

Supplemental

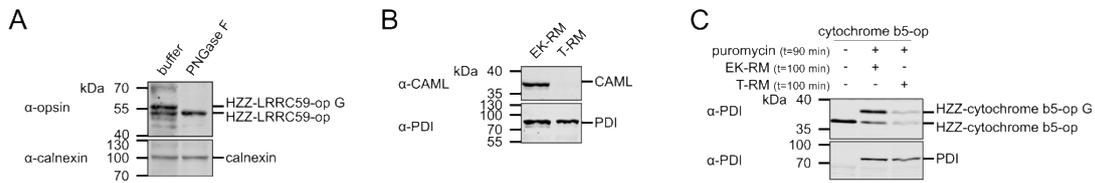


Figure S1. Posttranslational membrane insertion of LRRC59 (related to figures 2 and 3). (A) N-linked glycosylation of HZZ-LRRC59-op. HZZ-LRRC59-op was produced and modified as in figure 2B and subjected to treatment with PNGase F or buffer as indicated. Proteins were analyzed by SDS-PAGE and Western-blotting, using an antibody against the opsin-tag. Calnexin was used as a loading control. (B) Comparison of trypsin- (T-RM) and EDTA/high salt-treated (EK-RM) microsomes. Microsomes were mixed with SDS-loading buffer and analyzed by SDS-PAGE followed by Western-blotting, detecting CAML and, as loading control, PDI. (C) HZZ-cytochrome b5-opsin was produced in a coupled *in vitro* transcription/translation reaction and incubated with EDTA/high salt- (EK-RM) or trypsin- (T-RM) treated microsomes, with (+) or without (-) the addition of puromycin, as indicated. Proteins were analyzed by SDS-PAGE followed Western-blotting using antibodies against the opsin-tag and PDI.

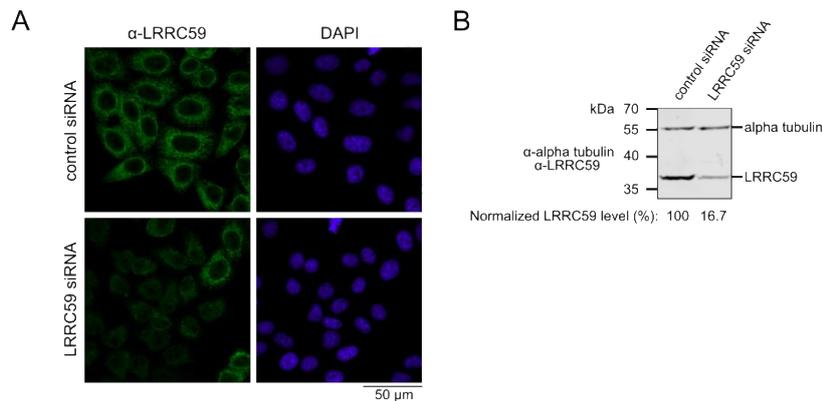


Figure S2. Specificity of the anti-LRRC59 antibody. HeLa cells were transfected with control siRNAs or siRNAs against LRRC59, fixed after 48 h and analyzed by indirect immunofluorescence using an antibody against LRRC59 (A) or analyzed by SDS-PAGE and immunoblotting using antibodies against LRRC59 and alpha-tubulin (B). Relative LRRC59-levels were normalized to alpha-tubulin.

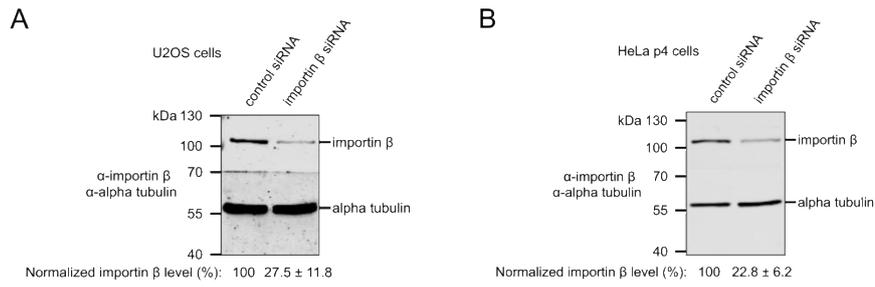


Figure S3. Knock-down of importin β (related to Figure 5). U2OS cells (A) or HeLa cells (B) were treated with control siRNAs or siRNAs against importin β and analyzed by SDS-PAGE followed by Western-blotting, detecting importin β and alpha-tubulin. The standard deviations from the mean of importin β levels in four (A) or nine (B) experiments, normalized to alpha-tubulin, are indicated.

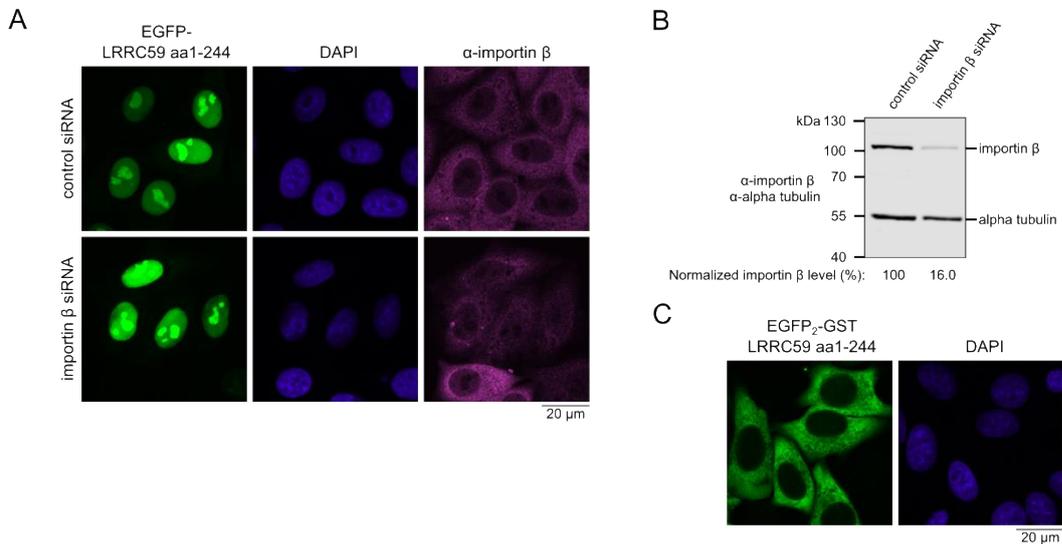


Figure S4. Size-dependent localization of LRRC59 lacking a TMD. (A) HeLa cells were transfected with a plasmid coding for EGFP-LRRC59 aa1-244 and control siRNAs or siRNAs against importin β . After 48 h, cells were fixed and analyzed by indirect immunofluorescence using antibodies against importin β . (B) Cells were treated as in (A) and analyzed by SDS-PAGE and Western-blotting, detecting importin β and alpha-tubulin. The importin β level was normalized to that of alpha-tubulin. (C) HeLa cells were transfected with a plasmid coding for EGFP₂-GST-LRRC59 aa1-244. After 48 h, cells were fixed and analyzed by fluorescence microscopy.