Absence of CX3CR1 impairs the internalization of Tau by microglia

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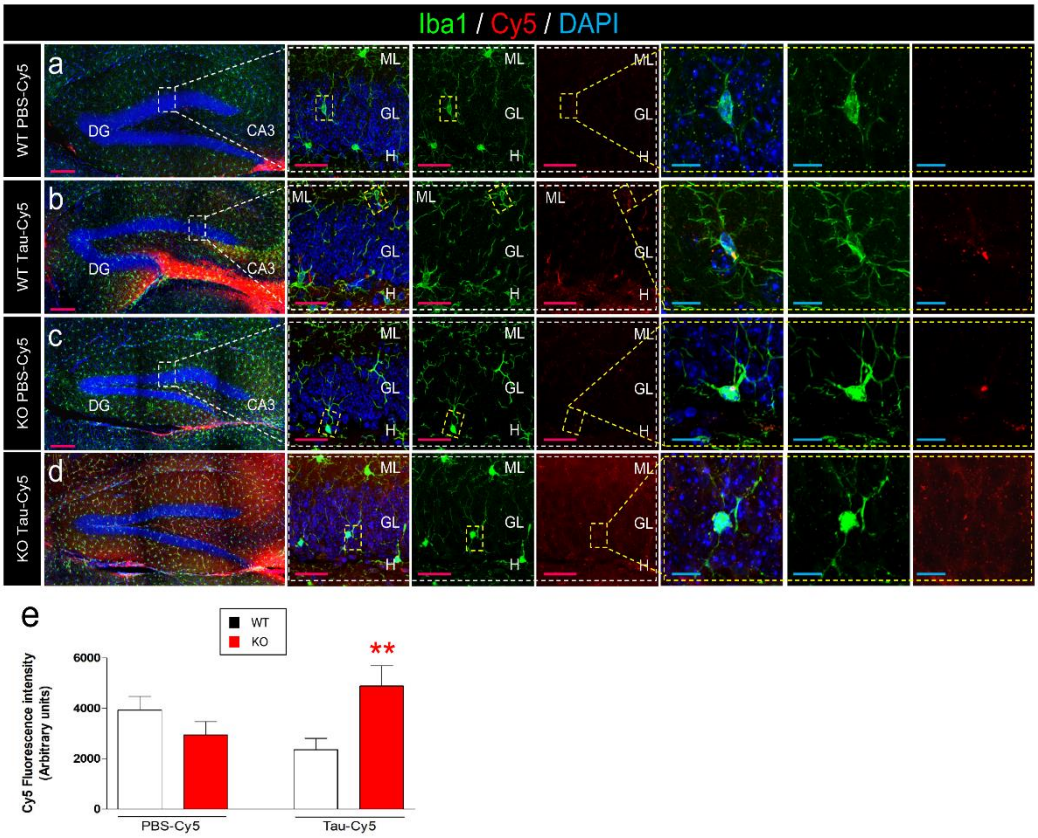
Additional files

Additional file 1. The absence of CX3CR1 led to a decrease in Tau internalization by microglia of the dentate gyrus (DG) *in vivo* after 8 weeks. Representative images of WT (a-b) and KO mice (c-d) stereotaxically injected with PBS-Cy5 or Tau-Cy5 in the DG and sacrificed 8 weeks later. Immunofluorescence images for Iba1 (green) and Cy5 (red) and high-power magnification are shown separately. (e) Quantification of Cy5 fluorescence intensity in mice injected with PBS-Cy5 or Tau-Cy5. (f) Colocalization between Iba1 and Cy5 in mice injected with PBS-Cy5 or Tau-Cy5 in the DG. Bars show means ± S.E. ∗∗p≤0.01; ∗∗∗p≤0.001. Purple scale bar: 50 µm, Blue scale bar: 100 µm. GL, granule layer; H, hilus; ML, molecular layer; CA3, cornu ammonis region 3; DG, dentate gyrus.



Additional file 2. Percentage of sequence identity between human Tau42 (a) and CX3CL1 (b) proteins. The sequences of human Tau42 and human CX3CL1 were obtained from NCBI. The amino acid sequences of human Tau42 (Reference Sequence: NP_005901.2) and human CX3CL1 (GenBank: CAG33707.1) were compared using the protein-protein BLAST tool (https://blast.ncbi.nlm.nih.gov). These proteins were found to share 37% sequence identity (c). Yellow: Human Tau identity sequences. Pink: CX3CL1 Tau identity sequences.

a)

Human Tau

MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPGSETSDAKSTPTAEDVTAPLVDEGAPGKQAAA
QPHTEIPEGTTAEEAGIGDTPSLEDEAAGHVTQEPESGKVVQEGFLREPGPPGLSHQLMSGMPGAPLLPEGPREATRQPSGTGPEDTEGGRHA
PELLKHQLLGDLHQEGPPLKGAGGKERPGSKEEVDEDRDVDESSPQDSPPSKASPAQDGRPPQTAAREATSIPGFPAEGAIPLPVDFLSKVSTEIP
ASEPDGPSVGRAKGQDAPLEFTFHVEITPNVQKEQAHSEEHLGRAAFPGAPGEGPEARGPSLGEDTKEADLPEPSEKQPAAAPRGKPVSRVPQL
KARMVSKSKDGTGSDDKKAKTSTRSSAKTLKNRPCLSPKHPTPGSSDPLIQPSSPAVCPEPPSSPKYVSSVTSRTGSSGAKEMKLKGADGKTKIATPR
GAAPPGQKGQANATRIPAKTPPAPKTPPSSGEPPKSGDRSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKSPSSAKSRLQTAPVPMPDL
KNVKSKIGSTENLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQVEVKSEKLDFKDRVQS
KIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDHGAEIVYKSPVVSGDTSPRHLSNVSSTGSIDMVDSPQLATLADEVSASLAKQGL

b)

Human CX3CL1

MAPISLSWLLRLATFCHLTVLLAGQHHGVTKCNITCSKMTSKIPVALLIHYQQNQASCGKRAIILETRQHRLFCADPKEQWVKDAMQHLDRQAAALT
RNGGTFEKQIGEVKPRTTPAAGGMDESVVLEPEATGESSSPEPTPSSQEAQRALGTSPELPTGVTGSSGTRLPPTPKAQDGGPVGTELFRVPPVST
AATWQSSAPHQPGPSLWAEAKTSEAPSTQDPSTQASTASSPAPEENAPSEGQRVWGQGQSPRPENSLEREEMGPVPAHTDAFQDWGPGSMAH
VSVVPVSSEGTPSREPVASGSWTPKAEEPIHATMDPQRLGVLITPVPDAQAATRRQAVGLLAFLGLLFCLGVAMFTYQSLQGCPRKMAGEMAEGLRY
IPRSCGSNSYVLVPV

c)

Human Tau- Human CX3CL1 blast

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VDLSKVTSKCGSLGNIHHK    human Tau
   SK+TSK       IH++    37% ident
ITCSKMTSKIPVALLIHYQ    human CX3CL1

SPGTPG-----SRSRTPSL-PTPPTREPKKVAVVRTPPKSPSSAKSR    human Tau
S GTP      S S TP   P   T +P+++ V+ TP     +A  R    37% ident
SEGTPSREPVASGSWTPKAEPIHATMDPQRLGVLITPVPDAQAATRR    human CX3CL1
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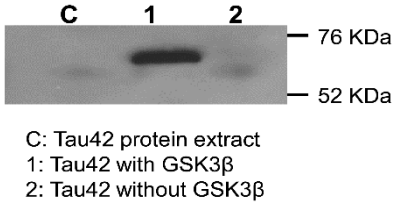
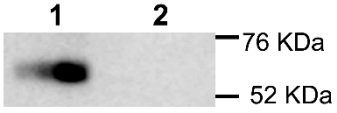
Additional file 3. Phosphorylation of Tau42 by GSK-3β. Western blot of phospho-Tau42 after enzymatic reaction with GSK3β. Anti-phospho-Tau (p-S396) antibody was used. C: Tau42 protein, control extract. 1: Tau42 phosphorylated by GSK3β following the methods described in the Methods section. 3: Tau42 without GSK3β (control of the enzymatic reaction).



C: Tau42 protein extract
1: Tau42 with GSK3β
2: Tau42 without GSK3β

Additional file 4. Phosphorylation of Tau42-Cy5 by GSK-3β. Western blot of phospho-Tau42 after enzymatic reaction with GSK3β. Anti-phospho-Tau (p-S396) antibody was used. 1: Tau42-Cy5 phosphorylated by GSK3β following the method described in the Methods section. 2: Tau42-Cy5 without GSK3β (control of the enzymatic reaction).



1: Tau42-cy5 with GSK3β
2: Tau42-cy5 without GSK3β