

### Transcript sequence

CTCCATCGTCTACCTGGAGATTGACAACCGGCAGTGTGTGCAGGCCTCCTCGCAGTGCTTCCAG  
AGTGCCACCGATGTGGCCGCATTCTGGGAGCGCTCGCCTCGCTGGGCAGCCTCAACATCCCC  
TACAAGATCGAGGCCGTGCAGAGCGGTCACAGCTGCCGGCCACCTGGGAACGAGGCGGTCACA  
GCTGCCGGTGGAGCCACCTGGGAACGAGTCCAGCCACGGGAAAGTGAGACCGTGGAGCCGCC  
CCCGCCGGCGCAGCTGCACTTCATGTACGTGGCGGGCGGCCCTTTGTGCTTCTGTTCTTCGTG  
GGCTGCGGGGTGCTGCTGTCCCAGCAAGCGCCGGCGGCAGCATGGCCAGCTCTGGTTCCTGA  
GGGCTTC

Green – exon 27 (E27)

Brown – insertion

Blue – exon 28 (E28)

### Genomic sequence

CTCCATCGTCTACCTGGAGATTGACAACCGGCAGTGTGTGCAGGCCTCCTCGCAGTGCTTCCAG  
AGTGCCACCGACGTGGCCGCATTCTGGGAGCGCTCGCCTCGCTGGGCAGCCTCAACATCCCC  
TACAAGATCGAGGCCGTGCAGAG**GT**AAGTGTGGCCCCATCCCGGGAACAGGCTCTGCCTGCAGG  
GGGTGCCATCCCCCGTGCACCAGACACGCTGGCTGTTTGTGCCAGTTGCTACCCACGGGTGT  
I27a GAGCGTTGCCGTCCGAGTTGGGGTAGGGCTTTTCTGGAATTTTCTGAATGGCACTCCGCCCCCA  
CCTGC**AG**GCGGTCACAGCTGCCGGCCACCTGGGAACGAGGCGGTCACAGCTGCCGGTGGAGC  
CACCTGGGAACGAGTCCAGCCACGGGAA**GT**GGGTGCCTGCTTCTCTCCCCACCCTTTCCTCCT  
GAATTTTCTTTGTTGGGTATTATTTCAAATCATTACGGCTTTTTTAAAGAAAAAAAAAAGAGAGAG  
AGAGAAGAATTGATCGGTGTCATGTGAAGTGTGAAGTTTGTATCTTGAAAATCCCTCTAAATCCT  
TTGTCTTAACAGCTCAGTGCAGTGCAGCGATTGAAGTTGACTAATCCTCCTTCTTAAAGGAG  
I28a AAAAAAGTAAAAGCCGTCTCCAGATAGAGTCGGCTGGTGCAGGAGAGAATTTAGCGATAGTTTG  
CAATTCTGATTAATCGCGTAGAAAATGACCTTATTTTGGAGGGCGGGATGGAGGAAATGGGGTTT  
AAGAAGGCCCCCGGACGCGAAGCCAGTCCGCCGCCCCCGGCCACCAGCCTGCTGCGTAGCC  
GCTGCCTGATGTCCGGGCACCTGCCCTGGCCCCCGTGCCCGC**AG**GTGAGACCGTGGAGCCG  
CCCCGCCGGCGCAGCTGCACTTCATGTACGTGGCGGGCGGCCCTTTGTGCTTCTGTTCTTC  
GTGGGCTGCGGGGTGCTGCTGTCCCAGCAAGCGCCGGCGGCAGCATGGCCAGCTCTGGTTCCT  
TGAGGGCTTCAAAGTGTCTGAGGCCAGCAAGAAGAAGCGGCGGGAGCCCCTCGGCGAGGACTC  
CGTGGGCCTCAA

Green – exon 27 (E27)

Brown – exon 27a

Blue – exon 28 (E28)

I27a – intron 27a

I28a – intron 28a

**Bold** - splice donor and acceptor sites

### Protein sequence encoded by the variant splice form

SIVYLEIDNRQCVQASSQCFQSATDVA AFLGALASLGS LNIPYKIEAVQ**SGHSCRPPGNEAVTAAGGA**  
**TWERVQPRE**SETVEPPPPAQLHFMVVA AVEPPPPAQLHFMVVA A

Brown – inserted region

**Supplementary Figure 3.** Transcript, genomic and protein sequence of the NOTCH1 variant splice form identified in the ACCx11 PDX tumor.