E07-10-0989 Hauri

Supplementary Figures

Figure S1

Specificity of antibodies against Surf4. (A) HeLa cell lysates were analyzed by Western blotting with crude antibodies (lane 1) and affinity-purified antibodies (lane 2) raised against human Surf4 (2). (B) HeLa cells were transfected with control siRNA or Surf4 siRNA and processed for indirect immunofluorescence with the affinity-purified anti-Surf4 antibody and anti-ERGIC-53 antibody. Bars, 10μm.

Figure S2

Purification of ERGIC membranes containing Surf4. HepG2 cells were treated with 10μg/ml BFA for 90 min. The cells were homongenized and the post-nuclear supernatant was loaded on a 13-29% Nycodenz gradient and centrifuged for 3h at 80'000xg as described previously (Breuza et al., 2004). Gradient fractions were collected and analyzed by Western blotting with antibodies against Surf4, BAP31, and ERGIC-53. The ERGIC fractions 9-12 were pooled and used for further analysis by Blue Native-PAGE.

Figure S3

Efficient knockdown of Surf4/ERGIC-53 or p25 does not affect protein levels of other proteins analyzed in this study. (A) HeLa cells transfected with control, Surf4/ERGIC-53 or p25 siRNA were lysed and equal protein amounts were processed for SDS-PAGE and analyzed by Western blotting using anti-p25, anti-Surf4 and anti-ERGIC-53. (B) Quantification of knockdown efficiency of p25, Surf4 and ERGIC-53. Three independent experiments were quantified. Results are means ± s.d. (C) Control, p25 and Surf4/ERGIC-53 siRNA treated HeLa cells were analyzed as in (A) using anti-Sec31, anti-β-COP, anti-tubulin and anti-KDEL-receptor antibodies. Shown are representative images of three independent experiments. Star indicates Sec31 protein.

Figure S4

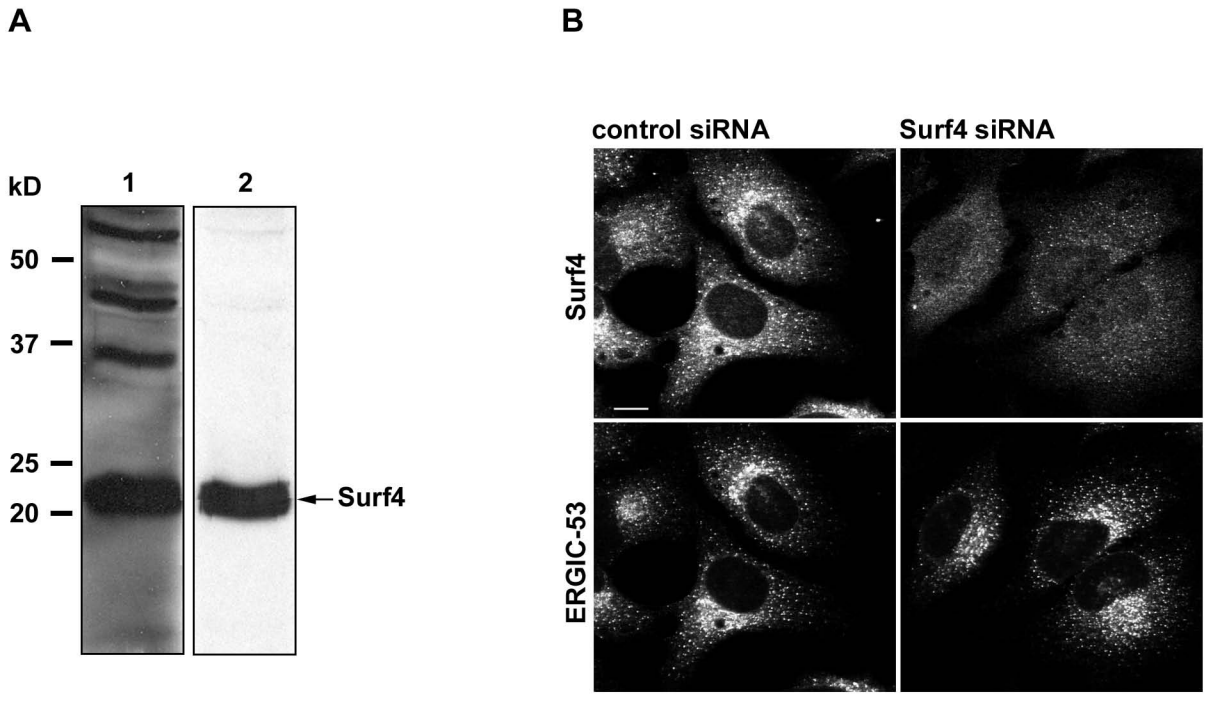
Single knockdowns of Surf4 and ERGIC-53 do not affect Golgi morphology or total protein secretion. (A) HeLa cells were transfected with control, Surf4 or ERGIC-53 siRNA. The Golgi was visualized by immunofluorescence using anti-giantin antibodies. Bar, 10μm. (B) HeLa cells were transfected with control siRNA, Surf4 siRNA or ERGIC-53 siRNA, or

double transfected wirh Surf4 and ERGIC-53 siRNAs for 4 days and subjected to pulse-chase analysis using [^{35}S]-methionine. Conditioned media were collected and assayed for incorporated radioactivity as described in Figure 5C. Results are means ± s.d. of at least three independent experiments.

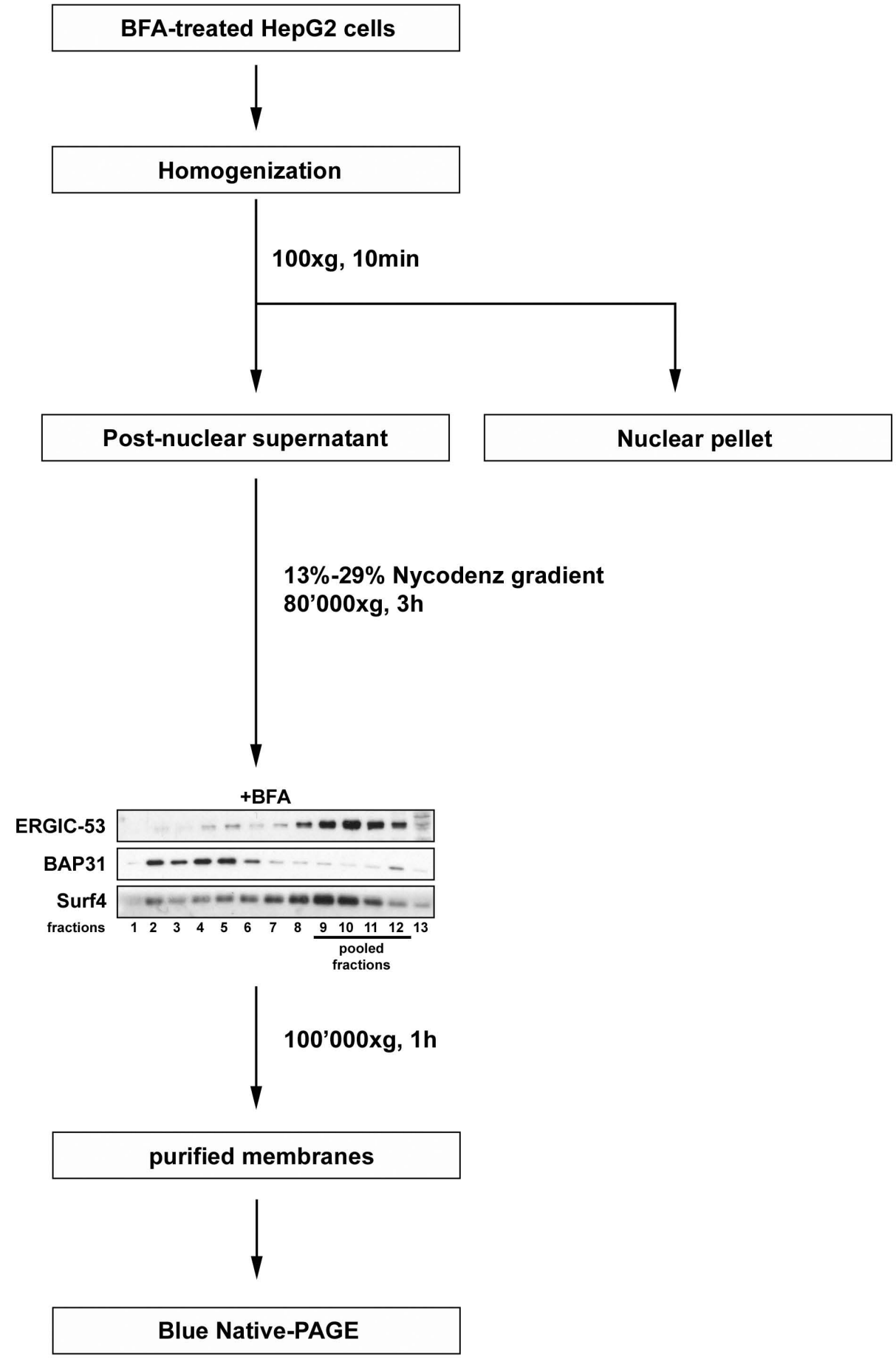
Movie 1. **Live imaging of GFP-ERGIC-53 in control cells.** Supplement to Fig. 6. HeLa cells stably expressing GFP-ERGIC-53 were treated with sodium butyrate overnight, transfected with control siRNA for 3 days and imaged every ~2 seconds for 4 minutes. The majority of the structures undergo little movement. Some fusion and splitting are seen. Movie is ×8 accelerated relative to real time.

Movie 2. Live imaging of GFP-ERGIC-53 in p25 knockdown cells. Supplement to Fig. 6. HeLa cells stably expressing GFP-ERGIC-53 were treated with sodium butyrate overnight, transfected with p25 siRNA for 3 days and imaged every ~2 seconds for 4 minutes. Several moving structures undergoing fusion, splitting, disappearance and re-emergence are seen. Movie is ×8 accelerated relative to real time.

Supplementary1

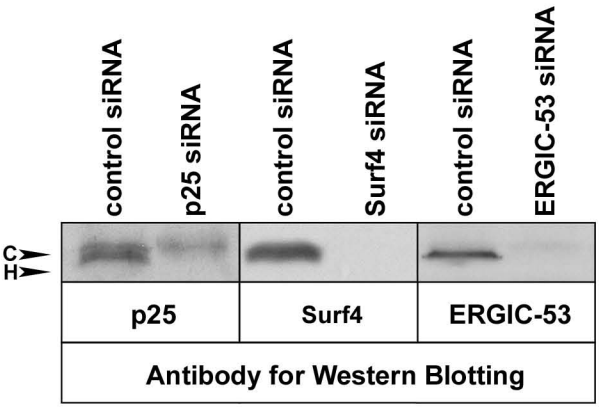


Supplementary2



Supplementary3

A



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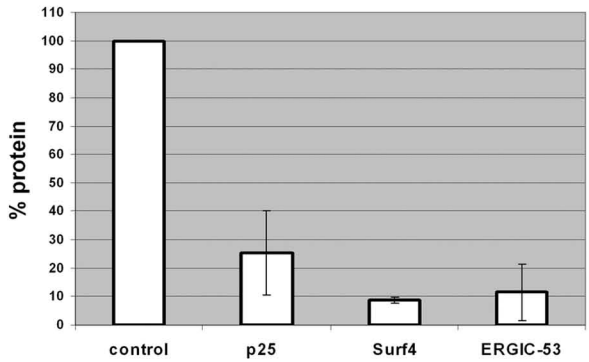

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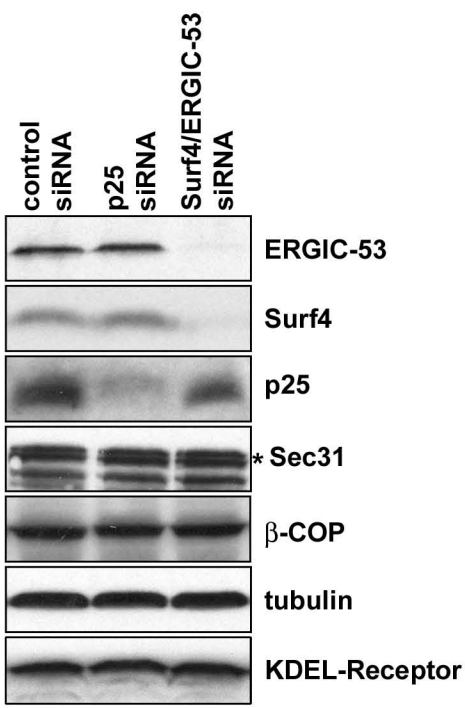

Supplementary4

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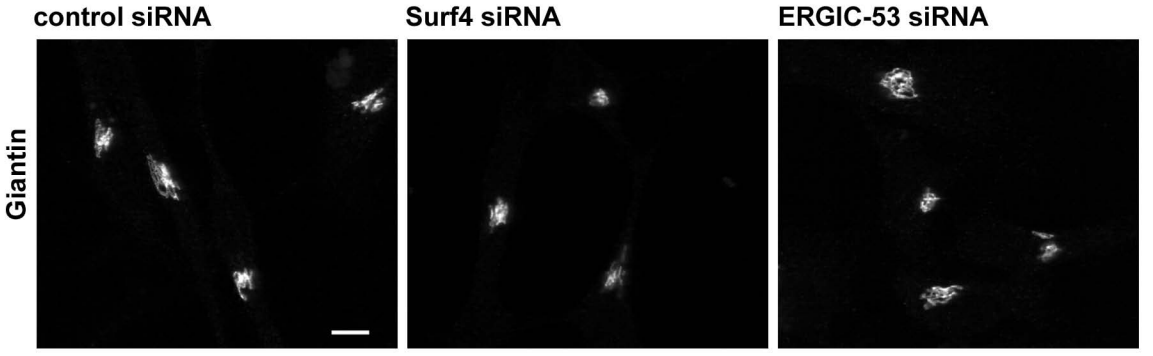

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