

The American Journal of Human Genetics, Volume 99

Supplemental Data

**Epileptic Encephalopathy Caused by Mutations
in the Guanine Nucleotide Exchange Factor *DENND5A***

Chanshuai Han, Reem Alkhater, Tawfiq Froukh, Arakel G. Minassian, Melissa Galati, Rui Han Liu, Maryam Fotouhi, Julia Sommerfeld, Ayman J. Albrook, Christian Marshall, Susan Walker, Peter Bauer, Stephen W. Scherer, Olaf Riess, Rebecca Buchert, Berge A. Minassian, and Peter S. McPherson

Supplemental Figures

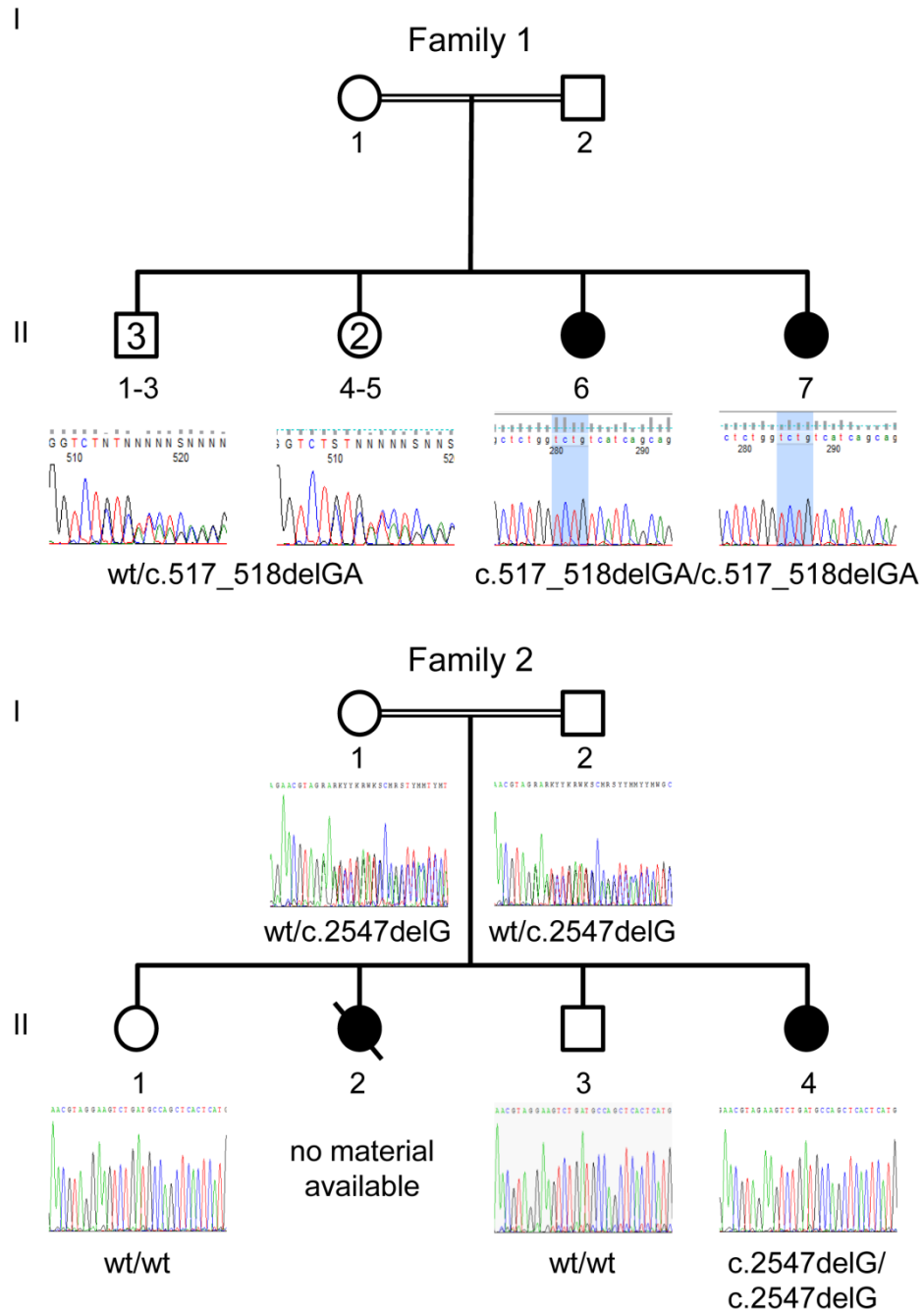


Figure S1: Sanger sequence traces of affected and non-affected family members

Sequence traces of affected and unaffected heterozygote siblings (Family 1) and heterozygote parents and wild-type siblings (Family 2).

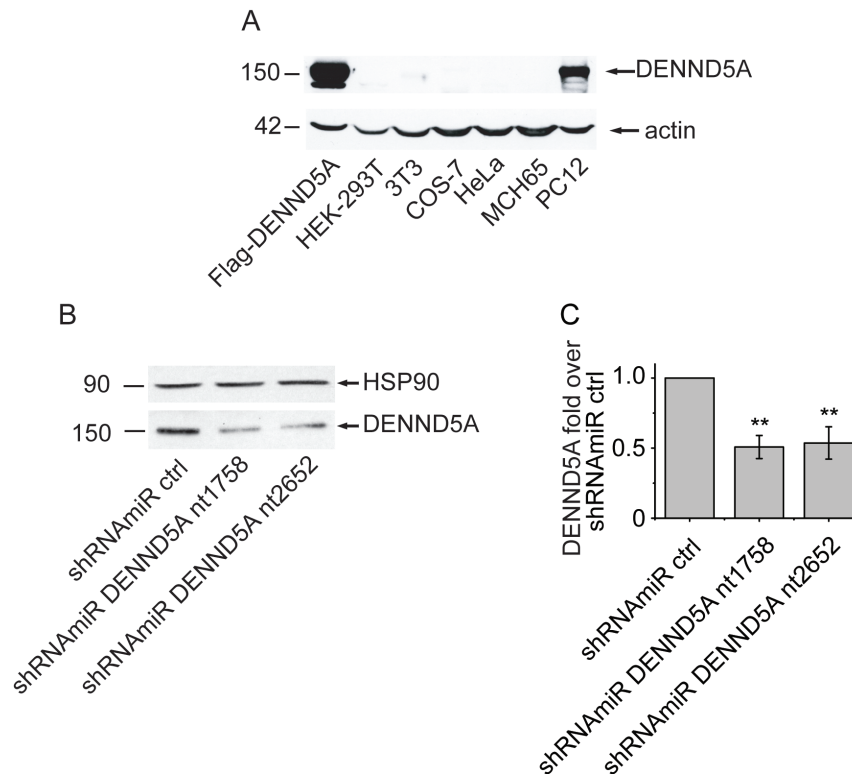


Figure S2: Knockdown of DENND5A

(A) Equal protein aliquots of lysates from cell lines were blotted with antibodies recognizing DENND5A (polyclonal antibody, Abcam) and actin (monoclonal antibody C4, Millipore) as indicated. Lysates from HEK-293T expressing Flag-tagged DENND5A (Flag-DENND5A) were included as a control. (B) PC12 cells were transduced with control lentivirus (shRNAmiR ctrl) or lentivirus to knockdown DENND5A (shRNAmiR DENND5A nt1758, shRNAmiR DENND5A nt2652). Equal protein aliquots of lysates from transduced PC12 cells were processed by Western blot for DENND5A and HSP90. (C) Quantification of DENND5A levels from experiments as in B normalized to HSP90 and expressed relative to the shRNAmiR ctrl cells. Error bars represent s.e.m. and statistical analysis employed a repeated-measure one-way ANOVA followed by a Dunnett's post-test. **, $p < 0.01$. N=5.

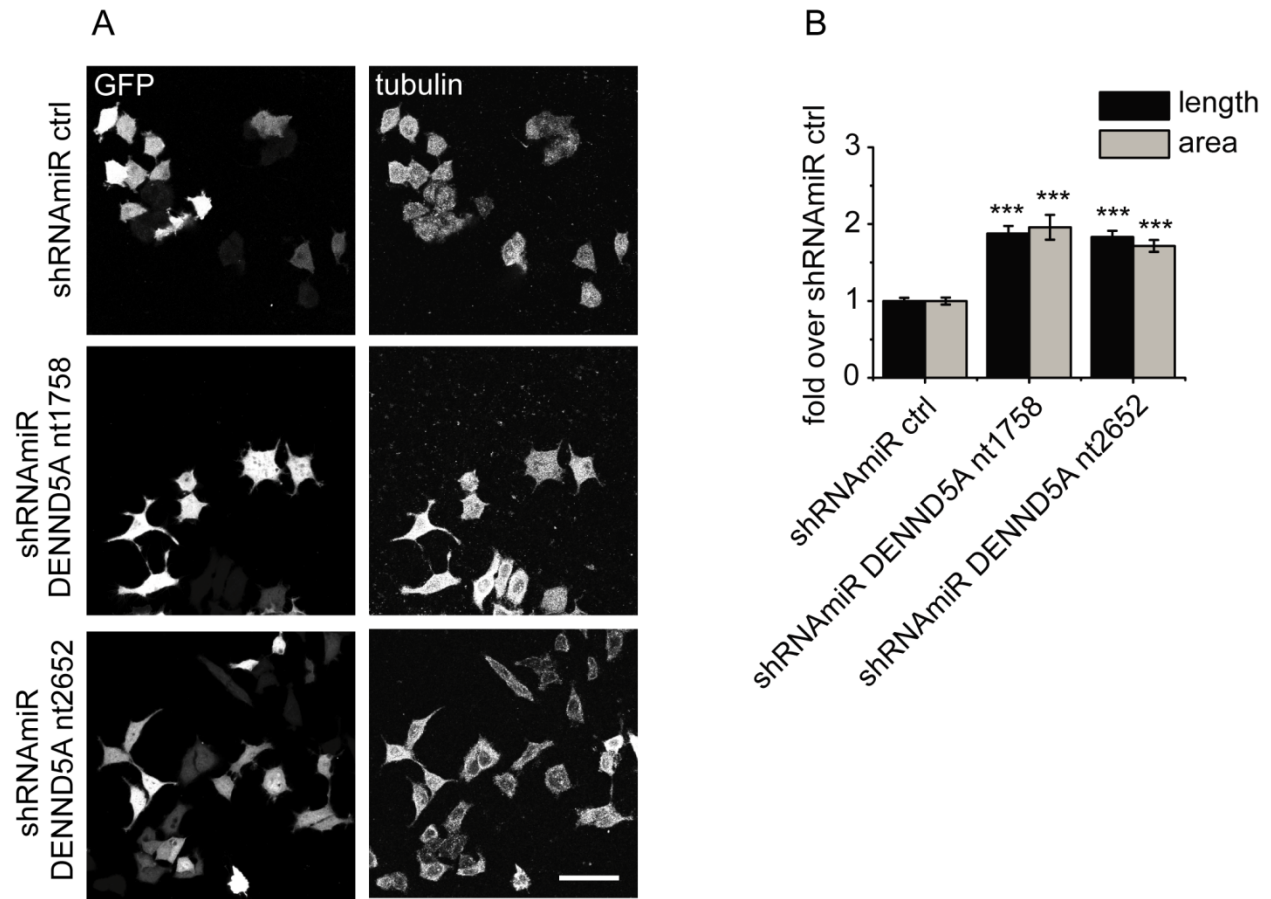


Figure S3: Knockdown of DENND5A enhances neurite outgrowth in the absence of NGF

(A) PC12 cells were transduced with control lentivirus (shRNA miR ctrl) or lentivirus to knockdown DENND5A (shRNA miR DENND5A nt1758, shRNA miR DENND5A nt2652). Transduced PC12 cells were serum starved for 24 h, fixed and processed for GFP fluorescence and for indirect immunofluorescence with antibody against tubulin. GFP is expressed as part of the viral expression cassette to verify transduction. The bar represents 50 μ m. (B) Quantification of length of the longest neurite or protrusion (length) and total area of neurites (area) for each cell from representative images as in A. The data show the mean \pm s.e.m. from two independent experiments measuring 30 cells per condition per experiment; a repeated-measure one-way ANOVA followed by a Dunnett's post-test revealed a significant difference between control and DENND5A knockdown cells. ***, $p < 0.001$.