

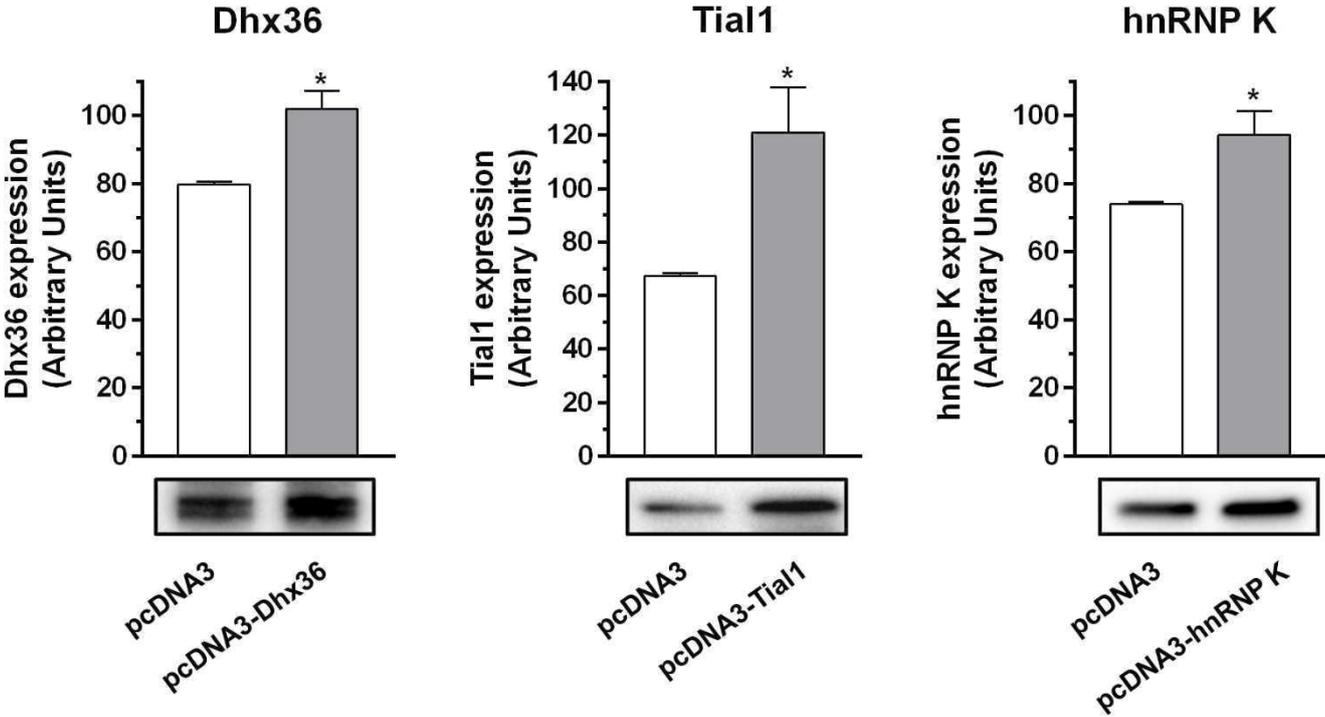
Supplementary Material

Post-Transcriptional Modulation of aENaC mRNA in Alveolar Epithelial Cells: Involvement of its 3' Untranslated Region

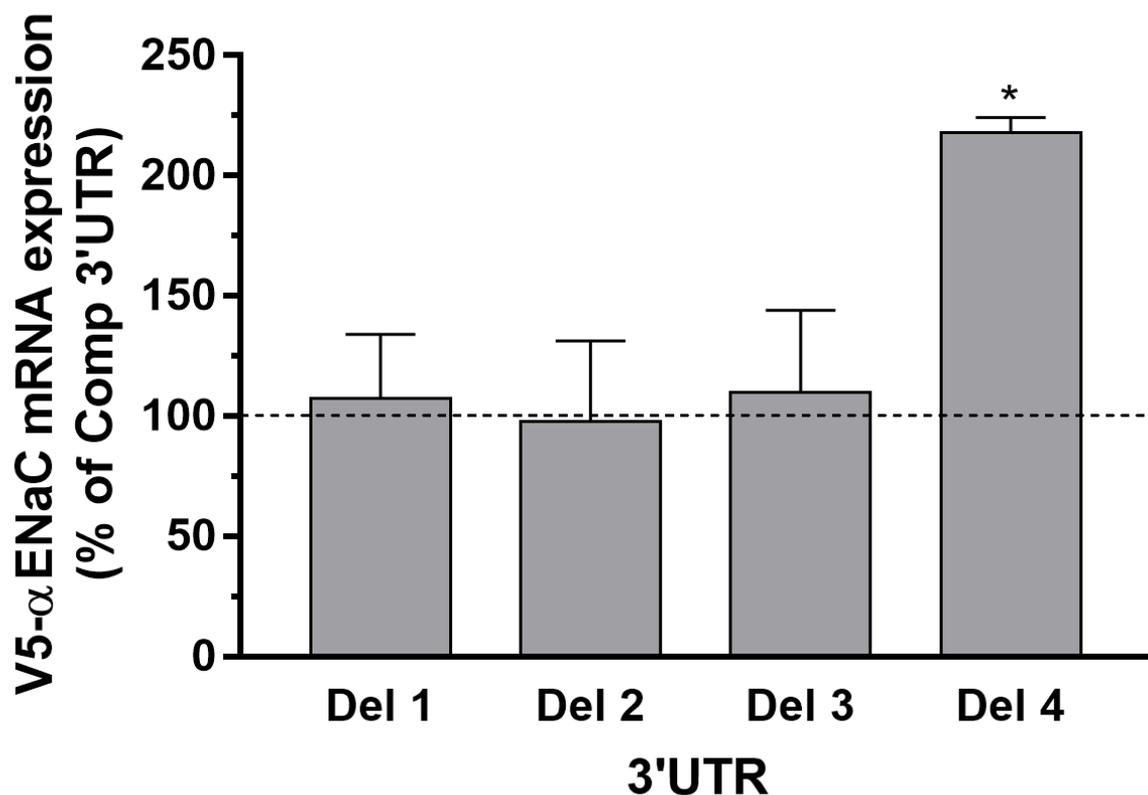
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Supplementary figure 1. Overexpression of Dhx36, hnRNP K and Tial1 proteins in alveolar epithelial cells. Alveolar epithelial cells were transfected with expression vector encoding for Dhx36, hnRNPK or Tial1 RBPs. Each RBP expression was increased significantly following transfection. *P<0.05 by unpaired t-test compared to pcDNA3; n=3 for each experimental condition.

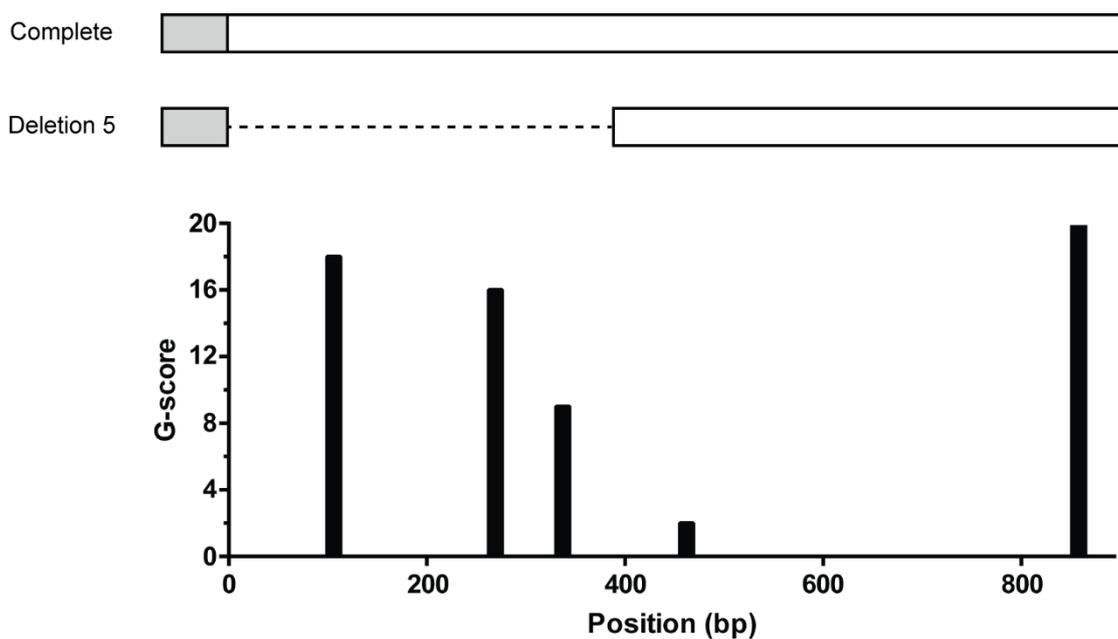


Supplementary figure 2. Impact of different deletions of the α ENaC 3'UTR on relative V5- α ENaC mRNA expression. Alveolar epithelial cells were transiently cotransfected with the pTRE-tight plasmid coding for V5- α ENaC mRNA with the different α ENaC 3'UTR deletion mutants along with pTet-Off plasmid that express tTA-Ad that allows the specific expression of the construct and its inhibition by doxycycline. Seventy-two h after transfection, expression of V5- α ENaC mRNA for each deletion was measured by quantitative RT-PCR and presented as percentage \pm SEM of V5- α ENaC mRNA expression of Comp 3'UTR after normalization with tTA-Ad. * $P < 0.05$ by one-sample t-tests compared to Comp 3'UTR ($n \geq 5$ for each experimental condition).



Supplementary figure 3. Presence of G-quadruplexes in the proximal region of the α ENaC 3'UTR.

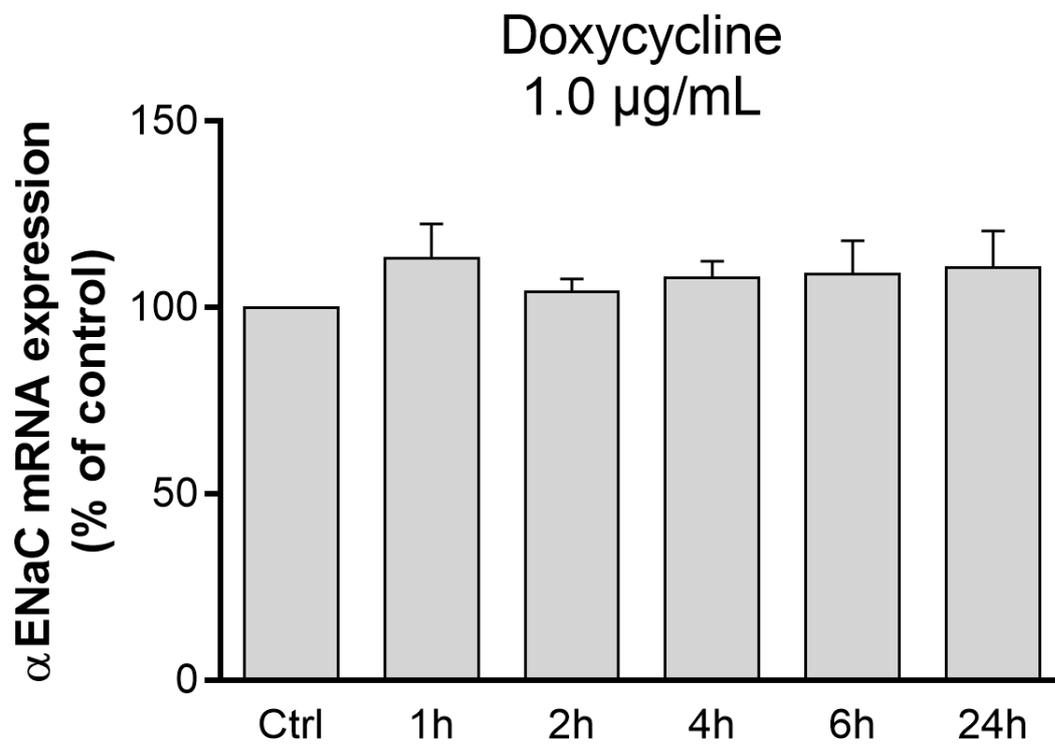
QGRS Mapper, a web-based server for predicting G-quadruplexes in nucleotide sequences, was used to identify the presence of G-quadruplexes in the α ENaC 3'UTR.



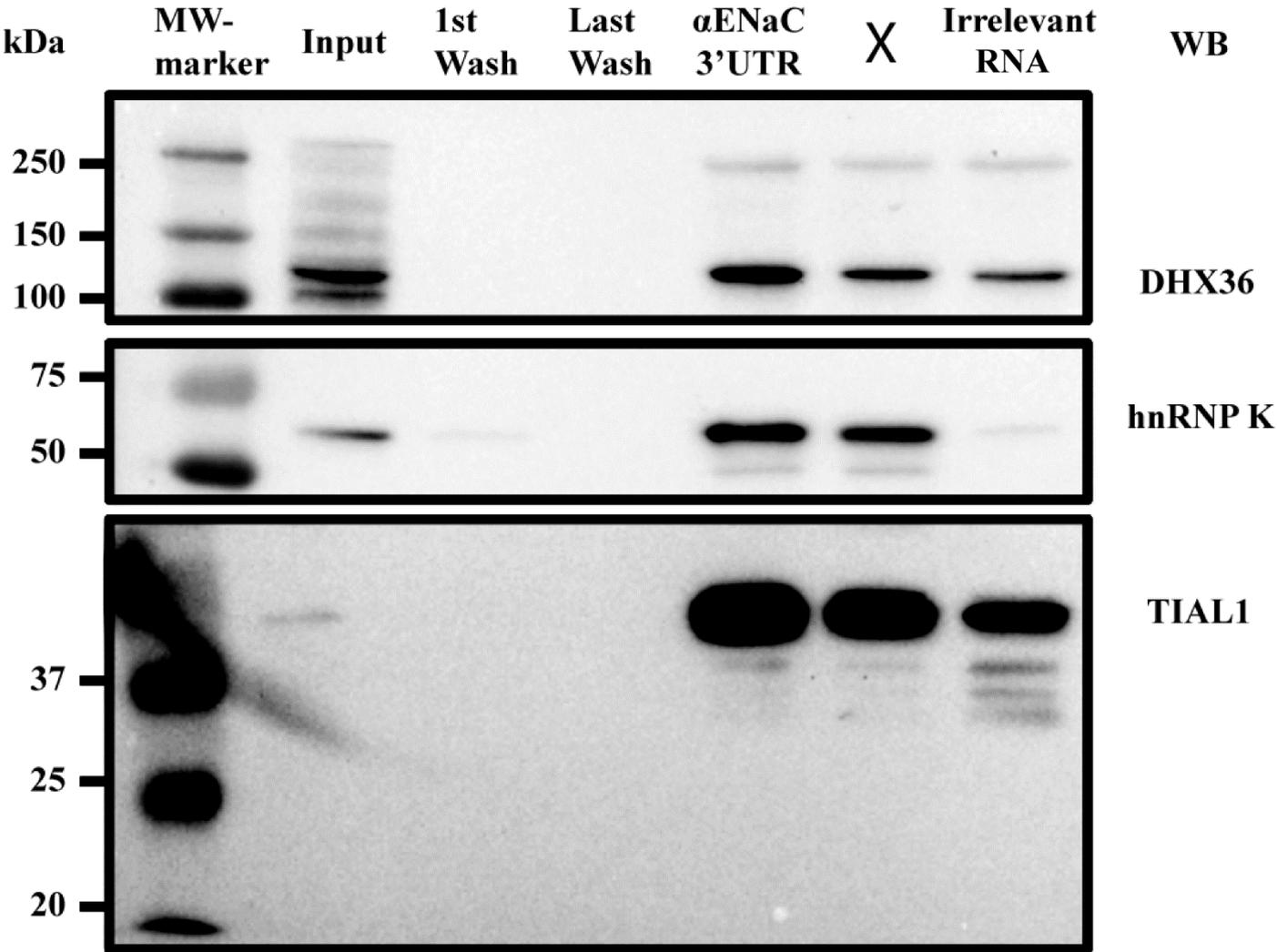
Position	Length	Sequence	G-Score
106	27	<u>GGGAACAGGCTGAGTAAGGGGCCCAGG</u>	18
269	26	<u>GGAACTTTCCTGGGAGAGGACCAAGG</u>	16
337	27	<u>GGCTGGCCTGGCCTCACTGCCTTCAGG</u>	9
462	29	<u>GGGTGGGGACTAGAGTAAGACAGACCAGG</u>	2
858	15	<u>GGACGGGTAGGCAGG</u>	20

Supplementary figure 4. Modulation of α ENaC mRNA by doxycycline in alveolar epithelial cells.

Alveolar epithelial cells were treated with 1.0 $\mu\text{g/ml}$ doxycycline for a period of 1 to 24 h. Expression of α ENaC mRNA was quantified by quantitative RT-PCR and expressed as expression of α ENaC mRNA \pm SEM compared to untreated cells (Ctrl; t = 0) after normalization with β -actin (One-way ANOVA, n = 4).



Supplementary figure 5. Identification of proteins bound to α ENaC 3'UTR by RNA affinity chromatography. Uncropped immunoblots from Figure 4B.



Supplementary figure 6. Posttranscriptional modulation of V5- α ENaC deletion mutants mRNA in cells that overexpress Dhx36 and Tial1. Alveolar epithelial cells were cotransfected with the different 3'UTR deletion mutants (Del 1-5) in pTRE-tight vectors along with pTet-Off plasmid and the expression vector for Dhx36 or Tial1 RBPs overexpression. V5- α ENaC mRNA expression was quantified by RT-qPCR 72 h posttransfection and expressed as percentage of V5- α ENaC mRNA compared to cells transfected with the complete 3'UTR and an empty vector (pcDNA3) \pm SEM after normalization with tTA-Ad. Overexpression of Dhx36 and Tial1 significantly inhibited V5- α ENaC mRNA expression for each construction except for V5- α ENaC-Del4, which lacks the 3'UTR sequences. * P <0.05 by Kruskal-Wallis tests and Dunn's post-hoc tests compared experimental vectors to the empty vector; $n \geq 6$ for each experimental condition.

