Table S1. Overview of primer sequences

Target	cDNA/gDNA	Forward 5' → 3'	Reverse 5' → 3'
Woodchuck hepatitis virus WPRE	gDNA	caattccgtggtgttgtcgg	gaaggtccgctggattgagg
Hs CTNS	cDNA	ccacaggcctacatgaactt	tccactggtcgttgttgtag
Hs β-actin	gDNA	tcacccacactgtgcccatctacga	cagcggaaccgctcattgccaatgg
Hs γ-actin	cDNA	cactgagcgaggctacagctt	ttgatgtcgcgcacgattt

Table S2. Overview of primary and secondary antibodies

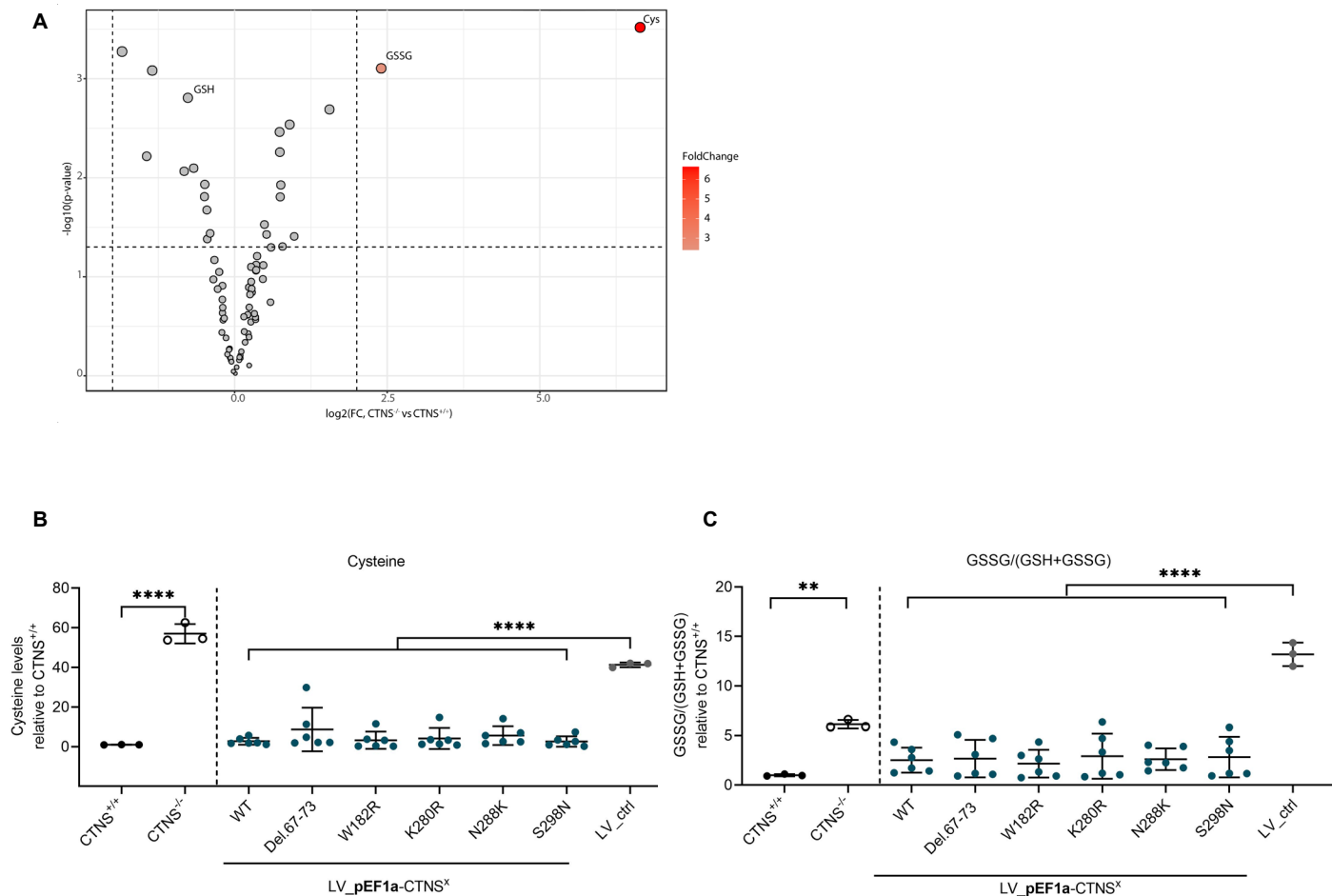| ANTIBODY	PROVIDER	SPECIES	DILUTION	APPLICATION
Anti-mouse Alexa 488	Invitrogen A11001	Goat	1/500	ICC
Anti-rabbit Alexa 555	Invitrogen A21429	Goat	1/500	ICC
Anti-mouse Alexa 633	Life Technologies A21050	Goat	1/500	ICC
Phalloidin Alexa 633	ThermoFisher Scientific 21840	NA	1/1000	ICC
Dapi	Life Technologies D1306	NA	1/1000	ICC
HA.11	BioLegend 901515	Mouse	1/1000	ICC
LA1	Cell Signaling 9091	Rabbit	1/200	ICC
HA.11	BioLegend 901515	Mouse	1/10 000	WB
HA (2-2.2.14)	Invitrogen 26183	Mouse	1/2000	WB
vinculin	Sigma V9131	Mouse	1/100 000	WB
anti-mouse polyclonal immunoglobulins/HRP	Agilent Dako P0260/P0447	Rabbit/goat	1/10 000	WB

A

B

C

Figure S1. *CTNS^WT* and *CTNS^mutant* cDNA addition after LV transduction in CRISPRed *CTNS^-/-* ciPTECs reduces the intracellular cysteine and redox levels.

(A) Volcano plot of metabolome changes in *CTNS^-/-* ciPTECs (Metaboanalyst 6.0). Red dots show the differentially produced metabolites. Fold change threshold =4.0 and P-value threshold = 0.05; unpaired t-test.

(B) Cysteine measurement (mass spectrometry) of *CTNS^-/-* ciPTECs transduced with either LV_pEF1a-CTNS^WT, LV_pEF1a-CTNS^mutant, or LV_ctrl. The data were normalized to protein content and are presented as the mean ± SD (n=3 or 6 independent metabolite extracts). Statistical testing was performed with a one-way Anova, Sidak's multiple comparison test.

(C) Redox measurement, represented as GSSG/(GSH+GSSG) (mass spectrometry) of *CTNS^-/-* ciPTECs transduced with either LV_pEF1a-CTNS^WT, LV_pEF1a-CTNS^mutant, or LV_ctrl. The data were normalized to protein content and are presented as the mean ± SD (n=3 or 6 independent metabolite extracts). Statistical testing was performed with a one-way Anova, Sidak's multiple comparison test.

LV, lentiviral vector; p, promoter; WT, wild-type; LV_ctrl, LV_pEF1a-eGFP; NT, non-transduced; Cys, cysteine; GSH, reduced glutathione; GSSG, oxidized glutathione; FC, foldchange; **, $p < 0.01$; ****, $p < 0.0001$.
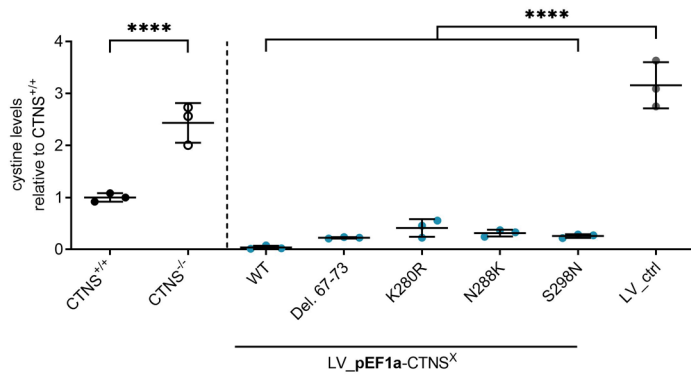
FIGURE S2

Figure S2. ***CTNS^WT and CTNS^mutant* cDNA addition after LV transduction in cystinosis patient-derived fibroblasts greatly reduces the intracellular cystine levels.** Cystine measurement (mass spectrometry) of CTNS^-/- fibroblasts transduced with either LV_pEF1a-CTNS^WT, LV_pEF1a-CTNS^mutant, or LV_ctrl. The data are presented as the mean ± SD (n=3 independent metabolite extracts). Cystine (μM) was normalized to protein content (μg/μl). Statistical testing was performed with a one-way Anova, Sidak's multiple comparison test. LV, lentiviral vector; p, promoter; WT, wild-type; LV_ctrl, LV_pEF1a-eGFP; ****, p<0.0001.

Figure S3. Weaker promoters leading to lower CTNS^WT expression are still able to rescue the cystinosis phenotype

(A) Effect of promoter differences on eGFP expression (flow cytometry) in CTNS-/- ciPTECs transduced with lentiviral vectors LV_pEF1a-eGFP, LV_pEFS-eGFP, LV_pCTNS-eGFP . Promoter activities as MFI for ~30 %eGFP positive cells (considered as one integrated copy).

(B) Quantification of integrated copies in CTNS^-/- ciPTECs transduced with lentiviral vectors LV_pEF1a-CTNS^WT, LV_pEFS-CTNS^WT, LV_pCTNS-CTNS^WT or LV_ctrl. Integrated copies were measured by quantification of the WPRE element present in the transgene construct. The data is normalized for total levels of *ACTB* and are presented as the mean ± SD (n=1 triplicates).

(C) Quantification of CTNS^WT-3HA protein expression in CTNS^-/- ciPTECs transduced with lentiviral vectors LV_pEF1a-CTNS^WT, LV_pEFS-CTNS^WT, LV_pCTNS-CTNS^WT or LV_ctrl (n=2-3). Samples normalized for total proteins of vinculin.

(D) Confocal microscopy images of the immunofluorescence signal of CTNS^WT-3HA and LAMP1 in CTNS^-/- ciPTECs transduced with either LV_pEF1a-CTNS^WT, LV_pEFS-CTNS^WT, LV_pCTNS-CTNS^WT or LV_ctrl. Nuclei were stained with DAPI. Scale bars are 10 μM.

(E) Cysteine measurement (mass spectrometry) of CTNS^-/- ciPTECs transduced with LV_pEF1a-CTNS^WT, LV_pEFS-CTNS^WT, LV_pCTNS-CTNS^WT or LV_ctrl. The data are presented as the mean ± SD (n=1, 3 or 6 independent metabolite extracts). Cysteine (Abundance) was normalized to protein content (μg/μl).

(F) Redox measurement, represented as GSSG/(GSH+GSSG) (mass spectrometry) of CTNS^-/- ciPTECs transduced with LV_pEF1a-CTNS^WT, LV_pEFS-CTNS^WT, LV_pCTNS-CTNS^WT or LV_ctrl. The data are presented as the mean ± SD (n=1, 3 or 6 independent metabolite extracts). GSSG/(GSH+GSSG) (Abundance) was normalized to protein content (μg/μl).

LV, lentiviral vector; p, promoter; MFI, median fluorescent intensity; WT, wild-type; LV_ctrl, LV_pCMV-dATP13A2; NT, non-transduced
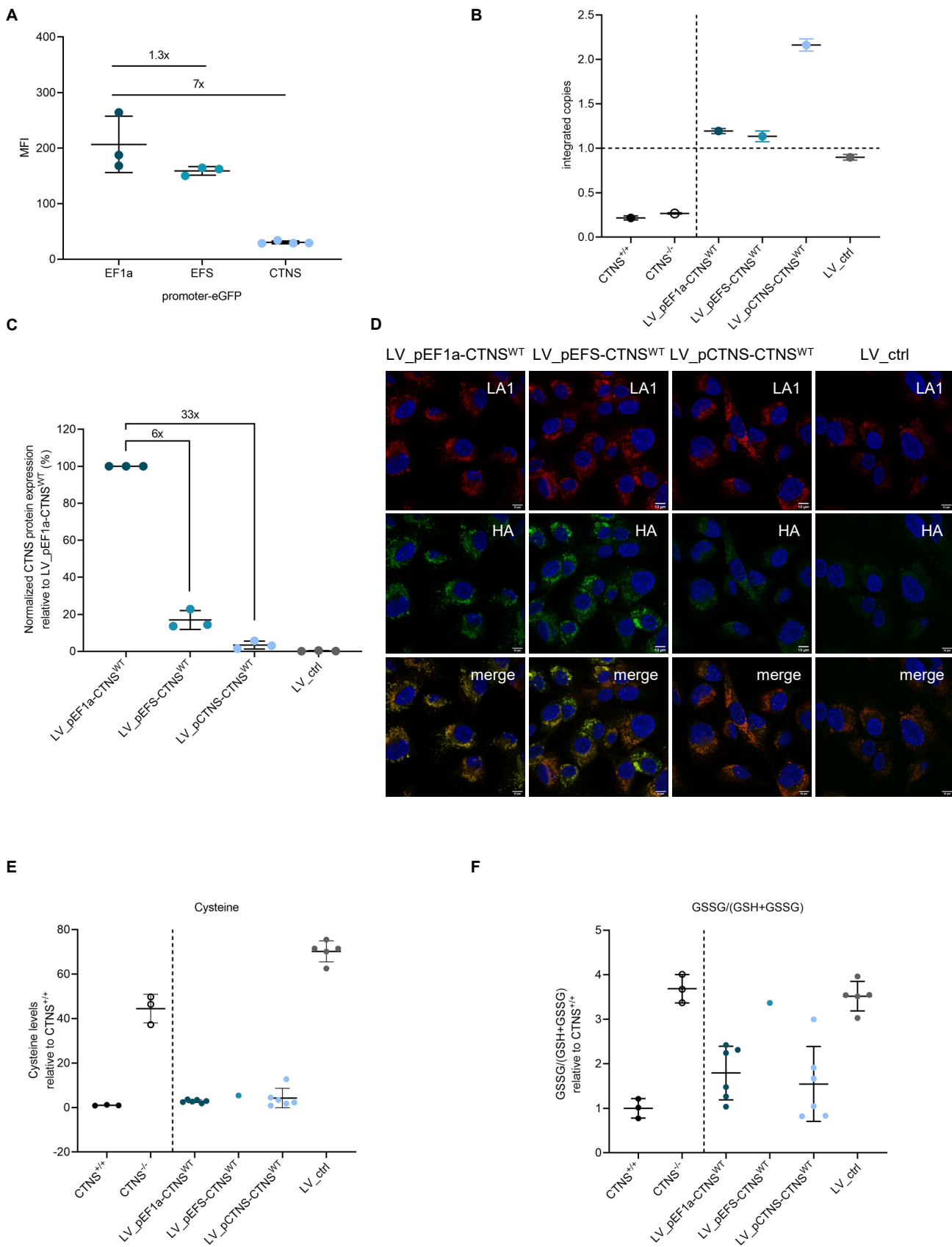
FIGURE S3

A

MFI (y-axis, 0–400)
promoter-eGFP (x-axis): EF1a, EFS, CTNS
1.3x
7x

B

integrated copies (y-axis, 0.0–2.5)
x-axis: CTNS$^{+/+}$, CTNS$^{-/-}$, LV_pEF1a-CTNS^{WT}, LV_pEFS-CTNS^{WT}, LV_pCTNS-CTNS^{WT}, LV_ctrl

C

Normalized CTNS protein expression relative to LV_pEF1a-CTNS^{WT} (%) (y-axis, 0–120)
x-axis: LV_pEF1a-CTNS^{WT}, LV_pEFS-CTNS^{WT}, LV_pCTNS-CTNS^{WT}, LV_ctrl
6x
33x

D

LV_pEF1a-CTNS^{WT} LV_pEFS-CTNS^{WT} LV_pCTNS-CTNS^{WT} LV_ctrl

LA1
HA
merge

E

Cysteine

Cysteine levels relative to CTNS$^{+/+}$ (y-axis, -20–80)
x-axis: CTNS$^{+/+}$, CTNS$^{-/-}$, LV_pEF1a-CTNS^{WT}, LV_pEFS-CTNS^{WT}, LV_pCTNS-CTNS^{WT}, LV_ctrl

F

GSSG/(GSH+GSSG)

GSSG/(GSH+GSSG) relative to CTNS$^{+/+}$ (y-axis, 0–5)
x-axis: CTNS$^{+/+}$, CTNS$^{-/-}$, LV_pEF1a-CTNS^{WT}, LV_pEFS-CTNS^{WT}, LV_pCTNS-CTNS^{WT}, LV_ctrl
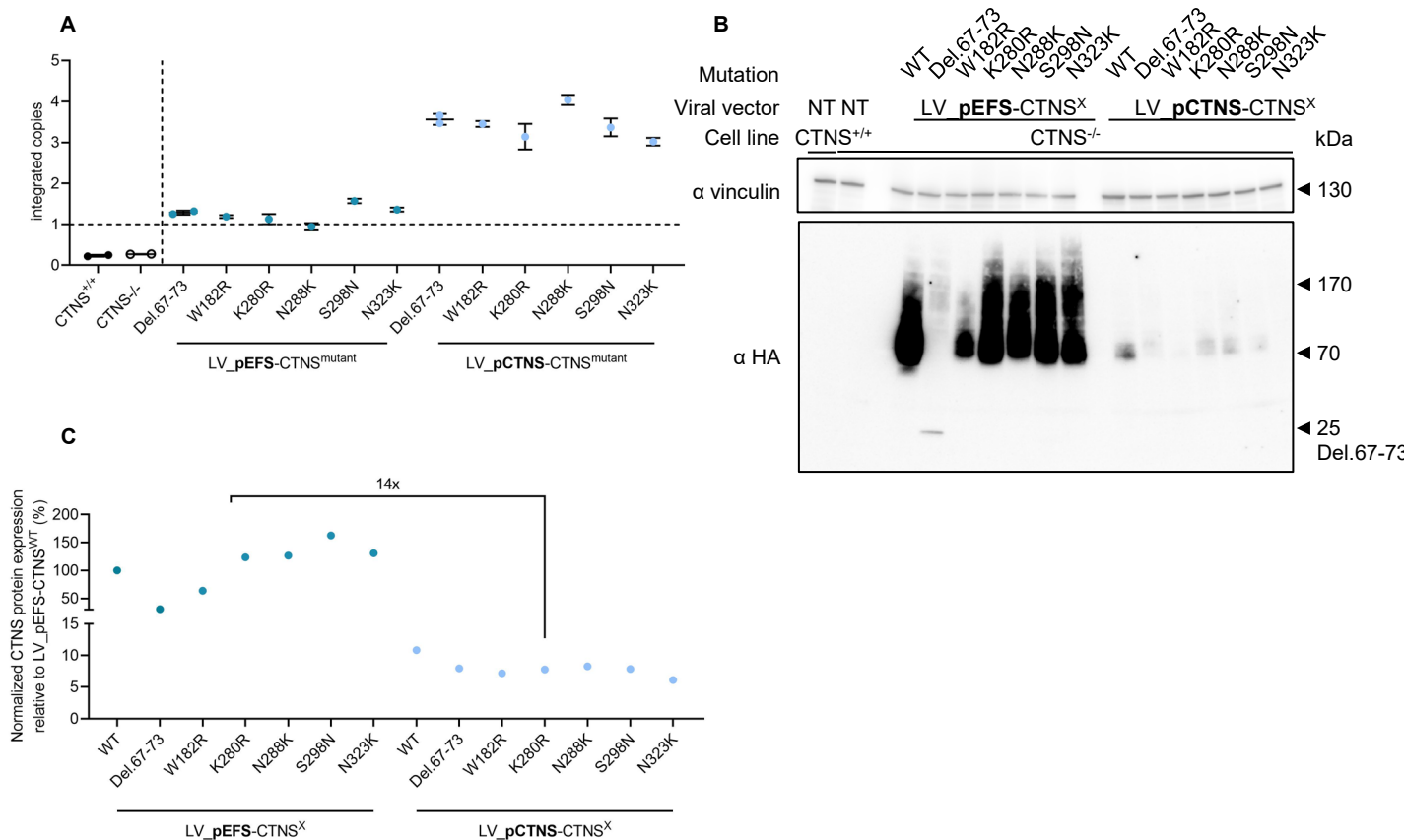
A

B

C

Figure S4. Different promoters showing rescue of mutants even with low activity

(A) Quantification of integrated copies in CTNS$^{-/-}$ ciPTECs transduced with lentiviral vectors LV_pEFS-CTNS^{mutant} LV_pCTNS-CTNS^{mutant} or LV_ctrl. Integrated copies were measured by quantification of the WPRE element present in the transgene construct. The data is normalized for total levels of *ACTB* and are presented as the mean ± SD (n=1 triplicates).

(B) Enhanced contrast of Figure 4B using ImageJ. Western blot analysis of CTNS$^{WT \ or \ mutant}$-3HA protein expression in CTNS$^{-/-}$ ciPTECs transduced with lentiviral vectors vectors LV_pEFS-CTNS$^{WT \ or \ mutant}$, LV_pCTNS-CTNS$^{WT \ or \ mutant}$ or LV_ctrl. Samples normalized for total proteins of vinculin.

(C) Quantification of CTNS^{mutants}-3HA protein expression via Western blot analysis of CTNS$^{WT \ or \ mutant}$-3HA protein expression in CTNS$^{-/-}$ ciPTECs transduced with lentiviral vectors vectors LV_pEFS-CTNS$^{WT \ or \ mutant}$, LV_pCTNS-CTNS$^{WT \ or \ mutant}$. or LV_ctrl (n=2). Samples normalized for total proteins of vinculin.

LV, lentiviral vector; p, promoter; WT, wild-type; LV_ctrl, LV_pCMV-dATP13A2; NT, non-transduced.
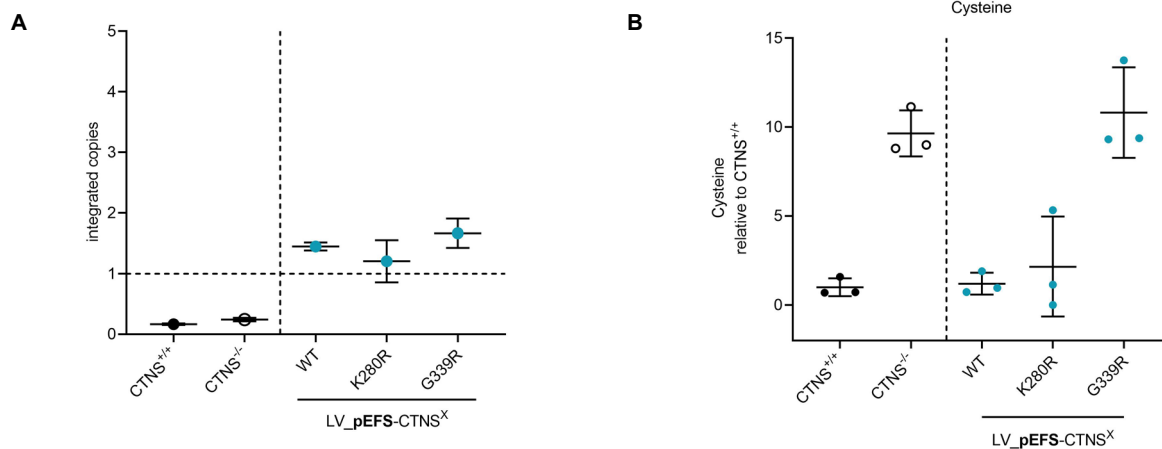
A

B

Cysteine

Figure 5. G339R mutant overexpression shows no rescue in cystine accumulation upon cDNA addition in CTNS$^{-/-}$ ciPTECs

(A)   Quantification of integrated copies in CTNS$^{-/-}$ ciPTECs transduced with lentiviral vectors LV_pEFS-CTNS^{WT} or LV_pEFS-CTNS$^{K280R \ or \ G339R}$. Integrated copies were measured by quantification of the WPRE element present in the transgene construct. The data is normalized for total levels of *ACTB* and are presented as the mean ± SD (n=1 triplicates).

(B) Cysteine measurement (mass spectrometry) of CTNS$^{-/-}$ ciPTECs transduced with LV_pEFS-CTNS^{WT} or LV_pEFS-CTNS$^{K280R \ or \ G339R}$. The data are presented as the mean ± SD (n=3 independent metabolite extracts). Cysteine (Abundance) was normalized to protein content (μg/μl).

LV, lentiviral vector; p, promoter; WT, wild-type; ***, p<0.001; ns, nonsignificant