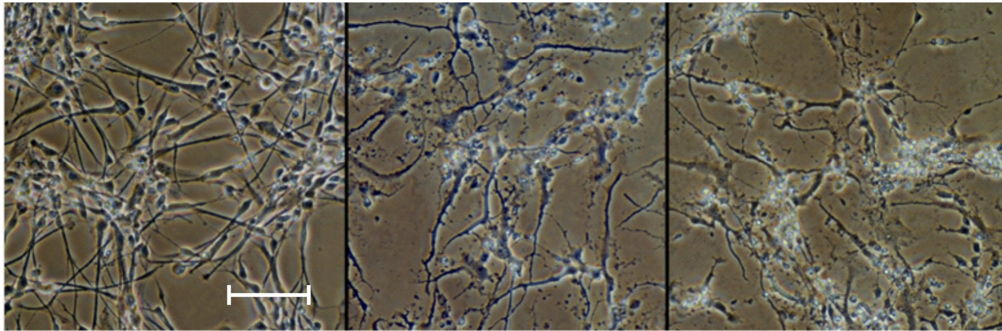
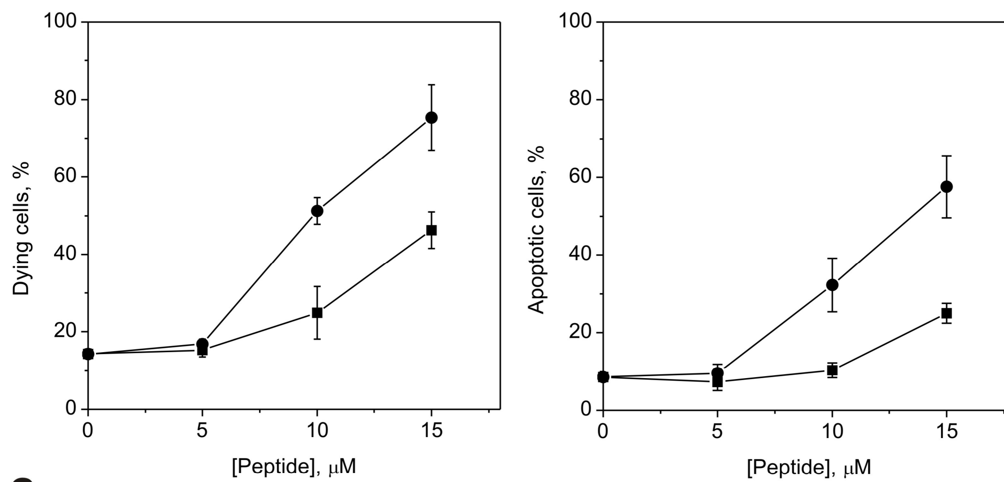
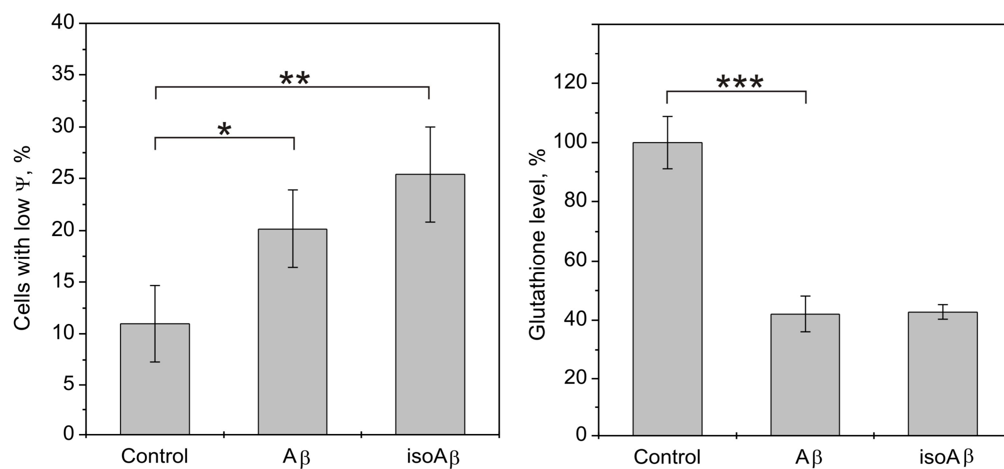
Supplementary information

Isomerization of Asp7 leads to increased toxic effect of amyloid-β42 on human neuronal cells

Supplementary Figure S1







(a) Action of the Aβ42 and isoAβ42 peptides on human neuronal cells NSC-hTERT (15 μM, 48 h). ***Left***, control. ***Center***, the action of Aβ42. ***Right***, the action of isoAβ42. Phase contrast, digital contrast enhancement. Scale length 100 μm. Synthetic peptides Aβ42: [H2N]-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-[COOH], and isoAβ42: [H2N]-DAEFRH [isoD] SGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-[COOH] were purchased from Biopeptide. Preliminary preparation of Aβ42 and isoAβ42 peptides was performed as described in [Klein W Neurochemistry International 2002, 41: 345-352]. Fresh 5 mM peptide solutions were prepared by adding 25 μL of 100% anhydrous DMSO (Sigma-Aldrich) to 0.56 mg peptide and incubating for 1 hour at room temperature. Neural stem cells NSC-hTERT, immortalized by introduction of the telomerase catalytic subunit (hTERT) gene in the cells of primary culture, [7] were cultured in DMEM/F12 supplemented with the 10 ng/ml human recombinant factors EGF and bFGF (Invitrogene), 2% serum substitute FetalCloneIII (Hyclone), 2 mM glutamine, N2 Supplement (Invitrogene), 0.11 mg/ml pyruvate of sodium and 40 units/ml of gentamicin. Culturing of cells was performed at 37° C in an atmosphere with 5% CO_2 and 4% O_2. Differentiation of cell culture was performed prior to treatment with peptides. Cells were seeded at 250 thousand/ml in wells of 24-well plates (Costar), and after they reached the monolayer, factors EGF and bFGF were removed from the cell medium. When cells developed neural network, the growth medium was replaced with serum-free medium containing peptides at various concentrations. Incubation of cells with peptides was performed for 48 h at 37° C in an atmosphere with 5% CO_2 and 4% O_2.

(b) The effect of different concentrations of the Aβ42 (squares) and isoAβ42 (circles) peptides on the amount of dying (***Left***) and apoptotic (***Right***) cells in a population of human neuronal cells NSC-hTERT after 48 h of incubation with peptides. The amount of apoptotic and dying cells is expressed as a percentage of the total number of cells. Analysis of the cells was performed on GALLIOS flow cytometer (BeckmanCoulter). Dying cells included cells

with damaged membrane (necrotic) and apoptotic cells. Cells with damaged membrane were determined according to staining with propidium iodide (PI) (Sigma) (Ex/Em = 535/617 nm). The apoptotic cells were detected using annexin V (Invitrogen), labeled with FITC (Ex/Em = 494/518 nm). Cells that were binding annexin V, but were not stained by PI, were characterized as apoptotic. Each value is the mean of at least three independent experiments with triplicate samples ± SD.

(c) Effect of the Aβ42 and isoAβ42 on the percentage of NSC-hTERT cells with low mitochondrial membrane potential (Ψ) (***Left***) and intracellular glutathione level (***Right***) after 48 h incubation with 10 μM peptides. Mitochondrial membrane potential (Ψ) was detected by MitoProbe $\mathrm{DilC_1(5)}$ (Ex/Em = 638/658 nm) (Invitrogen). $\mathrm{DilC_1(5)}$ stain intensity goes down when the mitochondrial membrane potential decreases. Assessment of the level of reduced glutathione was performed using the dye ThiolTracker Violet (Ex/Em = 405/525 nm) (Invitrogen). These parameters were recorded in the cells with intact membrane. Each value is the mean of at least three independent experiments with triplicate samples ± SD. The comparison of data groups was performed using Student's t-test; $p < 0.01$ was considered significant; * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0008$.