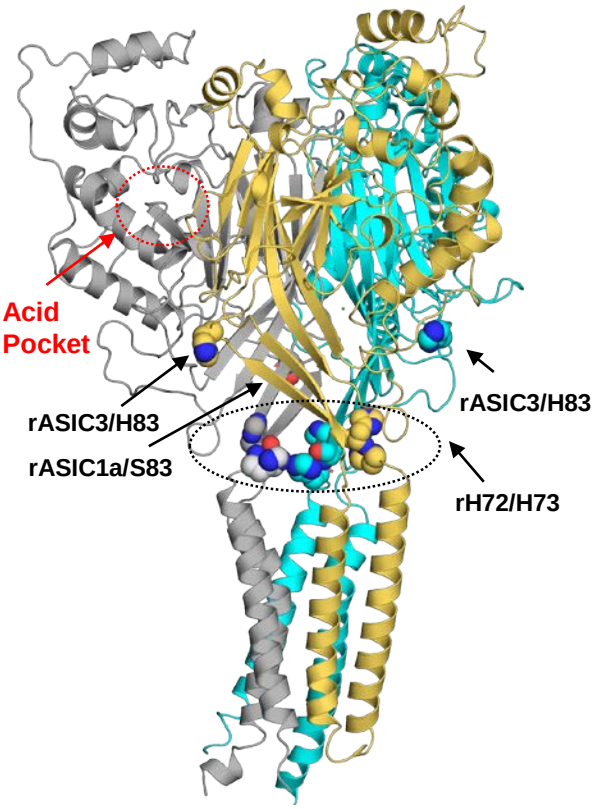
Supplementary Figure 1. Residues H72/H73 in rat ASIC1a, rat ASIC3 and H83 in rat ASIC3 are mapped onto the crystal structure of chicken ASIC1a. The three subunits in the heterotrimer are colored to represent one rASIC1a (grey) subunit and two rASIC3 subunits (cyan and yellow) in the ASIC1a/ASIC3 heterotrimeric channel. Based on the sequence alignment shown in Supplementary Figure 3, in the image shown here, P72 in 2QTS is mutated to H; T84 in subunit 1 is mutated to S; T84 in subunits 2 and 3 are mutated to H. The images are generated using PyMOL. r represents rat in the Figure.

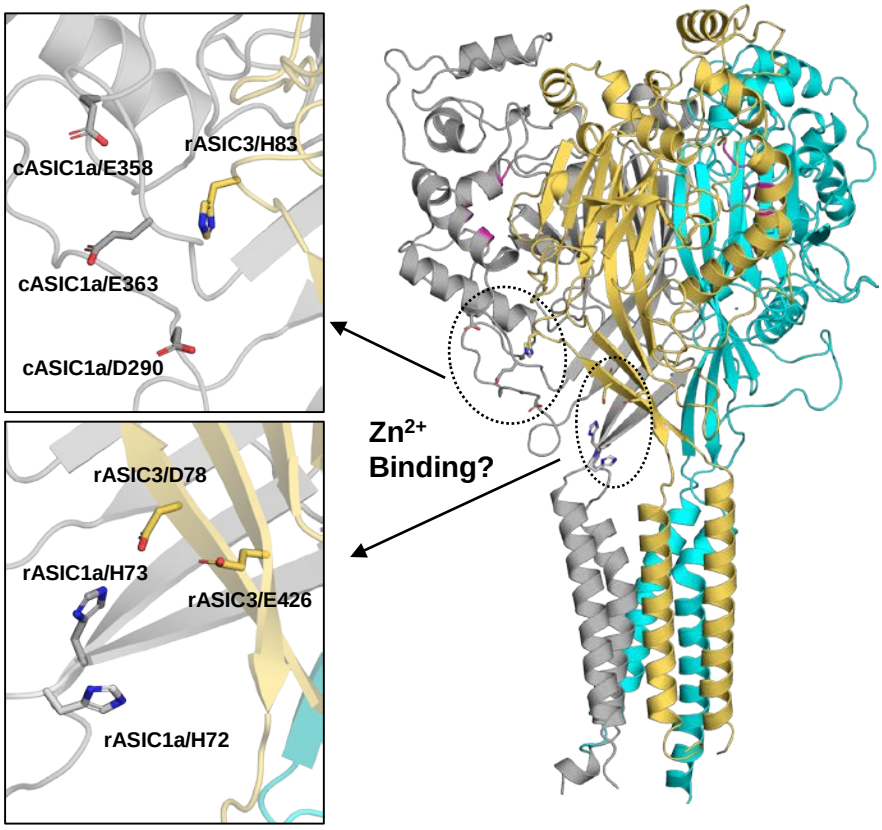
Supplementary Figure 2. Potential Zn^{2+} binding sites on heterotrimeric ASIC1a/3 channel mapped onto a crystal structure of the ASIC1a channel. The three subunits in the heterotrimer are colored to represent one rASIC1a (grey) subunit and two rASIC3 subunits (cyan and yellow) in the ASIC1a/3 heterotrimeric channel. Only sites at one inter-subunit interface are shown. r represents rat; c represent chicken in the Figure. The images are generated using PyMOL.

Supplementary Figure 3. Sequence alignment of rat ASIC1a, rat ASIC3 and the sequence of chicken ASIC1a, which is used to determine the crystal structure of ASIC1a channel. The alignment is performed using the serer PROMAL3D.

Supplementary Figure 1



Supplementary Figure 2



Colored PROMALS3D alignment (sequences in input order)

```
Conservation:          9      99    9   9  9999 9 99  999    9
ratASIC1a          1   -MELKTEEEEVGGVQPVSIQAFASSSTLHGLAHIFSYERL   39
ratASIC3           1   -MKPRSGLEEAQRRQASDIRVFASSCTMHGLGHIFGPGGL   39
chickenASIC1a      1   MMDLKVDEEEVDSGQPVSIQAFASSSTLHGISHIFSYERL   40
Consensus_aa:          .Mc.+s.bEEh...QsssIphFASStThHGltHIFt...L
Consensus_ss:                 hhh    hhhhhhhhh      ehhhhe

Conservation:          9 9  99     999         9  99    9 9 999
ratASIC1a         40   SLKRALWALCFLGSLAVLLCVCTERVQYYFCYHHVTKLDE   79
ratASIC3          40   TLRRGLWATAVLLSLAAFLYQVAERVRYYGEFHHKTTLDE   79
chickenASIC1a     41   SLKRVVWALCFMGSLALLALVCTNRIQYYFLYPHVTKLDE   80
Consensus_aa:          oL+RslWAhthh.SLAhhhh.hhpRlpYY..@.H.TpLDE
Consensus_ss:          hhhhhhhhhhhhhhhhhhhhhhhhhhhhhh   eeeeeee

Conservation:               9999999 99 9  9 9    999  99  9  9
ratASIC1a         80   VAASQLTFPAVTLCNLNEFRFSQVSKNDLYHAGELLALLN  119
ratASIC3          80   RESHQLTFPAVTLCNINPLRRSRLTPNDLHWAGTALLGLD  119
chickenASIC1a     81   VAATRLTFPAVTFCNLNEFRFSRVTKNDLYHAGELLALLN  120
Consensus_aa:          ..tppLTFPAVThCNlN.hR.Splo.NDL@@AGphLh.Ls
Consensus_ss:          eee eee   eee     hhhhhhhhhhhhhhhhhhhhh

Conservation:                        9  9        9     9 9   9
ratASIC1a        120   NRYEIPDTQMADEKQLEILQDKANFRSF-KPKPFNMREFY  158
ratASIC3         120   PAEHAA--------YLRALGQPPAPPGFMPSPTFDMAQLY  151
chickenASIC1a    121   NRYEIPDTQTADEKQLEILQDKANFRNF-KPKPFNMLEFY  159
Consensus_aa:          s.bchs.......bLchL.p.ss..sF..s.sFsM.phY
Consensus_ss:          hh          hhhhhhhhhh             hhhhh

Conservation:          9999    999 9  99  9  9 9 9 999 9 99999
ratASIC1a        159   DRAGHDIRDMLLSCHFRGEACSAEDFKVVFTRYGKCYTFN  198
ratASIC3         152   ARAGHSLEDMLLDCRYRGQPCGPENFTVIFTRMGQCYTFN  191
chickenASIC1a    160   DRAGHDIREMLLSCFFRGEQCSPEDFKVVFTRYGKCYTFN  199
Consensus_aa:          sRAGHslc-MLLsC.@RGp.CtsEsFpVlFTRhGpCYTFN
Consensus_ss:          hhh   hhhhhheeee  eee hhheeeee     eeee

Conservation:          9  9   9 9 999 999999999 99 9999 9    9
ratASIC1a        199   SGQDGRPRLKTMKGGTGNGLEIMLDIQQDEYLPVWGETDE  238
ratASIC3         192   SGAHGAELLTTPKGGAGNGLEIMLDVQQEEYLPIWKDMEE  231
chickenASIC1a    200   AGQDGKPRLITMKGGTGNGLEIMLDIQQDEYLPVWGETDE  239
Consensus_aa:          tG.cG...L.T.KGGhGNGLEIMLDlQQ-EYLPlW.-h-E
Consensus_ss:                eeee       eeeeeeee hhh
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Conservation:          9 99 99 9999999999 9999999 999 9999999 9
ratASIC1a       239    TSFEAGIKVQIHSQDEPPFIDQLGFGVAPGFQTFVSCQEQ  278
ratASIC3        232    TPFEVGIRVQIHSQDEPPAIDQLGFGAAPGHQTFVSCQQQ  271
chickenASIC1a   240    TSFEAGIKVQIHSQDEPPLIDQLGFGVAPGFQTFVSCQEQ  279
Consensus_aa:          TsFEhGI+VQIHSQDEPPhIDQLGFGhAPG@QTFVSCQpQ
Consensus_ss:                eeeeee           eeee   eeeeeeeeeh

Conservation:           9  99 999 9     9    9
ratASIC1a       279    RLIYLPSPWGTCNAVTMDSDFFD----------------S  302
ratASIC3        272    QLSFLPPPWGDCNTASLDPDDFDPEPSDPLGSPRPRPSPP  311
chickenASIC1a   280    RLIYLPPPWGDCKATTGDSEFYD----------------T  303
Consensus_aa:          pL.@LPsPWGsCphho.Ds-.@D.................s
Consensus_ss:          heee

Conservation:          99   99  99 99    9 999 9999  9 9 9 999
ratASIC1a       303    YSITACRIDCETRYLVENCNCRMVHMPGDAPYCTPEQYKE  342
ratASIC3        312    YSLIGCRLACESRYVARKCGCRMMHMPGNSPVCSPQQYKD  351
chickenASIC1a   304    YSITACRIDCETRYLVENCNCRMVHMPGDAPYCTPEQYKE  343
Consensus_aa:          YSlhtCRlsCEoRYlhcpCsCRMhHMPGstPhCoPpQYK-
Consensus_ss:          hhhhhhhhhhhhhhhhhh            ee  hhhhhh

Conservation:          99 9999    99   999  99  999 999999 999
ratASIC1a       343    CADPALDFLVEKDQEYCVCEMPCNLTRYGKELSMVKIPSK  382
ratASIC3        352    CASPALDAMLRKD--TCVCPNPCATTRYAKELSMVRIPSR  389
chickenASIC1a   344    CADPALDFLVEKDNEYCVCEMPCNVTRYGKELSMVKIPSK  383
Consensus_aa:          CAsPALDhhlcKD..hCVC..PCshTRYtKELSMV+IPS+
Consensus_ss:          hhhhhhhhhh             eeeeeeeeeee   h

Conservation:          999 999 9 9 99 99 99 99999999 9999  999
ratASIC1a       383    ASAKYLAKKFNKSEQYIGENILVLDIFFEVLNYETIEQKK  422
ratASIC3        390    ASARYLARKYNRSESYITENVLVLDIFFEALNYEAVEQKA  429
chickenASIC1a   384    ASAKYLAKKYNKSEQYIGENILVLDIFFEALNYETIEQKK  423
Consensus_aa:          ASA+YLA+K@N+SEpYIsENlLVLDIFFEhLNYEhlEQK.
Consensus_ss:          hhhhhhhhhh          eeeeeeee    eeeeeeee

Conservation:          999   9999999999999999 99 99  99  99   9
ratASIC1a       423    AYEIAGLLGDIGGQMGLFIGASILTVLELFDYAYEVIKHR  462
ratASIC3        430    AYEVSELLGDIGGQMGLFIGASLLTILEILDYLCEVFQDR  469
chickenASIC1a   424    AYEVAGLLGDIGGQMGLFIGASILTVLELFDYAYEVIKHR  463
Consensus_aa:          AYElt.LLGDIGGQMGLFIGAslLTlLElhDYhhEVhpcR
Consensus_ss:             hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
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Conservation:                                        9 9  99   9
ratASIC1a       463  LCRRGKCQKEAKRSSADKGVALSLDDVKRHNPCESLRGHP  502
ratASIC3        470  VLGYFWNRRSAQKRSGNTLLQEELNGHRTHVPHLSLGPRP  509
chickenASIC1a   464  LCRRGKCRKNHKRNNTDKGVALSMDDVKRHNPCESLRGHP  503
Consensus_aa:        lh...bsp+php+psssp.l.bphssh+pHsPhbSL.s+P
Consensus_ss:        hhhhhhhhh  hh

Conservation:                       9          9
ratASIC1a       503  AGMT--YAANILPHHPARGTFEDFTC------  526
ratASIC3        510  PTTPCAVTKTLSASHRT--------CYLVTRL  533
chickenASIC1a   504  AGMT--YAANILPHHPARGTFEDFTC------  527
Consensus_aa:        sshs..hh.sl.spH.h........C......
Consensus_ss:                                eeeee
```

Supplementary Table 1. pH_{50} and Hill coefficient of ASIC1a WT and histidine mutants in the extracellular domain of ASIC1a during activation.

	pH_{50}	Hill coefficient	Sample size	Statistics
ASIC1a WT	6.3 ± 0.05	2.7 ± 0.2	7	
ASIC1a-H72A	6.2 ± 0.05	2.8 ± 0.2	5	$p > 0.05$
ASIC1a-H73A	6.2 ± 0.06	2.6 ± 0.1	5	$p > 0.05$
ASIC1a-H110A	6.2 ± 0.09	2.8 ± 0.2	5	$p > 0.05$
ASIC1a-H163A	6.2 ± 0.05	2.7 ± 0.1	5	$p > 0.05$
ASIC1a-H173A	6.3 ± 0.04	2.7 ± 0.1	5	$p > 0.05$
ASIC1a-H250A	6.3 ± 0.06	2.6 ± 0.1	5	$p > 0.05$
ASIC1a-H327A	6.2 ± 0.07	2.8 ± 0.2	5	$p > 0.05$

pH_{50} and Hill value of each mutant were compared with ASIC1a WT. Data were presented as mean ± SEM.

Supplementary Table 2. pH_{50} and Hill coefficient of ASIC3 WT and histidine mutants in the extracellular domain of ASIC3 during activation of the channel

	pH_{50}	Hill coefficient	Sample size	Statistics
ASIC3 WT	6.8 ± 0.03	1.4 ± 0.1	6	
ASIC3-H72A	6.8 ± 0.02	1.3 ± 0.1	5	$p > 0.05$
ASIC3-H73A	6.8 ± 0.03	1.4 ± 0.1	5	$p > 0.05$
ASIC3-H83A	6.8 ± 0.03	1.3 ± 0.1	5	$p > 0.05$
ASIC3-H109A	6.9 ± 0.02	1.3 ± 0.1	5	$p > 0.05$
ASIC3-H123A	6.8 ± 0.03	1.4 ± 0.1	5	$p > 0.05$
ASIC3-H156A	6.8 ± 0.02	1.4 ± 0.1	5	$p > 0.05$
ASIC3-H195A	6.8 ± 0.03	1.4 ± 0.1	5	$p > 0.05$
ASIC3-H243A	6.9 ± 0.02	1.3 ± 0.1	5	$p > 0.05$
ASIC3-H262A	6.8 ± 0.03	1.4 ± 0.1	5	$p > 0.05$
ASIC3-H336A	6.8 ± 0.03	1.3 ± 0.1	5	$p > 0.05$

pH_{50} and Hill value of each mutant compared with ASIC1a WT. Data were presented as mean ± SEM.