

Supplemental Material and Methods

Table S1: List and details of secondary antibodies

Secondary antibodies*	Host	Working dilution	Cat n°
Anti-rabbit IgG (H+L), Alexa Fluor 488	Goat	1:1000	A-11008
Anti-rabbit IgG (H+L), Alexa Fluor 647	Goat	1:300	A-21245
Anti-mouse IgG (H+L), Alexa Fluor 555	Goat	1:300	A-21422
Anti-mouse IgG (H+L), Alexa Fluor 647	Goat	1:300	A-21235
Anti-chicken IgY (H+L), Alexa Fluor 555	Goat	1:300	A-32932
Anti-chicken IgY (H+L), Alexa Fluor 647	Goat	1:300	A-32933

* Thermo Fisher Scientific

Supplementary results (Figure S1)

The genetic background of C57BL/6J mice may possibly influence the response of dopamine (DA) neurons to fibril seeds. Thus, we compared the impact of F91 seeds in midbrain cultures generated with either C57BL/6J or Swiss mouse embryos. Specifically, we estimated the percentages of DA cell somas with large α Sa in three-week cultures exposed two weeks earlier to 0.1 or 0.5 μ M of F91. Our data revealed that the percentage of DA neurons with large somal α S inclusions was quite similar in the two types of cultures, at a same concentration of fibrils (Figure S1a). DA cell survival remained also unchanged in F91-treated midbrain cultures generated from Swiss mouse embryos, regardless of the concentration of fibrils used to seed α S aggregation (Figure S1b). These results are illustrated by microphotographs in figure S1c.

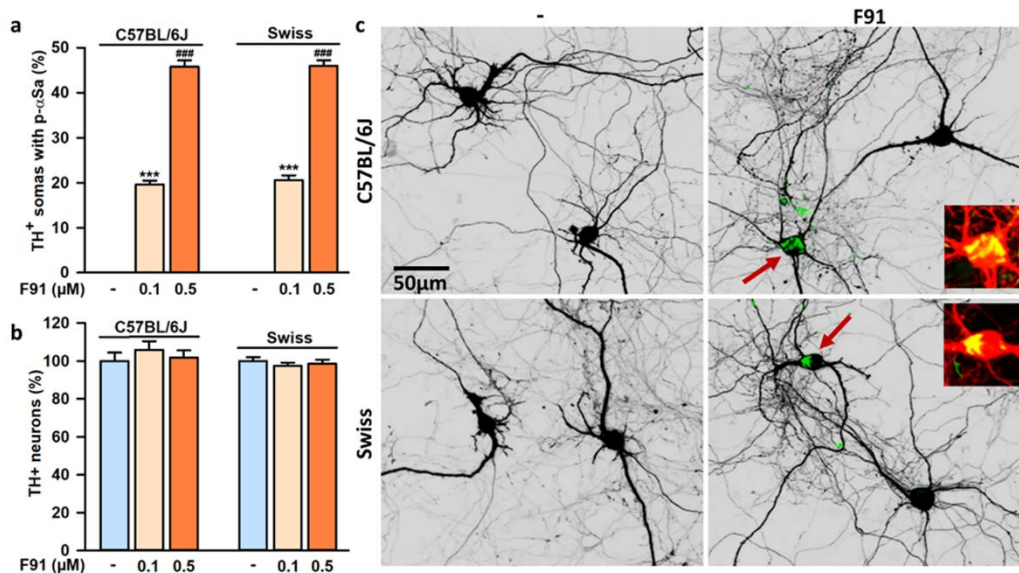


Figure S1: Impact of mouse lineage on α S seeded aggregation in midbrain DA neurons in culture. **(a)** Number of TH⁺ neurons with somal aggregates in three-week C57BL/6J and Swiss mouse midbrain cultures exposed two weeks earlier to 0.1 or 0.5 μ M F91. Data are means \pm SEM (n=5-16). ***p<0.001 vs. controls and ***p<0.001 vs. 0.1 μ M F91. One-way ANOVA followed by SNK test. **(b)** Survival of TH⁺ neurons in the same culture conditions as in **(a)** (n=5-16). **(c)** Representative microphotographs of TH⁺ neurons in three-week C57BL/6J and Swiss mouse midbrain cultures exposed 2 weeks earlier to 0.5 μ M F91. The immunosignal for p- α S corresponds to the green label and TH⁺ neurons are presented under an inverted black and white format. Red arrows point to DA cell bodies with somal α Sa. An enlarged version of TH⁺ somas (red) with p- α Sa (yellow after merging red and green) is shown in the inserts under a standard fluorescent format.