Supplementary figure 1. Sequence of the *GLI1AS* genomic region.

A. Sequence of the GLI1AS RNA, as detected by the 5' and 3' RACE analysis in Rh36, CCA and RMS13 rhabdomyosarcoma cells. The three exons of GLI1AS are shown in brown with the introns in black. Note, that the sequence of the third GLI1AS exon, apart from the first 8 and last 4 nucleotides, consists of an *AluSc* repeat, as determined by RepeatMasker (http://repeatmasker.org). The primers used to generate the exonic expression constructs in pCMV5: full-length GLI1AS, 5' GLI1AS and 3' GLI1AS are shown. The forward primers are in italics and the reverse primers are underlined.

B. Sequence of the genomic region encompassing the first exons of *GLI1* and *GLI1AS*. The exons are shown in brown, with the conserved GT intronic dinucleotides in bold. Note that GLI1AS first exon initiates 155 nucleotides upstream of the major transcription start site of GLI1 in an opposite orientation.

Supplementary figure 2. Transcription start sites in *GLI1* and *GLI1AS*.

Visualization of the CAGE data from the FANTOM5 Genome Browser indicate a multitude of transcription start sites in the sense orientation (green bars) and antisense orientation (purple bars) upstream of the first exon of *GLI1* (green line).

Supplementary figure 3. Expression pattern of the *GLI1AS/GLI1* genomic region according to the UCSC genome brower.

Visualization of transcription, H3K4me3 marks and RNA polymerase II density according to the ENCODE data in the genomic region encompassing *GLI1AS* and the flanking genes *INHBE* and *GLI1*. Note that in the GM12878 lymphocyte cell line

(GM78) there is expression of the *GLI1AS* region from the antisense strand (minus signal) in the poly A+ RNA from both the cytoplasmic and the nuclear fraction.

Supplementary figure 4. Position of real-time PCR primers sets for *INHBE*, *GLI1AS* and *GLI1*.

The position of the exonic and intronic primer sets for *INHBE*, *GLI1AS* and *GLI1* used in the gene expression analysis and the chromatin immunoprecipitation assays are shown. Red and green arrowheads indicate forward and reverse primers respectively in the schematic diagram of these genes.

Supplementary figure 5. Nuclear versus cytoplasmic distribution of GLI1AS in the RMS13 cell line.

Real-time RT/PCR analysis of the expression of GLI1AS in nuclear and cytoplasmic fractions of RMS13 cells. Data are represented as nuclear/cytoplasmic ratios, calculated by determining the $2^{-\Delta Ct}$ values in the nucleus and the cytoplasm, with the ΔCt obtained by subtracting the Ct value of GAPDH in the cytoplasmic fraction from all Ct values. Note that, as observed for Rh36 and CCA cells (Figure 4), the spliced forms of GLI1AS are preferentially retained in the cytoplasm (AS E1-2 and AS E2-3), similar to the exonic amplicons, GAPDH E1-3, HPRT E6-7 and ACTB E2-3, of the housekeeping genes used, while the opposite is true for the unspliced forms of GLI1AS (AS Int1 and AS Int2).

Supplementary figure 6. GLI1 and GLI1AS knock-downs in the CCA cell line.

Real-time RT/PCR analysis of the expression of GLI1 and GLI1AS transcripts in CCA cells, following knock-down of GLI1 or GLI1AS by a 48-hr treatment with si-

Gmix or si-ASmix, respectively. Data are represented as relative expression ($2^{-\Delta\Delta Ct}$ values), calculated by subtracting the Ct value of the housekeeping gene GAPDH from the Ct value of the GLI1 and GLI1AS transcripts (ΔCt), and normalized to the ΔCt value obtained by a control siRNA (si-Control), by subtracting this control ΔCt value from the ΔCt values of the si-Gmix and si-ASmix samples (ΔΔCt). Error bars indicate the standard deviation. Note that knock-down of GLI1 clearly reduces the level of AS E2-3.

Supplementary figure 7. Impact of GLI1AS intron 1 in modulating GLI1 expression / GLI1 target genes.

The expression of GLI1, PTCH1, PTCH2 and ADAR2 (negative control) in Rh36 cells, following transfection of pCMV5 expression constructs for 5'GLI1AS with (5′GLI1AS-Int1) or without (5′GLI1AS) inclusion of intron 1, is analyzed as in Figure 4A.

Supplementary figure 8. *INHBE* gene expression in Rh36 cells transfected with GLI1.

The RNA levels of INHBE in Rh36 cells following transfection of a pCMV5 expression construct for GLI1 (Materials and Methods) is analyzed. Data are represented as relative expression ($2^{-\Delta\Delta Ct}$ values), calculated by subtracting the Ct value of the housekeeping gene *TBP* from the Ct value of the INHBE mRNA (ΔCt), and normalized to the ΔCt value obtained following transfection of the pCMV5 control vector, by subtracting this control ΔCt value from the ΔCt values of all samples (ΔΔCt). Error bars indicate the standard deviation. *, Statistical significant, $P<0.01$ compared to control, calculated by the Student's t-test.

Note that overexpression of GLI11 does not increase, but in fact, decreases *INHBE* gene expression. This can be rationalized by the concomitant increase of GLI1AS (Figure 4F), which was shown to elicit suppressive effects on *INHBE* gene expression (Figure 4E).

Supplementary figure 9. H3K4me3 marks at the *INHBE / GLI1AS / GLI1* locus following GLI1AS overexpression

Chromatin immunoprecipitation assays for H3K4me3 in Rh36 cells 48 hrs after transfection of a pCMV5 expression construct for GLI1AS. For real-time PCR analysis, 8 primers sets (A to H) spanning the *INHBE* / *GLI1AS / GLI1* locus (Table 1, Supplementary figure 4) were used. Below the schematic diagram of the locus is a graph showing the immunoprecipitated signals relative to input control DNA for each interrogated segment. *, Statistical significant, $P<0.01$ compared to control, calculated by the Student's t-test

Supplementary figure 1A

*CTATTTCCCTCCCTCTTGCCGTTCT***GGAGGATGAGGCGGGGTAGCTGGAAGCAAGAGAAGGGGTGCCGGGCTTATTGCACCCCCAGGACCCCC**
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ATAATAGTGGAGGTAGGGTAGTCGCCTTTGGTTGGTGCTAACTGAGCATTCTGCCATCCTGTGGTTTATCTGCAG**GCCTCACCCTTTGGATGGAA**
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GCAAGGAGCAGCGGCTGAGGCTCTGGGTGCAGGGTGTGGTTCACGAGGTCACCAAGGAGCCCTGGGACTGAAGCCCCTTTCCCTAGAACCCC
*GGCTCTACGTCTCGAAGTTCTGGAG***GGTGCCGCCCCTCCGCACCACGTCACTGCAGGCTCTAGCTGCTCTTCCAGGCTTTCTGCTCCCTCTGCTGG**
CCGTTCTCACACATGACACCCGCTCGGAGTCGAAGCAGAAGAGCTGGGGAACTGAAGGAGGAGCCCAGTTGAAGGAGGAGCCCCTCACTAA
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GAAGCCTACAGAGTACACTTGAAGAACTGTTGCTCTGGACCATCACCCTAACCCTTCTTTATGACTCACATTTGTTGATTTTCTTTCTCCTTTTTATT
AAG**AAAGTATAAGCCAGGCGCGGTGGCTCACGCCTATAATCTCATCACTTCGGGAGGCCAAGTCGGGTGGATCAAGAGGTCAAGAGATTGA**
GACCATCCTGGCCAACATGTTGAAACCTCGTTTCTACTAAGAATACAAAAATTAGTGGGCGCCTGTAATCCCAGCTGCTCGGGAGGCTGAGGC
AGAAGAATCACTTGAACCCAGGAGACGGAGGTTGCAGTGAGCCAAGATCGTGTCACTGCACTCCAGCCTGGTGACAGAGCGAGACTCGATCT
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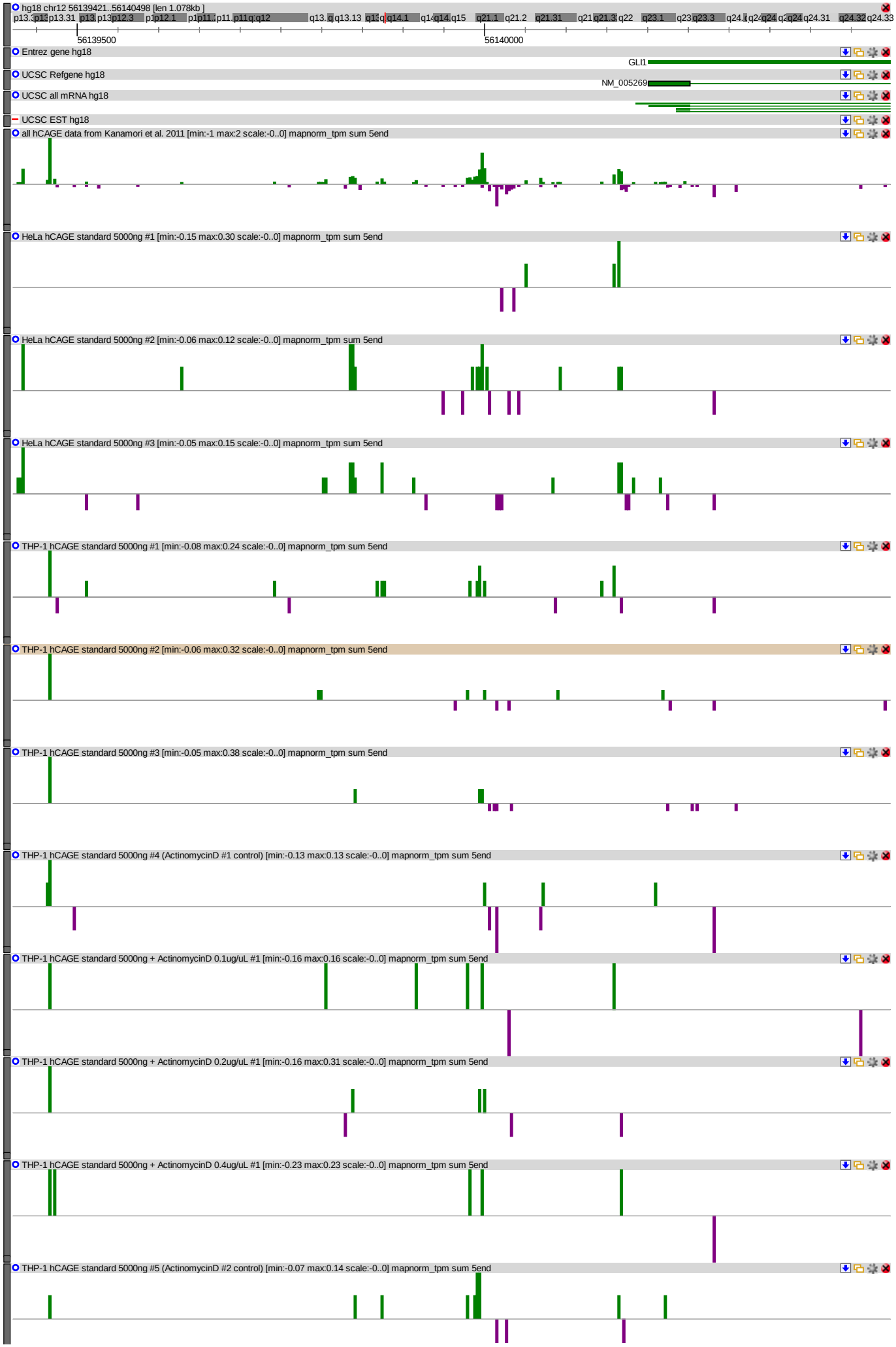
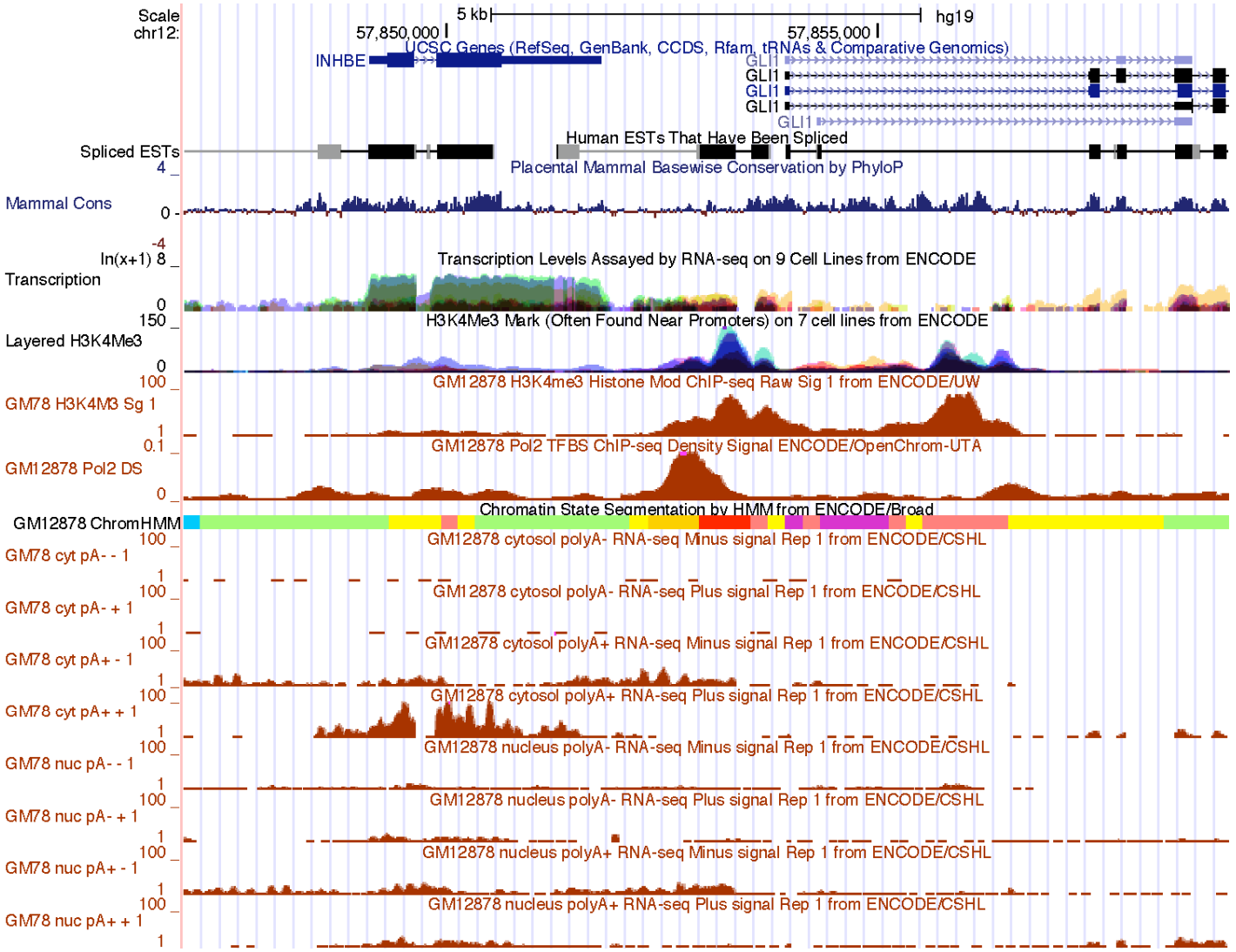
Supplementary figure 1B

Supplementary figure 2

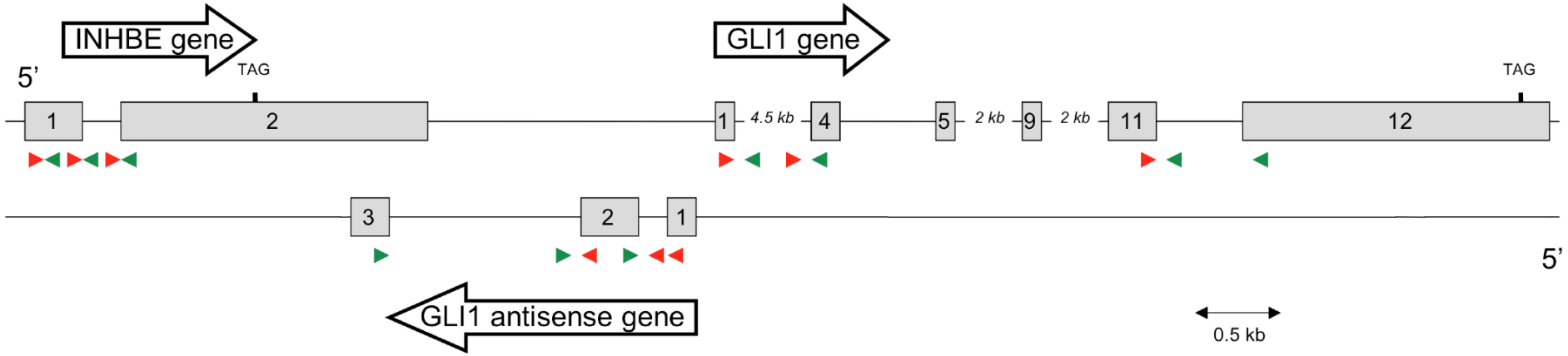


Supplementary figure 3

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly



Supplementary figure 4



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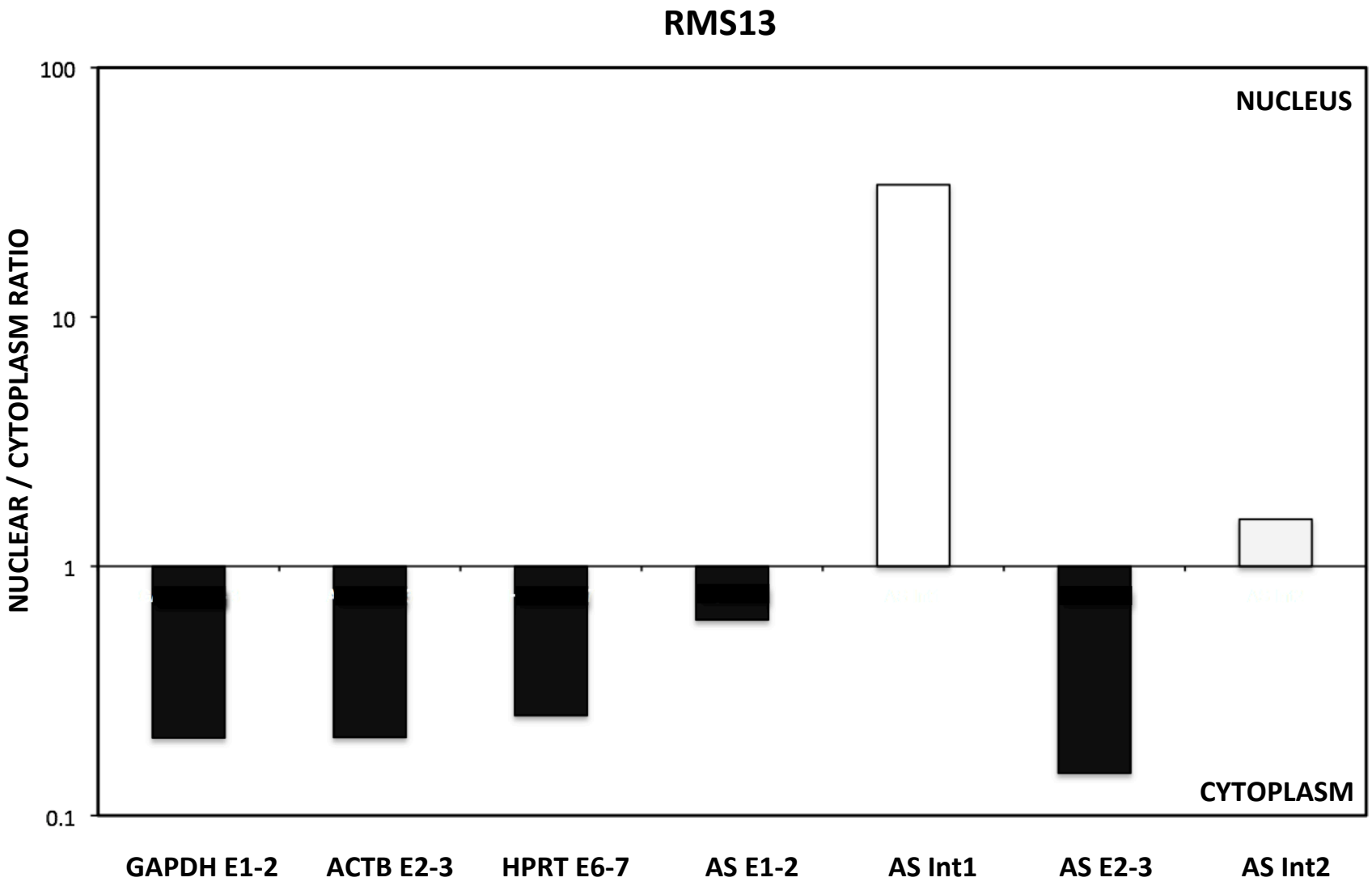
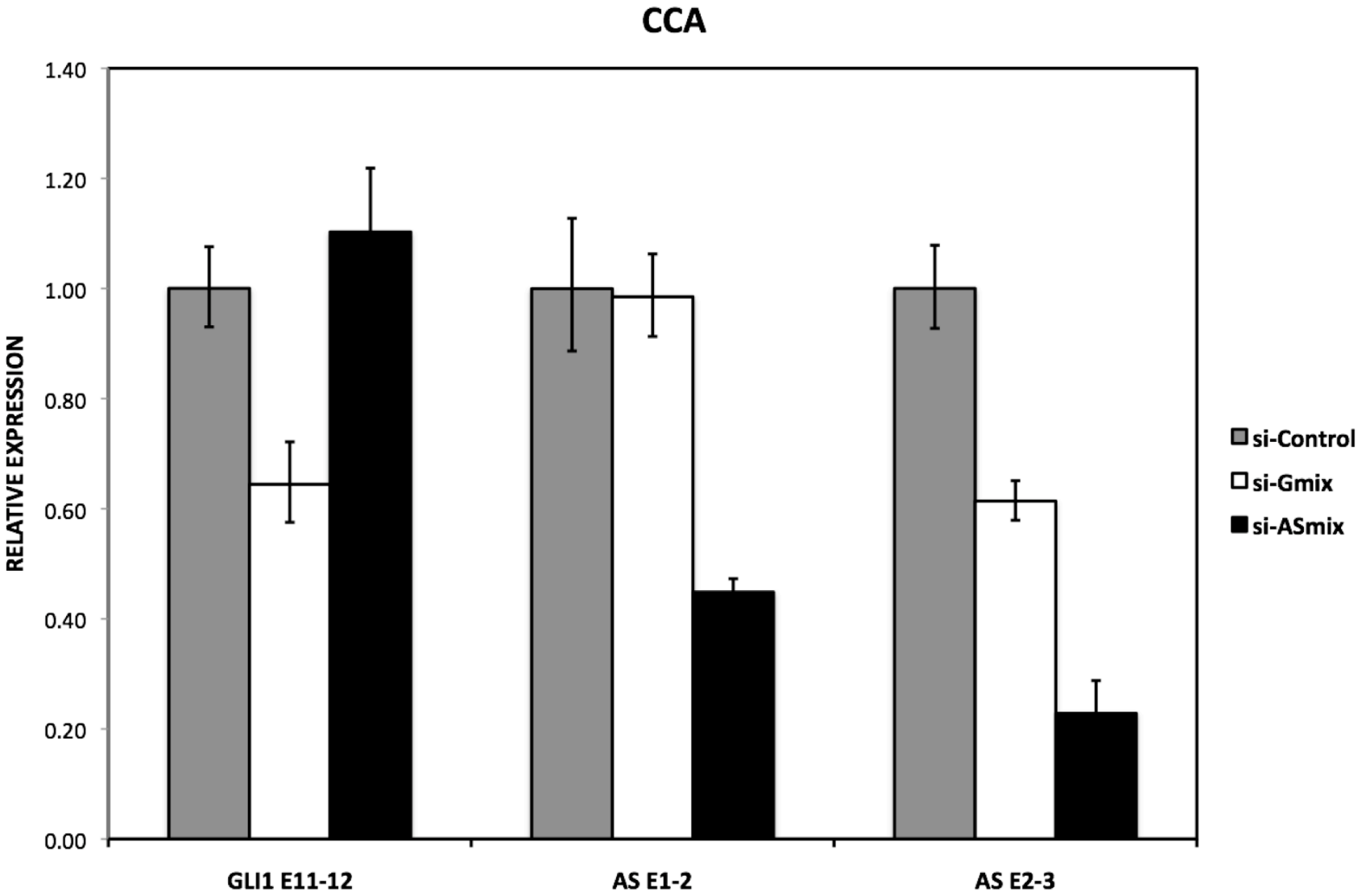


Supplementary figure 6



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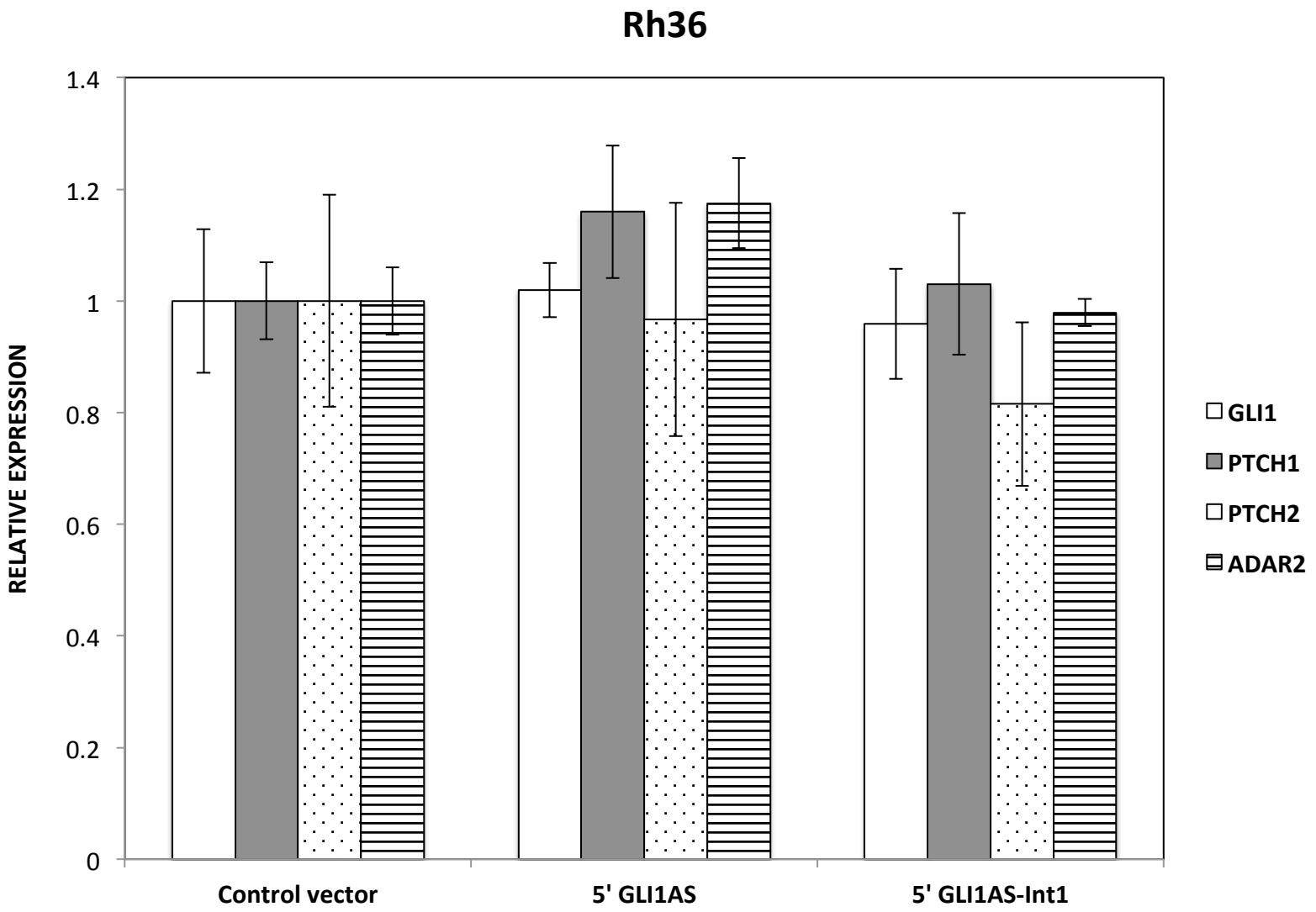
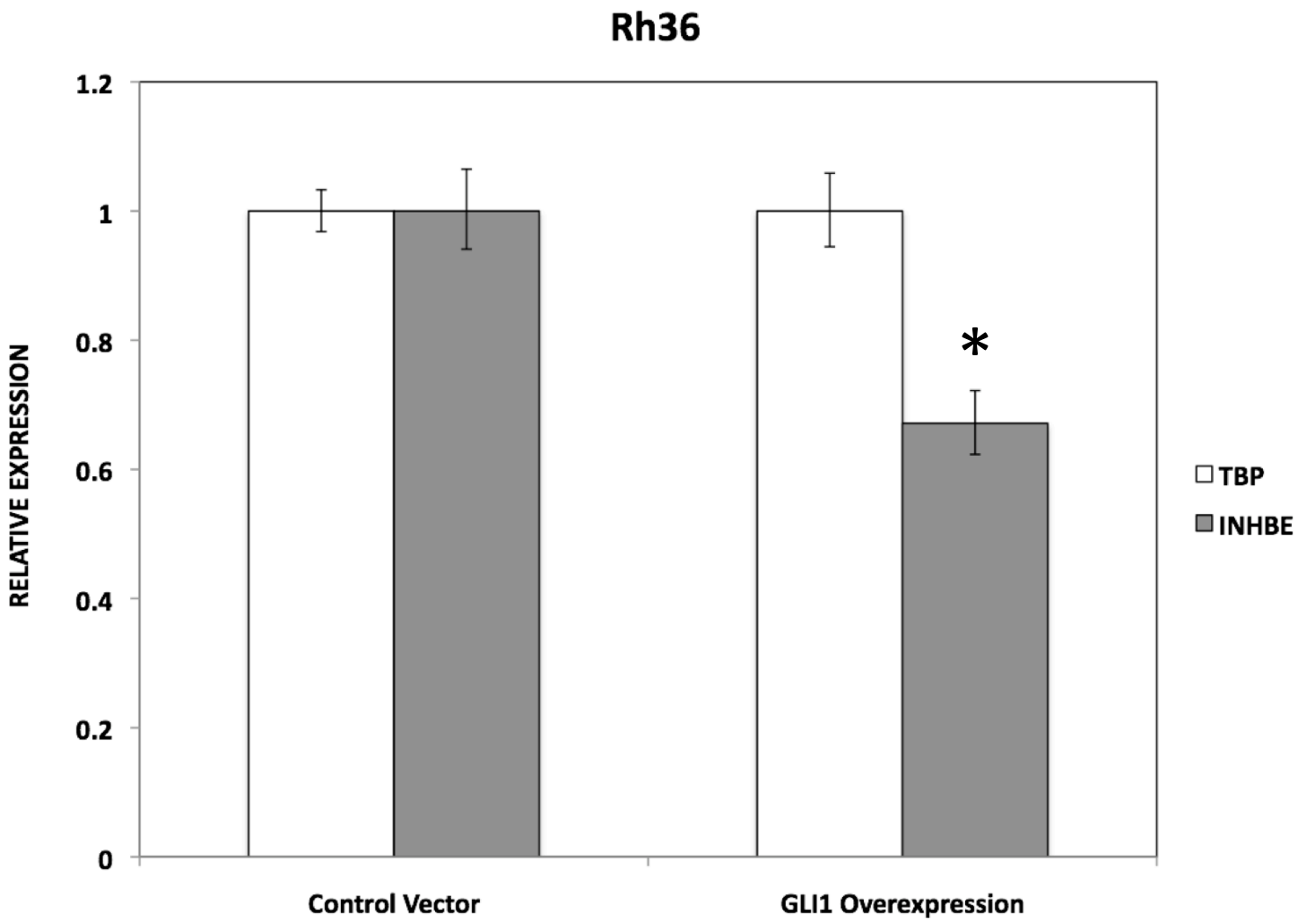
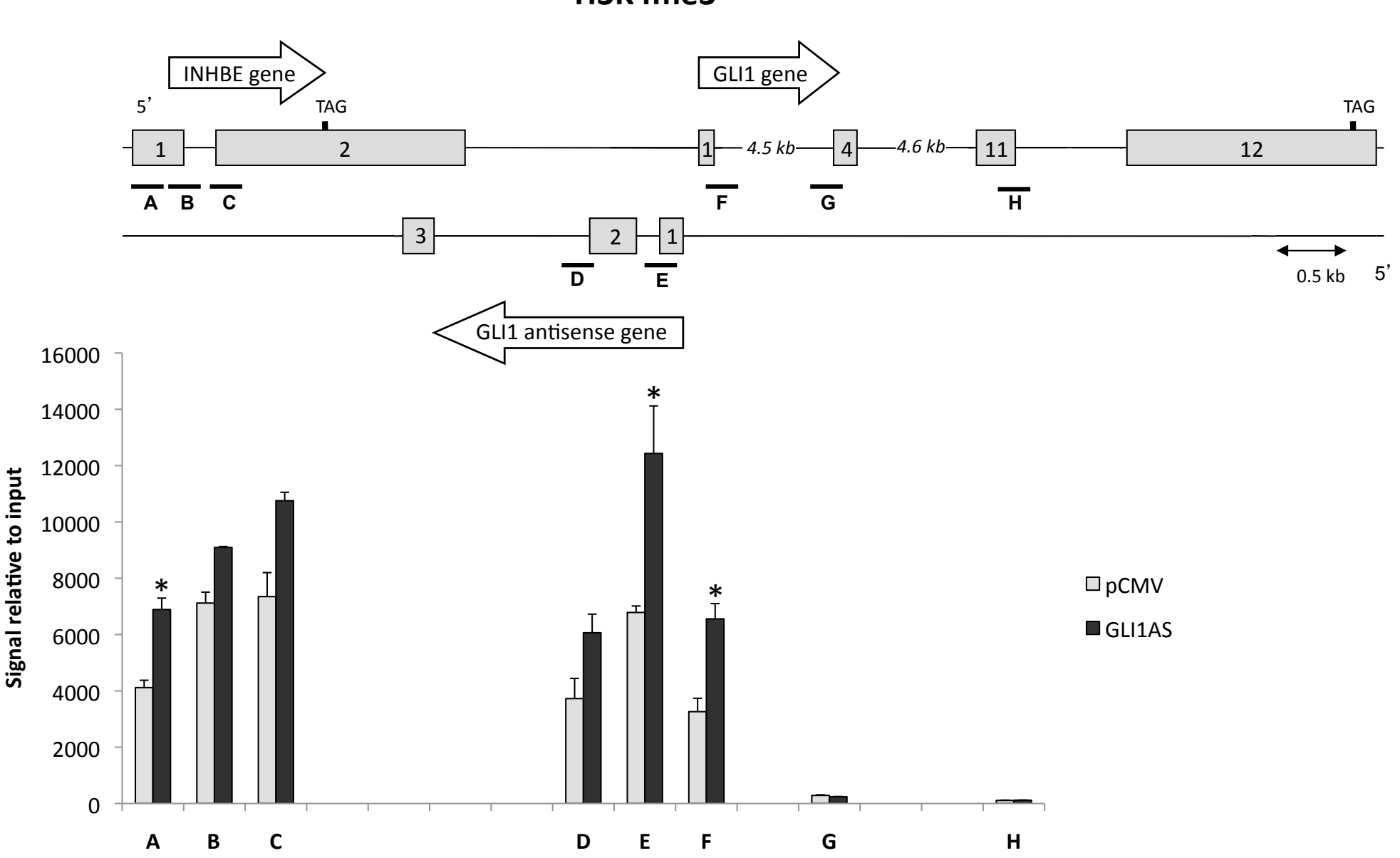


Supplementary figure 8



Supplementary figure 9



Supplementary table. Primers for RACE analysis

PRIMER NAME	SEQUENCE
Initial GLI1AS exons 1/2 forward	5’ CTGCTGTTGGCCTCACCCTTTGGA
Nested GLI1AS exons 2/3 forward	5’ CCCACTTAAAAGCCCAGGAGAAAGT
Initial GLI1AS exons 3/2 reverse	5’ CTTATACTTTCTCCTGGGCTTTTAAGTG
Nested GLI1AS exons 2/1 reverse	5’ AGGGTGAGGCCAACAGCAGCGTGT