

Supplementary information for:

Isorhamnetin attenuated the release of interleukin-6 from β -amyloid-activated microglia and mitigated interleukin-6-mediated neurotoxicity

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Supplementary figure legends

Supplementary Fig. 1. Time-response experiments of A β oligomers and

isorhamnetin in HMC3 cells. (A) Treatment with A β oligomers (200 nM) for 24 or 48 hours increased IL-6 release, whereas the secretions of IL-1 β and TNF- α were not affected by A β oligomers. (B) Experimental flowchart. HMC3 cells activated by A β were treated with isorhamnetin (10 μ M) for 24 or 48 hours. (C) In A β -activated HMC3 cells, treatment with isorhamnetin for 24 hours did not reduce the secretion of IL-6, while 48 hours treatment reduced the IL-6 secretion to baseline. Data were analyzed using one-way ANOVA with Bonferroni's post hoc test (* P < 0.05, ** P < 0.01 [control vs. A β]; ## P < 0.05 [A β vs. A β /ISN], n = 3; means \pm SEM). A β : A β oligomers; ISN: isorhamnetin.

Supplementary Fig. 2. NF- κ B inhibitor suppressed the A β -induced activation of

HMC3 cells. (A) Western blot in HMC3 cells treated with A β oligomers (200 nM) or EVP4593 (NF- κ B inhibitor, 1 μ M). Treatment with EVP4593 for 48 hours reduced phosphorylation of NF- κ B (B), the expression of CD11b (C), CD68 (D), IBA1(E), and IL-6 secretion (F). Data were analyzed using one-way ANOVA with Bonferroni's post hoc test or Student's t test (* P < 0.05, ** P < 0.01, *** P < 0.001 [control vs. A β]; # P < 0.05, ## P < 0.01, ### P < 0.001 [A β vs. A β /EVP4593], n = 3; means \pm SEM). A β : A β oligomers; EVP4593: NF- κ B inhibitor.

Supplementary Fig. 3. IL-6 IgG neutralized the effect of HMC3 activated-

conditional medium in SH-SY5Y-derived neurons. (A-B) IL-6 IgG (5 ng/ml for 48 hours) reduced the expression cleaved caspase 3 in SH-SY5Y-derived neurons treated with HMC3-conditioned medium. (C-D) IL-6 IgG reduced ROS production in SH-SY5Y-derived neurons treated with HMC3-conditioned medium. (E) The impaired

neurite outgrowth by HMC3 conditioned medium was rescued by IL-6 neutralizing antibody. Images were measured using MetaMorph software. Scale bar, 25 μ m. Data were analyzed using one-way ANOVA with Bonferroni's post hoc test (* $P < 0.05$, *** $P < 0.001$ [control vs. HMC3 C.M; control vs. HMC3 C.M/IL-6 IgG]; # $P < 0.05$ [HMC3 C.M vs. HMC3 C.M/IL-6 IgG], $n = 3$; means \pm SEM). HMC3 C.M: HMC3-conditioned medium; IL-6 IgG: IL-6 neutralized antibody.

Supplementary Fig. 4. TYK2 inhibitor suppressed IL-6 or HMC3 conditioned medium induced apoptosis in SH-SY5Y-derived neurons. (A-B) In SH-SY5Y-derived neurons, the upregulation of cleaved caspase 3 by IL-6 (350 pg/mL) or HMC3 conditioned medium was counteracted by treatment with TYK2 inhibitor (1 μ M) for 48 hours. Data were analyzed using one-way ANOVA with Bonferroni's post hoc test (** $P < 0.01$, *** $P < 0.001$ [control vs. IL-6; control vs. HMC3 C.M]; # $P < 0.05$ [IL-6 vs. IL-6/TYK2 inhibitor]; *** $P < 0.001$ [HMC3 C.M vs. HMC3 C.M/TYK2 inhibitor], $n = 3$; means \pm SEM). HMC3 C.M: HMC3 activated-conditioned medium.