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Supplementary Materials for

The Protease Omi Cleaves the Mitogen-Activated Protein Kinase Kinase MEK1 to Inhibit Microglial Activation

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Fig. S1. The amounts of pERK1/2 proteins in the brains of *mnd2* mice are increased compared with those of age-matched wild-type mice. Western blotting analysis was performed to show that pERK1/2 protein abundance is higher in the brains of *mnd2* mice than in those of WT mice of the same age. Twenty-five day-old *mnd2* mice and WT mouse brain lysates were subjected to Western blotting analysis with antibodies against the indicated proteins. The lower bar graph shows the band intensities of pERK1/2 relative to those of GAPDH. Values are the mean \pm SEM from three independent experiments. *P < 0.05 by one-way ANOVA (n = 3 pairs of mice).



Fig. S2. Omi protein abundance is unaffected by transfection or control siRNA. Western blotting analysis was performed to show knockdown of Omi in SH-SY5Y cells. SH-SY5Y cells were left untransfected as a control or were transfected with control siRNA or si-*Omi*. Forty-eight hours later, cell lysates were subjected to Western blotting analysis with antibodies against the indicated proteins. The bar graph shows the band intensities of Omi relative to those of GAPDH. Values are the mean \pm SEM from three independent experiments. *P < 0.05 by one-way ANOVA.



Fig. S3. The protease activity of Omi is required for the decrease in EGFP-MEK1 abundance. Western blotting analysis was performed to show that HA-Omi, but not HA alone or HA-Omi S276C, decreases EGFP-MEK1 protein abundance in N2a cells. N2a cells were cotransfected with plasmids encoding EGFP-MEK1 and HA, HA-Omi, or HA-Omi S276C. Twenty-four hours later, cell lysates were subjected to Western blotting analysis with antibodies against the indicated proteins. The bar graph shows the band intensities of EGFP-MEK1 relative to those of GAPDH. Values are the mean \pm SEM from three independent experiments. *P < 0.05 by one-way ANOVA.



Fig. S4. MEK1 directly interacts with Omi in vitro. In vitro pull-down assays were performed to show that GST-MEK1, but not GST alone, interacts with His-Omi.



Fig. S5. Omi cleaves the substrate β -casein in vitro. (A) In vitro cleavage assays show that β -casein, but not GST alone, is cleaved by His-Omi and not by His-Omi S276C. β -casein was incubated in vitro with 3 µg of WT Omi or 3 µg of protease-inactive S276C mutant Omi for 90 min in protease buffer at 37°C. (B) In vitro cleavage assays show that GST alone was not cleaved by His-Omi or His-Omi S276C. GST was incubated in vitro with 3 µg of WT Omi or 3 µg of protease-inactive S276C mutant Omi for 90 min and not by His-Omi or 3 µg of protease assays show that GST alone was not cleaved by His-Omi or His-Omi S276C. GST was incubated in vitro with 3 µg of WT Omi or 3 µg of protease-inactive S276C mutant Omi for 90 min in protease buffer at 37°C.



Fig. S6. PD98059 blocks the decrease in the abundance of I κ B α that is induced by knockdown of Omi. BV2 cells were transfected with si-NC or si-*Omi*. Twenty-four hours later, cells were treated with DMSO or 20 μ M PD98059 for a further 24 hours Cell lysates were subjected to Western blotting analysis with antibodies against the indicated proteins. The bar graph shows the band intensities of I κ B α relative to those of GAPDH. Values are the mean \pm SEM from three independent experiments. *P < 0.05 by one-way ANOVA.



Fig. S7. Analysis of cytokine mRNA abundances in the brains of *mnd2* mice. RT-PCR assays were performed to show that COX-2, IL-1 β , and TNF- α mRNA abundances are increased in the brains of *mnd2* mice compared to age-matched wild-type mice. Three independent experiments were performed.