

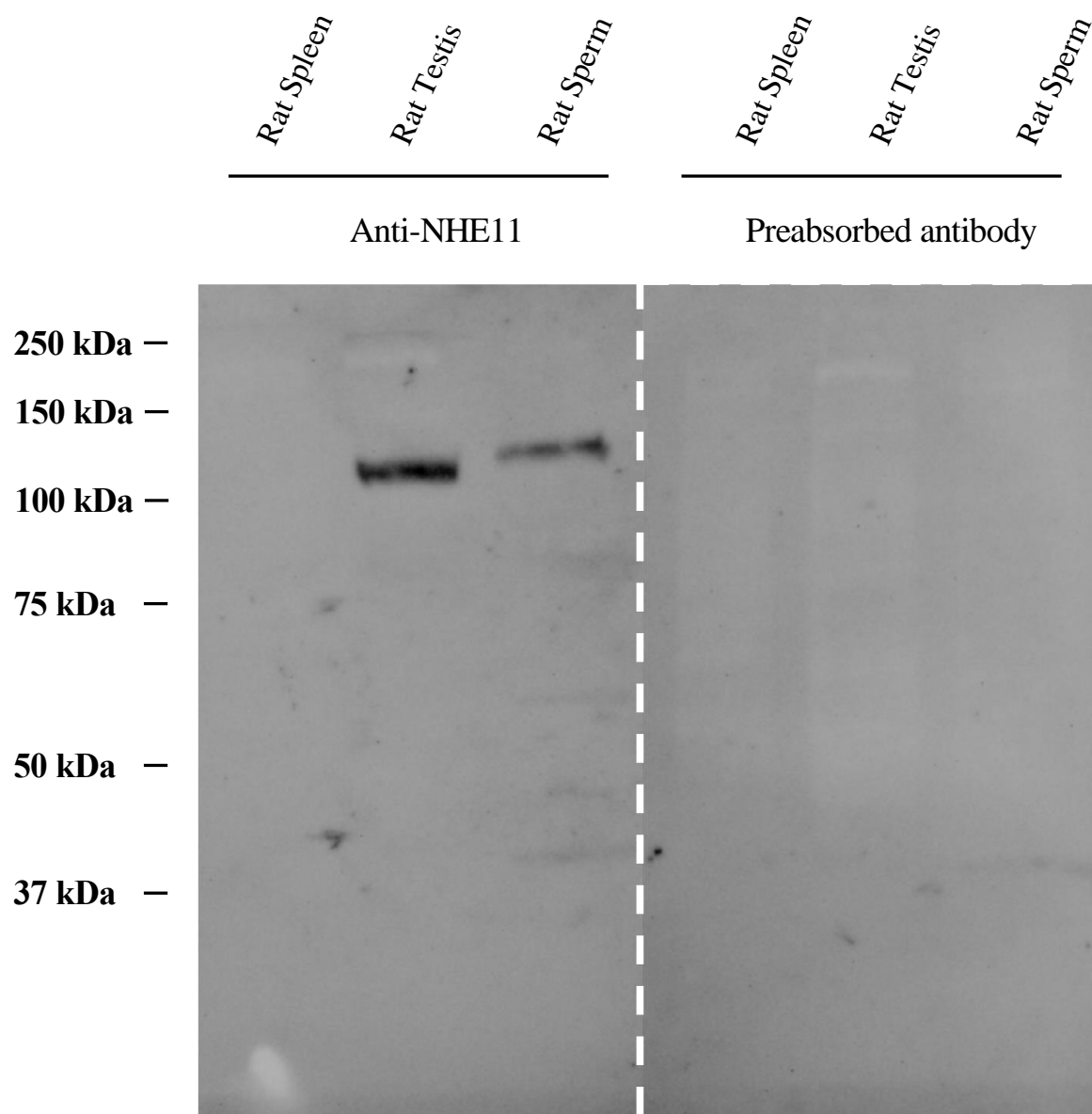
**The SLC9C2 gene product (Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 11; NHE11) is a testis-specific protein localized to the head of mature mammalian sperm**

Supplemental Material

rNHE11	1	MSNNFSFWVARNDNKPDLLCGRPADYFVVQKHFTILICATIVLGGFSRMVLKNSEVFVMIILSVSGFV
hNHE11	1	MSS---YFWAQNESNRPDLLCGQPADYLVEEKHFTTLVCFIVVLGGLLKMLKNCEVIVLTILSLSGFV
rNHE11	70	TGRLAYNFIEVHQIVYPLLKTPSFSLYCYFLPLIVFVAALDVDIFYVKNVFWQVLLTGLISFGTAFATI
hNHE11	67	IGHMAYNSVEVHQIVYPLLRTSFSFSLYSYFSLPIIFMVALDVEFYTLKKMFQVLLTGLISFSTASIII
rNHE11	139	SYVVLKFHEDKNDLQCCLLFSITLGIIDPLHSVNSLKMIGISKMYTDIIRGESLIICSFTCIFFGIFRG
hNHE11	136	GYVVIKFNKDSNDLQSCLLFSITLGIIDPLRSVNSLKTIGISKIYIDLIRGESLIICSIAIFFGNFRG
rNHE11	208	HTTNVSMFRELVIIIGLSFDIFGSAVCGYWCTRVIQIILTDLFSNTLTNVVLCFSLVYMTFFYLVEYFGM
hNHE11	205	NRIHFSIFRDLHVGIELSYDIIGSIIFGYWCAKIIQCILADVFSNMLTNIILCFSMVYMTFFIVEFLGM
rNHE11	277	SGIVALVTVGLNLDLSLFFKPRMEFIITKFLIMCSSVYEHLIYAFFGIVIGOGEEKYFRFHSLVFTVTLF
hNHE11	274	SGTLALAAVGLNLDLSLTFKPKIELVITKFLRIFSSVYEHLIYAFFGIVIGOGELSHYEFHTIPFIFILF
rNHE11	346	VTNVFVRLLTIIILVSPILMHSSYEYDWRWGCVITWSGIRGVFSLLLAPDIYNFSEYKLAAPHMCIFYIQ
hNHE11	343	TTVNLVRLLTIIILVSPILMHSSYEYNWRWGCVITWSGIRGVFNLLWAPDVYNLAERKVEVPQMFILYQ
rNHE11	415	ALSILTTGINSHMMLRSAKTLGLCALSLPRQMAVERNATEHIQEIIIRNTITLTKTEKILTNNVNVVEEK
hNHE11	412	VISLTMGINSYVMTQSARKLDLCVLSLPRQMIQINATQHIQEIVQNTITLTKTEKILTNNVNTLVEDK
rNHE11	484	AKIEYNPILKQARTVPLAHISHGP REESPTDEVLEEERLHVAIIQMSSFEKQCNIGILGVEAARILI
hNHE11	481	TRIEY-----IPFSHVSHNDMKTESTTDEALMEEARLHVAAIQMSSFEKQRNNGILEIEAARILI
rNHE11	552	GATKSYCPHKGFMSSIDVSTYVRARSLIKFKNMLTYLEYHKERVLFVTPGNKLLIFVYRIIISEEF
hNHE11	541	GAAKCYYSIQKGFMSYDVSTYMRTRSLIKFKNVLTFLCYIEKIHFIIPESNTFLTIFIFHIVFSEEF
rNHE11	621	EYATHVVTVMYIFPMIHLWPIARELNVSALISINYYMFLYTLESALKIILIKRKYFLQNWNTLDDFFI
hNHE11	610	EYTGQIINLIYIYPMIHLWPMARGLNVSALISINYYMFLYVLESTLKIILIKRKYFQCNWNTLEFFI
rNHE11	690	IVLGIVDILCMYFVKLRPDSLVLQFTTVVIGYLRVIRFLPIFKIIIPVLINMADVQIKKSLSLMYSITK
hNHE11	679	LVIGIIDIFCVYFVKLRPDNLALIQLTVIMGYLRIRFLPLFKIIPILIRIADVQIKKRLSLMYSITK
rNHE11	759	GYVKSQEDAKLLVRQISGRESIYQKLYDILERNKREAIRELGLIEHEGROVVIALKTKQTIRNVIAKAL
hNHE11	748	GYIKSQEDAKLLIKQIAVCESIYQKLCIEILETNKQDAVKELVLMHEGROVVIALKTKQAIRNVIAKAL
rNHE11	828	KNLTFLWSRGIIDKHGEGIEMNKVLLTKIKALNNFPMAIPPPTPDKYLPNIWVWENKDVLEFFKERTKL
hNHE11	817	KNLTFLCRGIIDKHEVIEINKVLLKKIKALNNFPKAIPPPTPDYILHNIWLEGGKDVLEDFKERAKL
rNHE11	897	TYFDYGDIIICREGEMPQGIYLIISGMASLHSSSATFGIDPSPTTDRQSKTMYTEYCTSGDVLGELSCL
hNHE11	886	ACFDSGDTICKGGEEMPQGIYLIISGMAILHSLSPTFGIESNQR CDRGSSEMETEFCTTGDIIIGELSCL
rNHE11	966	LKREIETYAICETTLQAFISLEDLYEGFDVFWPSLEYKMWLKLALSIAHQYFESSIAGEDLKFQKQVM
hNHE11	954	LKREIETYVICETSLQAFISLEDLYEGFDAFWPSLEYKMWLKLALSTAYQYFESSLIEDLRFQNCVM
rNHE11	1035	FNHAYVETLSSYNEMTLNHVNMKLIILVYGNVINSKTEETHVAPCIIPKSCEQIQGISDVSKLLVIPTTS
hNHE11	1023	FNQAYVETLSSYSMIIDNMNTMKFVIIIVYGSVIDTKTEEPYFAPCIIPPTTCEQVQGTSDLSKLLIIQAS
rNHE11	1104	EPVKKDATSHDV--VSTGLRTTKDYSWKRKDEFEGGHLFRKTY
hNHE11	1092	ELTQRNSNTNVMASVNTVFEQPGKNINGRQKMS-----

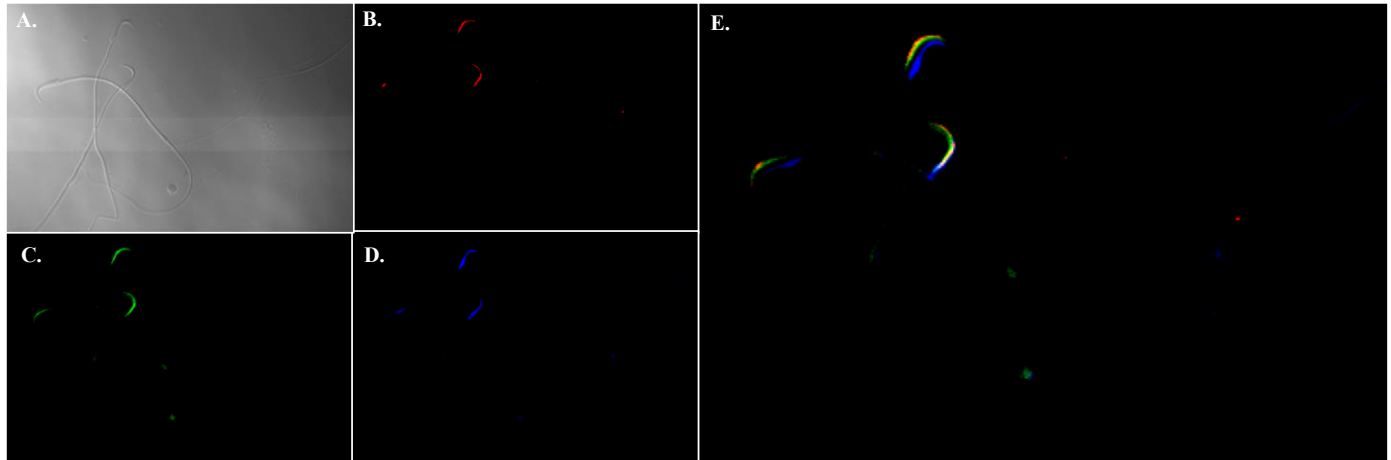
### Supplementary Figure S1. Rat and human NHE11 full-length amino acid sequence alignment.

The deduced rat and human NHE11 amino acid sequences from the cloned cDNAs were aligned. Rat and human NHE11 exhibit a 67.10% identity, 78.62% similarity to each other. Black font indicates identical amino acids.



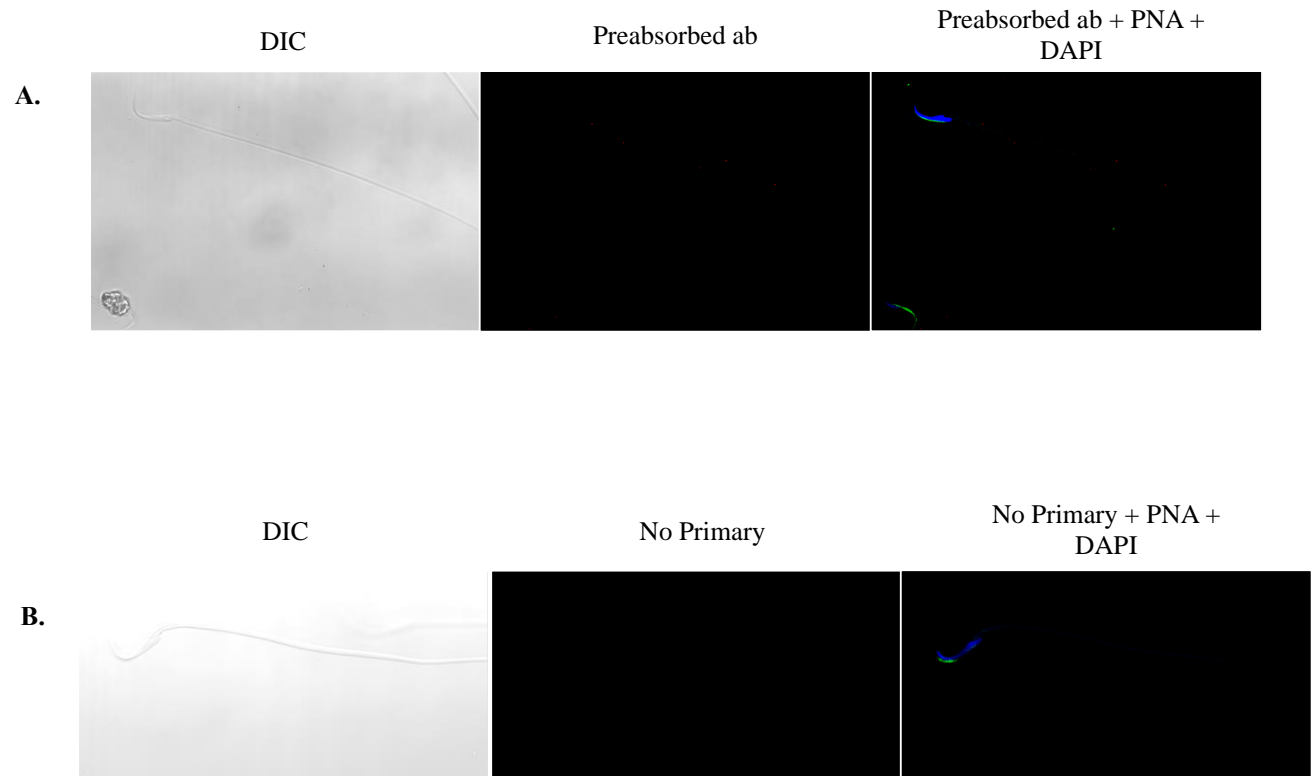
**Supplementary Figure S2. Anti-NHE11 antibody specificity.**

The full immunoblot from Figure 3 is shown here uncropped. A single band in the rat testis and sperm lanes are shown corresponding to the predicted size of NHE11. The NHE11 immunoreactive band was not seen if the anti-NHE11 antibody was preabsorbed with the NHE11 antigenic peptide prior to probing the blot, demonstrating the specificity of this anti-NHE11 antibody and that the immunoreactive band is not a result of non-specific secondary antibody interactions.



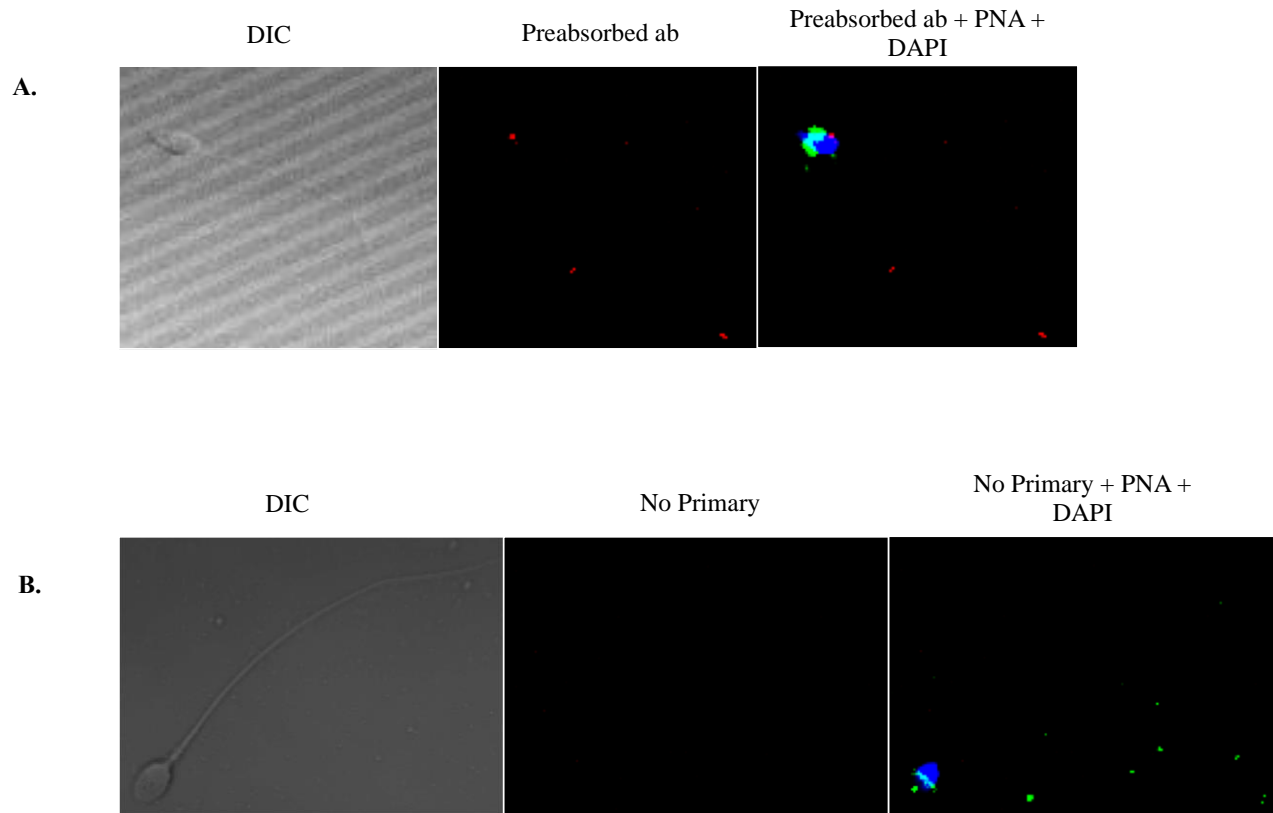
**Supplementary Figure S3. Anti-NHE11 immunocytochemistry on rat sperm cells demonstrates that NHE11 does not localize to the sperm flagellum.**

Immunolocalization of NHE11 in mature rat sperm cells was performed with our anti-NHE11 antibody. A) DIC image of rat sperm cells. B) Anti-NHE11 immunofluorescence (red). C) PNA-FITC staining of the acrosome (green). D) DAPI staining of cell nucleus (blue). E) Merged image of the three color channels (B, C, and D). NHE11 appears to localize to the plasma membrane overlaying the acrosomal region in mature rat sperm cells. There may be some colocalization of NHE11 to the acrosomal region (yellow). Note that there is no observed NHE11 staining of the rat sperm flagellum.



**Supplementary Figure S4. Negative controls for rat sperm immunocytochemistry experiments.**

For negative controls the rat sperm acrosomes and nuclei were stained with PNA-FITC and DAPI respectively, A) the anti-NHE11 antibody was preabsorbed with the antigenic NHE11 peptide and subsequently used for immunocytochemistry or B) the primary antibody was omitted entirely.



**Supplementary Figure S5. Negative controls for human sperm immunocytochemistry experiments.**

For negative controls the human sperm acrosomes and nuclei were stained with PNA-FITC and DAPI respectively, A) the anti-NHE11 antibody was preabsorbed with the immunizing NHE11 peptide and subsequently used for immunocytochemistry or B) the primary antibody was omitted entirely.