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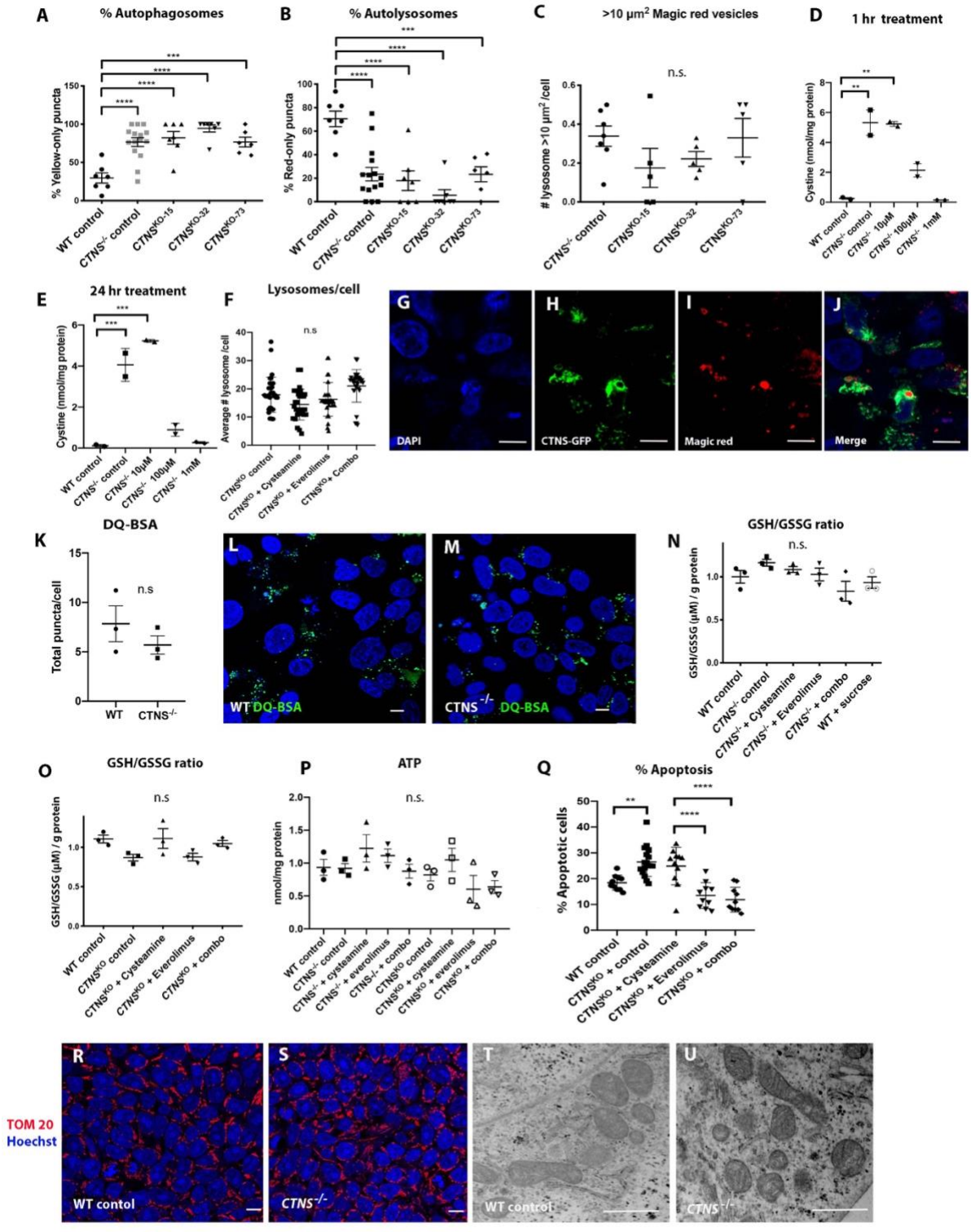
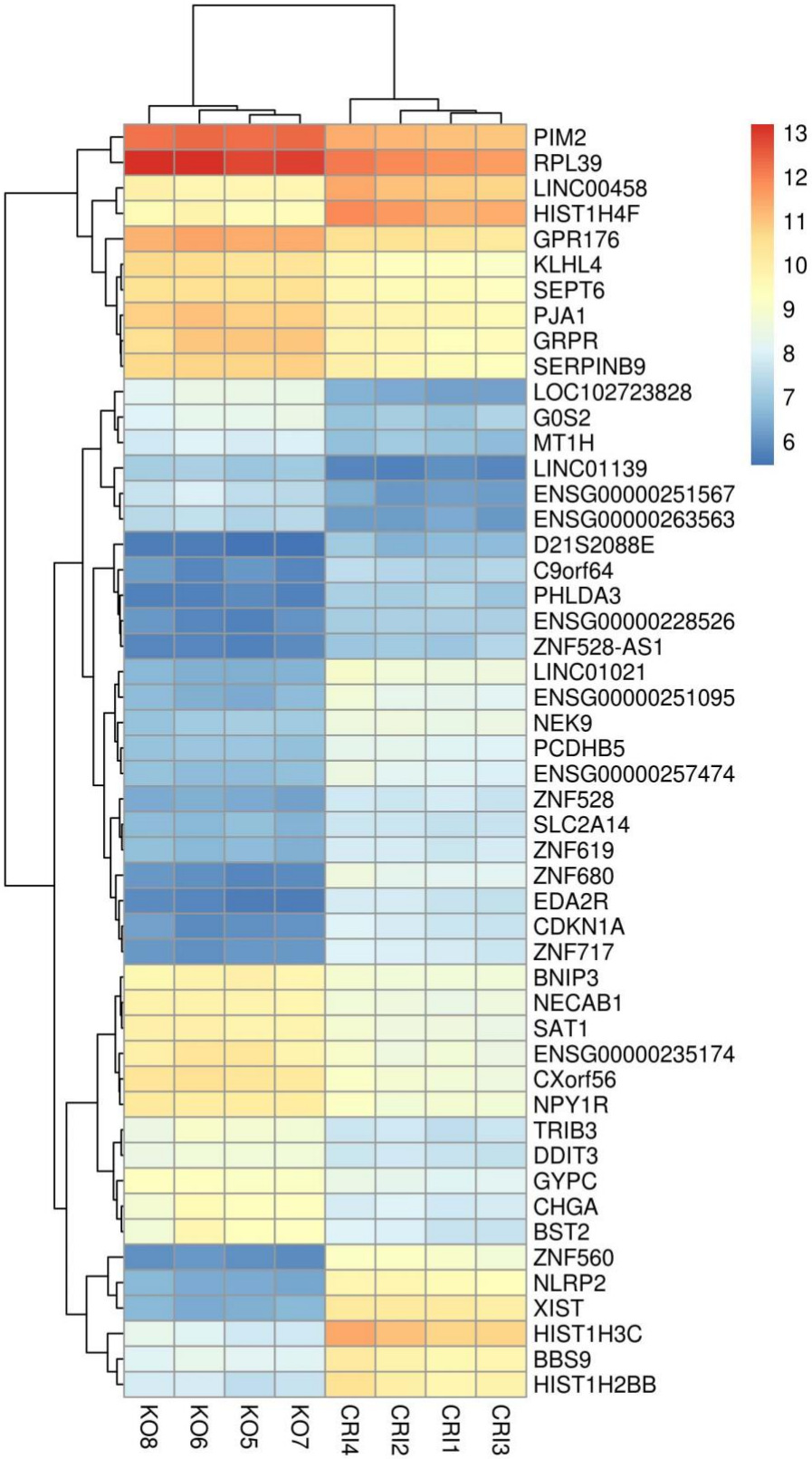
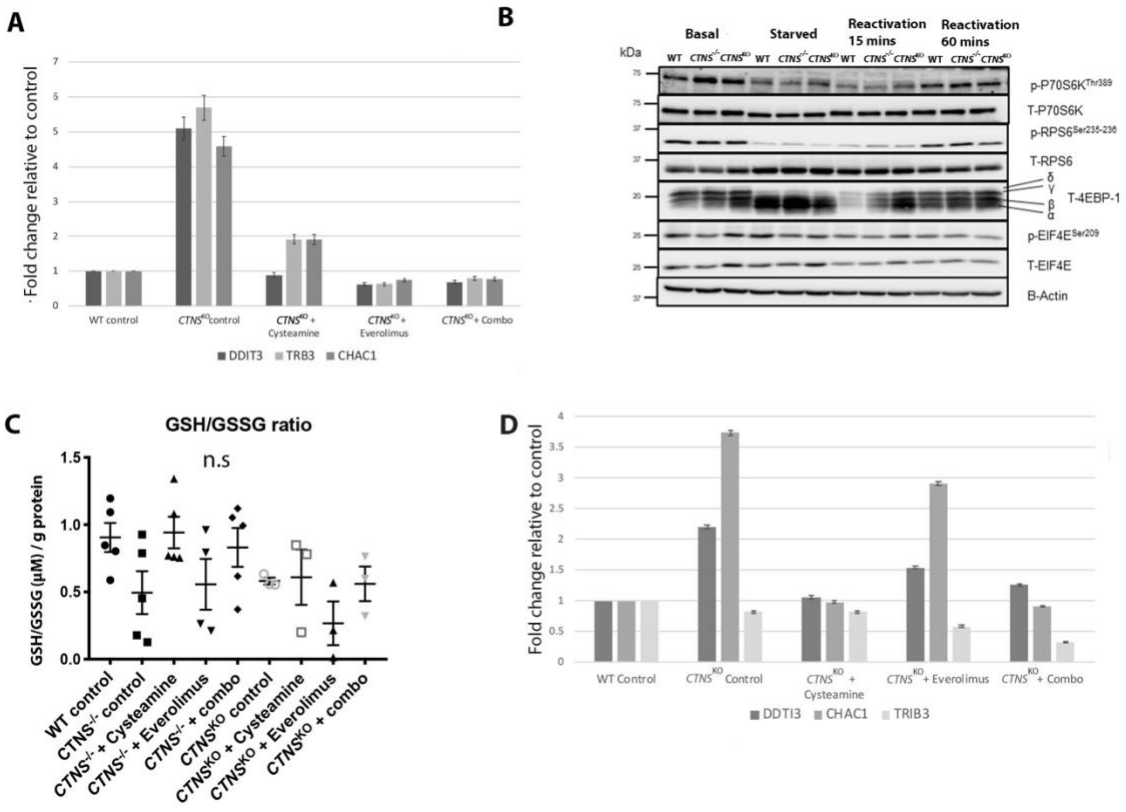
**Supplemental Figure S1**



**Supplemental Figure S2**



**Supplemental Figure S3**



**Supplemental Figure S4**

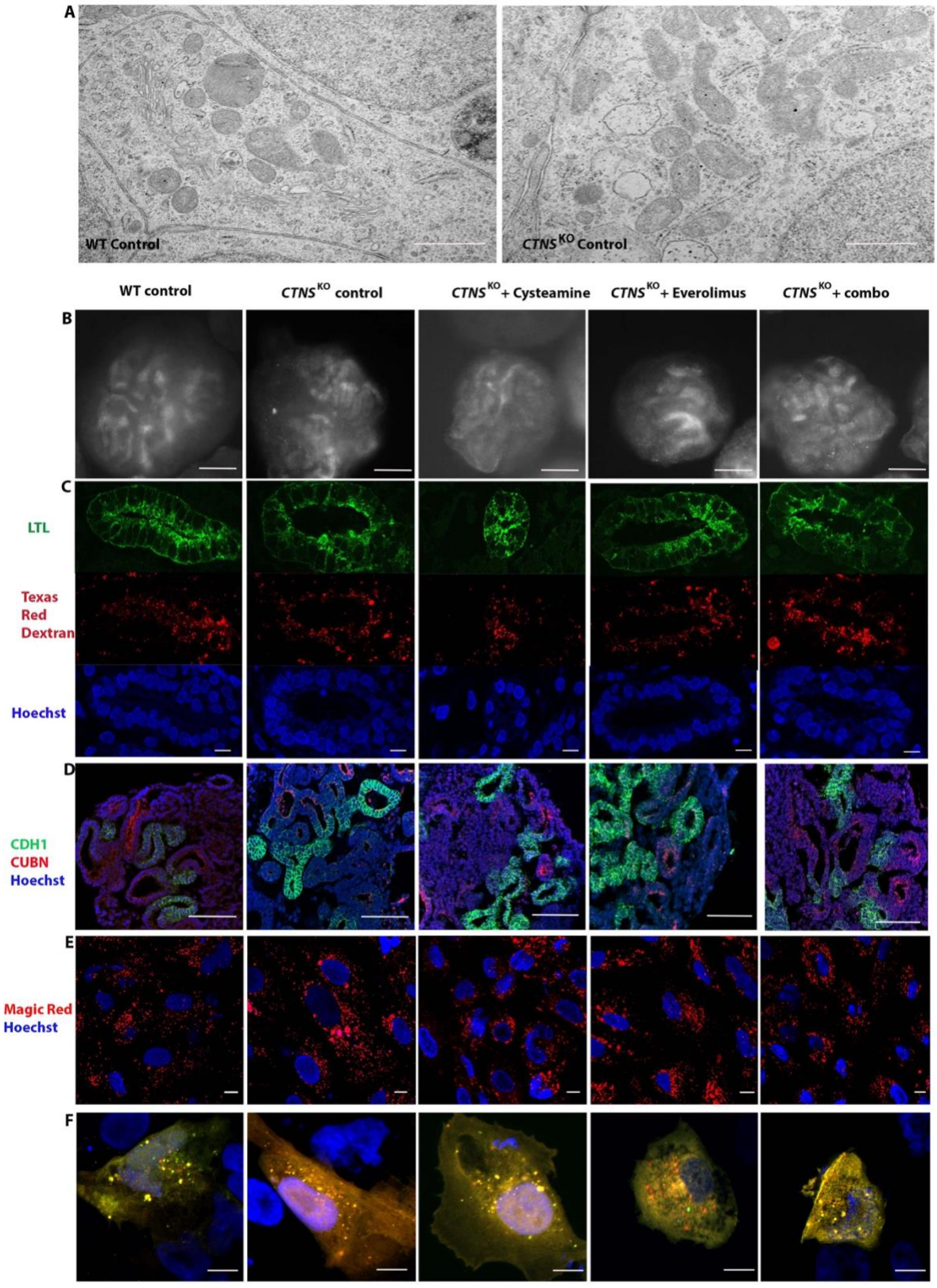


**Figure Legends:**

**Supplemental Figure S1**

**(A, B)** Percentage of yellow and red-only puncta in all three *CTNS*$^{KO}$ iPS cell lines compared to *CTNS*$^{-/-}$ and WT iPSCs. One-way ANOVA performed, **p<0.01, ***p<0.001, data is plotted as mean ± SEM, (n= 30 cells from 10 random fields per condition containing ~1-3 cells, 3 independent experiments). **(C)** Graph showing the average number of cells with lysosomes over 10 $\mu m^2$ in control *CTNS*$^{-/-}$ and all three *CTNS*$^{KO}$-iPSCs. One-way ANOVA performed, non-significant**,** data plotted as mean ± SEM, (n=300 cells from 5 random fields per condition, 20 cells/field, 3 independent experiments). **(D**) Amount of cystine (nmol/mg of protein) in WT and *CTNS*$^{-/-}$-iPSCs with 10 µM, 100 µM or 1 mM cysteamine treatment for 1 hr. One-way ANOVA performed, **p<0.01, data plotted as mean ± SEM, 2 independent experiments. **(E)** Amount of cystine (nmol/mg of protein) in WT and *CTNS*$^{-/-}$-iPSCs with 10 µM, 100 µM or 1 mM cysteamine treatment for 24 hr. One-way ANOVA performed, ***p<0.001, data plotted as mean ± SEM, 2 independent experiments. **(F)** Graph showing the average number of lysosomes per cell in *CTNS*$^{KO}$ control and *CTNS*$^{KO}$ treated with 1 mM Cysteamine, 100 nM Everolimus or Combo- 1 mM Cysteamine and 100nm Everolimus for 24 hrs. One-way ANOVA performed, non-significant**,** data plotted as mean ± SEM. **(G, H, I, J)** Representative images of fluorescent staining with Magic red in *CTNS*$^{KO}$-iPSCs over expressing Cystinosin-GFP showing overlap, individual channels of DAPI, Cystinosin-GFP, Magic red and merge shown respectively. Scale bar 10 µm. **(K)** Graph showing the total number of DQ-BSA$^{+}$ puncta in WT and *CTNS*-iPSCs incubated with 20 µg/ml working solution of DQ-BSA green for 3 hrs. One-way ANOVA performed, non-significant**,** data plotted as mean ± SEM. **(L, M)** Representative images of fluorescent staining with DQ-BSA in WT and *CTNS*-iPSCs. Scale bar 10 µm. **(N)** Ratio of GSH/GSSG (µM/g of protein) in WT and *CTNS*$^{-/-}$ iPSCs and **(O)** WT and *CTNS*$^{KO}$ iPS with various treatments (1 mM Cysteamine, 100 nM Everolimus, Combo - 1

mM Cysteamine and 100nM Everolimus or 50mM sucrose for 24 hrs). One-way ANOVA performed, non-significant**,** data plotted as mean ± SEM. **(P)** Graph showing the amount of ATP (nmol/mg of protein) in WT and *CTNS* iPSCs with various treatments (1 mM Cysteamine, 100 nM Everolimus, Combo - 1 mM Cysteamine and 100 nM Everolimus for 24 hrs). One-way ANOVA performed, non-significant**,** data plotted as mean ± SEM. **(Q)** Graph showing the degree of apoptosis as determined by the percentage of $GFP_+$ puncta in WT and *CTNS*$_{KO}$-iPSCs with various treatments (1 mM Cysteamine, 100 nM Everolimus, Combo - 1 mM Cysteamine and 100 nM Everolimus for 24 hrs). One-way ANOVA performed, **p<0.01, ****p<0.0001, data is plotted as mean ± SEM **(R, S)** Representative immunofluorescent staining with anti-TOM20 (red) in WT and *CTNS*$^{-/-}$ iPSCs. Scale bar 10 µm. **(T,U)** Transmission electron micrograph (TEM) of WT and *CTNS*$^{-/-}$-iPSCs mitochondria. Scale bars 1 µm. One-way ANOVA performed, n.s: non-significant**,** *p<0.5, **p<0.01, ***p<0.001, ****p<0.0001 data plotted as mean ± SEM.

**Supplemental Figure S2**. Top 50 differentially expressed genes in *CTNS*$_{KO}$-iPSCs and isogenic WT control cells. (FDR<0.05)

**Supplemental Figure S3. (A)** Quantitative PCR with various treatments (1 mM Cysteamine, 100nM Everolimus, Combo - 1 mM Cysteamine and 100 nM Everolimus for 24 hrs) in *CTNS*$_{KO}$ -iPSCs expressed as fold change relative to control, data plotted as mean ± SD **(B)** Representative Western blot against phosphorylated and total S6K, RPS6, 4EBP1, EIF4e under different feeding conditions in WT, *CTNS*$^{-/-}$ and *CTNS*$_{KO}$-iPSCs (representative of 3 independent experiments). **(C)** Ratio of GSH/GSSG (µM/g of protein) in WT and *CTNS*$^{-/-}$ and *CTNS*$_{KO}$ organoids with various treatments (1 mM Cysteamine, 100 nM Everolimus, Combo - 1 mM Cysteamine and 100 nM Everolimus for 24 hrs). One-way ANOVA performed, non-

significant**,** data plotted as mean ± SEM, n=3, 3 independent experiments. **(D)** Quantitative PCR with various treatments in *CTNS*KO organoids expressed as fold change relative to control, data plotted as mean ± SD.

**Supplemental Figure S4 (A)** Transmission electron micrograph (TEM) of WT and *CTNS*KO-kidney organoids showing mitochondria. Scale bars 1 µm. **(B)** Fluorescent whole-mount images of day 14 WT, *CTNS*KO control and *CTNS*KO kidney organoids treated with either 1 mM cysteamine, 100 nM Everolimus or combination for 24hrs incubated with 10 kDa Texas red-dextran for 48 hrs showing accumulation of dextran in tubules (representative of n=10 organoids). Scale bar 100 µm. **(C)** Cross-section of a kidney organoid following 10k Da Texas red-dextran incubation showing uptake of the dextran (red) into $LTL^+$ proximal tubules (green; representative of n=8 organoids). Scale bar 10 µm. **(D)** Immunofluorescent staining of paraffin sections of day 14 organoids showing CUBILIN (red) and CDH1 (green) labelled distal tubules. Nuclei stained with Hoechst 33342. Scale bar 100 µm. **(E)** Representative images of fluorescent staining with Magic red in WT control, and *CTNS*KO – kidney organoids with treatments (1 mM Cysteamine, 100 nM Everolimus, Combo - 1 mM Cysteamine and 100 nM Everolimus for 24 hrs). Scale bar 10 µm. **(F)** Dissociated organoids from WT control, *CTNS*KO control and *CTNS*KO – kidney organoids with treatments (1 mM Cysteamine, 100 nM Everolimus, Combo - 1 mM Cysteamine and 100 nM Everolimus for 24 hrs) transfected with tandem mCherry-LC3B-GFP plasmid showing red and yellow puncta. Scale bar 10 µm.

## Supplemental Information:

***Supplemental Table 1. Primary antibodies***

| Primary Antibody | Source | Product code | Dilution used |
|---|---|---|---|
| **Rabbit anti-LC3B** | Cell signalling | 3868S | 1:1000 |
| **Rabbit anti-P70 S6 kinase** | Cell signalling | 2708S | 1:1000 |
| **Rabbit anti-P-p70 S6 kinase (T389)** | Cell signalling | 9205S | 1:500 |
| **Rat anti-LAMP1** | Abcam | Ab25245 | 1:100 |
| **Mouse-anti β-actin** | Sigma | A1978 | 1:20,000 |
| **Rabbit anti-CUBILIN** | Abcam | Ab244274 | 1:500 |
| **Mouse anti- ECADHERIN** | BD Biosciences | 610181 | 1:300 |
| **Rabbit anti p-RPS6$^{Ser235-236}$** | Cell signalling | 4856 | 1:1000 |
| **Mouse anti-RPS6** | Cell signalling | 2317 | 1:1000 |
| **Rabbit anti-4EBP-1** | Cell signalling | 9644 | 1:1000 |
| **Rabbit anti p-EIF4e$^{Ser209}$** | Cell signalling | 9741 | 1:1000 |
| **Rabbit anti-EIF4e** | Cell signalling | 9742 | 1:2000 |

***Supplemental Table 2. Secondary antibodies for western blot and immunohistochemistry***

| Secondary Antibody | Source | Product code | Dilution |
|---|---|---|---|
| **Goat anti-Rabbit IgG-HRP** | Santa Cruz | Sc-2054 | 1:20,000 |
| **Anti-mouse IGg** | Sigma | A9044 | 1:20,000 |

| | | | |
|---|---|---|---|
| **Anti- rat Alexa Fluor 488** | Invitrogen | A-21210 | 1:500 |
| **Anti-Mouse Alexa Fluor 488** | Abcam | 96871 | 1:600 |
| **Anti-Rabbit Alexa Fluor 594** | Abcam | 96901 | 1:600 |
| **LTL** | Vector Labs | FL-1321 | 1:300 |

***Supplemental Table 3. List of primers for qPCR***

| **Gene** | ***Forward primer 5'-3'*** | ***Reverse primer 5'-3'*** |
|---|---|---|
| ***DDTI3*** | AGAACCAGGAAACGGAAACAGA | TCTCCTTCATGCGCTGCTTT |
| ***CHAC1*** | GCCCTGTGGATTTTCGGGTA | ATCTTGTCGCTGCCCCTATG |
| ***TRB3*** | CCCACCTACTGCTCCAGATCGTGCAA | CCTGGACGGGGTACACCTTGCAGGTATA |
| **CUBN** | CTTGCAGCAGACTGTTGACAA | TGGCAGCTCAAGGGTGTTC |
| **LRP2** | AAATTGAGCACAGCACCTTTGA | TCTGCTTTCCTGACTCGAATAATG |

***Supplemental Table 4. Average numerical values of cystine measurements and protein concentration of iPSCs and kidney organoids.***

| **Sample iPSCs** | **Cystine (nmol)** | **Protein (mg)** | **Cystine (nmol/mg of protein)** |
|---|---|---|---|
| **WT control** | 109.230 | 1750 | 0.04547 ± 0.01 |
| **WT + sucrose** | 79.282 | 1400 | 0.04224 ± 0.001 |
| **CTNS$^{-/-}$control** | 1949.671 | 1100 | 1.494 ± .325 |
| **CTNS$^{-/-}$ + Cysteamine** | 42.578 | 760 | 0.04933 ± 0.026 |
| **CTNS$^{-/-}$ + Everolimus** | 2586.966 | 800 | 2.340 ± 0.15 |
| **CTNS$^{-/-}$ + combo** | 43.825 | 600 | 0.05478 ± 0.006 |
| **CTNS$^{KO}$ control** | 4887.667 | 1500 | 2.454 ± 0.375 |
| **CTNS$^{KO}$ + Cysteamine** | 75.591 | 1400 | 0.04212 ± 0.0068 |
| **CTNS$^{KO}$ + Everolimus** | 4986.704 | 1500 | 2.354 ± 0.21 |
| **CTNS$^{KO}$ + combo** | 100.475 | 1600 | 0.04622 ± 0.004 |

| Sample Organoids | Cystine (nmol) | Protein (mg) | Cystine (nmol/mg of protein) |
|---|---|---|---|
| **WT control** | 907.974 | 3500 | 2.487 ± 0.89 |
| **CTNS$^{-/-}$control** | 763.384 | 900 | 9.154 ± 0.78 |
| **CTNS$^{-/-}$ + Cysteamine** | 112.879 | 900 | 1.320 ± .13 |
| **CTNS$^{-/-}$ + Everolimus** | 844.265 | 660 | 15.32 ± 2.01 |
| **CTNS$^{-/-}$ + combo** | 142.023 | 700 | 1.89 ± 0.27 |
| **CTNS$^{KO}$ control** | 2085.588 | 2500 | 8.983 ± 3.39 |
| **CTNS$^{KO}$ + Cysteamine** | 137.593 | 2000 | 0.6812 ± 0.03 |
| **CTNS$^{KO}$ + Everolimus** | 2965.880 | 2600 | 11.48 ± 0.51 |
| **CTNS$^{KO}$ + combo** | 141.991 | 1800 | 0.7866 ± 0.03 |