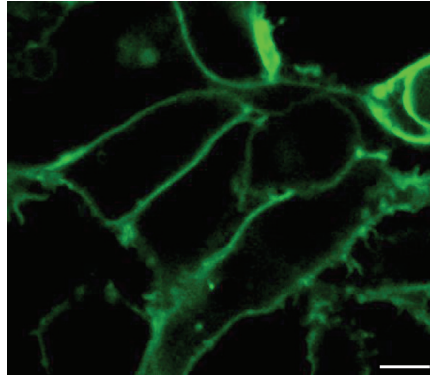


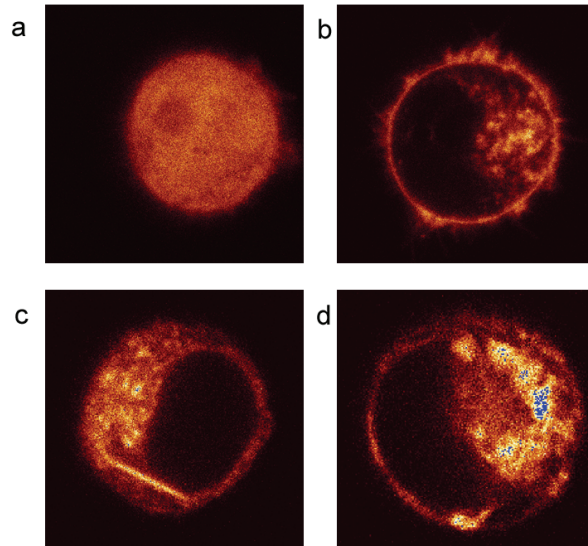
Supplementary Figure S1. Phylogenetic relationship between the proton channel (H_v1) and C15orf27 families and other VSD-containing proteins.

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 μ m.

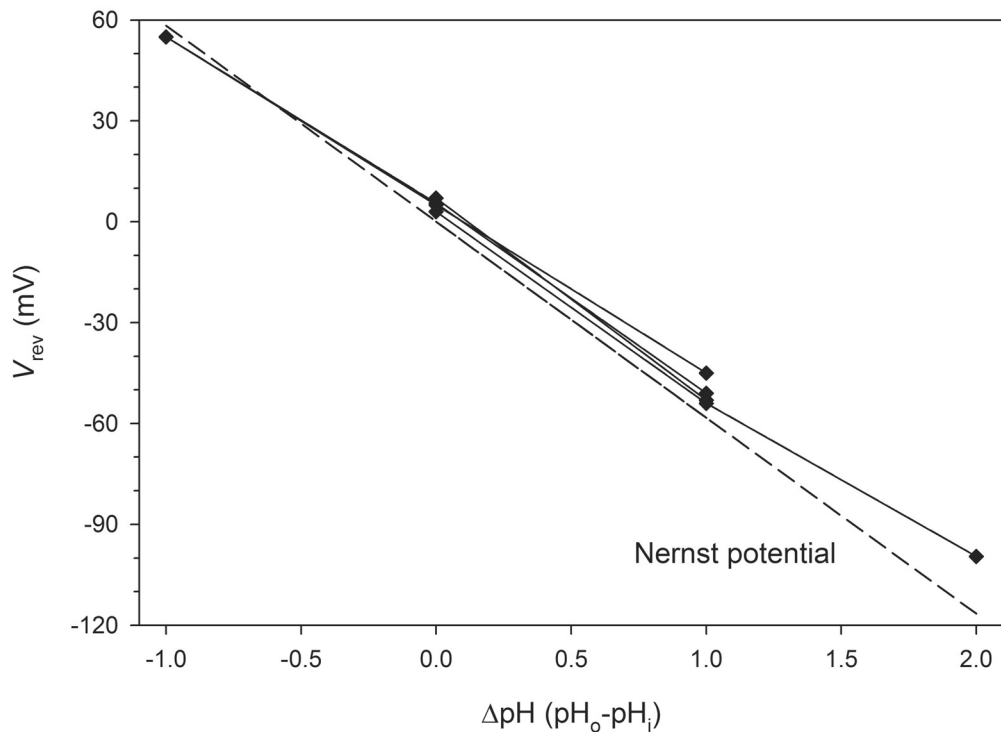
Supplementary Figure S3. GFP-tagged constructs appear to localize to the plasma membrane.



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 μ l 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.

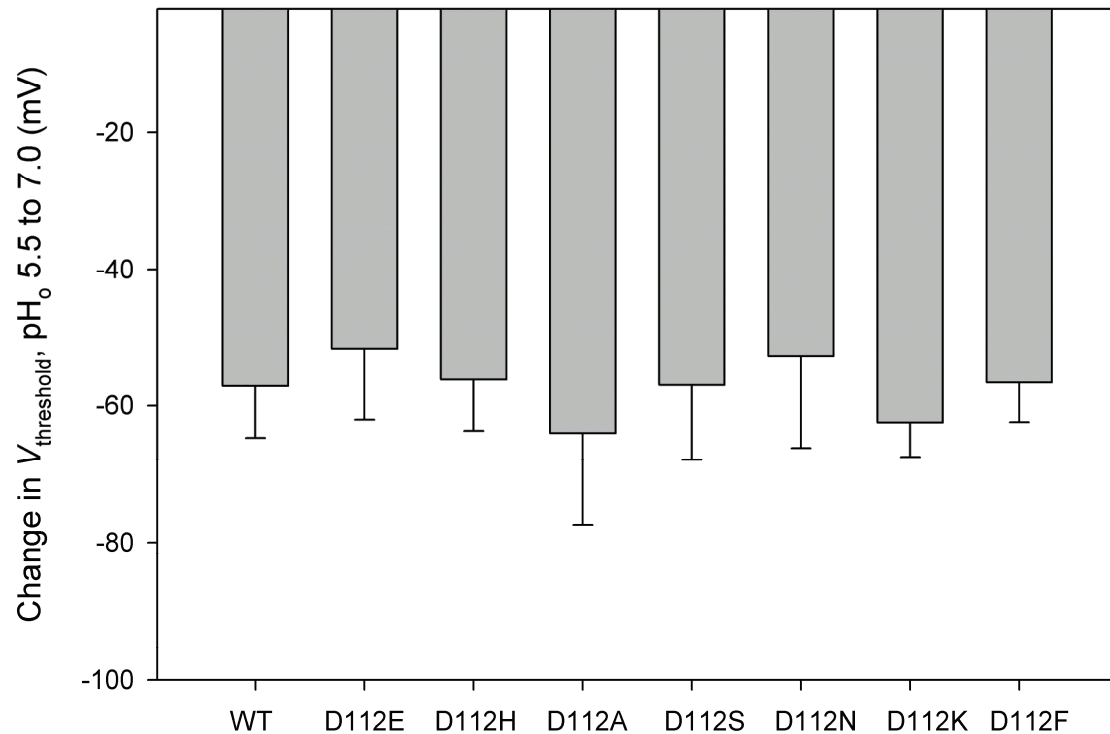


In inside-out patches of membrane from HEK-293 cells expressing H140A/H193A channels, V_{rev} was measured over a range of pH. Measurements in the same patch are connected by lines. This double mutant was used as a background for several of the Asp¹¹² mutants in order to validate that any currents detected 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 mV¹. The H140A/H193A mutation nearly eliminates inhibition by Zn^{2+} (refs. 2,3).

References

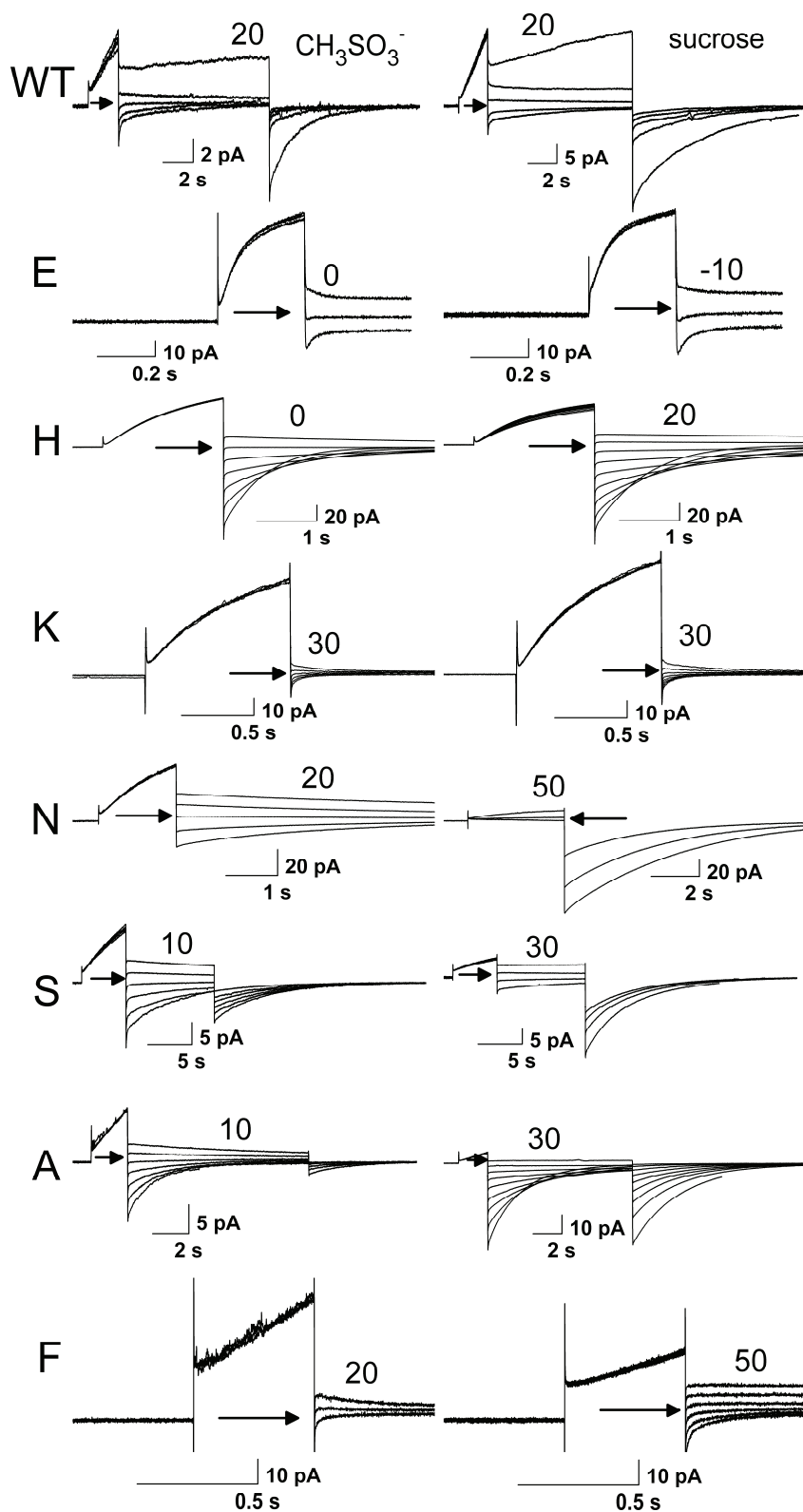
1. Cherny, V. V. & DeCoursey, T. E. pH-dependent inhibition of voltage-gated H^+ currents in rat alveolar epithelial cells by Zn^{2+} and other divalent cations. *J Gen Physiol* **114**, 819-38 (1999).
2. Musset, B. et al. Zinc inhibition of monomeric and dimeric proton channels suggests cooperative 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 Asp¹¹² does not eliminate the Δ pH dependence of gating.



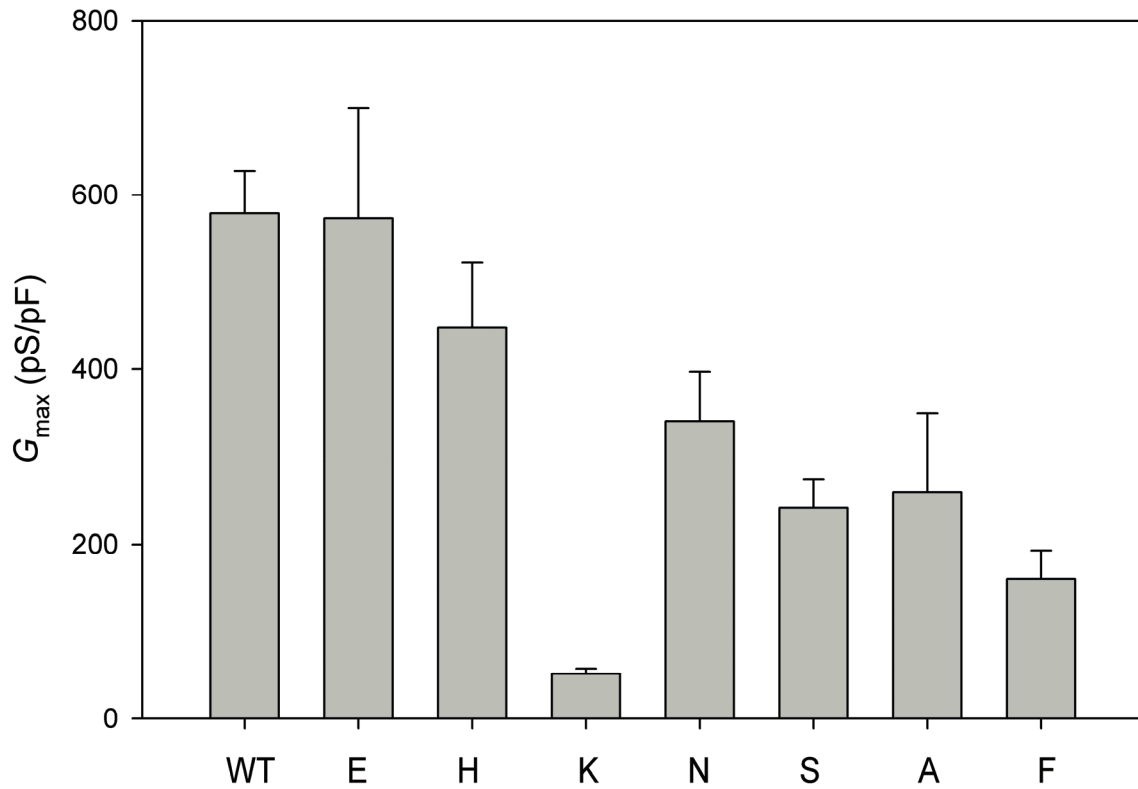
In whole-cell measurements like those in Fig. 2, $V_{\text{threshold}}$ was estimated as the voltage at which distinct conductance was activated, usually determined from tail currents, which are more sensitive. The graph shows the mean \pm SD shift of $V_{\text{threshold}}$ when pH_o 5.5 and 7.0 are 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 Asp¹¹² mutants are anion selective.

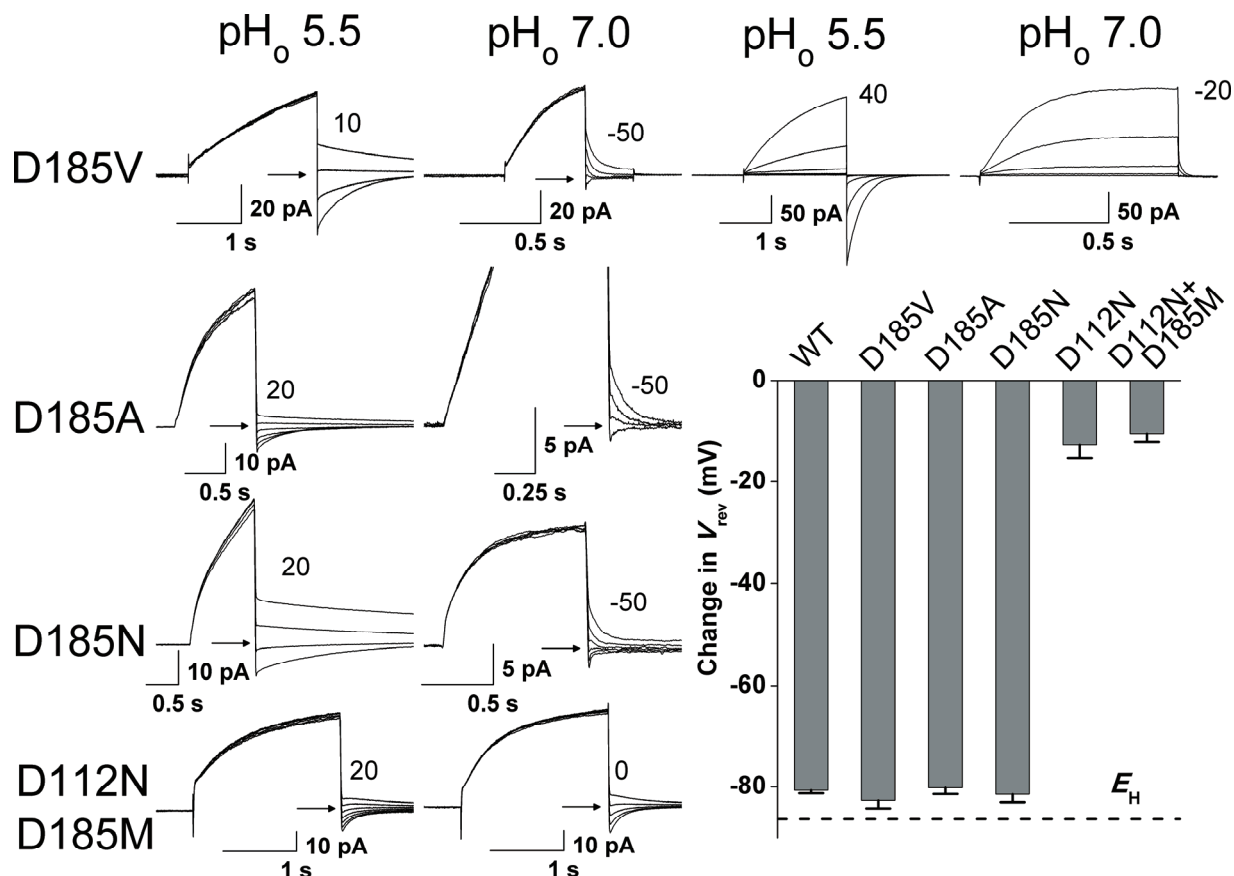


Measurement of V_{rev} by tail currents (or direct reversal of test current in D112N in sucrose) at pH 5.5//5.5 (CH_3SO_3^- solutions) is shown in the first column, and in the same cell after 90% dilution of the bath by isotonic sucrose in the second column. Arrows indicate zero current. Letters indicate 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 Asp¹¹² 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.

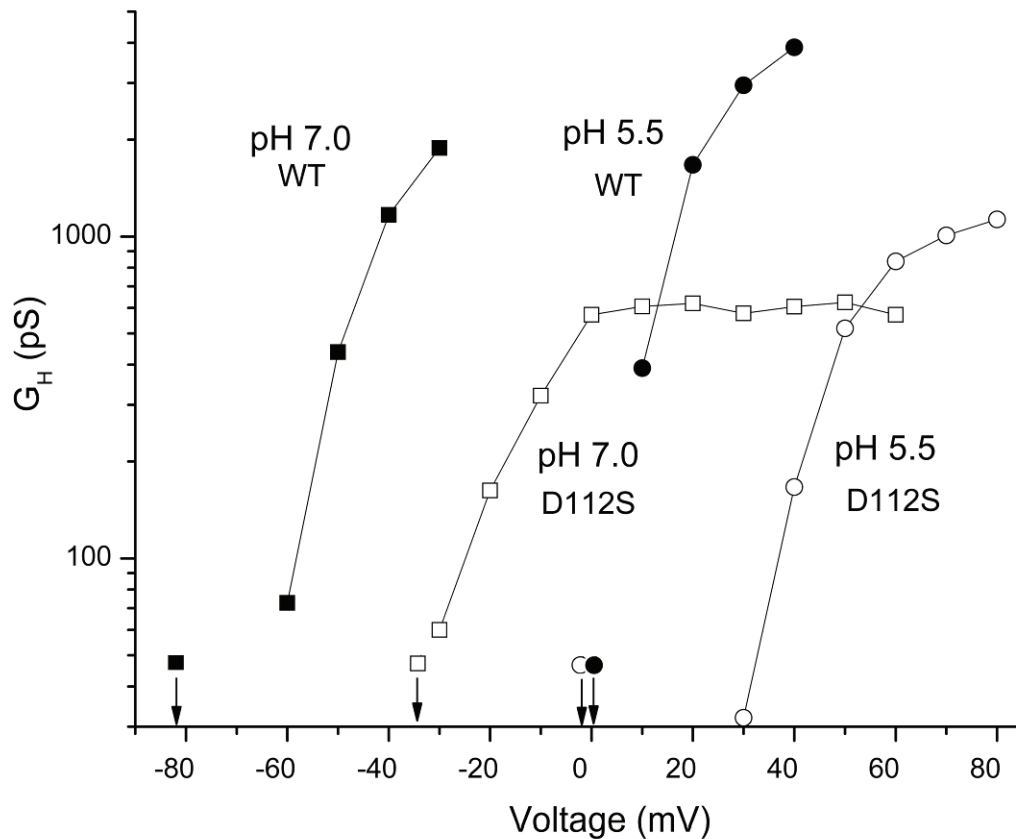
Supplementary Figure S8. Mutation of Asp¹⁸⁵ does not impair proton selectivity.

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 Asp¹⁸⁵ neutralization to an anion selective Asp¹¹² 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 Asp¹⁸⁵ 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 Asp¹¹² 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 $V_{\text{threshold}}$ (or the g - 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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IKVLVH-----PFFQLFILISVLIDCVFMSLTN-----
-----LPKW-----RPVLENTLLGIYT
FEILVKLFARGVWA-----GSFSFLGDPWNWLD FSVTVFEVIIRYSPLD-----
-----FIPTLQTARTLRILKIIPLN-----QGL-
KSLVGVLHCLKQ
>45 rabbit_CAC1C_repeat1 sp_P15381
ISIVEW-----KPF EIIILLTIFANCVALAIYIPFPED-----
-----DSNATNSN-----LERVEYLFLII FT
VEAFLKVIAYGLLF-----HPNAYLRNGWNLLDFIIVVVGLFSAILEQATK-AD-
-----GANALGGKGAGF-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSIIKAMV-
>46 mouse_CAC1S_repeat1 sp_Q02789
ISIVEW-----KPFETIILLTIFANCVALAVYLPMPED-----
-----DNNTLNLG-----LEKLEYFFLIVFS
IEAAMKIIAYGFLF-----HQDAYLRSGWNVLD FIIIVFLGVFTVILEQVNI IQT-
-----NTAPMSSKGAGL-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSIFKAML-
>47 mouse_CAC1F_repeat1 sp_Q9JIS7
ISIVEW-----KPF DILILLTIFANCVALGVYIPFPED-----
-----DSNTANHN-----LEQVEYVFLVI FT
VETVLKIVAYGLVL-----HPSAYIRNGWNLLDFIIVVVGLF SVLLEQGPGRPG-
-----DAPHTGGKPGGF-DVKALRAFRVLRPLRLVSGV-----PSL-
HIVVNSIMKALV-
>48 gallus_CAC1D_repeat1 sp_Q73700
ISLVEW-----KPF DIFILLSIFANCVALAVYIPFPED-----
-----DSNSTNHN-----LEKVEYAFLLI FT
VETFLKIIAYGLLL-----HPNAYVRNGWNLLDFVIVVVGLF SVILEQLTKETE-
-----GGSHSGGKPGGF-DVKALRAFRVLRPLRLVSGV-----PSL-
QVVLNSIIKAMV-
>49 homo_CACN_repeat1 gi|193788728|
ISIVEW-----KPF EIIILLTIFANCVALAIYIPFPED-----
-----DSNATNSN-----LERVEYLFLII FT

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VEAFKVIAYGLLF-----HPNAYLRNGWNLDFIIVVVGLFSAILEQATKADG-
 -----ANALGGKGAGF-DVKALRAFRVLRPLRLVSGV-----PSL-
 QVVLNSIIKAMVP
 >50 drosophila_CAC1D_repeat1 sp_Q24270
 IRIVEW-----KPFEFLLILLTIFANCIALAVYTPYPGS-----
 -----DSNVTNQT----LEKVEYVFLVIIFT
 AECVMKILAYGFVL-----HNGAYLRNGWNLDFIIVVIGAISTALSQMK----
 -----DAF-DVKALRAFRVLRPLRLVSGV-----PSL-
 QVVLNSILKAMV-
 >51 homo_CAC1A_repeat1 sp_O00555
 KKITEW-----PPFEYMILATIIANCIIVLALAQHLPDD-----
 -----DKTPMSER----LDDTEPYFIGIFC
 FEAGIKIIALGFAG-----HKGSYLRNGWNVMDVFFVVLTGILATVGTEF-----
 -----DLRTLRAVRVLRPLKLVSGI-----PSL-
 QVVLKSIMKAMIP
 >52 homo_CAC1B_repeat1 sp_Q00975
 KRITEW-----PPFEYMILATIIANCIIVLALAQHLPDG-----
 -----DKTPMSER----LDDTEPYFIGIFC
 FEAGIKIIALGFVF-----HKGSYLRNGWNVMDVFFVVLTGILATAGTDF-----
 -----DLRTLRAVRVLRPLKLVSGI-----PSL-
 QVVLKSIMKAMV-
 >53 rat_SCN11A_repeat3 sp_O88457
 YQIVKH-----SWFESFIIIFVILLSSGALIFEDVNLPS-----
 -----RPQVEKL----LRCTDNIFTFIFL
 LEMILKWVAFGF-----RRYFTSAWCWLDLIVVVSVLSLMNLP-----
 -----SLKSFRTLRLRPLRALSQF-----EGM-
 KVVVYALISAIPA
 >54 mouse_SCN11A_repeat3 sp_Q9R053
 YQIVKH-----SWFESFIIIFVILLSSGALIFEDVNLPS-----
 -----RPQVEKL----LKCTDNIFTFIFL
 LEMILKWVAFGF-----RKYFTSAWCWLDLIVVVSVLSLTNLP-----
 -----NLKSFRTLRLRPLRALSQF-----EGM-
 KVVVNALMSAIPA
 >55 rat_SCN9A_repeat3 sp_O08562
 YRIVEH-----SWFESFIVLMILLSSGALAFEDIYIEK-----
 -----KKTIIKII----LEYADKIIFTYIFI
 LEMLLKWVAYGY-----KTYFTNAWCWLDLIVDVSLVTLVANTLGYSDLG
 -----PIKSLRTLRLRPLRALSFR-----EGM-
 RVVVNALIGAIPS
 >56 rabbit_SCN9A_repeat3 sp_Q28644
 YRIVEH-----SWFESFIVLMILLSSGALAFEDIYIEK-----
 -----KKTIIKII----LEYADKIIFTYIFI
 LEMLLKWVAYGY-----KTYFTNAWCWLDLIVDVSLVTLVANTLGYSDLG
 -----PIKSLRTLRLRPLRALSFR-----EGM-
 RVVVNALIGAIPS
 >57 mouse_SCN9A_repeat3 uniprot_B7ZWN
 YRIVEH-----SWFESFIVLMILLSSGALAFEDIYIEK-----
 -----KKTIIKII----LEYADKIIFTYIFI
 LEMLLKWVAYGY-----KTYFTNAWCWLDLIVDVSLVTLVANTLGYSDLG
 -----PIKSLRTLRLRPLRALSFR-----EGM-
 RVVVNALIGAIPS
 >58 mouse_KCNH1 sp_Q60603
 -----TWDWIILILTFYTAILVPYNVFSK-----
 -----TRQNNVA----WLVDIVDVIFL
 VDIVLNFHTTFVGPAGEVISDPKLIRMNYLK-TWFVIDLLSCLPYDVINAFENVDEVSFAF
 MGDPGKIGFADQIPPLEGRESQGISSLFS-SLKVVRLRLRGRVARKLDHY-----IEY-
 GAAVLV-----
 >59 mouse_KCNH8 sp_P59111
 -----GWDWLILLATFYVAVTVPYNVCFIGN-----
 -----EDLSTTRS----TTVSDIAVEILFI
 IDIILNFRTTYVSKSGQVIFEARSICIHVVT-TWFIIDLIAALPFDLLYAFNVTV-----

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-----VSLVH-LLKTVRLLRLLRLLQKLDRY-----SQH-
STIVLTLLMSM--
>60 homo_KCNH3 sp_Q9ULD8
-----TWDFGILLATLYVAVTVPVYVVCVSTA-----
-----REPSAARGP----PSVCDLAVEVLF
LDIVLNFRTTFVSKSGQVVFAPKSI CLHYVT-TWFLLDVIAALPFDLLHAFKVVN-----
-----YFGAH-LLKTVRLLRLLRLLPRLDRY-----SQY-
SAVVL-----
>61 homo_CAC1G_repeat4 sp_O43497
HHLCTS-----HYLDLFIITGVIGLNVVVTMAMEHYQQ-----
-----PQILDEA----LKICNYIFTVIFV
LESVFKLVAFG-----FRRFFQDRWNQLDLAIVLLSIMGITLEEIEVNA--
-----SLPINPT-IIRIMRVLRIARVLKLLKMA-----VGM-
RALLDVTVMQALPQ
>62 mouse_SCN11A_repeat4 sp_Q9R053
FDLVTS-----QVFDVIILGLIVTNMIIMMAESEGO-----
-----PNEVKKI----FDILNIVFVVI FT
VECLIKVFALR-----QHYFTNGWNLFDCV VVVLSIISTLVSGLENSN--
-----VFPPT-LFRIVRLARIGRILRLVRAA-----RGI-
RTLLFALMMSLPS
>63 rat_SCN9A_repeat4 sp_O08562
FDLVTN-----QAFDITIMVLI CLNMVTMMVEKEGO-----
-----TEYMDYV----LHWINMVFIILFT
GECVLKLLISLR-----HYYFTVGWNIFDFV VVILSIVGMFLAEMIEKY--
-----FVSPT-LFRVIRLARIGRILRLIKGA-----KGI-
RTLLFALMMSLPA
>64 rat_SCN11A_repeat4 sp_O88457
FDLVTS-----QVFDVIILGLIVLNMIMMAESADQ-----
-----PKDVKKT----FDILNIAFVVI FT
IECLIKVFALR-----QHYFTNGWNLFDCV VVVLSIISTLVSRLEDS--
-----ISFPPT-LFRVVRLARIGRILRLVRAA-----RGI-
RTLLFALMMSLPS
>65 humo_CAC1G_repeat2 sp_O43497
RKIVDS-----KYFGRGIMIAIILVNTLSMGIEYHEQ-----
-----PEELTNA----LEISNIVFTSLFA
LEMLLKL LVYG-----PFGYIKNPYNIFDGVIVVISVWEIVGQGG-----
-----GLSVLRTFRLMRVLKLVRF-----PAL-
QRQLVVLMTMDN
>66 homo_CACNA1E_repeat_4 sp_Q15878
WHFVVS-----PSFEYTIMAMIALNTVVLMMKYISA-----
-----PCTYELA----LKYLNIAFTMVFS
LECVLKVIAFG-----FLNYFRDWNIFDFITVIGSITEI ILTDSKLVN--
-----TSGF-NMSFLKLFRAARLIKLLRQG-----YTI-
RILLWTFVQS FKA
>67 drosophila_CAC1A_repeat_4 sp_P91645
WRIVVS-----TPFEYFIMMLIVFNTLLMMKYHNQ-----
-----GDMYEKS----LKYINMGFTGMFS
VETVLKIIGFG-----VKNFFKDPWNIFDLITVLSIVDALWMEFGHDD--
-----SNSI-NVGFLRLFRAARLIKLLRQG-----YTI-
RILLWT-----
>68 homo_KCNV2 sp_Q8TDN2
WNLMEKPFSSVA AKAIGVASSTFVLVSVVALALNTVEEMQ-----QHSG
-----QEGE----GPDLRPI----LEHVEMLCMGFFT
LEYLLRLASTP-----DLRRFARSALNLVDLVAILPLYLQLLLECFTGEGHQ
-----RGQTVGSVGVKVGQ-VLRVMRLMRIFRILKLARHS-----TGL-
RAFGFTLRQCYQQ
>69 homo_KCNF1 sp_Q9H3M0_KCNF1
WKFLEKPESSCPARVVAVLSFLLIILVSSVVMCMGTIPELQ-----VLD-
-----AEG----NRVEHPT----LENVETACIGWFT
LEYLLRLFSSP-----NKLHFALSF MNIVDVLAILPFYVSLTLTHLGAR--
-----MMELTNVQQ-AVQALRIMRIARIFKLARHS-----SGL-

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QTLTYALKRSFKE
 >70 homo_KCNB1 sp_Q14721
 WDLLEKPNSSVAAKILAIISIMFIVLSTIALSLNTPPELQ-----SLD-
 -----EFG----QSTDNPQ----LAHVEAVCIAWFT
 MEYLLRFLSSP-----KKWKFFKGPLNAIDLLAILPYYVTIFLTESNKS---
 -----VLQFQNVRR-VVQIFRIMRILRILKLARHS-----TGL-
 QSLGFTLRRSYNE
 >71 canis_KCNB2 sp_Q95167
 RDLEKPNSSVAAKILAIIVSNLFIVLSTIALSLNTPPELQ-----EMD-
 -----EFG----QPNDNPQ----LAHVEAVCNAWFT
 MEYLLRFLSSP-----NKWKFFKGPLNVIDLLAILPYYVTIFLTESNKS---
 -----VLQFQNVRR-VVQIFRIMRILRILKLARHS-----TGL-
 QSLGFTLRRSYNE
 >72 drosophila_KCNAB sp_P17970
 WELLEKPNSTFAARVIAVISILFIVLSTIALTLNTPQLQ-----HIDN
 G-----TPQDNPQ----LAMVEAVCITWFT
 LEYILRFSASP-----DKWKFFKGGLNIIDLLAILPFVSLFLETNKN---
 -----ATDQFQDVRV-VVQVFRIMRILRVLKLARHS-----TGL-
 QSLGFTLRNSYKE
 >73 pongo_KCNV1 sp_Q5RC10
 WNILEKPGSSSTAARIFGVISIIIFVVVSIINMALMSAEL-----
 -----SWLDLQL----LEILEYVCISWFT
 GEFVLRFLCVR-----DRCRFLRKVPNIIDLLAILPFYITLLVESLSGSQ--
 -----TTQELENVGR-IVQVLRLLRMLKLGRHS-----TGL-
 RSLGMTITQCYEE
 >74 homo_KCNS3 sp_Q9BQ31
 WIRMENPAYCLSAKLIAISSLSVVLASIVAMCVHSMSEFQ-----NED-
 -----GEVDDPV----LEGVEIACIAWFT
 GELAVRLAAAP-----CQKKFWKNPLNIIDFVSIIPFYATLAVDTKEEE---
 -----SEDIENMGK-VVQILRLMRIFRILKLARHS-----VGL-
 RSLGATLRHSYHE
 >75 squirrelmonkey_KCNS1 sp_A4K2X4
 WLTMENPGYSLPSKLFSCVSIISVVLASIAAMCIHSLPEYQ-----AREA
 AAAVA-----AVAAGRSAE----GVRDDPV----LRRLEYFCIAWFS
 FEVSSRLLLAP-----STRNFFCHPLNLIIDIVSVLPFYLTLLAGAALGDQG-
 -----GTGGKEFGHLGK-VVQVFRMLRIFRVLKLARHS-----TGL-
 RSLGATLKHYSYRE
 >76 gallus_KCNG2 sp_O73606
 RDMVENPHSGIPGKIFACISISFVAITAVSLCISTMPDVR-----EEE-
 -----DRGE----CSQKCYD----IFVLETVCVAVWS
 FEFLLRISIQAE-----NKCAFLKTPLNIIIDILAILPFYISLIVDMASKNNS
 KP-----GGGAGNKYLERVGL-VLRFLRALRILYVMRLARHS-----LGL-
 QTLGLTVRRCTRE
 >77 homo_KCNG4 sp_Q8TDN1
 REMVENPQSGLPKVFACLSILFVATTAVSLCVSTMPDLR-----AEE-
 -----DQGE----CSRKCY----IFIVETICVAVWS
 LEFCLRFVQAQ-----DKCQFFQGPLNIIDILAI SPYVSLAVSEPPEDGE
 -----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
 WALFEDPYSSRAARFIAFASLFFILVSIITTFCLETHEAFN-----IVKN
 KTEPV-----INGTSVVLQY----EIETDPA----LTYVEGVCVVWFT
 FEFLVRIVFSP-----NKLEFIKNLLNIIDFVAILPFYLEVGLSGLSSK---
 -----AAKDVLG-FLRVVRFVRILRIFKLTRHF-----VGL-
 RVLGHTLRASTNE

>80 drosophila_KCNAW_sp_P17972
 WSLFDEPYSSNAAKTIGVSVVFFICISILSFCLKTHPDMR-----VPIV
 RNITVKT-----ANGSNGWFLDKT----QTNAHIA----FFYIECVNAWFT
 FEILVRFISSP-----NKWEFIKSSVNIIDYIATLSFYIDLVLQRFAS----
 -----HLENAD-ILEFFSIIRIMRLFKLTRHS-----SGL-
 KILIQTFRASAKE
 >81 homo_KCNA1_sp_Q09470
 WLLFEYPESSGPARGIAIVSVMVILISIVIFCLETLPELK-----DDKD
 F----TGT-----VHRIDNTTVIYN-----SNIFTDP----FFIVETLCIIWFS
 FELVVRFFACP-----SKTDFFRNIMNFIDIVAIIPYFITLGTETAEQE-G-
 -----NQGGEQATSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGQTLKASMRE
 >82 rat_KNCA6_sp_P17659
 WLLFEYPESSGPARGIAIVSVLVILISIVIFCLETLQPFRADGRGGSNEGSGTRMSPASR
 GSHEEEDDEDSDYAFPGSIPSGGLGTGGTSSSFSTLGGSFFTDP----FFLVETLCIVWFT
 FELLVRFSAACP-----SKAAFFRNIMNIIDLVAIFPYFITLGTETLVQRHEQQ
 PV-----SGGSGQNROQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGKTLQASMRE
 >83 homo_KCNA5_sp_P22460
 WLIFEYPESSGSARAIIVSVLVILISIIITFCLETLPEFRDEREL-----LRHPPAPHQ
 PPAPAPGANGS-----GVMAPPSGPTVAPLL----PRTLADP----FFIVETTCVIWFT
 FELLVRFACF-----SKAGFSRNIMNIIDVVAIFPYFITLGTETLAEQQPGG
 G-----GGGQNGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGKTLQASMRE
 >84 rat_KCNA3_sp_P15384
 WLLFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFR-----DEKD
 YPASPSQDV-----FEAANNSTSGASSG----ASSFSDP----FFVETLCIIWFS
 FELLVRFACF-----SKATFSRNIMNLIDIVAIIPYFITLGTETLAERQ---
 -----GNGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGQTLKASMRE
 >85 canis_Kv1.3_gi|57088651|
 WLLFEYPESSGPARGIAIVSVLVILVSVIVIFCLETLPEFR-----DDKD
 YAAAAQEQ-----PEAARNGTSGPPA----AAGFADP----FFVETLCIIWFS
 FELLVRFACF-----SKATFSRNIMNLIDIVAIIPYFITLGTETLAERQ---
 -----GNGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGQTLKASMRE
 >86 bovine_KCNA4_sp_Q05037
 WLLFEYPESSSPARGIAIVSVLVILISIVIFCLETLPEFR-----DDR
 LIMALSTGGHG-----GLLNDTSAPHPENSG----HTIFNDP----FFIVETVCIVWFS
 FEFVVRFCFACP-----SQALFFKNIMNIIDIVSILPYFITLGTDLAQQQGG-
 -----GNGQQQQAMSF A-ILRIIRLVRVFRIFKLSRHS-----KGL-
 QILGHTLRASMRE
 >87 homo_KCA10_sp_Q16322
 WLLFEYPESSSAARAVAVSVLVVVISITIFCLETLPEFR-----EDRE
 LKVVRD-----PNLNMSKTVLS----QTMFTDP----FFMVESTCIVWFT
 FELVLRVFCFACP-----SKTDFFRNIMNIIDIIISIIIPYFATLITELVQETE--
 -----PSAQQNMSLA-ILRIIRLVRVFRIFKLSRHS-----KGL-
 QILGQTLKASMRE
 >88 rat_Kv1.2_2R9R_b_vs_gi|16087779|
 WLLFEYPESSGPARIIAIVSVMVILISIVSFCLETLPIFR-----DENE
 DMHGGGVV-----FHTYSQSTIGYQQ----STSFTDP----FFIVETLCIIWFS
 FEFLVRFACF-----SKAGFFTNIMNIIDIVAIIPYVVTI--FLTESN---
 -----KSVLQFQNVRRVQIFRIMRILRIFKLSRHS-----KGL-
 Q-----
 >89 homo_Kv_gi|4826782|
 WLLFEYPESSGPARIIAIVSVMVILISIVSFCLETLPIFR-----DENE
 DMHGGGVV-----FHTYSNSTIGYQQ----STSFTDP----FFIVETLCIIWFS
 FEFLVRFACF-----SKAGFFTNIMNIIDIVAIIPYFITLGTETLAEKPED-
 -----AQQGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGQTLKASMRE
 >90 rat_Kv_pdb:2A79_chainb

WLLFEYPESGPARIIAIVSVMVILISIVSFCLETLPPIFR-----DENE
 DMHGGGVTF-----FHTYSNSTIGYQQ----STSFTDP----FFIVETLCIIWFS
 FEFLVRRFFACP-----SKAGFFTNIMNIIDIVAIIPYFITLGTLEAEKPED-
 -----AQQGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGQTLKASMRE
 >91 canis_KCNA2 sp_Q28293
 WLLFEYPESGPARIIAIVSVMVILISIVSFCLETLPPIFR-----DENE
 DMHGGGVTF-----FHTYSNSTIGYQQ----STSFTDP----FFIVETLCIIWFS
 FEFLVRRFFACP-----SKAGFFTNIMNIIDIVAIIPYFITLGTLEAEKPED-
 -----AQQGQQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGQTLKASMRE
 >92 drosophila shaker_Kchannel gi|288442|
 WLLFEYPESQAARVVAIISVVFVILLSIVIFCLETLPPEFK-----HYKV
 FNTTT-----NGTKIEEDE----VPDITDP----FFLIETLCIIWFT
 FELTVRFLACP-----NKLNFCRDVMNVIDIIAIIIPYFITLATVVAEEEDTL
 NLPK-----APVSPQDKSSNQAMSLA-ILRVIRLVRVFRIFKLSRHS-----KGL-
 QILGRTLKASMRE
 >93 rabbit_KCND3 sp_Q9TTT5
 WRAFENPHTSTLALVFYYVTGFFIAVSVITNVVETVPCGT-----VPGS
 -----KELPC----GERYSVA----FFCLDTACVMIFT
 VEYLLRLFAAP-----SRYRFIRSVMIIIDVVAIMPYYIGLVMTNNE-----
 -----DVSG-AFVTLRVFRVFRIFKFSRHS-----QGL-
 RILGYTLKSCASE
 >94 hum_CACNA1E repeat_2 sp_Q15878
 RHMVKS-----QVFWIVLSLVALNTACVAIVHHNQ-----

 -----PQWLTHL----LYYAEFLFLGLFL
 LEMSLKMYGMG-----PRLYFHSSFNCFDFGVTVGSIFEVWVAIFRP----
 -----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-----PFTELAITICIIIVNTVFLAMEHHNM-----
 -----DNSLKDI----LKIGNWVFTGIFI
 AEMCLKIIALD-----PYHYFRHGWNIFDSIVALVSLADVLFHKLSK----
 -----NLSFLASLRVLRVFKLAKSW-----PTL-
 NTLIKIIGHSVGA
 >97 rat_SCN11A repeat2 sp_O88457
 RTIMTD-----PFTELAITICIIINTVFLAVEHHNM-----
 -----DDNLKTI----LKIGNWVFTGIFI
 AEMCLKIIALD-----PYHYFRHGWNVFDSDIVALLSLADVLYNTL-----
 -----SDN-NRSFLASLRVLRVFKLAKSW-----PTL-
 NTLIKIIGHSVGA
 >98 rat_SCN9A repeat2 sp_O08562
 YFIVMD-----PFVDLAITICIVLNTLFMAMEHHPM-----
 -----TEEFKNV----LAVGNLIFTGIFA
 AEMVLKLIAMD-----PYEYFQVGWNIFDSLIVTSLIE-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-----AGFNCFLVACVILVVLLTLELLIDTK-----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|
WQVFLLS-----ASLNSFLVACVILVVILLTLELLIDTK-----LLQ-
-----FSN----AFQFAGV----IHWISLVILSVFF
SETVLRIVVLG-----IWDYIENKIEVFDGAVIILSLAPMVASTVANGP--
-----RSPWD-AISLIIMFRIWRVKRVIDAYVLPVKLEM-
EMVTQQYEKA---
>107 rat_C15orf27 gi|157817759|
WQVLLLS-----ASLNSFLVACVILVVILLTLELLIDIK-----LLQ-
-----FSS----AFQFAAV----IHWISLVILSVFF
SETILRIVVLG-----IWDYIENKIEVFDGAVIILSLAPMVASTVANGP--
-----RSPWD-AISLIIMFRIWRVKRVIDAYVLPVKLEM-
EMVTQQYEKA---
>108 ciona_C15orf gi|198433556|
RKILHS-----VAFYIYILISTFIVTLLLLAELLIDVG-----VINI
PSSPDTVVL-----NASALSTLKVQTP----AQKTSTI----LHWISFSFSLSLFF
IEIMFRLYAWK-----LNIIRSIVSVFDCSIVTMAIATNLAATLAAGS--
-----TSPFD-AISLLIILRFIRIHSLIQRCVSDSKQEIR
EKLTKTECS----
>109 methanococcus hyperpol Kv sp_Q57603
KKI-----MEVLSLIFTFEIVASFILSTY-----
-----NPPYQDL----LIKLDYISIMFFT
FEFIYNFYVED-----KAKFFKDIYNIVDAIVVIAFLLYSLQVIFY-SKA--
-----FLGLR-VINLLRILVLLRIIKLRKL-----EEN-
QALIN-----

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-----SRDVGGA-----PETVSLVISFFFL
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SKFLSAIAD----
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NILKTSN---SIK
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-----GLRFLRALRLIQFSEILQF-----L-
NILKTSN---SIK

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Supplementary Table S2. Gating kinetics of Asp¹¹² mutants at pH 5.5//5.5 (mean \pm 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 hHv1	1.1 \pm 0.4 (7)	1.4 \pm 0.6 (4)		0.81 \pm 0.07 (6)
D112E	0.2 \pm 0.09 (3)	*0.26 \pm 0.07 (6)	0.28 \pm 0.22 (3)	[†] *6.0 \pm 0.4 (4) 0.069 \pm 0.008 (4)
D112H		2.78 \pm 0.40 (8)	1.19 \pm 0.37 (4)	0.69 \pm 0.06 (14)
D112S		2.76 \pm 0.41 (9)	1.17 \pm 0.17 (5)	*3.35 \pm 0.25 (15)
D112A/A/A		3.03 \pm 0.99 (4)	2.47 \pm 0.18 (4)	*2.46 \pm 0.23 (8)
D112N/A/A		3.36 \pm 0.57 (7)	1.73 \pm 0.07 (5)	*2.46 \pm 0.21 (7)
D112K/A/A		1.06 \pm 0.19 (3)	1.46 \pm 0.29 (6)	*0.18 \pm 0.03 (7)
D112F/A/A		2.22 \pm 0.03 (3)	1.67 \pm 0.23 (8)	*0.028 \pm 0.0005 (6)

Mean \pm 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 $g_{\text{H}}-V$ relationship was shifted positively in most D112x mutants, as reported previously¹, 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)	$P_{\text{CH}_3\text{SO}_3}/P_{\text{Cl}}$ ($P_{\text{H}} = 0$)	$P_{\text{OH}}/P_{\text{Cl}}$ ($P_{\text{H}} = 0$)	$P_{\text{CH}_3\text{SO}_3}/P_{\text{Cl}}$ ($P_{\text{OH}} = 0$)	$P_{\text{H}}/P_{\text{Cl}}$ ($P_{\text{OH}} = 0$)
WT hH _v 1	-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 $P_{\text{CH}_3\text{SO}_3}/P_{\text{Cl}}$ and $P_{\text{OH}}/P_{\text{Cl}}$ 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 ($P_{\text{H}} = 0$). Columns 6 and 7 show analogous results, but assuming that the shift in V_{rev} in Column 2 is due mainly to H^+ permeation ($P_{\text{OH}} = 0$).

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 $[\text{OH}^-]$ is 32 times larger at pH_o 7.0, and $[\text{OH}^-]$ remains constant as other anions are diluted. The term $P_{\text{OH}}[\text{OH}^-]_o$ (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, respectively¹. 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 $[\text{H}^+]$ on the proximal side of the membrane. That deuterium reduced the conductance by 50% also supports H^+ permeation² 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 Asp¹¹² mutant channels.

Mutant	Ion	Raw ΔV_{rev} (mV)	V_{jet} 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.