10 Supplemental Figures

```
Trout_Nhe3a      1  MACRTCLWLLG.....TTFFTC......TVRLVVEGTEVGATQGSQP....................EPGVVGSNHSQAGGG..H
Trout_Nhe3b      1  MPA.....LWR.....STFVLV......MVLLAIS..........................RPSLASEATGN.GT
Zebrafish_Nhe3a  1  MASSTYICLLR.....AAFLVC......LVYPLVKGNSV.....................EVPNPHHHQTEN...
Zebrafish_Nhe3b  1  MAFSTLLL.........AFLVVS....GALHEAAAGLDFYGAEGKQN...................YSSRSSAEGSASSGNSSH
Human_NHE3       1  MWGLGARGPDR.....GLLLA.......LALGGLARAGGVEV.....................EPGGAHGESG...
Squalus_Nhe3     1  MGRDRSECAAR.....CALLTA......LGLLLCCPVAPSSVEEA.................EHGESHGESHGGNET
Human_NHE1       1  MVLRSGICGLSPHRIFPSLLVVVALVGLLPVLRSHGLQLSPTASTIRSSEPPRERSIGDVTTAPPEVTPESRPVNHSVTDHG...


Trout_Nhe3a     53  GSSISGIQIVTFKWHHVEAPYLIALWILVAGVSKM.VVELNHSLTSVIPESALLIIGWILGGIIYGADKMQTFTLTPIVFFFYL
Trout_Nhe3b     33  EHTITTLPIVIWKWEEIHFPYLVALWVLVSWLCKL.VFQLNPCLTRVIPESGLLICIGWVLGGIIYGADKVQTFRTQPFTFFFYL
Zebrafish_Nhe3a 41  ...LTGLPIVTFKWHHVETPYLVALWVFVAGLALSVIQLNHSIITGVFPESGLLIILGFILGGIVVGADKAQTFKTLPSTFFYYL
Zebrafish_Nhe3b 53  SATITTLPIVTWKWHHVETPYLVALWILTCWICKL.VTELNHNITSVIPESGLLIILGFILGGIVWGADKAQTFKTIPVNFFFYL
Human_NHE3      41  .....GFQVVTFEWAHVQDPYVIALWILVASLAKTI.GFHLSHKVTSVVPESALLIVLCLVLGGIVWAADHIASFTLTPTVFFFYL
Squalus_Nhe3    50  GDHEQGFHVVTFHWEHVQAPYVIALWILVASLAKTI.VFHLSEKVTSLVPESALLIVLCLILGGIVWAADHTTSFTLTPNVFFFYL
Human_NHE1      83  MKPRKAFPVLGIDYTHVRTPFEISLWILLACLMKTI.GFHVIPTISSIVPESCLLIVVCGLLVGGLIKGVGETPPF.LQSDVFFLFL


Trout_Nhe3a    137  LPQVILDAGYFMPNKLFFSNMGAILVYAVIGTCWNAATVGLGLWGCWLGGAMGDIDIGLLQFLLFGSLIAAVDPVAVIAVFEEVH
Trout_Nhe3b    117  LPQIVLDAGYSMPNKLFFGNLGAILVYAVIGTCWNAATLGLALWGCWLGGAMGNIDTGLLQLLLFGSLIAAVDPVAVIAVFEEVH
Zebrafish_Nhe3a 123 LPQIILDASYFMPNKLFFRNLGAILIYAIFGTCWNAAVGLSLWGCHEAGAMGDLNIGLLQFLLFGGLIAAVDPVAVIAVFEEVH
Zebrafish_Nhe3b 137 LPQIILDASYCMPNKLFFGNLGAILVHAIIGTCWNAGTVGIALWACYEGGAMGTLNIGCLQFLLFGALMSAVDPVAVIAVFEEVH
Human_NHE3     120  LPPIVLDAGYFMPNRLFFGNLGTILLYAVVGTVWNAATTGLSLYGVFLSGLMGDLQIGLLDFLLFGSLMAAVDPVAVIAVFEEVH
Squalus_Nhe3   134  LPPILDAGYFLPLRQFTENLGTILTYAVVGTVWNAATTGLSLYGVFLTGIMGDLNAGPLEFLLFGSLIAAVDPVAVIAVFEEVH
Human_NHE1     166  LPPIILDAGYFLPLRQFTENLGTILIFAVVGTLWNAFFLGGLMYAVCLVGGEQINNIGLLDNLLFGSIISAVDPVAVLAVFEEIH


Trout_Nhe3a    222  VNEVLEIMVFGESLLNDGVTVVLFNVFDAFVSLGGAEIDAVEIIKGIISFFVVAFGGSLVGIIFALLISLLTRCTKNIQIIEPGF
Trout_Nhe3b    202  VNEVLILVFGESLLNDGVTVVLFNVFDALVSLGGAQIDAVEIKGIISFFVVAGGGSLVGVVFALLSLLTRCTKNIQIIEPGF
Zebrafish_Nhe3a 208 VNEVLILVFGESLLNDGVTVVLYNVFDGLVSLGGPMIDAAEIFKGICSFVVVAFGGSFVGVAFAILLSLLTRCTKHVQIIEAGF
Zebrafish_Nhe3b 222 VNEVLILVFGESLLNDGVTVVLYNVFDALVSLGGPKINAAEIIKGIVSFAVVAFGGSFLGVFAVLTSMLTRVTKNVQIILAGF
Human_NHE3     205  VNEVLILIIVFGESLLNDAVTVVLYNVFESFVALGGDNVTGVDCVKGIVSFFVVSLGGILVCVVFAFLLSLVTRFTKHVRIIEPGF
Squalus_Nhe3   219  VNEVLIIVFGESLLNDAVTVVLYNVFESFEIGAGNIQGLDCFKGIVSFFVVSLGGTAIGIIFAFVLSLVTRFTKHVRIIEPGF
Human_NHE1     251  INELLHILVFGESLLNDAVTVVLYHLFEEFANY..EHVGIVDIFLGFLSFFVVALGGVLVGVVYGVIAAFTSRFTSHIRVIEPLF


Trout_Nhe3a    307  IFILGYLSYLTAEMLSLSAILSIVFCGICSQKYINANMDERSVITVRYVMKVFANGSETIIFVFLGISAIDPAVWVWNTGFILLT
Trout_Nhe3b    287  IFVLGYLSYLTAEMLSLSAILSCTFCGVCCQKYINANMDERSVSTVRSTMKVFANGSETIIFVFLGISAIDPSIWVWNTAFILLT
Zebrafish_Nhe3a 293 VFLVGYLSYLTVEMLSLSAILSTTFCGVCCQKYINANMDEKSVITVRHAMKMLANGSETMIFLFLGVTAIDTEIWVWNTGFILLT
Zebrafish_Nhe3b 307 IIVLGYLSYLTAEMLSLSAILSLTFCGVCCQKYINANMDEKSVTCLRYSLKVLANGSETMIFVFLGVSAIDQTIWVWNTGFILLT
Human_NHE3     290  VFIISYLSYLTSEMLSLSAILAIIFCGICCQKYVKANISEQSATIVRYTMKMLASSATIIIFMFLGISAVNPFFIWTINAFVLLT
Squalus_Nhe3   304  VFIISYLSYLTAEMLSLSAILAIIFCGICCQKYVKANLCEQSITIVRYAMKMLASGATIIFMFLGISAVDTTIWTWNPPFILLT
Human_NHE1     334  VFLYSYMAYLSAELFHLSGIMALIASGVVMRPYVEANISHRSHTTIKYFLKMWSSVSETLIFIFLGVSTVAGS.HHWNWTFVIST


Trout_Nhe3a    392  LLFIFVFRFIGVFLLTWILNKYRLVPLEFIDQVVMSYGGLRGAVAYGLVAMPGENKFKEKNLMVSTTLIVVYFTVMLQGITMKPL
Trout_Nhe3b    372  LLFIFVFRFIGVFLLTWILNQYRLVPLDFIDQLVMGYGGLRGAVAYGLVLLLDENKYKEKKFMISTTLIVVYFTVMLQGITMKPL
Zebrafish_Nhe3a 378 LLFIFIYRIIGVFMFTWMLNKFRLVPVELIDQVVMSYGGLRGAVAYGLAAMLDESTIPEKKFMISTTLIVVYFTVILQGTTMKPL
Zebrafish_Nhe3b 392 LLFIVVFRMMGVFFLNWILNQSRLIPIDLTDQLVMGYGGLRGAVAYGLAASLDENKIPEKNLMLGTTLIVVYFTVILQGITMKPL
Human_NHE3     375  LVFISVYRAIGVVLQTWLLNRYRMVQLEPIDQVVLSYGGLRGAVAFALVVLLDGDKVKEKNLFVSTTIVVFFTVLFQGLTIKPL
Squalus_Nhe3   389  LVFISVYRVIGVVIQTWILNRYRVVQLEIIDQVVMGYGGLRGAVAFALVVLLNKDTVKTRKLFVSTTIIVVYFTVLFQGLTIKPL
Human_NHE1     418  LLFCLIARVLGVLGLTWFINKFRIVKLTPKDQFIIAYGGLRGAIAFSLGYLLDKKHFPMCDLFLTAIITVIFFTVFVQGMTIRPL


Trout_Nhe3a    477  VTWLKVKRAAVTBLTLDAEKIQNRTFDHMLVAIEDISGQIGHNYMRDKWHNFEERWLCWFLMKPSARKSRDYIFNIFHQLNLKDAM
Trout_Nhe3b    457  VTWLKVKRAAVSERTLAEKIQNRTFDMLVAIEDISGQIGDNYMRDKWNNFEEKWLYWLLMTPIARKSCDPIFNIFHKLNIKDAT
Zebrafish_Nhe3a 463 VQWLKVKRAAHSDLKLVEKLNNRAFDLILAAVEDICGRIGDNWWTRGWNRFEEKYISWLLMKPKARKNRDYLFNIFHKLNLEDAM
Zebrafish_Nhe3b 477 VNWLKVKKATQSDLTLNGKLNNRVFETLTGMEDICGRMGDNWWTRHWNHFEEKYVCWLLMTSEARKRNDKILDAFHKLNVEDAT
Human_NHE3     460  VQWLKVKRSEHREPRLNEKLHGRAFDLILSAIEDISGQIGHNYLRDKWSHFDRKFLSRVLMRRSAQKSRDRILNVFHELNLKDAI
Squalus_Nhe3   474  VNWLKVKKSSQKDPLLNEKLHERAFDLILSAIEDISGQIGHNYLRDKWTNFDRKYLSKVMMRKSAQVSRDRILSVFRELNLKDAI
Human_NHE1     503  VDLLAVKKQETKRSINEEIHTQFLDRLLTGIEDICGHYGHHHWKDKLNRFNKKYVKKCLIAGE.RSKEPQLIAFYHKMEMKQAI
```

```
Trout_Nhe3a      562  SYVKEGERRGSLAFVRNETK...ADVDFNKKFRASFSEIMPDIMAD....NMGPDHVPLSSILQDIVPSVCLVMHEQDLT...MR
Trout_Nhe3b      542  SYVKEGERRGSLAFVRNENK...ADVDFNKKFGADFSEMMPYIMTE....NMGPDHVPVSSILRDSVPSVCLDMHEQDLK...MR
Zebrafish_Nhe3a  548  NYVSEGERQGSLAFIRNDEA...ANVDFKKQFDSEFADVMPDIMVDMSNYDFGIENISAAAIVKDTIPSVSLDIHGQDGK....N
Zebrafish_Nhe3b  562  KYVNEGESKGSLAFIRSYDS...ASVDFKKKLALEYADIIPDIMADMSEYDFDIDSVPVTSVMKNPIPSVSLDIHEQERR....G
Human_NHE3       545  SYVAEGERRGSLAFIRSPSTDNVVNVDFT...........P.........RSSTVEASVSYLLRENVSAVCLDMQSLEQRRRSIR
Squalus_Nhe3     559  SYVSEGERRGSLAFIRSSSD...VNVDFTG...........P.........RHSVVDSSVSAVLRESASEVCLDMHAVENRVKSVK
Human_NHE1       587  ELVES....GGMGKIPSAVS....TVSMQ...........P.........NIHPKSLPSERIL...PALSKDKEEEIRK.....


Trout_Nhe3a      637  ESEDFNAHHLLQQHLYRGRKQHRHRYSRSHLETNRDENEVQEIFQRTMRSRLESFKSAKMGVAPAKKIQTKHQKKDKPQKV....
Trout_Nhe3b      617  ESEDFDAHHLLQQHLYKGRKQHRHRYSRKQLEVNKDEHEVQEIFQRTMRSRLESFKSAKMGVAPPKKI.SKHLKKDQPQKM....
Zebrafish_Nhe3a  626  VVDDINAHRLLQQHLYRSRKHHHHKYSRSHFSINQSEEEVQEIFQRTMRSRLESFKSAKMGVNPTKKI.TKFHKKDPTDKM....
Zebrafish_Nhe3b  640  MSNDLNAHHLLEQHLYKSRRNNRATYSRSHYNTSENPDEMQEIFQRTMGNRLESFKSAKMGVNPAKKV.SKHPKKDAPHKP...
Human_NHE3       610  DAEDMVTHHTLQQYLYKPRQEYKHLYSRHELTPTEDEKQDREIFHRTMRKRLESFKSTKLGLNQNKKA.AKLYKRERAQKRRNS
Squalus_Nhe3     622  DREETVTHHMLQQHLYKPRQRYRLNYSRHKLMRNEDVKQDKEIFQRTMKKRLENFKSTKLGTNYNTKF..RNLKKERAAKKKHSD
Human_NHE1       634  ..L.....ILRNNLQKTRQRLR.SYNRHTLVADPYEEAWNQMLLRRQKARQLEQKINNYLTVPAHKL..L....DSPTMS....


Trout_Nhe3a      718  ...........................................RGEGTF.SPFL..J.....
Trout_Nhe3b      697  ..PNGKSTDNS..KSHAYGDEDFEFSEGDSASGYEAA........GGSFPMRAY..RGAGIV.NPAFMAEM..DTMDPMPPMQQIP
Zebrafish_Nhe3a  706  ..NGHTEGNG...YLYGD......EADSGVGADGA........ISSTFMRDTYKMGAGIE.NPVFMPDM..DSP..VHSPVHSP
Zebrafish_Nhe3b  720  ...NGKPADPN..RDHLYGDEDFEF.SADSASSSENT........CQFPRRVINTTGAGVD.NPAYIPEL..DT....SPSMRNP
Human_NHE3       693  SIPNGKLPMESPAQNFTIKEKDLELSDTEPPNYDEEMSGGIEFLASVTKDTASDSPAGID.NPVFSPDEALDR....SLLARLP
Squalus_Nhe3     705  AVPNGRLSTQS..VTFHVDK......DSDVEDNYDPS.DGGISFLITAPSEETDETRTGID.NPHFSAEE..DQ....SFYQMIP
Human_NHE1       698  ...RARIGSDP..LAYEPKE.DLPVITIDPASPQSPE.......SVDLVNEELKGKVLGLSRDPAKVAEE..DE.........


Trout_Nhe3a      728  .J.................RVRPHSHPLIGNVMCMLC......NFMLCHF....................
Trout_Nhe3b      766  SWLAETEVDSSNMVPSQRAQLRLPWTPSNLRRLAPLCLSTHSNDSFLLADSPATQEGISELPPPPPPQDGQGQGTH...K
Zebrafish_Nhe3a  766  PWLLETEM...SVAPSQRAQRRLPWTPN..RRLAPMRISTCSTDSFLMADVSSTLE.....VHEEQPQSDNGESVF...E
Zebrafish_Nhe3b  784  PWQTETGHN.TAVAPSQRAQGRLPWTPTNLRRLAPLRVSSRSTDSFAMPEPAADEE.....TPEEKPATHHTRL......
Human_NHE3       773  PWLSPGE....TVVPSQRARTQIPYSPGTFCRLMPFRLSSKSVDSFLQADGP.......EERPPAALPESTH...M
Squalus_Nhe3     774  PWMSNEE....TVVPSQRARLQIPASPTNFRRLTPLQLSSRSIDSFLLADIS..........DEHPLSFLPESS....M
Human_NHE1       757  .J.........DDDGGIMMRSKETSSPGTDDVFTPAPSDSPSSQRIQRCLSD..........PGPHPEPGEGEPFFPKGQ
```

Supplemental Figure 1. Alignment of NHE proteins. Alignment of trout, zebrafish, human NHE1, and NHE1 and Squalus NHE3 protein. Conserved amino acids are colored red and conserved regions are boxed. White amino acids boxed in red represent regions of identity. Representatives included are; *Oncorhynchus mykiss*, Nhe3a, NM_001130995; Nhe3b, FJ376630.1; *Danio rerio*, Nhe3a, EF591984.1; Nhe3B, EF591980.1; *Homo sapiens*, NHE3, NG_046804.1; *Squalus suckleyi*, Nhe3, KR297236.1; *Homo sapiens*, NHE1, NM_003047. Amino acid alignment was using the MAFFT program [75].
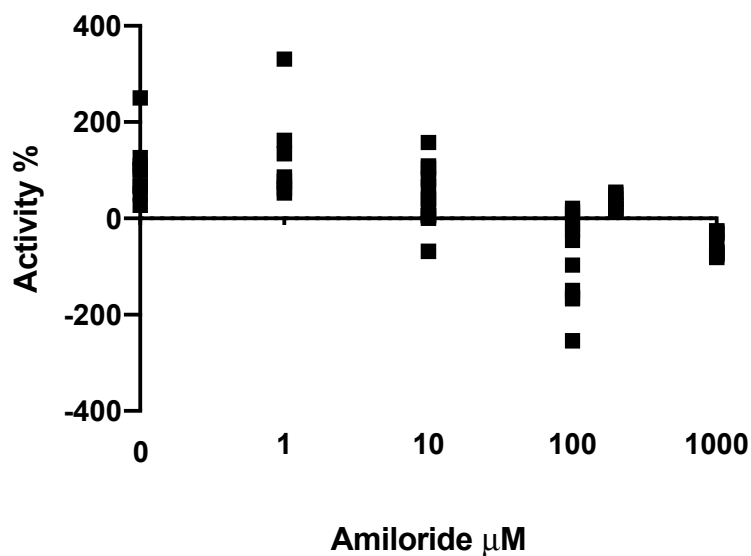
Supplemental Figure 2. Raw data points associated with Figure 5 (Effect of varying concentrations of amiloride on activity of tNhe3a). Each individual data point is relative to the mean of the control (DMSO) which was normalized to 100% activity.
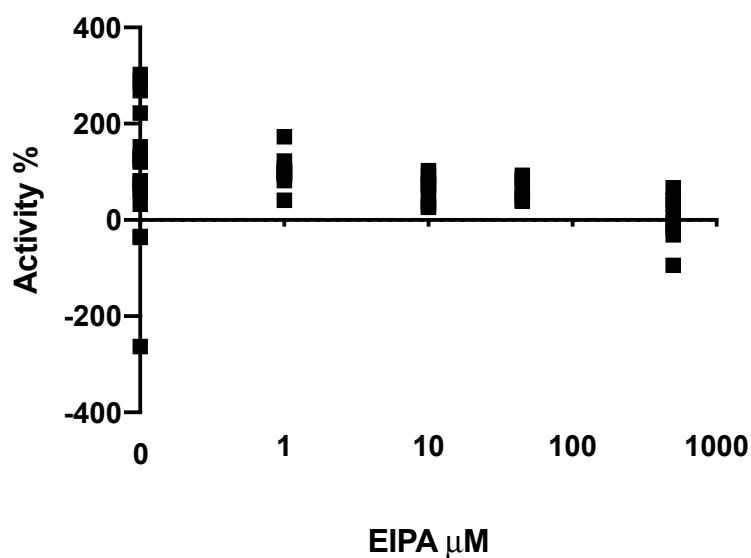
Supplemental Figure 3. Raw data points associated with Figure 6 (Effect of varying concentrations of amiloride on activity of tNhe3a). Each individual data point is relative to the mean of the control (DMSO) which was normalized to 100% activity.

Supplemental Tables

Supplemental Table S1. Sequences of primers and gene accession numbers used for cloning *nhe3a* and *nhe3b* from rainbow trout, *Oncorhynchus mykiss*. Restriction enzyme sites used in cloning are underlined.

Gene	Primer Set	Accession Number	Primer	Primer Sequence (5′– 3′)
nhe3a	1st set	NM_001130995.1	Forward	TACCCGGGATGGCATGCAGAACTTGC
			Reverse	CGCGTCGACAAAATGACATAACATGAAATTACAC
nhe3a	2nd set	NM_001130995.1	Forward	AAGAATTCGCTAGCGCCACCATGGCATGCAGAACTTGCCTCTG
			Reverse	CCGGAATTCACCGGTCCAAAATGACATAACATGAAATTACA
nhe3b	1st set	NM_001160482.1	Forward	CCAAGATTGATCAAAGCTCAAG
			Reverse	AAAGAGGTCAGTTTGTGGA
nhe3b	2nd set	NM_001160482.1	Forward	TACCCGGGATGCCAGCTCTG
			Reverse	CGTGTCGACCTTGTGGGTTCC
nhe3b	3rd set	NM_001160482.1	Forward	AAGAATTCGCTAGCGCCACCATGCCAGCTCTGTGGCGCTCCACTTTCG
			Reverse	CCGGAATTCGACCGGTCCCTTGTGGGTTCCCTGACCCTGTCCGTC

Supplemental Table S2. Percent identity of trout amino acid sequences Nhe3a and trout Nhe3b with other NHEs.

	Trout_Nhe3a	Trout_Nhe3b	Zebrafish_Nhe3a	Zebrafish_Nhe3b	Human_NHE3	Squalus_Nhe3	Human_NHE1
Trout_Nhe3a	100.00	78.77	66.44	61.80	57.66	55.48	35.19
Trout_Nhe3b		100.00	64.37	63.95	55.67	53.62	35.05
Zebrafish_Nhe3a			100.00	65.41	53.46	52.62	33.60
Zebrafish_Nhe3b				100.00	48.75	48.52	33.85
Human_NHE3					100.00	73.35	38.40
Squalus_Nhe3						100.00	38.52
Human_NHE1							100.00