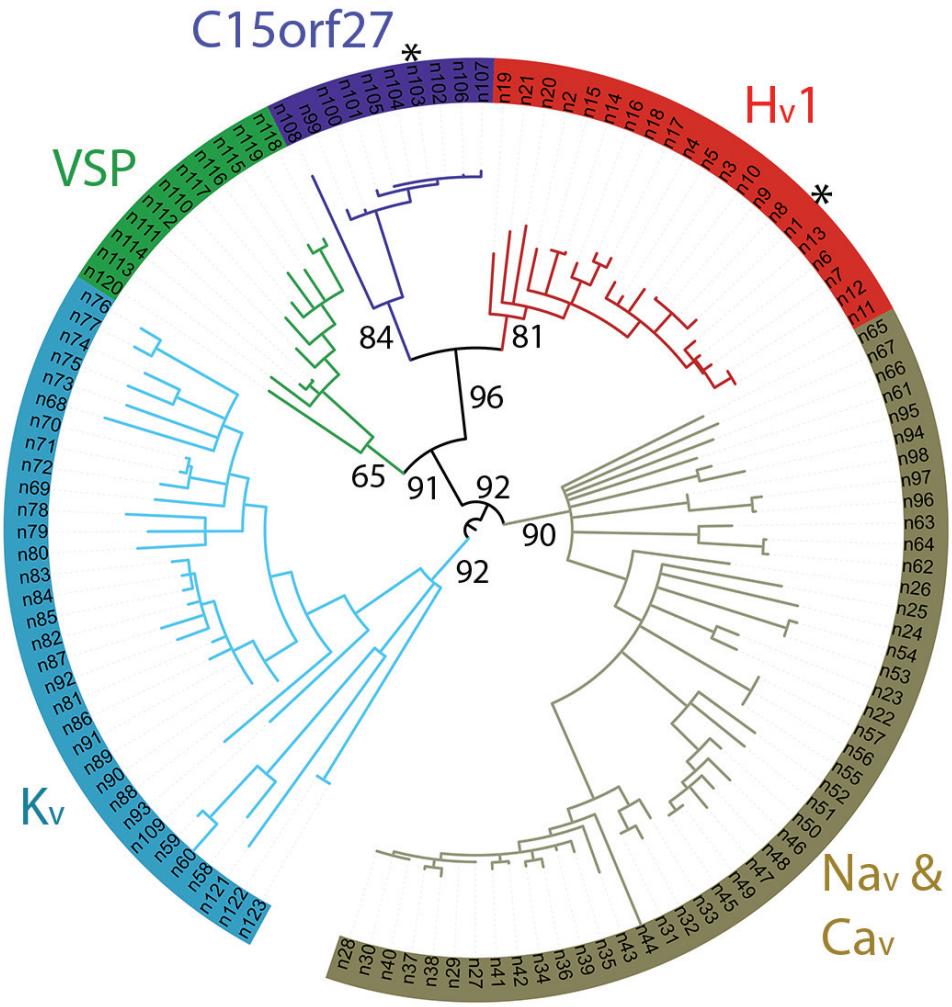
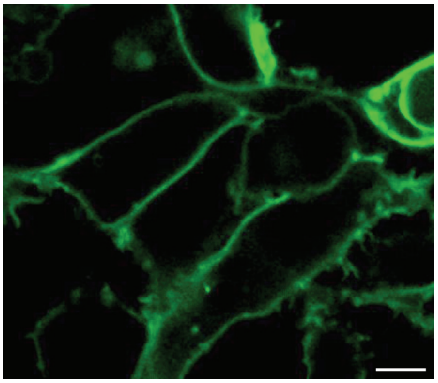


Supplementary Figure S1. Phylogenetic relationship between the proton channel (H_V1) and C15orf27 families and other VSD-containing proteins.

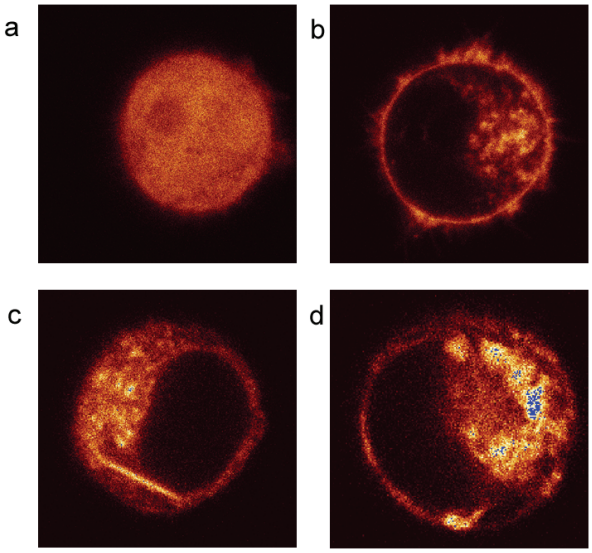
Unrooted phylogram from a maximum likelihood analysis of 122 VSDs (Table S1) shows that H_V1 sequences appear on a branch distinct from other VSDs. Sequences are colour coded: K_V = voltage gated potassium channel; Na_V = voltage gated sodium channel; Ca_V = voltage gated calcium channel; VSP = voltage sensitive phosphatase. Notably, the VSD homologs separate into three main branches, indicating that the VSDs of the H_V1/C15orf27/VSP group are phylogenetically distinct from VSDs of both K_V and also Na_V and Ca_V channels. Phylogenetic analysis was performed on VSD sequences only, and did not include sequences of channel pores. Branches with likelihood support values (a measure of confidence in a branch's appearance in a tree) <0.50 were collapsed. The range of support values shown is representative of the full range of all (non-collapsed) branches. *indicates hH_V1 and C15orf27 sequence positions.

Supplementary Figure S2. The c15orf27 protein localizes to the plasma membrane.



Human C15orf27 cDNA was subcloned into pEGFPN1 vector (Clontech, CA) with GFP fused to the C-terminus of the cDNA. HEK cells were transfected with C15orf27-GFP and mid-plane images were obtained 24 hours post-transfection by Laser scanning confocal microscopy. Scale bar is 5 μm.

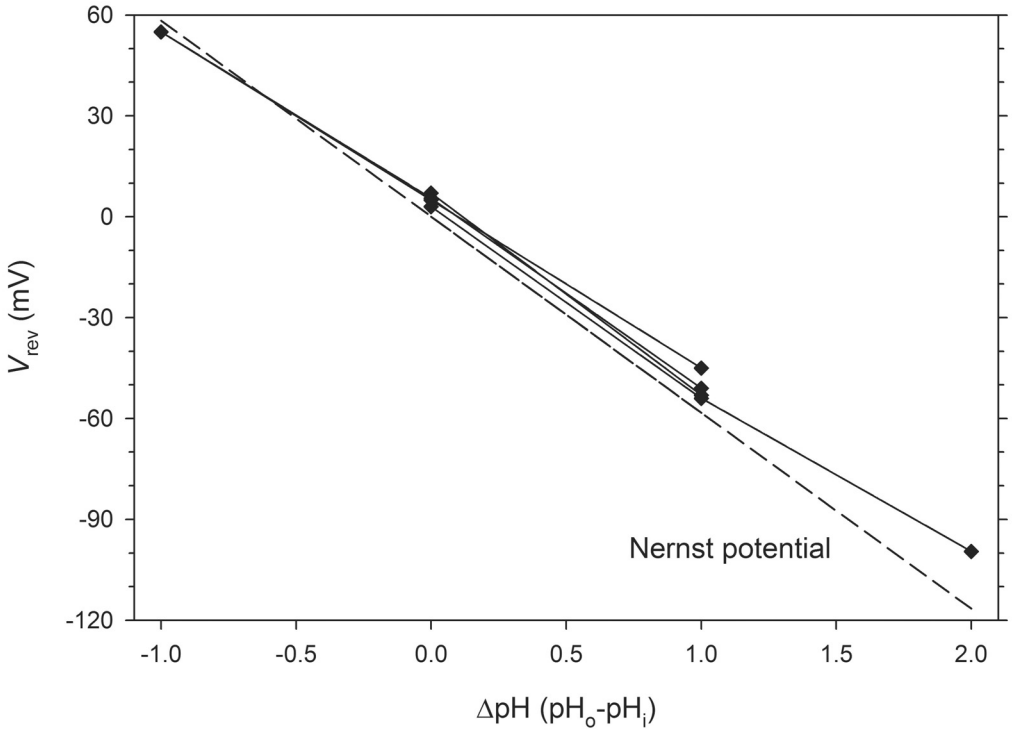
Supplementary Figure S3. GFP-tagged constructs appear to localize to the plasma membrane.



Pseudocolour images of GFP in representative COS-7 cells transfected with (a) GFP alone, (b) WT hH_V1 with GFP tag, (c) D112S with GFP tag, or (d) D112V with GFP tag. GFP alone is diffusely distributed throughout the cytoplasm. GFP-tagged hH_V1 constructs appear in the membrane and in intracellular compartments.

Methods - Transfected cells were cultured in 2 ml culture dishes in DMEM. The medium was removed by aspiration and the cells were detached by immersing monolayers in trypsin/EGTA solution (Sigma) for 3 min. Cells were washed in DMEM medium and suspended at a concentration of 2×10^6 cells/ml. An aliquot of the cell suspension was added to a measuring chamber containing 300 µl of Ringer's solution and cells were allowed to settle. Transfected cells were visualized by exciting at 488 nm and collecting emission at 490 - 560 nm using a Leica TCS SP2 confocal system (Leica Microsystems, Exton, PA, USA). Cells were imaged using the 40x water immersion lens and scanned at 400 Hz.

Supplementary Figure S4. The elimination of Zn^{2+} sensitivity by the H140A/H193A mutation does not detectably impair proton selectivity.

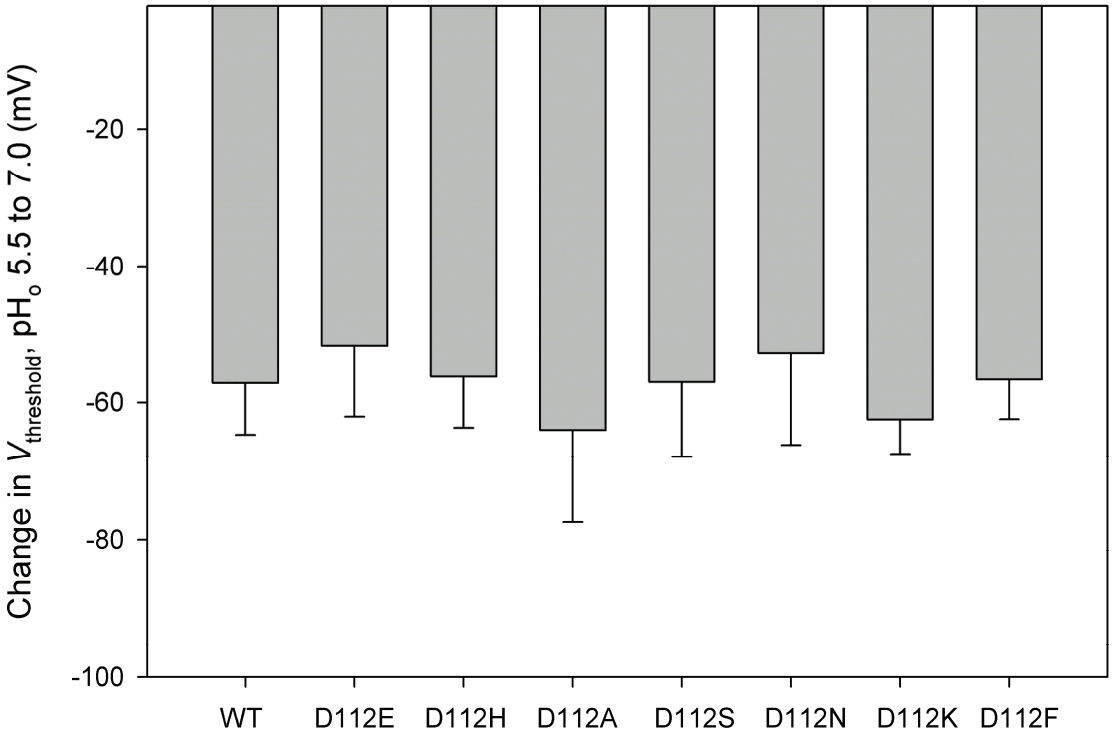


In inside-out patches of membrane from HEK-293 cells expressing H140A/H193A channels, V_{rev} was measured over a range of pH. Measurements in the same patch are connected by lines. This double mutant was used as a background for several of the Asp^{112} mutants in order to validate that any currents detected were due to the mutant channel, rather than native proton currents. In the presence of 100 μM Zn^{2+}, WT proton current activation is slowed ~15-fold, and the g_H-V relationship is shifted positively by ~60 mV[1]. The H140A/H193A mutation nearly eliminates inhibition by Zn^{2+} (refs. [2, 3]).

References

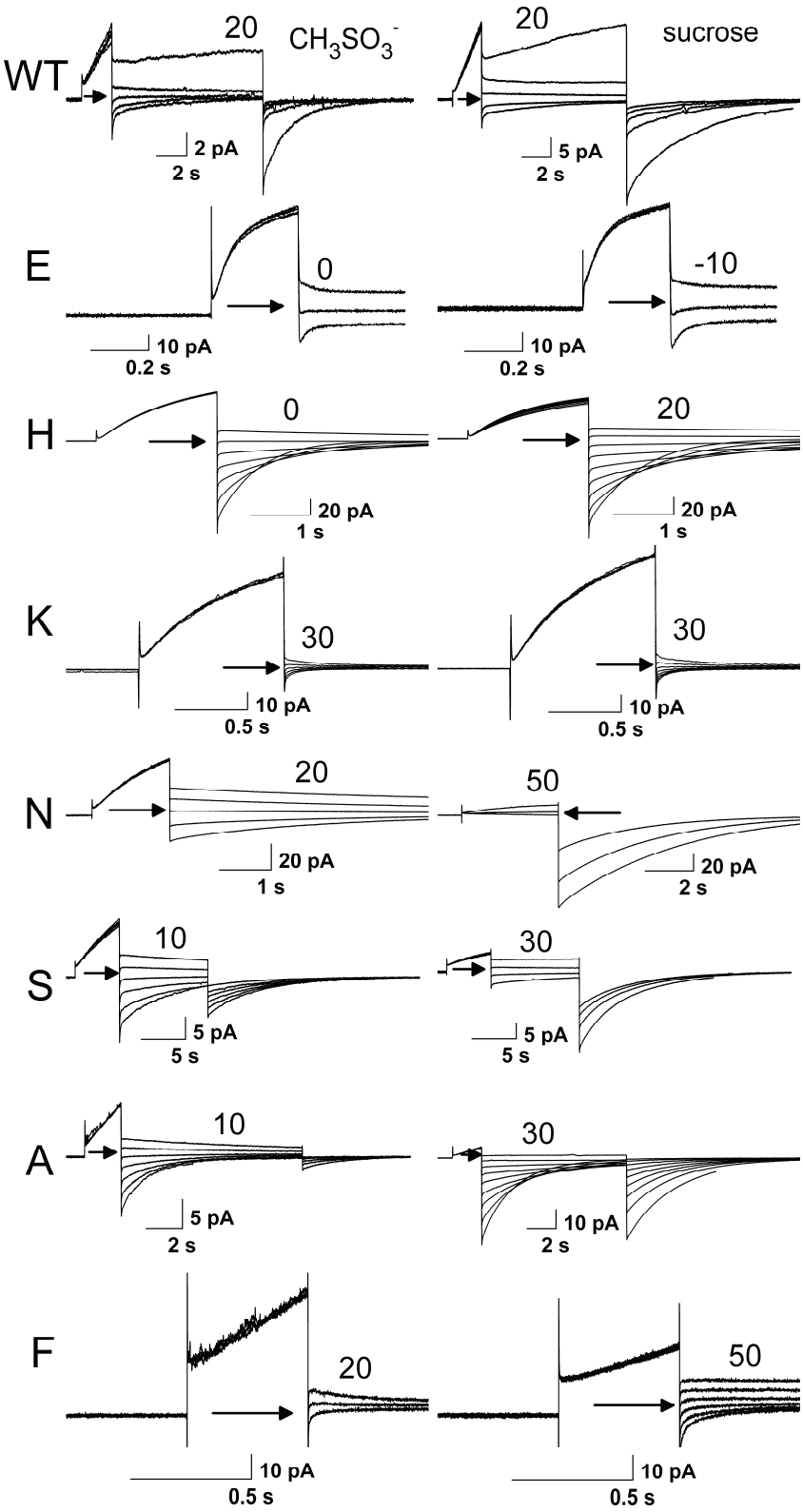
1. Cherny, V. V. & DeCoursey, T. E. pH-dependent inhibition of voltage-gated H^+ currents in rat alveolar epithelial cells by Zn^{2+} and other divalent cations. *J Gen Physiol* **114**, 819-38 (1999).
2. Musset, B. et al. Zinc inhibition of monomeric and dimeric proton channels suggests cooperative gating. *J Physiol* **588**, 1435-49 (2010).
3. Ramsey, I. S., Moran, M. M., Chong, J. A. & Clapham, D. E. A voltage-gated proton-selective channel lacking the pore domain. Nature **440**, 1213-6 (2006).

Supplementary Figure S5. Mutation of Asp^{112} does not eliminate the ΔpH dependence of gating.



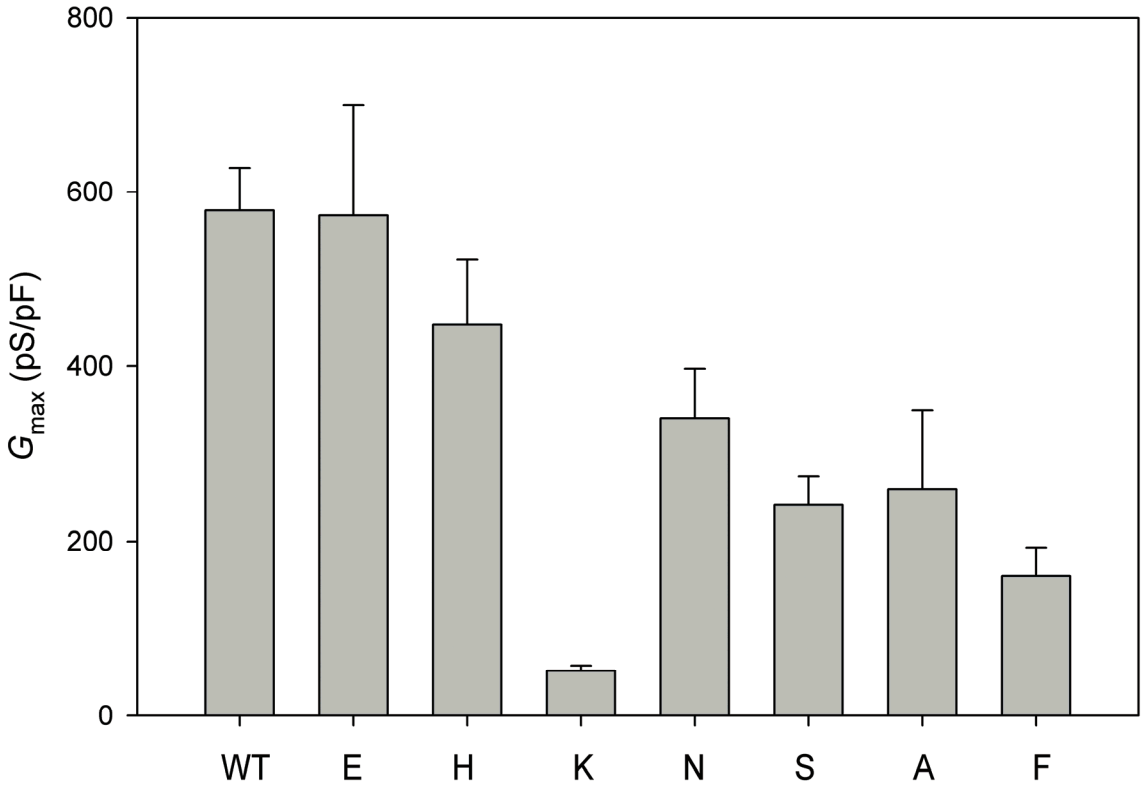
In whole-cell measurements like those in Fig. 2, $V_{threshold}$ was estimated as the voltage at which distinct conductance was activated, usually determined from tail currents, which are more sensitive. The graph shows the mean ± SD shift of $V_{threshold}$ when pH_o 5.5 and 7.0 are compared, both at pH_i 5.5. Numbers of cells range 3 to 11. None of the shifts for D112x mutants differed significantly from that in WT (p>0.28 for each).

Supplementary Figure S6. Dilution of ionic strength with isotonic sucrose reveals that most Asp[112] mutants are anion selective.



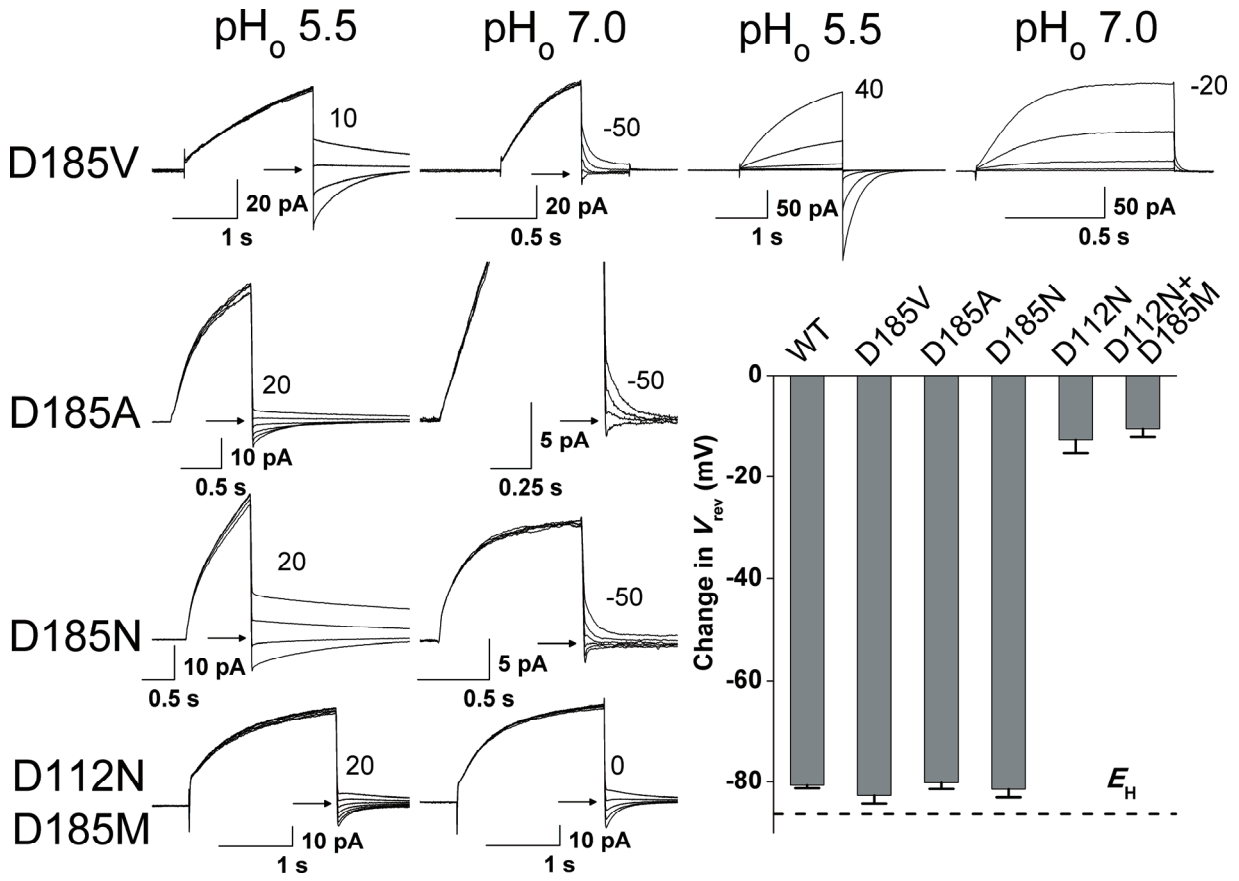
Measurement of V_{rev} by tail currents (or direct reversal of test current in D112N in sucrose) at pH 5.5//5.5 ($CH_3SO_3^-$ solutions) is shown in the first column, and in the same cell after 90% dilution of the bath by isotonic sucrose in the second column. Arrows indicate zero current. Letters indicate the amino acid substituted at position 112. The most positive voltage in each series is given, without correction for liquid junction potentials. V_{rev} of WT or D112E channels did not change, consistent with proton selectivity. For other mutants, except D112K, V_{rev} shifted positively, indicating anion selectivity. V_{hold} was -40 mV, or -20 mV for F. V_{pre} for control, sucrose was 30, 30 mV (WT); 20, 10 mV (E); 60, 60 (H); 140, 140 mV (K); 60 mV (N); 50, 40 mV (S); 50, 50 mV (A); and 100, 90 mV (F). The lack of a shift for D112K in $CH_3SO_3^-$ at pH_o 5.5 is anomalous, because sucrose did produce a positive shift at pH_o 5.5 in Cl^- solution, and at pH_o 7.0 in both Cl^- and $CH_3SO_3^-$ solutions (Fig. 3).

Supplementary Figure S7. Maximum conductance of Asp^{112} mutants expressed in COS-7 cells, at pH 5.5//5.5, normalized to capacity.



The maximum chord conductance G_{max} was calculated from the maximum current measured in each cell, using V_{rev} measured in the same solution. Mean of 8-14 cells for each mutant, with s.e. bars.

Supplementary Figure S8. Mutation of Asp^{185} does not impair proton selectivity.

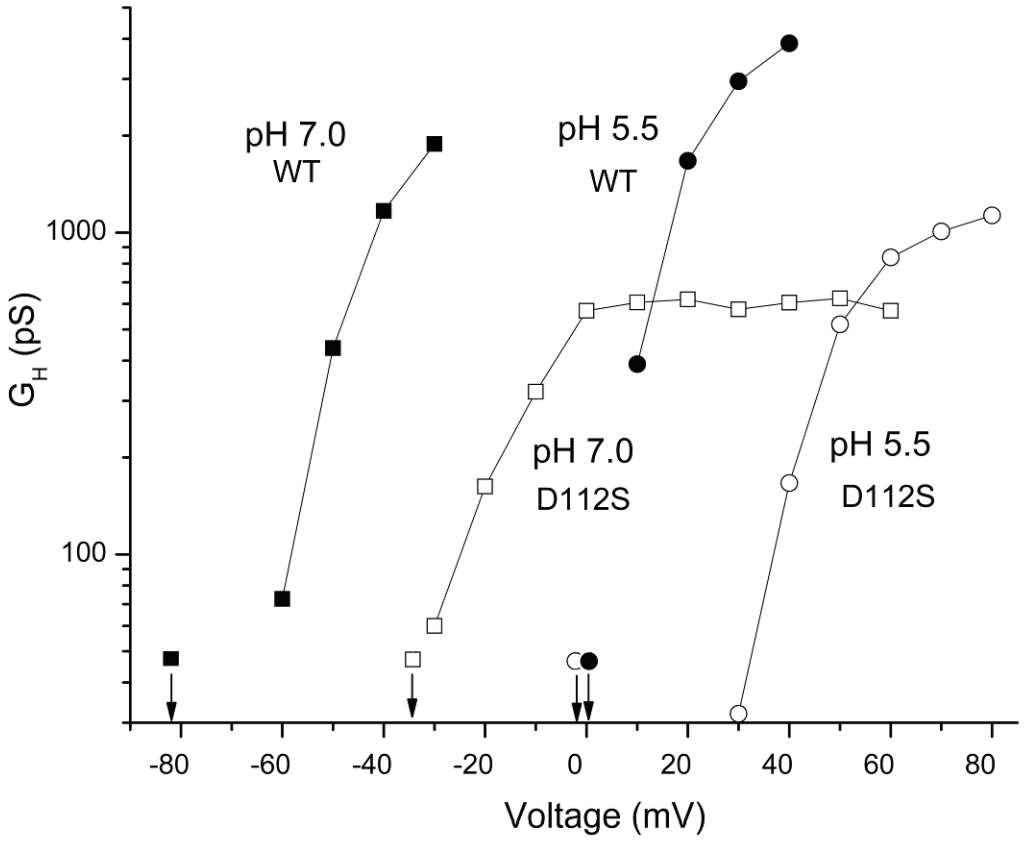


In whole-cell measurements like those in Fig. 2, V_{rev} was determined from tail currents at pH$_o$ 5.5 and pH$_o$ 7.0, both at pH$_i$ 5.5, as illustrated here. Families of currents are shown for D185V at pH$_o$ 5.5 and 7.0. The most positive voltage for tail currents or for families of pulses in 10 mV increments is labelled. For D185V tail currents, V_{hold} was -40 mV and V_{pre} was +30 mV at pH$_o$ 5.5, V_{hold} was -60 mV and V_{pre} was -30 mV at pH$_o$ 7.0. For families, V_{hold} was -40 mV and -70 mV at pH$_o$ 5.5 or pH$_o$ 7.0, respectively. For D185A, V_{hold} was -60 mV for both pH and V_{pre} was +75 or +15 mV, respectively, for pH$_o$ 5.5 and pH$_o$ 7.0. For D185N, V_{hold} was -60 mV for both pH and V_{pre} was +45 or -31 mV, respectively, for pH$_o$ 5.5 and pH$_o$ 7.0.

To determine whether the addition of Asp^{185} neutralization to an anion selective Asp^{112} mutant might produce additional effects, we also studied the double mutant D112N/D185M. Tail currents at pH$_o$ 5.5 and pH$_o$ 7.0 are shown, with V_{hold} -40 mV and V_{pre} +100 or +60 mV at pH$_o$ 5.5 and pH$_o$ 7.0, respectively.

The bar graph shows the change in V_{rev} when pH$_o$ was changed from 5.5 to 7.0 for these Asp^{185} mutants. For comparison, values from WT and D112N channels from Fig. 2b are replotted here. There is no difference between WT and D185V (n = 4), D185A (n = 4), or D185N (n = 4) or between D112N and D112N/D185M (n = 6).

Supplementary Figure S9. Dissociation of the relationship between V_{rev} and the *g-V* relationship in Asp[112] mutants.



For representative cells expressing WT hH_V1 (solid symbols) and D112S channels (open symbols), the conductance was calculated from the current amplitude extrapolated from a rising exponential fit, using V_{rev} measured in the relevant solution at pH_o 5.5 or pH_o 7.0, all at pH_i 5.5. The arrows indicate measured V_{rev} values. In both D112S and WT, the *g-V* relationship shifted negatively by ~60 mV at pH_o 7.0 compared to its position at pH_o 5.5, but V_{rev} shifted much less in D112S than in WT. In WT hH_V1, there is a linear relationship between V_{rev} and $V_{threshold}$ (or the g_H-*V* relationship) with a slope of ~40 mV/unit increase in ΔpH[1].

[1] Musset, B. *et al*. Detailed comparison of expressed and native voltage-gated proton channel currents. *J. Physiol.* **586**, 2477-2486(2008).

Supplementary Table 1. FASTA-formatted alignment of voltage sensor domain sequences.

This alignment was used to generate the phylogenetic tree in Fig. S1. Sequences are numbered as in the tree. One sequence from a protein that responds to hyperpolarizing potentials (109) was included. All other sequences were from protein families known to respond to depolarizing potentials, or from C15orf27 homologs. Accession numbers used are from NCBI (gi), Uniprot (uniprot or sp), or PDB (pdb) sequence databases.

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--------------------------QFE-ALGLLILLRLWRVARIINGI----IISV-
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>2 gallus_hvcn gi|71897219|
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>20 ciona_hvcn |gi_118344228|


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-------------------------EALS-GVGLLVVLRLWRIARIVNGI----ISSV-
KSQANDKIHHL--
>22 human_CACNA1E_repeat_3 gi|53832005|
HYIVNL-------RYFEMCILLVIAASSIALAAEDPVLT----------------------
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FEMVIKMIDQGLIL----------QDGSYFRDLWNILDFVVVVGALVAFALANALGTN--
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>25 homo_CAC1I_repeat_1 gi|51093859|
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-----------------------------NLSAIRTVRVLRPLKAINRV-----PSM-
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AEMTVKVVALGWCF----------GEQAYLRSSWNVLDGLLVLISVIDILVSMVSDSG--
------------------------TKILG-MLRVLRLLRTLRPLRVISRA-----QGL-
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-----------------------------NVSALRTFRVLRALKTISVI-----PGL-
KTIVGALIQSVKK
>40 canis_SCN_repeat1 gi|74004456|
IKILVH-------SLFNVLIMCTILTNCVFMTMSN------------------------
-----------------------------------PPDW----TKNVEYTFTGIYT
FESLIKILARGFCL----------EDFTFLRDPWNWLDFTVITFAYVTEFVDLG------
-----------------------------NVSALRTFRVLRALKTISVI-----PGL-
KTIVGA-------
>41 danio_SCN8AA_repeat1 sp_Q9DF53
IKILIH-------SVFSMFIMCTILTNCVFMTFSN------------------------
-----------------------------------PPEW----SKQVEYTFTGIYT
FESAVKIIARGFCI----------DGFTFLRDPWNWLDFMVISMAYVTEFVDLG------
-----------------------------NVSALRTFRVLRALKTISVI-----PGL-
KTIVGALIQSVKK
>42 mouse_SCN8A_repeat1 sp_Q9WTU3
IKILIH-------SVFSMIIMCTILTNCVFMTFSN------------------------
-----------------------------------PPEW----SKNVEYTFTGIYT
FESLVKIIARGFCI----------DGFTFLRDPWNWLDFSVIMMAYVTEFVDLG------
-----------------------------NVSALRTFRVLRALKTISVI-----PGL-
KTIVGALIQSVKK
>43 canis_SCNAA_repeat1 sp_O46669
IKVSVH-------SWFSLFITVTILVNCVGMTQTE------------------------
-----------------------------------LPD-------RIEYVFTVIYT
FEALIKILARGFCL----------NEFAYLRDPWDWLDFSVITLAYIGEATALR------
-----------------------------GISGLRTFRVLRALKTVSVI-----PGL-
KVIVGALIHSVRK
>44 homo_SCN7A_repeat1 sp_Q01118
IKVLVH-------PFFQLFILISVLIDCVFMSLTN------------------------
-----------------------------------LPKW----RPVLENTLLGIYT
FEILVKLFARGVWA----------GSFSFLGDPWNWLDFSVTVFEVIIRYSPLD------
-----------------------------FIPTLQTARTLRILKIIPLN-----QGL-
KSLVGVLIHCLKQ
>45 rabbit_CAC1C_repeat1 sp_P15381
ISIVEW-------KPFEIIILLTIFANCVALAIYIPFPED--------------------
-----------------------------------DSNATNSN----LERVEYLFLIIFT
VEAFLKVIAYGLLF----------HPNAYLRNGWNLLDFIIVVVGLFSAILEQATK-AD-
------------------GANALGGKGAGF-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSIIKAMV-
>46 mouse_CAC1S_repeat1 sp_Q02789
ISIVEW-------KPFETIILLTIFANCVALAVYLPMPED--------------------
-----------------------------------DNNTLNLG----LEKLEYFFLIVFS
IEAAMKIIAYGFLF----------HQDAYLRSGWNVLDFIIVFLGVFTVILEQVNIIQT-
------------------NTAPMSSKGAGL-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSIFKAML-
>47 mouse_CAC1F_repeat1 sp_Q9JIS7
ISIVEW-------KPFDILILLTIFANCVALGVYIPFPED--------------------
-----------------------------------DSNTANHN----LEQVEYVFLVIFT

VETVLKIVAYGLVL----------HPSAYIRNGWNLLDFIIVVVGLFSVLLEQGPGRPG-
------------------DAPHTGGKPGGF-DVKALRAFRVLRPLRLVSGV-----PSL-
HIVVNSIMKALV-
>48 gallus_CAC1D_repeat1 sp_O73700
ISLVEW-------KPFDIFILLSIFANCVALAVYIPFPED--------------------
-----------------------------------DSNSTNHN----LEKVEYAFLIIFT
VETFLKIIAYGLLL----------HPNAYVRNGWNLLDFVIVVVGLFSVILEQLTKETE-
------------------GGSHSGGKPGGF-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSIIKAMV-
>49 homo_CACN_repeat1  gi|193788728|
ISIVEW-------KPFEIIILLTIFANCVALAIYIPFPED--------------------
-----------------------------------DSNATNSN----LERVEYLFLIIFT
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VEAFLKVIAYGLLF----------HPNAYLRNGWNLLDFIIVVVGLFSAILEQATKADG-
------------------ANALGGKGAGF-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSIIKAMVP
>50 drosophila_CAC1D_repeat1 sp_Q24270
IRIVEW------KPFEFLILLTIFANCIALAVYTPYPGS--------------------
---------------------------------DSNVTNQT----LEKVEYVFLVIFT
AECVMKILAYGFVL----------HNGAYLRNGWNLLDFTIVVIGAISTALSQLMK----
--------------------------DAF-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSILKAMV-
>51 homo_CAC1A_repeat1 sp_O00555
KKITEW-------PPFEYMILATIIANCIVLALEQHLPDD--------------------
---------------------------------DKTPMSER----LDDTEPYFIGIFC
FEAGIKIIALGFAF----------HKGSYLRNGWNVMDFVVVLTGILATVGTEF------
------------------------------DLRTLRAVRVLRPLKLVSGI-----PSL-
QVVLKSIMKAMIP
>52 homo_CAC1B_repeat1 sp_Q00975
KRITEW-------PPFEYMILATIIANCIVLALEQHLPDG--------------------
---------------------------------DKTPMSER----LDDTEPYFIGIFC
FEAGIKIIALGFVF----------HKGSYLRNGWNVMDFVVVLTGILATAGTDF------
------------------------------DLRTLRAVRVLRPLKLVSGI-----PSL-
QVVLKSIMKAMV-
>53 rat_SCN11A_repeat3 sp_O88457
YQIVKH-------SWFESFIIFVILLSSGALIFEDVNLPS--------------------
---------------------------------RPQVEKL----LRCTDNIFTFIFL
LEMILKWVAFGF--------------RRYFTSAWCWLDFLIVVVSVLSLMNLP-------
------------------------------SLKSFRTLRALRPLRALSQF-----EGM-
KVVVYALISAIPA
>54 mouse_SCN11A_repeat3 sp_Q9R053
YQIVKH-------SWFESFIIFVILLSSGALIFEDVNLPS--------------------
---------------------------------RPQVEKL----LKCTDNIFTFIFL
LEMILKWVAFGF--------------RKYFTSAWCWLDFLIVVVSGLSLTNLP-------
------------------------------NLKSFRNLRALRPLRALSQF-----EGM-
KVVVNALMSAIPA
>55 rat_SCN9A_repeat3  sp_O08562
YRIVEH-------SWFESFIVLMILLSSGALAFEDIYIEK--------------------
---------------------------------KKTIKII----LEYADKIFTYIFI
LEMLLKWVAYGY--------------KTYFTNAWCWLDFLIVDVSLVTLVANTLGYSDLG
------------------------------PIKSLRTLRALRPLRALSRF-----EGM-
RVVVNALIGAIPS
>56 rabbit_SCN9A_repeat3 sp_Q28644
YRIVEH-------SWFESFIVLMILLSSGALAFEDIYIEK--------------------
---------------------------------KKTIKII----LEYADKIFTYIFI
LEMLLKWVAYGY--------------KTYFTNAWCWLDFLIVDVSLVTLVANTLGYSDLG
------------------------------PIKSLRTLRALRPLRALSRF-----EGM-
RVVVNALIGAIPS
>57 mouse_SCN9A_repeat3 uniprot_B7ZWN
YRIVEH-------SWFESFIVLMILLSSGALAFEDIYIEK--------------------
---------------------------------KKTIKII----LEYADKIFTYIFI
LEMLLKWVAYGY--------------KTYFTNAWCWLDFLIVDVSLVTLVANTLGYSDLG
------------------------------PIKSLRTLRALRPLRALSRF-----EGM-
RVVVNALIGAIPS
>58 mouse_KCNH1 sp_Q60603
--------------TWDWIILILTFYTAILVPYNVSFK----------------------
---------------------------------TRQNNVA----WLVVDSIVDVIFL
VDIVLNFHTTFVGPAGEVISDPKLIRMNYLK-TWFVIDLLSCLPYDVINAFENVDEVSAF
MGDPGKIGFADQIPPPLEGRESQGISSLFS-SLKVVRLLRLGRVARKLDHY-----IEY-
GAAVLV-------
>59 mouse_KCNH8 sp_P59111
--------------GWDWLILLATFYVAVTVPYNVCFIGN--------------------
---------------------------------EDLSTTRS-----TTVSDIAVEILFI
IDIILNFRTTYVSKSGQVIFEARSICIHYVT-TWFIIDLIAALPFDLLYAFNVTV-----
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------------------------VSLVH-LLKTVRLLRLLRLLQKLDRY-----SQH-
STIVLTLLMSM--
>60 homo_KCNH3 sp_Q9ULD8
--------------TWDGFILLATLYVAVTVPYSVCVSTA--------------------
---------------------------------REPSAARGP----PSVCDLAVEVLFI
LDIVLNFRTTFVSKSGQVVFAPKSICLHYVT-TWFLLDVIAALPFDLLHAFKVNV-----
------------------------YFGAH-LLKTVRLLRLLRLLPRLDRY-----SQY-
SAVVLT-------
>61 homo_CAC1G_repeat4 sp_O43497
HHLCTS-------HYLDLFITGVIGLNVVTMAMEHYQQ----------------------
-----------------------------------PQILDEA----LKICNYIFTVIFV
LESVFKLVAFG--------------FRRFFQDRWNQLDLAIVLLSIMGITLEEIEVNA--
----------------------SLPINPT-IIRIMRVLRIARVLKLLKMA-----VGM-
RALLDTVMQALPQ
>62 mouse_SCN11A_repeat4 sp_Q9R053
FDLVTS-------QVFDVIILGLIVTNMIIMMAESEGQ----------------------
-----------------------------------PNEVKKI----FDILNIVFVVIFT
VECLIKVFALR---------------QHYFTNGWNLFDCVVVVLSIISTLVSGLENSN--
------------------------VFPPT-LFRIVRLARIGRILRLVRAA-----RGI-
RTLLFALMMSLPS
>63 rat_SCN9A_repeat4 sp_O08562
FDLVTN-------QAFDITIMVLICLNMVTMMVEKEGQ----------------------
-----------------------------------TEYMDYV----LHWINMVFIILFT
GECVLKLISLR---------------HYYFTVGWNIFDFVVVILSIVGMFLAEMIEKY--
------------------------FVSPT-LFRVIRLARIGRILRLIKGA-----KGI-
RTLLFALMMSLPA
>64 rat_SCN11A_repeat4 sp_O88457
FDLVTS-------QVFDVIILGLIVLNMIIMMAESADQ----------------------
-----------------------------------PKDVKKT----FDILNIAFVVIFT
IECLIKVFALR---------------QHYFTNGWNLFDCVVVVLSIISTLVSRLEDSD--
------------------------ISFPPT-LFRVVRLARIGRILRLVRAA-----RGI-
RTLLFALMMSLPS
>65 humo_CAC1G_repeat2 sp_O43497
RKIVDS-------KYFGRGIMIAILVNTLSMGIEYHEQ----------------------
-----------------------------------PEELTNA----LEISNIVFTSLFA
LEMLLKLLVYG--------------PFGYIKNPYNIFDGVIVVISVWEIVGQQGG-----
-------------------------------GLSVLRTFRLMRVLKLVRFL-----PAL-
QRQLVVLMKTMDN
>66 homo_CACNA1E_repeat_4 sp_Q15878
WHFVVS-------PSFEYTIMAMIALNTVVLMMKYYSA----------------------
-----------------------------------PCTYELA----LKYLNIAFTMVFS
LECVLKVIAFG--------------FLNYFRDTWNIFDFITVIGSITEIILTDSKLVN--
------------------------TSGF-NMSFLKLFRAARLIKLLRQG-----YTI-
RILLWTFVQSFKA
>67 drosophila_CAC1A_repeat_4 sp_P91645
WRIVVS-------TPFEYFIMMLIVFNTLLLMMKYHNQ----------------------
-----------------------------------GDMYEKS----LKYINMGFTGMFS
VETVLKIIGFG--------------VKNFFKDPWNIFDLITVLGSIVDALWMEFGHDD--
-------------------------SNSI-NVGFLRLFRAARLIKLLRQG-----YTI-
RILLWT-------
>68 homo_KCNV2 sp_Q8TDN2
WNLMEKPFSSVAAKAIGVASSTFVLVSVVALALNTVEEMQ----------------QHSG
---------------------------QGEG----GPDLRPI----LEHVEMLCMGFFT
LEYLLRLASTP-------------DLRRFARSALNLVDLVAILPLYLQLLLECFTGEGHQ
-----------------RGQTVGSVGKVGQ-VLRVMRLMRIFRILKLARHS-----TGL-
RAFGFTLRQCYQQ
>69 homo_KCNF1 sp_Q9H3M0_KCNF1
WKFLEKPESSCPARVVAVLSFLLILVSSVVMCMGTIPELQ----------------VLD-
---------------------------AEG----NRVEHPT----LENVETACIGWFT
LEYLLRLFSSP-------------NKLHFALSFMNIVDVLAILPFYVSLTLTHLGAR---
---------------------MMELTNVQQ-AVQALRIMRIARIFKLARHS-----SGL-
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QTLTYALKRSFKE
>70 homo_KCNB1 sp_Q14721
WDLLEKPNSSVAAKILAIISIMFIVLSTIALSLNTLPELQ----------------SLD-
----------------------------EFG----QSTDNPQ----LAHVEAVCIAWFT
MEYLLRFLSSP------------KKWKFFKGPLNAIDLLAILPYYVTIFLTESNKS---
---------------------VLQFQNVRR-VVQIFRIMRILRILKLARHS-----TGL-
QSLGFTLRRSYNE
>71 canis_KCNB2 sp_Q95167
RDLLEKPNSSVAAKILAIVSNLFIVLSTIALSLNTLPELQ----------------EMD-
----------------------------EFG----QPNDNPQ----LAHVEAVCNAWFT
MEYLLRFLSSP------------NKWKFFKGPLNVIDLLAILPYYVTIFLTESNKS---
---------------------VLQFQNVRR-VVQIFRIMRILRILKLARHS-----TGL-
QSLGFTLRRSYNE
>72 drosophila_KCNAB sp_P17970
WELLEKPNTSFAARVIAVISILFIVLSTIALTLNTLPQLQ----------------HIDN
G-----------------------------------TPQDNPQ----LAMVEAVCITWFT
LEYILRFSASP------------DKWKFFKGGLNIIDLLAILPYFVSLFLLETNKN---
--------------------ATDQFQDVRR-VVQVFRIMRILRVLKLARHS-----TGL-
QSLGFTLRNSYKE
>73 pongo_KCNV1 sp_Q5RC10
WNILEKPGSSTAARIFGVISIIFVVVSIINMALMSAEL----------------------
-----------------------------------SWLDLQL----LEILEYVCISWFT
GEFVLRFLCVR------------DRCRFLRKVPNIIDLLAILPFYITLLVESLSGSQ--
--------------------TTQELENVGR-IVQVLRLLRALRMLKLGRHS-----TGL-
RSLGMTITQCYEE
>74 homo_KCNS3 sp_Q9BQ31
WIRMENPAYCLSAKLIAISSLSVVLASIVAMCVHSMSEFQ----------------NED-
-----------------------------------GEVDDPV----LEGVEIACIAWFT
GELAVRLAAAP------------CQKKFWKNPLNIIDFVSIIPFYATLAVDTKEEE---
---------------------SEDIENMGK-VVQILRLMRIFRILKLARHS-----VGL-
RSLGATLRHSYHE
>75 squirrelmonkey_KCNS1 sp_A4K2X4
WLTMENPGYSLPSKLFSCVSISVVLASIAAMCIHSLPEYQ----------------AREA
AAAVA------------------AVAAGRSAE----GVRDDPV----LRRLEYFCIAWFS
FEVSSRLLLAP------------STRNFFCHPLNLIDIVSVLPFYLTLLAGAALGDQG-
------------------GTGGKEFGHLGK-VVQVFRLMRIFRVLKLARHS-----TGL-
RSLGATLKHSYRE
>76 gallus_KCNG2 sp_O73606
RDMVENPHSGIPGKIFACISISFVAITAVSLCISTMPDVR----------------EEE-
----------------------------DRGE----CSQKCYD----IFVLETVCVAWFS
FEFLLRSIQAE------------NKCAFLKTPLNIIDILAILPFYISLIVDMASTKNSS
KP--------------GGGAGNKYLERVGL-VLRFLRALRILYVMRLARHS-----LGL-
QTLGLTVRRCTRE
>77 homo_KCNG4 sp_Q8TDN1
REMVENPQSGLPGKVFACLSILFVATTAVSLCVSTMPDLR----------------AEE-
----------------------------DQGE----CSRKCYY----IFIVETICVAWFS
LEFCLRFVQAQ------------DKCQFFQGPLNIIDILAISPYYVSLAVSEEPPEDGE
-----------------RPSGSSYLEKVGL-VLRVLRALRILYVMRLARHS-----LGL-
QTLGLTVRRCTRE
>78 rat_KCNC3 sp_Q01956_KCNC3
WALFEDPYSSRAARYVAFASLFFILISITTFCLETHEGFI----------------HISN
KTVTQASP-----------IPGAPPENITNV----EVETEPF----LTYVEGVCVVWFT
FEFLMRVTFCP------------DKVEFLKSSLNIIDCVAILPFYLEVGLSGLSSK---
----------------------AAKDVLG-FLRVVRFVRILRIFKLTRHF-----VGL-
RVLGHTLRASTNE
>79 homo_KCNC2 sp_Q96PR1
WALFEDPYSSRAARFIAFASLFFILVSITTFCLETHEAFN----------------IVKN
KTEPV-----------------INGTSVVLQY----EIETDPA----LTYVEGVCVVWFT
FEFLVRIVFSP------------NKLEFIKNLLNIIDFVAILPFYLEVGLSGLSSK---
----------------------AAKDVLG-FLRVVRFVRILRIFKLTRHF-----VGL-
RVLGHTLRASTNE
```

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>80 drosophila_KCNAW sp_P17972
WSLFDEPYSSNAAKTIGVVSVFFICISILSFCLKTHPDMR----------------VPIV
RNITVKT------------ANGSNGWFLDKT----QTNAHIA----FFYIECVCNAWFT
FEILVRFISSP------------NKWEFIKSSVNIIDYIATLSFYIDLVLQRFAS----
----------------------HLENAD-ILEFFSIIRIMRLFKLTRHS-----SGL-
KILIQTFRASAKE
>81 homo_KCNA1 sp_Q09470
WLLFEYPESSGPARVIAIVSVMVILISIVIFCLETLPELK----------------DDKD
F----TGT-----------VHRIDNTTVIYN-----SNIFTDP----FFIVETLCIIWFS
FELVVRFFACP------------SKTDFFKNIMNFIDIVAIIPYFITLGTEIAEQE-G-
-------------------NQKGEQATSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGQTLKASMRE
>82 rat_KNCA6 sp_P17659
WLLFEYPESSGPARGIAIVSVLVILISIVIFCLETLPQFRADGRGGSNEGSGTRMSPASR
GSHEEEDEDEDSYAFPGSIPSGGLGTGGTSSFSTLGGSFFTDP----FFLVETLCIVWFT
FELLVRFSACP------------SKAAFFRNIMNIIDLVAIFPYFITLGTELVQRHEQQ
PV------------SGGSGQNRQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGKTLQASMRE
>83 homo_KCNA5 sp_P22460
WLIFEYPESSGSARAIAIVSVLVILISIITFCLETLPEFRDEREL------LRHPPAPHQ
PPAPAPGANGS------GVMAPPSGPTVAPLL----PRTLADP----FFIVETTCVIWFT
FELLVRFFACP------------SKAGFSRNIMNIIDVVAIFPYFITLGTELAEQQPGG
G----------------GGGQNGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGKTLQASMRE
>84 rat_KCNA3 sp_P15384
WLLFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFR----------------DEKD
YPASPSQDV---------FEAANNSTSGASSG----ASSFSDP----FFVVETLCIIWFS
FELLVRFFACP------------SKATFSRNIMNLIDIVAIIPYFITLGTELAERQ---
--------------------GNGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGQTLKASMRE
>85 canis_Kv1.3 gi|57088651|
WLLFEYPESSGPARGIAIVSVLVILVSIVIFCLETLPEFR----------------DDKD
YAAAAQEQ-----------PEAARNGTSGPPA----AAGFADP----FFVVETLCIIWFS
FELLVRFFACP------------SKATFSRNIMNLIDIVAIIPYFITLGTELAERQ---
--------------------GNGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGQTLKASMRE
>86 bovine_KCNA4 sp_Q05037
WLLFEYPESSSPARGIAIVSVLVILISIVIFCLETLPEFR----------------DDRD
LIMALSTGGHG------GLLNDTSAPHPENSG----HTIFNDP----FFIVETVCIVWFS
FEFVVRCFACP------------SQALFFKNIMNIIDIVSILPYFITLGTDLAQQQGG-
------------------GNGQQQQAMSFA-ILRIIRLVRVFRIFKLSRHS-----KGL-
QILGHTLRASMRE
>87 homo_KCA10 sp_Q16322
WLLFEYPESSSAARAVAVVSVLVVVISITIFCLETLPEFR----------------EDRE
LKVVRD---------------PNLNMSKTVLS----QTMFTDP----FFMVESTCIVWFT
FELVLRFVVCP------------SKTDFFRNIMNIIDIISIIPYFATLITELVQETE--
--------------------PSAQQNMSLA-ILRIIRLVRVFRIFKLSRHS-----KGL-
QILGQTLKASMRE
>88 rat_Kv1.2 2R9R_b_vs gi|16087779|
WLLFEYPESSGPARIIAIVSVMVILISIVSFCLETLPIFR----------------DENE
DMHGGGVT-----------FHTYSQSTIGYQQ----STSFTDP----FFIVETLCIIWFS
FEFLVRFFACP------------SKAGFFTNIMNIIDIVAIIPYYVTI--FLTESN---
--------------------KSVLQFQNVRRVVQIFRIMRILRIFKLSRHS-----KGL-
Q------------
>89 homo_Kv gi|4826782|
WLLFEYPESSGPARIIAIVSVMVILISIVSFCLETLPIFR----------------DENE
DMHGSGVT-----------FHTYSNSTIGYQQ----STSFTDP----FFIVETLCIIWFS
FEFLVRFFACP------------SKAGFFTNIMNIIDIVAIIPYFITLGTELAEKPED-
-------------------AQQGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGQTLKASMRE
>90 rat_Kv pdb:2A79_chainb
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WLLFEYPESSGPARIIAIVSVMVILISIVSFCLETLPIFR----------------DENE
DMHGGGVT-----------FHTYSNSTIGYQQ----STSFTDP----FFIVETLCIIWFS
FEFLVRFFACP-------------SKAGFFTNIMNIIDIVAIIPYFITLGTELAEKPED-
-------------------AQQGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGQTLKASMRE
>91 canis_KCNA2 sp_Q28293
WLLFEYPESSGPARIIAIVSVMVILISIVSFCLETLPIFR----------------DENE
DMHGGGVT-----------FHTYSNSTIGYQQ----STSFTDP----FFIVETLCIIWFS
FEFLVRFFACP-------------SKAGFFTNIMNIIDIVAIIPYFITLGTELAEKPED-
-------------------AQQGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGQTLKASMRE
>92 drosophila_shaker_Kchannel gi|288442|
WLLFEYPESSQAARVVAIISVFVILLSIVIFCLETLPEFK----------------HYKV
FNTTT-----------------NGTKIEEDE----VPDITDP----FFLIETLCIIWFT
FELTVRFLACP-------------NKLNFCRDVMNVIDIIAIIPYFITLATVVAEEEDTL
NLPK---------APVSPQDKSSNQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
QILGRTLKASMRE
>93 rabbit_KCND3 sp_Q9TTT5
WRAFENPHTSTLALVFYYVTGFFIAVSVITNVVETVPCGT----------------VPGS
--------------------------KELPC----GERYSVA----FFCLDTACVMIFT
VEYLLRLFAAP-------------SRYRFIRSVMSIIDVVAIMPYYIGLVMTNNE-----
-------------------------DVSG-AFVTLRVFRVFRIFKFSRHS-----QGL-
RILGYTLKSCASE
>94 hum_CACNA1E_repeat_2_sp_Q15878
RHMVKS-------QVFYWIVLSLVALNTACVAIVHHNQ----------------------

-----------------------------------PQWLTHL----LYYAEFLFLGLFL
LEMSLKMYGMG--------------PRLYFHSSFNCFDFGVTVGSIFEVVWAIFRP----
-------------------------GTSF-GISVLRALRLLRIFKITKYW-----ASL-
RNLVVSLMSSMKS
>95 drosophila_CAC1A_repeat_2 sp_P91645
RHTVKT-------QWFYWFVIVLVFLNTVCVAVEHYGQ----------------------
-----------------------------------PSFLTEF----LYYAEFIFLGLFM
SEMFIKMYALG--------------PRIYFESSFNRFDCVVISGSIFEVIWSEVK-----
-------------------------GGSF-GLSVLRALRLLRIFKVTKYW-----SSL-
RNLVISLLNSMRS
>96 mouse_SCN11A_repeat2 sp_Q9R053
QTIMTD-------PFTELAITICIIVNTVFLAMEHHNM----------------------
-----------------------------------DNSLKDI----LKIGNWVFTGIFI
AEMCLKIIALD--------------PYHYFRHGWNIFDSIVALVSLADVLFHKLSK----
-------------------------------NLSFLASLRVLRVFKLAKSW-----PTL-
NTLIKIIGHSVGA
>97 rat_SCN11A_repeat2 sp_O88457
RTIMTD-------PFTELAITICIIINTVFLAVEHHNM----------------------
-----------------------------------DDNLKTI----LKIGNWVFTGIFI
AEMCLKIIALD--------------PYHYFRHGWNVFDSIVALLSLADVLYNTL------
--------------------------SDN-NRSFLASLRVLRVFKLAKSW-----PTL-
NTLIKIIGHSVGA
>98 rat_SCN9A_repeat2 sp_O08562
YFIVMD-------PFVDLAITICIVLNTLFMAMEHHPM----------------------
-----------------------------------TEEFKNV----LAVGNLIFTGIFA
AEMVLKLIAMD--------------PYEYFQVGWNIFDSLIVTLSLIE-LFLADVE----
-------------------------------GLSVLRSFRLLRVFKLAKSW-----PTL-
NMLIKIIGNSVGA


>99 ornitho_C15orf27 |gi_149410687|
WQVFLLS------ASLNSFLVACVILVVILLTLELLIDIK----------------LLQ-
---------------------------FSS----ASQFASV----VHWISLIILSVFF
TETILRIVVLG--------------IWDYIENKIEVFDGAVIILSLAPMVASTVANGP--
-------------------------SSPWD-AISLIITLRIWRVKRIIDAYVLPVKVEM-
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EMVIQQYEKA---
>100 danio_c15orf27 |gi_123703002|
WQVCLLS------AGFNCFLVACVILVVLLLTLELLIDTK----------------LLQ-
----------------------------FNN----AFQFACI----IHWISLVILSVFF
TETVFRIVVLG--------------IWDYIENKVEVFDGAVIVLSLAPMVASTVANGP--
-------------------------SSPWD-AISLIITLRIWRVKRIIDAYVLQVKVEM-
ELEIQQYEKS---
>101 monodelphis_C15orf27 gi|12627230|
WQVFLLS------ASLNSFLVACVILVVILLTLELLIDIK----------------SLQ-
----------------------------FSN----SSQFAGV----SHWISLVILSVFF
SETILRIVVLG--------------IWDYIENKIEVFDGAVIILSLAPMVASTIANGP--
-------------------------SSPWD-AISLTIALRIWRVKRIIDAYVLPVKVEL-
EMVIQQYEKA---
>102 sus_C15orf27 gi|194039682|
WQVFLLS------ASVNSFLVACVILVVILLTLELLIDIK----------------LLQ-
----------------------------FSS----AFQFAGV----IHWISLVILSVFF
SETVLRIVVLG--------------IWDYIENKIEVFDGAVIILSLAPMVASTVANGP--
-------------------------RSPWD-AISLIIMLRIWRVKRVIDAYVLPVKVEM-
EMVIQQYEKA---
>103 homo_C15orf27 |gi_118442841|
WQVFLLS------ASLNSFLVACVILVVILLTLELLIDIK----------------LLQ-
----------------------------FSS----AFQFAGV----IHWISLVILSVFF
SETVLRIVVLG--------------IWDYIENKIEVFDGAVIILSLAPMVASTVANGP--
-------------------------RSPWD-AISLIIMLRIWRVKRVIDAYVLPVKLEM-
EMVIQQYEKA---
>104 pan_C15orf27 |gi_114658268|
WQVFLLS------ASLNSFLVACVILVVILLTLELLIDIK----------------LLQ-
----------------------------FSS----AFQFAGV----IHWISLVILSVFF
SETVLRIVVLG--------------IWDYIENKIEVFDGAVIILSLAPMVASTVANGP--
-------------------------RSPWD-AISLIIMLRIWRVKRVIDAYVLPVKLEM-
EMVIQQYEKA---
>105 horse_C15orf27 |gi_149692210|
WQVFLLS------ASLNSFLVACVILVVILLTLELLIDIK----------------LLQ-
----------------------------FSS----AFQFAGV----IHWISLVILSVFF
SETVLRIVVLG--------------IWDYIENKIEVFDGAVIILSLAPMVASTVANGP--
-------------------------RSPWD-AISLIIMLRIWRVKRVIDAYVLPVKVEM-
EMVIQQYEKA---
>106 mus_C15orf27 gi|27370422|
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-------------------------FLGLR-VINLLRILVLLRIIKLRKL------EEN-
QALIN--------
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Supplementary Table S2. Gating kinetics of Asp^{112} mutants at pH 5.5//5.5 (mean ± s.e. (*n*)).

	τ_{act} at 40 mV (s)	τ_{act} at 60 mV (s)	τ_{act} at 80 mV (s)	τ_{tail} (s)
WT hH_V1	1.1 ± 0.4 (7)	1.4 ± 0.6 (4)		0.81 ± 0.07 (6)
D112E	0.2 ± 0.09 (3)	*0.26 ± 0.07 (6)	0.28 ± 0.22 (3)	†*6.0 ± 0.4 (4) 0.069 ± 0.008 (4)
D112H		2.78 ± 0.40 (8)	1.19 ±0.37 (4)	0.69 ± 0.06 (14)
D112S		2.76 ± 0.41 (9)	1.17 ± 0.17 (5)	*3.35 ± 0.25 (15)
D112A/A/A		3.03 ± 0.99 (4)	2.47 ± 0.18 (4)	*2.46 ± 0.23 (8)
D112N/A/A		3.36 ± 0.57 (7)	1.73 ± 0.07 (5)	*2.46 ± 0.21 (7)
D112K/A/A		1.06 ± 0.19 (3)	1.46 ± 0.29 (6)	*0.18 ± 0.03 (7)
D112F/A/A		2.22 ± 0.03 (3)	1.67 ± 0.23 (8)	*0.028 ± 0.0005 (6)

Mean ± s.e. (*n*) values of τ_{act} at several voltages 5 and τ_{tail} at -40 mV, all at pH 5.5//5.5 at room temperature (21°C). Because the g_H-V relationship was shifted positively in most D112x mutants, as reported previously[1], fittable currents were not present at all voltages. †Tail currents in D112E had two components that were measured at -60 mV, because at -40 mV channel closing was impracticably slow. For all mutants τ_{tail} differed significantly from WT ($p < 0.0001$) by Student's t-test. The time constant of activation (τ_{act}) of proton current during depolarizing pulses to +60 mV was obtained by fitting the current with a single rising exponential. The deactivation (channel closing, tail current) time constant (τ_{tail}) was obtained by fitting the tail current upon repolarization to a single decaying exponential. The D112E mutant had fast and slow components of tail current decay; we give the time constant of the dominant slower component. *Differs significantly from WT at $p<0.05$ (τ_{act}) by Student's t-test.

References

1. Ramsey, I. S. *et al.* An aqueous H^+ permeation pathway in the voltage-gated proton channel Hv1. *Nat. Struct. Mol. Biol.* **17**, 869-875 (2010).

Supplementary Table S3. Comparison of relative permeability values obtained from the GHK equation assuming OH^- or H^+ permeation in D112x mutants.

	V_{rev} pH 7.0 (mV)	V_{rev} pH 5.5 Cl^- (mV)	P_{CH3SO3}/P_{Cl} ($P_H = 0$)	P_{OH}/P_{Cl} ($P_H = 0$)	P_{CH3SO3}/P_{Cl} ($P_{OH} = 0$)	P_H/P_{Cl} ($P_{OH} = 0$)
WT hH_V1	-80.9	-0.3	-	-	-	-
D112E	-80.7	-0.1	-	-	-	-
D112H	-58.4	-37.5	0.14	3.0×10^6	0	1.6×10^4
D112K	-51.7	-20.6	0.35	4.6×10^6	0.04	2.6×10^4
D112N	-12.5	-33.1	0.23	0.3×10^6	0.13	0.5×10^4
D112S	-25.9	-40.8	0.15	0.5×10^6	0.04	0.6×10^4
D112A	-33.8	-28.8	0.27	1.4×10^6	0.07	1.3×10^4
D112F	-36.4	-33.5	0.20	1.2×10^6	0.04	1.1×10^4

Column 2 is the mean change in V_{rev} when pH_o was changed from 5.5 to 7.0, in $CH_3SO_3^-$ solutions (Fig. 2b). The third column is the change in V_{rev} measured when Cl^- replaced $CH_3SO_3^-$ at pH_o 5.5 (Fig. 3g). Columns 4 and 5 show P_{CH3SO3}/P_{Cl} and P_{OH}/P_{Cl} values obtained from the GHK equation (Eq. 1) by fitting the data in columns 2 and 3, assuming that the shift in V_{rev} in Column 2 is due mainly to OH^- permeation ($P_H = 0$). Columns 6 and 7 show analogous results, but assuming that the shift in V_{rev} in Column 2 is due mainly to H^+ permeation ($P_{OH} = 0$).

Although the data can be fitted assuming that either H^+ or OH^- is permeant, distinct predictions apply to sucrose dilution experiments at different pH_o. Sucrose effects should be larger at pH_o 5.5 than 7.0 if OH^- is permeant, because $[OH^-]$ is 32 times larger at pH_o 7.0, and $[OH^-]$ remains constant as other anions are diluted. The term $P_{OH}[OH^-]_o$ (in Eq. 1) will have a greater effect on V_{rev} at pH_o 7.0, and because E_{OH} does not change, V_{rev} will change less.

Consistent with OH^- permeability, sucrose produced a larger shift of V_{rev} at pH_o 5.5 than at pH_o 7.0 for all mutants except D112K (Fig. 3e & 3f). By similar reasoning, substituting Cl^- for $CH_3SO_3^-$ should shift V_{rev} more at pH_o 5.5 than at pH_o 7.0 if OH^- is permeant. Fig. 3g shows that this occurred in all six anion selective mutants. The data consistently point to a high OH^- permeability in D112x mutants.

Outward H^+ flux and inward OH^- flux both likely occur by a Grotthuss mechanism in a single-file channel, a more efficient permeation mechanism than diffusion used by other ions. H^+ moves in water by hopping from H_3O^+ to H_2O, whereas OH^- conduction occurs when OH^- extracts a proton from a nearby H_2O. The latter process involves proton transfer between neutral and negatively charged species, and thus seems more probable for an anion selective channel. Although we cannot rule out the possibility that H^+ carries some current, the sucrose dilution results indicate a distinct preference for anions, suggesting that OH^- permeation is more likely than H^+ permeation in the D112x mutant channels.

That WT hH_V1 conducts H^+ rather than OH^- is based mainly on the unitary conductance increasing at low pH_i. Lowering pH_i from 6.5 to 5.5 to 5.0 to 4.1 increased the conductance from 37 to 139 to 220 to 400 pS, respectively[1]. In contrast, changing pH_o from 7.5 to 6.5 had no effect. These results are consistent with increased conductance by increasing permeant ion concentration $[H^+]$ on the proximal side of the membrane. That deuterium reduced the conductance by 50% also supports H^+ permeation[2] through WT H_V1 channels.

References

1. Cherny, V. V., Murphy, R., Sokolov, V., Levis, R. A. & DeCoursey, T. E. Properties of single voltage-gated proton channels in human eosinophils estimated by noise analysis and by direct measurement. *J Gen Physiol* **121**, 615-28 (2003).
2. DeCoursey, T. E. & Cherny, V. V. Deuterium isotope effects on permeation and gating of proton channels in rat alveolar epithelium. *J Gen Physiol* **109**, 415-34 (1997).

Supplementary Table S4. Monovalent cation substitution does not change V_{rev} in anion selective Asp^{112} mutant channels.

Mutant	Ion	Raw ΔV_{rev} (mV)	V_{jct} correction (mV)	Corrected ΔV_{rev} (mV)
Various	Na^+	+0.5 ± 0.6 (6)	+1.3	+1.8
D112N/A/A	Na^+	-1.8 ± 0.6 (4)	+1.3	-0.5
D112N/A/A	TEA^+	+1.8 ± 0.6 (6)	-3.4	-1.6
D112N/A/A	K^+	-5.3 ± 1.7 (4)	+4.8	-0.5
D112N/A/A	$NMDG^+$	+6.6 ± 0.6 (4)	-6.0	+0.6

Mean ± s.e. (*n*) values of the change in V_{rev} relative to standard TMA^+ solution, when TMA^+ was replaced by the indicated cation (all at ~130 mM), all at pH 5.5//5.5 at room temperature (21°C). TEA^+ is tetraethylammonium$^+$, $NMDG^+$ is N-methyl-D-glucamine$^+$. Many COS-7 cells had endogenous K^+ conductances that prevented V_{rev} measurement. "Various" includes 1 D112H, 1 D112A, 1 D112S, and 3 D112N/D185M cells. The raw measured values are given (column 2), the correction for measured liquid junction potential differences (Column 3), and the corrected V_{rev} values (Column 4). The net values are of the same magnitude as the junction potential corrections, and well within the error of the measurements.