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Treatment with efavirenz extends survival in Creutzfeldt-Jakob disease model by regulating brain cholesterol metabolism

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Running title: EFV, an FDA-approved medication to treat human prion diseases

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Supplementary tables

Table S1: Details of mice at early clinical stage

Mouse ID	**sCJD Inoculation**	**Groups**	**Treatment**	**DPI**	**Early clinical stage**
2HsCJD-1-4065	-	sCJD	No treatment	176	Hunched posture, hindlimb clasping, rigid tail, rough coat, and weight loss
2HsCJD-1-4070	-	-	-	-	-
2HsCJD-1-4072	-	-	-	-	-
2HsCJD-1-4078	-	-	-	-	-
2HsCJD-1-4078	-	-	-	-	-
Mouse ID	**sCJD**	**sCJD+30 DPI**	**Oral treatment, EFV in drinking water**	**DPI**	**Early clinical stage**
2HsCJD-1-4110	-	-	-	176	Rigid tail, rough coat, mild hunched posture
2HsCJD-1-4111	-	-	-	-	-
2HsCJD-1-4112	-	-	-	-	-
2HsCJD-1-4120	-	-	-	-	-
2HsCJD-1-4124	-	-	-	-	-
Mouse ID	**sCJD**	**sCJD+130 DPI**	**Oral treatment, EFV in**	**DPI**	**Early clinical stage**

			drinking water		
2HsCJD-1-4128	-	-	-	176	Rigid tail, rough coat, mild hunched posture, and less weight loss
2HsCJD-1-4130	-	-	-	-	-
2HsCJD-1-4134	-	-	-	-	-
2HsCJD-1-4135	-	-	-	-	-
2HsCJD-1-4130	-	-	-	-	-

Table S2: Details of mice at terminal stage

Mouse ID	sCJD Inoculation	Groups	Treatment	DPI	Terminal stage of prion disease
2HsCJD-1-4066	-	sCJD	No treatment	181	Rough coat, rigid tail, imbalance, ataxia, hunched posture, hindlimb clasping, irresponsiveness, gait abnormalities, loss of righting reflex and weight loss
2HsCJD-1-4068	-	-	-	185	-
2HsCJD-1-4069	-	-	-	181	-
2HsCJD-1-4071	-	-	-	178	-
2HsCJD-1-4073	-	-	-	178	-
2HsCJD-1-4074	-	-	-	180	-
2HsCJD-1-4075	-	-	-	183	-
2HsCJD-1-4076	-	-	-	184	-
2HsCJD-1-4069	-	-	-	185	-

Mouse ID	sCJD	sCJD+ 30 DPI	Oral treatment, EFV in drinking water	DPI	Terminal stage of prion disease
2HsCJD-1-4112	-	-	-	197	Rough coat, rigid tail, imbalance, ataxia, hunched posture, hindlimb clasping, irresponsiveness, gait abnormalities, loss of righting reflex and weight loss

2HsCJD-1-4113	-	-	-	204	-
24sCJD-1-4114	-	-	-	187	-
2HsCJD-1-4115	-	-	-	197	-
2HsCJD-1-4116	-	-	-	211	-
2HsCJD-1-4117	-	-	-	194	-
2HsCJD-1-4118	-	-	-	208	-
2HsCJD-1-4119	-	-	-	166	Humane end point
2HsCJD-1-4120	-	-	-	210	-
2HsCJD-1-4121	-	-	-	185	-
2HsCJD-1-4123	-	-	-	219	-

Mouse ID	**sCJD**	**sCJD+ 130 DPI**	**Oral treatment, EFV in drinking water**	**DPI**	**Terminal stage of prion disease**
2HsCJD-1-4124	-	-	-	206	Rough coat, rigid tail, imbalance, ataxia, hunched posture, hindlimb clasping, irresponsiveness, gait abnormalities, loss of righting reflex and weight loss
2HsCJD-1-4125	-	-	-	210	-

2HsCJD-1-4126	-	-	-	191	-
24sCJD-1-4128	-	-	-	204	-
2HsCJD-1-4130	-	-	-	219	-
2HsCJD-1-4131	-	-	-	194	-
2HsCJD-1-4132	-	-	-	206	-
2HsCJD-1-4135	-	-	-	189	-
2HsCJD-1-4136	-	-	-	208	-
2HsCJD-1-4138	-	-	-	209	-

Table S3: Details of statistical analyses

Figures	Parameters analyzed	Groups	Statistical tests	p-Values	Post hoc analysis
Fig. 1	Animal survival	Non-treated VS treated	Log-rank (Montel-Cox) test	<0.0001	NA
		-	Gehan-Breslow-Wilcoxon test	<0.0001	-
Fig. 2B	Immunoblotting of PrP^{res} (3F4) at pre-clinical stage (176DPI)	-	Ordinary One-way ANOVA	<0.0092	-
Fig. 2C	IF of PrP^{res} (3F4)	-	-	<0.0062	-
Fig. 2D	Immunoblotting of PrP^{res} (4H11) in vitro	-	T-test (Unpaired t test)	<0.001	-
Fig. 2E	Immunoblotting of PrP^{res} (4H11) in vitro	-	T-test (Unpaired t test)	<0.05	-
Fig. 3A	Immunoblotting of CYP46A1	-	Ordinary One-way ANOVA	<0.0224	-
Fig. 3B	IF of CYP46A1	-	-	<0.0161	-
Fig. 3C	Brain 24SHC-ELISA	-	-	<0.0057	-
Fig. 3D	Serum 24SHC-ELISA	-	-	<0.0022	-
Fig. 3E	Media 24SHC-ELISA	-	T-test (Unpaired t test)	<0.1429	-
Fig. 3F	Media 24SHC-ELISA	-	T-test (Unpaired t test)	<0.001	-
Fig. 4A	Immunoblotting of SREBF2	-	Ordinary One-way ANOVA	<0.0002	-
Fig. 4B	IF of SREBF2	-	-	<0.0007	-
Fig. 4C	Filipin staining	-	-	<0.0018	-
Fig. 5A	IF of Perilipin	-	-	<0.0020	
Fig. 5B	IF of Perilipin	-	Two-way ANOVA	<0.0001	-
Fig. 5B	IF of GFAP	-	-	<0.0001	-
Fig. 4S	Immunoblotting of CYP46A1	-	T-test (Unpaired t test)	<0.0273	-
Fig. 6S	Brain 24SHC-ELISA	-	Ordinary One-way ANOVA	<0.0041	-
Fig. 7S	Media 24SHC-ELISA	-	T-test (Unpaired t test)	<0.05	-

Supplementary figures and legends

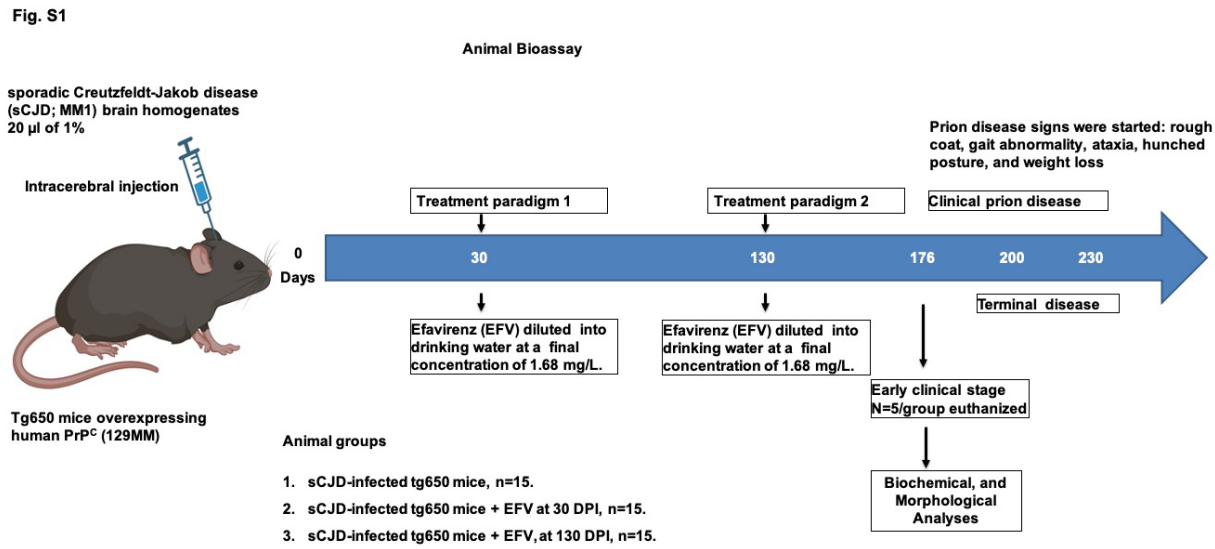


Fig S1. Schematic diagram illustrating the animal bioassay design and Efavirenz (EFV) treatment paradigms. The mouse and syringe images were created using BioRender.

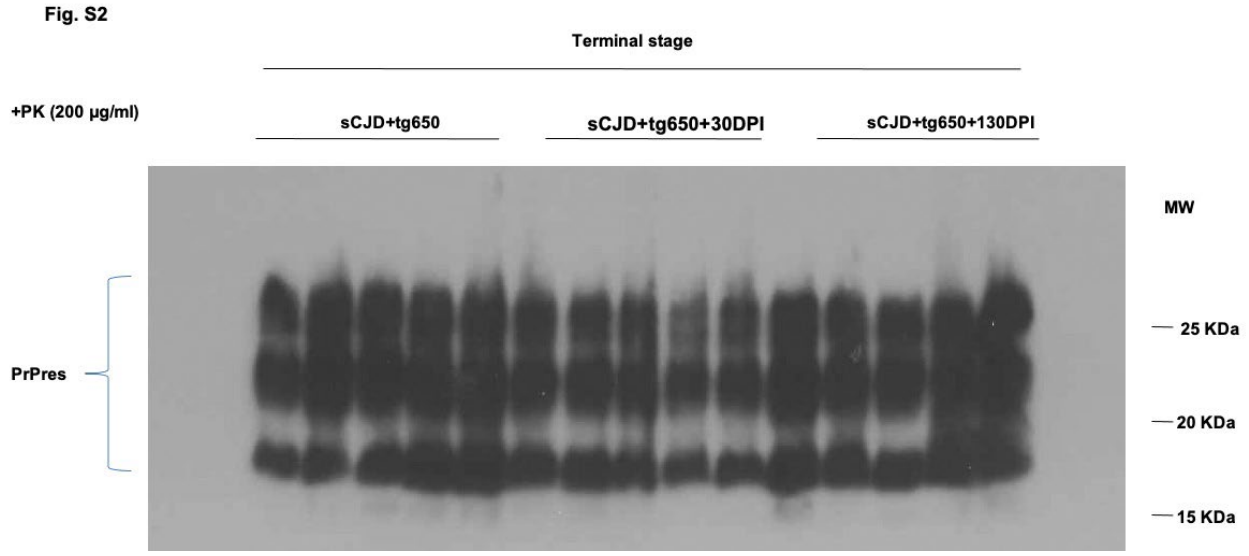


Fig. S2. PrP^{res} levels at the terminal stage of prion disease. Immunoblot analysis of uncropped PrPres using the 3F4 antibody in brain homogenates from non-treated sCJD-tg650 mice, and sCJD-tg650 mice EFV-treated at 30 DPI and 130 DPI.

Fig. S3

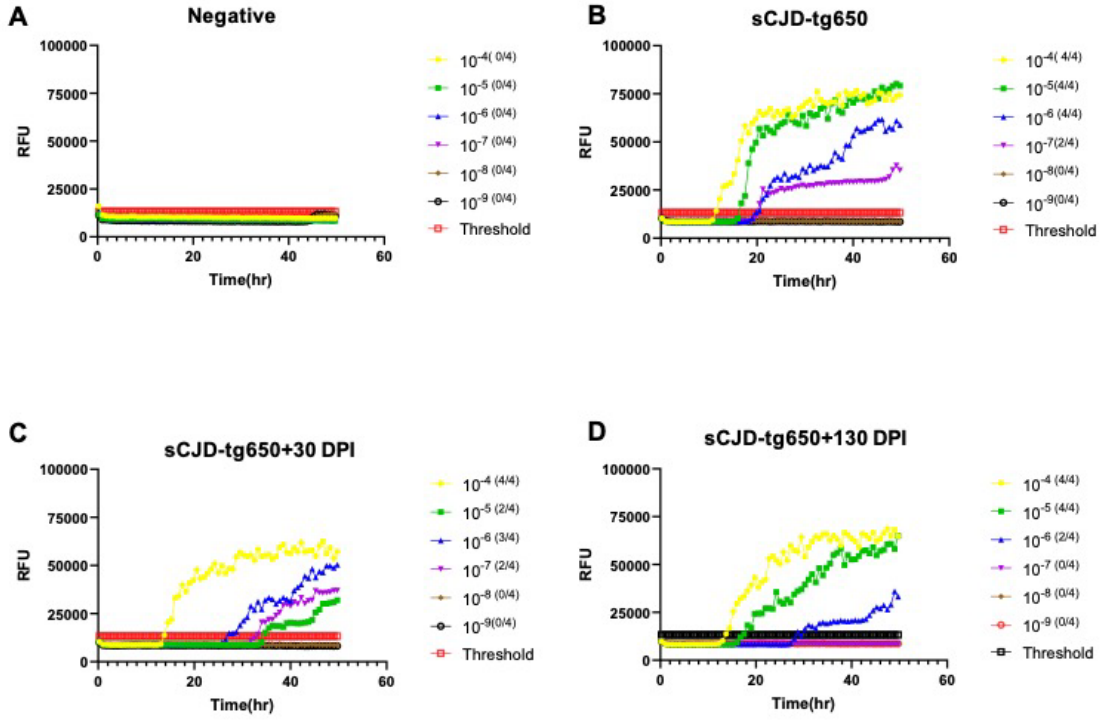


Fig. S3. Representative RT-QuIC graphs illustrating seeding activity in the brain homogenates at the early clinical stage (176 DPI) for all experimental groups including non-treated and treated (EFV treatment started at 30 DPI and 130 DPI) groups. The negative control consisted of brain tissue from age-matched, non-infected tg650 mice. Samples were considered positive if 2 out of 4 wells crossed the threshold, defined as the average RFU of the negative control group plus five times its standard deviation. The y-axis indicates RFU, while the x-axis represents time in hours (hr).

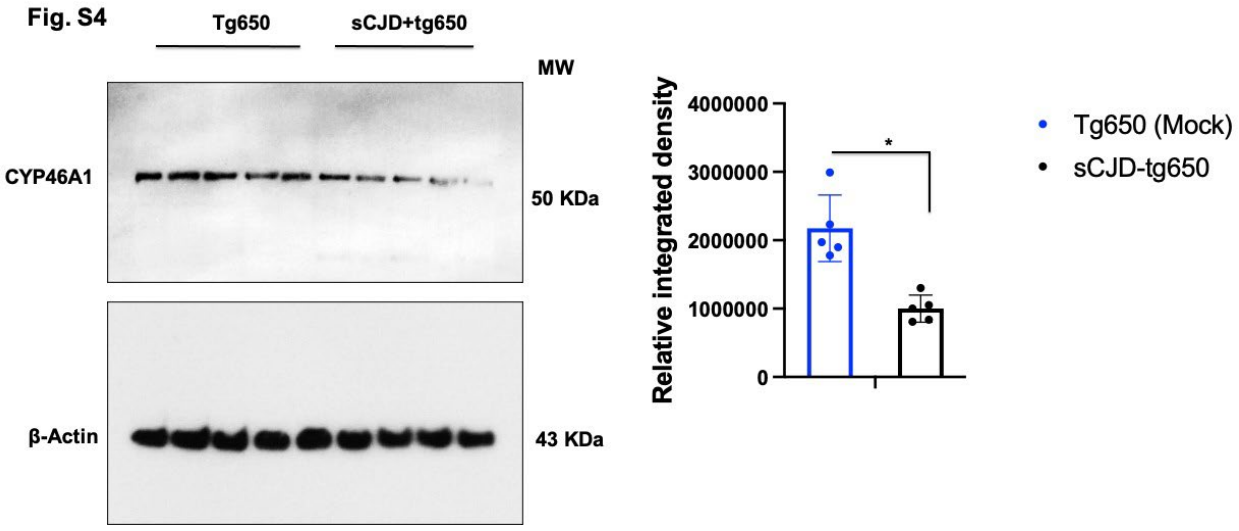


Fig. S4. Analysis of uncropped immunoblot and quantification of CYP46A1 in brain homogenates from five different non-infected (mock) tg650 mice and sCJD-tg650 mice at the terminal disease stage. The histograms are represented as the means ± SEM (n = 5 mice/group) of three independent experiments. T-test (unpaired t test) was performed, and statistical significance is $p<0.0273$.

Fig. S5

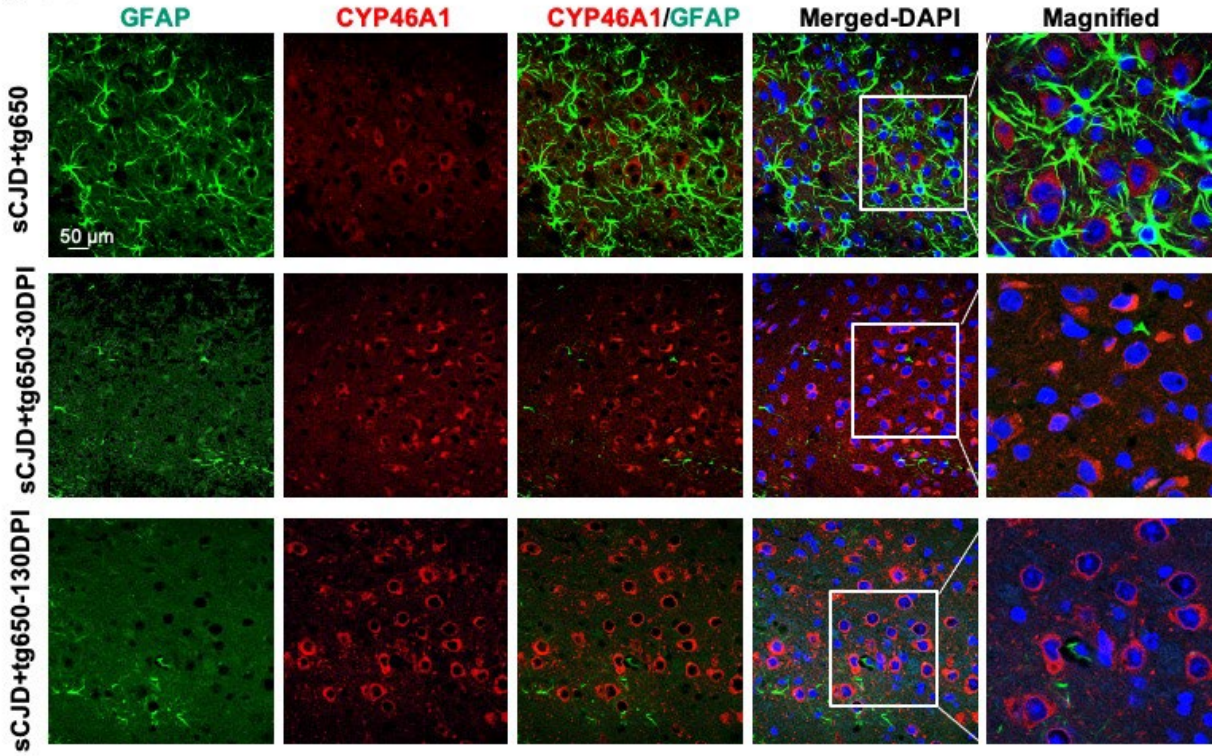


Fig. S5. Double immunofluorescence analysis of CYP46A1 and reactive astrocytes. Confocal images displaying double immunofluorescence staining for CYP46A1 (red) and GFAP (green) in the brains of sCJD-tg650, sCJD-tg650-30 DPI, and sCJD-tg650-130 DPI at pre-clinical stage (176 DPI). Magnification: 63X. Scale bar = 50 µm.

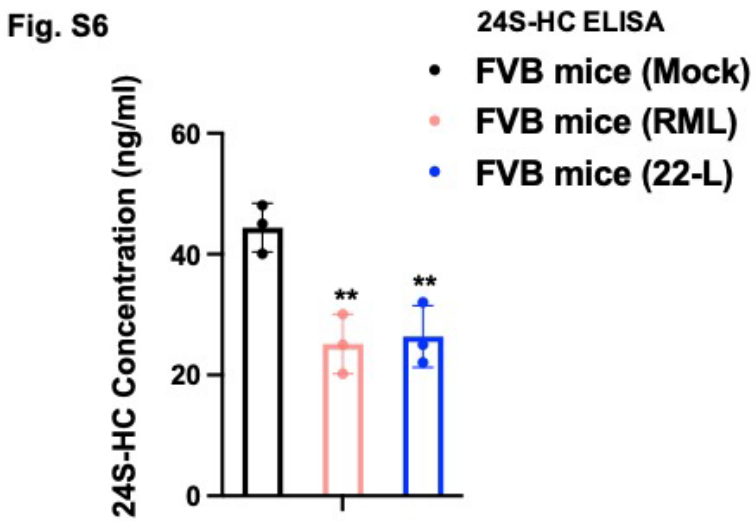
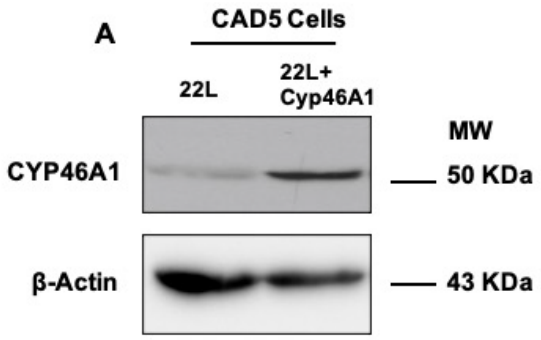


Fig. S6. ELISA quantification of the cholesterol metabolite 24S-hydroxycholesterol (24S-HC) in brain homogenates from FVB (non-infected, mock) mice and FVB mice infected with scrapie prions (RML and 22L). Histograms represent means ± SEM (n = 3 mice/group) from three independent experiments. Ordinary One-way ANOVA was performed, and statistical significance is $p<0.0041$.

Fig. S7



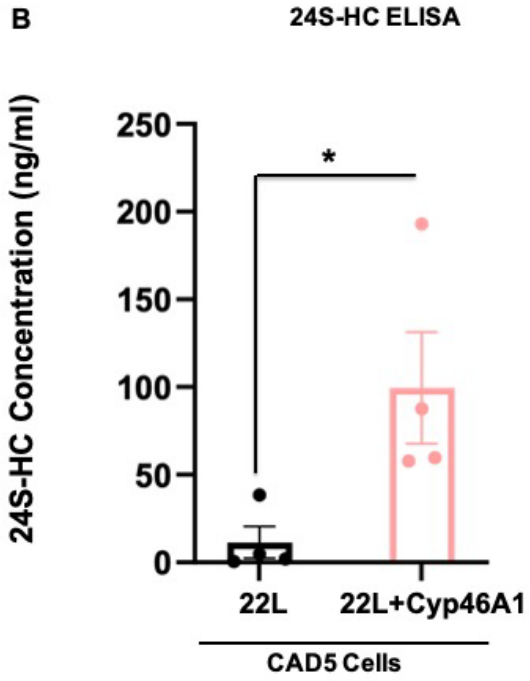


Fig. S7. (A) Immunoblot analysis of CYP46A1 in 22L-CAD cells and CYP46A1-overexpressing 22L-CAD5 cells. **(B)** ELISA quantification of the cholesterol metabolite 24S-hydroxycholesterol (24S-HC) in the media of 22L-CAD cells and CYP46A1-overexpressing 22L-CAD5 cells. The histograms represent the means ± SEM for n = 4 per group, obtained from 3 independent experiments. T-test (unpaired t test) was performed, and statistical significance is $p<0.05$.

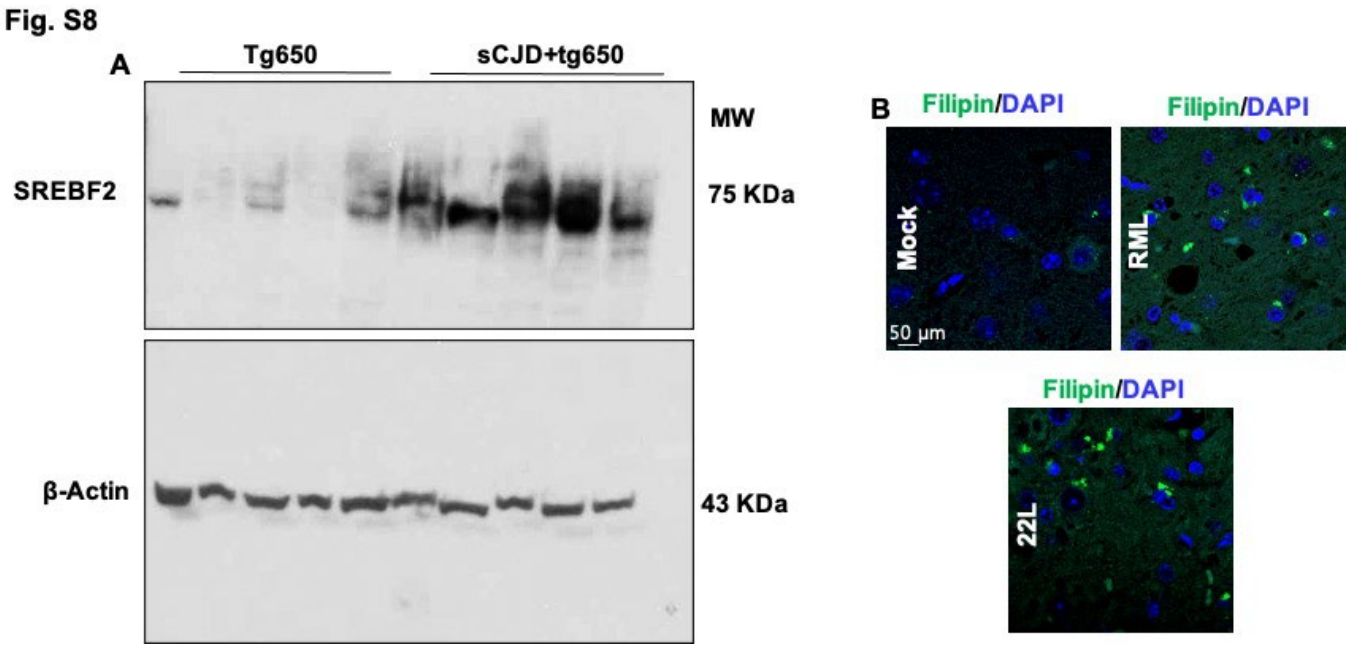


Fig. S8. (A) Analysis of uncropped immunoblot of SREBF2 protein in brain homogenates from five distinct non-infected (mock) tg650 mice and sCJD-tg650 mice at the terminal disease stage. **(B)** Filipin staining (green) and DAPI (blue) in brain sections from mock FVB mice and FVB mice infected with scrapie prions (RML and 22L). Magnification: 63X. Scale bar: 50 µm.