

**Allosteric links between the hydrophilic N-terminus and transmembrane core of human Na<sup>+</sup>/H<sup>+</sup> antiporter NHA2**

SUPPLEMENTARY MATERIAL

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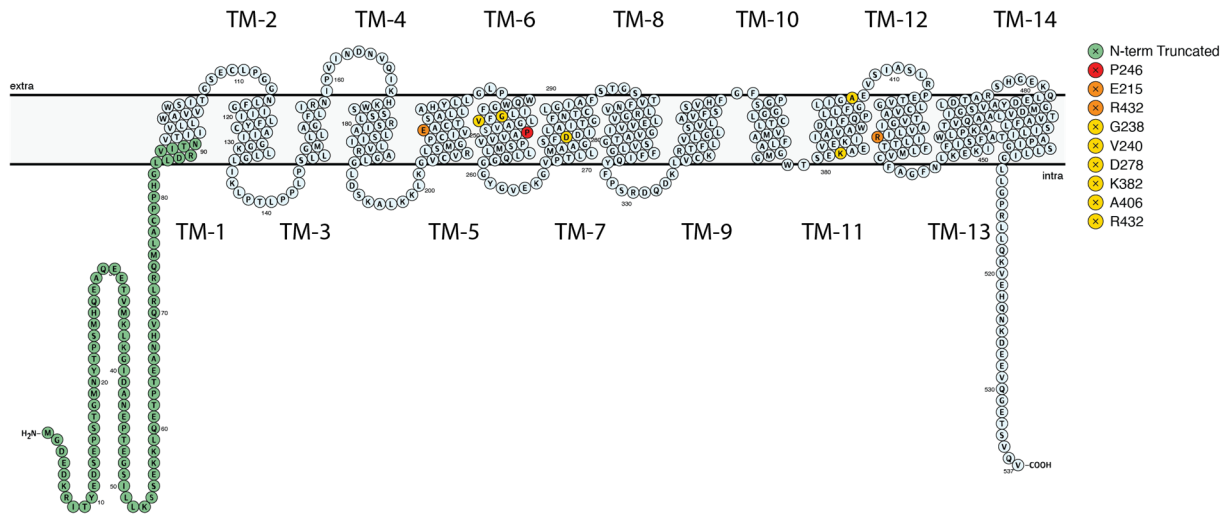
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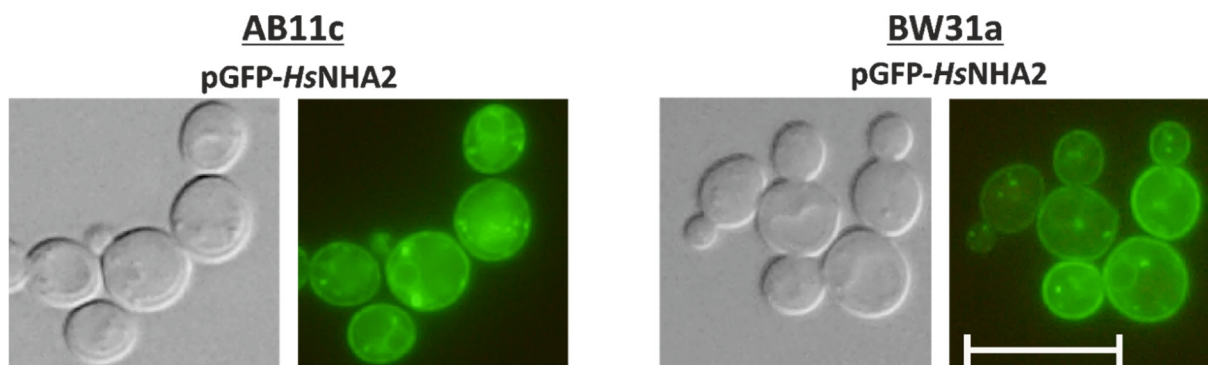
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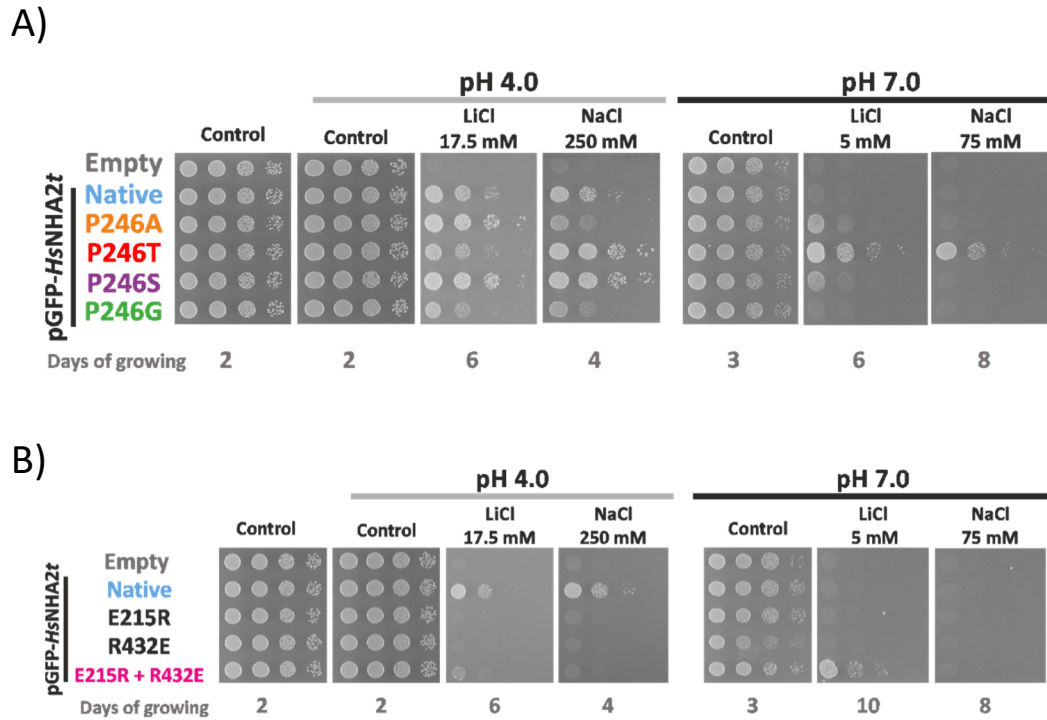
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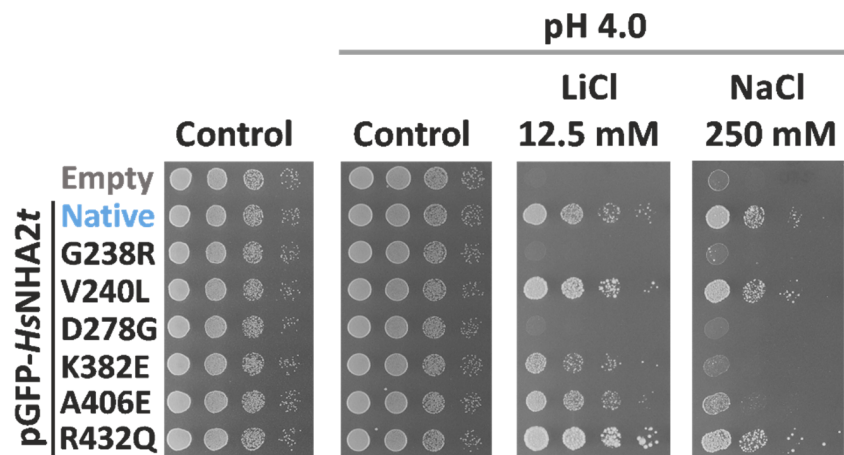
**Figure S1: The membrane topology of *HsNHA2*.** *HsNHA2* orientation in the membrane is shown in 2-dimensional representation with the extracellular matrix at the top and the cytoplasm at the bottom. Transmembrane helices are numbered 1-to-14. The N-terminus region, where various truncations were made, is colored green. The position of P246, which is involved in pH regulation, is highlighted in red, E215 and R432 that form a conserved salt bridge are colored orange, and the positions of other human variants are highlighted in yellow.



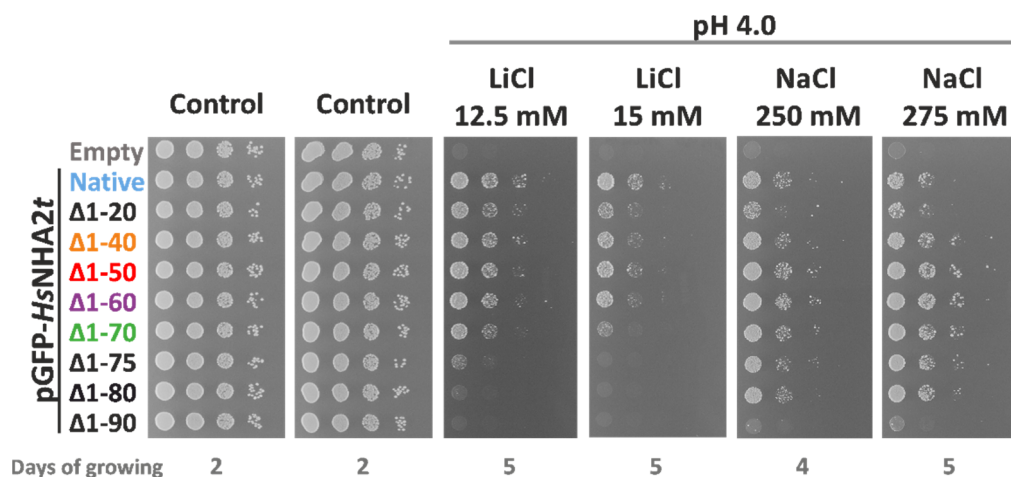
**Figure S2: Comparison of localization of GFP-HsNHA2 in two yeast backgrounds.** Transformants of AB11c (*ena1-4Δ nha1Δ nhx1Δ*) or BW31 (*ena1-4Δ nha1Δ*) expressing GFP-HsNHA2 from the pGFP-HsNHA2 plasmid were grown in YNB-Pro (4% glucose) to the exponential phase and observed under a fluorescence microscope (right). A Nomarski prism was used for whole-cell imaging (left). The scale bar corresponds to 10 μm.



**Figure S3: N-terminal GFP-tagging does not change substrate specificity, but increases activity of *HsNHA2* mutated versions.** Salt tolerance of *S. cerevisiae* BW31 cells containing empty vector or expressing GFP-*HsNHA2* or one of four GFP-*HsNHA2* versions with single point mutations P246A, T, S or G (A) or versions E215R, R432E or E215R + R432E (B) from pGFP-*HsNHA2t* plasmid. Cells were grown on non-buffered YNB-Pro plates with the pH adjusted to 4.0 or 7.0 and supplemented with LiCl or NaCl as indicated. Plates were incubated at 30 °C and photographed on the indicated day.



**Figure S4: N-terminal GFP-tagging does not change substrate specificity of *HsNHA2* versions with mutations that belong to known human SNPs.** The salt tolerance of *S. cerevisiae* BW31 cells containing the empty vector or expressing the native GFP-*HsNHA2* or one of six GFP-*HsNHA2* mutated versions - G238R, V240L, D278G, K382E, A406E and R432Q from the pGFP-*HsNHA2t* plasmid. Cells were grown on non-buffered YNB-Pro plates with the pH adjusted to 4.0 and supplemented with LiCl or NaCl as indicated. Growth was monitored for 2 (control) or 7 (LiCl or NaCl) days at 30°C.



**Figure S5: N-terminal GFP-tagging altered LiCl tolerance provided by *HsNHA2* versions truncated at N-terminus.** The salt tolerance of *S. cerevisiae* BW31 cells containing the empty vector or expressing the tagged GFP-*HsNHA2* or N-terminal truncated GFP-*HsNHA2* versions from the pGFP-*HsNHA2t*. Cells were grown on non-buffered YNB-Pro plates with the pH adjusted to 4.0 and supplemented with LiCl or NaCl as indicated. Plates were incubated at 30 °C and photographed on the indicated day.

Table S1. Oligonucleotides used in this study

Oligonucleotide	Sequence	Use
<i>Hs</i> NHA2-YEp-F	GTACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGGGGGATGAAGATAAAAG	<i>pHs</i> NHA2 and <i>pHs</i> NHA2t clonning
<i>pNHA1</i> -GFP-F	GTTTTTTGTACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGTCTAAAGGTGAAGAA TTATTC	<i>pGFP-Hs</i> NHA2 and <i>pGFP-Hs</i> NHA2t
<i>Hs</i> NHA2 TPS1 Term F	CAAATAAAGATGAAGAAGTTCAAGGAGAGACTTCTGTGCAAGTTTAGTGAACCCGATGCAAA TGAG	<i>pHs</i> NHA2t and <i>pGFP-Hs</i> NHA2t clonning
<i>Hs</i> NHA2_TPS1 Term R	GGACCAGGAATAGACGATCGTCTCATTGTCATCGGGTTCCTAACTTGCACAGAAGTCTC	<i>pHs</i> NHA2t and <i>pGFP-Hs</i> NHA2t clonning
TPS1_Term_Yep_R	GTCACGACGTTGTAACACGACGGCCAGTGCCAAGCTTGCATGTGTTTCAAGAAGAGATCAG	<i>pHs</i> NHA2t and <i>pGFP-Hs</i> NHA2t clonning
<i>Hs</i> NHA2-P246A_Fw	GGTGCTGTATCTGCAGCTGTTGTGGTG	Mutagenesis P246A
<i>Hs</i> NHA2-P246A_Rev	CACCACAACAGCTGCAGATACAGCACC	Mutagenesis P246A
<i>Hs</i> NHA2-P246T_Fw	GGTGCTGTATCTACAGCTGTTGTGGTG	Mutagenesis P246T
<i>Hs</i> NHA2-P246T_Rev	CACCACAACAGCTGTAGATACAGCACC	Mutagenesis P246T
<i>Hs</i> NHA2-P246S_Fw	GGTGCTGTATCTTACAGCTGTTGTGGTG	Mutagenesis P246S
<i>Hs</i> NHA2-P246S_Rev	CACCACAACAGCTGAAGATACAGCACC	Mutagenesis P246S
<i>Hs</i> NHA2_P246G_Fw	GGTGCTGTATCTGGAGCTGTTGTGGTG	Mutagenesis P246G
<i>Hs</i> NHA2_P246G_Rev	CACCACAACAGCTCCAGATACAGCACC	Mutagenesis P246G
<i>Hs</i> NHA2-E215R_Fw	CCCTGTATTGTGCGGGCGTGCACATCT	Mutagenesis E215R and E215R + R432E
<i>Hs</i> NHA2-E215R_Rev	AGATGTGCACGCCGACACAATACAGGG	Mutagenesis E215R and E215R + R432E
<i>Hs</i> NHA2-R432E_Fw	GCAGTATTGATAGAAATTTGACTACA	Mutagenesis R432E and E215R + R432E
<i>Hs</i> NHA2-R432E_Rev	TGTAGTCAAATTTCTATCAATACTGC	Mutagenesis E215R and E215R + R432E
<i>Hs</i> NHA2-G238R_Fw	GGATTTATACTGCGTTTTGTTTAGGT	Mutagenesis G238R
<i>Hs</i> NHA2-G238R_Rev	ACCTAAAACAAAACGCAGTATAAATCC	Mutagenesis G238R
<i>Hs</i> NHA2-V240L_Fw	ATACTGGGTTTTCTTTAGGTGCTGTA	Mutagenesis V240L
<i>Hs</i> NHA2-V240L_Rev	TACAGCACCTAAAAGAAAACCCAGTAT	Mutagenesis V240L
<i>Hs</i> NHA2-D278G_Fw	GCTGGCAGCTTCGGTGACATTCTGGCC	Mutagenesis D278G
<i>Hs</i> NHA2-D278G_Rev	GGCCAGAATGTCACCGAAGCTGCCAGC	Mutagenesis D278G
<i>Hs</i> NHA2-K382E_Fw	TGGACCAGCGAAGAGGCAGAGGTTGAA	Mutagenesis K382E
<i>Hs</i> NHA2-K382E_Rev	TTCAACCTCTGCCTCTTCGCTGGTCCA	Mutagenesis K382E
<i>Hs</i> NHA2-A406E_Fw	GGACTAATTGGAGAAGAGGTATCTATT	Mutagenesis A406E
<i>Hs</i> NHA2-A406E_Rev	AATAGATACCTCTTCTCCAATTAGTCC	Mutagenesis A406E
<i>Hs</i> NHA2-R432Q_Fw	GCAGTATTGATACAAATTTGACTACA	Mutagenesis R432Q
<i>Hs</i> NHA2-R432Q_Rev	TGTAGTCAAATTTGTATCAATACTGC	Mutagenesis R432Q
<i>Hs</i> NHA2 delta 1-20	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGTACACGCCCTCCATGCATCA	<i>pHs</i> NHA2t Δ1-20 clonning
<i>Hs</i> NHA2 delta 1-40	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGGATGCAAATGAACCAACAGAAGGA	<i>pHs</i> NHA2t Δ1-40 clonning
<i>Hs</i> NHA2 delta 1-50	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGCTTCTGAAAAGCAGTGAAAAAAG	<i>pHs</i> NHA2t Δ1-50 clonning
<i>Hs</i> NHA2 delta 1-60	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGGAAAACCAACTGAAGCAAATCAC	<i>pHs</i> NHA2t Δ1-60 clonning
<i>Hs</i> NHA2 delta 1-70 F	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGAGACTGAGACAAATGCTGGC	<i>pHs</i> NHA2t Δ1-70 clonning
<i>Hs</i> NHA2 delta 1-75	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGCTGGCTTGCCTCCACA	<i>pHs</i> NHA2t Δ1-75 clonning
<i>Hs</i> NHA2 delta 1-80	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGCATGGTTTACTGGACAGGGTC	<i>pHs</i> NHA2t Δ1-80 clonning
<i>Hs</i> NHA2 delta 1-90	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGGTTACCATCATTGTTCTTCTGTGG	<i>pHs</i> NHA2t Δ1-90 clonning
<i>Hs</i> NHA2 delta 1-110	ACATTATAAAAAAAAAATCCTGAACTTAGCTAGATATTATGCCTGGAGGAAACCTATTTGG	<i>pHs</i> NHA2t Δ1-110 clonning
<i>GFP-Hs</i> NHA2 delta 1-20 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGTACACGCCCTCCATGCATCA	<i>pGFP-Hs</i> NHA2t Δ1-20 clonning
<i>GFP-Hs</i> NHA2 delta 1-20 R	CTGTCTCCTCTGTGCTTCTTGATGCATGGAGGGCGTGTACATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-20 clonning
<i>GFP-Hs</i> NHA2 delta 1-40 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGGATGCAAATGAACCAACAGAAGGA	<i>pGFP-Hs</i> NHA2t Δ1-40 clonning
<i>GFP-Hs</i> NHA2 delta 1-40 R	TTTTCAGAAGAATACTTCTTCTGTTGGTTCAATTCATCCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-40 clonning
<i>GFP-Hs</i> NHA2 delta 1-50 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGCTTCTGAAAAGCAGTGAAAAAAG	<i>pGFP-Hs</i> NHA2t Δ1-50 clonning
<i>GFP-Hs</i> NHA2 delta 1-50 R	TTGGTGTCTTGTAGCTTTTTTCACTGCTTTTCAGAAGCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-50 clonning
<i>GFP- Hs</i> NHA2 delta 1-60 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGAAAACCAACTGAAGCAAATCAC	<i>pGFP-Hs</i> NHA2t Δ1-60 clonning
<i>GFP-Hs</i> NHA2 delta 1-60 R	TGTCTCAGTCTTTGTACGTGATTTGCTTCAGTTGGTGTTCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-60 clonning
<i>GFP Hs</i> NHA2 delta 1-70 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGAGACTGAGACAAATGCTGGC	<i>pGFP-Hs</i> NHA2t Δ1-70 clonning
<i>GFP Hs</i> NHA2 delta 1-70 R	GTAACCATGTGGAGGGCAAGCCAGCATTTGTCTCAGTCTCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-70 clonning
<i>GFP-Hs</i> NHA2 delta 1-75 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGCTGGCTTGCCTCCACA	<i>pGFP-Hs</i> NHA2t Δ1-75 clonning
<i>GFP-Hs</i> NHA2 delta 1-75 R	TTATGACCCTGTCCAGTAAACCATGTGGAGGGCAAGCCAGCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-75 clonning
<i>GFP-Hs</i> NHA2 delta 1-80 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGCATGGTTTACTGGACAGGGTC	<i>pGFP-Hs</i> NHA2t Δ1-80 clonning
<i>GFP-Hs</i> NHA2 delta 1-80 R	TGATGGTAACATTTGTTATGACCTGTCCAGTAAACCATGCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-80 clonning
<i>GFP-Hs</i> NHA2 delta 1-90 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGGTTACCATCATTGTTCTTCTGTGG	<i>pGFP-Hs</i> NHA2t Δ1-90 clonning
<i>GFP-Hs</i> NHA2 delta 1-90 R	TTGACCAAACACAGCCACAGAAGAACAATGATGGTAACCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-90 clonning
<i>GFP Hs</i> NHA2 delta 1-110 F	ATCCGGACTCAGATCTCGAGCTCAAGCTTCAATTCTATGCCTGGAGGAAACCTATTTGG	<i>pGFP-Hs</i> NHA2t Δ1-110 clonning
<i>GFP Hs</i> NHA2 delta 1-110 R	AATAGAACAGGATTATAAATCCAATAGTTTTCTCCAGGCATAGAATTCGAAGCTTGAGCT	<i>pGFP-Hs</i> NHA2t Δ1-110 clonning
<i>Hs</i> NHA2 E47A Fw	AATGAACCAACAGCAGGAAGTATTCTT	Mutagenesis E47A
<i>Hs</i> NHA2 E47A Rev	AAGAATACTTCTGCTGTTGGTTTATT	Mutagenesis E47A
<i>Hs</i> NHA2 E47K Fw	AATGAACCAACAAAAGGAAGTATTCTT	Mutagenesis E47K
<i>Hs</i> NHA2 E47K Rev	AAGAATACTTCTTTTGTGGTTTATT	Mutagenesis E47K
<i>Hs</i> NHA2 E56A Fw	CTGAAAAGCAGTGCAAAAAAGCTACAA	Mutagenesis E56A
<i>Hs</i> NHA2 E56A Rev	TTGTAGCTTTTTTGCAGTCTTTTTCAG	Mutagenesis E56A
<i>Hs</i> NHA2 E56K Fw	CTGAAAAGCAGTAAAAAAAAGCTACAA	Mutagenesis E56K
<i>Hs</i> NHA2 E56K Rev	TTGTAGCTTTTTTACTGCTTTTTCAG	Mutagenesis E56K
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<i>Hs</i> NHA2 KK57-58EE F	TATTCTTCTGAAAAGCAGTGAAGAAGAACTACAAGAAACACCAACTGAAG	Mutagenesis K57E + K58E
<i>Hs</i> NHA2 KK57-58EE R	CTTCAGTTGGTGTCTTCTGTAGTTCTTCTCACTGCTTTTTCAGAAGAATA	Mutagenesis K57E + K58E
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