Amplifying the heat shock response ameliorates ALS and FTD pathology in mouse and human models.

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Supplementary Materials and Methods

Immunohistochemistry

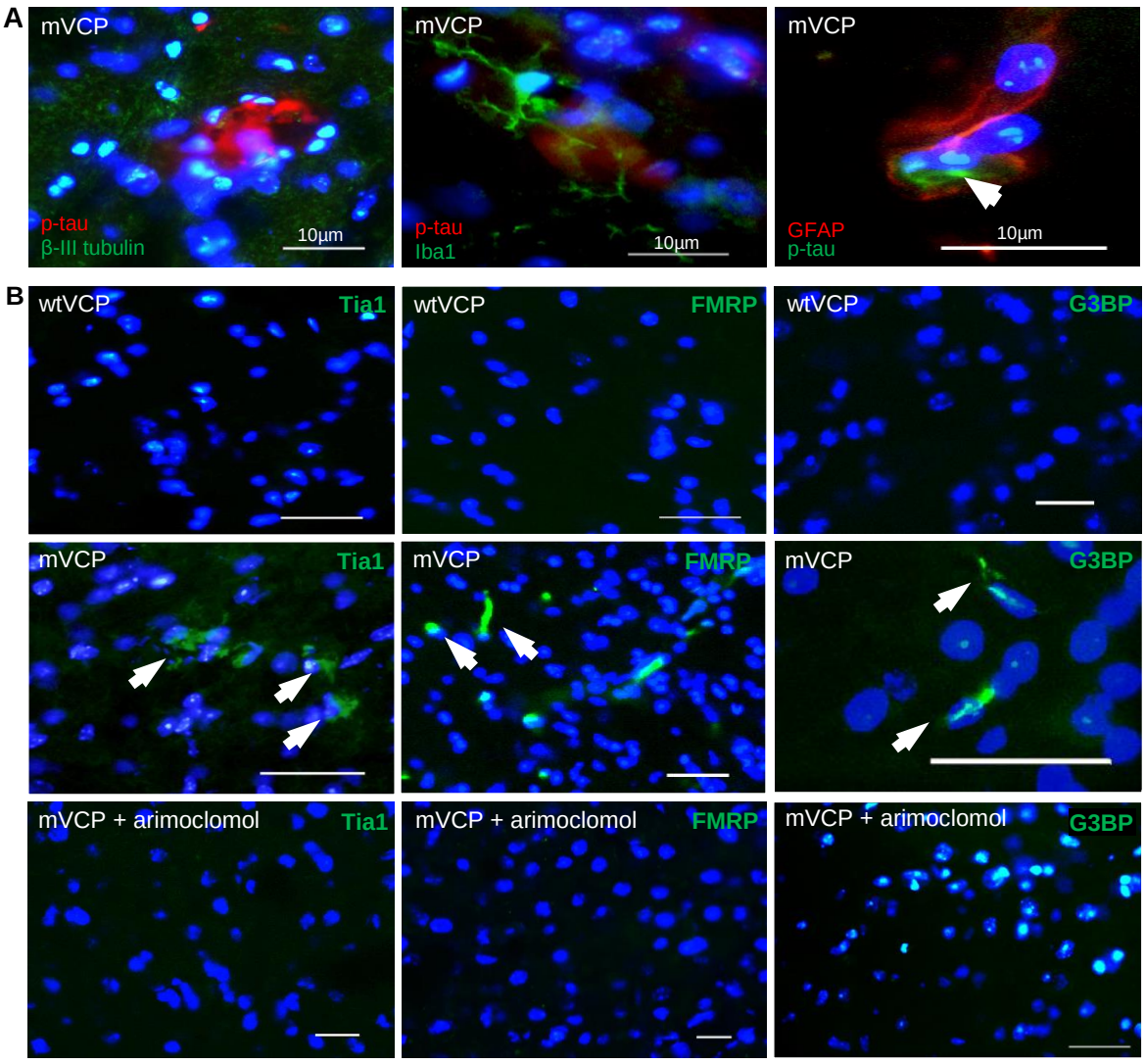
For immunofluorescent labelling, frozen sections were blocked for 1 hour at room temperature in blocking solution (10% normal goat serum in PBS + 0.1% Triton X-100), followed by incubation with primary antibodies against phosphorylated tau (AT8 Pierce Endogen Mouse monoclonal 1:100), Iba1 (Wako Chemicals 019-19741 Rabbit polyclonal 1:100), GFAP-Cy3 (Sigma G-A-5 Mouse monoclonal 1:1000), β-III tubulin (TUJ1, Cambridge Bioscience 3525-100 Rabbit polyclonal 1:100), Tia1 (Abcam ab205063 Rabbit polyclonal 1:50), G3BP (BD Bioscience Mouse monoclonal 1:200), FMRP (Abcam 1D10 Mouse monoclonal 1:200), for 1 hour at room temperature. Sections were washed in PBS and incubated for 2 hours at room temperature with the appropriate fluorescently labelled secondary antibodies and 4', 6-Diamidino-2-Phenylindole (DAPI; Sigma) to label nuclei. Sudan black was applied to sections for 10 minutes to quench autofluorescence prior to coverslip mounting. Tissue sections from three mice per experimental groups were assessed for each antibody tested and compared to negative controls run simultaneously. Final fluorescent images were visualised under a Leica fluorescent microscope and analysed using Leica Application Suite software (Leica Microsystems, Germany).

TUNEL Staining

The TACS® 2 TdT Fluorescein kit (Trevigen) was used for in situ detection of apoptosis by TUNEL in VCP patient fibroblasts using the manufacturer's instructions. Coverslips were counterstained with DAPI to label nuclei. Images were visualised using a Leica fluorescent microscope.

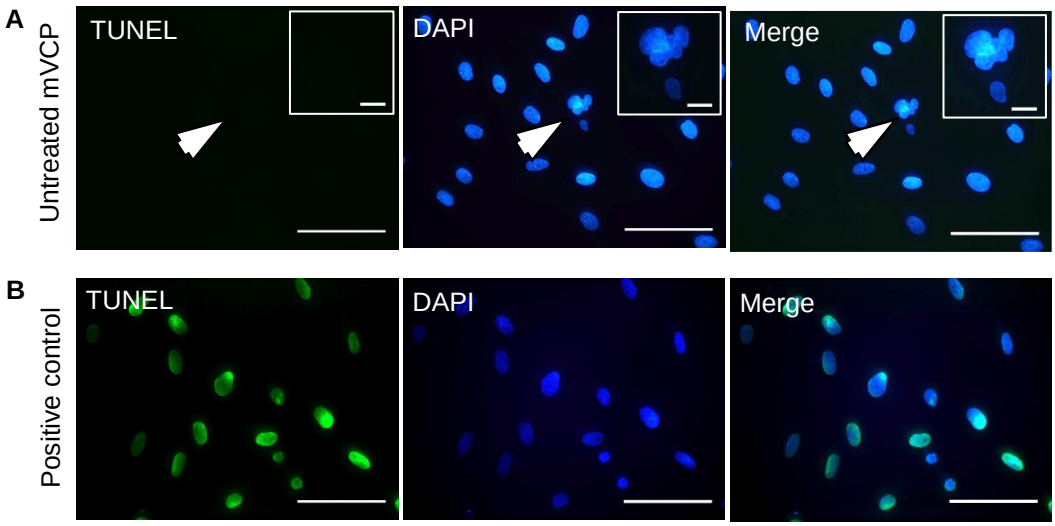
Supplementary Figures

Supplementary Fig 1



Sup Fig.1 Additional pathology in mouse brain study shows tau pathology and aggregation of stress granule markers. (**A**) Phosphorylated tau (p-tau) positive extracellular lesions in mVCP mouse brain co-immunostained with β-III tubulin, Iba1 and GFAP. (**B**) Representative images showing immunoreactivity of stress granule markers FMRP, Tia1 and G3BP in sagittal brain sections with and without arimoclomol treatment. DAPI labels nuclei (blue). Arrows indicate stress granule-positive aggregates. Scale bar = 20µm unless otherwise indicated.

Supplementary Fig 2.



Sup Fig 2. Abnormal nuclear morphology in mutant VCP patient fibroblasts show no evidence of apoptosis. TUNEL analysis of (**A**) untreated patient fibroblasts and (**B**) a TACS-Nuclease-treated positive control sample, co-stained with the nuclear marker DAPI (blue). Arrows indicate a disrupted nucleus that is TUNEL-negative. Scale bar= 50μm. Insets: higher magnification. scale bar= 10μm.

Supplementary Table 1. **FTD patient information**

Patient no.	Patient code	Age	Sex	PM delay (hours)	FTD sub-classification
1	P6/07	66	M	80.45	FTD associated with tau mutation
2	P65/10	62	M	93.00	FTD associated with mutation in TDP-43
3	P27/05	70	M	9.50	FTD with ubiquitinated inclusion bodies
4	P28/07	74	M	19.00	FTD associated with motor neuron disease
	Control code				
	P44/02	78	F	23.30	
	P47/11	79	F	78.50	
	P94/05	71	M	38.50	

Details of age, sex, post-mortem (PM) delay and diagnosed sub-classification for patients and healthy control from whom cortical brain samples were obtained for this study. No further information regarding the genetic diagnosis or phenotype was available for these patients.