Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology in vivo

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Supplemental Figure legends

Online Resource 1 Tau-seeding induced Tau-pathology in Tau transgenic mice

a Injections of Tau-seeds (upper panel) or PBS (lower panel) were performed in Tau transgenic mice at the age of 3 months and analyzed 6 months post-injection. Representative images of the frontal cortex at the site of injection, stained with anti-P-Tau (AT8; red) antibody shows robust induction of Tau-pathology in Tau-seeded, absent in age-matched PBS injected, Tau transgenic mice. Silver and ThioS staining demonstrate the presence of mature NFTs following injection of Tau seeds in Tau transgenic mice. **b** Comparison of injection of pre-aggregated Tau seeds (K18 agg), non-aggregated monomeric Tau (K18 mono) and PBS in frontal cortex of 3 months old Tau transgenic mice. Representative images of frontal cortex at the injection site, stained with anti-P-Tau (AT8; red) antibody, shows robust induction of Tau-pathology following injection of pre-aggregated Tau seeds (K18 agg), nearly absent following injection of non-aggregated monomeric Tau (K18 mono), and absent in age-matched PBS injected Tau transgenic mice. Quantitative analysis demonstrates significant induction of Tau-pathology following injection of pre-aggregated Tau compared to PBS injected Tau mice only (n=3, PBS; n=4, $Tau_{k18mono}$; n=4, Tau_{k18agg}; Kruskall-Wallis ANOVA with Dunn's multiple comparison post hoc, *p<0,05).

Online Resource 2 ASC deficiency reduces induction of Tau pathology following Tau-seeding in Tau transgenic mice (complementary to Figure 2)

a Immunohistological analysis of Tau-seed induced Tau pathology in brains of T+. ASC+/+ and T+.ASC-/- mice. Tau seeding was performed at 6 months of age and analyzed 3 months post-injection. Representative images of anti-P-Tau (AT8; red) staining in frontal cortex of T+. ASC+/+ and T+.ASC-/- mice are presented. Quantitative analysis of the area stained with AT8 reveals a significantly lower induction of Tau pathology in T+.ASC-/- compared to T+.ASC+/+ mice (n=6, T+.ASC+/+; n=7, T+.ASC-/-; Mann-Whitney t-test, *p<0,05). Silver (black) and This (green) staining demonstrate the presence of mature NFTs following Tau seeding in Tau transgenic mice, representative images for each staining of frontal cortex are shown. Tau-seeding induced Tau pathology (NFTs) assessed by silver and ThioS staining was significantly less in T+.ASC-/- compared to T+.ASC+/+ mice (n=6, T+.ASC+/+; n=7, T+.ASC-/-; Mann-Whitney t-test, *p<0,05). These results were obtained in a first cohort (cohort 1) used to evaluate the potential implication of inflammasome activation in Tau-seeded Tau pathology. The study was subsequently extended with a new cohort of mice presented in figure 2 of the manuscript. The implication of ASC in Tau-seeded Tau pathology has been identified in the 2 different cohorts analyzed independently. **b** Propagation of Tau pathology was assessed by analyzing Tau-seed induced Tau pathology at the contralateral side in brains of T+. ASC+/+ and T+.ASC-/- mice. Representative images of AT8 (red) staining in frontal cortex of the contralateral side in T+.ASC+/+ and T+.ASC-/- mice (data presented in figure 2) are presented. Quantitative analysis of the area stained with AT8 reveals a significantly lower induction of Tau pathology in T+.ASC-/- compared to T+.ASC+/+ mice (n=6, T+.ASC+/+; n=5, T+.ASC-/-; Mann-Whitney t-test, *p<0,05). **c** Biochemical analysis was performed on sarkosyl insoluble Tau fractions obtained from the frontal cortices of T+.ASC+/+ and T+.ASC-/- mice following Tau-seeding in frontal cortex. Representative samples of sarkosyl insoluble Tau following anti-P-Tau (AT8) immunoblotting are shown. Western blotting using AT8 on sarkosyl insoluble

fractions extracted from T+. ASC+/+ and T+.ASC-/- mice, revealed a significant decrease in sarkosyl insoluble Tau in T+.ASC-/- compared to T+. ASC+/+ mice following Tau-seeding in frontal cortex (n=5, T+.ASC+/+; n=4, T+.ASC-/-; Mann-Whitney t-test, *p<0,05).

Online Resource 3 Tau seeding induced microgliosis is decreased in ASC deficient Tau mice

Representative images of Iba1 (green) staining in brains of Tau-seeded T+.ASC+/+ compared to T+.ASC-/- are presented demonstrating increased microgliosis in Tau-seeded T+.ASC+/+ mice compared to T+.ASC-/- mice.

Online Resource 4 Uptake of Tau seeds in microglia and inflammasome activation in brains of Tau transgenic mice

a Immunofluorescent staining was performed on brain sections of aged-matched Tau transgenic (TPS) and wild-type (WT) mice. Co-immunostaining of anti-P-Tau (AT8; blue) and anti-ASC (green) or anti-NLRP3 (green) antibody, revealed increased and punctated staining in the brains of Tau transgenic mice, not detected in non-transgenic mice. Punctated staining is indicative for ASC and NLRP3 aggregate formation, supporting induction of inflammasome activation in brains of Tau transgenic mice. **b** Immunostaining of anti-P-Tau (AT8; blue) and anti-Iba1 (red) revealed microglial activation in the brains of Tau transgenic (TPS) mice not detected in non-transgenic (WT) mice. **c** Higher magnification of the staining presented in the upper panel, revealed the presence of NLRP3-positive punctae (green) within microglia (Iba1; red), in the brain of Tau transgenic (TPS) mice. **d** Higher magnification of the immunostaining presented in panel b, revealed the presence of anti-P-Tau (AT8; blue) staining within microglia (Iba1; red), indicating the uptake of Tau forms into the microglia, in the brain of Tau transgenic (TauP301S) mice.