

Supplemental material

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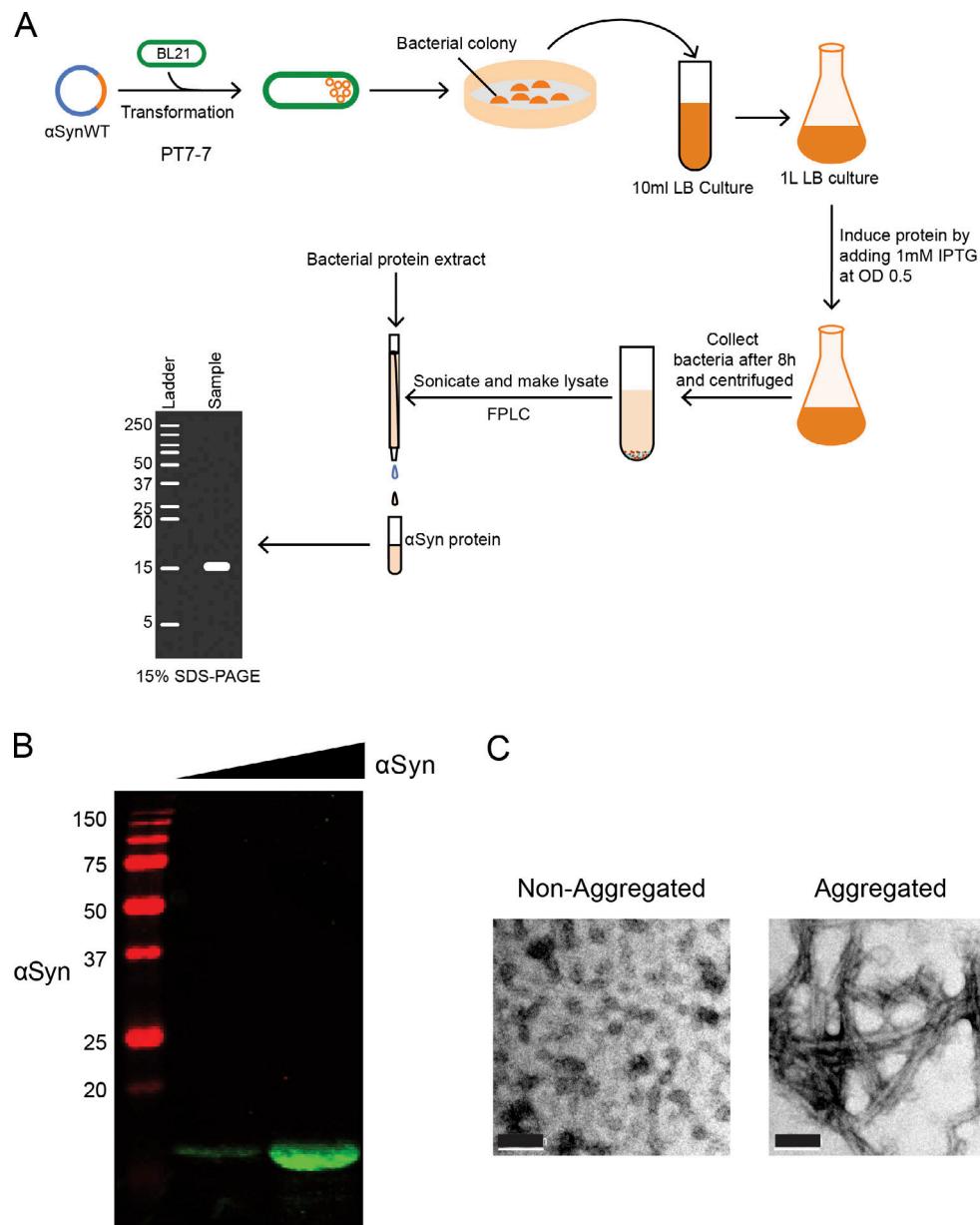


Figure S1. Obtaining and purifying human α Syn. **(A)** Human α Syn was obtained and purified as described in Materials and methods. FPLC, fast protein liquid chromatography; OD, optical density; IPTG, isopropyl β -D-1-thiogalactopyranoside. **(B)** Immunoblot for purified α Syn obtained after chromatography. **(C)** Electron microscopy pictures of unaggregated and aggregated α Syn. Scale bars, 100 nm.

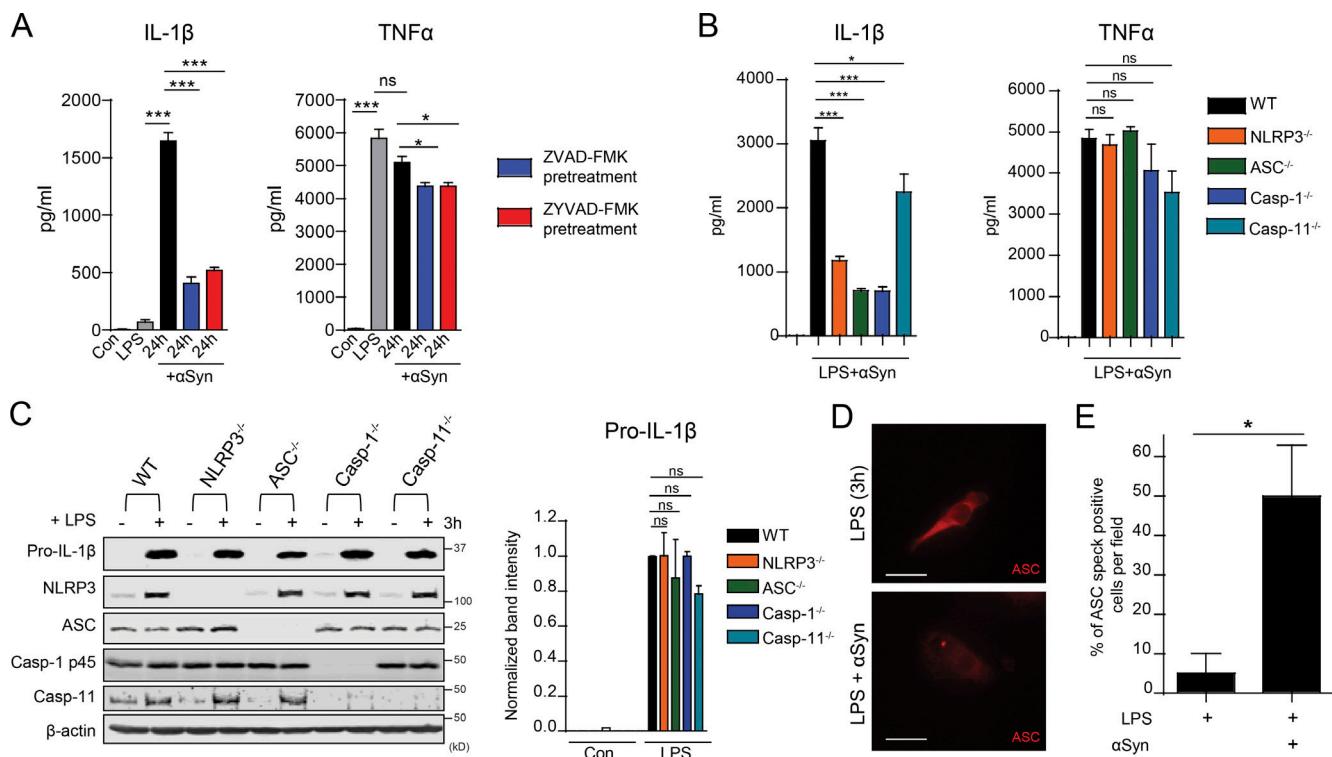


Figure S2. Aggregated αSyn acts as a danger signal to elicit NLRP3 inflammasome-dependent IL-1 β processing in LPS-primed microglia. (A) Luminex assay shows that αSyn elicits significant IL-1 β , but not TNF α production, 24 h after stimulation in LPS-primed microglia. Pretreatment of the cells with pan-caspase and Casp-1-specific inhibitors after priming but before αSyn treatment strongly attenuated the production of IL-1 β but minimally affected TNF α production. Error bars represent mean \pm SEM. One-way ANOVA followed by Tukey's post hoc test ($n = 3$). (B) Supernatant cytokine analysis from LPS-primed, αSyn-treated WT, NLRP3 $^{-/-}$, ASC $^{-/-}$, Casp-1 $^{-/-}$, and Casp-11 $^{-/-}$ BMDMs revealed strongly diminished IL-1 β , but not TNF α production, from NLRP3 $^{-/-}$, ASC $^{-/-}$, and Casp-1 $^{-/-}$ cells but minimally affected IL-1 β production from Casp-11 $^{-/-}$ cells, indicating that the canonical activation of the NLRP3 inflammasome was primarily responsible for the IL-1 β production in response to aggregated αSyn. Error bars represent mean \pm SEM. One-way ANOVA followed by Tukey's post hoc test ($n = 3$). (C) No discernible changes in LPS-induced pro-IL-1 β levels in WT, NLRP3 $^{-/-}$, ASC $^{-/-}$, Casp-1 $^{-/-}$, and Casp-11 $^{-/-}$ BMDMs. Error bars represent mean \pm SEM. One-way ANOVA followed by Tukey's post hoc test ($n = 3$). Con, control. (D and E) Aggregated αSyn treatment induced ASC speck formation in primary microglia. Error bars represent mean \pm SEM. Unpaired two-tailed t test ($n = 3$). Asterisks indicate the level of statistical significance: *, $P \leq 0.05$; ***, $P \leq 0.001$; ns, not significant. Scale bars, 15 μ m.

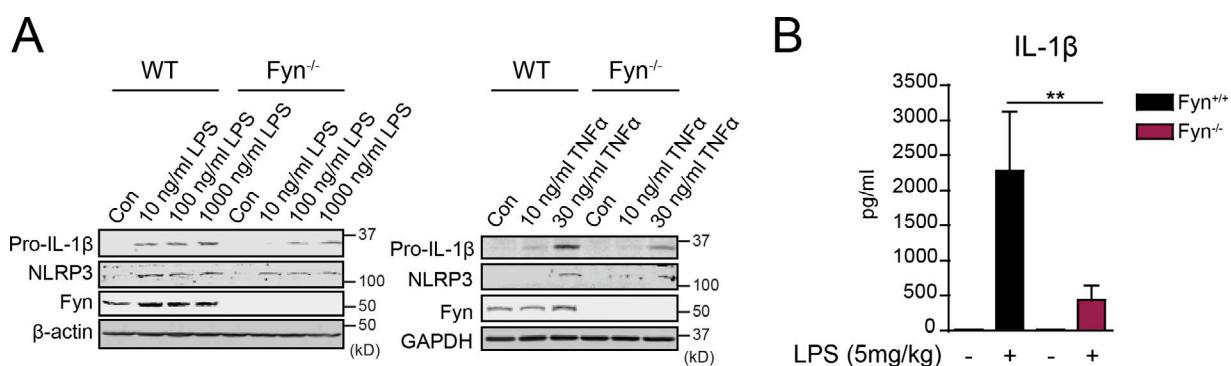


Figure S3. Fyn kinase contributes to the priming of the NLRP3 inflammasome in response to diverse inflammatogens. (A) WT and Fyn $^{-/-}$ microglia were treated with various doses of LPS (10, 100, and 1,000 ng/ml) and TNF α (10 and 30 ng/ml). Both inflammatogens elicited a dose-dependent induction of pro-IL-1 β and NLRP3 in WT microglia but did so to a significantly lesser extent in Fyn $^{-/-}$ microglia. Con, control. (B) Fyn $^{-/-}$ mice treated with LPS (5 mg/kg) for 24 h showed diminished serum secretion of IL-1 β when compared with Fyn $^{+/+}$ mice. Error bars represent mean \pm SEM. One-way ANOVA followed by Tukey's post hoc test ($n = 5$ mice per group). Asterisks indicate the level of statistical significance: **, $P \leq 0.01$.

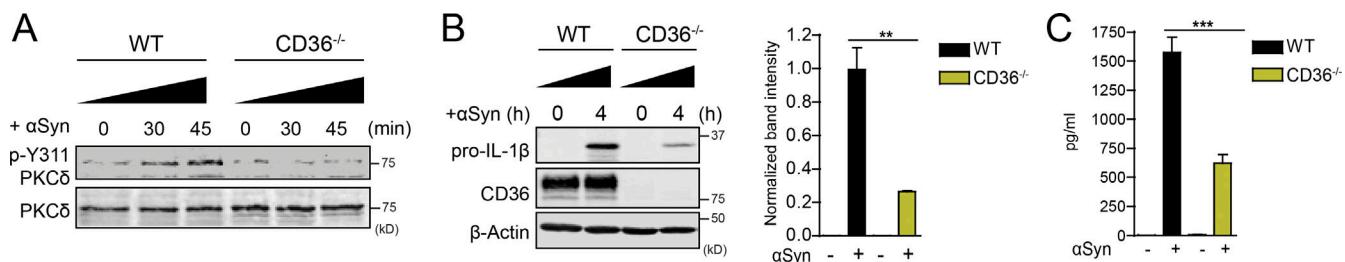


Figure S4. CD36 contributes to αSyn-mediated priming of the NLRP3 inflammasome. **(A–C)** Diminished αSyn induced p-Y311 PKCδ phosphorylation (A), pro-IL-1β induction (B), and IL-1β secretion (C) from CD36-deficient macrophages. One-way ANOVA followed by Tukey's post hoc test ($n = 3$). Asterisks indicate levels of statistical significance: **, $P \leq 0.01$; ***, $P \leq 0.001$.

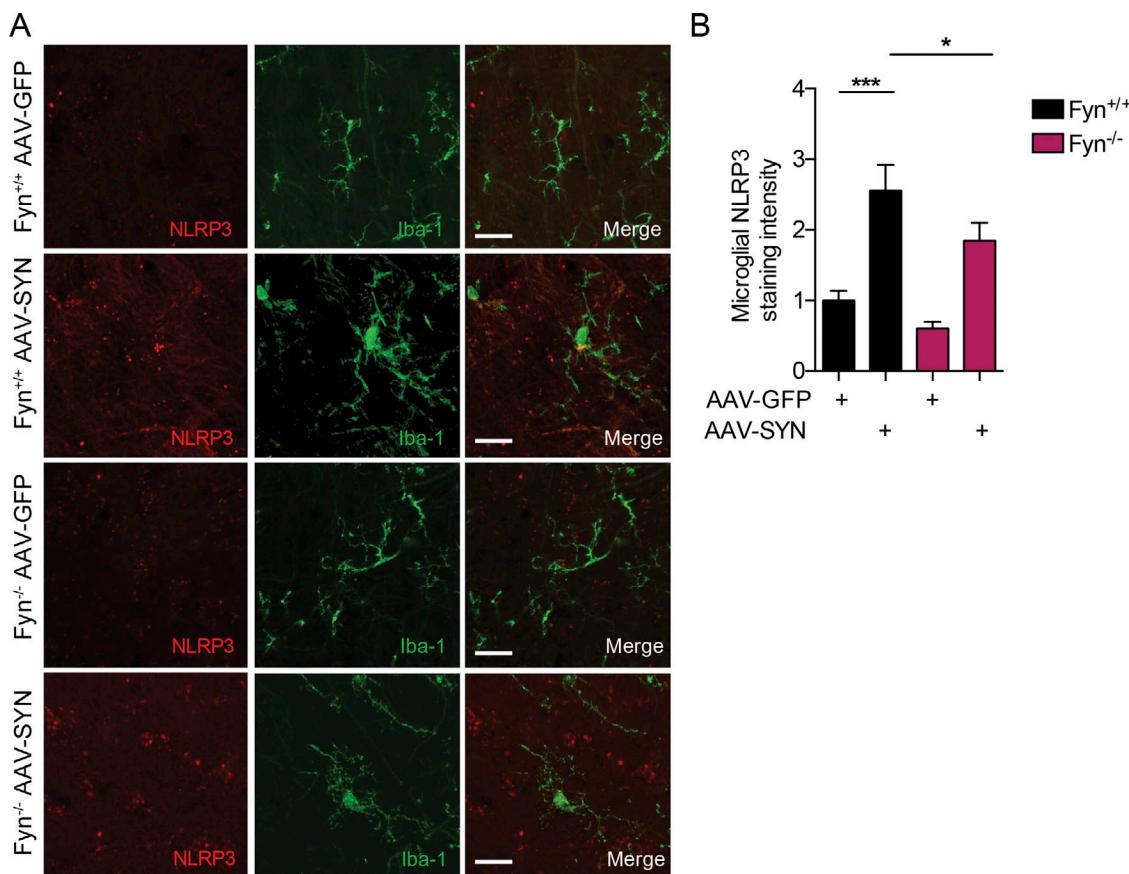


Figure S5. Reduced αSyn-induced microglial NLRP3 induction in Fyn-deficient mice. **(A)** AAV-SYN injection in Fyn^{+/+}, but not Fyn^{-/-}, mice elicits microglial NLRP3 induction ventral midbrain, as seen by double IHC for Iba-1 and NLRP3. Scale bars, 15 μm. **(B)** Quantification of the microglial NLRP3 expression upon AAV-GFP or AAV-SYN injection in Fyn^{+/+} and Fyn^{-/-} ventral midbrain sections. Error bars represent mean ± SEM. One-way ANOVA followed by Tukey's post hoc test ($n = 5$ mice per group). Asterisks indicate the level of statistical significance: *, $P \leq 0.05$; ***, $P \leq 0.001$.

Table S1. Details of postmortem PD and control SN brain samples

Sample number	FDX	CERAD	Braak	Age (yr)	Sex	Race	PMI	Brain region
2544	PD with dementia	B	4	89	M	W	16	SN
2545	PD, neurofib degen, cerebrovas (NC)	0	4	95	F	W	14	SN
2559	PD and dementia	B	5	81	M	W	8	SN
2570	PD, AD probable	B	4	70	M	W	5.5	SN
2572	PD with dementia, AD probable	B	1	79	F	W	7	SN
2573	PD with dementia, AD probable, mixed AD and PD	B	1	69	M	A	17	SN
0384	Control			68	M	W	14	SN
0430	Control			80	M	B	21	SN
0705	Control			73	M	W	9	SN
0710	Control			62	M	W	14	SN
0713	Control			73	F	W	9	SN
0989	Control			59	M	W	7	SN
HBBT_17_01	PD			73	M	AA		SN
HBCW_17_01	PD			88	M	C		SN
HBGK_17_08	PD			74	M	H		SN
HBBW_17_01	PD			79	M	C		SN
HAAW_18_07	PD			72	M	W		SN
HBFS_18_08	PD			91	M	W		SN
HctZV_17_13	Control			68	M	H		SN
HctYP_17_27	Control			75	M	C		SN
HctZZT_17_11	Control			85	M	C		SN
HctYY_17_08	Control			90	M	C		SN
HctZP_17_23	Control			77	M	C		SN
HCT16HDY_18_19	Control			91	F	W		SN

A, Asian; AA, African American; B, Black; C, Caucasian; CERAD, Consortium to Establish a Registry for Alzheimer's Disease neurocognitive battery test result; F, female; FDX, final diagnosis; H, Hispanic; M, male; PMI, postmortem interval (h); W, white; neurofib cerebrovas (NC), neurofibrillary degeneration (neurofibrillary tangles) not contributing to dementia.