SUPPLEMENTARY MATERIALS AND METHODS

Construction of the FE65 and FE65L1 targeting vectors

FE65 and FE65L1 targeting vectors were constructed by cloning two fragments of isogenic murine genomic DNA for each gene into restriction enzyme sites flanking a pol2neo cassette and a lacZ fused to a nuclear localization signal. A genomic DNA fragment of 8.77 kb starting and ending with the following sequences 5’-TGCAGCCAGTGATGAGGACTCAAGC… and … TTCCAAGTCTATTACCTGGGAAATGT-3’ and a 0.96 kb fragment starting and ending with 5’-TGTGTCCCTGGCACAGTGAGCCCC and … CCTCCTATTTGGCATGCGAAACAG-3’ corresponding to the FE65 gene were amplified from 129SV mouse liver DNA and cloned into the targeting vector backbone. Similarly, a 1.05 kb genomic DNA fragment starting and ending with the following sequences 5’-GAGATCACATGGGTCTGGATGGTC… and …GTTGGCACCCTGGCAGTTATG-3’ and a 6.72 kb genomic DNA fragment starting and ending with 5’-GATCCAGAACCTGGCACCAAGTGA… and …AGAGACTGCCATAGCCAGGGATCC -3’ corresponding to the FE65L1 gene were amplified from genomic DNA by PCR and cloned into the targeting vector backbone. For the FE65L1 gene the short arm was inserted upstream of a reading frame coding for the tau-LacZ fusion protein. The targeting vector backbones also contained two copies of the herpes simplex virus thymidine kinase gene allowing for negative selection of randomly integrated plasmids.

Detection of targeting vector homologous integration in the mouse genome

Linearized targeting vectors were electroporated into SM1 murine embryonic stem (ES) cells. Positive selection for ES clones bearing integrated targeting vector sequence was achieved with G418 (250 µg/ml) and negative selection against random integration was performed with ganciclovir (2.5 µM).
Homologous recombination of the targeting vector in the SM1 murine embryonic stem cell genome was detected by PCR. For detection of site specific integration at the FE65 locus, a primer in the genomic DNA upstream of the 0.96 kb FE65 fragment, 5’-GCAATGCAGTGGTTAAGGAAGGC-3’ and a primer recognizing the LacZ sequence in the targeting vector, 5’-CGTTGCAACCAGATGAAACGCCAGG-3’ was used to detect site specific integration. For detection of site specific integration at the FE65L1 locus, a primer in the genomic DNA upstream of the 1.1 kb FE65L1 fragment, 5’ GCCTGACCAGCGTACAGCCC-3’, and a primer recognizing the tau promoter sequence of the tauP-lacZ fusion, 5’CTCCTGGTCTTGCAGGTTAGCTTCC-3’ were used for PCR.

Southern blot analyses were performed on mouse tail genomic DNA to confirm the establishment of homologous recombination at the FE65 and FE65L1 loci. Wild type, FE65+/- and FE65−/− genomic DNAs were digested with PstI and probed with a 500 bp probe, generated by PCR from the region upstream of the 0.96 kb FE65 genomic fragment in the targeting vector using the primer pair 5’-ATTTCTGGGCTCCTGCTTCTTG-3’ and 5’-TAAGCCCACTCTACACTGGGACTAG-3’.

Wild type, FE65L1+/- and FE65L1−/− genomic DNAs were digested with NheI and probed with a 1.0 kb probe, generated by PCR from the region upstream of the 1.1 kb FE65L1 genomic fragment in the targeting vector using the primer pair 5’-GCTTCTTAGTCCCTTTCTTTACCTACTC-3’ and GAGCTGTCACAGCTTCTTAGCTTCCATTC-3’.

**Establishment of FE65+−, FE65L1−− and FE65+−;FE65L1−− mice**

Germline chimeras were derived according to standard protocols from two ES clones for each targeted gene (Willnow and Herz, 1994) and F1 heterozygous animals were mated to generate homozygous knockout animals. Compound knockouts were generated by crossing FE65−/− and FE65L1−/− mice, and their FE65+−;FE65L1+− progeny were crossed to generate mutants with three or four targeted alleles.
Subsequent matings to obtain FE65\textsuperscript{\textminus/\textminus};FE65L1\textsuperscript{\textminus/\textminus} mice or embryos were established between the mutants with three targeted alleles or between mutant females with three targeted alleles and FE65\textsuperscript{\textminus/\textminus};FE65L1\textsuperscript{\textminus/\textminus} male mice. The mice were maintained on a hybrid genetic strain background (C57BL/6 x 129SvEvBradley).

**Western Blot Analyses**

Brains were dissociated in 10 mM Tris pH 8.0, 140 mM NaCl, 5 mM EDTA and 1% Triton X-100 containing Complete EDTA-free protease inhibitor (Roche, Indianapolis, IN) using a tissue homogenizer and extracts were centrifuged 15 min at 12,000g. Protein concentrations of total cell lysates were determined by the BCA protein assay (Pierce, Rockford, IL). Proteins were subjected to electrophoresis on Tris-Glycine acrylamide gels, transferred to PVDF membrane (Millipore) and primary antibody incubations were followed by incubation with HRP-conjugated secondary antibody (Amersham Biosci.) followed by ECL (LumiGlo, Kirkegaard & Perry Laboratories, Inc.).

**In situ hybridization**

Probes specific for each of the FE65 gene family members were generated by PCR amplification of mouse DNA and cloned into pCR-II-Topo cloning vector (Invitrogen). The probes for FE65 (1013), FE65L1 (1014), and FE65L2 (1015) were generated using the following primers: 5’-AGCTGGGCCACCTTATCACAGG-3' and 5'-GGGCCAGCTCCTCTCGGTATC-3'; 5’-CCATGTTGCGGTATCAGAAGTGC -3' and 5'-AGTTGGCTAGGAAGGGTGTGGCCGC-3'; 5’-CCAGGAAGGGGAACAGAGGGG -3' and 5'-TGGCTTTTTCGCGGAACCATCACAGTG-3', respectively. Labeling reactions were carried out on all three templates (300 ng) using T7 RNA polymerase on templates linearized with HindIII for the sense probe or with SP6 RNA polymerase on
templates linearized with NotI for the antisense probe using 50 µCi [α-35S]-UTP (Amersham, Piscataway, NJ) and the MAXIscript in vitro transcription kit (Ambion, Austin, TX). Labeled probes were purified on NucAway Spin Columns (Ambion, Austin, TX). The expression patterns for FE65, FE65L1 and FE65L2 were determined in adult WT brains. FE65 expression was also examined in the FE65L1−/− brain, FE65L1 expression in the FE65−/− and FE65L2 expression in the FE65−/−;FE65L1−/− brain to determine whether expression levels of the different family members were altered to compensate for the absence of one or more of its homologs. In situ hybridization was performed on paraformaldehyde fixed tissue that was paraffin-embedded as previously described by Shelton et al. (2000; J Lipid Res 41, 532-7).