

Supplementary Material

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

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Supplementary Figures

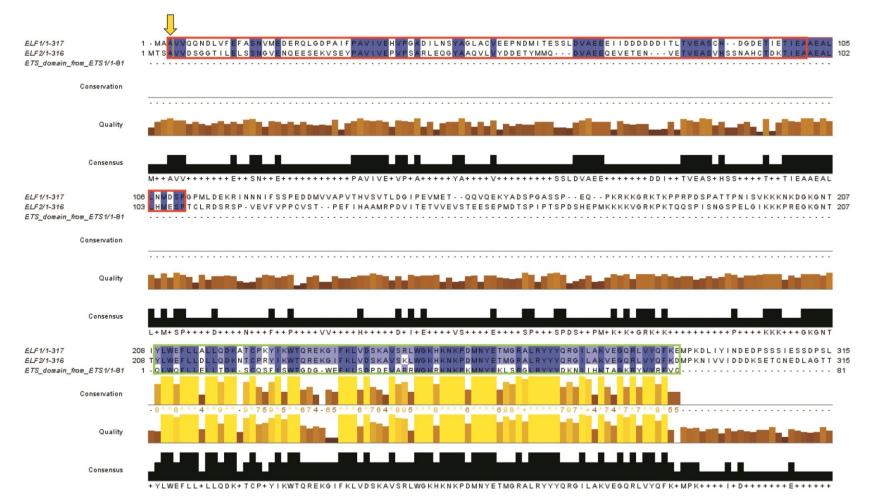
ELF2_HUMAN ELF2_PANTRE ELF2_MOUSE ELF2_XENTR ELF2_DANRE	GGCAACCATCAGTGAACCTTTAATTTCATTGATTAATAGCGTTTGAAGCTTCC-TCAG GGCAACCATCAGTGAACCTTTAATTTCATTGATTAATAGCGTTTGAAGCTTCC-TCAG GGCAATCGTCTGTGAGCCTT-AGTTCCGTTGCTTCACAGCATTTGAGACTTCCTCAGG GGGGTATGAGTCATGACTTCTGTGGTGCTTGTAGACAGTGGAGGAGCAGTGGT-
ELF2_HUMAN ELF2_PANTRE ELF2_MOUSE ELF2_XENTR ELF2_DANRE	GGAATAACAATGACATCA <mark>A</mark> CAGTGGTTGACAGTGGAGGTACTATTTTGGAGCTTTCCAGC GGAATAACAATGACATCAGCAGTGGTTGACAGTGGAGGTACTATTTTGGAGCTTTCCAGC GAACGC <mark>ACGATGGCATC</mark> GG <mark>QGGTGGTTGAC</mark> AGCGGAGGCTCTGCTCTGGAGCTTCCTAGC GG <mark>A</mark> GT <mark>ATG</mark> TC-ACT <mark>GCAGT</mark> CG <mark>ATGAC</mark> CATTTACCGGAGGAGGGTGGCGTGTATGAG
ELF2_HUMAN ELF2_PANTRE ELF2_MOUSE ELF2_XENTR ELF2_DANRE	AATGGAGTAGAAAATCAAGAGGAAAGTGAAAAGGTTTCTGAATATCCAGCAGTGATTGTG AATGGAGTAGAAAATCAAGAGGAAAGTGAAAAGGTTTCTGAATATCCAGCAGTGATTGTG GATGGAGGAGAAAATCAAGAGGGAGGTGACACGGGCCCTGACTGCCCGGCAGTGATTGTG GTGGAGGGTGAAATGGAGGGAGAGGTGGAGGGCGATGTGGAGTATCCAGCAGTGATTGTG
ELF2_HUMAN ELF2_PANTRE ELF2_MOUSE ELF2_XENTR ELF2_DANRE	GAGCCAGTTCCAAGTGCCAGATTAGAGCAGGGCTATGCAGCCCAGGTTCTGGTTTATGAT GAGCCAGTTCCAAGTGCCAGATTAGAGCAGGGCTATGCAGCCCAGGTTCTGGTTTATGAT GAGCCAGTTCCCAGTGCGAGGTTAGAGCAGGGCTATGCTGCCCAGGTGCTGGTCTATGAC GTCTGAATCTCCGCTTCCTCCTCTCTCCTGCCAATGTGAAGCTGAACCTC GAGCCAGTGCCCAGCGCGGGGGGGGGG
ELF2_HUMAN ELF2_PANTRE ELF2_MOUSE ELF2_XENTR ELF2_DANRE	GATGAGACTTATATGATGCAAGATGTGGCAGAAGAACAAGAAGTTGAGACCGAGAAT GATGAGACTTATATGATGCAAGATGTGGCAGAAGAACAAGAAGTTGAGACCGAGAAT GACGAGACTTATATGATGCAGGATGTGGCGGAAGAACAGGAAGTTGAGACGGAGAAT CGGGATAAATATGGCGACTTCGCTGCATGAGGGGACCTACCAACCA
ELF2_HUMAN ELF2_PANTRE ELF2_MOUSE ELF2_XENTR ELF2_DANRE	GTGGAAACAGTGGAAGCATCAGTTCACAGCAGTAATGCACACTGTACAGATAAGACAATT GTGGAAACAGTGGAAGCATCAGTTCATAGCAGTAATGCACACTGTACAGATAAGACAATT TCAGAAACAGTGGAAGCATCAGTTCACAGCAGTAATGCTCACTGTACGGATAAGACCATT ATCAGAGCCGTGGAAGCATCGGTTCATGGCAGCAATGTTCACTGCACGGATAAGACTATT CTGGAGACAGTGGAAGCATCAGTTCATGGTGTGCAATGCTCTGATAAAACCATC * ********** *** ***

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.

SP|Q15723|ELF2_HUMAN TR|H2QQ65|H2QQ65_PANTR SP|Q9JHC9|ELF2_MOUSE MAIA_VDSGGTILELSSNGVENQEES-EKVSEYPAVIVEPVPSARLEQGYAAQVLVYDDE SP|Q9JHC9|ELF2_MOUSE MAIA_VDSGGSALELPSDGGENQEGG-DTGPDCPAVIVEPVPSARLEQGYAAQVLVYDDE S9 TR|Q5XHJ7|Q5XHJ7_XENLA MT VDGGGSVGEFVSNGVEKLEQEEVQVTEYPAVIVEPVPSARLEQGYAAQVLVYDDE 60 TR Q6PHH1 Q6PHH1 DANRE SP|Q15723|ELF2_HUMAN TYMMQDVAEEQE-VETENVETVEASVHSSNAHCTDKTIEAAEALLHMESPTCLRDSRSPV 118 TR H2QQ65 H2QQ65_PANTR TYMMQDVAEEQE-VETENVETVEASVHSSNAHCTDKTIEAAEALLHMESPTCLRDSRS-- 116 SP|Q9JHC9|ELF2 MOUSE TYMMODVAEEQE-VETENSETVEASVHSSNAHCTDKTIEAAEALLHMESPTCLRDSRSPV 118 TR 05XHJ7 05XHJ7 XENLA TFMMODVAEEHE-VETESVEIVEASVHGSNVHCTDKTIEAAEALLHMESPTILRDTRSPV 119 TR|Q6PHH1|Q6PHH1_DANRE --MATSLHEGPANQLDLLIRAVEASVH--GVQCSDKTIEAAEALLHMDSPSSLRGDRSP- 55 * * * * * * * * * * * * * * * * * * ***** .: * SP|Q15723|ELF2 HUMAN EVFVPPCVSTPEFIHAAMRPDVITETVVEVSTEESEPMDTSPIPTSPDS--HEPMKKKKV 176 TR | H2QQ65 | H2QQ65_PANTR ------PEFIHAAMRPDVITETVVEVSTEESEPMDTSPIPTSPDS--HEPMKKKKV 164 EVFVPPCISTPEFIHAAMRPDVITETVVEVSTEESEPMDASPIPTSPDS--HEPMKKKKV 176 SP|Q9JHC9|ELF2_MOUSE TR|Q5XHJ7|Q5XHJ7_XENLA EVYVSPCVSTPEFIHAAMRPDVITETVVEVSTEDSEPMDSTSIPISIEL--GEPMKKKKA 177 TR 06PHH1 06PHH1 DANRE EVFVPPCVNTSEFLHAAMRPDVLTETVVEVSTEDMEPMEVVTVIQEPEMLDTEPSKRRKS 115 : . : SP|Q15723|ELF2_HUMAN GRKPKTQQSPISNGSPELGIKKKPREGKGNTTYLWEFLLDLLQDKNTCPRYIKWTQREKG 236 TR H2QQ65 H2QQ65_PANTR GRKPKTQQSPISNGSPELGIKKKPREGKGNTTYLWEFLLDLLQDKNTCPRYIKWTQREKG 224 SP|Q9JHC9|ELF2 MOUSE GRKPKTQQSPVSNGSPELGIKKKAREGKGNTTYLWEFLLDLLQDKNTCPRYIKWTQREKG 236 TR|Q5XHJ7|Q5XHJ7_XENLA GRKPKMQ0PAFSNGSPELGIKKKQREGKGNTTYLWEFLLDLLQDKNTCPRYIKWTQREKG 237 TR|Q6PHH1|Q6PHH1_DANRE_GRKPKP--HHISNGSPDMGIKKKSREGKGS-TYLWEFLLDLLQDKNTCPRYIKWTQREKG_172 ***** ***** SP|Q15723|ELF2_HUMAN IFKLVDSKAVSKLWGKHKNKPDMNYETMGRALRYYYQRGILAKVEGQRLVYQFKDMPKNI 296 TR H20065 H20065 PANTR IFKLVDSKAVSKLWGKHKNKPDMNYETMGRALRYYYORGILAKVEGORLVYOFKDMPKNI 284 SP|Q9JHC9|ELF2_MOUSE IFKLVDSKAVSKLWGKHKNKPDMNYETMGRALRYYYORGILAKVEGORLVYOFKDMPKNI 296 TR|Q5XHJ7|Q5XHJ7_XENLA IFKLVDSKAVSRLWGKHKNKPDMNYETMGRALRYYYQRGILAKVEGQRLVYQFKEMPKNI 297 TR|Q6PHH1|Q6PHH1_DANRE IFKLVDSKAVSKLWGKHKNKPDMNYETMGRALRYYYQRGILAKVEGQRLVYQFKEMPKDI 232 ******* WIDDDKSETCNEDLAGTTDEKSLERVSLSAESLLKAASSV------RSGKNSSPI 346 SP|015723|ELF2 HUMAN TR H20065 H20065 PANTR VVIDDDKSETCNEDLAGTTDEKSLERVSLSAESLLKAASSV------RSGKNSSPI 334 SP|Q9JHC9|ELF2_MOUSE WIDDDKSETCPEDLAAAADDKSLERVSLSAESLLKAATAV-----RGGKNSSPL 346 TR 05XHJ7 05XHJ7_XENLA VVIEEDKAEPCSEEMVSPIDDKSLERVSIPTEGIMKMAMPA------RLEKSPTK0 347 TR|Q6PHH1|Q6PHH1_DANRE_VVIDDDKCDPGDDVI----GEKTYERVPPSSDTLLTDLSKTPTILRAADRTVLHPGSPKA_288 . . * . *** * • • * * • : : :: ::. SP|Q15723|ELF2_HUMAN NCSRAEKGVARVVNITSPGHDASSRSPTTTASVSATAAPRTVRVAMQVPVVMT-SLGQKI 405 TR H2QQ65 H2QQ65_PANTR NCSRAEKGVARVVNITSPGHDASSRSPTTTASVSATAAPRTVRVAMQVPVVMT-SLGQKI 393 SP|09JHC9|ELF2 MOUSE NCSRAEKGVARVVNITSPTHDGSSRSPTTTAPVSAAAAPRTVRVAMOVPVVMT-SLGOKI 405 TR|Q5XHJ7|Q5XHJ7_XENLA SSNR----KPRLVSWSCTANEASPHS-----PTSTTPTQRTVRVAMQVPVVMT-SLGQKI 397 TR Q6PHH1 Q6PHH1 DANRE KAALSATPVQRTV------MVSTADPSHATIISNANAPRTVRVAMQVPVVMTNSLGQKI 341 * • • ******** STVAVQSVNA--GAPL-ITSTSPTTATSPKVVIQTIPTVMPASTENGDKITMQPAKIITI 462 SP|015723|ELF2 HUMAN TR H2QQ65 H2QQ65_PANTR STVAVQSVNA--GAPL-ITSTSPTTATSPKVVIQTIPTVMPASTENGDKITMQPAKIITI 450 SP|Q9JHC9|ELF2_MOUSE SAVAVQSVNAGTGSPL-ITSTSPASASSPKVVIQTVPTVMPASTENGDRITMQPAKIITI 464 TR Q5XHJ7 Q5XHJ7_XENLA STLAVQSMNP--GSPL-IANSSPTTASAPKVVIQTIPTMLPASSESGDKFTMQPAKIITI 454 TR 06PHH1 06PHH1 DANRE STVAVQSTNPSLLTTAPTNTGSPTGTNAPKVLIQTMPTMVPATAENGDKITVQLAKIITI 401 * . . * * * * **: : *** *** *** *** *** *** : SP|Q15723|ELF2_HUMAN PATQLAQCQLQTKSNLTGSGSINIVGTPLAVRALTPVSIAHGTPVMRLSMPTQ-QASG-- 519 TR H2QQ65 H2QQ65_PANTR PATQLAQCQLQTKSNLTGSGSINIVGTPLAVRALTPVSIAHGTPVMRLSMPTQ-QASG-- 507 SP|Q9JHC9|ELF2_MOUSE PATQLAQCQLQAKSNLTGSGSINIVGTPLAVRALTPVSIAHGTPVMRLSVPAQ-QASG-- 521 TR|Q5XHJ7|Q5XHJ7_XENLA PTSQLTQCQLQTKPGLSGSGGLNLVGTPLTVRALTPVSIAHGTPVMRLAMPAAHQARC-- 512 TR|Q6PHH1|Q6PHH1_DANRE PATQLTQCQLQAKPGT--PTGINLMGAPLTVRALTPMSVAPGTQVVRLAVPAQQSPVQAK 459 * • • * * • * * * * * • * * • * * • • * • SP|Q15723|ELF2_HUMAN -QTPPRVISAVIKGPE-VKSEAVAKK--QEHDVKTLQLVE-EKPADGNKTVTHVVVVSAP 574 TR/H20065/H20065 PANTR -OTPPRVISAVIKGPE-VKSEAVAKK--OEHDVKTLOLVE-EKPADGNKTVTHVVVVSAP 562 SP|Q9JHC9|ELF2_MOUSE -QTPPRVISALLKGPE-GKSE--AKK--QEHDVKTLQLVE-EKGADGNKTVTHVVVSAP 574 TR|Q5XHJ7|Q5XHJ7_XENLA -QTPPRVISTLIKGPE-IKSDMVVGR--QEGELKTFQFIKDENPADGGKTVTHVVVVSTP 568

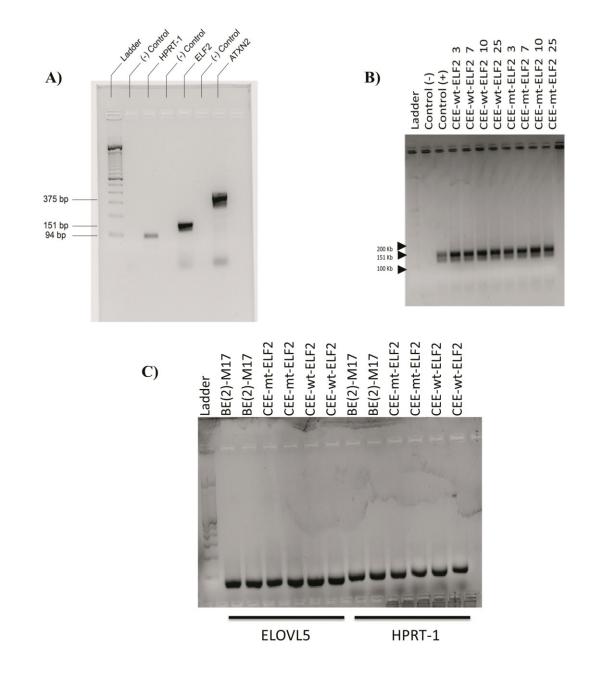
Supplementary Figure 2. elf-2 protein sequence compared across species showing the amino acid change. The elf-2 gene is conserved in human, chimpanzee, dog, cow, rat, chicken, and zebrafish. The position p.Ala4 is indicated with an arrow.





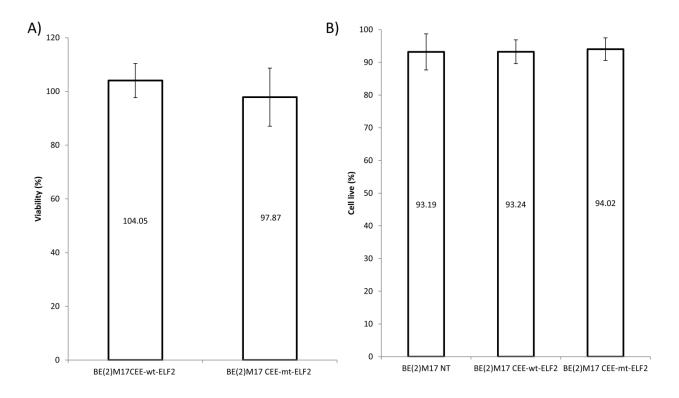
Supplementary Figure 3. Alignment between ELF2 protein sequence, ELF1 protein sequence and ETS domain sequence from ETS1 protein sequence. Alignment was made with ClustalO tool and managed in JalView Software. The relevant position in our study is marked with a yellow arrow at the beginning of the alignment (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 measure by A) mitochondrial activity (WST-1 assay) and B) percentage of live and dead cells.