

Supplementary Information

Spatial proteomics reveals secretory pathway disturbances caused by neuropathy-associated TECPR2

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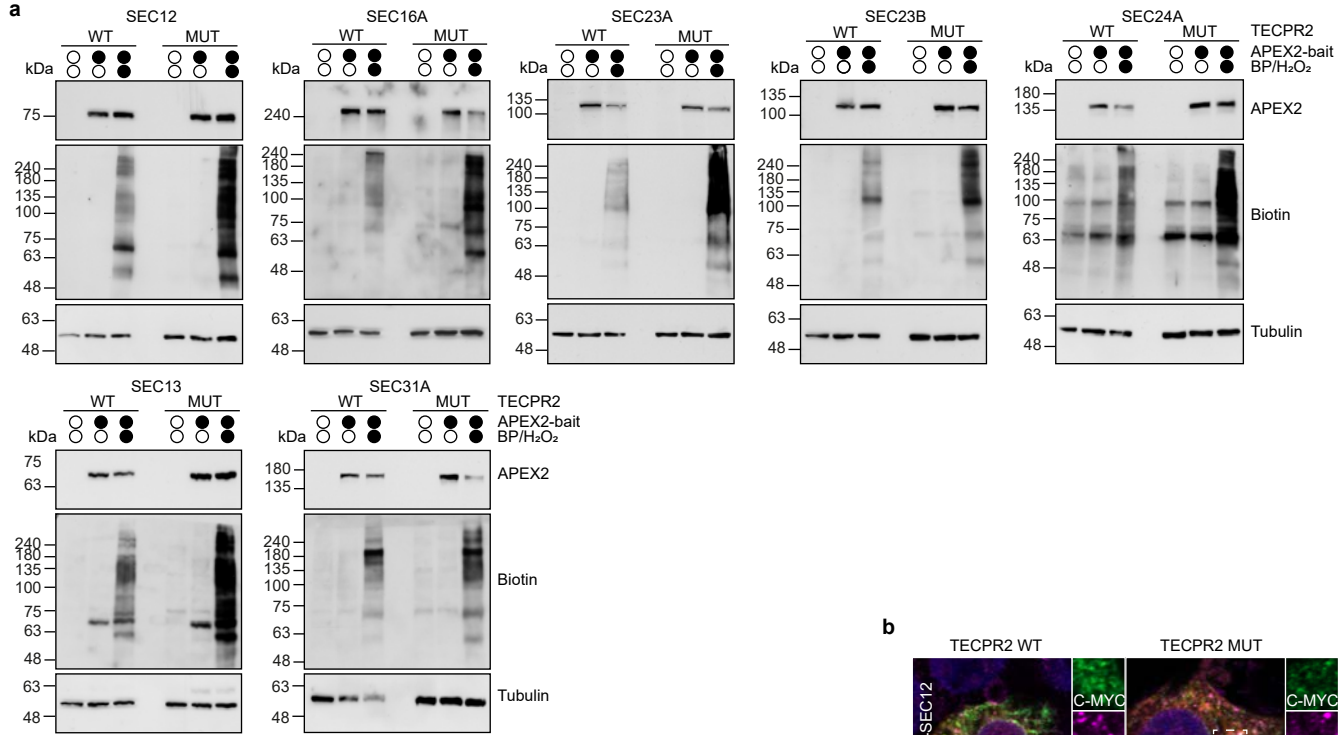
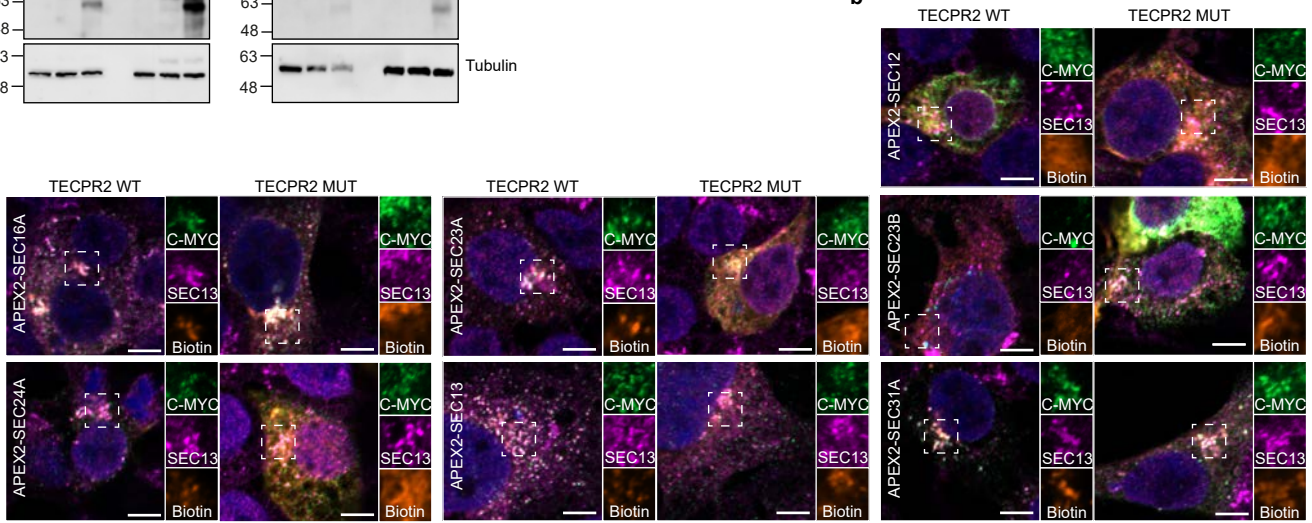
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a**b**

Supplementary Figure 1: **APEX2-ERES cell line evaluation.**

a, b TECPR2 WT and MUT cells expressing APEX2-SEC12, -SEC16A, -SEC23A, -SEC23B, -SEC24A, -SEC13 or -SEC31A were differentially treated with biotin-phenol (BP) and H₂O₂ to induce biotinylation followed by lysis and immunoblotting (**a**) or fixation and immunolabeling (**b**) with indicated antibodies. Insets show magnification of boxed areas. Scale bars 10 µm. Source data are provided in Source Data file.

a

		APEX2-		SEC12		SEC13		SEC16A		SEC23A		SEC23B		SEC24A		SEC31A		Empty	
TECPR2	WT	≥0.97	≥0.95	≥0.96	≥0.95	≥0.99	≥0.98	≥0.89	≥0.86										
	MUT	≥0.98	≥0.96	≥0.97	≥0.96	≥0.96	≥0.97	≥0.96	≥0.92										

Minimum r of 4 n, LFQ intensities

b

MS analysis: MaxQuant & Perseus:

3536 protein groups

↓ Filter for e.g. contaminants
Peptides ≥ 2 in at least 3/4 n per group

2570 protein groups

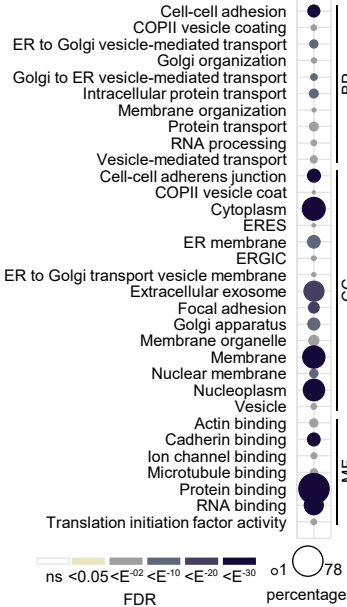
↓ Filter against unspecific background over all lines
by t-test p < 0.05, FDR < 0.05, log₂fc > 2

1070 protein groups

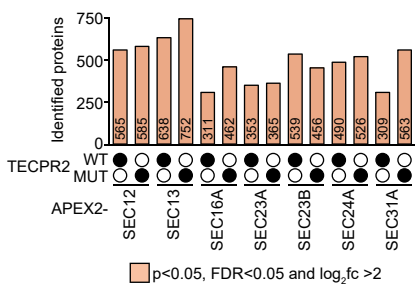
↓

Comparison TECPR2 WT vs MUT per cell line
by t-test p < 0.05, FDR < 0.05, log₂fc > 1 (class 1)
or t-test p < 0.05, log₂fc > 0.1 (class 2)

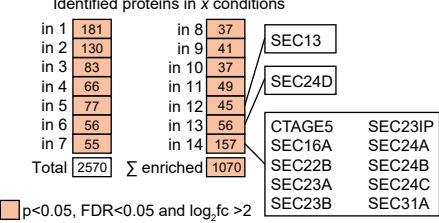
c



d

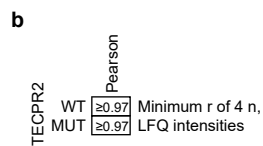
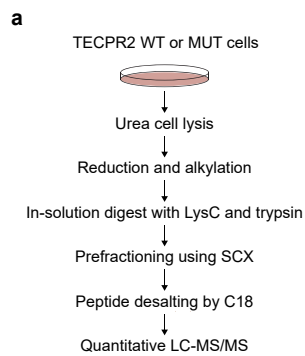


e



Supplementary Figure 2: **ERES proximity profiling.**

a Pearson correlation of four biological replicates based on LFQ intensities of identified and quantified proteins. **b** Schematic representation of proteomic data filtering and statistical analysis. **c** GO term enrichment of all proximity proteins that were significantly enriched compared to non-biotinylated control cells. Dot size correlates to the number of proteins, dot color to term enrichment (FDR) (two-sided t-test, n=4 independent experiments). **d** Number of identified proteins per APEX2 chimera in TECPR2 WT and MUT cells that were enriched over unspecific background (two-sided t-test, n=4 independent experiments). **e** Comparative analysis of identified proteins across different APEX2 chimeras in TECPR2 WT and MUT cells (two-sided t-test, n=4 independent experiments). Source data are provided in Source Data file.



c

MS analysis: MaxQuant & Perseus:

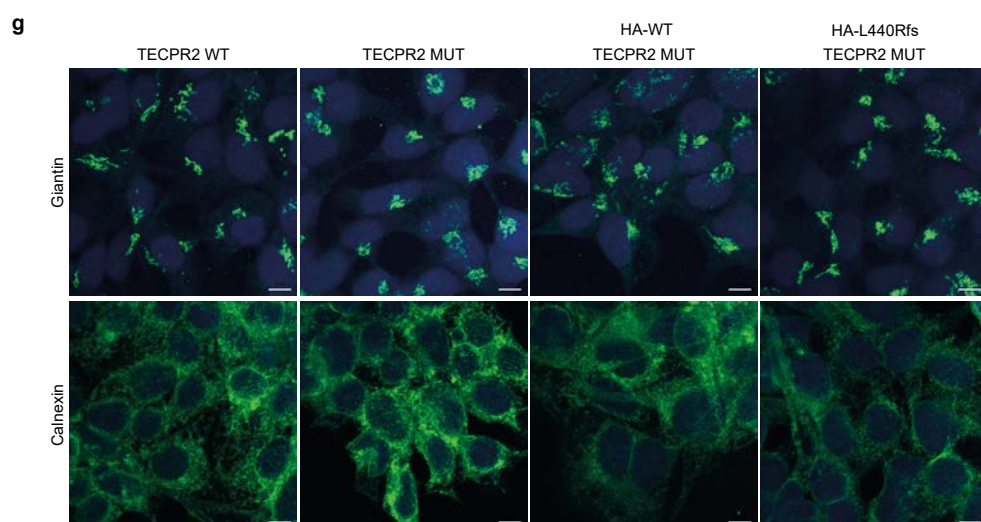
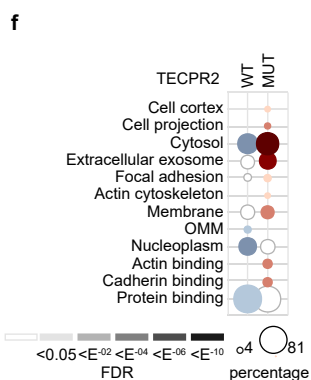
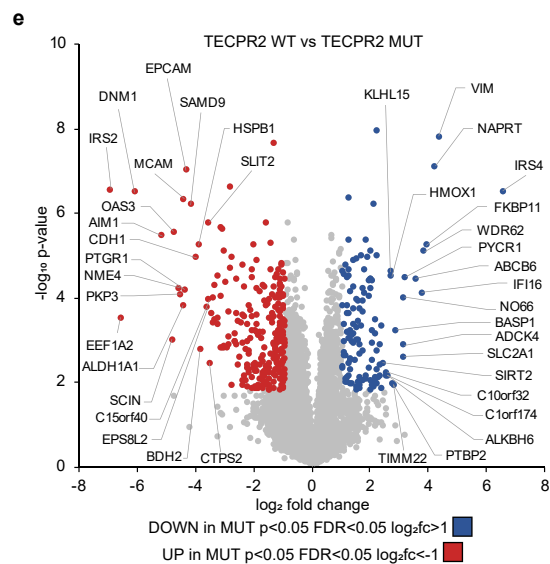
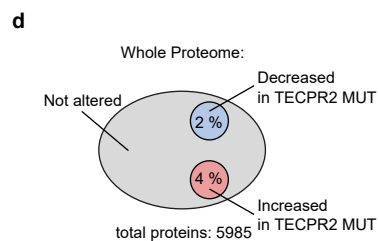
8882 protein groups

Filter for e.g. contaminants

Peptides ≥ 2 in at least 3/4 n per group

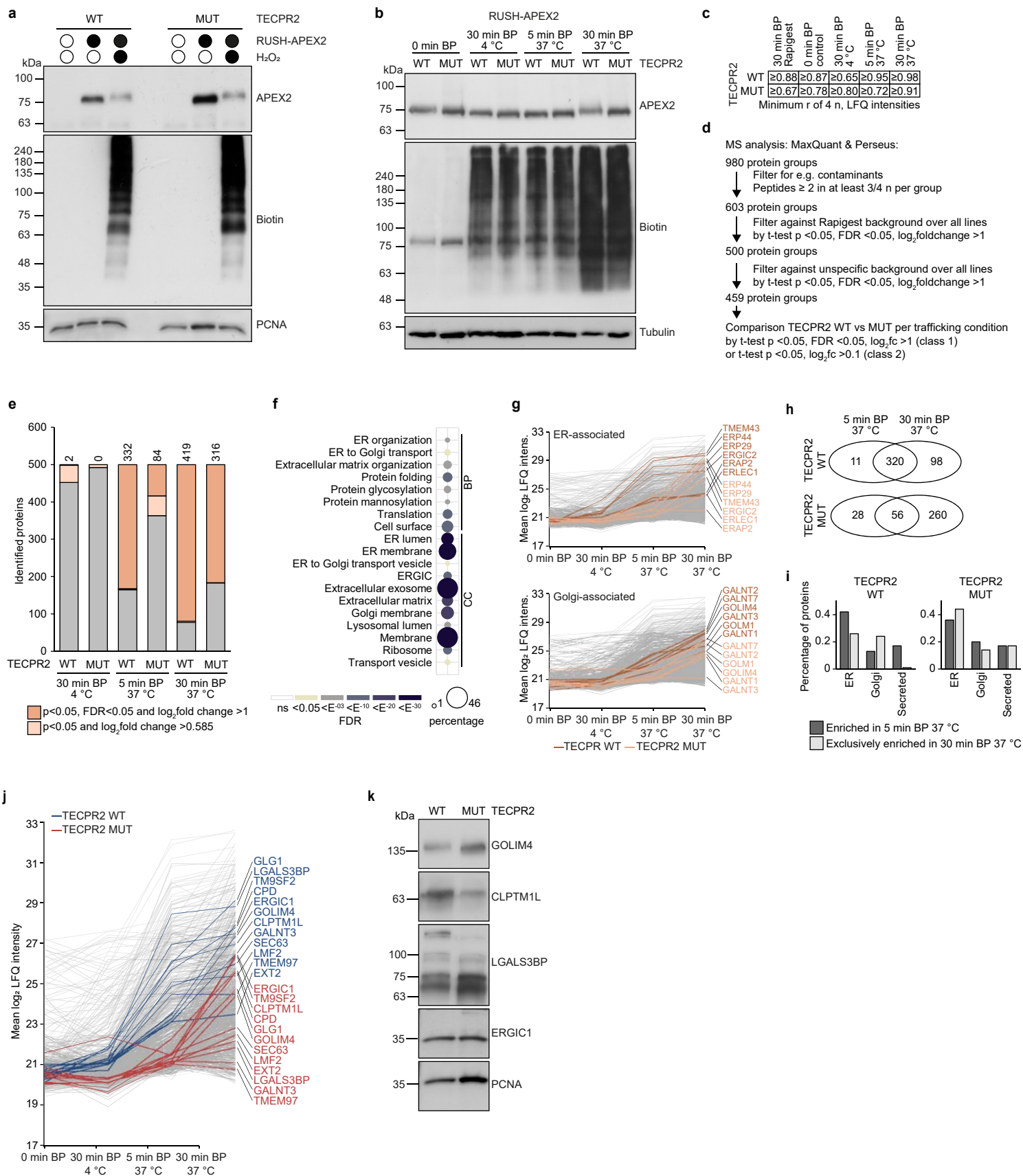
5985 protein groups

Comparison TECPR2 WT vs MUT by t-test $p < 0.05$, FDR < 0.05 , $\log_2 fc > 1$



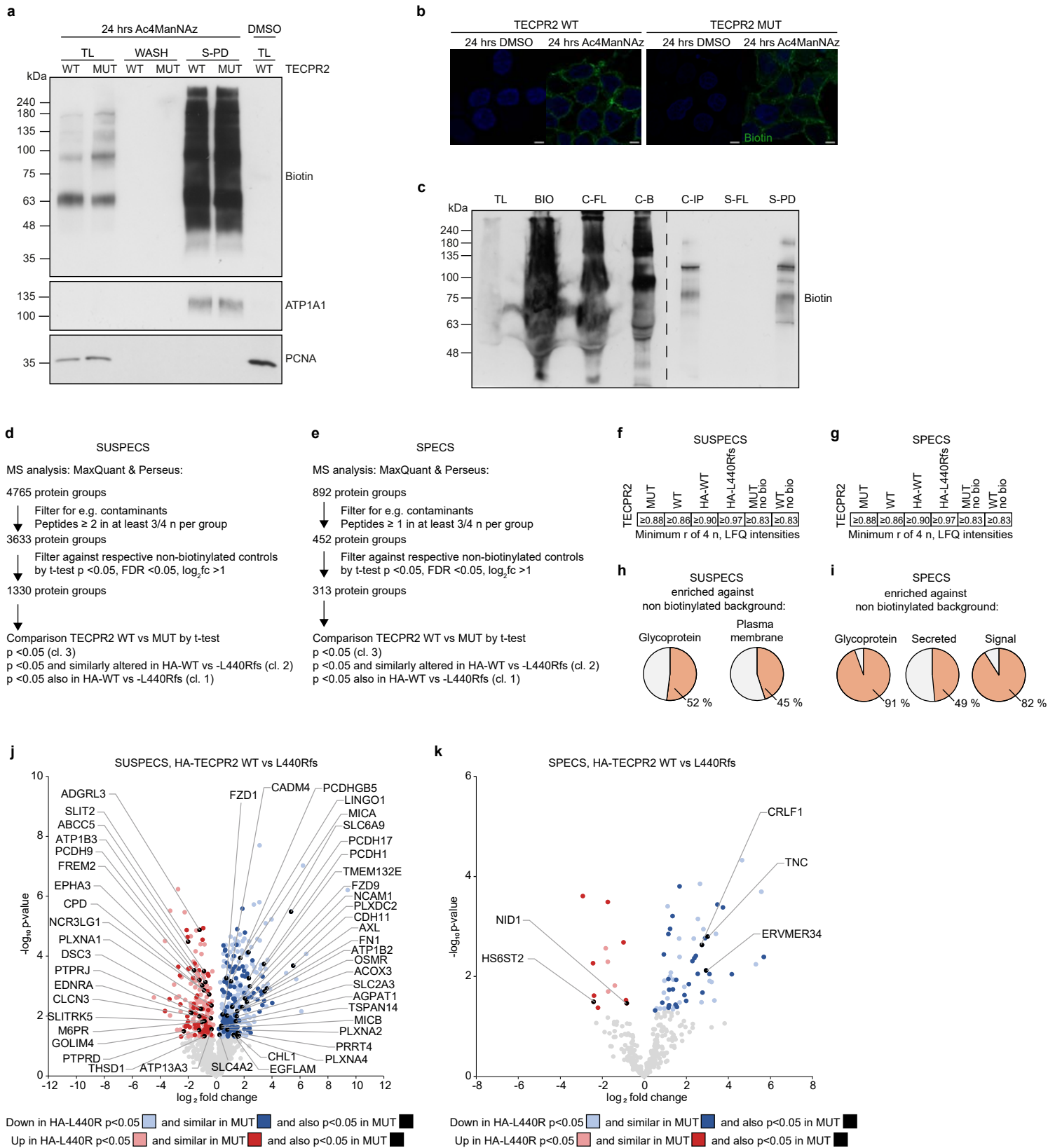
Supplementary Figure 3: **Global protein abundance profiling.**

a Whole proteome workflow. **b** Pearson correlation of 4 biological replicates based on LFQ intensities of identified proteins. **c** Schematic representation of whole proteome data filtering and statistical processing. **d** Venn graph-like summary of proteins decreased (blue), increased (red) or unchanged (grey) upon TECPR2 MUT compared to TECPR2 WT. **e** Volcano plot of the total proteome of TECPR2 WT compared to TECPR2 MUT cells. Proteins decreased in TECPR2 MUT cells are highlighted in blue, proteins increased in red and unchanged proteins in gray ($p < 0.05$, $FDR < 0.05$ and $\log_2$ fold change > 1 , two-sided t-test, $n=4$ independent experiments), respectively. **f** GO-Term analysis of candidates with altered total protein abundance in TECPR2 MUT cells. Dot size correlates to number of proteins, dot color to term enrichment (FDR) (two-sided t-test, $n=4$ independent experiments). **g** TECPR2 WT and MUT cells as well as TECPR2 MUT cells re-expressing TECPR2 WT and L440Rfs were fixed and immunostained with an anti-Giantin or anti-Calnexin antibody. Scale bars represent 10 μm . Source data are provided in Source Data file.



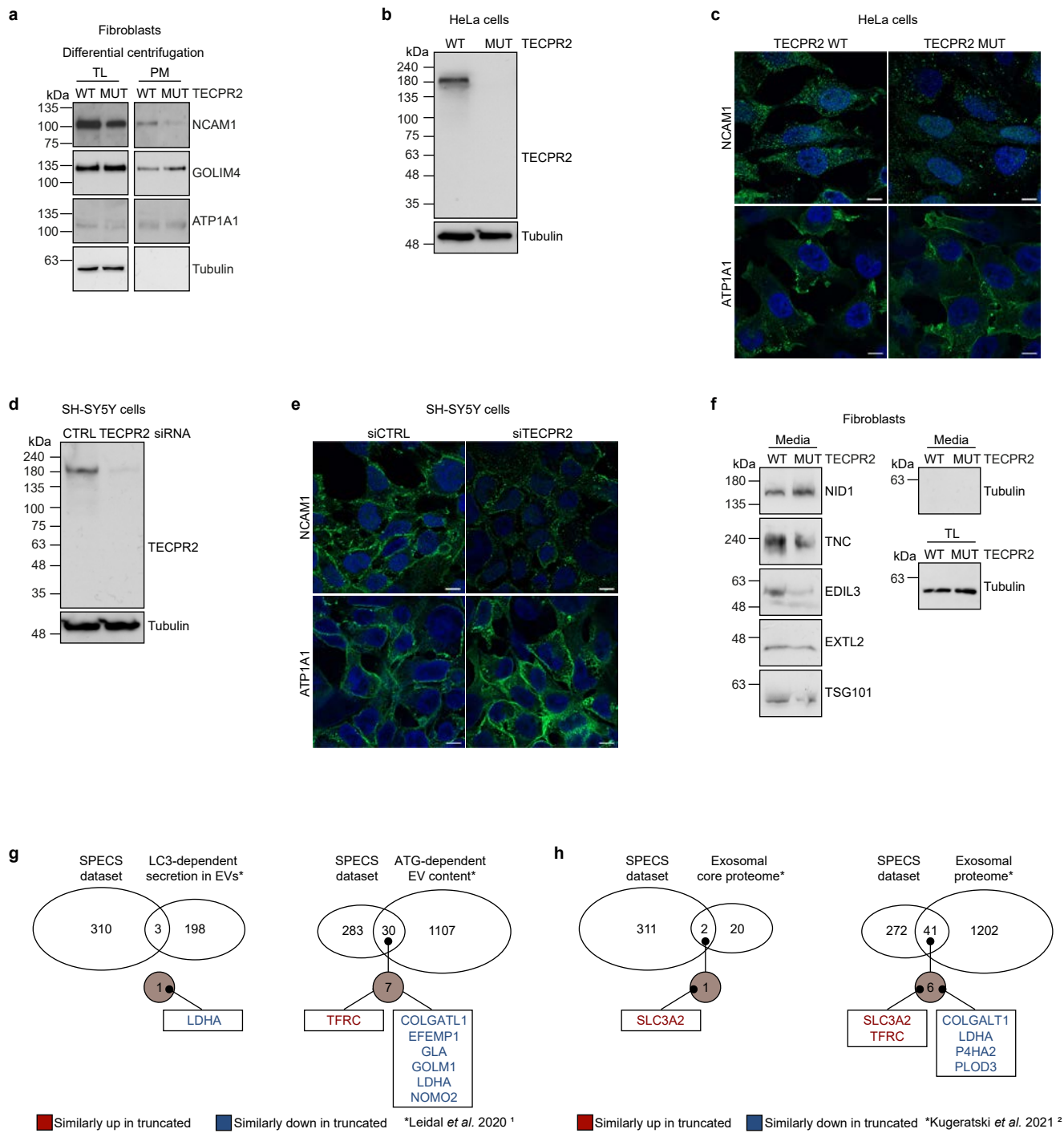
Supplementary Figure 4: **COPII cargo profiling by RUSH-APEX2.**

a Empty or MAN2A1-SBP-GFP (RUSH)-APEX2 expressing TECPR2 WT and MUT 293T cells grown in the presence of biotin-phenol (BP) were differentially treated with H₂O₂ and lysed for immunoblotting with anti-APEX2, -biotin and -PCNA antibodies. **b** TECPR2 WT and MUT cells expressing RUSH-APEX2 were treated for 0, 5 and 30 min with BP at 4 °C or 37 °C and pulsed with H₂O₂ prior to lysis and immunoblot analysis. **c** Pearson correlation of four biological replicates based on LFQ intensities of identified and quantified proteins. **d** Schematic representation of proximity proteomic data filtering and statistical analysis. **e** Bar graph of proteinase K protected proteins enriched after 5 min and 30 min BP treatment (orange) compared to untreated (0 min BP) control conditions (grey, unchanged) after prefiltering against proteins found in proteinase K- and RapiGest-treated background samples (two-sided t-test, n=4 independent experiments). **f** GO term enrichment of proximity proteins enriched over non-biotinylated control samples. Dot size correlates to number of proteins, dot color to term enrichment (FDR) (two-sided t-test, n=4 independent experiments). **g** Line graphs depicting mean log₂ LFQ intensities of selected known ER and Golgi proteins across different trafficking conditions in TECPR2 WT (brown) or MUT (orange) cells detected by protease protection-enhanced RUSH-APEX2 profiling. Grey lines represent all proteins enriched over non-biotinylated control samples. **h** Venn graph summarizing proteins enriched after 5 min and/or 30 min trafficking at 37 °C. **i** Distribution of enriched proteins annotated as ER-associated, Golgi-associated and secreted proteins (based on GO term keywords) in TECPR2 WT and MUT cells. Proteins considered for comparison were either enriched after 5 min trafficking at 37 °C (dark grey) or exclusively enriched after 30 min trafficking at 37 °C (light grey). **j** Line graphs depicting mean log₂ LFQ intensities of selected COPII cargo candidates across different trafficking conditions in TECPR2 WT (blue) and MUT (red) cells detected by protease protection-enhanced RUSH-APEX2 profiling. Grey lines represent all proteins enriched over non-biotinylated control samples. **k** Lysates from TECPR2 WT and MUT cells were analyzed by immunoblotting with indicated antibodies. PCNA served as loading control. Source data are provided in Source Data file.



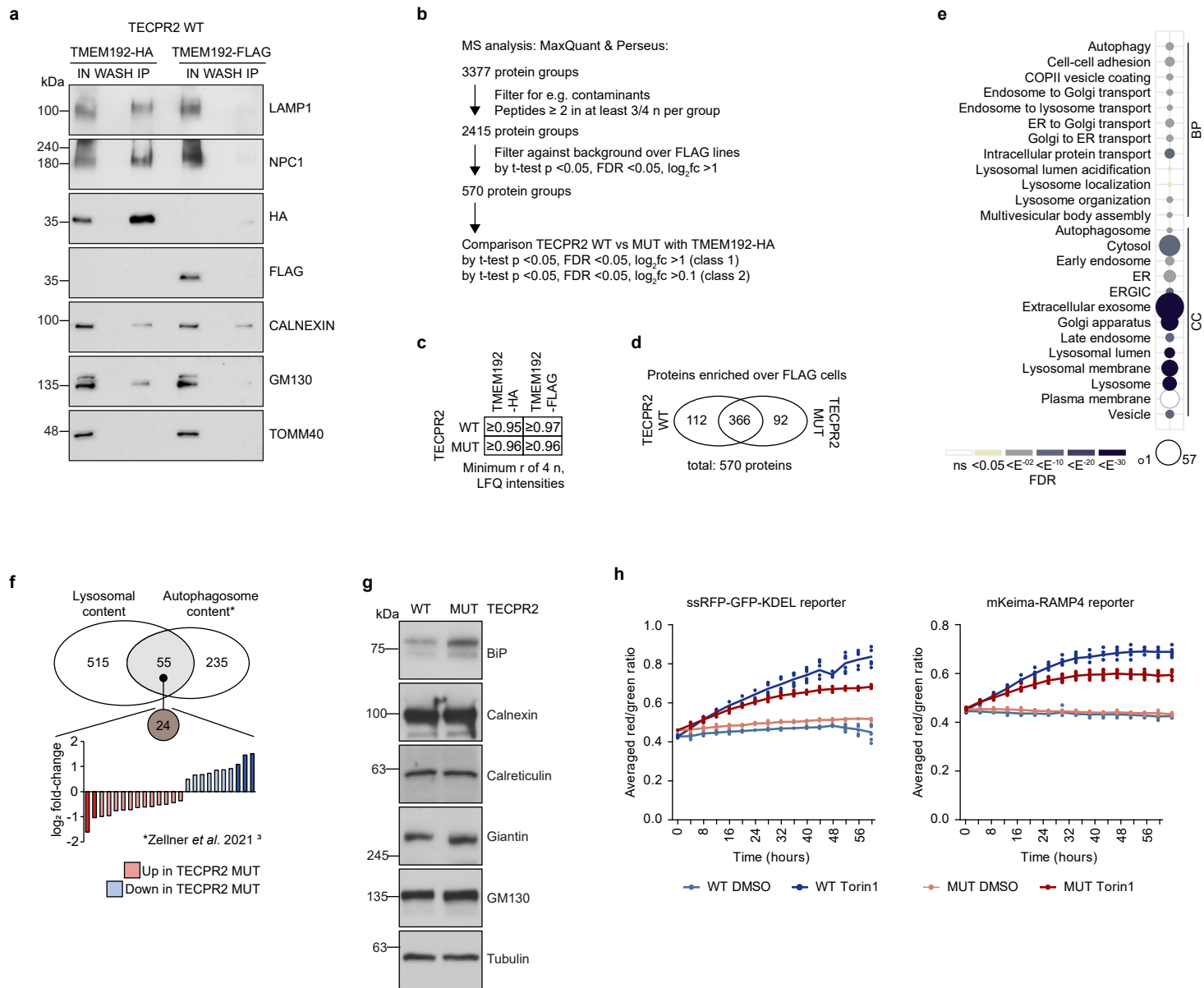
Supplementary Figure 5: **Cell surface proteome and secretome analysis.**

a, b TECPR2 WT and MUT 293T cells treated with DMSO or tetra-acetylated N-azidoacetylmannosamine (Ac₄-ManNAz) for 24 hrs and with sulfo-dibenzylcyclooctyne-biotin conjugate (DBCO) for 2 hrs at 4° C prior to denaturing lysis, streptavidin pulldown (S-PD) and immunoblotting (**a**) or to fixation without membrane permeabilization and immunolabeling with an anti-biotin antibody (**b**). TL, total lysate. ATP1A1 and PCNA served as loading controls. Scale bars 10 µm. **c** Immunoblot analysis of biotinylated protein enrichment from Ac₄ManNAz (24 hrs)- and DBCO (ON at 4° C)-treated 293T cell derived media that were enriched for glycoproteins with concanavalin A sepharose (2 hrs at 4 °C) BIO, fraction after biotinylation; C-FL, flow-through of concanavalin A sepharose; C-B, concanavalin A pulldown; C-IP, eluate after concanavalin A; S-FL, flow-through of streptavidin sepharose; S-IP, streptavidin pulldown. **d, e** Schematic representation of SUSPECS (**d**) and SPECS (**e**) proteomic data filtering and statistical processing. **f, g** Pearson correlation of four biological replicates based on LFQ intensities of identified proteins using SUSPECS (**f**) and SPECS (**g**) proteomics. **h, i** Distribution of or proteins identified by SPECS (**h**) and SUSPECS (**i**) with glycoprotein, plasma membrane, secreted or signal peptide GO term annotations enriched against non-biotinylated background. **j, k** Volcano plot of cell surface proteome (**j**) and secretome (**k**) alterations upon TECPR2 L440Rfs expression. Significantly altered proteins (two-sided t-test, n=4 independent experiments) are grouped as class III (light color), class II (dark color) or class I (black and annotated). Source data are provided in Source Data file.



Supplementary Figure 6: **Validation of cell surface proteome and secretome candidates.**

a Immunoblot of PM fractions obtained by differential centrifugation of homogenates from healthy individual- and patient-derived fibroblasts. ATP1A1 and tubulin served as loading controls. **b, c** TECPR2 WT and MUT HeLa cells were either lysed and analyzed by immunoblotted (**b**) or fixed and immunostained with an anti-NCAM1 antibody (**c**). Scale bars represent 10 μ m. **d, e** SH-SY5Y cells were reversely transfected with non-targeting siCTRL or siTECPR2 followed by lysis and immunoblotting (**d**) or fixation and immunostaining with an anti-NCAM1 antibody (**e**). Scale bars represent 10 μ m. **f** Media from healthy individual- and patient-derived fibroblasts were subjected to size-exclusion filtration and lectin-based immunoprecipitation followed by immunoblotting. EXTL2, TSG101 and tubulin served as loading controls. **g, h** Comparative analysis of all secreted proteins found in this study and those subjected to autophagy-dependent secretion ¹ (**g**) or exosome secretion ² (**h**). Brown circles indicate commonly altered proteins which are further broken down depending on their regulation in our SPECS dataset. Source data are provided in Source Data file.



Supplementary Figure 7: **Analysis of lysosome composition and ER-phagy.**

a Homogenates from 293T cells expressing C-terminally 3xHA or 2xFLAG-tagged TMEM192 were subjected to HA-IP followed by immunoblot analysis. LAMP1 and NPC1 served as lysosomal controls, calnexin, GM130 and TOMM40 as organelle markers. **b** Schematic representation of Lyso-IP proteomic data filtering and statistical processing. **c** Pearson correlation of four biological replicates based on LFQ intensities of proteins identified by LysolIP proteomics. **d** Venn graph of proteins enriched in TMEM192-3HA expressing cells compared to FLAG background. **e** GO term enrichment of proteins enriched in TMEM192-3HA expressing cells. Dot size correlates to number of proteins, dot color to term enrichment (FDR). **f** Comparative analysis of lysosomal proteins found in this study and previously mapped autophagosome content ³. Brown circles indicate commonly altered proteins for which log₂ fold changes upon expression of mutant TECPR2 are depicted as bar graph. **g** Lysates from TECPR2 WT and MUT 293T cells were analyzed by immunoblotting. PCNA served as loading control. **h** TECPR2 WT and MUT cells expressing ssRFP-GFP-KDEL or mKeima-RAMP4 reporter were seeded in 384-well plates in the presence of Doxycycline for 24 hours (KDEL) or 16 h without additional induction (RAMP4). Cells were subsequently treated with DMSO or 250 nM Torin1. Red and green signals were measured over a total period of 60 hrs using the IncuCyteS3. ER-phagy flux represented by the RFP/GFP (red/green) ratio (total fluorescent intensities). Data representative for two biological replicates, measured in three technical replicates each. Line graphs depict averaged values. Source data are provided in Source Data file.

a

TECPR2 WT	Empty
TECPR2 MUT	Empty
TECPR2 MUT + HA-TECPR2 WT	
TECPR2 MUT + HA-TECPR2 L440Rfs	
TECPR2 MUT + HA-FIP200	

MS analysis: MaxQuant & Perseus:

2828 protein groups

↓ Filter for e.g. contaminants

↓ Peptides ≥ 2 in at least 2 samples

2066 protein groups

↓ Keep when protein in at least 6/8 per condition

↓ Combine technical replicates and calculate LFQs

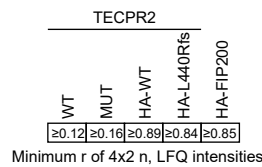
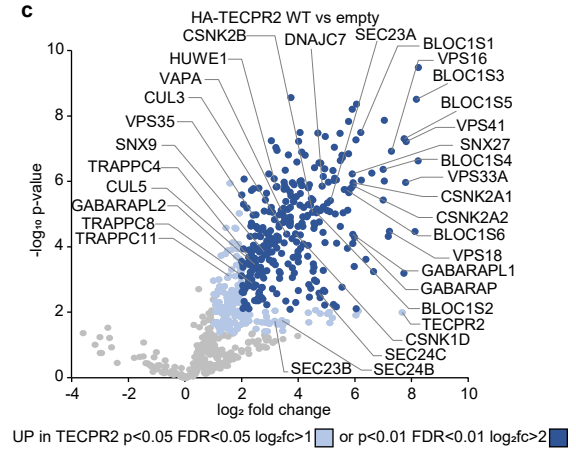
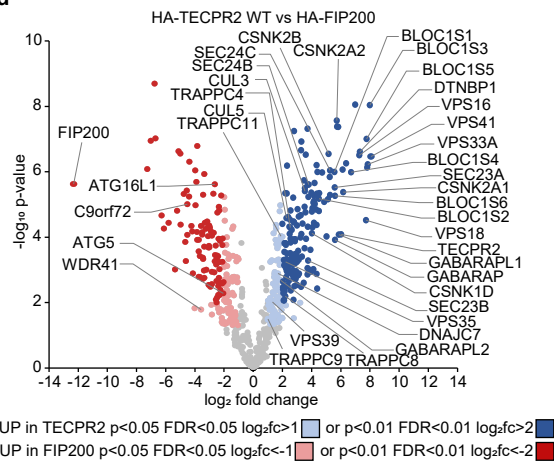
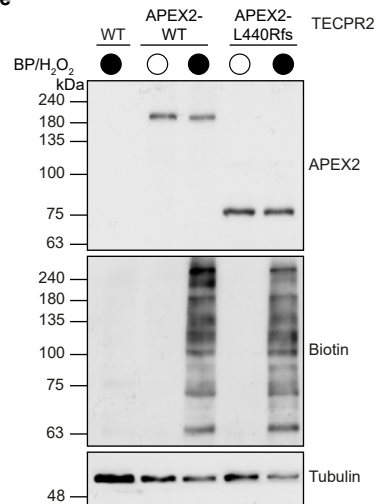
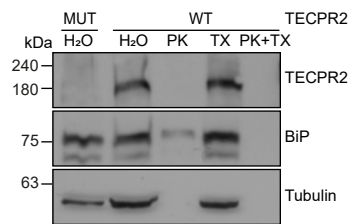
848 protein groups

↓ Filter against background of empty cells

↓ by t-test $p < 0.05$, FDR < 0.05 , $\log_2 \text{fc} > 1$

618 protein groups

↓ Comparison HA-TECPR2 WT vs L440Rfs vs HA-FIP200

by t-test $p < 0.05$, FDR < 0.05 , $\log_2 \text{fc} > 1$ (class 1)or t-test $p < 0.01$, FDR < 0.01 , $\log_2 \text{fc} > 2$ (class 2)**b****c****d****e****f**

Supplementary Figure 8: **TECPR2 interactome analysis.**

a Schematic representation of HA-IP proteomic data filtering and statistical processing. **b** Pearson correlation of 4 biological replicates based on LFQ intensities of identified proteins. **c**, **d** Volcano plot of candidate interacting proteins of HA-TECPR2 WT expressed in TECPR2 MUT cells compared to empty TECPR2 MUT cells (**c**) or HA-FIP200 expressed in TECPR2 MUT cells (**d**) identified by HA-IP proteomics. Enriched proteins were highlighted in light (p <0.05, FDR <0.05 and log2 fold change >1, two-sided t-test) or dark blue (p <0.01, FDR <0.01 and log2 fold change >2, two-sided t-test, n=4 independent experiments). **e** APEX2-TECPR2 WT or L440Rfs expressing 293T cells were labeled with biotin, lysed and analyzed by immunoblotting. Tubulin served as loading control. **f** Immunoblots of homogenates from TECPR2 WT and MUT cells left untreated (H₂O) or incubated with proteinase K (PK), Triton-X100 (TX) or both. BiP and Tubulin served as controls. Source data are provided in Source Data file.

Supplementary References:

- 1 Leidal, A. M. *et al.* The LC3-conjugation machinery specifies the loading of RNA-binding proteins into extracellular vesicles. *Nat Cell Biol* **22**, 187-199, doi:10.1038/s41556-019-0450-y (2020).
- 2 Kugeratski, F. G. *et al.* Quantitative proteomics identifies the core proteome of exosomes with syntenin-1 as the highest abundant protein and a putative universal biomarker. *Nat Cell Biol* **23**, 631-641, doi:10.1038/s41556-021-00693-y (2021).
- 3 Zellner, S., Schifferer, M. & Behrends, C. Systematically defining selective autophagy receptor-specific cargo using autophagosome content profiling. *Mol Cell* **81**, 1337-1354 e1338, doi:10.1016/j.molcel.2021.01.009 (2021).