

Figure S1

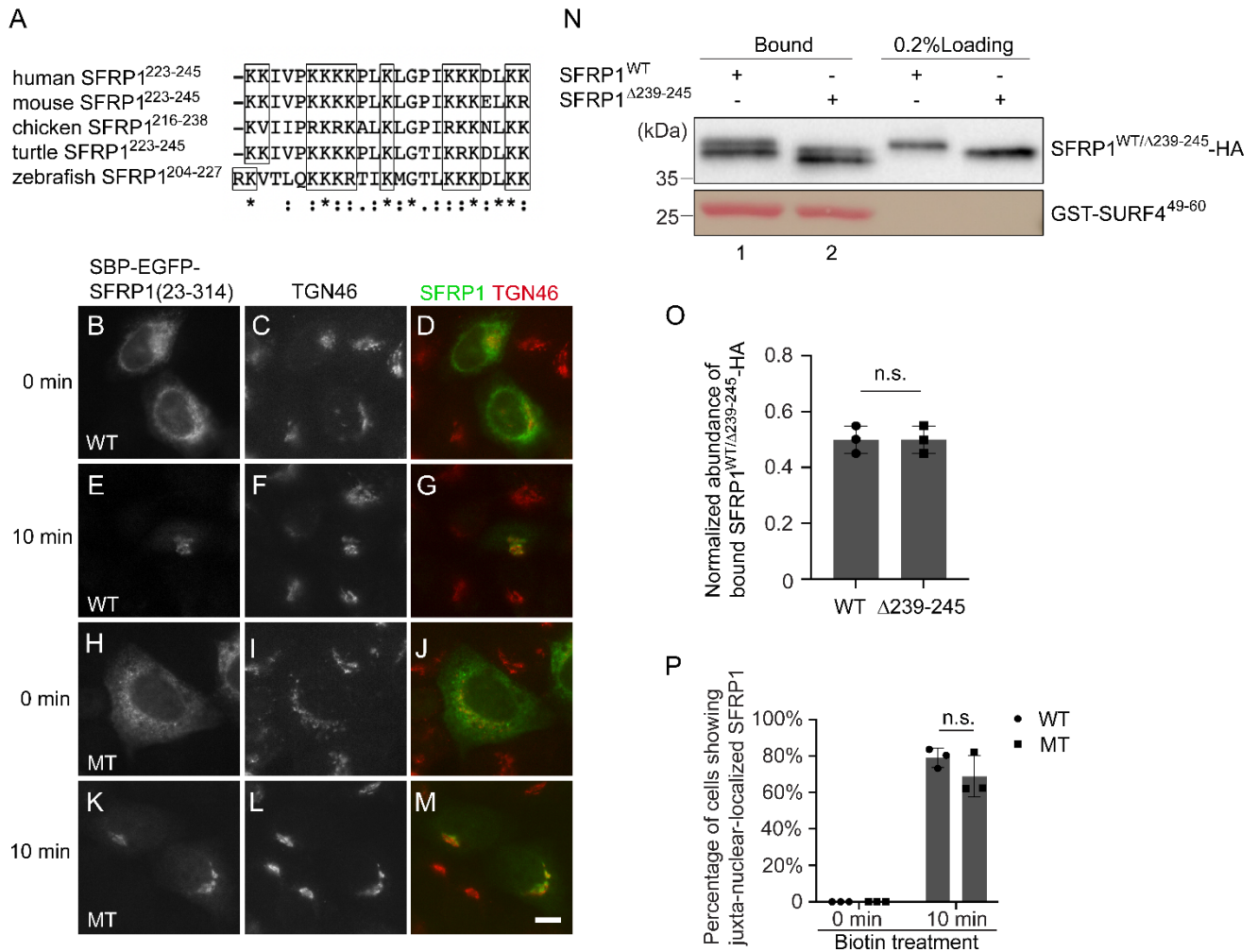


Fig. S1. Depleting (K/R)(K/R)(K/R)XX(K/R)(K/R) motif on SFRP1 did not cause defects on ER-to-Golgi transport of SFRP1. (A) Sequence alignment of SFRP1 across species. (B-M) HeLa cells were transfected with plasmids encoding Str-KDEL and SBP-EGFP-SFRP1 (B-G) or (K/R)(K/R)(K/R)XX(K/R)(K/R) motif mutated version of SFRP1 (H-M). Day 1 after transfection, the localizations of the different versions of RUSH constructs were analyzed after incubation with biotin for the indicated time (scale bar, 10 μ m). Magnification, 63 \times . (N) Purified GST-tagged human SURF4⁴⁹⁻⁶⁰ was incubated with lysates from HEK293T cells transfected with plasmids encoding wild-type or (K/R)(K/R)(K/R)XX(K/R)(K/R) motif-depleted SFRP1-HA. After incubation, the bound proteins were analyzed by immunoblotting with anti-HA antibodies. (O) Normalized abundances of SFRP1-HA bound to GST-SURF4⁴⁹⁻⁶⁰ were quantified (mean \pm SD; n = 3). The abundance of bound proteins was normalized to the bait protein GST-SURF4⁴⁹⁻⁶⁰, and this value was then normalized to the sum of the normalized abundance of wild-type SFRP1-HA and mutant SFRP1-HA bound to GST-SURF4⁴⁹⁻⁶⁰ in each experimental group. n.s., not significant. (P) Quantification of the percentage of cells showing juxta-nuclear-accumulated EGFP signal after incubation with biotin for the indicated time (mean \pm SD; n = 3; >50 cells counted for each time point). n.s., not significant.

Figure S2

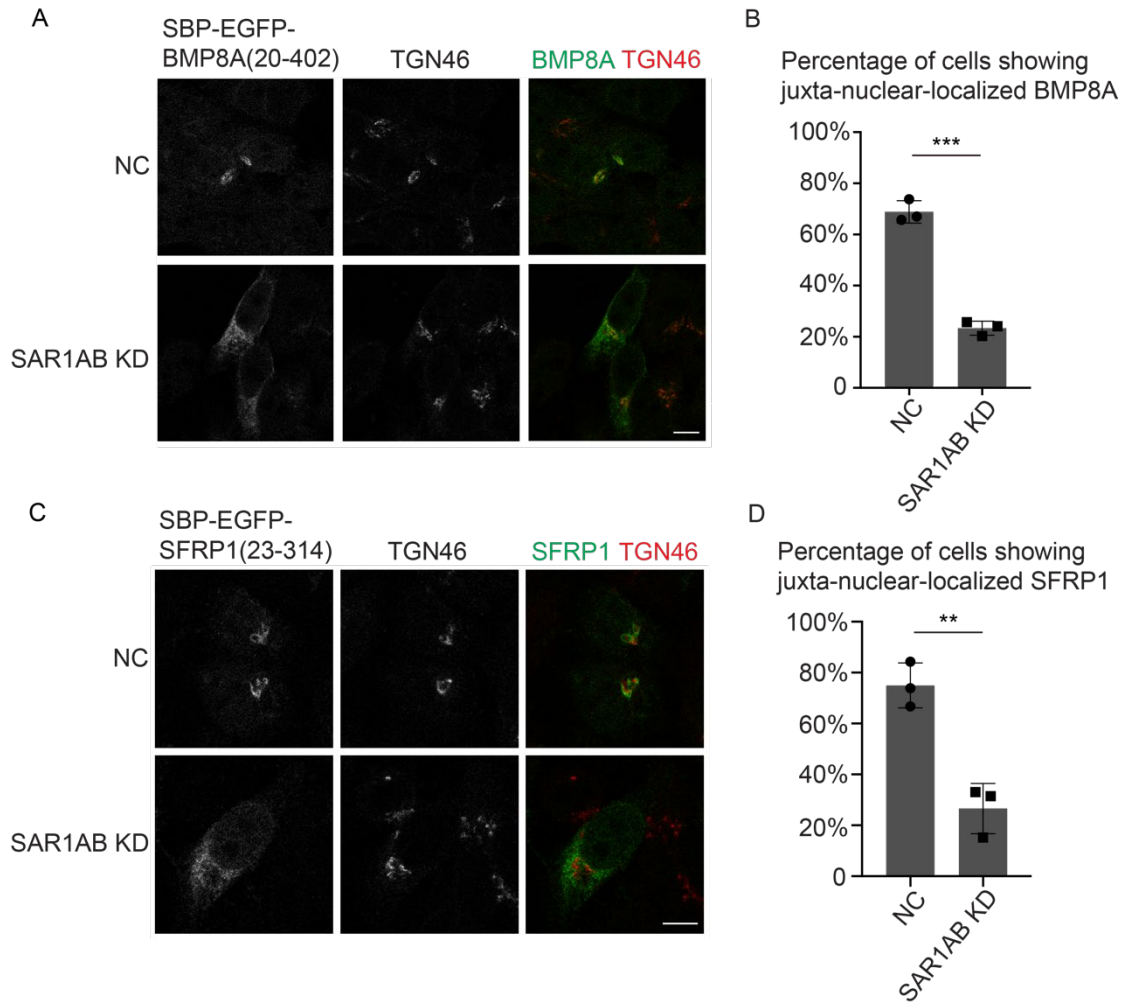


Figure S2. The ER-to-Golgi transport of BMP8A and SFRP1 is COPII-dependent. (A) and (C) HeLa cells were transfected with negative control (NC) siRNA or siRNA against SAR1A and SAR1B. At 48 h after transfection, cells were re-transfected with plasmids encoding Str-KDEL_SBP-EGFP-BMP8A (A) and Str-KDEL_SBP-EGFP-SFRP1 (C). On day 3 after knockdown, cells were incubated with biotin for 30 min (A) or 8 min (C), and the localization of BMP8A and SFRP1 was analyzed (scale bar, 10 μ m). Magnification, 63 \times . (B) and (D) Quantifications of the percentage of cells showing juxta-nuclear-accumulated EGFP signal (mean \pm SD; n = 3; >100 cells counted for each group). **P < 0.01; ***P < 0.001.