SUPPLEMENTARY INFORMATION TO

ClC-7 is a slowly voltage-gated $2Cl^-/1H^+$-exchanger and requires Ostm1 for transport activity

by

Lilia Leisle, Carmen F. Ludwig, Florian A. Wagner,
Thomas J. Jentsch and Tobias Stauber

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1, related to Figure 1B,C and Figure 4: ClC-7^{PM}/Ostm1 currents in mammalian cells and *Xenopus* oocytes. (**A**) Representative current traces of hClC-7^{PM} ('WT'), hClC-7^{PM}(E247A) and hClC-7^{PM}(E314A) obtained with a voltage-clamp protocol consisting of 20mV steps to voltages between -80 and +80 mV for 2 s (inset) from oocytes three days after co-injection with Ostm1; as shown for rClC-7 in **Figure 1C**. (**B**) Typical whole-cell current traces (voltage-step protocol in inset) of rClC-7^{PM}/Ostm1 expressed in HeLa and tsA201 cells, respectively. Note pronounced tail currents with 121 mM Cl^- in the pipette solution. (**C**), Current-voltage curves (protocol as in (**B**)) of rClC-7^{PM} co-expressed with Ostm1-GFP in HeLa cells in the presence and absence, respectively, of 2 mM ATP in the pipette. Values are mean current densities ± s.e.m. of 9 (with ATP) and 8 (without ATP) cells. (**D**), Current-voltage curve (protocol as in (**B**)) of rClC-7^{PM} expressed with GFP or with Ostm1-GFP in HeLa cells. Non-transfected (n.t.) cells served as control. Mean current densities ± s.e.m. of 9 (rClC-7^{PM} co-expressed with Ostm1-GFP; same cells as in (**C**) in the presence of ATP), 12 (rClC-7^{PM} with GFP) and 10 (untransfected) cells.

Supplementary Figure S2, related to Figure 4A-C: Ostm1 domains needed for ClC-7-dependent trafficking to lysosomes. HeLa cells were transfected with the five C-terminally GFP-tagged Ostm1/CD4 chimeras (COO, OCO, OOC, CCO and OCC; C for CD4 and O for Ostm1; the position in the name indicates N-terminal, trans-membrane and C-terminal domain, respectively) that are not shown in **Figure 4A,B,** either alone (**A**) or with rClC-7-HA (**B**). Immunodetection as in **Figure 4**. Only Ostm1/CD4 chimeras containing the TMD of Ostm1 are carried to lysosomes.

Supplementary Figure S3, related to Figure 4D: Partial plasma membrane localization of rClC-7^{PM} irrespective of Ostm1/CD4 co-expression in HeLa cells. HeLa cells were co-transfected with rClC-7^{PM} and C-terminally GFP-tagged Ostm1/CD4 chimeras (three-letter code: C for CD4 and O for Ostm1; the position in the name indicates N-terminal, trans-

membrane and C-terminal domain, respectively), and immunostained after 30 h for ClC-7 and the lysosome marker protein LAMP-2. In all cases, rClC-7^{PM} was detected at the plasma membrane.

Supplementary Figure S4, related to Figure 5: Localization of amino acids mutated in osteopetrosis in CLC structure. X-ray structure of CmClC (Feng *et al*, 2010) displaying the location of the osteopetrosis-causing ClC-7 mutations analyzed in this study. The transmembrane core-forming parts of the two identical subunits are shown in gray, CBS1 in yellow and CBS2 in orange, using darker colors for one subunit. Positions in the structure are based on the published alignment (Feng *et al*, 2010) of ClC-7 with CmClC and are only shown in one subunit. Color code of mutants as in **Figure 5A**: purple, mutants that localize to the endoplasmic reticulum in hClC7/Ostm1; red, mutants showing no or strongly reduced currents in hClC-7^{PM}/Ostm1; green, mutants with WT-like currents; blue, mutants with accelerated activation kinetics.

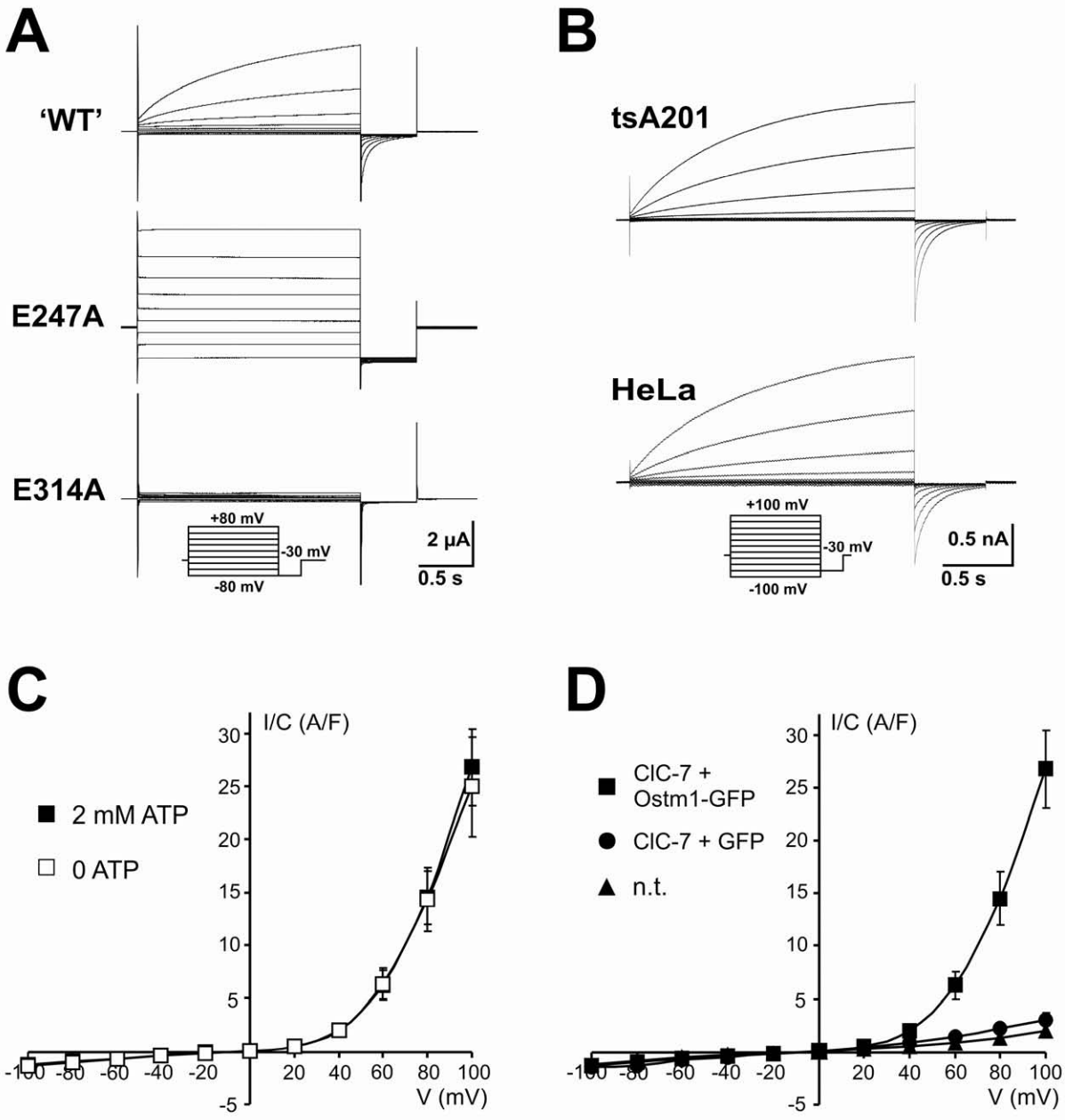
Supplementary Figure S5, related to Figure 5: Analysis of additional *CLCN7* mutations found in human osteopetrosis. (**A**) Typical current traces of *Xenopus* oocytes co-expressing the indicated mutants (in hClC-7^{PM}) together with Ostm1. (**B**) Subcellular localization of the same mutant (but in hClC-7) upon co-expression with Ostm1-GFP in HeLa cells. Experiments were performed as in **Figure 5C,D**.

Supplementary Figure S6, related to Figure 5: Expression level of osteopetrosis-causing mutations that yielded no currents. (**A**) Immunoblot showing the protein expression of hClC-7^{PM} ('WT' or selected osteopetrosis-causing mutations, which yielded no currents upon expression in *Xenopus* oocytes but localized with Ostm1-GFP to lysosomes when expressed in the wildtype background in HeLa cells) three days after co-injection of the respective cRNA with that of Ostm1. Whole-oocyte protein equivalent to 1.5 oocytes was probed on immunoblot with rabbit antibody against ClC-7 (7N4B, Kornak et al., 2001) and mouse antibody against α-tubulin (clone DM1A, Sigma). Signal detection used chemiluminescence and

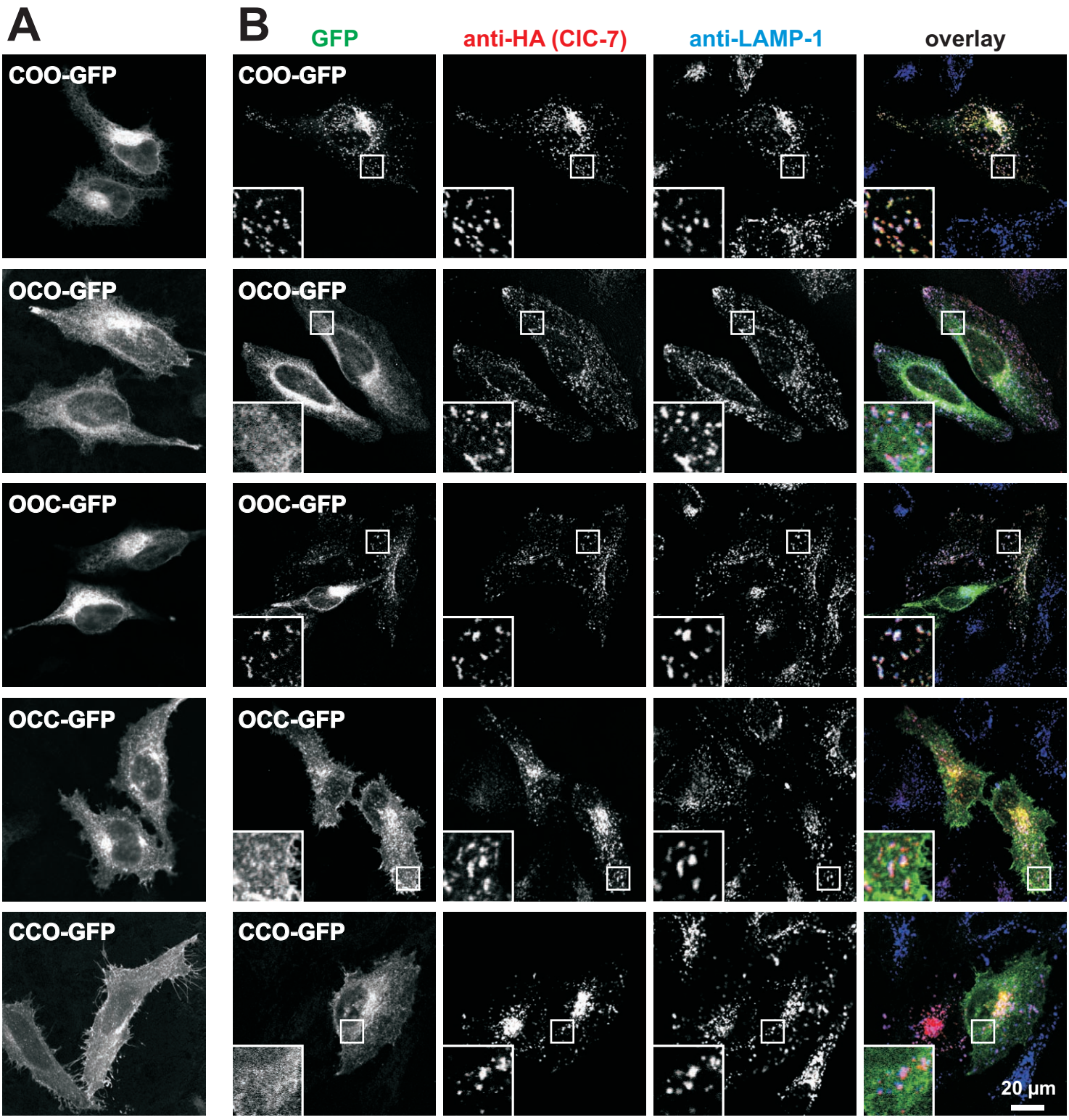
a camera system. (**B**) Signal intensities in immunoblots were quantified using the free software ImageJ and normalized to 'WT' after background subtraction. Mean values ± s.e.m. of three independent experiments are shown.

Supplementary Video 1, related to Figure 5B: Localization of 'accelerating' osteopetrosis mutations in the transmembrane-CBS interface.

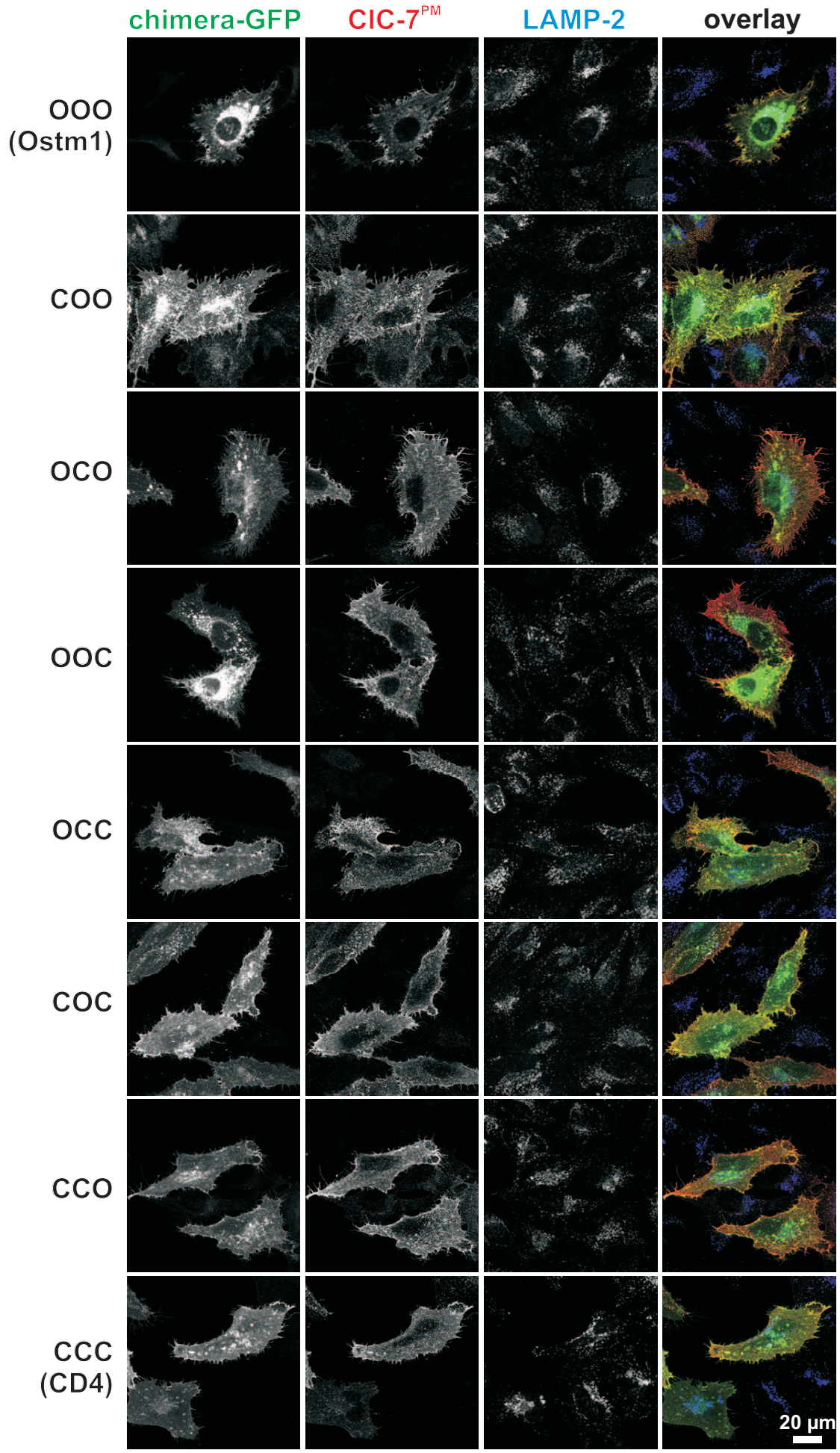
Rotation by 15° of a close-up of X-ray structure of CmClC (Feng *et al*, 2010) seen from a different perspective as in **Figure 5B** to better visualize the proximity of ClC-7 residues that accelerate gating when mutated. The transmembrane part of one subunit is shown in light gray, CBS1 in yellow, and CBS2 in orange (or pink). Dark gray and red helices on the left correspond to transmembrane part and CBS2, respectively, of the attached second subunit of the homodimer.



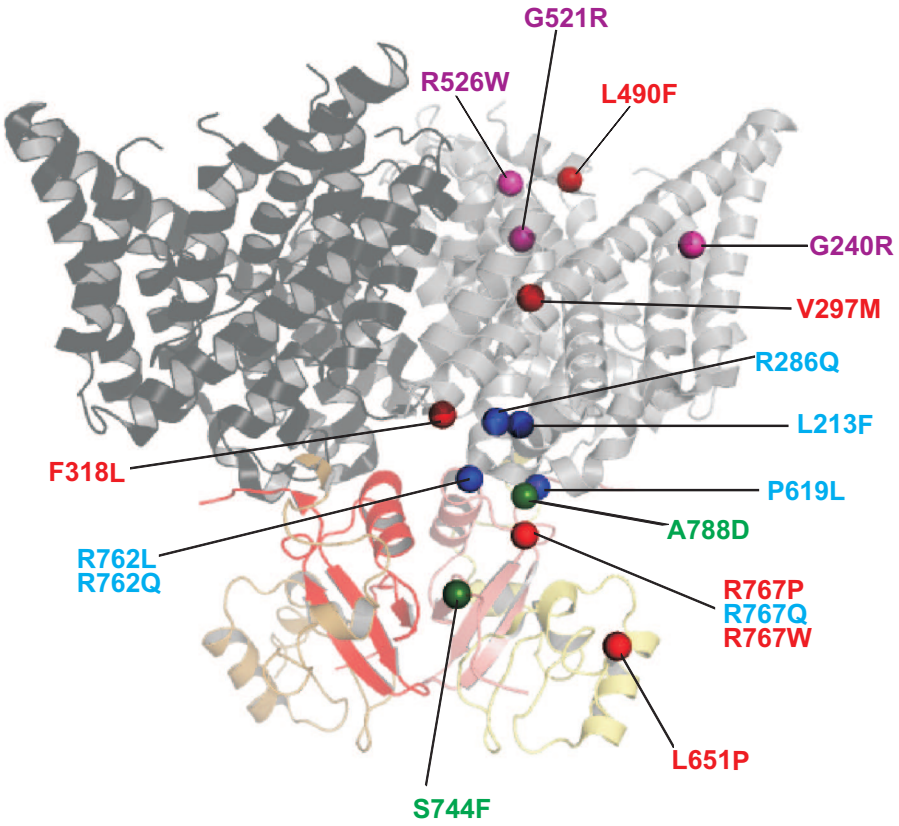
Supplementary Figure S1



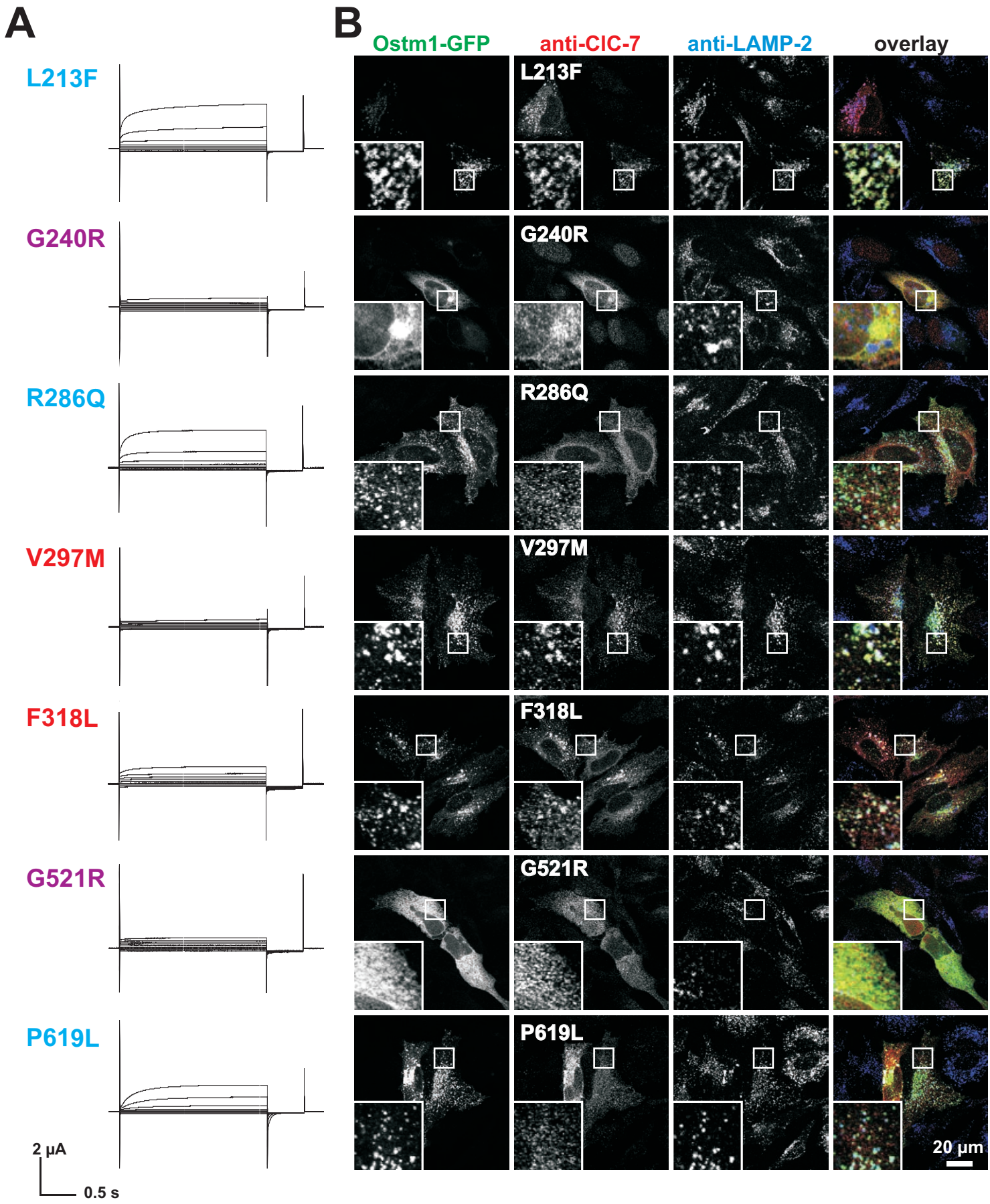
Supplementary Figure S2



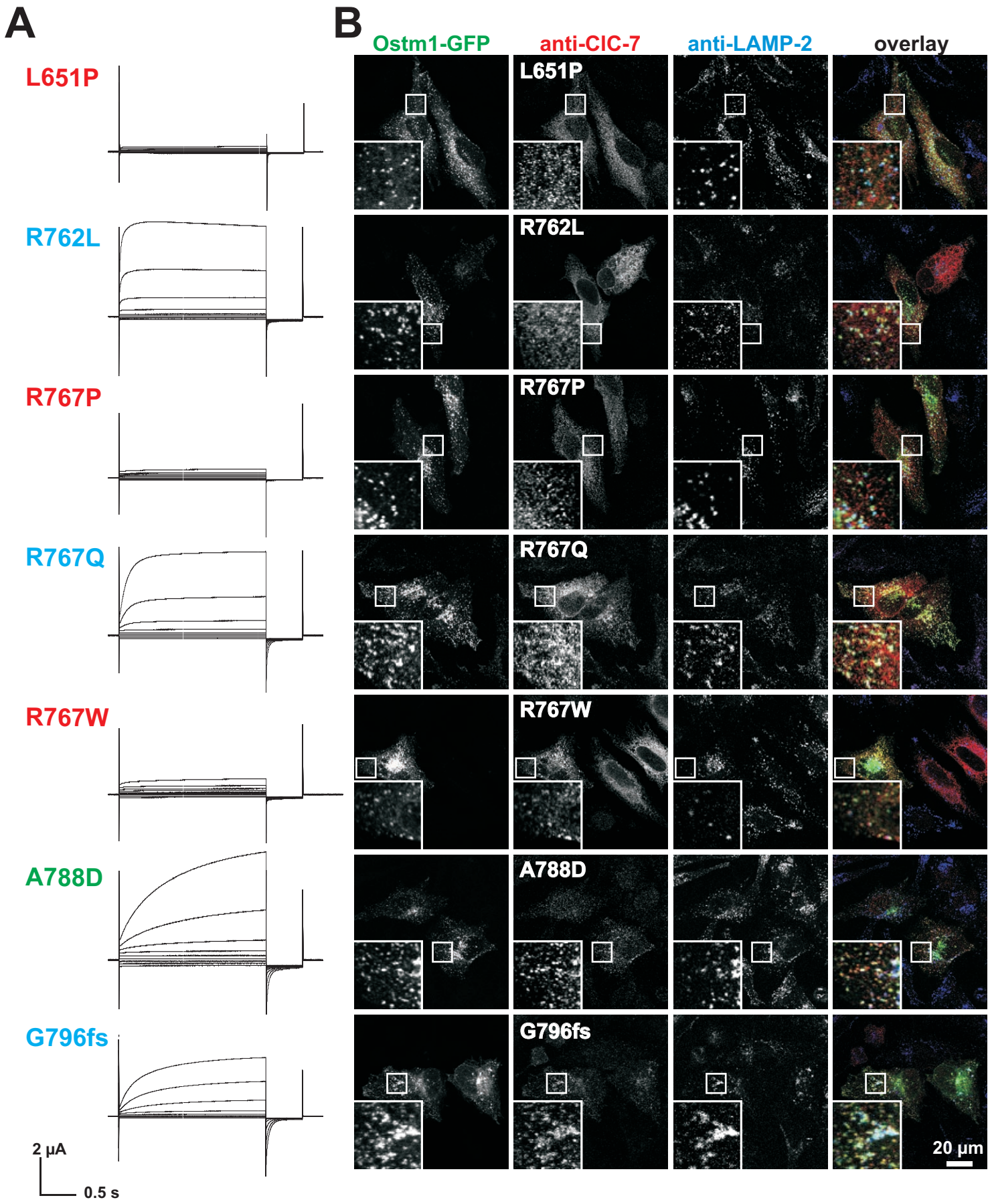
Supplementary Figure S3



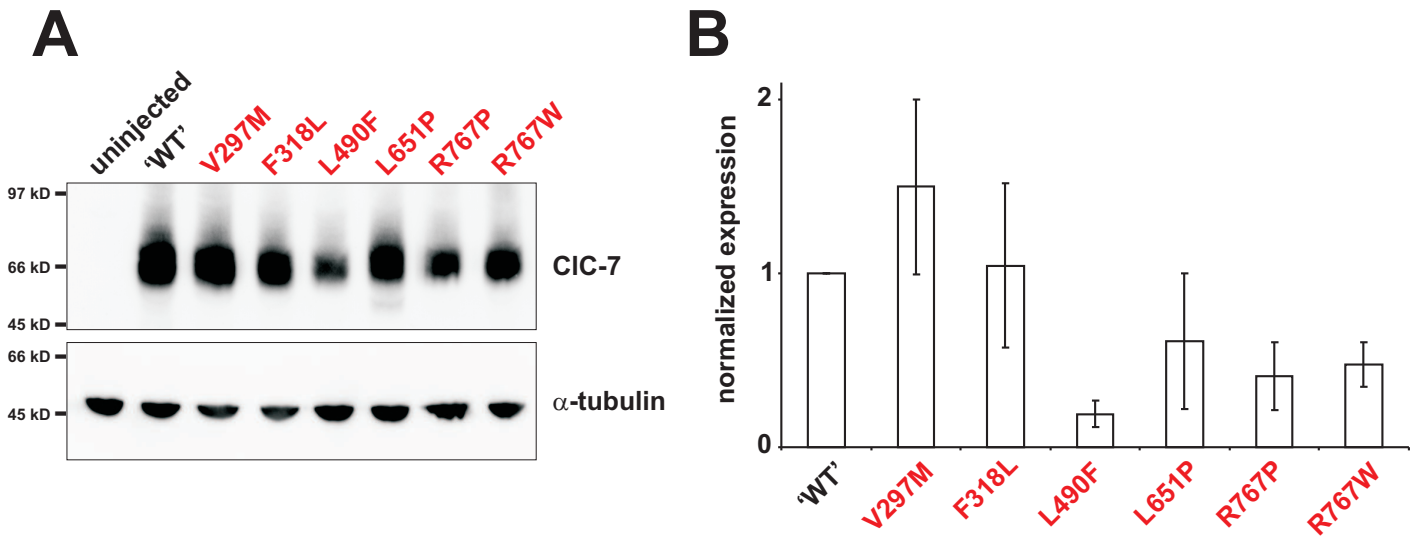
Supplementary Figure S4



Supplementary Figure S5, part 1



Supplementary Figure S5, part 2



Supplementary Figure S6