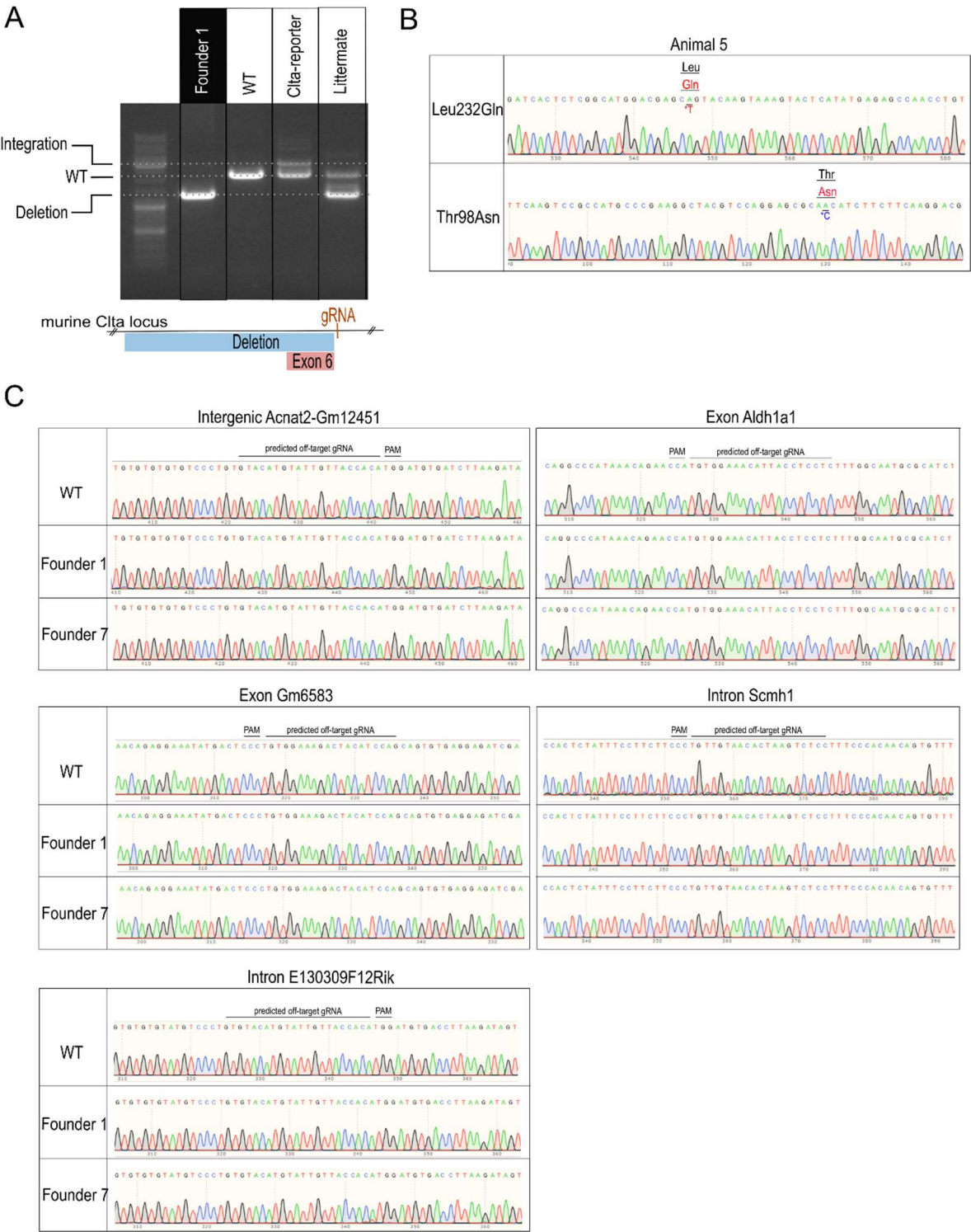


1 **Supporting information**

2 **S1 Figure**

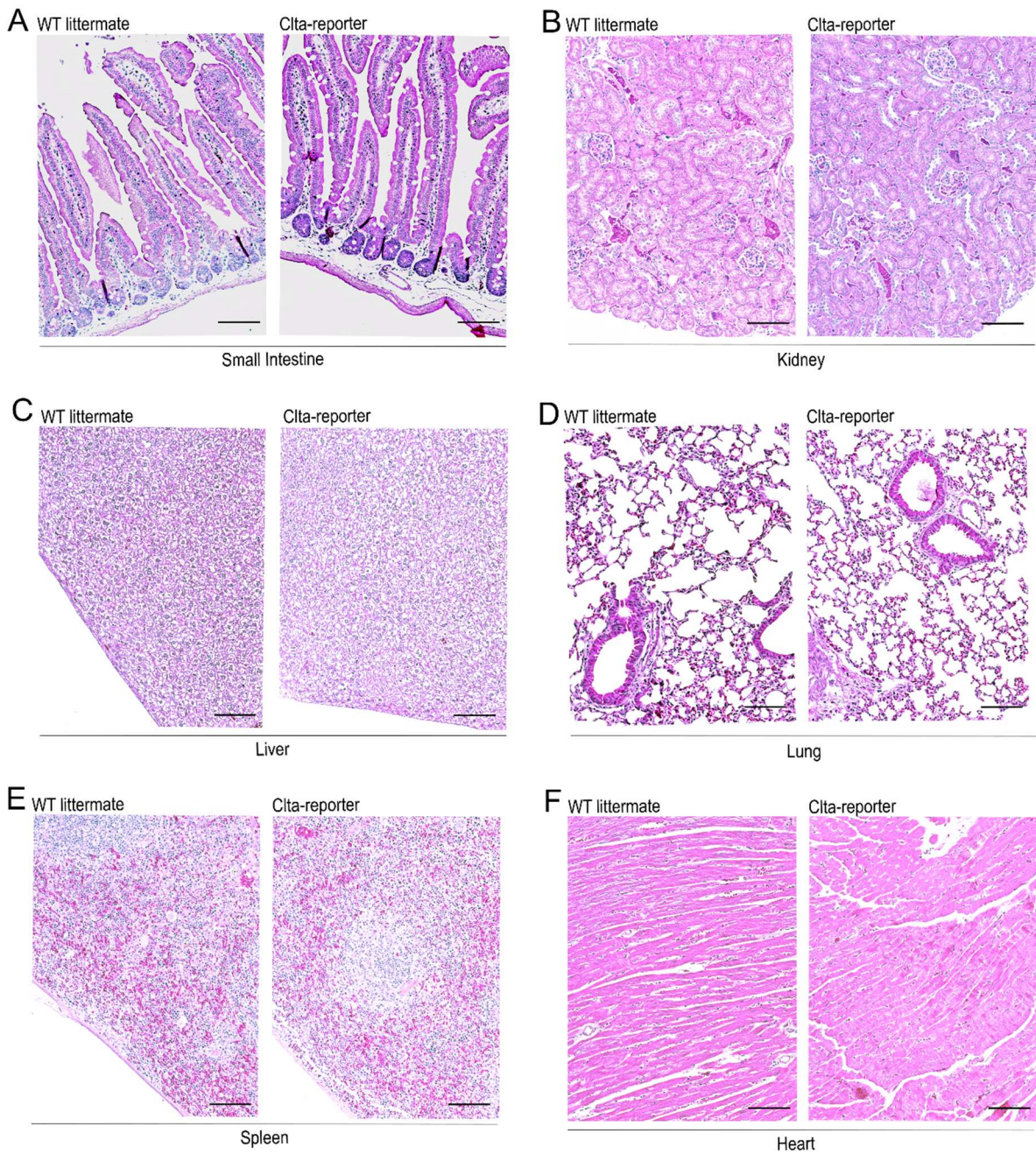


3

4 **S1 Fig. Genetic validation of the founder lines 1 and 7. (A)** PCR detecting the deletion on the

second allele in founder 1. An 855 bp deletion, downstream of the sgRNA-mediated cut site was detected. To detect the deletion a different forward primer (see Material and Methods section) was used. Integration band in founder 1 is hardly visible. **(B)** Sequencing confirmed 2 point mutations resulting in amino acid exchanges (Thr98Asn and Leu232Gln) in the eGFP sequence of Animal 5. **(C)** Off-target analysis in founder 1 and founder 7, only some predicted off-targets by CRISPOR.tefor.net were tested. PCR and subsequent sequencing revealed that none of the tested off-targets were edited (large deletions will not be detected with that approach).

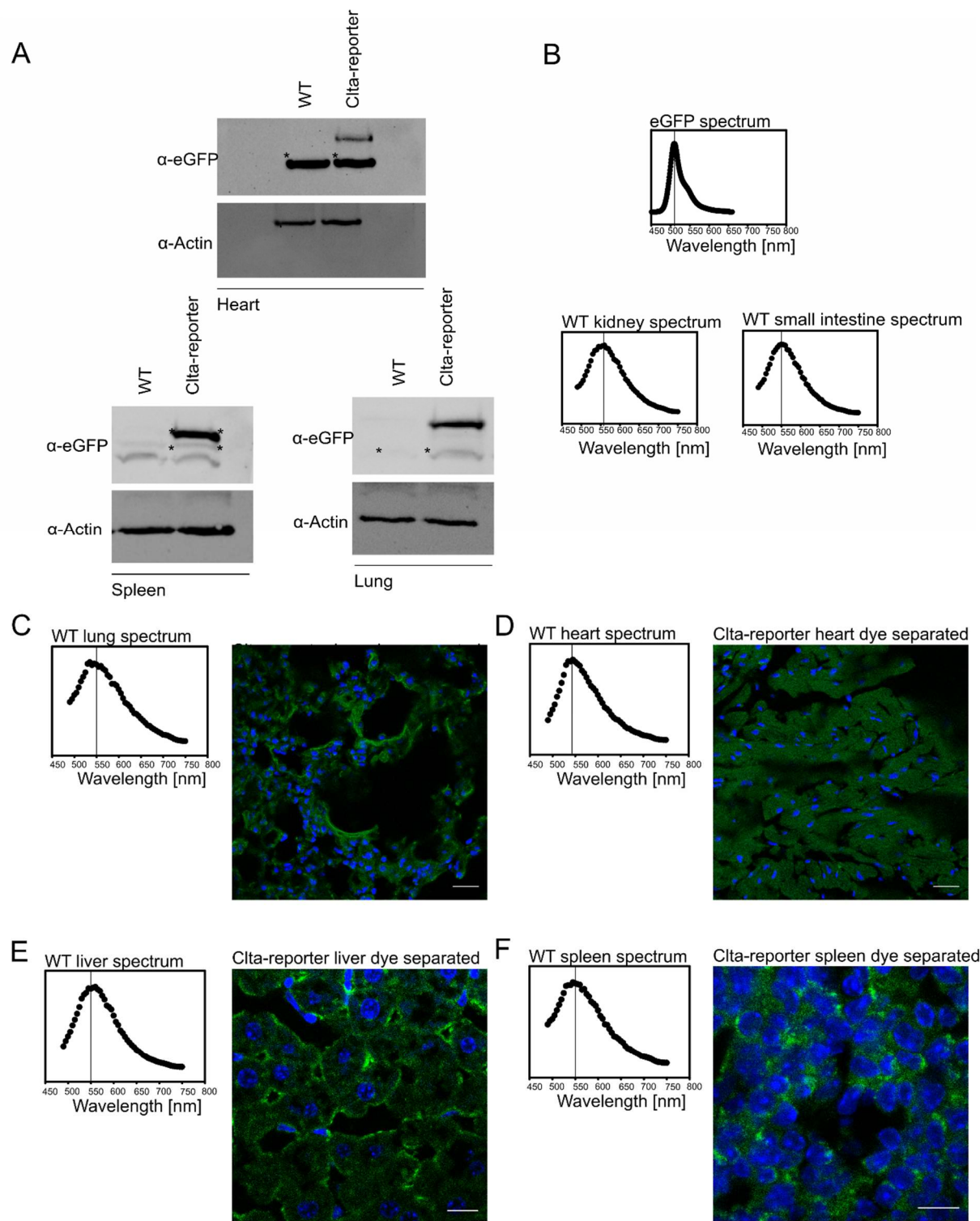
14 **S2 Figure**



15

16 **S2 Fig. Histological sections of Clta-reporter and WT.** Hematoxylin and Eosin (H&E)-staining
 17 of small intestine (A), kidney (B), liver (C), lung (D), spleen (E) and heart (F) did not reveal
 18 aberrant morphological alterations. Scale bar: 100 μm.

19



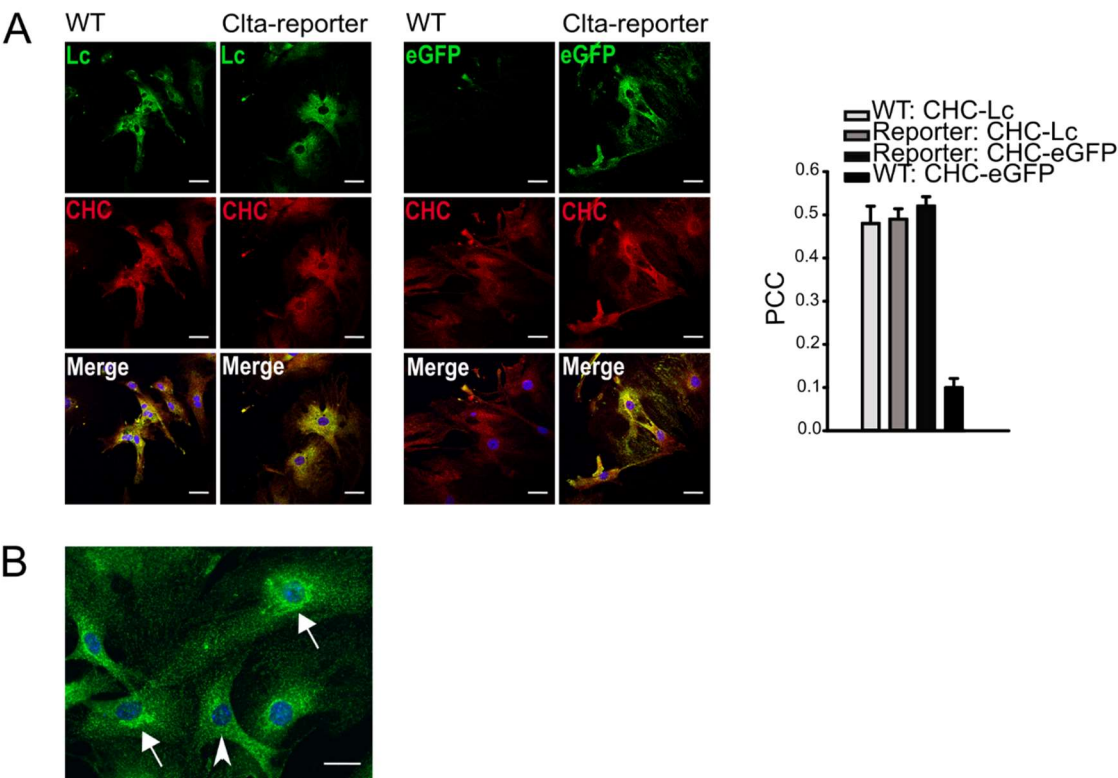
21

22 **S3 Fig. Expression of the Clta-eGFP fusion protein.** (A) Expression of the Clta-eGFP fusion pro-

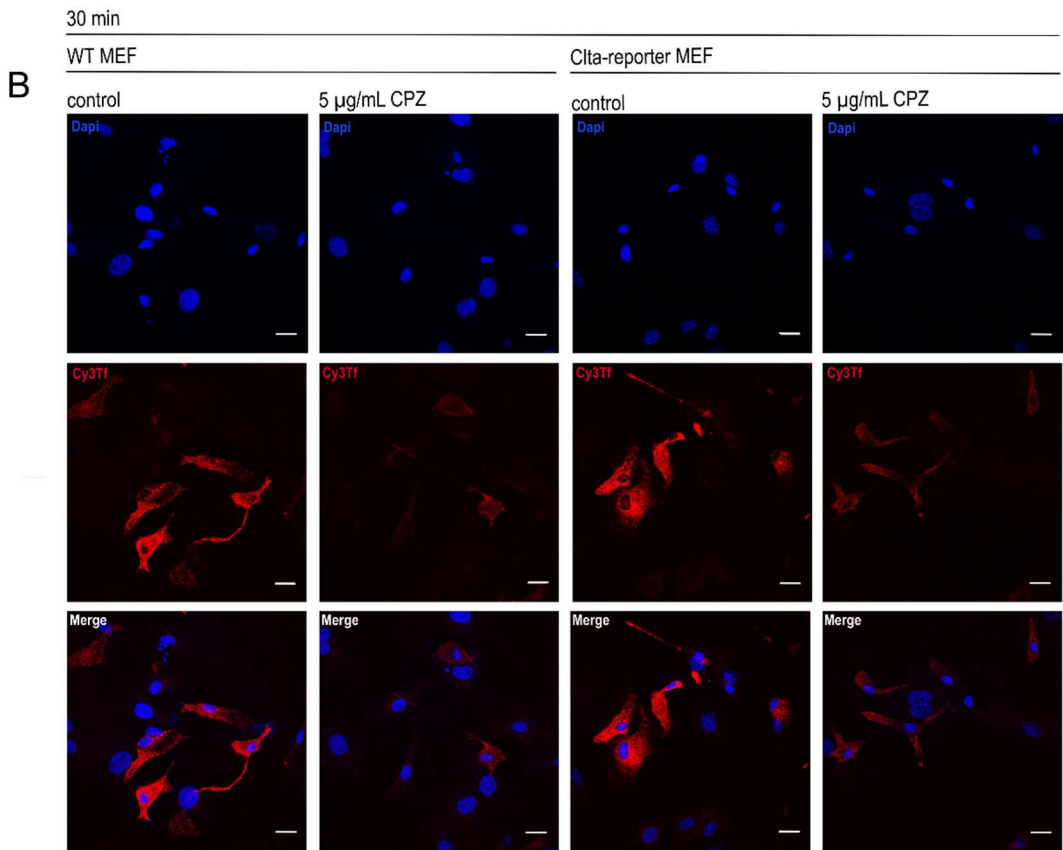
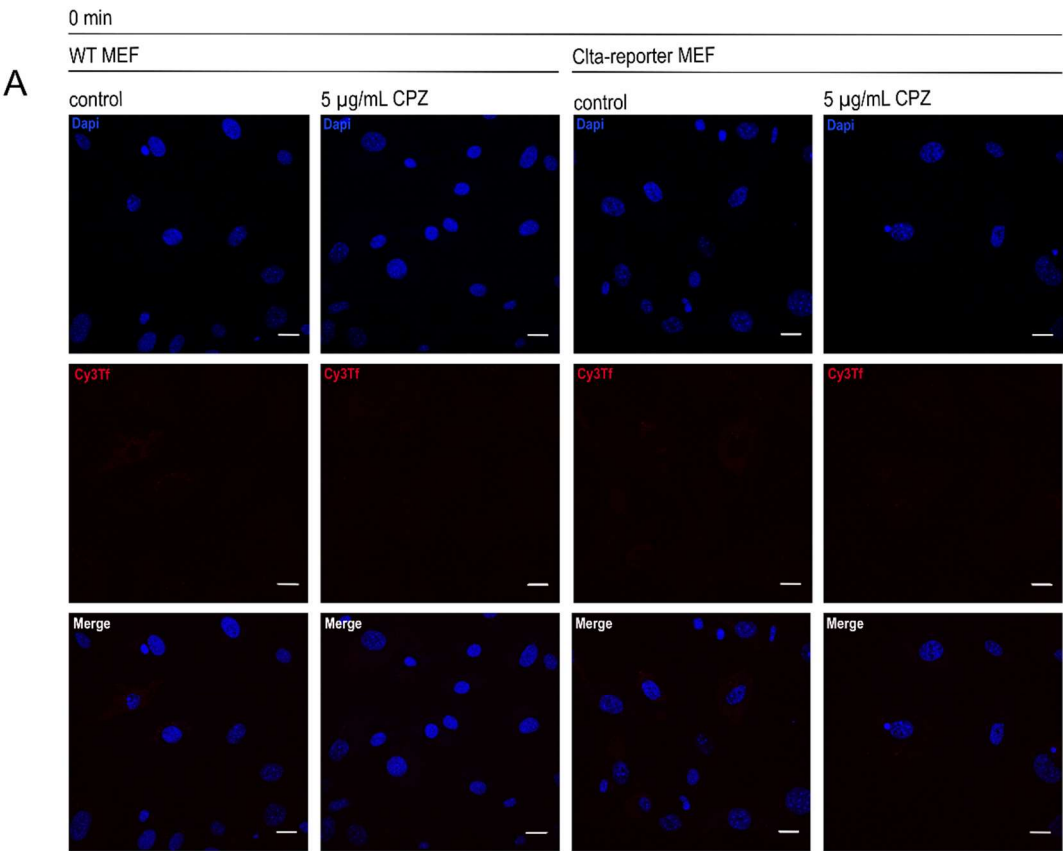
23 tein in heart, lung and spleen is demonstrated on Western Blot. *: unspecific cross reactivity

of the eGFP antibody. **(B)** Emission spectrum of eGFP and WT emission spectra in the green spectral range of kidney **(left)** and small intestine **(right)**. **(C-F)** WT emission spectrum in the green spectral range of lung; scale bar: 25 μm **(C)**, heart; scale bar: 25 μm **(D)**, liver; scale bar: 15 μm **(E)** and spleen; scale bar: 10 μm **(F)**, together with the resulting dye separated image of the Clta-eGFP reporter.

S4 Figure

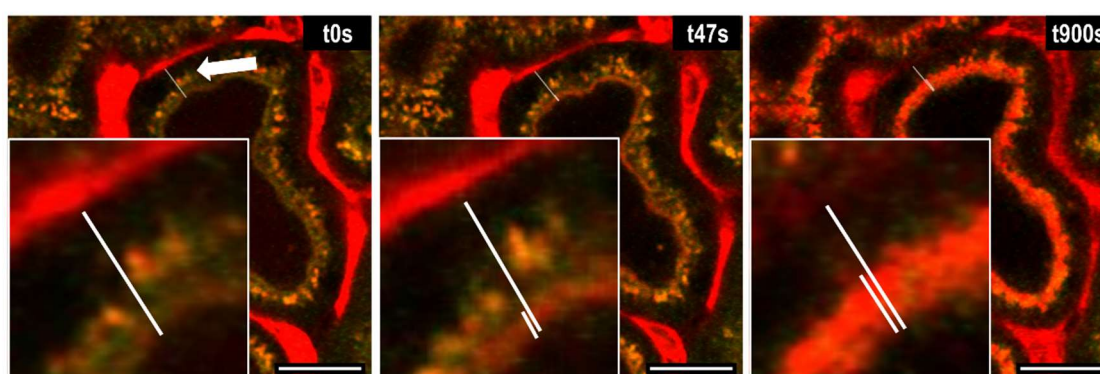


S4 Fig. A) Co-localization of CHC-Lc and CHC-Clta-eGFP double stainings in MEFs **(left)** and subsequent co-localization analysis by calculating Pearson's correlation coefficient **(right, students t-test)**. Scale bar: 50 μm . **B)** Primary Clta-eGFP MEFs stained with anti-GFP antibody and visualized with an epifluorescence microscope (Keyence 9000). Note some MEFs show prominent perinuclear staining (arrow) whereas others do not (arrow head). Scale bar: 50 μm .



S5 Fig. Cy3-Tf uptake in CPZ treated MEFs. WT and Clta-reporter MEF were treated 15 min prior and during the Cy3-Tf uptake with 5 $\mu\text{g}/\text{mL}$ of CPZ and compared with corresponding control-treated MEF (control). Confocal pictures at 0 min (**A**) and 30 min (**B**) after adding Cy3Tf are shown. Scale bar: 25 μm .

S6 Figure



S6 Fig. Determination of Alexa594-BSA uptake and intracellular trafficking dynamics. Ratiometric measurement between the apically labelled Alexa594-BSA region and the entire cellular cross-section length. It is possible to observe the progressive Alexa594-BSA uptake over time. Scale bar: 20 μm .

S1 Movie. TIRFM of Clta-eGFP in the cell shown in Fig. 5A-C. The cell was imaged at approx. 1 pfs. Clta-eGFP puncta show dynamic behaviour. In the boxed region at t=110 a circle highlights the appearance of a Clta-eGFP spot at t=113 seconds and persists over many frames before it gets weaker at t=135 seconds for 2 frames before it disappears.

S2 Movie. Time series of Alexa594-BSA uptake in WT kidney. Accumulation of Alexa594-BSA in the brush border of proximal tubule of murine kidney over time. Scale bar: 25 μm .

S3 Movie. Time series of Alexa594-BSA uptake in Clta-reporter kidney. Accumulation of Alexa594-BSA in the brush border of proximal tubule of murine kidney over time. Co-localization of Clta-eGFP and Alexa594-BSA in Clta-eGFP reporter animals results in the appearance of yellow color at the brush border. At late time points additional red fluorescence is observed a few micrometers below the brush border, which does not overlay with the Clta-eGFP reporter thus demonstrating endocytosed Alexa594-BSA, likely after uncoating of the clathrin cage. Scale bar: 25 μ m.

S1 File. Sequence of ssDNA donor. Black: homology arms, purple: additional *NdeI* and *ScaI* restriction sites, green: eGFP, red: GGSGSVWV-linker. The C-terminal coding sequence of the Clta exon 6 is underlined.