Presenilin 1 phosphorylation regulates amyloid-β degradation by microglia

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† In Memoriam

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Supplemental Figures Legends

Supplementary Figure 1: Lack of phosphorylation at serine 367 in PS1\(^{ki/ki}\) microglia. Number of microglial in Psen1\(^{ki/ki}\) mouse. FACS/Sorting gating strategy for isolating microglia from mouse brain.

(A) Total levels of PS1 in microglial cells from WT or Psen1\(^{ki/ki}\) mice was analyzed by Western blot. GAPDH was used as loading control. (B) Phosphorylation of PS1 at serine 367 in microglial cells from WT or Psen1\(^{ki/ki}\) mice was analyzed by Western blot using pS367-PS1 antibody. Total PS1 was immunoprecipitated from microglia lysates using an anti-PS1 antibody covalently bound to agarose beads, and immunoblotted with anti-PS1 pS367, stripped and rebotted against total PS1. (C) Schematic diagram showing the strategy used to verify microglial branching in Figure 1. Each segment is represented in different color (1-4), terminal endings are represented as number 4. (D) Confocal stack of microglia in the 2-month-old WT or Psen1\(^{ki/ki}\) mouse brain. Scale bar represents 50 \(\mu m\). Quantification of microglial density (cells / 10\(^{-3}\) mm\(^3\)), data represent means ± SEM \((n = 5\) mice per group). (E) Microglial cells were harvested from WT or Psen1\(^{ki/ki}\) mice and gated for CD45\(^+\), CD11B\(^+\), CX3CR1\(^+\), CSF1R\(^+\), C-KIT\(^-\), LY-6C\(^-\). N = 3-4 individual brains per group. (E) Quantitative RT-PCR performed for Psen1 (left panel) and Atp6v0a1 (middle panel) in control or Psen1\(^{ko}\) primary microglia culture (E) Lysosomal pH of control or Psen1\(^{ko}\) primary microglia culture was determined using LysoSensor Yellow/Blue dextran (450/535 nm) (right panel). Data represent means ± SEM \((n = 5\) independent cell culture preparations; ****\(P < 0.0001\), Student's t-test).

Supplementary Figure 2: Aβ levels, γ-secretase activity and gliosis in 5xFAD and 5xFAD\(^*\) Psen1\(^{ki/ki}\) mice.

(A) The levels of soluble Aβ40 or Aβ42 and insoluble Aβ40 or Aβ42 in 3-month-old mouse brains were analyzed by ELISA. (B) Exo-cell γ-secretase activity assay for recombinant APP substrate in 5xFAD or Psen1\(^{ki/ki}\) mouse. γ-Secretase activity is expressed as arbitrary units (for details see Methods). (C) Quantification of microglial density in 5xFAD and 5xFAD\(^*\) Psen1\(^{ki/ki}\) mice (cells / 10\(^{-3}\) mm\(^3\)). (D) Confocal stack of astrocytes (GFAP yellow) or (E) microglia (Iba1 cyan, Aβ plaque magenta, DNA yellow) in the 3-month-old mice brains. Scale bar represents 10 \(\mu m\). Quantification of GFAP\(^+\) cells (D right panel) or Iba-1 intensity (E right panel) in 5xFAD and
5xFAD* Psen1<sup>KI/KI</sup> mice using fluorescence intensity values. Data represent means ± SEM (n = 5 mice per group; **P < 0.01 ***P < 0.001, Student's t-test).

**Supplementary Figure 3: Quantification of synaptophysin and PSD95 in WT, Psen1<sup>KI/KI</sup>, 5xFAD and 5xFAD* Psen1<sup>KI/KI</sup> mice.**

(A) Quantification of synaptophysin and PSD95 in the hippocampal hilus and molecular layer related to Figure 5 (details described in methods). Mice were 3 months old. Data are represented as mean ± SEM. N = 8-12 microglia per group, 3 mice per group. *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA followed by Duncan's Method.

**Supplemental Table S1 content:**

- Gene expression of adult microglia, Related to Figure 2.
- Quantification of Aβ plaques using ClearMap, Related to Figure 4.

**Supplemental movie legends**

**Supplemental movie 1: WT versus Psen1<sup>KI/KI</sup> microglia activation upon injury using a highly localized laser-induced micro lesion, Related to Figure 1 A-B.**

After the micro lesion Psen1<sup>KI/KI</sup> microglial cells displayed a slower kinetic response compared to WT. For details see Methods section: Multiphoton microscopy.

**Supplemental movie 2: Three-dimensional reconstruction used for the analysis of microglial morphology, Related to Figure 1 C-E.**

For details see Methods section: Morphology tracing and volume images

**Supplemental movie 3: Confocal imaging of amyloid-β plaques using iDISCO, Related to Figure 4 D.**

Whole hemisphere labeled for amyloid-β plaques (Congo red) with Confocal Microscopy using iDISCO. For details see Methods section: iDISCO visualization and ClearMap quantitation of plaques.

**Supplemental movie 4: Three-dimensional reconstruction used for the analysis of synapses (synaptophysin – green and PSD95- red), Related to Figure 5 C-F.**