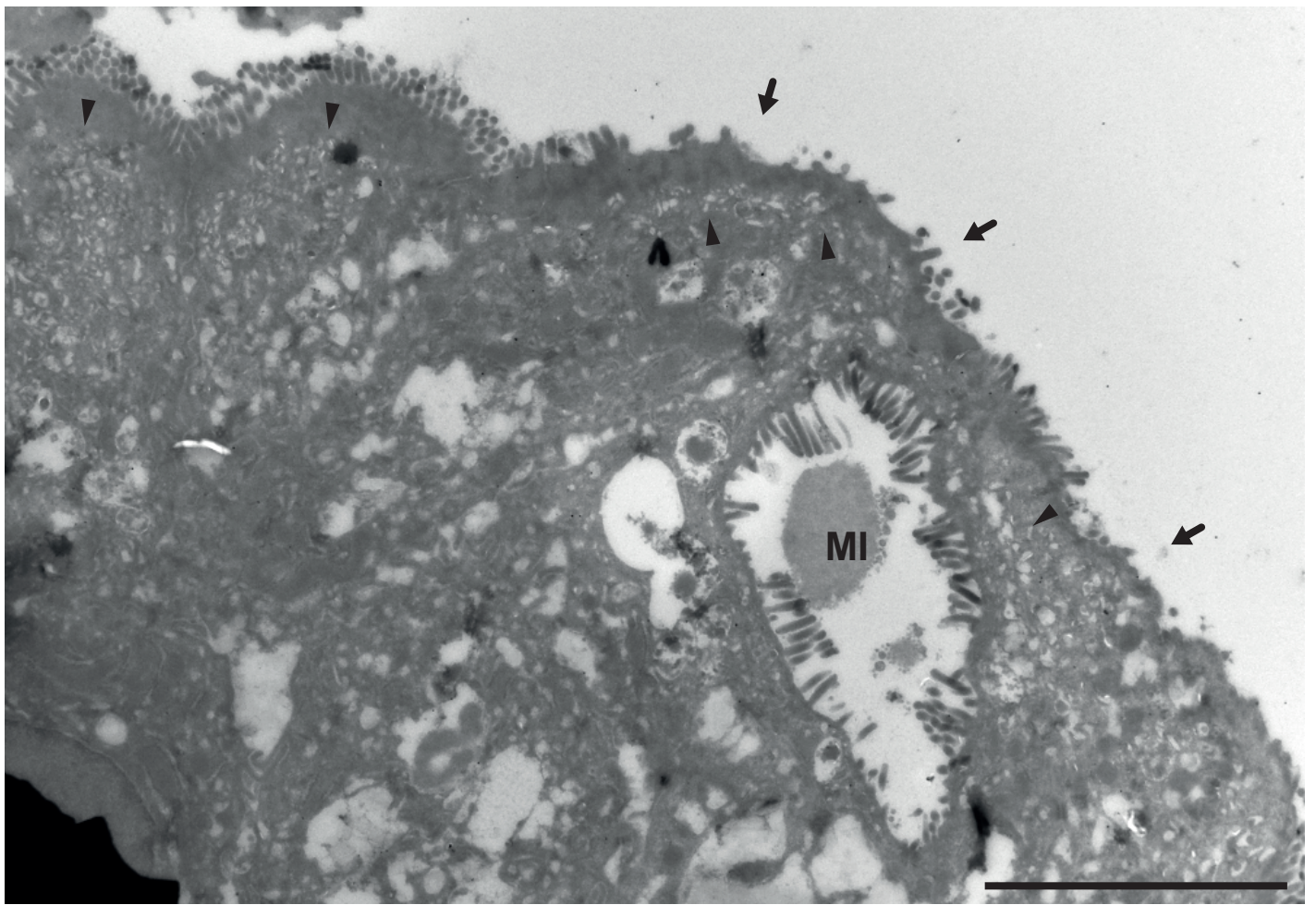
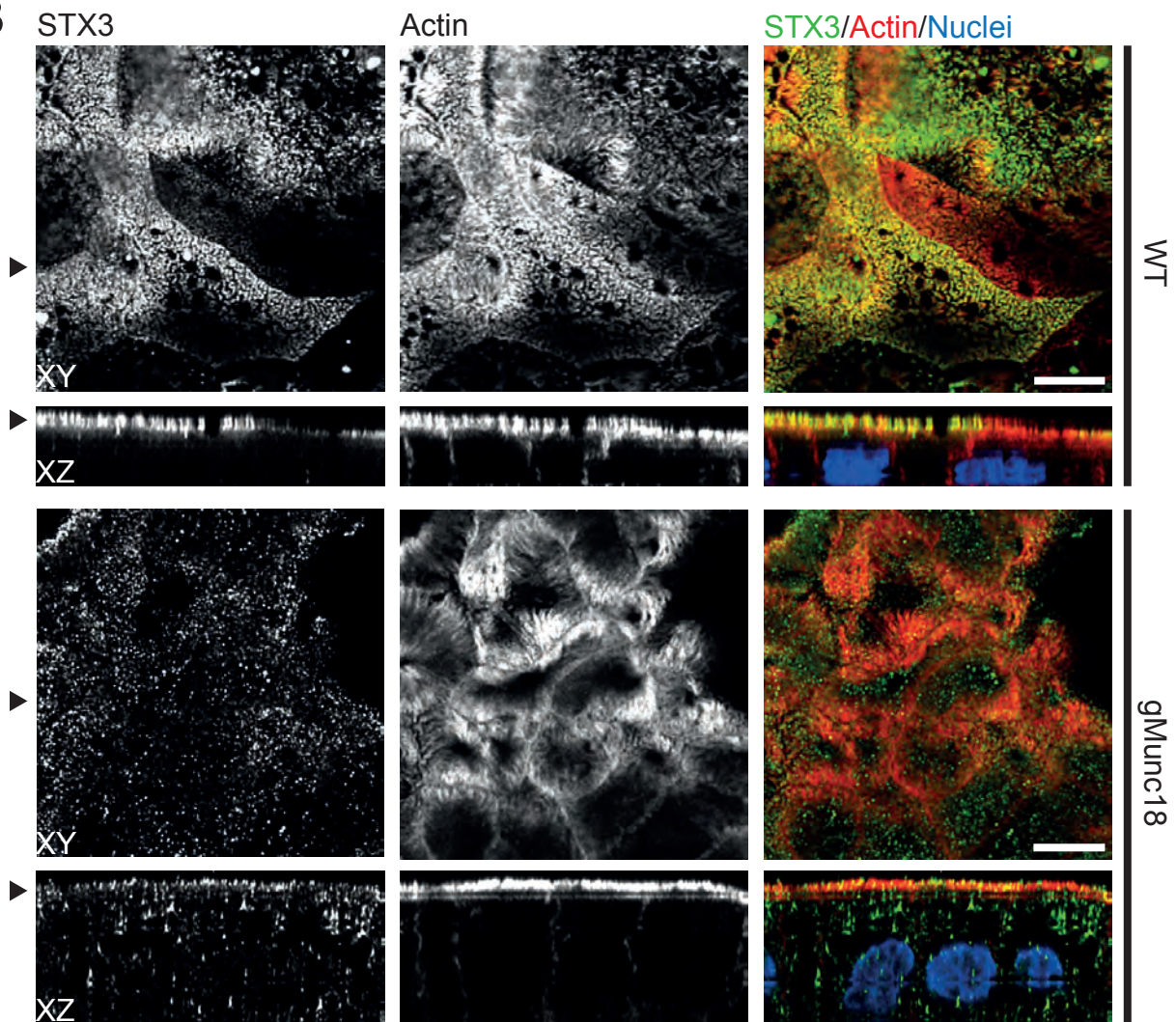


# Supplemental Figure 1

## A



## B

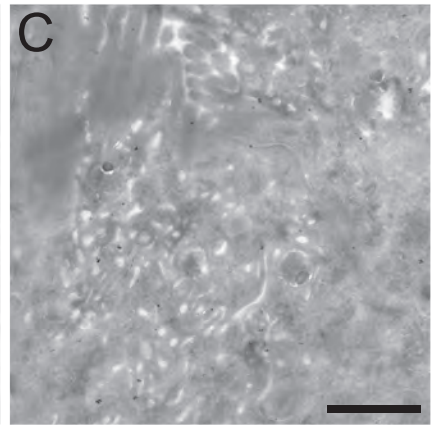
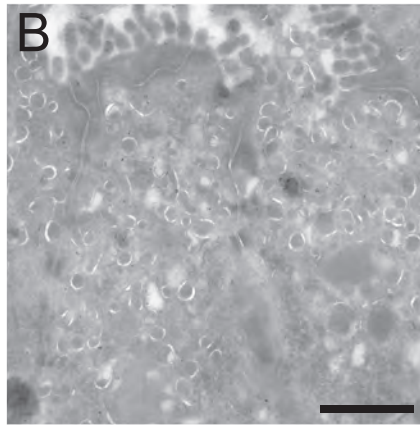
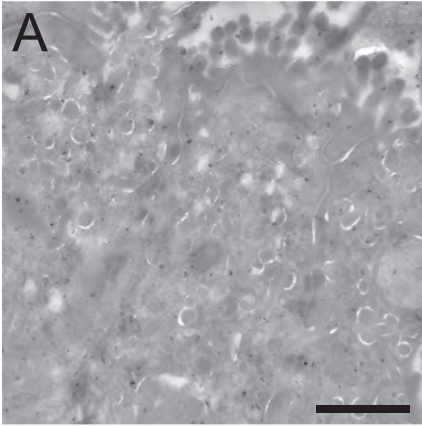


# Supplemental Figure 2

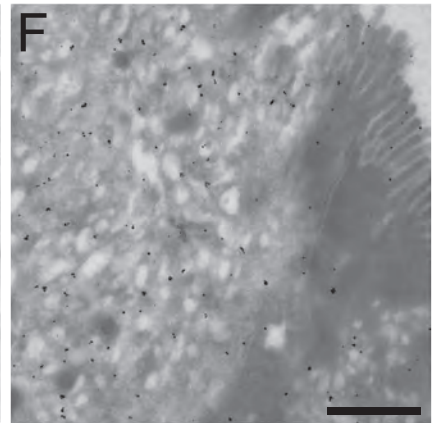
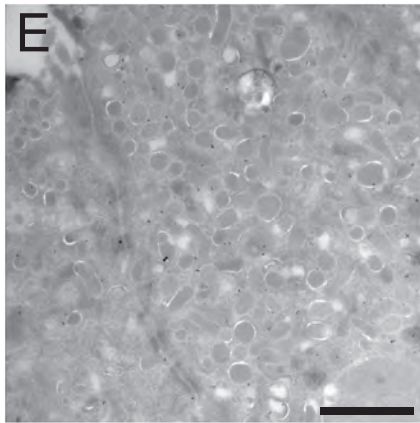
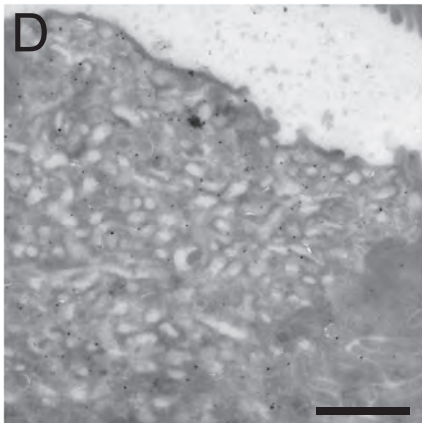
NHE3

Stx3

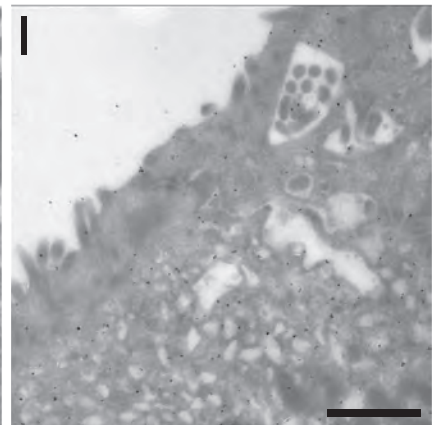
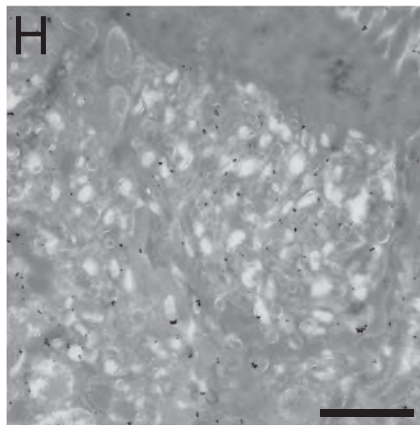
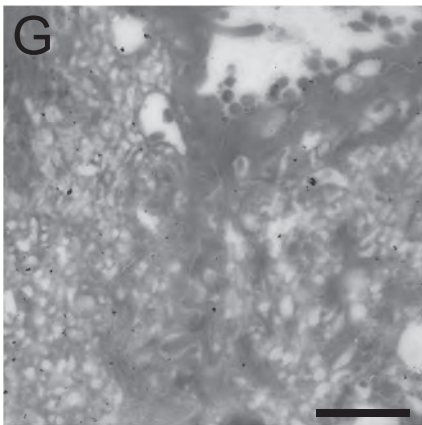
Rab11



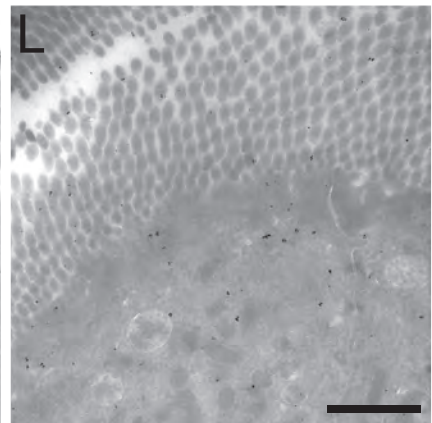
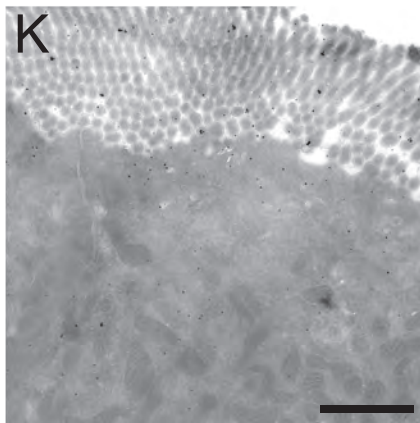
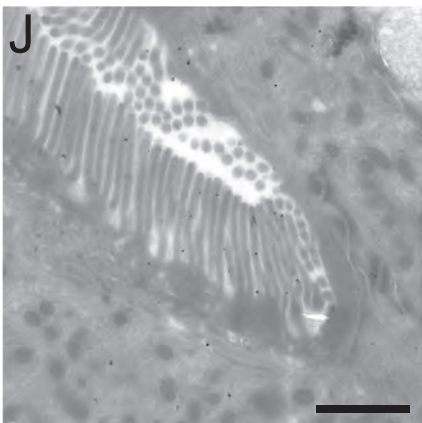
#1



#2



#4



ctrl.

Supplemental Table 1

Patient/sample	#1: FHL 5 biopsy	#2: FHL 5 biopsy see (12)	#3: FHL 5 cell monolayer from biopsy-derived organoid	#4: FHL 5 biopsy	CaCo2 cell model STXBP2 KO	MYO5B-MVID biopsy compiled from (17,31)	STX3-MVID biopsy see (17,18)
<b>Gene</b>	<i>STXBP2</i>	<i>STBP2</i>	<i>STXBP2</i>	<i>STBP2</i>	<i>STXBP2</i>	<i>MYO5B</i>	<i>STX3</i>
<b>Mutation</b>	c.37+1G>A	c.693_695delG AT	c.902+5G>A	c.1146delC	n.a.	c.1323-2A>G	c.372_373dup
<b>Clinics</b>	diarrhea, failure-to-thrive	pancytopenia, fever, diarrhea	pancytopenia, fever, diarrhea	diarrhea, failure-to-thrive	n.a.	diarrhea, failure-to-thrive	diarrhea, failure-to-thrive
<b>Facultative brush-border atrophy</b>	++	+++	-	+++	++	+ to +++	++
<b>Microvillus inclusions</b>	-	+	-	++	n.d.	+ to +++	+
<b>Basolateral microvilli</b>	+	++	+	++	n.d.	- to ++	++
<b>PAS-positive, subapical vesicles/tubules (historically: "secretory granules")</b>	+++	+++	++	+++	++	+++	+++
<b>NHE3, Rab11, STX3 labelling of subapical vesicles/tubules</b>	++	++	n.d.	++	++	++	++

+++ ... abundant

++ ... regular

+ ... sporadic

- ... not observed

n.a ... not applicable

n.d. ... not determined

### **Supplemental Figure 1:**

(A) Electron micrograph of villus enterocytes from patient#4 showing loss or shortening of apical microvilli (arrows), large clusters of subapical vesicles and tubules (arrowheads) and intracellular microvillus inclusions (MI); scale=5 $\mu$ m.

(B) The apical t-SNARE Stx3 mislocalises upon the deletion of Munc18 in CaCo2 cells. Brush border microvilli are indicated by actin staining; scale=10 $\mu$ m.

### **Supplemental Figure 2:**

Immunogold electron microscopy of thawed cryo-sections from duodenum biopsies of FHL 5 patients #1 (A-C), #2 (D-F), #4 (G-I) and a healthy control (J-K). NHE3, Stx3 and Rab11 mislocalize in FHL 5 patients predominantly to abnormal clusters of vesicles and tubules in the subapical cytoplasm of enterocytes, in contrast to their canonical distribution in controls. scale = 1 $\mu$ m.

### **Supplemental Table 1:**

Synoptic view on the here presented FHL5 patients and cell samples/models in comparison to published MVID-cases with mutated *MYO5B* or *STX3* summarizing clinical, electron microscopic and (immuno)cytochemical characteristics.