SUPPORTING INFORMATION

Assorted dysfunctions of endosomal alkali cation/proton exchanger *SLC9A6* variants linked to Christianson syndrome

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Running title: *Characterization of Christianson syndrome SLC9A6 variants*

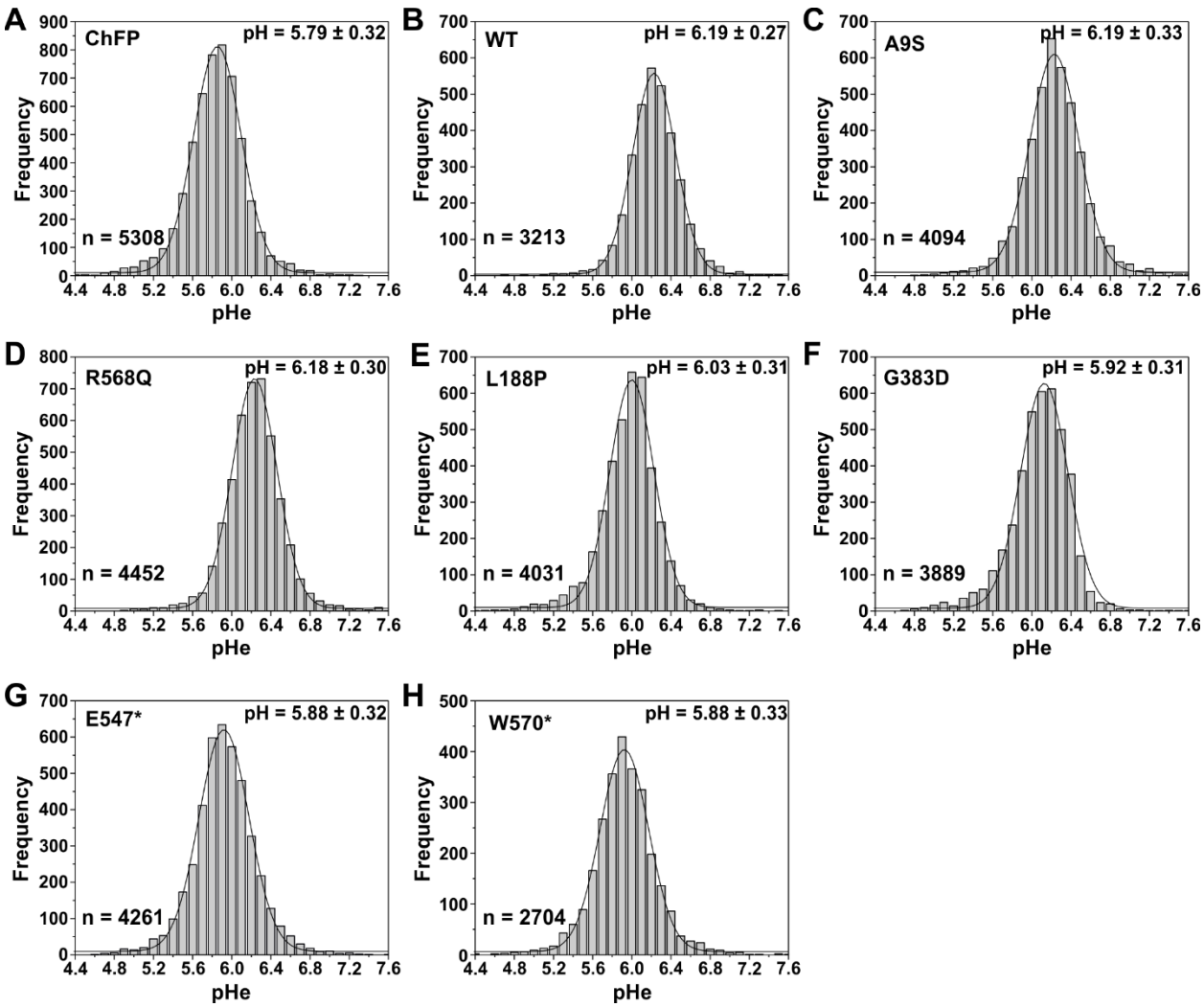

Figure S1. Assessment of recycling endosomal pH in AP-1 cells expressing NHE6 variants. Graphical plot of the intraluminal pH of recycling endosomes in AP-1 cells transiently transfected (48 h) with monomeric Cherry fluorescent protein (ChFP), ChFP-tagged NHE6 WT or CS-linked variants. Recycling endosomal pH (pH_e) was measured by fluorescence ratio image analysis of the internalized pH-sensitive probe fluorescein isothiocyanate-conjugated human transferrin (Tf-FITC). Data represent the frequency of vesicles as a function of their intraluminal pH_e analyzed from 24 to 36 cells per construct. The total number of vesicles analyzed (n) and the mean vesicular pH_e (mean ± S.D.) are indicated in the plots.

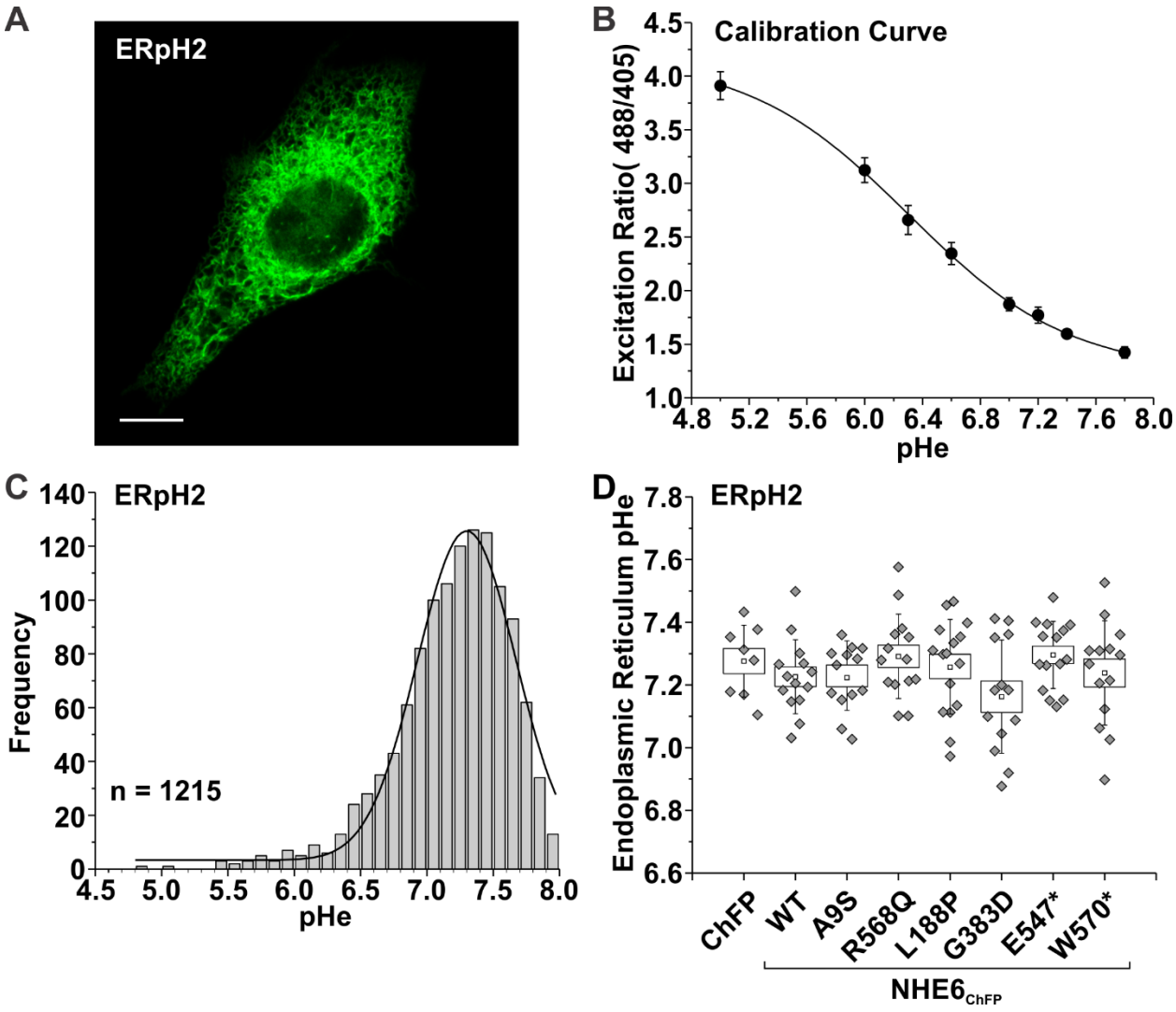

Figure S2. Assessment of endoplasmic reticulum pH in AP-1 cells expressing NHE6 variants. ***A***, Fluorescence image of AP-1 cells expressing endoplasmic reticulum-targeted pHluorin2 (ERpH2). ***B***, *In situ* calibration curve of ERpH2 fluorescence (excitation ratio 488/405) in AP-1 cells as a function of ER pH (pH_e) performed by *in situ* clamping intracellular pH between 5 and 7.8. Average intensities of fluorescent puncta or regions of interest (R.O.I., 0.3 to 3 µm in diameter; 150-200 R.O.I. per cell) were obtained for both the 405 and 488 channels using MetaXpress software and calculating the 488/405 ratios. ***C***, Graphical plot showing the frequency of R.O.I. as a function of their intraluminal pH_e analyzed from 8 AP-1 cells (n = the total number of R.O.I. analyzed). ***D***, Average ER pH_e/cell of AP-1 cells transiently expressing (48 h) ChFP, $NHE6_{ChFP}$ WT or CS-linked variants. Data are plotted as a box chart, with the central white square indicating the mean, the box representing the S.E.M. and the error bars showing the S.D. (n = 8-16 cells/construct). Significance was determined by one-way ANOVA with a *post-hoc* Tukey test; $p > 0.05$.

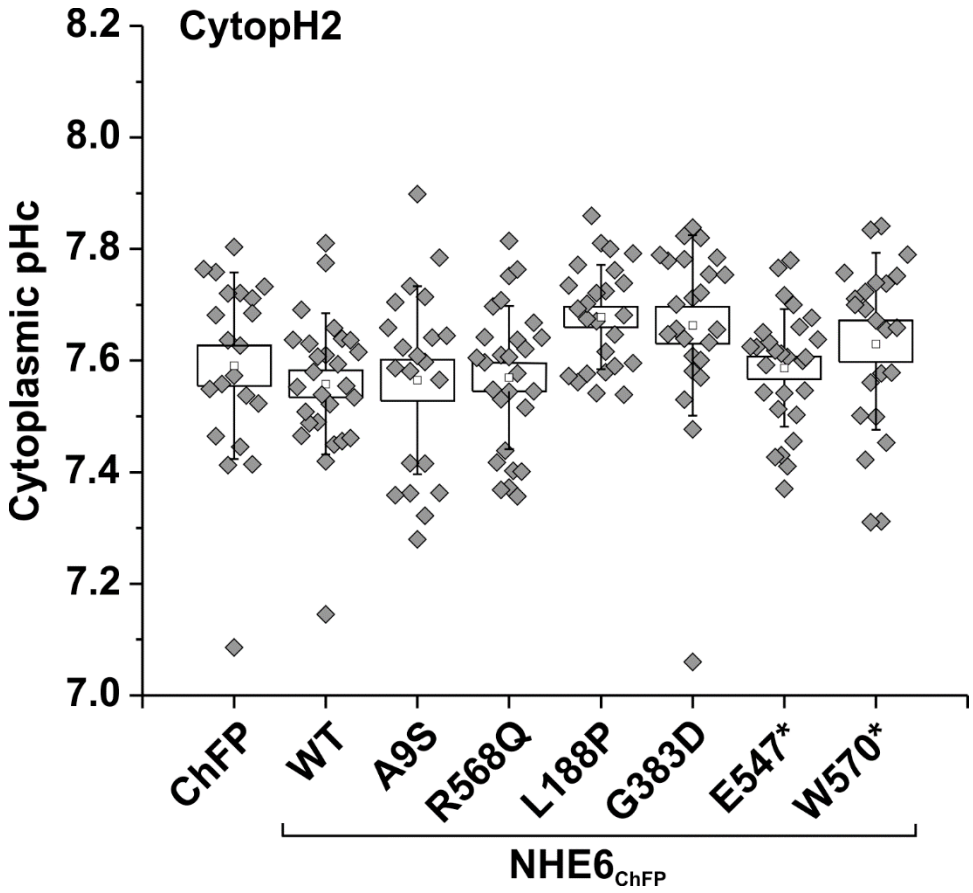

Figure S3. Assessment of cytoplasmic pH in AP-1 cells expressing NHE6 variants. Average cytoplasmic pH (pH_c)/cell of AP-1 cells transiently expressing soluble pHluorin2 (CytopH2) and ChFP, $NHE6_{ChFP}$ WT or CS-linked variants. Cytoplasmic pH_c was measured by fluorescence ratio image analysis of CytopH2 as described in "Experimental Procedures". Data are plotted as a box chart, with the central white square indicating the mean, the box representing the S.E.M. and the error bars showing the S.D. (n = 21-27 cells/construct). Significance was determined by one-way ANOVA with a *post-hoc* Tukey test; $p > 0.05$.