RAS signaling pathway is essential in regulating PIEZO1-mediated hepatic iron overload in dehydrated hereditary stomatocytosis.

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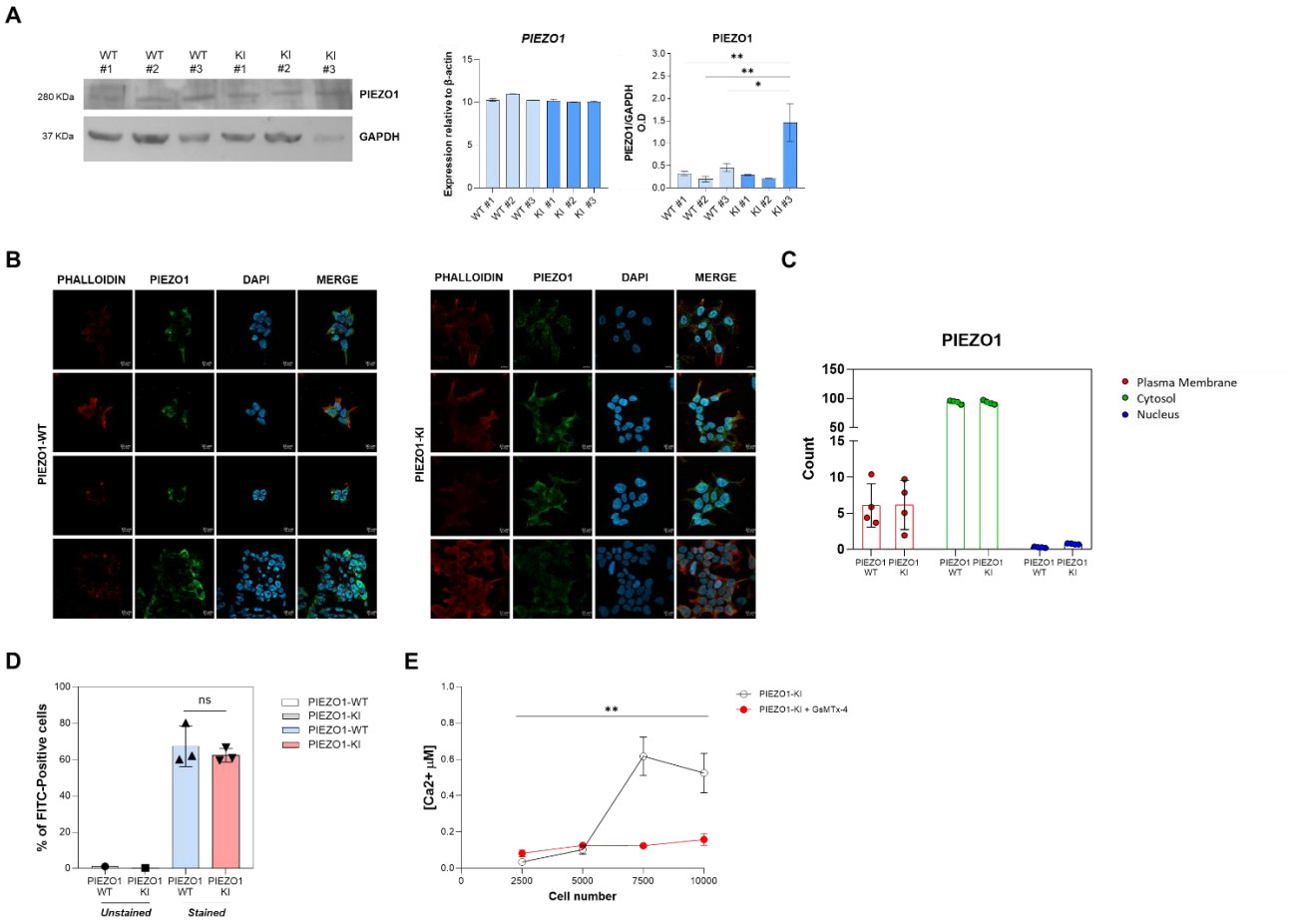
Supplementary Material contains:

Gene ID	**Forward Primer**	**Reverse Primer**
PIEZO1	TCCTCTGTCTGCCTCGGC	GGCATCCACATCCCTCTCAT
HAMP	ACAACAGACGGGACAACTTG	CAGCACATCCCACACTTTGA
SMAD6	AACTCCCTCATCACTGCTCC	GTGCTCCCAGTACGCCAC
R-RAS	CCATCCAGTTCATCCAGTCCT	TGCAGATCTTCGTGTAGGAGT
F2	GGATCCGCATCACTGACAAC	CCCCACTGTCACCTTCACA
ITGB4	AAGAAGGCCCCAGTGAAGAG	GGTCCCTGAACATCTCGTCT
RAC2	CTACACCACCAACGCCTTTC	GTTCACTGGCTTGCTGTCC
PDGFRB	TCAATGTCCCTGTCCGAGTG	ACTGTCTGTTCCCCACTGTC
r-ras	GAGTTTCAATGAGGTGGGCA	CTGCCTTGTTCCCAACCAAC
hamp	TTGCGATACCAATGCAGAAG	GGATGTGGCTCTAGGCTATGTT
	TaqMan Probe (Thermo Fisher Scientific)	
ID1	Hs03676575_s1	
ID3	Hs00171409_m1	

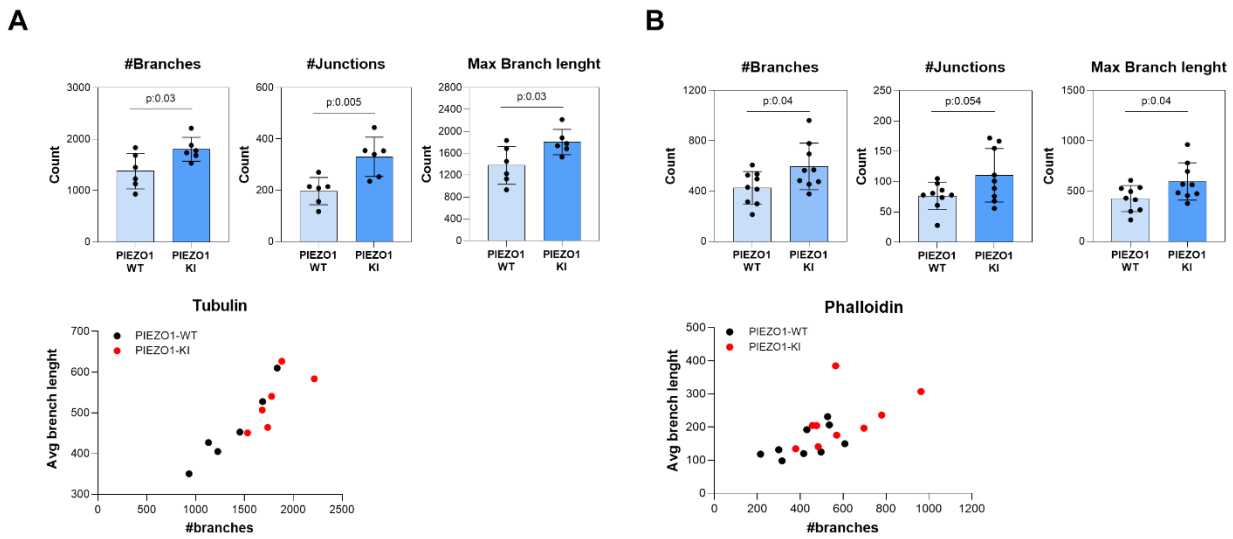
Supplementary table 1 *Oligonucleotides sequences used for qRT-PCR and TaqMan assays*

Reagent	**Volume**
Loading Buffer	
PowerLoad™ concentrate, 100X	100 µL
FluxOR™ reagent, reconstituted in DMSO	10 µL
Deionized water	8.8 mL
FluxOR™ assay buffer, 10X	1 mL
Probenecid, reconstituted in deionized water	100 µL
Assay Buffer (adjusted pH 7.4, with NaOH)	
Deionized water	8.7 mL
FluxOR™ assay buffer, 10X (Component B)	1 mL
1 M HEPES	200 µL
Probenecid, reconstituted in deionized water	100 µL
Stimulus Buffer	
Deionized water	2.5 mL
FluxOR™ chloride-free buffer, 5X	1 mL
K_2SO_4 concentrate	1 mL
Tl_2SO_4 concentrate	0.5 mL

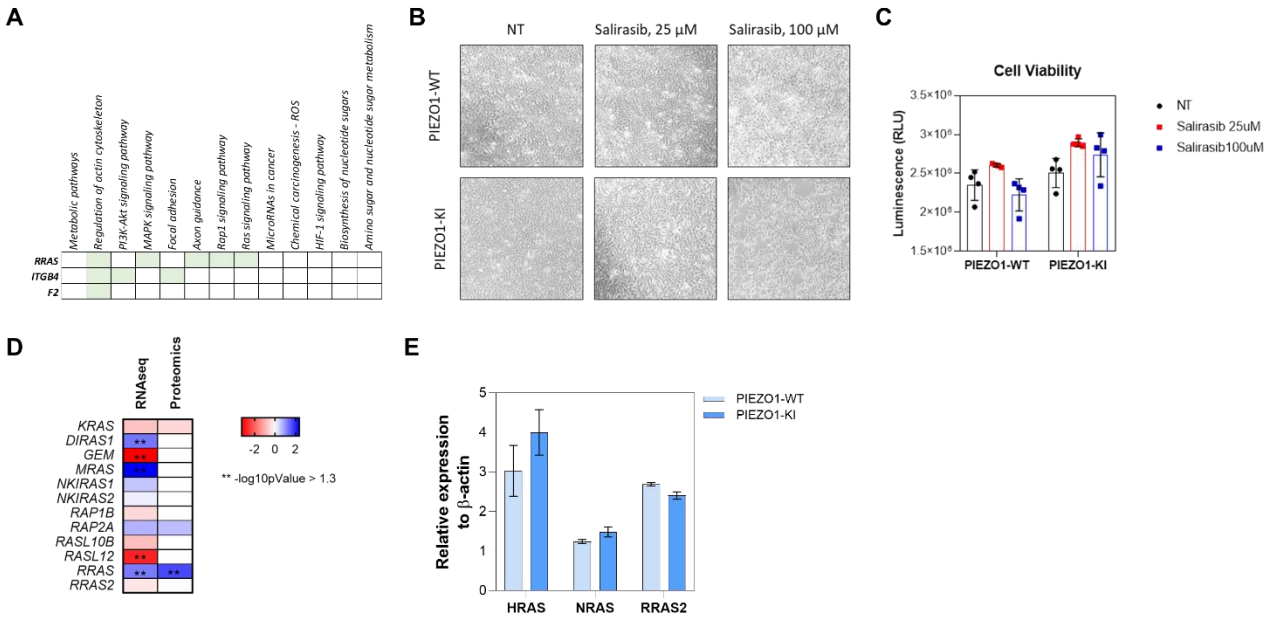
Supplementary table 2. Manufacturer's recipe of *FluxOR™ Potassium Ion Channel Assay* b*uffers.*



Supplementary figure 1. A) Left panel. Representative immunoblotting of PIEZO1 in three different single clones from PIEZO1-WT and PIEZO1-KI cells, respectively. GAPDH was used as the loading control. Left panel. PIEZO1 mRNA expression relative to β-actin and densitometric analysis normalized to GAPDH in three different single clones from PIEZO1-WT (light blue) and PIEZO1-KI cells (blue). *p < 0.05, **p < 0.01 by ANOVA test and post-hoc correction by Sidak's multiple comparison tests. **B)** Representative confocal imaging by ZEISS LSM 980 Airyscan 2 of PIEZO1-WT and PIEZO1-KI cells are shown. Rabbit anti-PIEZO1 antibody was used to stain the PIEZO1 protein (green). Phalloidin was used as a cytoskeleton marker (red), and DAPI was used as a nuclear marker (blue). Overlapping of both signals (MERGE) is shown on the right (yellow). Scale bar 10µm. Four representative visual fields are shown. **C)** Histograms showing quantification of PIEZO1 molecule stained in Plasma Membrane (red histograms), Cytoplasm (green histograms), and nucleus (blue histograms). **D)** Bar graph showing the parentage of Alexa488-positive cells in PIEZO1-WT and PIEZO1-KI cells stained or not (unstained) with PIEZO1-Alexa-Fluor 488. **p<0.01 by ANOVA test and post-hoc correction by Sidak's multiple comparison tests. PIEZO1-WT or PIEZO1-KI stained with PIEZO1-Alexa-Fluor 488 *vs* unstained cells. **E)** Quantification of total intracellular Ca2+ concentrations in PIEZO1-KI at increased cell confluence treated or not with PIEZO1 GsMTx-4 (red line). Data are means ± SD of three experiments and are normalized on protein concentrations (**p < 0.01, PIEZO1-KI 10000 cells vs 2500; 10000 cells vs 5000cells, by ANOVA test and post-hoc correction by Sidak's multiple comparison tests).



Supplementary figure 2. Histograms showing different parameters obtained from ImageJ analysis of stress fibers of the cytoskeleton in PIEZO1-WT (light blue) and PIEZO1-KI (dark blue) cells stained with Tubulin **(A)** and Phalloidin **(B)**. Data are means ± standard deviation of six different acquisitions. The number of Branches, Junctions, and Maximum branch length are expressed as Count. The #end-point and average branch length are related to the respective #branches. pValue calculated for each parameter by Unpaired t-test



Supplementary figure 3. A) Schematic representation of the presence of *RRAS*, *ITGB4*, and *F2* genes along deregulated pathways. **B)** Representative images of PIEZO1-WT and PIEZO1-KI cells treated or not with Salirasib (25 and 100 µm). **C)** Histogram showing cell viability of PIEZO1-WT and PIEZO1-KI cells treated or not with Salirasib (25µm, red columns and 100 µm, blue columns). Data are means ± SD of four independent measurements. **E)** Expression heatmap for upregulated (blue) and downregulated (red) genes and proteins belonging to RAS signaling super-pathway from PathCards. Data are presented as a mean of three replicates. **-log10(pValue) ≥ 1.3. **F)** Histograms showing mRNA levels of *HRAS*, *NRAS*, and *RRAS2* in PIEZO1-WT (light blue) and PIEZO1-KI (blue) cells. Data are presented as mean ± SD of three independent experiments.