Supplementary methods

TRIM46 cell based assay

COS7 cells transfected with plasma encoding full-length GFP tagged human TRIM46 (Clone ID, OHu04326C; vector, pcDNA3.1(+)-N-eGFP; GenScript). Post-transfection COS7 cells were incubated for 16-24 hours (37 degrees Celsius, in humidified atmosphere of 95% air/ 5% CO2). COS7 cells were then fixed (4% paraformaldehyde, 15 minutes) and permeabilized (0.2% Triton X-100, 10 minutes), and incubated with patient serum (1:200 dilution), CSF (1:10) or rabbit pan-TRIM46–specific IgG (1:200, ab254941, Abcam). After phosphate-buffered saline wash and incubation with secondary antibodies (1:200 tetramethylrhodamine-conjugated goat anti–rabbit IgG or goat anti–human IgG, Southern Biotechnology).

Indirect immunofluorescence assay (IFA) and microscopy

Patient serum and CSF and commercial antibodies were tested on a cryosectioned (4 μm) composite of adult mouse tissues: cerebellum, midbrain, cerebral cortex, hippocampus, kidney and gut. Sections were fixed using 4% paraformaldehyde for 1 minute, then permeabilized with 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 0.5%, in phosphate buffered saline (PBS, for 1 minute), and then blocked for 1 hour with normal goat serum (10% in PBS). After PBS-rinse, patient specimen was applied (serum was pre-absorbed with bovine liver powder, 1:240 dilution, and CSF was non-absorbed, 1:2 dilution). After 40 minutes, and PBS wash, a species-specific secondary antibody conjugated with fluorescein isothiocyanate (FITC, 1:100) or tetramethylrhodamine (TRITC, 1:100) was applied (Southern Biotechnology

Associates, Inc, Birmingham, AL, USA). Cover slips were mounted using ProLong Gold antifade medium (containing DAPI; Molecular Probes Thermo Fisher Scientific, USA). Fluorescence images were captured using Olympus BX51 polarizing microscope with Olympus DP73 high-performance Peltier-cooled, 17.28 megapixel camera. Patient specimens yielding positive results were titrated in doubling dilutions to determine the endpoint of autoantibody detection.

For dual staining on murine tissue, we applied patient serum (1:480) or CSF (1:20) and rabbit polyclonal TRIM46–specific IgG and secondary antibodies (1:200, tetramethylrhodamine-conjugated goat anti–rabbit IgG and goat anti–human IgG, Southern Biotechnology). Confocal images were captured using a LSM710 microscope (63 × or 40 × water immersion lens; Carl Zeiss Inc).

Histopathology of tumor tissue

Formalin-fixed paraffin-embedded 5 μm thick sections of breast adenocarcinoma (patient 3) were stained with hematoxylin and eosin (H&E). Immunohistochemistry was performed with the EnVisionTM FLEX immunohistochemistry system (Dako) after steam antigen retrieval with citric acid buffer pH 6.0 (Dako, Denmark). The primary antibody of TRIM46 (1:200, Abcam, USA) was incubated overnight at 4 degrees.