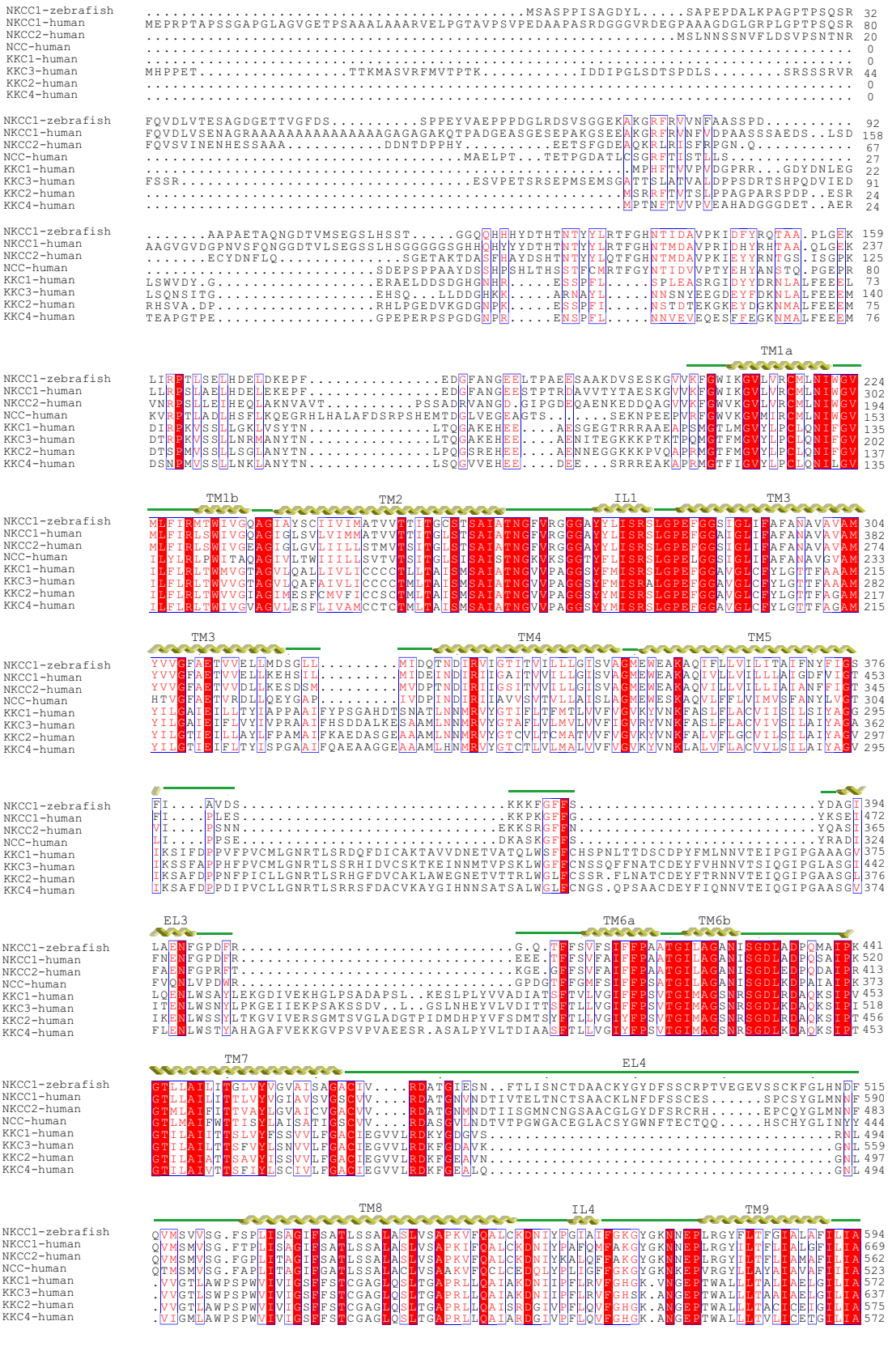
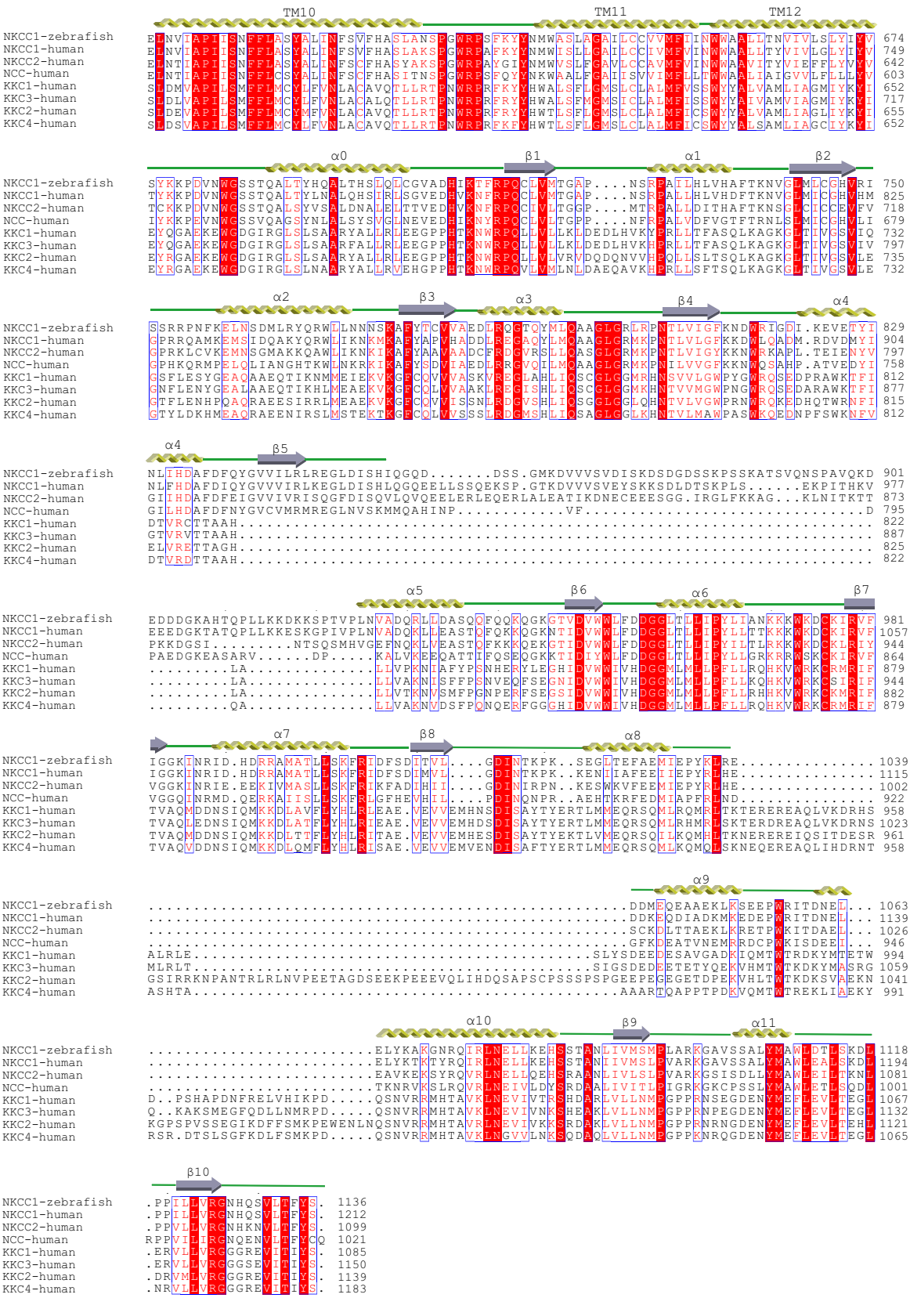
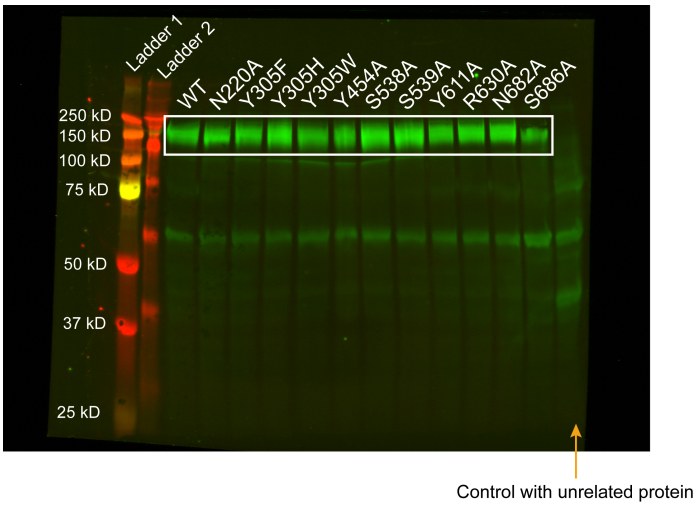

NKCC1-zebrafish
NKCC1-human
NKCC2-human
NCC-human
KKC1-human
KKC3-human
KKC2-human
KKC4-human
TM1a
TM1b
TM2
IL1
TM3
TM4
TM5
EL3
TM6a
TM6b
TM7
EL4
TM8
IL4
TM9



Supplementary Figure 1 | Sequence alignment. The protein sequences were aligned using Clustal Omega. The secondary structure elements are shown above the sequences.



Supplementary Figure 2 | Source image for gel electrophoresis in Extended Data Figure 6c.

Supplementary Table 1: Structural mapping of NCC mutations in Gitelman Syndrome [1] [40]

Human NCC Mutation	Zebrafish NKCC1	Location	References
Ion translocation pathway mutations			
R145C	R216	TM1	[65]
I154F	M225	TM1	[66]
L157P	I228	TM1	[67]
R158Q	R229	TM1	[67]
A226T	A297	TM3	[68]
H234Q	Y305	TM3	[69]
T235R	V306	TM3	[67]
S283Y	A354	TM5	[70]
P349L	P417	TM6	[71]
S350L	A418	TM6	[65]
N359K	N427	TM6	[72]
Y386C	Y454	TM7	[72]
G463E	S534	TM8	[73]
A464T	A535	TM8	[73]
K478E	K549	TM8	[74]
TM domain dimerization mutations			
L215P	F286	TM3	[68]
L542P	L613	TM10	[74]
L571P	L642	TM11	[75]
V578M	C649	TM11	[76]
Cap mutations			
A313V	K383	TM5-TM6 loop	[66]
S314F	K384	TM5-TM6 loop	[77]
G316V	G386	TM5-TM6 loop	[65]
R321W	D391	TM5-TM6 loop	[66]
R334W	R404	TM5-TM6 loop	[66]
T339I	F408	TM5-TM6 loop	[66]
R339C	R467	TM7-TM8 loop	[65]
S402F	T470	TM7-TM8 loop	[77]
N406H	S474	TM7-TM8 loop	[78]
C421R	C487	TM7-TM8 loop	[71]
N426K	D492	TM7-TM8 loop	[70]
C430G	C496	TM7-TM8 loop	[75]
G439S	G510	TM7-TM8 loop	[74]

N442S	N513	TM7-TM8 loop	[67]
TM-soluble interface mutations			
T194I	T265	TM2	[67]
G196V	G267	TM2-TM3 loop	[75]
R209Q	R280	TM2-TM3 loop	[66]
D486N	D557	TM8-TM9 loop	[71]
G496C	G567	TM8-TM9 loop	[71]
S555L	S626	TM10-TM11loop	[66]
P560H	P631	TM10-TM11 loop	[67]
N611S	N682	linker loop	[65]
G613S	G684	linker loop	[68]
S615L	S686	linker loop	[66]
L623P	Q694	C-term α0 helix	[79]
G630V	Q701	C-term α0 helix	[71]
N640S	T711	α0-β1 loop	[69]
R642G	R713	α0-β1 loop	[68]
S833T	Q941	α5-β6 loop	[67]
L858H	L966	α6 helix	[76]
R861C	N969	α5-β7 loop	[66]
T1026I	T1133	C-term	[66]
CTD dimerization mutations			
A728T	A799	α3-β4 loop	[71]
G729V	G800	α3-β4 loop	[66]
G731R	G802	α3-β4 loop	[74]
Structural mutations			
W172R	C243	TM2	[73]
G186D	G257	TM2	[74]
G264A	G335	TM4	[80]
T304P	S375	TM5	[81]
G374V	G442	TM7	[73]
P643L	P714	β1 strand	[66]
P735R	P806	α3-β4 loop	[67]
L738R	L809	β4 strand	[68]
G741R	G812	β4 strand	[82]
P751L	I822	α4 helix	[67]
L859P	I967	α6 helix	[71]
L892P	L1000	α7 helix	[69]
P947S	P1055	α9-α10 loop	[69]
Other TM mutations			
T163M	V234	TM1	[73]

S178L	A249	TM2	[66]
T180K	V251	TM2	[76]
I192T	I263	TM2	[67]
D259N	D330	TM4	[67]
R261H	R332	TM4	[68]
K284R	K355	TM5	[66]
A285G	A356	TM5	[83]
A459D	A530	TM8	[66]
C484W	C555	TM8	[65]
A523T	A594	TM9-TM10 loop	[65]
N534S	N605	TM10	[67]
A569E	A640	TM11	[76]
A570V	S641	TM11	[78]
A588V	A659V	TM12	[71]
Other CTD mutations			
T649M	T720	β1 strand	[69]
R655C	R726	α1 helix	[66]
M672I	M743	β2 strand	[84]
V677M	V748	β2 strand	[73]
R871H	R979	β7 strand	[70]
M881T	I989	α7 helix	[67]
R919C	E1027	α8 helix	[68]
R928C	K1036	α8-α9 loop	[70]

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