CLIC5: a novel ETV6 target gene in childhood acute lymphoblastic leukemia

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Supplemental methods

Constructs

pcDNA3.1 ETV6 contains the complete wild-type sequence of ETV6. pcDNA3.1 ETV6ΔETS was generated by creating KpnI restriction sites flanking the ETS domain by site-directed mutagenesis (QuickChange Multi-Site/XL Directed Mutagenesis kit; Stratagene) followed by enzymatic digestion and re-ligation. The SV40 Large T antigen Nuclear Localization Signal (NLS; PKKKRKV) was added to this mutant to assure its nuclear localization. ETV6ΔETS was amplified by PCR using the forward primer 5'-CC*GAATTC*GGCACGAGGAAACTTCTTA -3' and NLS containing (underlined) reverse primer 5'-CC*GAATTC*TCACACCTTCCTCTTCTTCTTCGGTTGTTCATCCAGCTCCTGGGACTC-3'. EcoRI digestion was used to clone the ETV6ΔETS_NLS PCR product into pcDNA3.1 vector. His tag was added in C-terminal to both ETV6 and ETV6ΔETS_NLS by a similar approach using His-tag containing reverse primers (**Table S5**) and EcoRI/NotI or BamHI/NotI, respectively. ETV6-His and ETV6ΔETS_NLS-His constructs were subcloned into Gateway compatible vector pENTR3C and transferred into pLENTI CMV puro DEST (w118-1) lentiviral vector (kindly provided by Dr. Christian Beauséjour) using LR clonase II reactions (Thermo Fisher Scientific). A C-terminal HA-tagged ETV6 was also generated. An oligomer containing 3 tandem HA tag repeats flanked by BSMI and NotI restriction sites (**Table S5**) was synthesized (Invitrogen), annealed and subcloned directly into pcDNA3.1 ETV6 (partial BSMI digestion). Both ETV6 and

ETV6-HA were subcloned into pCCL lentiviral vector (kindly provided by Dr. Christian Beauséjour). Briefly, GFP was excised from pCCL hPGK-GFP with AgeI/SalI digestion. ETV6 and ETV6-HA cDNA were obtained by enzymatic digestion. Extremities were filled-in for a subsequent blunt-end ligation. All ETV6 constructs are shown in **Figure S9**. pDONR221 CLIC5A was purchased from Harvard PlasmID Repository (clone # HsCD00044695) and transferred into pLENTI lentiviral vector as described previously.

Cell culture

Reh (ATCC ® CRL-8286™) and IM9 (ATCC® CCL159™) cell lines were maintained in RPMI 1640 (Wisent) 10% Fetal Bovine Serum (FBS; Wisent) in a 5% CO_2 incubator at 37°C.

Lentiviral production

$15X10^6$ HEK293T cells (kindly provided by Dr. Christian Beauséjour) were seeded into 15cm petri dishes in DMEM (Wisent) 10% FBS. The next day, cells were transfected with 9µg lentiviral expression plasmid (pLENTI or pCCL backbone) together with 6µg pRSV-Rev, 7.8µg pMD2.VSVG and 15µg pMDL third generation encapsidation plasmids (kindly provided by Dr. Christian Beauséjour) in fresh RPMI 1640 10% FBS medium using Polyethylenimine (Polysciences). Media was removed 16h post-transfection and replaced by fresh DMEM 10% FBS. After 30h, viral particles were either retrieved from media by ultracentrifugation (50 000g) and quantified by p24 antigen ELISA (Advanced

Bioscience Laboratories) or media was used directly for transduction (see lentiviral infection section).

Lentiviral infection

$1X10^6$ Reh or IM9 cells were seeded in 1mL of RPMI 1640 10% FBS medium. Either concentrated virus (100-1000ng p24) or 1mL of viral supernatant were added to cells. Polybrene was added to a final concentration of 5-8µg/mL. 24h post-infection, medium was changed with fresh RPMI 1640 10% FBS. The next day (48h post-infection), infected cells were selected with 1µg/mL puromycin (for pLENTI infected cells). These stably infected cells were maintained at least 2 weeks in culture before validating expression by western blotting (see related section) and carrying out further experiments.

Expression profiling by RNA-sequencing

Total RNA from two different Reh clones (generated in methylcellulose media) each stably expressing ETV6-His and ETV6ΔETS_NLS-His (and pLENTI control) was extracted (RNeasy^{TM} mini kit, QIAgen) and quantified by Nanodrop (Thermo Fisher scientific). RNA quality was assessed using the Agilent 2100 Bioanalyzer. Libraries were generated with 1µg total RNA (RIN ≥ 9.9) treated with DNAseI (Turbo DNA-free; Ambion) and processed through the TruSeq Stranded Total RNA protocol with ribosomal ribonucleic acid (rRNA) removal mix (Illumina). Two libraries per high throughput lane of HiSeq 2500 (Illumina) were sequenced for an average coverage of approximately 100 million reads per sample. Reads for each samples were mapped on hg19 reference genome using STAR with default settings and read counts per genes were determined using HTSeq-count. For the differentially expressed genes (DEG) analysis, the R bioconductor package edgeR was used with Benjamini Hochberg p-value adjustment. The two clones were considered as biological replicates.

The patient cohort used for RNA-sequencing was composed of 9 hyperdiploid and 9 t(12;21) patients. Using the mirVana Isolation kit (Ambion) according to the manufacturer's protocol, total RNA was extracted from leukemic bone marrow samples of all patients and from control pre-B cells (CD19+CD10+) isolated from healthy cord blood samples by Fluorescence Activated Cell Sorting (FACS). Following a DNAseI treatment, total or mature RNA samples were quantified by NanoDrop ND1000 (Thermo-Fisher Scientific, Waltham, MA) and RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). rRNA were depleted using the Invitrogen RiboMinus Eukaryote kit (Life Technologies). cDNA libraries were prepared using the SOLiD Total RNA-seq kit based on manufacturer's protocol and sequenced on the Life Technologies SOLiD 4/5500 System (paired-end: 50bp x 35bp). Reads were aligned to the hg19 reference genome and read counts per gene obtained using LifeScope Genomic Analysis Software (Whole Transcriptome Analysis pipeline, default parameters). Remaining ribosomal sequences were filtered out. Based on results obtained from the cell lines, further analysis were limited to a selection of the top 331 candidate genes (p-value < 0.05). DEG analysis was done as described above. Reads Per Kilobase per Million mapped reads (RPKM) were calculated for the identified genes using edgeR and a heatmap depicting the unsupervised clustering of samples based on RPKM values of the overexpressed candidate genes was generated using the heatmap.2 library of the gplots R package.

Quantitative real-time PCR

Total RNA was extracted and quantified as described above. 350ng of total RNA were retro-transcribed with M-MLV reverse transcriptase (Life Technologies). cDNA was then subjected to quantitative real-time PCR using the primer sets listed in **Table S1**. Reactions were performed in duplicate in a total volume of 25μL composed of 5μL cDNA (diluted 1:10), 5μL primers (0.2μM final concentration), 12.5μL SYBR Green PCR Master Mix (1X final concentration, Life technologies) and completed with H_2O. Amplification and detection were carried out on the ABI PRISM 7000 sequence detection system (Life Technologies) on the course of 40 cycles [95°C 15 sec; 60°C 1min]. Relative expression was determined by the $2^{-(\Delta\Delta Ct)}$ comparative method using GAPDH as the reference gene.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) have been performed on $10X10^6$ transduced Reh cells cross-linked directly in cell medium for 10min with 1% methanol-free formaldehyde (Polysciences). Cells were resuspended in TpA buffer (0.25% Triton, 10mM Tris pH 8.0, 10mM EDTA, 0.5mM EGTA, 1X protease inhibitor cocktail [Roche]) and incubated on ice for 5min. Pelleted cells were then resuspended in TpB buffer (200mM NaCl, 10mM Tris pH 8.0, 1mM EDTA, 0.5mM EGTA, 1X protease inhibitor cocktail) and incubated on ice for

30min. Fragilized nuclei were pelleted and resuspended in TpS buffer (0.5% SDS, 0.5% Triton, 10mM Tris pH 8.0, 140mM NaCl, 1mM EDTA, 0.5mM EGTA, 1X protease inhibitor cocktail) for sonication. Chromatin was sheared using a S2 ultrasonicator (Covaris) and cleared by centrifugation. Supernatant (cleared sheared chromatin) was combined with 1.4 volume TpS SDS-free and 2.5 volume of pre-washed anti-HA magnetic beads (Thermo Fisher Scientific) in ChIP dilution buffer (1% Triton, 10mM Tris pH 8.0, 150mM NaCl, 2mM EDTA) followed by a 4h incubation with rotation (4°C). A fraction (1:10) of supernatant was conserved for Inputs. Magnetic beads were washed twice with wash buffer (0.5% NP40, 150mM KCl, 10mM Tris pH 8.0, 1mM EDTA). To elute DNA-protein complexes from beads, we used a competitive approach using a solution of HA peptides (Thermo Fisher Scientific). Beads were eluted twice with HA peptides (2mg/mL in TBS) for 30min at 37°C. Reverse-crosslinking was performed for both eluates and Input DNA in TpE buffer (0.3% SDS, 50mM Tris pH 8.0, 10mM EDTA, 400mM NaCl) at 65°C overnight. 1.3 volume of RNase A mix (154µg/mL glycogen [Roche], 115µg/mL RNase A [Roche] in TE buffer) was added to all samples followed by a 2h incubation at 37°C. SDS and proteinase K (Thermo Fisher Scientific) were added to a final concentration of 0.3% and 8.4µg/mL, respectively, for an additional 2h incubation at 37°C. DNA was finally purified twice by standard phenol/chloroform/isoamyl alcohol (Sigma) extraction using MaXtract High Density gel columns (QIAgen) and ethanol precipitation. DNA pellets were washed with 70% ethanol, dried and resuspended in EB buffer (10mM Tris pH 8.0). This purified ChIP and Input DNA were quantified by qRT-

PCR using primers designed in the first 500bp upstream of genes' transcription start site (listed in **Table S2**).

Protein extraction

Whole cell extracts were prepared in RIPA buffer (50mM Tris pH 8.0, 150mM NaCl, Nonidet P-40 1%, 0.5% NaDOC, 0.1% SDS, 1X protease inhibitor cocktail) and mechanical disruption in 21G gauge needles (Becton Dickinson). For nuclear extracts, cells pellets were resuspended in A Buffer (10mM HEPES pH 7.9, 10mM KCl, 0.1mM EDTA, 0.1 mM EGTA, 1mM DTT, 0.5mM AEBSF, 1X protease inhibitor cocktail) for 15min followed by addition of Nonidet P-40. Nuclei were pelleted, resuspended in C buffer (20mM HEPES pH 7.9, 400mM NaCl, 1mM EDTA, 1mM EGTA, 1mM DTT, 1mM AEBSF, 1X protease inhibitor cocktail) and incubated with rotation to released nuclear proteins. After centrifugation, supernatant was collected. Both total and nuclear extracts were quantified on SpectraMax 190 (Molecular Devices) using the DC protein assay (BioRad) according to the manufacturer protocol.

Western blotting

20µg of nuclear extracts or 100µg of whole cell extracts were diluted in Laemmli buffer (final concentration of 63 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, 0.0025% bromophenol blue) prior to heat denaturation at 95°C for 3min. Samples were loaded and migrated on SDS-denaturating 10% polyacrylamide gels (37.5:1) and transferred at 4°C overnight on polyvinylidene difluoride membranes in transfer buffer (25mM Tris, 192mM glycine, 10%

methanol). Membranes were blocked in Blotto A solution (1X TBS, 5% milk and 0.05% Tween-20) prior to immunodetection. Antibodies (see **Table S6**) were diluted in Blotto A and membranes were blotted for 1h at room temperature. 3 washes of 5min in TBS-0.05% Tween-20 were done before the incubation with HRP-coupled secondary antibodies (see **Table S6**). Membranes were washed 3 times for 10-15min and assayed by enhanced chemiluminescence (ECL) detection with Western Lightning Plus-ECL (PerkinElmer) according to the manufacturer protocol.

Apoptosis assays

Cells were seeded at 2X10^5 cells/mL in RPMI 1640 10% FBS. Apoptosis was induced by treating cells for 20h with either hydrogen peroxide (PRXD; Sigma), camptothecin (CPT; Tocris Bioscience) or doxorubicin (DOXO; Sigma) with a concentration set to achieve approximately 70% cell death. Apoptosis was also modulated by pre-treating cells with different molecules prior to the addition of PRXD. With ferric ammonium citrate (FAC; Sigma), cells were treated for 24h and FAC-containing media was changed with fresh medium before apoptosis induction. For deferoxamine mesylate salt (DFO; Sigma), cells were treated for 2h before adding PRXD. Finally, CLIC-specific channel inhibitor IAA-94 (Sigma) was added 10min before PRXD. Apoptosis was assayed by Alexa Fluor 488-coupled Annexin V and propidium iodide (PI) double staining with Dead cell Apoptosis Kit (Thermo Fisher Scientific) according to manufacturer's protocol. 1X10^4 stained cells were analysed by flow cytometry on a LSRFortessa FACS (Becton Dickinson) using blue laser (488nm) with 505LP and 530/30 filters for

Alexa Fluor 488 detection and with 600LP and 610/20 filters for PI detection. Data were acquired with the FACSDiva software (Becton Dickinson). Total apoptosis includes Annexin V^+/ PI^- (early apoptotic), Annexin V^+/PI^+ (late apoptotic) and Annexin V^-/PI^+ (necrotic) cells.

Immunofluorescence microscopy

Reh cells were seeded at $2X10^6$ cells/mL in a poly-D-Lysine Hydrobromide (Sigma) treated glass 96-wells plate (Whatman) with or without Lysotracker Red DND-99 staining (Thermo Fisher Scientific) at 50nM final concentration. The plate was centrifuged and incubated for 2h at 37°C, 5% CO_2 to let cells attach to the glass surface. Media was removed and cells were fixed for 10min with freshly made 3.7% formaldehyde in phosphate-buffered saline (PBS). Fixing solution was removed and cells were washed with PTX (0.01% Triton in PBS). Blocking was performed for 1h at room temperature with a 5% Normal Goat Serum (NGS; Jackson Immunoresearch Laboratories) solution in PTX. Blocking solution was removed and immunostaining was performed overnight with CLIC5A antibody (ab191102 dil. 1:1000; Abcam) and transferrin receptor antibody (ab84036 dil. 1:200; Abcam) in 5% NGS solution. Cells were washed 4 times with PTX before adding secondary antibodies solution. Goat anti-mouse Alexa Fluor 488 (dil. 1:500; Thermo Fisher Scientific) was used to detect anti-CLIC5A and Goat anti-rabbit Alexa Fluor 546 (dil. 1:500; Thermo Fisher Scientific) was used to detect anti-transferrin receptor. Hoechst 33258 DNA stain (dil. 1:500; Thermo Fisher Scientific) was added to the mix to stain nuclei. After 1h incubation with secondary antibodies, cells were washed 4 times with PTX and mounted in

Fluoromount-G (SouthernBiotech). Cells were imaged using a 1.4NA plan-apochromat 100X oil immersion objective on an inverted microscope (DMI6000 B; Leica) fitted with an Ultraview Vox spinning disc confocal system (Perkin Elmer). Images were acquired using an Orca R2 charge-coupled device camera (Hamamatsu) controlled by Volocity 6 software (PerkinElmer/Improvision).

Statistical tests

Significance of observations were assessed using one or two-tailed Fisher's exact test or Mann-Whitney-U test when appropriate.

Ethics statement

The CHU Sainte-Justine Research Ethics Board approved the protocol. Informed consent was obtained from the parents of the patients to participate in this study and for publication of this report and any accompanying images.

Supplemental figures

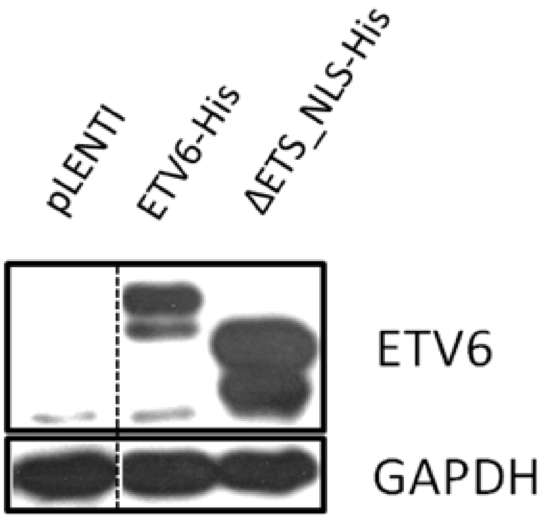

Figure S1: Protein expression levels of ETV6-His and ETV6ΔETS_NLS-His in Reh cells. Western blot of nuclear extracts from Reh cells infected with pLENTI empty vector, ETV6-His or ETV6ΔETS_NLS-His. Anti-ETV6 detects both ETV6 and ETV6ΔETS_NLS forms. GAPDH is used as a loading control. The result is shown for one given Reh clone. *Adjustments of brightness and contrast were applied to the whole image.*

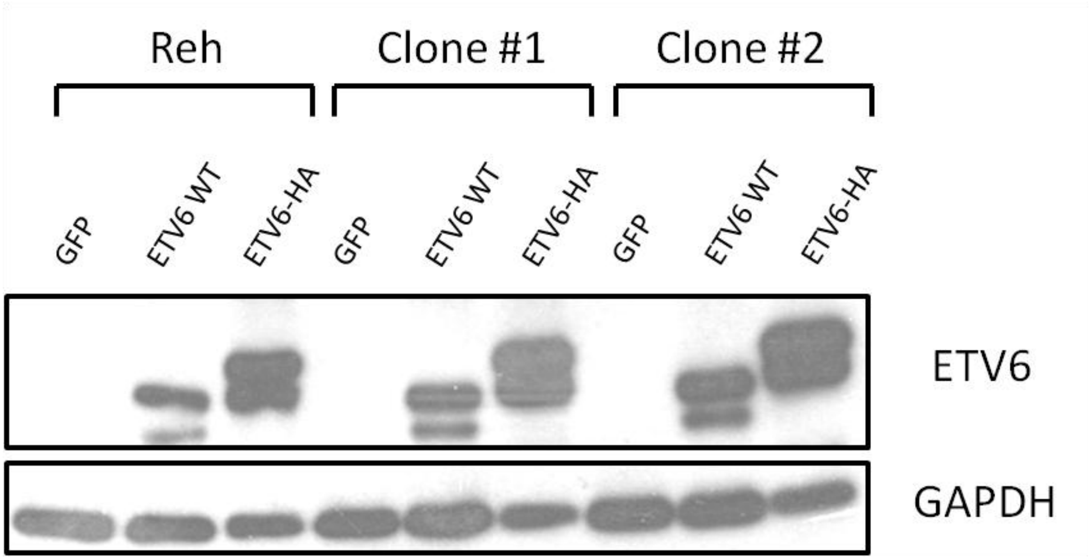

Figure S2: Expression of ETV6 and ETV6-HA proteins in Reh cells. Western blot analysis of nuclear extracts from Reh cells and both Reh clones infected with pCCL GFP control vector, Wild-type ETV6 (ETV6 WT) and HA-tagged ETV6 (ETV6-HA). ETV6 WT and ETV6-HA were detected with Anti-ETV6. GAPDH is used as a loading control. All clones used had similar expression patterns**.** *Adjustments of brightness and contrast were applied to the whole image.*

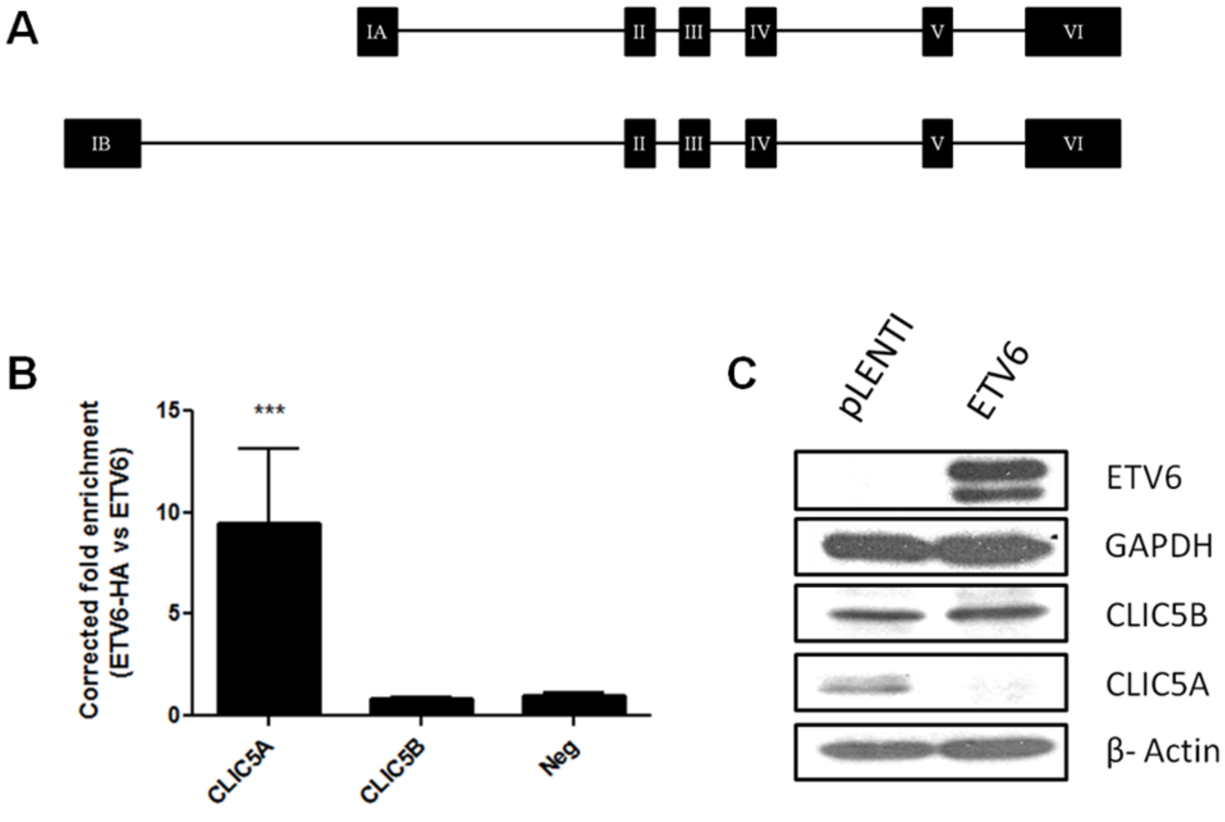

Figure S3: ETV6 specifically binds and represses the CLIC5A isoform. A) Representation of CLIC5A and CLIC5B isoforms at genomic location chr6:45866190-46048085. Protein products encoded by CLIC5A and CLIC5B contain 251(30 kDa) and 410 (55 kDa) amino acids, respectively. The last 234 amino acids encoded by exons 2 to 6 are identical. **B)** Chromatin immunoprecipitation was performed as previously described and enrichment for *CLIC5A* and *CLIC5B* promoters were calculated. *CLIC5A* proximal promoter is specifically enriched. **C)** Nuclear extracts (for ETV6 and GAPDH) or total extracts (CLIC5A/B and β-Actin) from Reh pLENTI control cells and Reh ETV6 cells were analyzed by western blotting. CLIC5A but not CLIC5B protein level is reduced with ETV6 overexpression. *Adjustments of brightness and contrast were applied to the whole image.*

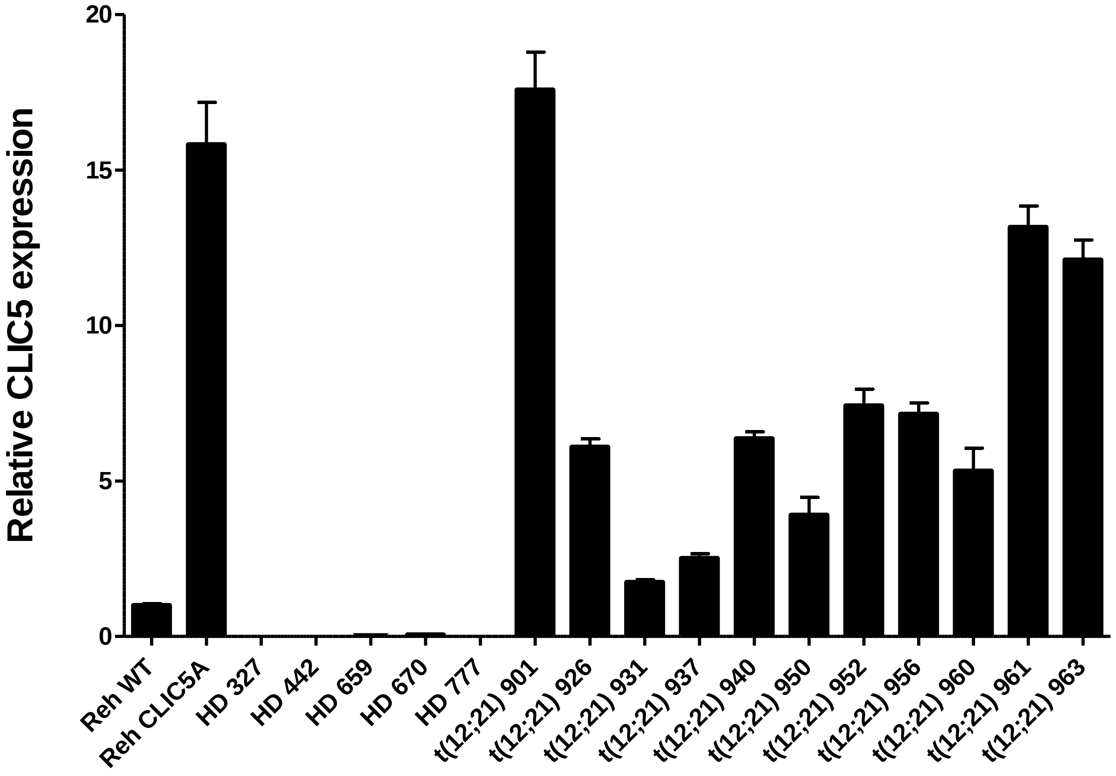

Figure S4: CLIC5 expression level in CLIC5A overexpressing Reh cells and leukemic patients samples. Total RNA was extracted from Reh WT and Reh CLIC5A cell lines and additional ALL patients cells for complementary DNA (cDNA) generation. This cDNA was submitted to qRT-PCR analysis to quantify relative expression of CLIC5. No or very low expression of CLIC5 was detected in hyperdiploid (HD) patients samples whereas a clear re-expression is observed in all t(12;21) samples. Additionally, CLIC5 expression in our Reh CLIC5A cells is very similar to what is seen in these patients. Error bars represent the standard deviation (n=3).

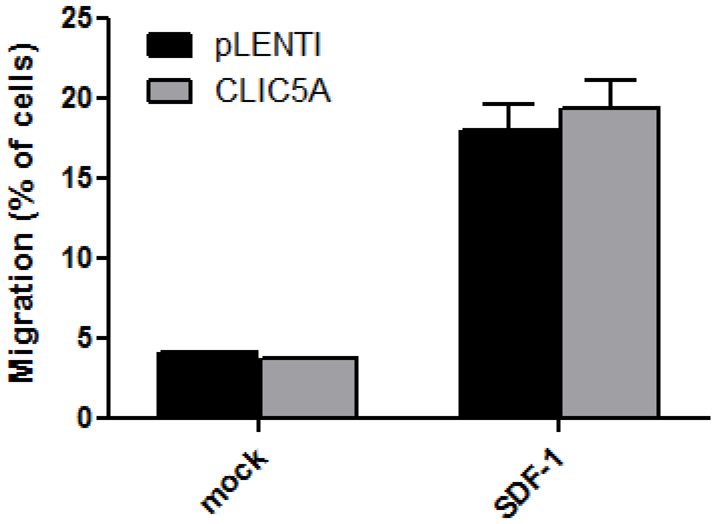

Figure S5: Migration of Reh cells to SDF-1: pLENTI control and CLIC5A overexpressing Reh cells were used in trans-well migration experiments with the SDF-1 chemokine. 200 000 cells were loaded on a 5µm-pore filter plate containing media with or without 10ng/mL SDF-1. Cells that migrated after 90min are present in the bottom chamber and were counted to calculate a migration percentage. SDF-1 induced migration of Reh cells but no difference was observed between CLIC5A overexpressing cells and control. This experiment was performed in duplicate for the SDF-1 condition. Error bars represent the standard deviation.

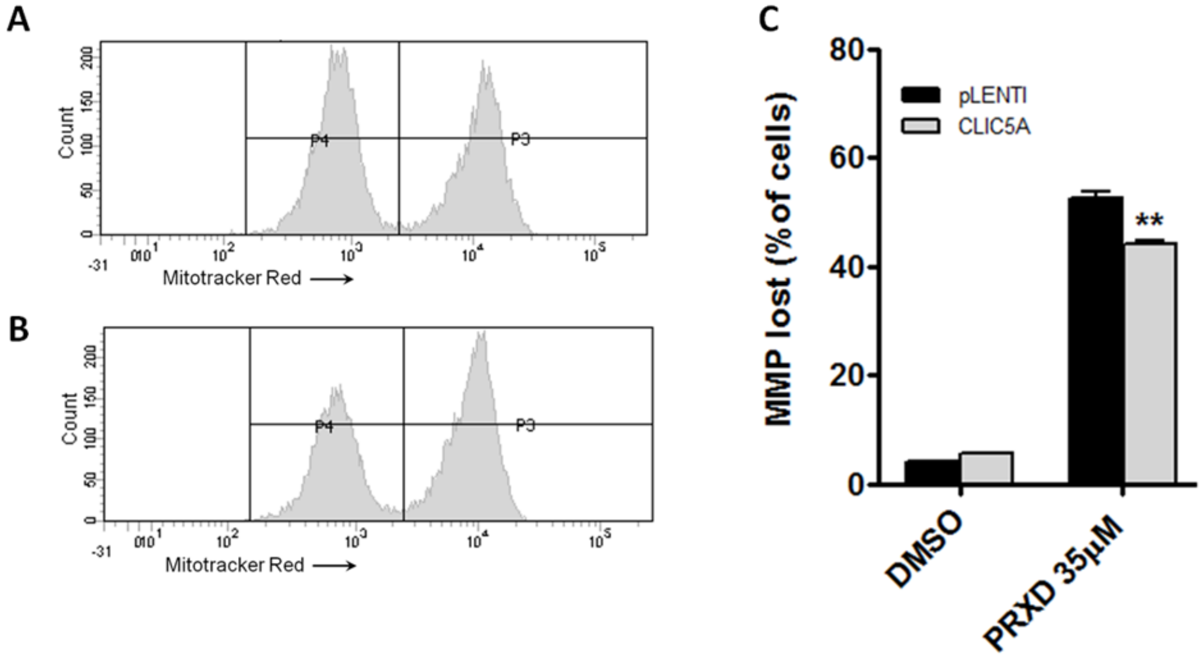

Figure S6: Mitochondrial membrane potential (MMP) in hydrogen peroxide-treated cells. pLENTI control and CLIC5A overexpressing Reh cells were treated for 20h with 35µM hydrogen peroxide (PRXD). Cells were then stained with Mitotracker Red CMXRos and analysed by flow cytometry using the PI detector. Mitotracker Red CMXRos only stains cells with positive MMP and thus is an interesting marker of mitochondria integrity. **A)** pLENTI control cells displayed a higher number of negatively stained cells **B)** compared to CLIC5A overexpressing cells, indicating that more mitochondria remain intact to a peroxide challenge with CLIC5A overexpression. **C)** This experiment was performed in triplicate. Error bars represent the standard deviation. Statistical significance is calculated by two-tailed Mann-Whitney-U test.

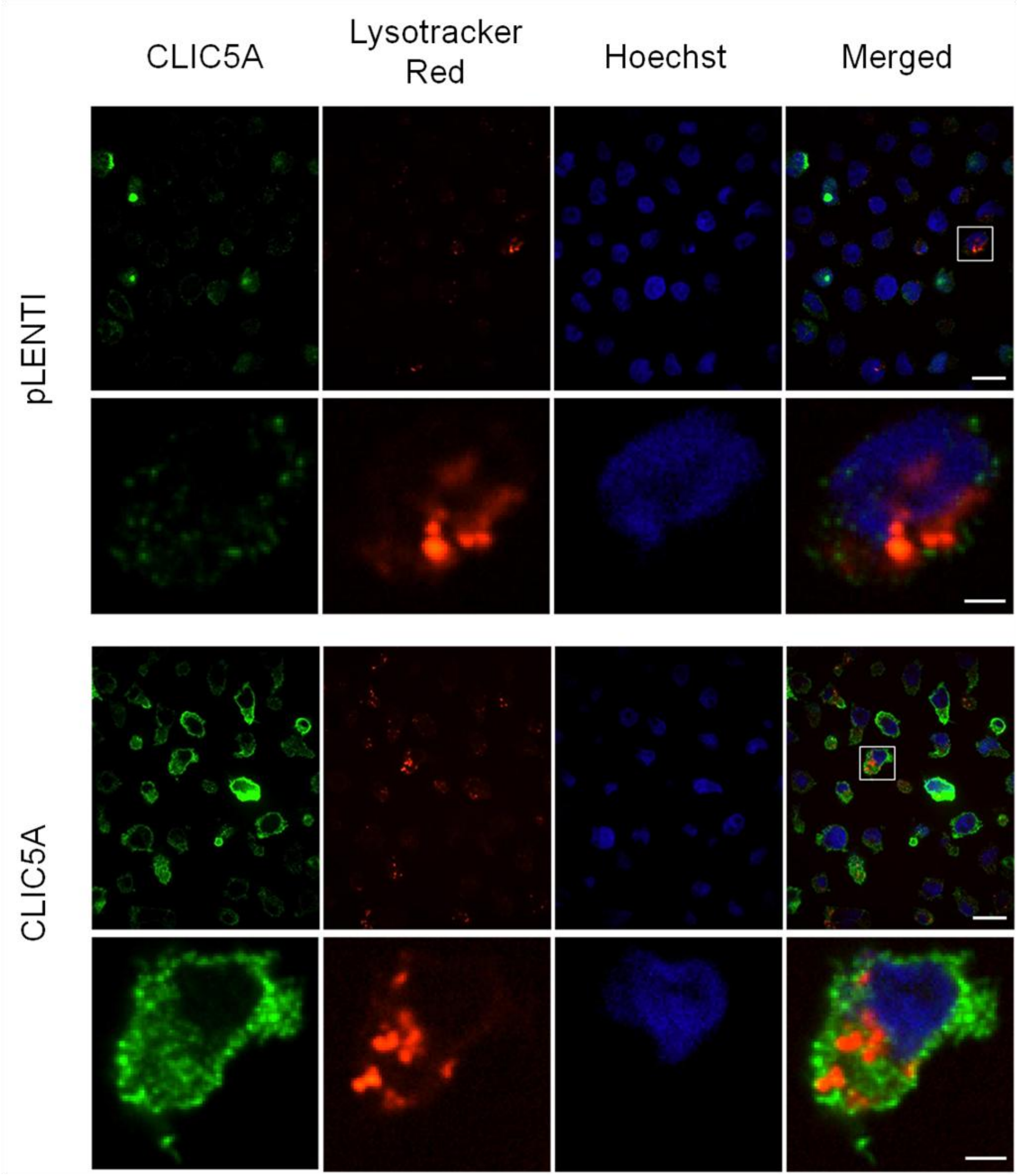

Figure S7: Localization of CLIC5A and lysosomes. pLENTI control and CLIC5A overexpressing Reh cells were stained with Lysotracker Red DND-99 dye prior to fixation and CLIC5A immunostaining. Hoechst DNA staining was also done. Results were obtained at 100X magnification (upper panels; scale bar = 10μm). No co-localization was observed between CLIC5A and lysosomes. Additional enlargement for the marked region of the initial image is presented in lower panels (scale bar = 2μm). A merged image was generated (right panels).

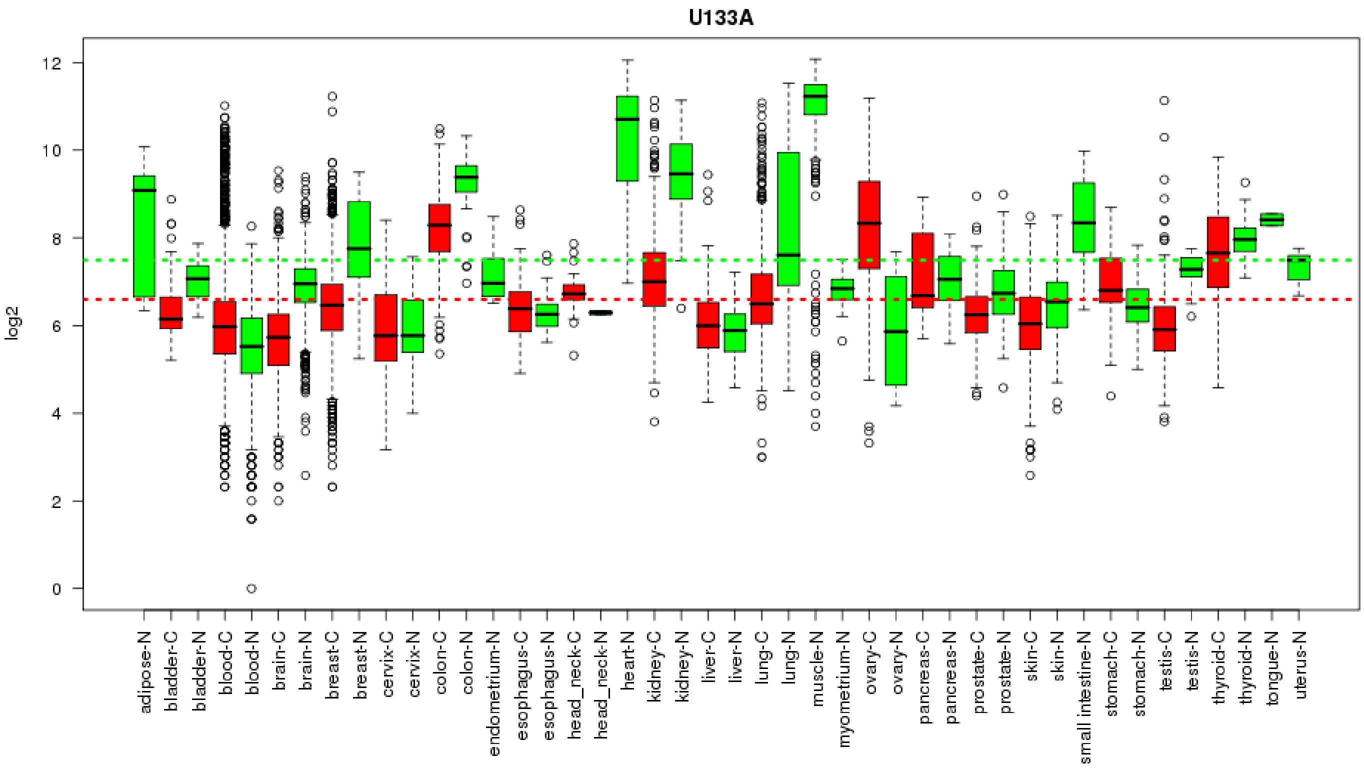

Figure S8: Expression profile of CLIC5 in normal and matched tumor tissue pairs. Affymetrix expression data (U133A array) for CLIC5 was obtained from the GENT database. Box plots of expression levels for normal (N; in green) and tumor (C; in red) tissues are shown. Notably, ovarian cancers overexpress CLIC5 compared their normal counterpart.

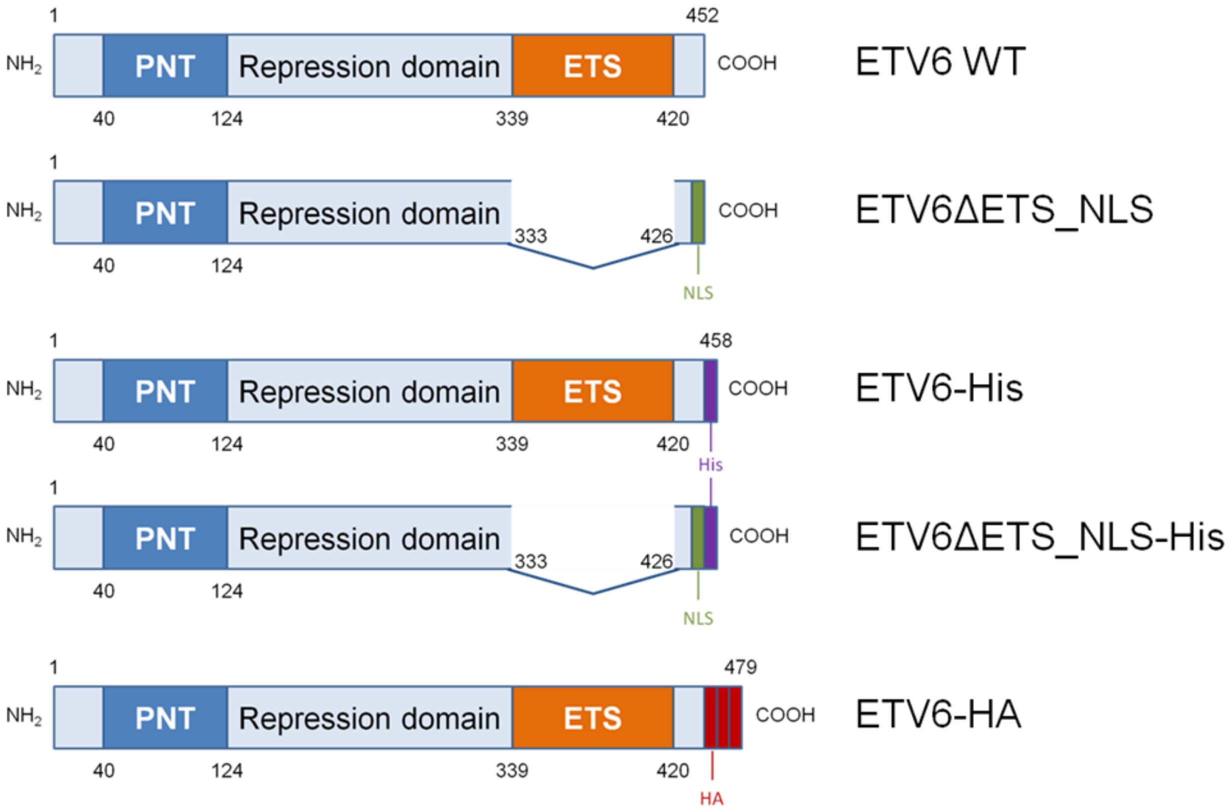

Figure S9: ETV6 constructs. Positions of ETV6 domains and deletions are indicated. Tags that were added at the C-terminus of the proteins are also indicated. Nuclear localization signal (NLS) in green, Poly-Histidine tag (His) in purple and hemagglutinin tag (HA) in red.

Supplemental tables

Table S1: **Oligonucleotide sequences used for quantitative real-time PCR validation**

Oligo Name	Sequence (5' to 3')
CLIC5_F	TGGCTGACTGCAATCTGTTG
CLIC5_R	CATCTCAGCCGGGATATCATAG
BIRC7_F	CTTCTTCCACACAGGCCATCA
BIRC7_R	GGAACTGACAGCTGGGGAA
ANGPTL2_F	GGTGACTCCTTTACATGGCAC
ANGPTL2_R	GTTATACCACCAGCCTCCCTT
WBP1L_F	CAATTACTCAGCGCTGCCAT
WBP1L_R	GAGGAGTTGGAGGTCGGTTC
LRRC4_F	ACGAAAAGGGTACAGGCTCA
LRRC4_R	TGCATGCGTGTCACTTGTAA
SLC51A_F	GGGCATCATTTCCCGTCAAG
SLC51A_R	CGTTGGCCAAGACTGAGAAG
GAPDH_F	GATCATCAGCAATGCCTC
GAPDH_R	TGTGGTCATGAGTCCTTCCA

Table S2: **Oligonucleotide sequences used for chromatin immunoprecipitation qPCR**

Oligo Name	Sequence (5' to 3')
ChIP_CLIC5_1_F	AGTTGGAGGAGGGAGCTCTA
ChIP_CLIC5_1_R	CCTCATTGGCAGTAACTCCA
ChIP_CLIC5_2_F	CCAGCACCCTACTCTCCTTT
ChIP_CLIC5_2_R	CTCCACTACCAGCCTTACCC
ChIP_BIRC7_F	GAGCCATCATCTCCAGCTCC
ChIP_BIRC7_R	TCTCTCTGAACAAGACGGGAA
ChIP_ANGPTL2_F	CTGCACGCACAGCTGGAG
ChIP_ANGPTL2_R	CCCTCTCCCCAAAGCTCAGG
ChIP_WBP1L_F	CTTCCGCCACACTCAAGATG
ChIP_WBP1L_R	TAAAATTCGTCCCAACCCGC
ChIP_SLC51A_F	CAAGACCTGTCAAATGCCCA
ChIP_SLC51A_R	TTCCCAGACCCCAGTTCATC
Neg_F	ATGGTTGCCACTGGGGATCT
Neg_R	TGCCAAAGCCTAGGGGAAGA

Table S3: **Downregulated transcripts in ETV6-His expressing Reh cells vs pLENTI cells**

Ensembl ID	Gene Symbol	logFC	logCPM	PValue	FDR
ENSG00000112782	CLIC5	-3,20	3,75	6,40E-39	8,09E-35
ENSG00000111816	FRK	-3,68	3,31	2,88E-30	1,82E-26
ENSG00000171403	KRT9	-5,94	-0,10	2,76E-16	1,16E-12
ENSG00000153993	SEMA3D	-2,54	2,70	3,18E-15	1,01E-11
ENSG00000218574	HNRNPA1P37	-5,11	0,15	6,22E-15	1,57E-11
ENSG00000136859	ANGPTL2	-1,68	4,37	3,34E-14	6,41E-11
ENSG00000168427	KLHL30	-3,34	1,35	2,79E-13	4,41E-10
ENSG00000030419	IKZF2	-1,78	6,64	6,03E-13	8,47E-10
ENSG00000178425	NT5DC1	-1,23	4,98	3,68E-12	4,23E-09
ENSG00000164920	OSR2	-2,79	2,25	4,94E-12	5,21E-09
ENSG00000145358	DDIT4L	-1,54	4,35	5,71E-12	5,55E-09
ENSG00000151012	SLC7A11	-1,62	5,51	1,01E-09	6,71E-07
ENSG00000166272	WBP1L	-1,10	5,18	1,06E-09	6,71E-07
ENSG00000123700	KCNJ2	-2,14	2,16	2,66E-09	1,60E-06
ENSG00000102471	NDFIP2	-3,60	-0,01	6,40E-09	3,68E-06
ENSG00000229164	TRAC	-2,12	2,03	1,05E-08	5,79E-06
ENSG00000173805	HAP1	-1,22	4,19	1,79E-08	9,45E-06
ENSG00000230138		-2,14	1,87	2,97E-08	1,50E-05
ENSG00000089902	RCOR1	-0,88	6,82	3,61E-08	1,75E-05
ENSG00000024422	EHD2	-2,48	1,69	6,89E-08	3,11E-05
ENSG00000177508	IRX3	-1,88	2,79	8,10E-08	3,53E-05
ENSG00000198400	NTRK1	-2,29	1,89	1,25E-07	5,29E-05
ENSG00000104888	SLC17A7	-2,20	1,16	3,76E-07	1,49E-04
ENSG00000154258	ABCA9	-1,37	4,95	5,89E-07	2,26E-04
ENSG00000165029	ABCA1	-1,50	4,44	8,21E-07	3,05E-04
ENSG00000224957		-5,90	-1,28	2,41E-06	8,48E-04
ENSG00000075213	SEMA3A	-2,49	1,94	2,85E-06	9,47E-04
ENSG00000102445	KIAA0226L	-1,53	2,44	3,27E-06	1,03E-03
ENSG00000157657	ZNF618	-0,96	4,87	3,54E-06	1,09E-03
ENSG00000128594	LRRC4	-1,16	3,23	3,99E-06	1,20E-03
ENSG00000211821	TRDV2	-2,38	0,68	4,51E-06	1,33E-03
ENSG00000173898	SPTBN2	-1,43	2,88	5,57E-06	1,60E-03
ENSG00000145147	SLIT2	-1,27	3,22	6,08E-06	1,67E-03
ENSG00000090104	RGS1	-3,18	1,03	6,67E-06	1,76E-03
ENSG00000050405	LIMA1	-0,79	4,95	8,28E-06	2,14E-03
ENSG00000100439	ABHD4	-1,24	3,08	8,82E-06	2,23E-03

ENSG00000176659	C20orf197	-2,71	0,00	1,16E-05	2,77E-03
ENSG00000185433	LINC00158	-2,30	1,20	1,21E-05	2,84E-03
ENSG00000163959	SLC51A	-1,50	2,43	1,45E-05	3,28E-03
ENSG00000081237	PTPRC	-0,73	7,67	1,51E-05	3,35E-03
ENSG00000163738	MTHFD2L	-0,78	5,87	1,69E-05	3,69E-03
ENSG00000130477	UNC13A	-1,99	2,30	2,08E-05	4,39E-03
ENSG00000101197	BIRC7	-1,39	3,02	2,30E-05	4,76E-03
ENSG00000169855	ROBO1	-3,94	2,65	2,51E-05	5,12E-03
ENSG00000141052	MYOCD	-0,83	4,51	3,71E-05	7,33E-03
ENSG00000228277		-3,29	-1,36	3,81E-05	7,40E-03
ENSG00000115232	ITGA4	-0,64	7,74	3,98E-05	7,62E-03
ENSG00000105851	PIK3CG	-0,72	5,31	5,02E-05	9,33E-03
ENSG00000169429	IL8	-2,08	0,77	5,60E-05	1,01E-02
ENSG00000112972	HMGCS1	-0,69	5,50	5,81E-05	1,02E-02
ENSG00000152580	IGSF10	-1,15	7,37	6,50E-05	1,11E-02
ENSG00000176887	SOX11	-0,97	4,93	6,91E-05	1,14E-02
ENSG00000132549	VPS13B	-0,83	5,95	6,79E-05	1,14E-02
ENSG00000076864	RAP1GAP	-1,98	0,65	7,28E-05	1,18E-02
ENSG00000136158	SPRY2	-0,99	3,40	8,28E-05	1,33E-02
ENSG00000120669	SOHLH2	-4,59	0,16	1,00E-04	1,59E-02
ENSG00000137193	PIM1	-0,75	4,87	1,08E-04	1,68E-02
ENSG00000136167	LCP1	-0,59	8,50	1,15E-04	1,75E-02
ENSG00000189325	C6orf222	-1,91	1,22	1,23E-04	1,81E-02
ENSG00000101752	MIB1	-0,59	8,81	1,25E-04	1,81E-02
ENSG00000177301	KCNA2	-2,00	0,37	1,52E-04	2,17E-02
ENSG00000027697	IFNGR1	-0,62	5,47	1,69E-04	2,35E-02
ENSG00000137441	FGFBP2	-2,47	-1,13	1,89E-04	2,58E-02
ENSG00000152413	HOMER1	-0,79	4,40	1,97E-04	2,65E-02
ENSG00000118308	LRMP	-0,70	6,27	2,06E-04	2,74E-02
ENSG00000058091	CDK14	-0,73	5,64	2,27E-04	2,98E-02
ENSG00000196954	CASP4	-2,00	0,35	2,84E-04	3,59E-02
ENSG00000235492		-1,92	1,08	2,87E-04	3,59E-02
ENSG00000007237	GAS7	-0,66	4,95	2,98E-04	3,63E-02
ENSG00000198053	SIRPA	-2,46	0,40	3,15E-04	3,76E-02
ENSG00000112245	PTP4A1	-0,76	5,70	3,48E-04	4,04E-02
ENSG00000169252	ADRB2	-1,96	-0,27	3,71E-04	4,23E-02
ENSG00000105409	ATP1A3	-1,11	4,19	3,97E-04	4,48E-02
ENSG00000078142	PIK3C3	-0,70	6,99	4,58E-04	5,08E-02
ENSG00000151276	MAGI1	-3,79	-0,62	4,75E-04	5,23E-02
ENSG00000183496	MEX3B	-1,23	2,04	5,00E-04	5,43E-02
ENSG00000153822	KCNJ16	-0,86	3,29	5,15E-04	5,52E-02

ENSG00000178752	FAM132B	-1,24	2,31	5,41E-04	5,75E-02
ENSG00000011105	TSPAN9	-0,89	3,30	5,91E-04	6,08E-02
ENSG00000080503	SMARCA2	-0,54	6,71	6,47E-04	6,54E-02
ENSG00000064666	CNN2	-1,00	4,18	6,71E-04	6,68E-02
ENSG00000173559	NABP1	-0,71	4,53	6,82E-04	6,70E-02
ENSG00000158201	ABHD3	-0,55	6,40	6,83E-04	6,70E-02
ENSG00000167094	TTC16	-1,29	1,98	7,62E-04	7,33E-02
ENSG00000188452	CERKL	-0,54	5,49	7,65E-04	7,33E-02
ENSG00000242265	PEG10	-0,62	6,06	7,89E-04	7,47E-02
ENSG00000160255	ITGB2	-1,43	2,02	8,43E-04	7,84E-02
ENSG00000241151		-2,22	0,00	9,10E-04	8,28E-02
ENSG00000234883	MIR155HG	-0,99	2,83	9,10E-04	8,28E-02
ENSG00000109861	CTSC	-0,61	5,47	9,36E-04	8,46E-02
ENSG00000152104	PTPN14	-1,77	0,44	1,03E-03	9,05E-02
ENSG00000100097	LGALS1	-0,92	2,88	1,06E-03	9,20E-02
ENSG00000134762	DSC3	-0,89	6,98	1,14E-03	9,80E-02

Table S4: **Downregulated transcripts in ETV6-His expressing Reh cells vs ETV6ΔETS_NLS-His cells**

Ensembl ID	Gene Symbol	logFC	logCPM	PValue	FDR
ENSG00000112782	CLIC5	-3,23	4,66	7,00E-52	9,59E-48
ENSG00000101197	BIRC7	-1,93	4,02	3,44E-11	1,45E-07
ENSG00000145358	DDIT4L	-1,35	5,04	3,84E-11	1,45E-07
ENSG00000136859	ANGPTL2	-1,31	5,00	4,24E-11	1,45E-07
ENSG00000218574	HNRNPA1P37	-3,99	0,66	6,67E-11	1,83E-07
ENSG00000139083	ETV6	-1,66	4,49	4,27E-09	8,36E-06
ENSG00000111816	FRK	-1,78	3,60	1,20E-08	2,05E-05
ENSG00000102471	NDFIP2	-3,05	0,66	1,08E-07	1,65E-04
ENSG00000230138		-1,66	2,49	7,65E-06	9,18E-03
ENSG00000166272	WBP1L	-0,79	5,78	8,04E-06	9,18E-03
ENSG00000153993	SEMA3D	-1,35	3,12	9,81E-06	1,03E-02
ENSG00000101198	NKAIN4	-1,85	1,68	5,42E-05	4,95E-02
ENSG00000099365	STX1B	-2,08	-0,11	5,82E-05	4,99E-02
ENSG00000250366	LINC00617	-2,10	-0,69	7,42E-05	5,64E-02
ENSG00000128594	LRRC4	-1,02	3,90	9,18E-05	6,29E-02
ENSG00000178425	NT5DC1	-0,62	5,50	8,74E-05	6,29E-02
ENSG00000024422	EHD2	-1,66	2,22	1,06E-04	6,60E-02
ENSG00000163959	SLC51A	-1,31	3,11	1,14E-04	6,79E-02
ENSG00000099250	NRP1	-1,48	1,77	1,58E-04	9,04E-02

Table S5: **Oligonucleotide sequences used for cloning**

Oligo Name	Sequence (5' to 3')
ETV6_F	GGGAATTCGGCACGAGGAAACT
ETV6**His**_R	CCGCGGCCGCTCA**ATGATGATGATGATGATG**GCATTCATCTTCTTGGTATATTTGTT
ETV6deltaETS_NLS_F	**CCGGATCC**AATTCGGCACGAGGAAACT
ETS_NLS**His**_R	CCGCGGCCGCTCA**ATGATGATGATGATGATG**CACCTTCCTCTTCTTCTTCGGTTG
Oligo**_HA**$_3$	GAATGCTTA**TACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGATTACGCT**TGAGCGGCCG

Table S6: **Antibodies used in western blotting**

Primary antibodies	Host	Antibody # (Provider)	Working dilution
ETV6	mouse	ab54705 (Abcam)	1:1000
GAPDH	goat	sc-31915 (Santa Cruz)	1:1000
CLIC5	rabbit	ab75948 (Abcam)	1:100
Beta-Actin	mouse	sc-81178 (Santa Cruz)	1:1000
Secondary antibodies (HRP-coupled)			
Anti-mouse	rabbit	sc-358914 (Santa Cruz)	1:5000
Anti-goat	chicken	sc-2961 (Santa Cruz)	1:5000
Anti-rabbit	donkey	sc-2077 (Santa Cruz)	1:5000