially expressed RNA-binding proteins were shown by VN plots (P<0.01). After conducting a comprehensive analysis of gene expression, disparities and current research progress, the RPL9 gene was identified as the focal point for our subsequent investigation. RPL9, ribosomal protein L9; HCC, hepatocellular carcinoma; CA, serum exosomes proteins derived from HCC patients with TNM stage I/II; CB, serum exosomes proteins derived from HCC patients with TNM stage III/IV; N, serum exosomes proteins from patients with benign liver disease.

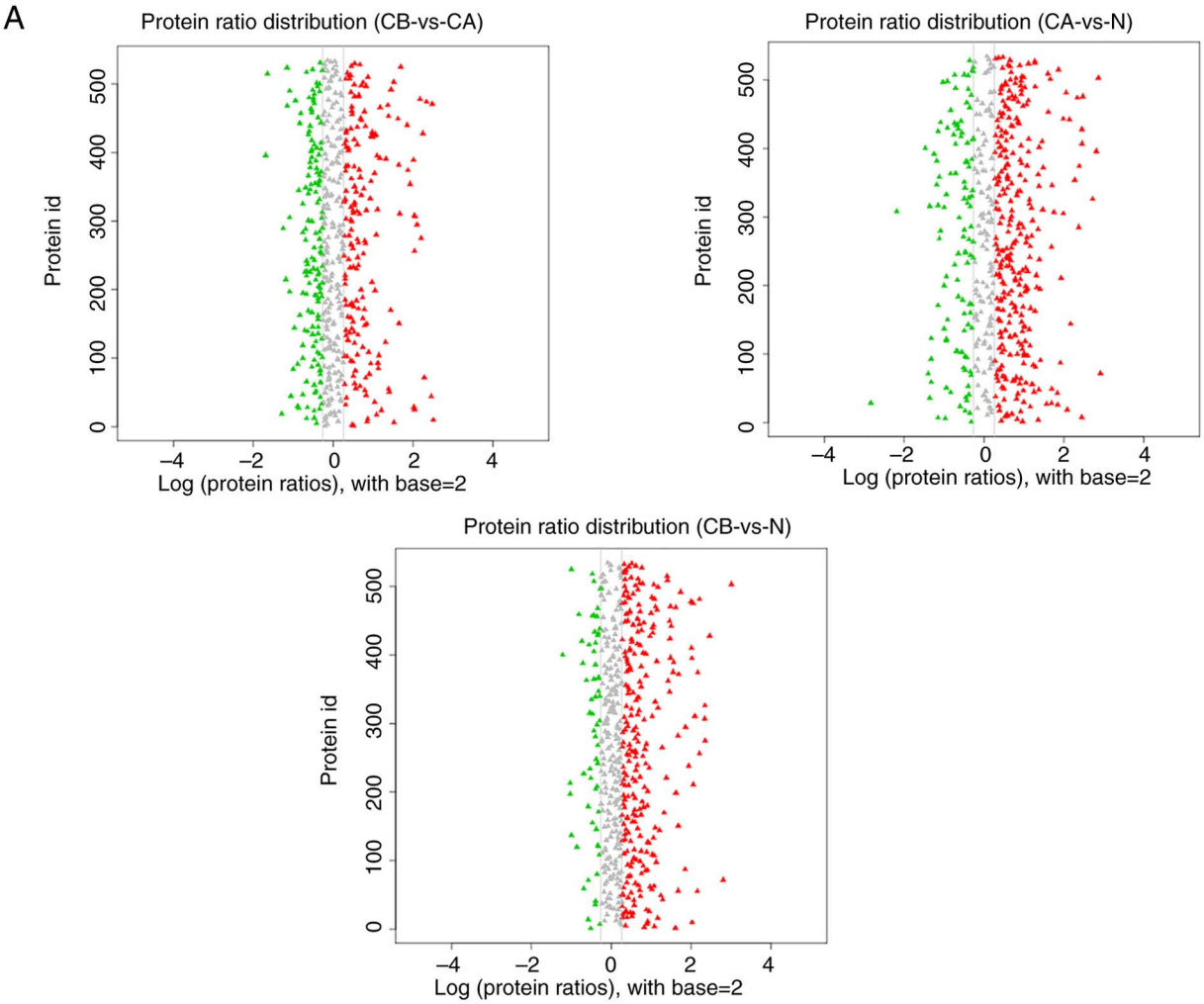


Figure S2. Downregulation of RPL9 expression causes alterations in certain miRNAs. miRNA microarray technique identified 19 statistically significant miRNAs. The present study downregulated the expression of RPL9 in MHCC97H cells and found that the differences of these miRNAs were inconsistent between cells and exosomes. (A and C) The content of miR-24-3p and miR-185-5p in cells and exosomes were decreased and it was hypothesized that they might be more related to RPL9 (nsP>0.05, *P<0.05, **P<0.01, ***P<0.001). (B and D) Some miRNAs were increased or unchanged in cells but decreased in exosomes, suggesting that downregulation of RPL9 may inhibit the trafficking of miRNAs from cells to exosomes. This experiment was subsequently repeated with Huh7 cells with similar results (*P<0.05, **P<0.01, ***P<0.001). RPL9, ribosomal protein L9; miRNA/miR, microRNA; ns, no significance.

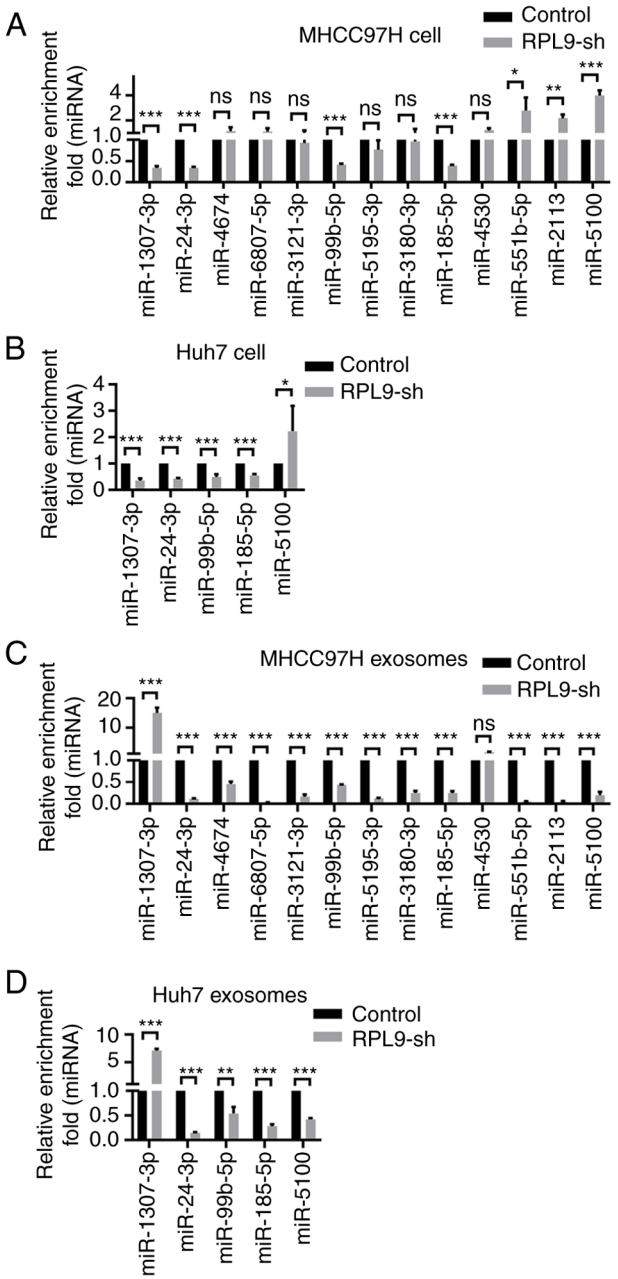


Figure S3. miRNAs that can bind to RPL9. (A) RIP assays were performed using serum exosomes from HCC patients as lysis substrates, RT-qPCR showed significant differences between several miRNAs bound by RPL9 antibody and those bound by IgG antibody (nsP>0.05, *P<0.05, ***P<0.001). (B and C) RIP experiments were performed using MHCC97H cells (B) or Huh7 cells (C) as substrate and RT-qPCR showed significant differences between several miRNAs bound by RPL9 antibody and those bound by IgG antibody (nsP>0.05, *P<0.05, **P<0.01, ***P<0.001). miRNA/miR, microRNA; RPL9, ribosomal protein L9; RIP, RNA immunoprecipitation; HCC, hepatocellular carcinoma; RT-qPCR, reverse transcription-quantitative PCR; ns, no significance.

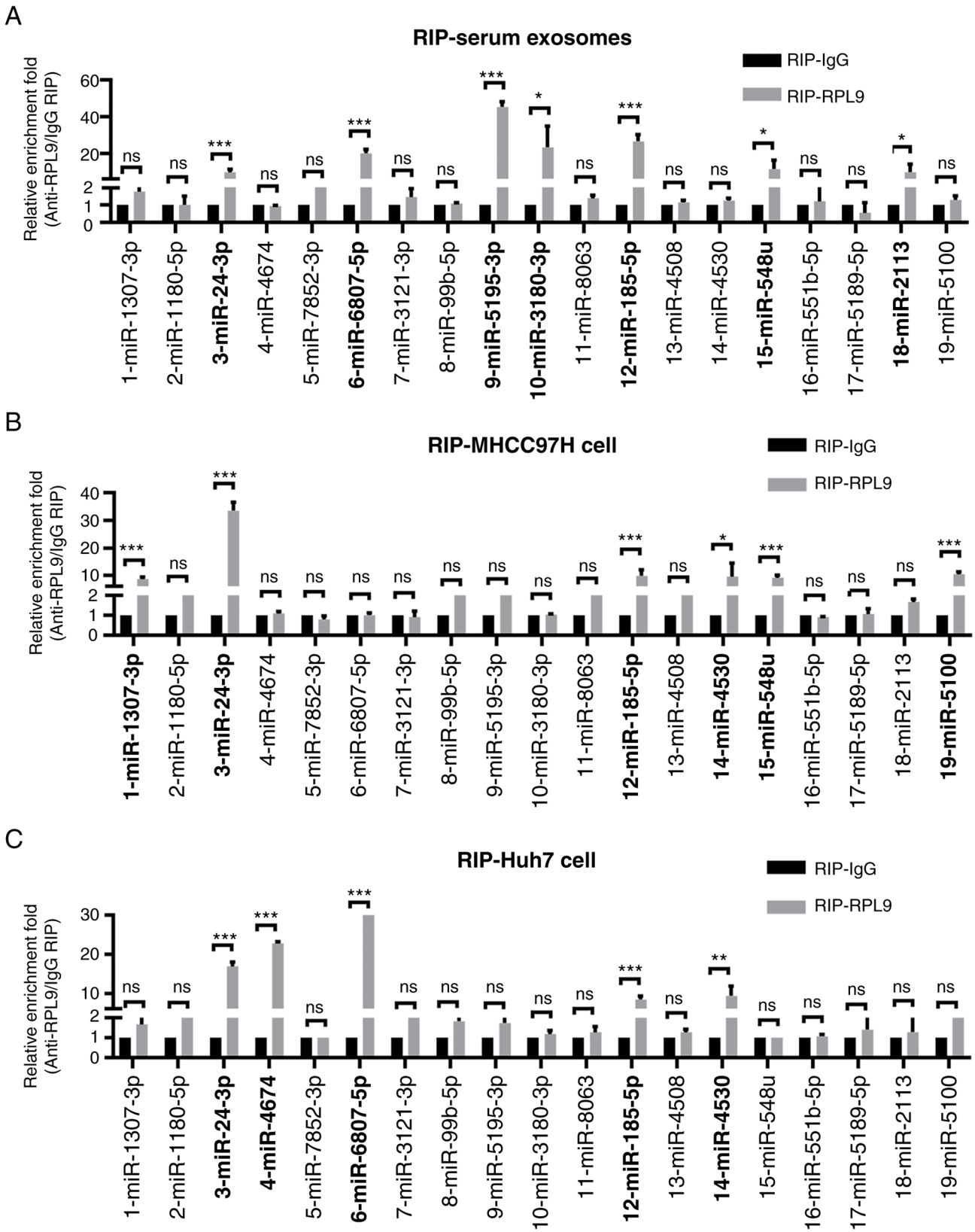
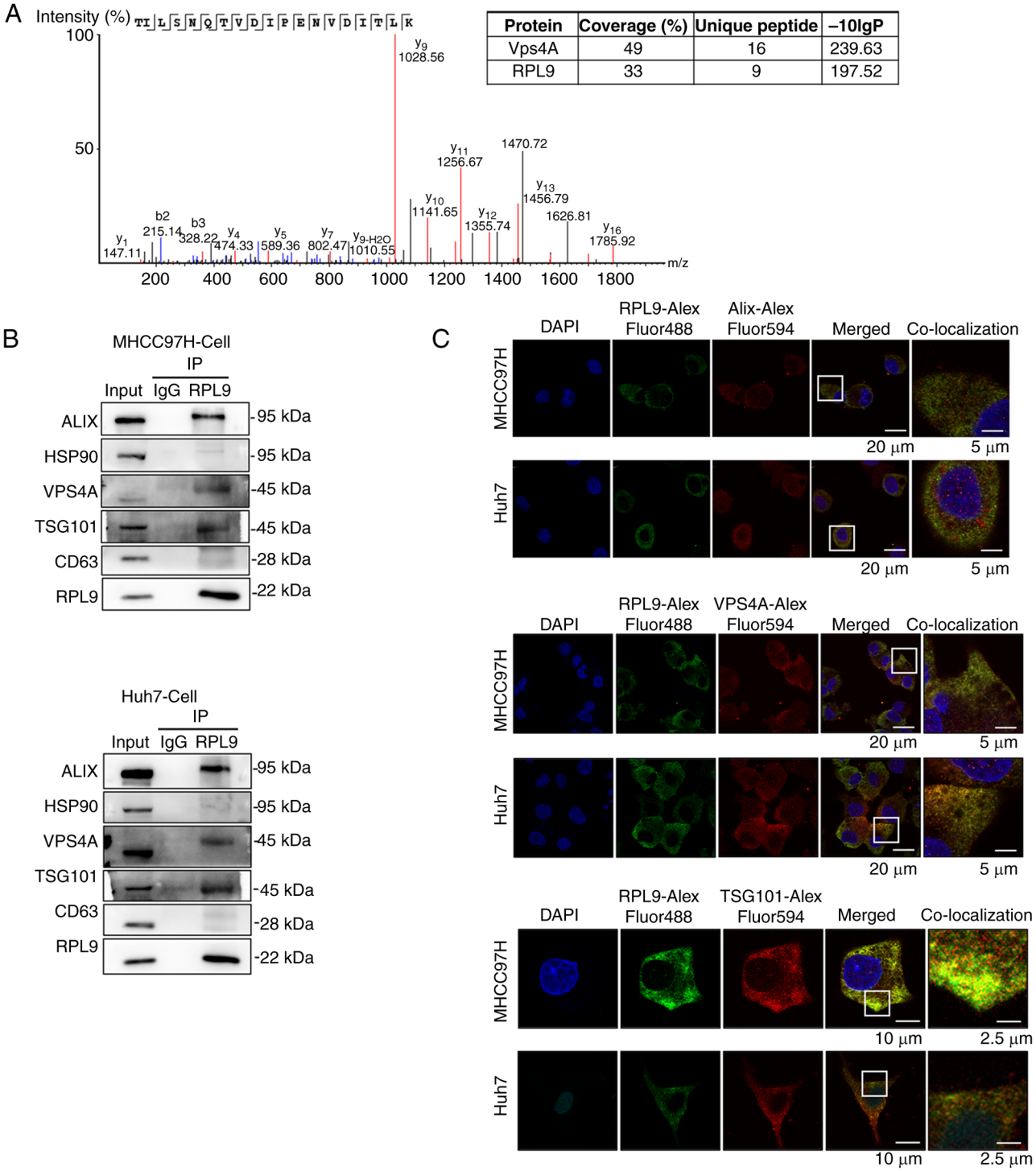


Figure S4. ESCRT-related proteins are capable of interacting with RPL9. The present study conducted further investigation into the potential mechanism by which RPL9 enters exosomes. (A) We have previously reported that Vps4A can induce changes in the miRNA profile of liver cancer exosomes and contribute to the packaging of proteins into exosomes. In that experiment, we used the Huh7 cell line for Vps4A IP combined with mass spectrometry analysis and successfully identified multiple proteins that interacted with Vps4A, including RPL9. (B) IP-western blotting experiments using the RPL9 antibody found that RPL9 not only interacts with Vps4A *in vitro* but also binds to the components of ESCRT-I (TSG101) and the ESCRT-III-associated protein (Alix). (C) Immunofluorescence experiments in Huh7 and MHCC97H cells, demonstrated the co-localization of RPL9 with Vps4A, TSG101 and Alix. Based on these findings, it was hypothesized that Alix, Vps4A and TSG101 may play crucial roles in regulating the entry of RPL9 into exosomes. ESCRT, endosomal sorting complexes required for transport; RPL9, ribosomal protein L9; miRNA/miR, microRNA; IP, immunoprecipitation.



Protein	Coverage (%)	Unique peptide	−10lgP
Vps4A	49	16	239.63
RPL9	33	9	197.52

Figure S5. When silencing ESCRT-related genes, the quantity of RPL9 protein in exosomes significantly decreases. siRNA (CON, Alix-si, Vps4A-si, TSG101-si) was designed to investigate the effect of protein expression levels on RPL9 content in exosomes. (A) Reverse transcription-quantitative PCR (*P<0.05, **P<0.01, ***P<0.001) and (B) western blotting confirmed significant inhibition of Alix, Vps4A and TSG101 at both mRNA and protein levels. At this point, there was only a slight increase in RPL9 within cells. It was hypothesized that as a ribosome-binding protein, RPL9 has a higher basal expression level and represents only a small proportion when it enters exosomes as cargo. When Alix, Vps4A or TSG101 was inhibited in the cells, the entry of RPL9 into exosomes was blocked and RPL9 accumulates in the cells. (C) In exosomes, differential changes in RPL9 protein content was observed in exosomes secreted by MHCC97H and Huh7 cells after knockdown of Alix, TSG101 and Vps4A gene expression. With the silencing of Alix, TSG101 and Vps4A genes, the amount of RPL9 protein in exosomes was significantly reduced. It was hypothesized that Alix, Vps4A and TSG101 may be key proteins regulating the entry of RPL9 into exosomes. ESCRT, endosomal sorting complexes required for transport; RPL9, ribosomal protein L9; miRNA/miR, microRNA; Con, control; Exo, exosomes; si, short interfering.