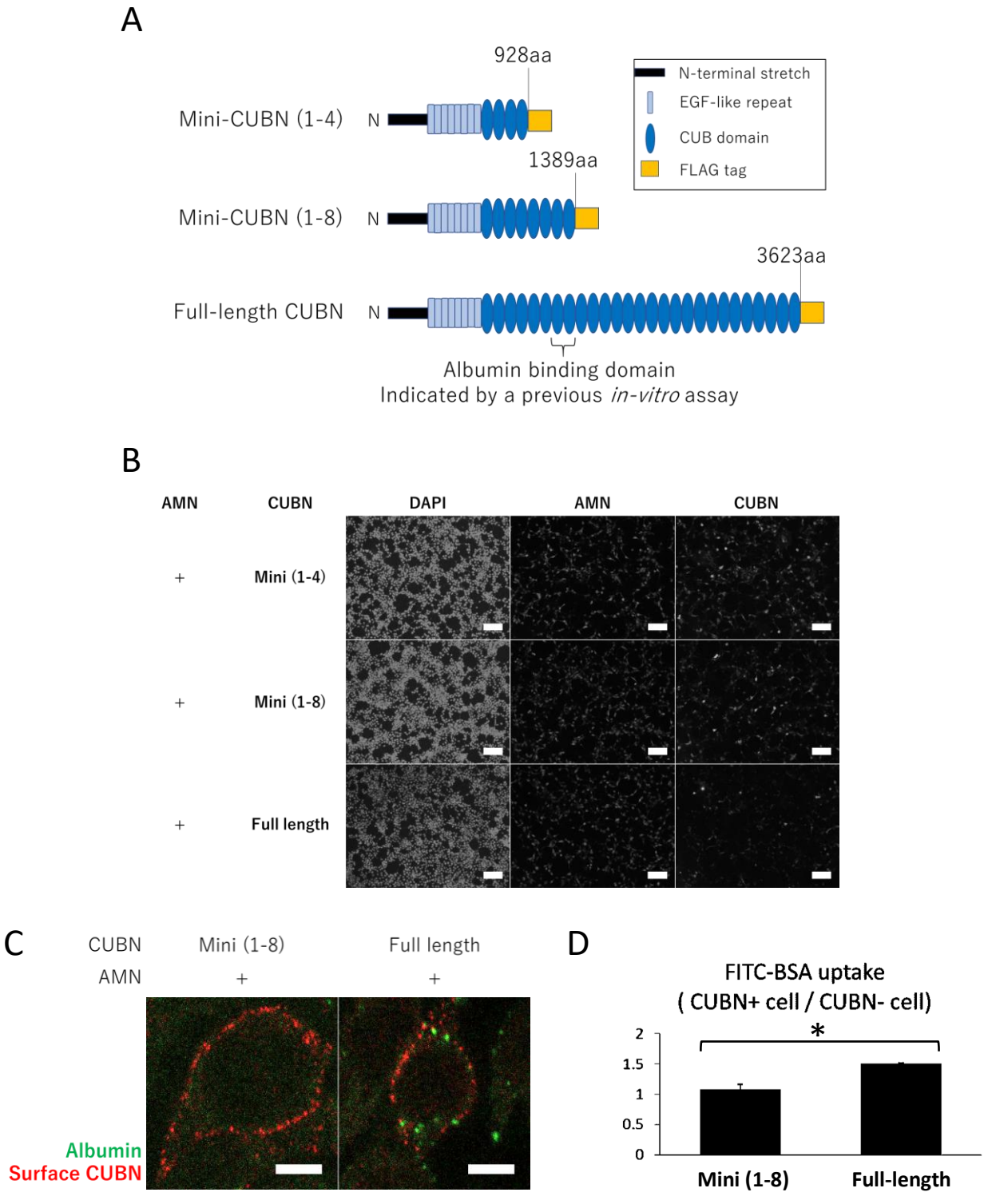

Figure S1. A CUBN segment stretching from the N-terminus to CUB 8 domain fails to facilitate cellular albumin uptake.

A Schematic representation of mini-CUBN (1–4), mini-CUBN (1–8), and full-length CUBN.

B Expression of FLAG-tagged mini-CUBN (1–4), mini-CUBN (1–8), and full-length CUBN co-expressed with myc-GFP-tagged AMN in HEK293T cells. Scale bar = 100 μm.

C FITC-albumin uptake by HEK293T cells expressing mini-CUBN (1–8) or full-length CUBN with AMN. Cells were immunostained for surface CUBN after FITC-albumin uptake. Scale bar = 5 μm.

D Flow cytometric quantification of FITC-albumin uptake by HEK293T cells expressing mini-CUBN (1–8) or full-length CUBN on their surface as in Figure 1E. Data are shown as mean ± s. e. m., n = 3. *$P < 0.05$, two-sided *t*-test.

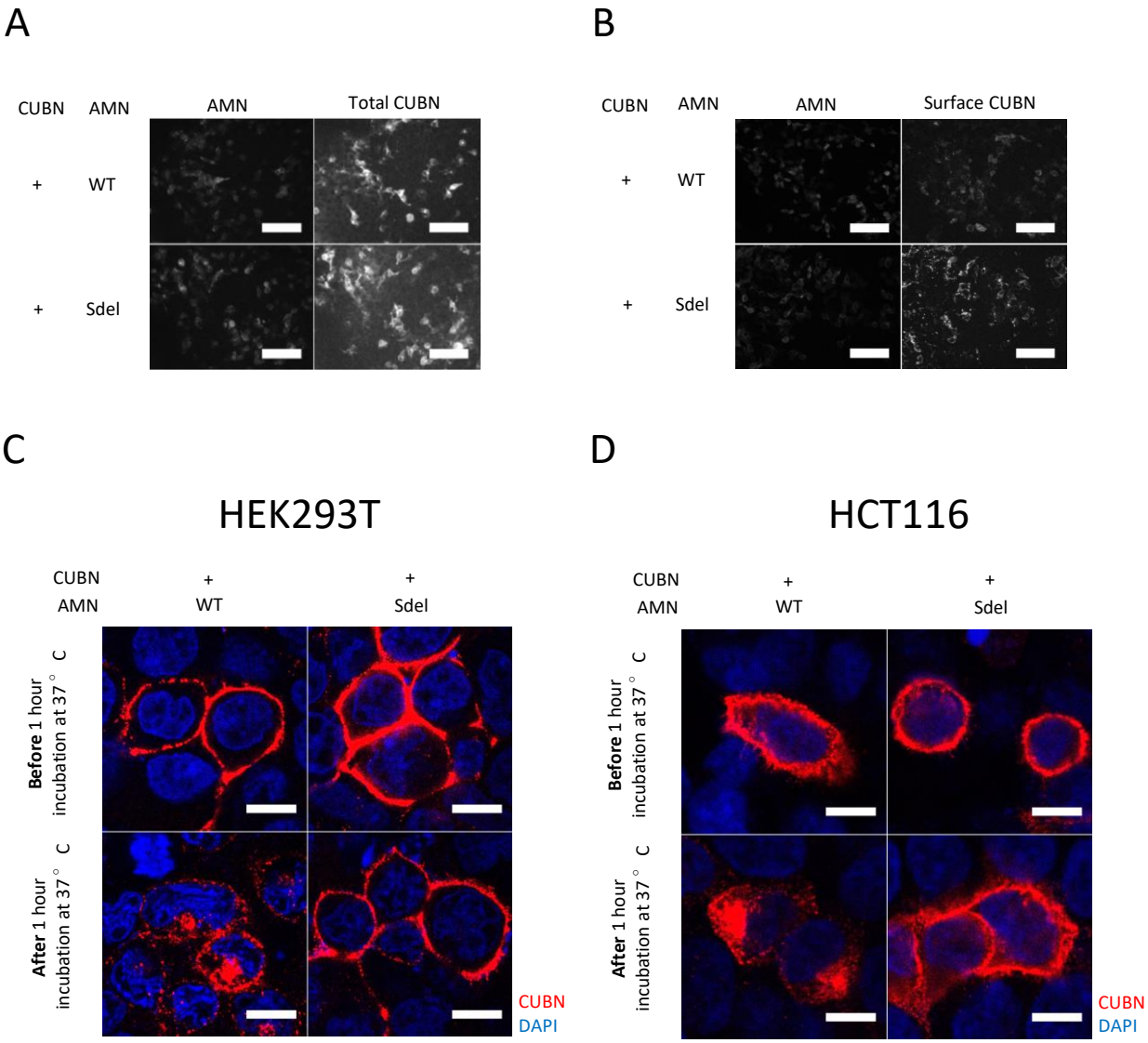

Figure S2. Signal motifs of AMN are critical for CUBN endocytosis in cells with or without endogenous megalin.
A, B Total expression (A) and membrane expression (B) of FLAG-tagged full-length CUBN in HEK293T cells co-expressing myc-GFP-tagged WT-AMN or Sdel-AMN. Scale bar = 100 μm.
C, D HEK293T (C) or HCT116 (D) cells were co-transfected with FLAG-tagged CUBN and myc-tagged WT-AMN or Sdel-AMN. FLAG-CUBN on the cell surface was detected before or after the endocytosis period (37° C, 1 h). Scale bar = 10 μm.

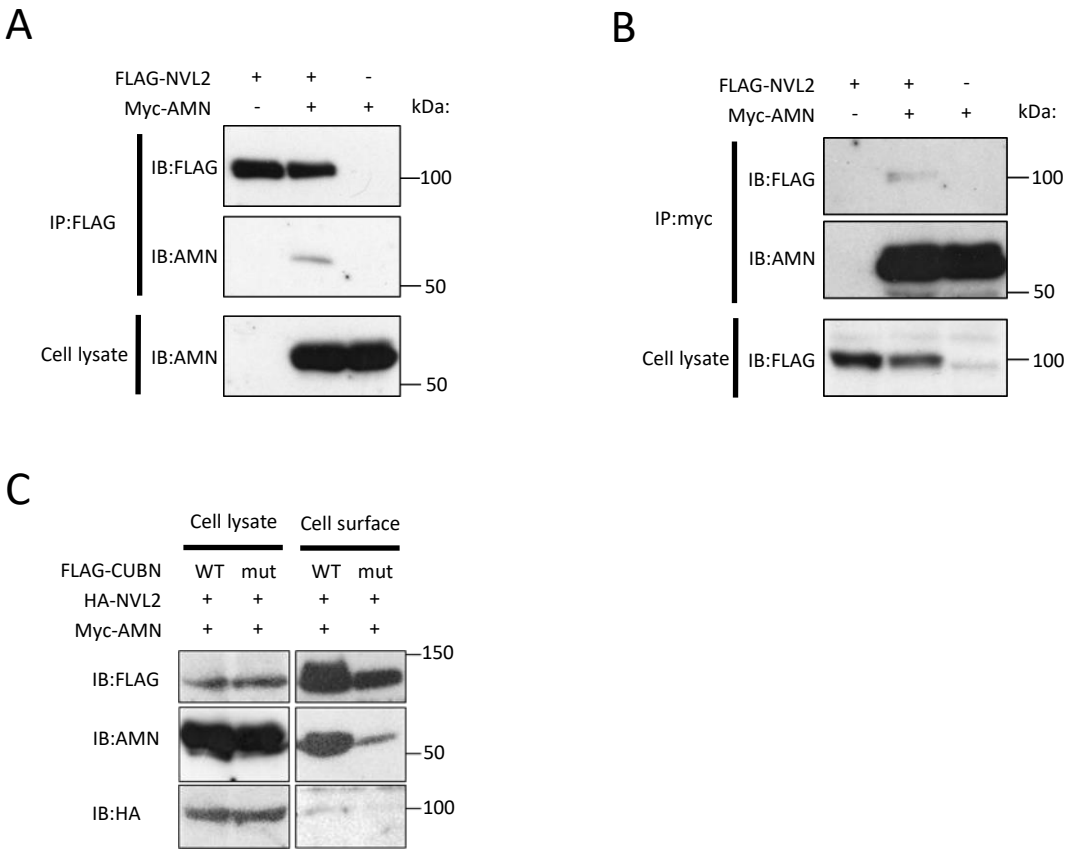

Figure S3. NVL2 interacts with AMN and CUBAM.

A, B Coimmunoprecipitation of myc-tagged AMN and FLAG-tagged NVL2 exogenously expressed in HEK293T cells.

C Immunoprecipitation of WT or mutant (G653R) CUBN expressed on the cell surface. FLAG-tagged CUBN on cell surface was labelled with anti-FLAG antibody in cultured medium and its bound proteins were analyzed by western blot.

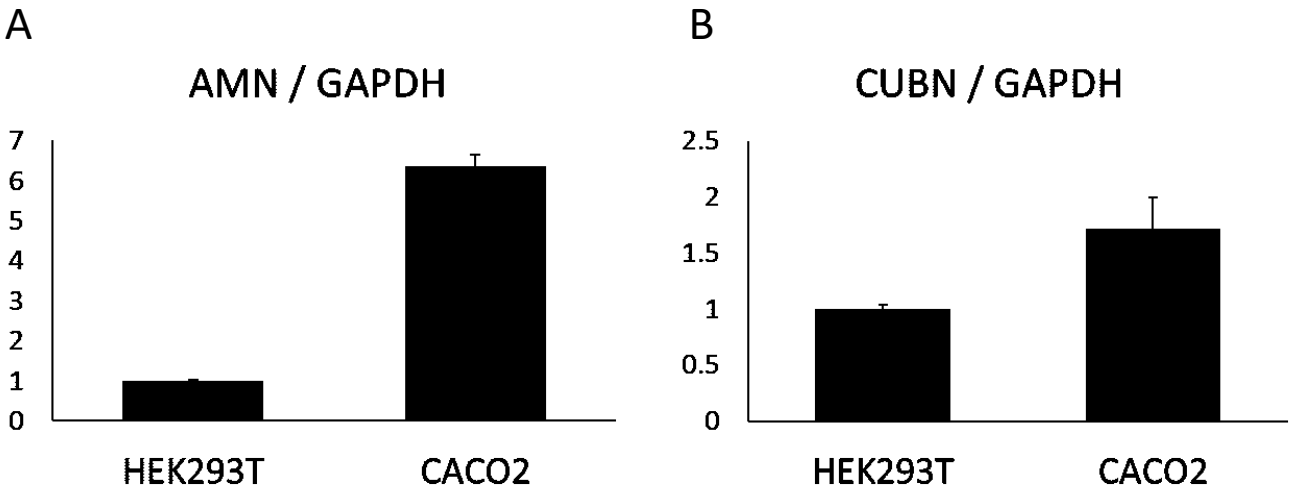

Figure S4. CACO2 cells, but not HEK293T cells, express endogenous AMN and CUBN mRNA.
A, B AMN (A) and CUBN (B) mRNA levels normalized to GAPDH mRNA in HEK293T and CACO2 cells were evaluated by qPCR.

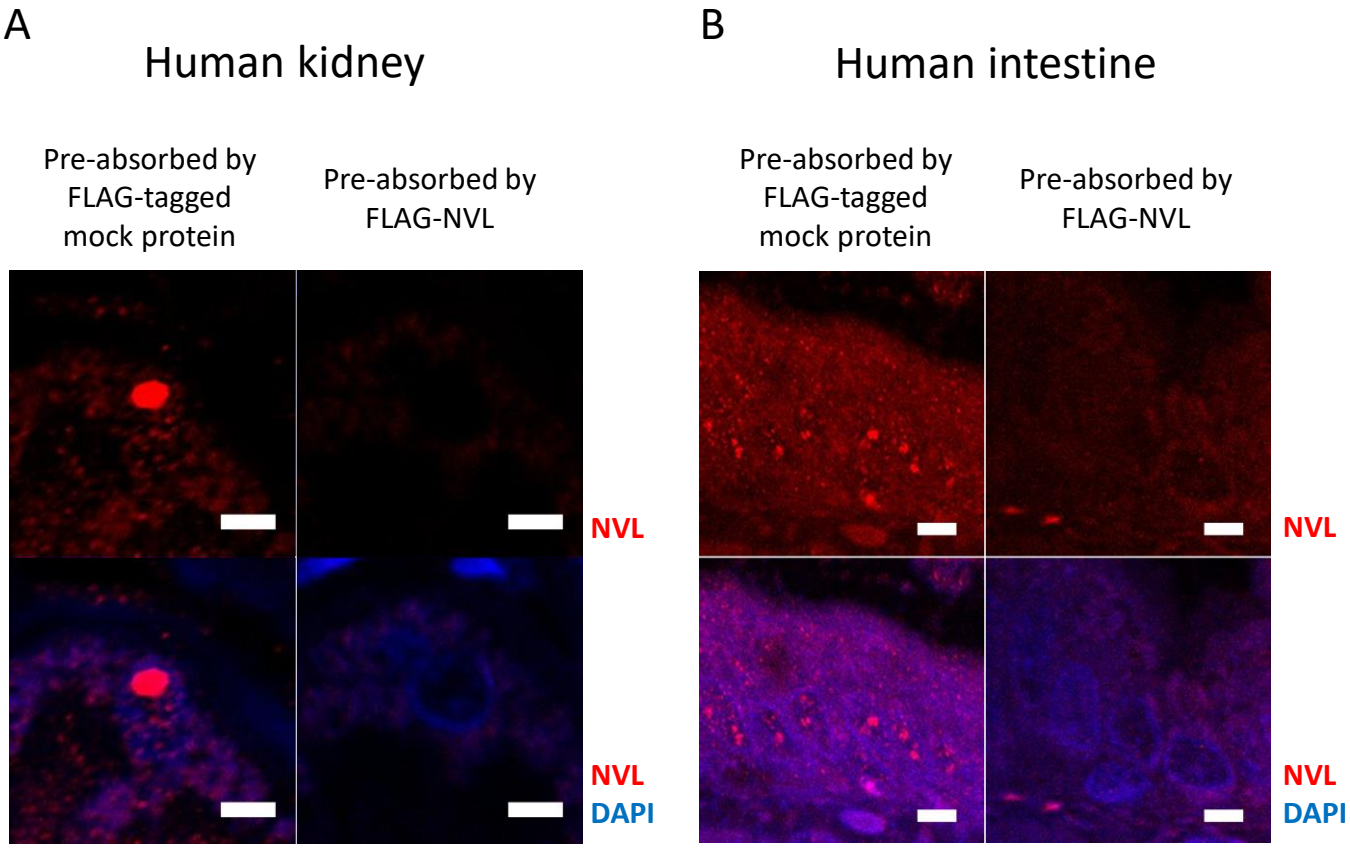

Figure S5. Immunofluorescence signals are specificity of signals for NVL in human kidney and intestine.
A, B Human kidney(A) and intestine(B) sections were immunostained with anti-NVL antibody pre-absorbed by anti-FLAG immunoprecipitates from lysates of HEK293T cells expressing FLAG-tagged negative control protein (FLAG-KLHL3) or FLAG-NVL2. Scale bar = 5 μm

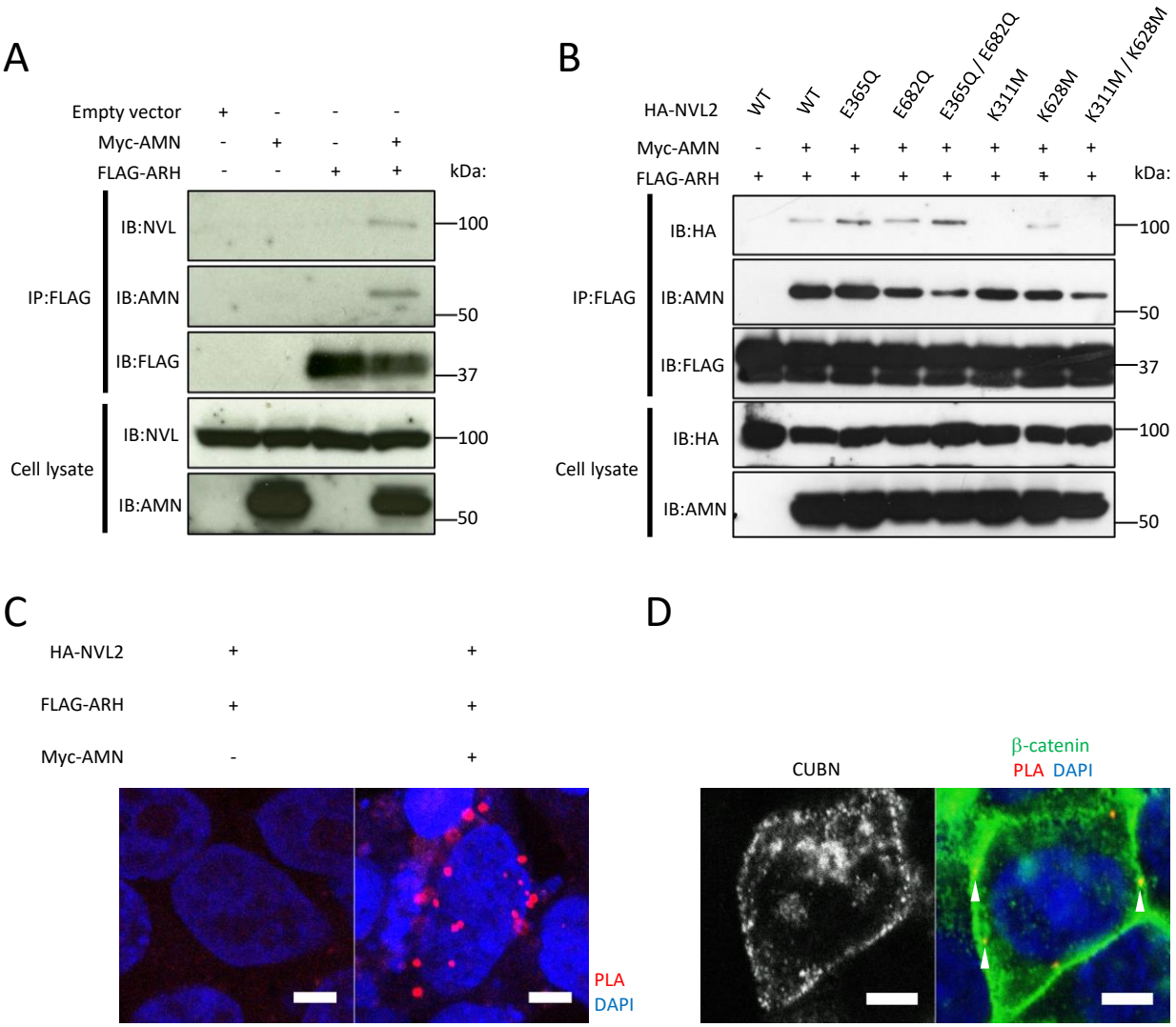

Figure S6. NVL2 forms a trimeric complex with ARH and AMN.

A Co-immunoprecipitation of endogenous NVL2 with exogenously expressed AMN and ARH in HEK293T. Cell lysates and anti-FLAG immunoprecipitates were analyzed using the indicated antibodies.

B Co-immunoprecipitation of HA-tagged WT or mutant NVL2 with AMN and ARH exogenously expressed in HEK 293T cells.

C In situ PLA of HA-tagged NVL2 and FLAG-tagged ARH in HEK293T cells with or without AMN coexpression. Scale bar = 5 μm.

D In situ situ PLA of HA-tagged NVL2 and FLAG-tagged ARH in HEK293T cells coexpressing myc-tagged AMN and GFP-tagged CUBN. Cells were immunostained for β-catenin after the PLAs. Scale bar = 5 μm.

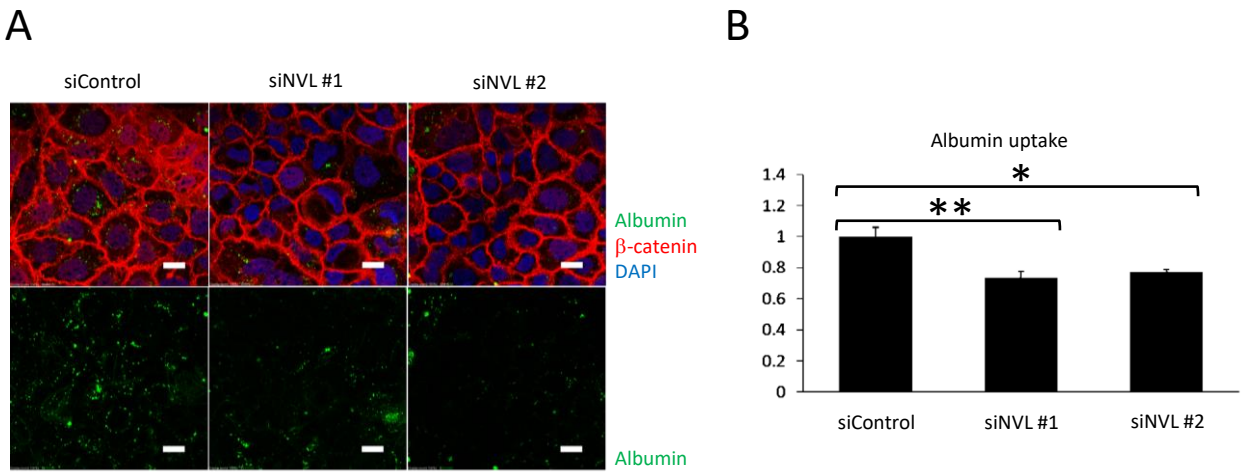

Figure S7. NVL depletion reduces albumin uptake in CACO2 cells.
A FITC-albumin uptake by CACO2 cells transfected with the indicated siRNAs. Cells were immunostained for b-catenin after FITC-albumin uptake. Scale bar = 20 μm.
B Quantification of FITC-albumin uptake by CACO2 cells transfected with the indicated siRNAs. Median FITC signal intensity of each cell group was measured by flow cytometry. Data are shown as mean ± s. e. m., n = 6. **P*<0.05, ***P*<0.01, two-sided *t*-test.

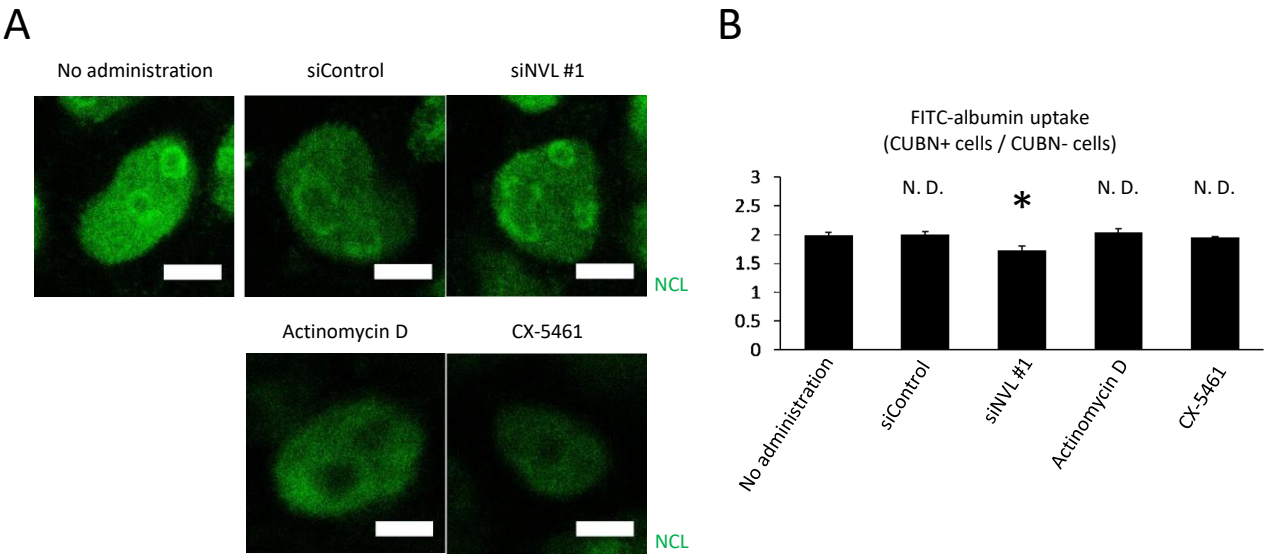

Figure S8. NVL2 promotes albumin endocytosis independently of nucleolar stress.
A Localization of nucleolin (NCL) in HEK293T cells transfected with siNVL, or incubated with media containing actinomycin D or CX-5461. Scale bar = 5 μm.
B Flow cytometric quantification of FITC-albumin uptake in HEK293T cells transfected with siNVL, or incubated with media containing actinomycin D or CX-5461 as in Figure 1E. Data are shown as mean ± s. e. m., n=3. N. D. = no difference, *P < 0.05, two-sided t-test.