PNAS

Supporting Information for

Early death in a mouse model of Alzheimer's disease exacerbated by microglial loss of TAM receptor signaling

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Other supporting materials for this manuscript include the following:

Movies S1 and S2

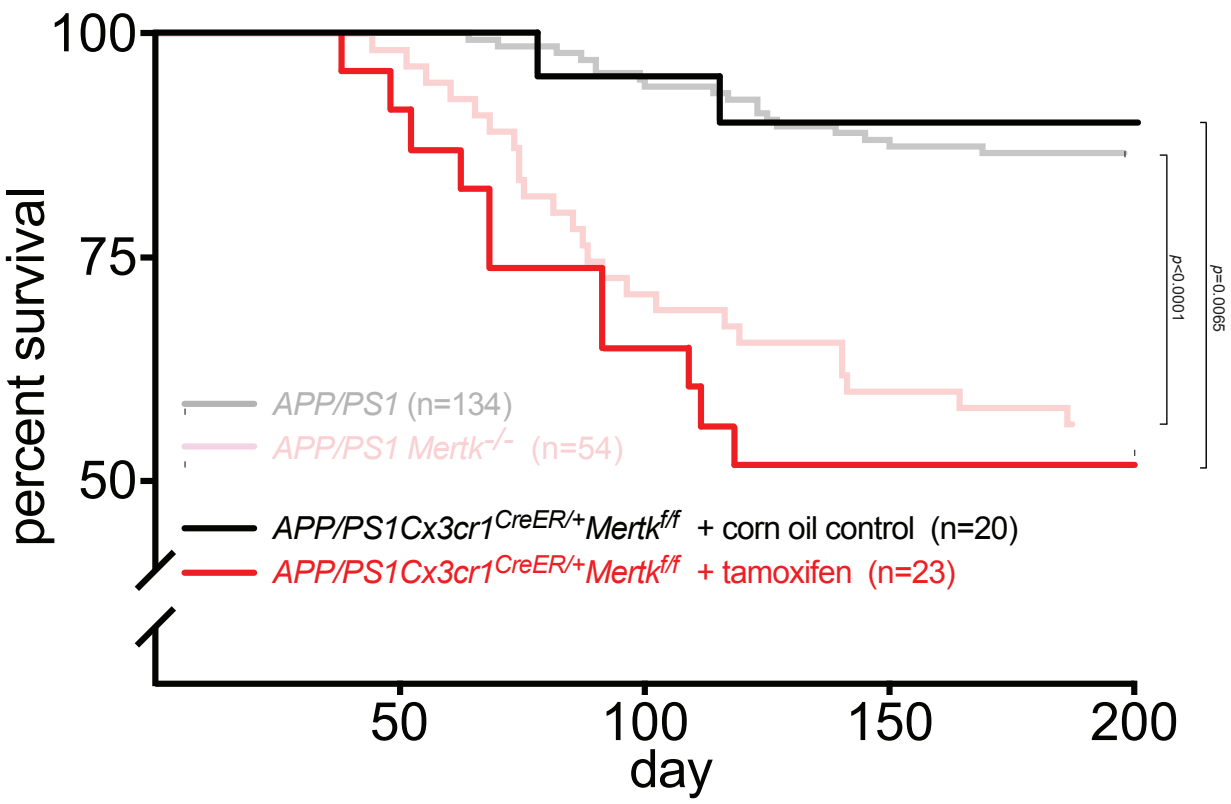

Fig. S1. Loss of Mer specifically in microglia accounts for increased lethality in *APP/PS1* compound mutants. Percent survival of mouse populations of the indicated genotypes and indicated sizes (n = number of mice monitored) as a function of age (postnatal day). *APP/PS1* and *APP/PS1Mertk*$^{-/-}$ data are reproduced from Fig. 1 for comparison. Intraperitoneal injections of corn oil or tamoxifen into *APP/PS1 Cx3cr1*$^{CreER/+}$*Mertk*$^{f/f}$ mice were initiated after weaning, at P30-40. Log-rank test.

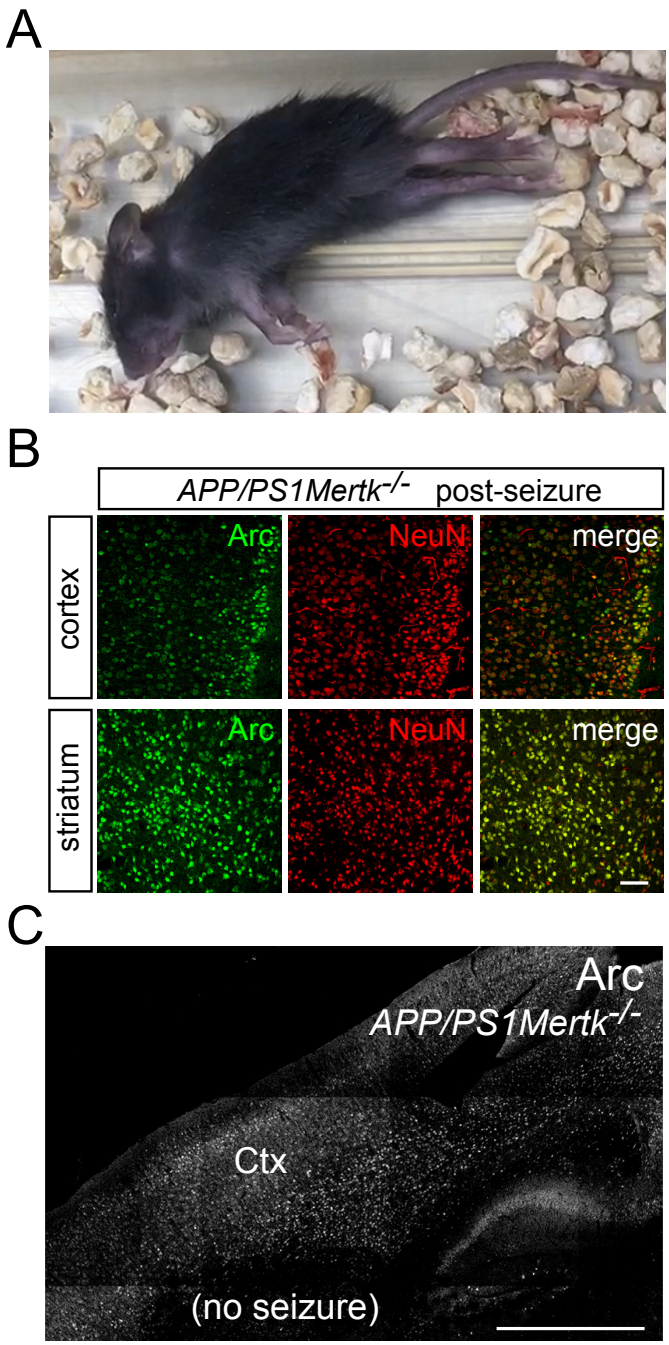

Fig. S2. Seizure phenotypes in Mer-deficient *APP/PS1* mice. (A) A very young (18 d) *APP/PS1Mertk*$^{-/-}$ mouse immediately after death from seizure. Note the distended hindlimbs, which are a typical posture subsequent to this event. (B) Nuclear Arc expression (green, left panels) and expression of the neuronal nuclear marker NeuN (red, middle panels) in the cortex (top panels) and striatum (bottom panels) of the *APP/PS1Mertk*$^{-/-}$ brain shown in Fig. 2A (top) are co-incident (right panels). (C) High power image of Arc expression (white) in the cortex (Ctx) in an *APP/PS1Mertk*$^{-/-}$ mouse in the absence of apparent seizure. Scale bars, (B) 100 μm, (C) 1mm.

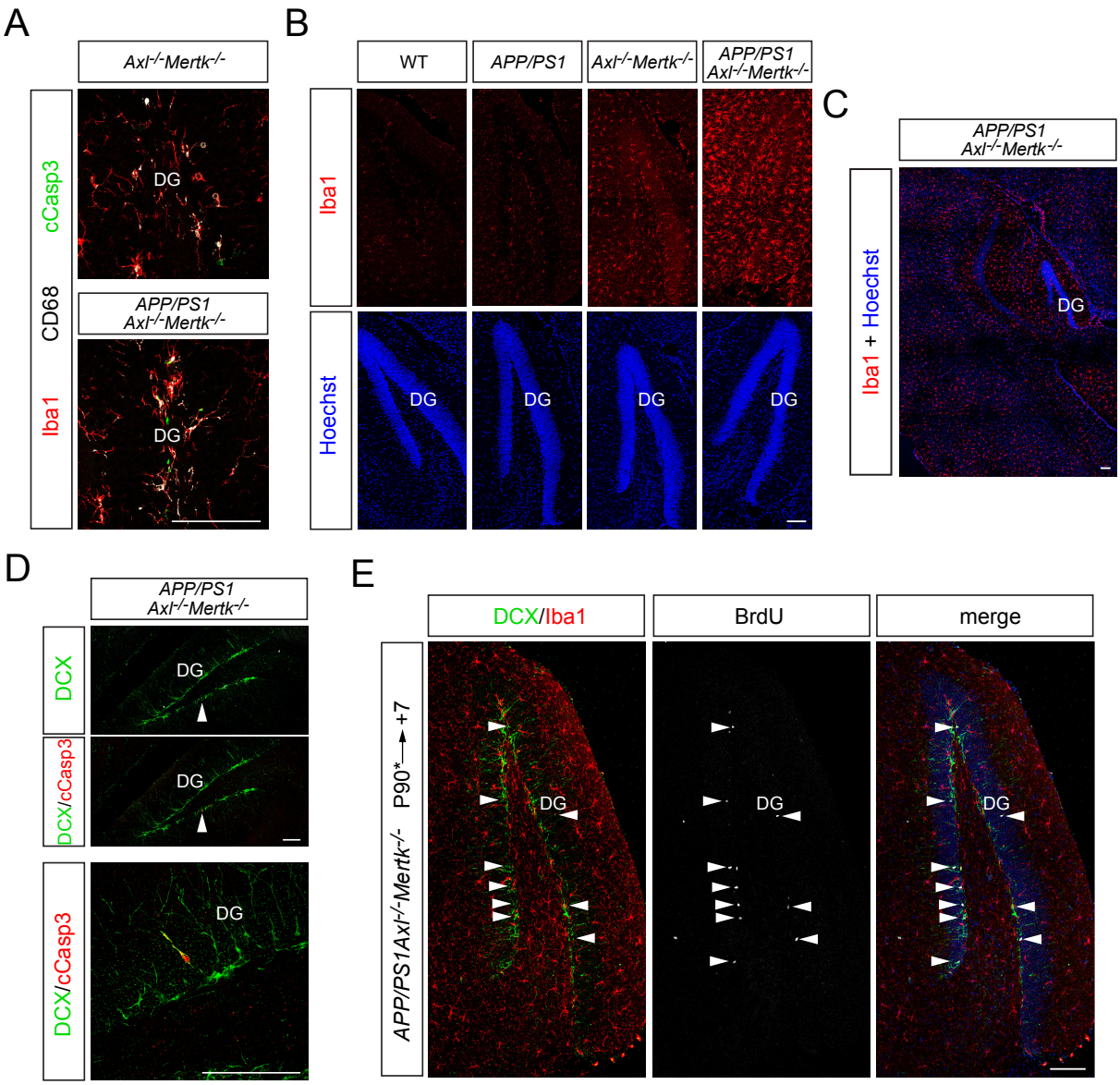

Fig. S3. Apoptotic cells, BrdU-labeled cells, neural stem cells, and microglia in the dentate gyrus (DG). (A) High power view of CD68$^+$ cells (white), Iba1$^+$ cells (red), and cCasp3$^+$ cells (green) in *Axl$^{-/-}$Mertk$^{-/-}$* (top) and *APP/PS1Axl$^{-/-}$Mertk$^{-/-}$* (bottom) DG at P30. (B) Relative Iba1 expression (red, top panels) in the P30 DG of the indicated genotypes. DNA staining of the same sections (Hoechst, lower panels) outlines the V-shaped DG. (C) Lower power view of the P30 hippocampus and cortex illustrating relative enhancement of Iba1 expression (red) in the area of the former. (D) The cCasp3$^+$ ACs that appear in the P90 *APP/PS1Axl$^{-/-}$Mertk$^{-/-}$* DG are, consistent with the literature, doublecortin-positive (DCX$^+$) neural stem cells. (E) The BrdU$^+$ cells labeled in the P90 *APP/PS1Axl$^{-/-}$Mertk$^{-/-}$* DG 7 days after a pulse are all DCX+ cells and none are Iba1$^+$ microglia. Scale bars, all panels 100 μm.

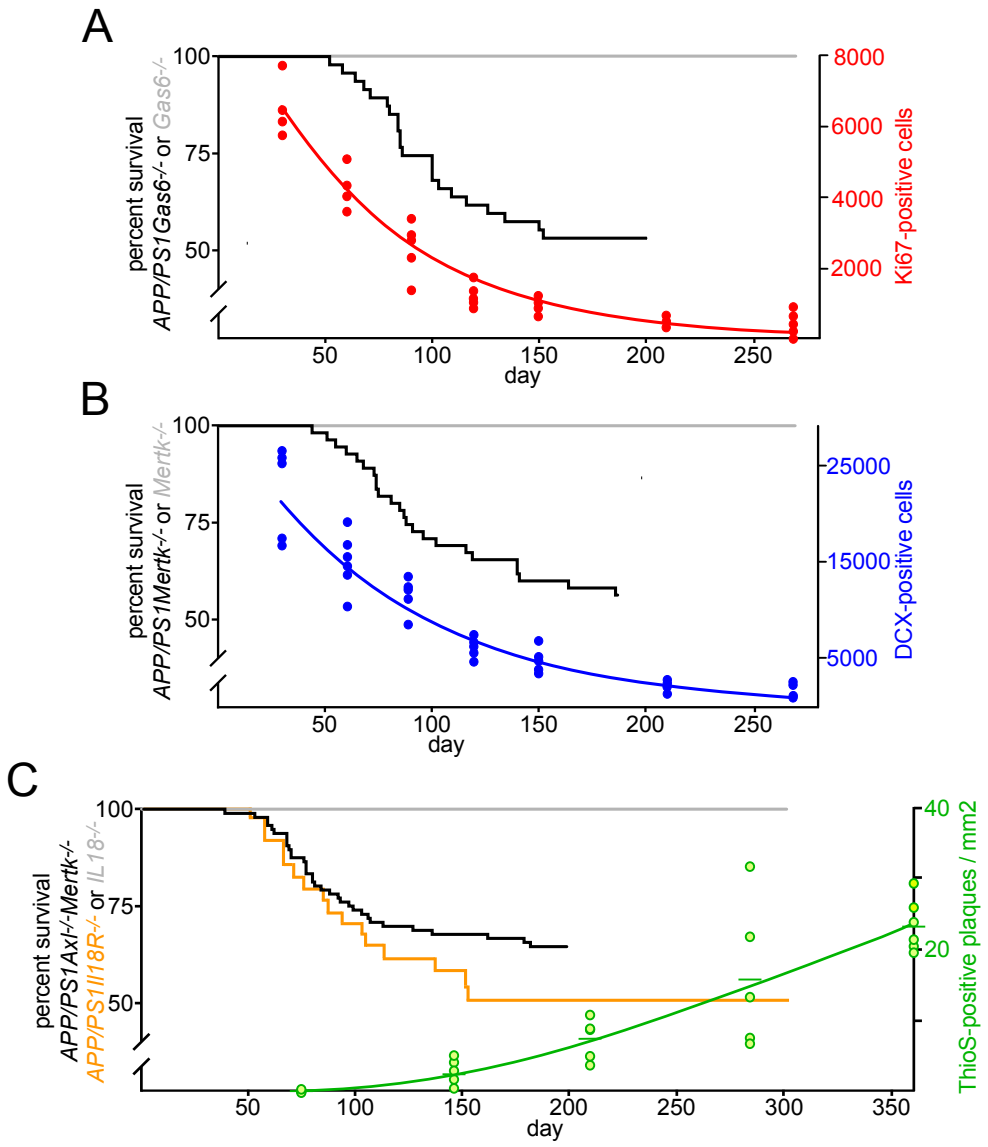

Fig. S4. Sudden death in compound *APP/PS1* mutants relative to adult neurogenesis and dense-core plaque deposition. (A) Sudden death in *APP/PS1Gas6*$^{-/-}$ mice (black, from Fig. 1) plotted relative to the number of proliferating Ki67^{+} cells (red) in the C57Bl/6J dentate gyrus over time (postnatal day), as previously determined by Ben Abdallah and colleagues (*1*). *Gas6*$^{-/-}$ mice (gray) are 100% viable. (B) Sudden death in *APP/PS1Mertk*$^{-/-}$ mice (black, from Fig. 1) plotted relative to the number of newborn doublecortin (DCX)$^{+}$ neurons (blue) in the C57Bl/6J dentate gyrus over time, as determined by Ben Abdallah and colleagues (*1*). *Mertk*$^{-/-}$ mice (gray) are 100% viable. (C) Sudden death in *APP/PS1Axl*$^{-/-}$*Mertk*$^{-/-}$ mice (black, from Fig. 1) and *APP/PS1IL18R*$^{-/-}$ mice (orange, as previously quantified by Tzeng and colleagues (*2*)) plotted relative to the density of ThioS^{+} dense-core Aβ plaques in the cortex (green, quantified as described previously (*3*)) over time (postnatal day). *Il18*$^{-/-}$ mice (gray) are 100% viable.

Movie S1 (separate file). Continuous home cage monitoring set-up. A representative video clip illustrating continuous top-down recording of home cage activity of a mouse cohort. Recording for this sequence was performed during dark cycle housing, under red light illumination (see Materials and Methods). In this example, an *APP/PS1Mertk*$^{-/-}$ mouse (age 2.5 mo) in the cage at the lower right displays a series of abnormal, seizure-like motor behaviors that led to its death shortly after the end of the clip.

Movie S2 (separate file). *APP/PS1Mertk*$^{-/-}$ mutants develop lethal seizures. A video montage of distinct seizure activities exhibited by two different mice. First, an *APP/PS1Mertk*$^{-/-}$ mouse (P18), the animal shown in Fig. S3A, at the end of a lethal seizure. Second, an *APP/PS1Axl*$^{-/-}$*Mertk*$^{-/-}$ mouse (P20) in a tonic-clonic seizure. Third, the same mouse alternating between frozen distended postures in which its body is elevated. Fourth, the same mouse exhibiting tremor and pronounced hyperactivity. This mouse died from seizure after the recording.

SI References

1. N. M. Ben Abdallah, L. Slomianka, A. L. Vyssotski, H. P. Lipp, Early age-related changes in adult hippocampal neurogenesis in C57 mice. *Neurobiology of aging* **31**, 151-161 (2010).
2. T. C. Tzeng *et al.*, Inflammasome-derived cytokine IL18 suppresses amyloid-induced seizures in Alzheimer-prone mice. *Proc Natl Acad Sci U S A* **115**, 9002-9007 (2018).
3. Y. Huang *et al.*, Microglia use TAM receptors to detect and engulf amyloid beta plaques. *Nat Immunol* **22**, 586-594 (2021).