Cell Reports, Volume *36*

Supplemental information

Neuronal VCP loss of function recapitulates FTLD-TDP pathology

Abubakar Wani, Jiang Zhu, Jason D. Ulrich, Abdallah Eteleeb, Andrew D. Sauerbeck, Sydney J. Reitz, Khalid Arhzaouy, Chiseko Ikenaga, Carla M. Yuede, Sara K. Pittman, Feng Wang, Shan Li, Bruno A. Benitez, Carlos Cruchaga, Terrance T. Kummer, Oscar Harari, Tsui-Fen Chou, Rolf Schröder, Christoph S. Clemen, and Conrad C. Weihl

Supplementary Figure 1

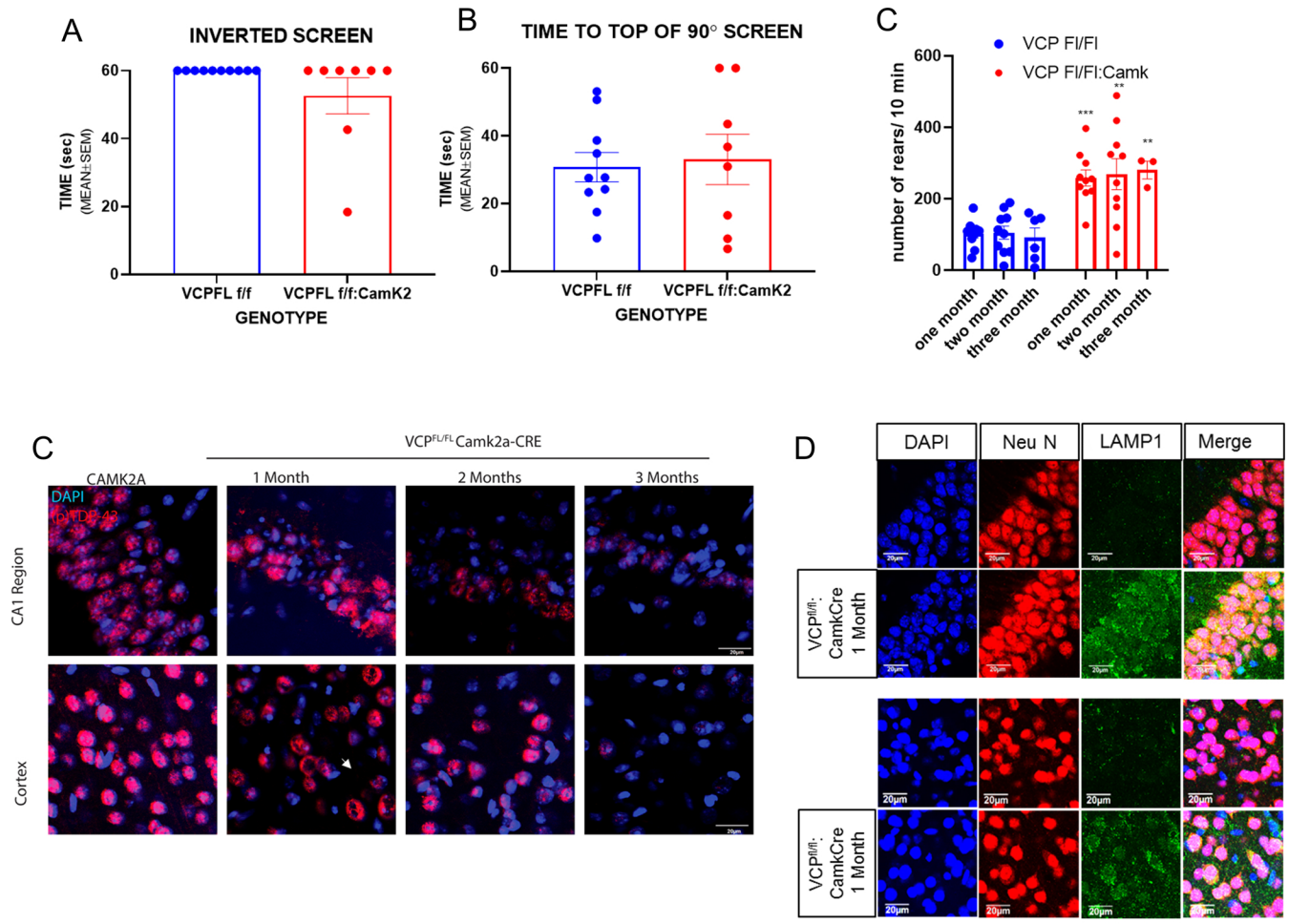

Figure S1. VCP cKO phenotypic data. Related to Figure 1, 2 and 3 (A) Graph of the latency to fall from an inverted screen or (B) time to climb to the top of a 90 degree screen for 2 month old control or VCP cKO mice. (C) Number of rears/10 min in VCP cKO mice are significantly higher than control at one, two and three months of age. (Paired T-test between genotype at the age performed ***p < 0.001 or **p < 0.01). Data represent mean ± SEM (error bars) (n=10 mice/group). Each dot represents an individual animal . Immunohistochemistry of cVCP KO mice. (D) Representative images of pTDP-43 (red) immunofluorescence and DAPI nuclear (blue) fluorescence of the cortex and CA1 region of the hippocampus from control (Camk2A) and 1, 2 and 3 month old VCP cKO mice. Closed arrows indicate cytosolic TDP-43. Scale bar is 20 uM. (E) Lamp1 (green) and NeuN (red) immunofluorescence and DAPI nuclear (blue) fluorescence of the cortex and CA1 region of the hippocampus from control (C57) and 1 month old VCP cKO mice. Scale bar is 20 uM.

Supplementary Figure 2

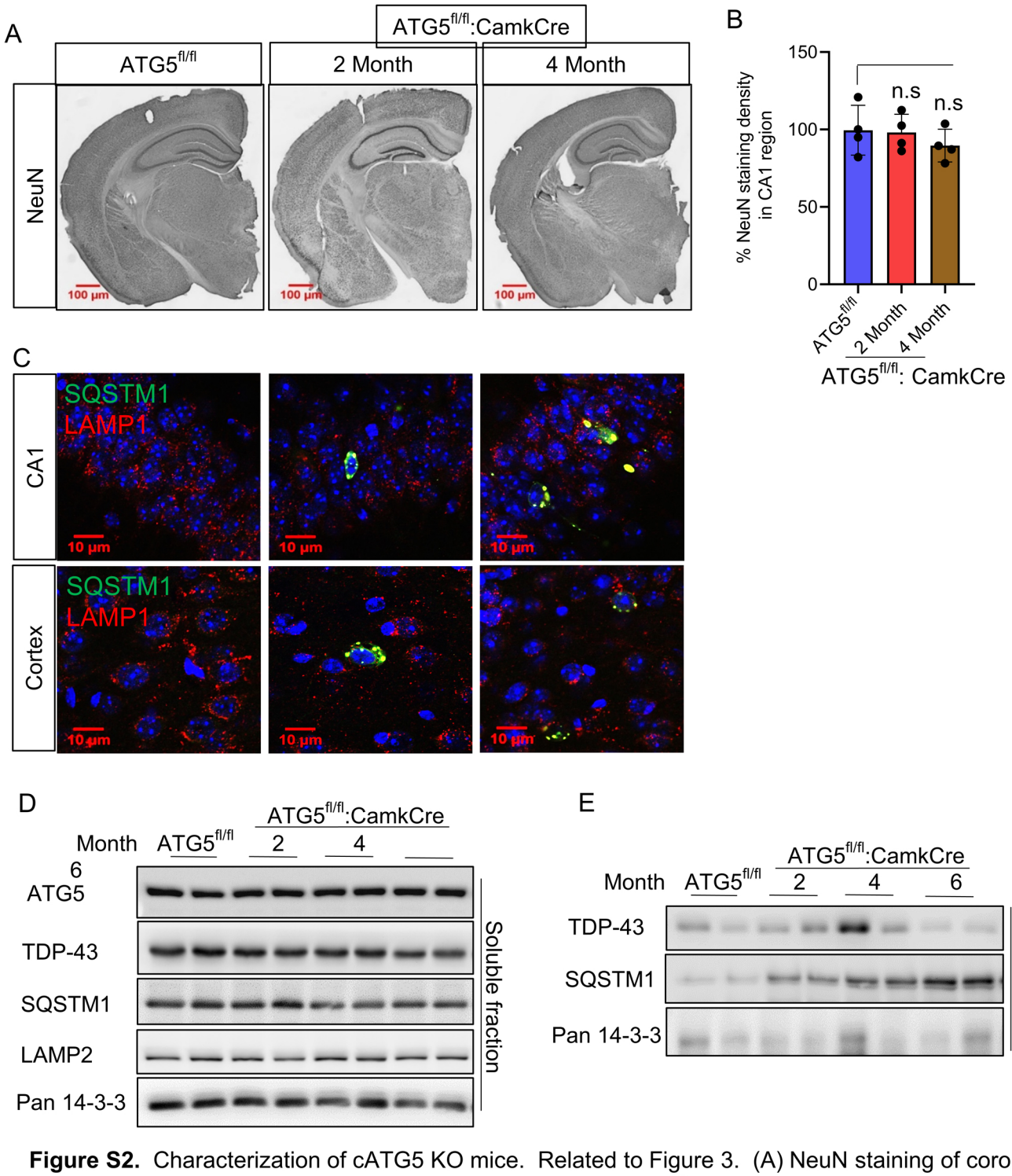

Figure S2. Characterization of cATG5 KO mice. Related to Figure 3. (A) NeuN staining of coronal sections through the cortex and hippocampus of control ($ATG5^{fl/fl}$) or 2, and 4 month old ATG5 cKO mice ($ATG5^{fl/fl}$:CamkCre). Scale bar is 100 uM. (B) Quantitation of NeuN staining in the CA1 region of the hippocampus from control or 2, and 4 month old ATG5 cKO mice. Data represent mean ± SD (error bars) (n= 3 slices from 4 mice per group). Unpaired t test were used for statistical comparisons, n.s = not significant. (C) SQSTM1 (green) and Lamp1 (red) immunofluorescence and DAPI nuclear (blue) fluorescence of the cortex and CA1 region of the hippocampus from control or 2, and 4 month old ATG5 cKO mice. Scale bar is 10 uM. (D) Fractionation of soluble and insoluble proteins from brain lysates of control or 2, 4, and 6 month old ATG5 cKO mice were immunoblotted with antibodies to ATG5, Lamp2, TDP-43, SQSTM1, and 14-3-3 (loading control).

Supplementary Figure 3

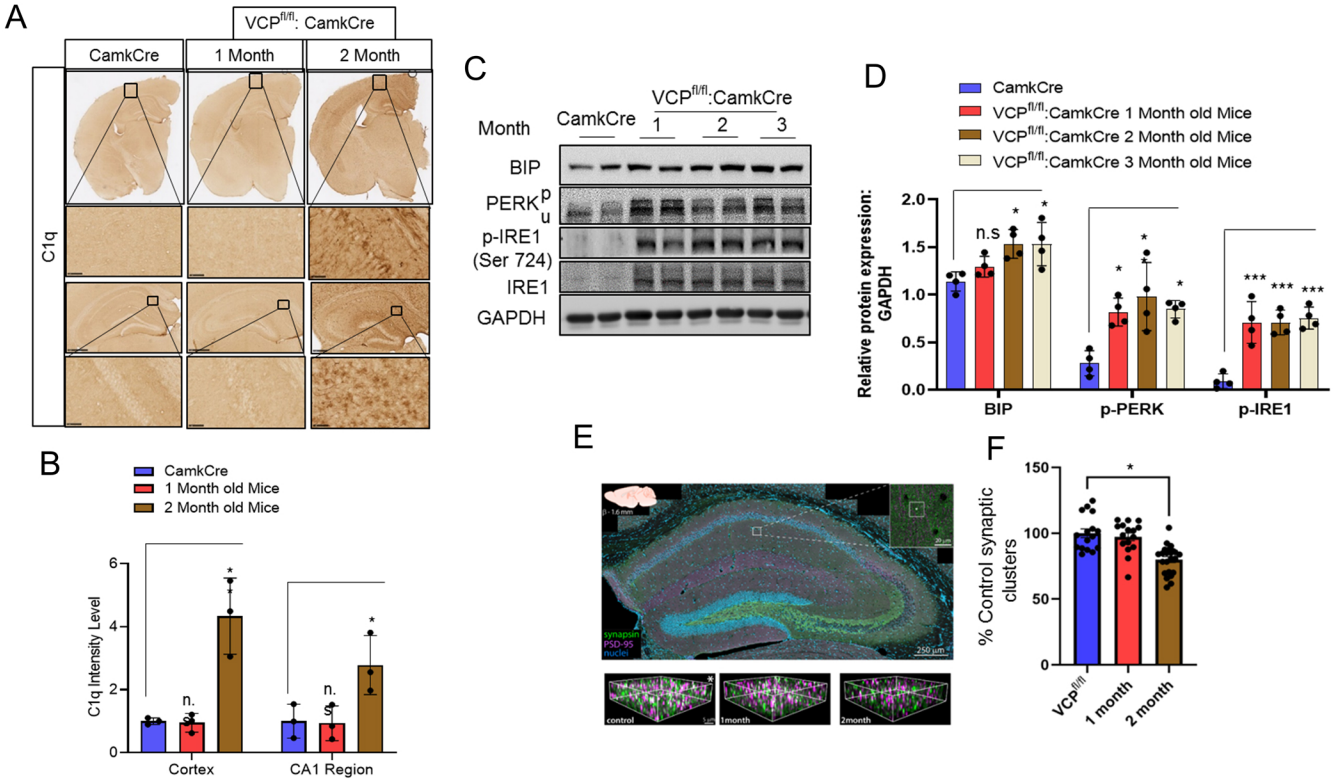

Figure S3. Proteomic and transcriptomic validation. Related to Figure 4. (A) C1q immunostaining of coronal sections through the cortex and hippocampus of control (Camkcre) or 1, 2 and 3 month old VCP cKO mice. Scale bar is 60 µm and 500 µm respectively. Insets and arrows denote the cortex or CA1 region of the hippocampus. Scale bar is 50 µm. (B) Quantitation of C1q staining in the cortex or CA1 region from control or 1, 2 and 4 month old VCP cKO mice, Data represent mean ± SD (error bars) (data points represent mouse) (n= 2 slices from 3 mice per group). A one-way ANOVA was used for statistical testing treating two slices from three mice as independent sample, one way ANOVA for cortex is $F(3, 8)= 12.21$ p=0.0023, for CA1 region is $F(3, 8)= 6.804$ p=0.0136. Post hoc comparisons using Tukey **p < 0.01. Data analyses was performed using GraphPad Prism, version 8.0. (C) Immunoblot for BIP, PERK, IRE1, pIRE1 and GAPDH (loading control) of brain lysates from control (CamkCre) and 1, 2 or 3 month old VCP cKO mice. (D) Quantitation band intensities of BIP, PERK, pIRE1 relative to GAPDH in control (CamkCre) and 1, 2 or 3 month old VCP cKO mice brains. Data represent mean ± SD (error bars) (data points represent mouse) (n=4 animals per group). A one-way ANOVA was used for statistical testing treating mice brains as independent sample, one way ANOVA for BIP is $F(3,12)= 6.176$ p<0.0088, for p-PERK is $F(3,12)= 8.708$ p=0.0024, for p-IRE1 is $F(3,12)= 19.18$ p<0.0001. Post hoc comparisons using tukey test *p < 0.05. Data analyses was performed using GraphPad Prism, version 8.0. (E) SEQUIN analysis of synaptic density across control, 1, and 2 month old VCP cKO mouse brains. Pre- and postsynaptic structures were labeled against synapsin 1/2, and PSD-95, respectively (see overview, from a control animal), and imaged at super-resolution in the CA1 stratum radiatum (see panels below). (F) SEQUIN quantification of synaptic loci by measurement of marker separation in 3-dimensions revealed a loss of synaptic loci at 2 months in VCP cKO animals. Data represent mean ± SEM (error bars) (data points represent fields). A one-way ANOVA was used for statistical testing treating each hemisphere as independent sample, one way ANOVA is $F(2,15)=4.484$ p=0.03. Post hoc comparisons using Tukey *p<0.02. Data analyses was performed using GraphPad Prism, version 8.0.

Supplementary Figure 4

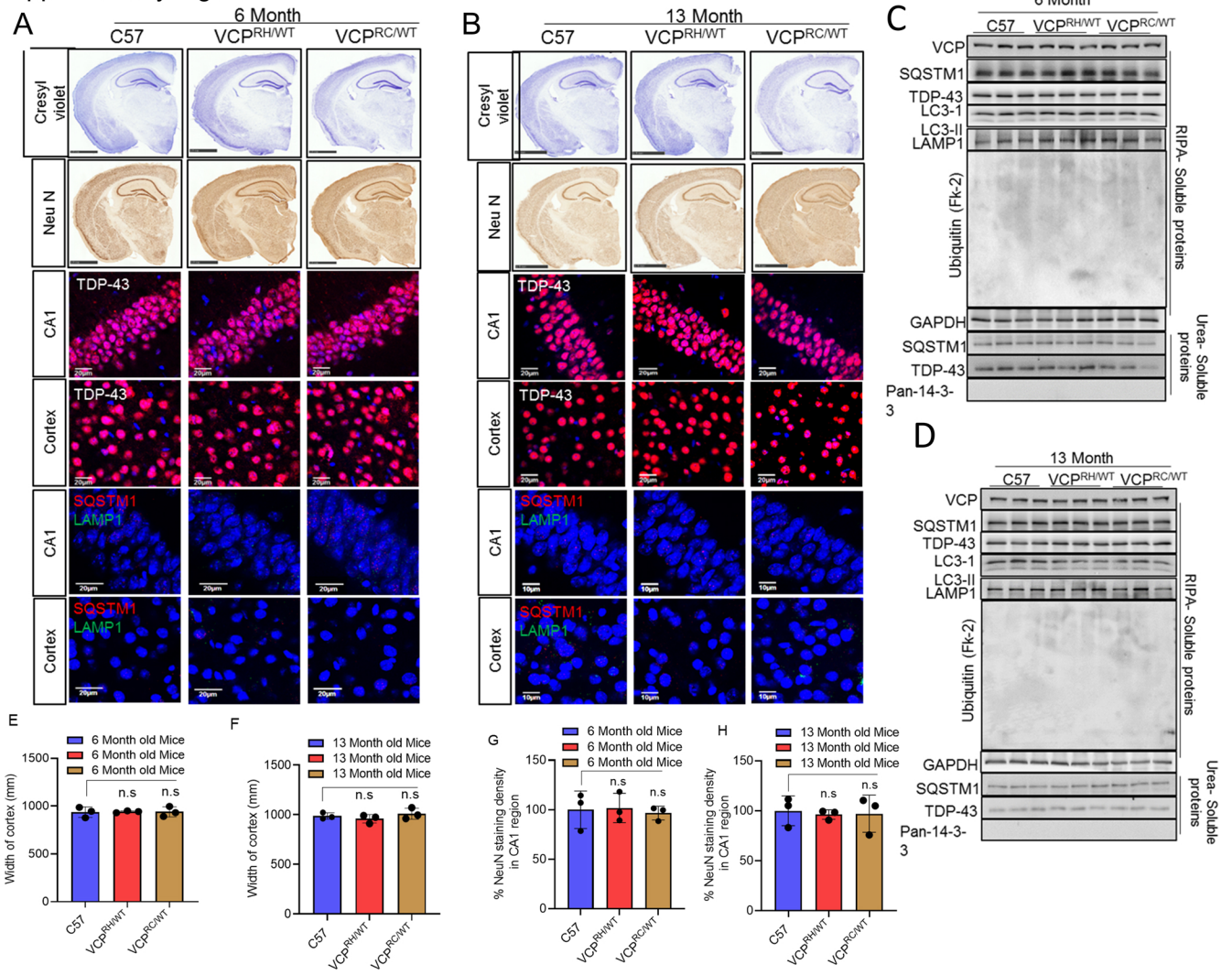

Figure S4. Characterization of VCP mutant mouse models. Related to Figure 5 and 6. (A) Coronal sections from 6 and (B) 13 month old C57 (control), VCP-R155H heterozygous ($VCP^{RH/WT}$) and VCP-R155C heterozygous ($VCP^{RC/WT}$) mouse brains. Sections were stained with cresyl violet, immunostained for NeuN (Scale bar is 2.5 mm) or immunofluorescence with TDP-43 (red) and DAPI nuclear (blue) fluorescence or SQSTM1 (red) and Lamp1 (green) immunofluorescence and DAPI (blue) fluorescence of the cortex and CA1 region of the hippocampus. Scale bar is 20 uM. (C) Fractionation of soluble and insoluble proteins from brain lysates of 6 and (D) 13 month old C57, $VCP^{RH/WT}$ and $VCP^{RC/WT}$ mouse brains were immunoblotted with antibodies to VCP, Lamp1, TDP-43, SQSTM1, LC3, ubiquitin conjugates (FK2), GAPDH (loading control) and 14-3-3 (loading control). E-F) Bar graph of cortical width from 6 and 13 month old control C57, $VCP^{RH/WT}$ and $VCP^{RC/WT}$ mouse brains. Data represent mean ± SD (error bars) (data points represent mouse) (n= 2 slices from 3 mice per group). A one-way ANOVA was used for statistical testing treating two slices from three mice as independent sample, one way ANOVA for 6 months is $F(2, 6)= 0.00846$ p=0.9916. 9 months $F(2, 6)= 0.9934$ p=0.4240 Post hoc comparisons using Tukey **p < 0.01. Data analyses was performed using GraphPad Prism, version 8.0. (G-H) Quantitation of NeuN staining in the CA1 region of the hippocampus from control C57, $VCP^{RH/WT}$ and $VCP^{RC/WT}$ mouse brains. Data represent mean ± SD (error bars) (n= 2 slices from 3 mice per group) . Data represent mean ± SD (error bars) (data points represent mouse) (n= 2 slices from 3 mice per group). A one-way ANOVA was used for statistical testing treating two slices from three mice as independent sample, one way ANOVA for 6 months is $F(2, 6)= 0.09791$ p=0.9081. 9 months $F(2, 6)= 0.06095$ p=0.9414 Post hoc comparisons using Tukey **p < 0.01. Data analyses was performed using GraphPad Prism, version 8.0

Supplementary Figure 5

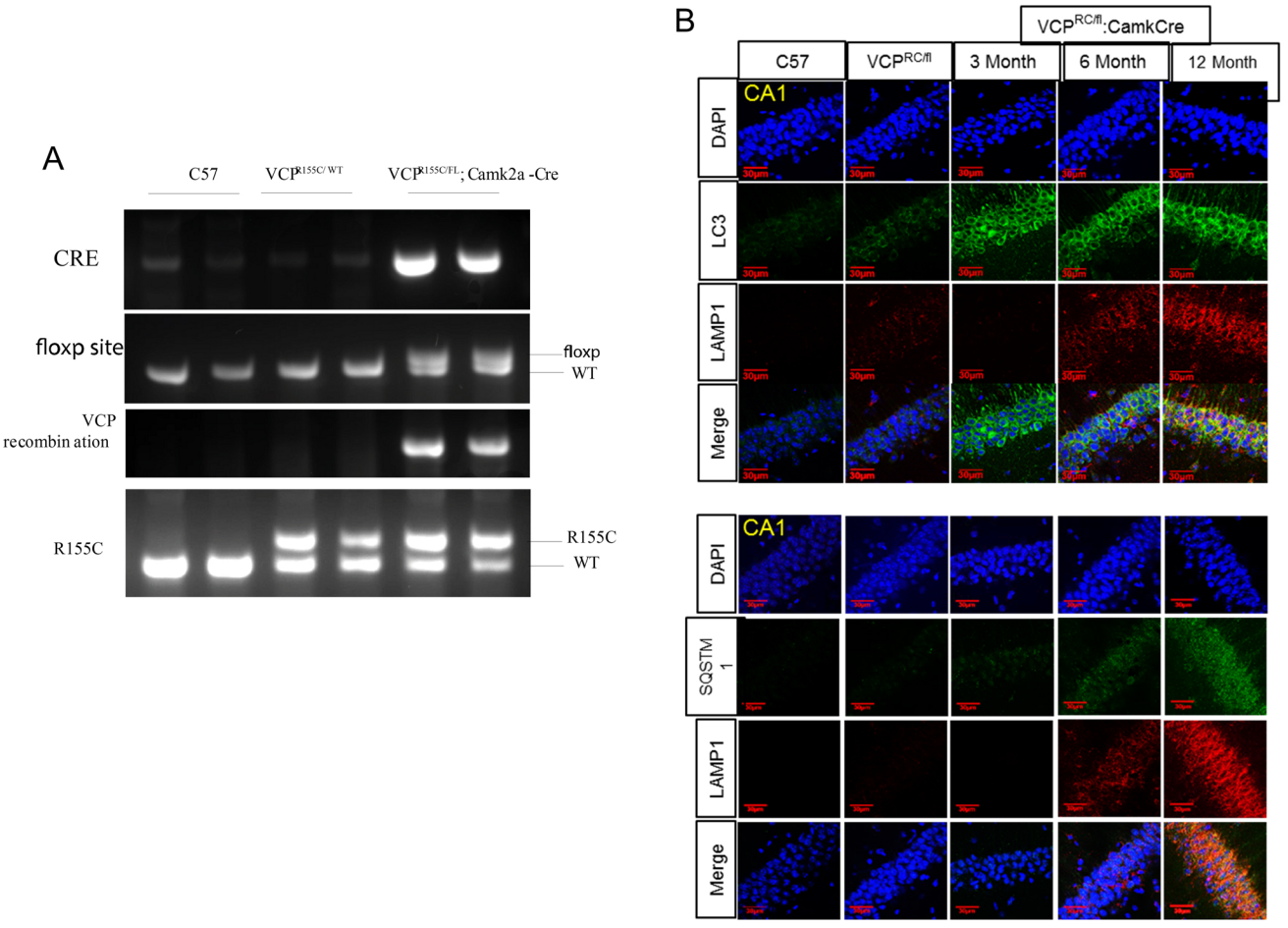

Figure S5. Characterization of VCP cRC mice. Related to Figure 5 and 6. (A) Agarose gels of cortical brain genomic DNA PCR amplification of cre, VCP loxP allele, evidence LoxP recombination and the VCP RC allele. Note that only in the VCP cRC mice does both VCP recombination of the floxed allele and presence of the VCP RC allele remain. (B) LC3 (green), Lamp1 (red) immunofluorescence and DAPI nuclear (blue) fluorescence of the CA1 region of the hippocampus from nine month old control (C57), nine month old VCP-R155C heterozygous ($VCP^{RC/FL}$), or VCP cRC ($VCP^{RC/FL}$:CamkCre) mice at 3, 6 and 12 months of age. Scale bar is 30 uM. (B) SQSTM1 (green), Lamp1 (red) immunofluorescence and DAPI nuclear (blue) fluorescence of the CA1 region of the hippocampus from nine month old control (C57), nine month old VCP-R155C heterozygous ($VCP^{RC/FL}$), or VCP cRC ($VCP^{RC/FL}$:CamkCre) mice at 3, 6 and 12 months of age. Scale bar is 30 uM.

Supplementary Figure 6

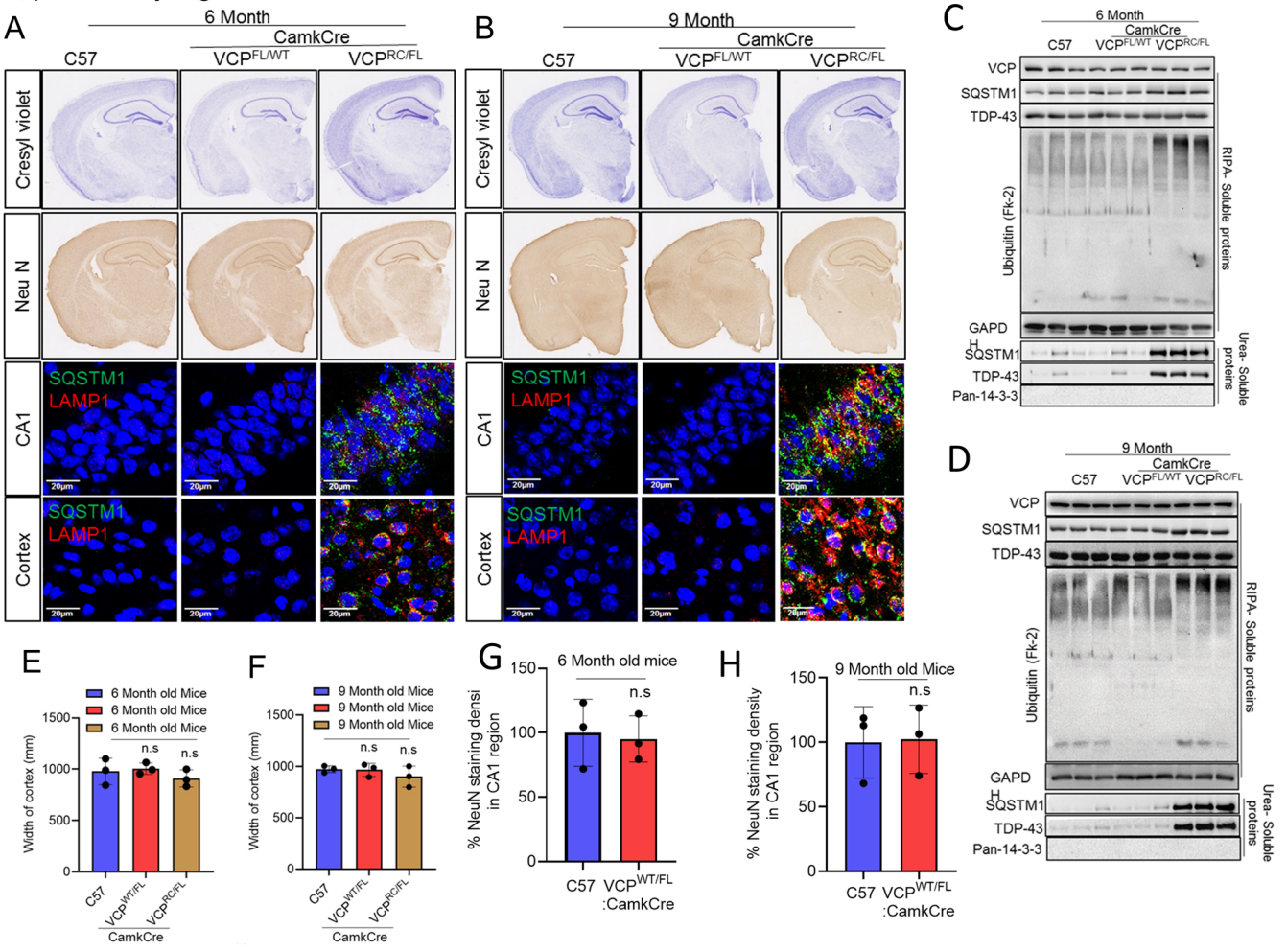

Figure S6. Characterization of hemizygous cVCP KO. Related to Figure 5 and 6. (A) Coronal sections from 6 and (B) 9 month old C57 (control), VCP hemi-floxed ($VCP^{WT/FL}$:CamkCre), and VCP cRC ($VCP^{RC/FL}$:CamkCre) mouse brains. Sections were stained with cresyl violet, immunostained for NeuN (Scale bar is 2.5 mm) or immunofluorescence with TDP-43 (red) and DAPI nuclear (blue) fluorescence or SQSTM1 (red) and Lamp1 (green) immunofluorescence and DAPI (blue) fluorescence of the cortex and CA1 region of the hippocampus. Scale bar is 20 uM. (C) Fractionation of soluble and insoluble proteins from brain lysates of 6 and (D) 9 month old C57, VCP hemi-floxed ($VCP^{WT/FL}$:CamkCre), and VCP cRC mouse brains were immunoblotted with antibodies to VCP, Lamp1, TDP-43, SQSTM1, LC3, ubiquitin conjugates (FK2), GAPDH (loading control) and 14-3-3 (loading control). (E-F) Bar graph of cortical width from 6 and 9 month old C57, VCP hemi-floxed ($VCP^{WT/FL}$:CamkCre), and VCP cRC mouse brains. Data represent mean ± SD (error bars) (data points represent mouse) (n= 2 slices from 3 mice per group). A one-way ANOVA was used for statistical testing treating two slices from three mice as independent sample, one way ANOVA for 6 months is $F(2, 6)= 0.8112$ $p=0.4877$. 9 months $F(2, 6)= 0.9155$ $p=0.4496$ Post hoc comparisons using Tukey $^{**}p < 0.01$. Data analyses was performed using GraphPad Prism, version 8.0. (G-H) Quantitation of NeuN staining in the CA1 region of the hippocampus from 6 and 9 month old C57, VCP hemi-floxed ($VCP^{WT/FL}$:CamkCre) mouse brains. Data represent mean ± SD (error bars) (n= 2 slices from 3 mice per group). One-way ANOVA test followed by tukey was used for statistical comparisons, *** $p< 0.001$; **** $p < 0.0001$; n.s = not significant. $P=0.8076$ with unpaired t test for 6 and 9 months against C57 Data analyses was performed using GraphPad Prism, version 8.0.

Supplementary Figure 7

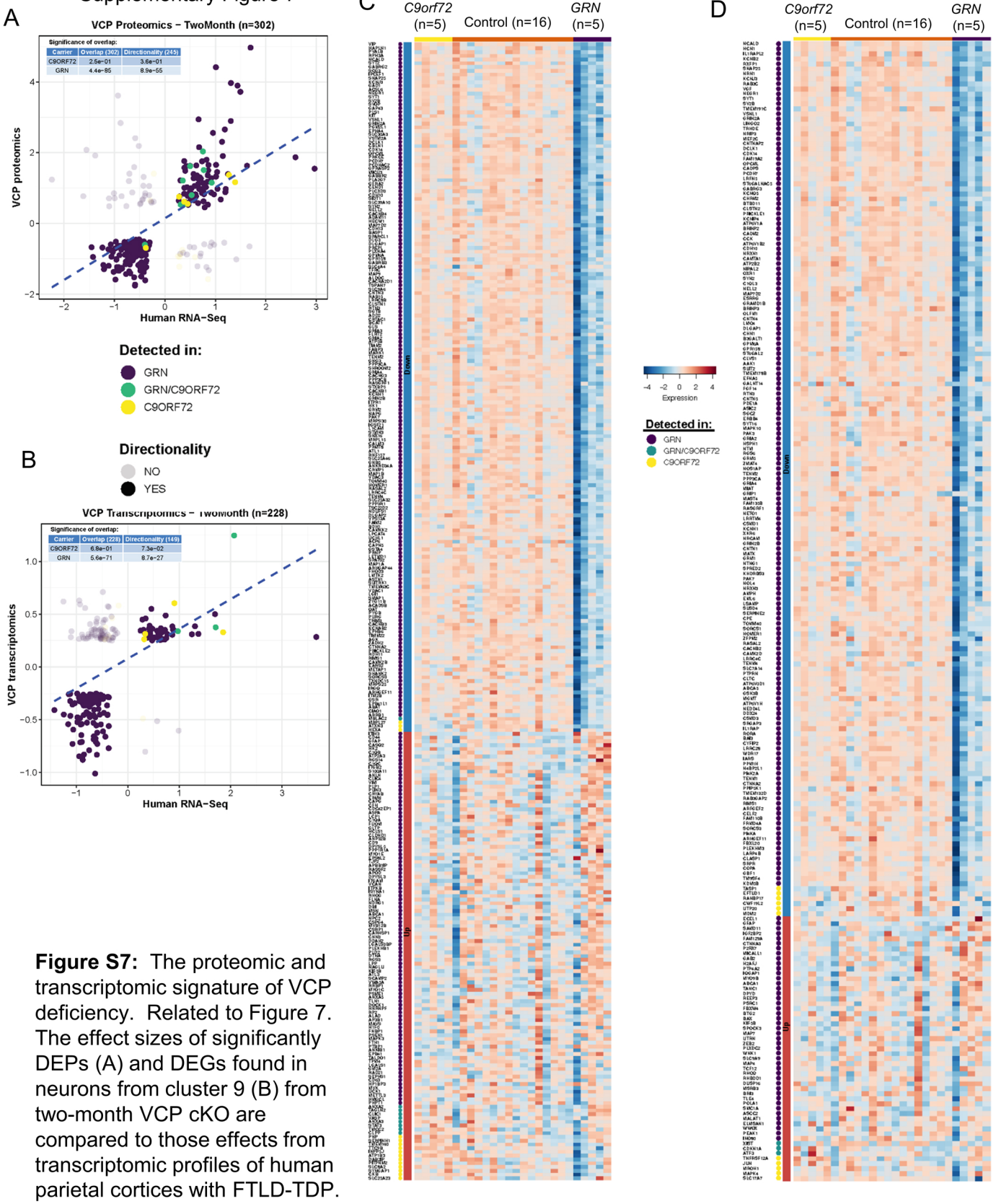

Figure S7: The proteomic and transcriptomic signature of VCP deficiency. Related to Figure 7. The effect sizes of significantly DEPs (A) and DEGs found in neurons from cluster 9 (B) from two-month VCP cKO are compared to those effects from transcriptomic profiles of human parietal cortices with FTLD-TDP. (C) A heatmap representing the gene expression in TPM for significant up/down-regulated genes overlapping between VCP cKO DEPs and FTLD-TDP *GRN* and *C9orf72* carriers indicated in panel A. The genes are ordered by their genetic strata and logFC. (D) Same as panel C, but showing the genes overlapping between VCP cKO neuronal DEGs and brain transcriptomics from FTLD-TDP *GRN* and *C9orf72* carriers reported in panel B.

Table S1: Demographics and RNA technical details for patients with *GRN, C9ORF72* variants, and control individuals. Related to Methods Human brain sample RNA Sequencing section.

	GRN	C9orf72	Control
Sample size	5	5	16
Age	71.41 ± 12.58	74.74 ± 8.86	87.21 ± 10.82
PMI	20.62 ± 6.10	9.15 ± 5.54	9.86 ± 5.35
Male %	60	80	31
RIN	4.82 ± 1.44	6.66 ± 0.63	6.41 ± 1.40
DV200	82.8 ± 6.97	89.6 ±1.34	91.06 ± 2.48
CDR	2.4 ± 1.34	2.5 ± 1.11	0.18 ± 0.25
CDR < 1	1	1	16
CDR >=1	4	4	0
Number of Total Reads (Million)	56.57 ± 3.17	53.52 ± 9.79	59.04 ± 3.46
Uniquely Mapped Reads %	67.89 ± 14.26	77.97 ± 4.33	77.58 ± 4.75
Mapped to Multiple Loci Reads %	22.59 ± 10.42	15.16 ± 2.41	15.13 ± 2.66