

Figure 1

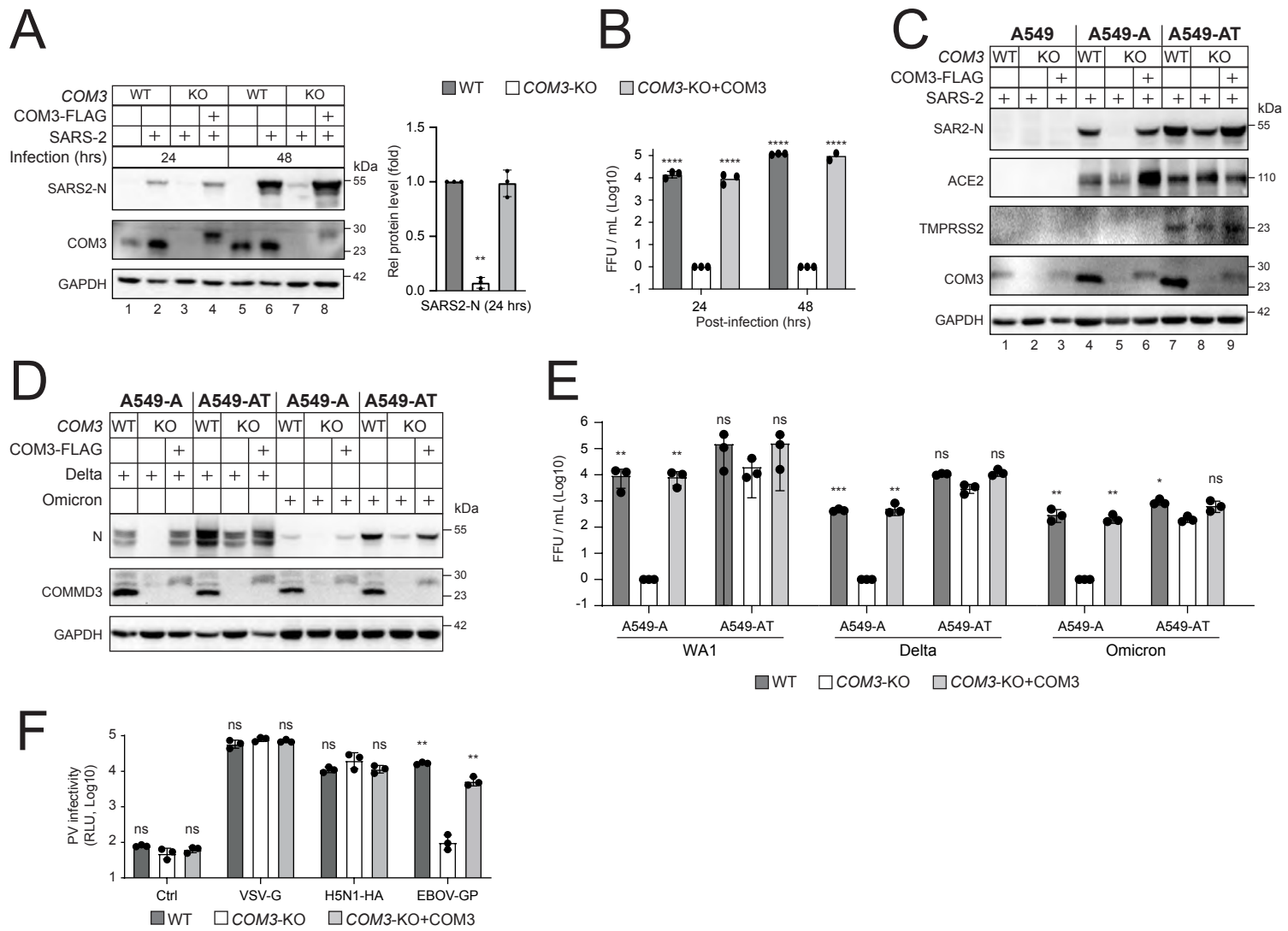


Figure 2

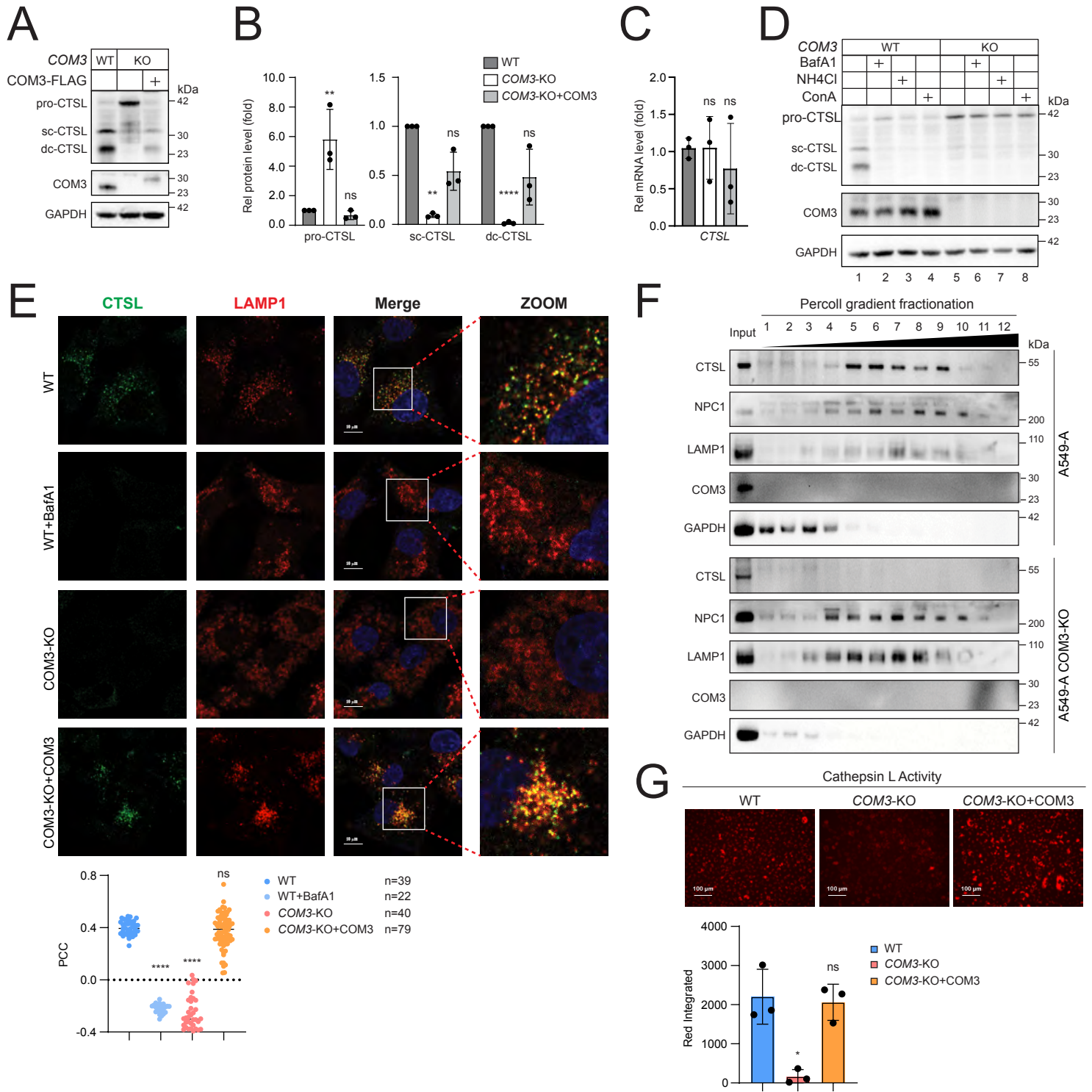




Figure 3

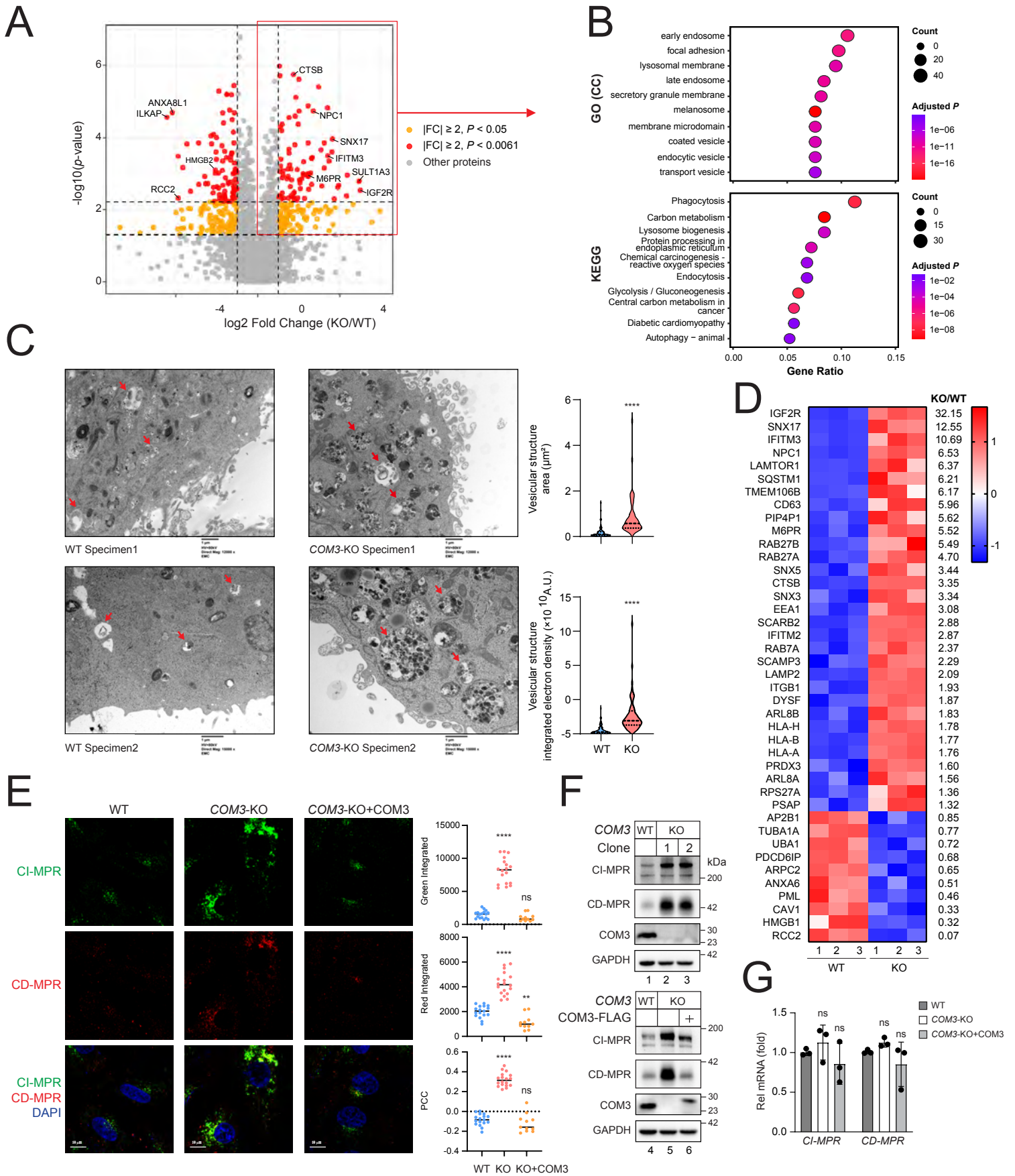


Figure 4

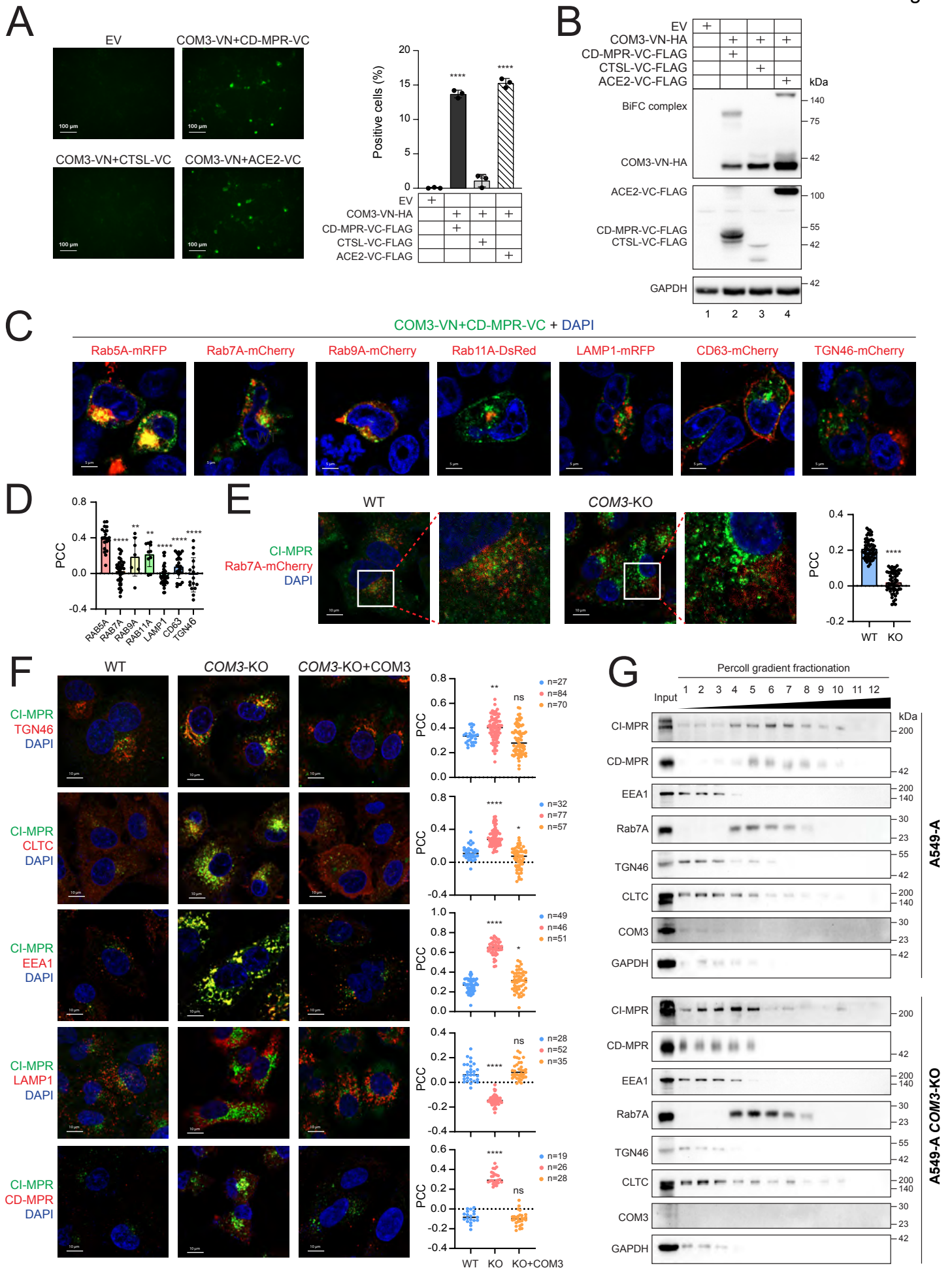


Figure 5

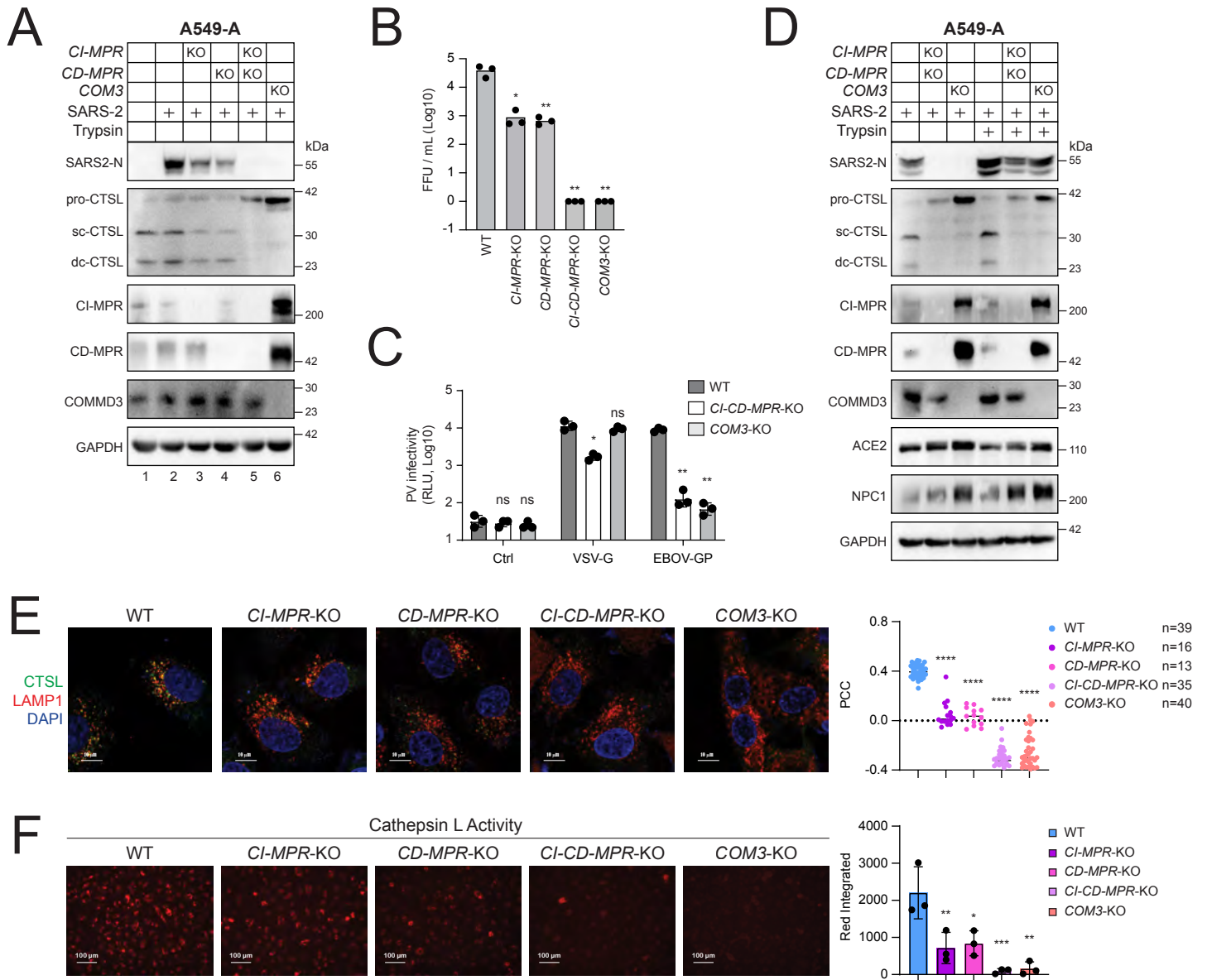




Figure 6

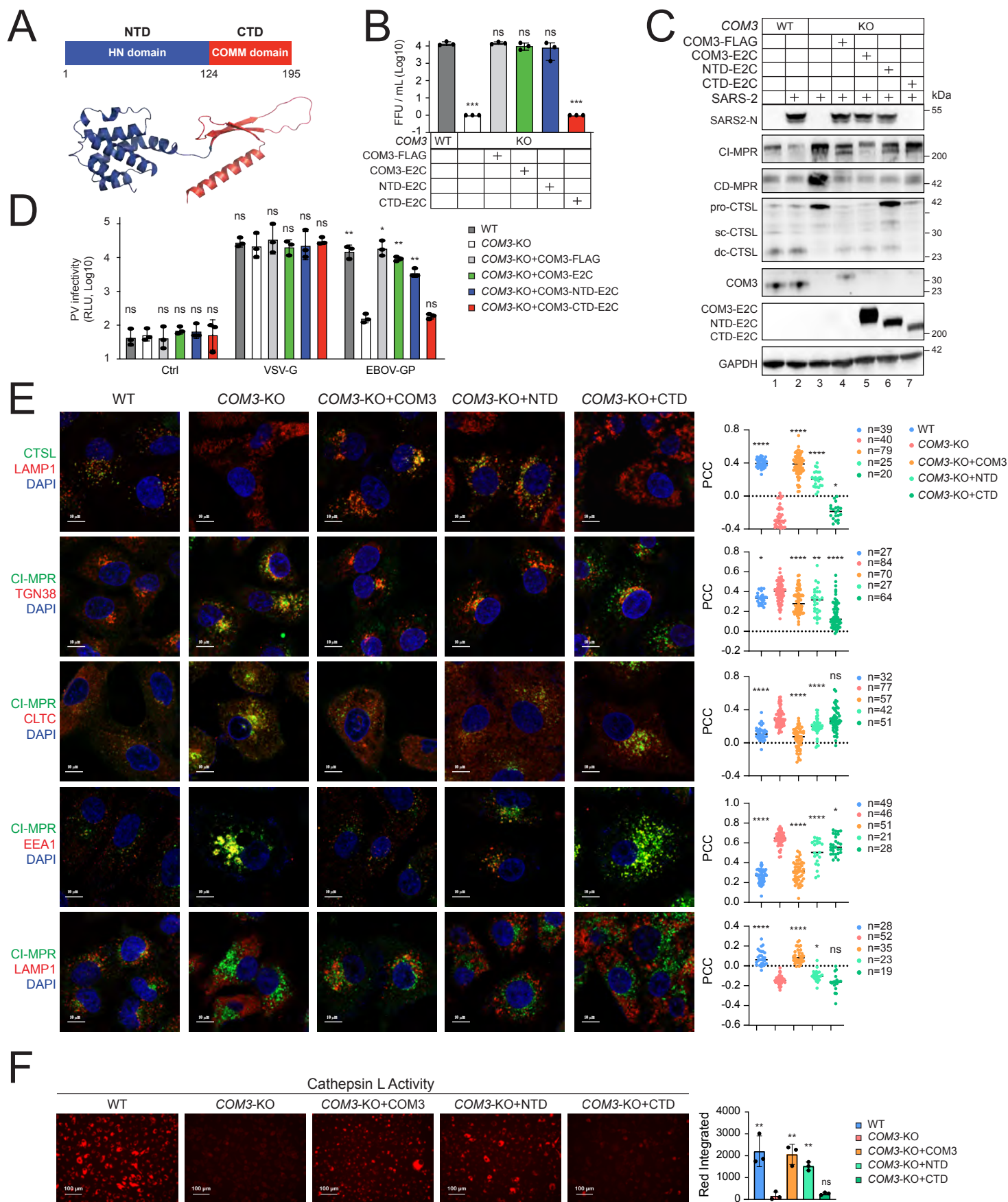
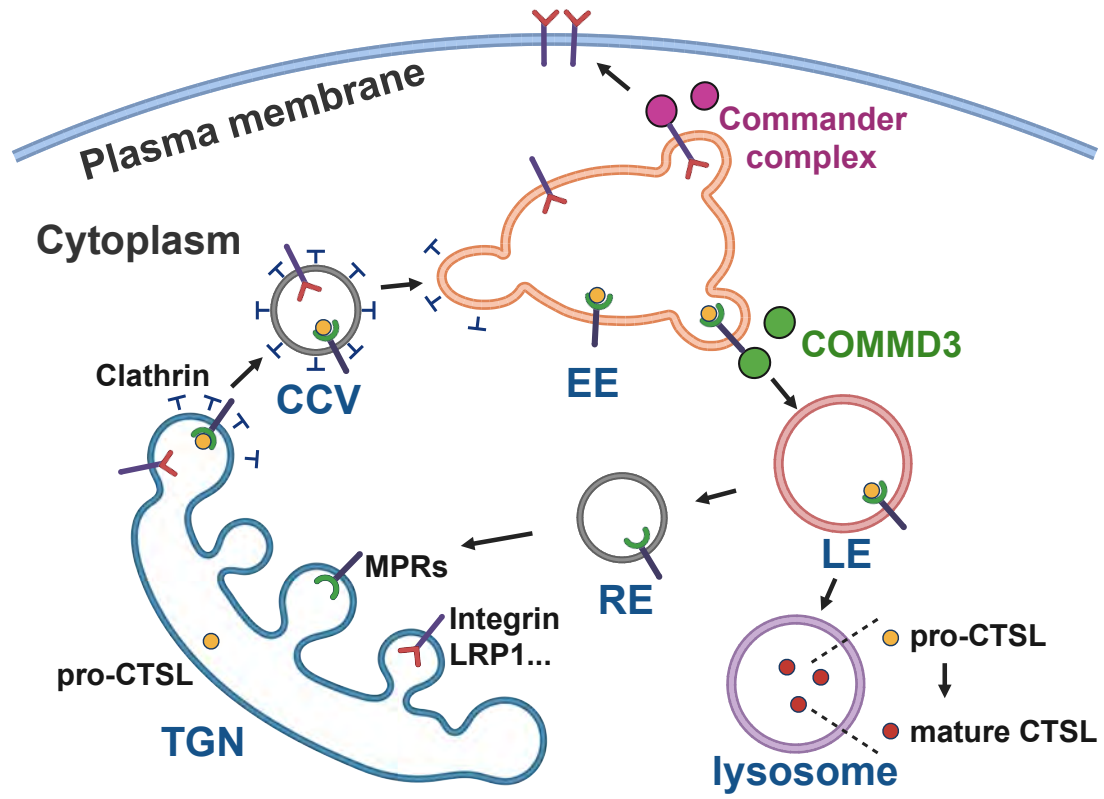
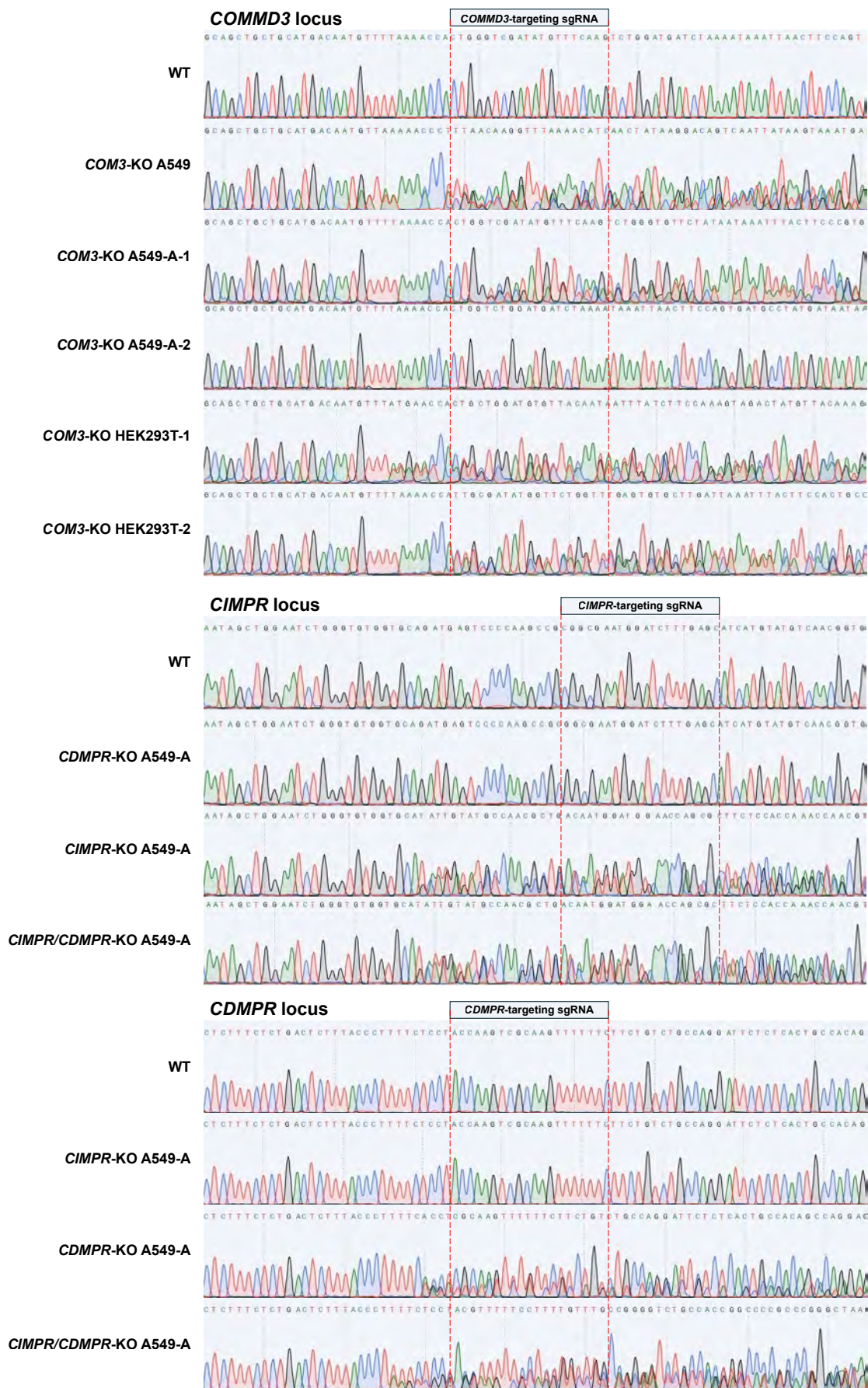


Figure 7

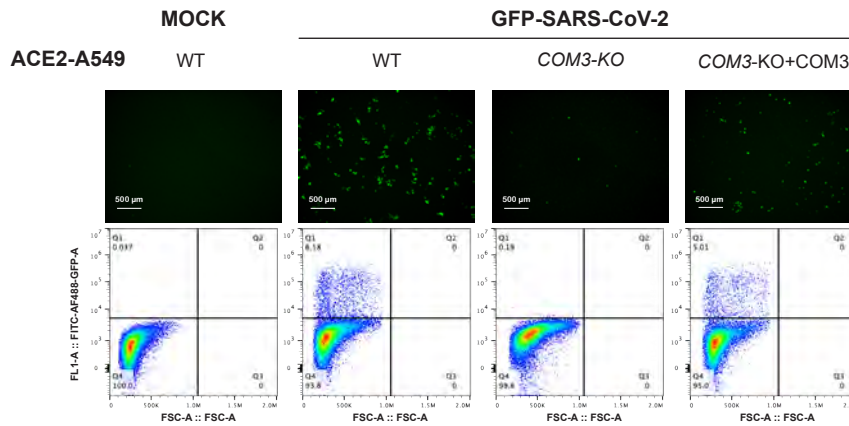




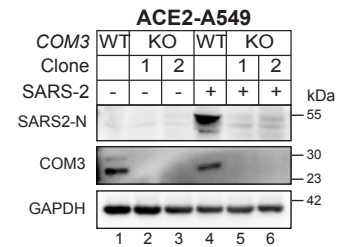
**Fig. S1. Sanger sequencing chromatograms of indicated knockout cell lines.**



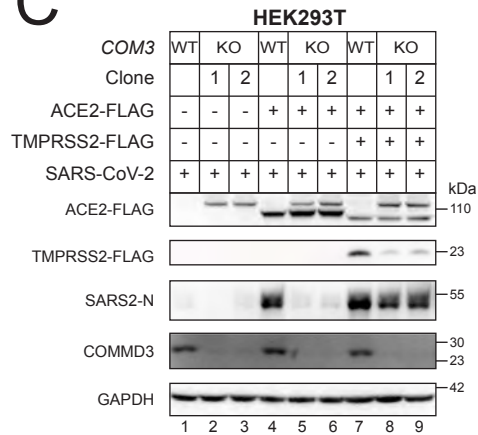
A



B



C

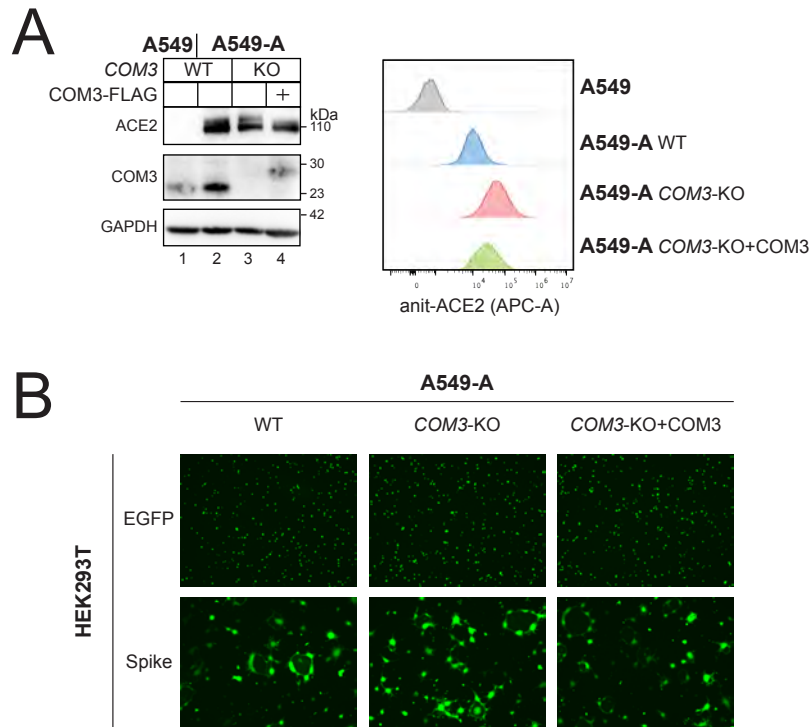


**Fig. S2. COMMD3 knockout impaired SARS-CoV-2 endocytic entry.**

**A.** Representative fluorescence images of WT, *COM3*-KO, and rescued A549-A cells infected with GFP-expressing SARS-CoV-2 for 24 hours are shown. The percentage of GFP-positive cells quantified by flow cytometry is shown below.

**B.** WT and two independent *COM3*-KO A549-A cells clones were infected with SARS2 WA1 strain at an MOI of 0.1 and harvested at 24, followed by western blot validation of SARS2 N protein, CI-MPR, and CD-MPR expression.

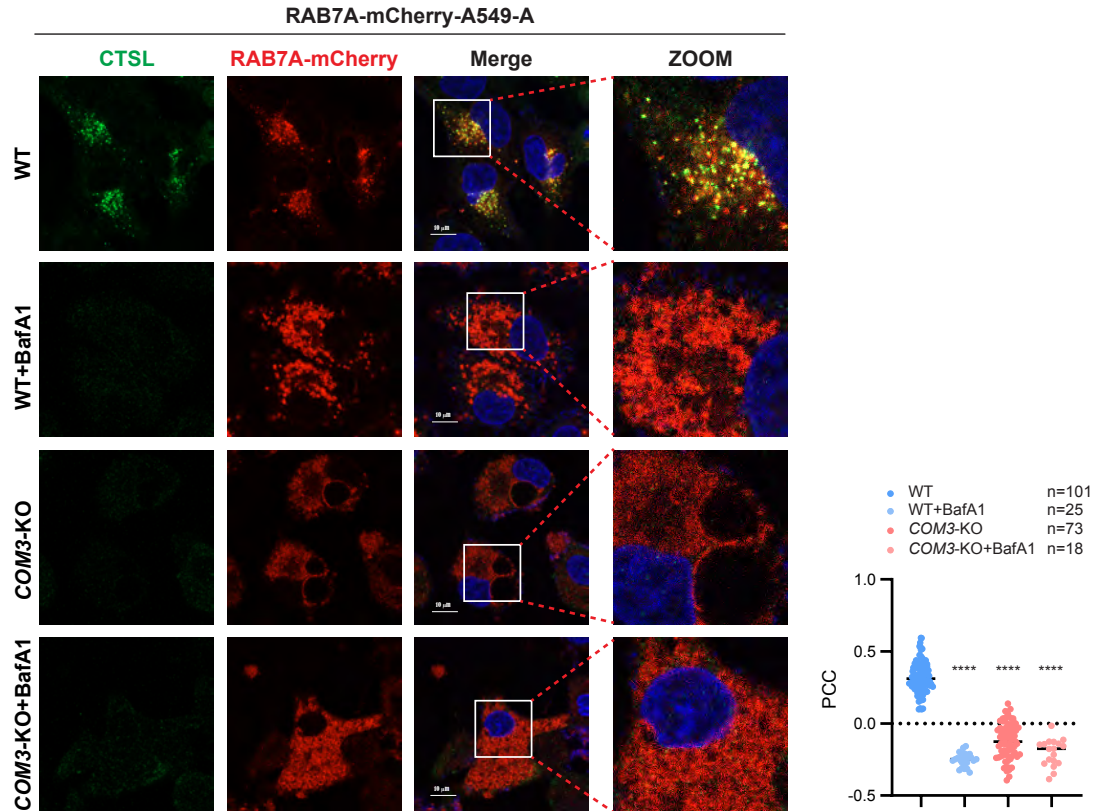
**C.** WT and two independent *COM3*-KO HEK293T cells clones were transfected with an ACE2 construct alone or together with a TMPRSS2 construct for 36 h, followed by infection with the SARS2 WA1 strain at an MOI of 0.1 for 24 h. SARS2 N protein expression was validated by western blotting.



**Fig. S3. COMMD3 knockout does not affect levels of the total or cell-surface ACE2 expression.**

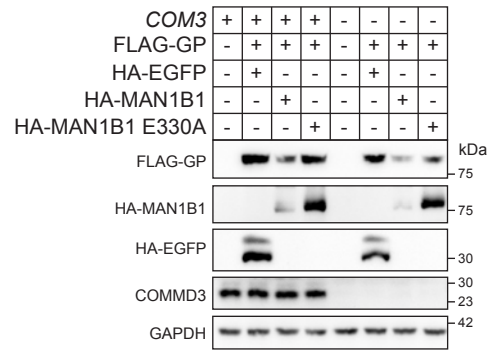
**A.** Western blot validation of ACE2 expression in WT, *COM3*-KO, and rescued A549-A cells, as well as WT A549 cells. The cell-surface ACE2 expression quantified by flow cytometry is shown on the right.

**B.** Representative fluorescence images of the cell-to-cell fusion assay in WT, *COM3*-KO, and rescued A549-A cells. HEK293T cells were transfected with an EGFP-expression vector alone or together with a SARS-CoV-2 Spike-expression vector for 36 h, followed by co-culture with the A549-A cells for 9 h.



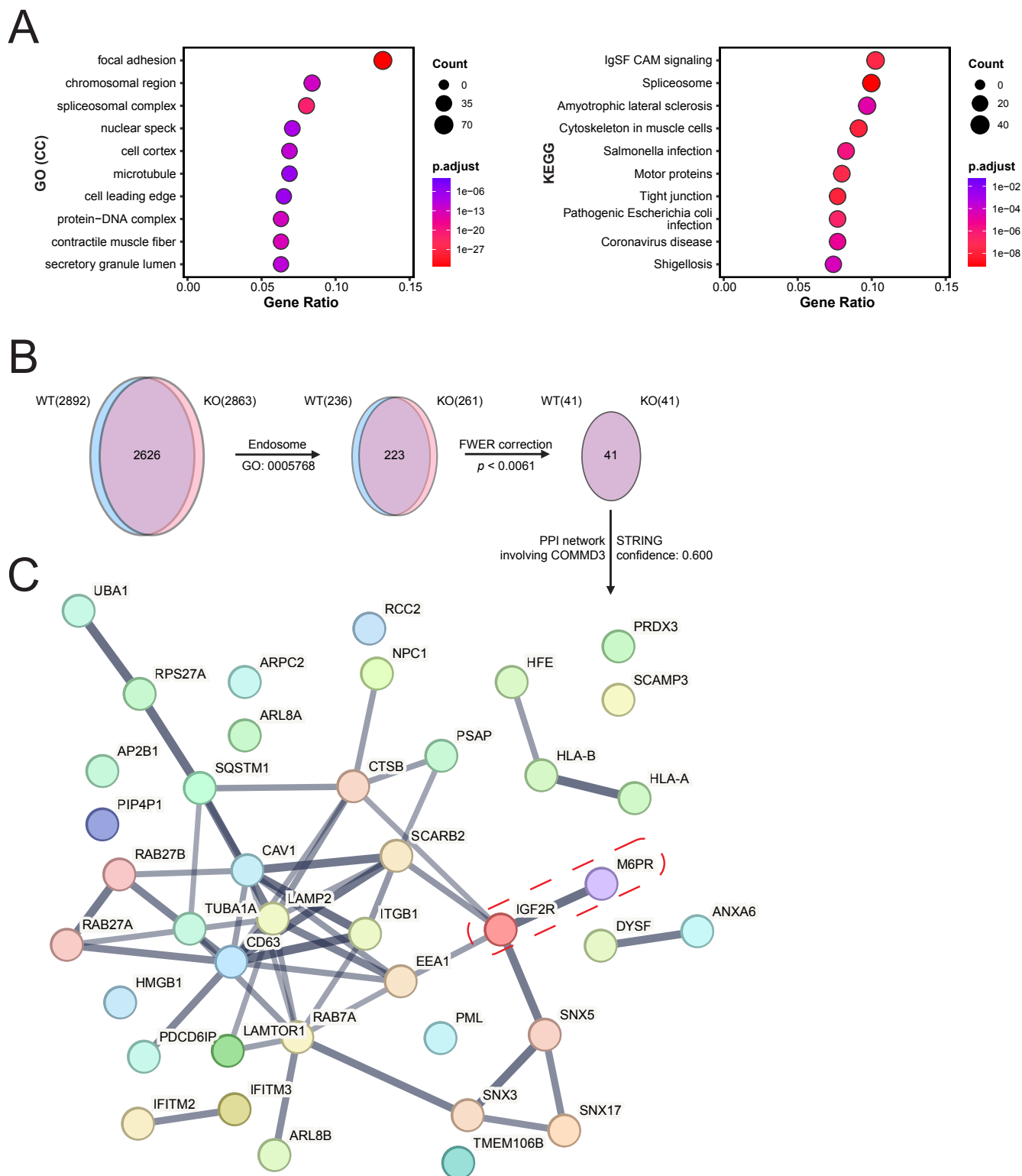
**Fig. S4. COMMD3 knockout alters CTSL colocalization with RAB7A.**

Representative immunofluorescence images of WT and *COM3*-KO RAB7A-mCherry-expressing A549-A cells with or without BafA1 treatment. Green, CTSL; red, RAB7A-mCherry; blue, DAPI. PCC quantification of CTSL colocalization with LAMP1 in indicated cells is shown on the right. PCC was calculated using an intensity threshold of 0.05. Each dot represents one field of view. Sample sizes are indicated in the figure. One-way ANOVA followed by Dunnett's test using the WT group as the control. \*\* $P < 0.01$ .



**Fig. S5. MAN1B1-dependent lysosomal degradation is preserved in COM3-KO cells.**  
 HEK293T cells were transfected with the indicated expression vectors and harvested at 36 h after transfection, followed by western blot validation.



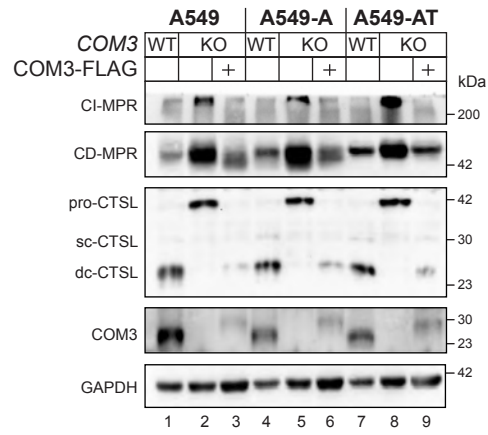


**Fig. S6. Proteomic identification of endosome-associated proteins regulated by COMMD3.**

**A.** Gene Ontology (GO) Cellular Component (CC) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of significantly downregulated proteins ( $P < 0.05$  in *COM3*-KO compared with WT).

**B.** Schematic of the filtering strategy used to identify endosome-associated proteins.

**C.** STRING protein-protein interaction (PPI) network of the 41 filtered proteins involving (confidence score  $\geq 0.600$ ).



**Fig. S7. COMMD3 knockout enhances MPRs expression and blocks CTSL maturation.**

Western blot analysis of CI-MPR, CD-MPR and CTSL expression in WT, COM3-KO, and rescued A549, A549-A and A549-AT cells.