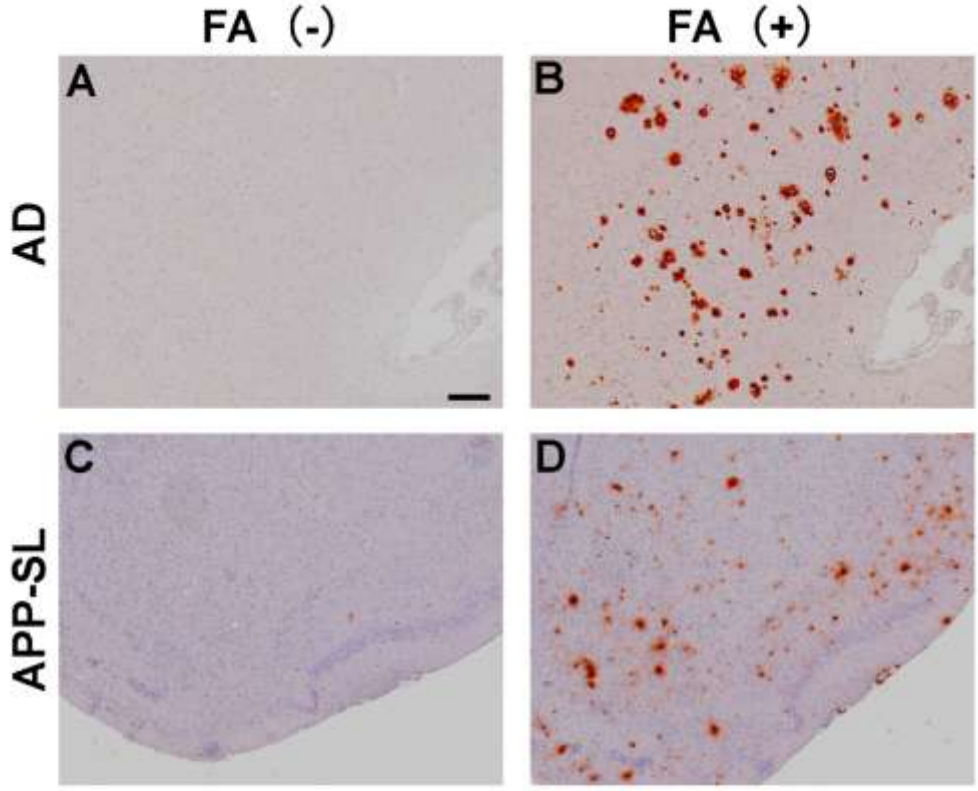
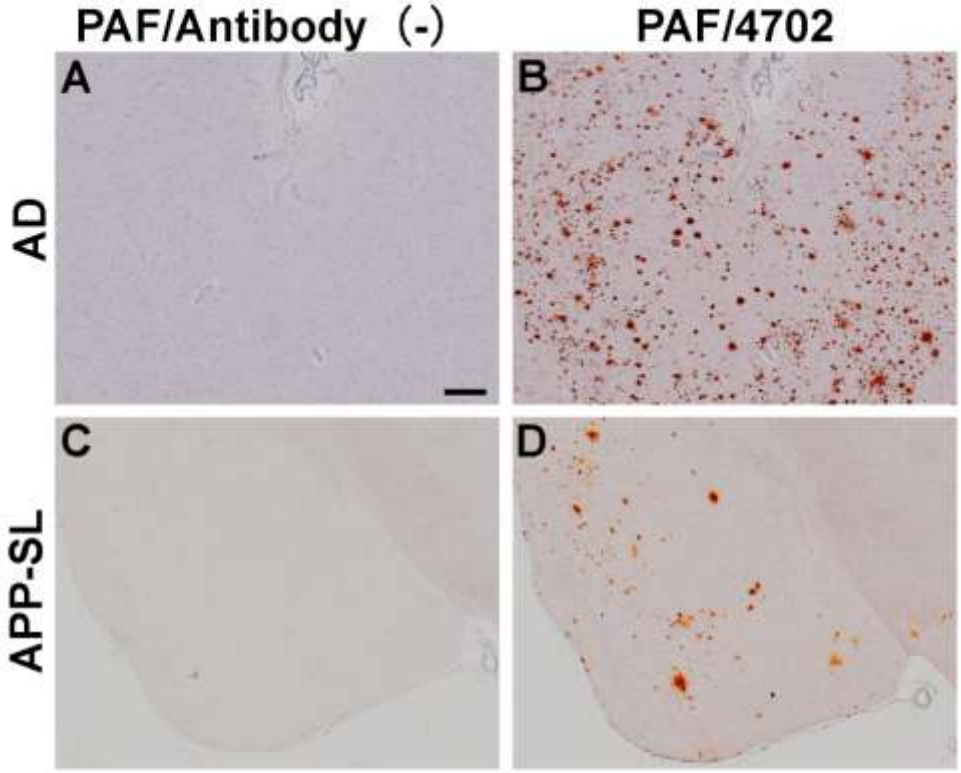


Supplementary Figure 1

Supplementary Figure 1:

Antigen retrieval by the formic acid (FA) method for Aβ-IHC.

(A-D) Brain tissue sections from an AD patient (A and B) or from an APP-SL mouse of the line 7-5 (C and D) were examined by Aβ-IHC using the 4702 antibody without (FA -) (A and C) or with (FA+) (B and D) the FA method. Note that a massive deposition of Aβ plaques was detected with the FA method. The images shown are of the same region from the cerebral cortex of a 72-year-old female (A and B) and from the forebrain around a piriform cortex of a 16.1month-old mouse (C and D), respectively. Scale bar, 200 μm.

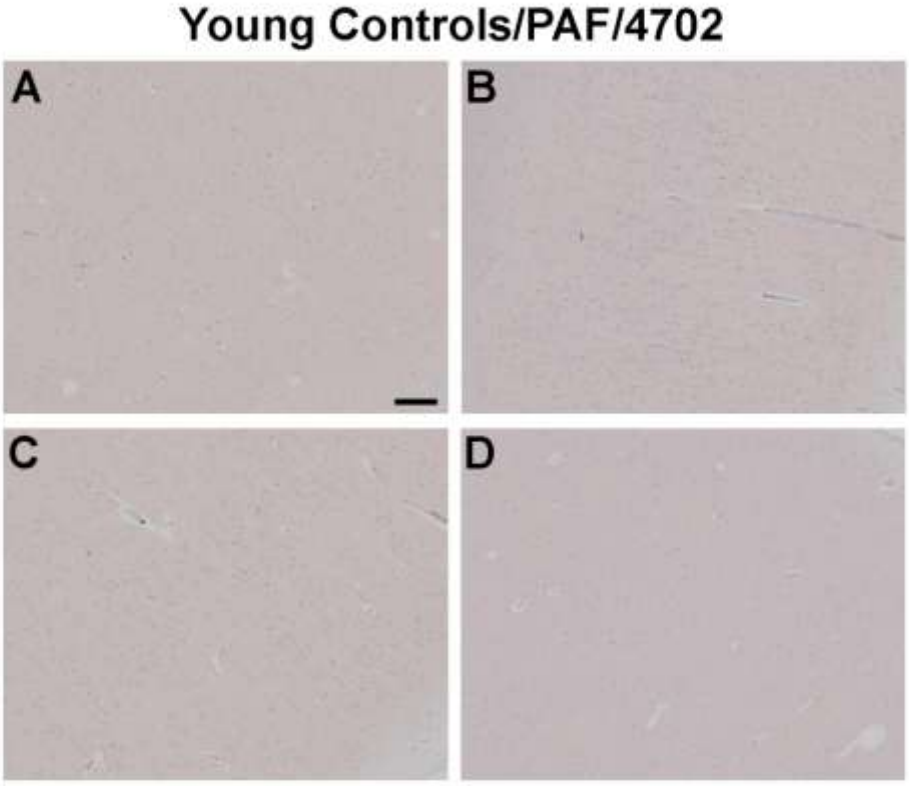


Supplementary Figure 2

Supplementary Figure 2:

The PAF method does not create non-specific staining.

(A-D) Brain tissue sections from an AD patient (A and B) and from an APP-SL mouse of the line 7-5 (C and D) were immunostained by application of the PAF method with (PAF/Antibody -) (A and C) or without (PAF/4072) (B and D), omitting incubation with the primary antibody. Note that no staining was created without the primary antibody. The images shown are of the same region from the frontal cortex of a 78-year-old female AD patient (A and B) or from the forebrain around a piriform cortex of a 16.3 month-old APP-SL line 7-5 mouse (C and D), respectively. Scale bar, 200 μm.



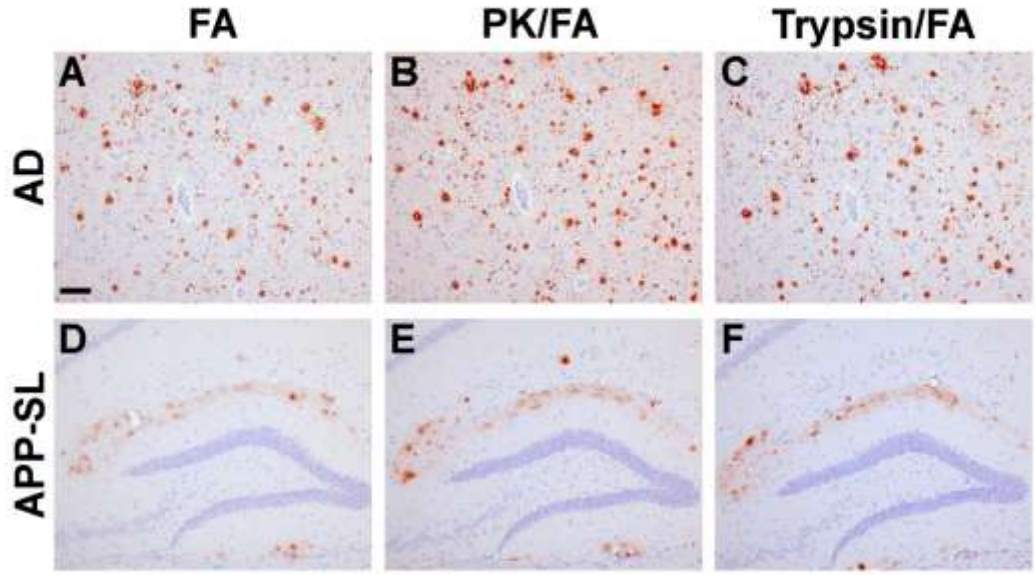
Supplementary Figure 3

Supplementary Figure 3:

The PAF method does not create any Aβ-associated structures in young human

controls.

(A-D) Brain tissue sections from male controls of 35 (A), 27 (B), 28 (C) and 21 (D) years of age were immunostained with the antibody 4702 by application of the PAF method. Note that no Aβ plaques or Aβ-associated structures are visible. Scale bar, 200 μm.

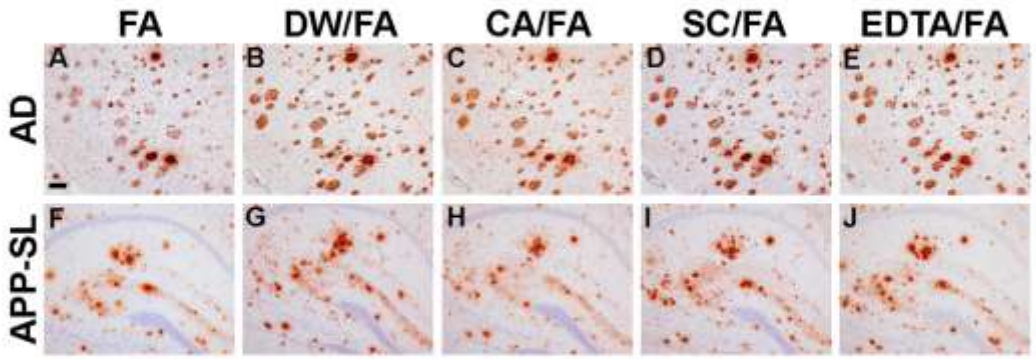


Supplementary Figure 4

Supplementary Figure 4:

AR efficacy of PK vs. trypsin in the double combination of enzymatic digestion and the FA method.

(A-F) Brain tissue sections were treated by the FA method only (FA) (A and D), or by incubation with PK (PK/FA) (B and E) or trypsin (Trypsin/FA) (C and F), respectively, prior to applying the FA method, and then were immunostained with the 4702 antibody. The images shown are of the same region from the frontal cortex of 78-year-old female patient with AD (the same patient shown in Supplementary Figure 2, A and B) (A-C) and the hippocampus of a 16.1 month-old APP-SL line 7-5 mouse (D-F), respectively. Scale bar, 200 μm.

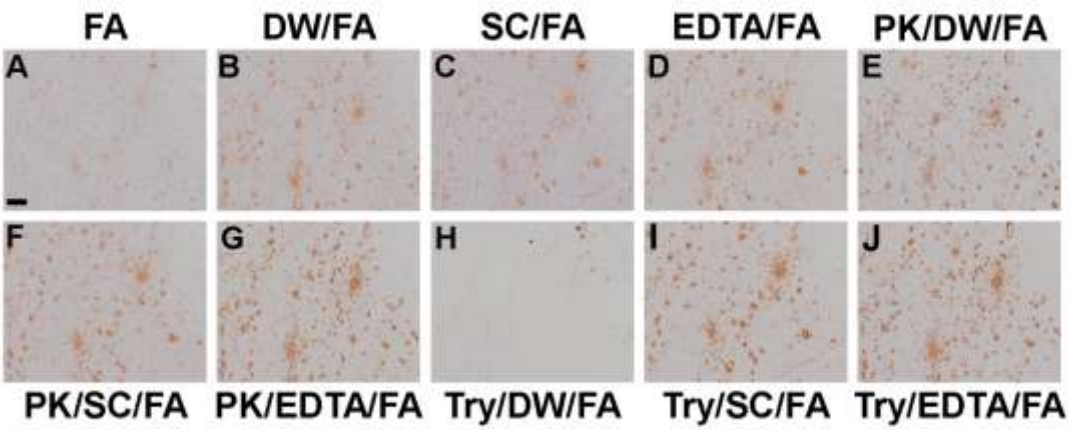


Supplementary Figure 5

Supplementary Figure 5:

AR efficacy of several solutions used for the autoclave heating in the double combination with the FA method.

(A-J) Brain tissue sections were treated by the FA method only (FA) (A and F), or by autoclave heating in DW (pH 10.0, 105C) (DW/FA) (B and G), citraconic anhydride (pH 7.4, 105C) (CA/FA) (C and H), sodium citrate (pH 7.2, 105C) (SC/FA) (D and I) or EDTA (pH 6.0, 121C) (EDTA/FA) (E and J), respectively, prior to applying the FA method, and then were immunostained with the 4702 antibody. The images shown are of the same region from the temporal cortex of a 70-year-old female patient with AD (A-E) or the hippocampus of a 16.1-month-old APP-SL line 7-5 mouse (F-J). Scale bar represents 100 μm.

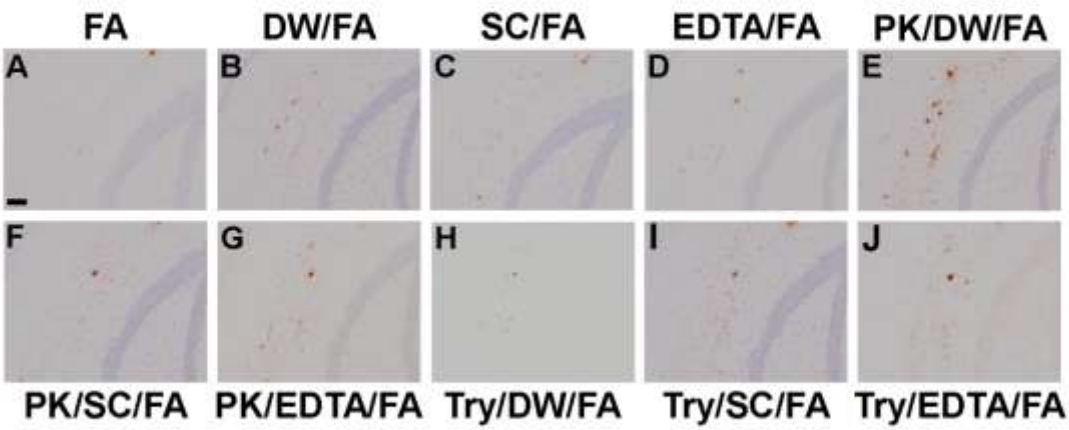


Supplementary Figure 6

Supplementary Figure 6:

AR efficacy of double combinations including several autoclaving procedures and of triple combinations including PK or trypsin digestion and several autoclaving procedures in the AD brain.

(A-J) Serial brain sections were treated by the FA method only (A), two steps-comprised AR procedures (B-D) or three steps-comprised AR procedures (E-J). The two steps-comprised AR procedures consist of autoclave heating either in DW (pH 10.0, 105C) (DW/FA) (B), sodium citrate (pH 7.2, 105C) (SC/FA) (C) or EDTA (pH 6.0, 121C) (EDTA/FA) (D), and the FA method. The AR procedures consisting of three steps are the following: PK-digestion and autoclaving in DW (pH 10.0, 105C) (PK/DW/FA) (E), PK-digestion and autoclaving in sodium citrate (pH 7.2, 105C) (PK/SC/FA) (F), PK-digestion and autoclaving in EDTA (pH 6.0, 121C) (PK/EDTA/FA) (G), trypsin-digestion and autoclaving in DW (pH 10.0, 105C) (Try/DW/FA) (H), trypsin-digestion and autoclaving in sodium citrate (pH 7.2, 105C) (Try/SC/FA) (I), and trypsin-digestion and autoclaving in EDTA (pH 6.0, 121C) (Try/EDTA/FA) (J), all of which were followed by the FA method. The sections were then immunostained with the primary antibody 4702. The images shown are of the same region from the frontal cortex of 78-year-old female with AD. Scale bar, 100 μm.



Supplementary Figure 7

Supplementary Figure 7:

AR efficacy of double combinations including several autoclaving procedures and of triple combinations including PK or trypsin digestion and several autoclaving procedures in the AD mouse model brain.

(A-J) Serial brain sections were treated by the FA method only (A), two steps-comprised AR procedures (B-D) or the three-step AR procedures (E-J). The two-steps AR procedures consist of autoclave heating either in DW (pH 10.0, 105C) (DW/FA) (B), sodium citrate (pH 7.2, 105C) (SC/FA) (C) or EDTA (pH 6.0, 121C) (EDTA/FA) (D), and the FA method. The three steps AR procedures consist of PK-digestion and autoclaving in DW (pH 10.0, 105C) (PK/DW/FA) (E), PK-digestion and autoclaving in sodium citrate (pH 7.2, 105C) (PK/SC/FA) (F), PK-digestion and autoclaving in EDTA (pH 6.0, 121C) (PK/EDTA/FA) (G), trypsin-digestion and autoclaving in DW (pH 10.0, 105C) (Try/DW/FA) (H), trypsin-digestion and autoclaving in sodium citrate (pH 7.2, 105C) (Try/SC/FA) (I), and trypsin-digestion and autoclaving in EDTA (pH 6.0, 121C) (Try/EDTA/FA) (J), all of which were followed by the FA method. The sections were then immunostained with the primary antibody 4702. The images shown are of the same region from the hippocampus of a 12.1-month-old APP-SL line 7-5 mouse. Scale bar, 100 μm.