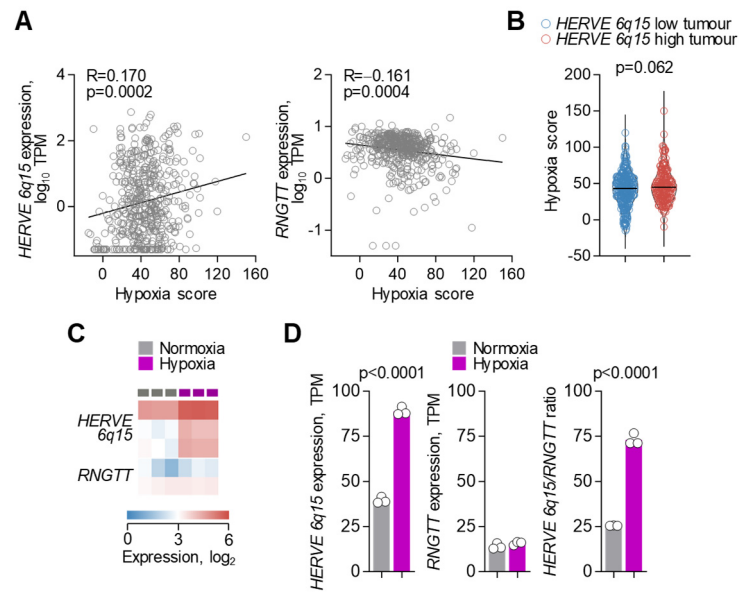
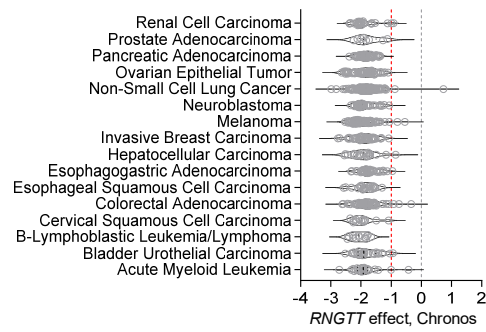
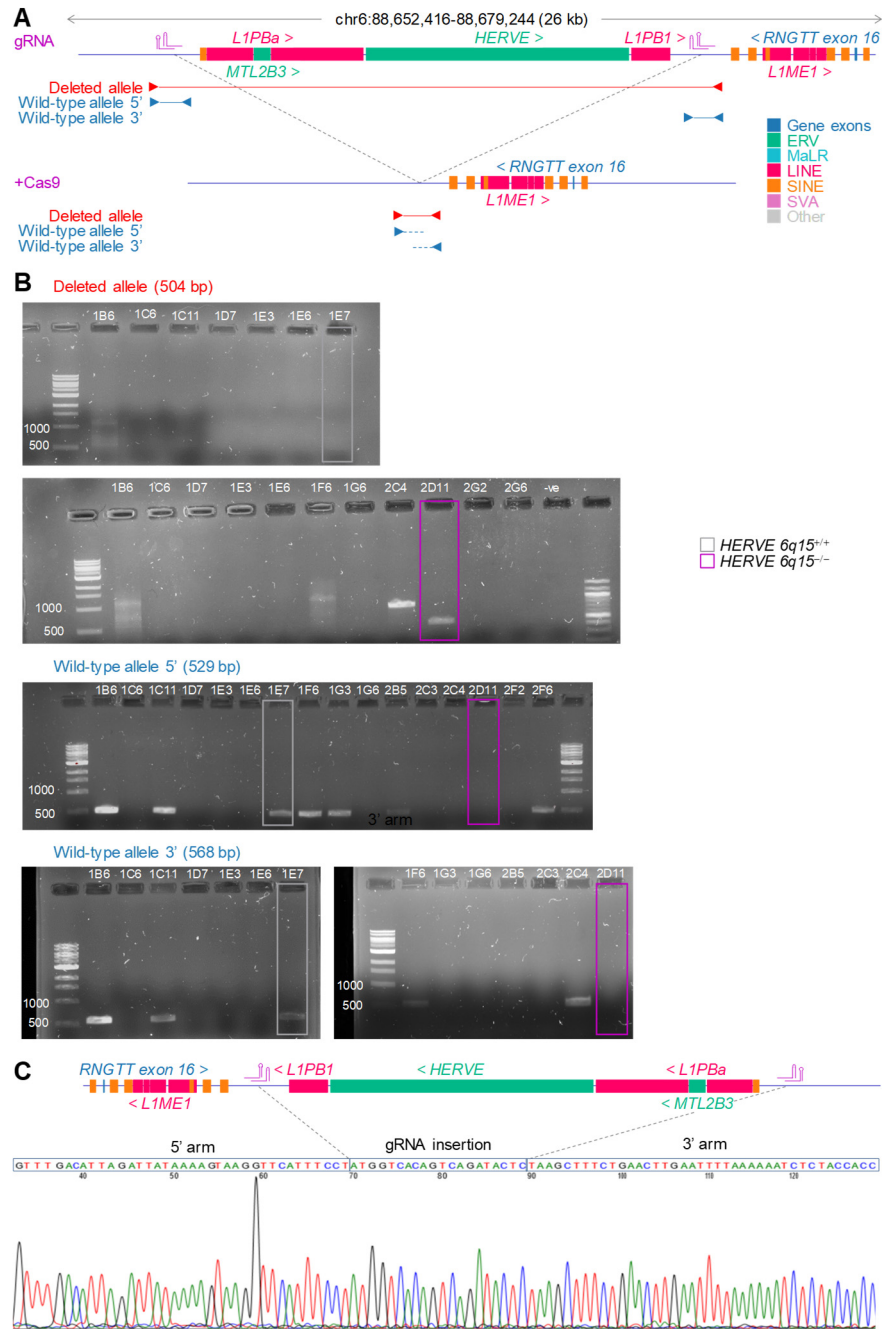




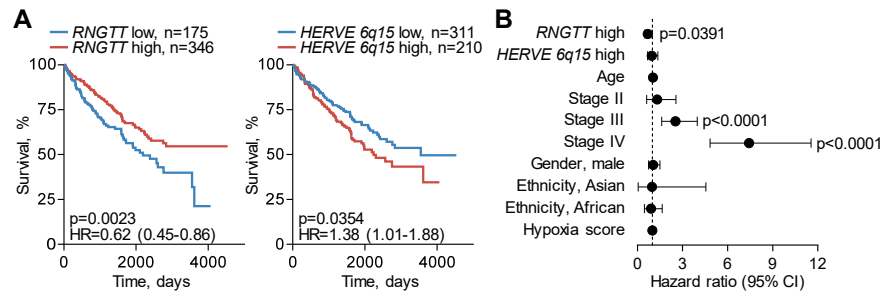
**Fig. S1. *HERVE 6q15* responsiveness to hypoxia.** **(A)** *HERVE 6q15* and *RNGTT* expression (TPM) in KIRC samples (n=485) according to their hypoxia scores (p values calculated with linear regression). **(B)** Hypoxia score in KIRC samples with low (n=285) and high (n=200) *HERVE 6q15* expression (p value calculated with Mann-Whitney test). **(C)** Expression of transcripts overlapping *HERVE 6q15* or the canonical *RNGTT* in RNA-seq data (GSE120887) from VHL-sufficient RCC4 cells grown in normoxic or hypoxic conditions [20]. **(D)** *HERVE 6q15* and *RNGTT* expression (TPM), and ratio of *HERVE 6q15* to *RNGTT* expression in the same cells as in c (p values calculated with two-tailed Student's t-tests).



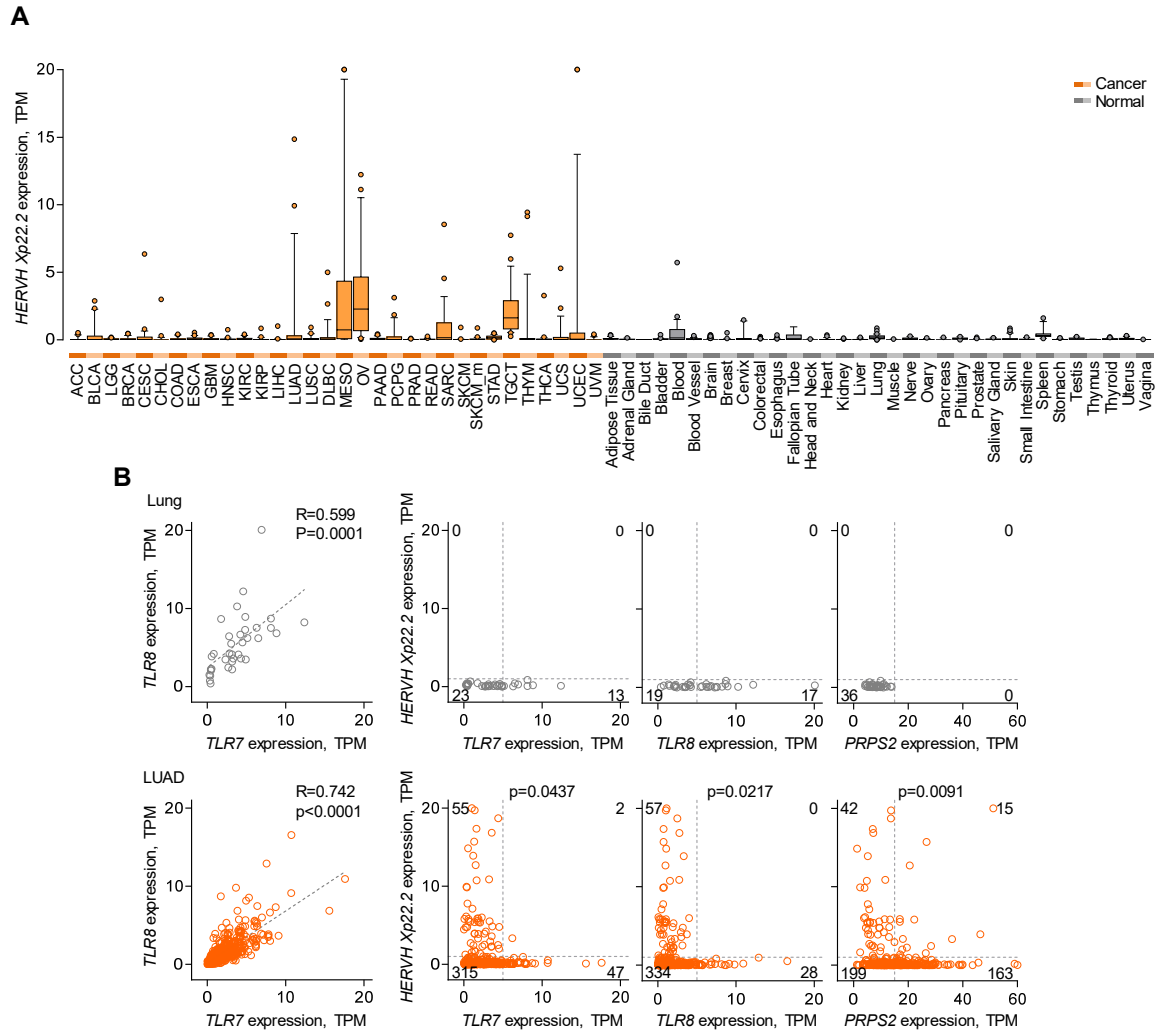
**Fig. S2. Essential function of *RRGTT* in cancer cell lines.** Effect of *RRGTT* deletion (Chronos score) on the growth of cancer cell lines of the indicated origin. Red dashed line represents the threshold for a significant effect. Data downloaded from the Dependency Map (DepMap) portal [35].



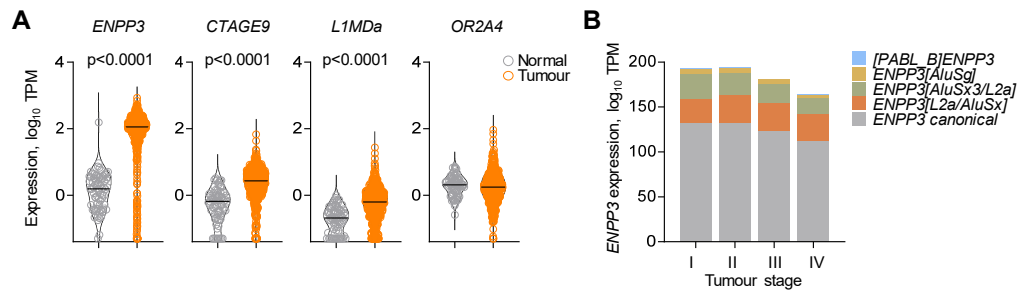
**Fig. S3. CRISPR/Cas9-mediated deletion of the *HERVE 6q15* provirus. (A)** RTE content in the intronic region of *HERVE 6q15* integration, position of guide RNA (gRNA) molecules, and position of the PCR primers used for the detection of the wild-type and deleted alleles. **(B)** Gel electrophoresis of amplicons from A498 clones with wild-type or deleted alleles. **(C)** Sanger sequencing of PCR amplicon of the deleted allele in *HERVE 6q15*<sup>-/-</sup> clone 2D11. Also noted is the insertion of one gRNA sequence in site of the deletion.



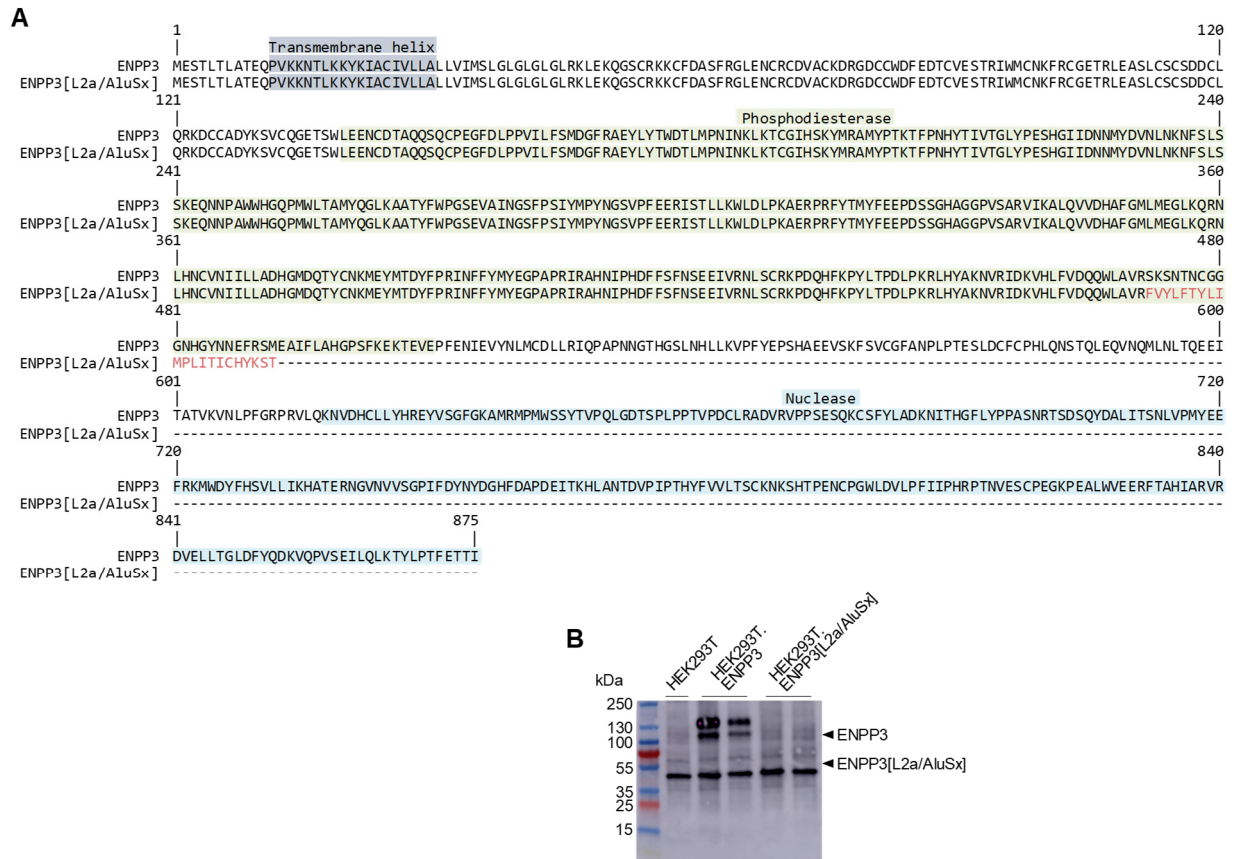
**Fig. S4. Correlation of *RRGTT* and *HERVE 6q15* expression with survival in KIRC. (A)** Overall survival of KIRC patients, stratified by expression levels of *RRGTT* (left) or *HERVE 6q15* (right) (p values calculated with log-rank tests). **(B)** Overall survival hazard ratios (HRs) for the indicated variables in KIRC patients (*RRGTT* high n=346, reference low n=175; *HERVE 6q15* high n=210, reference low n=311; Age n=521; Stage II n=56, III n=123, IV n=82, reference I n=257; Gender, male n=338, reference female n=183; Ethnicity, Asian n=8, African n=55, reference White n=450; Hypoxia score n=470). Error bars represent 95% confidence intervals (CIs) (p values calculated with Cox proportional hazards regression).



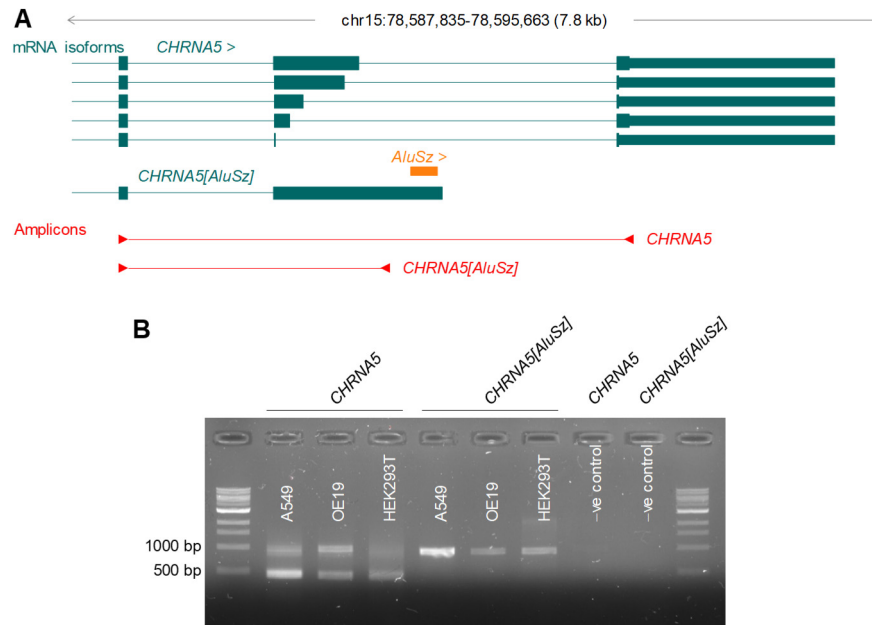




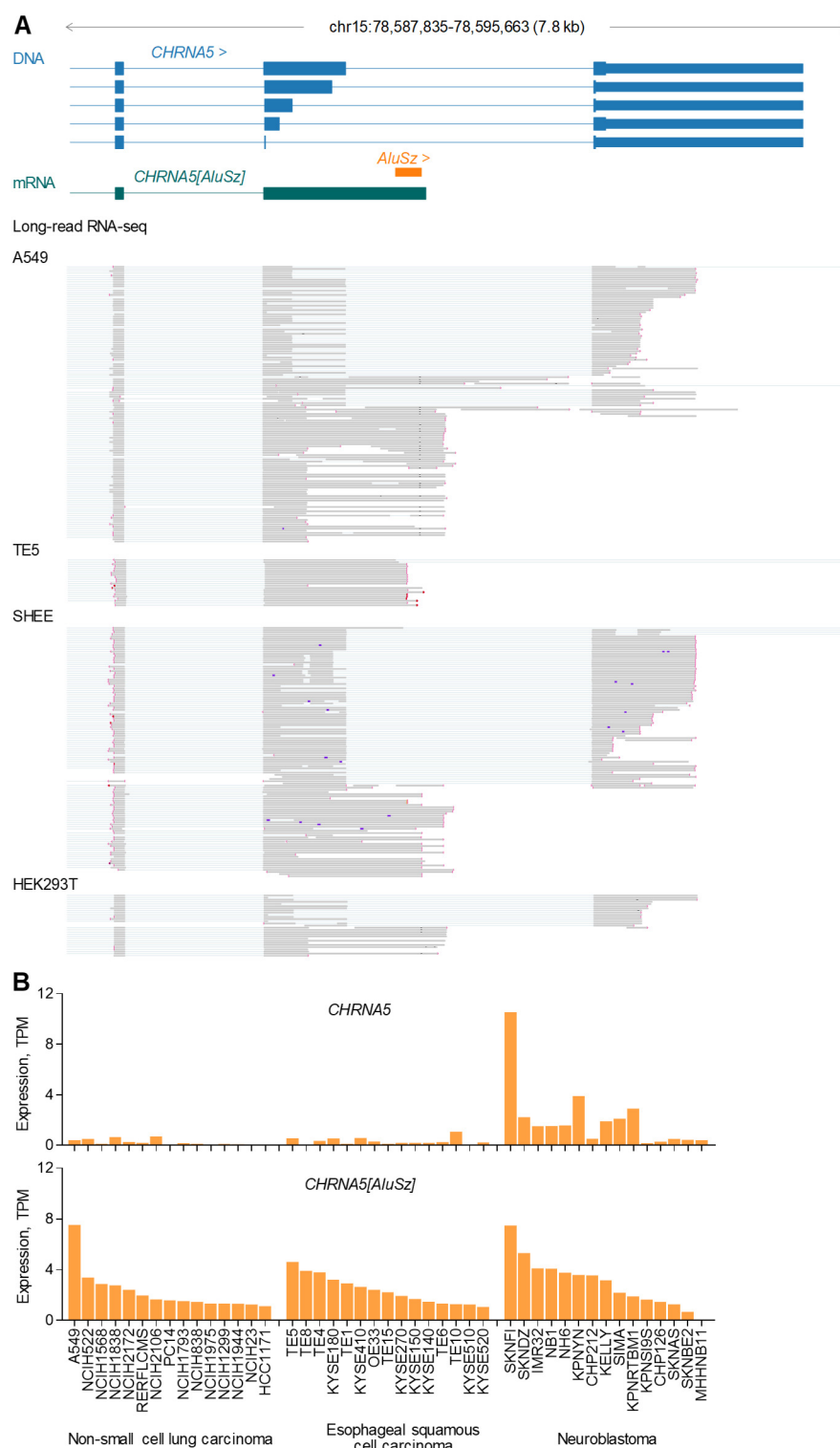
**Fig. S7. Cancer specificity of *ENPP3*, and overlapping gene and RTE expression in KIRC. (A)** Expression of canonical *ENPP3* or overlapping genes and RTEs in normal kidney tissue (n=71) and KIRC samples (n=538) (p values calculated with Mann-Whitney tests). **(B)** *ENPP3* isoform expression (TPM) in KIRC samples according to tumour stage (I n=271, II n=59, III n=123, IV n=82).



**Fig. S8. Instability of the *ENPP3*[L2a/AluSx] protein product. (A)** Amino acid sequence alignment of canonical *ENPP3* and the *ENPP3*[L2a/AluSx] isoform. Amino acid residues in red denote the sequence in *ENPP3*[L2a/AluSx] that replaces the end of the phosphodiesterase and all of the nuclease domain. **(B)** Western blot for *ENPP3* in lysates from parental HEK293T cells and cells transduced to express *ENPP3* and *ENPP3*[L2a/AluSx] (HEK293T.*ENPP3* and HEK293T.*ENPP3*[L2a/AluSx], respectively, run in duplicate). Arrows show the theoretical mass of each isoform.

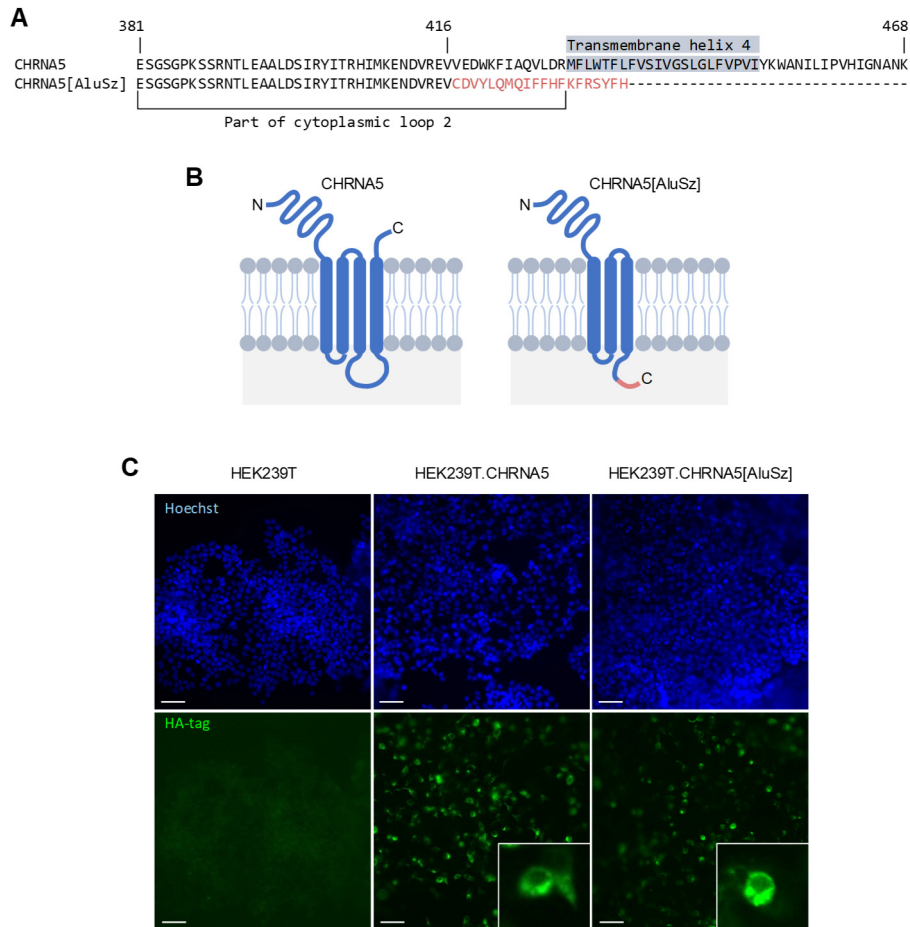


**Fig. S9. PCR validation of the *CHRNA5[AluSz]* isoform. (A)** Annotated and assembled *CHRNA5* transcripts (exons 4-6 only), location of exonised *AluSz*, and amplicons used for transcript validation. **(B)** Gel electrophoresis of amplicons of *CHRNA5[AluSz]* or canonical *CHRNA5* isoforms amplified from the indicated cell lines. *CHRNA5* amplicons of different sizes correspond to canonical isoforms utilising different splice donor sites in exon 5.

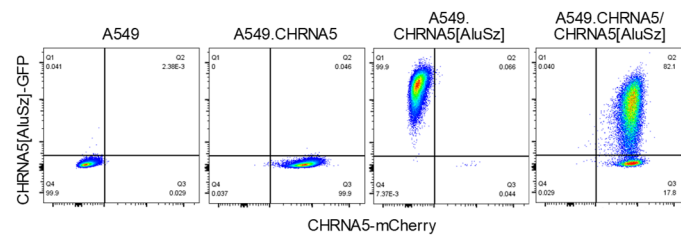


**Fig. S10. Balance of *CHRNA5* isoform expression in cancer cell lines. (A)** Gene structure (exons 4-6 only), location of exonised *AluSz*, assembled *CHRNA5[AluSz]* transcript, and long-read RNA-seq data from HEK293T and A549 cells [22], and esophageal squamous cell carcinoma TE5 and normal immortalized esophageal squamous epithelial SHEE cells (PRJNA515570) [21]. **(B)** *CHRNA5* and *CHRNA5[AluSz]* isoform expression (TPM) in RNA-seq data from the indicated cell lines in CCLE.

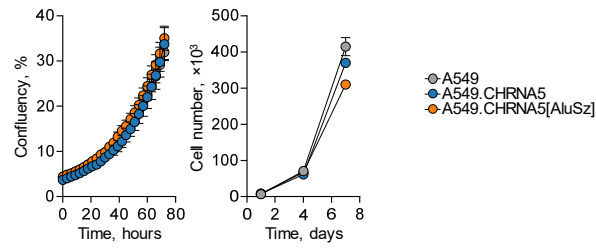




**Fig. S12. Sequence and expression of the CHRNA5[AluSz] protein. (A)** Amino acid sequence alignment of canonical CHRNA5 (last 88 amino acids only) and the CHRNA5[AluSz] isoform. Amino acid residues in red denote the sequence in CHRNA5[AluSz] that replaces the end of the last transmembrane helix and extracellular C-terminal sequence of the canonical isoform. **(B)** Schematic representation of canonical CHRNA5 and the CHRNA5[AluSz] isoform. **(C)** Immunofluorescence detection of cell surface expression (in non-permeabilised cells) of HA-tagged canonical CHRNA5 and CHRNA5[AluSz] isoforms, overexpressed in HEK293T cells. Images are from a single experiment (scale bar=100µm, insets 5× magnification).



**Fig. S13. Establishment of A549 cells expressing *CHRNA5* and *CHRNA5[AluSz]*.** Flow cytometric detection of fluorescent reporter expression in parental A549 cells and A549 cells expressing the canonical *CHRNA5* isoform and mCherry reporter, the *CHRNA5[AluSz]* isoform and GFP reporter or both isoforms.



**Fig. S14. Effect of *CHRNA5* and *CHRNA5[AluSz]* expression on A549 *in vitro* growth.** Mean confluency ( $\pm$ SEM, n=6 from 1 experiment) (*left*) and mean cell number ( $\pm$ SEM, n=2 from 1 experiment) (*right*) of parental A549 cell cultures and those of A549 cells expressing the canonical *CHRNA5* or the *CHRNA5[AluSz]* isoform.