Scharaw et al., https://doi.org/10.1083/jcb.201601090

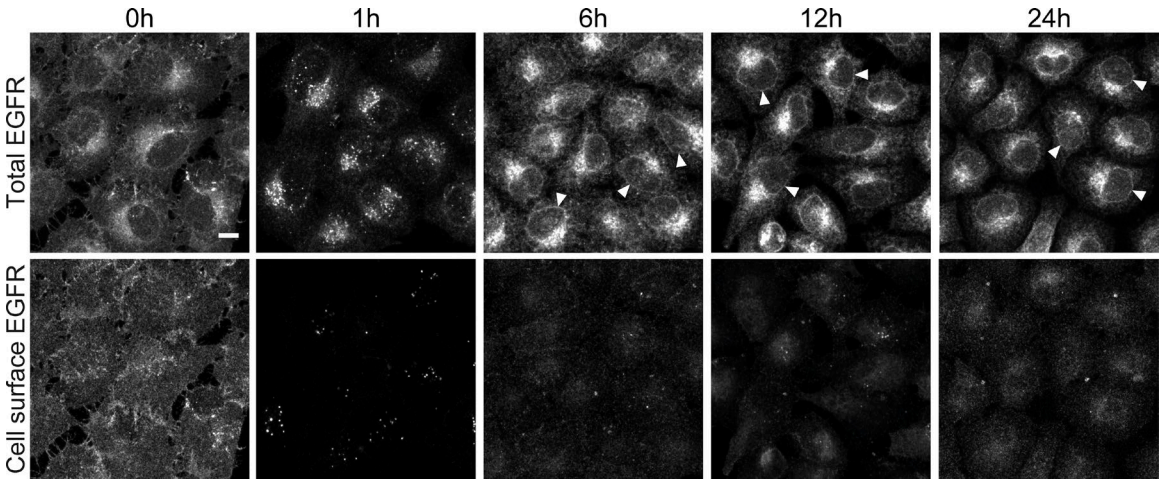

Figure S1. **Long-term EGF stimulation depletes cell surface EGFR.** Endogenous EGFR protein localization was investigated by confocal microscopy in HeLa cells nontreated or treated with 200 ng/ml EGF for various times. EGFR was either localized both intracellularly and at the cell surface (total EGFR) or at the cell surface only. Arrowheads point to the nuclear envelope in example cells. Images are sum projections of z stacks covering the entire cell volume, acquired on a confocal laser-scanning microscope. Bar, 10 µm.

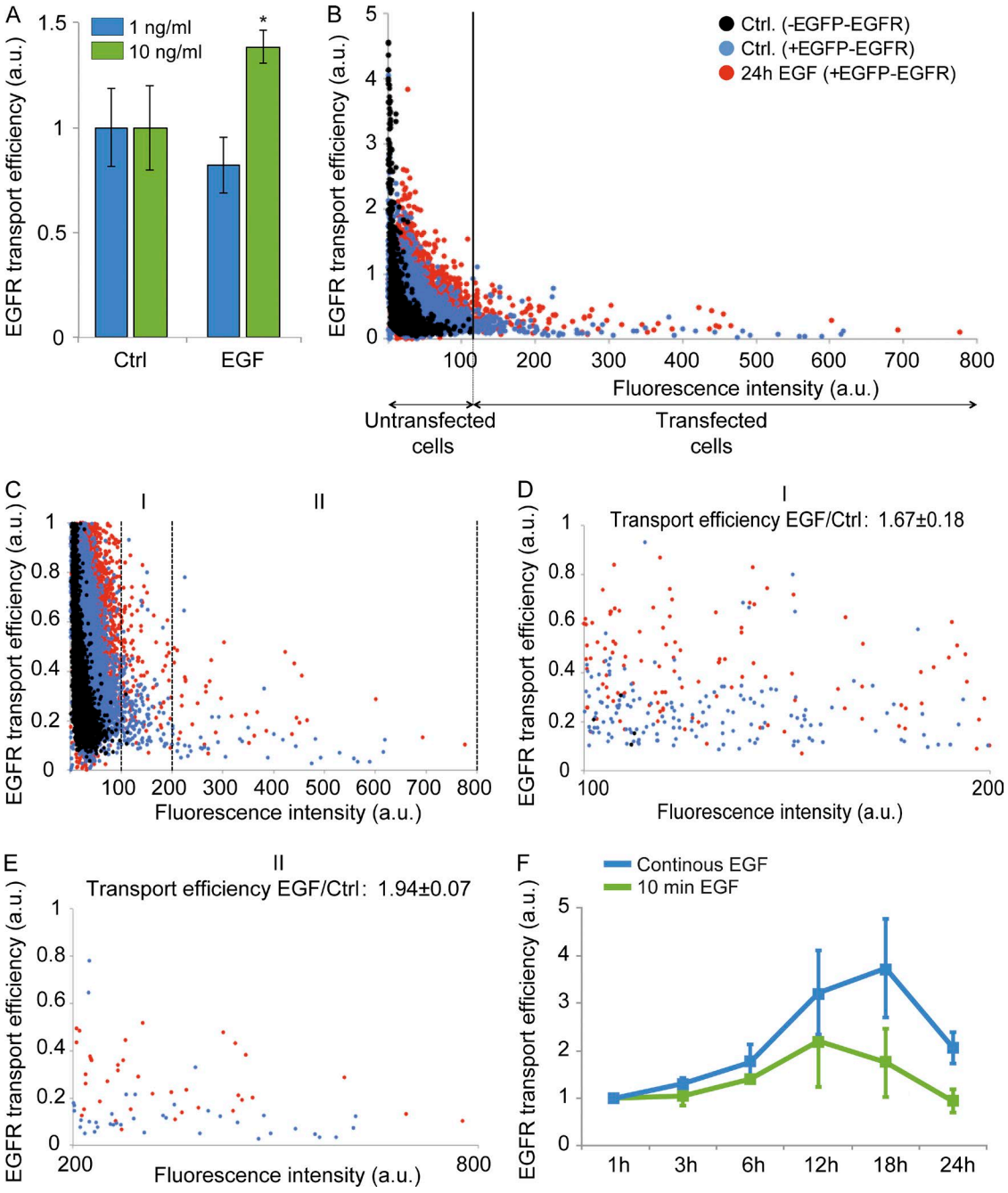

Figure S2. **Long-term 10-ng/ml and 200-ng/ml EGF stimulation increases EGFP-EGFR transport efficiency.** (A) EGFP-EGFR transport efficiency was investigated by performing the RUSH transport assay in HeLa cells nontreated or stimulated for 18 h with 1 or 10 ng/ml EGF. Data are means ± SEM (n = 3; t test: *, $P < 0.05$). (B) Graphical representation of the EGFP-EGFR transport efficiency dependence on the fluorescence intensity. Data points represent the transport efficiency in individual HeLa cells in the absence (untransfected) or presence (transfected) of EGFP-EGFR expression. Cells are shown for nontreated conditions or for 24-h treatment with 200 ng/ml EGF. (C–E) Graphical separation of transfected cells into low (I), medium, and high (II) EGFP-EGFR expression intensity intervals (dashed vertical lines). The mean EGFP-EGFR transport efficiency of EGF-stimulated cells compared with nontreated cells was calculated for each interval, excluding untransfected cells. (F) HeLa cells were stimulated with 200 ng/ml EGF either continuously or for a 10-min pulse and incubated for various times. EGFP-EGFR transport efficiency was quantified using the RUSH transport assay. Data are means ± SEM (n = 3). a.u., arbitrary units.

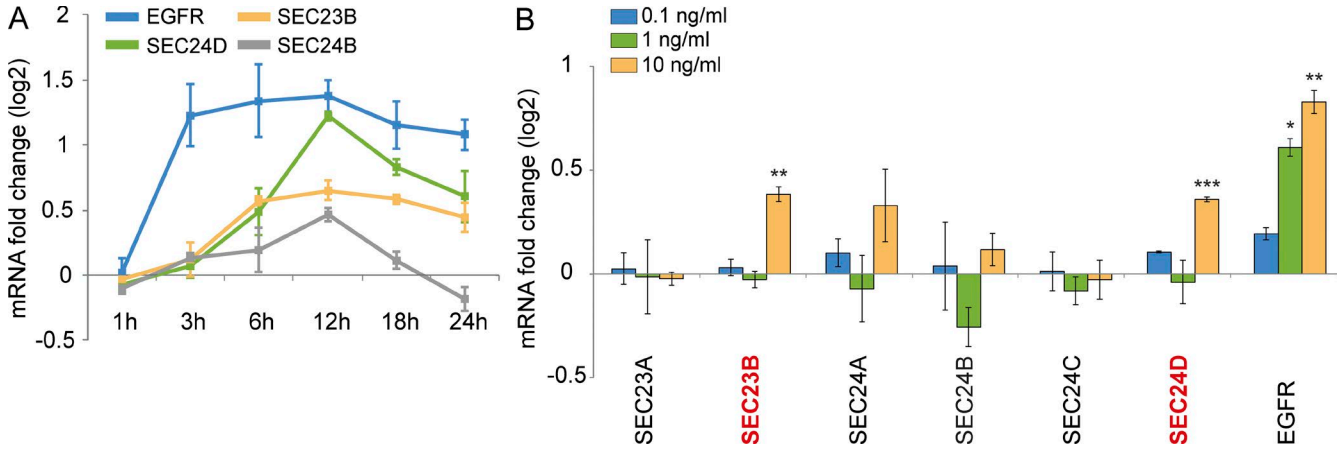

Figure S3. **Long-term 10-ng/ml and 200-ng/ml EGF stimulation up-regulates mRNA levels of inner COPII paralogues and EGFR.** HeLa cells were stimulated with EGF and the effect on inner COPII paralogues and EGFR mRNA levels was investigated by Q-RT-PCR. Results were normalized to nontreated cells and GAPDH mRNA levels. (A) Log2 mRNA fold changes of HeLa cells stimulated with 200 ng/ml EGF for various times (n = 4). (B) Log2 mRNA fold changes of HeLa cells stimulated for 12 h with 0.1, 1, or 10 ng/ml EGF (n = 3). All data are means ± SEM (t test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

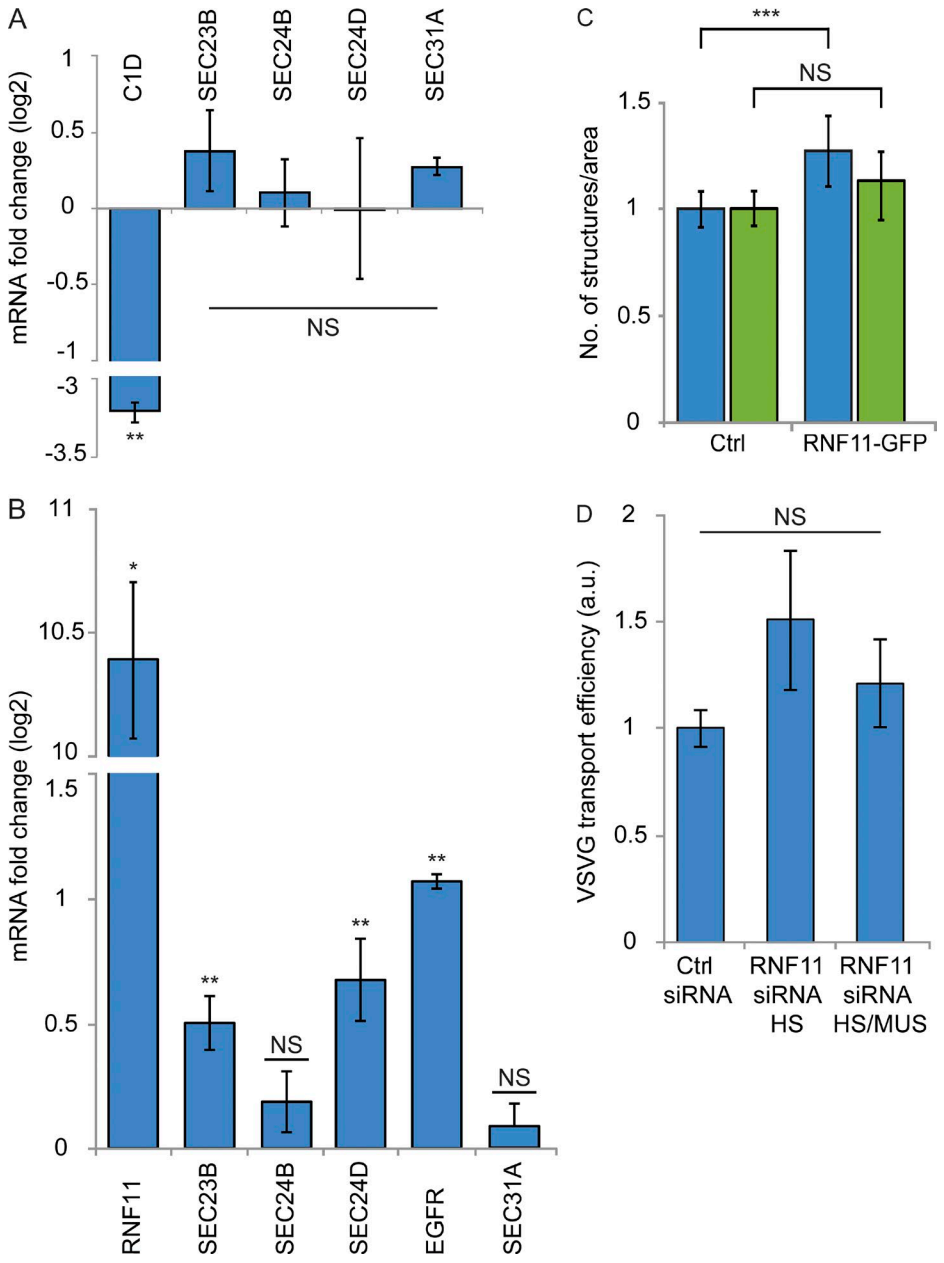

Figure S4. **RNF11, but not C1D, is required for expression changes of inner COPII paralogues, and RNF11 is dispensable for EGFP-VSVG transport efficiency.** (A) The predicted transcriptional regulation of SEC23B, SEC24B, and SEC24D by C1D was experimentally tested in HeLa cells. Q-RT-PCR experiments were performed, and the log2 mRNA fold change of COPII paralogues was investigated after 48 h of siRNA-mediated C1D knockdown (n = 3). (B) RNF11-GFP was overexpressed for 24 h in HeLa cells (RNF11-GFP transfection efficiency was 30%). Q-RT-PCR experiments were performed, and the log2 mRNA fold change of COPII paralogues and EGFR was investigated (n = 5). The results in A and B were normalized to nontreated cells and GAP DH mRNA levels. (C) The RNF11 overexpression effect on the number of SEC24B- and SEC31A-labeled ER exit sites was quantified based on confocal microscopy (n = 3 with each 10 analyzed cells). (D) Using the RUSH transport assay, the effect of 48-h siRNA-mediated RNF11 knockdown on EGFP-VSVG transport efficiency was quantified in HeLa cells (n = 3). All data are means ± SEM (t test: *, P < 0.05; **, P < 0.01; ***, P < 0.001). a.u., arbitrary units.

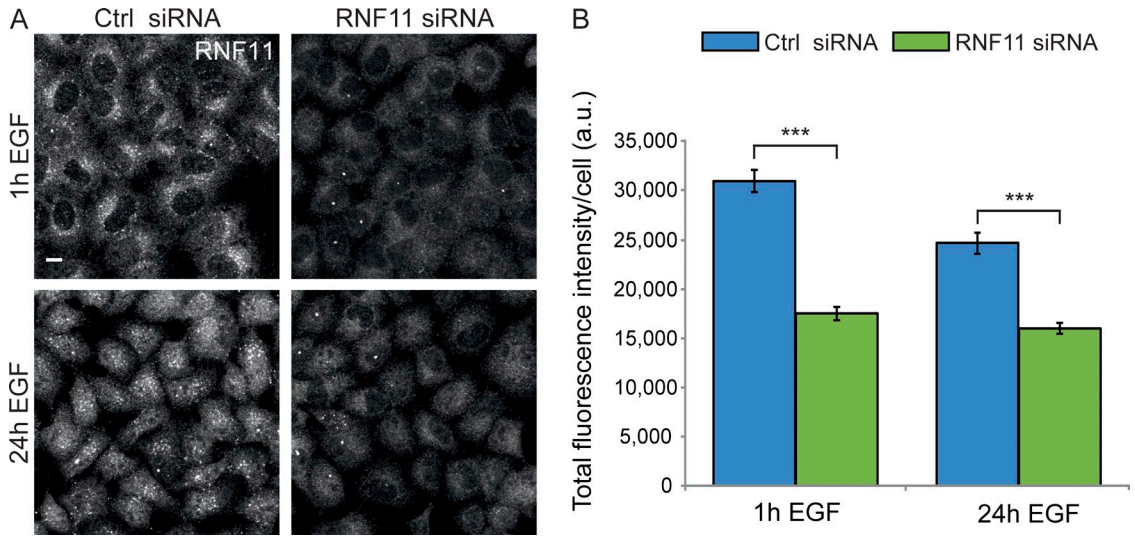

Figure S5. **RNF11 knockdown decreases endogenous RNF11 staining.** The specificity of endogenous RNF11 staining was investigated by confocal microscopy after 48 h of siRNA-mediated RNF11 knockdown, followed by 200-ng/ml EGF stimulation for 1 h or 24 h. Results were compared with cells treated with nonsilencing negative control siRNA, followed by 200-ng/ml EGF stimulation for 1 h or 24 h. (A) Representative cells showing endogenous RNF11 localization. Images are maximum projections of z stacks covering the entire cell volume, acquired on a confocal laser-scanning microscope. Bar, 10 µm. (B) Quantification of the total RNF11 fluorescence intensity per cell. Data are means ± SEM (n = 3 with each 30 analyzed cells; t test: ***, $P < 0.001$). a.u., arbitrary units.

Table S1. **siRNA sequences used for RNAi experiments**

Gene	Class	Sense siRNA sequence (5′–3′)	Antisense siRNA sequence (5′–3′)	Targeted transcripts
SEC23A	COPII	GCCAUCAAGUCGACUGGAATT	UUCCAGUCGACUUGAUGGCCA	9/11
SEC23B	COPII	CCAAGGAUUUAACUGCAAATT	UUUGCAGUUAAAUCCUUGGTC	5/6
SEC24A	COPII	GCCAGAGUUUGUUAGACAATT	UUGUCUAACAAACUCUGGCAA	3/3
SEC24B	COPII	GCAGGUUCCAUCUGGAUAUTT	AUAUCCAGAUGGAACCUGCAT	3/3
SEC24C	COPII	GCCUCUGAAGAGCGUCUAATT	UUAGACGCUCUUCAGAGGCTC	5/6
SEC24D	COPII	GGAGAAGUCUUUGUUCCUUTT	AAGGAACAAAGACUUCUCCAA	5/6
RNF11 HS	TR	CCUCCUUGCCUCUUGUCUUTT	AAGACAAGAGGCAAGGAGGTC	1/1 (3′ UTR)
RNF11 HS/MUS	TR	GGAAGAGAUGGAUCAGAAATT	UUUCUGAUCCAUCUCUUCCAG	1/1
XWNeg9 (s444246)	Control	UACGACCGGUCUAUCGUAGtt	CUACGAUAGACCGGUCGUAtt	
Scramble (s229174)	Control	UUCUCCGAACGUGUCACGUtt	ACGUGACACGUUCGGAGAAtt	

Table S2. **Primer sequences used for Q-RT-PCR experiments**

Gene	Class	Forward primer (5′–3′)	Reverse primer (5′–3′)
COPA	COPI	TGCTCACCCTAACCTTAACCTC	TCCCGTTCCAGCTTAAACAC
COPB1	COPI	GTGAAGCCATTCAGCGCTTT	AGGATCCATAATGCTCCTCGG
COPB2	COPI	GAATTGGGTTGTGACAGGAGC	GGATGAACAGCAATACAGCGA
COPD	COPI	CCATCATTGAAACTGATAAACCAA	CCTTTCCTTTGGCTCCAAGT
COPE	COPI	GAACGCCTTCTACATCGGCA	ACGTCTCTCTCTGGGCTTGA
COPG1	COPI	CCTGGGATGAGGTAGGGGAT	CTCACAAGGGTGCATTCCCA
COPG2	COPI	TCCGAATTGCCAGTCGCTTA	TCGCAAGCAGCTCTCAATGAA
COPZ1	COPI	GAGGGGTGATCCTAGAGAGTGAT	CCGTAAGGGGGACATCTTC
ARF1	COPI	GATCGTGACCACCATTCCCA	TCCCACACAGTGAAGCTGAT
SEC16A	COPII	CCTGCCATGGAGCAAGTT	CACAGACATGAGGCACTGAAA
SAR1A	COPII	TTGATCTTGGTGGGCACGAG	GGATTCCACGAGGCGAGAAT
SAR1B	COPII	CCATTGCTGGCATGACGTTT	TGCCATTGATAGCAGGAAGGT
SEC12	COPII	GTTGTACGCGCTTCAGGTC	GCGCCCATTAATCAGCTCTA
SEC23A	COPII	GGTGGCACATGTCAGTGGAA	ACGCCCTCCTTGAGGAATTG
SEC23B	COPII	TCTTCCGAGGGACCAAGGAT	AAGCAAAAGGGTGCTCCTGT
SEC24A	COPII	GAATTCAGTTTGCCAGAGTTTGT	CAAATGTTATGAAGCCAATTTTTG
SEC24B	COPII	ATGACTCCTGTTTGGGCTCC	CAGTAAAGCCTGTGTCTGTGGA
SEC24C	COPII	GCGTCTCCTCCTTCAGTCAG	GCCTCGAACCTTCTTGGACA
SEC24D	COPII	TGTGAAGAACTGAAGACCATGC	TGCAGACGTCTCTTCTTGCT
SEC31A	COPII	CTCAAGATGGAAGCCACCCT	TGGCTTGAGTGAGTTGCACA
SEC31B	COPII	GGCACAAGCACCACTGATAC	GGAGGCCAAAAGTAACCAGC
SEC13	COPII	GGGAAGGCCAATGGGAAGTA	CGATGGGTGGTCTATGAGGC
RNF11	TR	GCTTCACGAGTCTCAGTCCG	GGCTAGGTGTTGGGTGGTAG
GAPDH	Control	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT

Table S3 is provided as an Excel file and shows assays for targeted proteomics analysis.
Table S4 is provided as an Excel file and shows TRs coexpressed with SEC23B, SEC24B, and SEC24D.