

Supplementary Material

Clinical and functional characterization of a novel missense ELF2 variant in a CANVAS family

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Supplementary Figures

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ELF2_HUMAN      GGCAACCATCAGTGAACCTTTAATTC--ATTGATTAATAGCGTTTGAAGCTTCC-TCAG
ELF2_PANTRE     GGCAACCATCAGTGAACCTTTAATTC--ATTGATTAATAGCGTTTGAAGCTTCC-TCAG
ELF2_MOUSE      GGCAATCGTCTGTGAGCCTT-AGTTCC--GTTGCTTACAGCATTTGAGACTTCCTCAGG
ELF2_XENTR      -----
ELF2_DANRE      GT----GGGGTATGAGTCATGACTTCTGTGGTGCTTGTAGACAGTGGAGGAGCAGTGGT-

ELF2_HUMAN      GGAATAACAATGACATCAACAGTGGTTGACAGTGGAGGTACTATTTTGGAGCTTCCAGC
ELF2_PANTRE     GGAATAACAATGACATCAGCAGTGGTTGACAGTGGAGGTACTATTTTGGAGCTTCCAGC
ELF2_MOUSE      GAACGCACGATGGCATCGGCGGTGGTTGACAGCGGAGGCTCTGCTCTGGAGCTTCCTAGC
ELF2_XENTR      -----
ELF2_DANRE      ---GGAGTATGTC-ACTGCAGTCCATGACCATTTACCGGAGGAGGTTGGCGTGTATGAG

ELF2_HUMAN      AATGGAGTAGAAAATCAAGAGGAAAGTGAAGGTTTCTGAATATCCAGCAGTGATTGTG
ELF2_PANTRE     AATGGAGTAGAAAATCAAGAGGAAAGTGAAGGTTTCTGAATATCCAGCAGTGATTGTG
ELF2_MOUSE      GATGGAGGAGAAAATCAAGAGGGAGGTGACACGGGCCCTGACTGCCCGGCAGTGATTGTG
ELF2_XENTR      -----
ELF2_DANRE      GTGGAGGGTGAATGGAGGGAGAGGTGGAGGGCGATGTGGAGTATCCAGCAGTGATTGTG

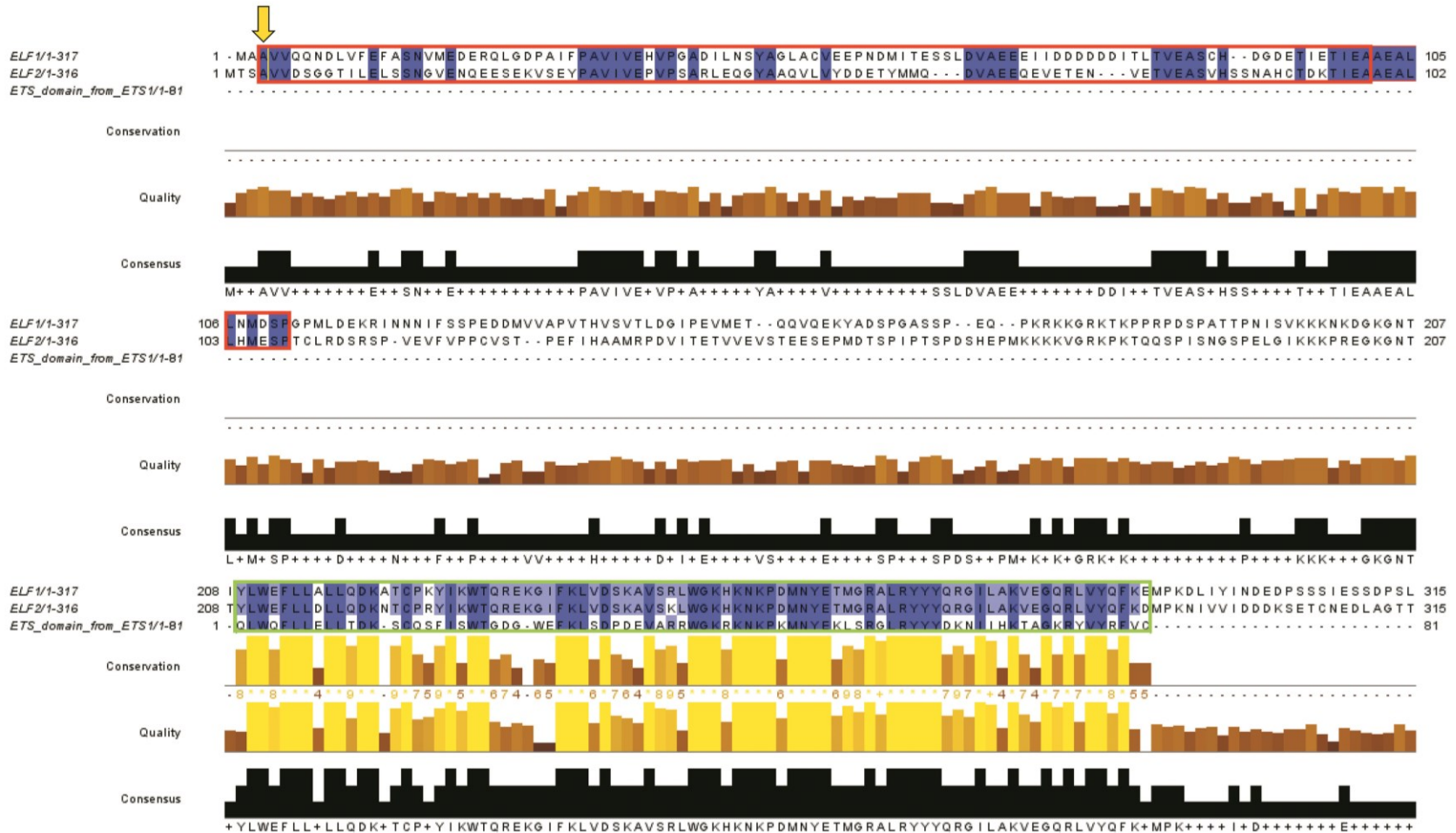
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ELF2_MOUSE      GAGCCAGTTCCCAGTGCAGGTTAGAGCAGGGCTATGCTGCCAGGTGCTGGTCTATGAC
ELF2_XENTR      -----GTCTGAATCTCCGTTCC---TCCTCTCTCCTGCCAATGTGAAGCTGAACCTC
ELF2_DANRE      GAGCCAGTGCCAGCGCGGATGGAGCAAGGCTTGTCTGCTCAGGTGCTGGTGTACGAT
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ELF2_HUMAN      GATGAGACTTATATGATGCAAGATGTGG---CAGAAGAACAAGAAGTTGAGACCGAGAAT
ELF2_PANTRE     GATGAGACTTATATGATGCAAGATGTGG---CAGAAGAACAAGAAGTTGAGACCGAGAAT
ELF2_MOUSE      GACGAGACTTATATGATGCAAGATGTGG---CGGAAGAACAAGAAGTTGAGACCGAGAAT
ELF2_XENTR      CGGGAT--AAATATGGCGACTTCGCTGCATGAGGGACCTACCAACCAGTTGATCTGCTC
ELF2_DANRE      GATGAGACATACCTGATGCAGGGAGTAGCTGAGG---AGCAGGAGGTGGAGACAGAGGTG
                ** * ** * * * * *

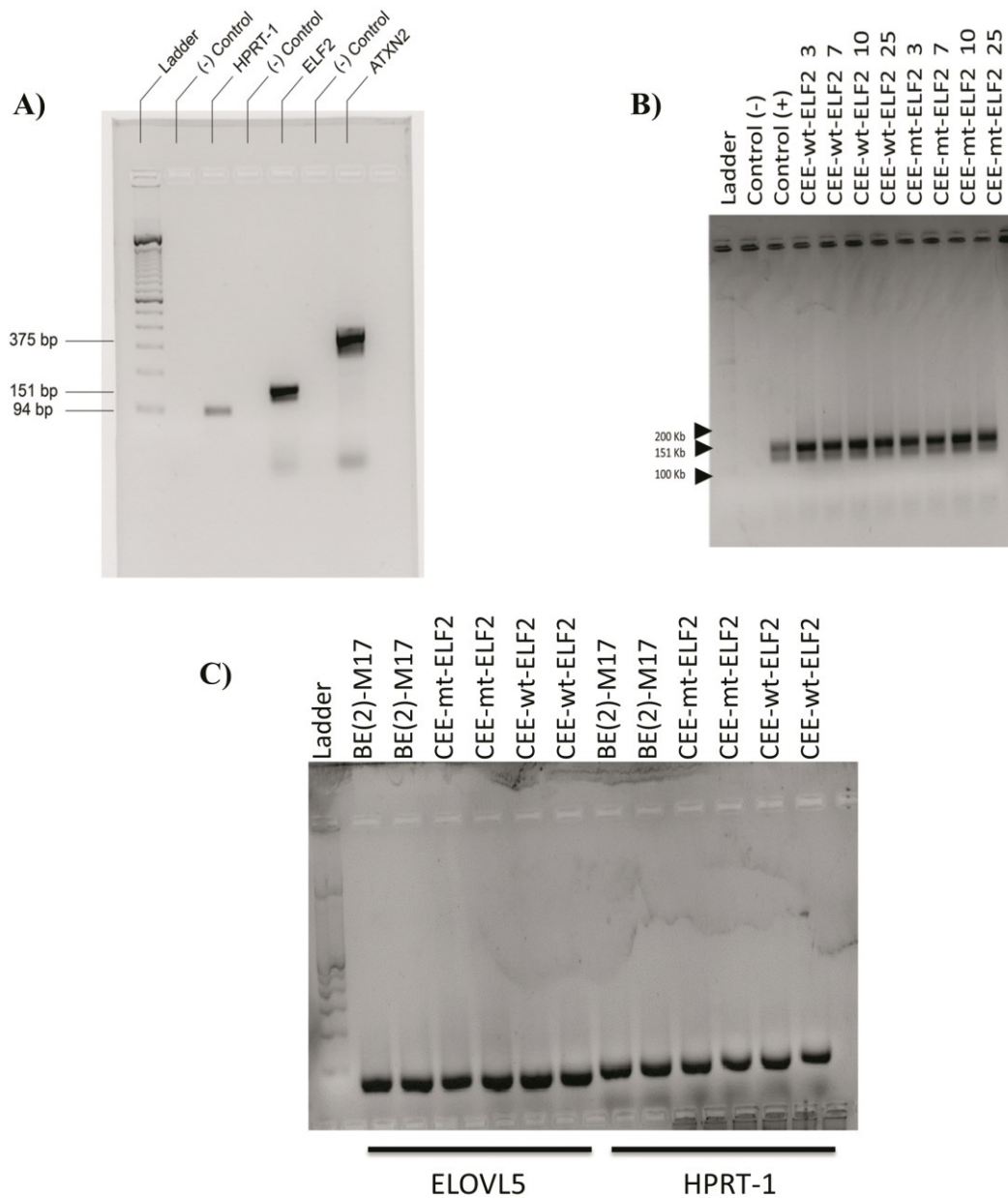
ELF2_HUMAN      GTGGAACAGTGAAGCATCAGTTCACAGCAGTAATGCACACTGTACAGATAAGACAATT
ELF2_PANTRE     GTGGAACAGTGAAGCATCAGTTCATAGCAGTAATGCACACTGTACAGATAAGACAATT
ELF2_MOUSE      TCAGAAACAGTGAAGCATCAGTTCACAGCAGTAATGCTCACTGTACGGATAAGACCATT
ELF2_XENTR      ATCAGAGCCGTGGAAGCATCGGTTTCATGGCAGCAATGTTCACTGCACGGATAAGACTATT
ELF2_DANRE      CTGGAGACAGTGAAGCATCAGTTC-----ATGGTGTGCAATGCTCTGATAAAACCATC
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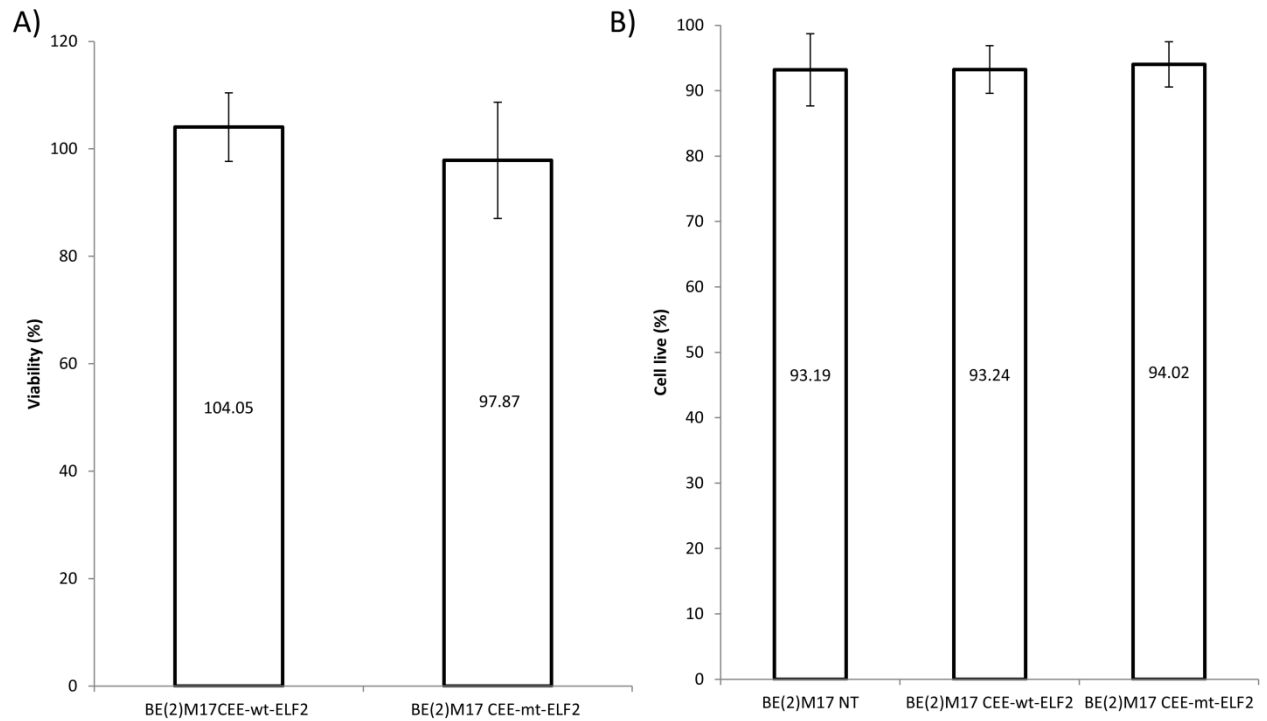
Supplementary Figure 1. ELF2 gene DNA sequence compared across species showing the base change. The ELF2 gene is conserved in human, chimpanzee, dog, cow, rat, chicken, and zebrafish. The variant chr4: g.140058846 C>T, c.10A>G.



Supplementary Figure 3. Alignment between ELF2 protein sequence, ELF1 protein sequence and ETS domain sequence from ETS1 protein sequence. Alignment was made with ClustalO tool and managed in JalView Software. The relevant position in our study is marked with a yellow arrow at the beginning of the alignment (p.Ala4). This position is highly conserved between three sequences. ETS domain sequence alignment is marked in green box. ELF-1 transcription binding domain is marked in a red box. Highly conserved amino acids between sequences in both domains are highlighted in dark blue.



Supplementary Figure 4. A) Constitutive expression of ELF2 and ATXN2 genes in the neuroblastoma cell-line BE (2) M17. B) PCR of CEE-mt-ELF2 and CEE-wt-ELF2 in BE(2)M17 cells over time. Vector remained stable at day 3, 7, 10 and 25 after transduction. C) Constitutive expression of ELOVL5 and HPRT1 genes in BE (2) M17 cells.



Supplementary Figure 5. Viability after transduction. The cell-line BE(2)M17 was transduced with CEE-mt-ELF2 and CEE-wt-ELF. Proliferation and survival rate were measured at 24h, 48h, 3, 7, 10 and 14 days. Viability after transduction was measured by A) mitochondrial activity (WST-1 assay) and B) percentage of live and dead cells.