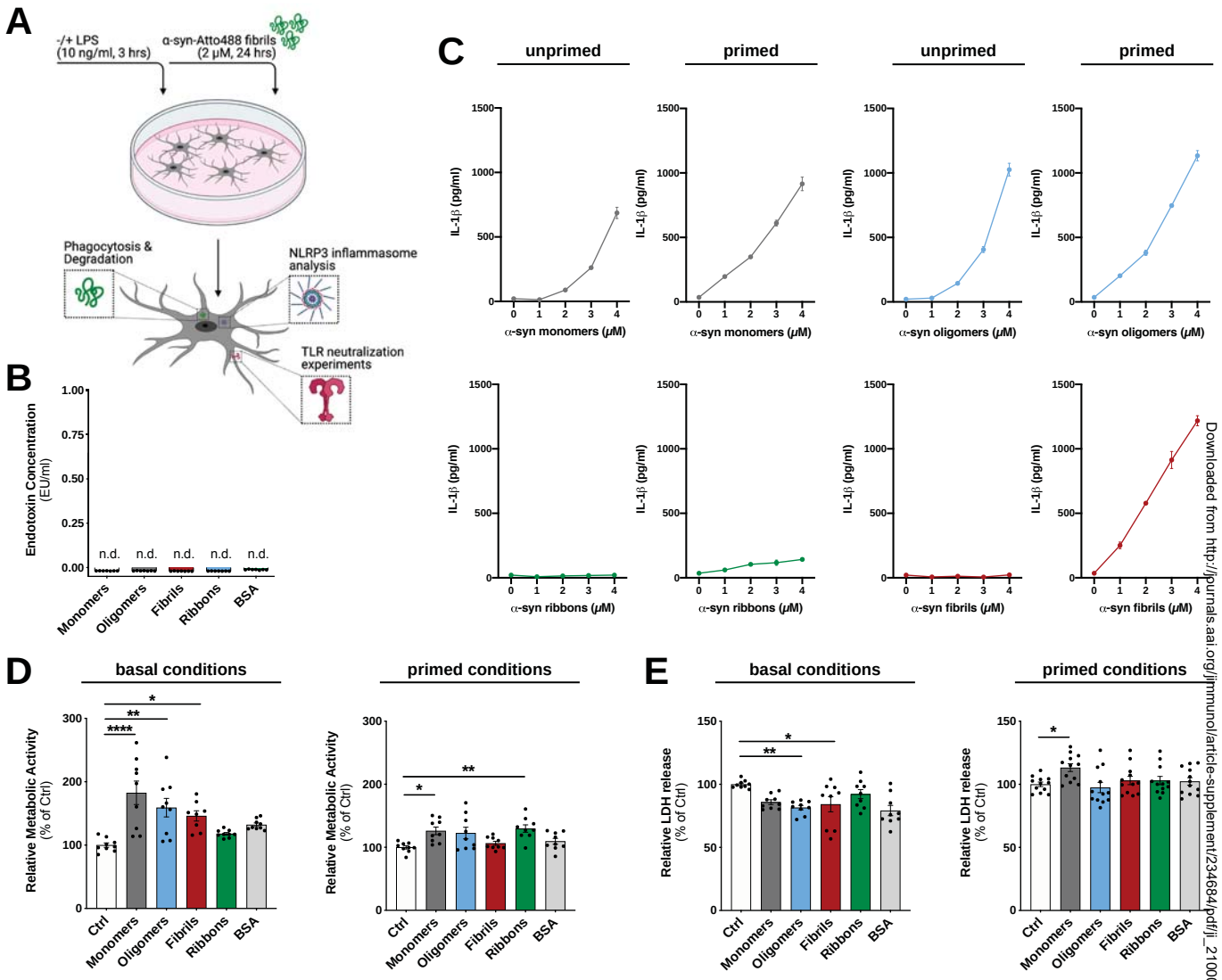

Figure S1

(A) Schematic drawing of the experimental setup used in this study. **(B)** Quantification of the different α-syn forms for endotoxin verify the preparations as endotoxin-free. (n=3 independent experiments with triplicated measurements). **(C)** Quantification of IL-1β levels in conditioned medium of primary microglia treated for 24 hrs with different concentrations of distinct α-syn forms. (n=4 independent experiments with dublicated measurements). Quantification of the relative metabolic activity **(D)** and the relative LDH release **(E)** by microglia treated with 2 μM α-syn under basal conditions or after pre-exposure to LPS. See also Fig. 1. All graphs are presented as mean ± SEM and were analysed by one-way ANOVA followed by Tukey's multiple comparison post hoc test. Levels of significance are: ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

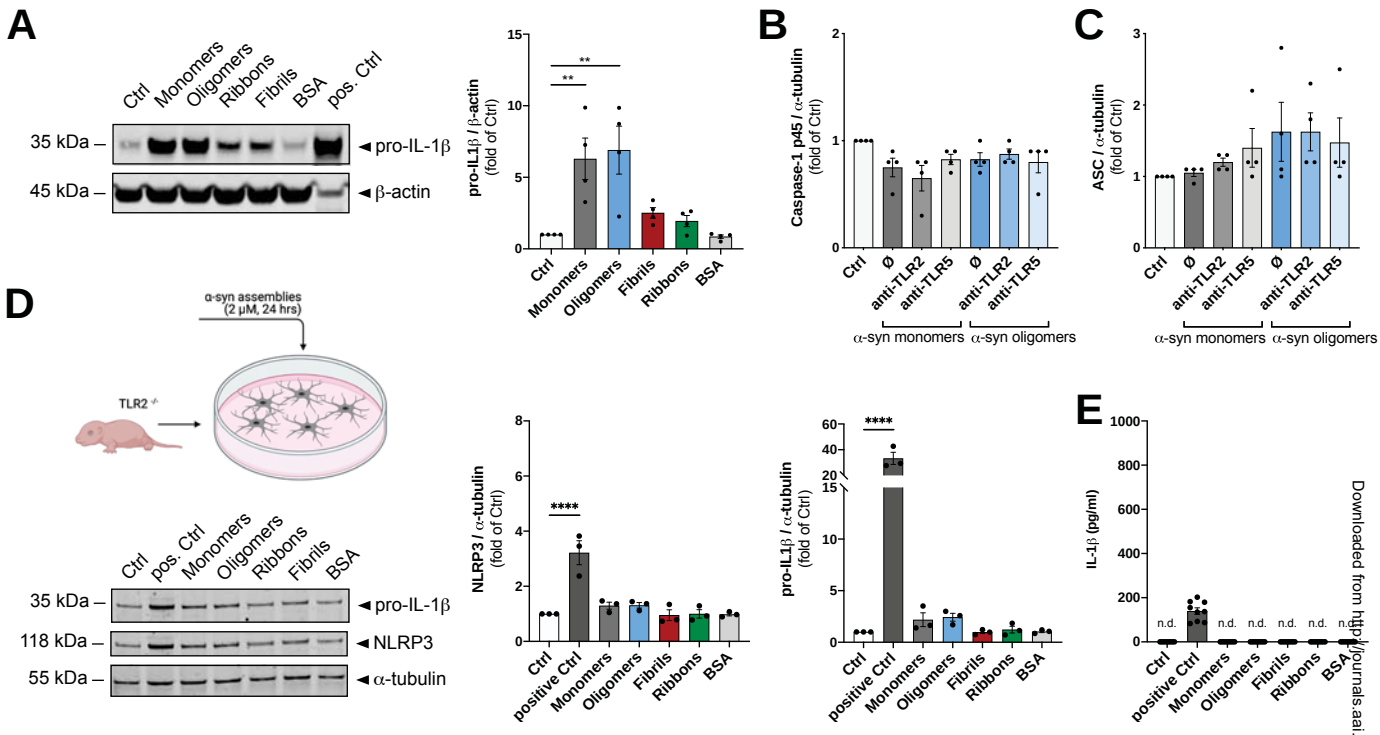

Figure S2

(A) Immunoblot analysis and quantifications of pro-IL1β levels in microglia treated with different α-syn assemblies. Quantification of caspase-1 p45 **(B)** and ASC **(C)** expression upon treatment with TLR2 and TLR5 neutralizing antibodies. (n=4 independent experiments). **(D)** Immunoblot analysis and quantifications of pro-IL1β and NLRP3 levels in microglia derived from TLR2$^{-/-}$ mice treated with different α-syn assemblies. (n=3 independent experiments). **(E)** Quantification of IL-1β levels in conditioned medium of primary microglia derived from TLR2$^{-/-}$ mice treated for 24 hrs with 2 μM distinct α-syn forms and 200 nM CRID3. (n=3 independent experiments with triplicated measurements). See also Fig. 2. All graphs are presented as mean ± SEM and were analysed by one-way ANOVA followed by Tukey's multiple comparison post hoc test **(A, D-E)** or Kruskal-Wallis test for nonparametric data **(B-C)**. Levels of significance are: ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

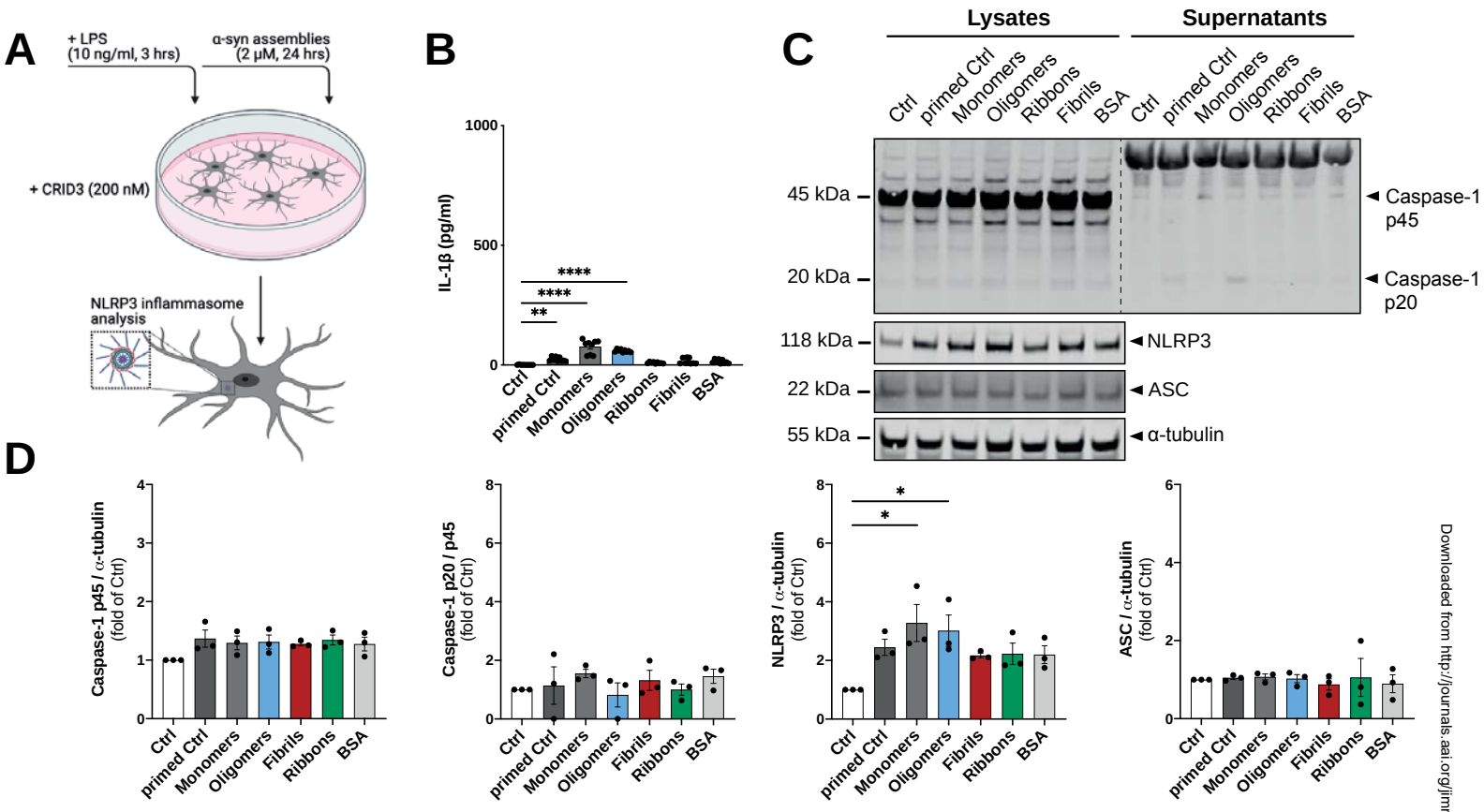

Figure S3

(A) Schematic drawing of the experimental setup used for the following experiments **(B-D)**. **(B)** Quantification of IL-1β levels in conditioned medium of LPS-primed primary microglia treated for 24 hrs with 2 μM distinct α-syn forms and 200 nM CRID3. (n=3 independent experiments with triplicated measurements). **(C-D)** Immunoblot and quantification of primed microglia cell lysates and supernatants exposed to 2 μM α-syn and 200 nM CRID3 for 24 hrs, stained for caspase-1, NLRP3, ASC and α-tubulin. All graphs are presented as mean ± SEM and were analysed by one-way ANOVA followed by Tukey's multiple comparison post hoc test. Levels of significance are: ****p < 0.0001, **p < 0.01, *p < 0.05.

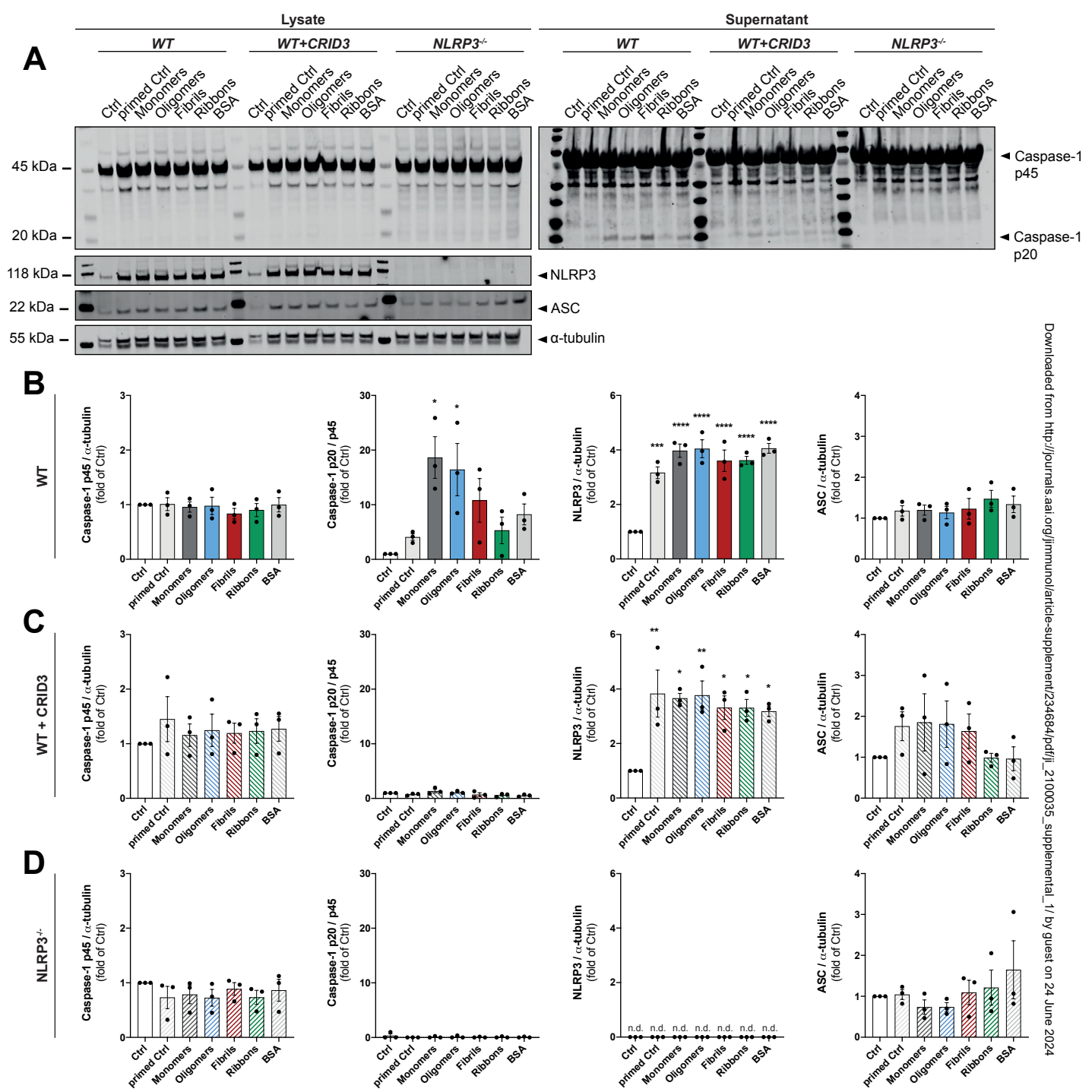

Figure S4

(A) Immunoblot of cell lysates (left) and supernatants (right) of primary microglia derived from WT or NLRP3$^{-/-}$ mice exposed to 2 µM α-syn and 200 nM CRID3 for 24 hrs. Blots were stained for caspase-1, NLRP3, ASC and α-tubulin. Quantification of Caspase-1 p45, cleaved Caspase-1 p20, NLRP3, and ASC in WT **(B)**, WT+CRID3 **(C)**, and NLRP3-/- **(D)**. n=3 independent experiments. All graphs are presented as mean ± SEM and were analysed by one-way ANOVA followed by Tukey's multiple comparison post hoc test. Levels of significance are: ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

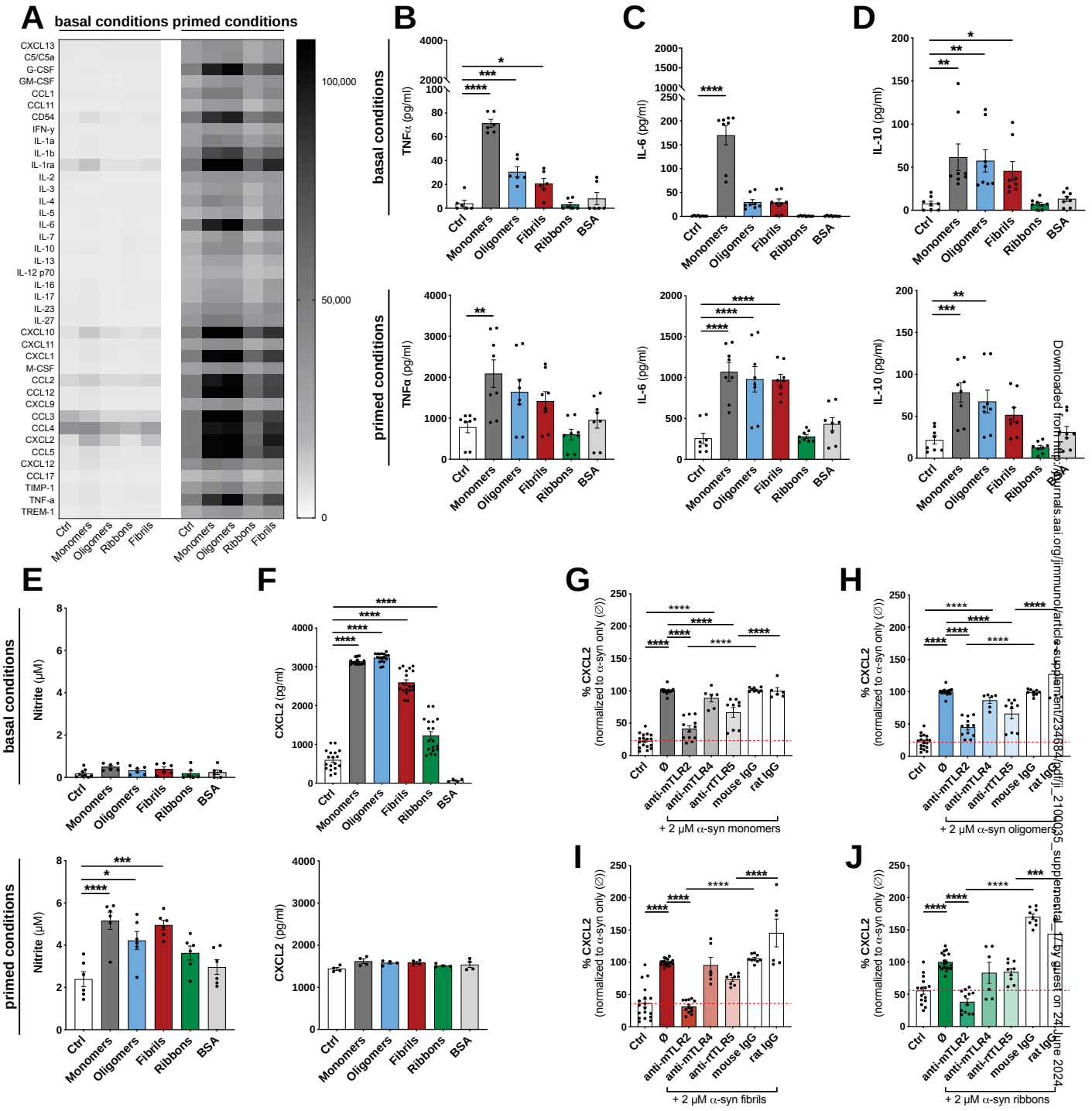

Figure S5

(A) Proteome profiling array on supernatants of primary microglia treated with 2 μM distinct α-syn forms without or with pre-exposure to LPS for 3 hrs. Quantification of TNFα **(B)**, IL-6 **(C)**, IL-10 **(D)**, Nitrite **(E)**, and CXCL2 **(F)** in supernatants of primary microglia treated with 2 μM distinct α-syn forms without (upper panel) or with (lower panel) pre-exposure to LPS for 3 hrs. (n=3-4 independent experiments with single, duplicate or triplicate measurements). CXCL2 levels in conditioned medium of unprimed primary microglia treated for 24 hrs with TLR2-, TLR4, and TLR5-neutralizing antibodies or the respective isotype controls in parallel to stimulation with α-syn monomers **(G)**, oligomers **(H)**, fibrils **(I)** and ribbons **(J)**. (n=4-6 independent experiments with duplicate or triplicate measurements). All graphs are presented as mean ± SEM and were analysed by one-way ANOVA followed by Tukey's multiple comparison post hoc test. Levels of significance are: ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

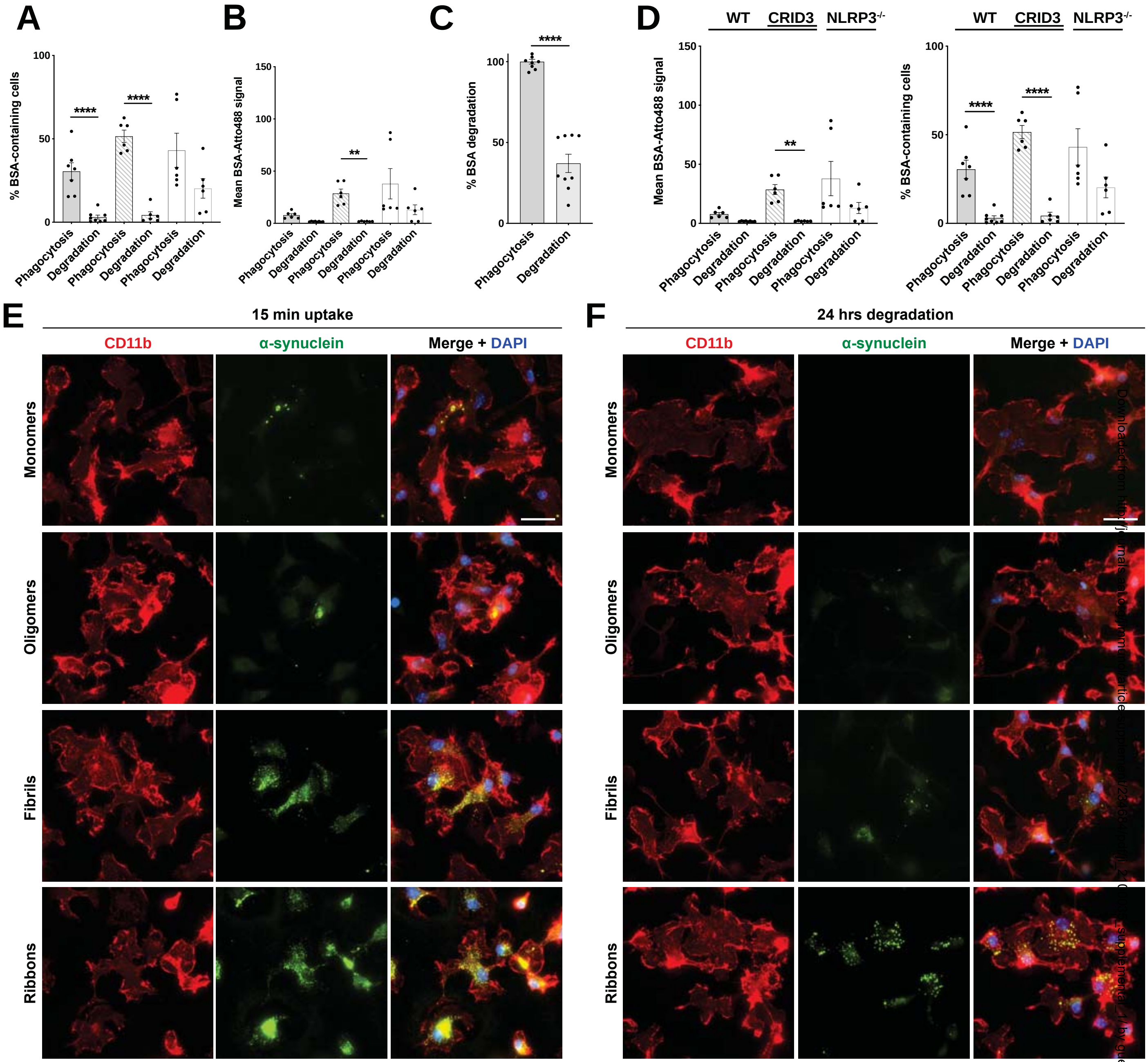

Figure S6

Quantification of the percent of phagocytic cells **(A)**, the phagocytic index **(B)**, and the percentage of degradation **(C)** of primary microglia exposed to BSA as protein control. (n=5-6 independent experiments). For comparison see also Fig. 4 and 5. **(D)** Quantification and comparison of phagocytosis and degradation of BSA by WT microglia (filled bars), WT microglia treated with the NLRP3 inflammasome inhibitor CRID3 (striped bars), or NLRP3-deficient microglia (empty bars). Representative immunostainings for CD11b-positive microglia (red) and distinct forms of α-syn-Atto488 (green) after 15 minutes of phagocytosis **(E)** or 24 hours of degradation **(F)**. All graphs are presented as mean ± SEM and were analysed by one-way ANOVA followed by Tukey's multiple comparison post hoc test. Levels of significance are: ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05. Scale bar: 20 µm.