Figure S2.

Determination of the GISP start codon. A, Met 1 indicates the first methionine of predicted open reading frame. Met2, Met 3 and Met 4 indicate the successive methionines in the GISP sequence. Immunogen is the protein used to raise the anti-GISP antibody. B, Deletion mutant GISP constructs starting from the different potential start codons indicated in A were transfected into HEK cells resolved on 5% gel and analysed by immunoblotting with anti-GISP antibody. M1 to M4, methionine 1 to 4; BE, rat brain extract. C, Mapping the GISP antibody recognition site. GST fusion proteins of GISP peptides encompassing the antigen sequence used to raise the GISP antibody. D, The purified peptides shown in C were tested for blockade of GISP antibody binding to the M1 protein illustrated in A and brain extract (BE). The preincubation with the 47-103 peptide prevented GISP antibody binding.

