Supplemental material

Bassaganyas et al., https://doi.org/10.1083/jcb.201902028

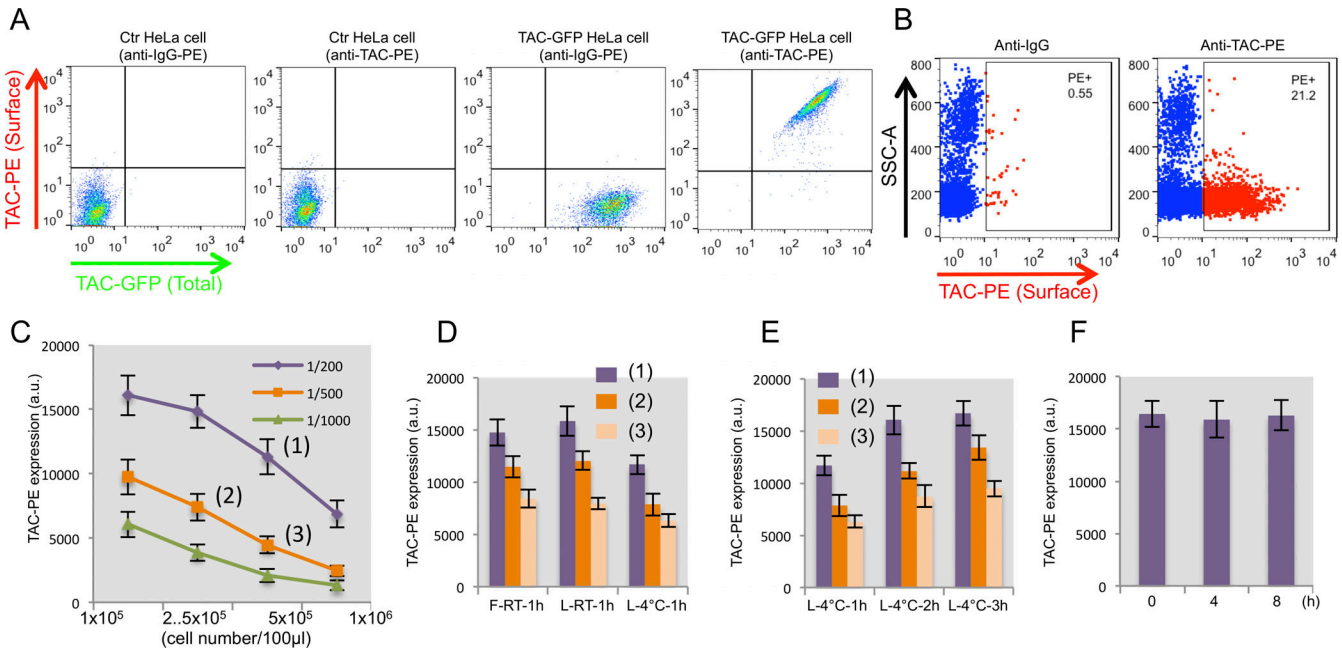

Figure S1. **Dual fluorescent reporter for protein transport. (A)** Surface and total TAC expression were analyzed by flow cytometry in control HeLa cells and in cells stably expressing TAC-GFP. **(B)** TAC protein is endogenously expressed by immune cells such as lymphocytes. To test further the specificity of the anti-TAC antibody, TAC surface expression was analyzed by flow cytometry in nucleated cells from blood sample. **(C–F)** Optimization of experimental conditions to monitor TAC expression by flow cytometry. **(C)** TAC surface expression in HeLa TAC-GFP cells (10^5–10^6 cells/100 µl) were analyzed by flow cytometry using increasing concentrations of anti–TAC-PE antibody (mean of $n = 3 \pm$ SEM). **(D)** Using conditions established in C, TAC surface expression was analyzed by flow cytometry on fixed (F) and Lived (L) cells by incubating cells with the anti–TAC-PE antibody during 1 h at room temperature (RT) or at 4°C (mean of $n = 3 \pm$ SEM). **(E)** Using conditions established in C and D, TAC surface expression was analyzed by flow cytometry on Lived (L) cells by incubating cells with the anti–TAC-PE antibody for 1, 2, or 3 h at 4°C (mean of $n = 3 \pm$ SEM). **(F)** Lived HeLa TAC-GFP cells were incubated for 2 h at 4°C with the anti–TAC-PE antibody, followed by flow cytometry analysis at T = 0, or after 4 and 8 h. Results demonstrate that TAC surface expression on lived cells maintained at 4°C after staining remain stable for 8 h (mean of $n = 3 \pm$ SEM). This experiment establishes that TAC-PE signal is stable over time for FACS sorting.

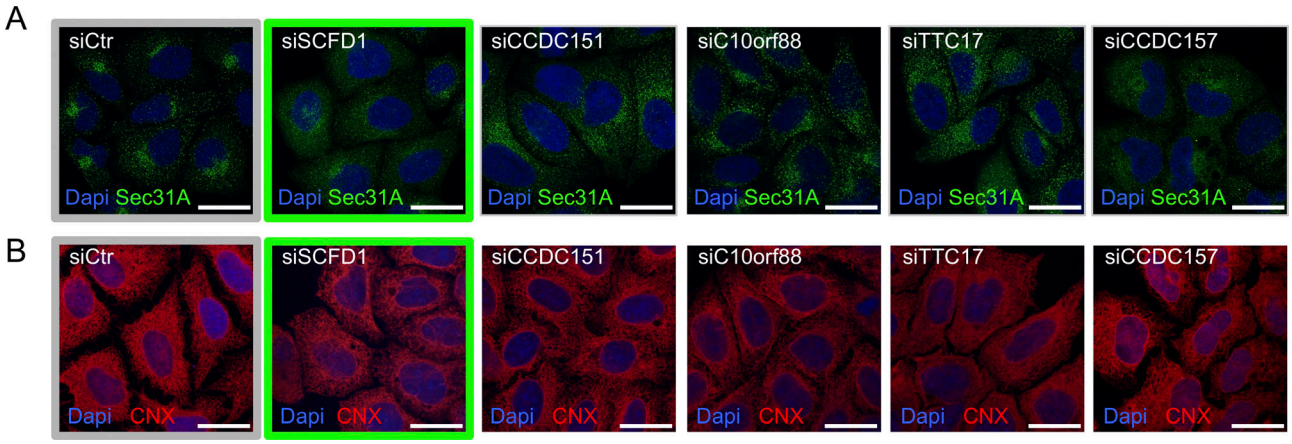

Figure S2. **ER exit site and ER organization in cells knockdown for newly identified factors. (A and B)** HeLa cells were transfected with the indicated specific individual siRNAs and after 3 d were analyzed by immunofluorescence microscopy using antibodies against Sec31A (A) and CNX (B), respectively. Nuclei were stained with DAPI. Scale bars, 25 µm.

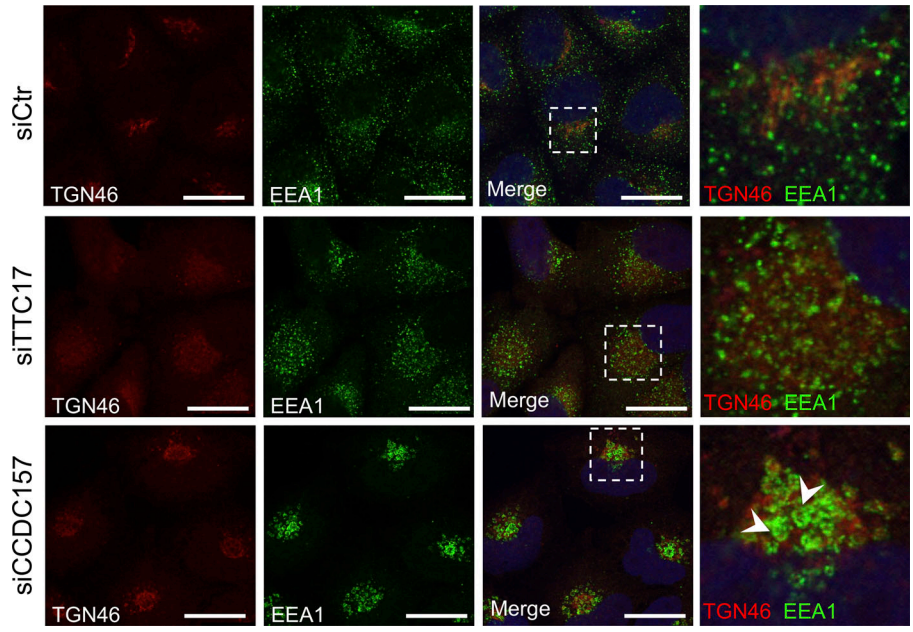

Figure S3. **EEA1 distribution is altered in CCDC157-depleted cells.** Control HeLa cells or cells depleted for CCDC157 and TTC17 were analyzed by immunofluorescence microscopy using anti-EEA1 and anti-TGN46 antibodies. Arrowheads point at enlarged EEA1-positive vesicles, surrounding by Golgi membranes. Nuclei were stained with DAPI. Scale bars, 25 µm.

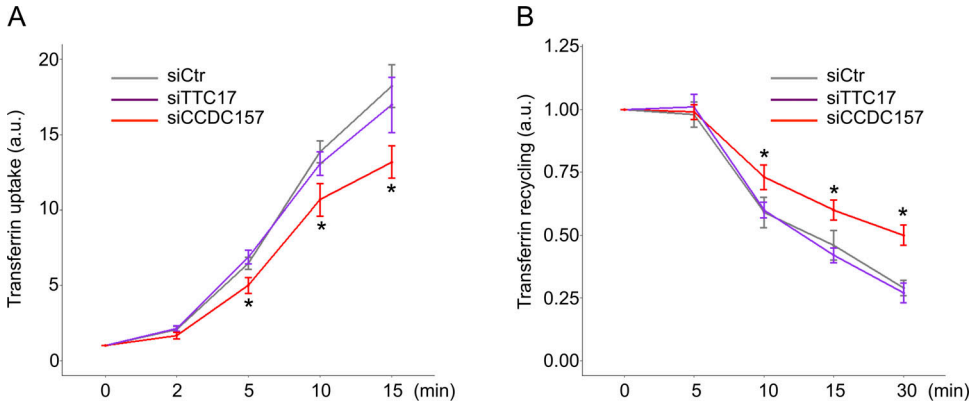

Figure S4. **The uptake and recycling of Tf is inhibited in CCDC157-depleted cells. (A)** Control HeLa cells and cells knocked down for TTC17 and CCDC157 were incubated at 4°C for 30 min in presence of 50 µg/ml Tf conjugated with Alexa Fluor 647 and then incubated at 37°C. At the indicated type points, Tf internalization was stopped by placing cells on ice for 10 min, and cells were analyzed by flow cytometry for Tf uptake (mean of $n = 3 \pm$ SEM; two-tailed Student's t test for comparison to control condition; *, $P < 0.05$). **(B)** Control HeLa cells and cells knockdown for TTC17 and CCDC157 were incubated with 50 µg/ml Tf conjugated with Alexa Fluor 647 for 30 min at 37°C, washed, and incubated at 37°C with 100 µg/ml unlabeled Tf for the indicated time points. Cells were then analyzed by flow cytometry for Tf recycling (mean of $n = 3 \pm$ SEM; two-tailed Student's t test for comparison to control condition; *, $P < 0.05$).

Provided online are two tables in Excel.

Table S1. Pooled genome-wide CRISPRi screen results. Combined analysis of CRISPRi screen replicates where read counts of sgRNAs obtained by deep sequencing were analyzed using MAGeCK (Li et al., 2014). Columns list the gene name, P value, false discovery rate (fdc), rank position, and lfc. Genes are listed following their negative rank, meaning that the first ranked genes strongly inhibit TAC transport. For a same gene, alternative promoters can be targeted by different sgRNAs of the CRISPRi-v2 library. In such a case, the gene name is followed by _P1 or _P2. If a single promoter per gene is targeted, the gene name is followed by _P1P2.

Table S2. Candidates genes selected from the CRISPRi screen and analyzed for HRP secretion. The secondary screen use a secretion assay based on the detection of ss-HRP by chemiluminescence after the knockdown of selected genes. Columns for the secondary screen list the HRP signal from cell lysate and medium normalized to control condition and the ratio medium/lysate HRP signal. Values of each replicate have been averaged. Cells transfected with siRNA targeting SCFD1 (in red) were used as positive controls. Genes selected for further analysis (TTC17, CCDC157, C10orf88, and CCDC151) are highlighted in green.

Reference

Li, W., H. Xu, T. Xiao, L. Cong, M.I. Love, F. Zhang, R.A. Irizarry, J.S. Liu, M. Brown, and X.S. Liu. 2014. MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. *Genome Biol.* 15:554. https://doi.org/10.1186/s13059-014-0554-4