

Supplemental Data

Figure S1

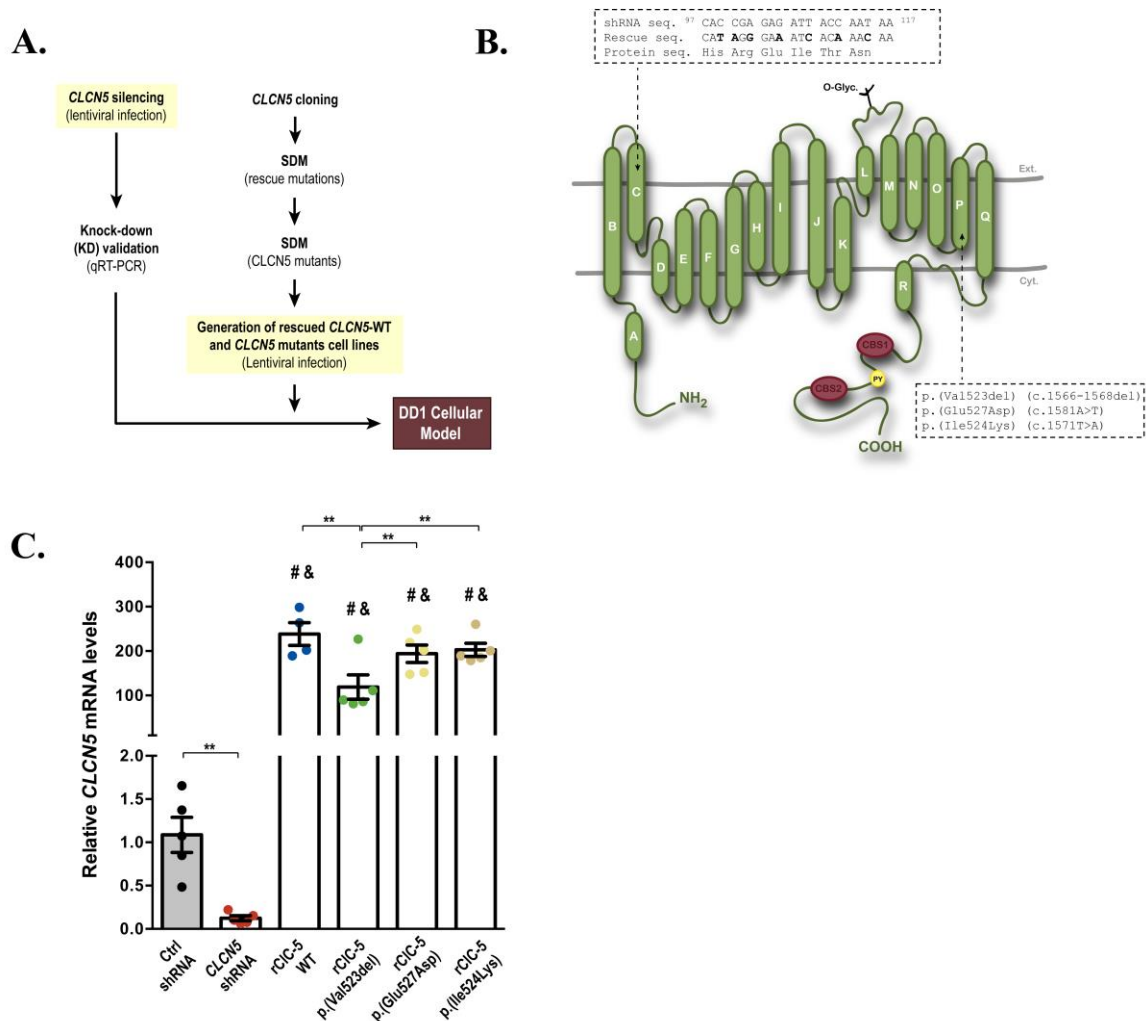
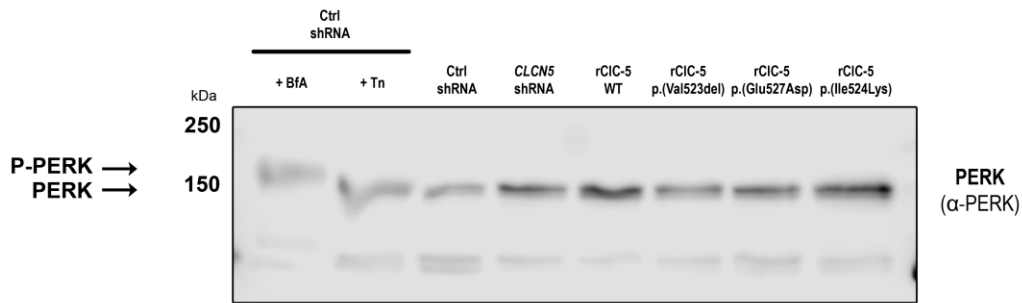


Figure S1. Generation of Dent disease 1 cell models. To explore the molecular mechanisms underlying PTCs dysfunction in DD1, we have generated stable RPTEC/TERT1 cell lines silenced for *CLCN5* gene or carrying the pathogenic *CIC-5* mutations p.(Val523del), p.(Glu527Asp) or p.(Ile524Lys). (A) *CLCN5* was initially silenced in RPTEC/TERT1 cells using lentiviral shRNA vectors, and cells carrying *CLCN5* silencing were selected with the antibiotic puromycin. To re-introduce wild-type (WT) or mutant *CIC-5*, we introduced silent mutations in the shRNA target sequence to prevent RISC-mediated degradation. Subsequently, we mutated *CIC-5* residues Val523, Glu527 and Ile524 and we transduced the previously *CIC-5* silenced cells. Cells carrying both *CLCN5* shRNA and re-introduced *CIC-5* forms were isolated using dual antibiotic selection (puromycin and

hygromycin). (B) Scheme depicting CIC-5 the canonical 746-amino acid CIC-5 protein with its 18 membrane spanning α -helices, and the localization of shRNA target sequences and mutations p.(Val523del), p.(Glu527Asp) and p.(Ile524Lys) within the helix P of CIC-5. (C) mRNA levels of total (endogenous and exogenous) *CLCN5* from control cells (Ctrl shRNA), *CLCN5*-silenced cells (*CLCN5* shRNA), and *CLCN5*-silenced cells carrying WT (rCIC-5 WT) and mutant (rCIC-5 p.(Val523del), rCIC-5 p.(Glu527Asp) and rCIC-5 p.(Ile524Lys)) CIC-5 were measured by RT-qPCR using a commercial TaqMan probe, as indicated in Methods. Statistical significance was determined using an unpaired t test (two-tailed) for Ctrl shRNA vs *CLCN5* shRNA comparison, and one-way ANOVA followed by Tukey's multiple comparisons test for the rest of comparisons. All values were normalized to Ctrl shRNA levels. ** $p < 0.01$; # $p < 0.01$ compared to Ctrl shRNA; & $p < 0.01$ compared to *CLCN5* shRNA.

Figure S2

A.



B.

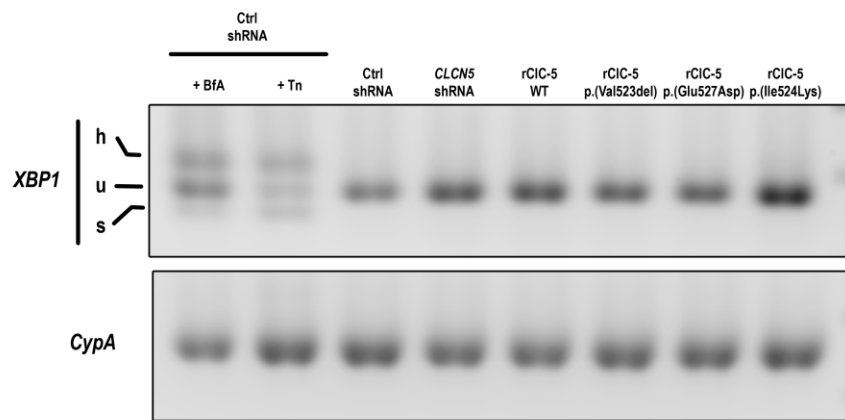


Figure S2. Expression of mutant CIC-5 proteins does not induce ER stress in RPTEC/TERT1 cells. To investigate whether the expression of CIC-5 mutants could be inducing the Unfolded Protein Response (UPR) and ER stress as a result of their accumulation in the ER, we checked the activation status of markers of the main branches of ER stress, i.e, PERK and IRE1 α pathways. As positive controls, cells were treated with the well-established ER stress inducers brefeldin-A (BfA) or Tunicamycin (Tn). (A) Western blots showing that only BfA, but not Tn, *CLCN5* silencing or expression of CIC-5 mutants induced a shift in the molecular weight of PERK, which has been associated with increased phosphorylation and activation of this protein kinase. (B) The activation of the IRE1 α pathway was assessed by monitoring the splicing of XBP-1 mRNA in cells expressing WT or mutant CIC-5 (h-hybrid, u-unspliced and s-spliced refer to the forms of XBP-1 mRNA identified in agarose gels). In this case, both BfA and Tn induced XBP-1 cleavage. On the other hand, neither *CLCN5* silencing nor any of the CIC-5 mutants induced detectable cleavage of XBP-1.

Figure S3

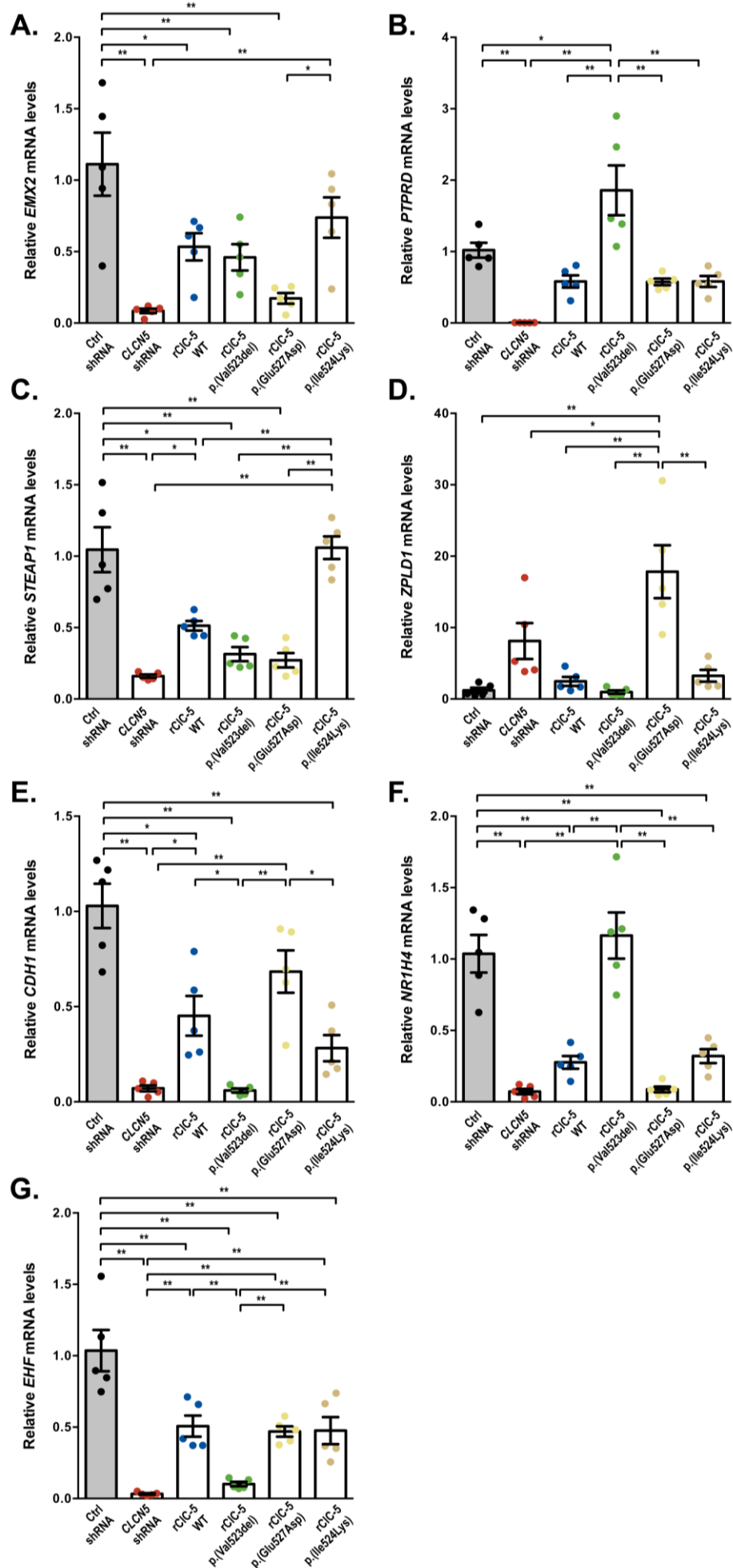


Figure S3. mRNA expression levels of DNA microarray validation genes. To validate the reliability of the results obtained from the DNA microarray, the expression levels of *EMX2*, *PTPRD*, *STEAP1*, *ZPLD1*, *CDH1*, *NR1H4* and *EHF* genes were analyzed by qRT-PCR. Validation genes were selected among those that meet the following requirements: i) their expression was altered by some of the mutations compared to the WT condition and, ii) its expression was also modified by the silencing of *CLCN5* and totally or partially restored by *CLC-5* WT re-introduction. All genes showed an expression pattern that correlated with that observed in the DNA microarray. Dots represent individual values and columns indicate the mean \pm SEM, n = 5. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparisons post hoc test. *, p < 0.05; **, p < 0.01.

Supplementary Tables

Table S1. Top 20 down-regulated genes by *CLCN5* silencing

Gene Symbol	<i>CLCN5</i> shRNA vs Ctrl shRNA		rCIC-5 WT vs <i>CLCN5</i> shRNA	
	logFC	adj.p.val.	logFC	adj.p.val.
SLPI	-5,012258677	7,25872E-18	2,490688757	1,48614E-10
PTPRD	-4,774660354	5,73261E-18	4,076780087	4,04796E-16
MUC1	-4,725636323	1,63254E-20	2,641888478	1,76224E-14
SOSTDC1	-4,167052862	4,70927E-14	1,301344707	0,000249537
EHF	-4,10574689	4,93531E-19	3,279045767	2,02258E-16
SLC34A2	-4,012080704	3,34897E-16	3,063994515	2,63198E-13
ARHGEF38	-3,797136664	7,14438E-15	0,983246057	0,000762179
LIX1	-3,7193965	9,11766E-14	0,883886992	0,005773676
CDH1	-3,697591042	7,14438E-15	2,466205116	1,48614E-10
CXCL6	-3,547100112	5,63081E-12	2,069829383	7,08157E-07
ANPEP	-3,497712417	2,57939E-14	3,384013567	1,46952E-13
C3	-3,431960618	7,14438E-15	1,977200085	3,2444E-09
PTGFR	-3,339062198	4,36867E-13	0,641977911	0,040806607
CDH3	-3,31371677	5,73261E-18	0,515618763	0,004751952
LOC101927630	-3,312773819	3,67966E-14	1,846828299	2,36354E-08
DPP4	-3,30097477	1,0374E-17	2,391987241	4,87344E-14
MAOA	-3,287033568	1,28465E-14	1,55734935	1,80241E-07
CSGALNACT1	-3,242946306	1,80999E-13	0,723010169	0,012523965
KRT7	-3,191449694	1,43739E-14	1,843985356	5,77037E-09
EMP1	-3,03942192	4,08616E-14	1,579039068	9,30668E-08

Table S2. Top 20 up-regulated genes by *CLCN5* silencing

Gene Symbol	<i>CLCN5</i> shRNA vs Ctrl shRNA		rClC-5 WT vs <i>CLCN5</i> shRNA	
	logFC	adj.p.val.	logFC	adj.p.val.
SLC14A1	5,08104134	4,79114E-14	-3,977038387	4,18868E-11
TTC29	3,679206623	1,14704E-12	-1,067780535	0,002680413
TMEM178A	3,350740312	3,79059E-16	-2,614100467	2,12546E-13
ACSM3	3,04935039	3,89094E-14	-3,482219987	7,56065E-15
DIRAS2	3,014635521	3,92929E-15	-1,807712049	6,97955E-10
RAB3B	2,969127618	7,25872E-18	-1,363054288	6,97955E-10
LINC01508	2,871168794	5,11238E-11	-1,560351427	1,15104E-05
ADAMTS16	2,856340255	2,26768E-10	-2,292388697	6,32122E-08
ITGA11	2,820938391	1,54507E-14	-2,796221655	5,08676E-14
KIT	2,784145733	5,37046E-11	-1,970813304	1,67632E-07
NTM	2,766697018	4,97022E-13	-1,45799613	5,89012E-07
FAM189A1	2,617138339	2,24867E-15	-1,498680451	1,08268E-09
SLC16A10	2,541950339	3,98234E-11	-1,243742469	4,16723E-05
ST8SIA2	2,44775401	7,32502E-14	-1,116925328	1,39984E-06
PGM5	2,44469443	1,80999E-13	-1,476028667	2,36354E-08
ZPLD1	2,429899703	1,65216E-12	-1,687245584	1,20979E-08
MAP3K7CL	2,357575992	2,55733E-11	-1,568185814	2,48828E-07
SFRP4	2,331536894	7,59206E-14	-1,353862223	2,35953E-08
HMCN1	2,26960979	1,77002E-10	-2,34963613	4,42059E-10
ZNF385D	2,161982015	1,33712E-12	-1,526276707	6,79872E-09

Table S3. Top 20 down-regulated genes by re-introduction of p.(Val523del) CIC-5 mutant

Gene Symbol	rCIC-5 p.(Val523del) vs rCIC-5 WT	
	logFC	adj.p.val.
CDH1	-2,2699238	2,1101E-09
MFAP5	-2,1515998	2,1095E-08
C3	-2,0811644	2,9962E-09
LUM	-1,8057315	5,6068E-07
KRT7	-1,7459962	2,9746E-08
OPRPN	-1,6387219	1,2899E-05
EHF	-1,5793078	3,4844E-09
WNT7A	-1,5521822	2,1101E-09
SLCO2B1	-1,4213937	4,7274E-06
ADGRF1	-1,4152315	1,2046E-09
FABP3	-1,3842962	4,9308E-06
MAP1B	-1,3263065	8,4725E-07
MUC1	-1,3139873	7,0926E-08
ENTPD1	-1,3088421	8,5295E-06
MACC1	-1,296171	7,5965E-09
LCN2	-1,2896225	0,00088447
TAGLN	-1,2727844	1,6752E-05
UCA1	-1,2380877	5,3125E-08
PDE1C	-1,2030441	7,0256E-06
ZDHHC15	-1,1699512	3,5396E-05

Table S4. Top 20 up-regulated genes by re-introduction of p.(Val523del) CIC-5 mutant

Gene Symbol	rCIC-5 p.(Val523del) vs rCIC-5 WT	
	logFC	adj.p.val.
SLC17A1	3,04935497	7,574E-09
SLC17A3	2,81283694	1,4946E-08
NR1H4	2,59213551	7,3806E-11
CLDN2	2,44095847	3,5125E-07
NR1D1	2,35073551	2,1101E-09
HES1	2,31656296	5,3125E-08
CXCL6	2,11838805	6,8979E-07
APCDD1L-AS1	1,9331687	2,7107E-08
RPL22L1	1,88554225	4,4577E-13
SLC27A2	1,81289443	2,919E-07
KCNJ15	1,77286807	0,00010917
EFHB	1,71213441	3,5743E-07
LINC01291	1,70389896	1,4025E-07
TCF4	1,67893034	2,8791E-08
KLF10	1,6788553	1,941E-09
MATN2	1,59817147	1,2046E-09
PTPRD	1,54984402	4,1356E-07
PRKAR2B	1,54571457	5,1072E-07
ANPEP	1,51082471	2,1612E-06
RELN	1,49928918	2,328E-05

Table S5. Top 20 down-regulated genes by re-introduction of p.(Glu527Asp) CIC-5 mutant

Gene Symbol	rCIC-5 p.(Glu527Asp) vs rCIC-5 WT	
	logFC	adj.p.val.
MFAP5	-2,3750789	8,8178E-09
FLG	-2,0583374	5,6017E-09
NETO1	-1,8661723	4,6023E-06
EMX2	-1,5523818	7,3078E-08
CPT1A	-1,5493772	2,704E-08
LCP1	-1,4606835	3,044E-05
THY1	-1,4440417	1,1259E-06
C3	-1,3513745	5,6331E-06
ABCB5	-1,3061963	0,00024186
NR1H4	-1,2872162	1,2428E-05
NPAS2	-1,2807504	1,4478E-09
KHDRBS3	-1,2806112	3,1426E-08
SGIP1	-1,1928716	1,8136E-07
ADAMTS9	-1,1748411	1,4828E-06
CACNA2D3	-1,1701907	1,0215E-07
HS3ST2	-1,1226335	0,00024107
CLDN10	-1,0989468	0,00021468
GREM1	-1,0892441	0,00012516
MLPH	-1,0785273	5,6162E-08
CDH13	-1,0721972	0,00017979

Table S6. Top 20 up-regulated genes by re-introduction of p.(Glu527Asp) CIC-5 mutant

Gene Symbol	rCIC-5 p.(Glu527Asp) vs rCIC-5 WT	
	logFC	adj.p.val.
ZPLD1	2,24788773	5,697E-10
TNNT1	1,90773131	1,1518E-08
HLA-DRB1	1,79629489	8,8178E-09
SELENOP	1,7741324	8,6929E-07
CLDN2	1,69304491	0,00011843
HLA-DMA	1,55848284	7,3078E-08
GPX3	1,52380486	3,3773E-06
MAPRE3	1,51464658	2,8941E-06
NDST3	1,49303626	5,3534E-07
LINC01508	1,45530881	6,5544E-05
HLA-DPA1	1,44850854	1,029E-06
SOSTDC1	1,42354969	0,00014937
CORO2A	1,38018793	5,8778E-06
PAQR5	1,3748296	0,00038225
LUCAT1	1,31447518	3,0936E-06
SLC17A1	1,3092692	0,00193727
SNAI1	1,29662188	0,00063583
COBL	1,29648308	3,9939E-06
EFCAB13	1,2842711	0,00010262
SPNS2	1,25758919	5,6162E-08

Table S7. Top 20 down-regulated genes by re-introduction of p.(Ile524Lys) CIC-5 mutant

Gene Symbol	rCIC-5 p.(Ile524Lys) vs rCIC-5 WT	
	logFC	adj.p.val.
CDH1	-1,10357	0,022233
KRT7	-0,74643	0,044569
CATSPER1	-0,66144	0,022233
CLYBL-AS2	-0,60521	0,044569
SLC38A8	-0,58758	0,044569
CNGA2	-0,51673	0,022233

Table S8. Top 20 up-regulated genes by re-introduction of p.(Ile524Lys) CIC-5 mutant

Gene Symbol	rCIC-5 p.(Ile524Lys) vs rCIC-5 WT	
	logFC	adj.p.val.
PREX1	1,05644	0,022233
CP	1,031158	0,022233
TMEM71	0,902159	0,022233
STEAP1	0,752778	0,022233
EXOSC8	0,70184	0,024853
TCIM	0,662846	0,035405
IQCD	0,660759	0,024853
DYNC2H1	0,660272	0,02306
LIN54	0,655919	0,023526
LRTOMT	0,629516	0,022233
ZRANB3	0,628185	0,024853
PLAG1	0,624192	0,022233
TNIK	0,614592	0,023526
TEX2	0,58021	0,024853
MIS12	0,572103	0,022233
STEAP1B	0,567719	0,044569
NUFIP1	0,558783	0,022233
PXYLP1	0,555539	0,044569
CHCHD7	0,554923	0,022233
RAB38	0,55239	0,044569