A combined computational and functional approach identifies new residues involved in pH-dependent gating of ASIC1a

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Supplemental Table S1: Analysis of the quality of homology models

Model	Cα RMSD with the template (Å) (sequence identity %)	Number of residues in favorable regions of the Ramachandran plot	Number of residues in the generously and additionally allowed regions of the Ramachandran plot	Number of residues in the disallowed regions of the Ramachandran plot
Model based on 2QTS template	0.5 (90%)	1048 (92.4%)	84 (7.5%)	2 (0.2%)
Model based on 3HGC template	0.4 (90%)	992 (91.1%)	95 (8.7%)	2 (0.2%)

The table indicates the parameters determined by PROCHECK (1) of the two homology models.

1. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) *J. Appl. Cryst.* **26**, 283-291

Supplemental Table S2. pKa values and accessible surface area of Asp, Glu and His residues

		unprotonat	ted models	stepwise	accessible
				protonation	surface area
residue	position	2QTS	3HGC	2QTS	%
E63	1	18.35	19.97	-3.81	21
	2	19.77	18.22	р	
H70		1.86	4.50	-2.48	33
H72		4.08	0.17	3.85	20
H73		5.14	5.26	4.65	13
D78	1	2.11	9.98	-5.79	11
	2	8.42	2.61	р	
E79	1	10.69	12.36	р	1
	2	6.30	11.62	2.10	
E97	1	11.03	11.43	3.88	2
	2	10.94	11.54	р	
D107	1	3.65	5.09	2.81	0
	2	0.57	3.08	-0.45	
H110		4.62	4.56	4.63	10
E113	1	6.43	4.89	6.34	14
	2	6.17	2.72	6.07	
E123	1	3.76	4.51	3.76	31
	2	3.78	1.79	3.80	
D126	1	3.78	3.29	3.80	43
	2	3.79	4.23	3.80	
D131	1	4.01	3.47	4.00	34
	2	4.05	3.42	4.05	
E132	1	3.81	4.33	3.80	39
	2	3.79	4.35	3.80	
E136	1	5.39	4.69	5.53	33
_	2	5.07	4.71	5.04	
D140	1	4.38	4.30	4.94	32
	2	5.17	4.25	4.51	
E156	1	4.44	6.18	4.18	23
	2	5.01	4.46	4.91	
D159	1	1.63	1.13	1.22	20
	2	3.70	2.70	3.50	
H163	- <u> </u>	2.95	-2.57	-0.15	0
D164	1	-0.82	-1.04	-1.49	10
	2	5.59	4.94	5.48	
D167	1	3.74	3.85	2.85	23
	2	3.71	3.70	2.87	
H1/3		4.93	5.63	4.52	20
E1//	1	8.93	3.81	2.46	24
F 4 9 6	2	10.59	3.56	p	
E182	1	4.34	3.61	4.13	44
D 400	2	4.42	4.02	4.13	
183	1	5.12	4.78	5.11	14
	2	3.00	4.78	4.75	

Supplemental Table S2, page 2

		unprotonat	ted models	stepwise	accessible
				protonation	surface area
residue	position	2QTS	3HGC	2QTS	%
D202	1	4.58	3.93	4.56	31
	2	4.25	3.93	4.78	
D212	1	6.18	4.72	3.93	0
	2	5.87	6.34	3.78	
E219	1	7.46	7.25	4.87	18
	2	9.43	8.32	4.90	
D223	1	6.91	5.65	5.46	0
	2	5.73	5.76	5.45	
D227	1	1.54	3.34	-0.85	33
	2	5.23	0.47	3.72	
E228	1	7.50	6.47	7.39	6
	2	5.25	4.17	5.13	
E235	1	5.30	3.54	5.20	29
	2	1.84	-1.01	1.86	
D237	1	8.50	6.07	2.26	21
	2	10.06	7.49	-1.20	
E238	1	12.95	6.23	1.14	2
	2	16.76	13.21	р	
E242	1	6.53	6.46	2.64	4
	2	8.35	6.81	р	
H250		4.49	2.30	3.34	2
D253	1	5.98	4.09	5.08	28
	2	5.11	4.08	5.03	
E254	1	5.95	2.86	5.83	4
	2	5.95	5.07	5.89	
D259	1	4.23	4.13	4.08	13
	2	4.27	3.27	4.13	
E277	1	6.97	9.61	3.05	1
	2	8.51	9.12	р	
D296	1	3.94	8.53	3.93	41
	2	3.94	6.28	3.94	
D298	1	4.54	8.33	3.49	42
	2	5.02	6.77	4.99	
D300	1	4.12	6.84	4.10	36
	2	4.18	10.18	4.20	
D303	1	4.14	6.90	3.94	32
	2	5.62	5.51	5.63	
D313	1	5.73	12.32	2.77	6
	2	5.04	16.96	4.60	
E315	1	12.13	9.09	р	1
	2	14.38	9.34	6.41	
E321	1	5.09	4.25	4.99	43
	2	5.15	4.25	5.05	
H329		0.33	0.84	-1.52	9

Supplemental Table S2, page 3

			pKa values	5	
		unprotonat	ted models	stepwise	accessible
				protonation	surface area
residue	position	2QTS	3HGC	2QTS	%
D333	1	3.78	4.21	3.79	55
	2	3.79	4.26	3.79	
E340	1	4.87	3.55	4.60	25
	2	5.02	3.55	4.63	
E344	1	6.09	4.19	5.93	41
	2	4.78	4.19	5.49	
D347	1	12.00	8.90	6.35	2
	2	15.48	8.04	6.03	
D351	1	7.61	3.67	3.92	9
	2	10.64	7.27	р	
E355	1	6.46	5.20	5.88	14
	2	5.78	6.68	5.44	
D357	1	1.55	7.60	-0.11	0
	2	2.41	2.38	-1.14	
E359	1	4.30	4.36	4.28	42
	2	4.30	4.36	4.28	
E364	1	4.73	5.67	4.66	26
	2	4.73	5.47	4.69	
E375	1	5.87	7.53	4.53	7
	2	5.47	5.53	4.20	
E398	1	4.65	4.48	4.58	24
	2	3.36	1.33	3.31	
E403	1	3.96	-0.48	3.57	11
	2	1.79	4.37	1.52	
D409	1	10.45	10.08	1.15	0
	2	11.58	10.44	р	
E413	1	7.15	1.54	6.23	5
	2	7.05	5.10	6.40	
E418	1	8.76	10.80	6.97	5
	2	8.76	10.30	7.21	
E421	1	4.38	3.42	4.17	15
	2	3.49	4.30	3.25	
E427	1	4.16	7.38	3.90	26
	2	4.16	3.59	3.88	
D434	1	24.64	43.92	р	18
	2	15.44	36.43	-7.02	

pKa values were calculated as described in the text, for the two oxygen atoms of each Asp and Glu; His residues have 1 pKa value. Stepwise protonation, as described in the text; residues marked "p" were protonated in the model, and the pKa of the remaining residues was re-calculated. Iterations of protonation and re-calculation of pKa values of remaining, non-protonated side chains were done until all remaining side chains had a pKa < 5. For residues with pKa between 5 and 8, the pKa calculated in the round just before their own protonation is indicated.

Accessible surface area was calculated from the 2QTS-based model by using the the COOR SEARCH function implemented in the CHARMM c34 molecular mechanics software. The relative accessible surface of acidic residues is shown as percentage of the surface of the isolated residue.

Supplemental Table S3

		distance	relative orientation
residue1	residue2	0-0* (Å)	
E79	E418	5.56	towards each other
E97	E238	5.15	same orientation, 97 behind 238
E136	D140	4.03	~parallel
D164	D167	4.58	~parallel
E219	D409	2.95	on two anti-parallel beta-sheets, ~parallel
D227	E228	5.05	at ~70° Angle
E355	D351	3.73	parallel
D357	E359	6.85	similar orientation, 357 behind 359
E375	E413	4.96	towards each other, angle 120°

Distance and relative orientation of simple pairs of Asp and Glu residues

*, distances were calculated in Chimera between the closest side-chain oxygen residues of the two acidic side chains from the human ASIC1a model based on the chicken ASIC1 structure 2QTS.

Supplemental Figure S1

		* 180 *		200	*	220	*	240	
hASICla	:			MELKAEEEEVGGV-		-QPVSIQAFAS	3		: 23
cASIC1	:		M	MDLKVDEEEVDSG-		-QPVSIQAFAS	3		: 24
rASIC1b	:			MEAGSELDEGDD		-SPRDLVAFAN	1		: 22
hASIC2a	:			MDLKESPSEGS		LQPSSIQIFAN	1		: 22
hASIC3	:			MKPTSGPEEARR		-PASDIRVFAS	3		: 22
rASIC2b	:	MS	RSGG	ARLPATALSGPGRFI	RMAR	EQPAPVAVAAA	RQPGGDR	SGDPA	: 47
hASIC4	:	GLLAREGQGREALASPSSRGQMPIEIVCKI	KFAE:	EDAKPKEK <mark>E</mark> AGDEQ:	SLLGAVAPG.	AAPRDLATFAS	3		: 174
hENaC-alph	:	MEGNKLEEQDSSPPQSTPGLMKGNKR	EEQG	LGPEPAAPQQPTAE	EEALIEFHR	SYRELFEFFCN	1		: 64
MEC4_C.ele	:	PYKASLATSVDLVKRTLSAFDGAMGKAGGN	KDHE	EEREVVTEPPTTPA	PTTKPARRR	GKRDLSGA <mark>F</mark> FE	PG		: 230
PPK-dros.m	:	IREDEEEKKSGISILPGPELLALPGFDTRA	SIAS	AALSDVPSDVIIKS	RIRYGSPLS	ACKGL <mark>LE</mark> YAK	[: 71
						a			
				TM1		රි	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	?	
		* 260 *		280			*	320	
hASICla	:	SSTLH <mark>G</mark> LAHIFSYER	LS	LKRALWALCFLG	SL <mark>AVLL</mark> CVC'	IERVQYYFHYH	IHVTKLDE	VAASQ	: 84
cASIC1	:	SSTLH <mark>G</mark> ISHIFSYER	LS	LKRVVWALCFMG	SL <mark>ALLALVC</mark> '	INRIQYYFLYP	HVTKLDE	VAATR	: 85
rASIC1b	:	SCTLH <mark>G</mark> ASHVFVEGG	PG	PRQALWAVAFVI	ALGAFLCQV	GDRVAYYLSYP	HVTLLDE	VATSE	: 83
hASIC2a	:	TSTLH <mark>G</mark> IRHIFVYGP	LT	IRRVLWAVAFVG	SL <mark>GLLLVES</mark>	SERVSYYFSYQ	₽UTKVDE	VVAQS	: 83
hASIC3	:	NCSMHGLGHVFGPGS	LS	LRRGMWAAAVVL	SV <mark>ATFL</mark> YQV.	AERVRYYREFH	IHQTAL DE	RESHR	: 83
rASIC2b	:	LQGPGVARRGRPSLSRTKLHGLRHMCAGRT	AAGG	SFQRRALWVLAFCT	SL <mark>GLLL</mark> SWS	SNRLLYWLSFP	SHTRVHR	EWSRQ	: 127
hASIC4	:	TSTLH <mark>G</mark> LGRACGPGP	HG	LRRTLWALALLT:	SL <mark>AAFL</mark> YQA	AGLARGYLTRP	HLVAMDP.	AAPAP	: 235
hENaC-alph	:	NTTIH <mark>G</mark> AIRLVCSQH	NR	MKTAFWAVLWLC	TFGMMYWQF	GLLFGEYFSYP	VSLNIN-	-LNSD	: 123
MEC4_C.ele	:	FARCLCGSQ <mark>G</mark> SSEQEDKDE	EKEE	ELLETTTKKVFNINI	DADEEWDGM	E <mark>E</mark> YDNEHYENY	DVEATTG	MNMME	: 299
PPK-dros.m	:	STTIH <mark>G</mark> IRYIFEVHR	PI	YEKLY <mark>W</mark> LFFTCI	sv <mark>yfav</mark> sli	W <mark>D</mark> TYLKWQESP	VILGF <mark>DE</mark>	LVPV	: 132
		hG		W					
		\$						No.	
		* 340 *	_	360	*	380	*	400	
hASICla	:	LTFPAVTLCNLNEFRFSQ	VSKN	DI	LYHAG			- <mark>E</mark> LLA	: 116
cASIC1	:	LTFPAVTFCNLNEFRFSR	VTKN	DI	LYHAG			- <mark>E</mark> LLA	: 117
rASIC1b	:	LVFPAVTFCNTNAVRLSQ	LSYP	DI	LLYLA			-PMLG	: 115
hASIC2a	:	LVFPAVTLCNLNGFRFSR	LTTN	DI	LYHAG			- <mark>E</mark> LLA	: 115
hASIC3	:	LIFPAVTLCNINPLRRSR	LTPN	DI	LHWAGS			- <mark>A</mark> LLG	: 116
rASIC2b	:	LPFPAVTVCNNNPLRFPR	LSKG	DI	LYYAG			-HWLG	: 159
hASIC4	:	VAGFPAVTLCNINRFRHSA	LSDA	Di	IFHLA			-NLTG	: 268
hENaC-alph	:	KLVFPAVTICTLNPYRYPE	IKEE:	LEELDRITEQTLFD	LYKYS			-SFTT	: 169
MEC4_C.ele	:	ECQSERTKFDEPTGFDDRCICAFDRSTHDA	WPCF:	LNGTWETTECDTCN	EHAFCTKDN	KTAKGHRSPCI	CAPSRFC	VAYNG	: 379
PPK-dros.m	:	HKIPFPTITICPEIKMERNV	FDYTI	NVSRQLWE	EYKQNG			-NISD	: 174
		Fpat C r							
		× * * * * * * * * * * * * * * * * * * *							
		* 420 *	_	440	*	460	*	480	
hASICla	:	LLNNRYEIPDTQMADEKQLEILQ	D	KANFRSFKP-KPFN					: 153
cASIC1	:	LLNNRYEIPDTQTADEKQIEILQ	D	KANFRNFKP-KPFN					: 154
rASICID	:	LDESDDPGVPLAP	P	GPEAFSG-EPFN					: 140
hASIC2a	:	LLDVNLQIPDPHLADPSVLEALR	Q	KANFKHYKP-KQFS					: 152
hASIC3	:	DPAEHAAF RALG	R.	PPAPPGFMPSPTFD	~				: 145
rASIC2D	:	LLEPNRTARPLVSELLRGDEPRROWFR	K.	LADERLELLPPRHFE(G				: 202
hASIC4	:		Y.	PEPD					: 288
neNaC-aiph	:	VAGSRSRRDLRGTLPHPLQRLR	V.	PPPPHGARRARSVA;	SSLRDNNPQ	VDWKDWKIGFQ	DCNQNKS	DCFYQ	: 239
MEC4_C.ele	:	KTPPIEIWTYLQGGTPTEDPNFLEAMG	F(QGMTDEVALVTKAKI	EN				: 423
PPK-dros.m	:	DDEDLARMAVAMHICDSEVVQRFTPLL	SQLN.	PPNVDVTQTLIDLS.	ISKNETGPF	CKWNG			: 234
		1 d		N	1 0				
		* 500 *		<u>б</u> соо 🔨	× ×	F 4 0	+	FCO	
hagral-		~ 5UU *					" TENGADO		
mASICIA	•	MREFYD	RAGH		VCSAE-DEK	VVFT-RYGKCY		JKPKL	· 20/
CASICI	:	MLEFYD			CON NEW	VVFI-RIGKCY		JAPK	· 208
LASICID	•	LHRFYN	KSCH.	N JEDMILLICSNCGG	FCGPH-NFS	VVFI-RYGKCY	MENCORD	JKPKL	· 194
hasicza	:					TVFI-KIGKCY	TENCOND	JAPL	· 200
MADICS	:	TO A DATE			COUL NET	TIFI-RMGKCY	MENCORD	JALL	· 199
LASICZD	:	ISAAFMD	DIDGH		LCGPH-NES	SVFI-RIGKCY		JAPL	· 25/
HASIC4	•		TEEH		CONOR NES	VVII-RIGKCY	TEN-AD.	CNIL HIM	· 340
MECA C al-	:	IIBBGVUAVKEWIKFHIINILSKLPETLPS	LEED	TUGNE LEACREINQV		THIP TO A THE PARTY OF THE PART			· JUL
MEC4_C.ere	:		TENOT			INTUPAPGSCF	TEMHNKT.		· 405
FFK-ULOS.M	•	KFIFCDKIFDFVAIDEGICYQ	r NGL				fn fn	UDIGF	· 305
			±	c o g	C TO	y c	L 1 1		

		A A		. S.	COO	640	
hASICla :	KTMKDGTGNGLE I	✓ 580✓ MLDIQODEYLPVWG	ETDETSFEAGIK	VOIHSODEPPFIL	620 QLGFGVAP <mark>G</mark> FOTF	640 VACQ	: 276
cASIC1 :	ITMKGGTGNGLEI	ML <mark>D</mark> IQQ <mark>DE</mark> YLPVWG-·	<mark>ETDETSFE</mark> AGI <mark>K</mark>	VQIHSQ <mark>D</mark> EPPLII	QLGF <mark>GVA</mark> P <mark>G</mark> FQTF	VSCQ	: 277
rASIC1b :	KTMKGGTGNGLEI	MLDIQQDEYLPVWG	ETDETSFEAGIK	VQIHSQDEPPFII	QLGFGVAPGFQTF	VSCQ	: 263 · 275
hASIC2a :	TTTRGGMGNGLEL	MLDIQQDEYLPIWG	DNEETPFEAGVK	VQIHSQSEPPFIQ	OLGIGVSPGYOTE	VATQ	· 2/5
rASIC2b :	TTVKGGTGNGLEI	MLDIOODEYLPIWG-	ETEETTFEAGVK	VOIHSOSEPPFIC	ELGFGVAPGFOTF	VATO	: 326
hASIC4 :	PSRAGGMGSGLEI	ML <mark>DIQQ</mark> EEYLPIWR-·	<mark>ETNETSFE</mark> AGIR	VQIHSQEEPPYIH	IQLGFGV <mark>S</mark> P G FQTF	vscõ	: 409
hENaC-alph :	SSMPG-INNGLSL	ML <mark>RAEQND</mark> FIPLLS-	<mark>TVT</mark> GAR	VMVHGQDE PAFMI	DGGFNLRPGVETS	ISMR	: 380
MEC4_C.ele :	TSIRAGPMYGLRM	LVYVNASDYMPTTE-·		NEINSDE SVDI TT	TFGYSAPTGYVSS	FGLR	: 547 · 295
PPR-dros.m ·	d dl d	q 5 p	f q 4	6 6hsq epp	g pG	VSVLPAIVVS	• 303
	<u>^</u>	1 1	<u>_</u> ~	1 S	A		
	√ *	660	* 680	* 2	? 00 *	720	
hASICla :	EQRLIYLPPPWGT	KAVTMDSD	LDFFDSYSI	TACRIDCETRYLV	ENCNCRMVHMPG-	-DAPYCTPEQ	: 341 · 240
rASIC1 :	EORLIYLPSPWGD	CNAVTMDS	DFFDSYST	TACRIDCETRIL	ENCNCRMVHMPG-	-DAPICIPEQ	· 340 : 326
hASIC2a :	EQRLTYLPPPWGE	CRSSEMGL	DFFPVYSI	TACRIDCETRYIV	ENCNCRMVHMPG-	-DAPF <mark>C</mark> TPEQ	: 338
hASIC3 :	QQQ <mark>L</mark> SFLPPPWGD	C <mark>SSASLNPNYEPEPS</mark> I	DPLGSPSPSPS <mark>PS</mark> PPYTL	MG <mark>CRLACE</mark> TRYVA	RKCGCRMVYMPG-	-DVPV <mark>C</mark> SPQQ	: 346
rASIC2b :	EQRLTYLPPPWGE	CRSSEMGL	DFFPVYSI	TACRIDCETRYIV	ENCNCRMVHMPG-	-DAPFCTPEQ	: 389
hENaC-alph :	EQRETYLPQPWGN	CRAESELREPE	LQGYSAYSV I.VDSKVTC	OVCINCEREE	QRCHCRMVHMPG-	ONVEYODYRK	: 4/6 : 449
MEC4 C.ele :	LRKMSRLPAPYGD	CVPDGKTSDY	IYSNYEYSV	EGCYRSCFOOLVI	KECRCGDPRFPVP	ENARHCDAAD	: 615
PPK-dros.m :	TONLHEITPEKRQ	CLFDDERS	LRFF <mark>R</mark> SYSQ	SNCQTECLANYT	SKCGCAKFWMPKP	lgtpv <mark>c</mark> glkd	: 451
	б бррд	C	¥3	C C	CC mP	C	
	3 ⁴ * *		* 760	*	780 *	800	
hASIC1a :	YKEC	ADPALDFLVEKD	DEYCVCEMPCNLTRYG	KELSMVKIPSKAS	AKYLAKKFNKSEQ	YIGENI	: 405
cASIC1 :	YKEC	ADPALDFLVEKDI	JEYCV <mark>C</mark> EMP <mark>C</mark> NVTR <mark>Y</mark> G	KELSMV <mark>K</mark> IPSKAS	akyl <mark>akkyn</mark> kseq	YIGENI	: 404
rASIC1b :	YKEC	ADPALDFLVEKD	QEYCVCEMPCNLTR <mark>Y</mark> G	KELSMVKIPSKAS	AKYL <mark>AKKFN</mark> KSEQ	YIGENI	: 390
hASIC2a :		AEPALGLLAEKD	SNYCLORTPONLIRYN	KELSMVKIPSKTS	AKYLEKKFNKSEK	YISENI	: 402
rASIC2b :	HKEC	AEPALGLLAEKD	SCACPNPCASIRIA SNYCLCRTPCNLTRYN	KELSMVKIPSKAP IKELSMVKIPSKTS	AKYLEKKENKSEK	YISENI	· 408
hASIC4 :	YIEC	ADHTLDSLGGGPI	EGPCF <mark>C</mark> PTP <mark>C</mark> NLTR <mark>Y</mark> G	KEISMV <mark>R</mark> IP <mark>NRG</mark> S	ARYL <mark>ARKYN</mark> R <mark>NET</mark>	YIRENF	: 540
hENaC-alph :	HSSWG	YCYYKLQVDFSSDHLO	GCFTK <mark>C</mark> RKP <mark>C</mark> SVTS <mark>Y</mark> Q	LSAGYS <mark>RWPSVT</mark> S	QEWV <mark>FQMLS</mark> RQNN	YTVNNKRNGV	: 521
MEC4_C.ele :	PIAR	KCLDARMNDLGGLHG	SFRCRCQQPCRQSIYS	SVTYSPAKWPSLSI	QIQ GSCNGTAVE	CNKHYKEN-G	: 685
PPR-dros.m ·			Y DG DAGMONGNEISLENN	es 4 ps	IRAFREEIEHI-D.	vi n	• 525
	3 2 2		TM2			1	
	* * *	820		*	860 *	880	
hASICIa :	LVLDIFFEVLNYE	FIEQKKAYEIAGLLG	DIGGQMGLFIGASILT	VLELFDYAYEVIK	HKLCRRGKCQKE-	AKRSSA	· 480
rASIC1 :	LVLDIFFEMLNYE	TIEOKKAYEIAGLLG	DIGGOMGLFIGASILI	VLELFDIAIEVIK	HRLCRRGKCOKE-	AKRSSA	· 465
hASIC2a :	LVLDIFFEALNYE	TIEQKKAYEV <mark>AALL</mark> G	DIGGQMGLFIGASILT	ILELFDY IY ELIK	EKLLDLLGKE-	EDEGSH	: 475
hASIC3 :	LA <mark>LDIFFE</mark> ALNYE	TVEQKKAYEM <mark>SE</mark> LLG	D <mark>IGGQMGLFIG</mark> ASLLT	ILEILDY <mark>LC</mark> EVFF	DKVLGYFWNRQH-	SQRHSS	: 483
rASIC2b :	LVLDIFFEALNYE	TIEQKKAYEVAALLG	DIGGQMGLFIGASLLT	ILELFDYIYELIK	EKLLDLLGKE-	EEEGSH	: 526
hENaC-alph :	AKVNIFFKELNYK	AMEORAA IGLSALLG TNSESPSVTMVTLLS	JLGGOMGLFIGASILI JLGSOWSLWEGSSVLS	VVEMAELVEDIJA	TMFLMLLRRFRSR	YWSPGRGGRG	· 615
MEC4_C.ele :	AMVEVFYEQLNFE	MLTESEAYGFVNLLA	D <mark>F</mark> GGQLGLW <mark>C</mark> GISFLT	CCEFVFLFLETAY	MSAEHNYSLYKK-	KKAEK	: 759
PPK-dros.m :	L <mark>SVYF</mark> KEHQFT	AIKRTILFGVST <mark>LI</mark> S	1 <mark>CGGICGLFMGISC</mark> LS	FLELIYFFCMRIC	GSCRDRRKHKIQ-	QQNSV	: 597
	6 655e ln e	a 161	L Ggq gL5 G S L3	E e			
	*	900	* 920	*	940		
hASICla :	DKGVALSLDDVKR	HNPCESLRGHPA	MTYAANILPHHPARG	TFEDFTC	:	528	
cASIC1 :	DKGVALSMDDVKR	HNPCESLRGHPA	GMTYAANILPHHPARG	TFEDFTC	:	527	
rASICID :	DKGVALSLDDVKR	HNPCESLRGHPA	JMIYAANILPHHPARG	TFEDFTC	:	513 512	
hASIC3 :	TNLLOEGLGSHRT	OVPHLSLGPRPP'	TPPCAVTKTLSASHRT	CYLVTOL		531	
rASIC2b :	DENMST	CDTMPNHSE	TISHTVN-VPLQTALG	TLEEIAC	:	563	
hASIC4 :	GGISTLGLQELKE	QSPCPSRGRVEGGGV	SSLLPNHHHPHGPPGG	LFEDFAC	:	666	
hENaC-alph :	AQEVASTLASSPP	SHFCPHPMSLSLSQP	GPAPSPALTAPPPAYA	TLGPRPSPGGSAG	ASSSTCPLGGP :	669 769	
PPK-dros m :	DLPEENSEN					606	

Supplemental Figure S1. Alignment of ASICs and other family members. Alignment of ASIC1-4 and ENaC α (human), MEC-4 (C. elegans) and Pickpocket (PPK, drosophila), indicating Glu and Asp residues with pKa > 8 in blue, with 8 > pKa > 5 in red. Numbers of the amino acid residue in hASIC1a is indicated above the columns and is framed if the residue is conserved within the pH-gated ASIC subunits (ASIC1a, -1b, 2a and 3). GenBank accession numbers are AAB48981.1 (hASIC1a), AAY28986 (cASIC1), EDL86974 (rASIC1b), AAH75042 (hASIC2a), AAC64188 (hASIC3), EDM05432 (rASIC2b), EAW70762 (hASIC4), BAG65217 (ENaC- α), AAC47265 (MEC-4), and AAF53394 (PPK).

Supplemental Figure S2. Absence of window current in the E413Q/E418Q double mutant



Supplemental Figure S2. Absence of window current in the E413Q/E418Q double mutant.

A, Typical traces in response to acidification to the pH indicated, during the time indicated by the black bar. B, Activation and SSIN curves of hASIC1a wt and the mutant E413Q/E418Q. For the pH range 7.1 to 6.4 (black lines, left axis). In grey, the sustained current amplitude, normalized to the peak current amplitude at pH 4.5, is indicated on a 100-fold expanded scale (right axis, n=5-8). This figure shows that in the two mutants represented, the currents inactivate as wt, and that there is no sustained current different from wt.

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Supplemental Table S4. pKa values calculated from the 3HGC-based model in the absence or presence of bound Cs⁺ ions

proximity	of	Cs 467			proximity	of (Cs 468		
residue		3HGC	3HGC-Cs	delta	residue		3HGC	3HGC-Cs	delta
E97	1	11.43	11.42	-0.01	D296	1	8.53	8.53	0.00
	2	11.54	11.53	-0.01		2	6.28	6.29	0.01
E219	1	7.25	7.07	-0.18	D298	1	8.33	8.33	0.00
	2	8.32	8.01	-0.31		2	6.77	6.77	0.00
E235	1	3.54	3.54	0.00	D300	1	6.84	6.84	0.00
	2	-1.01	-1.01	0.00		2	10.18	10.18	0.00
D237	1	6.07	6.08	0.01	D303	1	6.90	6.90	0.00
	2	7.49	7.49	0.00		2	5.51	5.51	0.00
E242	1	6.46	6.40	-0.07	D313	1	12.32	12.32	0.00
	2	6.81	6.73	-0.08		2	16.96	16.97	0.01
D259	1	4.13	4.12	-0.01					
	2	3.27	3.27	0.00					
D347	1	8.90	8.90	0.00	proximity	of of	Cs 469/47	70	
	2	8.04	8.04	0.00	residue		3HGC	3HGC-Cs	delta
D351	1	3.67	3.67	0.00	E427	1	7.38	4.46	-2.92
	2	7.27	7.27	0.00		2	3.59	1.42	-2.17
E355	1	5.20	5.20	0.00	D434	1	43.92	0.99	-42.93
	2	6.68	6.68	0.00		2	36.43	1.47	-34.96
					E63	1	19.97	14.78	-5.19
				_		•	40.00	10.50	E 0.4



)		2	3.59	1.42	-2.17
)	D434	1	43.92	0.99	-42.93
)		2	36.43	1.47	-34.96
	E63	1	19.97	14.78	-5.19
		2	18.22	12.59	-5.64
	D78	1	9.98	9.95	-0.02
		2	2.61	2.58	-0.02
	H70		4.50	4.03	-0.47
	H72		0.17	-0.03	-0.19
	H73		5.26	5.18	-0.08

Supplemental Table S4. pKa values calculated from the 3HGC-based model in the absence or presence of bound Cs⁺ ions.

pKa values were calculated for residues in the proximity of Cs⁺ ions in the 3IJ4 structure. The calculation was performed in the hASIC1a model derived from the 3HGC structure, in which the Cs⁺ ions had been placed. The table shows the pKa values for the 3HGC model, for the 3HGC model containing the Cs⁺ ions, and the column "delta" displays their difference. The inserted figure represents the 3IJ4 structure and indicates the position of Cs⁺ ions 467 and 468 of one subunit, and of Cs⁺ ions 469 and 470 (all shown in purple) in the channel pore. The Cl⁻ ions are shown in green.

Supplemental Table S5. Comparison of pH50-shifts relative to wt in this compared to previous studies

Residue	pH50-shift	compared mutation	pH50-shift	Reference
	relative to		relative to wt	
	wt in this			
	study			
E113	< 0.1	ASIC2a E112Q	< 0.1	(1)
E136	< 0.1	ASIC2a E135Q	-0.13	(1)
D164	-0.10	ASIC2a D163N	< 0.1	(1)
D183	-0.10	ASIC2a D182N	+0.34	(1)
D223	< 0.1	rASIC1a D223N	< 0.1	(2)
E228	-0.13	rASIC1a E228Q	< 0.1	(2)
E235	< 0.1 ^b	rASIC1a E235Q	-0.2	(2)
E254	< 0.1	rASIC1a E254Q	< 0.1	(2)
E344	< 0.1	rASIC1a E342Q	< 0.1	(2)
D347	-0.19 ^a	cASIC1 D346N	-0.3 ^c	(3)
E413Q	-0.10	rASIC1a E411Q	-0.1	(2)
E418Q	-0.19 ^a	rASIC1a E418Q	-0.15	(2)

The pH50 values of mutants of D303, E315, E321 and E355 have not been determined previously, and SSIN has been analyzed for none of these residues to our knowledge. pH50 data are reported from ASIC1 where available, and for some mutants from ASIC2a. rASIC1a, rat ASIC1a ; cASIC1, chicken ASIC1. ^a, difference from wt statistically tested (p < 0.05). ^b, nH, Hill coefficient significantly different from wt (p < 0.05). ^c, for this mutant a marked decrease in the Hill coefficient was reported.

Residue in		Cited study	
this study	Position	Species ^a	Reference
Y71	72	r	(4)
H72	72	r	(2)
H73	73	r	(2)
H73	72	ASIC2a	(5)
D78	78	r	(2)
E79	79	rASIC3	(6)
K105	105	r	(2)
N106	106	r	(2)
D107	107	r	(2)
R190	190	r	(2)
E235	235	r	(2)
D237	237	r	(2)
E238	238	r	(2)
D253	253	r	(2)
E254	254	r	(2)
W287	288	r	(4)
D347	346	С	(3)
D351	350	С	(3)
D357	357	h	(7)
D357	355	r	(2)
Q358	358	r	(8)
Q358	358	h	(7)
E359	359	r	(8)
E413	411	r	(2)
E418	416	r	(2)

Supplemental Table S6. Numbering and species of ASIC residues cited in the discussion

^a, and indication of ASIC isoform, if different from ASIC1a. r, rat; c, chicken, h, human.

References for Tables S5 and S6

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