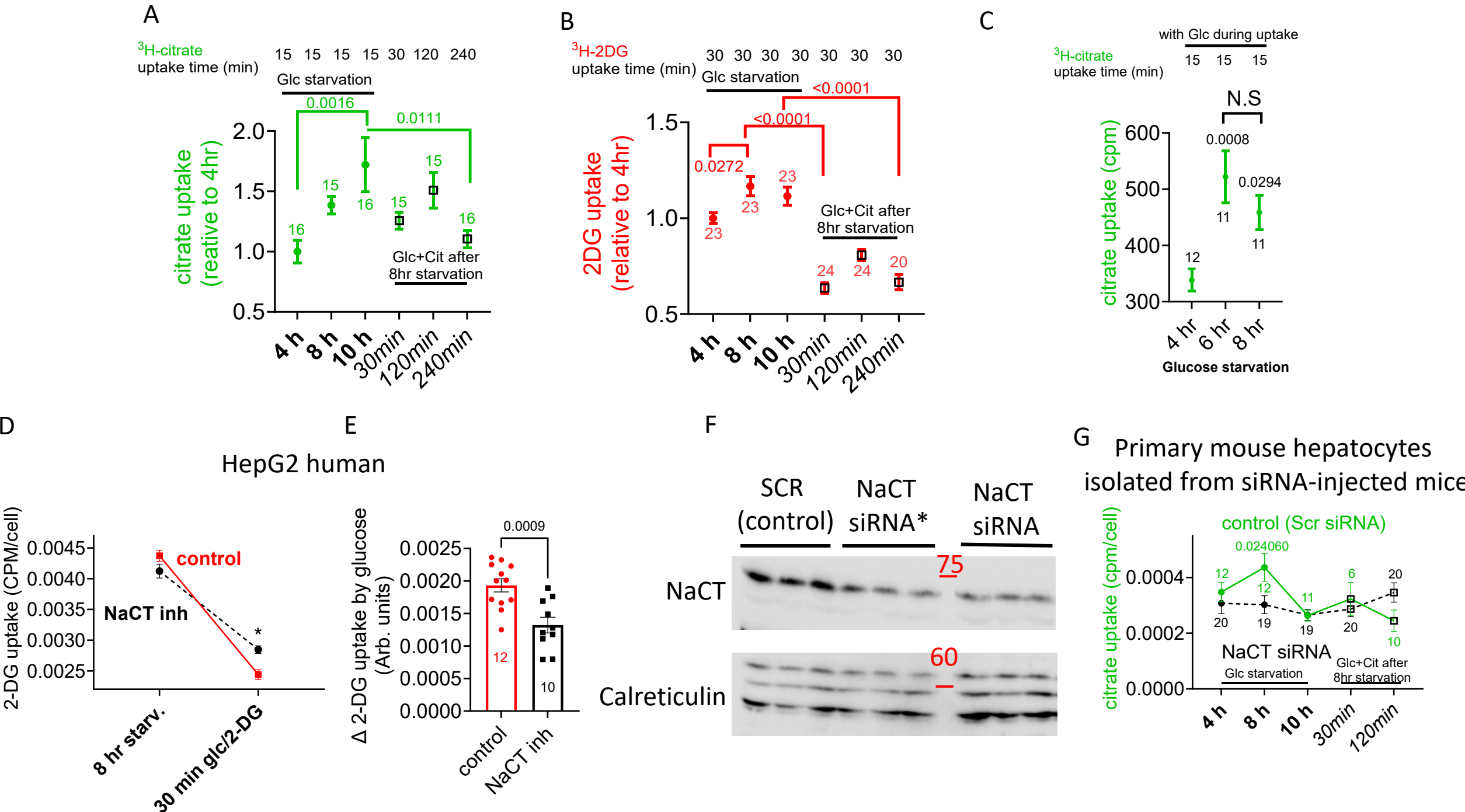
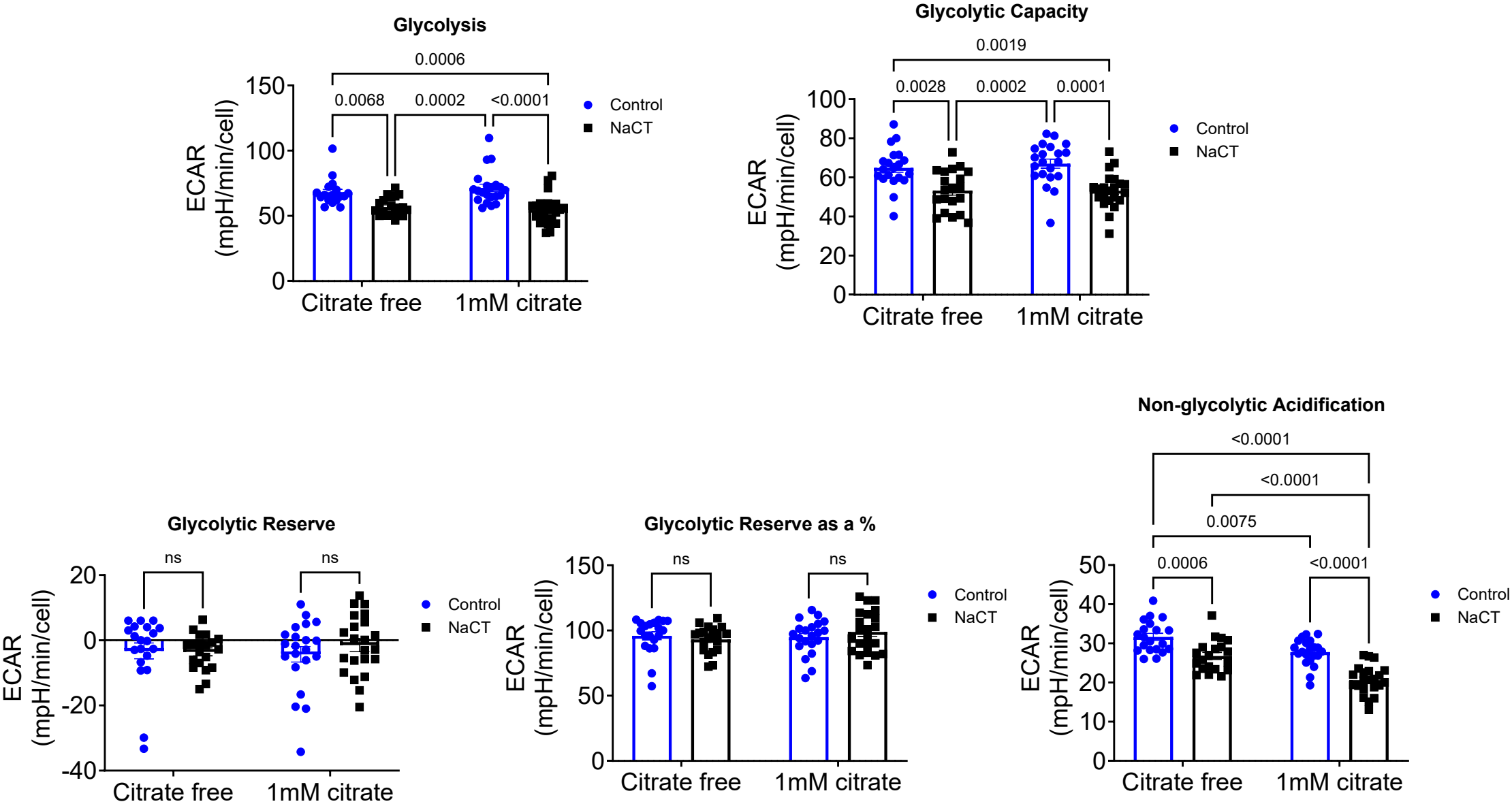




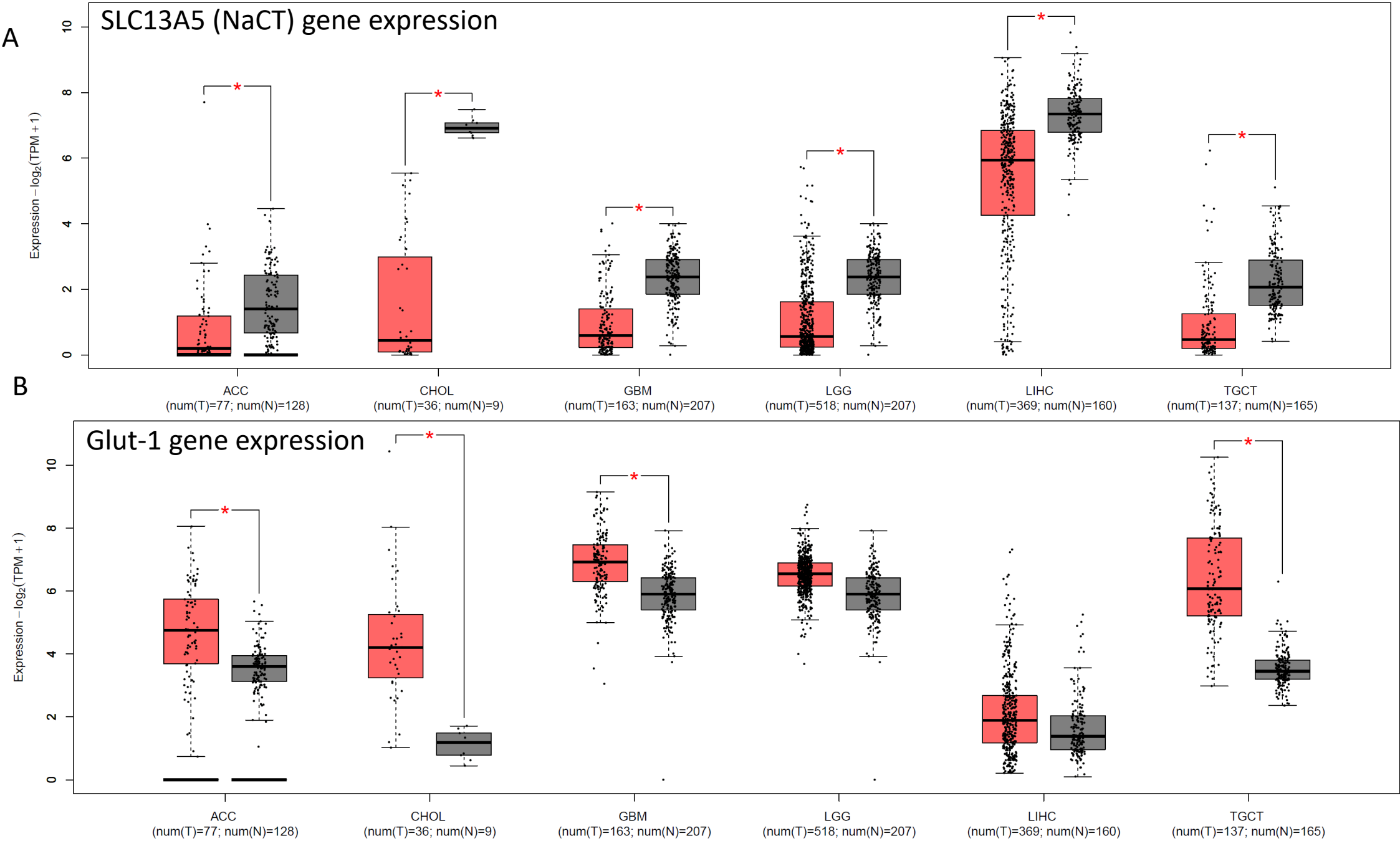
Supp. Fig. 1: **Citrate and glucose uptake in human hepatocyte cultures or mouse primary hepatocytes.** (A and B) The experimental protocol described in Fig. 1B and C was performed using THLE2 human hepatocytes as indicated in the Figure. (C) 15 min ^3H -citrate uptake into primary mouse hepatocytes monitored in the presence of 10 mM citrate and 5 mM glucose after 4, 6, and 8 h of glucose starvation. (D) Human hepatocyte cells (HepG2) were cultured and incubated in the absence of glucose (8 h), after which glucose was applied. 2-DG uptake was monitored with or without the NaCT inhibitor, as indicated. The summary in (E) describes changes in 2-DG uptake between treatments (30 min glc/2-DG values were subtracted from the average of 8 h starv. values per experiment). (F) The expression of NaCT in mouse AML12 hepatocytes transfected with either Scr siRNA, NaCT siRNA* (previously reported) or NaCT siRNA (used for the *in vivo* injections described in Fig. 1). (G) Analysis and summary ^3H -citrate uptake in primary mouse hepatocyte cultures used for the measurements of ^3H -2DG uptake in Fig. 1H after 4, 8 and 10 h of glucose starvation (closed circles) or After 8 h of starvation and application of 10 mM citrate and 5 mM glucose for 30, and 120 min (open squares) in mice injected with either scrambled siRNA (Scr) (3 mice) or NaCT-siRNA (5 mice). In (A-C) and (E), N is the number of uptake measurements in individual wells of cultured hepatocytes.

Supp. Fig. 2

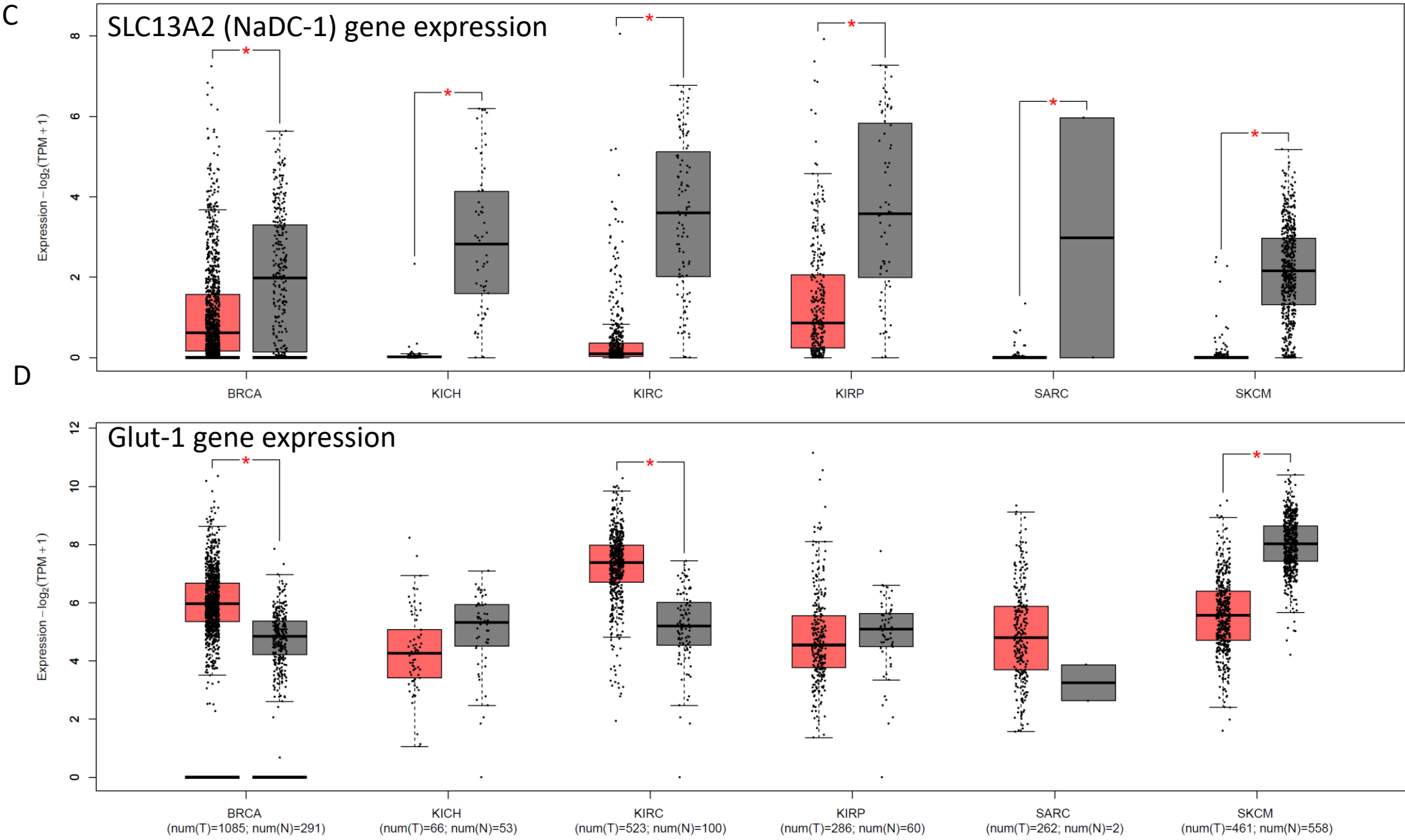


Supplementary Fig. 2: **Analysis of seahorse analyzer measurements.** The analysis was performed for the glycostress assay measurements in Fig. 1I. Three independent experiments were performed. N is the number of individual HEK293 culture wells for which glucostress measurements were performed. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test to determine significant differences between groups.

Supp. Fig. 3



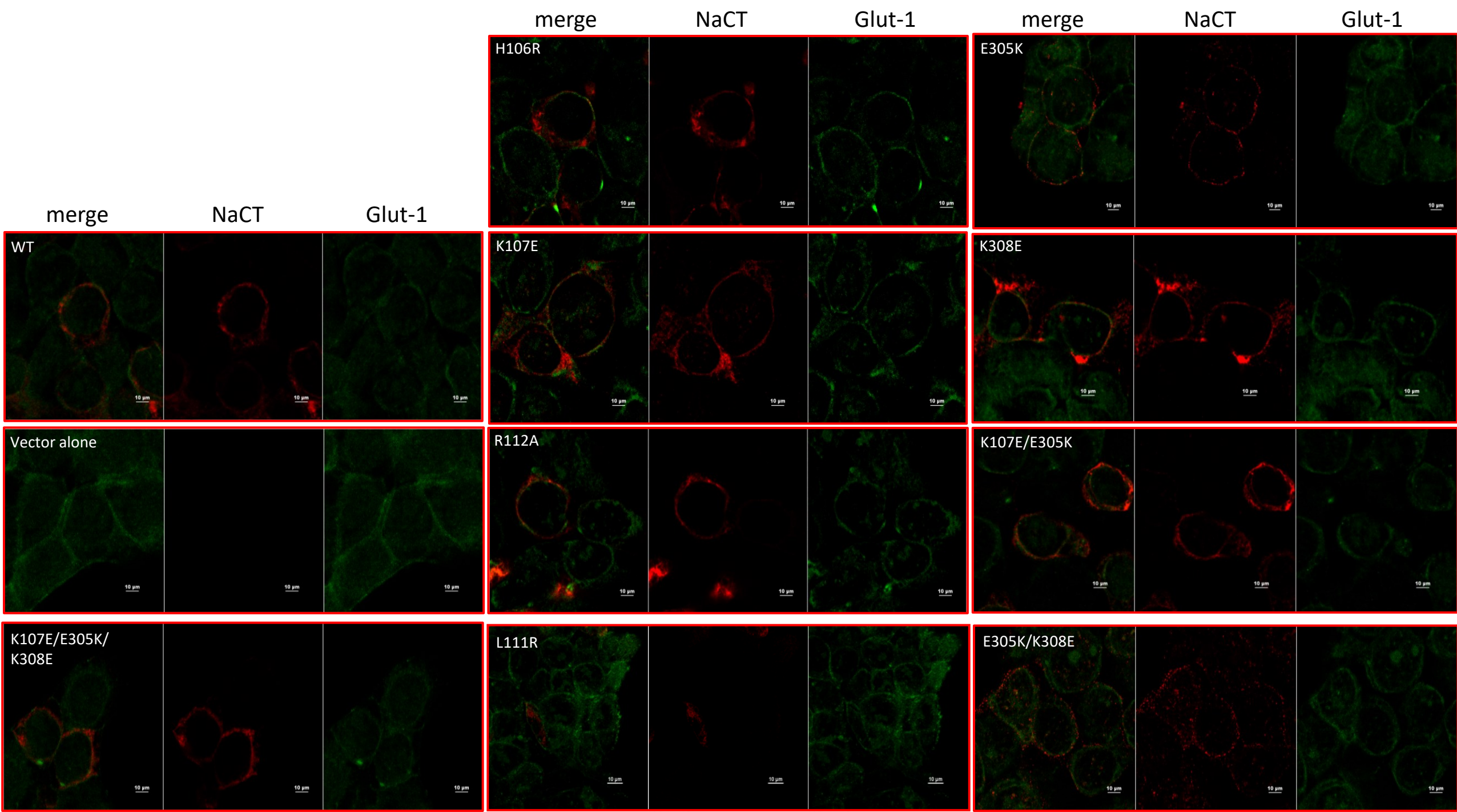
Supp. Fig. 3



Supplementary Fig. 3: SLC13 and Glut-1 gene expression in different cancer types.

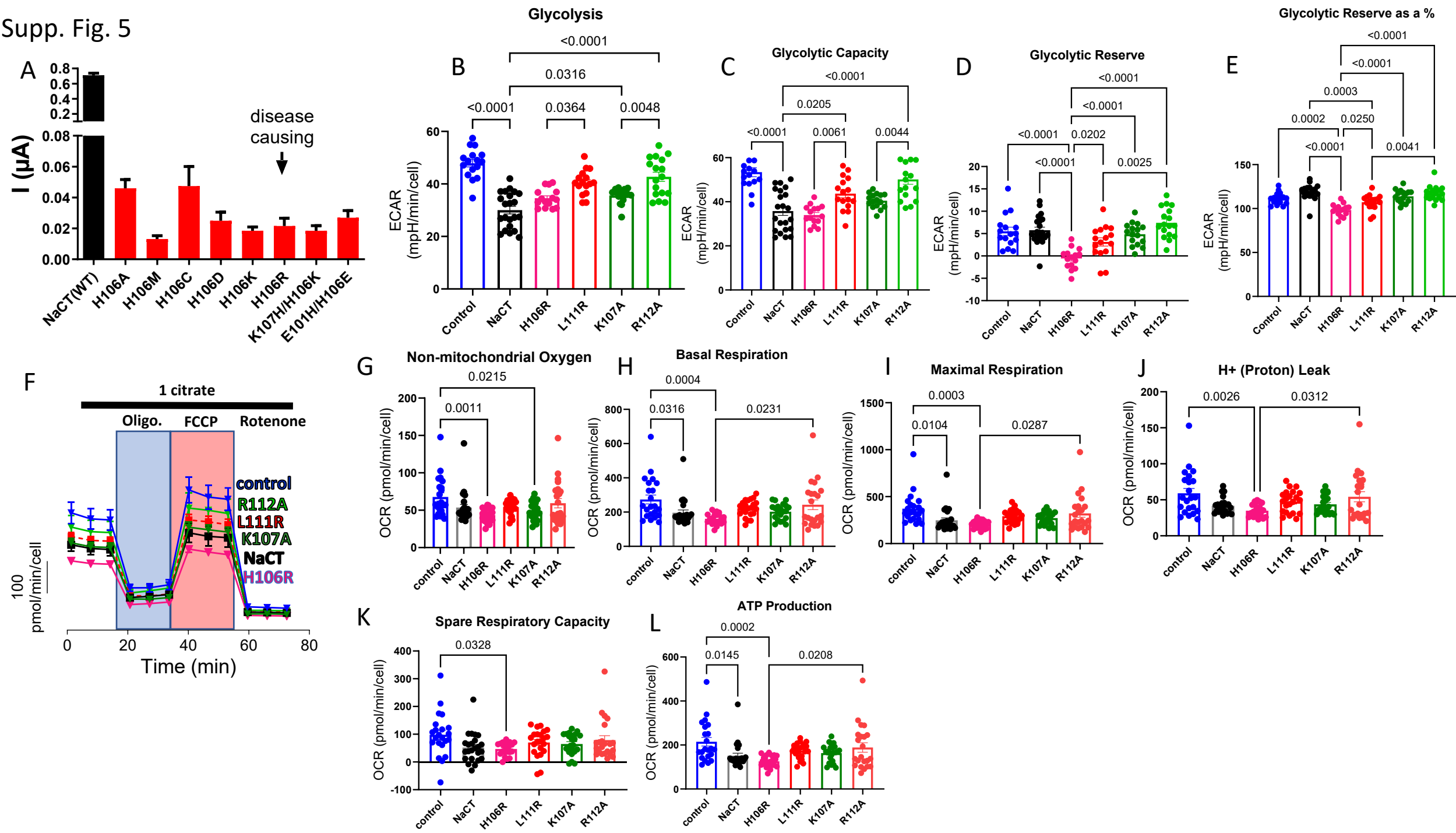
Database analysis of gene expression data from human cancer (red) or healthy (gray) tissues using the GEPIA 2 website shows SLC13 (A and C) and Glut-1 (B and D) expression in tissues that endogenously express SLC13A2 or SLC13A5 in healthy individuals. ACC-Adrenocortical carcinoma; CHOL-Cholangio carcinoma; GBM-Glioblastoma multiforme; LGG-Brain Lower Grade Glioma; LIHC-Liver hepatocellular carcinoma; TGCT- Testicular Germ Cell Tumors; BRCA-Breast invasive carcinoma; KICH-Kidney Chromophobe; KIRC-Kidney renal clear cell carcinoma; KIRP-Kidney renal papillary cell carcinoma; SARC-Sarcoma; SKCM-Skin Cutaneous Melanoma.

Supp. Fig. 4



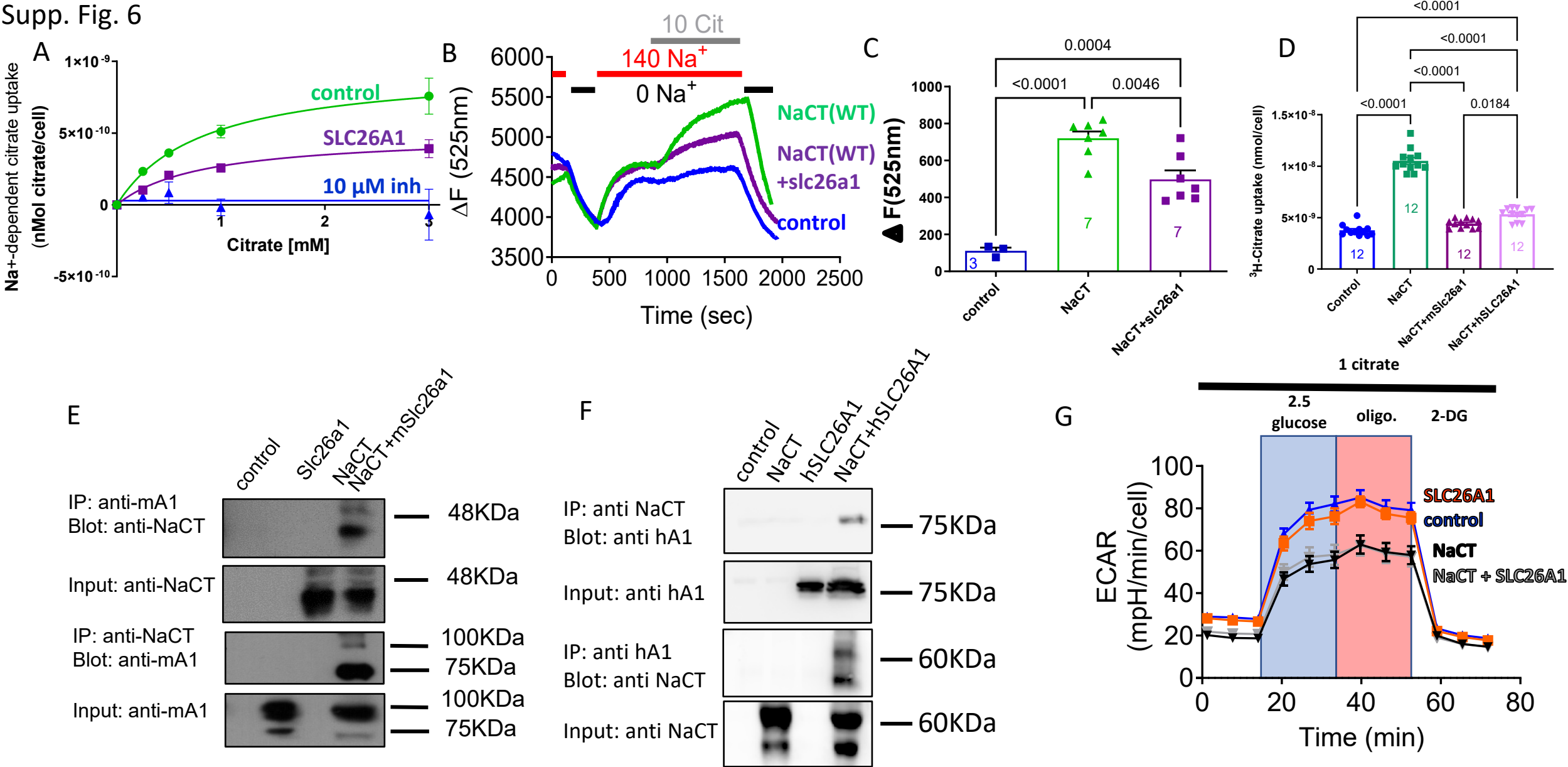
Supplementary Fig. 4: **The NaCT H4c/H6b residues control NaCT trafficking.** The expression pattern of mKate-tagged NaCT and mutations described in Figs 2,5, and 6.

Supp. Fig. 5

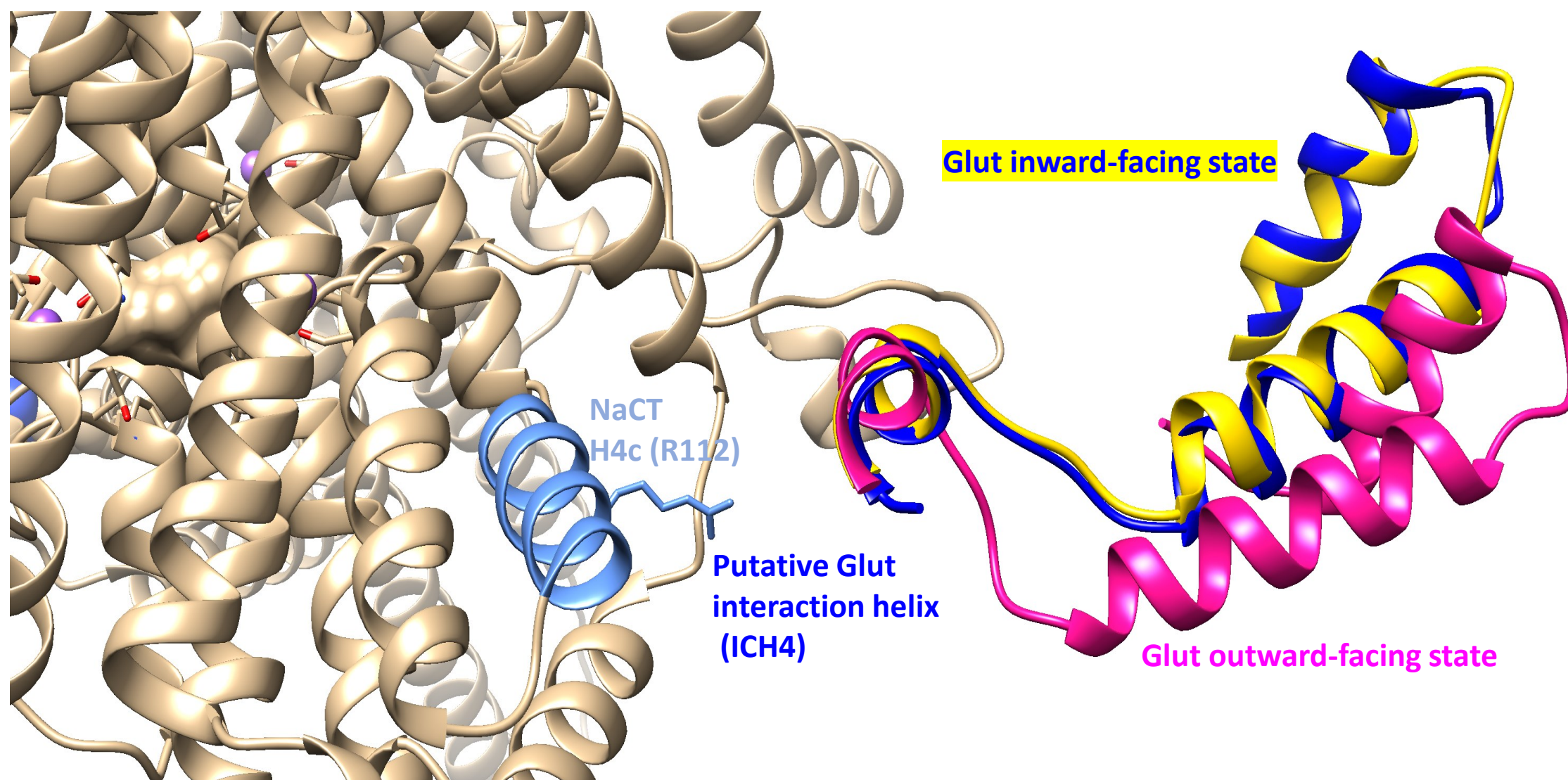


Supplementary Fig. 5: Analyses of NaCT function and seahorse analyzer measurements in cells expressing mutant NaCT constructs. (A) Charge swap and hydrophobic mutations of the H106 residue. (B-E) Glycostress analysis for the assay measurements in Figs 2G and 3F. (F) Mitostress assay protocol was performed using HEK293-T cell cultures expressing either NaCT WT or mutant constructs as indicated. (G-L) The analysis for the mitostress assay measurements in F. N is the number of individual HEK293 culture wells for which glucostress or mitostress measurements were performed. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test to determine significant differences between groups.

Supp. Fig. 6

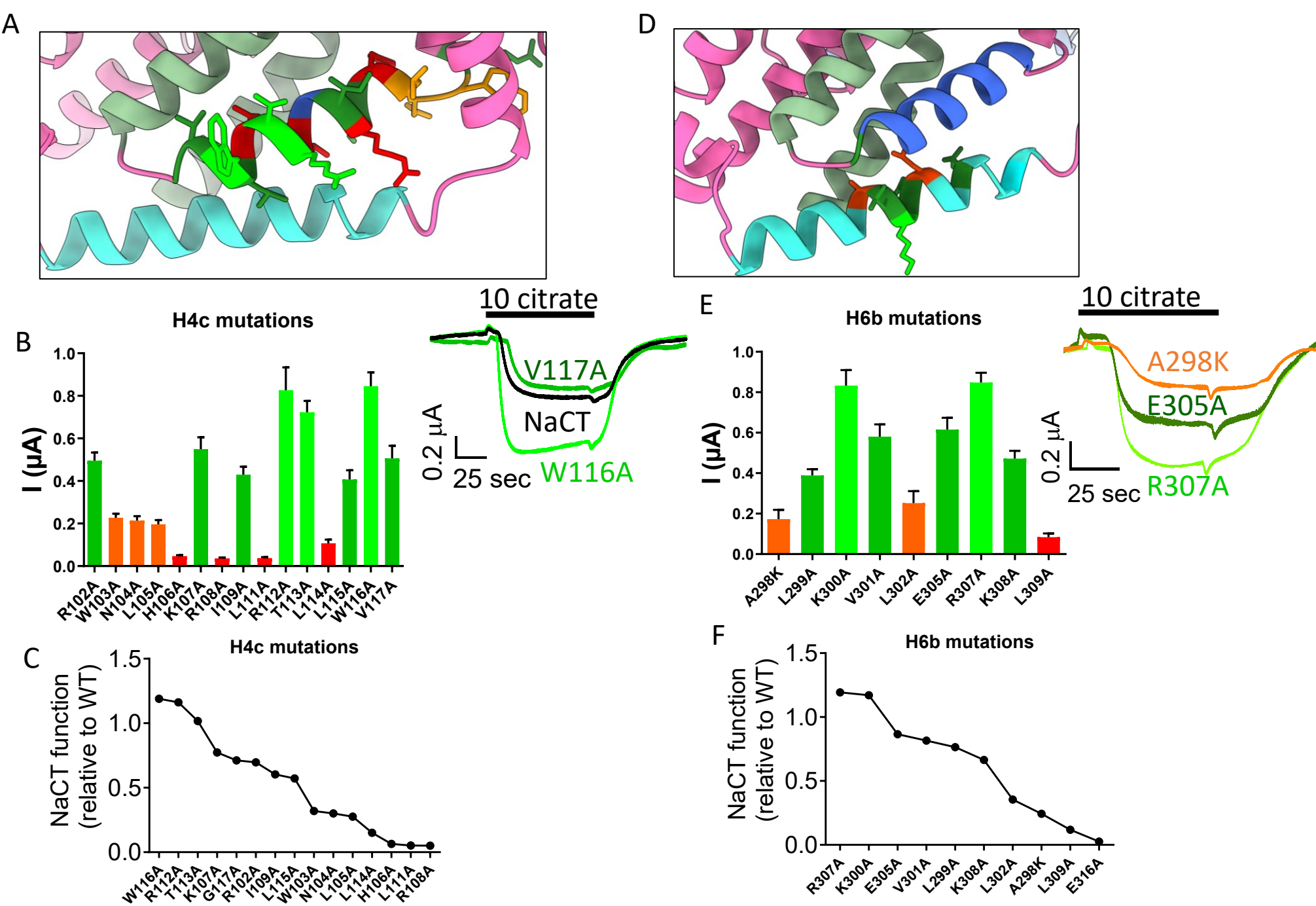


Supplementary Fig. 6: **The hepatic anion/bicarbonate/oxalate transporter, SLC26A1, inhibits NaCT-mediated citrate transport, which does not affect glycolysis regulation by NaCT.** (A) ^3H -citrate uptake was monitored in the presence or absence of Na^+ in HepG2 hepatocytes transfected with either GFP (control), NaCT, or treated with 10 μM of the human NaCT inhibitor BI01383298. (B) representative traces and (C) summary measurements of NaCT function in mammalian HEK293-T cells transfected with either an empty vector, NaCT and SLC26A1, as indicated. Real-time fluorescent measurements were performed in cells loaded with the ING-2 Na^+ sensitive dye, as described in the figure and the methods section. (D) ^3H -citrate uptake in HEK293-T cells transfected with NaCT, mSlc26a1 (mouse) or hSLC26A1 (human). Representative WB analyses of a CoIP assay showing the interaction between human NaCT and either mSlc26a1 (mA1-mouse) (E) or hSLC26A1 (hA1-human) (F). (G) Glycostress protocols performed using HEK293-T cell cultures expressing WT NaCT with or without hSLC26A1 constructs. *N* is the number of culture wells for which uptake measurements were performed. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test to determine significant differences between groups.



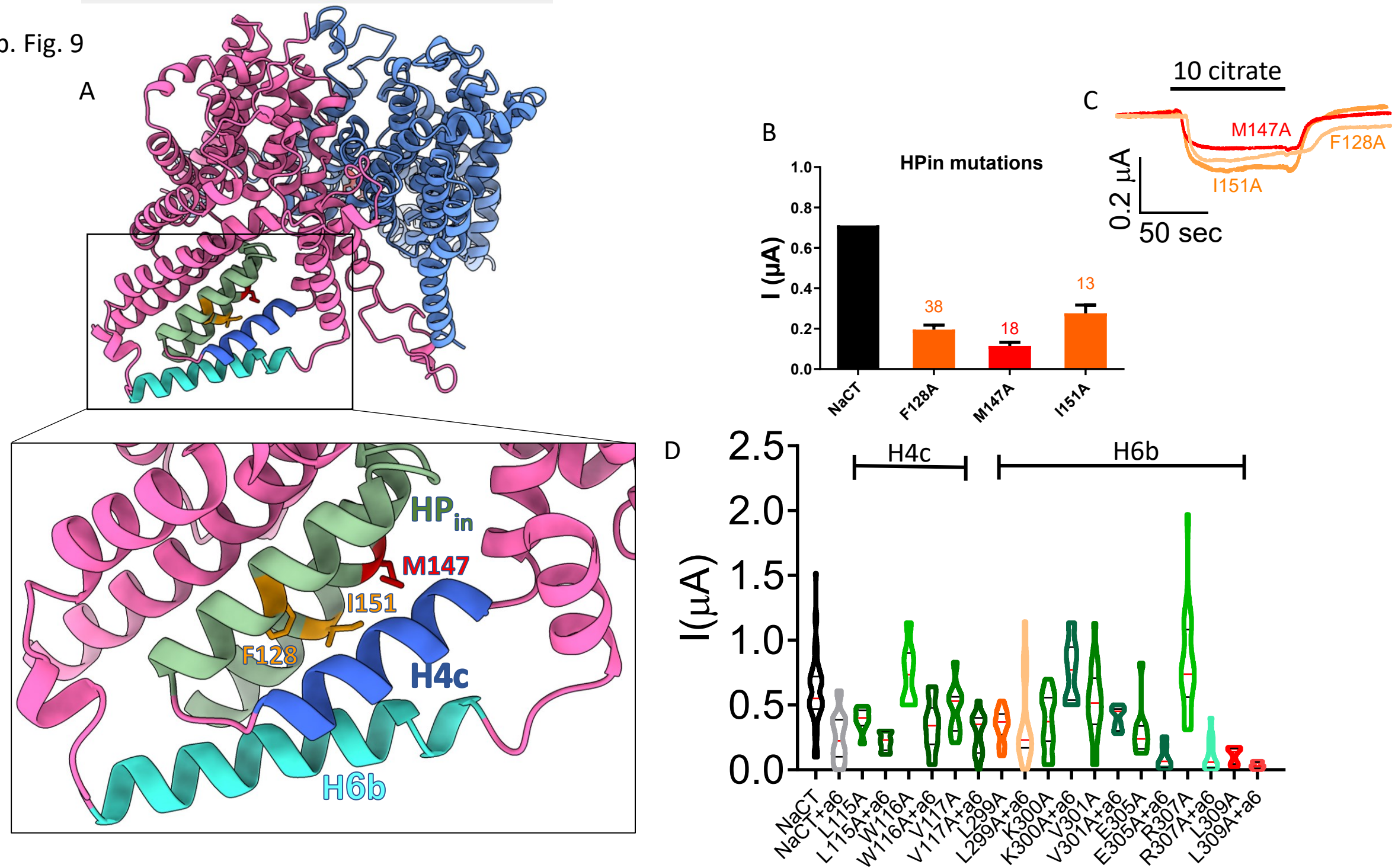
Supplementary Fig. 7. **The NaCT H4c domain R112 is predicted to interact with residues of Glut ICH4.** The Glut3 outward conformation (purple, PDB 4ZWB) and Glut1 inward conformation (yellow, PDB 4PYP) models were compared to the NaCT-Glut2 putative model (blue). The ICH region shows high dynamics, while the interaction site at ICH4 is stable to facilitate interaction with NaCT H4c R112.

Supp. Fig. 8



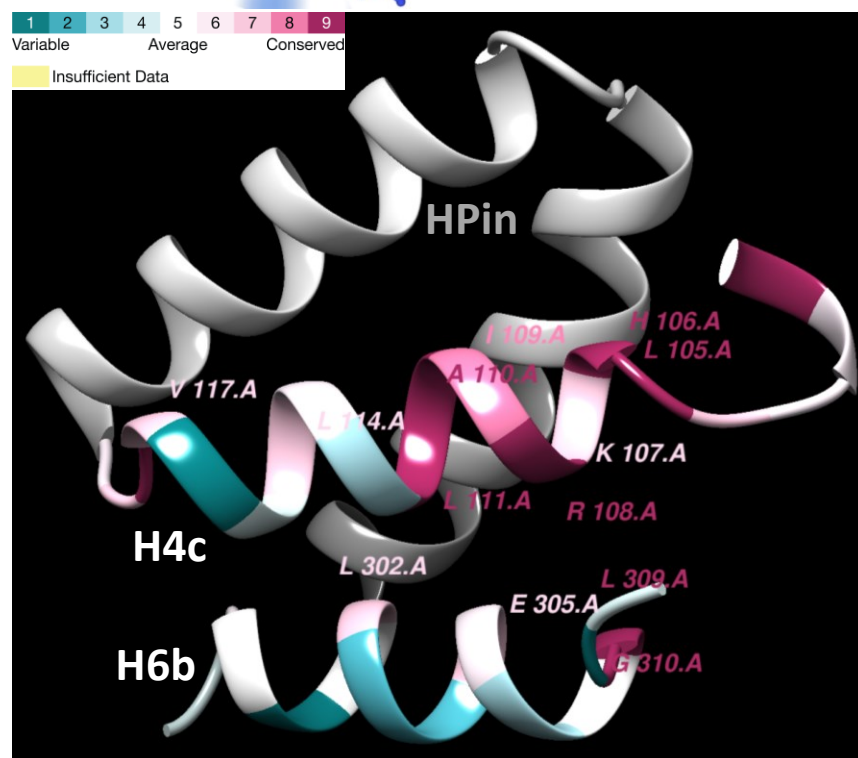
Supplementary Fig. 8: **Similar effects of mutations in H4c or H6b residues on NaCT dynamic range.** (A) Functional map and NaCT-mediated current measurements (B and C) of mutations in H4c residues. (D) Functional map and NaCT-mediated functional measurements (F and G) of mutations in H6b residues. Note: The data in Fig. 6E and F was re-plotted to generate the results in this figure. Statistics: see the statistical analysis description in the methods section.

Supp. Fig. 9



Supplementary Fig. 9: **NaCT function is significantly impaired by mutations in HP_{in} residues predicted to interact with H4c or H6b residues during the turnover cycle.** (A) Functional map of HP_{in} residues. (B) Summary and (C) representative currents of the indicated HP_{in} mutations. (D) NaCT currents are monitored for the indicated H4c and H6b mutations in the presence or absence of SLC26A6, which strongly inhibits NaCT function. Statistics: see the statistical analysis description in the methods section. Statistics: Error bars Mean $\pm$ SEM.

A



C

[illegible]

Supplementary Fig. 10: **The H4c-H6b interaction site and residue conservation.** (A) residues within the proximity of NaCT K107 residue (AlphaFold structure is shown and is identical to the NaCT cryo-EM structure). (B) Conservation analysis of H4c and H6b residues using Consurf software. (C) Multiple sequence alignment and amino acid conservation between mammalian citrate/succinate transporters of the SLC13 family (NaDC-1, NaDC-3, and NaCT).

Fig. 1B (citrate uptake)

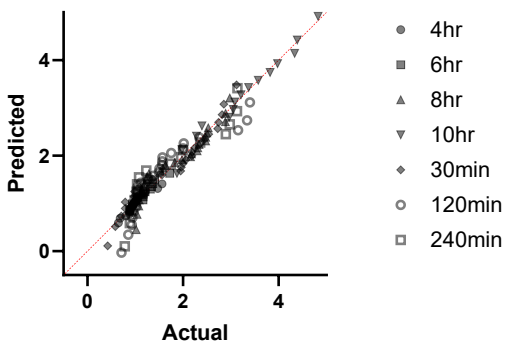


Fig. 1C (2DG uptake)

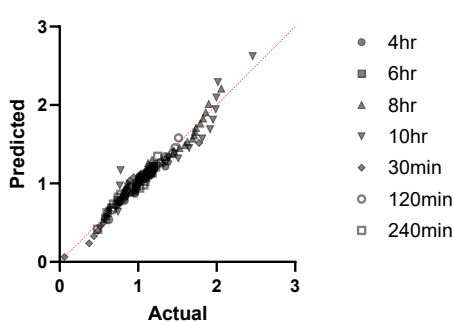


Fig. 1D (2DG uptake)

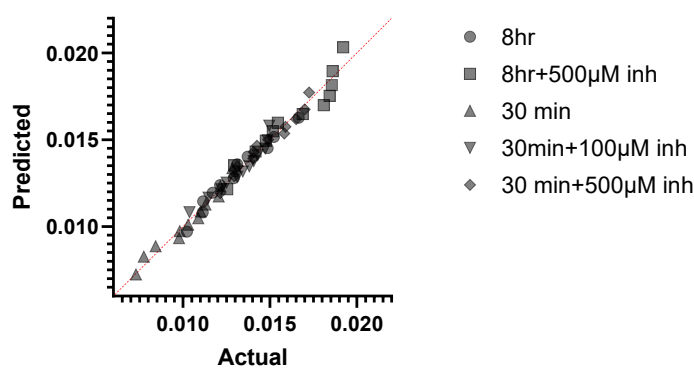


Fig. 3B

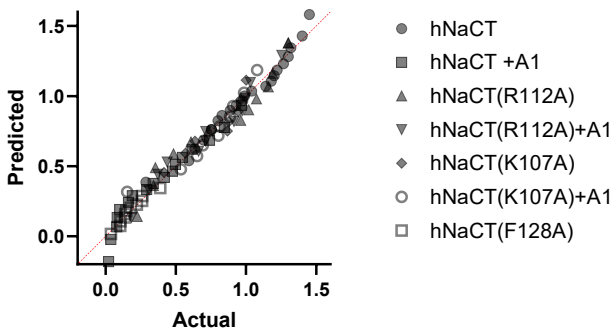


Fig. 3D

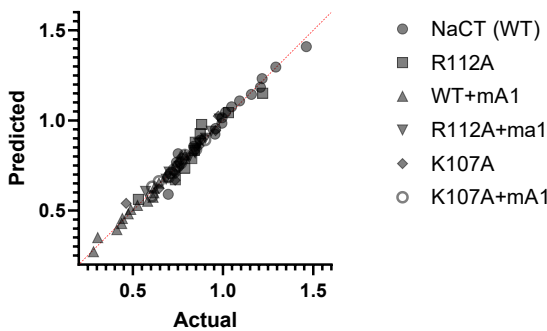


Fig. 4C (ECAR blue)

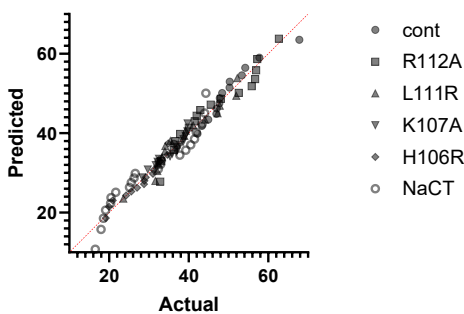
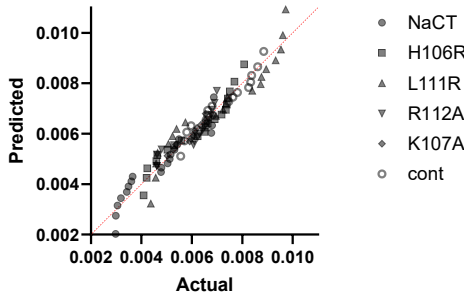
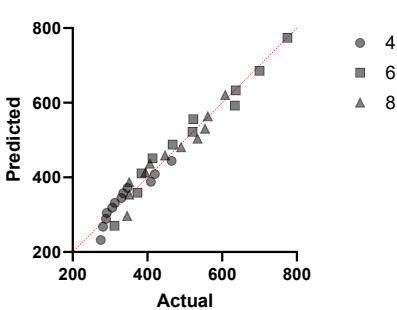


Fig. 4C (2DG uptake red)



Supp. Fig. 1C



Supp. Fig. 6C

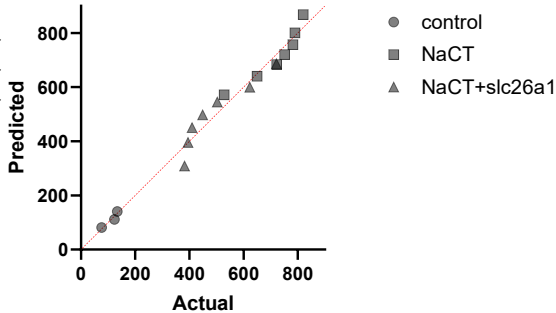


Fig. 4A

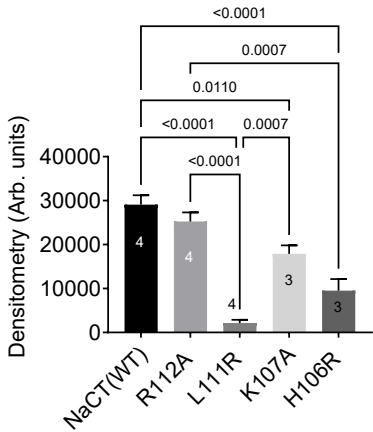


Fig. 4H

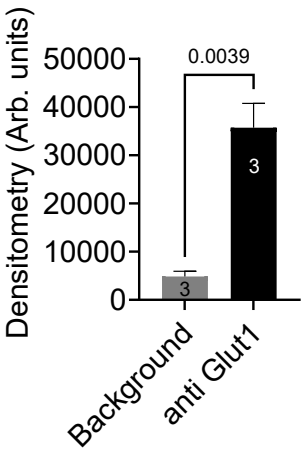


Fig. 4J

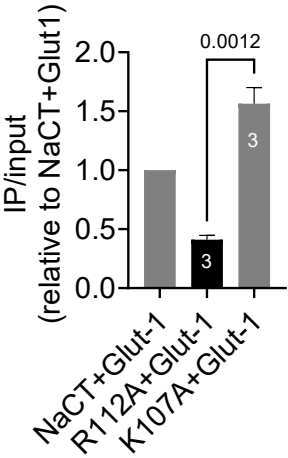
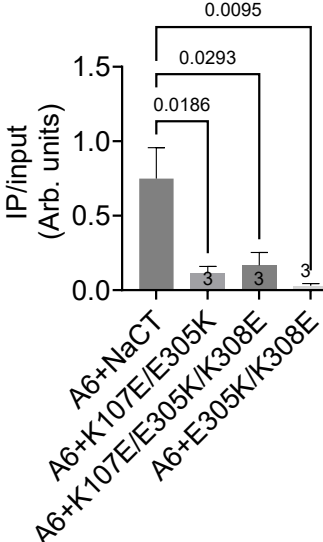
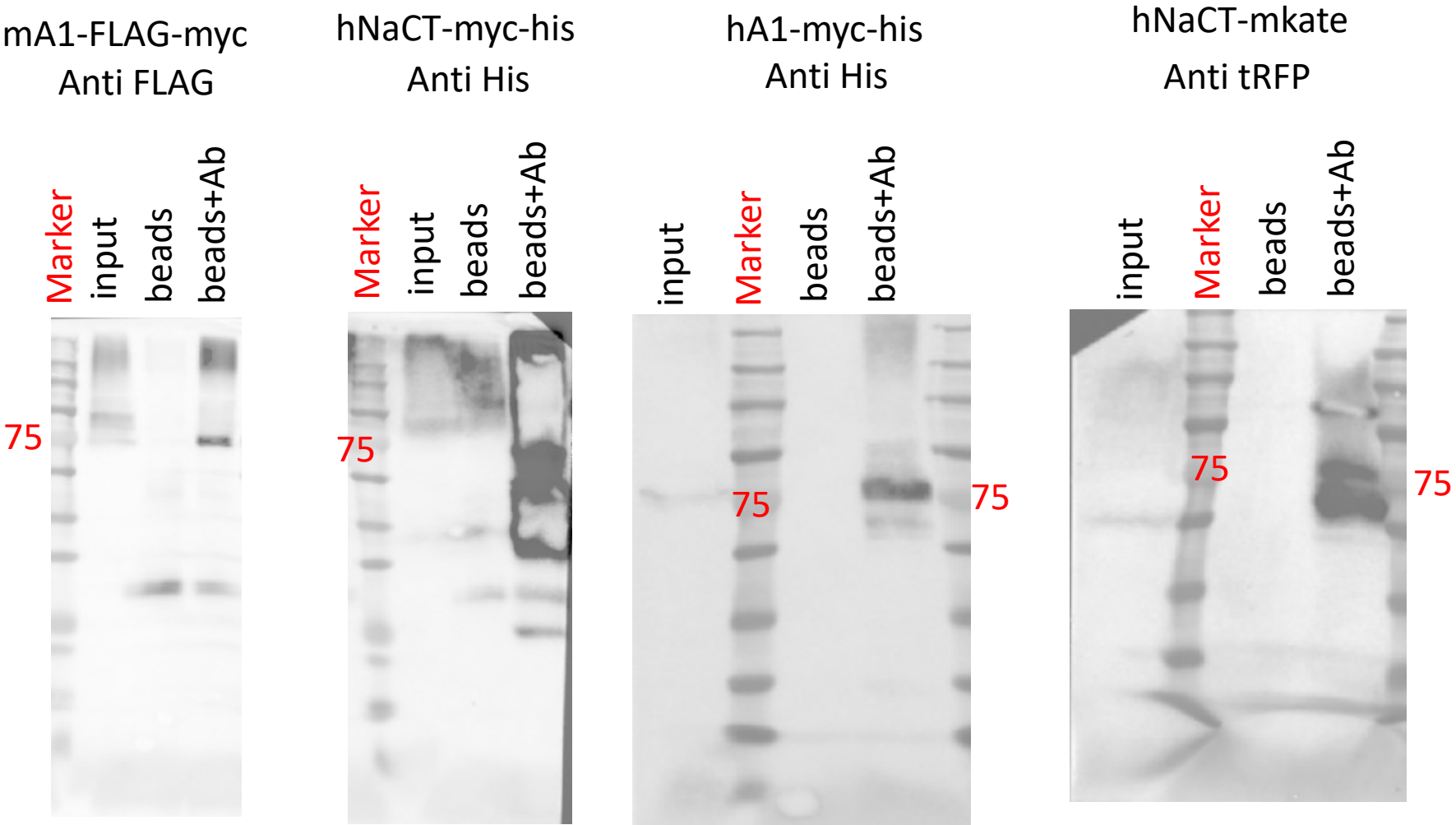


Fig. 5D



Supplementary Fig. 11: **Q-Q plots used to assess normal distribution for subsequent ANOVA multiple comparison tests and western blot densitometry analysis.** The designated figures that show the analyzed data are indicated above. N is the number of individual densitometry measurements from 3 independent experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test to determine significant differences between groups.

Supp. Fig. 12



Supplementary Fig. 12: (A) **Protein enrichments by Pull-down assays for all antibodies used in this study.**

Supplementary Table 1: Antibodies Used

Target / Tag	Supplier	Catalog #	Clone	Lot #	Dilution	Host	Application(s)	Validation Source
SLC13A5 (NaCT)	LS-Bio (CA, USA)	LS-C501192	Polyclonal	240882	1:1000 (WB)	Rabbit	WB (mouse hepatocytes)	Manufacturer datasheet / prior literature
NaCT	Santa Cruz Biotechnology	SC-293277	Monoclonal	D1625	1:500 (WB) 1:25 (IF)	Mouse	WB (biotinylation) (IF)	Manufacturer datasheet / prior literature
Calreticulin	Affinity BioReagents	PA3-900	Polyclonal	NA	1:500 (WB)	Rabbit	WB	Manufacturer datasheet
HisX6 tag	Thermo Fisher Scientific	MA1-21315	Monoclonal	WB317615	1:1000 (WB) 1:100 (Co-IP)	Mouse	WB, Co-IP	Manufacturer datasheet
tRFP	Evrogen	AB233	polyclonal	23301241269	1:3000 (WB) 1:100 (Co-IP)	Rabbit	WB, Co-IP	Manufacturer / prior publications
Glut1	Cell Signaling Technology	D3J3A	Monoclonal	12939	1:50	Rabbit	Co-IP, Immunofluorescence	Manufacturer datasheet
FLAG (HRP-conjugated)	Sigma–Aldrich	A8592	Monoclonal	SLCH6307	1:1000 (WB) 1:100 (Co-IP)	Mouse	WB, Co-IP	Manufacturer datasheet
anti-mouse IgG-HRP	Thermo Fisher Scientific	G21040	Polyclonal	2810843	1:1000	Goat	Secondary antibody	Manufacturer datasheet

anti-rabbit IgG-HRP	Thermo Fisher Scientific	G21234	polyclonal	2720402	1:2000	Goat	Secondary antibody	Manufacturer datasheet
Alexa488 anti-mouse	Jackson ImmunoResearch	715-545-151	polyclonal	132977	1:100 (IF)	Donkey (secondary)	Immunofluorescence	Manufacturer datasheet
Cy3 anti-rabbit	Jackson ImmunoResearch	711-165-152	Polyclonal	155995	1:1000 (IF)	Donkey (secondary)	Immunofluorescence	Manufacturer datasheet
Cy5 anti-rabbit	Jackson ImmunoResearch	111-605-144	Polyclonal	134014	1:100	Goat (secondary)	Immunofluorescence	Manufacturer datasheet
FLAG	Sigma–Aldrich	F1804	Monoclonal	0000308125	1:100	Mouse	IP	Manufacturer datasheet

Supplementary Table 2: Eukaryotic Cell Lines and Primary Cells

Cell Type / Species	Source / Description	Application(s)	Catalog / ID (if applicable)	Sex (if applicable)	Mycoplasma Test Status	Misidentification Status
HepG2 (human hepatoma)	ATCC	Immunofluorescence, uptake assays	HB-8065	Male	Tested negative	No
HEK293-T (human embryonic kidney)	ATCC	Transfection, imaging, Seahorse, uptake	CRL-11268	Male	Tested negative	No
THLE2 (human liver epithelial)	ATCC	Uptake and inhibition assays	CRL-2706	Male	Tested negative	No
AML-12 (mouse hepatocyte line)	ATCC	siRNA knockdown, protein analysis	CRL-2254	Male	Tested negative	No
Primary hepatocytes (C57BL/6 mouse)	Isolated in-house via collagenase perfusion	Uptake, metabolic assays	N/A	Male	Freshly isolated	No
Xenopus laevis oocytes	Lab sourced	Expression, electrophysiology	N/A	Female	Not applicable	No

Supplementary Table 3: Plasmids and Constructs

Construct Name	Accession No	Vector / Tag	Species	Description / Use Case	Notes
NaCT (WT)		pCMV6-AC + mKate or Myc-His-tag	Human	Imaging, uptake, functional assays	Commercial and site-mutated
NaCT mutants		pCMV6-AC + mKate or Myc-His-tag	Human	Structure-function studies	H4c domain mutants
SLC26A1	NM_006516.3	pcDNA3.1-C-(k)DYK (FLAG-tagged)	Human	Co-expression, protein interaction	
SLC26A6	NM_134420	pCMV6-AC-mKate	Mouse	Co-expression, imaging	
NaCT (WT) - pC4-TOPO	BC_104795	pC4-TOPO	Human	Injection to Xenopus oocytes	Used in electrophysiological assays