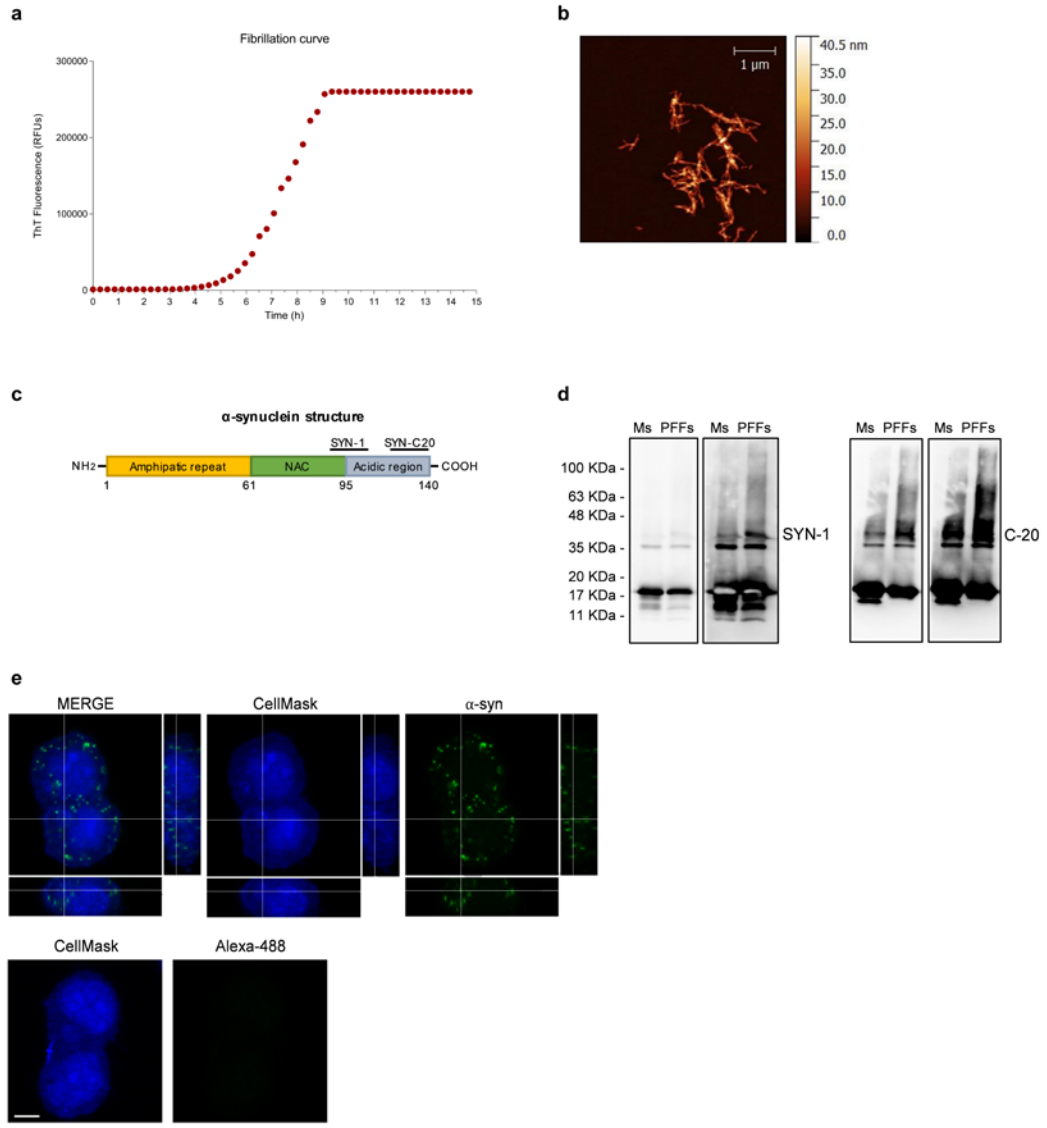
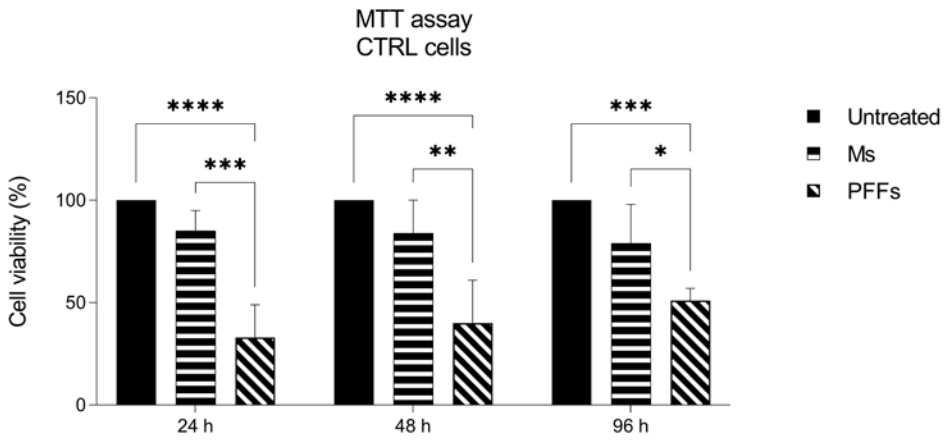
ADDITIONAL INFORMATION:

Supplementary Figure 1



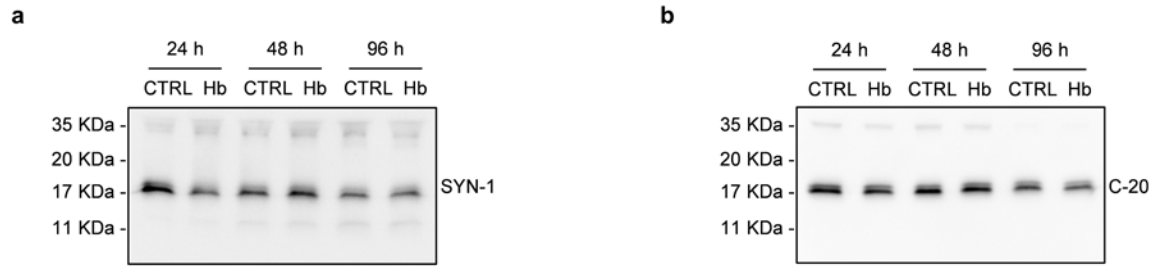
Biochemical analysis and structural characterization of α-syn preparations. Fibrillation curve of recombinant human α-syn protein analysed using ThT fluorescence assay. Mean of three wells is represented (**a**). AFM analysis of fibrillary human α-syn aggregates after 5 min of sonication (**b**). Schematic representation of the domains structure of human α-syn protein and epitopes of anti-α-syn antibodies used for the study (**c**). Ms and PFFs preparations were analysed by western blot with SYN-1 and C20 antibodies. The same membrane was exposed for a longer period in order to detect high molecular weight species (**d**). Sonicated α-syn fibrils internalization after quenching with Trypan Blue. Representative confocal microscopy images of Hb cells treated with Alexa-488 labelled PFFs for 24 h, after Trypan Blue quenching. Cells not incubated with labelled PFFs were used to establish autofluorescence levels. Entire cells were labelled by CellMask (**e**). Scale bar 10 µm.

Supplementary Figure 2



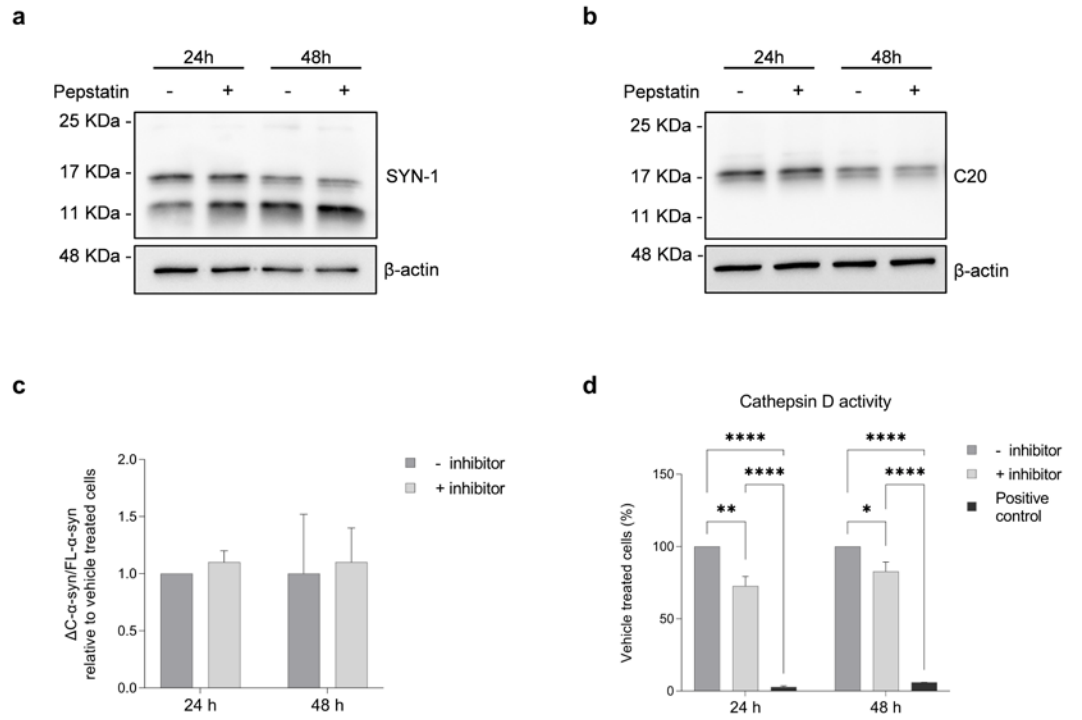
Cytotoxicity of α-syn preparations in iMN9D cells. Cells were treated with Ms and PFFs preparations for 24 h, 48 h, 96 h and cell viability was determined by MTT assay. Results are mean ± standard deviation of three independent experiments, each performed in four replicas and are expressed as percentage of untreated CTRL cells. Statistical analysis was performed with one-way Anova. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$; ns, not significant.

Supplementary Figure 3



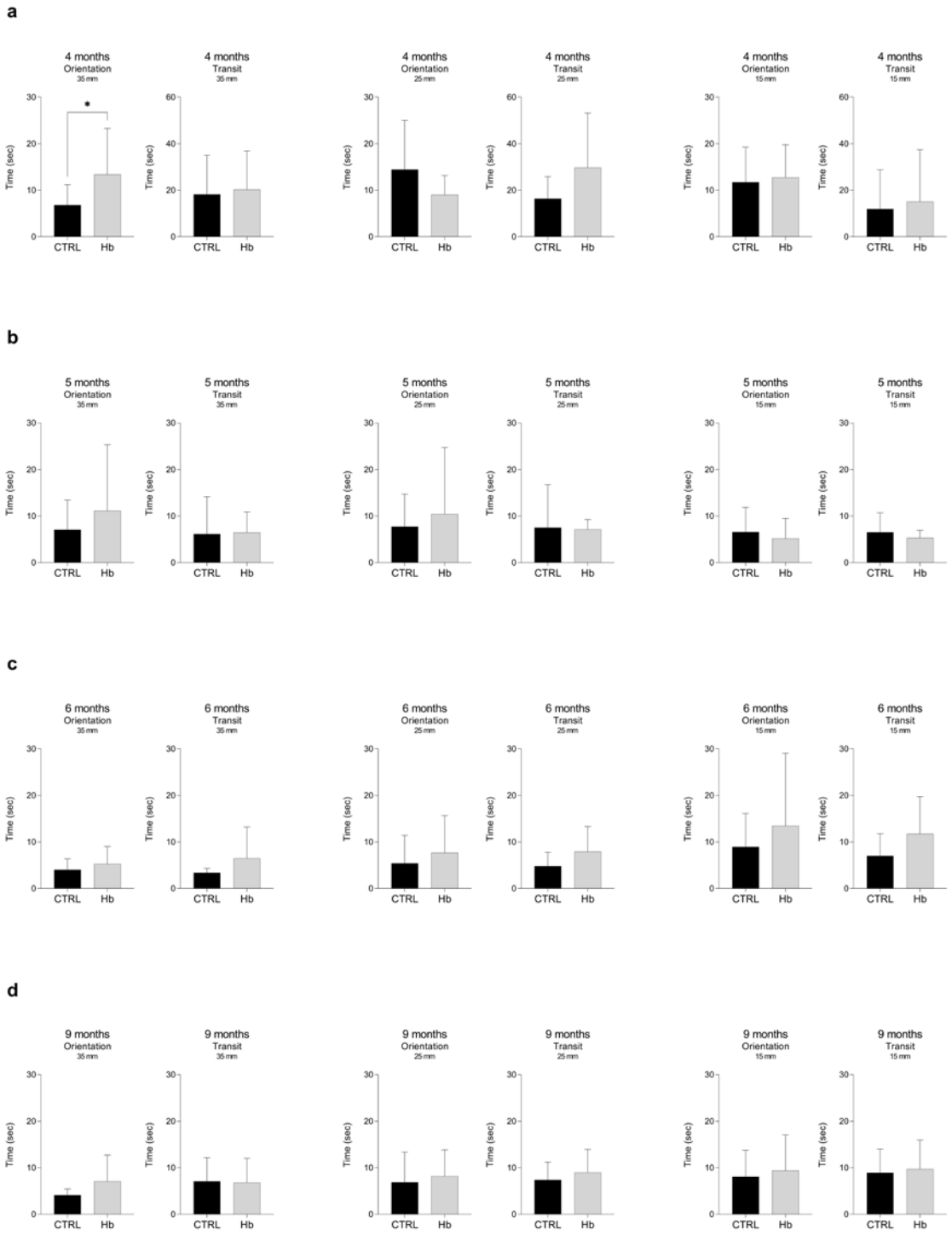
C-terminal truncated α-syn accumulation in the presence of Hb in cell media. CTRL and Hb cells were treated with α-syn amyloids and cell medium were collected at the indicated time points and analysed by immunoblotting with SYN-1 and C-20 antibodies.

Supplementary Figure 4



Effect of Cathepsin D inhibition on α-syn C-terminal truncated species accumulation in Hb cells. Cell lysates of Hb cells treated with DMSO (-) and pepstatin (+) were analysed by immunoblotting with SYN-1 (**a**) and C-20 (**b**) antibodies. Band intensity corresponding to ΔC-α-syn and FL-α-syn was quantified and the ratio was calculated. Data represent means ± SEM and are representative of five independent experiments. Statistical analysis was performed with one-way Anova. *, p ≤ 0.05; **, p ≤ 0.01; ***, p≤ 0.001; ****, p ≤ 0.0001; ns, not significant (**c**). Cell lysates of Hb cells treated with DMSO (-) and pepstatin (+) were analysed by Cathepsin D activity assay. Data represent means ± SEM and are representative of two independent experiments, each performed in three replicas and are expressed as percentage of vehicle treated cells. Statistical analysis was performed with one-way Anova. *, p ≤ 0.05; **, p ≤ 0.01; ***, p≤ 0.001; ****, p ≤ 0.0001; ns, not significant (**d**).

Supplementary Figure 5



Static rods test on AAV9-CTRL and AAV9-Hb mice. AAV9-CTRL (n=15) and AAV9-Hb (n=15) were assessed in static rods test measuring two parameters, transit time and orientation time (seconds) and different time points. Different diameters of the rods were used, 35, 25 and 15mm. The time points evaluated in this test were 4 (a), 5 (b), 6 (c) and 9 months (d) after injection. Data represent means ± SEM. Statistical analysis was performed with unpaired t test with Welch's correction. *, $p \leq 0.05$.