

1 **Supplementary Figure 1**

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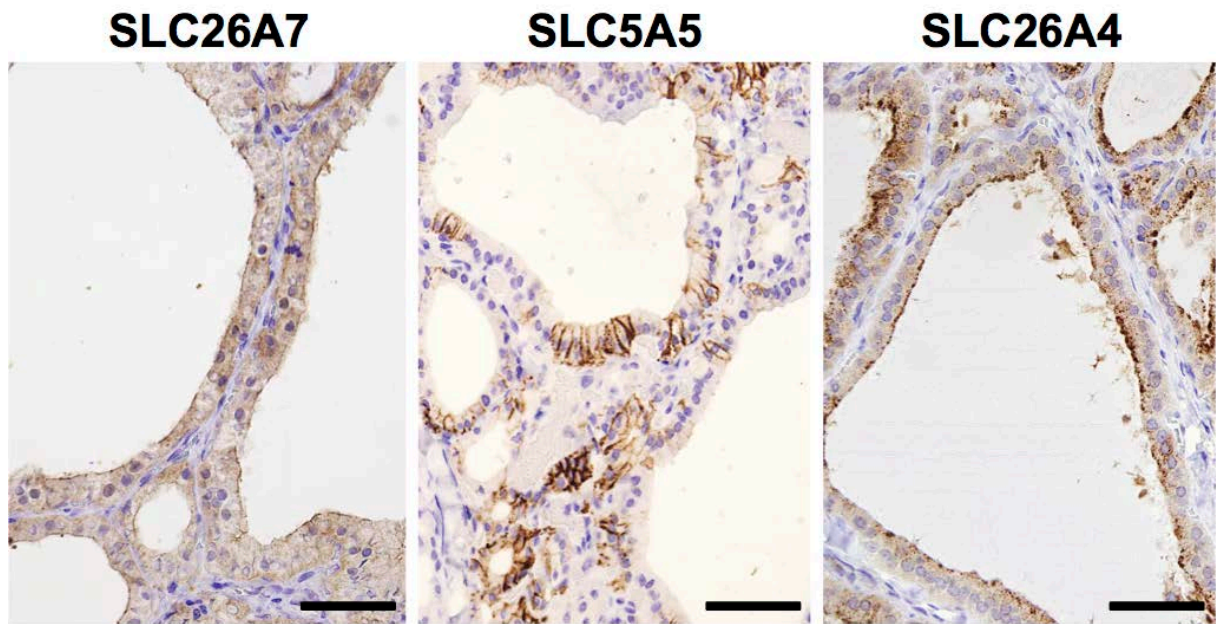
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16 Supplementary Figure 1. Immunohistochemical staining of SLC26A7 (left), SLC5A5

17 (centre), and SLC26A4 (right) in thyroid tissues. Scale bar, 50  $\mu$ m.

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19 **Supplementary Figure 2**

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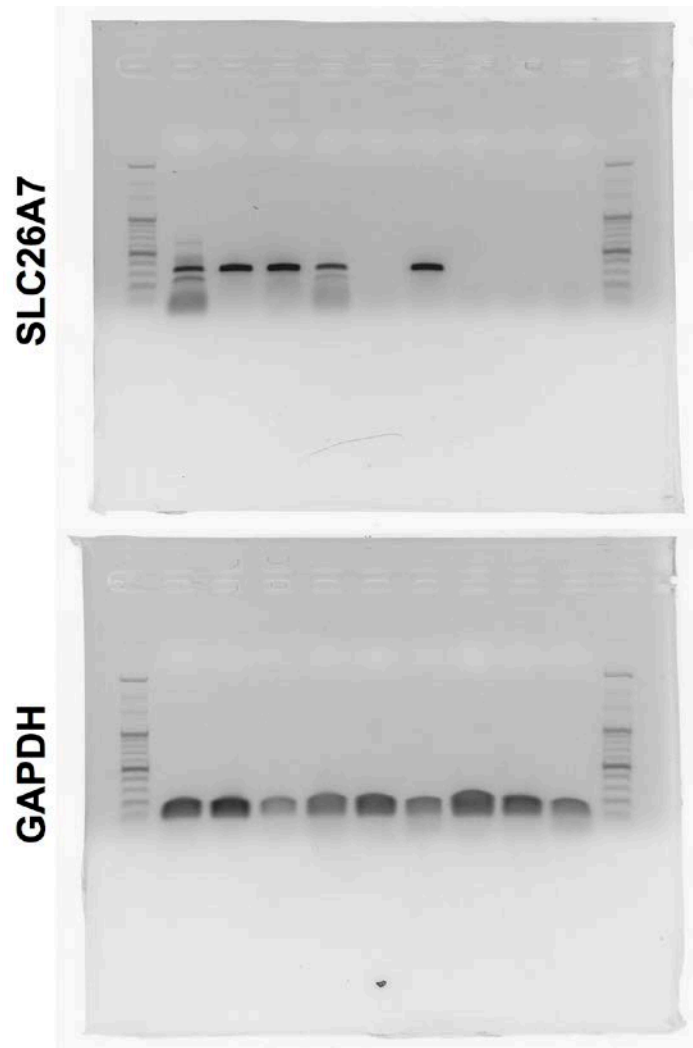
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42 **Supplementary Figure 2. Uncropped gel image in Figure 1b.**

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44 **Supplementary Figure 3**

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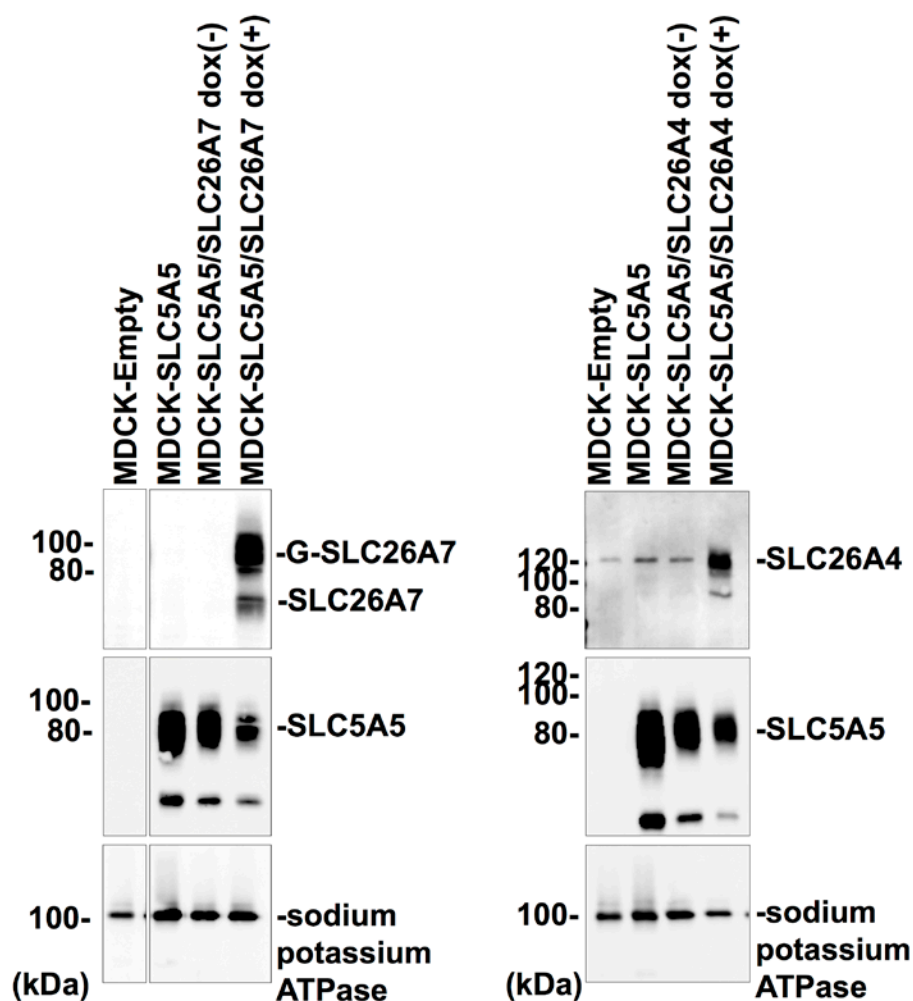
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67 Supplementary Figure 3. Immunoblot analysis to confirm the gene transduction of *SLC26A7*

68 and *SLC26A4* and *SLC5A5* in MDCK cells. Sodium potassium ATPase served as an internal

69 control. dox, doxycycline; G-SLC26A7, glycosylated SLC26A7.

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71 **Supplementary Figure 4**

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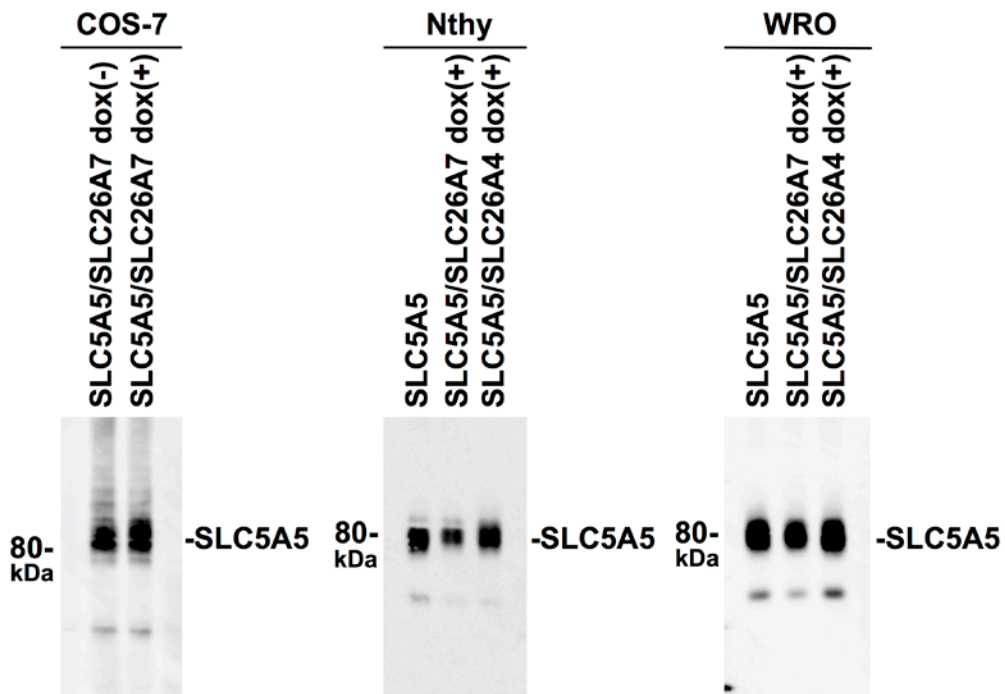
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87 Supplementary Figure 4. Immunoblot analysis of SLC5A5 in SLC5A5 or SLC5A5 and  
88 SLC26A7 (SLC5A5/SLC26A7), or SLC5A5 and SLC26A4 (SLC5A5/ SLC26A4) in  
89 inducible COS-7, Nthy and WRO cells in the absence or presence of the inducer  
90 doxycycline (dox(-), dox(+)) respectively).

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92 **Supplementary Figure 5**

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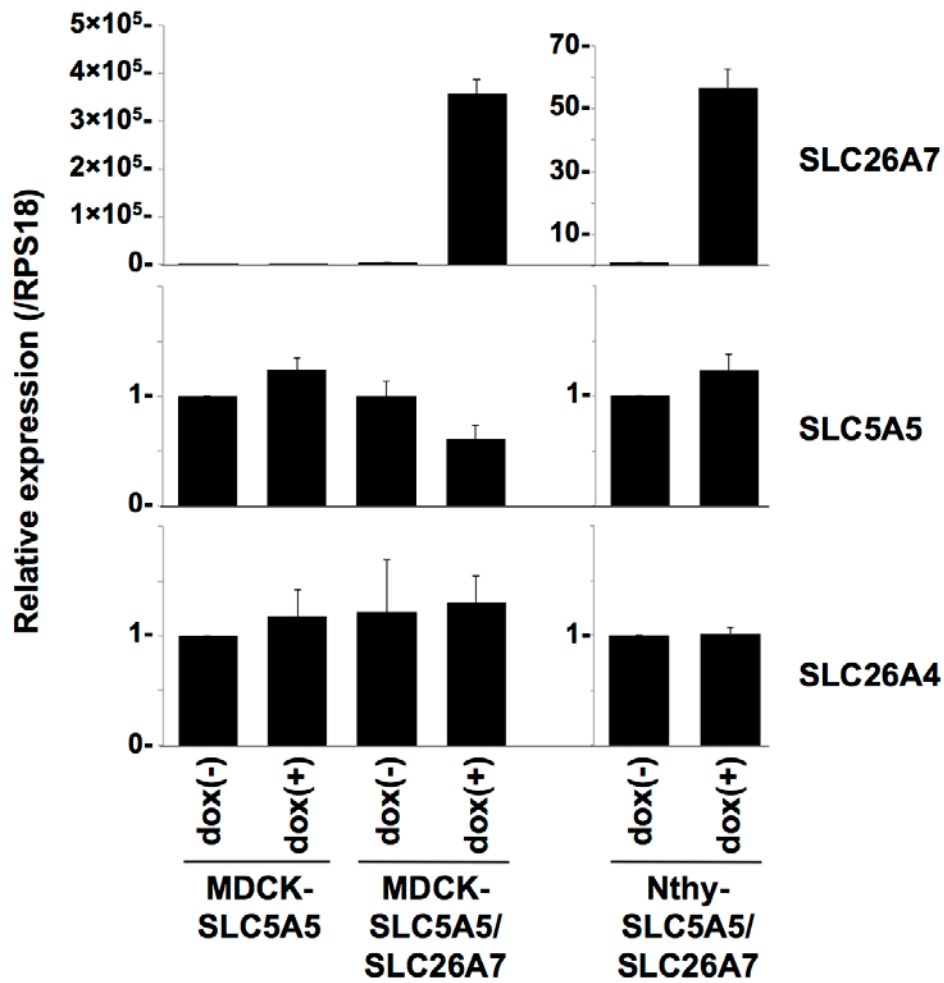
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114 Supplementary Figure 5. Semiquantitative RT-PCR analysis of SLC26A7, SLC5A5, and  
 115 SLC26A4 mRNA expression in SLC5A5-inducible or SLC5A5 and SLC26A7  
 116 (SLC5A5/SLC26A7)-inducible MDCK and Nthy cells. Data are shown as means±SD. dox,  
 117 doxycycline. SLC5A5 and SLC26A4 expression were not greatly affected by *SLC26A7*  
 118 induction.

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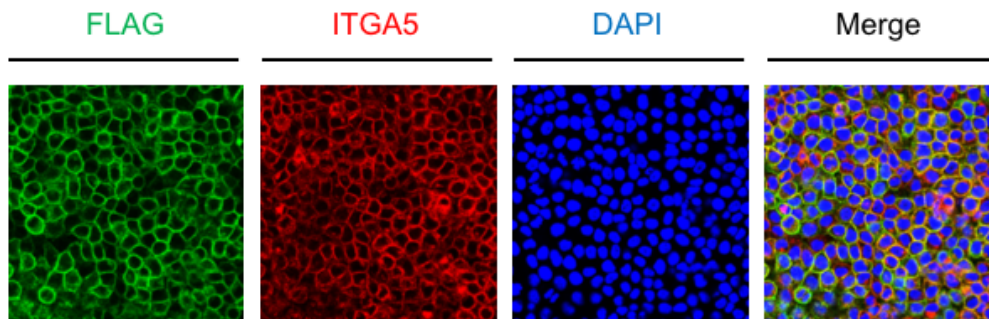
120 **Supplementary Figure 6**

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134 Supplementary Figure 6. Immunofluorescence analysis of FLAG (green) and ITGA5

135 (integrin subunit alpha 5, red) in FLAG-tagged SLC26A7 WT-transfected HeLa cells.

136 4',6-diamidino-2-phenylindole, DAPI (blue) marks cell nuclei. Note SLC26A7 WT protein

137 colocalized with the plasma membrane protein IGTA5.

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139 **Supplementary Figure 7**

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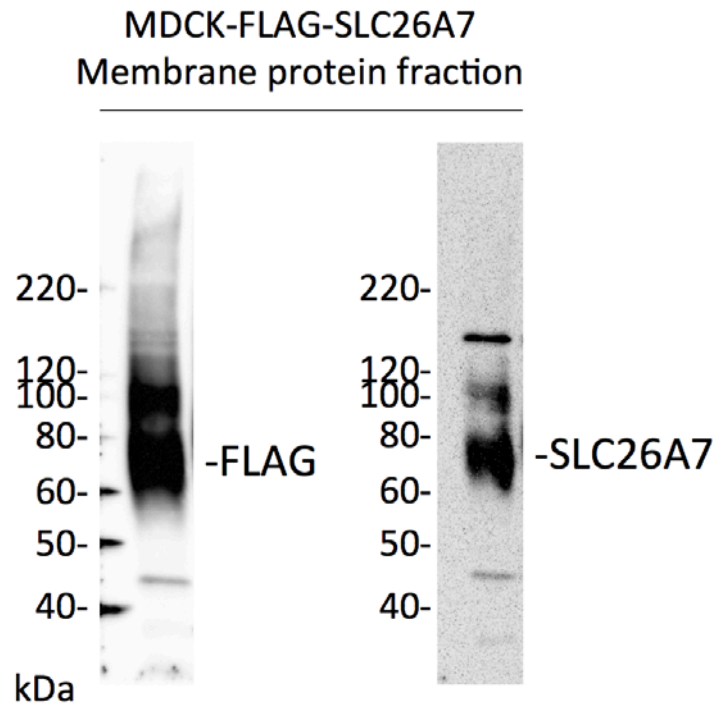
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156 Supplementary Figure 7. Immunoblot analysis to confirm whether SLC26A7 is expressed

157 on the cell membrane. Membrane protein fraction was extracted using a membrane protein

158 isolation kit (Pierce Cell Surface Protein Isolation Kit, Thermo Fisher Scientific) from

159 FLAG-tagged SLC26A7-inducible MDCK cells. FLAG-tagged SLC26A7 was detected in

160 membrane protein fraction.

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162 **Supplementary Figure 8**

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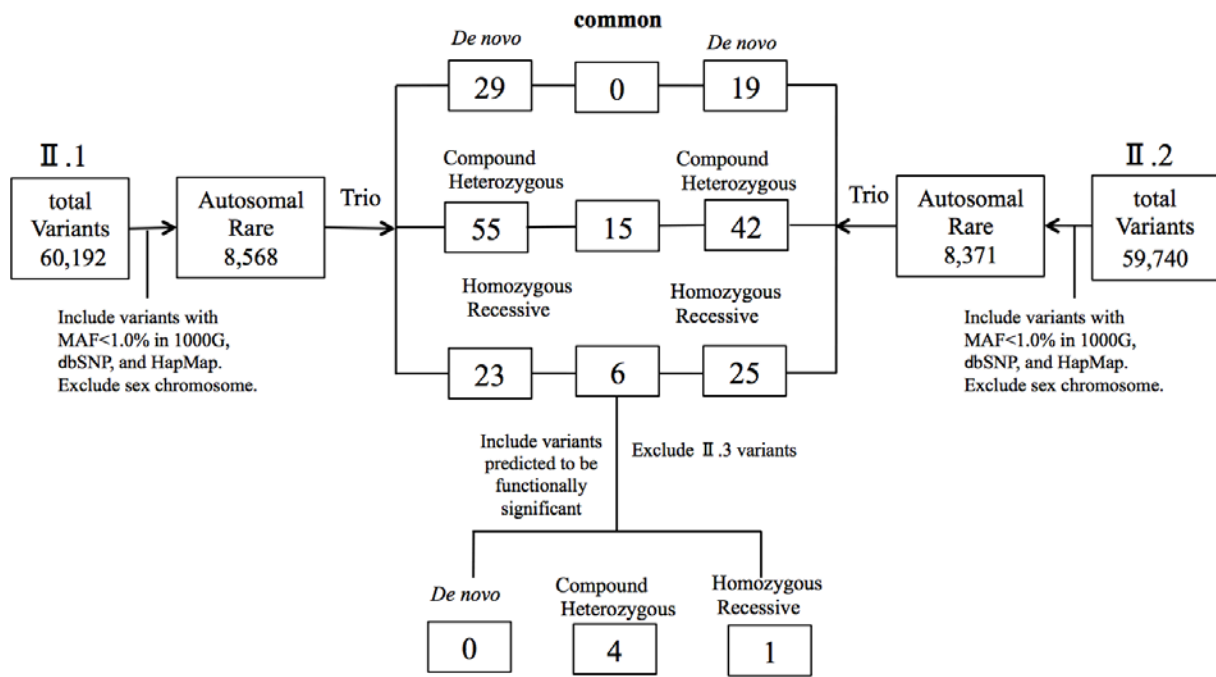
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179 Supplementary Figure 8. Schematic for identifying candidate homozygous or compound

180 heterozygous rare variants in genes.

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Supplementary Figure 8. Schematic for identifying candidate homozygous or compound heterozygous rare variants in genes.



182 **Supplementary Figure 9**

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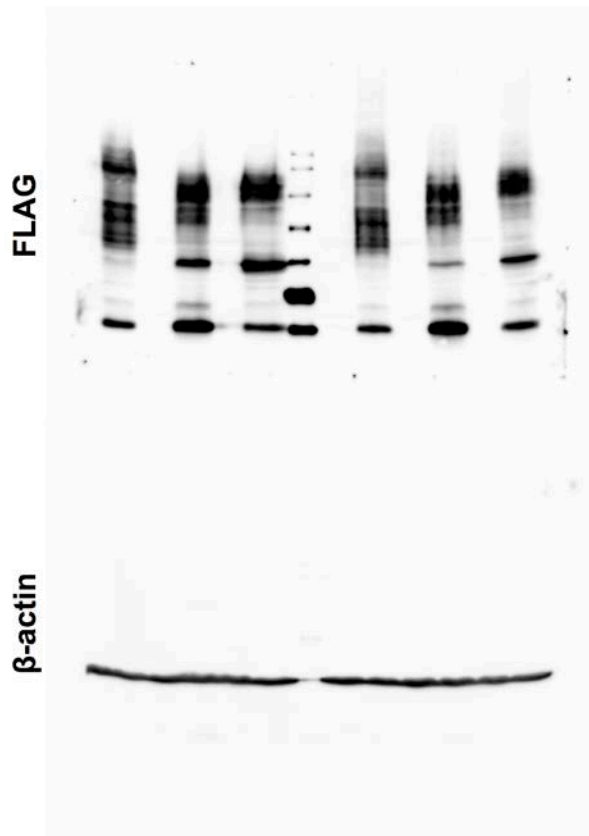
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200 Supplementary Figure 9. Uncropped blot image in Figure 5a.

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202 **Supplementary Figure 10**

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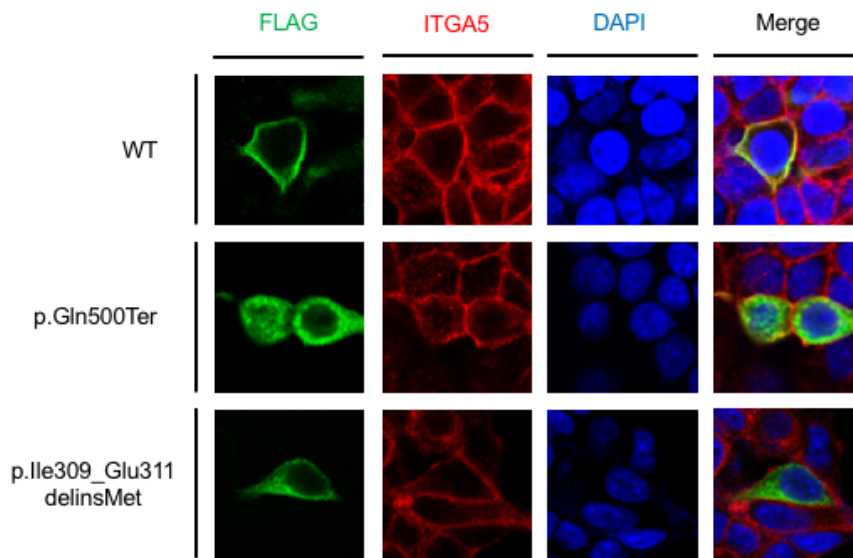
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218 Supplementary figure 10. Immunofluorescence analysis of FLAG (green) and ITGA5 (red)  
219 in FLAG-tagged wild type (WT), p.Gln500Ter mutant or p.Ile309\_Glu311delinsMet mutant  
220 SLC26A7 in transiently transfected HEK293T cells. 4',6-diamidino-2-phenylindole, DAPI  
221 (blue) marks cell nuclei. WT protein colocalises with the plasma membrane protein IGTA5,  
222 however mutant proteins show predominantly cytosolic localisation.

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224 **Supplementary Figure 11**

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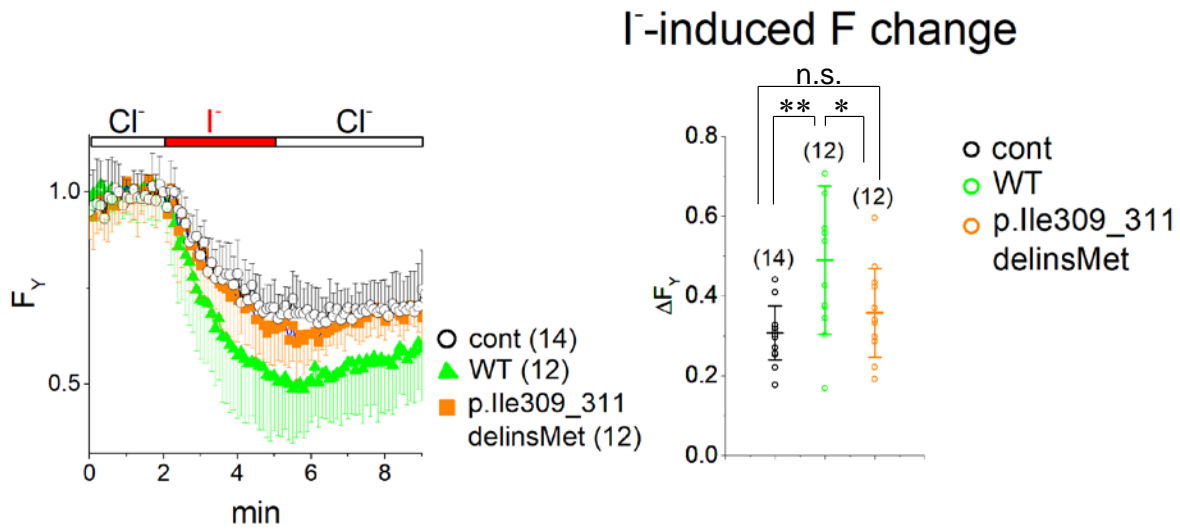
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237 Supplementary Figure 11. (Left panel) The accumulation of  $I^-$ . Shown are time-lapse  
 238 changes in the normalised fluorescence intensity of YFP-HQ/IL ( $F_Y$ ) in cells expressing the  
 239 mutant YFP-HQ/IL alone (open circles) or YFP-HQ/IL with WT (green triangles) or  
 240 p.Ile309\_Glu311delinsMet mutant SLC26A7 (orange squares). Symbols and error bars  
 241 represent mean and S.D., respectively The NaI bath solution was perfused for 3 minutes, as  
 242 indicated by red bars. Numbers of cells are indicated in parentheses.(Right panel) Summary  
 243 of the  $F_Y$  change. Circles represent changes in  $F_Y$  in each cell at 3 minutes after the NaI  
 244 perfusion. Bars and error bars represent mean and S.D., respectively. Numbers of cells are  
 245 indicated in parentheses. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; n.s., not significant (WT against control:  
 246  $P = 0.0020$ , Tukey's test; WT against p.II309\_Glu311delinsMet mutant:  $P = 0.0359$ , Tukey's  
 247 test)

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249 **Supplementary Figure 12**

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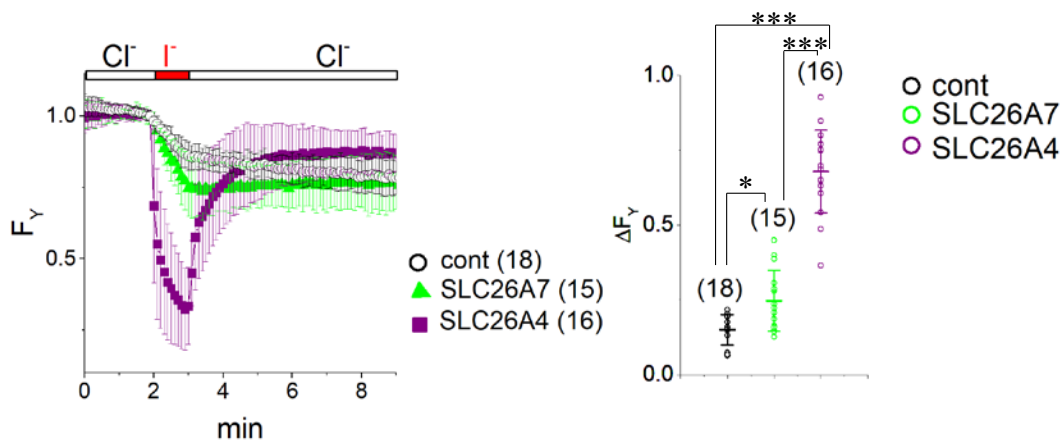
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**I<sup>-</sup>-induced F change**



262 Supplementary Figure 12. (Left panel) The accumulation of I<sup>-</sup> is compared in cells  
263 expressing the mutant YFP-HQ/IL alone or YFP-HQ/IL with SLC26A7 WT (green  
264 triangles) or SLC26A4 WT (violet squares). Symbols and error bars represent mean and  
265 S.D., respectively. The NaI bath solution was perfused for 1 minute, as indicated by red bars.  
266 Numbers of cells are indicated in parentheses. (Right panel) Summary of the F<sub>Y</sub> change.  
267 Circles represent changes in F<sub>Y</sub> in each cell at 1 minute after the NaI perfusion. Bars and  
268 error bars represent mean and S.D., respectively. Numbers of cells are indicated in  
269 parentheses. \*, p < 0.05; \*\*\*, p < 0.001 (Tukey's test)

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271 **Supplementary Table 1**

272 SLC26A7 expression in thyroid tissues. Abbreviations: +, positive; -, negative.

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<b>SLC26A7 immunohistochemistry</b>			<b>SLC26A7 RT-PCR</b>	
<b>Tissue</b>	<b>Cytosol</b>	<b>Apical side</b>	<b>Tissue</b>	<b>SLC26A7 RT-PCR</b>
Thyroid-1	+	+	Thyroid-4	+
Thyroid-2	+	+	Thyroid-5	+
Thyroid-3	+	-	Thyroid-7	+
Thyroid-4	+	-	Thyroid-10	+
Thyroid-5	+	+	Kidney-1	+
Thyroid-6	+	+	Kidney-2	+
Thyroid-7	+	+	Kidney-3	-
Thyroid-8	+	-	Kidney-4	-
Thyroid-9	+	-	Kidney-5	+
Thyroid-10	+	+	Kidney-6	+
			Kidney-7	+

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276 **Supplementary Table 2**

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Cell line	Total iodine in cells (µg/L)
COS-7-SLC26A7 dox(-)	<25
COS-7-SLC26A7 dox(+)	<25
COS-7-SLC26A7 dox(-) SLC5A5 transfected	520
COS-7-SLC26A7 dox(+) SLC5A5 transfected	425
Nthy-SLC26A7 dox(-)	<25
Nthy-SLC26A7 dox(+)	<25
Nthy-SLC26A7 dox(-) SLC5A5 transfected	178
Nthy-SLC26A7 dox(+) SLC5A5 transfected	120

dox, doxycycline

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