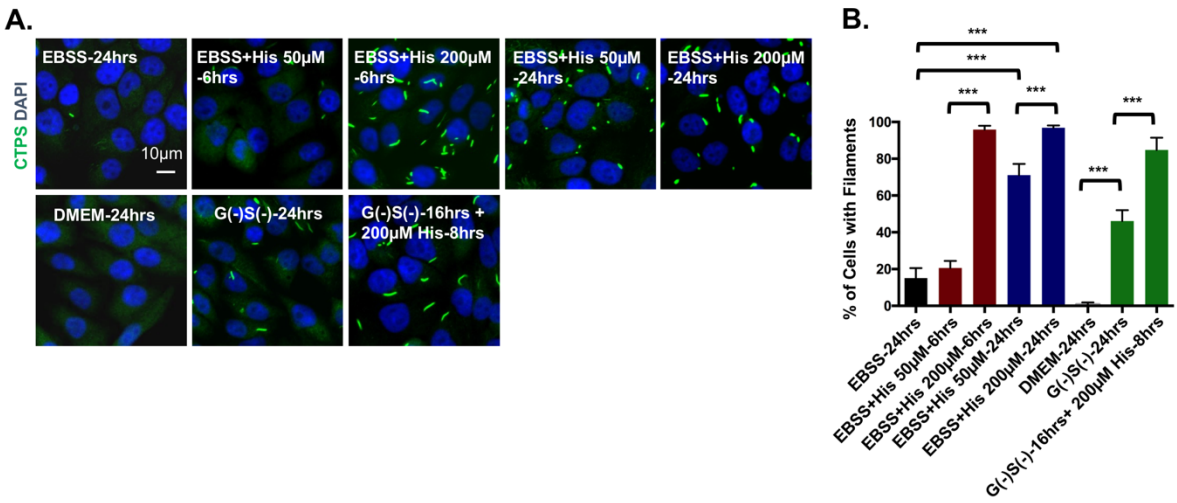


Fig. S1: Histidine (His) induced CTPS filaments during nutrient starvation in HEp-2 cells

(A-B) HEp-2 cells were incubated with two different concentrations (50 μM & 200 μM) of His for 6 h and 24 h in EBSS medium and then immunostained with anti-CTPS (green) antibodies and DAPI (blue). After 16 h, HEp-2 cells incubated in G(-)S(-) conditions were stimulated with 200 μM His for another 8 h to induce CTPS filaments. The percentage of cells bearing CTPS filaments was calculated for 3 independent experiments. Results are mean±s.d. ***$P < 0.001$ (Student's t-test).

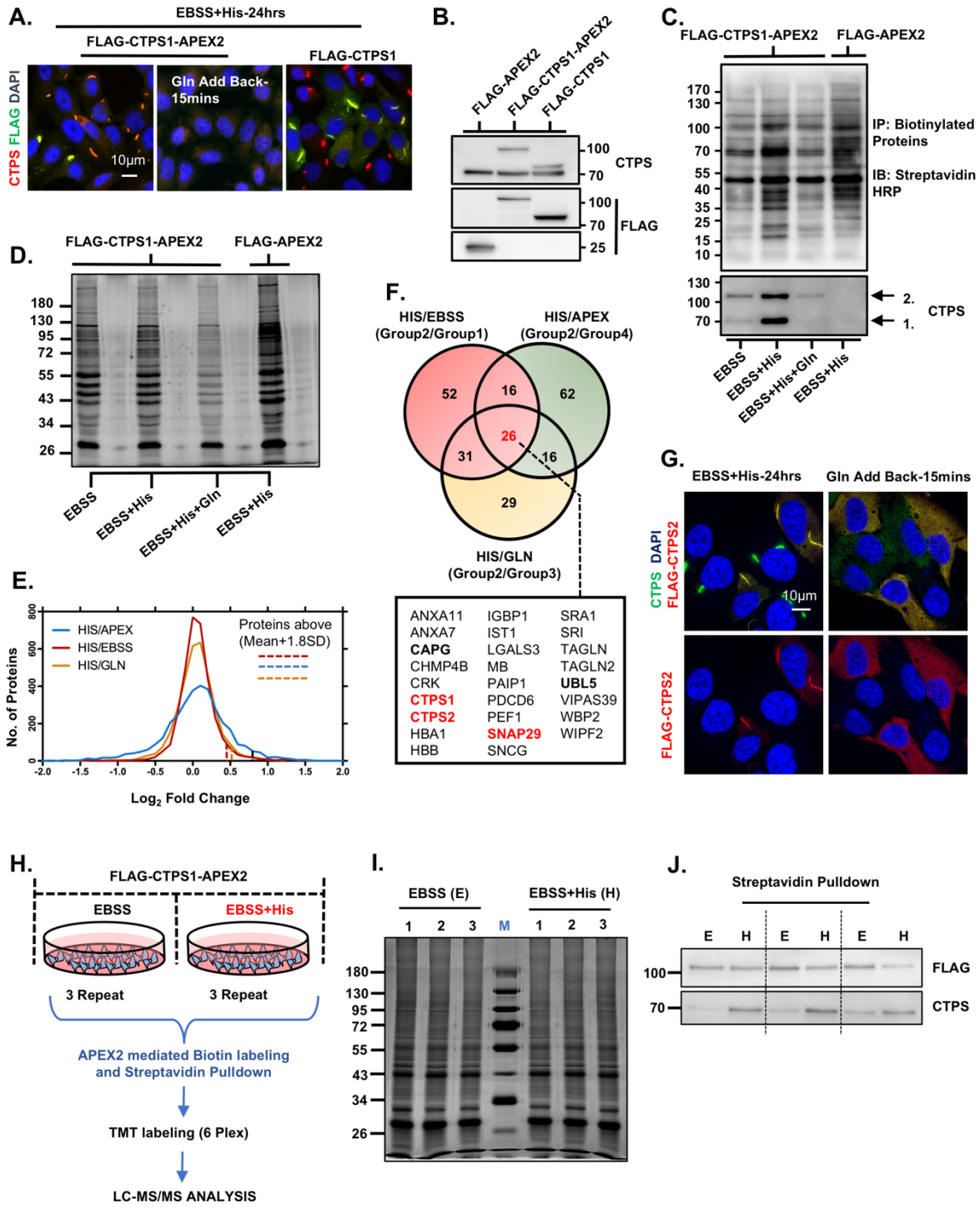


Fig. S2: Discovery of candidate genes important for CTPS filament formation

(A) HEp-2 cells expressing FLAG-CTPS1-APEX2 and FLAG-CTPS1 were incubated in His-containing EBSS medium for 24 h and further immunostained with anti-Flag (green), anti-CTPS (red) antibodies and DAPI (blue). FLAG-CTPS1-APEX2 filaments dissembled within 15 min upon 4 mM Gln treatment. **(B)** Western blotting of exogenous FLAG-CTPS1, FLAG-

CTPS1-APEX2 and FLAG-APEX2 proteins of HEp-2 cells in DMEM. **(C)** Western blotting analysis of biotinylated proteins after immunoprecipitation (IP) using streptavidin-conjugated magnetic beads. Streptavidin-horseradish peroxidase (HRP) was used to detect the level of biotinylation, and anti-CTPS antibodies were used to detect endogenous and exogenous CTPS enriched in each group after streptavidin pulldown. **(D)** Silver staining of the biotinylated proteins after streptavidin IP from each group, i.e., FLAG-CTPS1-APEX2 in EBSS (group 1), EBSS+His (group 2), EBSS+His+Gln (group 3), and FLAG-APEX2 in EBSS+His (group 4). **(E)** Distribution of genes over the iTRAQ ratios $\log_2(115/117)$, i.e., HIS/APEX (Group 2/Group 4), $\log_2(115/114)$, i.e., HIS/EBSS (Group 2/Group 1) and $\log_2(115/116)$, i.e., HIS/GLN (Group 2/Group 3). In total, 3417 proteins were quantified with cut-off criteria of unique peptides ≥ 2, ratio counts ≥ 2 and a false discovery rate (FDR) < 0.01. To minimize the false positives, mean+1.8 SD (standard deviation) was set as the cutoff. **(F)** Venn diagram showing 26 proteins common among HIS/EBSS (Group 2/Group 1), HIS/APEX (Group 2/Group 4) and HIS/GLN (Group 2/Group 3). KRT genes enriched in HIS/EBSS are KRT10, 2, 1 and 9. KRT genes enriched in HIS/APEX are KRT16, 10, 2, 1, 19, 6B, 18, 8 and 9. KRT genes enriched in HIS/GLN are KRT16, 19 and 8. **(G)** HEp-2 cells expressing FLAG-CTPS2 were incubated in EBSS+His conditions for 24 h. Cells were further treated with 4 mM Gln for 15 min to dissemble the CTPS filaments. Exogenous CTPS2 was immunostained with anti-Flag (red) antibodies; endogenous CTPS was immunostained with anti-CTPS1 (green) antibodies; and nuclei were immunostained with DAPI (blue). **(H)** Schematic representation of the strategy used for biotinylation of CTPS1 filaments in the TMT labeling assay. HEp-2 cells were transfected with FLAG-CTPS1-APEX2 and subjected to EBSS and EBSS+His conditions for 6 h. Each group had three triplicates. **(I)** Silver staining of the biotinylated proteins after streptavidin IP from HEp-2 cells expressing FLAG-CTPS1-APEX2 in EBSS and EBSS+His conditions at 6 h. **(J)** Western blotting analysis of biotinylated proteins after immunoprecipitation (IP) using streptavidin-conjugated magnetic beads. One-tenth of the IP product was used to confirm CTPS enrichment in histidine-induced groups before proceeding to TMT labeling. Anti-Flag antibodies and anti-CTPS antibodies were used to detect FLAG-CTPS1-APEX2 (exogenous) and endogenous CTPS respectively.

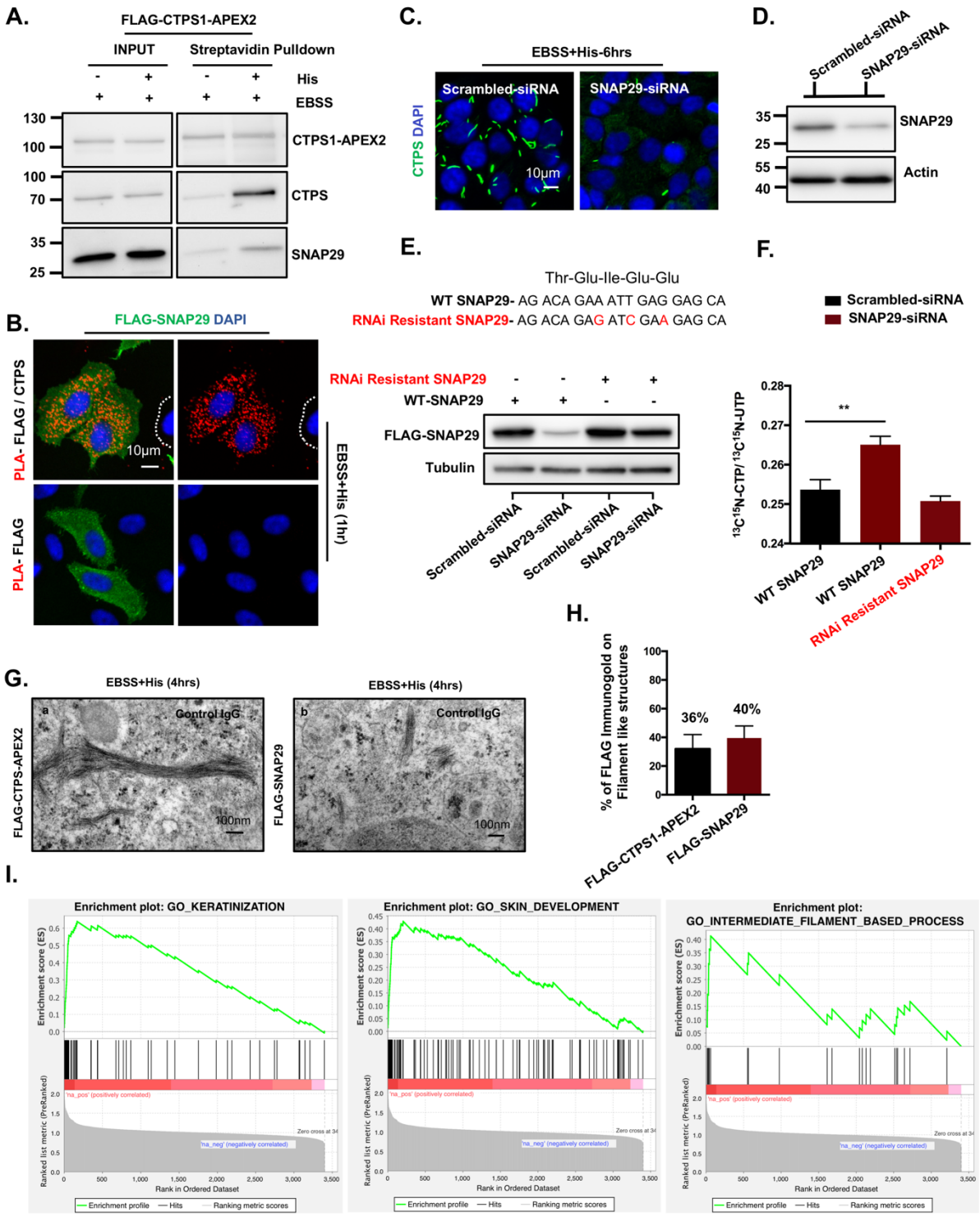


Figure S3: SNAP29 is required for CTPS filament formation

(A) APEX2 biotinylation IP confirming the enrichment of SNAP29 in the EBSS+His group at 6 h. **(B)** Proximity ligation assay (PLA) between CTPS and FLAG in EBSS+His conditions at 1 h. HEp-2 cells were transfected with FLAG-SNAP29 for 24 h before replacing the medium

with EBSS+His conditional medium. After PLA, cells were immunostained for 30 mins with the secondary antibody (green) to detect FLAG signal. **(C-D)** CTPS was immunostained with anti-CTPS (green) antibodies to reconfirm the reduced formation of CTPS filaments in SNAP29 knockdown HEp-2 cells at 6 h. SNAP29 knockdown was confirmed using western blotting. **(E)** RNAi-resistant SNAP29 was made by site-directed mutagenesis of three nucleotides encoding for the amino acids glutamate and isoleucine on the siRNA target region for SNAP29. Western blotting images show that HEp-2 cells transfected with RNAi-resistant SNAP29 transgene show a lesser knockdown effect than wild-type (WT) SNAP29 overexpression. **(F)** SNAP29 knockdown HEp-2 cells expressing WT-SNAP29 and RNAi-resistant SNAP29 were cultured in EBSS+His conditions for 5 h followed by treatment with $^{13}C^{15}N$-uridine (100 µM) for 1 h in the same medium. The ratio of labeled CTP to labeled UTP is shown. Results are mean±s.d. **P < 0.01 (Student's *t*-test). **(G)** Electron micrograph of a section of HEp-2 cells transfected with FLAG-CTPS1-APEX2 and FLAG-SNAP29. HEp-2 Cells were transfected with FLAG-SNAP29 and FLAG-CTPS1-APEX2 separately for 24 h before replacing the medium with EBSS+His conditional medium for 4 h to induce CTPS filaments. ImmunoEM revealed no gold particle deposition for control antibody labeling. **(H)** The percentage of FLAG immunogold on filament-like structures was quantified for HEp-2 cells expressing FLAG-CTPS-APEX2 and FLAG-SNAP29 in EBSS+His conditional medium at 4 h. Ten images were used to quantify each group from three independent experiments. **(I)** Gene set enrichment analysis of proteins identified by TMT assay showed enrichment for keratin-involved pathways during filament formation.

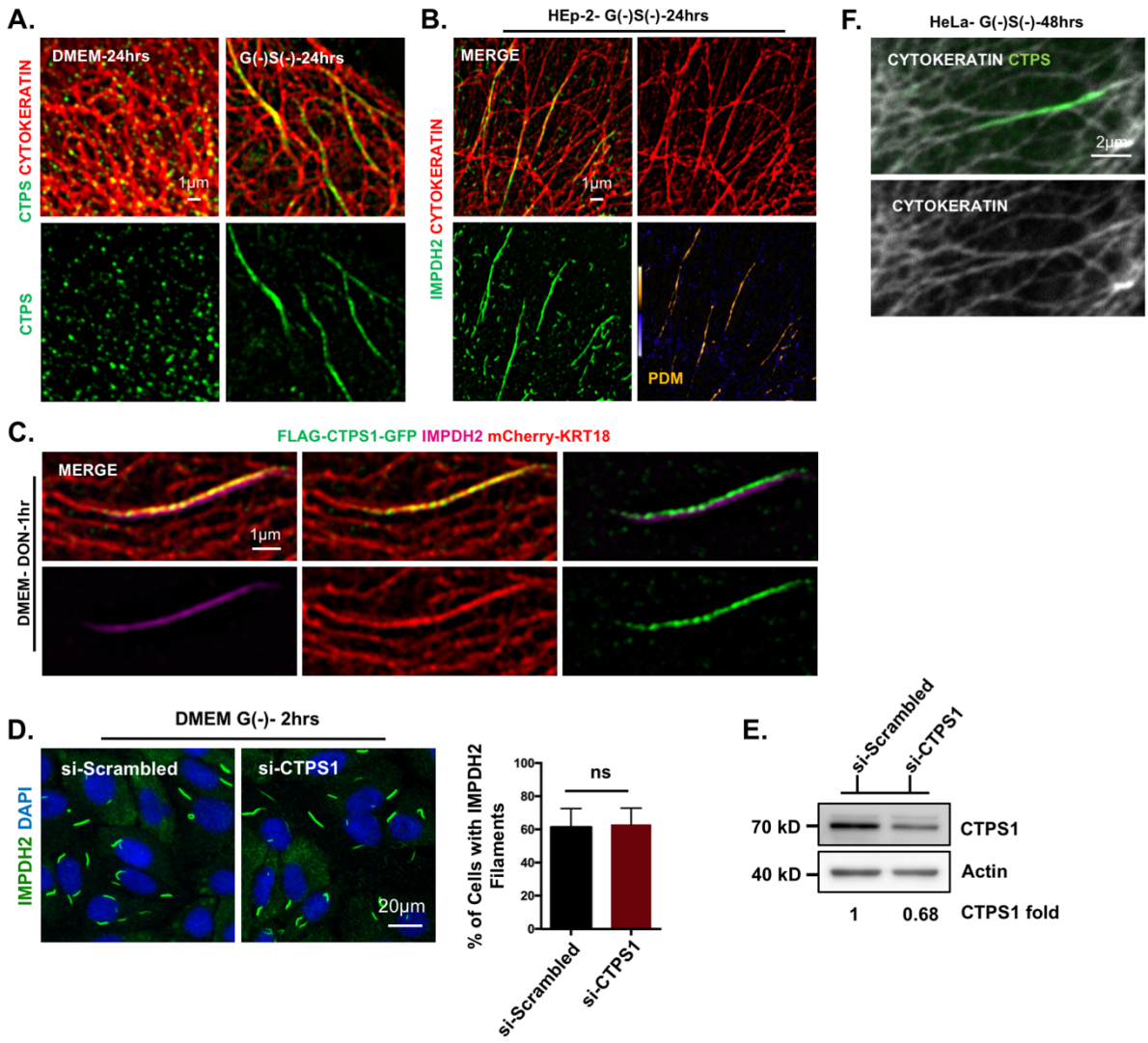


Figure S4: CTPS and IMPDH filaments co-align along the cytokeratin network

(A) Super-resolution images of CTPS and cytokeratin in DMEM and G(-)S(-) conditions at 24 h. **(B)** Super-resolution image of IMPDH2 filaments along the cytokeratin network in the G(-)S(-) condition at 24 h. HEp-2 cells were immunostained with anti-IMPDH2 (green) and anti-pan cytokeratin (red) antibodies. **(C)** CTPS and IMPDH2 are colocalized on the cytokeratin network in HEp-2 cells after 1 mM DON treatment for 1 h. HEp-2 cells were transfected with FLAG-CTPS1-GFP (green) and mCherry-KRT18 (red) and immunostained with anti-IMPDH antibody (purple). **(D-E)** Partial Knockdown of CTPS1 in HEp-2 cells didn't reduced the IMPDH2 filaments. IMPDH2 filaments were immunostained with anti-IMPDH2 (green) and nucleus with DAPI (blue). Results are mean±s.d. ns, not significant (Student's *t*-test) **(F)** CTPS filaments colocalized with cytokeratin in HeLa cells. CTPS filaments were induced in the G(-)S(-) condition for 48 h and then immunostained with anti-CTPS (green) and anti-pan-cytokeratin (white) antibodies.

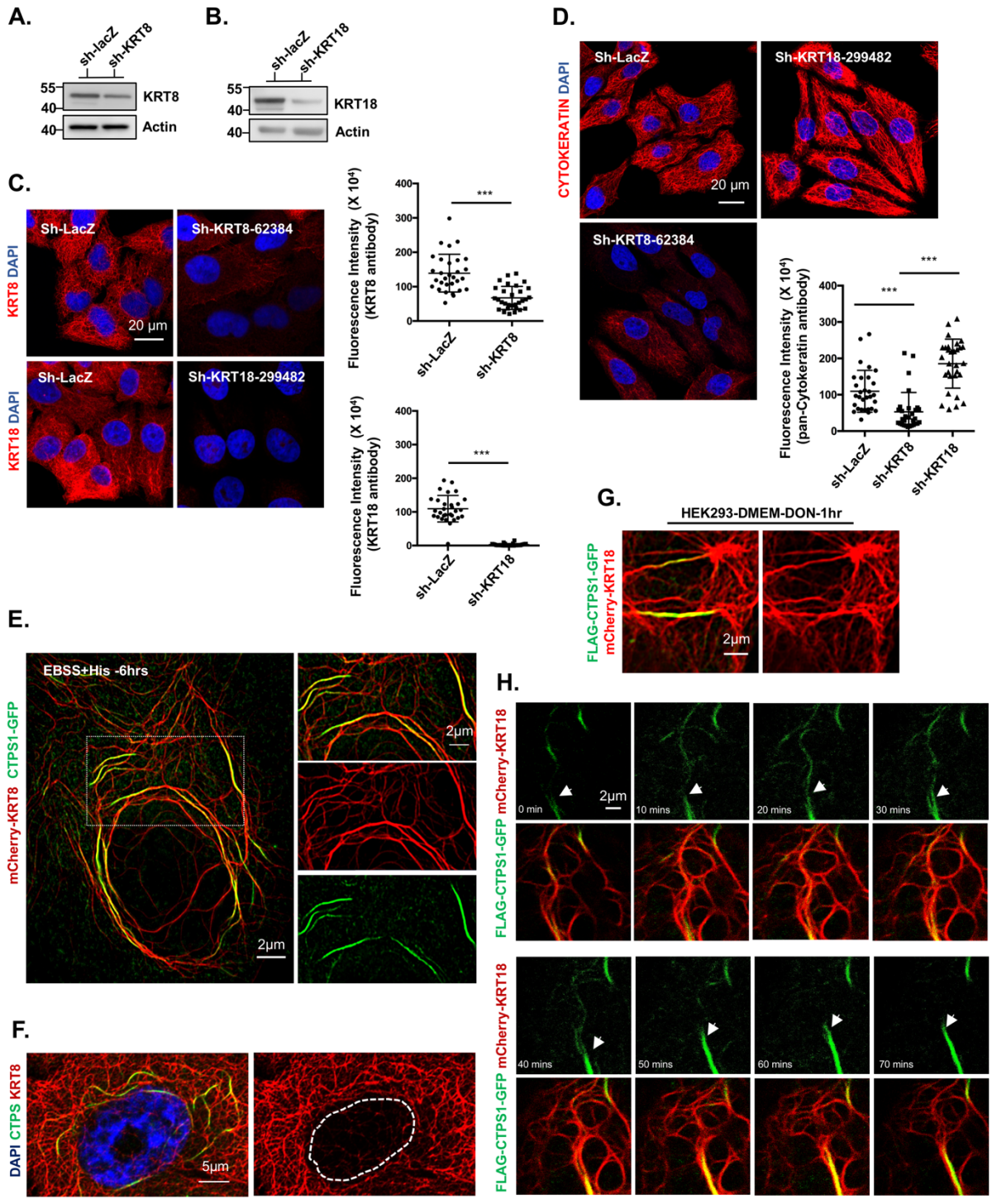


Figure S5: The cytokeratin network serves as the track for CTPS filament assembly

(A-C) ShRNA-mediated knockdown of KRT8 and KRT18 in HEp-2 cells cultured in DMEM were confirmed using western blotting (A-B). The fluorescent intensity for KRT8 and KRT18 was significantly reduced in HEp-2 cells targeting shRNA for KRT8 and KRT18, respectively (C). To measure fluorescence intensity, 30 cells cultured in DMEM were randomly quantified from each group from 3 independent experiments. Results are mean±s.d. ***P < 0.001

(Student's *t*-test). **(D)** Pan cytokeratin fluorescent intensity was significantly reduced in HEp-2 cells targeting shRNA for KRT8. To measure fluorescence intensity, 30 cells cultured in DMEM were randomly quantified from each group from 3 independent experiments. Results are mean±s.d. ***P < 0.001 (Student's *t*-test). **(E)** Super-resolution images of the GFP-tagged CTPS and mCherry-tagged KRT8 in HEp-2 cells. The square box represents the enlarged region. **(F)** CTPS filaments colocalized with KRT8 in EBSS+His conditions at 6 h. CTPS was immunostained with anti-CTPS (green) antibodies; KRT8 was immunostained with anti-KRT8 (red) antibodies; and nuclei were immunostained with DAPI (blue). **(G)** Super-resolution images of the GFP-tagged CTPS and mCherry-tagged KRT18 in HEK 293T cells. CTPS filaments were induced in DMEM under 1 mM DON treatment for 1 h. **(H)** Zoomed images of HEp-2 cells expressing FLAG-CTPS-GFP (green) and mCherry-KRT18 (red) were obtained by LSM780 confocal microscopy every 30 seconds after 2 h of incubation with His-containing EBSS medium (Movie 3). Arrowhead points to the enrichment of CTPS along the cytokeratin.

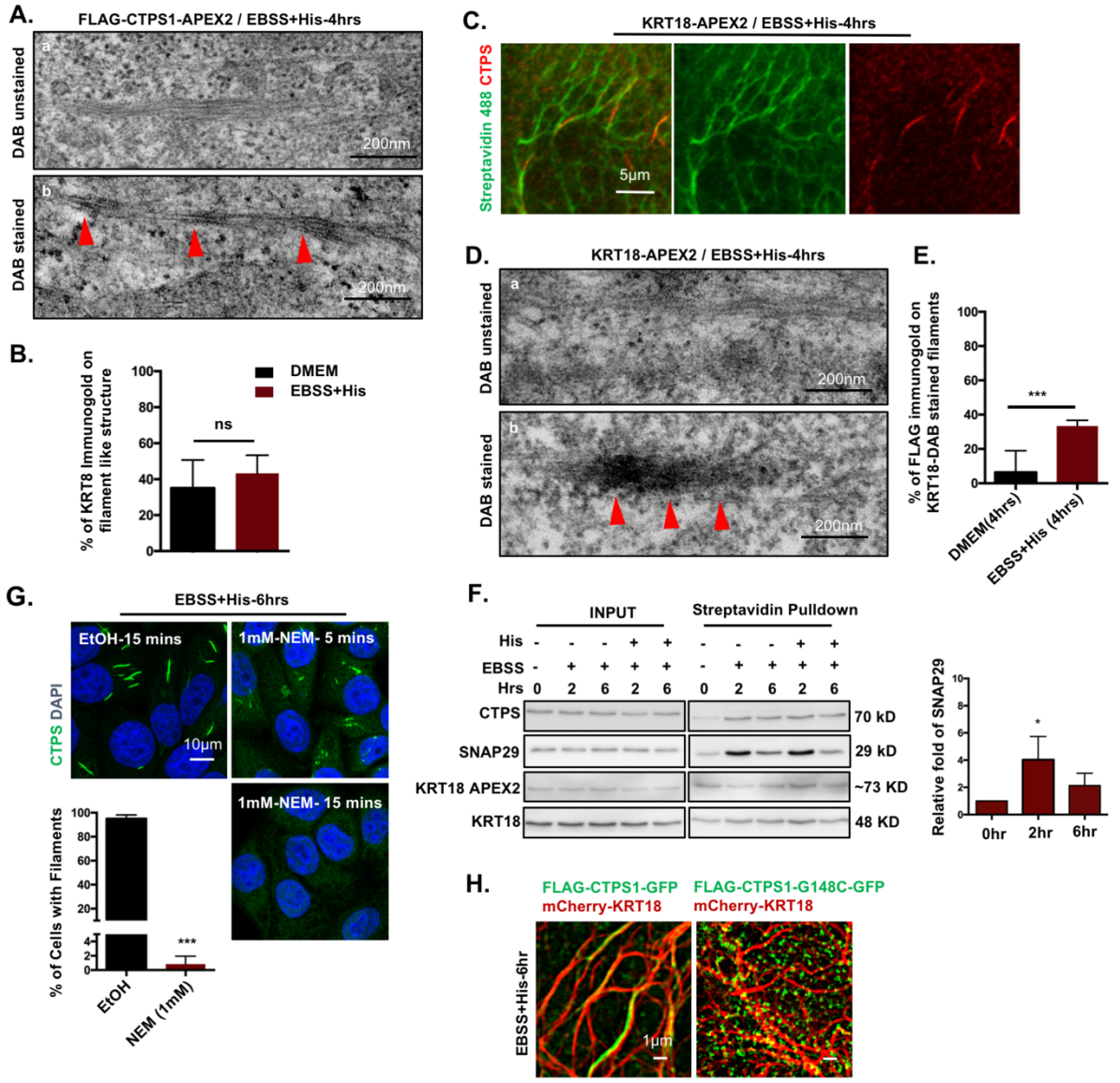
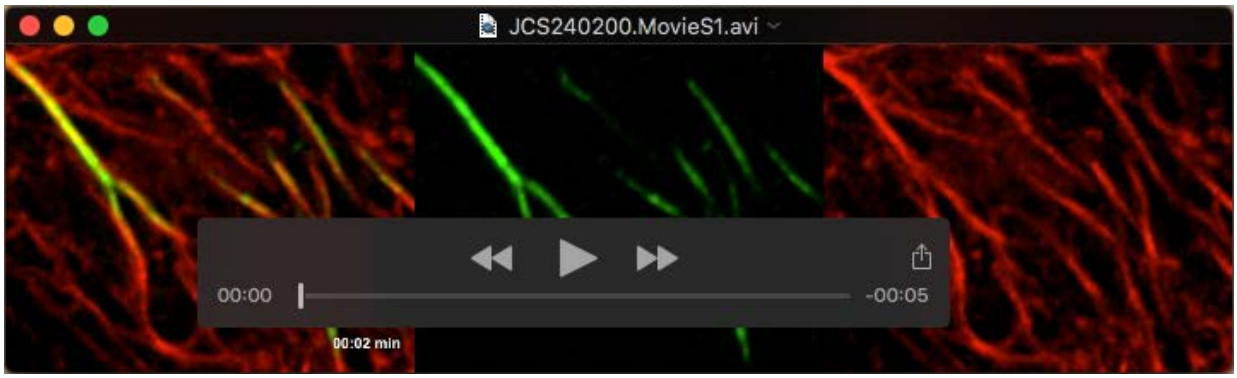


Figure S6: APEX2-mediated proximity labeling of the cytokeratin network

(A) Electron micrograph of DAB-stained CTPS1-APEX2 filaments in EBSS+His conditions. DAB-stained filaments (red arrow) were darker then the unstained filaments. **(B)** The percentage of KRT8 immunogold on filament-like structures was quantified for HEp-2 cells expressing FLAG-CTPS-APEX2 in DMEM and EBSS+His conditional medium at 4 h. 10 images were used to quantify each group from three independent experiments. Results are mean±s.d. ns, not significant (Student's *t*-test). **(C)** Streptavidin staining of HEp-2 cells expressing KRT18-APEX2 in EBSS+His conditions after 4 h of incubation. Biotinylation signals were detected using streptavidin-Alexa Fluor 488 conjugate (green); CTPS was detected with anti-CTPS (red) antibodies. **(D)** Electron micrograph of DAB-stained KRT18-

APEX2 filaments in EBSS+His conditions. DAB-stained filaments (red arrow) were darker then the unstained filaments. **(E)** The percentage of FLAG immunogold on DAB-stained KRT18 filaments was quantified for HEp-2 cells expressing KRT18-APEX2 and FLAG-CTPS-GFP in DMEM and EBSS+His conditional medium at 4 h. 10 images were used to quantify each group from three independent experiments. Results are mean±s.d. ***P < 0.001 (Student's *t*-test). **(F)** Time-dependent enrichment of SNAP29 in EBSS and EBSS+His. HEp-2 cells were transfected with KRT18-APEX2. Cells were incubated in conditional medium, and biotinylated proteins at 0 h, 2 h and 6 h were immunoprecipitated (IP) using streptavidin-conjugated magnetic beads. Band intensity of the streptavidin pulldown of SNAP29 was normalized against that of streptavidin pulldown of KRT18-APEX2, and the relative fold of SNAP29 was measured in EBSS/or EBSS+His by normalizing all groups (0 h, 2 h and 6 h) against 0 h. The percentage of relative SNAP29 fold was calculated for 3 independent experiments. Results are mean±s.d. *P < 0.05 (Student's *t*-test). **(G)** NEM treatment dissembled CTPS filaments within 5 min. HEp-2 cells were incubated in EBSS+His conditions for 6 h and further treated with NEM in the same medium for 5 min and 15 min. The percentage of cells bearing CTPS filaments after 15 min of 1 mM NEM treatment was calculated for 3 independent experiments. Results are mean±s.d. ***P < 0.001 (Student's *t*-test). **(H)** Super-resolution images of the GFP-tagged tetrameric mutant of CTPS (G148C) and mCherry-tagged KRT18 in EBSS+His conditions at 6 h.



Movie 1: CTPS filaments aligned along the cytokeratin network

HEp-2 cells expressing FLAG-CTPS1-GFP (green) and mCherry-KRT18 (red) were imaged with DeltaVision Ultra microscopy after 1 hr of incubation with His containing EBSS medium for 3 min. Images were acquired every 2 seconds. All images were deconvoluted after acquiring.

Movie 2: CTPS filaments assembling along KRT8

HEp-2 cells expressing FLAG-CTPS1-GFP (green) and mCherry-KRT8 (red) were imaged with Nikon Ti2 Dragonfly High Speed confocal platform. Images were acquired every 5 minutes for 65 minutes. Prior to imaging cells were incubated in EBSS+ His condition for 1hr. All images were deconvoluted after acquiring.

Movie 3: CTPS filaments assembling along KRT18

HEp-2 cells expressing FLAG-CTPS-GFP (green) and mCherry-KRT18 (red) were imaged with LSM780 confocal microscopy after 2 h of incubation with His containing EBSS medium for 1 hour 30 mins. Images were acquired every 30 seconds.

Table S1: List of candidate proteins identified by iTRAQ analysis

A collated list of proteins identified from comparison of HIS/EBSS, HIS/APEX2 and HIS/GLN above mean+1.8 SD (standard deviation). 26 common proteins are marked in Red. Fold change values are represented in black if above mean+1.8 SD.

Click here to Download Table S1

Table S2: List of proteins identified by TMT analysis

List of proteins identified from three repeats of HIS/EBSS by TMT analysis, above mean+2 SD (standard deviation) and p Value <0.05.

Click here to Download Table S2

Table S3: GSEA analysis of proteins identified in TMT analysis

Click here to Download Table S3

Table S4: List of antibodies used in the experiments.

Antibody	Dilution
anti-pan Cytokeratin antibody *(Abcam, cat.no. ab86734)*	Immunostaining: 1:100
anti-Tubulin antibody *(Abcam, cat.no. ab6160)*	Immunostaining: 1:100
anti-SNAP29 antibody *(GeneTex, cat.no. GTX131028)*	Western Blotting: 1:1000
anti-SNAP29 antibody *(Abcam, cat.no. ab181151)*	Western Blotting: 1:1000 Immunostaining: 1:100
anti-IMPDH2 antibody *(Proteintech, cat.no: 12948-1-AP)*	Western Blotting: 1:1000 Immunostaining: 1:500
anti-CTP synthase antibody *(Santa Cruz Biotechnology, cat.no. sc-134457)*	Immunostaining: 1:50
anti-CTPS antibody *(GeneTex, cat.no. GTX105265)*	Western Blotting: 1:1000 Immunostaining: 1:300
anti-KRT18 antibody *(Santa Cruz Biotechnology, cat.no. sc-6259)*	Western Blotting: 1:1000 Immunostaining: 1:50
anti-KRT8 antibody *(Santa Cruz Biotechnology, cat.no. sc-8020)*	Western Blotting: 1:1000 Immunostaining: 1:50
anti-KRT8 antibody *(Proteintech, cat.no. 10384-1-AP)*	Immuno EM: 1:20
Monoclonal ANTI-FLAG® M2 antibody *(Sigma-Aldrich, cat.no. F1804)*	Western Blotting: 1:1000 Immunostaining: 1:500
Monoclonal ANTI-FLAG® M2 antibody *(Sigma-Aldrich, cat.no. F3165)*	Immuno EM: 1:10
Alexa Fluor 488 Phalloidin *(Thermo Scientific, cat.no. A12379)*	Immunostaining: 1:100
EasyBlot anti Rabbit IgG HRP *(GeneTex, cat.no. GTX221666-01)*	Western Blotting: 1:1000
Streptavidin-Alexa Fluor 488 conjugate *(Thermo Scientific, cat.no. S11223)*	Immunostaining: 1:500
Streptavidin-HRP *(Thermo Scientific, cat. no. 21126)*	Western Blotting: 1:1000